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Total Synthesis of Farnesyl Lipid I/II Analogs
and
Contributions to the Total Synthesis of Vancoresmycin

Dissertation

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Publikationen

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[2] A. Babczyk, L. M. Wingen (Co-First Authorship), D. Menche, *Eur. J. Org. Chem.* **2020**, 6645–6648.

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Abbreviations

Ac	Acetyl
Abu	Aminobutyric acid
Ala	Alanine
Alloc	Allyloxycarbonyl
Bn	Benzyl
Boc	*tert*-Butyloxycarbonyl
brsm	Based on recovered starting material
calcd	Calculated
CDI	1,1'-Carbonyldiimidazole
***c*Hex**	Cyclohexyl
COSY	Correlation spectroscopy
CSA	Camphorsulfonic acid
2CTC	2-Chlorotrityl chloride
CyH	Cyclohexane
d	day
DCC	*N*,*N*'-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
Dha	Dehydroalanine
Dhb	Dehydrobutyrine
DIBAL-H	Diisobutylaluminiumhydrid
DIPEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMP	Dess-Martin periodinane
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dr	Diastereomeric ratio
ee	Enantiomeric excess
EI	Electron ionization
ESI	Electrospray ionization
equiv	Equivalent
et al.	And others
Et	Ethyl
Et_2O	Diethyl ether
EtOAc	Ethyl acetate
Fmoc	Fluorenylmethyloxycarbonyl
GlcNAc	*N*-Acetylglucosamine
Glu	Glutamic acid
Gly	Glycine
h	Hour
HBTU	Hexafluorophosphate benzo-triazole tetramethyl uranium
HFIP	Hexafluoro-2-propanol
HMBC	Heteronuclear multiple bond correlation
HOBt	Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High-resolution mass spectrometry
HSQC	Heteronuclear single quantum correlation
HWE	Horner-Wadsworth-Emmons
Hz	Hertz
IBX	2-Iodoxybenzoic acid
Ipc	Isopinocampheyl

KHMDS	Potassium bis(trimethylsilyl)amide
LLS	Longest linear sequence
Lys	Lysine
***m*-CPBA**	*meta*-Chloroperoxybenzoic acid
Me	Methyl
MeCN	Acetonitrile
MeOH	Methanol
MIC	Minimum inhibitory concentration
min	Minute
MNBA	2-Methyl-6-nitrobenzoic anhydride
MS	Molecular sieve or mass spectrometry
MTPA	α-Methoxy-α-trifluoromethylphenylacetic acid
MurNAc	*N*-Acetylmuramic acid
NADPH	Nicotinamide adenine dinucleotide phosphate
NBS	*N*-Bromosuccinimide
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser enhancement spectroscopy
PBP	Penicillin-binding protein
PGN	Peptidoglycan
Ph	Phenyl
PMB	*p*-Methoxybenzyl
ppm	Parts per million
***p*-TsOH**	*p*-Toluenesulfonic acid
py	Pyridine
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
rt	Room temperature
SAD	Sharpless asymmetric dihydroxylation
SAE	Sharpless asymmetric epoxidation
SAR	Structure-activity relationship
SPPS	Solid-phase peptide synthesis
TBAF	Tetra-*n*-butylammonium fluoride
TBAP	Tetrabutylammonium phosphate
TBS	*tert*-Butyldimethylsilyl
TCA	Trichloroacetonitril
Teoc	2-(Trimethylsilyl)ethoxycarbonyl
TFA	Trifluoroacetic acid
TFFH	Tetramethylfluoroformamidinium hexafluorophosphate
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMSE	2-(Trimethylsilyl)ethyl
UDP	Uridine diphosphate
UMP	Uridine monophosphate
WHO	World Health Organization

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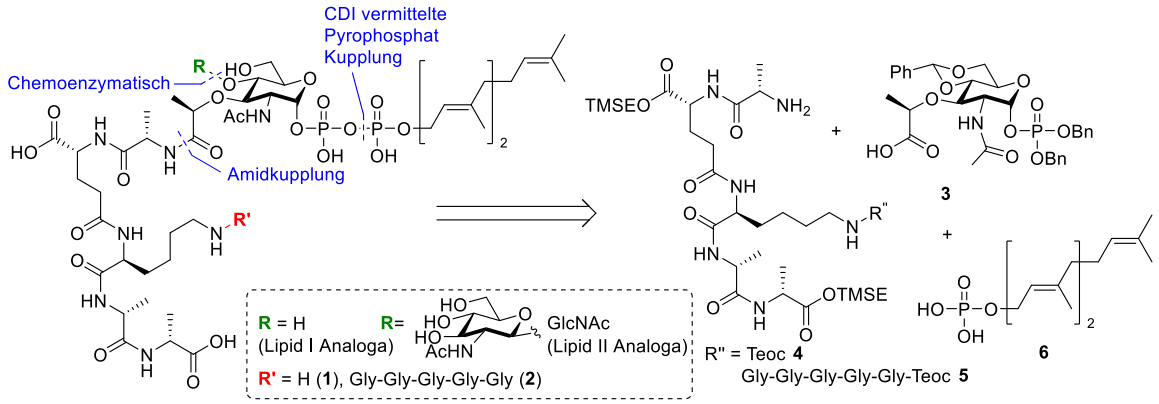
1 Kurzzusammenfassung/Abstract

Kurzzusammenfassung Teil I: Totalsynthese von Farnesyl Lipid I/II Analoga

Der erste Teil dieser Dissertation befasst sich mit der Totalsynthese von Farnesyl Lipid I Analoga **1** und **2** (**Schema 1**). Diese Verbindungen stellen Analoga des natürlichen Zellwandvorläufers Lipid I dar, in dem anstelle des Farnesylrestes ein Undecaprenylrest vorhanden ist. Dieser Undecaprenylrest des natürlichen Peptidoglykanbausteins erschwert strukturelle und biologische Untersuchungen aufgrund ungünstiger physikalisch-chemischer Eigenschaften wie Aggregation und komplexer spektroskopischer Charakteristika.[1] Dennoch ist es wichtig, die Forschung mit diesen Zellwandbausteinen fortzusetzen, da sie eines der Hauptangriffsziele für Antibiotika sind.[2] Die verkürzten Farnesyl Lipid I Analoga sollten substanzielle Untersuchungen ohne die Nachteile des langen Lipidrestes ermöglichen. Neben der Synthese des Lipid I Analogon **1** wird diese Arbeit auch über die erste Totalsynthese des Lipid I Analogon **2** berichten, welches die *S. aureus* spezifische Peptidmodifikation aufweist, bei der sich ein Pentaglycinrest an der Seitenkette des Lysins befindet.[3]



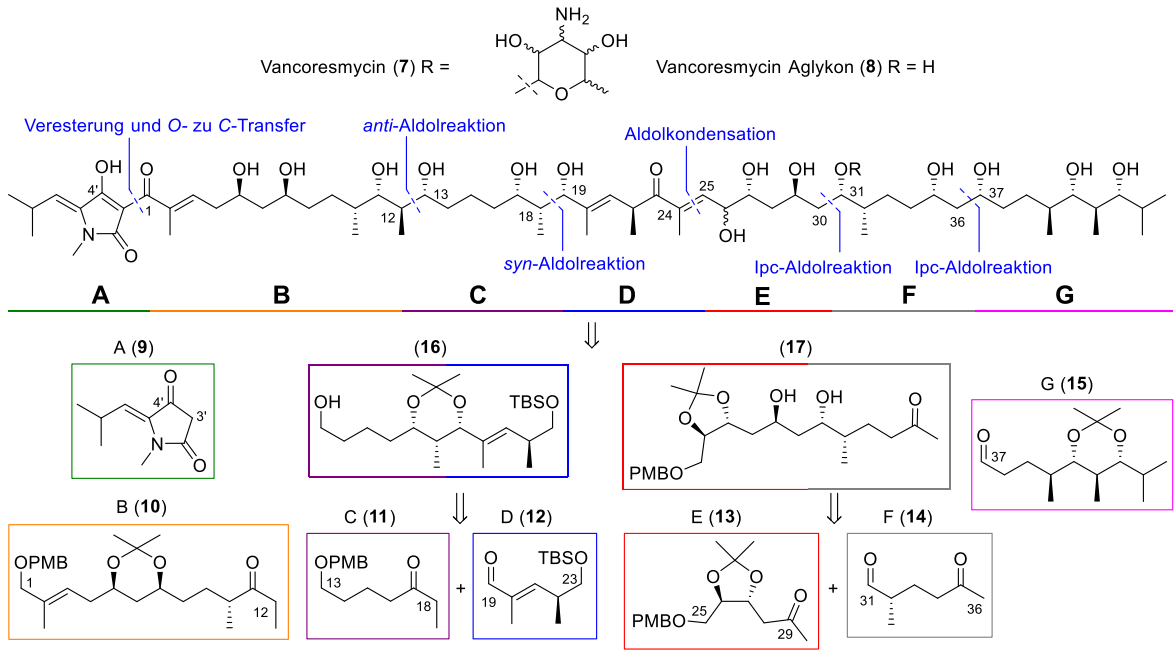
***Schema 1*:** Synthetisierte Lipid I Analoga (**1**, **2**) und ihre retrosynthetische Analyse.

Im retrosynthetischen Ansatz wurde eine konvergente und modulare Synthesestrategie verfolgt, bei der die Lipid I Analoga in die drei Bausteine **3**, **4** (**5**) und **6** unterteilt wurden. Um das jeweilige Peptid **4** oder **5** an das Kohlenhydratgerüst **3** zu kuppeln, wurde eine PyBOP vermittelte Amidbildung vorgesehen. Für die Kupplung des daraus resultierenden Glykopeptids mit dem Farnesylphosphat **6** wurde eine CDI-vermittelte Pyrophosphatkupplung als Schlüsselschritt gewählt. Die Implementierung einer neuartigen, hoch ergiebigen Festphasen-peptidsynthese für Peptid **4** ermöglichte die Synthese des Lipid I Analogons **1** in einer Ausbeute von 19% über die längste lineare Sequenz (LLS) von 16 Stufen. Darüber hinaus wurde eine

modifizierte Festphasenpeptidsynthese entwickelt, um das Decapeptid **5** zu gewinnen, das für die Totalsynthese des Lipid I Analogons **2** verwendet wurde und was zu einer Gesamtausbeute von 8% über 26 Schritte in der LLS führte. Nach erfolgreichem Syntheseabschluss der beiden Lipid I Analoga wurden mehrere Kooperationen initiiert, von denen sich neue Einblicke in die Zellwandbiosynthese und Antibiotika-Interaktionen erhofft werden. Unter anderem wurde eine Zusammenarbeit mit der Gruppe von Prof. Dr. *Tanja Schneider* gestartet. Mittels dieser Kooperation wurden die bereitgestellten Lipid I Analoga (**1** und **2**) chemoenzymatisch in ihre jeweiligen Lipid II Analoga (R = GlcNAc) überführt.

Kurzzusammenfassung Teil II: Beiträge zur Totalsynthese von Vancoresmycin

Der zweite Teil dieser Arbeit befasst sich mit Vancoresmycin (**7**, **Schema 2**), welches erstmals 2002 aus der Fermentation des Aktinomyceten *Amycolatopsis* sp. ST 101170 isoliert wurde.[4] Dieser natürliche Metabolit ist durch eine hoch funktionalisierte Tetramsäure charakterisiert, die mit einer langen Polyketidkette verbunden ist, welche eine Vielzahl an stereogenen Zentren sowie einen Kohlenhydratrest aufweist. Neben der faszinierenden Struktur und den bisher nur vorgeschlagenen stereogenen Zentren wurden mehrere potente antibiotische Aktivitäten gegen pathogene Bakterien bestimmt, was Vancoresmycin (**7**) und das entsprechende Aglykon **8** zu einem attraktiven Ziel für eine Totalsynthese macht.[5]



***Schema 2*:** Retrosynthese von Vancoresmycin Aglykon **8** und die resultierenden Fragmente A-G (**9**-**15**).

Diese Dissertation beschreibt die erste Retrosynthese von Vancoresmycin Aglykon **8**, in der die Verbindung in sieben Fragmente A-G (**9**-**15**, **Schema 2**) unterteilt wurde. Nach Planung eines hochkonvergenten und leicht modifizierbaren Synthesewegs konnten in dieser Arbeit die Fragmente A-E (**9**-**13**) erfolgreich aufgebaut werden, während die Fragmente F (**14**) und G (**15**) von *Stefanie Spindler* synthetisiert wurden. Für jedes Fragment wurde die Gesamtausbeute, die längste lineare Sequenz und ausgewählte Schlüsselschritte in **Tabelle 1** zusammengefasst. Zusätzlich wurden die Fragmente C (**11**) und D (**12**) sowie E (**13**) und F (**14**) bereits gekuppelt und modifiziert, um die Verbindungen **16** und **17** zu erhalten.

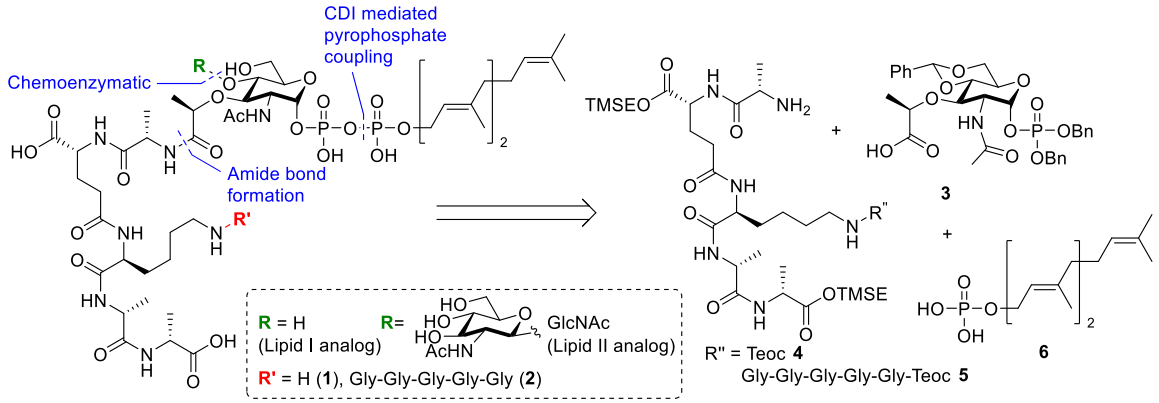
Tabelle 1: Synthetisierte Fragmente mit Schlüsselschritten, Ausbeuten und LLS.

Fragment	**Schlüsselschritte**	**Ausbeute [%]**	**LSS**
A (**9**)	Aldolkondensation	79	3
B (**10**)	HWE-Reaktion, *Paterson*-Aldolreaktion, *Narasaka-Prasad*-Reduktion	3	14
C (**11**)	*Grignard*-Reaktion	58	4
D (**12**)	HWE-Reaktion	52	6
E (**13**)	HWE-Reaktion, *Sharpless*-Dihydroxylierung	29	11
16	*syn*-Aldolreaktion, *Narasaka-Prasad*-Reduktion	5	10
17	Domino-Aldolkupplung/Reduktionssequenz	4	12

Darüber hinaus wurden mit Fragment A (**9**) Veresterungsreaktionen durchgeführt, um verschiedene Reste an der 4'-OH-Position einzuführen. Ein anschließender Sauerstoff-Kohlenstoff-Transfer ergab mehrere Vancoresmycin-ähnliche 3-Acyltetramsäuren, die von der Gruppe um Prof. Dr. *Tanja Schneider* auf ihre antibiotischen Eigenschaften getestet wurden. Interessanterweise zeigten einige der Verbindungen, welche am authentischsten zum Naturstoff waren, bereits antibiotische Aktivität. Daher wurde angenommen, dass die Tetramsäureeinheit einen wichtigen Bestandteil des Pharmakophors von Vancoresmycin bildet.[6] Erstaunlicherweise zeigte ein NMR-Vergleich zwischen ausgewählten Vancoresmycin-fragmenten und dem Naturstoff signifikante Diskrepanzen in der C-38–C-45-Region. Aufgrund dieser Unstimmigkeit wurde eine umfangreiche bioinformatische Analyse eingeleitet, die einen neuartigen Vorschlag für die Vancoresmycin-Stereostruktur ergab.[7]

Abstract Part I: Total Synthesis of Farnesyl Lipid I/II Analogs

The first part of this dissertation deals with the total synthesis of farnesyl lipid I analogs **1** and **2** (**Scheme 1**). These compounds represent analogs of the natural cell wall precursor lipid I in which an undecaprenyl tail is present instead of the farnesyl residue. This full length 55-carbon lipid tail of the natural peptidoglycan building block hampers structural and biological studies due to unfavorable physicochemical properties, including precipitation, aggregation and complex spectroscopic characteristics.[1] Nevertheless, it is vital to continue research with these cell wall building blocks, as they are one of the prime targets for antibiotics.[2] Thus, the farnesyl lipid I analogs should allow substantial studies without the drawbacks of the full length lipid tail. Besides the generation of the more general lipid I analog **1**, this work also reports on the first total synthesis of lipid I analog **2**, which includes the *S. aureus* specific peptide modification, being the attached pentaglycine linked to the lysine side chain.[3]



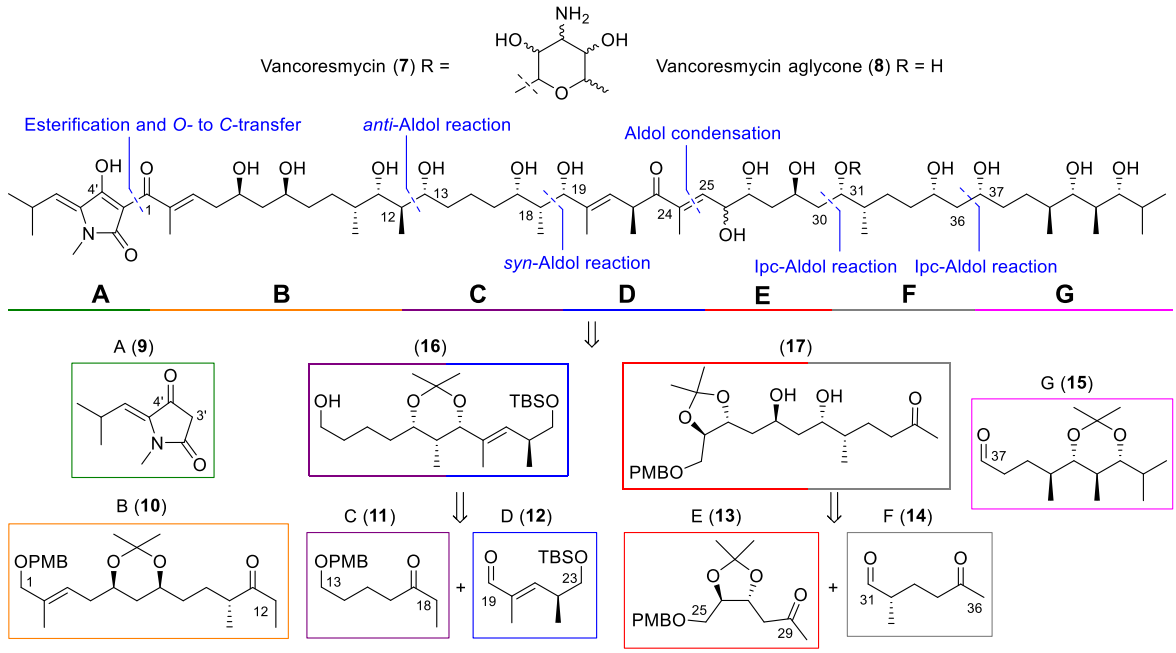
***Scheme 1*:** Synthesized lipid I analogs (**1**, **2**) and their retrosynthetic analysis.

In the retrosynthetic approach, the lipid I analogs were dissected into the three building blocks **3**, **4** (**5**) and **6**, which support a convergent and modular synthesis strategy. Key steps for their connection were an amide bond formation using PyBOP to attach the respective peptide **4** or **5** to the carbohydrate **3** as well as a CDI mediated pyrophosphate coupling to link the farnesyl phosphate **6** to the generated glycopeptide. The implementation of a novel high yielding solid-phase peptide synthesis (SPPS) for **4** allowed the generation of lipid I analog **1** in 19% yield over the longest linear sequence (LLS) of 16 steps. In addition, a modified SPSS was developed to yield decapeptide **5**, which was used for the total synthesis of lipid I analog **2** leading to an overall yield of 8% over 26 steps in its LLS.

After the successful synthesis of the two lipid I analogs, several cooperations, which are believed to give novel insights into the cell wall biosynthesis and antibiotic interactions, were initiated. Among others, a cooperation with the group of Prof. Dr. *Tanja Schneider* was started, who was able to convert the provided lipid I analogs (**1** and **2**) chemoenzymatically into their respective lipid II analogs (R = GlcNAc).

Abstract Part II: Contributions to the Total Synthesis of Vancoresmycin

The second part of this thesis addresses vancoresmycin (**7**, **Scheme 2**), which was first isolated in 2002 from the fermentation broth of the actinomycete *Amycolatopsis* sp. ST 101170.[4] This natural metabolite is characterized by a densely functionalized tetramic acid connected to a large polyketide chain bearing a plethora of stereogenic centers and also a carbohydrate moiety. In addition to the intriguing structure and the so far only proposed stereogenic centers, several potent antibiotic activities were determined against pathogenic bacteria, making vancoresmycin (**7**) and the respective aglycone **8** an attractive target for a total synthesis.[5]



***Scheme 2*:** Retrosynthetic analysis of vancoresmycin aglycone **8** and the resulting fragments A-G (**9-15**).

This dissertation reports on the first retrosynthetic analysis of vancoresmycin aglycone **8**, in which the compound was divided into seven fragments A-G (**9-15**, **Scheme 2**). After planning the highly convergent and also easily modifiable synthesis, it was possible to successfully build up the fragments A-E (**9-13**) during this work, while fragments F (**14**) and G (**15**) have been synthesized by *Stefanie Spindler*. The overall yield, the longest linear sequence and selected

key steps for each fragment are summarized in **Table 1**. Additionally, the fragments C (**11**) and D (**12**) as well as E (**13**) and F (**14**) were already coupled and modified to give compounds **16** and **17**.

Table 1: Synthesized fragments with key steps, yields and LLS.

Fragment	Key steps	Yield [%]	LSS
A (**9**)	aldol condensation	79	3
B (**10**)	HWE reaction, *Paterson* aldol reaction, *Narasaka-Prasad* reduction	3	14
C (**11**)	*Grignard* reaction	58	4
D (**12**)	HWE reaction	52	6
E (**13**)	HWE reaction, *Sharpless* asymmetric dihydroxylation	29	11
16	*syn*-aldol reaction, *Narasaka-Prasad* reduction	5	10
17	domino aldol-coupling/reduction sequence	4	12

Furthermore, fragment A (**9**) was submitted to esterification reactions to introduce various residues at the 4'-OH position. A subsequent oxygen to carbon transfer yielded several vancoresmycin-type, 3-acyl tetramic acids, which were already tested for their antibiotic properties by the group of Prof. Dr. *Tanja Schneider*. Interestingly, some of the tested compounds, which were most authentic to the natural product, already showed antibiotic activity. Thus, it was assumed that a part of the vancoresmycin pharmacophore resides at the tetramic acid moiety.[6]

Astonishingly, NMR shift comparison between elaborate vancoresmycin fragments and the natural product showed significant discrepancies in the C-38–C-45 region. Thus, an extensive bioinformatic analysis was initiated, which revealed a novel proposal for the vancoresmycin stereostructure.[7]

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2 Antibiotic Resistance Crisis

Rapidly occurring bacterial resistance against approved medication is steadily increasing and represents an enormous threat for the human health in all parts of the world.[1,2] Even multidrug resistant bacteria or the so called “superbugs”, which are mostly drug resistant, are surrounding humanity and should be an alarming signal to fuel research in the field of antibiotics.[3–5] Although, these resistances can happen spontaneously e.g. caused by random mutations, or by one species which is transferring resistance to another species, the excessive or wrong use of antibiotics in health care or in the food industry accelerates the process.[2,4] After a golden era in the period between 1940 and 1960, following the origin of antibiotic research being the discovery of penicillin by *Alexander Fleming* in the late 1920s, stagnation in antibiotic discovery and increasing antibiotic resistances led to a discovery void.[6] Among others, a bacterial infection can cause diseases like pneumonia, tuberculosis and gonorrhea, ranking infectious diseases as the second leading killer in the world.[4,6] The European Centre for Disease Prevention and Control reported that around 672000 infections by antibiotic-resistant bacteria within 33000 being lethal happened in year 2015 only in Europe.[1] The World Health Organization (WHO) recognized the discrepancy in the discovery void and increasing resistance and warned that a post-antibiotic era will go along with plenty of infections and small injuries causing deaths if humanity fails to intervene this problem. [4,7] Therefore, WHO published antimicrobial action plans and strategies in 2011.[7]

Despite the menace, the pharmaceutical industry slowed down antibiotic development drastically, due to inadequate political decisions, exhausting research and no or poor return of investment.[2,6] Finally, in 2020 the threat was targeted and an antimicrobial action fund was launched by the pharmaceutical industry in collaboration with the WHO, which should support the development of novel antibiotics.[8]

Recently, the virus namely COVID-19 causing a worldwide state of emergency resulting in dramatic impairment of human health and economy, but we should keep in mind that this circumstances can also happen due to an outbreak of resistant bacteria.[9,10]

3 General Remarks

Based on the burden of antibiotic resistance and its impact, described in **Chapter 2**, this work is dedicated to antibiotic research. From this point, the present work is divided into two parts, which both focus on the fight against pathogenic bacteria.

Spotlight of part one will be the total synthesis of farnesyl lipid I/II analogs, which represent key building blocks of the bacterial cell wall biosynthesis and being therefore essential targets of many common antibiotics.

Part two will then focus on the total synthesis of vancoresmycin, a novel highly potent antibiotic, which structure is not fully elucidated.

Furthermore, a third topic was explored during the time as doctoral researcher. Here, a new improved route was developed to synthesize a carbasugar, which had fascinating antibiotic properties in previous studies.[11] The results of this area have already been published in full[12] and are not discussed further in this work.

4 Part I: Total Synthesis of Farnesyl Lipid I/II Analogs

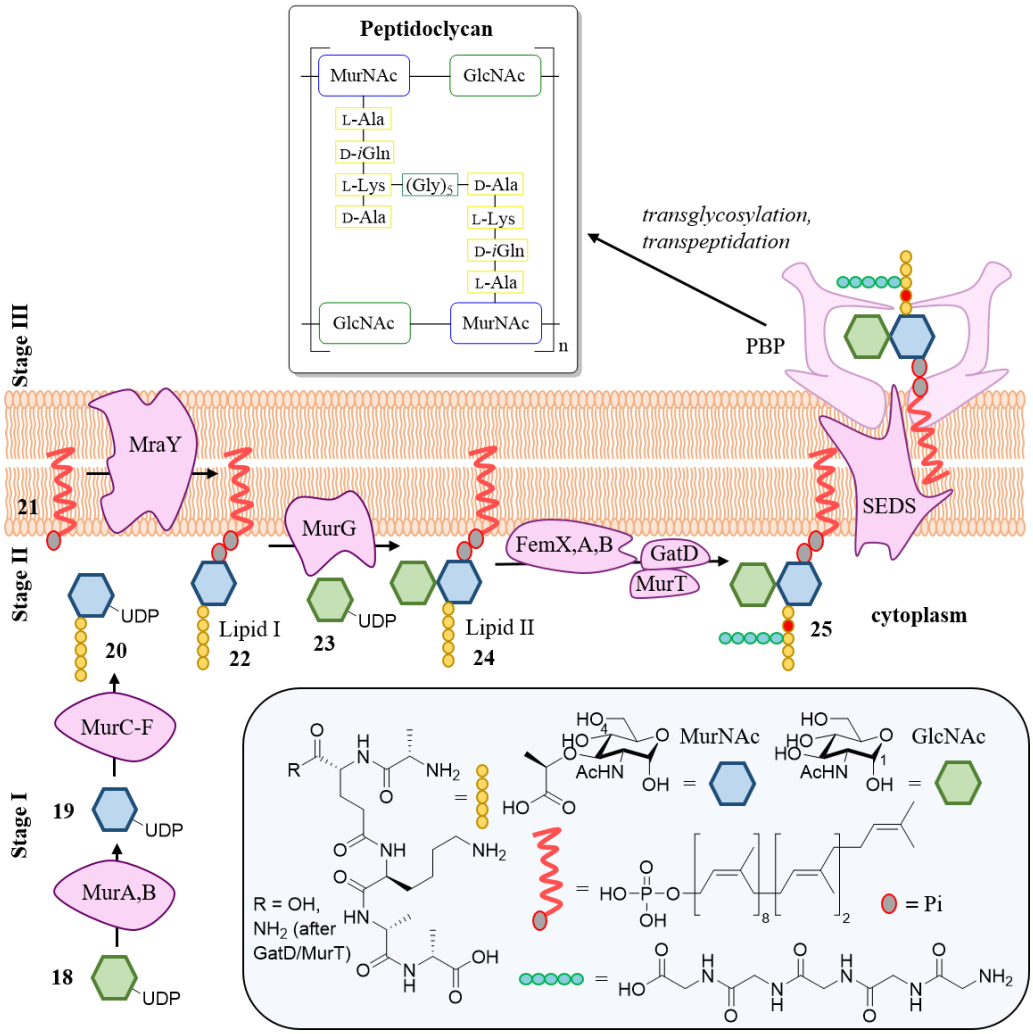
4.1 Introduction

4.1.1 Bacterial Peptidoglycan Biosynthesis and Lipid II as its Achilles Heel

Bacterial cell membranes are surrounded by a peptidoglycan (PGN) mesh, which represents an essential hull protecting bacteria from lysing under high osmotic pressure and ensuring cell integrity.[13,14] Peptidoglycans are constructed out of polysaccharide strands in which β-1,4-connected *N*-acetylglucoseamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) are alternating. The lactyl group of MurNAc features a peptide, which is able to perform crosslinks in the PGN network. [14–18] Both, gram-negative and gram-positive bacteria are surrounded by a PGN layer, although this layer is considerably thinner and covered by an outer membrane in gram-negative organisms.[14]

In general, PGN biosynthesis is subdivided into three stages (**Scheme 4.1.1-1**).[16,19] Cytosolic stage I represents the synthesis of UDP-MurNAc-pentapeptide (**20**) out of UDP-GlcNAc (**18**). First, enolpyruvyl transferase MurA and the NADPH-dependent reductase MurB are introducing the lactyl residue to the 3-position, which then serves as linkage for the pentapeptide (commonly: L-Ala-D-γ-Glu-L-Lys-D-Ala-D-Ala, for modifications see **Figure 4.1.1-1**) catalyzed by the ligases MurC-F.[16,19–22] Stage II is located at the cytoplasmic surface of the bacterial membrane and includes two enzymes. The integral membrane protein MraY is required to carry out a pyrophosphate exchange reaction. Therefore, this enzyme attaches a membrane-anchored 55-carbon undecaprenyl phosphate (**21**) to the anomeric position of phosphor-MurNAc-pentapeptide (**20**) yielding the so named lipid I (**22**, undecaprenyl-pyrophosphoryl-muramyl-pentapeptide) under ejection of UMP.[14,23,24] Finally, the glycosyltransferase MurG catalyzes the transfer of GlcNAc from UDP-GlcNAc (**23**) to the C4-hydroxyl group of lipid I (**22**) yielding the key precursor lipid II (**24**), which carries a complete

PGN subunit.[14,16,19,23] In several bacteria, peptidic modifications (see **Figure 4.1.1-1**) occur at the terminal amino group of the lysine residue leading to different interpeptidic bridges in PGN.[25]. In *S. aureus* for example, five glycines are attached by the peptidyltransferases FemX,A,B. Furthermore, it is reported that in *S. aureus* the bi-enzyme complex MurT/GatD amidates the glutamate of the stem peptide generating a glutamine at this position.[18,26]



***Scheme 4.1.1-1*:** Schematic representation of key steps in PGN biosynthesis and structures of the building blocks. In *S. aureus*, lipid II is further modified by a pentaglycine attachment catalyzed by FemX,A,B enzymes and the glutamate is amidated to glutamine by MurT/GatD. (Created with Power Point 2013 and ChemBioDraw 14.0)

Stage III completes PGN biosynthesis by translocation of (modified) lipid II (**25**), which is expected to be mediated by SEDS (shape, elongation, division and sporulation) proteins or MOP (multidrug/oligosaccharidyl-lipid/polysaccharide) exporters[27–31] to the extracytoplasmic site of the cell membrane followed by subsequent transglycosylation and transpeptidation

through the activity of penicillin-binding proteins (PBPs) to ensure polymerization to a complete PGN network also called murein sacculus.[16,21,32]

As already mentioned, variations of the key building block lipid II are strain-dependent and mostly restricted towards modifications of the stem peptide. Although these modifications can have an impact on antibiotic resistance, the core structure is highly conserved and cannot be easily adapted. **Figure 4.1.1-1** displays the structure of natural lipid II (**24**) and common species-specific modifications.[14,15]

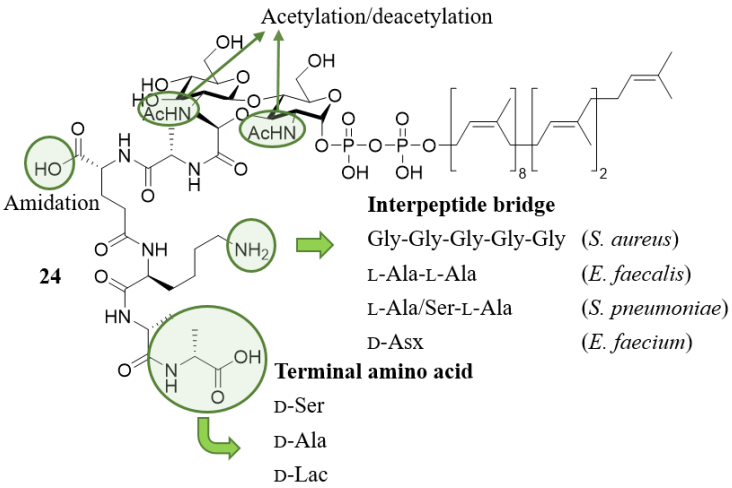


***Figure 4.1.1-1*:** Structure of natural lipid II (**24**) and main modifications.

The importance of PGN biosynthesis makes the highly conserved precursor lipid II one of the most promising targets for antibiotics.[14,33] There are several additional advantages of attacking lipid II as a hot spot for antimicrobial compounds. It is present at the outside of the cytoplasmic membrane making it readily attackable and similar structures and their respective pathways are lacking in eukaryotic cells leading to a higher target specificity and a decrease of side effects.[14] Furthermore, lipid II offers various possible binding sites and its amount is very limited in the bacterial cell requiring a recycling machinery.[33] More recently, lipid II was also found to play influential roles in bacterial signal transduction pathways giving rise to an extra advantage of addressing lipid II as a weak point of bacterial cells.[33] Consequently, blocking lipid II can trigger diverse malfunctions in bacteria resulting in an imperfect cell wall or a disturbed signal transduction pathway ultimately leading to bacterial death.

Antimicrobial drugs such as vancomycin and nisin, which bind to lipid II, are already indispensable in areas like agriculture, clinics and the food industry.[14,34–38] Several interactions between antibiotics and the PGN precursor have already been studied and reviewed (**Figure 4.1.1-2**, **A** displays discovered binding sites).[14,15,39] As example, vancomycin (for structure see **Figure 5.1.1-1**), the most popular glycopeptide antibiotic, is known to form five distinct hydrogen bonds between the acyl-D-Ala-D-Ala terminus of lipid II and its glycopeptide core leading to a relatively stable complex and thus prevents an uptake of the building block into the PGN network.[40] An exchange of the terminal D-Ala with D-Lac or D-Ser (see **Figure 4.1.1-1**) by target modification leads to a loss of hydrogen bond interaction and reduces the binding affinity dramatically generating vancomycin-resistant staphylococci (VRSA) or enterococci (VRE).[36–38] Despite the fact that the development of vancomycin resistance took decades, the pyrophosphate group of lipid I and lipid II seems to be even harder to alter for bacteria. [14,36–38] Fortunately, this group is also addressed by a variety of antibiotics like lantibiotics,[41–45] defensins,[46–51] ramoplanin[52–59] and teixobactin[60]. For example nisin (**26**, **Figure 4.1.1-2**, **B**), also referred as the prototype lantibiotic, which is produced by *Lactococcus lactis* subsp. *lactis* and was first described in 1928, is effective against a plethora of gram-positive bacteria.[34,35,61,62] The reported double mode of action for nisin (**26**) makes it a highly potent antibiotic. On the one hand, it forms a complex with the highly conserved lipid II and thus leads to an inhibition of peptidoglycan synthesis, but on the other hand, it uses lipid II as a docking site to form pores in the cytoplasmic membrane (**Figure 4.1.1-2**, **C**).[43,44,56,63–65] Previous structural analyses showed that the thioether rings A and B of nisin are relevant for binding lipid II or also lipid I at the pyrophosphate moiety.[41,42] Additionally, it is believed that parts of MurNAc and the undecaprenyl chain of lipid I/II are involved in the interaction with lantibiotics.[43–45]

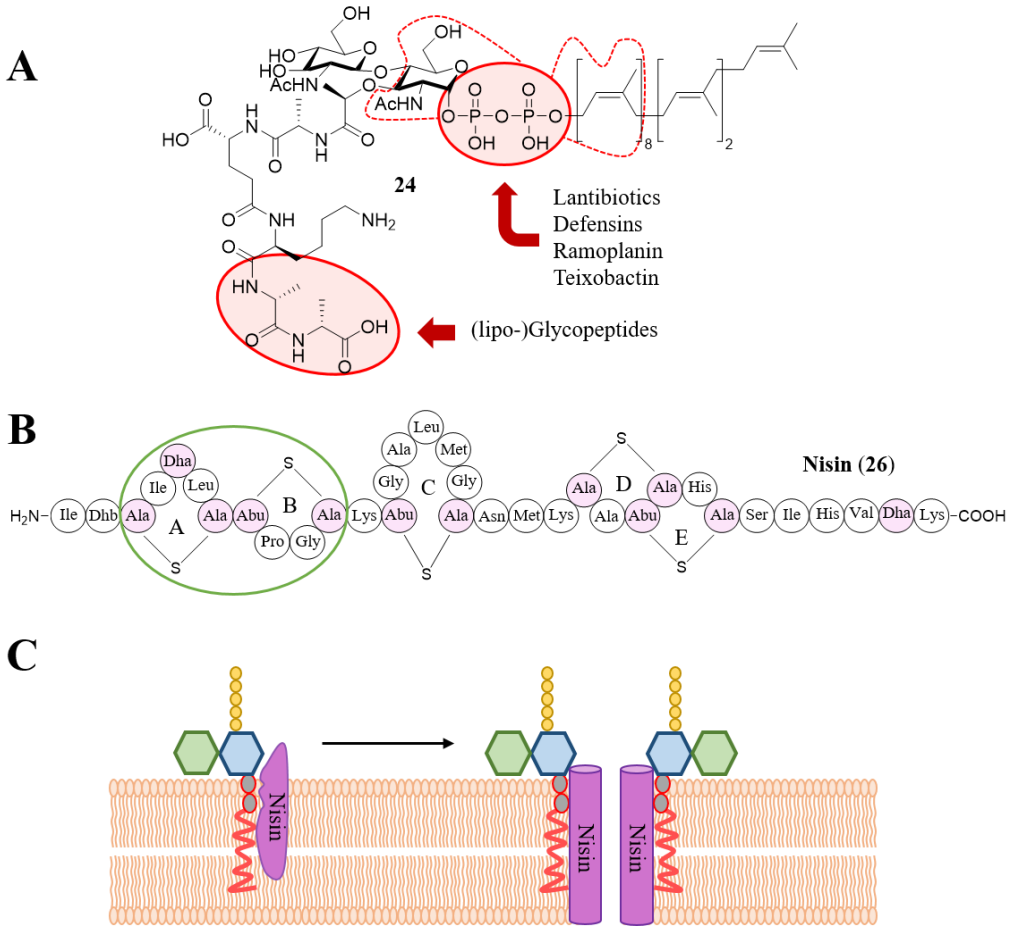


***Figure 4.1.1-2*: (A)** Structure of natural lipid II (**24**) with the known binding sites (red) for prominent antibiotics. The dotted red lines indicate structural parts of lipid II, which are probably also involved in the antibiotic interaction. (**B**) Primary structure of nisin. The proposed, most important binding motif is highlighted in green and amino acids that are post-translationally modified are marked in rose. Dha = dehydroalanine, Dhb = dehydrobutyrine, Abu = aminobutyric acid. (**C**) Illustration of the pore formation by nisin. After blocking lipid II and structural rearrangements, the final pore is formed out of eight nisin and four lipid II molecules.[64]

Although certain progress was made in the past, rising resistance to antibiotics requires a better understanding of bacterial cell wall biosynthesis and the mode of action of established antibiotics, leading in the best case to the rational design of novel, potent compounds. One of the main problems is that structural and functional studies are severely hampered by the limited supply of adequate amounts of natural components such as lipid I and lipid II. Isolation from natural sources is often very tedious and in addition, structural studies with the authentic full-length building blocks are complicated by unfavorable physicochemical properties, including aggregation, precipitation and complex spectroscopic characteristics, which are mainly caused by the lipophilic undecaprenyl tails.[16,66] In recent years, it has become more and more apparent

that these tails can be dramatically shortened and existing biochemical studies with truncated analogs suggest that these surrogates are mostly compatible with the authentic molecules.[16,41,67,68] This renders the synthesis of lipid I/II analogs an essential task for the combat against pathogenic bacteria.

4.2 State of Knowledge Concerning Chemical Synthesis of Lipid I/II

In the years around the millennium change, impressive progress was generated in the synthesis of lipid I (**22**) and II (**24**). Especially 2001 could be described as the golden year of PGN precursor synthesis. Milestones in the chemical synthesis of lipid I/II are illustrated in the timeline displayed in **Figure 4.2-1** and summarized in this chapter.

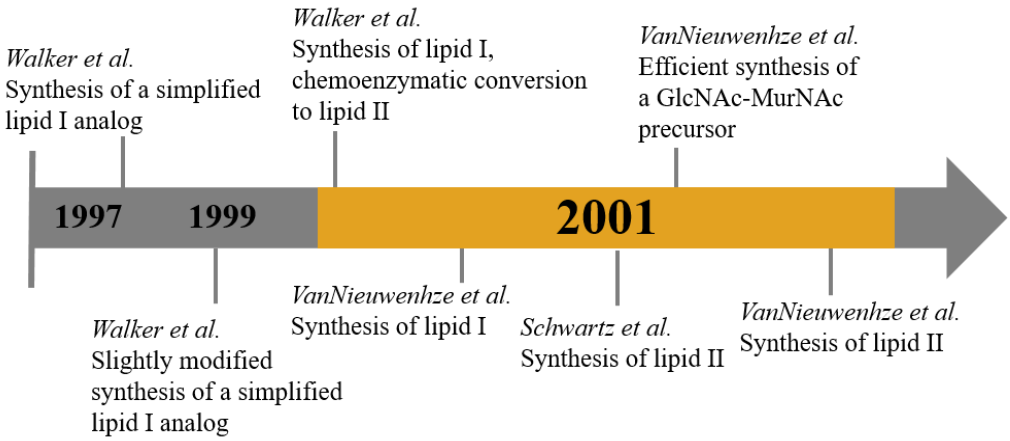
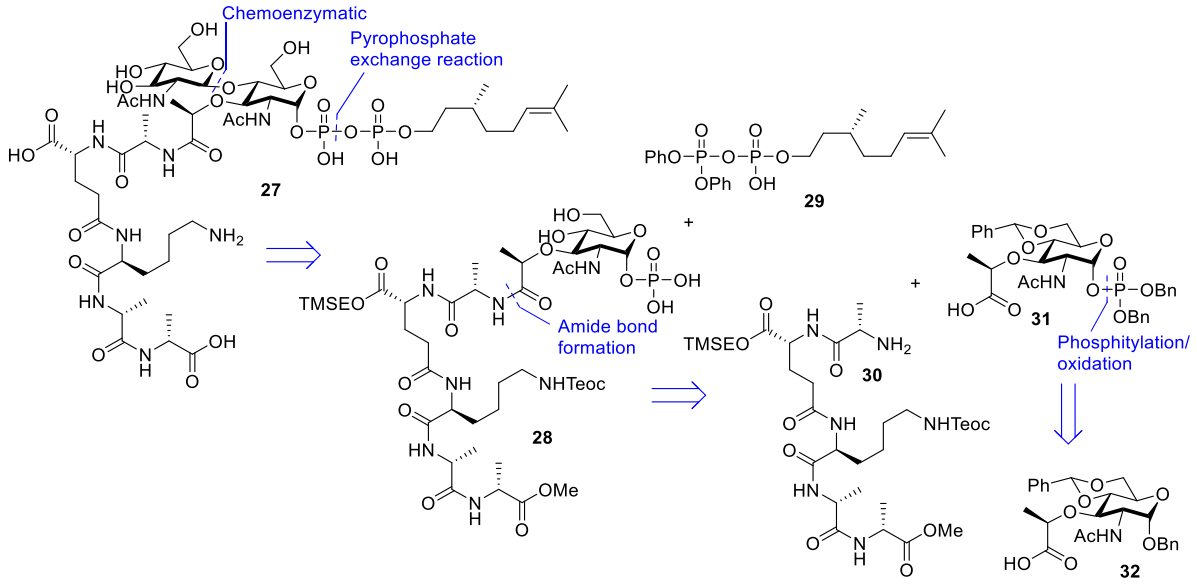


***Figure 4.2-1*:** Timeline with highlighted milestones in the synthesis of lipid I (**22**) and II (**24**) under the use of the submission date of the respective manuscript.[16,19,66–70]

4.2.1 Synthesis of Lipid I and Chemoenzymatic Conversion to Lipid II by *Walker et al.*

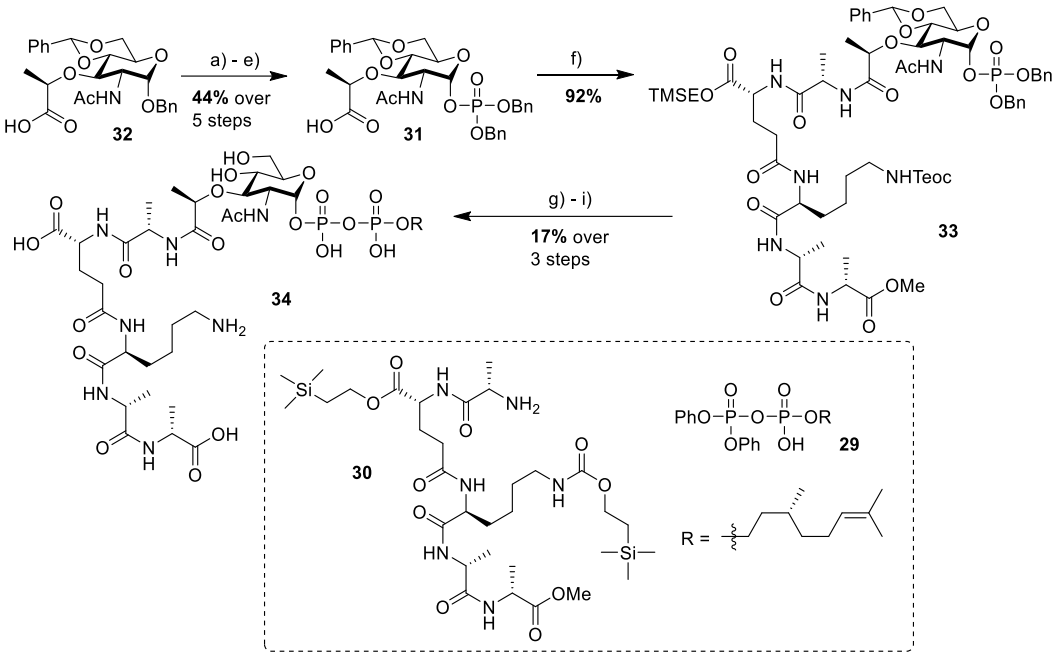
Walker et al. submitted a manuscript in late 1997, which was published 1998 and described the synthesis of a lipid I analog **34** (**Scheme 4.2.1-2**).[67] Being the first synthetic approach for such an analog, the group of *Walker* decided to replace the natural occurring 55-carbon undecaprenol chain by the 10-carbon chain of citronellol. This switch of the lipid chain should facilitate their synthesis due to better solubility properties, easier structural analysis and also the citronellol analog did not feature an allylic pyrophosphate, which was known to be unstable. Fortunately, *Walker et al.* tested the unnatural substrate in a developed, direct assay for MurG activity and

showed that MurG accepted their compound, although the lipid chain was dramatically shortened. This conversion should in principle be able to generate lipid II (**27**) out of lipid I (**34**) and UDP-GlcNAc but no isolated product or analytical data were reported in this manuscript.[67] Despite a not reported retrosynthetic analysis, a retrosynthetic perspective of their route is shown in **Scheme 4.2.1-1**.



***Scheme 4.2.1-1*:** Retrosynthetic analysis by *Walker et al.*

Thus, lipid II analog **27** was planned with a chemoenzymatic conversion of its respective lipid I analog (**34**), which should be accessible out of diphenyl citronellol pyrophosphate (**29**) and glycosyl pentapeptide (**28**), using a pyrophosphate exchange reaction. The latter was planned to be built up using standard amide bond formation out of protected pentapeptide **30** and monosaccharide **31**. While solid-phase peptide synthesis should yield pentapeptide **30**, the carbohydrate fragment was aimed to be synthesized out of commercially available compound **32** after application of a protection scheme and a phosphitylation/oxidation procedure.[67]

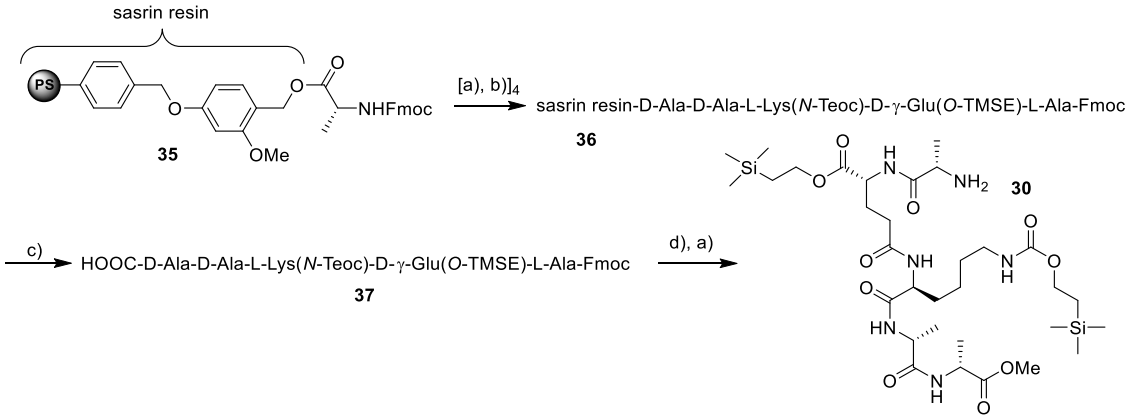


***Scheme 4.2.1-2*:** Synthesis of lipid I analog **34** by *Walker et al.* Reaction conditions: a) CCl_3CH_2OH (2.0 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), THF, rt, 4 h, **80%**; b) H_2, 10% Pd/C, EtOAc, rt, 30 min; c) $PhCH(OCH_3)_2$ (10 equiv), *p*-TsOH (0.1 equiv), DMF, rt, 10 h, **81%** over 2 steps; d) $(BnO)_2PN(i\text{-}Pr)_2$ (2.0 equiv), 1*H*-tetrazole (4.0 equiv), DCM, −20 °C to rt, 30 min, then *m*-CPBA (5.0 equiv), −40 °C to rt, 2 h, **75%**; e) Zn dust, 90% $AcOH/H_2O$, rt, 1 h, **91%**; f) **30** (1.4 equiv), PyBOP (2.0 equiv), HOBt (2.0 equiv), DIPEA, DMF, 0 °C, 30 min, **92%**; g) H_2, 10% Pd/C, MeOH, rt, 30 min; h) **29** (2.0 equiv), pyridine, DMF, rt, 48 h, **30%** over 2 steps; i) TBAF (20 equiv), DMF, rt, 24 h, **57%**.

As a first step, the group used commercially acquired carbohydrate **32** and protected the lactyl residue in a *Steglich* esterification reaction in 80% yield followed by hydrogenolysis of the anomeric benzyl group (**Scheme 4.2.1-2**). During this reaction, the acetal group was cleaved as well, which was subsequently reattached in a yield of 81% over these two steps. Afterwards, the free hydroxyl group was phosphitylated and directly oxidized to the respective phosphate in a one-pot procedure. Unmasking the lactyl group with zinc dust in an acidic environment furnished compound **32** in 91% yield for this step. Carbohydrate **31** featured the free carboxyl group, which served as linkage for the peptide coupling. Therefore, protected pentapeptide **30** (for synthesis see **Scheme 4.2.1-3**) was coupled using a PyBOP/HOBt protocol and proceeded in an excellent yield of 92%. Hydrogenolytic deprotection rendered the free phosphate, which was coupled in a pyrophosphate exchange reaction with diphenyl citronellol pyrophosphate **29**. *Walker* and co-workers claimed, that the acetal protecting group of the MurNAc derivative was cleaved as well after hydrogenolysis, which could not be reproduced by others and also created

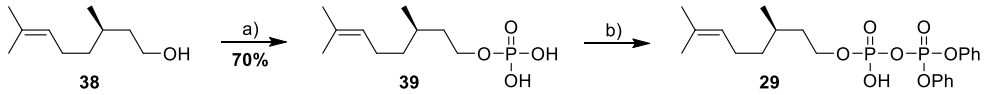
issues (see **Chapter 4.4.7**), which were fortunately solved, in this work.[25] However, a final global deprotection with fluoride ions yielded lipid I analog **34** in an overall yield of 7% starting from monosaccharide **32** and 2% out of modified sasrin resin **35** (**Scheme 4.2.1-3**). Unusually, the terminal D-alanine methyl ester was reported to be cleaved in the same deprotection reaction under TBAF conditions. Unfortunately, no experimental details beyond the reactants and equivalents were given in this publication.[67]

The used protected pentapeptide **30** (**Scheme 4.2.1-3**) was synthesized with solid-phase peptide synthesis in 15% yield over 11 steps starting from sasrin-D-Ala-Fmoc and using the respective, previously protected amino acids.[67]



***Scheme 4.2.1-3*:** Synthesis of pentapeptide **30** by *Walker et al.* Reaction conditions: a) 55% piperidine/NMP, rt, 30 min; b) Respective amino acid (HO-D-Ala-Fmoc, HO-L-Lys(*N*-Teoc)-Fmoc, HO-D-γ-Glu(*O*-TMSE)-Fmoc or HO-L-Ala-Fmoc), HBTU/HOBt, DIPEA, NMP, rt, 2 h, equivalents were not reported; c) 1% TFA/DCM, rt, 5x2 min; d) CH_3I (50 equiv), $KHCO_3$, DMF, rt, 2 h, overall yield **15%**.

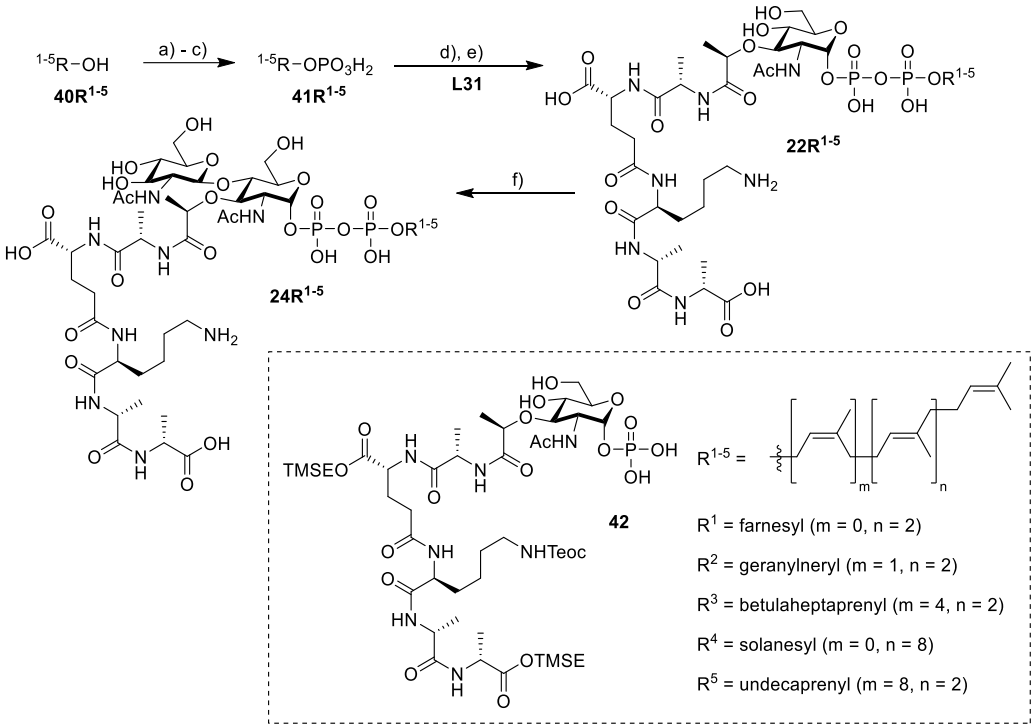
Diphenyl citronellol pyrophosphate **29** was synthesized out of citronellol (**38**) in two steps (**Scheme 4.2.1-4**). First, a monophosphate was introduced by the treatment of citronellol with phosphorus oxychloride, which was extended in the second step to the pyrophosphate with addition of diphenyl chlorophosphate. The product **29** was directly used for the following reaction.[67]



***Scheme 4.2.1-4*:** Synthesis of diphenyl citronellol pyrophosphate **29** by *Walker et al.* Reaction conditions: a) $POCl_3$ (5.0 equiv), NEt_3, hexane, rt, 1 h; then acetone/H_2O/NEt_3 (85:10:5), rt, 10 h, **70%**; b) diphenyl chlorophosphate (1.5 equiv), DIPEA, DCM, −20 °C to rt, 1 h, generated in situ.

In 1999, a second publication by *Walker et al.* reported on the synthesis of the same lipid I analog **34** and this time more synthetic details were given.[16] While the route stayed more or less the same, the used pentapeptide **30** differed in the protection group of the terminal D-Ala (TMSE ester instead of methyl ester). One possible reason for the change of the protection strategy could be that the deprotection of the previously used methyl ester with TBAF was not as straight forward as thought. Unfortunately, no further information were given than that the pentapeptide was synthesized using standard HOBt/HBTU procedures with Fmoc-protected amino acids. Even more confusing is the reported synthesis of an amino acid building block (Z-D-Glu(OH)-OTMSE), which does not feature Fmoc protection and is therefore not useful for their claimed peptide synthesis using Fmoc protected amino acids.[16]

Finally, in 2001, *Walker et al.* managed to synthesize various lipid II analogs **24R$^{1-4}$** (**Scheme 4.2.1-5**) and also the natural lipid II (**24R^{5}**), containing the 55 carbon undecaprenyl chain, using chemoenzymatic conversion of the synthesized lipid I analogs with the glycosyltransferase MurG and the carbohydrate donor UDP-GlcNAc. Additionally, the group described some changes in their strategy, which are illustrated in **Scheme 4.2.1-5**.[68]

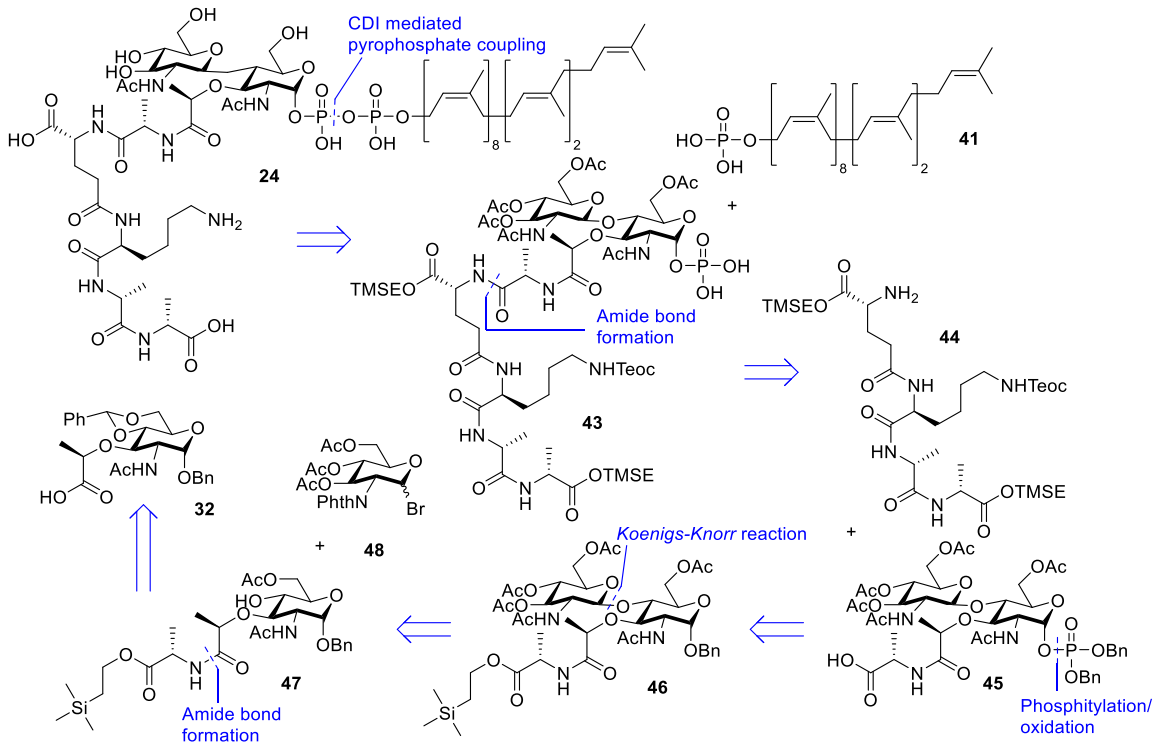


***Scheme 4.2.1-5*:** Synthesis of natural lipid I/II and analogs **22R$^{1-5}$**, **24R$^{1-5}$** by *Walker et al.* Reaction conditions: a) Bis(2-cyanoethoxy)-*N*,*N*-diisopropylaminophosphine (1.7 equiv), THF, 0 °C, then 1*H*-tetrazole (4.0 equiv), rt, 1 h; b) $NaIO_4$ (excess), pyridine, 0 °C to rt, 2 h; c) NaOMe (excess), MeOH, rt, 24 h; d) CDI (5.1 equiv), THF, rt, 30 min, then **42**, DMSO/THF, rt, 24 h; e) TBAF (20 equiv), DMF, rt, 24 h. Yield over 5 steps: for R^1 = **28%**, R^2 = **28%**, R^3 = **23%**, R^4 = **15%**, R^5 = **11%**; f) MurG, UDP-GlcNAc (2.0 equiv), 50 mM HEPES-5, $MgCl_2$, pH 7-9, rt, 1 h, no yields were given for this conversion.

Employing the previous conditions, which were used to activate citronellyl phosphate (**Scheme 4.2.1-2**) for the coupling to pentapeptide-carbohydrate phosphate **42**, resulted in extensive decomposition in the case of the less stable allylic phosphates. Thus, a previously reported pyrophosphate coupling using CDI was applied after synthesizing the monophosphates **41R$^{1-5}$** out of the respective alcohols **40R$^{1-5}$** using bis(2-cyanoethoxy)-*N*,*N*-diisopropyl-aminophosphine.[71,72] All silyl protecting groups of the pentapeptide were cleaved using an excess of TBAF yielding lipid I analogs **22R$^{1-5}$** in the range of 11-28% yield over five steps starting from **40R$^{1-5}$**. Fortunately, MurG accepted all synthesized lipid I analogs **22R$^{1-5}$**, although differences in reaction speed and isolated amounts were noticed but not reported in detail.[68]

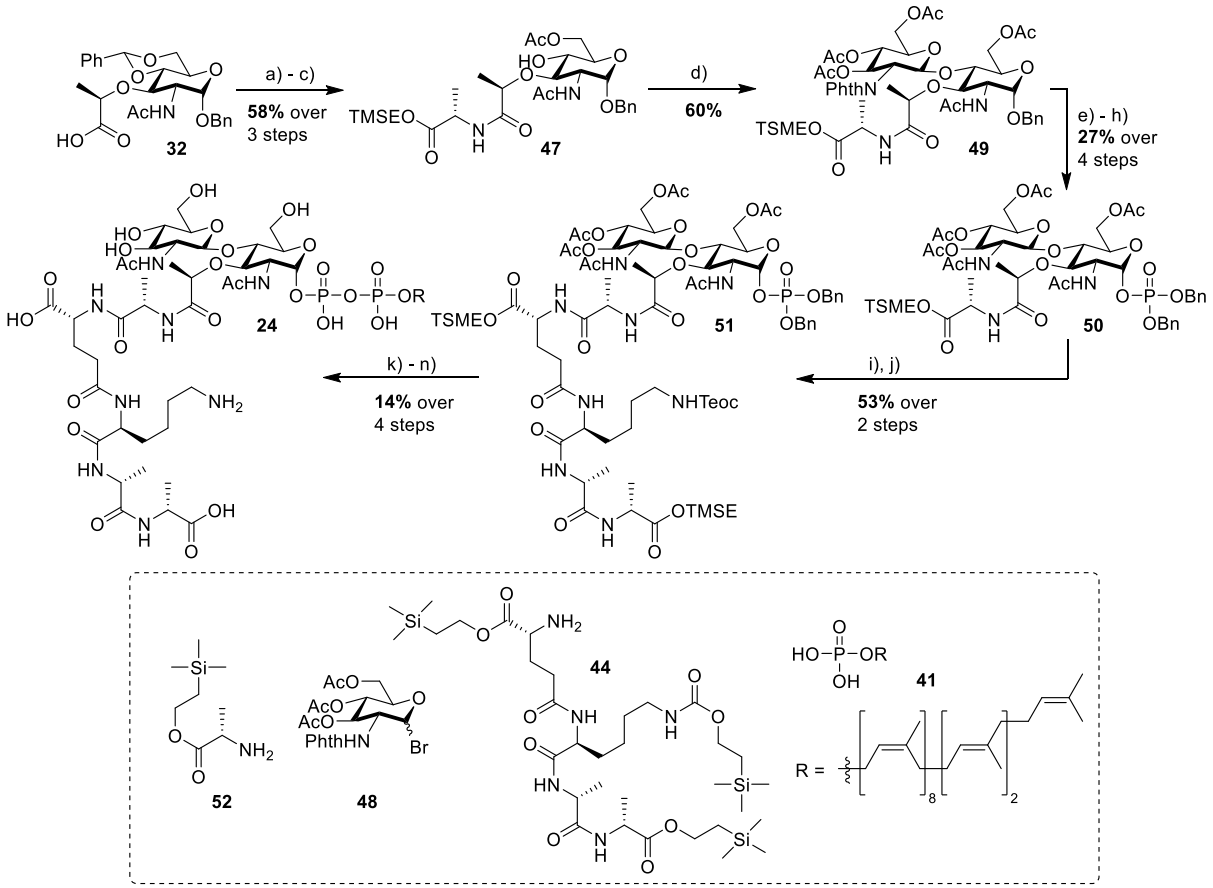
4.2.2 Total Chemical Synthesis of Lipid II by *Schwartz et al.*

In July 2001, *Schwartz et al.* reported on the chemical synthesis of lipid II (**24**).[66] Contrary to *Walker et al.*, the group of *Schwartz* chose a fully synthetic approach without a chemoenzymatic conversion.[68] In a retrosynthetic perspective (**Scheme 4.2.2-1**), they divided lipid II (**24**) into commercially available undecaprenyl monophosphate **41** and disaccharide-pentapeptide **43**, which was chosen to be protected with silyl groups at the amino acid side chains to allow a joint deprotection using fluoride ions. Furthermore, the free hydroxyl groups of glycopeptide **43** should be masked with acetates. Compound **43** could be constructed out of silyl protected tetrapeptide **44** and carbohydrate building block **45** using standard peptide coupling reactions like the PyBOP/HOBt procedure. Carbohydrate **45** should be accessible using a suitable phosphitylation reaction followed by oxidation. Disaccharide **46** was further subdivided into the monosaccharides **47** and **48**, which should be connected via *Koenigs-Knorr* conditions. A possible introduction of L-Ala, masked as trimethylsilyl ethyl ester, should lead back to commercially available carbohydrate **32** as a starting point for their synthesis.[66]



***Scheme 4.2.2-1*:** Retrosynthetic analysis by *Schwartz et al.*

Schwartz et al. started from commercially available MurNAc derivative **32**, which was coupled to protected L-alanine **52** followed by deprotection of the acetal and monoacylation of the resulting primary alcohol using one equivalent of AcCl rendering compound **47** in 58% yield over these three steps (**Scheme 4.2.2-2**). *Schwartz* and co-workers reported that they introduced the alanine because they were worried about an undesired cyclization, which occurred when the lactate was protected as an ester. Disaccharide **49** was then generated in 60% yield by coupling of compound **47** via the free hydroxyl group at C4 with glycosyl donor **48** utilizing previously described conditions (AgOTf) to activate the glycosyl bromide.[73] Hence, the desired β-1,4 linkage was built due to the directing effect of the phthalimido group in 2-position. Subsequent cleavage of the phthalimido group using an ethylenediamine-derivatized resin[74] followed by acetylation with acetic anhydride proceeded in 53% yield over these two steps. Hydrogenolysis of the benzyl group gave the free anomeric hydroxyl group, which was further modified by the introduction of the phosphate. This was achieved in an one-pot, two-step procedure in which an initial introduced phosphite was subsequently oxidized with *m*-CPBA[75,76] furnishing compound **50** in a yield of 27%. After unmasking the alanine with TBAF, the free carboxyl group served as linkage for the coupling with the protected tetrapeptide **44** giving disaccharyl pentapeptide **51** in 53% yield over the last two steps. The synthesis of the required tetrapeptide **44** was outsourced to a peptide supplier (New England Peptide, Fitchburg, MA). Thus, compound **44** was purchased and no experimental details were given. The free phosphate was obtained after hydrogenolysis, activated with CDI and coupled to undecaprenyl phosphate. Two multiple deprotection reactions to remove the peptidyl silyl and the hydroxyl acetate groups rendered lipid II (**24**) in 14% over the last four steps and in an overall yield of 0.7% starting from carbohydrate **32**.[66]



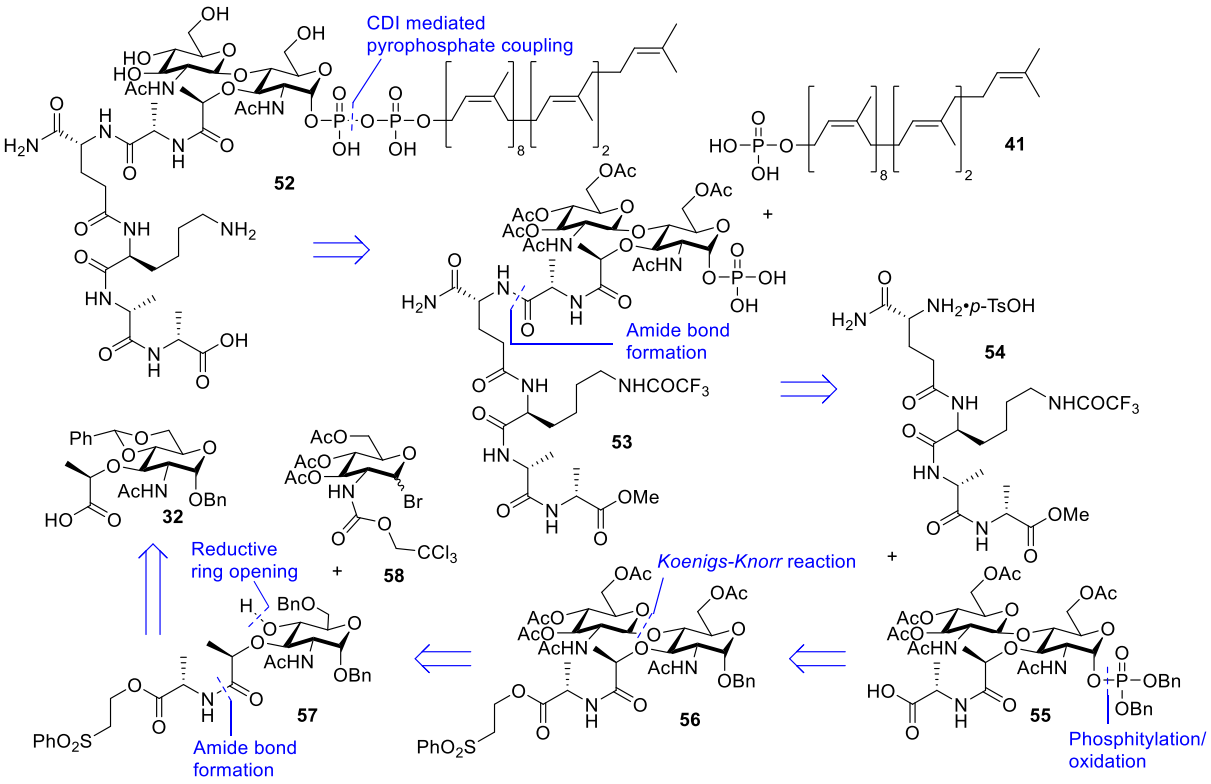
***Scheme 4.2.2-2*:** Synthesis of lipid II (**24**) by *Schwartz et al.* Reaction conditions: a) **52** (0.96 equiv), PyBOP (1.03 equiv), HOBt (1.03 equiv), DIPEA (2.0 equiv), THF/DCM, rt, 2 h, **81%**; b) TsOH (cat.), MeOH, 75 °C, 30 min, **89%**; c) AcCl (1.0 equiv), pyridine (2.0 equiv), DCM, −30 °C, 15 min, then warm to rt, **80%**; d) **48** (2.0 equiv), AgOTf (3.0 equiv), 4 Å MS, DCM, −40 °C, on, **60%**; e) diaminoethylene-derivatized *Merrifield* resin, 4 Å MS, butanol, 85 °C, 24 h; f) Ac_2O/pyridine, rt, on, **53%** over 2 steps; g) 10% Pd/C, H_2, MeOH, rt, 1 h, **93%**; h) 1*H*-tetrazole (3.0 equiv), $(BnO)_2PN(i\text{-}Pr)_2$, DCM, −30 °C to rt, 1 h, then *m*-CPBA (7.3 equiv), −40 °C to rt, 1 h, **55%**; i) TBAF (3.7 equiv), THF, rt, 45 min, **87%**; j) **44** (1.5 equiv), PyBOP (1.6 equiv), HOBt (1.6 equiv), DIPEA (3.0 equiv), THF/DCM, rt, 2 h, **61%**; k) 10% Pd/C, H_2, MeOH, 1 h, **quant.**; l) CDI (5.1 equiv), DMF, rt, 4 h, then MeOH (9.3 equiv), then **41** (0.8 equiv), rt, 2 d, **39%**; m) TBAF (114 equiv), DMF, rt, 24 h; n) 3% NaOMe (9.0 equiv), MeOH/DCM, 0 °C, 1 h, **35%**.

4.2.3 Total Chemical Synthesis of Lipid II by *VanNieuwenhze et al.*

VanNieuwenhze et al. published a chemical synthesis of natural lipid II (**52**) in October 2001, which featured the occurring peptide modification naturally catalyzed by MurT/GatD.[19] Thus, this lipid II (**52**) contained a glutamine amino acid in the stem pentapeptide instead of

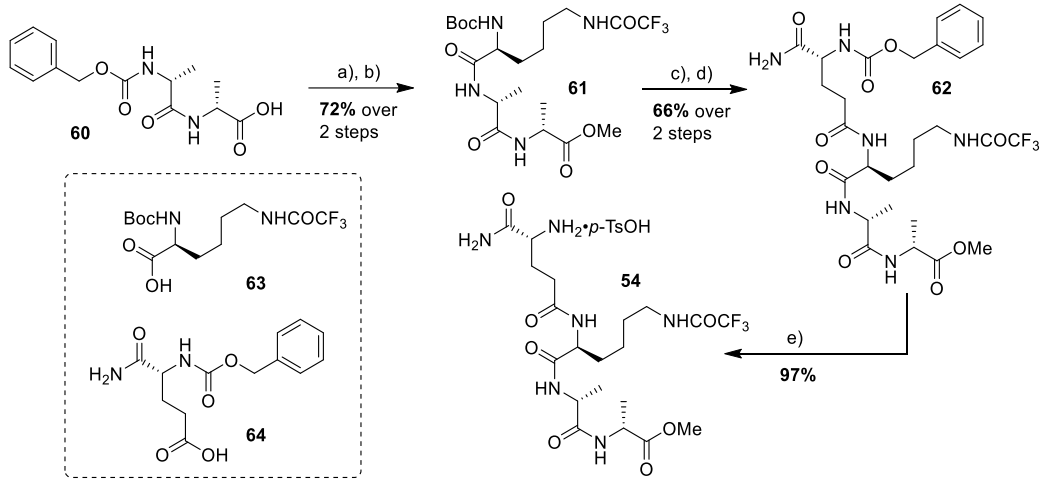
glutamate. Their retrosynthetic analysis is shown in **Scheme 4.2.3-1**. Being the most labile moiety in lipid I and lipid II, the prenyl-linked diphosphate was planned to be constructed at a late stage in the synthesis, which also prevents solubility issues due to the lipophilic 55-carbon tail. The supposed instability under acidic conditions of the anomeric and allylic diphosphate also triggered their use of base-cleavable protecting groups. Thus, acetate groups were chosen to mask free hydroxyl groups, while trifluoroacetate should protect the side chain amino group of L-lysine. The terminal carboxy function of D-alanine should be blocked by the respective methyl ester. This rendered the possibility of a global deprotection using hydroxide ions.

With the knowledge of their previous results from the synthesis of lipid I (not shown), which was published in May 2001,[69] *VanNieuwenhze et al.* decided to adopt an analogous disconnection for the synthesis of lipid II (**52**). Hence, lipid II (**52**) was divided into disaccharyl pentapeptide **53** and undecaprenyl monophosphate **41**. While compound **41** was commercially acquired, the disaccharyl pentapeptide **53** was further divided into a suitable protected tetrapeptide **54** and the carbohydrate building block **55**. Compound **55** ensured triple orthogonality, allowing selective unmasking of the functional groups prior to coupling with the tetrapeptide **54** or the undecaprenyl chain **41**. Carbohydrate building block **55** was envisaged to derive from compound **56** using a phosphitylation/oxidation sequence for the stereo-selective introduction of the anomeric phosphate followed by removal of the carboxyl protecting group. Previously, the needed GlcNAc-MurNAc subunit **56** was synthesized and published in September 2001.[70] Here, *VanNieuwenhze et al.* established the synthesis of carbohydrate **56** using a *Koenigs-Knorr* type reaction to yield the desired β-1,4-glycosidic bond out of the fragments **57** and **58**.[19]



***Scheme 4.2.3-1*:** Retrosynthetic analysis by *VanNieuwenhze et al.*

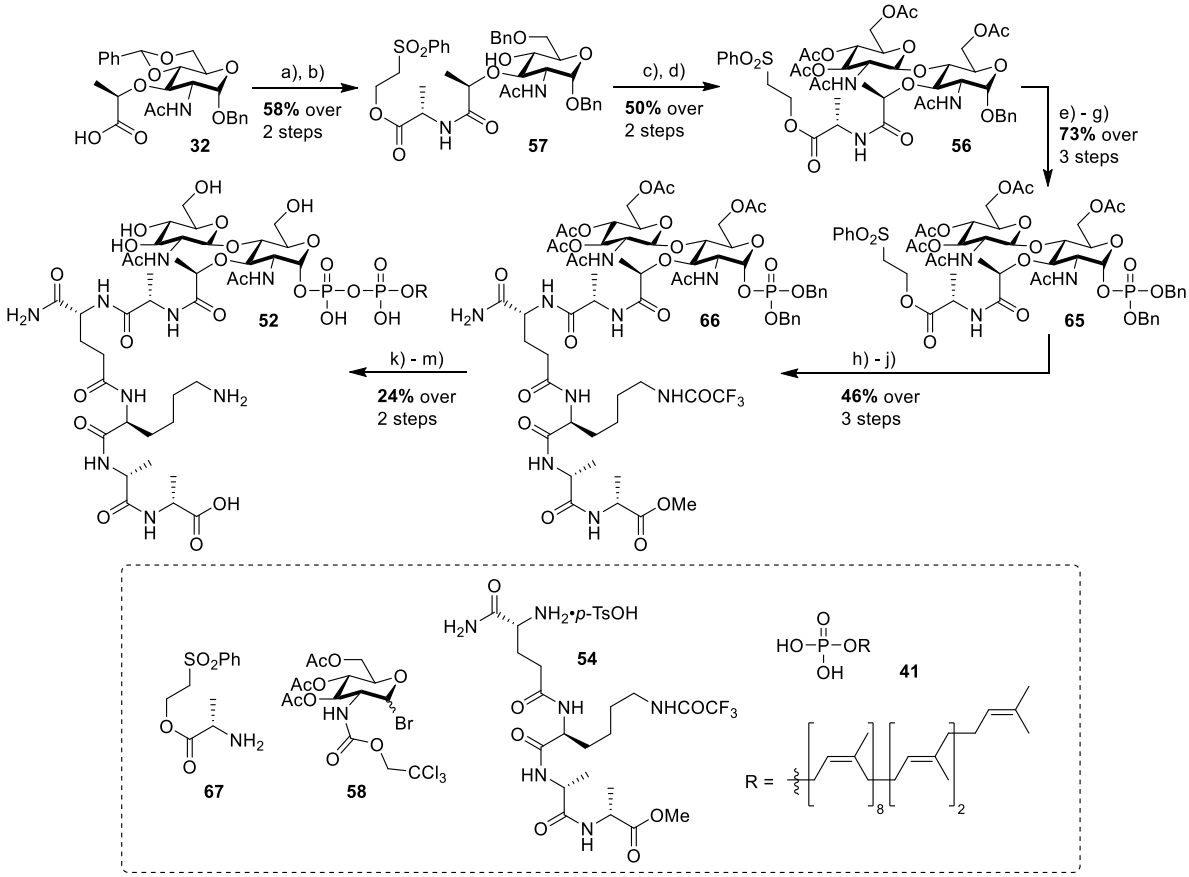
The synthesis of the desired tetrapeptide **54** is illustrated in **Scheme 4.2.3-2** and proceeded with a yield of 46% starting from commercially acquired Cbz-D-Ala-D-Ala **60**. Therefore, the peptide sequence started with the cleavage of the benzyl carbamate protection using hydrogenolysis followed by coupling of the protected L-lysine **63**, which was activated to the analogous NHS-ester (72% from compound **60**). After TFA-mediated cleavage of the Boc-protective group, the free amine was connected to D-glutamine derivative **64** using again activation with DCC and NHS to furnish peptide **62** in 66% yield over these two steps. Finally, hydrogenolysis of the Cbz-protecting group, which proceeded in an expected excellent yield of 97%, gave compound **54** ready for coupling with fragment **55**.[19,69]



***Scheme 4.2.3-2*:** Synthesis of tetrapeptide **54** by *VanNieuwenhze et al.* Reaction conditions: a) $Pd(OH)_2$ (0.9 equiv), TFA (1.0 equiv), H_2, MeOH, rt, 2 h; b) **63** (1.03 equiv), DCC (1.03 equiv), NHS (1.03 equiv), DIPEA (0.2 equiv) THF, 0 °C to rt, 3 h, **72%** over 2 steps; c) TFA/DCM, rt, 2.5 h; d) EDC (2.0 equiv), NHS (2.0 equiv), **64** (1.6 equiv), DIPEA (2.3 equiv), DMF, rt, 75 h, **66%**; e) *p*-TsOH (1.0 equiv), 5% Pd/C, H_2 (60 psi), rt, 1.5 h, **97%**.

To fulfill the total synthesis of lipid II (**52**), *VanNieuwenhze et al.* started from commercially available carbohydrate **32** and introduced the first amino acid to the lactyl group of MurNAc (**Scheme 4.2.3-3**). Therefore, phenylsulfonylethyl ester protected L-alanine **67** was attached in 95% yield. Previously reported reductive ring opening conditions (TFA, triethylsilane)[70] rendered compound **57**, which offered a free hydroxyl group at C4. With respect to the results of *Wong et al.* regarding glycosyl donors, *VanNieuwenhze* and co-workers also decided to use a trichloroethoxycarbonyl (Troc) protected glycosyl donor **58**.[77] *Koenigs-Knorr* conditions (AgOTf) were chosen to generate the β-1,4-linkage between the two carbohydrates followed by a protection scheme generating compound **56** in 50% yield over the glycosylation and the protection steps. Hydrogenolysis of the anomeric benzyl group worked with an excellent yield of 94% and paved the way for the phosphitylation/oxidation sequence. Here, the group of *VanNieuwenhze* relied on the experience gained by their lipid I synthesis, selecting again a nucleophilic carbohydrate and an electrophilic phosphorus agent.[69] Fortunately, α-phosphate **65** was obtained in 78% yield after introducing the phosphorus species with dibenzyl-*N*,*N*'-diethyl-phosphoramidite and 1*H*-tetrazole followed by oxidation using 30% hydrogen peroxide. For the elaboration of the pentapeptide, the lactyl phenylsulfonylethyl ester was unmasked by DBU, furnishing the free carboxyl group, which was activated prior coupling to the tetrapeptide **54** using the respective NHS-ester (46% overall yield from compound **65**).

To set the stage for the final coupling and deprotection sequence, the remaining benzyl groups of the phosphate were cleaved by hydrogenolysis rendering the unmasked phosphate, which was coupled to the commercially acquired undecaprenyl monophosphate **41** using a literature known phosphoro-imidazolidate method, previously employed in their lipid I synthesis.[69,78,79] Finally, global deprotection ensured by exposure to aqueous NaOH gave lipid II (**52**) in 24% yield over the last three steps from compound **66**. In total, lipid II (**52**) was synthesized in 2% yield over a 13 step linear sequence starting from commercially available carbohydrate **32**.



***Scheme 4.2.3-3*:** Synthesis of lipid II (**52**) by *VanNieuwenhze et al.* Reaction conditions: a) *N*-methylmorpholine (NMM, 2.0 equiv), 2-chloro-4,6-dimethoxy-1,3,5-triazine (1.2 equiv), **67** (1.2 equiv), DCM, 0 °C to rt, 3 d, **95%**; b) triethylsilane (5.0 equiv), TFA (8.0 equiv), DCM, 0 °C, on, **61%**; c) AgOTf (3.0 equiv), **58** (3.0 equiv), 4 Å MS, DCM, rt, 20 h, **74%**; d) $ZnCl_2$ (10 equiv), Zn dust (10 equiv), THF/Ac_2O/AcOH, rt, 24 h, **67%**; e) 10% Pd/C, hydrogenation apparatus (50 psi), THF/MeOH, rt, 3 h, **94%**; f) 1*H*-tetrazole (4.7 equiv), dibenzyl *N*,*N*'-diethyl-phosphoramidite (3.0 equiv), DCM, rt, 2 h; g) hydrogen peroxide, THF, −78 °C to rt, 2 h, **78%** over 2 steps; h) DBU (1.0 equiv), DCM, rt, 15 min; i) NHS (1.03 equiv), EDC (1.03 equiv), DMF, rt, 18 h; j) **54** (1.06 equiv, for synthesis see **Scheme 4.2.3-2**), DIPEA (3.2 equiv), DMF, rt, 18 h, **46%** over 3 steps; k) 10% Pd/C, H_2, MeOH, rt,

1.5 h; l) CDI (4.6 equiv), **41** (0.7 equiv), 1*H*-tetrazole (0.9 equiv), DMF/THF, rt, 4 d; m) 1 N NaOH, 1,4-dioxane/water, rt, 2 h, **24%** over 3 steps.

4.2.4 Concluding Words about the Chemical Syntheses of Lipid I/II and Analogs

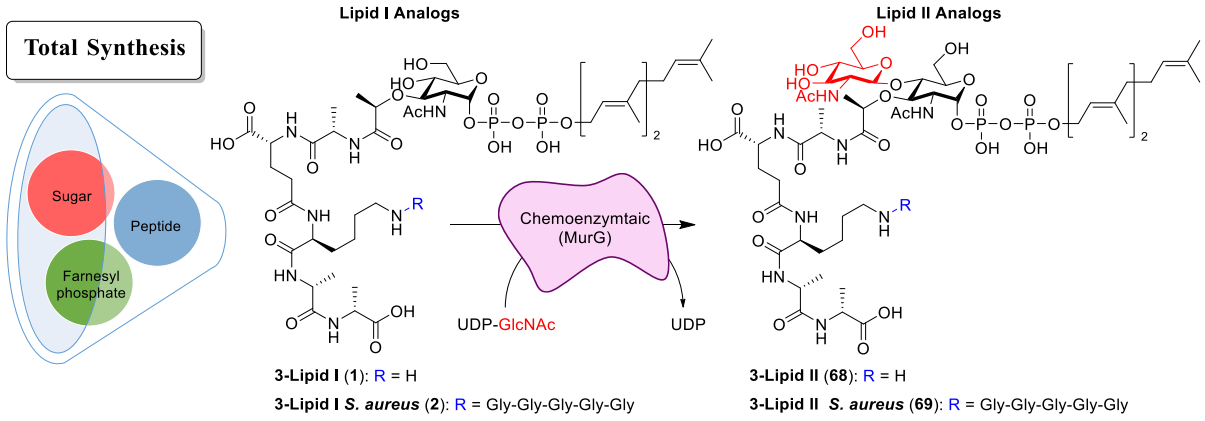
Despite the progress made in the above elucidated syntheses of lipid I/II and their analogs, only limited experimental details as well as not reported or not well resolved NMR spectra, which do also not allow to determine final/stereochemical purity, were provided. Furthermore, the described procedures left room for improvements in terms of yield, costs and time. Even though several useful and advantageous procedures were published in recent years, the supply issue of these highly valuable and essential compounds could not be solved yet.[25,80–83] Additionally, the established routes were not able to yield lipid I/II analogs featuring a strain dependent peptide modification at the lysine side chain, which would allow to further explore the interpeptidic crosslinks.

4.3 Aim of the Project

Detailed structural and functional studies of interactions between antibiotics and the main cell wall precursor's lipid I/II are severely hampered by the limited supply of these precious bacterial building blocks. Additionally, biochemical and structural studies with the full length lipid I/II are complicated by unfavorable physicochemical properties, including aggregation, precipitation and complex spectroscopic characteristics, which are mainly caused by the lipophilic undecaprenyl tail.[16,67] Previous studies showed that the natural C_{55} tail can be dramatically shortened and existing biochemical investigations with truncated analogs, such as farnesyl lipid I/II analogs **1** and **68**, suggested that these surrogates are mostly compatible with the authentic cell wall precursors.[16,25,67,76,84,85] Thus, the main task for this project was to supply the collaborative research center (TRR261, Cellular Mechanisms of Antibiotic Action; acronym: ANTIBIOTIC CellMAP) with sufficient amounts of shortened lipid I analog **1**, which could be transferred to the respective lipid II analog **68** chemoenzymatically. For this final step, a cooperation with the group of Prof. Dr. *Tanja Schneider*, who have an outstanding expertise in this field, should be established. For the total synthesis of lipid I analog **1**, an optimized, efficient, reproducible and scalable route should be developed to gain satisfactory yields of these valuable compounds, which would allow various further microbiological research within the TRR261.

If this main objective can be achieved, the following goal would be the development of a route to access species dependent interpeptidic bridge modifications.[84] Here, the initial focus should be on the first total synthesis of a shortened lipid I analog **2**, containing the *S. aureus* pentaglycine bridge modification, which was recognized to be essential for this pathogenic bacteria.[84] This novel compound was also planned to be transferred to its lipid II analog **69** chemoenzymatically and these surrogates are expected to be powerful tools to generate progress in antibiotic research.

In case satisfactory amounts of cell wall precursors can be synthesized, initial, extended NMR studies should be carried out in cooperation with Dr. *Senada Nozinovic* to get a better insight regarding the structures, stability and antibiotic interactions.



***Scheme 4.3-1*:** Target compounds of the project, which should be achieved by total synthesis (lipid I analogs **1**, **2**) and chemoenzymatic conversion (Lipid II analogs **68**, **69**).

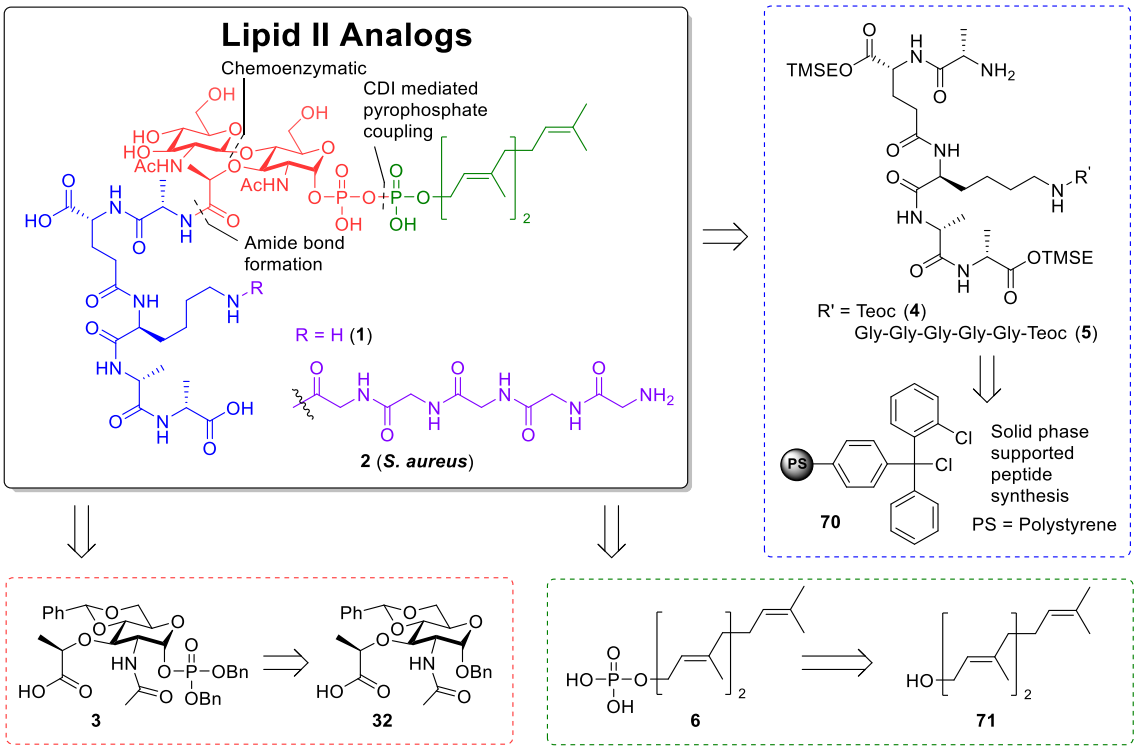
4.4 Results & Discussion

4.4.1 Retrosynthetic Analysis

As lipid II has three structural diverse components in its general composition, a retrosynthetic approach is more or less predetermined. Due to a cooperation with the *Schneider* group in Bonn and their outstanding know-how regarding cell wall biosynthesis, a chemoenzymatic conversion of synthesized lipid I analogs to their respective lipid II analogs, using the glycosyltransferase MurG and UDP-GlcNAc as a carbohydrate donor, was considered. Similar to *Walker et al.*,[16,67] the lipid I analogs **1** and **2** should then be synthesized from carbohydrate **3**, peptide **4** (or **5** for *S. aureus* modification) and the phospholipid **6** (**Scheme 4.4.1-1**). This approach is highly modular and gives the possibility to synthesize lipid I analog **2**, containing the pentaglycine bridge modification, which is known for *S. aureus*, without changing the overall procedure. While monosaccharide **3** should be gained after an only slightly modified, previously reported procedure from commercially acquired compound **32**,[16] the peptide fragment **4** was reconsidered. Thus, a novel solid phase supported synthesis was planned, giving access to the stem pentapeptide in high yield and quality. As a solid phase, the 2-chlorotrityl chloride (2CTC) resin **70** was chosen to allow facile peptide cleavage under mild conditions (hexafluoroisopropanol, HFIP), which would be compatible with the silyl protecting groups chosen for the two terminal carboxylates (D-Ala, L-Glu) and the side chain amine of lysine. This identical choice of protecting groups would also allow for a joint removal at a late stage of the synthesis. Peptide coupling should then be realized using the respective synthesized silyl and Fmoc protected amino acids. Furthermore, the synthesis of the peptide was planned in such a way that only slight modifications allow access to stem-specific interpeptidic bridges, like the pentaglycine in analog **2**. As in previous procedures and in view of the instability of an allylic pyrophosphate moiety, a late stage carbodiimidazole mediated coupling was envisaged for the introduction of farnesyl phosphate **6**, which in turn should be yielded out of farnesol (**71**) using a more recently reported one-pot procedure.[80]

In accordance to *Walker et al.*, the farnesyl phosphate **6** was planned to be activated as a phosphoimidazole,[69] which should be added to the pentapeptide-carbohydrate-phosphate fragment and not vice versa like the reported procedures by *Schwartz et al.* or *VanNieuwenhze*

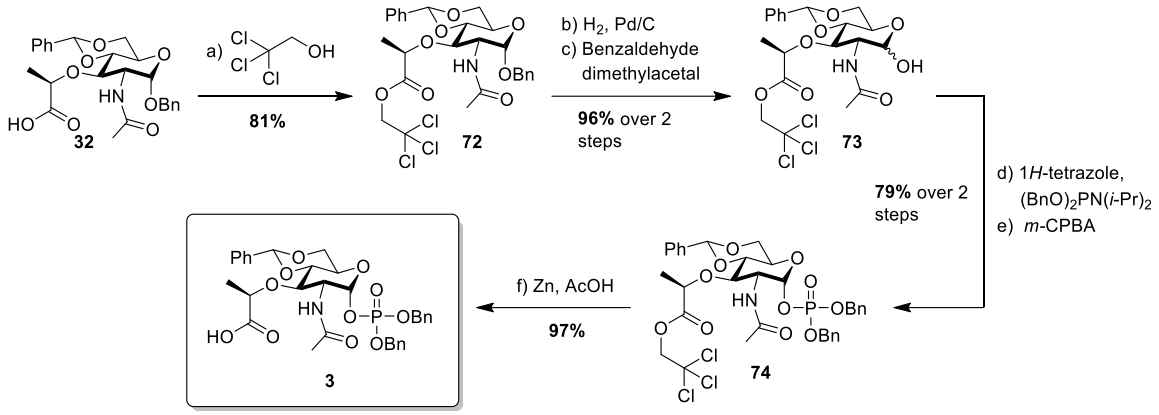
et al.[19,66] This order was chosen due to an expected facile synthesis of the farnesyl phosphate **6**, which could be used in an excess in the pyrophosphate coupling.



***Scheme 4.4.1-1*:** Retrosynthetic analysis. The three main components and the interpeptidic side chain modification known for *S. aureus* are colored. Carbohydrate = red, peptide = blue, farnesyl phosphate = green and interpeptidic side chain = purple. The second sugar (GlcNAc) was planned to be attached by a chemoenzymatic conversion of lipid I to lipid II analogs in a last step.

4.4.2 Synthesis of the Carbohydrate Fragment 3

An overview of the synthesis towards carbohydrate fragment **3** is shown in **Scheme 4.4.2-1**. The route in general was adopted from previous described procedures (see **Chapter 4.2** for overview) using slightly more technical modifications to facilitate methods and raise the overall yield.



***Scheme 4.4.2-1*:** Synthetic route towards carbohydrate fragment **3**. Reaction conditions: a) CCl_3CH_2OH (2.4 equiv), DCC (1.5 equiv), DMAP (0.1 equiv), THF, rt, 5 h, **81%**; b) H_2, 10% Pd/C, EtOAc, rt, 20 min; c) $PhCH(OCH_3)_2$ (1.5 equiv), *p*-TsOH (0.3 equiv), MeCN, rt, 4 h, **96%** over 2 steps; d) 1*H*-tetrazole (3.8 equiv), $(BnO)_2PN(i\text{-}Pr)_2$ (2.5 equiv), DCM, −40 °C to rt, 1 h; e) *m*-CPBA (3.0 equiv), −60 °C to rt, 2 h, **79%** over 2 steps; e) Zn dust, 90% $AcOH/H_2O$, rt, 1 h, **97%**.

Starting from commercially acquired carbohydrate **32** the first reaction proceeded like reported in literature with an identical yield of 81% for the *Steglich* esterification to protect the free carboxyl group as a trichloroethyl ester.[16] A trimethylsilyl ester was considered for protection as well, since they are easily cleavable with fluoride ions, but this idea was dropped due to the successful reproduced protection scheme. A mechanistic point of view regarding the *Steglich* esterification is described in **Chapter 4.4.4** caused by a more eventfully reaction process. The fully protected carbohydrate **72** was then exposed to palladium in a hydrogen atmosphere to cleave the anomeric benzyl group. Being not fully inert to this method, the acetal group was also cleaved during this reaction. *Walker et al.* noticed the missing protecting group and reattached it using benzaldehyde dimethylacetal and catalytic amounts of *p*-toluenesulfonic acid in DMF.[16] However, this method proved to be more difficult and modifications were considered. Thus, the solvent was exchanged to MeCN, which can be easier removed under reduced pressure and the amount of *p*-toluenesulfonic acid was slightly increased from 0.1 to 0.3 equiv. These modifications resulted in an increase of the yield from 81% to almost quantitative 96% and shortened the required reaction time from 10 h to 4 h. Additionally, the reaction worked in the same way even if the amount of benzaldehyde dimethylacetal was dramatically reduced from the reported high excess of 10 to 1.5 equiv. For alcohol **73**, a 4:1 α:β mixture was observed in the ^1H NMR spectrum measured in CD_2Cl_2 at room temperature being in accordance to *Walker et al.*, who recognized a similar ratio.[16] The favored α-species can be

explained by the anomeric effect, which was introduced by *Lemieux* and *Chu* in 1958.[86] Since then several approaches have been published, which explain the preference of the axial position of the substituent at C1, as here in the α-anomer, in contrast to the presumed more favorable equatorial position. For example, minimization of the dipole character and a beneficial hyperconjugation are often mentioned to explain the unusual axial position.[86] Although it is reported that the mixture can be used for the phosphorylation, a closer analysis of the anomeric mixture was done during this work. Hence, HPLC was performed (for HPLC details see experimental section), which was able to guarantee a baseline separation (**Figure 4.4.2-1**). Afterwards, the two resulting fractions were analyzed by NMR spectroscopy. While the separated α-fraction showed pure NMR spectra (CD_2Cl_2, room temperature), the β-fraction revealed again a mixture of anomers (α:β = 1:2). These observations suggest that the β-anomer partially equilibrates back to the α-anomer while the separated α-anomer remains stable under the used conditions. The structure of carbohydrate **73** can be confirmed using NMR spectroscopy and is exemplarily shown for the predominant α-anomer (**Figure 4.4.2-1**).

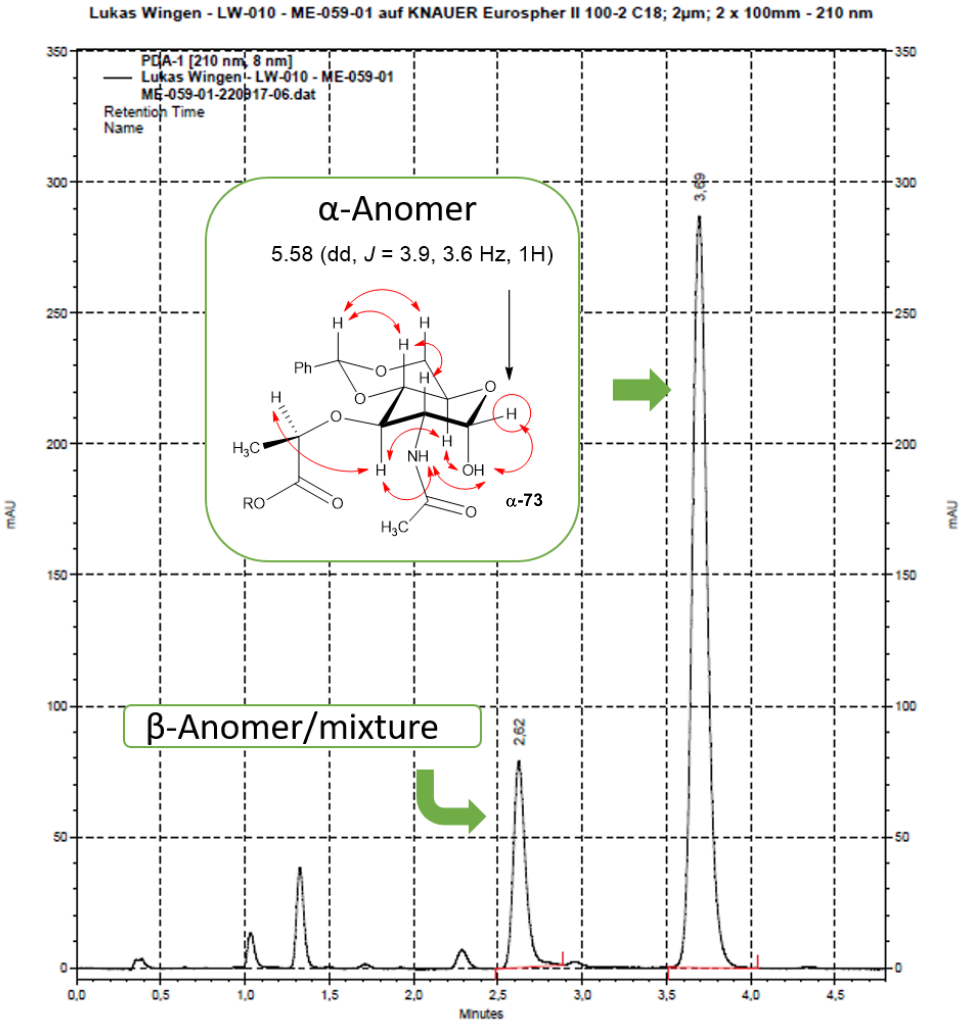
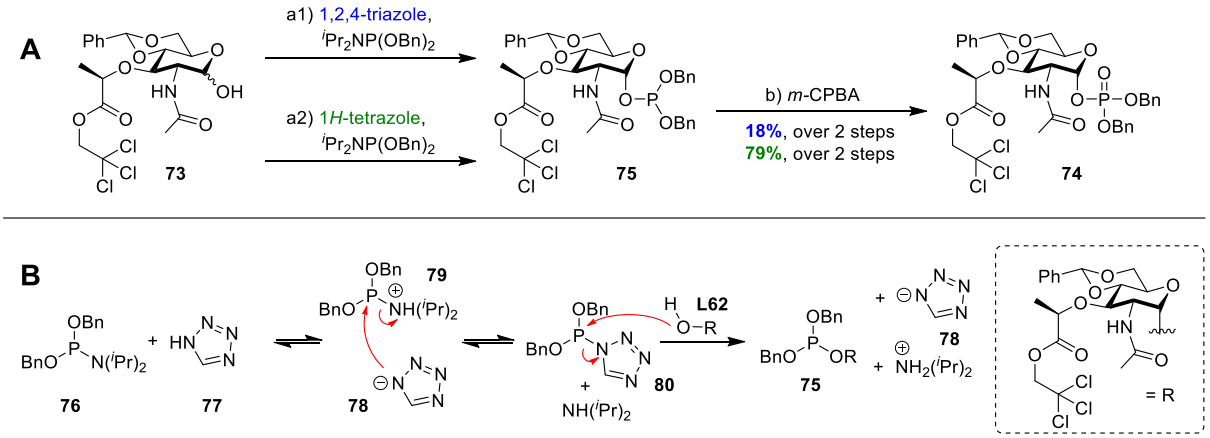


***Figure 4.4.2-1*:** HPLC chromatogram from an analytical separation of the anomeric mixture of compound **73**. For the predominant α-anomer, selected NOESY signals (red arrows) and the signal for H-1 are shown, which were used for the determination of the absolute structure.

Larger coupling constants ($J \approx 10 - 12$ Hz) were observed for *trans*-couplings in the case of H-2, 3, 4 and 5, while the signal for H-1 (5.58 ppm, dd, $J = 3.9, 3.6$ Hz), which was used to assign the α-position, showed only a small coupling constant due to the *cis*-configuration. Additionally, NOESY experiments were able to give more insights in structural analysis and therefore approved the general structure of the molecule with its two chairlike ring systems (**Figure 4.4.2-1**).

The free lactol **73** paved the way for the introduction of the anomeric phosphate, which was performed based on previous results,[16,66,69] using the 4:1 α:β mixture of compound **73**. In general, preparation of glycosyl monophosphates can be divided into two classes. The carbohydrate can act either as nucleophilic or as electrophilic species. In the latter case, it is

reported that a carbohydrate, featuring a functional group capable of neighboring group participation at the C2-position, generally favors the formation of a 1,2-*trans* linked glycosyl phosphate, which will result in a product with undesired anomeric stereochemistry.[87] Hence, a literature reported procedure,[16,69,88] where the carbohydrate serves as a nucleophile and using an electrophilic phosphorus reagent, was applied. Therefore, treatment of compound **73** with dibenzyl *N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole was used, yielding only the desired anomeric phosphite, which was directly oxidized with *m*-CPBA, rendering carbohydrate **74** in 79% yield over these two steps. Again, the stereochemistry was controlled by the respective coupling constants and NOESY signals. It is believed that the capture by the α-hydroxyl group occurs faster than anomerization and attack by the β-anomer, so that only the α-substitution is observed.[69] A direct oxidation was chosen due to a literature reported instability of anomeric phosphites.[89] Caused by a supply issue of 1*H*-tetrazole, the reaction was first tried using 1,2,4-triazole, which also worked for phosphitylation procedures in the past.[69,75,89] Even though compound **74** was obtained using 1,2,4-triazole, the yield was much lower (18% for the two steps) than using 1*H*-tetrazole, which was directly used after supply (for comparison see **Scheme 4.4.2-2**, **A**). This discrepancy can be explained by considering the mechanism (**Scheme 4.4.2-2**, **B**).



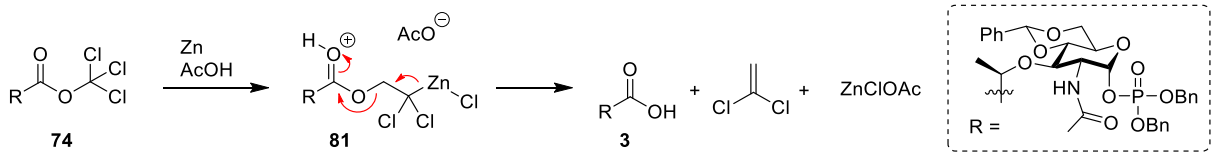
***Scheme 4.4.2-2*: A**: Comparison of methods. In blue the reaction using 1,2,4-triazole and in green using 1*H*-tetrazole. Reaction conditions: a1) 1*H*-tetrazole (3.8 equiv), $(BnO)_2PN(i\text{-}Pr)_2$ (2.5 equiv), DCM, −40 °C to rt, 1 h; a2) 1,2,4-triazole (3.8 equiv), $(BnO)_2PN(i\text{-}Pr)_2$ (2.5 equiv), DCM, −40 °C to rt, 1 h; b) *m*-CPBA (3.0 equiv), DCM, −60 °C to rt, 2 h, for yields see scheme. **B**: Proposed reaction mechanism using 1*H*-tetrazole.

It is proposed that as a first step, the nitrogen of the phosphoramidite **76** is protonated by 1*H*-tetrazole (**77**) resulting in a longer and weaker phosphorus nitrogen bond.[88] Secondly, the

protonated phosphorus species **79** is attacked by the nucleophilic tetrazole **78** and diisopropylamine is displaced. This renders the active species **80**, which is finally attacked by the anomeric hydroxyl of carbohydrate **73**. Thus, for an efficient activation of the phosphoramidite, an acid is needed to protonate the phosphoramidite nitrogen but the corresponding base has to serve as a good nucleophile as well, to allow rapid conversion to the activated intermediate **80**. Furthermore, an additional good leaving group tendency to facilitate the formation of the phosphite triester **75** is required.[88]

Since the used tetrazole is more acidic (pK_a = 4.9) than triazole (pK_a = 10), it is believed that the initiating protonation step proceeds smoother ensuring a superior reaction outcome.[69]

To fulfill the synthesis of carbohydrate fragment **3** and to ensure a possible coupling of the peptides **4** or **5**, the carboxylic acid of the lactyl moiety had to be unmasked. For this purpose, zinc powder in acidic condition was utilized, rendering the final monosaccharide scaffold **3** in an excellent yield of 97% for the deprotection.



***Scheme 4.4.2-3*:** Mechanism of the trichloroethyl ester cleavage.

The reductive cleavage of ester **74** generally includes two steps and is initiated by a metal-halogen-exchange reaction resulting in the formation of β-metallated ester **81**. As a second step, species **81** collapses inevitably through 1,2-elimination giving 1,1-dichloroethane and the desired carbohydrate **3**, which features the free carboxylic acid.[90] Even though the used conditions seems to be harsh (90% AcOH), the reaction could be performed flawlessly generating acid **3** in high yield and purity. Notably, milder cleavage opportunities like SmI_2 and Cp_2TiCl_2 have also been reported.[90,91]

In total, carbohydrate fragment **3** was synthesized in an overall yield of 60% over six steps from commercially available compound **32**, which compared favorably to the literature reported yield of 41%.[16] Remarkably, reactions b and c as well as d and e (see **Scheme 4.4.2-1**) can be performed as one-pot reactions.

4.4.3 Synthesis of Amino Acid Building Blocks

To synthesize the stem peptide of lipid I/II and to pave the way for the introduction of species dependent side chain modifications like the pentaglycine, known for *S. aureus*, several amino acid building blocks were required. While some of the amino acids were commercially available, others had to be synthesized to feature suitable protecting groups for solid phase supported peptide coupling and to allow convenient deprotection schemes. Furthermore, silyl protecting groups were installed at amino acid side chains and the carboxylate of the terminal D-Ala to ensure a global deprotection in the endgame strategy. In **Figure 4.4.3-1** all amino acids, which were used for either synthesizing pentapeptide **4** or decapeptide **5** (see **Scheme 4.4.1-1**), are shown.

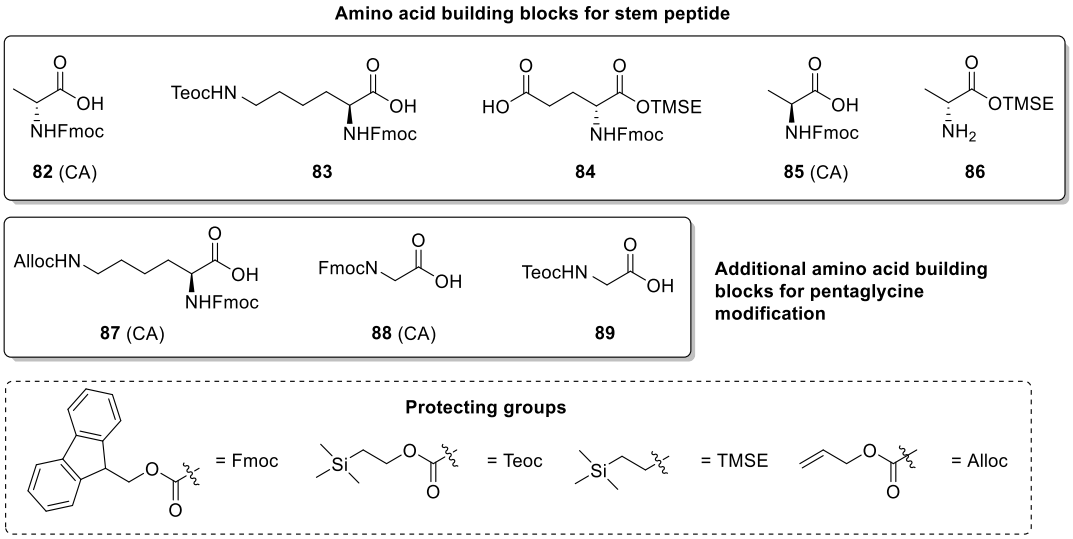
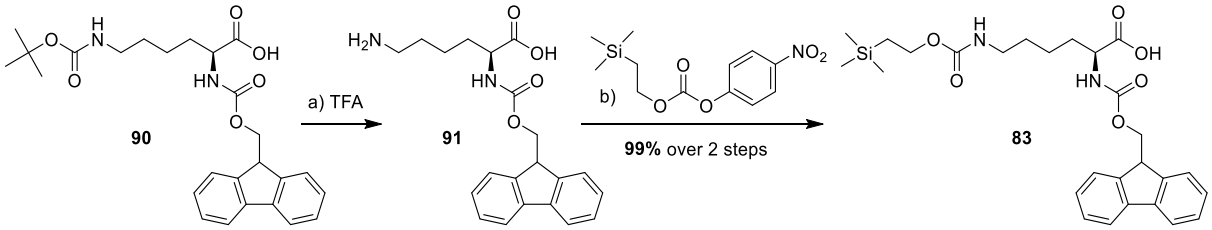


***Figure 4.4.3-1*:** Top: Amino acid building blocks, which were used for the synthesis of the stem peptide. Middle: Additional amino acid blocks for the introduction of the interpeptidic pentaglycine modifications, known for *S. aureus*. Bottom: Used protecting groups. CA = commercially acquired.

For the synthesis of the stem pentapeptide **4**, the suitable amino acid building blocks **82** and **85** were readily available and commercially purchased. However, the lysine, glutaminic acid and the alanine derivatives **83**, **84** and **86** had to be synthesized prior to the utilization in the planned solid phase supported synthesis.

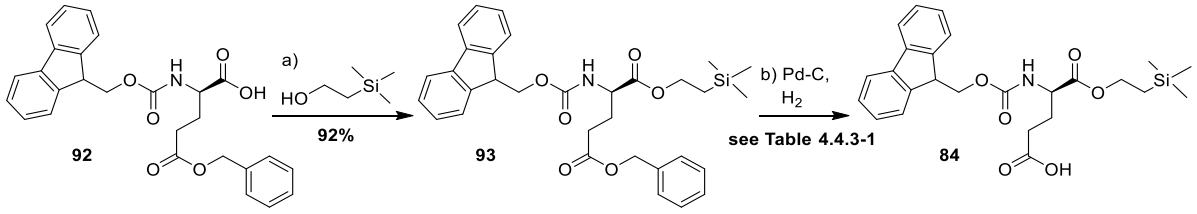
Fmoc-L-Lys(*N*-Teoc)-OH (**83**) was synthesized using a one-pot, two-step procedure starting from acid **90** (**Scheme 4.4.3-1**).[16]



***Scheme 4.4.3-1*:** Synthesis of lysine building block **83**. Reaction conditions: a) TFA/DCM (1:1), rt, 20 min; b) 2-(Trimethylsilyl)ethyl *p*-nitrophenyl carbonate (1.2 equiv), DIPEA (5.0 equiv), DMF, rt, 2 h, **99%** over two steps.

First, the Boc-protecting group was cleaved using acidic conditions, which generated the free amine **91** ready for the attachment of a silyl protecting group. Thus, TFA and the solvent were removed under reduced pressure and crude compound **91** was directly used in the same vessel to install a Teoc protecting group generating lysine derivative **83** in a quantitative yield of 99% over two steps.

Fmoc-D-Glu(OH)-TMSE (**84**) was first synthesized using a literature reported synthesis[67] and as a first step, the free carboxylic acid **92** was masked as a 2-(trimethylsilyl)ethyl (TMSE) ester under the use of *Steglich* conditions. Afterwards, the benzyl ester of the side chain was hydrogenolized to warrant a coupling via the side chain, as it is found in natural lipid I/II. While the *Steglich* esterification generated fully protected **93** in a facile and high yielding (92%) fashion, the hydrogenolysis was not as unchallenging as expected.



***Scheme 4.4.3-2*:** Synthesis of glutaminic acid building block **84**. Reaction conditions: a) DCC (1.2 equiv), DMAP (0.1 equiv), 2-(trimethylsilyl)ethanol (1.2 equiv), EtOAc, rt, 2 h, **92%**; b) H_2, 10% Pd/C, MeOH, rt, 10 min, for yield and additive see **Table 4.4.3-1**.

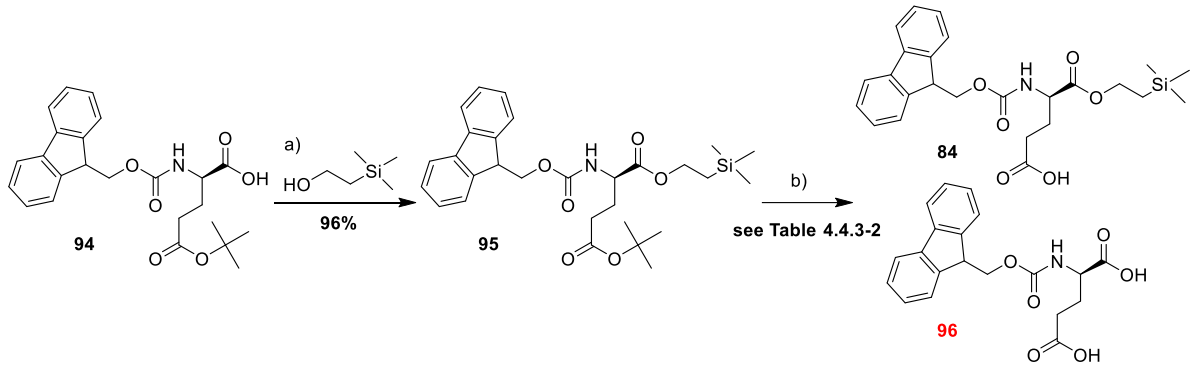
In a first attempt, only 23% yield was achieved for compound **84** and NMR analysis showed that the Fmoc group did not withstand the applied conditions for the hydrogenolysis (**Table 4.4.3-1**). It was expected that the benzyl group is more labile and maybe shorter reaction times would prevent Fmoc cleavage and thus raise the yield. With a halved reaction time, a higher

product yield of 41% was isolated but the timing remained a major drawback and rendered reaction control by thin layer chromatography, which at least takes some minutes, impractical. In general, the Fmoc group is known to be cleaved under basic conditions involving β-elimination and it was found that the addition of $CaCl_2$ can increase the lifetime of the Fmoc protective group, due to prevention of basic conditions.[92] Also in this case, addition of $CaCl_2$ to a final concentration of 0.25 M showed a positive effect and the desired product was isolated in 54% yield. Finally, an expected stability of compound **84** towards a slightly acidic environment motivated to add a few drops (≈ 100 µL) of acetic acid and the yield was further increased to 84%.

Table 4.4.3-1: Screened conditions for the synthesis of compound **84**.

Entry	Reaction time [min]	Additive	Yield [%]
1	20	-	23
2	10	-	41
3	10	0.25 M $CaCl_2$	54
4	10	0.03 M AcOH	**84**

Cooling and solvent exchange was also considered but in the meantime a different, higher yielding, preparative less laborious and more economical strategy to yield compound **84** was found. For this purpose, amino acid **94** (5 g = 95 €, Iris Biotech GmbH, May 06, 2020) was purchased, which represents a more cost-efficient starting point compared to compound **92** (5 g = 180 €, Iris Biotech GmbH, May 06, 2020). To protect the free carboxyl group of acid **94** as a TMSE ester, the same *Steglich* conditions were used and the reaction remained unproblematic with a yield of 96%.



***Scheme 4.4.3-3.*:** Novel approach for glutaminic acid building block **84**. Reaction conditions: a) DCC (1.2 equiv), DMAP (0.2 equiv), 2-(trimethylsilyl)ethanol (1.5 equiv), EtOAc, rt, 2 h, **92%**; b) see **Table 4.4.3-2**.

To achieve the optimal conditions for the *tert*-butyl ester cleavage, a small screening was started (**Table 4.4.3-2**).

Table 4.4.3-2: Screened conditions for the synthesis of **84**.

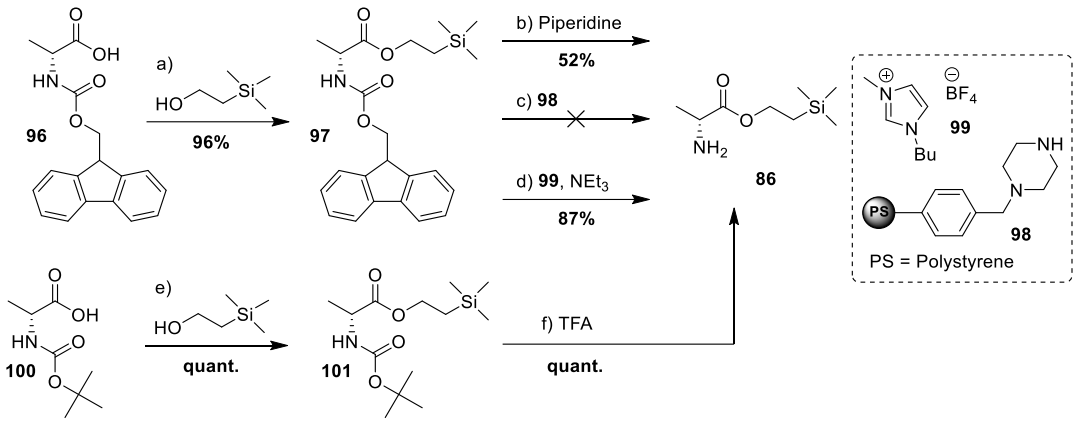
Entry	Conditions	Result
1	TFA/DCM (1:1), rt, 30 min	**84**/**96** (1:4)
2	TFA/DCM (1:9), rt, 1.5 h	Unselective
3	Et_3SiH (2.5 equiv), TFA/DCM (1:4), rt, 1 h	Unselective
4	$ZnBr_2$ (12 equiv), DCM, rt, 24 h	Only **96**
5	H_3PO_4/DCM (1:1), rt, 5 h	No conversion
6	TMSOTf (10 equiv), 2,6-lutidine (20 equiv), DCM, 0 °C to rt, 1 h	**97%** isolated **84**

The major task was to find reactants which can cleave the *tert*-butyl ester without damaging the TMSE ester. As a first attempt, standard acidic conditions were used (**Table 4.4.3-2**, entry 1) but after removal of the volatiles, NMR analysis showed a mixture of compounds **84**/**96** in a 1:4 ratio, so that in a next reaction a milder TFA/DCM mixture was chosen (entry 2).[93,94] Full conversion was observed but the TLC showed again a mixture of the two compounds **84** and **96**. Literature describes triethylsilane as a carbocation scavenger in the deprotection of *tert*-butyl esters, but this additive did not circumvent missing selectivity (entry 3).[95] A reported procedure using an excess of $ZnBr_2$ furnished only compound **96** (entry 4) and using a previously described aqueous phosphoric acid procedure as much milder alternative showed no

reaction at all (entry 5).[96,97] Finally, an excess of TMSOTf and 2,6-lutidine was able to unmask the side chain carboxylic acid in a selective manner rendering glutaminic acid building block **84** in 97% yield (entry 6).[98] Notably, no column chromatography was necessary for this step and simple extractions were adequate to give the desired compound in high purity. The novel two-step procedure towards compound **84** compared favorably to the reported method.[67] The yield was only moderately increased from 77% to 93%, but the new procedure starts from a half priced starting material, uses cheap reactants, is easier reproducible and has no need of chromatography in the second step.

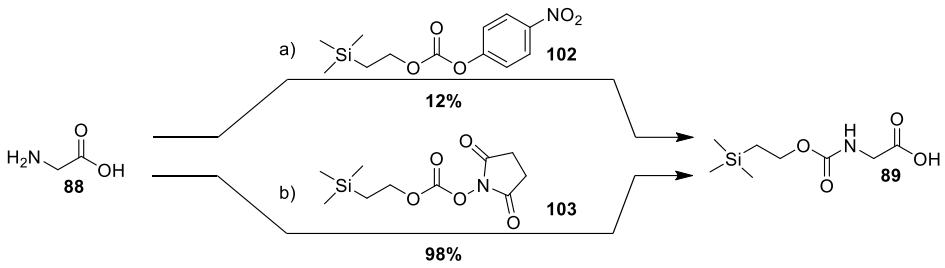
The amino acid building block H_2N-D-Ala-TMSE (**86**) was also synthesized in a two-step procedure with a *Steglich* esterification to protect the carboxylic acid as a first step (**Scheme 4.4.3-4**). Starting from Fmoc-D-Ala-COOH (**96**), the respective protected ester **97** was gained in an excellent yield of 96%. Subsequent Fmoc cleavage using a piperidine/DMF mixture rendered building block **86**. For this step, a moderate yield was achieved. It was assumed that the reaction was unproblematic and TLC also confirmed a well-functioning reaction process. As a consequence, the yield loss was attributed to the work up. Although various work up conditions like extractions, adding NEt_3 to the chromatography solvent and trying different chromatography materials (aluminumoxide different pH, silica) were employed, an increase of the yield over 52% was not realized. Hence, two more recent methods for the removal of Fmoc were tried. A procedure published by *Chin et al.* in 2012 reported on a Fmoc removal with the use of polymer-bound piperazine **98** allowing a work up without column chromatography.[99] Unfortunately, this technique was not suitable for protected alanine **97** and no conversion to unprotected amine **86** was observed. Secondly, a procedure using an ionic liquid was envisioned, which worked for a plethora of substrates reported by *Gioia et al*.[100] Ionics liquids gained more attention in recent years due to a possible eco-friendly alternative to replace organic solvents. Additionally, advantageous properties of ionic liquids, such as non-inflammability, low volatility, reusability and high thermal stability, are described. *Gioia et al.* postulated a stabilizing effect of ionic liquid **99** on charged intermediates and also a somehow assistance to the abstraction of the fluorenyl proton.[100] Thus, the group recognized an intense improvement for the deprotection reaction by using the ionic liquid instead of organic solvents.[100] The reported methodology, which allowed facile purification using extractions, worked and yielded compound **86** in 87%. In contrast to the reported short reaction times (5-15 min), a complete conversion was monitored after 2 d, which is a considerable

disadvantage also in view of a possible isomerization under the used basic conditions. Therefore, the search for a better access to compound **86** was continued. Starting from Boc protected D-Ala **100**, the TMSE protection yielded quantitative amounts of alanine derivative **101**[101] followed by deprotection of the Boc group using a 25% TFA/DCM mixture, which in this case selectively cleaved the carbamate. After evaporation of all volatiles, amine **86** was synthesized in a quantitative yield over two steps without using column chromatography for the Boc cleavage.



***Scheme 4.4.3-4*:** Synthesis of alanine building block **86**. Reaction conditions: a) DCC (1.2 equiv), DMAP (0.1 equiv), 2-(trimethylsilyl)ethanol (1.2 equiv), EtOAc, rt, 40 min, **96%**; b) 20% Piperidine/DMF, rt, 1 h, **52%**; c) Piperazine polymer-bound (**98**, 4.0 equiv), DCM, rt, on; d) NEt_3 (3.0 equiv), **99** as solvent, rt, 2 d, **87%**, e) DCC (1.2 equiv), DMAP (0.2 equiv), 2-(trimethylsilyl)ethanol (1.5 equiv), EtOAc, rt, 2 h, **quant.**; f) 20% TFA/DCM, rt, 1 h, **quant**.

After the successful synthesis of the stem peptide **4** (see **Chapter 4.4.4**), a possible way to synthesize peptides, which feature species specific interpeptidic modifications, was searched. To introduce a respective pentaglycine chain in a solid phase supported synthesis, three additional amino acid building blocks were necessary (see **Figure 4.4.3-1**). While orthogonal protected lysine derivative **87** and Fmoc-Gly (**88**) were purchased, the needed glycine featuring a Teoc protection **89** was synthesized (**Scheme 4.4.3-5**).



***Scheme 4.4.3-5*:** Synthesis of glycine building block **89**. Reaction conditions: a) 2-(Trimethylsilyl)ethyl *p*-nitrophenyl carbonate (**102**, 1.2 equiv), DIPEA (3.0 equiv), DMF, rt, 3 h, **12%**; b) *N*-[2-(Trimethylsilyl)ethoxycarbonyloxy]succinimide (**103**, 1.2 equiv), NEt_3 (3.0 equiv), 1,4-dioxane/water (1:1), rt, on, **98%**.

The Teoc protecting procedure, which was also used for the preparation of lysine building block **83**, yielded protected glycine **89** in poor yield (12%) but a protecting method using succinimide **103** and a solvent mixture of 1,4-dioxane/water gave desired glycine derivative **89** in an excellent yield of 98%.
In conclusion, a high yielding and facile method was found for all required amino acid building blocks.

4.4.4 Synthesis of Pentapeptide 4

Having a reliable, scalable and high yielding strategy for the synthesis of the respective pentapeptide **4** is one of the most crucial aspects in the development of lipid I/II analogs. Therefore, a novel route was designed, which relied on solid phase peptide synthesis using the 2-chlorotrityl-chloride (2CTC) resin to allow for facile peptide cleavage under mild conditions (hexafluoroisopropanol, HFIP).[102] This mild cleavage opportunity was expected to be compatible with the silyl protecting groups chosen for the carboxylates in D-alanine **86** and L-glutamic acid **84** as well as the side chain amine of L-lysine **83** (**Figure 4.4.3-1**). Once a solid phase synthesis protocol was designed, which includes functionalization of the resin, deprotections, couplings, washings and cleavage from the resin, the actual handling was more or less simple if the developed protocol was strictly followed (see experimental section for details). Thus, a frit containing syringe (**Figure 4.4.4-1**) was loaded with the resin and the resin was swelled for 20 min in DCM.

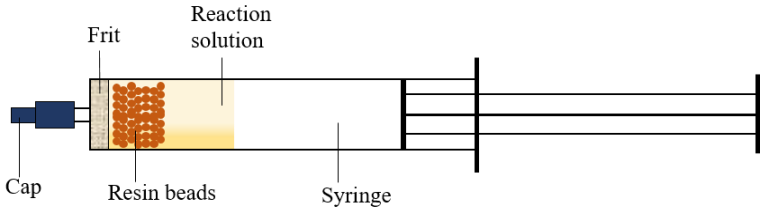
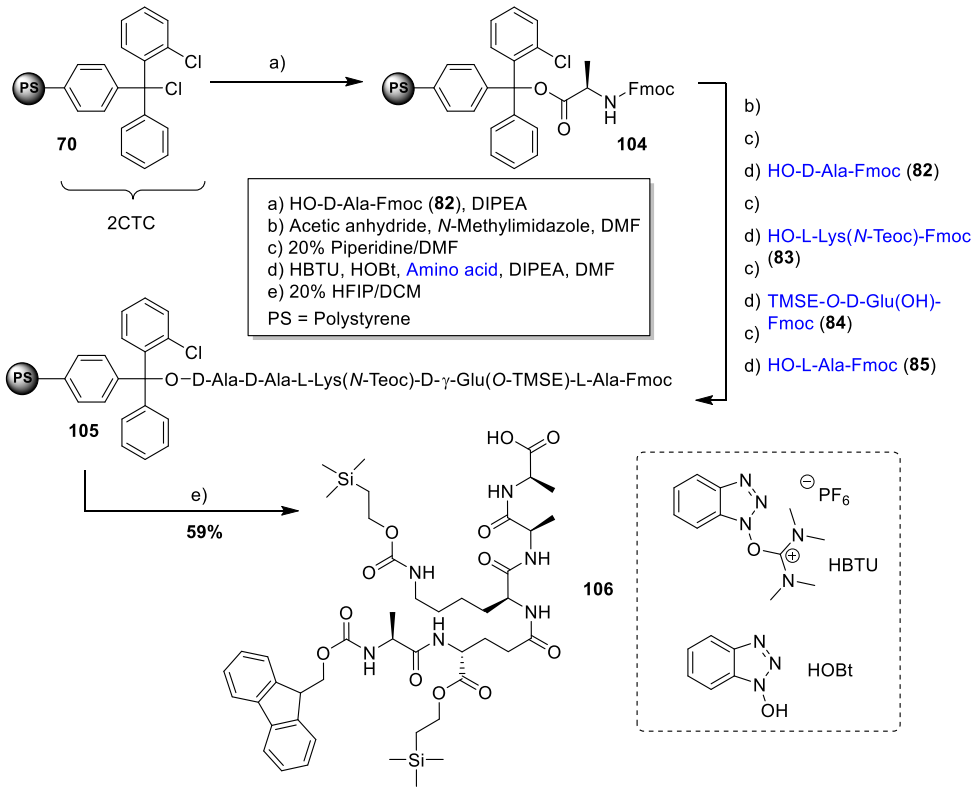


***Figure 4.4.4-1*:** Schematic construction of the syringe which served as reaction vessel.

The syringe was placed in a shaker to ensure sufficient mixing and after draining of the swelling solvent the first amino acid was coupled to the resin. Thus, a solution of HO-D-Ala-Fmoc (**82**) and DIPEA in DMF was drawn into the syringe (**Scheme 4.4.4-1**, a).



***Scheme 4.4.4-1*:** Synthesis of pentapeptide **106**. For details regarding equivalents, time as well as the exact procedure see experimental section.

The syringe containing the functionalization solution was shaken for 1 h at room temperature before draining. In this step, only 0.5 equivalents of the amino acid building block were used to reduce the initial resin loading from 2.1 mmol/g to 1 mmol/g. This reduction of the loading

was done because it was expected that the chain growth of the peptide was more unhindered if the resin loading was lower (see **Figure 4.4.4-2** for visualization).

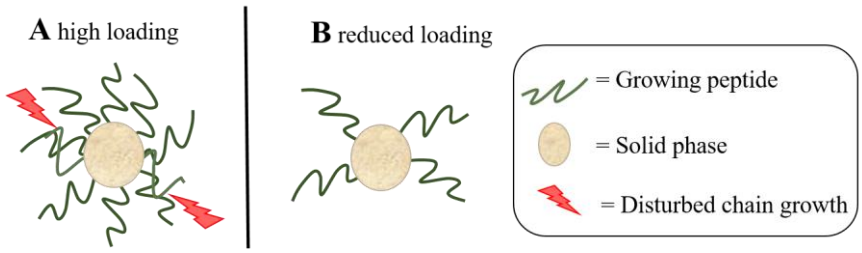
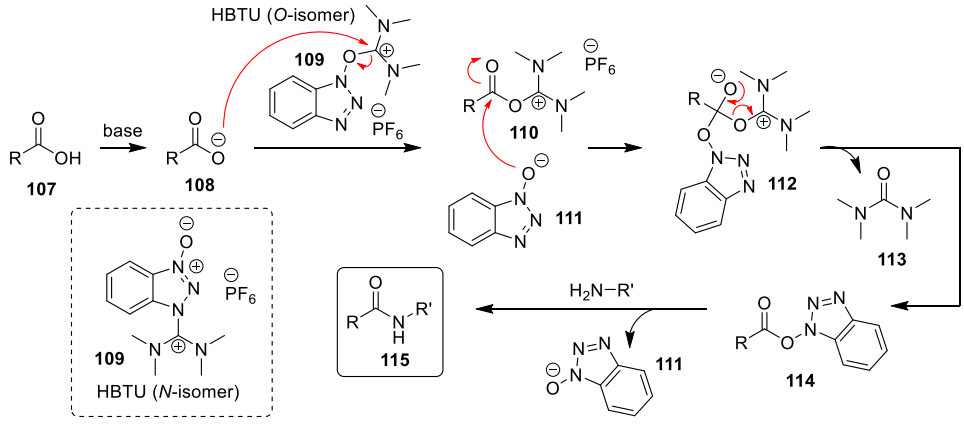


***Figure 4.4.4-2*:** Schematic illustration of the peptide growth with a high loading of the resin (A) and if the loading is reduced (B).

As shown in **Figure 4.4.4-2** (A), a high loading of the resin beads may result in unfavorably interactions of the peptide chains, while a reduced loading (**Figure 4.4.4-2**, B) ensures an unhindered peptide growth. After reduction of the resin loading and functionalization with the first amino acid, the unreacted 2CTC-functionalities had to be capped which was done using a mixture of acetic anhydride and *N*-methylimidazole in DMF (**Scheme 4.4.4-1**, b). After washings with DMF, the Fmoc group of resin bound amino acid **104** was cleaved using 20% piperidine/DMF (**Scheme 4.4.4-1**, c) followed by coupling of the next amino acid building block **82** using a HBTU/HOBt coupling method (**Scheme 4.4.4-1**, d). DMF and DCM rinses between the steps ensured that unreacted reagents were flushed out and the use of an excess of reagents as well as repetition of each coupling and deprotection step using a fresh reaction solution should guarantee complete conversions. An accepted activation mechanism of carboxylic acids using HBTU is displayed in **Scheme 4.4.4-2**.



***Scheme 4.4.4-2*:** Mechanism of amide bond formation using carboxylic acid activation by HBTU.

First, the carboxylic acid **107** is deprotonated by a base (DIPEA) and the resulting carboxylate **108** attacks the carbeniumion of HBTU (**109**). Subsequently, the displaced benzotriazole *N*-oxide (**111**) attacks the carbonyl group of compound **110** furnishing tetrahedral intermediate **112**, which collapses to the activated ester **114** and tetramethyl urea **113** as byproduct. Finally, aminolysis yields the respective amide **115** and benzotriazole *N*-oxide (**111**) is ejected.[103,104] Noteworthy, X-ray crystallography showed that HBTU and related coupling reagents crystallize as an aminium salt (*N*-isomer) rather than the corresponding uronium salt (*O*-isomer).[105]

HOBt was added to the coupling solution caused by reported advantageous effects like acceleration of the coupling process and decrease of the epimerization level. [103,104]

Repetitive deprotection and couplings of the remaining amino acid building blocks (**82**, **83**, **84** and **85**) yielded resin bound pentapeptide **105** (**Scheme 4.4.4-1**), which was liberated from the solid phase using the weak acid HFIP. After precipitation in Et_2O, pentapeptide **106** was isolated in 59% yield.

During solid phase synthesis the reaction processes cannot be tracked by standard methods like TLC, mass spectrometry or NMR analysis. In order to get indications for successful reactions, test cleavages can be carried out or the deprotection solution after Fmoc removals can be examined UV-metrically. The latter was done during the sequence towards pentapeptide **106** and after every Fmoc cleavage, the solution was collected in a volumetric flask, filled up with 20% piperidine/DMF and a dilution series was prepared, which was analyzed using a UV/VIS-spectrometer and the following formula:

$$loading\ [mmol/g] = \frac{\mathrm{E} * \mathrm{V}_{stock} * \mathrm{D}}{\varepsilon * \mathrm{m} * \mathrm{l}}$$

With:

E = Absorption of the sample solution at 301 nm

V_{Stock} = Volume of the stock solution [mL]

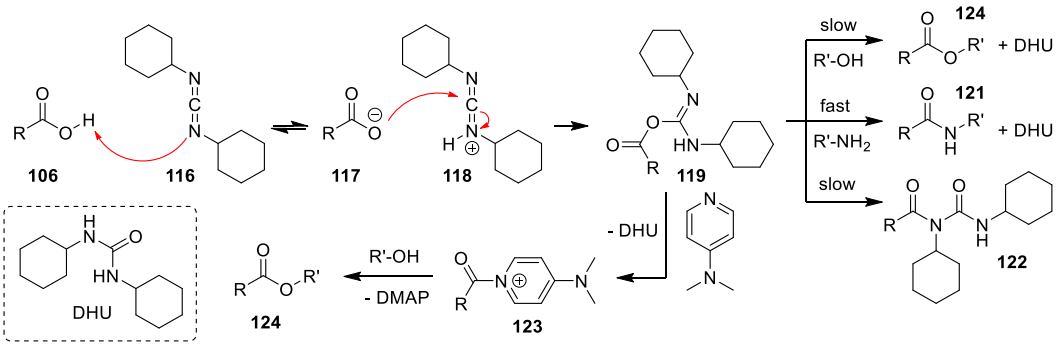
D = Dilution factor

ε = Molar absorption coefficient at 301 nm = 6054 [L mol^{-1} cm^{-1}]

m = sample weight of the resin [mg]

l = optical path length of the cell = 1 [cm]

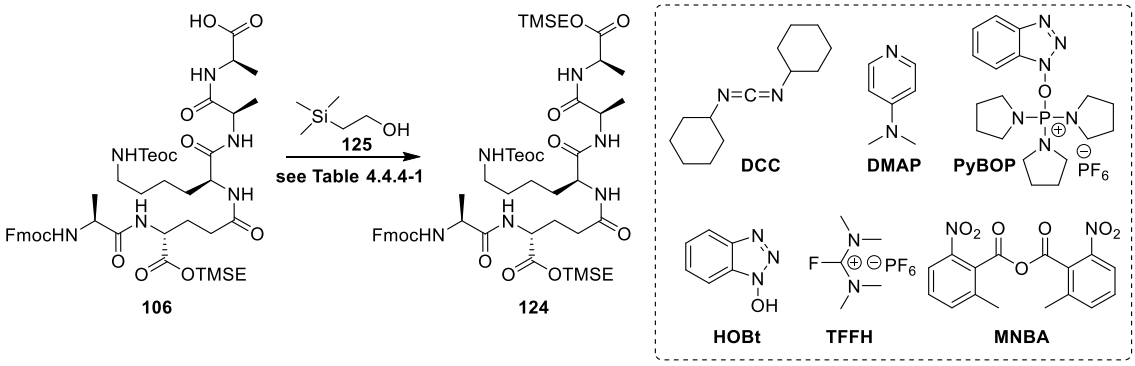
Overall, the procedure to pentapeptide **106** can be realized in less than 15 h and a subsequent two-step protection modification should yield the protected, terminal carboxyl group and the free amine function for coupling with the carbohydrate fragment **3**. To protect the free, terminal carboxyl group, *Steglich* esterification with 2-(trimethylsilyl)ethanol, which was also used for the amino acid building blocks, was carried out but unfortunately the procedure did not turn out as planned. The reaction suffered from long reaction times, incomplete conversions and epimerization. In a usual *Steglich* esterification, the carboxylic acid (here pentapeptide **106**) and the carbodiimide (**116**, DCC) are able to form an *O*-acylisourea intermediate **119** (**Scheme 4.4.4-3**). This intermediate can react with an amine to the respective amide **121** or with an alcohol to form ester **124**. The latter is a comparably slow process due to the less nucleophilic alcohol. It is also possible that *O*-acylisourea **119** undergoes an intramolecular rearrangement to form the *N*-acylurea byproduct **122**. To allow an accelerated ester formation and to avoid the generation of byproduct **122**, DMAP is often added. DMAP reacts rapidly with the *O*-acylisourea **119** and generates an acyl pyridinium species **123**, which is incapable of intramolecular byproduct formation and can react smoothly with the alcohol to yield the ester **124** and to gain back DMAP.[106–108]



***Scheme 4.4.4-3*:** Mechanism of the *Steglich* esterification with DCC and DMAP.

As already mentioned, *Steglich* esterification worked satisfactorily for the amino acid building blocks but not for the protection of pentapeptide **106**. One possible reason could be that the generated *O*-acylisourea **119** is stabilized by the various hydrogen bond possibilities of the peptide. Mass spectrometry showed an accumulated pentapeptide cyclohexylcarbodiimide species, which could either be the *O*-acylisourea **119** or even the *N*-acylurea byproduct **122** due to the same exact mass. Additionally, epimerization was indicated by HPLC analyses. Hence,

the reaction was not further investigated and other esterification methods were considered instead (**Scheme 4.4.4-4**, **Table 4.4.4-1**).[106,109]



***Scheme 4.4.4-4*:** Esterification reaction towards **124** and used reagents. For reaction conditions, see the following table.

Table 4.4.4-1: Screened conditions for the synthesis of **124**.

Entry	Conditions
1	**125** (1.3 equiv), DCC (1.2 equiv), DMAP (0.1 equiv), DMF, rt, 6 h
2	**125** (2.0 equiv), PyBOP (1.5 equiv), HOBt (1.5 equiv), DIPEA (5.0 equiv), DMF, rt, 1 h
3	**125** (10 equiv), TFFH (1.2 equiv), DIPEA (3.0 equiv), DMF, rt, 1 h
4	**125** (2.0 equiv), MNBA (1.0 equiv), DMAP (0.1 equiv), DIPEA (3.0 equiv), DMF, rt, 1 h

With exception of the *Shiina* esterification (**Table 4.4.4-1**, entry 4),[106,109] all other methods showed the formation of desired, protected pentapeptide **124** but HPLC analyses indicated an occurring epimerization for all conditions used. A typical HPLC chromatogram (**Figure 4.4.4-3**) for an occurring epimerization is shown for the conditions used in entry 2 (**Table 4.4.4-1**).

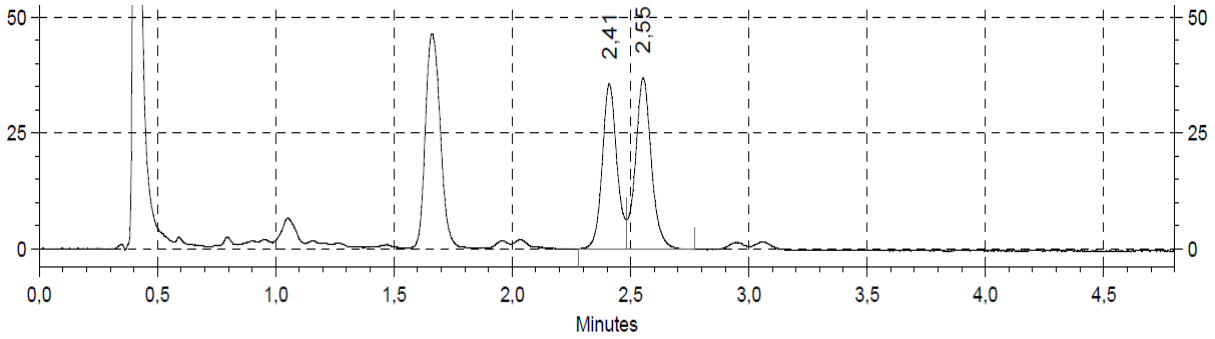


***Figure 4.4.4-3*:** HPLC chromatogram indicating epimerization after applying the PyBOP/HOBt conditions (**Table 4.4.4-1**, entry 2)

The peaks at 2.41 min and 2.55 min were separated and the resulting fractions analyzed. While mass spectrometry showed for both fractions the desired product mass, closer examination of the NMR spectra (**Figure 4.4.4-4**) showed small differences, especially in the region of the terminal stereogenic center. In addition, the rotation values were different (see experimental section for details). These observations confirmed the suspicion of epimerization, which will probably follow the reported mechanism (**Scheme 4.4.4-5**).[103]

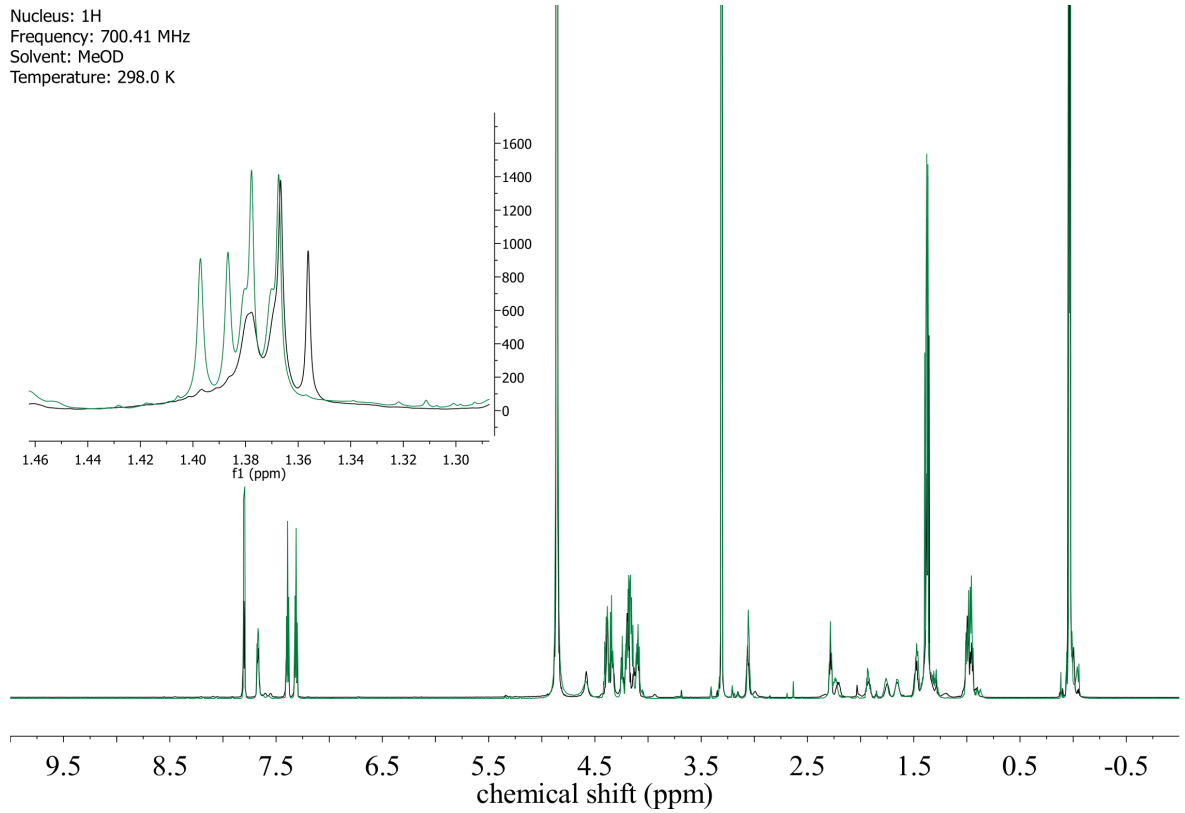
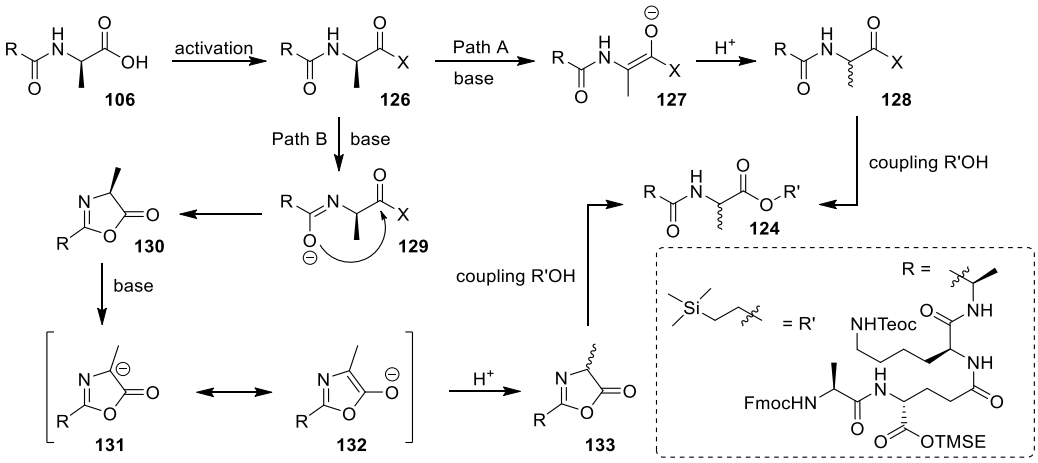


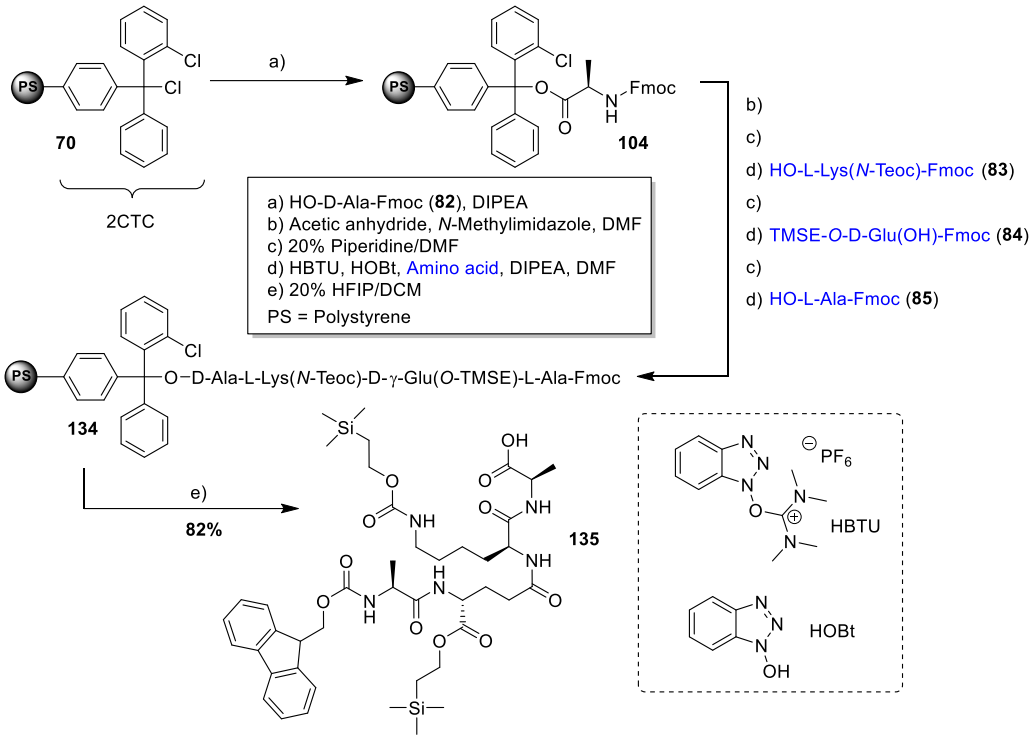
***Figure 4.4.4-4*:** Superimposed ^{1}H spectrum of the two fractions with zoomed-in area of the terminal stereogenic center.

Activation of the carboxylic acid **106** is a crucial step for the esterification (**Scheme 4.4.4-5**). Unfortunately, this activation step can result in a loss of the configuration at the α-position. Additionally, it is assumed that hydrogen bonds of the peptide **106** may stabilize the respective active ester and 2-(trimethylsilyl)ethanol is a rather weak nucleophile compared to amines. In literature, two major pathways of the epimerization can be found. The formation of an activated ester **126** often increases the acidity of the α-proton, which results in deprotonation (Path A) or favors formation of an oxazole (Path B). The deprotonation of the oxazole **130** is considered to be the most prominent pathway for the loss of configuration.[103]



***Scheme 4.4.4-5*:** Possible pathways of the epimerization process.

The described drawbacks of the esterification fueled the search for a better alternative. In general, amines exhibit a stronger nucleophilicity compared to alcohols and it was expected that the use of an amine is superior. Therefore H_2N-D-Ala-TMSE (**86**) as an amino acid building block was synthesized (for synthesis see **Chapter 4.4.3**). It was considered that a connection of the free amine in H_2N-D-Ala-TMSE (**86**) and a corresponding tetrapeptide **135** operates more satisfactorily than the performed esterification of the pentapeptide **106**. For this purpose, tetrapeptide **135** was synthesized using a similar solid phase protocol (**Scheme 4.4.4-6**). Only the coupling/deprotection of the second D-alanine was skipped. The missing D-alanine was planned to be attached as silyl protected ester **86** in solution to circumvent racemization due to the higher nucleophilicity of the amine.



***Scheme 4.4.4-6*:** Synthesis of tetrapeptide **135**. For details regarding equivalents, time as well as the exact procedure see experimental section.

After cleavage from the solid phase, tetrapeptide **135** was obtained in an excellent yield of 82% over nine steps starting from resin **70**. The planned introduction of the remaining amino acid D-Ala **86**, which already carried the desired silyl protection, was performed using PyBOP/HOBt coupling (**Scheme 4.4.4-7**) and fortunately avoided unfavorable epimerization. This conversion to the fully protected pentapeptide **124** proceeded in an excellent yield of 95% and the maintenance of the stereo information could be confirmed using NMR and HPLC analyses. Thus, the HPLC chromatogram after this reaction showed only one product signal in contrast to the chromatogram in **Figure 4.4.4-3**, which showed the formation of two epimers.

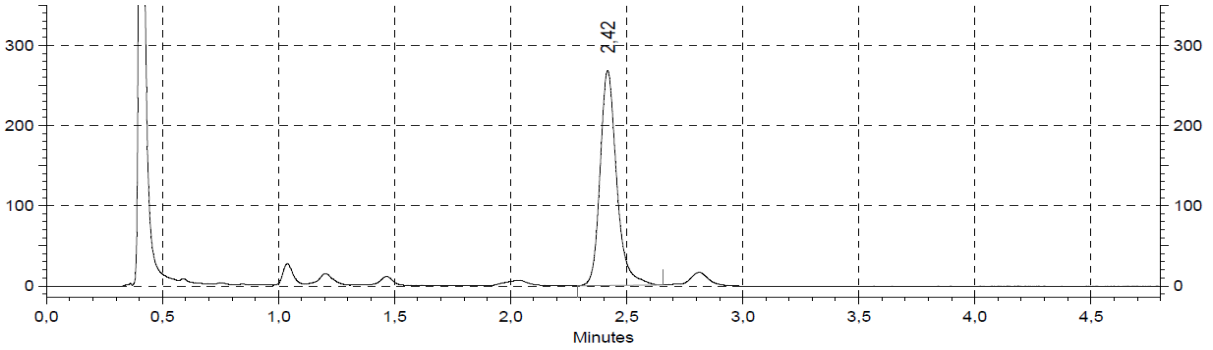
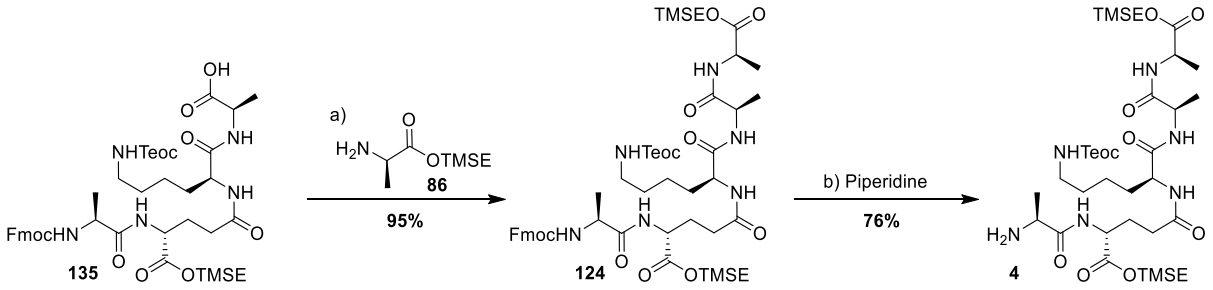


***Figure 4.4.4-5*:** HPLC chromatogram after the reaction of tetrapeptide **135** and amino acid **86**.

Finally, the Fmoc group was removed by piperidine in DMF furnishing desired pentapeptide **4** in 76% yield out of fully protected compound **124** (**Scheme 4.4.4-7**). The novel, established route towards the stem peptide **4** is reliable, scalable, high yielding and fast. Previously reported procedures suffer from lengthy solution phase synthesis[25,69] or solid phase procedures giving low yields (15%)[67]. The purity and the high overall yield of 59% over eleven steps compared favorably to the existing methods.



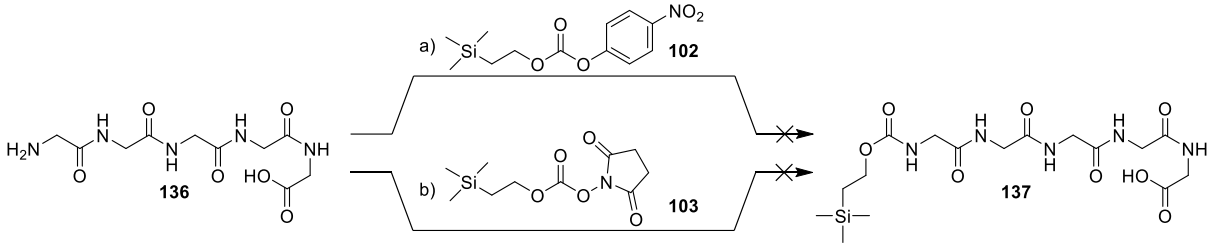
***Scheme 4.4.4-7*:** Final steps towards stem peptide **4**. Reaction conditions: a) PyBOP (1.5 equiv), HOBt (1.5 equiv), DIPEA (5.0 equiv), **86** (1.4 equiv), DMF, rt, 25 min, **95%**; b) 20% Piperidine/DMF, rt, 30 min, **76%**.

The free amine in peptide **4** will serve as connection point to build the required amide bond, which will unite peptide fragment **4** and the carbohydrate fragment **3** (see **Chapter 4.4.7**).

4.4.5 Synthesis of Decapeptide 5

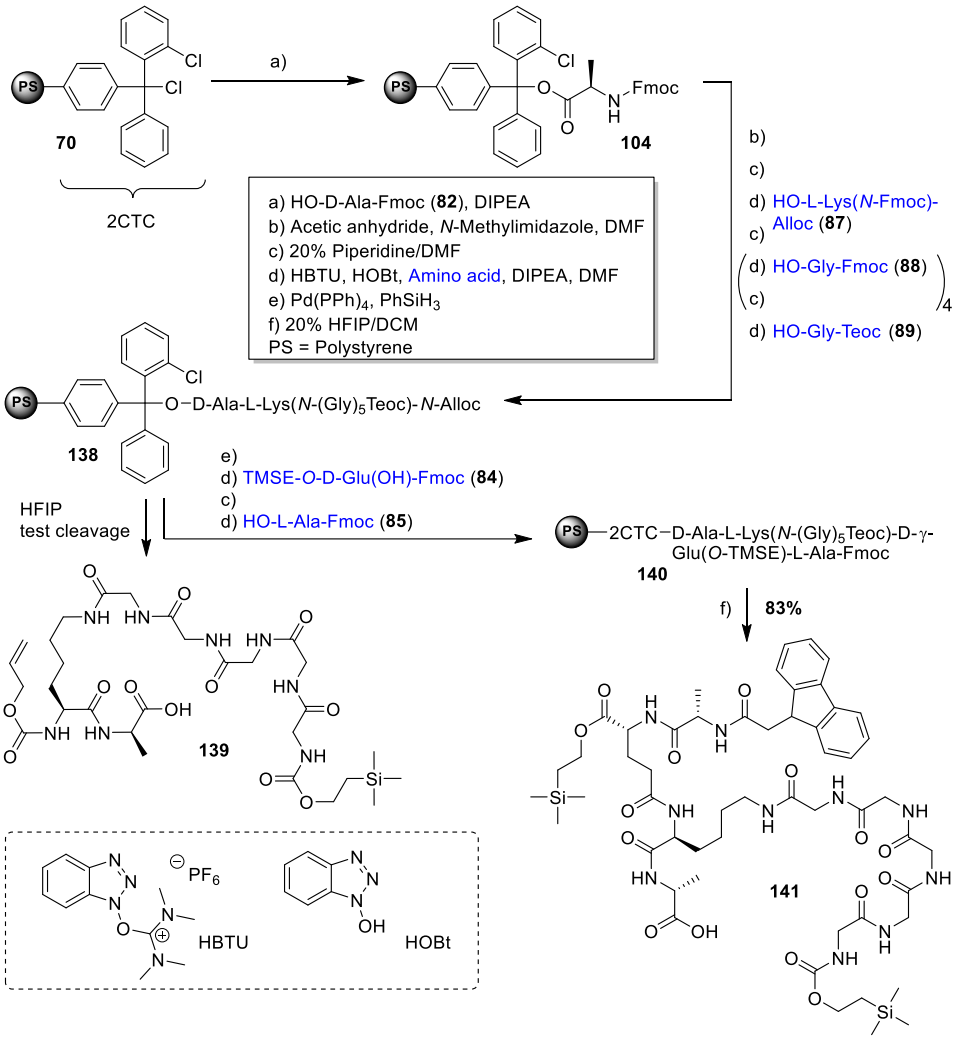
In a first approach to synthesize the desired lipid I analog **2** containing the pentaglycine side chain, known for *S. aureus*, it was tried to introduce the previously used Teoc protecting group to commercially available pentaglycine (**136**, **Scheme 4.4.5-1**). It was supposed that with the

use of the resulting silyl protected pentaglycine, a late stage attachment of the interpeptidic side chain would be possible.



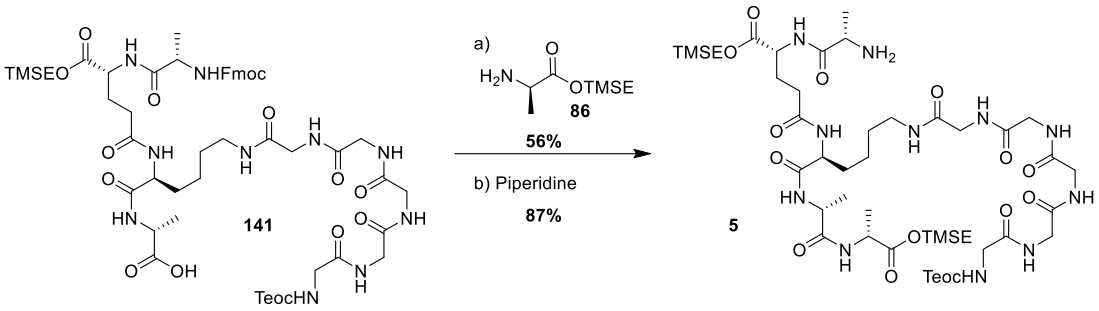
***Scheme 4.4.5-1*:** Attempted synthesis of Teoc protected pentaglycine **137**. Reaction conditions: a) 2-(Trimethylsilyl)ethyl *p*-nitrophenyl carbonate (**102**, 1.2 equiv), DIPEA (4.0 equiv), DMF, rt, on; b) *N*-[2-(Trimethylsilyl)-ethoxycarbonyloxy]succinimide (**103**, 1.1 equiv), NEt_3 (3.0 equiv), 1,4-dioxane/water (1:1), rt, on.

Unfortunately, desired product **137** could not be generated using the reaction conditions in **Scheme 4.4.5-1**, which was mainly attributed to the poor solubility of pentaglycine (**136**). However, protecting a single glycine with Teoc was possible (synthesis described in **Chapter 4.4.3**). Under the use of this protected amino acid block and the design of a novel solid phase supported synthesis, it was possible to synthesize nonapeptide **141** (**Scheme 4.4.5-2**). Using the gained knowledge from the designed and successful solid phase synthesis for the stem pentapeptide **4** (described in **Chapter 4.4.4**), the remaining silyl protected D-alanine **86** should again be attached in solution. Thus, the solid-phase route similarly relied on attachment of the second D-Ala amino acid to a chlorotrityl resin and stepwise elaboration using Fmoc protected amino acids. In contrast to the sequence for the stem pentapeptide **4**, a modified lysine building block **87** was utilized with the Fmoc group being at the side chain amine, while the α-amine was protected in an orthogonal fashion with an allyloxycarbonyl (alloc) moiety. After coupling of this lysine derivative and removal of the terminal Fmoc group, four glycine residues (**88**) were then attached in an iterative fashion using standard coupling and deprotection protocols. The final glycine was then introduced as its silyl protected building block **89**. To verify the success of the procedure so far, a test cleavage was carried out resulting in heptapeptide **139**. This confirmed the route and the peptide was further elaborated. After cleavage of the alloc protecting group with tetrakis(triphenylphosphine)-palladium(0) ($Pd(PPh)_4$) and phenylsilane ($PhSiH_3$), amino acids **84** and **85** were attached, before the resulting nonapeptide was cleaved from the resin giving peptide **141** in excellent yield (83%) over these nineteen steps on the solid phase support.



***Scheme 4.4.5-2*:** Synthesis of nonapeptide **141**. For details regarding equivalents, time as well as the exact procedure see experimental section.

Finally, remaining (TMSE) protected D-alanine **86** was coupled in solution (**Scheme 4.4.5-3**) and the Fmoc group was cleaved using piperidine. In total, desired decapeptide **5** was obtained in high overall yield (40%) over 21 steps starting from commercially available resin **70**.

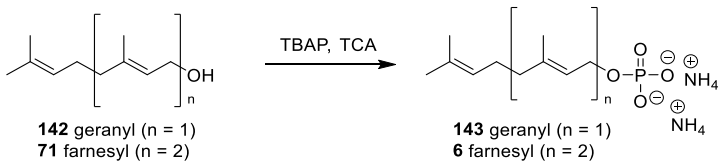


***Scheme 4.4.5-3*:** Final steps towards decapeptide **5**. Reaction conditions: a) PyBOP (1.5 equiv), HOBt (1.5 equiv), DIPEA (4.0 equiv), **86** (1.4 equiv), DMF, rt, 40 min, **95%**; b) 20% Piperidine/DMF, rt, 30 min, **76%**.

Notably, this sequence is the first protocol to obtain lipid I/II interpeptidic modifications and due to its variability it should be applicable to other pathogen specific interpeptidic analogs (described in **Chapter 4.1.1**).[110]

4.4.6 Synthesis of Organophosphate Monoesters

A method for the synthesis of farnesyl phosphate **6** was published 2013 by *Wessjohann et al.*[80] and was included in the synthetic strategy towards lipid I analogs (**Scheme 4.4.6-1**). This procedure allowed the simple one-pot conversion of corresponding alcohols to phosphates using tetrabutylammonium hydrogen phosphate (TBAP) and trichloroacetonitrile (TCA), followed by flash chromatography on silica gel (solvent system: 10% water/20% NH_3 (conc.)/isopropanol) as an unusual method and a final cation exchange chromatography with Dowex® 50WX8. The last step is not only to exchange tetrabutylammonium to ammonium but also to remove dissolved silica.



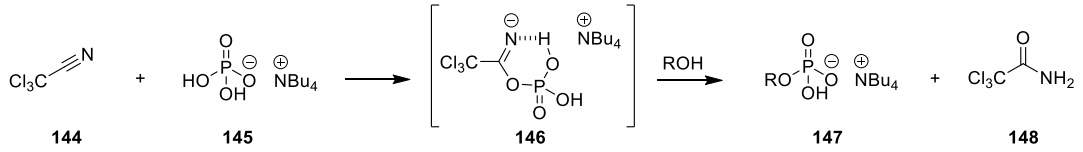
142 geranyl (n = 1)
71 farnesyl (n = 2)

143 geranyl (n = 1)
6 farnesyl (n = 2)

***Scheme 4.4.6-1*:** Synthesis of geranyl phosphate **143** and farnesyl phosphate **6**. Reaction conditions: for **143**: TCA (5.0 equiv), TBAB (3.0 equiv) addition over 1 h via syringe pump, MeCN, rt, 6 h, **62%**; for **6**: TCA (2.4 equiv), TBAB (2.0 equiv) addition over 1 h via syringe pump, MeCN, rt, 7 h, **71%**.

First, the reaction was tested with geraniol (**142**), as this starting material was already available in the working group. Encouraged by the outcome of this reaction, farnesol (**71**) was ordered and submitted to the method yielding the desired farnesyl phosphate **6** in 71% yield, which was used for the synthesis of farnesyl lipid I analogs **1** and **2**.

Wessjohann et al. proposed that TCA (**144**) reacts with TBAP (**145**) to yield the reactive phosphorylated trichloroacetimidate **146** (**Scheme 4.4.6-2**), which represents a mixed anhydride-like activated phosphate. Nucleophilic attack by an alcohol yields the desired organophosphate monoester **147** and trichloroacetamide (**148**).[80]

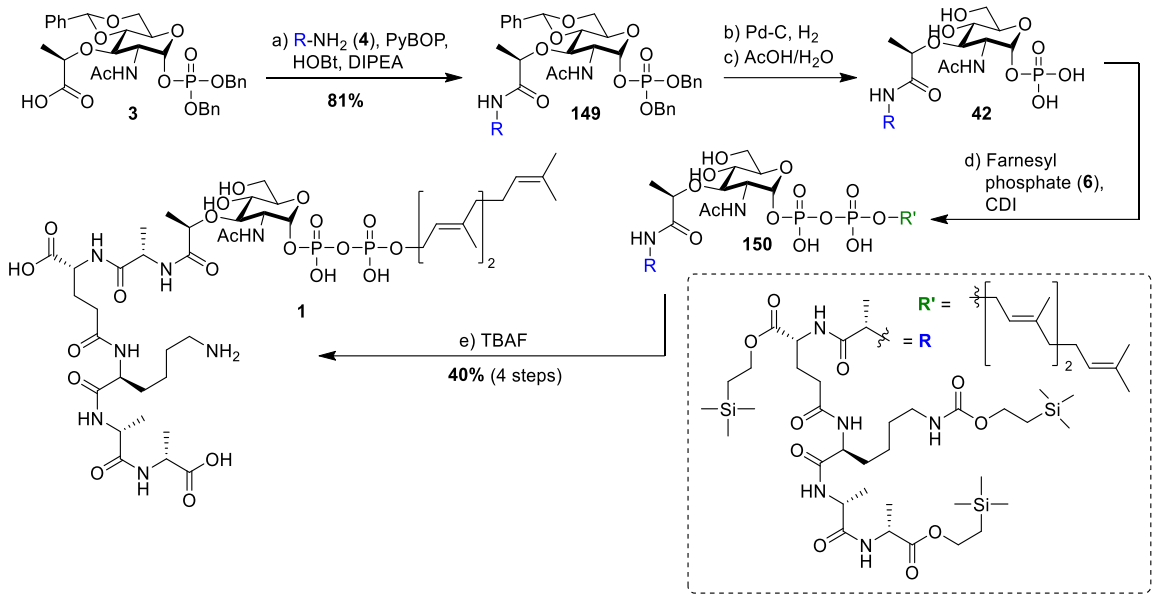


***Scheme 4.4.6-2*:** Proposed reaction mechanism by *Wessjohann et al.*[80]

The described method is ideal for the synthesis of lipid I analogs and its consideration drastically simplified the synthesis and improved the overall yield.

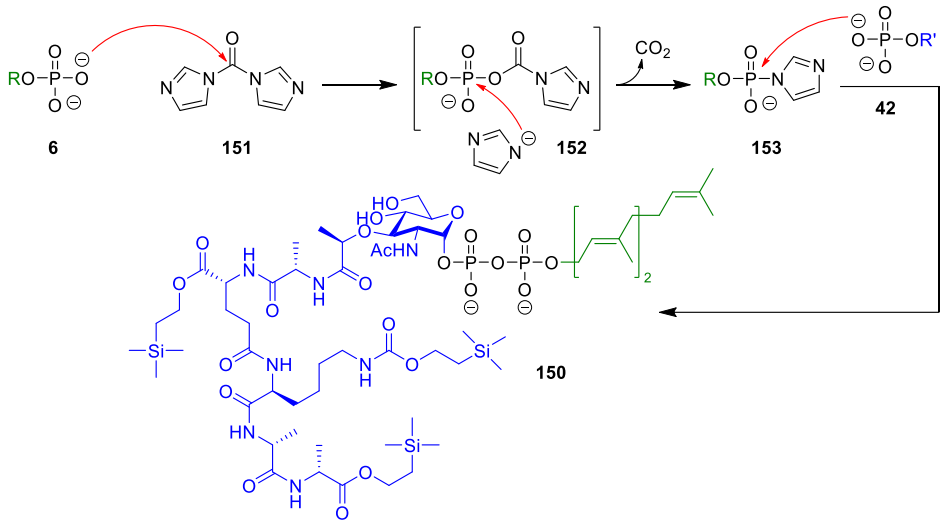
4.4.7 Coupling of Fragments/Endgame of Farnesyl Lipid I Analog 1

After the synthesis of the three main fragments being carbohydrate fragment **3**, peptide fragment **4** and farnesyl phosphate **6**, coupling and deprotection using previously reported methods should complete the synthesis of farnesyl lipid I analog **1** (**Scheme 4.4.7-1**).[66,76,111,112]



***Scheme 4.4.7-1*:** Fragment coupling and final steps towards lipid I analog **1**. Reaction conditions: a) **4** (1.3 equiv), PyBOP (1.5 equiv), HOBt (1.5 equiv), DIPEA (5.0 equiv), DMF, rt, 45 min, **81%**; b) H_2, 10% Pd/C, MeOH, rt, 30 min; c) 80% AcOH/water, rt, 2 d; d) Farnesyl phosphate (**6**, 1.0 equiv), CDI (4.0 equiv), DMF/THF, rt, 3 d; e) TBAF (23 equiv), DMF, rt, 2 d, **40%** over 4 steps.

Therefore, carbohydrate **3** and peptide **4** were coupled in a standard amide bond formation with the use of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 1-hydroxybenzotriazole (HOBt) yielding glycopeptide **149** in high yield (81%). As reported by *Walker et al.*, a joint removal of the benzyl groups and the acetal moiety using hydrogenolysis could not be realized.[16] Even an increase of the reaction time from literature stated 30 min to 5 h did only furnish the free phosphate without cleavage of the acetal group. However, the joint removal of the protecting groups also seemed to be not reproducible in other working groups. Thus, *Arimoto et al.* described the use of a two-step procedure for their depsi-lipid I analog.[25] While the phosphate group was freed from benzyl protection using again hydrogenolysis, the acetal was cleaved using acidic conditions, which also worked for glycopeptide **149**. Pyrophosphate linkage of farnesyl phosphate **6** and phosphate **42** was achieved using carbonyldiimidazole coupling.



***Scheme 4.4.7-2*:** Mechanism for CDI mediated pyrophosphate coupling to yield compound **150**.

With implementation of the literature reported mechanism[113] farnesyl phosphate **6** and CDI (**151**) are supposed to give a mixed anhydride species **152**, which spontaneously degrades to imidazolide **153** under release of CO_2 (**Scheme 4.4.7-2**). The formation of compound **153** was also proved in this study using mass spectrometry. This activated phosphate can react with various nucleophiles such as amines, alcohols and phosphates. In this case, addition of glycopeptide phosphate **42** led to the formation of desired pyrophosphate **150**.[113][114]

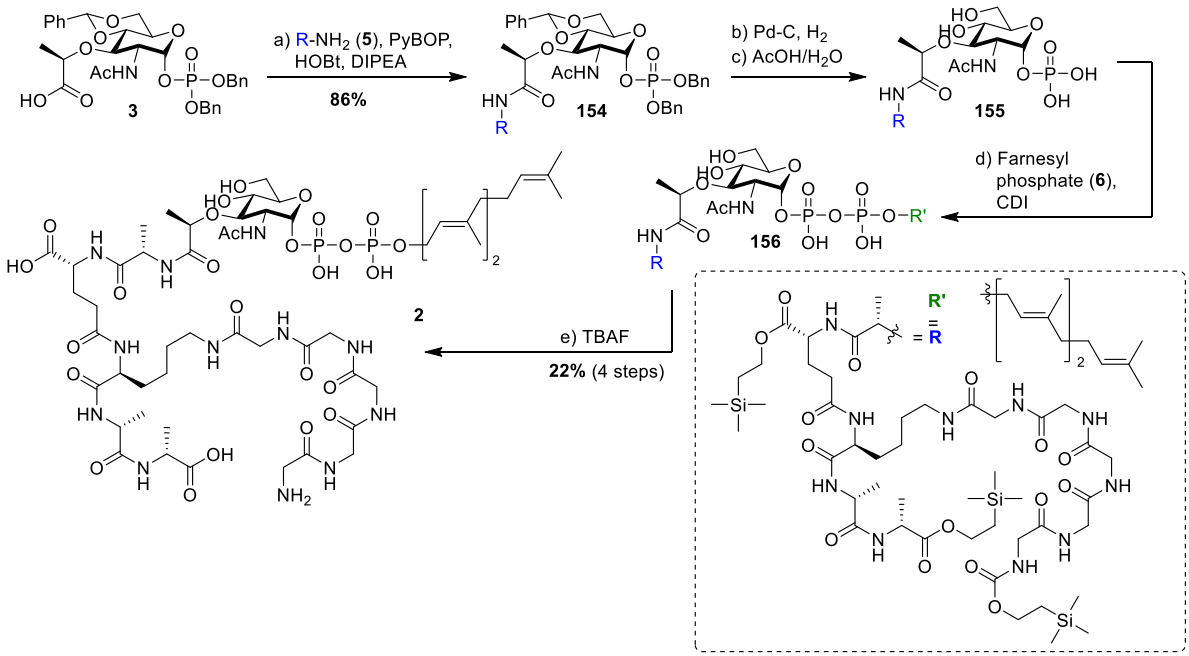
As a final step and as a proof of synthetic design, all silyl protecting groups were cleaved in a joint fashion using tetrabutylammonium fluoride. For the purification of farnesyl lipid I analog **1**, a three step method was developed. First, gel filtration with Sephadex® LH-20 was applied followed by ion-exchange chromatography (Dowex® 50WX8) to substitute residual tetrabutylammonium counterions with ammonium, which was also advantageous due to the fact that the huge disturbing signals, caused by the tetrabutyl residues, vanished in NMR spectra.

Final HPLC separation yielded highly pure farnesyl lipid I analog **1** in 40% yield over the four steps starting from glycopeptide **149**. Even though HPLC separation was carried out using a NH_4HCO_3 buffer, tetrabutyl ammonium counterions were still stuck to the product making the ion exchange before HPLC separation an essential step. In total, farnesyl lipid I **1** was obtained in pure form in 16 steps in the longest linear sequence from commercially available HO-D-Ala-Fmoc (**82**) with an excellent overall yield of 19%, which compared favorably to the previous synthetic route (≈ 3% yield starting from sasrin resin-D-Ala-Fmoc **35**, see

Chapter 4.2.1).[16,67,76] The sequence proved well scalable and a first batch of 11 mg highly pure 3-lipid I (**1**) was readily obtained.

4.4.8 Coupling of Fragments/Endgame of Farnesyl Lipid I Analog 2, Containing the *S. aureus* Pentaglycine Bridge

Encouraged by the successful final steps towards farnesyl lipid I analog **1**, an analogous three component sequence was applied to complete the first total synthesis of a lipid I analog **2**, which includes an interpeptidic bridge (**Scheme 4.4.8-1**).



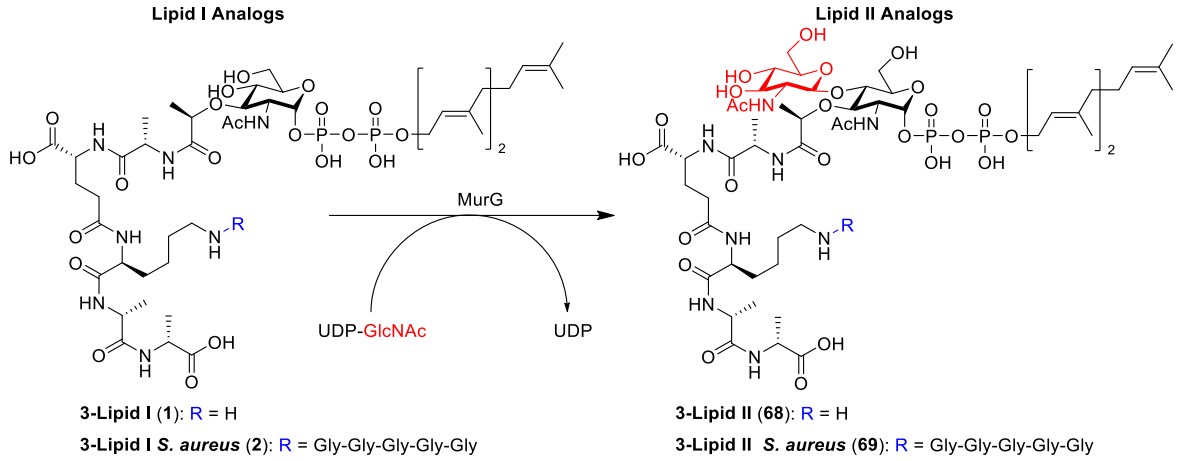
***Scheme 4.4.8-1*:** Fragment coupling and final steps towards lipid I analog **2** containing the *S. aureus* pentaglycine bridge. Reaction conditions: a) **5** (1.1 equiv), PyBOP (1.5 equiv), HOBt (1.5 equiv), DIPEA (4.0 equiv), DMF, rt, 45 min, **81%**; b) H_2, 10% Pd/C, MeOH, rt, 30 min; c) 80% AcOH/water, rt, 2 d; d) Farnesyl phosphate (**6**, 1.0 equiv), CDI (4.0 equiv), DMF/THF, rt, 3 d; e) TBAF (23 equiv), DMF, rt, 2 d, **22%** over 4 steps.

Hence, novel decapeptide **5** was coupled to the carbohydrate component **3** using the established PyBOP/HOBt procedure to give glycopeptide **154** in 86% yield, which was even slightly higher than the yield after coupling with the stem pentapeptide **4** (81%). Caused by the longer peptide chain, solubility in solvents like EtOAc and Et_2O decreased, which can cause problems in

handling and reactions but turned out to be advantageous, since precipitation led to facile purification. Hydrogenolysis of the phosphate benzyl groups, acidic cleavage of the acetal and CDI mediated pyrophosphate coupling with farnesyl phosphate **6** gave silyl protected **156**, which was semi-purified using Sephadex® LH-20. Again, final, global deprotection using TBAF freed the peptide from its silyl groups and farnesyl lipid I analog **2**, containing the *S. aureus* pentaglycine, was gained in pure form after utilization of the developed three step purification, consisting out of gel filtration with Sephadex® LH-20, ion-exchange chromatography (Dowex® 50WX8) and a final HPLC purification. Lipid I analog **2** was obtained in an overall yield of 8% in its longest linear sequence over 26 steps starting from HO-D-Ala-Fmoc (**82**) and represents a key tool to answer questions about the function and necessity of the pentaglycine bridge modification known for *S. aureus*, which was recently found to be essential for its bacterial cell integrity.[115] Notably, the route towards the interpeptidic bridge should be applicable to other pathogen specific interpeptidic analogs (described in **Chapter 4.1.1**). So far, the pentaglycine modification seemed to be the most synthetically challenging but also the most interesting and important analog among the interpeptidic modifications.[15]

4.4.9 Chemoenzymatic Conversion of Lipid I (1, 2) to Lipid II Analogs (68, 69)

As part of the cooperation within the collaborative research center (TRR261, Cellular Mechanisms of Antibiotic Action; acronym: ANTIBIOTIC CellMAP), the synthesized lipid I analogs **1** and **2** were transferred to the research team of Prof. Dr. *Tanja Schneider*, who has an outstanding microbiological knowledge of antibiotics. Here, Dr. *Marvin Rausch* performed the attachment of β-1-4-linked *N*-acetylglucosamine chemoenzymatically using the glycosyltransferase MurG to access the respective farnesyl lipid II analogs **68** and **69**.



***Scheme 4.4.9-1*:** Chemoenzymatic conversion of lipid I analogs **1** and **2** to lipid II analog **68** and **69**.

Fortunately, the used MurG accepted both analogs **1** and **2** and the resulting lipid II analogs **68** and **69** were analyzed using mass spectrometry. Impressively, this also represented the first chemoenzymatic conversion of a lipid I (**2**) to lipid II (**69**) analog containing the pentaglycine bridge known for *S. aureus*. For the previously reported lipid I to lipid II conversion of analog **1**, a synthesis on bigger scale was performed.[76] Thus, 1 mg of lipid II analog **68** was obtained in pure form with a useful yield of 55% from lipid I analog **1**, which represented a sufficient amount for characterization including the assignment of NMR signals. After full characterization, this lipid II analog **68** was handed over to the group of Prof. Dr. *Thilo Stehle*, who is also a member of the collaborative research center (TRR261). With lipid II analog **68** and the excellent know-how of this group, it is believed to gain a better insight into the bi-enzyme complex MurT/GatD, which in nature amidates the glutamate of the peptide generating a glutamine at this position.

4.4.10 Initial NMR Studies of Farnesyl Lipid I Analog 1

Based on the novel, efficient route, a relatively high amount (11 mg) of farnesyl lipid I analog **1** was synthesized. This amount fueled additional NMR experiments besides to the recorded 2D spectra (HH-COSY, HSQC, HMBC, NOESY and TOCSY measured for analogs **1**, **2** and **68**, shown in **Chapter 7.1**). Thus, different temperatures were applied to ^{1}H (**Figure 4.4.10-1**) and ^{31}P (**Figure 4.4.10-2**) measurements.

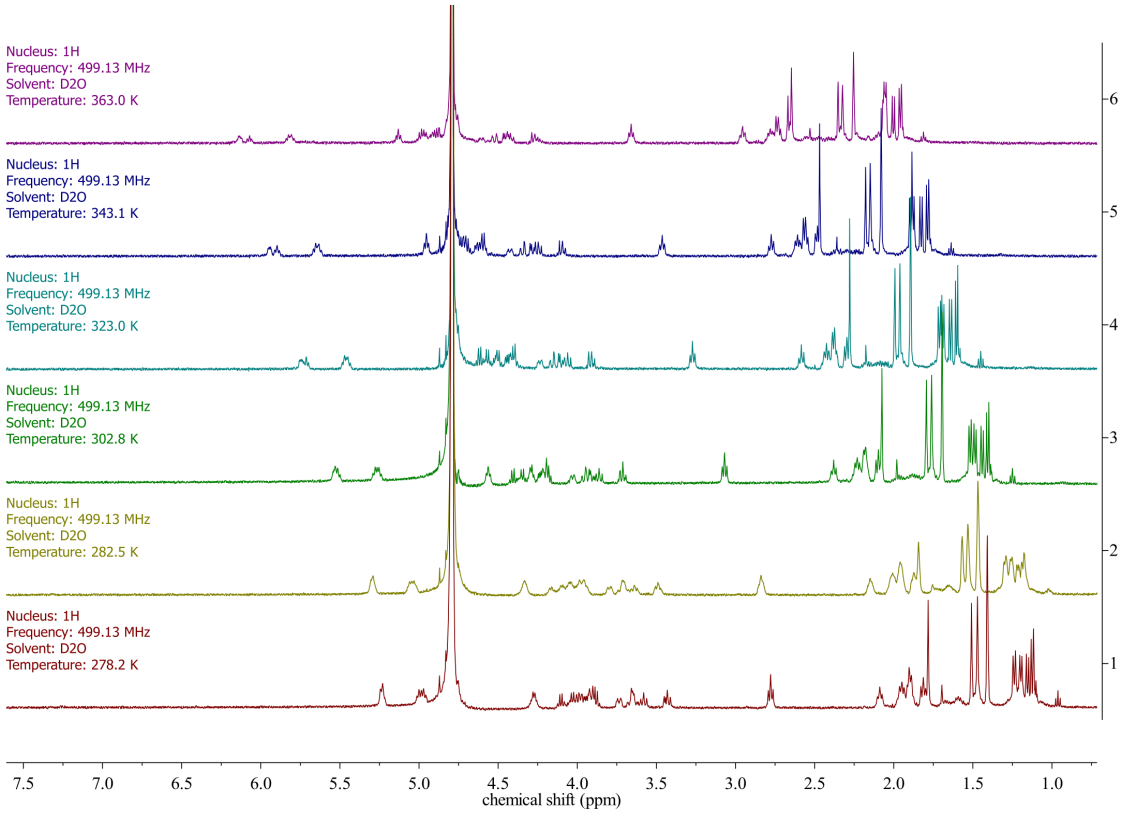


***Figure 4.4.10-1*:** ^{1}H spectra of farnesyl lipid I analog **1** at different temperatures. Increasing temperature from bottom (red, 278 K) to top (purple, 363 K).

Temperature dependences in NMR spectroscopy can give insights into effects like hydrogen bonding, stability of the compound and conformational changes.[116] As shown in **Figure 4.4.10-1**, there are detectable differences in the chemical shifts at various temperatures. With increasing temperature, a downfield shift was observed. Additionally, broadening of the signals was seen especially for the temperature shift from 278 K to 282 K. If the temperature was increased further, the signals got sharp again. ^{31}P NMR spectra were recorded at the same temperatures as well (**Figure 4.4.10-2**). These measurements showed an even more interesting effect.

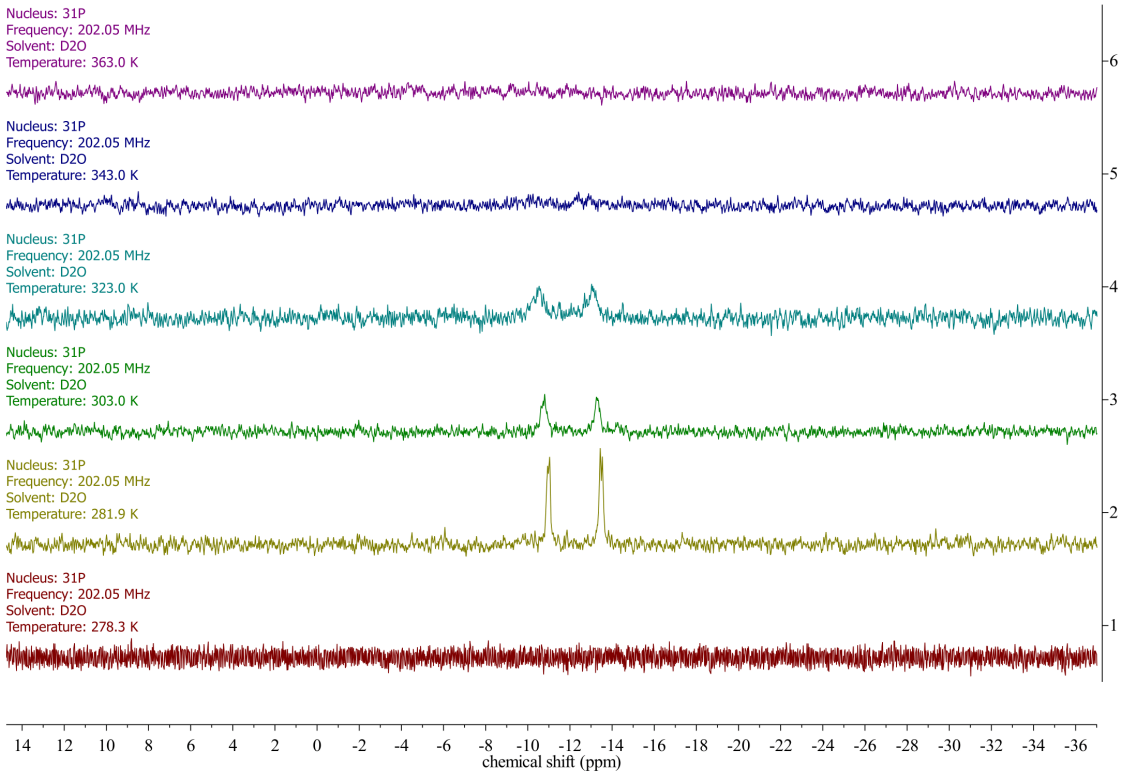


***Figure 4.4.10-2*:** ^{31}P spectra of farnesyl lipid I analog **1** at different temperatures. Increasing temperature from bottom (red, 278 K) to top (purple, 363 K).

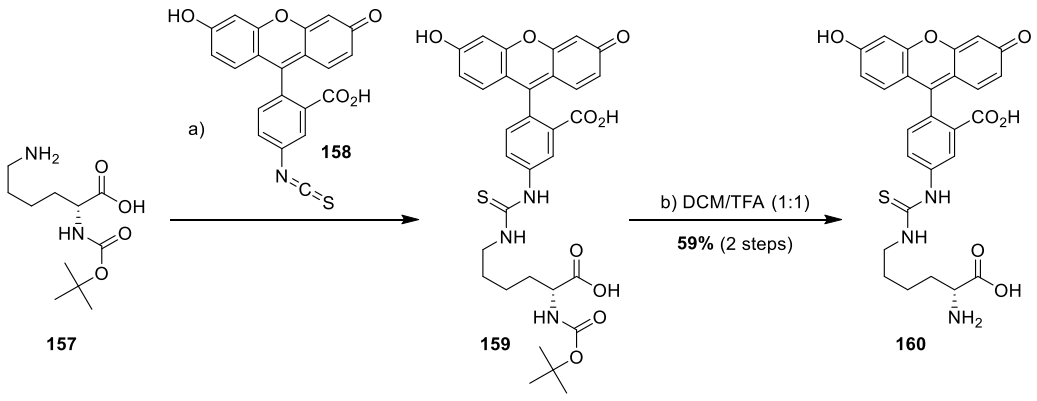
While the signals for the pyrophosphate were not visible at 278 K, sharp signals appeared at 282 K, which broadened again and vanished completely if the temperature was increased further. Surprisingly, this observation is the opposite of the ^{1}H spectra in which the signals at 282 K were the most broadened. The changes in the signals can be caused by various effects like conformational changes, exchange processes or temperature dependent hydrogen bonding. Possibly, the phosphate counter ions also have an effect of causing the disappearing signals, especially at lower temperatures. However, further experiments have to be done to proof and clarify initial assumptions, which may also play a crucial role in antibiotic binding to cell wall precursors.

When the temperature measurements were started, it was expected that due to the complex structure, the synthesized farnesyl lipid I analog **1** would not be stable at elevated temperatures. The ^{1}H spectra at 363 K (**Figure 4.4.10-1**) showed no significant degradation. Only partial decay of the compound was observed after cooling back to room temperature.

Furthermore, a NMR titration experiment of a compound (not shown due to unpublished results of a different working group), which is supposed to bind to the cell wall precursors lipid I and II, was performed. Unfortunately, the compound was not soluble in D_2O, which is the solvent of choice for lipid I analog **1** and was supposed to be a useful solvent to represent the natural medium. Hence, it was tried to titrate a solution of the ligand dissolved in deuterated DMSO to a solution of farnesyl lipid I analog **1** in D_2O. However, the solubility of the ligand was poor and only a 1:1 mixture of deuterated water/DMSO led to respective ligand signals, which resulted in poorly resolved signals for the lipid analog **1**. The discrepancy of solubility made a determination of the binding using NMR spectroscopy impossible. In future, more water soluble lipid I/II-binding compounds will be tested and also the previously described application of micelles as a membrane-like environment will be investigated.[117–119]

4.4.11 Synthesis of Labeled Compounds to Trace Cell Wall Biosynthesis

In order to be able to track processes in the cell wall biosynthesis, differently labeled compounds were synthesized and provided to the collaborative research center (**Scheme 4.4.11-1** and **Scheme 4.4.11-2**).



***Scheme 4.4.11-1*:** Synthesis of fluorescent D-lysine **160**. Reaction conditions: a) **158** (0.8 equiv), DMF, rt, 4 h; b) DCM/TFA (1:1), rt, 30 min, **59%** (over two steps).

The fluorescent D-lysine **160**, which is shown in **Scheme 4.4.11-1**, makes it e.g. possible to track the activity of penicillin-binding proteins (PBPs) using fluorescence.[120] For the

synthesis, a previously described two-step procedure was used, which consisted out of formation of compound **159** under the use of fluorescein isothiocyanate (**158**, FITC) and subsequent Boc deprotection.[121] After HPLC purification, the desired, modified lysine **160** was gained in 49% yield over two steps.

Preliminary results from the group of Dr. *Fabian Grein* showed that *S. aureus* incorporated the synthesized fluorescent D-lysine **160** (**Figure 4.4.11-1**).

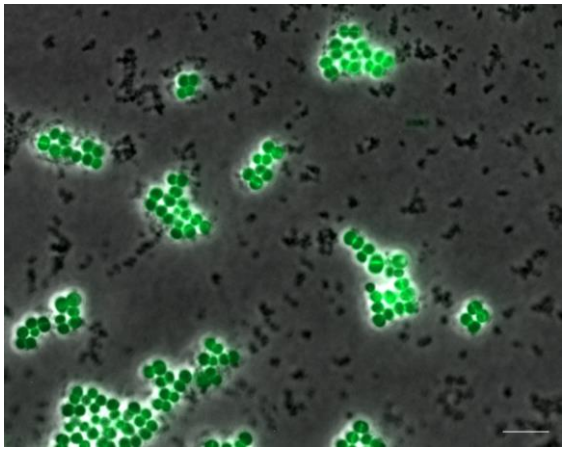
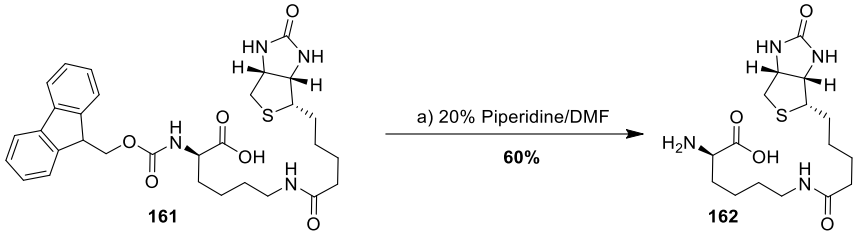


***Figure 4.4.11-1*:** Fluorescence microscopic image of *S. aureus* with the incorporated, labeled lysine **160**. The image was provided by the group of Dr. *Fabian Grein*.

In addition, biotin-D-lysine (**162**) was prepared out of commercially available lysine derivative **161** using Fmoc deprotection as reported in literature.[120] This labeled substrate allows to trace the size of glycan strands by using western blotting and streptavidin binding, which will be carried out by the group of Prof. Dr. *Tanja Schneider*.[122]



***Scheme 4.4.11-2*:** Synthesis of biotin-D-lysine **162**. Reaction conditions: a) 20% Piperidine/DMF, toluene, rt, 45 min, **60%**.

4.5 Conclusion

Within this work an optimized, efficient, scalable and reproducible route to farnesyl lipid I analog **1** was developed. For this purpose, the syntheses of the three components being carbohydrate **3**, peptide **4** and farnesyl phosphate **6** were closely examined. Regarding carbohydrate **3**, various technical modifications and optimizations, for example by exchange of the reaction solvent or by adjusting the reagent equivalents, led to a considerable increase of the overall yield from literature reported 41% to 60% over six steps (**Scheme 4.5-1**).[16]

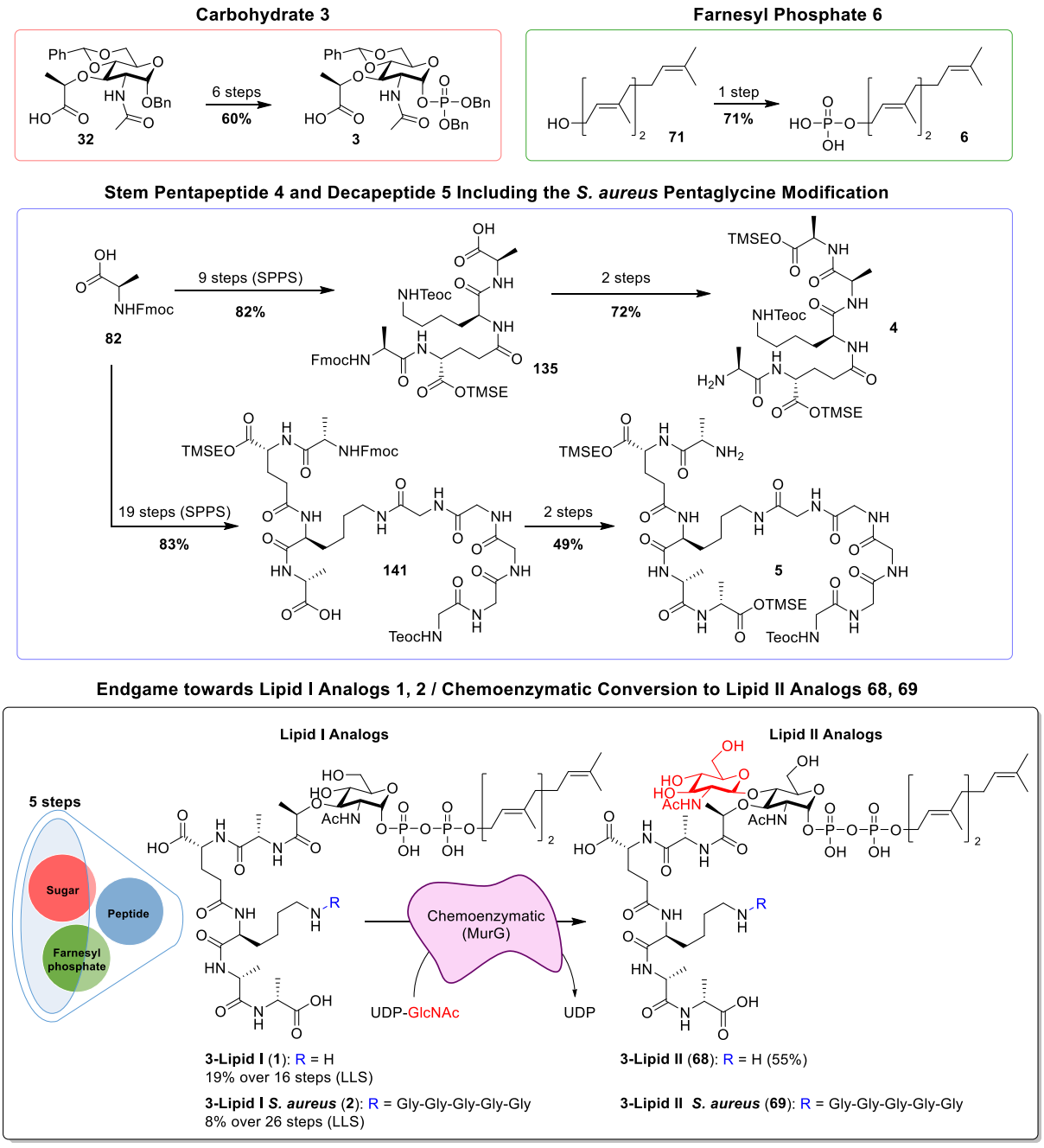
Existing routes for the stem pentapeptide suffered from lengthy solution phase sequences as well as a low reported yield (15%) for a solid phase approach.[16,19,25,66,67,69] During this work, a novel, efficient and scalable solid phase peptide synthesis was developed. This optimized route relied on preparation of the tetrapeptide HO-D-Ala-L-Lys(*N*-Teoc)-D-γ-Glu(*O*-TMSE)-L-Ala-Fmoc (**135**) using 2-chlorotrityl chloride (2CTC) solid phase support and subsequent attachment of the remaining silyl protected D-Ala **86** as well as a final Fmoc cleavage in solution phase. This approach was crucial to avoid an unfavorable epimerization, which occurred during the attachment of the required TMSE protecting group to the terminal D-Ala carboxylate of a corresponding pentapeptide. Following the novel route, a synthesis of pentapeptide **4**, which is equipped with three silyl protecting groups, allowing a joint removal in the endgame, was possible in an excellent yield of 59% over 11 steps.[123]

Including a recently reported one-pot procedure for the conversion of alcohols to their phosphates[80] allowed to further simplify the route towards the precious cell wall precursors. Thus, farnesyl phosphate **6** was synthesized out of farnesol (**71**) in 71% yield using only one step.

After coupling of the fragments and deprotection, farnesyl lipid I analog **1** was purified with a newly developed three step purification including gel filtration (Sephadex® LH-20), ion exchange chromatography (Dowex® 50WX8) and HPLC separation giving the desired product **1** in high purity over 16 steps (longest linear sequence) starting from HO-D-Ala-Fmoc (**82**). This sequence proved well scalable and a batch of 11 mg lipid I analog **1** was readily obtained in high purity.[123]

After the successful optimization of the route towards lipid I analogs, emphasis was placed on the development of a procedure for an interpeptide bridge containing lipid I analog. Therefore,

the pentaglycine modification found in *S. aureus* was targeted. This synthetically challenging modification was considered to be the most interesting and important among the interpeptidic variations described for specific pathogens.[15] While the overall procedure was adopted from the route towards farnesyl lipid I analog **1**, a novel solid phase sequence was designed to allow for the introduction of interpeptidic modifications. This route enabled the synthesis of decapeptide **5** in 40% yield over 21 steps, which was used in an analogous three component coupling sequence to complete the first total synthesis of an interpeptidic lipid I analog **2** in an overall yield of 8% in its longest linear sequence over 26 steps starting from HO-D-Ala-Fmoc (**82**).[123] Notably, this sequence is variable and should be applicable to other pathogen specific interpeptidic analogs.



***Scheme 4.5-1*:** Reaction overview towards the synthetic target structures **1** and **2** and their chemoenzymatic transfer to **68** and **69**. SPPS = solid phase peptide synthesis. LLS = longest linear sequence.

The synthesized farnesyl lipid I analogs **1** and **2** represent valuable tools to address biochemical questions and to gain new insights into the important field of antibiotic research. Several cooperations were already started based on the present work. Together with the NMR expert Dr. *Senada Nozinovic*, initial advanced NMR studies regarding stability, temperature influence and binding affinities were started. Additionally, the compounds were further used within the

collaborative research center (TRR261). In cooperation with the group of Prof. Dr. *Tanja Schneider*, who have an excellent biochemical know-how, both of the synthesized lipid I analogs **1** and **2** were transferred to the corresponding lipid II analogs **68** and **69**.[123] This transformation was carried out chemoenzymatically using the natural glycosyltransferase MurG, which fortunately also accepted the lipid I analog **2** containing the pentaglycine modification. Chemoenzymatic conversion of farnesyl lipid I analog **1** was already performed on a bigger scale giving 1 mg of farnesyl lipid II analog **68** after purification (55% yield from lipid I analog **1**).[123] This compound was finally transferred to the group of Prof. Dr. *Thilo Stehle* and will be used as substrate to gain a better understanding of the bi-enzyme complex MurT/GatD, which in nature amidates the glutamate of the peptide generating a glutamine at this position.[18,26]

4.6 Outlook

Using the synthesis developed in this work, it should be possible to generate various lipid I analogs containing other species dependent interpeptidic bridges (**Figure 4.6-1**). These compounds are supposed to be useful tools to generate a further knowledge about their function in bacteria.

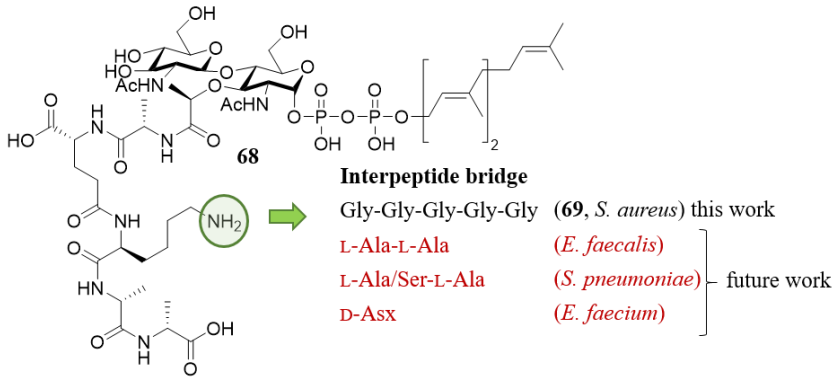


***Figure 4.6-1*:** Farnesyl Lipid II (**68**) with *S. aureus* pentaglycine modification (**69**) and possible other interpeptidic variations found among different bacterial species.

Due to the fact that analogs of the main cell wall precursors lipid I/II are essential for antibiotic research, it would be interesting to see if the routes are even adoptable for a gram scale synthesis.

Furthermore, a continuation of the initially started NMR investigations is highly recommended. Together with suitable antibiotic compounds, which bind to the cell wall precursors, NMR measurements can determine binding sites, affinities and they can give further insights into the resulting complexes. Results of these NMR experiments will be of high interest for antibiotic researchers and in longer terms for human health.

5 Part II: Contributions to the Total Synthesis of Vancoresmycin

5.1 Introduction and State of Knowledge

5.1.1 Amycolatopsis

Amycolatopsis, first discovered by *Lechevalier* in 1986, is a genus of bacteria which belongs to the family of pseudonocardiaceae and therefore to the order of actinomycetales.[124–126] Over 65 species have been reported from a variety of environments, including soil, rock, ocean sediment and clinical sources.[125,127] Their secondary metabolites are known for a wide application range in medicine and agriculture.[127,128] One of the most prominent examples is the antibiotic vancomycin (**163**, **Figure 5.1.1-1**), which was isolated from the species *Amycolatopsis orientalis*.[127,129] In general, *Amycolatopsis* are gram-positive, aerobic, non-endospore-forming bacteria featuring a high G+C-content in the genomic DNA, which is usually above 60%.[125,130] They form long chains of aerial and substrate mycelia and are further characterized by its cell wall, which contains *meso*-diaminopimelic acid and lacks mycolic acid.[125,130]

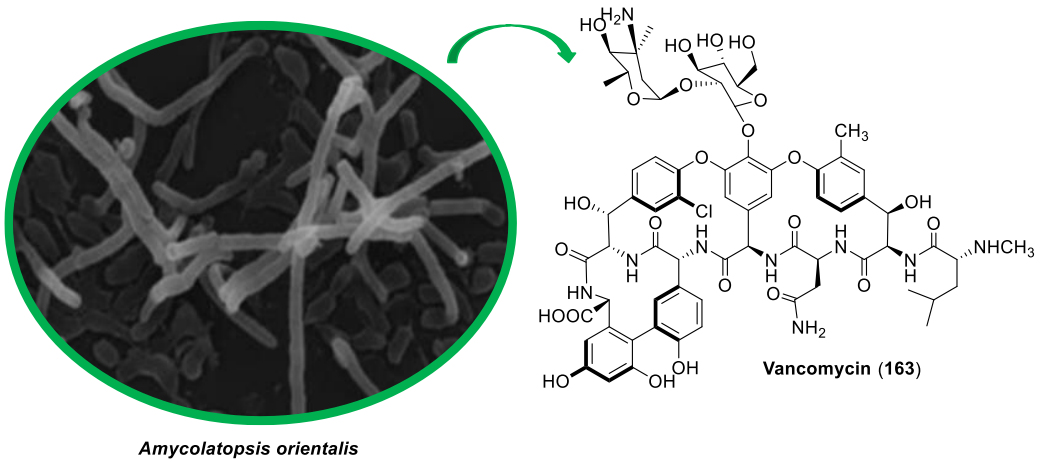


***Figure 5.1.1-1*:** Scanning electron micrograph of *A. orientalis* HCCB10007 and chemical structure of vancomycin (**163**).[129]

5.1.2 Vancoresmycin

Vancoresmycin (**7**, **Figure 5.1.2-1**) was first isolated in 2002 from the fermentation broth of the actinomycete *Amycolatopsis* sp. ST 101170 and was originally reported without the assignment of stereogenic centers.[131] The natural product presents a structurally unique metabolite that is characterized by a densely functionalized acyl tetramic acid fused to an extended polyketide chain with a plethora of hydroxyl- and methyl-bearing stereogenic centers and also includes an aminoglycoside moiety.[131] In 2018, a heterologous expression of vancoresmycin was reported and the structure was further elucidated including a domain analysis of the ketoreductase domains making it possible to propose a stereochemical assignment for most of the unknown configurations (**Figure 5.1.2-1**).[132]

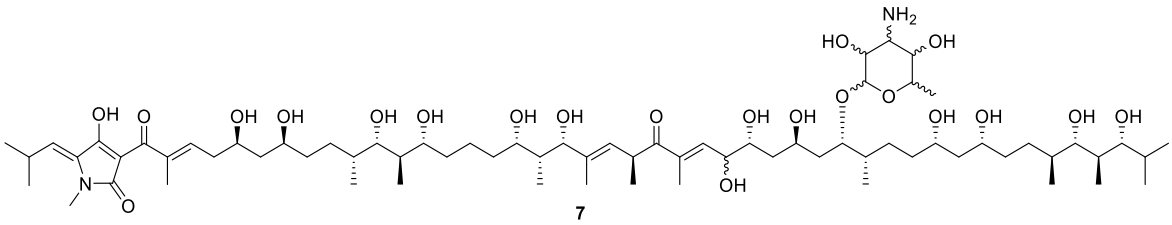


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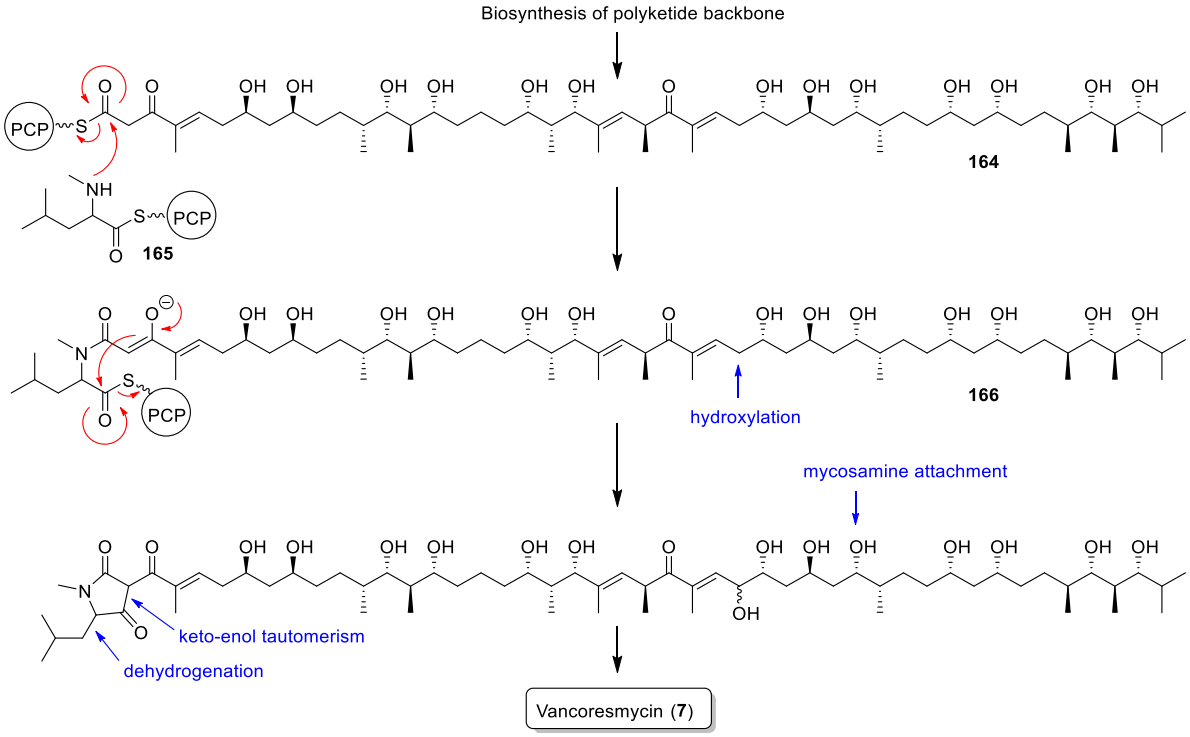
***Figure 5.1.2-1*:** Structure of vancoresmycin (**7**) with the proposed stereoinformation.[132]

Additionally, a biosynthetic pathway was proposed by *Allenby et al.*[132] The group reported that the vancoresmycin biosynthetic gene cluster contains 38 genes in which eight genes were classified as polyketide synthases (PKS) type I. All other genes are supposed to be involved in the synthesis of the tetramic acid, late stage tailoring effects and mycosamine biosynthesis. The eight type I PKS genes revealed a total of 101 enzymatic domains, which are organized into 23 modules. Furthermore, it was proposed that the biosynthesis of the polyketide starts with loading of an isobutyrate unit followed by subsequent condensation of 10 malonyl-CoA, 13 methylmalonyl-CoA units and respective PKS modifications. *Allenby et al.* suggested that the tetramic acid moiety is derived from *N*-methylated leucine, which is condensed with the polyketide backbone and afterwards cyclized. The biosynthetic pathway is completed by hydroxylation, mycosamine attachment, a non-enzymatical keto-enol tautomerization and dehydrogenation of the tetramic acid side chain (positions marked in **Scheme 5.1.2-1**) to furnish vancoresmycin (**7**).



***Scheme 5.1.2-1*:** Proposed biosynthetic completion of vancoresmycin (**7**) after biosynthesis of the polyketide backbone by PKS.[132]

The novel natural product vancoresmycin (**7**) showed extremely potent minimal inhibitory concentration (MIC) values ranging from 0.125 to 2 µg/mL against a variety of pathogens, including multidrug resistant gram-positive bacteria. No antimicrobial activity was observed against gram-negative bacteria like *Escherichia coli*, which means that vancoresmycin (**7**) may act selectively.[131,132]

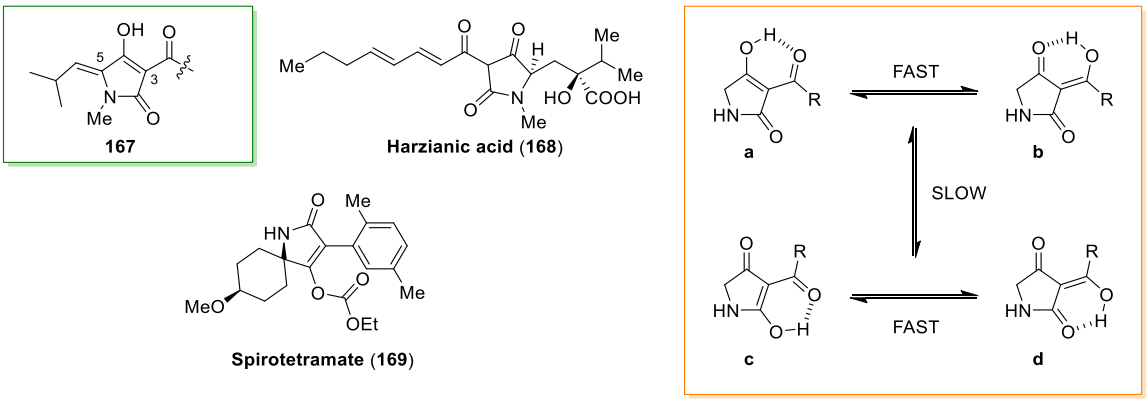
Furthermore, biochemical investigations revealed that vancoresmycin (**7**) targets the cytoplasmic membrane and it was proposed that the mode of action is a non-pore-forming, concentration-dependent depolarization of bacterial membranes.[132]

Both, its intriguing structure and the biological activity make vancoresmycin a desirable compound and lead structure that is worth to synthesize. Furthermore, a synthesis can give access to modified or simplified compounds retaining biological activity and the confirmation of unknown as well as only proposed configuration should be possible. Until now, no synthesis or retrosynthesis of vancoresmycin (**7**) or a vancoresmycin fragment has been published. Only the tetramic acid fragment derived from vancoresmycin was published based on results of this work.[133]

5.1.3 Tetramic Acids

An outstanding moiety of vancoresmycin is the highly substituted 3-acyl tetramic acid (**167**, **Scheme 5.1.3-1**). Compared to the novel *Amycolatopsis* metabolite, tetramic acids have already been extensively researched and reviewed.[134–136] However, a vancoresmycin-like tetramic acid (**167**) was not reported so far.

Tetramic acids and their often occurring 3-acyl derivatives can be found in a multitude of terrestrial and marine species[136] and a broad range of bioactivities like antibacterial,[137] antiviral[138] and antitumoral[139] potencies have been identified. Most of these heterocyclic moieties are biosynthesized by mixed polyketide synthase and non-ribosomal peptide synthetase pathways, while the residue at position 5 is often attributed to the biosynthetically used amino acid.[136] The particular structure of these compounds enables them to complex various metal ions like Mg^{2+}, Fe^{2+}, Zn^{2+} and Cu^{2+} and in some cases this type of chelation is even essential for the biological activity.[140,141] Harzianic acid (**168**), first isolated from a culture filtrate of *Trichoderma harzianum* SY-307, is in its active Zn^{2+} complex a potent serine/threonine phosphatase type 2A (PP2A) inhibitor (IC_{50} = 10 µg/mL), while in absence of Zn^{2+}, a complete loss of activity was observed.[141,142] Although tetramic acids have a broad spectrum of bioactivities, there are no representatives in clinical application so far. Notably, synthetic spirotetramate (**169**) was marketed under the name Movento® and is used as an insecticide.[143]



***Scheme 5.1.3-1*:** Structures of vancoresmycin-type tetramic acid (**167**), harzianic acid (**168**) and spirotetramate (**169**). Tautomerism of 3-acyltetramic acids (orange box).

Besides the comparably high acidity ($pK_s \approx 3$)[143] of 3-acyl tetramic acids, they are known for extended tautomerism (**Scheme 5.1.3-1**). In solution, four tautomeric forms (**a**, **b**, **c**, **d**) can be observed, which may be classified as a pair of 'external' (**ab**/**cd**) and a pair of 'internal' (**a**/**b**; **c**/**d**) tautomers. Due to the C-C bond rotation of the 3-acyl group, the interconversion between the 'external' isomers is a rather slow process compared to the NMR time scale resulting in separate NMR signals in non-polar solvents (e.g. CD_2Cl_2).[144,145]

The described extraordinary features render the tetramic acid scaffold a fascinating heterocycle but also complicate the synthesis and analysis.

5.2 Aim of the Project

Vancoresmycin (**7, Figure 5.2-1**) represents a structurally unique compound that is characterized by a large polyketide chain, which has a plethora of hydroxyl- and methyl-bearing stereogenic centers, connected to a densely functionalized tetramic acid. Furthermore, an aminoglycoside moiety is linked to the hydroxyl group at position 31. The fascinating structure alone justifies a total synthesis and makes it a challenging task. It is assumed that innovative, attractive synthetic methods can be found and established during a total synthesis of this complex natural product. Moreover, a synthetic effort combined with suitable analytical methods can lead to stereochemical clarification and confirmation. In addition, vancoresmycin (**7**) has shown highly potent antibiotic properties in the past.[132] Here, a total synthesis could be helpful to provide further material for an advanced biological investigation and to furnish derivatives to gain insights into a structure-activity relationship (SAR), which in the best case leads to even more potent and easier synthesizable compounds.

Of course, the ultimate goal is the total synthesis of vancoresmycin (**7**) in its natural form and with determination/confirmation of all stereogenic centers but due to the short remaining time after the performed total syntheses of lipid analogs (**Chapter 4** = part I) and the high complexity of the molecule with not confirmed or determined stereogenic centers as well as no preliminary synthetic effort, feasible goals have been set in this work.

The primary objective was to design the first retrosynthetic approach, which should serve as guideline for the project. Here, the main focus should be on the vancoresmycin aglycone **8** since no stereochemical information of the carbohydrate moiety was available. Furthermore, it was considered very useful to aim for the aglycone **8** to gain knowledge about the carbohydrate impact in the bioactivity.

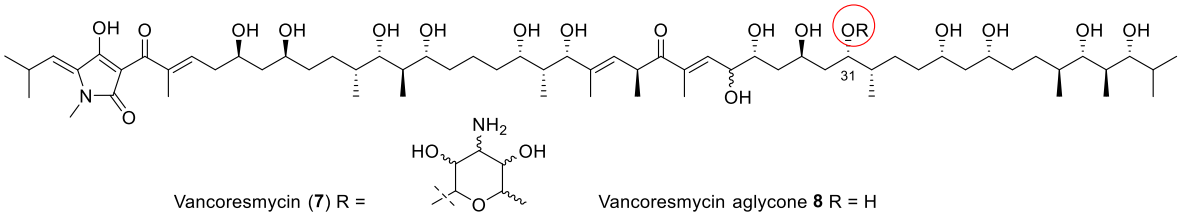


***Figure 5.2-1*:** Vancoresmycin (**7**) and vancoresmycin aglycone **8**.

If a promising theoretical approach to vancoresmycin aglycone **8** can be found, the syntheses of first fragments should be started. After generating suitable fragments, first couplings should be performed, so that larger parts of the target structure **8** may be available. Furthermore, the project could give insights whether fragments of vancoresmycin (**7**) already exhibit biological activity in order to identify the pharmacophore and possibly use smaller, easier obtainable fragments as novel lead structures for antibiotic research.

