Development of *Ortho*-Substituted Aromatic (Thio)ureas and Derived Heterocycles as Modulators of P-Glycoprotein and Multidrug Resistance-Associated Protein 1

Dissertation

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Contents

Abbreviations

ABC	ATP binding cassette
Ac	acetyl
ACE	angiotensin converting enzyme
AChE	acetylcholinesterase
Anal.	combustion elemental analysis
ATP	adenosine 5′-triphosphate
AUC	area under the curve
BCRP	breast cancer resistance protein
Bn	benzyl
Bz	benzoyl
calcd	calculated
calcein AM	acetoxymethyl ester of calcein
CDI	N,N'-carbonyldiimidazole
CEase	cholesterol esterase
c-Hex	cyclohexyl
compd	compound
concd	concentrated
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EC_{50}	effective concentration required to induce a 50% effect
equiv	equivalent
Et	ethyl
FT	Fourier transform
HB	hydrogen bond
HLE	human leukocyte elastase
HMBC	heteronuclear multiple bond correlation
HMQC	heteronuclear multiple quantum correlation
IC_{50}	concentration required for 50% inhibition
i-Pr	isopropyl
IR	infrared
lit.	literature value
M	molecular mass
MDR	multidrug resistance
Me	methyl

mp	melting point
MRP	multidrug resistance-associated protein
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
NBD	nucleotide-binding domain
ni	no inhibition
p53	tumor protein 53
PET	positron emission tomography
P-gp	P-glycoprotein
Ph	phenyl
PPA	polyphosphoric acid
ppm	parts per million
R_F	retardation factor
ref.	reference
rt	room temperature
SD	standard deviation
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
TMD	transmembrane domain

CHAPTER 1

Introduction

1.1 ATP-Dependent Transporters and Multidrug Resistance

Failure to respond to chemotherapy is a serious impediment in the treatment of cancer. In this regard, the development of multidrug resistance (MDR) is frequently observed, which leads to a reduced sensibility of cancer cells toward a broad spectrum of cytostatics. The most commonly observed mechanism of MDR is associated with the overexpression of transporters of the ATP binding cassette (ABC) family. These efflux pumps are localized in the cell membrane and reduce the intracellular concentration of multiple structurally and functionally unrelated drugs by an active extrusion at the cost of ATP hydrolysis.[1–5]

Forty eight functional proteins within the human genome belong to the superfamily of ABC transporters. They can be divided into seven subfamilies (ABCA–ABCG) on the basis of their gene structure, order of domains, and sequence homology. The proteins are typically organized as two homologous halves, each containing a highly conserved nucleotide-binding domain (NBD) at the cytoplasmic face of the membrane and a transmembrane domain (TMD). The transmembrane sequences consist of α-helices, separated by hydrophilic loops. In the tertiary structure, the two halves are closely associated to form an internal cavity through which substrates are transported (Figure 1.1).[5–7]

The mechanisms of this transport are not fully elucidated; however, they can be described by widely accepted concepts, *e.g.*, the "hydrophobic vacuum cleaner" and the "flippase" models by Higgins and Gottesman.[8] In the former concept, the transporter pulls the substrate out of the cell membrane and expels it to the extracellular compartment. The latter model suggests that the substrate is translocated from the cytoplasmic to the extracellular leaflet of the membrane bilayer, where it is exposed to the extracellular medium. The catalytic cycle starts with the binding of ATP at the nucleotide-binding sites and the substrate in distinct regions of the TMDs. The protein is then assumed to change its conformation in the transmembrane regions induced by ATP hydrolysis followed by a release of the substrate. The cycle is finished by a resetting to the basal conformation.[5,8–10]

Besides their role in drug resistance of cancer cells, ABC transport proteins are expressed in several non-malignant tissues, among them, important pharmacological barriers of the

human body. For example, various transporters have been identified in the brush border membrane of intestinal cells, the canalicular membrane of hepatocytes, and the blood–brain barrier indicating their pivotal role in detoxification and protection against xenobiotics.[1,3,7] Additionally, ABC transporters can significantly affect bioavailability, accumulation, disposition, and elimination of drugs. Consequently, the evaluation of transport susceptibility of new drugs as well as drug–drug interactions due to inhibition of efflux pumps have become important in drug development and safety.[1,11,12]

While 15 ABC transporters have been observed to export chemotherapeutics *in vitro*, only a few have been implicated so far as major contributors to clinical relevant MDR. These are P-glycoprotein (P-gp/ABCB1), the multidrug resistance-associated proteins 1 and 2 (MRP1/ABCC1 and MRP2/ABCC2), and the breast cancer resistance protein (BCRP/ABCG2). However, the high degree of sequence homology indicates that further ABC transporters might interact with cytostatic drugs.[1,3,5]

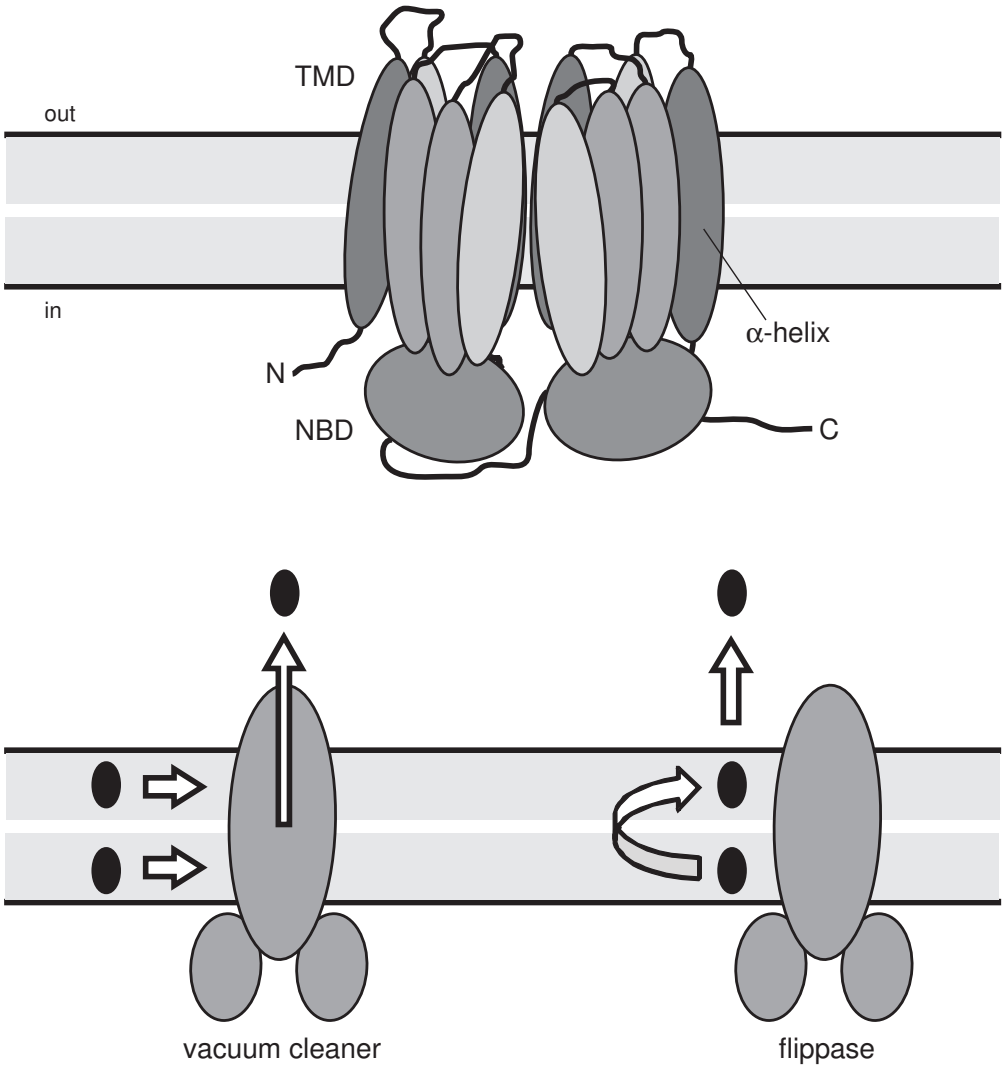


Figure 1.1: Domain organization of ABC transporters in the cell membrane (top) and models for the action of multidrug efflux pumps (bottom; modified from ref.[5]).

1.2 Comparison of the Drug Efflux Pumps P-gp, MRP1/2, and BCRP

P-glycoprotein was the first discovered and is the most extensively studied ABC transporter. In 1976, Juliano and Ling found it to be responsible for colchicine resistance in mutated Chinese hamster ovary cells.[13] The *ABCB1* gene mapped to chromosome 7q21 encodes for this 170 kDa protein which possesses the typical architecture of ABC efflux pumps with two TMDs, each comprised of six α-helices, and two cytoplasmic NBDs (Figure 1.2).[3,14]

P-gp is present at low levels in many human tissues, but is generally highly expressed in epithelial cell layers, for example, in the apical cells of the proximal tubules and the villous membranes of the intestines. In the endothelial cell of the blood–brain barrier it is exclusively orientated toward the blood. It can therefore be assumed that P-gp is involved in restricting drug entry, excreting metabolites, or preventing access to sensitive organs such as the brain.[5,15] High expression levels of P-gp are detectable in tumors derived from cells normally possessing P-gp such as renal and colon carcinomas, however, the protein is also found in tumors derived from cell types with no intrinsic P-gp expression, *e.g.*, bladder and ovarian cancer as well as certain sarcomas.[3,16]

All transporters involved in MDR exhibit unusually broad substrate specificities. P-gp preferentially transports neutral or positively charged hydrophobic substrates, besides other pharmaceuticals, anticancer agents such as anthracyclines, taxanes, epipodophyllotoxins, and *Vinca* alkaloids.[3,5] Radioligand-binding data and functional binding studies indicate that there are different substrate-binding sites within the transmembrane domains of P-gp. The molecular basis for a substrate "polyspecificity" was recently revealed by the three-dimensional structure of mouse P-gp (87% sequence homology to human P-gp). Distinct binding sites in the internal cavity of two additional X-ray structures with co-crystallized cyclic peptide inhibitors could be identified.[10,14,17]

Subsequent to the discovery of P-gp, MRP1 was described in 1992 as the first member of the ABCC subfamily.[18] The 190 kDa membrane-spanning protein is encoded by the *ABCC1* gene located on chromosome 16p13.1. MRP1 shares the common structural motif of ABC proteins. Similar to P-gp, MRP1 is composed of the large core segment containing the nucleotide binding sites and two TMDs but additionally possesses a third transmembrane domain with five helices and an extracellular *N*-terminus (Figure 1.2).[7,19,20] Its amino acid sequence resembles that of P-gp only to a modest extent of ~15%.[15]

MRP1 is broadly expressed in human tissues, *e.g.*, in endothelial cells of brain capillaries, epithelial cells of the digestive, urogenital, and respiratory tracts, endocrine glands, and the hematopoietic system.[15,20] Since its discovery in the H69AR cancer cell line, MRP1 has been found in multidrug-resistant cell lines derived from different tissues and tumor types, for example, in lung, colon, breast, bladder, and prostate cancer, as well as leukemia.[18,21–23] Like P-gp, it is capable of transporting a wide variety of anticancer agents, *e.g.*, anthracyclines, *Vinca* alkaloids, and methotrexate. In contrast to P-gp, MRP1 is facilitating the extrusion of negatively charged compounds such as numerous glucuronate, sulfate, and glutathione conjugates, including the eicosanoid leukotriene C_4.[19,20,24–26]

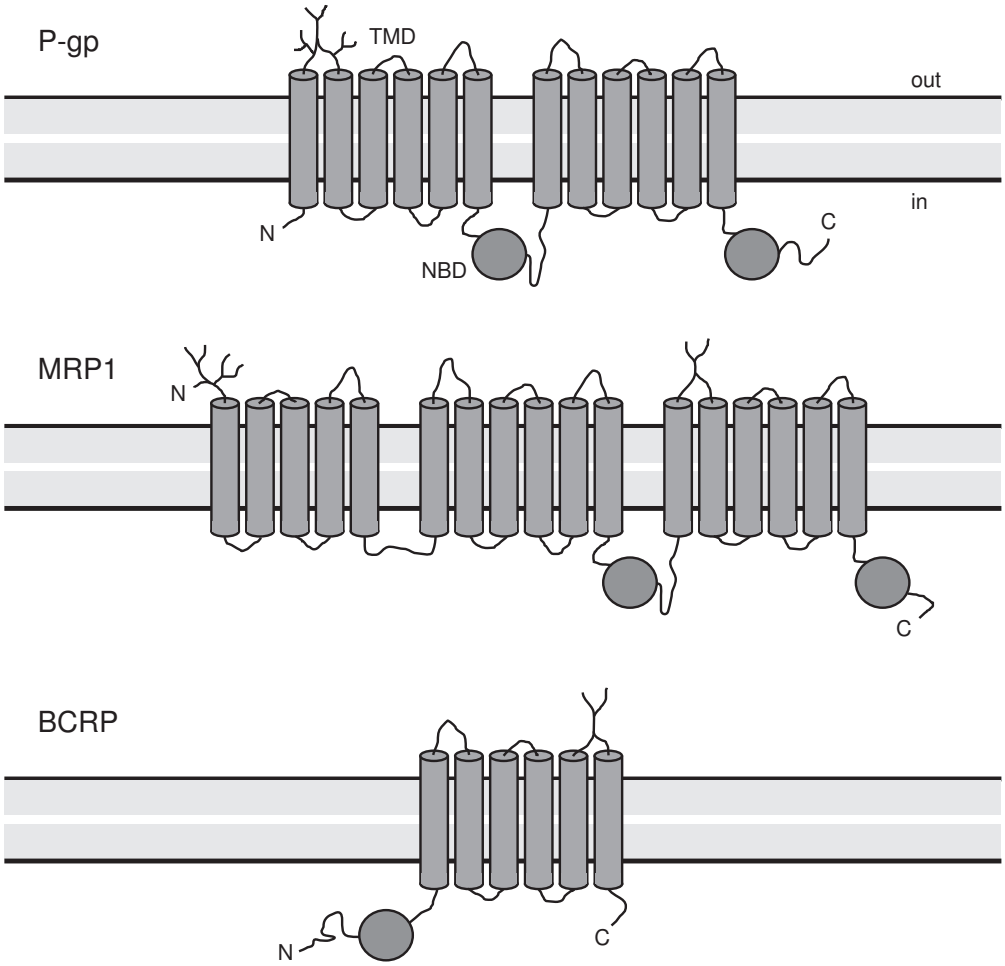


Figure 1.2: Topological models of the ABC efflux pumps P-gp (top), MRP1 (middle), and BCRP (bottom; modified from ref.[5]).

Mayer *et al.* demonstrated the expression of MRP2 and its selective absence in transport-deficient mutant hepatocytes in 1995.[27] Structurally, MRP2 is similar to MRP1 containing 17 transmembrane segments organized in three TMDs and two NBDs, the corresponding *ABCC2* gene has been localized to chromosome 10q24. In contrast to most ABCC subfamily members, which are expressed on basolateral membranes, MRP2 is located in the apical membranes of polarized cells, such as hepatocytes and enterocytes. So, the most important physiological role of this efflux pump is facilitating the export of organic anions, bile acids, and xenobiotics into the bile.[1,11,15,28] Although MRP2 was detected in renal, lung, colorectal and hepatocellular carcinomas, further work is required to define its functional significance in chemotherapeutic drug treatment. The nature of MRP2 substrates is very close to that handled by MRP1. Interestingly, MRP2 (and MRP1) are able to efflux platinum drugs as glutathione complexes.[11,28,29]

The identification of a new member of the ABC transporter family by Doyle *et al.* in a human breast cancer subline suggested that this protein was responsible for MDR in tumor cells without detectable amounts of P-gp and/or MRPs.[30] Those resistances were characterized by decreased accumulation of anticancer drugs such as mitoxantrone and anthracyclines.

BCRP, the *ABCG2* gene product, is a 72 kDa protein with an *N*-terminal NBD and a *C*-terminal TMD (Figure 1.2). Presumably, the half-transporter functions as a homodimer.[11,31] *ABCG2* mRNA expression analyses indicated high levels in the placenta, brain, liver, and intestines. Given its high expression in the placenta, BCRP might protect the fetus from toxins. In addition, BCRP contributes to limiting brain permeation of various compounds.[31] BCRP is overexpressed in several drug resistant cancer cells, *e.g.*, breast, colon, myeloma and fibrosarcoma cell lines, however, its contribution to MDR in cancer should be further evaluated.[1,5,31] The substrate specificity of BCRP is widely overlapping with P-gp and MRP1, although there are some important differences. Like MRP1, BCRP also transports negatively charged compounds and conjugates. On the other hand, it cannot transport *Vinca* alkaloids (substrates of P-gp and MRP1), as well as taxols and verapamil (P-gp subtrates).[5,31]

1.3 Modulators of P-gp and MRP1

There is a wide variety of published compounds that can reverse MDR mediated by ABC transporters. These inhibitors, modulators, or chemosensitizers interfere with the ability of the transporter to efflux drugs *in vitro* and can therefore enhance intracellular accumulation of cytostatics.[5,32] On the molecular level, their mode of action is not well understood. Most inhibitors are likely to bind to the substrate-binding sites mentioned above. There they can effectively compete with the substrates for binding. In many cases, the modulators themselves are transported out by the efflux-pump, followed by a rapid translocation to the inner leaflet of the membrane, thus resulting in a transport cycle under ATP hydrolysis. On the other hand, binding of inhibitors was also described at different regions of the nucleotide binding sites.[5,7,32]

P-gp directed MDR modulators belong to different chemical and pharmacological classes, *e.g.*, representatives of calcium channel blockers, immunosuppressants, calmodulin antagonists, steroids, flavonoids, and anthranilamides.[3,5,33,34] Additionally, they have been categorized into a first, second, and third generation. The first generation was typically identified from registered pharmaceuticals, such as verapamil (Figure 1.3), which was found to overcome P-gp mediated vincristine resistance in leukemia cells (Tsurio *et al.*, 1981).[35] Subsequent studies demonstrated that representatives of other calcium antagonist classes, for instance, dihydropyridines and benzothiazepines also shared the MDR reversing properties of verapamil. Until the beginning of the 1990s, examples from other drug categories followed: the antimalarial quinine, the macrolide antibiotic erythromycin, the antipsychotic chlorpromazine, and the immunosuppressant cyclosporin A, which is one of the most effective first generation MDR modulators. In general, those drugs inhibited ABC transporters at much higher concentrations than those required for their individual therapeutic activity and thus led to severe side effects. Consequently, structural analogs of first generation agents were developed as second generation modulators. For example, the less cardiotoxic dexverapamil (*R*-enantiomer of verapamil) was found to be equally effective in modulating P-gp mediated drug transport. In addition to a reduced toxicity, some compounds such as PSC 833, a non-immunosuppressive analog of cyclosporin A, demonstrated a 10- to 20-fold better efficacy. Moreover, VX-710, a simplified analog of tacrolimus, was developed as an inhibitor of P-gp and MRP1 to treat tumor cells expressing a combination of both transporters.[3,5,32,36]

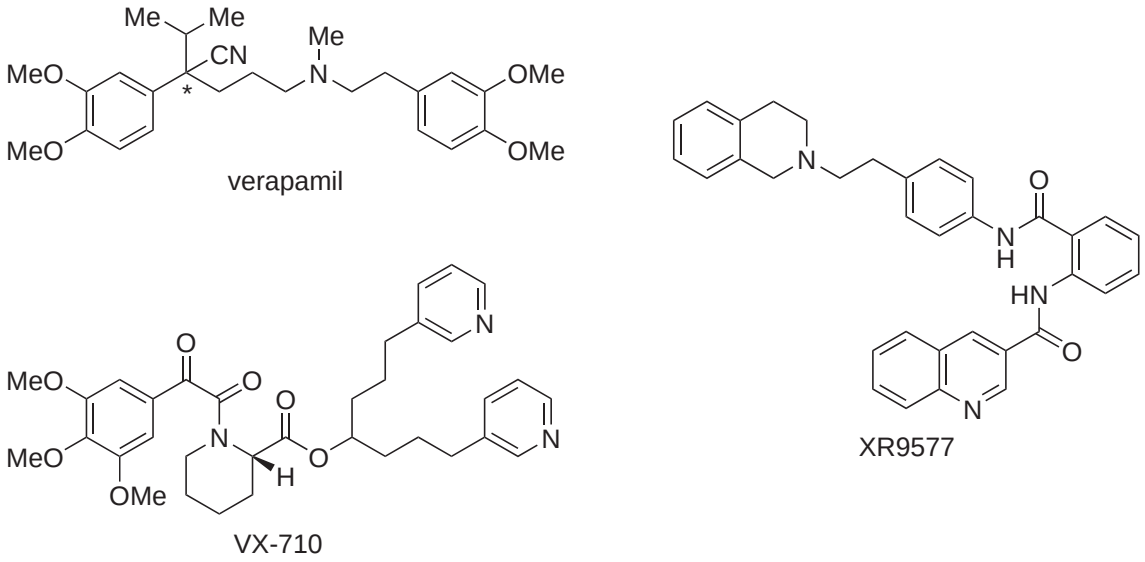


Figure 1.3: Representatives of 1st to 3rd generation P-gp modulators.

The last generation of P-gp inhibiting drugs is characterized by a high selectivity for P-gp and a further increased potency with effective concentrations in the nanomolar range. This group includes the anthranilamide tariquidar and derivatives like WK-X-34 and XR9577 (Figure 1.3).[37–40] Although the lead compound entered two Phase III clinical trials in patients with non-small cell lung cancer, studies were stopped due to chemotherapy-related toxicity.[36] Laniquidar, a 6,11-dihydro-5*H*-imidazo[2,1-*b*][3]benzazepine, was developed as an orally active MDR inhibitor. The ^{11}C labeled derivative [^{11}C]laniquidar was recently evaluated for its feasibility as a PET tracer for imaging P-gp in rats.[41] One of the most potent P-gp inhibitors described, the dibenzosuberane derivative zosuquidar, is still under clinical development.[36]

Chemical structures of selected modulators from the recent literature are depicted in Figure 1.4. Colabufo *et al.* reported on P-gp modulators with a 2-substituted 3-(methyloxyphenylethyl)-phenyl scaffold. Introduction of a *N*-butyltetrahydroisoquinoline side chain (compound **I**) resulted in an effective inhibition of the P-gp mediated [^{3}H]vinblastine transport in Caco-2 cells (IC_{50} = 0.08 μM), whereas transport of [^{3}H]mithoxantrone by BCRP was not affected.[42] The same group evaluated a panel of 2-aryloxazoles and 2-arylthiazoles toward P-gp, BCRP, and MRP1. Although P-gp inhibition dropped in comparison to the reference compounds, the 2-aryloxazole moiety was found to be a versatile scaffold for new BCRP or MRP1 inhibitors. Furthermore, structural features for the modulation of both ABC transporters were presented.[43] Two series of [1,4]thiazino[3,4-*c*]oxadiazol-3-ones were derived from the calcium channel modulator diltiazem.[44] For example, compound **II** reduced the IC_{50} value of doxorubicin in A2780/DX3 cell by ~40%. On the basis of the so-called "frozen-analog" approach, a *N*,*N*-bis-arylalkylamine moiety was substituted by a *N*,*N*-bis(cyclohexanol)amine scaffold. The *trans*/*cis* isomer **III** inhibited pirarubucin efflux from doxorubicin-resistant K562/DIX leukemia cells with an IC_{50} of 0.01 μM. Moreover, it was able to inhibit basal ATPase activity and reversed doxorubicin resistance by factor 36.[45] Little structural variation of rhodamine derivatives resulted in an unexpected alteration of ATPase activity.[46] Exchange of a tertiary amide for a thioamide group at the thienyl moiety of compounds like **IV** caused

ATPase stimulation for the former and effective inhibition for the latter. In MDCKII-MDR1 cells, the thioamide-derivative **IV** promoted calcein AM uptake and inhibited vinblastine efflux with IC_{50} values around ~2 μM, thus in the range of cyclosporin A. Das *et al.* reported on a novel class of cytotoxic agents which reverse MDR.[47] The quinoxaline 1,4-dioxides **V** inhibited proliferation in a number of human tumor cell lines, especially leukemic and breast cancer cells. Interaction with P-gp was measured by intracellular accumulation of rhodamine 123 in *ABCB1*-transfected L-5178 lymphoma cells. The best compounds increased fluorescent intensities of rhodamine 123 comparable to the reference verapamil.

Particularly for P-gp, *in silico* models have been successfully applied to identify new lead structures[33,48,49] and pharmacophore patterns of P-gp ligands addressing different binding sites have been derived.[50–52]

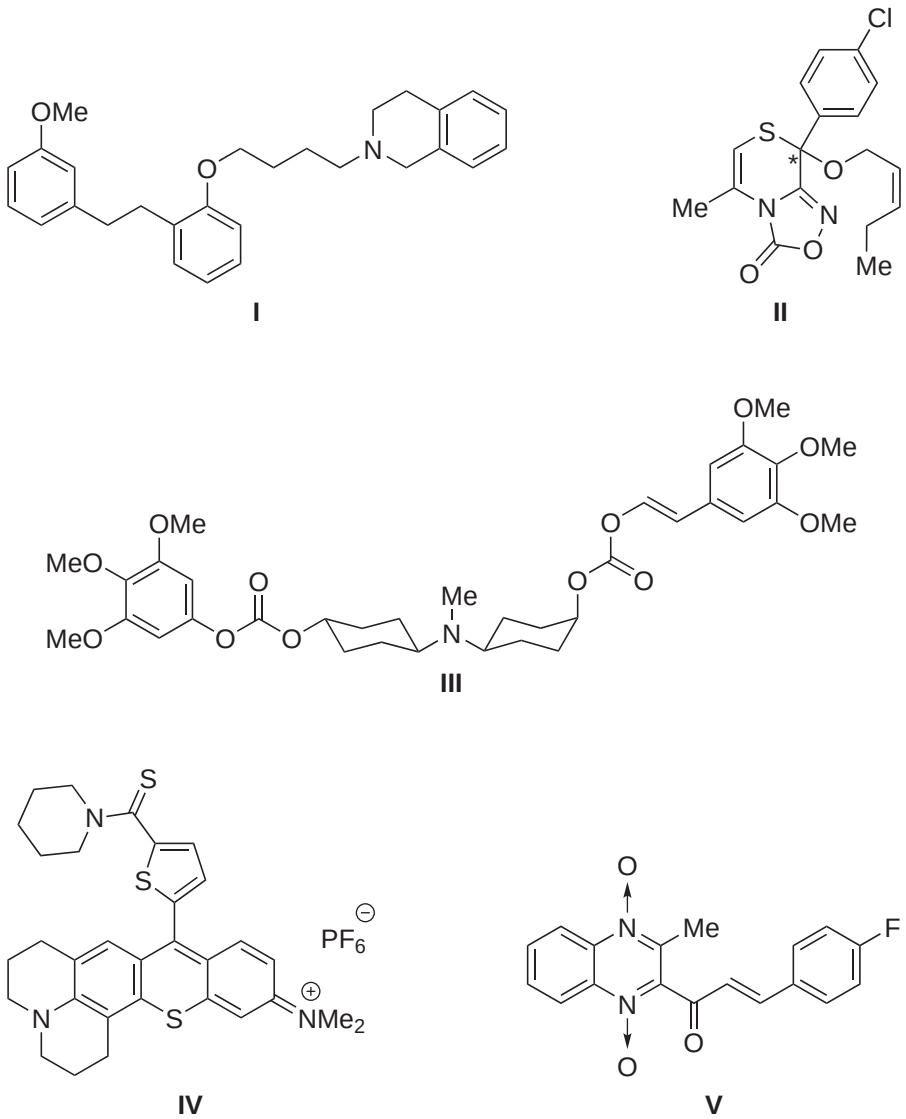
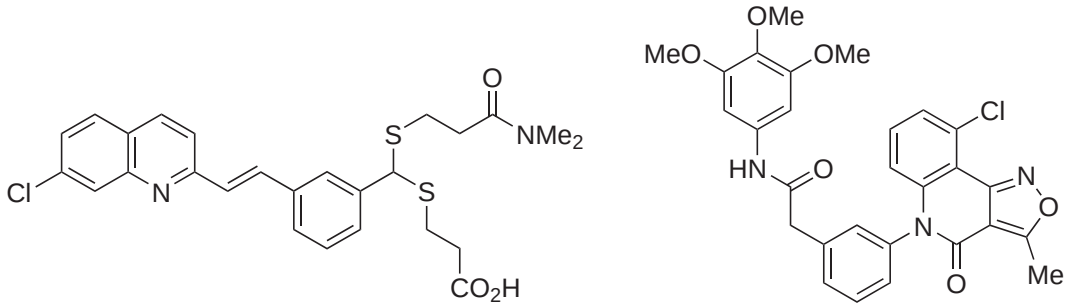


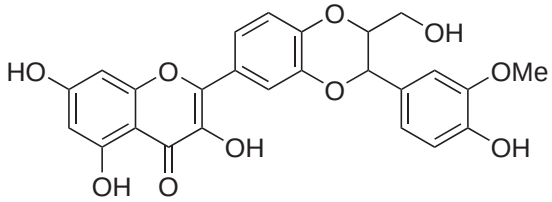
Figure 1.4: Structures of P-gp modulators from recent literature.

Much effort has been focused on the finding of inhibitors for MRP1, but the number of published compounds is considerably lower than those available for P-gp.[3,53,54] Whereas most P-gp inhibitors failed to affect MRP1, cyclosporin A and verapamil proved active against MRP1. Additionally, several specific inhibitors have been described within the last 15 years. The leukotriene D_4 receptor antagonist MK 571 inhibited the leukotriene C_4 transport by membrane vesicles from MRP1-transfected cells,[55,56] and it was frequently used in recent pharmacological studies (Figure 1.5).[57–64] MK 571 exhibited an K_i value of 0.6 μM in MRP1-mediated leukotriene C_4 transport,[55] IC_{50} values of 3.3 μM in ATP-dependent [^{3}H] *para*-aminohippurate transport,[65] and 7.6 μM in the calcein AM assay.[66]

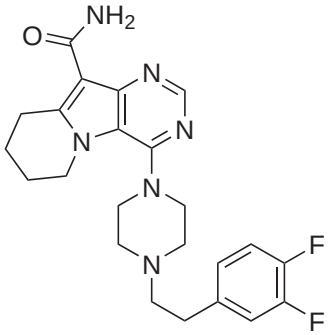


MK 571

LY402913



Dehydrosilybin



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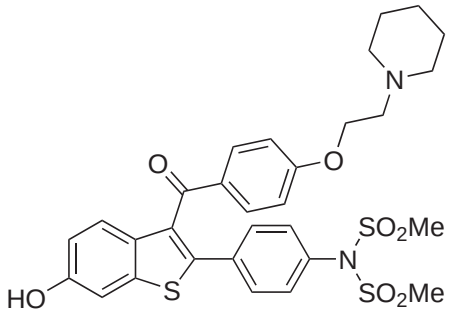
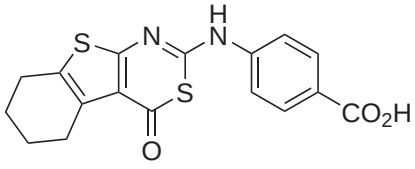


LY329146



VI

Figure 1.5: Structures of known MRP1 inhibitors.

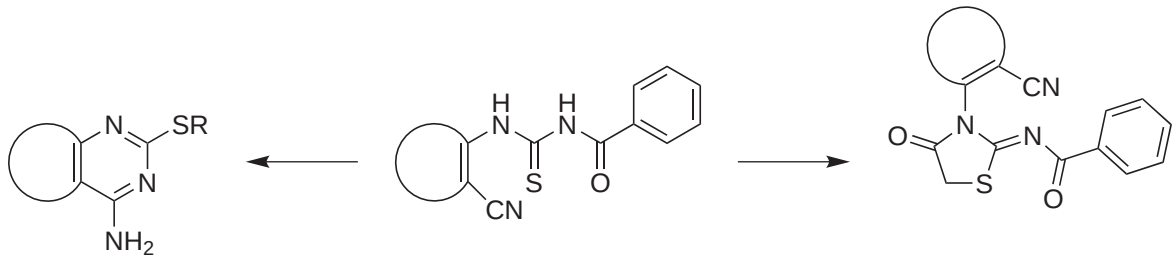
Among various flavonoids,[67] dehydrosilybin was a particularly active inhibitor of leukotriene C_4 transport.[68] Recently, Wong *et al.* demonstrated that (ethylene glycol)-linked flavonoid homodimers effectively restored doxorubicin and etoposid accumulation in 2008/MRP1 cells.[69] The selective estrogen receptor modulator raloxifene was used as a lead structure to conceive inhibitors of MRP1-mediated MDR. The analogue LY329146 with a bis(methylsulfonyl)amino group in place of one hydroxyl group in raloxifene showed improved activity.[70] The tricyclic isoxazole LY402913 was identified as a potent and selective modulator by reversing drug resistance to different MRP1 substrates in HeLa-T5 and A2780 cells. Furthermore, LY402913 delayed tumor progression in a xenograft mouse model.[71,72] Another series of selective and highly efficient MRP1 inhibitors derived from a pyrrolo[3,2-*d*]pyrimidine template was reported by Wang *et al.* Structural modifications led to pyrrolo- and indolopyrimidines, exhibiting IC_{50} values of approximately 0.1 μM. Some derivatives such as XR12890 displayed good pharmacokinetic profiles, *e.g.*, high oral bioavailability and limited interactions with cytochromes P450.[73,74] Recently, we identified the thieno[2,3-*d*][1,3]thiazin-4-one derivative **VI** as being active at MRP1 and 2.[66]

1.4 Objectives of the Dissertation

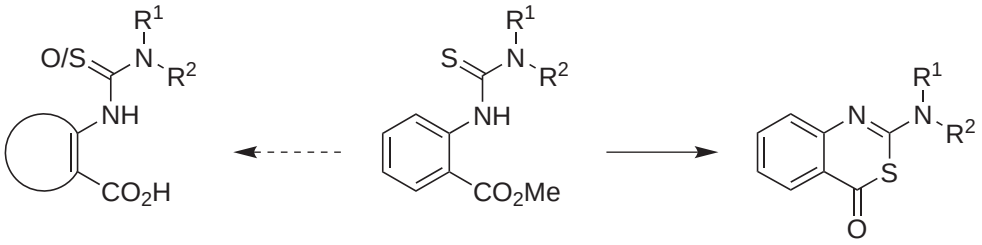
The aim of the presented studies was the synthesis and analytical characterization of potential bioactive molecules, especially *ortho*-substituted aromatic (thio)ureas and derived cyclization products. The main focus was on establishing new synthetic routes to the target compounds and on making them available for various biological testings.

In a first project, it was intended to transform *N*-benzoyl-*N*′-(*o*-cyanoaryl)thioureas to annelated 4-aminothieno[2,3-*d*]pyrimidines and 4-aminoquinazolines with various 2-alkylsulfanyl substituents (Scheme 1.1). The newly prepared heterocycles as well as substances from the compound library of our group were to be tested in the group of Prof. Dr. M. Wiese for their interaction with P-glycoprotein.

An alternative cyclization of the aforementioned *N*-benzoyl-*N*′-(*o*-cyanoaryl)thioureas to 2-(benzoylimino)-3-(*o*-cyanoaryl)thiazolidin-4-ones by an adaption of the reaction conditions was to be investigated (Scheme 1.1). Special attention was to be paid to the influence of the ring size of the *ortho*-cyanoaryl substituent on the occurrence of atropisomerism due to rotational barriers within the molecules.



Scheme 1.1: Transformation of *N*-benzoyl-*N*′-(*o*-cyanoaryl)thioureas to (hetero)fused 4-aminopyrimidines (left) and thiazolidin-4-ones (right).



Scheme 1.2: Aromatic 2-(thio)ureidocarboxylic acids as new modulators of MRP1 (left) and a potential synthetic entry to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones (right).

Another major objective of this dissertation was aromatic carboxylic acids bearing either a substituted urea or thiourea moiety at the neighboring position to the carboxyl group (Scheme 1.2). This structural feature was identified in a screening for new inhibitors of multidrug resistance-associated protein 1 in collaboration with the group of Prof. Dr. M. Wiese. To derive structure–activity relationships for this new class of MRP1 modulators, it was intended to provide four series of *ortho*-(thio)ureidocarboxylic acids with a benzene or thiophene ring as aromatic scaffold. In addition to substances that were taken from the compound library, further derivatives had to be synthesized, such as a set of 2-[3,3-(dialkyl)thioureido]benzoic acids and selected 2-[3,3-(dialkyl)thioureido]thiophen-3-carboxylic acids.