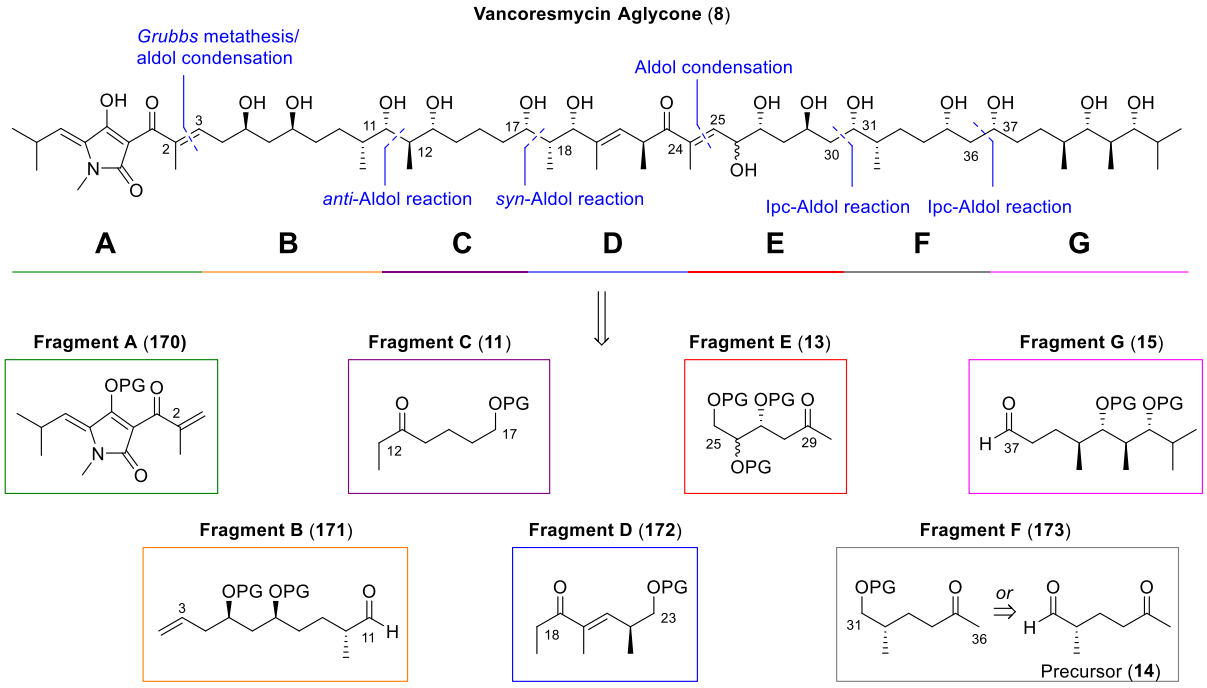
5.3 Results & Discussion

5.3.1 Retrosynthetic Analysis

So far, no retrosynthetic analysis can be found in literature. Therefore, the first retrosynthetic approach towards vancoresmycin was developed (first generation) in cooperation with *Stefanie Spindler*. Since some of the planned reactions proved to be problematic, the retrosynthetic plan was also adapted. Based on the novel gained knowledge, a current, second generation retrosynthetic approach was designed (**Chapter 5.3.1.2**). In general, the work initially focused on the synthesis of the vancoresmycin aglycone **8**. This was considered reasonable, since no stereochemical information on the carbohydrate moiety is available yet. In future, the stereogenic centers of the sugar may be determined by degradation experiments and subsequent spectroscopic methods. Furthermore, it was considered very useful to have a kind of structure-activity relationship in which vancoresmycin aglycone **8** is tested for biological activity. Due to the reported late stage attachment of the carbohydrate in the biosynthetic pathway, it may be possible to add the sugar moiety chemoenzymatically to the respective aglycone **8** as it was the case for lipid I analogs (**Chapter 4.4.9**).[132] Additionally, it should be noted that all stereocenters were only proposed and have not yet been adequately proven.[132] Therefore, the synthesis was planned in a way that allows a quick stereochemical adaption for example by changing enantiomeric ligands for the performed aldol reactions.

5.3.1.1 First Generation

In the first retrosynthetic approach, the vancoresmycin aglycone **8** was divided into seven fragments (**Scheme 5.3.1.1-1**, Fragments A-G) of approximately same sizes.

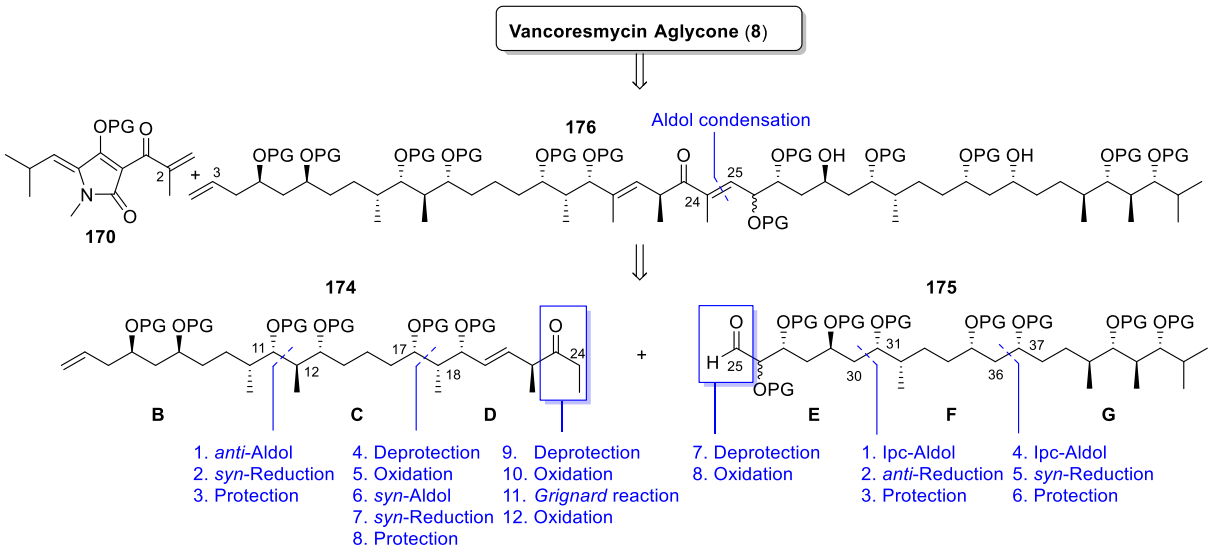


***Scheme 5.3.1.1-1*:** Retrosynthetic division of vancoresmycin aglycone **8** into the seven fragments A-G.

Due to the long polyketide chain and the frequently occurring 1,3-diols, aldol reactions with subsequent *syn*- or *anti*-reductions were often planned for the coupling of the fragments. However, to couple fragment A (**170**), which contains the tetramic acid moiety, to fragment B (**171**), a *Grubbs* metathesis was envisioned. Alternatively, an aldol condensation with an only slightly modified fragment A and B seems also feasible.[146,147] Linkage of the C-11 in fragment B (**171**) and C-12 in fragment C (**11**) should be possible by an *anti*-aldol reaction. Here, $[cHex]_2BCl$ and a small base like NEt_3 should ensure the formation of an (*E*)-enole borinate, resulting in the generation of the desired *anti*-product.[148] The usage of (+)-$[Ipc]_2BOTf$ and the more sterically hindered base DIPEA was considered for the generation of the (*Z*)-enole borinate out of fragment D (**172**), which should result in the formation of the desired stereogenic centers in a *syn*-aldol addition with deprotected and oxidized fragment C (**11**).[149] In order to generate C-24, which should serve as connecting point in the aldol condensation with fragment E (**13**), a deprotection, oxidation, *Grignard* elongation and a second oxidation to yield the respective ethyl ketone had to be performed prior to the planned condensation. For the linkage of fragment E (**13**) and F (**173**), (+)-$[Ipc]_2BCl$ mediated *Paterson* aldol coupling was envisioned, which would be performed after appropriate deprotection and oxidization of fragment F or directly with the precursor **14** if a connection between fragment E (**13**) and F (**14**) is prioritized over the linkage of fragment F (**173**) and G (**15**).[149]

A (+)-$[Ipc]_2BCl$ mediated *Paterson* aldol coupling could be used to connect C-36 of fragment F (**173**) to fragment G (**15**).[149] After the performed aldol additions, *Narasaka-Prasad* or *Evans-Saksena* reductions were planned to yield the 1,3-*syn* or 1,3-*anti* diols.[150–154]

While the order of couplings could be varied for some fragments, a strict coupling order was scheduled for others. If fragment D (**172**) and E (**13**) would be coupled first via the aldol condensation, a ketone would be present at C-23. With the neighbored enone on the left side of C-22, the stereogenic methyl group would be prone to racemization. Hence, fragment D (**172**) should first be coupled to fragment C (**11**) to get rid of the enone function. Furthermore, the coupling between fragment D (**172**) and E (**13**) within the polyketide chain should be performed at a later stage, otherwise the ketone at C-23 may cause regioselectivity problems in future reductions. In an exemplary, proposed coupling strategy (**Scheme 5.3.1.1-2**), the fragments B (**171**) and C (**11**) could be fused first via the $[cHex]_2BCl$ mediated *anti*-aldol reaction. *Narasaka-Prasad* reduction was planned for the synthesis of the *syn*-1,3-diol, which should be protected as acetonide. In general, acetonide protection was envisioned for all 1,3-diols, which should also allow to confirm the *syn*- and *anti*-configurations.[155–158] TBS ethers were chosen to mask primary hydroxyl groups, which could be cleaved selectively using fluoride ions but also in a joint, global deprotection, together with the acetonide protecting groups using acidic conditions. After the connection of fragment B (**171**) and C (**11**), deprotection of the primary hydroxyl group followed by an oxidation should give the respective aldehyde, which could be coupled to fragment D (**172**) via a *syn*-aldol reaction and subsequent *syn*-reduction as well as acetonide protection. After combining fragment B (**171**), C (**11**) and D (**172**), the ethyl ketone synthesis was planned at C-23, which consists out of a deprotection, oxidation, *Grignard* reaction and a second oxidation. This should furnish compound **174**, representing the left half of the polyketide chain.

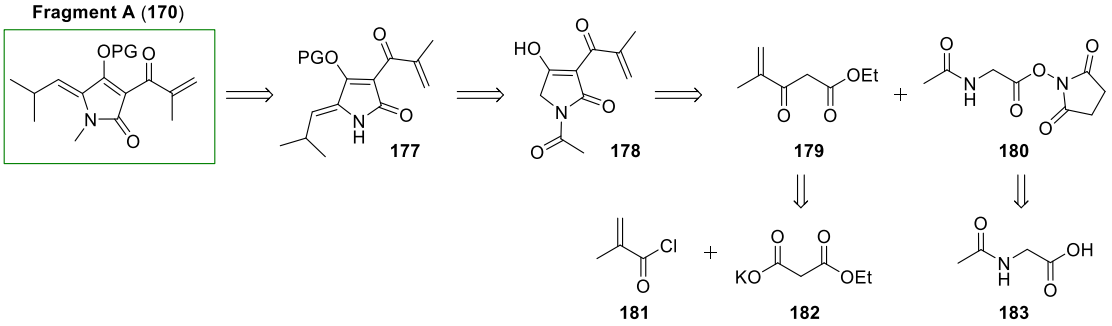


***Scheme 5.3.1.1-2*:** Retrosynthetic approach and linkage plan.

For the right side of the polyketide chain, which consists of fragment E (**13**), F (**173**) and G (**15**), there is no direct need of a coupling order. Thus, fragment F (**173**) could be attached to fragment G (**15**) or E (**13**) first. A coupling between fragment E (**168**) and F (**173**) may be preferred, since the required aldehyde (see **Scheme 5.3.1.1-1**, **14**) is a precursor of fragment F (**173**) and the longest linear sequence in the synthetic route could be shortened. Following the (+)-$[Ipc]_2BCl$-mediated aldol reaction between fragment E (**13**) and the precursor of fragment F (**14**), the *anti*-diol could be accessible using an *Evans-Saksena* reduction, which ensures an intramolecular hydride delivery in a six-membered transition state and therefore, should not affect the ketone at C-35.[153,154] After acetonide protection of the resulting 1,3-*anti*-diol, a second (+)-$[Ipc]_2BCl$-mediated aldol reaction could be used for the connection to fragment G (**15**). Subsequent *syn*-reduction using *Narasaka-Prasad* conditions and protection of the diol followed by deprotection and oxidation of the primary alcohol at C-25 should yield aldehyde **175**. With the left part of the polyketide chain **174** and the right part **175**, an aldol condensation could give the desired vancoresmycin chain **176**, which could then be coupled to the tetramic acid **170** (Fragment A) using *Grubbs* metathesis and global deprotection with acidic conditions should furnish the vancoresmycin aglycone **8**.

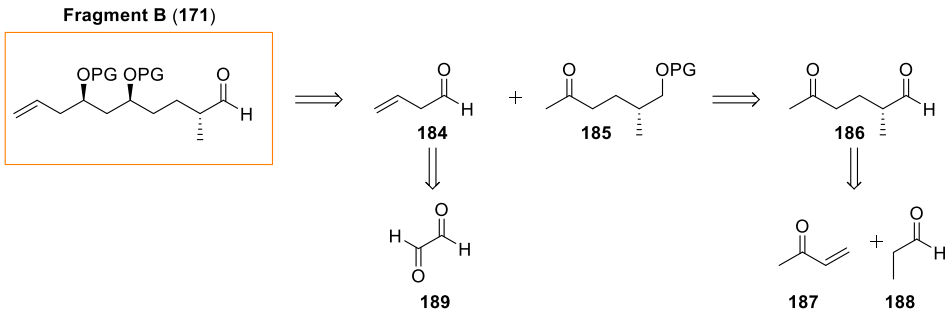
The first retrosynthetic approach for fragment A (**170**) is shown in **Scheme 5.3.1.1-3**. Tetramic acid fragment **170** derives from tetramic acid **177** using *N*-methylation. Pyrrolidin derivative **177** should be obtained by condensation of imide **178** with isobutyraldehyde and

simultaneous deacylation as well as subsequent protection of the hydroxyl function. Methylene activated diketone **179** was planned to attack active ester **180** followed by an intramolecular ring closure to yield tetramic acid **178**. This procedure is reported in literature for other methylene activated compounds.[159] Compounds **179** and **180** could be synthesized out of commercially available compounds **181**, **182** and **183** as described in literature.[160–162]



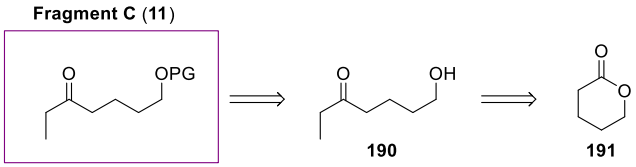
***Scheme 5.3.1.1-3*:** First generation retrosynthetic approach for fragment A (**170**).

Accessibility to fragment B (**171**) was envisioned by an Ipc-mediated aldol reaction between but-3-enal (**184**) and ketone **185** (**Scheme 5.3.1.1-4**). The aldehyde **184** could be generated out of glyoxal (**189**) by a literature known procedure.[163,164] Ketone **185** could be synthesized out of aldehyde **186** using a reduction, primary protection and oxidation sequence, while aldehyde **186** could be gained applying a literature described, enantioselective *Michael* addition with a phenylalanine derived imidazolidinone as organocatalyst.[165–167] For a detailed description of the reactions and occurring problems, an insight into the dissertation of *Stefanie Spindler* is recommended.[167] The synthesis of fragment B was adapted in the later course (see **Chapter 5.3.1.2** for retrosynthesis) and was also synthesized during this work (**Chapter 5.3.3**).



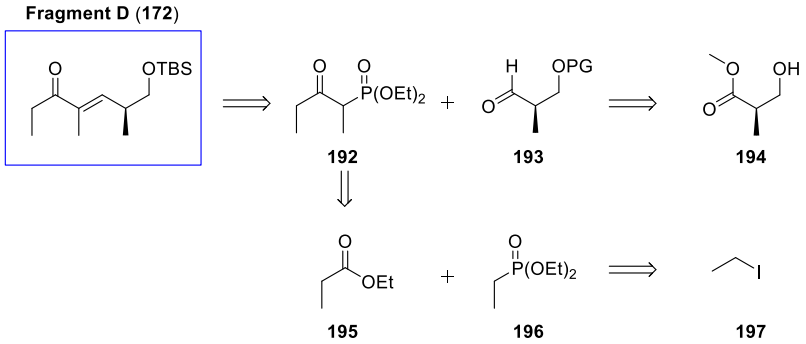
***Scheme 5.3.1.1-4*:** First generation retrosynthetic approach for fragment B (**171**).

For fragment C (**11**), a literature known two-step procedure was planned (**Scheme 5.3.1.1-5**).[168] Starting from δ-valerolactone (**191**), ring opening by *Weinreb* amide formation followed by *Grignard* reaction should yield ketone **190**, which should give fragment C (**11**) after protection of the hydroxyl group. The synthesis of fragment C (**11**) out of δ-valerolactone (**191**) can be found in the dissertation of *Stefanie Spindler* and a novel approach (see **Chapter 5.3.1.2** for retrosynthesis) can be found in this work (**Chapter 5.3.4**).[167]



***Scheme 5.3.1.1-5*:** First generation retrosynthetic approach for fragment C (**11**).

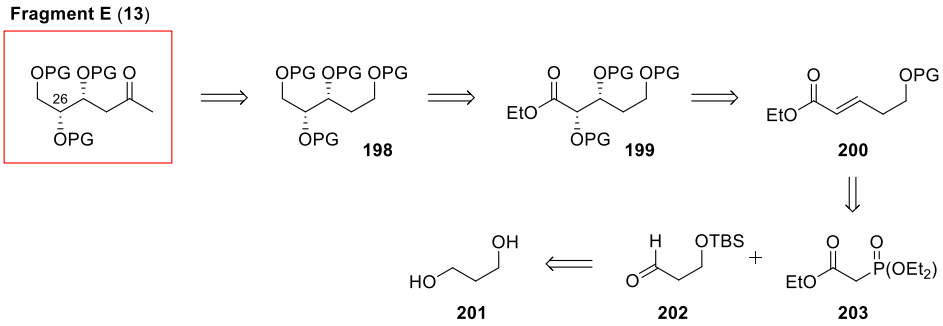
For fragment D (**172**), the first retrosynthetic approach is shown in **Scheme 5.3.1.1-6**. Here, it was envisioned to get access to fragment D (**172**), with the desired (*E*)-double bond, by a *Horner-Wadsworth-Emmons* (HWE) reaction from phosphonate **192** and aldehyde **193**. The aldehyde **193** was planned to be synthesized out of (*R*)-*Roche* ester (**194**) using protection of the primary alcohol and reduction of the ester function.[169,170] Phosphonate **192** could be gained from ethyl propionate (**195**) and phosphonate **196**, which should be accessible by an *Arbuzov* reaction out of ethyl iodide (**197**).[171,172]



***Scheme 5.3.1.1-6*:** First generation retrosynthetic approach for fragment D (**172**).

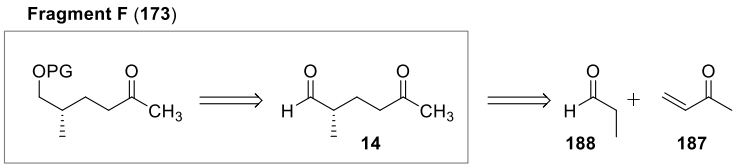
It was planned to obtain fragment E (**13**, **Scheme 5.3.1.1-7**) from protected polyol **198** by cleavage of the right, primary hydroxyl protecting group followed by subsequent oxidation, *Grignard* reaction and a second oxidation to obtain the ketone. Here, three orthogonal protecting groups are required. While the right sided, primary hydroxyl group should be masked

as silyl ether (e.g. TBS), the left sided primary alcohol should be protected as PMB ether. The 1,2-diol could be protected like the 1,3-diols using the respective acetonide formation, which should allow for a joint acetonide removal at a late stage using acidic conditions. The protected polyol **198** could be obtained after ester reduction and PMB-protection of ester **199**. Since no configuration of C-26 was proposed so far, an initial *Sharpless* asymmetric dihydroxylation (SAD) with AD-mix-β was chosen. Notably, the other 1,2-configurations of the diol should be easily accessible using AD-mix-α for the SAD or stereoselective asymmetric epoxidation (e.g. *Sharpless*, SAE) and selective epoxide opening.[173–179] However, a literature known sequence for compound **199** was planned, which should furnish ester **199** out of HWE product **200** by SAD and acetonide protection.[180] The (*E*)-double bond in compound **200** should be available by a HWE reaction of aldehyde **202** and commercially available phosphonate **203**. Aldehyde **202** could easily be synthesized from 1,3-propanediol (**201**) by the help of a monoprotection and oxidation on the other side.[180]



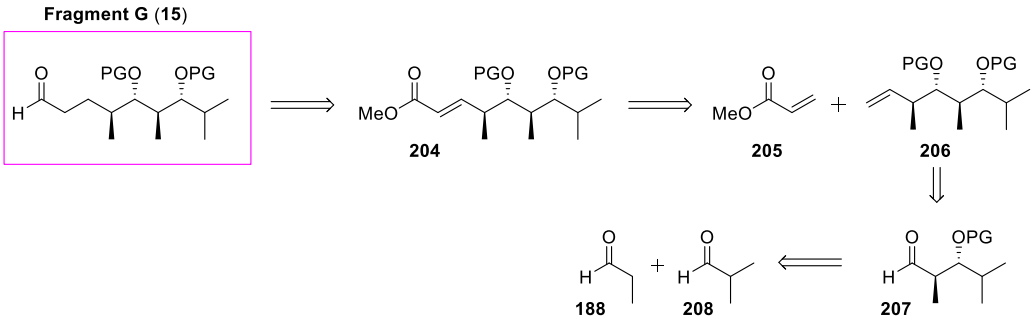
***Scheme 5.3.1.1-7*:** First generation retrosynthetic approach for fragment E (**13**).

Fragment F (**173**, **Scheme 5.3.1.1-8**) represents the enantiomer of ketone **185** (**Scheme 5.3.1.1-4**) and thus, it is obvious to synthesize it analogously, by the previously reported enantioselective *Michael* addition with the other enantiomeric phenylalanine derived organocatalyst.[165–167] Again, propionaldehyde (**188**) and methyl vinyl ketone (**187**) could be used as starting materials. Notably, if a coupling of fragment F (**173**) and fragment E (**13**) will be prioritized, the precursor **14** could be used, which already has the needed aldehyde moiety. Therefore, this coupling order would save the three steps from aldehyde **14** to compound **173**, which are reduction, selective protection of the primary alcohol and oxidation to the ketone.



***Scheme 5.3.1.1-8*:** First generation retrosynthetic approach for fragment F (**173**).

The retrosynthetic approach for fragment G (**15**), which completes the vancoresmycin aglycone **8** on the right side, is shown in **Scheme 5.3.1.1-9**. The aldehyde functionality in compound **15** is needed due to the desired coupling with fragment F (**173**). Fragment G (**15**) could be generated out of ester **204** by reduction of the double bond and the methyl ester. If a conventional Pd/C and H_2 reduction of the double bond would fail, a reduction using *Strykers* reagent ($[(PPh_3)CuH]_6$) was envisioned, while the methyl ester could be reduced using DIBAL-H.[181–183] For the generation of unsaturated ester **204**, a *Grubbs* metathesis was planned using methyl acrylate (**205**) and protected diol **206,** which could be accessible by a *Brown* crotylation of aldehyde **207** and subsequent protection.[184] A literature known synthesis of aldehyde **207** was considered using a proline catalyzed aldol reaction of isobutyraldehyde (**208**) and propionaldehyde (**188**) followed by protection of the hydroxyl function.[185,186]



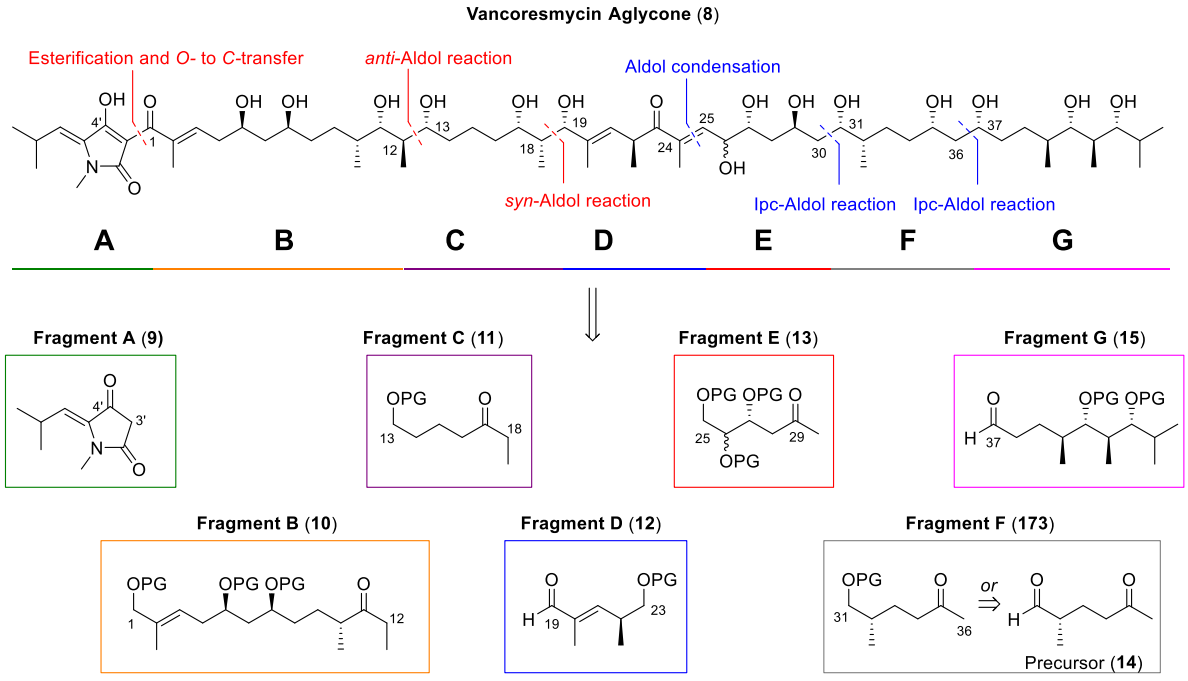
***Scheme 5.3.1.1-9*:** First generation retrosynthetic approach for fragment G (**15**).

5.3.1.2 Second (current) Generation

More knowledge about the vancoresmycin aglycone **8**, its fragments and their coupling was gained during the synthetic work. It became apparent that vancoresmycin-type tetramic acids exhibited strong tautomerism, metal chelating properties and instability for some compounds (see **Chapter 5.3.2**). Thus, the synthesis and the retrosynthesis were modified. Therefore, it

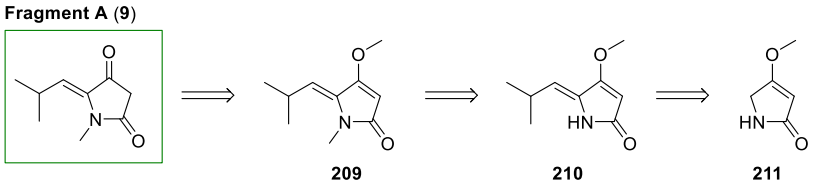
was planned to couple the respective polyketide chain in a late stage to the tetramic acid fragment **9** (**Scheme 5.3.1.2-1**) via an esterification reaction at HO-4' and a subsequent oxygen to carbon (C-3') transfer.[144,187,188] A PMB-protection was envisioned for the alcohol at C-1 (fragment B, **10**), which was planned to be removed prior to oxidation and esterification with fragment A (**9**). Additionally, the planned *syn*-aldol coupling of fragment C (**11**) and D (**172**), which was envisioned in the first generation, was not promising and it was assumed that the deprotonation of the enone **172** followed by a stereoselective aldol addition was difficult (**Chapter 5.3.6**). Thus, the aldol reaction was planned in a reverse manner with fragment C (**11**) containing the ketone and fragment D (**12**) the aldehyde moiety. Also the dissection point for the *anti*-aldol coupling was shifted by one C-atom, which allowed preservation of the same fragment C (**11**) as previously described (**Scheme 5.3.1.1-5**) only turning the representation by 180 degrees in the current approach (**Scheme 5.3.1.2-1**). However, if a satisfactory stereoselectivity cannot be achieved with the *anti*-aldol coupling, the fragments should be easily modifiable.

Again, acetonide protection was chosen to protect 1,3-diols as well as the 1,2-diol at C-26 and C-27. These protective groups are required for the determination of relative configurations and a joint removal in the end. Primary hydroxyl groups were chosen to be mainly protected by TBS ethers or a PMB ether to allow orthogonal cleavages.



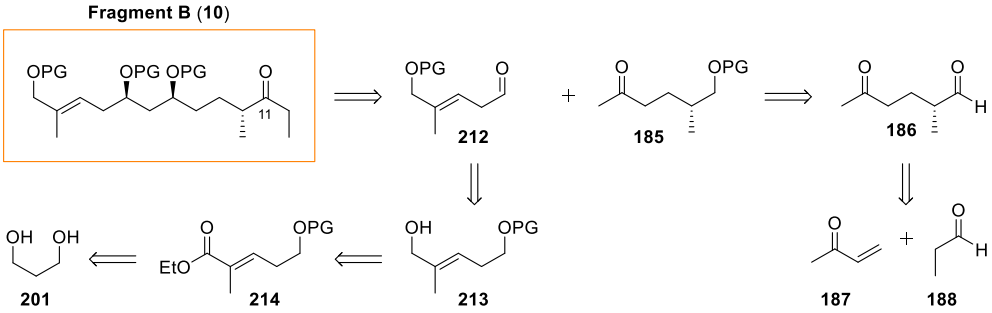
***Scheme 5.3.1.2-1*:** Modified retrosynthetic division of vancoresmycin aglycone **8** into the seven fragments A-G.

The novel retrosynthetic approach of fragment A (**9**) is shown in **Scheme 5.3.1.2-2**. In order to avoid the 3-acyl tetramic acids, which were difficult to handle due to tautomerism, chelating properties and instability, the acyl residue was shifted to fragment B (**10**), furnishing compound **9** as novel fragment A. Tetramic acid **9** could be gained by demethylation of compound **209** using acidic conditions, while compound **209** should be accessible by methylation of the ring nitrogen. Starting from commercially available 4-methoxy-1,5-dihydro-2*H*-pyrrol-2-on (**211**), a literature described procedure should allow the attachment of the isobutyl moiety under basic conditions.[189]



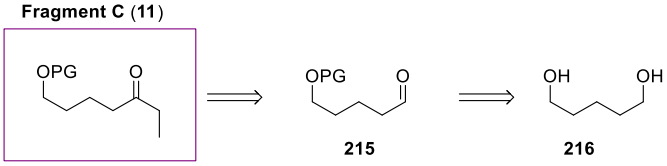
***Scheme 5.3.1.2-2*:** Second generation retrosynthetic approach for fragment A (**9**).

While the right part of fragment B (**10**), synthesized by *Stefanie Spindler*, remained the same as in the first generation, the left side was modified leading to aldehyde **212** as precursor (**Scheme 5.3.1.2-3**).[167] Thus, an (+)-[Ipc]$_2$BCl mediated *Paterson* aldol coupling was planned to couple aldehyde **212** and previously envisioned ketone **185**, which should yield the novel fragment B (**10**) after *syn*-reduction, acetonide protection and modification at C-11. The novel aldehyde **212** could be accessible from compound **213** by protection of the free alcohol moiety (e.g. with PMBCl) as well as selective deprotection and oxidation of the other hydroxyl group. Alcohol **213** should be synthesizable by reduction of ester **214**, which in turn should be accessible from 1,3-propanediol (**201**) by monoprotection, oxidation and a HWE-reaction.[190]



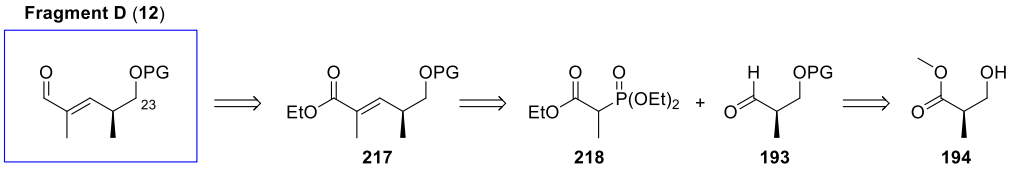
***Scheme 5.3.1.2-3*:** Second generation retrosynthetic approach for fragment B (**10**).

Since the route of the first generation towards fragment C (**11**) suffered from reproducibility and unsatisfactory yields (see dissertation of *Stefanie Spindler*),[167] a novel route was considered (**Scheme 5.3.1.2-4**). Fragment C (**11**) should be accessible out of aldehyde **215** with a *Grignard* reaction followed by oxidation. Using monoprotection and oxidation, aldehyde **215** could be gained out of 1,5-pentaneediol (**216**), which represents a low priced starting material.[191] As the sequence involves only preparative simple steps, a higher yield and reproducibility of fragment C (**11**) was expected with this new route.



***Scheme 5.3.1.2-4*:** Second generation retrosynthetic approach for fragment C (**11**).

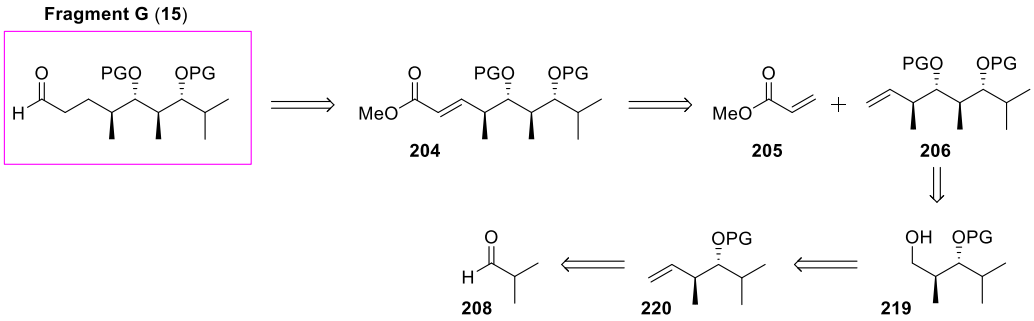
Fragment D (**12**) was also slightly adapted in the second generation as it should contain an aldehyde function instead of the previous ketone (**Scheme 5.3.1.2-5**). Aldehyde **12** could be generated from α,β-unsaturated ester **217** using a DIBAL-H reduction.[192] Compound **217** should be accessible by a HWE reaction of commercially available phosphonate **218** and previously described aldehyde **193** (see **Chapter 5.3.1.1**).[169,170,192] Coupling of fragment D (**12**) to fragment E (**13**) would then further require deprotection, oxidation, *Grignard* reaction and re-oxidation at C-23.



***Scheme 5.3.1.2-5*:** Second generation retrosynthetic approach for fragment D (**12**).

Since the retrosyntheses of fragment E (**13**) and F (**173**) have not been modified, they are not shown again (see **Chapter 5.3.1.1**). Fragment G (**15**) remained unchanged as well but due to difficulties (described in the dissertation of *Stefanie Spindler*),[167] an adapted route was developed (**Scheme 5.3.1.2-6**). According to the first generation retrosynthetic approach, fragment G (**15**) should be accessible via ester **204**, which could be gained after a *Grubbs*

metathesis of methyl acrylate (**205**) and protected diol **206**. While the last steps to fragment G (**15**) were kept, the synthesis of compound **206** was modified, by utilization of alcohol **219** and *Krische* crotylation.[193,194] Application of this procedure seemed to be advantageous, because it could use the more stable alcohol **219** instead of the previously used aldehyde **207** (**Scheme 5.3.1.1-9**). For the synthesis of alcohol **219**, an ozonolysis/reduction sequence of the terminal double bond at alkene **220** was envisioned. Starting from inexpensive isobutyraldehyde (**208**), a crotylation reaction like the *Brown* procedure and subsequent protection was planned to yield compound **220**.[184]

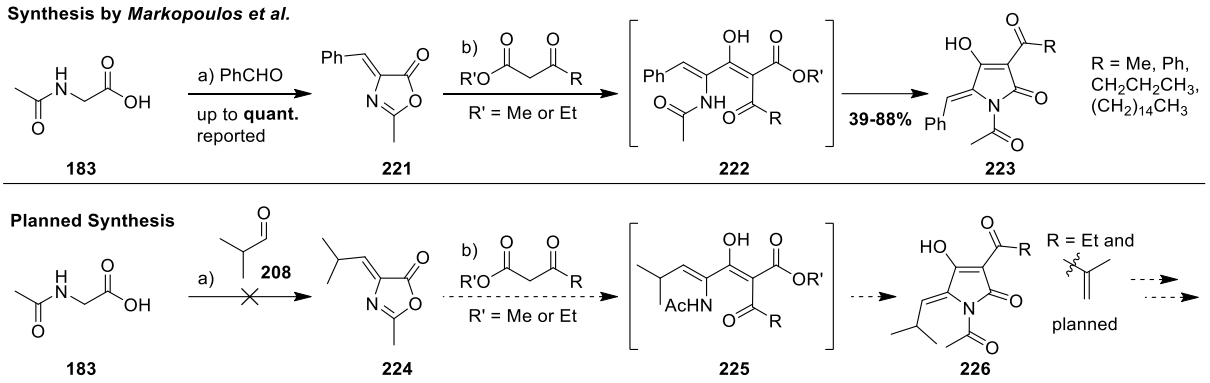


***Scheme 5.3.1.2-6*:** Second generation retrosynthetic approach for fragment G (**15**)

In summary, the first retrosynthetic approach for vancoresmycin aglycone **8** was developed (first generation) and already optimized (second generation). The planned route has been designed in such a way that the stereogenic centers can be interchanged easily, since their absolute configuration has not been confirmed yet. In addition, attention has been given to ensure that the fragments can be simply adapted to allow a quick exchange of the coupling order if necessary.

5.3.2 Synthesis of Fragment A (Tetramic Acid)

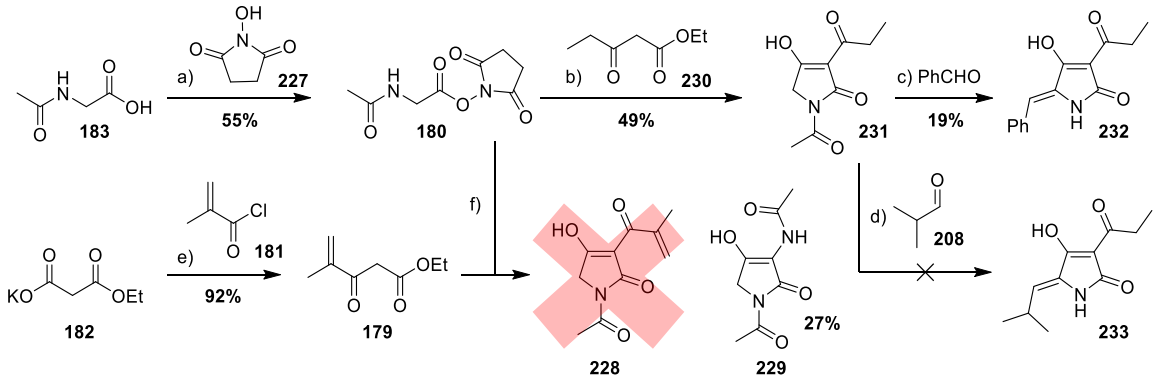
A sequence towards 3-acyl tetramic acids was developed by *Markopoulos et al.*[195] and was investigated as a first approach. The group was able to synthesize various tetramic acid derivatives featuring a 3-acyl and a 5-benzylidene group using only two steps (**Scheme 5.3.2-1**).



***Scheme 5.3.2-1*:** Reported synthesis by *Markopoulos et al.* and planned but failed synthesis towards fragment A. Reaction conditions: a) Respective aldehyde (1.5 equiv), NaOAc (1.5 equiv), Ac_2O (2.5 equiv), 120 °C, 7 h; b) NaH (2.0 equiv), respective activated methylene compound (2.0 equiv), THF, 0 °C to rt, 2 h.

The synthesis of oxazolone **221** is an established method also known as *Erlenmeyer-Plöchl azlactone synthesis* and up to quantitative yields have been reported for this compound.[196] Additionally, it is known that this reaction does not proceed with aliphatic aldehydes, which feature a α-hydrogen, since they often give enol acetates and diacetates when heated with acetic anhydride.[176] However, due to the very short route towards 3-acyl tetramic acids and the readily available starting materials the reaction was tried with isobutyraldehyde (**208**). As expected, the reaction towards compound **224** turned out to be problematic and the synthetic route was not further investigated.

Using the same starting material **183**, a respective active ester **180** could be synthesized with application of a reported method (**Scheme 5.3.2-2**).[160,161]

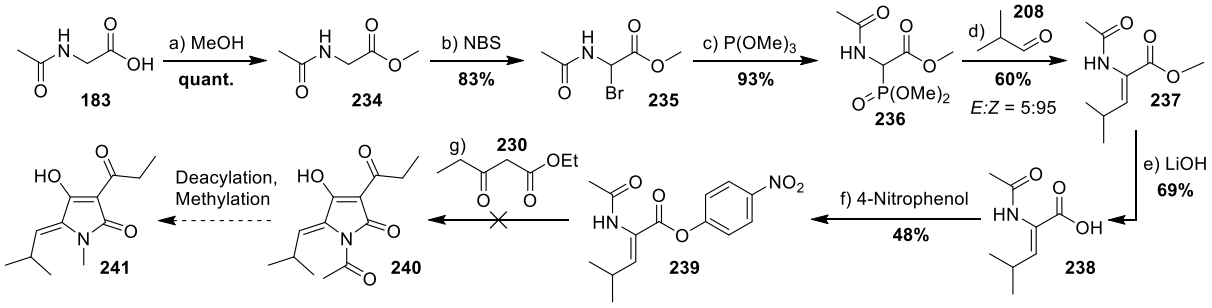


***Scheme 5.3.2-2*:** Carried out reactions to yield tetramic acid derivatives. Reaction conditions: a) NHS (**227**, 1.0 equiv), DCC (1.0 equiv), 1,4-dioxane, 3 °C, on, **55%**; b) NaH (2.0 equiv), **230** (3.0 equiv), toluene, rt, 3 h, **49%**; c) PhCHO (2.0 equiv), 8% HCl/Ethanol, 90 °C, 3 h, **19%**; d) Isobutyraldehyde (**208**, 2.0 equiv), 8% HCl/Ethanol, 90 °C, 3 h; e) $MgCl_2$ (1.2 equiv), NEt_3 (2.2 equiv), **181** (0.8 equiv), MeCN, 0 °C, 30 min, **92%**; f) NaH (3.0 equiv), **179** (3.0 equiv), THF, 0 °C to rt, 3 h, **27%** of **229**.

With deprotonated, methylene activated compound **230** it was possible to attack the active ester **180** and subsequent intramolecular ring closure yielded tetramic acid **231** in a fair yield. A method described for other 3-acyl tetramic acid derivatives allowed the attachment of a benzylidene group under acidic conditions, which simultaneously cleaved the *N*-acetate.[159] In accordance to previously obtained 3-acyl tetramic acids,[134–136,197] the novel compounds also showed extended tautomerism (described in **Chapter 5.1.3**) complicating handling and NMR analysis. In unpolar solvents (CD_2Cl_2), at least two data sets were observed due to the rather slow interconversion between the external tautomers compared to the NMR timescale. In polar solvents ($(CD_3)_2SO$), usually only one data set could be observed but the signals often showed broadening.[144,145] To simplify **Scheme 5.3.2-2** and also following schemes, only one possible tautomer is displayed for 3-acyl tetramic acids. The established procedure for compound **232** could not be adopted for tetramic acid **233**, which is probably caused by the α-hydrogen of isobutyraldehyde (**208**).

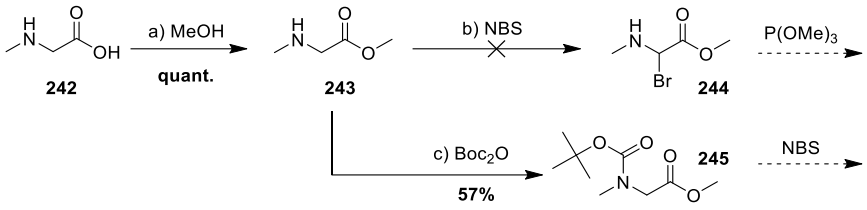
In order to introduce a terminal double bond, which may allow concatenation of the tetramic acid to a polyketide backbone with *Grubbs* catalysts, methylene activated **179** was synthesized as previously described.[162] Similar reaction conditions like they were used for the synthesis of tetramic acid **231** were applied to yield compound **228**. However, only product **229** could be isolated, which results out of a reaction between two active esters **180**.[198] Therefore, it was assumed that compound **179**, which contains an α,β-double bond, is unsuitable for the desired reaction due to its *Michael* acceptor properties.

Caused by the problematic introduction of the leucine derived side chain after the formation of the tetramic acid scaffold (**Scheme 5.3.2-2**), initial introduction of the side chain and subsequent ring closure seemed more promising (**Scheme 5.3.2-3**).



***Scheme 5.3.2-3*:** Approach towards tetramic acid **241**. Reaction conditions: a) $SOCl_2$ (2.0 equiv), MeOH, rt, on, **quant.**; b) *N*-Bromosuccinimide (NBS, 1.1 equiv), CCl_4, 110 °C, sunlamp irradiation, 5 h, **83%**; c) Trimethyl phosphite (1.0 equiv), EtOAc, rt, on, **93%**; d) 1,1,3,3-tetramethylguanidine (1.2 equiv), isobutyraldehyde (**208**, 1.2 equiv), THF, −78 °C to rt, 2.5 h, *E/Z* = 5:95, **60%**; e) 1 M LiOH, THF, rt, on, **69%**; f) DCC (1.2 equiv), DMAP (0.1 equiv), 4-nitrophenol (1.2 equiv), DCM, rt, 1 h, **48%**; g) NaH (2.0 equiv), **230** (3.0 equiv), toluene, rt, 3 h.

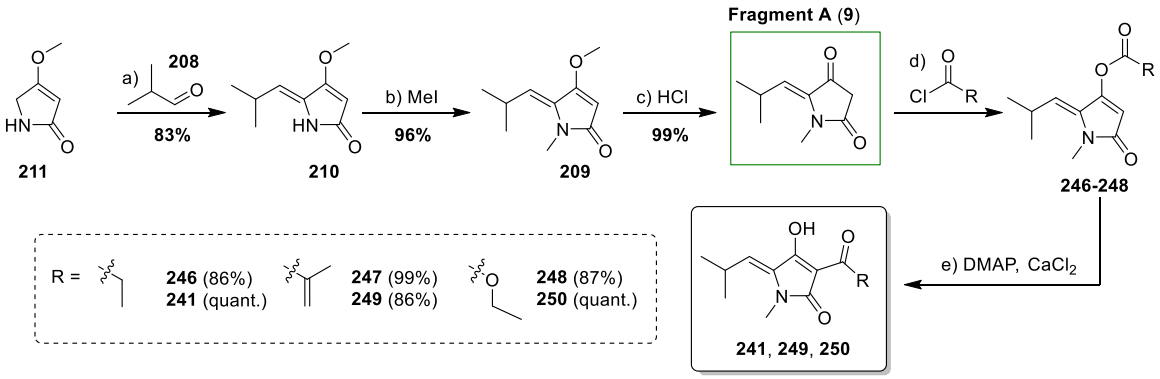
To yield compound **237**, a sequence reported by *Davies et al.* was applied.[199] Thus, glycine derivative **183** was transferred into its methyl ester **234** in quantitative yield followed by α-bromination using NBS, CCl_4, reflux and sunlamp irradiation. A subsequent *Arbuzov*-reaction yielded phosphonate **236** in 93% yield, which allowed olefination using isobutyraldehyde (**208**) giving compound **237** in excellent *E*/*Z* selectivity (5:95). Saponification yielded the free acid **238**, which was converted to active ester **239** in a moderate yield. A 4-nitrophenolester was chosen due to UV activity and the successful reported use of related (without side chain) compounds in tetramic acid synthesis.[198] Thus, active ester **239** was reacted with methylene activated compound **230**. Although product formation was indicated in GC-MS analyses, it was not possible to isolate the desired tetramic acid **240** using column chromatography or HPLC. Simultaneously, it was tried to use the same sequence shown in **Scheme 5.3.2-3** starting from sarcosine (**242**, **Scheme 5.3.2-4**). A successful application of this sequence would avoid the deacylation and methylation in the end.



***Scheme 5.3.2-4*:** Approach towards tetramic acid **241** starting from sarcosine (**242**). Reaction conditions: a) $SOCl_2$ (2.0 equiv), MeOH, rt, on, **quant.**; b) *N*-Bromosuccinimide (NBS, 1.1 equiv), CCl_4, 110 °C, sunlamp irradiation, 5 h; c) Boc_2O (1.4 equiv), NEt_3 (2.0 equiv), DCM, rt, 2 d, **57%**.

Hence, the methyl ester **243** was generated from sarcosine (**242**) in quantitative yield. Using the same conditions as previously used for **234** (**Scheme 5.3.2-3**), the desired bromination was not achieved and thus, the secondary amine was Boc protected to yield compound **245** in 57% yield. The Boc protecting group should be easily removable, using for example TFA, and possibly has an advantageous effect in the bromination step.

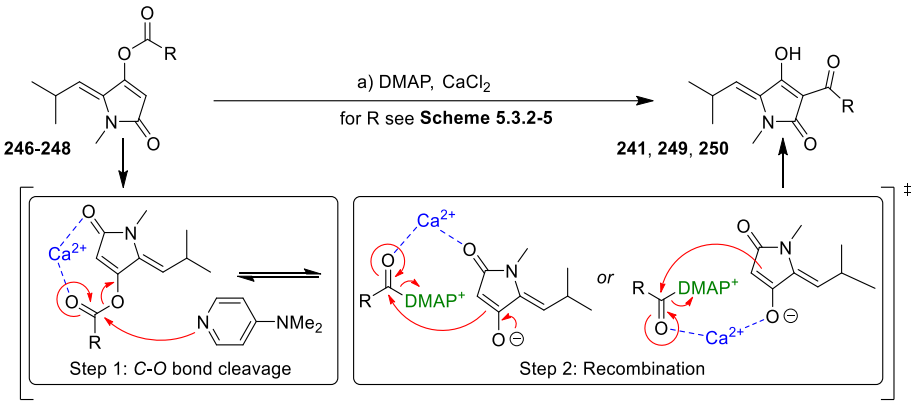
However, the described routes were not further studied although they might have been successful if they had been continued. In the meantime, a very efficient and convenient route towards the desired tetramic acids was found (**Scheme 5.3.2-5**) and therefore, there was initially no necessity to further investigate other routes.



***Scheme 5.3.2-5*:** Synthesis of tetramic acids **241**, **249**, **250**. Reaction conditions: a) Isobutyraldehyde (**208**, 1.0 equiv), NaOH, 60 °C, 7 h, **83%**; b) NaH (1.1 equiv), MeI (1.1 equiv), DMF, rt, 3 h, **96%**; c) Conc. HCl, rt, 8 h, **99%**; d) Acid chloride (1.2 equiv), NEt_3 (1.4 equiv), DCM, −5 °C, 30 min, **86-99%**; e) $CaCl_2$ (1.5 equiv), DMAP (0.3 equiv), NEt_3 (1.4 equiv), DCM, rt, 4-7 h, **86%-quant**.

The successful route started with the introduction of the 5-substituent to commercially available 4-methoxy-3-pyrrolin-2-one (**211**). Here, it was possible to adopt a previously reported procedure and isobutyraldehyde (**208**) was added in basic conditions to give compound **210** in

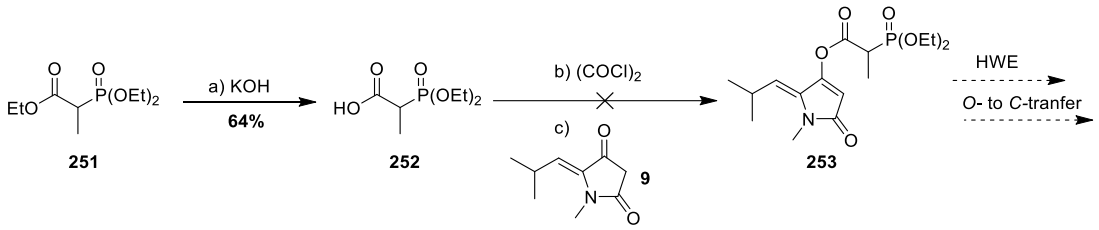
83% yield.[189] *N*-Methylation with iodomethane yielded compound **209** and cleavage of the methoxy group under acidic conditions furnished key compound **9** (fragment A) in 79% over three steps without the requirement of column chromatography. However, any intermediate can be purified by using column chromatography if needed. With fragment A (**9**) in hand, it was possible to introduce various esters at the 4-position using acid chlorides and compounds **246**-**248** were gained in up to 99% yield. A known rearrangement by using $CaCl_2$ in the presence of DMAP and triethylamine ensured the required oxygen-to-carbon transfer for the novel esters, which efficiently realized the synthesis of 3-acyl tetramic acids **241**, **249** and **250** in excellent yields.[144,187,188] The acylation reagents were chosen to be authentic to vancoresmycin (**7**) and also to allow following transformations like aldol condensation, cross metathesis or a nucleophilic attack of the ester at the 3-position. *Yoda et al.*, who tested several metal salts, found that $CaCl_2$ was the best additive for the oxygen-to-carbon rearrangement and proposed that the mechanism consist out of two fundamental steps, namely, a *C*-*O* bond cleavage and the recombination of the resulting ion pairs (**Scheme 5.3.2-6**). The group supposed that the metal salt helps in stabilization of the generated ion pairs by coulombic attraction, which keeps the ion pairs together and thus, facilitates the formation of 3-acyl tetramic acids.[187,200] However, Ca^{2+} may also play a role in the first step and activate the carbonyl group for a nucleophilic attack.[200]



***Scheme 5.3.2-6*:** Proposed mechanism for the oxygen-to-carbon rearrangement.[187,200] Reaction conditions: a) $CaCl_2$ (1.5 equiv), DMAP (0.3 equiv), NEt_3 (1.4 equiv), DCM, rt, 4-7 h.

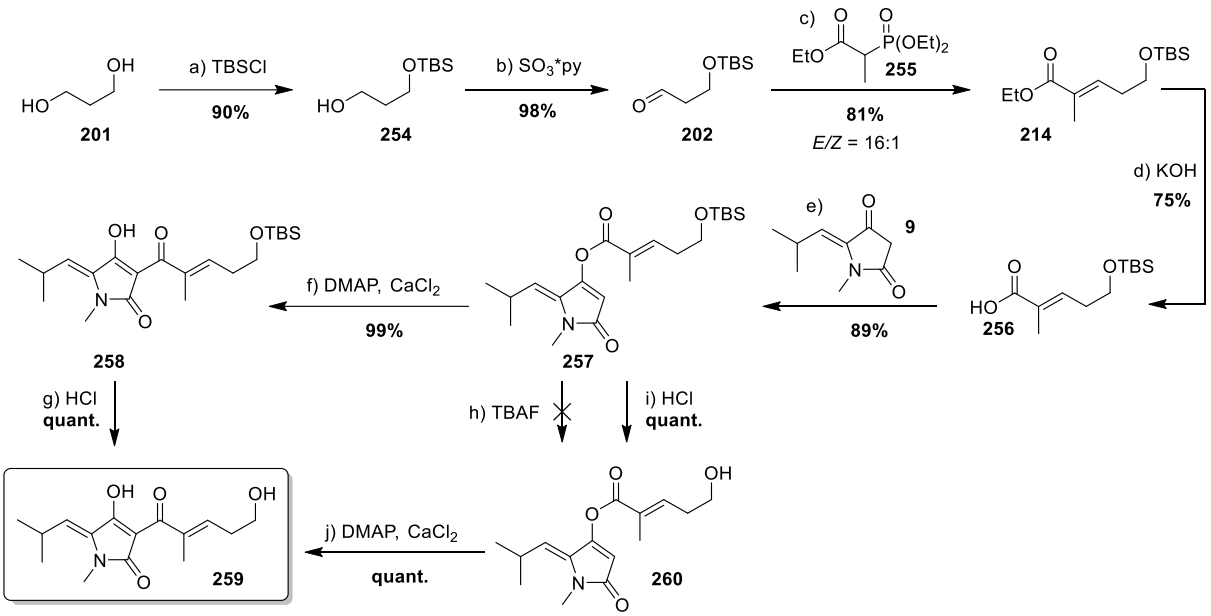
To allow for *Horner-Wadsworth-Emmons* (HWE) couplings, it was investigated to introduce phosphonate **252** to the tetramic acid core **9** (**Scheme 5.3.2-7**). Saponification of commercially available phosphonate **251** has been carried out to furnish compound **252** featuring a free

carboxyl group. In an initial experiment, it was tried to couple this phosphonate using in situ activation to the respective acid chloride. Unfortunately, desired product **253** was not gained. Milder coupling strategies like a DCC procedure will be tested in future.



***Scheme 5.3.2-7*:** Attempted synthetic route towards a phosphonate modified tetramic acid. Reaction conditions: a) KOH (1.2 equiv), ethanol/water, rt, on, **64%**; b) $(COCl)_2$ (1.2 equiv), DCM, rt, 5 h; c) **9** (0.9 equiv), NEt_3 (1.2 equiv), DCM, −5 °C, 3 h.

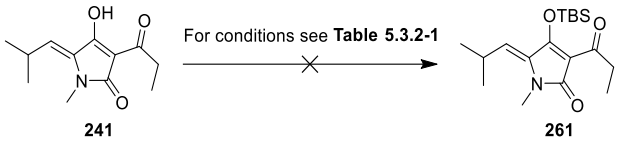
To create a vancoresmycin-derived tetramic acid, which features a first part of the polyketide chain, compound **214** (**Scheme 5.3.2-8**) was synthesized using a modified reported procedure from 1,3-propanediol (**201**).[190] In detail, a *Parikh-Doering* oxidation was used instead of a *Swern* reaction and a different HWE procedure was applied for better *E*/*Z* selectivity. After saponification, compound **256** was isolated in 54% yield over four steps, which was used for a DCC mediated coupling with fragment A (**9**), followed by the oxygen-to-carbon transfer to yield tetramic acid **258** with an excellent yield of 88% over two steps. The TBS protecting group can be cleaved with acidic conditions either at ester **257** to furnish alcohol **260** or as a final step to yield compound **259**. It was also tried to cleave the TBS group in compound **257** using TBAF but this resulted in degradation of the compound and an almost quantitative amount of tetramic acid precursor **9** was isolated, which suggests that **257** is an easily cleavable ester making this compound more or less unsuitable for the elongation of the polyketide chain at this stage.



***Scheme 5.3.2-8*:** Synthesis of tetramic acid **259**. Reaction conditions: a) Imidazole (1.1 equiv), TBSCl (1.0 equiv), THF, 0 °C, 30 min, **90%**; b) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1.5 h, **98%**; c) $Ba(OH)_2$ (3.5 equiv), **255** (1.4 equiv), THF, rt, on, **81%**; d) KOH (1.2 equiv), ethanol/water, rt, 6 h, **75%**; e) **9** (1.0 equiv), DCC (1.2 equiv), DMAP (0.2 equiv), DCM, rt, 6 h, **89%**; f) $CaCl_2$ (1.5 equiv), DMAP (0.3 equiv), NEt_3 (1.4 equiv), DCM, rt, 7 h, **99%**; g) 1 M HCl (1.7 equiv), MeOH, rt, 20 min, **quant.**; h) TBAF (1.5 equiv), THF, 0 °C, 2 h; i) 1 M HCl (1.7 equiv), MeOH, rt, 1 h, **quant.**; j) $CaCl_2$ (1.5 equiv), DMAP (0.3 equiv), NEt_3 (1.4 equiv), DCM, rt, 8 h, **quant**.

Notably, the successful synthesized tetramic acid **259** represents the largest part of the natural product vancoresmycin without a chiral center. Similar to the other 3-acyl tetramic acids, tautomerism was observed if CD_2Cl_2 was used as solvent in NMR measurements but this time NMR studies using deuterated DMSO or MeCN evidenced instability of the compound in these solvents. Additionally, it was observed that a batch of **259** decomposed when stored over weeks even at low temperatures (−20 °C) under argon.

Protection and trapping of the possible tetramic acid tautomers was also examined. First, a TBS protection of tetramic acid **241** (**Scheme 5.3.2-9**) using different conditions (**Table 5.3.2-1**) was investigated.

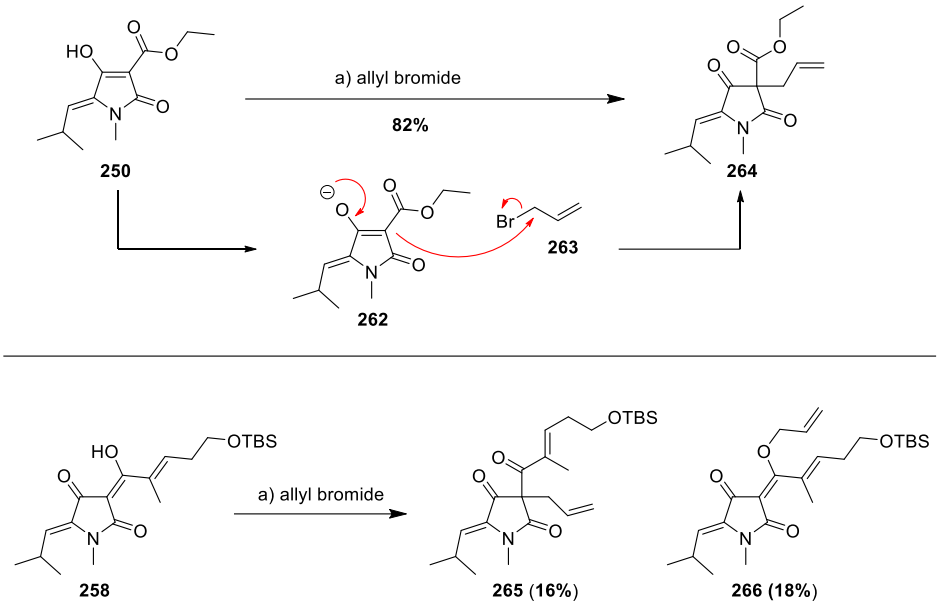


***Scheme 5.3.2-9*:** Attempted but unsuccessful synthesis of silyl protected **261**.

Table 5.3.2-1: Screened but unsuccessful conditions for the TBS protection of **241**.

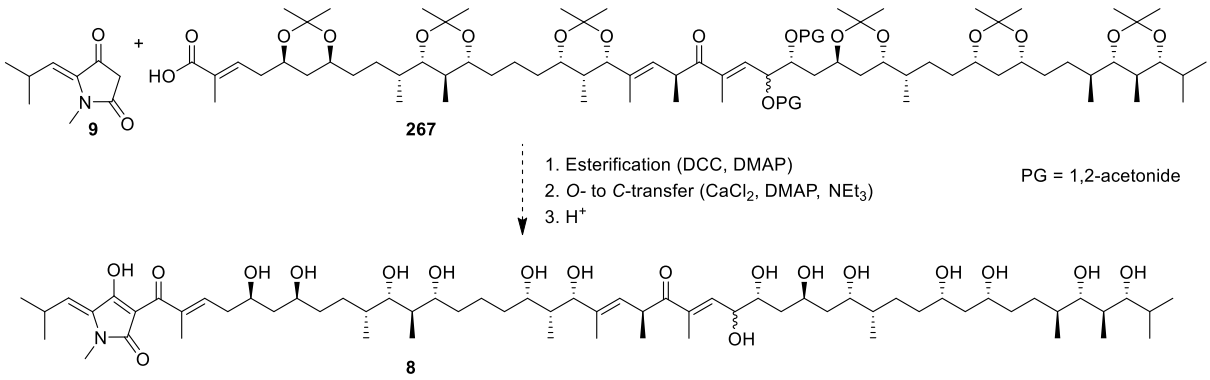
Entry	Conditions
1	TBSCl (1.2 equiv), imidazole (2.0 equiv), DCM, rt, on
2	TBSCl (1.1 equiv), imidazole (1.2 equiv), MeCN, rt, 3 h
3	TBSOTf (1.5 equiv), 2,6-lutidine (2.0 equiv), DMF, rt, 3 h
4	TBSOTf (1.2 equiv), NEt_3 (1.6 equiv), DCM, −78 °C to rt, on

Unfortunately, none of the listed conditions led to the isolation of a silyl protected tetramic acid tautomer. Therefore, protection of compound **250** (**Scheme 5.3.2-10**) was evaluated as this representative is characterized by a lower degree of tautomerization, which became apparent in NMR measurements where only traces of a second data set appeared. This indicates a stabilization of the double bond system by the ester group. In addition, an allylic protection was chosen as silyl enol ethers would be expected to be more labile. However, in the initial experiments, the allyl group was attached to the 3-position instead of protecting a hydroxyl and compound **264** was isolated as a racemic mixture, which was proven by HPLC on chiral phase. Future investigations may lead to suitable reaction conditions and may show whether the alkylation is tunable with respect to the oxygen to carbon regioselectivity. By applying the same reaction conditions but changing the substrate to **258**, which contains a longer part of the vancoresmycin chain, it was possible to isolate one of the protected tautomers **266** in addition to the racemic *C*-alkylated compound **265**. The shift towards the *O*-alkylation is probably attributed to the additional steric hindrance of the substrate **258** but electronic properties like the additional conjugated double bound and tautomerism may also play a role in the *O*- vs. *C*-selectivity.



***Scheme 5.3.2-10*:** Top: Allyl attachment to yield **264** and proposed mechanism. Bottom: Allyl attachment to yield **265** and **266**. Reaction conditions: a) Allyl bromide (1.3 equiv), K_2CO_3 (1.5 equiv), DMF, 45-55 °C, 5-6 h, for yields see scheme.

Combining all the lessons learned from the synthesis of vancoresmycin-type tetramic acids together with the known extended tautomerism and chelation properties of these compounds, it is believed that the best strategy for a total synthesis of vancoresmycin aglycone **8** is to introduce the tetramic acid framework at a late stage (**Scheme 5.3.2-11**).

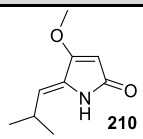
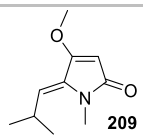
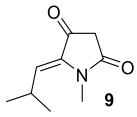
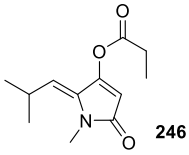
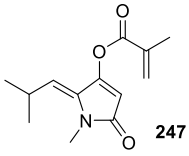
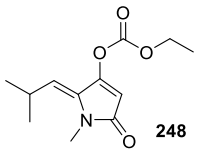
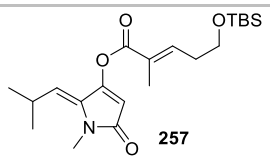


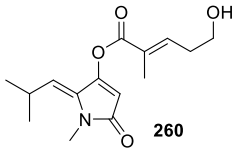
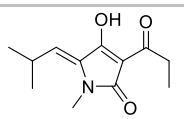
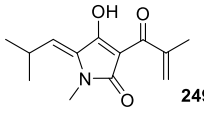
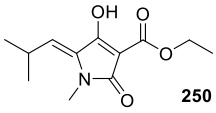
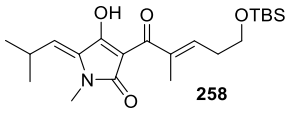
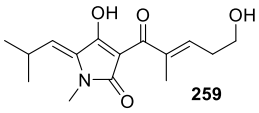
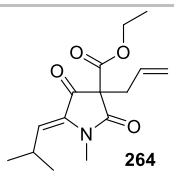
***Scheme 5.3.2-11*:** Planned final steps to yield deglycosylated vancoresmycin **8**.

A plethora of new tetramic acids and especially vancoresmycin type tetramic acids have been synthesized in this work. As previously mentioned in **Chapter 5.1.3**, tetramic acids showed a variety of biological activities.[137–139] With the known antibiotic potency of vancoresmycin, it

is very interesting to know whether the tetramic acid moiety is a part of the pharmacophore of this complex natural product. A cooperation with the group of Prof. Dr. *Tanja Schneider* was started and 14 vancoresmycin type tetramic acids have already been tested for their antibiotic properties. For these compounds, the minimal inhibitory concentration (MIC) values against gram-positive *S. aureus* SG511 and gram-negative *E. coli* K12 were determined (**Table 5.3.2-2**).

Table 5.3.2-2: MIC values for the tested tetramic acids.

Compound	**MIC [µg/mL]** ***E. coli* K12**	**MIC [µg/mL]** ***S. aureus* SG511**
 **210**	> 128	> 128
 **209**	> 128	> 128
 **9**	> 128	128
 **246**	> 128	128
 **247**	> 128	128
 **248**	> 128	> 128
 **257**	> 128	> 128

 **260**	> 128	128
 **241**	> 128	64
 **249**	> 128	32
 **250**	> 128	> 128
 **258**	> 128	> 128
 **259**	> 128	64
 **264**	> 128	> 128

Some of the synthesized tetramic acids showed inhibitory effects against *S. aureus*. Compounds **241**, **249** and **259** were the most potent and these candidates also have the highest structural similarities compared to vancoresmycin (**7**). Although tetramic acid **259** is the longest and most authentic compound, it was less potent (MIC: 64 µg/mL) as tetramic acid **249** (MIC: 32 µg/mL). An explanation for this discrepancy may be found in the lower degree of stability for tetramic acid **259** (already described in this chapter). For gram-negative *E. coli*, no activities were observed, which is in agreement with the reported selective mode of action for vancoresmycin.[132]

Furthermore, the group of Prof. Dr. *Tanja Schneider* screened the compounds in a *lial-lux* bioreporter assay to see if they interact with the bacterial cell wall machinery. This assay shows luminescence in case of an antibiotic that interferes with the lipid II biosynthesis cycle and thus

causes cell wall stress. As shown in **Figure 5.3.2-1** and in line with the MIC values, the compounds **241**, **249** and **259** induced the *Bacillus subtilis lial-lux* bioreporter, resulting in measurable luminescence, which reveals interference with the cell wall biosynthesis and thus substantiates an antibiotic activity.

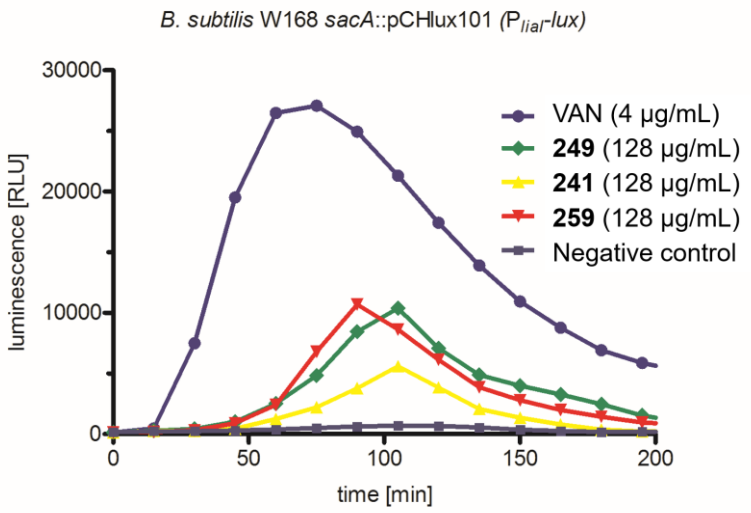


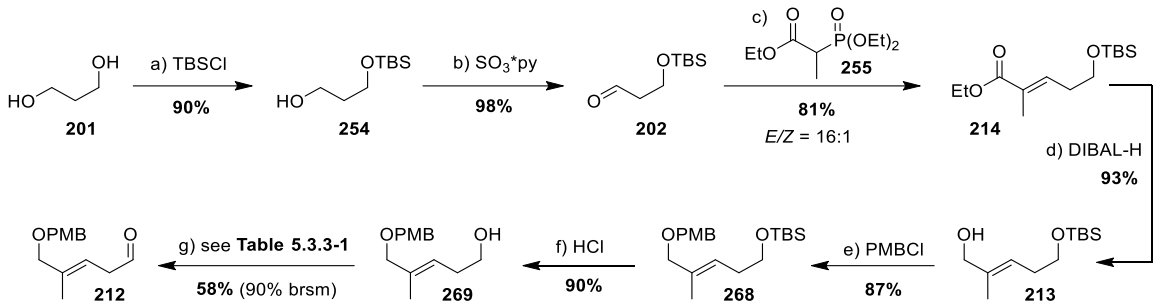
***Figure 5.3.2-1*:** Results of the *lial-lux* bioreporter assay. VAN = Vancomycin. In the negative control, no antibiotic compound was added to the assay. The other tetramic acids found in **Table 5.3.2-2** did not induce the assay and are therefore not shown.

These activities suggest that a first part of the vancoresmycin pharmacophore was found and synthesized.[133]

5.3.3 Synthesis of Fragment B

For the synthesis of fragment B (**10**), aldehyde **212** (**Scheme 5.3.3-1**) was targeted, which was synthesized out of commercially available 1,3-propanediol (**201**) with an overall yield of 30% over seven steps. The sequence from 1,3-propanediol (**201**) to ester **214** was already established for a tetramic acid derivative. Consequently, ester **214** found a double application and its synthesis is further described in **Chapter 5.3.2**.[190] DIBAL-H mediated reduction of ester **214**, followed by PMB protection of the resulting free hydroxyl group and cleavage of the TBS protecting group gave unproblematic access to alcohol **269**. For the planned Ipc-mediated aldol

reaction, the respective aldehyde **212** was always prepared freshly and stored over molecular sieves for one night.



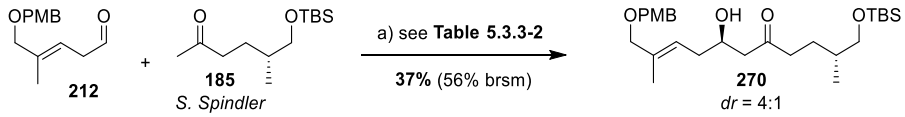
***Scheme 5.3.3-1*:** Synthesis of aldehyde **212**. Reaction conditions: a) Imidazole (1.1 equiv), TBSCl (1.0 equiv), THF, 0 °C, 30 min, **90%**; b) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1.5 h, **98%**; c) $Ba(OH)_2$ (3.5 equiv), **255** (1.4 equiv), THF, rt, on, **81%**; d) DIBAL-H (2.2 equiv), THF, −78 °C, 1 h, **93%**; e) PMBCl (1.1 equiv), NaH (1.1 equiv), DMF, 0 °C, 3 h, **87%**; f) 1 M HCl (1.7 equiv), MeOH, 0 °C, 1 h, **90%**; g) see **Table 5.3.3-1**.

Table 5.3.3-1: Screened conditions for oxidation of **269**.

Entry	Conditions	Yield [%]
1	SO_3*py (3.0 equiv), DIPEA (4.0 equiv), DMSO (10 equiv), DCM, rt, 1.5 h	32
2	IBX (1.5 equiv), DMSO, rt, 1 h	58 (90 brsm)

Two different conditions were investigated (**Table 5.3.3-1**) for the oxidation to aldehyde **212**. A *Parikh-Doering* oxidation (entry 1) was performed in the first approach, which had already worked perfectly for the synthesis of aldehyde **202**. Utilization of alcohol **269** as substrate gave only 32% product. An IBX oxidation proceeded with a moderately higher yield of 58% (90% brsm, entry 2).

In the next step, aldol reactions were investigated (**Scheme 5.3.3-2**). The required ketone **185** was provided by *Stefanie Spindler*, who was able to synthesize this compound under the use of a literature known, organocatalyzed and enantioselective *Michael*-addition as key step.[165–167]

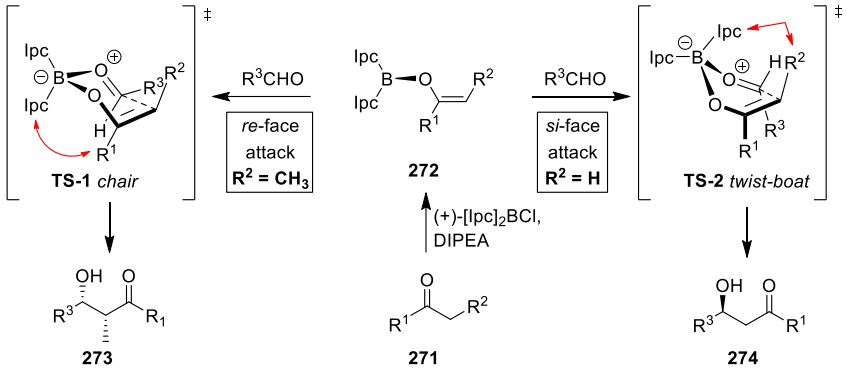


***Scheme 5.3.3-2*:** Aldol reaction to yield **270**. For reaction conditions see **Table 5.3.3-2**.

Table 5.3.3-2: Screened conditions for the aldol reaction using **212** and **185**.

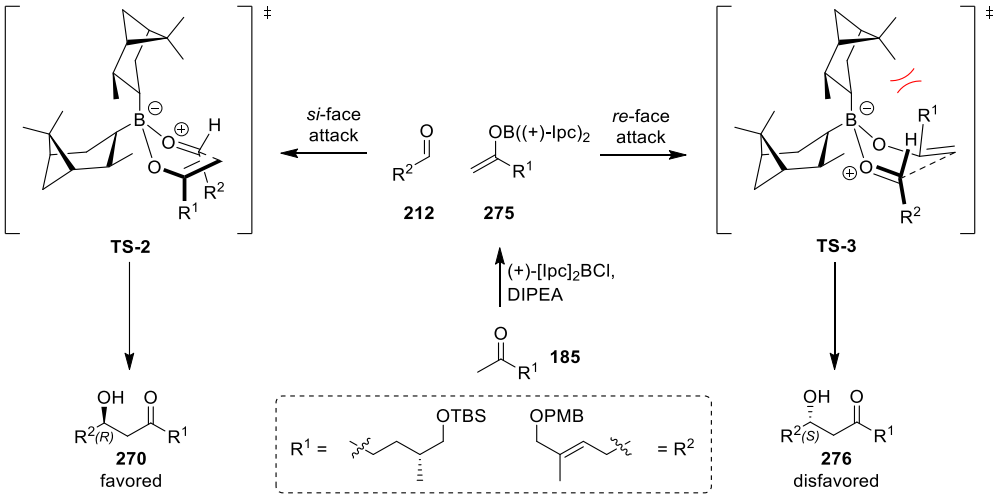
Entry	Conditions	Yield [%]	*dr*
1	(+)-$[Ipc]_2BCl$ (1.1 equiv), aldehyde **212** (1.2 equiv), DIPEA (2.0 equiv), DCM, −78 °C to −15 °C	37 (56 brsm)	4:1
2	(+)-$[Ipc]_2BOTf$ (1.1 equiv), aldehyde **212** (1.3 equiv), DIPEA (2.0 equiv), DCM, −78 °C to −15 °C	13 (27 brsm)	2.5:1

With aldehyde **212** and ketone **185**, a *Paterson* aldol addition for methyl ketones was performed under the use of commercially available (+)-$[Ipc]_2BCl$. Due to the lack of stereogenic control from the substrate, this chiral boron reagent was chosen to introduce the asymmetric information. Here, the (+)-Ipc-ligand yielded the desired (*R*)-configured β-hydroxyketone **270** as a major product. First, enolization of ketone **185** was ensured by DIPEA and (+)-$[Ipc]_2BCl$ to generate the respective enol borinate. Due to the methyl ketone, an *E* vs. *Z* selectivity of the enolization had not to be taken into account. The stereogenic outcome of the reaction can be understood by looking at the transition states (**Scheme 5.3.3-3**). In contrast to ethyl ketones, the usual *Zimmermann-Traxler* transition state (**TS-1**) is not formed. Instead, it is assumed that a twist-boat arrangement (**TS-2**) is favored for the aldol addition using a methyl ketone.[149,201] This leads to a different enantiofacial selectivity in the reaction using a methyl or an ethyl ketone (**Scheme 5.3.3-3**). In a methyl ketone derived enolate (R^2 = H), the twist-boat type transition state **TS-2** is favored, as it avoids steric interactions between R^1 and a bulky Ipc-moiety in the chair structure **TS-1**. However, in an aldol reaction using an ethyl ketone ($R^2 = CH_3$), the chair transition state **TS-1** is favored due to the avoidance of the more serious interaction between R^2 and an Ipc group, which would occur in the twist-boat structure **TS-2**.[149,201]



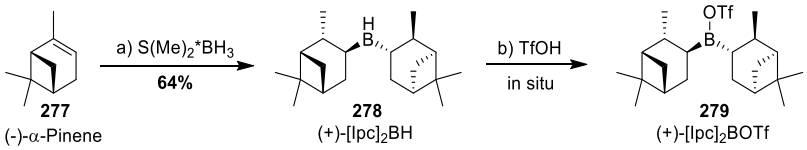
Scheme 5.3.3-3*: *Paterson aldol addition for an ethyl ketone ($R^2 = CH_3$) and a methyl ketone ($R^2 = H$). Different residues for R^1 and R^3 have been tested by the group of *Paterson*. Steric interactions in the transition states are shown in red.[149,201]

Scheme 5.3.3-4 displays the two predominant twist-boat transition states **TS-2** and **TS-3** for the performed aldol addition. In general, two further transition states are conceivable, in which R^2 points upwards but this orientation of the aldehyde will be of much higher energy due to steric interaction between R^2 and an Ipc-ligand. From the two predominant arrangements transition state **TS-3** is less favored due to steric interactions between R^1 and an Ipc-moiety. For this reason, the reaction proceeded preferably via transition state **TS-2** in which the aldehyde was attacked from the *si*-face and the (*R*)-configured β-hydroxyketone **270** was yielded as a major product (*dr* = 4:1).



***Scheme 5.3.3-4*: Mechanism** of the performed Ipc-mediated aldol addition.

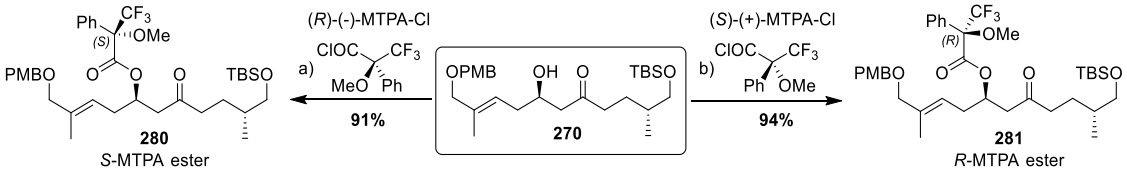
To optimize the aldol addition in terms of yield and diastereoselectivity, fresh (+)-[Ipc]$_2$BOTf (**279**) was prepared by hydroboration of (-)-α-pinene (**277**) to yield (+)-[Ipc]$_2$BH (**278**). Compound **278** was then stored in the glove box and the respective (+)-[Ipc]$_2$BOTf (**279**) was prepared in situ directly before usage.[149,202]



***Scheme 5.3.3-5*:** Synthesis of (+)-[Ipc]$_2$BOTf (**279**). Reaction conditions: a) Borane-dimethyl sulfide (0.5 equiv), THF, 0-4 °C, 46 h, **64%**; b) TfOH (1.0 equiv), hexane, rt, 2 h.

Compared to commercially purchased (+)-[Ipc]$_2$BCl, freshly prepared (+)-[Ipc]$_2$BOTf (**279**) showed no improvement in the aldol addition (see **Table 5.3.3-2**).Thus, usage of the former is superior and also more convenient.

To determine the absolute configuration of the new secondary alcohol, the *Mosher* ester analysis was implemented (**Scheme 5.3.3-6**). β-Hydroxyketone **270** was treated once with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride ((*R*)-(−)-MTPA-Cl) to yield (*S*)-ester **280** and once with (*S*)-(+)-MTPA-Cl to give (*R*)-ester **281**.



***Scheme 5.3.3-6*:** Synthesis of *S*-MTPA ester **280** and *R*-MTPA ester **281**. Reaction conditions: a) (−)-(*R*)-MTPA-Cl (6.0 equiv), pyridine, rt, 2 h, **91%**; b) (+)-(*S*)-MTPA-Cl (6.0 equiv), pyridine, rt, 2 h, **94%**.

Using both diastereomeric esters **280** and **281** and the method developed by *Mosher* and coworkers, it was possible to deduce the absolute configuration at the methine carbon.[203–205] It is assumed that the conformation of MTPA-esters, which dominates the spectroscopic features, is the one in which the ester adopts the usual *s-trans* arrangement, leading to a *syn*-coplanar order of the trifluoromethyl (CF_3), the methine proton of the secondary alcohol and the carbonyl group (**Figure 5.3.3-1**). Aryl groups (like the phenyl residue in the MTPA esters)

are known for their anisotropic effect. This leads to a magnetic shielding and causes an upfield chemical shift in the NMR spectrum for protons residing above or below the plane of the ring.[203–205]

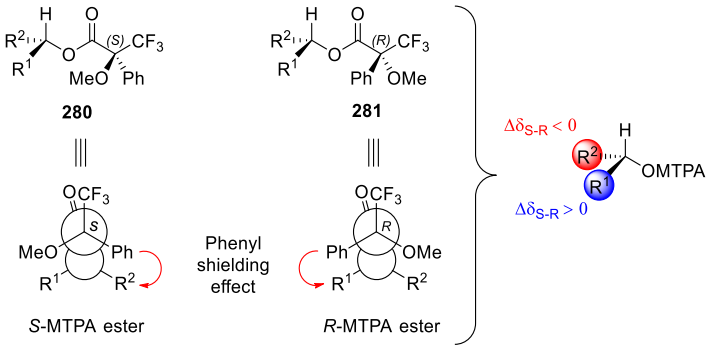
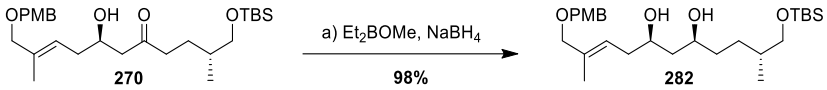


***Figure 5.3.3-1*:** Determination of the absolute configuration using *Mosher* ester analysis.

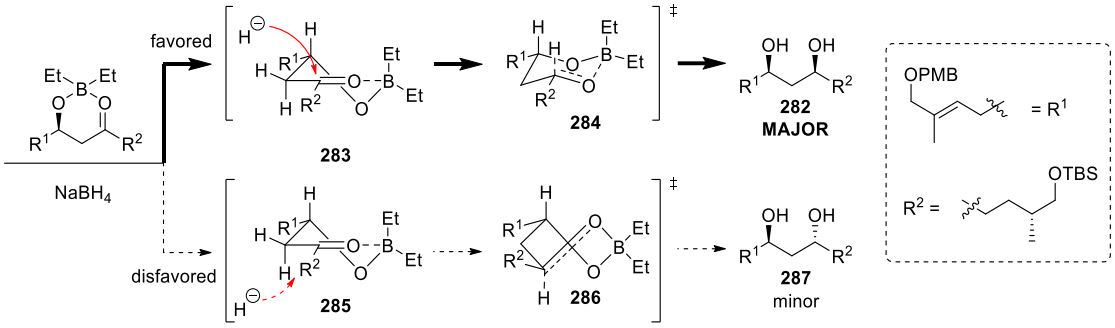
In the *S-Mosher* ester **280** the R^2 group and in the *R-Mosher* ester **281** the R^1 group is shielded. Following the procedure, the differences in chemical shifts between the *S*- and *R-Mosher* ester ($\Delta\delta_{S\text{-}R}$) have to be calculated for the same protons in the NMR spectrum. Every proton resulting in negative $\Delta\delta_{S\text{-}R}$-values should reside at R^2 (**Figure 5.3.3-1**, red) and should therefore be behind the paper plane. Vice versa all protons resulting in positive $\Delta\delta_{S\text{-}R}$-values should be addressed to R^1 (**Figure 5.3.3-1**, blue) pointing towards the reader.[203–205]
Using this method, the new stereogenic center of **270** was determined as *R*-configured. A detailed evaluation including a table containing significant chemical shifts is shown in the experimental section **6.3.2**. Applying the same procedure, the stereogenic secondary alcohol of the minor diastereomer, being the *S*-configured β-hydroxyketone **276**, was also confirmed. Furthermore, the integral ratio of the two diastereomeric *Mosher* esters allowed determination of the diastereomeric ratio, which resulted from the aldol addition (*dr* = 4:1, see experimental section **6.3.2**).

After the successful synthesis of β-hydroxyketone **270**, a *Narasaka-Prasad*-reduction was performed to give *syn*-1,3-diol **282** with an excellent yield of 98% (**Scheme 5.3.3-7**). This reaction is highly stereoselective and a respective *anti*-1,3-diol was not obtained.



***Scheme 5.3.3-7*:** Performed *Narasaka-Prasad*-reduction. Reaction conditions: a) Et_2BOMe (1.1 equiv), $NaBH_4$ (1.1 equiv), THF/MeOH, −78 °C, 4 h, then $NaOH/H_2O_2$, 0 °C, 3 h, **98%**.

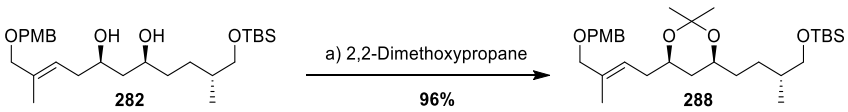
The high stereoselectivity is based on the chelation of the β-hydroxyketone **270** with the dialkylborane species forming a cyclic, six membered transition state in half chair conformation in which both of the substituents R^1 and R^2 occupy an equatorial position (**Scheme 5.3.3-8**).[150–152]



***Scheme 5.3.3-8*:** Mechanism of the *Narasaka-Prasad* reduction.

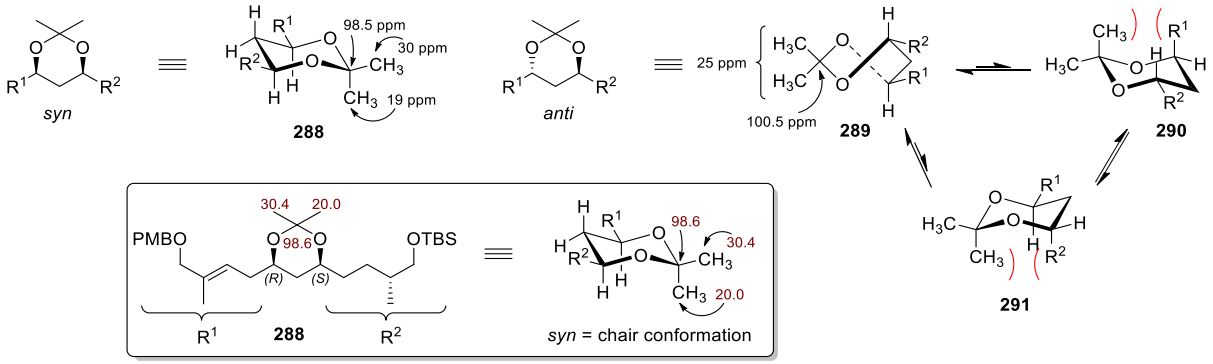
After formation of transition state **283**, the hydride favorably attacks the carbonyl group from the less hindered top face following the *Bürgi-Dunitz* trajectory, leading to *syn*-diol **282** as major product. In addition, a hydride attack from the bottom would lead to a twist-boat like transition state **286**, which is of higher energy compared to the chair like transition state **284**.[150–152]

In order to protect 1,3-diol **282** and to simultaneously confirm the *syn*-configuration, acetonide **288** was synthesized (**Scheme 5.3.3-9**).



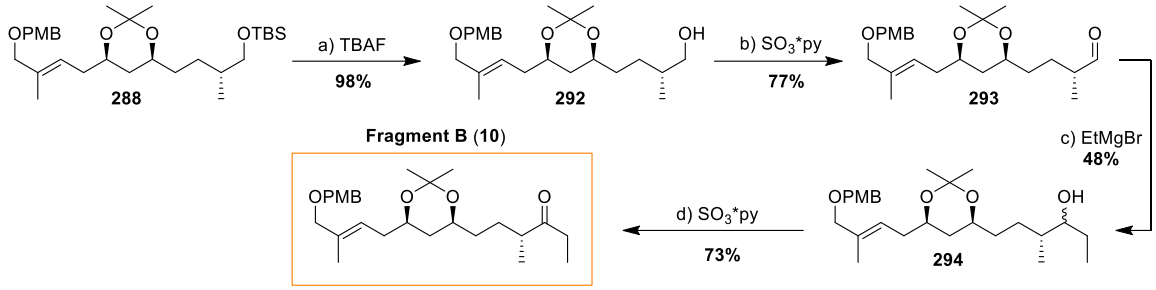
***Scheme 5.3.3-9*:** Performed acetonide protection. Reaction conditions: a) CSA (0.01 equiv), 2,2-dimethoxypropane, rt, 1.5 h, **96%**.

Syn-acetonides occupy a chair conformation in which the methyl groups of the acetonide are present in an axial and equatorial position **288** (**Scheme 5.3.3-10**). In contrast, *anti*-acetonides are preferentially present in a twist-boat conformation, resulting from 1,3-diaxial interactions in chair conformations (**290** and **291**). *Syn*- and *anti*-acetonides differ from each other in the chemical shifts of the ketal group in their ^{13}C-NMR-spectra. Ketal carbons in *syn*-acetonides show a signal closely to 98.5 ppm and the two methyl groups appear in signals at 19 ppm (axial) and 30 ppm (equatorial). *Anti*-acetonides show signals for the two methyl groups at 25 ppm and a signal for the ketal carbon at 100.5 ppm. Thus, acetonide formation makes it possible to distinguish between *syn*- and *anti*-configurations. In the case of the synthesized acetonide **288**, the assumed *syn* configuration was confirmed by the respective ^{13}C-NMR signals (see **Scheme 5.3.3-10**).[155–158]



***Scheme 5.3.3-10*:** Differentiation of 1,3-*syn*- and 1,3-*anti*-diols using the acetonide method. Box: Measured ketal signals for **288**, which confirmed the *syn*-configuration.

Four remaining steps had to be performed for the completion of fragment B (**10**, **Scheme 5.3.3-11**).

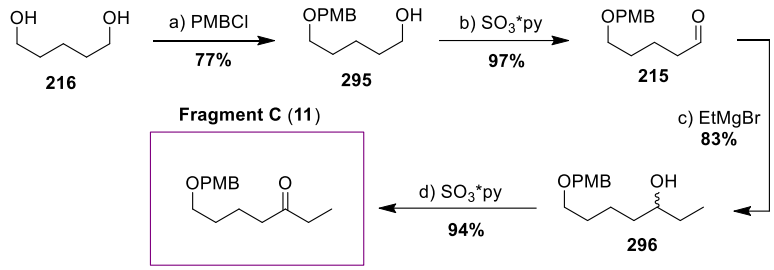


***Scheme 5.3.3-11*:** Final steps for the completion of fragment B (**10**). Reaction conditions: a) TBAF (1.5 equiv), THF, rt, 3 h, **98%**; b) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, 0 °C, 20 min, **77%**; c) EtMgBr (1.2 equiv), THF, rt, 3 h, **48%**; d) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, 0 °C, 20 min, **73%**.

First, the TBS protecting group of acetonide **288** was cleaved to give alcohol **292** with 98% yield, followed by *Parikh-Doering*-oxidation and subsequent chain elongation with a *Grignard*-reaction. The new set stereogenic center from the *Grignard*-reaction has been more or less ignored, due to the following oxidation to the respective ketone **10** by *Parikh-Doering*-conditions. In the last three steps, the yields, especially for the *Grignard*-reaction (48%), could be improved, which are probably lower due to the performed scale in a lower milligram range. In summary, fragment B (**10**) was synthesized in 14 linear steps from commercially available 1,3-propane diol (**201**) with an overall yield of 2.8%. Reproducibility has already been confirmed by re-synthesis, which was carried out by *Max Schönenbroicher*.[206] With the ketone function at the right side of the molecule **10**, it should be possible to perform aldol reactions and after deprotection of the PMB group and oxidation to the carboxylic acid at the left side, a connection with the tetramic acid moiety **9** could be achieved.

5.3.4 Synthesis of Fragment C

The rather small fragment C (**11**) was synthesized from 1,5-pentanediol (**216**) over four linear steps with an overall yield of 58% (**Scheme 5.3.4-1**).

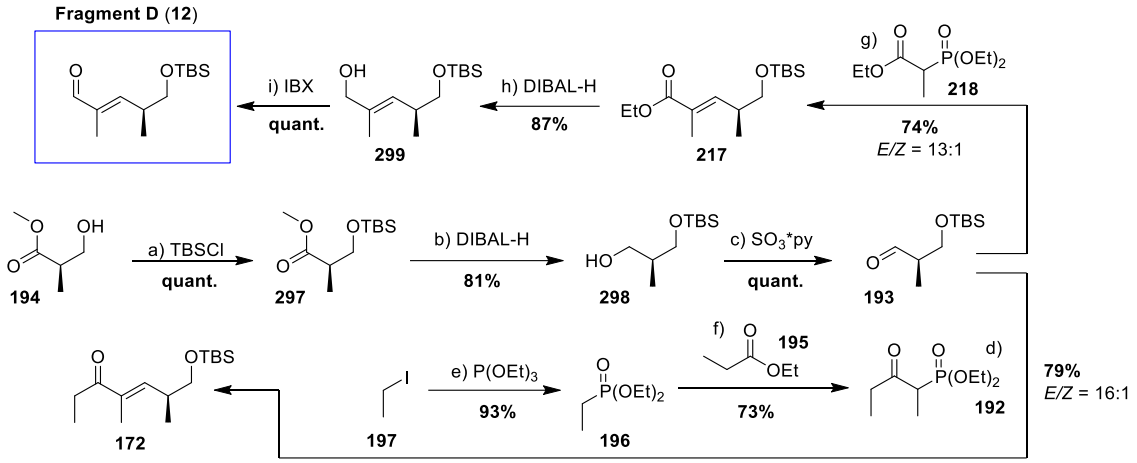


***Scheme 5.3.4-1*:** Synthesis of fragment C (**11**). Reaction conditions: a) NaH (1.2 equiv), PMBCl (0.5 equiv), DMF, rt, 2 h, **77%**; b) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1 h, **97%**; c) EtMgBr (1.2 equiv), THF, 0 °C, 30 min, **83%**; d) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1 h, **94%**.

Starting from diol **216**, mono PMB protection yielded PMB ether **295** with 77% yield. Despite the adjustment of the protective reagent equivalents, 11% of the double protected alcohol was isolated as side product.[191] The residual alcohol function was oxidized using the *Parikh-Doering*-reaction. Resulting aldehyde **215** was gained in an excellent yield of 97% and first aldol couplings with this aldehyde and fragment D (**172**) were tried but failed (see **Chapter 5.3.6**). This dissatisfaction caused the reconstruction of the fragments so that fragment C should contain the ketone (**11**) and fragment D the aldehyde functionality (**12**), which will then be coupled in a vice versa aldol reaction. Therefore, an uneventfully *Grignard*-reaction and subsequent *Parikh-Doering*-oxidation were carried out to gain fragment C (**11**) with the ketone functionality.

5.3.5 Synthesis of Fragment D

For the synthesis of fragment D (**12**), with the desired stereogenic center at the methyl group, (*R*)-*Roche* ester (**194**) was used as starting material (**Scheme 5.3.5-1**). TBS protection of the free hydroxyl group, DIBAL-H mediated reduction of the ester and reoxidation of the alcohol yielded aldehyde **193** with 81% yield over three steps.[169,170] Oxidation of alcohol **298** in the last step was also achieved using *Dess-Martin* periodinane (DMP) as oxidant, which gave a comparable yield of 96% (see experimental section **6.3.4** for details). It is worth mentioning that DIBAL-H reductions can be stopped at the aldehyde stage, but as these are often unstable, the respective alcohols have been stored and freshly oxidized when used.[207,208]



***Scheme 5.3.5-1*:** Synthesis of fragment D (**12**). Reaction conditions: a) Imidazole (2.0 equiv), TBSCl (1.2 equiv), DCM, rt, 4 h, **quant.**; b) DIBAL-H (3.0 equiv), THF, −78 °C to rt, 2.5 h, **81%**; c) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1 h, **quant.**; d) $Ba(OH)_2$ (3.5 equiv), **192** (1.4 equiv), THF/water, rt, on, **79%**; e) $P(OEt)_3$ (1.0 equiv), 115 °C, 5 h, **93%**; f) *n*-BuLi (1.2 equiv), **195** (1.3 equiv), THF, −78 °C, 2 h, **73%**; g) $Ba(OH)_2$ (3.5 equiv), **218** (1.4 equiv), THF/water, rt, on, **74%**; h) DIBAL-H (2.5 equiv), THF, −78 °C, 1 h, **87%**; i) IBX (2.0 equiv), DMSO, rt, 1.5 h, **quant.**

Initially, aldehyde **193** was used in a HWE reaction with phosphonate **192**, readily available from ethyl iodide (**197**) using a *Michael-Arbuzov* reaction and subsequent modification of the phosphonate **196** with *n*-BuLi and ethyl propionate (**195**).[171,172] For the HWE reaction, two different conditions were investigated (**Table 5.3.5-1**).

Table 5.3.5-1: Screened conditions for the HWE reaction to yield **172**.

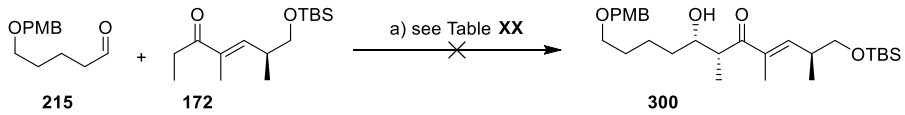
Entry	Conditions	Yield [%]	*E/Z*
1	*n*-BuLi (1.4 equiv), DMPU (1.5 equiv), phosphonate **192** (1.5 equiv), THF, 0 °C, 4 h	68	2:1
2	$Ba(OH)_2$ (3.5 equiv), phosphonate **192** (1.4 equiv), THF/water, rt, on	79	16:1

Utilization of *n*-BuLi and DMPU gave the desired product **172** with a fair yield, but with bad *E/Z* selectivity (entry 1). Thus, a second approach was examined using $Ba(OH)_2$ as base and stirring overnight at room temperature, which was convenient due to the freshly prepared aldehyde at the same day (entry 2). With 79% yield and 16:1 *E/Z* selectivity, this method was more advantageous. Synthesized enone **172** turned out to be unsuitable for the planned aldol coupling and fragment D had to be reconsidered (see **Chapter 6.3.5**). Therefore, aldehyde **193**

was submitted to a HWE reaction under the use of a different phosphonate **218**, which was commercially acquired.[192] Once again, the olefination procedure using $Ba(OH)_2$ provided a good yield (74%) and a high *E/Z* selectivity of 13:1. Determination of the *E* and *Z* configurations for enone **172** as well as ester **217** was done with NOESY NMR measurements and can be found in the experimental section. The gained α,β-unsaturated ester **217** was reduced to the alcohol **299** using DIBAL-H and the alcohol function was re-oxidized by IBX to the respective aldehyde **12** with 87% yield starting from ester **217** (two steps).[192] In summary, fragment D (**12**) has been synthesized in six steps and in an overall yield of 52% before it was used in aldol reactions with fragment C (**11**).

5.3.6 Coupling of Fragment C and D

Syn-aldol coupling of fragment C and D was first investigated by utilization of fragment C (**215**) as aldehyde electrophile and fragment D (**172**) as enolate (**Scheme 5.3.6-1**). Different reaction conditions have been evaluated, but the desired product **300** was not obtained (**Table 5.3.6-1**). Most of the starting material **172** could be reisolated after the performed approaches.



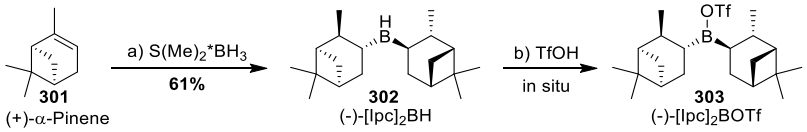
***Scheme 5.3.6-1*:** Failed aldol coupling between **215** and **172**. For screened reaction conditions see **Table 5.3.6-1**.

Table 5.3.6-1: Screened but unsuccessful conditions for the aldol addition of **215** and **172**.

Entry	Conditions
1	(+)-$[Ipc]_2BCl$ → (+)-$[Ipc]_2BOTf$ (1.1 equiv), DIPEA (2.1 equiv), aldehyde **215** (1.4 equiv), DCM, −78 °C to −15 °C, overnight
2	(+)-$[Ipc]_2BH$ → (+)-$[Ipc]_2BOTf$ (1.1 equiv), DIPEA (2.1 equiv), aldehyde **215** (1.4 equiv), DCM, −78 °C to −15 °C, overnight
3	(+)-$[Ipc]_2BCl$ (1.1 equiv), DIPEA (2.1 equiv), aldehyde **215** (1.4 equiv), Et_2O, −78 °C to −15 °C, overnight

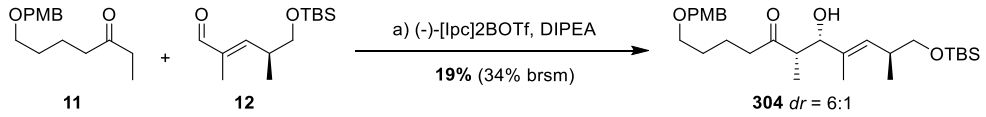
4	(+)-$[Ipc]_2BCl$ (1.1 equiv), KHMDS (1.3 equiv), aldehyde **215** (1.4 equiv), Et_2O, −78 °C, overnight

First, it was tried to transform commercially acquired (+)-$[Ipc]_2BCl$ to (+)-$[Ipc]_2BOTf$ in situ as the triflate was described to give good *syn:anti* ratios for aldol adducts (entry 1).[149] As this procedure failed, (+)-$[Ipc]_2BOTf$, which was freshly prepared from (+)-$[Ipc]_2BH$ (see **Chapter 5.3.3**), was used (entry 2). Again, the formation of desired product **300** could not be detected. In order to exclude the possibility of failed boron reagent synthesis, the reaction was carried out with (+)-$[Ipc]_2BCl$, as this led to success in the synthesis of fragment B (**10**, **Chapter 5.3.3**). Only the starting materials were recovered and it was suspected that the used base (DIPEA) was too weak to deprotonate the enone **172**. Utilization of KHDMS as stronger base also did not lead to success (entry 4). As the screening of aldol conditions proved to be rather laborious, the approach was reconsidered. It was assumed that the difficulty was related to the enone **172**. To bypass this problem, the aldol reaction was set up in reverse, so that fragment C contains the ketone (**11**) and fragment D the aldehyde functionality (**12**, see **Chapter 5.3.4** and **5.3.5** for fragment synthesis). Due to the inversed system, the boron reagent had to be adjusted. In the latter case (−)-$[Ipc]_2BOTf$ (**303**) was needed. This enantiomeric reagent was synthesized analogously to (+)-$[Ipc]_2BOTf$ (**Chapter 5.3.3**) with (+)-α-pinene (**301**) as starting material (**Scheme 5.3.6-2**).



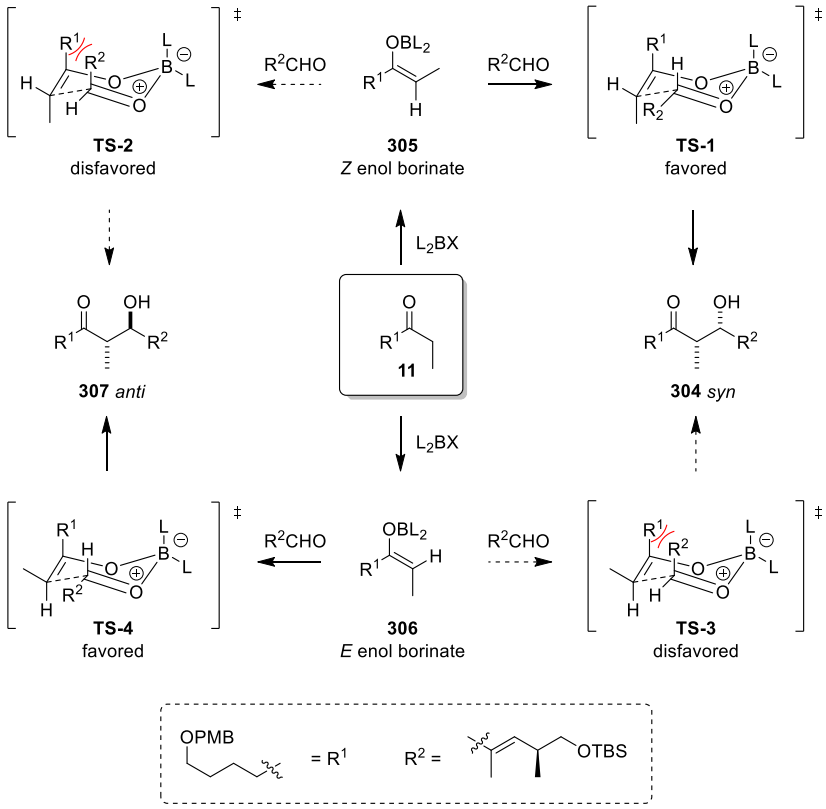
***Scheme 5.3.6-2*:** Synthesis of (−)-$[Ipc]_2BOTf$ (**303**). Reaction conditions: a) Borane-dimethyl sulfide (0.5 equiv), THF, 0-4 °C, 46 h, **64%**; b) TfOH (1.0 equiv), hexane, rt, 2 h.

After the synthesis of the required materials, the stage was set for the aldol coupling (**Scheme 5.3.6-3**).



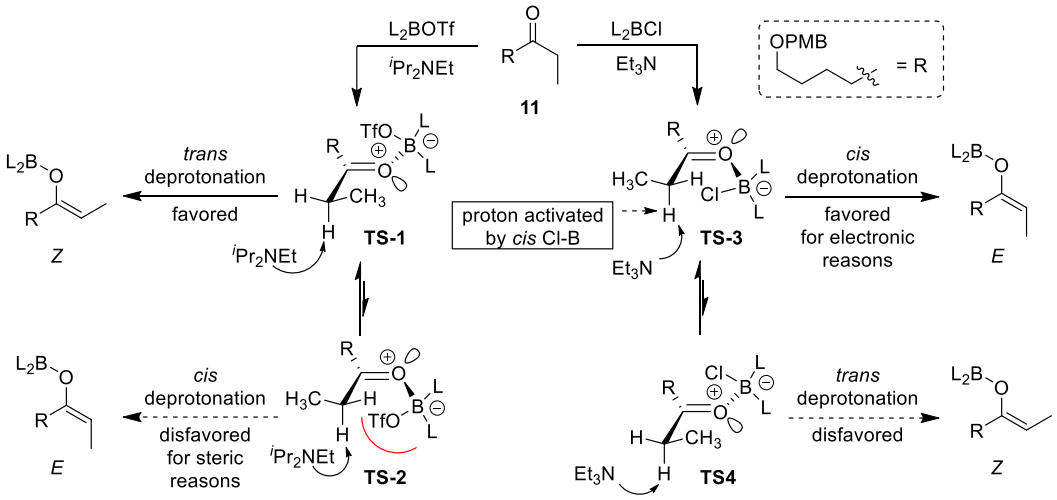
***Scheme 5.3.6-3*:** Synthesis of **304** via an aldol coupling. Reaction conditions: a) (−)-$[Ipc]_2BH$ → (−)-$[Ipc]_2BOTf$ (1.1 equiv), DIPEA (2.1 equiv), aldehyde **12** (1.2 equiv), DCM, −78 °C to −15 °C, on, **19%** (34% brsm).

First attempts using the novel aldol system were already successful and the desired product **304** could be obtained. Due to separation problems of aldol adduct **304** from the Ipc-byproduct, a HPLC separation was performed, which gave the product in 19% (34% brsm) yield. A diastereomeric ratio of 6:1 could be determined directly using the ^1H NMR spectrum of the product (see **Chapter 6.3.5**). The 1,2-*syn*-selectivity is comprehensible by considering the chair like transition states (**Scheme 5.3.6-4**) described by *Zimmerman* and *Traxler*.[209] Thus, the reaction proceeds via the transition state which minimizes the 1,3-pseudoaxial repulsion. For an (*E*)-configured enol **306**, the favored transition state would therefore be **TS-4** resulting in a 1,2-*anti*-configuration (**307**). After formation of a (*Z*)-configured enol borinate **305**, the reaction should prefer transition state **TS-1** furnishing the desired 1,2-*syn*-product **304**. Consequently, the formation of the (*Z*)-enolate is essential and was ensured with the use of (−)-$[Ipc]_2BOTf$ and DIPEA.[149,209,210]



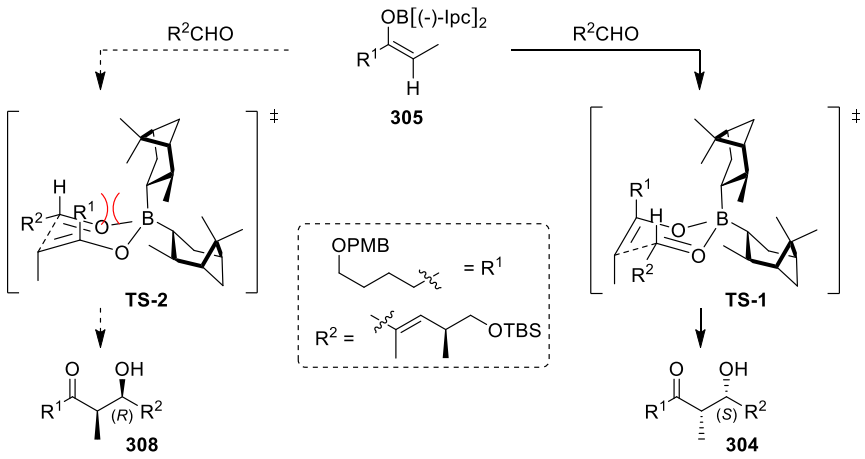
***Scheme 5.3.6-4*:** Relevant transition states in an aldol reaction using a (*Z*)-configured or (*E*)-configured enol borinate, which result from the use of a suitable boron reagent L_2BX (L = alkyl ligand, X = electronegative group) and base. 1,3-Repulsions are shown in red.[149,209,210]

The synthesis of the kinetic enol borinate is influenced by the steric demand of the boron reagent, the tertiary amine base and the substrate structure. *Paterson et al.* described an observed contrasting regio- and stereoselectivity for the use of dialkyl boron chloride and triflate reagents with bulky ligands like Ipc or ^{c}Hex (**Scheme 5.3.6-5**).[148] By a generalized anomeric effect, electron density is shifted from the n_O carbonyl oxygen into the σ^*_{B-X} orbital, causing the *Lewis* acid to adopt a favored *cis*-position. Using L_2BCl, the short distance between the chloride and the α-proton induces a partial negative charge at the α-carbon atom of the carbonyl group and thus, activates the *cis*-side of the transition state **TS-3** for sterically unhindered bases such as NEt_3 to form preferentially the *E*-enol borinate. If L_2BOTf is used, it is supposed that the steric bulk of the triflate group forces the X-B-O=C angle to increase, which reduces the electronic preference for the *Lewis* acid to be *cis* to the less substituted group. Thus, *trans*-deprotonation via transition state **TS-1** is favored due to steric repulsion in transition state **TS-2**. This results in the preferred formation of the *Z*-enol, which was also desired for the performed aldol coupling.[148]



***Scheme 5.3.6-5*:** Enolization of ketone **11** using L_2BOTf and DIPEA or L_2BCl and NEt_3. L = alkyl ligand.[148]

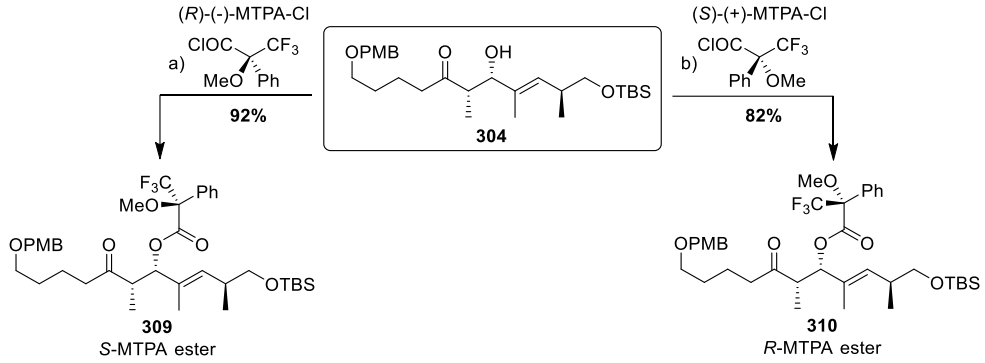
The absolute stereochemistry of the aldol product **304** was influenced by the use of the chiral diisopinocampheyl ligands from (−)-$[Ipc]_2BOTf$. Using chair like transition states, the two pathways shown in **Scheme 5.3.6-6** can be used to make the stereogenic output intelligible.[149,211]



***Scheme 5.3.6-6*:** Transition states of the aldol reaction determining the absolute configuration.[149,211]

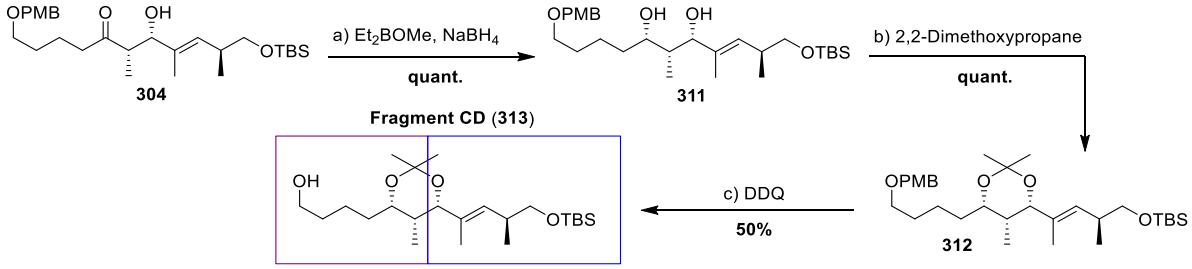
Due to steric interactions of R^1 with the bulky Ipc ligand in transition state **TS-2**, the reaction prefers to proceed via transition state **TS-1** in which the aldehyde is attacked from the *si*-face forming the desired (*S*)-configured hydroxyl group in compound **304**,[149,211] which was confirmed using *Mosher* ester analysis (**Scheme 5.3.6-7**). For a background regarding *Mosher*

ester analysis see **Chapter 5.3.3** and for experimental details as well as a table containing significant chemical shifts see experimental section **6.3.5**.



***Scheme 5.3.6-7*:** Synthesis of *S*-MTPA ester **309** and *R*-MTPA ester **310**. Reaction conditions: a) (−)-(*R*)-MTPA-Cl (6.0 equiv), pyridine, rt, 1 h, **92%**; b) (+)-(*S*)-MTPA-Cl (6.0 equiv), pyridine, rt, 2 h, **82%**.

After confirmation of the stereogenic center of the new hydroxyl group, three final steps have been performed for the synthesis of fragment CD (**313**, **Scheme 5.3.6-8**).



***Scheme 5.3.6-8*:** Final steps towards fragment CD (**313**). Reaction conditions: a) Et_2BOMe (1.1 equiv), $NaBH_4$ (1.1 equiv), THF/MeOH, −78 °C, 4 h, then $NaOH/H_2O_2$, 0 °C, 4 h, **quant.**; b) CSA (0.01 equiv), 2,2-Dimethoxypropane, rt, 3 h, **quant.**; c) DDQ (2.0 equiv.), DCM/pH7 buffer, rt, 1.5 h, **50%**.

By utilization of the *Narasaka-Prasad* reduction, β-hydroxyketone **304** was transferred into the respective 1,3-*syn*-diol **311**. A background, which explains the high stereoselectivity of this reaction, can be found in **Chapter 5.3.3**. The reaction proceeded in quantitative yield and at this stage, a separation of the diastereomeric mixture (*dr* = 6:1), which resulted out of the aldol addition, was possible. In consequence diol **311** was used in pure form for the next steps. Analogously to fragment B (**10**), compound **311** was also protected as acetonide. The protecting group was chosen not only to mask the hydroxyl groups but also to determine the relative configuration of the methyl group and the new hydroxyl group, which is possible due to the

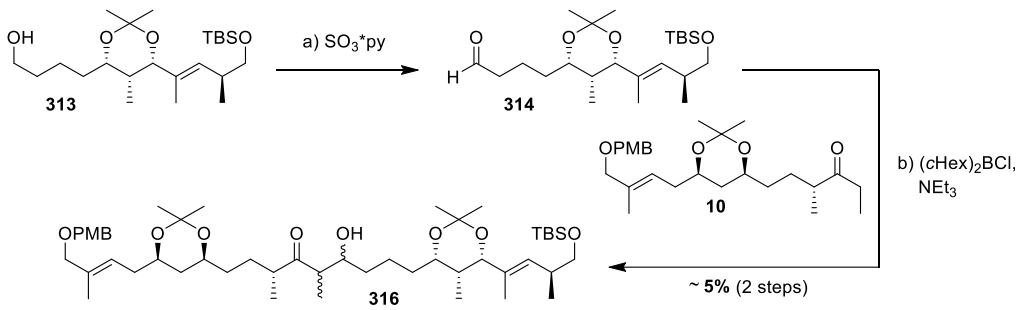
chair conformation of the acetonide. As explained in **Chapter 5.3.3**, the 1,3-*syn*-configuration was confirmed using the ^{13}C-NMR spectrum and additionally, the 1,2-*syn*-configuration could also be confirmed using the respective ^{1}H-NMR signal and NOESY correlations (see experimental section **6.3.5** for details).

Deprotection of the PMB group with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) yielded fragment CD (**313**) with 50% yield. This last step was only performed once in a very low scale which may explains the moderate yield.

In conclusion, fragment CD (**313**) has been synthesized with 5% yield over ten steps as longest linear sequence, starting from (*R*)-*Roche* ester (**194**). Reliability of the developed route was confirmed by *Maximilian Seul*, who was able to reproduce fragment CD (**313**).[212] This fragment was planned to be oxidized freshly to enable an aldol coupling with fragment B (**10**). After a successful fusion of fragment B (**10**) and CD (**313**), as well as required modifications, the TBS group can be cleaved prior to further elongation of the polyketide chain.

5.3.7 Coupling of Fragment B and CD

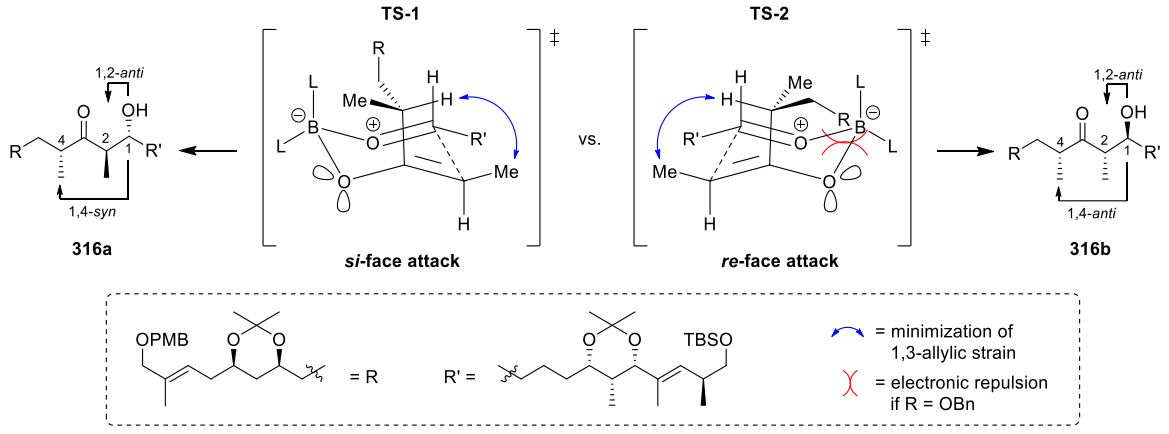
With the support of *Maximilian Seul* and *Max Schönenbroicher*,[206,212] who were able to re-synthesize the compounds **313** and **10**, based on the developed and already performed routes out of this work, it was possible to try an initial coupling of fragment B (**10**) and fragment CD (**313**). Therefore, alcohol **313** was oxidized in a small scale (7 mg) by *Parikh-Doering* conditions and directly used for the *anti*-aldol reaction (**Scheme 5.3.7-1**).[206,212]



***Scheme 5.3.7-1*:** Coupling of fragment B (**10**) and CD (**313**). Reaction conditions: a) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1 h; b) **10** (2.1 equiv), (*c*Hex)$_2$BCl (3.2 equiv), NEt_3 (4.0 equiv), Et_2O, −78 °C, 2 h and −15 °C, on,**~5%** over two steps.

As previously described (see **Chapter 5.3.6**), the usage of (*c*Hex)$_2$BCl and NEt$_3$ should ensure the formation of the *E*-enol borinate out of ketone **10**, which should result in an 1,2-*anti*-configuration of the product. Although the isolation of the target compound was successful and the constitution could be confirmed, an analysis of the selectivity and the absolute configuration was not possible, due to the small performed scale.

Previous studies about *anti*-aldol reactions with chiral, α-methyl substituted ethyl ketones, which were performed by *Paterson et al.*, indicated that the undesired absolute configuration **316b** could probably be preferred (**Scheme 5.3.7-2**).[213–215] The group postulated two chair like transition states (**TS-1** and **TS-2**) for an *anti*-aldol reaction of the given type, in which the 1,3-allyl strain is minimized. Thus, the hydrogen atom from the α-stereogenic center covers the double bond of the enol borinate in both transition states. If the residue (R) features a group, which is linked by an oxygen for example a benzyl ether, the desired 1,2-*anti*-1,4-*syn* pattern is yielded after *si*-face attack, due to additional electronic repulsion in transition state **TS-2**.[213–215] As the used ketone lacked this type of oxygen substitution in the performed aldol reaction, a *re*-face attack might be favored leading to the unwanted 1,2-*anti*-1,4-*anti* pattern but clarity can only be achieved experimentally.



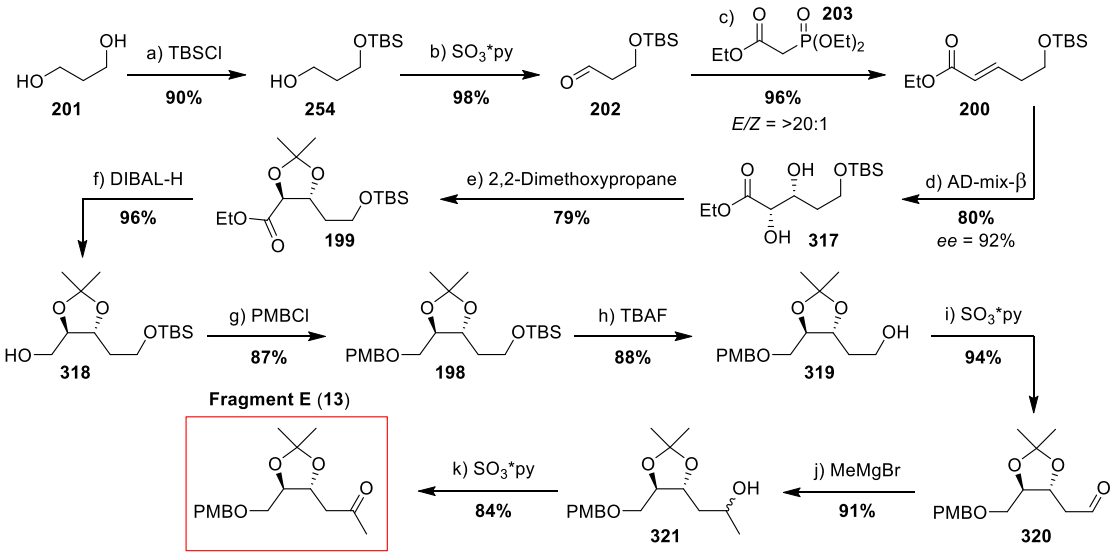
***Scheme 5.3.7-2*:** Postulated chair-like transition states for aldol reactions with α-methyl substituted (*E*)-enolates.[213–215]

In case of bad or unwanted selectivity, the use of chiral boron reagents like [Ipc]$_2$BCl together with NEt$_3$ will be investigated in future approaches. If the desirable stereogenic outcome cannot be achieved, it is also easily possible to invert the aldol coupling. In the latter case the α-stereogenic center would reside at the respective aldehyde. This aldehyde **293** (see **Chapter 5.3.3**)

has already been synthesized as a precursor for ketone **10** and only the aldehyde of fragment CD (**314**) has to be converted to the corresponding ketone, which should be simply possible by an ethyl *Grignard* reaction and oxidation. Subsequently, the aldol coupling can be carried out in a vice versa manner. For a screening of the most suitable coupling procedure, the use of a test system is recommended so that the valuable fragments will not be wasted.

5.3.8 Synthesis of Fragment E

The synthesis of fragment E (**13**) started with a literature described sequence to obtain 1,2-acetonide **199** (**Scheme 5.3.8-1**).[180] 1,3-propanediol (**201**) was used as starting material and monoprotected by a TBS group. The other hydroxyl group was oxidized to the aldehyde with *Parikh-Doering*-conditions. Protected aldehyde **202** was gained with excellent 88% yield over two steps and was transformed in a HWE reaction, furnishing compound **200** in 96% yield and a high *E*/*Z* selectivity so that only the desired *E*-product was obtained.

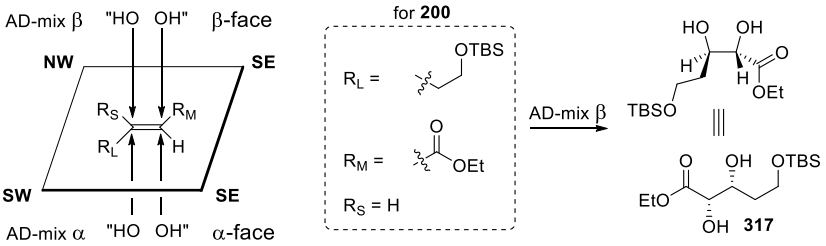


***Scheme 5.3.8-1*:** Synthesis of fragment E (**13**). Reaction conditions: a) Imidazole (1.1 equiv), TBSCl (1.0 equiv), THF, 0 °C, 30 min, **90%**; b) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, rt, 1.5 h, **98%**; c) **203** (1.2 equiv), NaH (1.2 equiv), THF, rt, on, **96%** ; d) AD-mix-β (1.4 g/mmol), methanesulfonamide (1.0 equiv), *tert*-butanol/water, rt, 48 h, **80%**; e) 2,2-dimethoxypropane (6.0 equiv), *p*TsOH (0.01 equiv), acetone, rt, on, **79%**; f) DIBAL-H (2.5 equiv), THF, −78 °C to rt, 1.5 h, **96%**; g) PMBCl (1.1 equiv), NaH (1.1 equiv), DMF, 0 °C to rt, 4 h, **87%**; h) TBAF (1.5 equiv), THF, rt, 3 h, **88%**; i) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv),

DCM, 0 °C, 30 min, **94%**; c) MeMgBr (1.2 equiv), Et_2O, 0 °C, 20 min, **91%**; d) SO_3*py (3.0 equiv), DMSO (10 equiv), DIPEA (4.0 equiv), DCM, 0 °C, 30 min, **84%**.

Subsequent *Sharpless* asymmetric dihydroxylation (SAD) furnished 1,2-diol **317** in 80% yield. In literature, an enantiomeric excess (*ee*) of 92% was reported for this compound using the stated conditions. Thus, the *ee* was not determined again and only the rotation values were compared, which showed a satisfactory accordance.[180]

The high *ee* can be explained by the mechanism of the SAD. For the asymmetric *cis*-*vic*-dihydroxylation, catalytic amounts of a suitable osmium-species, stoichiometric amounts of $K_3Fe(CN)_6$, an enantiomerically pure ligand (L*) and a base (K_2CO_3) are required. Conveniently, mixtures of these reagents are commercially available and are called AD-mix-α or AD-mix-β, depending on the chiral ligand. These ligands determine from which side (α-face or β-face) the catalytically active osmium (VIII) species attacks. Thus, both enantiomers of the diol can be obtained with high selectivity. An empirical model, the so-called mnemonic device (**Scheme 5.3.8-2**), was developed by *Sharpless* and co-workers to allow predictions of the enantiofacial selectivity. Therefore, the substituents have to be ranked (R_S = small, R_M = medium and R_L = large) and the largest residue has to be placed in the southwestern corner (SW). For a dihydroxylation from the bottom face (α –face), AD-mix-α and from the top face (β-face), AD-mix-β should be used.[173–176]



***Scheme 5.3.8-2*:** The mnemonic device and its application in the performed reaction with **200**.[173]

The ligands, which are added to the premixes are $(DHQ)_2PHAL$ (**322**) in AD-mix-α and $(DHQD)_2PHAL$ (**323**) in AD-mix-β (**Figure 5.3.8-1**).[173,176]

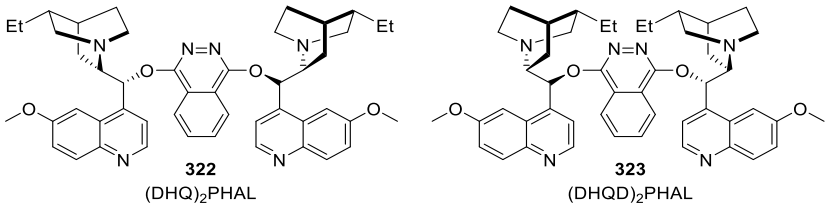
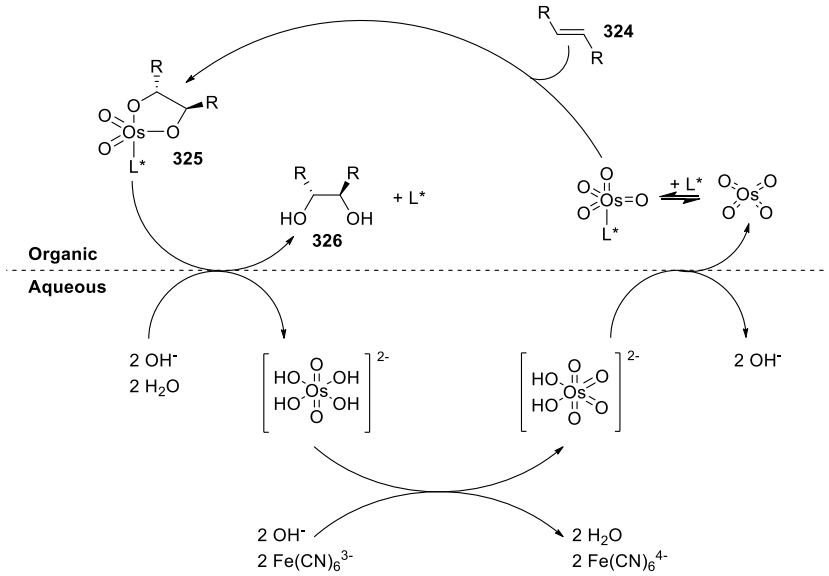


***Figure 5.3.8-1*:** Structures of the chiral ligands $(DHQ)_2PHAL$ (**322**) and $(DHQD)_2PHAL$ (**323**).[173,176]

Besides the chiral ligand, AD-mixes also contain $K_2OsO_2(OH)_4$, which is an osmium (VI)-species and represents the precursor to the catalytically active osmium (VIII)-species. The precursor is used, because $Os(VIII)O_4$ is volatile and toxic. To generate the active species in situ, $K_3Fe(CN)_6$ is added to the mixture as cooxidant. The reaction is carried out in a two-phase system (*tert*-butanol/water). This is important because *Sharpless et al.* discovered that in a single-phase system a second catalytic cycle can take place, which has less or poor enantioselectivity. The actual dihydroxylation takes place in the organic phase and the regeneration of the active catalyst in the aqueous phase (**Scheme 5.3.8-3**). First, the ligand reversibly coordinates to the OsO_4, which is faster than the reaction of OsO_4 and the alkene. The ligand binding to the OsO_4 was found to accelerate the reaction, which is called ligand-accelerated catalysis.[174] Thus, L*-OsO_4 reacts faster with the alkene **324** than OsO_4, which is relevant to achieve high stereoselectivity. The ligand binds with the tertiary nitrogen of a DHQ- or DHQD-moiety to OsO_4 and induces a negative charge at an osmium oxygen resulting in the formation of a chiral binding pocket for the alkene. The alkene fits into the binding pocket so that steric interactions are minimized leading to stereoselective dihydroxylations. Methanesulfonamide is normally added as an additive to the reaction as it was found to accelerate the hydrolysis of the osmium(VI) glyconate (**325**), which can lead to 50 fold reduced reaction times.[173–176]

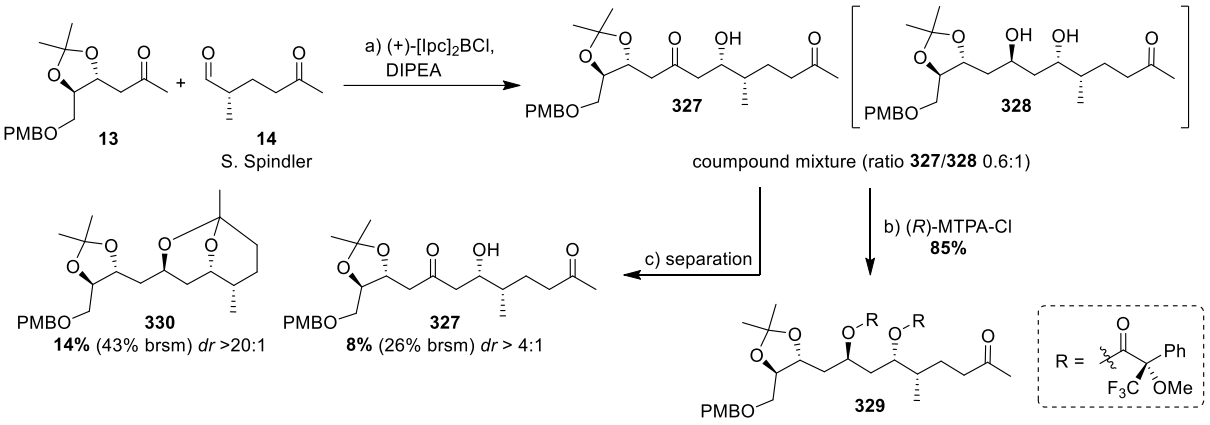


***Scheme 5.3.8-3*:** Catalytic cycle of the *Sharpless* asymmetric dihydroxylation.[173]

Following the dihydroxylation, the newly formed diol **317** was protected using 2,2-dimethoxypropane and 1,2-acetonide **199** was obtained with 79% yield (**Scheme 5.3.8-1**). Ester reduction to alcohol **318** gave an excellent yield of 96%. Compound **318** could be oxidized to the respective aldehyde for investigations of aldol condensations on the left side of the molecule. However, it was decided to first couple fragment E (**13**) and fragment F (**14**) and therefore, the hydroxyl group was protected as PMB ether furnishing compound **198** in 88% yield. The TBS group was cleaved and the free alcohol oxidized to aldehyde **320**. Subsequent *Grignard*-reaction with MeMgBr and reoxidation using again *Parikh-Doering*-conditions gave fragment E (**13**) in an overall yield of 28% over 11 steps starting from 1,3-propanediol (**201**). Fragment E contained the ketone, which was planned to serve as functional group for an aldol coupling with fragment F (**14**).

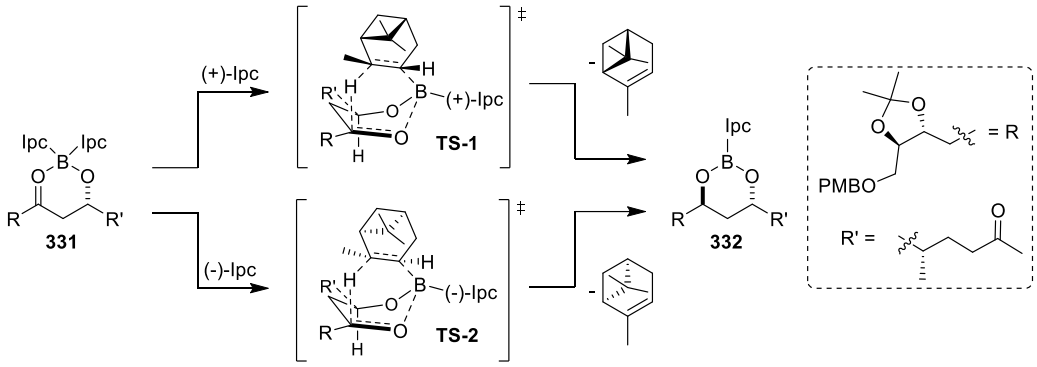
5.3.9 Coupling of Fragment E and F

The synthesized fragment E (**13**) was already coupled with fragment F (**14**), provided by *Stefanie Spindler*, who was able to synthesize this compound under the use of a literature known, organocatalyzed and enantioselective *Michael*-addition in one step from commercially available and inexpensive starting materials.[165–167]



***Scheme 5.3.9-1*:** Coupling of fragment E (**13**) and F (**14**). Reaction conditions: a) (+)-[Ipc]$_2$BCl (1.1 equiv), DIPEA (2.0 equiv), aldehyde **14** (2.0 equiv), DCM, −78 °C to −15 °C, for yields see c); b) (−)-(*R*)-MTPA-Cl (6.0 equiv), pyridine, rt, 2 h, **85%**, based on the 0.6:1 ratio of **327** and **328**; c) chromatography (80% EtOAc/hexane) of the compound mixture and removal of the solvents at 40 °C, **8% 327**, **14% 330**.

For the coupling of fragment E (**13**) and F (**14**), a (+)-[Ipc]$_2$BCl mediated *Paterson* aldol reaction for methyl ketones was utilized.[149] This type of aldol reaction was already used for fragment B and has been mechanistically explained in this section (**Chapter 5.3.3**). After the aldol reaction, a mixture of β-hydroxyketone **327** and 1,3-diol **328** was obtained and the 0.6:1 product ratio was determined by the ^{1}H NMR spectra. The presence of 1,3-diol **328** was further confirmed by mass analysis (**HRMS (ESI)** *m/z*: calcd for $C_{24}H_{38}O_7Na$ $[M + Na]^+$: 461.2510, found: 461.2506) and the mixture was submitted to an esterification reaction with (−)-(*R*)-MTPA-Cl, which in fact yielded the double *Mosher* ester **329**. Formation of *anti*-1,3-diols after an aldol coupling with the described reagents had already been observed by *Menche* and *Dieckmann*, who described the stereoselective synthesis of 1,3-*anti* diols by an Ipc-mediated domino aldol-coupling/reduction sequence.[216] Thus, the same postulated mechanism was assumed for the performed reaction leading to the *anti*-configuration in **328**, which still has to be confirmed for example by acetonide analysis.



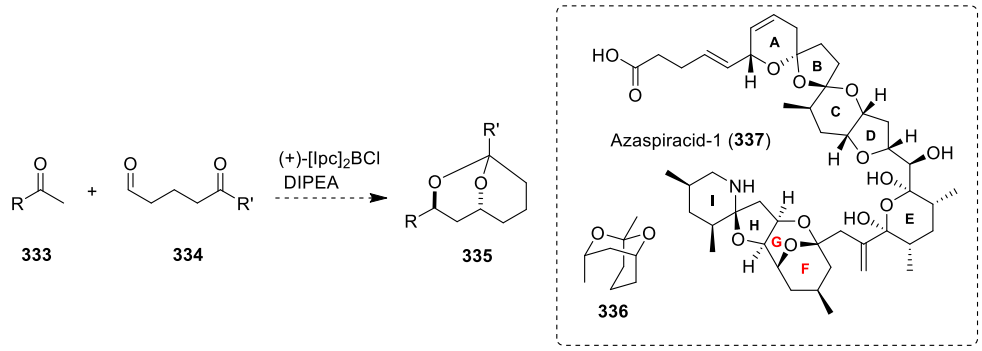
***Scheme 5.3.9-2*:** Proposed mechanism for the intramolecular Ipc-mediated reduction to cyclic boronate **332**.[216]

In agreement to the transition states for methyl ketones proposed by *Paterson et al.*, it was assumed that the Ipc-mediated reduction also proceeds via a six-membered, twist-boat like transition state (**TS-1** or **TS-2**, **Scheme 5.3.9-2**).[149,216] An intramolecular Ipc-induced hydrogen transfer gives the cyclic, *anti*-configured boronate **332** under extrusion of α-pinene. Furthermore, it was reported that the *anti*-reduction is independent of the Ipc isomer used. Oxidative removal of the boronate then leads to the corresponding *anti*-diols.[216] Notably, this domino reaction generated two new stereogenic centers in highly concise fashion and was therefore very welcome, due to the possible avoidance of an *Evans-Saksena anti*-reduction, which shortens the route by one step.[153,154]

It is believed that the domino reaction towards **328** can be optimized by an elevated temperature (rt), as this was also described to be superior for the substrates in literature.[216]

However, if the mixture of **327** and **328** was separated by a second column chromatography, a new highly interesting compound could be identified after solvent evaporation. Besides β-hydroxyketone **327**, compound **330** was isolated and 1,3-diol **328** could no longer be detected. After confirmation of compound **330** by NMR spectroscopy and mass spectrometry, two consequences were made. First, the 1,3-diols **328** seemed to be stabilized in the compound mixture with β-hydroxyketone **327**. Second, spontaneous intramolecular acetalization with the internal ketone occurs when the compound is isolated and the solvent is removed. This is a fascinating reaction and it could extend the developed domino reaction of *Menche* and *Dieckmann* one step further. Additionally, only one diastereomer was observed which means that a third stereogenic center was selectively generated. Unfortunately, the configuration could not be determined unambiguously until now, but this will be part of future investigation. Thus,

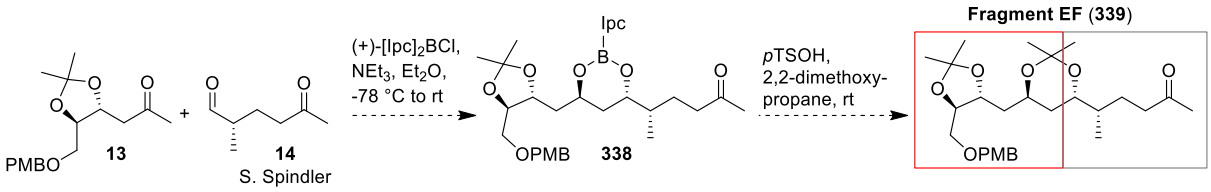
formation of the two-ring system and generation of three stereogenic centers would be possible by applying only one step to easily accessible starting materials (**Scheme 5.3.9-3**).



***Scheme 5.3.9-3*:** General cascade reaction to **335**. Various residues R and R' will be tested in future. Box = naturally occurring compounds, which show a **335** like moiety.

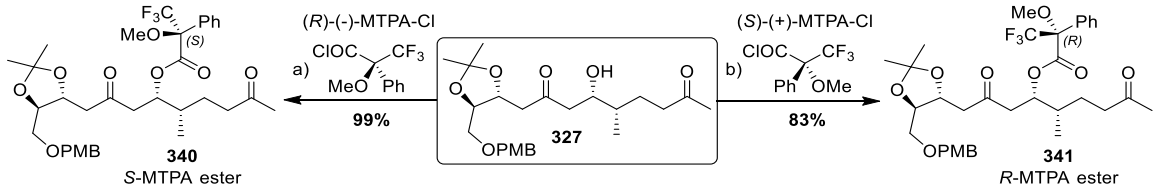
This cascade reaction is of high interest, since the shown double ring framework occurs in natural products. For example compound **336** was isolated from the Norway Spruce *Picea abies* infested by the ambrosia beetle *Trypdendron lineatum*.[217–219] This natural product would thus be easily synthesized in one step if R, R'= CH_3. Furthermore, the cascade reaction could also be used in even more complex natural molecules such as the group of azaspiracids, which are polycyclic ethers and marine algal toxins produced by the dinoflagellate *Azadinium spinosum*.[220,221] Here, the developed sequence could be helpful for the synthesis of the G and F ring framework (**Scheme 5.3.9-3**). Besides the determination and confirmation of the stereogenic outcome after the cascade reaction, different residues will be tested in future.

With regard to the synthesis of vancoresmycin, the described procedure by *Menche* and *Dieckmann* could be used. Here, the cyclic boronate **338** (**Scheme 5.3.9-4**) will be isolated (no oxidative work up) and directly submitted to the acetonide protection, which will hopefully prevent the intramolecular cyclization and will allow the desired protection of the diol and confirmation of the 1,3-*anti*-configuration.



***Scheme 5.3.9-4*:** Planned synthesis for fragment EF (**339**)

With the isolated β-hydroxyketone **327**, a *dr* > 4:1 was roughly determined by ^{1}H NMR due to overlapping signals. Notably, the isolation of only one diastereomer for **330** may indicate that the *dr* could be even higher in favor to the desired configuration and only the *S*-configured β-hydroxyketone was implemented in the domino process. The *S*-configuration of **327** was confirmed using *Mosher* ester analysis.



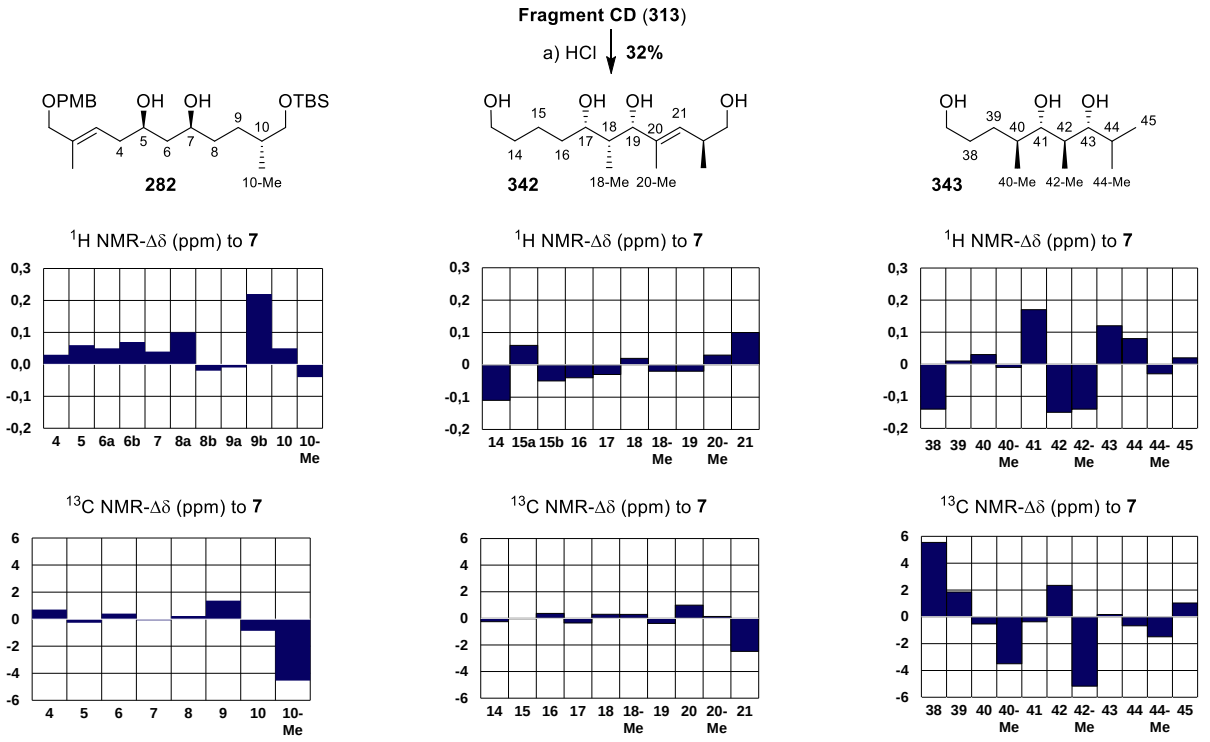
***Scheme 5.3.9-5*:** Synthesis of *S*-MTPA ester **340** and *R*-MTPA ester **341**. Reaction conditions: a) (−)-(*R*)-MTPA-Cl (6.0 equiv), pyridine, rt, 2 h, **99%**; b) (+)-(*S*)-MTPA-Cl (6.0 equiv), pyridine, rt, 2 h, **83%**.

For a background regarding *Mosher* ester analysis see **Chapter 5.3.3** and for experimental details as well as a table containing significant chemical shifts see experimental section **6.3.8**.

5.3.10 NMR Comparison and Bioinformatic Analysis Leading to a Revised Vancoresmycin Stereochemistry

Using vancoresmycin fragment CD (**313**, for synthesis see **Chapter 5.3.6**), *Maximilian Seul* was able to yield compound **342** by cleavage of the protective groups.[212] Together with diol **282** (for synthesis see **Chapter 5.3.3**) and compound **343** (synthesized by *Stefanie Spindler*),[167] a comparison of the ^{1}H and ^{13}C NMR chemical shifts with those obtained for the natural product vancoresmycin (**7**) was performed to validate the proposed stereogenic centers by *Allenby et al.*[132] For compounds **342** and **282**, the compared and calculated differences of the chemical shifts showed good agreements (**Scheme 5.3.10-1**), confirming the proposed

relative configuration of these fragments. However, stronger discrepancies were observed for compound **343**. Especially, the shifts of C42 and 42-Me are strongly deviating questioning the proposed (40,41)-*anti*, (42,43)-*anti* (*S*, *S*, *R*, *R*)-configuration.



***Scheme 5.3.10-1*:** Differences between adjusted ^{1}H and ^{13}C chemical shifts (in ppm, MeOD) of fragments **282** (500/125 MHz), **342** (700/175 MHz) and **343** (400/100 MHz) to vancoresmycin (**7**) data. Reaction conditions: a) 1 M HCl (2.0 equiv), MeOH, rt, 1.5 h, **32%**.

Within the collaborative research center (TRR261) and based on the observed discrepancies, an extensive bioinformatic analysis was carried out by the group of Prof. Dr. *Nadine Ziemert*. Including methods developed by *McDaniel*,[222] *Caffrey*,[223] *Leadlay*,[224] *Keatinge-Clay*,[225] *Kitsche*[226] and *Kalesse*,[226] a revised configurational assignment of vancoresmycin was proposed (**344**, **Figure 5.3.10-1**). Remarkably, the novel proposed structure is consistent with the performed NMR comparison (**Scheme 5.3.10-1**).[227]

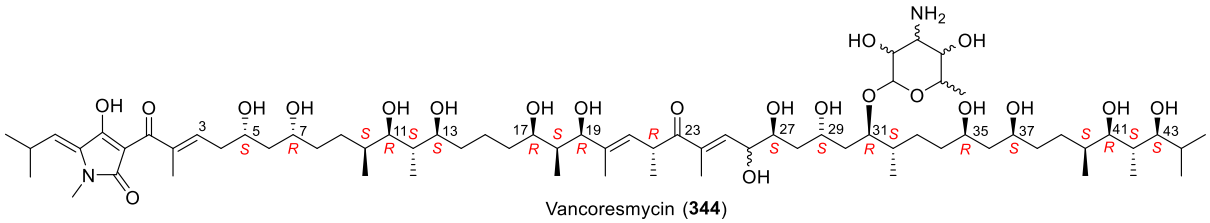


***Figure 5.3.10-1*:** Revised proposal for the stereostructure of vancoresmycin (**344**).

Notably, the synthesis of vancoresmycin was planned in a way that the stereochemistry can be easily adapted. Thus, the developed and approved routes described in this work can be used by application of the enantiomeric reagents and starting materials. Efforts can now be directed towards confirmation of the novel vancoresmycin stereostructure (**344**).

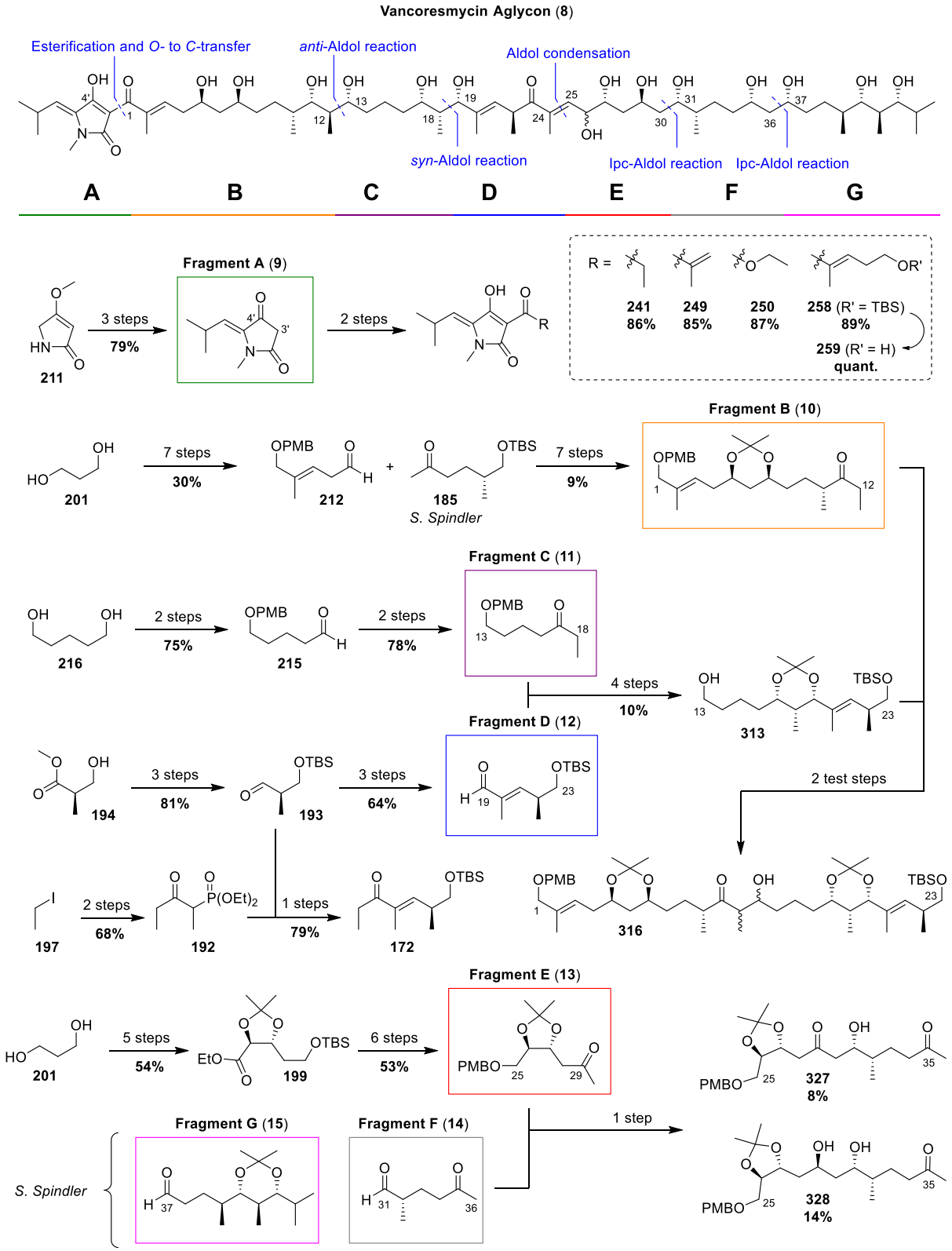
5.4 Conclusion

During this work and together with the project partner *Stefanie Spindler*, the first retrosynthetic approach towards vancoresmycin aglycone (**8**) was designed (first generation) and already optimized (second generation).

Based on the novel retrosynthesis, aglycone **8** was divided into seven fragments (A-G, **9**-**15**, **Scheme 5.4-1**) of approximately the same size. Amazingly, all fragments could be synthesized, which proved the design of the novel routes. While fragments F (**14**), G (**15**) and a part of B (**185**), have been synthesized by *Stefanie Spindler*, the other fragments (A (**9**), B (**10**), C (**11**), D (**12**), E (**13**)) resulted from the synthetic effort of this work.

Different sequences were investigated for the synthesis of fragment A. However, these results were not very promising and sometimes included many steps. Fortunately, a sequence was found, which yielded the key tetramic acid **9** (fragment A) with a high yield of 79% in only three steps and without the need of chromatographic purification. Applying two more steps, an esterification at 4'-OH and an oxygen to carbon transfer, yielded vancoresmycin-type, 3-acyl tetramic acids (**241**, **249**, **250**, **258** and **259**) in a concise fashion.[133] Different residues at the acyl position have been chosen to be authentic to vancoresmycin and to allow further modifications. During this study more and more knowledge was gained about the fascinating properties of 3-acyl tetramic acids, which are characterized by an extended tautomerism and metal chelating possibilities. Additionally, interesting bioactivities can be found in literature for different tetramic acids.[137–139] Thus, it was decided to start a cooperation with the group of Prof. Dr. *Tanja Schneider* and the novel vancoresmycin-type tetramic acids have been tested for their antibiotic activities. Fortunately, some of the tested compounds already showed moderate antibiotic effects against *S. aureus*, while no activity was seen for *E. coli*.[133] These results are in agreement with the reported data for vancoresmycin.[132] Furthermore, the compounds with the highest similarity to the natural product showed the highest potencies. A MIC of 32 µg/mL for compound **249** and 64 µg/mL for tetramic acids **241** and **259** have been determined. Hence, it is believed that the first part of the vancoresmycin pharmacophore was found.[133]

Fragment B (**10**) was synthesized out of aldehyde **212** and ketone **185**. While ketone **185** was provided by *Stefanie Spindler*, aldehyde **212** was synthesized in this work. Starting from 1,3-propanediol (**201**), seven steps were required and a HWE reaction was chosen as a key step. Subsequently, a *Paterson* aldol reaction was performed and after confirmation of the desired, new stereogenic center (*dr* = 4:1), fragment B (**10**) was generated in six further steps including a highly stereoselective *Narasaka-Prasad* reduction and an acetonide protection, which also allowed the analysis of the 1,3-*syn*-configuration. In conclusion, fragment B (**10**) was synthesized out of 1,3-propanediol (**201**) over 14 linear steps in an overall yield of 2.8%.



***Scheme 5.4-1*:** Vancoresmycin aglycone **8** with the planned coupling reactions. Overview of the performed syntheses.

For fragment C (**11**), a straightforward sequence was performed, which involved four linear steps, yielding the desired ketone **11** in a yield of 58% out of inexpensive 1,5-pentandiol (**216**). In the originally planned route, precursor **215** was intended as fragment C and should be coupled with ketone **172** (fragment D in first generation retrosynthetic approach) using a *syn*-aldol reaction. Therefore, ketone **172** was prepared by a HWE reaction of phosphonate **192**, which was synthesized in two steps with a 68% yield, and aldehyde **193**, which was gained in 81% yield after a three-step sequence out of (*R*)-*Roche* ester (**194**). However, several trials of the envisioned *syn*-aldol reaction only led to the reisolation of starting materials and it was assumed that a vice versa *syn*-aldol reaction in which the ketone function is attached to fragment C and the aldehyde to fragment D is more promising. Hence, previously synthesized aldehyde **193** was converted to the final fragment D (**12**) in three steps and again with the use of a HWE reaction. Thus, fragment D (**12**) was synthesized in six linear steps and 52% yield. With the use of the inverted set up, the *syn*-aldol reaction was successful (*dr* = 6:1) and further three steps being *Narasaka-Prasad* reduction, acetonide protection and deprotection of the hydroxyl group at C-13 yielded alcohol **313** in an overall yield of 5% over ten steps starting from (*R*)-*Roche* ester (**194**).

In addition, it was possible to oxidize compound **313** to the respective aldehyde, which was directly used for a test *anti*-aldol coupling with fragment B (**10**). Thereby, compound **316** could be isolated, which represents a very large part of the natural product vancoresmycin. However, an exact outcome of the reaction and stereogenic analysis was not done yet due to the low scale in the test reaction.

Fragment E (**13**) has been synthesized, applying a developed eleven step sequence, which included a HWE reaction and a *Sharpless* asymmetric dihydroxylation as key steps. This sequence allowed the generation of fragment E (**13**) in an overall yield of 29% and in addition, fragment E (**13**) was already coupled with fragment F (**14**), which was provided by *Stefanie Spindler*. The here performed *Paterson* aldol reaction yielded a mixture of β-hydroxyketone **327** and 1,3-diol **328**. After isolation, diol **328** showed an additionally fascinating, spontaneous, intramolecular acetalization (with ketone at C-35), which might lead to the development of a cascade reaction in which three stereogenic centers and two ring systems could be selectively formed.

In summary, the development and optimization of the first reported retrosynthesis as well as the successful synthesis of all planned fragments laid the foundation for a synthetic access to

vancoresmycin aglycone **8**. Together with the already performed fragment couplings, a total synthesis seems to become possible and closer. In addition, a part of the vancoresmycin pharmacophore has already been identified and published, so that these compounds could be used as novel lead structures.[133]

NMR comparison between vancoresmycin fragments and the natural product revealed good agreements for the C-4–C-9 and C-14–C-21 region but significant discrepancies for the terminal C-38–C-45 region (**see Chapter 5.3.10**). Thus, a cooperation with the group of Prof. Dr. *Nadine Ziemert* was started, who were able to reveal a novel proposal of the vancoresmycin stereostructure (**344**, **Figure 5.4-1**) after an intense bioinformatic analysis.[227]

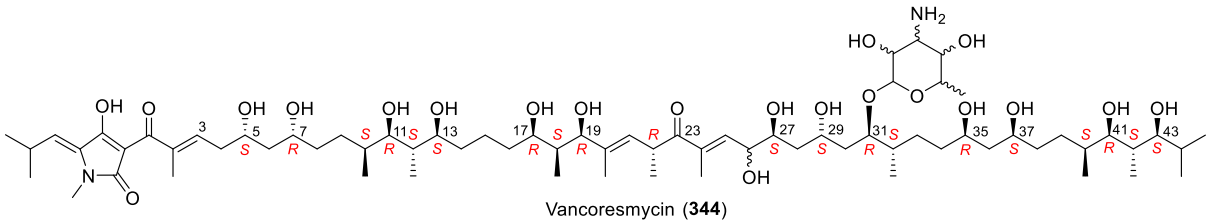


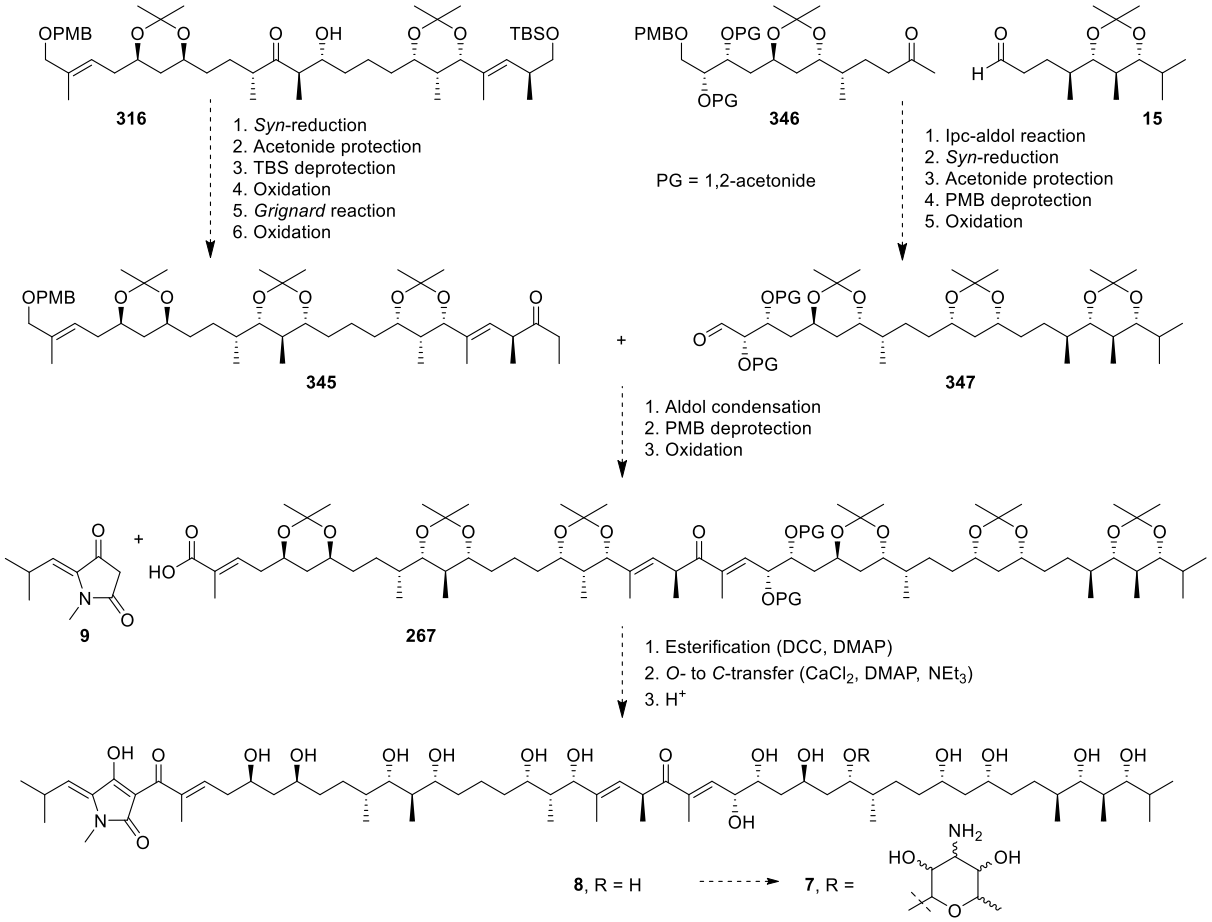
***Figure 5.4-1*:** Revised proposal for the stereostructure of vancoresmycin (**344**).

Now, emphasis should first be placed on the confirmation of this novel stereostructure. Ideally, the synthesis has already been planned in such a way that it can be easily modified with respect to the stereogenic centers. Thus, the synthetic routes from this work can be used by only adapting the chiral reagents and starting materials.

5.5 Outlook

Based on the developed route and the already performed synthesis of all fragments as well as already carried out fragment couplings, the first total synthesis of vancoresmycin aglycone **8** could be completed in the near future (**Scheme 5.5-1**). For this purpose, *syn*-reduction has to be performed with β-hydroxyketone **316**. Subsequent acetonide protection of the 1,3-diol followed by TBS cleavage and formation of the ethyl ketone should yield fragment BCD (**345**). On the other side, fragment EF (**346**) could be coupled to fragment G (**15**) via the planned Ipc-mediated aldol reaction. *Syn*-reduction, acetonide protection, PMB deprotection and oxidation should furnish fragment EFG (**347**), which could be coupled to fragment BCD (**345**) using an aldol condensation. Cleavage of the PMB group and oxidation should give polyketide **267** (fragment BCDEFG) featuring the free carboxylic acid, which should be used for the esterification with tetramic acid **9** (fragment A). Final oxygen to carbon transfer and acidic deprotection could complete the first total synthesis of vancoresmycin aglycone **8**.

Due to the late stage attachment of the carbohydrate moiety in the biosynthesis of vancoresmycin a chemoenzymatic conversion of vancoresmycin aglycone **8** to vancoresmycin (**7**) may be possible.



***Scheme 5.5-1*:** Final steps to complete the total synthesis of vancoresmycin aglycone **8**.

However, since the vancoresmycin stereostructure has been revised during this work (**see Chapter 5.3.10**), emphasis should be placed on the confirmation of this novel proposal (**344**, **Figure 5.5-1**), which was revealed after NMR comparison of vancoresmycin fragments and the natural product as well as extensive bioinformatic analysis.

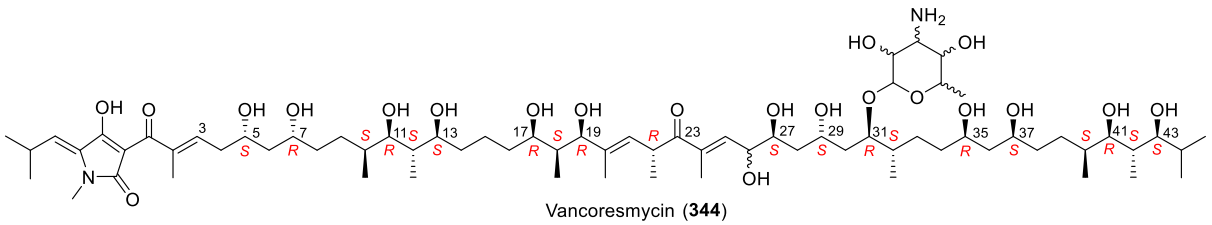


***Figure 5.5-1*:** Revised proposal for the stereostructure of vancoresmycin (**344**).

In general, the synthetic routes, established during this thesis, can be used for the synthesis of vancoresmycin fragments, which include the novel absolute configuration, by adapting chiral

reagents and starting materials. These fragments should then be compared with the natural product by NMR analysis to validate the novel, proposed stereostructure.

Looking back at the route, which has been performed so far, it becomes apparent that the yield limiting steps can be linked to aldol reactions. If later on an optimization of the route towards vancoresmycin is intended, different, selective 1,3-diol formations like a procedure reported by *Gansäuer et al.* could be considered.[228] This procedure was able to give all stereoisomers of a selected 1,3-diol in high yield and selectivity, using cuprate addition, *Sharpless* asymmetric epoxidation and subsequent S_N2 reaction.[228]

Since the novel vancoresmycin-type tetramic acids, which have been synthesized in this work (see **Chapter 5.3.2**), already showed antibiotic characteristics, an extension of the structure activity relationship would be reasonable. Furthermore, it is recommended to screen the synthesized 3-acyl tetramic acids for other bioactivities, due to reported antiviral[138] and antitumoral[139] properties of similar compounds. In addition, metal chelating tendencies could be examined, as these were found to be sometimes essential for the bioactivity.[141,142]

In addition, an investigation and optimization of the discovered cascade reaction in **Chapter 5.3.9** is supposed to be fruitful as this framework can be found in natural products and three reactions including the formation of three stereogenic centers can be performed in one step.

6 Experimental Section

6.1 General Methods

Reaction conditions: All reagents were purchased from commercial suppliers (*Sigma-Aldrich, TCI, Acros, AlfaAesar, Iris Biotech*) in the highest purity grade available and used without further purification. Anhydrous solvents (THF, DCM, MeCN, Et_2O, toluene) were obtained from a solvent drying system MB SPS-800 (*MBraun*) and stored over molecular sieves (3 or 4 Å). Unless stated otherwise, all non-aqueous reactions were performed under an argon atmosphere in flame-dried glassware. The reactants were handled using standard *Schlenk* techniques. Temperatures above rt (23 °C) refer to oil bath or heat-on temperatures, which were controlled by a temperature modulator. For cooling, the following baths were used: water/ice (0 °C), MeCN/dry ice (−40 °C) and acetone/dry ice (−78 °C) as well as a *Huber* TC100E-F-NR cryostat.

TLC monitoring was performed with silica gel 60_{F254} pre-coated polyester sheets (0.2 mm silica gel, *Macherey-Nagel*) and visualized using UV light and staining with a solution of CAM (1.0 g $Ce(SO_4)_2$), 2.5 g $(NH_4)_6Mo_7O_{24}$, 8 mL conc. H_2SO_4 in 100 mL H_2O) and subsequent heating.

Purification methods: For column chromatography, silica gel (pore size 60 Å, 40-63 µm) was used from *Merck* or *Aldrich*. Compounds were eluated using the stated mixtures under pressure of nitrogen or air. Solvents for column chromatography were distilled prior to use. For ion exchange chromatography, Dowex® 50WX8, 100–200 mesh by *Acros* was used. Gel permeation chromatography was performed using Sephadex™ LH–20 purchased from *GE Healthcare*.

Preparative thin-layer chromatography was performed on alumina backed silica gel sheets (20x20 cm, pore size 60 Å) coated with a fluorescent indicator purchased from *Merck*. The crude product was applied as a solution in DCM (~ 20 µL) and using a *Hamilton* syringe. Detection was carried out under UV light and the desired areas were scrapped off and stirred 1 h with EtOAc or DCM. After filtration, the silica gel was washed (EtOAc or DCM) and the solvent was removed under reduced pressure.

Semi-preparative and analytical HPLC analyses were performed on *Knauer Wissenschaftliche Geräte GmbH* systems with support from *Andreas J. Schneider*. The solvents for HPLC were purchased in HPLC grade. The chromatograms were recorded by UV-detection at 240, 215, 210 and 205 nm.

Table 6.1-1: HPLC configuration for analytical HPLC.

	System A	**System B**	**System C**
series	PLATINblue	Azura	PLATINblue
pumps	binary, HPG P1 system, 10 mL	binary, HPG P 6.1L, 10 mL	binary, HPG P1 system, 5 mL
pressure	750 bar	700 bar	1000 bar
autosampler	AS1 with 10 µL injection loop	3950 with 100 µL injection loop	AS1 with 10 µL injection loop
mixing chamber	static, SmartMix 350 µL	integrated static mixing chamber, 100 µL	static, SmartMix 100 µL
column heater	T1	CT 2.1	T1
detection type	PDA UV/VIS detection PDA1, D_2/Hg halogen lamps, 190-1000 nm	PDA UV/VIS detection DAD 6.1L, D_2/Hg halogen lamps, 190-1020 nm	PDA UV/VIS detection PDA1, D_2/Hg halogen lamps, 190-1000 nm
degasser	analytical 2-channel-online-degasser	analytical 2-channel-online-degasser	analytical 2-channel-online-degasser

Table 6.1-2: HPLC configuration for preparative HPLC.

	System D	**System E**
series	Azura	Smartline
pumps	binary, HPG P 6.1L, 50 mL	binary, S-1800, 100 mL
pressure	200-300 bar	400 bar
autosampler	assistant ASM 2.1L with a feed pump P 2.1S	assistant 6000 with a feed pump S-100
mixing chamber	integrated static mixing chamber, 200 µL	static, SmartMix 350 µL
detection type	MWL 2.1L, 190-700 nm	UV detector S-2550, 190-600 nm
degasser	preparative 2-channel-online-degasser	preparative 2-channel-online-degasser

Analytical methods: All NMR spectra were recorded on *Bruker* spectrometers at the University of Bonn under supervision of Dr. *Senada Nozinovic* with operating frequencies of 125 (^{13}C), 150 (^{13}C), 175 (^{13}C), 400 (^{1}H), 500 (^{1}H), 600 (^{1}H) and 700 MHz (^{1}H) in deuterated solvents obtained from *Deutero* and *Carl Roth*. Spectra were measured at room temperature unless stated otherwise and chemical shifts are reported in ppm relative to $(Me)_4Si$ (δ = 0.00 ppm) and were calibrated to the residual signal of undeuterated solvents.[229] Data for ^{1}H-NMR spectra are reported as follows: chemical shift (multiplicity, coupling constants (*J*) reported in hertz, number of hydrogens, assignment). Abbreviations used are: s (singlet), d (doublet), t (triplet), q (quartet), quint (quintet), m (multiplet), br (broad). Overlapped and not resolved signals are reported as multiplets. Atom numbering was chosen randomly and is shown for the isolated products in the respective schemes.

Mass spectra (MS) and high resolution mass spectra (HRMS) were recorded on the documented systems in **Table 6.1-3** at the University of Bonn under supervision of Dr. *Marianne Engeser*.

Table 6.1-3: Used MS systems for MS and HRMS.

	manufacturer	**measurements**
MAT 95 XL	*Thermo Finnigan* (Bremen)	EI
MAT 90	*Thermo Finnigan* (Bremen)	EI, LIFDI, CI, FAB
MALDI autoflex II TOF/TOF	*Bruker Daltonik* (Bremen)	MALDI
micrOTOF-Q	*Bruker Daltonik* (Bremen)	ESI, APCI, nano-ESI, MS/MS, LC-MS, DC-MS
Orbitrap XL	*Thermo Fisher Scientific* (Bremen)	ESI, APCI, APPI, nano-ESI
Apex IV FT-ICR	*Bruker Daltonik* (Bremen)	ESI, nano-ESI, MALDI, EI, CI

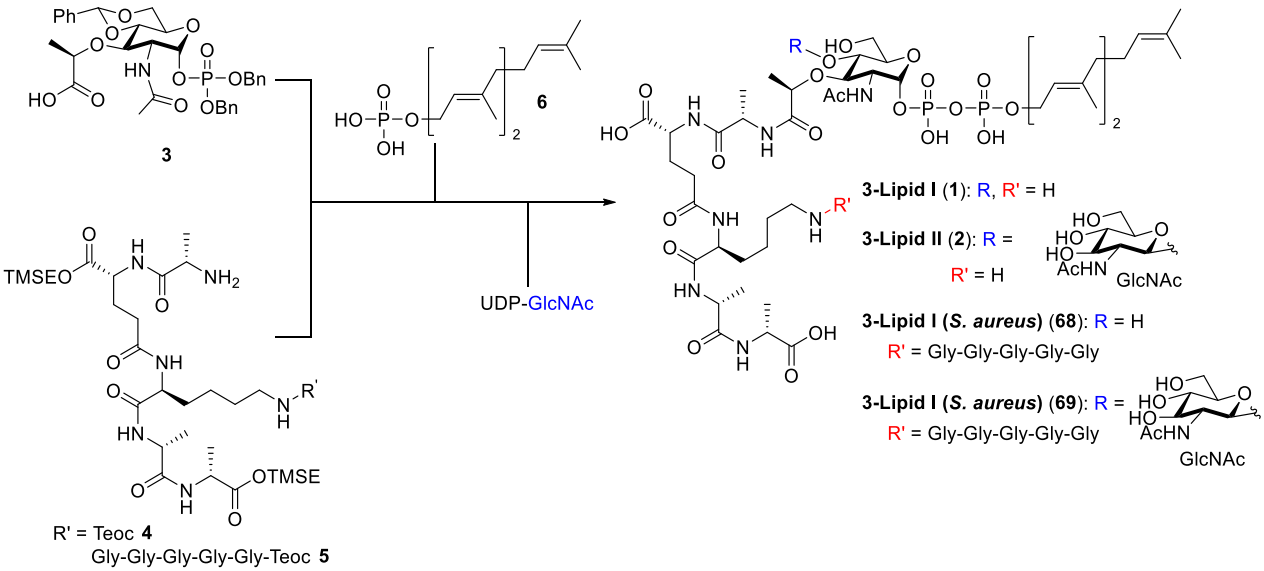
Optical rotation data were measured using an *Anton Paar* modular compact polarimeter MCP 150, with either a 10 mm cuvette (0.2 ml) or a 100 mm cuvette (0.7 ml), using a LED (589 nm) as a light source. The optical rotation was measured autonomously five times and the specific rotation was calculated.

$$[\alpha]_D^T = \frac{\alpha_D^T}{l * c}$$

[α]: specific rotation, D: light source (589 nm), T: temperature, α: optical rotation, l: path length of the cuvette, c: concentration [g/100 mL]

6.2 Total Synthesis of Farnesyl Lipid I/II analogs

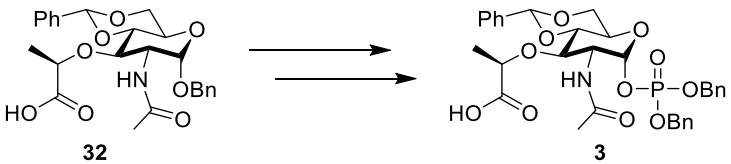
In this chapter, all experimental details for the total synthesis of farnesyl lipid I/II analogs **1**/**68** and **2**/**69** are described. As shown in **Scheme 6.2-1**, the synthesis is based on three components being the monosaccharide **3**, peptide **4** and accordingly **5** for the synthesis of analog **2**, and terpene **6**. A late stage chemoenzymatic attachment of *N*-acetylglucoseamine yielded the final farnesyl lipid II analogs **68** and **69**.



***Scheme 6.2-1*:** Synthesis of lipid I/II analogs **1**/**68** and **2**/**69** and the three main fragments **3**, **4** (**5**) and **6**.

6.2.1 Synthesis of the Carbohydrate Fragment 3

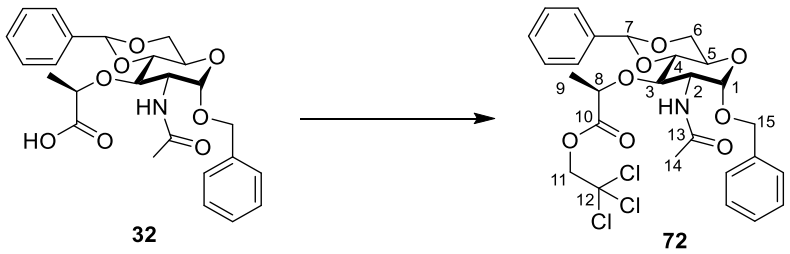
Carbohydrate fragment **3** can be synthesized in six steps from commercially available **32**.



***Scheme 6.2.1-1*:** Synthesis of the carbohydrate fragment **3** starting from **32**.

Synthesis of Compound 72

2,2,2-Trichloroethyl (*R*)-2-(((2*R*,4a*R*,6*S*,7*R*,8*R*,8a*S*)-7-acetamido-6-(benzyloxy)-2-phenyl-hexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)-propanoate



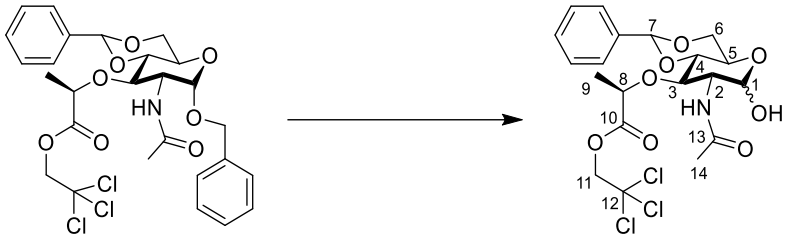
$C_{27}H_{30}Cl_3NO_8$
M = 602.89 g/mol

To a solution of acid **32** (1.00 g, 2.12 mmol, 1.00 equiv) and 4-(dimethylamino)-pyridine (DMAP) (25.9 mg, 212 µmol, 0.10 equiv) in THF (16 mL) was added 2,2,2-trichloroethanol (0.48 mL, 4.24 mmol, 2.35 equiv) and *N,N'*-dicyclohexylcarbodiimide (656 mg, 3.18 mmol, 1.50 equiv). After stirring for 5 h at rt, the mixture was filtered over a cotton plug and the precipitate was washed with EtOAc (2x20 mL). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (15% EtOAc/CH_2Cl_2) to yield ester **72** as a colorless solid (1.03 g, 1.71 mmol, 81%).

$\mathbf{R_f}$ 0.44 (15% EtOAc/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +92.7° (*c* = 0.90 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.46 – 7.28 (m, 10H, $H_{arom.}$), 7.07 (d, *J* = 5.5 Hz, 1H, NH), 5.58 (s, 1H, H-7), 5.34 (d, *J* = 3.4 Hz, 1H, H-1), 4.97 (d, *J* = 11.9 Hz, 1H, H-11), 4.68 (d, *J* = 11.8 Hz, 1H, H-15), 4.66 (q, *J* = 7.1 Hz, 1H, H-8), 4.60 (d, *J* = 11.9 Hz, 1H, H-11'), 4.52 (d, *J* = 11.9 Hz, 1H, H-15'), 4.21 (dd, *J* = 10.1, 4.6 Hz, 1H, H-6), 4.01 – 3.98 (m, 2H, H-3, H-5), 3.76 (dd, *J* = 10.3 Hz, 10.3 Hz, 1H, H-6'), 3.73 (dd, *J* = 9.2 Hz, 9.2 Hz, 1H, H-4), 2.03 (s, 3H, CH_3-14), 1.50 (d, *J* = 7.0 Hz, 3H, CH_3-9). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 173.7 (C-10), 170.9 (C-13), 137.5 ($C_{arom.}$), 137.3 ($C_{arom.}$), 129.2 ($C_{arom.}$), 128.6 ($C_{arom.}$), 128.5 ($C_{arom.}$), 128.1 ($C_{arom.}$), 128.0 ($C_{arom.}$), 126.0 ($C_{arom.}$), 101.6 (C-7), 97.5 (C-1), 94.6 (C-12), 83.4 (C-4), 75.2 (C-8), 75.1 (C-3), 74.3 (C-11), 70.5 (C-15), 69.1 (C-6), 63.1 (C-5), 54.2 (C-2), 23.3 (C-14), 18.8 (C-9). **HRMS (ESI)** *m/z*: calcd for $C_{27}H_{30}Cl_3NO_8Na$ $[M + Na]^+$: 624.0929, found: 624.0932. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 73

2,2,2-Trichloroethyl (*R*)-2-(((2*R*,4a*R*,7*R*,8*R*,8a*S*)-7-acetamido-6-hydroxy-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propanoate



To a solution of carbohydrate **72** (500 mg, 829 µmol, 1.00 equiv) in EtOAc (40 mL) was added Pd–C (650 mg, 10% Pd), the reaction vessel was filled with hydrogen and the reaction stirred for 20 min at rt. The suspension was filtered over celite® and the precipitate was washed with MeOH (2x20 mL). The solvent was removed under reduced pressure and MeCN (30 mL) was added followed by benzaldehyde dimethyl acetal (187 µL, 1.24 mmol, 1.50 equiv) and a solution of *p*-TsOH in MeCN (1 mL of a 232 mM solution, 232 µmol, 0.30 equiv), dried over 3 Å MS. After stirring for 4 h at rt, the reaction was neutralized with NEt_3 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (90% EtOAc/CyH) to yield alcohol **73** as a colorless solid (409 mg, 798 µmol, 96%) and as a mixture of α- and β-anomers (ratio α:β = 4:1). The mixture was used for the next reactions without separation. For analytical purpose, separation of the α and β anomer was achieved with HPLC (50% MeCN/water, retention time 11.5 min (β anomer), 16.1 min (α anomer), using a KNAUER Eurospher II 100-5 C18; 5 µm; 250 x 16 mm + precolumn 30 x 16 mm, 210 nm).

$\mathbf{R}_f$ (α anomer) 0.33, $\mathbf{R}_f$ (β anomer) 0.26 (90% EtOAc/CyH).

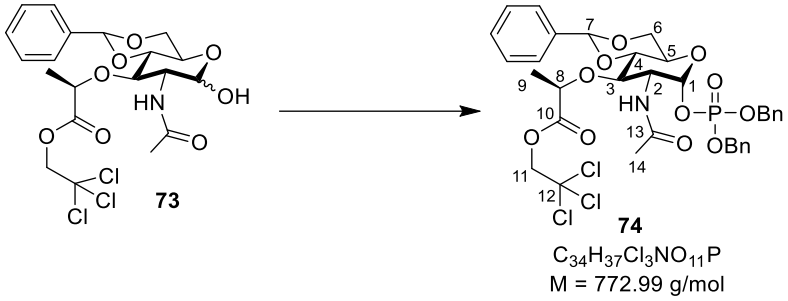
α anomer:

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +58.9° (*c* = 0.73 in CH_2Cl_2). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 7.47 – 7.45 (m, 2H, $H_{arom.}$), 7.40 – 7.36 (m, 3H, $H_{arom.}$), 7.19 (d, *J* = 4.5 Hz, 1H, NH), 5.60 (s, 1H, H-7), 5.58 (dd, *J* = 3.9 Hz, 3.6 Hz, 1H, H-1), 5.01 (d, *J* = 12.0 Hz, 1H, H-11), 4.68 (q, *J* = 7.1 Hz, 1H, H-8), 4.65 (d, *J* = 12.0 Hz, 1H, H-11‘), 4.24 (dd, *J* = 10.3, 5.0 Hz, 1H, H-6), 4.03 (td, *J* = 10.3 Hz, 5.0 Hz, 1H, H-5), 3.94 – 3.86 (m, 1H, H-3), 3.87 – 3.83 (m, 1H, H-2), 3.75 (dd, *J* = 10.3 Hz, 10.3 Hz, 1H, H-6‘), 3.73 (dd, *J* = 10.3 Hz, 10.3 Hz, 1H, H-4), 3.68 (dd, *J* = 3.9 Hz, 1.0 Hz, 1H, OH),

2.01 (s, 3H, CH_3-14), 1.51 (d, *J* = 7.1 Hz, 3H, CH_3-9). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 174.3 (C-10), 171.5 (C-13), 138.1 ($C_{arom.}$), 129.5 ($C_{arom.}$), 128.8 ($C_{arom.}$), 126.5 ($C_{arom.}$), 101.9 (C-7), 95.1 (C-12), 92.3 (C-1), 83.8 (C-4), 75.7 (C-8), 75.2 (C-3), 74.7 (C-11), 69.6 (C-6), 63.3 (C-5), 55.2 (C-2), 23.5 (C-14), 19.1 (C-9). **HRMS(ESI)** *m/z*: calcd for $C_{20}H_{24}Cl_3NO_8Na$ $[M + Na]^+$: 534.0460, found: 534.0463. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 74

2,2,2-Trichloroethyl (*R*)-2-(((2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7-acet-amido-6-((bis(ben-zyloxy)phosphoryl)oxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propano-ate



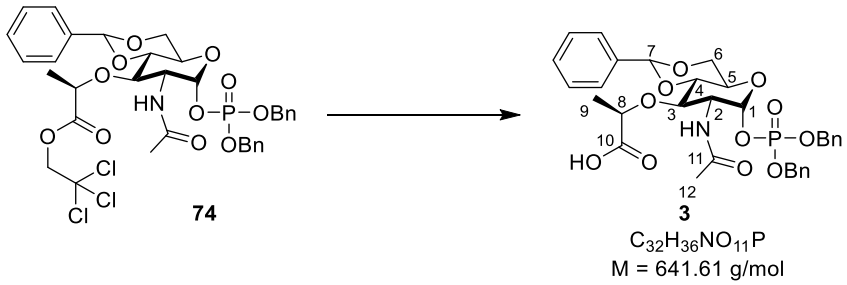
Alcohol **73** (100 mg, 195 µmol, 1.00 equiv) was dissolved in dry CH_2Cl_2 (5 mL) and a 0.45 M solution of 1*H*-tetrazole in MeCN (1.63 mL, 731 µmol, 3.75 equiv) was added. The reaction was cooled to –40 °C and dibenzyl(*N*,*N*-diisopropyl)phosphoramidite (164 µL, 488 µmol, 2.50 equiv) was added. After 1 h warming to rt, the reaction was stirred for another hour. Then, *m*-CPBA (101 mg, 585 µmol, 3.00 equiv) was added at –60 °C and the reaction was stirred for 30 min at 0 °C followed by 30 min stirring at rt. The mixture was diluted with CH_2Cl_2 (5 mL) and washed with aq. Na_2SO_3 (2x10 mL, 10%), aq. sat. $NaHCO_3$ (2x10 mL) and water (2x10 mL). After drying over $MgSO_4$, the mixture was filtered, concentrated and purified by flash chromatography (65% EtOAc/CyH) to yield organophosphate **74** as a colorless solid (101 mg, 585 µmol, 79%).

R_f 0.22 (70% EtOAc/CyH). **$[\alpha]_D^{25}$** = +66.5° (*c* = 0.66 in CH_2Cl_2). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 7.45 – 7.44 (m, 2H, $H_{arom.}$), 7.42 – 7.33 (m, 13H, $H_{arom.}$), 7.15 (d, *J* = 4.9 Hz, 1H, NH), 6.04 (dd, *J* = 6.1, 3.2 Hz, 1H, H-1), 5.57 (s, 1H, H-7), 5.06 (d, *J* = 8.3 Hz, 4H, 2xCH_2-Ph), 5.01 (d, *J* = 12.0 Hz, 1H, H-11), 4.65 (q, *J* = 7.0 Hz, 1H, H-8), 4.62 (d, *J* = 12.0 Hz, 1H, H-11‘), 4.08

(dd, *J* = 10.3, 4.9 Hz, 1H, H-6), 4.00 – 3.96 (m, 1H, H-2), 3.92 (ddd, *J* = 10.3, 10.3, 4.9 Hz, 1H, H-5), 3.82 (dd, *J* = 9.9, 9.9 Hz, 1H, H-3), 3.77 (dd, *J* = 10.3 Hz, 9.9 Hz, 1H, H-4), 3.72 (dd, *J* = 10.3, 10.3 Hz, 1H, H-6‘), 1.87 (s, 3H, H-14), 1.51 (d, *J* = 7.1 Hz, 3H, H-9). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 174.2 (C-10), 171.3 (C-13), 137.8 ($C_{arom.,quart.}$-Ph), 136.4 (d, *J* = 7.2 Hz, $C_{arom.,quart.}$-Bn), 136.4 (d, *J* = 7.2 Hz, $C_{arom.,quart.}$-Bn), 129.6 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.1 ($C_{arom.}$), 128.8 ($C_{arom.}$), 128.4 ($C_{arom.}$), 128.4 ($C_{arom.}$), 126.5 ($C_{arom.}$), 102.1 (C-7), 96.6 (d, *J* = 6.7 Hz, C-1), 95.0 (C-12), 82.9 (C-4), 75.8 (C-8), 74.8 (C-11), 74.7 (C-3), 70.0 (d, *J* = 4.1 Hz, CH_2-Ph), 70.0 (d, *J* = 4.1 Hz, CH_2-Ph), 69.0 (C-6), 65.1 (C-5), 54.5 (C-2), 23.2 (C-14), 19.0 (C-9). **^{31}P NMR** (284 MHz, CD_2Cl_2) δ = –2.4. **HRMS(ESI)** *m/z*: calcd for $C_{34}H_{37}Cl_3NO_{11}PH$ $[M + H]^+$: 772.1243, found: 772.1236. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 3

(*R*)-2-(((2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7-Acetamido-6-((bis(benzyl-oxy)phosphoryl)oxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propanoic acid



Carbohydrate **74** (160 mg, 207 µmol, 1.00 equiv) was dissolved in a mixture of 90% AcOH/water (15 mL) and zinc powder (120 mg, 1.84 mmol, 8.89 equiv) was added. The suspension was stirred vigorously for 3 h at rt. After filtering and washing with MeOH, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (0.1% AcOH/10% MeOH/CH_2Cl_2) to yield carboxylic acid **3** as a colorless solid (129 mg, 201 µmol, 97%).

$\mathbf{R_f}$ 0.29 (0.1% AcOH/10% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +61.1° (*c* = 0.46 in MeOH). **^{1}H NMR** (700 MHz, CD_3OD) δ = 7.48 – 7.45 (m, 2H, $H_{arom.}$), 7.41 – 7.35 (m, 13H, $H_{arom.}$), 6.06 (dd, *J* = 6.0, 3.3 Hz, 1H, H-1), 5.62 (s, 1H, H-7), 5.11 – 5.08 (m, 4H, 2xCH_2-Ph), 4.42 (q, *J* = 7.1 Hz, 1H, H-8), 4.03 – 4.01 (m, 1H, H-6), 3.87 – 3.84 (m, 1H, H-2), 3.79 – 3.71 (m, 4H, H-3, H-4, H-5, H-6’), 1.87 (s, 3H, H-12), 1.37 (d, *J* = 7.1 Hz, 3H, H-9). **^{13}C NMR** (176 MHz, CD_3OD) δ =

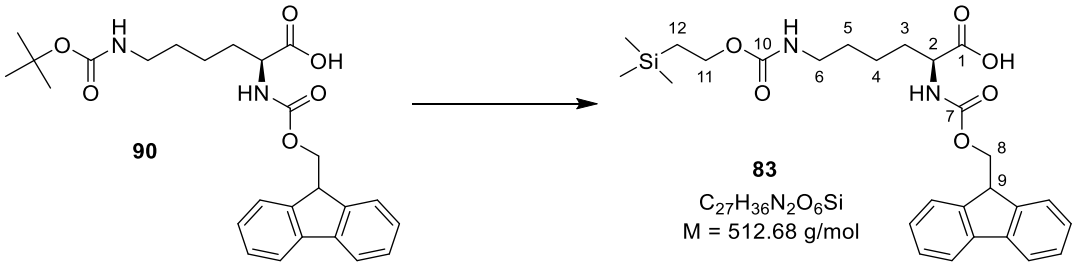
178.4 (C-10, not resolved but HMBC correlation), 173.9 (C-11), 138.9 ($C_{arom.,quart.}$-Ph), 137.0 (d, J = 2.4 Hz, $C_{arom.,quart.}$-Bn), 137.0 (d, J = 2.4 Hz, $C_{arom.,quart.}$-Bn), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 128.4 ($C_{arom.}$), 127.2 ($C_{arom.}$), 102.7 (C-7), 97.3 (d, J = 6.7, C-1), 83.0 (C-4), 77.0 (C-8), 75.1 (C-3), 71.2 (d, J = 2.2 Hz, CH_2-Ph), 71.1 (d, J = 2.3 Hz, CH_2-Ph), 69.1 (C-6), 66.1 (C-5), 55.8 (C-2), 22.6 (C-12), 19.2 (C-9). **^{31}P NMR** (284 MHz, CD_2Cl_2) δ = –3.0. **HRMS(ESI)** *m/z*: calcd for $C_{32}H_{36}NO_{11}PNa$ $[M + Na]^+$: 664.1918, found: 664.1914. The spectroscopic data were in agreement with those previously reported.[16]

6.2.2 Synthesis of Amino Acid Building Blocks 83, 84, 86 and 89

This chapter reports on the experimental details of the amino acid building blocks (**83**, **84**, **86** and **89**), which were used in the synthesis of pentapeptide **4** and decapeptide **5**.

Synthesis of Compound 83

N^2-(((9*H*-Fluoren-9-yl)methoxy)carbonyl)-N^6-((2-(trimethylsilyl)ethoxy)carbonyl)-L-lysine

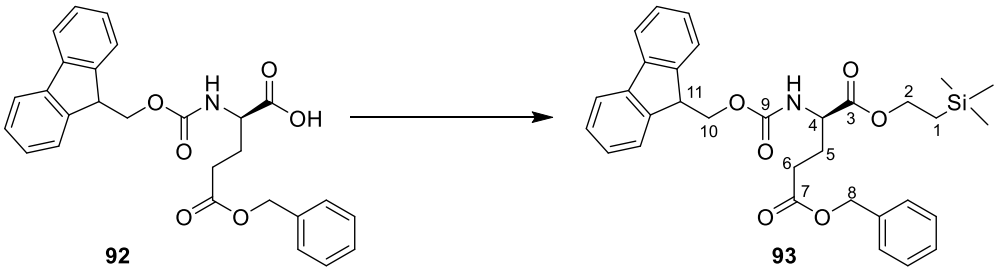


Fmoc-L-Lys(*N*-Boc)-OH (**90**, 1.50 g, 3.20 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (20 mL) and TFA (20 mL) was added. The mixture was stirred for 20 min at rt. After removing the solvent under reduced pressure, DMF (20 mL) and diisopropylethylamine (2.72 ml, 16.0 mmol, 5.00 equiv) were added. 2-(Trimethylsilyl)ethyl *p*-nitrophenyl carbonate (1.09 g, 3.84 mmol, 1.20 equiv) was dissolved in DMF (6 mL) and transferred to the reaction solution. After stirring for 2 h at rt, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (100% EtOAc → 10% MeOH/CH_2Cl_2/0.1% AcOH) to yield Teoc-protected lysine **83** as a colorless solid (1.79 g, 3.81 mmol, 99%).

$\mathbf{R_f}$ 0.42 (10% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = –1.1° (*c* = 2.42 in MeOH). **^{1}H NMR** (500 MHz, CD_3OD) δ = 7.80 (d, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.67 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.39 (t, *J* = 7.3 Hz, 2H, $H_{arom.}$), 7.31 (td, *J* = 7.5, 1.1 Hz, 2H, $H_{arom.}$), 4.37 – 4.34 (m, 2H, H-8), 4.23 (t, *J* = 6.9 Hz, 1H, H-9), 4.18 – 4.08 (m, 3H, H-11, H-2), 3.09 (t, *J* = 6.9 Hz, 2H, H-6), 1.89 – 1.82 (m, 1H, H-3), 1.73 – 1.65 (m, 1H, H-3), 1.55 – 1.48 (m, 2H, H-5), 1.45 – 1.39 (m, 2H, H-4), 0.95 (t, *J* = 8.6 Hz, 2H, H-12), 0.02 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (126 MHz, CD_3OD) δ = 159.3 (C-10), 158.7 (C-7), 145.4 ($C_{arom.}$), 142.6 ($C_{arom.}$), 128.8 ($C_{arom.}$), 128.2 ($C_{arom.}$), 126.3 ($C_{arom.}$), 120.9 ($C_{arom.}$), 67.9 (C-8), 63.7 (C-11), 55.9 (C-2), 48.5 (C-9), 41.4 (C-6), 32.7 (C-3), 30.6 (C-5), 24.1 (C-4), 18.7 (C-12), –1.5 (3xCH_3). **HRMS(ESI)** *m/z*: calcd for $C_{27}H_{36}N_2O_6SiNa$ $[M + Na]^+$: 535.2235, found: 535.2231. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 93

5-Benzyl 1-(2-(trimethylsilyl)ethyl) (((9*H*-fluoren-9-yl)methoxy)-carbonyl)-D-glutamate



Fmoc-D-Glu(O-Bzl)-OH (**92**, 510 mg, 1.11 mmol, 1.00 equiv) and DMAP (13.6 mg, 111 µmol, 0.10 equiv) were dissolved in EtOAc (15 mL). DCC (285 mg, 1.33 mmol, 1.20 equiv) and 2-(trimethylsilyl)ethanol (191 µL, 1.33 mmol, 1.20 equiv) were added. After stirring for 2 h at rt, the reaction was filtered over celite® and the residue was washed with EtOAc. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (15% EtOAc/CyH) to yield protected glutamic acid **93** as a colorless solid (573 mg, 1.02 mmol, 92%).

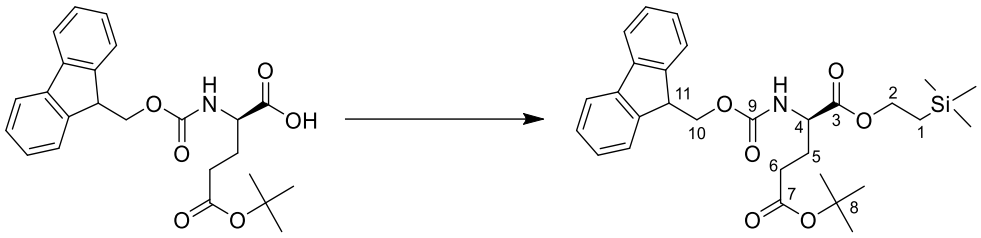
$\boldsymbol{R_f}$ 0.20 (15% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +13.0° (*c* = 0.92 in MeOH).

^{1}H NMR (500 MHz, CD_3OD) δ = 7.78 (d, *J* = 7.6 Hz, 2H, $H_{arom.}$), 7.65 (t, *J* = 8.1 Hz, 2H, $H_{arom.}$), 7.40 – 7.25 (m, 9H, $H_{arom.}$), 5.11 (s, 2H, H-8), 4.35 (dd, *J* = 6.9, 2.5 Hz, 2H, H-10), 4.25

– 4.16 (m, 4H, H-2, H-4, H-11), 2.46 (t, *J* = 7.5 Hz, 2H, H-6), 2.22 – 2.15 (m, 1H, H-5), 1.97 – 1.90 (m, 1H, H-5), 1.00 – 0.97 (m, 2H, H-1), 0.03 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (126 MHz, CD_3OD) δ = 174.0 (C-7), 173.7 (C-3), 158.6 (C-9), 145.3 ($C_{arom.}$), 145.1 ($C_{arom.}$), 142.6 ($C_{arom.}$), 137.5 ($C_{arom.}$), 129.5 ($C_{arom.}$), 129.2 ($C_{arom.}$), 128.8 ($C_{arom.}$), 128.1 ($C_{arom.}$), 126.2 ($C_{arom.}$), 120.9 ($C_{arom.}$), 67.9 (C-10), 67.4 (C-8), 64.7 (C-2), 54.8 (C-4), 48.4 (C-11), 31.3 (C-6), 27.7 (C-5), 18.2 (C-1), –1.5 ($3xCH_3$). **HRMS(ESI)** calcd for $C_{32}H_{37}NO_6SiNa$ $[M + Na]^+$: 582.2285, found: 582.2283. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 95

5-(*tert*-Butyl) 1-(2-(trimethylsilyl)ethyl) (((9*H*-fluoren-9-yl)methoxy)carbonyl)-D-glutamate



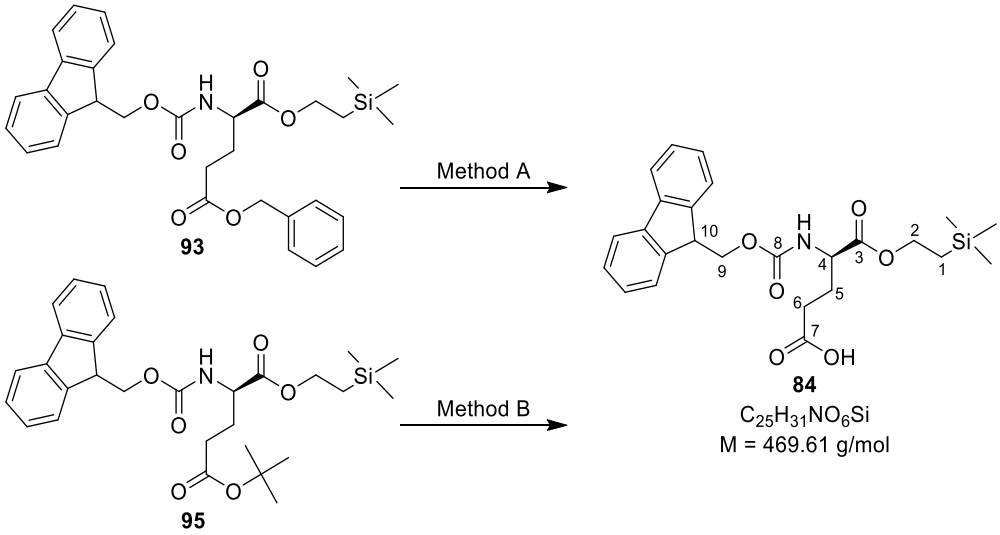
Fmoc-D-Glu(O-*t*Bu)-OH (**94**, 1.50 g, 3.53 mmol, 1.00 equiv) and DMAP (86.1 mg, 705 µmol, 0.20 equiv) were dissolved in EtOAc (20 mL) and cooled to 0 °C. 2-(Trimethylsilyl)ethanol (758 µL, 5.29 mmol, 1.50 equiv) and DCC (1 M in CH_2Cl_2, 4.23 mL, 4.23 mmol, 1.20 equiv) were added. After stirring for 2 h at rt, the reaction was filtered over celite® and the residue was washed with EtOAc (2x20 mL). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (15% EtOAc/CyH) to yield protected glutamic acid **95** as a colorless solid (1.77 g, 3.37 mmol, 96%).

$\mathbf{R_f}$ 0.32 (15% EtOAc/CyH). $[\boldsymbol{\alpha}]_D^{25}$ = +13.6° (*c* = 1.98 in MeOH). **^{1}H NMR** (700 MHz, CD_3OD) δ = 7.79 (d, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.66 (t, *J* = 8.3 Hz, 2H, $H_{arom.}$), 7.38 (t, *J* = 7.2 Hz, 2H, $H_{arom.}$), 7.30 (t, *J* = 8.0 Hz, 2H, $H_{arom.}$), 4.38 – 4.32 (m, 2H, H-10), 4.23 – 4.19 (m, 4H, H-2, H-4, H-11), 2.33 (t, *J* = 7.0 Hz, 2H, H-6), 2.15 – 2.07 (m, 1H, H-5), 1.92 – 1.83 (m, 1H, H-5), 1.44 (s, 9H, $3xCH_3$-Boc), 1.04 – 0.96 (m, 2H, H-1), 0.04 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, CD_3OD) δ = 173.8 (C-7), 173.7 (C-3), 158.6 (C-9), 145.3 ($C_{arom.}$), 145.2 ($C_{arom.}$), 142.6

($C_{arom.}$), 128.8 ($C_{arom.}$), 128.2 ($C_{arom.}$), 128.1 ($C_{arom.}$), 126.3 ($C_{arom.}$), 126.2 ($C_{arom.}$), 120.9 ($C_{arom.}$), 81.8 (C-8), 68.0 (C-10), 64.6 (C-2), 54.8 (C-4), 48.4 (C-11), 32.6 (C-6), 28.4 (3xCH_3-Boc), 27.8 (C-5), 18.2 (C-1), –1.5 (3xCH_3). **HRMS(ESI)** *m/z*: calcd for $C_{29}H_{40}NO_6Si$ $[M + H]^+$: 526.2619, found: 526.2598.

Synthesis of Compound 84

(*R*)-4-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-5-oxo-5-(2-(trimethylsilyl)ethoxy)pentanoic acid



Method A:

To a solution of Fmoc-D-Glu(O-Bzl)-OTMSE (**93**, 2.60 g, 4.65 mmol, 1.00 equiv) in MeOH (60 mL) and AcOH (≈ 100 µL) was added 10% Pd–C (1 g). The reaction vessel was filled with hydrogen. After stirring for 10 min at rt, the suspension was filtered over celite® and the catalyst was washed with MeOH. The solvent of the filtrate was removed under reduced pressure and the crude product was purified by flash chromatography (10% EtOAc/CH_2Cl_2 → 10% MeOH/0.1% AcOH/CH_2Cl_2) to yield carboxylic acid **84** as a colorless solid (1.83 g, 3.90 mmol, 84%).

Method B:

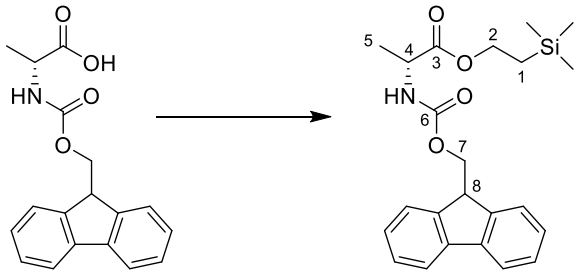
Fmoc-D-Glu(O-*t*Bu)-OTMSE (**95**, 1.00 g, 1.90 mmol, 1.00 equiv) was dissolved in CH_2Cl_2 (30 mL). 2,6-Lutidine (4.42 mL, 38.0 mmol, 20.0 equiv) and trimethylsilyl trifluoromethanesulfonate (TMSOTf, 3.44 mL, 19.0 mmol, 10.0 equiv) were added at 0 °C and stirred for 30 min.

The reaction was stirred for further 30 min at rt followed by slow addition of aq. sat. NH_4Cl (30 mL). The aqueous phase was extracted with EtOAc (3x40 mL), the organic phase was washed with 30% AcOH/water, dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield carboxylic acid **84** as a colorless solid (866 mg, 1.84 mmol, 97%).

$\mathbf{R_f}$ 0.44 (10% EtOAc/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +19.2° (*c* = 1.04 in MeOH). **^{1}H NMR** (500 MHz, CD_3OD) δ = 7.79 (d, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.67 (t, *J* = 6.3 Hz, 2H, $H_{arom.}$), 7.39 (t, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.31 (td, *J* = 7.5, 1.1 Hz, 2H, $H_{arom.}$), 4.39 – 4.32 (m, 2H, H-9), 4.24 – 4.19 (m, 4H, H-2, H-4, H-10), 2.40 (t, *J* = 7.4 Hz, 2H, H-6), 2.19 – 2.12 (m, 1H, H-5), 1.95 – 1.88 (m, 1H, H-5), 1.02 – 0.99 (m, 2H, H-1), 0.04 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (126 MHz, CD_3OD) δ = 176.3 (C-7), 173.8 (C-3), 158.6 (C-8), 145.3 ($C_{arom.}$), 145.2 ($C_{arom.}$), 142.6 ($C_{arom.}$), 128.2 ($C_{arom.}$), 128.1 ($C_{arom.}$), 126.3 ($C_{arom.}$), 126.2 ($C_{arom.}$), 120.9 ($C_{arom.}$), 68.0 (C-9), 64.7 (C-2), 55.0 (C-4), 48.4 (C-10), 31.1 (C-6), 27.7 (C-5), 18.2 (C-1), –1.5 (3xCH_3). **HRMS(ESI)** *m/z*: calcd for $C_{25}H_{31}NO_6SiNa$ $[M + Na]^+$: 492.1813, found: 492.1817. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 97

2-(Trimethylsilyl)ethyl (((9*H*-fluoren-9-yl)methoxy)carbonyl)-D-alaninate



96 **97**

$C_{23}H_{29}NO_4Si$

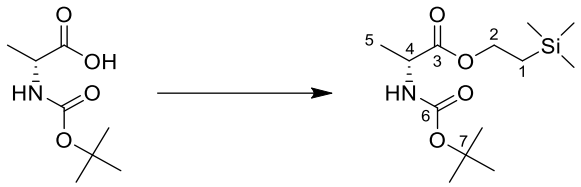
M = 411.57 g/mol

Fmoc-D-Ala-OH (**96**, 1.00 g, 3.21 mmol, 1.00 equiv) and DMAP (39.2 mg, 321 µmol, 0.10 equiv) were dissolved in EtOAc (30 mL). DCC (795 mg, 3.85 mmol, 1.20 equiv) and 2-(trimethylsilyl)ethanol (553 µL, 3.85 mmol, 1.20 equiv) were added. After stirring for 40 min at rt, the reaction was filtered over celite® and the residue was washed with EtOAc. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (20% EtOAc/CyH) to yield protected alanine **97** as a colorless solid (1.27 g, 3.09 mmol, 96%).

R_f 0.35 (20% EtOAc/CyH). $[\alpha]_D^{25}$ = +20.2° (*c* = 1.48 in MeOH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.77 (d, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.62 – 7.60 (m, 2H, $H_{arom.}$), 7.40 (t, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.32 (td, *J* = 7.5, 1.1 Hz, 1H $H_{arom.}$), 5.40 (d, *J* = 7.3 Hz, 1H, NH), 4.43 – 4.36 (m, 3H, H-4, H-7), 4.27 – 4.22 (m, 3H, H-2, H-8), 1.44 (d, *J* = 7.1 Hz, 3H, H-5), 1.04 – 1.01 (m, 2H, H-1), 0.06 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 173.3 (C-3), 155.7 (C-6), 144.1 ($C_{arom.}$), 143.9 ($C_{arom.}$), 141.4 ($C_{arom.}$), 127.8 ($C_{arom.}$), 127.2 ($C_{arom.}$), 125.2 ($C_{arom.}$), 120.1 ($C_{arom.}$), 67.1 (C-7), 64.1 (C-2), 49.9 (C-4), 47.3 (C-8), 18.9 (C-5), 17.5 (C-1), –1.4 (3xCH_3). **HRMS(ESI)** calcd for $C_{23}H_{29}NO_4SiNa$ $[M + Na]^+$: 434.1758, found: 434.1765.

Synthesis of Compound 101

2-(Trimethylsilyl)ethyl (*tert*-butoxycarbonyl)-D-alaninate

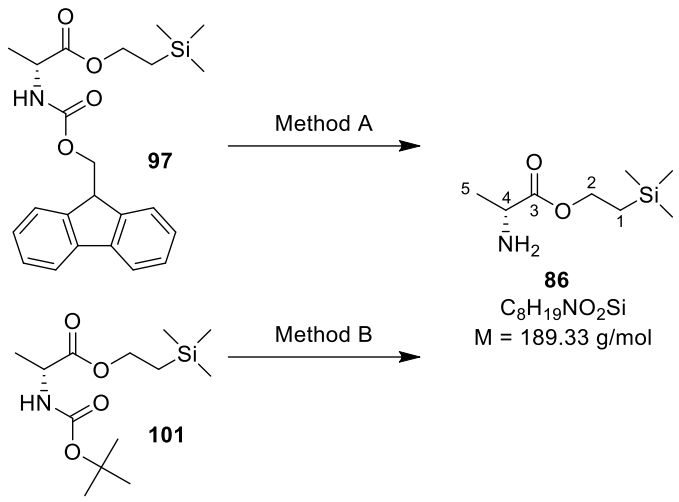


Boc-D-Ala-OH (**100**, 1.50 g, 7.93 mmol, 1.00 equiv) and DMAP (194 mg, 1.59 mmol, 0.20 equiv) were dissolved in EtOAc (40 mL) and cooled to 0 °C. 2-(Trimethylsilyl)ethanol (1.70 mL, 11.9 mmol, 1.50 equiv) and DCC (1 M in CH_2Cl_2, 9.51 mL, 9.51 mmol, 1.20 equiv) were added. After stirring for 2 h at rt, the reaction was filtered over celite® and the residue was washed with EtOAc (2x20 mL). The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (10% EtOAc/CyH) to yield ester **101** as a colorless solid (2.27 g, 7.84 mmol, 99%).

R_f 0.17 (10% EtOAc/CyH). $[\alpha]_D^{25}$ = +30.5° (*c* = 2.00 in MeOH). **^{1}H NMR** (700 MHz, CD_3OD) δ = 4.25 – 4.18 (m, 2H, H-2), 4.09 (q, *J* = 7.2 Hz, 1H, H-4), 1.44 (s, 9H, 3xCH_3-Boc), 1.33 (d, *J* = 7.2 Hz, 3H, H-5), 1.04 – 1.00 (m, 2H, H-1), 0.06 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, CD_3OD) δ = 175.2 (C-3), 157.9 (C-6), 80.5 (C-7), 64.4 (C-2), 50.8 (C-4), 28.7 (3xCH_3-Boc), 18.2 (C-1), 17.7 (C-5), –1.5 (3xCH_3). **HRMS(ESI)** *m/z*: calcd for $C_{13}H_{27}NO_4SiNa$ $[M + Na]^+$: 312.1597, found: 312.1602. The spectroscopic data were in agreement with those previously reported.[101]

Synthesis of Compound 86

2-(Trimethylsilyl)ethyl-D-alaninate



Method A:

Fmoc-D-Ala-TMSE (**97**, 470 mg, 1.14 mmol, 1.00 equiv) was dissolved in 20% piperidine/ DMF (10 mL). After stirring for 1 h at rt, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (100% EtOAc → 10% MeOH/CH_2Cl_2) to yield amine **86** as colorless solid (112 mg, 592 µmol, 52%).

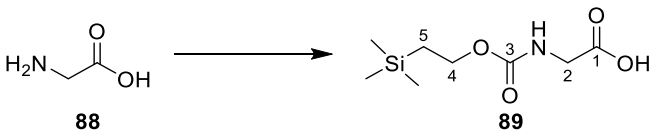
Method B:

Boc-D Ala-TMSE (**101**, 220 mg, 760 µmol, 1.00 equiv) was dissolved in 20% TFA/CH_2Cl_2 (4.5 mL). After stirring for 1 h at rt, toluene (15 mL) was added and the solvent was removed under reduced pressure to yield amine **86** as colorless solid (144 mg, 760 µmol, quant.).

$\mathbf{R_f}$ 0.16 (10% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +1.2° (*c* = 0.82 in MeOH). **^{1}H NMR** (500 MHz, CD_3OD) δ = 4.35 – 4.31 (m, 2H, H-2), 4.00 (q, *J* = 7.2 Hz, 1H, H-4), 1.51 (d, *J* = 7.2 Hz, 3H, H-5), 1.09 – 1.06 (m, 2H, H-1), 0.07 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (126 MHz, CD_3OD) δ = 171.7 (C-3), 65.8 (C-2), 50.0 (C-4), 18.2 (C-1), 16.6 (C-5), –1.6 ($3xCH_3$). **HRMS(ESI)** *m/z*: calcd for $C_8H_{20}NO_2Si$ $[M + H]^+$: 190.1258, found: 190.1263.

Synthesis of Compound 89

(2-(Trimethylsilyl)ethoxy)carbonyl)glycine



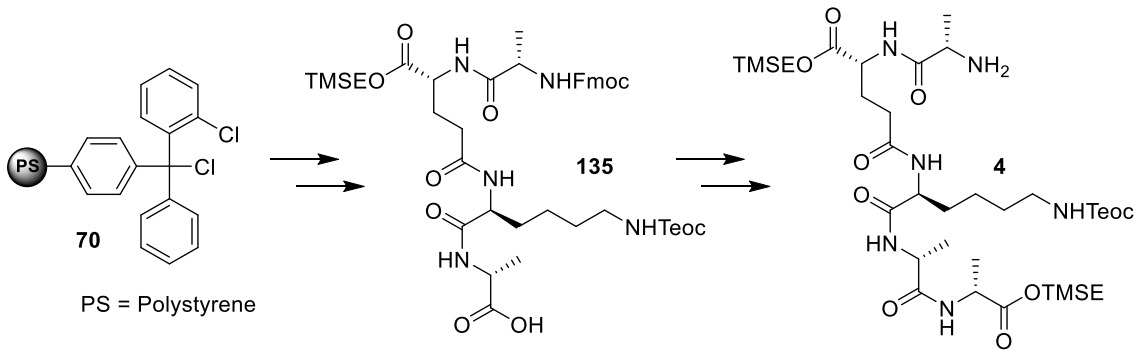
$C_8H_{17}NO_4Si$
M = 219.31 g/mol

Glycine (**88**, 250 mg, 3.33 mmol, 1.00 equiv) was dissolved in 50% dioxane/water (8 mL) and NEt_3 (1.38 mL, 9.99 mmol, 3.00 equiv) and *N*-(2-trimethylsilyl)ethoxycarbonyloxy)succinimide (950 mg, 3.66 mmol, 1.10 equiv) were added. The solution was stirred overnight at rt and diluted with water (20 mL). Citric acid (0.5 M, aq.) was added until the pH turned 4. The aqueous phase was extracted with diethyl ether (3x40 mL) and the combined ether layers were washed twice with water (15 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield protected glycine **89** as a colorless solid (714 mg, 3.26 mmol, 98%).

R_f 0.41 (10% MeOH/0.1% AcOH/CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_3OD) δ = 4.18 – 4.14 (m, 2H, H-4), 3.80 (s, 2H, H-2), 1.02 – 0.99 (m, 2H, H-5), 0.05 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (126 MHz, CD_3OD) δ = 173.7 (C-1), 159.4 (C-3), 64.2 (C-4), 43.0 (C-2), 18.6 (C-5), –1.5 (3xCH_3). **HRMS(ESI)** *m/z*: calcd for $C_8H_{16}NO_4Si$ $[M - H]^-$: 218.0854, found: 218.0851.

6.2.3 Synthesis of the Pentapeptide 4

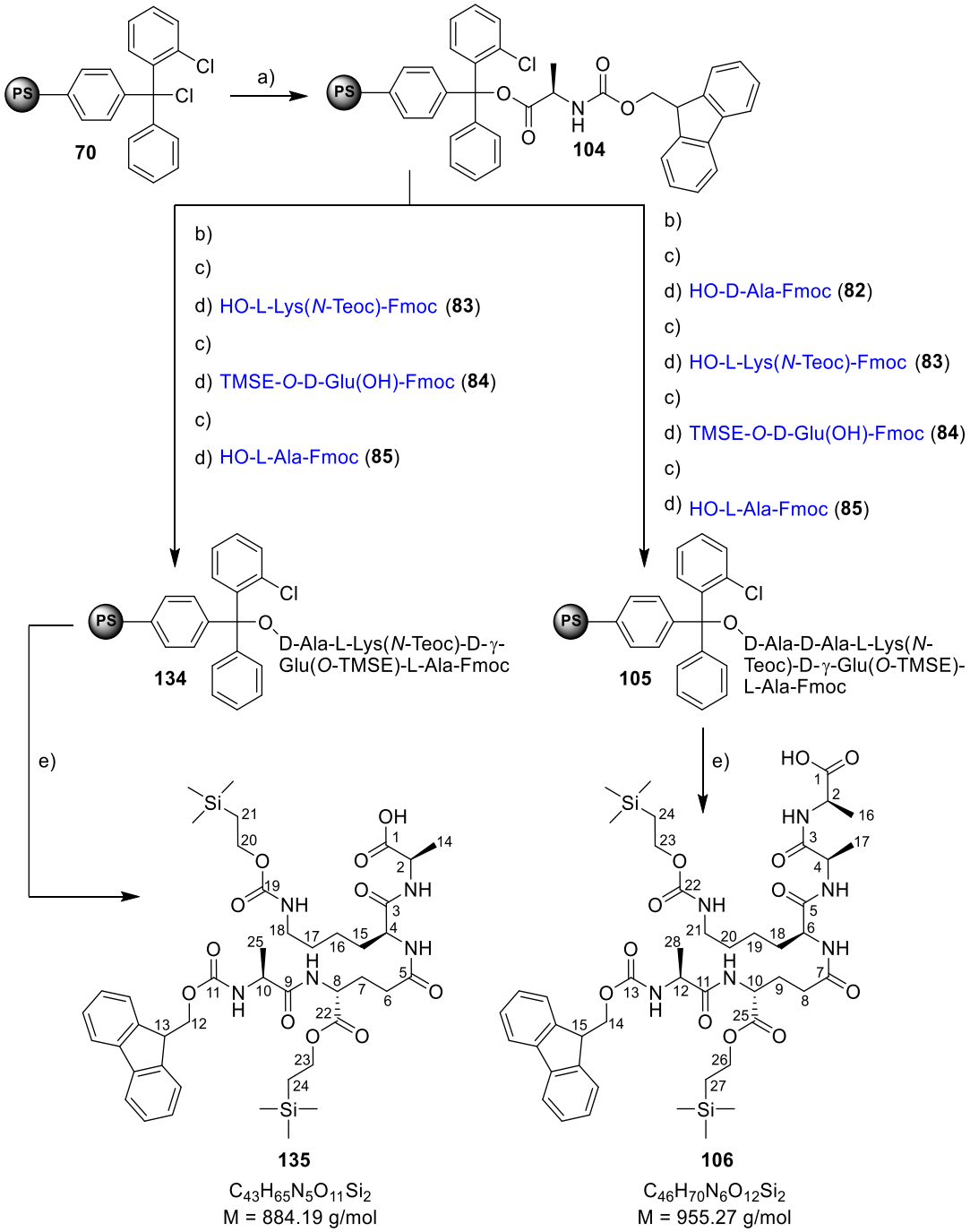
With use of the synthesized amino acid building blocks, tetrapeptide **4** was synthesized in 11 linear steps using solid phase support starting from 2-chlorotrityl chloride resin **70**. The last two steps to pentapeptide **4** were performed in solution. This approach was crucial to avoid an unfavorable epimerization processes that was observed otherwise during the attachment of the required TMSE protecting group on the terminal D-Ala-carboxylate of the corresponding pentapeptide **106** (see **Chapter 4.4.4** for more details).



***Scheme 6.2.3-1*:** Synthesis of the pentapeptide **4** starting from solid phase **70**.

Synthesis of Compound 135 and 106

N^2-((*R*)-4-((*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-propanamido)-5-oxo-5-(2-(trimethylsilyl)ethoxy)pentanoyl)-N^6-((2-(trimethylsilyl)ethoxy)carbonyl)-L-lysyl-D-alanine (tetrapeptide 135) and N^2-((*R*)-4-((*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-5-oxo-5-(2-(trimethylsilyl)ethoxy)pentanoyl)-N^6-((2-(trimethylsilyl)ethoxy)carbonyl)-L-lysyl-D-alanyl-D-alanine (pentapeptide 106)



a) Functionalization of the resin

A frit containing syringe was loaded with 2-chlorotrityl chloride resin (280 mg for tetrapeptide **135**, 100 mg for pentapeptide **106**, initial loading 2.1 mmol/g). The resin was swelled for 20 min in CH_2Cl_2 (3 mL). The solvent was removed and a solution of Fmoc-D-Ala-OH (0.50 equiv) and DIPEA (2.00 equiv) in CH_2Cl_2 (2 mL) was added. The syringe was shaken for 1 h at rt. After draining the solvent, the resin was washed with DMF (5 x 1 min shaking with 2 mL). The

initial resin loading (2.1 mmol/g) was reduced in this step to 1.0 mmol/g. The new loading was determined by UV-metric Fmoc analysis after the first deprotection. The used amounts for the coupling solutions used in *c)* were calculated with the new reduced loading of 1.0 mmol/g, which was determined as described in *c2)*.

b) Capping of free reaction sites

A solution of *N*-methylimidazol (600 µL) and acetanhydride (300 µL) in DMF (900 µL) was added to the resin and the syringe was shaken for 45 min at rt. The reaction solution was removed and the resin was washed with DMF (5 x 1 min shaking with 2 mL).

c) Deprotection (Removal of Fmoc)

The resin was shaken 5 min at rt with 20% piperidine/DMF (2 mL). The deprotection solution was removed and the resin was loaded again with 20% piperidine/DMF (2 mL) and was shaken for 15 min at rt. After removal of the deprotection solution, the resin was washed with DMF (4x1 min shaking with 2 mL), CH_2Cl_2 (3x1 min shaking with 2 mL) and again with DMF (3x1 min shaking with 2 mL).

c2) Deprotection analysis

To get the new loading after *a)* or to get predictions about the couplings from *d)* the deprotection solutions after *a)* or couplings *d)* were collected in a 5 mL volumetric flask and filled up with 20% piperidine/DMF. A dilution series was prepared and the loadings were determined using a UV/VIS-spectrometer and the following formula:

$$loading\ [mmol/g] = \frac{\mathrm{E} * \mathrm{V}_{stock} * \mathrm{D}}{\varepsilon * \mathrm{m} * \mathrm{l}}$$

With:

E = Absorption of the sample solution at 301 nm

V_{Stock} = Volume of the stock solution [mL]

D = Dilution factor

ε = Molar absorption coefficient at 301 nm = 6054 [L mol^{-1} cm^{-1}]

m = sample weight of the resin [mg]

l = optical path length of the cell = 1 [cm]

d) Coupling

The resin was loaded with a solution of amino acid (for Fmoc-L-Lys(*N*-Teoc)-OH, Fmoc-D-Glu(OH)-TMSE: 1.50 equiv and Fmoc-D-Ala-OH, Fmoc-L-Ala-OH: 4.00 equiv), HBTU (same equiv as the used amino acid), HOBt (same equiv as the used amino acid) and DIPEA (twice the equiv of the used amino acid) in DMF (2 mL). The resin was shaken 40 min at rt, washed with DMF (3x1 min shaking with 2 mL) and the resin was loaded again with a fresh reaction solution of the same amino acid and was shaken for 40 min. After this double coupling procedure, the resin was washed with DMF (5x1 min shaking with 2 mL).

e) Cleavage from the solid phase

A solution of 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in CH_2Cl_2 (3 mL) was added to the dry resin and shaken for 3 h at rt. The solvent was collected and the resin was washed further two times with 20% HFIP/CH_2Cl_2. The solvent was removed under reduced pressure and the residue dissolved in *t*BuOH/water and lyophilized to yield tetrapeptide **135** (219 mg, 229 µmol, 82%) as a colorless solid. For pentapeptide **106**, a yield of 59% (56.7 mg, 59.3 µmol) was achieved.

Analytical data for **135** (tetrapeptide):

$\mathbf{R_f}$ 0.19 (10% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = –9.0° (*c* = 0.44 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 12.48 (br, s, 1H, COOH), 8.25 (d, *J* = 7.7 Hz, 1H, NH(Glu)), 8.17 – 8.13 (m, 1H, NH(Ala)), 7.90 – 7.88 (m, 3H, 2x $H_{arom.}$, NH(Lys)), 7.75 – 7.69 (m, 2H, $H_{arom.}$), 7.47 (d, *J* = 7.9 Hz, 1H, NH(Ala)), 7.41 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.32 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 6.95 – 6.93 (m, 1H, NH(Teoc)), 4.28 – 4.24 (m, 3H, H-4, H-12), 4.22 – 4.16 (m, 4H, H-8, H-13, H-10, H-2), 4.14 – 4.10 (m, 2H, H-23), 4.01 – 3.98 (m, 2H, H-20), 2.93 – 2.89 (m, 2H, H-18), 2.22 – 2.13 (m, 2H, H-6), 1.99 – 1.92 (m, 1H, H-7), 1.81 – 1.73 (m, 1H, H-7), 1.61 – 1.55 (m, 1H, H-15), 1.47 – 1.43 (m, 1H, H-15), 1.36 – 1.32 (m, 2H, H-17), 1.24 (d, *J* = 7.2 Hz, 3H, H-14), 1.24 (d, *J* = 7.2 Hz, 3H, H-25), 1.22 – 1.17 (m, 2H, H-16), 0.95 – 0.93 (m, 2H, H-24), 0.91 – 0.88 (m, 2H, H-21), 0.01 (s, 9H, $Si(CH_3)_3$), -0.00 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 174.0 (C-1), 172.4 (C-9), 171.5 (C-22), 171.1 (C-5), 170.8 (C-3), 156.0 (C-19), 155.3 (C-11), 149.3 ($C_{arom.}$), 140.4 ($C_{arom.}$), 127.3 ($C_{arom.}H$), 126.8 ($C_{arom.}H$), 125.0 ($C_{arom.}H$), 119.8 ($C_{arom.}H$), 65.4 (C-12), 62.3 (C-23), 61.0 (C-20), 51.9 (C-4), 51.5 (C-8), 49.6 (C-10), 47.2 (C-2), 46.4 (C-13), 39.8 (C-18), 31.8 (C-15), 31.0 (C-6), 28.9 (C-17), 26.9 (C-7), 22.3 (C-16), 18.4

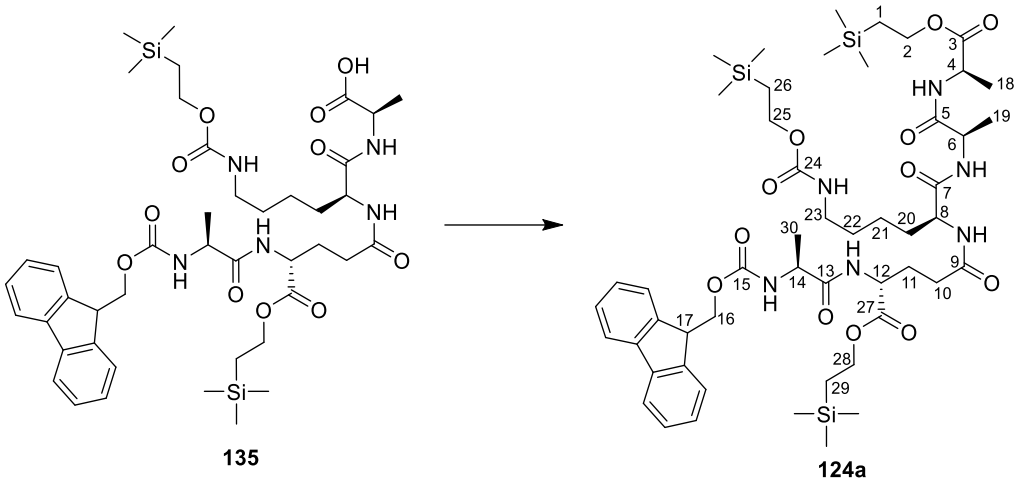
(C-14), 17.1 (C-25), 16.9 (C-21), 16.5 (C-24), –1.4 ((Lys)Si(CH_3)$_3$), –1.5 ((Glu)Si(CH_3)$_3$). **HRMS(ESI)** *m/z*: calcd for $C_{43}H_{66}N_5O_{11}Si_2$ $[M + H]^+$: 884.4279, found: 884.4279.

Analytical data for **106** (pentapeptide):

$\mathbf{R_f}$ 0.27 (10% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = –6.6° (*c* = 0.75 in MeOH). **^{1}H NMR** (500 MHz, (CD_3)$_2$SO) δ = 12.42 (br, s, 1H, COOH), 8.30 (d, *J* = 6.7 Hz, 1H, NH(Glu)), 8.12 (d, *J* = 7.8 Hz, 1H, NH(Ala)), 8.06 – 8.03 (m, 1H, NH(Ala)), 8.00 – 7.97 (m, 1H, NH(Lys)), 7.88 (d, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.72 (t, *J* = 7.8 Hz, 2H, $H_{arom.}$), 7.55 (br s, 1H, NH(Ala)), 7.41 (t, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.32 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 6.95 – 6.92 (m, 1H, NH(Teoc)), 4.32 – 4.08 (m, 12H, H-2, H-4, H-6, H-10, H-12, H-14, H-15, H-26), 4.02 – 3.98 (m, 2H, H-23), 2.94 – 2.89 (m, 2H, H-21), 2.21 – 2.14 (m, 2H, H-8), 1.96 – 1.91 (m, 1H, H-9), 1.81 – 1.78 (m, 1H, H-9), 1.60 – 1.53 (m, 1H, H-18), 1.50 – 1.43 (m, 1H, H-18), 1.35 – 1.32 (m, 2H, H-20), 1.28 – 1.23 (m, 8H, H-17, H-19, H-28), 1.19 (d, *J* = 7.1 Hz, 3H, H-16), 0.95 – 0.87 (m, 4H, H-24, H-27), 0.01 (s, 18H, Si(CH_3)$_3$). **^{13}C NMR** (126 MHz, (CD_3)$_2$SO) δ = 173.9 (C-1), 173.9 (C-3), 172.7 (C-11), 171.7 (C-25), 171.4 (C-7), 169.4 (C-5), 156.3 (C-22), 155.6 (C-13), 143.9 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}$H), 127.1 ($C_{arom.}$H), 125.3 ($C_{arom.}$H), 120.1 ($C_{arom.}$H), 65.7 (C-14), 62.6 (C-26), 61.3 (C-23), 52.7 (C-6), 51.7 (C-10), 49.9 (C-12), 47.6 (C-2), 47.5 (C-4), 46.6 (C-15), 39.5 (C-21), 31.5 (C-18), 31.2 (C-8), 29.2 (C-20), 27.0 (C-9), 22.6 (C-19), 18.6 (Ala-CH_3), 18.1 (Ala-CH_3), 17.4 (C-28), 17.1 (C-24), 16.8 (C-27), –1.4 ((Lys)Si(CH_3)$_3$), –1.5 ((Glu)Si(CH_3)$_3$). **HRMS(ESI)** *m/z*: calcd for $C_{46}H_{70}N_6O_{12}Si_2Na$ $[M + Na]^+$: 977.4482, found: 977.4478.

Synthesis of Compound 124a

2-(Trimethylsilyl)ethyl (5*S*,8*R*,13*S*,16*R*,19*R*)-1-(9*H*-fluoren-9-yl)-5,16,19-trimethyl-3,6,11,14,17-pentaoxo-8-((2-(trimethylsilyl)-ethoxy)carbonyl)-13-(4-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)butyl)-2-oxa-4,7,12,15,18-pentaazaicosan-20-oate



135 **124a**

$C_{51}H_{82}N_6O_{12}Si_3$
M = 1055.50 g/mol

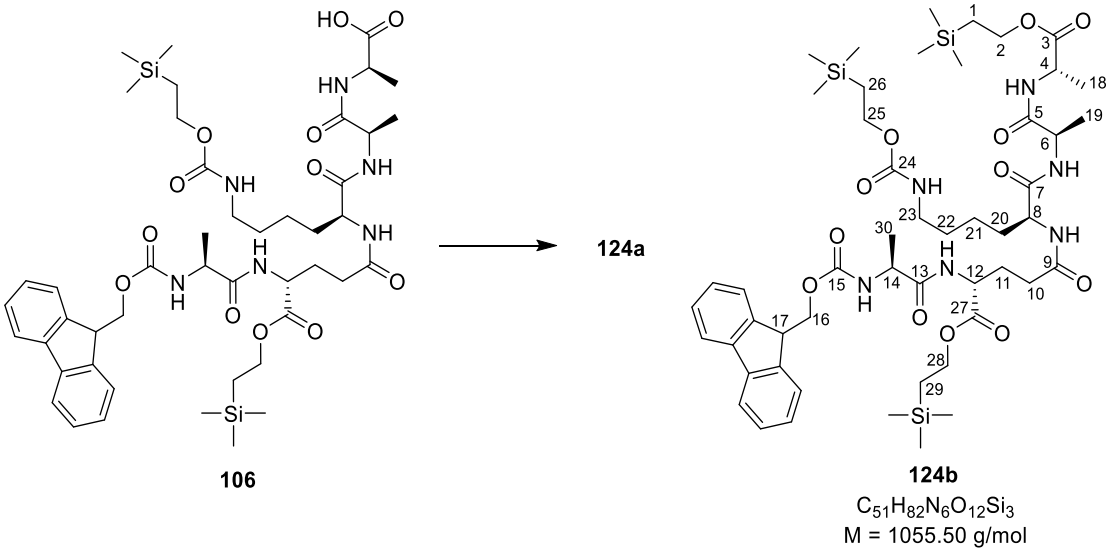
To a solution of Fmoc-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys(*N*-Teoc)-D-Ala-COOH (**135**, 25.0 mg, 28.7 µmol, 1.00 equiv), PyBOP (22.1 mg, 42.4 µmol, 1.50 equiv) and HOBt (5.73 mg, 42.4 µmol 1.50 equiv) in DMF (0.5 mL) was added dropwise a solution of H_2N-D-Ala-O-TMSE (**86**, 7.49 mg, 39.6 µmol, 1.40 equiv), DIPEA (24.0 µL, 141 µmol, 5.00 equiv) in DMF (0.5 mL). After stirring for 25 min at rt, the solvent was removed under reduced pressure and the crude product was purified by HPLC (85% MeCN/water, retention time 14.6 min, using a KNAUER Eurospher II 100-5 C18; 5 µm; 250 x 16 mm + precolumn 30 x 16 mm, 265 nm) to yield compound **124a** as a colorless solid (28.3 mg, 26.8 µmol, 95%).

R_f 0.34 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +10.2° (*c* = 0.60 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 8.24 (d, *J* = 7.7 Hz, 1H, NH(Glu)), 8.16 (d, *J* = 7.5 Hz, 1H, NH(D-Ala)), 8.14 (d, *J* = 7.5 Hz, 1H, NH(D-Ala)), 8.00 (d, *J* = 7.3 Hz, 1H, NH(Lys)), 7.89 (d, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.75 – 7.69 (m, 2H, $H_{arom.}$), 7.47 (d, *J* = 7.5 Hz, 1H, NH(L-Ala)), 7.41 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.32 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 6.94 (t, *J* = 5.4 Hz, 1H, NH(Teoc)), 4.30 (q, *J* = 7.5 Hz, 1H, H-6), 4.24 (d, *J* = 7.0 Hz, 2H, H-16), 4.21 – 4.07 (m, 12H, H-12, H-8, H-4, H-14, H-2, H-17, H-28), 4.01 – 3.99 (m, 2H, H-25), 2.92 – 2.90 (m, 2H, H-23), 2.21 – 2.12 (m, 2H, H-10), 1.95 – 1.90 (m, 1H, H-11), 1.80 – 1.75 (m, 1H, H-11), 1.59 – 1.54 (m, 1H, H-20), 1.47 – 1.44 (m, 1H, H-20), 1.35 (p, *J* = 6.8 Hz, 2H, H-22), 1.28 (d, *J* = 7.3 Hz, 3H, H-18), 1.23 (d, *J* = 7.5 Hz, 3H, H-30), 1.19 (d, *J* = 7.0 Hz, 3H, H-19), 1.26 – 1.15 (m, 2H, H-21), 0.94 – 0.88 (m, 6H, H-1, H-26, H-28), 0.01 (s, 9H, $Si(CH_3)_3$), 0.01 (s, 9H, $Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 172.2 (C-13), 171.9 (C-3), 171.3 (C-5), 171.0 (C-27), 171.0 (C-7), 171.0 (C-9), 156.2 (C-24), 155.4 (C-15), 143.6 ($C_{arom.}$), 140.6 ($C_{arom.}$), 127.5

($C_{arom.}H$), 126.9 ($C_{arom.}H$), 125.2 ($C_{arom.}H$), 120.0 ($C_{arom.}H$), 65.5 (C-16), 62.4 (C-28), 62.3 (C-2), 61.2 (C-25), 52.7 (C-8), 51.6 (C-12), 49.7 (C-14), 47.6 (C-4), 47.4 (C-6), 46.5 (C-17), 39.7 (C-23), 31.3 (C-20), 31.1 (C-11), 29.1 (C-22), 26.9 (C-11), 22.5 (C-21), 18.5 (C-30), 17.9 (C-19), 17.3 (C-26), 16.7 (C-29), 16.6 (C-1), 16.6 (C-18), –1.6 ($Si(CH_3)_3$), –1.6 ($Si(CH_3)_3$), –1.7 ($Si(CH_3)_3$). **HRMS(ESI)** *m/z*: calcd for $C_{51}H_{81}N_6O_{12}Si_3Na$ $[M + Na]^+$: 1077.5191, found: 1077.5195.

Synthesis of Compound 124b

2-(Trimethylsilyl)ethyl (5*S*,8*R*,13*S*,16*R*,19*R*)-1-(9*H*-fluoren-9-yl)-5,16,19-trimethyl-3,6,11,14,17-pentaoxo-8-((2-(trimethylsilyl)-ethoxy)carbonyl)-13-(4-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)butyl)-2-oxa-4,7,12,15,18-pentaazaicosan-20-oate



To a solution of Fmoc-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys(*N*-Teoc)-D-Ala-D-Ala-COOH (**106**, 30.0 mg, 31.4 µmol, 1.00 equiv), PyBOP (24.5 mg, 47.1 µmol, 1.50 equiv) and HOBt (6.37 mg, 47.1 µmol 1.50 equiv) in DMF (0.7 mL) was added dropwise 2-(trimethylsilyl)ethanol (8.95 µL, 62.8 µmol, 2.00 equiv) and DIPEA (26.7 µL, 157 µmol, 5.00 equiv). After stirring for 1 h at rt, the solvent was removed under reduced pressure and the crude product was purified by HPLC (85% MeCN/water, retention time 14.6 min, using a KNAUER Eurospher II 100-5 C18; 5 µm; 250 x 16 mm + precolumn 30 x 16 mm, 265 nm) to yield compound **124a** (9.50 mg, 9.00 µmol, 29%) and the diastereomer **124b** (6.80 mg, 6.44 µmol, 21%) as colorless solids.

Analytical data for compound **124a** were already given in the reaction above. Comparison of the ^{1}H NMR spectra in CD_3OD can be found in **Chapter 4.4.4**.

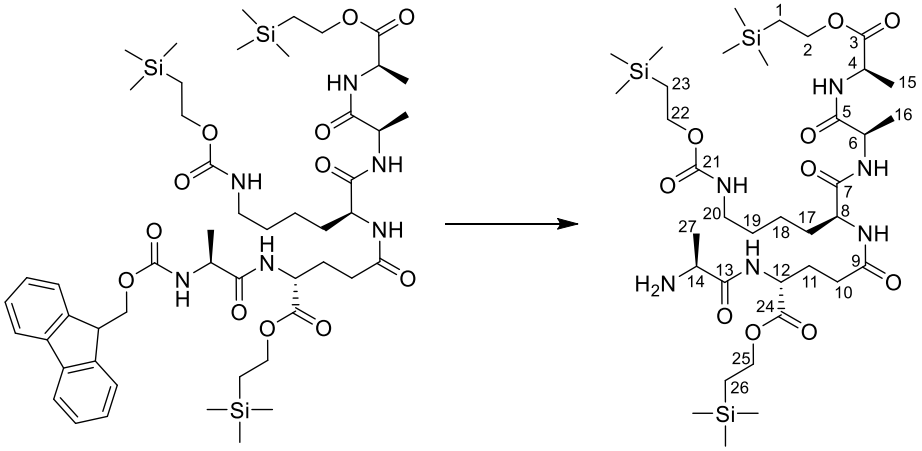
Analytical data for **124b**:

R_f 0.34 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +1.3° (*c* = 0.77 in MeOH). **^{1}H NMR** (700 MHz, CD_3OD) δ = 7.80 (d, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.68 – 7.66 (m, 2H, $H_{arom.}$), 7.39 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.32 – 7.30 (m, 2H, $H_{arom.}$), 4.39 – 4.09 (m, 14H, H-2, H-4, H-6, H-8 H-12, H-14, H-17, H-16, H-25, H-28), 3.07 – 3.05 (m, 2H, H-23), 2.28 (t, *J* = 7.0 Hz, 2H, H-10), 2.23 – 2.18 (m, 1H, H-11), 1.95 – 1.90 (m, 1H, H-11), 1.78 – 1.73 (m, 1H, H-20), 1.68 – 1.63 (m, 1H, H-20), 1.48 (p, *J* = 7.1 Hz, 2H, H-22), 1.40 – 1.36 (m, 11H, 3xAla-CH_3, H-21), 0.04 (s, 9H, $Si(CH_3)_3$), 0.03 (s, 18H, 2x$Si(CH_3)_3$). **^{13}C NMR** (176 MHz, CD_3OD) δ = 175.7 (C=O), 175.0 (C=O), 174.7 (C=O), 174.6 (C=O), 174.2 (C=O), 172.9 (C=O), 159.3 (C-24), 158.4 (C-15), 145.3 ($C_{arom.}$), 142.6 ($C_{arom.}$), 128.8 ($C_{arom.}$H), 128.2 ($C_{arom.}$H), 126.2 ($C_{arom.}$H), 121.0 ($C_{arom.}$H), 68.1 (C-4/C-6/C-14), 64.7 (C-2/C-28), 64.6 (C-2/C-28), 63.7 (C-25), 55.8 (C-16), 53.1 (C-12), 52.3 (C-4/C-6/C-14), 50.3 (C-8), 49.8 (C-4/C-6/C-14), 48.4 (C-17), 41.3 (C-23), 32.4 (C-10), 32.0 (C-20), 30.6 (C-22), 28.2 (C-11), 24.1 (C-21), 18.7 (C-26), 18.3 (CH_3), 18.3 (C-29), 18.2 (C-1), 17.9 (CH_3), 17.5 (CH_3), –1.4 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$).

HRMS(ESI) *m/z*: calcd for $C_{51}H_{81}N_6O_{12}Si_3Na$ $[M + Na]^+$: 1077.5191, found: 1077.5186.

Synthesis of Compound 4

2-(Trimethylsilyl)ethyl (12*S*,15*R*,18*R*)-12-((*R*)-4-((*S*)-2-amino-propanamido)-5-oxo-5-(2-(trimethylsilyl)ethoxy)pentanamido)-2,2,15,18-tetramethyl-6,13,16-trioxo-5-oxa-7,14,17-triaza-2-silanonadecan-19-oate



124a **4**

$C_{36}H_{72}N_6O_{10}Si_3$
M = 833.26 g/mol

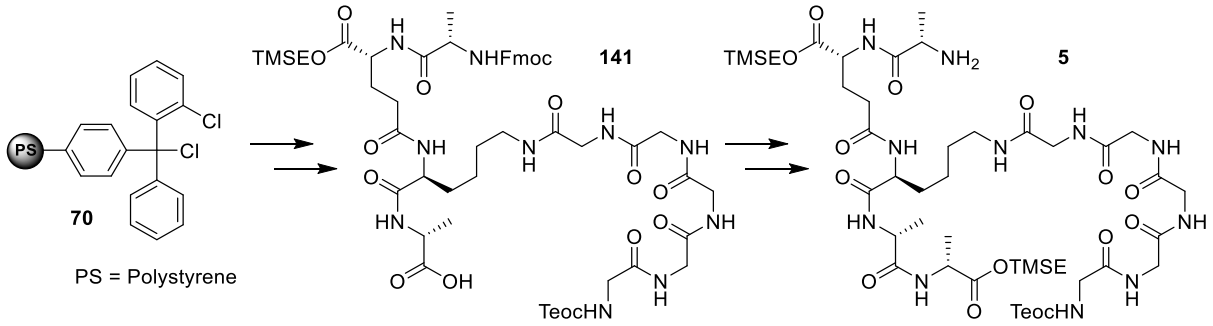
Fmoc-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys(*N*-Teoc)-D-Ala-D-Ala-O-TMSE (**124a**, 100 mg, 94.7 µmol, 1.00 equiv) was dissolved in 20% piperidine/DMF (1 mL). After stirring for 30 min at rt, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (10% MeOH/CH_2Cl_2) to yield peptide **4** as a colorless solid (60.3 mg, 72.4 µmol, 76%).

R_f 0.23 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +22.7° (*c* = 0.44 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 8.15 (d, *J* = 7.9 Hz, 1H, NH(D-Ala)), 8.14 (d, *J* = 7.9 Hz, 1H, NH(D-Ala)), 8.08 (d, *J* = 6.8 Hz, 1H, NH(Glu)), 8.00 (d, *J* = 7.3 Hz, 1H, NH(Lys)), 6.94 (t, *J* = 5.5 Hz, 1H, NH(Teoc)), 4.31 – 4.27 (m, 1H, H-6), 4.21 – 4.17 (m, 2H, H-4, H-12), 4.17 – 4.08 (m, 5H, H-8, H-2, H-25), 4.02 – 3.97 (m, 2H, H-22), 3.29 (q, *J* = 6.9 Hz, 1H, H-14), 2.91 (dt, *J* = 6.8 Hz, 2H, H-20), 2.52 (m, 2H, NH_2), 2.21 – 2.13 (m, 2H, H-10), 1.97 – 1.92 (m, 1H, H-11), 1.81 – 1.76 (m, 1H, H-11), 1.59 – 1.54 (m, 1H, H-17), 1.49 – 1.44 (m, 1H, H-17), 1.35 (p, *J* = 7.5 Hz, 2H, H-19), 1.28 (d, *J* = 7.3 Hz, 3H, H-15), 1.26 – 1.22 (m, 2H, H-18), 1.19 (d, *J* = 7.1 Hz, 3H, H-16), 1.11 (d, *J* = 6.9 Hz, 3H, H-27), 0.96 – 0.88 (m, 6H, H-1, H-23, H-26), 0.02 (s, 18H, 2xSi$(CH_3)_3$), 0.01 (s, 9H, Si$(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 175.7 (C-13), 172.1 (C-3), 171.7 (C-5), 171.6 (C-24), 171.2 (C-9), 171.1 (C-7), 156.0 (C-21) 62.3 (C-25), 62.2

(C-2), 61.0 (C-22), 52.6 (C-8), 51.1 (C-12), 49.8 (C-14), 47.4 (C-4), 47.2 (C-6), 39.7 (C-20), 31.2 (C-17), 31.0 (C-10), 28.9 (C-19), 26.9 (C-11), 22.3 (C-18), 21.3 (C-27), 17.7 (C-16), 17.1 (C-23), 16.5 (C-26), 16.5 (C-1), 16.4 (C-15), –1.7 (2xSi(CH_3)$_3$), –1.8 (Si(CH_3)$_3$), –1.8 (Si(CH_3)$_3$). **HRMS(ESI)** *m/z*: calcd for $C_{36}H_{73}N_6O_{10}Si_3$ $[M + H]^+$: 833.4690, found: 833.4690. The spectroscopic data were in agreement with those previously reported.[230]

6.2.4 Synthesis of Decapeptide 5 Including the Pentaglycine Side Chain known for *S. aureus*

This section gives experimental details towards the synthesis of decapeptide **5**, which was yielded after 21 linear steps, using solid phase supported synthesis and starting from the 2-chlorotrityl chloride resin **70**. Again, the last two steps from nonapeptide **141** to decapeptide **5** were performed in solution.



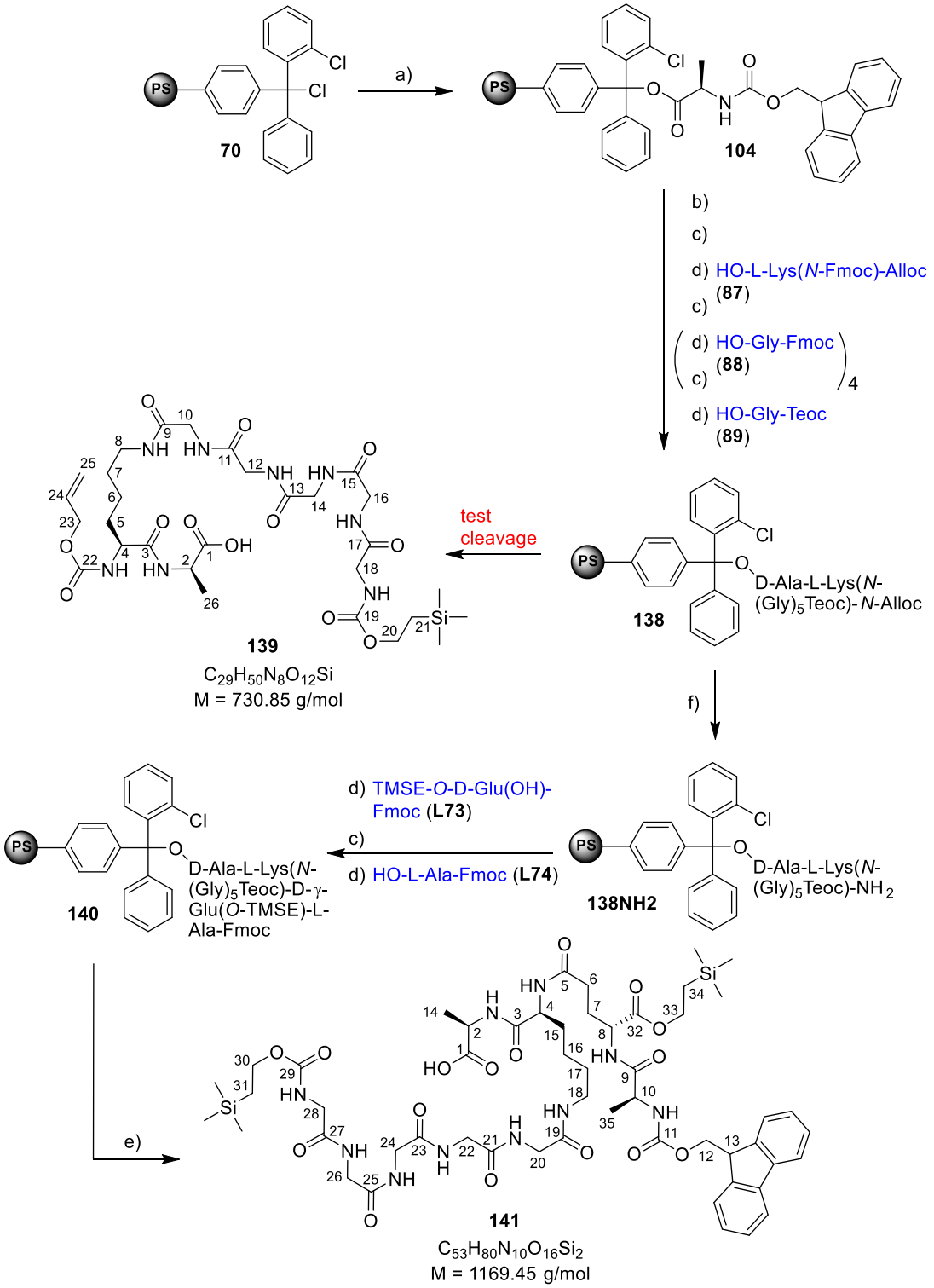
***Scheme 6.2.4.-1*:** Synthesis of the decapeptide **5** starting from solid phase **70**.

Synthesis of Compound 139 and 141

N^2-((Allyloxy)carbonyl)-N^6-((2-(trimethylsilyl)ethoxy)-carbonyl)glycylglycylglycyl-glycylglycyl-L-lysyl-D-alanine (139)

and

N^2-((*R*)-4-((*S*)-2-((((9*H*-Fluoren-9-yl)methoxy)carbonyl)amino)-propanamido)-5-oxo-5-(2-(trimethylsilyl)ethoxy)pentanoyl)-N^6-((2-(trimethylsilyl)-ethoxy)carbonyl)glycyl-glycylglycylglycylglycyl-L-lysyl-D-alanine (141)



a) Functionalization of the resin

A frit containing syringe was loaded with 2-chlorotrityl chloride resin (137 mg, initial loading 2.1 mmol/g). The resin was swelled for 20 min in CH_2Cl_2 (2 mL). The solvent was removed and a solution of Fmoc-D-Ala-OH (44.7 mg, 142 µmol, 0.50 equiv) and DIPEA (98.3 µL,

0.57 mmol, 2.00 equiv) in CH_2Cl_2 (1 mL) was added. The syringe was shaken for 1 h at rt. After draining the solvent, the resin was washed with DMF (5x1 min shaking with 2 mL). The initial resin loading 2.1 mmol/g was reduced in this step to 1.0 mmol/g. The new loading was determined by UV-metric Fmoc analysis after the first deprotection. The used amounts for the coupling solutions used in *c)* were calculated with the new reduced loading of 1.0 mmol/g, which was determined as described in *c2)*.

b) Capping of free reaction sites

A solution of *N*-methylimidazol (300 µL) and acetanhydride (150 µL) in DMF (450 µL) was added to the resin and the syringe was shaken for 45 min at rt. The reaction solution was removed and the resin was washed with DMF (5x1 min shaking with 2 mL).

c) Deprotection (Removal of Fmoc)

The resin was shaken 5 min at rt with 20% piperidine/DMF (2 mL). The deprotection solution was removed and the resin was loaded again with 20% piperidine/DMF (2 mL) and was shaken for 15 min at rt. After removal of the deprotection solution, the resin was washed with DMF (4x1 min shaking with 2 mL), CH_2Cl_2 (3x1 min shaking with 2 mL) and again with DMF (3x1 min shaking with 2 mL).

c2) Deprotection analysis

To get the new loading after *a)* or to get predictions about the couplings from *d)* the deprotection solutions after *a)* or couplings *d)* were collected in a 5 mL volumetric flask and filled up with 20% piperidine/DMF. A dilution series was prepared and the loadings were determined using a UV/VIS-spectrometer and the following formula:

$$loading\ [mmol/g] = \frac{\mathrm{E} * \mathrm{V}_{stock} * \mathrm{D}}{\varepsilon * \mathrm{m} * \mathrm{l}}$$

With:

E = Absorption of the sample solution at 301 nm

V_{Stock} = Volume of the stock solution [mL]

D = Dilution factor

ε = Molar absorption coefficient at 301 nm = 6054 [L mol^{-1} cm^{-1}]

m = sample weight of the resin [mg]
l = optical path length of the cell = 1 [cm]

d) Coupling

The resin was loaded with a solution of amino acid (for Alloc-L-Lys(*N*-Fmoc)-OH, Fmoc-D-Glu(OH)-TMSE: 1.50 equiv and Fmoc-Gly-OH, Teoc-Gly-OH, Fmoc-L-Ala-OH: 4.00 equiv), HBTU (same equiv as the used amino acid), HOBt (same equiv as the used amino acid) and DIPEA (twice the equiv of the used amino acid) in DMF (2 mL). The resin was shaken 40 min at rt, washed with DMF (3x1 min shaking with 2 mL) and the resin was loaded again with a fresh reaction solution of the same amino acid and was shaken again for 40 min. After this double coupling procedure, the resin was washed with DMF (5x1 min shaking with 2 mL).

e) Cleavage from the solid phase

A solution of 20% 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) in CH_2Cl_2 (3 mL) was added to the dry resin and shaken for 3 h at rt. The solvent was collected and the resin was washed further two times with 20% HFIP/CH_2Cl_2. The solvent was added slowly to cold ether resulting in precipitation and after centrifugation, the supernatant solvent was discarded and the precipitate dissolved in *t*BuOH/water and lyophilized to yield nonapeptide **141** as a colorless solid (133 mg, 114 µmol, 83%). After the glycine couplings and before the alloc removal, a test cleavage from the resin was performed with 15.0 mg to yield analytical amounts of the alloc protected heptapeptide **139** as a colorless solid.

f) Deprotection (Removal of alloc)

The peptide containing resin was dried at hv and flushed with argon. CH_2Cl_2 (0.5 mL) were added and the suspension was stirred 15 min at rt. Then, phenylsilane (591 µL, 4.79 mmol) in CH_2Cl_2 (1 mL) and $Pd(PPh_3)_4$ (57.6 mg, 49.9 µmol) in CH_2Cl_2 (3 mL) were added and the reaction stirred for 2 h at rt. The suspension was filled back to a frit containing syringe and the solvent was drained. The resin was washed with CH_2Cl_2 (6x1 min shaking with 3 mL), DMF (4x1 min shaking with 3 mL) and again with CH_2Cl_2 (4x1 min shaking with 3 mL). Before the next coupling, the resin was swollen again with DMF (2 mL) for 20 min at rt and the solvent was drained.

Characterization data for compound 139 (testcleavage)

$\mathbf{R_f}$ 0.30 (20% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = –3.5° (*c* = 0.28 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 8.22 – 8.16 (br m, 2H, 2xNH(Gly)), 8.10 (t, *J* = 5.2 Hz, 2H, 2xNH(Gly)), 8.04 (s, 1H, NH(Ala)), 7.73 (t, *J* = 5.6 Hz, 1H, NH(Lys)), 7.27 – 7.23 (m, 2H, NH(Lys), NH(Teoc)), 5.93 – 5.87 (m, 1H, H-24), 5.29 (dd, *J* = 17.2 Hz (*trans* coupling), 1.5 Hz, 1H, H-25), 5.17 (d, *J* = 10.5 Hz (*cis* coupling), 1.2 Hz, 1H, H-25), 4.46 (d, *J* = 5.2 Hz, 2H, H-23), 4.17 – 4.12 (m, 1H, H-2), 4.05 – 4.02 (m, 2H, H-20), 3.98 – 3.96 (m, 1H, H-4), 3.75 (d, *J* = 5.7 Hz, 2H, H-14), 3.75 (d, *J* = 5.7 Hz, 2H, H-16), 3.73 (d, *J* = 5.8 Hz, 2H, H-12), 3.65 (d, *J* = 5.9 Hz, 2H, H-10), 3.62 (d, *J* = 6.0 Hz, 2H, H-18), 3.02 (q, *J* = 6.7 Hz, 2H, H-8), 1.63 – 1.54 (m, 1H, H-5), 1.52 – 1.47 (m, 1H, H-5), 1.40 – 1.34 (m, 2H, H-7), 1.31 – 1.26 (m, 2H, H-6), 1.24 (d, *J* = 7.2 Hz, 3H, H-26), 0.93 – 0.91 (m, 2H, H-21), 0.02 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 174.0 (C-1), 171.5 (C-3), 169.7 (C-17), 169.3 (C-15), 169.3 (C-13), 169.0 (C-11), 168.4 (C-9), 156.7 (C-19), 155.7 (C-22), 133.6 (C-24), 116.9 (C-25), 64.4 (C-23), 61.9 (C-20), 54.4 (C-4), 47.7 (C-2), 43.5 (C-18), 42.1 (C-16), 42.0 (C-14), 42.0 (C-12), 42.0 (C-10), 38.3 (C-8), 31.8 (C-5), 28.6 (C-7), 22.7 (C-6), 17.6 (C-21), 17.3 (C-26), –1.4 ($Si(CH_3)_3$). **HRMS(ESI)** *m/z*: calcd for $C_{29}H_{49}N_8O_{12}Si$ $[M + H]^+$: 729.3245, found: 729.3241.

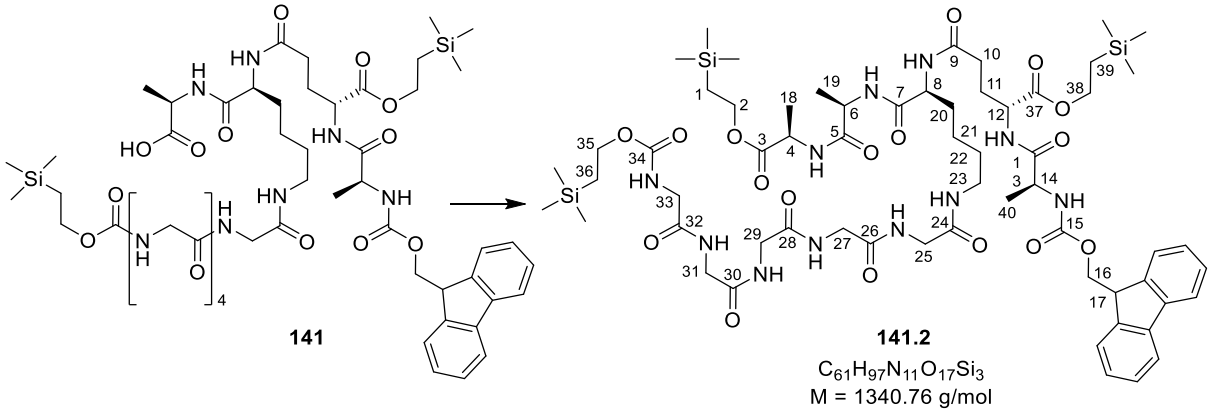
Characterization data for compound 141

$\mathbf{R_f}$ 0.21 (15% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = –2.8° (*c* = 0.70 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 8.26 (d, *J* = 7.5 Hz, 1H, NH(Glu)), 8.19 (br s, 2H, 2xNH(Gly)), 8.10 – 8.07 (m, 3H, 2xNH(Gly), NH-D-Ala), 7.91 (d, *J* = 8.2 Hz, 1H, NH(Lys)), 7.89 (d, *J* = 7.5 Hz, 2H, $H_{arom.}$), 7.74 – 7.71 (m, 3H, $NH(Lys_{sidechain})$, 2x$H_{arom.}$), 7.48 (d, *J* = 7.0 Hz, 1H, NH-L-Ala), 7.41 (t, *J* = 7.8 Hz, 2H, $H_{arom.}$), 7.32 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.23 (t, *J* = 5.9 Hz, 1H, NH(Teoc)), 4.25 – 4.10 (m, 9H, H-33, H-12, H-13, H-10, H-2, H-4, H-8), 4.04 – 4.02 (m, 2H, H-30), 3.75 (d, *J* = 5.7 Hz, 2H, H-24), 3.75 (d, *J* = 5.7 Hz, 2H, H-26), 3.73 (d, *J* = 5.8 Hz, 2H, H-22), 3.65 (d, *J* = 5.8 Hz, 2H, H-20), 3.62 (d, *J* = 5.9 Hz, 2H, H-28), 3.01 (q, *J* = 6.4 Hz, 2H, H-18), 2.22 – 2.14 (m, 2H, H-6), 1.98 – 1.93 (m, 1H, H-7), 1.81 – 1.75 (m, 1H, H-7), 1.62 – 1.57 (m, 1H, H-15), 1.47 – 1.46 (m, 1H, H-15), 1.36 (p, *J* = 7.1 Hz, 2H, H-17), 1.24 (d, *J* = 7.0 Hz, 3H, H-35), 1.23 (d, *J* = 7.3 Hz, 3H, H-14), 1.28 – 1.19 (m, 2H, H-16), 0.95 – 0.90 (m, 4H, H-31, H-34), 0.02 (s, 9H, $Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 174.0 (C-1), 172.7 (C-9), 171.7 (C-3), 171.3 (C-32), 171.1 (C-5), 169.7 (C-27), 169.3 (C-25), 169.3 (C-23), 169.0 (C-21), 168.4 (C-19), 156.7 (C-29), 155.6 (C-11), 143.8 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}H$),

127.1 ($C_{arom.}H$), 125.3 ($C_{arom.}H$), 120.1 ($C_{arom.}H$), 65.7 (C-12), 62.6 (C-33), 61.9 (C-30), 52.3 (C-4), 51.7 (C-8), 49.8 (C-10), 47.6 (C-2) 46.6 (C-13), 43.5 (C-28), 42.1 (C-24), 42.1 (C-22), 42.0 (C-26), 42.0 (C-20), 38.4 (C-18), 31.9 (C-15), 31.3 (C-6) 28.7 (C-17), 27.1 (C-7), 22.6 (C-16), 18.6 (C-14), 17.3 (C-35), 16.8 (C-31), 16.8 (C-34), –1.4 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$). **HRMS(ESI)** *m/z*: calcd for $C_{53}H_{79}N_{10}O_{16}Si_2$ $[M + H]^+$: 1167.5220, found: 1167.5216.

Synthesis of Compound 141.2

2-(Trimethylsilyl)ethyl N^2-((((9*H*-fluoren-9-yl)methoxy)-carbonyl)-L-alanyl)-N^5-((*S*)-1-oxo-1-(((*R*)-1-oxo-1-(((*R*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)amino)-6-(2-(2-(2-(2-(2-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)acet-amido)acet-amido)-acetamido)acetamido)acetamido)hexan-2-yl)-D-glutaminate



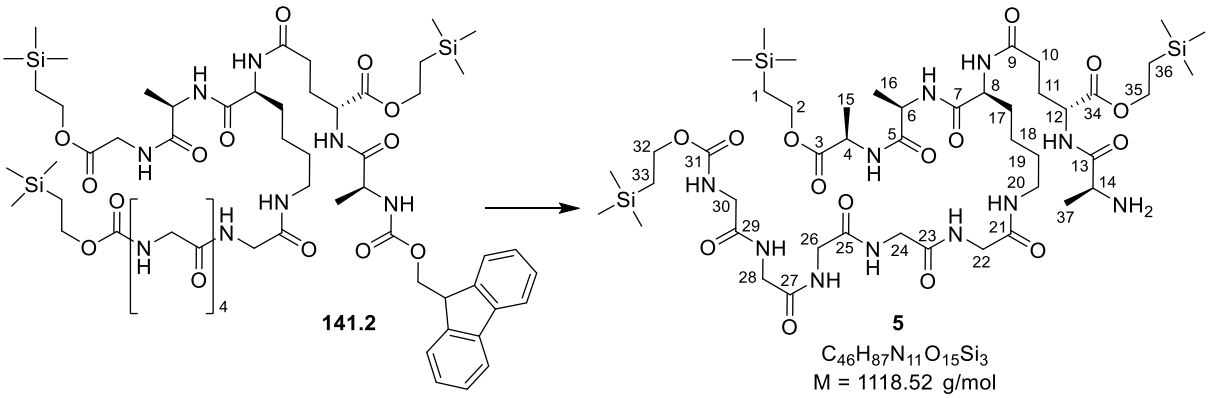
Fmoc-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys((Gly)$_5$-Teoc)-D-Ala-COOH (**141**, 60.0 mg, 51.3 µmol, 1.00 equiv), PyBOP (40.1 mg, 76.9 µmol, 1.50 equiv), HOBt (10.4 mg, 76.9 µmol, 1.50 equiv) and H_2N-D-Ala-*O*-TMSE (**86**) (13.6 mg, 71.8 µmol, 1.40 equiv) were dissolved in DMF (2 mL) and DIPEA (34.9 µL, 205 µmol, 4.00 equiv) was added immediately. After stirring for 40 min at rt, the solvent was removed under reduced pressure and the crude product was dissolved in a minimum DMF and added to ice cold diethyl ether. The formed precipitate was filtered and washed with cold ether and DCM, redissolved in MeOH and the solvent was removed under reduced pressure to yield peptide **141.2** as a colorless solid (38.5 mg, 28.7 µmol, 56%).

$\mathbf{R_f}$ 0.27 (10% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +7.4° (*c* = 0.68 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 8.24 (d, *J* = 7.6 Hz, 1H, NH(Glu)), 8.17 – 8.12 (m, 4H, 2xNH-D-Ala, 2xNH(Gly)), 8.08 – 8.04 (m, 2H, 2xNH(Gly)), 8.01 (d, *J* = 7.4 Hz, 1H, NH(Lys)), 7.89 (d, *J* =

7.6 Hz, 2H, $H_{arom.}$), 7.73 – 7.70 (m, 3H, NH($Lys_{sidechain}$), 2x$H_{arom.}$), 7.47 (d, *J* = 7.0 Hz, 1H, NH-L-Ala), 7.41 (t, *J* = 7.4 Hz, 2H, $H_{arom.}$), 7.32 (t, *J* = 7.3 Hz, 2H, $H_{arom.}$), 7.22 (t, *J* = 6.0 Hz, 1H, NH(Teoc)), 4.31 (q, *J* = 7.6 Hz, 1H, H-6), 4.25 – 4.07 (m, 11H, H-2, H-38, H-16, H-17, H-14, H-12, H-8, H-4), 4.04 – 4.02 (m, 2H, H-35), 3.75 (d, *J* = 5.6 Hz, 2H, H-31), 3.75 (d, *J* = 5.6 Hz, 2H, H-29), 3.73 (d, *J* = 5.8 Hz, 2H, H-27), 3.65 (d, *J* = 5.8 Hz, 2H, H-25), 3.62 (d, *J* = 6.0 Hz, 2H, H-33), 3.02 – 2.99 (m, 2H, H-23), 2.22 – 2.12 (m, 2H, H-10), 1.95 – 1.90 (m, 1H, H-11), 1.80 – 1.76 (m, 1H, H-11'), 1.60 – 1.56 (m, 1H, H-20), 1.50 – 1.45 (m, 1H, H-20'), 1.39 – 1.35 (m, 2H, H-22), 1.28 (d, *J* = 7.3 Hz, 3H, H-18), 1.27 – 1.20 (m, 2H, H-21), 1.23 (d, *J* = 7.1 Hz, 3H, H-40), 1.19 (d, *J* = 7.1 Hz, 3H, H-19), 0.94 – 0.90 (m, 6H, H-1, H-36, H-39), 0.02 (s, 9H, $Si(CH_3)_3$), 0.01 (s, 9H, $Si(CH_3)_3$), 0.00 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 172.7 (C-13), 172.4 (C-3), 172.0 (C-5), 171.7 (C-7), 171.5 (C-37), 171.4 (C-9), 169.7 (C-32), 169.3 (C-30), 169.3 (C-28), 169.0 (C-26), 168.4 (C-24), 156.7 (C-34), 155.6 (C-15), 143.8 ($C_{arom.}$), 140.7 ($C_{arom.}$), 127.6 ($C_{arom.}$), 127.1 ($C_{arom.}$), 125.3 ($C_{arom.}$), 120.1 ($C_{arom.}$), 65.7 (C-16), 62.6 (C-38), 62.5 (C-2), 61.9 (C-35), 52.8 (C-8), 51.7 (C-12), 49.8 (C-14), 47.7 (C-4), 47.5 (C-6), 46.6 (C-17), 43.5 (C-33), 42.1 (C-29), 42.1 (C-27), 42.0 (C-31), 42.0 (C-25), 38.4 (C-23), 31.4 (C-20), 31.2 (C-10), 28.8 (C-22), 27.0 (C-11), 22.7 (C-21), 18.7 (C-40), 18.1 (C-19), 17.3 (C-18), 16.8 (C-36), 16.7 (C-1), 16.7 (C-39), –1.4 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$). **HRMS(ESI)** *m/z*: calcd for $C_{61}H_{97}N_{11}O_{17}Si_3Na$ $[M + Na]^+$: 1362.6264, found: 1362.6238.

Synthesis of Compound 5

2-(Trimethylsilyl)ethyl-*N*^{2}-(L-alanyl)-*N*^{5}-((*S*)-1-oxo-1-(((*R*)-1-oxo-1-(((*R*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)amino)-6-(2-(2-(2-(2-(2-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)acetamido)acetamido)acetamido)acetamido)-acetamido)hexan-2-yl)-D-glutaminate



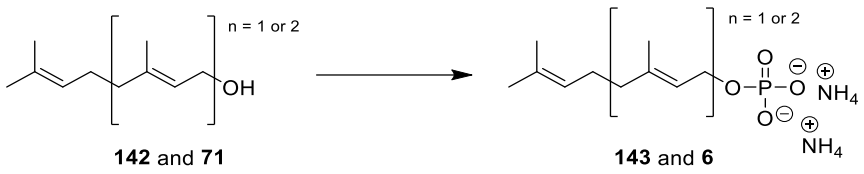
Fmoc-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys((Gly)$_5$-Teoc)-D-Ala-D-Ala-*O*-TMSE (**141.2**, 35.0 mg, 26.1 µmol, 1.00 equiv) was dissolved in 20% piperidine/DMF (2 mL). After stirring for 30 min at rt, the solvent was removed under reduced pressure. Cold diethyl ether was added and the resulting precipitate was filtered off, washed with cold diethyl ether, dissolved in MeOH and the solvent was removed under reduced pressure to yield peptide **5** as a colorless solid (25.5 mg, 22.8 µmol, 87%).

R$_f$ 0.53 (22% MeOH/CH_2Cl_2). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +10.1° (*c* = 0.99 in MeOH). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 8.22 – 8.11 (m, 5H, NH(Glu), 2xNH-D-Ala, 2xNH(Gly)), 8.07 (dt, *J* = 11.3, 5.7 Hz, 2H, 2xNH(Gly)), 8.01 (d, *J* = 7.4 Hz, 1H, NH(Lys)), 7.73 (t, *J* = 5.5 Hz, 1H, $NH(Lys_{sidechain})$), 7.22 (t, *J* = 5.9 Hz, 1H, NH(Teoc)), 4.29 (p, *J* = 7.1 Hz, 1H, H-6), 4.20 – 4.08 (m, 7H, H-2, H-4, H-8, H-12, H-35), 4.06 – 3.99 (m, 2H, H-32), 3.75 (d, *J* = 5.5 Hz, 2H, H-28), 3.75 (d, *J* = 5.5 Hz, 2H, H-26), 3.73 (d, *J* = 5.8 Hz, 2H, H-24), 3.65 (d, *J* = 5.8 Hz, 2H, H-22), 3.62 (d, *J* = 6.0 Hz, 2H, H-30), 3.29 (q, *J* = 6.9 Hz, 1H, H-14), 3.01 (q, *J* = 6.9 Hz, 2H, H-20), 2.52 (m. 2H, NH_2), 2.20 – 2.14 (m, 2H, H-10), 1.97 – 1.95 (m, 1H, H-11), 1.80 – 1.78 (m, 1H, H-11'), 1.61 – 1.56 (m, 1H, H-17), 1.49 – 1.46 (m, 1H, H-17'), 1.39 – 1.34 (m, 2H, H-19), 1.28 (d, *J* = 7.3 Hz, 3H, H-15), 1.28 – 1.19 (m, 2H, H-18), 1.19 (d, *J* = 7.1 Hz, 3H, H-16), 1.16 (d, *J* = 6.9 Hz, 3H, H-37), 0.96 – 0.91 (m, 6H, H-1, H-26, H-33), 0.02 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 172.4 (C-3), 172.0 (C-5), 171.8 (C-7), 171.5 (C-34), 171.4 (C-9), 169.7 (C-29), 169.3 (C-27), 169.3 (C-25), 169.0 (C-23), 168.4 (C-21), 156.7 (C-31), 62.7 (C-35), 62.5 (C-2), 61.9 (C-32), 52.8 (C-8), 51.5 (C-12), 49.8 (C-14), 47.7 (C-4), 47.5 (C-6), 43.5 (C-30), 42.1 (C-26), 42.1 (C-24), 42.0 (C-28), 42.0 (C-22), 38.4 (C-20), 31.4 (C-17), 31.2 (C-10), 28.8 (C-19), 27.1 (C-11), 22.7 (C-18), 20.9

(C-37) 18.0 (C-16), 17.3 (C-15), 16.8 (C-33), 16.8 (C-1), 16.7 (C-39), –1.4 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$). **HRMS(ESI)** *m/z*: calcd for $C_{46}H_{88}N_{11}O_{15}Si_3$ $[M + H]^+$: 1118.5764, found: 1118.5750.

6.2.5 Synthesis of Phospholipids 6 and 143

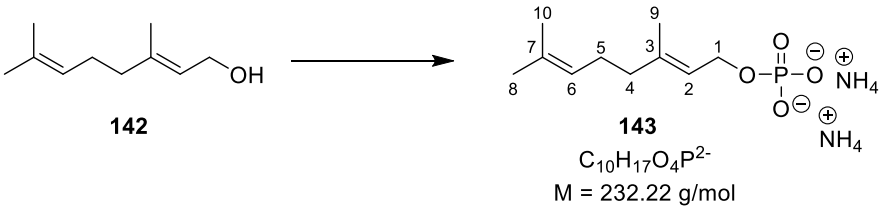
This section gives experimental details regarding the synthesis of phospholipids out of the respective, commercially available alcohols.



***Scheme 6.2.5-1*:** Synthesis of the terpene phosphates **143** and **6**.

Synthesis of Compound 143

(*E*)-3,7-Dimethylocta-2,6-dien-1-yl phosphate

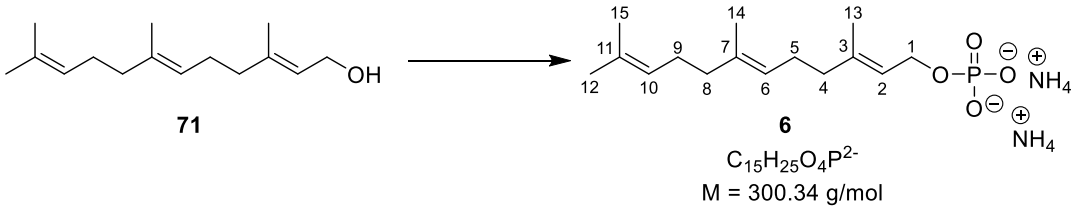


Trichloroacetonitrile (TCA, 650 µL, 6.48 mmol, 5.00 equiv) was added to geraniol (**142**, 225 µL, 1.30 mmol, 1.00 equiv). To this stirred solution was added tetrabutylammoniumphosphate (TBAP, 0.4 M in MeCN, 9.72 mL, 3.89 mmol, 3.00 equiv) over 1 h via syringe pump. The reaction was stirred for further 6 h at rt and the solvent was removed under reduced pressure. The crude product was first purified by flash chromatography (10% water/20% conc. aq. NH_3/isopropanol) and then percolated through Dowex® 50WX8 with a 0.025 M NH_4HCO_3 solution. (Dowex® 50WX8 was washed before usage with 3:1 NH_3/water and 0.025 M NH_4HCO_3 solution until the pH of the eluent turned 8.) The solvent was removed by lyophilization to yield phosphate **143** as a colorless powder (188 mg, 803 µmol, 62%).

R_f 0.12 (10% water/20% NH_3 (conc., aq.)/isopropanol). **^{1}H NMR** (700 MHz, D_2O) δ = 5.45 (tq, *J* = 1.2, 7.5 Hz, 1H, H-2), 5.21 – 5.19 (m, 1H, H-6), 4.47 (t, *J* = 6.8 Hz, 2H, H-1), 2.17 – 2.14 (m, 2H, H-5), 2.10 – 2.08 (m, 2H, H-4), 1.71 (s, 3H, H-9), 1.68 (s, 3H, H-10), 1.62 (s, 3H, H-8). **^{13}C NMR** (176 MHz, D_2O) δ = 142.7 (C-3), 133.7 (C-7), 124.1 (C-6), 119.8 (d, *J* = 8.5 Hz, C-2), 62.6 (d, *J* = 5.5 Hz, C-1), 38.7 (C-4), 25.5 (C-5), 24.7 (C-10), 16.9 (C-8), 15.5 (C-9). **^{31}P NMR** (284 MHz, D_2O) δ = 2.66. **HRMS(ESI)** *m/z*: calcd for $C_{10}H_{18}O_4P$ $[M-H]^-$: 233.0948, found: 233.0950. The spectroscopic data were in agreement with those previously reported.[80]

Synthesis of Compound 6

(2*E*,6*E*)-3,7,11-Trimethyldodeca-2,6,10-trien-1-yl phosphate



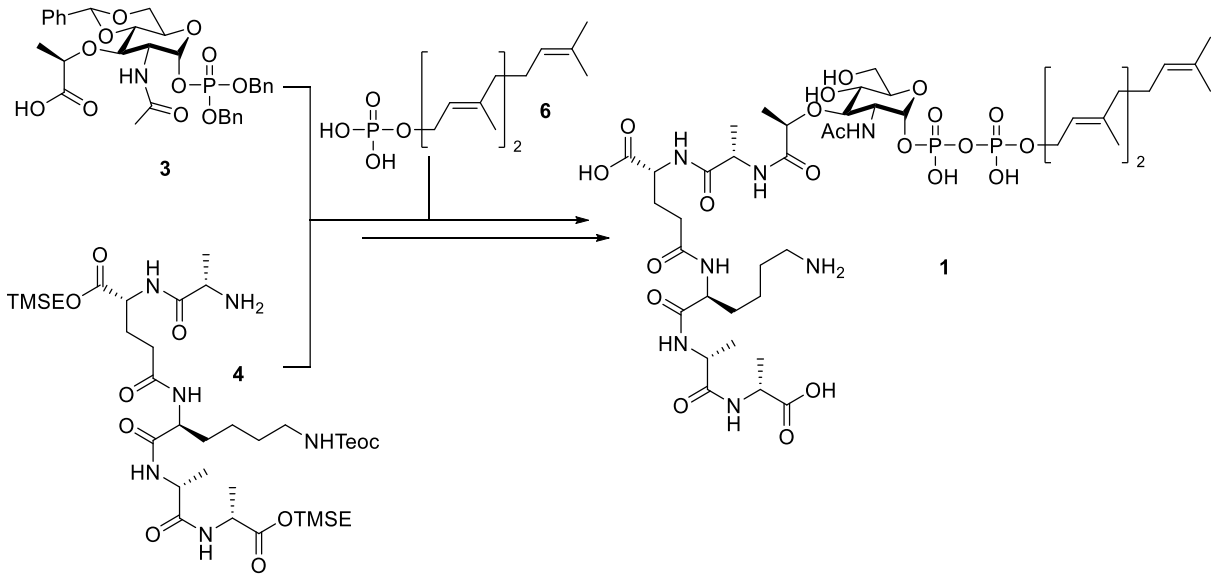
Trichloroacetonitrile (TCA, 433 µL, 4.32 mmol, 2.40 equiv) was added to farnesol (**71**, 449 µL, 1.80 mmol, 1.00 equiv). To this stirred solution was added tetrabutylammoniumphosphate (TBAP, 0.4 M in MeCN, 8.99 mL, 3.60 mmol, 2.00 equiv) over 1 h via syringe pump. The reaction was stirred for further 7 h at rt and the solvent was removed under reduced pressure. The crude product was first purified by flash chromatography (10% water/20% conc. aq. NH_3/isopropanol) and then percolated through Dowex® 50WX8 with a 0.025 M NH_4HCO_3 solution. (Dowex® 50WX8 was washed before usage with 3:1 NH_3/water and 0.025 M NH_4HCO_3 solution until the pH of the eluent turned 8.) The solvent was removed by lyophilization to yield phosphate **6** as a colorless powder (425 mg, 1.27 mmol, 71%).

R_f 0.14 (10% water/20% NH_3 (conc., aq.)/isopropanol). **^{1}H NMR** (500 MHz, D_2O) δ = 5.40 (t, *J* = 6.4 Hz, 1H, H-2), 5.12 (t, *J* = 7.5 Hz, 1H, H-6), 5.08 (t, *J* = 6.8 Hz, 1H, H-10), 4.38 (s, 2H, H-1), 2.14 – 1.88 (m, 8H, H-4, H-5, H-8, H-9), 1.69 (s, 3H, H-13), 1.64 (s, 3H, H-15), 1.58 (s, 3H, H-14), 1.56 (s, 3H, H-12). **^{13}C NMR** (126 MHz, D_2O) δ = 141.4 (C-3), 135.0 (C-7), 130.8 (C-11), 124.5 (C-10), 124.1 (C-6), 120.2 (d, *J* = 7.3 Hz, C-2), 61.9 (d, *J* = 4.8 Hz, C-1), 39.6 (C-4), 39.5 (C-5), 26.6 (C-8), 26.4 (C-9), 25.2 (C-15), 17.2 (C-12), 15.9 (C-13), 15.6 (C-14). **^{31}P NMR** (202 MHz, D_2O) δ = 0.76. **HRMS(ESI)** *m/z*: calcd for $C_{15}H_{26}O_4P$ $[M-H]^-$: 176

301.1574, found: 301.1575. The spectroscopic data were in agreement with those previously reported.[80]

6.2.6 Fragment Coupling/Endgame of Farnesyl Lipid I Analog 1

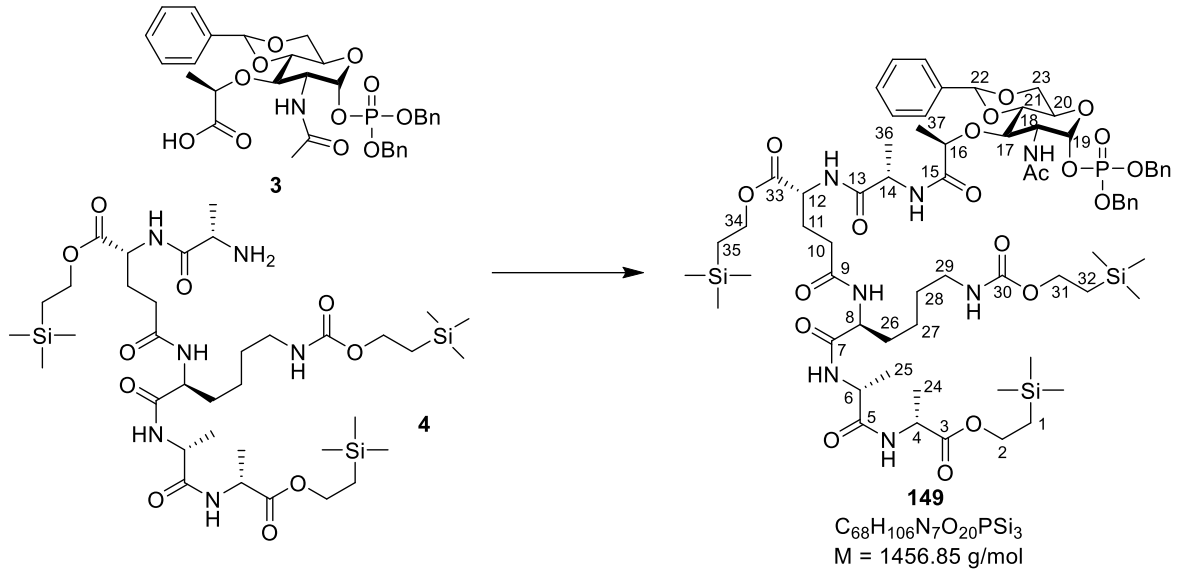
This section describes the experimental details for the connection of the three fragments (**3**, **4** and **6**) and the final modifications towards farnesyl lipid I analog **1**.



***Scheme 6.2.6-1*:** Synthesis of lipid I analog **1**.

Synthesis of Compound 149

2-(Trimethylsilyl)ethyl (2*R*,5*R*,8*S*,13*R*,16*S*,19*R*)-19-(((2*R*,4a*R*,-6*R*,7*R*,8*R*,8a*S*)-7-acetamido-6-((bis(benzyloxy)phosphoryl)oxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)-2,5,16-trimethyl-4,7,10,15,18-pentaoxo-13-((2-(trimethylsilyl)ethoxy)carbonyl)-8-(4-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)butyl)-3,6,9,14,17-pentaazaicosanoate



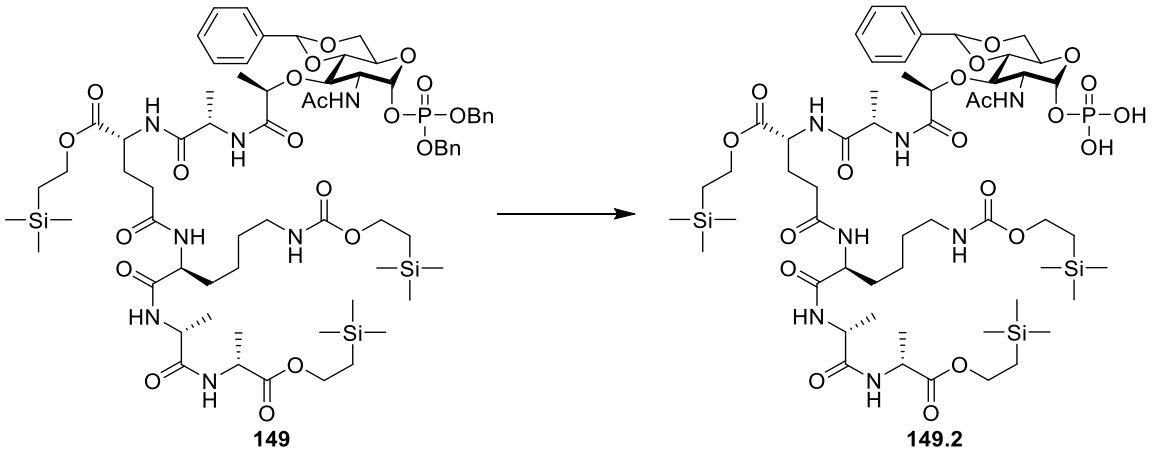
H_2N-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys(*N*-Teoc)-D-Ala-D-Ala-*O*-TMSE (**4**, 45.6 mg, 54.7 µmol, 1.30 equiv), PyBOP (32.9 mg, 63.1 µmol, 1.50 equiv), HOBt (8.53 mg, 63.1 µmol, 1.50 equiv) and carbohydrate **3** (27.0 mg, 42.1 µmol, 1.00 equiv) were dissolved in DMF (2 mL) and DIPEA (35.8 µL, 210 µmol, 5.00 equiv) was added immediately. After stirring for 45 min at rt, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (2.5% MeOH/CH_2Cl_2) to yield glycopeptide **149** as a colorless solid (49.8 mg, 34.2 µmol, 81%).

R_f 0.35 (10% MeOH/CH_2Cl_2). $[\alpha]_D^{25}$ = +40.6° (*c* = 0.32 in MeOH). **^{1}H NMR** (700 MHz, CD_3OD) δ = 7.49 – 7.48 (m, 2H, $H_{arom.}$), 7.39 – 7.33 (m, 13H, $H_{arom.}$), 5.85 (dd, *J* = 5.9, 3.5 Hz, 1H, H-19), 5.63 (s, 1H, H-22), 5.15 – 5.08 (m, 4H, 2xCH_2-Ph), 4.38 (q, *J* = 7.1 Hz, 1H, H-16), 4.38 (q, *J* = 7.1 Hz, 1H, H-6), 4.36 – 4.31 (m, 3H, H-4, H-12, H-14), 4.20 – 4.11 (m, 8H, H-2, H-8, H-18, H-31, H-34), 4.04 (dd, *J* = 9.9, 4.1 Hz, 1H, H-23), 3.85 – 3.79 (m, 3H, H-17, H-20, H-21), 3.77 (dd, *J* = 9.9, 9.9 Hz, 1H, H-23), 3.11 – 3.07 (m, 2H, H-29), 2.26 (t, *J* = 7.2 Hz, 2H, H-10), 2.22 – 2.15 (m, 1H, H-11), 1.93 – 1.89 (m, 1H, H-11‘), 1.85 (s, 3H, CH_3-NHAc), 1.79 – 1.74 (m, 1H, H-26), 1.69 – 1.63 (m, 1H, H-26), 1.48 (p, *J* = 7.0 Hz, 2H, H-28), 1.39 (d, *J* = 7.3 Hz, 3H, CH_3), 1.37 (d, *J* = 7.1 Hz, 3H, CH_3), 1.36 (d, *J* = 6.8 Hz 3H, CH_3), 1.35 (d, *J* = 7.2 Hz, 1H, CH_3), 1.37 – 1.32 (m, 2H, H-27), 1.02 – 0.95 (m, 6H, H-1, H-32, H-35), 0.04 (s, 9H, $Si(CH_3)_3$), 0.04 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, CD_3OD) δ = 175.6 (C=O), 174.9 (C=O), 174.7 (C=O), 174.6 (C=O), 174.4 (C=O), 174.1 (C=O), 173.8 (NHAc-C=O), 172.9 (C=O), 159.5 (not resolved in ^{13}C-cpd but HMBC correlation, C-40), 138.9 ($C_{arom.,quart.}$-Ph), 137.0 (d, *J* = 6.5 Hz, $C_{arom.,quart.}$-Bn), 137.0 (d, *J* = 6.5 Hz, $C_{arom.,quart.}$-

Bn), 130.0 ($C_{arom.}$), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.3 ($C_{arom.}$), 129.2($C_{arom.}$), 129.2 ($C_{arom.}$), 127.3 ($C_{arom.}$), 102.8 (C-22), 97.9 (C-19), 82.3 (C-21), 78.3 (C-16), 76.4 (C-17), 71.2 (d, *J* = 6.0 Hz, CH_2-Ph), 71.1 (d, *J* = 6.0 Hz, CH_2-Ph), 69.1 (C-23), 66.0 (C-20), 64.7 (C-2), 64.5 (C-34), 63.7 (C-31), 55.4 (C-18), 55.1 (C-8) 53.1 (C-12), 50.4 (C-14/C-4/C-6), 50.1 (C-4/C-14/C-6), 49.7 (C-6/C-14/C-4), 41.3 (C-29) 32.4 (C-10), 32.2 (C-26), 30.6 (C-28), 28.2 (C-11), 24.1 (C-27), 22.9 (NHAc-CH_3), 19.9 (CH_3), 18.7 (C-32), 18.5 (CH_3), 18.3 (C-35), 18.2 (C-1), 17.9 (CH_3), 17.4 (CH_3), –1.4 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$). **^{31}P NMR** (202 MHz, CD_3OD) δ = –3.0. **HRMS(ESI)** *m/z*: calcd for $C_{68}H_{106}N_7O_{20}Si_3PNa$ $[M + Na]^+$: 1478.6430, found: 1478.6431. The spectroscopic data were in agreement with those previously reported.[16]

Synthesis of Compound 149.2

2-(Trimethylsilyl)ethyl (2*R*,5*R*,8*S*,13*R*,16*S*,19*R*)-19-(((2*R*,3*R*,-4*R*,5*S*,6*R*)-3-acetamido-5-hydroxy-6-(hydroxymethyl)-2-(phosphonooxy)-tetrahydro-2*H*-pyran-4-yl)oxy)-2,5,16-trimethyl-4,7,10,15,18-pentaoxo-13-((2-(trimethylsilyl)ethoxy)carbonyl)-8-(4-(((2-(trimethylsilyl)ethoxy)-carbonyl)amino)butyl)-3,6,9,14,17-pentaazaicosanoate



149 → **149.2**

$C_{54}H_{94}N_7O_{20}PSi_3$

M = 1276.60 g/mol

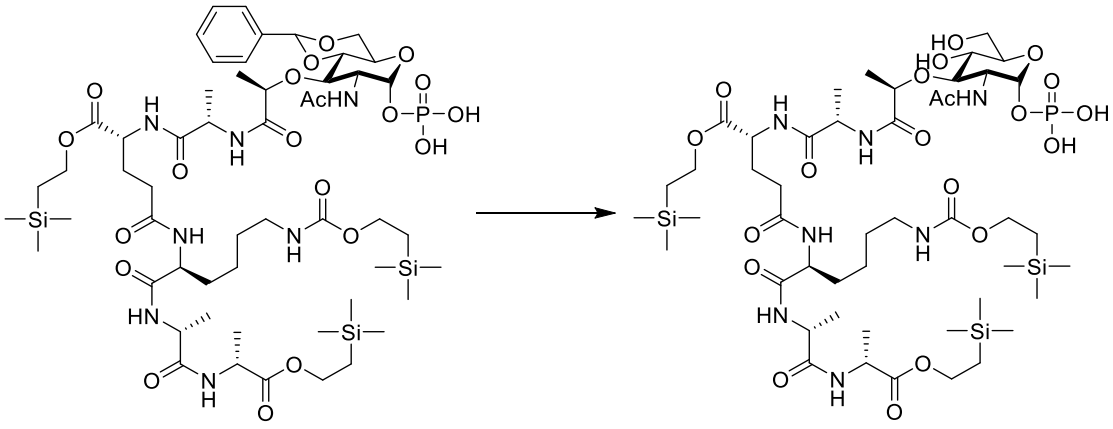
To a solution of glycopeptide **149** (35.6 mg, 24.4 µmol, 1.00 equiv) in MeOH (2.5 mL) was added Pd–C (40.0 mg, Pd-10%). The reaction vessel was filled with hydrogen. After stirring for 30 min at rt, full conversion was detected by mass spectrometry and the suspension was filtered over celite®. The precipitate was washed with MeOH and the solvent was removed

under reduced pressure to yield phosphate **149.2** as a colorless solid, which was used for the next reaction without further purification or analysis (29.0 mg, 24.4 µmol, quant.).

HRMS(ESI) *m/z*: calcd for $C_{54}H_{93}N_7O_{20}Si_3P$ [M – H]$^-$: 1274.5526, found: 1274.5532.

Synthesis of Compound 42

2-(Trimethylsilyl)ethyl (2*R*,5*R*,8*S*,13*R*,16*S*,19*R*)-19-(((2*R*,3*R*,-4*R*,5*S*,6*R*)-3-acetamido-5-hydroxy-6-(hydroxymethyl)-2-(phosphonooxy)tetrahydro-2*H*-pyran-4-yl)oxy)-2,5,16-trimethyl-4,7,10,15,18-pentaoxo-13-((2-(trimethylsilyl)ethoxy)carbonyl)-8-(4-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)butyl)-3,6,9,14,17-pentaazaicosanoate

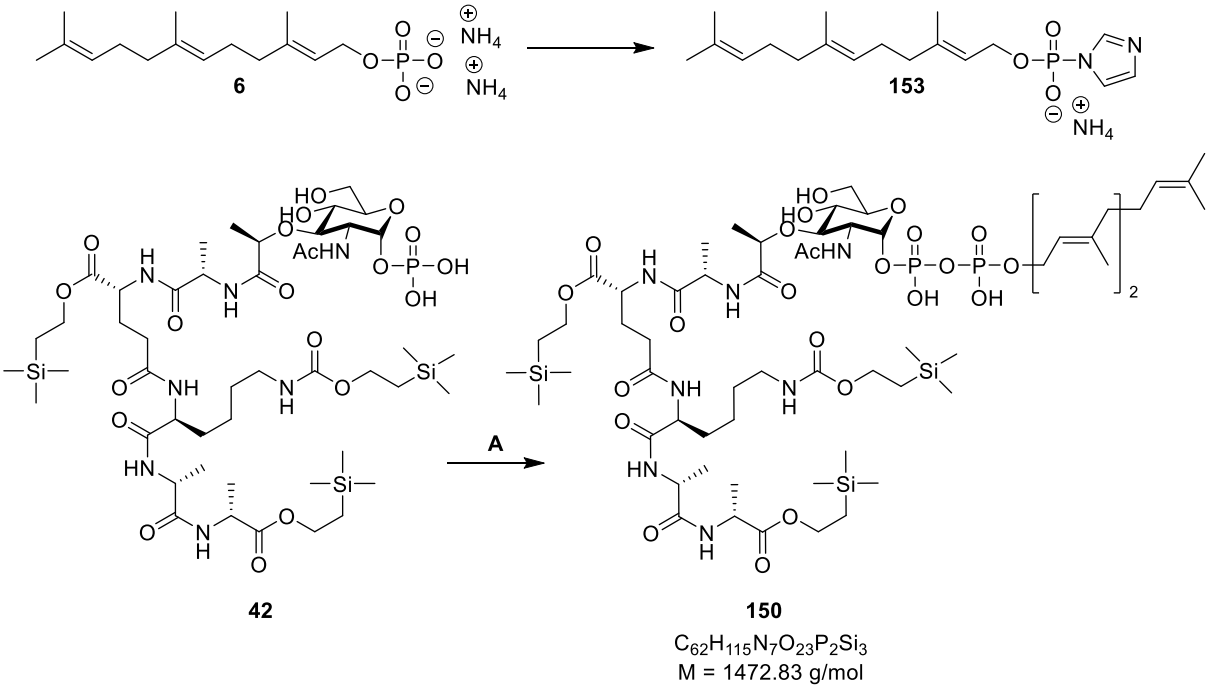


Phosphate **149.2** (27.0 mg, 21.2 µmol, 1.00 equiv) was dissolved in 80% AcOH/water (3 mL) and stirred for 2 d at rt. Mass spectrometry showed full conversion at this point and toluene (20 mL) was added. The solvent was removed under reduced pressure to yield compound **42** as a colorless solid, which was used for the next reaction without further purification or analysis (25.1 mg, 21.1 µmol, quant.).

HRMS(ESI) *m/z*: calcd for $C_{47}H_{89}N_7O_{20}Si_3P$ [M – H]$^-$: 1186.5213, found: 1186.5334.

Synthesis of Compound 150

2-(Trimethylsilyl)ethyl (2*R*,5*R*,8*S*,13*R*,16*S*,19*R*)-19-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-5-hydroxy-2-((hydroxy((hydroxy(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)phosphoryl)oxy)phosphoryl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)-2,5,16-trimethyl-4,7,10,15,18-pentaoxo-13-((2-(trimethylsilyl)ethoxy)carbonyl)-8-(4-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)butyl)-3,6,9,14,17-pentaaza-icosanoate



Farnesyl phosphate **6** (60.0 mg, 200 µmol, 1.00 equiv) was coevaporated two times from toluene (1 mL) under argon and dissolved in 50% DMF/THF (1.4 mL). Carbonyldiimidazol (CDI, 130 mg, 799 µmol, 4.00 equiv) was dissolved in 50% DMF/THF (1 ml) and added to the farnesyl phosphate solution. The reaction was stirred 2 h at rt and MeOH (35 µL) was added before stirring for further 45 min. The solvents were removed under reduced pressure and the synthesized phosphoimidazol intermediate **153** was coevaporated twice from toluene (1 mL) under argon before the addition of DMF (1 mL). Mass spectrometry showed the successfully synthesized compound **153**.

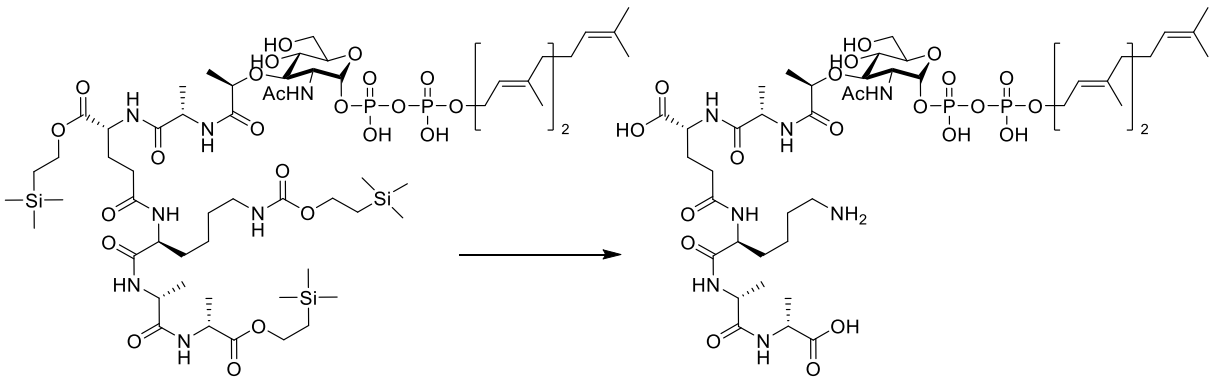
HRMS(ESI) *m/z*: calcd for $C_{18}H_{28}N_2O_3P$ $[M - H]^-$: 351.1843, found: 351.1835.

Glycopeptide **42** (28.0 mg, 23.6 µmol, 1.00 equiv) was coevaporated first from pyridine (200 µL) and then, twice from toluene (1 mL) under argon. DMF (1 mL) and the freshly prepared phosphoimidazole solution were added and the mixture was stirred 3 d at rt. The solvent was removed under reduced pressure and the crude product was semi purified by gel permeation chromatography (Sephadex LH-20, GE Healthcare, 260 x 20 mm, MeOH) to yield compound **150** as a colorless solid, which was used for the next reaction without further purification or analysis (26.8 mg, <18.2 µmol, <77%).

R_f 0.80 (4 MeOH/2 $CHCl_3$/0.5 water). **HRMS(ESI)** *m/z*: calcd for $C_{62}H_{114}N_7O_{23}Si_3P_2$ $[M - H]^-$: 1470.6755, found: 1470.6750.

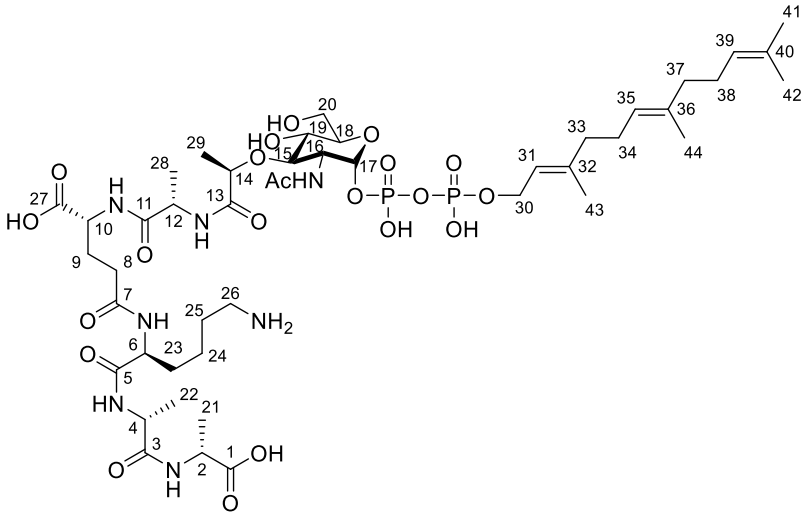
Synthesis of Compound 1

N^2-(((2*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-Acetamido-5-hydroxy-2-((hydroxy((hydroxy(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)phosphoryl)oxy)phosphoryl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((*S*)-6-amino-1-(((*R*)-1-(((*R*)-1-carboxy-ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)-D-glutamine



Protected, semi pure glycopeptide **150** (26.8 mg, <18.2 µmol, 1.00 equiv) was coevaporated from toluene (1 mL) twice and dissolved in DMF (0.5 mL). The solution was cooled to 0 °C and TBAF (1 M in THF, 419 µL, 419 µmol, 23.0 equiv) was added. The reaction mixture was allowed to warm to rt and was stirred 2 d at rt before removing the solvent under reduced pressure. The crude product was purified by gel permeation chromatography (Sephadex LH-20, GE

Healthcare, 260 x 20 mm, MeOH) and the tetrabutylammonium counterions were exchanged to ammonium ions using percolation through Dowex® 50WX8 with a 0.02 M NH_4HCO_3 solution. (Dowex® 50WX8 was washed before usage with 3:1 NH_3/water and 0.02 M NH_4HCO_3 solution until pH of the eluent turned 8.) The solvent was removed by lyophilization to yield 20.3 mg (<18.0 µmol) of the semi purified lipid I analog **1**. Final purification was achieved by HPLC (25% – 50 % NH_4HCO_3 (0.1% aq.)/MeOH, retention time 8.9 min, using a KNAUER Eurospher II 100-5 C8; 5 µm; 250 x 16 mm + precolumn 30 x 16 mm, 205 nm) to yield farnesyl lipid I analog **1** as a colorless solid (10.7 mg, 7.28 µmol, 40% over four steps).

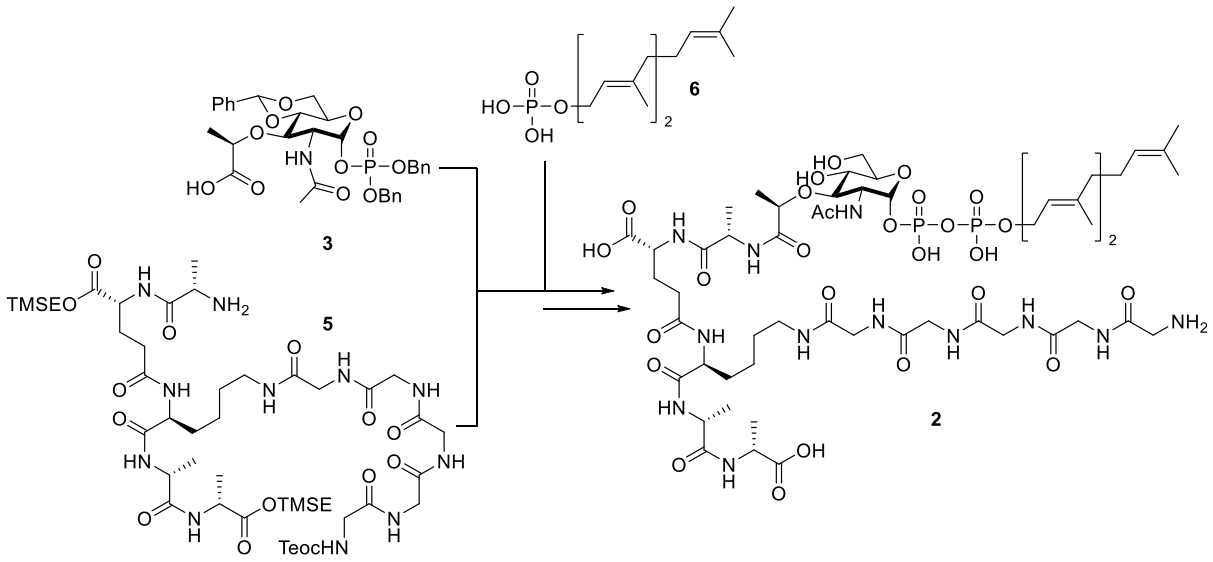


R_f 0.46 (3 MeOH/3 $CHCl_3$/1 water). $[\alpha]_D^{25}$ = +58.8° (*c* = 0.51 in MeOH). **^{1}H NMR** (500 MHz, D_2O) δ = 5.50 – 5.47 (m, 1H, H-17), 5.46 (t, *J* = 7.1 Hz, 1H, H-31), 5.22 (t, 1H, *J* = 6.5 Hz, H-35), 5.20 (t, *J* = 7.8 Hz, 1H, H-39), 4.54 – 4.49 (m, 2H, H-30), 4.35 (q, *J* = 7.2 Hz, 1H, H-4), 4.29 (q, *J* = 7.2 Hz, 1H, H-12), 4.25 – 4.22 (m, 2H, H-6, H-14), 4.20 – 4.10 (m, 3H, H-2, H-10, H-16), 3.98 – 3.96 (m, 1H, H-18), 3.92 – 3.84 (m, 2H, H-20), 3.80 (dd, *J* = 9.6, 9.6 Hz, 1H, H-15), 3.66 (dd, *J* = 9.6, 9.6 Hz, 1H, H-19), 3.02 (t, *J* = 7.5 Hz, 2H, H-26), 2.32 (t, *J* = 7.8 Hz, 2H, H-8), 2.20 – 2.15 (m, 3H, H-9, H-34), 2.15 – 2.11 (m, 4H, H-33, H-38), 2.04 (t, *J* = 7.1 Hz, 2H, H-37), 2.02 (s, 3H, CH_3-NHAc), 1.93 – 1.89 (m, 1H, H-9), 1.87 – 1.77 (m, 2H, H-23), 1.74 (s, 3H, H-43), 1.72 – 1.70 (m, 2H, H-25), 1.70 (s, 3H, H-42), 1.64 (s, 6H, H-41, H-44), 1.52 – 1.47 (m, 2H, H-24), 1.46 (d, *J* = 7.3 Hz, 3H, H-28), 1.43 (d, *J* = 6.8 Hz, 3H, H-29), 1.39 (d, *J* = 7.2 Hz, 3H, H-22), 1.35 (d, *J* = 7.2 Hz, 3H, H-21). **^{13}C NMR** (176 MHz, D_2O) δ =179.8 (C-1), 177.6 (C-27), 175.8 (C-11), 175.7 (C-7), 174.1 (C-5), 174.1 (NHAc-C=O), 174.0 (C-3), 173.6 (C-13), 143.2 (C-32), 136.7 (C-36), 133.4 (C-40), 124.4 (C-39), 124.2 (C-35), 119.3 (C-31), 94.7 (C-17), 79.9 (C-15), 78.0 (C-4), 73.0 (C-18), 68.0 (C-19), 63.1 (C-30), 60.3 (C-20), 54.2

(C-10), 54.2 (C-6) 53.4 (C-16), 51.0 (C-2), 49.9 (C-12), 49.6 (C-14), 39.1 (C-26), 38.8 (C-33), 38.8 (C-37), 31.7 (C-8), 30.5 (C-23), 28.1 (C-9), 26.3 (C-25), 25.8 (C-38), 25.5 (C-34), 24.9 (C-42), 22.2 (NHAc-CH_3), 22.0 (C-24), 18.6 (C-22), 17.4 (C-21), 17.0 (C-41), 16.8 (C-28), 16.5 (C-29), 15.7 (C-43), 15.3 (C-44). **^{31}P NMR** (284 MHz, D_2O) δ = –10.9 (d, *J* = 14.0 Hz), –13.4 (d, *J* = 14.0 Hz). **HRMS(ESI)** *m/z*: calcd for $C_{46}H_{78}N_7O_{21}P_2$ $[M - H]^-$: 1126.4731, found: 1126.4715. The spectroscopic data were in agreement with those previously reported.[68]

6.2.7 Fragment Coupling/Endgame of Farnesyl Lipid I Analog 2, Containing the Pentaglycine Side Chain known for *S. aureus*

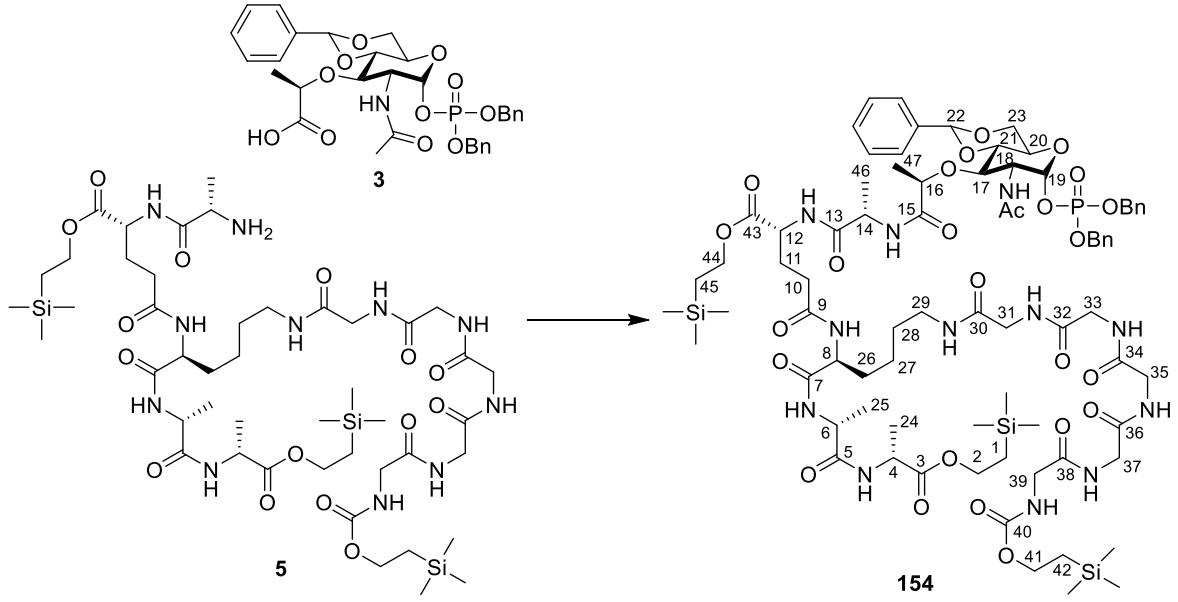
This section gives the experimental details for the connection of the three fragments (**3**, **5** and **6**) and the final modifications towards farnesyl lipid I analog **2**, which includes the interpeptidic pentaglycine modification known from *S. aureus*.



***Scheme 6.2.7-1*:** Synthesis of lipid I analog **2**.

Synthesis of Compound 154

N^2-(((*R*)-2-(((2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7-Acetamido-6-((bis(benzyloxy)phosphoryl)oxy)-2-phenylhexahydropyrano-[3,2-*d*][1,3]dioxin-8-yl)oxy)propanoyl)-L-alanyl)-N^5-((*S*)-1-oxo-1-(((*R*)-1-oxo-1-(((*R*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)amino)-6-(2-(2-(2-(2-(2-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)acet-amido)acet-amido)acetamido)acetamido)acetamido)hexan-2-yl)-D-glutaminate



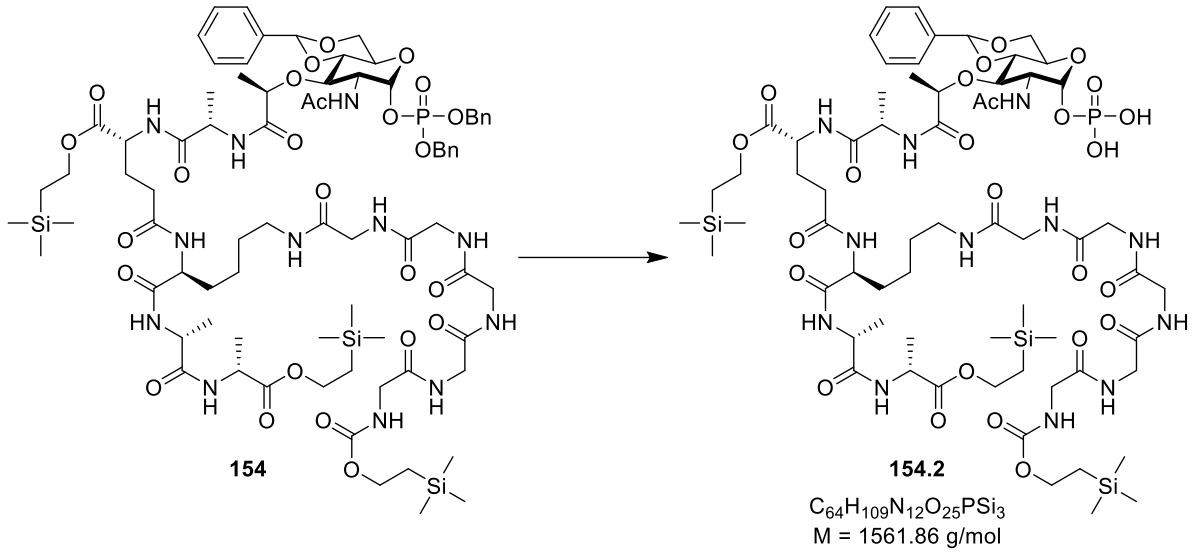
H_2N-L-Ala-γ-D-Glu(*O*-TMSE)-L-Lys((Gly)$_5$-Teoc)-D-Ala-D-Ala-*O*-TMSE (**5**, 21.1 mg, 18.9 µmol, 1.10 equiv), PyBOP (13.4 mg, 25.7 µmol, 1.50 equiv), HOBt (3,47 mg, 25.7 µmol, 1.50 equiv) and acid **3** (11.0 mg, 17.4 µmol, 1.00 equiv) were dissolved in DMF (1.5 mL) and DIPEA (11.7 µL, 68.6 µmol, 4.00 equiv) was added immediately. After stirring for 45 min at rt, the solvent was removed under reduced pressure. Cold EtOAc was added and the resulted precipitate was filtered off, washed with cold EtOAc, dissolved in MeOH and the solvent was removed under reduced pressure to yield glycopeptide **154** as a colorless solid (26.0 mg, 14.9 µmol, 86%).

R_f 0.27 (10% MeOH/CH_2Cl_2). **$[\alpha]_D^{25}$** = +25.0° (*c* = 0.36 in MeOH). **^{1}H NMR** (700 MHz, CD_3OD) δ = 7.49 – 7.47 (m, 2H, $H_{arom.}$), 7.43 – 7.34 (m, 13H, $H_{arom.}$), 5.84 (dd, *J* = 5.8, 3.6 Hz, 1H, H-19), 5.63 (s, 1H, H-22), 5.15 – 5.08 (m, 4H, 2xCH_2-Ph), 4.38 (q, *J* = 7.1 Hz, 1H, H-16), 4.38 (q, *J* = 7.1 Hz, 1H, H-6), 4.35 – 4.30 (m, 3H, H-4, H-12, H-14), 4.20 – 4.12 (m, 8H, H-2,

H-8, H-18, H-41, H-44), 4.03 (dd, *J* = 9.8, 4.0 Hz, 1H, H-23), 3.92 – 3.77 (m, 15H, H-17, H-20, H-21, H-23, H-31, H-33, H-35, H-37, H-39), 3.21 – 3.19 (m, 2H, H-29), 2.28 (t, *J* = 7.2 Hz, 2H, H-10), 2.19 – 2.12 (m, 1H, H-11), 1.91 – 1.87 (m, 1H, H-11‘), 1.85 (s, 3H, CH_3-NHAc), 1.77 – 1.72 (m, 1H, H-26), 1.68 – 1.62 (m, 1H, H-26‘), 1.56 – 1.49 (m, 2H, H-28), 1.40 (d, *J* = 7.2 Hz, 3H, CH_3), 1.37 (d, *J* = 7.2 Hz, 3H, CH_3), 1.37 – 1.32 (m, 2H, H-27) 1.35 (d, *J* = 7.2 Hz, 3H, CH_3), 1.34 (d, *J* = 6.8 Hz, 3H, CH_3), 1.02 – 0.94 (m, 6H, H-1, H-42, H-45), 0.05 (s, 9H, $Si(CH_3)_3$), 0.04 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$). **^{13}C NMR** (176 MHz, CD_3OD) δ = 175.6 (C=O), 174.9 (C=O), 174.7 (C=O), 174.6 (C=O), 174.5 (C=O), 174.1 (C=O), 173.8 (NHAc-C=O), 173.4 (C=O), 172.9 (C=O), 172.7 (C=O), 172.7 (C=O), 172.1 (C=O), 171.5 (C=O), 159.4 (C-40), 138.9 ($C_{arom.,quart.}$-Ph), 137.0 (d, *J* = 6.5 Hz, $C_{arom.,quart.}$-Bn), 137.0 (d, *J* = 6.5 Hz, $C_{arom.,quart.}$-Bn), 130.1 ($C_{arom.}$), 130.0 ($C_{arom.}$), 129.9 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.8 ($C_{arom.}$), 129.3 ($C_{arom.}$), 129.2 ($C_{arom.}$), 129.2 ($C_{arom.}$), 127.3 ($C_{arom.}$), 102.8 (C-22), 97.9 (C-19), 82.3 (C-21), 78.4 (C-16), 76.5 (C-17), 71.2 (d, *J* = 6.0 Hz, CH_2-Ph), 71.1 (d, *J* = 6.0 Hz, CH_2-Ph), 69.1 (C-23), 66.0 (C-20), 64.7 (C-2), 64.5 (C-44), 64.5 (C-41), 55.5 (C-18), 55.0 (C-8), 53.2 (C-12), 50.3 (C-14/C-4/C-6), 50.1 (C-4/C-14/C-6), 49.7 (C-6/C-14/C-4), 45.0 (CH_2-Gly), 44.0 (CH_2-Gly), 43.9 (CH_2-Gly), 43.8 (CH_2-Gly), 43.7 (CH_2-Gly), 40.0 (C-29), 32.4 (C-10), 32.2 (C-26), 29.8 (C-28), 28.2 (C-11), 24.1 (C-27), 22.9 (NHAc-CH_3), 19.9 (CH_3), 18.7 (C-42), 18.5 (CH_3), 18.3 (C-45), 18.2 (C-1), 18.0 (CH_3), 17.4 (CH_3), –1.4 ($Si(CH_3)_3$), –1.4 ($Si(CH_3)_3$), –1.5 ($Si(CH_3)_3$). **^{31}P NMR** (284 MHz, CD_3OD) δ = –2.6. **HRMS(ESI)** *m/z*: calcd for $C_{78}H_{121}N_{12}O_{25}Si_3PNa$ $[M + Na]^+$: 1763.7503, found: 1763.7525.

Synthesis of Compound 154.2

2-(Trimethylsilyl)ethyl *N*^{2}-(((*R*)-2-(((2*R*,4a*R*,6*R*,7*R*,8*R*,8a*S*)-7-acetamido-2-phenyl-6-(phosphonooxy)hexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propanoyl)-L-alanyl)-*N*^{5}-((*S*)-1-oxo-1-(((*R*)-1-oxo-1-(((*R*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)amino)-6-(2-(2-(2-(2-(2-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)acetamido)acet-amido)-acetamido)acetamido)acetamido)hexan-2-yl)-D-glutaminate

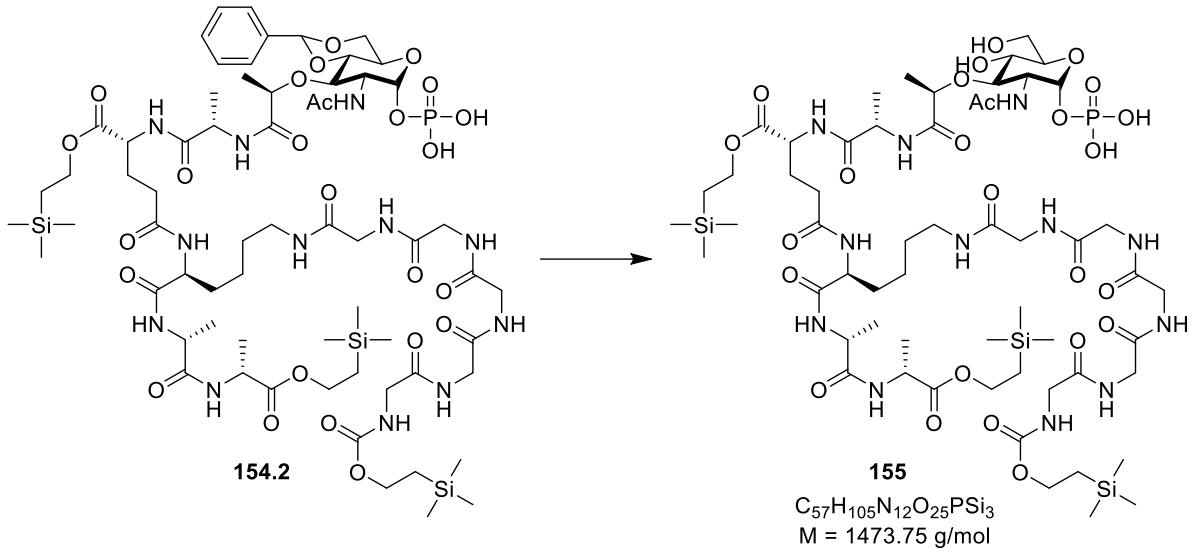


To a solution of glycopeptide **154** (24.2 mg, 13.9 µmol, 1.00 equiv) in MeOH (2 mL) was added Pd–C (25.0 mg, Pd-10%). The reaction vessel was filled with hydrogen. After stirring for 30 min at rt, full conversion was detected by mass spectrometry and the suspension was filtered over celite®. The catalyst was washed with MeOH and the solvent was removed under reduced pressure to yield phosphate **154.2** as a colorless solid, which was used for the next reaction without further purification or analysis (19.5 mg, 12.5 µmol, 90%).

HRMS(ESI) *m/z*: calcd for $C_{64}H_{108}N_{12}O_{25}Si_3P$ $[M - H]^-$: 1559.6599, found: 1559.6516.

Synthesis of Compound 155

2-(Trimethylsilyl)ethyl N^2-(((*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-5-hydroxy-6-(hydroxymethyl)-2-(phosphonooxy)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((*S*)-1-oxo-1-(((*R*)-1-oxo-1-(((*R*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)propan-2-yl)amino)-6-(2-(2-(2-(2-(2-(((2-(trimethylsilyl)ethoxy)carbonyl)amino)acetamido)-acetamido)acetamido)acetamido)acetamido)hexan-2-yl)-D-glutaminate

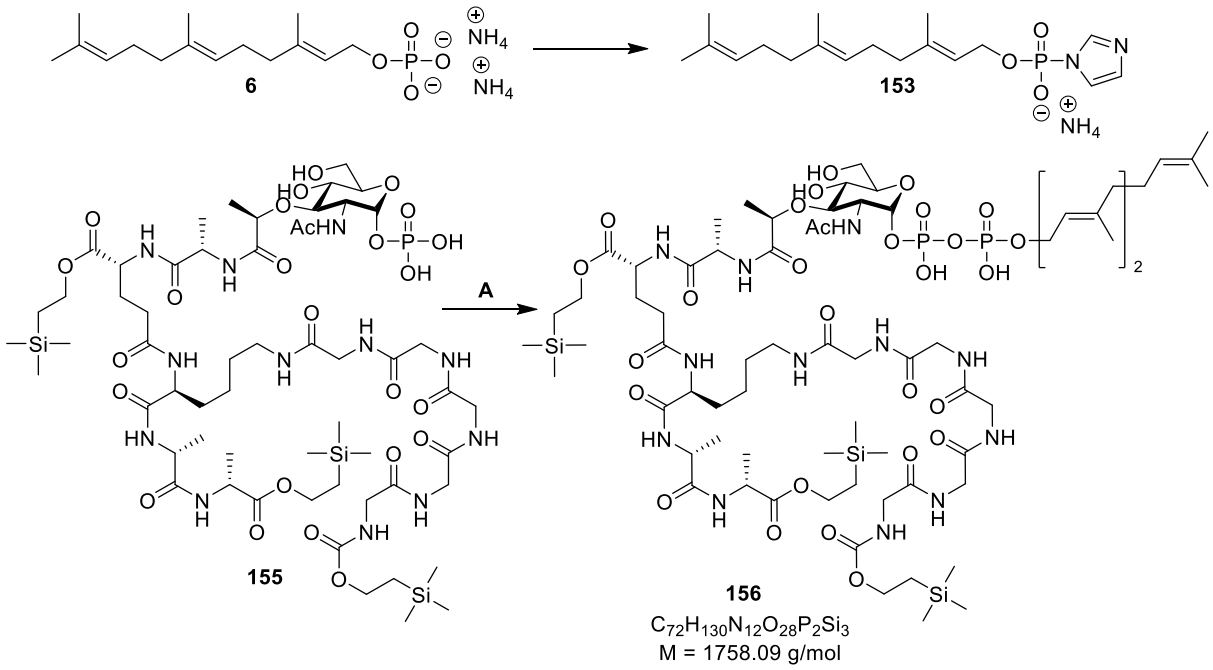


Acetal **154.2** (16.7 mg, 10.7 µmol, 1.00 equiv) was dissolved in 80% AcOH/water (3 mL) and stirred for 2 d at rt. Mass spectrometry showed full conversion at this point and toluene (30 mL) was added. The solvent was removed under reduced pressure to yield compound **155** as a colorless solid, which was used for the next reaction without further purification or analysis (15.7 mg, 10.7 µmol, quant.).

HRMS(ESI) *m/z*: calcd for $C_{57}H_{104}N_{12}O_{25}Si_3P$ $[M - H]^-$: 1471.6286, found: 1471.6201.

Synthesis of Compound 156

2-(Trimethylsilyl)ethyl N^2-(((2*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-5-hydroxy-2-((hydroxyl-((hydroxy(((2*E*,6*E*)-3,7,11-trimethyl-dodeca-2,6,10-trien-1-yl)oxy)phosphoryl)oxy)-phosphoryl)oxy)-6-(hydroxyl-methyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((S)-1-oxo-1-(((*R*)-1-oxo-1-(((*R*)-1-oxo-1-(2-(trimethylsilyl)ethoxy)propan-2-yl)amino)-propan-2-yl)amino)-6-(2-(2-(2-(2-(2-(((2-(trimethylsilyl)ethoxy)carbonyl)-amino)acetamido)acetamido)acetamido)acetamido)acetamido)hexan-2-yl)-D-glutaminate



Farnesyl phosphate **6** (27.7 mg, 92.1 µmol, 1.00 equiv) was coevaporated from toluene (1 mL) two times and dissolved in 50% DMF/THF (1.4 ml). Carbonyldiimidazol (CDI, 59.7 mg, 368 µmol, 4.00 equiv) was dissolved in 50% DMF/THF (1 ml) and added to the farnesyl phosphate solution. The reaction was stirred 2 h at rt and MeOH (16 µL) was added before stirring further 45 min. The solvents were removed under reduced pressure and the synthesized phosphoimidazol intermediate **153** was coevaporated from toluene (1 mL) twice before the addition of DMF (1 mL). Mass spectrometry showed the successfully synthesized compound **153**.

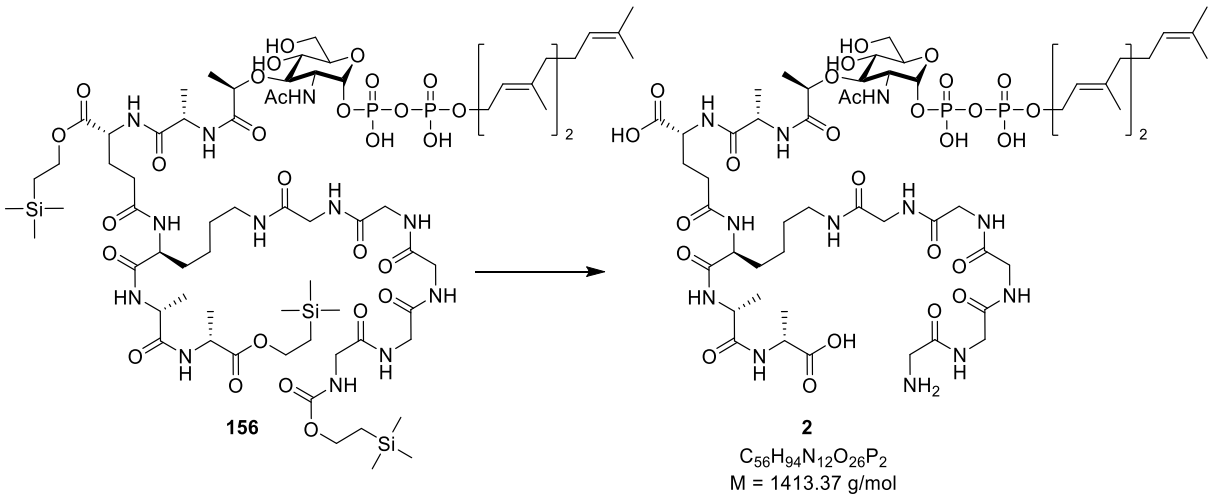
HRMS(ESI) *m/z*: calcd for $C_{18}H_{28}N_2O_3P$ $[M - H]^-$: 351.1843, found: 351.1835.

In the meantime, phosphate **155** (15.7 mg, 10.7 µmol, 1.00 equiv) was coevaporated first from pyridine (95 µL) and then twice from toluene (1 mL) under argon. DMF (0.5 mL) and the freshly prepared phosphoimidazole solution were added and the reaction was stirred 3 d at rt. The solvent was removed under reduced pressure and the crude product was semi purified by gel permeation chromatography (Sephadex LH-20, GE Healthcare, 260 x 20 mm, MeOH) to yield compound **156** as a colorless solid, which was used for the next reaction without further purification or analysis (10.9 mg, <6.20 µmol, <58%).

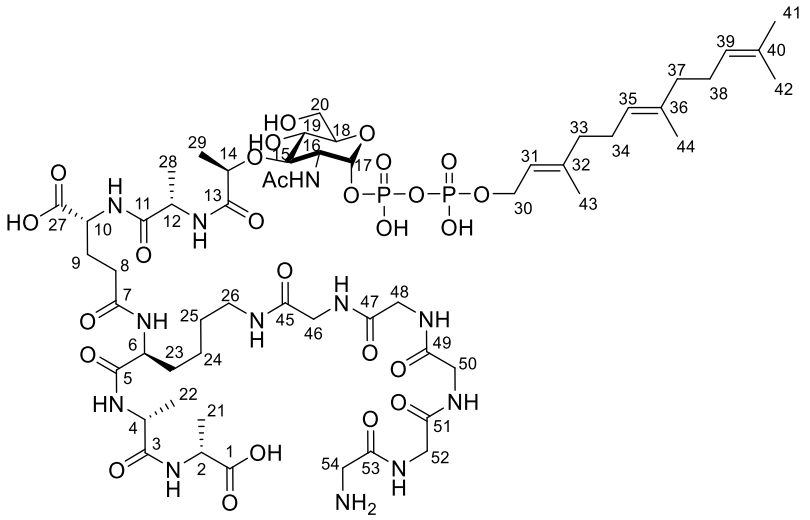
$\mathbf{R_f}$ 0.62 (4 MeOH/2 $CHCl_3$/0.5 water). **HRMS(ESI)** *m/z*: calcd for $C_{72}H_{129}N_{12}O_{28}Si_3P_2$ $[M - H]^-$: 1755.7828, found: 1755.7826, calcd for $C_{72}H_{128}N_{12}O_{28}Si_3P_2$ $[M - 2H]^{2-}$: 877.3877, found: 877.3879.

Synthesis of Compound 2

N^2-(((2*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-Acetamido-5-hydroxy-2-((hydroxy((hydroxy(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)-phosphoryl)oxy)phosphoryl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((*S*)-6-(2-(2-(2-(2-(2-aminoacetamido)-acetamido)acetamido)acetamido)acetamido)-1-(((*R*)-1-(((*R*)-1-carboxy-ethyl)amino)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)-D-glutamine



Protected, semi pure compound **156** (10.9 mg, <6.20 µmol, 1.00 equiv) was coevaporated twice from toluene (1 mL) under argon and dissolved in DMF (0.7 mL). The solution was cooled to 0 °C and TBAF (1 M in THF, 116 µL, 116 µmol, 23.0 equiv) was added. The reaction mixture was allowed to warm to rt and stirred 2 d at rt before removing the solvent under reduced pressure. The crude product was purified by gel permeation chromatography (Sephadex LH-20, GE Healthcare, 260 x 20 mm, MeOH) and the tetrabutylammonium counterions were exchanged to ammonium using percolation through Dowex® 50WX8 with a 0.02 M NH_4HCO_3 solution. (Dowex® 50WX8 was washed before usage with 3:1 NH_3/water and 0.02 M NH_4HCO_3 solution until pH of the eluent turned 8.) The solvent was removed by lyophilization to yield 8.20 mg (<5.80 µmol) of the semi purified lipid I analog **2**. Final purification was achieved using HPLC (25% – 50% NH_4HCO_3 (0.1% aq.)/MeOH, retention time 8.1 min, using a KNAUER Eurospher II 100-5 C8; 5 µm; 250 x 16 mm + precolumn 30 x 16 mm, 205 nm) to yield farnesyl lipid I analog **2** as a colorless solid (4.4 mg, 3.04 µmol, 22% over four steps).



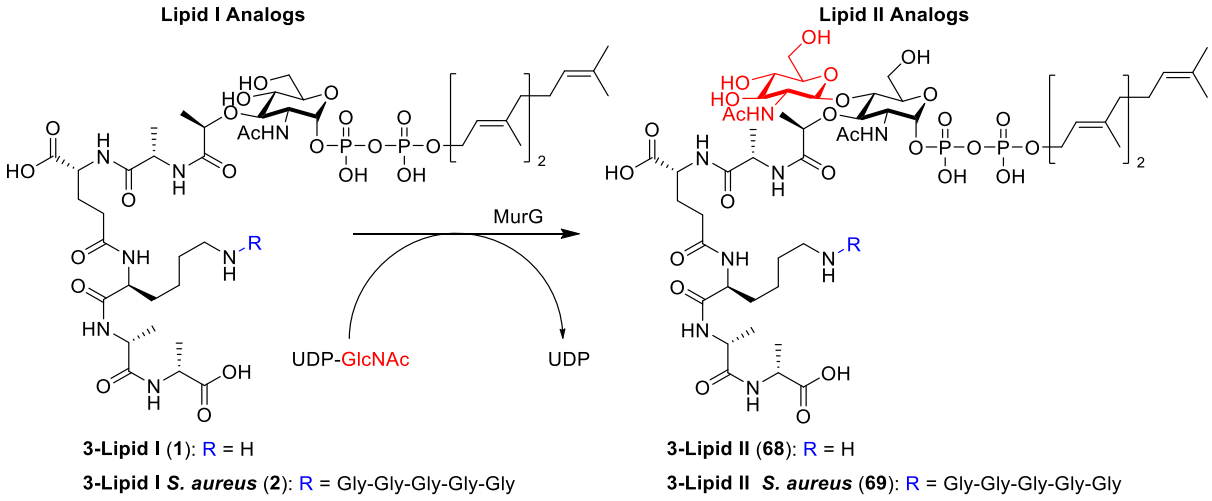
$\mathbf{R}_f$ 0.38 (3 MeOH/3 $CHCl_3$/1 water). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +20.5° (*c* = 0.44 in MeOH).

^{1}H NMR (700 MHz, D_2O) δ =5.50 – 5.48 (m, 1H, H-17), 5.46 (t, *J* = 6.7 Hz, 1H, H-31), 5.22 (t, *J* = 6.7 Hz, 1H, H-35), 5.20 (t, *J* = 6.7 Hz, 1H, H-39), 4.54 – 4.49 (m, 2H, H-30), 4.37 (q, *J* = 7.2 Hz, 1H, H-14), 4.30 (q, *J* = 7.2 Hz, 1H, H-4), 4.26 – 4.17 (m, 3H, H-6, H-10, H-12), 4.16 – 4.10 (m, 2H, H-2, H-16), 4.08 (s, 2H, H-52), 4.03 (s, 2H, H-48/H-50), 4.01 (s, 2H, H-48/H-50), 3.98 – 3.96 (m, 1H, H-18), 3.91 (s, 2H, H-46), 3.90 – 3.83 (m, 2H, H-20), 3.82 (s, 2H, H-45), 3.80 (dd, *J* = 9.6 Hz, 9.6 Hz, 1H, H-15), 3.66 (dd, *J* = 9.6 Hz, 9.6 Hz, 1H, H-19), 3.23 (t, *J* = 6.7 Hz, 2H, H-26), 2.37 – 2.28 (m, 2H, H-8), 2.20 – 2.16 (m, 3H, H-9, H-34), 2.15 – 2.11 (m, 4H, H-33, H-38), 2.04 (t, *J* = 7.1 Hz, 2H, H-37), 2.02 (s, 3H, CH_3-NHAc), 1.93 – 1.88 (m, 1H, H-9), 1.83 – 1.73 (m, 2H, H-23), 1.74 (s, 3H, H-43), 1.72 – 1.70 (m, 2H, H-25), 1.71 (s, 3H, H-42), 1.64 (s, 6H, H-41, H-44), 1.56 – 1.51 (m, 2H, H-24), 1.46 (d, *J* = 7.2 Hz, 3H, H-22), 1.43 (d, *J* = 6.8 Hz, 3H, H-28), 1.38 (d, *J* = 7.2 Hz, 3H, H-29), 1.35 (d, *J* = 7.2 Hz, 3H, H-21). **^{13}C NMR** (176 MHz, D_2O) δ =179.9 (C-1), 177.7 (C-27), 175.7 (C-11), 175.6 (C-7), 174.2 (C-5), 174.1 (NHAc-C=O), 174.0 (C-3), 173.6 (C-13), 172.2 (C-47/C-49/C-51), 172.1 (C-47/C-49/C-51), 172.0 (C-47/C-49/C-51), 171.0 (C-45), 169.9 (C-53) 143.2 (C-32), 136.7 (C-36), 133.4 (C-40), 124.4 (C-39), 124.2 (C-35), 119.3 (C-31), 94.7 (C-17), 79.9 (C-15), 78.0 (C-12), 73.0 (C-18), 68.0 (C-19), 63.2 (C-30), 60.3 (C-20), 54.3 (C-10), 54.3 (C-6), 53.4 (C-16), 51.0 (C-2), 49.8 (C-4), 49.5 (C-14), 42.6 (C-48/C-50), 42.5 (C-48/C-50), 42.5 (C-46), 42.4 (C-52), 41.2 (C-54), 39.0 (C-26), 38.8 (C-33), 38.8 (C-37), 31.8 (C-8), 30.7 (C-23), 28.1 (C-9), 27.8 (C-25), 25.8 (C-38), 25.5 (C-34), 24.9 (C-42), 22.4 (NHAc-CH_3), 22.2 (C-24), 18.7 (C-28), 17.4 (C-21), 17.0 (C-41), 16.9 (C-22), 16.6 (C-29), 15.7 (C-43), 15.3 (C-44). **^{31}P NMR**

(162 MHz, D_2O) δ = –10.8 (d, *J* = 15.4 Hz), –13.3 (d, *J* = 15.4 Hz). **HRMS(ESI)** *m/z*: calcd for $C_{56}H_{93}N_{12}O_{26}P_2$ $[M - H]^-$: 1411.5805, found: 1411.5810, calcd for $C_{56}H_{92}N_{12}O_{26}P_2$ $[M - 2H]^{2-}$: 705.2866, found: 705.2877.

6.2.8 Chemoenzymatic Conversion of Lipid I to Lipid II Analogs

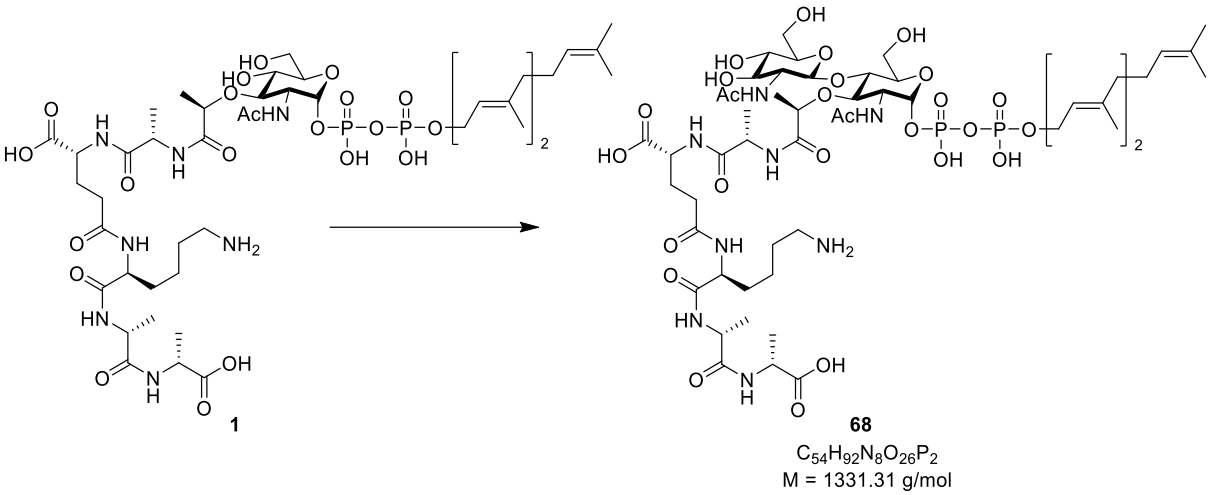
The second sugar (*N*-acetylglucoseamine) was attached to the synthesized lipid I analogs **1** and **2** chemoenzymatically by Dr. *Marvin Rausch* and purified and analyzed during this work. Experimental details for these final modifications can be found in this section.



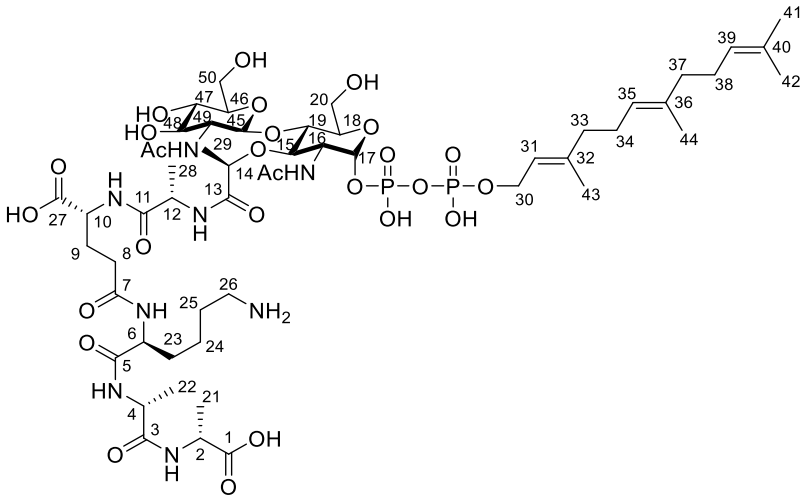
***Scheme 6.2.8-1*:** Chemoenzymatic synthesis of lipid II analogs **68** and **69**.

Synthesis of Compound 68

N^2-(((2*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-Acetamido-5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-2-((hydroxy((hydroxy(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)phosphoryl)oxy)phosphoryl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((*S*)-6-amino-1-(((*R*)-1-(((*R*)-1-carboxyethyl)amino)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)-D-glutamine



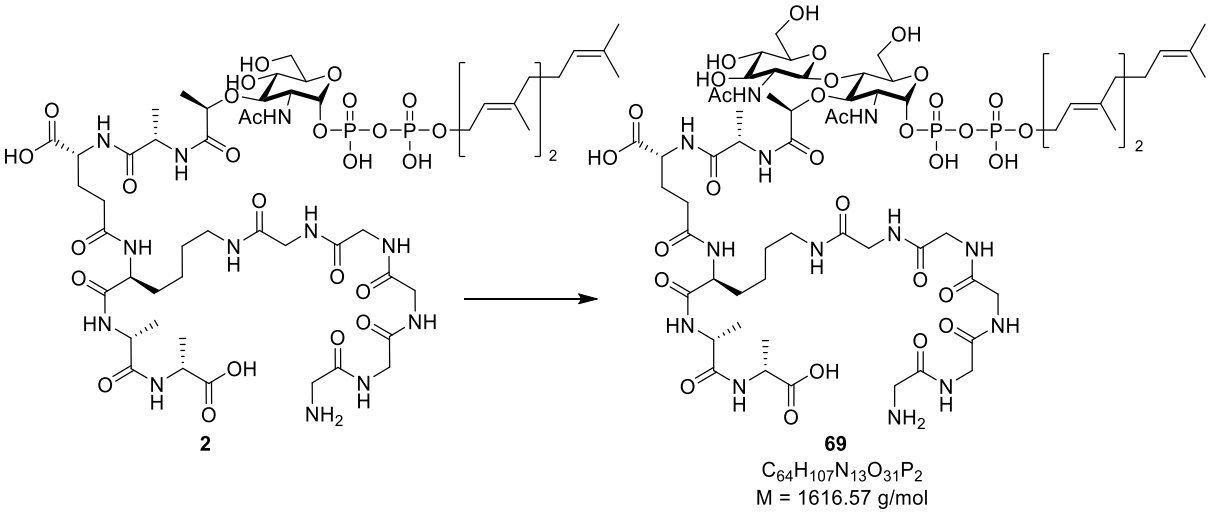
In vitro synthesis of compound **68** was performed in a total volume of 4.5 mL (aq.) containing 1.50 mg (1.33 µmol) compound **1**, 2 mM UDP-D-GlcNAc, 50 mM NaP_i and 0.5 mM $MgCl_2$, pH 6.5. The reaction was initiated by the addition of 300 µg of MurG-His_6. After incubation for 16 h at 30 °C, the reaction was quenched by the addition of MeOH (3 mL) and evaporated to dryness. The crude product was purified by HPLC (35% – 50% NH_4HCO_3 (0.1% aq.)/MeOH, retention time 8.0 min, using a KNAUER Eurospher II 100-5 C8; 5 µm; 250 x 16 mm + pre-column 30 x 16 mm, 205 nm) to yield farnesyl lipid II analog **68** as a colorless solid (0.98 mg, 0.73 µmol, 55%). Recombinant MurG-His_6 enzyme was overexpressed and purified as described for MurG[231] and dialized against 10 mM NaP_i buffer, pH 7.0.



$[\alpha]_D^{25}$ = +11.1° (*c* = 0.18 in H_2O). **^{1}H NMR** (700 MHz, D_2O) δ = 5.50 – 5.47 (m, 1H, H-17), 5.46 (t, *J* = 6.9 Hz, 1H, H-31), 5.23 (t, *J* = 6.4 Hz, 1H, H-35), 5.20 (t, *J* = 6.4 Hz, 1H, H-39), 4.63 (d, *J* = 8.3 Hz, 1H, H-45), 4.51 – 4.49 (m, 2H, H-30), 4.35 (q, *J* = 7.1 Hz, 1H, H-4), 4.32 – 4.27 (m, 2H, H-12, H-14), 4.24 – 4.20 (m, 2H, H-6, H-10), 4.15 – 4.12 (m, 3H, H-2, H-16), 3.98 – 3.89 (m, 4H, H-18, H-19, H-20, H-50), 3.86 – 3.81 (m (pt), 1H, H-15), 3.79 – 3.74 (m, 3H, H-20‘, H-49, H-50‘), 3.58 (pt (dd), *J* = 8.6, 8.6 Hz, 1H, H-48), 3.46 – 3.91 (m, 2H, H-46, H-47), 2.97 (t, *J* = 7.0 Hz, 2H, H-26), 2.34 – 2.33 (m, 2H, H-8), 2.19 – 2.17 (m, 3H, H-9, H-34), 2.14 – 2.11 (m, 4H, H-33, H-38), 2.07 (s, 3H, CH_3-NHAc), 2.05 (t, *J* = 7.7 Hz, 2H, H-37), 2.01 (s, 3H, CH_3-NHAc), 1.93 – 1.89 (m, 1H, H-9‘), 1.86 – 1.82 (m, 1H, H-23), 1.81 – 1.79 (m, 1H, H-23‘), 1.74 (s, 3H, H-43), 1.71 (s, 3H, H-42), 1.69 – 1.66 (m, 2H, H-25), 1.64 (s, 6H, H-41, H-44), 1.47 (d, *J* = 6.9 Hz, H-28), 1.46 (d, *J* = 6.8 Hz, 3H, H-29), 1.45 – 1.39 (m, 2H, H-24), 1.40 (d, *J* = 7.2 Hz, 3H, H-22), 1.35 (d, *J* = 7.2 Hz, 3H, H-21). **^{13}C NMR** (176 MHz, D_2O) δ = 179.8 (C-1), 177.6 (C-27), 175.7 (C-11), 175.7 (C-7), 174.4 (NHAc-C=O), 174.2 (NHAc-C=O), 174.1 (C-5), 174.0 (C-3), 173.6 (C-13), 143.2 (C-32), 136.7 (C-36), 133.5 (C-40), 124.4 (C-39), 124.2 (C-35), 119.3 (C-31), 100.1 (C-45), 94.2 (C-17), 78.4 (C-14), 77.9 (C-15), 75.9 (C-46), 74.0 (C-48), 73.8 (C-18), 72.4 (C-19), 70.3 (C-47), 63.1 (C-30), 61.1 (C-50/C-20), 59.7 (C-50/C-20), 56.1 (C-49), 54.3 (C-6), 53.7 (C-10), 53.5 (C-16), 51.0 (C-2), 50.0 (C-12), 49.6 (C-4), 39.2 (C-26), 38.8 (C-33), 38.8 (C-37), 31.8 (C-8), 30.5 (C-23), 28.2 (C-9), 26.5 (C-25), 25.8 (C-38), 25.6 (C-34), 24.9 (C-42), 22.2 (C-24), 22.2 (NHAc-CH_3), 22.1 (NHAc-CH_3), 18.7 (C-22), 17.4 (C-21), 17.0 (C-41), 16.9 (C-28), 16.5 (C-29), 15.7 (C-43), 15.3 (C-44). **^{31}P NMR** (284 MHz, D_2O) δ = –10.9 (d, *J* = 20.7 Hz), –13.4 (d, *J* = 19.2 Hz). **HRMS(ESI)** *m/z*: calcd for $C_{54}H_{90}N_8O_{26}P_2$ $[M – 2H]^{2–}$: 664.2726, found: 664.2719, calcd for $C_{54}H_{89}N_8O_{26}P_2$ $[M – 3H]^{3–}$: 442.5127, found: 442.5117. The spectroscopic data were in agreement with those previously reported.[68]

Synthesis of Compound 69

N^2-(((2*R*)-2-(((2*R*,3*R*,4*R*,5*S*,6*R*)-3-Acetamido-5-(((2*S*,3*R*,4*R*,5*S*,6*R*)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl)oxy)-2-((hydroxy((hydroxy(((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trien-1-yl)oxy)phosphoryl)oxy)phosphoryl)oxy)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((S)-6-(2-(2-(2-(2-(2-aminoacetamido)acetamido)acetamido)acetamido)acetamido)-1-(((*R*)-1-(((*R*)-1-carboxyethyl)amino)-1-oxopropan-2-yl)amino)-1-oxohexan-2-yl)-D-glutamine



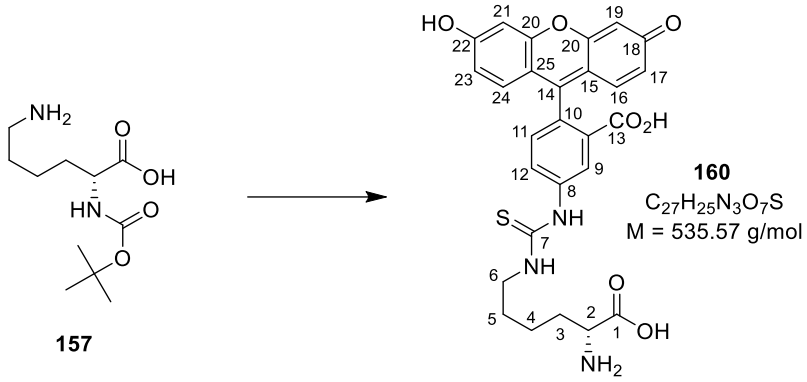
In vitro synthesis of compound **69** was performed in a total volume of 30 µl (aq.) containing 10 µg compound **2**, 2 mM UDP-D-GlcNAc, 50 mM NaP_i and 0.5 mM $MgCl_2$, pH 6.5. The reaction was initiated by the addition of 2 µg of MurG-His_6. After incubation for 4 h at 30 °C, the reaction was quenched by the addition of MeOH (60 µl) and evaporated to dryness. Dried samples were dissolved in distilled water for mass spectrometric analysis. Recombinant MurG-His_6 enzyme was overexpressed and purified as described for MurG[231] and dialized against 10 mM NaP_i buffer, pH 7.0.

HRMS(ESI) *m/z*: calcd for $C_{64}H_{105}N_{13}O_{31}P_2$ $[M - 2H]^{2-}$: 806.8263, found: 806.8255; calcd for $C_{64}H_{104}N_{13}O_{31}P_2Na$ $[M - 3H + Na]^{2-}$: 817.8173, found: 817.8166; calcd for $C_{64}H_{104}N_{13}O_{31}P_2$ $[M - 3H]^{3-}$: 537.5484, found: 537.5475.

6.2.9 Synthesis of Labeled Compounds to Trace Cell Wall Biosynthesis

Synthesis of Compound 160

(*R*)-5-(3-(5-Amino-5-carboxypentyl)thioureido)-2-(6-hydroxy-3-oxo-3*H*-xanthen-9-yl)benzoic acid



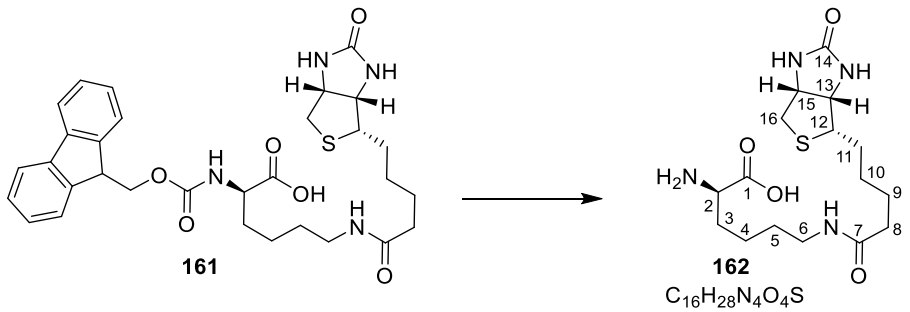
To a solution of Boc-D-lysine **157** (38.6 mg, 157 µmol, 1.00 equiv) in DMF (1.2 mL) was added fluorescein isothiocyanate (50.7 mg, 130 µmol, 0.83 equiv). The reaction mixture was stirred for 4 h at rt and the solvent was removed under reduced pressure. Afterwards, the residue was redissolved in EtOAc (29 mL), washed with 1 M aq. HCl (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed under reduced pressure. DCM/TFA (1:1, 5 mL) was added to the residue and the mixture was stirred for 30 min at rt. All volatiles were removed under reduced pressure and the crude product was purified by HPLC (20% – 40% MeCN/water, retention time 9.6 min, using a KNAUER Eurospher II 100-5 C18; 5 µm; 250 x 16 mm + precolumn 30 x 16 mm, 225, 270, 455 nm) to yield compound **160** as a yellow solid (41.4 mg, 77.3 µmol, 59%).

R_f 0.26 (20% MeOH/DCM). $[\alpha]_D^{20} = -1.6°$ ($c = 0.62$ in CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_3OD) δ = 8.14 (d, J = 1.7 Hz, 1H, H-9), 7.76 (dd, J = 8.2, 1.7 Hz, 1H, H-12), 7.18 (d, J = 8.3 Hz, 1H, H-11), 6.71 – 6.69 (m, 4H, H-16, H-19, H-21, H-24), 6.56 (dd, J = 8.7, 2.4 Hz, 2H, H-17, H-23), 3.67 (br s, 2H, H-6), 3.60 (t, J = 5.9 Hz, 1H, H-2), 1.98 – 1.86 (m, 2H, H-3), 1.79 – 1.70 (m, 2H, H-5), 1.58 – 1.51 (m, 2H, H-4). **^{13}C NMR** (126 MHz, CD_3OD) δ = 182.9 (C-18), 174.3 (C-1), 171.2 (C-7), 161.6 (C-13), 154.2 (C-20), 142.4 ($C_{arom.}$-COOH), 131.8 (C-12), 130.3 (C-16, C-24), 129.1 (C-10), 125.7 (C-11), 120.0 (C-9), 113.7 (C-17), 111.5 (C-15), 103.5 (C-21,

C-19), 56.1 (C-2), 45.2 (C-6), 32.0 (C-3), 29.6 (C-5), 23.6 (C-4). **HRMS (ESI)** *m/z*: calcd for $C_{27}H_{25}N_3O_7SH$ $[M + H]^+$: 536.1486, found: 536.1486. The spectroscopic data were in agreement with those previously reported.[121]

Synthesis of Compound 162

N^6-(5-((3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl)pentanoyl)-D-lysine

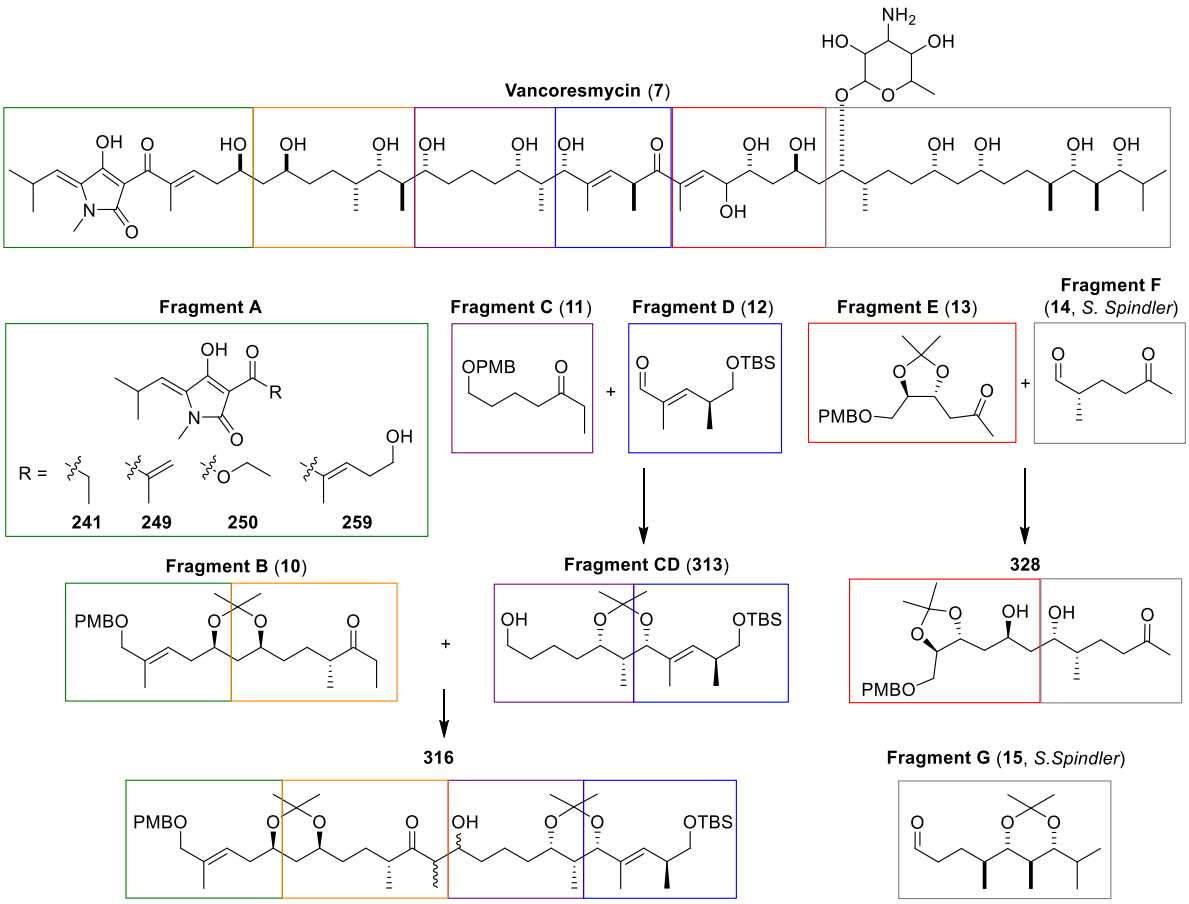


Lysine derivative **161** (500 mg, 841 µmol, 1.00 equiv) was dissolved in 20% piperidine/DMF (15 mL) and toluene (2 mL). The reaction mixture was stirred 45 min at rt and all volatiles were removed under reduced pressure. Water (10 mL) was added to the residue, the resulting suspension was filtered and the precipitate was washed with water. The filtrate was concentrated to yield the title compound **162** as a white solid (187 mg, 502 µmol, 60%).[120]

Due to the polarity of the compound, a R_f value was not determined. $[\alpha]_D^{20}$ = +32.6° (*c* = 0.46 in H_2O). **^{1}H NMR** (500 MHz, D_2O) δ = 4.63 (dd, *J* = 7.9, 4.9 Hz, 1H, H-15), 4.44 (dd, *J* = 7.9, 4.5 Hz, 1H, H-13), 3.74 (t, *J* = 6.5 Hz, 1H, H-2), 3.38 – 3.34 (m, 1H, H-12), 3.21 (t, *J* = 6.9 Hz, 2H, H-6), 3.02 (dd, *J* = 13.1, 5.0 Hz, 1H, H-16), 2.80 (d, *J* = 13.1 Hz, 1H, H-16'), 2.27 (t, *J* = 7.3 Hz, 2H, H-8), 1.95 – 1.82 (m, 2H, H-3), 1.77 – 1.55 (m, 6H, H-5, H-9, H-10), 1.49 – 1.37 (m, 4H, H-4, H-11). **^{13}C NMR** (126 MHz, D_2O) δ = 176.8 (C-7), 174.9 (C-1), 165.5 (C-14), 62.2 (C-13), 60.3 (C-15), 55.4 (C-12), 54.8 (C-2), 39.8 (C-16), 39.0 (C-6), 35.6 (C-8), 30.2 (C-3), 28.2 (C-5), 27.9 (C-11), 27.7 (C-10), 25.2 (C-9), 21.9 (C-4). **HRMS (ESI)** *m/z*: calcd for $C_{16}H_{28}N_4O_4SH$ $[M + H]^+$: 373.1904, found: 373.1902.

6.3 Contributions to the Total Synthesis of Vancoresmycin

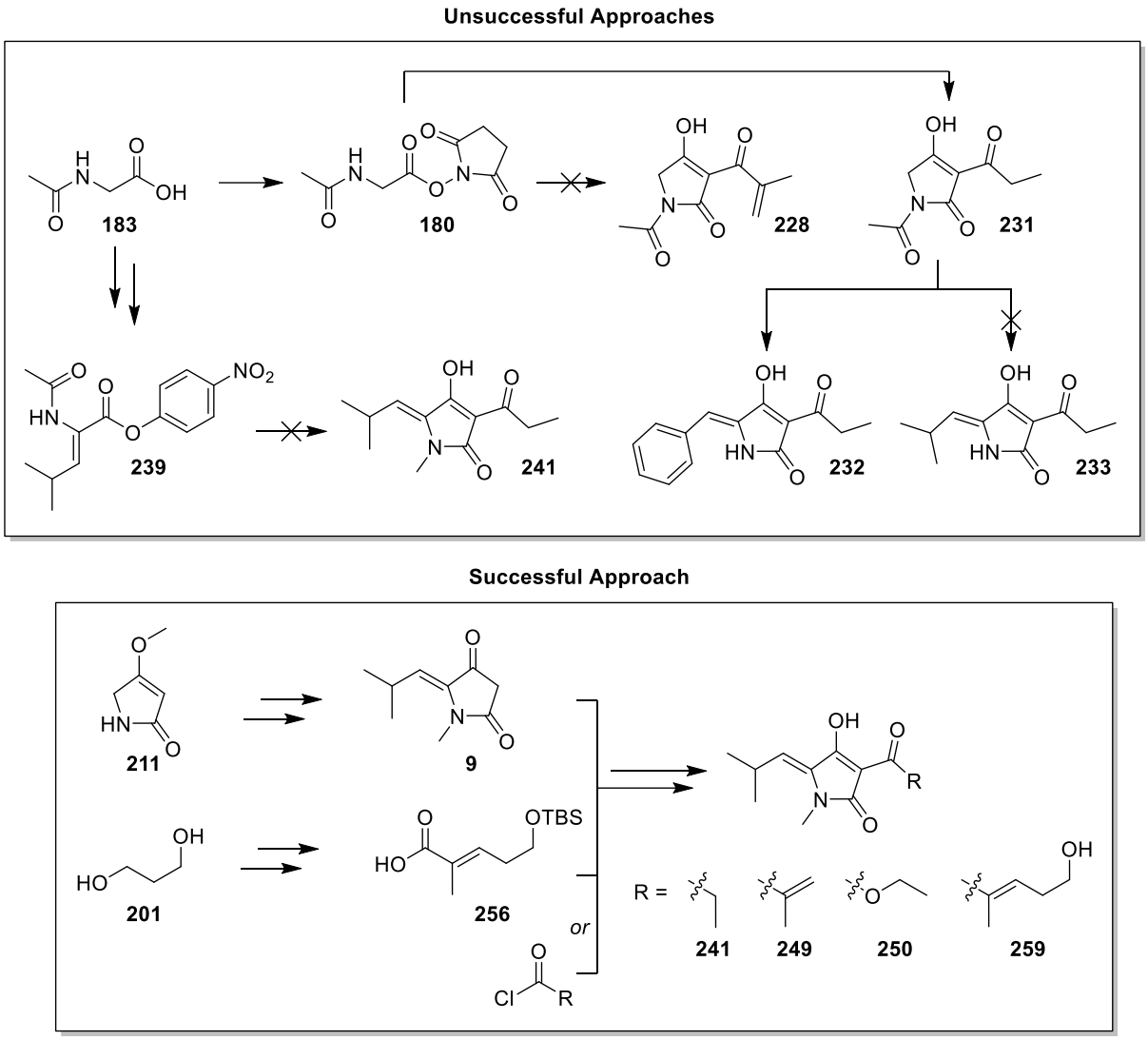
In this chapter, experimental details are given for the synthesized fragments, which are suitable for a total synthesis of vancoresmycin (**7**). **Scheme 6.3-1** gives an overview of the synthesized fragments.



***Scheme 6.3-1*:** Vancoresmycin (**7**) and the synthesized fragments.

6.3.1 Synthesis of Fragment A (Tetramic Acid Derivatives)

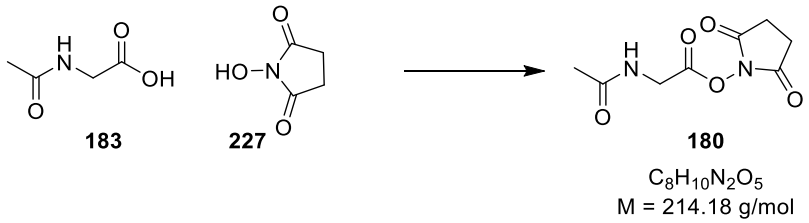
First, experimental details are described for substances, which were yielded during approaches not being capable to gain excess to fully vancoresmycin substituted tetramic acids. Then, experimental details are given for a successful route to different accurate derivatives. The longest synthesized authentic vancoresmycin fragment **259** was gained after seven linear steps out of propanediol **201**.



***Scheme 6.3.1-1*:** Approaches to vancoresmycin derived tetramic acids (fragment A).

Synthesis of Compound 180

2,5-Dioxopyrrolidin-1-yl acetylglycinate



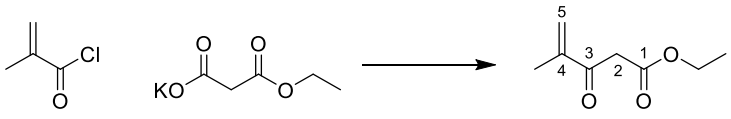
N-Hydroxysuccinimide (**227**, 7.00 g, 60.8 mmol, 1.00 equiv) and *N*-acetylglycine (**183**, 7.12 g, 60.8 mmol, 1.00 equiv) were dissolved in 1,4-dioxane (120 mL). The solution was cooled to

0 °C and DCC (12.6 g, 60.8 mmol, 1.00 equiv) was added. After standing overnight in the fridge (3 °C), the reaction mixture was filtered over celite® and the residue was washed with 1,4-dioxane. The solvent was removed under reduced pressure and the crude product was washed with Et_2O. Final recrystallization in DCM yielded the title compound **180**, which was used without further purification, as a colorless solid (7.22 g, 33.7 mmol, 55%).

R_f 0.33 (10% MeOH/DCM). **^{1}H NMR** (500 MHz, $(CD_3)_2SO$) δ = 8.52 (t, *J* = 5.8 Hz, 1H, NH), 4.23 (d, *J* = 5.8 Hz, 2H, CH_2), 2.81 (s, 4H, $(CH_2)_2$), 1.89 (s, 3H, CH_3). **^{13}C NMR** (126 MHz, $(CD_3)_2SO$) δ = 170.0 (2xC=O), 166.5 (C=O), 38.2 (CH_2), 25.4 (2xCH_2), 22.1 (CH_3). **MS (ESI)** *m/z*: calcd for $C_8H_{10}N_2O_5Na$ $[M + Na]^+$: 237.049, found: 237.049. The spectroscopic data were in agreement with those previously reported.[160,161]

Synthesis of Compound 179

Ethyl 4-methyl-3-oxopent-4-enoate



181 **182** **179**

$C_8H_{12}O_3$

M = 156.18 g/mol

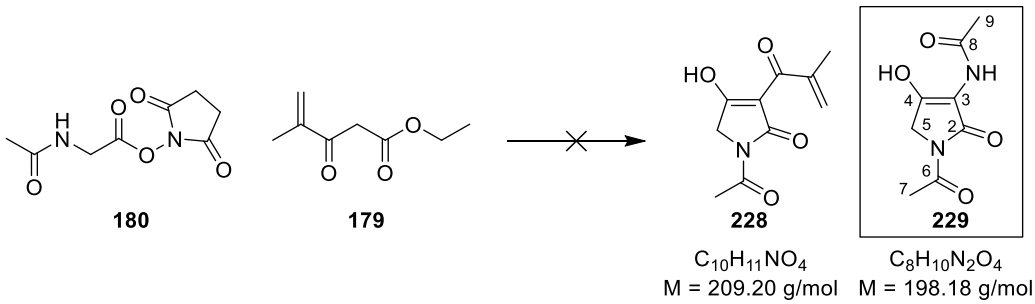
To a solution of monoethyl potassium malonate (**182**, 4.88 g, 28.7 mmol, 2.00 equiv) and $MgCl_2$ (3.01 g, 31.6 mmol, 2.20 equiv) in MeCN (50 mL) was added NEt_3 (8.75 mL, 63.1 mmol, 4.40 equiv) dropwise. The reaction mixture was cooled to 0 °C and methacryloyl chloride (1.40 mL, 14.4 mmol, 1.00 equiv) was added slowly. After stirring for 30 min at 0 °C, aq. HCl (2 M, 10 mL) was added and the solution was poured into ice water (50 mL) and extracted with EtOAc (3x50 mL). The combined organic layers were washed with water (100 mL), sat. aq. $NaHCO_3$ solution (100 mL) and brine (100 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (100% DCM) to yield the title compound **179** as a colorless oil (2.05 g, 13.1 mmol, 92%).

R_f 0.34 (DCM). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 5.95 (q, *J* = 0.8 Hz, 1H, H-5), 5.89 (q, *J* = 1.5 Hz, 1H, H-5'), 4.16 (q, *J* = 7.1 Hz, 2H, OEt), 3.71 (s, 2H, H-2), 1.87 (dd, *J* = 1.5, 0.8 Hz, 3H, 4-CH_3), 1.25 (t, *J* = 7.1 Hz, 3H, OEt). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 194.8 (C-3), 168.2 (C-1), 144.7 (C-4), 127.0 (C-5), 61.8 (OEt), 45.6 (C-2), 17.6 (4-CH_3), 14.4 (OEt). **HRMS (ESI)**

m/z: calcd for $C_8H_{12}O_3Na$ [M + Na]$^+$: 179.0679, found: 179.0674. The spectroscopic data were in agreement with those previously reported.[162]

Synthesis of Compound 229. Failed Synthesis of Compound 228

N-(1-Acetyl-4-hydroxy-2-oxo-2,5-dihydro-1*H*-pyrrol-3-yl)acetamide (229)

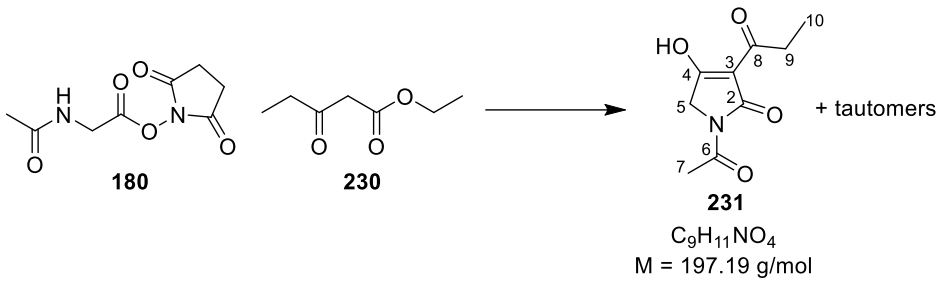


Ethyl 4-methyl-3-oxopent-4-enoate (**179**, 328 mg, 2.10 mmol, 3.00 equiv) was added dropwise to a suspension of NaH (84.0 mg, 2.10 mmol, 3.00 equiv, 60% dispersion in mineral oil) in THF (5 mL) at 0 °C. After stirring for 1 h at 0 °C, glycine derivative **180** (150 mg, 700 µmol, 1.00 equiv) was added. The reaction mixture was stirred 2.5 h at 0 °C and 30 min at rt. Et_2O (5 mL) and water (5 mL) were added, the phases separated and the aqueous layer was acidified to pH = 1 using 10% aq. HCl. The aqueous layer was extracted with DCM (3x5 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The solid was washed with Et_2O to yield the title compound **229** as a colorless solid (18.5 mg, 93.4 µmol, 27%). Planned compound **228** could not be isolated.

R_f 0.49 (10% MeOH/DCM). **^{1}H NMR** (500 MHz, $(CD_3)_2SO$) δ = 12.62 (br s, 1H, OH), 9.73 (s, 1H, NH), 4.15 (s, 2H, H-5), 2.39 (s, 3H, H-7), 2.06 (s, 3H, H-9). **^{13}C NMR** (126 MHz, $(CD_3)_2SO$) δ = 171.4 (C-8), 168.2 (C-6), 166.2 (C-2), 160.8 (C-4), 104.2 (C-3), 45.6 (C-5), 24.3 (C-7), 22.0 (C-9). **MS (ESI)** *m/z*: calcd for $C_8H_{10}N_2O_4Na$ [M + Na]$^+$: 221.053, found: 221.053. The spectroscopic data were in agreement with those previously reported.[198]

Synthesis of Compound 231

1-Acetyl-4-hydroxy-3-propionyl-1,5-dihydro-2*H*-pyrrol-2-one



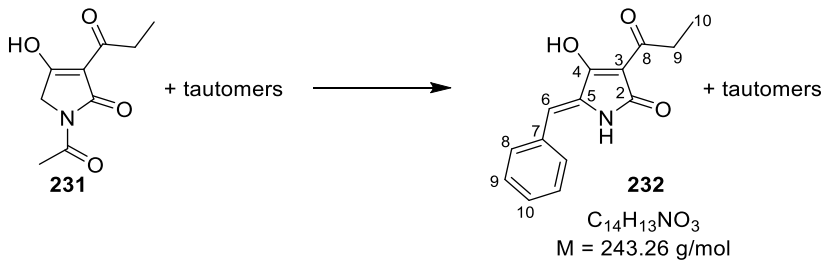
$C_9H_{11}NO_4$
M = 197.19 g/mol

To a suspension of NaH (56.0 mg, 1.40 mmol, 2.00 equiv, 60% dispersion in mineral oil) in toluene (2 mL) was added ethyl propionylacetate (**230**, 299 µL, 2.10 mmol, 3.00 equiv) dropwise at rt. The thick colorless slurry was stirred 1.5 h at rt followed by the addition of ester **180** (150 mg, 700 µmol, 1.00 equiv). After stirring for further 3 h at rt, the reaction was cooled to 0 °C and water (5 mL) was added. The aqueous layer was separated, cooled to 0 °C, acidified to pH = 1 with aq. 2 M HCl and extracted with DCM (3x10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Finally, the crude product was washed with water (5 mL), petroleum ether (2x2 mL) and dried in vacuo to give the title compound **231** as a yellow solid (67.2 mg, 341 µmol, 49%).

R_f 0.62 (15% MeOH/DCM). **^{1}H NMR** (500 MHz, $(CD_3)_2SO$) δ = 7.10 (s, 1H, OH), 4.13 (s, 1H, H-5), 2.79 (q, *J* = 7.4 Hz, 2H, H-9), 2.42 (s, 3H, H-7), 1.02 (t, *J* = 7.4 Hz, 3H, H-10). **^{13}C NMR** (126 MHz, $(CD_3)_2SO$) δ = 194.6 (C-8), 185.8 (C-4), 168.7 (C-6), 168.1 (C-2), 104.5 (C-3), 49.2 (C-5), 31.6 (C-9), 24.7 (C-7), 8.4 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_9H_{10}NO_4$ $[M - H]^-$: 196.0615, found: 196.0613.

Synthesis of Compound 232

(*Z*)-5-Benzylidene-4-hydroxy-3-propionyl-1,5-dihydro-2*H*-pyrrol-2-one



$C_{14}H_{13}NO_3$
M = 243.26 g/mol

Tetramic acid derivative **231** (47.5 mg, 241 µmol, 1.00 equiv) was stirred in 8% HCl/Ethanol (4 mL) at rt. Stirring was continued until complete solvation of the starting material was achieved and benzaldehyde (48.7 µL, 482 µmol, 2.00 equiv) was added. The reaction mixture was refluxed (~90 °C) for 3 h and left stirring overnight at rt. The formed precipitate was filtered off, washed with cold Et_2O (2x2 mL), redissolved in DCM and the solvent was removed under reduced pressure to yield the title compound **232** as a yellow solid (10.9 mg, 44.8 µmol, 19%). $\mathbf{R_f}$ 0.41 (10% MeOH/DCM). Due to extended tautomerism and thus a broadening of the signals, an exact assignment was not unambiguous. **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 10.48 (br s, 1H, NH), 7.64 (d, *J* = 7.5 Hz, 2H, H-8), 7.40 (dd, *J* = 7.5, 7.5 Hz, 2H, H-9), 7.34 (d, *J* = 7.5 Hz, 1H, H-10), 6.47 (s, 1H, H-6), 2.89 (q, *J* = 7.5 Hz, 2H, H-9), 1.12 (t, *J* = 7.5 Hz, 3H, H-10). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 192.8 (C-4), 184.1 (C-8), 166.9 (C-2), 133.2 (C-7), 129.7 (C-8), 128.8 (C-9), 128.4 (C-10), 122.4 (C-5), 108.3 (C-6), 101.3 (C-3), 26.3 (C-9), 9.0 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{13}NO_3H$ $[M + H]^+$: 244.0968, found: 244.0967.

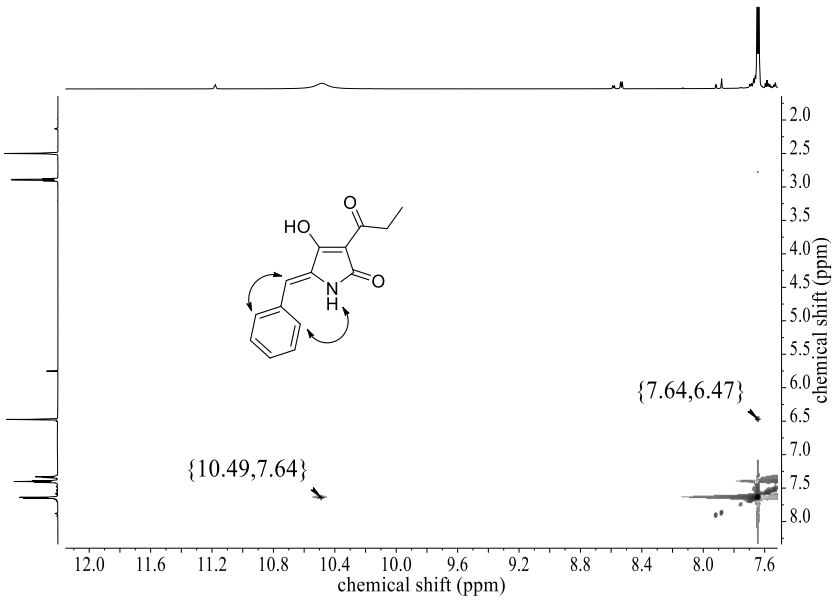
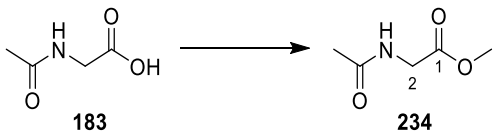


Figure 6.3.1-1: Selected NOESY signals of compound **232**, which were used for the determination of the *Z*-configuration.

Synthesis of Compound 234

Methyl acetylglycinate

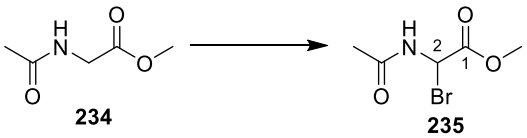


To a suspension of *N*-acetylglycine (**183**, 11.0 g, 93.9 mmol, 1.00 equiv) in MeOH (60 mL) was added thionyl chloride (13.6 mL, 187.9 mmol, 2.00 equiv) at 0 °C. The solution was stirred overnight at rt. All volatiles were removed under reduced pressure to yield ester **234** as a colorless solid (12.3 g, 93.8 mmol, quant.).

R_f 0.22 (10% MeOH/DCM). **^{1}H NMR** (400 MHz, CD_3OD) δ = 3.94 (s, 2H, H-2), 3.72 (s, 3H, O-CH_3), 2.02 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, CD_3OD) δ = 174.1 (NHC=O), 171.7 (C-1), 52.6 (O-CH_3), 42.0 (C-2), 22.1 (CH_3). **MS (ESI)** *m/z*: calcd for $C_5H_9NO_3H$ $[M + H]^+$: 132.066, found: 132.065. The spectroscopic data were in agreement with those previously reported.[199]

Synthesis of Compound 235

Methyl 2-acetamido-2-bromoacetate



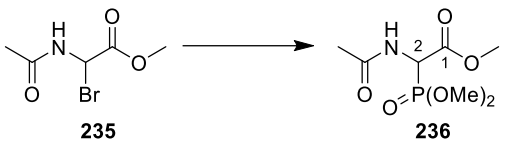
Methyl acetylglycinate (**234**, 4.10 g, 31.3 mmol, 1.00 equiv) and *N*-bromosuccinimide (6.12 g, 34.4 mmol, 1.10 equiv) were dissolved in tetrachloromethane (220 mL). The reaction mixture was refluxed (110 °C), whilst being irradiated with a sunlamp, for 5 h. After cooling to rt, the mixture was filtered over a cotton plug and the solvent was removed under reduced pressure to yield the title compound **235** as a yellow solid (5.47 g, 26.0 mmol, 83%).

R_f 0.28 (10% MeOH/DCM). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.87 (br d, 1H, NH), 6.46 (d, *J* = 10.2 Hz, 1H, H-2), 3.87 (s, 3H, O-CH_3), 2.09 (s, 3H, CH_3). **^{13}C NMR** (101 MHz, $CDCl_3$) δ = 169.0 (NHC=O), 167.4 (C-1), 53.9 (O-CH_3), 49.1 (C-2), 23.5 (CH_3). The compound was not

detectable in MS (ESI), which was also not reported in the literature. NMR data were in agreement with those previously reported.[199]

Synthesis of Compound 236

Methyl 2-acetamido-2-(dimethoxyphosphoryl)acetate



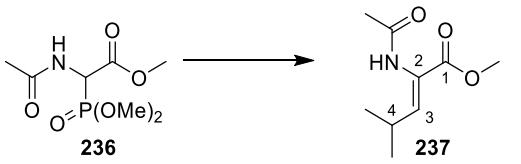
$C_7H_{14}NO_6P$
M = 239.16 g/mol

Methyl 2-acetamido-2-bromoacetate (**235**, 4.94 g, 23.5 mmol, 1.00 equiv) was dissolved in EtOAc (60 mL) and trimethyl phosphite (2.78 mL, 23.5 mmol, 1.00 equiv) was added. The reaction mixture was stirred overnight at rt and the solvent was removed under reduced pressure to yield title compound **236** as a yellow solid (5.25 g, 22.0 mmol, 93%).

R_f 0.37 (10% MeOH/DCM). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 6.43 (br d, *J* = 7.9 Hz, 1H, NH), 5.23 (dd, *J* = 22.2, 8.9 Hz, 1H, H-2), 3.83 (d, *J* = 8.5 Hz, 3H, $POCH_3$), 3.83 (d, *J* = 0.5 Hz, 3H, O-CH_3), 3.81 (d, *J* = 8.5 Hz, 3H, $POCH_3$), 2.08 (d, *J* = 0.8 Hz, 3H, CH_3). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 169.7 (d, *J* = 8.5 Hz, NHC=O), 167.3 (d, *J* = 2.2 Hz, C-1), 54.4 (d, *J* = 6.5 Hz, $POCH_3$), 54.1 (d, *J* = 6.5 Hz, $POCH_3$), 53.5 (O-CH_3), 50.2 (d, *J* = 147.9 Hz, C-2), 23.0 (CH_3). **^{31}P NMR** (202 MHz, $CDCl_3$) δ = 18.6. **MS (ESI)** *m/z*: calcd for $C_7H_{14}NO_6H$ $[M + H]^+$: 240.064, found: 240.063. The spectroscopic data were in agreement with those previously reported.[199]

Synthesis of Compound 237

Methyl (*Z*)-2-acetamido-4-methylpent-2-enoate



$C_9H_{15}NO_3$
M = 185.22 g/mol

Methyl 2-acetamido-2-(dimethoxyphosphoryl)acetate (**236**, 5.06 g, 21.2 mmol, 1.00 equiv) was dissolved in THF (60 mL) and 1,1,3,3-tetramethylguanidine (3.18 mL, 25.4 mmol, 1.20 equiv)

was added at −78 °C. The reaction mixture was stirred 15 min at this temperature, isobutyraldehyde (2.22 mL, 24.3 mmol, 1.15 equiv) was added and stirring was continued for further 2 h at −78 °C. After stirring at rt for 30 min, EtOAc (30 mL) and aq. sat. NH_4Cl solution (50 mL) were added. The aqueous phase was extracted with EtOAc (3x100 mL), the combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (70% EtOAc/CyH) to yield the title compound **237** as a colorless solid (2.34 g, 12.6 mmol, 60%, *E*/*Z* = 5:95).

R_f 0.19 (70% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.73 (br d, 1H, NH), 6.52 (d, *J* = 10.3 Hz, 1H, H-3), 3.76 (s, 3H, O-CH_3), 2.65 – 2.53 (m, 1H, H-4), 2.11 (s, 3H, CH_3), 1.05 (d, *J* = 6.6 Hz, 6H, 4-$(CH_3)_2$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 169.0 (NHC=O), 165.6 (C-1), 145.9 (C-3), 123.0 (C-2), 52.5 (O-CH_3), 28.3 (C-4), 23.5 (CH_3), 21.7 (4-$(CH_3)_2$). **MS (ESI)** *m/z*: calcd for $C_9H_{15}NO_3H$ $[M + H]^+$: 186.113, found: 186.112. The spectroscopic data were in agreement with those previously reported.[199]

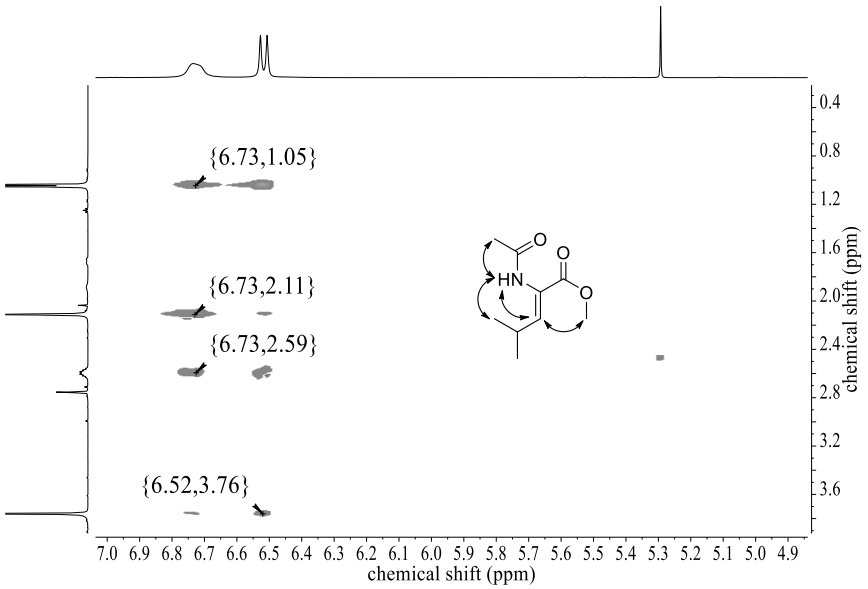
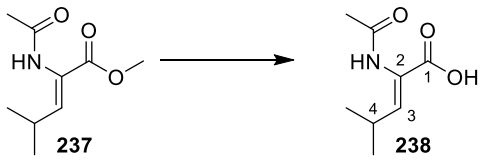


Figure 6.3.1-2: Selected NOESY signals of compound **237**, which were used for the determination of the *Z*-configuration.

Synthesis of Compound 238

(*Z*)-2-Acetamido-4-methylpent-2-enoic acid



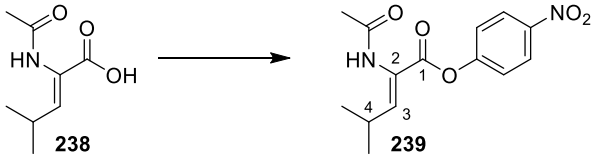
$C_8H_{13}NO_3$
M = 171.20 g/mol

Methyl (*Z*)-2-acetamido-4-methylpent-2-enoate (**237**, 819 mg, 4.37 mmol, 1.00 equiv) was dissolved in THF (30 mL). MeOH (4 mL) and 1 M aq. LiOH solution (5.5 mL) were added. The reaction mixture was stirred overnight at rt, acidified to pH = 1 with 1 M aq. HCl and brine (10 mL) was added. After separation of the organic phase, the aqueous phase was extracted with EtOAc (3x70 mL), dried over $MgSO_4$ and all volatiles were removed under reduced pressure to yield the title compound **238** as a colorless solid (514 mg, 3.00 mmol, 69%).

R_f 0.55 (20% MeOH/DCM). **^{1}H NMR** (700 MHz, $(CD_3)_2SO$) δ = 12.35 (br s, 1H, OH), 8.99 (s, 1H, NH), 6.20 (d, *J* = 10.3 Hz, 1H, H-3), 2.56 – 2.52 (m, 1H, H-4), 1.91 (s, 3H, CH_3), 0.96 (d, *J* = 6.6 Hz, 6H, 4-$(CH_3)_2$). **^{13}C NMR** (176 MHz, $(CD_3)_2SO$) δ = 168.6 (NHC=O), 165.9 (C-1), 142.4 (C-3), 126.1 (C-2), 26.6 (C-4), 22.4 (CH_3), 21.6 (4-$(CH_3)_2$). **HRMS (ESI)** *m/z*: calcd for $C_8H_{13}NO_3H$ $[M + H]^+$: 172.0968, found: 172.0968.

Synthesis of Compound 239

4-Nitrophenyl (*Z*)-2-acetamido-4-methylpent-2-enoate



$C_{14}H_{16}N_2O_5$
M = 292.29 g/mol

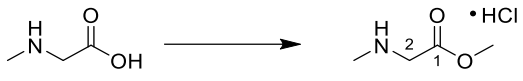
(*Z*)-2-Acetamido-4-methylpent-2-enoic acid (**238**, 50.0 mg, 292 µmol, 1.00 equiv), DMAP (3.57 mg, 29.2 µmol, 0.10 equiv) and 4-nitrophenol (48.8 mg, 351 µmol, 1.20 equiv) were dissolved in DCM (1 mL). DCC (1 M in DCM, 351 µL, 351 µmol, 1.20 equiv) was added at 0 °C and the reaction mixture was stirred for 1 h at rt. The mixture was filtered over celite® and the crude product was purified by flash chromatography (50% EtOAc/CyH) to yield the title

compound **239** as a colorless solid, which was used without further purification (40.9 mg, 140 µmol, 48%).

R_f 0.18 (50% EtOAc/CyH). **^{1}H NMR** (400 MHz, CD_2Cl_2) δ = 8.27 (d, *J* = 9.2 Hz, 2H, $H_{arom.}$), 7.33 (d, *J* = 9.2 Hz, 2H, $H_{arom.}$), 6.78 (s, 1H, NH), 6.69 (d, *J* = 10.3 Hz, 1H, H-3), 2.74 – 2.65 (m, 1H, H-4), 2.12 (s, 3H, CH_3), 1.11 (d, *J* = 6.6 Hz, 6H, 4-$(CH_3)_2$). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 169.7 (NHC=O), 163.3 (C-1), 156.3 ($C_{arom.}$), 148.1 (C-3), 146.0 ($C_{arom.}$), 125.7 ($C_{arom.}$H), 123.7 (C-2), 123.1 ($C_{arom.}$H), 28.6 (C-4), 23.5 (CH_3), 21.9 (4-$(CH_3)_2$). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{16}N_2O_5H$ $[M + H]^+$: 293.1130, found: 293.1130.

Synthesis of Compound 243

Methyl methylglycinate

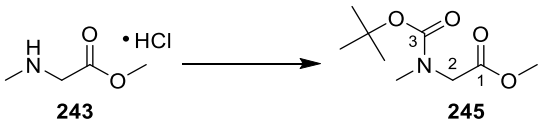


To a suspension of sarcosine (**242**, 10.0 g, 112 mmol, 1.00 equiv) in MeOH (60 mL) was added thionyl chloride (16.3 mL, 224 µmol, 2.00 equiv) dropwise at 0 °C. The reaction mixture was stirred overnight at rt and refluxed for 4 h. All volatiles were removed under reduced pressure to yield the title compound **243** as a colorless solid (15.6 g, 112 mmol, quant.).

R_f 0.19 (15% MeOH/DCM). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 9.86 (s, 2H, NH_2), 3.87 (t, *J* = 5.6 Hz, 2H, H-2), 3.83 (s, 3H, O-CH_3), 2.84 (t, *J* = 5.6 Hz, 3H, N-CH_3). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 166.6 (C-1), 53.3 (O-CH_3), 48.9 (C-2), 33.4 (N-CH_3). **MS (ESI)** *m/z*: calcd for $C_4H_9NO_2H$ $[M + H]^+$: 104.071, found: 104.071. The spectroscopic data were in agreement with those previously reported.[232]

Synthesis of Compound 245

Methyl *N*-(*tert*-butoxycarbonyl)-*N*-methylglycinate



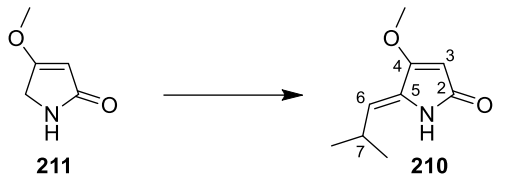
$C_9H_{17}NO_4$
M = 203.24 g/mol

Sarcosine methylester (**243**, 1.00 g, 7.16 mmol, 1.00 equiv) was dissolved in DCM (16 mL), Boc anhydride (2.15 mL, 10.0 mmol, 1.40 equiv) was added and the mixture was cooled to 0 °C. Triethylamine (1.99 mL, 14.3 mmol, 2.00 equiv) was added and the reaction mixture was stirred for 2 days at rt. 1 M aq. HCl was added (until pH = 2), the organic layer was separated, washed with water (15 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (15% EtOAc/CyH) to yield the title compound **245** as a colorless paste (1.46 g, 4.07 mmol, 57%).

R_f 0.17 (15% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 3.94/3.89 (2xs, 2H, H-2), 3.72/3.71 (2xs, 3H, O-CH_3), 2.88 (s, 6H, N-CH_3), 1.45/1.39 (2xs, 9H, $(CH_3)_3$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 171.0 (C-1), 156.5/155.8 (C-3), 80.4 ($\underline{C}(CH_3)_3$), 52.3 (O-CH_3), 51.4/50.6 (C-2), 35.9/35.8 (NCH_3), 28.6/28.5 ($(CH_3)_3$). **MS (ESI)** *m/z*: calcd for $C_9H_{17}NO_4Na$ $[M + Na]^+$: 226.106, found: 226.105. The spectroscopic data were in agreement with those previously reported.[232]

Synthesis of Compound 210

(*Z*)-4-Methoxy-5-(2-methylpropylidene)-1,5-dihydro-2*H*-pyrrol-2-one



$C_9H_{13}NO_2$
M = 167.21 g/mol

To a solution of compound **211** (3.00 g, 26.5 mmol, 1.00 equiv) in aq. NaOH (30 mL, 1 M) was added isobutyraldehyde (2.42 mL, 26.5 mmol, 1.00 equiv). The reaction mixture was stirred for 7 h at 60 °C. The formed precipitate was filtered, washed with cold water, redissolved in

DCM, dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the title compound **210** as a colorless solid (3.70 g, 22.1 mmol, 83%).

R_f 0.14 (50% EtOAc/CyH). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 7.35 (s, 1H, NH), 5.25 (d, *J* = 9.9 Hz, 1H, H-6), 5.07 (d, *J* = 1.7 Hz, 1H, H-3), 3.82 (s, 3H, O-CH_3), 2.56 – 2.50 (m, 1H, H-7), 1.08 (d, *J* = 6.7 Hz, 6H, 7-(CH_3)$_2$). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 171.5 (C-2), 167.2 (C-4), 131.7 (C-5), 116.9 (C-6), 93.2 (C-3), 58.6 (O-CH_3), 27.7 (C-7), 23.0 (CH($\underline{C}H_3$)$_2$). **HRMS (ESI)** *m/z*: calcd for $C_9H_{13}NO_2H$ $[M + H]^+$: 168.1019, found: 168.1017. The spectroscopic data were in agreement with those previously reported.[189]

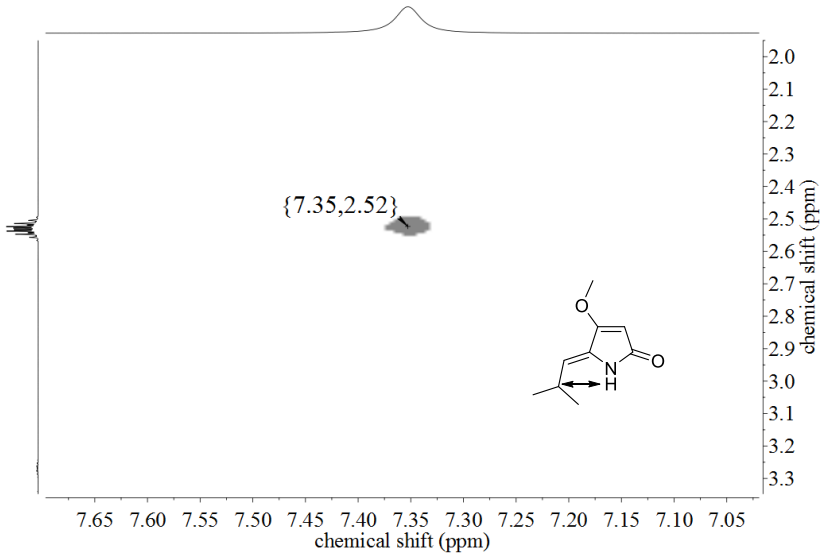
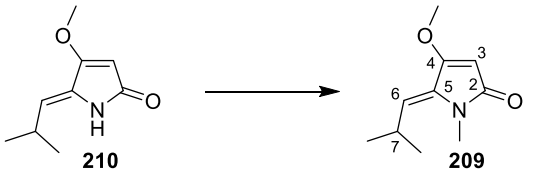


Figure 6.3.1-3: Selected NOESY signal of compound **210**, which was used for the determination of the *Z*-configuration.

Synthesis of Compound 209

(*Z*)-4-Methoxy-1-methyl-5-(2-methylpropylidene)-1,5-dihydro-2*H*-pyrrol-2-one



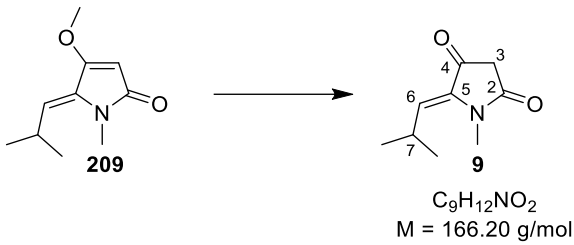
$C_{10}H_{15}NO_2$
M = 181.24 g/mol

A solution of compound **210** (3.50 g, 20.9 mmol, 1.00 equiv) in DMF (30 mL) was cooled to 0 °C and NaH (921 mg, 23.0 mmol, 1.10 equiv, 60% dispersion in mineral oil) was added. The reaction mixture was stirred 15 min at rt before cooling again to 0 °C. Iodomethane (1.43 mL, 23.0 mmol, 1.10 equiv) was added and the mixture was stirred 3 h at rt. Water (20 mL) and aq. sat. NH_4Cl solution (10 mL) were added. After separation of the organic phase, the aqueous phase was extracted with DCM (3x30 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (50% EtOAc/CyH) to yield the title compound **209** as a colorless solid (3.66 g, 20.2 mmol, 97%).

R_f 0.24 (50% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 5.23 (d, *J* = 10.7 Hz, 1H, H-6), 5.02 (s, 1H, H-3), 3.79 (s, 3H, O-CH_3), 3.22 (s, 3H, N-CH_3), 3.15 – 2.99 (m, 1H, H-7), 1.09 (d, *J* = 6.6 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 170.7 (C-2), 166.8 (C-4), 132.9 (C-5), 117.7 (C-6), 91.5 (C-3), 58.4 (O-CH_3), 28.0 (N-CH_3), 26.1 (C-7), 24.1 (CH($\underline{C}H_3$)$_2$). **HRMS (ESI)** *m/z*: calcd for $C_{10}H_{15}NO_2H$ $[M + H]^+$: 182.1176, found: 182.1175.

Synthesis of Compound 9

(*Z*)-1-Methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione

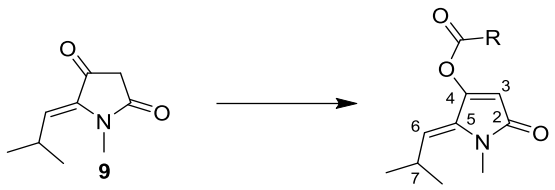


A solution of compound **209** (50.0 mg, 276 µmol, 1.00 equiv) in conc. HCl (1.5 mL) was stirred at rt for 8 h. The reaction mixture was cooled to 0 °C and diluted with water (20 mL). The aqueous layer was extracted with DCM (3x20 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the title compound **9** as a yellow solid (45.8 mg, 274 µmol, 99%). If necessary, the product can be further purified by flash chromatography (50% EtOAc/CyH).

R_f 0.32 (50% EtOAc/CyH). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 5.01 (d, *J* = 9.9 Hz, 1H, H-6), 3.67 – 3.57 (m, 1H, H-7), 3.01 (s, 3H, N-CH_3), 3.01 (s, 2H, H-3), 1.04 (d, *J* = 6.7 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 194.9 (C-4), 168.0 (C-2), 135.7 (C-5), 123.7

(C-6), 41.5 (C-3), 26.2 (N-CH_3), 25.7 (C-7), 23.6 (CH($\underline{C}H_3$)$_2$). **HRMS (ESI)** *m/z*: calcd for $C_9H_{13}NO_2H$ [M + H]$^+$: 168.1019, found: 168.1017.

General procedure for acylations using acid chlorides (246, 247, 248)

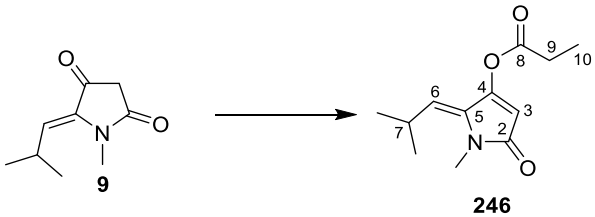


246, **247**, **248**

A solution of compound **9** (1.00 equiv) in DCM (0.25 M) was cooled to –5 °C. The appropriate acid chloride (1.15 equiv) and NEt_3 (1.40 equiv) were added dropwise. The reaction mixture was stirred for 30 min at –5 °C. 5% aq. $NaHCO_3$ solution and water were added followed by extraction of the aqueous layer with DCM. The combined organic layers were washed with aq. HCl (0.5 M), dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (30% EtOAc/CyH).

Synthesis of Compound 246

(*Z*)-1-Methyl-2-(2-methylpropylidene)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl propionate



246

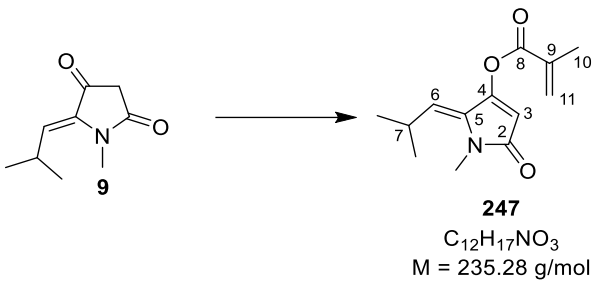
$C_{12}H_{17}NO_3$

M = 223.27 g/mol

Yield/Appearance: 86%/yellow solid. $\mathbf{R_f}$ 0.22 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 6.12 (d, *J* = 1.4 Hz, 1H, H-3), 5.21 (dd, *J* = 10.0, 1.4 Hz, 1H, H-6), 3.31 – 3.20 (m, 1H, H-7), 3.00 (s, 3H, N-CH_3), 2.59 (q, *J* = 7.5 Hz, 2H, H-9), 1.23 (t, *J* = 7.5 Hz, 3H, H-10), 1.10 (d, *J* = 6.7 Hz, 6H, 7-(CH_3)$_2$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 170.6 (C-8), 168.0 (C-2), 154.9 (C-4), 133.6 (C-5), 123.0 (C-6), 108.2 (C-3), 28.7 (C-9), 26.8 (C-7), 25.2 (N-CH_3), 24.1 (CH($\underline{C}H_3$)$_2$), 9.0 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_{12}H_{17}NO_3H$ [M + H]$^+$: 224.1281, found: 224.1280.

Synthesis of Compound 247

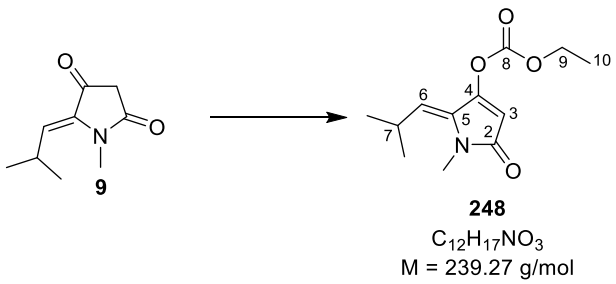
(*Z*)-1-Methyl-2-(2-methylpropylidene)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl methacrylate



Yield/Appearance: 99%/yellow solid. $\mathbf{R_f}$ 0.21 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 6.29 – 6.26 (m, 1H, H-11), 6.17 (d, *J* = 1.4 Hz, 1H, H-3), 5.86 – 5.83 (m, 1H, H-11), 5.24 (dd, *J* = 10.1, 1.4 Hz, 1H, H-6), 3.32 – 3.20 (m, 1H, H-7), 3.02 (s, 3H, N-CH_3), 2.05 (s, 3H, H-10), 1.10 (d, *J* = 6.7 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 167.9 (C-2), 163.8 (C-8), 154.9 (C-4), 135.8 (C-9), 133.7 (C-5), 129.0 (C-11), 122.9 (C-6), 108.6 (C-3), 26.8 (C-7), 25.2 (N-CH_3), 24.1 (CH($\underline{C}H_3)_2$), 18.6 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_{13}H_{17}NO_3H$ $[M + H]^+$: 236.1281, found: 236.1282.

Synthesis of Compound 248

(*Z*)-1-Methyl-2-(2-methylpropylidene)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl methacrylate

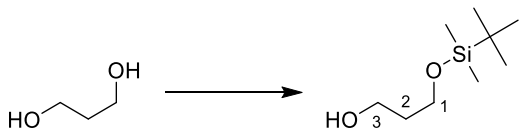


Yield/Appearance: 87%/yellow solid. $\mathbf{R_f}$ 0.17 (30% EtOAc/CyH). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 6.09 (d, *J* = 1.4 Hz, 1H, H-3), 5.22 (dd, *J* = 10.2, 1.4 Hz, 1H, H-6), 4.34 (q, *J* = 7.1 Hz, 2H, H-9), 3.31 – 3.21 (m, 1H, H-7), 3.00 (s, 3H, N-CH_3), 1.38 (t, *J* = 7.1 Hz, 3H, H-10), 1.09 (d, *J* = 6.6 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 167.5 (C-2), 155.5 (C-4), 151.5 (C-8), 133.0 (C-5), 123.5 (C-6), 107.4 (C-3), 66.4 (C-9), 26.7 (C-7), 25.2 (N-CH_3), 24.1

(CH($\underline{C}$H$_3$)$_2$), 14.5 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_{12}H_{17}NO_4H$ $[M + H]^+$: 240.1230, found: 240.1228.

Synthesis of Compound 254

3-((*tert*-Butyldimethylsilyl)oxy)propan-1-ol



201 **254**

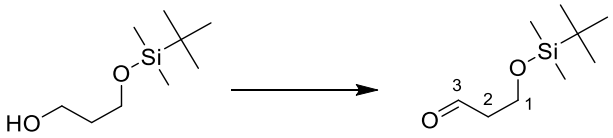
$C_9H_{22}O_2Si$
M = 190.36 g/mol

A solution of diol **201** (7.21 mL, 99.5 mmol, 5.00 equiv) and 1*H*-imidazole (1.49 g, 21.9 mmol, 1.10 equiv) in THF (40 mL) was cooled to 0 °C. *tert*-Butyldimethylsilyl chloride (3.00 g, 19.9 mmol, 1.00 equiv) was added and the reaction mixture was stirred 30 min at 0 °C and overnight at rt. Sat. aq. NH_4Cl solution (20 mL) was added and the aqueous phase was extracted with Et_2O (3x60 mL). The combined organic layers were washed with water (30 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (30% EtOAc/CyH) to yield the title compound **254** as a colorless oil (3.40 g, 17.9 mmol, 90%).

R_f 0.34 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 3.83 (t, *J* = 5.7 Hz, 2H, H-1), 3.80 (t, *J* = 5.7 Hz, 2H, H-3), 2.07 (s, 1H, OH), 1.78 (p, *J* = 5.5 Hz, 2H, H-2), 0.90 (s, 9H, TBS), 0.08 (s, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 63.1 (C-1), 62.6 (C-3), 34.3 (C-2), 26.0 (TBS), 18.3 (TBS), –5.4 (TBS). **MS (EI)** *m/z*: calcd for $C_5H_{13}O_2Si^{+\bullet}$ $[M - {}^tBu]^{+\bullet}$: 133.0, found: 133.0. The spectroscopic data were in agreement with those previously reported.[180]

Synthesis of Compound 202

3-((*tert*-Butyldimethylsilyl)oxy)propanal



254 **202**

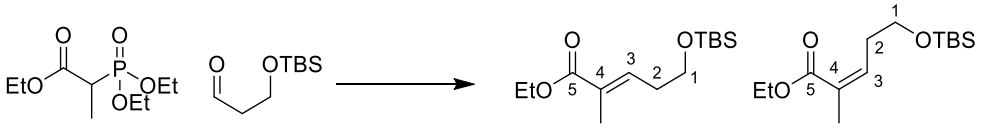
$C_9H_{20}O_2Si$
M = 188.34 g/mol

DCM (45 mL) was added to sulfur trioxide pyridine complex (8.78 g, 55.2 mmol, 3.00 equiv) followed by the addition of *N*,*N*-diisopropylethylamine (12.5 mL, 73.6 mmol, 4.00 equiv) and DMSO (13.1 mL, 164 mmol, 10.0 equiv). The reaction mixture was cooled to 0 °C and alcohol **254** (3.50 g, 18.4 mmol, 1.00 equiv) was added. After stirring at rt for 1.5 h, sat. aq. $NaHCO_3$ solution (150 mL) was added and the aqueous phase was extracted with DCM (3x150 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x150 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the title compound **202** as a colorless oil, which was used without further purification (3.40 g, 18.0 mmol, 98%).

R_f 0.41 (20% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 9.80 (t, *J* = 2.1 Hz, 1H, H-3), 3.98 (t, *J* = 6.0 Hz, 2H, H-1), 2.59 (td, *J* = 6.0, 2.1 Hz, 2H, H-2), 0.88 (s, 9H, TBS), 0.06 (s, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 202.2 (C-3), 57.6 (C-1), 46.7 (C-2), 26.0 (TBS), 18.4 (TBS), –5.3 (TBS). **MS (ESI)** *m/z*: calcd for $C_9H_{20}O_2SiH$ $[M + H]^+$: 189.131, found: 189.130. The spectroscopic data were in agreement with those previously reported.[233]

Synthesis of Compound 214*E* and 214*Z*

Ethyl (*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-2-enoate (214*E*) and Ethyl (*Z*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-2-enoate (214*Z*)



255 **202** **214*E*** **214*Z***

$C_{14}H_{28}O_3Si$
M = 272.46 g/mol

$C_{14}H_{28}O_3Si$
M = 272.46 g/mol

$Ba(OH)_2$ (1.37 g, 7.99 mmol, 3.50 equiv) was dried for 5 h at 120 °C using high vacuum. After cooling to rt, THF (14 mL) was added followed by phosphonate **255** (686 µL, 3.20 mmol, 1.40 equiv). The suspension was stirred for 30 min at rt. Freshly prepared aldehyde **202** (430 mg, 2.28 mmol, 1.00 equiv, in 40:1 THF/water (7 mL)) was added and the reaction was stirred overnight at rt. The suspension was filtered over celite® and washed with Et_2O. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (3% Et_2O/CyH) to yield the title compounds **214*E*** (472 mg, 1.73 mmol, 76%, *E*/*Z* = 16:1) and **214*Z*** (29.5 mg, 108 µmol, 5%, *E*/*Z* = 16:1) as colorless oils.

Analytical data for **214*E***:

R_f 0.22 (5% Et_2O /CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 6.78 (tq, *J* = 6.8, 1.3 Hz 1H, H-3), 4.19 (q, *J* = 7.1 Hz, 2H, OEt), 3.70 (t, *J* = 6.7 Hz, 2H, H-1), 2.40 (dt, *J* = 7.2, 6.8 Hz, 2H, H-2), 1.85 (s, 3H, 4-CH_3), 1.29 (t, *J* = 7.1 Hz, 3H, OEt), 0.89 (s, 9H, TBS), 0.05 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 168.2 (C-5), 138.6 (C-4), 129.4 (C-3), 61.9 (C-1), 60.6 (OEt), 32.5 (C-2), 26.0 (TBS), 18.5 (TBS), 14.4 (OEt), 12.7 (4-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{28}O_3SiH$ $[M + H]^+$: 273.1880, found: 273.1880. The spectroscopic data were in agreement with those previously reported.[190]

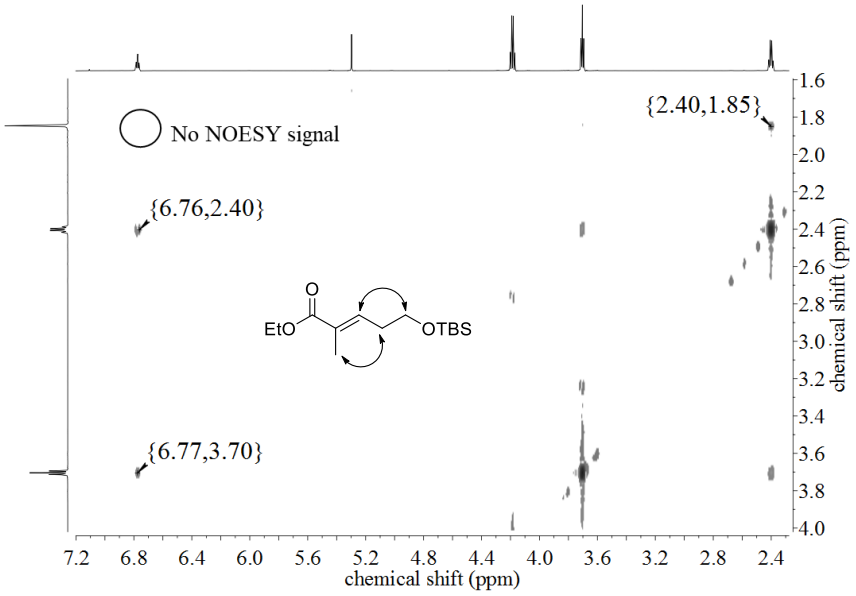


Figure 6.3.1-4: Selected NOESY signals of compound **214*E***, which were used for the determination of the *E*-configuration. No NOESY signal could be detected between H-3 and 4-CH_3.

Analytical data for **214*Z***:

R_f 0.27 (5% Et_2O/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.01 (tq, *J* = 7.3, 1.6 Hz 1H, H-3), 4.19 (q, *J* = 7.1 Hz, 2H, OEt), 3.68 (t, *J* = 6.5 Hz, 2H, H-1), 2.71 – 2.64 (m, 2H, H-2), 1.91 (q, *J* = 1.6 Hz, 3H, 4-CH_3), 1.30 (t, *J* = 7.1 Hz, 3H, OEt), 0.89 (s, 9H, TBS), 0.05 (s, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 168.2 (C-5), 139.4 (C-3), 128.7 (C-4), 62.7 (C-1), 60.2 (OEt), 33.3 (C-2), 26.1 (TBS), 20.8 (4-CH_3), 18.5 (TBS), 14.4 (OEt), –5.1 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{28}O_3SiH$ $[M + H]^+$: 273.1880, found: 273.1883. The spectroscopic data were in agreement with those previously reported.[190]

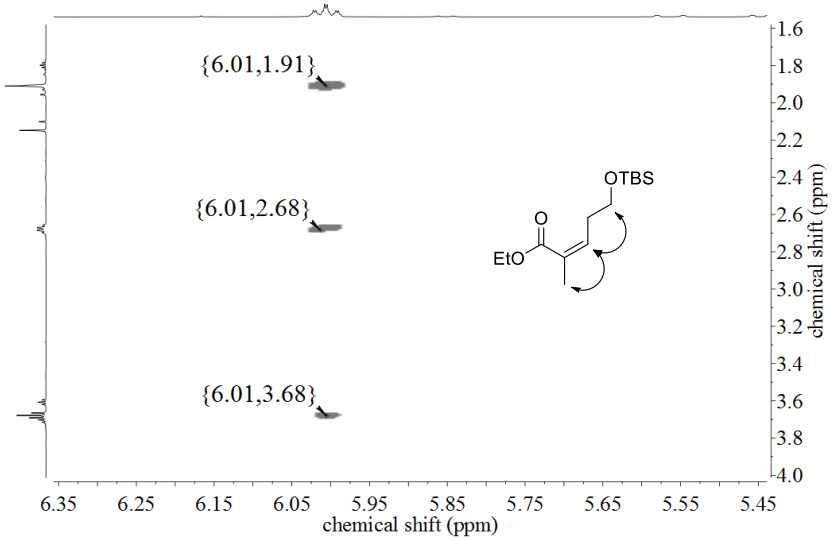
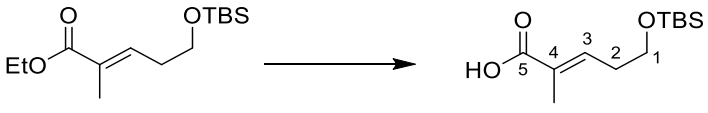


Figure 6.3.1-5: Selected NOESY signals of compound **214*Z***, which were used for the determination of the *Z*-configuration.

Synthesis of Compound 256

(*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-2-methylpent-2-enoic acid



214*E* **256**

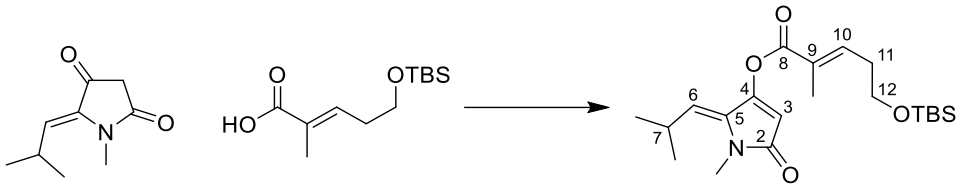
$C_{12}H_{24}O_3Si$
M = 244.41 g/mol

KOH (41.5 mg, 740 µmol, 1.20 equiv) was dissolved in water (40 µL) and ethanol (100 µL). The resulting solution was added to the ester **214*E*** (168 mg, 617 µmol, 1.00 equiv) and the reaction was stirred for 6 h at rt. Et_2O (10 mL) and water (10 mL) were added and the aqueous phase was acidified with aq. 1 M HCl until the pH turned 5-6. The organic layer was separated and the aqueous layer was extracted with Et_2O (3x20 mL). The combined organic layers were dried over $MgSO_4$, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (20% EtOAc/CyH) to yield the title compound **256** as a colorless oil (113 mg, 464 µmol, 75%).

R_f 0.37 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.94 – 6.88 (m, 1H, H-3), 3.72 (t, *J* = 6.7 Hz, 2H, H-1), 2.43 (dt, *J* = 7.2, 6.7 Hz, 2H, H-2), 1.86 (s, 3H, 4-CH_3), 0.89 (s, 9H, TBS), 0.06 (s, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 172.7 (C-5), 141.7 (C-3), 128.6 (C-4), 61.7 (C-1), 32.8 (C-2), 26.0 (TBS), 18.4 (TBS), 12.4 (4-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{12}H_{24}O_3SiH$ $[M + H]^+$: 245.1567, found: 245.1563. The spectroscopic data were in agreement with those previously reported.[234]

Synthesis of Compound 257

(*Z*)-1-Methyl-2-(2-methylpropylidene)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl (*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-2-enoate



9 **256** **257**

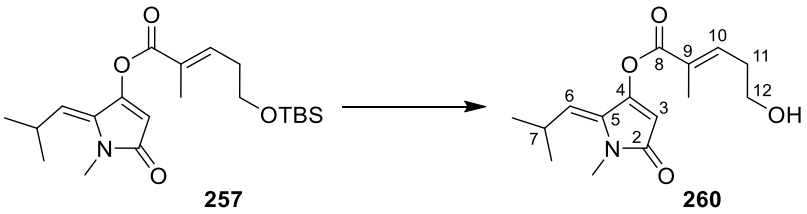
$C_{21}H_{35}NO_4Si$
M = 393.60 g/mol

Acid **256** (439 mg, 1.79 mmol, 1.20 equiv) was dissolved in DCM (12 mL) and cooled to 0 °C. DCC (1.79 mL, 1.79 mmol, 1.20 equiv, 1 M in DCM) and DMAP (36.5 mg, 299 µmol, 0.20 equiv) were added and the reaction was stirred 5 min at 0 °C. Tetramic acid **9** (250 mg, 1.50 mmol, 1.00 equiv) was added and the reaction was allowed to warm to rt and stirred for 6 h. The resulting suspension was filtered over celite®, washed with EtOAc and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (20% EtOAc/CyH) to yield the title compound **257** as a yellow oil (522 mg, 1.33 mmol, 89%).

R_f 0.28 (30% EtOAc/CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.00 – 6.97 (tq, *J* = 7.4, 1.3 Hz, 1H, H-10), 6.21 (d, *J* = 1.4 Hz, 1H, H-3), 5.20 (dd, *J* = 10.0, 1.3 Hz, 1H, H-6), 3.75 (t, *J* = 6.4 Hz, 1H, H-12), 3.27 – 3.21 (m, 1H, H-7), 3.06 (s, 3H, N-CH_3), 2.49 (dt, *J* = 7.4, 6.4 Hz, 2H, H-11), 1.95 (s, 3H, 9-CH_3), 1.11 (d, *J* = 6.7 Hz, 6H, 7-$(CH_3)_2$), 0.89 (s, 9H, TBS), 0.06 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 168.0 (C-2), 163.9 (C-8), 154.6 (C-4), 143.4 (C-10), 133.6 (C-5), 128.2 (C-9), 122.5 (C-6), 108.6 (C-3), 61.6 (C-12), 33.0 (C-11), 26.3 (C-7), 26.0 (TBS), 25.1 (N-CH_3), 24.1 (7-$(CH_3)_2$), 18.4 (TBS), 12.8 (9-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{21}H_{35}NO_4SiH$ $[M + H]^+$: 394.2408, found: 394.2415.

Synthesis of Compound 260

(*Z*)-1-Methyl-2-(2-methylpropylidene)-5-oxo-2,5-dihydro-1*H*-pyrrol-3-yl (*E*)-5-hydroxy-2-methylpent-2-enoate



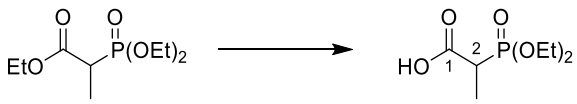
Tetramic acid derivative **257** (40.0 mg, 102 µmol, 1.00 equiv) was dissolved in MeOH (2 mL) and 1 M aq. HCl (173 µL, 173 µmol, 1.70 equiv) was added dropwise at 0 °C. The reaction mixture was stirred for 1 h at rt. DCM (10 mL) and water (10 mL) were added and the aqueous layer was extracted with DCM (3x10 mL). The combined organic layers were dried over Na_2SO_4, the solvent was removed under reduced pressure and the crude product was purified by flash chromatography (80% EtOAc/CyH) to yield the title compound **260** as a colorless solid (28.3 mg, 101 µmol, quant.).

R_f 0.28 (80% EtOAc/CyH). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 7.02 (tq, *J* = 7.4, 1.3 Hz, 1H, H-10), 6.24 (d, *J* = 1.4 Hz, 1H, H-3), 5.21 (dd, *J* = 10.1, 1.4 Hz, 1H, H-6), 3.81 (dt, *J* = 5.9, 5.9 Hz, 2H, H-12), 3.35 – 3.18 (m, 1H, H-7), 3.05 (s, 3H, N-CH_3), 2.60 – 2.50 (m, 2H, H-11), 1.97 (q, *J* = 1.1 Hz, 3H, 9-CH_3), 1.62 (t, *J* = 5.4 Hz, 1H, OH), 1.11 (d, *J* = 6.7 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 168.0 (C-2), 163.7 (C-8), 154.5 (C-4), 142.7 (C-10), 133.5 (C-5), 128.7 (C-9), 122.6 (C-6), 108.4 (C-3), 61.3 (C-12), 32.5 (C-11), 26.4 (C-7), 25.1

(N-CH_3), 24.1 (7-(CH_3)$_2$), 12.9 (9-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{21}NO_4H$ $[M + H]^+$: 280.1543, found: 280.1539.

Synthesis of Compound 252

2-(Diethoxyphosphoryl)propanoic acid



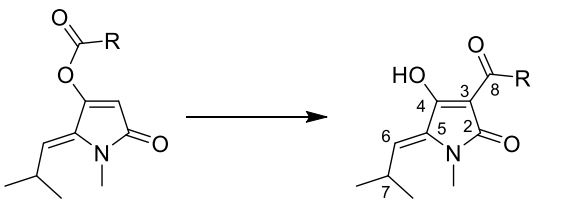
251 **252**

$C_7H_{15}O_5P$
M = 210.17

A solution of KOH (565 mg, 10.1 mmol, 1.20 equiv) in water (0.5 mL) and ethanol (1.25 mL) was prepared and added to ester **251** (2.00 g, 8.40 mmol, 1.00 equiv). The reaction mixture was stirred overnight at rt and water (20 mL) was added. The aqueous layer was acidified to pH = 1 using 6 N aq. HCl, extracted with DCM (3x20 mL), dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the title compound **252** as a colorless solid (1.13 g, 5.38 mmol, 64%).

R_f 0.38 (10% MeOH/DCM). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 8.93 (br s, 1H, COOH), 4.23 – 4.17 (m, 4H, OEt), 3.06 (2xq, *J* = 7.2 Hz, 1H, H-2), 1.43 (2xd, *J* = 7.2 Hz, 3H, 2-CH_3), 1.34 (t, *J* = 7.1 Hz, 6H, OEt). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 171.7 (d, *J* = 3.6 Hz, C-1), 63.4 (d, *J* = 67.4 Hz, OEt), 39.4 (d, *J* = 133.0 Hz, C-2), 16.4 (d, *J* = 6.0 Hz, OEt), 11.7 (d, *J* = 6.6 Hz, 2-CH_3). **^{31}P NMR** (162 MHz, $CDCl_3$) δ = 24.9. **HRMS (ESI)** *m/z*: calcd for $C_7H_{14}O_5P$ $[M – H]^–$: 209.0584, found: 209.0578. The spectroscopic data were in agreement with those previously reported.[235]

General procedure for the *O*- to *C*-rearrangement (241, 249, 250, 258, 259)

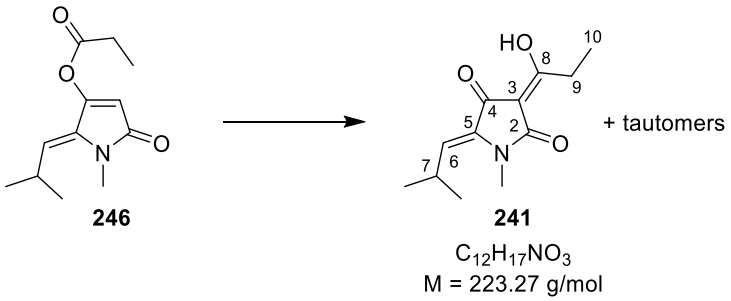


246, **247**, **248**, **257**, **260** **241**, **249**, **250**, **258**, **259**

Appropriate ester **246**, **247**, **248**, **257** or **260** (1.00 equiv) was dissolved in DCM (0.15 M). $CaCl_2$ (1.50 equiv) and DMAP (0.30 equiv) were added followed by NEt_3 (1.40 equiv). The reaction mixture was stirred for 4-7 h at rt until TLC showed complete conversion. DCM and aq. 1 M HCl were added and the aqueous layer was extracted with DCM. The combined organic layers were washed with water, dried over Na_2SO_4 and the solvent was removed under reduced pressure.

Synthesis of Compound 241

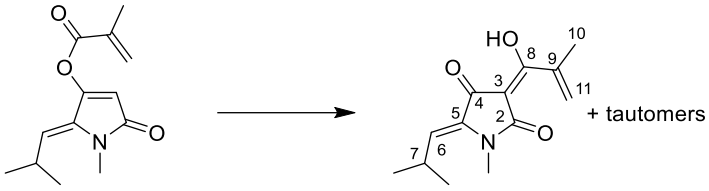
(3*E*,5*Z*)-3-(1-Hydroxypropylidene)-1-methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione



Yield/Appearance: quant./orange solid. $\mathbf{R_f}$ 0.24 (50% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 13.6 (br s, 1H, OH), 5.25/5.13 (2xd, *J* = 10.1 Hz, 1H, H-6), 3.86 – 3.61/3.70 – 3.61 (2xm, 1H, H-7), 3.02/3.00 (2xs, 3H, N-CH_3), 2.92/2.87 (2xq, *J* = 7.5 Hz, 2H, H-9), 1.20/1.19 (2xt, 3H, H-10), 1.08/1.05 (d, *J* = 6.6 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 194.5/187.6 (C-8), 186.4/183.0 (C-4), 171.3/164.5 (C-2), 134.8/132.9 (C-5), 125.9/124.9 (C-6), 104.6/101.9 (C-3), 28.5/26.3 (C-9), 26.3/25.3 (C-7), 25.2/25.1 (N-CH_3), 23.7/23.6 (C-10), 10.3/9.6 (7-$(CH_3)_2$). **HRMS (ESI)** *m/z*: calcd for $C_{12}H_{17}NO_3H$ $[M + H]^+$: 224.1281, found: 224.1285.

Synthesis of Compound 249

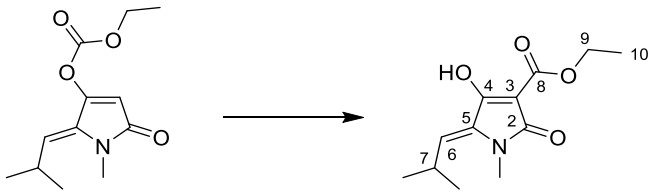
(3*E*,5*Z*)-3-(1-Hydroxy-2-methylallylidene)-1-methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione



Yield/Appearance: 86%/yellow solid. $\mathbf{R_f}$ 0.27 (50% EtOAc/CyH). Due to extended tautomerism only signals of two major tautomers were picked. **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 6.44/6.40 (2xs, 1H, H-11), 5.85/5.79 (2xs, 1H, H-11), 5.26/5.16 (2xd, *J* = 9.9 Hz, 1H, H-6), 3.84 – 3.79/3.71 – 3.66 (2xm, 1H, H-7), 3.06/3.01 (2xs, 3H, N-CH_3), 2.04 (s, 3H, H-10), 1.10/1.05 (2xd, *J* = 6.7 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 188.5/181.0 (C-8), 187.9/181.0 (C-4) 172.7/163.7 (C-2), 138.8/137.6 (C-9), 134.3/132.5 (C-5), 128.9/127.2 (C-11), 125.8/125.2 (C-6), 103.9/101.2 (C-3), 26.3/25.2 (C-7), 25.5/25.4 (N-CH_3), 23.7/23.5 (7-$(CH_3)_2$), 19.2/19.0 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_{13}H_{17}NO_3H$ $[M + H]^+$: 236.1281, found: 236.1279.

Synthesis of Compound 250

Ethyl (*Z*)-4-hydroxy-1-methyl-5-(2-methylpropylidene)-2-oxo-2,5-dihydro-1*H*-pyrrole-3-carboxylate

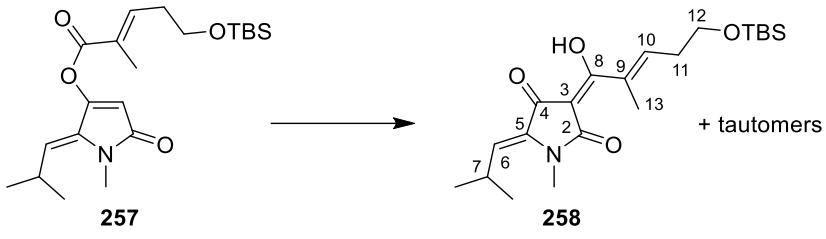


Yield/Appearance: quant./red oil. $\mathbf{R_f}$ 0.26 (50% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 11.58 (br s, 1H, OH), 5.40 (d, *J* = 10.1 Hz, 1H, H-6), 4.37 (q, *J* = 7.1 Hz, 2H, H-9), 3.50 – 3.43 (m, 1H, H-7), 2.98 (s, 3H, N-CH_3), 1.37 (t, *J* = 7.1 Hz, 3H, H-10), 1.11 (d, *J* = 6.6 Hz, 6H,

7-(CH_3)$_2$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 175.2 (C-6), 168.5 (C-8), 163.4 (C-2), 131.5 (C-5), 127.3 (C-6), 98.2 (C-3), 61.9 (C-9), 27.0 (C-7), 25.2 (N-CH_3), 23.8 (7-(CH_3)$_2$), 14.6 (C-10). **HRMS (ESI)** *m/z*: calcd for $C_{12}H_{17}NO_4H$ [M + H]$^+$: 240.1230, found: 240.1229.

Synthesis of Compound 258

(3*E*,5*Z*)-3-((*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-1-hydroxy-2-methylpent-2-en-1-ylidene)-1-methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione



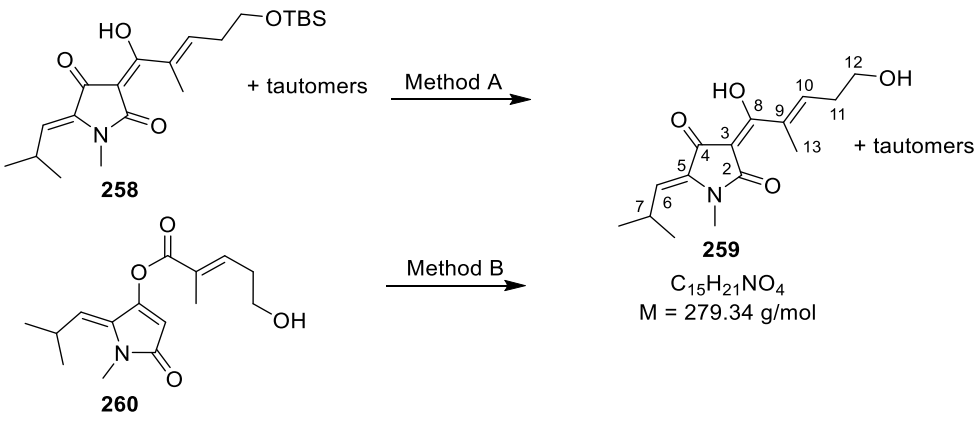
257 **258**

$C_{21}H_{35}NO_4Si$
M = 393.60 g/mol

Yield/Appearance: 99%/yellow oil. **R_f** 0.18 (30% EtOAc/CyH). Due to extended tautomerism only signals of two major tautomers were picked. **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 7.00/6.98 (2xt, *J* = 7.2 Hz, 1H, H-10), 5.23/5.13 (d, *J* = 9.9 Hz, 1H, H-6), 3.87 – 3.79/3.73 – 3.64 (2xm, 1H, H-7), 3.77/3.75 (2xt, *J* = 6.7 Hz, 2H, H-12), 3.05/2.99 (2xs, 3H, N-CH_3), 2.52 (dt, *J* = 6.7, 6.6 Hz, 2H, H-11), 1.94/1.93 (2xs, 3H, H-13), 1.09/1.04 (2xd, *J* = 6.6 Hz, 6H, 7-(CH_3)$_2$), 0.89/0.88 (2xs, 9H, TBS), 0.06/0.06 (2xs, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 189.0/182.7 (C-8), 188.4/180.9 (C-4), 173.1/163.9 (C-2), 143.7/141.8 (C-10), 134.4/132.7 (C-5), 131.9/130.5 (C-9), 125.2/124.7 (C-6), 103.4/100.3 (C-3), 62.1 (C-12), 33.5/33.2 (C-11), 26.2 (TBS), 26.2/25.2 (C-7), 25.5/25.3 (N-CH_3), 23.8/23.6 (7-(CH_3)$_2$), 18.7 (TBS), 13.2/13.1 (C-13), -5.1 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{21}H_{35}NO_4SiH$ [M + H]$^+$: 394.2408, found: 394.2406.

Synthesis of Compound 259

(3*E*,5*Z*)-3-((*E*)-1,5-Dihydroxy-2-methylpent-2-en-1-ylidene)-1-methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione



Method A:

Tetramic acid derivative **258** (24.5 mg, 62.3 µmol, 1.00 equiv) was dissolved in MeOH (1 mL) and 1 M aq. HCl (106 µL, 106 µmol, 1.70 equiv) was added dropwise at 0 °C. The reaction mixture was stirred for 20 min at rt. DCM (20 mL) and water (20 mL) were added and the aqueous layer was extracted with DCM (3x60 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield the title compound **259** as an orange oil (17.3 mg, 61.9 µmol, quant.).

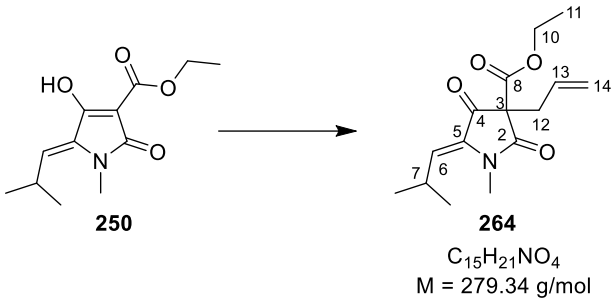
Method B:

See general procedure for the *O*- to *C*-rearrangement (Yield: quant.)

R_f 0.40 (10% MeOH/DCM). Due to extended tautomerism only signals of two major tautomers were picked. **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 6.60/6.54 (2xt, *J* = 7.4 Hz, 1H, H-10), 5.31/5.19 (2xd, *J* = 9.9 Hz, 1H, H-6), 3.79/3.76 (2xt, *J* = 5.6 Hz, 2H, H-12), 3.73 – 3.66/3.64 – 3.59 (2xm, 1H, H-7), 3.05/3.02 (2xs, 3H, N-CH_3), 2.50 (dt, *J* = 6.3 Hz, 2H, H-11), 1.92 (s, 3H, H-13), 1.10/1.04 (2xd, *J* = 6.6 Hz, 6H, 7-$(CH_3)_2$). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 189.8/184.1 (C-8), 188.4/181.9 (C-4), 172.6/164.6 (C-2), 142.7/141.2 (C-10), 134.6/132.3 (C-5), 131.1/130.2 (C-9), 126.5/125.9/123.8 (C-6), 103.5/100.8 (C-3), 61.7/61.6 (C-12), 33.0/32.9 (C-11), 26.3/25.3 (C-7), 25.7/25.3 (N-CH_3), 23.7/23.5 (7-$(CH_3)_2$), 12.8/12.7 (C-13). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{21}NO_4H$ $[M + H]^+$: 280.1543, found: 280.1544.

Synthesis of Compound 264

Ethyl (*Z*)-3-allyl-1-methyl-5-(2-methylpropylidene)-2,4-dioxopyrrolidine-3-carboxylate

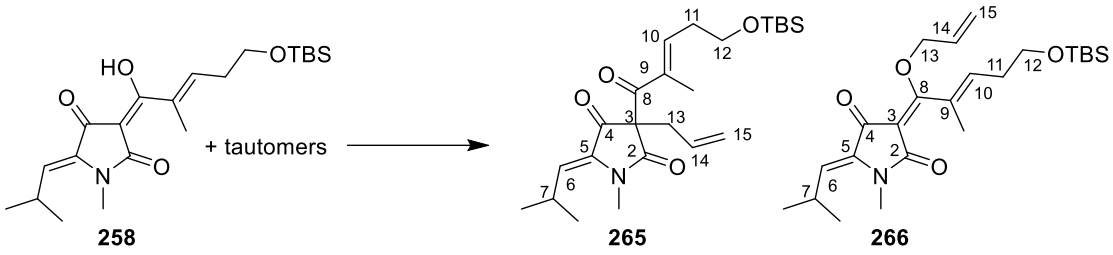


DMF (8 mL) was added to tetramic acid derivative **250** (105 mg, 439 µmol, 1.00 equiv) and K_2CO_3 (91.0 mg, 658 µmol, 1.50 equiv). Allyl bromide (49.3 µL, 571 µmol, 1.30 equiv) was added and the reaction mixture was stirred 5 h at 45 °C. DCM (20 mL), aq. sat. $NaHCO_3$ solution (10 mL) and water (20 mL) were added, the phases were separated and the aqueous layer was extracted with DCM (3x20 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (30% EtOAc/CyH) to yield the racemic title compound **264** as a colorless solid (100 mg, 358 µmol, 82%).

R_f 0.28 (30% EtOAc/CyH). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 5.54 – 5.48 (m, 1H, H-13), 5.14 – 5.10 (m, 2H, H-14), 5.04 (d, *J* = 10.1 Hz, 1H, H-6), 4.19 – 4.10 (m, 2H, H-10), 3.63 – 3.58 (m, 1H, H-7), 3.06 (s, 3H, N-CH_3), 2.83 – 2.81 (m, 2H, H-12), 1.18 (t, *J* = 7.1 Hz, 3H, H-11), 1.06 (d, *J* = 6.7 Hz, 3H, 7-$(CH_3)_2$), 1.04 (d, *J* = 6.7 Hz, 3H, 7-$(CH_3)_2$). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 193.7 (C-4), 167.7 (C-2), 165.6 (C-8), 134.3 (C-5), 130.8 (C-13), 125.6 (C-6), 120.8 (C-14), 63.0 (C-10), 62.9 (C-3), 35.8 (C-12), 26.7 (N-CH_3), 26.0 (C-7), 23.5 (7-$(CH_3)_2$), 14.2 (C-11). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{21}NO_4H$ $[M + H]^+$: 280.1543, found: 280.1541. Analytical, chiral HPLC showed the existence of the two enantiomers (98% *n*-hexane/ethanol, retention time 2.57 min and 2.76 min, using a DAICEL Chiralpak IG-U <2 µm; 3.0 x 100 mm).

Synthesis of Compound 265 and 266

(*Z*)-3-Allyl-3-((*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-2-enoyl)-1-methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione (265) and (3*E*,5*Z*)-3-((*E*)-1-(allyloxy)-5-((*tert*-butyldimethylsilyl)oxy)-2-methylpent-2-en-1-ylidene)-1-methyl-5-(2-methylpropylidene)pyrrolidine-2,4-dione (266)



DMF (2 mL) was added to tetramic acid derivative **258** (37.0 mg, 94.0 µmol, 1.00 equiv) and K_2CO_3 (19.5 mg, 141 µmol, 1.50 equiv). Allyl bromide (10.6 µL, 122 µmol, 1.30 equiv) was added and the reaction mixture was stirred 6 h at 55 °C. DCM (10 mL), aq. sat. $NaHCO_3$ solution (5 mL) and water (5 mL) were added, the phases were separated and the aqueous layer was extracted with DCM (3x15 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (10% EtOAc/CyH) to yield racemic **265** (6.50 mg, 15.0 µmol, 16%) and **266** (7.20 mg, 16.6 µmol, 18%) as yellow oils.

Analytical data for **265**:

R_f 0.34 (20% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.00 – 5.97 (tq, *J* = 6.9, 1.2 Hz 1H, H-10), 5.54 – 5.46 (m, 1H, H-14), 5.15 (d, *J* = 10.0 Hz, 1H, H-6), 5.13 – 5.10 (m, 1H, H-15), 5.04 – 5.02 (m, 1H, H-15'), 3.70 – 3.63 (m, 1H, H-7), 3.58 (t, *J* = 6.7 Hz, 2H, H-12), 3.09 (s, 3H, NCH_3), 2.91 – 2.83 (m, 2H, H-13), 2.36 (dt, *J* = 6.9, 6.8 Hz, 2H, H-11), 1.77 (s, 3H, 9-CH_3), 1.06 (d, *J* = 6.7 Hz, 3H, 7-CH_3), 1.05 (d, *J* = 6.7 Hz, 3H, 7-CH_3), 0.87 (s, 9H, TBS), 0.03 (s, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 194.4 (C-4), 191.2 (C-8), 169.2 (C-2), 138.7 (C-10), 137.3 (C-9), 133.9 (C-5), 130.3 (C-14), 125.6 (C-6), 120.8 (C-15), 67.2 (C-3), 61.6 (C-12), 38.7 (C-13), 33.1 (C-11), 26.0 (N-CH_3), 25.5 (C-7), 23.5 (7-CH_3), 23.4 (7-CH_3), 18.5 (TBS), 13.1 (TBS), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{24}H_{39}NO_4SiH$ $[M + H]^+$: 434.2721, found: 434.2720.

Analytical data for **266**:

R_f 0.19 (20% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 6.67 (t, *J* = 7.0 Hz, 1H, H-10), 5.93 – 5.85 (m, 1H, H-14), 5.33 – 5.24 (m, 2H, H-15), 5.18 (d, *J* = 9.9 Hz, 1H, H-6), 4.48 (d, *J* = 5.4 Hz, 2H, H-13), 3.72 (t, *J* = 6.3 Hz, 2H, H-12), 3.41 – 3.35 (m, 1H, H-7), 2.99 (s, 3H, N-CH_3), 2.52 (dt, *J* = 6.6, 6.3 Hz, 2H, H-11), 1.90 (s, 3H, 9-CH_3), 1.07 (d, *J* = 6.7 Hz, 6H, 7-$(CH_3)_2$), 0.84 (s, 9H, TBS), 0.02 (s, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 194.2 (C-4),

166.5 (C-2), 160.9 (C-8), 146.8 (C-10), 139.9 (C-9), 132.6 (C-5), 131.7 (C-14), 123.6 (C-6), 118.8 (C-15), 109.4 (C-3), 73.5 (C-13), 61.4 (C-12), 33.0 (C-11), 26.1 (C-7), 25.9 (TBS), 25.3 (N-CH_3), 23.8 (7-$(CH_3)_2$), 18.3 (TBS), 11.6 (9-CH_3), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{24}H_{39}NO_4SiH$ $[M + H]^+$: 434.2721, found: 434.2725.