Methyl 2-[3,3-(dialkyl)thioureido]benzoates, that served as precursors for the corresponding benzoic acids mentioned above, were employed to explore synthetic entries to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones (Scheme 1.2). An alternative access from 2-(methylthio)-4*H*-3,1-benzothiazin-4-one and intermediates of the interconversions were also to be studied. To assess possible biological activities, a series of benzothiazinones had to be provided for testing on a panel of eight different proteases and esterases in our group.

The structural characterization of three related salts derived from tetrafluorophthalic acid and isopropyl amine was the focus of the last project. Among the structures, an anomalous salt with two dibasic acid molecules per cation was identified (Figure 1.6). In order to distinguish the anomalous salt from the other structures, crystallographic data and the results of different spectroscopic methods, such as solution and solid-state NMR, were to be combined. Furthermore, a possible involvement of fluorine in hydrogen bonding of the crystal structures had to be elucidated.

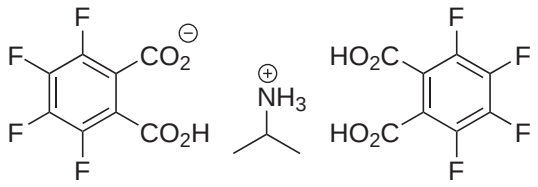


Figure 1.6: Anomalous salt derived from tetrafluorophthalic acid and isopropyl amine.

CHAPTER 2

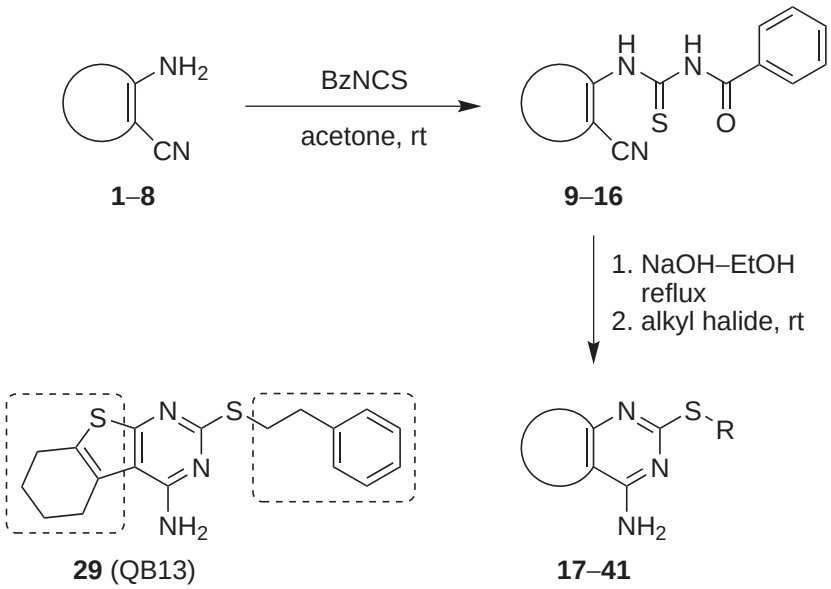
Fused 4-Aminopyrimidines as Modulators of P-Glycoprotein Substrate Specificity

2.1 Substrate-Dependent Bidirectional Modulation of P-gp

In contrast to the wide variety of published P-pg inhibitors, a smaller number of substances showed a reversed effect. They were able to protect cells from distinct cytostatics by stimulation of the efflux pump. Shapiro and Ling[75] identified two positively cooperative drug-binding sites of P-gp. Hoechst 33342 and rhodamine 123 activated the P-gp-mediated transport of each other. Moreover, different groups of cytostatics stimulated either Hoechst 33342 or rhodamine 123 transport. A comparable effect has also been discussed for flavonoids.[76–78] Recently, erlotinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, was found to be a substrate-dependent bidirectional modulator of P-gp.[79]

Kondratov *et al.*[80] investigated a diverse set of p53 inhibitors in a cell-based assay with different P-gp substrates, *e.g.*, doxorubicin, daunorubicin, rhodamine 123, vinblastine, and taxol. The addition of these small molecules improved survival of Con A cells in the presence of certain cytotoxic substrates, while pump activity was decreased for others, thus changing the cross-resistance pattern of P-gp. For example, QB102, a tricyclic tetrahydrobenzimidazo[2,1-*b*]thiazole, increased resistance to the anthracyclines doxorubicin and daunorubicin, but showed the opposite effect for taxol and vinblastine. On the basis of daunorubicin accumulation, other active substances such as the triazacyclopentaindene QB11 and the thieno[2,3-*d*]pyrimidine QB13 (Scheme 2.1) were supposed to behave similar to QB102. This group of compounds was also included in the derivation of a pharmacophore pattern for the Hoechst 33342 binding site of P-gp.[51] In accordance with the biological results, QB11 and QB13 produced similar overlays as QB102. QB13 complied with the pharmacophore in four hydrophobic aromatic centers, one hydrogen bond (HB) acceptor point, and one HB donor point.

It was intended to modify the lead structure QB13 to further characterize the interaction of the analog substances with P-gp. Therefore, the annelated tetrahydrobenzothieno moiety and the phenethylsulfanyl residue of QB13 were varied to compose two sets of target compounds (Scheme 2.1, dashed boxes).



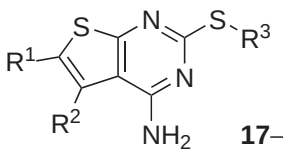
Scheme 2.1: Structure of the lead compound **29** (QB13) and synthetic route to compounds **17**–**41**.

2.2 Thieno[2,3-*d*]pyrimidines and Quinazolines as Analogs of QB13

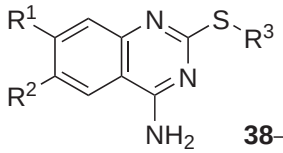
In the first set (Table 2.1, **18**–**31**), the sulfanyl substituent in position 2 of the heterosystem was diversified for dimethyl substituted (**18**–**24**) and tetrahydrobenzothieno[2,3-*d*]pyrimidines (**25**–**31**). Besides the phenethyl residue of **29** (QB13), small alkyl (Me, Et), polar aliphatic ($(CH_2)_2OH$, CH_2CO_2Et) and aromatic substituents (CH_2COPh, $CH(Ph)CO_2H$) were included. Within this set, seven out of 14 compounds were newly prepared, the others were taken from the substance library (see Table 2.1). The second set (**32**–**41**) was intended to discover possible influences of the ring system fused with the aminopyrimidine. Three tricyclic thienopyrimidine templates (**32**–**37**) as well as two quinazolines (**38**–**41**) were combined with the parent phenethylsulfanyl residue ($(CH_2)_2Ph$) or the polar ester function (CH_2CO_2Et) in position 2.

The synthesis of the target compounds was accomplished in two or three steps (Scheme 2.1). 2-Aminothiophenes (**2**–**6**) were prepared by Gewald synthesis.[81] For this purpose, a one-pot version including the condensation of an appropriate ketone, malononitrile and sulfur in the presence of diethylamine was applied.[82] A direct transformation of acetaldehyde by this one-pot technique is not possible,[83] therefore, 2-aminothiophene-3-carbonitrile (**1**) was generated from 1,4-dithiane-2,5-diol.[84] Subsequently, **1**–**6** as well as commercially available *o*-aminobenzonitriles **7**, **8** were reacted with benzoyl isothiocyanate to afford benzoylthioureas **9**–**16** (Table 2.1). Thermal ring closure of these intermediates was effected by sodium hydroxide in an ethanol–water mixture, followed by the addition of alkyl halides.[85] The conditions of the last reaction step were slightly adopted, *i.e.*, prolongation of heating, to restrain the formation of alternative cyclization products (see Chapter 3).

Table 2.1: Thieno[2,3-*d*]pyrimidines **17**–**37** and quinazolines **38**–**41** with corresponding yields and EC_{50} values in the daunorubicin accumulation assay.



17–**37**



38–**41**

Compd	R^1	R^2	R^3	Yield[a] (%)	Prepared From	$EC_{50} \pm SD$[b] (μM)
17	H	H	CH_2CO_2Et	58	**9**	nd[c]
18*	|	|	Me			1.31 ± 0.49
19	|	|	Et	51	**10**	4.59 ± 0.94
20*	|	|	$(CH_2)_2OH$			nd[d]
21	Me	Me	CH_2CO_2Et	42	**10**	0.90 ± 0.27
22	|	|	$(CH_2)_2Ph$	32	**10**	2.25 ± 0.89
23	|	|	CH_2COPh	84	**10**	1.31 ± 0.63
24*	|	|	$CH(Ph)CO_2H$			> 100
25*		|	Me			2.65 ± 0.80
26*		|	Et			0.46 ± 0.14
27*		|	$(CH_2)_2OH$			1.92 ± 0.14
28		$-(CH_2)_4-$	CH_2CO_2Et	48	**12**	2.24 ± 0.43
29		|	$(CH_2)_2Ph$	72	**12**	1.02 ± 0.48
30		|	CH_2COPh	77	**12**	0.64 ± 0.12
31*		|	$CH(Ph)CO_2H$			> 100
32		$-(CH_2)_3-$	CH_2CO_2Et	65	**11**	4.15 ± 1.59
33		$-(CH_2)_3-$	$(CH_2)_2Ph$	61	**11**	0.97 ± 0.21
34		$-CH_2-O-(CH_2)_2-$	CH_2CO_2Et	74	**13**	1.55 ± 0.42
35		$-CH_2-O-(CH_2)_2-$	$(CH_2)_2Ph$	47	**13**	0.56 ± 0.04
36		$-CH_2-N(Bn)-(CH_2)_2-$	CH_2CO_2Et	49	**14**	0.64 ± 0.19
37		$-CH_2-N(Bn)-(CH_2)_2-$	$(CH_2)_2Ph$	25	**14**	nd[d]
38	H	H	CH_2CO_2Et	55	**15**	3.98 ± 1.02
39	H	H	$(CH_2)_2Ph$	69	**15**	1.10 ± 0.33
40	OMe	OMe	CH_2CO_2Et	68	**16**	1.21 ± 0.60
41	OMe	OMe	$(CH_2)_2Ph$	67	**16**	1.52 ± 0.35

* Compound was taken from the substance library.
[a] Yields of products after recrystallization.
[b] Values are means of at least three experiments.
[c] Compound was not included in the biological evaluation.
[d] Compound was not determined due to limited solubility.

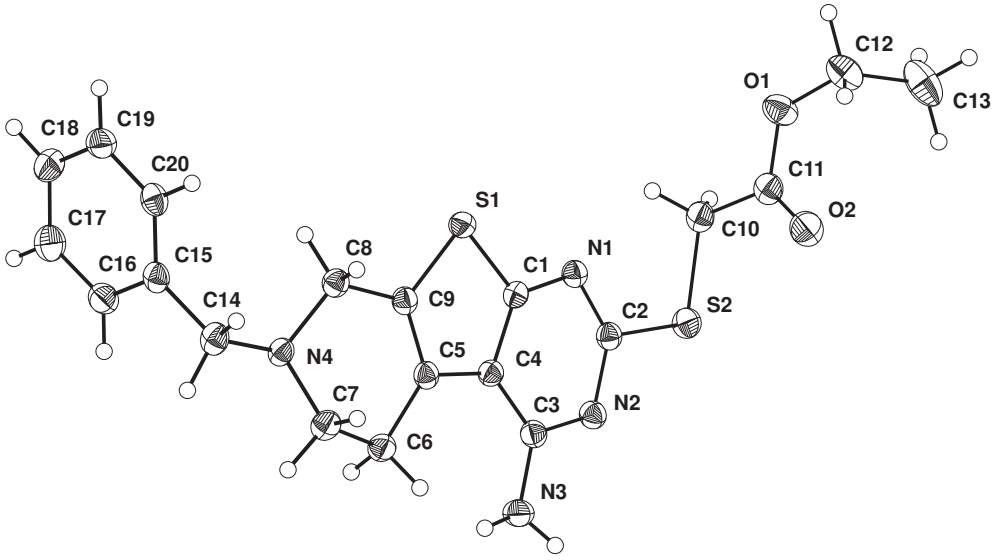


Figure 2.1: Molecular plot of **36** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level for the non-H atoms. H atoms are depicted as small circles of arbitrary radii.[86]

Structure and purity of the products were confirmed by NMR and elemental analyses. Two-dimensional NMR experiments (HMQC, HMBC) were carried out for the thieno[2,3-*d*]pyrimidine **19** and the quinazoline **38** (see Appendix and Supplementary Information of ref.[87]). To the best of our knowledge, ^{13}C assignments of (hetero)-annelated [*d*]pyrimidines are only reported for (hetero)aryl substituted 4-aminoquinazolines and 4-aminopyrazolo[3,4-*d*]pyrimidines.[88,89] Values obtained, *e.g.*, for compound **38** (C-4, 161.3 ppm; C-2, 165.6 ppm) are in accordance with selected C=N signals given for 4-amino-2-(2-thienyl)quinazoline (160.0 and 164.8 ppm). In the thieno[2,3-*d*]pyrimidines, the sequence of both signals was reversed (for example, **21**: C-2, 163.0 ppm; C-4, 165.4 ppm). To complete structural elucidation, **36** was subjected to X-ray diffraction analysis (Figure 2.1).[86]

2.3 Daunorubicin Accumulation and Cell Viability Assays

Biological evaluations were performed by A. de la Haye and K. Sterz from the group of Prof. Dr. M. Wiese.[90] At first, the effects of the substances on daunorubicin accumulation in the human ovarian cancer cell line A2780 and its P-gp overexpressing counterpart A2780 Adr were investigated. Cells were pre-incubated with the compounds and daunorubicin was added as fluorescent substrate of P-gp. The intracellular fluorescence was measured using a Becton Dickinson FACS-calibur. Details of the assay and the statistical analyses are given elsewhere.[38,87,90]

Most compounds showed effects in the daunorubicin accumulation assay. Those reduced intracellular fluorescence in A2780 Adr cells with EC_{50} values between 0.5 and 5 μM (Table 2.1, Figure 2.2), thus behaved similarly as activators of P-gp. This is in accordance with the effect of QB13 on the accumulation of daunorubicin in Con A cells.[80]

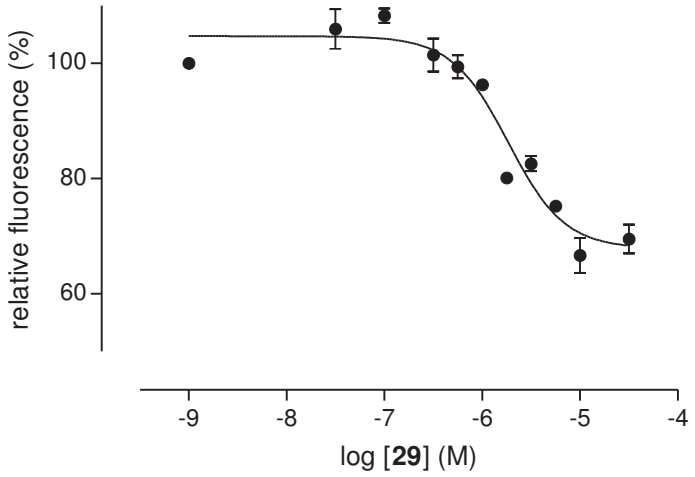


Figure 2.2: Concentration–effect curve of **29** (QB13) in the daunorubicin accumulation assay. The curve represents the average of three independent experiments.

When considering pairs with the same sulfanyl moiety within the first set (**18**–**31**), a clear effect of the dimethyl–tetramethylene exchange (**18**–**24** *vs.* **25**–**31**) in the thieno[2,3-*d*]pyrimidines was not observed. Three out of seven compounds with aliphatic side chains (**18**, 1.31 μM, **21**, 0.90 μM, and **26**, 0.46 μM) had activities better or comparable to the lead compound **29** (1.02 μM). The two phenacyl derivatives **23** (1.31 μM) and **30** (0.64 μM) were the most potent aromatic representatives. Substituted phenylacetic acids (**24**, **31**) showed no effect on daunorubicin accumulation. This is in agreement with literature data, as P-gp generally does not transport negatively charged compounds.

In the second set (**32**–**41**), most phenethyl derivatives were superior to analogous ethyl acetates. Only the dimethoxyquinazolines **40**, **41** had comparable EC_{50} values. The pyrano derivative **35** (0.56 μM) and the *N*-benzyl-pyrido annelated ester **36** (0.64 μM) were the most potent substances of this set. A bioisosteric exchange of thiophene for benzene gave three active quinazolines (**39**–**41**) with EC_{50} values in the range of the lead structure **29**.

Taken together, the somewhat higher activities of compounds with aryl substituents in the sulfanyl side chain might be explained by a further hydrophobic aromatic interaction, as suggested by the pharmacophore model.[51] However, structural modifications of the lead compound **29** seemed to be well tolerated by P-gp. For example, truncation of the phenethyl to an ethyl residue led to the most potent compound **26**. An additional carbonyl function in the phenacyl residue of **30** also gained activity. Moreover, the introduction of heteroatoms (**35**) and larger substituents (**36**) within the annelated tetrahydrobenzothieno moiety resulted in active representatives as well.

To assess transport profiles of different P-gp substrates, cell viability (MTT) assays were performed. Different concentrations (10–100 μM) of a cytostatic drug (daunorubicin, vinblastine or colchicine) were combined with a fixed nontoxic concentration (31.6 μM) of **21**, **22**, **28**, or **29**.[91] The four selected compounds showed similar effects in the MTT assay (Figure 2.3 and Supplementary Information of ref.[87]). In agreement with the accumulation assay, toxic effects of daunorubicin were reduced. However, for vinblastine, a potentiation of the cytotoxicity was observed. The thienopyrimidines showed no effect on colchicine transport. Therefore, we

could demonstrate a modulation of P-gp substrate specificity for **29** (QB13) and analogous thieno[2,3-*d*]pyrimidines. With respect to the transport profile, this family of substances corresponded to QB102.[80] Moreover, the results agree with the overlays of QB11, QB13 and QB102 in the pharmacophore pattern of the Hoechst 33342 binding site of P-gp.[51]

Sterz and Möllmann *et al.* recently reported on a series of imidazo[1,2-*a*]benzimidazoles and tetrahydroimidazo[2,1-*b*]benzothiazoles structurally related to QB11 and QB102, respectively.[92] In conformity with the results obtained for the QB13 analogs, derivatives of both heterocycles stimulated daunorubicin (and rhodamine 123) efflux in A2780 Adr cells. On the other hand, selected compounds were able to increase toxicity of vinblastine as well as colchicine in the MTT assay.

In summary, we evaluated a panel of thieno[2,3-*d*]pyrimidines and quinazolines as activators of P-gp mediated daunorubicin transport. Structural modifications in different positions of the scaffolds were well tolerated by the transporter. Moreover, we could demonstrate a bidirectional modulation of P-gp activity for selected compounds in combination with different cytostatic drugs.

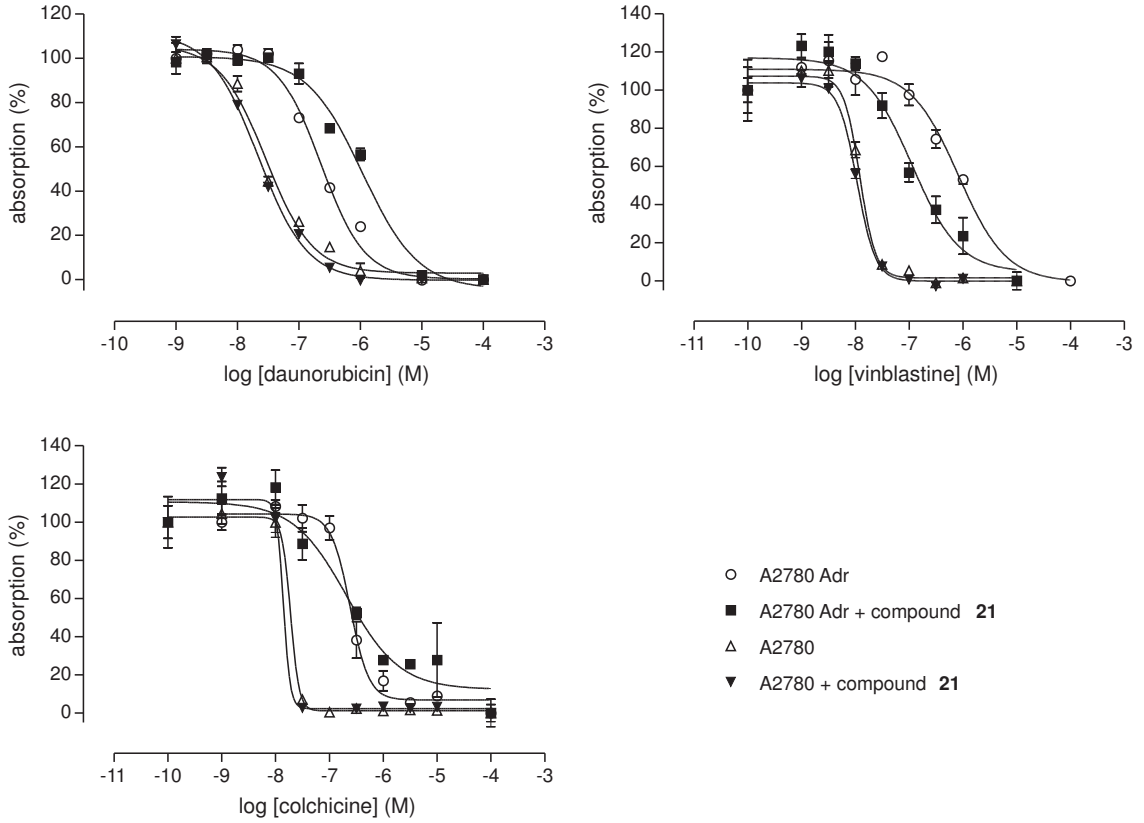


Figure 2.3: Concentration–effect curves of daunorubicin (top left), vinblastine (top right), and colchicine (bottom left) in P-gp-overexpressing cells (A2780 Adr), P-gp-overexpressing cells + compound **21** (31.6 μM), wild-type cells (A2780), and wild-type cells + **21** (31.6 μM). Curves show a representative experiment with three replicates.

CHAPTER 3

Formation of 2-(Benzoylimino)thiazolidin-4-ones by an Alternative Ring Closure

3.1 Alternative Cyclization of Benzoylthioureas

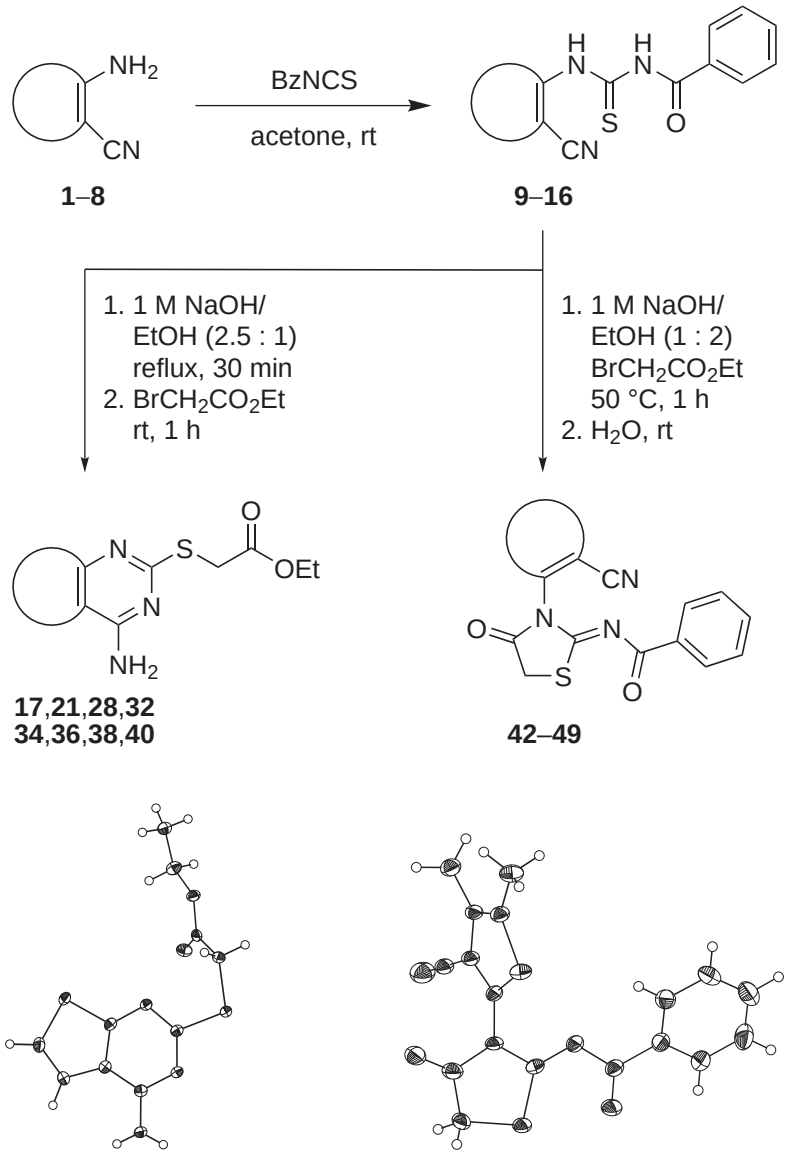
Several 2-(alkylsulfanyl)-4-aminothieno[2,3-*d*]pyrimidines (**17**–**37**) as well as corresponding quinazolines (**38**–**41**) were accessible by thermal ring closure of benzoylthioureas (**9**–**16**) in aqueous sodium hydroxide solution, followed by the reaction with suitable alkyl halides (see Chapter 2). However, when this one-pot procedure was applied in the reaction of benzoylthiourea **10** with ethyl bromoacetate as the alkylating agent, a side product was formed in addition to the expected thienopyrimidine **21** (Scheme 3.1, Table 3.1). As NMR and IR spectra of the side product revealed an unaffected nitrile function, the presence of a benzoyl group and the loss of ethanol, an alternative heterocyclization to a 2-iminothiazolidin-4-one was assumed. To elucidate which nitrogen of the starting thiourea was incorporated into the five-membered ring, crystals of the side product were subjected to X-ray diffraction analysis, and structure **43** was determined (Scheme 3.1).[93] The formation of **43** includes alkylation of the thiourea sulfur *prior* to acylation of the nitrogen atom bound to the thiophene ring. In reactions of unsymmetrical thioureas with α-halo esters, regiocontrol in the cyclization step was influenced by electronic factors and by a possible conjugative stabilization of the isothiourea imine through a (hetero)aryl substituent and could be promoted by hydrogen bond interactions.[94,95] The finding that the aryl-substituted, and not the electron-poor, benzoylated nitrogen atom was incorporated into heterocycle **43** is in accordance with literature reports.[96–98]

Various biological effects of 2-iminothiazolidin-4-ones have been reported.[99–107] FR171113, for example, a 2-(aroylimino)-3-arylthiazolidin-4-one derivative, is a thrombin receptor antagonist with potent antiplatelet activity.[108]

3.2 Preparation and Characterization of Thiazolidin-4-ones

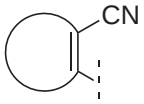
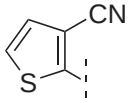
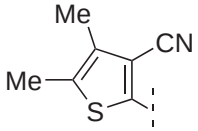
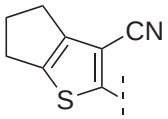
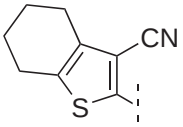
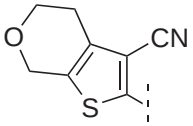
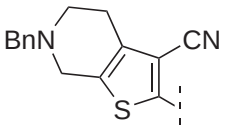
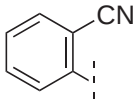
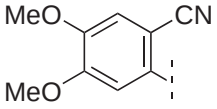
A small library of 2-(benzoylimino)thiazolidin-4-ones (**42**–**49**) was generated from benzoylthioureas **9**–**16** (Table 3.1). Slightly different reaction conditions led to both heterocycles

(Scheme 3.1). The direct addition of ethyl bromoacetate, a lowered temperature, an increased amount of ethanol, and the reduction of sodium hydroxide suppressed the formation of thieno[2,3-*d*]pyrimidines in favor of corresponding thiazolidin-4-ones **42**–**49**. The new 2-(benzoylimino)thiazolidin-4-ones could be purified by a single recrystallization step and were obtained in mostly good yields. Conversion of **9**–**16** into **42**–**49** did not affect the nitrile function, whereas different behavior was obvious for thiazolidin-4-ones with an unsubstituted imino function in position 2.[109] When such compounds were formed as intermediates, they underwent a pyrimidine cyclization, and subsequent ethanolysis of the thiazolidin-4-one ring produced 4-aminopyrimidines. In this way, Gewald *et al.* have prepared fused pyrimidines **21**, **28**, and **38** from aromatic *o*-(chloroacetylamino)nitriles.[109]



Scheme 3.1: Formation of alternative cyclization products from benzoylthioureas **9**–**16**; molecular plots of compounds **17** (left) and **43** (right).[93]

Table 3.1: Substitution patterns of thiazolidin-4-ones with corresponding yields.

	Compd	Yield[a] (%)	Prepared From
	**42**	71	**9**
	**43**	63	**10**
	**44**	66	**11**
	**45**	58	**12**
	**46**	72	**13**
	**47**	19[b]	**14**
	**48**	70	**15**
	**49**	66	**16**

[a] Yields of products after recrystallization.
[b] Yield after purification by column chromatography.

The structures of compounds **42**–**49** were confirmed by ^{1}H and ^{13}C NMR spectroscopy. In contrast to the ^{1}H NMR spectra of thiophene derivatives **42**–**47**, those of benzonitriles **48**, **49** showed splitting of the thiazolidin-4-one methylene signal into two doublets with a coupling constant of 18.6 Hz. This could be attributed to hindered rotation around the N–C_{aryl} bond, thus leading to atropisomerism.[110] The diastereotopic nature of the protons gave rise to geminal coupling in the spectra. Atropisomerism about *N*-aryl bonds, *e.g.*, in thiazolidines or thiazolidin-4-ones, where an *ortho* substituent of the aryl group accounted for steric hindrance, was recently reported.[111,112] In contrast to these investigations focusing

on the dimension and electronic properties of that *ortho* substituent, in our case the size of the aryl moiety seemed to be crucial for the occurrence of atropisomerism. Increasing the aryl size from a five-membered thiophene to a six-membered benzene ring led to a reduction of the gap between the *o*-nitrile function and the carbonyl and benzoylimino groups of the adjacent thiazolidin-4-one.[113] This is illustrated by comparison of the angles θ in the crystal structures[93] of **42** and **43** (125.9°, 124.2°) with that of **48** (120.1°) as representatives of the two ring sizes (Figure 3.1). In the three structures, the benzoylimino groups are almost in plane with the thiazolidin-4-one system. Compounds **42**, **43**, and **48** display a *Z*-configuration about the C=N bonds and an *s-cis* conformation about the N_{imine}–$C_{carbonyl}$ bonds (Scheme 3.1). The non-bonded intramolecular distance between the thiazolidin-4-one sulfur and the benzoyl oxygen atoms of 2.61–2.65 Å indicates a polar interaction between the O and S atoms.[114,115]

3.3 Analysis of Rotational Barriers

Theoretical calculations were performed by Dr. P. W. Elsinghorst to affirm the hypothesis that an internal rotational barrier between the nitrile group and the carbonyl and acylimino function of the thiazolidin-4-one led to atropisomers in case of the anthranilonitrile derivatives **48** and **49**. In this section, a brief summary of the approach and the main findings are outlined, a detailed description is given in ref.[116] and citations therein. On the basis of their crystal structures, **42**, **43** and **48** were subjected to a complete rotation about the dihedral angle φ regarding the C–N bond connecting the cyanoarene and the thiazolidin-4-one (Figure 3.1). Two rotational barriers were observed, corresponding to the steric proximity of the cyano and carbonyl group and a clash between the cyano and imine group, respectively. Compared to **42** and **43**, the corresponding energy values of compound **48** increased significantly (64/59 and 66/67 *versus* 87/95 kJ/mol). This was mainly attributed to the decreased θ value for **48** (124.2 and 125.9 *versus* 120.1°; Figure 3.1), thus resulting in hindered rotation of **48**. Calculated rotational barriers of **48** were in accordance with literature data. For 2-(arylimino)-4-methyl-3-(*o*-substituted phenyl)thiazolines, theoretical calculations as well as racemization studies using HPLC on chiral support resulted in energy values between 85 and 122 kJ/mol.[111] Enantiomers of 2-(arylimino)thiazolidin-4-ones with an *o*-tolyl, *o*-methoxyphenyl, *o*-chlorophenyl, or α-naphthyl substituent at position 3 were resolved and subjected to thermal racemization to obtain rotational barriers between 98 and 114 kJ/mol.[112]

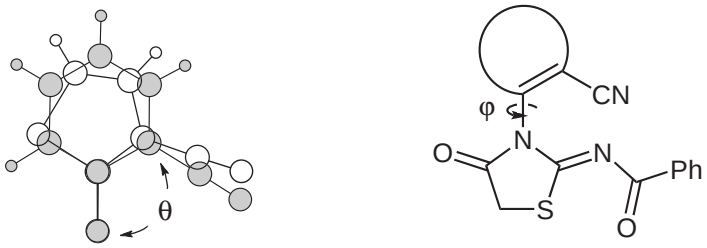


Figure 3.1: (a) Comparison of the angle θ in the crystal structures of **42** (transparent) and **48** (gray).[93] (b) Thiazolidin-4-ones **42**, **43**, and **48** with the rotation angle φ.

The calculations for **42**, **43**, and **48** were consistent with the spectroscopic data of the thiazolidin-4-ones. On the one hand, the five-membered cyanothienyl derivatives **42**–**47** did not form stable atropisomers at room temperature, since the larger angle θ allowed the substituents to pass each other. On the other hand, the six-membered cyanophenyl derivatives **48**, **49** with smaller θ values had sufficient steric repulsion for hindered rotation and were axially chiral racemates (Figure 3.2). Accordingly, **48**, **49** had diastereotopic methylene protons in their ^{1}H NMR spectra. NMR experiments showed that rotation in **42** could not be restricted at lower temperatures down to −40 ℃, and that, *vice versa*, rotation was not allowed in **48** at higher temperatures up to 70 ℃.

Replacement of one *ortho* substituent by hydrogen in tri-*o*-substituted biaryls mostly results in the loss of axial chirality.[110] We prepared the truncated compounds **50** and **51** (Figure 3.2). As expected, the ethylene protons of **50** showed a simple coupling pattern of two triplets (^3J = 7.7 Hz). Diastereotopic protons in the spectrum of **51** were not observed either, and the two methylene groups gave rise to two triplets (^4J = 1.0 Hz). This can be explained by long-range coupling through the eclipsing lone pair of the sulfur atom.[117–122] The long-range coupling in the spectrum of **51** is independent of the presence of the cyano group, since it was also found for compound **53** (^4J = 1.0 Hz).

Thiazolidin-4-ones **51** and **53** were obtained by the reaction of anthranilonitrile or aniline with thioglycolic acid and aqueous formaldehyde according to patent literature.[123,124] Compound **50** was prepared from *N*-benzoyl-*N*′-(2-cyanophenyl)thiourea (**15**) and 1,2-dibromoethane in DMF in the presence of sodium acetate at room temperature. Thiazolidin-4-one derivative **52** could be synthesized by virtually the same reaction conditions as those used for **42**–**49**. In both **50** and **52**, the aniline nitrogen was incorporated into the five-membered ring. This could be confirmed by ^{13}C NMR spectroscopy, in particular when the influence of the (benzoylimino)thiazolidine substituent on the *ipso* position in benzene or benzonitrile was considered.

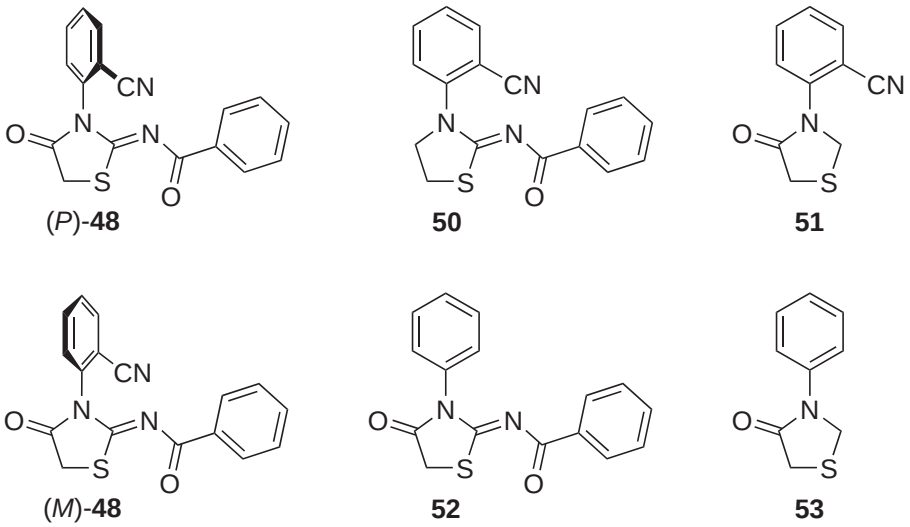


Figure 3.2: The two stereoisomers of **48**, not interconvertible at room temperature, and the truncated structures **50**–**53**.