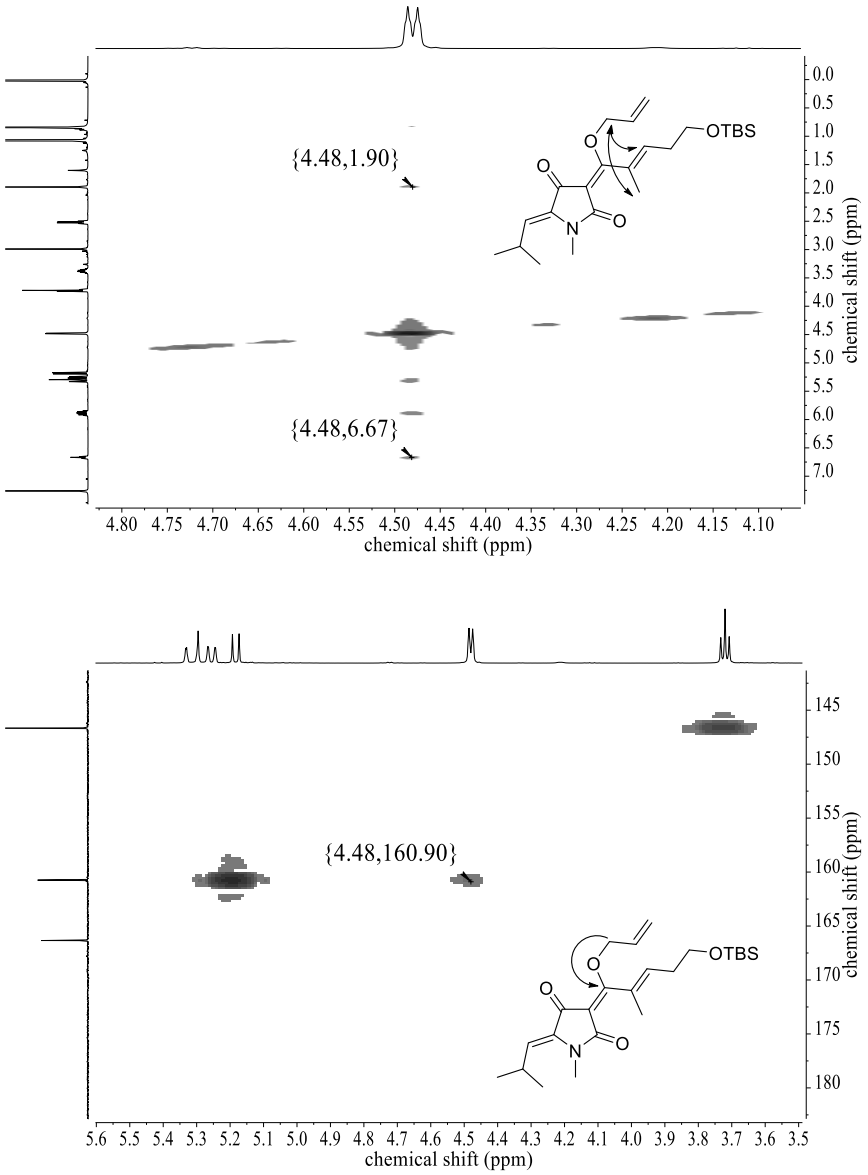
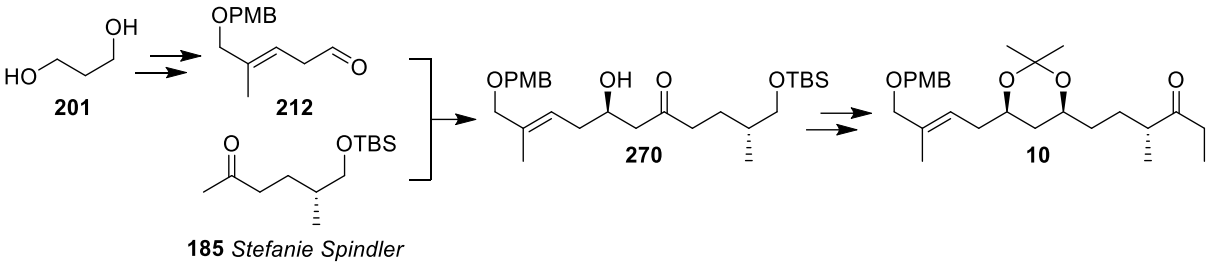


Figure 6.3.1-6: Selected NOESY (top) and HMBC (bottom) signals of compound **266**, which were used to determine the regioisomer of the allyl protection.

6.3.2 Synthesis of Fragment B (10)

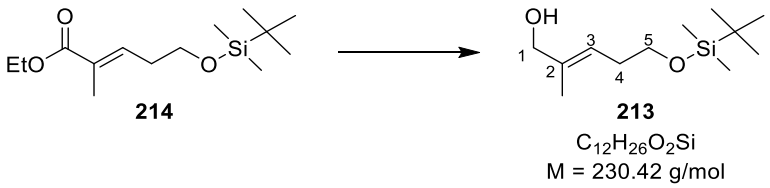
Experimental details are given for the synthesis of fragment B (**10**), which was synthesized in 14 linear steps from diol **201** (the used compound **185** was synthesized by *Stefanie Spindler*).



***Scheme 6.3.2-1*:** Synthesis towards fragment B (**10**).

Synthesis of Compound 213

(*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-2-methylpent-2-en-1-ol



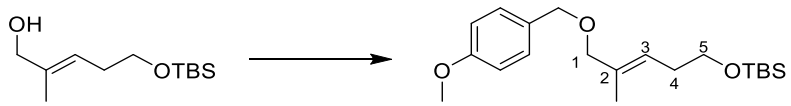
Ester **214** (1.00 g, 3.67 mmol, 1.00 equiv, for synthesis see **Chapter 6.3.1**) was dissolved in THF (6 mL), the solution was cooled to −78 °C and DIBAL-H (8.07 mL, 8.07 mmol, 2.20 equiv, 1 M in CyH) was added slowly. After stirring 1 h at −78 °C, MeOH (1.5 mL) and sat. aq. Rochelle salt solution (5.8 mL) were added. The mixture was allowed to warm to rt and stirred until clearly two phases appeared (2 h). The phases were separated, the aqueous layer was extracted with Et_2O (3x30 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (30% EtOAc/CyH) to yield the title compound **213** as a colorless oil (787 mg, 3.42 mmol, 93%).

R_f 0.25 (20% EtOAc/CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 5.42 (tq, *J* = 7.1, 1.3 Hz, 1H, H-3), 4.01 (s, 2H, H-1), 3.62 (t, *J* = 7.0 Hz, 2H, H-5), 2.29 (dt, *J* = 7.1, 7.0 Hz, 2H, H-4), 1.68 (s, 3H, 2-CH_3), 1.30 (br s, 1H, OH), 0.89 (s, 9H, TBS), 0.05 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 136.8 (C-2), 122.3 (C-3), 69.0 (C-1), 62.9 (C-5), 31.6 (C-4), 26.1 (TBS), 18.5

(TBS), 13.9 (2-CH_3), –5.1 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{12}H_{26}O_2SiH$ $[M + H]^+$: 231.1775, found: 231.1772. The spectroscopic data were in agreement with those previously reported.[236]

Synthesis of Compound 268

(*E*)-*tert*-Butyl((5-((4-methoxybenzyl)oxy)-4-methylpent-3-en-1-yl)oxy)dimethylsilane



$C_{20}H_{34}O_3Si$
M = 350.57 g/mol

Alcohol **213** (600 mg, 2.60 mmol, 1.00 equiv) was dissolved in DMF (3 mL), cooled to 0 °C and 4-methoxybenzyl chloride (388 µL, 2.86 mmol, 1.10 equiv) was added. NaH (115 mg, 2.86 mmol, 1.10 equiv, 60% dispersion in mineral oil) was added over the period of 20 min and the reaction mixture was stirred for 3 h at 0° C followed by the addition of sat. aq. NH_4Cl solution (5 mL), DCM (15 mL) and water (10 mL). The phases were separated, the aqueous phase was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (5% EtOAc/CyH) to yield the title compound **268** as a colorless oil (792 mg, 2.26 mmol, 87%).

R_f 0.18 (5% EtOAc/CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.43 (tq, *J* = 7.1, 1.3 Hz, 1H, H-3), 4.38 (s, 2H, Ar-CH_2), 3.88 (s, 2H, H-1), 3.80 (s, 3H, O-CH_3), 3.62 (t, *J* = 7.0 Hz, 2H, H-5), 2.29 (dt, *J* = 7.1, 7.0 Hz, 2H, H-4), 1.68 (s, 3H, 2-CH_3), 0.89 (s, 9H, TBS), 0.05 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.2 ($C_{arom.}O$), 134.2 (C-2), 130.9 ($C_{arom.}$), 129.5 ($C_{arom.}H$), 124.4 (C-3), 113.9 ($C_{arom.}H$), 76.0 (C-1), 71.3 (Ar-CH_2), 62.9 (C-5), 55.4 (O-CH_3), 31.7 (C-4), 26.1 (TBS), 18.5 (TBS), 14.2 (2-CH_3), –5.1 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{20}H_{34}O_3SiH$ $[M + H]^+$: 351.2350, found: 351.2348.

Synthesis of Compound 269

(*E*)-5-((4-Methoxybenzyl)oxy)-4-methylpent-3-en-1-ol

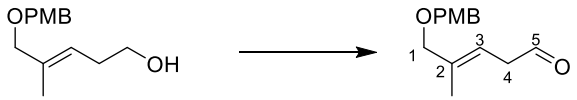
$C_{14}H_{20}O_3$
M = 236.31 g/mol

Compound **268** (630 mg, 1.80 mmol, 1.00 equiv) was dissolved in MeOH (20 mL), cooled to 0 °C and 1 M aq. HCl (3.05 mL, 3.05 mmol, 1.70 equiv) was added. The reaction mixture was stirred 1 h at 0 °C followed by the addition of DCM (10 mL) and water (10 mL). The organic layer was separated, the aqueous layer was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (40% EtOAc/CyH) to yield the title compound **269** as a colorless oil (383 mg, 1.62 mmol, 90%).

R_f 0.18 (40% EtOAc/CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.45 (tq, *J* = 7.1, 1.3 Hz, 1H, H-3), 4.40 (s, 2H, Ar-CH_2), 3.89 (s, 2H, H-1), 3.80 (s, 3H, O-CH_3), 3.66 (t, *J* = 6.5 Hz, 2H, H-5), 2.34 (dt, *J* = 7.1, 6.5 Hz, 2H, H-4), 1.70 (s, 3H, 2-CH_3), 1.53 (br s, 1H, OH). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}O$), 135.6 (C-2), 130.7 ($C_{arom.}$), 129.5 ($C_{arom.}H$), 123.6 (C-3), 113.9 ($C_{arom.}H$), 75.8 (C-1), 71.6 (Ar-CH_2), 62.4 (C-5), 55.4 (O-CH_3), 31.4 (C-4), 14.3 (2-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{20}O_3H$ $[M + H]^+$: 237.1485, found: 237.1484. The spectroscopic data were in agreement with those previously reported.[237]

Synthesis of Compound 212

(*E*)-5-((4-Methoxybenzyl)oxy)-4-methylpent-3-enal



$C_{14}H_{18}O_3$
M = 234.30 g/mol

Parikh-Doering oxidation:

DCM (1.5 mL) was added to sulfur trioxide pyridine complex (303 mg, 1.90 mmol, 3.00 equiv) followed by the addition of *N*,*N*-diisopropylethylamine (432 µL, 2.54 mmol, 4.00 equiv) and DMSO (451 µL, 6.35 mmol, 10.0 equiv). The reaction mixture was cooled to 0 °C and alcohol **269** (150 mg, 635 µmol, 1.00 equiv) was added. After stirring at rt for 1.5 h, sat. aq. $NaHCO_3$ solution (4 mL) was added and the aqueous phase was extracted with DCM (3x10 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x10 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) to yield the title compound **212** as a yellowish oil (47.3 mg, 202 µmol, 32%).

IBX oxidation:

Alcohol **269** (138 mg, 584 µmol, 1.00 equiv) was dissolved in DMSO (1 mL) and 2-iodoxybenzoic acid (IBX, 245 mg, 876 µmol, 1.50 equiv) was added. After stirring 1 h at rt, DCM (5 mL) and water (5 mL) were added and the mixture was filtered over celite®. The organic layer was separated, the aqueous layer was extracted with DCM (3x10 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (35% EtOAc/CyH) giving back starting material **269** (48.8 mg, 207 µmol, 35%) and to yield the title compound **212** as a yellowish oil (137 mg, 340 µmol, 58%, 90% brsm).

R_f 0.16 (20% EtOAc/CyH). **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 9.68 (t, *J* = 1.9 Hz, 1H, H-5), 7.27 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.65 (tq, *J* = 7.2, 1.4 Hz, 1H, H-3), 4.42 (s, 2H, Ar-CH_2), 3.93 (s, 2H, H-1), 3.81 (s, 3H, O-CH_3), 3.23 – 3.19 (m, 2H, H-4), 1.69 (s, 3H, 2-CH_3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 199.6 (C-5), 159.4 ($C_{arom.}$O), 138.1 (C-2), 130.5 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 116.2 (C-3), 113.9 ($C_{arom.}$H), 75.2 (C-1), 71.7 (Ar-CH_2), 55.4 (O-CH_3), 43.1 (C-4), 14.5 (2-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{18}O_3H$ $[M + H]^+$: 235.1329, found: 235.1329.

Synthesis of Compound 270

(2*R*,7*R*,*E*)-1-((*tert*-Butyldimethylsilyl)oxy)-7-hydroxy-11-((4-methoxybenzyl)oxy)-2,10-dimethylundec-9-en-5-one

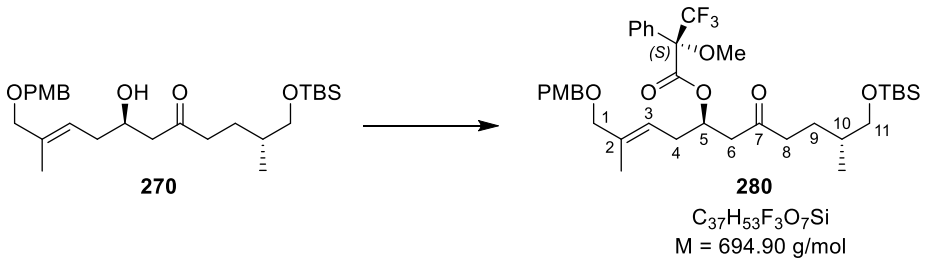
(+)-[Ipc]$_2$BCl (102 mg, 316 µmol, 1.10 equiv) was dissolved in DCM (1 mL). After cooling to −78 °C, DIPEA (97.8 µL, 575 µmol, 2.00 equiv) and ketone **185** (synthesized by *Stefanie Spindler*,[167] 70.3 mg, 288 µmol, 1.00 equiv, stored over 3 Å MS in 2 mL DCM) were added dropwise. The reaction mixture was stirred 3 h at −78 °C and freshly prepared aldehyde **212** (70.3 mg, 288 µmol, 1.15 equiv, stored 14 h over 3 Å MS in 2 mL DCM) was added dropwise. After stirring 1 h at −78 °C, the reaction vessel was placed in a deep freezer (−15 °C) overnight. MeOH (3.3 mL), pH 7 buffer (1.8 mL) and H_2O_2 (1.8 mL, 35%) were added to quench the reaction. After stirring 1 h at rt, brine (8 mL) was added, the phases were separated, the aqueous layer was extracted with DCM (3x10 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) giving back starting material **185** (24.1 mg, 98.7 µmol, 34%) and yielding the title compound **270** as a colorless oil (50.7 mg, 106 µmol, 37%, 56% brsm, *dr* = 4:1). Separation of the diastereomers was not possible.

$\mathbf{R_f}$ 0.15 (40% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = −3.6° (*c* = 1.40 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.47 (tq, *J* = 7.4, 1.1 Hz, 1H, H-3), 4.39 (s, 2H, Ar-CH_2), 4.12 – 4.08 (m, 1H, H-5), 3.89 (s, 2H, H-1), 3.80 (s, 3H, O-CH_3), 3.41 (d, *J* = 6.0 Hz, 2H, H-11), 3.03 (d, *J* = 3.2 Hz, 1H, OH), 2.63 – 2.60 (m, 1H, H-6), 2.54 – 2.41 (m, 3H, H-6', H-8), 2.32 – 2.28 (m, 1H, H-4), 2.24 – 2.20 (m, 1H, H-4'), 1.72 – 1.68 (m, 1H, H-9), 1.68 (s, 3H, 2-CH_3), 1.59 – 1.54 (m, 1H, H-10), 1.41 – 1.35 (m, 1H, H-9'), 0.89 (s, 9H, TBS), 0.86 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.03 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 212.4 (C-7), 159.3 ($C_{arom.}$O), 135.5 (C-2), 130.7 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 123.1 (C-3), 113.9 ($C_{arom.}$H), 75.9 (C-1), 71.6 (Ar-CH_2), 68.1 (C-11), 67.7 (C-5), 55.4 (O-CH_3), 48.4 (C-6), 41.6 (C-8), 35.4 (C-10), 34.9 (C-4), 27.2 (C-9), 26.1 (TBS), 18.5 (TBS), 16.7 (10-CH_3), 14.4 (2-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{27}H_{46}O_5SiH$ $[M + H]^+$: 479.3187,

found: 479.3193, calcd for $C_{27}H_{46}O_5SiNH_4$ [M + NH_4]$^+$: 496.3453, found: 496.3458, calcd for $C_{27}H_{46}O_5SiNa$ [M + Na]$^+$: 501.3007, found: 501.3009.

Synthesis of Compound 280

(5*R*,10*R*,*E*)-11-((*tert*-Butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)oxy)-2,10-dimethyl-7-oxoundec-2-en-5-yl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate



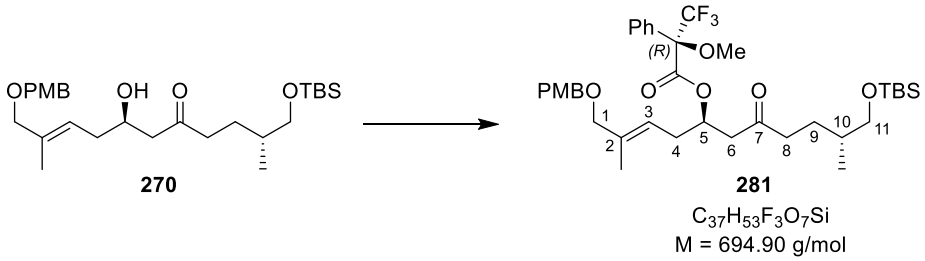
Alcohol **270** (5.00 mg, 10.4 µmol, 1.00 equiv) was dissolved in pyridine (120 µL) and (−)-(*R*)-MTPA-Cl (11.7 µL, 62.7 µmol, 6.00 equiv) was added at 0°C. The reaction mixture was stirred 2 h at rt followed by the addition of sat. aq. NH_4Cl solution (1 mL) and EtOAc (2 mL). After separation of the organic layer, the aqueous phase was extracted with EtOAc (3x2 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification of the crude product was achieved by preparative TLC (20% EtOAc/CyH) and HPLC (90% MeCN/water, retention time 20.4 min, using a MA-CHEREY-NAGEL Nucleodur 110-5 Gravity C18; 5 µm; 250 x 10 mm) to yield the title compound **280** as a colorless oil (6.60 mg, 9.50 µmol, 91%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

R_f 0.37 (20% EtOAc/CyH). **$[\alpha]_D^{20}$** = −15.9° (*c* = 0.44 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.49 – 7.47 (m, 2H, $H_{arom., MTPA}$), 7.37 – 7.35 (m, 3H, $H_{arom., MTPA}$), 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.60 – 5.56 (m, 1H, H-5), 5.42 (t, *J* = 7.3 Hz, 1H, H-3), 4.37 (s, 2H, Ar-CH_2), 3.85 (s, 2H, H-1), 3.80 (s, 3H, O-CH_3), 3.51 (s, 3H, O-$CH_{3, MTPA}$), 3.38 – 3.37 (m, 2H, H-11), 2.79 (dd, *J* = 17.1, 7.9 Hz, 1H, H-6), 2.57 (dd, *J* = 17.1, 4.8 Hz, 1H, H-6'), 2.53 – 2.49 (m, 1H, H-4), 2.47 – 2.43 (m, 1H, H-4'), 2.41 – 2.35 (m, 1H, H-8), 2.28 – 2.24 (m, 1H, H-8'), 1.66 (s, 3H, 2-CH_3), 1.61 – 1.57 (m, 1H, H-9), 1.53 – 1.48 (m, 1H, H-10), 1.30 – 1.26 (m, 1H, H-9'), 0.88 (s, 9H, TBS), 0.82 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.03 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 207.2 (C-7), 165.9 ($C{=}O_{MTPA}$), 159.3 ($C_{arom.}O$), 136.8 (C-2), 132.4 ($C_{arom.,MTPA}$), 130.5 ($C_{arom.}$), 129.6 ($C_{arom.}H_{MTPA}$), 129.4 ($C_{arom.}H$), 128.5 ($C_{arom.}H_{MTPA}$),

127.5 ($C_{arom.}H_{MTPA}$), 123.4 (q, *J* = 283.7 Hz, CF_3), 120.8 (C-3), 113.9 ($C_{arom.}H$), 75.4 (C-1), 72.7 (C-5), 71.7 (Ar-CH_2), 68.1 (C-11), 55.4 (O-CH_3), 45.5 (C-6), 41.4 (C-8), 35.3 (C-10), 32.1 (C-4), 27.0 (C-9), 26.1 (TBS), 18.5 (TBS), 16.6 (10-CH_3), 14.3 (2-CH_3), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{37}H_{53}F_3O_7SiH$ $[M + H]^+$: 695.3585, found: 695.3591.

Synthesis of Compound 281

(5*R*,10*R*,*E*)-11-((*tert*-Butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)oxy)-2,10-dimethyl-7-oxoundec-2-en-5-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate



Alcohol **270** (5.00 mg, 10.4 µmol, 1.00 equiv) was dissolved in pyridine (120 µL) and (+)-(*S*)-MTPA-Cl (11.7 µL, 62.7 µmol, 6.00 equiv) was added at 0 °C. The reaction mixture was stirred 2 h at rt followed by the addition of sat. aq. NH_4Cl solution (1 mL) and EtOAc (2 mL). After separation of the organic layer, the aqueous phase was extracted with EtOAc (3x2 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification of the crude product was achieved by preparative TLC (15% EtOAc/CyH) and HPLC (90% MeCN/water, retention time 20.4 min, using a MA-CHEREY-NAGEL Nucleodur 110-5 Gravity C18; 5 µm; 250 x 10 mm) to yield the title compound **281** as a colorless oil (6.80 mg, 9.79 µmol, 94%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

$\mathbf{R_f}$ 0.26 (15% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = +11.8° (*c* = 0.68 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.49 – 7.47 (m, 2H, $H_{arom., MTPA}$), 7.37 – 7.35 (m, 3H, $H_{arom., MTPA}$), 7.24 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 5.57 – 5.53 (m, 1H, H-5), 5.30 (t, *J* = 6.9 Hz, 1H, H-3), 4.35 (s, 2H, Ar-CH_2), 3.81 (s, 5H, H-1, O-CH_3), 3.48 (s, 3H, O-$CH_{3, MTPA}$), 3.38 (d, *J* = 6.0 Hz, 2H, H-11), 2.82 (dd, *J* = 17.2, 8.4 Hz, 1H, H-6), 2.62 (dd, *J* = 17.2, 4.3 Hz, 1H, H-6'), 2.47 – 2.42 (m, 2H, H-4, H-8), 2.39 – 2.33 (m, 2H, H-4',H-8'), 1.68 – 1.63 (m, 1H, H-9), 1.63 (s, 3H, 2-CH_3), 1.56 – 1.49 (m, 1H, H-10), 1.36 – 1.32 (m, 1H, H-9'), 0.88 (s, 9H, TBS), 0.83 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.02 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 207.4 (C-7), 166.0

(C=O_{MTPA}), 159.3 ($C_{arom.}O$), 136.7 (C-2), 132.4 ($C_{arom.,MTPA}$), 130.6 ($C_{arom.}$), 129.7 ($C_{arom.}H_{MTPA}$), 129.4 ($C_{arom.}H$), 128.5 ($C_{arom.}H_{MTPA}$), 127.6 ($C_{arom.}H_{MTPA}$), 123.4 (q, *J* = 285.9 Hz, CF_3), 120.7 (C-3), 113.9 ($C_{arom.}H$), 75.5 (C-1), 72.5 (C-5), 71.6 (Ar-CH_2), 68.1 (C-11), 55.4 (O-CH_3), 45.5 (C-6), 41.4 (C-8), 35.3 (C-10), 31.8 (C-4), 27.1 (C-9), 26.1 (TBS), 18.5 (TBS), 16.6 (10-CH_3), 14.3 (2-CH_3), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{37}H_{53}F_3O_7SiH$ $[M + H]^+$: 695.3585, found: 695.3585.

Mosher Ester Analysis for Compound 270

Determination of the absolute configuration was accomplished using the *Mosher* ester analysis. Two *Mosher* ester derivatives **280** and **281** have been synthesized and the respective NMR-data have been compared as shown in **Table 6.3.2-1**. The resulting difference between the ^{1}H NMR data of the (*S*)- and (*R*)-MTPA ester led to the determination of the absolute configuration as shown in **Figure 6.3.2-1**. For a detailed background, see **Chapter 5.3.3** or the corresponding literature.[203,204]

Main diastereomer:

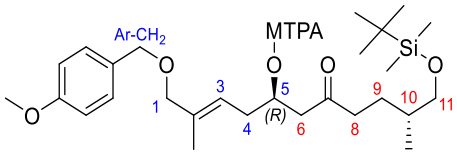


Table 6.3.2-1: Significant ^{1}H NMR signals of **280** and **281** (major diastereomer, *dr* = 4:1) and their use in the *Mosher* ester analysis.

position	δ_H [ppm] (**280**) (*S*)	δ_H [ppm] (**281**) (*R*)	$\Delta\delta^{S-R}$
8	2.26	2.37	-0.11
8‘	2.38	2.45	-0.07
9	1.59	1.66	-0.07
9‘	1.28	1.34	-0.06
6	2.57	2.62	-0.05
6‘	2.79	2.82	-0.03
10	1.51	1.53	-0.02
10-CH_3	0.82	0.83	-0.01

11	3.37	3.38	-0.01
Ar-CH_2	4.37	4.35	0.02
2-CH_3	1.66	1.63	0.03
5	5.58	5.55	0.03
4	2.50	2.45	0.05
4‘	2.46	2.37	0.09
3	5.42	5.30	0.12

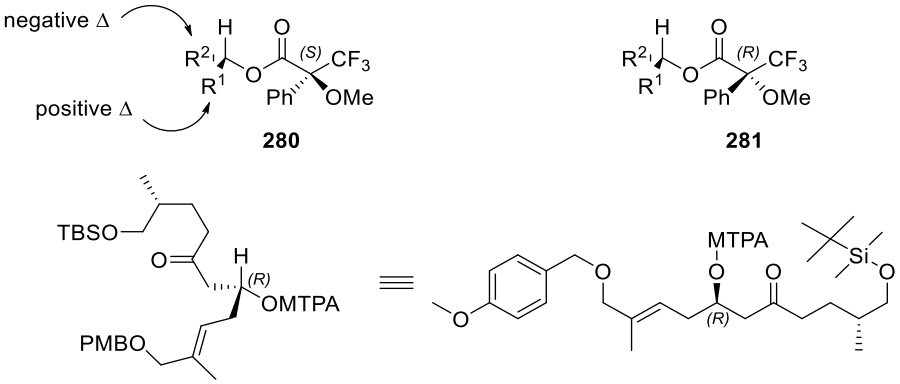


Figure 6.3.2-1: Proposed configuration of the major diastereomer (*dr* = 4:1) under the use of the *Mosher* ester analysis.

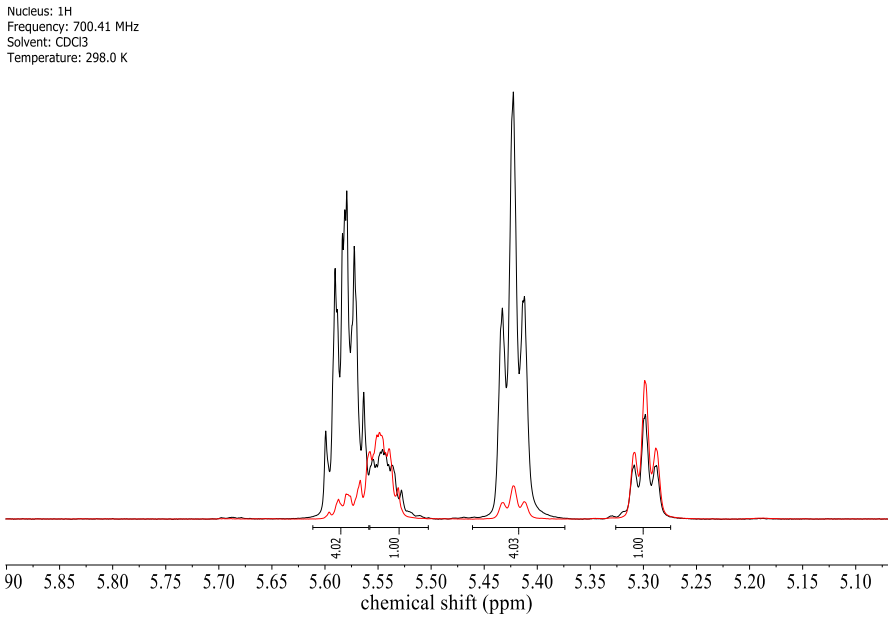


Figure 6.3.2-2: Superimposed ^{1}H NMR spectra of the two *Mosher* esters showing the *dr* of 4:1, resulting out of the aldol reaction to compound **270**.

Minor diastereomer:

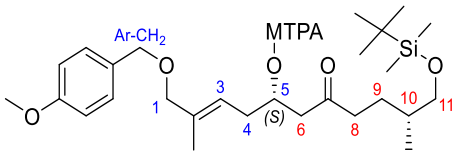


Table 6.3.2-2: Significant ^{1}H NMR signals of **280** and **281** (minor diastereomer, *dr* = 4:1) and their use in the *Mosher* ester analysis.

position	δ_H [ppm] (**280**) (*S*)	δ_H [ppm] (**281**) (*R*)	$\Delta\delta^{S-R}$
9‘	1.38	1.34	0.04
6	2.62	2.57	0.05
6‘	2.82	2.79	0.03
10-CH_3	0.84	0.82	0.02
11	3.38	3.37	0.01
Ar-CH_2	4.35	4.37	-0.02
2-CH_3	1.63	1.66	-0.03
5	5.54	5.58	-0.04
3	5.30	5.42	-0.12

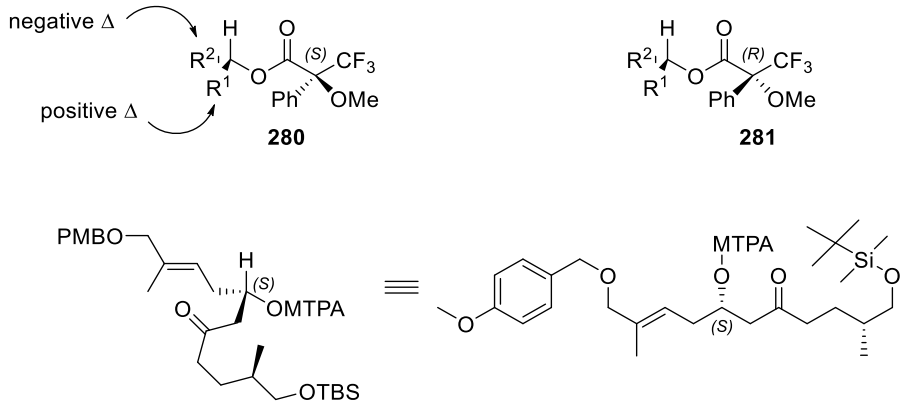
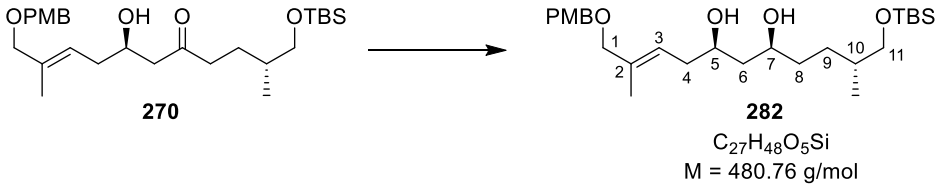


Figure 6.3.2-3: Proposed configuration of the minor diastereomer (*dr* = 4:1) under the use of the *Mosher* ester analysis.

Synthesis of Compound 282

(5*R*,7*S*,10*R*,*E*)-11-((*tert*-Butyldimethylsilyl)oxy)-1-((4-methoxybenzyl)oxy)-2,10-dimethylundec-2-ene-5,7-diol



270 **282**

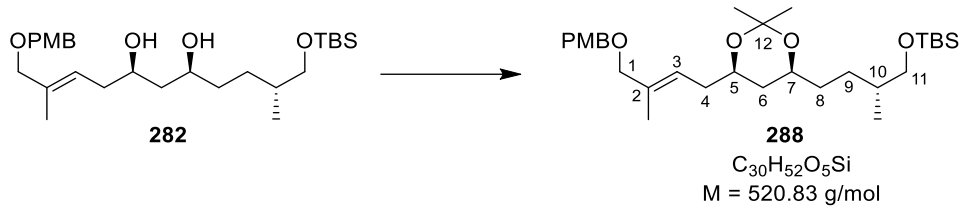
$C_{27}H_{48}O_5Si$
M = 480.76 g/mol

Alcohol **270** (29.0 mg, 60.6 µmol, 1.00 equiv) was dissolved in THF/MeOH (4:1, 0.75 mL) and cooled to −78 °C. Et_2BOMe (66.6 µL, 66.6 µmol, 1.10 equiv, 1 M in THF) was added slowly and the mixture was stirred 15 min at −78 °C followed by the addition of $NaBH_4$ (2.52 mg, 66.6 µmol, 1.10 equiv) and further stirring for 4 h at −78 °C. The reaction mixture was allowed to warm to 0 °C and quenched with aq. NaOH (0.6 mL, 3 M) and H_2O_2 (0.8 mL, 35%). After stirring 3 h at 0 °C, water (1.5 mL) and Et_2O (4 mL) were added, the phases separated. The aqueous phase was extracted with Et_2O (3x5 mL), the combined organic phases were washed with water (10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (40% EtOAc/CyH) to yield the title compound **282** as a colorless oil (28.5 mg, 59.3 µmol, 98%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

$\mathbf{R_f}$ 0.20 (40% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = +2.9° (*c* = 1.02 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 5.48 (tq, *J* = 7.4, 1.1 Hz, 1H, H-3), 4.40 (s, 2H, Ar-CH_2), 3.92 – 3.88 (m, 1H, H-5), 3.90 (s, 2H, H-1), 3.85 – 3.82 (m, 1H, H-7), 3.80 (s, 3H, O-CH_3), 3.45 – 3.38 (m, 2H, H-11), 3.00 (br s, 2H, 2xOH), 2.31 – 2.21 (m, 1H, H-4), 2.26 – 2.18 (m, 1H, H-4'), 1.69 (s, 3H, 2-CH_3), 1.65 – 1.43 (m, 7H, H-6, H-8, H-9, H-10), 1.16 – 1.07 (m, 1H, H-9'), 0.89 (s, 9H, TBS), 0.88 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.03 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}O$), 135.8 (C-2), 130.7 ($C_{arom.}$), 129.5 ($C_{arom.}H$), 123.1 (C-3), 113.9 ($C_{arom.}H$), 75.9 (C-1), 73.6 (C-7), 72.9 (C-5), 71.6 (Ar-CH_2), 68.3 (C-11), 55.4 (O-CH_3), 42.6 (C-6), 36.6 (C-4), 35.9 (C-10), 35.6 (C-8), 29.0 (C-9), 26.1 (TBS), 18.5 (TBS), 17.0 (10-CH_3), 14.4 (2-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{27}H_{48}O_5SiH$ $[M + H]^+$: 481.3344, found: 481.3342.

Synthesis of Compound 288

tert-Butyl((_R_)-4-((4_S_,6_R_)-6-((_E_)-4-((4-methoxybenzyl)oxy)-3-methylbut-2-en-1-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methylbutoxy)dimethylsilane



2,2-Dimethoxypropane (1 mL) was added to diol **282** (28.5 mg, 59.3 µmol, 1.00 equiv) followed by the addition of CSA (12.8 µL, 0.59 µmol, 0.01 equiv, 43 mM in DCM). The reaction mixture was stirred 1.5 h at rt and quenched with the addition of NEt_3 (20 µL). All volatiles were removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (10% EtOAc/CyH) to yield the title compound **288** as a colorless oil (29.5 mg, 56.6 µmol, 96%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

$\mathbf{R_f}$ 0.24 (10% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +1.1° (*c* = 0.94 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.45 (tq, *J* = 7.4, 1.1 Hz, 1H, H-3), 4.38 (s, 2H, Ar-CH_2), 3.88 (s, 2H, H-1), 3.84 – 3.82 (m, 1H, H-5), 3.80 (s, 3H, O-CH_3), 3.77 – 3.73 (m, 1H, H-7), 3.43 (dd, *J* = 9.8, 6.0 Hz, 1H, H-11), 3.35 (dd, *J* = 9.8, 6.6 Hz, 1H, H-11'), 2.31 – 2.27 (m, 1H, H-4), 2.21 – 2.16 (m, 1H, H-4'), 1.67 (s, 3H, 2-CH_3), 1.57 – 1.43 (m, 5H, H-6, H-8, H-9, H-10), 1.42 (s, 3H, 12-CH_3), 1.39 (s, 3H, 12-CH_3), 1.13 – 1.02 (m, 2H, H-6', H-9'), 0.89 (s, 9H, TBS), 0.87 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.03 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}O$), 134.6 (C-2), 130.8 ($C_{arom.}$), 129.5 ($C_{arom.}H$), 123.4 (C-3), 113.9 ($C_{arom.}H$), 98.6 (C-12), 76.0 (C-1), 71.3 (Ar-CH_2), 69.6 (C-7), 69.1 (C-5), 68.4 (C-11), 55.4 (O-CH_3), 36.7 (C-6), 35.9 (C-10), 35.1 (C-4), 34.0 (C-8), 30.4 (12-CH_3), 28.5 (C-9), 26.1 (TBS), 20.0 (12-CH_3), 18.5 (TBS), 16.8 (10-CH_3), 14.4 (2-CH_3), –5.2 (TBS)5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{30}H_{52}O_5SiNH_4$ $[M + NH_4]^+$: 538.3922, found: 538.3923.

Acetonide Analysis for Compound 288

The determination of the correct relative stereochemistry of diol **282** (stereogenic center at position 7) was accomplished by the accepted model for the analysis of the corresponding acetonide **288**. Thus, the ^{13}C NMR spectrum was used and analyzed showing different chemical shifts for the two methyl groups of the acetonide, which indicates a chair conformation, resulting out of the *syn*-1,3-diol acetonide. A more detailed theoretical background can be found in **Chapter 5.3.3** and in literature.[155–158]

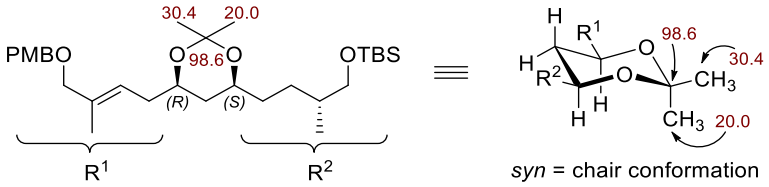
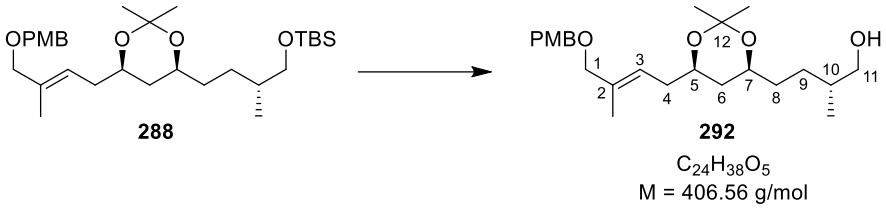


Figure 6.3.2-4: Relevant ^{13}C NMR signals used for the determination of the 1,3-*syn*-diol acetonide **288**.

Synthesis of Compound 292

(*R*)-4-((4*S*,6*R*)-6-((*E*)-4-((4-Methoxybenzyl)oxy)-3-methylbut-2-en-1-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methylbutan-1-ol



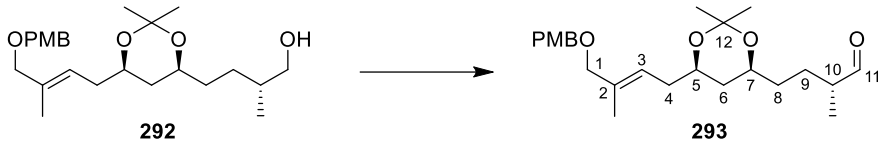
Acetonide **288** (29.5 mg, 56.6 µmol, 1.00 equiv) was dissolved in THF (4 mL) and cooled to 0 °C. TBAF (85.0 µL, 85.0 µmol, 1.50 equiv, 1 M in THF) was added and after stirring for 20 min at 0 °C, the reaction mixture was stirred further 3 h at rt. Et_2O (10 mL) and water (10 mL) were added, the phases were separated, the aqueous phase was extracted with Et_2O (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (50% EtOAc/CyH) to yield the title compound **292** as a colorless oil (22.5 mg, 55.3 µmol, 98%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

R_f 0.27 (50% EtOAc/CyH). **$[\alpha]_D^{20}$** = +1.0° (*c* = 1.02 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 5.44 (t, *J* = 7.4 Hz, 1H,

H-3), 4.38 (s, 2H, Ar-CH_2), 3.88 (s, 2H, H-1), 3.86 – 3.82 (m, 1H, H-5), 3.80 (s, 3H, O-CH_3), 3.78 – 3.75 (m, 1H, H-7), 3.50 – 3.42 (m, 2H, H-11), 2.32 – 2.27 (m, 1H, H-4), 2.21 – 2.15 (m, 1H, H-4'), 1.67 (s, 3H, 2-CH_3), 1.63 – 1.45 (m, 5H, H-6, H-8, H-9, H-10), 1.43 (s, 3H, 12-CH_3), 1.39 (s, 3H, 12-CH_3), 1.16 – 1.11 (m, 2H, H-6', H-9'), 0.91 (d, *J* = 6.7 Hz, 3H, 10-CH_3). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}O$), 134.6 (C-2), 130.8 ($C_{arom.}$), 129.5 ($C_{arom.}H$), 123.3 (C-3), 113.9 ($C_{arom.}H$), 98.6 (C-12), 75.9 (C-1), 71.3 (Ar-CH_2), 69.6 (C-7), 69.1 (C-5), 68.2 (C-11), 55.4 (O-CH_3), 36.7 (C-6), 35.9 (C-10), 35.0 (C-4), 33.9 (C-8), 30.4 (12-CH_3), 28.5 (C-9), 20.0 (12-CH_3), 16.8 (10-CH_3), 14.4 (2-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{24}H_{38}O_5NH_4$ $[M + NH_4]^+$: 424.3057, found: 424.3068.

Synthesis of Compound 293

(*R*)-4-((4*S*,6*R*)-6-((*E*)-4-((4-Methoxybenzyl)oxy)-3-methylbut-2-en-1-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-2-methylbutanal



292 **293**

$C_{24}H_{36}O_5$
M = 404.55 g/mol

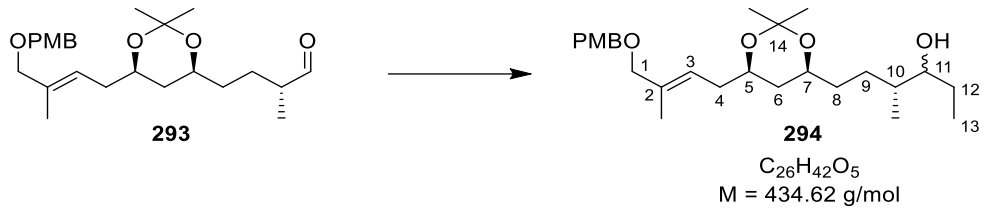
DCM (0.5 mL) was added to sulfur trioxide pyridine complex (26.4 mg, 166 µmol, 3.00 equiv) followed by the addition of *N*,*N*-diisopropylethylamine (37.7 µL, 221 µmol, 4.00 equiv) and DMSO (39.3 µL, 553 µmol, 10.0 equiv). The reaction mixture was cooled to 0 °C and alcohol **292** (22.5 mg, 55.3 µmol, 1.00 equiv, in 1 mL DCM) was added. After stirring for 20 min at 0 °C, sat. aq. $NaHCO_3$ solution (4 mL) was added and the aqueous phase was extracted with DCM (3x10 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x15 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the title compound **293** as a colorless oil (17.3 mg, 42.8 µmol, 77%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

R_f 0.36 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 9.62 (t, *J* = 1.9 Hz, 1H, H-11), 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 5.44 (t, *J* = 7.4 Hz, 1H, H-3), 4.39 (s, 2H, Ar-CH_2), 3.88 (s, 2H, H-1), 3.86 – 3.82 (m, 1H, H-5), 3.81 (s, 3H, O-CH_3), 3.79 – 3.76 (m, 1H, H-7), 2.37 – 2.20 (m, 1H, H-4), 1.87 – 1.20 (m, 7H, H-6, H-8, H-9, H-10) 1.67 (s, 3H, 2-CH_3), 1.42 (s, 3H, 12-CH_3), 1.39 (s, 3H, 12-CH_3), 1.10 (d, *J* = 7.0 Hz, 3H, 10-CH_3).

HRMS (ESI) *m/z*: calcd for $C_{24}H_{36}O_5NH_4$ $[M + NH_4]^+$: 422.2901, found: 422.2903. Due to instability, the compound was freshly prepared and used for the next reaction without further analysis.

Synthesis of Compound 294

(4*R*)-6-((4*S*,6*R*)-6-((*E*)-4-((4-Methoxybenzyl)oxy)-3-methylbut-2-en-1-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-4-methylhexan-3-ol



Aldehyde **293** (13.0 mg, 32.1 µmol, 1.00 equiv) was dissolved in THF (1 mL), cooled to 0 °C and EtMgBr (42.9 µL, 38.6 µmol, 1.20 equiv, 0.9 M in THF) was added. After stirring for 3 h at rt, EtOAc (10 mL) and aq. sat. NH_4Cl (10 mL) were added, the phases separated, the aqueous phase was extracted with EtOAc (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (30% EtOAc/CyH) to yield the title compound **294** as a colorless oil (6.70 mg, 15.4 µmol, 48%, *dr* = 16:8:2:1 resulting of a *dr* = 2:1 out of this reaction and a *dr* = 4:1 out of the aldol reaction to compound **270**).

$\mathbf{R_f}$ 0.21 (30% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = +3.8° (*c* = 0.78 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 5.45 (t, *J* = 7.4 Hz, 1H, H-3), 4.39 (s, 2H, Ar-CH_2), 3.88 (s, 2H, H-1), 3.84 – 3.83 (m, 1H, H-5), 3.80 (s, 3H, O-CH_3), 3.78 – 3.75 (m, 1H, H-7), 3.45 – 3.33 (m, 1H, H-11), 2.31 – 2.28 (m, 1H, H-4), 2.22 – 2.16 (m, 1H, H-4'), 1.67 (s, 3H, 2-CH_3), 1.58 – 1.44 (m, 7H, H-6, H-8, H-9, H-10, H-12), 1.43 (s, 3H, 14-CH_3), 1.39 (s, 3H, 14-CH_3), 1.17 – 1.10 (m, 2H, H-6', H-9'), 0.97 – 0.93 (m, 3H, H-13), 0.89 – 0.86 (m, 3H, 10-CH_3). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3, 134.6, 130.8, 129.5, 123.3, 113.9, 98.6, 76.5, 76.4, 75.9, 71.3, 69.7, 69.4, 69.1, 55.4, 38.7, 38.4, 38.0, 37.8, 36.8, 36.7, 35.0, 34.4, 34.3, 34.2, 30.4, 28.9, 28.7, 27.4, 27.2, 27.1, 26.5, 20.0, 20.0, 15.7, 14.4, 13.7, 13.5, 10.8, 10.7, 10.5. Due to the extended stereoisomerism, the carbon atoms were not assigned at this stage. **HRMS (ESI)** *m/z*: calcd for $C_{26}H_{42}O_5H$ $[M + H]^+$: 435.3105, found: 435.3106.

Synthesis of Compound 10

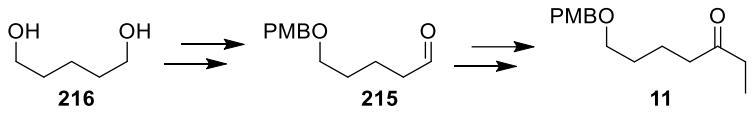
(*R*)-6-((4*S*,6*R*)-6-((*E*)-4-((4-Methoxybenzyl)oxy)-3-methylbut-2-en-1-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-4-methylhexan-3-one

DCM (0.2 mL) was added to sulfur trioxide pyridine complex (4.39 mg, 27.6 µmol, 3.00 equiv) followed by the addition of *N*,*N*-diisopropylethylamine (6.26 µL, 36.8 µmol, 4.00 equiv) and DMSO (6.54 µL, 92.0 µmol, 10.0 equiv). The reaction mixture was cooled to 0 °C and alcohol **294** (4.00 mg, 9.2 µmol, 1.00 equiv, in 1 mL DCM) was added. After stirring for 20 min at 0 °C, the reaction mixture was diluted with DCM (10 mL) and water (10 mL), the phases were separated, the aqueous phase was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) to yield the title compound **10** as a colorless oil (2.90 mg, 6.70 µmol, 73%, *dr* = 4:1 resulting out of the aldol reaction to compound **270**).

$\mathbf{R_f}$ 0.20 (20% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = −6.9° (*c* = 0.58 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 5.44 (t, *J* = 7.4 Hz, 1H, H-3), 4.38 (s, 2H, Ar-CH_2), 3.88 (s, 2H, H-1), 3.85 – 3.82 (m, 1H, H-5), 3.81 (s, 3H, O-CH_3), 3.77 – 3.73 (m, 1H, H-7), 2.54 – 2.41 (m, 3H, H-10, H-12), 2.31 – 2.27 (m, 1H, H-4), 2.20 – 2.16 (m, 1H, H-4'), 1.75 – 1.71 (m, 1H, H-9), 1.64 – 1.28 (m, 5H, H-6, H-8, H-9'), 1.67 (s, 3H, 2-CH_3), 1.41 (s, 3H, 14-CH_3), 1.38 (s, 3H, 14-CH_3), 1.07 (d, *J* = 6.9 Hz, 3H, 10-CH_3), 1.04 (t, *J* = 7.3 Hz, 3H, H-13). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 215.4 (C-11), 159.3 ($C_{arom.}$O), 134.7 (C-2), 130.8 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 123.2 (C-3), 113.9 ($C_{arom.}$H), 98.6 (C-14), 75.9 (C-1), 71.3 (Ar-CH_2), 69.1 (C-7), 69.0 (C-5), 55.4 (O-CH_3), 46.1 (C-10), 36.6 (C-6), 35.0 (C-4), 34.4 (C-12), 34.2 (C-8), 30.4 (14-CH_3), 28.4 (C-9), 20.0 (14-CH_3), 16.7 (10-CH_3), 14.4 (2-CH_3), 7.9 (C-13). **HRMS (ESI)** *m/z*: calcd for $C_{26}H_{40}O_5NH_4$ $[M + NH_4]^+$: 450.3214, found: 450.3217.

6.3.3 Synthesis of Fragment C (11)

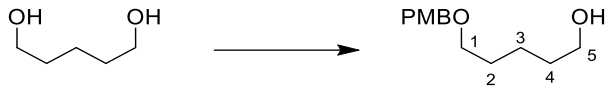
This section describes experimental details for the synthetic route to gain fragment C (**11**). Both **215** and **11** were used for coupling reactions with fragment D (**172** or **12**), while the coupling of compound **11** and **12** was more promising in the screened aldol reactions (see **Chapter 5.3.6** for details). Compound **11** was synthesized in four linear steps out of pentanediol **216**.



***Scheme 6.3.3-1*:** Synthesis towards fragment C (**11**).

Synthesis of Compound 295

5-((4-Methoxybenzyl)oxy)pentan-1-ol



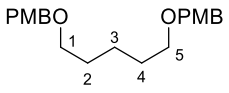
$C_{13}H_{20}O_3$
M = 224.30 g/mol

Diol **216** (3.02 mL, 28.9 mmol, 2.00 equiv) was dissolved in DMF (40 mL), cooled to 0 °C and NaH (1.27 mg, 31.8 mmol, 2.20 equiv, 60% dispersion in mineral oil) was added. The reaction mixture was stirred for 1 h at rt, cooled again to 0 °C, 4-methoxybenzyl chloride (1.95 mL, 14.4 mmol, 1.00 equiv) was added and the reaction was stirred 2 h at rt. Water (30 mL) was added slowly and the aqueous phase was extracted with EtOAc (3x40 mL). The combined organic phases were washed with aq. sat. NH_4Cl solution (25 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (30-40% EtOAc/CyH) to yield the title compound **295** as a colorless oil (2.50 g, 11.2 mmol, 77%) and the double protected diol **295b** as a side product (557 mg, 1.62 mmol, 11%).

R_f 0.10 (30% EtOAc/CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 4.43 (s, 2H, Ar-CH_2), 3.80 (s, 3H, O-CH_3), 3.63 (t, *J* = 6.5 Hz, 2H, H-5), 3.45 (t, *J* = 6.5 Hz, 2H, H-1), 1.63 (dt, *J* = 14.7, 6.5 Hz, 2H, H-2), 1.58 (dt, *J* = 14.7, 6.5 Hz, 2H, H-4), 1.46 – 1.43 (m, 2H, H-3), 1.37 (br s, 1H, OH). **^{13}C NMR** (176 MHz, $CDCl_3$)

δ = 159.3 ($C_{arom.}O$), 130.8 ($C_{arom.}$), 129.4 ($C_{arom.}H$), 113.9 ($C_{arom.}H$), 72.7 (Ar-CH_2), 70.1 (C-1), 63.0 (C-5), 55.4 (O-CH_3), 32.7 (C-4), 29.6 (C-2), 22.6 (C-3). **MS (EI)** *m/z*: calcd for $C_{13}H_{20}O_3^{+\bullet}$ $[M]^{+\bullet}$: 224.2, found: 224.2. The spectroscopic data were in agreement with those previously reported.[191]

1,5-Bis((4-methoxybenzyl)oxy)pentane



295b

$C_{21}H_{28}O_4$

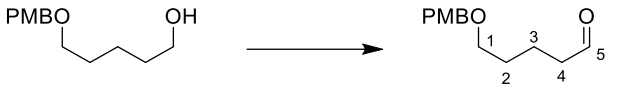
M = 344.45 g/mol

Compound **295b** was isolated as side product.

R_f 0.38 (30% EtOAc/CyH). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.25 (d, *J* = 8.6 Hz, 4H, $H_{arom.}$), 6.87 (d, *J* = 8.6 Hz, 4H, $H_{arom.}$), 4.42 (s, 4H, 2xAr-CH_2), 3.80 (s, 6H, 2xO-CH_3), 3.44 (t, *J* = 6.6 Hz, 4H, H-1, H-5), 1.62 (dt, *J* = 14.5, 6.7 Hz, 4H, H-2, H-4), 1.45 – 1.41 (m, 2H, H-3). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.2 (2x$C_{arom.}O$), 130.9 (2x$C_{arom.}$), 129.4 ($C_{arom.}H$), 113.9 ($C_{arom.}H$), 72.7 (2xAr-CH_2), 70.2 (C-1, C-5), 55.4 (2xO-CH_3), 29.7 (C-2, C-4), 23.0 (C-3). **HRMS (ESI)** *m/z*: calcd for $C_{21}H_{28}O_4Na$ $[M + Na]^+$: 367.1880, found: 367.1880.

Synthesis of Compound 215

5-((4-Methoxybenzyl)oxy)pentanal



295 **215**

$C_{13}H_{18}O_3$

M = 222.28 g/mol

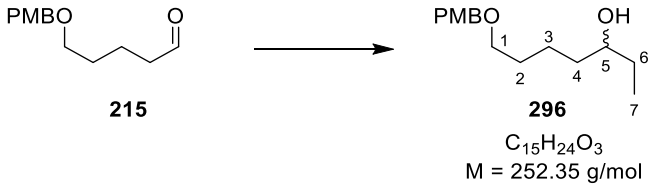
DCM (3.5 mL) was added to sulfur trioxide pyridine complex (532 mg, 3.34 mmol, 3.00 equiv) and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (758 µL, 4.46 mmol, 4.00 equiv), DMSO (792 µL, 11.2 mmol, 10.0 equiv) and alcohol **295** (250 mg, 1.11 µmol, 1.00 equiv, in 1 mL DCM) were added. After stirring 1 h at rt, sat. aq. $NaHCO_3$ solution (10 mL) was added and the aqueous phase was extracted with DCM (3x10 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x70 mL). The organic phase was

dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the title compound **215** as a colorless oil (241 mg, 1.08 mmol, 97%).

R_f 0.23 (30% EtOAc/CyH). **^{1}H NMR** (300 MHz, $CDCl_3$) δ = 9.76 – 9.74 (m, 1H, H-5), 7.25 (d, *J* = 8.5 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.5 Hz, 2H, $H_{arom.}$), 4.42 (s, 2H, Ar-CH_2), 3.80 (s, 3H, O-CH_3), 3.46 (t, *J* = 6.0 Hz, 2H, H-1), 2.44 (t, *J* = 7.1 Hz, 2H, H-4), 1.78 – 1.59 (m, 1H, H-2, H-3). **^{13}C NMR** (75 MHz, $CDCl_3$) δ = 202.7 (C-5), 159.3 ($C_{arom.}$O), 130.7 ($C_{arom.}$), 129.4 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 72.7 (Ar-CH_2), 69.6 (C-1), 55.4 (O-CH_3), 43.7 (C-4), 29.3 (C-2), 19.1 (C-3). **HRMS (ESI)** *m/z*: calcd for $C_{13}H_{18}O_3Na$ $[M + Na]^+$: 245.1148, found: 245.1148. The spectroscopic data were in agreement with those previously reported.[238]

Synthesis of Compound 296

7-((4-Methoxybenzyl)oxy)heptan-3-ol

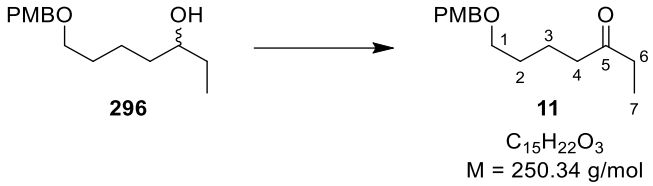


Aldehyde **215** (1.00 g, 4.50 mmol, 1.00 equiv) was dissolved in THF (40 mL), cooled to 0 °C and EtMgBr (5.40 mL, 5.40 mmol, 1.20 equiv, 1 M in THF) was added. After stirring for 30 min at 0 °C, EtOAc (20 mL) and aq. sat. NH_4Cl (25 mL) were added, the phases separated, the aqueous phase was extracted with EtOAc (3x25 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (30-40% EtOAc/CyH) to yield the racemic title compound **296** as a colorless oil (946 mg, 3.75 mmol, 83%).

R_f 0.19 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 4.43 (s, 2H, Ar-CH_2), 3.80 (s, 3H, O-CH_3), 3.54 – 3.50 (m, 1H, H-5), 3.45 (t, *J* = 6.5 Hz, 2H, H-1), 1.66 – 1.39 (m, 8H, H-2, H-3, H-4, H-6), 0.93 (t, *J* = 7.4 Hz, 3H, H-7). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}$O), 130.8 ($C_{arom.}$), 129.4 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 73.4 (C-5), 72.7 (Ar-CH_2), 70.1 (C-1), 55.4 (O-CH_3), 36.8 (C-4), 30.3 (C-6), 29.9 (C-2), 22.5 (C-3), 10.0 (C-7). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{24}O_3H$ $[M + H]^+$: 253.1798, found: 253.1801.

Synthesis of Compound 11

7-((4-Methoxybenzyl)oxy)heptan-3-one

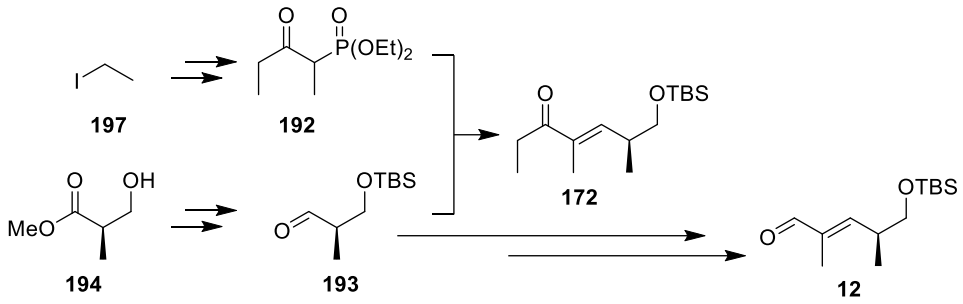


DCM (14 mL) was added to sulfur trioxide pyridine complex (1.78 g, 11.2 mmol, 3.00 equiv) and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (2.53 mL, 14.9 mmol, 4.00 equiv), DMSO (2.65 mL, 37.3 mmol, 10.0 equiv) and alcohol **296** (940 mg, 3.72 mmol, 1.00 equiv, in 1 mL DCM) were added. After stirring 1 h at rt, sat. aq. $NaHCO_3$ solution (10 mL) was added and the aqueous phase was extracted with DCM (3x20 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x70 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) to yield the title compound **11** as a colorless oil (874 mg, 3.49 mmol, 94%).

R_f 0.36 (30% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.25 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.42 (s, 2H, Ar-CH_2), 3.80 (s, 3H, O-CH_3), 3.44 (t, *J* = 6.2 Hz, 3H, H-1), 2.43 – 2.38 (m, 4H, H-4, H-6), 1.69 – 1.58 (m, 4H, H-2, H-3), 1.04 (t, *J* = 7.4 Hz, 3H, H-7). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 211.7 (C-5), 159.3 ($C_{arom.}$O), 130.8 ($C_{arom.}$), 129.4 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 72.7 (Ar-CH_2), 69.8 (C-1), 55.4 (O-CH_3), 42.2 (C-4), 36.0 (C-6), 29.4 (C-2), 20.8 (C-3), 8.0 (C-7). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{22}O_3Na$ $[M + Na]^+$: 273.1461, found: 273.1459.

6.3.4 Synthesis of Fragment D (12)

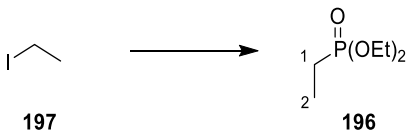
Experimental details for the synthesis of fragment D (**12**) are given in this section. Both **172** and **12** were used for coupling reactions with fragment C (**215** or **11**), while it was only possible to yield a desired aldol product using compound **12** and **11** (see **Chapter 5.3.6** for details). Compound **172** was synthesized in four linear steps and compound **12** in six steps.



***Scheme 6.3.4-1*:** Synthesis towards fragment D (**12**).

Synthesis of Compound 196

Diethyl ethylphosphonate



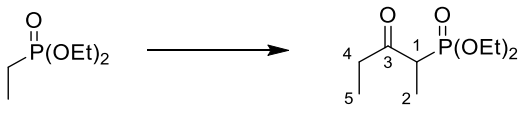
$C_6H_{15}O_3P$
M = 166.16 g/mol

A mixture of triethyl phosphite (10.4 mL, 60.2 mmol, 1.00 equiv) and iodoethane (**197**, 4.84 mL, 60.2 mmol, 1.00 equiv) was refluxed (115 °C) for 5 h. Distillation of the mixture (5 mbar at 75 °C) yielded phosphonate **196** as a colorless oil (9.29 g, 55.9 mmol, 93%).

R_f 0.10 (50% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 4.08 – 3.98 (m, 4H, ($OC\underline{H_2}CH_3)_2$), 1.69 (dq, *J* = 18.1, 7.7 Hz, 2H, H-1), 1.29 (t, *J* = 7.1 Hz, 6H, ($OCH_2\underline{CH_3})_2$), 1.11 (dt, *J* = 19.9, 7.7 Hz, 3H, H-2). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 61.9 (d, *J* = 6.6 Hz, ($O\underline{C}H_2CH_3)_2$), 19.3 (d, *J* = 143.5 Hz, C-1), 16.9 (d, *J* = 5.9 Hz, ($OCH_2\underline{C}H_3)_2$), 6.9 (d, *J* = 6.8 Hz, C-2). **^{31}P NMR** (202 MHz, CD_2Cl_2) δ = 33.1. **HRMS (ESI)** *m/z*: calcd for $C_6H_{15}O_3PH$ $[M + H]^+$: 167.0832, found: 167.0832. The spectroscopic data were in agreement with those previously reported.[171]

Synthesis of Compound 192

Diethyl (3-oxopentan-2-yl)phosphonate



196 **192**

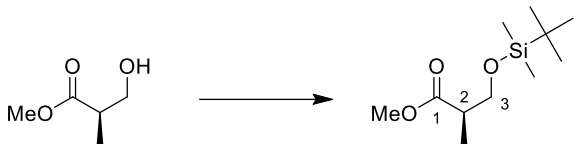
$C_9H_{19}O_4P$
M = 222.22 g/mol

Diethyl ethylphosphonate (**196**, 1.00 g, 6.02 mmol, 1.00 equiv) was dissolved in THF (3 mL) and cooled to −78 °C. *n*-BuLi (2.89 mL, 7.22 mmol, 1.20 equiv, 2.5 M in hexanes) was added and the reaction mixture was stirred 1 h at −78 °C followed by the addition of ethyl propionate (898 µL, 7.82 mmol, 1.30 equiv, in 2 mL THF). The reaction mixture was stirred again for 1 h at −78 °C and further 45 min at rt. EtOAc (15 mL) and water (20 mL) were added, the phases separated, the aqueous phase was extracted with EtOAc (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (50% EtOAc/CyH) to yield the title compound **192** as a colorless oil (970 mg, 4.37 mmol, 73%).

R_f 0.13 (50% EtOAc/CyH). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 4.11 – 4.04 (m, 4H, (OCH_2CH_3)$_2$), 3.19 (dq, *J* = 24.9, 7.1 Hz, 1H, H-1), 2.80 (dq, *J* = 18.3, 7.2 Hz, 1H, H-4), 2.49 (dq, *J* = 18.3, 7.2 Hz, 1H, H-4'), 1.36 – 1.21 (m, 9H, (OCH_2CH_3)$_2$, H-2), 1.02 (t, *J* = 7.2 Hz, 1H, H-5). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 207.0 (C-3), 63.0 (dd, *J* = 17.7, 6.6 Hz, (OCH_2CH_3)$_2$), 46.9 (d, *J* = 127.4 Hz, C-1), 36.9 (C-4), 16.8 (d, *J* = 5.9 Hz, (OCH_2CH_3)$_2$), 11.4 (d, *J* = 6.6 Hz, C-2), 8.0 (C-5). **^{31}P NMR** (202 MHz, CD_2Cl_2) δ = 23.6. **HRMS (ESI)** *m/z*: calcd for $C_9H_{19}O_4PH$ $[M + H]^+$: 223.1094, found: 223.1100. The spectroscopic data were in agreement with those previously reported.[172]

Synthesis of Compound 297

Methyl (*R*)-3-((*tert*-butyldimethylsilyl)oxy)-2-methylpropanoate



194 **297**

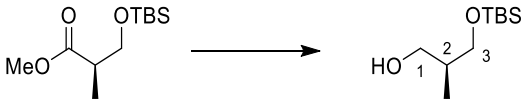
$C_{11}H_{24}O_3Si$
M = 232.40 g/mol

(*R*)-*Roche* ester (**194**, 467 mL, 4.23 mmol, 1.00 equiv) was dissolved in DCM (2.5 mL), imidazole (576 mg, 8.47 mmol, 2.00 equiv, in 2 mL DCM) was added and the reaction mixture was cooled to 0 °C before addition of TBSCl (766 mg, 5.08 mmol, 1.20 equiv). After stirring 4 h at rt, aq. sat. NH_4Cl solution (10 mL) was added, the phases separated, the aqueous phase was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (100% DCM) to yield the title compound **297** as a colorless oil (981 mg, 4.22 mmol, quant.).

$\mathbf{R_f}$ 0.56 (DCM). $[\alpha]_D^{20}$ = −19.8° (*c* = 0.86 in CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 3.75 (dd, *J* = 9.7, 6.9 Hz, 1H, H-3), 3.69 – 3.61 (dd, *J* = 9.7, 6.9 Hz, 1H, H-3'), 3.64 (s, 3H, O-CH_3), 2.65 – 2.58 (m, 1H, H-2), 1.11 (d, *J* = 7.0 Hz, 3H, 2-CH_3), 0.87 (s, 9H, TBS), 0.04 (2xs, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 175.8 (C-1), 65.9 (C-3), 51.9 (O-CH_3), 43.1 (C-2), 26.1 (TBS), 18.7 (TBS), 13.8 (2-CH_3), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{11}H_{24}O_3SiH$ $[M + H]^+$: 233.1567, found: 233.1567. The spectroscopic data were in agreement with those previously reported.[169]

Synthesis of Compound 298

(*S*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-methylpropan-1-ol



297 **298**

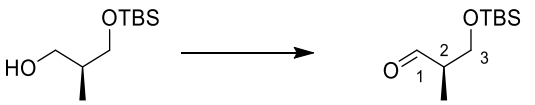
$C_{10}H_{24}O_2Si$
M = 204.39 g/mol

Ester **297** (200 mg, 861 µmol, 1.00 equiv) was dissolved in THF (1 mL), the solution was cooled to −78 °C and DIBAL-H (2.58 mL, 2.58 mmol, 3.00 equiv, 1 M in CyH) was added slowly. After stirring 2 h at −78 °C and 30 min at rt, the reaction mixture was cooled again to −78 °C before addition of EtOAc (1.5 mL) and sat. aq. Rochelle salt solution (3 mL). The mixture was allowed to warm to rt and stirred until clearly two phases appeared (2 h). The phases were separated, the aqueous layer was extracted with EtOAc (3x10 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (100% DCM) to yield the title compound **298** as a colorless oil (142 mg, 659 µmol, 81%).

R_f 0.15 (DCM). **$[\alpha]_D^{20}$** = −9.0° (*c* = 0.67 in CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 3.70 (dd, *J* = 9.9, 4.8 Hz, 1H, H-3), 3.57 – 3.52 (m, 3H, H-1, H-3'), 2.45 (t, *J* = 5.7 Hz, 1H, OH), 1.89 – 1.84 (m, 1H, H-2), 0.90 (s, 9H, TBS), 0.83 (d, *J* = 6.9 Hz, 3H, 2-CH_3), 0.07 (s, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 68.7 (C-3), 68.2 (C-1), 37.9 (C-2), 26.2 (TBS), 18.6 (TBS), 13.5 (2-CH_3), –5.3 (TBS). **MS (EI)** *m/z*: calcd for $C_6H_{15}O_2Si^{+\bullet}$ $[M - {}^tBu]^{+\bullet}$: 147.1, found: 147.1. The spectroscopic data were in agreement with those previously reported.[169]

Synthesis of Compound 193

(*R*)-3-((*tert*-Butyldimethylsilyl)oxy)-2-methylpropanal



298 **193**

$C_{10}H_{22}O_2Si$
M = 202.37 g/mol

DMP oxidation:

Alcohol **298** (70.0 mg, 343 µmol, 1.00 equiv) was dissolved in DCM (4 mL) and DMP (218 mg, 514 µmol, 1.50 equiv) was added. After stirring 2 h at rt, the reaction mixture was concentrated, Et_2O was added and the suspension was filtered over celite®. The solvent was removed under reduced pressure and the crude product was purified using flash chromatography (100% DCM) to yield the title compound **193** as a colorless oil (66.4 mg, 328 µmol, 96%).

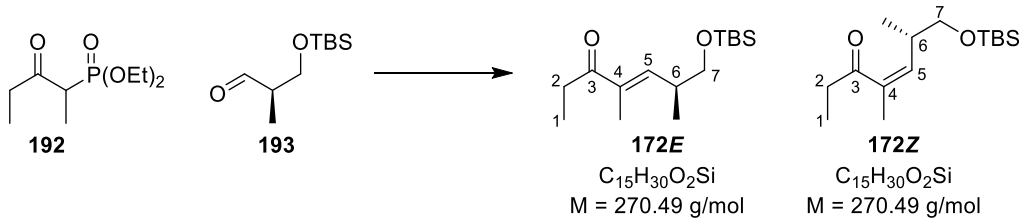
Parikh-Doering oxidation:

DCM (30 mL) was added to sulfur trioxide pyridine complex (4.67 g, 29.4 mmol, 3.00 equiv) and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (6.66 mL, 39.1 mmol, 4.00 equiv), DMSO (6.95 mL, 97.9 mmol, 10.0 equiv) and alcohol **298** (2.00 g, 9.79 mmol, 1.00 equiv) were added. After stirring 1 h at rt, sat. aq. $NaHCO_3$ solution (20 mL) was added and the aqueous phase was extracted with DCM (3x50 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x150 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the title compound **193** as a colorless oil (1.97 g, 9.73 mmol, quant.).

$\mathbf{R_f}$ 0.51 (DCM). $[\alpha]_D^{20}$ = −34.4° (*c* = 0.84 in CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 9.70 (d, *J* = 1.6 Hz, 1H, H-1), 3.87 (dd, *J* = 10.2, 5.0 Hz, 1H, H-3), 3.80 (dd, *J* = 10.2, 6.3 Hz, 1H, H-3'), 2.53 – 2.46 (m, 1H, H-2), 1.06 (d, *J* = 7.0 Hz, 3H, 2-CH3), 0.88 (s, 9H, TBS), 0.06 (s, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 205.0 (C-1), 64.0 (C-3), 49.4 (C-2), 26.1 (TBS), 18.7 (TBS), 10.6 (2-CH_3), -5.3 (TBS). **MS (EI)** *m/z*: calcd for $C_{10}H_{21}O_2Si^{+\bullet}$ $[M]^{+\bullet}$: 201.2, found: 201.2. The spectroscopic data were in agreement with those previously reported.[170]

Synthesis of Compounds 172*E* and 172*Z*

(*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-4,6-dimethylhept-4-en-3-one (172*E*) and (*S*,*E*)-7-((*tert*-Butyldimethylsilyl)oxy)-4,6-dimethylhept-4-en-3-one (172*Z*)



$Ba(OH)_2$ (178 mg, 1.04 mmol, 3.50 equiv) was dried for 5 h at 120 °C using high vacuum. After cooling to rt, THF (2 mL) was added followed by phosphonate **192** (92.2 mg, 415 µmol, 1.40 equiv). The suspension was stirred for 30 min at rt. Freshly prepared aldehyde **193** (60.0 mg, 297 µmol, 1.00 equiv, in 40:1 THF/water (1 mL)) was added and the reaction was stirred overnight at rt. The suspension was filtered over celite® and washed with Et_2O. The

solvent was removed under reduced pressure and the crude product was purified by flash chromatography (2% Et_2O /CyH) to yield the title compounds **172*E*** (59.2 mg, 219 µmol, 74%, *E*/*Z* = 16:1) and **172*Z*** (3.7 mg, 13.7 µmol, 5%, *E*/*Z* = 16:1) as colorless oils.

Analytical data for **172*E***:

$\mathbf{R_f}$ 0.28 (5% Et_2O/CyH). $[\alpha]_D^{20}$ = +4.6° (*c* = 0.65 in CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 6.43 (dq, *J* = 9.4, 1.5 Hz, 1H, H-5), 3.56 – 3.52 (m, 2H, H-7), 3.79 – 2.72 (m, 2H, H-6), 2.66 (qd, *J* = 7.3, 2.5 Hz, 2H, H-2), 1.78 (d, *J* = 1.5 Hz, 3H, 4-CH_3), 1.05 (t, *J* = 7.3 Hz, 3H, H-1), 1.02 (d, *J* = 6.7 Hz, 3H, 6-CH_3), 0.88 (s, 9H, TBS), 0.04 (s, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 202.9 (C-3), 145.0 (C-5), 137.2 (C-4), 67.7 (C-7), 37.0 (C-6), 30.8 (C-2), 26.2 (TBS), 18.7 (TBS), 16.5 (6-CH_3), 12.0 (4-CH_3), 9.2 (C-1), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{30}O_2SiH$ $[M + H]^+$: 271.2088, found: 271.2086.

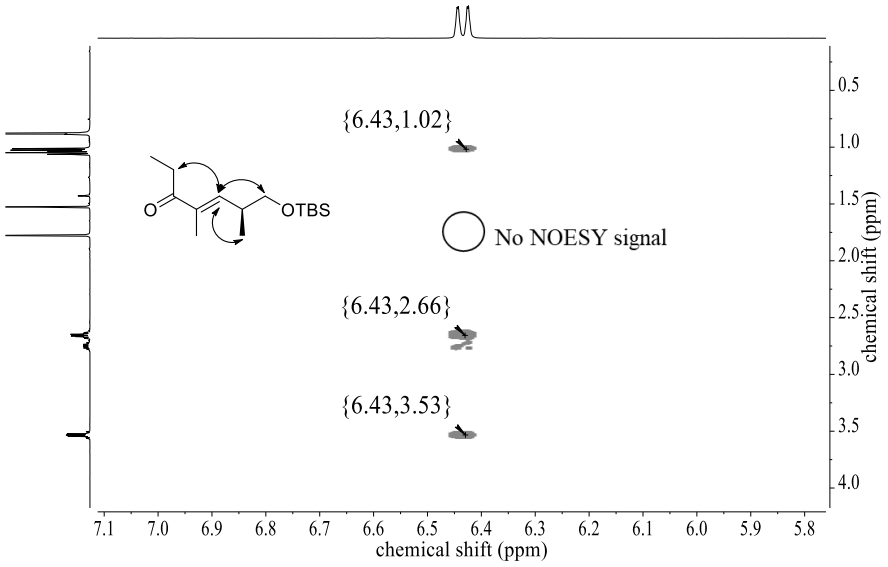


Figure 6.3.4-1: Selected NOESY signals of compound **172*E***, which were used for the determination of the *E*-configuration. No NOESY signal could be detected between H-5 and 4-CH_3.

Analytical data for **172*Z***:

$\mathbf{R_f}$ 0.34 (5% Et_2O/CyH). $[\alpha]_D^{20}$ = +18.2° (*c* = 1.32 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 5.41 (dq, *J* = 10.1, 1.5 Hz, 1H, H-5), 3.47 – 3.42 (m, 2H, H-7), 2.83 – 2.79 (m, 2H, H-6), 2.55 (q, *J* = 7.3 Hz, 2H, H-2), 1.91 (d, *J* = 1.5 Hz, 3H, 4-CH_3), 1.07 (t, *J* = 7.3 Hz, 3H, H-1), 0.97 (d, *J* = 6.7 Hz, 3H, 6-CH_3), 0.87 (s, 9H, TBS), 0.02 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 206.9 (C-3), 139.6 (C-5), 136.0 (C-4), 68.0 (C-7), 36.6 (C-6), 35.2 (C-2), 26.0 (TBS), 21.0

(4-CH_3), 18.5 (TBS), 17.2 (6-CH_3), 7.9 (C-1), 5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{30}O_2SiH$ $[M + H]^+$: 271.2088, found: 271.2101.

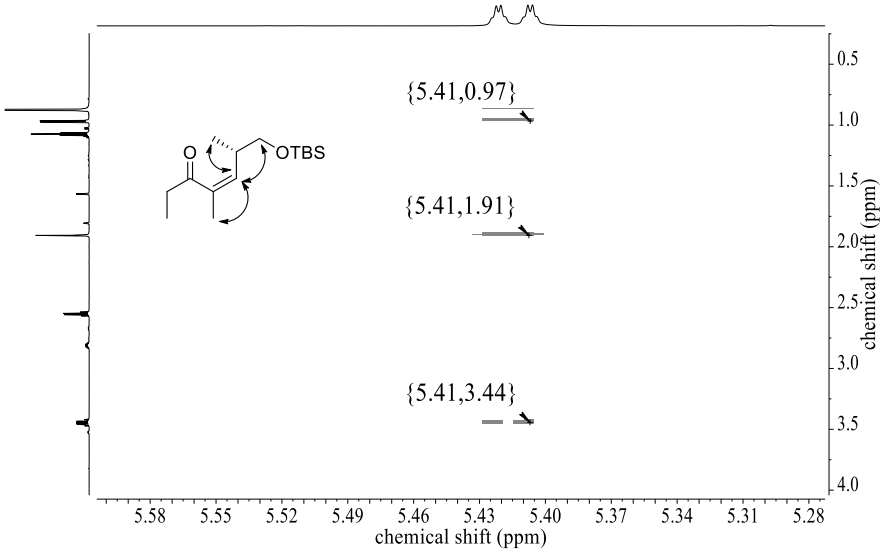
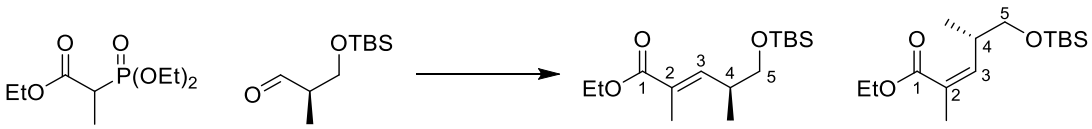


Figure 6.3.4-2: Selected NOESY signals of compound **172*Z***, which were used for the determination of the *Z*-configuration.

Synthesis of Compounds 217*E* and 217*Z*

Ethyl (*S*,*E*)-5-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethylpent-2-enoate (217*E*) and Ethyl (*S*,*Z*)-5-((*tert*-butyldimethylsilyl)oxy)-2,4-dimethylpent-2-enoate (217*Z*)



$Ba(OH)_2$ (178 mg, 1.04 mmol, 3.50 equiv) was dried for 6 h at 120 °C using high vacuum. After cooling to rt, THF (50 mL) was added followed by phosphonate **218** (2.82 mL, 13.1 mmol, 1.40 equiv). The suspension was stirred for 30 min at rt. Freshly prepared aldehyde **193** (1.90 g, 9.39 mmol, 1.00 equiv, in 40:1 THF/water (28 mL)) was added and the reaction was stirred overnight at rt. The suspension was filtered over celite® and washed with Et_2O. The

solvent was removed under reduced pressure and the crude product was purified by flash chromatography (2% Et_2O /CyH) to yield the title compounds **217*E*** (1.85 g, 6.46 mmol, 69%, *E*/*Z* = 13:1) and **217*Z*** (142 mg, 496 µmol, 5%, *E*/*Z* = 13:1) as colorless oils.

Analytical data for **217*E***:

$\mathbf{R_f}$ 0.25 (5% Et_2O/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = −4.4° (*c* = 0.90 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 6.55 (dq, *J* = 9.9, 1.4 Hz, 1H, H-3), 4.22 – 4.15 (m, 2H, O$\underline{CH_2}CH_3$), 3.52 – 3.47 (m, 2H, H-5), 2.72 – 2.66 (m, 1H, H-4), 1.86 (d, *J* = 1.5 Hz, 3H, 2-CH_3), 1.29 (t, *J* = 7.1 Hz, 3H, $OCH_2\underline{CH_3}$), 1.01 (d, *J* = 6.7 Hz, 3H, 4-CH_3), 0.88 (s, 9H, TBS), 0.03 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 168.4 (C-1), 144.7 (C-3), 128.1 (C-2), 67.3 (C-5), 60.6 (O$\underline{CH_2}CH_3$), 36.4 (C-4), 26.0 (TBS), 18.5 (TBS), 16.4 (4-CH_3), 14.4 ($OCH_2\underline{CH_3}$), 12.8 (2-CH_3), –5.2 (TBS), 5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{30}O_3SiH$ $[M + H]^+$: 287.2037, found: 287.2035. The spectroscopic data were in agreement with those previously reported.[192]

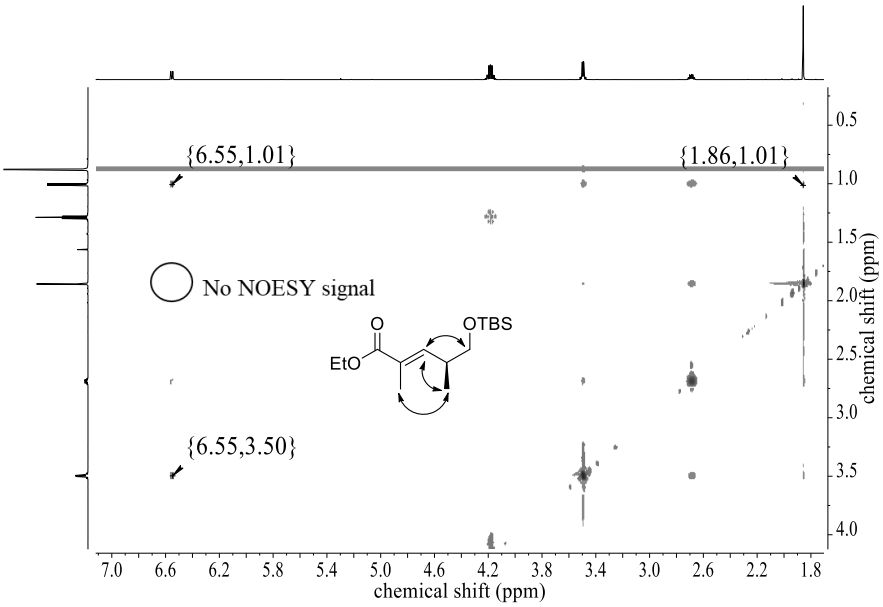


Figure 6.3.4-3: Selected NOESY signals of compound **217*E***, which were used for the determination of the *E*-configuration. No NOESY signal could be detected between H-3 and 2-CH_3.

Analytical data for **217*Z***:

$\mathbf{R_f}$ 0.33 (5% Et_2O/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +27.8° (*c* = 0.72 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 5.74 (dq, *J* = 8.3, 1.5 Hz, 1H, H-3), 4.19 (q, *J* = 7.1 Hz, 2H, O$\underline{CH_2}CH_3$), 3.51 – 3.44 (m, 2H, H-5), 3.28 – 3.23 (m, 1H, H-4), 1.89 (d, *J* = 1.4 Hz, 3H, 2-CH_3), 1.30 (t, *J* = 7.1 Hz, 3H, $OCH_2\underline{CH_3}$), 0.99 (d, *J* = 6.7 Hz, 3H, 4-CH_3), 0.88 (s, 9H, TBS), 0.03 (2xs, 6H, TBS). **^{13}C NMR**

(176 MHz, $CDCl_3$) δ = 168.3 (C-1), 145.1 (C-3), 127.4 (C-2), 67.8 (C-5), 60.2 ($O\underline{CH_2}CH_3$), 36.4 (C-4), 26.0 (TBS), 21.0 (2-CH_3), 18.5 (TBS), 17.0 (4-CH_3), 14.4 ($OCH_2\underline{CH_3}$), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{15}H_{30}O_3SiH$ $[M + H]^+$: 287.2037, found: 287.2036.

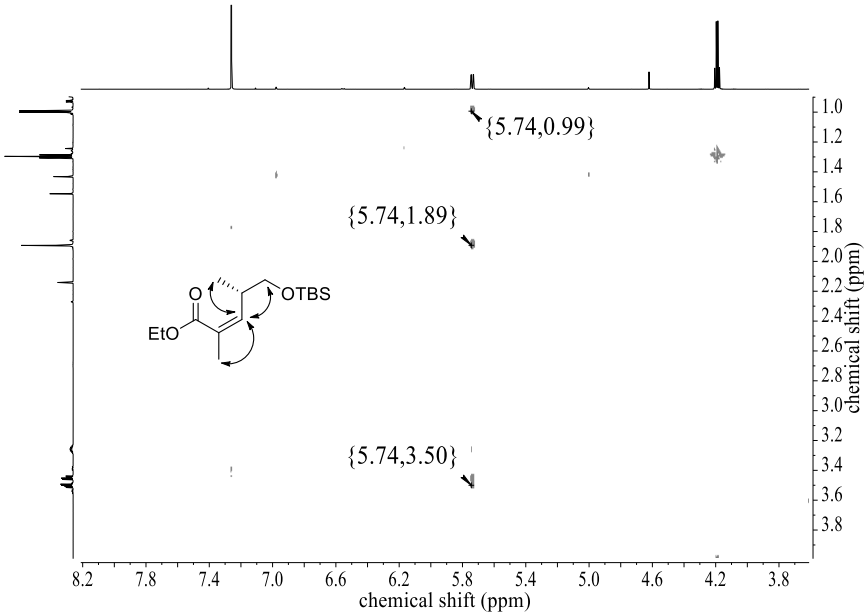
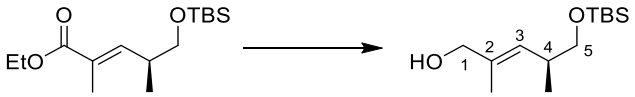


Figure 6.3.4-4: Selected NOESY signals of compound **217*Z***, which were used for the determination of the *Z* configuration.

Synthesis of Compound 299

(*S*,*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-2,4-dimethylpent-2-en-1-ol



217*E* **299**

$C_{13}H_{28}O_2Si$

M = 244.45 g/mol

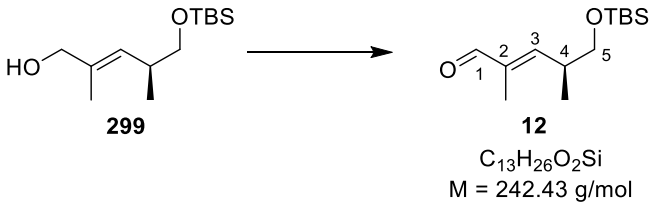
Ester **217*E*** (1.86 g, 6.49 mmol, 1.00 equiv) was dissolved in THF (27 mL), the solution was cooled to −78 °C and DIBAL-H (14.8 mL, 16.2 mmol, 2.50 equiv, 1.1 M in CyH) was added slowly. After stirring 1 h at −78 °C, the reaction solution was poured into a sat. aq. Rochelle salt solution (80 mL). The mixture was stirred until clearly two phases appeared (2 h). The phases were separated, the aqueous layer was extracted with Et_2O (3x70 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure.

Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) to yield the title compound **299** as a colorless oil (1.37 g, 5.62 mmol, 87%).

R_f 0.24 (20% EtOAc/CyH). **$[\alpha]_D^{20}$** = +13.2° (*c* = 0.76 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 5.19 (d, *J* = 10.0 Hz, 1H, H-3), 4.00 (d, *J* = 5.6 Hz, 2H, H-1), 3.47 – 3.35 (m, 2H, H-5), 2.62 – 2.54 (m, 1H, H-4), 1.69 (s, 3H, 2-CH_3), 1.28 (t, *J* = 5.6 Hz, 1H, OH), 0.96 (d, *J* = 6.7 Hz, 3H, 4-CH_3), 0.89 (s, 9H, TBS), 0.04 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 135.3 (C-2), 129.1 (C-3), 69.1 (C-1), 68.0 (C-5), 35.3 (C-4), 26.1 (TBS), 18.5 (TBS), 17.3 (4-CH_3), 14.1 (2-CH_3), –5.1 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{13}H_{28}O_2SiH$ $[M + H]^+$: 245.1928, found: 245.1928. The spectroscopic data were in agreement with those previously reported.[192]

Synthesis of Compound 12

(*S*,*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-2,4-dimethylpent-2-enal

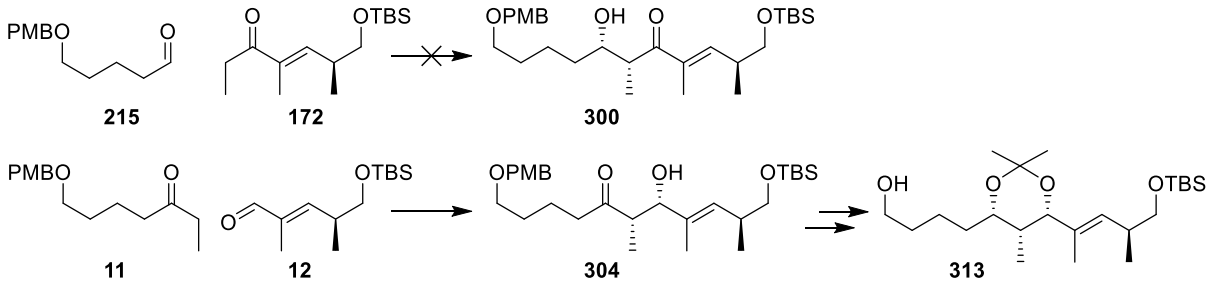


Alcohol **299** (260 mg, 1.06 mmol, 1.00 equiv) was dissolved in DMSO (1.3 mL) and 2-iodoxybenzoic acid (IBX, 596 mg, 2.13 mmol, 2.00 equiv) was added. After stirring 1.5 h at rt, DCM (10 mL) and water (10 mL) were added and the mixture was filtered over celite®. The organic layer was separated, the aqueous layer was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (10% EtOAc/CyH) to yield the title compound **12** as a colorless oil (257 mg, 1.06 mmol, quant.)

R_f 0.39 (10% EtOAc/CyH). **$[\alpha]_D^{20}$** = +8.3° (*c* = 0.84 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 9.40 (s, 1H, H-1), 6.32 (dq, *J* = 9.7, 1.3 Hz, 1H, H-3), 3.61 – 3.53 (m, 2H, H-5), 2.93 – 2.85 (m, 1H, H-4), 1.77 (d, *J* = 1.3 Hz, 3H, 2-CH_3), 1.06 (d, *J* = 6.8 Hz, 2H, 4-CH_3), 0.87 (s, 9H, TBS), 0.03 (2xs, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 195.7 (C-1), 157.4 (C-3), 139.4 (C-2), 67.0 (C-5), 36.6 (C-4), 26.0 (TBS), 18.4 (TBS), 16.2 (4-CH_3), 9.6 (2-CH_3), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{13}H_{26}O_2SiH$ $[M + H]^+$: 243.1775, found: 243.1775. The spectroscopic data were in agreement with those previously reported.[192]

6.3.5 Fragment Coupling (Fragment C+D)

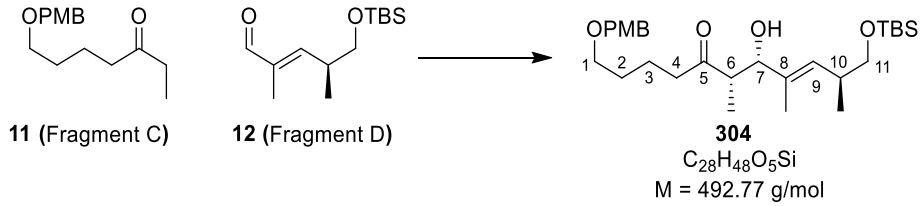
Concatenation of **215** and **172** was not successful. As an alternative, this section reports on experimental details for the coupling of **11** and **12** and further modifications to gain fragment C+D (**313**), which could be synthesized in ten linear steps out of (*R*)-*Roche* ester **194**.



***Scheme 6.3.5-1*:** Synthesis towards fragment C+D (**313**).

Synthesis of Compound 304

(6*S*,7*S*,10*S*,*E*)-11-((*tert*-Butyldimethylsilyl)oxy)-7-hydroxy-1-((4-methoxybenzyl)oxy)-6,8,10-trimethylundec-8-en-5-one



Preparation of (−)-[Ipc]$_2$BH: (−)-[Ipc]$_2$BH was synthesized using the literature described hydroboration.[149,239] Thus, BH_3*SMe_2 (3.87 mL, 40.8 mmol, 1.00 equiv) was added to THF (40 mL). This solution was cooled to 0 °C and (α)-(+)-pinene (13.0 mL, 81.6 mmol, 2.00 equiv) was added over the period of 20 min via syringe pump. Afterwards, the reaction vessel was stored 46 h at 0 °C under argon. The reaction mixture was allowed to warm to rt and the supernatant was removed from the formed precipitate using a syringe. Finally, the precipitate was washed with Et_2O (3x40 mL) under argon atmosphere, dried at high vacuum and the yielded (−)-[Ipc]$_2$BH (6.99 g, 24.4 mmol, 60%) was stored in the glove box fridge and was used over the period of two months.

Preparation of (−)-[Ipc]$_2$BOTf: (−)-[Ipc]$_2$BOTf was always prepared freshly and in situ before usage in the corresponding aldol reaction. For this purpose, hexane (0.8 mL) was added to (−)-[Ipc]$_2$BH (1.00 g, 3.49 mmol, 1.00 equiv) and the mixture was cooled to 0 °C. Triflic acid (308 µL, 3.49 mmol, 1.00 equiv) was added carefully and the mixture was stirred at rt until complete disappearance of (−)-[Ipc]$_2$BH crystals (2-3 h), resulting in a two phase system. Due to the literature described conversion of 60%,[149] a 1.9 M stock solution of (−)-[Ipc]$_2$BOTf in hexane (upper layer of the two phase system) was assumed.

(−)-[Ipc]$_2$BOTf (486 µL, 923 µmol, 1.10 equiv, 1.9 M in hexane) was dissolved in DCM (3.5 mL). After cooling to −78 °C, DIPEA (300 µL, 1.76 mmol, 2.10 equiv) and ketone **11** (210 mg, 839 µmol, 1.00 equiv, stored over 3 Å MS in 2 mL DCM) were added dropwise. The reaction mixture was stirred 2 h at −78 °C and freshly prepared aldehyde **12** (244 mg, 1.01 mmol, 1.20 equiv, stored 14 h over 3 Å MS in 2 mL DCM) was added dropwise. After stirring 1 h at −78 °C, the reaction vessel was placed in a deep freezer (−15 °C) overnight. MeOH (1.8 mL), pH 7 buffer (2.7 mL) and H_2O_2 (1.4 mL, 35%) were added to quench the reaction. After stirring 1 h at rt, DCM (15 mL) and brine (8 mL) were added, the phases were separated, the aqueous layer was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) and HPLC (60%-100% MeCN/water, retention time 17.2 min, using a KNAUER Eurospher II 100-5 C18; 5 µm; 250 x 60 mm) to give back ketone **11** (93.6 mg, 374 µmol, 45%) and to yield the title compound **304** as a colorless oil (77.6 mg, 158 µmol, 19%, 34% brsm, *dr* = 6:1).

$\mathbf{R_f}$ 0.18 (20% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = −2.1° (*c* = 0.94 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.25 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.26 (dq, *J* = 9.4, 1.2 Hz, 1H, H-9), 4.42 (s, 2H, Ar-CH_2), 4.28 (br t, 1H, H-7), 3.80 (s, 3H, O-CH_3), 3.45 – 3.33 (m, 4H, H-1, H-11), 2.69 (qd, *J* = 7.1, 4.1 Hz, 1H, H-6), 2.59 (d, *J* = 2.8 Hz, 1H, OH), 2.57 – 2.53 (m, 1H, H-10), 2.52 – 2.40 (m, 2H, H-4), 1.67 – 1.56 (m, 4H, H-2, H-3), 1.59 (d, *J* = 1.2 Hz, 3H, 8-CH_3), 1.04 (d, *J* = 7.1 Hz, 3H, 6-CH_3), 0.93 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.88 (s, 9H, TBS), 0.03 (2xs, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 215.4 (C-5), 159.3 ($C_{arom.}$O), 134.0 ($C_{arom.}$), 130.8 (C-9), 129.6 (C-8), 129.4 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 75.4 (C-7), 72.7 (Ar-CH_2), 69.9 (C-1), 68.1 (C-11), 55.4 (O-CH_3), 48.4 (C-6), 41.7 (C-4), 35.4 (C-10), 29.3 (C-2), 26.1 (TBS), 20.5 (C-3), 18.5 (TBS), 17.3 (10-CH_3), 13.7 (8-CH_3), 10.3 (6-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{28}H_{48}O_5SiNa$ $[M + Na]^+$: 515.3163, found: 515.3166.

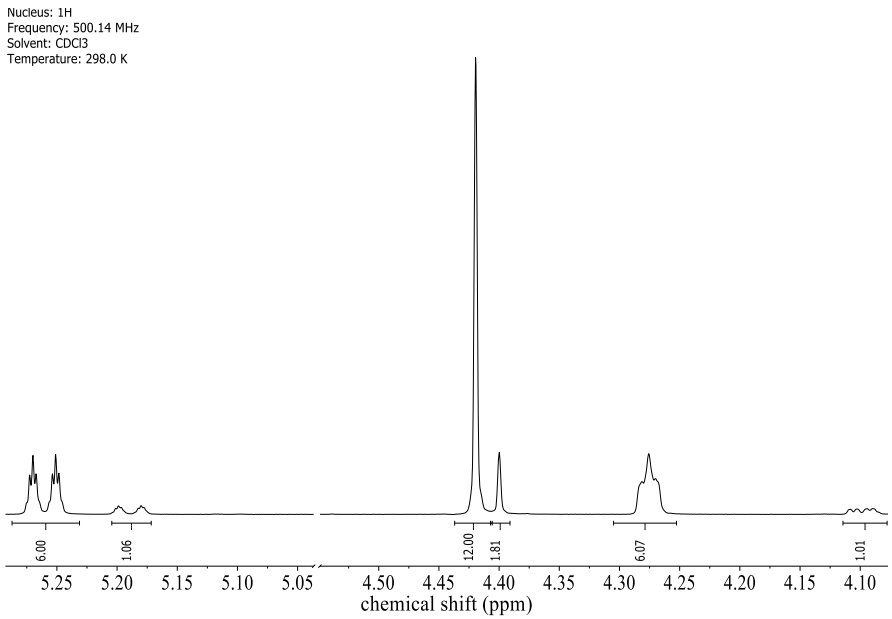
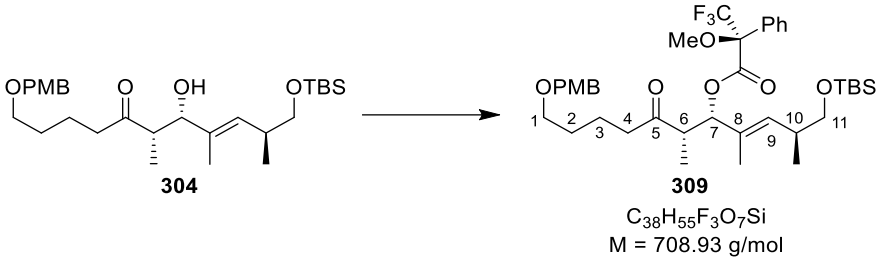


Figure 6.3.5-1: Cutout of the ^{1}H NMR spectrum to show the *dr* of 6:1, resulting out of the aldol reaction to compound **304**.

Synthesis of Compound 309

(2*S*,5*S*,6*S*,*E*)-1-((*tert*-Butyldimethylsilyl)oxy)-11-((4-methoxybenzyl)oxy)-2,4,6-trimethyl-7-oxoundec-3-en-5-yl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate

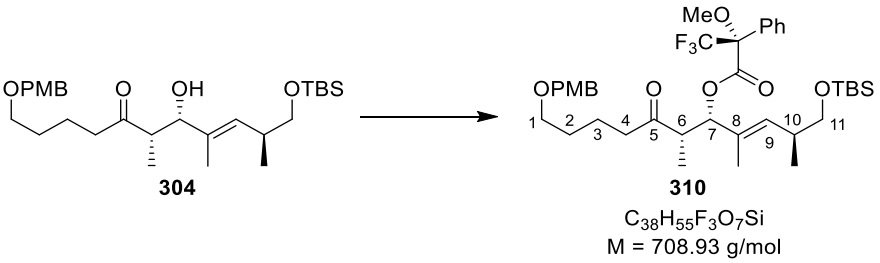


Alcohol **304** (6.00 mg, 12.2 µmol, 1.00 equiv) was dissolved in pyridine (140 µL) and (−)-(*R*)-MTPA-Cl (13.7 µL, 73.1 µmol, 6.00 equiv) was added at 0 °C. The reaction mixture was stirred 1 h at rt followed by the addition of sat. aq. NH_4Cl solution (1 mL) and DCM (2 mL). After separation of the organic layer, the aqueous phase was extracted with DCM (3x2 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (15% EtOAc/CyH) to yield the title compound **309** as a colorless oil (7.90 mg, 11.1 µmol, 92%, *dr* = 6:1 resulting out of the aldol reaction to compound **304**).

$\mathbf{R_f}$ 0.32 (20% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = −25.3° (*c* = 1.58 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.49 – 7.47 (m, 2H, $H_{arom., MTPA}$), 7.38 – 7.37 (m, 3H, $H_{arom., MTPA}$), 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.57 (d, *J* = 7.8 Hz, 1H, H-7), 5.31 (d, *J* = 9.5 Hz, 1H, H-9), 4.41 (s, 2H, Ar-CH_2), 3.80 (s, 3H, O-CH_3), 3.51 (s, 3H, O-$CH_{3, MTPA}$), 3.43 – 3.40 (m, 3H, H-1, H-11), 3.35 (dd, *J* = 9.7, 7.1 Hz, 1H, H-11'), 2.90 – 2.86 (m, 1H, H-6), 2.54 – 2.49 (m, 1H, H-10), 2.42 – 2.37 (m, 2H, H-4), 1.65 (d, *J* = 1.3 Hz, 3H, 8-CH_3), 1.60 – 1.52 (m, 4H, H-2, H-3), 0.99 (d, *J* = 7.0 Hz, 3H, 6-CH_3), 0.89 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.87 (s, 9H, TBS), 0.02 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 210.5 (C-5), 165.7 (C=O_{MTPA}), 159.3 ($C_{arom.}O$), 134.2 (C-9), 132.6 ($C_{arom., MTPA}$), 131.1 (C-8), 130.8 ($C_{arom.}$), 129.7 ($C_{arom.}H_{MTPA}$), 129.4 ($C_{arom.}H$), 128.4 ($C_{arom.}H_{MTPA}$), 127.6 ($C_{arom.}H_{MTPA}$), 123.5 (q, *J* = 283.7 Hz, CF_3), 113.9 ($C_{arom.}H$), 81.3 (C-7), 72.7 (Ar-CH_2), 69.8 (C-1), 67.6 (C-11), 55.4 (O-CH_3), 48.6 (C-6), 41.8 (C-4), 35.5 (C-10), 29.3 (C-2), 26.1 (TBS), 20.3 (C-3), 18.5 (TBS), 17.0 (10-CH_3), 13.4 (8-CH_3), 12.6 (6-CH_3), –5.2 (TBS), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{38}H_{55}F_3O_7SiNa$ $[M + Na]^+$: 731.3561, found: 731.3567, calcd for $C_{38}H_{55}F_3O_7SiNH_4$ $[M + NH_4]^+$: 726.4007, found: 726.4013.

Synthesis of Compound 310

(2*S*,5*S*,6*S*,*E*)-1-((*tert*-Butyldimethylsilyl)oxy)-11-((4-methoxybenzyl)oxy)-2,4,6-trimethyl-7-oxoundec-3-en-5-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate



Alcohol **304** (5.70 mg, 11.6 µmol, 1.00 equiv) was dissolved in pyridine (140 µL) and (+)-(*S*)-MTPA-Cl (13.0 µL, 69.4 µmol, 6.00 equiv) was added at 0 °C. The reaction mixture was stirred 2 h at rt followed by the addition of sat. aq. NH_4Cl solution (1 mL) and DCM (2 mL). After separation of the organic layer, the aqueous phase was extracted with DCM (3x2 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (15%

EtOAc/CyH) to yield the title compound **310** as a colorless oil (6.70 mg, 9.45 µmol, 82%, *dr* = 6:1 resulting out of the aldol reaction to compound **304**).

$\mathbf{R}_f$ 0.31 (20% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +8.0° (*c* = 1.12 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.49 – 7.46 (m, 2H, $H_{arom., MTPA}$), 7.39 – 7.33 (m, 3H, $H_{arom., MTPA}$), 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.53 (d, *J* = 6.8 Hz, 1H, H-7), 5.14 (d, *J* = 9.5 Hz, 1H, H-9), 4.41 (s, 2H, Ar-CH_2), 3.80 (s, 3H, O-CH_3), 3.52 (s, 3H, O-$CH_{3, MTPA}$), 3.42 (t, *J* = 6.2 Hz, 2H, H-1), 3.37 (dd, *J* = 9.7, 6.1 Hz, 1H, H-11), 3.31 (dd, *J* = 9.7, 7.2 Hz, 1H, H-11'), 2.88 – 2.84 (m, 1H, H-6), 2.50 – 2.40 (m, 3H, H-4, H-10), 1.62 – 1.54 (m, 4H, H-2, H-3), 1.54 (d, *J* = 1.2 Hz, 3H, 8-CH_3), 1.09 (d, *J* = 7.0 Hz, 3H, 6-CH_3), 0.87 (s, 9H, TBS), 0.83 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.02 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 210.6 (C-5), 165.6 ($C{=}O_{MTPA}$), 159.3 ($C_{arom.}O$), 133.2 (C-9), 132.4 ($C_{arom.,MTPA}$), 130.8 (C-8), 130.8 ($C_{arom.}$), 129.7 ($C_{arom.}H_{MTPA}$), 129.4 ($C_{arom.}H$), 128.5 ($C_{arom.}H_{MTPA}$), 127.6 ($C_{arom.}H_{MTPA}$), 123.5 (q, *J* = 283.7 Hz, CF_3), 113.9 ($C_{arom.}H$), 80.9 (C-7), 72.7 (Ar-CH_2), 69.8 (C-1), 67.6 (C-11), 55.4 (O-CH_3), 48.3 (C-6), 41.7 (C-4), 35.4 (C-10), 29.3 (C-2), 26.1 (TBS), 20.4 (C-3), 18.5 (TBS), 16.9 (10-CH_3), 13.4 (8-CH_3), 12.2 (6-CH_3), –5.2 (TBS), –5.3 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{38}H_{55}F_3O_7SiNa$ $[M + Na]^+$: 731.3561, found: 731.3558.

Mosher Ester Analysis for Compound 304

Determination of the absolute configuration was accomplished using the *Mosher* ester analysis. Two *Mosher* ester derivatives **309** and **310** have been synthesized and the respective NMR data have been compared as shown in **Table 6.3.5-1**. The resulting difference between the ^{1}H NMR data of the (*S*)- and (*R*)-MTPA ester led to the determination of the absolute configuration as shown in **Figure 6.3.5-1**. For a detailed background, see **Chapter 5.3.3** or the corresponding literature.[203,204]

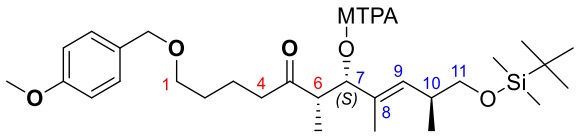


Table 6.3.5-1: Significant ^{1}H NMR signals of **309** and **310** and their use in the *Mosher* ester analysis.

position	δ_H [ppm] (**309**) (*S*)	δ_H [ppm] (**310**) (*R*)	$\Delta\delta^{S-R}$
1	3.41	3.52	-0.11
6-CH_3	0.99	1.09	-0.10
4	2.40	2.45	-0.05
7	5.57	5.53	0.04
11	3.41	3.37	0.04
11‘	3.35	3.31	0.04
10	2.52	2.47	0.05
10-CH_3	0.89	0.83	0.06
8-CH_3	1.65	1.54	0.11
9	5.31	5.14	0.17

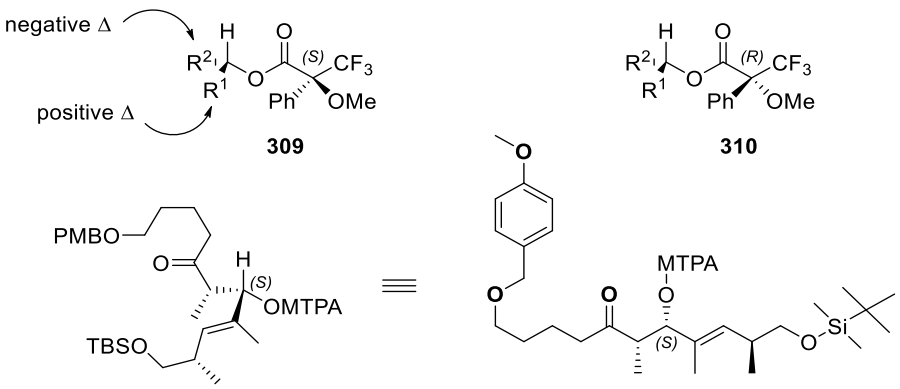
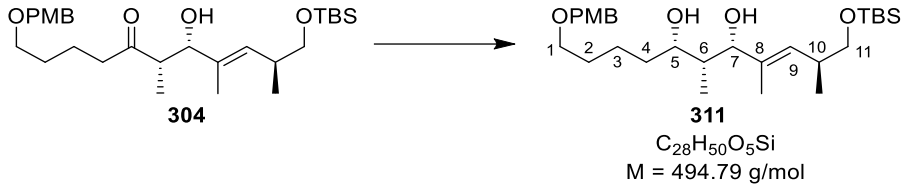


Figure 6.3.5-1: Proposed configuration of **304** under the use of the *Mosher* ester analysis.

Synthesis of Compound 311

(2*S*,5*S*,6*R*,7*S*,*E*)-1-((*tert*-Butyldimethylsilyl)oxy)-11-((4-methoxybenzyl)oxy)-2,4,6-trimethylundec-3-ene-5,7-diol



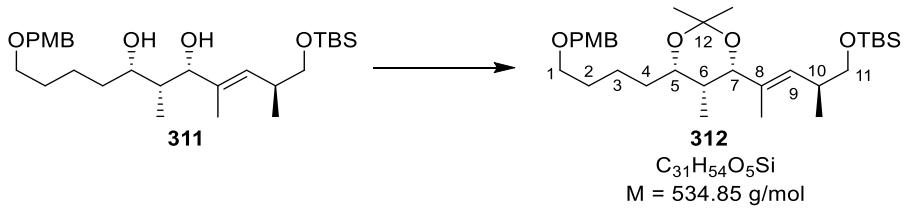
Alcohol **304** (31.7 mg, 64.3 µmol, 1.00 equiv) was dissolved in THF/MeOH (4:1, 0.8 mL) and cooled to −78 °C. Et_2BOMe (70.8 µL, 70.8 µmol, 1.10 equiv, 1 M in THF) was added slowly and the mixture was stirred 15 min at −78 °C followed by the addition of $NaBH_4$ (2.68 mg, 70.8 µmol, 1.10 equiv) and further stirring for 4 h at −78 °C. The reaction mixture was allowed to warm to 0 °C and quenched with aq. NaOH (0.6 mL, 3 M) and H_2O_2 (0.9 mL, 35%). After stirring 4 h at 0 °C, water (1.5 mL) and Et_2O (4 mL) were added, the phases were separated, the aqueous phase was extracted with Et_2O (3x5 mL), the combined organic phases were washed with water (10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (30% EtOAc/CyH) to yield the title compound **311** as a colorless oil (31.8 mg, 64.3 µmol, quant.). Furthermore, the separation of the diastereomeric mixture (*dr* = 6:1, resulting out of the aldol reaction to compound **304**) was possible, using the described flash chromatography, and only the major diastereomer was used for following reactions.

$\mathbf{R_f}$ 0.31 (40% EtOAc/CyH). $[\alpha]_D^{20}$ = +2.3° (*c* = 1.28 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.24 (dq, *J* = 9.4, 1.3 Hz, 1H, H-9), 4.43 (s, 2H, Ar-CH_2), 4.18 (br d, *J* = 2.3 Hz, 1H, H-7), 3.85 – 3.83 (m, 1H, H-5), 3.80 (s, 3H, O-CH_3), 3.47 – 3.44 (m, 3H, H-1, H-11), 3.39 (dd, *J* = 9.7, 7.2 Hz, 1H, H-11'), 2.67 (s, 1H, 5-OH), 2.63 – 2.55 (m, 1H, H-10), 2.39 (s, 1H, 7-OH), 1.67 – 1.61 (m, 3H, H-2, H-6), 1.58 (d, *J* = 1.3 Hz, 3H, 8-CH_3), 1.56 – 1.35 (m, 4H, H-3, H-4), 0.96 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.89 (s, 9H, TBS), 0.80 (d, *J* = 7.1 Hz, 3H, 6-CH_3), 0.04 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}$O), 135.9 (C-8), 130.8 ($C_{arom.}$), 129.4 ($C_{arom.}$H), 127.8 (C-9), 113.9 ($C_{arom.}$H), 80.7 (C-7), 76.2 (C-5), 72.7 (Ar-CH_2), 70.1 (C-1), 68.1 (C-11), 55.4 (O-CH_3), 39.0 (C-6), 35.4 (C-4), 35.3 (C-10), 29.8 (C-2), 26.1 (TBS), 23.1 (C-3), 18.5 (TBS), 17.5 (10-CH_3),

14.3 (8-CH_3), 4.8 (6-CH_3), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{28}H_{50}O_5SiH$ $[M + H]^+$: 495.3500, found: 495.3503.

Synthesis of Compound 312

***tert*-Butyl(((*S*,*E*)-4-((4*S*,5*R*,6*S*)-6-(4-((4-methoxybenzyl)oxy)butyl)-2,2,5-trimethyl-1,3-dioxan-4-yl)-2-methylpent-3-en-1-yl)oxy)dimethylsilane**



2,2-Dimethoxypropane (0.5 mL) was added to diol **311** (9.50 mg, 19.2 µmol, 1.00 equiv) followed by the addition of CSA (4.47 µL, 0.19 µmol, 0.01 equiv, 43 mM in DCM). The reaction mixture was stirred 3 h at rt and quenched with the addition of NEt_3 (7 µL). All volatiles were removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (15% EtOAc/CyH) to yield the title compound **312** as a colorless oil (10.2 mg, 19.1 µmol, quant.).

R_f 0.45 (20% EtOAc/CyH). $[\alpha]_D^{20}$ = −2.7° (*c* = 1.46 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.27 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.24 (dq, *J* = 9.3, 1.0 Hz, 1H, H-9), 4.43 (s, 2H, Ar-CH_2), 4.19 (br s, 1H, H-7), 3.89 – 3.87 (m, 1H, H-5), 3.80 (s, 3H, O-CH_3), 3.47 – 3.44 (m, 3H, H-1, H-11), 3.36 (dd, *J* = 9.7, 7.5 Hz, 1H, H-11'), 2.62 – 2.56 (m, 1H, H-10), 1.65 – 1.61 (m, 2H, H-2), 1.55 (d, *J* = 1.0 Hz, 3H, 8-CH_3), 1.53 – 1.43 (m, 3H, H-3, H-4, H-6), 1.42 (s, 3H, 12-CH_3), 1.42 (s, 3H, 12-CH_3), 1.39 – 1.32 (m, 2H, H-3',H-4'), 0.97 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.89 (s, 9H, TBS), 0.67 (d, *J* = 6.9 Hz, 3H, 6-CH_3), 0.04 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}$O), 132.9 (C-8), 130.9 ($C_{arom.}$), 129.4 ($C_{arom.}$H), 127.0 (C-9), 113.9 ($C_{arom.}$H), 98.9 (C-12), 75.9 (C-7), 73.2 (C-5), 72.7 (Ar-CH_2), 70.1 (C-1), 68.3 (C-11), 55.4 (O-CH_3), 35.3 (C-10), 33.5 (C-6), 33.0 (C-4), 30.2 (12-CH_3), 29.9 (C-2), 26.1 (TBS), 22.3 (C-3), 19.8 (12-CH_3), 18.5 (TBS), 17.5 (10-CH_3), 14.0 (8-CH_3), 5.1 (6-CH_3), –5.1 (TBS), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{31}H_{54}O_5SiNH_4$ $[M + NH_4]^+$: 552.4079, found: 552.4077.

Acetonide Analysis for Compound 312

The determination of the correct relative stereochemistry of diol **311** (stereogenic center at position 5 and 6) was accomplished by the accepted model for the analysis of the corresponding acetonide **312**. Thus, the ^{13}C NMR spectrum was used and analyzed showing different chemical shifts for the two methyl groups of the acetonide, which indicates a chair conformation, resulting out of the *syn*-1,3-diol acetonide. In the ^{1}H NMR spectrum, a qdd was identified for H-6 although the signal is in the multiplet region of 1.53 – 1.43 ppm. The coupling constant of 6.9 Hz results from the coupling with the 6-CH_3 group and the two coupling constants of 2.3 Hz indicate a *cis* coupling to H-5 and H-7. The *cis*-hydrogens were also confirmed with NOESY correlations as shown in **Figure 6.3.5-2**. A more detailed theoretical background can be found in **Chapter 5.3.3** and in literature.[155–158]

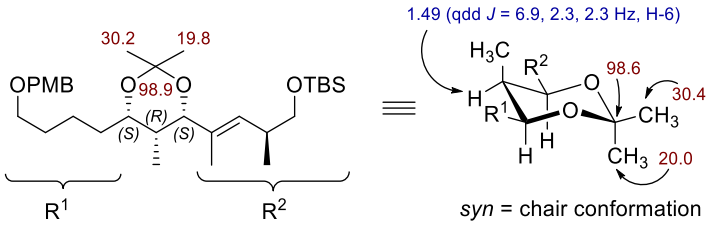


Figure 6.3.5-2: Relevant ^{13}C NMR signals (brown) and the ^{1}H NMR signal for H-6 used for the determination of the 1,3-*syn*-diol acetonide and the relative configuration of 6-CH_3.

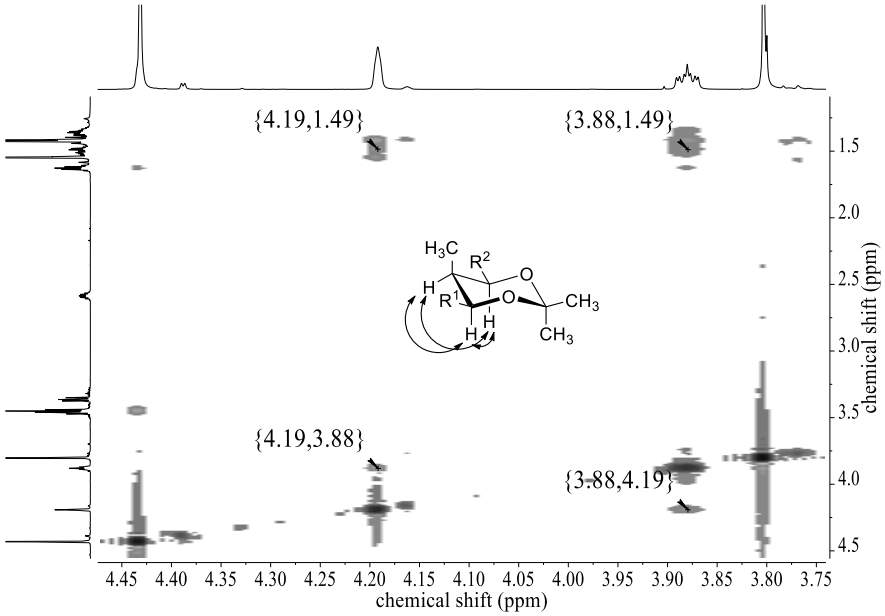
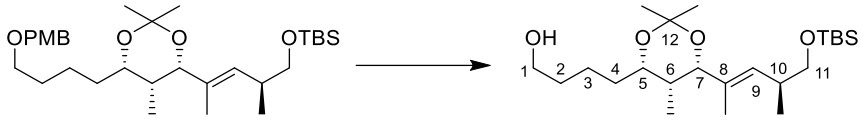


Figure 6.3.5-3: Selected NOESY signals of compound **312**, which were used for the determination of the relative configuration of 6-CH_3.

Synthesis of Compound 313

4-((4*S*,5*R*,6*S*)-6-((*S*,*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-4-methylpent-2-en-2-yl)-2,2,5-trimethyl-1,3-dioxan-4-yl)butan-1-ol



312 **313**

$C_{23}H_{46}O_4Si$

M = 414.70 g/mol

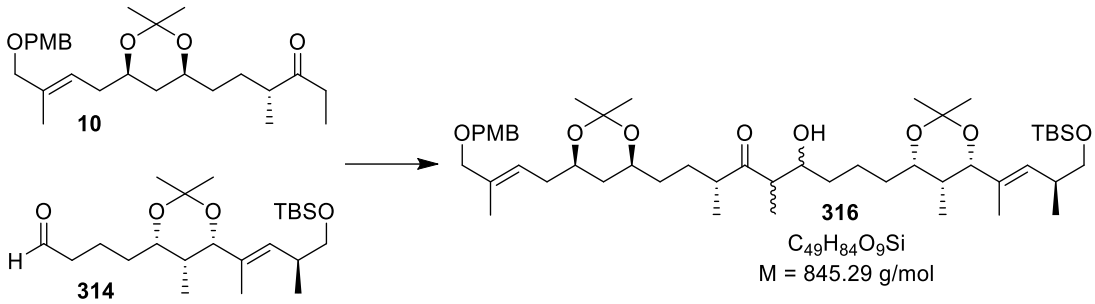
Acetonide **312** (9.60 mg, 18.0 µmol, 1.00 equiv) was dissolved in DCM (0.7 mL) and pH7 buffer (70 µL) was added. After adding DDQ (8.15 mg, 35.9 µmol, 2.00 equiv), the reaction mixture was stirred 90 min at rt. DCM (4 mL) and water (3 mL) were added, the phases separated, the aqueous phase was extracted with DCM (3x4 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) to yield the title compound **313** as a colorless oil (3.70 mg, 8.92 µmol, 50%).

R_f 0.21 (20% EtOAc/CyH). $[\alpha]_D^{20}$ = −2.1° (*c* = 0.48 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 5.25 (dq, *J* = 9.3, 1.0 Hz, 1H, H-9), 4.20 (br s, 1H, H-7), 3.91 – 3.89 (m, 1H, H-5), 3.67 (t, *J* = 6.5 Hz, 2H, H-1), 3.46 (dd, *J* = 9.7, 6.0 Hz, 1H, H-11), 3.36 (dd, *J* = 9.7, 7.5 Hz, 1H, H-11'), 2.62 – 2.57 (m, 1H, H-10), 1.64 – 1.58 (m, 3H, H-2), 1.55 (d, *J* = 1.0 Hz, 3H, 8-CH_3), 1.51 – 1.45 (m, 3H, H-3, H-4, H-6), 1.43 (s, 3H, 12-CH_3), 1.42 (s, 3H, 12-CH_3), 1.40 – 1.58 (m, 2H, H-3',H-4'), 0.97 (d, *J* = 6.7 Hz, 3H, 10-CH_3), 0.68 (d, *J* = 6.9 Hz, 3H, 6-CH_3), 0.04 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 132.8 (C-8), 127.1 (C-9), 98.9 (C-12), 75.9 (C-7), 73.3 (C-5), 68.3 (C-11), 63.0 (C-1), 35.3 (C-10), 33.7 (C-6), 33.0 (C-4), 32.8 (C-2), 30.2 (12-CH_3), 26.1 (TBS), 22.1 (C-3), 19.8 (12-CH_3), 18.5 (TBS), 17.5 (10-CH_3), 14.0 (8-CH_3), 5.1 (6-CH_3), –5.1 (TBS), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{23}H_{46}O_4SiNH_4$ $[M + NH_4]^+$: 432.3504, found: 432.3513.

6.3.6 Fragment Coupling (Fragment B+CD)

Synthesis of Compound 316

(3*R*)-9-((4*S*,5*R*,6*S*)-6-((*S*,*E*)-5-((*tert*-Butyldimethylsilyl)oxy)-4-methylpent-2-en-2-yl)-2,2,5-trimethyl-1,3-dioxan-4-yl)-6-hydroxy-1-((4*S*,6*R*)-6-((*E*)-4-((4-methoxybenzyl)oxy)-3-methylbut-2-en-1-yl)-2,2-dimethyl-1,3-dioxan-4-yl)-3,5-dimethylnonan-4-one

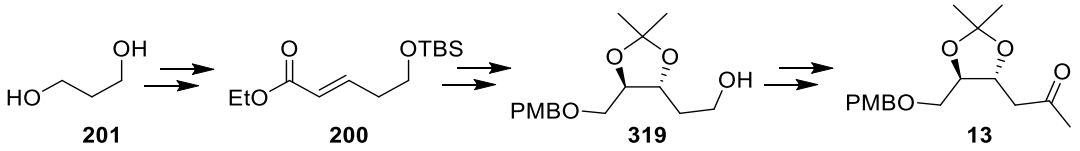


Ketone **10** (15.4 mg, 35.6 µmol, 2.10 equiv, dried over 3 Å MS in 0.3 mL Et_2O) was added to Et_2O (0.3 mL) and cooled to −78 °C followed by the addition of (cHex)$_2$BCl (53.4 µL, 53.4 µmol, 3.15 equiv, 1 M in hexane) and NEt_3 (9.46 µL, 67.9 µmol, 4.00 equiv). The reaction mixture was allowed to warm to 0 °C for 2 h before cooling again to −78 °C. Aldehyde **314** (7.00 mg, 17.0 µmol, 1.00 equiv, dried over 3 Å MS in 0.6 mL Et_2O, synthesized out of **313** by *Maximilian Seul*, using *Parikh-Doering* conditions and **314** was directly used without further analysis)[212] was added and the reaction mixture stirred for 2 h at −78 °C and overnight at −20 °C. MeOH (0.3 mL), pH 7 buffer (0.7 mL) and H_2O_2 (0.3 mL) were added at 0 °C and the mixture was stirred 2.5 h at rt. Water (5 mL) was added, the phases were separated, the aqueous layer was extracted with Et_2O (3 x 10 mL), the combined organic layers were washed with brine (25 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (20% EtOAc/CyH) to yield the semi pure title compound **316** as a colorless oil (0.67 mg, 0.79 µmol,~5%).

R_f 0.31 (30% EtOAc/CyH). **HRMS (ESI)** *m/z*: calcd for $C_{49}H_{84}O_9SiNH_4$ $[M + NH_4]^+$: 862.6223, found: 862.6220. NMR data indicated the formation of desired compound **316** but due to impurities and the low quantity an assignment could not be made unambiguously.

6.3.7 Synthesis of Fragment E (13)

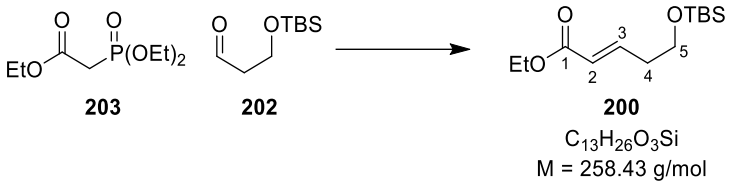
This section describes the experimental details to yield fragment E (**13**), which was synthesized in eleven linear steps out of propanediol **201**.



***Scheme 6.3.7-1*:** Synthesis towards fragment E (**13**).

Synthesis of Compound 200

Ethyl (*E*)-5-((*tert*-Butyldimethylsilyl)oxy)pent-2-enoate



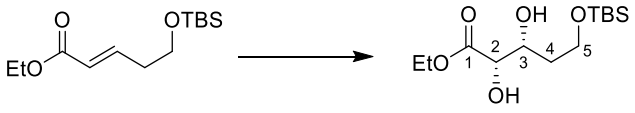
A solution of triethyl phosphonoacetate (**203**, 143 mg, 637 µmol, 1.20 equiv) in THF (3.5 mL) was cooled to 0 °C and NaH (25.5 mg, 637 µmol, 1.20 equiv; 60% dispersion in mineral oil) was added over the period of 5 min. The reaction mixture was stirred 30 min at rt before cooling again to 0 °C. Aldehyde **202** (100 mg, 531 µmol, 1.00 equiv; in 1 mL THF, for synthesis see **Chapter 6.3.1**) was added and stirring was continued overnight at rt. Aq. sat. NH_4Cl solution (6 mL) and EtOAc (10 mL) were added, the phases separated, the aqueous phase was extracted with EtOAc (3x10 mL), the combined organic phases were washed with water (10 mL) and brine (10 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (5% EtOAc/CyH) to yield the title compound **200** as a colorless oil (132 mg, 510 µmol, 96%, only the *E* isomer was obtained, *E*/*Z* > 20:1).

R_f 0.20 (5% EtOAc/CyH). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 6.96 (dt, *J* = 15.7, 7.1 Hz, 1H, H-3), 5.86 (dt, *J* = 15.7, 1.5 Hz, 1H, H-2), 4.19 (q, *J* = 7.1 Hz, 2H, O$\underline{CH_2}CH_3$), 3.72 (t, *J* = 6.5 Hz, 2H, H-5), 2.41 (dtd, *J* = 7.1, 6.5, 1.5 Hz, 2H, H-4), 1.28 (t, *J* = 7.1 Hz, 3H, $OCH_2\underline{CH_3}$), 0.89 (s, 9H, TBS), 0.05 (s, 6H, TBS). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 166.6 (C-1), 146.0 (C-3), 123.1 (C-2), 61.7 (C-5), 60.3 (O$\underline{CH_2}CH_3$), 35.8 (C-4), 26.0 (TBS), 18.4 (TBS), 14.4

($OCH_2\underline{CH_3}$), –5.2 (TBS). **MS (ESI)** *m/z*: calcd for $C_{13}H_{26}O_3SiH$ $[M + H]^+$: 259.173, found: 259.172. The large coupling constant of 15.7 Hz between H-2 and H-3 indicated a *trans*-coupling confirming the *E* double bond. The spectroscopic data were in agreement with those previously reported.[180]

Synthesis of Compound 317

Ethyl (2*S*,3*R*)-5-((*tert*-butyldimethylsilyl)oxy)-2,3-dihydroxypentanoate



200 **317**

$C_{13}H_{28}O_5Si$

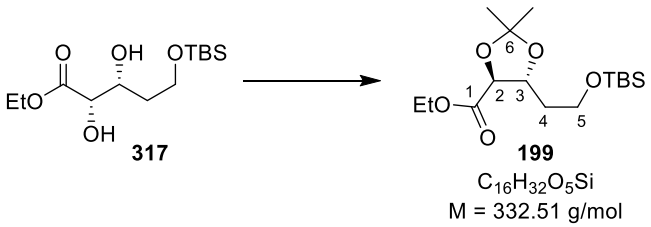
M = 292.45 g/mol

A solution of ester **200** (365 mg, 1.41 mmol, 1.00 equiv) in 1:1 *tert*-butanol/water (3.3 mL) was added to a 0 °C cooled solution of methanesulfonamide (135 mg, 1.41 mmol, 1.00 equiv) and AD-mix-β (1.97 g, 1.40 g per mmol starting material) in 1:1 *tert*-butanol/water (1.4 mL). The mixture was stirred for 48 h at rt, Na_2SO_3 (1.78 g, 14.1 mmol, 10.0 equiv) was added and stirring was continued for 3 h at rt. EtOAc (30 mL) and water (30 mL) were added, the were phases separated, the aqueous phase was extracted with EtOAc (3x30 mL), the combined organic phases were washed with brine (30 mL), dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (35% EtOAc/CyH) to yield the title compound **317** as a colorless oil (330 mg, 1.13 mmol, 80%).

$\mathbf{R_f}$ 0.34 (40% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = −2.2° (*c* = 0.90 in $CHCl_3$). **^{1}H NMR** (400 MHz, $CDCl_3$) δ = 4.29 (q, *J* = 7.1 Hz, 2H, $O\underline{CH_2}CH_3$), 4.20 – 4.17 (br m, 1H, H-3), 4.06 (br s, 1H, H-2), 3.94 – 3.82 (m, 2H, H-5), 3.20 – 3.01 (m, 2H, 2-OH, 3-OH), 2.00 – 1.91 (m, 1H, H-4), 1.77 – 1.71 (m, 1H, H-4'), 1.32 (t, *J* = 7.1 Hz, 3H, $OCH_2\underline{CH_3}$), 0.90 (s, 9H, TBS), 0.08 (s, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 173.3 (C-1), 73.8 (C-2), 72.3 (C-3), 62.0 ($O\underline{CH_2}CH_3$), 61.8 (C-5), 35.4 (C-4), 26.0 (TBS), 18.3 (TBS), 14.3 ($OCH_2\underline{CH_3}$), –5.4 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{13}H_{28}O_5SiH$ $[M + H]^+$: 293.1779, found: 293.1778. Due to the similarity of the rotation values (literature: $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = −3.6° (*c* = 0.90 in $CHCl_3$) and the literature known procedure to product **317**, the *ee* was not checked and the literature provided *ee* of 92% was assumed. The spectroscopic data were in agreement with those previously reported.[180]

Synthesis of Compound 199

Ethyl (4*S*,5*R*)-5-(2-((*tert*-butyldimethylsilyl)oxy)ethyl)-2,2-dimethyl-1,3-dioxolane-4-carboxylate

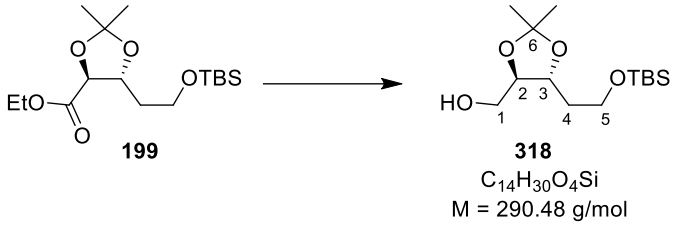


Diol **317** (60.0 mg, 205 µmol, 1.00 equiv) was dissolved in acetone (1 mL) and 2,2-dimethoxypropane (151 µL, 1.23 mmol, 6.00 equiv) and *p*TsOH (200 µL, 2.40 µmol, 0.01 equiv, 12 mM solution in acetone) were added. The reaction mixture was stirred overnight at rt followed by the addition of solid $NaHCO_3$ (10 mg) and further stirring for 15 min at rt. After filtration of the mixture over a short pad of silica, all volatiles were removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (15% EtOAc/CyH) to yield the title compound **199** as a colorless oil (53.7 mg, 162 µmol, 79%).

$\mathbf{R_f}$ 0.59 (40% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{25}}$ = +11.8° (*c* = 1.36 in $CHCl_3$). **^{1}H NMR** (700 MHz, CD_2Cl_2) δ = 4.24 (td, *J* = 7.7, 3.7 Hz, 1H, H-3), 4.19 (q, *J* = 7.1 Hz, 2H, O$\underline{CH_2}CH_3$), 4.16 (d, *J* = 7.7 Hz, 1H, H-2), 3.79 – 3.72 (m, 2H, H-5), 1.42 (s, 3H, 6-CH_3), 1.39 (s, 3H, 6-CH_3), 1.27 (t, *J* = 7.1 Hz, 3H, $OCH_2\underline{CH_3}$), 0.89 (s, 9H, TBS), 0.05 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, CD_2Cl_2) δ = 171.2 (C-1), 111.1 (C-6), 79.6 (C-2), 76.6 (C-3), 61.7 (O$\underline{CH_2}CH_3$), 60.0 (C-5), 37.2 (C-4), 27.5 (6-CH_3), 26.2 (TBS), 26.0 (6-CH_3), 18.7 (TBS), 14.5 ($OCH_2\underline{CH_3}$), –5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{16}H_{32}O_5SiH$ $[M + H]^+$: 333.2092, found: 333.2093. The spectroscopic data were in agreement with those previously reported.[180]

Synthesis of Compound 318

((4*R*,5*R*)-5-(2-((*tert*-Butyldimethylsilyl)oxy)ethyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methanol

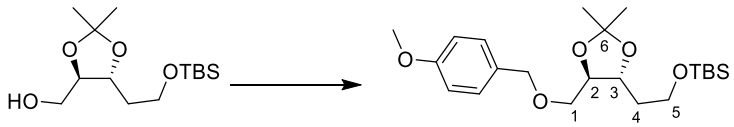


Ester **199** (270 mg, 812 µmol, 1.00 equiv) was dissolved in THF (3.5 mL), the solution was cooled to −78 °C and DIBAL-H (2.03 mL, 2.03 mmol, 2.50 equiv, 1 M in THF) was added slowly. After stirring 1 h at −78 °C and 30 min at rt, the reaction solution was poured into sat. aq. Rochelle salt solution (15 mL). The mixture was stirred until clearly two phases appeared (2 h). The phases were separated, the aqueous layer was extracted with Et_2O (3x30 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (40% EtOAc/CyH) to yield the title compound **318** as a colorless oil (225 mg, 775 µmol, 96%).

$\mathbf{R_f}$ 0.24 (30% EtOAc/CyH). $[\alpha]_D^{25}$ = +17.8° (*c* = 0.90 in CH_2Cl_2). **^{1}H NMR** (500 MHz, CD_2Cl_2) δ = 4.00 – 3.95 (m, 1H, H-3), 3.80 – 3.70 (m, 4H, H-1, H-2, H-5), 3.62 – 3.57 (m, 1H, H-1'), 1.96 (t, *J* = 6.3 Hz, 1H, OH), 1.18 – 1.76 (m, 2H, H-4), 1.38 (s, 3H, 6-CH_3), 1.36 (s, 3H, 6-CH_3), 0.89 (s, 9H, TBS), 0.06 (2xs, 6H, TBS). **^{13}C NMR** (126 MHz, CD_2Cl_2) δ = 108.9 (C-6), 82.0 (C-2), 75.0 (C-3), 62.6 (C-1), 60.5 (C-5), 36.8 (C-4), 27.7 (6-CH_3), 27.3 (6-CH_3), 26.2 (TBS), 18.7 (TBS), –5.1 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{14}H_{30}O_4SiH$ $[M + H]^+$: 291.1986, found: 291.1984.

Synthesis of Compound 198

***tert*-Butyl(2-((4*R*,5*R*)-5-(((4-methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)ethoxy)dimethylsilane**



318 **198**

$C_{22}H_{38}O_5Si$
M = 410.63 g/mol

Alcohol **318** (225 mg, 775 µmol, 1.00 equiv) was dissolved in DMF (1.5 mL), cooled to 0 °C and 4-methoxybenzyl chloride (115 µL, 852 µmol, 1.10 equiv) was added. NaH (34.1 mg, 852 µmol, 1.10 equiv, 60% dispersion in mineral oil) was added over the period of 5 min and the reaction mixture was stirred for 2 h at 0° C and 2 h at rt. DCM (15 mL) and water (10 mL) were added at 0 °C, the phases separated, the aqueous phase extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (25% EtOAc/CyH) to yield the title compound **198** as a colorless oil (276 mg, 672 µmol, 87%).
$\mathbf{R_f}$ 0.45 (30% EtOAc/CyH). $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ = +10.7° (*c* = 1.22 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.6 Hz, 2H, $H_{arom.}$), 4.57 – 4.49 (m, 2H, Ar-CH_2), 3.92 (td, *J* = 8.3, 3.6 Hz, 1H, H-3), 3.87 (dt, *J* = 8.3, 4.8 Hz, 1H, H-2), 3.80 (s, 3H, O-CH_3), 3.77 – 3.74 (m, 1H, H-5), 3.73 – 3.69 (m, 1H, H-5'), 3.54 (d, *J* = 4.8 Hz, 2H, H-1), 1.83 – 1.79 (m, 1H, H-4), 1.77 – 1.72 (m, 1H, H-4'), 1.40 (s, 3H, 6-CH_3), 1.38 (s, 3H, 6-CH_3), 0.88 (s, 9H, TBS), 0.04 (2xs, 6H, TBS). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.3 ($C_{arom.}$O), 130.3 ($C_{arom.}$), 129.4 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 108.9 (C-6), 80.2 (C-2), 75.4 (C-3), 73.3 (Ar-CH_2), 70.4 (C-1), 60.0 (C-5), 55.4 (O-CH_3), 36.5 (C-4), 27.5 (6-CH_3), 27.1 (6-CH_3), 26.1 (TBS), 18.4 (TBS), -5.2 (TBS). **HRMS (ESI)** *m/z*: calcd for $C_{22}H_{38}O_5SiNH_4$ $[M + NH_4]^+$: 428.2827, found: 428.2834.

Synthesis of Compound 319

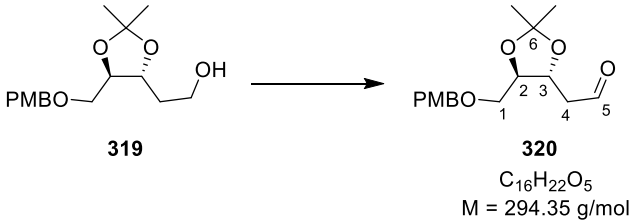
2-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)ethan-1-ol

Acetonide **198** (270 mg, 658 µmol, 1.00 equiv) was dissolved in THF (12 mL) and cooled to 0 °C. TBAF (986µL, 986 µmol, 1.50 equiv, 1 M in THF) was added and after stirring for 20 min at 0 °C, the reaction mixture was stirred further 3 h at rt. Et_2O (15 mL) and water (15 mL) were added, the phases were separated, the aqueous phase was extracted with Et_2O (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (60% EtOAc/CyH) to yield the title compound **319** as a colorless oil (171 mg, 577 µmol, 88%).

$\mathbf{R_f}$ 0.29 (60% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +5.4° (*c* = 0.74 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.25 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.53 – 4.49 (m, 2H, Ar-CH_2), 3.97 (td, *J* = 8.1, 4.0 Hz, 1H, H-3), 3.91 (dt, *J* = 8.2, 5.1 Hz, 1H, H-2), 3.81 (s, 3H, O-CH_3), 3.78 (t, *J* = 5.6 Hz, 1H, H-5), 3.60 (dd, *J* = 10.1, 5.3 Hz, 1H, H-1), 3.52 (dd, *J* = 10.1, 5.1 Hz, 1H, H-1'), 2.37 (br s, 1H, OH), 1.89 – 1.80 (m, 2H, H-4), 1.41 (s, 3H, 6-CH_3), 1.39 (s, 3H, 6-CH_3). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.5 ($C_{arom.}$O), 129.9 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 114.0 ($C_{arom.}$H), 109.2 (C-6), 79.8 (C-2), 78.4 (C-3), 73.5 (Ar-CH_2), 70.3 (C-1), 60.9 (C-5), 55.4 (O-CH_3), 35.5 (C-4), 27.3 (6-CH_3), 27.1 (6-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{16}H_{24}O_5H$ $[M + H]^+$: 297.1697, found: 297.1696.

Synthesis of Compound 320

2-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)acetaldehyde

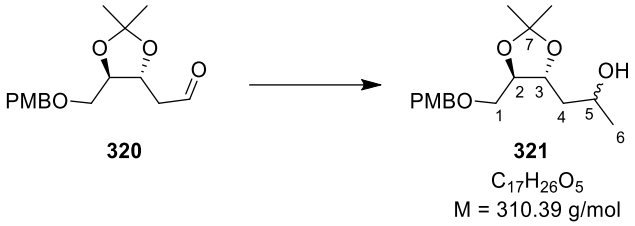


DCM (0.5 mL) was added to sulfur trioxide pyridine complex (182 mg, 1.44 mmol, 3.00 equiv) followed by the addition of *N*,*N*-diisopropylethylamine (260 µL, 1.53 mmol, 4.00 equiv) and DMSO (271 µL, 3.81 mmol, 10.0 equiv). The reaction mixture was cooled to 0 °C and alcohol **319** (113 mg, 381 µmol, 1.00 equiv, in 2 mL DCM) was added. After stirring for 30 min at 0 °C, sat. aq. $NaHCO_3$ solution (10 mL) was added and the aqueous phase was extracted with DCM (3x15 mL). The combined organic layers were washed with $CuSO_4$, NH_4Cl and brine solution (each aq. sat. 2x30 mL). The organic phase was dried over $MgSO_4$ and the solvent was removed under reduced pressure to yield the title compound **320** as a colorless oil (105 mg, 358 µmol, 94%).

R_f 0.50 (60% EtOAc/CyH). $[\alpha]_D^{20}$ = +1.3° (*c* = 1.50 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 9.79 (t, *J* = 2.0 Hz, 1H, H-5), 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.88 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.49 (s, 2H, Ar-CH_2), 4.29 (td, *J* = 7.7, 4.5 Hz, 1H, H-3), 3.87 (dt, *J* = 8.2, 5.3 Hz, 1H, H-2), 3.81 (s, 3H, O-CH_3), 3.64 (dd, *J* = 10.0, 5.1 Hz, 1H, H-1), 3.53 (dd, *J* = 10.0, 5.5 Hz, 1H, H-1'), 2.73 – 2.66 (m, 2H, H-4), 1.41 (s, 6H, 2x6-CH_3). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 200.1 (C-5), 159.5 ($C_{arom.}$O), 129.9 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 114.0 ($C_{arom.}$H), 109.7 (C-6), 79.4 (C-2), 74.0 (C-3), 73.5 (Ar-CH_2), 69.9 (C-1), 55.4 (O-CH_3), 47.0 (C-4), 27.2 (6-CH_3), 27.0 (6-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{16}H_{22}O_5NH_4$ $[M + NH_4]^+$: 312.1805, found: 312.1802. The spectroscopic data were in agreement with those previously reported.[240]

Synthesis of Compound 321

1-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-ol

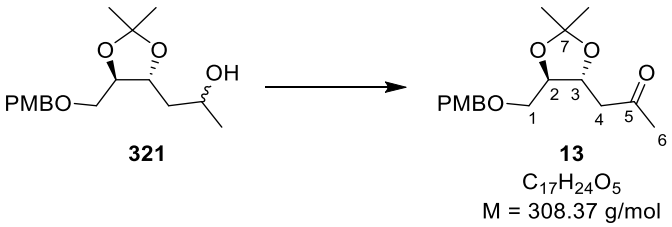


Aldehyde **320** (100 mg, 340 µmol, 1.00 equiv) was dissolved in Et_2O (5 mL), cooled to 0 °C and MeMgBr (136 µL, 408 µmol, 1.20 equiv, 3 M in Et_2O) was added. After stirring for 20 min at 0 °C, Et_2O (10 mL) and aq. sat. NH_4Cl (15 mL) were added, the phases separated, the aqueous phase was extracted with Et_2O (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (60% EtOAc/CyH) to yield the title compound **321** as a colorless oil (95.6 mg, 308 µmol, 91%, *dr* = 1.5:1 but the stereogenic center at C-5 was removed in the next step).

R_f 0.33 (60% EtOAc/CyH). $[\alpha]_D^{20}$ = +5.4° (*c* = 1.06 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 – 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.89 – 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.54 – 4.48 (s, 2H, Ar-CH_2), 4.06 – 3.83 (m, 3H, H-2, H-3, H-5), 3.81 (s, 3H, O-CH_3), 3.60 – 3.57 (m, 1H, H-1), 3.53 – 3.50 (m, 1H, H-1'), 1.74 – 1.61 (m, 2H, H-4), 1.42 – 1.39 (m, 6H, 2x7-CH_3), 1.21/1.19 (2xd, *J* = 6.3 Hz, 3H, H-6). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.5/159.5 ($C_{arom.}O$), 130.0/130.0 ($C_{arom.}$), 129.5/129.5 ($C_{arom.}H$), 114.0/114.0 ($C_{arom.}H$), 109.6/109.1 (C-7), 80.4/79.6 (C-2), 78.8/76.6 (C-3), 73.4/73.4 (Ar-CH_2), 70.3/69.9 (C-1), 67.4/65.3 (C-5), 55.4 (O-CH_3), 42.0/41.3 (C-4), 27.4/27.3 (7-CH_3), 27.1/27.1 (7-CH_3), 23.8/23.4 (C-6). **HRMS (ESI)** *m/z*: calcd for $C_{17}H_{26}O_5H$ $[M + H]^+$: 311.1853, found: 311.1852.

Synthesis of Compound 13

1-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)propan-2-one

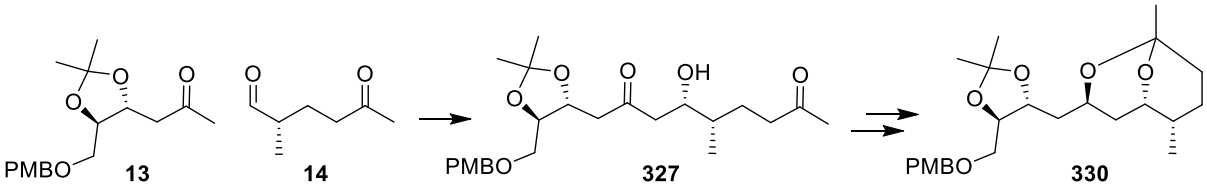


DCM (1.5 mL) was added to sulfur trioxide pyridine complex (147 mg, 924 µmol, 3.00 equiv) followed by the addition of *N*,*N*-diisopropylethylamine (210 µL, 1.23 mmol, 4.00 equiv) and DMSO (219 µL, 3.08 mmol, 10.0 equiv). The reaction mixture was cooled to 0 °C and alcohol **321** (95.6 mg, 308 µmol, 1.00 equiv, in 1.5 mL DCM) was added. After stirring for 30 min at 0 °C, the reaction mixture was diluted with DCM (10 mL) and water (10 mL), the phases were separated, the aqueous phase was extracted with DCM (3x15 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (40% EtOAc/CyH) to yield the title compound **13** as a colorless oil (79.6 mg, 258 µmol, 84%).

$\mathbf{R_f}$ 0.23 (40% EtOAc/CyH). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = +4.5° (*c* = 0.66 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.49 (d, *J* = 3.3 Hz, 2H, Ar-CH_2), 4.24 (td, *J* = 8.2, 4.1 Hz, 1H, H-3), 3.83 (dt, *J* = 8.2, 5.3 Hz, 1H, H-2), 3.80 (s, 2H, O-CH_3), 3.61 (dd, *J* = 10.0, 5.4 Hz, 1H, H-1), 3.52 (dd, *J* = 10.0, 5.1 Hz, 1H, H-1'), 2.73 (dd, *J* = 16.2, 7.9 Hz, 1H, H-4), 2.66 (dd, *J* = 16.2, 4.1 Hz, 1H, H-4'), 2.18 (s, 3H, H-6), 1.39 (s, 6H, 2x7-CH_3). **^{13}C NMR** (126 MHz, $CDCl_3$) δ = 206.3 (C-5), 159.4 ($C_{arom.}$O), 130.1 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 109.4 (C-7), 79.6 (C-2), 74.9 (C-3), 73.4 (Ar-CH_2), 70.2 (C-1), 55.4 (O-CH_3), 47.2 (C-4), 30.9 (C-6), 27.3 (7-CH_3), 27.1 (7-CH_3). **HRMS (ESI)** *m/z*: calcd for $C_{17}H_{24}O_5Na$ $[M + Na]^+$: 331.1516, found: 331.1513.

6.3.8 Fragment Coupling (Fragment E+F)

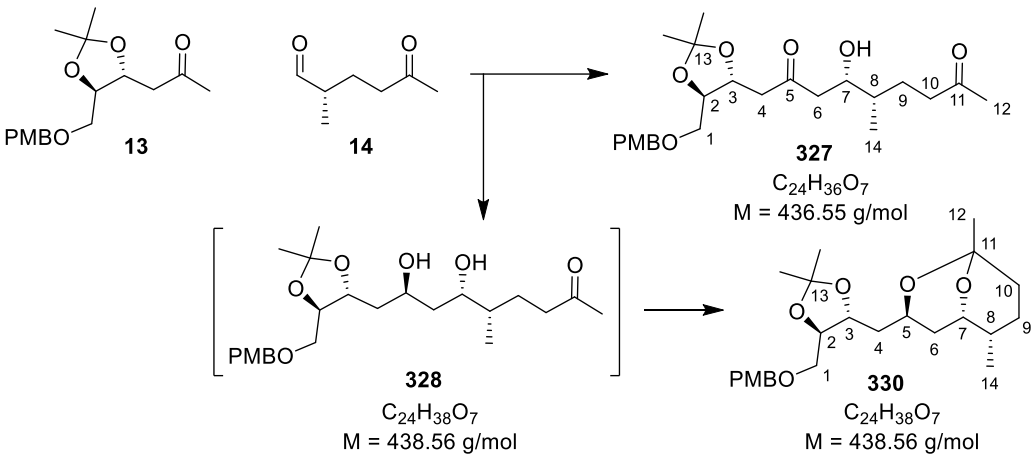
This section reports on the coupling of Fragment E (**13**, synthetic details in **Chapter 6.3.7**) and Fragment F (**14**, synthesized by *Stefanie Spindler*).[167] Furthermore, an astonishing cascade reaction to double ring system **330** was observed.



***Scheme 6.3.8-1*:** Coupling of Fragment E+F (**13**+**14**) and cascade reaction to **330**.

Synthesis of Compounds 327 and 330

(4*S*,5*S*)-4-Hydroxy-1-((4*R*,5*R*)-5-(((4-methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-5-methylnonane-2,8-dione (327) and (3*S*,5*S*,6*S*)-3-(((4*R*,5*R*)-5-(((4-methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)methyl)-1,6-dimethyl-2,9-dioxabicyclo[3.3.1]nonane (330)



(+)-[Ipc]$_2$BCl (80.1 mg, 250 µmol, 1.10 equiv) was dissolved in DCM (1 mL). After cooling to −78 °C, DIPEA (77.2 µL, 454 µmol, 2.00 equiv) and ketone **13** (70.0 mg, 227 µmol, 1.00 equiv, stored over 3 Å MS in 2 mL DCM) were added dropwise. The reaction mixture was stirred 2.5 h at −78 °C and aldehyde **14** (58.2 mg, 454 µmol, 2.00 equiv, stored 14 h over 3 Å MS in 2 mL DCM, synthesized by *Stefanie Spindler*)[167] was added dropwise. After stirring 1 h at

−78 °C, the reaction vessel was placed in a deep freezer (−15 °C) overnight. MeOH (2.8 mL), pH 7 buffer (1.6 mL) and H_2O_2 (1.6 mL, 35%) were added to quench the reaction. After stirring 1 h at rt, water (8 mL) was added, the phases were separated, the aqueous layer was extracted with DCM (3x10 mL), the combined organic phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (50-80% EtOAc/CyH) to yield a mixture of the title compound **327** and 1,3-diol **328** as a colorless oil (25.2 mg, ratio = 0.6:1 determined by NMR).

The presence of 1,3-diol **328** was confirmed by NMR analysis of the compound mixture, mass analysis (**HRMS (ESI)** *m/z*: calcd for $C_{24}H_{38}O_7Na$ $[M + Na]^+$: 461.2510, found: 461.2506) and 1.80 mg of this mixture was submitted to a *Mosher* esterification, which in fact led to the formation of two ester functions (see synthesis of compound **329**). Due to reported Ipc-mediated reductions after aldol coupling, an *anti*-configuration has been assumed, which has to be confirmed (see **Chapter 5.3.9**).[216]

With a second flash chromatography (80% EtOAc/hexane) it was possible to separate the mixture to yield compound **327** (7.80 mg, 17.9 µmol, 8% (26% brsm), *dr* > 4:1). After separation, the diol **328** spontaneously formed a double ring system in an intramolecular acetalization and **330** was yielded (12.9 mg, 30.7 µmol, 14% (43% brsm), the new stereogenic centers were not proven or determined so far). The diastereomeric ratio (> 4:1) given for compound **327** has been roughly determined by the ^{1}H NMR analysis (overlapping signals). Notably, only one diastereomer was found for **330**, which led to the assumption that the *dr* could be even higher in favor to the desired *S*-configuration at C-7.

Analytical data for **327**:

$\mathbf{R_f}$ 0.35 (80% EtOAc/hexane). $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ = −8.7° (*c* = 0.92 in CH_2Cl_2). **^{1}H NMR** (500 MHz, $CDCl_3$) δ = 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.49 (d, *J* = 3.0 Hz, 2H, Ar-CH_2), 4.26 – 4.22 (m, 1H, H-3), 3.98 – 3.93 (m, 1H, H-7), 3.85 – 3.80 (m, 1H, H-2), 3.80 (s, 3H, O-CH_3), 3.63 – 3.50 (m, 2H, H-1), 2.79 – 2.68 (m, 2H, H-4), 2.64 – 2.54 (m, 2H, H-6), 2.53 – 2.44 (m, 2H, H-10), 2.15 (s, 3H, H-12), 1.81 – 1.74 (m, 1H, H-9), 1.52 – 1.46 (m, 1H, H-8), 1.42 – 1.40 (m, 1H, H-9'), 1.39 (s, 6H, 2x13-CH_3), 0.88 (d, *J* = 6.7 Hz, 3H, H-12).

^{13}C NMR (126 MHz, $CDCl_3$) δ = 209.7 (C-5), 209.3 (C-11), 159.4 ($C_{arom.}$), 130.0 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 114.0 ($C_{arom.}$H), 109.6 (C-13), 79.4 (C-2), 74.8 (C-3), 73.4 (Ar-CH_2), 70.2 (C-7), 70.1 (C-1), 55.4 (O-CH_3), 47.3 (C-6), 47.1 (C-4), 41.6 (C-10), 37.5 (C-8), 30.1 (C-12), 27.3

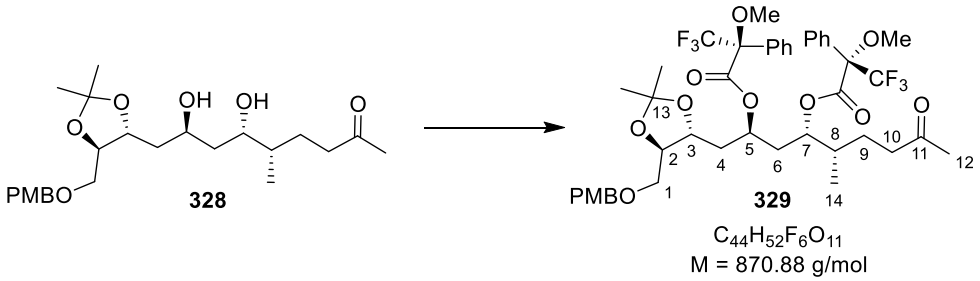
(13-CH_3), 27.1 (13-CH_3), 26.6 (C-9), 14.4 (C-14). **HRMS (ESI)** *m/z*: calcd for $C_{24}H_{36}O_7NH_4$ $[M + NH_4]^+$: 454.2799, found: 454.2796.

Analytical data for **330**:

$\mathbf{R_f}$ 0.4 (40% EtOAc/hexane). $[\alpha]_D^{20}$ = +11.2° (*c* = 0.80 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.26 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 4.56 – 4.47 (m, 3H, Ar-CH_2, H-5), 3.99 – 3.96 (m, 1H, H-2), 3.90 – 3.87 (m, 1H, H-3), 3.83 – 3.81 (m, 1H, H-7), 3.81 (s, 3H, O-CH_3), 3.56 – 3.50 (m, 2H, H-1), 2.09 – 2.04 (m, 1H, H-9), 1.88 – 1.84 (m, 2H, H-4, H-6, H-10), 1.73 – 1.69 (m, 2H, H-8, H-10'), 1.66 – 1.62 (m, 1H, H-4'), 1.51 (ddd, *J* = 13.3, 3.7, 1.1 Hz, 1H, H-6'), 1.41 (s, 3H, 13-CH_3), 1.39 (s, 3H, 13-CH_3), 1.27 (s, 3H, H-12), 1.12 (d, *J* = 7.0 Hz, 3H, H-14).**^{13}C NMR** (176 MHz, $CDCl_3$) δ = 159.4 ($C_{arom.}$), 130.3 ($C_{arom.}$), 129.5 ($C_{arom.}$H), 113.9 ($C_{arom.}$H), 108.9 (C-13), 95.4 (C-11), 80.1 (C-2), 74.5 (C-3), 73.3 (C-7), 73.3 (Ar-CH_2), 70.2 (C-1), 65.1 (C-5), 55.4 (O-CH_3), 41.0 (C-4), 35.2 (C-6), 31.5 (C-8), 30.8 (C-10), 30.1 (C-12), 27.4 (13-CH_3), 27.2 (13-CH_3), 26.2 (C-9), 20.6 (C-14). **HRMS (ESI)** *m/z*: calcd for $C_{24}H_{36}O_6H$ $[M + H]^+$: 421.2585, found: 421.2584.

Synthesis of Compound 329

(2*S*,4*S*,5*S*)-1-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-5-methyl-8-oxononane-2,4-diyl (2*S*,2'*S*)-bis(3,3,3-trifluoro-2-methoxy-2-phenylpropanoate)



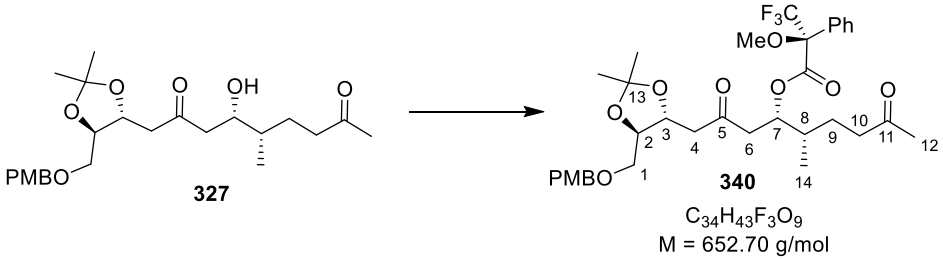
1.80 mg of the compound mixture (**327** and **328**) from the reaction above was used for *Mosher* esterification. The mixture was dissolved in pyridine (120 µL) and (−)-(*R*)-MTPA-Cl (4.63 µL, 24.7 µmol, 6.00 equiv) was added at 0 °C. The reaction mixture was stirred 2 h at rt followed by the addition of sat. aq. NH_4Cl solution (1 mL) and DCM (2 mL). After separation of the organic layer, the aqueous phase was extracted with DCM (3x2 mL), the combined organic

phases were dried over Na_2SO_4 and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (40% EtOAc/hexane) to yield the title compound **329** as a colorless oil (2.10 mg, 2.41 µmol, 85%, based on the 0.6:1 ratio of **327** and **328** in the starting material).

$\mathbf{R_f}$ 0.44 (40% EtOAc/hexane). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = −2.4° (*c* = 0.42 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.55 – 7.52 (m, 4H, $H_{arom., MTPA}$), 7.44 – 7.38 (m, 6H, $H_{arom., MTPA}$), 7.22 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.86 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.23 – 5.19 (m, 1H, H-5), 5.02 – 5.00 (m, 1H, H-7), 4.47 (s, 2H, Ar-CH_2), 3.79 (s, 3H, O-CH_3), 3.81 – 3.78 (m, 1H, H-3), 3.77 – 3.74 (m, 1H, H-2), 3.55 (s, 3H, O-$CH_{3, MTPA}$), 3.51 (dd, *J* = 10.2, 5.3 Hz, 1H, H-1), 3.47 (s, 3H, O-$CH_{3, MTPA}$), 3.42 (dd, *J* = 10.2, 4.6 Hz, 1H, H-1'), 2.40 – 2.28 (m, 2H, H-10), 2.05 (s, 3H, H-12), 1.97 – 1.93 (m, 1H, H-6), 1.88 (dd, *J* = 5.8, 5.8 Hz, 2H, H-4), 1.86 – 1.82 (m, 1H, H-6'), 1.82 – 1.79 (m, 1H, H-8), 1.42 – 1.37 (m, 2H, H-9), 1.30 (s, 3H, 13-CH_3), 1.28 (s, 3H, 13-CH_3), 0.70 (d, *J* = 6.9 Hz, 3H, H-14). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 208.2 (C-11), 166.5 ($C{=}O_{MTPA}$), 166.3 ($C{=}O_{MTPA}$), 159.4 ($C_{arom.}$), 132.2 ($C_{arom.,MTPA}$), 131.7 ($C_{arom.,MTPA}$), 130.1 ($C_{arom.}$), 129.9 ($C_{arom.}H_{MTPA}$), 129.8 ($C_{arom.}H_{MTPA}$), 129.5 ($C_{arom.}H$), 128.8 ($C_{arom.}H_{MTPA}$), 128.7 ($C_{arom.}H_{MTPA}$), 128.0 ($C_{arom.,MTPA}$), 127.5 ($C_{arom.,MTPA}$), 114.0 ($C_{arom.}H$), 109.5 (C-13), 79.7 (C-2), 76.0 (C-7), 74.9 (C-3), 73.4 (Ar-CH_2), 71.8 (C-5), 70.0 (C-1), 55.7 (O-$CH_{3, MTPA}$), 55.4 (O-$CH_{3, MTPA}$), 55.4 (O-CH_3), 40.9 (C-10), 37.2 (C-4), 35.4 (C-8), 32.9 (C-6), 30.0 (C-12), 27.2 (13-CH_3), 27.0 (13-CH_3), 26.1 (C-9), 14.1 (C-14). **HRMS (ESI)** *m/z*: calcd for $C_{44}H_{52}F_6O_{11}NH_4$ $[M + NH_4]^+$: 888.3752, found: 888.3751.

Synthesis of Compound 340

(4*S*,5*S*)-1-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-5-methyl-2,8-dioxononan-4-yl (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate

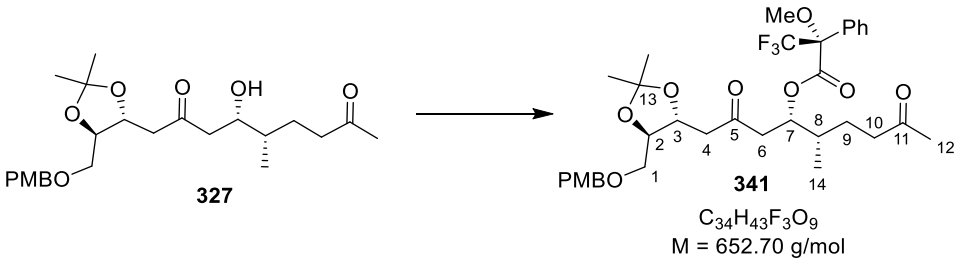


Alcohol **327** (2.90 mg, 6.64 µmol, 1.00 equiv) was dissolved in pyridine (200 µL) and (−)-(*R*)-MTPA-Cl (7.46 µL, 39.9 µmol, 6.00 equiv) was added at 0 °C. The reaction mixture

was stirred 2 h at rt followed by the addition of sat. aq. NH_4Cl solution (2 mL) and DCM (2 mL). After separation of the organic layer, the aqueous phase was extracted with DCM (3x2 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (50% EtOAc/hexane) to yield the title compound **340** as a colorless oil (4.30 mg, 6.59 µmol, 99%) $\mathbf{R_f}$ 0.46 (50% EtOAc/hexane). $[\boldsymbol{\alpha}]_D^{\mathbf{20}}$ = −4.7° (*c* = 0.86 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.58 – 7.51 (m, 2H, $H_{arom., MTPA}$), 7.44 – 7.36 (m, 3H, $H_{arom., MTPA}$), 7.24 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.56 – 5.53 (m, 1H, H-7), 4.49 (d, *J* = 5.1 Hz, 2H, Ar-CH_2), 4.14 – 4.12 (m, 1H, H-3), 3.81 – 3.80 (m, 1H, H-2), 3.80 (s, 3H, O-CH_3), 3.59 (dd, *J* = 10.0, 5.3 Hz, 1H, H-1), 3.51 – 3.46 (m, 3H, H-1', O-$CH_{3, MTPA}$), 2.84 (dd, *J* = 17.5, 7.7 Hz, 1H, H-6), 2.65 (dd, *J* = 17.5, 4.7 Hz, 1H, H-6'), 2.62 (d, *J* = 6.4 Hz, 1H, H-4), 2.49 (t, *J* = 7.6 Hz, 2H, H-10), 2.13 (s, 3H, H-12), 1.79 – 1.76 (m, 1H, H-8), 1.66 – 1.61 (m, 1H, H-9), 1.44 – 1.39 (m, 1H, H-6'), 1.37 (s, 3H, 13-CH_3), 1.35 (s, 3H, 13-CH_3), 0.89 (d, *J* = 6.9 Hz, 3H, H-14). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 208.3 (C-11), 204.5 (C-5), 166.1 (C=O_{MTPA}), 159.4 ($C_{arom.}$), 132.1 ($C_{arom.,MTPA}$), 130.0 ($C_{arom.}$), 129.7 ($C_{arom.}H_{MTPA}$), 129.5 ($C_{arom.}H$), 128.8 ($C_{arom.}H_{MTPA}$), 128.6 ($C_{arom.}H_{MTPA}$), 127.8 ($C_{arom.,MTPA}$), 114.0 ($C_{arom.}H$), 109.5 (C-13), 79.5 (C-2), 74.8 (C-3), 74.7 (C-7), 73.4 (Ar-CH_2), 70.0 (C-1), 55.5 (O-$CH_{3, MTPA}$), 55.4 (O-CH_3), 46.6 (C-4), 44.4 (C-6), 41.2 (C-10), 35.8 (C-8), 30.1 (C-12), 27.3 (13-CH_3), 27.0 (13-CH_3), 26.5 (C-9), 14.6 (C-14). **HRMS (ESI)** *m/z*: calcd for $C_{34}H_{43}F_3O_9NH_4$ $[M + NH_4]^+$: 670.3197, found: 670.3199.

Synthesis of Compound 341

(4*S*,5*S*)-1-((4*R*,5*R*)-5-(((4-Methoxybenzyl)oxy)methyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-5-methyl-2,8-dioxononan-4-yl (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate



Alcohol **327** (1.70 mg, 3.89 µmol, 1.00 equiv) was dissolved in pyridine (200 µL) and (+)-(*S*)-MTPA-Cl (4.37 µL, 23.4 µmol, 6.00 equiv) was added at 0 °C. The reaction mixture

was stirred 2 h at rt followed by the addition of sat. aq. NH_4Cl solution (2 mL) and DCM (2 mL). After separation of the organic layer, the aqueous phase was extracted with DCM (3x2 mL), the combined organic phases were dried over $MgSO_4$ and the solvent was removed under reduced pressure. Purification of the crude product was achieved by flash chromatography (50% EtOAc/hexane) to yield the title compound **341** as a colorless oil (2.10 mg, 3.22 µmol, 83%). $\mathbf{R_f}$ 0.40 (50% EtOAc/hexane). $[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +19.0° (*c* = 0.42 in CH_2Cl_2). **^{1}H NMR** (700 MHz, $CDCl_3$) δ = 7.58 – 7.49 (m, 2H, $H_{arom., MTPA}$), 7.42 – 7.37 (m, 3H, $H_{arom., MTPA}$), 7.23 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 6.87 (d, *J* = 8.7 Hz, 2H, $H_{arom.}$), 5.55 – 5.53 (m, 1H, H-7), 4.48 (d, *J* = 3.7 Hz, 2H, Ar-CH_2), 4.21 – 4.15 (m, 1H, H-3), 3.86 – 3.81 (m, 1H, H-2), 3.80 (s, 3H, O-CH_3), 3.60 (dd, *J* = 10.0, 5.3 Hz, 1H, H-1), 3.51 – 3.45 (m, 3H, H-1', O-$CH_{3, MTPA}$), 2.86 (dd, *J* = 17.6, 8.4 Hz, 1H, H-6), 2.69 – 2.66 (m, 3H, H-4, H-6'), 2.35 (t, *J* = 7.6 Hz, 2H, H-10), 2.08 (s, 3H, H-12), 1.76 – 1.69 (m, 1H, H-8), 1.49 – 1.44 (m, 1H, H-9), 1.37 (s, 3H, 13-CH_3), 1.35 (s, 3H, 13-CH_3), 1.33 – 1.28 (m, 1H, H-9'), 0.83 (d, *J* = 6.9 Hz, 3H, H-14). **^{13}C NMR** (176 MHz, $CDCl_3$) δ = 208.3 (C-11), 204.7 (C-5), 166.1 ($C{=}O_{MTPA}$), 159.5 ($C_{arom.}$), 132.4 ($C_{arom.,MTPA}$), 130.0 ($C_{arom.}$), 129.8 ($C_{arom.}H_{MTPA}$), 129.5 ($C_{arom.}H$), 128.8 ($C_{arom.}H_{MTPA}$), 127.8 ($C_{arom.}H_{MTPA}$), 114.0 ($C_{arom.}H$), 109.5 (C-13), 79.4 (C-2), 74.8 (C-3), 74.5 (C-7), 73.4 (Ar-CH_2), 70.1 (C-1), 55.7 (O-$CH_{3, MTPA}$), 55.4 (O-CH_3), 46.6 (C-4), 44.1 (C-6), 41.3 (C-10), 35.8 (C-8), 30.0 (C-12), 27.3 (13-CH_3), 27.0 (13-CH_3), 26.0 (C-9), 14.7 (C-14). **HRMS (ESI)** *m/z*: calcd for $C_{34}H_{43}F_3O_9NH_4$ $[M + NH_4]^+$: 670.3197, found: 670.3193.

Mosher Ester Analysis for Compound 327

Determination of the absolute configuration was accomplished using the *Mosher* ester analysis. Two *Mosher* ester derivatives **340** and **341** have been synthesized and the respective NMR data have been compared as shown in **Table 6.3.8-1**. The resulting difference between the ^{1}H NMR data of the (*S*)- and (*R*)-MTPA ester led to the determination of the absolute configuration as shown in **Figure 6.3.8-1**. For a detailed background, see **Chapter 5.3.3** or the corresponding literature.[203,204]

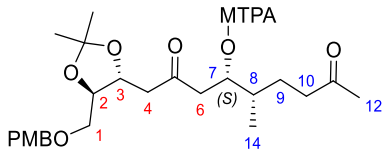


Table 6.3.8-1: Significant ^{1}H NMR signals of **340** and **341** and their use in the *Mosher* ester analysis.

position	δ_H [ppm] (**340**) (*S*)	δ_H [ppm] (**341**) (*R*)	$\Delta\delta^{S-R}$
3	4.13	4.18	-0.05
4	2.64	2.67	-0.03
6’	2.65	2.68	-0.03
6	2.84	2.86	-0.02
2	3.80	3.82	-0.02
1	3.58	3.60	-0.02
7	5.55	5.54	0.01
12	2.13	2.08	0.05
8	1.77	1.72	0.05
14	0.89	0.83	0.06
10	2.44	2.35	0.09
9	1.46	1.30	0.16
9’	1.63	1.46	0.17

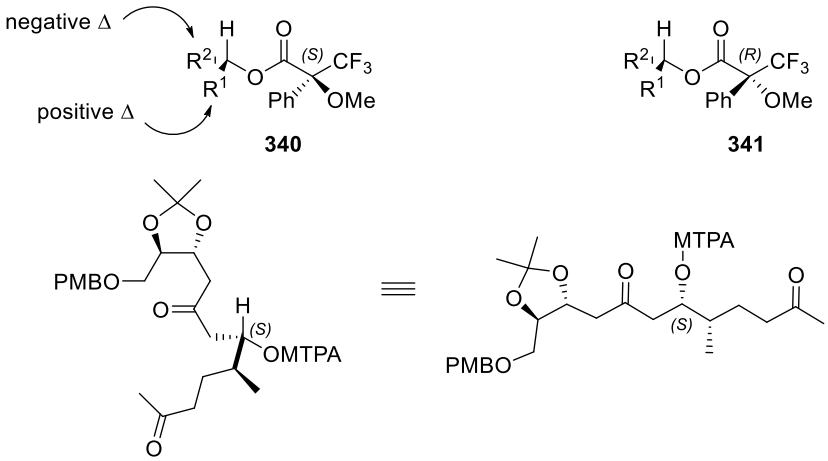


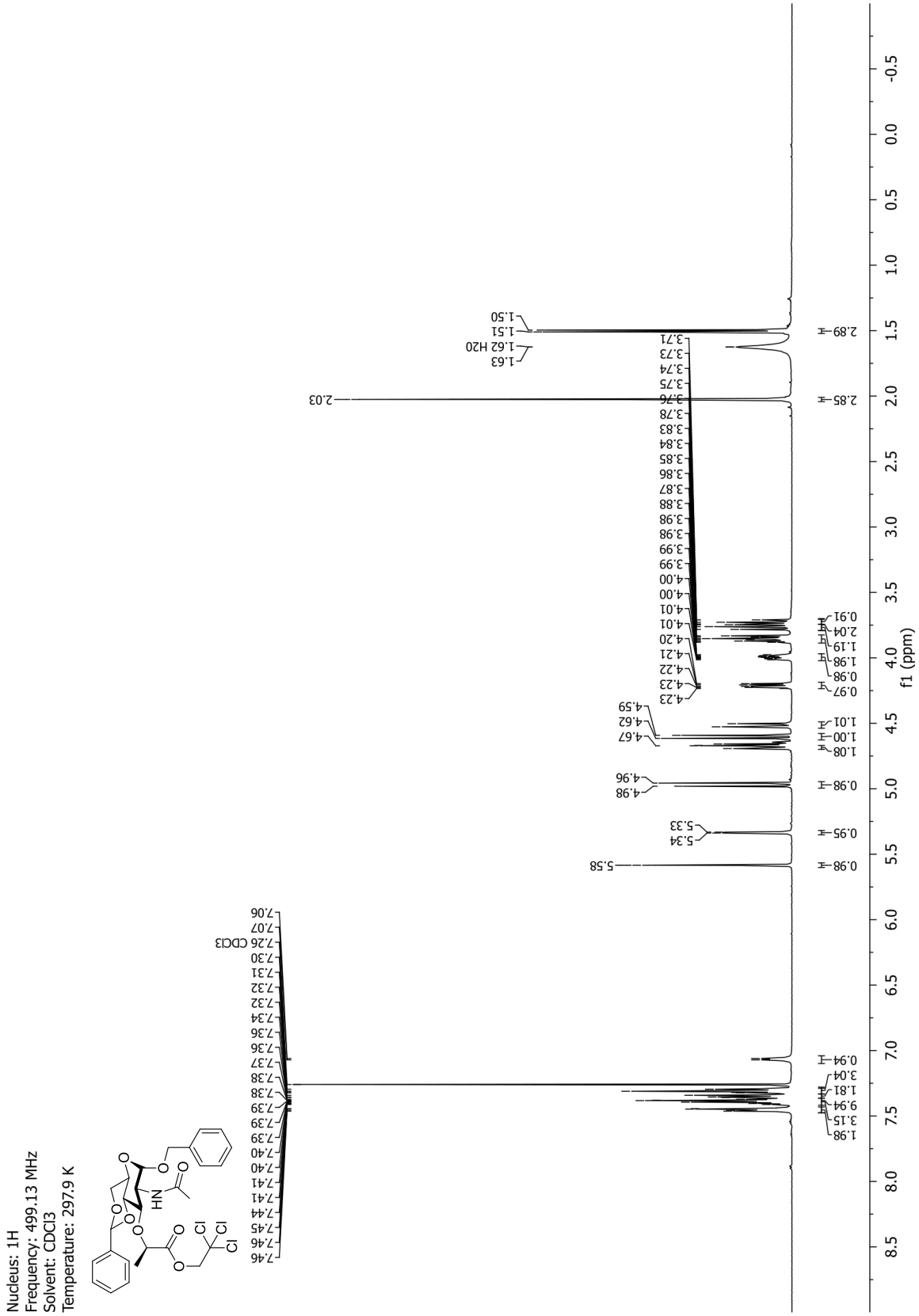
Figure 6.3.8-1: Proposed configuration under the use of the *Mosher* ester analysis.

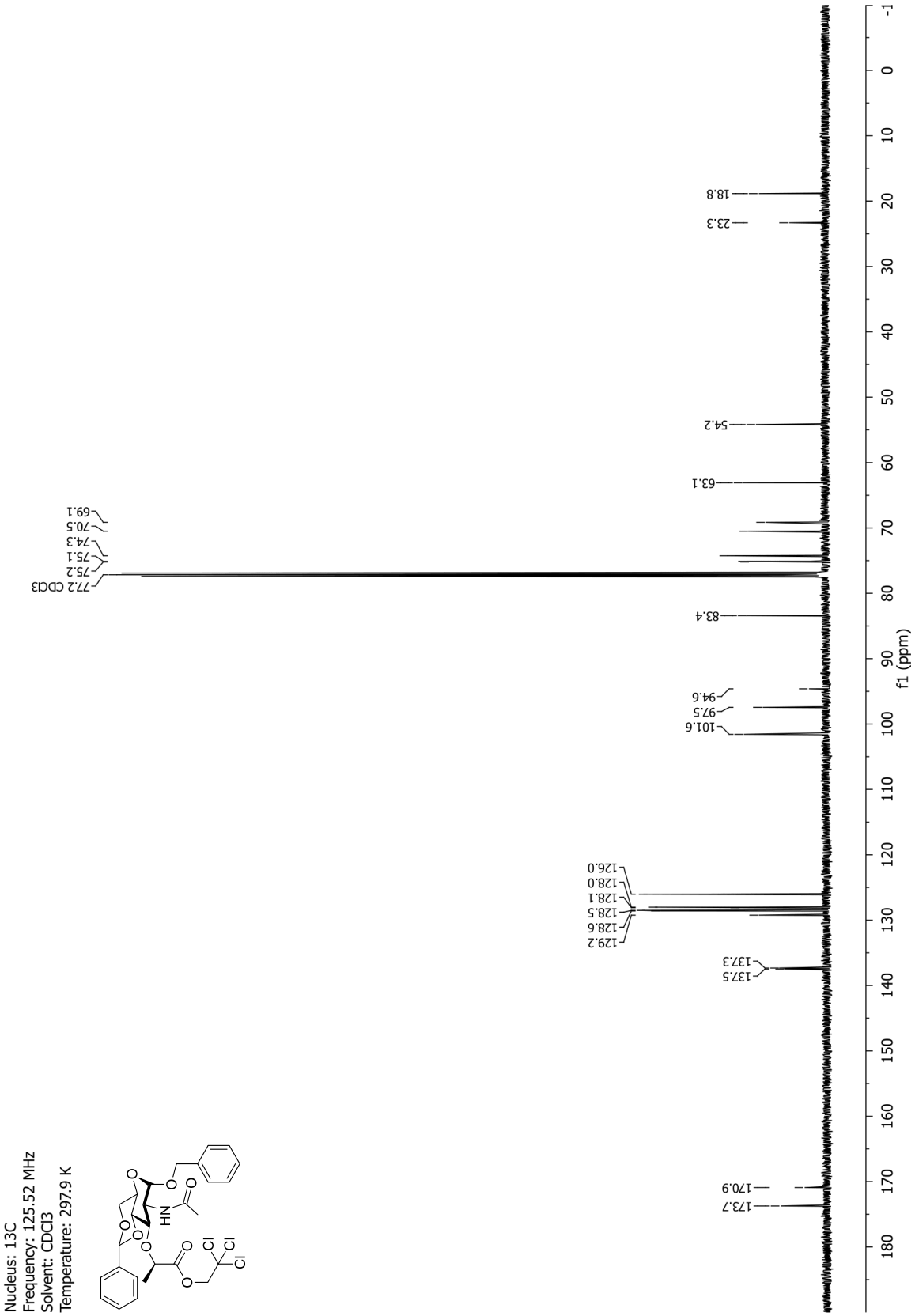
7 Copies of NMR Spectra

7.1 Copies of NMR Spectra (Synthesis of Farnesyl Lipid I/II Analogs)

– NMR Spectra of the synthesized compounds starting at the next page –

NMR-Spectra for Compound 72

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Solvent: CDCl3
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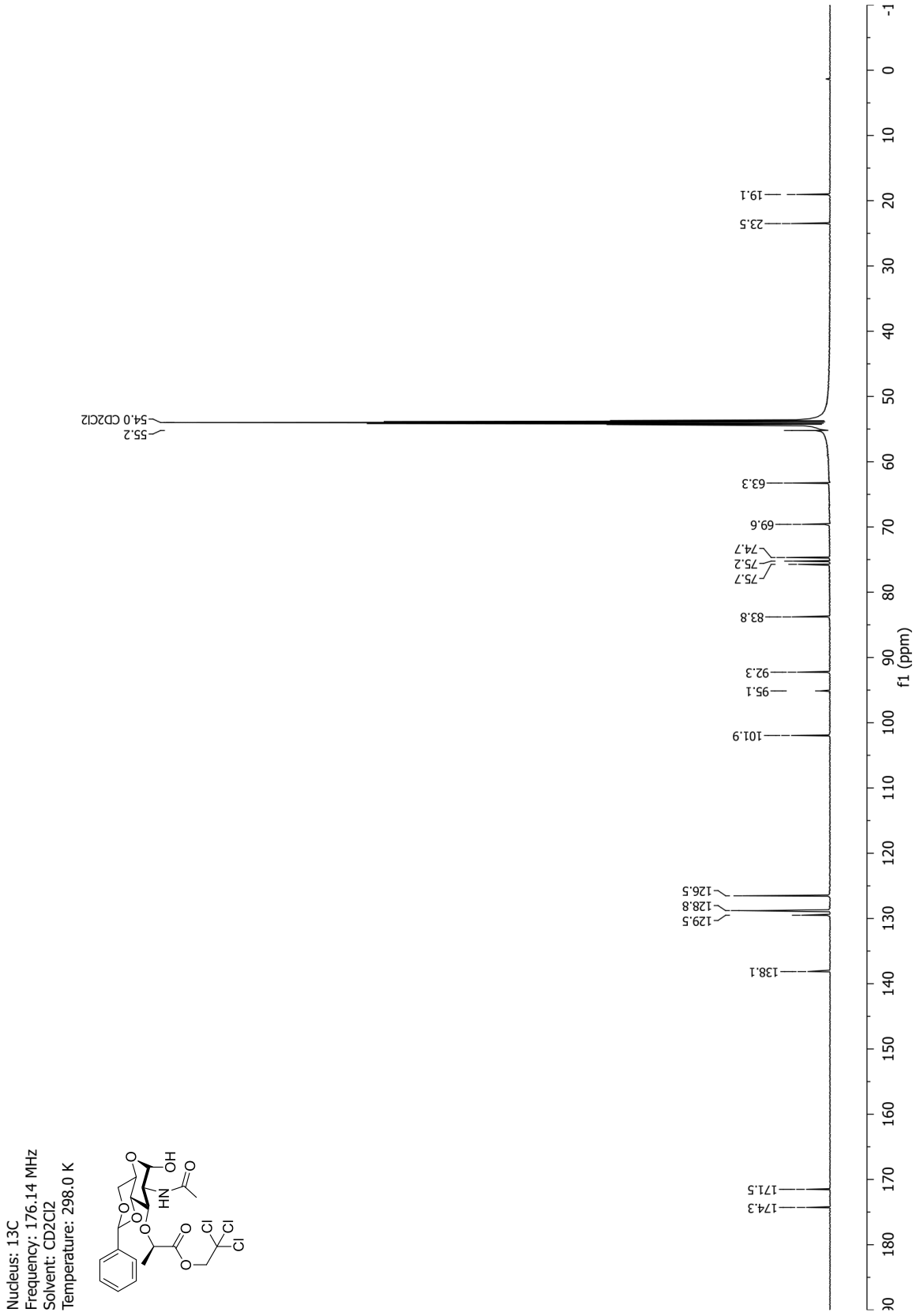
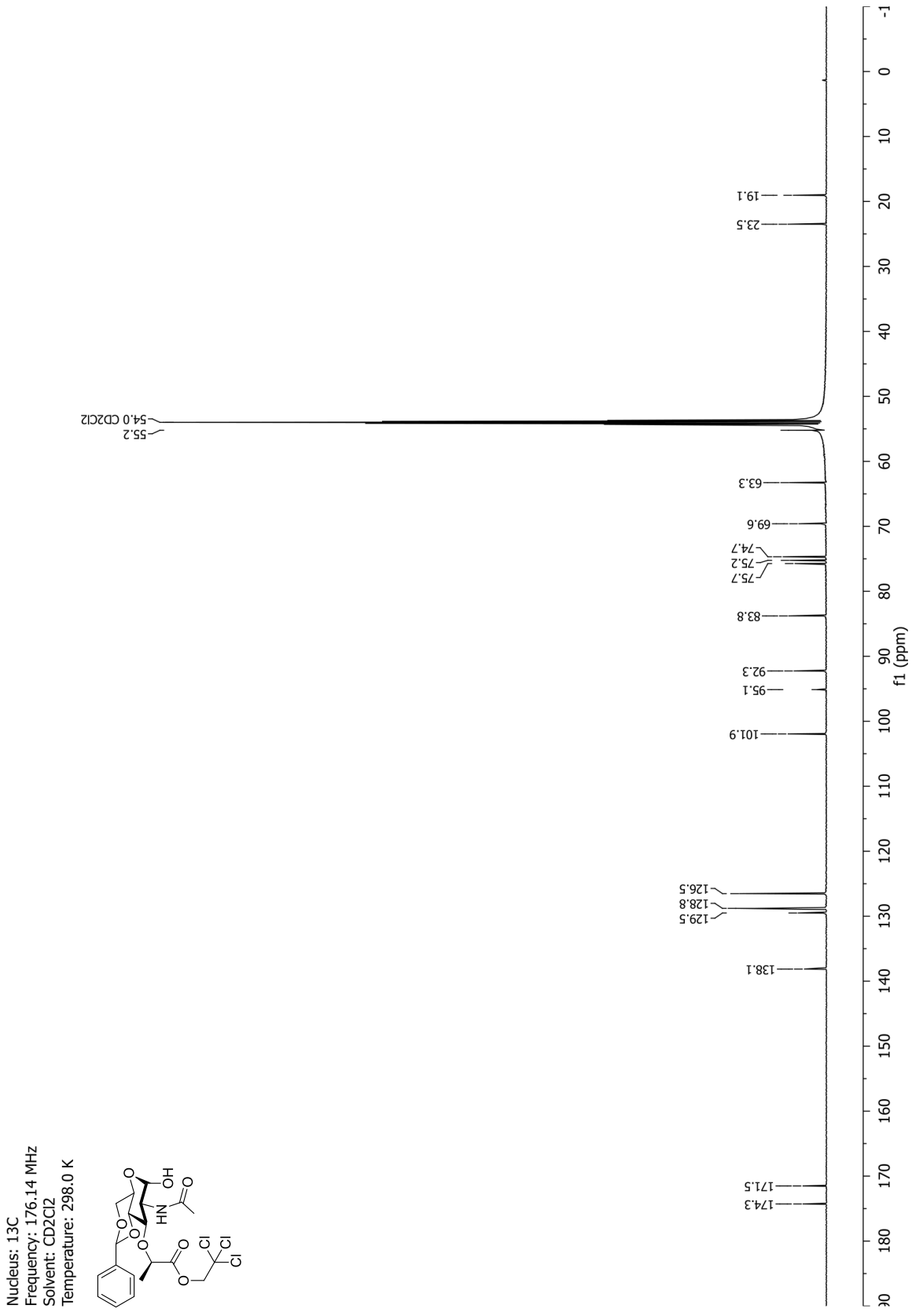
170.9
173.7
137.3
137.5
129.2
128.6
128.5
128.1
128.0
126.0
101.6
97.5
94.6
83.4
77.2 CDCl3
75.2
75.1
74.3
70.5
69.1
63.1
54.2
23.3
18.8

NMR-Spectra for Compound 73

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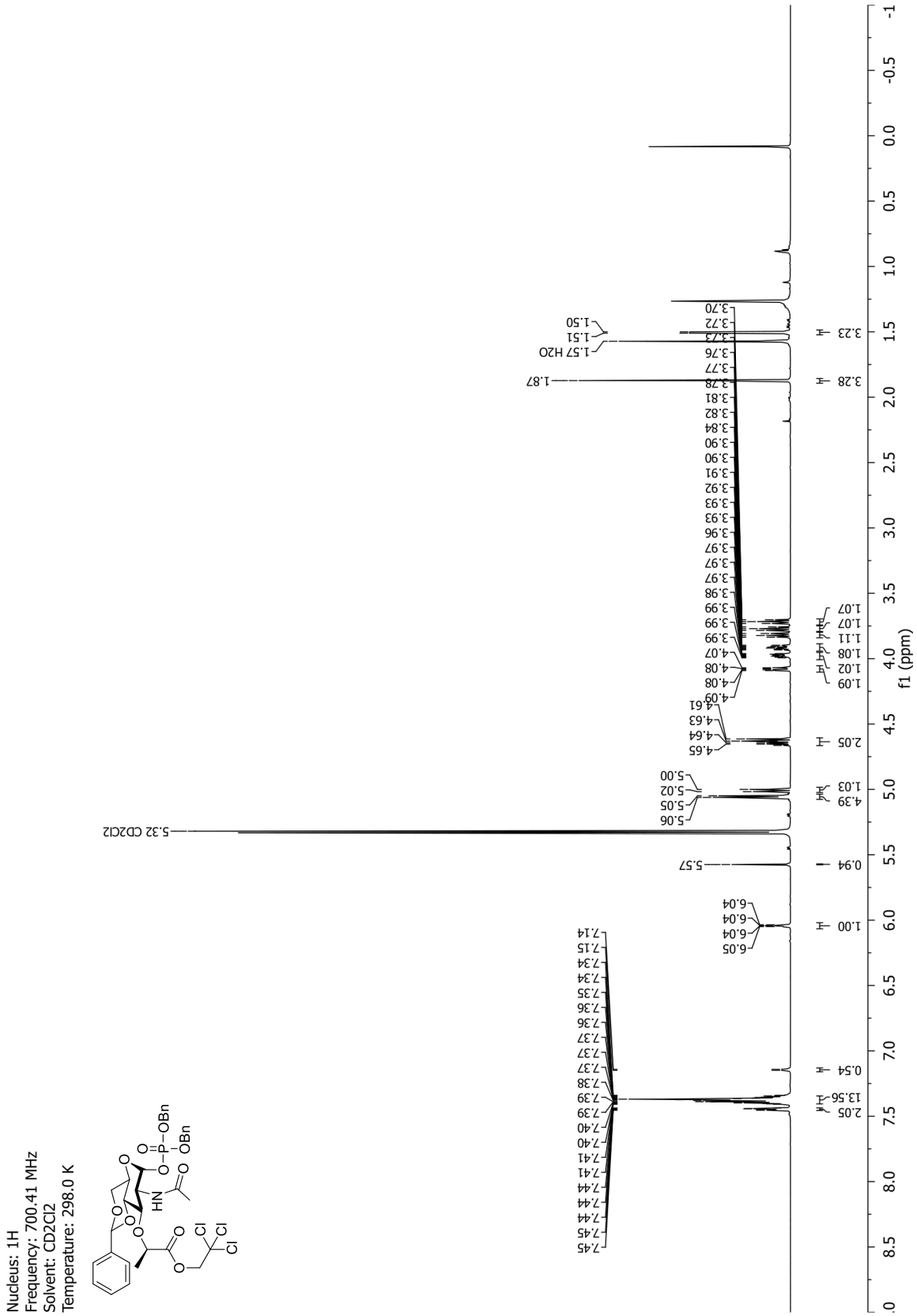
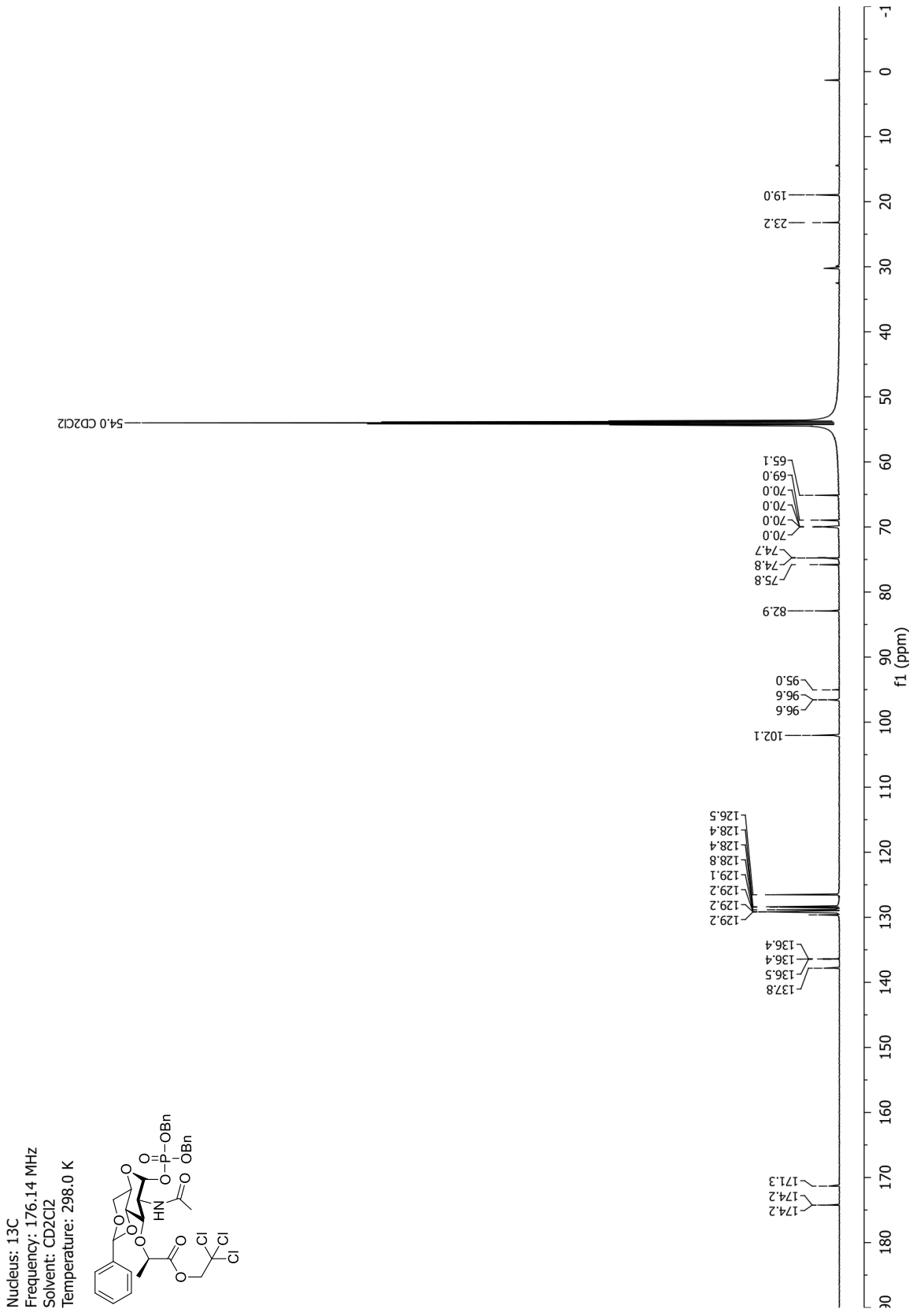
Frequency: 176.14 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
19.1
23.5
54.0 CD2Cl2
55.2
63.3
69.6
74.7
75.2
75.7
83.8
92.3
95.1
101.9
126.5
128.8
129.5
138.1
171.5
174.3
f1 (ppm)

NMR-Spectra for Compound 74




Nucleus: 13C
Frequency: 176.14 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
f1 (ppm)
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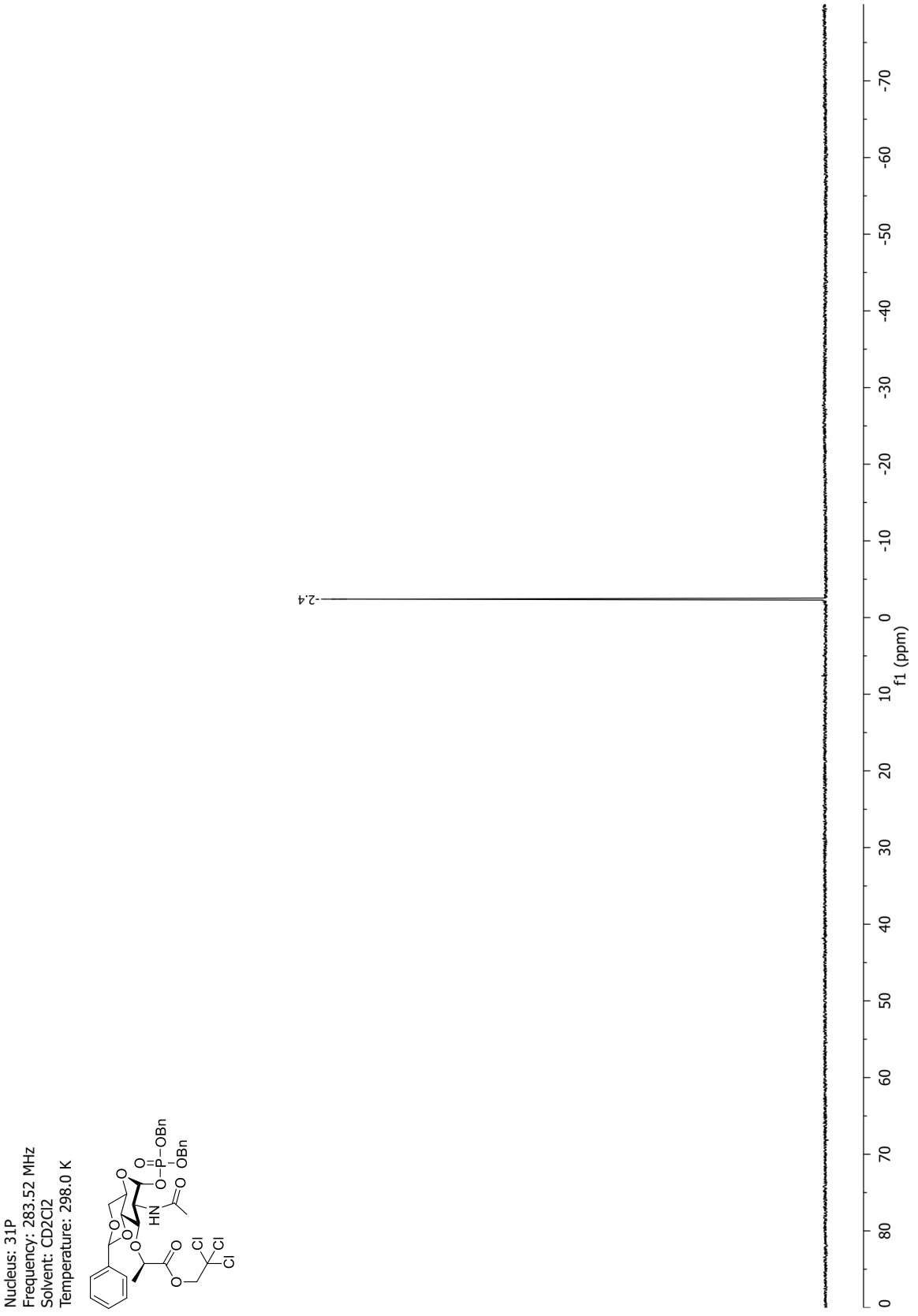
19.0
23.2
65.1
69.0
70.0
70.0
70.0
70.0
74.7
74.8
75.8
82.9
95.0
96.6
96.6
102.1
126.5
128.4
128.4
128.8
129.1
129.2
129.2
129.2
136.4
136.4
136.5
137.8
171.3
174.2
174.2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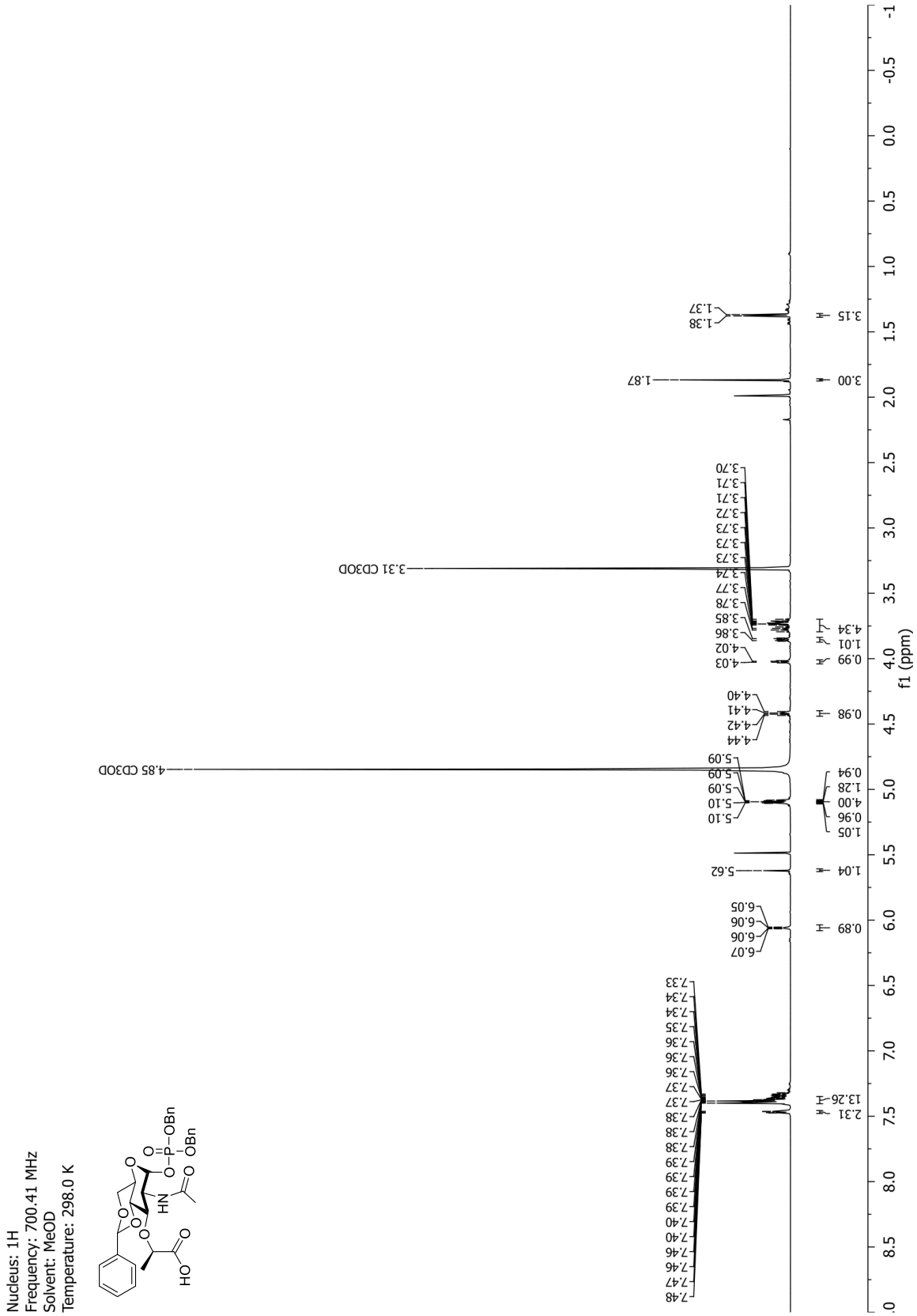
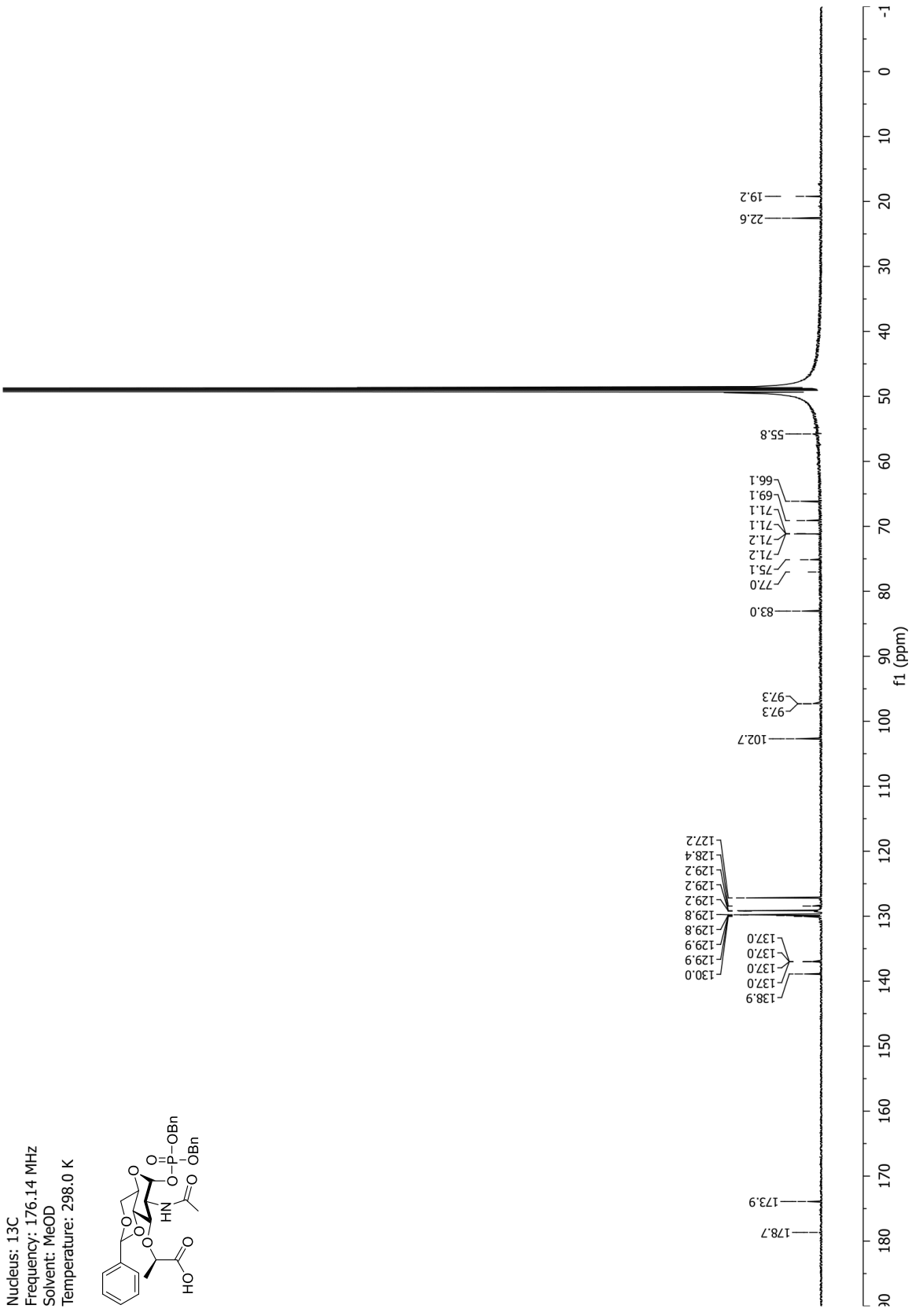
Frequency: 283.52 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
2.4
f1 (ppm)

NMR-Spectra for Compound 3

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Frequency: 176.14 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)
19.2
22.6
55.8
66.1
69.1
71.1
71.1
71.2
71.2
75.1
77.0
83.0
97.3
97.3
102.7
127.2
128.4
129.2
129.2
129.2
129.8
129.8
129.9
129.9
130.0
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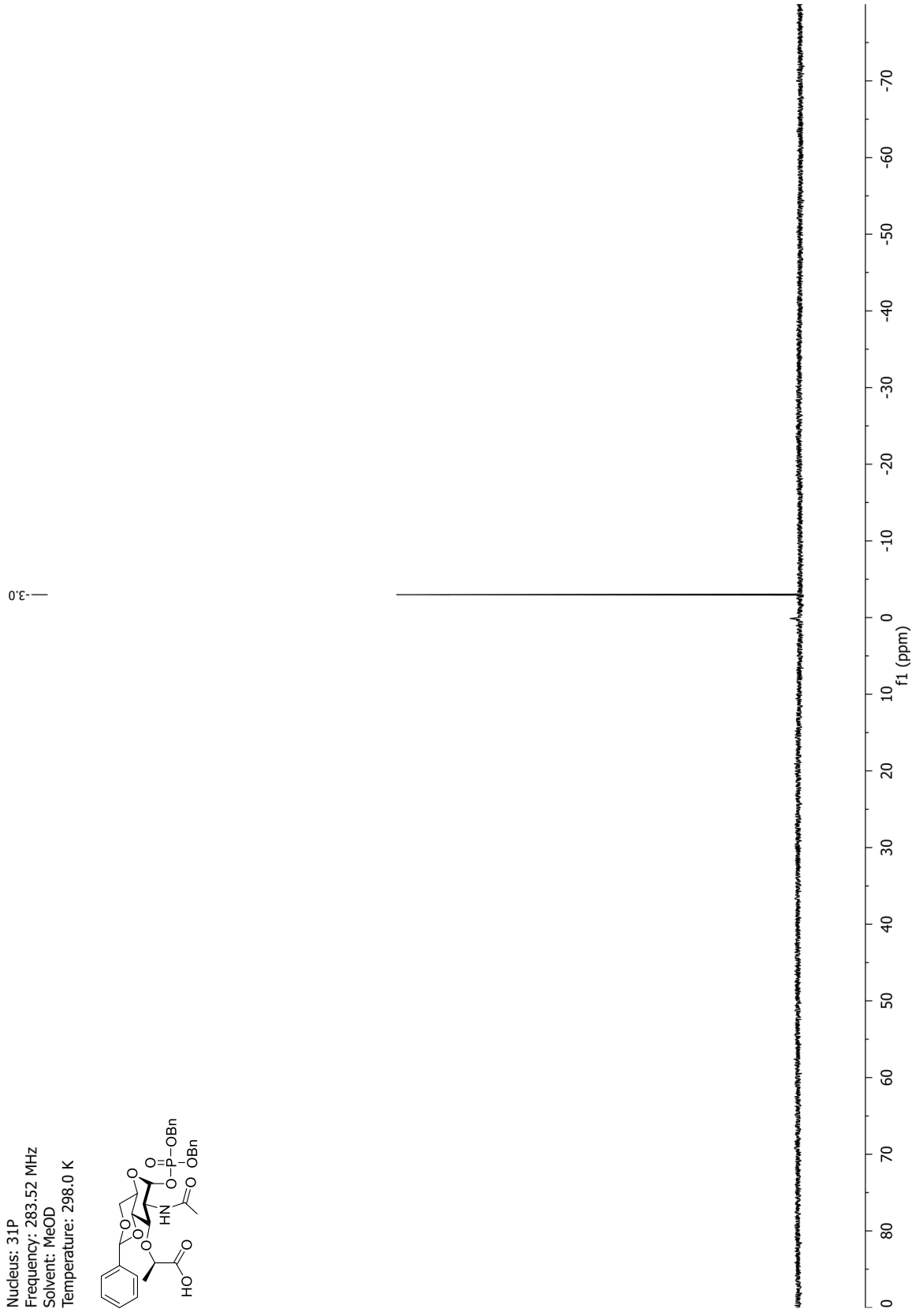
138.9
173.9
178.7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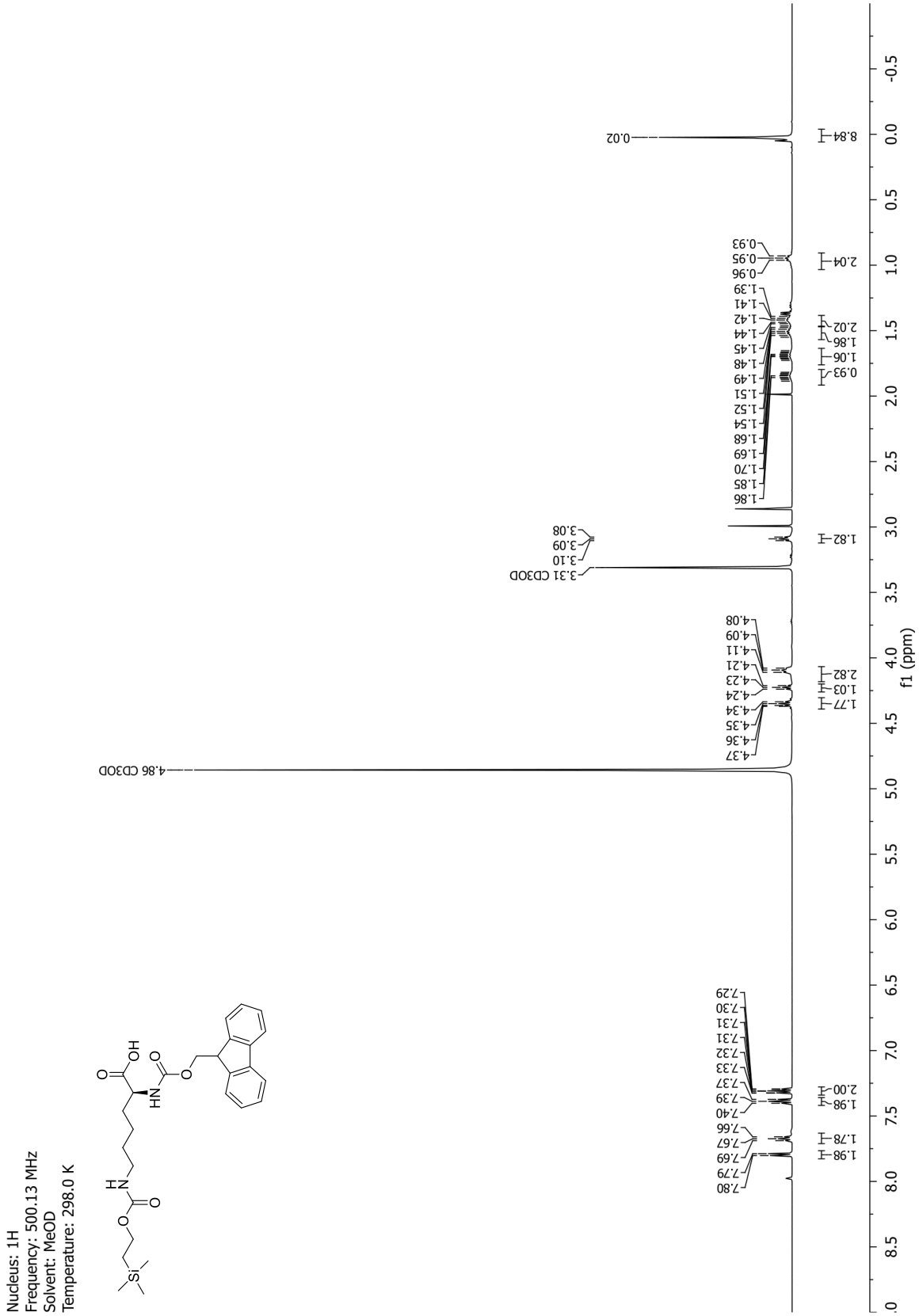
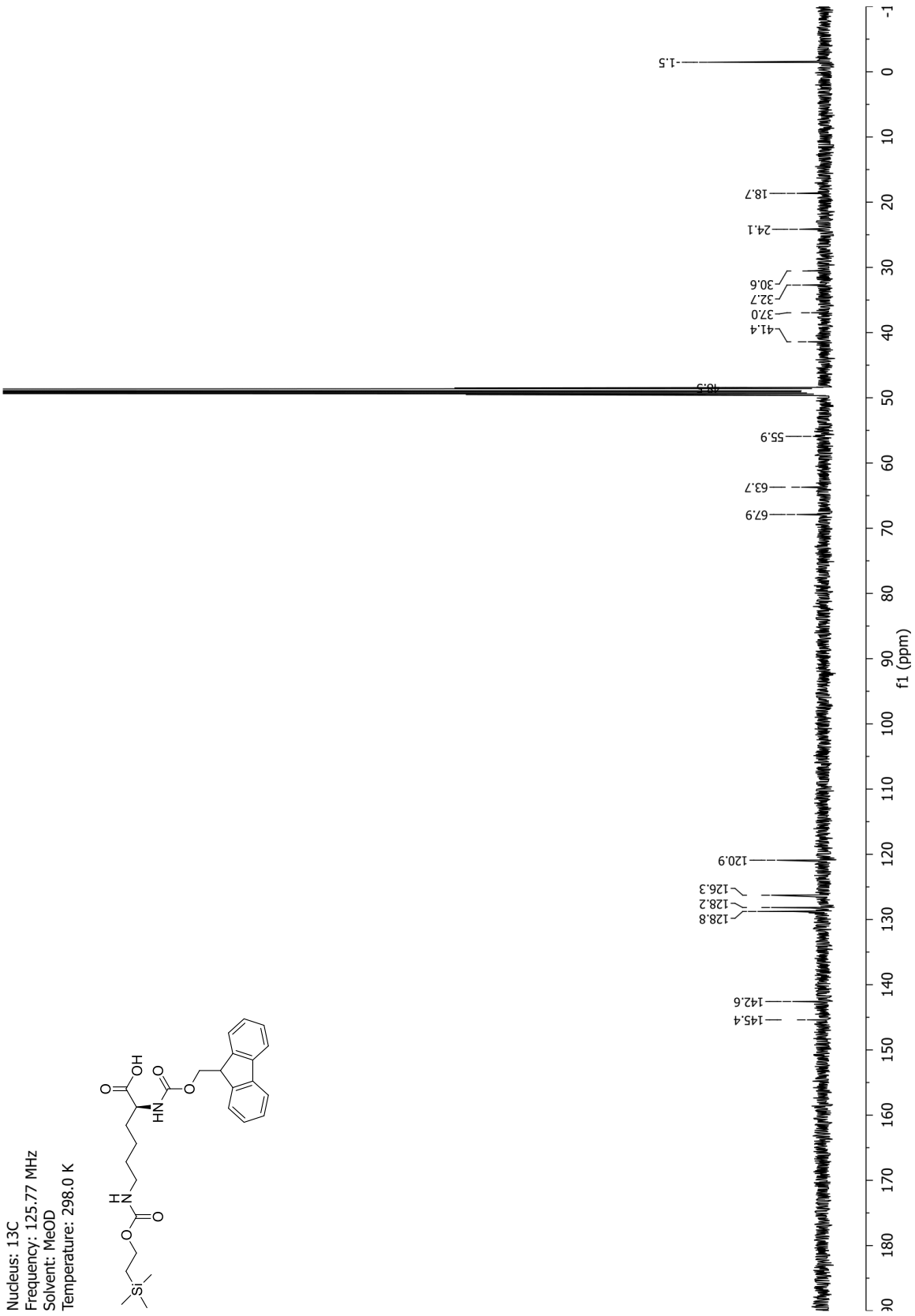
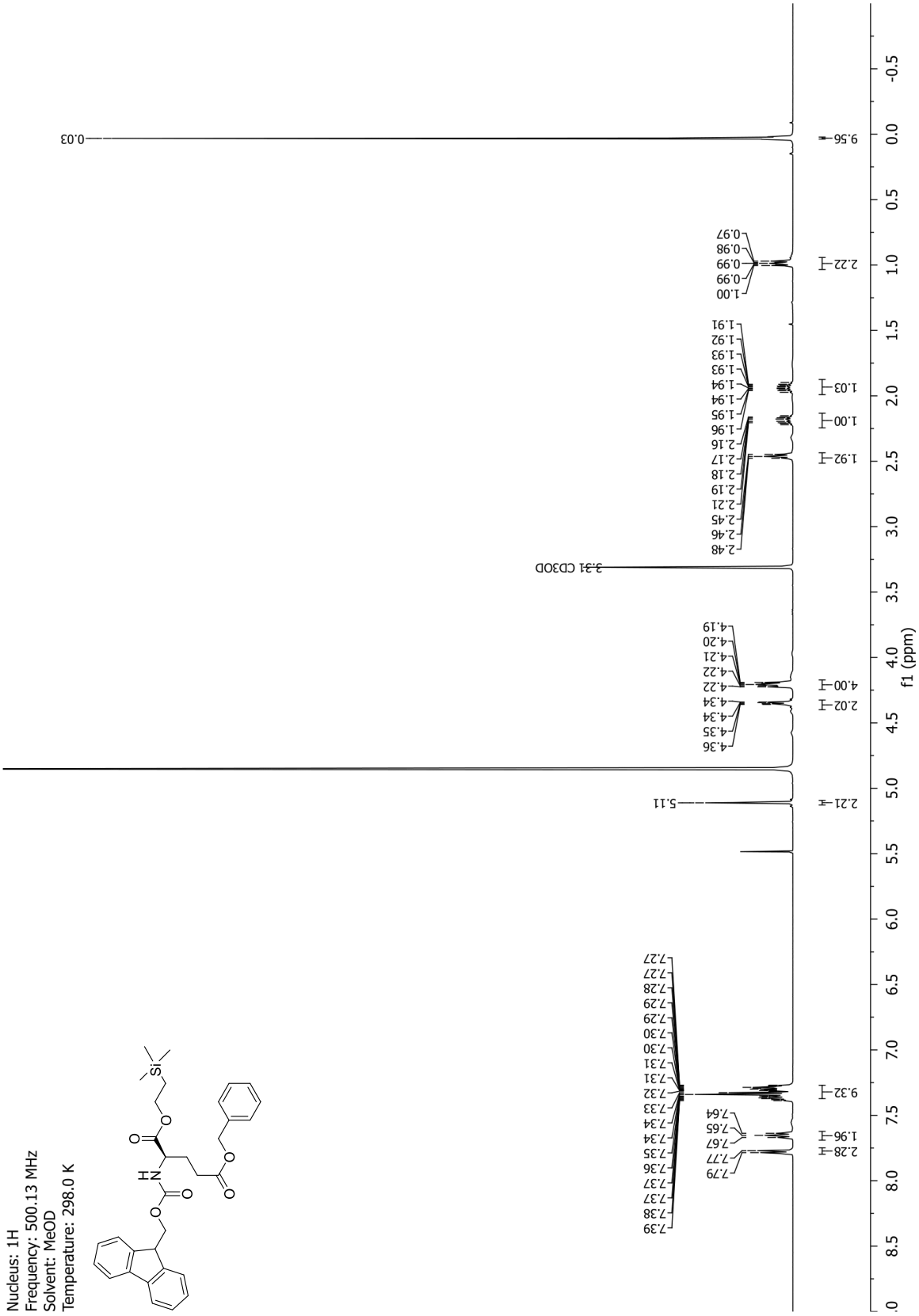
Frequency: 283.52 MHz
Solvent: MeOD
Temperature: 298.0 K
-3.0
f1 (ppm)

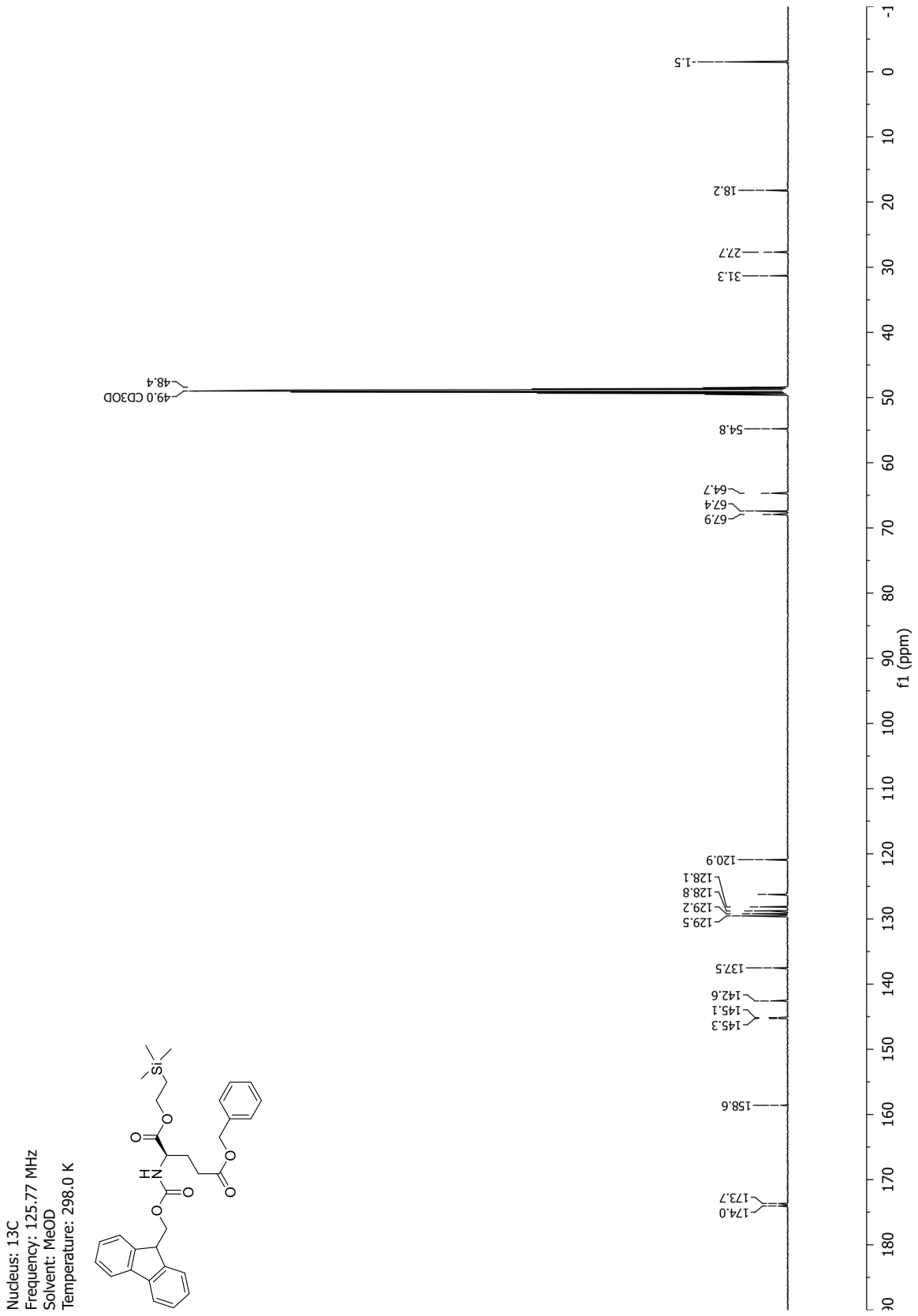
NMR-Spectra for Compound 83



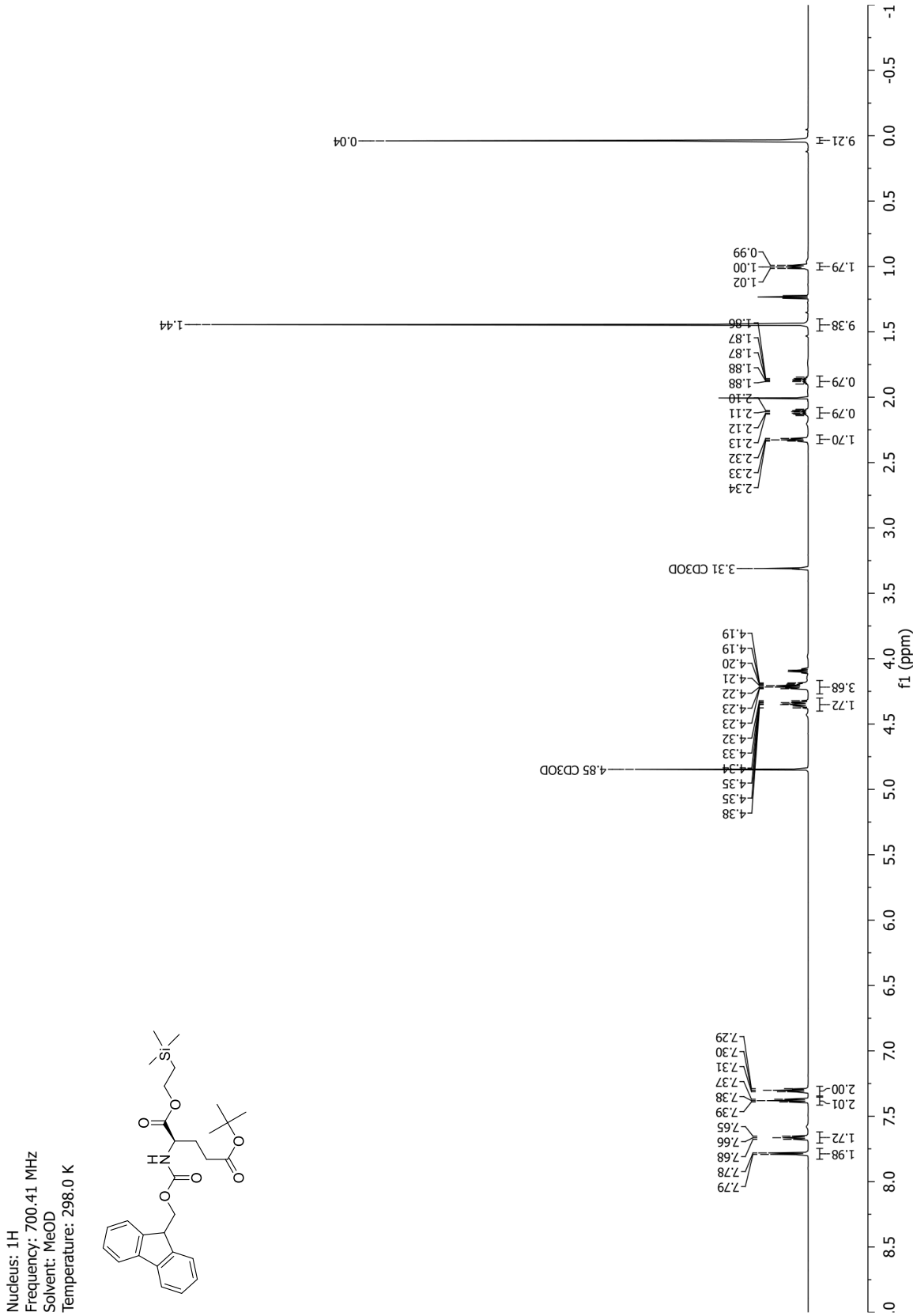

Nucleus: 13C
Frequency: 125.77 MHz
Solvent: MeOD
Temperature: 298.0 K
-1.5
18.7
24.1
30.6
32.7
37.0
41.4
48.5
55.9
63.7
67.9
120.9
126.3
128.2
128.8
142.6
145.4
f1 (ppm)