An alternative method to prepare 2-iminothiazolidin-4-ones is the treatment of thioureas with haloacetyl halides.[94,100,102] Instead of ethyl bromoacetate, bromoacetyl bromide was applied for the conversion of **15** into **48**. However, the reaction did not proceed uniformly as reported for other *N*-aroyl-*N*′-arylthioureas.[102] The ^{13}C NMR spectrum revealed an additional signal set obviously caused by the corresponding regioisomer. Klika *et al.* have proposed a mechanism for the reaction of *N*-(anthracen-9-yl)-*N*′-ethylthiourea with bromoacetyl bromide involving S-acylation, acetyl migration, and S-alkylation to produce two isomeric 2-iminothiazolidin-4-ones (Figure 3.3).[94]

In summary, we have elaborated optimized reaction conditions for the selective preparation of substituted thiazolidin-4-ones **42**–**49** from *N*-benzoyl-*N*′-(*o*-cyanoaryl)thioureas **9**–**16** and ethyl bromoacetate. In view of the diverse biological activities of thiazolidin-4-ones, compounds **42**–**49** can be tested for their potency but might also be a useful scaffold for further chemical transformations, *e.g.*, modifications of the cyano group or a condensation with appropriate aromatic aldehydes. Whereas the size of sterically demanding substituents has frequently been reported to influence atropisomerization,[110–112] we investigated the less common influence of ring size by means of NMR measurements and theoretical calculations. In contrast to (*o*-cyanothienyl)-substituted thiazolidin-4-ones **42**–**47**, (*o*-cyanophenyl)-substituted derivatives **48**, **49** cannot overcome internal rotational barriers at room temperature and were characterized as axially chiral racemates.

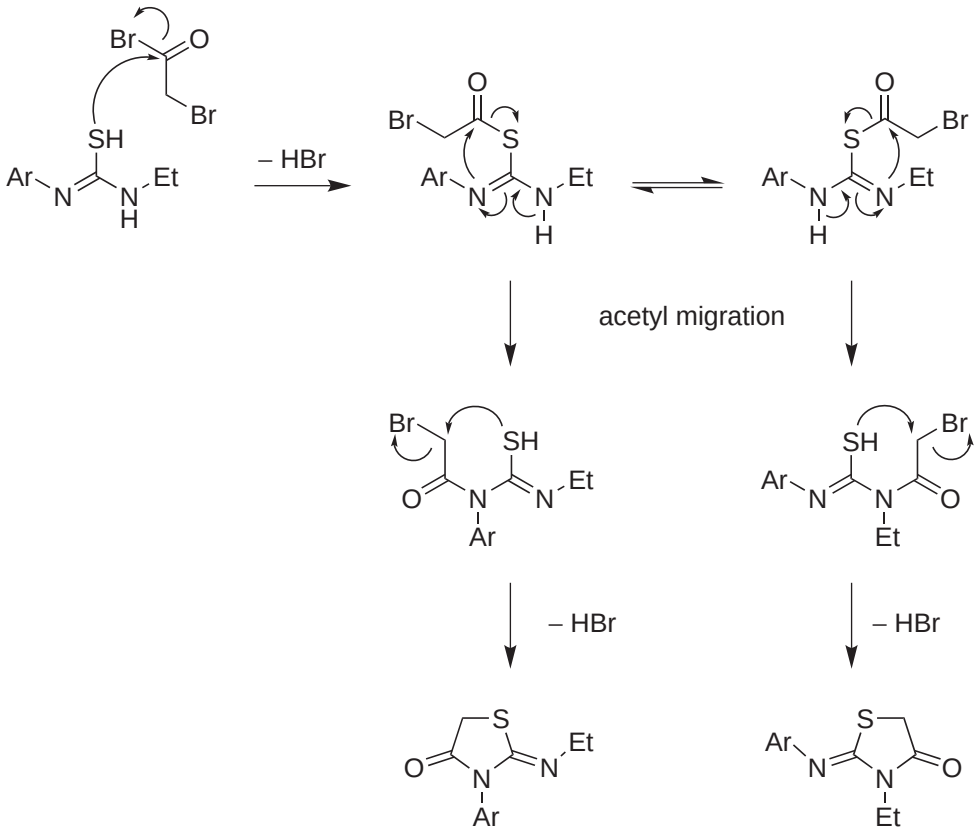


Figure 3.3: Mechanism for the reaction of *N*-(anthracen-9-yl)-*N*′-ethylthiourea with bromoacetyl bromide (modified from ref.[94]).

CHAPTER 4

Aromatic Carboxylic Acids as Multidrug Resistance-Associated Protein 1 Modulators

4.1 Chemistry of Aromatic 2-(Thio)ureidocarboxylic Acids

The starting point for this study was the evaluation of low molecular weight compounds from the substance library of our group for modulating effects on MRP1 in a fluorometric calcein AM assay.[66,125] Initially, the selection was focused on carboxylic acids with respect to the anionic nature of the MRP1 substrates. More than 75 structurally diverse carboxylic acids were inspected (see Supplementary Information of ref.[66]). Among them, some aromatic 2-(thio)ureidocarboxylic acids showed modulating activity on MRP1. On the basis of these initial results, it was intended to provide four series of aromatic carboxylic acids (Figure 4.1). All compounds bear either a substituted urea or thiourea moiety at the neighboring position to the carboxyl group. As an aromatic scaffold, either a (substituted) benzene or thiophene ring was chosen.

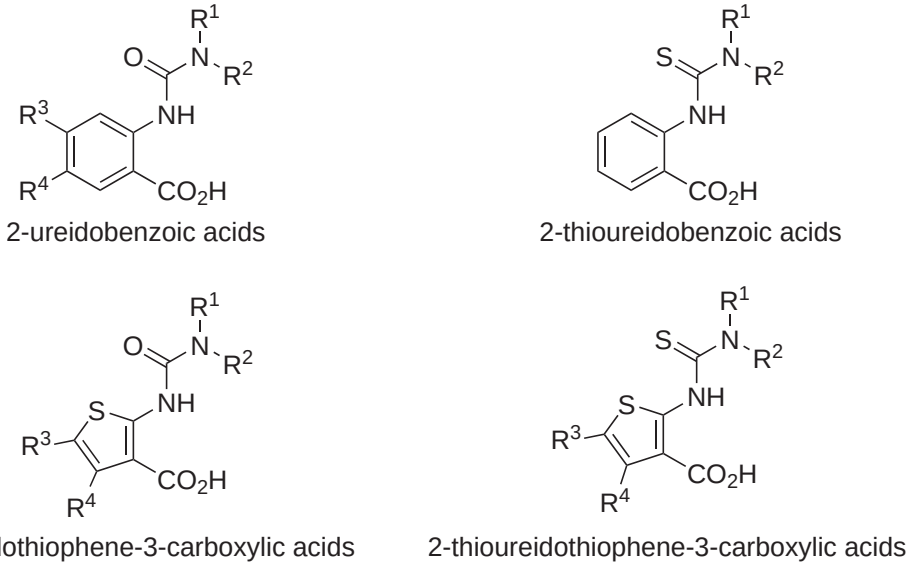


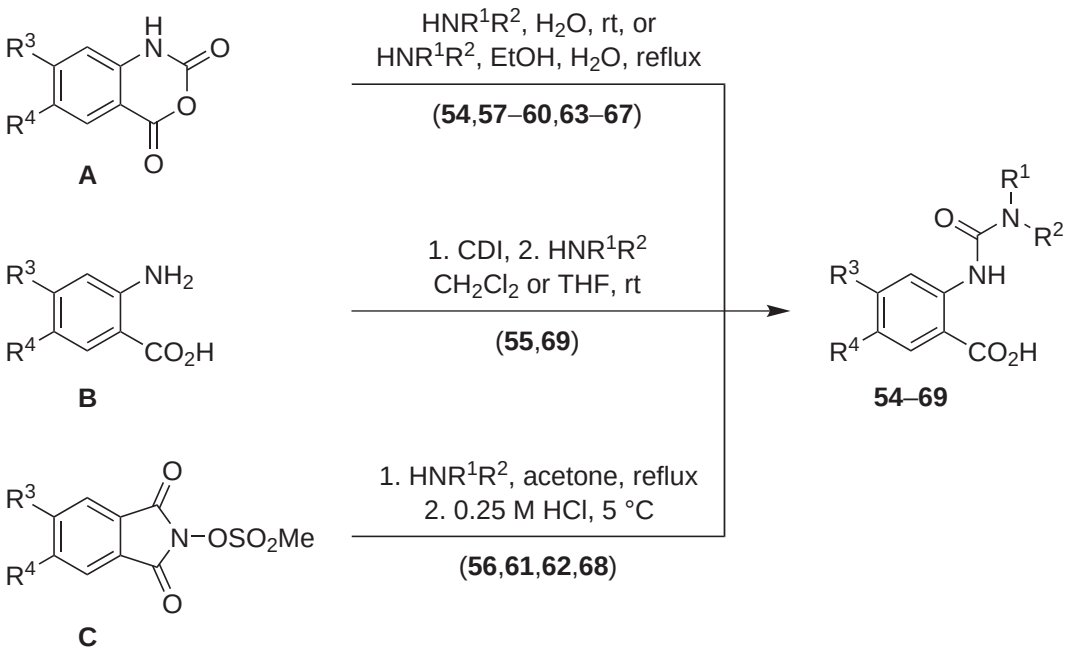
Figure 4.1: Different series of aromatic carboxylic acids.

Most compounds from the 2-ureidobenzoic acid series (**54**–**69**, Table 4.1) were taken from the substance library. They were accessible either from the reaction of isatoic anhydrides **A** with benzylamine or appropriate secondary amines,[126] or by reacting *N*-(mesyloxy)phthalimides **C** with secondary amines (Scheme 4.1).[127] Moreover, a new synthetic route was introduced: To prepare 2-(3,3-diethylureido)benzoic acid (**55**), anthranilic acid (**B**, $R^3 = R^4 = H$) was pretreated with *N*,*N*′-carbonyldiimidazole followed by the addition of diethylamine. Similarly, the naphthalene derivative **69** was obtained from 3-amino-2-naphthalenecarboxylic acid (**B**, R^3R^4 = –CH=CH–CH=CH–).

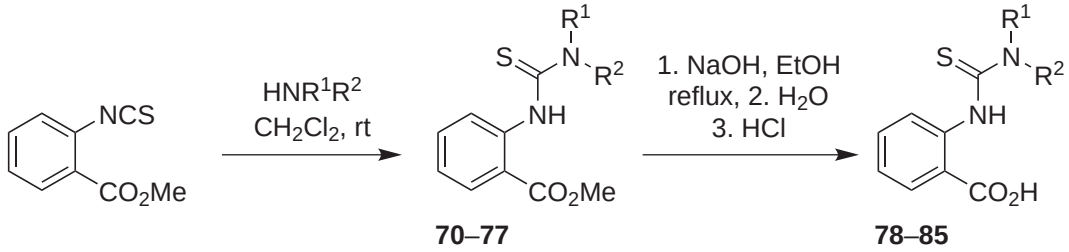
The two-step synthetic route to the new 2-thioureidobenzoic acids **78**–**85** (Table 4.2) is depicted in Scheme 4.2. The methyl benzoate precursors **70**–**77** were prepared from secondary amines and methyl 2-isothiocyanatobenzoate[128] in dichloromethane and subsequently saponified with aqueous ethanolic sodium hydroxide. Representatives of both groups were also applied for the preparation of 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones. A detailed description of the synthesis and interconversion of these heterocycles is given in Chapter 5.

2-Ureidothiophene-3-carboxylic acids **86**–**89** (Table 4.3) were synthesized by two alternative methods (Scheme 4.3). Thieno[2,3-*d*][1,3]oxazine-4-ones **D** underwent ring-opening upon treatment with sodium hydroxide. Because a disubstituted amino group prevented Dimroth rearrangement to pyrimidinediones,[129,130] the desired non-cyclized ureas **86**, **88**, and **89** were obtained. According to this literature method, **88** was prepared in 72% yield, whereas the other compounds of this series were taken from the substance library. Trifluoroacetic acid promoted the cleavage of the *tert*-butyl ester group in **E** to give the corresponding isopropylurea **87**. Both routes started with the Gewald thiophene synthesis[81,82] to alkyl 2-aminothiophene-3-carboxylates. In contrast to the preparation of 2-aminothiophene-3-carbonitriles, which were employed as starting materials for thienopyrimidines (Chapter 2) and thiazolidin-4-ones (Chapter 3), cyanoacetic esters are used instead of malononitrile to obtain the corresponding 2-aminothiophene-3-carboxylates. Those were converted either with isopropyl isocyanate to **E** or in five reaction steps to the heterocycles **D**.[131] In analogy to 2-ureidobenzoic acids, *o*-ureidothiophenecarboxylic acids are accessible from thiaisatoic anhydrides, the thiophene analogs of isatoic anhydride.[132,133] The latter entry was not applied in the course of this work.

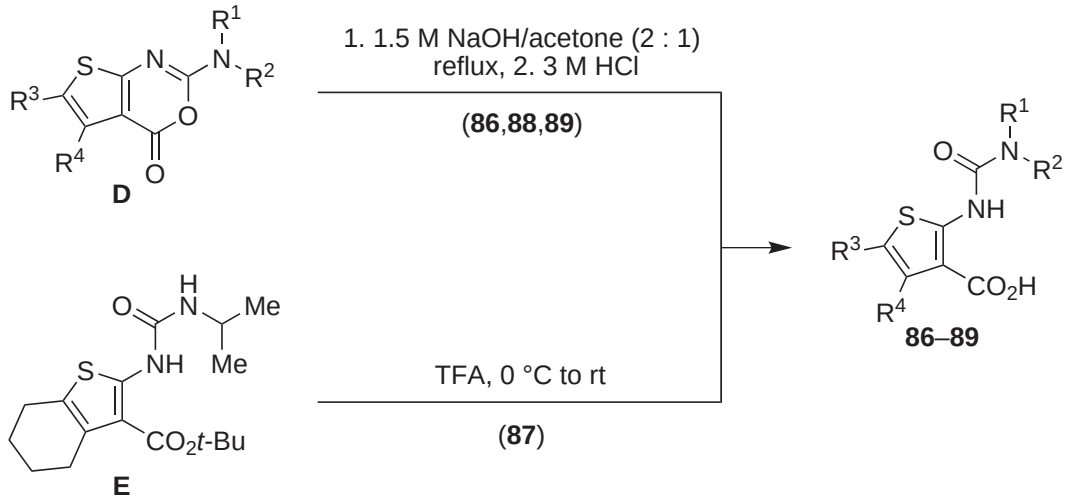
Synthetic pathways to thiophene-3-carboxylic acids with a thiourea moiety at position 2 (**102**–**122**, Table 4.4) are shown in Scheme 4.4. Fourteen out of 21 compounds were taken from the substance library. Those were obtained by sodium hydroxide-promoted cleavage of the thiazinone ring of compounds **H**.[131] Similar to the preparation of 2-thioureidobenzoic acids **78**–**85**, a direct saponification of the ethyl esters **G** was applied for compounds **103**, **105**–**107**, and **120**–**122**. With respect to R^3 and R^4, either non-, mono-, and disubstituted thiophenes (**102**–**111**) or fused compounds (**112**–**122**) were included. All substances were accessible by an initial Gewald reaction. To obtain the aromatic fused isothiocyanate **94**, three additional steps were necessary (Scheme 4.5).[131,134] The final compound **101** (Scheme 4.4) was achieved following the first mentioned route, *i.e.*, conversion of methyl 3-aminothiophene-2-carboxylate to the isothiocyanate and the diethylthioureido derivative, which was cyclized with concentrated sulfuric acid to the corresponding 4*H*-thieno[3,2-*d*][1,3]thiazin-4-one and reopened to **101**.[131]



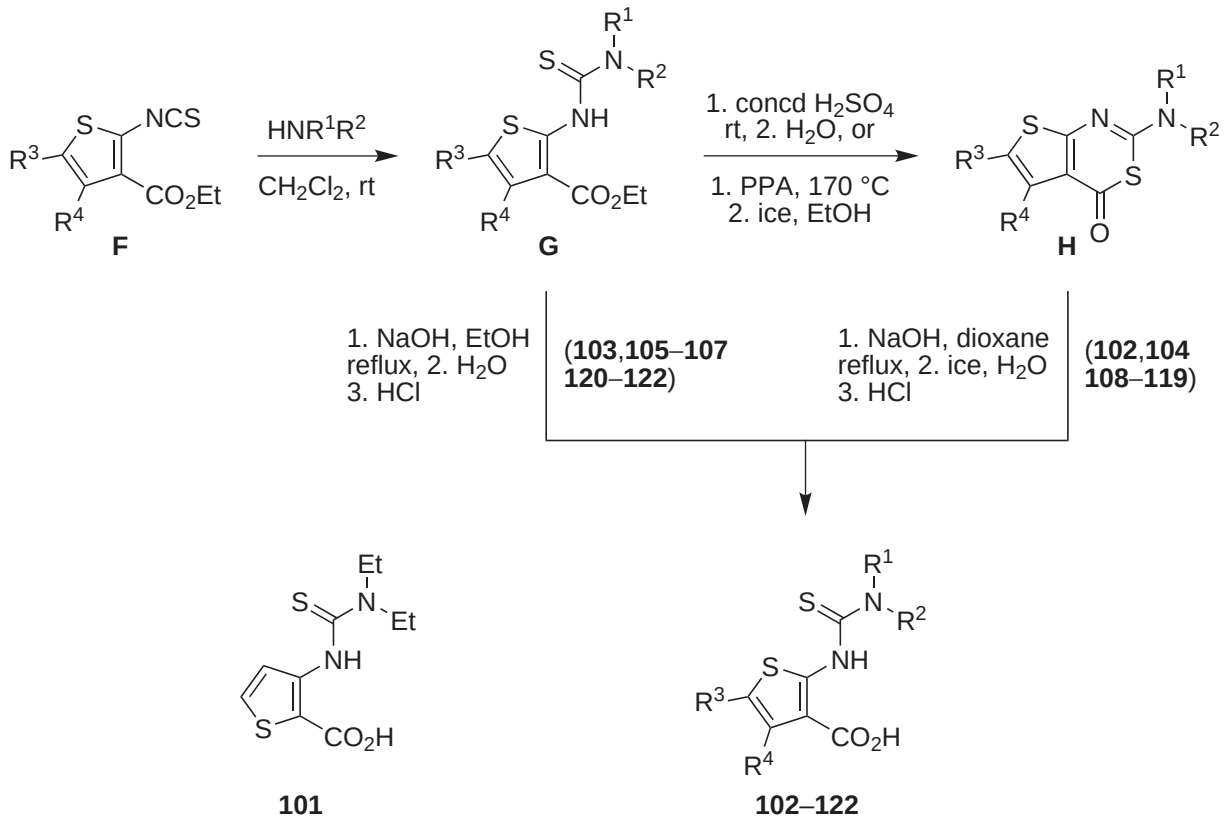
Scheme 4.1: Synthetic routes to 2-ureidobenzoic acids **54**–**69**.



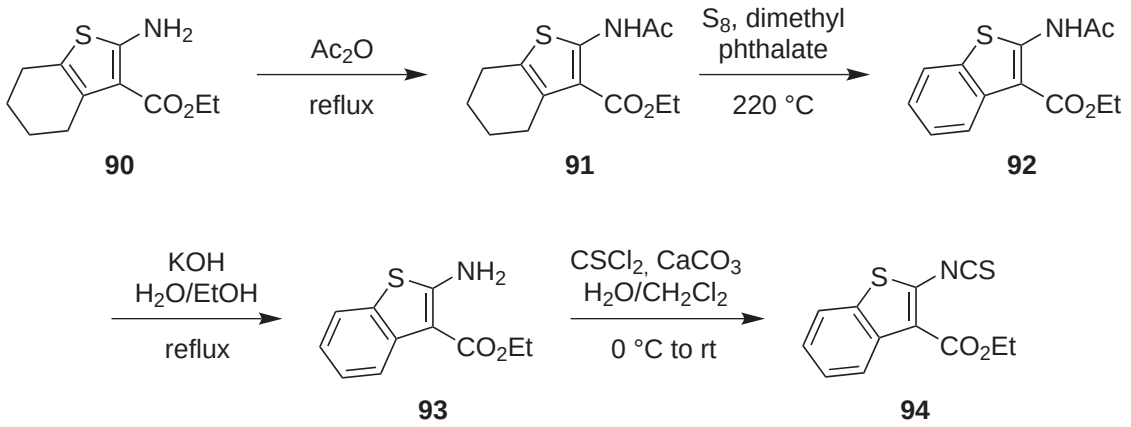
Scheme 4.2: Preparation of 2-thioureidobenzoic acids **78**–**85**.



Scheme 4.3: Synthetic routes to 2-ureidothiophene-3-carboxylic acids **86**–**89**.

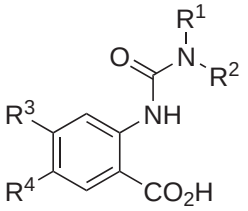


Scheme 4.4: 3-(Diethylthioureido)thiophene-2-carboxylic acid (**101**) and synthetic routes to 2-thioureidothiophene-3-carboxylic acids **102**–**122**.



Scheme 4.5: Preparation of ethyl 2-isothiocyanatobenzo[*b*]thiophene-3-carboxylate (**94**).

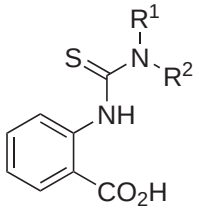
Table 4.1: 2-Ureidobenzoic acids **54**–**69** with corresponding IC_{50} values at MRP1.



Compd	NR^1R^2	R^3	R^4	Yield (%)	Prepared from	IC_{50} ± SD (μM)
54*	NHBn	H	H		**A**	ni[d]
55	$N(Et)_2$	H	H	54	**B**	26.4 ± 10.1
56*	$N(Me)CH_2CO_2Me$	H	H		**C**	ni
57*[a,b,c]	N(Me)cyclohexyl	H	H		**A**	12.3 ± 2.8
58*	N(Me)Ph	H	H		**A**	ni
59*	N(Me)Bn	H	H		**A**	42.6 ± 1.6
60*	$N(Me)CH_2CH_2Ph$	H	H		**A**	ni
61*	1-pyrrolidinyl	H	H		**C**	43.1 ± 11.1
62*	4-morpholinyl	H	H		**C**	ni
63*	N(Me)cyclohexyl	H	Me		**A**	22.8 ± 6.2
64*	N(Me)Ph	H	Me		**A**	ni
65*	N(Me)Bn	H	Me		**A**	ni
66*	$N(Me)CH_2CH_2Ph$	H	Me		**A**	ni
67*	4-morpholinyl	H	Me		**A**	ni
68*	4-morpholinyl	Me	Me		**C**	43.4 ± 13.3
69[a,b,c]	$N(Et)_2$	–CH=CH–CH=CH–		68	**B**	5.38 ± 0.68

* Compound was taken from the substance library.
[a] Compound showing no effect in the P-gp assay.
[b] Compound (at 31.6 μM) inhibited BCRP between 25 and 65% relative to the inhibitory effect of XR9577 (at 10 μM).
[c] No cytotoxicity ($IC_{50} > 100$ μM).
[d] ni, no inhibition of the MRP1-mediated transport.

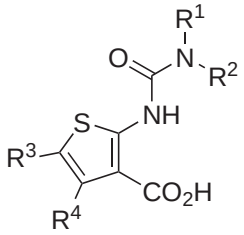
Table 4.2: 2-Thioureidobenzoic acids **78**–**85** with corresponding IC_{50} values at MRP1.



Compd	NR^1R^2	Yield (%)	Prepared from	$IC_{50} \pm SD$ (μM)
78	$N(Et)_2$	78	**70**	21.6 ± 5.3
79[a,b,c]	N(Me)cyclohexyl	90	**71**	16.2 ± 4.2
80	N(Me)Ph	66	**72**	198 ± 29
81	N(Me)Bn	61	**73**	29.1 ± 9.1
82	$N(Me)CH_2CH_2Ph$	81	**74**	24.0 ± 6.9
83	1-pyrrolidinyl	85	**75**	18.3 ± 4.6
84	1-piperidinyl	84	**76**	20.7 ± 2.5
85	4-morpholinyl	75	**77**	ni[d]

[a] Compounds showing no effect in the P-gp assay.
[b] Compound (at 31.6 μM) inhibited BCRP less than 20% relative to the inhibitory effect of XR9577 (at 10 μM).
[c] No cytotoxicity ($IC_{50} > 100$ μM).
[d] ni, no inhibition of the MRP1-mediated transport.

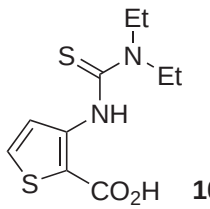
Table 4.3: 2-Ureidothiophene-3-carboxylic acids **86**–**89** with IC_{50} values at MRP1.



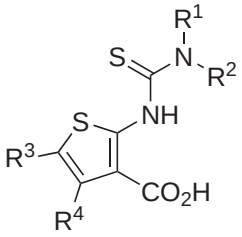
Compd	NR^1R^2	R^3 R^4	Yield (%)	Prepared from	$IC_{50} \pm SD$ (μM)
86[*,a,b,c]	$N(Et)_2$	$-(CH_2)_3-$		**D**	11.6 ± 3.1
87[*]	$NHCH(Me)_2$	$-(CH_2)_4-$		**E**	ni[d]
88[a,b,c]	$N(Et)_2$	$-(CH_2)_4-$	72	**D**	8.29 ± 4.22
89[*]	4-morpholinyl	$-(CH_2)_4-$		**D**	ni

[*] Compound was taken from the substance library.
[a] Compounds showing no effect in the P-gp assay.
[b] Compounds (at 31.6 μM) inhibited BCRP between 25 and 65% relative to the inhibitory effect of XR9577 (at 10 μM).
[c] No cytotoxicity ($IC_{50} > 100$ μM).
[d] ni, no inhibition of the MRP1-mediated transport.

Table 4.4: 3-(Diethylthioureido)thiophene-2-carboxylic acid **101** and 2-(thioureido)-thiophene-3-carboxylic acids **102**–**122** with corresponding IC_{50} values at MRP1.



101



102–**122**

Compd	NR^1R^2	R^3	R^4	Yield (%)	Prepared from	IC_{50} ± SD (μM)
101[*,a,b,c]						4.18 ± 0.70
102[*,a,b,c]	$N(Et)_2$	H	H		**H**	4.46 ± 0.58
103[a,c,d]	N(Me)cyclohexyl	H	H	78	**G** (**95**)	4.51 ± 0.49
104[*]	$N(Et)_2$	H	$CH(Me)_2$		**H**	14.5 ± 5.5
105	4-morpholinyl	H	Ph	84	**G**	ni[h]
106[c,e]	$N(Et)_2$	Ph	H	90	**G** (**96**)	14.3 ± 4.7
107	4-morpholinyl	Ph	H	73	**G** (**97**)	27.5 ± 3.3
108[*]	$N(Et)_2$	Me	Me		**H**	20.4 ± 2.1
109[*,a,b,c]	N(Me)cyclohexyl	Me	Me		**H**	4.19 ± 1.51
110[*]	4-morpholinyl	Me	Me		**H**	18.3 ± 4.5
111[*]	$N(Et)_2$	$CONH_2$	Me		**H**	> 100
112[*,a,b,c]	$N(Et)_2$	$-(CH_2)_3-$			**H**	2.56 ± 1.11
113[*]	$N(Me)_2$	$-(CH_2)_4-$			**H**	ni
114[*,a,b,c]	$N(Et)_2$	$-(CH_2)_4-$			**H**	2.71 ± 0.35
115[*]	N(Me)cyclohexyl	$-(CH_2)_4-$			**H**	nd[i]
116[*]	4-morpholinyl	$-(CH_2)_4-$			**H**	ni
117[*]	4-methyl-1-piperazinyl	$-(CH_2)_4-$			**H**	ni
118[*,a,c,f]	$N(Et)_2$	$-(CH_2)_3CH(CH_3)-$			**H**	4.06 ± 1.05
119[*,a,b,c]	$N(Et)_2$	$-(CH_2)_5-$			**H**	5.97 ± 3.83
120[a,c,g]	$N(Et)_2$	–CH=CH–CH=CH–		41	**G** (**98**)	0.932 ± 0.041
121[a,b,c]	N(Me)cyclohexyl	–CH=CH–CH=CH–		81	**G** (**99**)	1.23 ± 0.29
122	4-morpholinyl	–CH=CH–CH=CH–		65	**G** (**100**)	ni

[*] Compound was taken from the substance library.
[a] Compound showing no effect in the P-gp assay.
[b] Compound (31.6 μM) inhibited BCRP between 25 and 65% relative to the inhibition of XR9577 (10 μM).
[c] No cytotoxicity (IC_{50} > 100 μM).
[d] Partial inhibition of BCRP with IC_{50app} = 10.2 ±3.6 μM.
[e] 20% inhibition at 31.6 μM, 40% inhibition at 100 μM in the P-gp assay.
[f] Partial inhibition of BCRP with IC_{50app} = 9.10 ±1.71 μM.
[g] Partial inhibition of BCRP with IC_{50app} = 6.16 ±1.36 μM.
[h] ni, no inhibition of the MRP1-mediated transport.
[i] nd, not detectable, concentration-dependent decrease of fluorescence in 2008 WT and 2008 MRP1 cells.

4.2 Results from Calcein AM, Hoechst, and Cell Viability Assays

Biological evaluations were carried out by Dr. S. Leyers, J. Wiendlocha, and D. Baumert from the group of Prof. Dr. M. Wiese. This section outlines the general approach as well as the main findings from the biological assays, for a detailed description see refs.[135–137]

An accumulation assay using the MRP1 substrate calcein AM was performed to determine the influence of the test compounds on the transporter protein MRP1.[54,66,125] Calcein AM is a lipophilic, non-fluorescent acetoxymethyl ester of calcein that easily penetrates cellular membranes. Intracellular calcein AM can either be transported out of the cell by MRP1 or cleaved by unspecific cytosolic esterases to produce the fluorescent calcein. The human ovarian cancer cell line 2008 stably expressing MRP1 was used. In 2008 MRP1 cells, the efflux of calcein AM leads to a decreased intracellular fluorescence, compared to the corresponding wild-type cell line, 2008 WT. An MRP1 inhibitor blocks calcein AM efflux, leading to an enhancement of calcein concentration and intracellular fluorescence (Figure 4.2). In contrast, as the wild type does not exhibit MRP1, addition of an inhibitor to 2008 WT cells has no effect on fluorescence (Figure 4.3). When performing the assay, XR9577 (Figure 1.3),[138] a selective blocker of P-gp and BCRP, having no effect on MRP1,[39] was added to inhibit P-gp, which is present to a minor degree in both MRP1-expressing and wild-type cells. The assay medium contained cobalt ions to quench extracellular calcein fluorescence so that only the fluorescence of intracellular calcein is measured.

A subset of active compounds was defined and tested for the ability to affect P-gp in a similar calcein AM assay using P-gp overexpressing A2780/ADR cells. Furthermore, these compounds were investigated for modulation of BCRP in MCF-7 MX cells in an accumulation assay with the fluorescent substrate Hoechst 33342.[139] Cytotoxicity of the subset of compounds was determined in 2008 WT cells by measuring viability *via* MTT assay.[91] The MTT assay was also used to assess potentiating effects of two exemplary 2-ureidothiophene-3-carboxylic acids (**103**, **120**) in combination with the cytotoxic drugs vinblastine and daunorubicin.

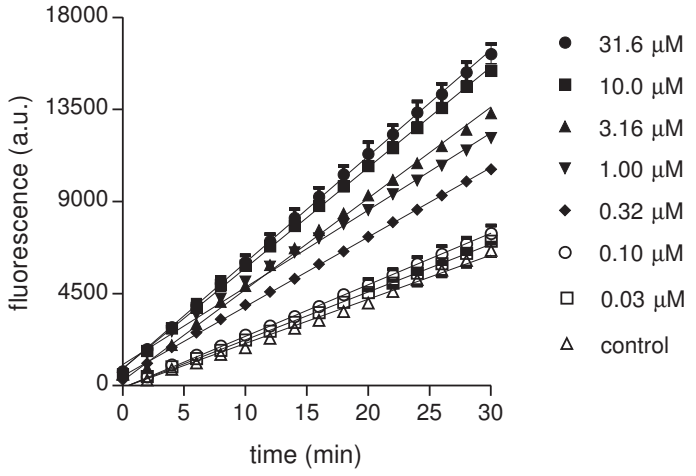


Figure 4.2: Fluorescence–time curves for different concentrations of **120** determined with the calcein AM assay in 2008 MRP1 cells. Data are averages from a typical experiment with two replicates belonging to a series of three independent experiments.

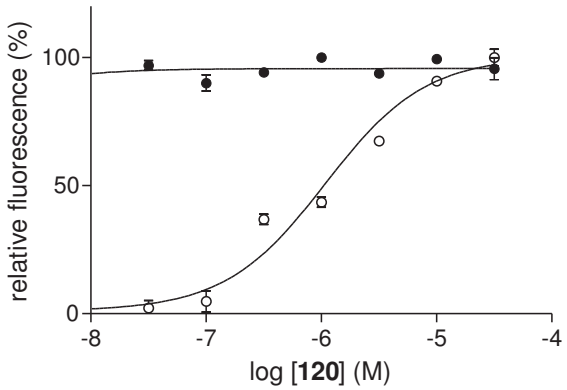


Figure 4.3: Concentration–effect curve of compound **120** in MRP1-transfected cells (open circles) in comparison to the wild-type cells (closed circles). Curves represent the average of at least five independent experiments. For normalization of data, slopes from fluorescence–time curves were transformed to relative units by subtracting the lowest determined single value from all other data and thus setting it to 0%. The highest measured single value was defined as 100%.

In search of MRP1 inhibitors, we identified active representatives of 2-ureidobenzoic acids. All 2-ureidobenzoic acids (**54**–**69**) investigated are outlined in Table 4.1. The presence of a diethylureido and a cyclohexylmethylureido moiety provided active compounds (**55**, **57**, **63**, **69**). A replacement of the cyclohexyl residue by a phenyl, benzyl, or phenethyl group mostly led to a loss of potency (**57** *versus* **58**, **60**; **63** *versus* **64**–**66**). While the introduction of a 5-methyl substituent (R^4 = Me) did not improve biological activity, an additional fused benzene ring was advantageous (**55** *versus* **69**). The naphthalene derivative **69** (IC_{50} = 5.38 μM) was the only potent substance among the 2-ureidobenzoic acids. Compound **69** did not inhibit P-gp, showed a weak inhibition of BCRP and was not cytotoxic. Whereas seven of the 16 2-ureidobenzoic acids inhibited MRP1, no active compound was identified from a series of 13 related anthranilic acid derivatives (see Supplementary Information of ref.[137]), *i.e.*, benzoic acids with a 2-alkylamino, 2-arylamino, 2-acylamino, 2-alkoxycarbonylamino, or 2-(3-aroylureido) substituent.

Compounds **78**–**85** are benzoic acids with a thiourea moiety at position 2 (Table 4.2). As in the ureidobenzoic acid series, a cyclohexylmethylureido moiety in **79** (IC_{50} = 16.2 μM) accounted for activity. This substance did not inhibit P-gp but showed also a weak inhibition of BCRP. No cytotoxicity was observed for **79**. Toward MRP1, all compounds, except of **80** and **85**, had IC_{50} values below 30 μM. When comparing analogous ureas with thioureas, a clear effect of the oxygen–sulfur exchange could not be observed (**55**, **57**–**62** *versus* **78**–**83**, **85**). Five benzoic acids with a 2-(3-aroylthioureido) residue and optional aromatic substituents have also been investigated and proved to be inactive. The replacement of the carboxyl group in **78** by a cyano function abolished activity, indicating the importance of this moiety (see Supplementary Information of ref.[137]).

IC_{50} values of 2-ureidothiophene-3-carboxylic acids **86**–**89** are in given in Table 4.3. A 2-(3-isopropylureido) or 2-[(4-morpholinylcarbonyl)amino] residue gave inactive compounds. The activities of the two diethylurea derivatives **86** and **88** (IC_{50} = 11.6 and 8.29 μM) were between those of the two benzoic acid-derived diethylureas **55** and **69**. Both compounds,

86 and **88**, did not affect P-gp-mediated transport but were also weak inhibitors of BCRP. They did not exhibit cytotoxicity. Four thiophene-3-carboxylic acids with 2-acylamino or 2-alkoxycarbonylamino groups in place of the 2-ureido function were also evaluated but had no effect on MRP1-mediated transport (see Supplementary Information of ref.[137]).

Thiophenecarboxylic acids **101**–**122** with an *o*-thioureido substituent are outlined in Table 4.4. Again, the carboxyl group was required for activity as the corresponding methyl ester of **101** and the corresponding carbonitrile of **114** did not affect MRP1 (see Supplementary Information of ref.[137]). Within the thioureido residue, a diethylamino or cyclohexylmethylamino substitution pattern was advantageous over a more polar one, *i.e.*, morpholino or methylpiperazino. The two isomeric thiophenes **101** and **102** were equipotent.

Starting from 2-(3-diethylthioureido)thiophene-3-carboxylic acid (**102**), the influence of substituents in positions 4 and 5 was examined. The potency was somewhat diminished by introducing two methyl groups (**102** *versus* **108**), or an isopropyl group (**104**), but was lost by replacing the 5-methyl by a polar carboxamide moiety (**111**). A fused cycloaliphatic ring was tolerated in most cases and provided slightly improved inhibitors, *i.e.*, the tri- and tetramethylene compounds (**102** *versus* **112** and **114**). Introduction of a methyl group into the tetramethylene chain of **114** resulted in a slight decrease of activity (**118**). As in the 2-ureidobenzoic acid series (compound **69**) a bicyclic aromatic system was advantageous. An additional fused benzene ring in the benzothiophene **120** accounted for the most potent MRP1 inhibitor of our study with an IC_{50} value of 0.932 μM. Increased potency of **120** compared to the analogous cycloaliphatic derivative **114** could be due to planarity of the fused aromatic system. Removing the steric fixation of the annelated benzene ring in favor of a substituted benzene ring considerably dropped activity (**120** *versus* **106**).

Although it was demonstrated by means of X-ray crystallography that the biaryl structure in **106** is nearly in plane ($\varphi = 16°$, Figure 4.4), the extended aromatic system might lead to steric repulsion. An exchange of the diethylamino group in **120** for a cyclohexylmethylamino substituent in the thioureido residue led to the other potent benzothiophene **121** ($IC_{50} = 1.23$ μM). Again, the exchange of the dialkyl moiety for a polar morpholino substituent in **122** resulted in a loss of activity. When comparing the MRP1-inhibiting properties of analogously substituted thiophenes (**86**, **88** *versus* **112**, **114**), thioureas were superior to ureas. The bioisosteric benzene–thiophene exchange improved the activity as can be seen from the comparison of the parent diethylthioureidocarboxylic acids (**78** *versus* **101** and **102**).

Selected MRP1 inhibitors of this series (Table 4.4) were evaluated as modulators of P-gp, but only **106** showed a weak inhibitory activity. However, inhibition of BCRP was observed in all these cases. Compounds **103**, **118**, and **120** behaved as partial inhibitors of BCRP with a maximum inhibitory effect of 60% relative to XR9577 and apparent IC_{50} values between 6 and 10 μM. The selected MRP1 inhibitors of this series were not cytotoxic. In the modified MTT assay, MRP1-expressing cells were treated with **103** or **120** (in a fixed concentration of 31.6 μM) and vinblastine or daunorubicin (in different concentrations). These combinations did not result in an increased cytotoxicity of vinblastine and daunorubicin, respectively (see Supplementary Information of ref.[137]).

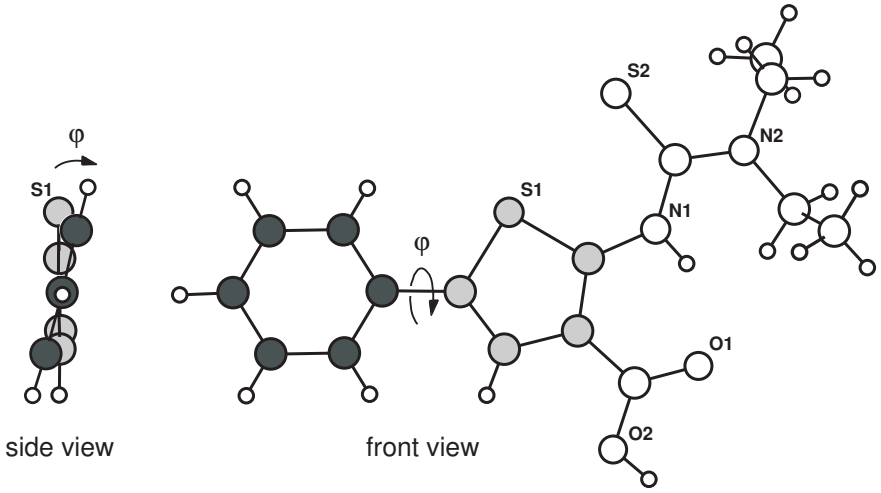


Figure 4.4: Torsion angle φ between phenyl (dark gray) and thiophene (light gray) planes in the crystal structure of **106**. Heteroatoms are designated according to the atom-labeling scheme.[140]

4.3 Structural Features for Bioactivity

On the basis of the structure–activity relationships detailed above, we combined the advantageous structural features as can be deduced from the biological activities of the four series of aromatic carboxylic acids (Figure 4.5). The best MRP1 inhibitors of the present series are more potent than verapamil, indomethacin, MK 571, and cyclosporin A for which IC_{50} values between 5 and 12 μM had been determined in the calcein AM assay (Table 4.5).[66] They have the same order of activity as dehydrosilybin (IC_{50} = 1.1 μM),[53] LY329146 (IC_{50} = 0.8 μM),[53] LY402913 (IC_{50} = 1.8 μM),[141] and compound **VI** (IC_{50} = 1.2 μM)[66] but are less potent than the recently reported pyrrolo- and indolopyrimidines such as XR12890[73,74] (Figure 1.5). The structural features derived for the aromatic carboxylic acids of this study (Figure 4.5) reflects their differences to other classes of MRP1 inhibitors. The essential components are a carboxyl group at a (hetero)aromatic scaffold with a (thio)urea function in *ortho*-position. The terminal nitrogen of the (thio)urea should be disubstituted, preferentially with two alkyl groups (*e.g.*, diethyl or cyclohexylmethyl). The (hetero)aromatic ring (A) consists of benzene or thiophene, while the orientation of the sulfur seems to have minor influence on activity. An additional fused benzene ring (B) improves the inhibitory activity against MRP1.

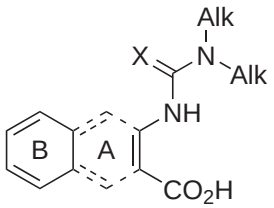


Figure 4.5: Structural features for bioactive aromatic 2-(thio)ureidocarboxylic acids.

Table 4.5: Standard inhibitors with corresponding IC_{50} values at MRP1, P-gp, and BCRP determined in the calcein AM/Hoechst assays (modified from ref.[66]).

Compd	IC_{50}[a] (μM)		
	MRP1	P-gp	BCRP
Verapamil	9.66 ± 2.79	5.42 ± 1.33	525 ± 73[c]
Indomethacin	12.0 ± 3.5	ni[b]	nd[d]
MK571	7.57 ± 1.10	ni	nd
Cyclosporin A	4.78 ± 0.61	4.92 ± 0.20	63.1 ± 14.7[c]

[a] Values are means ±SD of at least three independent experiments carried out on different occasions.
[b] ni, no inhibition.
[c] taken from ref.[139]
[d] nd, not detected.

In addition to the calcein AM accumulation assays in 2008 MRP1 cells, toxicity for 16 selected compounds was evaluated in corresponding wild-type cells (Table 4.1–Table 4.4) and no cytotoxic properties were observed. However, compounds **103** and **120** failed to reverse multidrug resistance of MRP1-expressing cells against vinblastine and daunorubicin, respectively. Although different transport mechanisms of substrates are well characterized for MRP1, the mechanism of inhibition is poorly understood.[2,5,53,54,142] Perrotton *et al.* investigated modulating effects of (*R*)- and (*S*)-verapamil on MRP1.[143] Both enatiomers induced an increase in calcein AM accumulation in MRP1-overexpressing cells, thus implying effective inhibition of the efflux pump. In potentiation assays, only (*R*)-verapamil reverted resistance of MRP1-BHK-21 cells to vincristine. As **103** and **120** in our case, (*S*)-verapamil did not show a cytotoxicity-potentiating effect. The different modulation of MRP1 by both enatiomers was discussed as explanation of controversial results of verapamil in reversion of chemoresistance.[61,143–145]

The most potent representatives of the four series were evaluated for modulating effects on BCRP- and P-gp mediated transport. All selected substances showed a weak inhibition of Hoechst 33342 efflux mediated by BCRP. Overlapping inhibitors of different ABC transporter are discussed in the literature.[3,7,12,31,43] Our finding that carboxylic acids are capable of an interaction with BCRP is in agreement with a recently published report revealing MK 571 to be a BCRP inhibitor.[12] On the other hand, all MRP1 inhibitors with IC_{50} values less than 10 μM did not affect P-gp. As P-gp generally does not transport negatively charged compounds, it might be concluded that the presence of a carboxylic acid moiety accounts for this selectivity.

Structural modifications of the title compounds might be useful for further studies, *e.g.*, an exchange of rings A and/or B for other heteroaromatics, additional substituents at N-3 of the (thio)urea (cyclohexylethyl or di(iso)propyl), and a bioisosteric replacement of the carboxyl group against a tetrazole moiety.[146]

CHAPTER 5

Synthesis and Interconversion of 2-Substituted 4*H*-3,1-Benzothiazin-4-ones

5.1 Biological Activities of Fused 3,1-Benzothiazin-4-ones

It was the aim of this study to search for synthetic entries to 4*H*-3,1-benzothiazin-4-ones with amino or alkylthio substituents at position 2. Representatives of this heterocyclic class are assumed to possess biological activities since they might provide four heteroatoms as potential hydrogen bond acceptors and the fused phenyl ring for possible π–π interactions. Analogous 4*H*-3,1-benzoxazin-4-ones have attracted considerable attention as serine hydrolase inhibitors. Their interaction with serine hydrolases involves the acylation of the active-site serine due to enzymatic ring cleavage, followed by slow deacylation of the acyl-enzyme intermediate.[147] 2-Amino and 2-alkylthio substituted 4*H*-3,1-benzoxazin-4-ones have been characterized as potent inhibitors of human leukocyte elastase (HLE),[148–151] cathepsin G,[152,153] chymase,[154] C1r serine protease of the complement system,[155,156] thrombin,[157] and human cytomegalovirus protease.[158] 6-Methyl-2-*p*-tolylamino-4*H*-3,1-benzoxazin-4-one (URB754) was identified as a potent inhibitor of the endocannabinoid-deactivating enzyme monoacylglycerol lipase.[159] 2-Aryl substituted 4*H*-3,1-benzoxazin-4-ones have been evaluated as specific inhibitors of the tissue factor/factor VIIa-induced pathway of coagulation.[160]

Biological activities of 4*H*-3,1-benzothiazin-4-ones and hetero-fused analogues have been investigated less extensively.[161] Examples include 6-thiaoxanosine, an imidazo[1,5-*a*][1,3]thiazin-7(3*H*)-one riboside with strong antiviral and anticancer properties[162] and the antiproliferative compound 2-(2,4-dihydroxyphenyl)-4*H*-3,1-benzothiazin-4-one.[163] 2-Arylamino substituted thieno[1,3]thiazin-4-ones and analogous [1,3]thiazino[5,4-*b*]indole-4-ones have been reported as inhibitors of HLE.[164,165]

5.2 Chemistry of 2-*sec*-Amino-4*H*-3,1-benzothiazin-4-ones

Our initial approach to produce 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones was the treatment of methyl 2-thioureidobenzoates **70**–**77** (see Chapter 4) with concentrated sulfuric acid. This

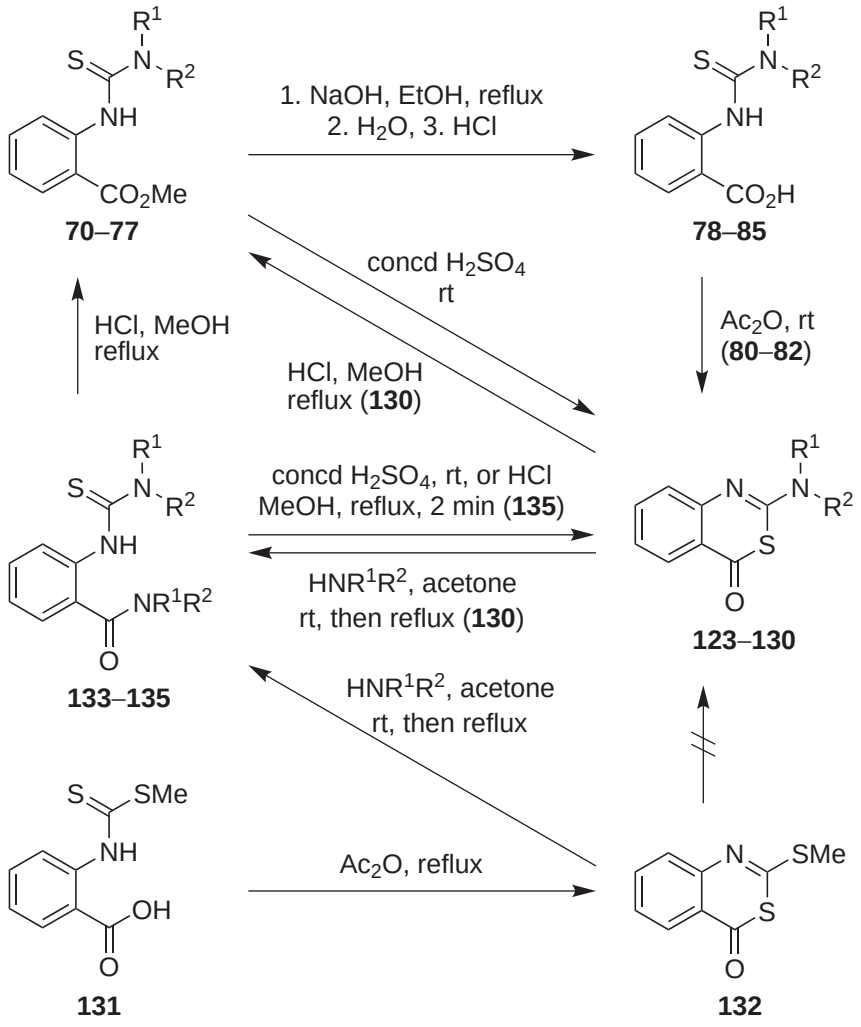
procedure was introduced to prepare 2-aminothieno[2,3-*d*][1,3]thiazin-4-ones,[166] for example, the *p*-aminobenzoic acid derivative **VI** (Figure 1.5),[66] and successfully applied to other heterocyclic systems.[164,165,167–169] Accordingly, Tarzia *et al.* have prepared the benzothiazine analogue of URB754 that way.[170] Ring closure to 4*H*-3,1-benzothiazin-4-ones was also achieved by treatment of 2-benzoylaminothiobenzamide with concentrated sulfuric acid.[171]

The reaction of compounds **70**, **71**, and **75**–**77** with concentrated sulfuric acid at room temperature conveniently afforded the desired benzothiazinones **123**, **124**, and **128**–**130** (Scheme 5.1). The benzyl(methyl)thiourea derivative **73** was not converted to **126** due to *N*-debenzylation under the strong acidic conditions used. The methyl(phenyl)thiourea **72** gave the corresponding benzothiazinone **125** in only 20% yield, and the methyl-(2-phenylethyl)thiourea **74** could not be transformed to **127**. Therefore, an alternative synthetic route was chosen. Benzoic acid derivatives **80**–**82**, which were hydrolyzed from the corresponding methyl benzoates **72**–**74** (see Chapter 4), were cyclized with acetic anhydride[172,173] to yield **125**–**127**, thus allowing the facile introduction of aromatic structures within the 2-substituent of **123**–**130**.

A synthetic access to 2-(methylthio)-4*H*-3,1-benzothiazin-4-one (**132**) was envisaged *via* the dithiocarbamate **131**, which was prepared from anthranilic acid, carbon disulfide and methyl iodide. This intermediate underwent an easy cyclocondensation upon treatment with acetic anhydride. Only one representative of this heterocyclic class, *i.e.*, 6,7-difluoro-2-(methylthio)-4*H*-3,1-benzothiazin-4-one, has already been described by Mazuoka *et al.*[174] The preparation of further 2-alkylthio-4*H*-3,1-benzothiazinones as well as their biological investigation are outlined in refs.[175,176]

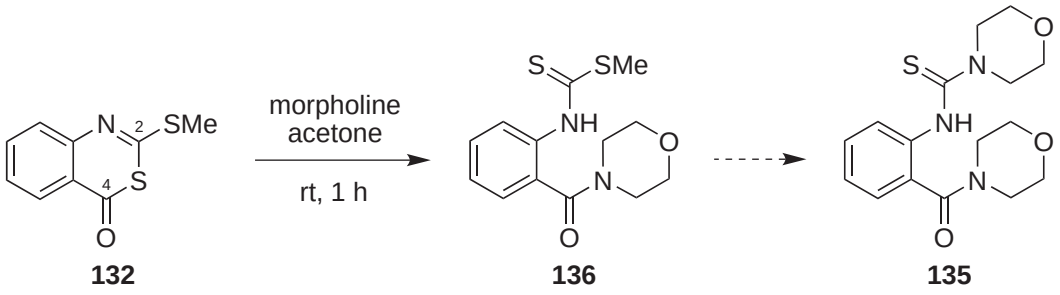
To explore an alternative entry to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones, the *S*-methyl derivative **132** was reacted with secondary amines. However, the corresponding 2-aminobenzothiazinones were not formed and instead, we obtained 2-thioureidobenzamides **133**–**135**. The attack of an amine on **132** might either occur at the C-2 or C-4 carbons. An attack at C-2 followed by C-2–S-3 bond breakage would not lead to **133**–**135**. The nucleophilic substitution of the methanethiol group would generate 2-aminobenzothiazinones **123**–**130**. Such intermediates could subsequently undergo ring cleavage due to the attack of the amine at C-4 to produce **133**–**135**. When treating the 2-morpholinobenzothiazinone **130** with morpholine under the conditions used for the conversion of **132** to **133**–**135**, compound **135** was indeed obtained. However, a different mechanism was proposed based on the isolation of the intermediate **136** in the reaction of **132** with morpholine (Scheme 5.2). Hence, the secondary amine attacks the 2-(methylthio)benzothiazinone **132** at C-4, followed by ring opening and subsequent transformation of the dithiocarbamate substituent into a thiourea. Leistner and Wagner reported on a similar formation of 2-thioureido*thio*benzamides when reacting 2-(methylthio)-4*H*-3,1-benzothiazin-4-*thione* with secondary amines.[177]

With the novel 2-thioureidobenzamides **133**–**135** in hand, we also investigated their utility as precursors to **123**–**130**. Indeed, the corresponding 2-aminobenzothiazinones **123**, **128** and **130** were obtained in quantitative yield and high purity by reacting the benzamide derivatives **133**–**135** with concentrated sulfuric acid (Scheme 5.1).



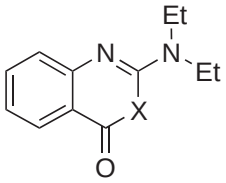
70,**78**,**123**,**133**	$R^1 = R^2 = Et$	**74**,**82**,**127**	$R^1 = Me$, $R^2 = (CH_2)_2Ph$
71,**79**,**124**	$R^1 = Me$, $R^2 = c$-Hex	**75**,**83**,**128**,**134**	NR^1R^2 = 1-pyrrolidinyl
72,**80**,**125**	$R^1 = Me$, $R^2 = Ph$	**76**,**84**,**129**	NR^1R^2 = 1-piperidinyl
73,**81**,**126**	$R^1 = Me$, $R^2 = Bn$	**77**,**85**,**130**,**135**	NR^1R^2 = 4-morpholinyl

Scheme 5.1: Synthesis and interconversion of 2-amino-4*H*-3,1-benzothiazin-4-ones **123**–**130**.

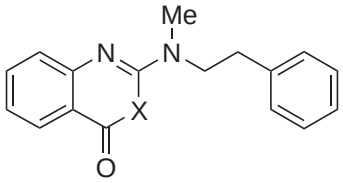


Scheme 5.2: Reaction pathway from **132** to **135**.

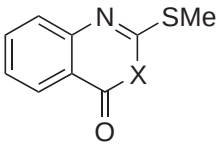
Table 5.1: Cyclization reactions of benzoic acid derivatives **78**, **82**, and **131** with acetic anhydride and trifluoroacetic anhydride, respectively.



123 X = S
137 X = O



127 X = S
138 X = O



132 X = S
139 X = O

Educt	Reagent	Thiazinone (X = S)	Yield[a] (%)	Oxazinone (X = O)	Yield[a] (%)
78	TFAA	**123**	26	**137**	65
82	Ac_2O	**127**	65[b]	—	
82	TFAA	**127**	28	**138**	68
131	Ac_2O	**132**	93[b]	—	
131	TFAA	**132**	91	**139**	2

[a] Yields of products after purification by column chromatography.
[b] Yield after recrystallization.

Heating the 2-thioureidobenzamides **133**–**135** in methanolic hydrochloric acid yielded methyl thioureidobenzoates **70**, **75**, and **77** (Scheme 5.1). This transformation is formally an acid-catalyzed amide alcoholysis under conditions where a simple benzamide such as 4-benzoylmorpholine did not react.[127] A ring closure–reopening mechanism operative in the conversion of **133**–**135** to the corresponding methyl 2-thioureidobenzoates is initiated by the rapid cyclocondensation to intermediate 2-aminobenzothiazinones **123**–**127**. This could be concluded as the product **130** was identified after short-time treatment of **135** with methanolic hydrochloric acid. Prolonged heating of **130** then led to formation of the methyl thioureidobenzoate **77**.

In the course of this study, acetic anhydride was successfully used in cyclocondensations to convert the benzoic acid derivatives **80**–**82** and **131** to benzothiazinones **125**–**127** and **132**, respectively. Unexpectedly, the replacement of acetic anhydride by trifluoroacetic anhydride (TFAA) produced different results (Table 5.1). The treatment of 2-*thioureido*benzoic acids (**78**/**82**) with this reagent gave mixtures of the corresponding benzothiazinones (**123**/**127**) and benzoxazinones (**137**/**138**) with the latter compounds being the dominant products. On the other hand, the benzothiazinone **132** was the main product of the reaction of the *dithiocarbamate* **131** with TFAA while the corresponding benzoxazinone **139** was only formed in traces. The formation of **137** and **138** is envisaged to occur by a nucleophilic attack of the carboxyl oxygen at the activated thiocarbonyl carbon.[129,178–182] Further investigations are needed to clarify the mechanism of this desulfurisation–cyclization.

In the ^{13}C NMR spectra of the benzothiazinone representatives **123** and **132** the characteristic signals for C-2/C-4 appeared at 155/185 ppm (**123**) and 164/182 ppm (**132**). The other benzothiazinones had similar NMR data. The corresponding chemical shifts of the benzoxazinones were observed at 153/160 ppm (**137**) and 164/159 ppm (**139**), respectively.

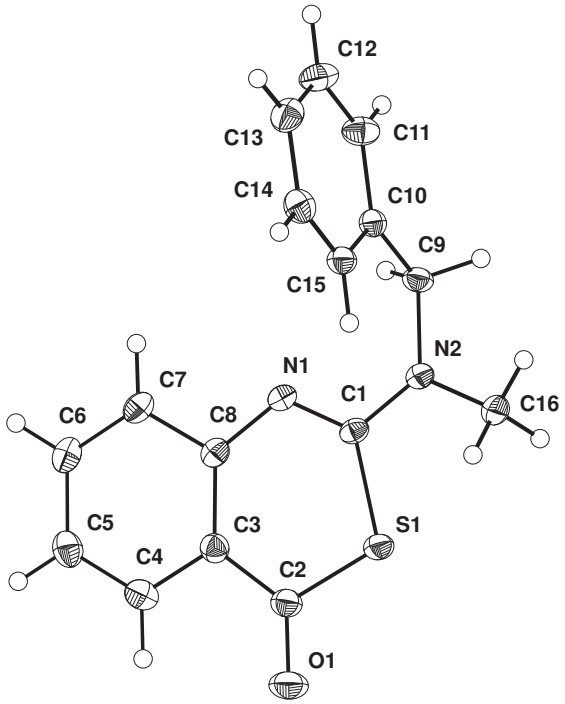


Figure 5.1: Molecular plot of **126** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level for the non-H atoms. H atoms are depicted as small circles of arbitrary radii.[183]

These values were in accordance with literature data for 4*H*-3,1-benzoxazin-4-ones.[160,179,184–186] A similar influence of the sulfur–oxygen exchange on the chemical shift of the C-4 carbon was observed for pairs of 2-thien-2-yl and 2-cyano substituted 4*H*-3,1-benzothiazin(oxazin)-4-ones.[179,187] The structure of the title compounds was furthermore confirmed by X-ray crystal structure analysis of **126** (Figure 5.1).[183] The thiazinone rings adopt an almost planar conformation with the largest deviation from the least square planes defined by the six atoms of the heterocyclic ring being 0.022 Å.

5.3 Enzyme Inhibition Studies

2-Aminobenzothiazinones **123**–**130** and the 2-methylthio derivative **132** were evaluated as potential inhibitors of HLE[188] (Table 5.2). Other representative members of serine proteases (human cathepsin G, bovine chymotrypsin and bovine trypsin) were also investigated. The compounds were furthermore assessed toward the cysteine protease human cathepsin L and the metalloprotease angiotensin converting enzyme (ACE). Two serine esterases, acetylcholinesterase (AChE) and cholesterol esterase (CEase), which share the acyl transfer mechanism with serine proteases were also included in the inhibition studies. Biological investigations were carried out by J. Zhou and S. Hautmann. Detailed descriptions of the assays as well as the calculations of IC_{50} values are given in ref.[176] and citations therein.

Table 5.2: Enzyme inhibitory activities of 2-substituted 4*H*-3,1-benzothiazin-4-ones.

	IC_{50} values (μM)							
Compd	HLE	Cathepsin G	Chymotrypsin	Trypsin	Cathepsin L	ACE	AChE	CEase
123	> 100[a]	>100	> 25	> 100	> 50	> 100	>25	> 25
124	> 100	> 50	> 100	> 100	8.93 ± 1.58[e]	> 100	> 50	> 50
125	> 25	> 100	10.4 ± 0.5[c]	> 100	> 50	> 100	> 100	> 50
126	> 25	> 100	22[d]	> 100	22[f]	> 100	> 50	25[g]
127	> 25	> 100	> 50	> 100	> 50	> 100	> 100	> 50
128	> 100	> 100	> 100	> 100	> 100	> 100	> 50	> 100
129	> 100	> 100	> 25	> 100	> 50	> 100	> 25	> 50
130	> 100	> 100	> 50	> 100	> 25	> 100	> 25	> 25
132	3.31 ± 0.24[b]	> 100	> 100	> 100	> 100	> 100	> 50	> 25

[a] Limits were calculated from duplicate measurements at one or two inhibitor concentrations.
[b] Duplicate measurement at five different inhibitor concentrations.
[c] Duplicate measurement at five different inhibitor concentrations, see Supplementary Information of ref.[176]
[d] Duplicate measurement at one inhibitor concentration (10 μM).
[e] Triplicate measurement at five different inhibitor concentrations, see Supplementary Information of ref.[176]
[f] Duplicate measurement at two inhibitor concentrations (10 and 20 μM).
[g] Quadruplicate measurement at one inhibitor concentration (5 μM).

None of the investigated 2-aminobenzothiazinones inhibited HLE. As 2-amino-substituted 4*H*-3,1-benzoxazin-4-ones are potent inhibitors of HLE, a replacement of the ring oxygen by sulfur resulted in a loss of activity, which can be attributed to the increased intrinsic stability of the benzothiazinones. The second order rate constant for the alkaline hydrolysis of **130** (1.7 $M^{-1}s^{-1}$) was significantly lower than that of the analogous 2-(morpholin-4-yl)-4*H*-3,1-benzoxazin-4-one (28 $M^{-1}s^{-1}$).[189] 2-(*N*-Cyclohexyl-*N*-methylamino)-4*H*-3,1-benzothiazin-4-one (**124**) exhibited a remarkable inhibitory capacity against human cathepsin L.[190] This compound was selective for cathepsin L with respect to the other enzymes investigated in this study. It might therefore serve as a lead structure for cysteine protease inhibitors. Further investigations are needed to inspect selectivity among cysteine proteases.

2-(Methylthio)-4*H*-3,1-benzothiazin-4-one (**132**) was identified as HLE inhibitor with an IC_{50} value in the low micromolar range. This compound carries a 2-substituent with the least steric demand among all the benzothiazinones tested. HLE has a primary substrate specificity for small aliphatic amino acid residues at P^1 position. It can therefore be assumed, that the methylthio moiety is accommodated by the S^1 subsite of HLE. The concentration-dependent inhibition by **132** is presented in Figure 5.2. The progress curves of the HLE-catalyzed substrate consumption were linear over the 10-min time course. Thus, the time-independent inhibition indicated a non-covalent interaction of **132** with HLE. Provided that **132** behaved kinetically as a competitive inhibitor, a K_i value of 1.2 μM corresponds to the IC_{50} value of 3.3 μM.[191] Noteworthy, the 2-methylthiobenzothiazinone **132** did not inhibit any of the other enzymes studied here.

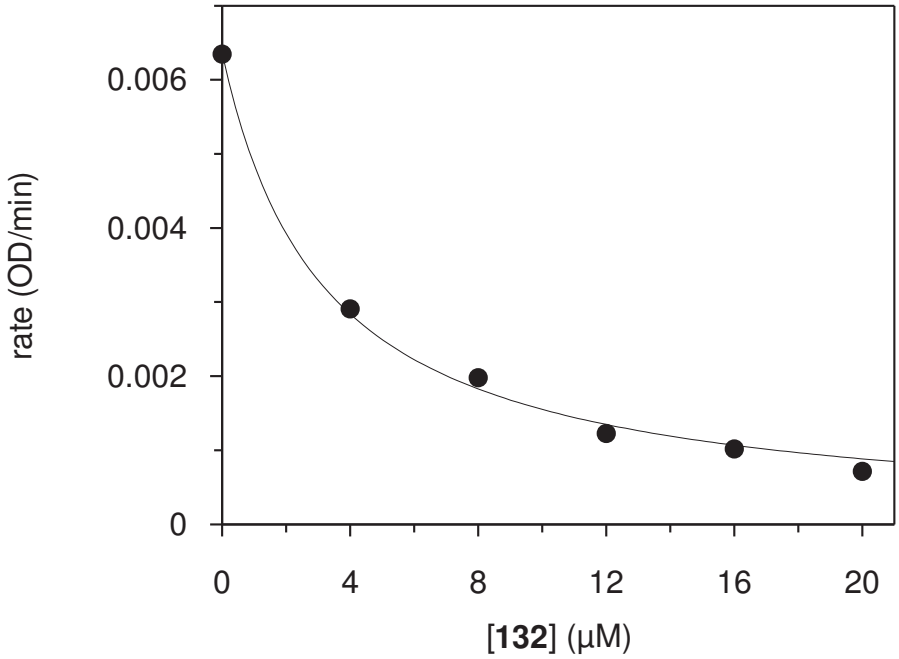


Figure 5.2: Plot of the steady-state rates *versus* inhibitor concentration for the inhibition of HLE by compound **132**.

In summary, different routes to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones **123**–**130** have been explored. A particularly versatile method involved the acetic anhydride-promoted cyclocondensation of 2-thioureidobenzoic acids **80**–**82**, which were readily accessible by saponification of the corresponding methyl 2-thioureidobenzoates (**72**–**74**). The preparation of 2-(methylthio)-4*H*-3,1-benzothiazin-4-one (**132**) from anthranilic acid was demonstrated using a two-step procedure. We could also show that **132** was ring-opened to 2-thioureidobenzamides **133**–**135**, which on their own proved to be further precursors to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones **123**–**130**. Unexpectedly, one 2-aminobenzothiazinone, **124**, inhibited human cathepsin L, a cysteine protease of therapeutic importance. In the course of this study, biological activities of 2-alkylthio-4*H*-3,1-benzothiazin-4-ones have been evaluated for the first time, and compound **132** was identified as an inhibitor of human leukocyte elastase.

CHAPTER 6

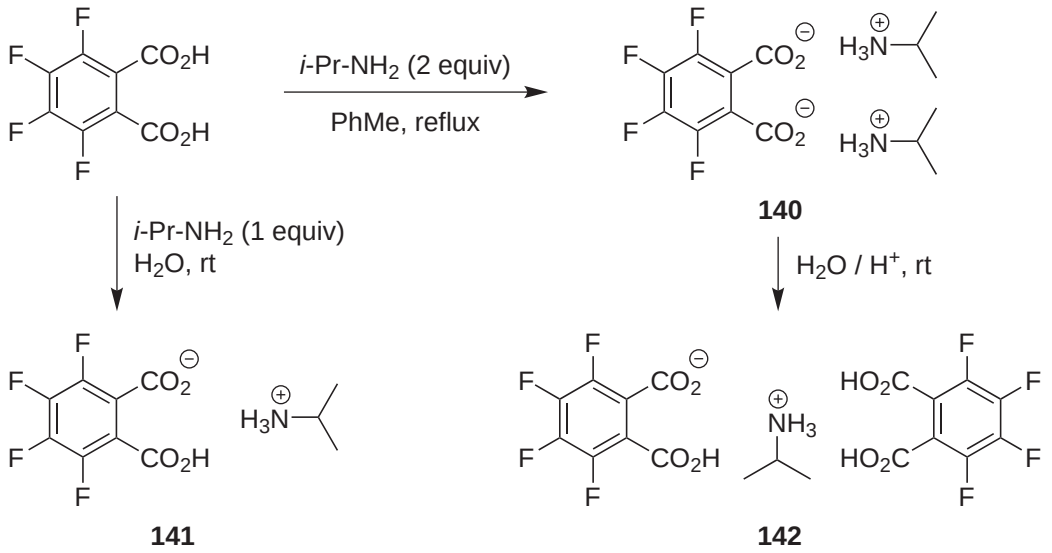
Structural Characterization of Salts From Tetrafluorophthalic Acid and Isopropylamine

6.1 Carbon-Bound Fluorine in Medicinal Chemistry

The ability of covalently bound fluorine to accept hydrogen bonds has been the subject of a scientific debate.[192–194] This issue has attracted much interest in analyses of crystal structures of fluorine-containing molecules,[195–198] particularly in cases of aromatic carbon-bound fluorine-mediated hydrogen bonds.[199] The introduction of fluorine is a commonly used strategy in medicinal chemistry to improve properties of bioactive compounds.[200,201] Although a hydrogen–fluorine exchange results in only minor steric changes, the high electronegativity of fluorine and the resulting polarization of the C–F bond remarkably alter the physicochemical properties of molecules.[193,199] Therefore, the introduction of fluorine can lead to enhanced binding interactions, increased metabolic stability or selective reactivity.[202] Favorable (fluorophilic) and unfavorable (fluorophobic) environments within target proteins have been determined. Such approaches have been exploited to develop inhibitors of serine and cysteine proteases[192–194,203–206] or to design potent inhibitors of angiogenesis by fluorination of phthalic acid derivatives.[207–210] In the course of studies on salts of tetrafluorophthalic acid, one has to face limitations by the absence of aromatic protons in ^{1}H NMR, and fluorine–carbon couplings in ^{13}C NMR spectroscopy. The structural characterization is additionally complicated as a dibasic acid (H_2Y) is able to form salts with a monoacid base (R) in different stoichiometric ratios. Besides a neutral (R_2Y) and a normal acid salt (RHY), various anomalous salts (*e.g.*, RH_3Y_2, $R_2H_4Y_3$) can be formed.[211–213]

6.2 Preparation of Isopropylammonium Tetrafluorophthalates

The reaction of tetrafluorophthalic acid with two equivalents of isopropylamine in boiling toluene gave the expected neutral salt bis(isopropylammonium) tetrafluorophthalate (**140**) in quantitative yield (Scheme 6.1). Equimolar amounts of isopropylamine and tetrafluorophthalic acid led to the normal acid salt **141**. When **140** was kept at room temperature in aqueous



Scheme 6.1: Preparation of tetrafluorophthalates **140**–**142**.

acidic solution, crystals of a different compound were obtained. This material was not the expected tetrafluorophthalic acid, but an anomalous acid salt with two dibasic acid molecules per cation,[211,212,214] compound **142**. The exact assignment of the stoichiometry of the three salts was not possible by ^{13}C NMR measurements in solution. However, the structures were assumed from elemental analysis and confirmed by X-ray crystallography as well as solid-state NMR experiments.