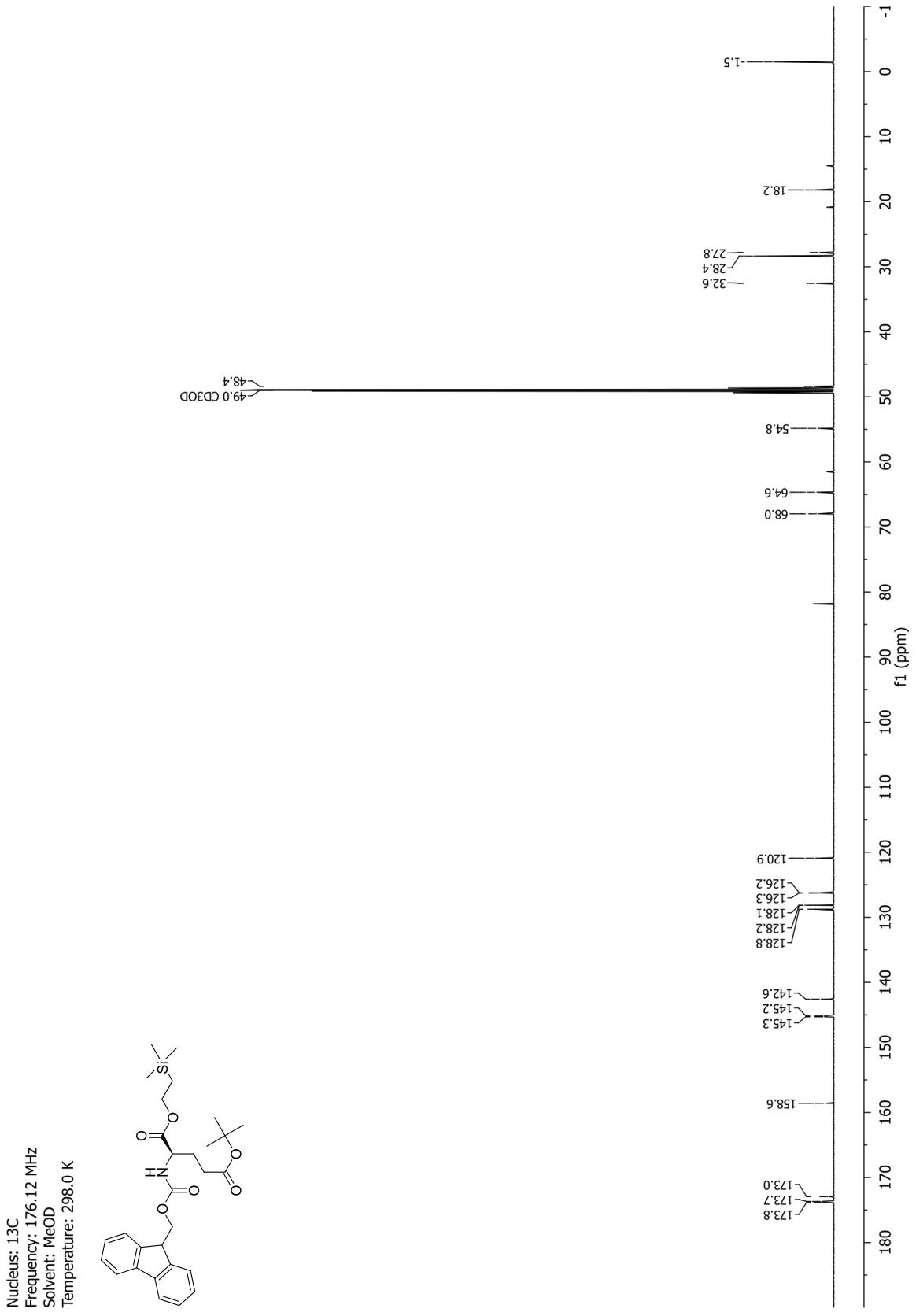
NMR-Spectra for Compound 93




Nucleus: 13C
Frequency: 125.77 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)
-1.5
18.2
27.7
31.3
48.4
49.0 CD3OD
54.8
64.7
67.4
67.9
120.9
128.1
128.8
129.2
129.5
137.5
142.6
145.1
145.3
158.6
173.7
174.0

NMR-Spectra for Compound 95

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Frequency: 176.12 MHz
Solvent: MeOD
Temperature: 298.0 K
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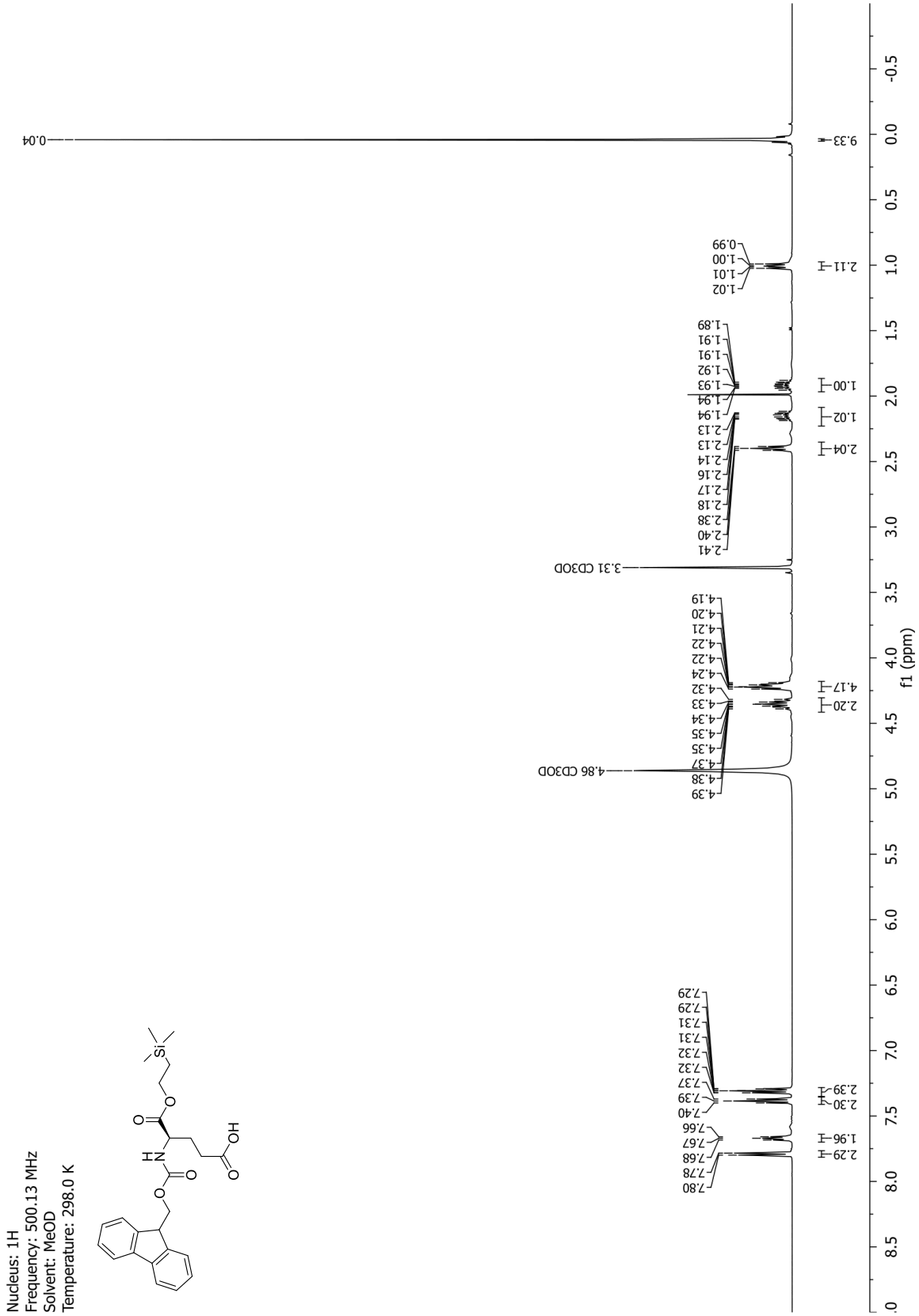
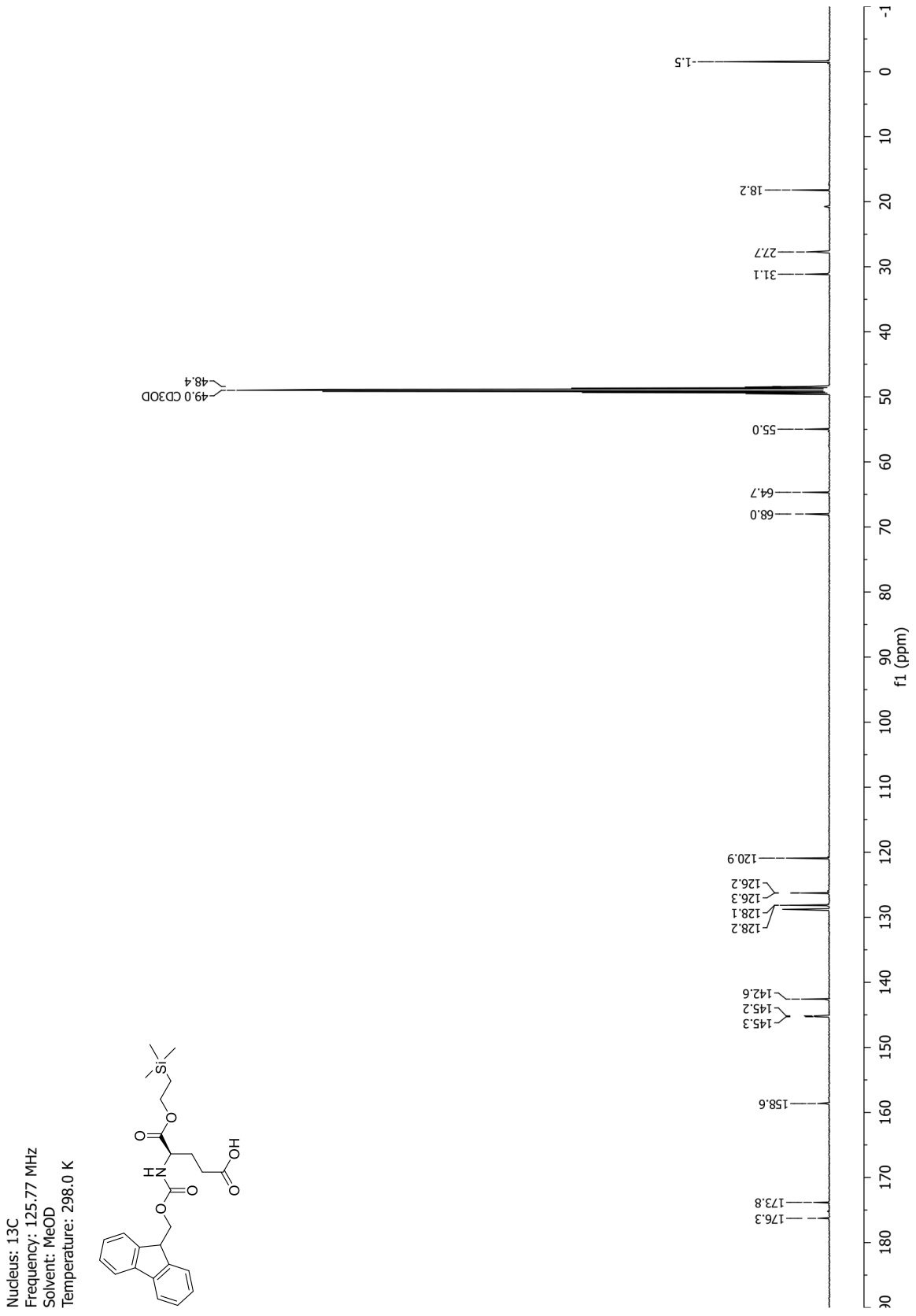
18.2
27.8
28.4
32.6
48.4
49.0 CD3OD
54.8
64.6
68.0
120.9
126.2
126.3
128.1
128.2
128.8
142.6
145.2
145.3
158.6
173.0
173.7
173.8
f1 (ppm)

NMR-Spectra for Compound 84

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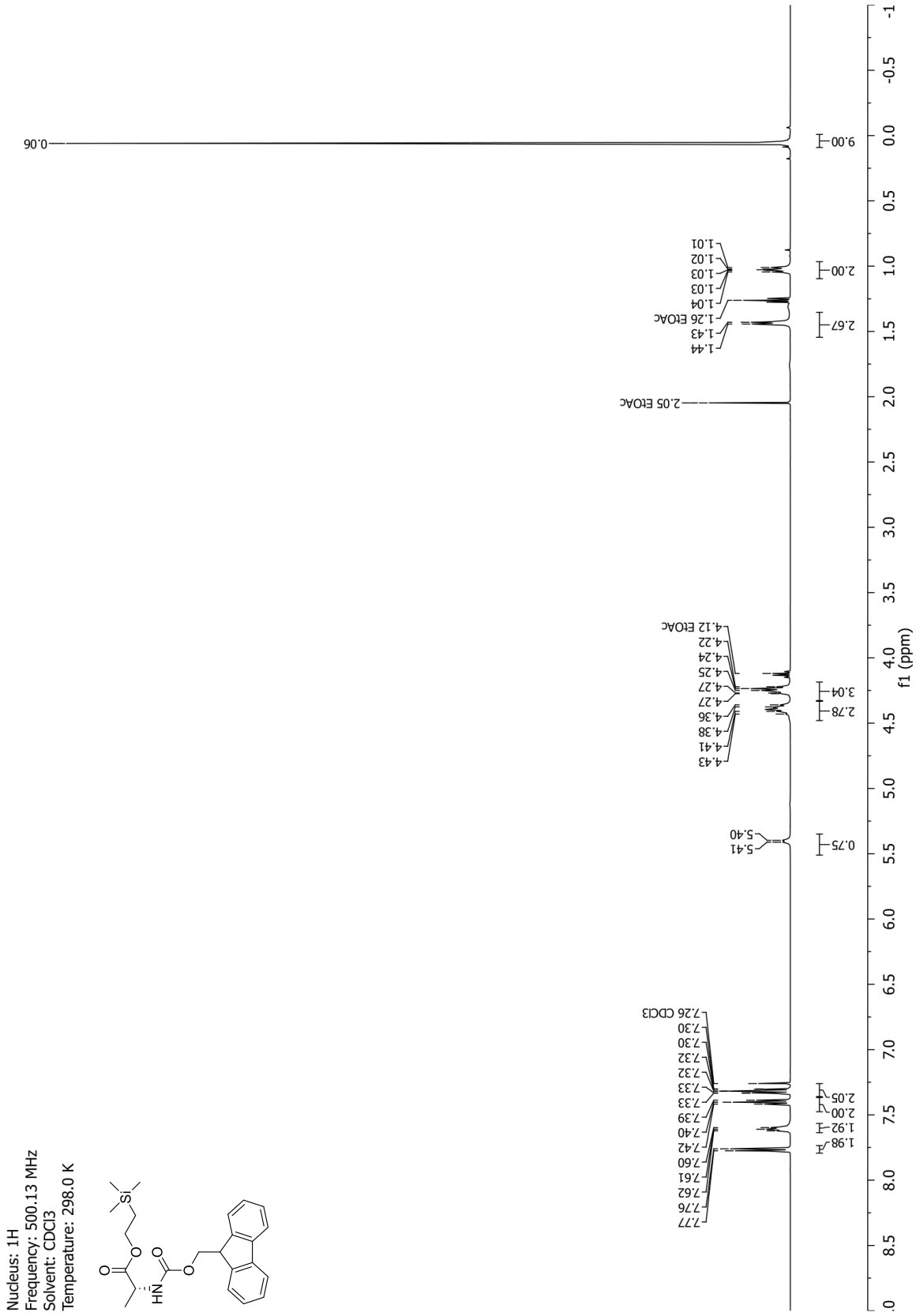
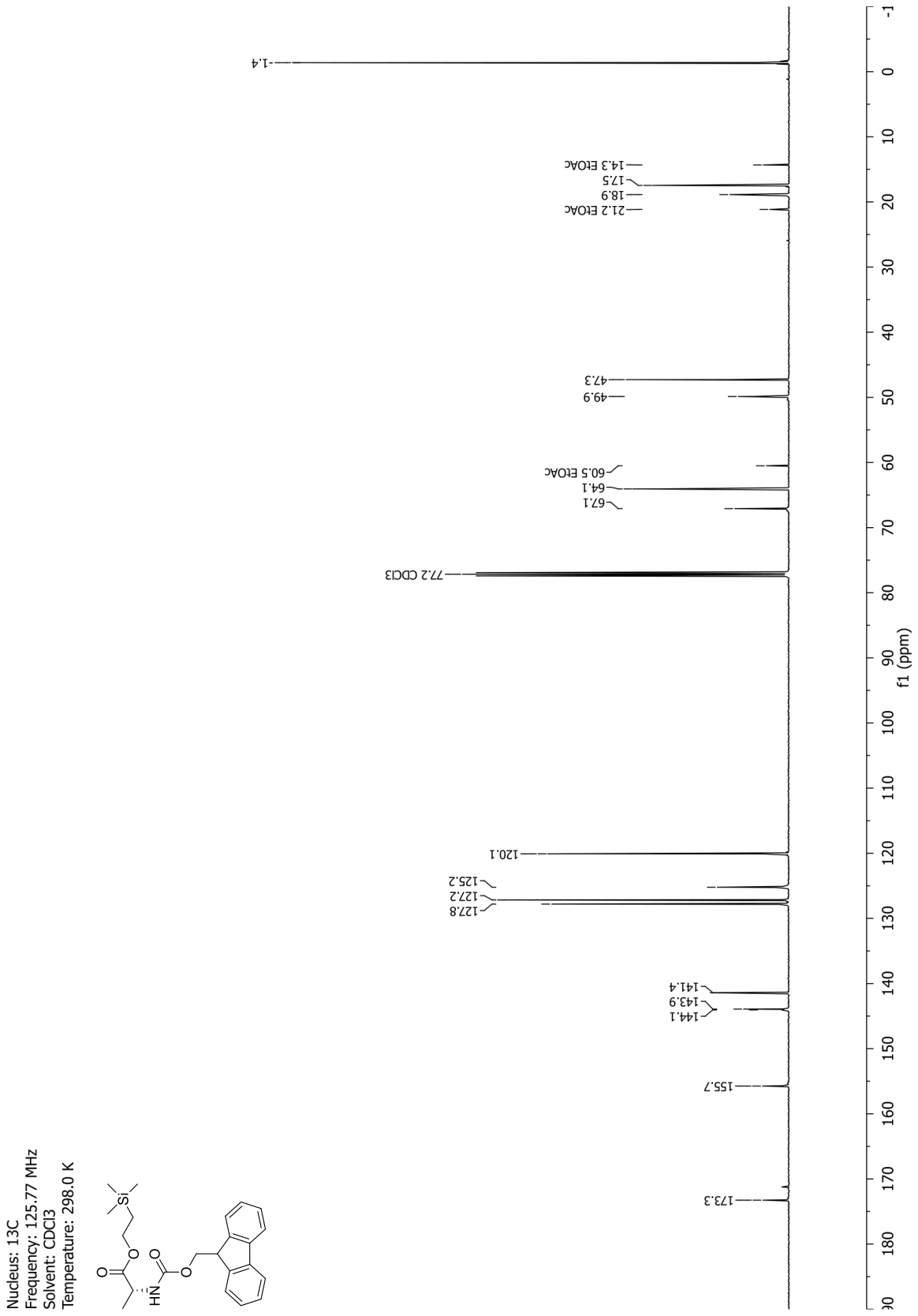
Frequency: 125.77 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)
-1.5
18.2
27.7
31.1
48.4
49.0 CD3OD
55.0
64.7
68.0
120.9
126.2
126.3
128.1
128.2
142.6
145.2
145.3
158.6
173.8
176.3

NMR-Spectra for Compound 97

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Frequency: 125.77 MHz
Solvent: CDCl3
Temperature: 298.0 K
Si
O
O
HN
O
O
-1.4
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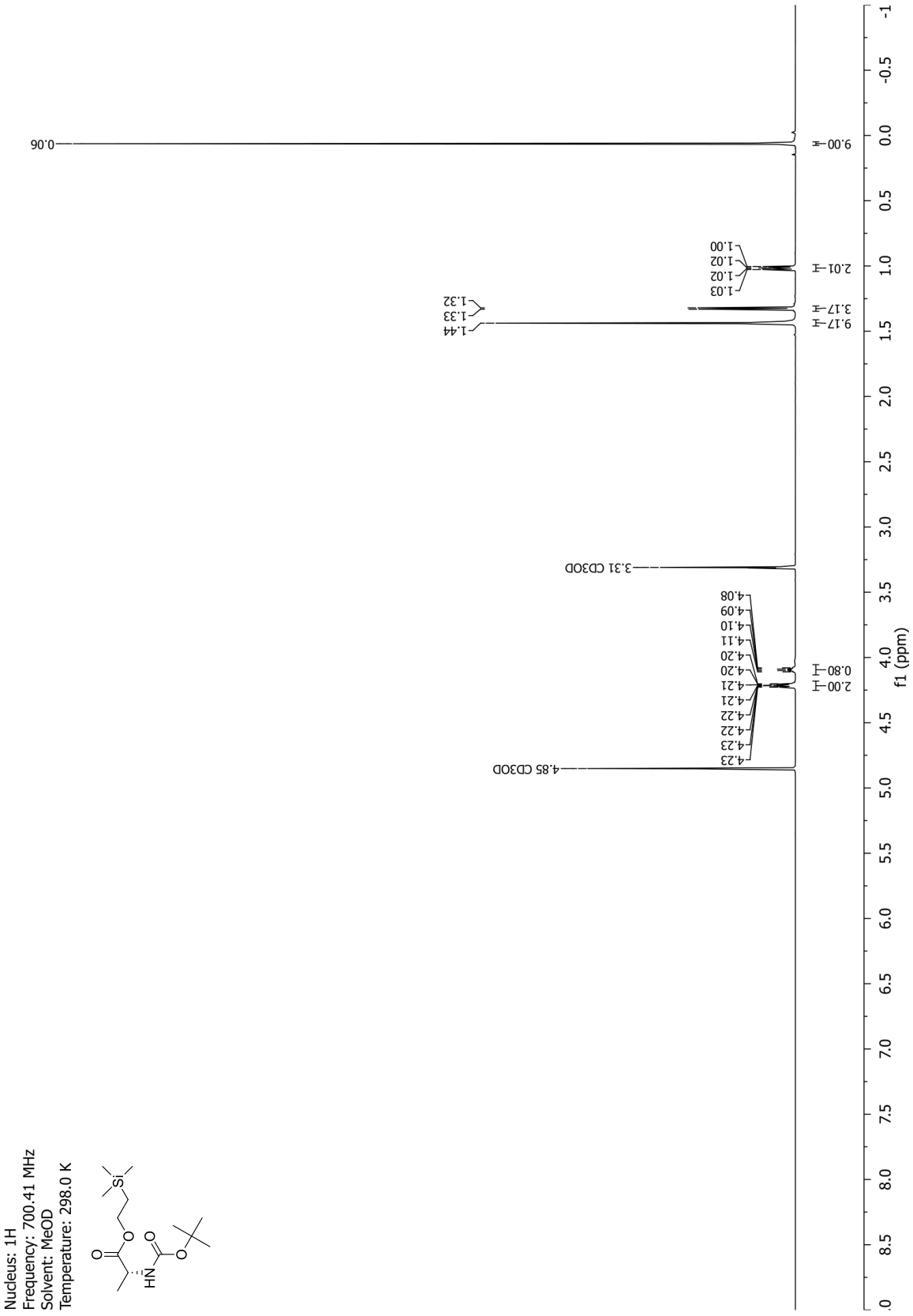
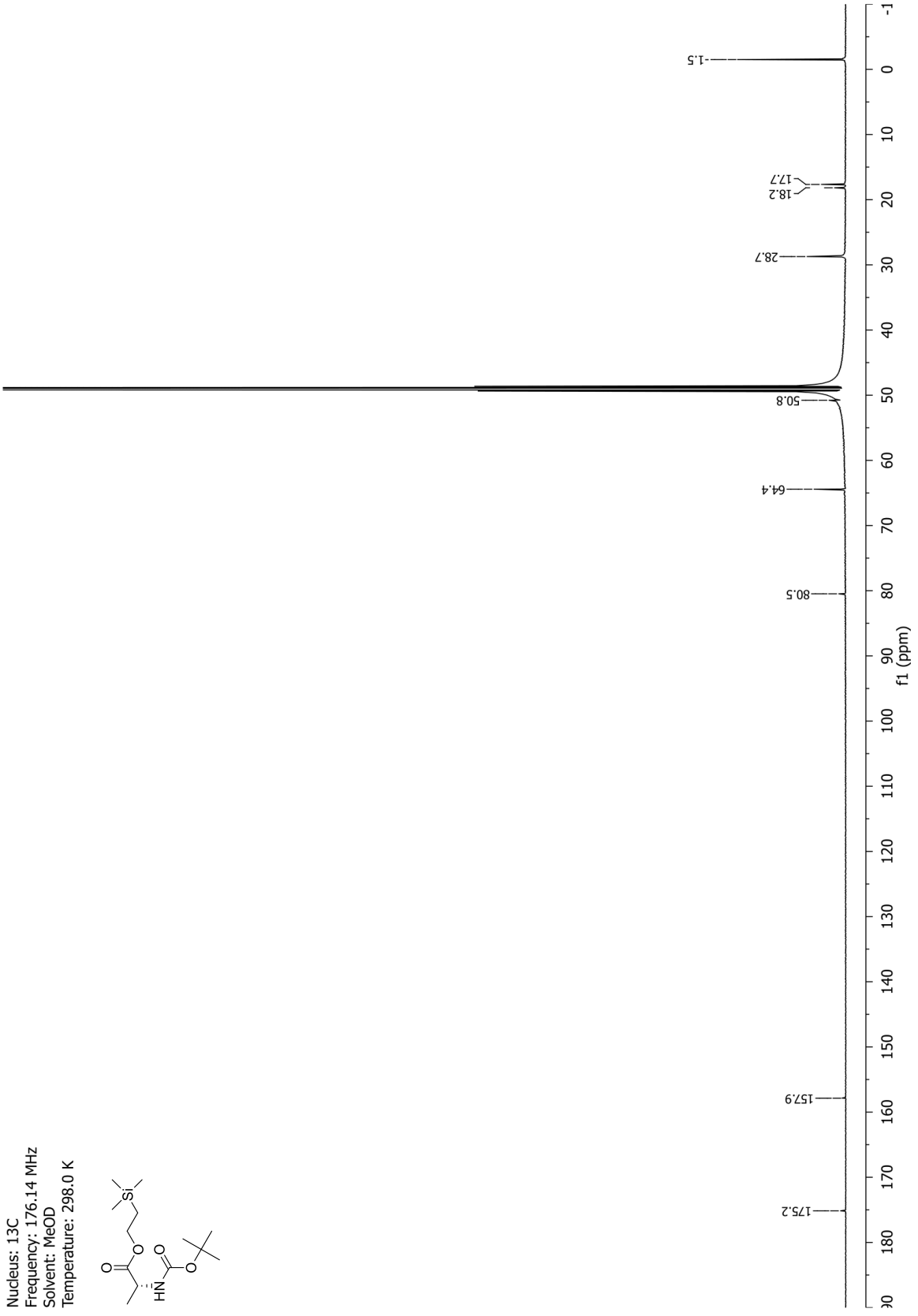
17.5
18.9
21.2 EtOAc
47.3
49.9
60.5 EtOAc
64.1
67.1
77.2 CDCl3
120.1
125.2
127.2
127.8
141.4
143.9
144.1
155.7
173.3
f1 (ppm)

NMR-Spectra for Compound 101




Nucleus: 13C
Frequency: 176.14 MHz
Solvent: MeOD
Temperature: 298.0 K
-1.5
17.7
18.2
28.7
50.8
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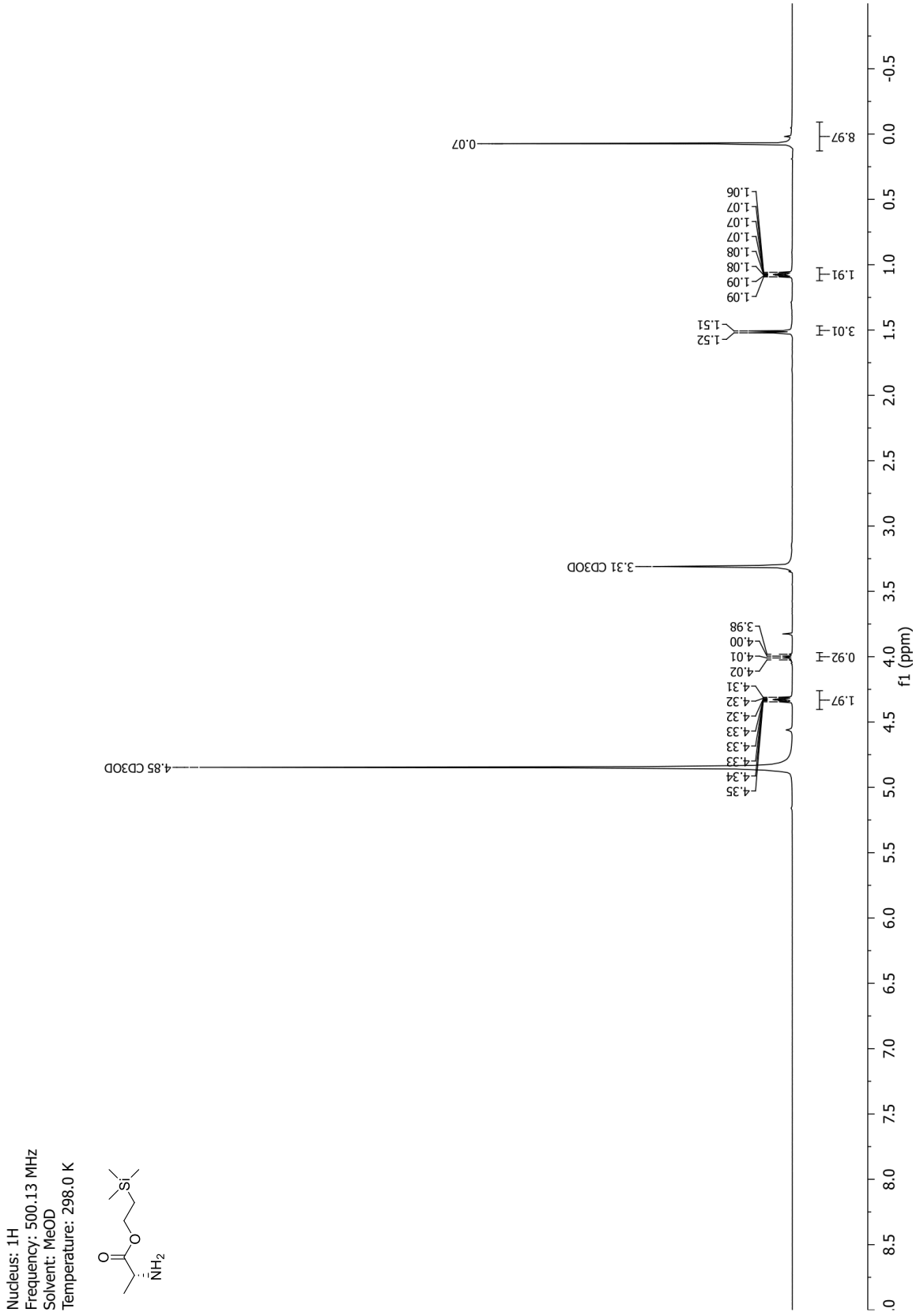
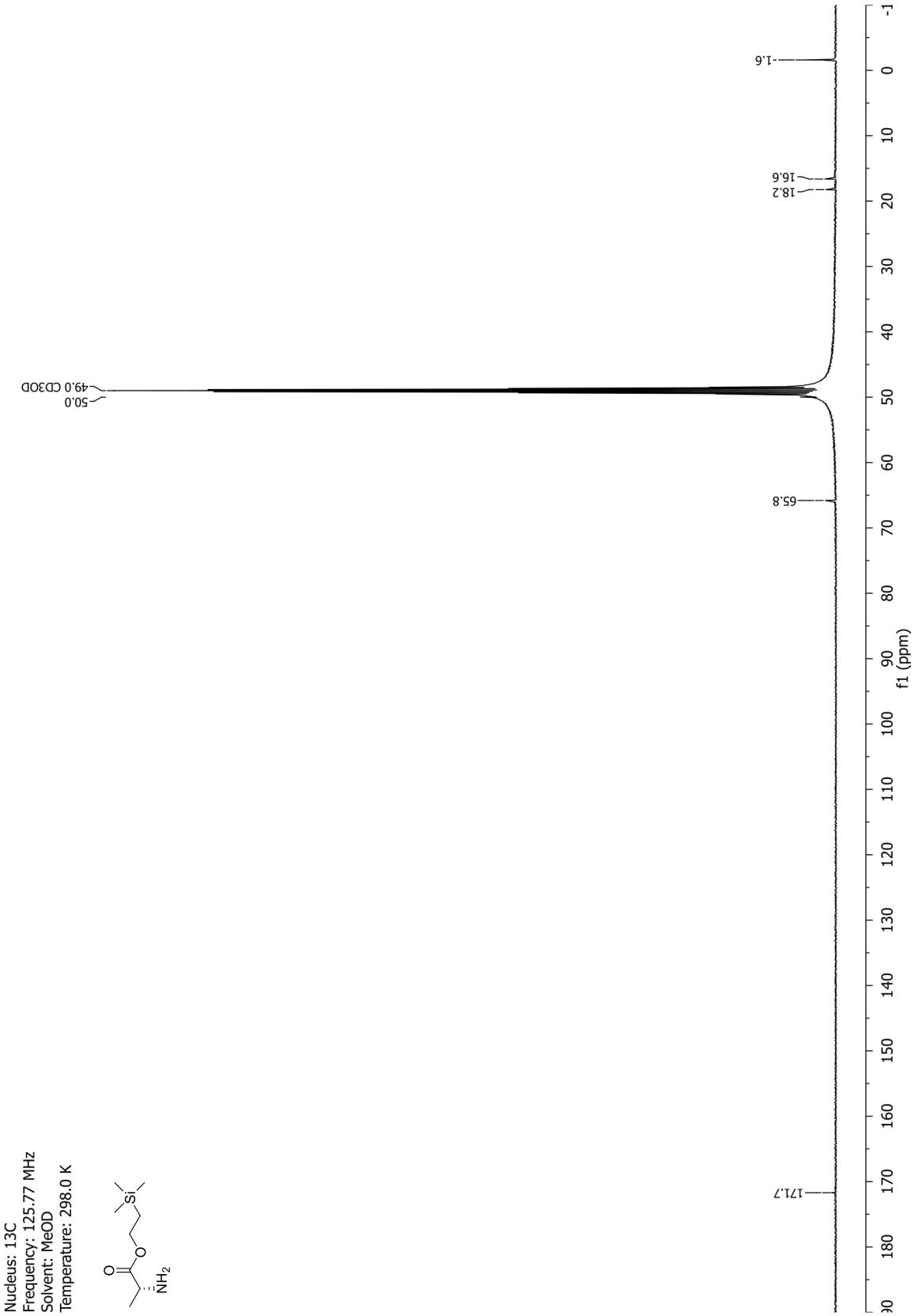
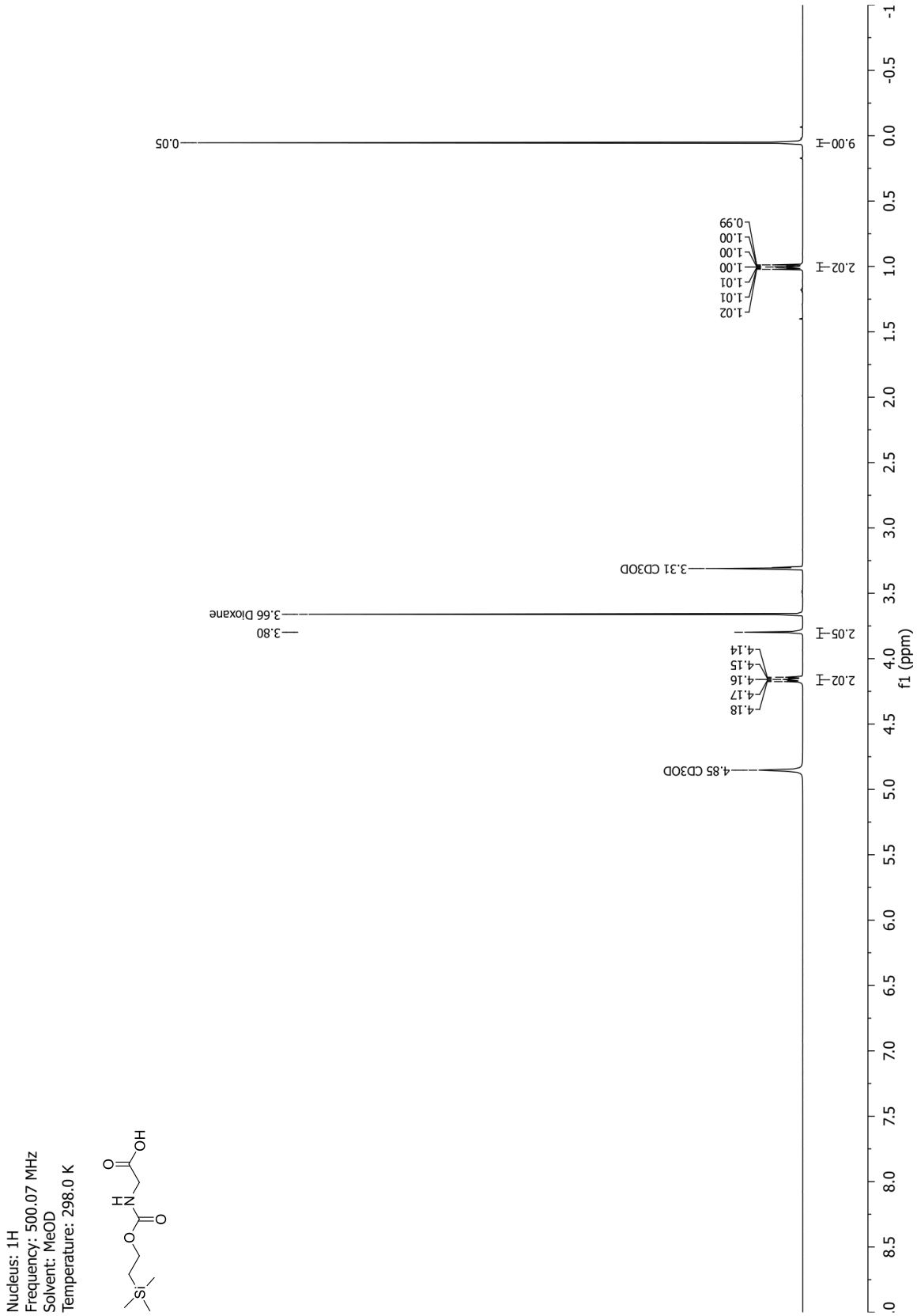
157.9
175.2
f1 (ppm)

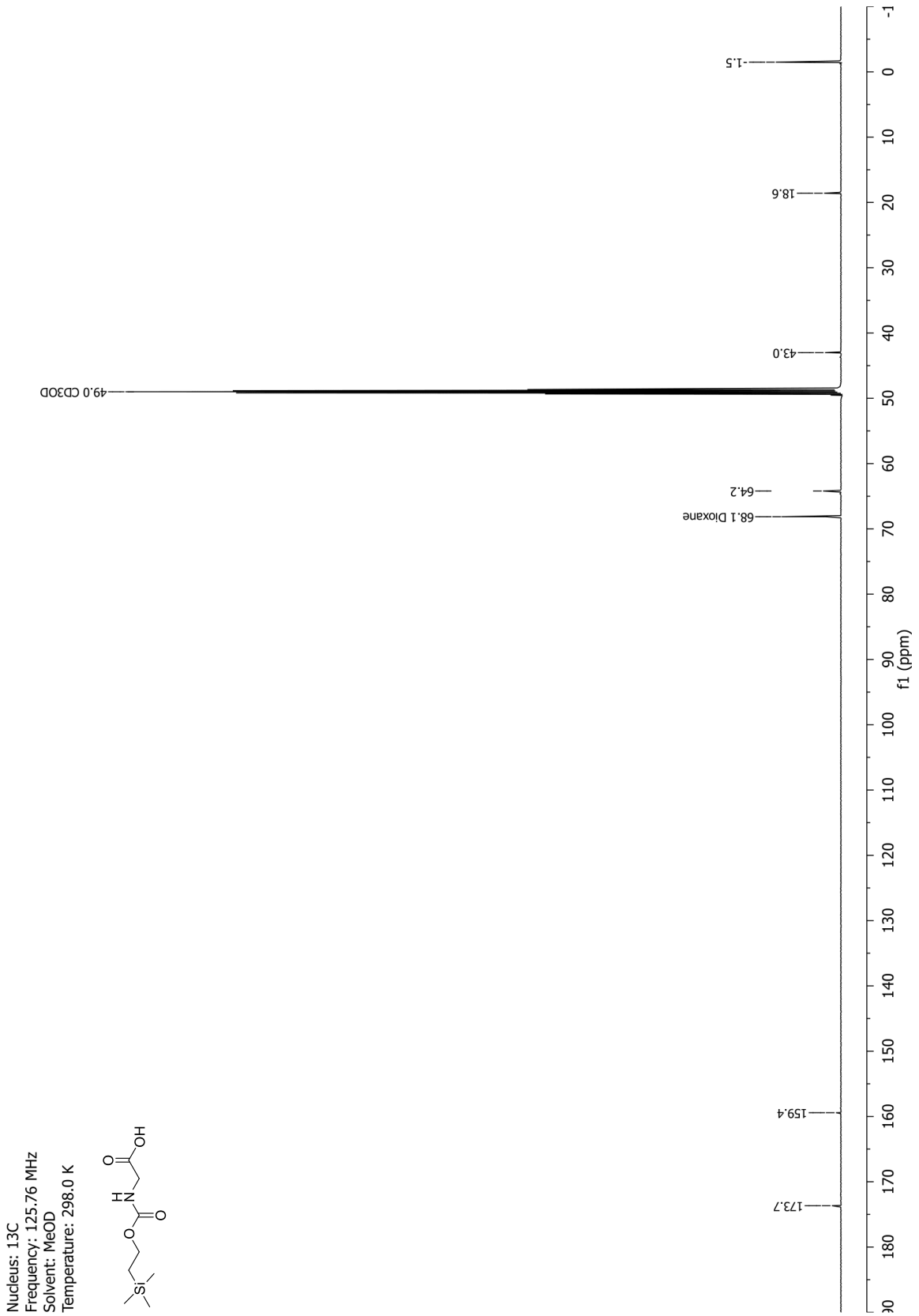
NMR-Spectra for Compound 86



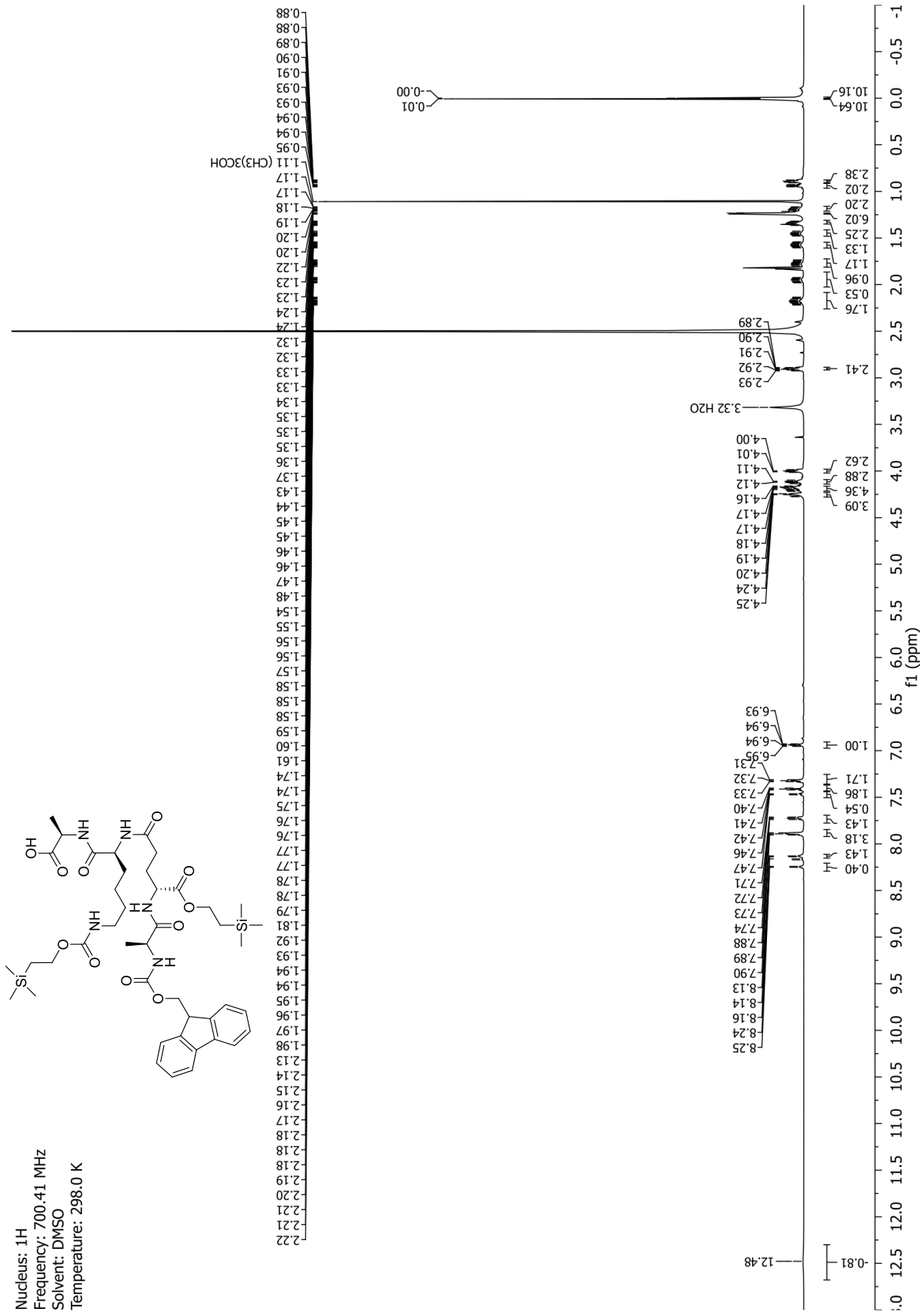

Nucleus: 13C
Frequency: 125.77 MHz
Solvent: MeOD
Temperature: 298.0 K
Si
O
O
NH2
-1.6
16.6
18.2
49.0 CD3OD
50.0
65.8
171.7
f1 (ppm)

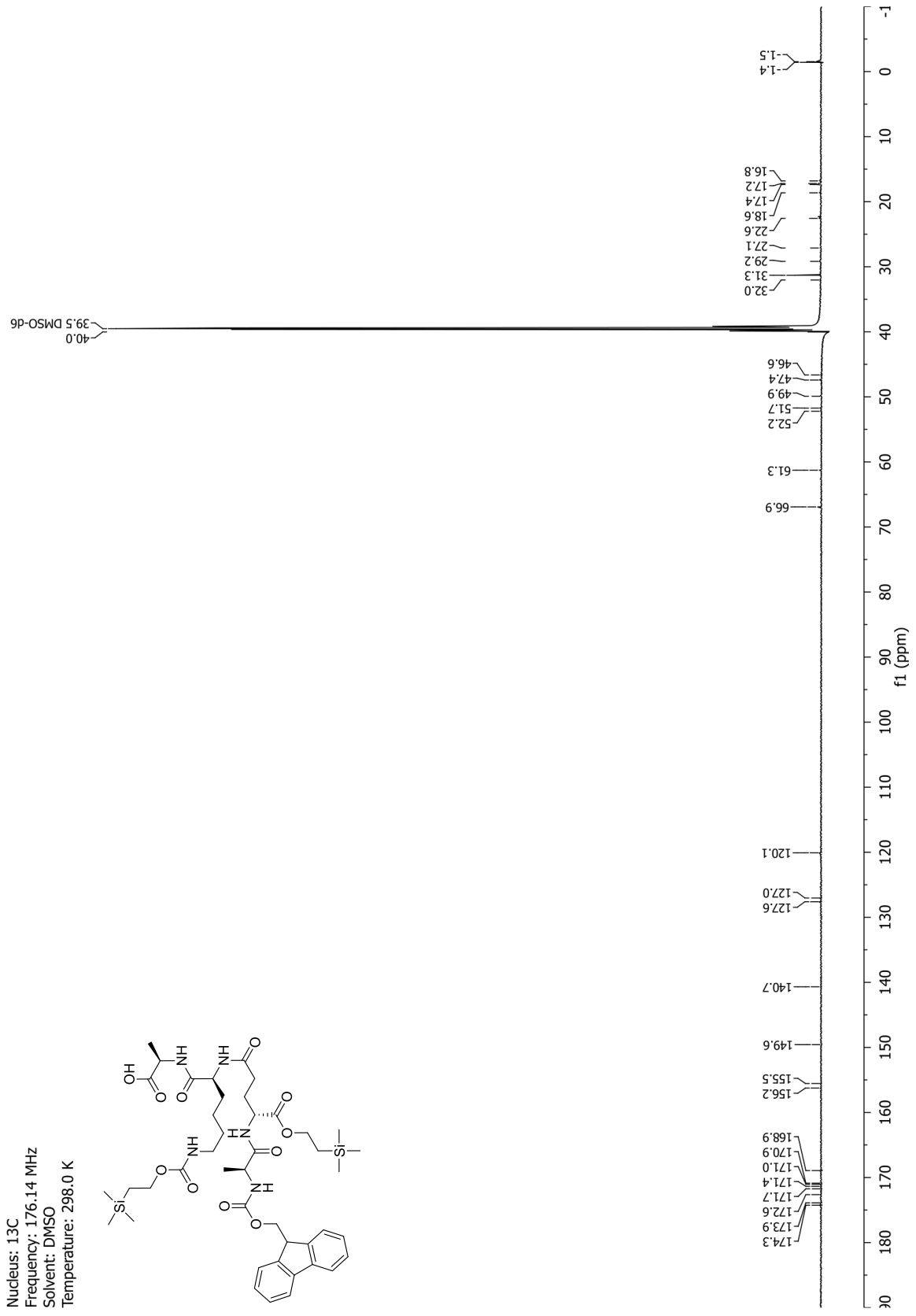
NMR-Spectra for Compound 89



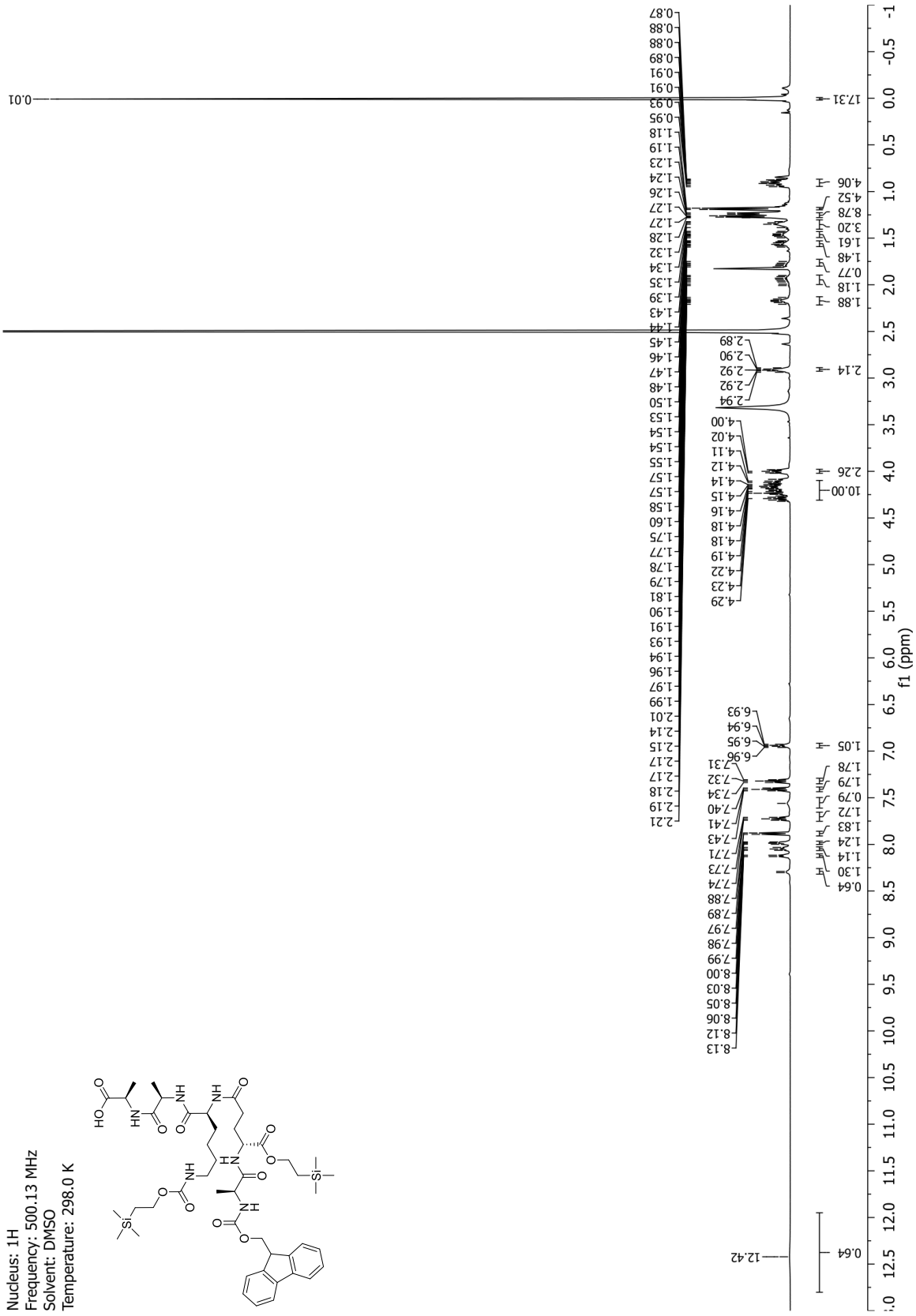

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: MeOD
Temperature: 298.0 K
-1.5
18.6
43.0
49.0 CD3OD
64.2
68.1 Dioxane
159.4
173.7
f1 (ppm)

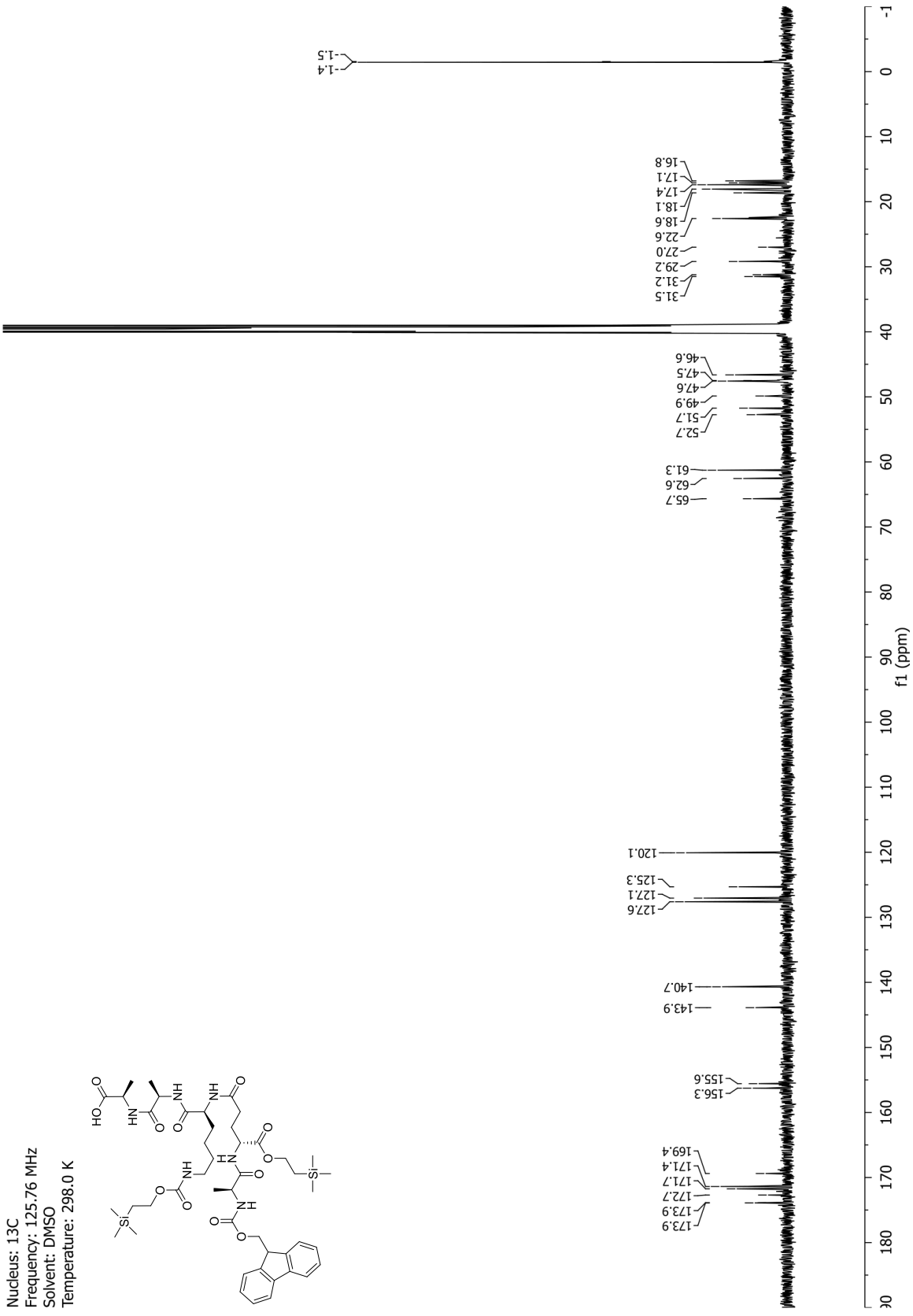
NMR-Spectra for Compound 135



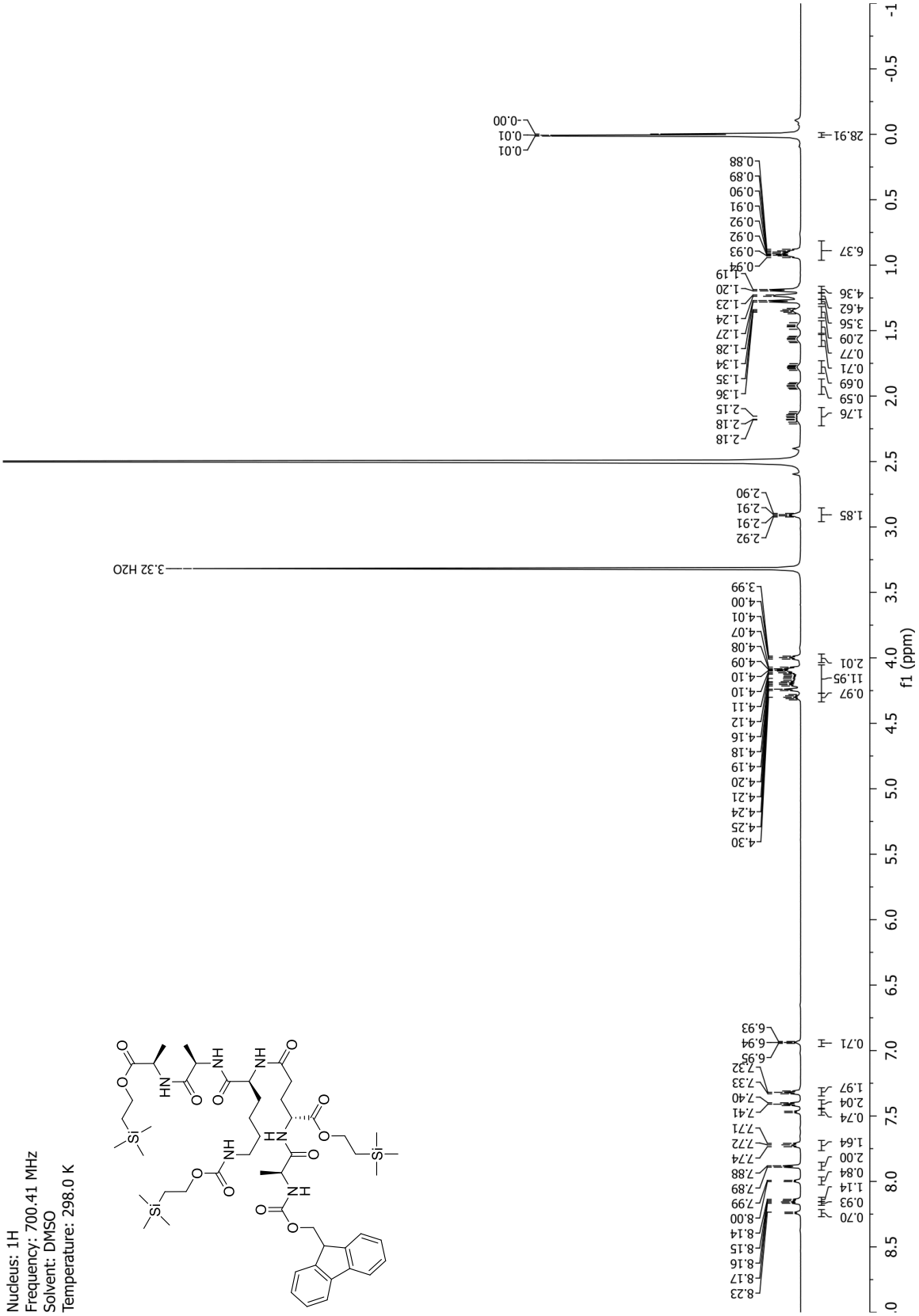

Nucleus: 13C
Frequency: 176.14 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)
-1.4
-1.5
16.8
17.2
17.4
18.6
22.6
27.1
29.2
31.3
32.0
39.5 DMSO-d6
40.0
46.6
47.4
49.9
51.7
52.2
61.3
66.9
120.1
127.6
127.0
140.7
149.6
155.5
156.2
168.9
170.9
171.0
171.4
171.7
172.6
173.9
174.3

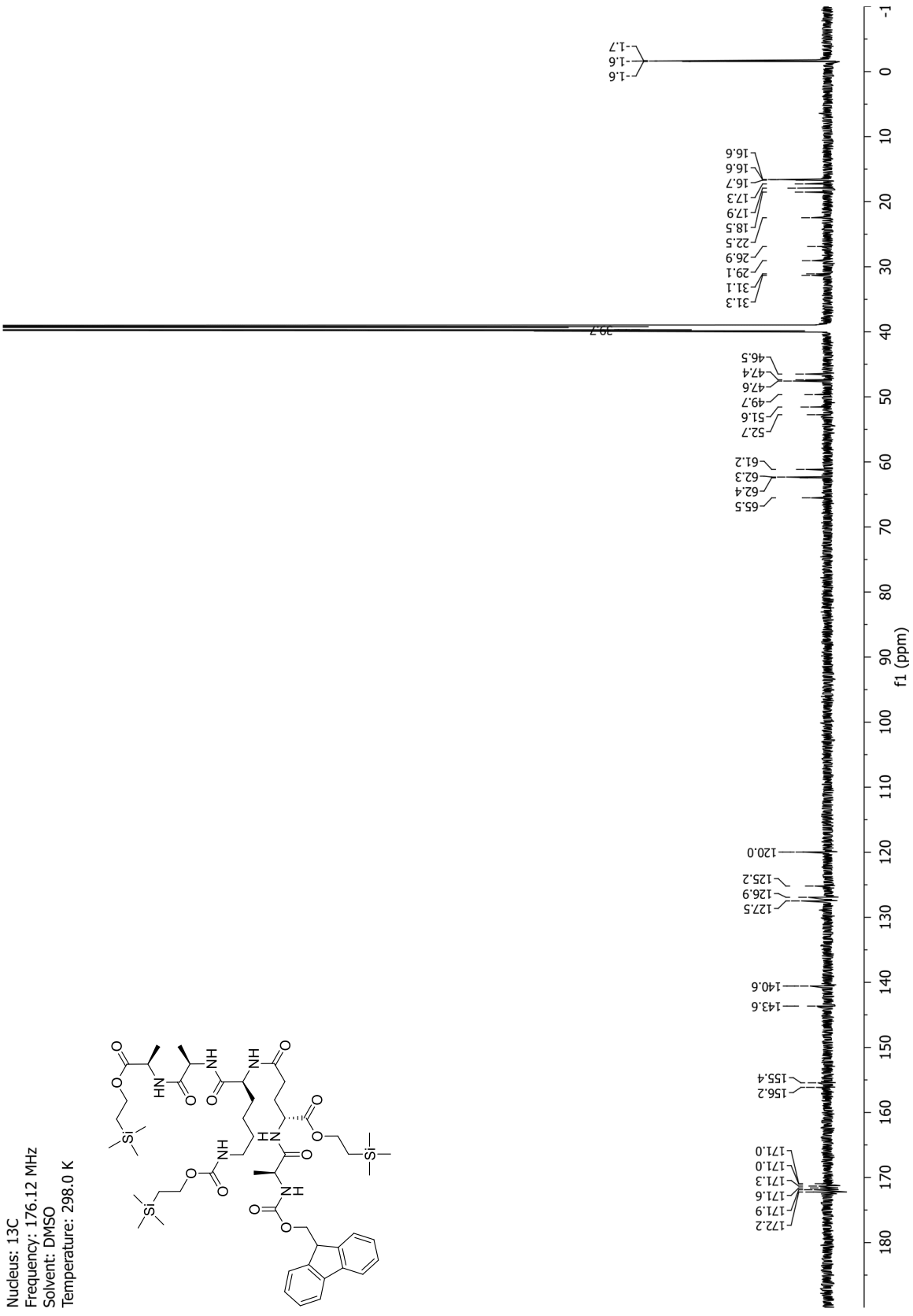
NMR-Spectra for Compound 106



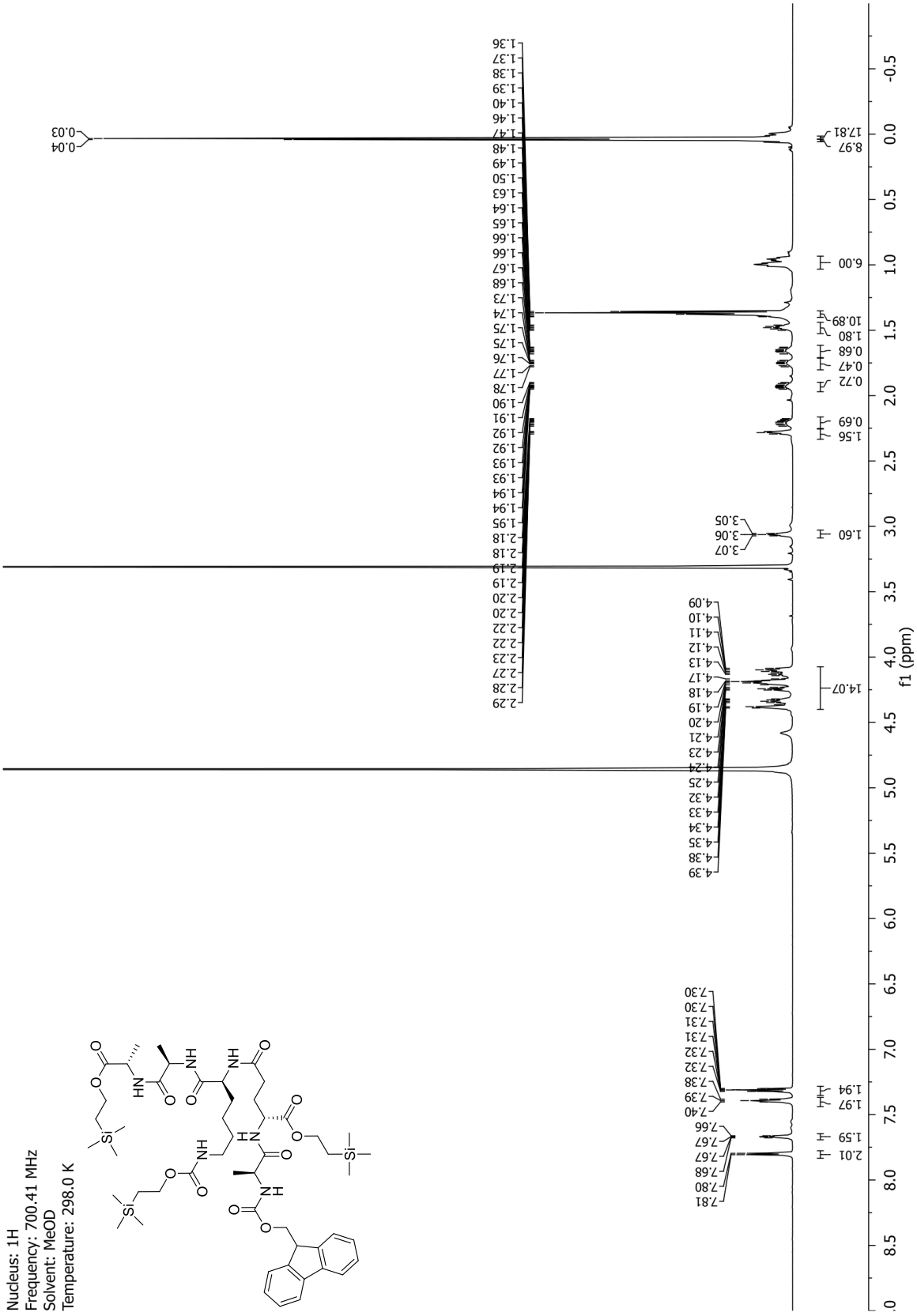

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)
-1.4
-1.5
16.8
17.1
17.4
18.1
18.6
22.6
27.0
29.2
31.2
31.5
46.6
47.5
47.6
49.9
51.7
52.7
61.3
62.6
65.7
120.1
125.3
127.1
127.6
140.7
143.9
155.6
156.3
169.4
171.4
171.7
172.7
173.9
173.9

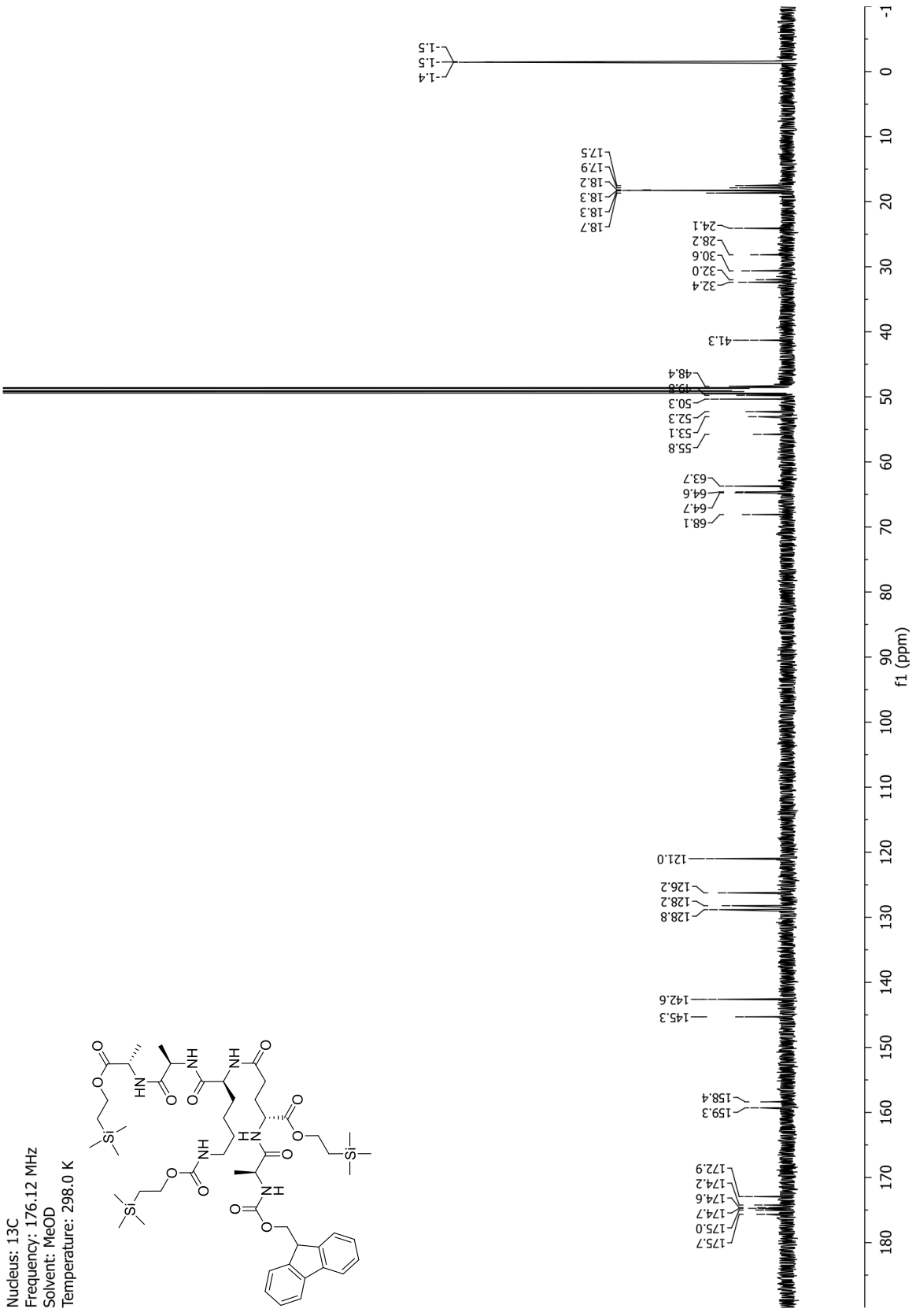
NMR-Spectra for Compound 124a



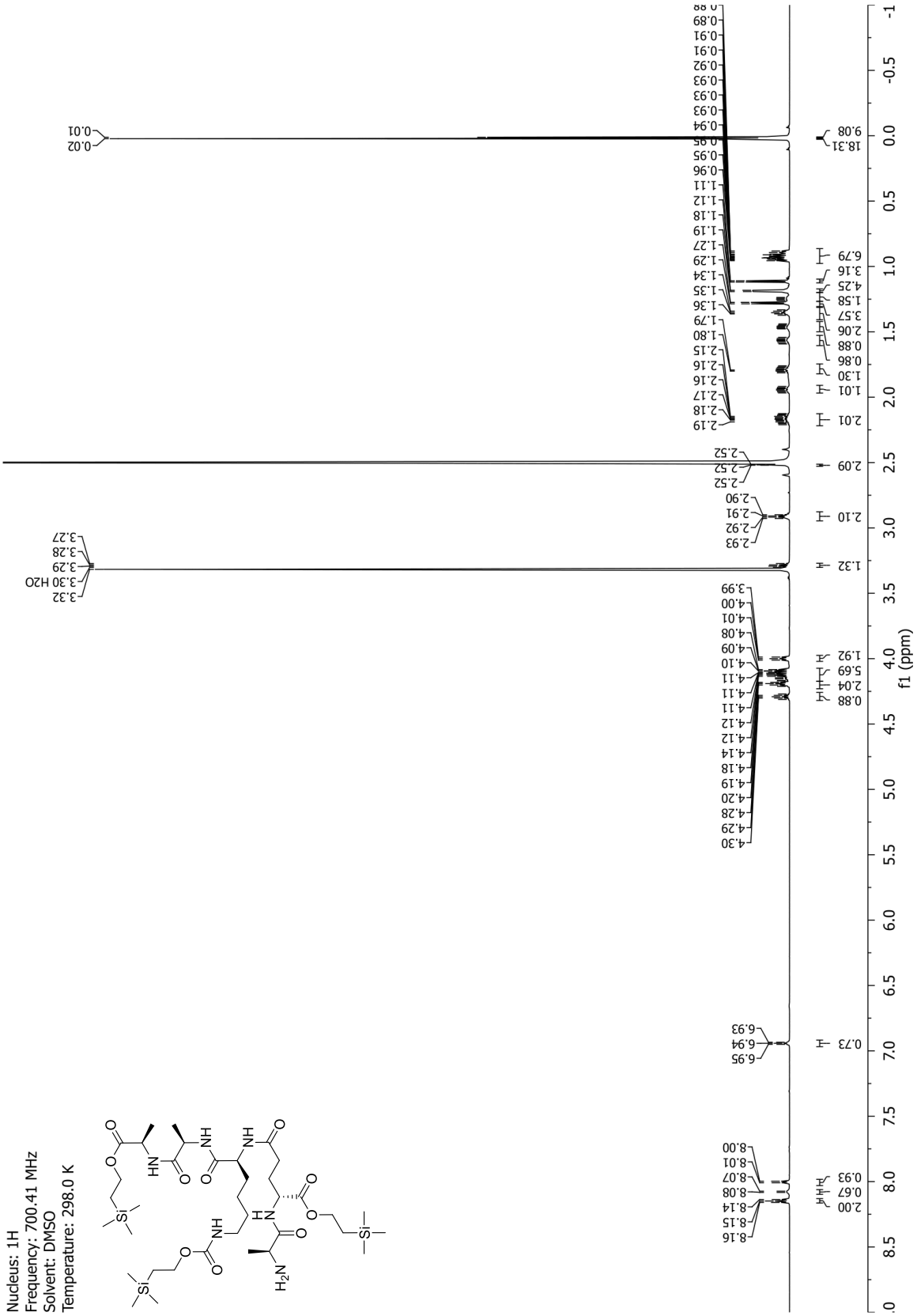

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)

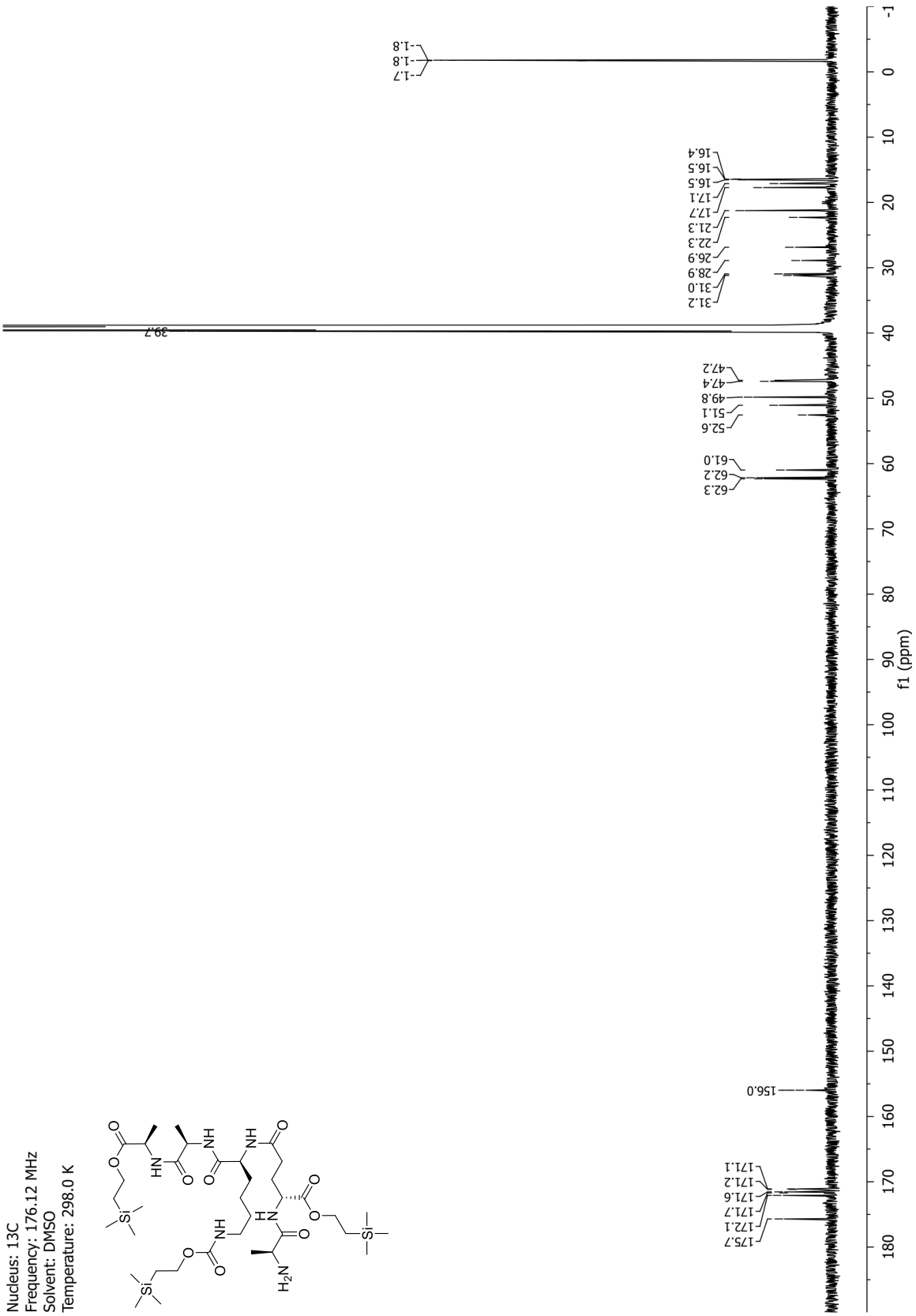
NMR-Spectra for Compound 124b



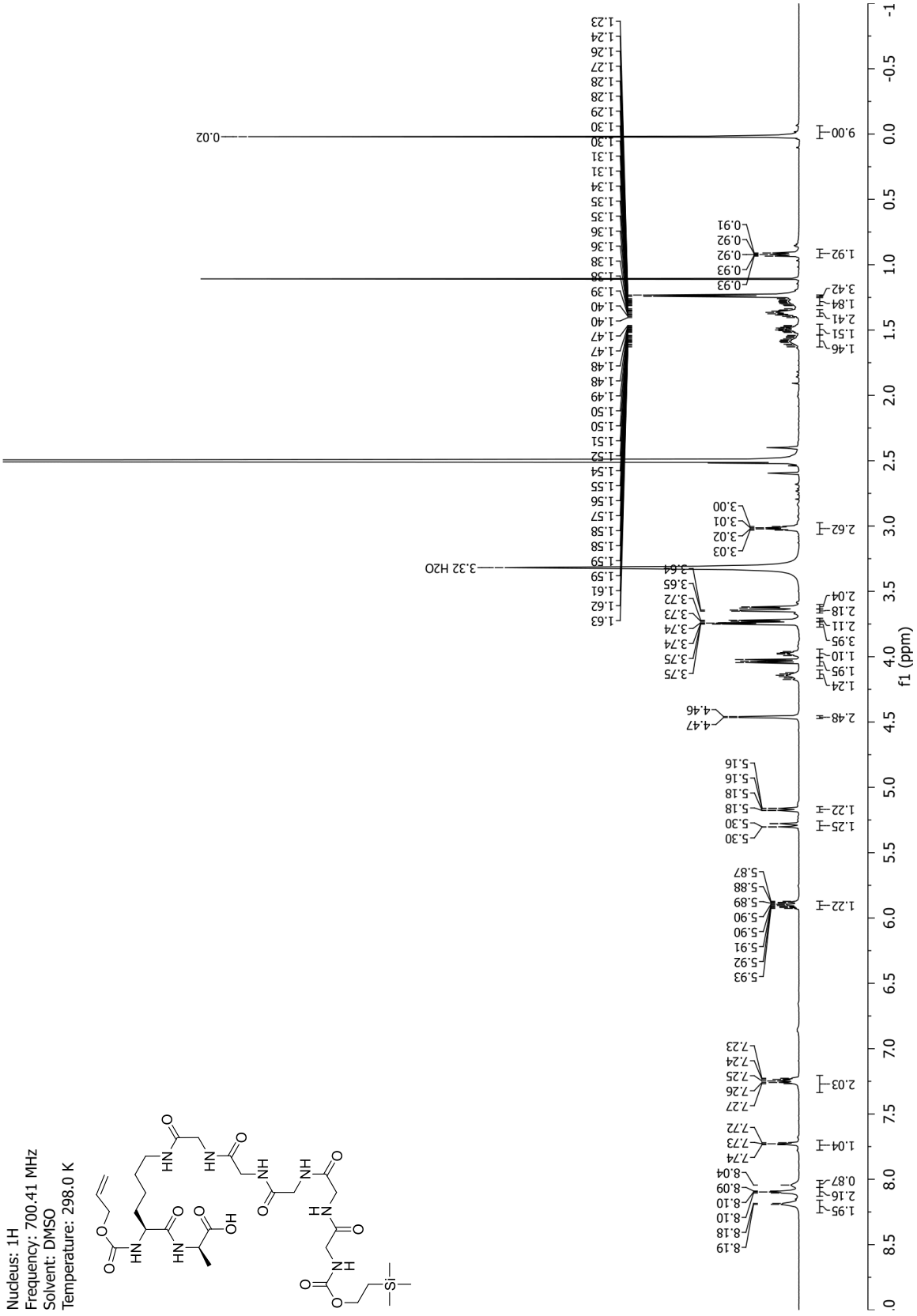

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)

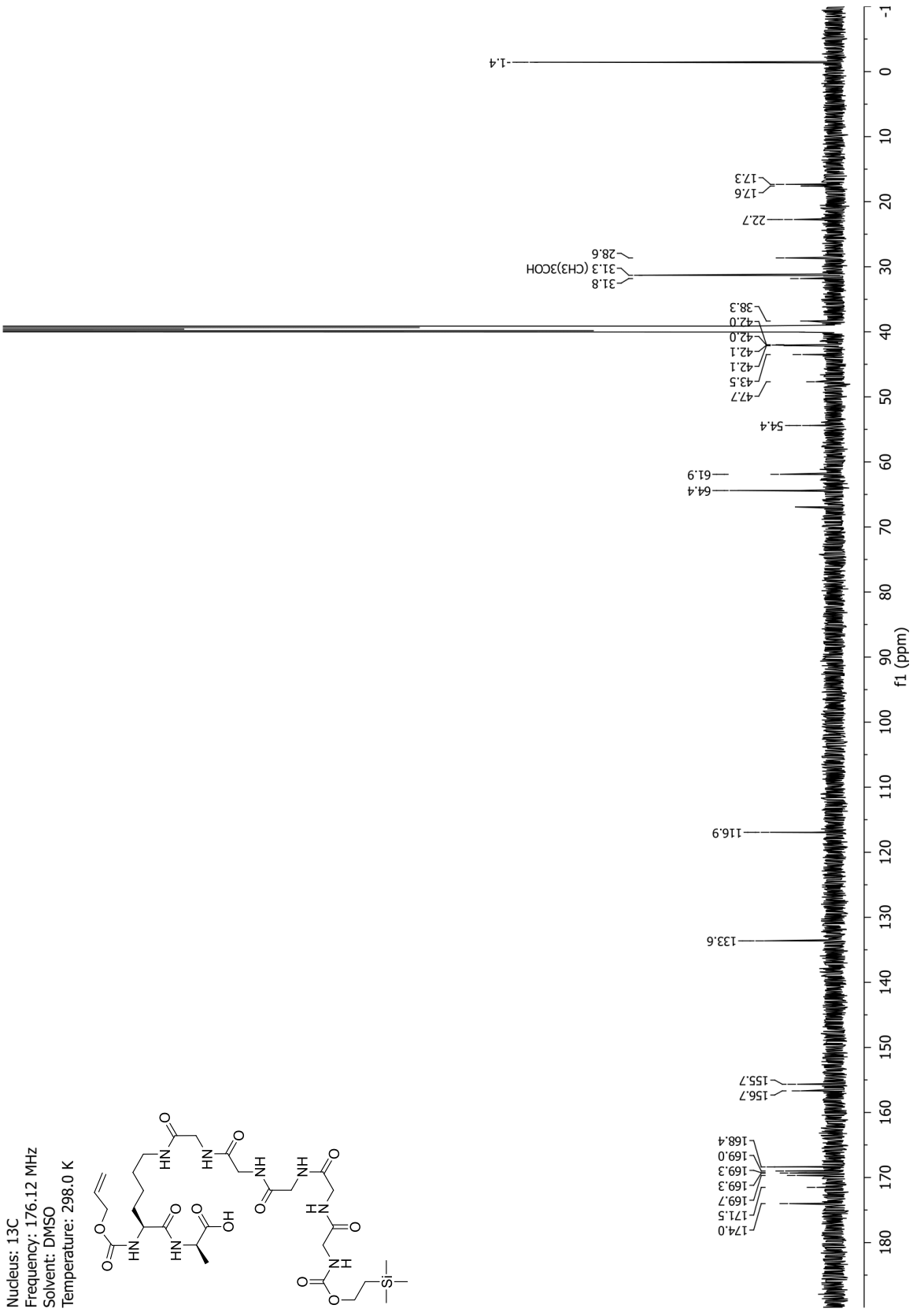
NMR-Spectra for Compound 4



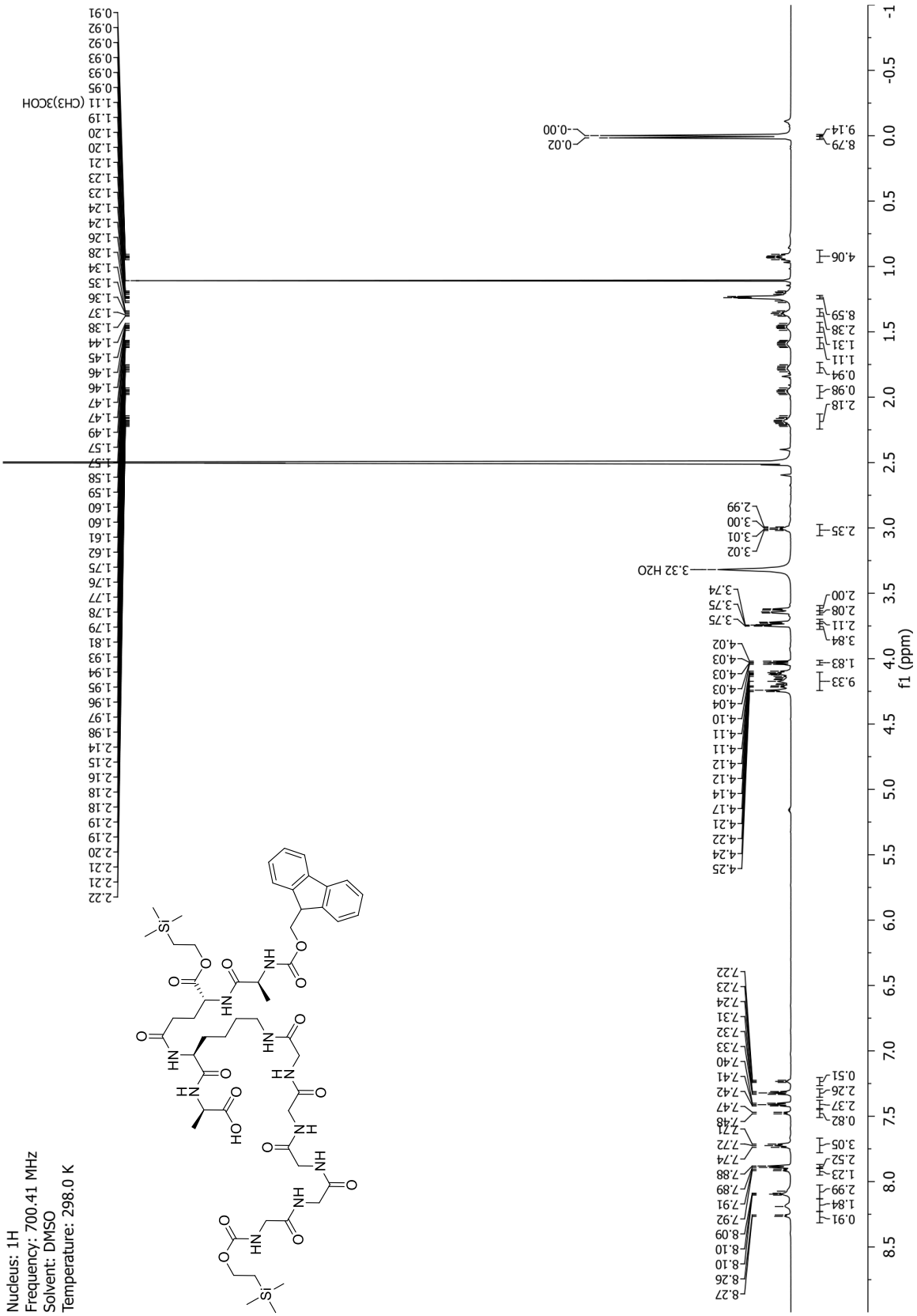

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)
-1.7
-1.8
-1.8
16.4
16.5
16.5
17.1
17.7
21.3
22.3
26.9
28.9
31.0
31.2
39.7
47.2
47.4
49.8
51.1
52.6
61.0
62.2
62.3
156.0
171.1
171.2
171.6
171.7
172.1
175.7

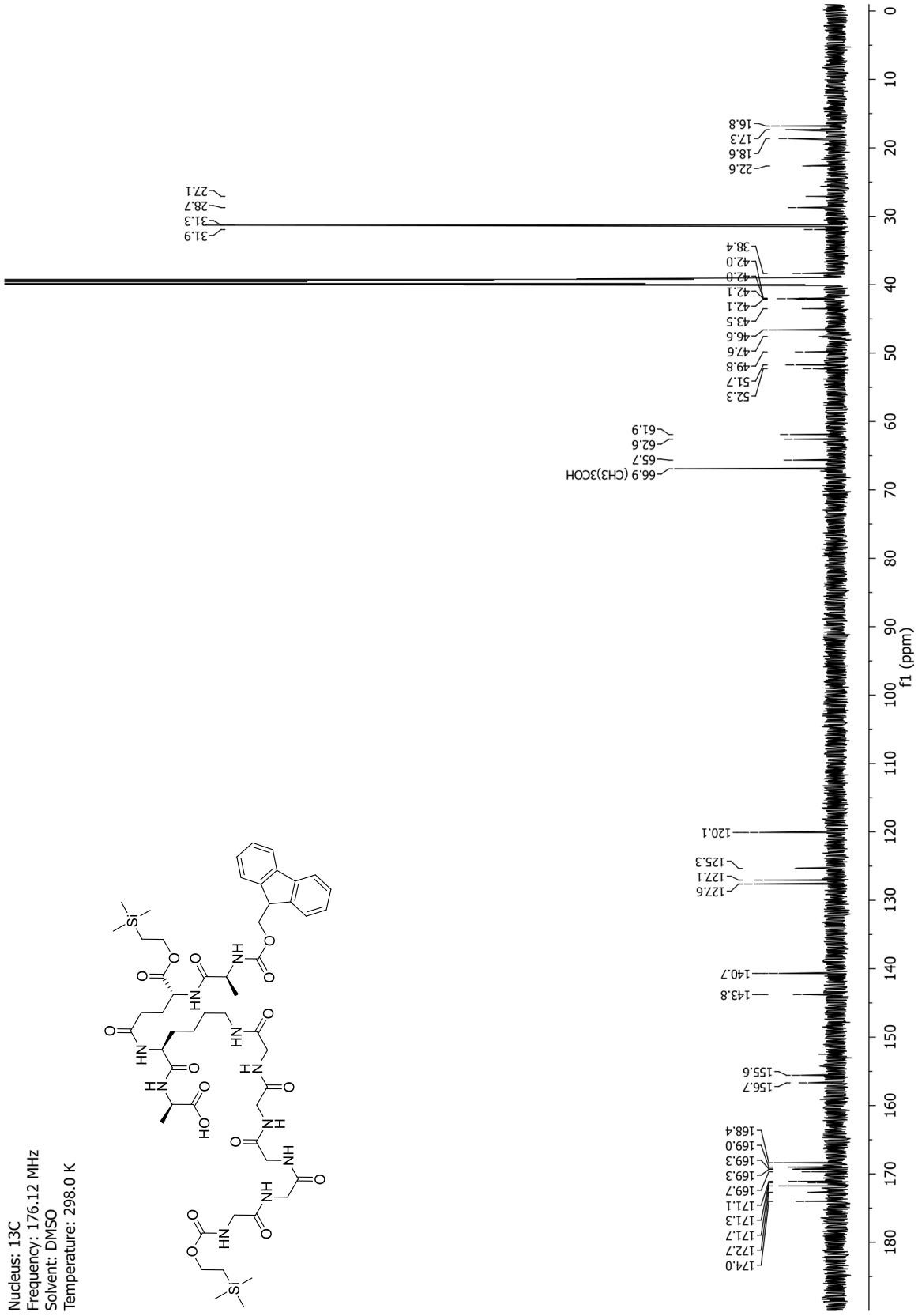
NMR-Spectra for Compound 139



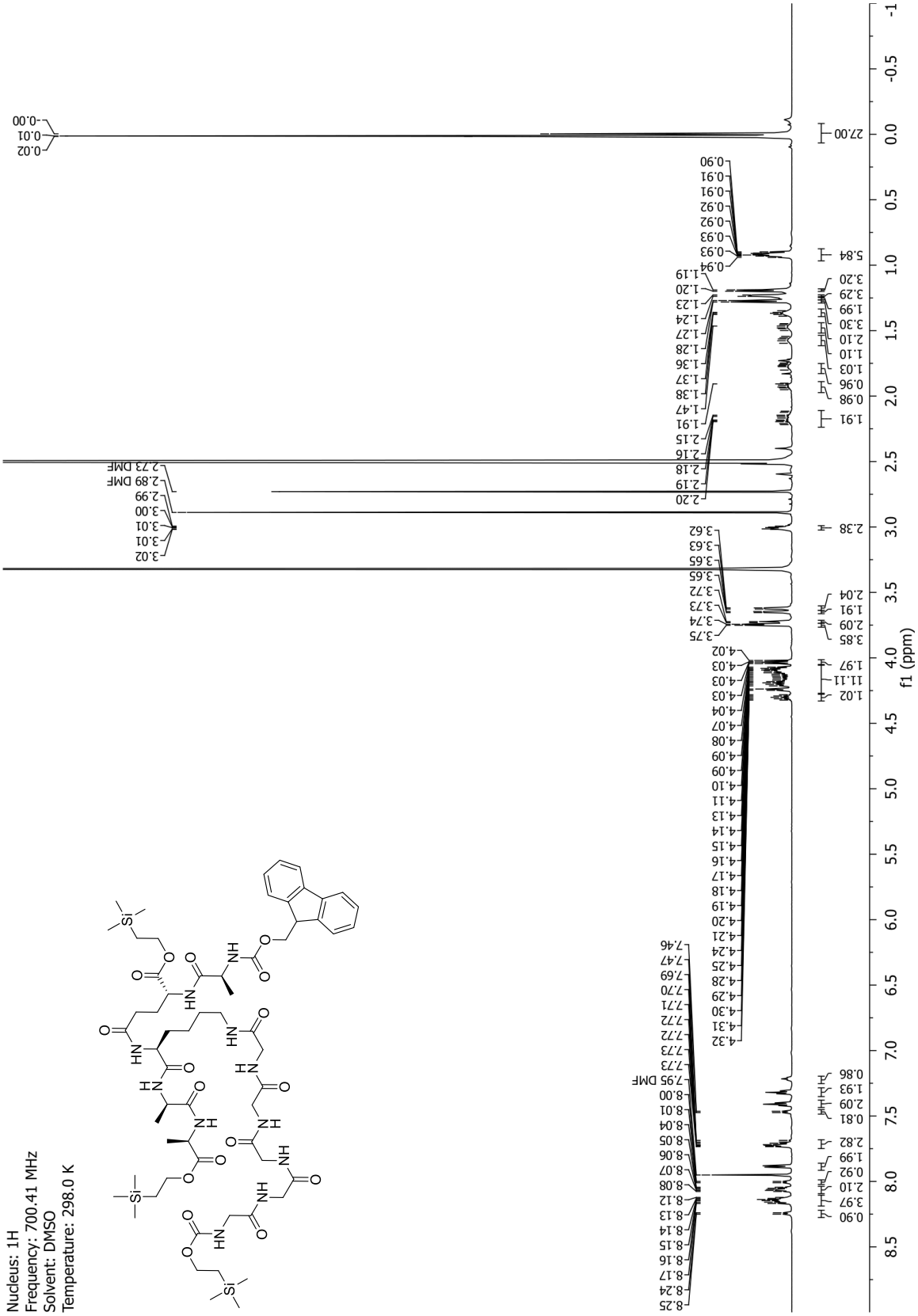

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)
-1.4
17.3
17.6
22.7
28.6
31.3 (CH3)3COH
31.8
38.3
42.0
42.0
42.1
42.1
43.5
47.7
54.4
61.9
64.4
116.9
133.6
155.7
156.7
168.4
169.0
169.3
169.3
169.7
171.5
174.0

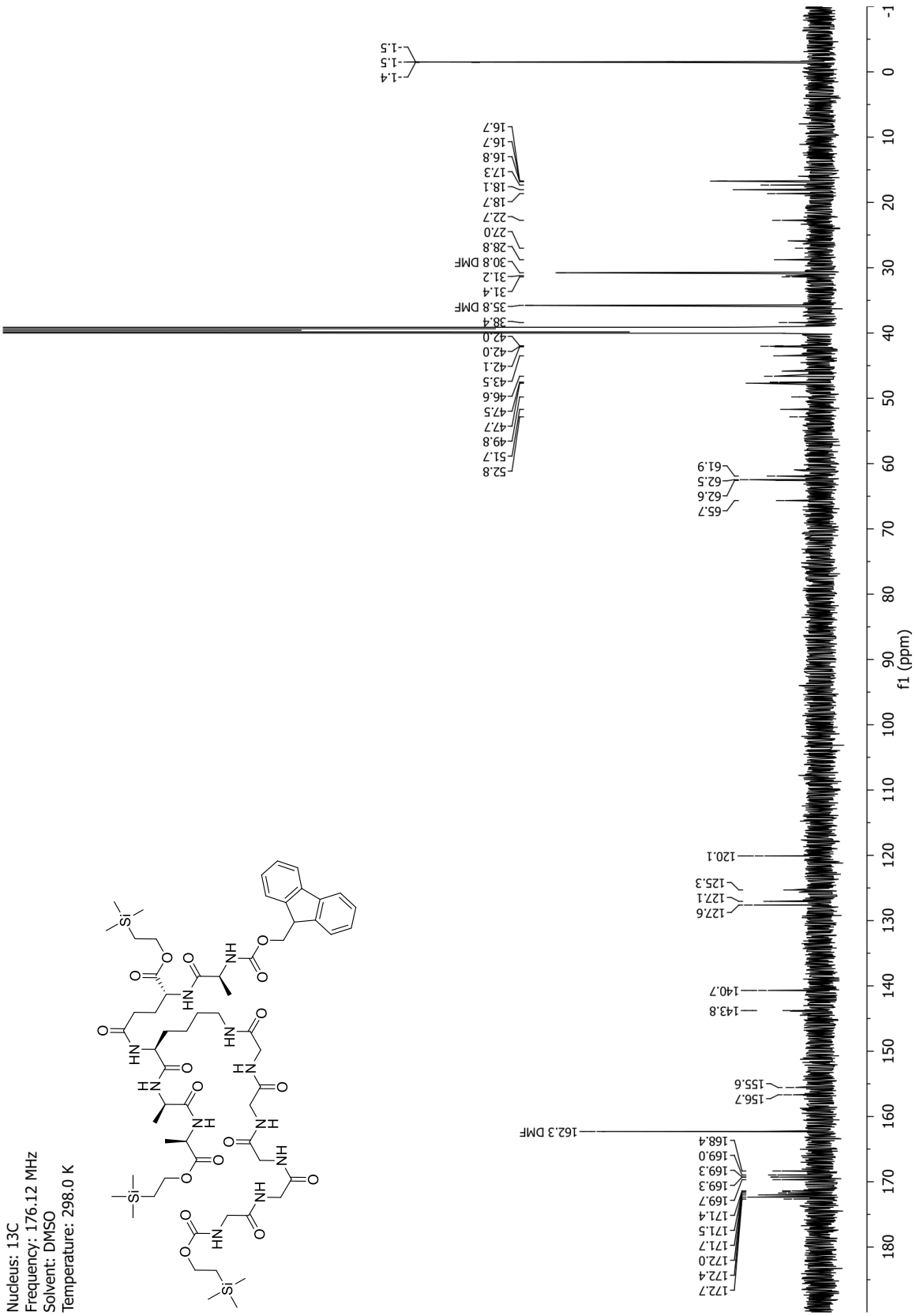
NMR-Spectra for Compound 141



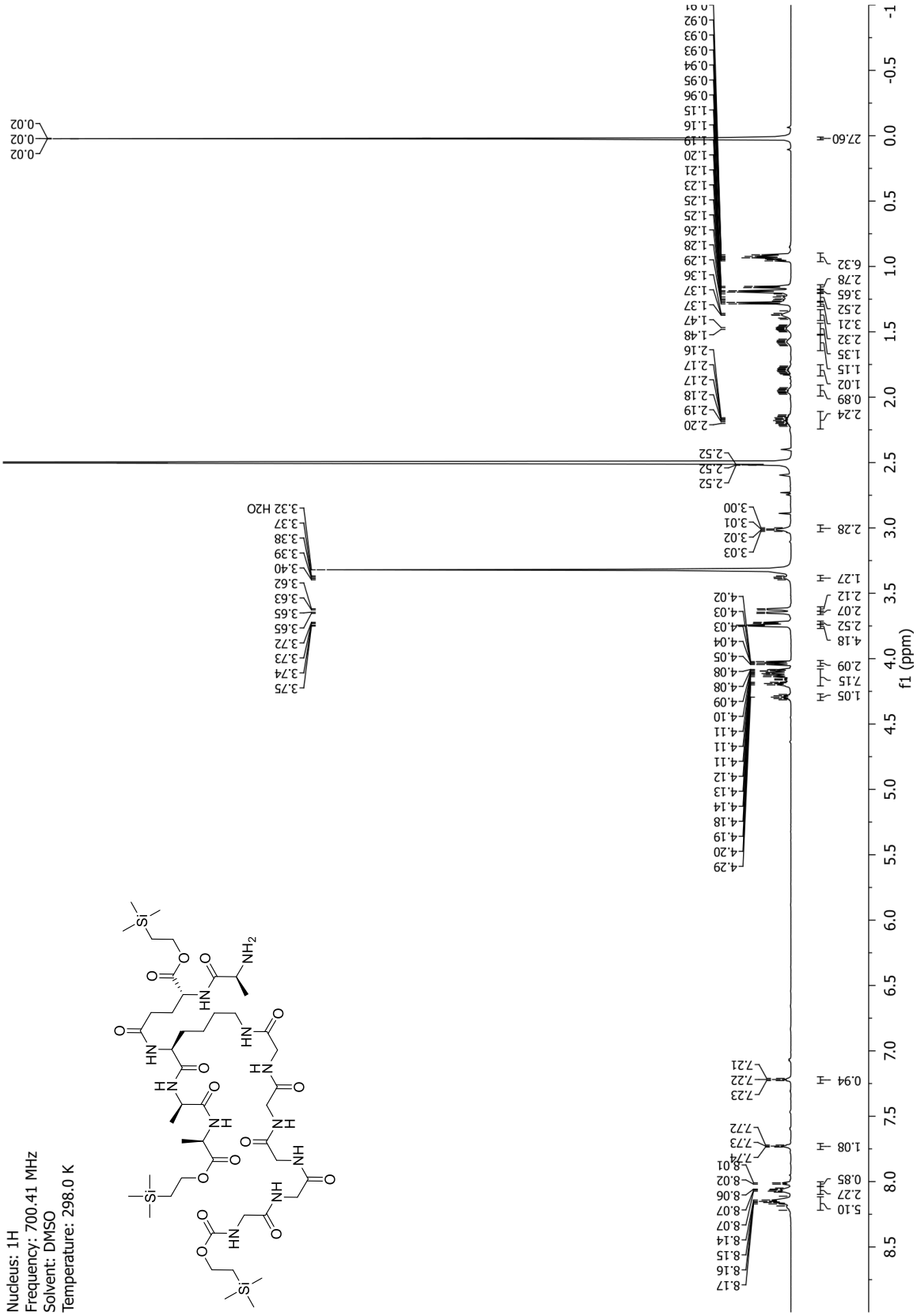

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)
16.8
17.3
18.6
22.6
27.1
28.7
31.3
31.9
38.4
42.0
42.0
42.1
42.1
43.5
46.6
47.6
49.8
51.7
52.3
61.9
62.6
65.7
66.9 (CH3)3COH
120.1
125.3
127.1
127.6
140.7
143.8
155.6
156.7
168.4
169.0
169.3
169.3
169.7
171.1
171.3
171.7
172.7
174.0

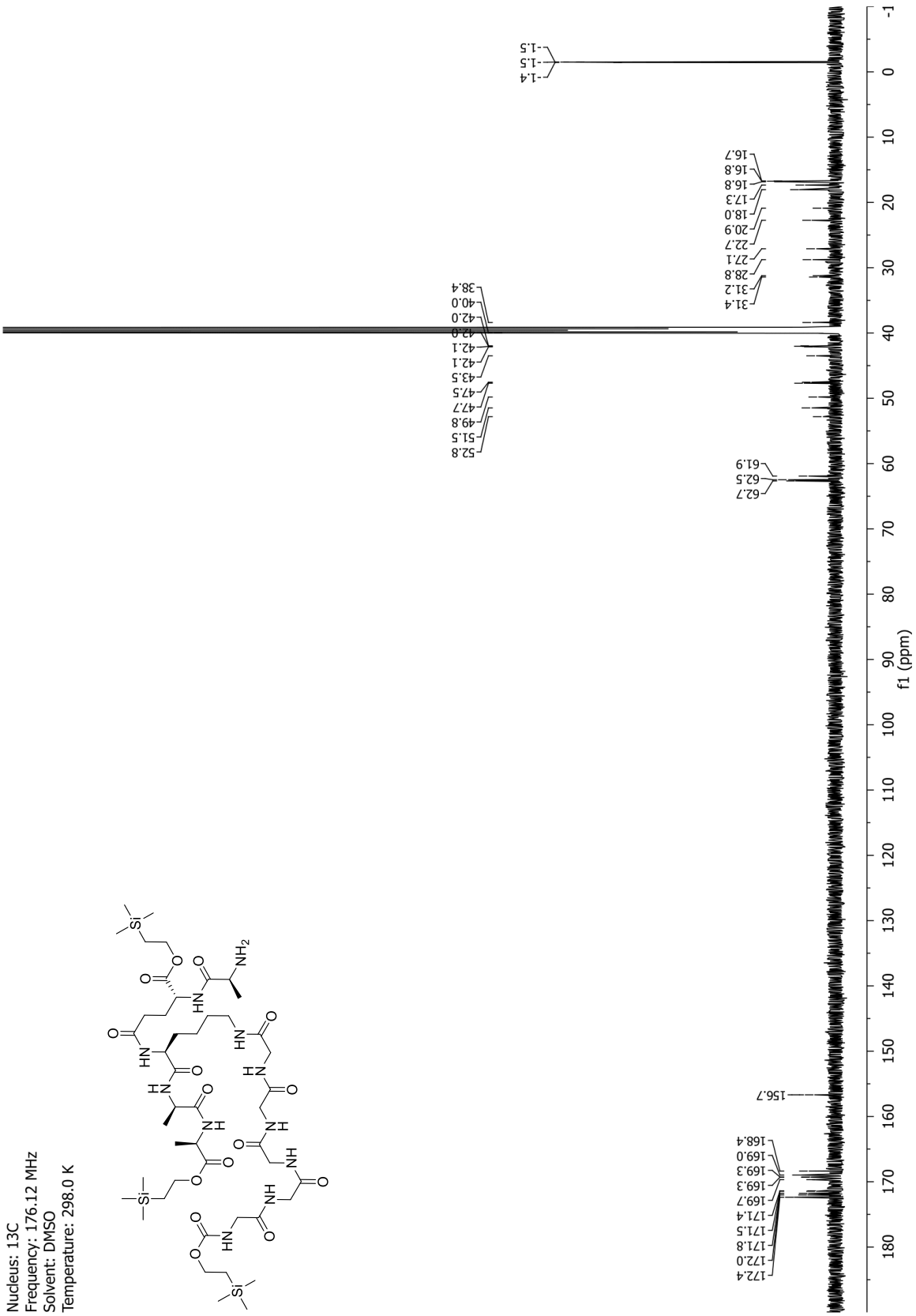
NMR-Spectra for Compound 141.2



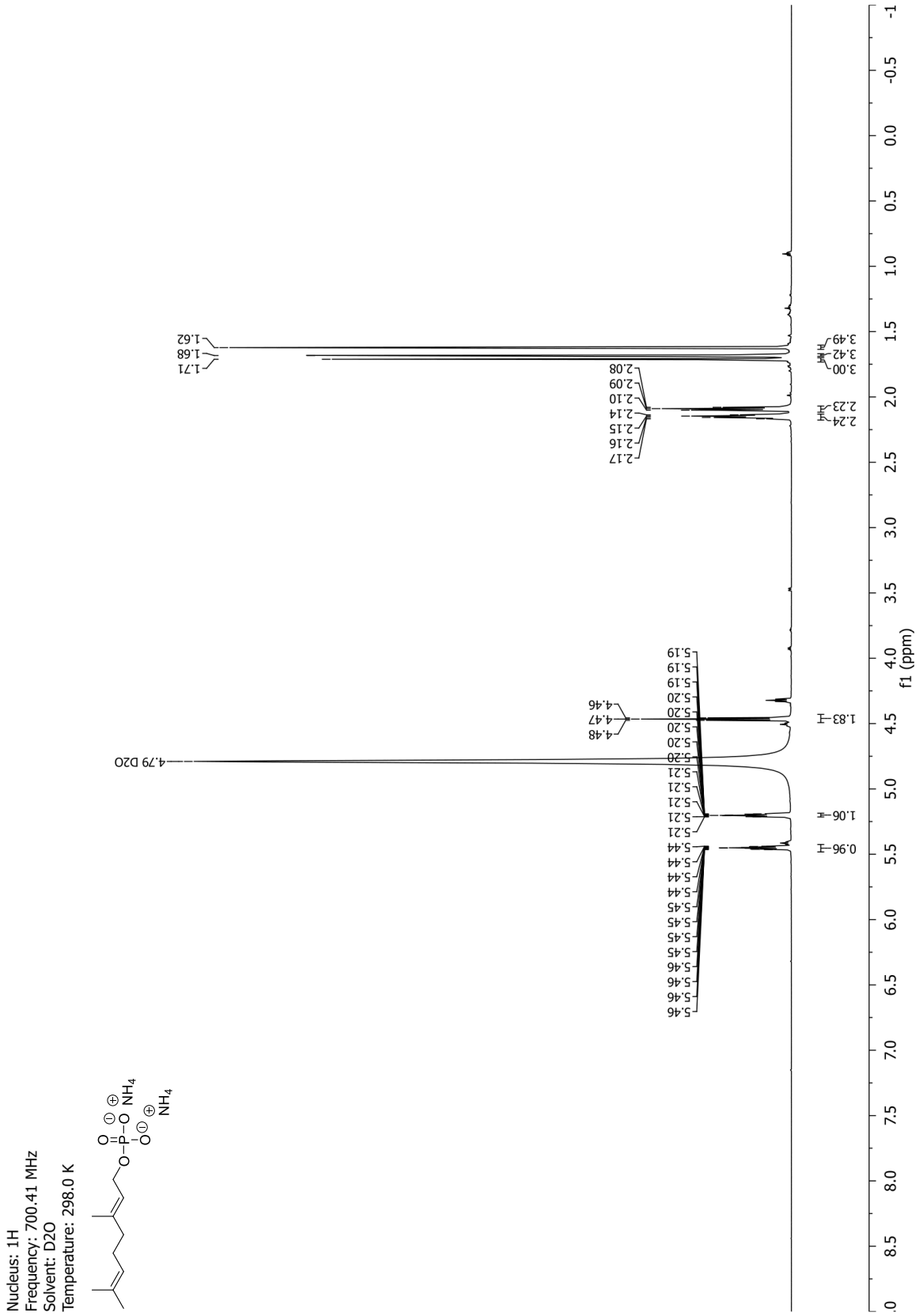

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)
-1.4
-1.5
-1.5
16.7
16.7
16.8
17.3
18.1
18.7
22.7
27.0
28.8
30.8 DMF
31.2
31.4
35.8 DMF
38.4
42.0
42.0
42.1
43.5
46.6
47.5
47.7
49.8
51.7
52.8
61.9
62.5
62.6
65.7
120.1
125.3
127.1
127.6
140.7
143.8
155.6
156.7
162.3 DMF
168.4
169.0
169.3
169.3
169.7
171.4
171.5
171.7
172.0
172.4
172.7

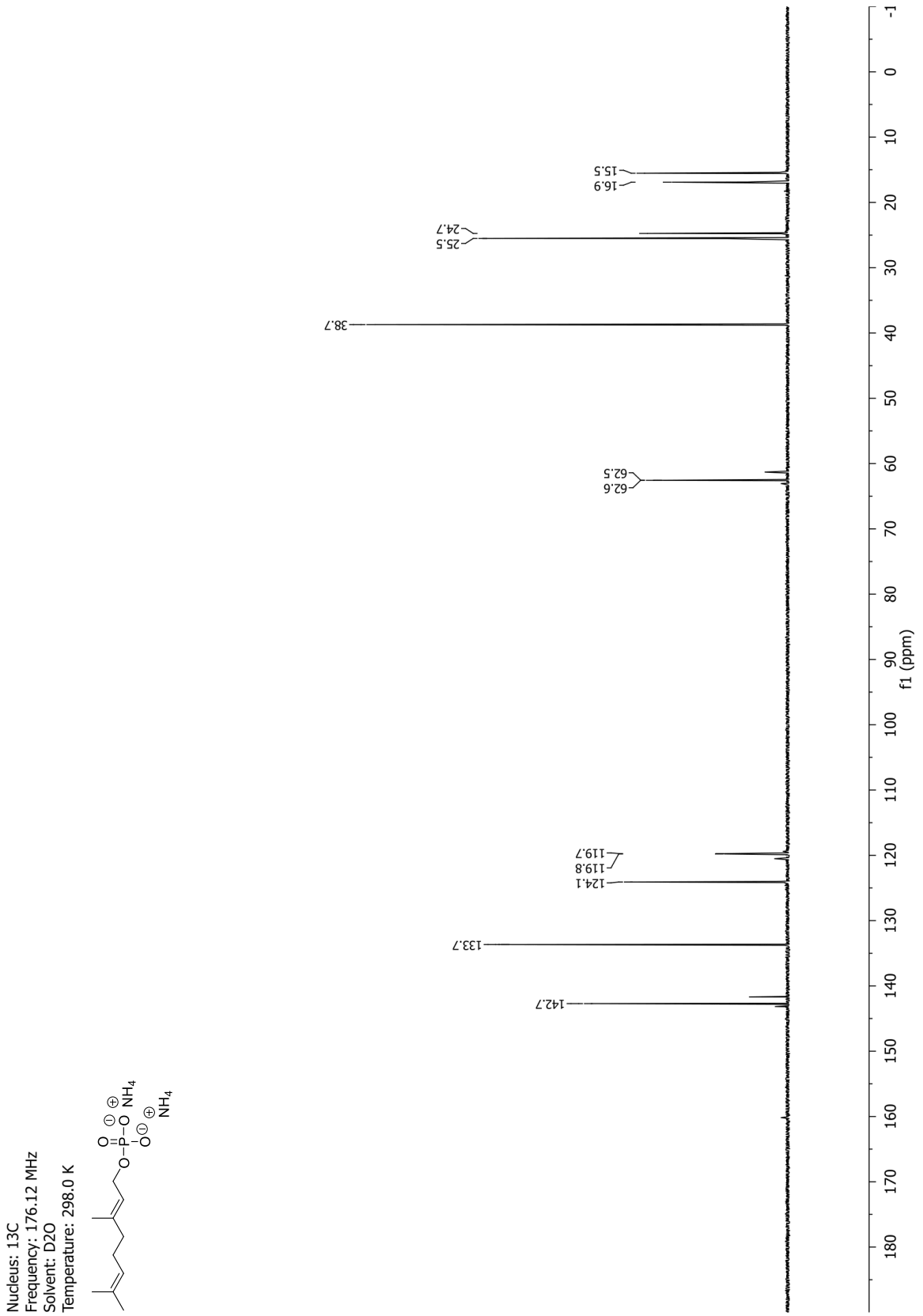
NMR-Spectra for Compound 5

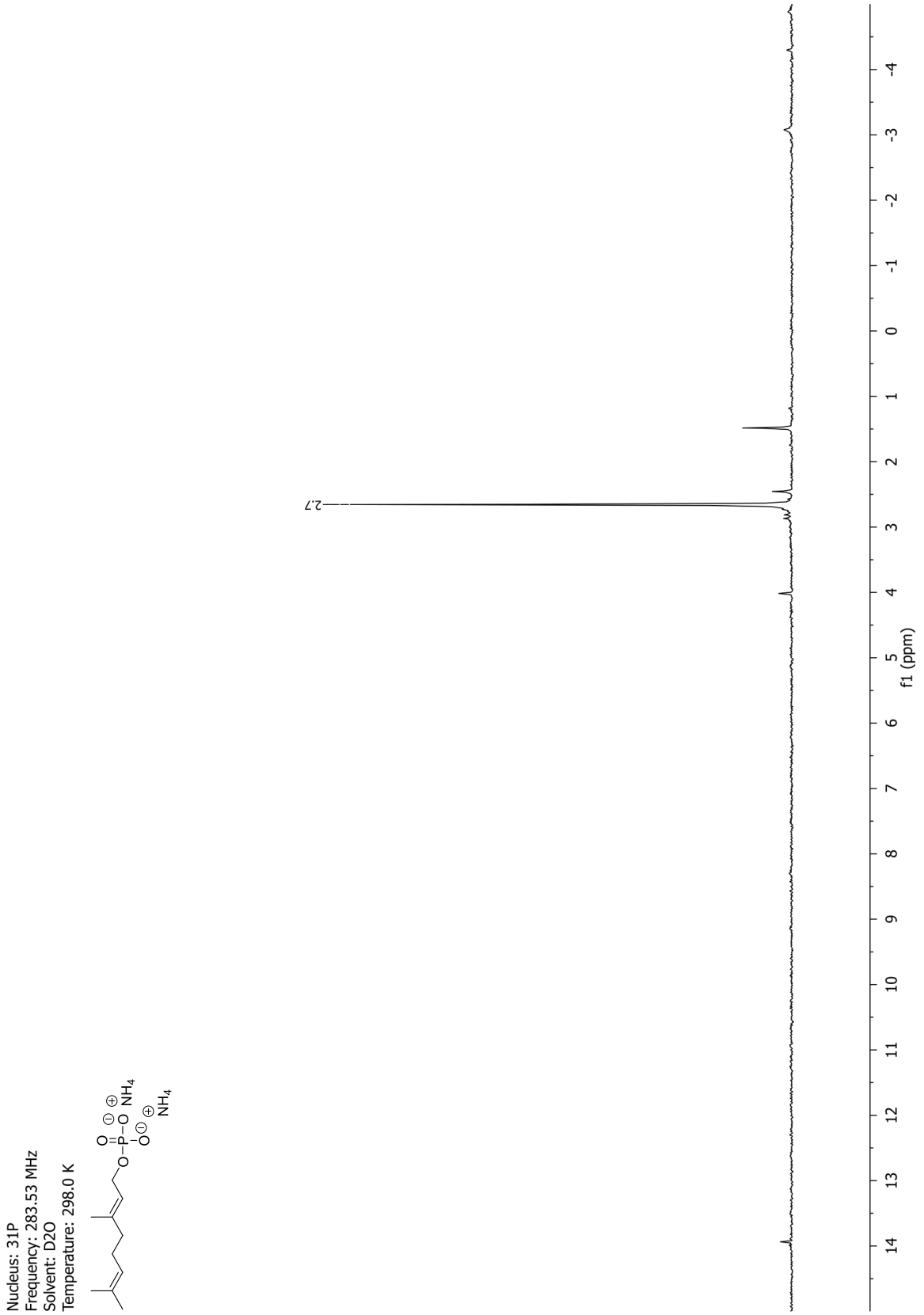



Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
f1 (ppm)

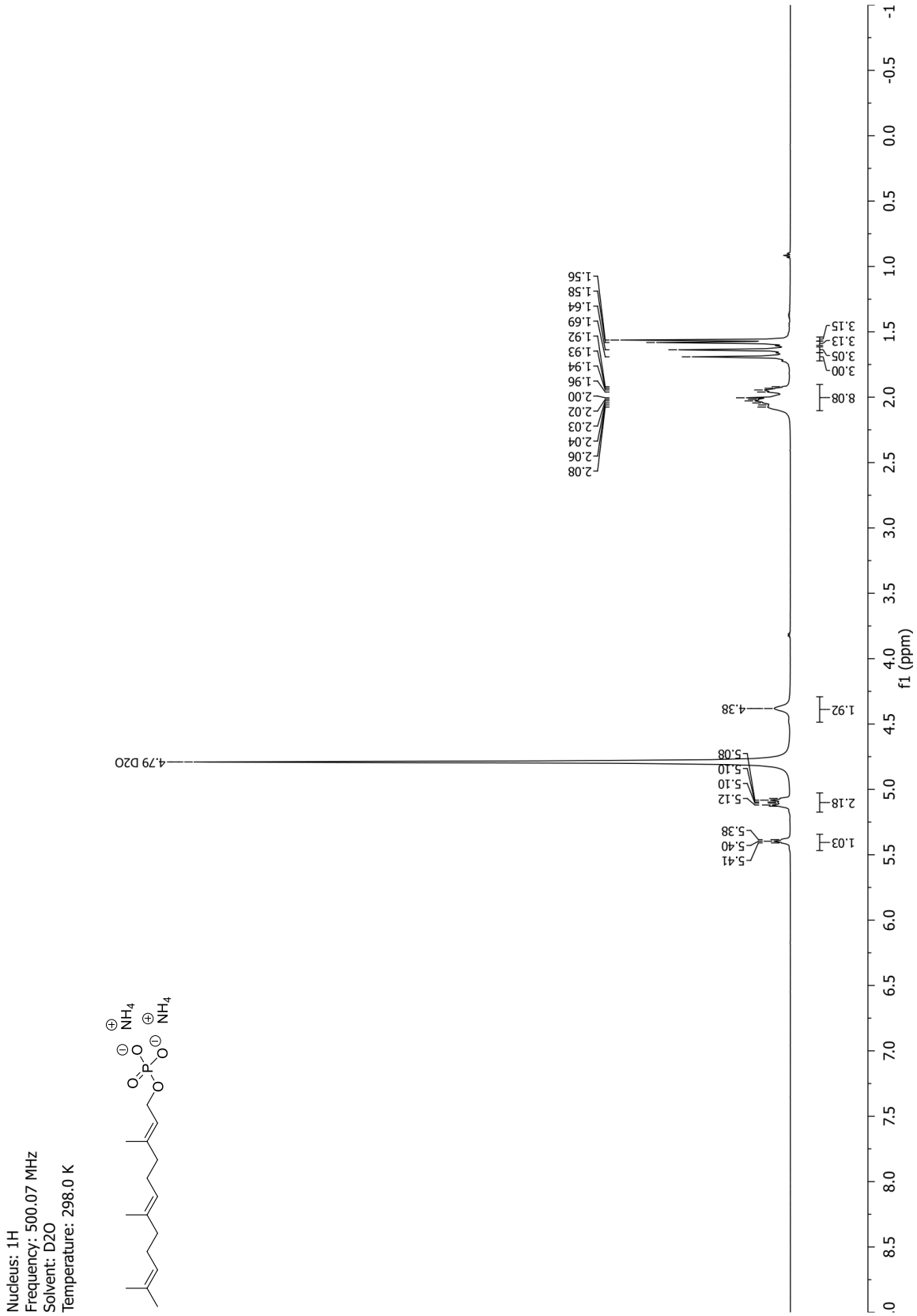
NMR-Spectra for Compound 143

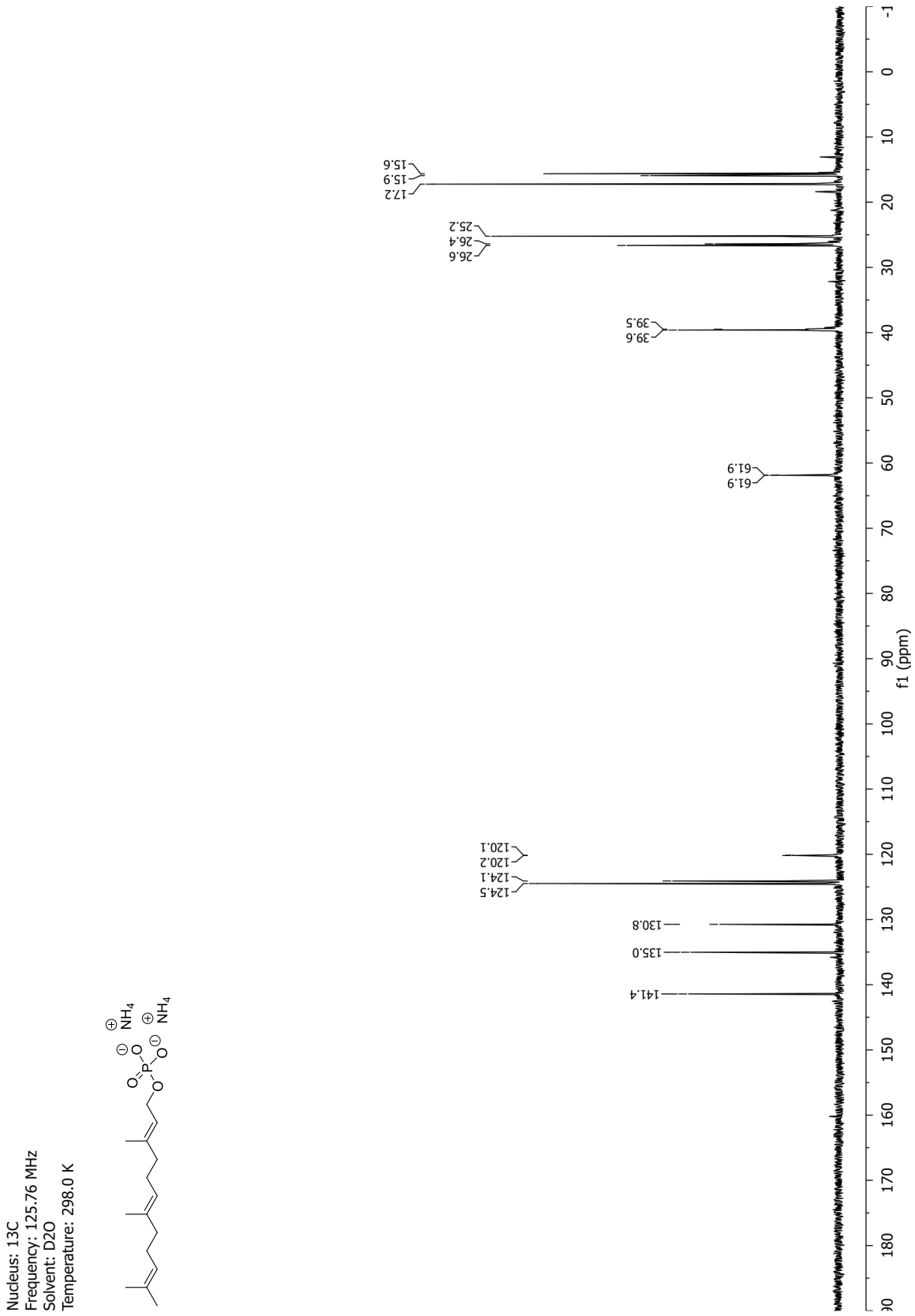


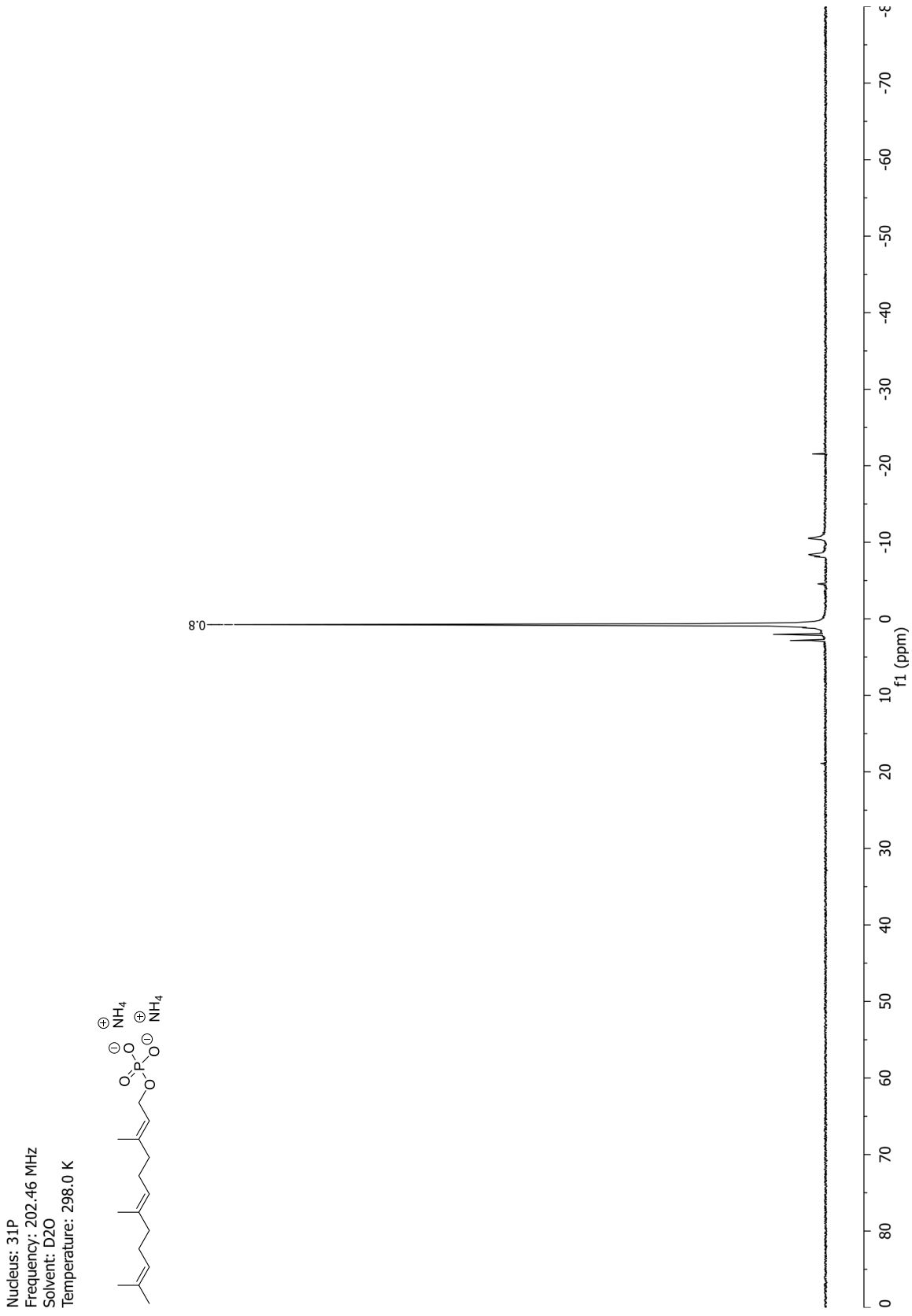

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: D2O
Temperature: 298.0 K
142.7
133.7
124.1
119.8
119.7
62.6
62.5
38.7
25.5
24.7
16.9
15.5
f1 (ppm)


Nucleus: 31P
Frequency: 283.53 MHz
Solvent: D2O
Temperature: 298.0 K
2.7
f1 (ppm)

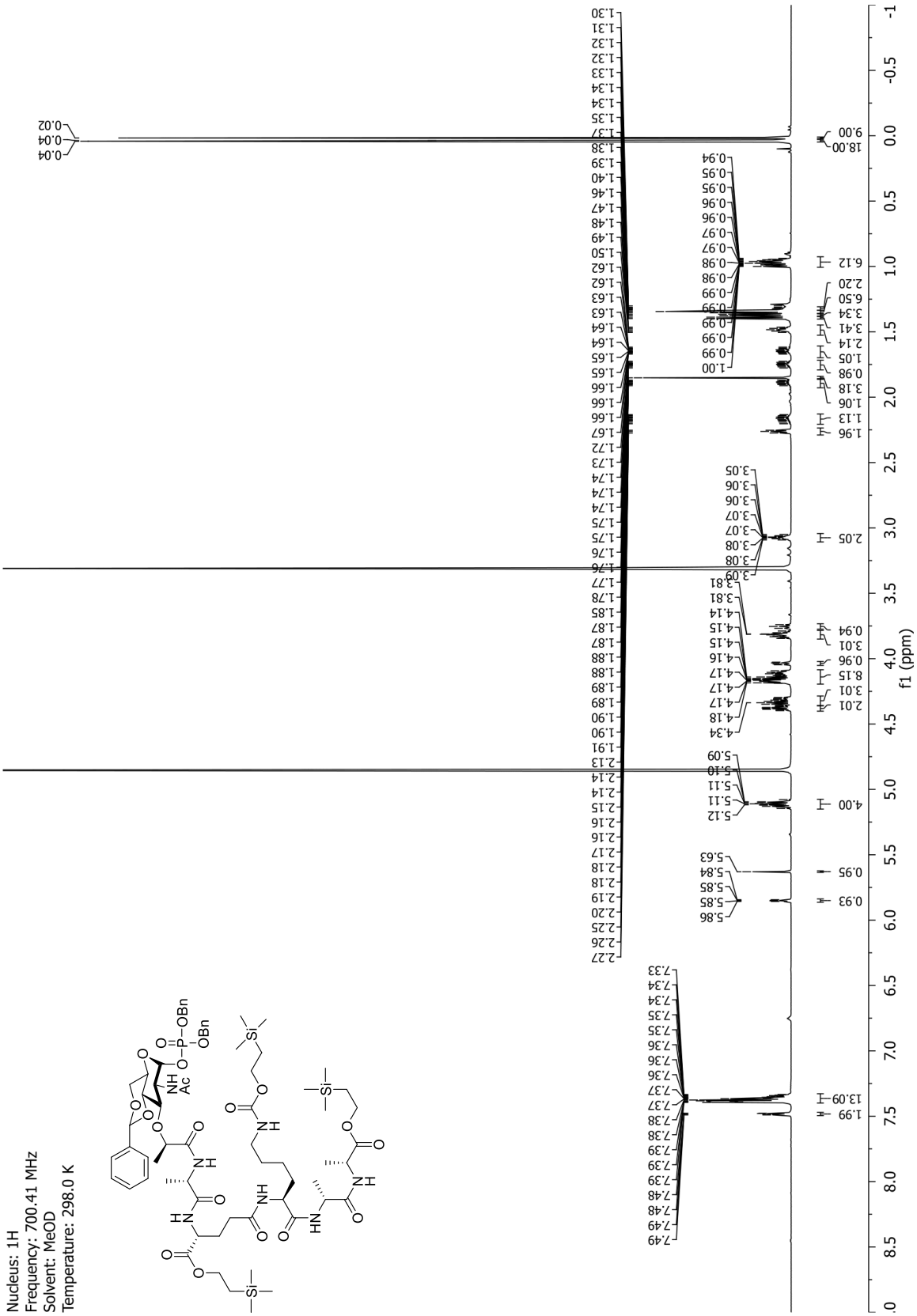
NMR-Spectra for Compound 6

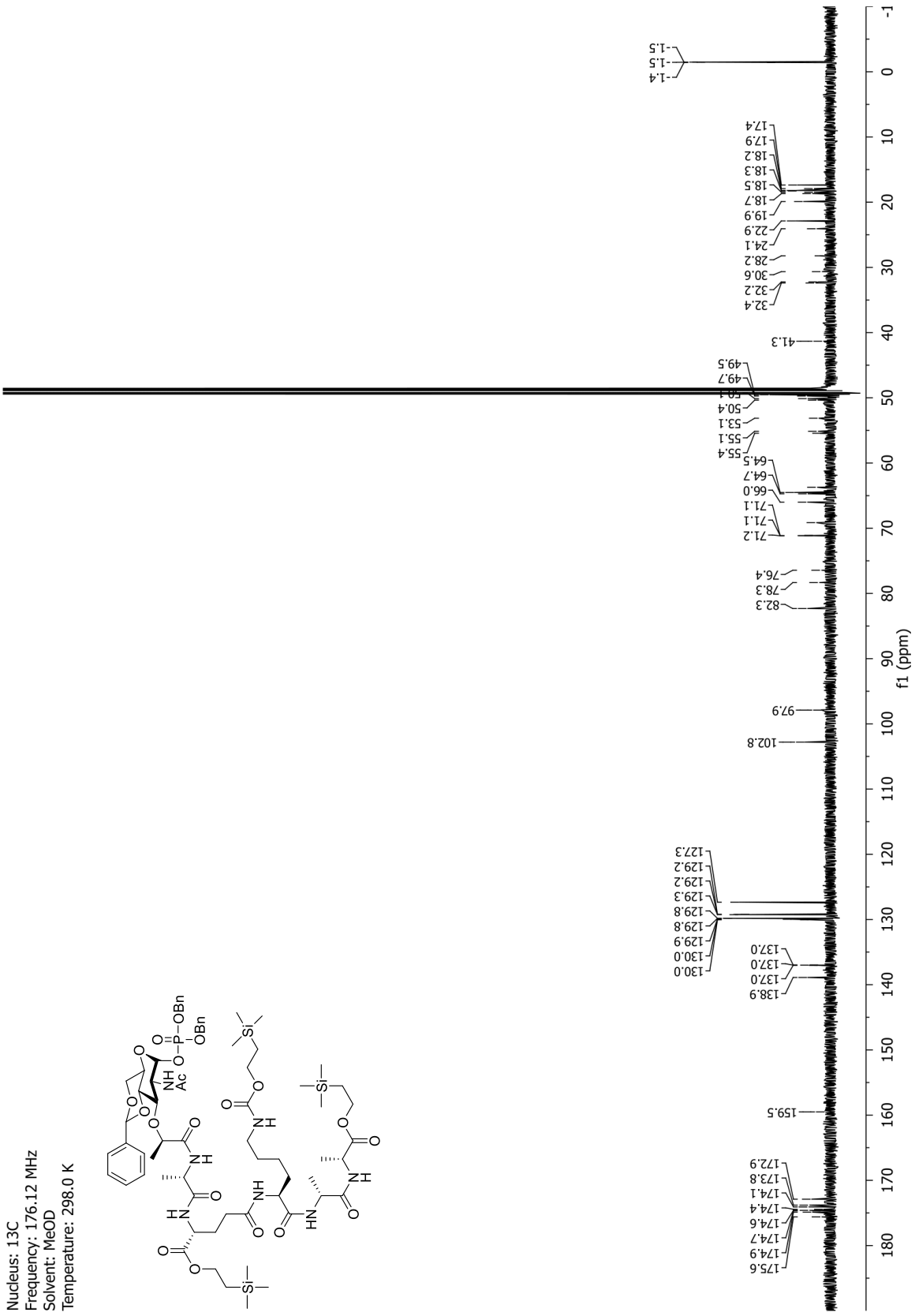


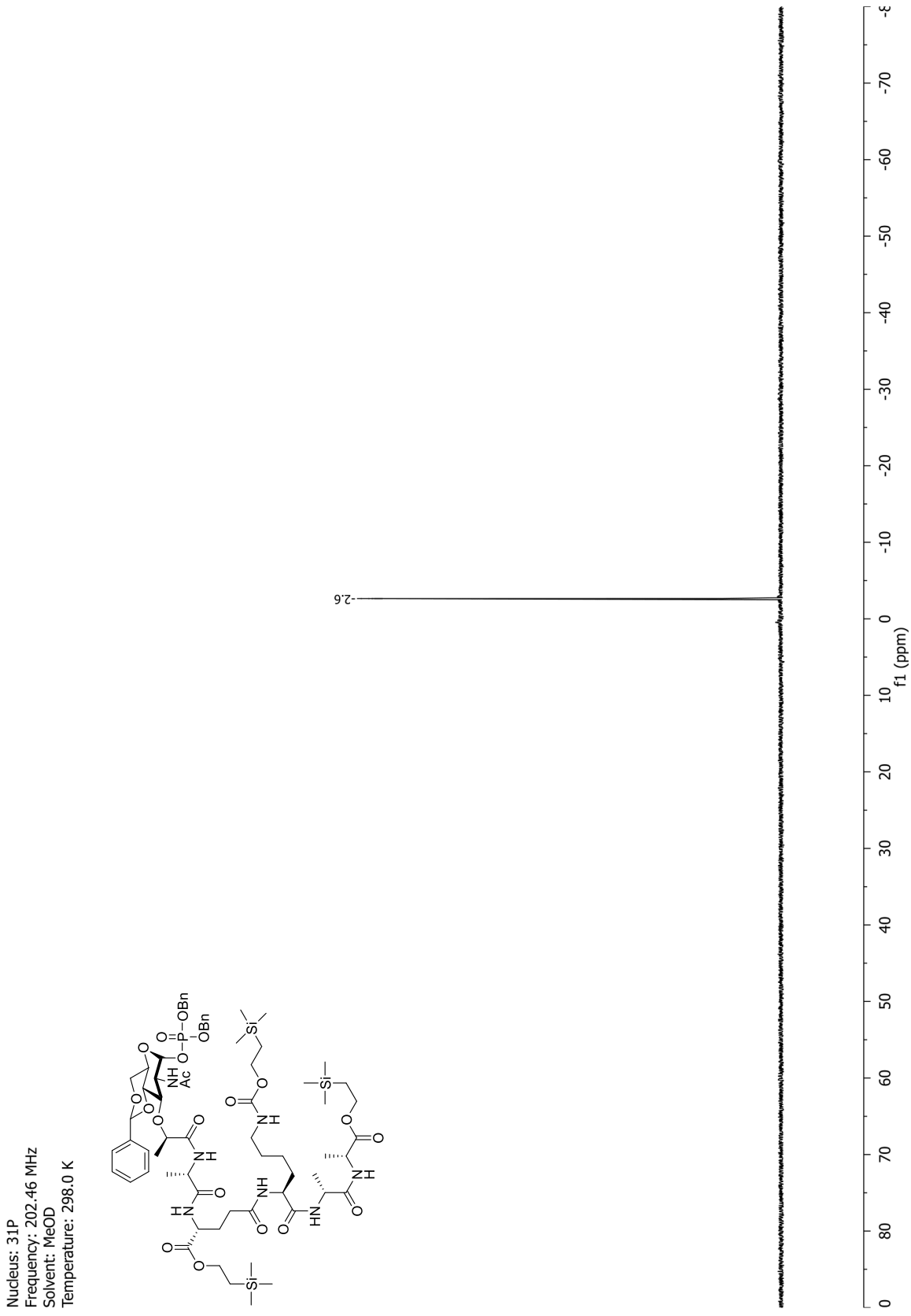

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: D2O
Temperature: 298.0 K
NH4
NH4
15.6
15.9
17.2
25.2
26.4
26.6
39.5
39.6
61.9
61.9
120.1
120.2
124.1
124.5
130.8
135.0
141.4
f1 (ppm)


Nucleus: 31P
Frequency: 202.46 MHz
Solvent: D2O
Temperature: 298.0 K
8.0
f1 (ppm)

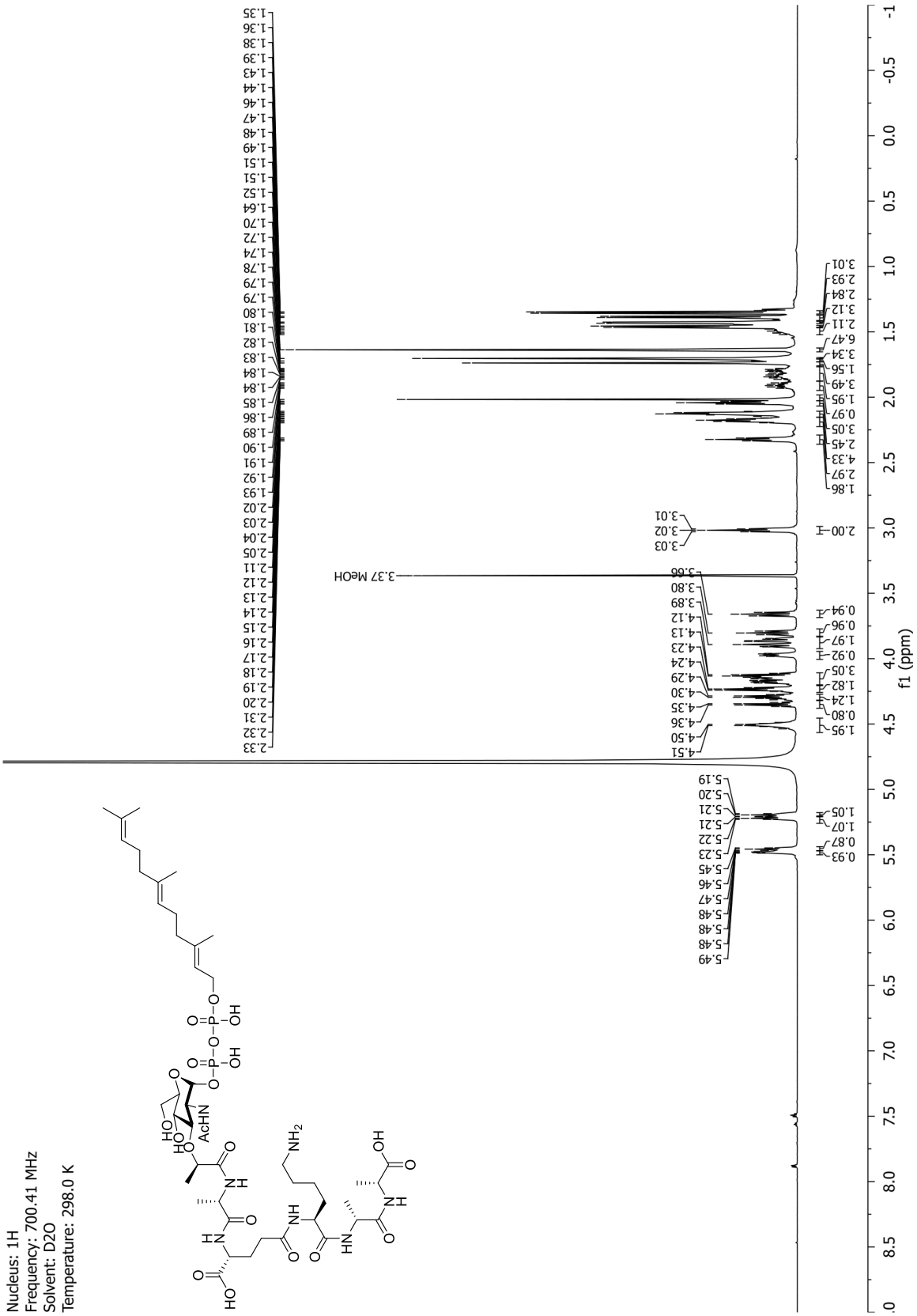
NMR-Spectra for Compound 149

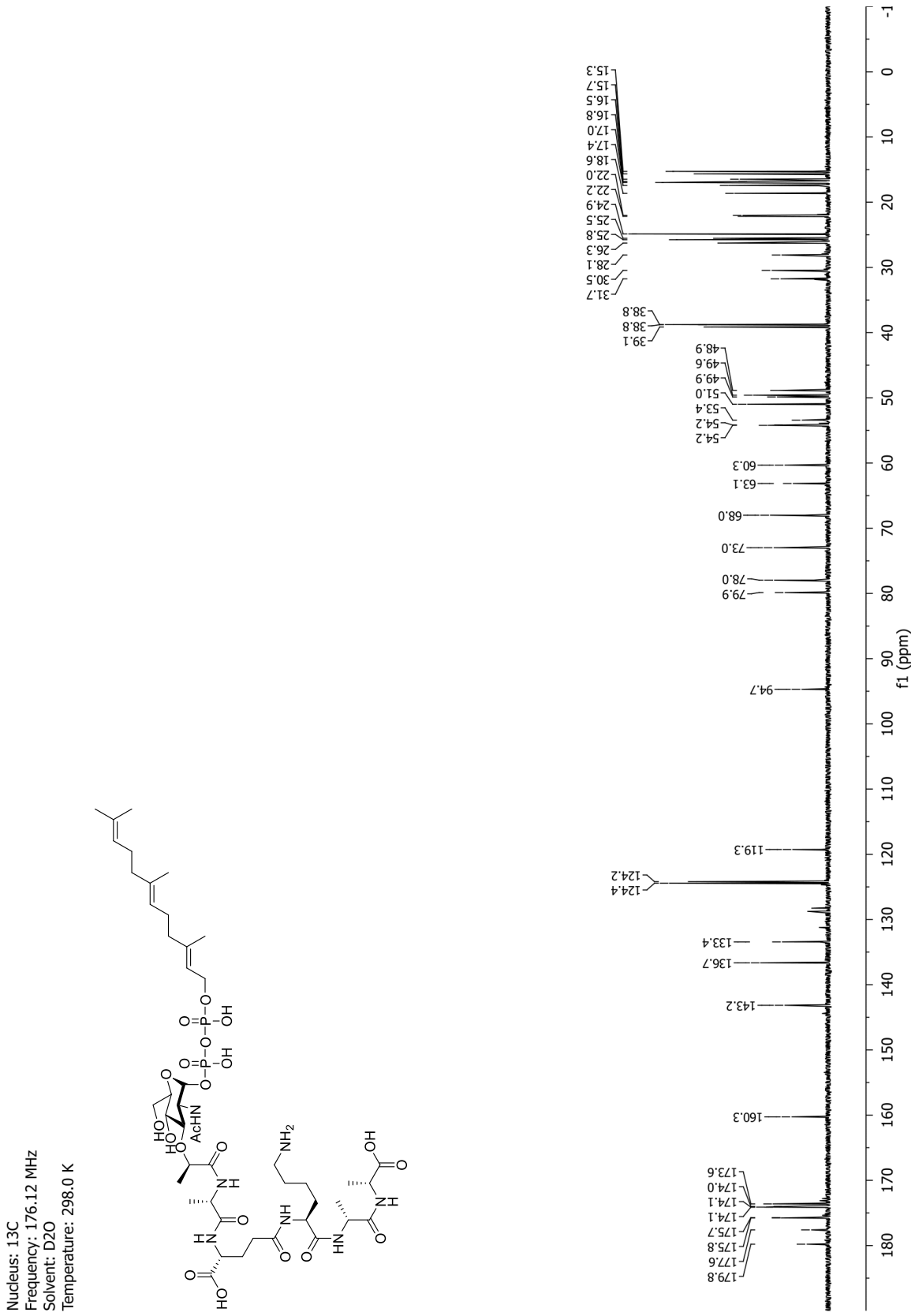


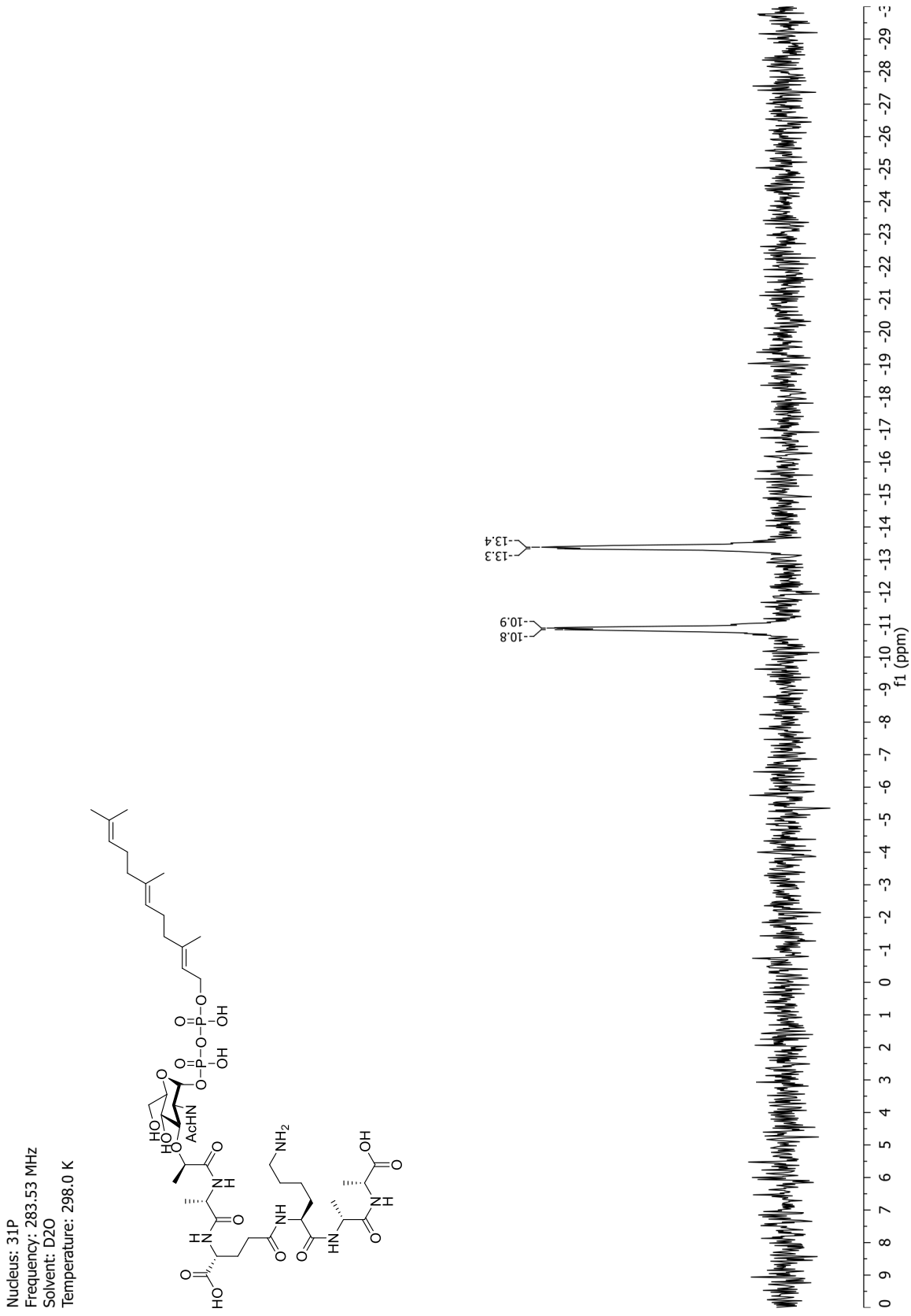

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)

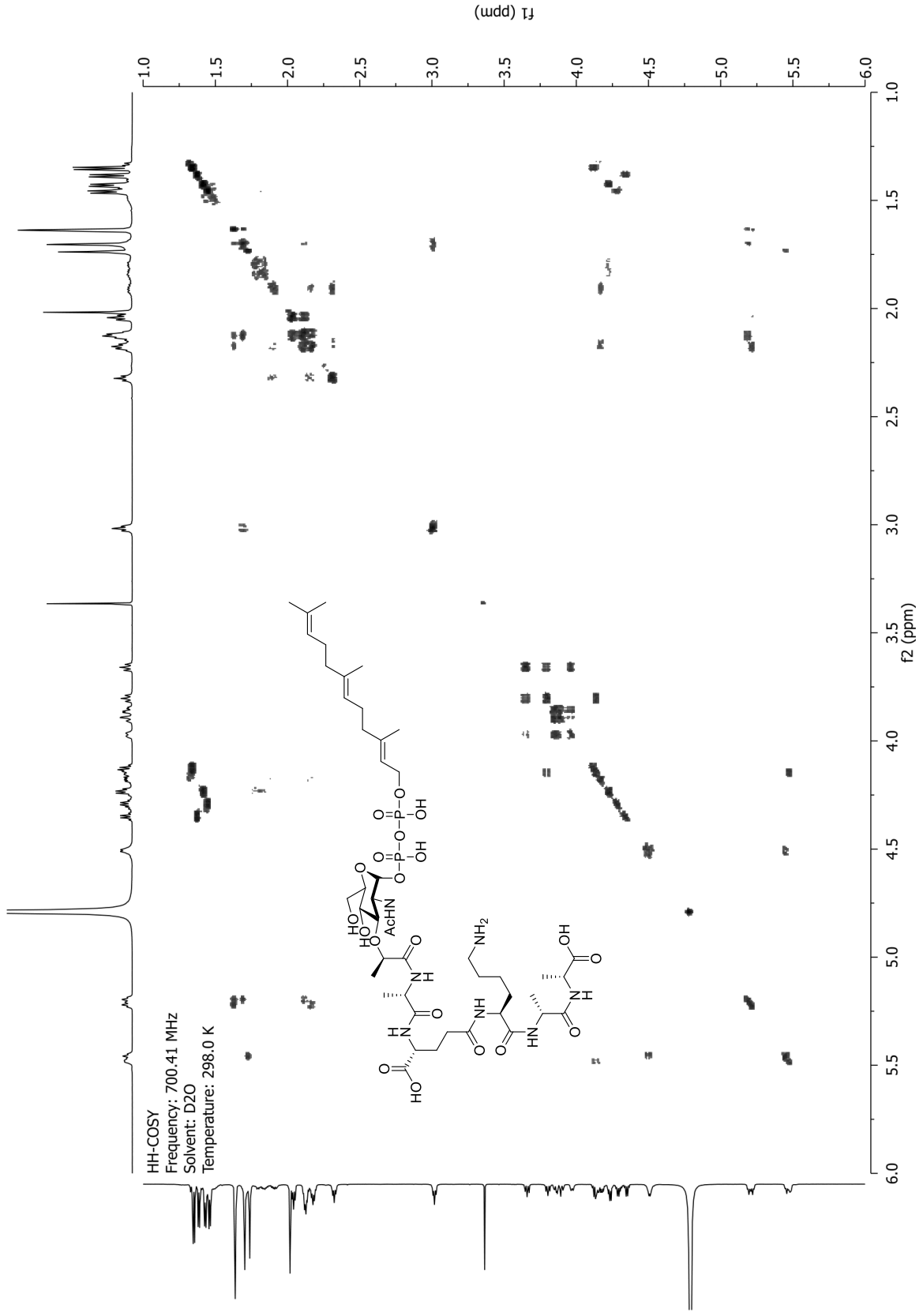

Nucleus: 31P
Frequency: 202.46 MHz
Solvent: MeOD
Temperature: 298.0 K
-2.6
f1 (ppm)

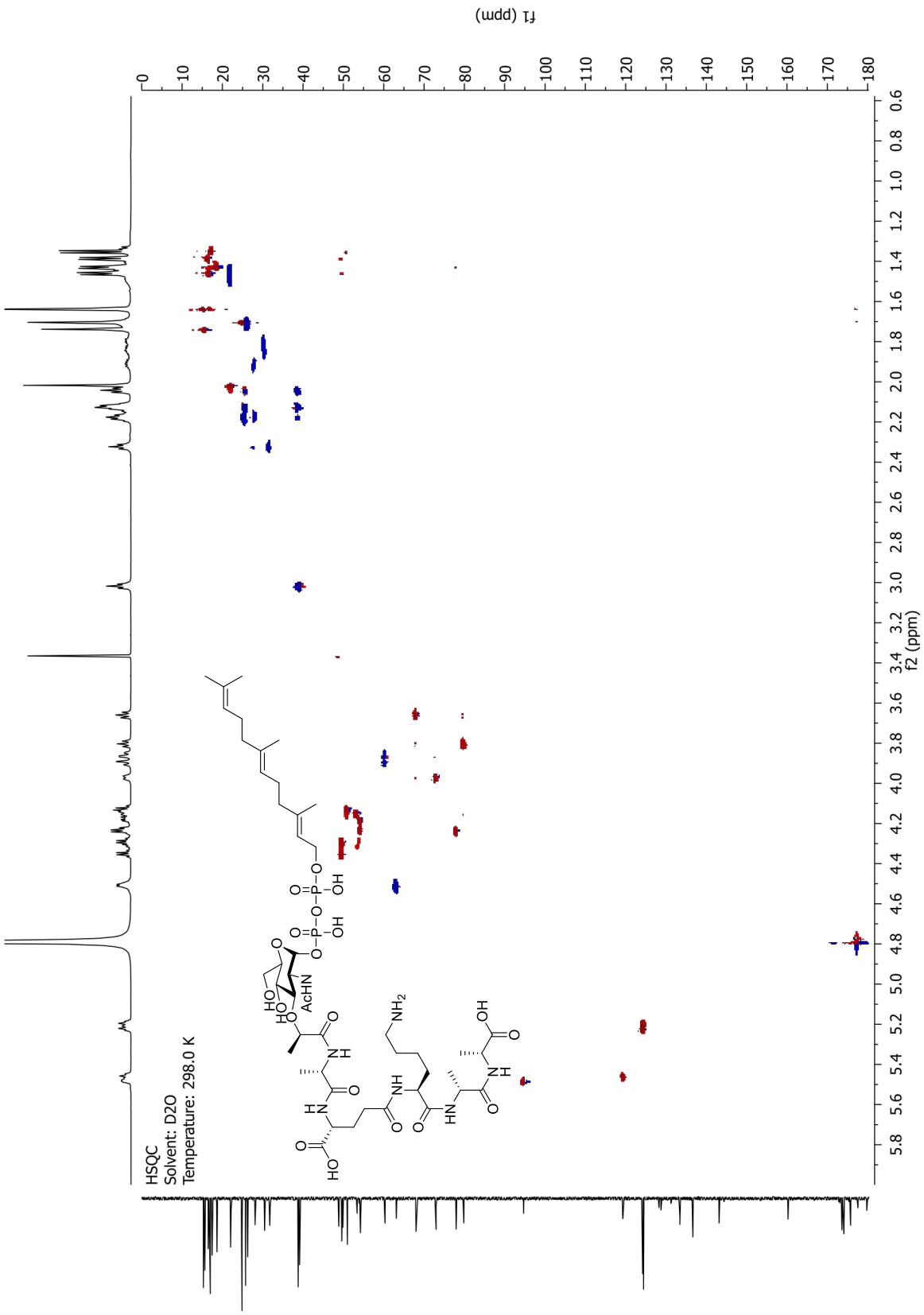
NMR-Spectra for Compound 1

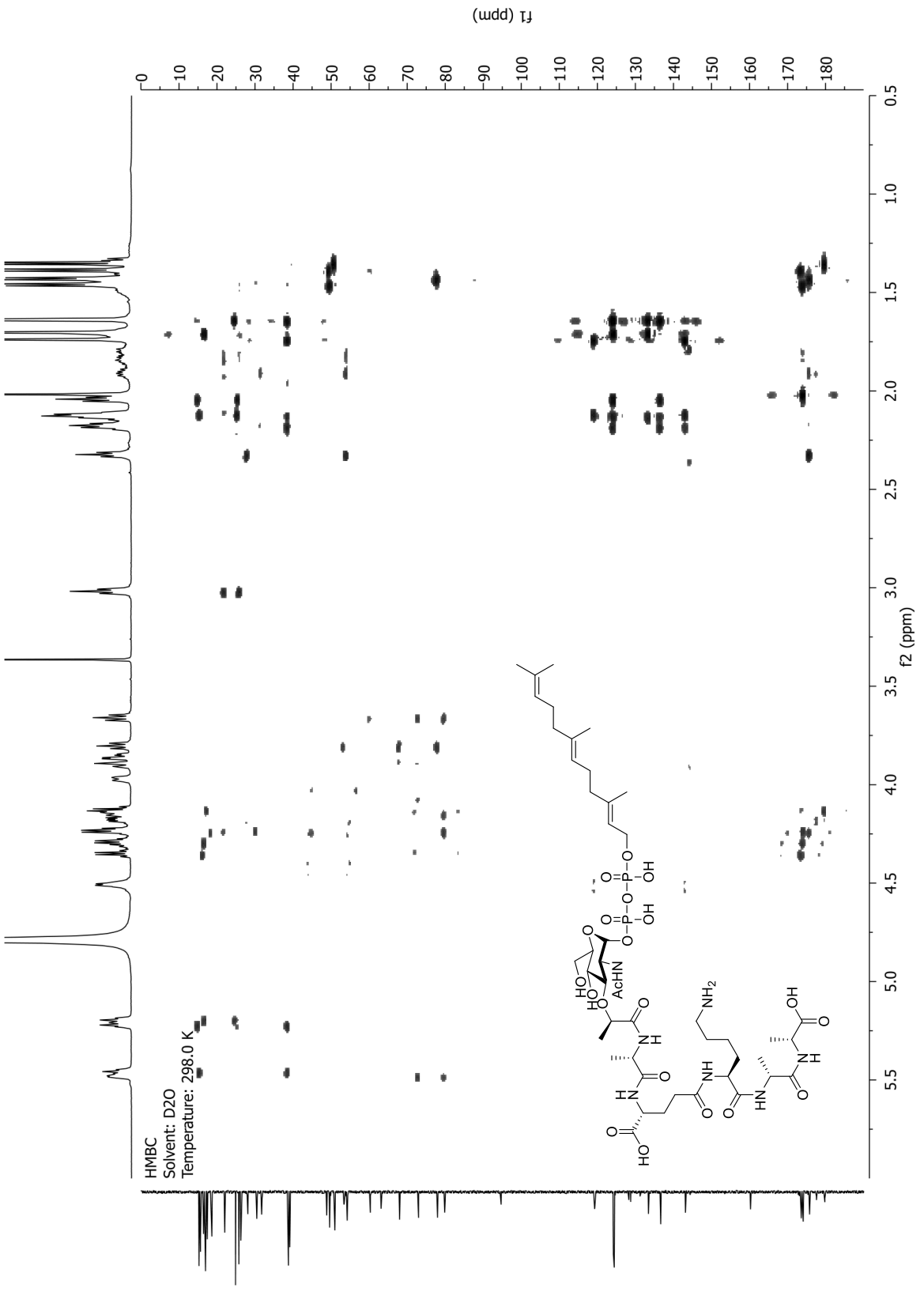


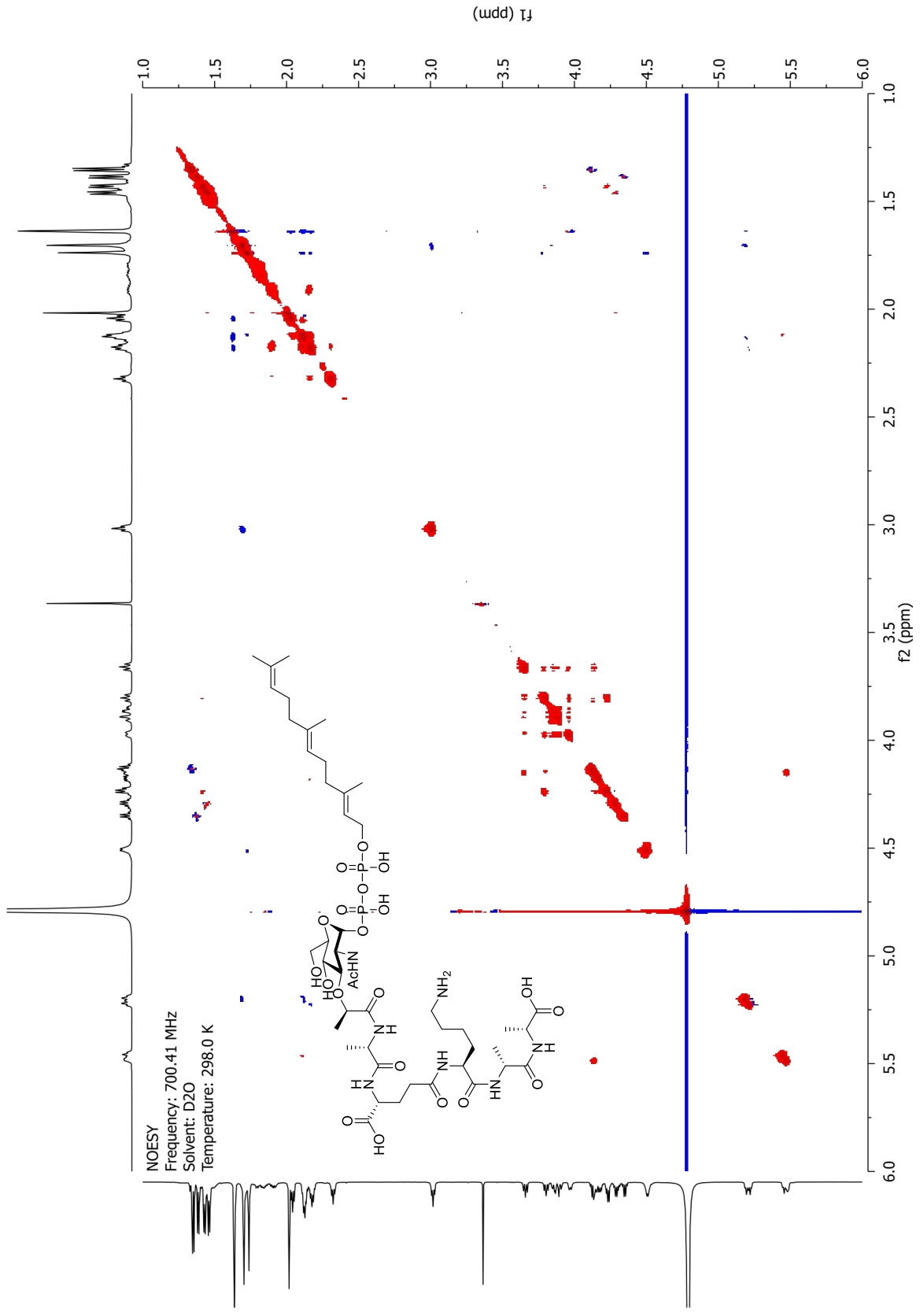

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)

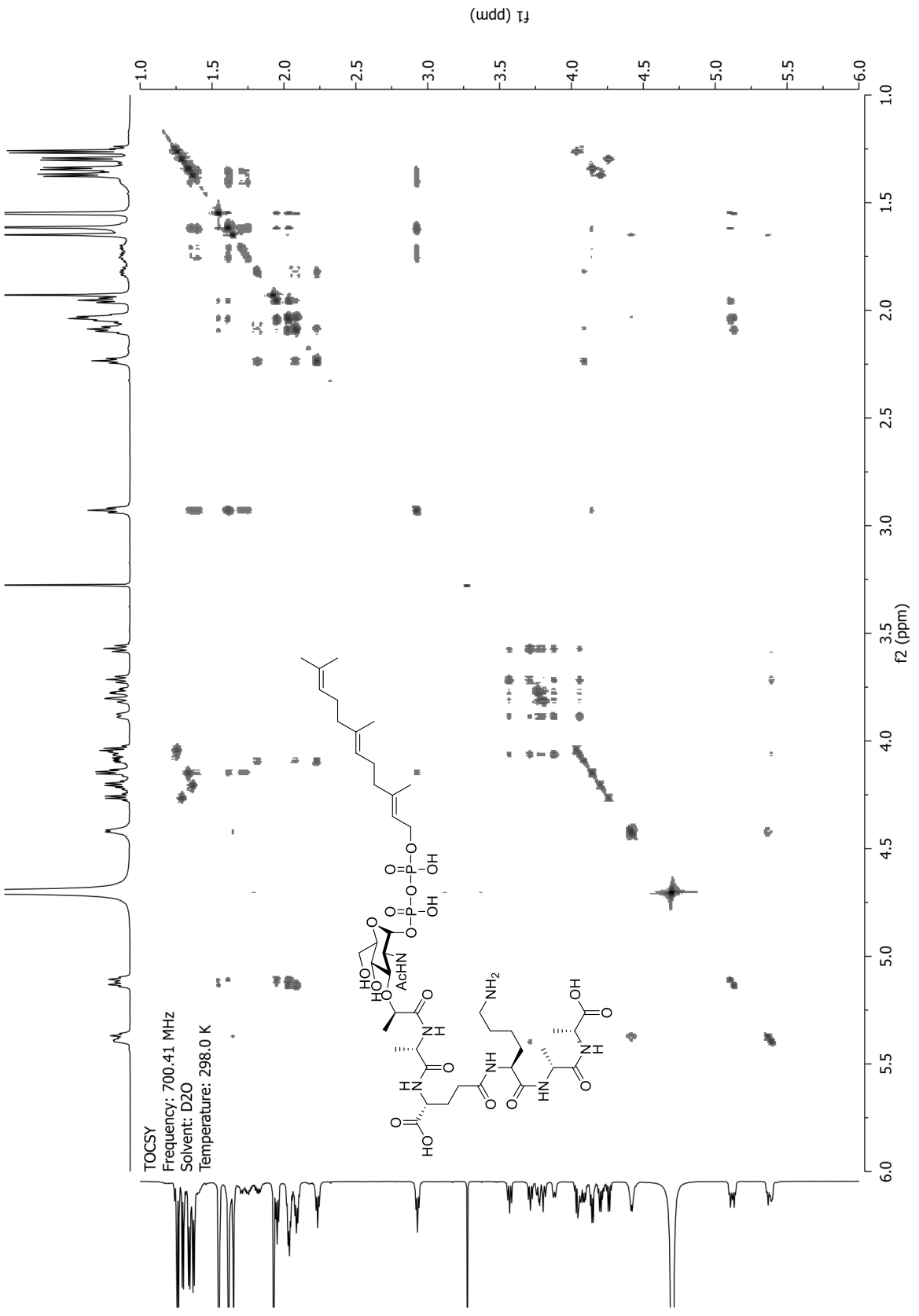

Nucleus: 31P
Frequency: 283.53 MHz
Solvent: D2O
Temperature: 298.0 K
-13.4
-13.3
-10.9
-10.8
f1 (ppm)


HH-COSY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f2 (ppm)
f1 (ppm)

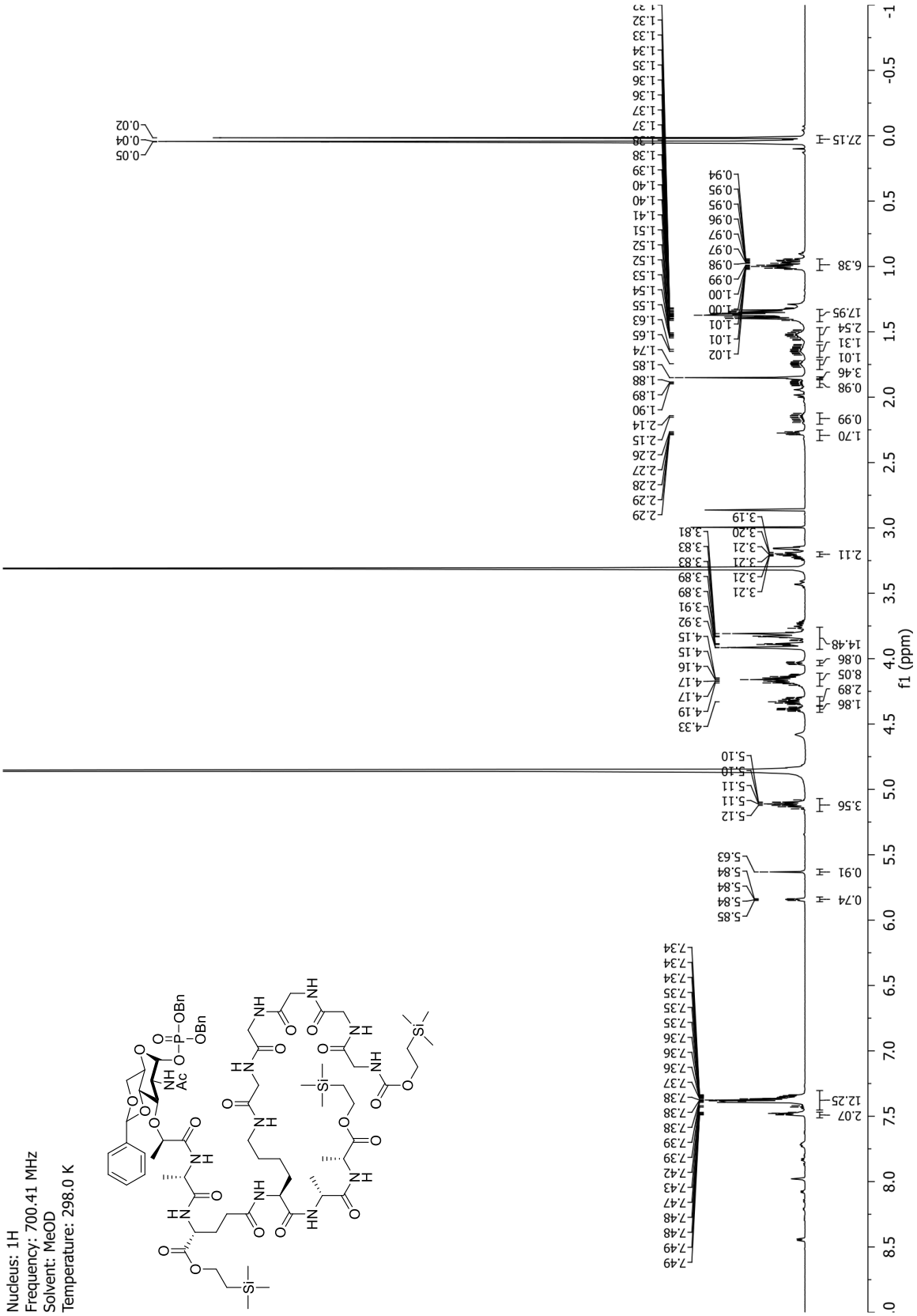

HSQC
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)

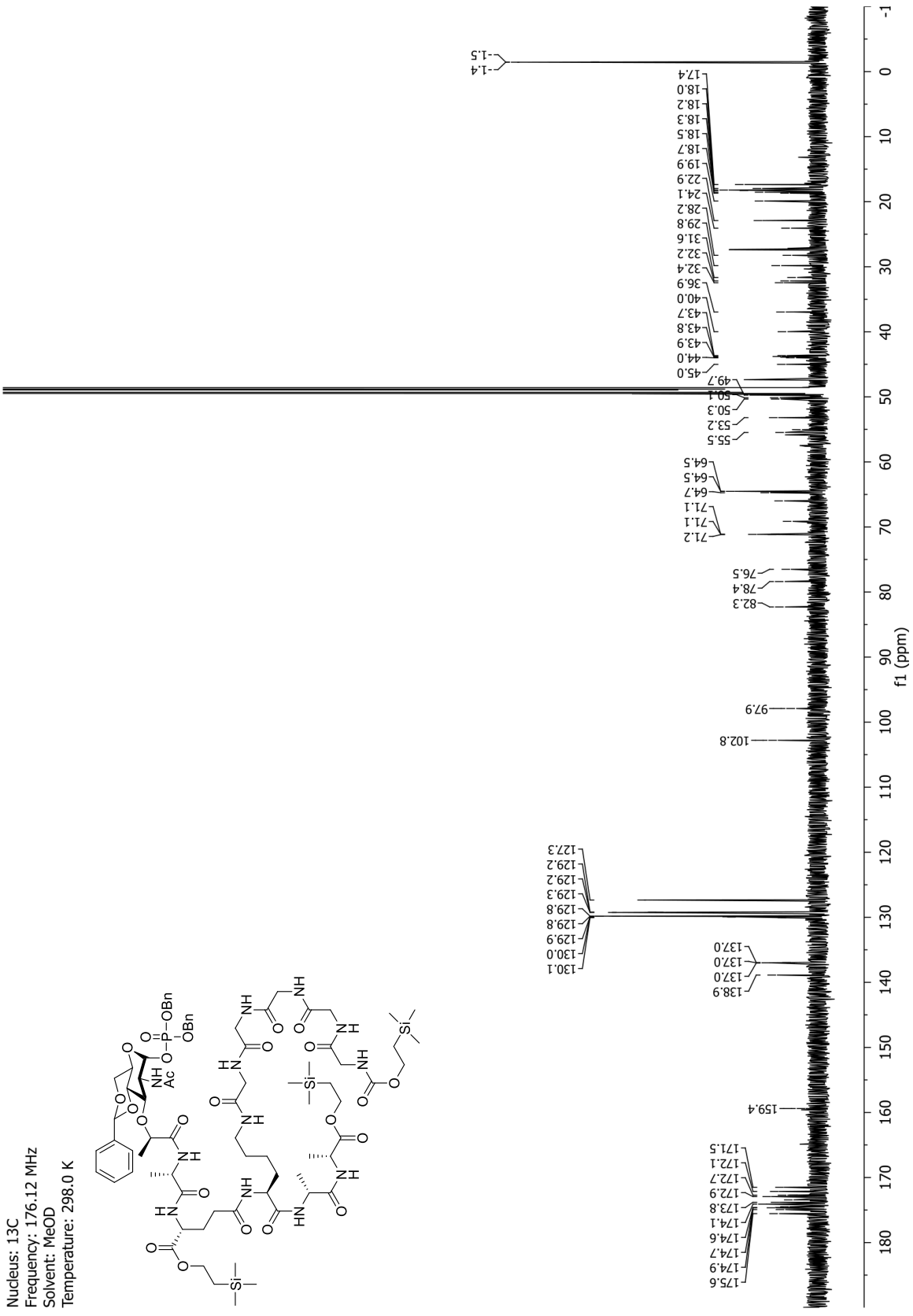

f1 (ppm)
f2 (ppm)
HMBC
Solvent: D2O
Temperature: 298.0 K

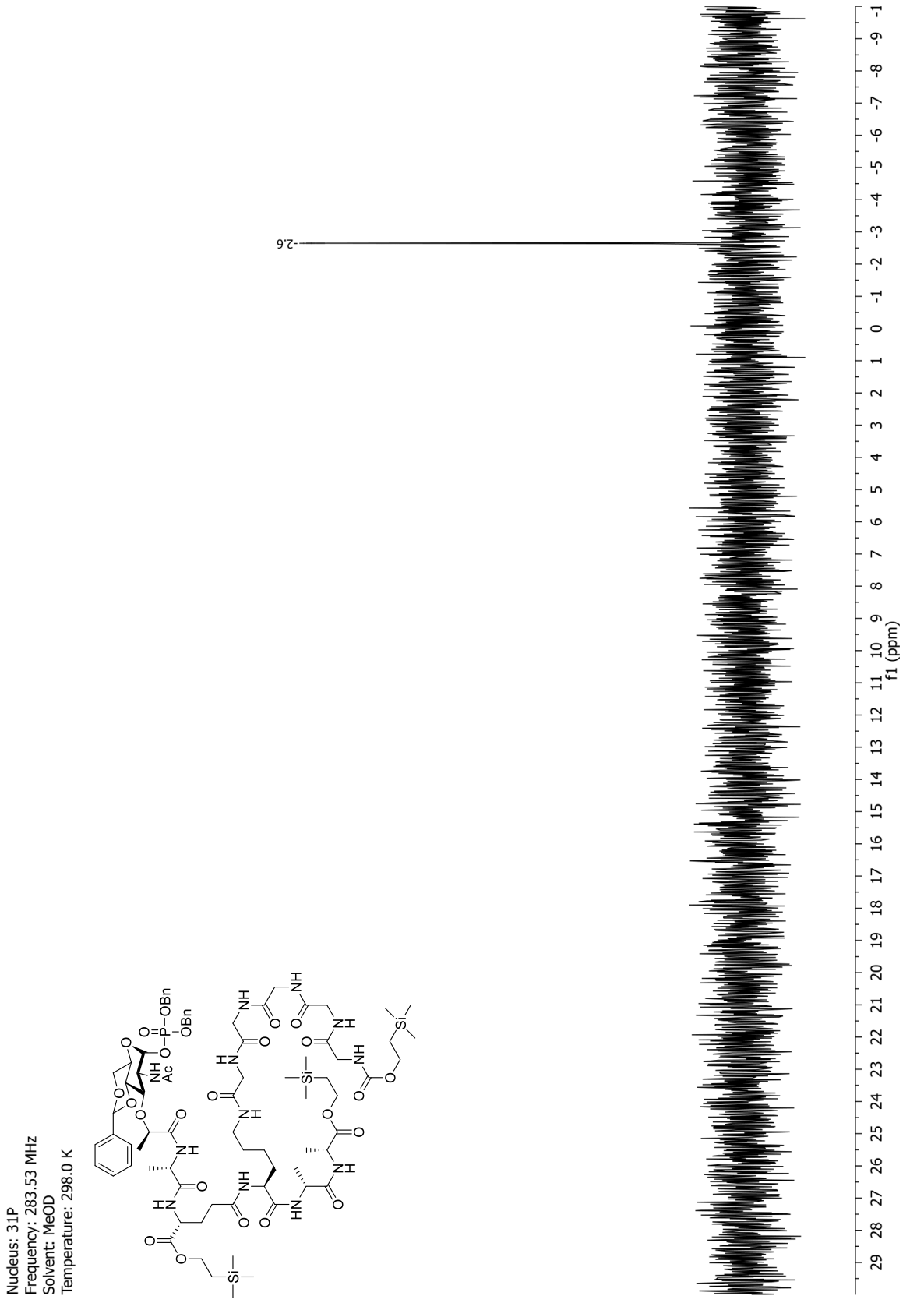

NOESY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
AcHN
NH2
OH
HO


TOCSY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f2 (ppm)
f1 (ppm)

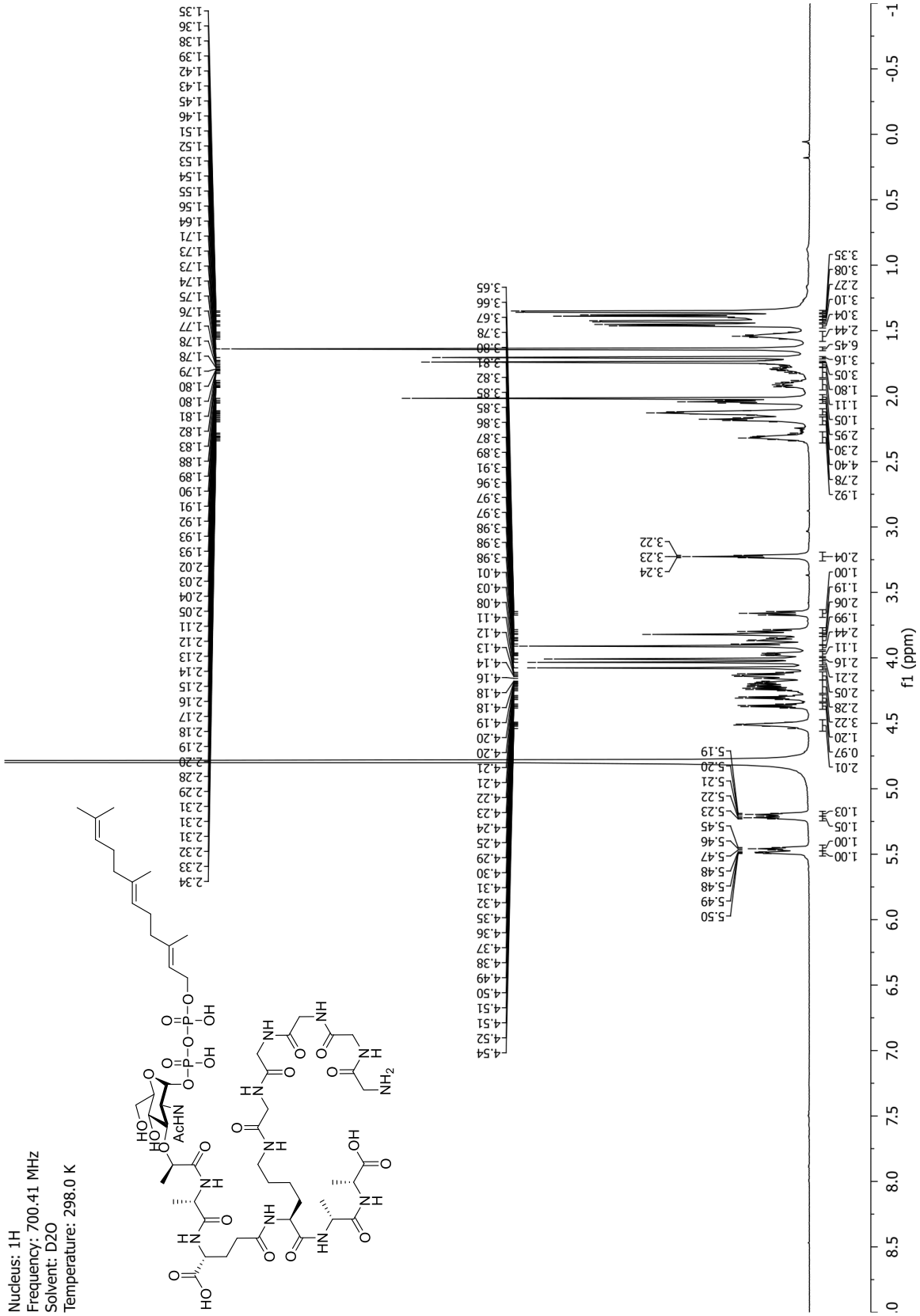
NMR-Spectra for Compound 154

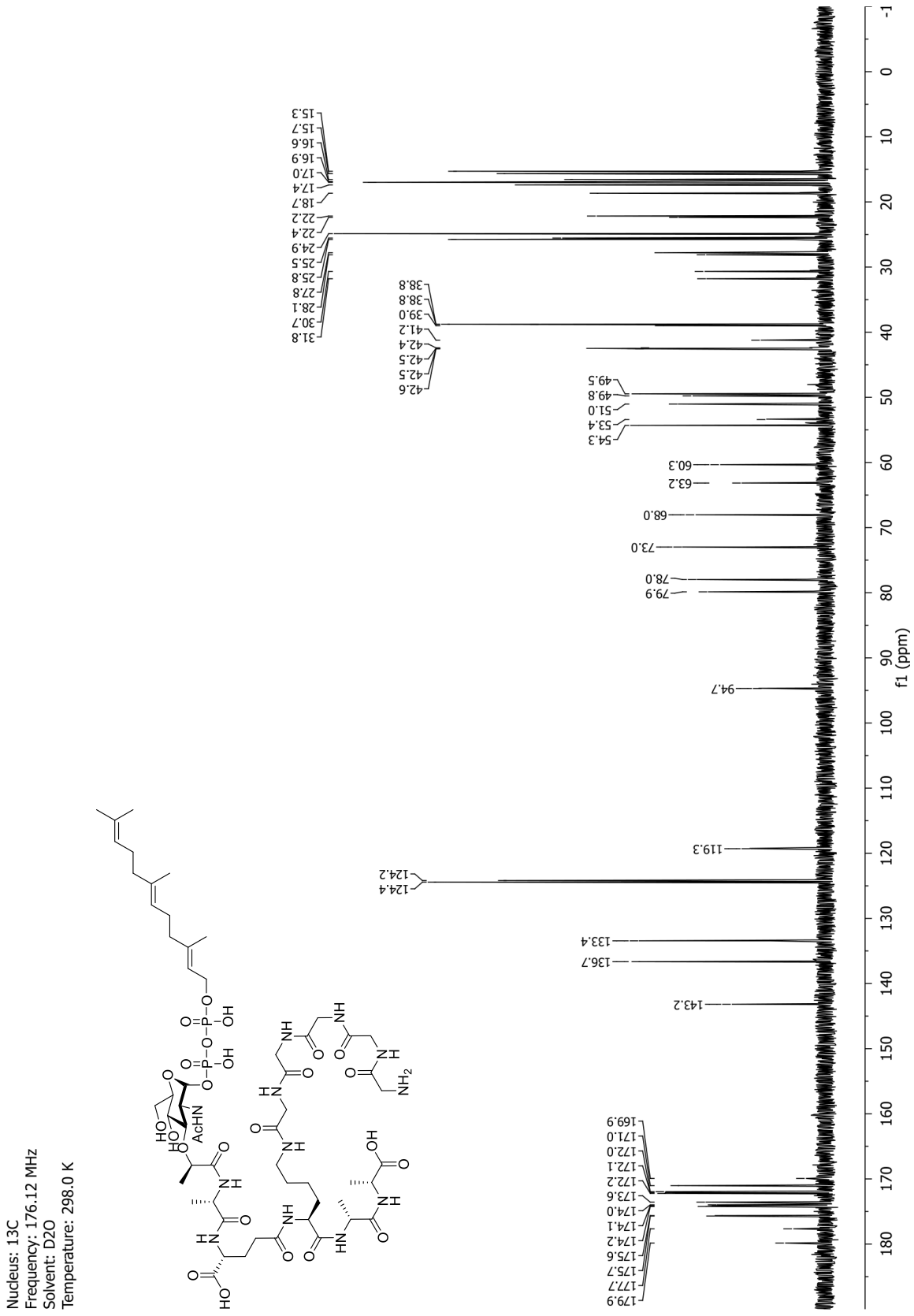


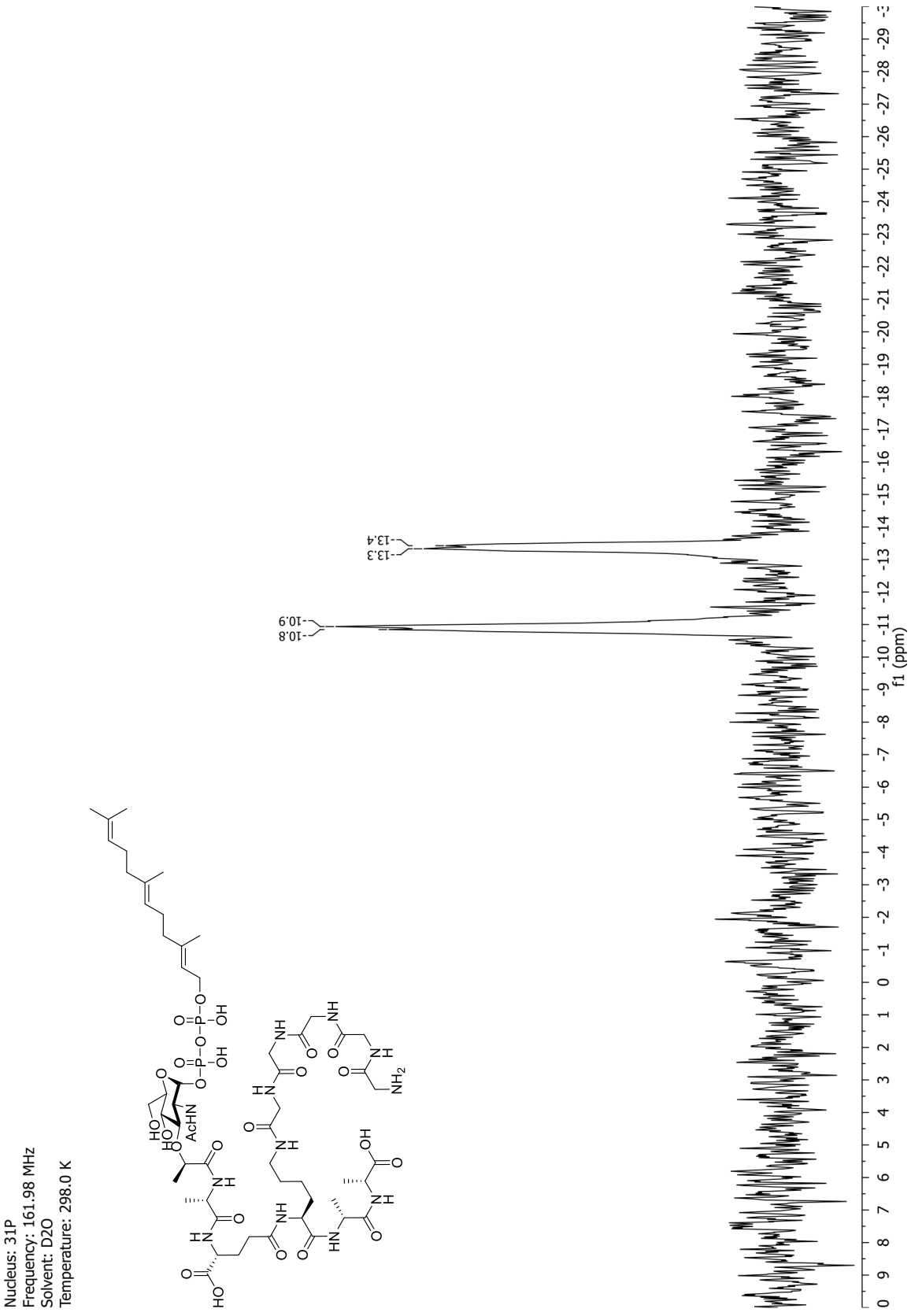

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)

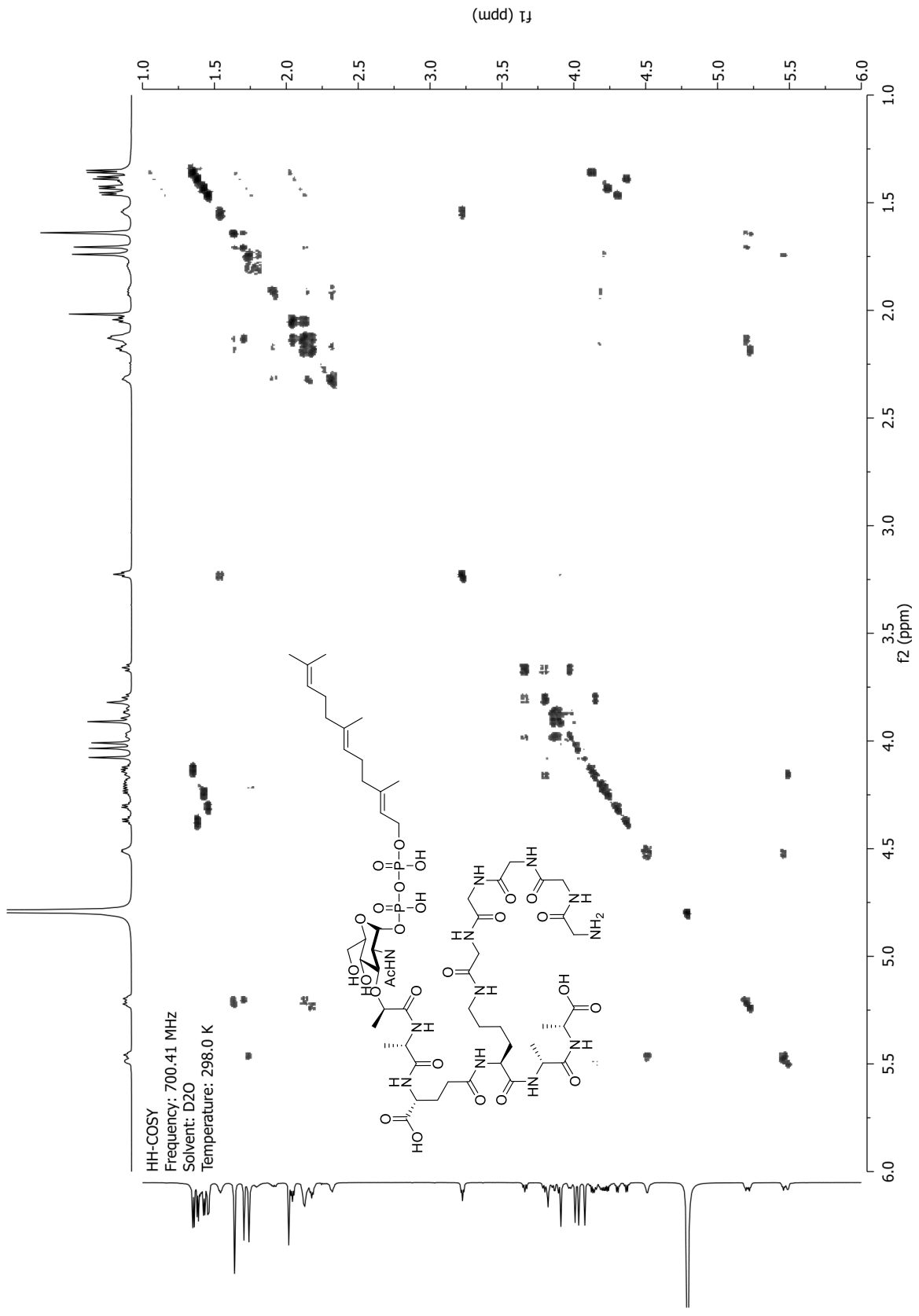

Nucleus: 31P
Frequency: 283.53 MHz
Solvent: MeOD
Temperature: 298.0 K
-2.6
f1 (ppm)

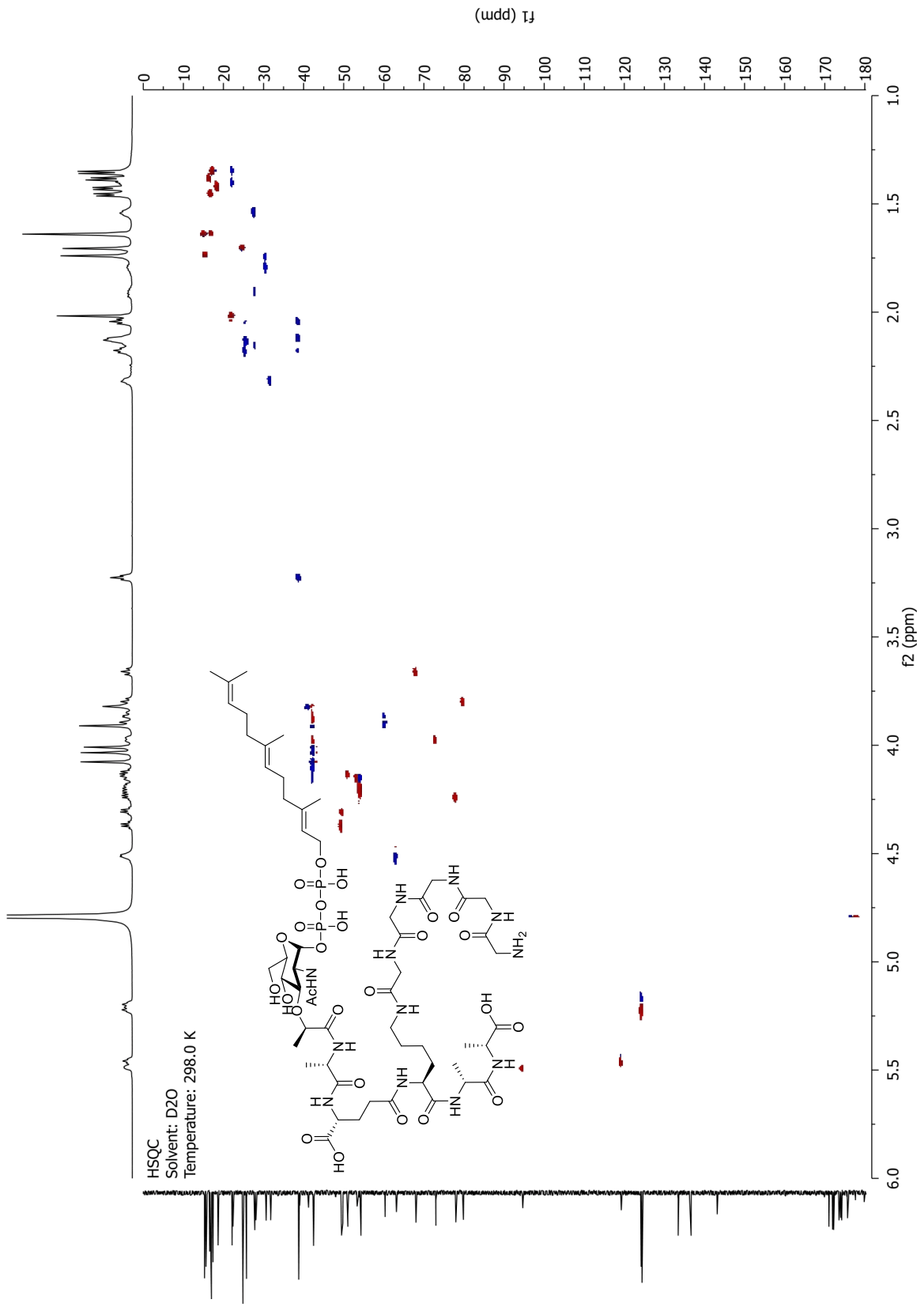
NMR-Spectra for Compound 2

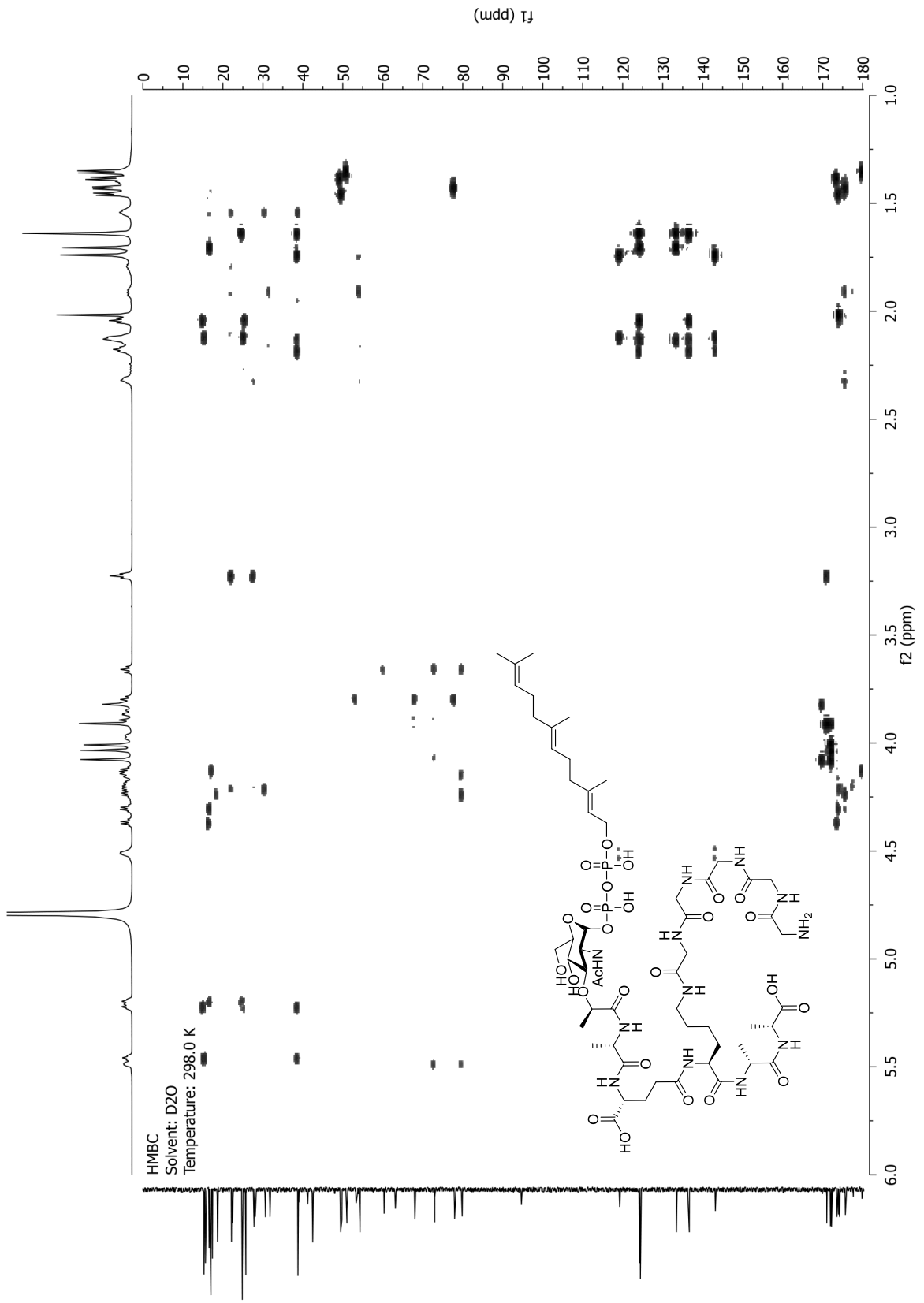


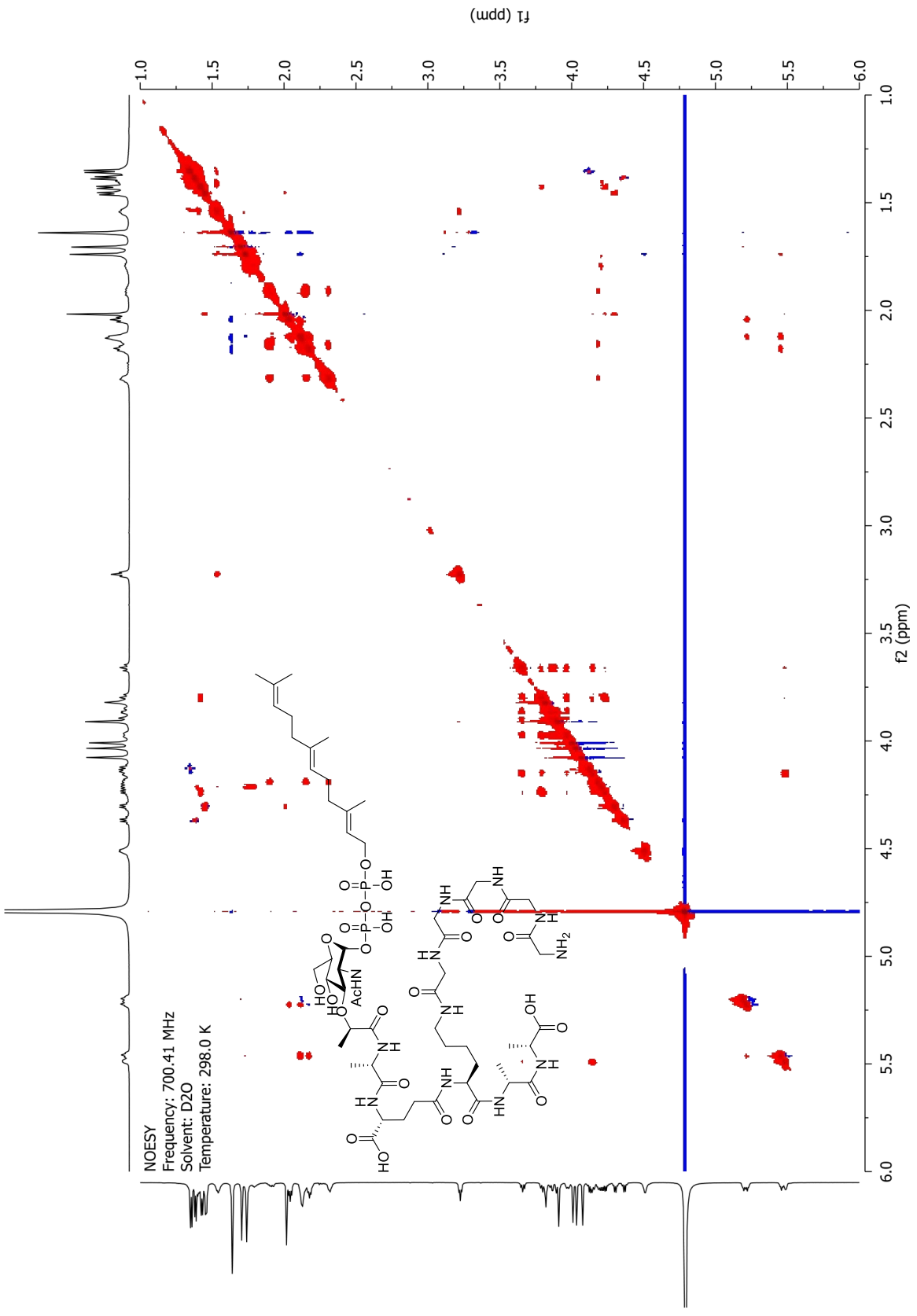

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)

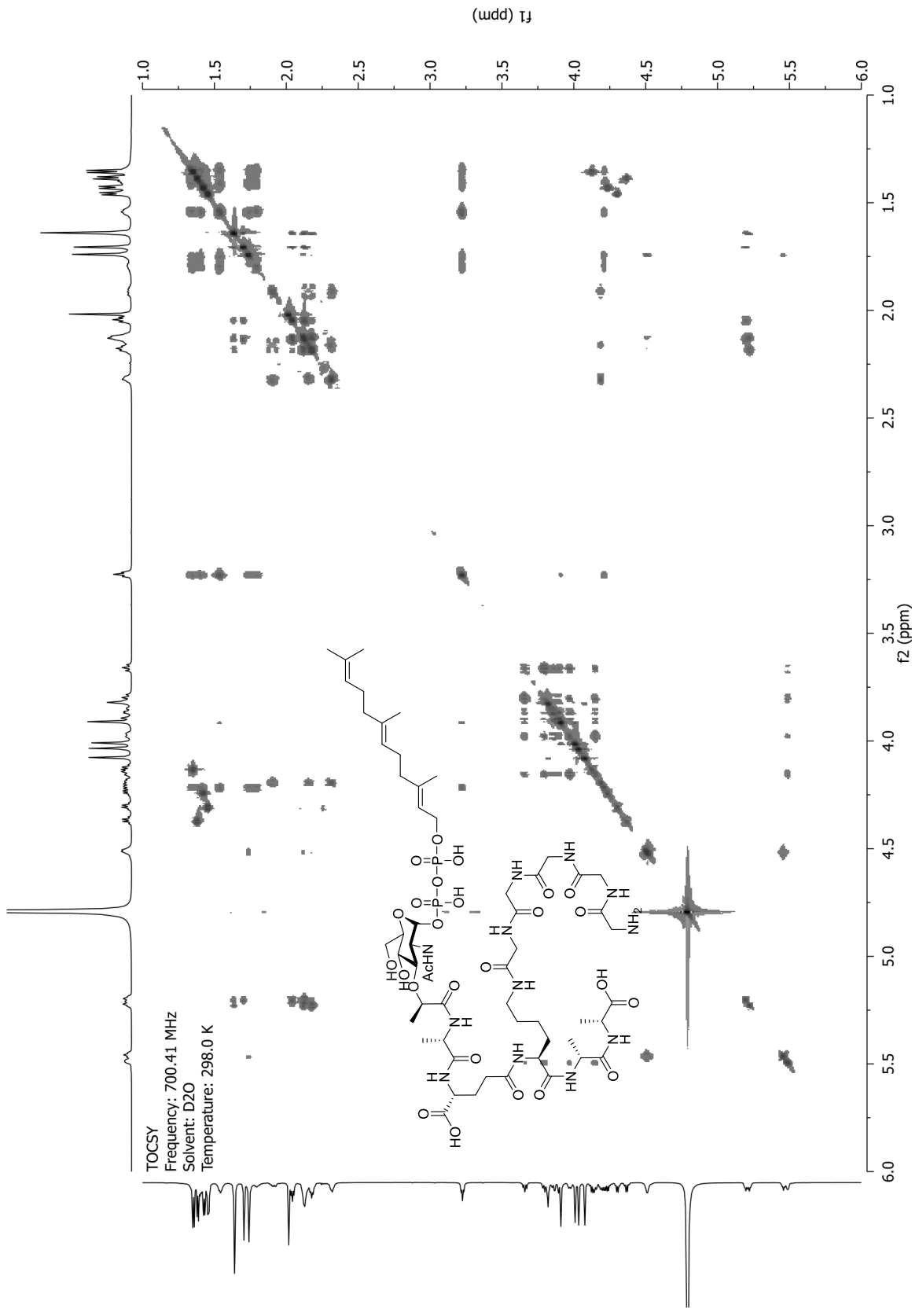

Nucleus: 31P
Frequency: 161.98 MHz
Solvent: D2O
Temperature: 298.0 K
-10.8
-10.9
-13.3
-13.4
f1 (ppm)


HH-COSY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
AcHN
NH2

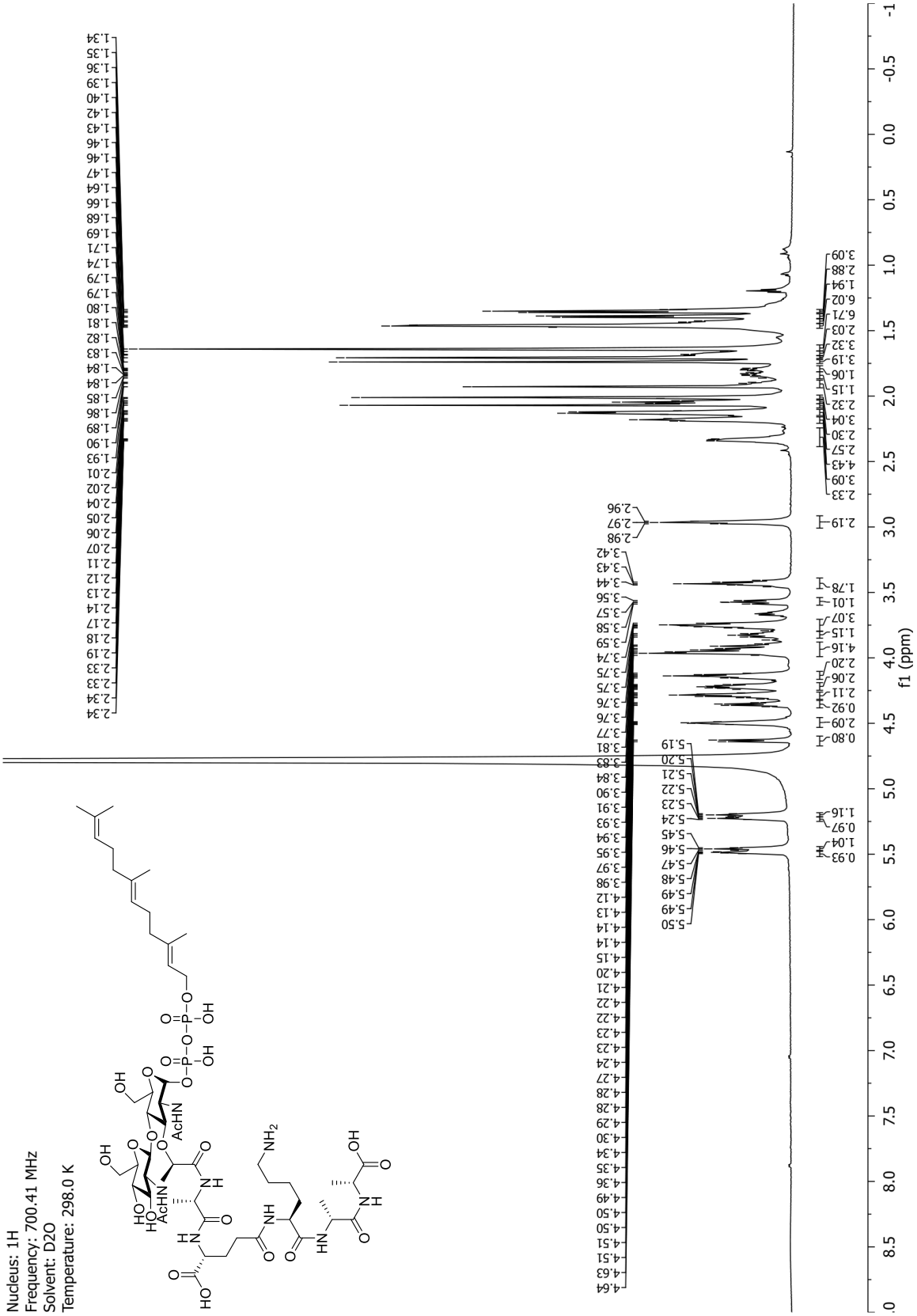

f1 (ppm)
f2 (ppm)
HSQC
Solvent: D2O
Temperature: 298.0 K

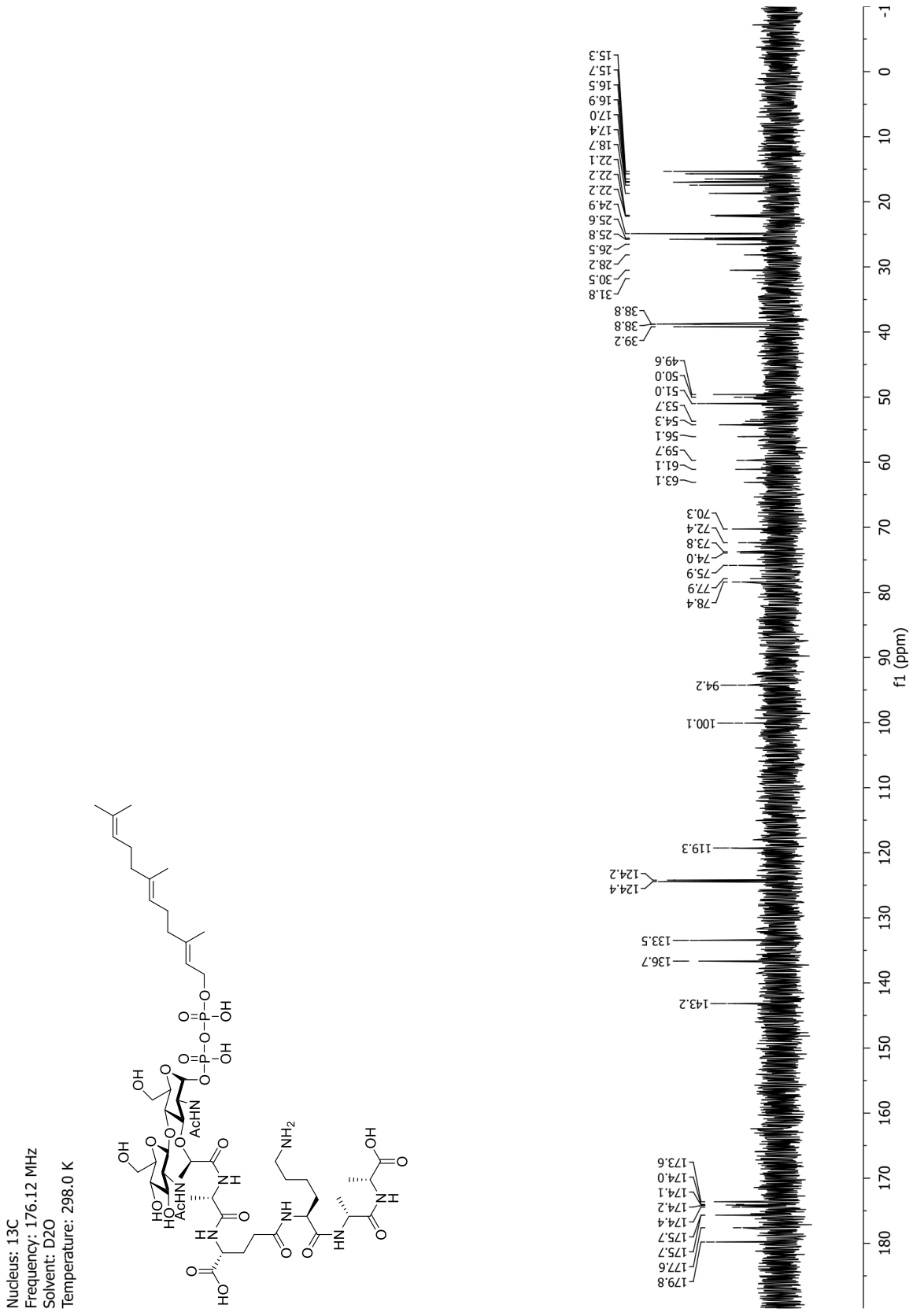

f1 (ppm)
f2 (ppm)
HMBC
Solvent: D2O
Temperature: 298.0 K

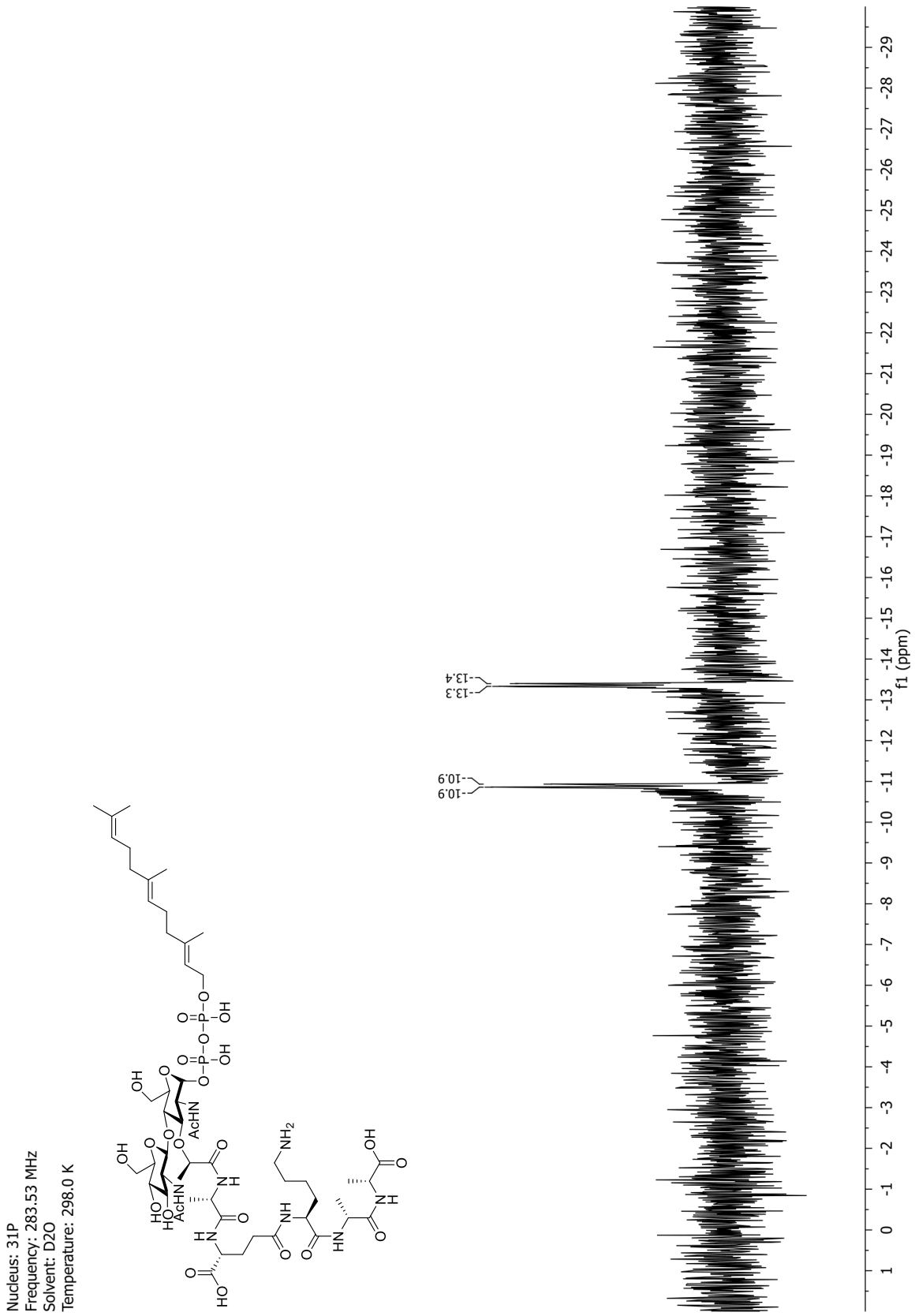

NOESY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
AcHN
NH2

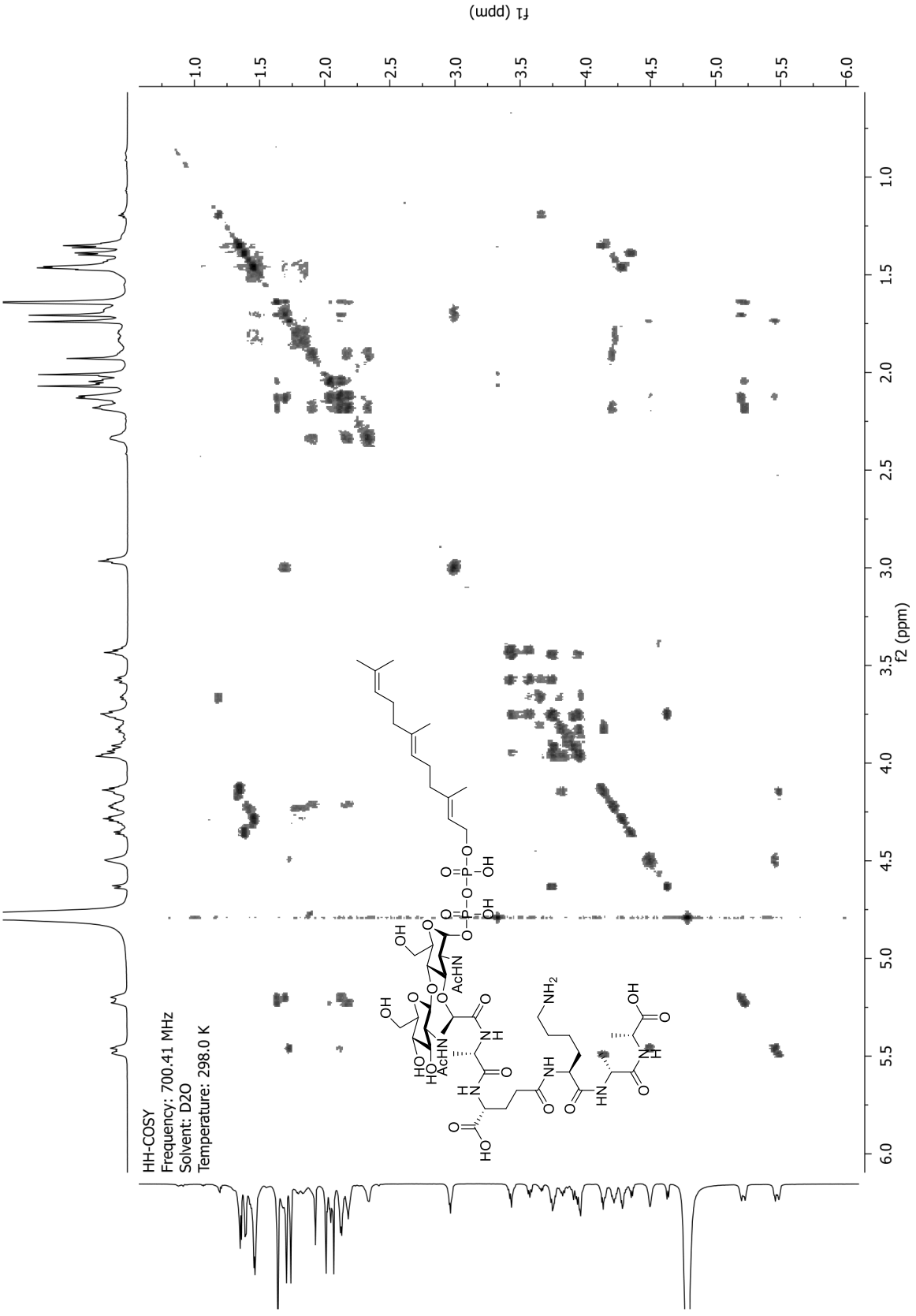

TOCSY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
AcHN
HO
NH
NH2
OH

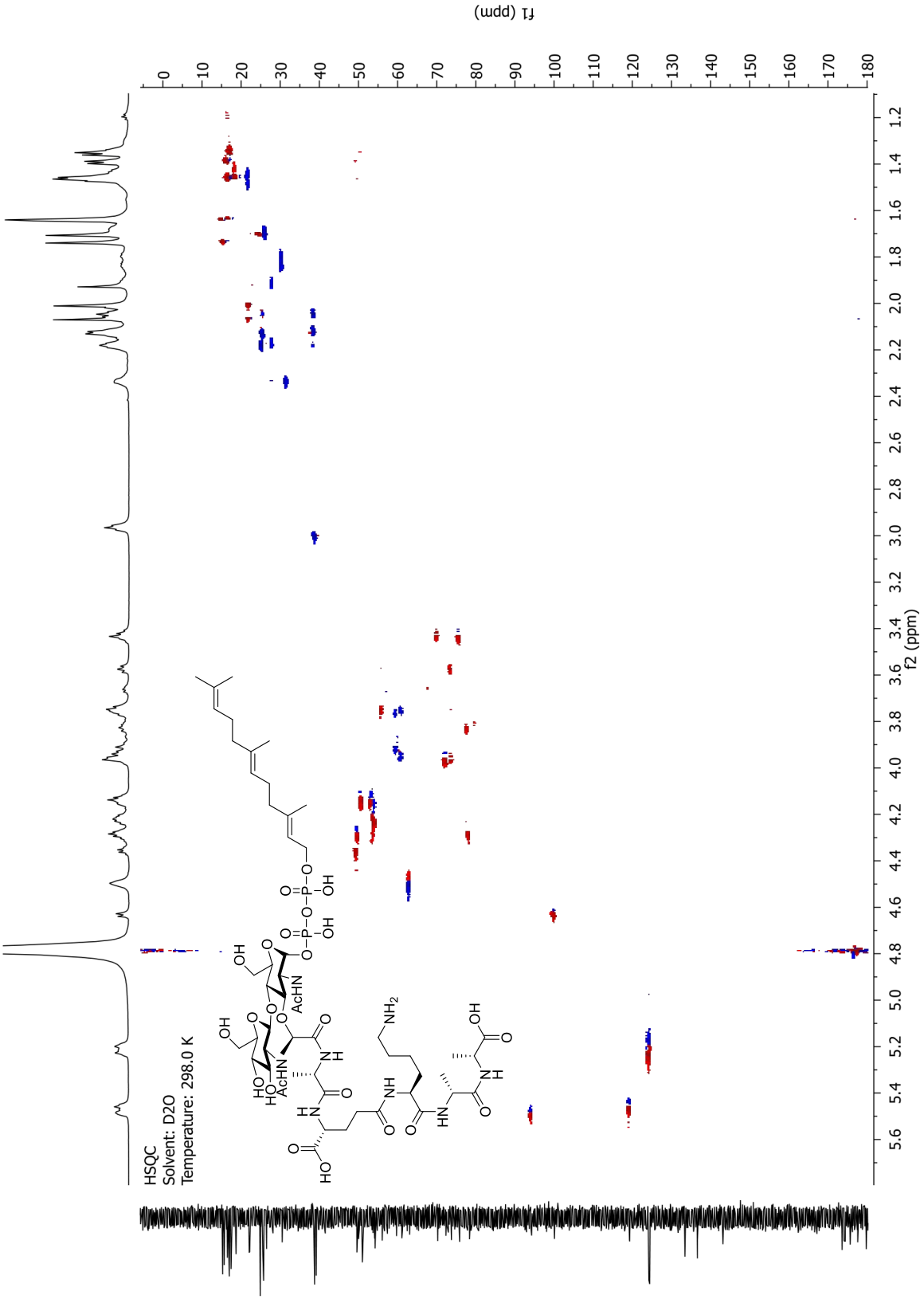
NMR-Spectra for Compound 68

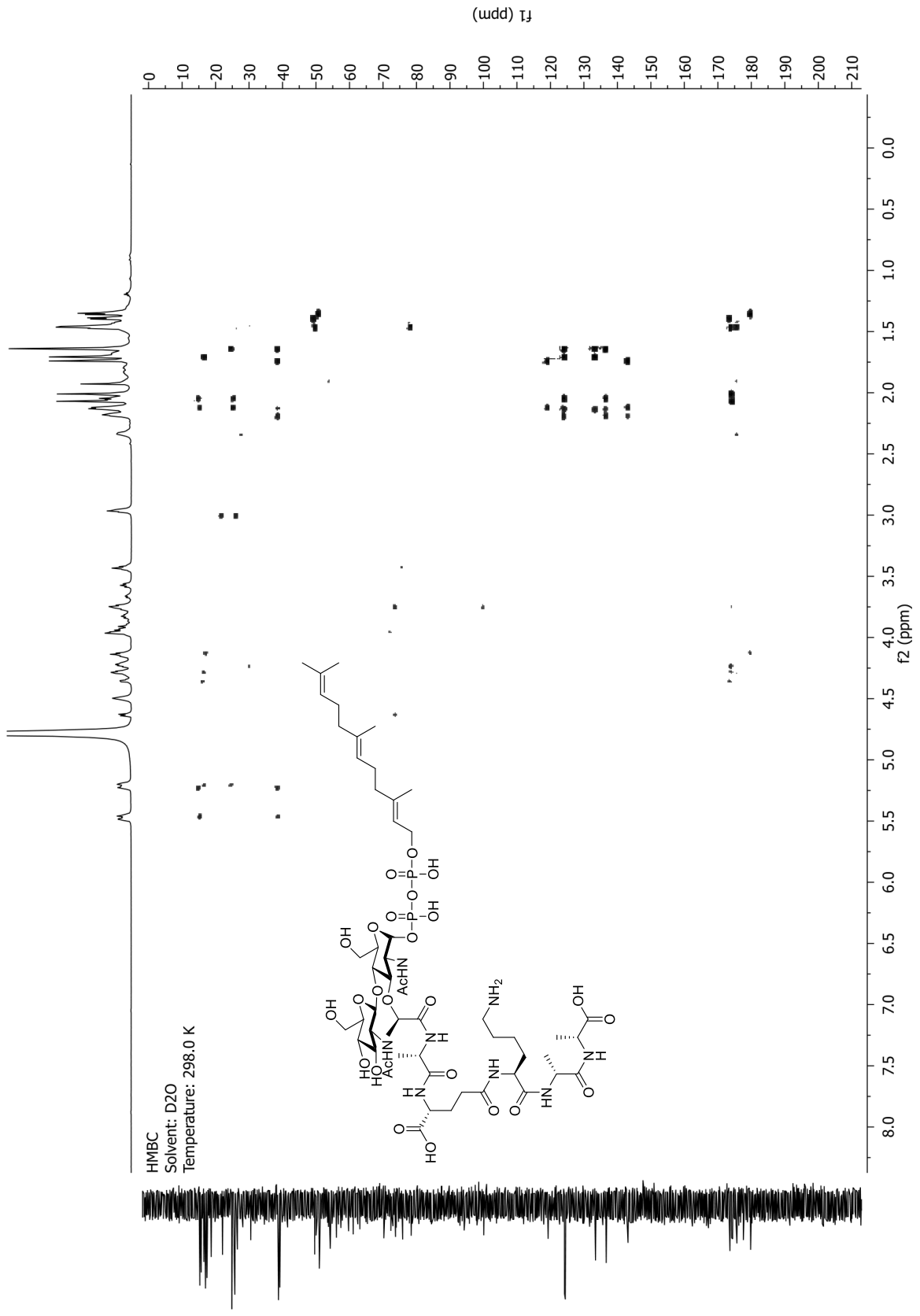


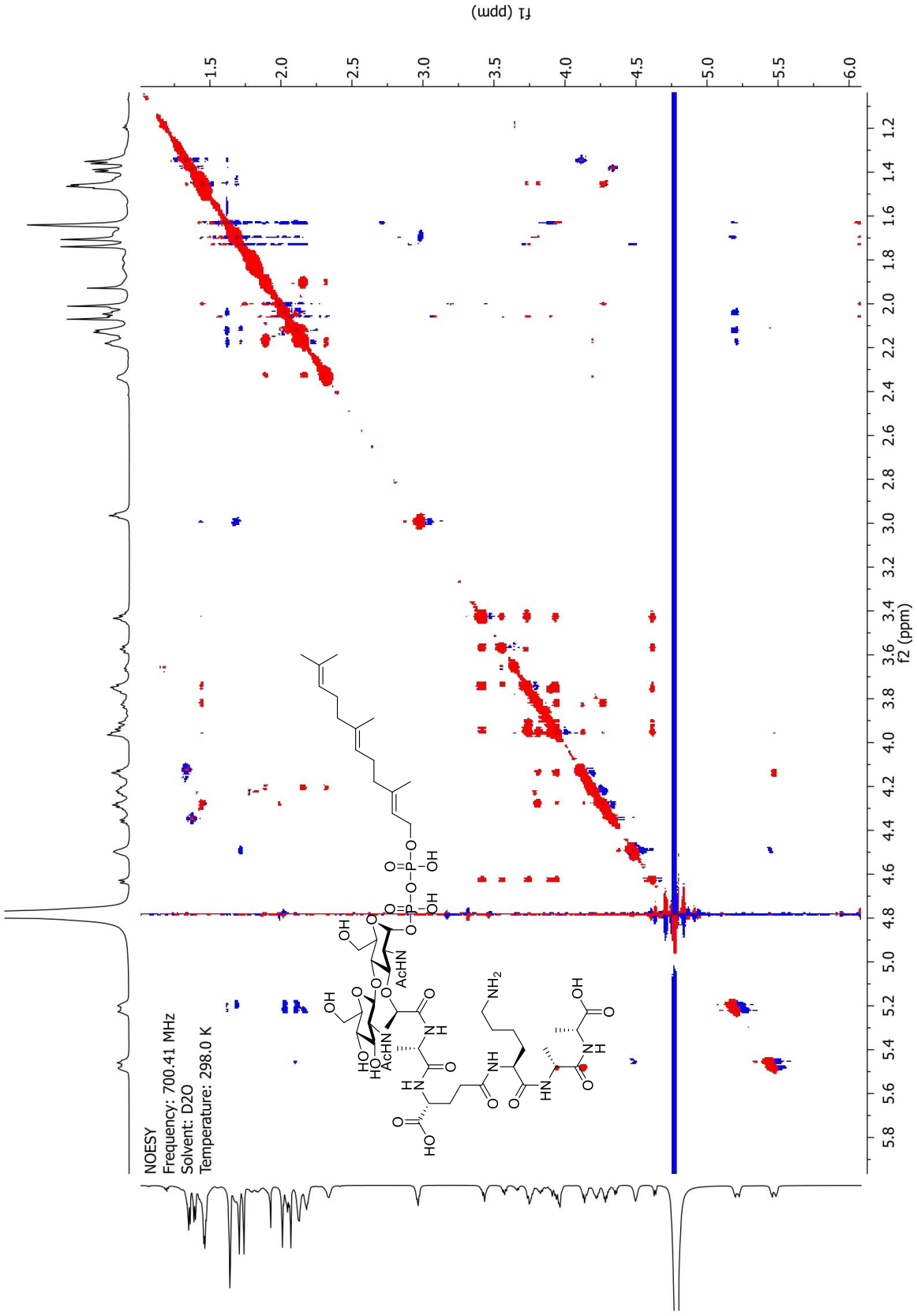

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)

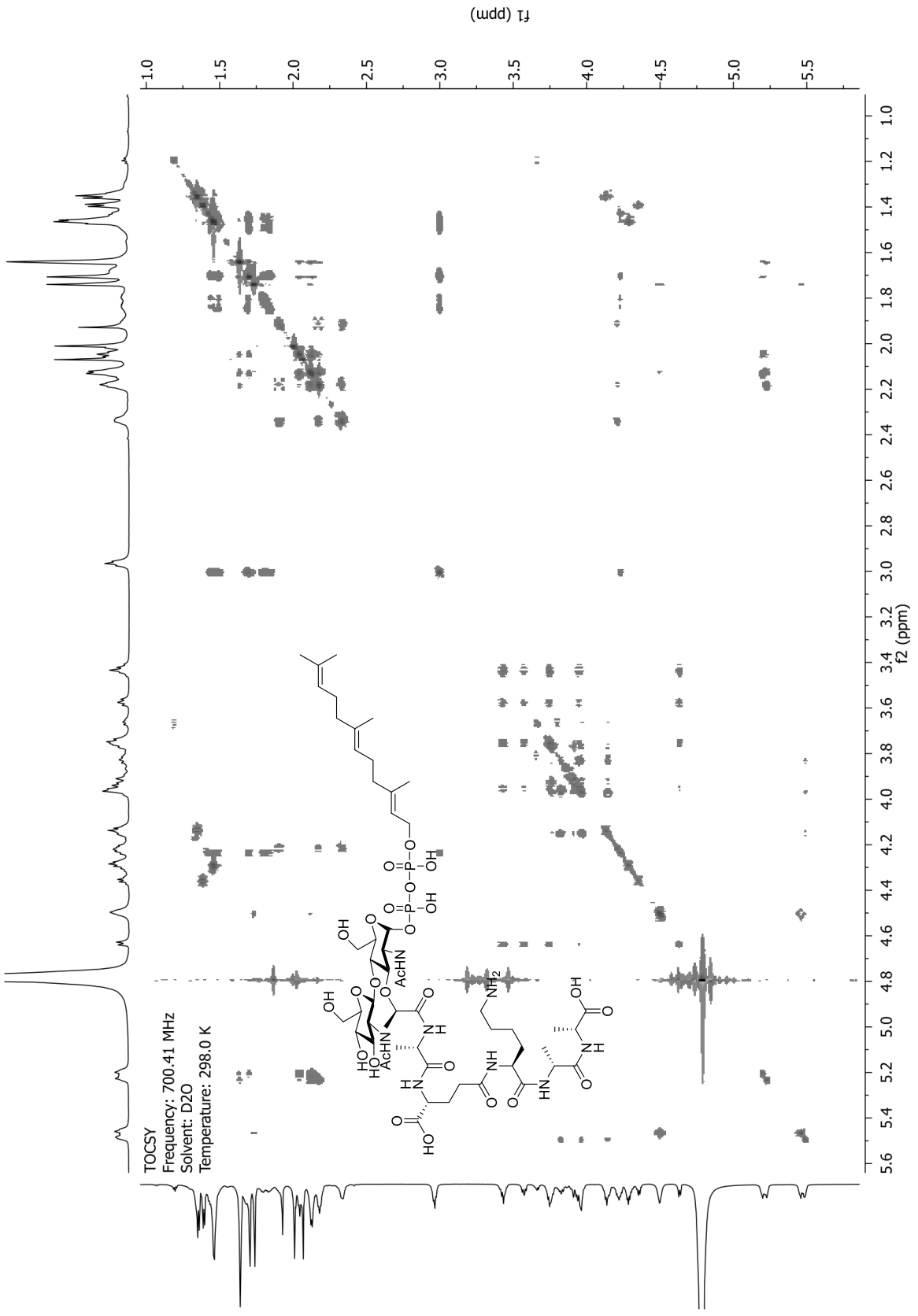

Nucleus: 31P
Frequency: 283.53 MHz
Solvent: D2O
Temperature: 298.0 K
-10.9
-10.9
-13.3
-13.4
f1 (ppm)


HH-COSY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0

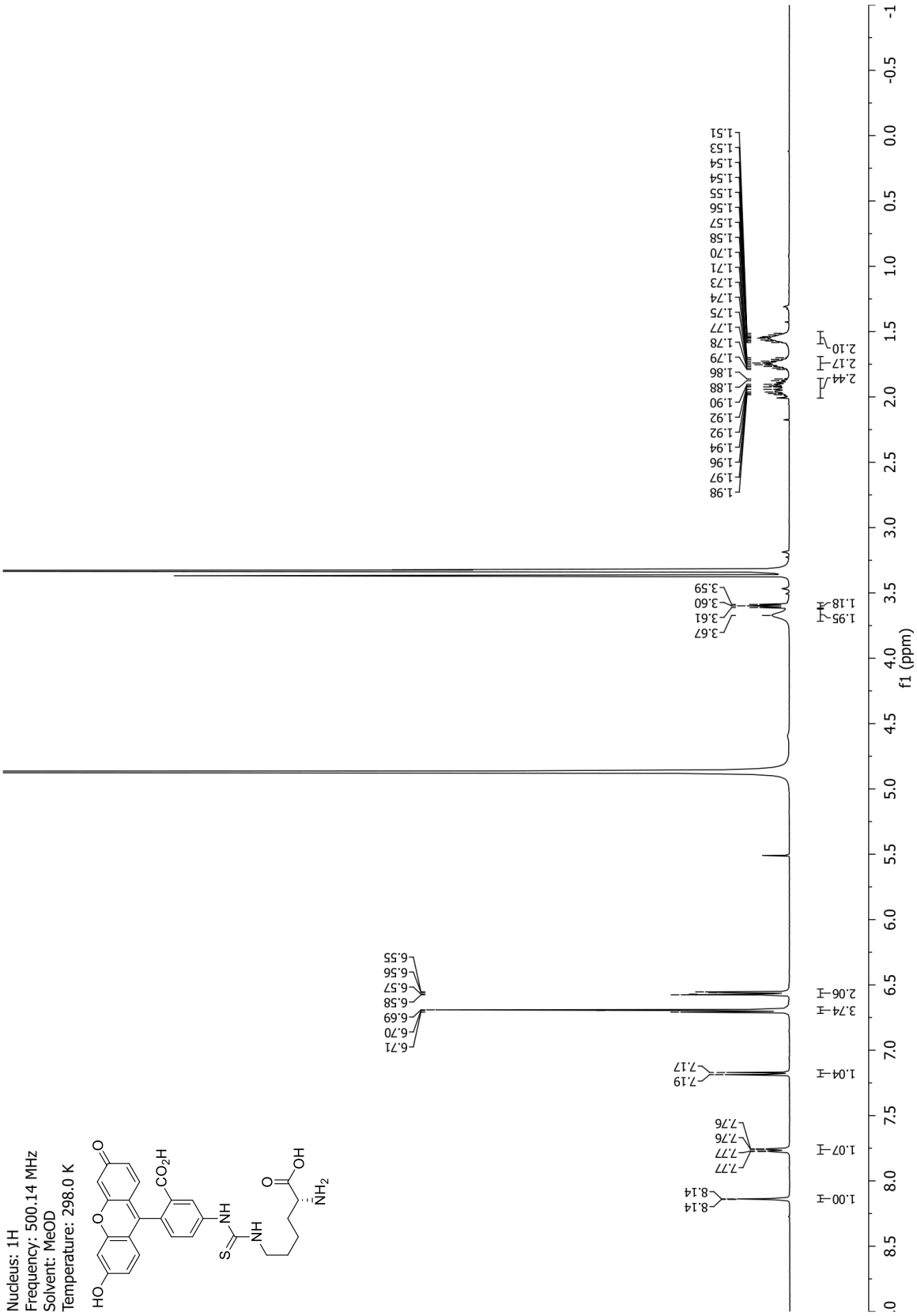

HSQC
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)

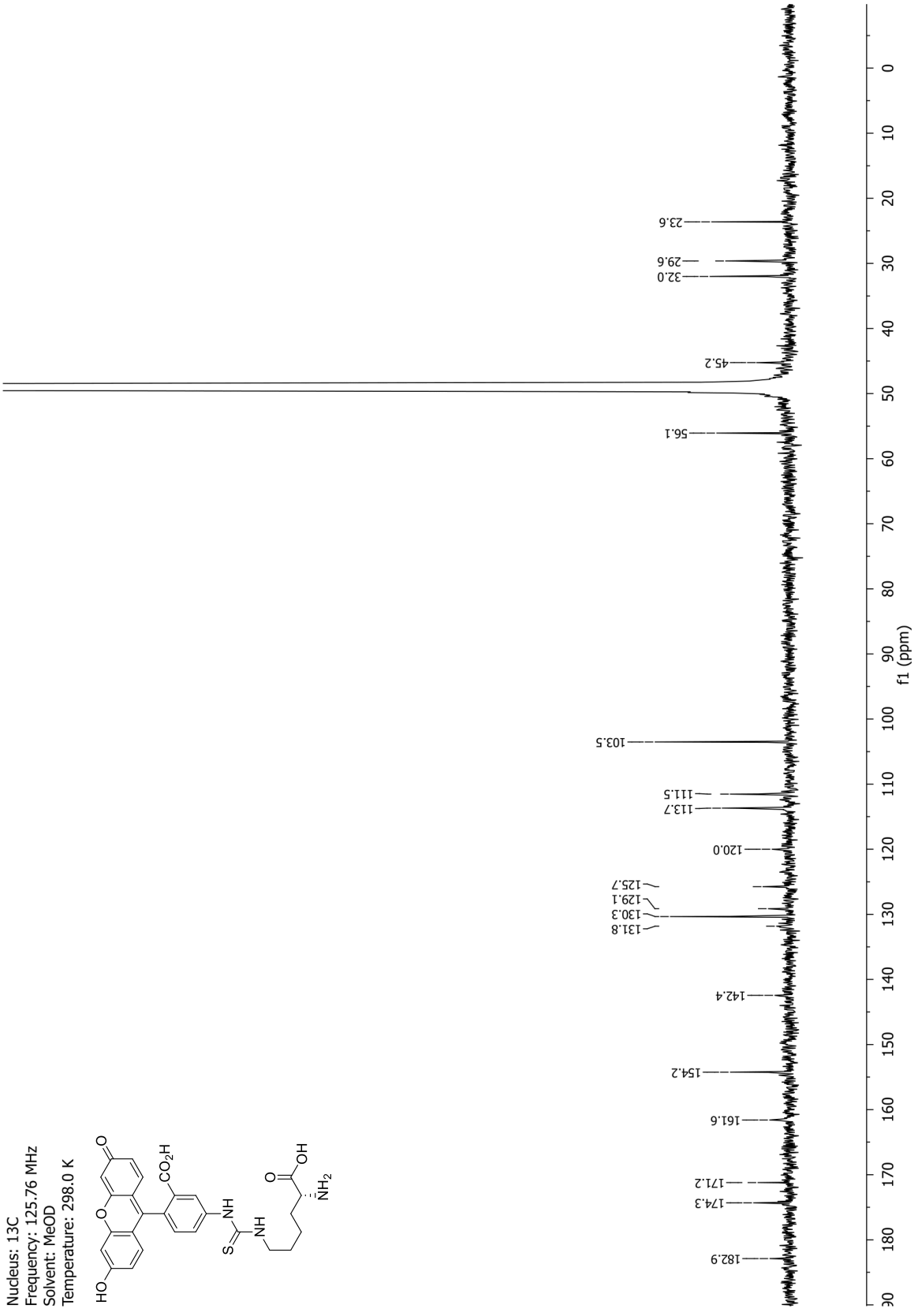

HMBC
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)


NOESY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)

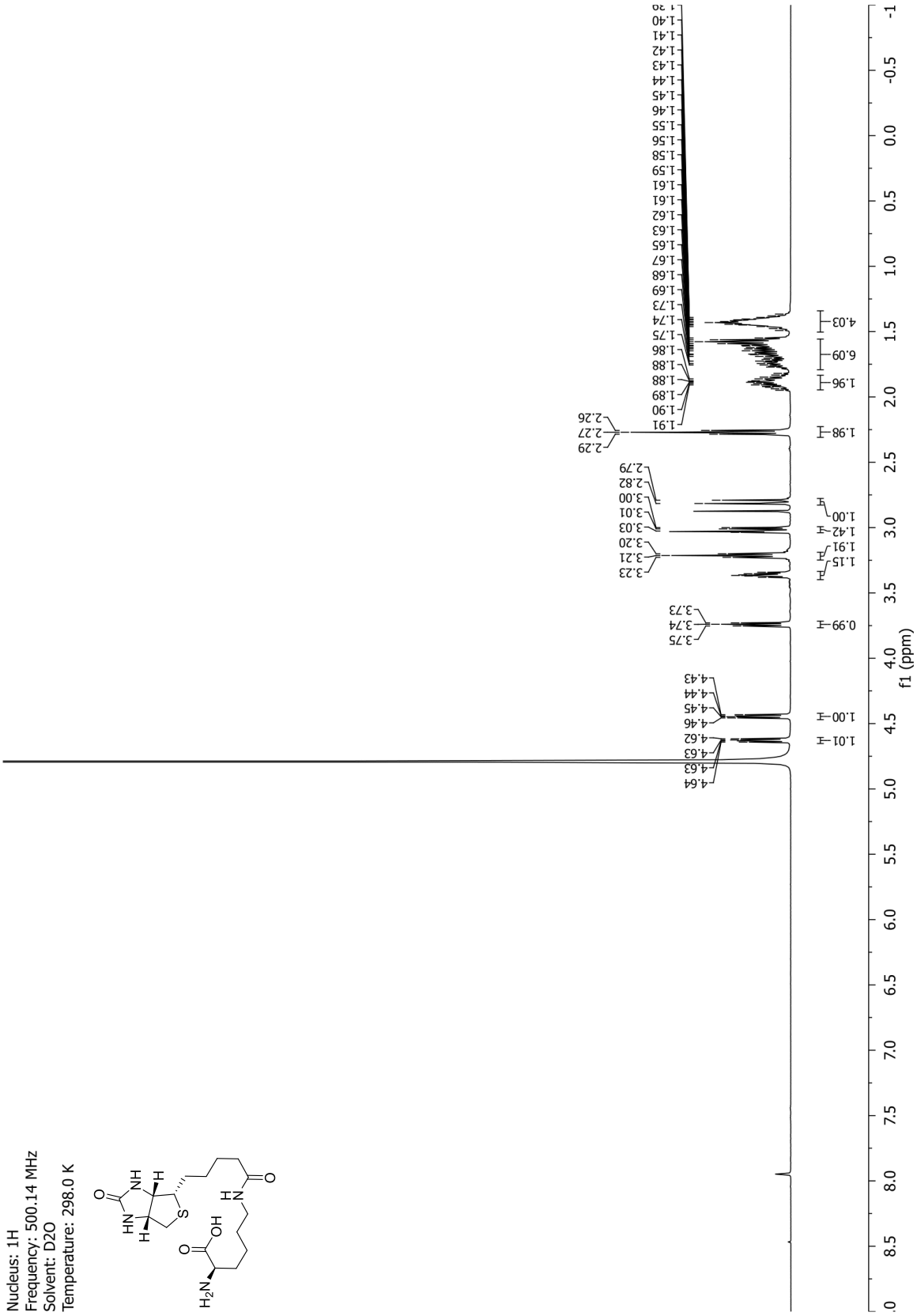

TOCSY
Frequency: 700.41 MHz
Solvent: D2O
Temperature: 298.0 K
f1 (ppm)
f2 (ppm)

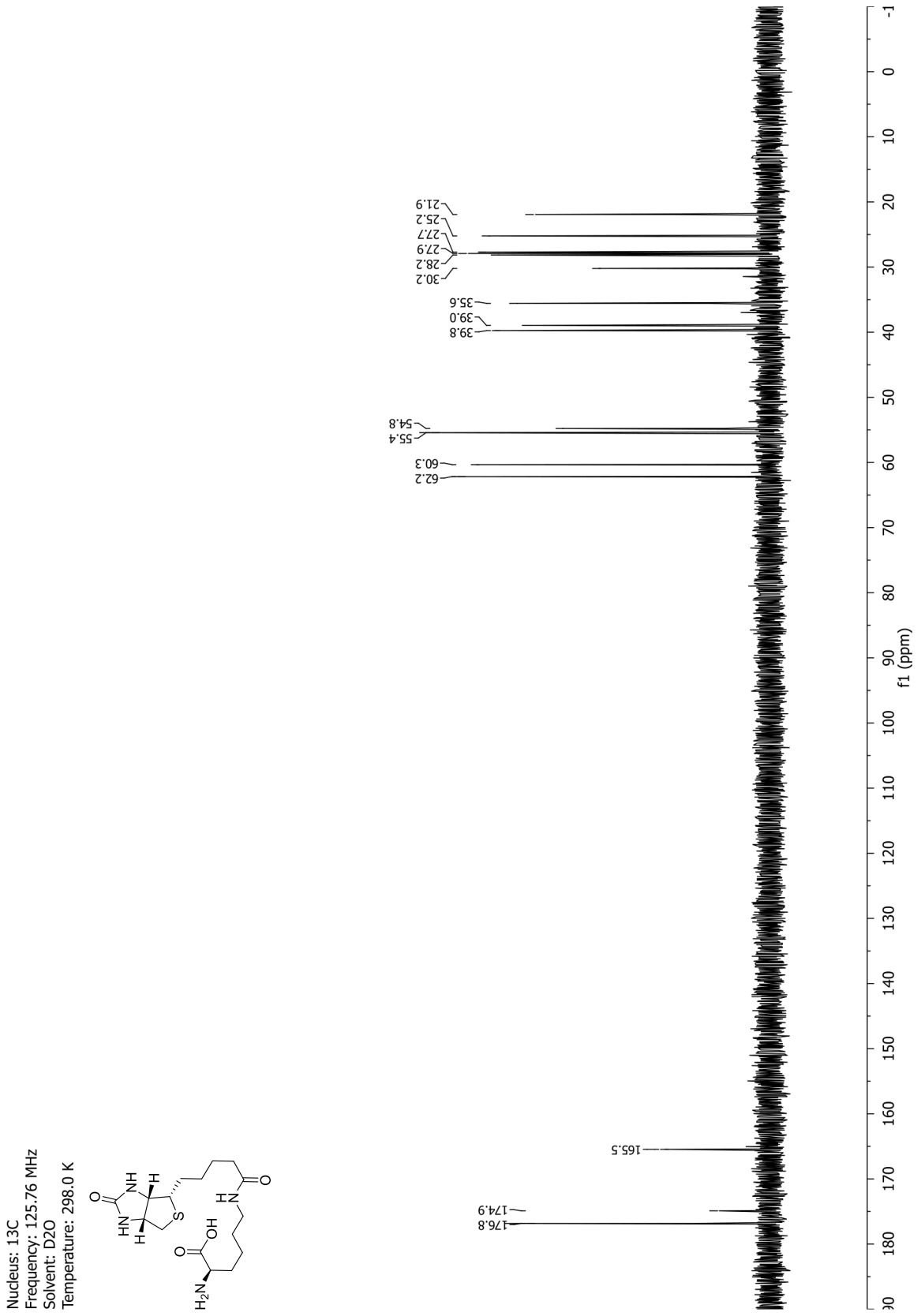
NMR-Spectra for Compound 160




Nucleus: 13C
Frequency: 125.76 MHz
Solvent: MeOD
Temperature: 298.0 K
f1 (ppm)
182.9
174.3
171.2
161.6
154.2
142.4
131.8
130.3
129.1
125.7
120.0
113.7
111.5
103.5
56.1
45.2
32.0
29.6
23.6

NMR-Spectra for Compound 162

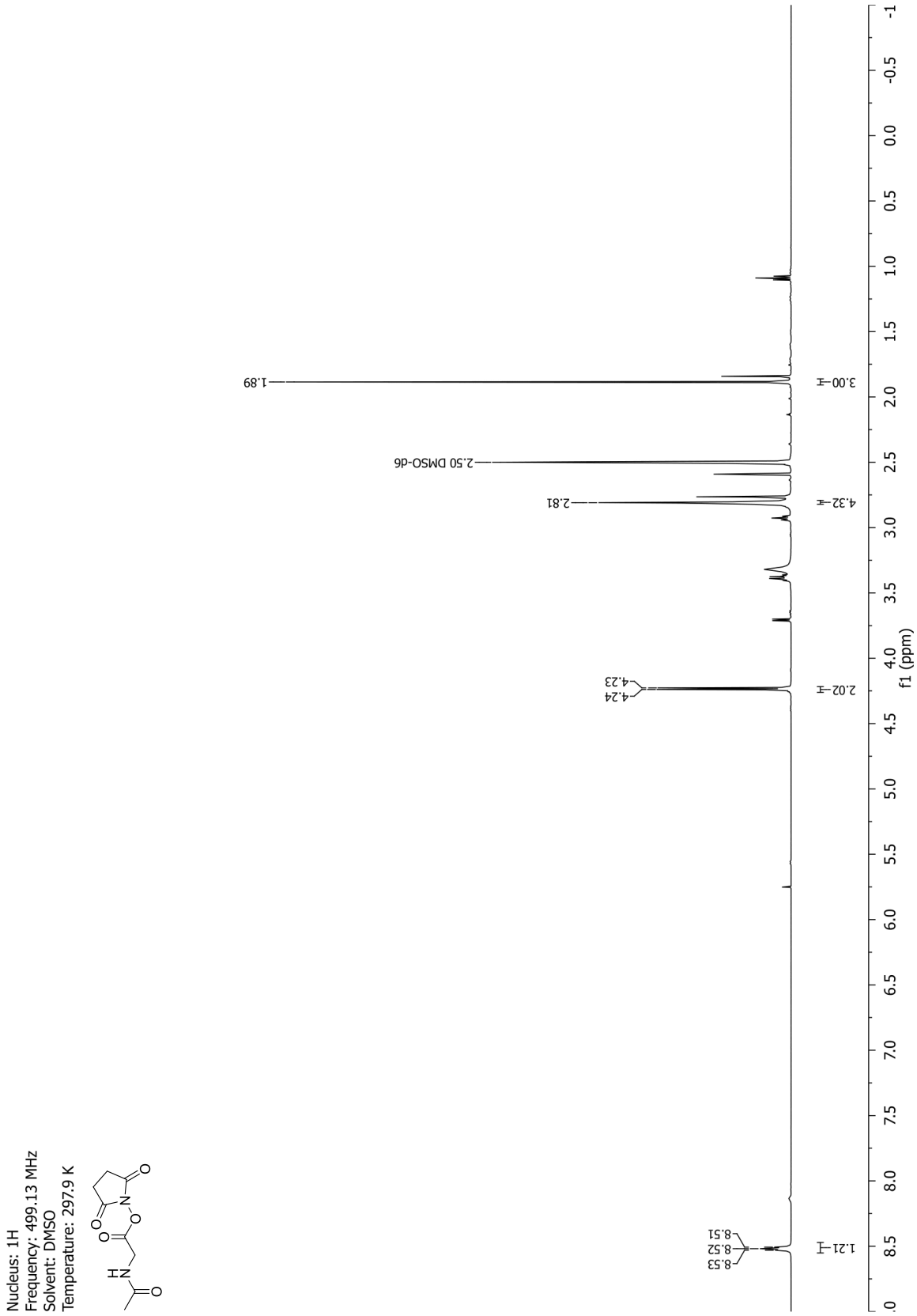


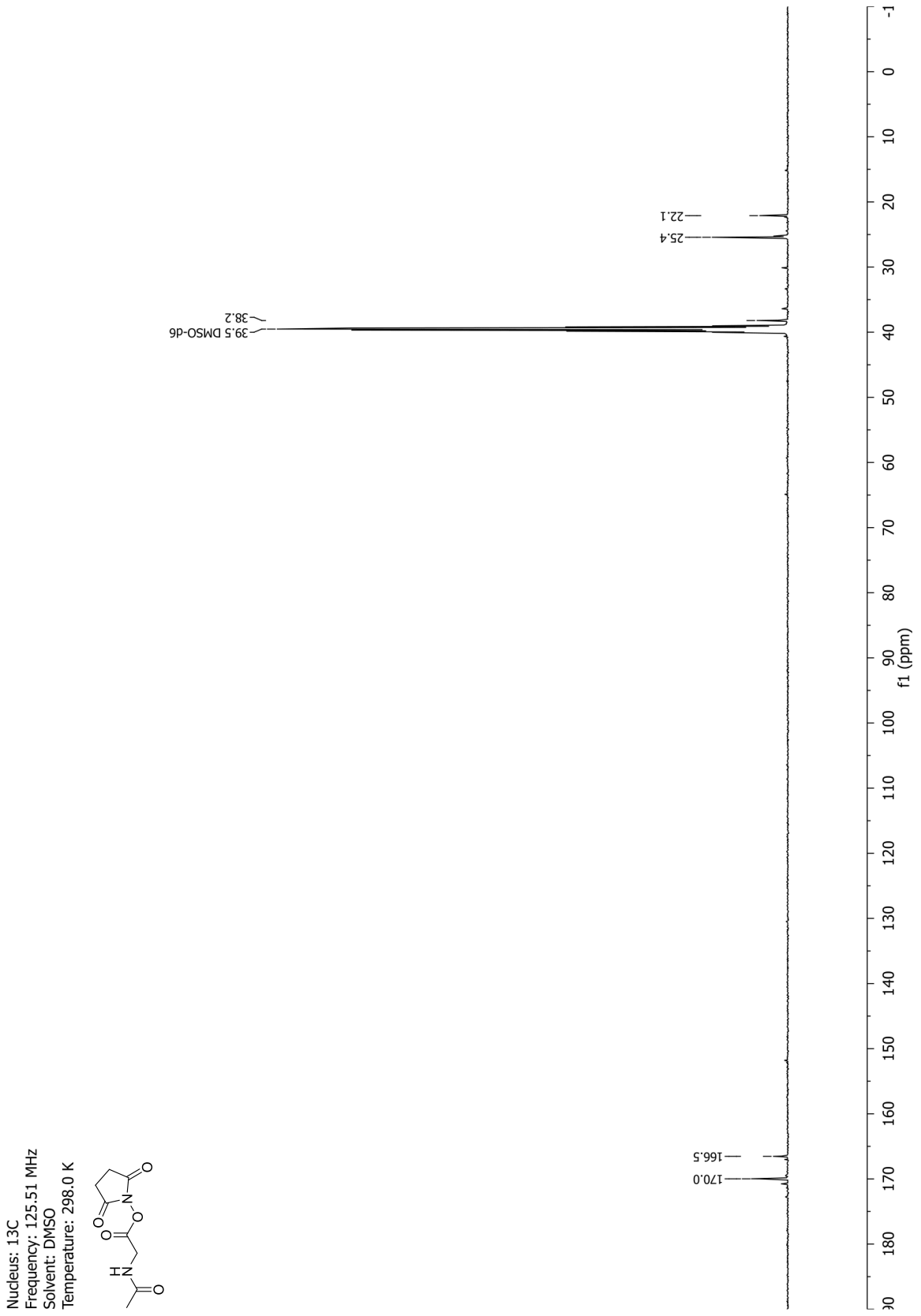

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: D2O
Temperature: 298.0 K
21.9
25.2
27.7
27.9
28.2
30.2
35.6
39.0
39.8
54.8
55.4
60.3
62.2
165.5
174.9
176.8
f1 (ppm)

7.2 Copies of NMR Spectra (Contributions to the Total Synthesis of Vancoresmycin)

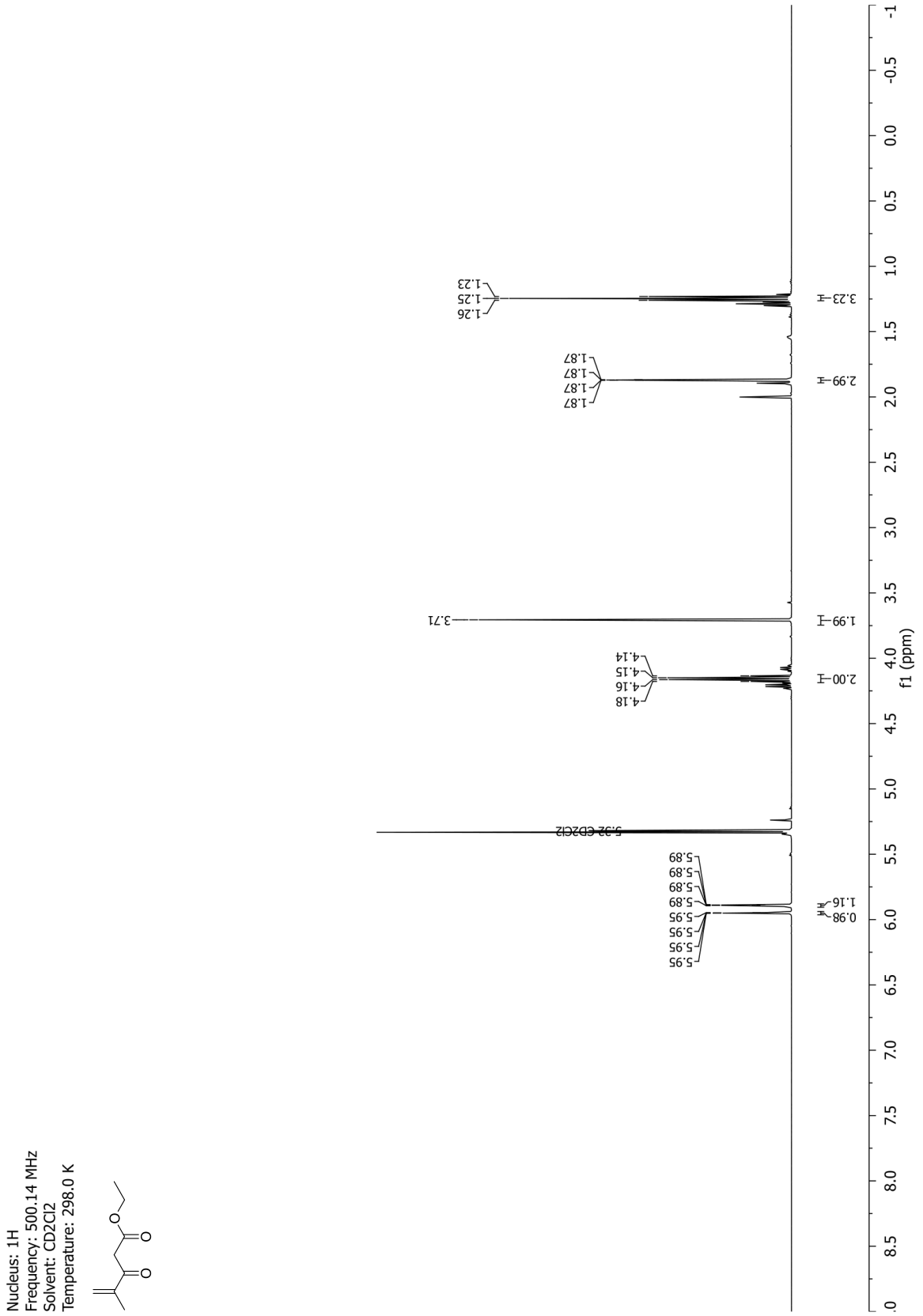
– NMR Spectra of the synthesized compounds starting at the next page –

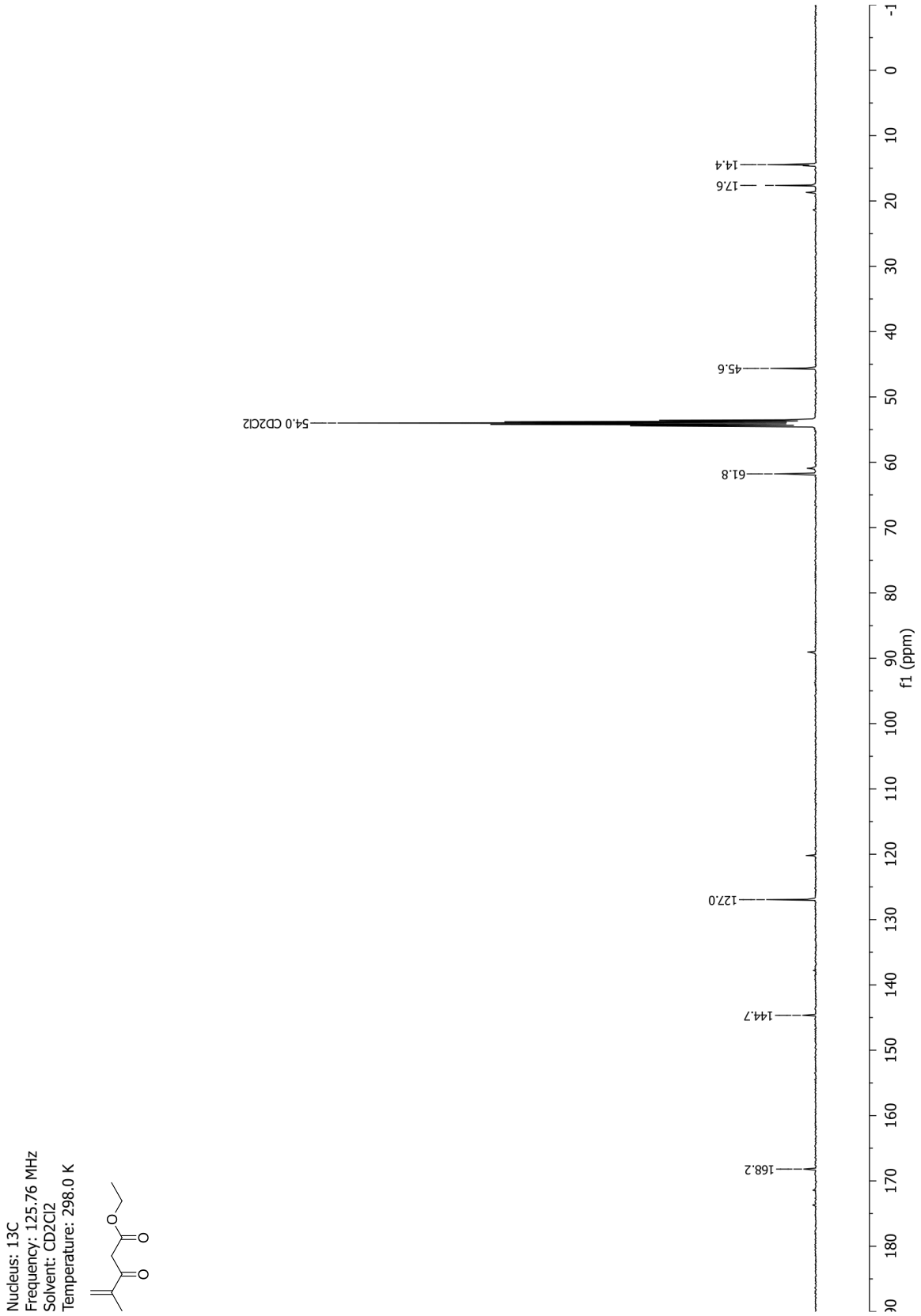
NMR-Spectra for Compound 180



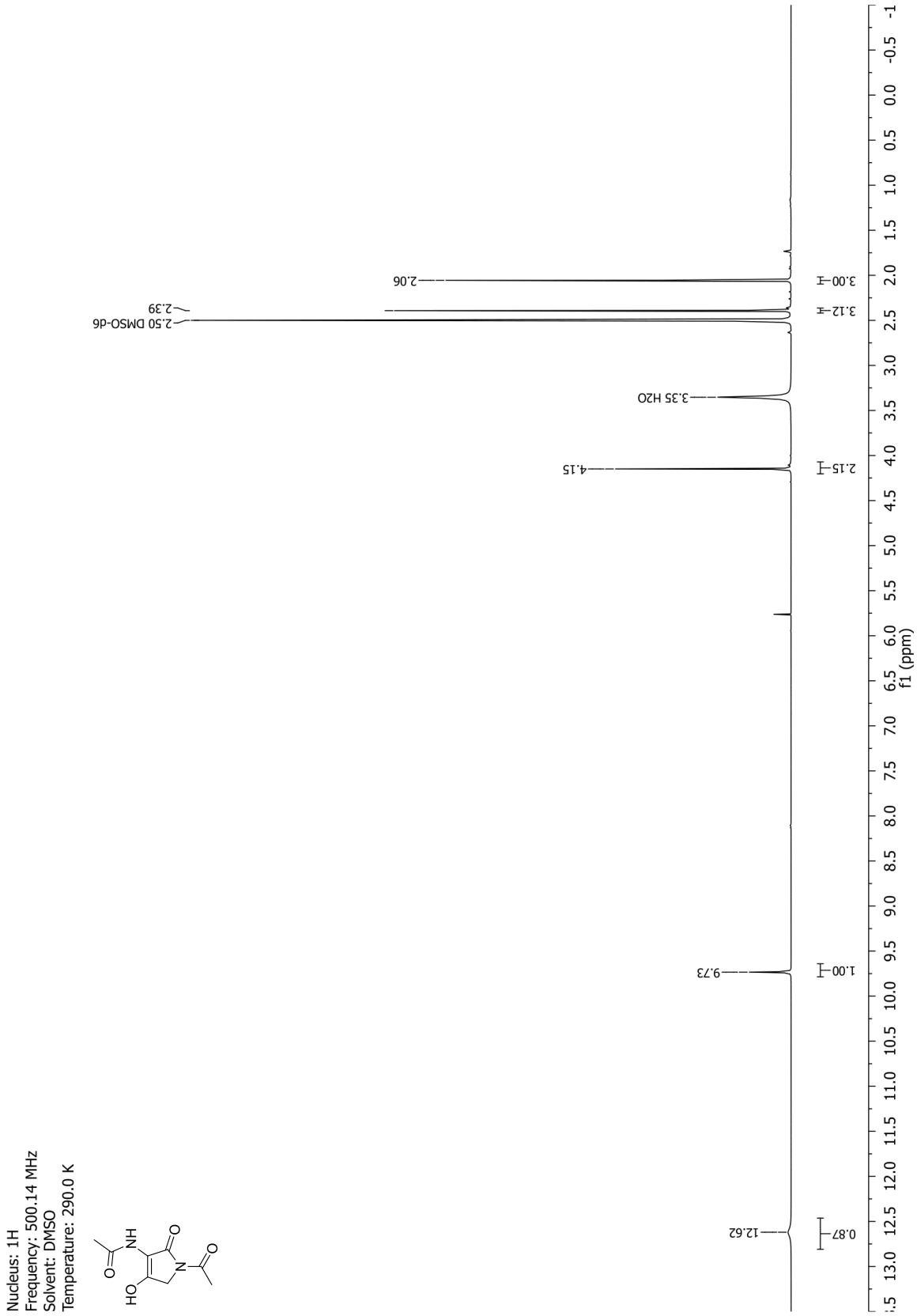

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: DMSO
Temperature: 298.0 K
166.5
170.0
22.1
25.4
38.2
39.5 DMSO-d6
f1 (ppm)

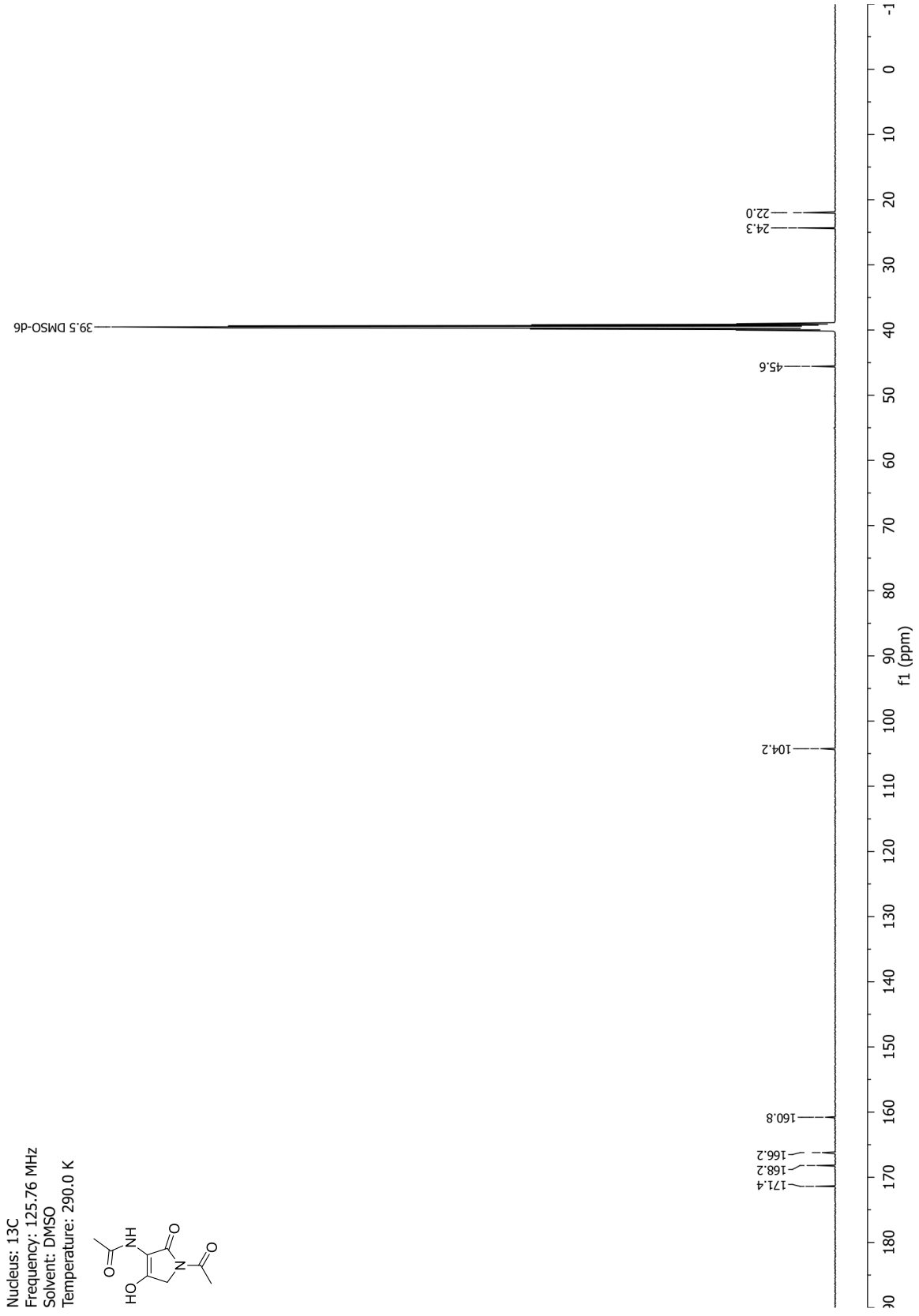
NMR-Spectra for Compound 179



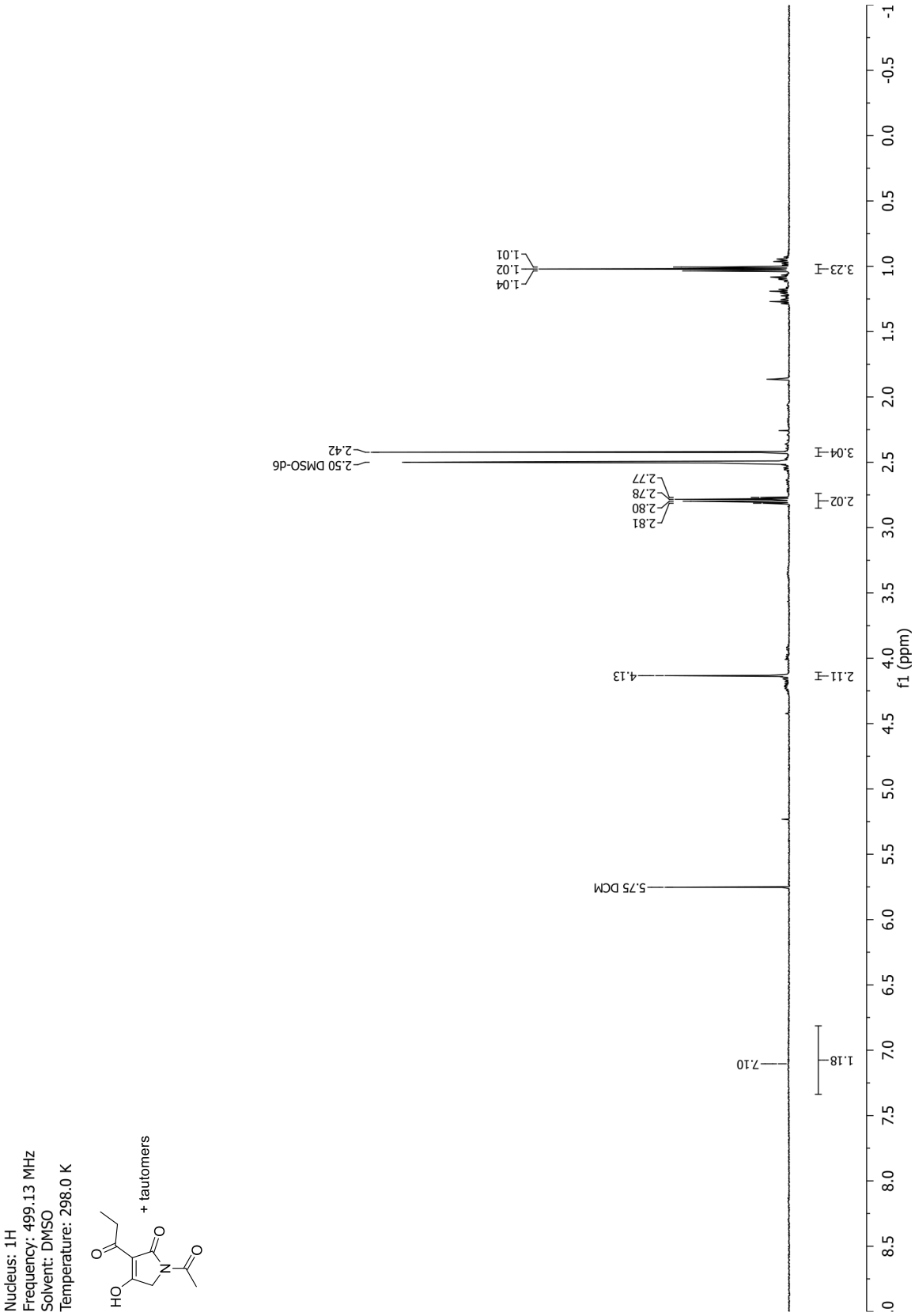

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
14.4
17.6
45.6
54.0 CD2Cl2
61.8
127.0
144.7
168.2
f1 (ppm)

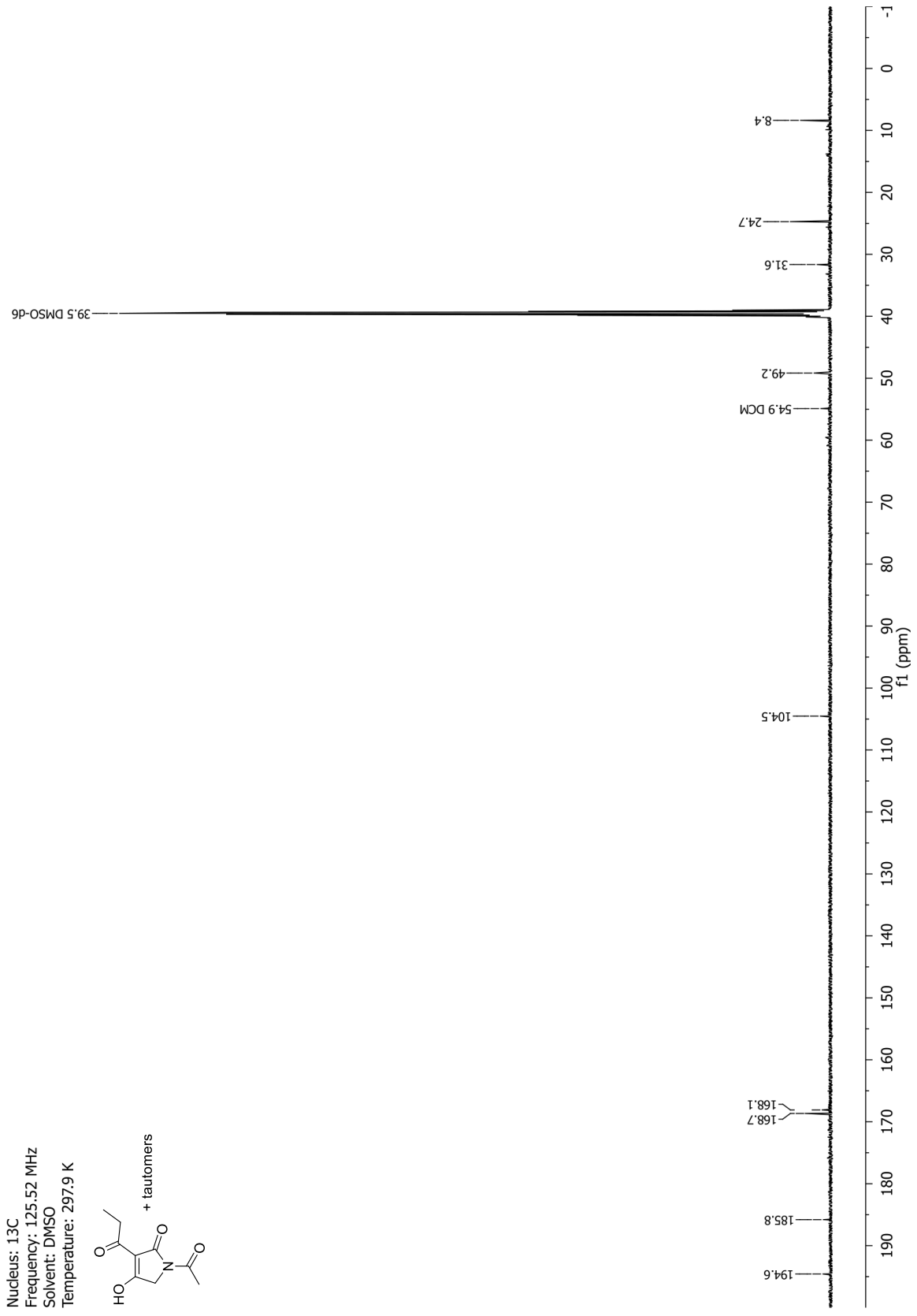
NMR-Spectra for Compound 228



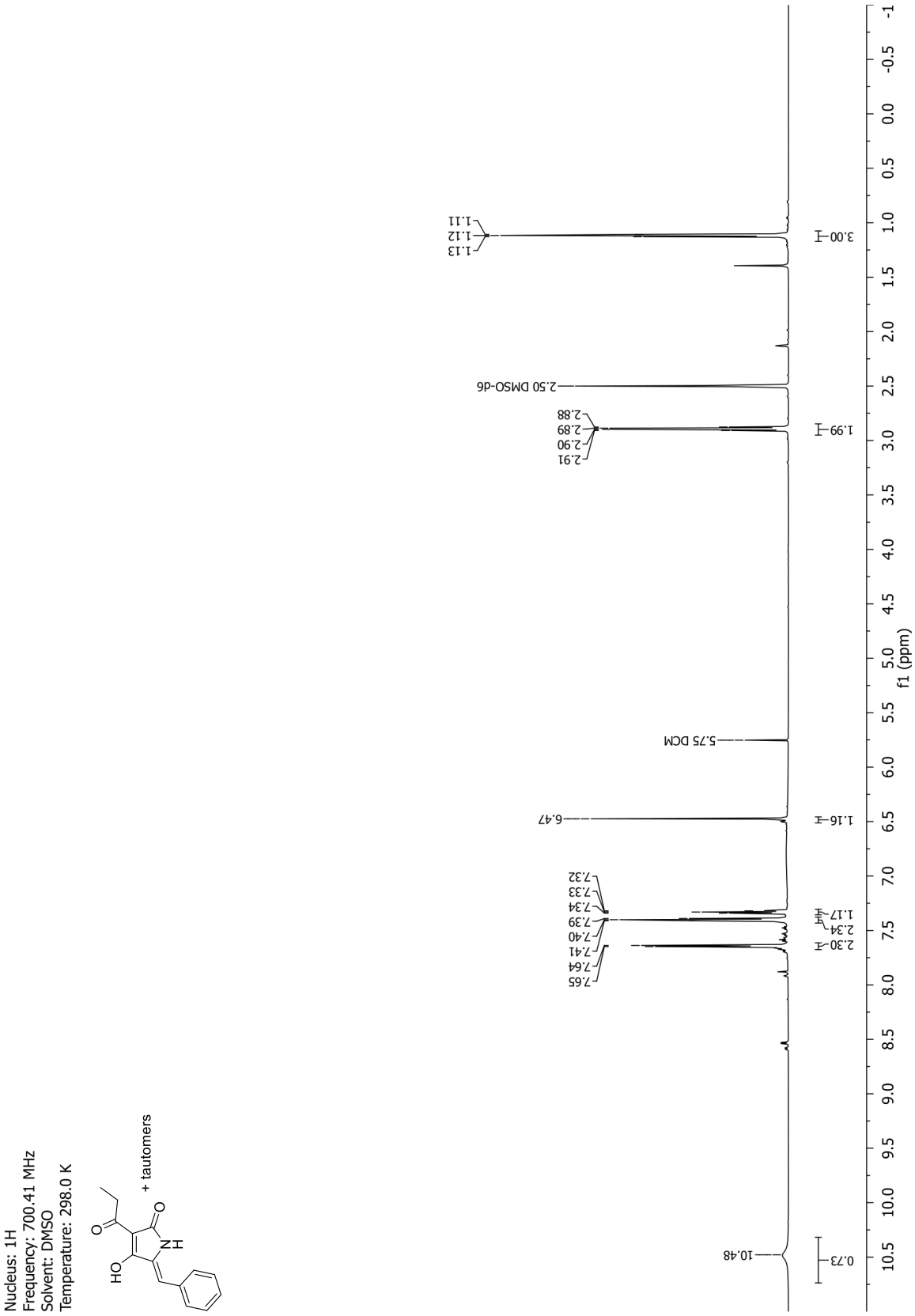

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: DMSO
Temperature: 290.0 K
22.0
24.3
39.5 DMSO-d6
45.6
104.2
160.8
166.2
168.2
171.4
f1 (ppm)

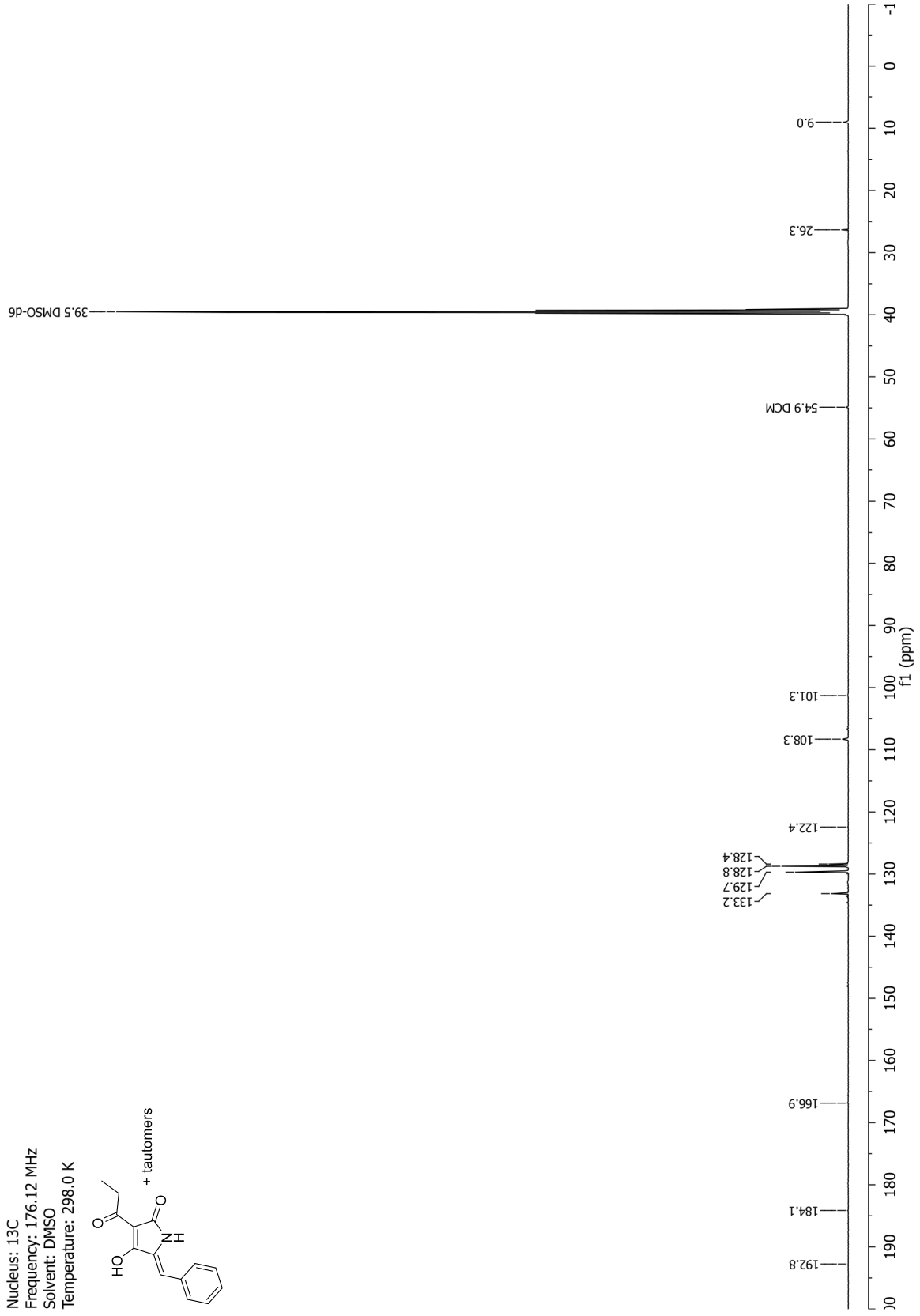
NMR-Spectra for Compound 231



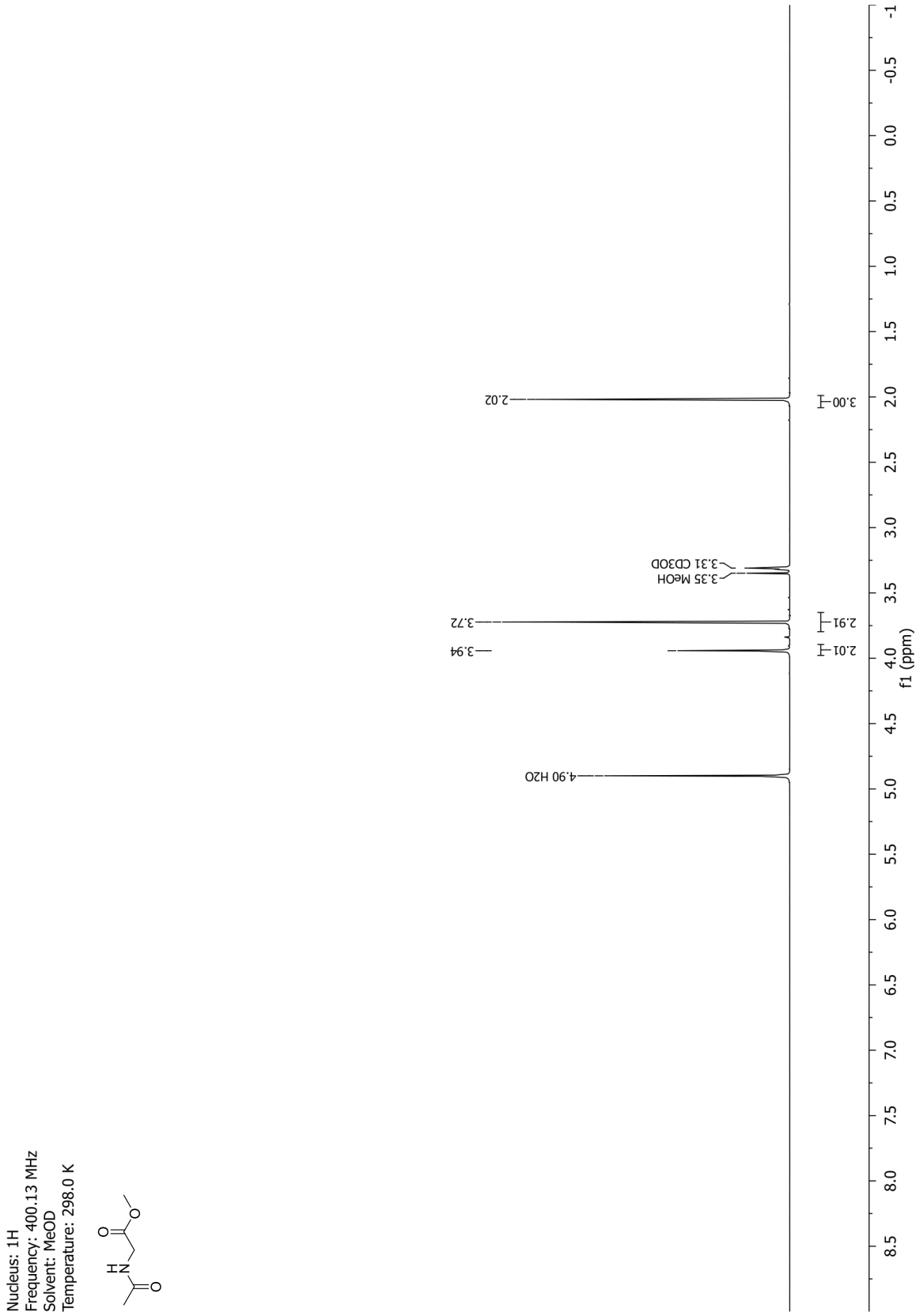

Nucleus: 13C
Frequency: 125.52 MHz
Solvent: DMSO
Temperature: 297.9 K
+ tautomers
HO
O
O
N
O
8.4
24.7
31.6
39.5 DMSO-d6
49.2
54.9 DCM
104.5
168.7
168.1
185.8
194.6
f1 (ppm)

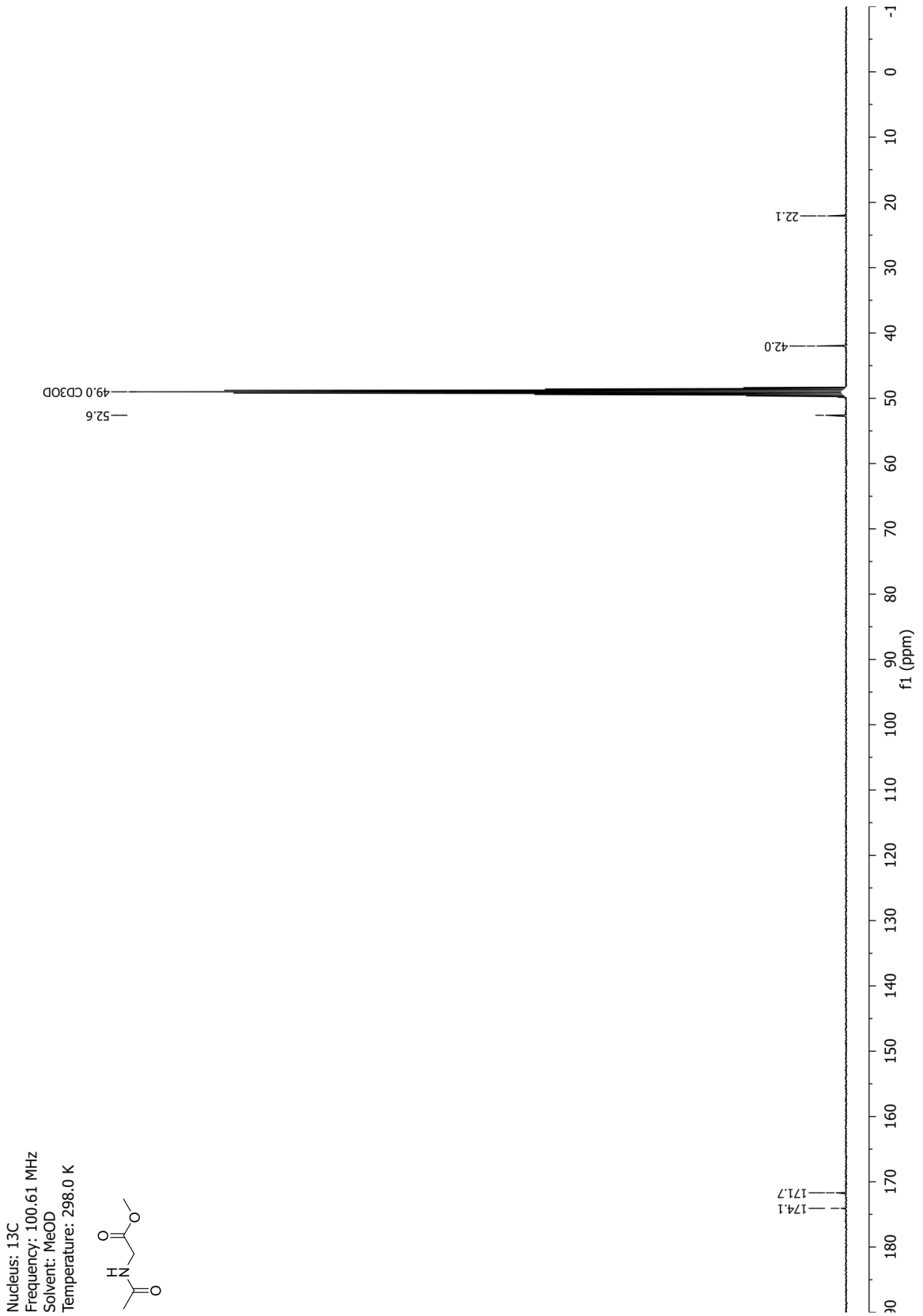
NMR-Spectra for Compound 232



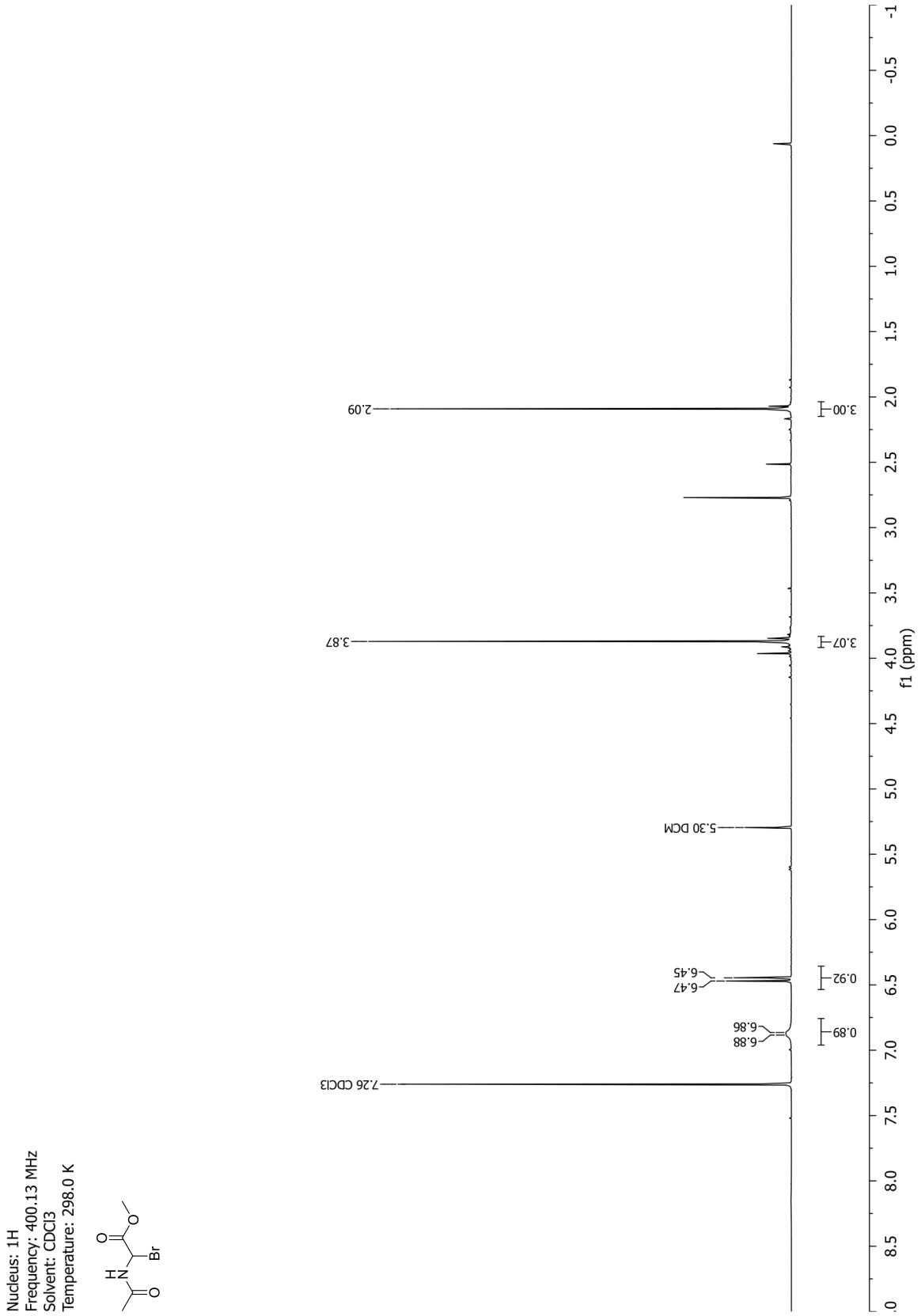

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
+ tautomers
O
O
NH
HO
9.0
26.3
39.5 DMSO-d6
54.9 DCM
101.3
108.3
122.4
128.4
128.8
129.7
133.2
166.9
184.1
192.8
f1 (ppm)

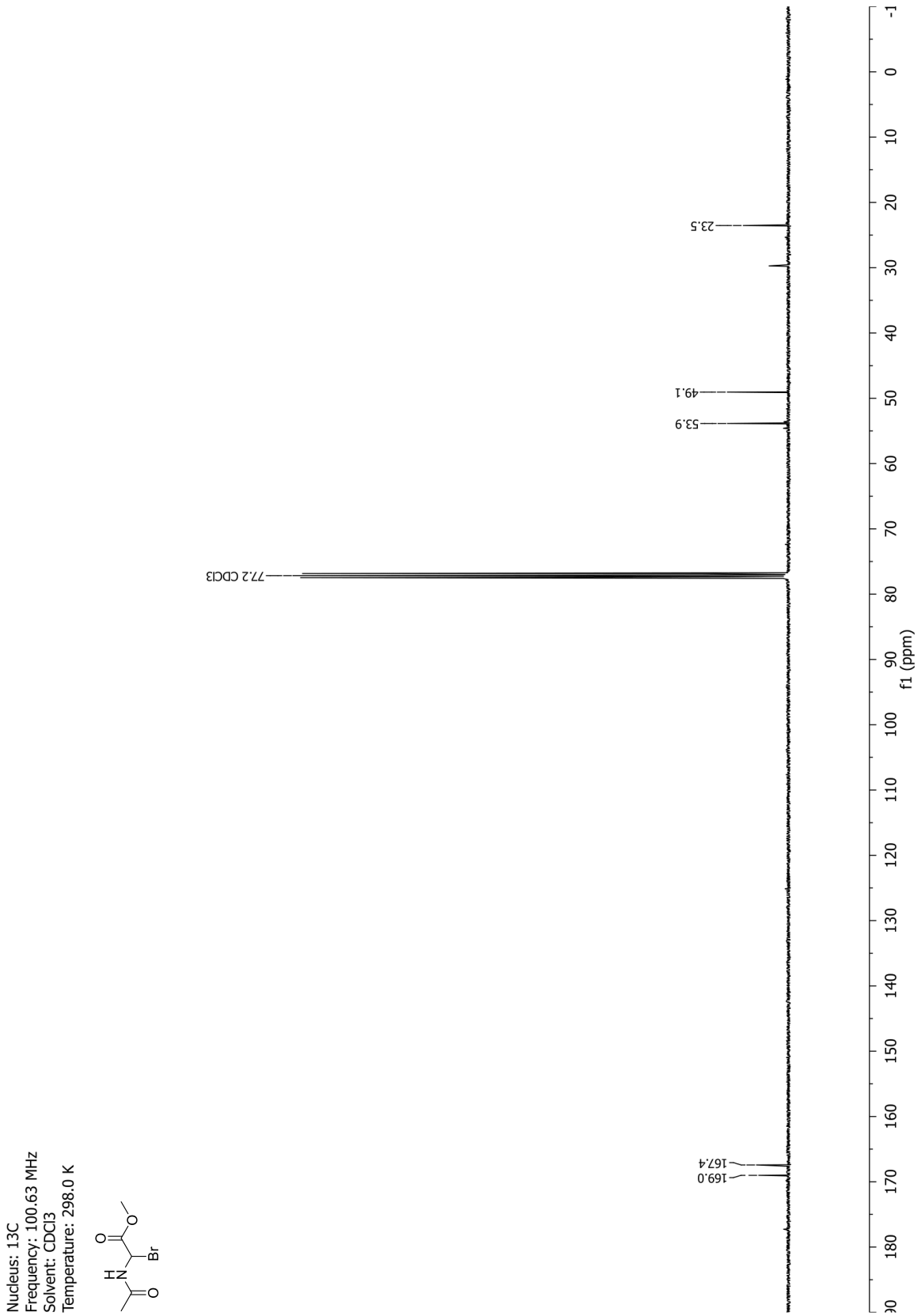
NMR-Spectra for Compound 234



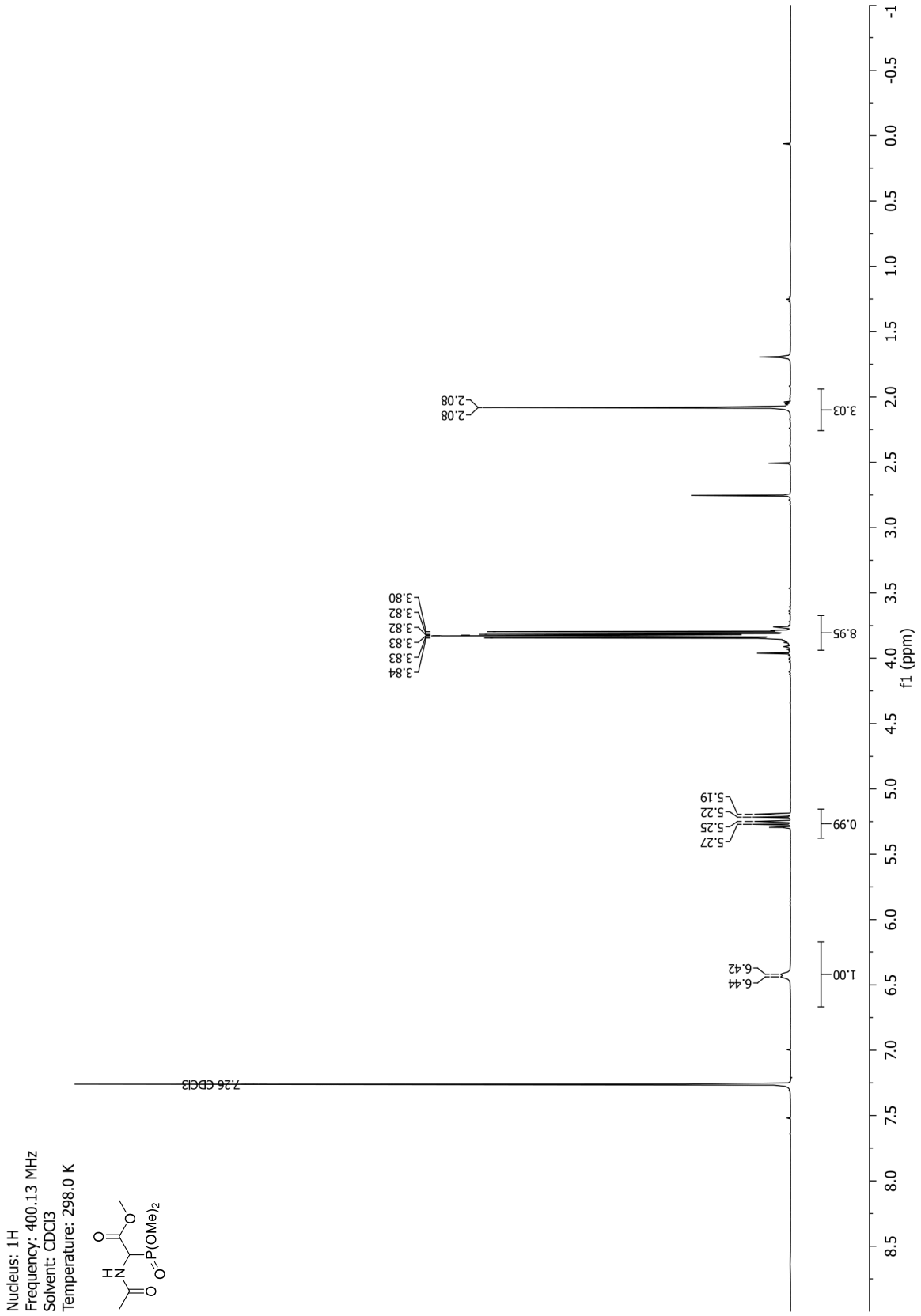

Nucleus: 13C
Frequency: 100.61 MHz
Solvent: MeOD
Temperature: 298.0 K
22.1
42.0
52.6
49.0 CD3OD
171.7
174.1
f1 (ppm)

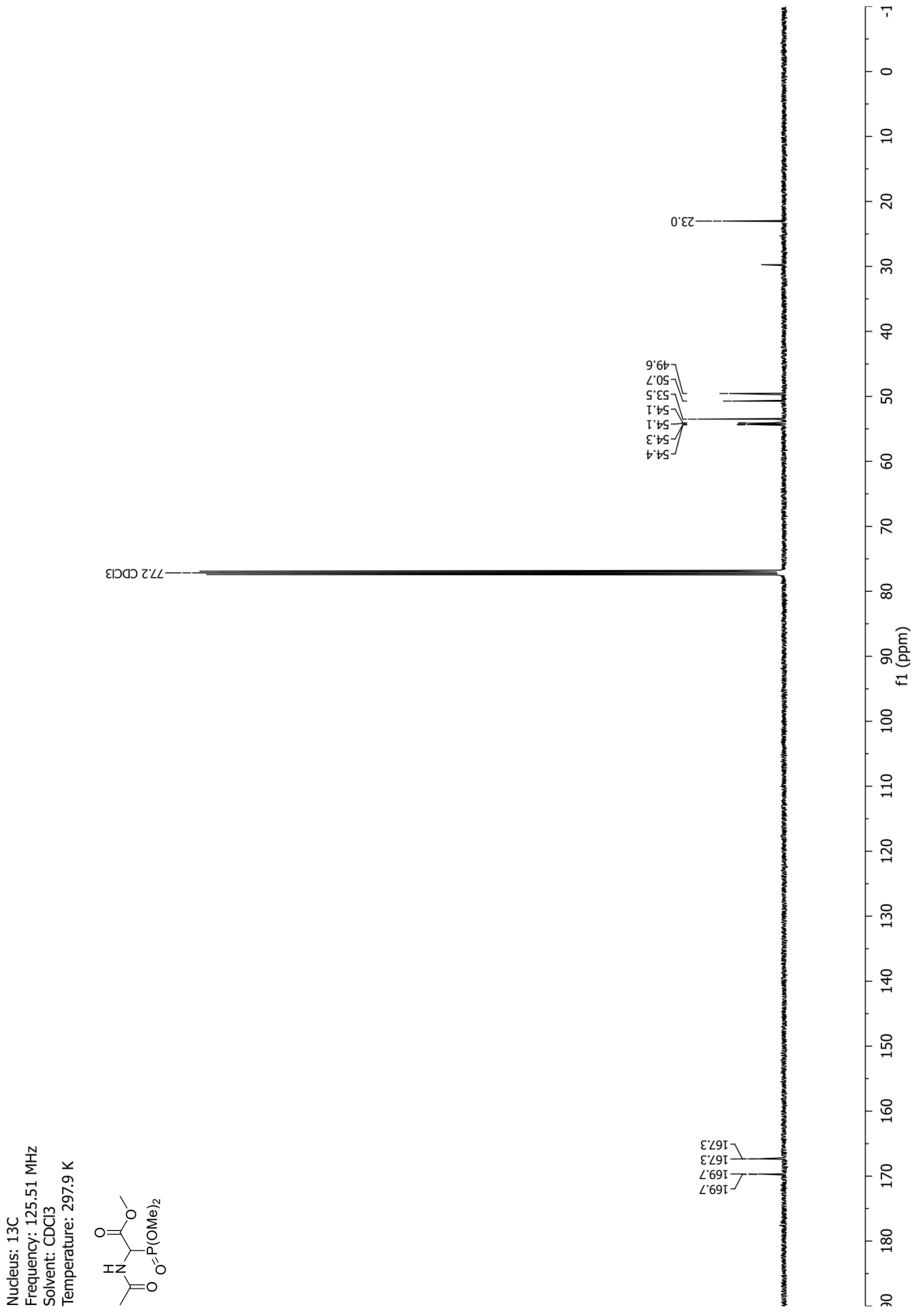
NMR-Spectra for Compound 235

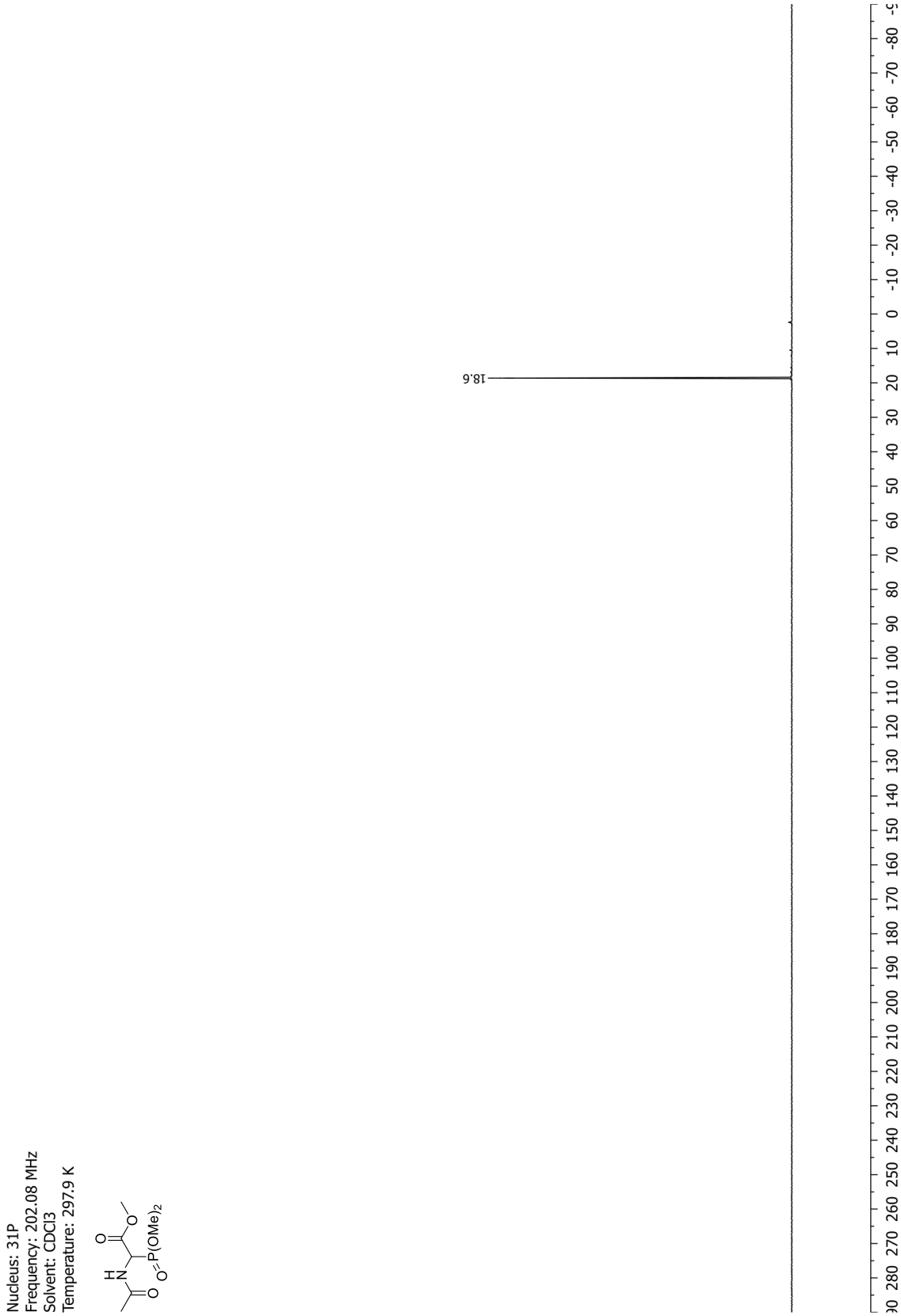



Nucleus: 13C
Frequency: 100.63 MHz
Solvent: CDCl3
Temperature: 298.0 K
77.2 CDCl3
169.0
167.4
53.9
49.1
23.5
f1 (ppm)

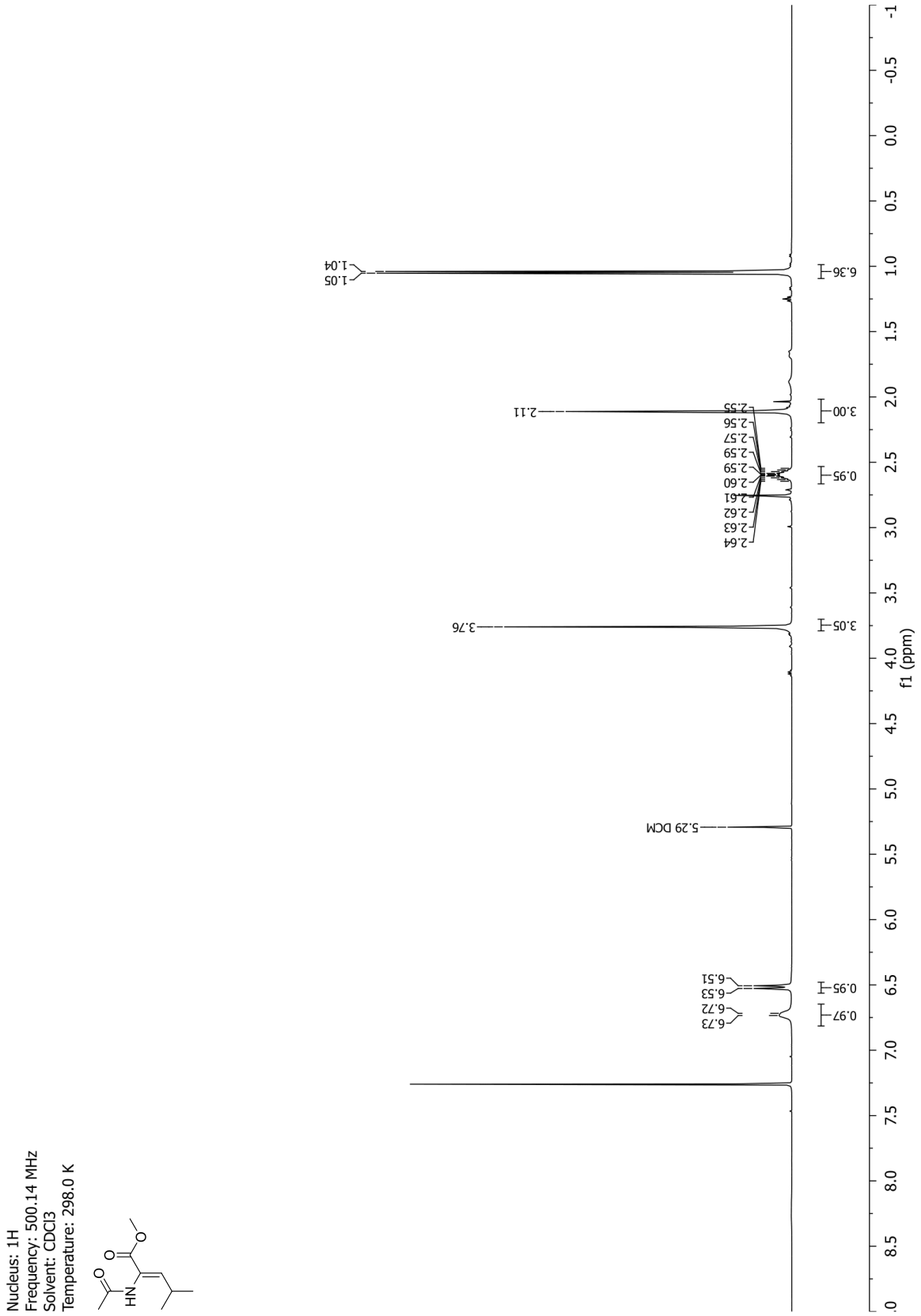
NMR-Spectra for Compound 236

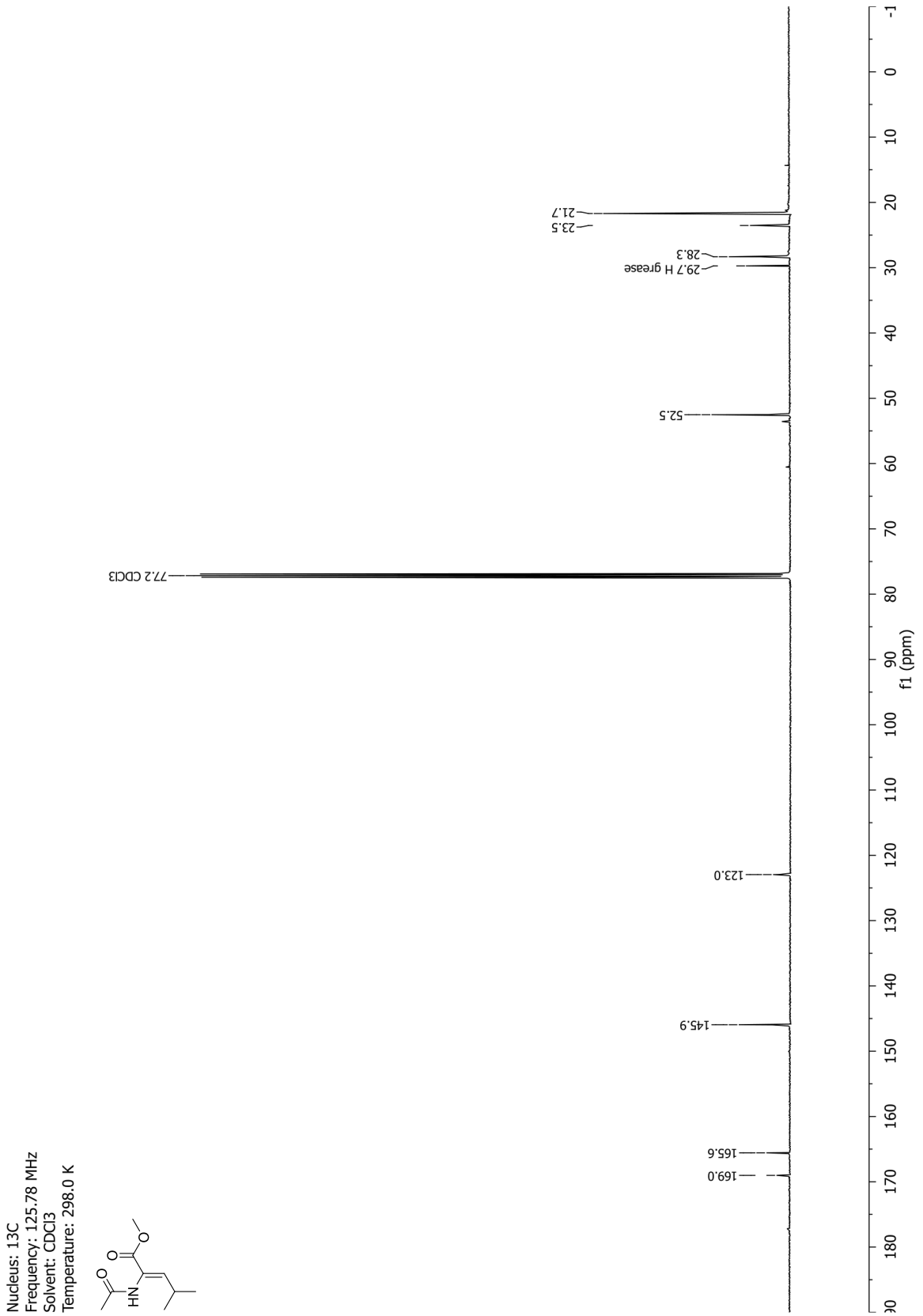



Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 297.9 K
77.2 CDCl3
169.7
169.7
167.3
167.3
54.4
54.3
54.1
54.1
53.5
50.7
49.6
23.0
f1 (ppm)

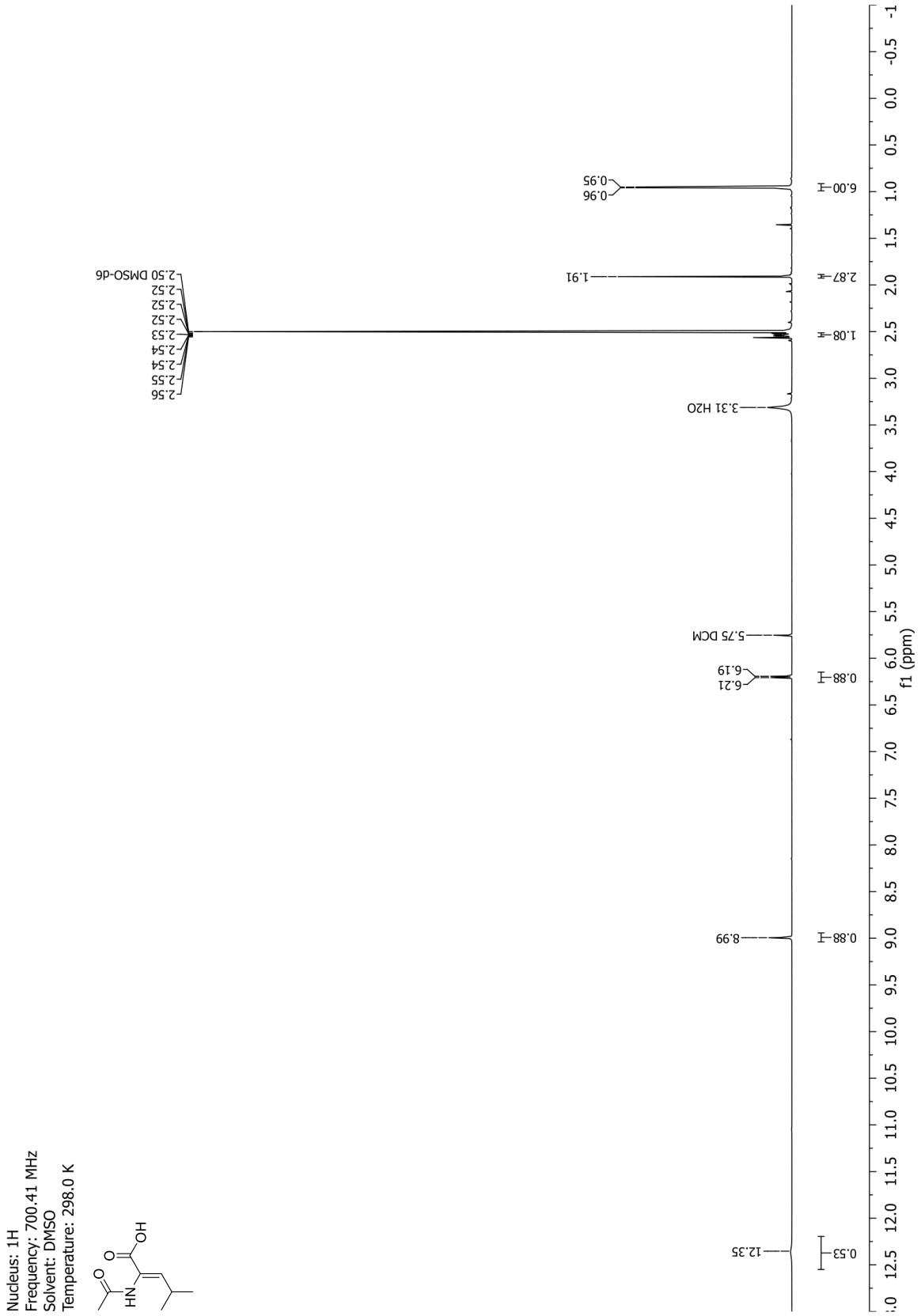

Nucleus: 31P
Frequency: 202.08 MHz
Solvent: CDCl3
Temperature: 297.9 K
18.6
f1 (ppm)

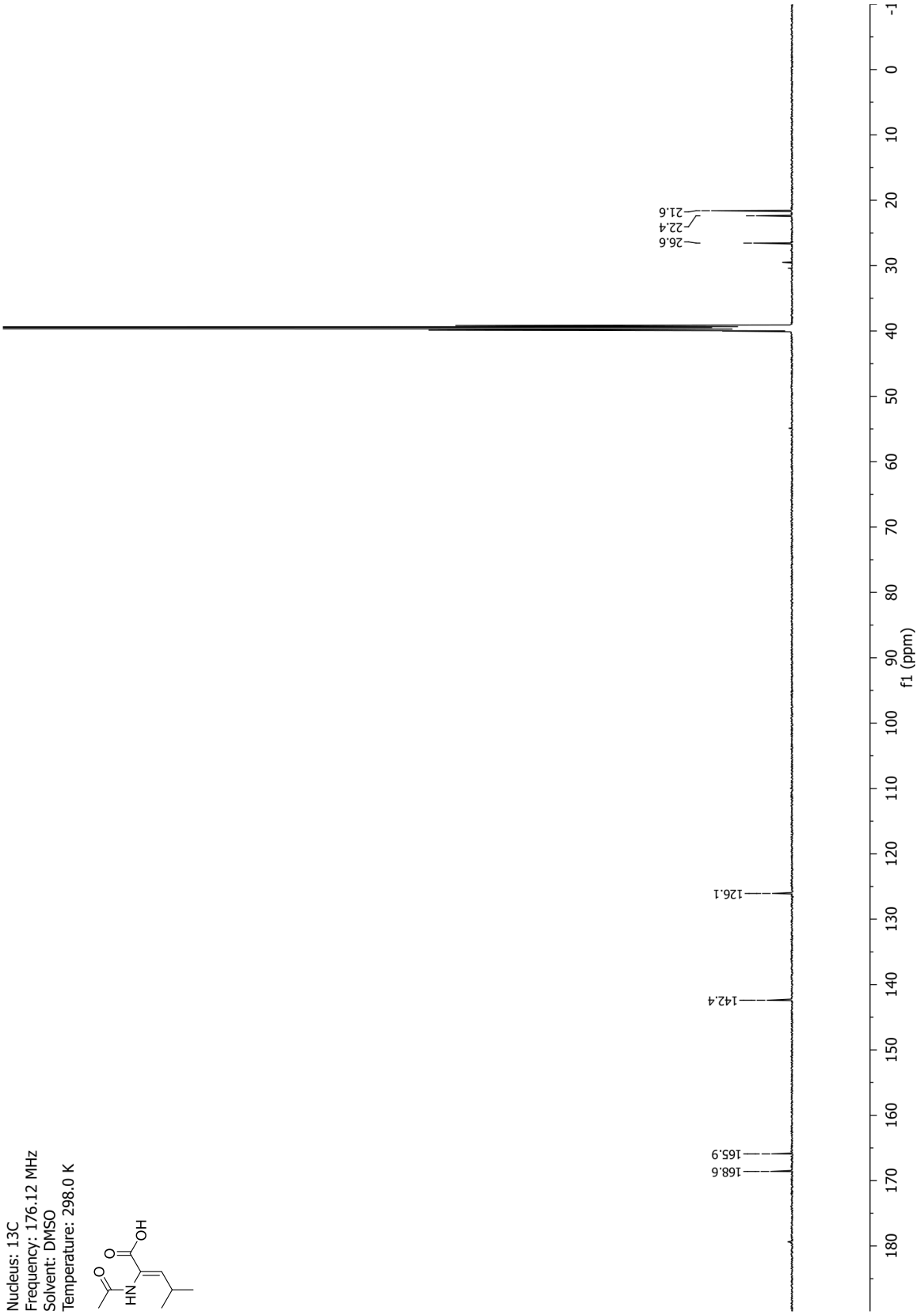
NMR-Spectra for Compound 237



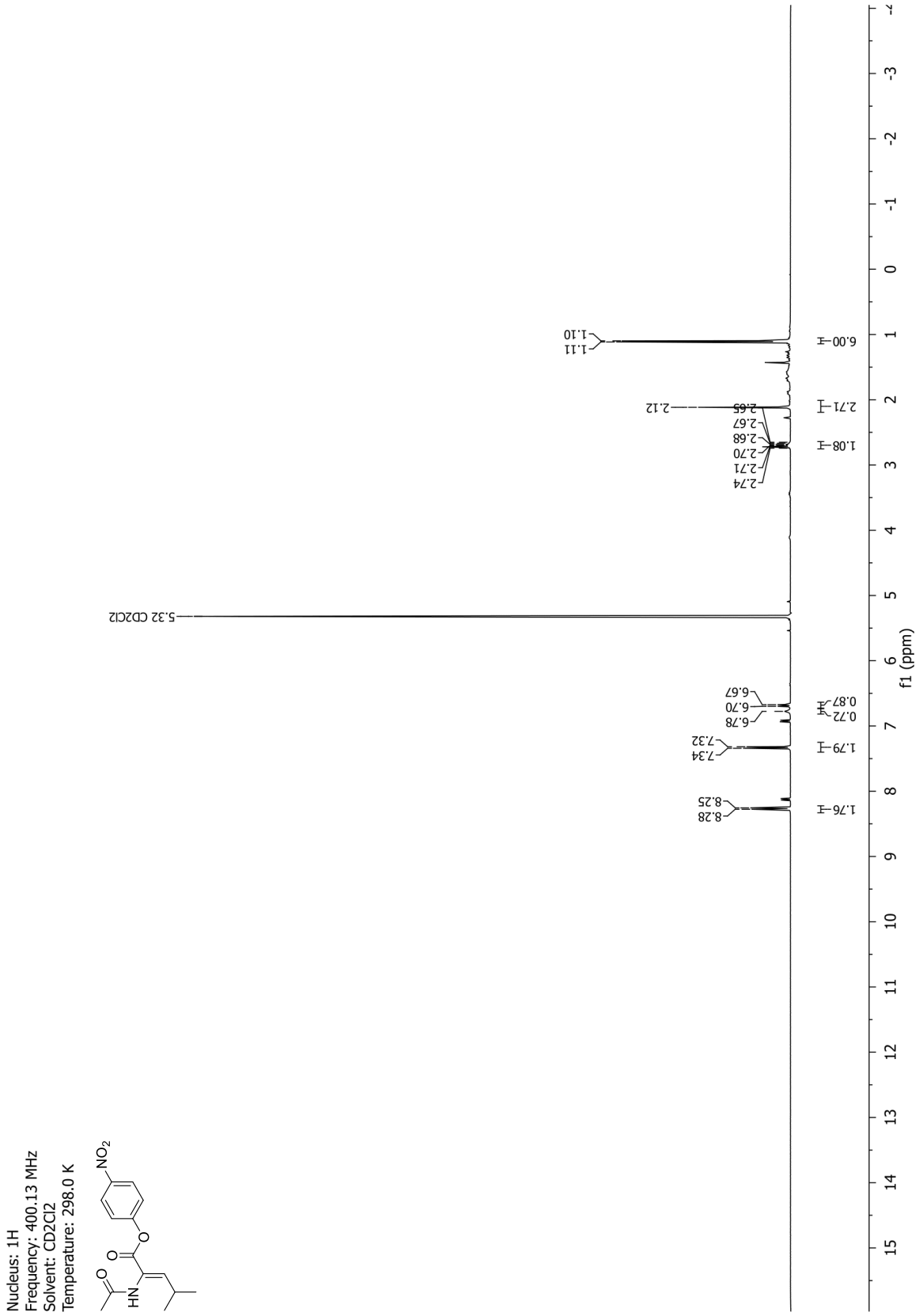

Nucleus: 13C
Frequency: 125.78 MHz
Solvent: CDCl3
Temperature: 298.0 K
HN
O
O
O
169.0
165.6
145.9
123.0
77.2 CDCl3
52.5
29.7 H grease
28.3
23.5
21.7
f1 (ppm)

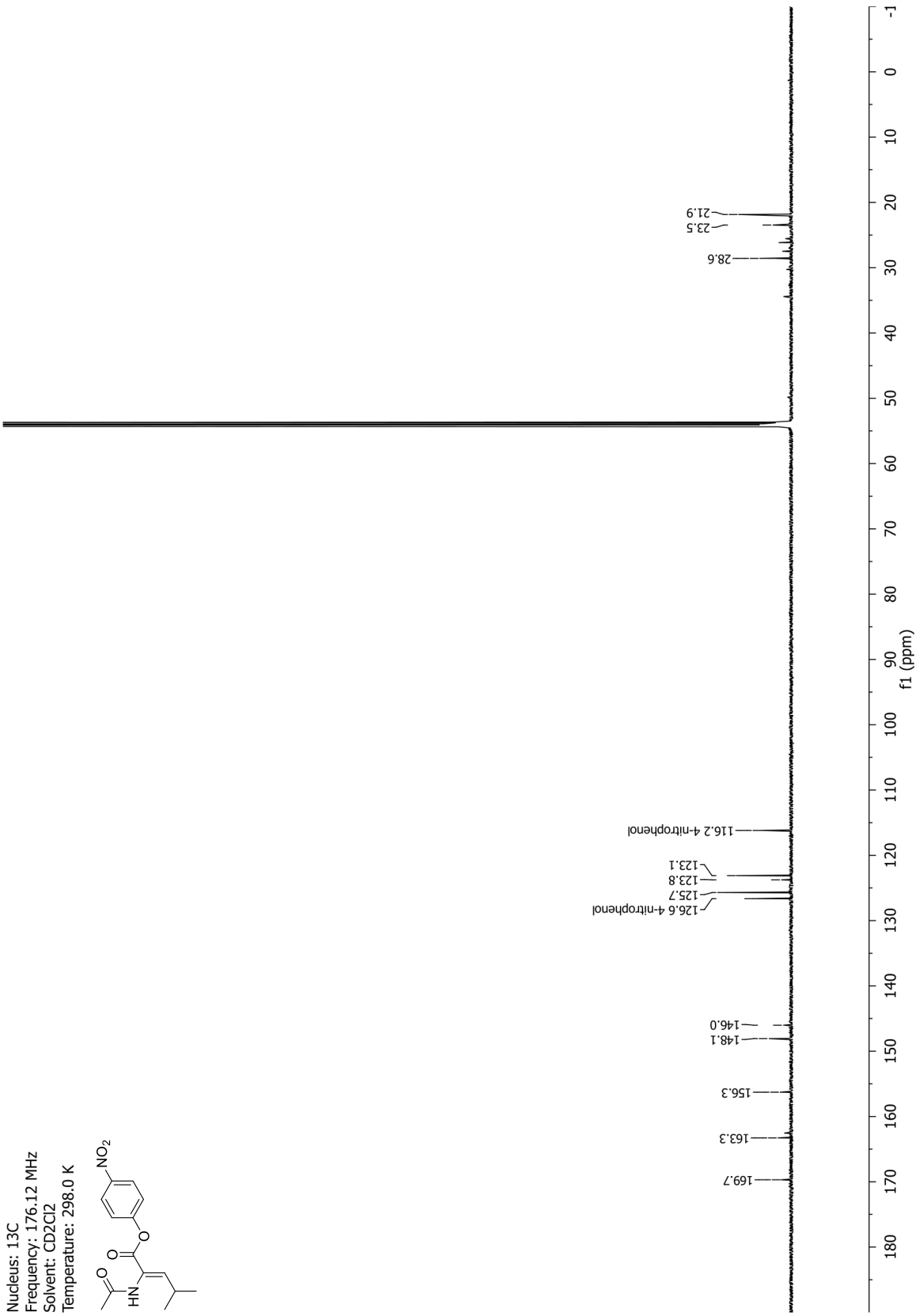
NMR-Spectra for Compound 238



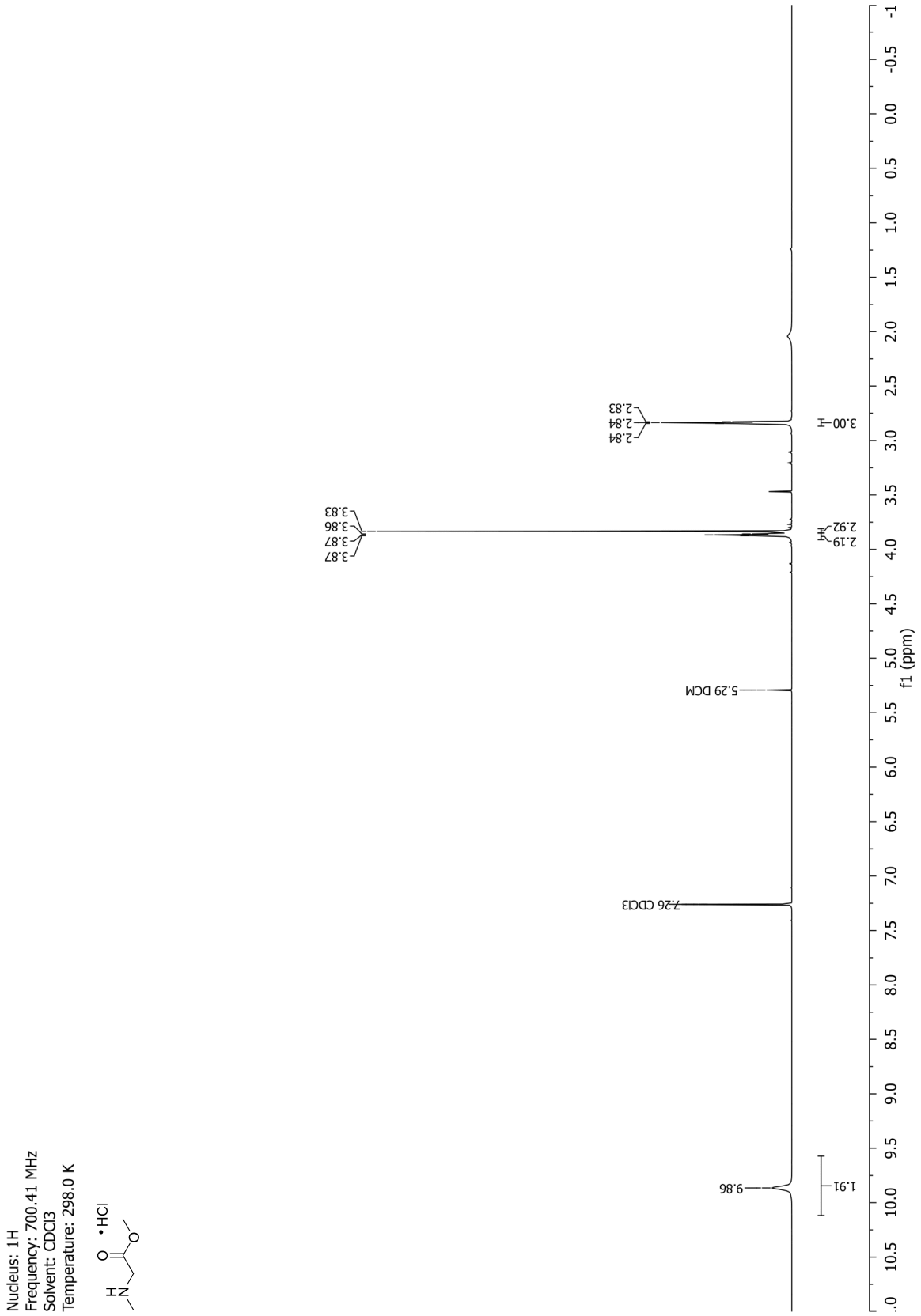

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: DMSO
Temperature: 298.0 K
21.6
22.4
26.6
126.1
142.4
165.9
168.6
f1 (ppm)

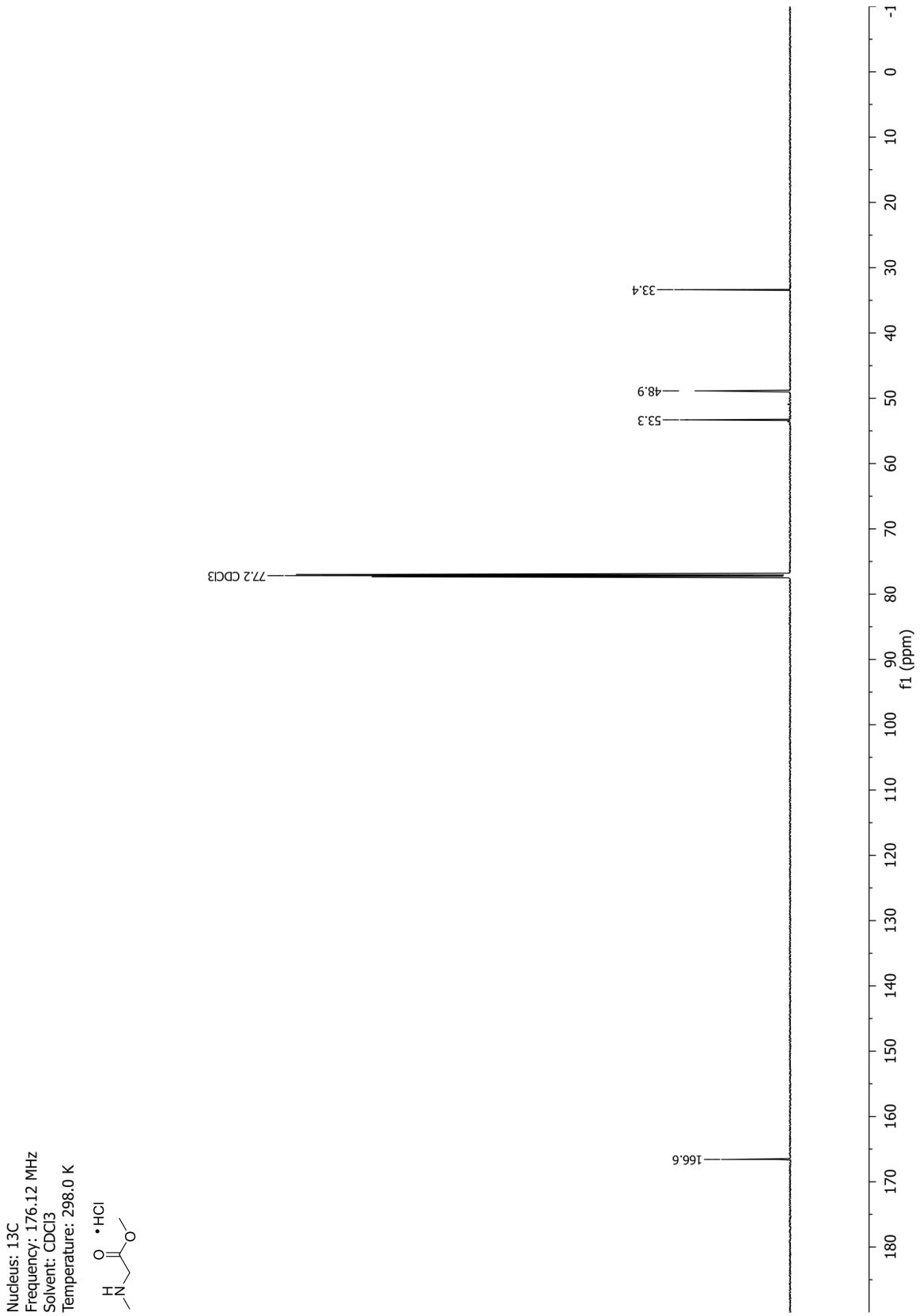
NMR-Spectra for Compound 239



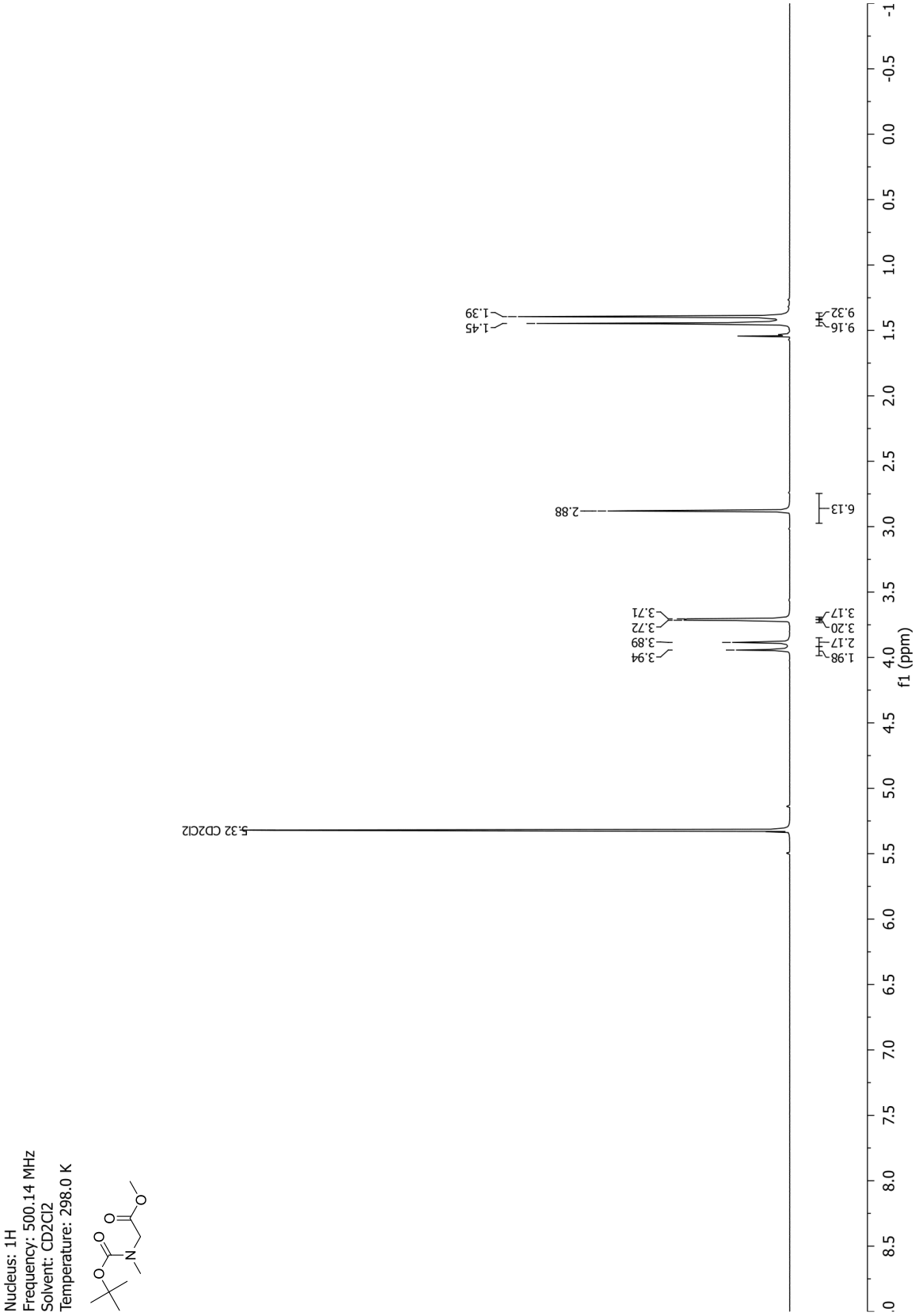

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
NO2
O
O
HN
O
169.7
163.3
156.3
148.1
146.0
126.6 4-nitrophenol
125.7
123.8
123.1
116.2 4-nitrophenol
28.6
23.5
21.9
f1 (ppm)

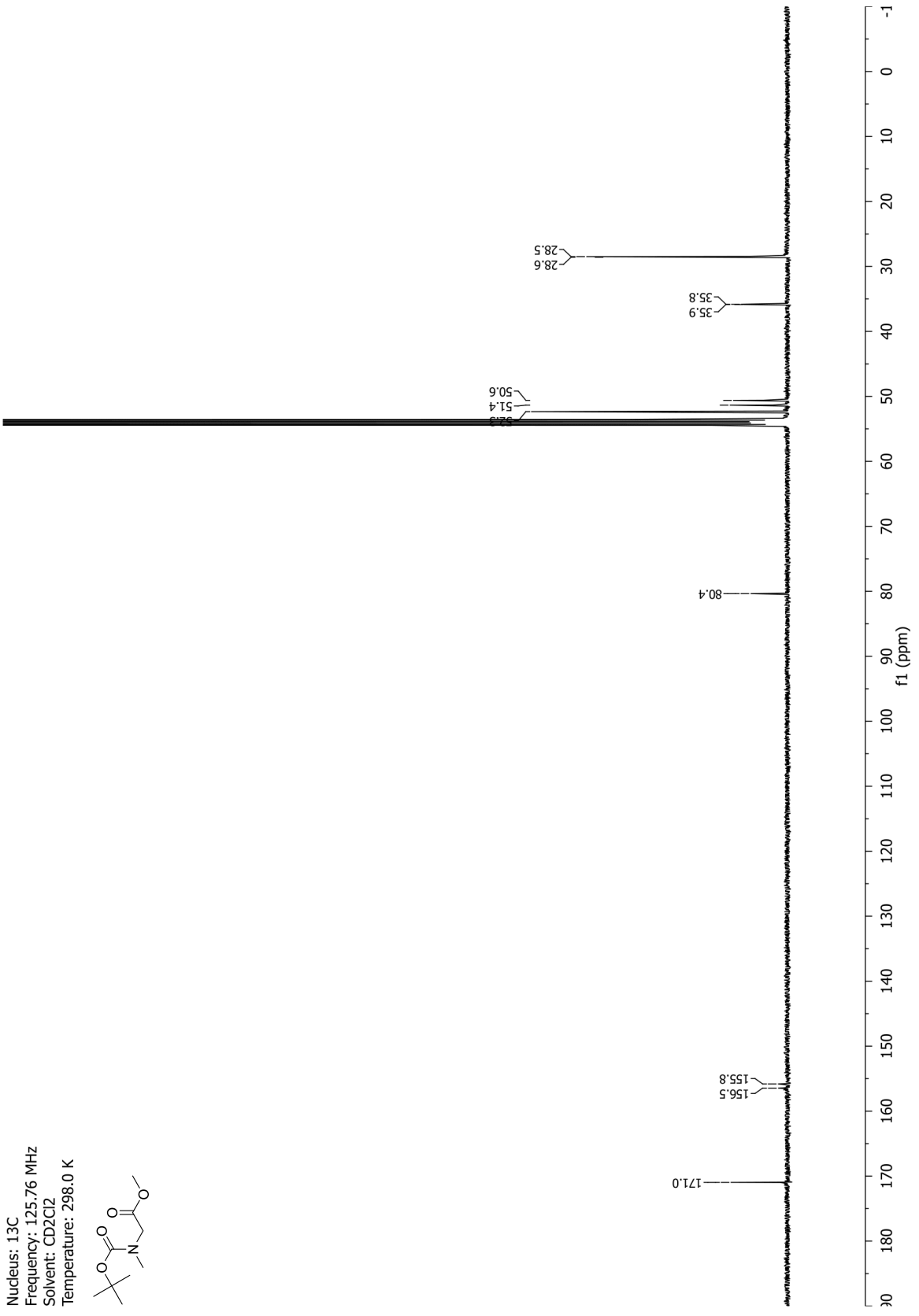
NMR-Spectra for Compound 243



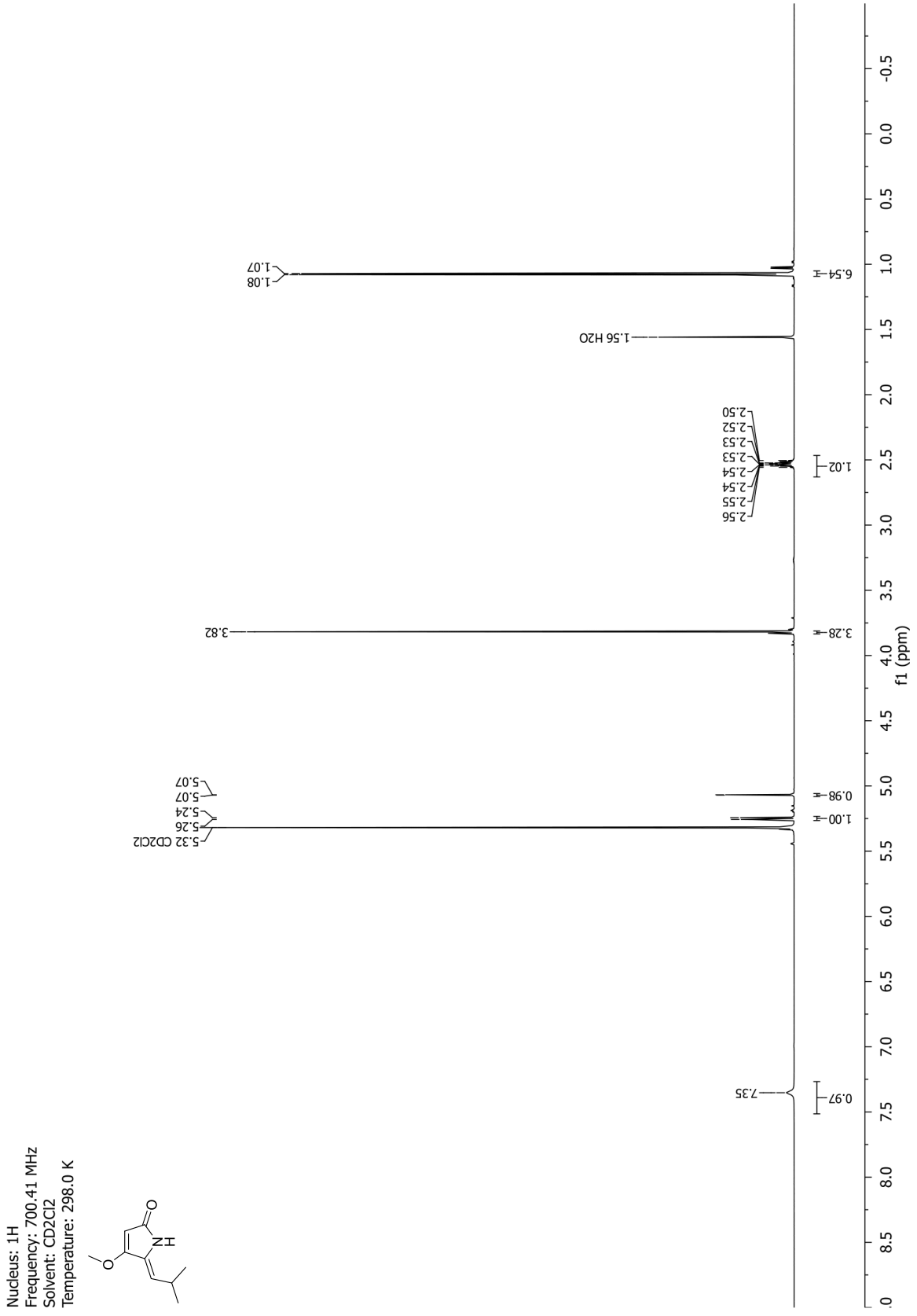

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
• HCl
166.6
77.2 CDCl3
53.3
48.9
33.4
f1 (ppm)

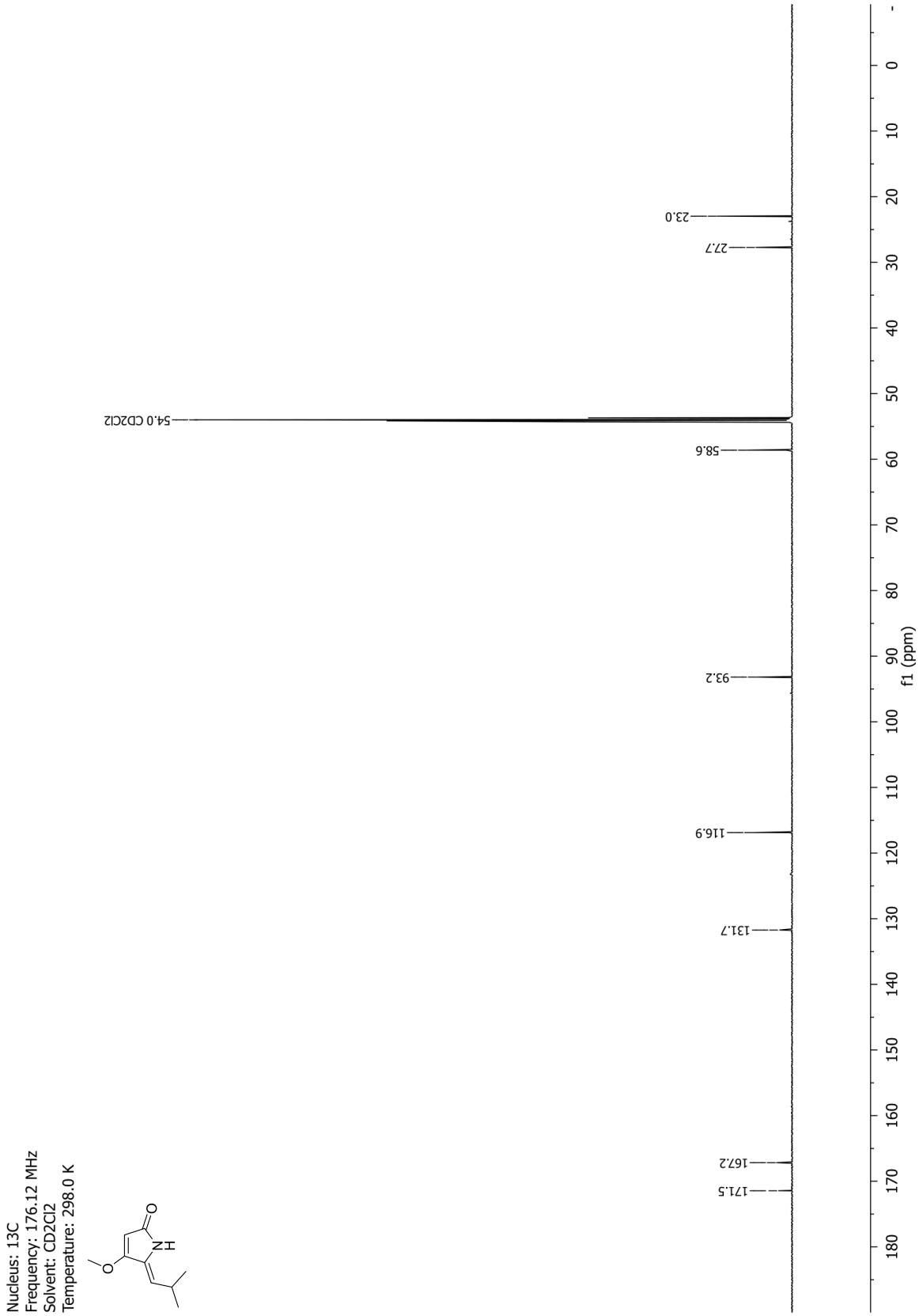
NMR-Spectra for Compound 245



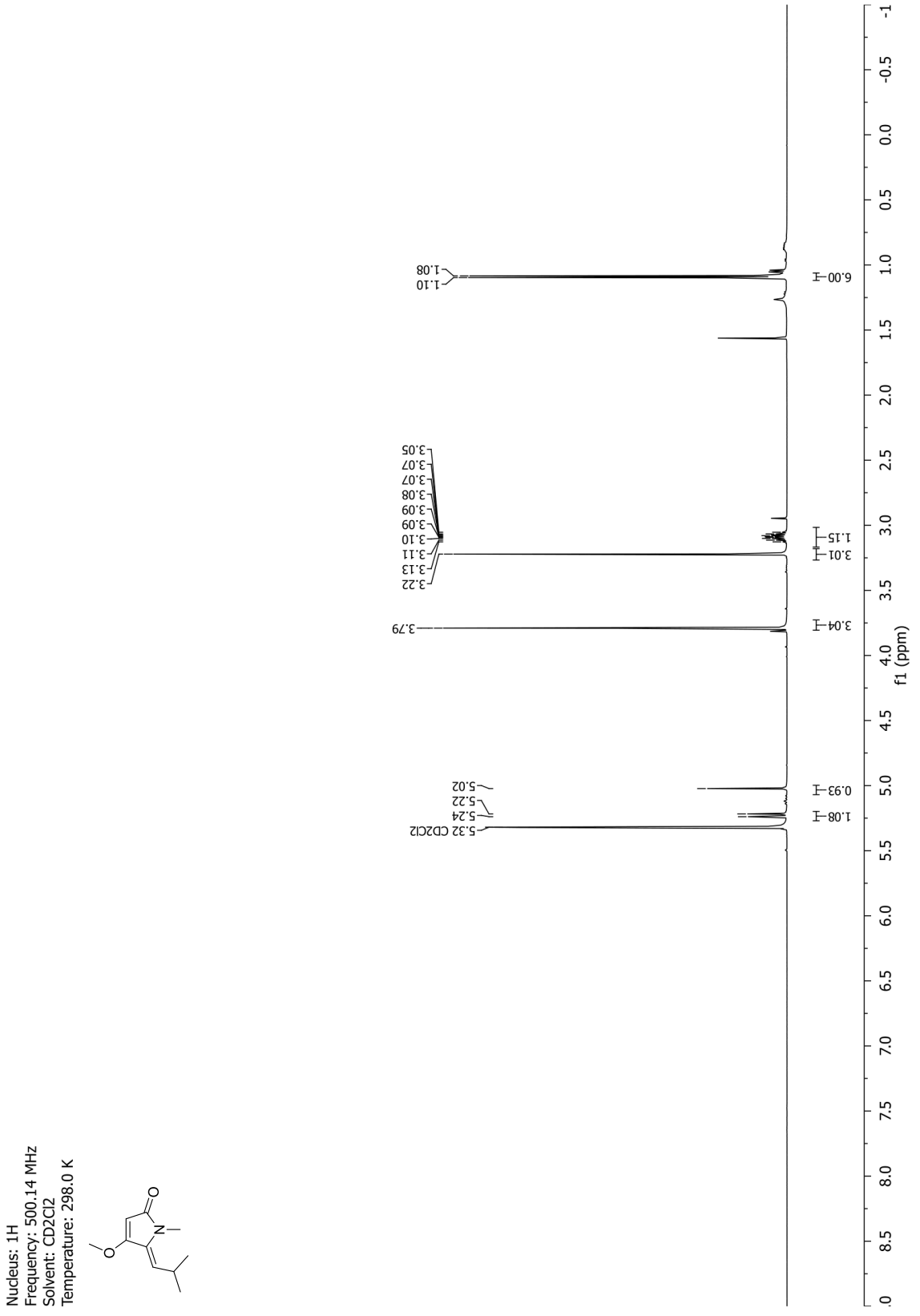

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
28.6
28.5
35.9
35.8
50.6
51.4
80.4
156.5
155.8
171.0
f1 (ppm)

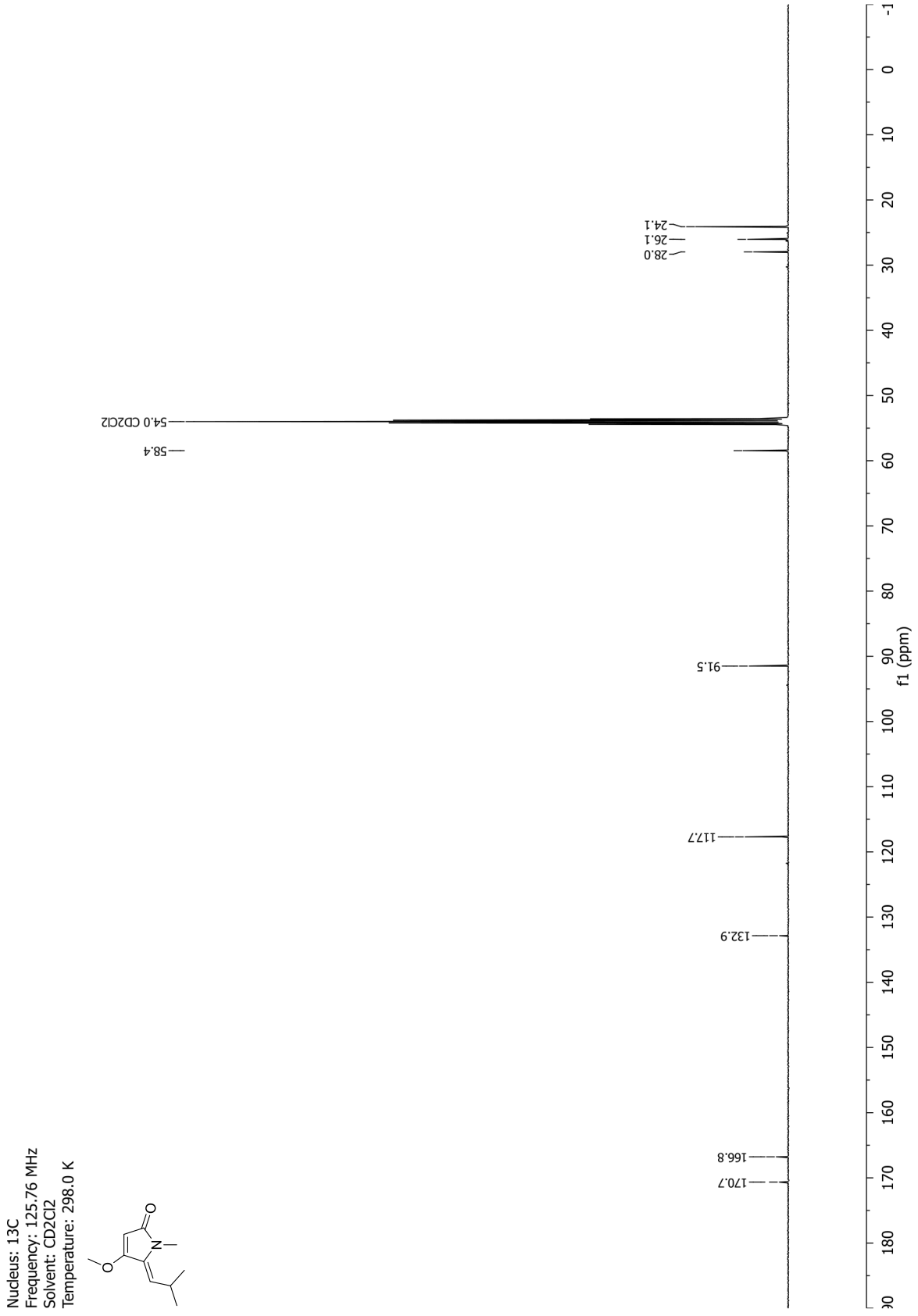
NMR-Spectra for Compound 210



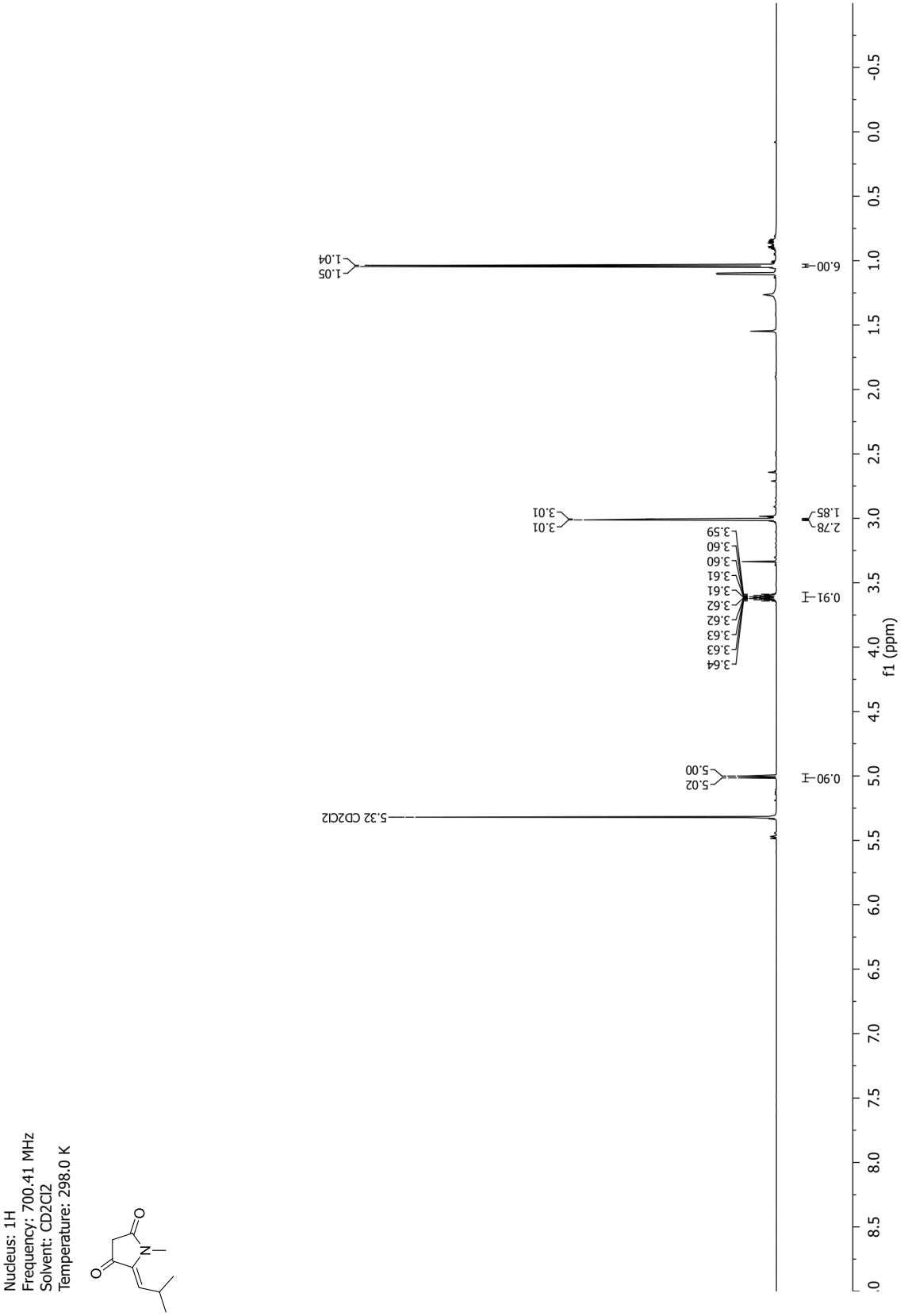

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
23.0
27.7
54.0 CD2Cl2
58.6
93.2
116.9
131.7
167.2
171.5
f1 (ppm)

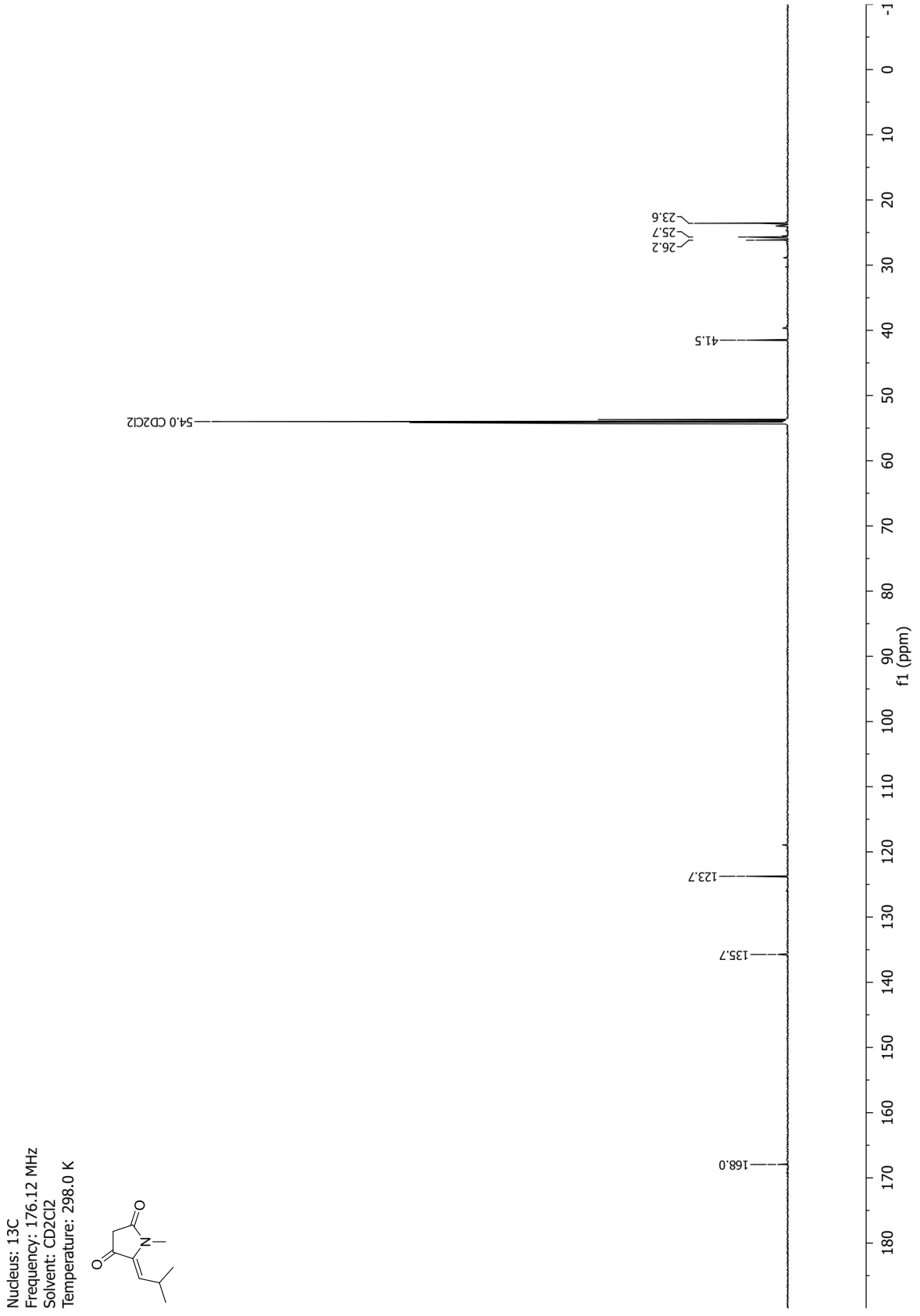
NMR-Spectra for Compound 209



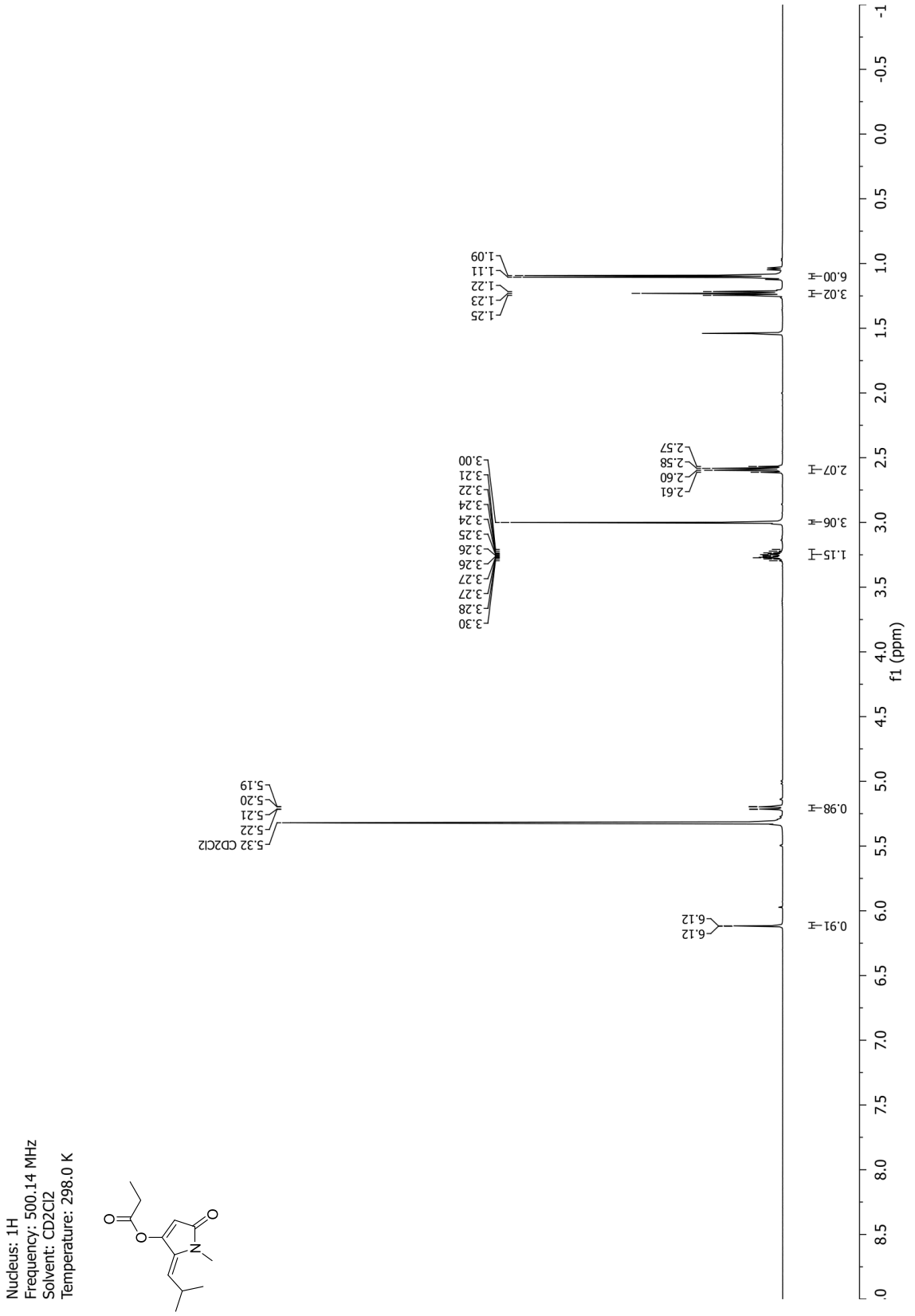

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
24.1
26.1
28.0
54.0 CD2Cl2
58.4
91.5
117.7
132.9
166.8
170.7
f1 (ppm)

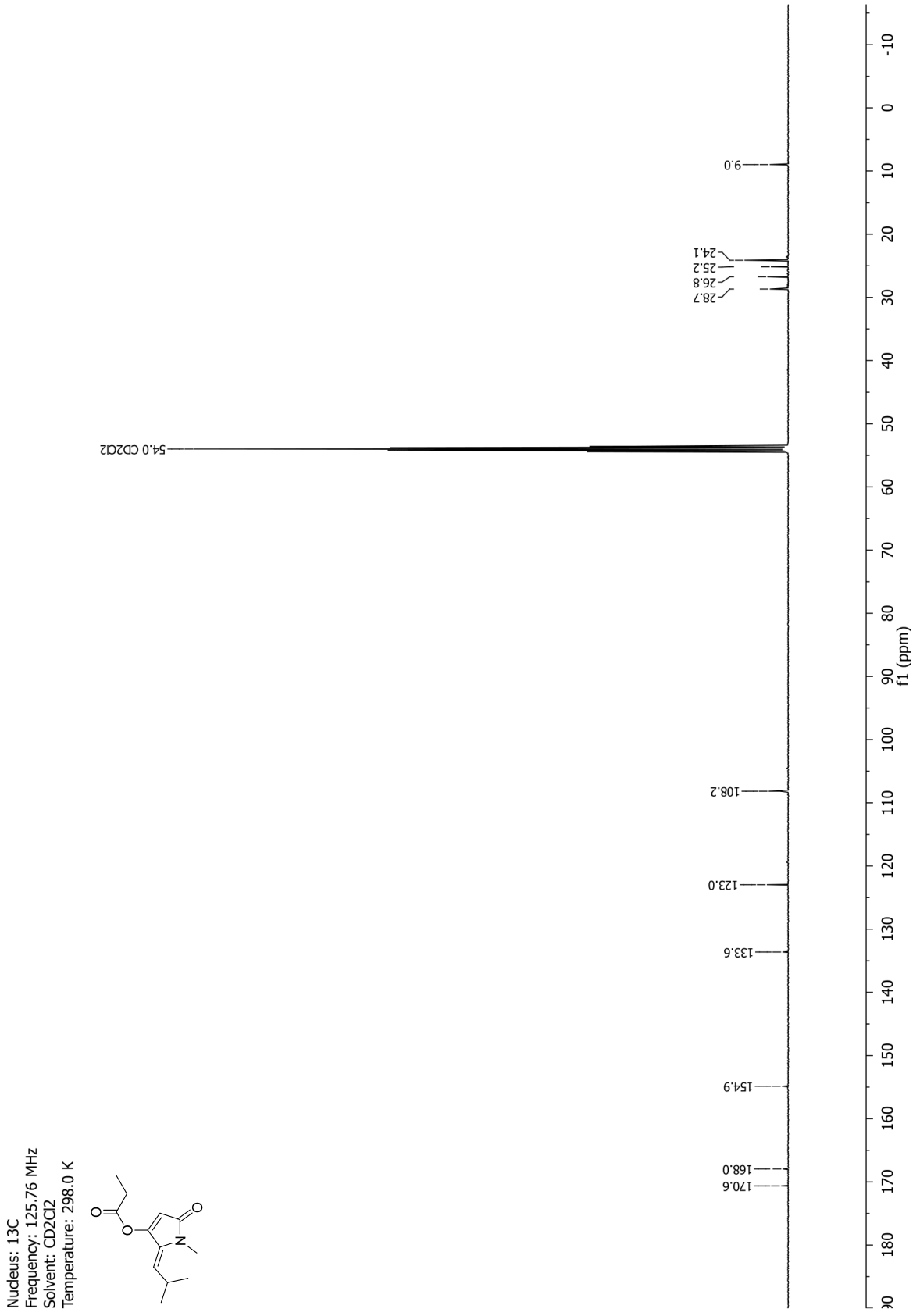
NMR-Spectra for Compound 9



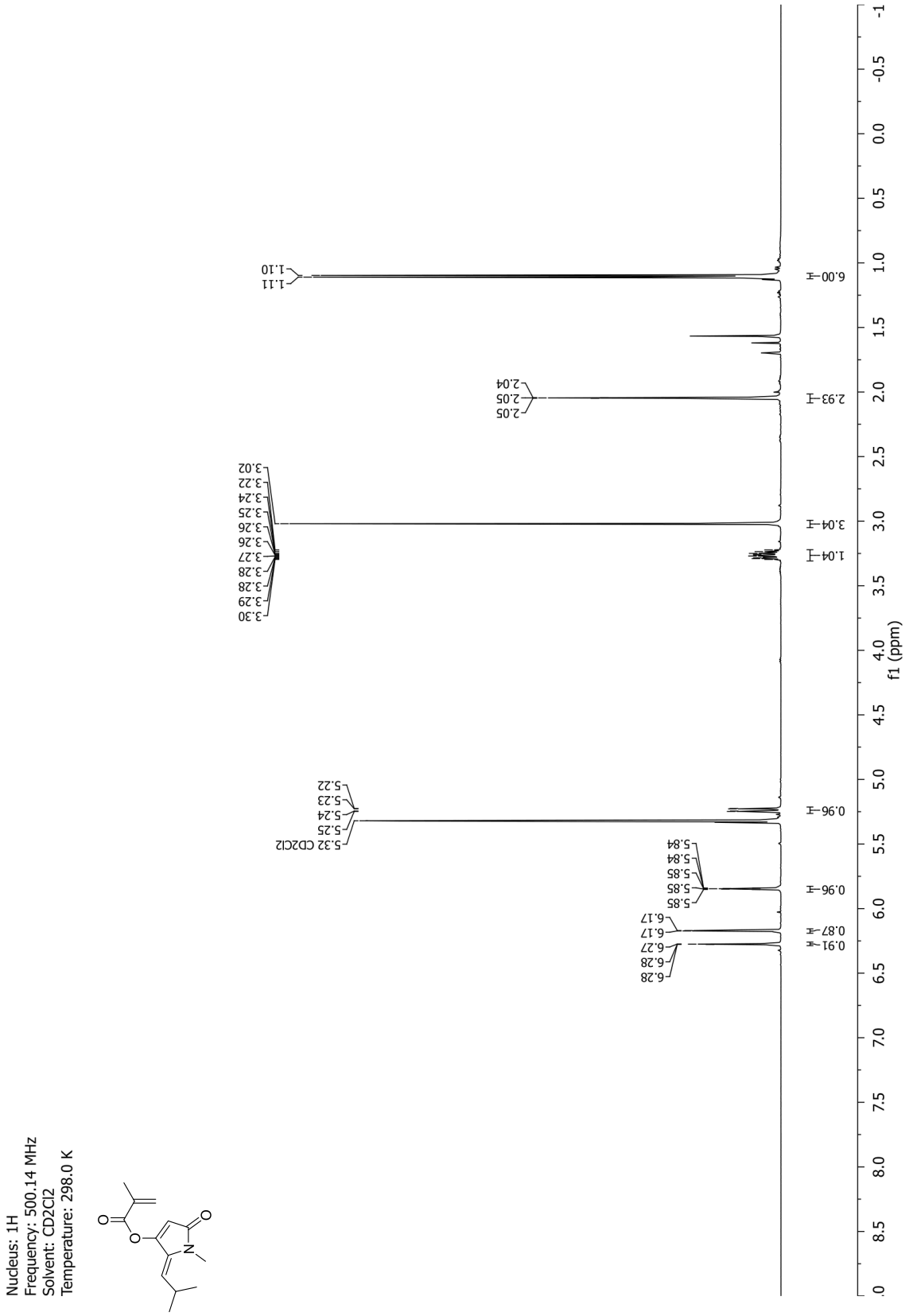

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
168.0
135.7
123.7
54.0 CD2Cl2
41.5
26.2
25.7
23.6
f1 (ppm)

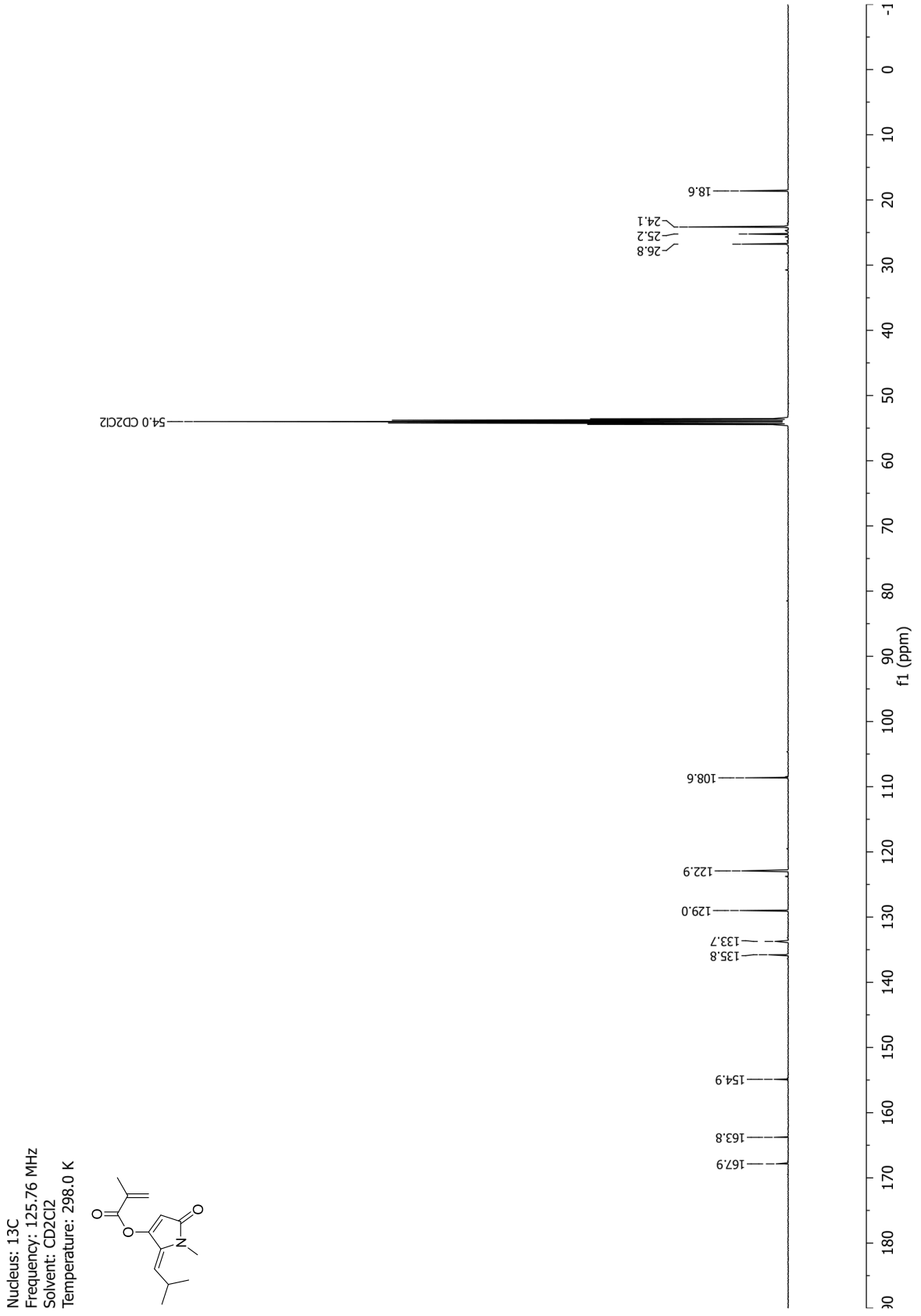
NMR-Spectra for Compound 246



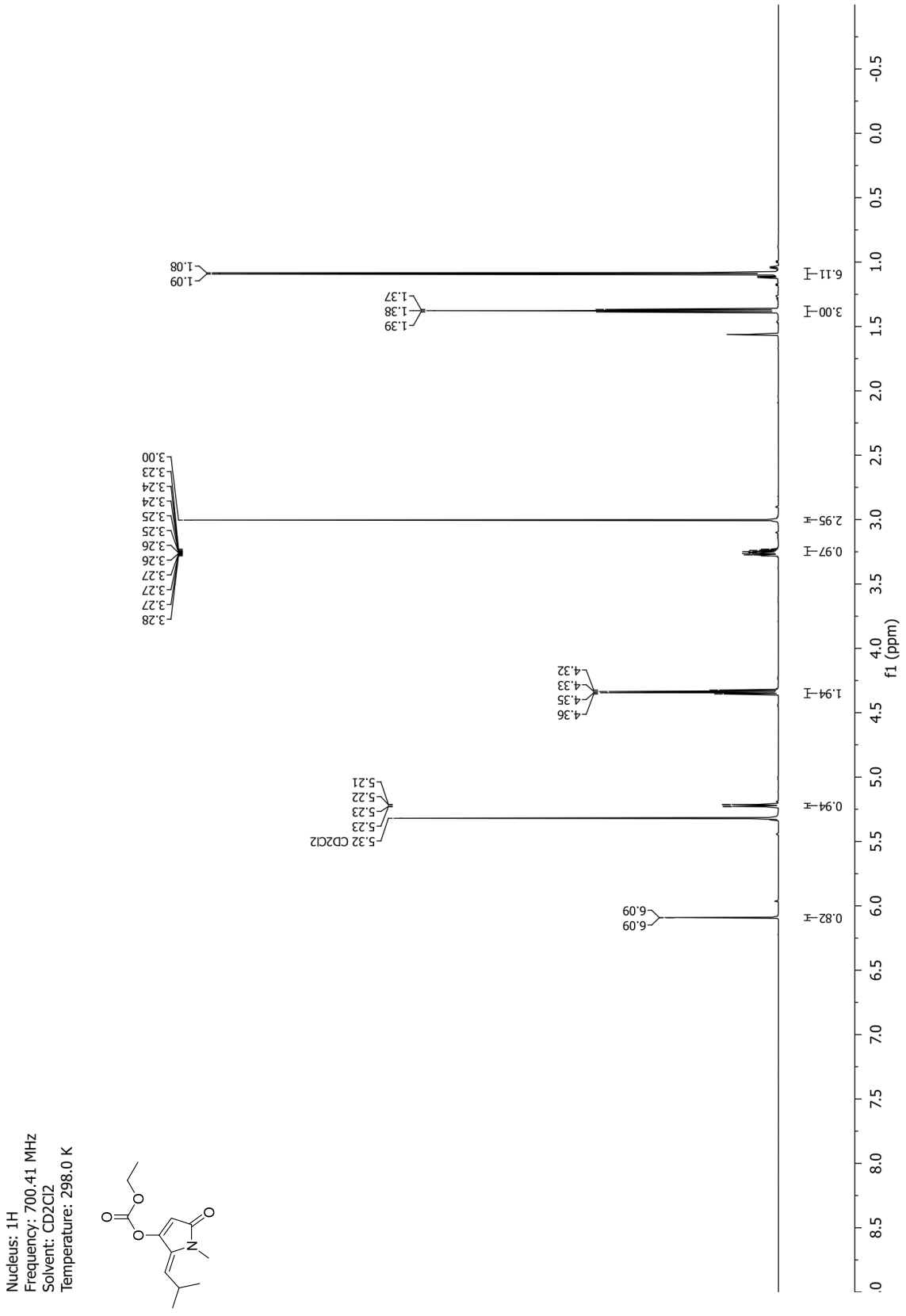

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
9.0
24.1
25.2
26.8
28.7
54.0 CD2Cl2
108.2
123.0
133.6
154.9
168.0
170.6
f1 (ppm)

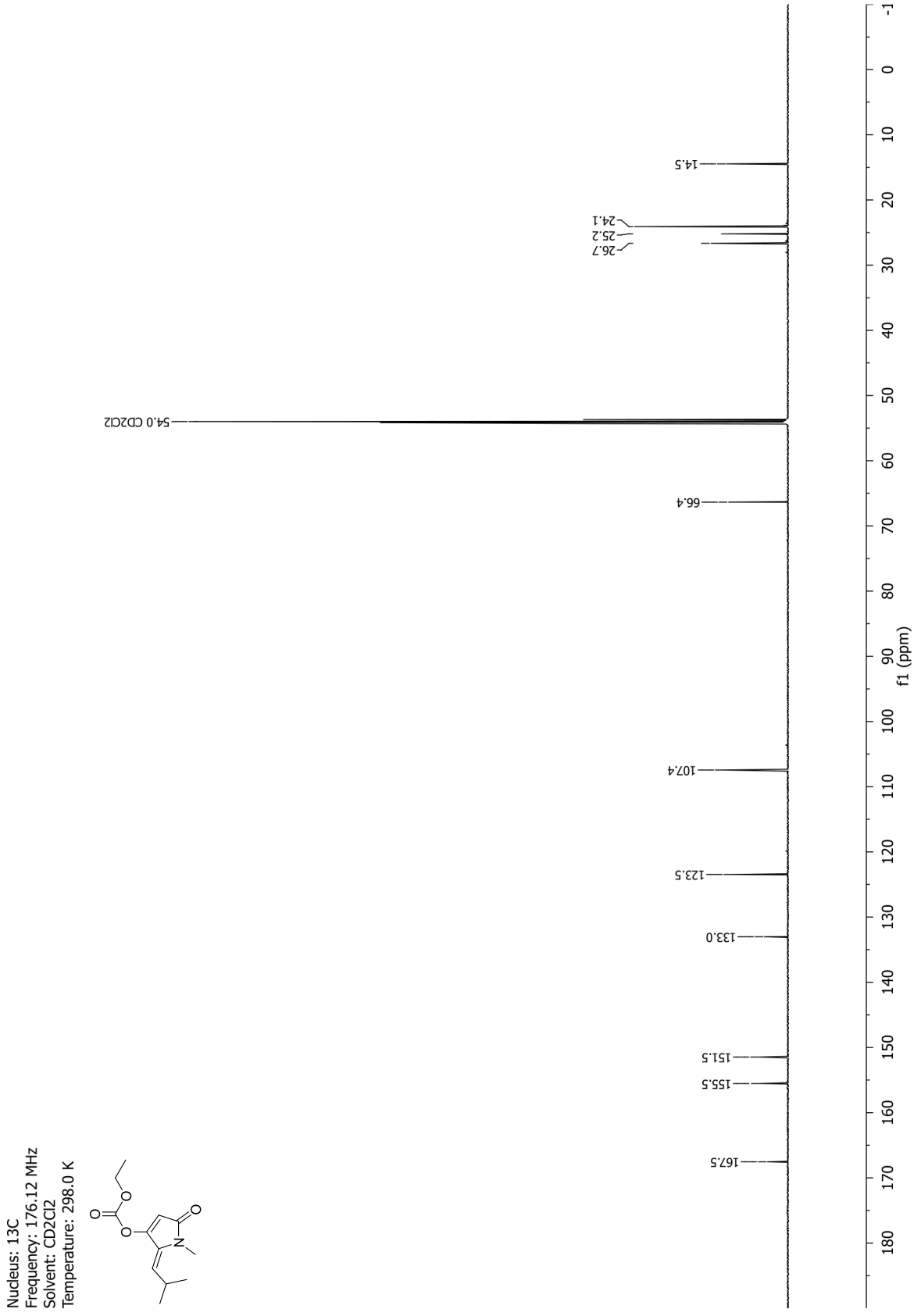
NMR-Spectra for Compound 247



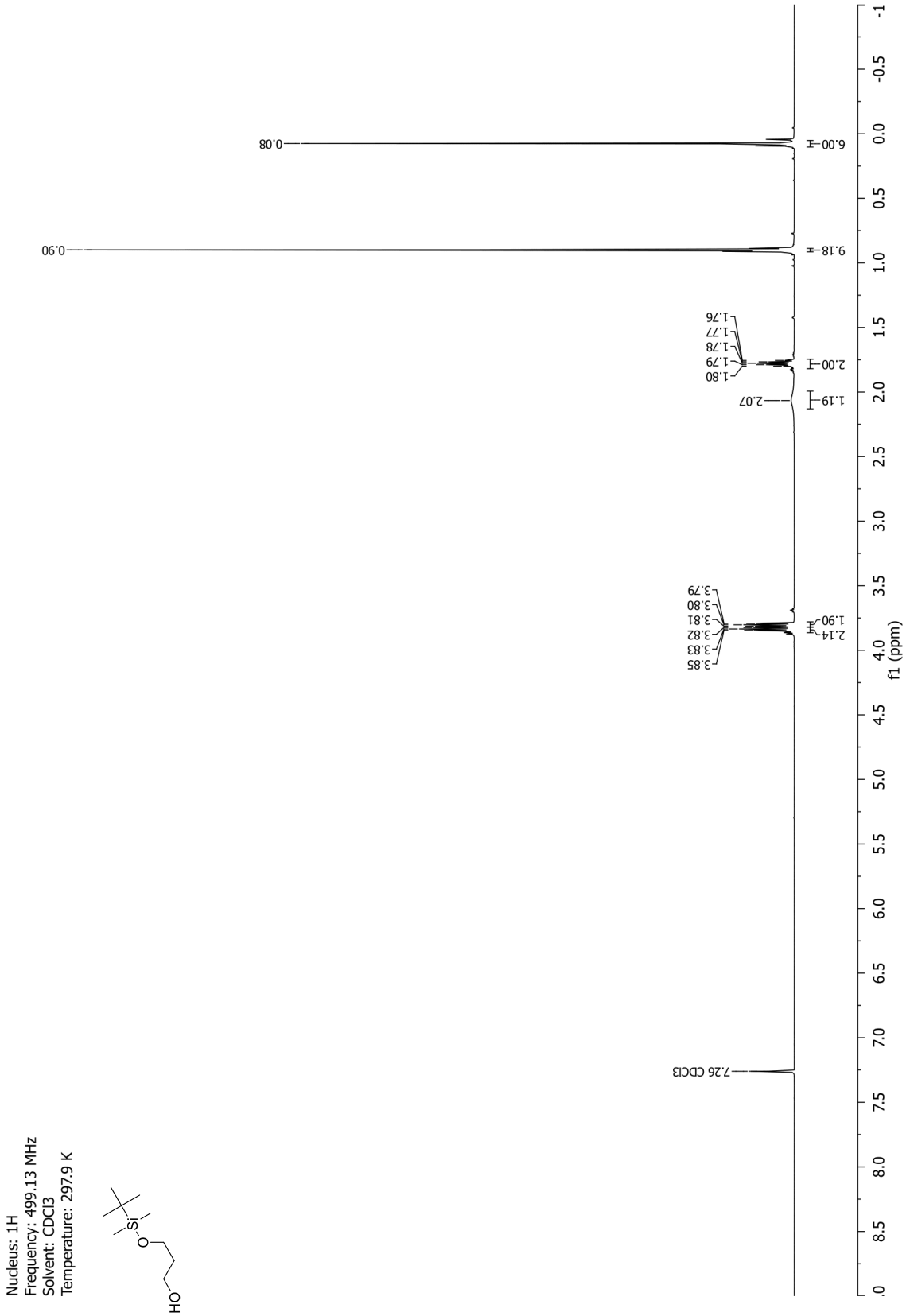

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
18.6
24.1
25.2
26.8
54.0 CD2Cl2
108.6
122.9
129.0
133.7
135.8
154.9
163.8
167.9
f1 (ppm)

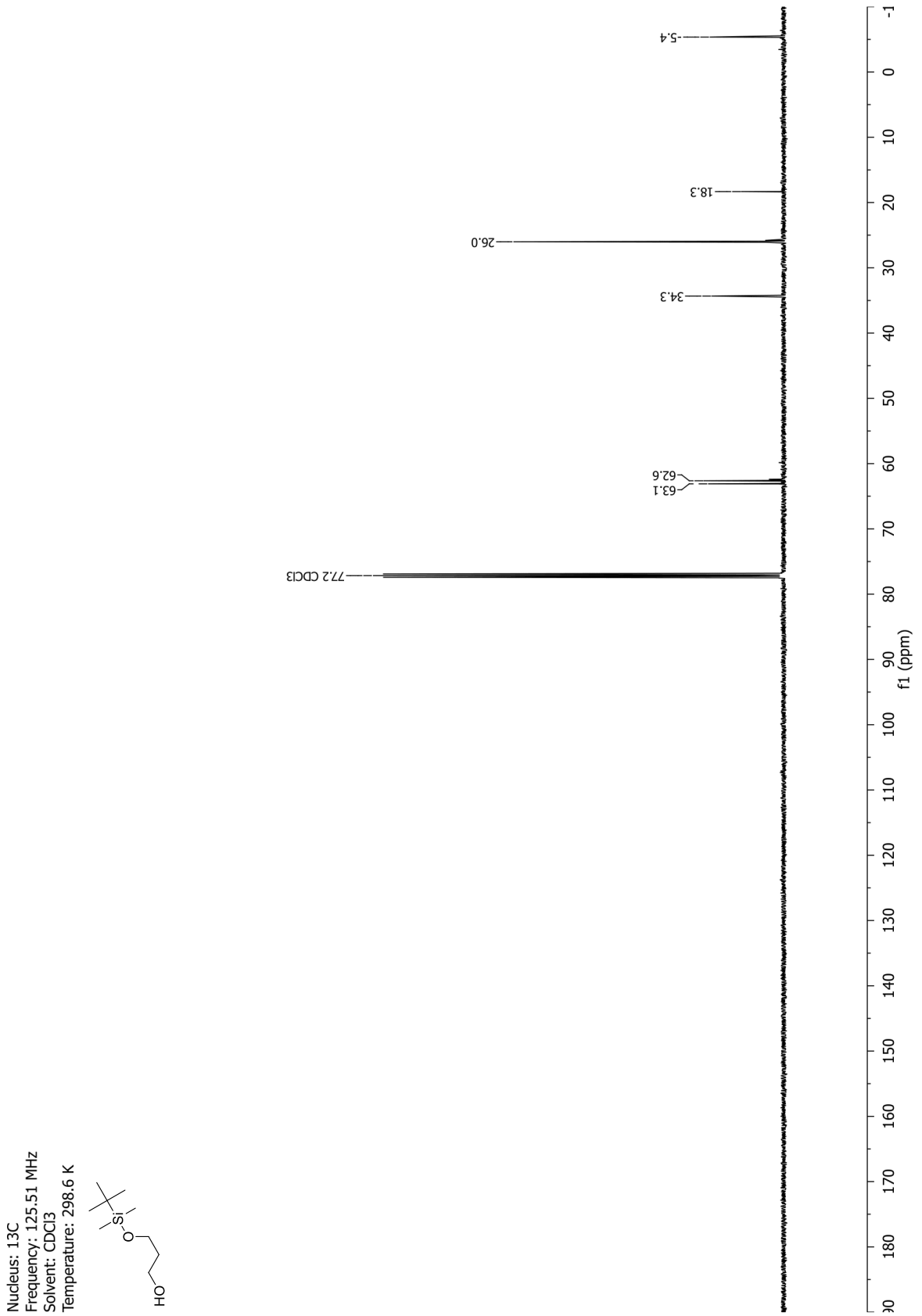
NMR-Spectra for Compound 248



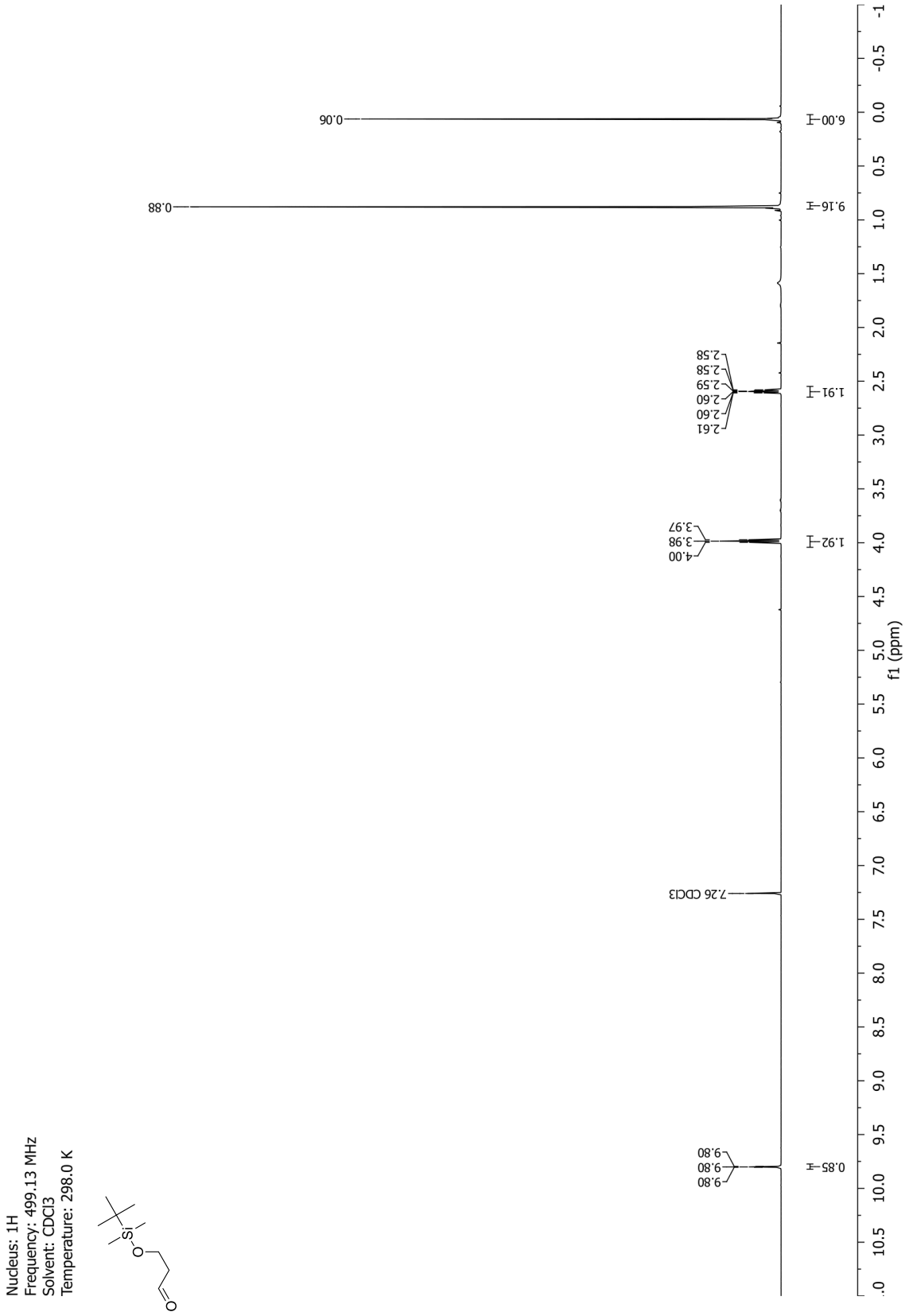

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
14.5
24.1
25.2
26.7
54.0 CD2Cl2
66.4
107.4
123.5
133.0
151.5
155.5
167.5
f1 (ppm)

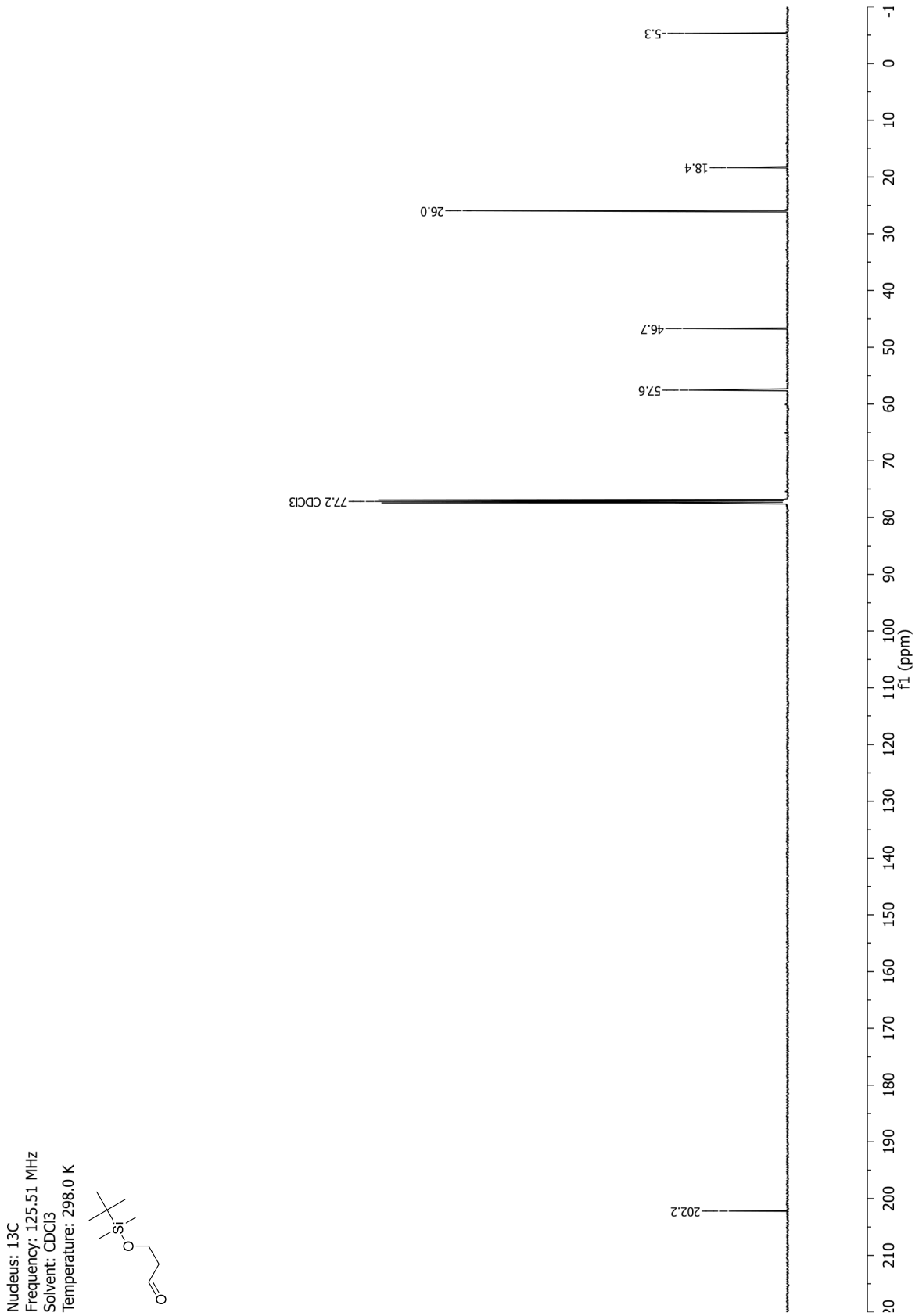
NMR-Spectra for Compound 254



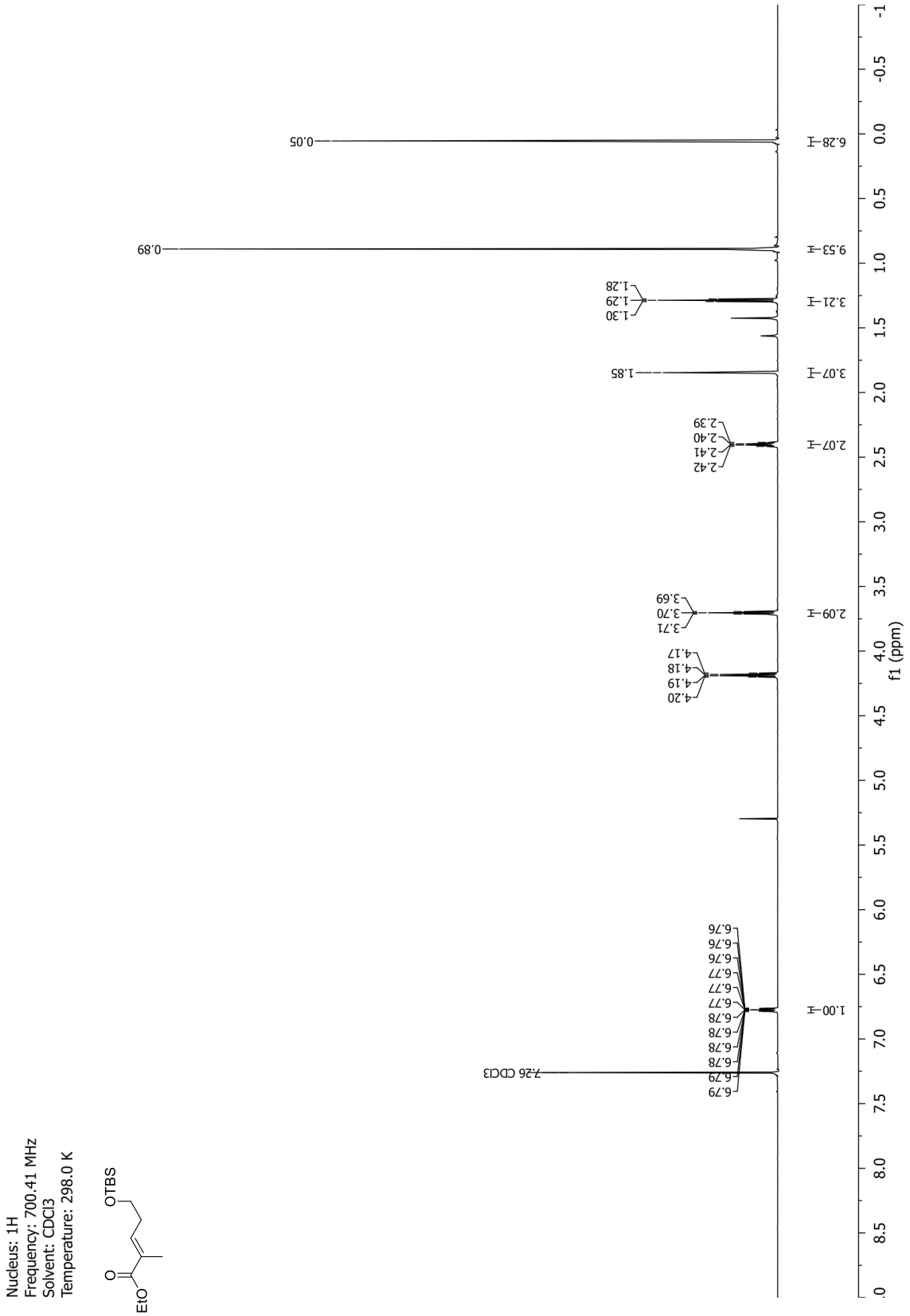

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 298.6 K
Si
O
HO
-5.4
18.3
26.0
34.3
62.6
63.1
77.2 CDCl3
f1 (ppm)

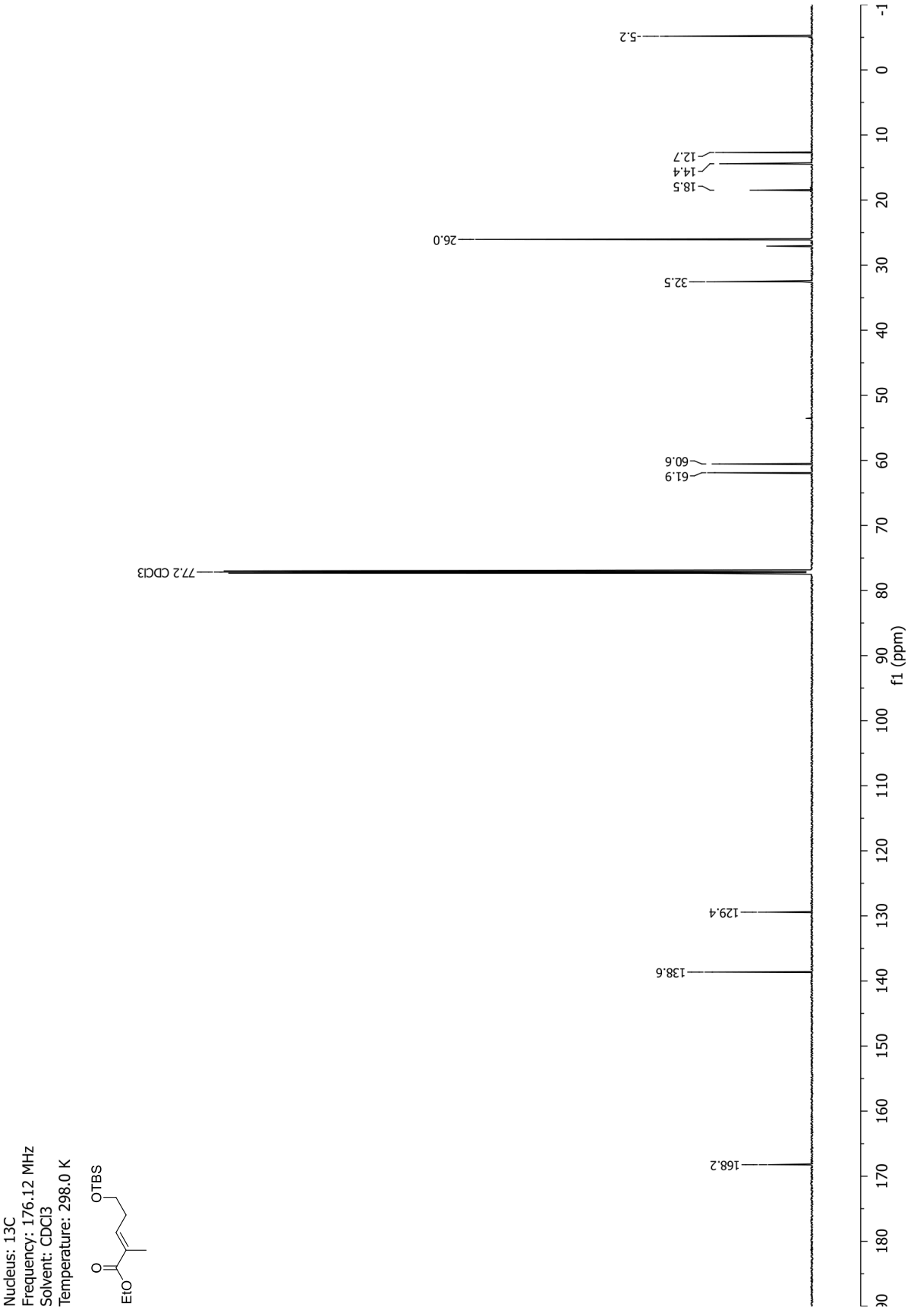
NMR-Spectra for Compound 202



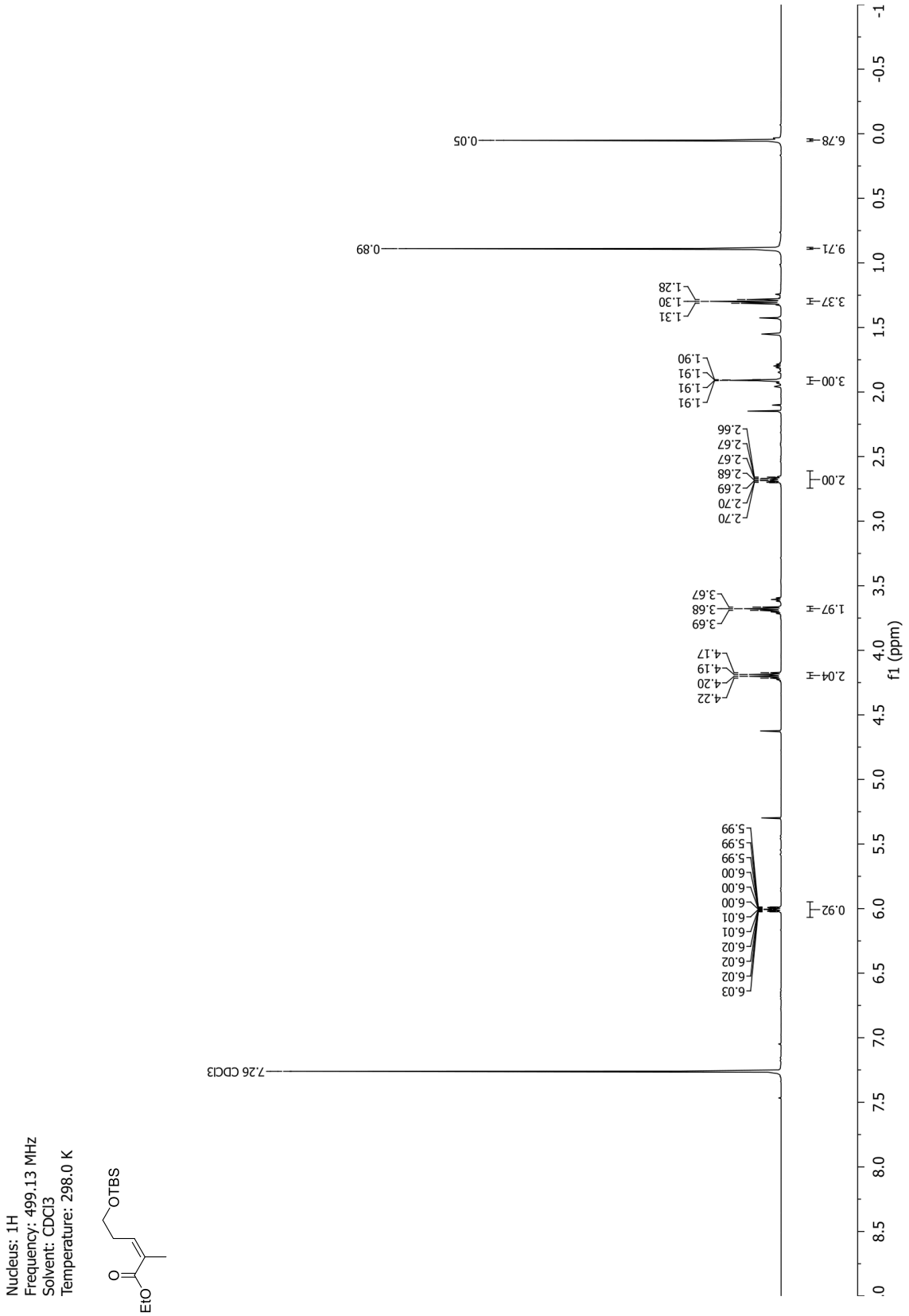

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 298.0 K
Si
O
O
202.2
77.2 CDCl3
57.6
46.7
26.0
18.4
-5.3
f1 (ppm)

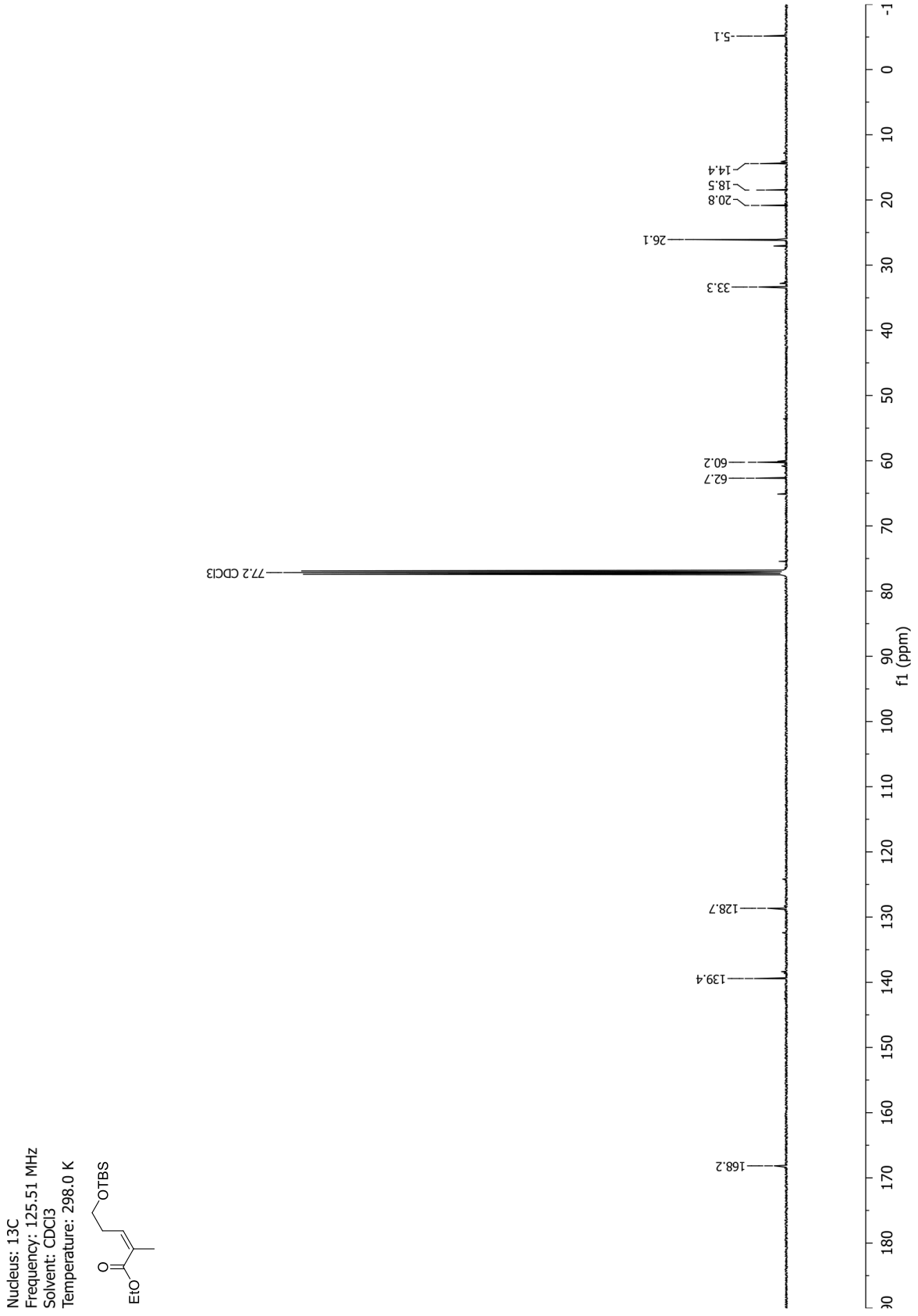
NMR-Spectra for Compound 214*E*



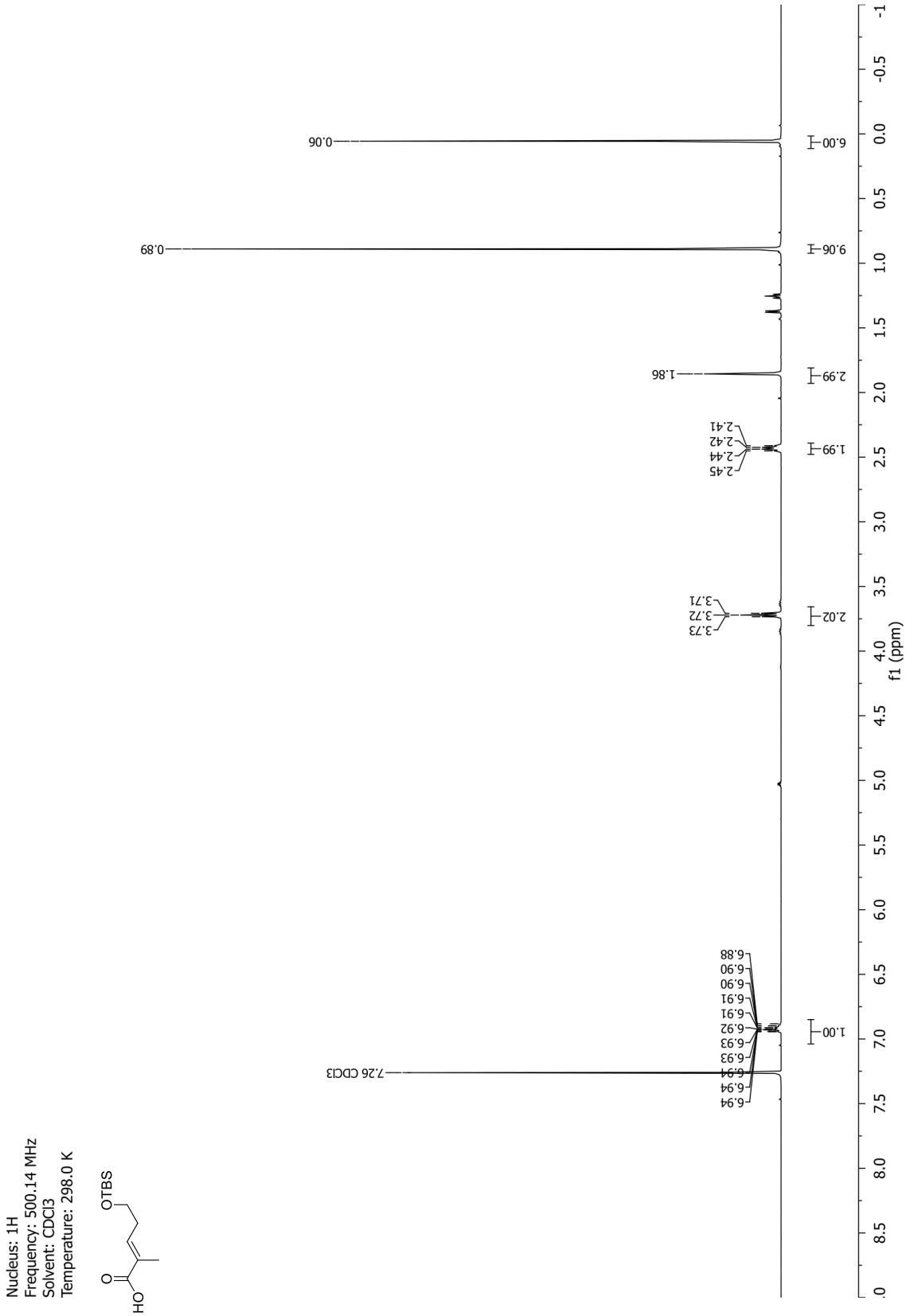

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
EtO
168.2
138.6
129.4
77.2 CDCl3
61.9
60.6
32.5
26.0
18.5
14.4
12.7
-5.2
f1 (ppm)

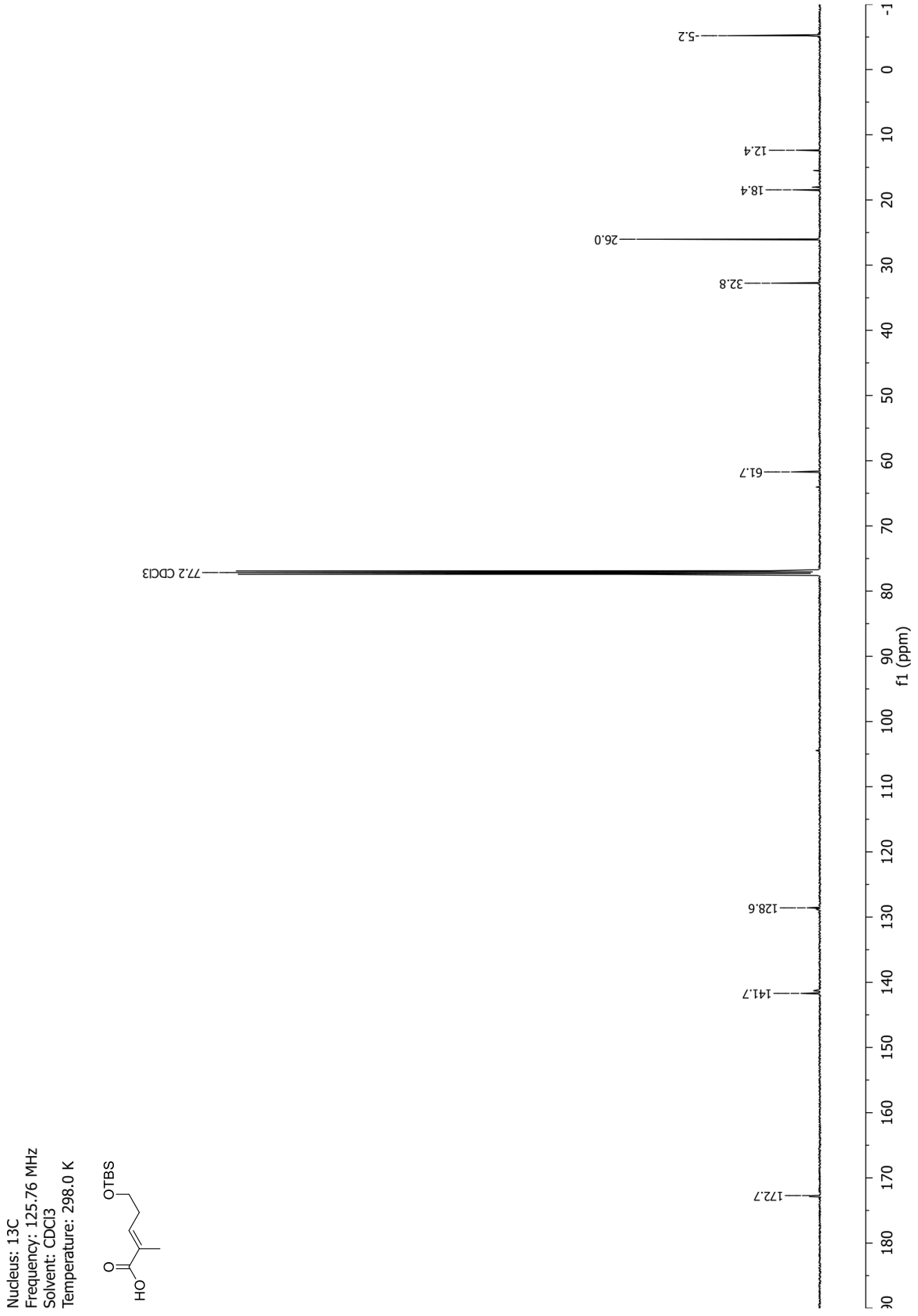
NMR-Spectra for Compound 214*Z*



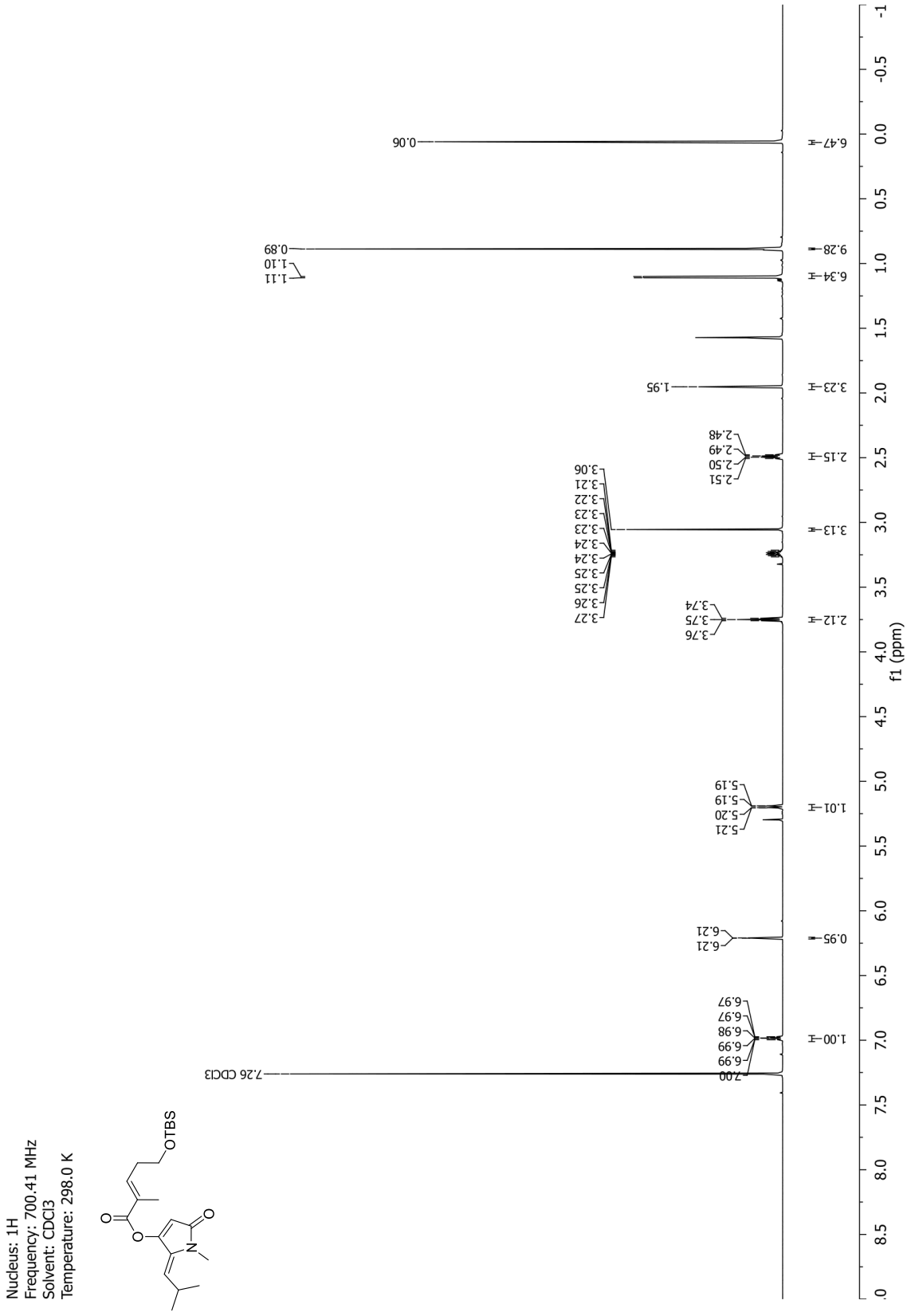

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
EtO
168.2
139.4
128.7
77.2 CDCl3
62.7
60.2
33.3
26.1
20.8
18.5
14.4
-5.1
f1 (ppm)

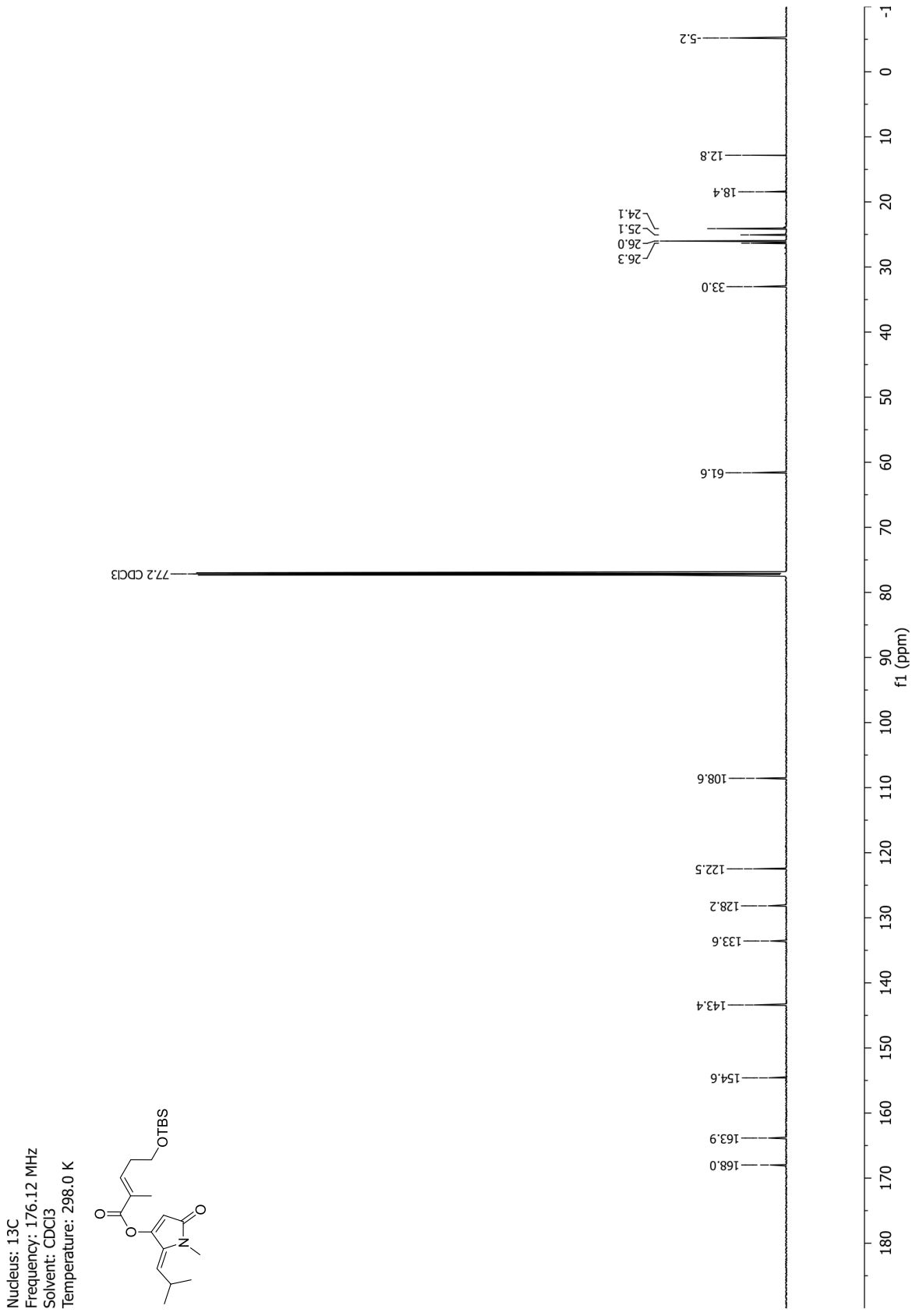
NMR-Spectra for Compound 256



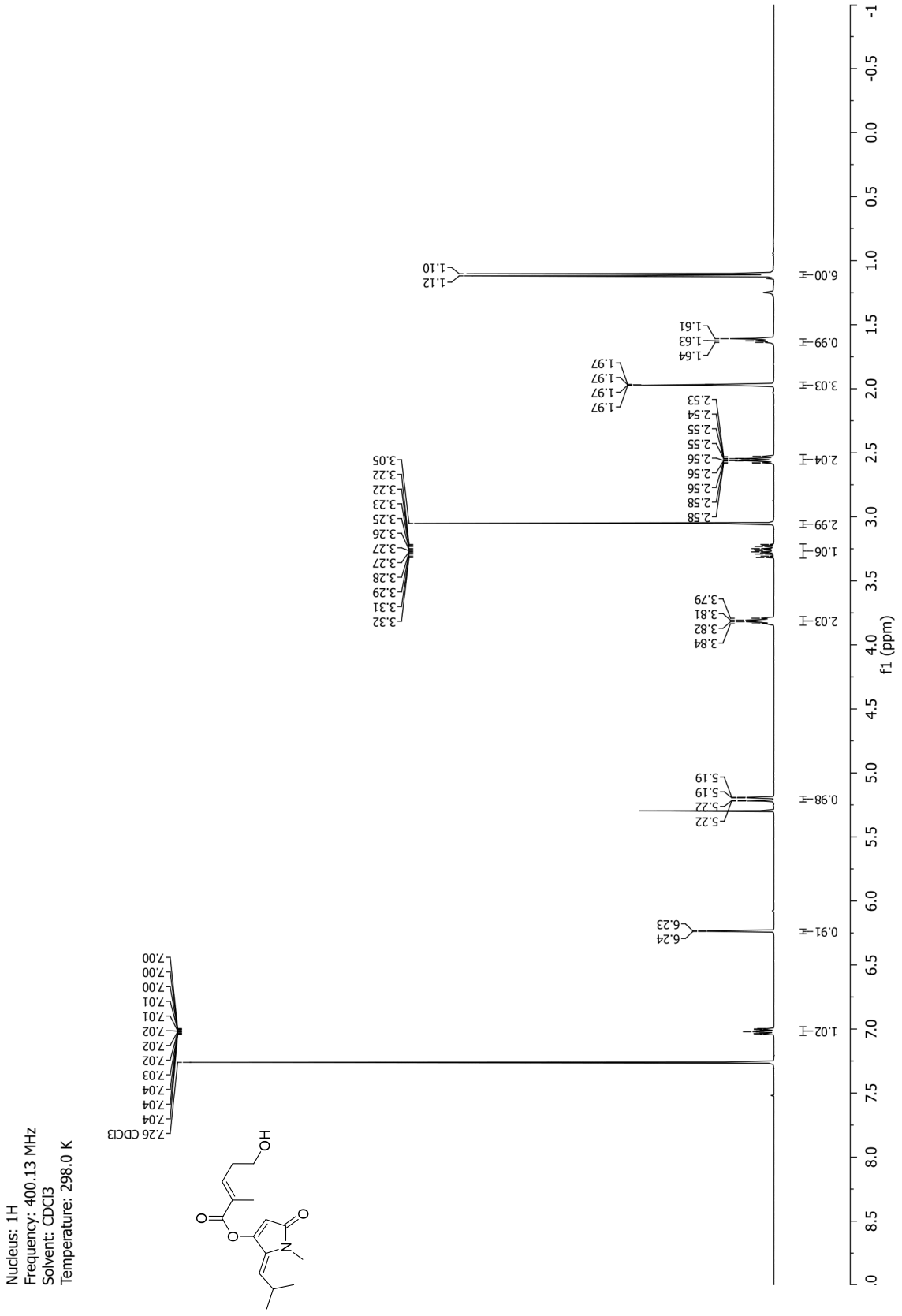

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
HO
172.7
141.7
128.6
77.2 CDCl3
61.7
32.8
26.0
18.4
12.4
-5.2
f1 (ppm)