6.3 Crystal Structures and Hydrogen Bonding Patterns

There are only few literature reports on X-ray crystal structures of tetrafluorophthalic acid and its salts. Examples are the structures of tetrafluorophthalic acid,[215] L-histidinium tetrafluorohydrogenphthalate[216] and dipotassium tetrafluorophthalate.[217,218] For compounds **140**–**142**, selected bond lengths and angles from the crystal data are listed in Table 6.1, hydrogen bonding parameters are presented in Table 6.2.[219]

The three structures crystallized in the monoclinic system with space group $C2/c$ (**140**) and $P2_1/n$ (**141**, **142**), respectively. As has been shown for dipotassium tetrafluorophthalate,[218] molecules of compound **140** (Figure 6.1) have a crystallographic C2-symmetry with the twofold rotation axis through the center of the C1–C1# as well as the C3–C3# bonds. The dihedral angle between the planes of both carboxylate fragments (O1/C7/O2 and O1#/C7#/O2#) is 58.8° and 59.3° between a carboxylate group and the tetrafluorobenzene ring. This differs from the conformation in dipotassium tetrafluorophthalate, in which a carboxylate group is nearly perpendicular (89.1°) to the other and inclined at an angle of 82.0° to the benzene ring.[218] In the crystal structure of **141** (Figure 6.2), the dihedral angle between the planes of the carboxylate fragment (O1/C7/O2) and the protonated carboxyl group (O3/C8/O4) is 56.4°. The angles of both groups relative to the tetrafluorobenzene ring are 50.8° (O1/C7/O2) and 58.6° (O3/C8/O4), respectively.

Table 6.1: Selected bond lengths (Å) and angles (°) for **140**–**142**.

140			
C7–O1	1.265(2)		
C7–O2	1.238(1)		
O1–C7–O2	126.0(1)		
C2–C1–C7–O1	120.5(1)		
C2–C1–C7–O2	−59.8(2)		
141			
C7–O1	1.2636(15)		
C7–O2	1.2389(15)		
C8–O3	1.3104(16)		
C8–O4	1.2130(16)		
O1–C7–O2	128.11(12)		
O3–C8–O4	125.38(12)		
C6–C1–C7–O1	−52.54(16)		
C6–C1–C7–O2	126.81(13)		
C3–C2–C8–O3	120.54(12)		
C3–C2–C8–O4	−56.65(17)		
142			
C7–O1	1.212(4)	C7′–O1′	1.210(4)
C7–O2	1.310(5)	C7′–O2′	1.316(4)
C8–O3	1.233(4)	C8′–O3′	1.212(4)
C8–O4	1.264(4)	C8′–O4′	1.311(4)
O1–C7–O2	125.4(3)	O1′–C7′–O2′	125.2(3)
O3–C8–O4	126.1(3)	O3′–C8′–O4′	124.8(3)
C6–C1–C7–O1	71.3(5)	C6′–C1′–C7′–O1′	99.5(4)
C6–C1–C7–O2	−103.6(4)	C6′–C1′–C7′–O2′	−74.2(4)
C3–C2–C8–O3	−149.4(3)	C3′–C2′–C8′–O3′	166.7(3)
C3–C2–C8–O4	29.8(5)	C3′–C2′–C8′–O4′	−13.0(5)

Table 6.2: Hydrogen bonding parameters for **140**–**142**.

D–H···A	D–H (Å)	H···A (Å)	D···A (Å)	D–H···A (°)
140				
N1–HN1A···O1[a]	0.95(2)	1.83(2)	2.775(2)	173(1)
N1–HN1B···O2	0.96(2)	2.08(2)	2.826(1)	134(1)
N1–HN1B···O2[b]	0.96(2)	2.23(2)	2.886(2)	125(1)
N1–HN1C···O1[c]	0.92(2)	1.90(2)	2.807(1)	171(1)
141				
O3–HO3···O1[d]	0.99(2)	1.62(2)	2.551(1)	155(2)
N1–HN1A···O1[e]	0.91(2)	2.02(2)	2.929(1)	178(1)
N1–HN1B···O2[f]	0.91(2)	1.87(2)	2.771(2)	170(2)
N1–HN1C···O4[g]	0.93(2)	2.02(2)	2.859(2)	148(2)
N1–HN1C···F3[h]	0.93(2)	2.42(2)	3.029(1)	123(1)
C11–H11B···O3[e]	0.98(2)	2.50(2)	3.388(2)	150(1)
142				
N1–H1A···O1	0.91(1)	1.95(2)	2.840(4)	168(4)
N1–H1B···O1′	0.91(1)	2.02(2)	2.851(4)	152(4)
N1–H1B···O3′	0.91(1)	2.62(3)	3.237(4)	126(3)
N1–H1C···O2′[i]	0.91(1)	2.41(2)	3.248(4)	154(4)
O2–H2···O3[j]	0.84(1)	1.79(2)	2.588(4)	157(4)
O2′–H2′···O4[j]	0.84(1)	1.77(1)	2.612(3)	174(5)
O4′–H4′···O4[k]	0.84(1)	1.77(1)	2.603(4)	175(5)
C9–H9···O2[j]	1.00	2.54	3.424(5)	148
C11–H11a···O3	0.98	2.50	3.313(5)	140

Symmetry transformations used to generate equivalent atoms:

[a] $x,\ -y+1,\ z+1/2$
[b] $-x,\ y,\ -z+1/2$
[c] $x,\ y,\ z+1$
[d] $x+1/2,\ -y+5/2,\ z+1/2$
[e] $-x+1/2,\ y-1/2,\ -z+3/2$
[f] $-x,\ -y+2,\ -z+2$
[g] $-x+1/2,\ y-1/2,\ -z+5/2$
[h] $x+1/2,\ -y+3/2,\ z+1/2$
[i] $-x+1,\ -y+2,\ -z+1$
[j] $-x+1,\ -y+1,\ -z+1$
[k] $x,\ y+1,\ z$

Molecules of **142** contain a tetrafluorophthalic acid component (Figure 6.3, left) and a tetrafluorohydrogenphthalate unit (Figure 6.3, right). The two protonated carboxyl groups of the tetrafluorophthalic acid component are twisted to each other by 74.6° and have dihedral angles relative to the tetrafluorobenzene ring of 75.3° (O1′/C7′/O2′) and 12.0° (O3′/C8′/O4′). A resembling conformation was observed for the crystals from the sole tetrafluorophthalic acid with corresponding dihedral angles between the carboxyl groups and the aromatic ring of 81.0° and 13.4°, respectively.[215] In the tetrafluorohydrogenphthalate unit of **142**, the planes of the deprotonated and protonated carboxyl groups have a dihedral angle of 66.3°, and each of them is inclined to the tetrafluorobenzene ring by 30.4° (O3/C8/O4) and 75.6° (O1/C7/O2). The latter two values show the same tendency compared to those observed in **141**, but differ from typical orientations in hydrogenphthalates with angles between carboxylate fragments and phenyl planes in the range of 65–85° and smaller angles (5–40°) between carboxyl groups and benzene rings.[220]

Only N–H···O hydrogen bonds have been observed in the crystal structure of compound **140** (Figure 6.1). All the three hydrogen atoms of the isopropylammonium group are part of hydrogen bonds including two between the nitrogen and two O1 atoms of different molecules as well as one bifurcated interaction to connect the nitrogen with two O2 atoms of different molecules (Table 6.2). The hydrogen-acceptor distances of the two-center hydrogen bonds (1.83, 1.90 Å) were in accordance with literature data (mean value 1.84 Å, between mono-substituted ammonium groups and *carboxylate* oxygens).[221] The distances of the three-center (bifurcated) hydrogen bond are longer, and their angles have typical values (134, 125°) for intermolecular three-center hydrogen bonds.[222] As expected,[222,223] the major component of the three-center bond (*i.e.*, the one with the shorter H···O distance) is more linear than the minor one.

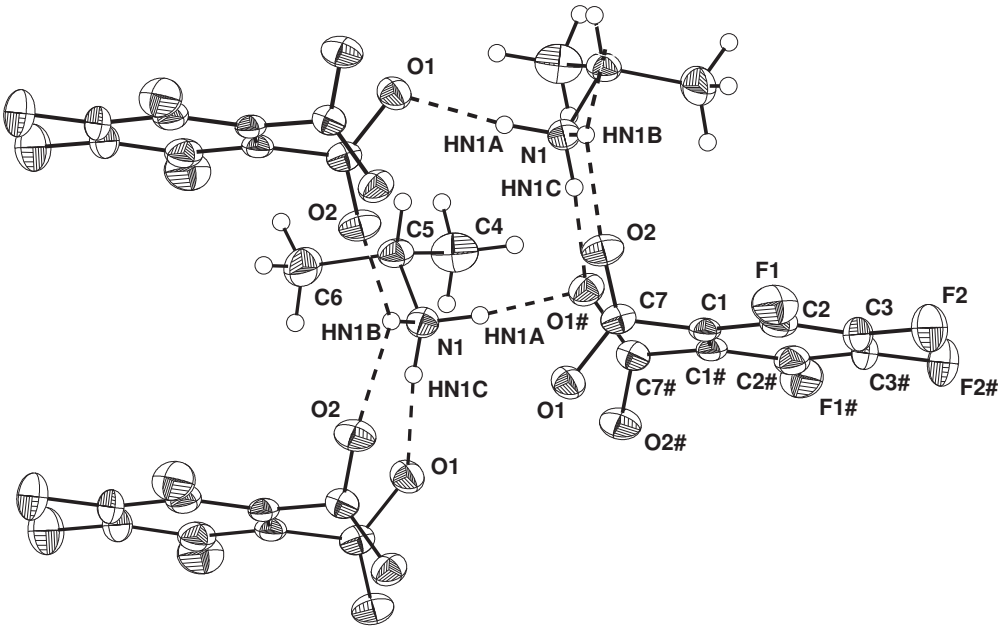


Figure 6.1: Molecular plot of **140** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level for the non-H atoms. H atoms are depicted as small circles of arbitrary radii and dashed lines represent hydrogen bonds.[219]

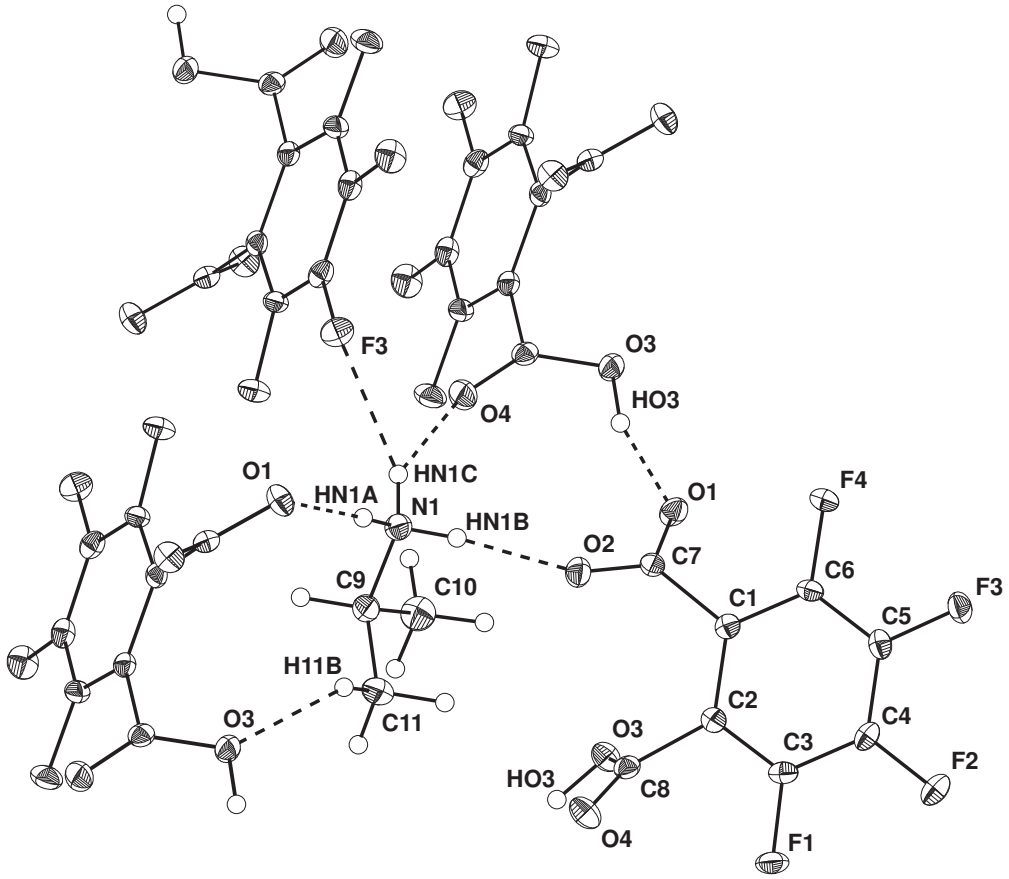


Figure 6.2: Molecular plot of **141** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level for the non-H atoms. H atoms are depicted as small circles of arbitrary radii and dashed lines represent hydrogen bonds.[219]

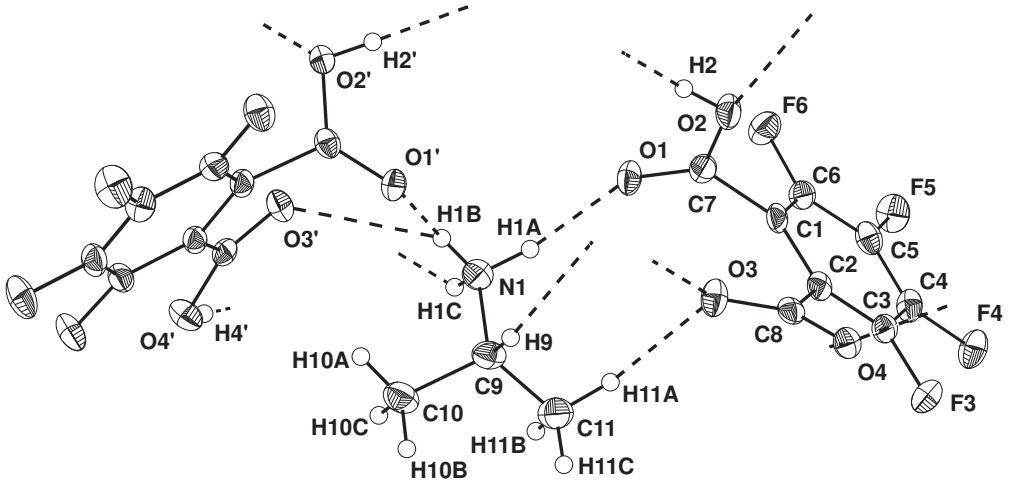


Figure 6.3: Molecular plot of **142** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level for the non-H atoms. H atoms are depicted as small circles of arbitrary radii and dashed lines represent hydrogen bonds.[219]

Different types of hydrogen bonds were found in compound **141**. Besides N–H···O, O–H···O, and C–H···O bonds, one fluorine of the aromatic ring was incorporated into a hydrogen bond of type N–H···F. All three ammonium hydrogen atoms are part of hydrogen bonds with four different tetrafluorohydrogenphthalate molecules. The hydrogen bond network is shown in Figure 6.2. As in structure **140** two hydrogens are incorporated in two-center N–H···O hydrogen bonds. In the presented structure **141**, both contacts are formed with oxygens of carboxylate fragments. The HN1B···O2 distance (1.87 Å) is in accordance with literature value mentioned above,[221] but the HN1A···O1 bond is somewhat longer (2.02 Å).

In contrast to **140** and **142**, a fluorine atom, *i.e.*, F3 in *para*-position to the protonated carboxyl group, is involved in the hydrogen bonding network of structure **141**. The hydrogen atom HN1C is forming a three-center (bifurcated) hydrogen bond to acceptors fluorine F3 and oxygen O4 from two different tetrafluorohydrogenphthalate molecules. The major component of this unsymmetrical bifurcated hydrogen bond is directed to the oxygen, as it is more linear (148° *versus* 123°) and has a shorter hydrogen-acceptor distance in comparison to the minor one directed to the fluorine (2.02 Å *versus* 2.43 Å). There are structures reported with bifurcated hydrogen bonds to fluorine and oxygen, but the donors/acceptors do not belong to three different molecules in such cases.[196,224] The protonated oxygen O3 of the carboxyl group is part of an intermolecular O–H···O bond to oxygen O1 of the carboxylate fragment. Such strong contacts (O···O distance 2.55 Å) were also observed in crystals of other hydrogenphthalates.[220,225–227] Moreover, the carboxyl oxygen O3 accepts a C–H···O hydrogen bond from a methyl group of the isopropyl ammonium molecule.

In the structure of **142**, N–H···O, O–H···O, and C–H···O hydrogen bonds were observed, all of them being intermolecular (Figure 6.3). As in structures **140** and **142**, two two-center and one three-center N–H···O hydrogen bonds were formed (Table 6.2). The hydrogen-acceptor distance (1.95 Å) of the two-center hydrogen bond to the carbonyl oxygen of a protonated carboxyl group (N1–H1A···O1) agreed with published data (mean value 1.94 Å, between mono-substituted ammonium groups and *carboxyl* oxygens).[221] Again, the bifurcated hydrogen bond has a major and a minor component. Their H···O distances are longer (2.02, 2.62 Å) and their angles are smaller (152, 126°) compared to the above-mentioned two-center bond. Further hydrogen bonds were formed with protonated carboxyl groups as donors, which again can be classified as strong contacts.[228] Only the oxygens of the carboxylate group (O3, O4) are multiple acceptors[229] for two hydrogen bonds each, with O and C as hydrogen donating atoms. Benedict *et al.* reported on the structure of potassium hydrogen diphthalatedihydrate where a hydrogen phthalate dimer is bridge by two symmetry-related H atoms, each with half-site occupancies.[230] As discussed above, also **142** can be regarded as an anomalous acid salt with two dibasic acid molecules per cation. Such anomalous acid salts have been the objective of studies regarding their solid structure and the nature of hydrogen bonds.[211,212,214]

In total four N–H···O hydrogen bonds are directed toward the carboxylate oxygens of **140**, the carboxylate fragment of **142** accepts four hydrogen bonds from N–H or C–H donors, whereas three hydrogen bonds from O–H or N–H are directed toward the carboxylate oxygens of **141**. Taken together, the three crystal structures of **140**, **142** and **141** show different binding geometries and hydrogen bonding arrangements. Only in the structure of **141**, a fluorine atom acts as a hydrogen bond acceptor.

6.4 Spectroscopic Characteristics

^{1}H and ^{13}C NMR spectra of compounds **140**–**142** were recorded in DMSO-d_6. The ^{1}H NMR spectra of the three salts are similar, showing the expected signals for the aliphatic protons. The shifts for the aromatic carbons were assigned on the basis of ^{13}C–^{19}F coupling constants and substituent increments.[231] $^{2}J_{\mathrm{C–F}}$ coupling constants for carbons 1 and 2 were 14 and 16 Hz, respectively, and $^{1}J_{\mathrm{C–F}}$ coupling constants for carbons 3–6 were in the range of 236–253 Hz. In all cases, only four different signals for the carbons of the tetrafluorophthalic acid components appeared in the spectra. Noteworthy, different positions relative to the protonated or deprotonated carboxyl moieties in **141** and **142** were not reflected, additionally, the two tetrafluorophthalic acid units of **142** were not distinguishable. This can be attributed to a rapid proton migration in solution. Similar observations have been made for related proton transfers in 1,3-dicarbonyl compounds[232] and dicarboxylic acids.[233]

When comparing the salts **140**–**142**, we noticed substantially different ^{13}C chemical shifts for the CO carbons and for those bearing the COO(H) groups (*ipso*-C). The values correlate with the ratio of carboxylate fragments to carboxyl groups. The neutral salt **140** with exclusively carboxylate fragments provides shifts of 164.5 ppm (CO) and 125.9 ppm (*ipso*-C), respectively. The values of the normal acid salt **141** with COO^{-} and COOH in equal amounts are 163.1 ppm (CO) and 122.2 ppm (*ipso*-C). A COO^{-}/COOH ratio of 1:3 in the anomalous salt **142** yields 162.9 ppm (CO) and 120.3 ppm (*ipso*-C).

The signals of compound **142** are close to the values of tetrafluorophthalic acid. We have recorded the ^{13}C NMR spectrum of tetrafluorophthalic acid under the same conditions to obtain chemical shifts in agreement with reported values[234] and coupling constants as follows: 118.27 (d, $^{2}J_{\mathrm{C–F}} = 15$ Hz, C-1/2), 141.33 (dtd, $^{1}J_{\mathrm{C–F}} = 254$ Hz, $^{2}J_{\mathrm{C–F}} = 15$ Hz, $^{3}J_{\mathrm{C–F}} = 5$ Hz, C-4/5), 144.47 (dd, $^{1}J_{\mathrm{C–F}} = 252$ Hz, $^{2}J_{\mathrm{C–F}} = 10$ Hz), 162.8 (CO). Thus, the *ipso*-carbon signal in tetrafluorophthalic acid is further upfield shifted by 2.07 ppm, compared to the one of compound **142**. In particular, the value for the *ipso*-position (substituent increment[231] for COONa: 8.4, for COOH: 2.1) indicates the stoichiometric ratio, whereas the shifts of the other benzene carbons are inappropriate because of carbon–fluorine couplings.

In accordance with the stoichiometric ratio, the peak intensity of the carbonyl singlet relative to the aliphatic signals is larger in **141** than in **140**. However, similar ratios were observed for the normal acid (**141**) and the anomalous salt (**142**).

Further NMR experiments were performed to characterize the structure of the anomalous acid salt **142**. In solution, only one set of ^{13}C NMR signals was observed and thus, all carboxyl groups appear to be equivalent. This might be attributed to fast proton transfer processes which are facilitated by intermolecular N–H···O and O–H···O hydrogen bonds. Due to the rapid proton migration,[232,233] even at lower temperature the corresponding carbons of **142** could not be differentiated by NMR measurements in solution. Spectra of **142** recorded in MeOD in the range of 293–213 K are given in Figure 6.4. A temperature-dependent shift of the signals[231] occurred, but a splitting of the carbon signals was not observed. Explicitly, a single resonance for the carboxylate and protonated carboxyl groups, as well as a single signal for the *ipso*-carbons remained.

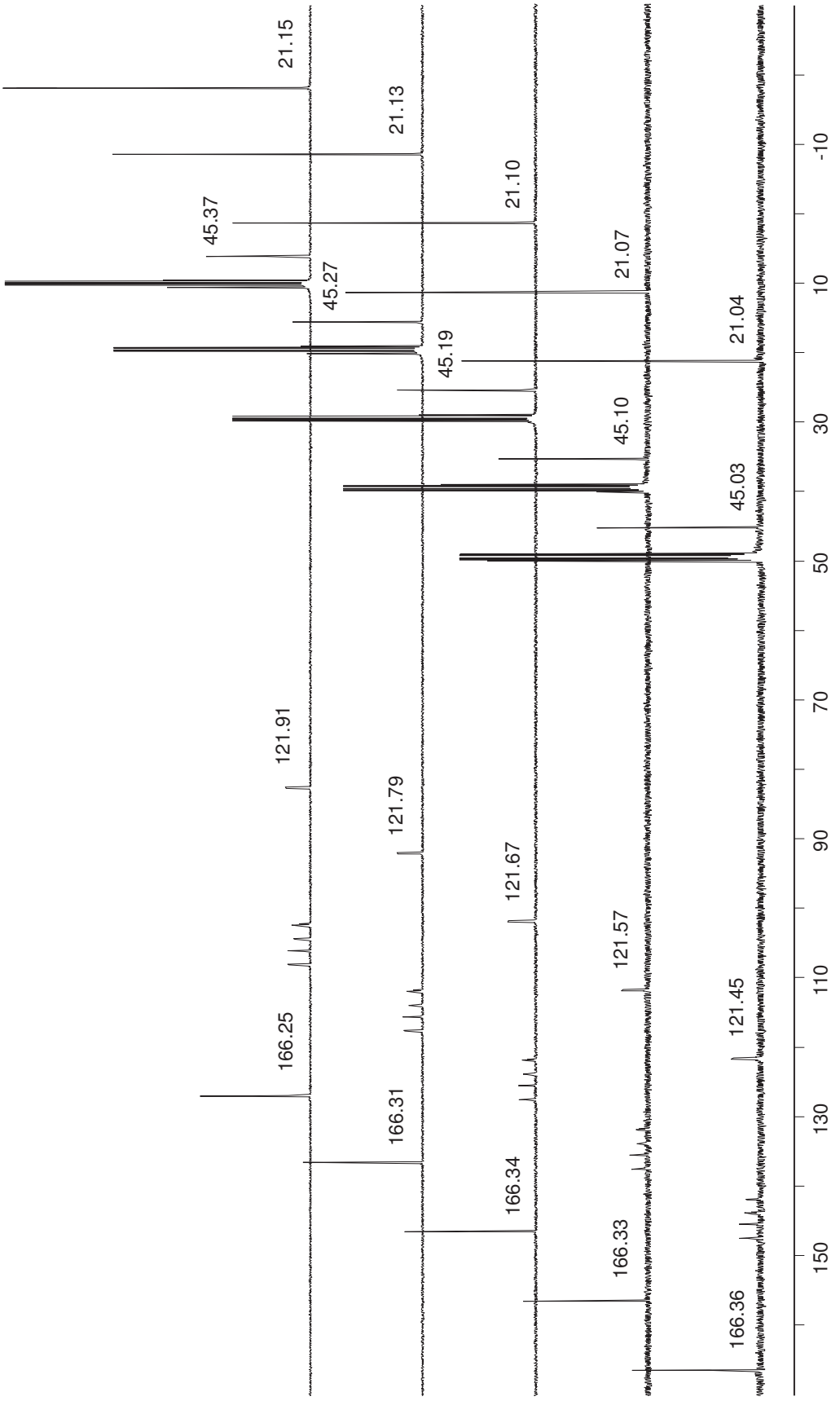


Figure 6.4: Temperature-dependent ^{13}C NMR measurements of the anomalous acid salt **142**, recorded in MeOD at 293 K (upper spectrum), 273, 253, 233, and 213 K. The two lower spectra were recorded with a reduced number of scans.

Anomalous acid salts and proton transfers have been investigated using ^{13}C solid-state NMR techniques.[212,214,232] In the course of our study, solid-state NMR experiments were carried out by Dr. W. Hoffbauer from the Institute of Inorganic Chemistry, University of Bonn. In the spectra of **140**, **141**, and **142** (Figure 6.5), chemical shifts of fluorine-substituted carbons appear together as broad signals (136–149, 135–149, and 138–151 ppm, respectively). As expected, the spectrum of bis(isopropylammonium) tetrafluorophthalate (**140**) did not show a splitting in the carbon signals, since the corresponding carbons are equivalent.

The anomalous acid salt **142**, however, provided a solid-state ^{13}C NMR spectrum significantly different from that recorded in solution. A splitting into three resonances arising from the *ipso*-carbons was observed. Thus, the *ipso*-carbons (C1, C2) of the tetrafluorohydrogenphthalate unit (114.2, 120.3 ppm) became distinguishable from the *ipso*-carbons (C1′, C2′) of the tetrafluorophthalic acid component. The signal for the latter two equivalent carbons (122.3 ppm) could be assigned on the basis of its integration (twofold higher AUC). The asymmetry of the normal acid salt **141**, induced by the different protonation, is also reflected by two signals for the *ipso*-carbons. Integration of the corresponding peak areas gave similar values (3.1 *versus* 3.6).

Vila *et al.* studied 1,3-diphenyl-propane-1,3-dione and noticed splitting of the *ipso*-carbon and carbonyl carbon signals.[232] In contrast, the solid-state ^{13}C NMR spectra of **141** and **142** do not reveal a dissimilarity of the CO carbons, since their signals are not separated. The occurrence of one common signal for COO^- and COOH carbons is in agreement with the relatively low sensitivity of the ^{13}C isotropic chemical shift to protonation, as can be explained by relations between main components δ_{22} and δ_{11} of the ^{13}C chemical shift tensor.[235] However, as a matter of principle, such a differentiation is possible. Ilczyszyn *et al.* have determined the structure of a sarcosine–maleic acid (1:1) complex by X-ray diffraction and distinguished the charged and uncharged carboxyl(ate) groups by means of solid-state ^{13}C NMR spectroscopy.[236] Barry *et al.* characterized normal and anomalous tetramethylammonium salts of dicarboxylic acids and observed different ^{13}C NMR shifts for nonequivalent carboxyl(ate) carbons in solid state.[214] In the cases of **141** and **142**, crystal and bonding forces which influence bond angles and distances and thus the resulting ^{13}C chemical shift[212] might be responsible for a coalescence of the CO signals.

In summary, we have characterized three salts derived from tetrafluorophthalic acid and isopropylamine. Crystallographic data revealed that a fluorine atom is involved in the hydrogen bonding network of structure **141**. Molecules of the anomalous salt **142** contain a tetrafluorophthalic acid component and a tetrafluorohydrogenphthalate unit. The compounds were also characterized by means of solution and solid-state NMR. The *ipso*-carbons of **141** and **142**, whose signals are equivalent in solution, could be distinguished in the solid state, thus reflecting the asymmetric nature of **141** and the anomalous salt structure of **142**.

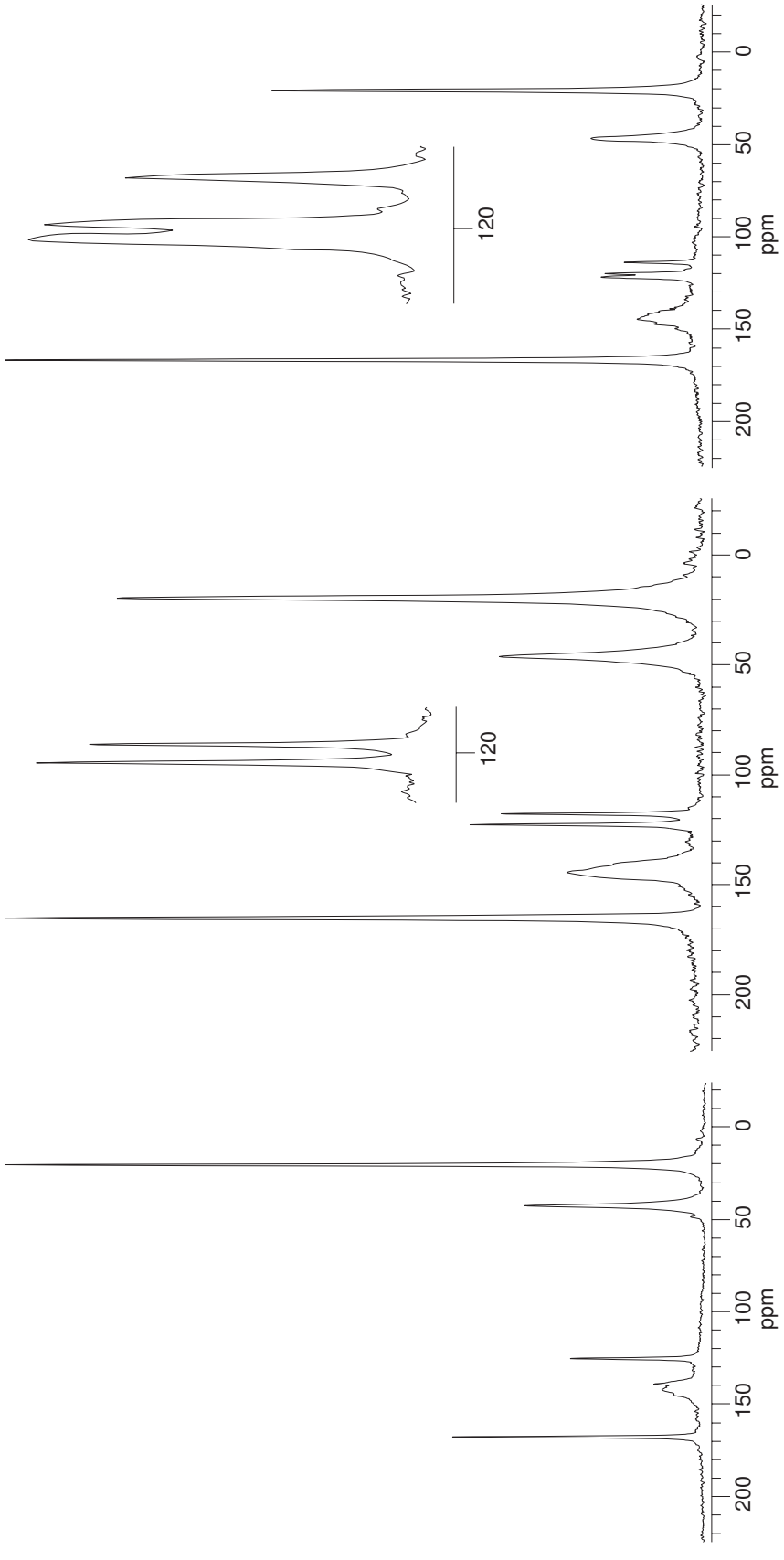


Figure 6.5: ^{13}C CP-MAS spectra of **140** (left), **141** (center), and **142** (right). Expansions of the *ipso*-carbon regions are given as insets for **141** and **142**, respectively.

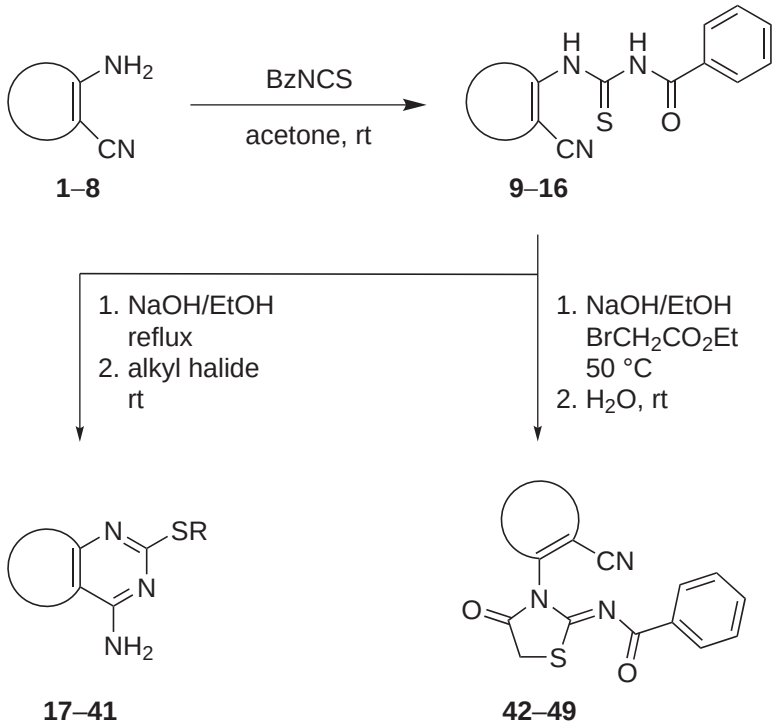
CHAPTER 7

Summary

In this work, different series of *ortho*-substituted aromatic (thio)ureas and derived heterocycles were synthesized for various biological testings. The studies included the establishment of new synthetic routes and the analytical characterization of the target compounds.

A panel of 2-alkylsulfanyl-substituted 4-aminothieno[2,3-*d*]pyrimidines and 4-aminoquinazolines was devised on the basis of a lead structure that was described as a modulator of the ABC transporter P-glycoprotein. As depicted in Scheme 7.1, the synthesis of new representatives included the preparation of various *N*-benzoyl-*N*′-(*o*-cyanoaryl)thioureas from aromatic *o*-aminonitriles and their subsequent transformation with different alkyl halides. In addition to the confirmation of structure and purity, ^{13}C signals of the heterocycles were assigned on the basis of two-dimensional NMR experiments. The substances were evaluated for their ability to affect P-glycoprotein in the group of Prof. Dr. M. Wiese. At first, daunorubicin accumulation was measured in human ovarian cancer cells that overexpressed the drug-efflux transporter. In accordance with the lead compound, most (hetero)fused 4-aminopyrimidines activated daunorubicin efflux from the cells. Additionally, a bidirectional modulation of P-glycoprotein activity could be demonstrated by the combination of different cytostatics and selected compounds in cell viability measurements. The cells showed an increased resistance to daunorubicin, while, in contrast, a potentiation of vinblastine cytotoxicity was observed.