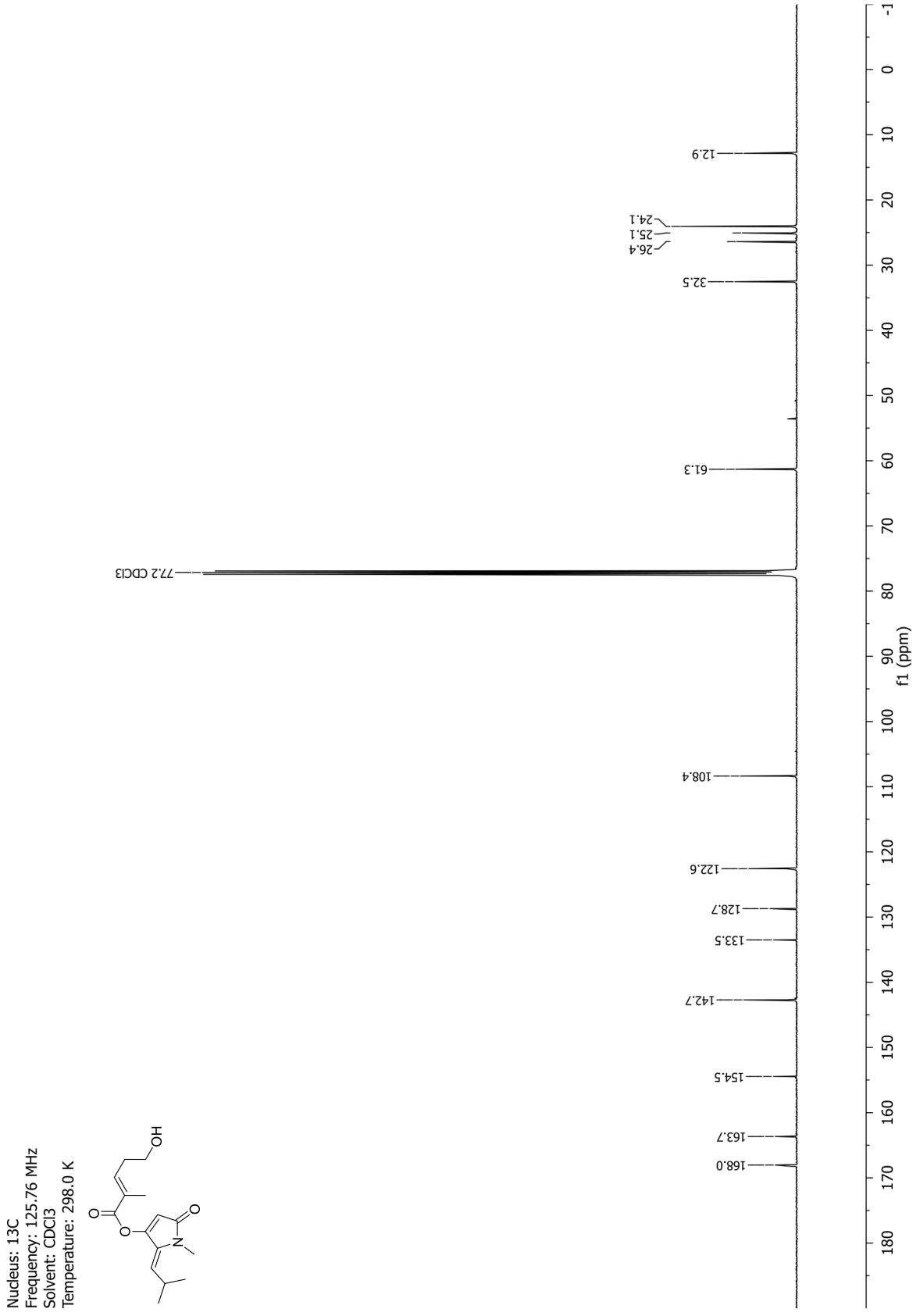
NMR-Spectra for Compound 257



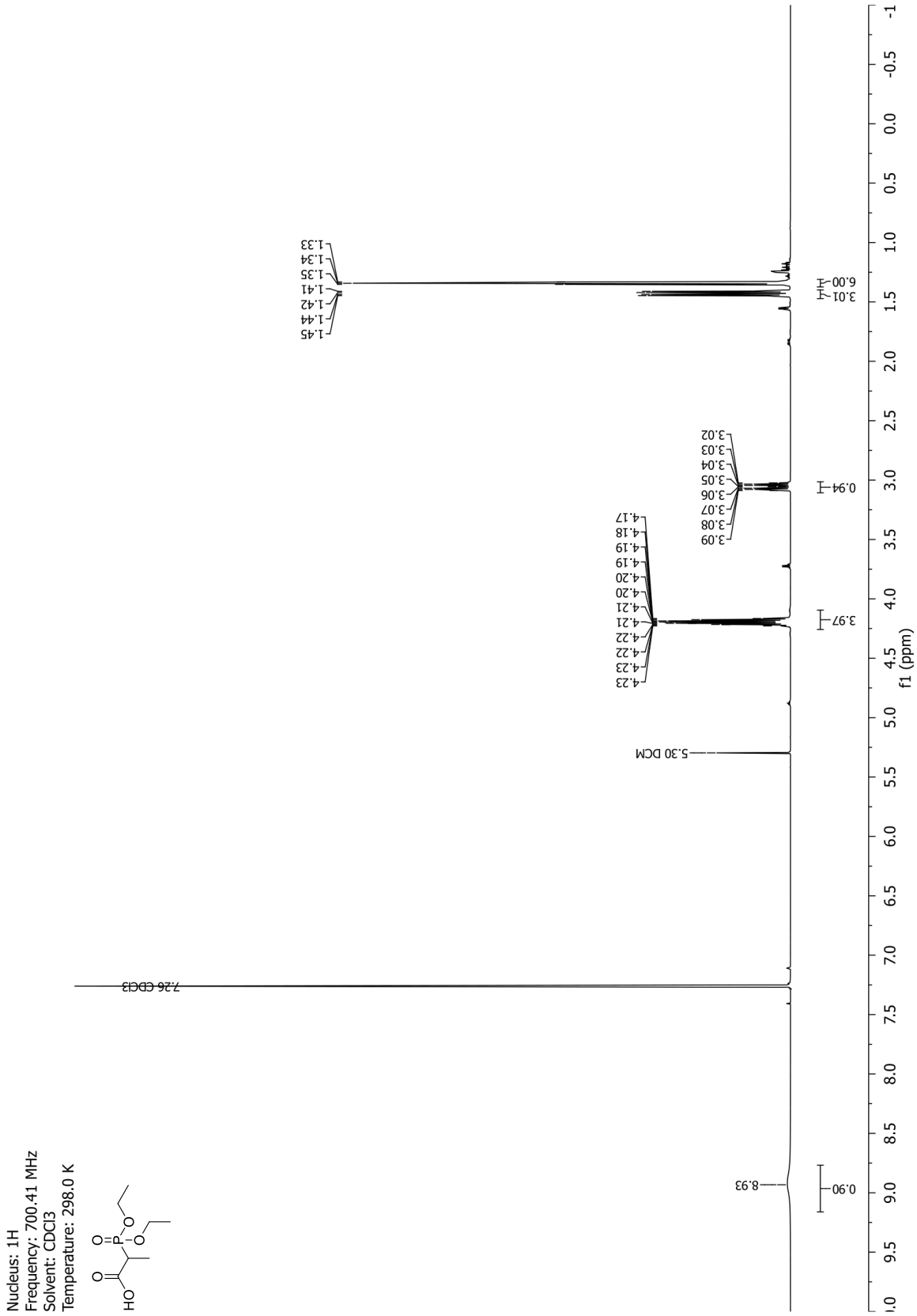

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
O
O
N
-5.2
12.8
18.4
24.1
25.1
26.0
26.3
33.0
61.6
77.2 CDCl3
108.6
122.5
128.2
133.6
143.4
154.6
163.9
168.0
-1
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
f1 (ppm)

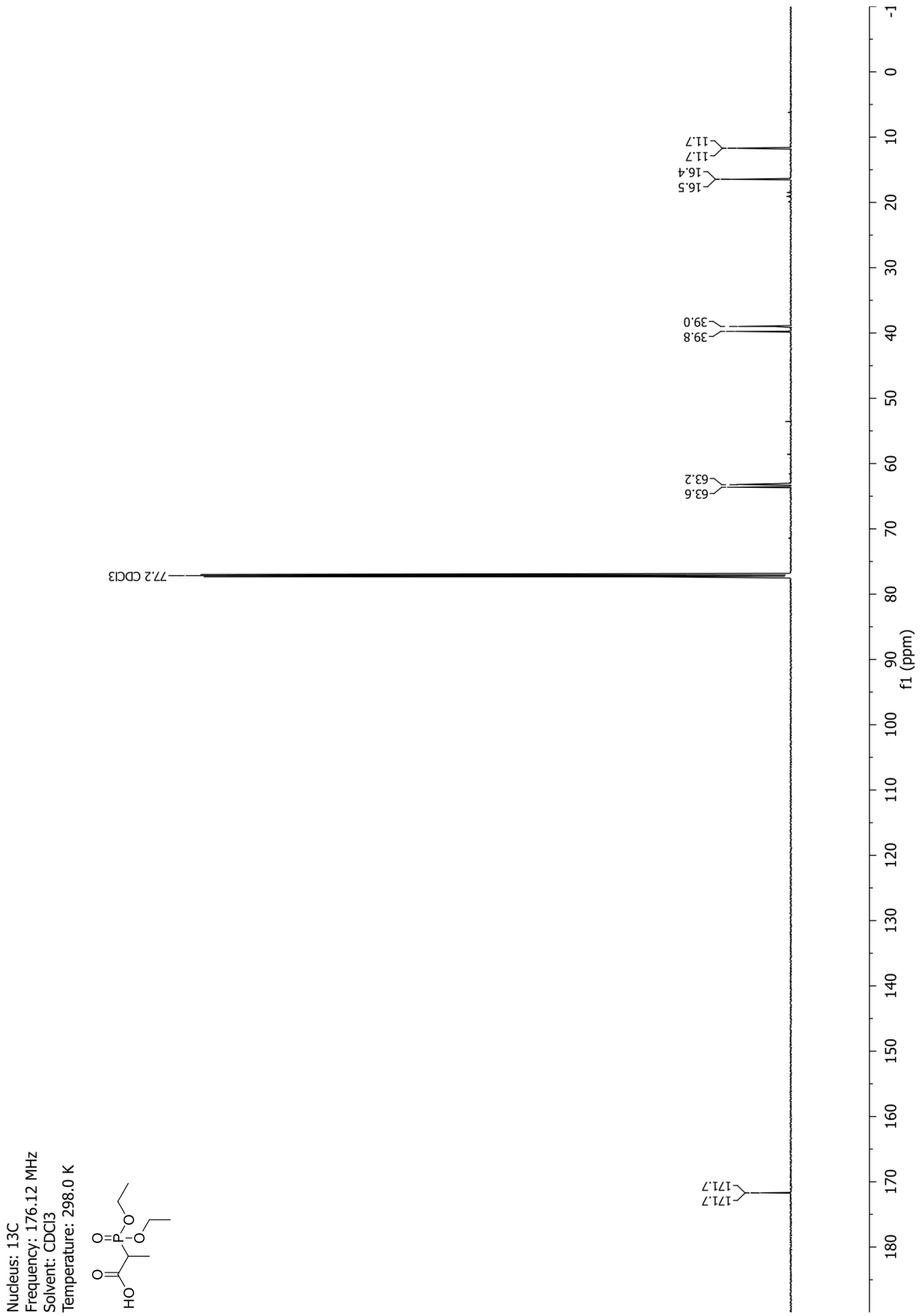
NMR-Spectra for Compound 260

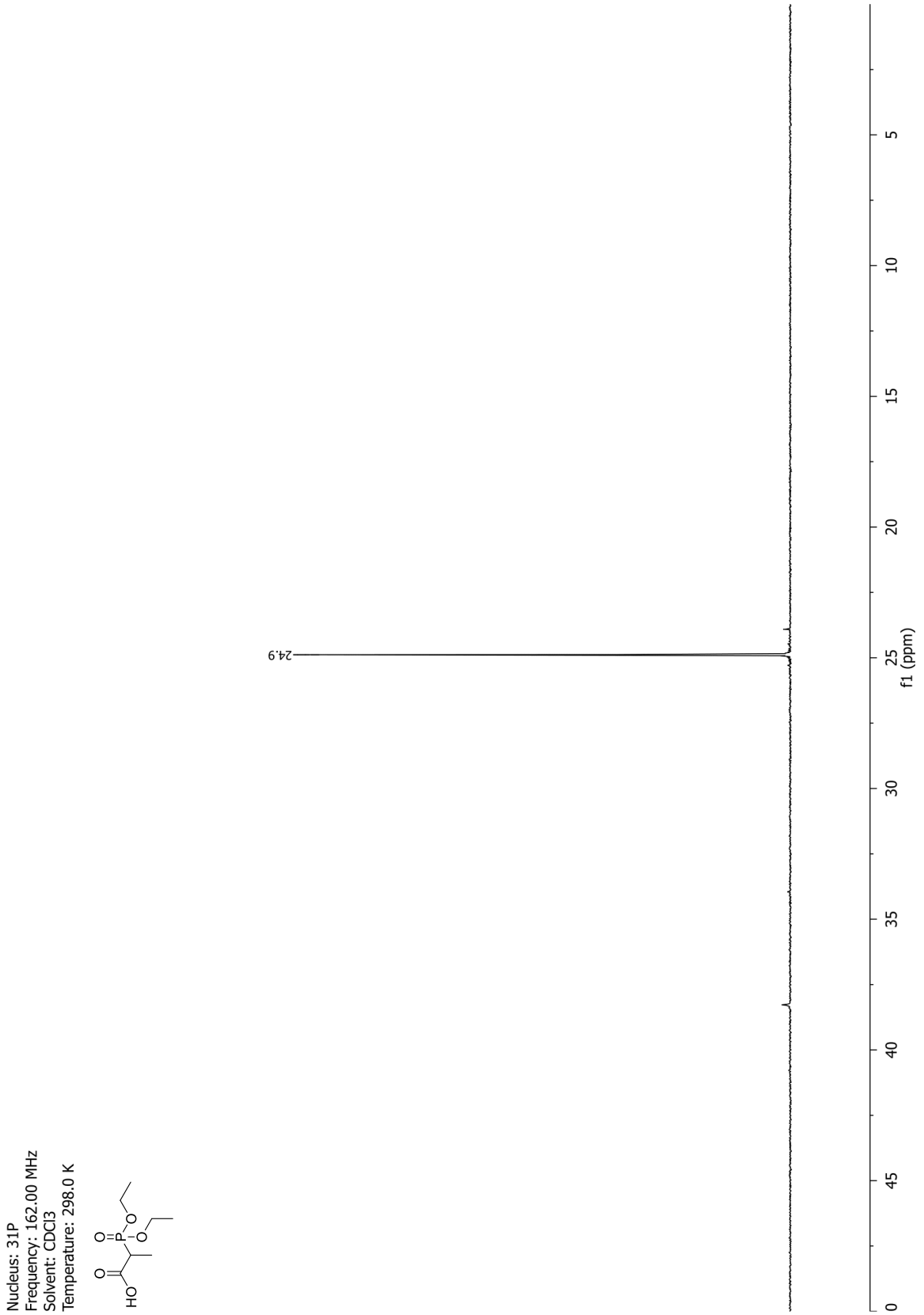



Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
168.0
163.7
154.5
142.7
133.5
128.7
122.6
108.4
77.2 CDCl3
61.3
32.5
26.4
25.1
24.1
12.9
f1 (ppm)

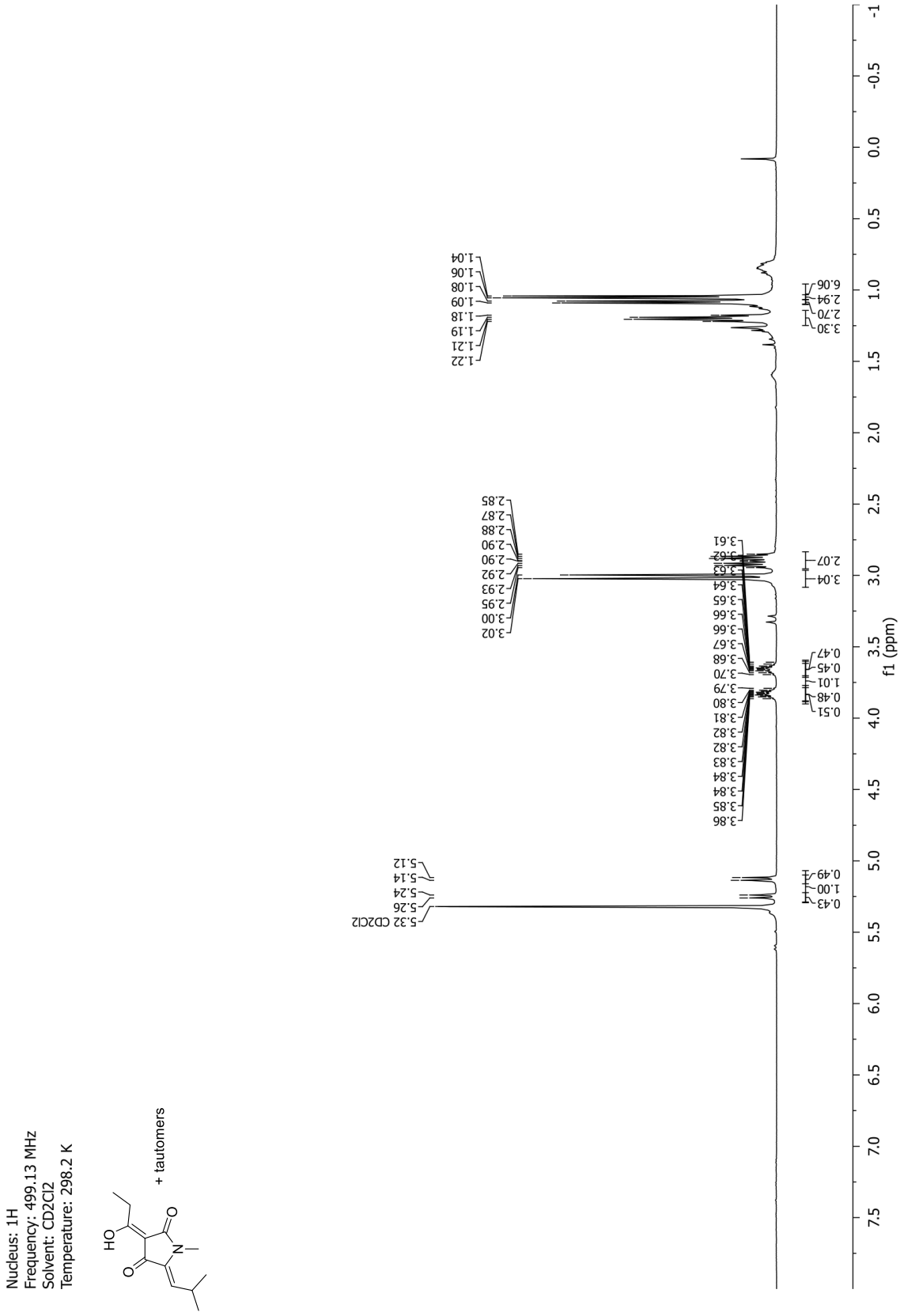
NMR-Spectra for Compound 252

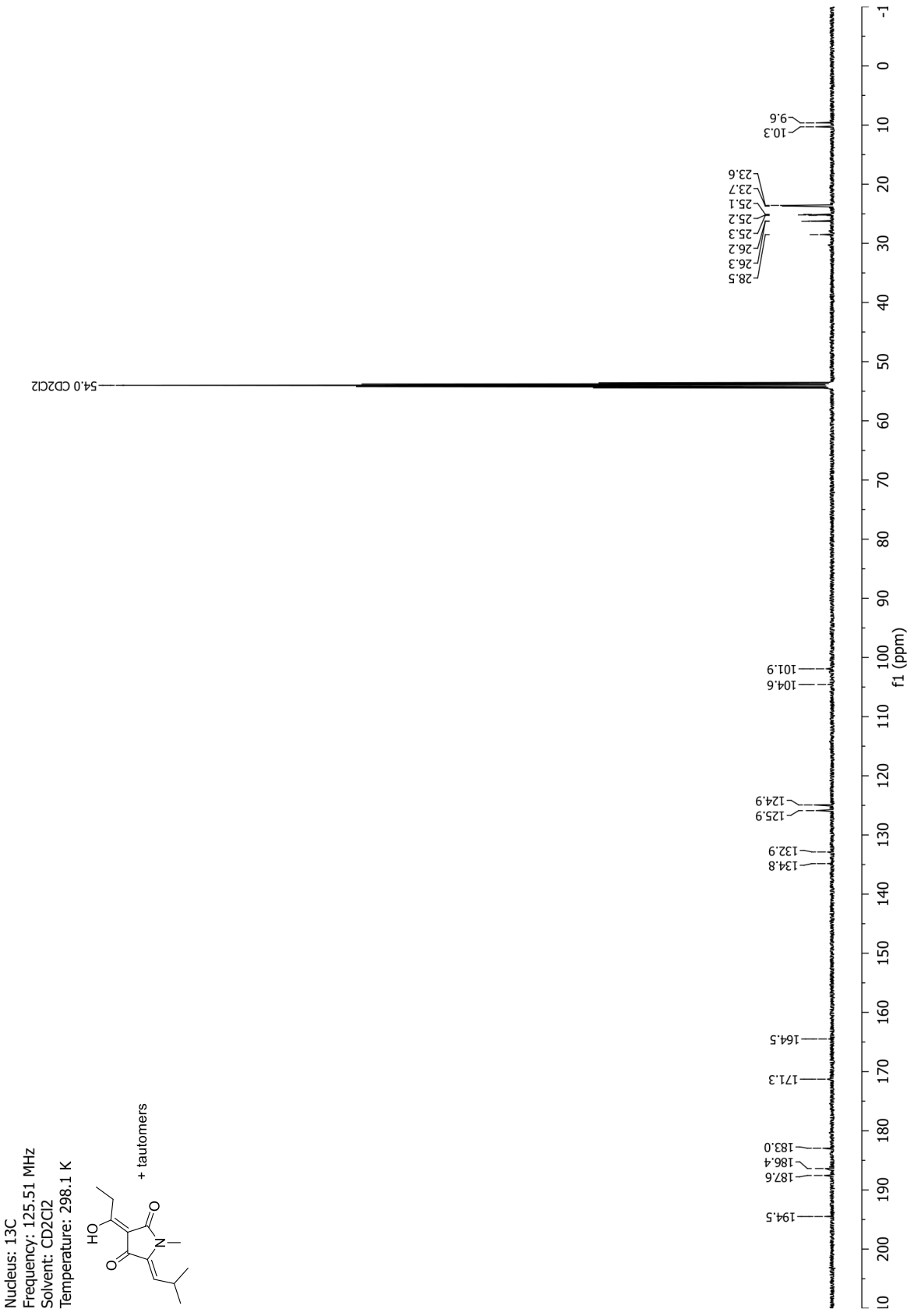



Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
HO
O
O
P
O
O
171.7
171.7
77.2 CDCl3
63.6
63.2
39.8
39.0
16.5
16.4
11.7
11.7
f1 (ppm)

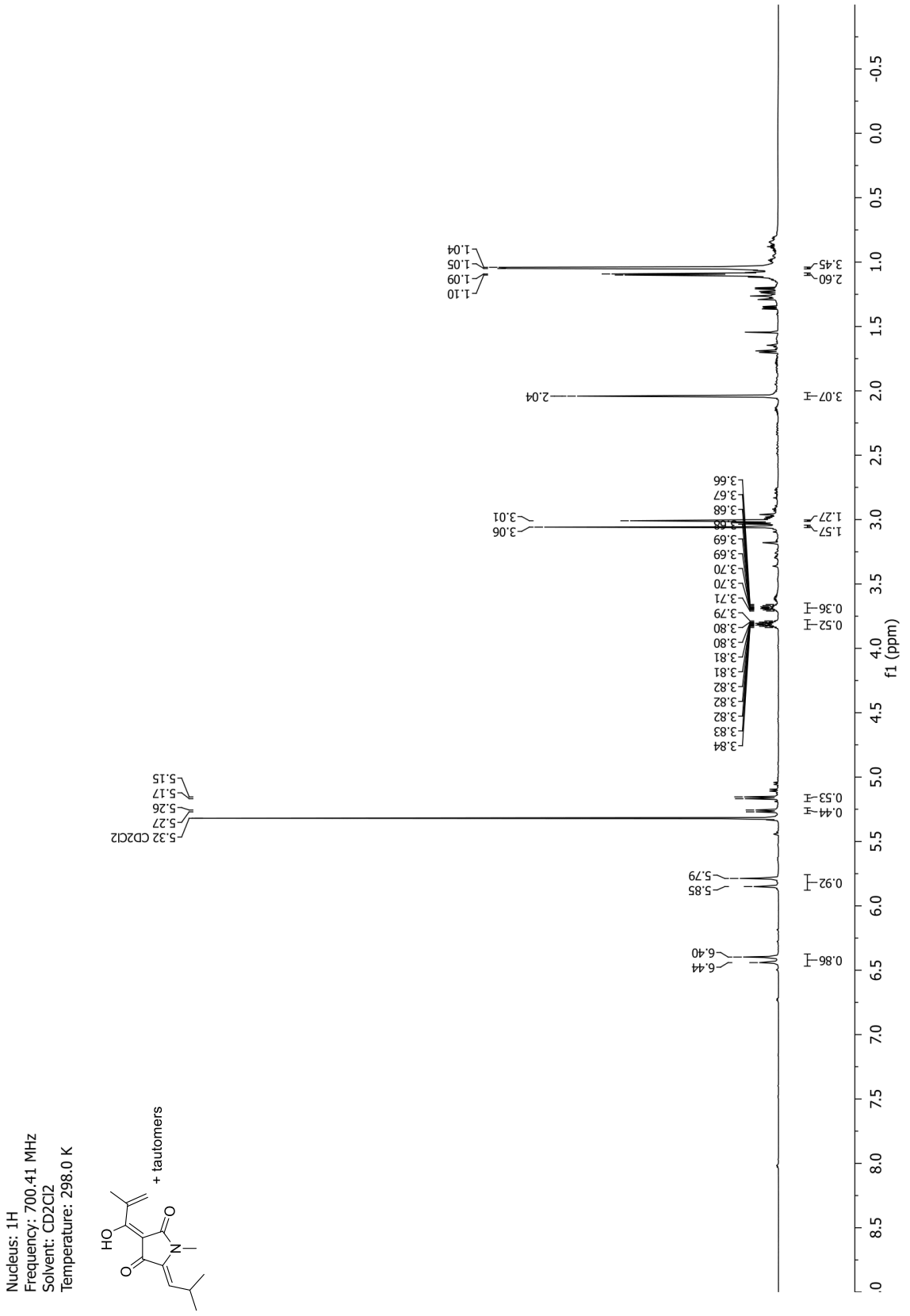

Nucleus: 31P
Frequency: 162.00 MHz
Solvent: CDCl3
Temperature: 298.0 K
24.9
f1 (ppm)
0
5
10
15
20
25
30
35
40
45

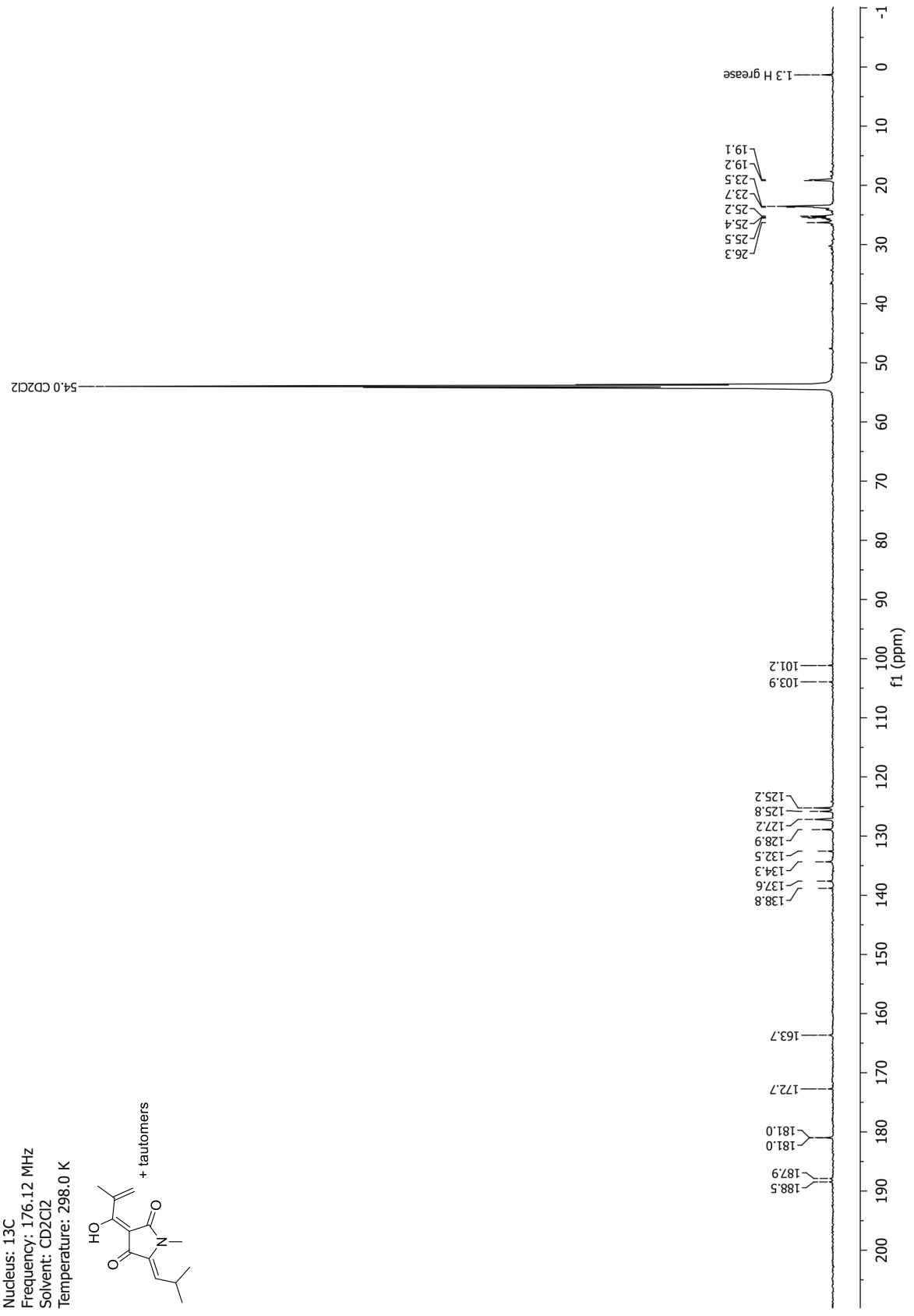
NMR-Spectra for Compound 241



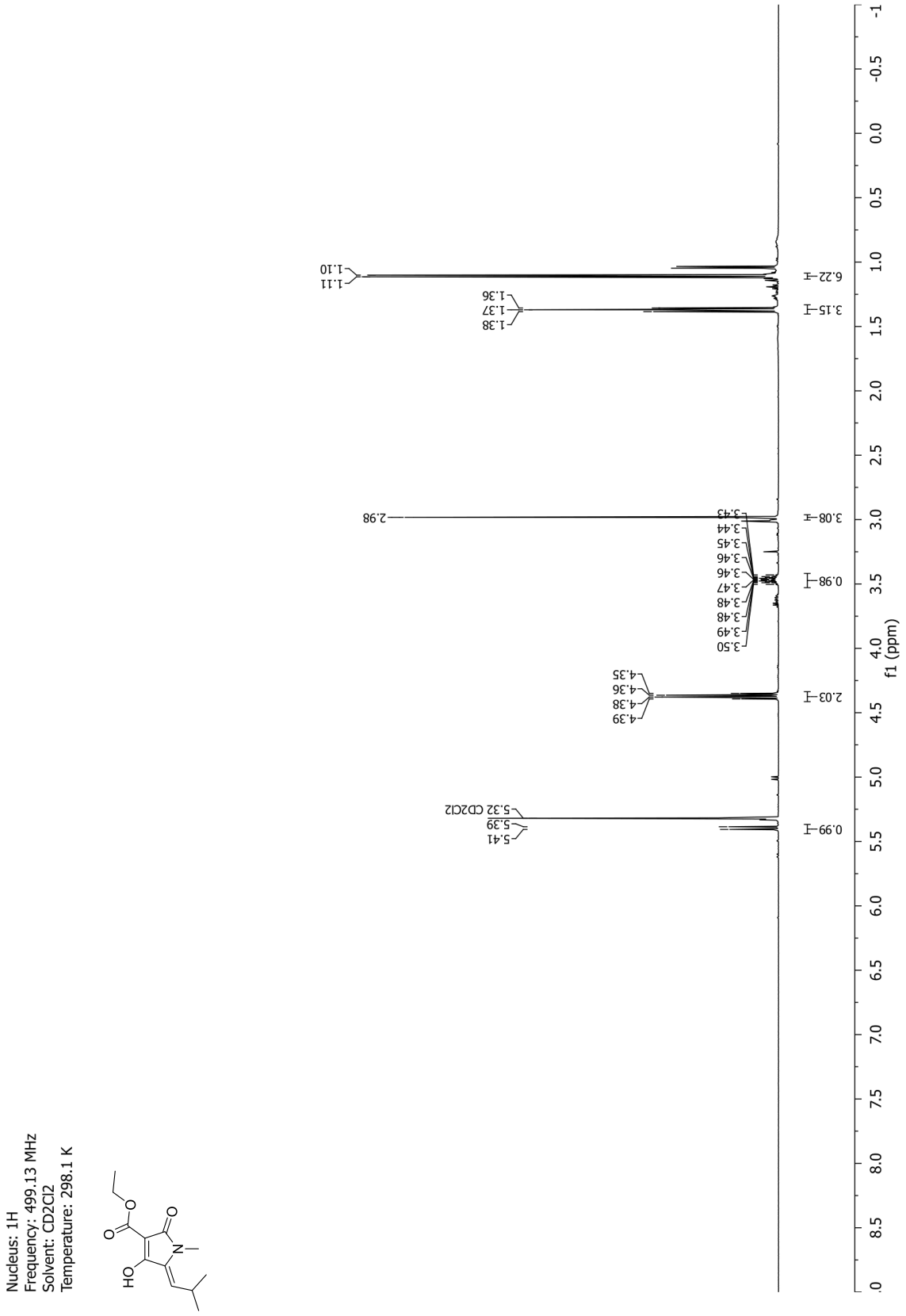

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CD2Cl2
Temperature: 298.1 K
+ tautomers
HO
O
O
N
54.0 CD2Cl2
9.6
10.3
23.6
23.7
25.1
25.2
25.3
26.2
26.3
28.5
101.9
104.6
124.9
125.9
132.9
134.8
164.5
171.3
183.0
186.4
187.6
194.5
f1 (ppm)

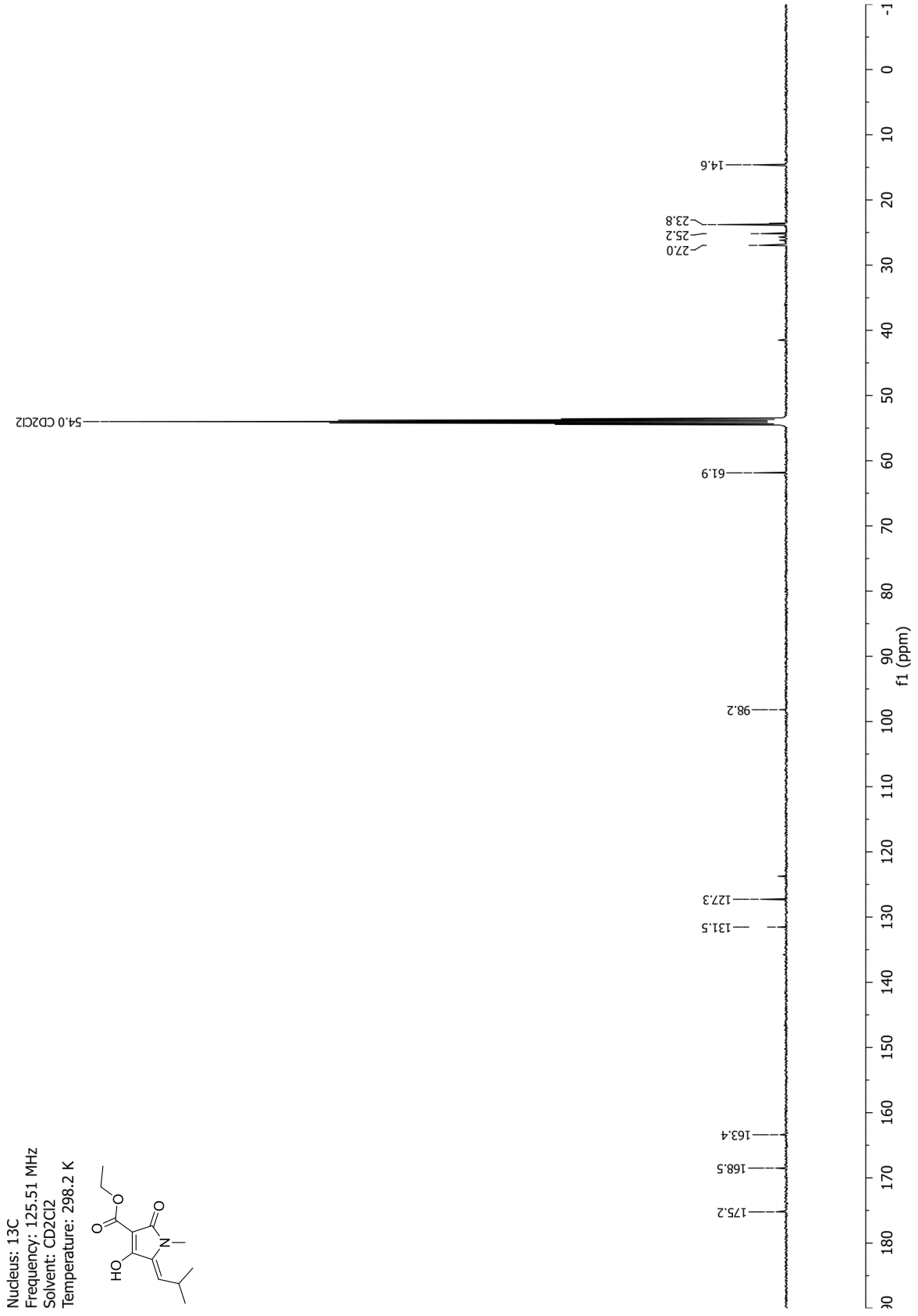
NMR-Spectra for Compound 249



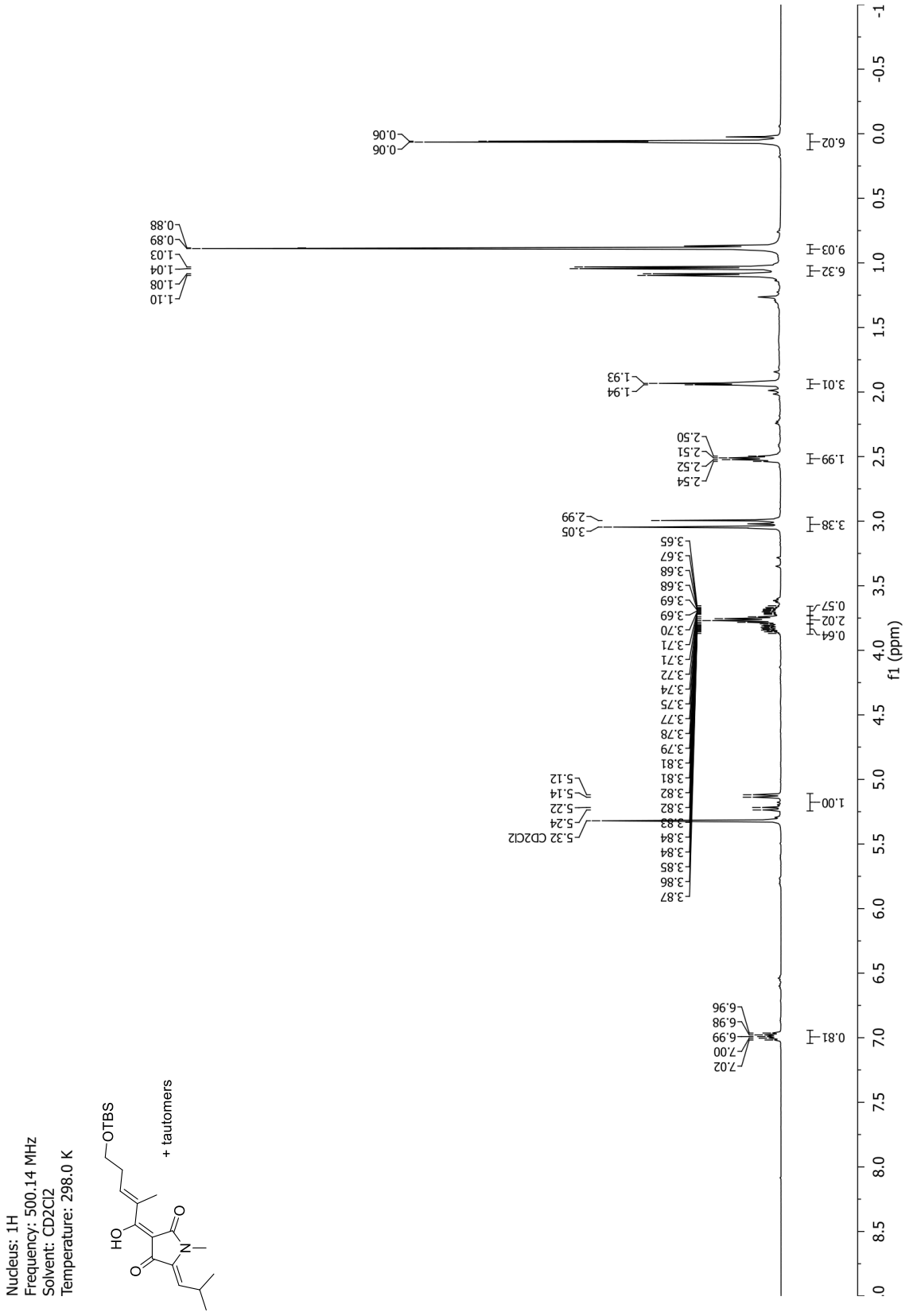

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
+ tautomers
HO
O
O
N
54.0 CD2Cl2
1.3 H grease
19.1
19.2
23.5
23.7
25.2
25.4
25.5
26.3
101.2
103.9
125.2
125.8
127.2
128.9
132.5
134.3
137.6
138.8
163.7
172.7
181.0
181.0
187.9
188.5
f1 (ppm)

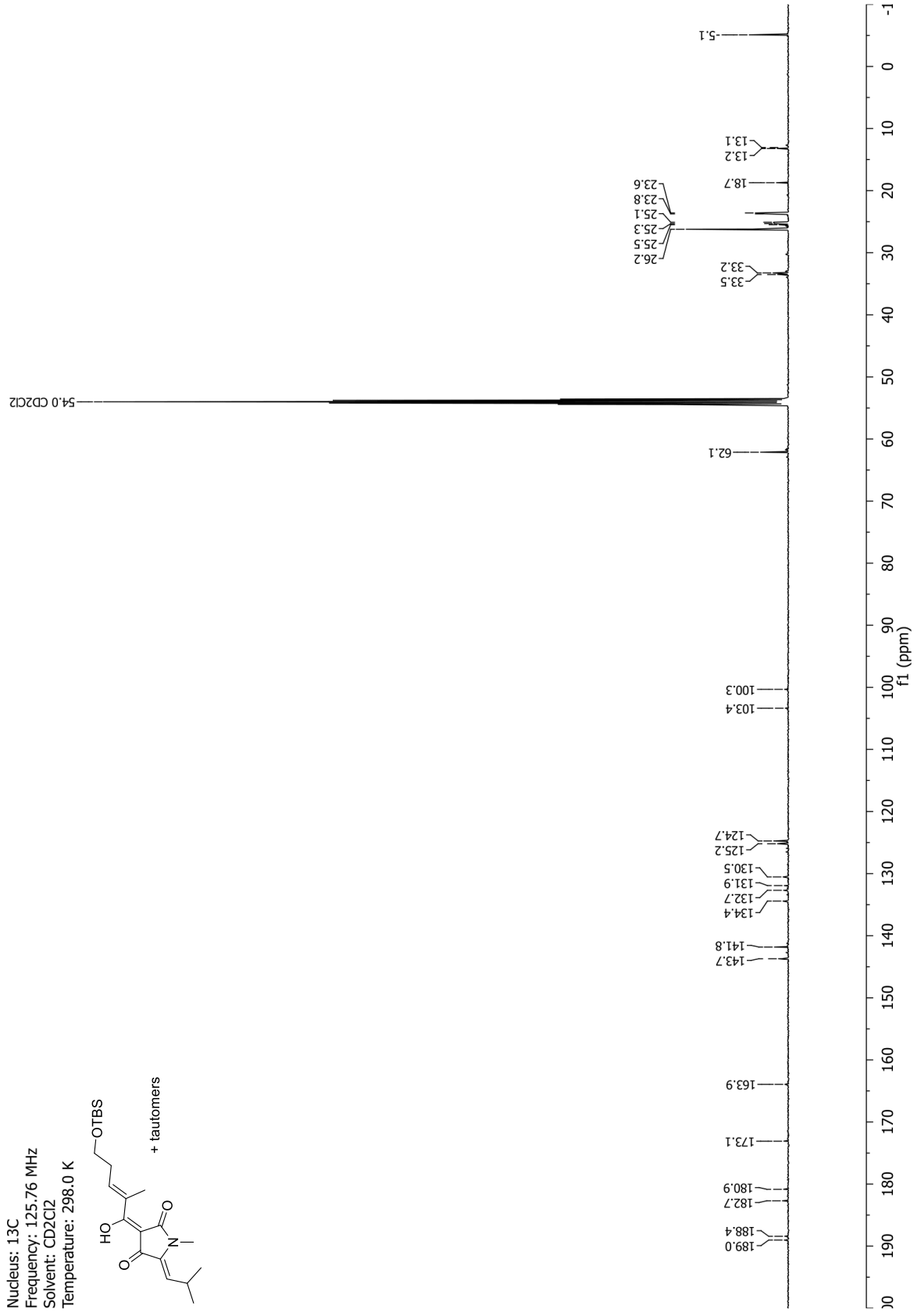
NMR-Spectra for Compound 250



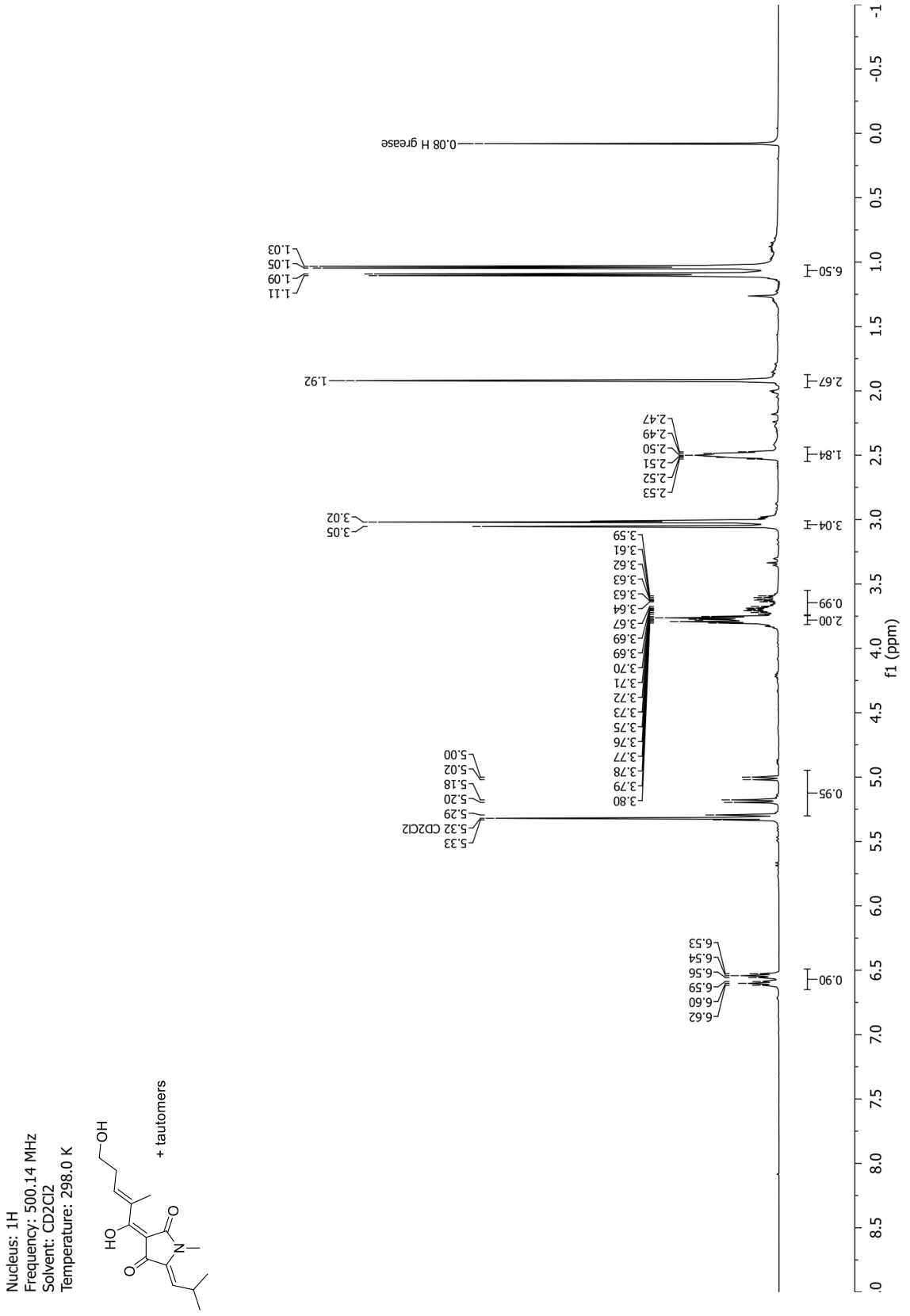

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CD2Cl2
Temperature: 298.2 K
14.6
23.8
25.2
27.0
54.0 CD2Cl2
61.9
98.2
127.3
131.5
163.4
168.5
175.2
f1 (ppm)

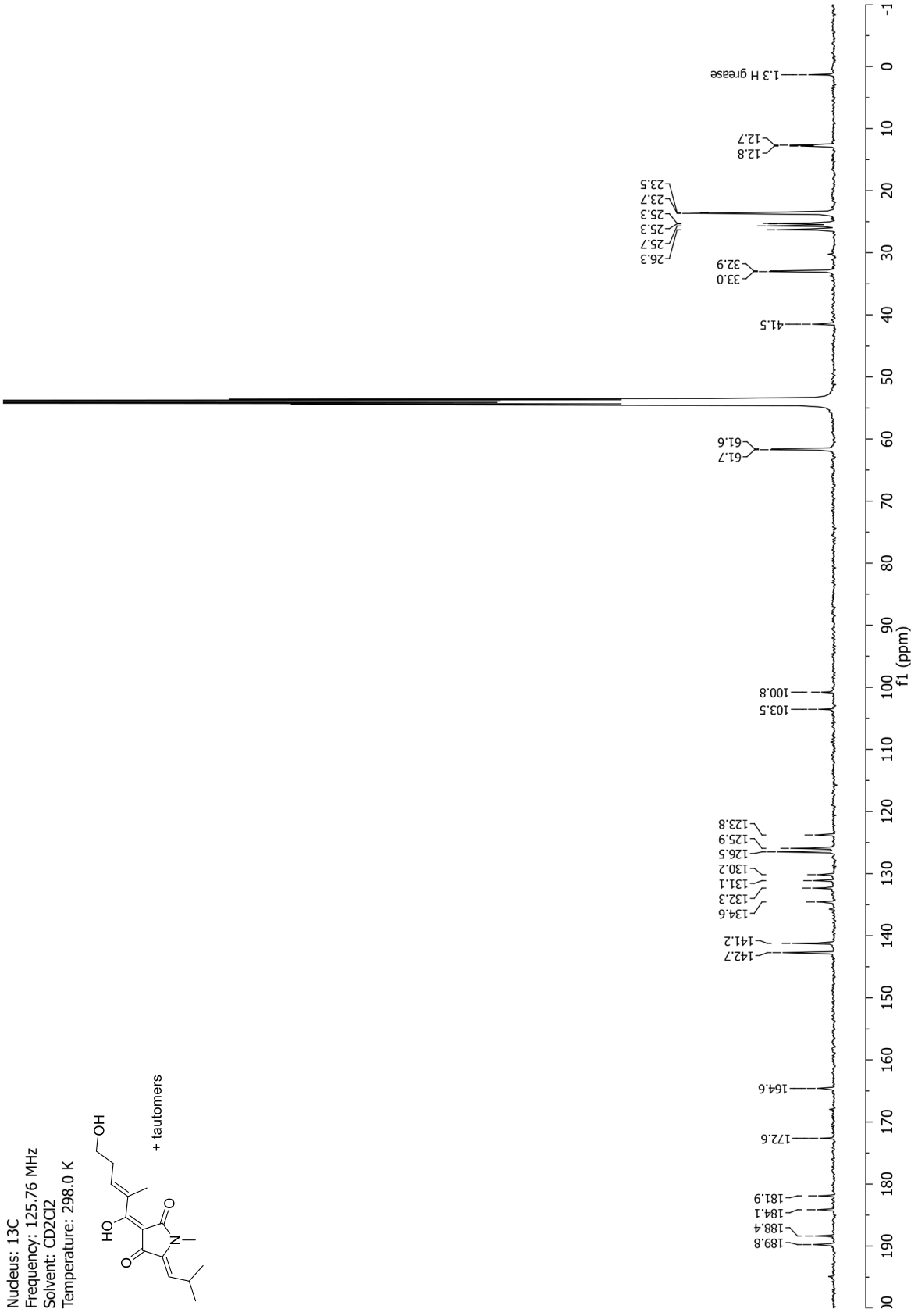
NMR-Spectra for Compound 258



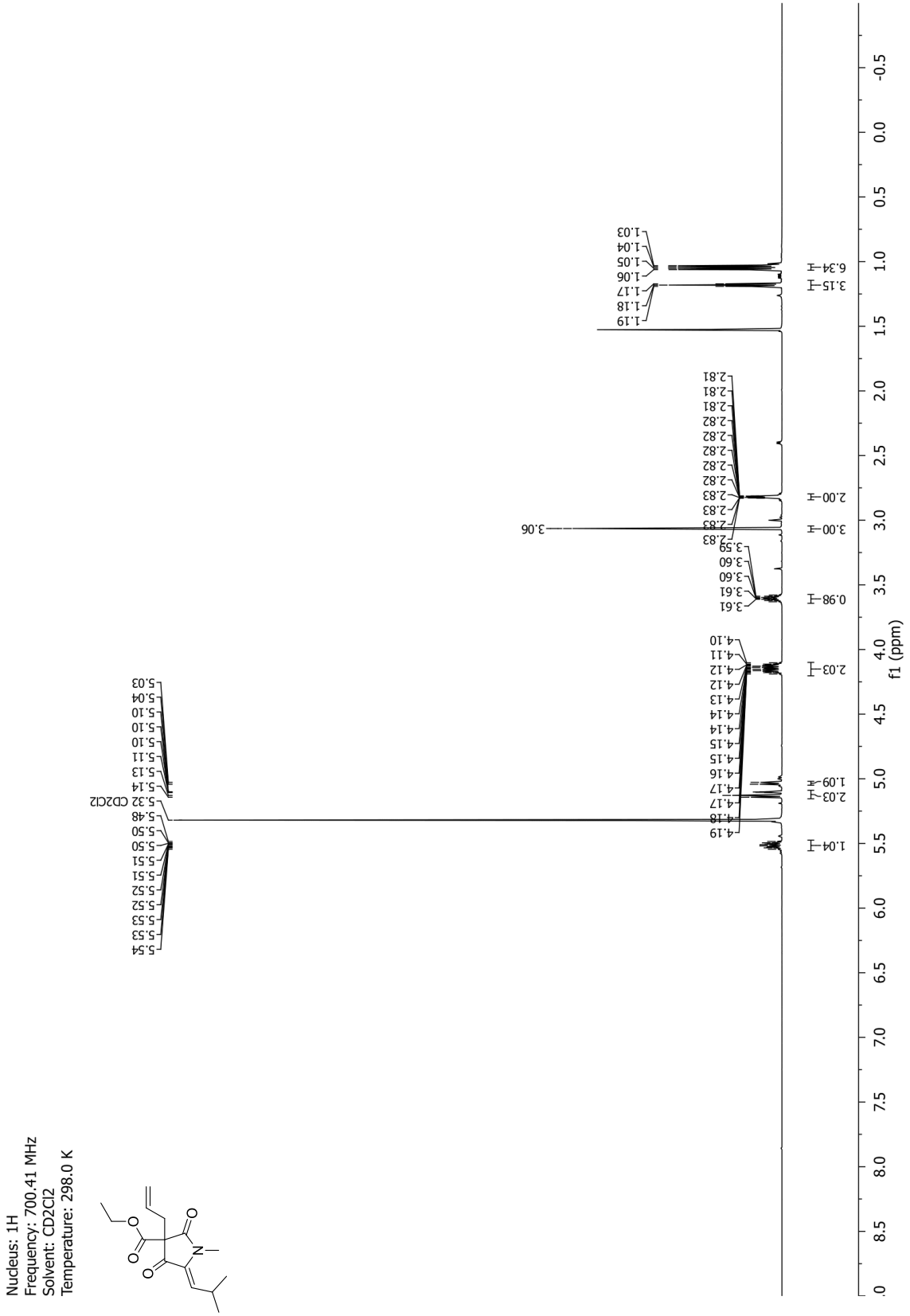

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
OTBS
HO
O
O
N
+ tautomers
-5.1
13.1
13.2
18.7
23.6
23.8
25.1
25.3
25.5
26.2
33.2
33.5
54.0 CD2Cl2
62.1
100.3
103.4
124.7
125.2
130.5
131.9
132.7
134.4
141.8
143.7
163.9
173.1
180.9
182.7
188.4
189.0
f1 (ppm)

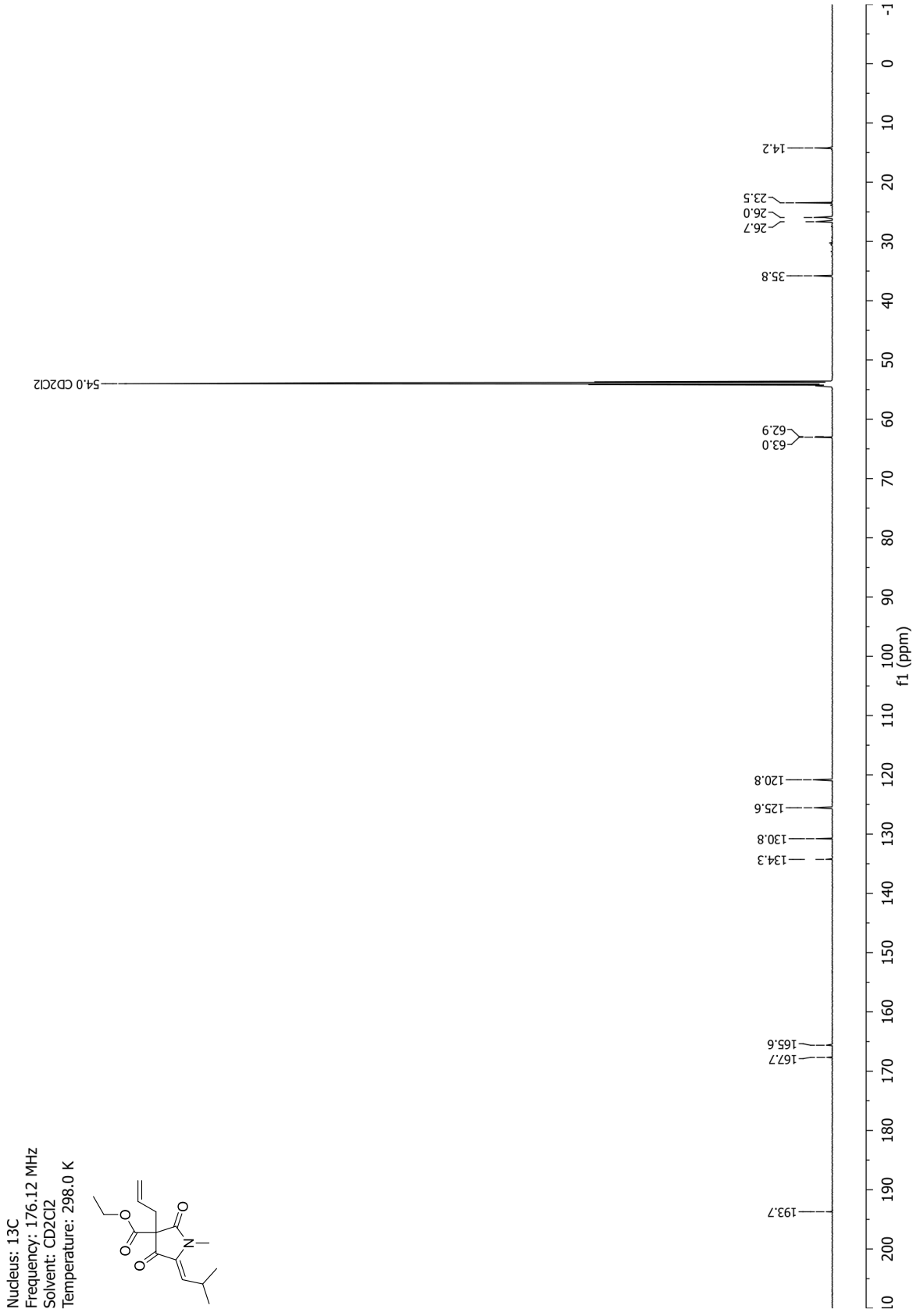
NMR-Spectra for Compound 259



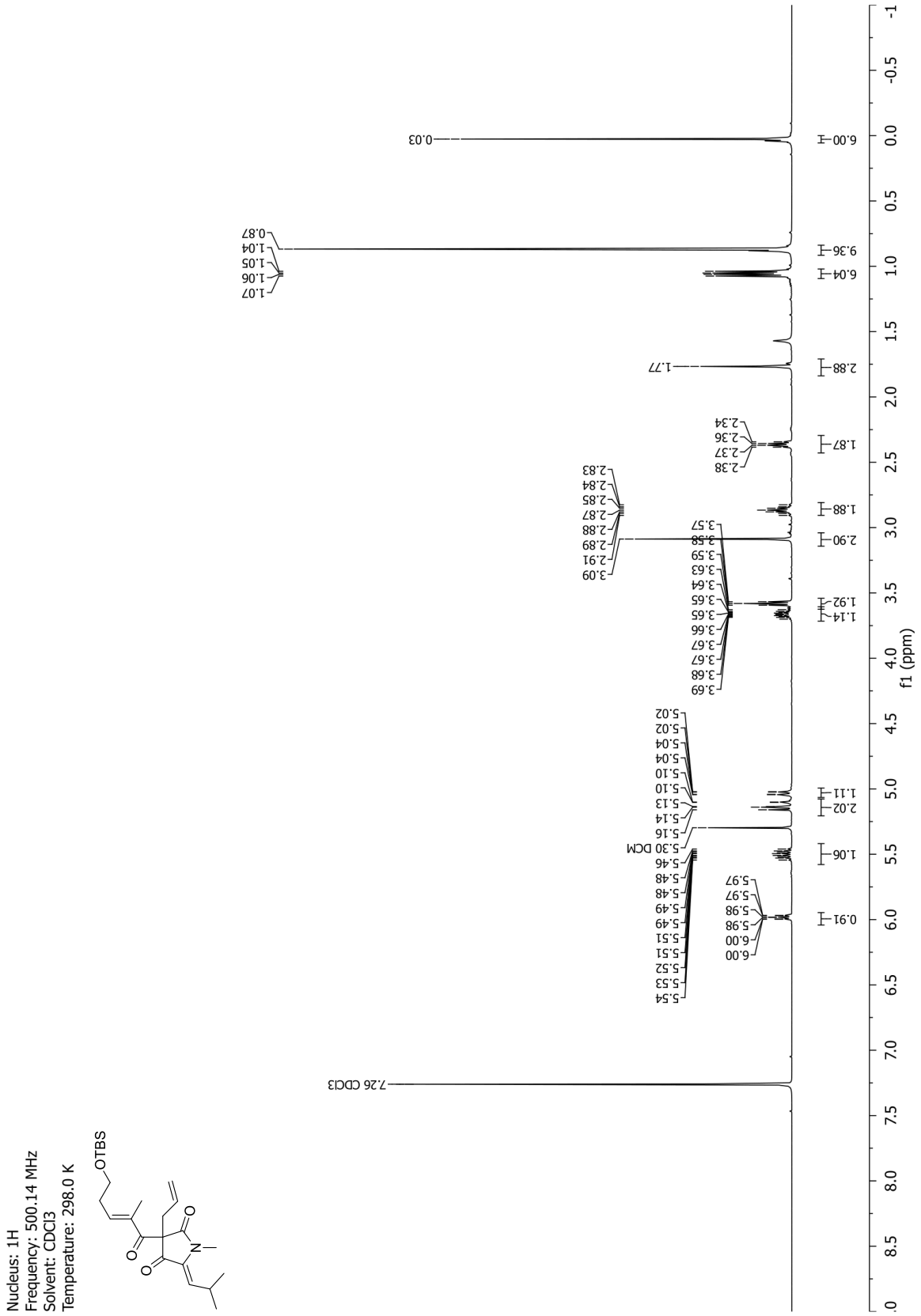

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
+ tautomers
OH
HO
O
O
N
1.3 H grease
12.8
12.7
23.5
23.7
25.3
25.3
25.7
26.3
32.9
33.0
41.5
61.7
61.6
100.8
103.5
123.8
125.9
126.5
130.2
131.1
132.3
134.6
141.2
142.7
164.6
172.6
181.9
184.1
188.4
189.8
f1 (ppm)

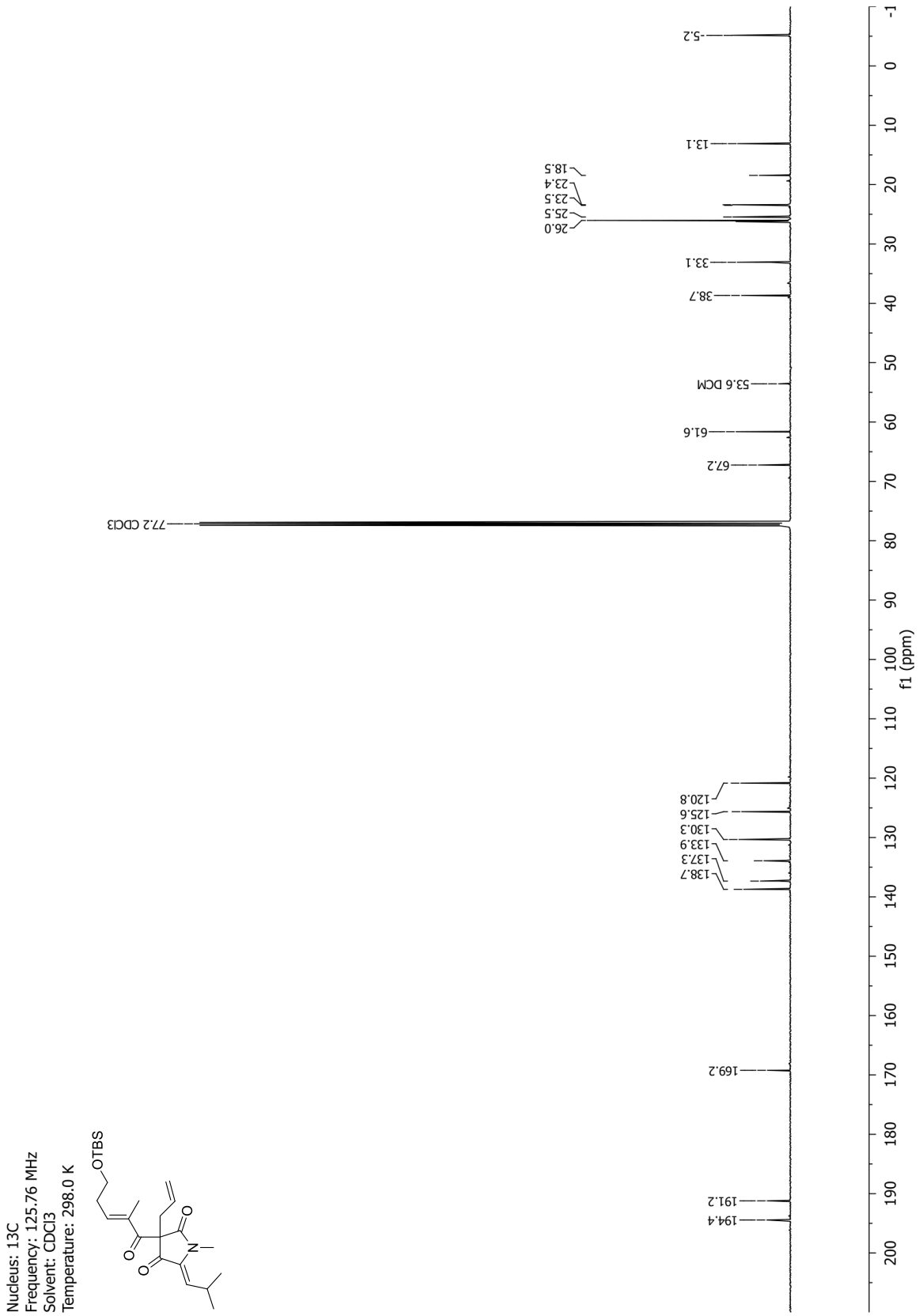
NMR-Spectra for Compound 264



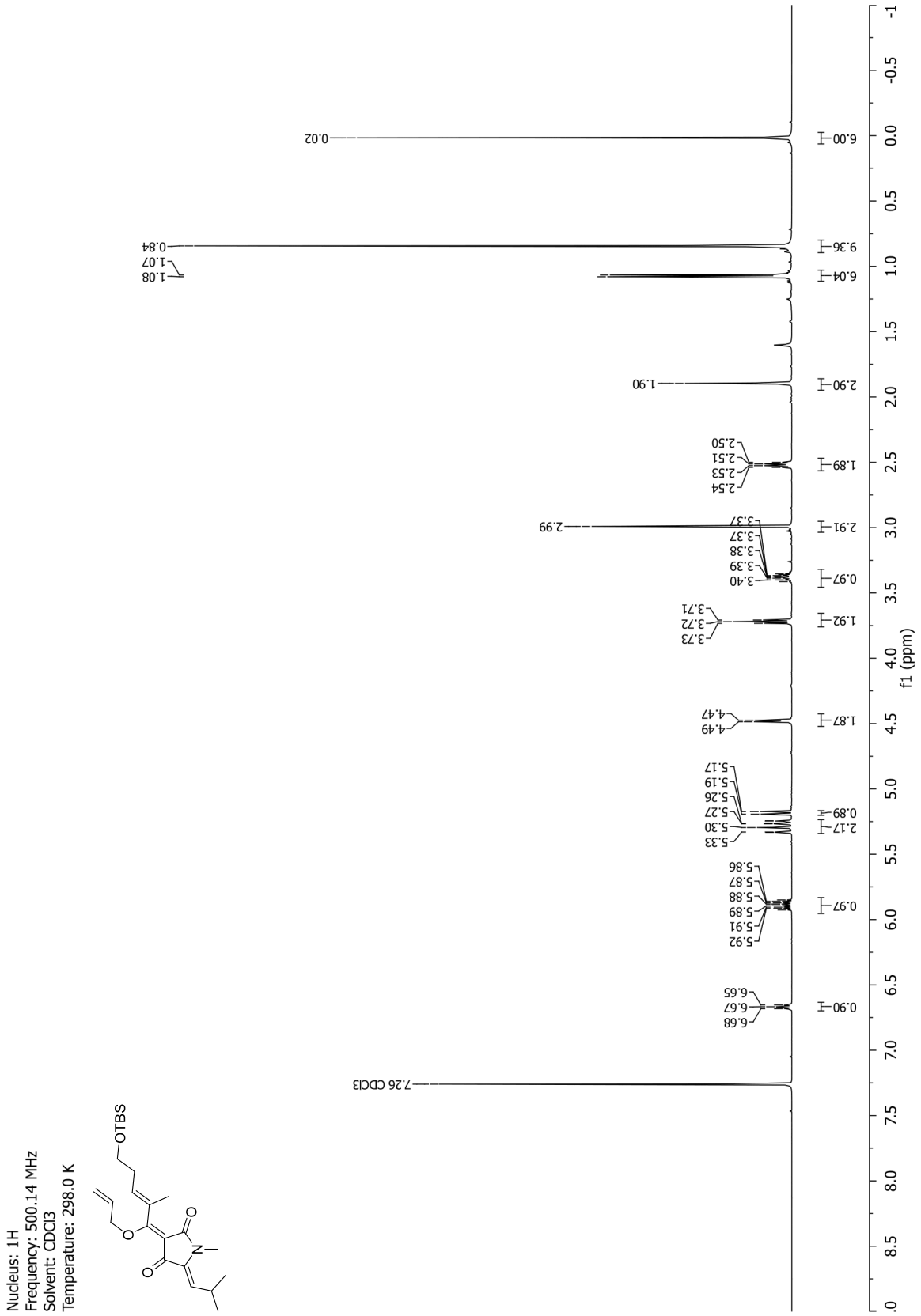

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
14.2
23.5
26.0
26.7
35.8
54.0 CD2Cl2
62.9
63.0
120.8
125.6
130.8
134.3
165.6
167.7
193.7
f1 (ppm)

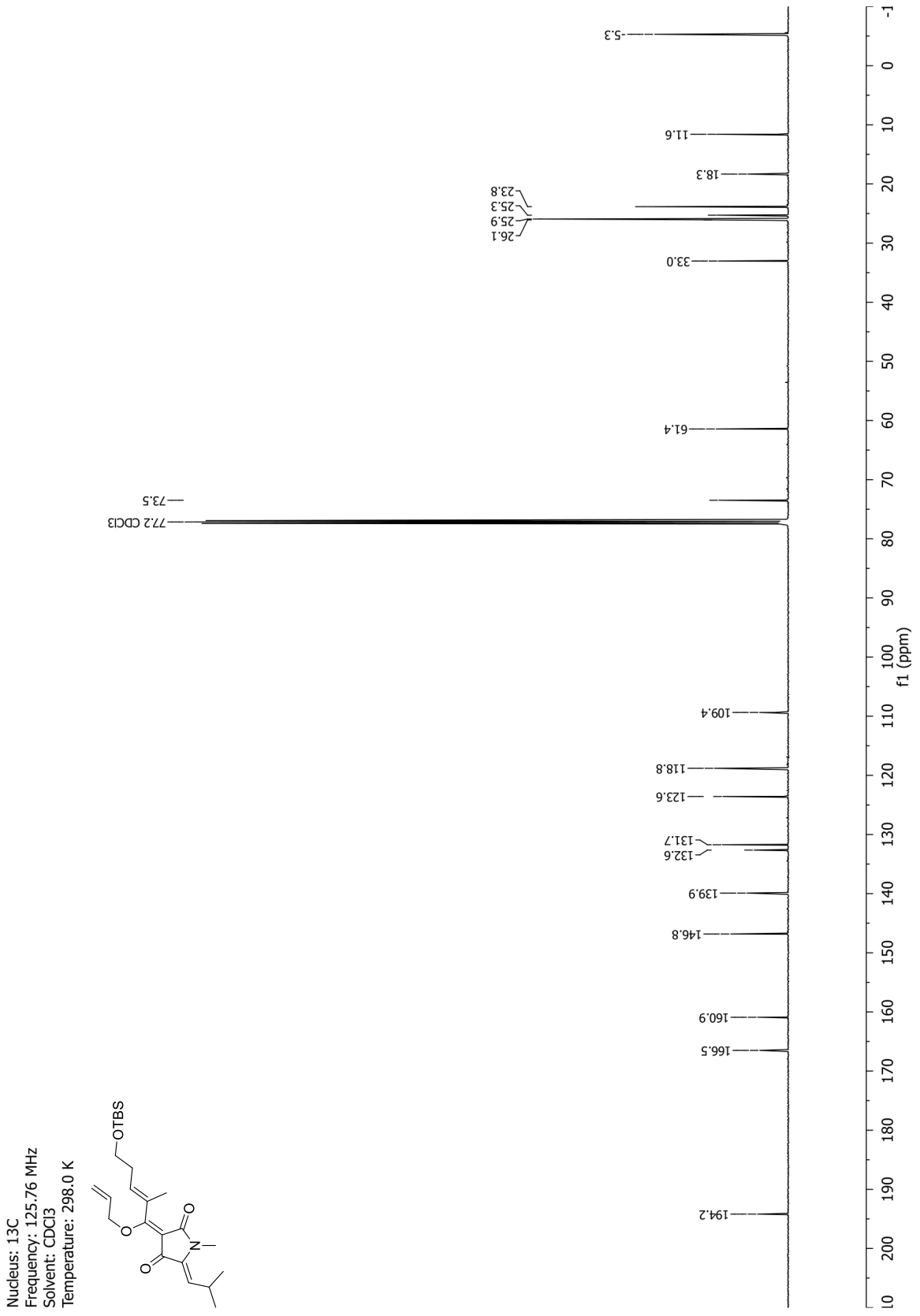
NMR-Spectra for Compound 265



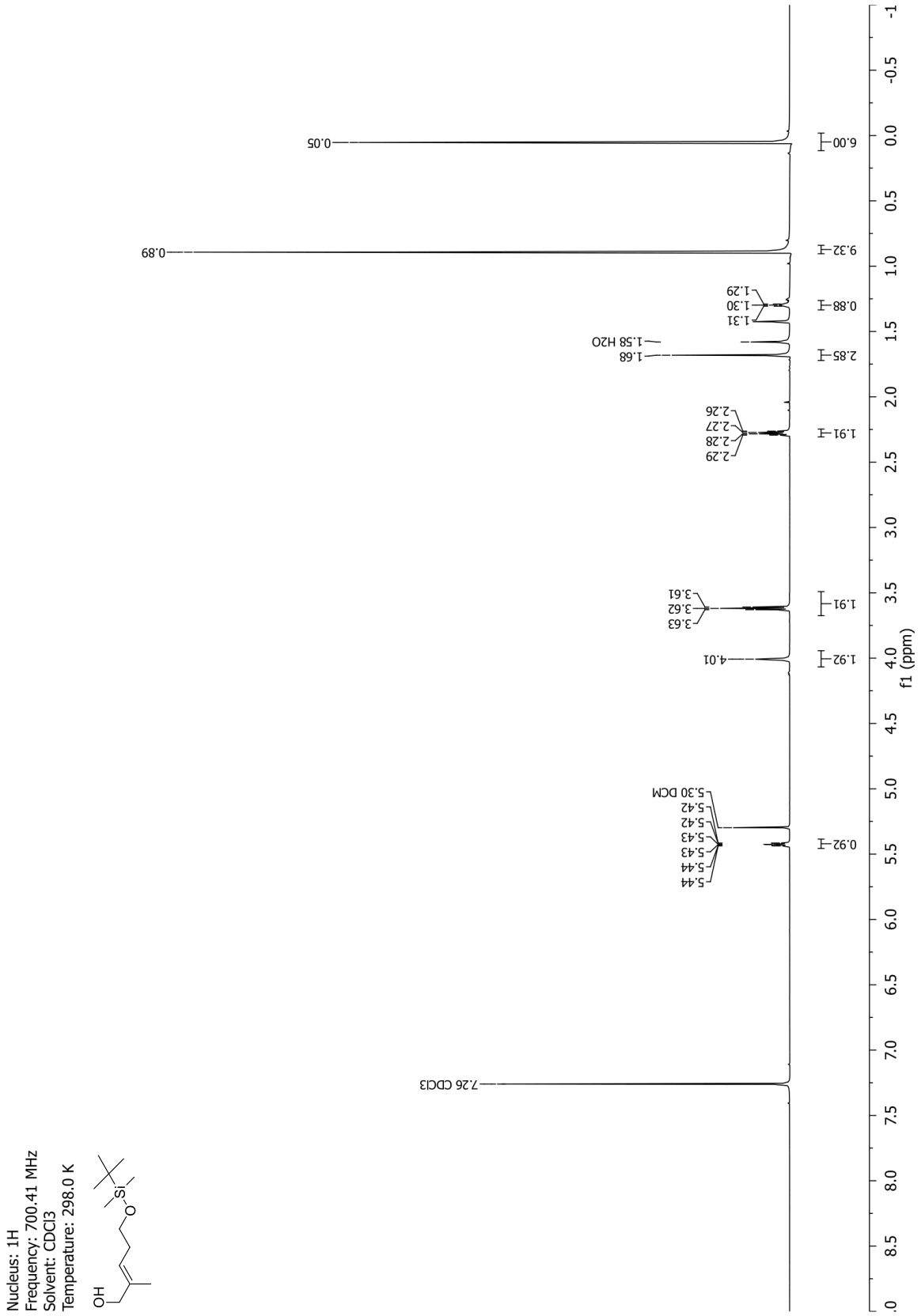

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
O
N
O
-5.2
13.1
18.5
23.4
23.5
25.5
26.0
33.1
38.7
53.6 DCM
61.6
67.2
77.2 CDCl3
120.8
125.6
130.3
133.9
137.3
138.7
169.2
191.2
194.4
-1
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
f1 (ppm)

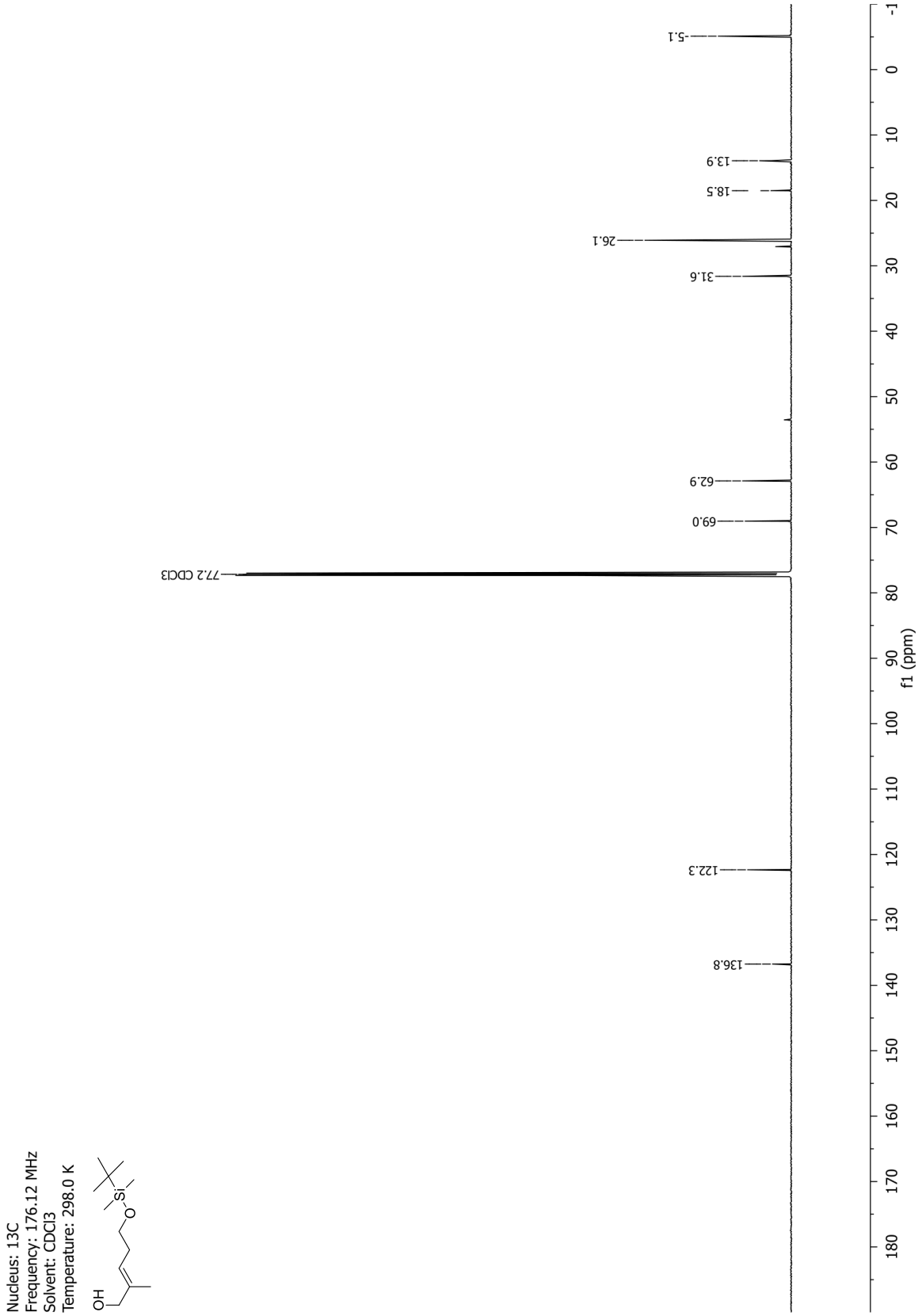
NMR-Spectra for Compound 266



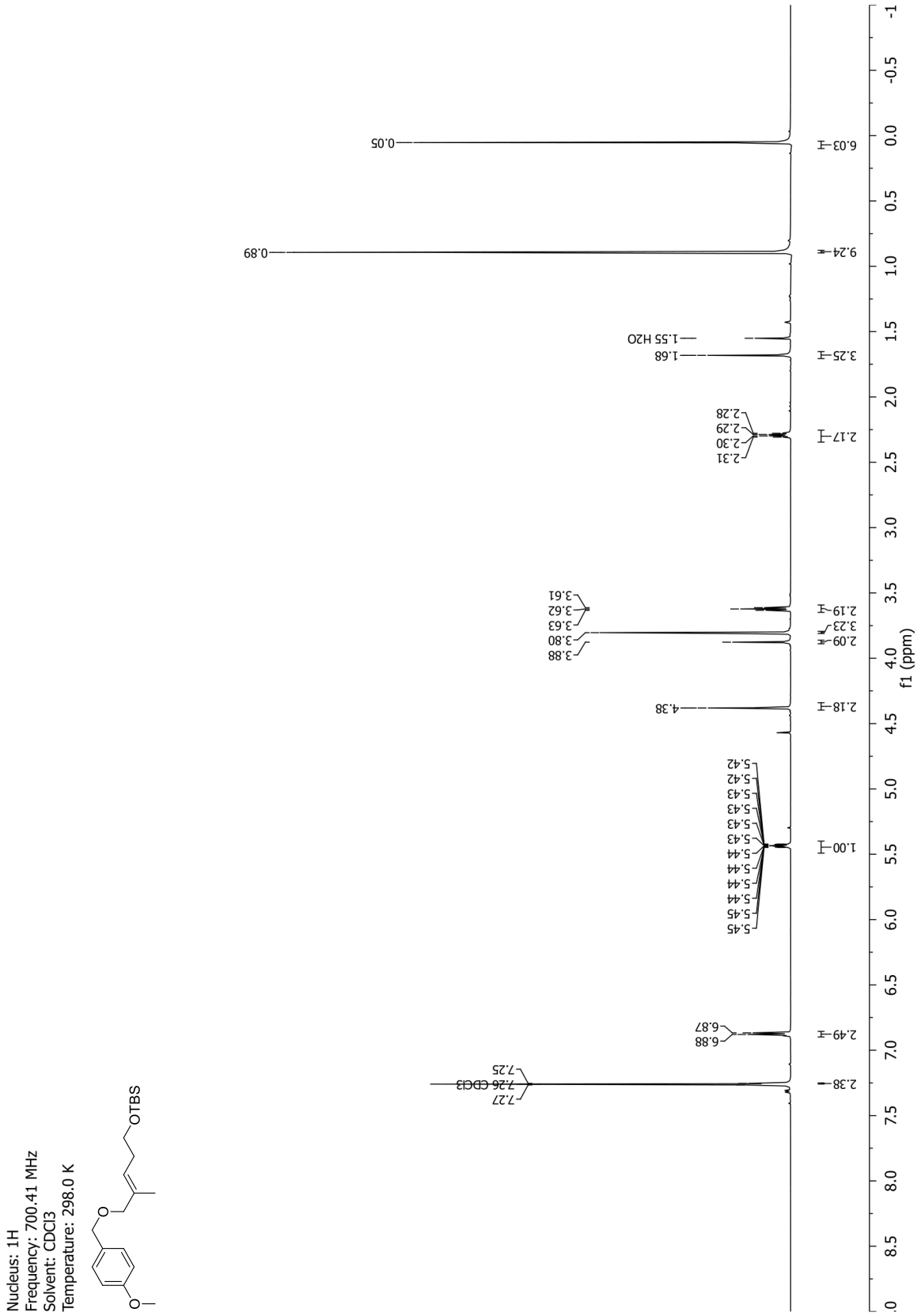

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
N
O
O
-5.3
11.6
18.3
23.8
25.3
25.9
26.1
33.0
61.4
73.5
77.2 CDCl3
109.4
118.8
123.6
131.7
132.6
139.9
146.8
160.9
166.5
194.2
f1 (ppm)

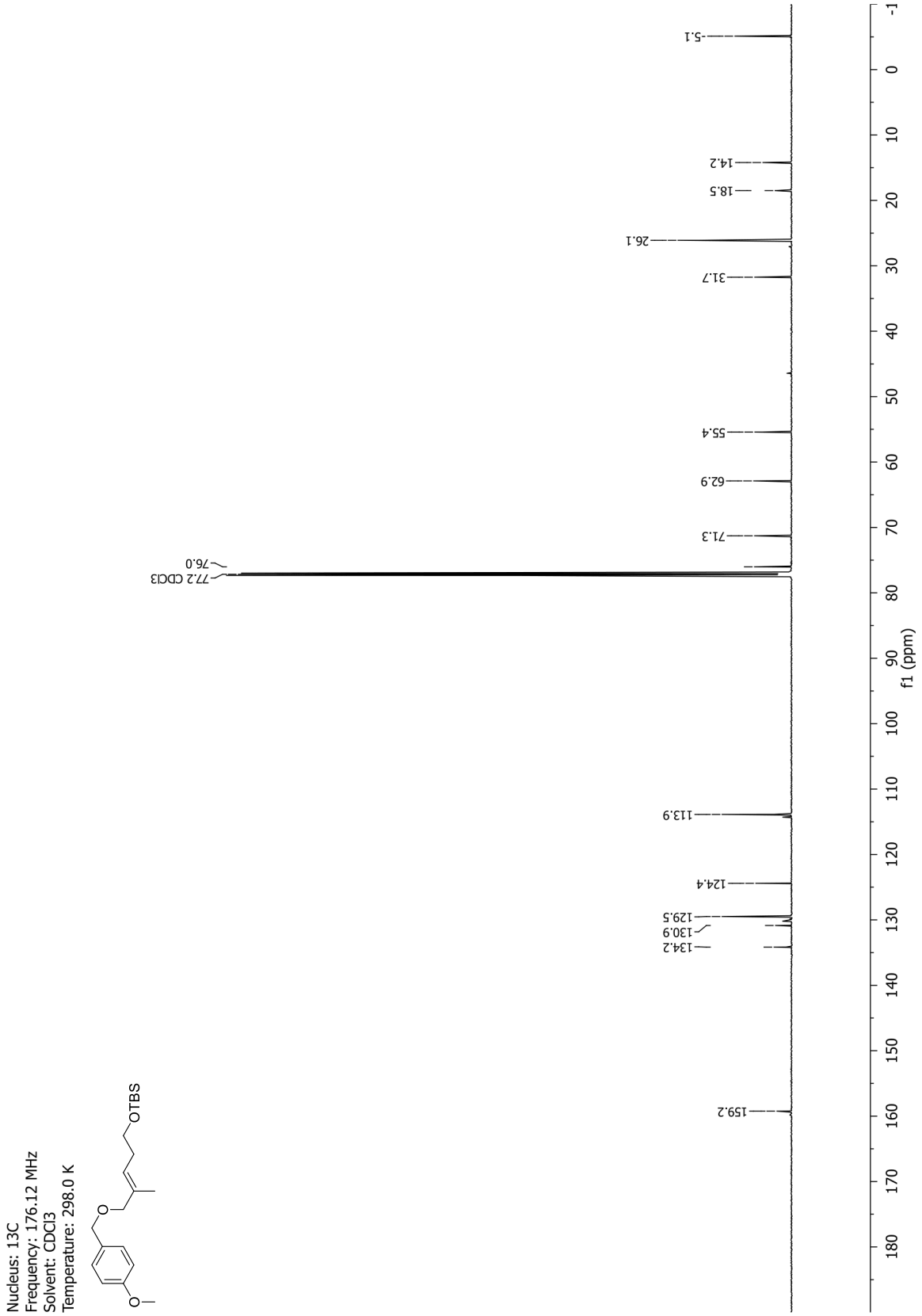
NMR-Spectra for Compound 213



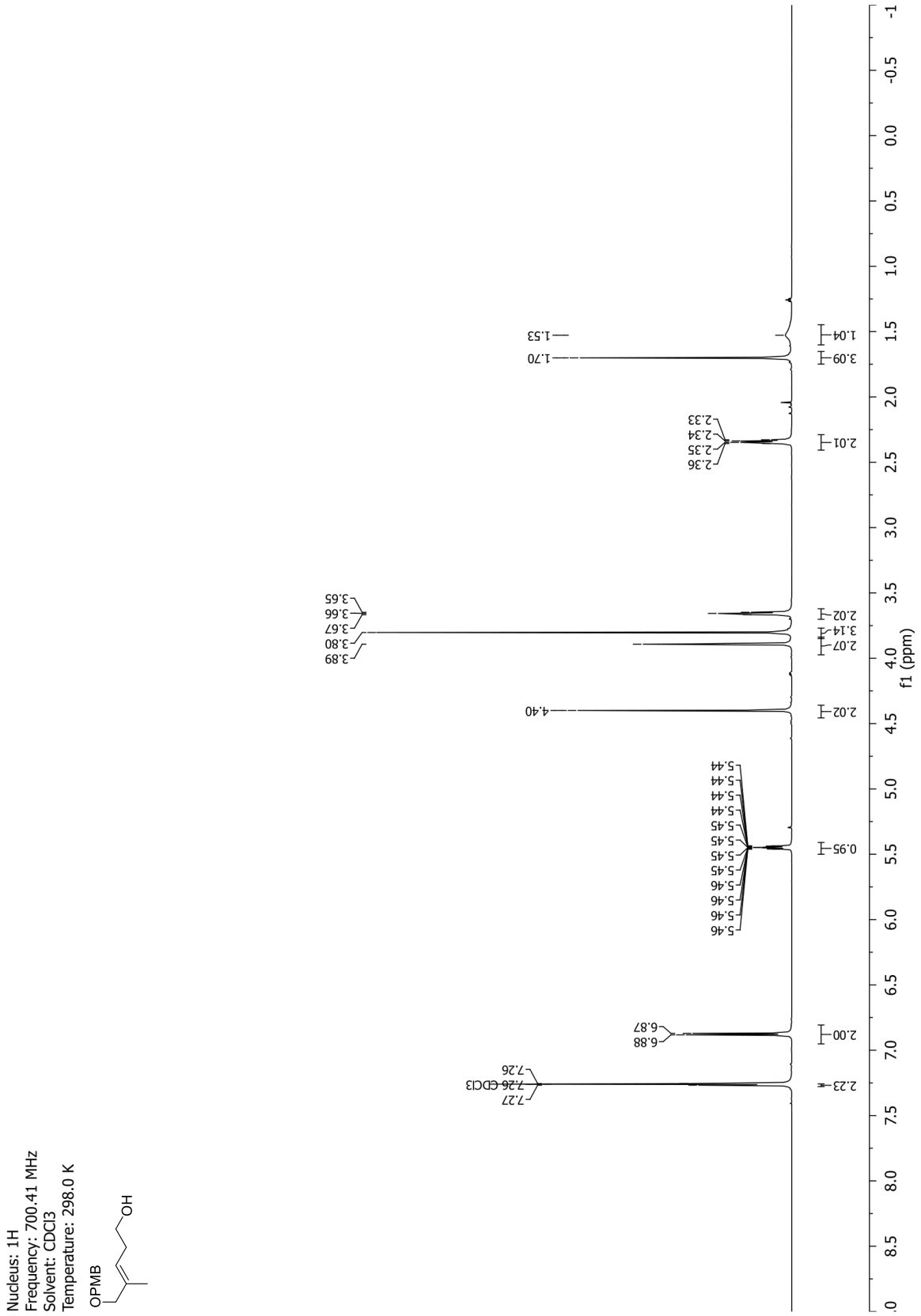

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OH
Si
O
-5.1
13.9
18.5
26.1
31.6
62.9
69.0
77.2 CDCl3
122.3
136.8
f1 (ppm)

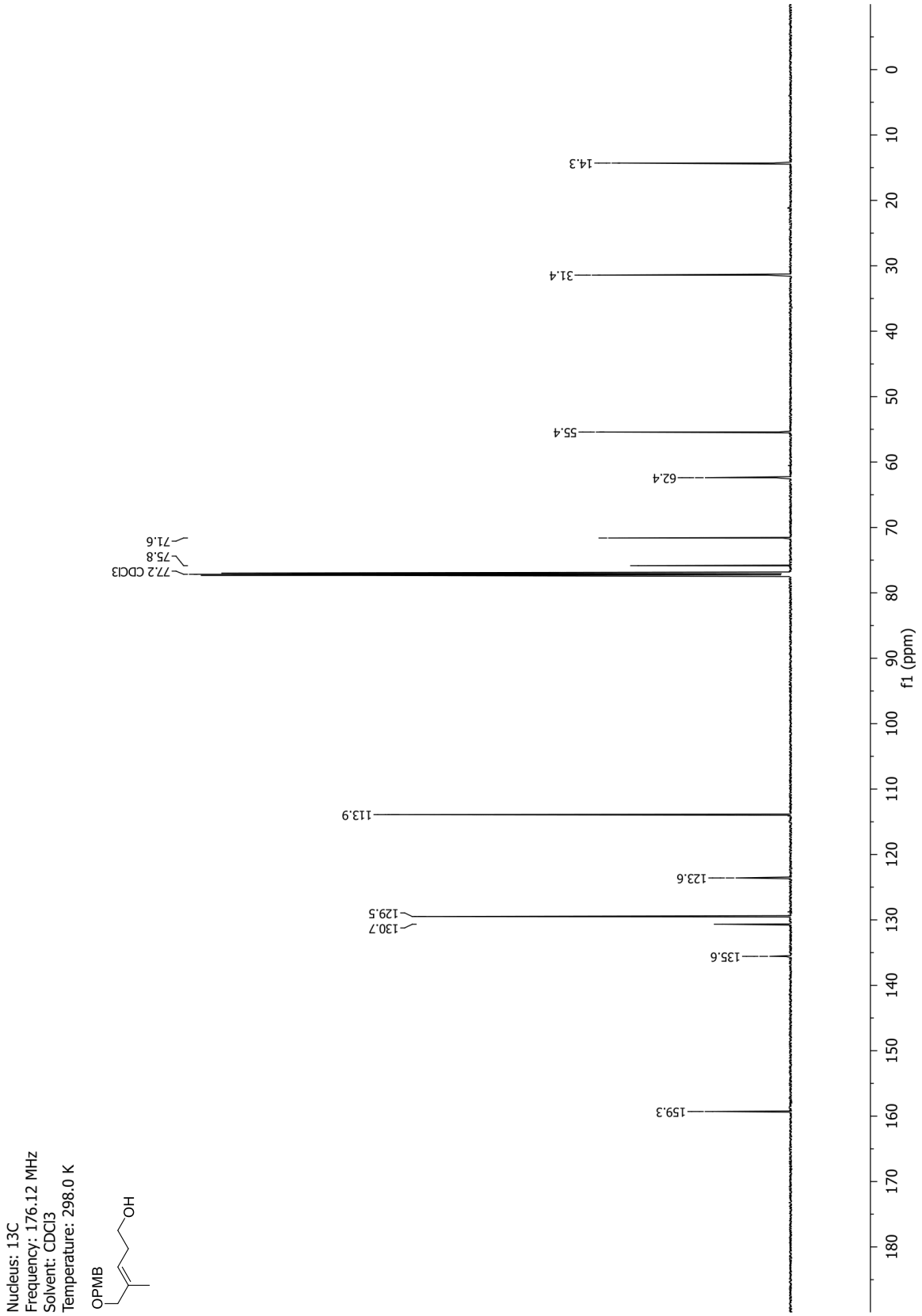
NMR-Spectra for Compound 268



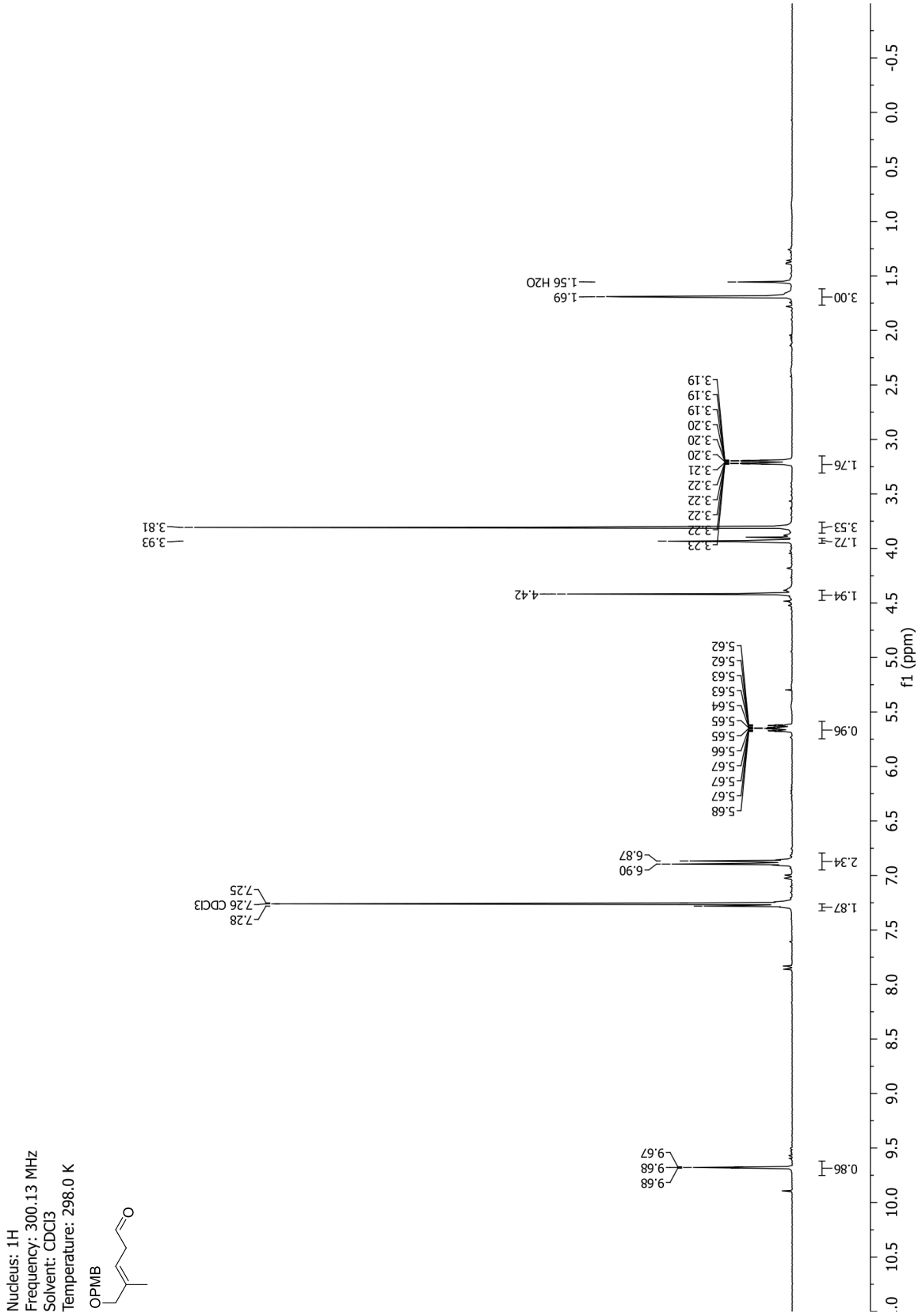

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
O
-5.1
14.2
18.5
26.1
31.7
55.4
62.9
71.3
76.0
77.2 CDCl3
113.9
124.4
129.5
130.9
134.2
159.2
f1 (ppm)

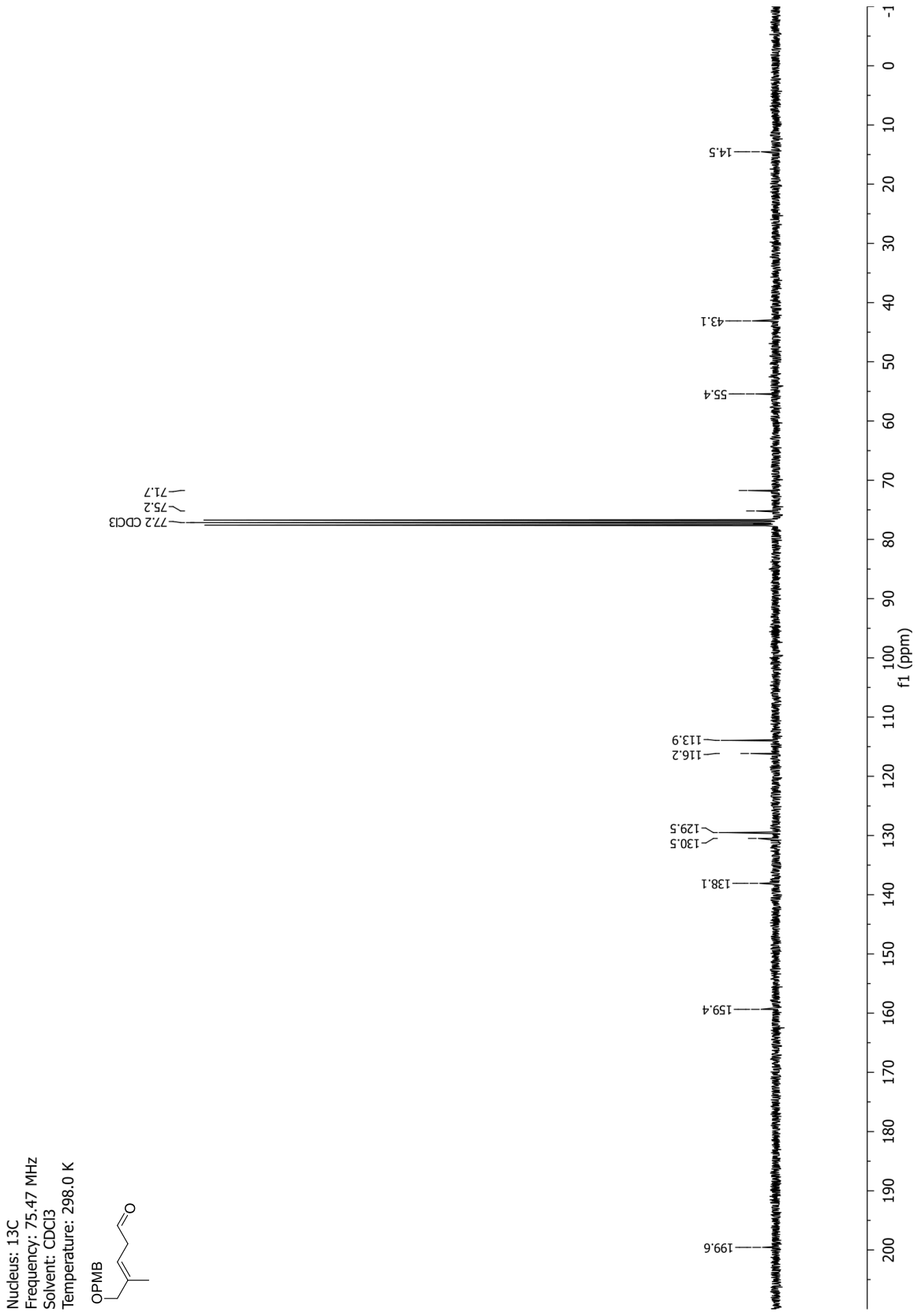
NMR-Spectra for Compound 269



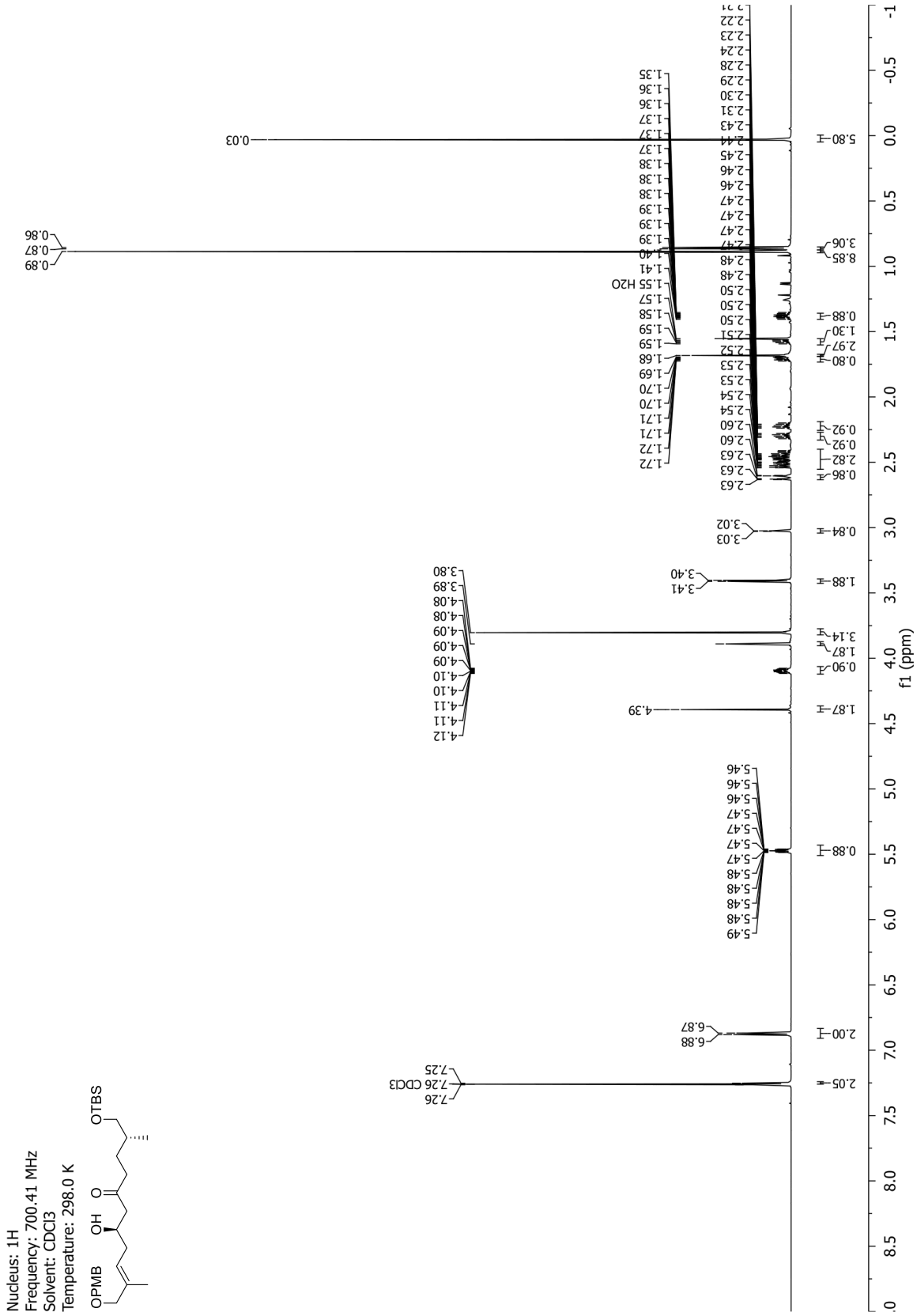

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OPMB
OH
14.3
31.4
55.4
62.4
71.6
75.8
77.2 CDCl3
113.9
123.6
129.5
130.7
135.6
159.3
f1 (ppm)

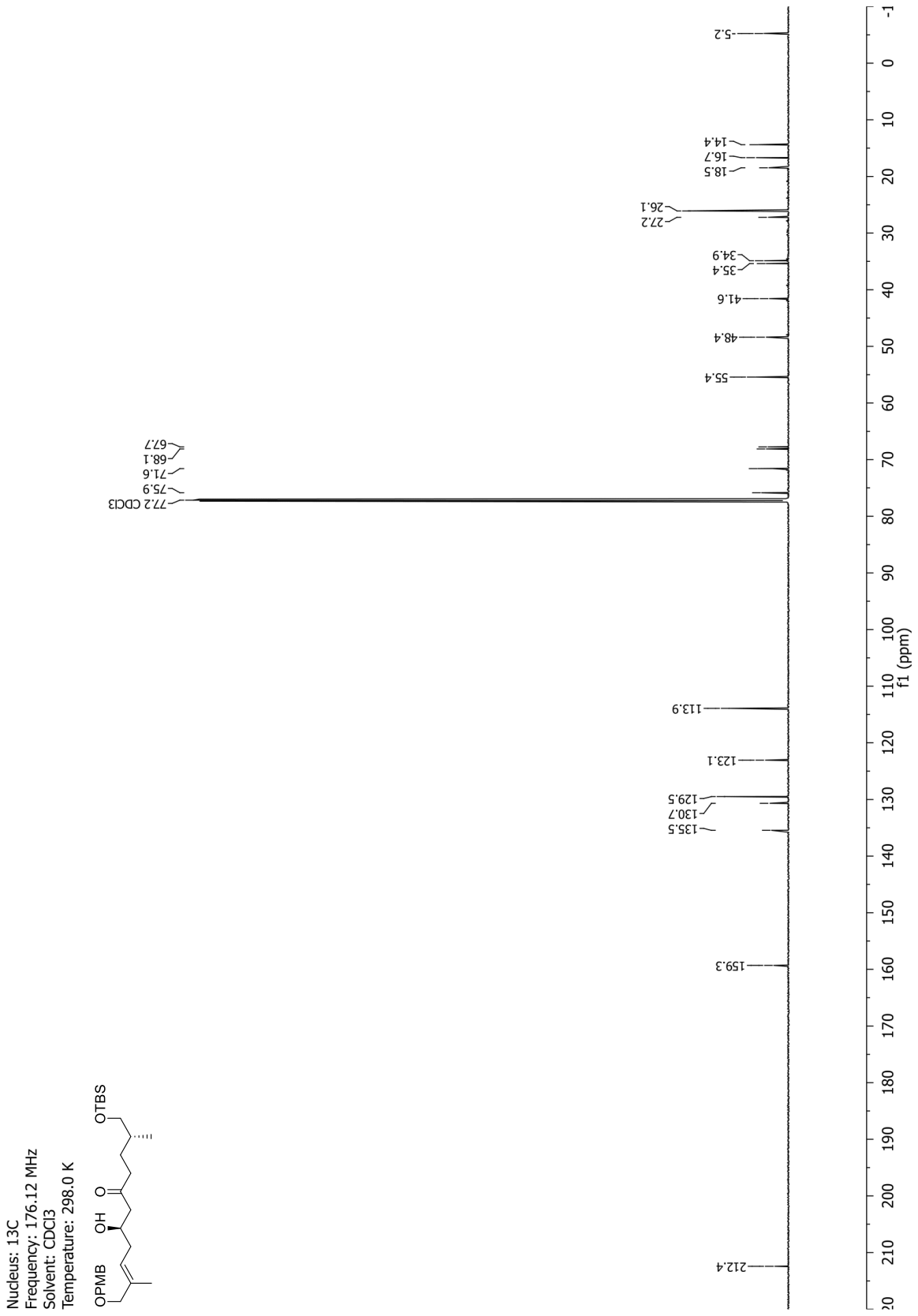
NMR-Spectra for Compound 212



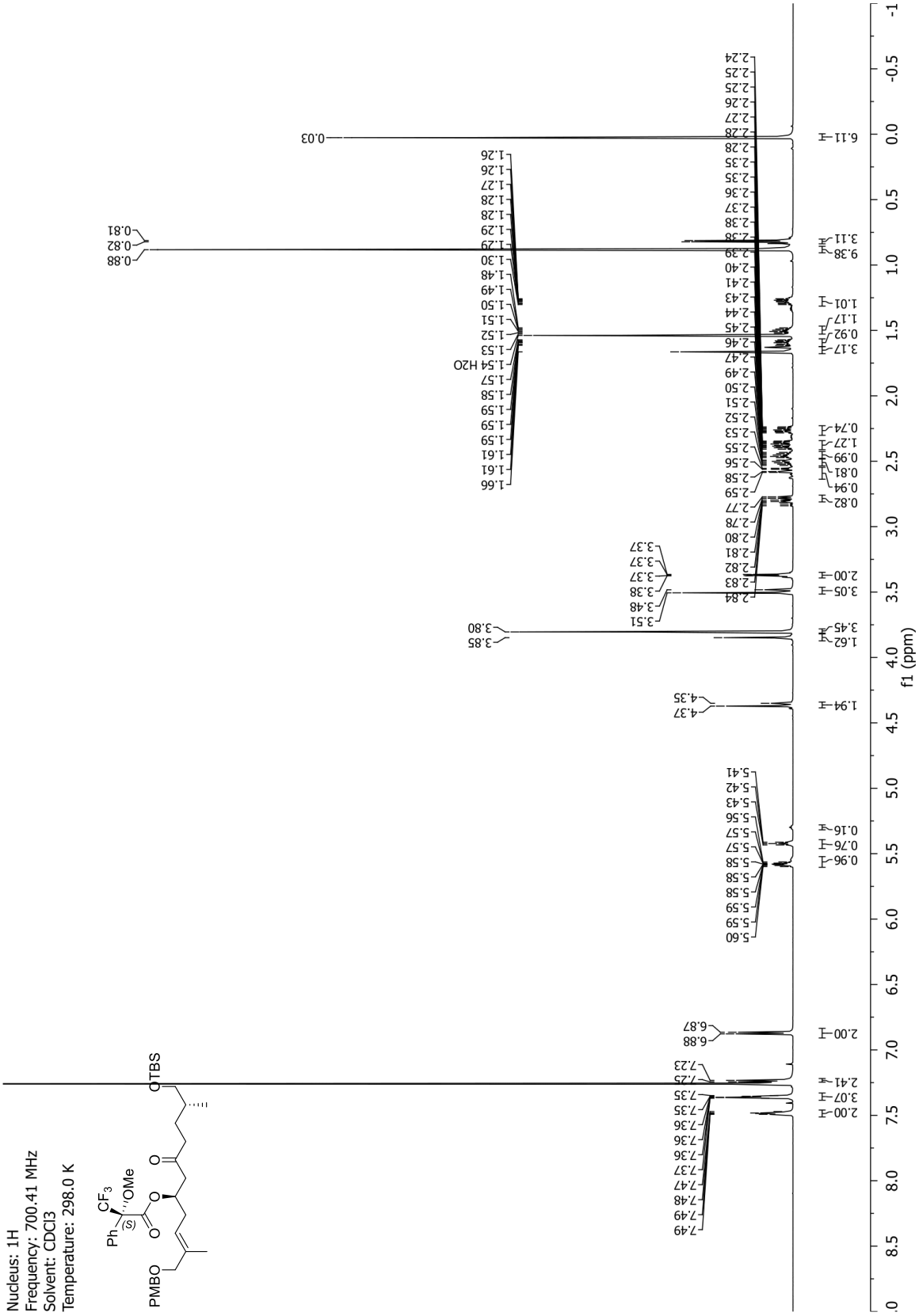

Nucleus: 13C
Frequency: 75.47 MHz
Solvent: CDCl3
Temperature: 298.0 K
OPMB
O
199.6
159.4
138.1
130.5
129.5
116.2
113.9
77.2 CDCl3
75.2
71.7
55.4
43.1
14.5
f1 (ppm)

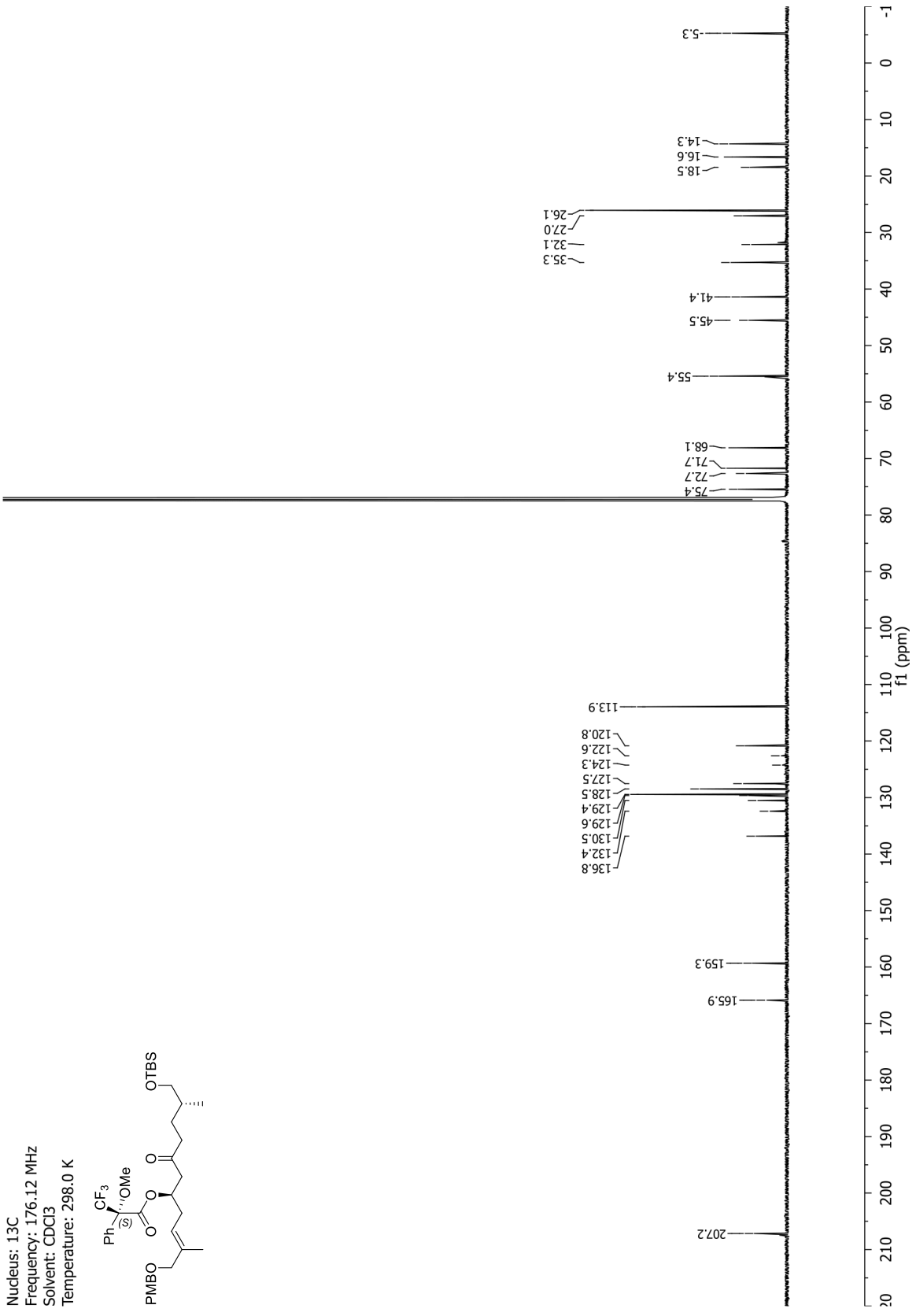
NMR-Spectra for Compound 270



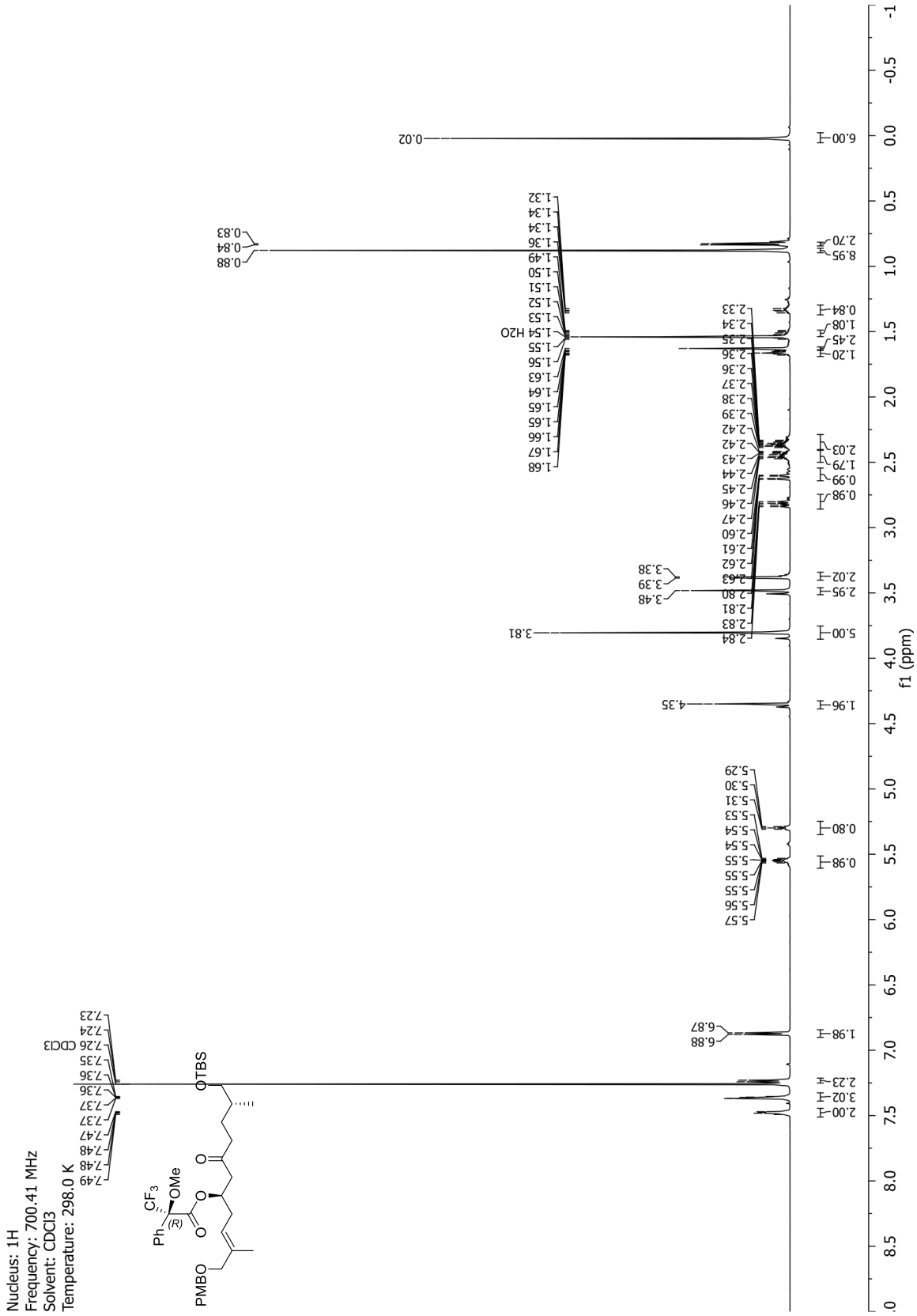

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OPMB
OH
O
OTBS
-5.2
14.4
16.7
18.5
26.1
27.2
34.9
35.4
41.6
48.4
55.4
67.7
68.1
71.6
75.9
77.2 CDCl3
113.9
123.1
129.5
130.7
135.5
159.3
212.4
f1 (ppm)

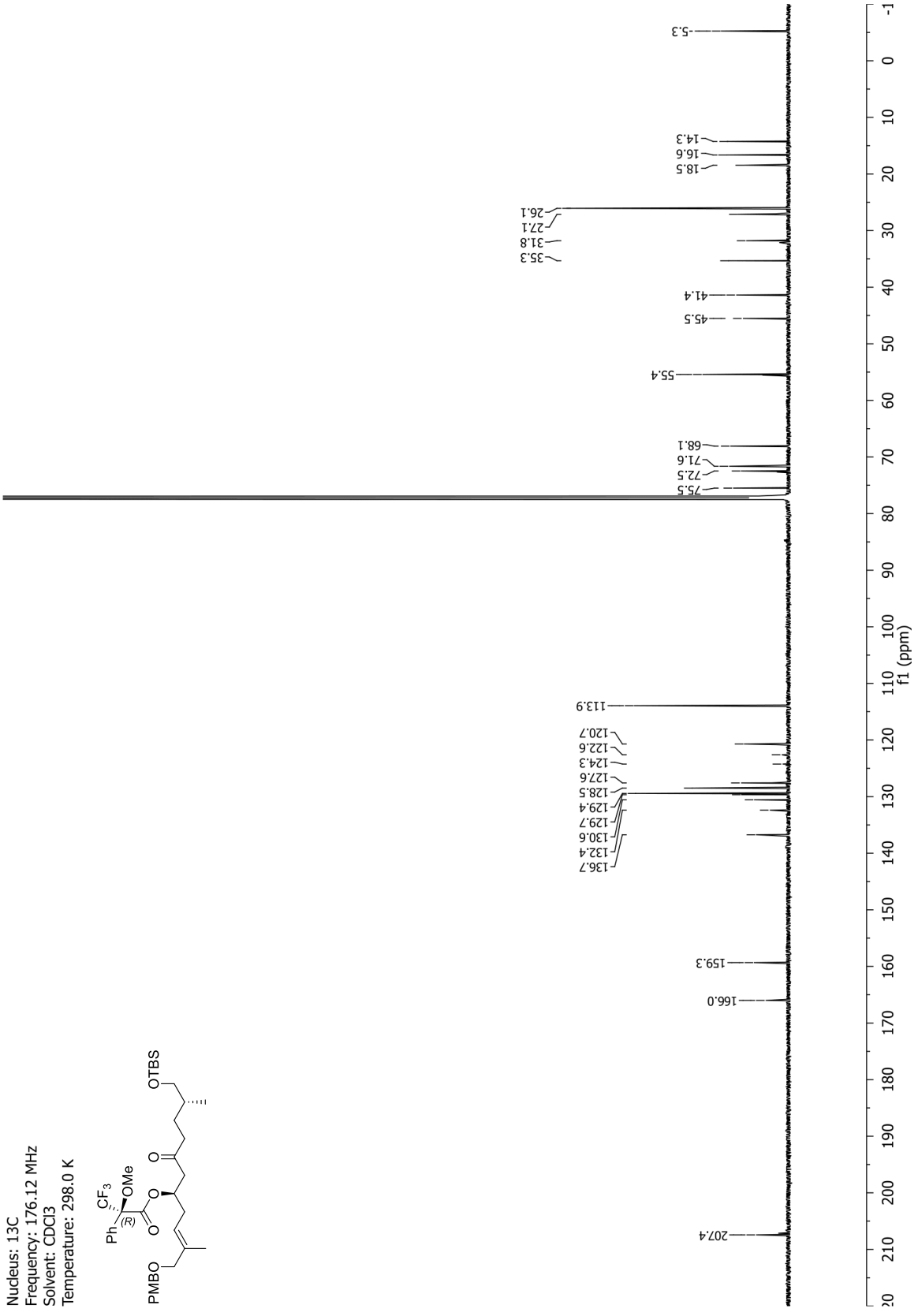
NMR-Spectra for Compound 280



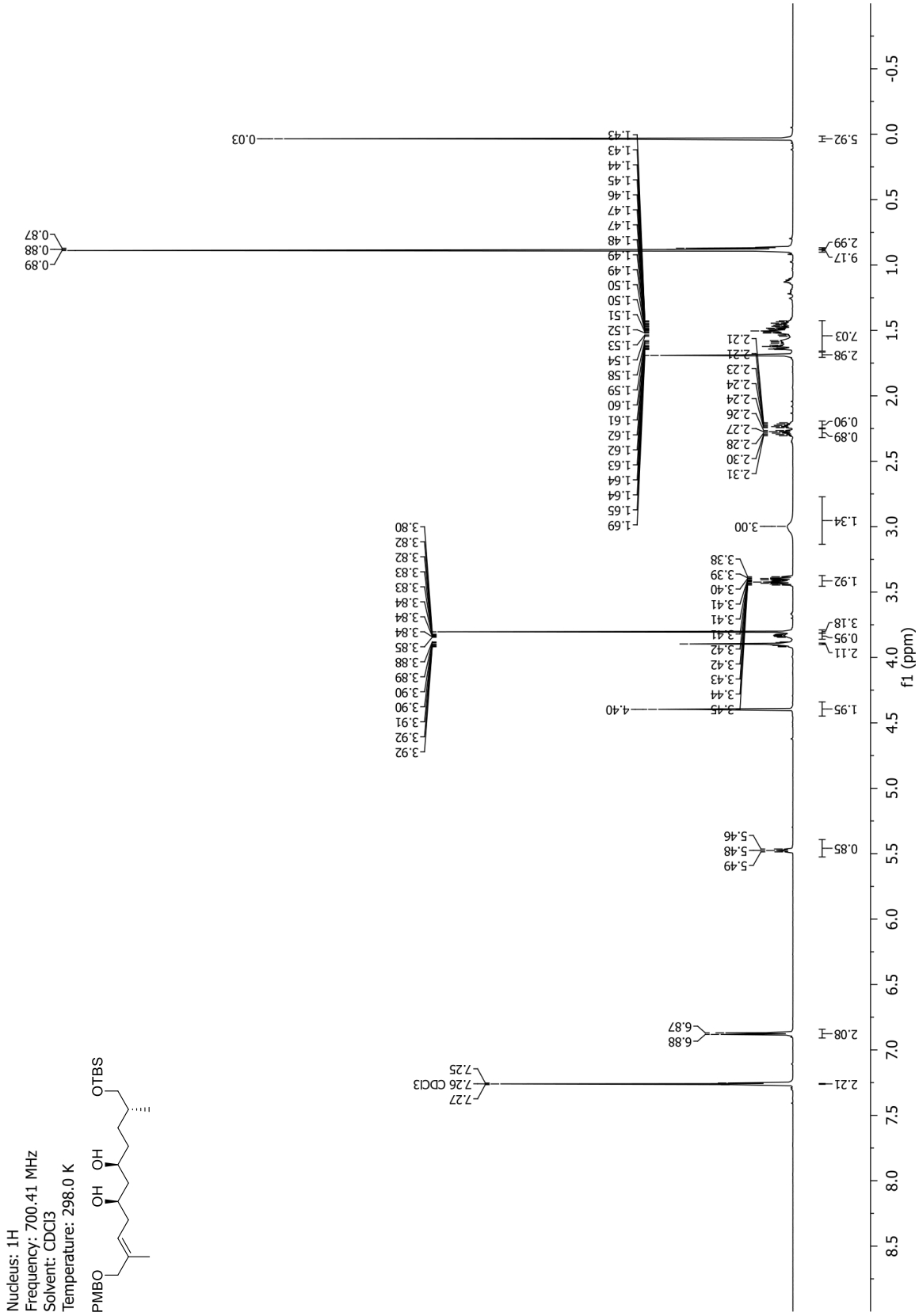

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
PMBO
Ph
CF3
OMe
(S)
f1 (ppm)

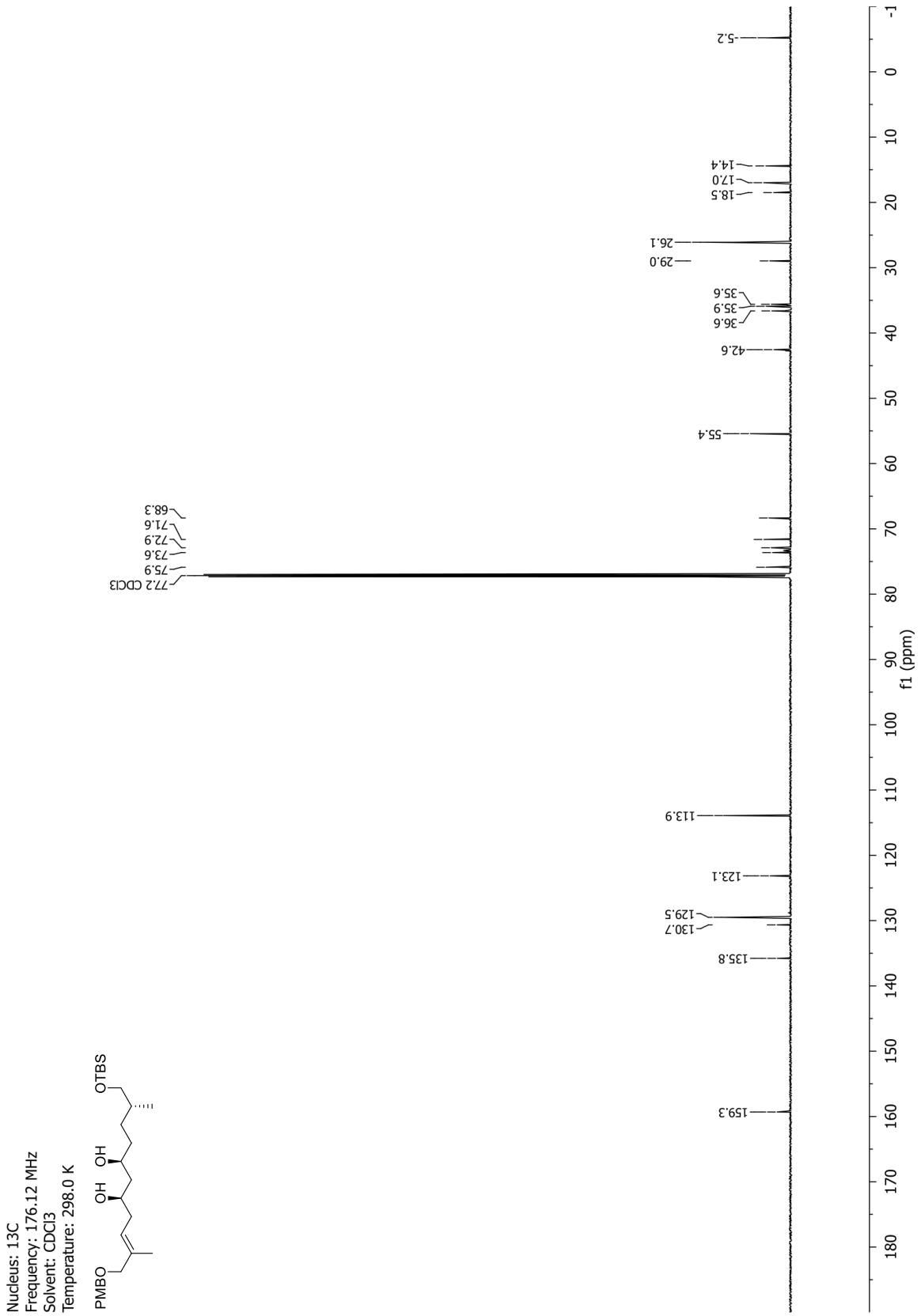
NMR-Spectra for Compound 281



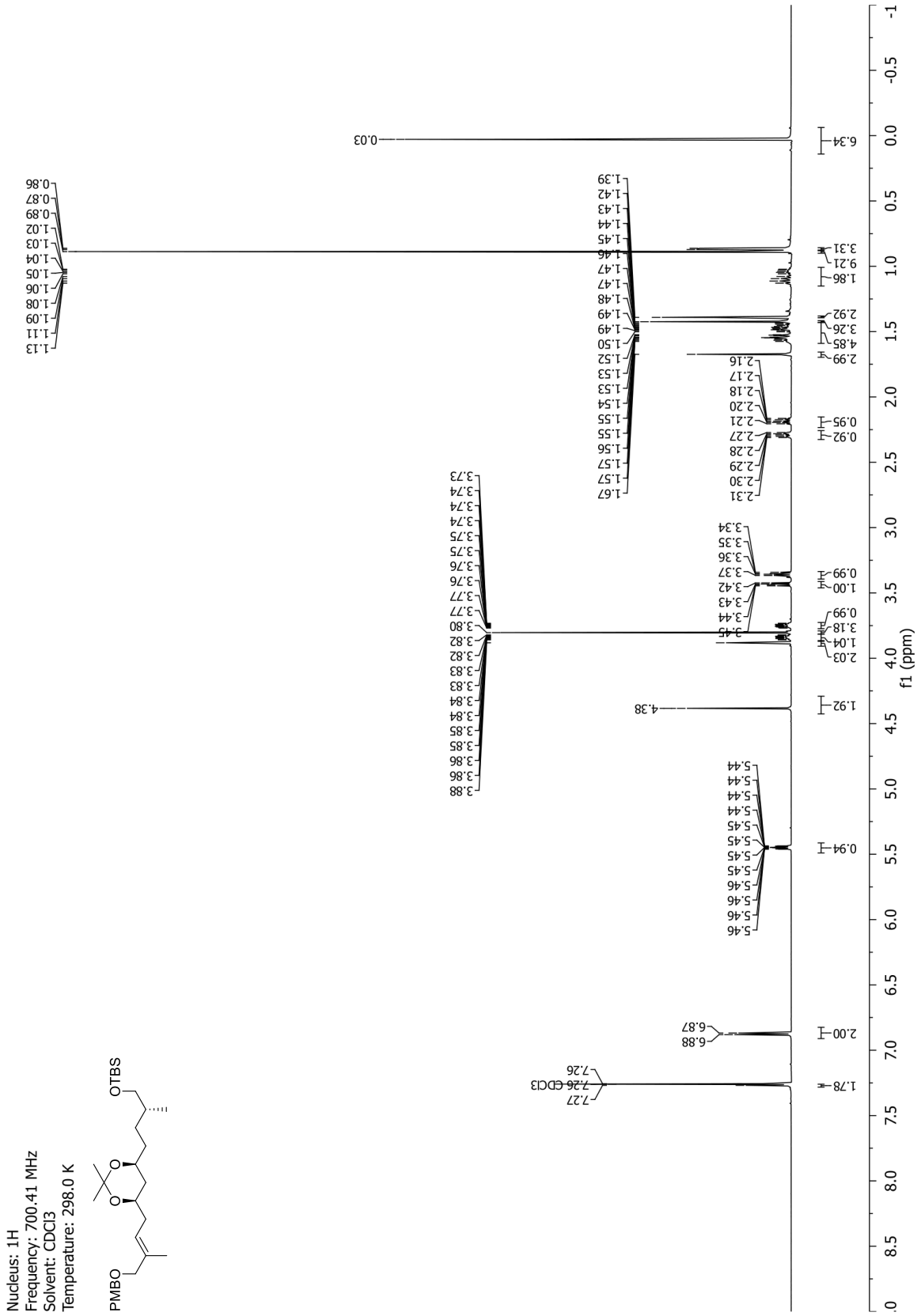

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
Ph
CF3
OMe
(R)
O
O
O
O
OTBS
PMBO
-5.3
14.3
16.6
18.5
26.1
27.1
31.8
35.3
41.4
45.5
55.4
68.1
71.6
72.5
75.5
113.9
120.7
122.6
124.3
127.6
128.5
129.4
129.7
130.6
132.4
136.7
159.3
166.0
207.4
f1 (ppm)

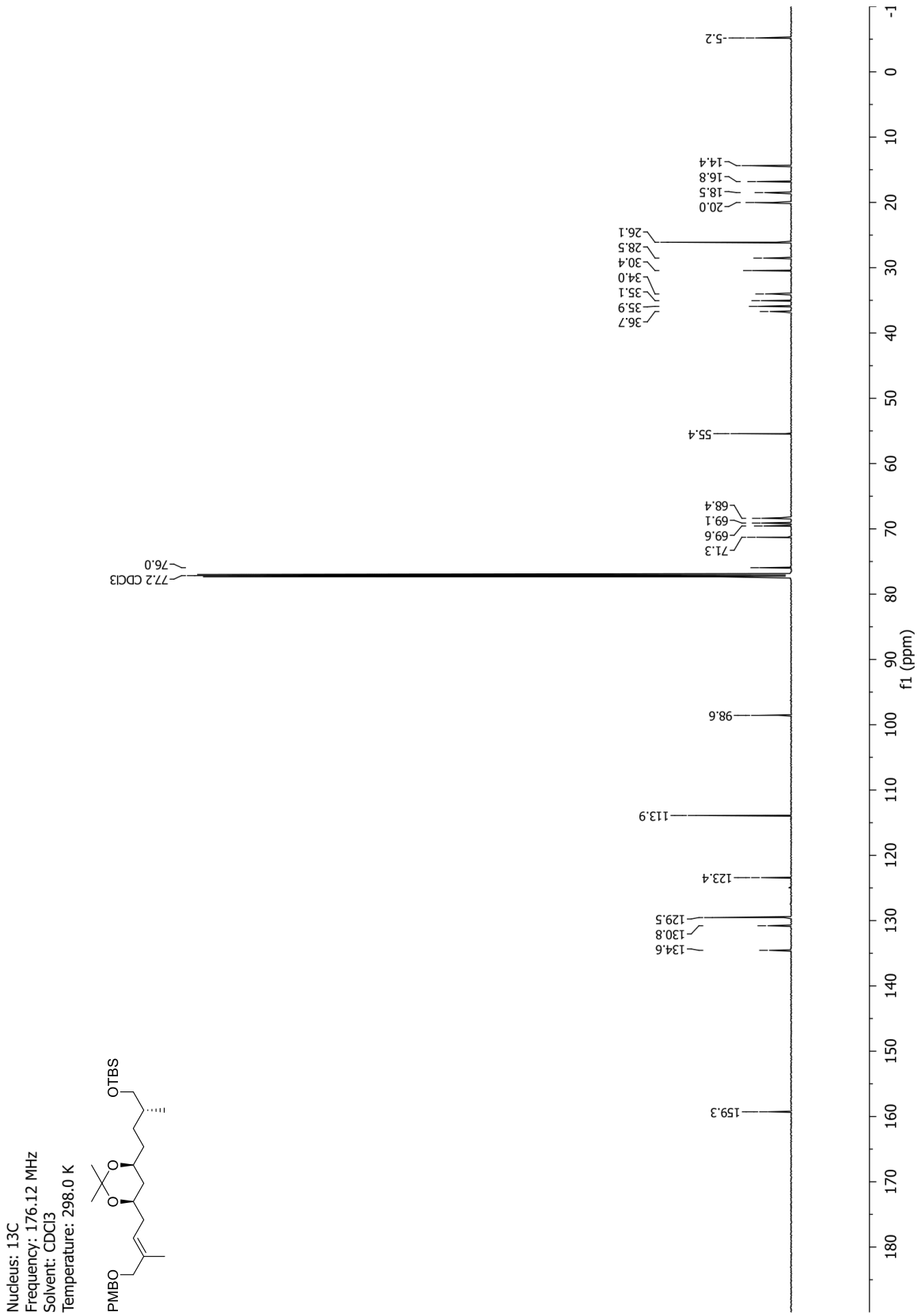
NMR-Spectra for Compound 282



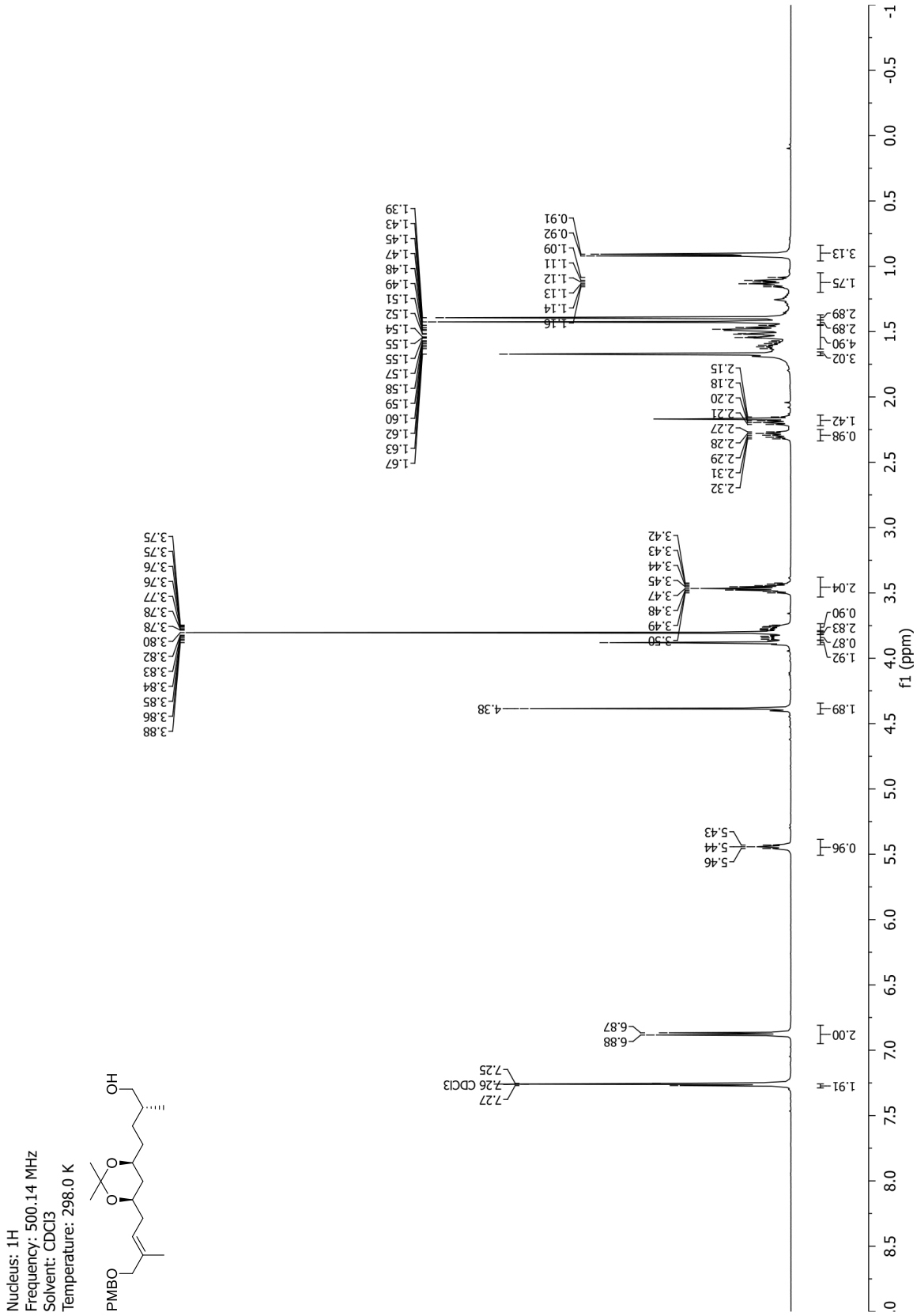

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OH
OH
OTBS
f1 (ppm)
-5.2
14.4
17.0
18.5
26.1
29.0
35.6
35.9
36.6
42.6
55.4
68.3
71.6
72.9
73.6
75.9
77.2 CDCl3
113.9
123.1
129.5
130.7
135.8
159.3

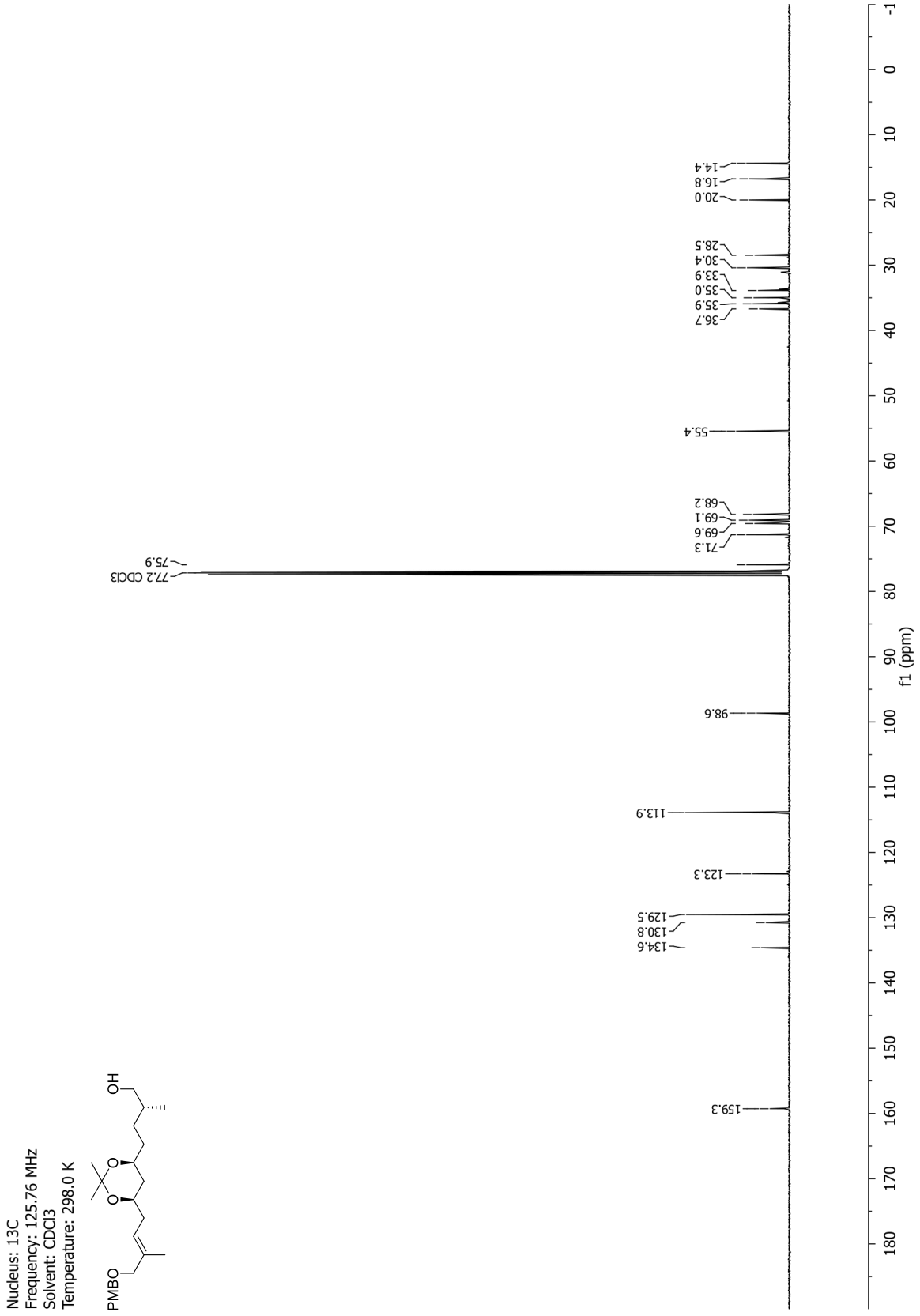
NMR-Spectra for Compound 288



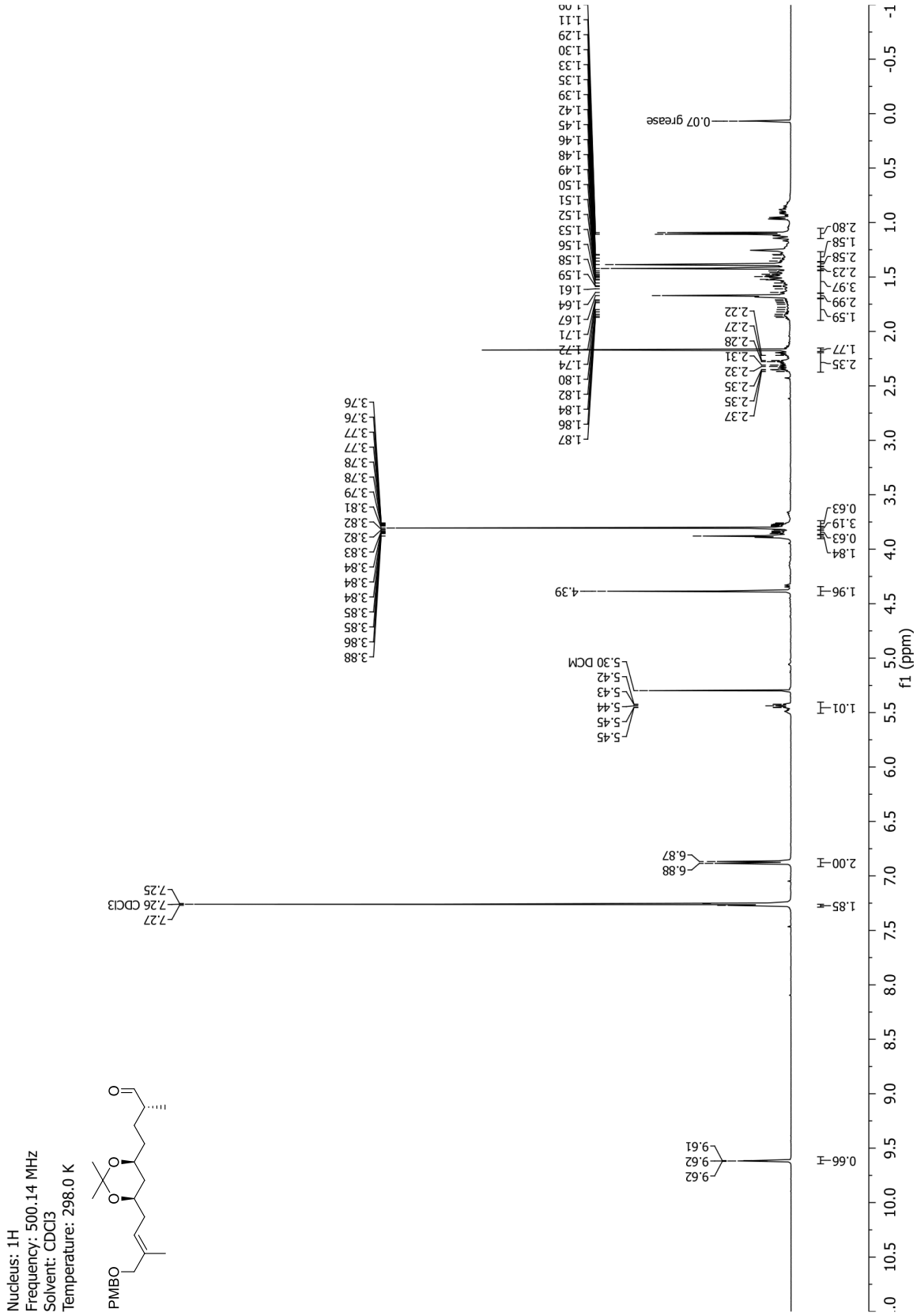

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OTBS
f1 (ppm)
-5.2
14.4
16.8
18.5
20.0
26.1
28.5
30.4
34.0
35.1
35.9
36.7
55.4
68.4
69.1
69.6
71.3
76.0
77.2 CDCl3
98.6
113.9
123.4
129.5
130.8
134.6
159.3

NMR-Spectra for Compound 292

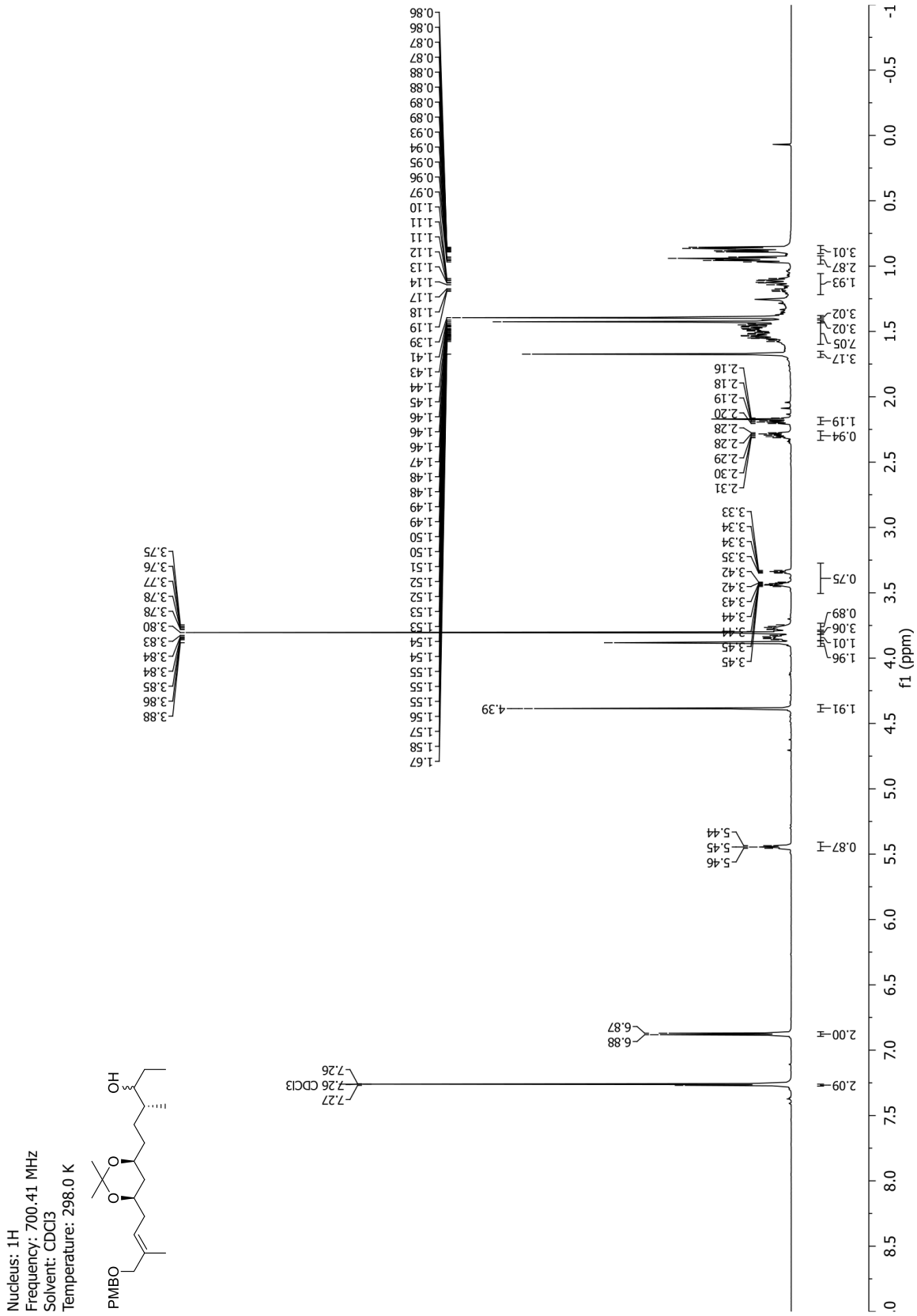


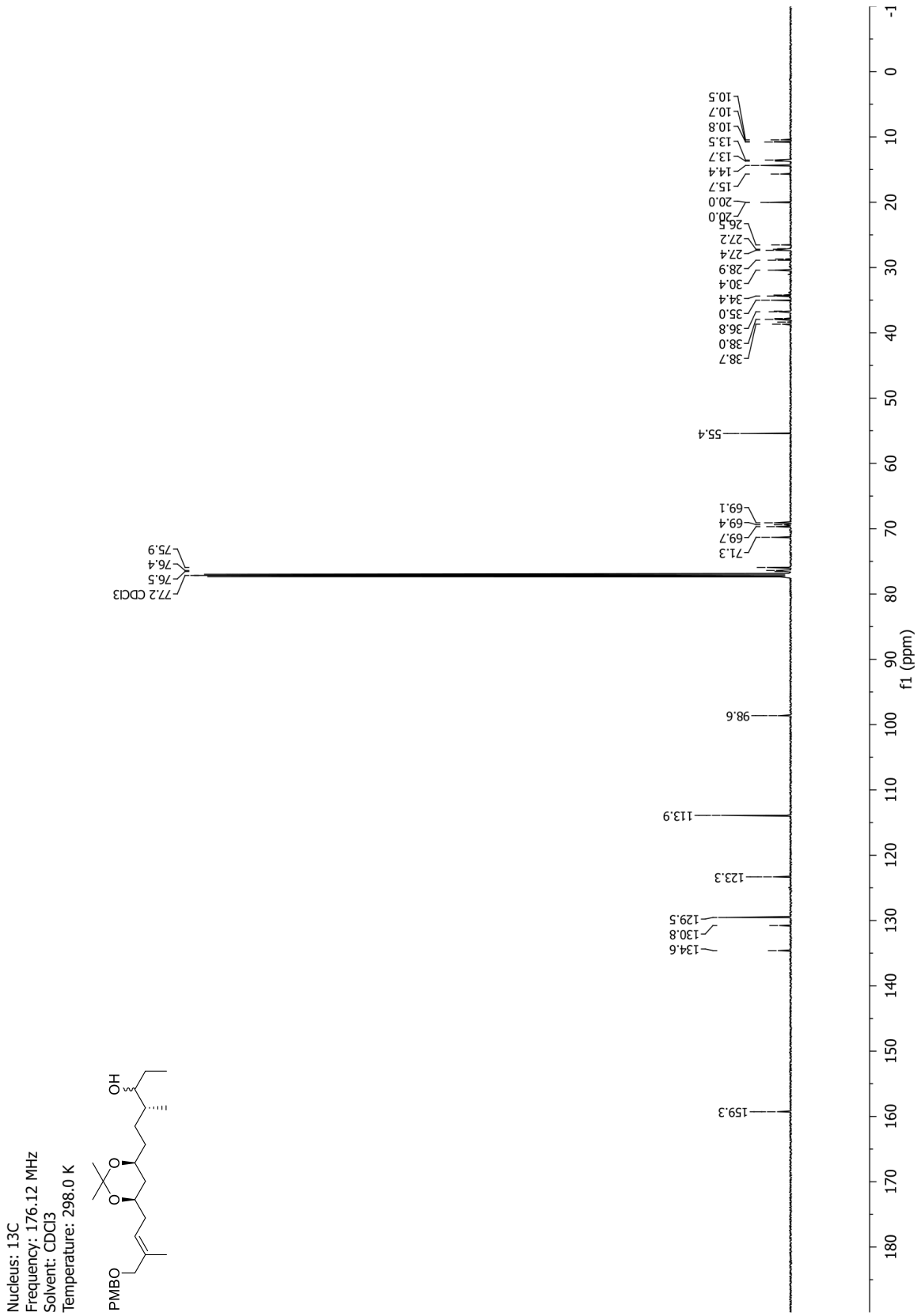

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OH
O
O
14.4
16.8
20.0
28.5
30.4
33.9
35.0
35.9
36.7
55.4
68.2
69.1
69.6
71.3
75.9
77.2 CDCl3
98.6
113.9
123.3
129.5
130.8
134.6
159.3
f1 (ppm)

NMR-Spectra for Compound 293

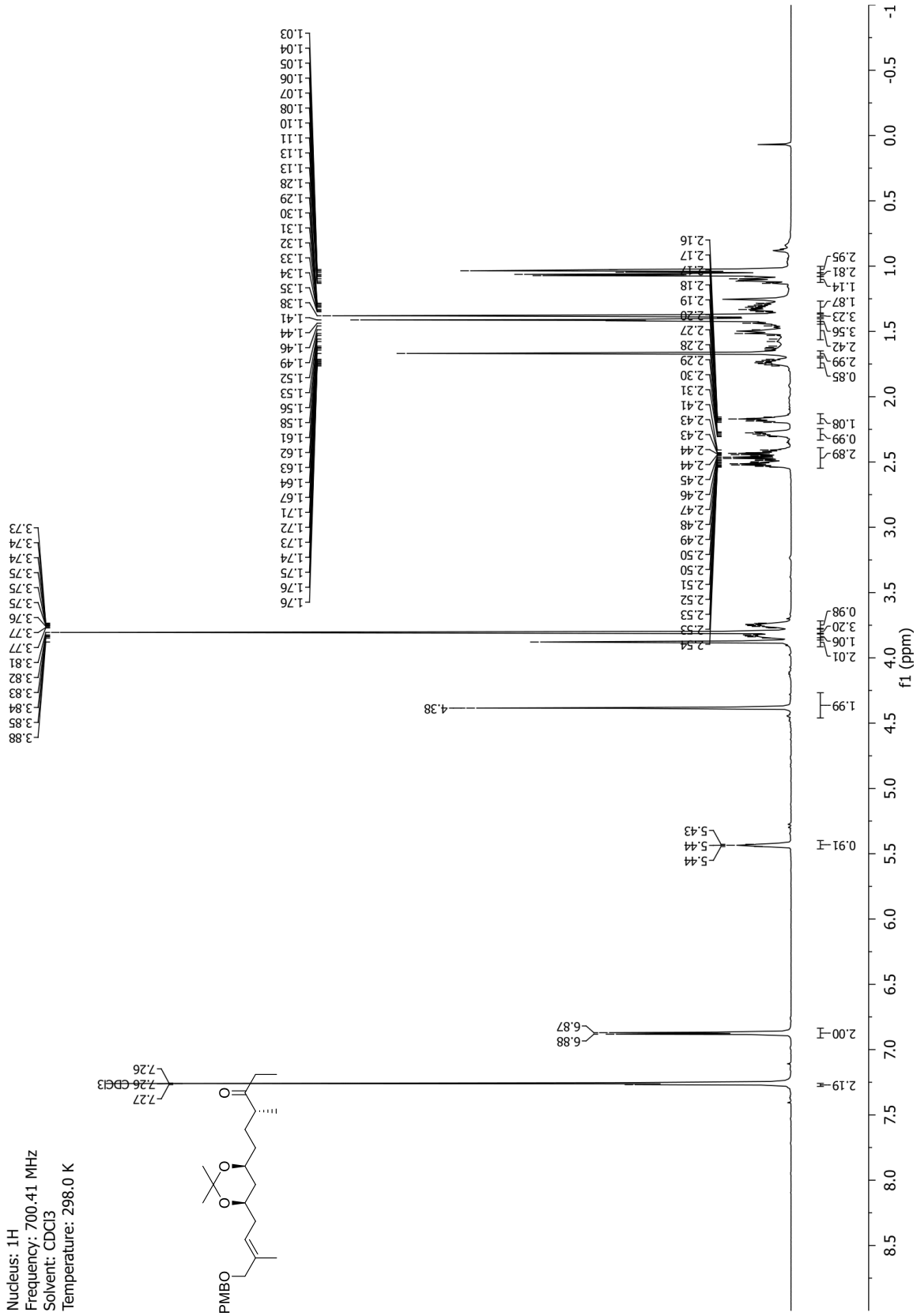


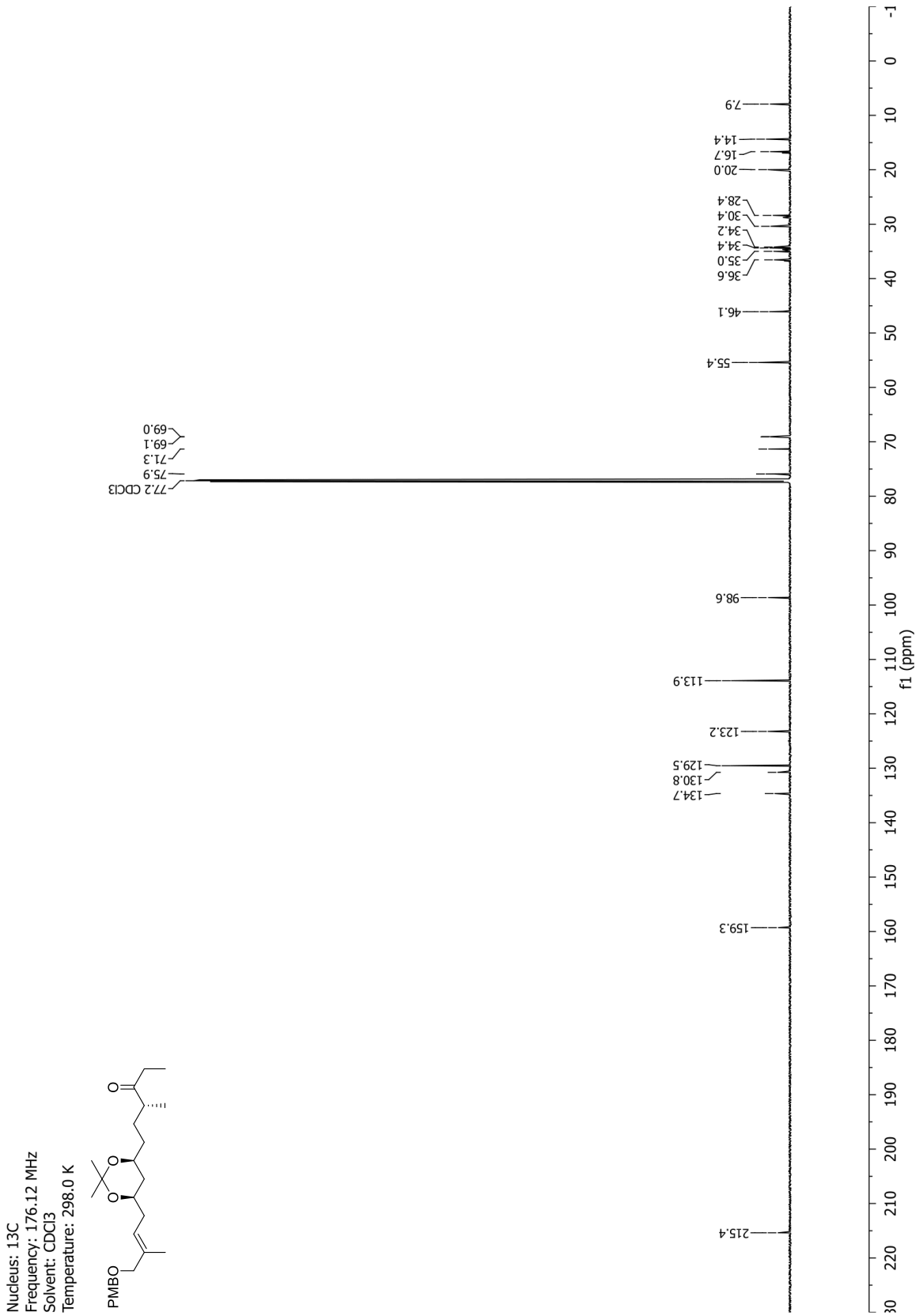
NMR-Spectra for Compound 294



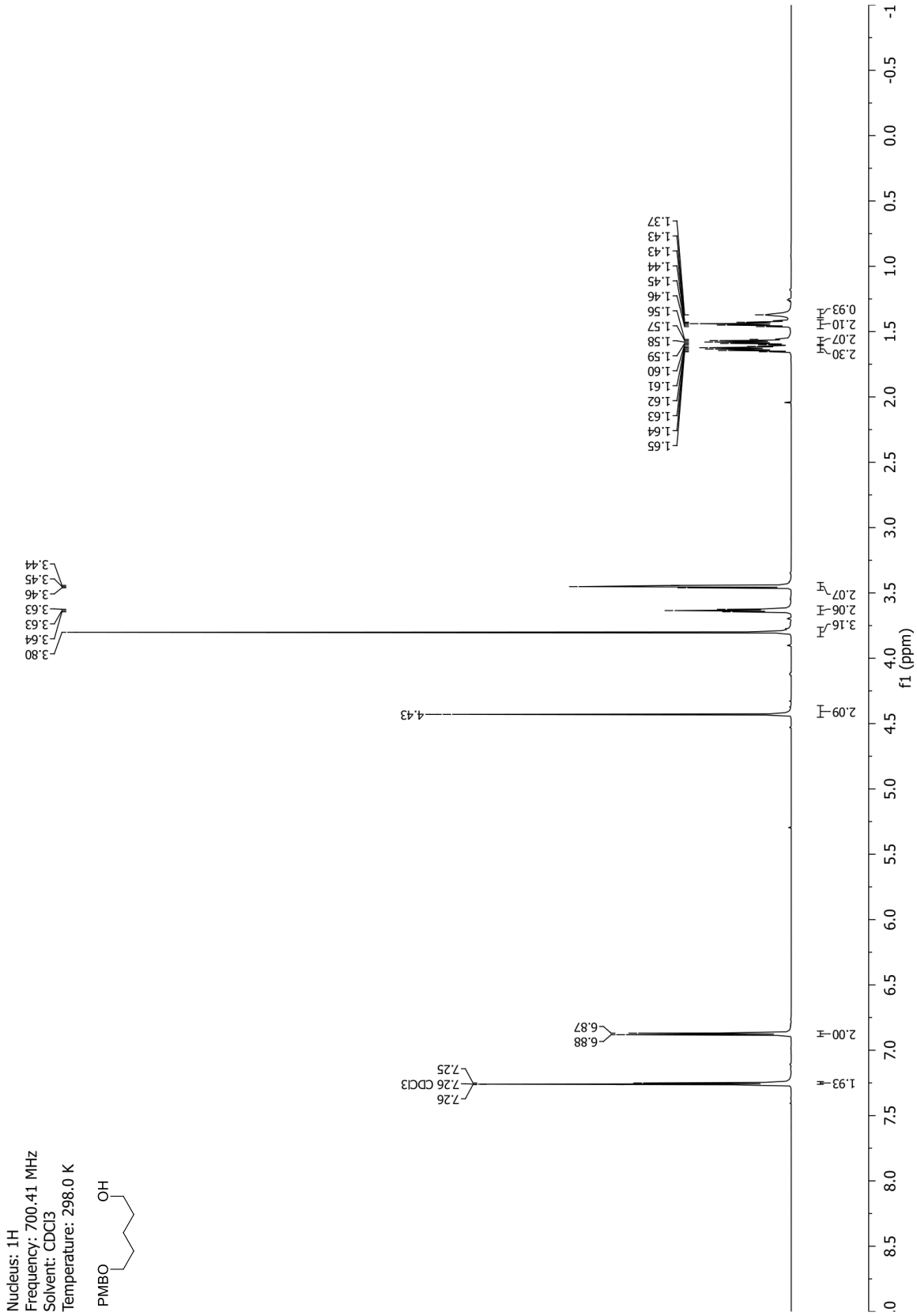

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OH
O
O
10.5
10.7
10.8
13.5
13.7
14.4
15.7
20.0
20.0
26.5
27.2
27.4
28.9
30.4
34.4
35.0
36.8
38.0
38.7
55.4
69.1
69.4
69.7
71.3
75.9
76.4
76.5
77.2 CDCl3
98.6
113.9
123.3
129.5
130.8
134.6
159.3
f1 (ppm)

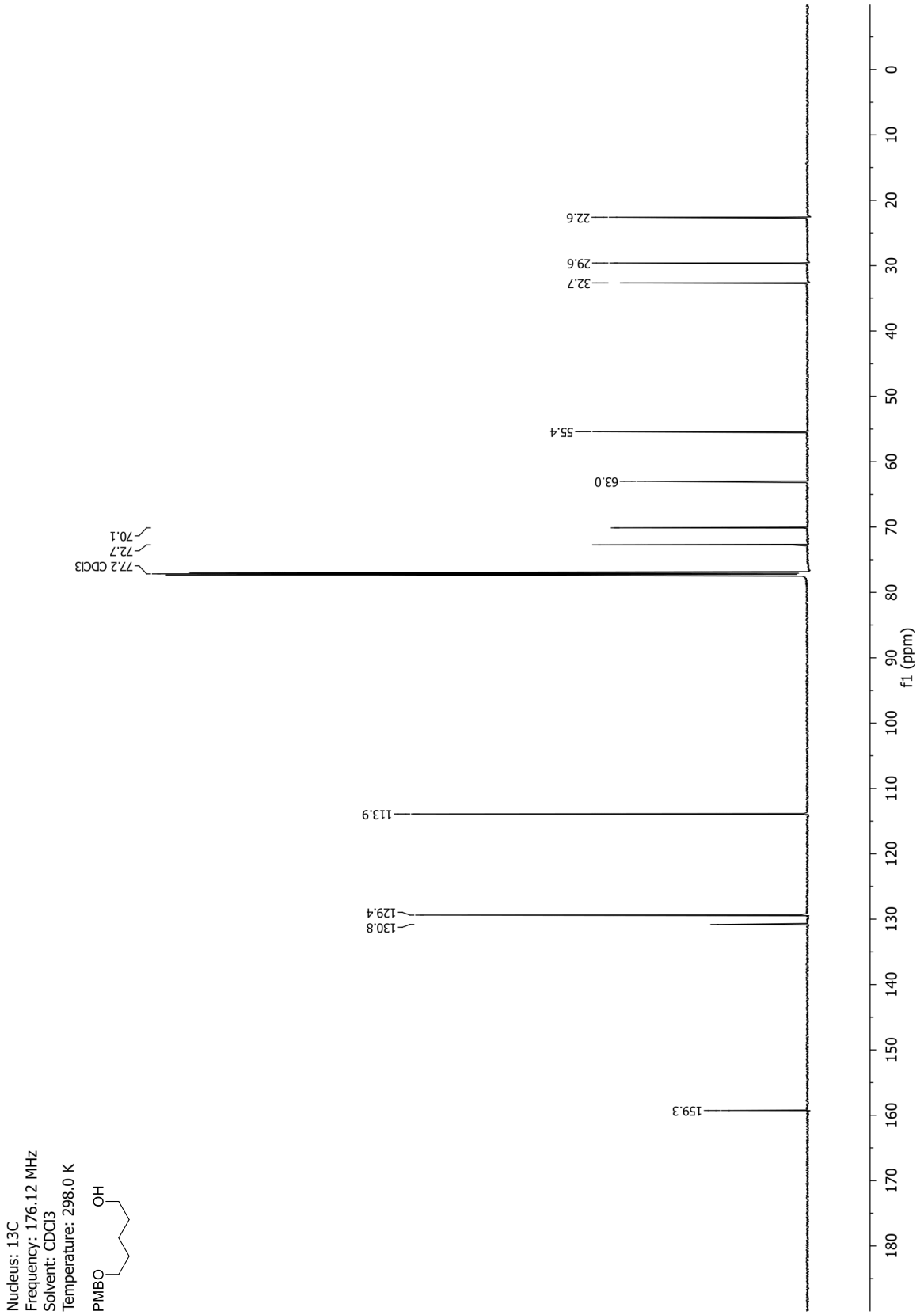
NMR-Spectra for Compound 10



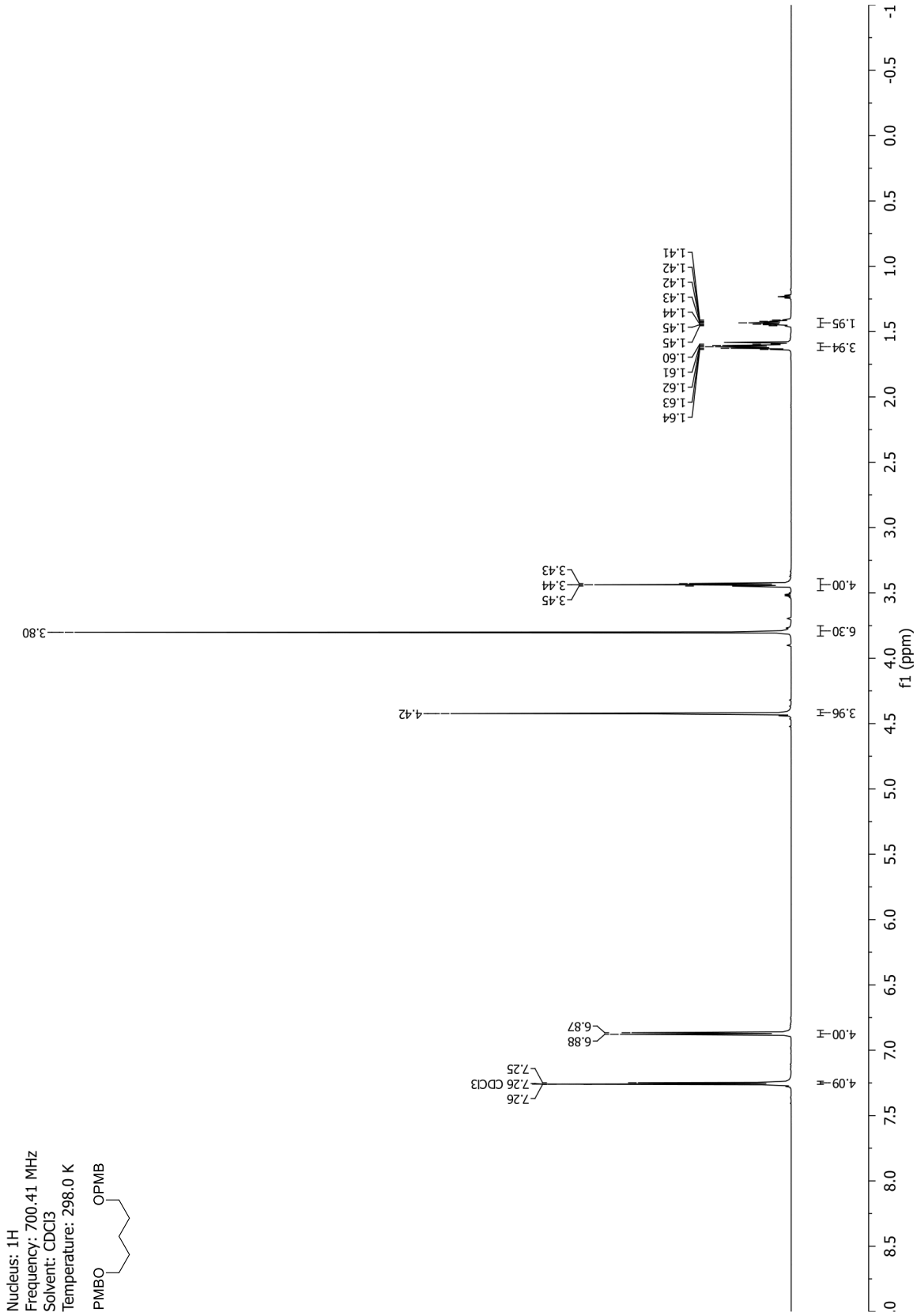

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
O
O
O
7.9
14.4
16.7
20.0
28.4
30.4
34.2
34.4
35.0
36.6
46.1
55.4
69.0
69.1
71.3
75.9
77.2 CDCl3
98.6
113.9
123.2
129.5
130.8
134.7
159.3
215.4
f1 (ppm)

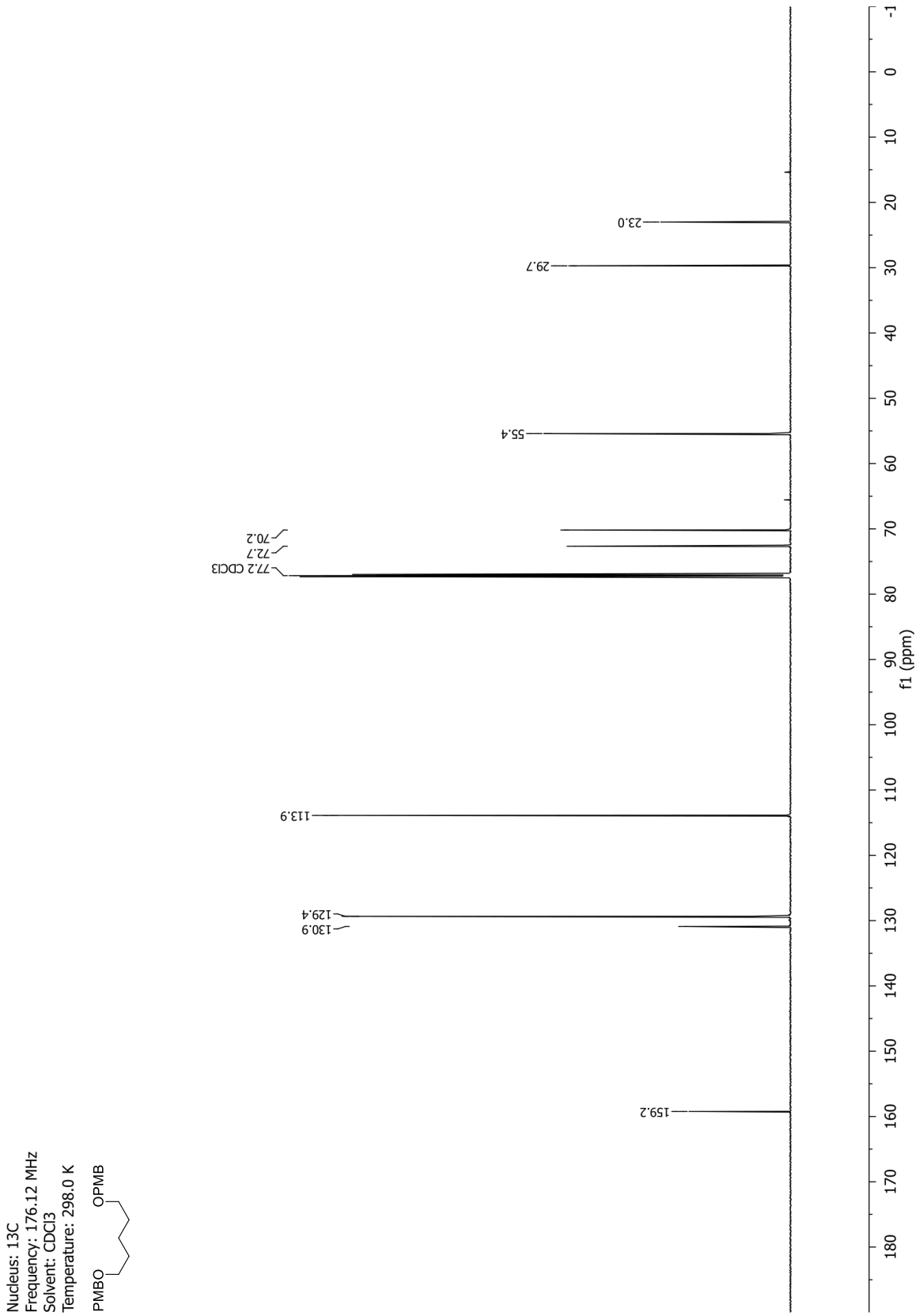
NMR-Spectra for Compound 295



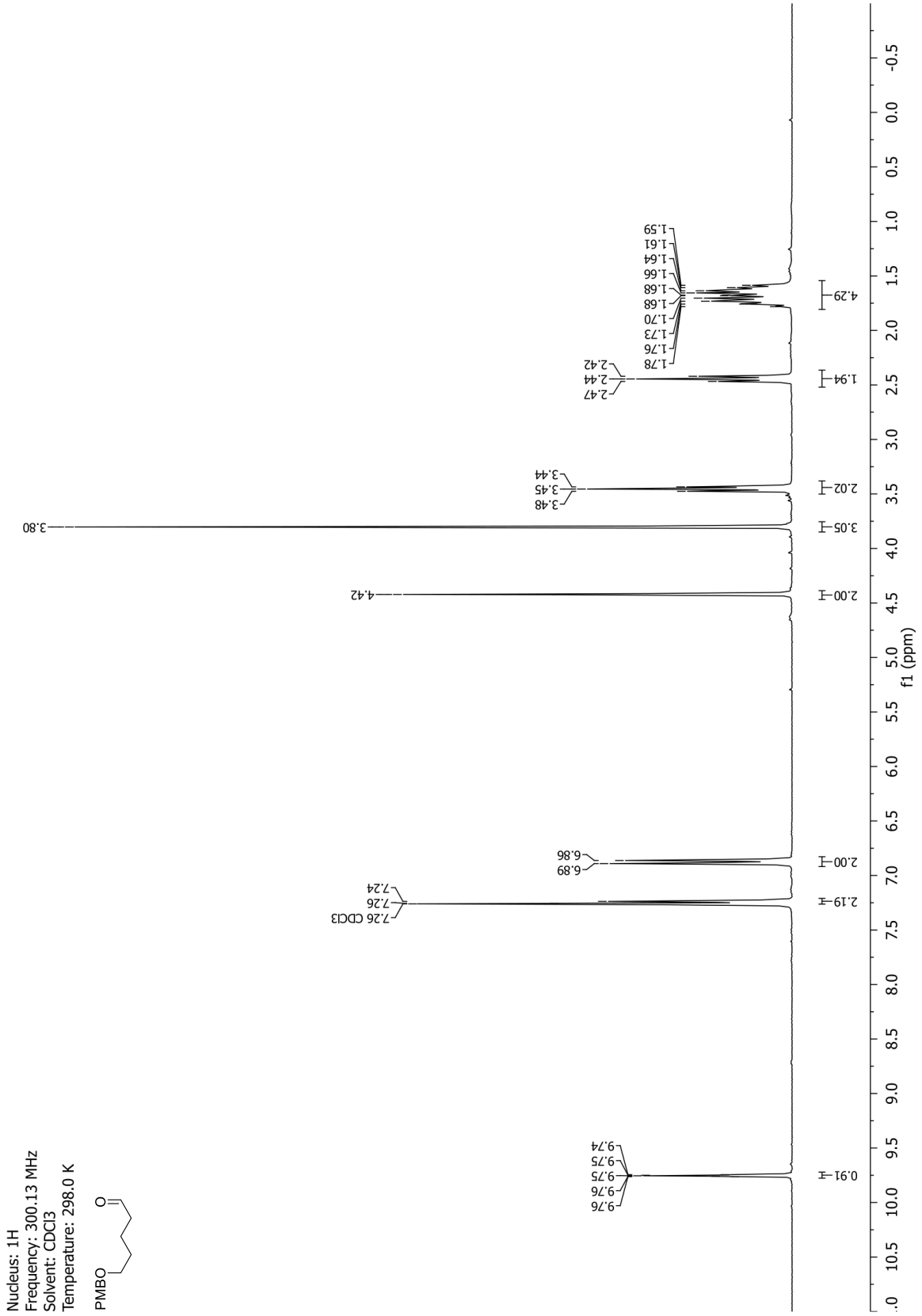

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OH
159.3
130.8
129.4
113.9
77.2 CDCl3
72.7
70.1
63.0
55.4
32.7
29.6
22.6
f1 (ppm)

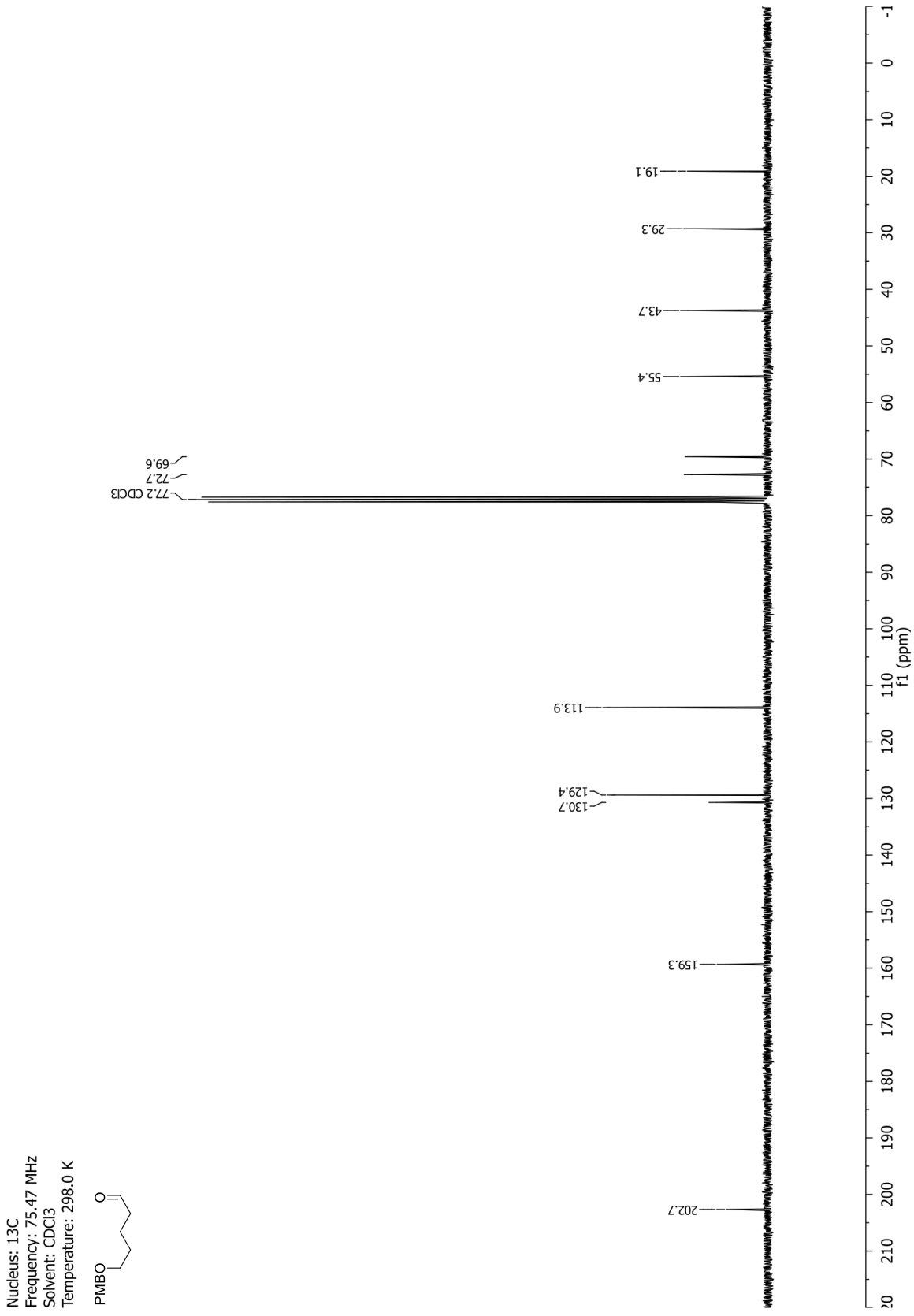
NMR-Spectra for Compound 295b



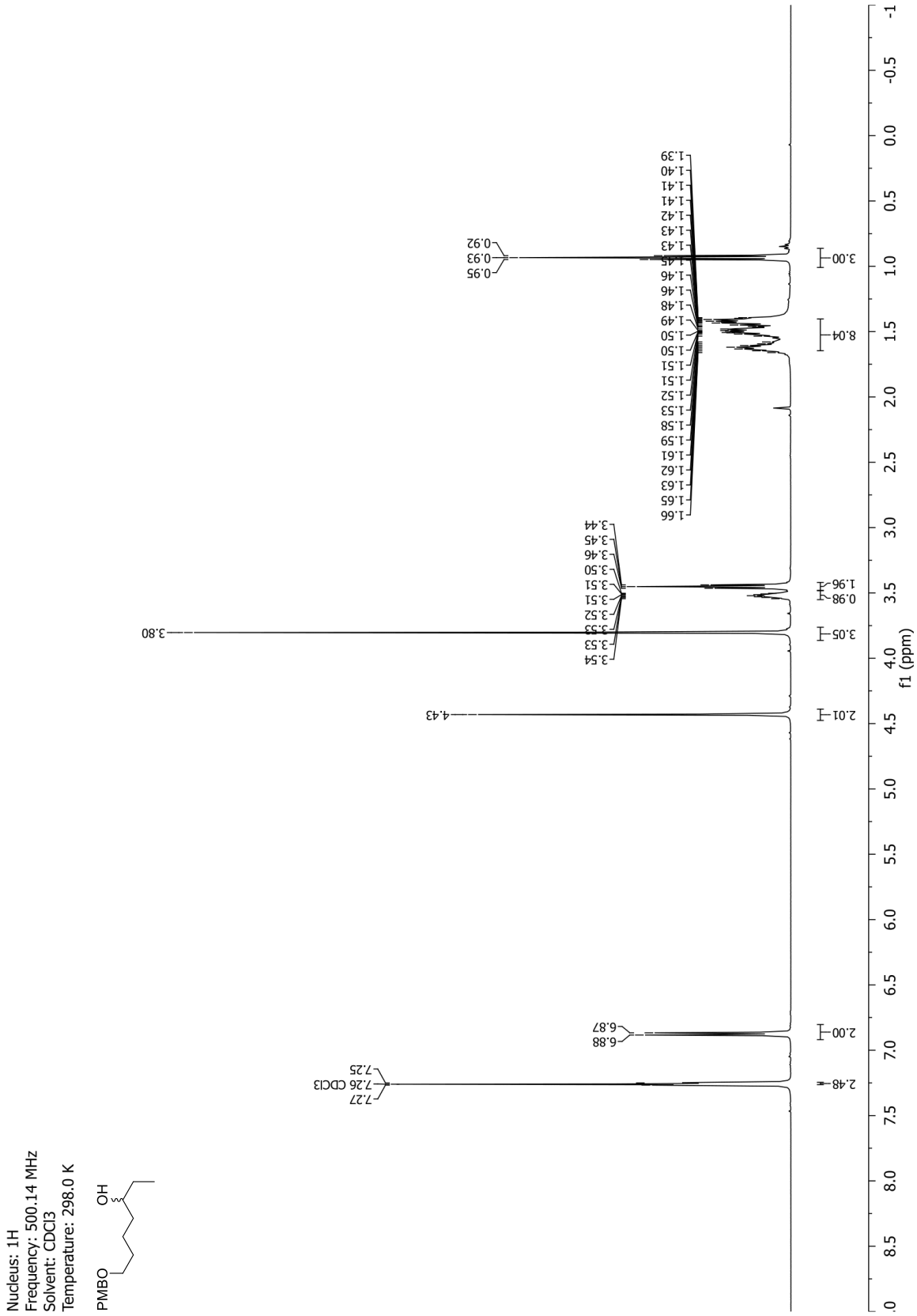

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OPMB
159.2
130.9
129.4
113.9
77.2 CDCl3
72.7
70.2
55.4
29.7
23.0
f1 (ppm)

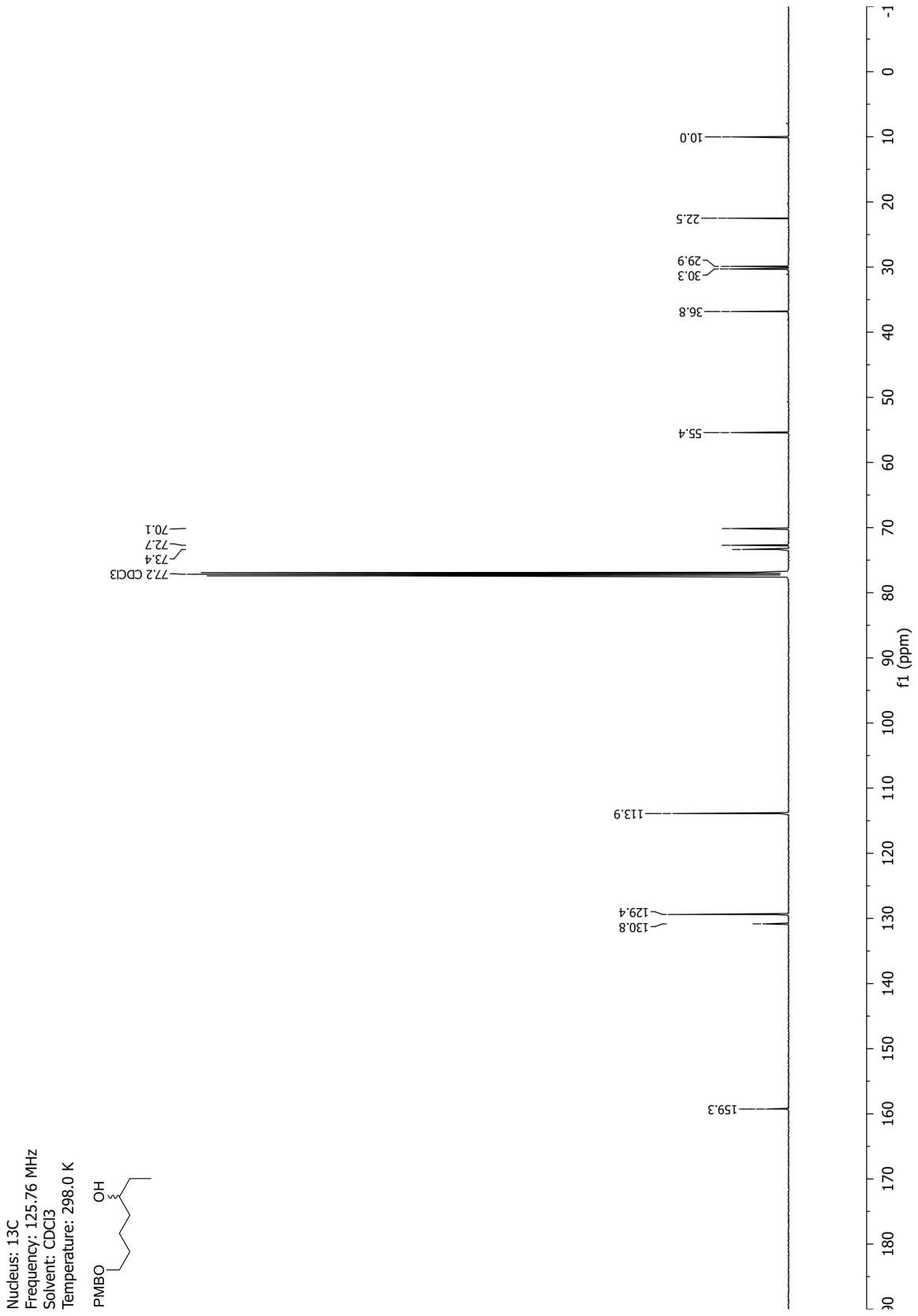
NMR-Spectra for Compound 215



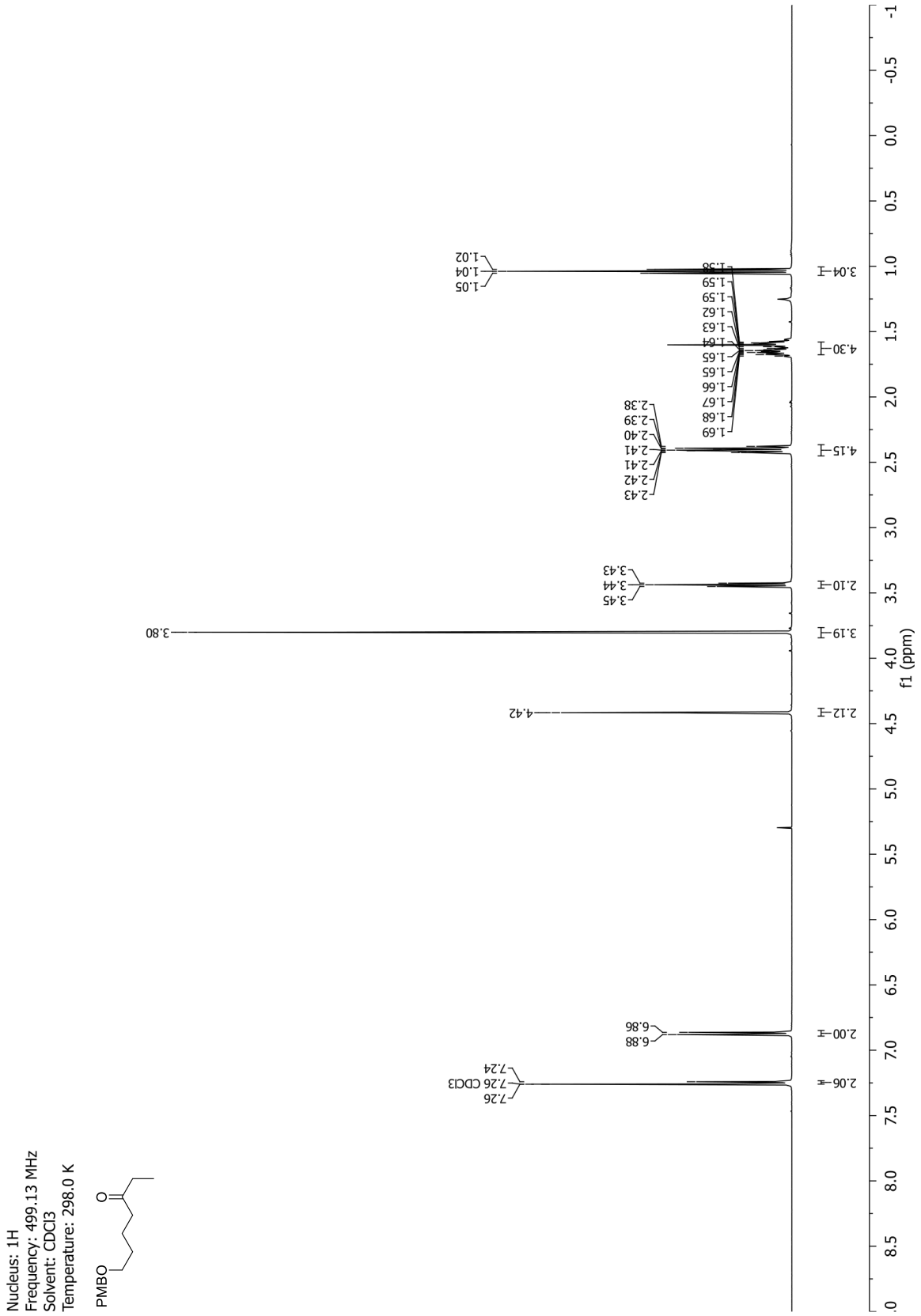

Nucleus: 13C
Frequency: 75.47 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
O
202.7
159.3
130.7
129.4
113.9
77.2 CDCl3
72.7
69.6
55.4
43.7
29.3
19.1
f1 (ppm)

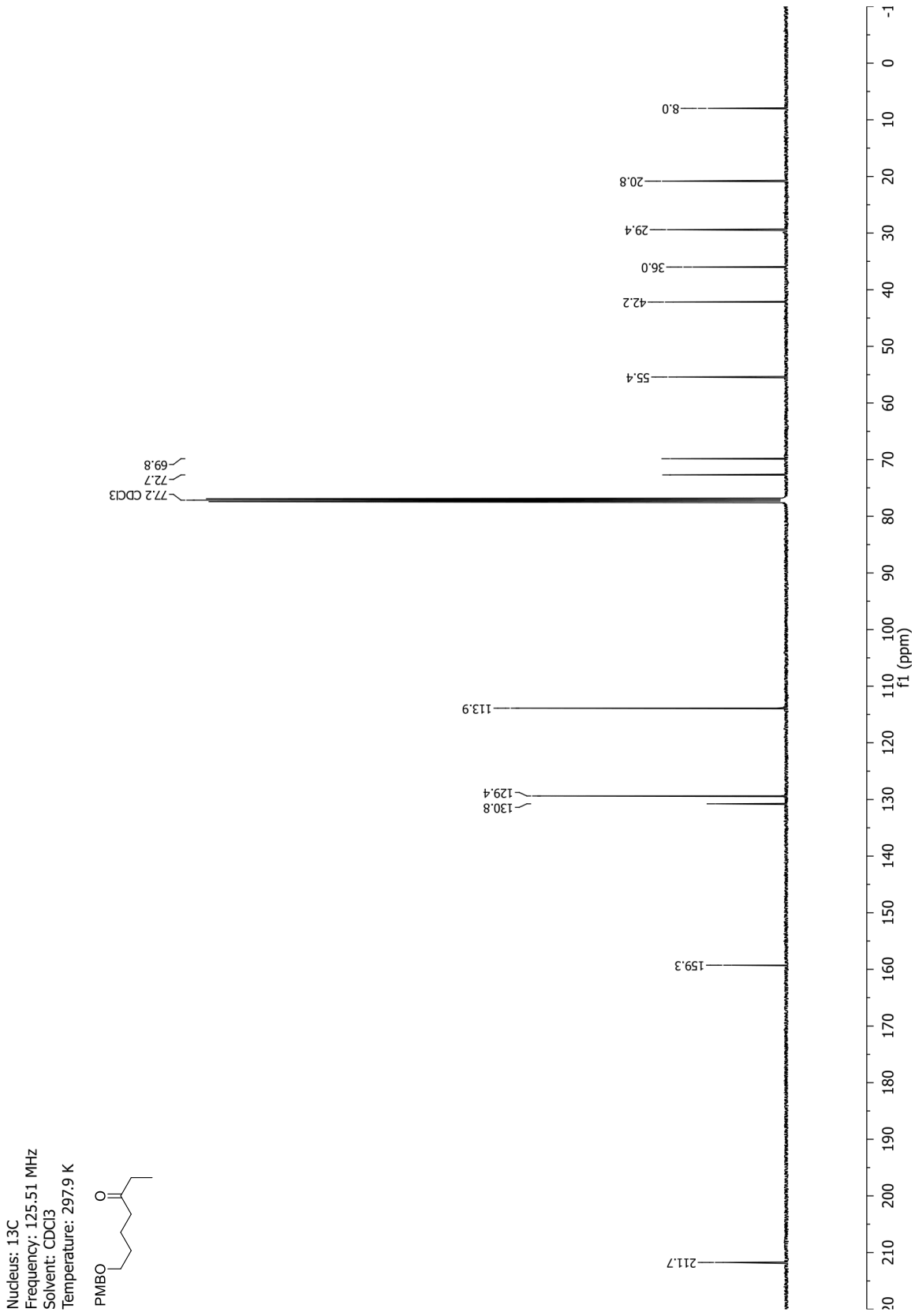
NMR-Spectra for Compound 296



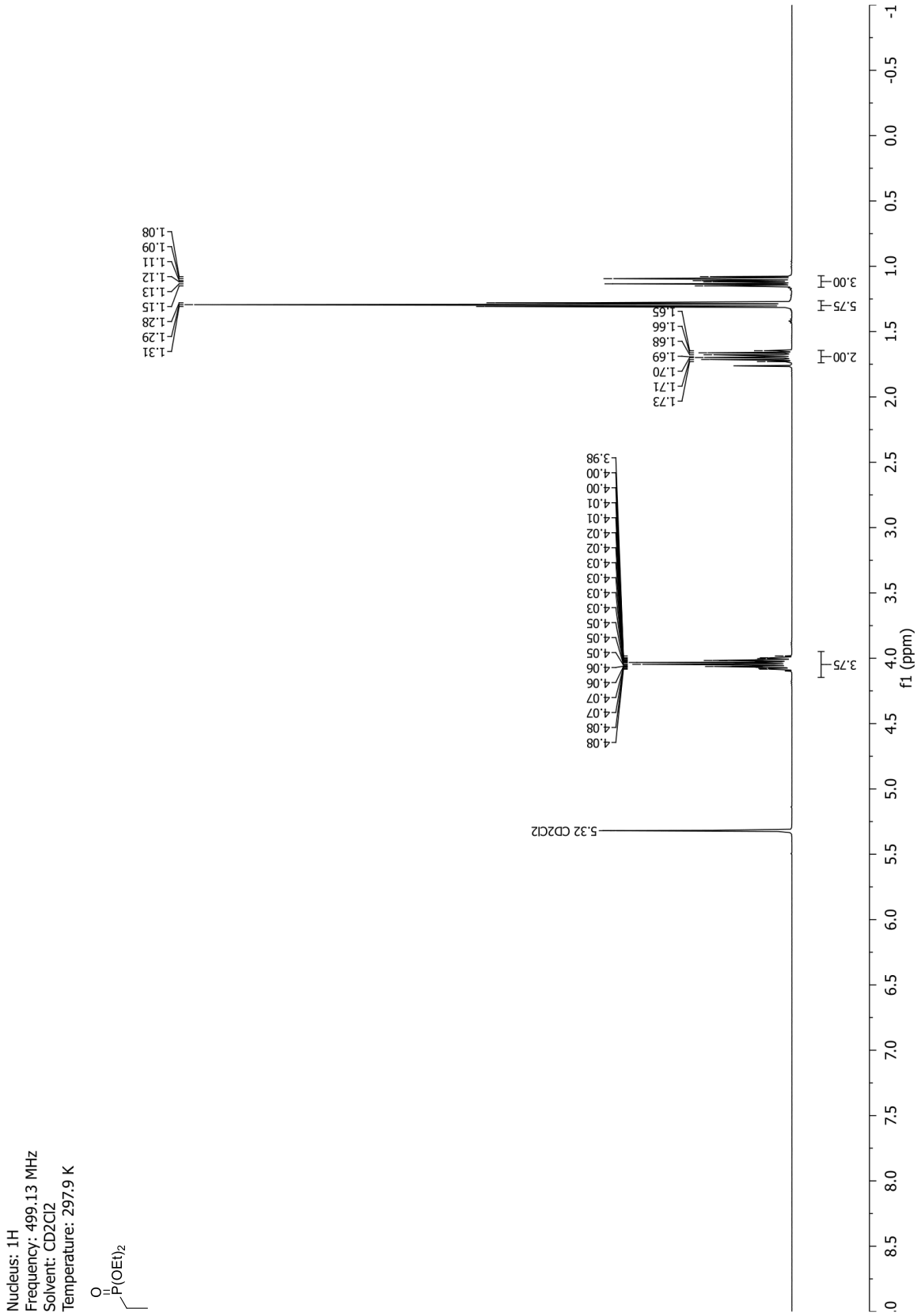

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OH
10.0
22.5
29.9
30.3
36.8
55.4
70.1
72.7
73.4
77.2 CDCl3
113.9
129.4
130.8
159.3
f1 (ppm)

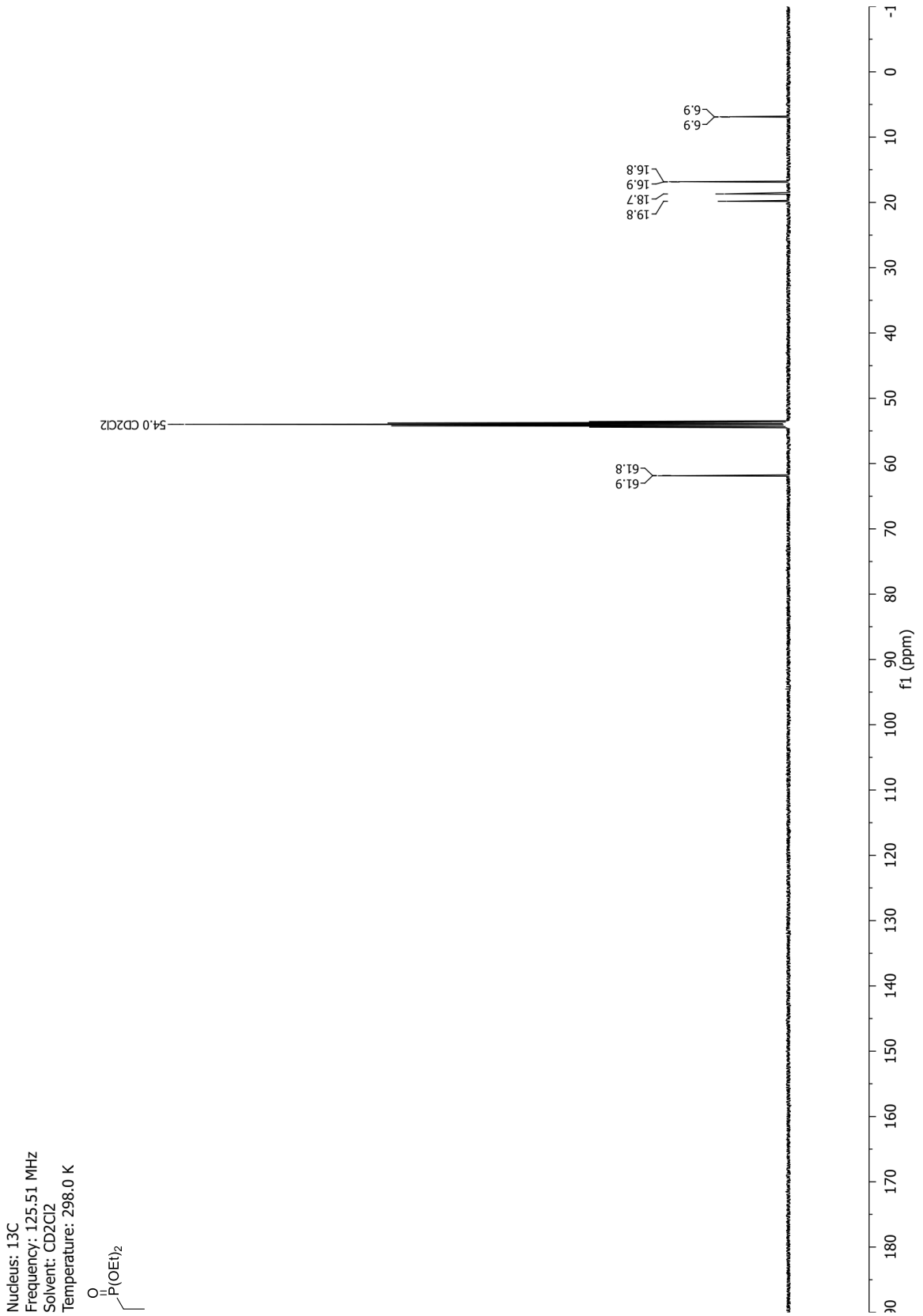
NMR-Spectra for Compound 11

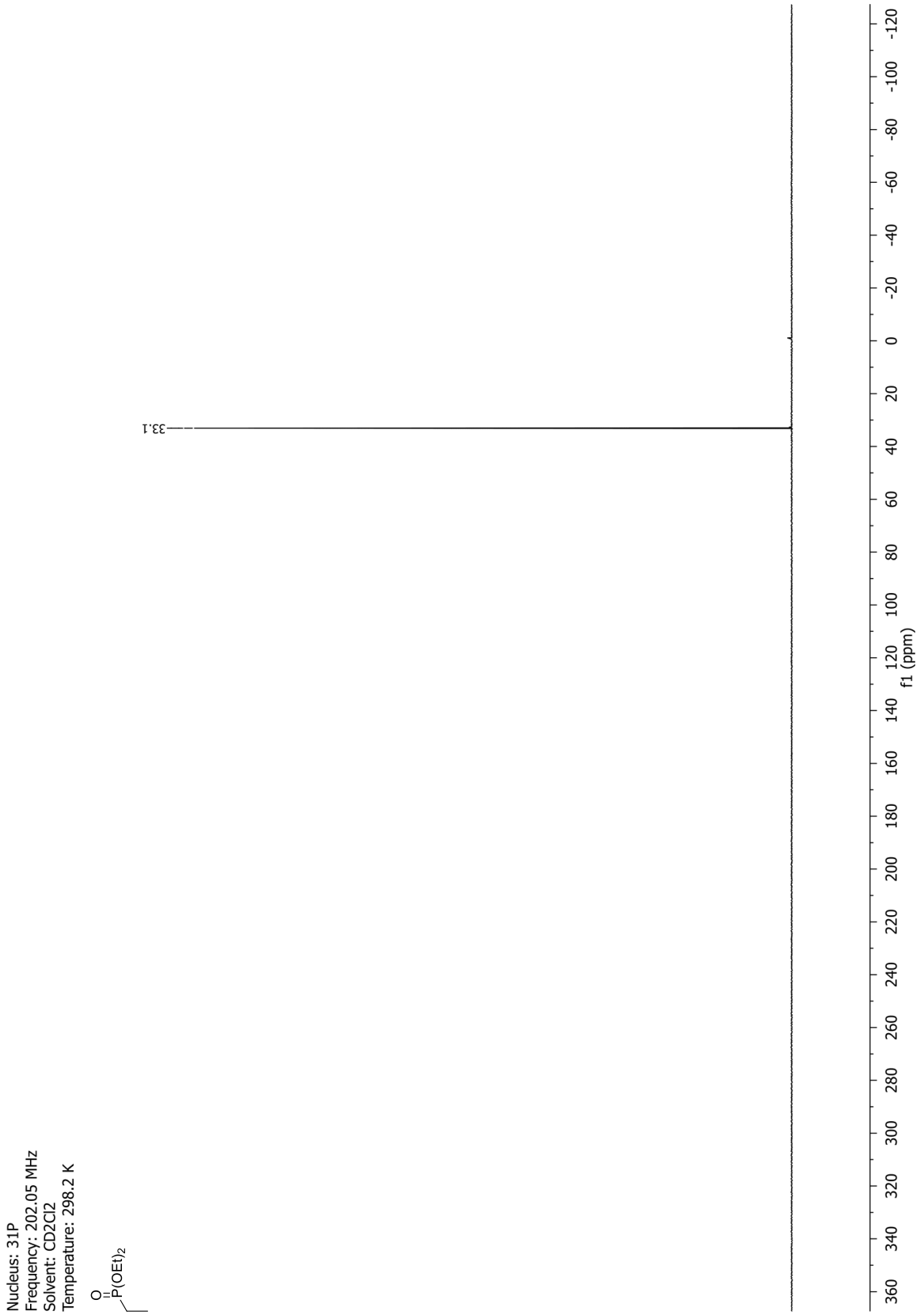



Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 297.9 K
PMBO
O
211.7
159.3
130.8
129.4
113.9
77.2 CDCl3
72.7
69.8
55.4
42.2
36.0
29.4
20.8
8.0
f1 (ppm)