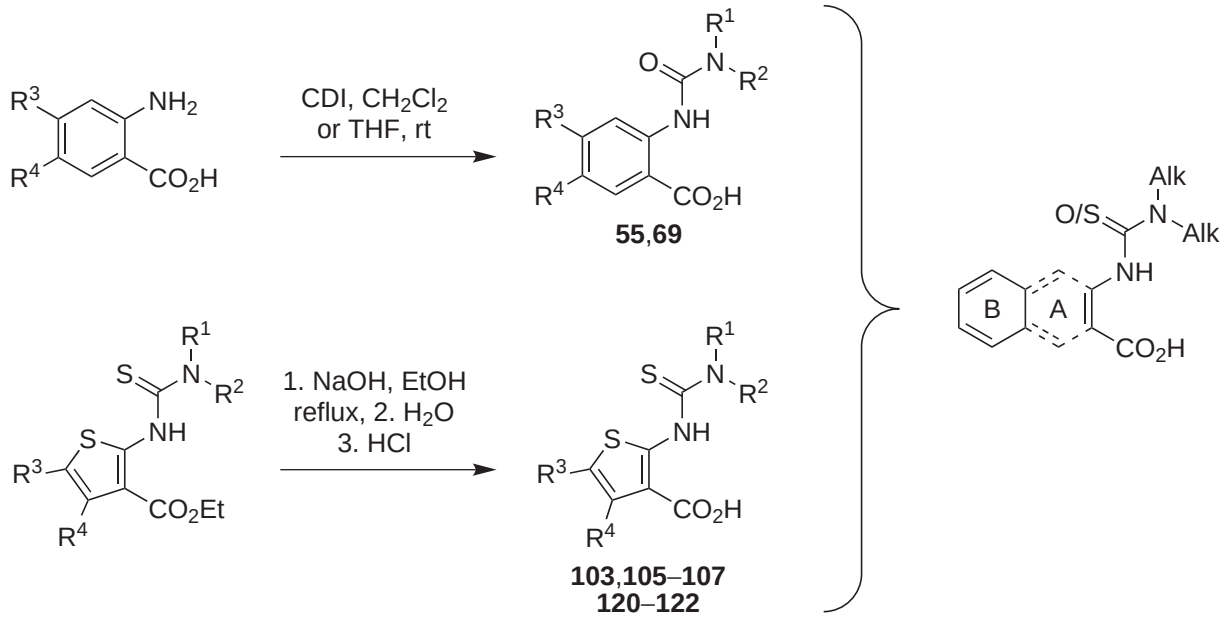
In a second project, the cyclization of *N*-benzoyl-*N*′-(*o*-cyanoaryl)thioureas with ethyl bromoacetate under alkaline conditions was examined in detail. Optimized reaction conditions were elaborated for the selective preparation of either fused 4-aminopyrimidines or 2-(benzoylimino)thiazolidin-4-ones (Scheme 7.1). Again, a small library of the latter heterocycles was generated from the aforementioned intermediates. Atropisomerism in the thiazolidin-4-ones was found to be influenced by the size of the *o*-cyanoaryl ring. This phenomenon was investigated by means of NMR measurements and X-ray crystallography and further supported by theoretical calculations. In contrast to (*o*-cyanothienyl)-substituted thiazolidin-4-ones, (*o*-cyanophenyl)-substituted derivatives cannot overcome internal rotational barriers at room temperature and were characterized as axially chiral racemates.



Scheme 7.1: Preparation of (hetero)fused 2-alkylsulfanyl-4-aminopyrimidines **17**–**41** and 2-(benzoylimino)-3-(*o*-cyanoaryl)thiazolidin-4-ones **42**–**49**.

The development of new inhibitors of multidrug resistance-associated protein 1 in collaboration with the group of Prof. Dr. M. Wiese was another main objective of the dissertation. On the basis of an initial screening, four series of aromatic benzoic and thiophenecarboxylic acids with a substituted urea or thiourea moiety at the neighboring position to the carboxyl group were provided. In addition to compounds from the substance library of our group, new (hetero)aromatic carboxylic acids were synthesized. This included the application of novel synthetic routes, such as the *N*,*N*′-carbonyldiimidazole-based conversion of anthranilic acids to 2-(3,3-dialkyl)ureidobenzoic acids or the direct saponification of methyl 2-thioureidobenzoates and ethyl 2-thioureidothiophene-3-carboxylates to the corresponding carboxylic acids (Scheme 7.2 and Scheme 7.3).

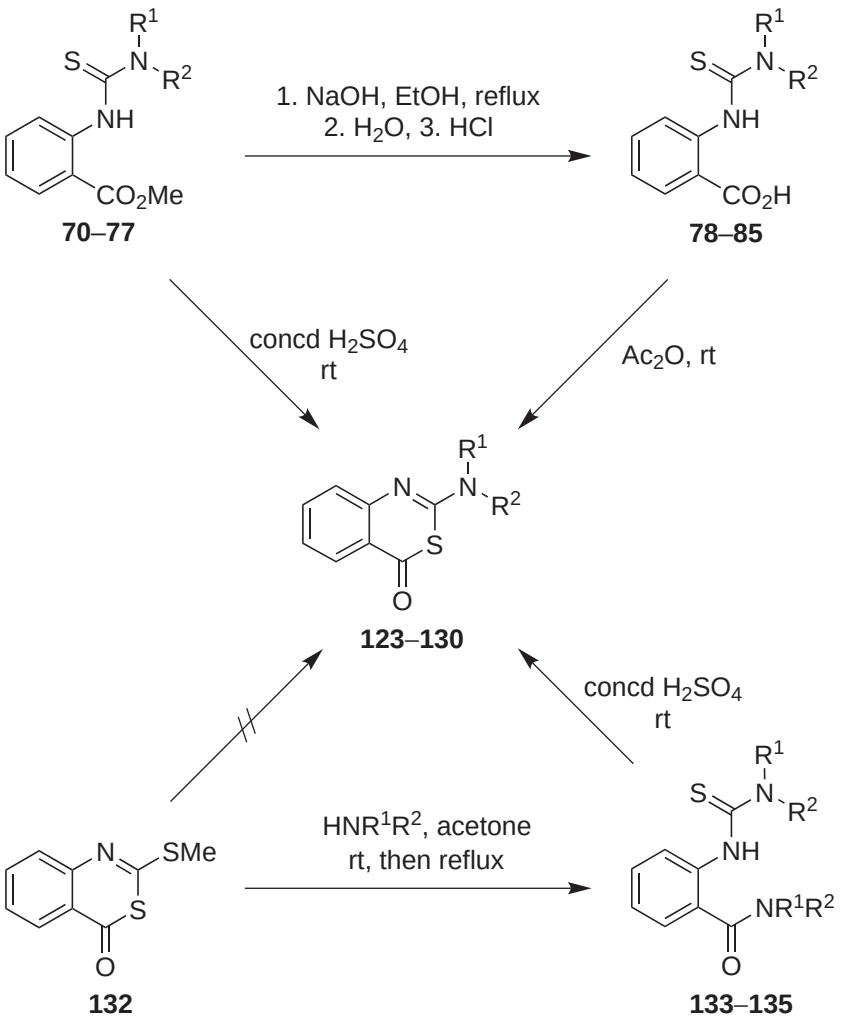
In the group of Prof. Dr. M. Wiese, the four different compound series were evaluated as inhibitors of multidrug resistance-associated protein 1 and selected substances were examined toward P-glycoprotein and breast cancer resistance protein to assess selectivity. Two 2-thioureidobenzo[*b*]thiophene-3-carboxylic acids were identified as particularly potent inhibitors of the former efflux transporter with IC_{50} values of around 1 μM. Additionally, structure–activity relationships were derived from the biological data. The essential components of this new family of nontoxic multidrug resistance-associated protein 1 inhibitors are a carboxyl group at a (hetero)aromatic scaffold with a (thio)urea function in *ortho*-position (Scheme 7.2). The terminal nitrogen of the (thio)urea should be disubstituted, preferentially with two alkyl groups. The (hetero)aromatic ring consists of benzene or thiophene, while an additional fused benzene ring improves the inhibitory activity.



Scheme 7.2: Novel synthetic pathways to aromatic *o*-(thio)ureidocarboxylic acids (left) and structural features for the inhibition of multidrug resistance-associated protein 1 (right).

Different routes that were explored to produce 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones are shown in Scheme 7.3. Those compounds were accessible from the aforementioned methyl 2-thioureidobenzoates as well as the corresponding carboxylic acids. The preparation of 2-(methylthio)-4*H*-3,1-benzothiazin-4-one from anthranilic acid was accomplished by a two-step procedure. Its direct conversion to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones failed, however, a ring-opening reaction to novel 2-thioureidobenzamides was found, which on their own proved to be further precursors to the target compounds. In addition, interconversion pathways were elucidated, for example, by isolation and characterization of intermediates. In the course of this study, biological activities of 4*H*-3,1-benzothiazin-4-ones were evaluated on a panel of eight proteases and esterases in our group. 2-(*N*-Cyclohexyl-*N*-methylamino)- and 2-(methylthio)-4*H*-3,1-benzothiazin-4-one were identified as selective inhibitors of cathepsin L and human leukocyte elastase, respectively.

Another project focused on the structural characterization of three related salts derived from tetrafluorophthalic acid and isopropylamine. Among them, an anomalous salt with two dibasic acid molecules per cation was identified (Figure 7.1). Data from X-ray crystallography were employed to describe geometrical arrangements as well as hydrogen-bonding networks of the salts. In the crystal structure of isopropylammonium tetrafluorohydrogenphthalate, an aromatic carbon-bound fluorine was incorporated into a N–H···F hydrogen bond. The compounds were furthermore characterized by means of solution and solid-state NMR spectroscopy. In the solid-state spectra of two salts, a signal splitting of the *ipso*-carbons bearing the carboxyl/carboxylate groups occurred. This observation was in agreement with the anomalous salt structure of isopropylammonium tetrafluorohydrogenphthalate × tetrafluorophthalic acid and reflected the asymmetric nature of isopropylammonium tetrafluorohydrogenphthalate as well.



Scheme 7.3: Synthetic routes to 2-*sec*-amino-4*H*-3,1-benzothiazin-4-ones.

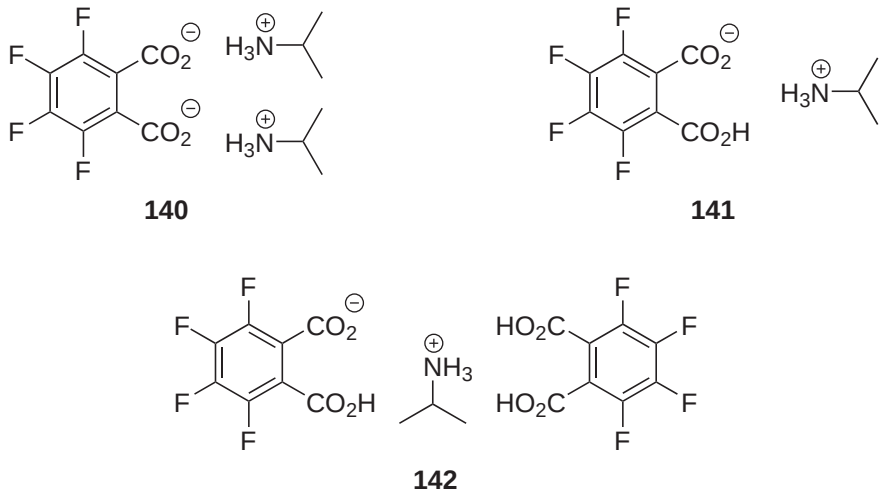


Figure 7.1: Different salts derived from tetrafluorophthalic acid and isopropylamine.

Major parts of the results presented herein were published in the following articles:

Pietsch, M.; Häcker, H.-G.; Schnakenburg, G.; Hoffbauer, W.; Nieger, M.; Gütschow, M. Structural characterization of two salts derived from tetrafluorophthalic acid and isopropylamine. *J. Mol. Struct.* **2008**, *878*, 131–138.

Leyers, S.; Häcker, H.-G.; Wiendlocha, J.; Gütschow, M.; Wiese, M. A 4-aminobenzoic acid derivative as novel lead for selective inhibitors of multidrug resistance-associated proteins. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4761–4763.

Häcker, H.-G.; Grundmann, F.; Lohr, F.; Ottersbach, P. A.; Zhou, J.; Schnakenburg, G.; Gütschow, M. 2-Amino- and 2-alkylthio-4*H*-3,1-benzothiazin-4-ones: synthesis, interconversion and enzyme inhibitory activities. *Molecules* **2009**, *14*, 378–402.

Häcker, H.-G.; Elsinghorst, P. W.; Michels, S.; Daniels, J.; Schnakenburg, G.; Gütschow, M. 2-(Benzoylimino)thiazolidin-4-ones: formation by an alternative ring closure and analysis of rotational barriers. *Synthesis* **2009**, 1195–1203.

Häcker, H.-G.; Leyers, S.; Wiendlocha, J.; Gütschow, M.; Wiese, M. Aromatic 2-(thio)ureidocarboxylic acids as a new family of modulators of multidrug resistance-associated protein 1: synthesis, biological evaluation, and structure–activity relationships. *J. Med. Chem.* **2009**, *52*, 4586–4595.

Häcker, H.-G.; Schnakenburg, G.; Hoffbauer, W.; Daniels, J.; Pietsch, M.; Gütschow, M. Isopropylammonium tetrafluorohydrogenphthalate: structural characterization and comparison to two related salts with different stoichiometric ratios. *J. Mol. Struct.* **2009**, *934*, 23–27.

Häcker, H.-G.; de la Haye, A.; Sterz, K.; Schnakenburg, G.; Wiese, M.; Gütschow, M. Analogs of a 4-aminothieno[2,3-*d*]pyrimidine lead (QB13) as modulators of P-glycoprotein substrate specificity. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6102–6105.

CHAPTER 8

Experimental

8.1 General Methods and Materials

Thin-layer chromatography was carried out on Merck aluminum sheets, silica gel 60 F_{254}. Preparative column chromatography was performed on Merck silica gel 60 (70–230 mesh).

Melting points were determined on a Boëtius hot stage microscope apparatus (PHMK, VEB Wägetechnik Rapido, Radebeul, Germany). IR spectra were obtained on a Bruker Tensor 27 FT-IR spectrometer.

^{1}H NMR (500 MHz) and ^{13}C NMR spectra (125 MHz) were recorded on a Bruker Avance DRX 500 in $CDCl_3$ at 298 K, in DMSO-d_6 at 303 K, or in MeOD at different temperatures. Chemical shifts δ are given in ppm referring to the signal center using the solvent peaks for reference: $CDCl_3$ 7.26/77.0 ppm, DMSO-d_6 2.49/39.7 ppm, MeOD 3.35/49.3 ppm. To characterize spin multiplicities, the following abbreviation were used within the compounds descriptions: *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet; *quint*, quintet; *sept*, septet. Broadened signals are designated as *br*.

Solid-state, magic-angle spinning (MAS) NMR experiments were carried out by Dr. W. Hoffbauer (Institute of Inorganic Chemistry, University of Bonn) on a Varian Infinity+ spectrometer equipped with a commercial 4 mm MAS-NMR double-resonance probe. The magnetic field strength was 9.4 T corresponding to a ^{13}C and ^{1}H resonance frequency of 100.98 and 401.52 MHz, respectively. The ^{13}C MAS-NMR spectra were acquired with a ramped $^{13}C\{^1H\}$ cross-polarization experiment. The spectra shown were obtained in 1 h with a repetition delay of 5 s at rt. At 12 kHz spinning frequency the ^{13}C pulse lengths was 2.5 µs, 100 kHz spectral width and 800 transients. A line broadening of 50 Hz was used in data processing. The ^{13}C chemical shifts refer to tetramethylsilane. Values for the spectral parameters (integral, full width half maximum) were achieved from the least square fitting of the experimental spectrum by the spectrometer software.

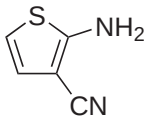
Elemental analyses were performed with a Vario EL apparatus. All values are given as percentages.

Crystal structure data were collected on a Nonius KappaCCD diffractometer equipped with a low-temperature device (Cryostream, Oxford Cryosystems) at the Institute of Inorganic Chemistry, University of Bonn. The structures were solved by Dr. J. Daniels (**17**, **42**, **48**, **106**, **141**), Dr. M. Nieger (**142**), and Dr. G. Schnakenburg (**36**, **43**, **126**, **140**). For all compounds, a summary of data collection, structure refinement, and the corresponding CCDC-number is given in the Appendix. The supplementary crystallographic data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Solvents and reagents were obtained from Acros (Geel, Belgium), Alfa Aesar (Karlsruhe, Germany), Fluka (Taufkirchen, Germany), Merck Schuchardt (Hohenbrunn, Germany), or Sigma-Aldrich (Steinheim, Germany).

8.2 2-Aminothiophene-3-carbonitriles and 2-Aminobenzonitriles

2-Aminothiophene-3-carbonitrile (**1**)



$M = 124.16$ g/mol

Compound **1** was prepared by a method according to Hallas and Towns.[84] Diethylamine (3.18 g, 4.48 mL, 44.0 mmol) was slowly added to a stirred, ice-cooled suspension of 1,4-dithiane-2,5-diol (3.00 g, 19.0 mmol) and malononitrile (2.61 g, 39.0 mmol) in MeOH (50 mL). The mixture was stirred at 0 ℃ for further 15 min. After evaporation of the solvent, the crude material was recrystallized from PhMe to give **1** as orange prisms; yield: 2.60 g (54%), mp 102–104 ℃ (PhMe; lit.[84] 103.5–104 ℃).

^{1}H NMR (500 MHz, DMSO-d_6) δ 6.37 (d, $J = 5.8$ Hz, 1H, 4/5-H), 6.70 (d, $J = 5.7$ Hz, 1H, 4/5-H), 7.05 (br s, 2H; NH_2).

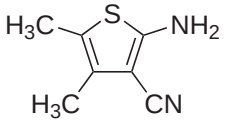
^{13}C NMR (125 MHz, DMSO-d_6) δ 83.40 (C-3), 108.76 (C-4/5), 116.56 (CN), 125.63 (C-4/5), 165.23 (C-2).

Anal. Calcd for $C_5H_4N_2S$: C, 48.37; H, 3.25; N, 22.56. Found: C, 48.54; H, 3.21; N, 22.63.

2-Aminothiophene-3-carbonitriles **2–6**; General Procedure

Compounds **2**–**6** were prepared by a procedure described by Gewald *et al.*[81] Diethylamine (7.10 g, 10.0 mL, 97.1 mmol) was added within 10 min to a stirred mixture of the appropriate ketone (100 mmol), malononitrile (6.61 g, 100 mmol) and sulfur (3.21 g, 100 mmol) in EtOH (50 mL). If temperature raised above 50 ℃ the mixture was cooled with an ice bath. After 3 h the reaction mixture was poured into water (150 mL) and cooled to 5 ℃. The precipitate was removed by suction filtration and recrystallized from EtOH.

2-Amino-4,5-dimethylthiophene-3-carbonitrile (**2**)



$M = 152.22$ g/mol

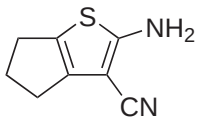
Conversion of butan-2-one according to the aforementioned procedure gave **2** as orange needles; yield: 3.54 g (24%), mp 138–140 ℃ (EtOH; lit.[81] 141–142 ℃).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.93 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 6.82 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.17 (CH_3), 12.59 (CH_3), 85.61 (C-3), 113.80 (C-4/5), 116.72 (CN), 128.54 (C-4/5), 162.03 (C-2).

Anal. Calcd for $C_7H_8N_2S$: C, 55.23; H, 5.30; N, 18.40. Found: C, 54.98; H, 5.64; N, 17.95.

2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile (**3**)



M = 164.23 g/mol

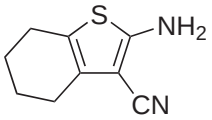
Conversion of cyclopentanone gave **3** as pale yellow needles; yield: 6.90 g (42%), mp 150–151 °C (EtOH; lit.[237] 154 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (quint, J = 7.3 Hz, 2H, 5-H), 2.55 (tt, J = 7.3, 1.9 Hz, 2H, 4/6-H), 2.64 (tt, J = 7.3, 1.9 Hz, 2H, 4/6-H), 6.98 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 26.88 (C-4–6), 28.14 (C-4–6), 29.03 (C-4–6), 79.00 (C-3), 116.55 (CN), 121.51 (C-6a), 141.14 (C-3a), 168.74 (C-2).

Anal. Calcd for $C_8H_8N_2S$: C, 58.51; H, 4.91; N, 17.06. Found: C, 58.32; H, 4.95; N, 17.22.

2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**4**)



M = 178.25 g/mol

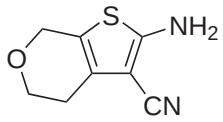
Conversion of cyclohexanone gave **4** as white needles; yield: 14.1 g (79%), mp 144–145 °C (EtOH; lit.[81] 147–148 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.64–1.73 (m, 4H, 5/6-H), 2.30–2.34 (m, 2H, 4/7-H), 2.37–2.41 (m, 2H, 4/7-H), 6.89 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.95 (C-4–7), 23.11 (C-4–7), 23.63 (C-4–7), 24.16 (C-4–7), 83.44 (C-3), 116.28 (C-7a/CN), 117.00 (C-7a/CN), 131.25 (C-3a), 162.86 (C-2).

Anal. Calcd for $C_9H_{10}N_2S$: C, 60.64; H, 5.65; N, 15.72. Found: C, 60.04; H, 5.63; N, 15.45.

2-Amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carbonitrile (**5**)



$M = 180.23$ g/mol

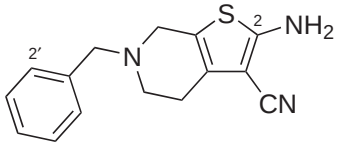
Conversion of tetrahydro-4*H*-pyran-4-one gave **5** as pale yellow prisms; yield: 15.3 g (85%), mp 238–240 °C (decomposition).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.42 (tt, J = 5.7, 1.9 Hz, 2H, 4-H), 3.81 (t, J = 5.7 Hz, 2H, 5-H), 4.41 (t, J = 1.9 Hz, 2H, 7-H), 7.07 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.70 (C-4), 63.82 (C-5/7), 63.92 (C-5/7), 83.03 (C-3), 114.62 (C-7a/CN), 115.87 (C-7a/CN), 129.31 (C-3a), 163.70 (C-2).

Anal. Calcd for $C_8H_8N_2OS$: C, 53.31; H, 4.47; N, 15.54. Found: C, 53.22; H, 4.62; N, 15.28.

2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carbonitrile (**6**)



$M = 269.36$ g/mol

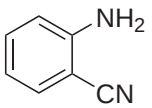
Conversion of *N*-benzyl-4-piperidone gave **6** as yellow prisms; yield: 20.1 g (75%), mp 155–156 °C (EtOH; lit.[238] 147–150 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.41 (t, J = 5.7 Hz, 2H, 4-H), 2.68 (t, J = 6.0 Hz, 2H, 5-H), 3.28 (t, J = 1.9 Hz, 2H, 7-H), 3.62 (s, 2H, C$\underline{H}_2$Ph), 6.99 (br s, 2H, NH_2), 7.21–7.28 (m, 1H, 4′-H), 7.30–7.34 (m, 4H, 2′/3′/5′/6′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.33 (C-4), 49.13 (C-5/7), 50.43 (C-5/7), 61.84 ($\underline{C}H_2$Ph), 82.92 (C-3), 114.68 (C-7a), 116.08 (CN), 127.16 (C-4′), 128.37 (C-2′/3′/5′/6′), 128.87 (C-2′/3′/5′/6′), 130.01 (C-3a′), 138.44 (C-1′), 163.43 (C-2).

Anal. Calcd for $C_{15}H_{15}N_3S$: C, 66.88; H, 5.61; N, 15.60. Found: C, 66.39; H, 5.83; N, 15.18.

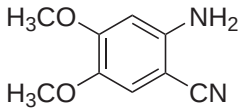
2-Aminobenzonitrile (**7**)



$M = 118.14$ g/mol

This compound was obtained from Acros (Geel, Belgium).

2-Amino-4,5-dimethoxybenzonitrile (**8**)



$M = 178.19$ g/mol

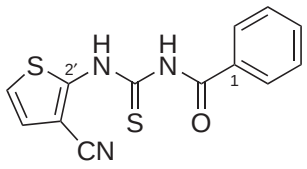
This compound was obtained from Fluka (Taufkirchen, Germany).

8.3 *N*-Benzoyl-*N*'-(*o*-cyanoaryl)thioureas

N-Benzoyl-*N*′-(*o*-cyanoaryl)thioureas **9–16**; General Procedure

Benzoyl chloride (8.43 g, 6.96 mL, 60.0 mmol) was added dropwise to a stirred solution of NH_4SCN (5.18 g, 68.0 mmol) in anhydrous acetone (20 mL) at 0 ℃. The mixture was refluxed for 5 min and then cooled to rt, and the precipitated NH_4Cl was removed by suction filtration. Subsequently, the resulting BzNCS solution was cooled (ice) and a solution of the appropriate (*o*-aminoaryl)carbonitrile (**1**–**8**, 50.0 mmol) in acetone was added dropwise. Upon complete addition, the reaction mixture was allowed to warm to rt and stirred for 3 h. The precipitated product was collected by filtration and washed with acetone (−20 ℃, 30 mL). Additional substance was obtained by cooling the remaining solution overnight (8 ℃). The material was used without further purification.

N-Benzoyl-*N*′-(3-cyano-2-thienyl)thiourea (**9**)



M = 278.36 g/mol

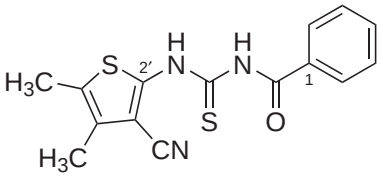
Pale yellow solid; yield: 11.9 g (83%); mp 199–202 ℃; R_F = 0.56 (petroleum ether–EtOAc, 2:1). IR (KBr): 3231, 3114, 2217, 1673 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.28 (d, J = 5.7 Hz, 1H, 4′/5′-H), 7.33 (d, J = 5.7 Hz, 1H, 4′/5′-H), 7.53–7.57 (m, 2H, 3/5-H), 7.66–7.69 (m, 1H, 4-H), 8.00–8.02 (m, 2H, 2/6-H), 12.26 (br s, 1H, NH), 14.50 (br s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 97.10 (C-3′), 114.20 (CN), 120.04 (C-4′/5′), 124.64 (C-4′/5′), 128.60 (C-2/3/5/6), 129.06 (C-2/3/5/6), 131.65 (C-1), 133.64 (C-4), 150.07 (C-2′), 169.51 (CO), 176.09 (CS).

Anal. Calcd for $C_{13}H_9N_3OS_2$: C, 54.34; H, 3.16; N, 14.62. Found: C, 53.80; H, 3.16; N, 14.53.

N-Benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**)



M = 315.41 g/mol

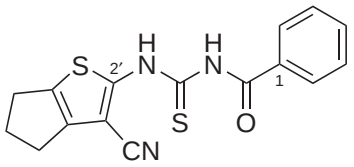
Off-white solid; yield: 12.9 g (82%); mp 208–210 ℃ (lit.[85] 208–210 ℃); R_F = 0.59 (toluene–EtOAc, 4:1). IR (KBr): 3274, 2917, 2217, 1672 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.28 (s, 3H, CH_3), 7.53–7.58 (m, 2H, 3/5-H), 7.65–7.71 (m, 1H, 4-H), 7.97–8.03 (m, 2H, 2/6-H), 12.19 (br s, 1H, NH), 14.40 (br s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.18 (CH_3), 12.26 (CH_3), 99.13 (C-3′), 114.14 (CN), 126.17 (C-4′/5′), 128.60 (C-2/3/5/6), 128.98 (C-4′/5′), 129.06 (C-2/3/5/6), 131.68 (C-1), 133.63 (C-4), 145.96 (C-2′), 169.57 (CO), 175.43 (CS).

Anal. Calcd for $C_{15}H_{13}N_3OS_2$: C, 57.12; H, 4.15; N, 13.32. Found: C, 56.50; H, 4.38; N, 12.75.

N-Benzoyl-*N*′-(3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)thiourea (**11**)



M = 327.42 g/mol

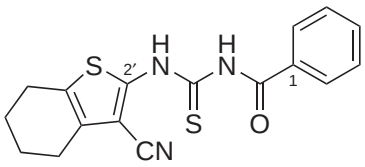
Pale yellow solid; yield: 15.3 g (93%); mp 213–214 °C; R_F = 0.71 (toluene–EtOAc, 4:1). IR (KBr): 2859, 2216, 1670 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.49 (quint, J = 7.3 Hz, 2H, 5′-H), 2.90 (tt, J = 7.3, 1.6 Hz, 2H, 4′-H), 2.95 (tt, J = 7.3, 1.6 Hz, 2H, 6′-H), 7.56–7.60 (m, 2H, 3/5-H), 7.68–7.72 (m, 1H, 4-H), 7.95–7.80 (m, 2H, 2/6-H), 9.14 (br s, 1H, NH), 14.20 (br s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 28.08 (C-4′/5′), 28.11 (C-4′/5′), 29.55 (C-6′), 94.42 (C-3′), 113.97 (CN), 127.72 (C-2/3/5/6), 129.30 (C-2/3/5/6), 130.88 (C-1), 134.14 (C-4), 136.32 (C-6a′), 141.69 (C-3a′), 151.58 (C-2′), 167.08 (CO), 173.90 (CS).

Anal. Calcd for $C_{16}H_{13}N_3OS_2$: C, 58.69; H, 4.00; N, 12.83. Found: C, 58.65; H, 4.17; N, 12.74.

N-Benzoyl-*N*′-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)thiourea (**12**)



M = 341.45 g/mol

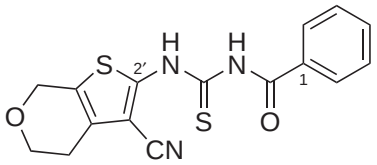
Yellow solid; yield: 15.9 g (93%); mp 219–220 °C (lit.[85] 214–216 °C); R_F = 0.89 (toluene–EtOAc, 4:1). IR (KBr): 3289, 2921, 2212, 1670 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.79–1.86 (m, 4H, 5′/6′-H), 2.62–2.68 (m, 4H, 4′/7′-H), 7.51–7.55 (m, 2H, 3/5-H), 7.63–7.66 (m, 1H, 4-H), 7.90–7.93 (m, 2H, 2/6-H), 9.12 (br s, 1H, NH), 14.16 (br s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 22.01 (C-4′–7′), 22.99 (C-4′–7′), 23.86 (C-4′–7′), 24.07 (C-4′–7′), 98.61 (C-3′), 113.71 (CN), 127.68 (C-2/3/5/6), 129.23 (C-2/3/5/6), 129.75 (C-7a′), 130.79 (C-1/3a′), 131.80 (C-1/3a′), 134.07 (C-4), 146.83 (C-2′), 167.04 (CO), 174.02 (CS).

Anal. Calcd for $C_{17}H_{15}N_3OS_2$: C, 59.80; H, 4.43; N, 12.31. Found: C, 59.69; H, 4.62; N, 12.15.

N-Benzoyl-*N*′-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)thiourea (**13**)



M = 343.42 g/mol

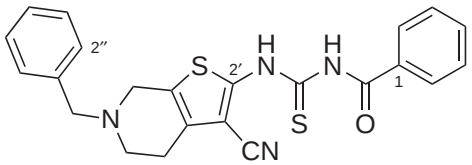
Pale yellow solid; yield: 16.7 g (97%); mp 218–220 °C; R_F = 0.66 (toluene–EtOAc, 4:1). IR (KBr): 3312, 2841, 2217, 1672 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.67 (tt, J = 5.7, 1.6 Hz, 2H, 4′-H), 3.89 (t, J = 5.7 Hz, 2H, 5′-H), 4.66 (t, J = 1.6 Hz, 2H, 7′-H), 7.53–7.57 (m, 2H, 3/5-H), 7.66–7.70 (m, 1H, 4-H), 7.99–8.02 (m, 2H, 2/6-H), 12.25 (br s, 1H, NH), 14.48 (br s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 23.90 (C-4′), 63.86 (C-5′/7′), 63.91 (C-5′/7′), 96.85 (C-3′), 113.36 (CN), 126.66 (C-7a′), 128.60 (C-2/3/5/6), 129.06 (C-3a′), 129.08 (C-2/3/5/6), 131.64 (C-1), 133.66 (C-4), 148.08 (C-2′), 169.60 (CO), 175.64 (CS).

Anal. Calcd for $C_{16}H_{13}N_3O_2S_2$: C, 55.96; H, 3.82; N, 12.24. Found: C, 55.59; H, 3.95; N, 12.09.

N-Benzoyl-*N*′-(6-benzyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)thiourea (**14**)



M = 432.56 g/mol

Off-white solid; yield: 19.3 g (89%); mp 138–140 °C; R_F = 0.56 (petroleum ether–EtOAc, 2:1). IR (KBr): 3287, 2830, 2219, 1668 cm^{-1}.

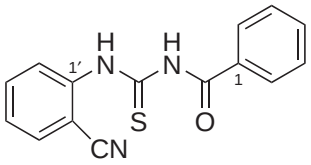
^{1}H NMR (500 MHz, $CDCl_3$) δ 2.76 (t, J = 5.4 Hz, 2H, 4′-H), 2.84 (t, J = 5.6 Hz, 2H, 5′-H), 3.55 (t, J = 1.6 Hz, 2H, 7′-H), 3.71 (s, 2H, C$\underline{H}_2$Ph), 7.25–7.28 (m, 1H, 4″-H), 7.31–7.35 (m, 4H, 2″/3″/5″/6″-H), 7.51–7.55 (m, 2H, 3/5-H), 7.63–7.66 (m, 1H, 4-H), 7.90–7.93 (m, 2H, 2/6-H), 9.16 (br s, 1H, NH), 14.23 (br s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 24.07 (C-4′), 49.39 (C-5′/7′), 50.72 (C-5′/7′), 61.69 (C$\underline{H}_2$Ph), 98.01 (C-3′), 113.48 (CN), 127.21 (C-7a′/4″), 127.41 (C-7a′/4″), 127.72 (C-2/3/5/6), 128.44

(C-2″/3″/5″/6″), 129.03 (C-2″/3″/5″/6″), 129.26 (C-2/3/5/6), 130.36 (C-3a′), 130.74 (C-1), 134.15 (C-4), 137.69 (C-1″), 147.64 (C-2′), 167.16 (CO), 174.07 (CS).

Anal. Calcd for $C_{23}H_{20}N_4OS_2$: C, 63.86; H, 4.66; N, 12.95. Found: C, 63.58; H, 4.86; N, 12.67.

N-Benzoyl-*N*′-(2-cyanophenyl)thiourea (**15**)



M = 281.33 g/mol

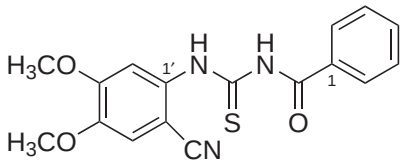
White solid; yield: 11.1 g (79%); mp 169–172 °C (lit.[239] 172–173 °C); R_F = 0.64 (toluene–EtOAc, 4:1). IR (KBr): 3120, 2229, 1675 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.48–7.52 (m, 1H, 4′/5′/6′-H), 7.53–7.57 (m, 2H, 3/5-H), 7.65–7.69 (m, 1H, 4-H), 7.74–7.80 (m, 2H, 4′/5′/6′-H), 7.89–7.91 (m, 1H, 3′-H), 7.99–8.03 (m, 2H, 2/6-H), 11.85 (br s, 1H, NH), 12.56 (br s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 110.64 (C-2′), 116.56 (CN), 127.90 (C-3′–6′), 128.64 (C-2/3/5/6), 128.93 (C-2/3/5/6), 132.03 (C-1), 133.24 (C-3′–6′), 133.45 (C-3′–6′), 133.85 (C-4), 141.32 (C-1′), 168.37 (CO), 181.54 (CS).

Anal. Calcd for $C_{15}H_{11}N_3OS$: C, 64.04; H, 3.94; N, 14.94. Found: C, 64.01; H, 4.03; N, 14.72.

N-Benzoyl-*N*′-(2-cyano-4,5-dimethoxyphenyl)thiourea (**16**)



M = 341.38 g/mol

Yellow solid; yield: 16.4 g (96%); mp 201–204 °C; R_F = 0.55 (toluene–EtOAc, 4:1). IR (KBr): 3073, 2229, 1761, 1676 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.83 (s, 3H, CH_3), 3.83 (s, 3H, CH_3), 7.40 (s, 1H, 3′/5′-H), 7.42 (s, 1H, 3′/5′-H), 7.53–7.57 (m, 2H, 3/5-H), 7.65–7.69 (m, 1H, 4-H), 7.98–8.01 (m, 2H, 2/6-H), 11.83 (br s, 1H, NH), 12.47 (br s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 56.28 (CH_3), 56.31 (CH_3), 101.58 (C-2′), 111.83 (C-3′/6′/CN), 114.38 (C-3′/6′/CN), 116.86 (C-3′/6′/CN), 128.64 (C-2/3/5/6), 128.89 (C-2/3/5/6), 132.06 (C-1), 133.43 (C-4), 135.90 (C-1′), 147.77 (C-4′/5′), 152.66 (C-4′/5′), 168.50 (CO), 181.57 (CS).

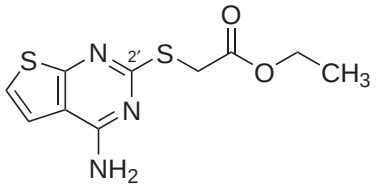
Anal. Calcd for $C_{17}H_{15}N_3O_3S$: C, 59.81; H, 4.43; N, 12.31. Found: C, 59.79; H, 4.64; N, 12.18.

8.4 4-Aminothieno[2,3-*d*]pyrimidines and 4-Aminoquinazolines

Thieno[2,3-*d*]pyrimidines **17–37**, and Quinazolines **38–41**; General Procedure

The appropriate *N*-benzoyl-*N*′-(*o*-cyanoaryl)thiourea (**9**–**16**, 3.00 mmol) was heated under reflux for 30 min in a mixture of EtOH (3.0 mL) and NaOH (1 M, 7.5 mL). The solution was cooled to rt and the appropriate alkyl halide (3.60 mmol, 1.2 equiv) was slowly added. After 1 h, the solid was removed by suction filtration, washed with H_2O (100 mL) and dried under reduced pressure. Pure material was obtained by recrystallization from EtOH, if not stated otherwise.

Ethyl [(4-Aminothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]acetate (**17**)



M = 269.34 g/mol

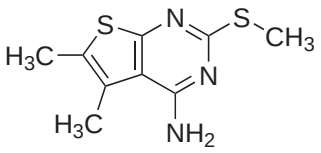
Reaction of *N*-benzoyl-*N*′-(3-cyano-2-thienyl)thiourea (**9**) and ethyl bromoacetate gave **17** as colorless plates; yield: 468 mg (58%); mp 116–117 ℃ (EtOH); R_F = 0.45 (petroleum ether–EtOAc, 2:1). IR (KBr): 3388, 3162, 2903, 1735, 1647 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.95 (s, 2H, SCH_2), 4.11 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 7.37 (d, J = 6.0 Hz, 1H, 5′/6′-H), 7.47 (d, J = 6.0 Hz, 1H, 5′/6′-H), 7.57 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.23 ($CH_2\underline{C}H_3$), 32.82 (SCH_2), 60.88 ($\underline{C}H_2CH_3$), 113.23 (C-4a′), 119.69 (C-5′/6′), 120.69(C-5′/6′) , 158.06 (C-7a′), 164.46 (C-2′), 166.89 (C-4′), 169.36 (CO).

Anal. Calcd for $C_{10}H_{11}N_3O_2S_2$: C, 44.59; H, 4.12; N, 15.60. Found: C, 44.41; H, 4.17; N, 15.47.

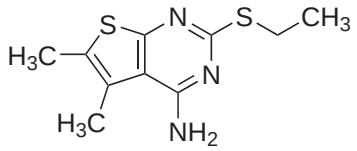
5,6-Dimethyl-2-(methylsulfanyl)thieno[2,3-*d*]pyrimidin-4-amine (**18**)[85]



M = 225.33 g/mol

Compound was taken from the substance library.

2-(Ethylsulfanyl)-5,6-dimethylthieno[2,3-*d*]pyrimidin-4-amine (**19**)



$M = 239.36$ g/mol

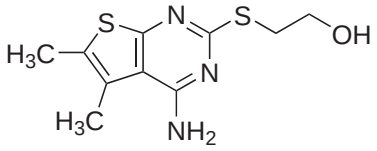
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**) and ethyl bromide gave **19** as colorless plates; yield: 366 mg (51%); mp 216–218 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.28 (t, $J = 7.4$ Hz, 3H, $CH_2C\underline{H}_3$), 2.32 (s, 3H, CH_3), 3.35 (s, 3H, CH_3), 3.04 (q, $J = 7.3$ Hz, 2H, $C\underline{H}_2CH_3$), 6.88 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.95 (CH_3), 13.91 (CH_3), 14.96 ($CH_2\underline{C}H_3$), 24.21 ($\underline{C}H_2CH_3$), 113.33 (C-4a), 124.79 (C-5/6), 125.82 (C-5/6), 157.90 (C-7a), 164.26 (C-2), 165.68 (C-4).

Anal. Calcd for $C_{10}H_{13}N_2S_2$: C, 50.18; H, 5.47; N, 17.56. Found: C, 49.98; H, 5.56; N, 17.16.

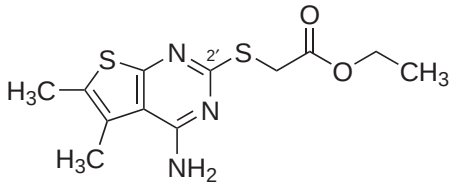
2-[(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]ethanol (**20**)[85]



$M = 255.36$ g/mol

Compound was taken from the substance library.

Ethyl [(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]acetate (**21**)



$M = 297.40$ g/mol

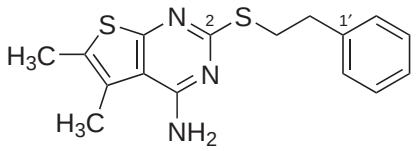
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**) and ethyl bromoacetate gave **21** as colorless plates; yield: 375 mg (42%); mp 149–151 °C (EtOH; lit.[109] 148–151 °C); $R_F = 0.17$ (toluene–EtOAc, 4:1). IR (KBr): 3506, 3298, 3140, 2978, 1733, 1644 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, $J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$), 2.33 (s, 3H, CH_3), 2.35 (s, 3H, CH_3), 3.93 (s, 2H, SCH_2), 4.10 (q, $J = 7.1$ Hz, 2H, $C\underline{H}_2CH_3$), 6.98 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.95 (CH_3), 13.89 (CH_3), 14.25 ($CH_2\underline{C}H_3$), 32.75 (SCH_2), 60.88 ($\underline{C}H_2CH_3$), 113.49 (C-4a′), 124.82 (C-5′/6′), 126.23 (C-5′/6′), 157.91 (C-7a′), 162.97 (C-2′), 165.40 (C-4′), 169.38 (CO).

Anal. Calcd for $C_{12}H_{15}N_3O_2S_2$: C, 48.46; H, 5.08; N, 14.13. Found: C, 47.97; H, 5.22; N, 13.84.

5,6-Dimethyl-2-[(2-phenylethyl)sulfanyl]thieno[2,3-*d*]pyrimidin-4-amine (**22**)



M = 315.46 g/mol

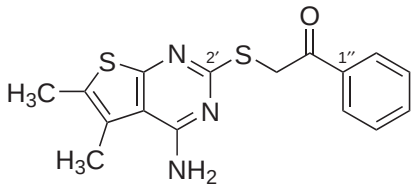
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**) and phenethyl bromide gave **22** as colorless needles; yield: 300 mg (32%); mp 166–167 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.33 (s, 3H, CH_3), 2.36 (s, 3H, CH_3), 2.93 (t, J = 7.7 Hz, 2H, CH_2), 3.29 (t, J = 7.7 Hz, 2H, CH_2), 6.91 (br s, 2H, NH_2), 7.18–7.22 (m, 1H, 4′-H), 7.26–7.32 (m, 4H, 2′/3′/5′/6′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.96 (CH_3), 13.93 (CH_3), 31.44 (CH_2), 35.44 (CH_2), 113.39 (C-4a), 124.82 (C-5/6), 125.90 (C-5/6), 126.31 (C-4′), 128.44 (C-2′/3′/5′/6′), 128.73 (C-2′/3′/5′/6′), 140.67 (C-1′), 157.93 (C-7a), 164.07 (C-2), 165.69 (C-4).

Anal. Calcd for $C_{16}H_{17}N_3S_2$: C, 60.92; H, 5.43; N, 13.32. Found: C, 60.82; H, 5.49; N, 13.12.

2-[(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-1-phenylethanone (**23**)



M = 329.44 g/mol

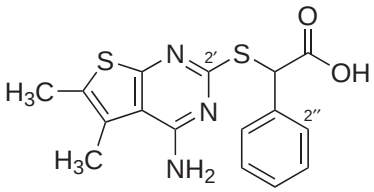
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**) and phenacyl bromide gave **23** as colorless needles; yield: 830 mg (84%); mp 154–156 °C (EtOH; lit.[85] 152–153 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.30 (s, 3H, CH_3), 3.34 (s, 3H, CH_3), 4.70 (s, 2H, SCH_2), 6.92 (br s, 2H, NH_2), 7.52–7.57 (m, 2H, 3″/5″-H), 7.63–7.68 (m, 1H, 4″-H), 8.02–8.05 (m, 2H, 2″/6″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.93 (CH_3), 13.87 (CH_3), 37.69 (SCH_2), 113.46 (C-4a′), 124.78 (C-5′/6′), 126.16 (C-5′/6′), 128.42 (C-2″/3″/5″/6″), 128.83 (C-2″/3″/5″/6″), 133.42 (C-4″), 136.43 (C-1″), 157.87 (C-7a′), 163.08 (C-2′), 165.36 (C-4′), 194.89 (CO).

Anal. Calcd for $C_{16}H_{15}N_3OS_2$: C, 58.33; H, 4.59; N, 12.76. Found: C, 58.34; H, 4.47; N, 12.62.

[(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl](phenyl)acetic Acid (**24**)[240]



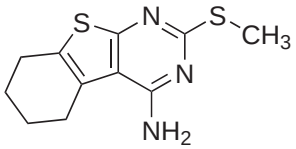
M = 345.44 g/mol

Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.32 (s, 3H, CH_3), 3.34 (s, 3H, CH_3), 5.52 (s, 1H, CH), 6.95 (br s, 2H, NH_2), 7.24–7.28 (m, 1H, 4″-H), 7.29–7.34 (m, 2H, 3″/5″-H), 7.48–7.52 (m, 2H, 2″/6″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.97 (CH_3), 13.89 (CH_3), 52.69 (CH), 113.51 (C-4a′), 124.81 (C-5′/6′), 126.17 (C-5′/6′), 127.75 (C-4″), 128.54 (C-2″/3″/5″/6″), 128.57 (C-2″/3″/5″/6″), 137.61 (C-1″), 157.84 (C-7a′), 163.31 (C-2′), 165.41 (C-4′), 171.45 (CO_2H).

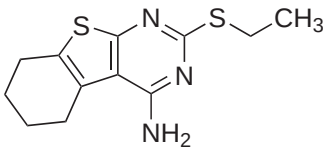
2-(Methylsulfanyl)-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-4-amine (**25**)[85]



M = 251.37 g/mol

Compound was taken from the substance library.

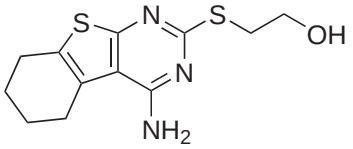
2-(Ethylsulfanyl)-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-4-amine (**26**)[85]



M = 265.40 g/mol

Compound was taken from the substance library.

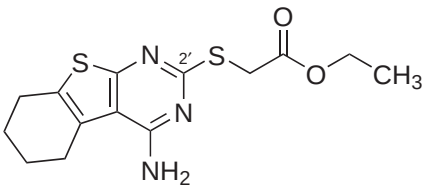
2-[(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]ethanol (**27**)[85]



M = 281.40 g/mol

Compound was taken from the substance library.

Ethyl [(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]acetate (**28**)



M = 323.43 g/mol

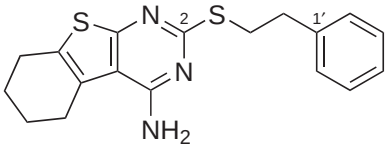
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)thiourea (**12**) and ethyl bromoacetate gave **28** as white needles; yield: 468 mg (48%); mp 139–140 °C (EtOH; lit.[109] 138–141 °C); R_F = 0.22 (toluene–EtOAc, 4:1). IR (KBr): 3511, 3296, 3130, 2930, 1740, 1632 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.75–1.79 (m, 4H, 6′/7′-H), 2.69–2.70 (m, 2H, 5′/8′-H), 2.83–2.84 (m, 2H, 5′/8′-H), 3.93 (s, 2H, CH_2), 4.10 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.86 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.26 ($CH_2\underline{C}H_3$), 22.06 (C-6′/7′), 22.37 (C-6′/7′), 24.83 (C-5′/8′), 25.43 (C-5′/8′), 32.76 (SCH_2), 60.88 ($\underline{C}H_2CH_3$), 112.45 (C-4a′), 126.96 (C-4b′/8a′), 129.48 (C-4b′/8a′), 157.76 (C-9a′), 163.09 (C-2′), 166.09 (C-4′), 169.37 (CO).

Anal. Calcd for $C_{14}H_{17}N_3O_2S_2$: C, 51.99; H, 5.30; N, 12.99. Found: C, 51.69; H, 5.57; N, 12.59.

2-[(2-Phenylethyl)sulfanyl]-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-4-amine (**29**)



M = 341.49 g/mol

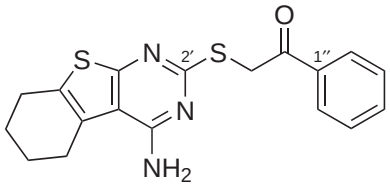
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)thiourea (**12**) and phenethyl bromide gave **29** as white needles; yield: 740 mg (72%); mp 159–162 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.77–1.80 (m, 4H, 6/7-H), 2.68–2.72 (m, 2H, 5/8-H), 2.83–2.87 (m, 2H, 5/8-H), 2.93 (t, J = 7.7 Hz, 2H, CH_2), 3.29 (t, J = 7.7 Hz, 2H, CH_2), 6.91 (br s, 2H, NH_2), 7.18–7.22 (m, 1H, 4′-H), 7.27–7.32 (m, 4H, 2′/3′/5′/6′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 22.09 (C-6/7), 22.40 (C-6/7), 24.85 (C-5/8), 25.47 (C-5/8), 31.46 (CH_2), 35.42 (CH_2), 112.32 (C-4a), 126.31 (C-4′), 126.96 (C-4b/8a), 128.44 (C-2′/3′/5′/6′), 128.73 (C-2′/3′/5′/6′), 129.16 (C-4b/8a), 140.66 (C-1′), 157.79 (C-9a), 164.18 (C-2), 166.38 (C-4).

Anal. Calcd for $C_{18}H_{19}N_3S_2$: C, 63.31; H, 5.61; N, 12.30. Found: C, 63.35; H, 5.59; N, 12.24.

2-[(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-1-phenylethanone (**30**)



M = 355.48 g/mol

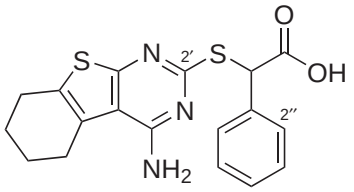
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)thiourea (**12**) and phenacyl bromide gave **30** as white needles; yield: 820 mg (77%); mp 176–179 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.75–1.78 (m, 4H, 6′/7′-H), 2.65–2.68 (m, 2H, 5′/8′-H), 2.81–2.85 (m, 2H, 5′/8′-H), 4.70 (s, 2H, SCH_2), 6.85 (br s, 2H, NH_2), 7.51–7.56 (m, 2H, 3″/5″-H), 7.63–7.67 (m, 1H, 4″-H), 8.02–8.05 (m, 2H, 2″/6″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 22.05 (C-6′/7′), 22.36 (C-6′/7′), 24.81 (C-5′/8′), 25.41 (C-5′/8′), 37.70 (SCH_2), 112.40 (C-4a′), 126.91 (C-4b′/8a′), 128.42 (C-2″/3″/5″/6″), 128.83 (C-2″/3″/5″/6″), 129.40 (C-4b′/8a′), 133.42 (C-4″), 136.42 (C-1″), 157.72 (C-9a′), 163.19 (C-2′), 166.05 (C-4′), 194.89 (CO).

Anal. Calcd for $C_{18}H_{17}N_3OS_2$: C, 60.82; H, 4.82; N, 11.82. Found: C, 60.69; H, 4.92; N, 11.47.

[(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl](phenyl)acetic Acid (**31**)[240]



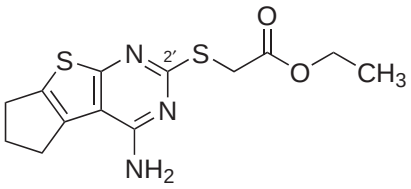
M = 371.48 g/mol

Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.75–1.80 (m, 4H, 6′/7′-H), 2.68–2.72 (m, 2H, 5′/8′-H), 2.82–2.86 (m, 2H, 5′/8′-H), 5.55 (s, 1H, CH), 6.91 (br s, 2H, NH_2), 7.26–7.32 (m, 1H, 4′-H), 7.33–7.37 (m, 2H, 3″/5″-H), 7.47–7.51 (m, 2H, 2″/6″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 22.06 (C-6′/7′), 22.37 (C-6′/7′), 24.85 (C-5′/8′), 25.42 (C-5′/8′), 52.01 (CH), 112.55 (C-4a′), 126.98 (C-4b′/8a′), 128.09 (C-4″), 128.55 (C-2″/3″/5″/6″), 128.76 (C-2″/3″/5″/6″), 129.57 (C-4b′/8a′), 136.57 (C-1″), 157.73 (C-9a′), 163.04 (C-2′), 166.05 (C-4′), 171.42 (CO_2H).

Ethyl [(4-Amino-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-acetate (**32**)



M = 309.41 g/mol

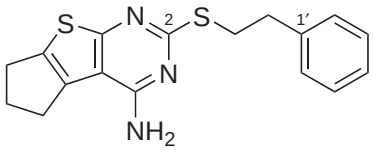
Reaction of *N*-benzoyl-*N*′-(3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)thiourea (**11**) and ethyl bromoacetate gave **32** as colorless plates; yield: 599 mg (65%); mp 180–182 °C (EtOH); R_F = 0.19 (toluene–EtOAc, 4:1). IR (KBr): 3490, 3290, 3119, 2906, 1734, 1641 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.34–2.39 (m, 2H, 6′-H), 2.85–2.89 (m, 2H, 5′/7′-H), 2.96–2.99 (m, 2H, 5′/7′-H), 3.94 (s, 2H, SCH_2), 4.10 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.96 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.25 ($CH_2\underline{C}H_3$), 27.34 (C-6), 28.81 (C-5/7), 29.11 (C-5/7), 32.81 (SCH_2), 60.90 ($\underline{C}H_2CH_3$), 109.55 (C-4a), 134.63 (C-4b/7a), 136.02 (C-4b/7a), 157.39 (C-8a), 163.09 (C-2), 169.38 (CO), 171.42 (C-4).

Anal. Calcd for $C_{13}H_{15}N_3O_2S_2$: C, 50.46; H, 4.89; N, 13.58. Found: C, 50.20; H, 4.94; N, 13.44.

2-[(2-Phenylethyl)sulfanyl]-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**33**)



M = 327.47 g/mol

Reaction of *N*-benzoyl-*N*′-(3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)thiourea (**11**) and phenethyl bromide gave **33** as white needles; yield: 599 mg (61%); mp 176-177 °C (EtOH).

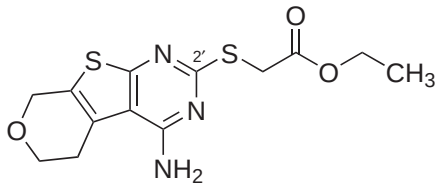
^{1}H NMR (500 MHz, DMSO-d_6) δ 2.35–2.41 (m, 2H, 6-H), 2.85–2.90 (m, 2H, 5/7-H), 2.94 (t, J = 7.7 Hz, 2H, CH_2), 2.96–3.01 (m, 2H, 5/7-H), 3.30 (t, J = 7.7 Hz, 2H, CH_2), 6.92 (br s, 2H, NH_2), 7.18–7.22 (m, 1H, 4′-H), 7.27–7.31 (m, 4H, 2′/3′/5′/6′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 27.33 (C-6), 28.83 (C-5/7), 29.12 (C-5/7), 31.48 (CH_2), 35.38 (CH_2), 109.41 (C-4a), 126.32 (C-4′), 128.44 (C-2′/3′/5′/6′), 128.73 (C-2′/3′/5′/6′),

134.26 (C-4b/7a), 136.03 (C-4b/7a), 140.66 (C-1′), 157.40 (C-8a), 164.20 (C-2), 171.76 (C-4).

Anal. Calcd for $C_{17}H_{17}N_3S_2$: C, 62.35; H, 5.23; N, 12.81. Found: C, 61.17; H, 5.34; N, 12.61.

Ethyl [(4-Amino-5,8-dihydro-6*H*-pyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-acetate (**34**)



M = 325.41 g/mol

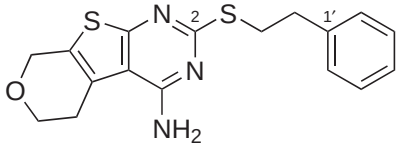
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)thiourea (**13**) and ethyl bromoacetate gave **34** as colorless blocks; yield: 726 mg (74%); mp 192–193 °C (EtOH); R_F = 0.40 (toluene–EtOAc, 4:1). IR (KBr): 3489, 3294, 3161, 2968, 1727, 1631 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.95 (tt, J = 5.5, 1.9 Hz, 2H, 5′-H), 3.91 (t, J = 5.5 Hz, 2H, 6′-H), 3.95 (s, 2H, SCH_2), 4.10 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 4.71 (t, J = 1.9 Hz, 2H, 8′-H), 6.97 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.25 ($CH_2\underline{C}H_3$), 26.02 (C-5), 32.80 (SCH_2), 60.90 ($\underline{C}H_2CH_3$), 63.94 (C-6/8), 64.58 (C-6/8), 111.89 (C-4a), 124.86 (C-4b/8a), 127.23 (C-4b/8a), 157.81 (C-9a), 163.60 (C-2), 166.60 (C-4), 169.32 (CO).

Anal. Calcd for $C_{13}H_{15}N_3O_3S_2$: C, 47.98; H, 4.65; N, 12.91. Found: C, 47.89; H, 4.64; N, 12.86.

2-[(2-Phenylethyl)sulfanyl]-5,8-dihydro-6*H*-pyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-amine (**35**)



M = 343.47 g/mol

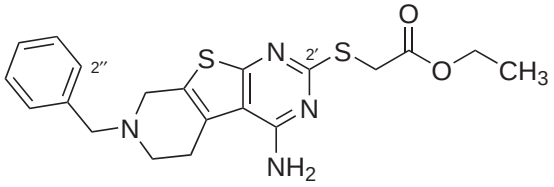
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)- thiourea (**13**) and phenethyl bromide gave **35** as white needles; yield: 488 mg (47%); mp 179–181 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.92–2.98 (m, 4H, 5-H/CH_2), 3.29–3.32 (m, 2H, CH_2), 3.92 (t, J = 5.5 Hz, 2H, 6-H), 4.72 (t, J = 1.9 Hz, 2H, 8-H), 6.93 (br s, 2H, NH_2), 7.18–7.22 (m, 1H, 4′-H), 7.26–7.32 (m, 4H, 2′/3′/5′/6′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 26.07 (C-5), 31.48 (CH_2), 35.39 (CH_2), 63.97 (C-6/8), 64.61 (C-6/8), 111.76 (C-4a), 124.86 (C-4b/8a), 126.33 (C-4′), 126.91 (C-4b/8a), 128.44 (C-2′/3′/5′/6′), 128.73 (C-2′/3′/5′/6′), 140.63 (C-1′), 157.84 (C-9a), 164.69 (C-2), 166.89 (C-4).

Anal. Calcd for $C_{17}H_{17}N_3OS_2$: C, 59.45; H, 4.99; N, 12.23. Found: C, 59.23; H, 4.94; N, 12.08.

Ethyl [(4-Amino-7-benzyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-2-yl)-sulfanyl]acetate (**36**)



M = 414.54 g/mol

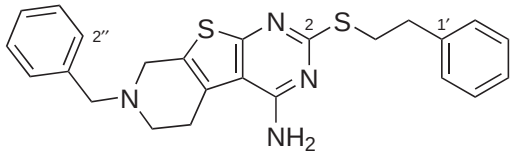
Reaction of *N*-benzoyl-*N*′-(6-benzyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)thiourea (**14**) and ethyl bromoacetate gave **36** as yellow needles; yield: 611 mg (49%); mp 143–144 °C (EtOH); R_F = 0.24 (toluene–EtOAc, 4:1). IR (KBr): 3501, 3283, 3107, 2911, 1741, 1634 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 2.77 (t, J = 5.7 Hz, 2H, 5′/6′-H), 2.94 (t, J = 5.7 Hz, 2H, 5′/6′-H), 3.60 (s, 2H, 8′-H), 3.70 (s, 2H, $C\underline{H}_2C_6H_5$), 3.96 (s, 2H, SCH_2), 4.11 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 6.94 (br s, 2H, NH_2), 7.26–7.30 (m, 1H, 4″-H), 7.33–7.38 (m, 4H, 2″/3″/5″/6″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.25 ($CH_2\underline{C}H_3$), 25.65 (C-5′), 32.78 (SCH_2), 48.97 (C-6′/8′), 51.39 (C-6′/8′), 60.75 ($\underline{C}H_2C_6H_5$), 60.89 ($\underline{C}H_2CH_3$), 111.95 (C-4a′), 125.61 (C-4b′/8a′/4″), 127.11 (C-4b′/8a′/4″), 127.23 (C-4b′/8a′/4″), 128.40 (C-2″/3″/5″/6″), 128.94 (C-2″/3″/5″/6″), 138.29 (C-1″), 157.80 (C-9a′), 163.44 (C-2′), 166.43 (C-4′), 169.33 (CO).

Anal. Calcd for $C_{20}H_{22}N_4O_2S_2$: C, 57.95; H, 5.35; N, 13.52. Found: C, 57.70; H, 5.56; N, 13.35.

7-Benzyl-2-[(2-phenylethyl)sulfanyl]-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]-pyrimidin-4-amine (**37**)



M = 432.60 g/mol

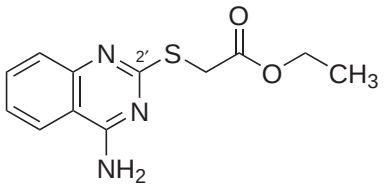
Reaction of *N*-benzoyl-*N*′-(6-benzyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)thiourea (**14**) and phenethyl bromide gave **37** as an off-white solid; yield: 323 mg (25%); mp 172–173 °C (acetone).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.76 (t, J = 5.7 Hz, 2H, 5/6-H), 2.91–2.96 (m, 4H, 5/6-H/CH_2), 3.30 (t, J = 7.7 Hz, 2H, CH_2), 3.59 (s, 2H, 8-H), 3.69 (s, 2H, $C\underline{H}_2C_6H_5$), 3.96 (s, 2H, CH_2), 6.88 (br s, 2H, NH_2), 7.18–7.22 (m, 1H, 4′-H), 7.26–7.37 (m, 9H, 2′/3′/5′/6′/2″–6″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 25.69 (C-5), 31.47 (CH_2), 35.39 (CH_2), 49.01 (C-6/8), 51.42 (C-6/8), 60.77 ($\underline{C}H_2C_6H_5$), 111.82 (C-4a), 125.62 (C-4b/8a), 126.32 (C-4′), 126.79 (C-4b/8a), 127.23 (C-4″), 128.41 (C-2″/3″/5″/6″), 128.44 (C-2′/3′/5′/6′), 128.73 (C-2′/3′/5′/6′), 128.94 (C-2″/3″/5″/6″), 138.31 (C-1″), 140.64 (C-1′), 157.82 (C-9a), 164.54 (C-2), 166.72 (C-4).

Anal. Calcd for $C_{24}H_{24}N_4OS_2$: C, 66.63; H, 5.59; N, 12.95. Found: C, 66.54; H, 5.75; N, 12.82.

Ethyl [(4-Aminoquinazolin-2-yl)sulfanyl]acetate (**38**)



M = 263.32 g/mol

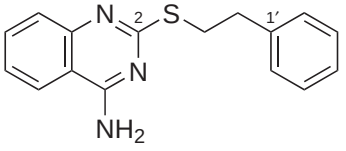
Reaction of N-benzoyl-N'-(2-cyanophenyl)thiourea (**15**) and ethyl bromoacetate gave **38** as white needles; yield: 438 mg (55%); mp 153–154 °C (EtOH; lit.[109] 151–152 °C); R_F = 0.88 (EtOAc). IR (KBr): 3406, 3173, 2973, 1735, 1646 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.19 (t, J = 7.0 Hz, 3H, $CH_2C\underline{H}_3$), 3.98 (s, 2H, SCH_2), 4.11 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 7.36 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 6′-H), 7.45 (dd, J = 8.2, 1.3 Hz, 1H, 8′-H), 7.69 (ddd, J = 7.9, 7.1, 1.6 Hz, 1H, 7′-H), 7.85 (br s, 2H, NH_2), 8.13 (dd, J = 8.2, 1.6 Hz, 1H, 5′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.26 ($CH_2\underline{C}H_3$), 32.79 (SCH_2), 60.86 ($\underline{C}H_2CH_3$), 112.70 (C-4a′), 123.92 (C-5′), 124.55 (C-6′), 126.09 (C-8′), 133.41 (C-7′), 150.04 (C-8a′), 161.34 (C-4′), 165.58 (C-2′), 169.54 (CO).

Anal. Calcd for $C_{12}H_{13}N_3O_2S$: C, 54.74; H, 4.98; N, 15.96. Found: C, 54.45; H, 5.10; N, 15.54.

2-[(2-Phenylethyl)sulfanyl]quinazolin-4-amine (**39**)



M = 281.38 g/mol

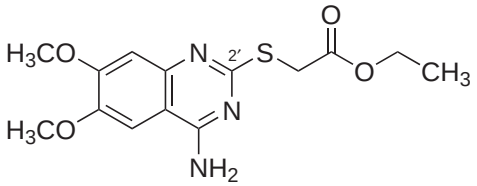
Reaction of N-benzoyl-N'-(2-cyanophenyl)thiourea (**15**) and phenethyl bromide gave **39** as colorless rhombes; yield: 582 mg (69%); mp 143–146 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.98 (t, J = 7.7 Hz, 2H, CH_2), 3.33 (t, J = 7.8 Hz, 2H, CH_2), 7.19–7.23 (m, 1H, 4′-H), 7.29–7.33 (m, 4H, 2′/3′/5′/6′-H), 7.36 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 6-H), 7.54 (dd, J = 8.2, 1.3 Hz, 1H, 8-H), 7.70 (ddd, J = 8.4, 6.9, 1.4 Hz, 1H, 7-H), 7.80 (br s, 2H, NH_2), 8.13 (dd, J = 8.2, 1.3 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 31.44 (CH_2), 35.65 (CH_2), 112.72 (C-4a), 123.90 (C-5), 124.30 (C-6), 126.20 (C-8), 126.29 (C-4′), 128.47 (C-2′/3′/5′/6′), 128.74 (C-2′/3′/5′/6′), 133.29 (C-7), 140.88 (C-1′), 150.33 (C-8a), 161.26 (C-4), 166.67 (C-2).

Anal. Calcd for $C_{16}H_{15}N_3S$: C, 68.30; H, 5.37; N, 14.93. Found: C, 68.24; H, 5.29; N, 14.81.

Ethyl [(4-Amino-6,7-dimethoxyquinazolin-2-yl)sulfanyl]acetate (**40**)



M = 323.37 g/mol

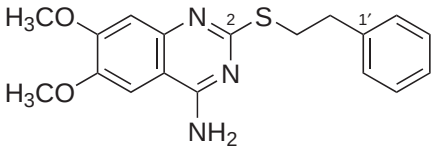
Reaction of *N*-benzoyl-*N*′-(2-cyano-4,5-dimethoxyphenyl)thiourea (**16**) and ethyl bromoacetate gave **40** as white needles; yield: 659 mg (68%); mp 178–181 °C (EtOH); R_F = 0.27 (toluene–EtOAc, 4:1). IR (KBr): 3108, 2888, 1725, 1661 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.20 (t, J = 7.0 Hz, 3H, $CH_2C\underline{H}_3$), 3.01 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.97 (s, 2H, SCH_2), 4.12 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 6.87 (s, 1H, 5′/8′-H), 7.51 (br s, 2H, NH_2), 7.52 (s, 1H, 5′/8′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.25 ($CH_2\underline{C}H_3$), 32.65 (SCH_2), 55.76 (OCH_3), 56.09 (OCH_3), 60.81 ($\underline{C}H_2CH_3$), 103.34 (C-5′/8′), 105.87 (C-4a′), 106.01 (C-5′/8′), 147.13 (C-6′/7′), 147.56 (C-6′/7′), 154.49 (C-8a′), 160.32 (C-4′), 163.25 (C-2′), 169.66 (CO).

Anal. Calcd for $C_{14}H_{17}N_3O_4S$: C, 52.00; H, 5.30; N, 12.99. Found: C, 52.14; H, 5.30; N, 13.00.

6,7-Dimethoxy-2-[(2-phenylethyl)sulfanyl]quinazolin-4-amine (**41**)



M = 341.43 g/mol

Reaction of *N*-benzoyl-*N*′-(2-cyano-4,5-dimethoxyphenyl)thiourea (**16**) and phenethyl bromide, followed by recrystallization from MeOH gave **41** as orange needles; yield: 682 mg (67%); mp 165–167 °C (MeOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.97 (t, J = 7.7 Hz, 2H, CH_2), 3.32 (t, J = 7.7 Hz, 2H, CH_2), 3.83 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.95 (s, 1H, 5/8-H), 7.18–7.23 (m, 1H, 4′-H), 7.28–7.33 (m, 4H, 2′/3′/5′/6′-H), 7.46 (br s, 2H, NH_2), 7.52 (s, 1H, 5/8-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 31.34 (CH_2), 35.57 (CH_2), 55.81 (OCH_3), 56.08 (OCH_3), 103.34 (C-5/8), 105.87 (C-4a), 106.13 (C-5/8), 126.27 (C-4′), 128.45 (C-2′/3′/5′/6′), 128.76 (C-2′/3′/5′/6′), 140.92 (C-1′), 147.43 (C-6/7), 154.46 (C-8a), 160.26 (C-4), 164.46 (C-2).

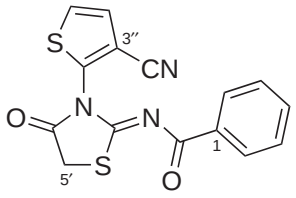
Anal. Calcd for $C_{18}H_{19}N_3O_2S$: C, 63.32; H, 5.61; N, 12.31. Found: C, 63.09; H, 5.71; N, 11.99.

8.5 2-(Benzoylimino)thiazolidin-4-ones

N-[3-(*o*-Cyanoaryl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamides **42–49**; General Procedure

The appropriate *N*-benzoyl-*N*′-(*o*-cyanoaryl)thiourea (**9**–**16**, 1.00 mmol) was dissolved in a mixture of EtOH (3.0 mL) and NaOH (1 M, 1.5 mL). The solution was heated to 50 °C and ethyl bromoacetate (200 mg, 133 μL, 1.20 mmol) was added within 1 min. If turbidity was observed, a few drops of 1 M NaOH were added to prevent precipitation. After heating at 50 °C for 1 h, the mixture was cooled to rt and H_2O (5 mL) was added. The precipitate was removed by suction filtration, washed with H_2O (50 mL), and dried *in vacuo*. Pure material was obtained by recrystallization from EtOH, if not stated otherwise.