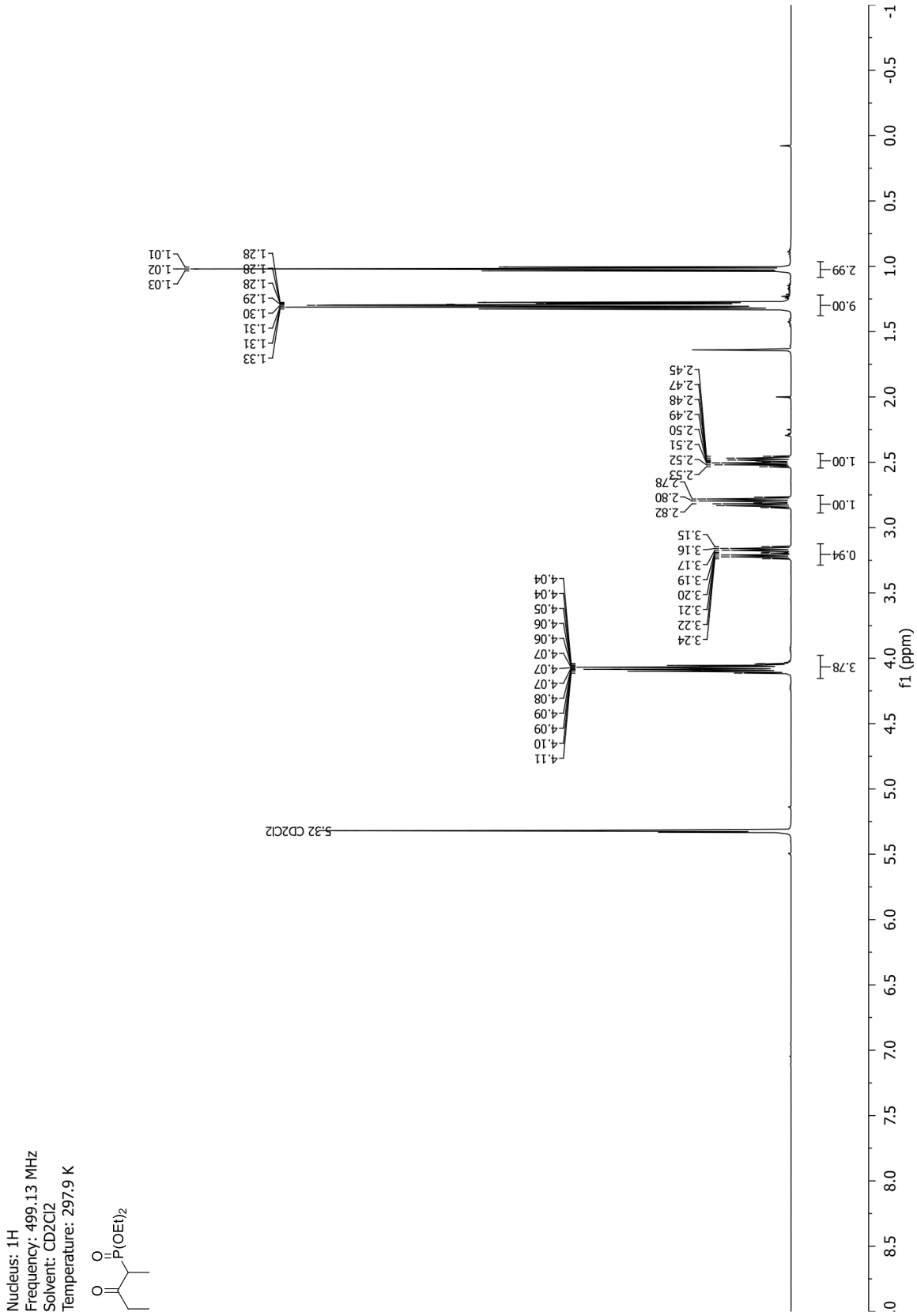
NMR-Spectra for Compound 196

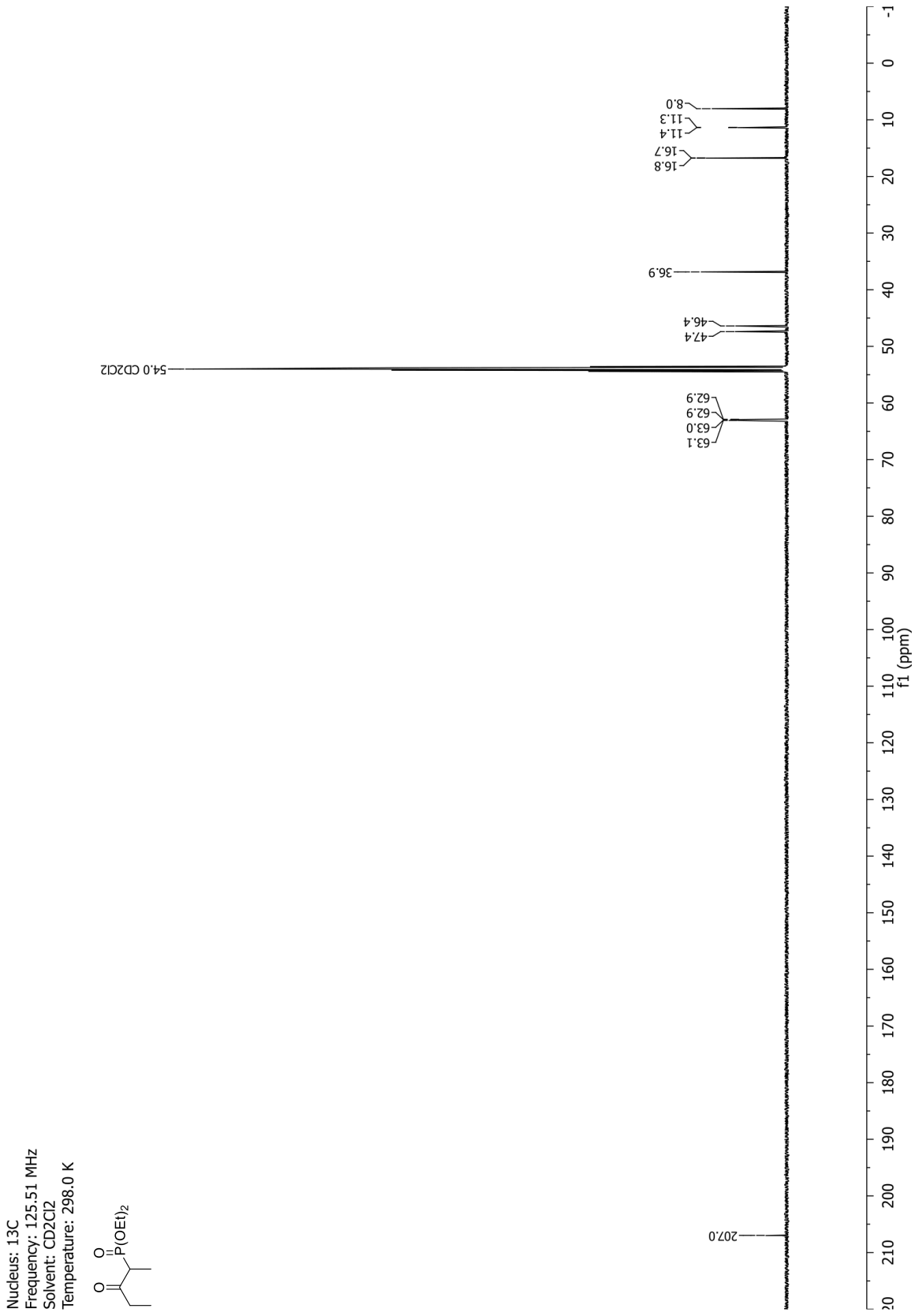


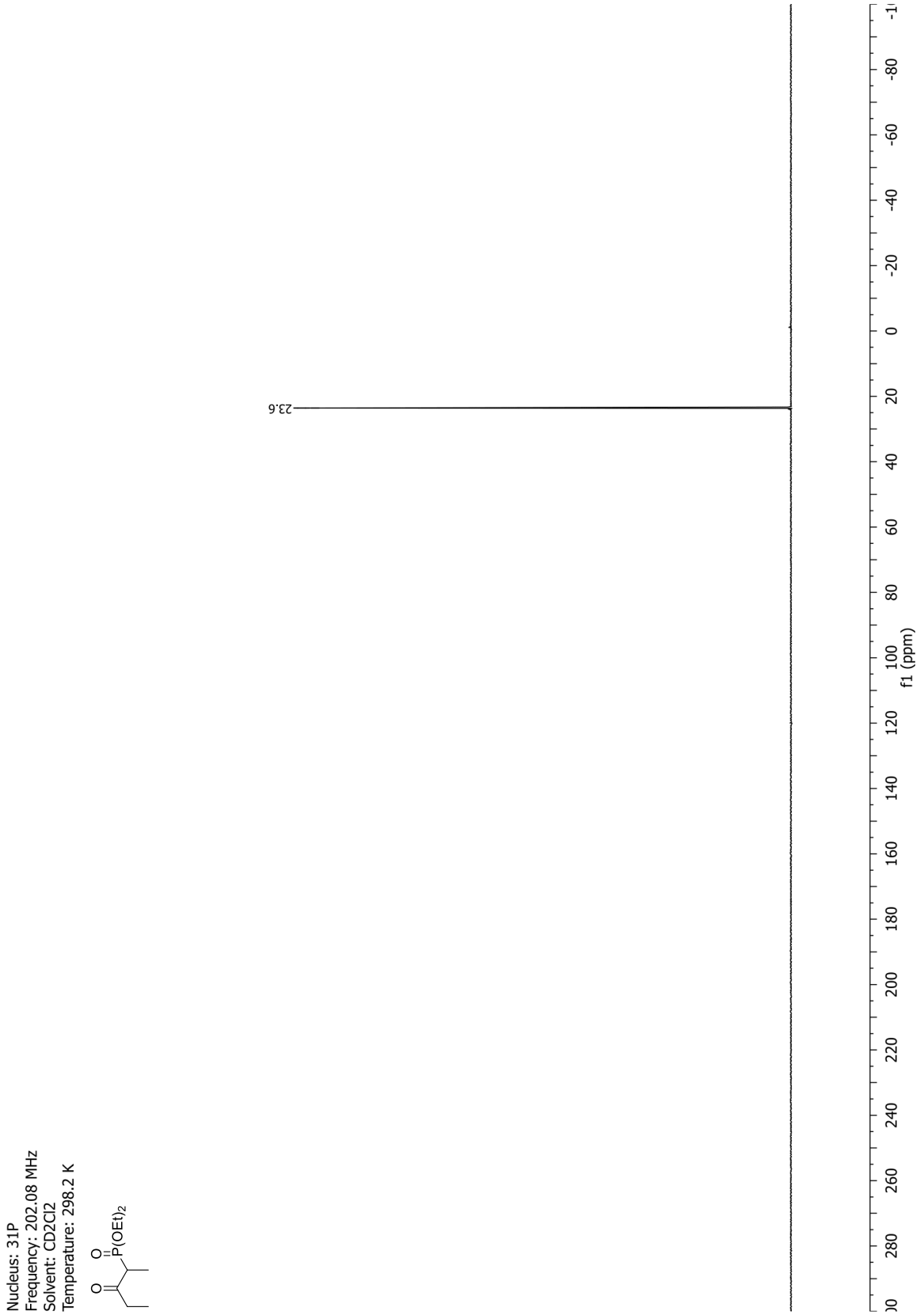

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
O=P(OEt)2
6.9
6.9
16.8
16.9
18.7
19.8
54.0 CD2Cl2
61.8
61.9
f1 (ppm)


Nucleus: 31P
Frequency: 202.05 MHz
Solvent: CD2Cl2
Temperature: 298.2 K
O
P(OEt)2
33.1
360 340 320 300 280 260 240 220 200 180 160 140 120 100 80 60 40 20 0 -20 -40 -60 -80 -100 -120
f1 (ppm)

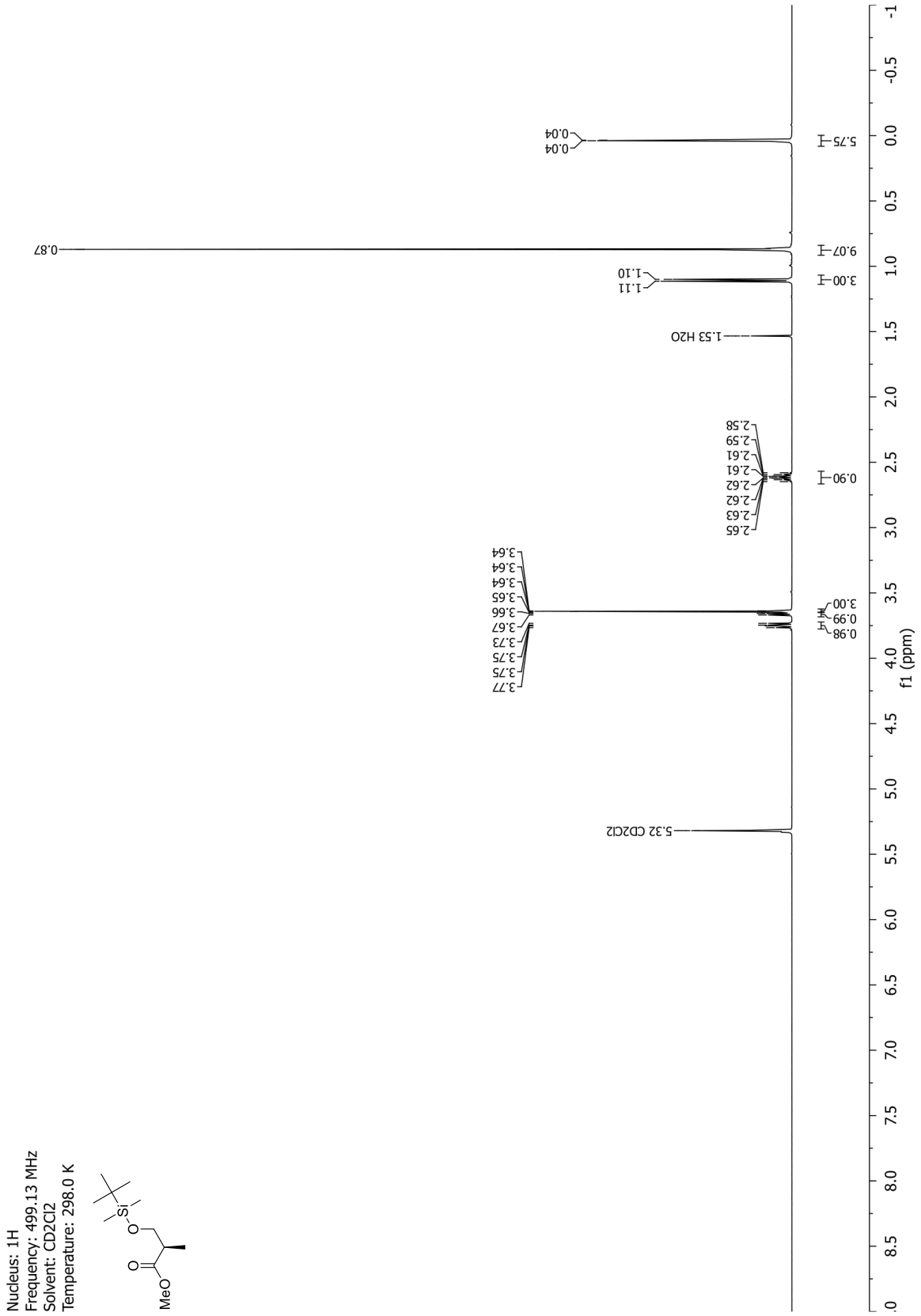
NMR-Spectra for Compound 192

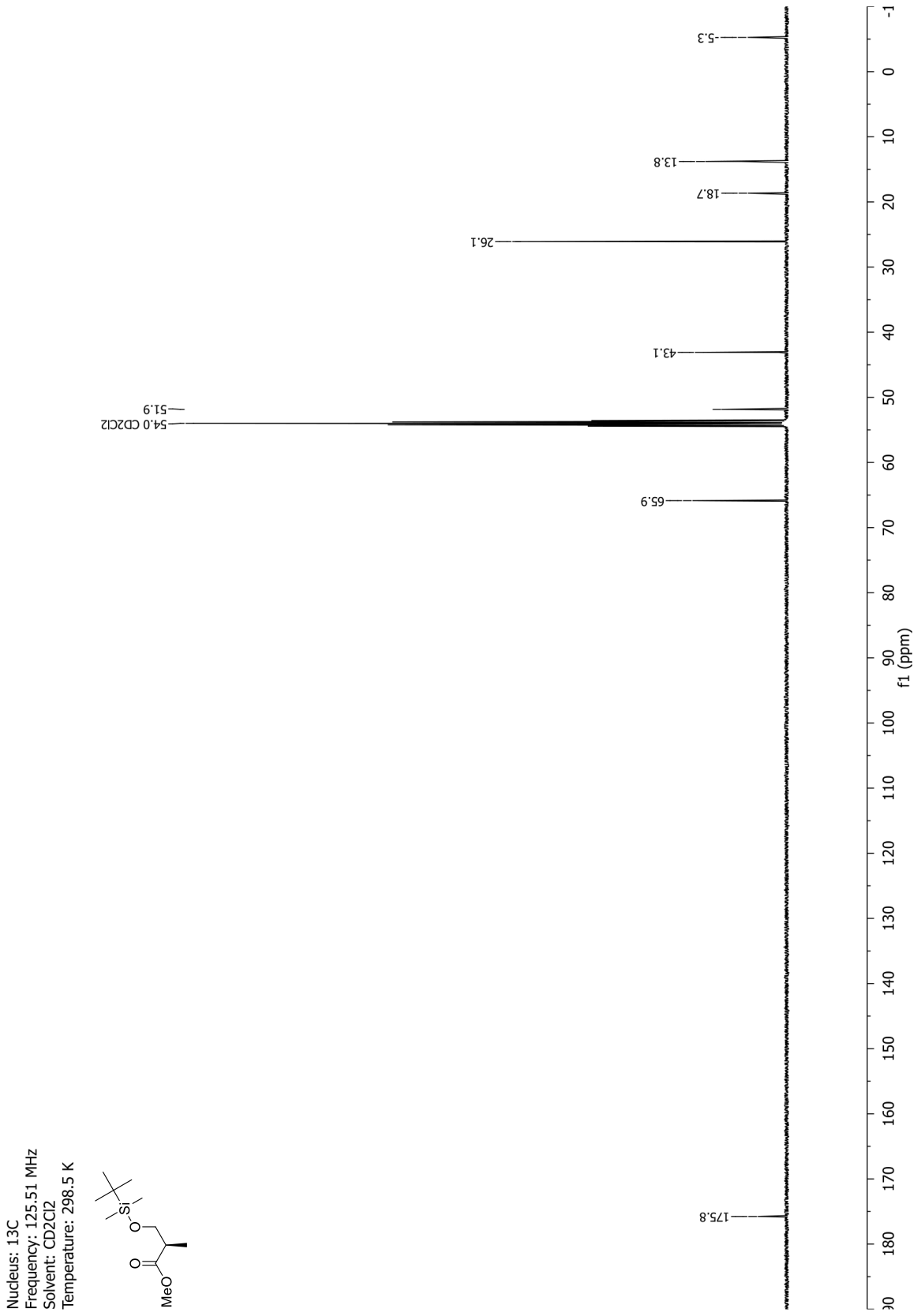



Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
O
O
P(OEt)2
207.0
63.1
62.9
62.9
63.0
54.0 CD2Cl2
47.4
46.4
36.9
16.8
16.7
11.4
11.3
8.0
f1 (ppm)

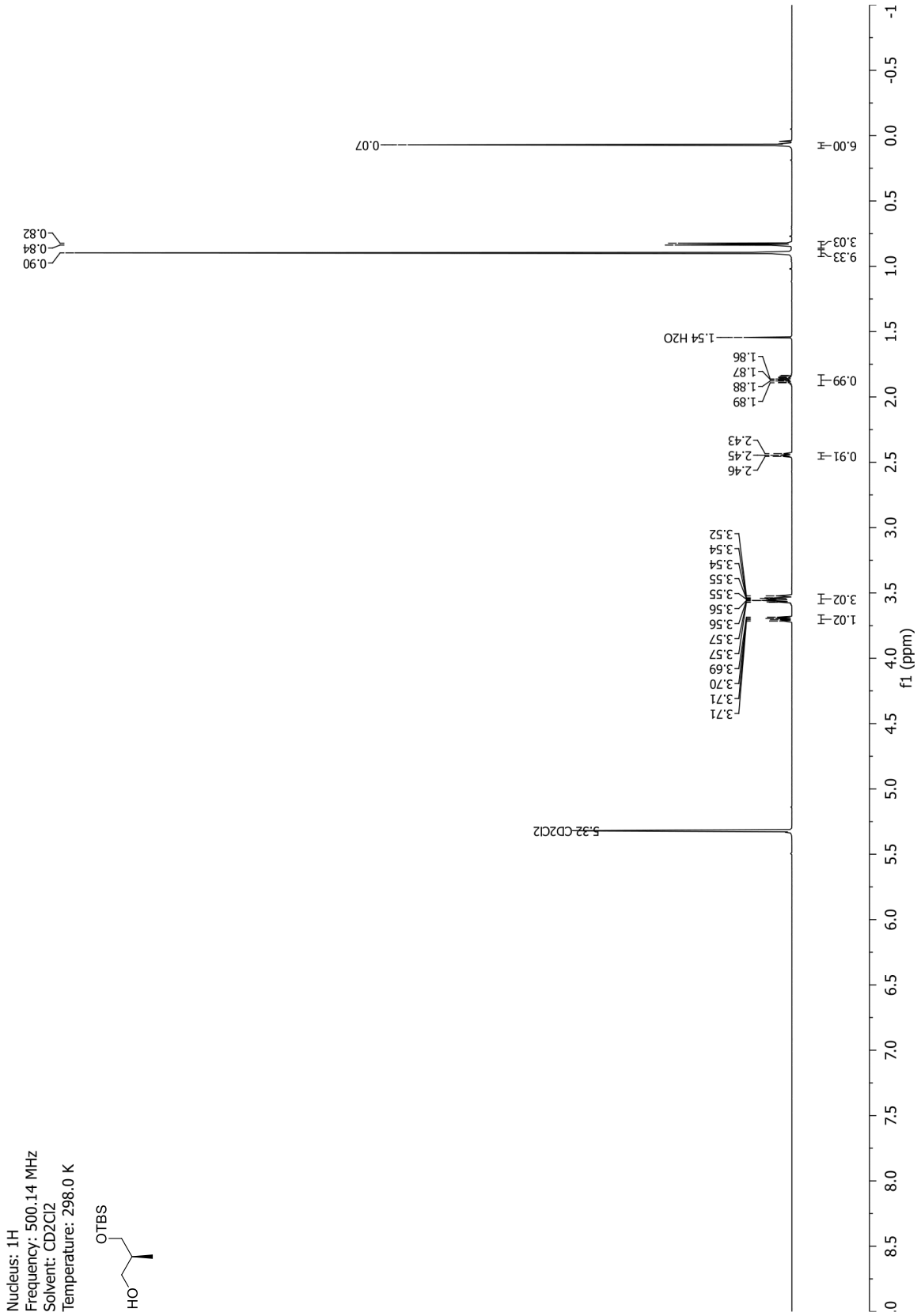

Nucleus: 31P
Frequency: 202.08 MHz
Solvent: CD2Cl2
Temperature: 298.2 K
O
O
P(OEt)2
23.6
f1 (ppm)

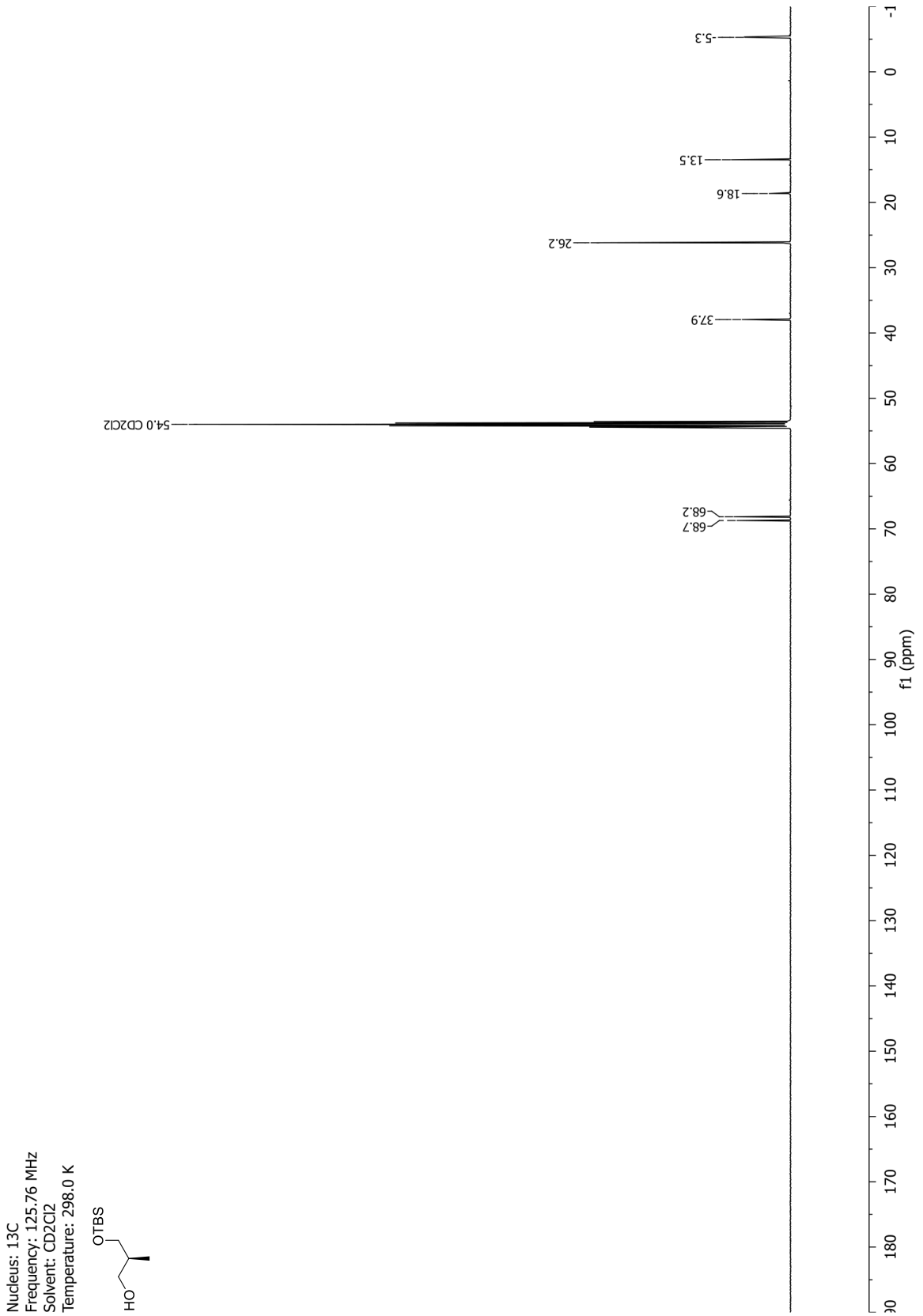
NMR-Spectra for Compound 297



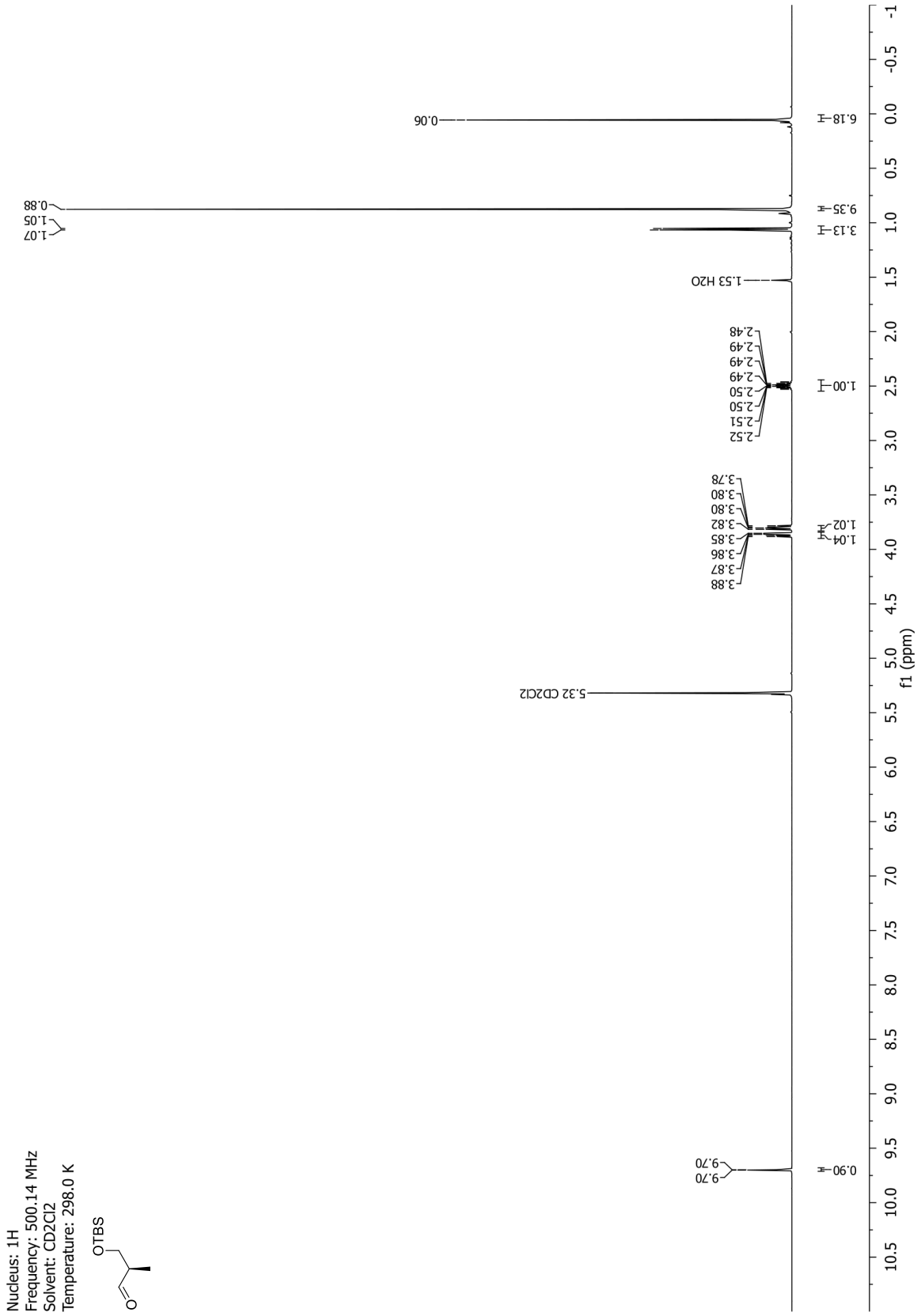

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CD2Cl2
Temperature: 298.5 K
Si
O
O
MeO
175.8
65.9
54.0 CD2Cl2
51.9
43.1
26.1
18.7
13.8
-5.3
f1 (ppm)

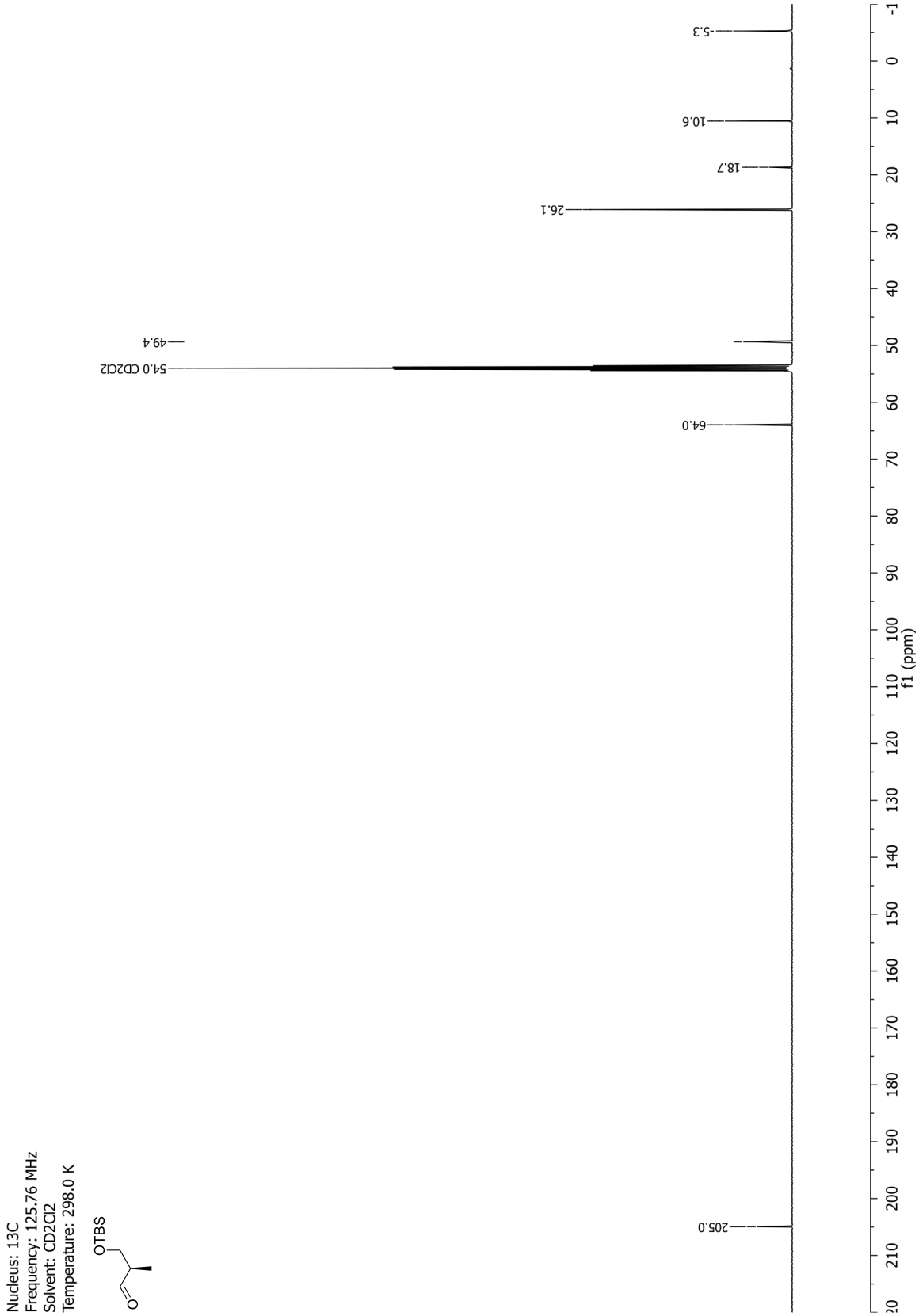
NMR-Spectra for Compound 298



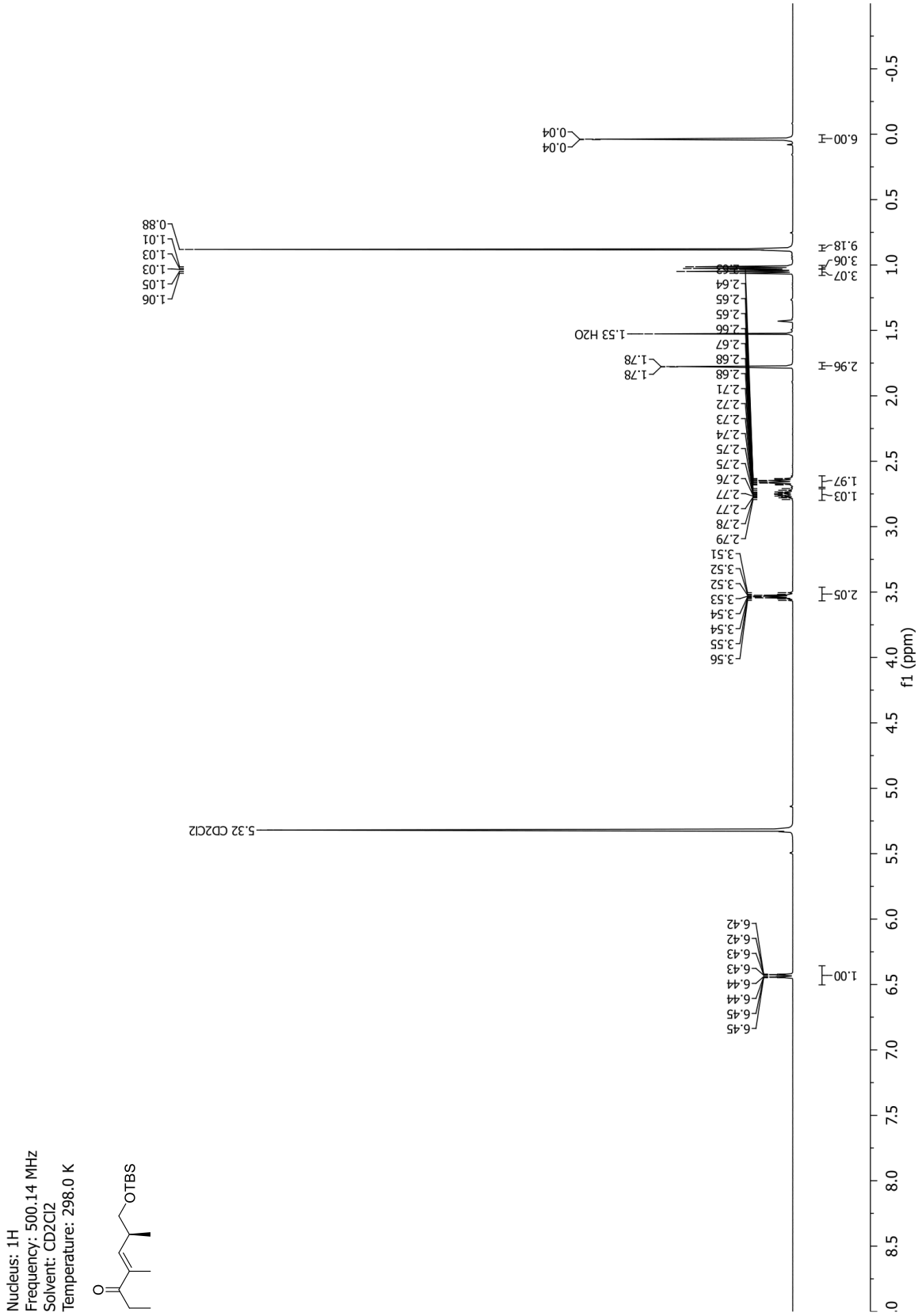

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
OTBS
HO
-5.3
13.5
18.6
26.2
37.9
54.0 CD2Cl2
68.2
68.7
f1 (ppm)

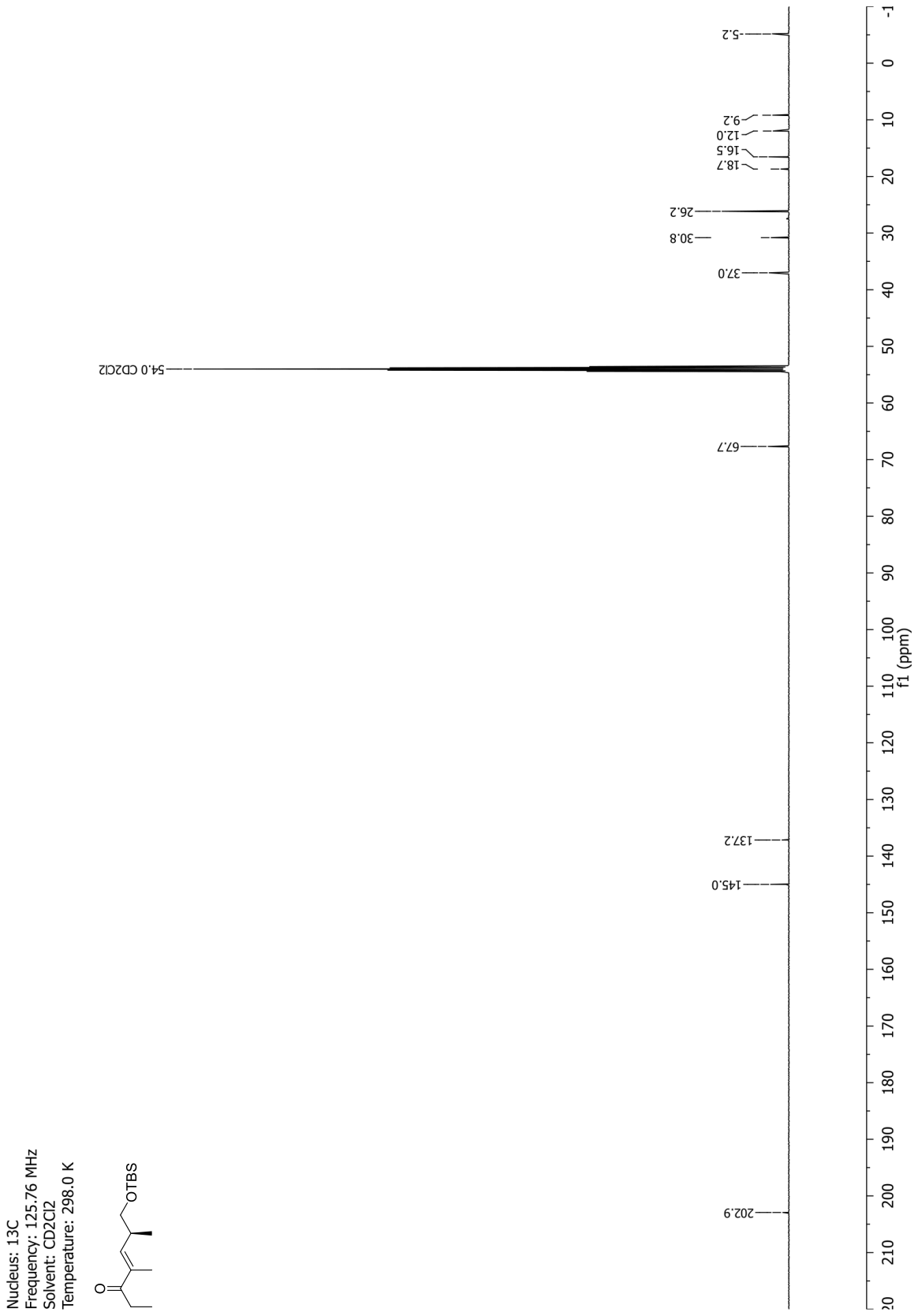
NMR-Spectra for Compound 193



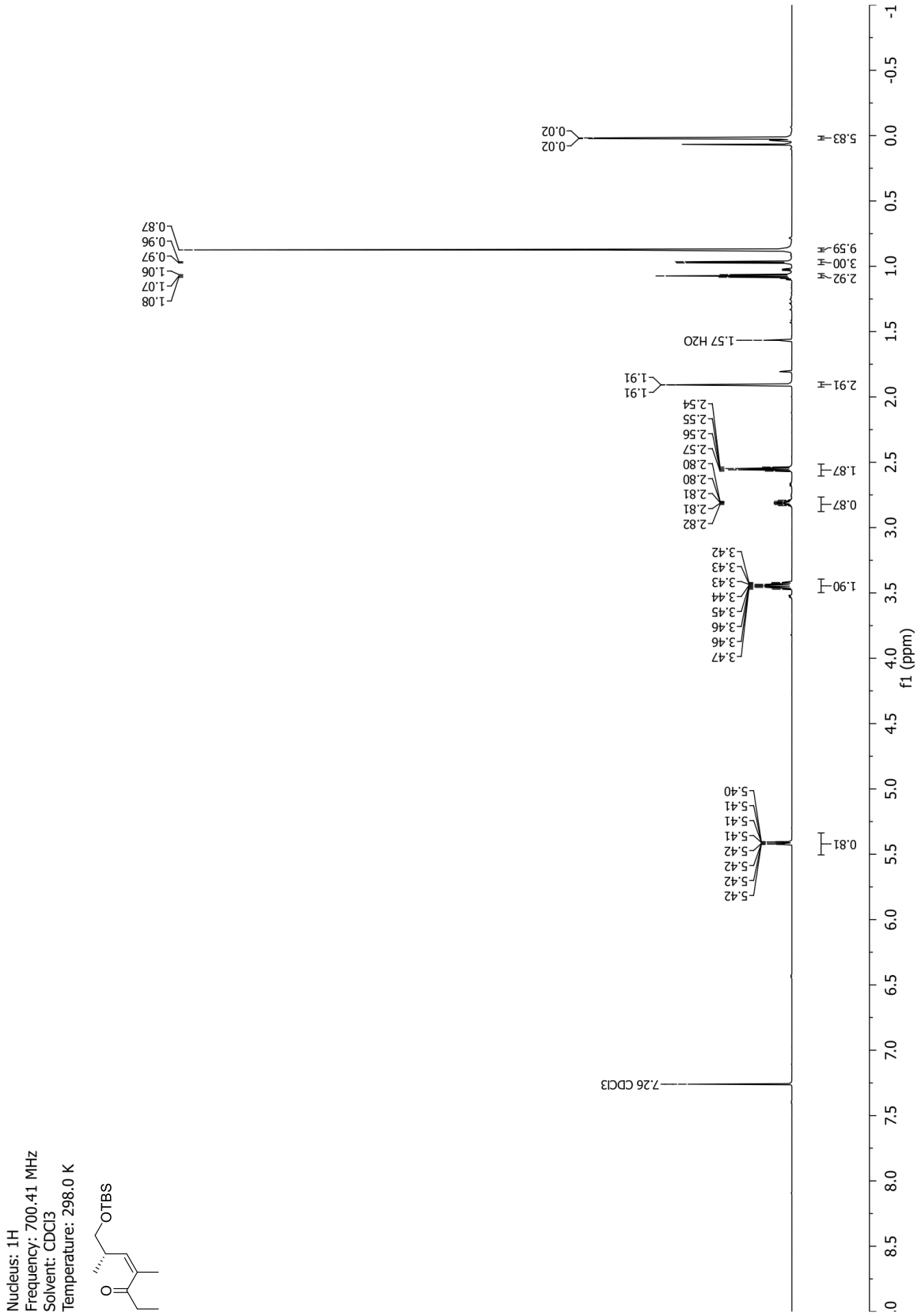

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
OTBS
O
205.0
64.0
54.0 CD2Cl2
49.4
26.1
18.7
10.6
-5.3
f1 (ppm)

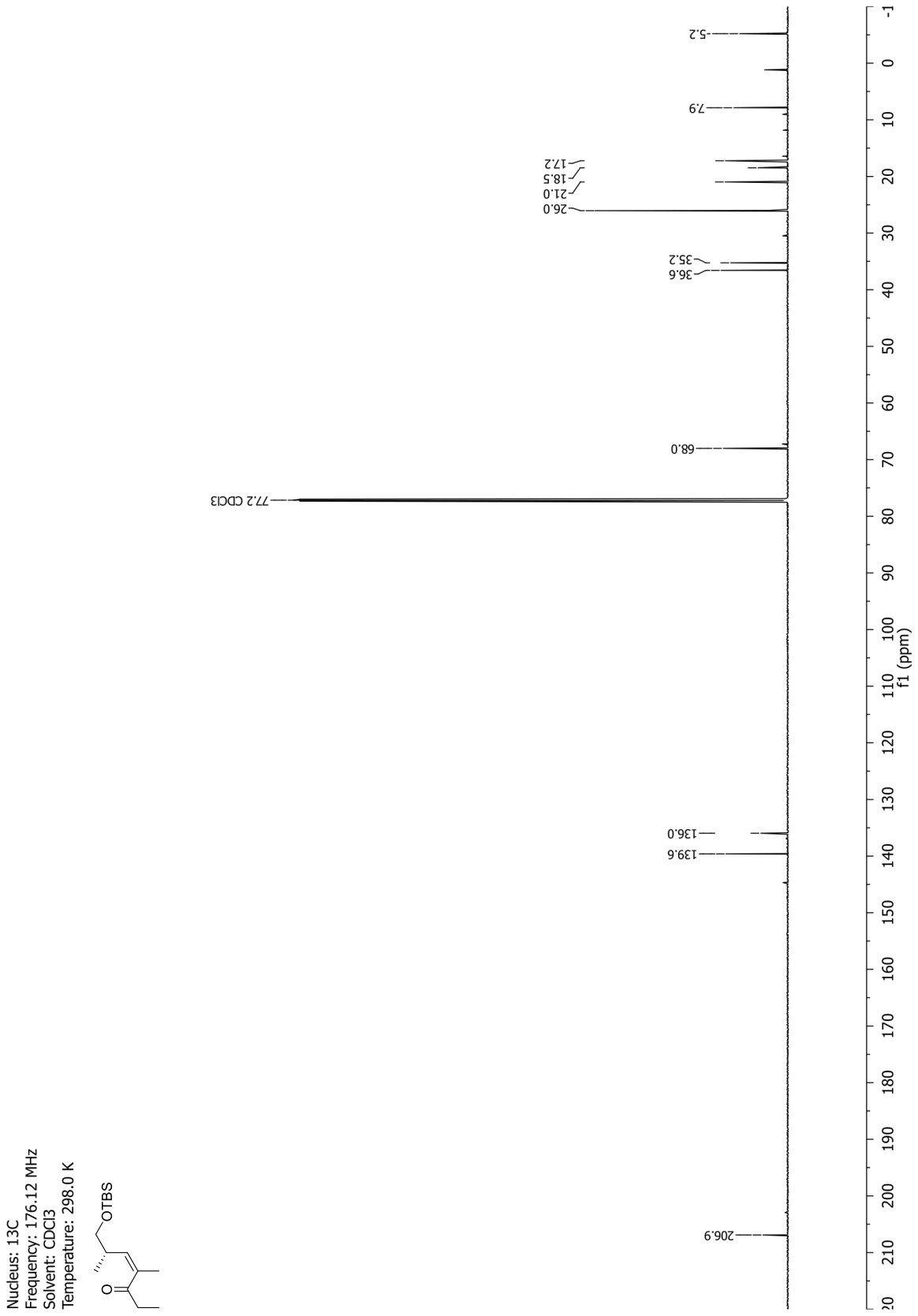
NMR-Spectra for Compound 172*E*



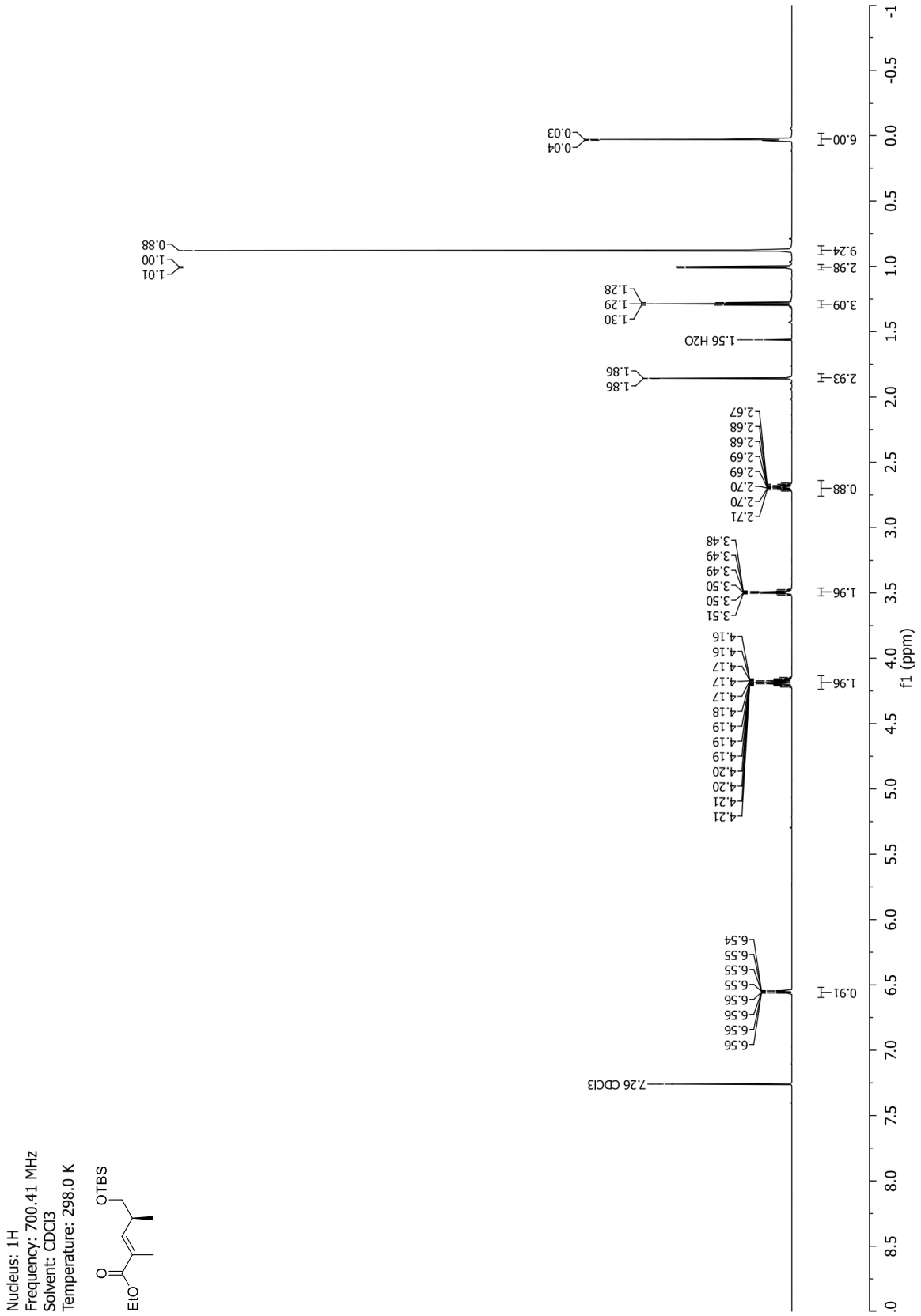

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
OTBS
O
-5.2
9.2
12.0
16.5
18.7
26.2
30.8
37.0
54.0 CD2Cl2
67.7
137.2
145.0
202.9
f1 (ppm)

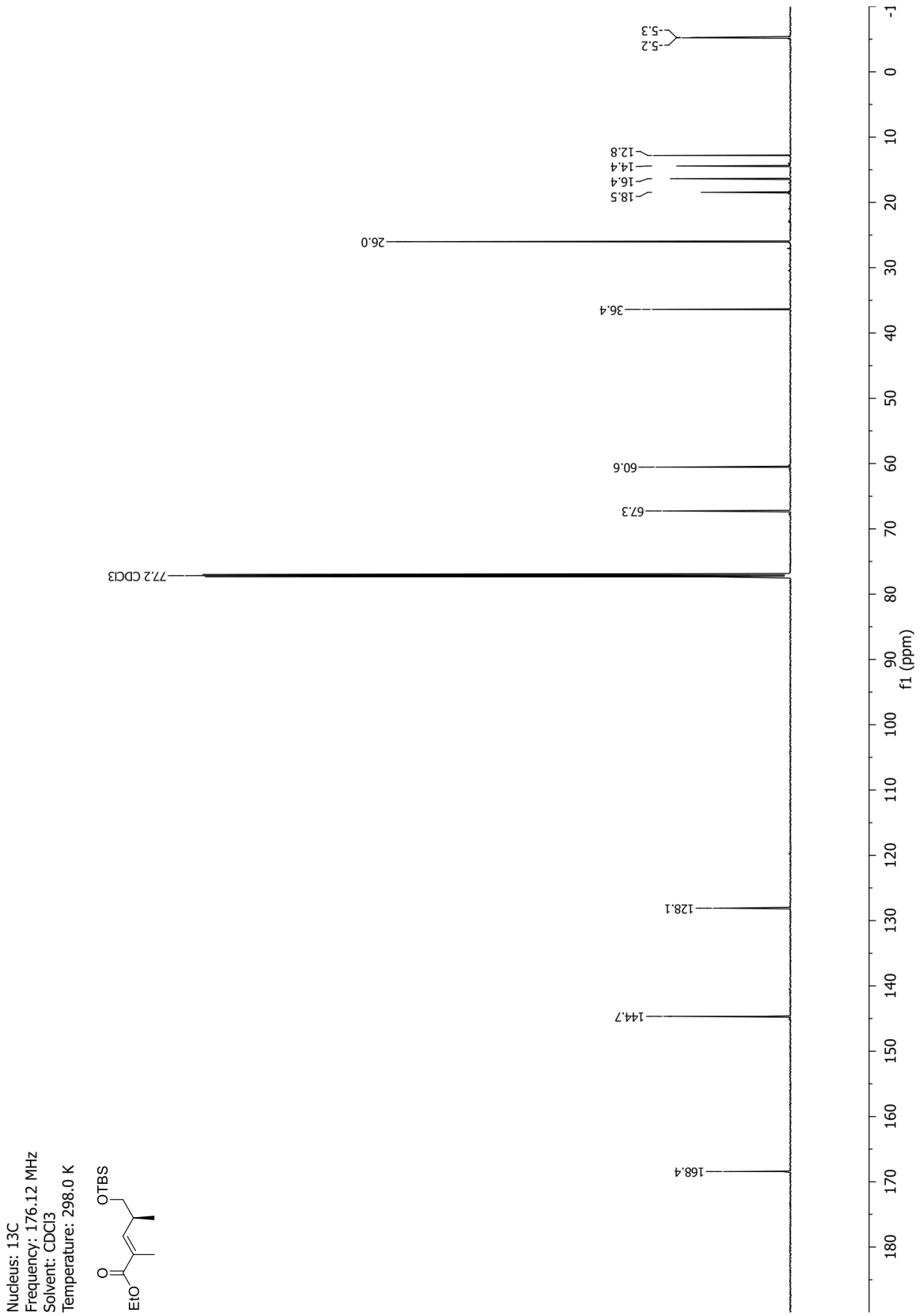
NMR-Spectra for Compound 172*Z*



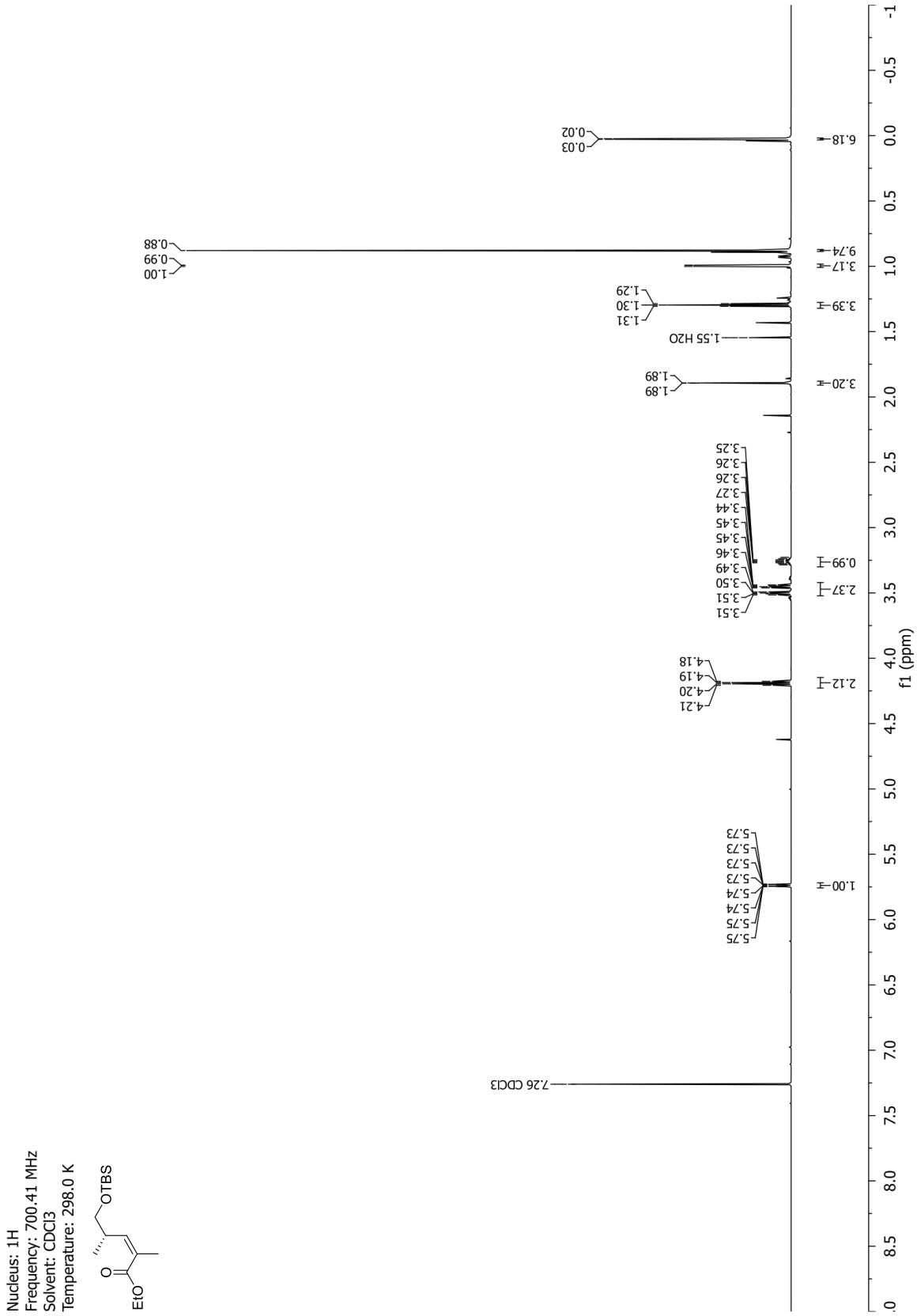

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
206.9
139.6
136.0
77.2 CDCl3
68.0
36.6
35.2
26.0
21.0
18.5
17.2
7.9
-5.2
f1 (ppm)

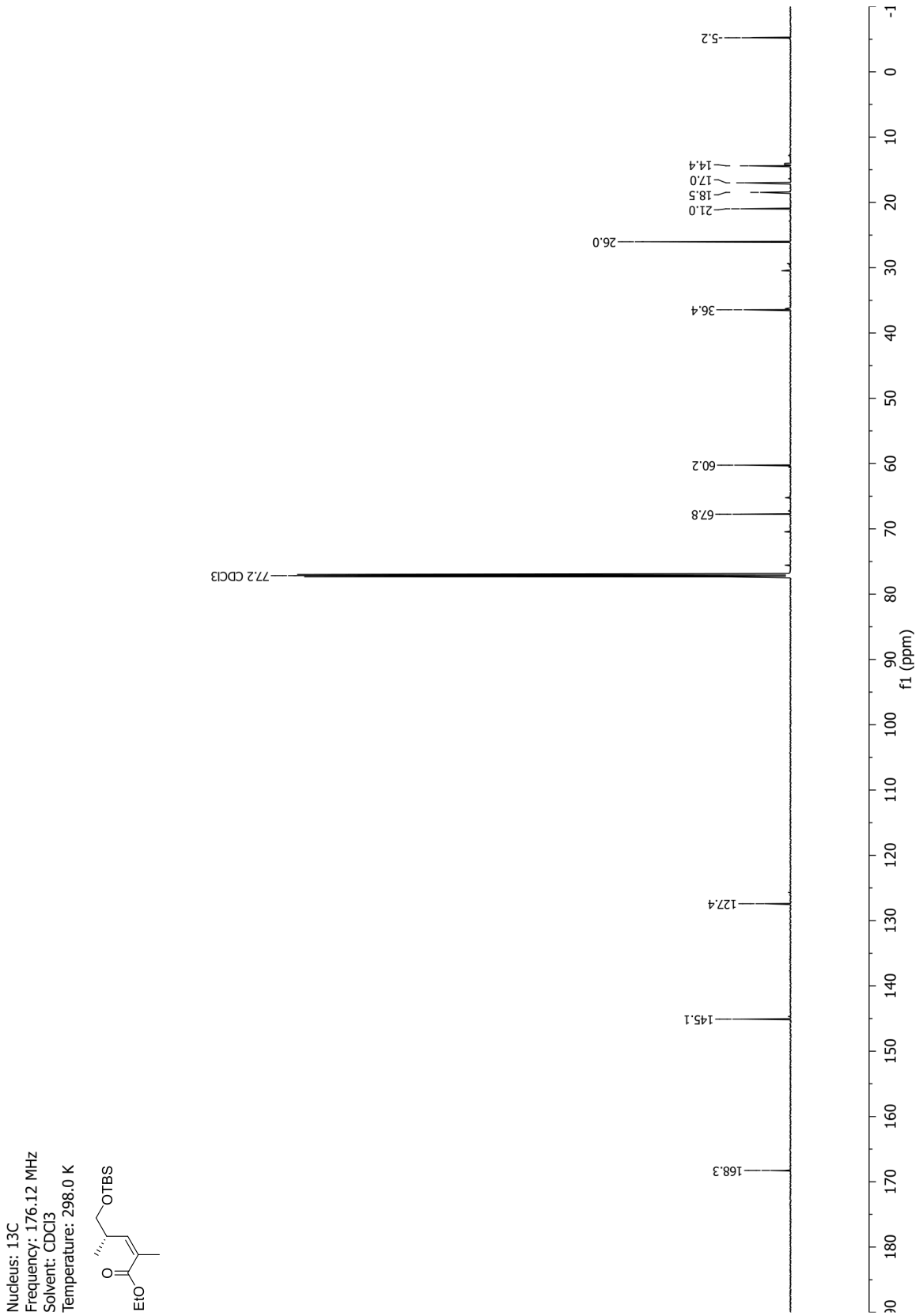
NMR-Spectra for Compound 217*E*



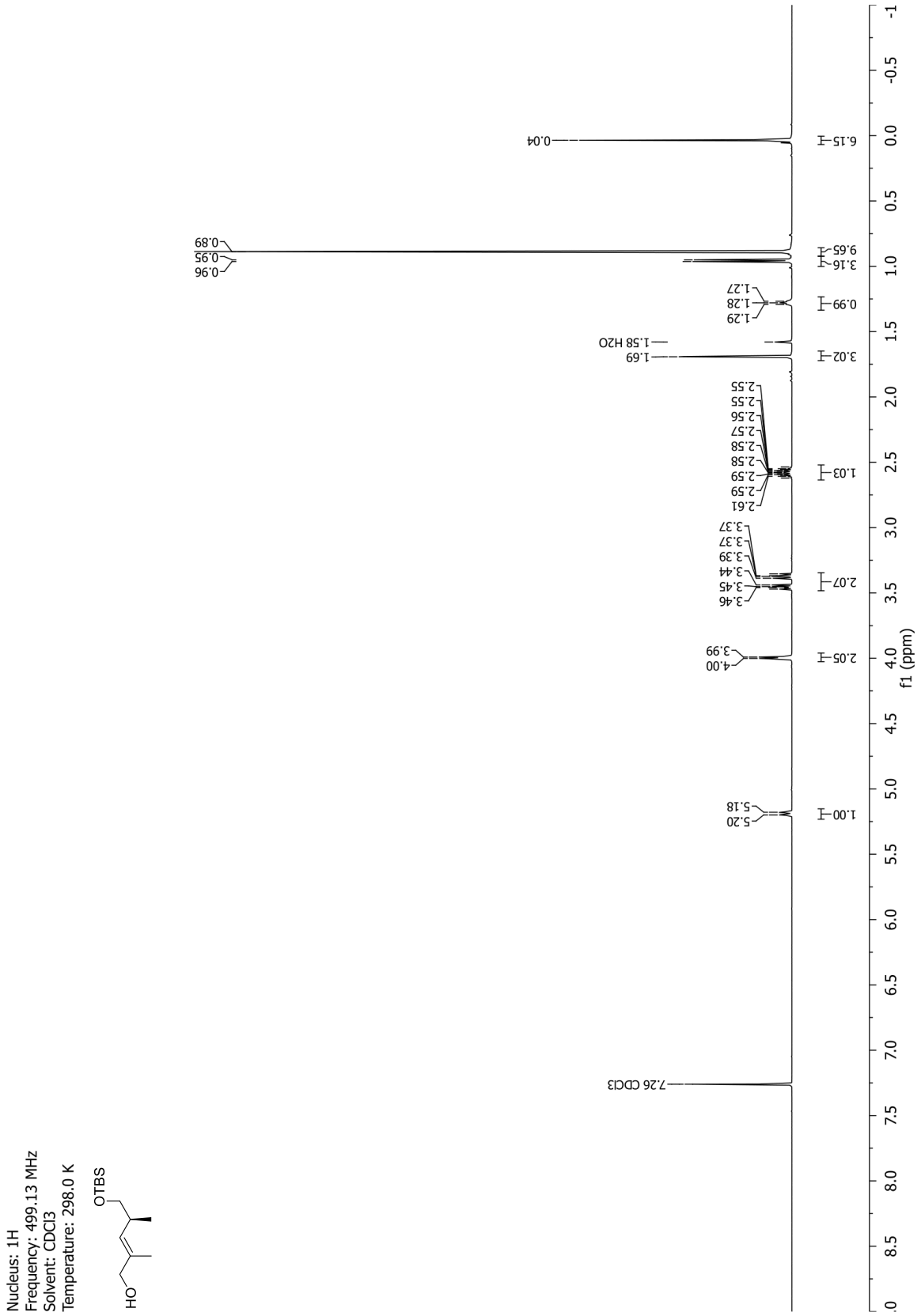

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
EtO
168.4
144.7
128.1
77.2 CDCl3
67.3
60.6
36.4
26.0
18.5
16.4
14.4
12.8
-5.2
-5.3
f1 (ppm)

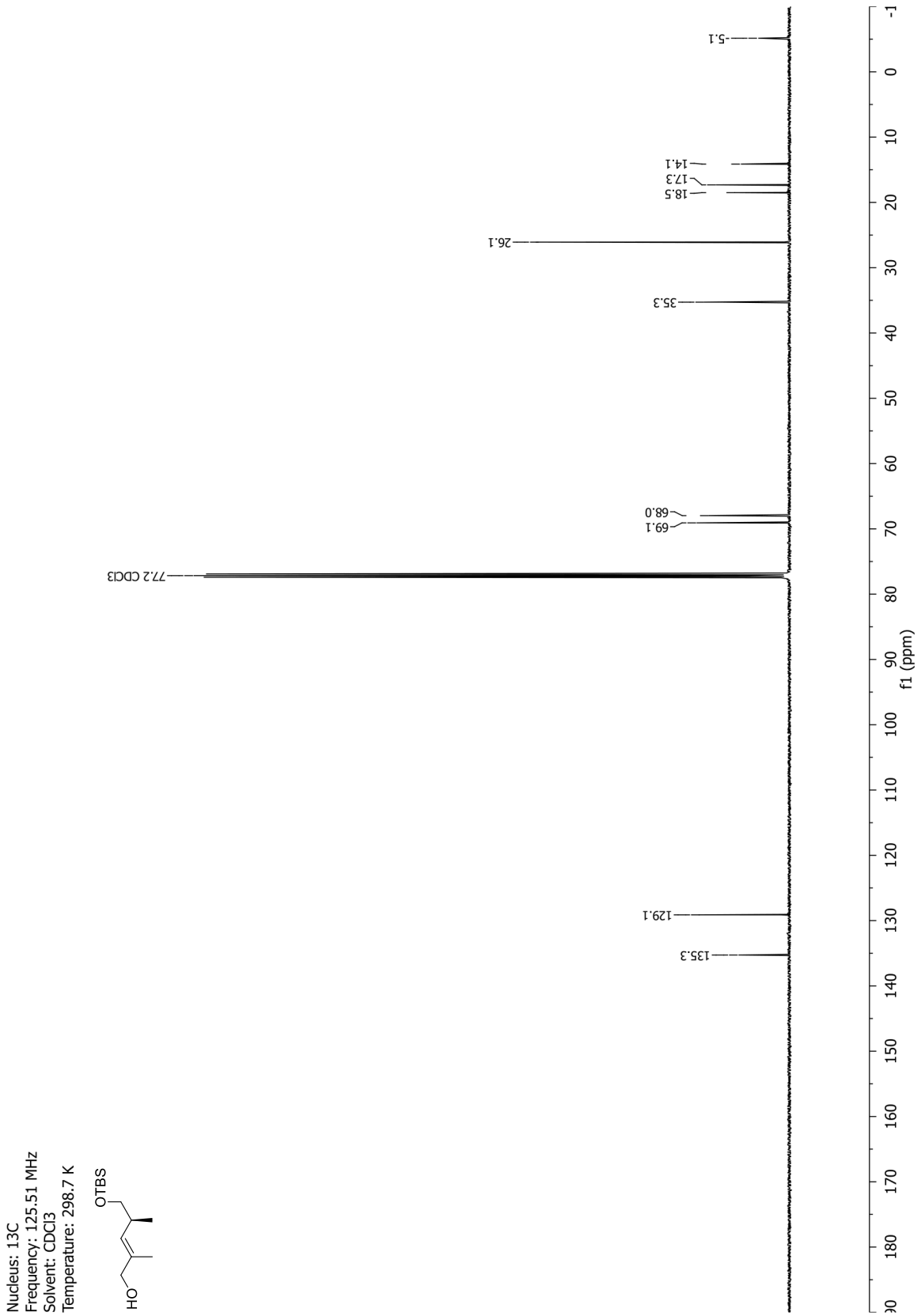
NMR-Spectra for Compound 217Z



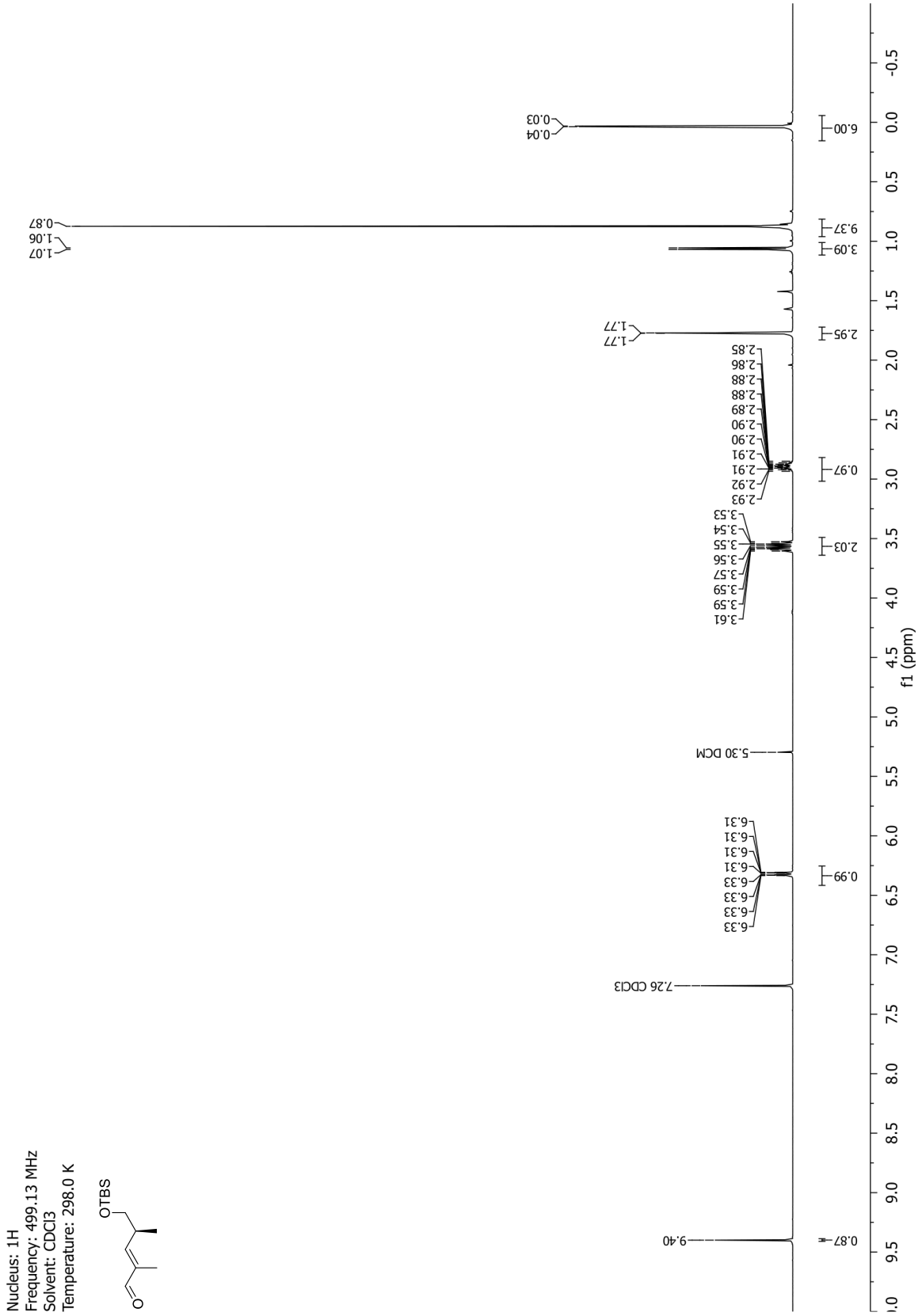

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
EtO
-5.2
14.4
17.0
18.5
21.0
26.0
36.4
60.2
67.8
77.2 CDCl3
127.4
145.1
168.3
f1 (ppm)

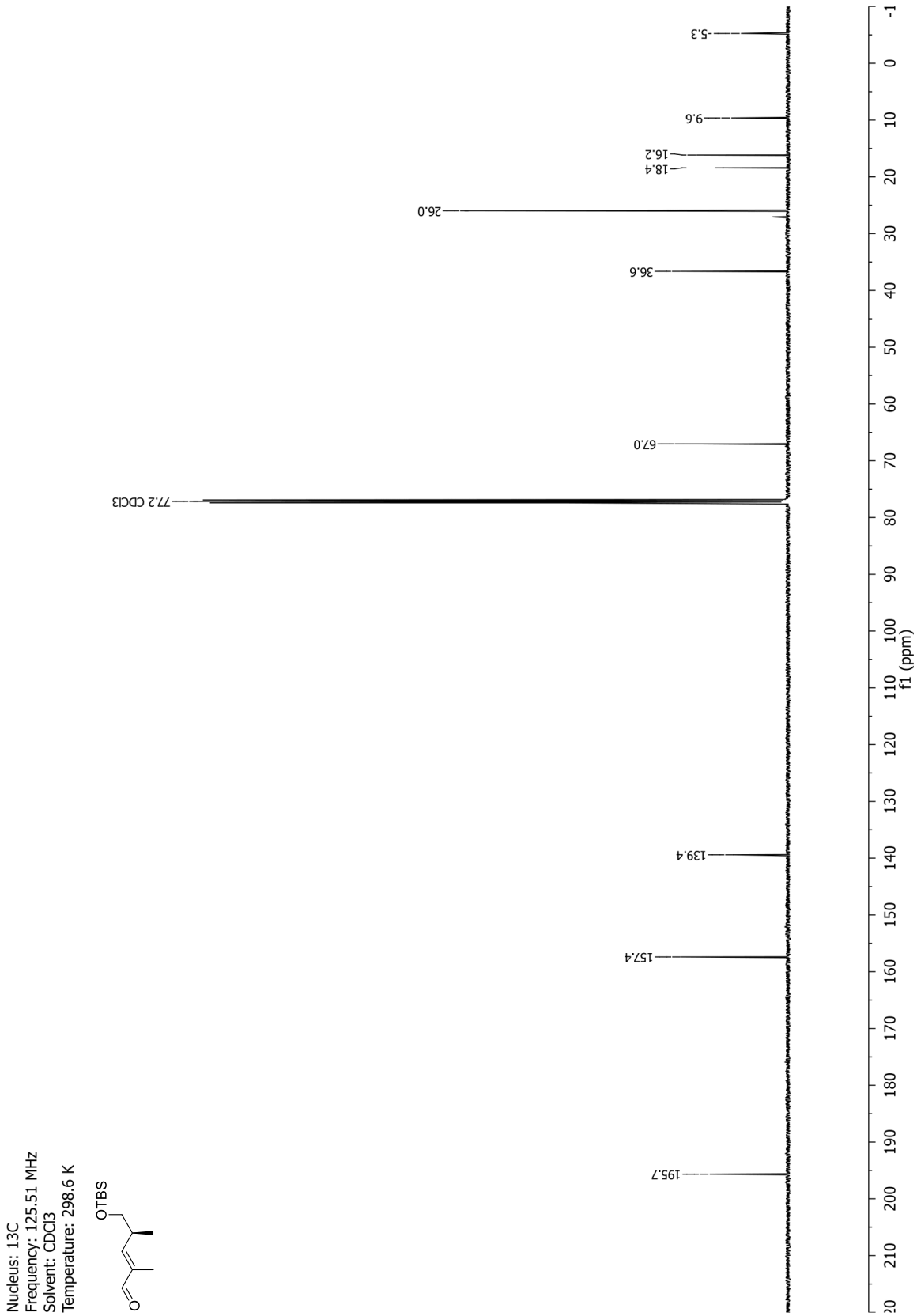
NMR-Spectra for Compound 299



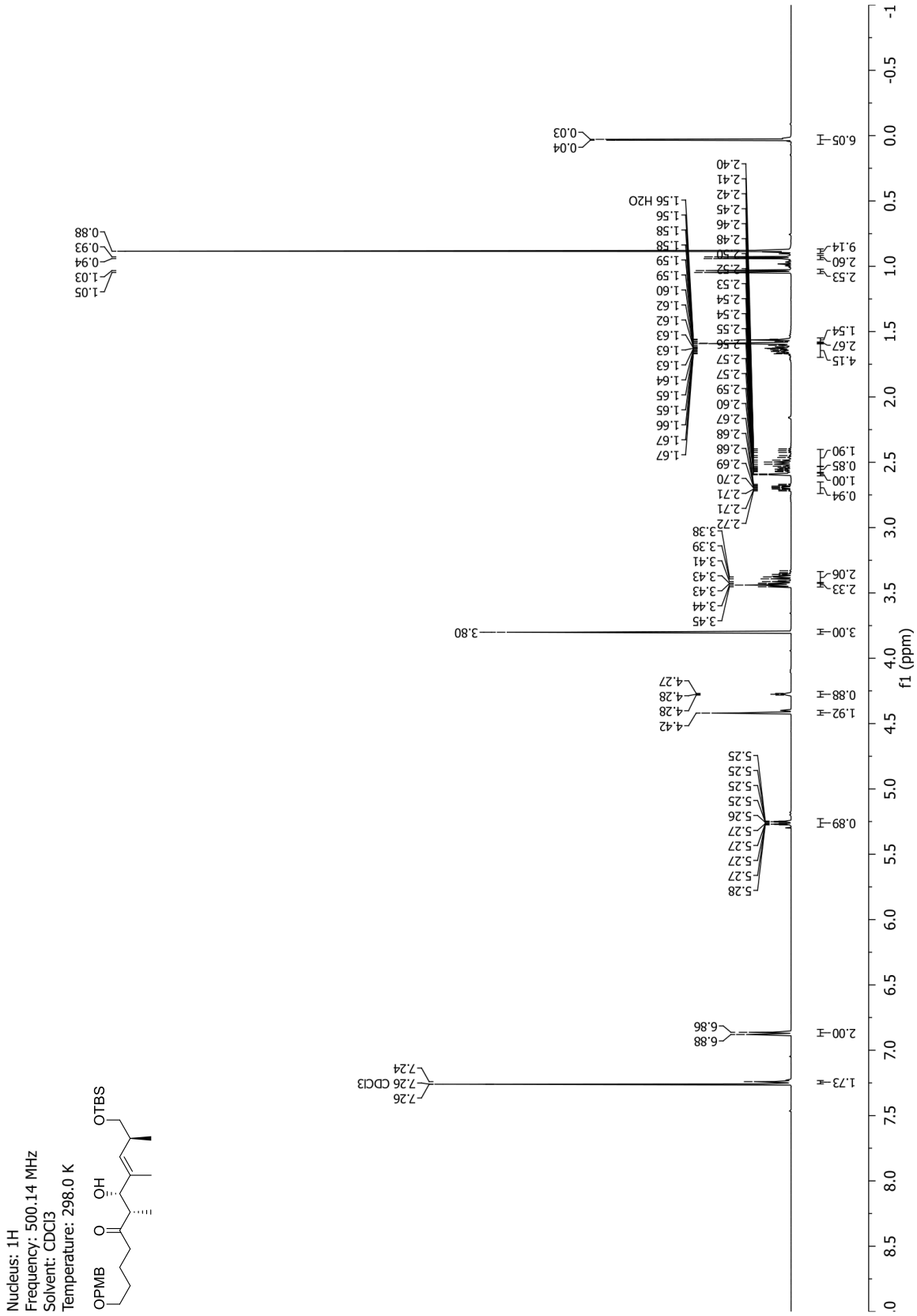

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 298.7 K
OTBS
HO
-5.1
14.1
17.3
18.5
26.1
35.3
68.0
69.1
77.2 CDCl3
129.1
135.3
f1 (ppm)

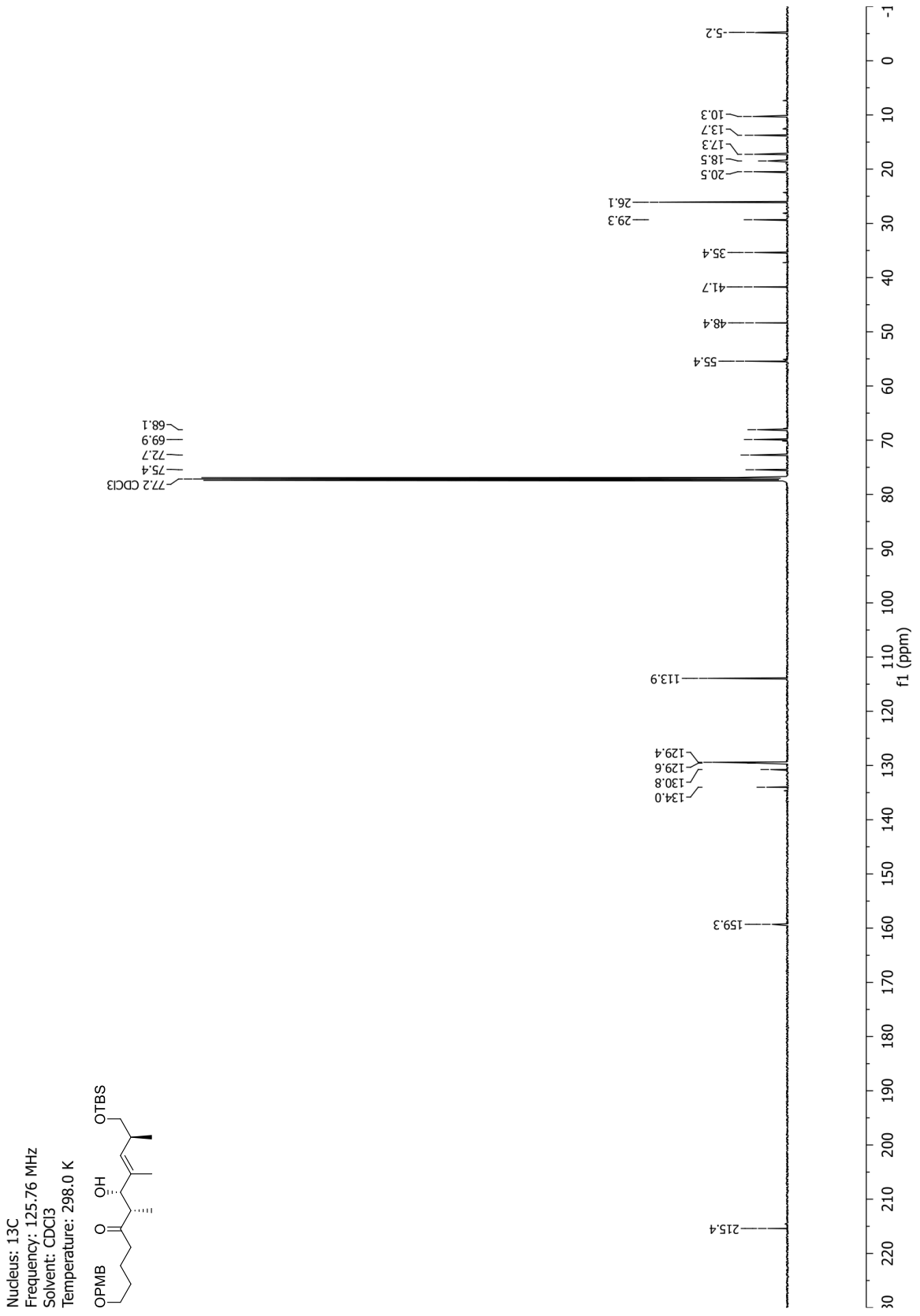
NMR-Spectra for Compound 12



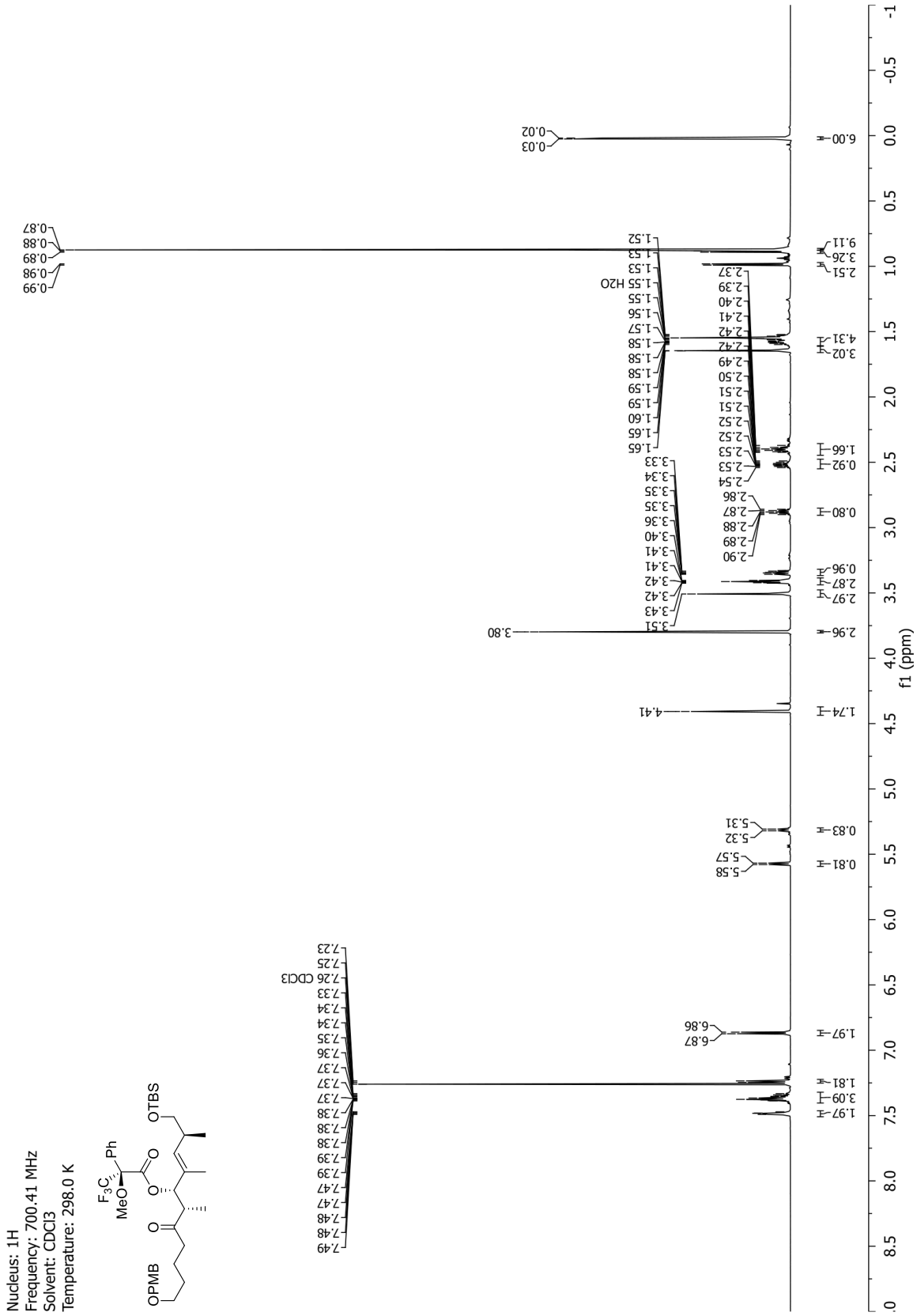

Nucleus: 13C
Frequency: 125.51 MHz
Solvent: CDCl3
Temperature: 298.6 K
OTBS
O
195.7
157.4
139.4
77.2 CDCl3
67.0
36.6
26.0
18.4
16.2
9.6
-5.3
f1 (ppm)

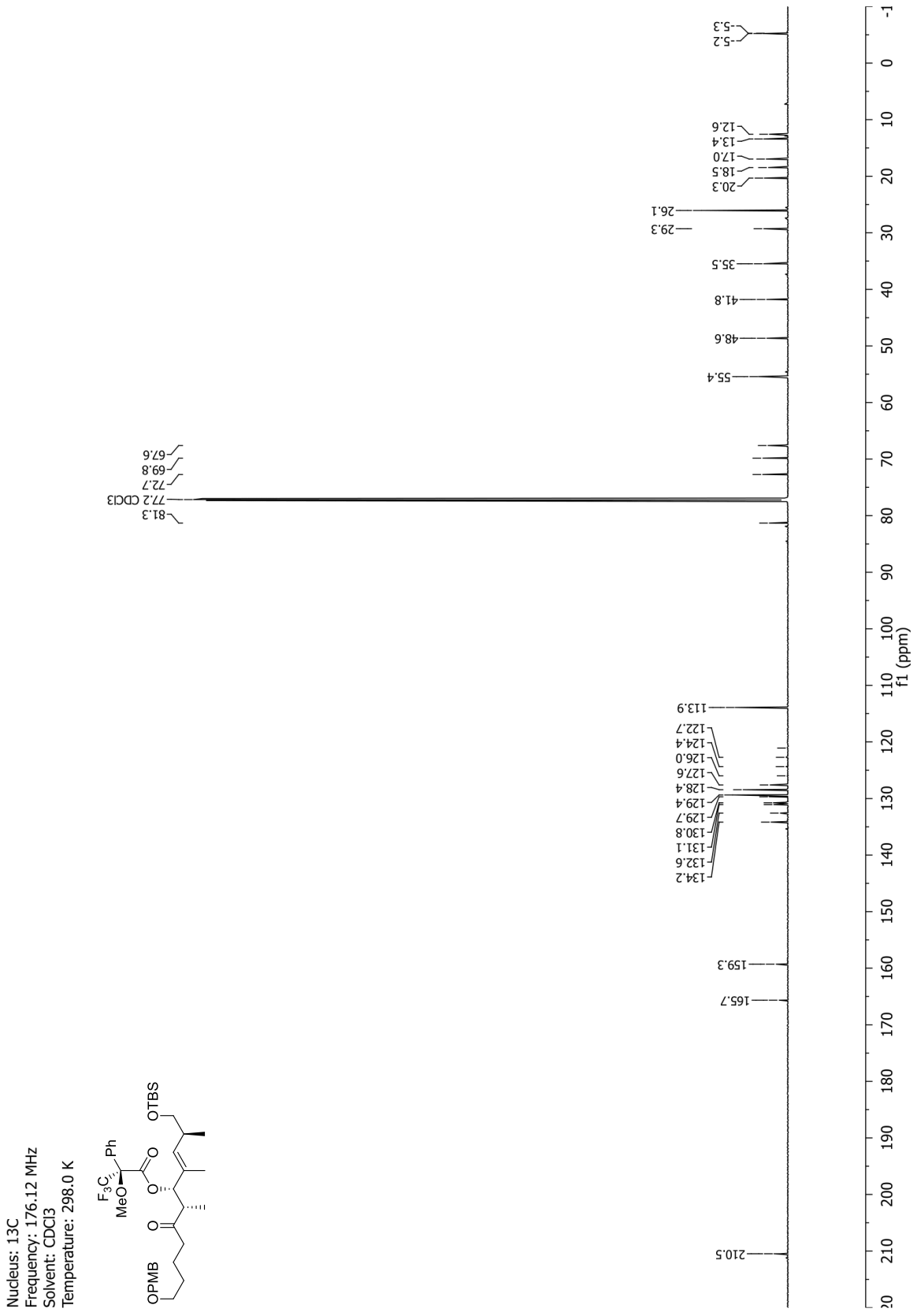
NMR-Spectra for Compound 304



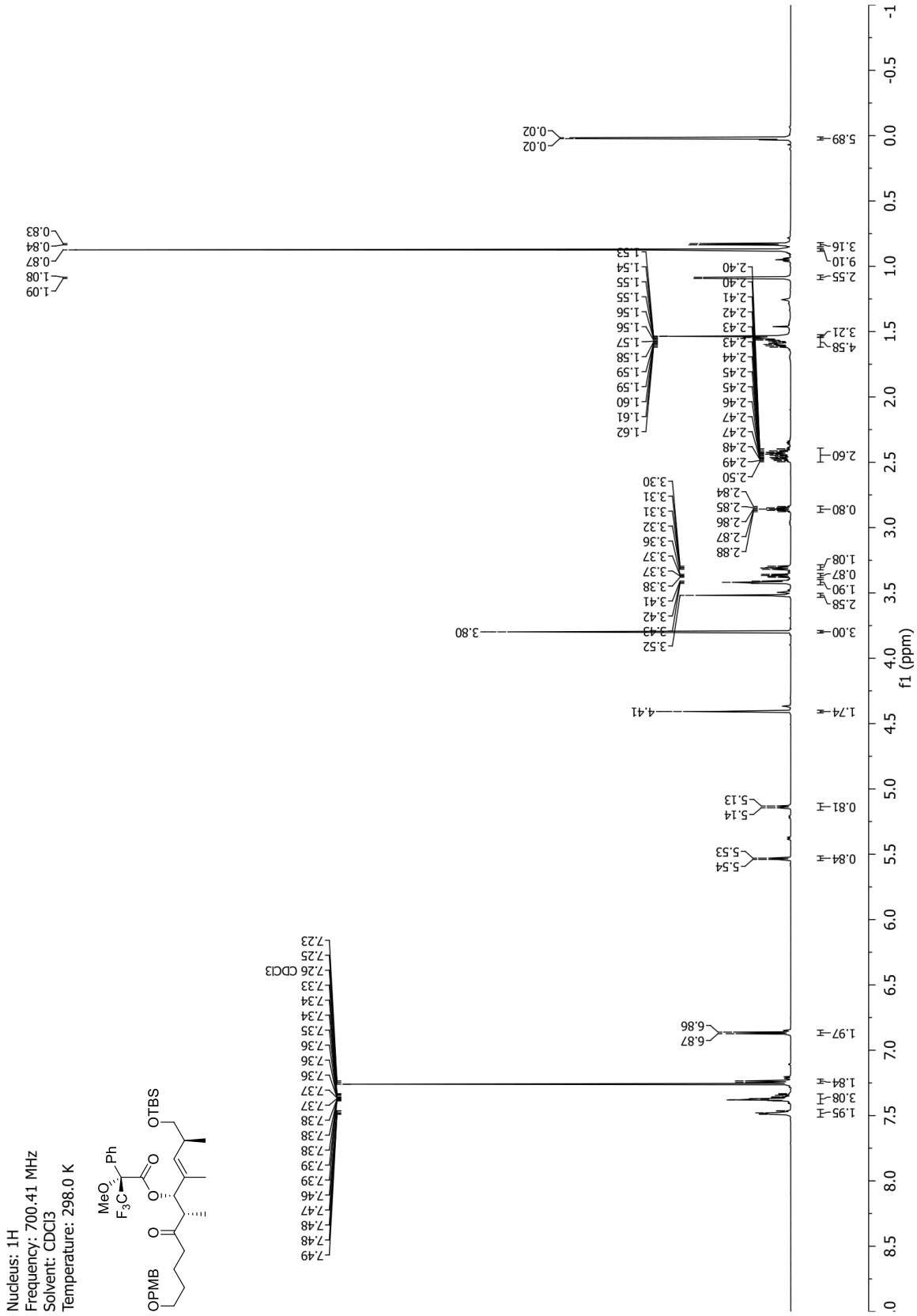

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
OPMB
O
OH
OTBS
-5.2
10.3
13.7
17.3
18.5
20.5
26.1
29.3
35.4
41.7
48.4
55.4
68.1
69.9
72.7
75.4
77.2 CDCl3
113.9
129.4
129.6
130.8
134.0
159.3
215.4
f1 (ppm)

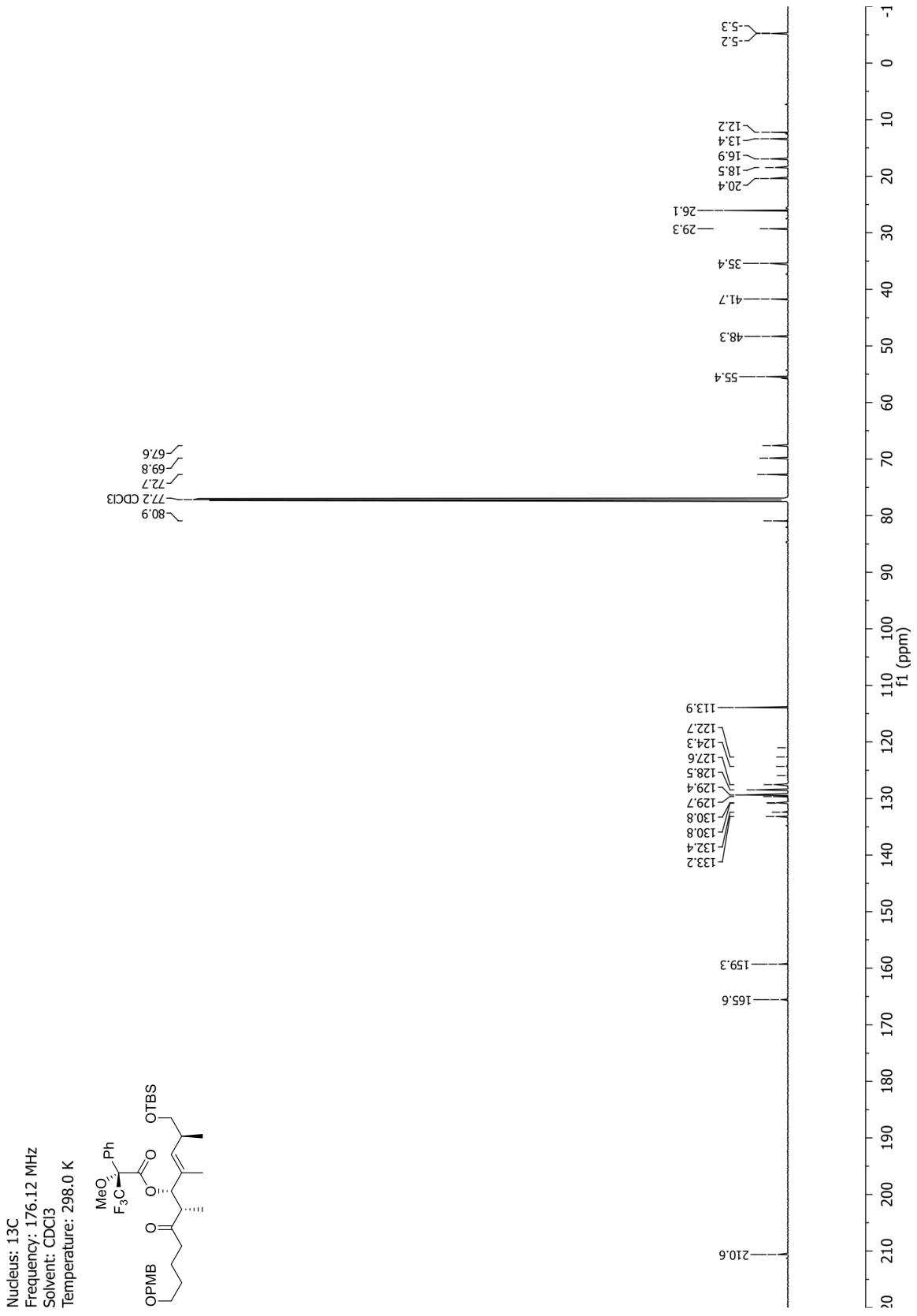
NMR-Spectra for Compound 309



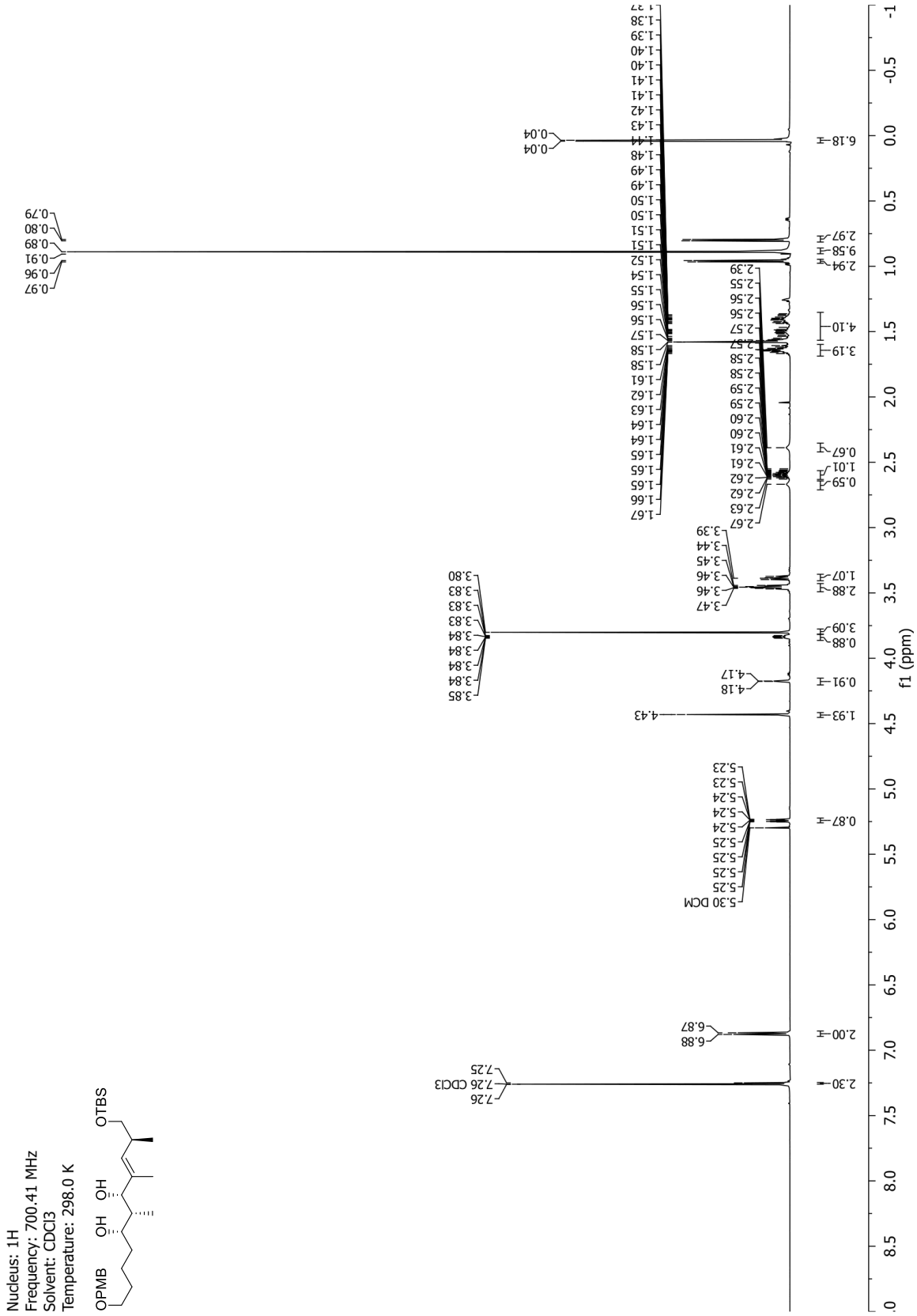

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
OPMB
Ph
MeO
F3C
-5.3
-5.2
12.6
13.4
17.0
18.5
20.3
26.1
29.3
35.5
41.8
48.6
55.4
67.6
69.8
72.7
77.2 CDCl3
81.3
113.9
122.7
124.4
126.0
127.6
128.4
129.4
129.7
130.8
131.1
132.6
134.2
159.3
165.7
210.5
f1 (ppm)

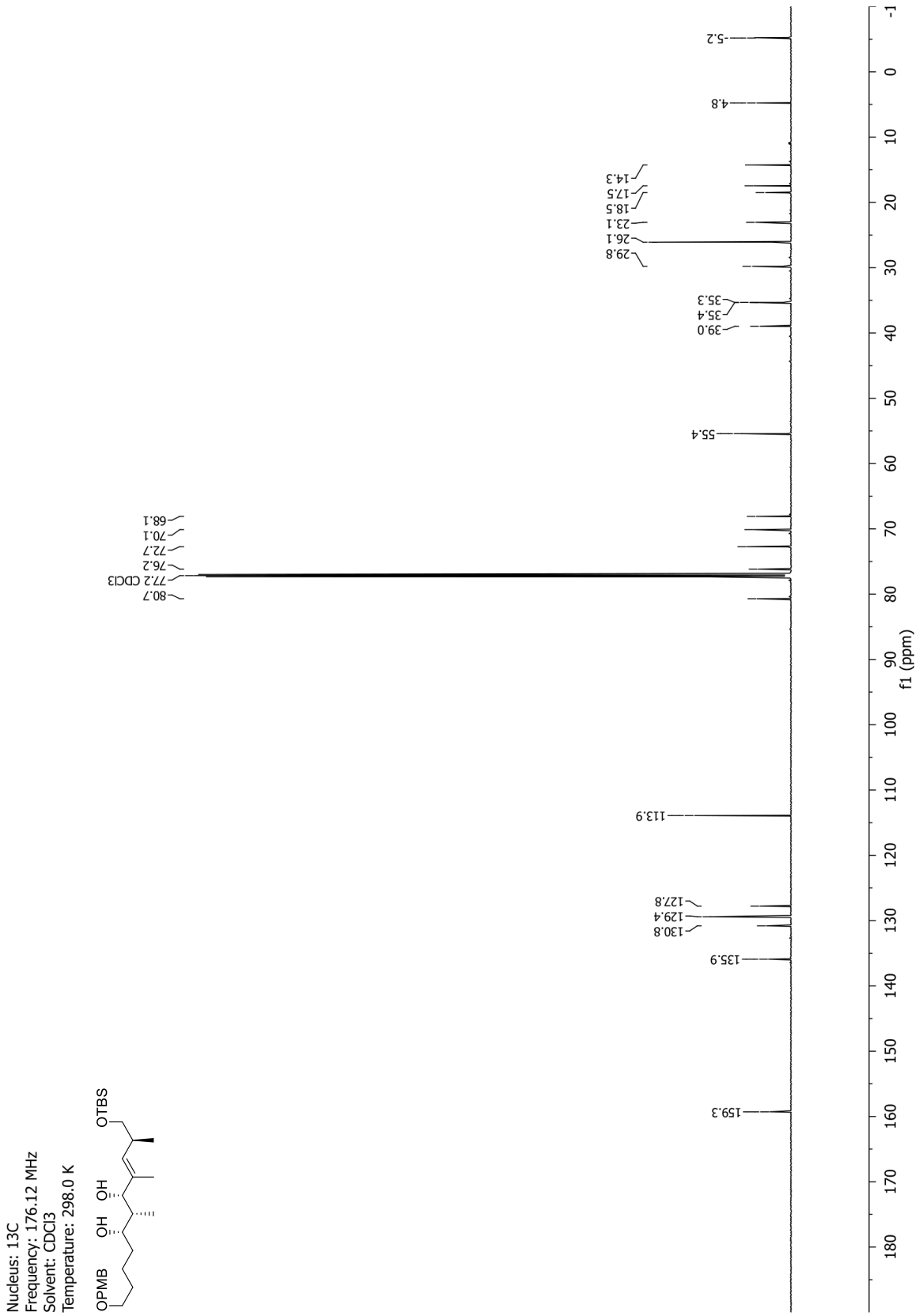
NMR-Spectra for Compound 310



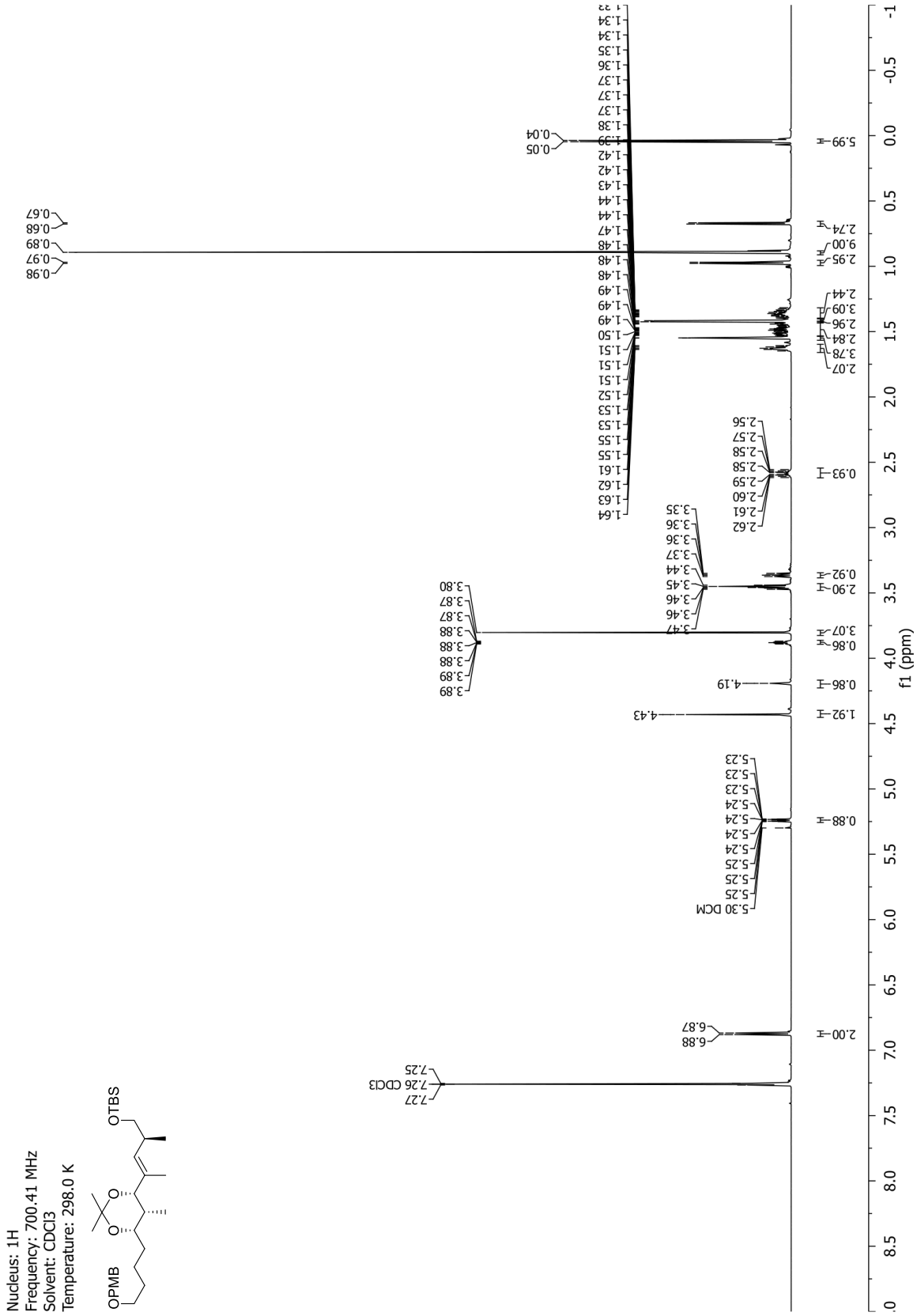

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
OPMB
MeO
Ph
F3C
-5.3
-5.2
12.2
13.4
16.9
18.5
20.4
26.1
29.3
35.4
41.7
48.3
55.4
67.6
69.8
72.7
77.2 CDCl3
80.9
113.9
122.7
124.3
127.6
128.5
129.4
129.7
130.8
130.8
132.4
133.2
159.3
165.6
210.6
f1 (ppm)

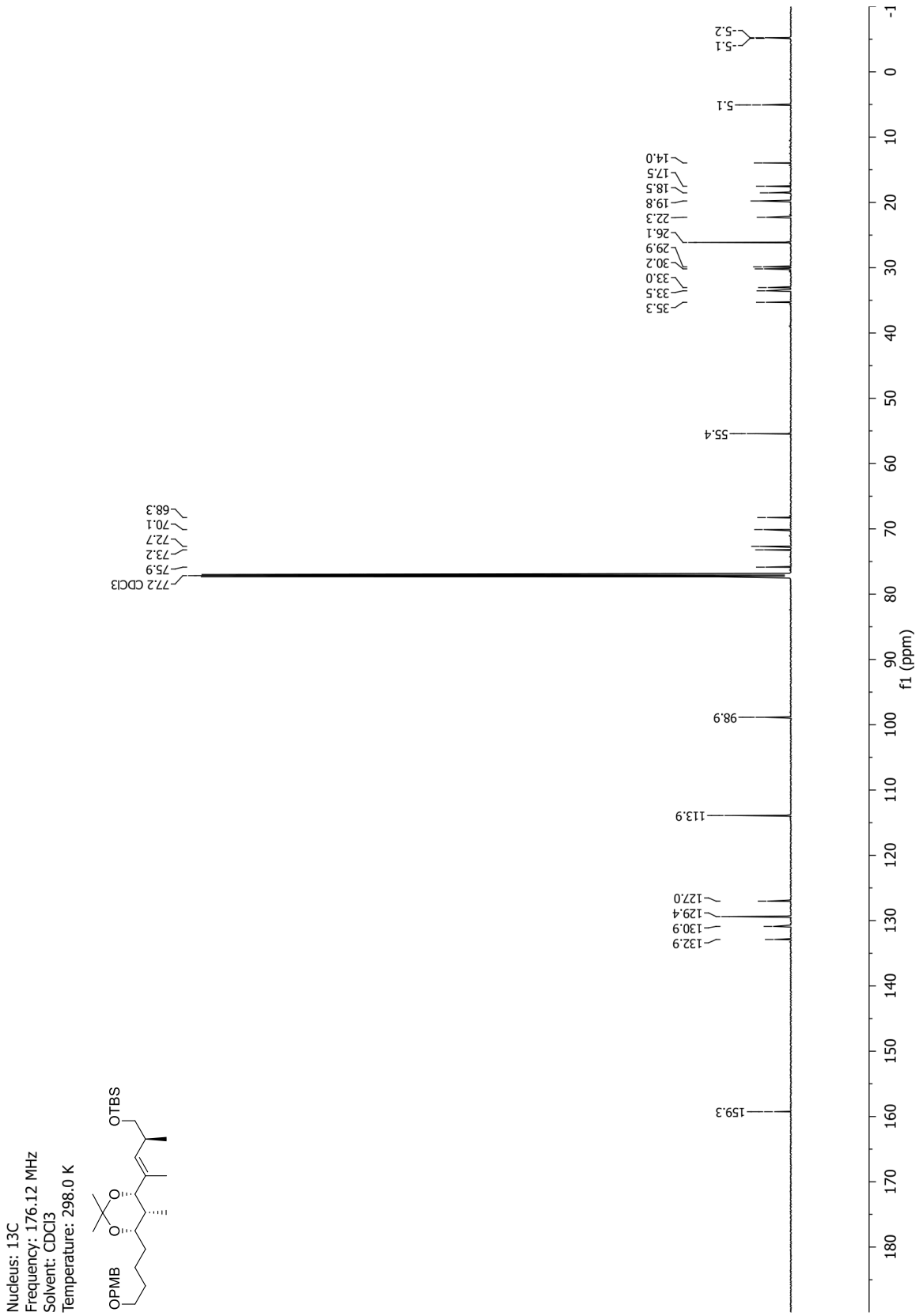
NMR-Spectra for Compound 311



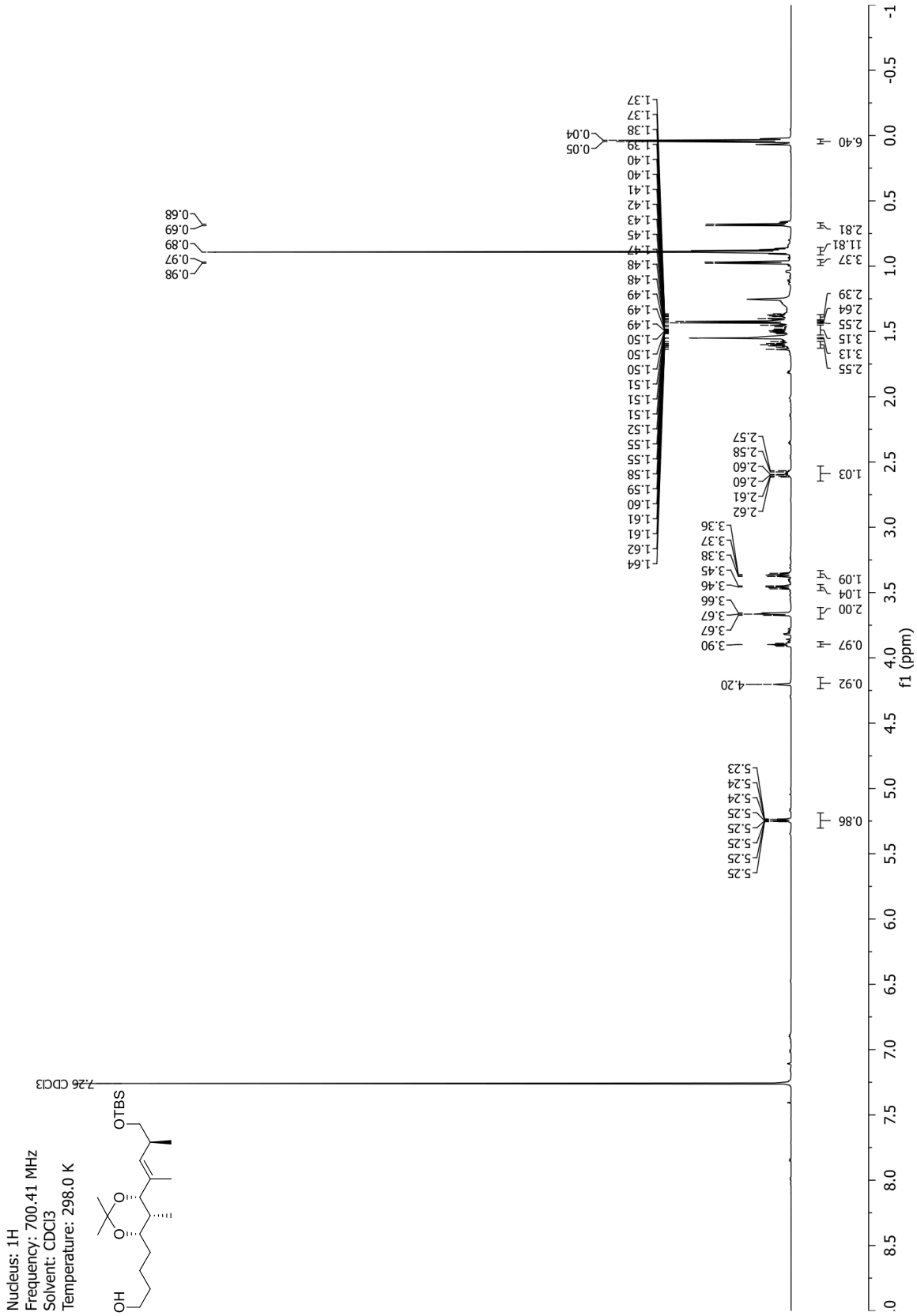

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OPMB
OH
OH
OTBS
f1 (ppm)
-5.2
4.8
14.3
17.5
18.5
23.1
26.1
29.8
35.3
35.4
39.0
55.4
68.1
70.1
72.7
76.2
77.2 CDCl3
80.7
113.9
127.8
129.4
130.8
135.9
159.3

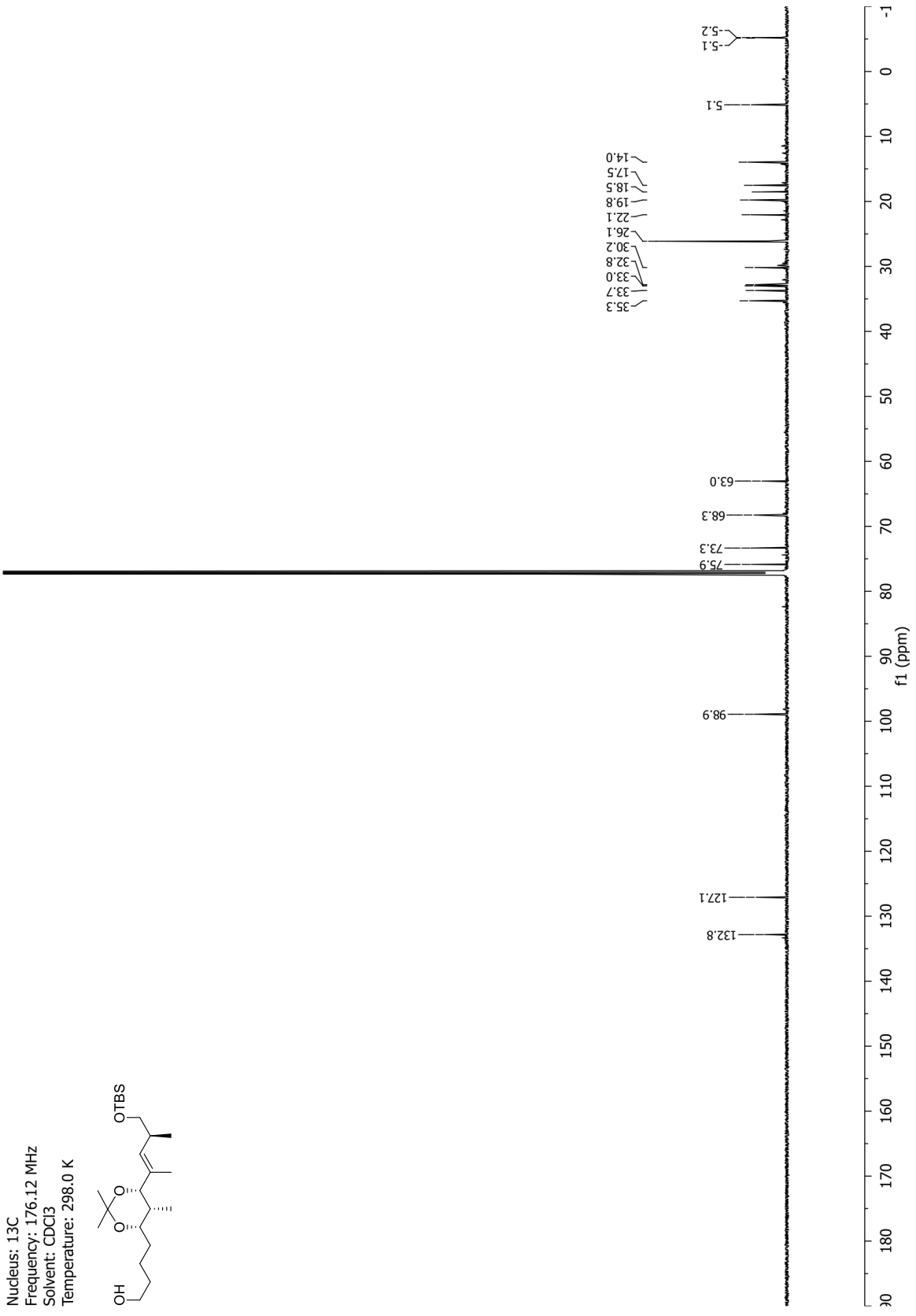
NMR-Spectra for Compound 312



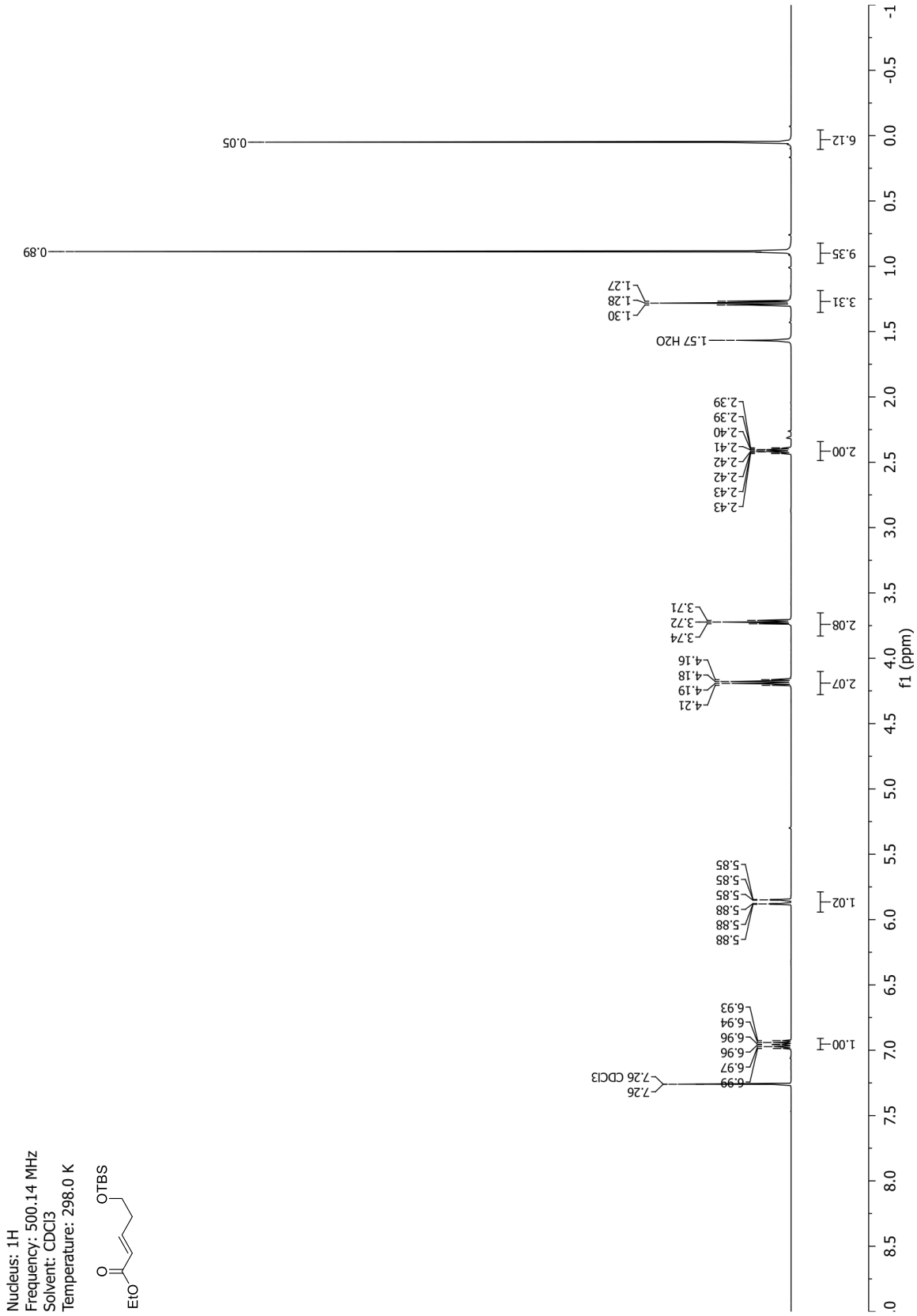

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
OPMB
O
O
-5.2
-5.1
5.1
14.0
17.5
18.5
19.8
22.3
26.1
29.9
30.2
33.0
33.5
35.3
55.4
68.3
70.1
72.7
73.2
75.9
77.2 CDCl3
98.9
113.9
127.0
129.4
130.9
132.9
159.3
f1 (ppm)

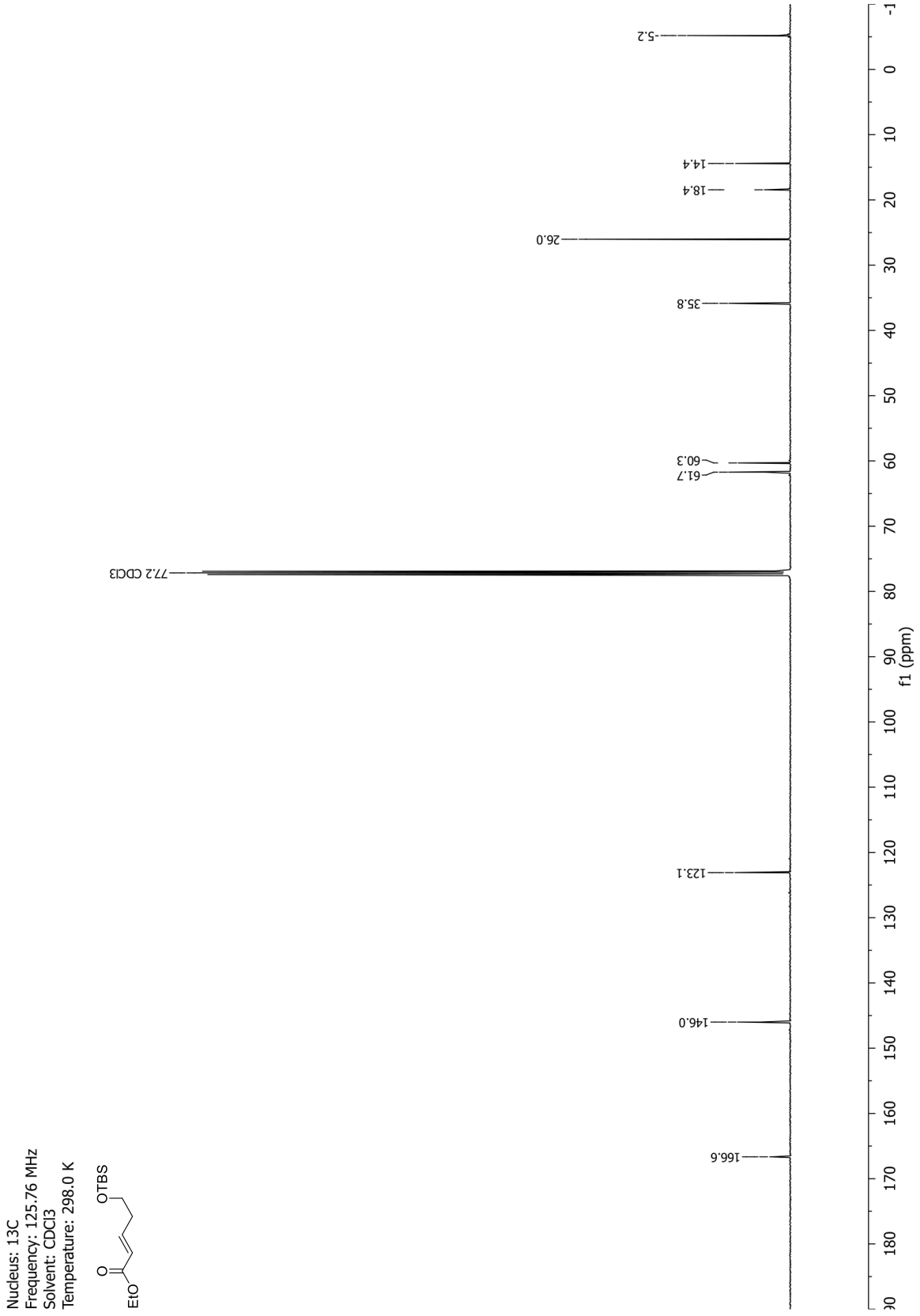
NMR-Spectra for Compound 313



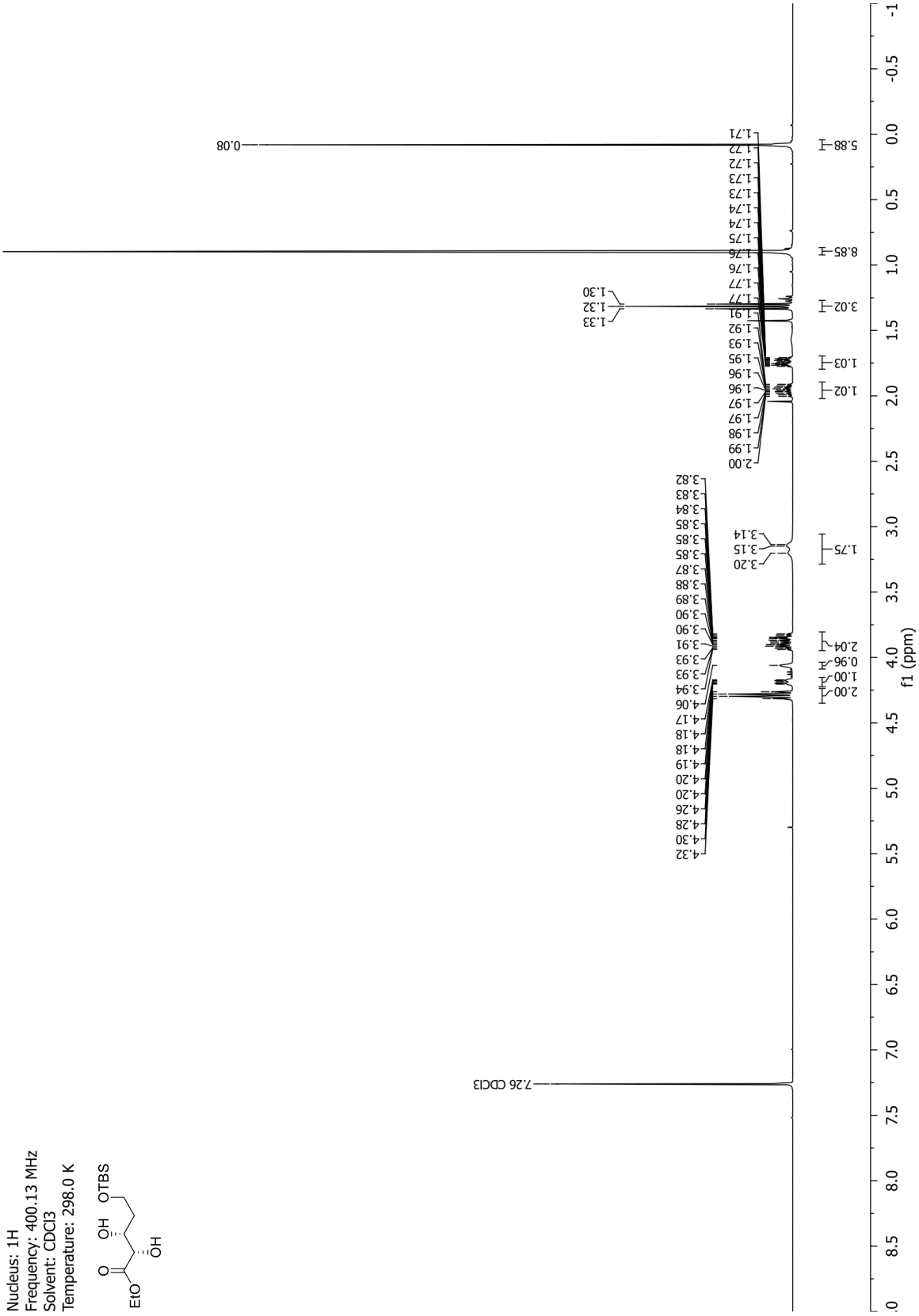

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
OH
O
O
-5.2
-5.1
5.1
14.0
17.5
18.5
19.8
22.1
26.1
30.2
32.8
33.0
33.7
35.3
63.0
68.3
73.3
75.9
98.9
127.1
132.8
f1 (ppm)

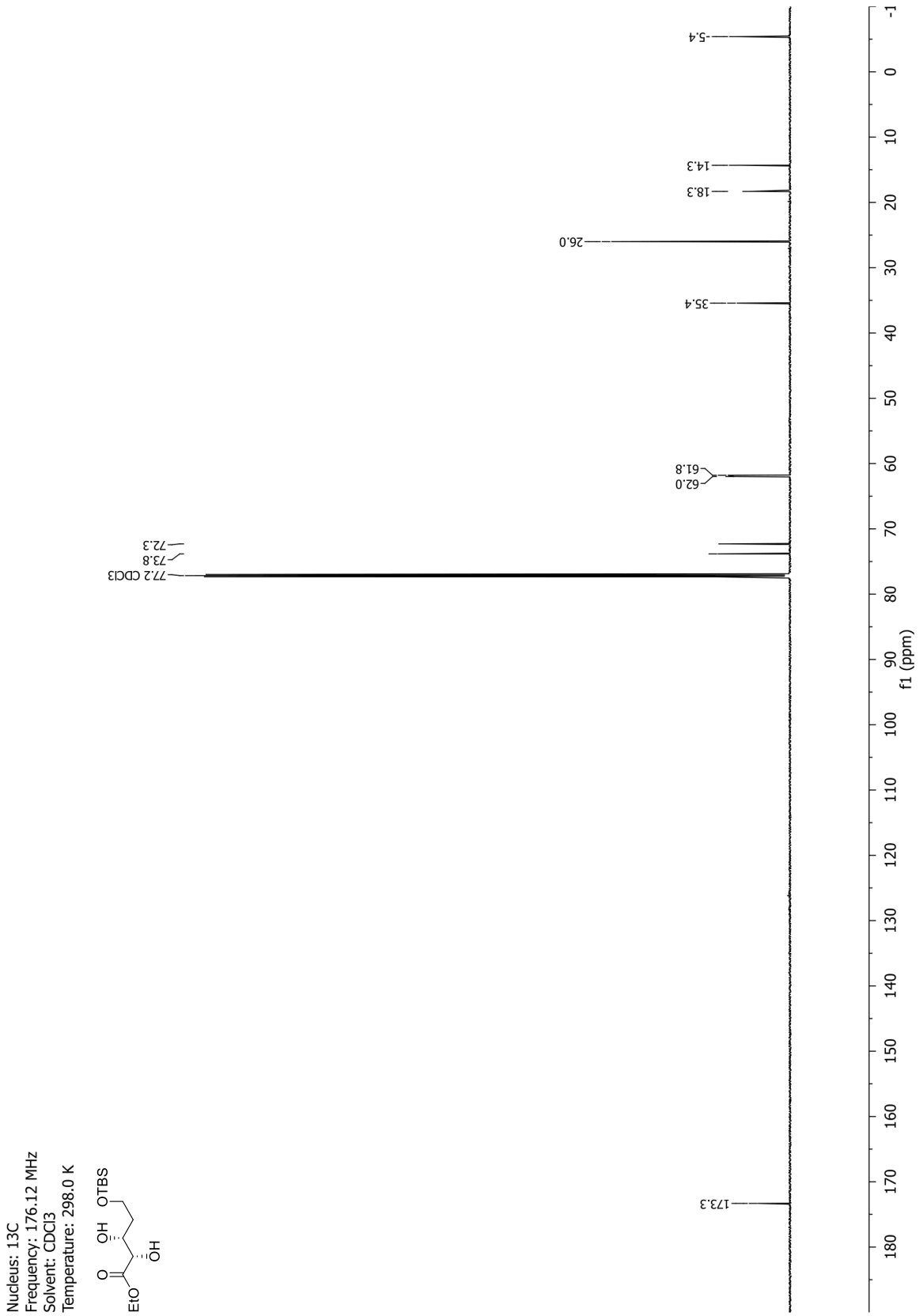
NMR-Spectra for Compound 200



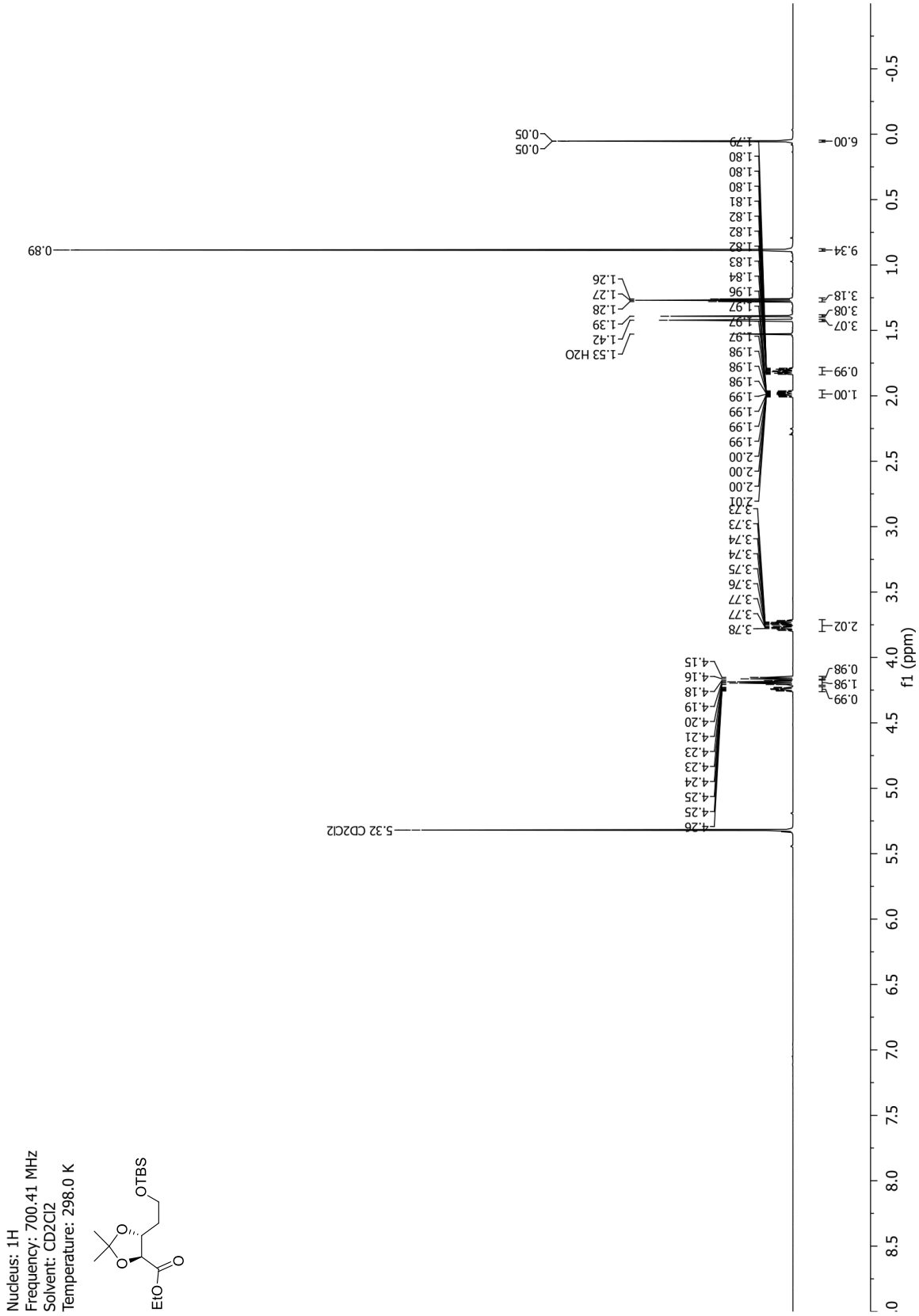

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
O
EtO
166.6
146.0
123.1
77.2 CDCl3
61.7
60.3
35.8
26.0
18.4
14.4
-5.2
f1 (ppm)

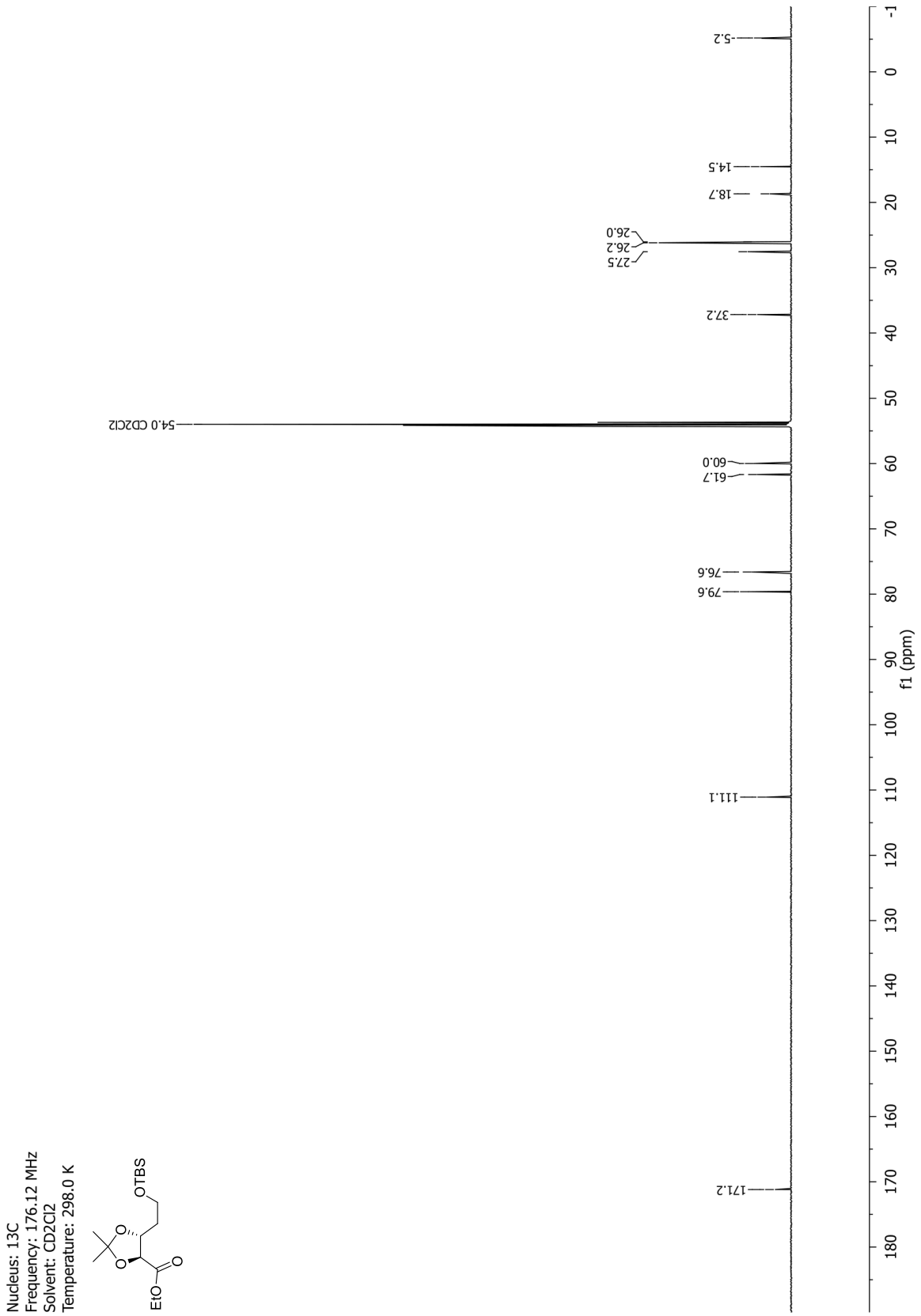
NMR-Spectra for Compound 317



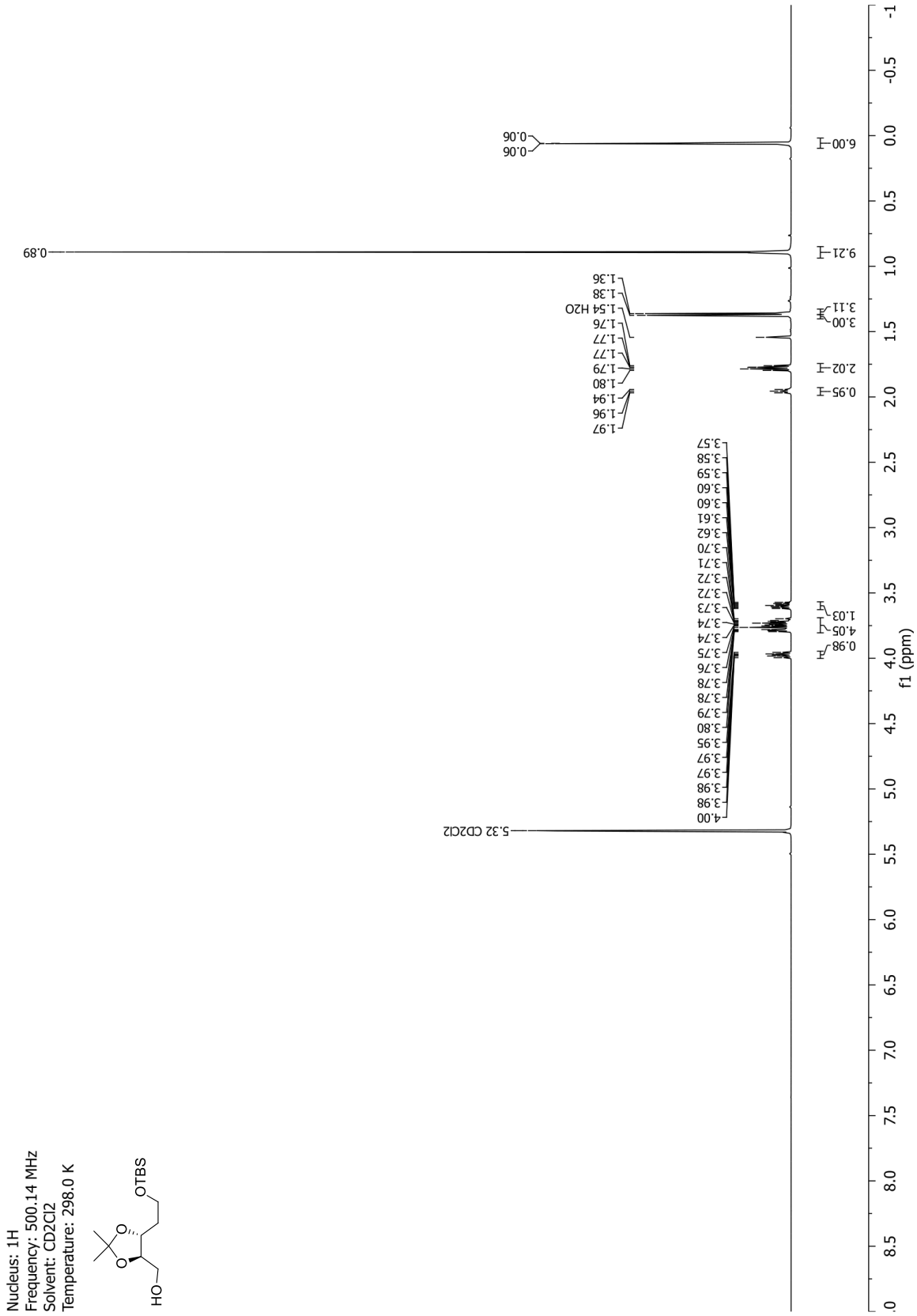

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
EtO
O
OH
OTBS
HO
-5.4
14.3
18.3
26.0
35.4
61.8
62.0
72.3
73.8
77.2 CDCl3
173.3
f1 (ppm)

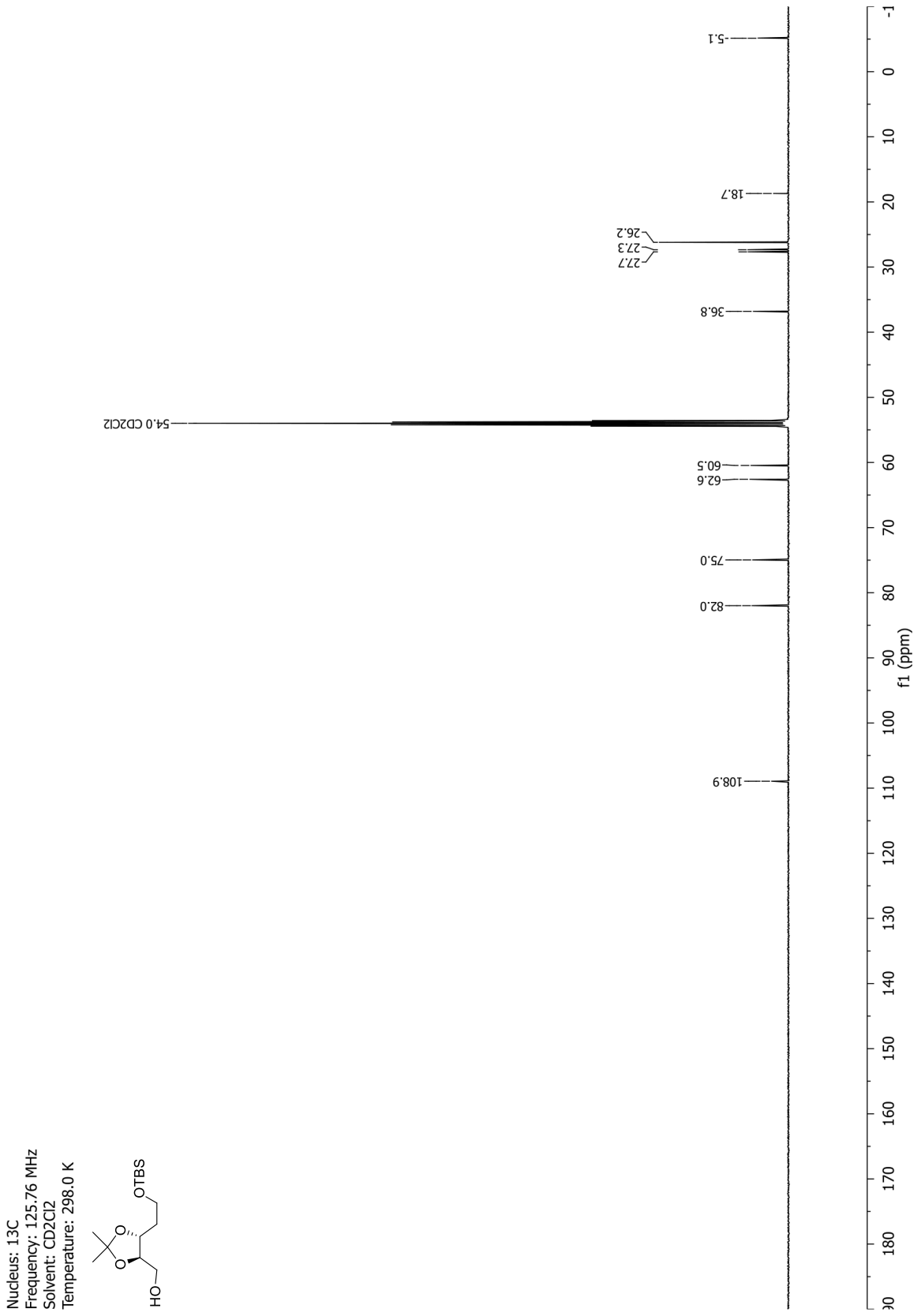
NMR-Spectra for Compound 199



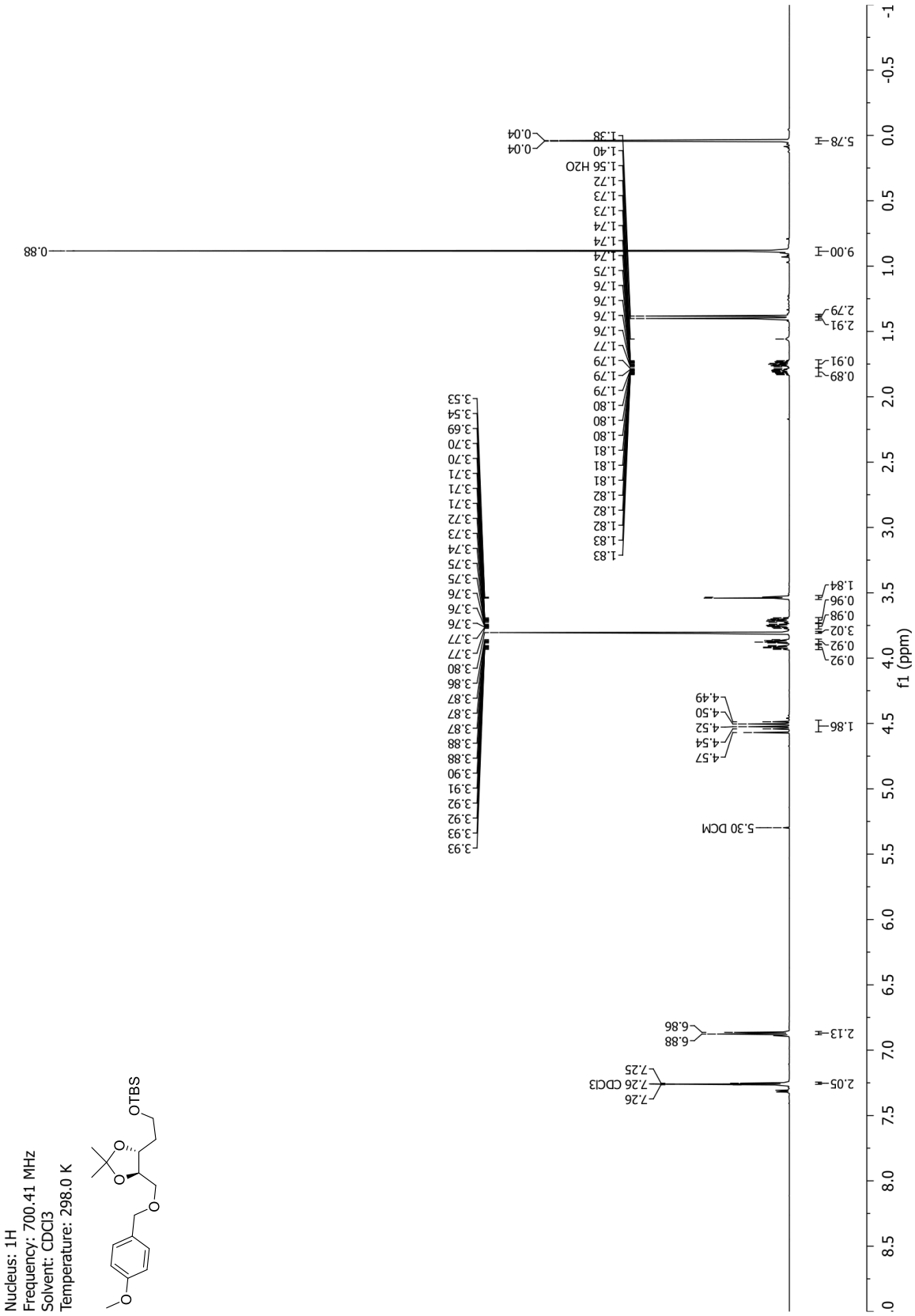

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
OTBS
O
O
O
EtO
-5.2
14.5
18.7
26.0
26.2
27.5
37.2
54.0 CD2Cl2
60.0
61.7
76.6
79.6
111.1
171.2
f1 (ppm)

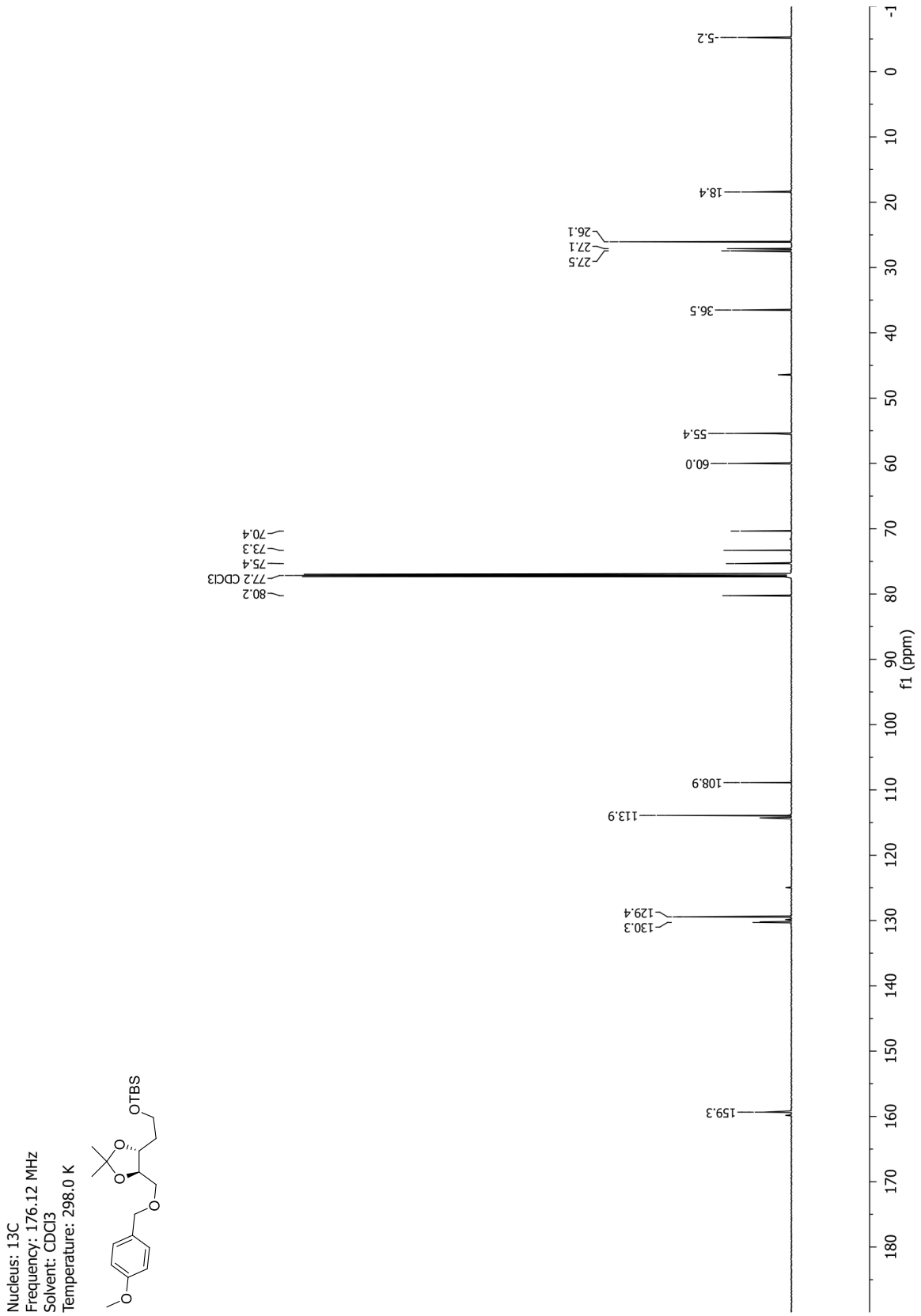
NMR-Spectra for Compound 318



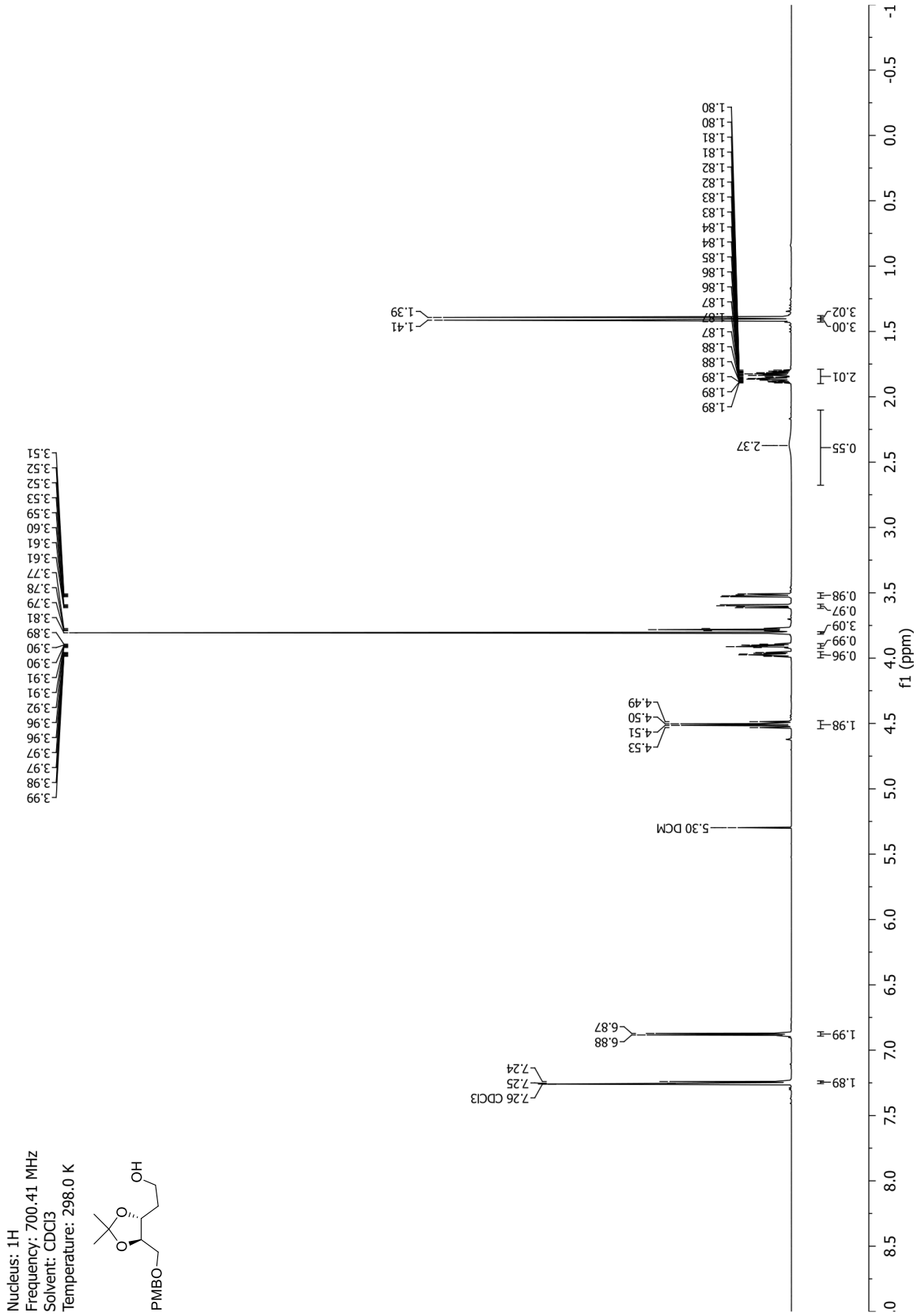

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CD2Cl2
Temperature: 298.0 K
OTBS
HO
-5.1
18.7
26.2
27.3
27.7
36.8
54.0 CD2Cl2
60.5
62.6
75.0
82.0
108.9
f1 (ppm)

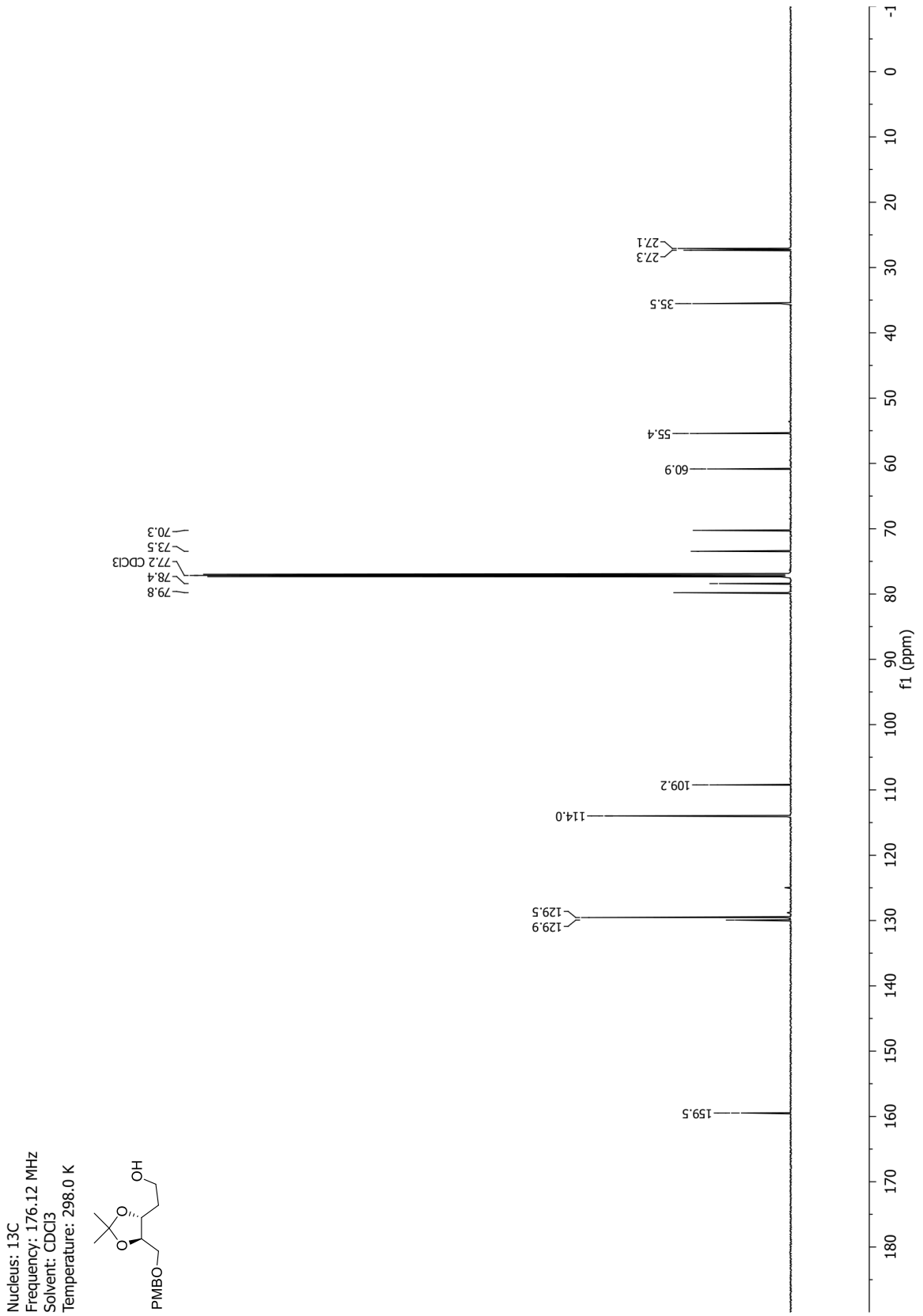
NMR-Spectra for Compound 198



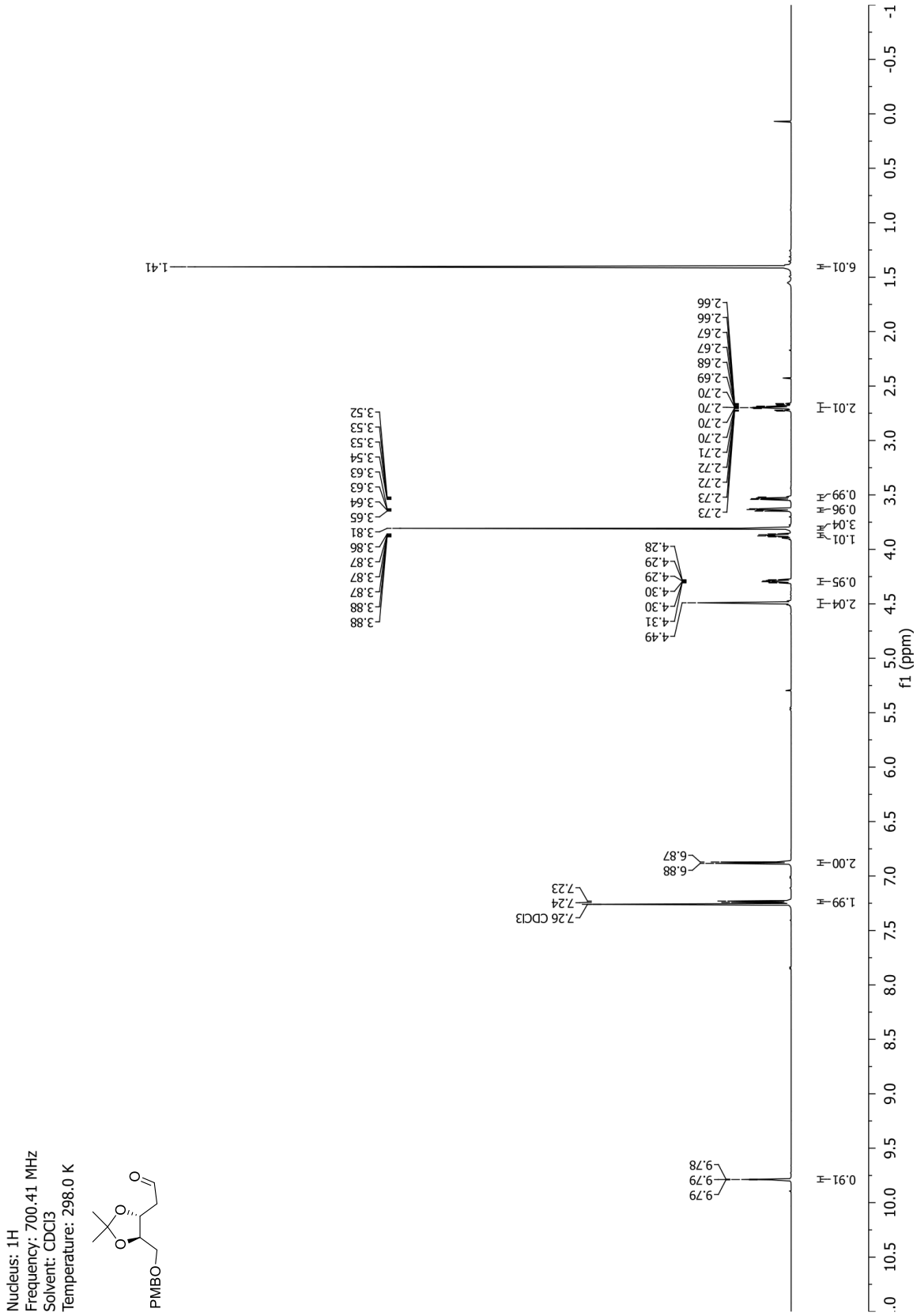

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OTBS
-5.2
18.4
26.1
27.1
27.5
36.5
55.4
60.0
70.4
73.3
75.4
77.2 CDCl3
80.2
108.9
113.9
129.4
130.3
159.3
f1 (ppm)

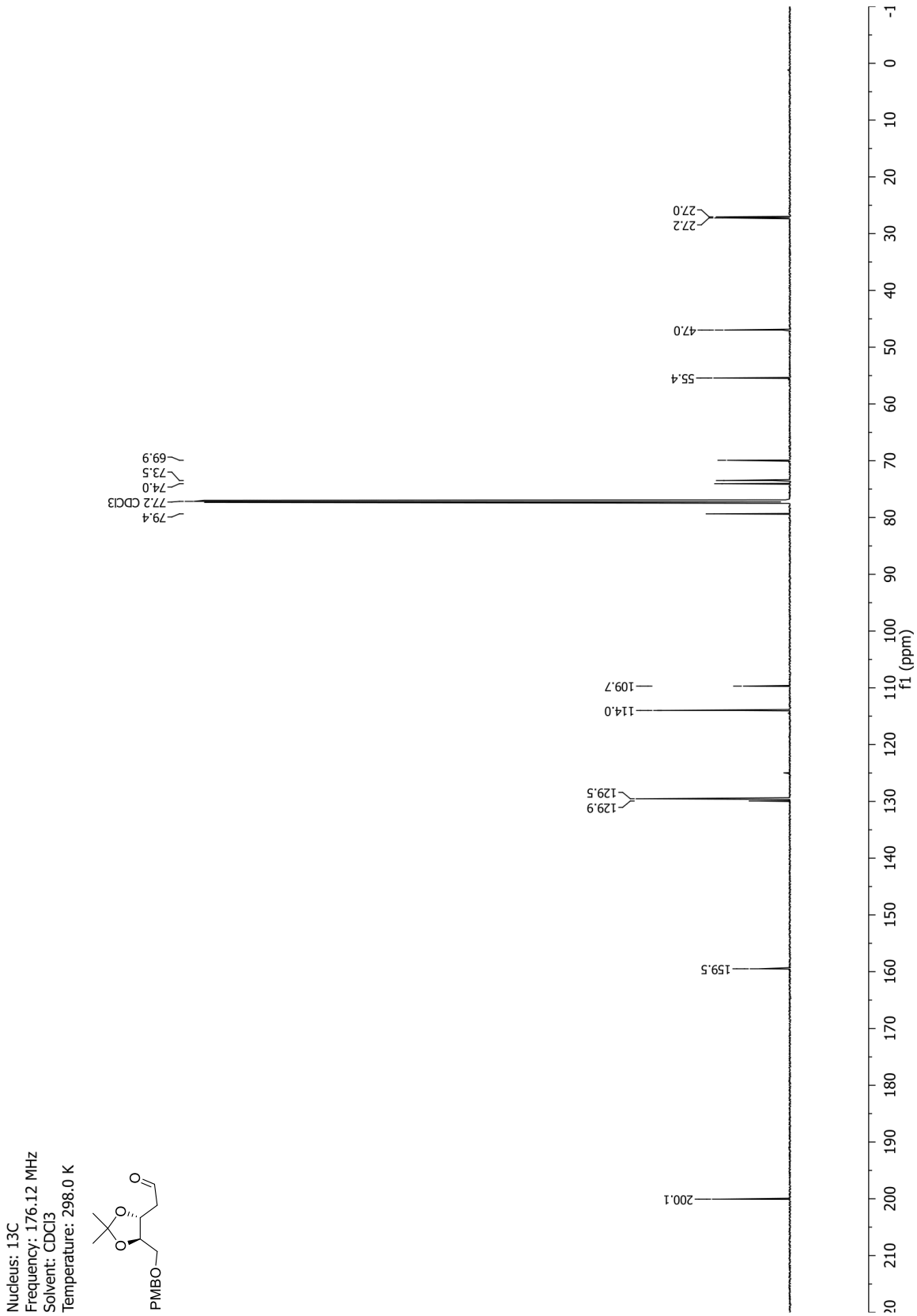
NMR-Spectra for Compound 319



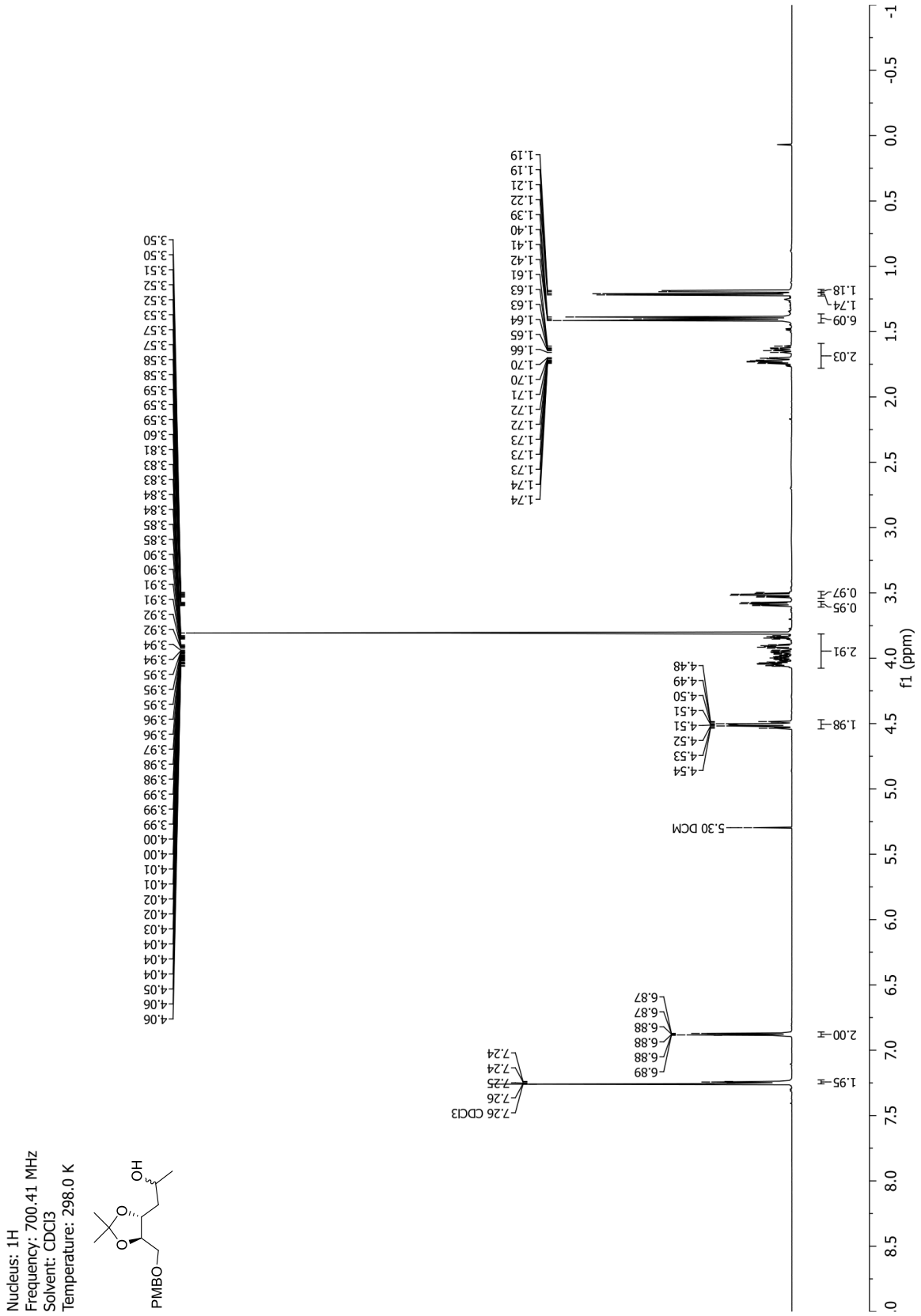

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OH
O
O
PMBO
159.5
129.9
129.5
114.0
109.2
79.8
78.4
77.2 CDCl3
73.5
70.3
60.9
55.4
35.5
27.3
27.1
f1 (ppm)

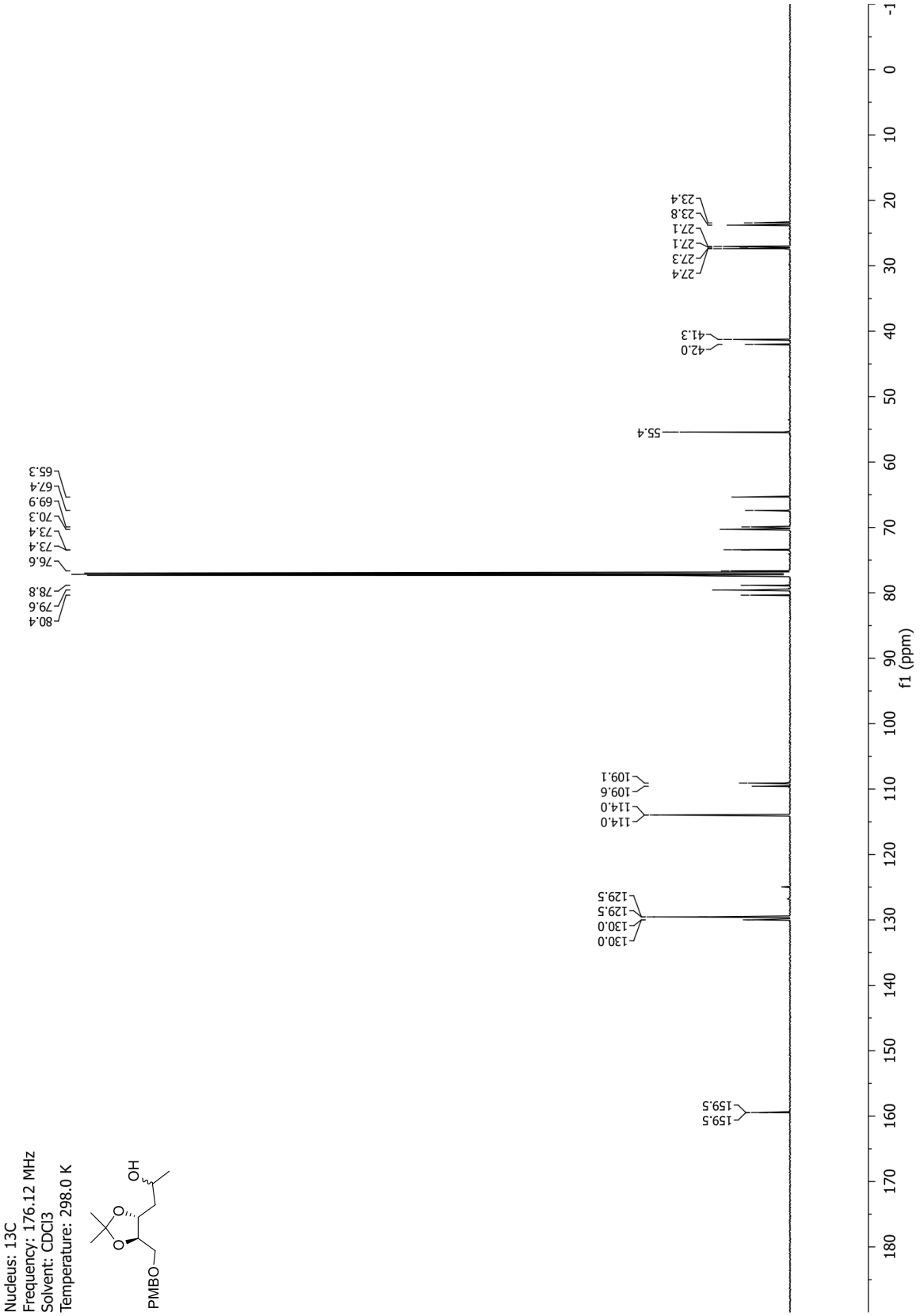
NMR-Spectra for Compound 320



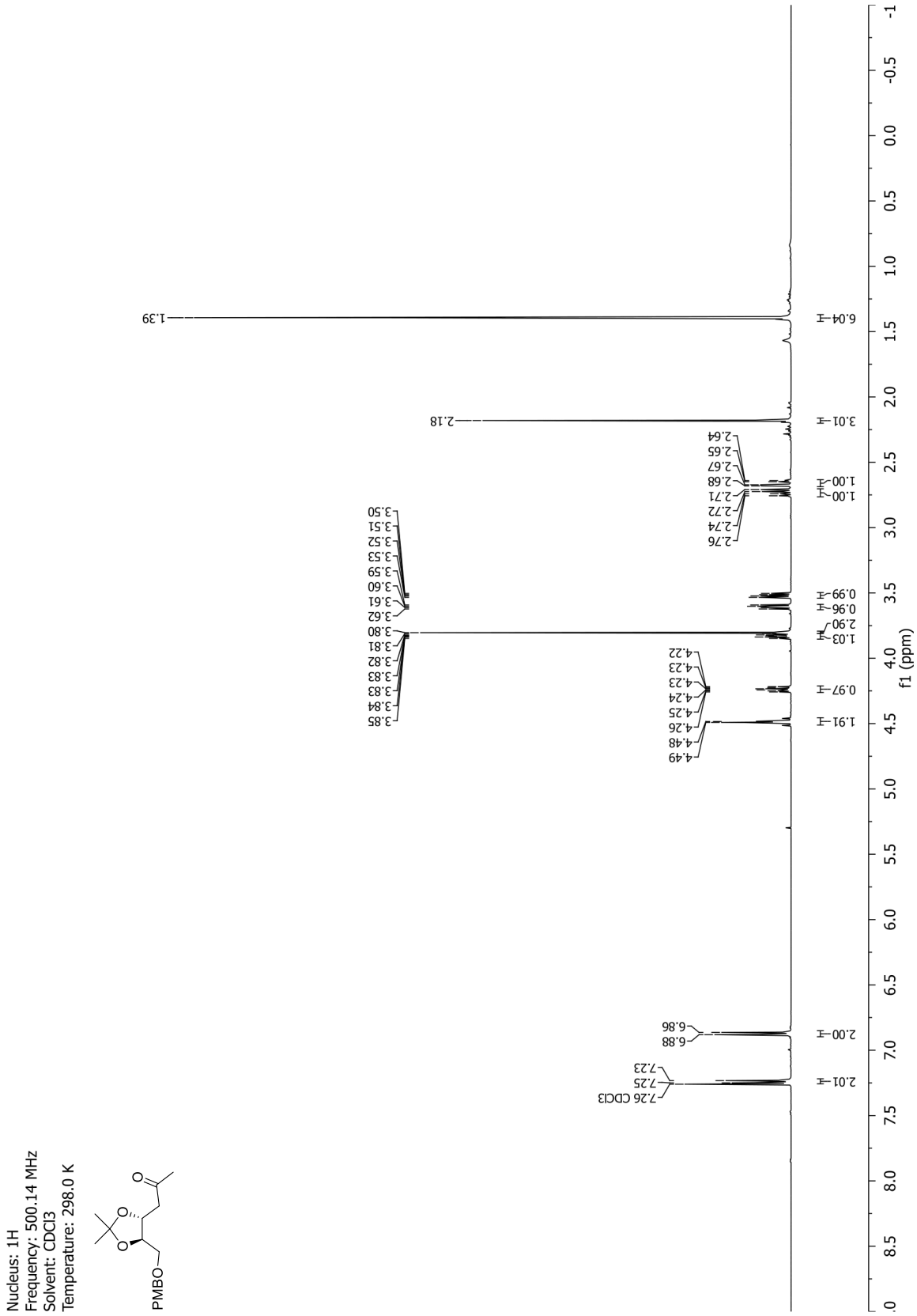

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
200.1
159.5
129.9
129.5
114.0
109.7
79.4
77.2 CDCl3
74.0
73.5
69.9
55.4
47.0
27.2
27.0
f1 (ppm)

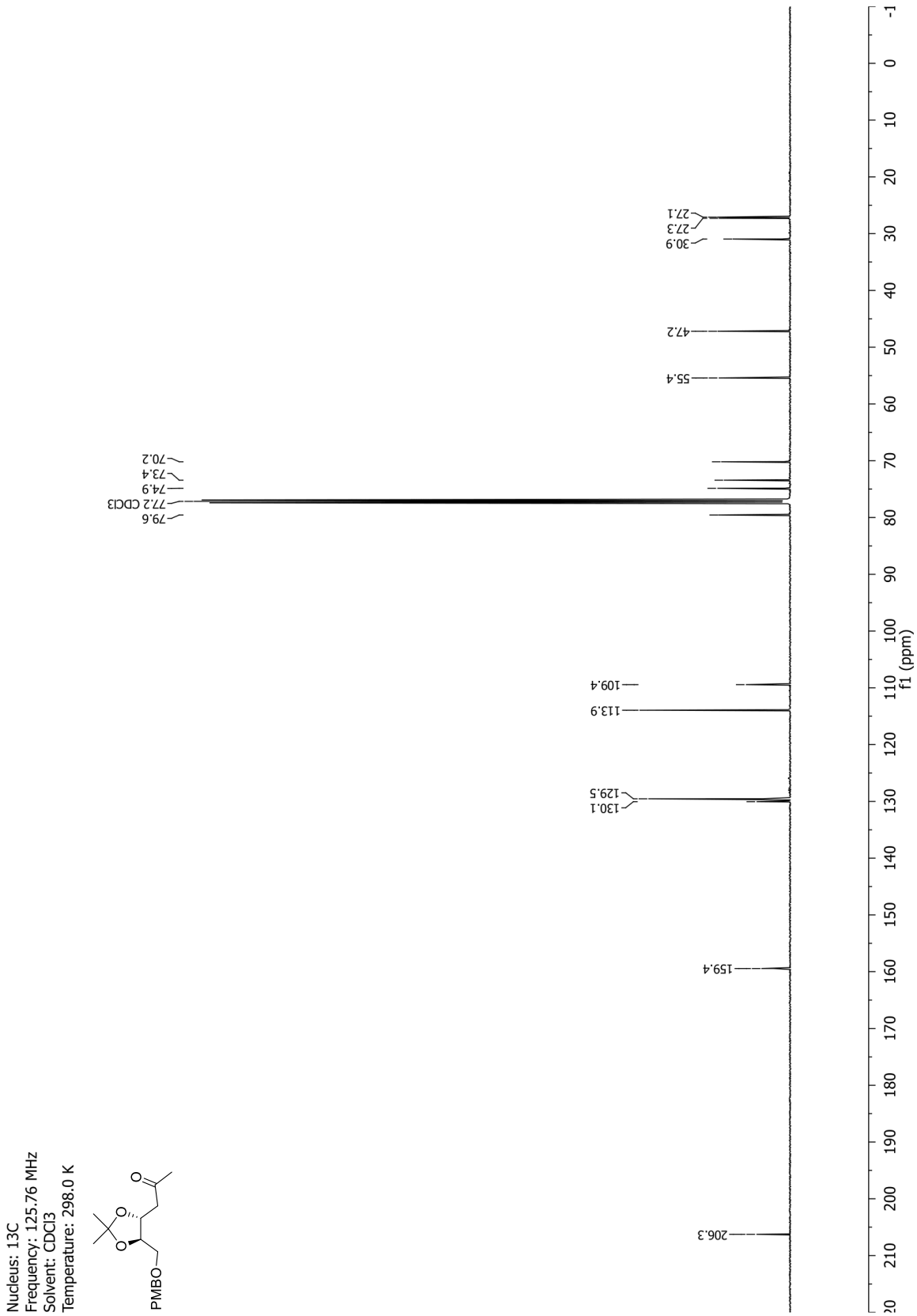
NMR-Spectra for Compound 321



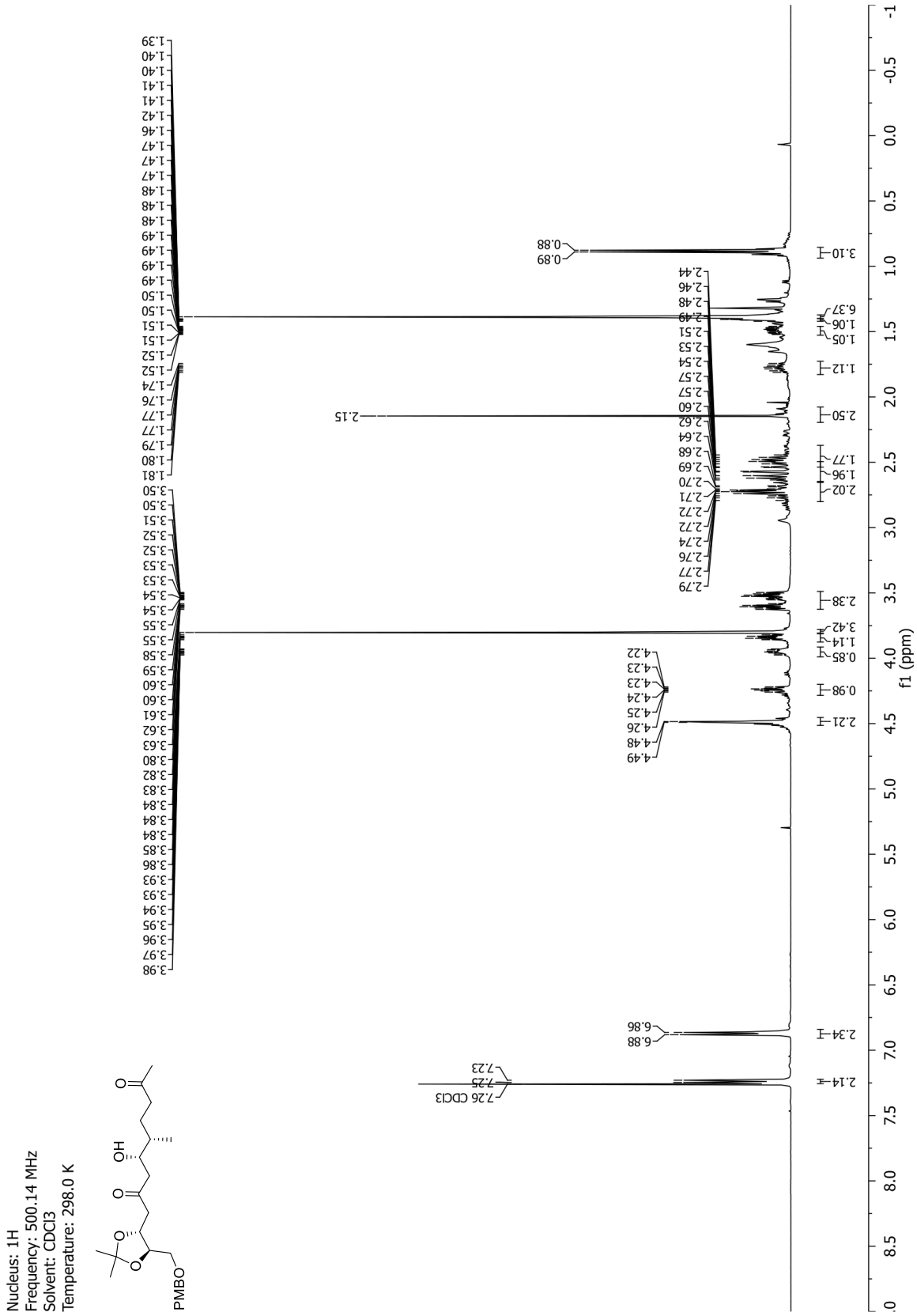

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OH
O
O
PMBO
159.5
159.5
130.0
130.0
129.5
129.5
114.0
114.0
109.6
109.1
55.4
42.0
41.3
27.4
27.3
27.1
27.1
23.8
23.4
80.4
79.6
78.8
76.6
73.4
73.4
70.3
69.9
67.4
65.3
f1 (ppm)

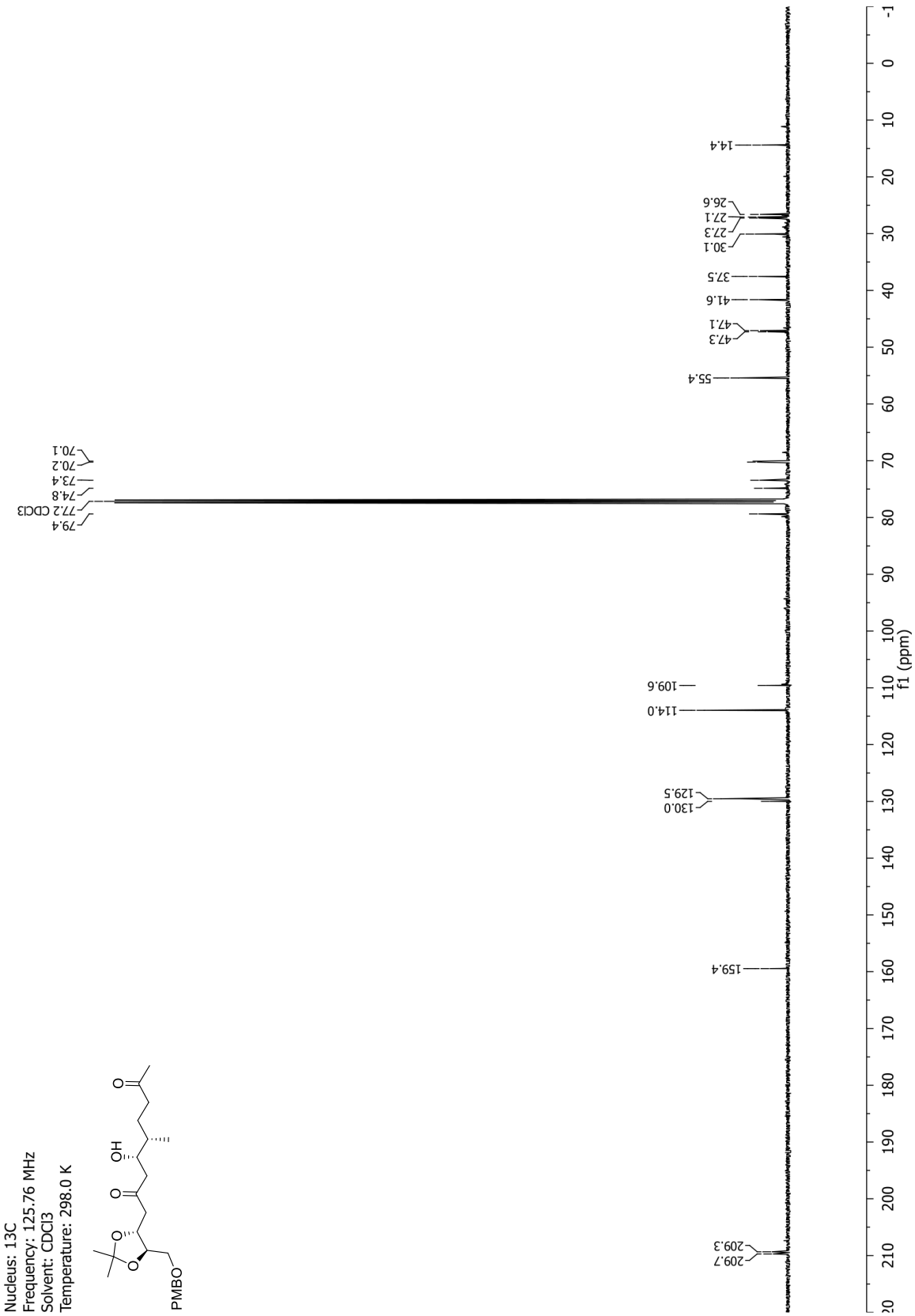
NMR-Spectra for Compound 13



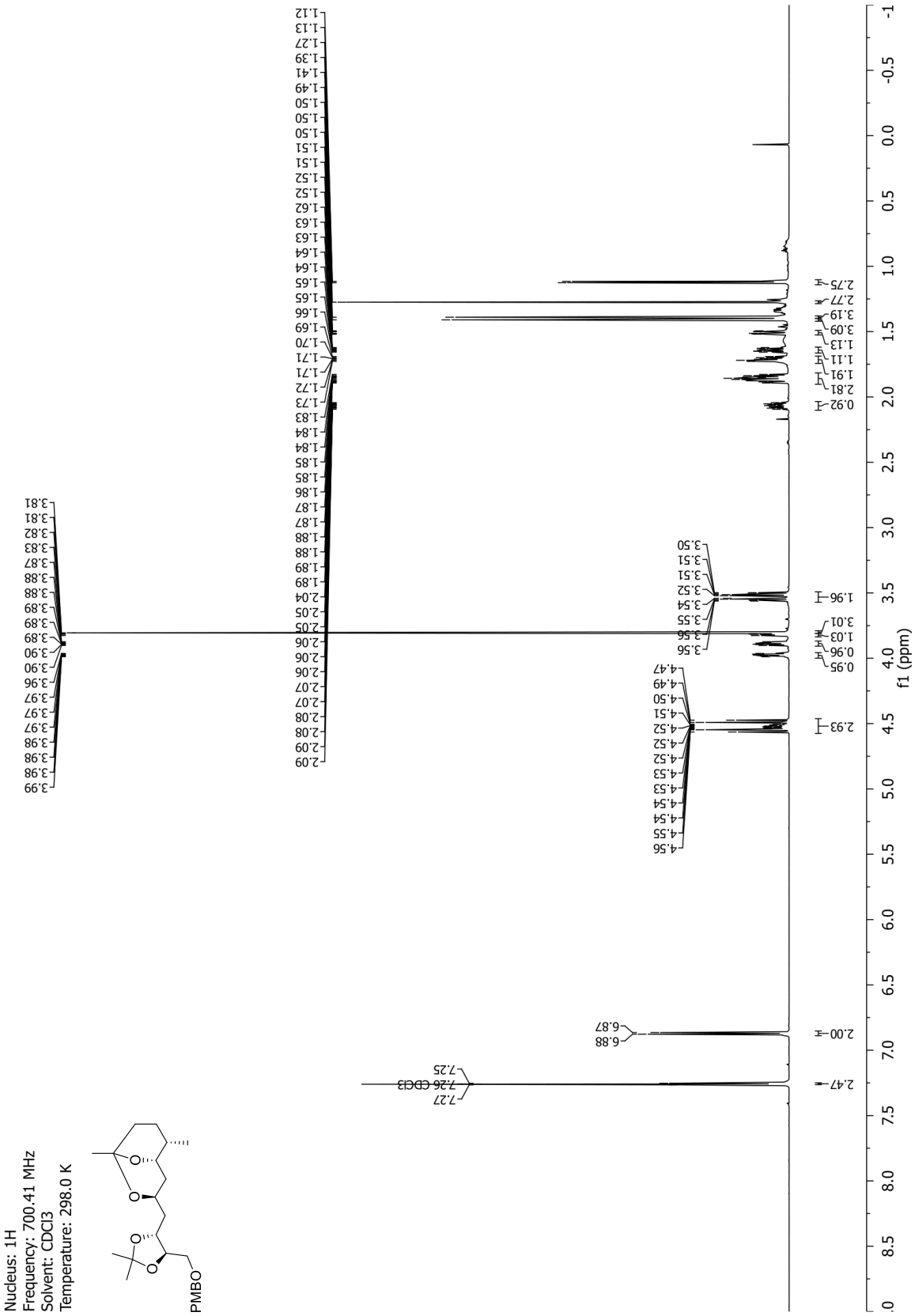

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
206.3
159.4
130.1
129.5
113.9
109.4
79.6
77.2 CDCl3
74.9
73.4
70.2
55.4
47.2
30.9
27.3
27.1
f1 (ppm)

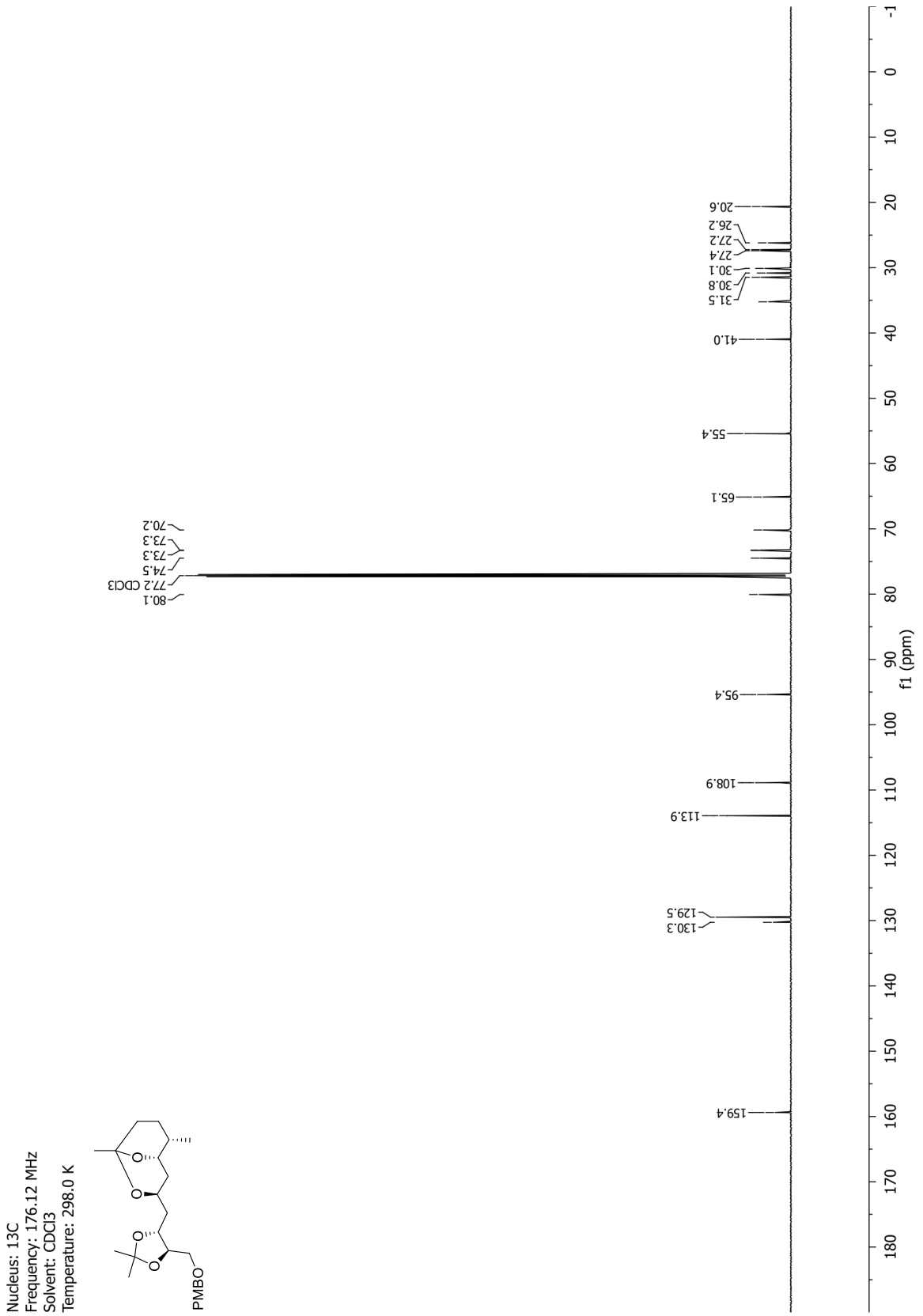
NMR-Spectra for Compound 327



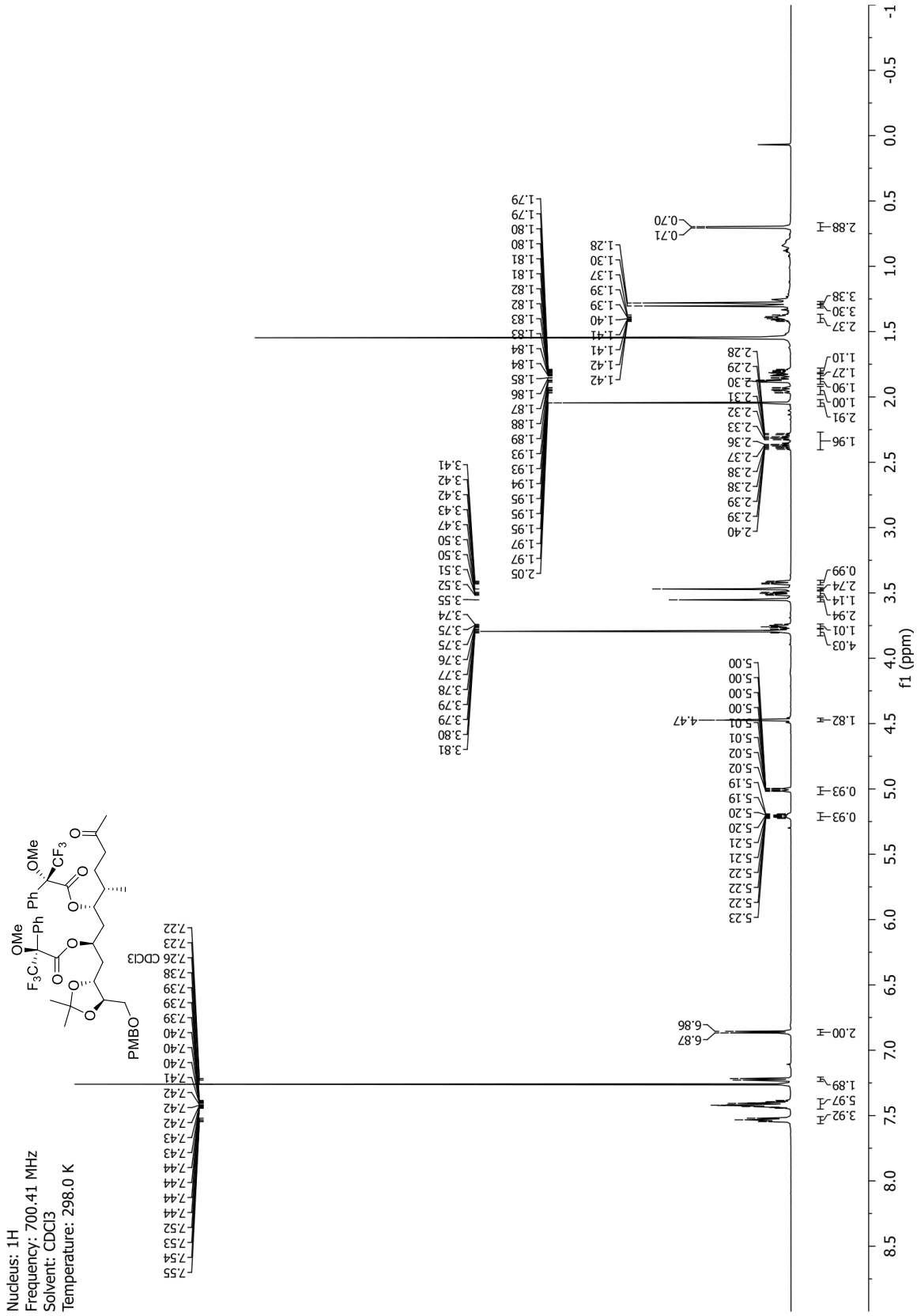

Nucleus: 13C
Frequency: 125.76 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
OH
O
209.7
209.3
159.4
130.0
129.5
114.0
109.6
79.4
77.2 CDCl3
74.8
73.4
70.2
70.1
55.4
47.3
47.1
41.6
37.5
30.1
27.3
27.1
26.6
14.4
f1 (ppm)

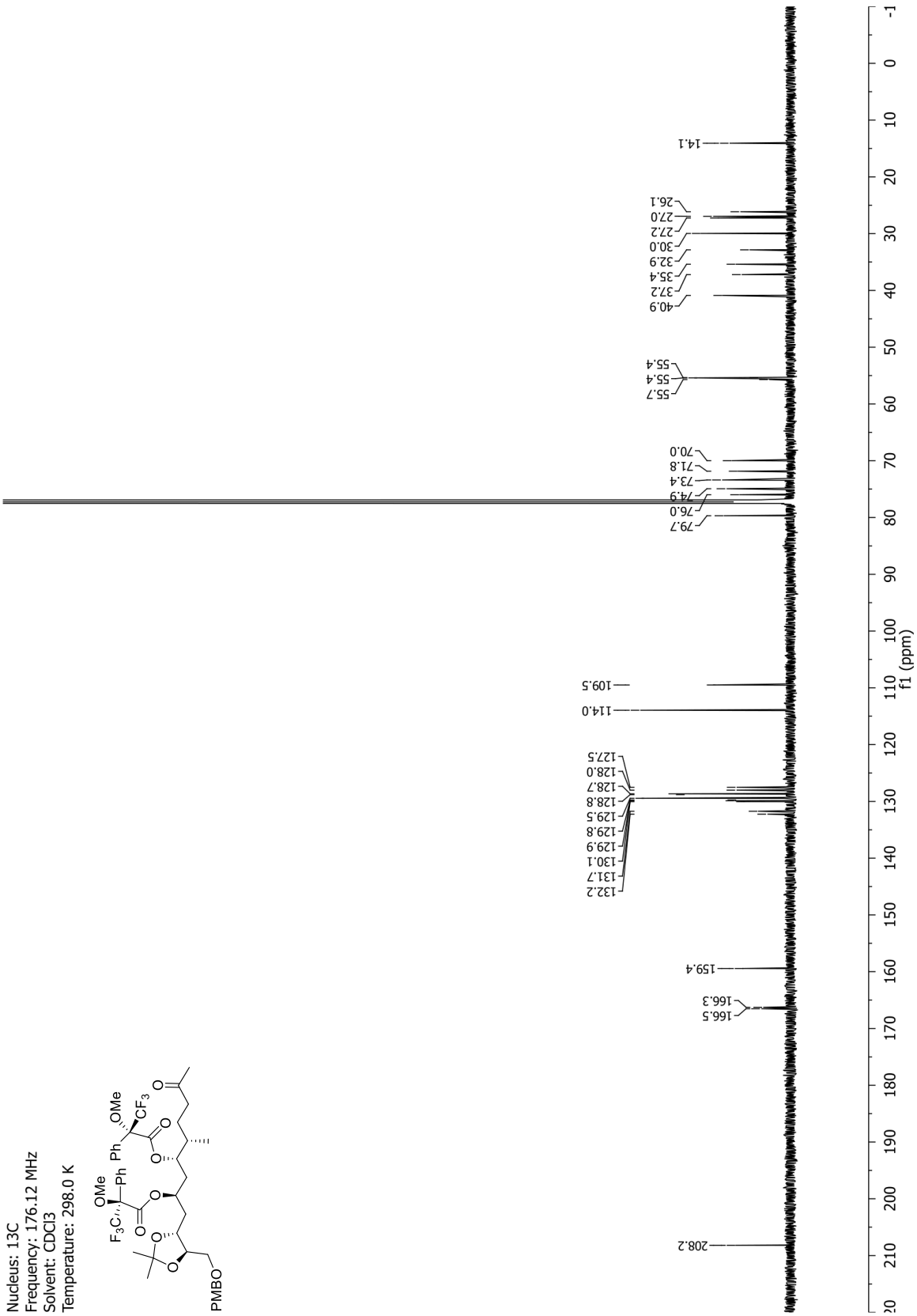
NMR-Spectra for Compound 330



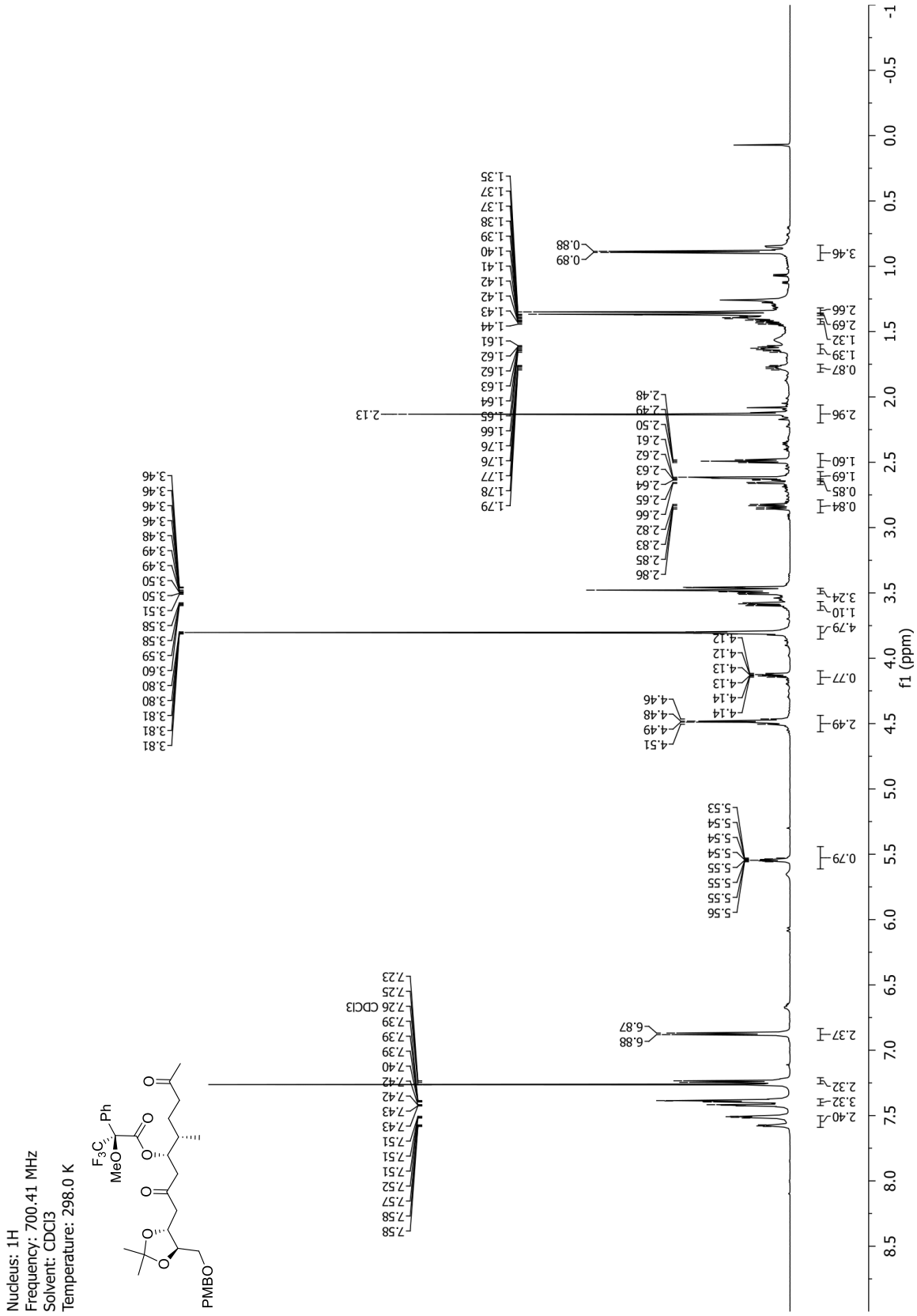

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
PMBO
159.4
130.3
129.5
113.9
108.9
95.4
80.1
77.2 CDCl3
74.5
73.3
73.3
70.2
65.1
55.4
41.0
31.5
30.8
30.1
27.4
27.2
26.2
20.6
f1 (ppm)

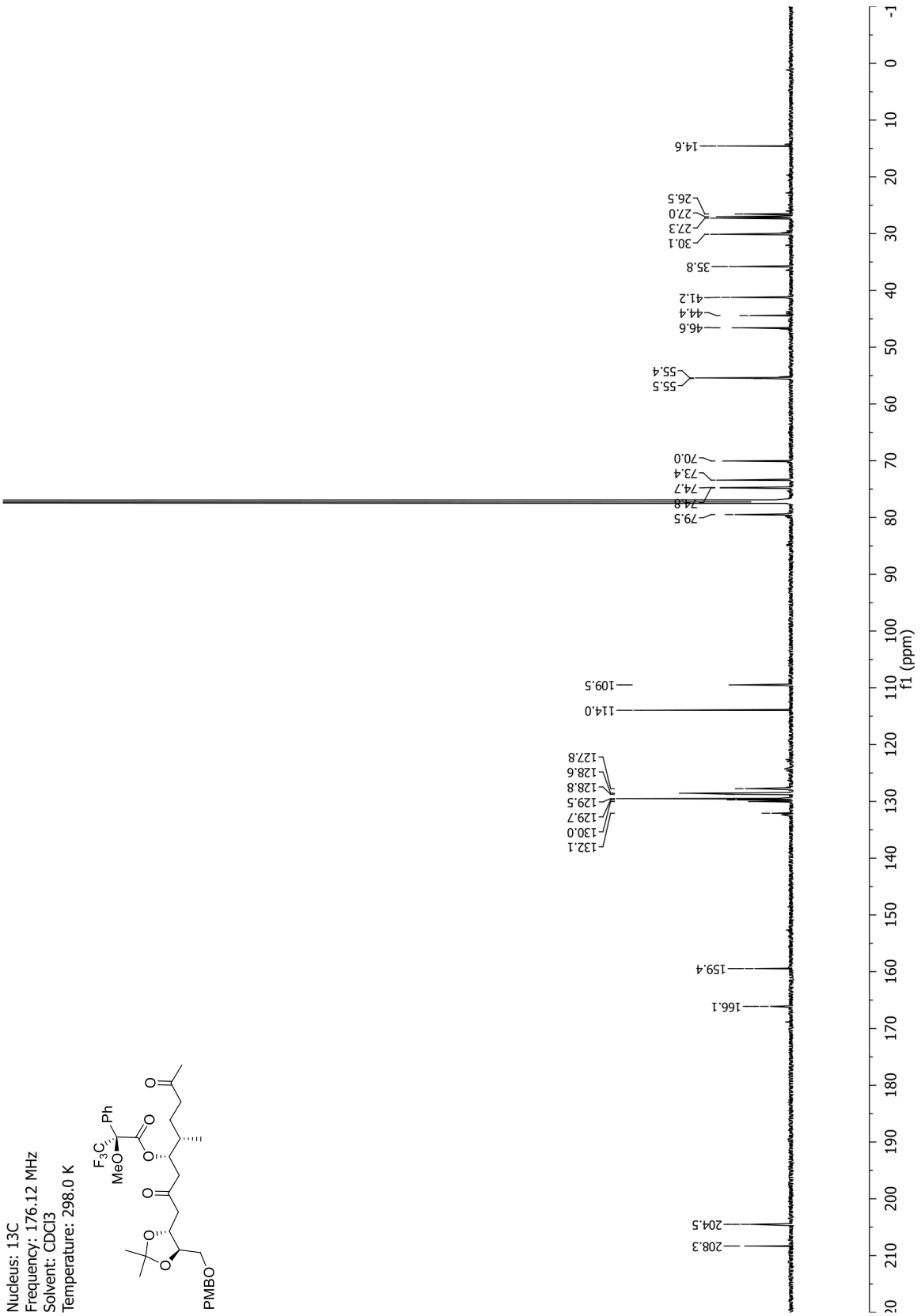
NMR-Spectra for Compound 329



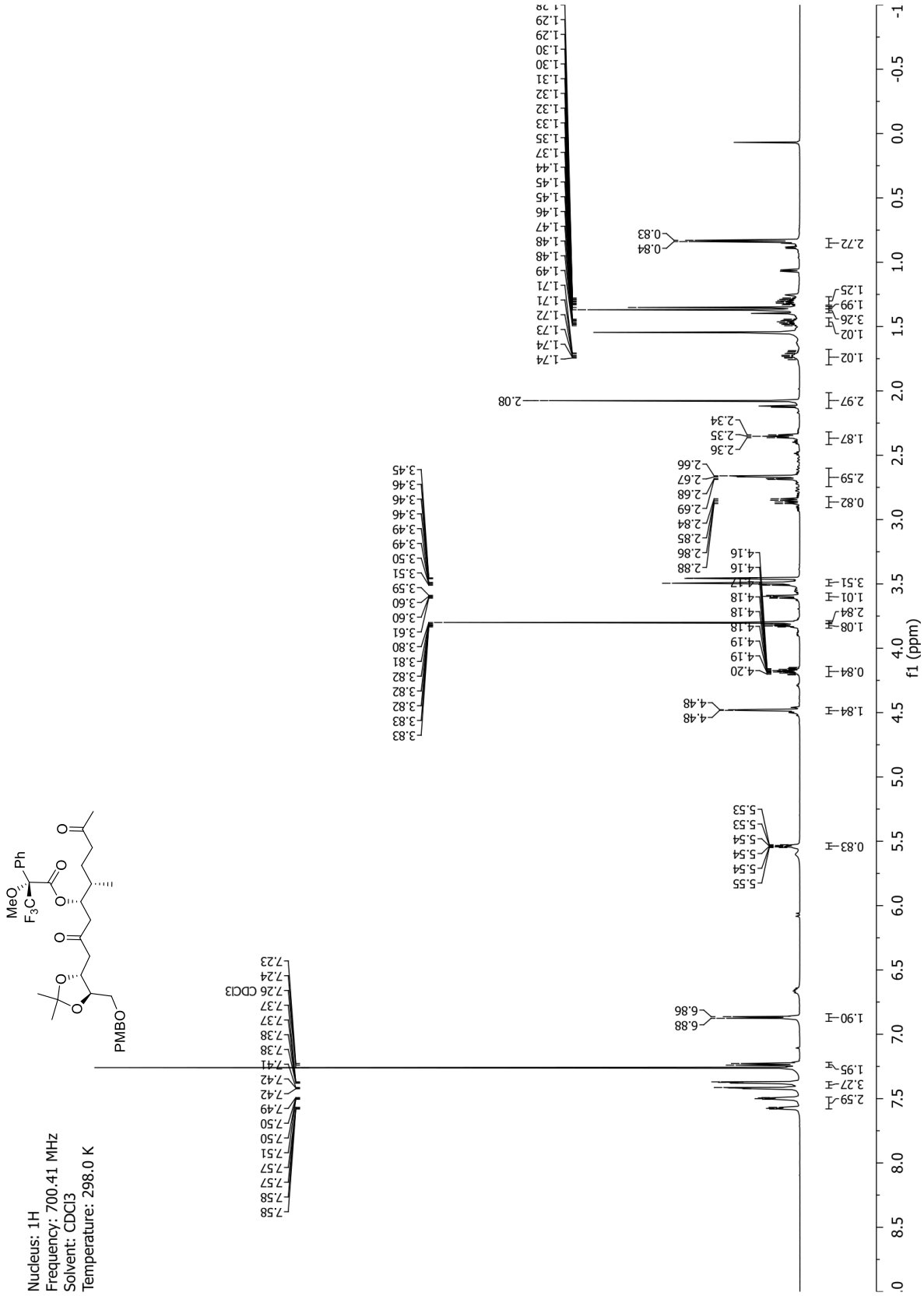

Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
OMe
Ph
F3C
O
O
O
O
O
O
O
OMe
Ph
CF3
O
O
PMBO
208.2
166.5
166.3
159.4
132.2
131.7
130.1
129.9
129.8
129.5
128.8
128.7
128.0
127.5
114.0
109.5
79.7
76.0
74.9
73.4
71.8
70.0
55.7
55.4
55.4
40.9
37.2
35.4
32.9
30.0
27.2
27.0
26.1
14.1
f1 (ppm)

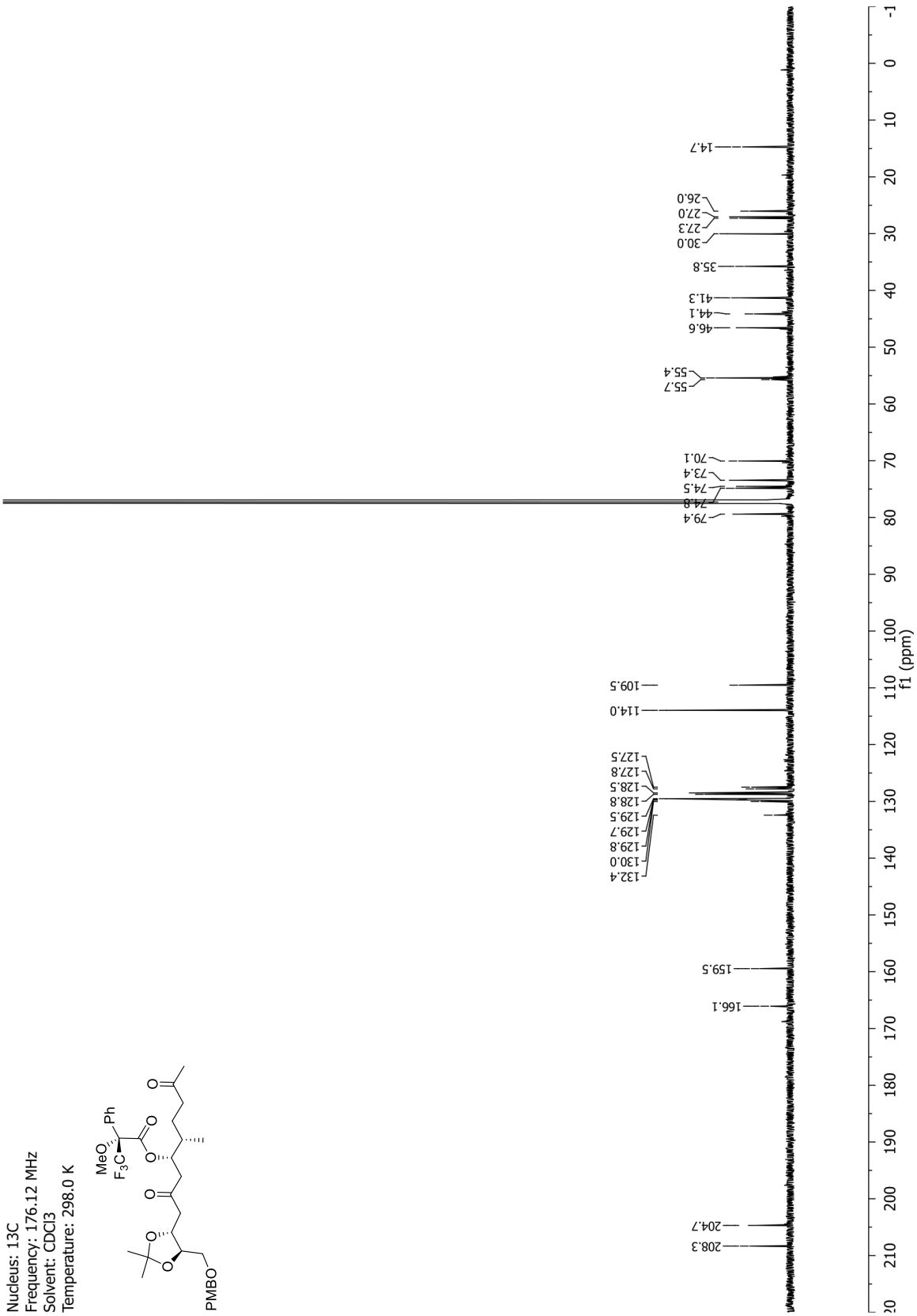
NMR-Spectra for Compound 340




Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
F3C
Ph
MeO
PMBO
14.6
26.5
27.0
27.3
30.1
35.8
41.2
44.4
46.6
55.4
55.5
70.0
73.4
74.7
74.8
79.5
109.5
114.0
127.8
128.6
128.8
129.5
129.7
130.0
132.1
159.4
166.1
204.5
208.3
f1 (ppm)

NMR-Spectra for Compound 341




Nucleus: 13C
Frequency: 176.12 MHz
Solvent: CDCl3
Temperature: 298.0 K
MeO
Ph
F3C
PMBO
14.7
26.0
27.0
27.3
30.0
35.8
41.3
44.1
46.6
55.4
55.7
70.1
73.4
74.5
74.8
79.4
109.5
114.0
127.5
127.8
128.5
128.8
129.5
129.7
129.8
130.0
132.4
159.5
166.1
204.7
208.3
f1 (ppm)

8 References

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