N-[3-(3-Cyano-2-thienyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**42**)



M = 327.38 g/mol

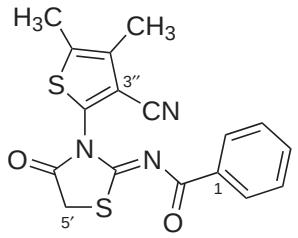
Reaction of *N*-benzoyl-*N*′-(3-cyano-2-thienyl)thiourea (**9**) and ethyl bromoacetate gave **42** as white needles; yield: 245 mg (71%); mp 191–192 °C (EtOH); R_F = 0.40 (petroleum ether–EtOAc, 2:1). IR (KBr): 3111, 2970, 2234, 1755, 1648 cm^{-1}. Material for X-ray crystallography was recrystallized from EtOAc.

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.29 (s, 2H, 5′-H), 7.46–7.51 (m, 2H, 3/5-H), 7.59–7.63 (m, 2H, 4/4″/5″-H), 7.93–7.96 (m, 2H, 2/6-H), 7.99 (d, J = 5.7 Hz, 1H, 4″/5″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 33.58 (C-5′), 109.71 (C-3″), 113.25 (CN), 127.15 (C-4″/5″), 128.86 (C-2/3/5/6), 129.52 (C-2/3/5/6), 129.77 (C-4″/5″), 133.71 (C-4), 134.61 (C-1), 143.04 (C-2″), 171.48 (C-2′/CO), 172.66 (C-2′/CO), 176.12 (CO).

Anal. Calcd for $C_{15}H_9N_3O_2S_2 \bullet H_2O$: C, 52.16; H, 3.21; N, 12.17. Found: C, 52.25; H, 3.25; N, 12.18.

N-[3-(3-Cyano-4,5-dimethyl-2-thienyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**43**)



$M = 355.43$ g/mol

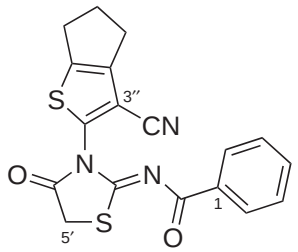
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**) and ethyl bromoacetate gave **43** as pale yellow plates; yield: 223 mg (63%); mp 218–221 °C (EtOH); $R_F = 0.62$ (toluene–EtOAc, 4:1). IR (KBr): 2230, 1751, 1649 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.26 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.27 (s, 2H, 5′-H), 7.48–7.52 (m, 2H, 3/5-H), 7.60–7.62 (m, 1H, 4-H), 7.96–7.98 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.56 (CH_3), 13.24 (CH_3), 33.44 (C-5′), 111.67 (C-3″), 113.24 (CN), 128.89 (C-2/3/5/6), 129.57 (C-2/3/5/6), 132.05 (C-4″/5″), 133.68 (C-4), 134.60 (C-1), 136.16 (C-4″/5″), 138.21 (C-2″), 171.49 (C-2′/CO), 172.41 (C-2′/CO), 176.25 (CO).

Anal. Calcd for $C_{17}H_{13}N_3O_2S_2$: C, 57.45; H, 3.69; N, 11.82. Found: C, 57.02; H, 3.92; N, 11.53.

N-[3-(3-Cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]-benzamide (**44**)



$M = 367.44$ g/mol

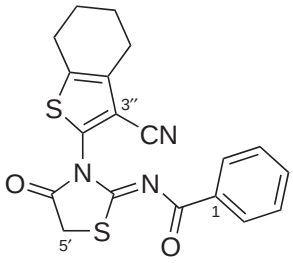
Reaction of *N*-benzoyl-*N*′-(3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)thiourea (**11**) and ethyl bromoacetate gave **44** as colorless needles; yield: 243 mg (66%); mp 175–176 °C (EtOH); $R_F = 0.49$ (petroleum ether–EtOAc, 2:1). IR (KBr): 2934, 2224, 1746, 1654 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.40–2.47 (m, 2H, 5″-H), 2.87–2.92 (m, 2H, 4″/6″-H), 3.01–3.06 (m, 2H, 4″/6″-H), 4.27 (s, 2H, 5′-H), 7.48–7.51 (m, 2H, 3/5-H), 7.60–7.62 (m, 1H, 4-H), 7.96–7.99 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 27.37 (C-4″/5″), 27.82 (C-4″/5″), 30.04 (C-6″), 33.43 (C-5′), 105.62 (C-3″), 113.08 (CN), 128.87 (C-2/3/5/6), 129.58 (C-2/3/5/6), 133.68 (C-4), 134.57 (C-1), 143.32 (C-2″/3a″/6a″), 143.39 (C-2″/3a″/6a″), 144.16 (C-2″/3a″/6a″), 171.56 (C-2′/CO), 172.43 (C-2′/CO), 176.28 (CO).

Anal. Calcd for $C_{18}H_{13}N_3O_2S_2$: C, 58.84; H, 3.57; N, 11.44. Found: C, 58.87; H, 3.60; N, 10.97.

N-[3-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]-benzamide (**45**)



M = 381.47 g/mol

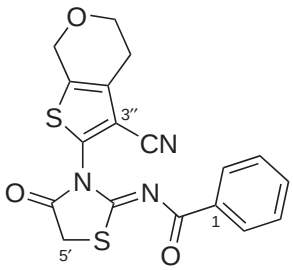
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)thiourea (**12**) and ethyl bromoacetate gave **45** as colorless plates; yield: 223 mg (58%); mp 194–197 °C (EtOH); R_F = 0.76 (toluene–EtOAc, 4:1). IR (KBr): 2934, 2227, 1754, 1642 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.82–1.86 (m, 4H, 5″/6″-H), 2.65–2.69 (m, 2H, 4″/7″-H), 2.80–2.84 (m, 2H, 4″/7″-H), 4.27 (s, 2H, 5′-H), 7.48–7.52 (m, 2H, 3/5-H), 7.60–7.64 (m, 1H, 4-H), 7.97–7.99 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 21.54 (C-4″–7″), 22.46 (C-4″–7″), 23.88 (C-4″–7″), 24.35 (C-4″–7″), 33.43 (C-5′), 109.84 (C-3″), 112.77 (CN), 128.88 (C-2/3/5/6), 129.60 (C-2/3/5/6), 133.67 (C-4), 133.98 (C-3a″), 134.56 (C-1), 138.61 (C-2″/7a″), 139.28 (C-2″/7a″), 171.43 (C-2′/CO), 172.17 (C-2′/CO), 176.27 (CO).

Anal. Calcd for $C_{19}H_{15}N_3O_2S_2$: C, 59.82; H, 3.96; N, 11.02. Found: C, 59.92; H, 4.18; N, 10.82.

N-[3-(3-Cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]-benzamide (**46**)



M = 383.44 g/mol

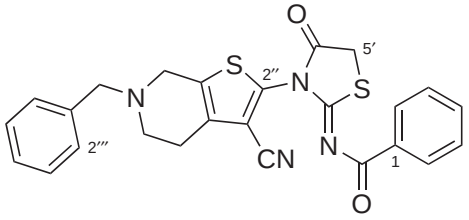
Reaction of *N*-benzoyl-*N*′-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)thiourea (**13**) and ethyl bromoacetate gave **46** as colorless needles; yield: 277 mg (72%); mp 181–182 °C (EtOH); R_F = 0.42 (toluene–EtOAc, 4:1). IR (KBr): 2926, 2230, 1756, 1645 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.75–2.83 (m, 2H, 4″-H), 3.98 (t, J = 5.4 Hz, 2H, 5″-H), 4.28 (s, 2H, 5′-H), 4.81 (t, J = 1.6 Hz, 2H, 7″-H), 7.48–7.52 (m, 2H, 3/5-H), 7.61–7.63 (m, 1H, 4-H), 7.97–8.00 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.28 (C-4″), 33.50 (C-5′), 63.87 (C-5″/7″), 64.30 (C-5″/7″), 109.88 (C-3″), 112.41 (CN), 128.88 (C-2/3/5/6), 129.61 (C-2/3/5/6), 131.78 (C-3a″), 133.68 (C-4), 134.55 (C-1), 136.26 (C-2″/7a″), 140.61 (C-2″/7a″), 171.40 (C-2′/CO), 172.31 (C-2′/CO), 176.24 (CO).

Anal. Calcd for $C_{18}H_{13}N_3O_3S_2$: C, 56.38; H, 3.42; N, 10.96. Found: C, 56.30; H, 3.66; N, 10.70.

N-[3-(6-Benzyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**47**)



M = 472.58 g/mol

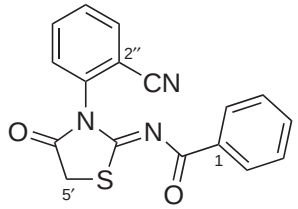
N-Benzoyl-*N*′-(6-benzyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)thiourea (**14**) and ethyl bromoacetate were reacted according to the aforementioned procedure. Compound **47** was obtained as an orange solid after purification by column chromatography (silica gel, petroleum ether–EtOAc, 2:1); yield: 105 mg (19%); mp 86–89 °C; R_F = 0.40 (toluene–EtOAc, 4:1). IR (KBr): 2924, 2228, 1756, 1646 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.73–2.89 (m, 4H, 4″/5″-H), 3.69–3.81 (m, 4H, 7″-H, CH_2), 4.27 (s, 2H, 5′-H), 7.26–7.30 (m, 1H, 4‴-H), 7.33–7.38 (m, 4H, 2‴/3‴/5‴/6‴-H), 7.48–7.51 (m, 2H, 3/5-H), 7.60–7.63 (m, 1H, 4-H), 7.97–8.00 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 23.84 (C-4″), 33.48 (C-5′), 48.62 (C-5″/7″), 50.71 (C-5″/7″), 60.45 (CH_2), 109.41 (C-3″), 112.58 (CN), 127.36 (C-4‴), 128.47 (C-2‴/3‴/5‴/6‴), 128.87 (C-2/3/5/6), 129.00 (C-2‴/3‴/5‴/6‴), 129.60 (C-2/3/5/6), 132.59 (C-3a″), 133.68 (C-4), 134.57 (C-1), 136.09 (C-2″/7a″), 137.88 (C-1‴), 140.17 (C-2″/7a″), 171.42 (C-2′/CO), 172.26 (C-2′/CO), 176.26 (CO).

Anal. Calcd for $C_{25}H_{20}N_4O_2S_2$ • EtOAc: C, 62.12; H, 5.03; N, 9.99. Found: C, 62.02; H, 5.19; N, 9.69.

N-[3-(2-Cyanophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**48**)



M = 321.35 g/mol

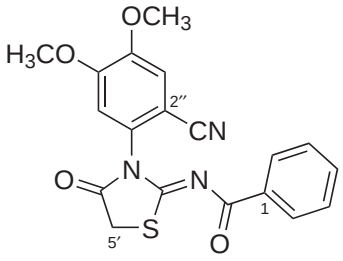
Reaction of *N*-benzoyl-*N*′-(2-cyanophenyl)thiourea (**15**) and ethyl bromoacetate gave **48** as yellow plates; yield: 226 mg (70%); mp 196–198 °C (EtOH); R_F = 0.51 (toluene–EtOAc, 4:1). IR (KBr): 2240, 1758, 1632 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.26 (d, J = 18.6 Hz, 1H, 5′-H), 4.42 (d, J = 18.6 Hz, 1H, 5′-H), 7.40–7.46 (m, 2H, 3/5-H), 7.55–7.59 (m, 1H, 4-H), 7.76–7.79 (m, 2H, 4″/6″-H), 7.80–7.83 (m, 2H, 2/6-H), 7.95–7.99 (m, 1H, 5″-H), 8.12–8.15 (m, 1H, 3″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 33.75 (C-5′), 111.84 (C-2″), 115.80 (CN), 128.79 (C-2/3/5/6), 129.35 (C-2/3/5/6), 130.26 (C-4″/6″), 130.52 (C-4″/6″), 133.58 (C-4), 133.74 (C-3″), 134.67 (C-1), 134.75 (C-5″), 137.50 (C-1″), 172.34 (C-2′/CO), 173.03 (C-2′/CO), 176.11 (CO).

Anal. Calcd for $C_{17}H_{11}N_3O_2S$: C, 63.54; H, 3.45; N, 13.08. Found: C, 63.27; H, 3.41; N, 12.90.

N-[3-(2-Cyano-4,5-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**49**)



M = 381.41 g/mol

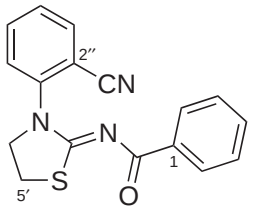
Reaction of *N*-benzoyl-*N*′-(2-cyano-4,5-dimethoxyphenyl)thiourea (**16**) and ethyl bromoacetate gave **49** as orange cubes; yield: 251 mg (66%); mp 216–218 °C (EtOH); R_F = 0.29 (toluene–EtOAc, 4:1). IR (KBr): 2940, 2225, 1755, 1642 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.22 (d, J = 18.6 Hz, 1H, 5′-H), 4.40 (d, J = 18.6 Hz, 1H, 5′-H), 7.41 (s, 1H, 3″/6″-H), 7.44–7.47 (m, 2H, 3/5-H), 7.57–7.59 (m, 1H, 4-H), 7.65 (s, 1H, 3″/6″-H), 7.86–7.89 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 33.50 (C-5′), 56.50 (2 × CH_3), 103.21 (C-2″), 113.11 (C-3″/6″/CN), 114.78 (C-3″/6″/CN), 116.17 (C-3″/6″/CN), 128.81 (C-2/3/5/6), 129.42 (C-2/3/5/6), 131.95 (C-1″), 133.54 (C-4), 134.76 (C-1), 149.38 (C-4″/5″), 153.10 (C-4″/5″), 172.37 (C-2′/CO), 172.93 (C-2′/CO), 176.27 (CO).

Anal. Calcd for $C_{19}H_{15}N_3O_4S$: C, 59.83; H, 3.96; N, 11.02. Found: C, 59.81; H, 3.98; N, 11.63.

N-[3-(2-Cyanophenyl)-1,3-thiazolidin-2-ylidene]benzamide (**50**)



M = 307.37 g/mol

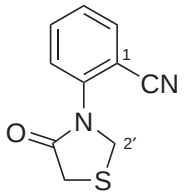
A mixture of *N*-benzoyl-*N*′-(2-cyanophenyl)thiourea (**15**; 281 mg, 1.00 mmol) and NaOAc (197 mg, 2.40 mmol) in DMF (10 mL) was treated dropwise with 1,2-dibromoethane (225 mg, 103 µL, 1.20 mmol). The reaction was stirred overnight at rt. A precipitate was removed by filtration and the filtrate was evaporated *in vacuo*. The crude material was purified by column chromatography (silica gel, toluene–EtOAc, 4:1) to provide **50** as a white solid; yield: 85 mg (28%); mp 130–132 °C; R_F = 0.28 (toluene–EtOAc, 4:1). IR (KBr): 3059, 2231, 1617 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.43 (t, J = 7.7 Hz, 2H, 5′-H), 4.19 (t, J = 7.7 Hz, 2H, 4′-H), 7.36–7.40 (m, 2H, 3/5-H), 7.47–7.51 (m, 1H, 4-H), 7.59–7.63 (m, 1H, 4″-H), 7.72 (dd, J = 8.2, 1.0 Hz, 1H, 6″-H), 7.84–7.89 (m, 3H, 2/6/5″-H), 8.02–8.05 (dd, J = 7.9, 1.6 Hz, 1H, 3″-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 27.66 (C-5′), 51.76 (C-4′), 111.44 (C-2″), 116.66 (CN), 127.13 (C-6″), 128.35 (C-2/3/5/6), 128.58 (C-4″), 129.09 (C-2/3/5/6), 132.36 (C-3″/5″), 133.71 (C-4), 134.58 (C-1), 136.03 (C-3″/5″), 142.75 (C-1″), 172.38 (C-2′/CO), 174.45 (C-2′/CO).

Anal. Calcd for $C_{17}H_{13}N_3OS$: C, 66.43; H, 4.26; N, 13.67. Found: C, 66.23; H, 4.19; N, 13.65.

2-(4-Oxo-1,3-thiazolidin-3-yl)benzonitrile (**51**)



M = 204.25 g/mol

Compound **51** was prepared by a method according to Brouwer *et al.*[123] An aqueous formaldehyde solution (37 wt%, 4.5 mL) was added dropwise to a solution of anthranilonitrile (5.91 g, 50.0 mmol) and thioglycolic acid (4.61 g, 3.48 mL, 50.0 mmol) in EtOH (15 mL). After stirring for 4 h at rt, the mixture was poured into ice water (75 mL). Precipitated 2-[(2-cyanophenylamino)methylsulfanyl]acetic acid (9.85 g, 44.3 mmol) was recovered by

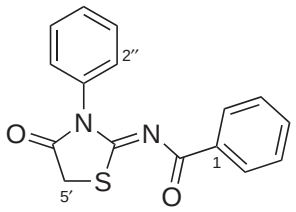
suction filtration, washed with H_2O (100 mL), and dried under reduced pressure. It was then redissolved in *p*-xylene (60 mL) and refluxed for 6 h using a water trap. The resulting solution was filtered and the solvent was removed under reduced pressure. Recrystallization from EtOH afforded **51** as white needles; yield: 3.47 g (43%, 2 steps); mp 124–126 ℃ (EtOH; lit.[123] 120–122 ℃); R_F = 0.21 (toluene–EtOAc, 4:1). IR (KBr): 2233, 1683 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.73 (t, J = 1.0 Hz, 2H, 5′-H), 4.85 (t, J = 1.0 Hz, 2H, 2′-H), 7.53–7.57 (m, 1H, 5-H), 7.62 (dd, J = 8.2, 1.0 Hz, 1H, 3-H), 7.78–7.82 (m, 1H, 4-H), 7.93 (dd, J = 7.9, 1.6 Hz, 1H, 6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 31.70 (C-5′), 48.86 (C-2′), 111.12 (C-1), 116.41 (CN), 127.52 (C-3), 128.62 (C-5), 133.80 (C-6), 134.50 (C-4), 141.11 (C-2), 170.98 (CO).

Anal. Calcd for $C_{10}H_8N_2OS$: C, 58.80; H, 3.95; N, 13.72. Found: C, 58.92; H, 3.97; N, 13.73.

N-(4-Oxo-3-phenyl-1,3-thiazolidin-2-ylidene)benzamide (**52**)



M = 296.34 g/mol

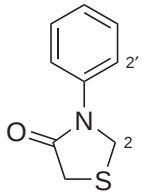
N-Benzoyl-*N*′-phenylthiourea (256 mg, 1.00 mmol), which was prepared according to Rasmussen *et al.*,[241] was dissolved in a mixture of EtOH (5.0 mL) and NaOH (1 M, 1.5 mL). The solution was heated to 50 ℃ and ethyl bromoacetate (200 mg, 133 µL, 1.20 mmol) was added within 1 min. After heating at 50 ℃ for 1 h, the mixture was cooled to ambient temperature and H_2O (8 mL) was added. The resulting precipitate was removed by suction filtration, washed with H_2O (50 mL), and dried *in vacuo*. Recrystallization from EtOAc afforded **52** as colorless needles; yield: 255 mg (86%); mp 221–222 ℃ (EtOAc; lit.[96] 224 ℃); R_F = 0.54 (petroleum ether–EtOAc, 2:1). IR (KBr): 2968, 1733, 1637 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.16 (s, 2H, 5′-H), 7.40–7.45 (m, 4H, 3/5/2″/6″-H), 7.49–7.59 (m, 4H, 4/3″–5″-H), 7.83–7.86 (m, 2H, 2/6-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 33.57 (C-5′), 128.32 (C-2″/3″/5″/6″), 128.67 (C-2/3/5/6), 129.01 (C-4″), 129.08 (C-2″/3″/5″/6″), 129.36 (C-2/3/5/6), 133.30 (C-4), 135.07 (C-1/1″), 135.39 (C-1/1″), 173.04 (C-2′/CO), 173.88 (C-2′/CO), 176.24 (CO).

Anal. Calcd for $C_{16}H_{12}N_2O_2S$: C, 64.85; H, 4.08; N, 9.45. Found: C, 64.56; H, 4.04; N, 9.41.

3-Phenyl-1,3-thiazolidin-4-one (**53**)



$M = 179.24$ g/mol

Compound **53** was prepared by a procedure described by Kay *et al.*[124] Thioglycolic acid (5.68 g, 4.29 mL, 61.7 mmol) was added within 1 min to a solution of aniline (5.75 g, 5.62 mL, 61.7 mmol) in PhMe (120 mL). After 10 min, the solution was treated dropwise with an aqueous formaldehyde solution (37 wt%, 4.8 mL), followed by the addition of *p*-toluenesulfonic acid monohydrate (10 mg, 53 μmol). The mixture was refluxed for 4 h using a water trap. After the mixture had cooled to rt and been washed with saturated aqueous $NaHCO_3$ (2 × 50 mL), the solvent was removed under reduced pressure. After purification by column chromatography (silica gel, toluene–EtOAc, 4:1), **53** was obtained as a white solid; yield: 6.01 g (54%); mp 110–112 °C (EtOH; lit.[123] 116–119 °C, lit.[242] 110–114 °C); $R_F = 0.35$ (petroleum ether–EtOAc, 4:1). IR (KBr): 3058, 1677 cm^{-1}.

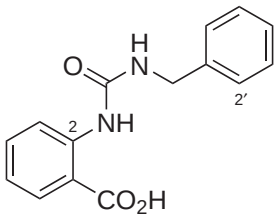
^{1}H NMR (500 MHz, DMSO-d_6) δ 3.70 (t, $J = 1.0$ Hz, 2H, 2/5-H), 4.89 (t, $J = 1.0$ Hz, 2H, 2/5-H), 7.21–7.25 (m, 1H, 4′-H), 7.38–7.42 (m, 2H, 3′/5′-H), 7.48–7.52 (m, 2H, 2′/6′-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 32.66 (C-5), 48.71 (C-2), 122.57 (C-2′/6′), 125.84 (C-4′), 128.94 (C-3′/5′), 139.05 (C-1′), 170.40 (CO).

Anal. Calcd for C_9H_9NOS: C, 60.31; H, 5.06; N, 7.81. Found: C, 60.25; H, 5.36; N, 7.86.

8.6 2-Ureidobenzoic Acids

2-(3-Benzylureido)benzoic Acid (**54**)[126]



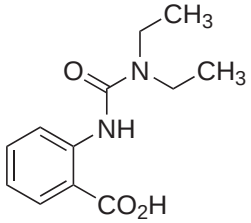
M = 270.28 g/mol

Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 4.28 (d, J = 5.7 Hz, 2H, C$\underline{H}_2$Ph), 6.95 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 5-H), 7.21–7.24 (m, 1H, 4′-H), 7.28–7.32 (m, 4H, 2′/3′/5′/6′-H), 7.46 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 4-H), 7.90 (dd, J = 8.2, 1.6 Hz, 1H, 6-H), 7.94–7.97 (m, 1H, NH), 8.39 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.19 (s, 1H, NH), 13.23 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 43.01 (C$\underline{H}_2$Ph), 114.71 (C-1), 119.30 (C-3/5), 120.22 (C-3/5), 126.83 (C-4′), 127.38 (C-2′/3′/5′/6′), 128.38 (C-2′/3′/5′/6′), 131.04 (C-6), 133.81 (C-4), 140.28 (C-1′), 143.23 (C-2), 154.91 (NHCO), 169.61 (CO_2H).

2-(3,3-Diethylureido)benzoic Acid (**55**)



M = 236.27 g/mol

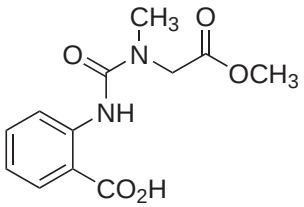
Anthranilic acid (688 mg, 5.00 mmol) was added in portions to a stirring solution of N,N'-carbonyldiimidazole (811 mg, 5.00 mmol) in CH_2Cl_2 (50 mL). After 30 min, diethylamine (731 mg, 1.03 mL, 10.0 mmol) was added dropwise and the mixture was stirred overnight. The organic layer was washed with HCl (1 M, 2 × 5 mL), filtered, and evaporated to dryness to obtain **55** as a white solid; yield: 637 mg (54%); mp 144–148 °C (lit.[127] 151 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.24 (t, J = 7.3 Hz, 6H, $CH_2C\underline{H}_3$), 3.43 (q, J = 7.3 Hz, 4H, C$\underline{H}_2CH_3$), 6.96 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H, 5-H), 7.52 (ddd, J = 8.5, 7.2, 1.6 Hz, 1H, 4-H), 8.05 (dd, J = 8.2, 1.6 Hz, 1H, 6-H), 8.62 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.50 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 13.67 ($CH_2\underline{C}H_3$), 41.73 ($\underline{C}H_2CH_3$), 112.60 (C-1), 119.56 (C-3/5), 120.52 (C-3/5), 131.58 (C-6), 135.56 (C-4), 144.40 (C-2), 154.55 (NHCO), 172.83 (CO_2H).

Anal. Calcd for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 60.78; H, 7.02; N, 11.63.

2-[3-(Methoxycarbonylmethyl)-3-methylureido]benzoic Acid (**56**)



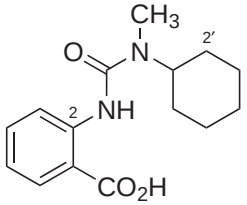
$M = 266.25$ g/mol

Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.07 (s, 3H, NCH_3), 3.66 (s, 3H, OCH_3), 4.16 (s, 2H, CH_2), 7.02 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 5-H), 7.52 (ddd, J = 8.5, 7.2, 1.6 Hz, 1H, 4-H), 7.96 (dd, J = 8.0, 1.4 Hz, 1H, 6-H), 8.42 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.67 (s, 1H, NH), 13.56 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 35.30 (NCH_3), 50.01 (CH_2/OCH_3), 51.89 (CH_2/OCH_3), 114.69 (C-1), 118.65 (C-3/5), 120.92 (C-3/5), 131.15 (C-6), 134.28 (C-4), 143.04 (C-2), 154.89 (NHCO), 170.45 (CO/CO_2H), 170.49 (CO/CO_2H).

2-(3-Cyclohexyl-3-methylureido)benzoic Acid (**57**)



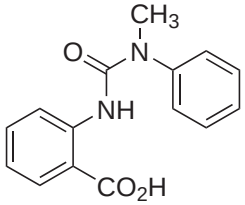
$M = 276.33$ g/mol

Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.05–1.18 (m, 10H, 2′–6′-H), 2.83 (s, 3H, NCH_3), 3.90–3.98 (m, 1H, 1′-H), 6.97 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 5-H), 7.50 (ddd, J = 8.5, 7.2, 1.6 Hz, 1H, 4-H), 7.94 (dd, J = 8.2, 1.6 Hz, 1H, 6-H), 8.47 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.83 (s, 1H, NH), 13.51 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 25.09 (C-4′), 25.58 (C-3′/5′), 28.12 (NCH_3), 30.07 (C-2′/6′), 53.79 (C-1′), 114.44 (C-1), 118.80 (C-3/5), 120.39 (C-3/5), 131.11 (C-6), 134.16 (C-4), 143.67 (C-2), 154.27 (NHCO), 170.56 (CO_2H).

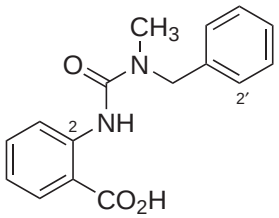
2-(3-Methyl-3-phenylureido)benzoic Acid (**58**)[154]



$M = 270.28$ g/mol

Compound was taken from the substance library.

2-(3-Benzyl-3-methylureido)benzoic Acid (**59**)



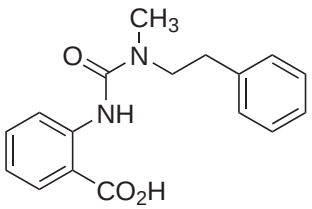
$M = 284.31$ g/mol

Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.98 (s, 3H, NCH_3), 4.57 (s, 2H, $C\underline{H}_2Ph$), 7.00 (ddd, $J = 8.2$, 7.1, 1.3 Hz, 1H, 5-H), 7.23–7.28 (m, 3H, 2′/4′/6′-H), 7.32–7.36 (m, 2H, 3′/5′-H), 7.53 (ddd, $J = 8.5$, 7.3, 1.6 Hz, 1H, 4-H), 7.95 (dd, $J = 7.9$, 1.6 Hz, 1H, 6-H), 8.51 (dd, $J = 8.5$, 1.0 Hz, 1H, 3-H), 10.97 (s, 1H, NH), 13.51 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 34.23 (NCH_3), 51.33 ($\underline{C}H_2Ph$), 114.66 (C-1), 118.81 (C-3/5), 120.69 (C-3/5), 127.19 (C-4′), 127.29 (C-2′/3′/5′/6′), 128.66 (C-2′/3′/5′/6′), 131.13 (C-6), 134.22 (C-4), 138.14 (C-1′), 143.39 (C-2), 154.83 (NHCO), 170.46 (CO_2H).

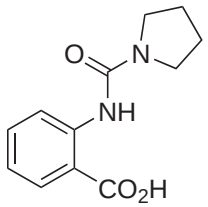
2-[3-Methyl-3-(2-phenylethyl)ureido]benzoic Acid (**60**)[154]



$M = 298.34$ g/mol

Compound was taken from the substance library.

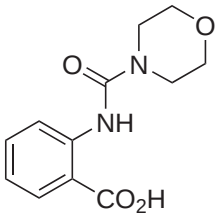
2-[(1-Pyrrolidinylcarbonyl)amino]benzoic Acid (**61**)[127]



$M = 234.25$ g/mol

Compound was taken from the substance library.

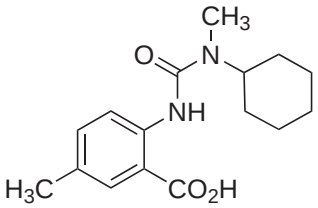
2-[(4-Morpholinylcarbonyl)amino]benzoic Acid (**62**)[127]



$M = 250.25$ g/mol

Compound was taken from the substance library.

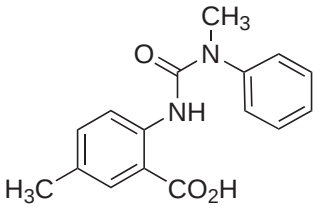
2-(3-Cyclohexyl-3-methylureido)-5-methylbenzoic Acid (**63**)[154]



$M = 290.36$ g/mol

Compound was taken from the substance library.

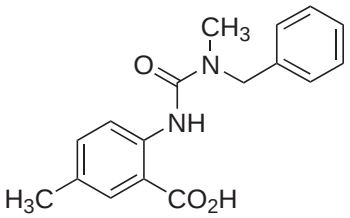
5-Methyl-2-(3-methyl-3-phenylureido)benzoic Acid (**64**)[154]



$M = 284.31$ g/mol

Compound was taken from the substance library.

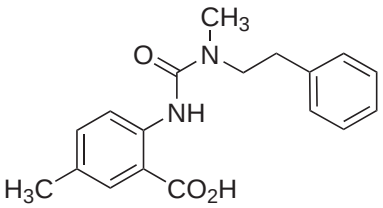
2-(3-Benzyl-3-methylureido)-5-methylbenzoic Acid (**65**)[154]



$M = 298.34$ g/mol

Compound was taken from the substance library.

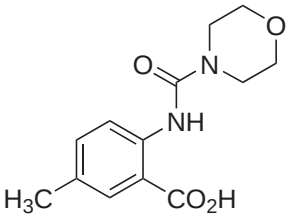
5-Methyl-2-[3-methyl-3-(2-phenylethyl)ureido]benzoic Acid (**66**)[154]



$M = 312.36$ g/mol

Compound was taken from the substance library.

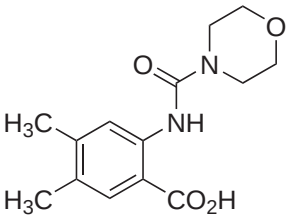
5-Methyl-2-[(4-morpholinylcarbonyl)amino]benzoic Acid (**67**)[154]



$M = 264.28$ g/mol

Compound was taken from the substance library.

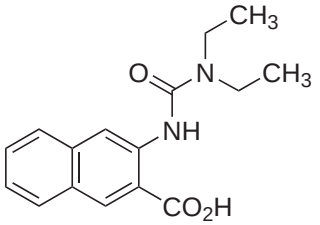
4,5-Dimethyl-2-[(4-morpholinylcarbonyl)amino]benzoic Acid (**68**)[127]



$M = 278.30$ g/mol

Compound was taken from the substance library.

3-(3,3-Diethylureido)-2-naphthoic Acid (**69**)



$M = 286.33$ g/mol

N,*N*′-Carbonyldiimidazole (487 mg, 3.00 mmol) was added in portions to a solution of 3-amino-2-naphthalenecarboxylic acid (562 mg, 3.00 mmol) in anhydrous THF (30 mL). After 2 h, diethylamine (439 mg, 618 µL, 6.00 mmol) was added dropwise, and the mixture was stirred overnight at rt. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (20 mL). The organic layer was washed with HCl (1 M, 2 × 5 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. The crude material was recrystallized from MeOH to obtain **69** as yellowish plates; yield: 586 mg (68%); mp 166–169 °C (MeOH).

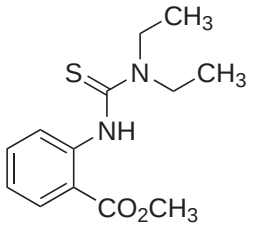
^{1}H NMR (500 MHz, DMSO-d_6) δ 1.17 (t, $J = 7.1$ Hz, 6H, $CH_2C\underline{H}_3$), 3.38 (q, $J = 7.0$ Hz, 4H, $C\underline{H}_2CH_3$), 7.38 (ddd, $J = 8.0, 6.9, 1.3$ Hz, 1H, 6/7-H), 7.54 (ddd, $J = 8.0, 6.8, 1.3$ Hz, 1H, 6/7-H), 7.76 (d, $J = 7.8$ Hz, 1H, 5/8-H), 7.95 (d, $J = 8.2$ Hz, 1H, 5/8-H), 8.66 (s, 1H, 1/4-H), 8.88 (s, 1H, 1/4-H), 10.74 (s, 1H, NH), 13.80 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 13.90 ($CH_2\underline{C}H_3$), 41.08 ($\underline{C}H_2CH_3$), 114.40 (C-4), 116.23 (C-2), 124.58 (C-1/5–8), 126.84 (C-1/5–8), 127.11 (C-8a), 129.16 (C-1/5–8), 129.19 (C-1/5–8), 133.18 (C-1/5–8), 136.21 (C-3/4a), 138.60 (C-3/4a), 153.93 (NHCO), 170.55 (CO_2H).

Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 66.79; H, 6.32; N, 9.59.

8.7 Methyl 2-Thioureidobenzoates and 2-Thioureidobenzoic Acids

Methyl 2-(3,3-Diethylthioureido)benzoate (**70**)



M = 266.36 g/mol

Method 1: Diethylamine (476 mg, 670 µL, 6.50 mmol) was added dropwise to a stirring solution of methyl 2-isothiocyanatobenzoate (966 mg, 5.00 mmol), that was prepared according to Carpenter *et al.*,[128] in CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 3 h. The organic layer was washed with HCl (0.5 M, 2 × 5 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. Recrystallization from EtOH yielded **70** (0.946 g, 71%) as colorless needles, mp 85–87 °C (EtOH).

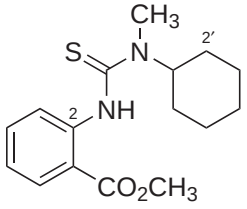
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.34 (t, J = 6.9 Hz, 6H, $CH_2C\underline{H}_3$), 3.82 (q, J = 6.9 Hz, 4H, $C\underline{H}_2CH_3$), 3.88 (s, 3H, CO_2CH_3), 7.03 (ddd, J = 8.2, 7.3, 1.3 Hz, 1H, 5-H), 7.48 (ddd, J = 8.6, 7.3, 1.6 Hz, 1H, 4-H), 7.93 (dd, J = 8.2, 1.6 Hz, 1H, 6-H), 8.73 (dd, J = 8.6, 1.3 Hz, 1H, 3-H), 10.66 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 12.46 ($CH_2\underline{C}H_3$), 45.68 ($\underline{C}H_2CH_3$), 52.31 ($CO_2\underline{C}H_3$), 116.86 (C-1), 122.22 (C-5), 123.62 (C-3), 130.22 (C-6), 132.82 (C-4), 143.28 (C-2), 168.99 ($\underline{C}O_2CH_3$), 179.23 (NHCS).

Anal. Calcd for $C_{13}H_{18}N_2O_2S$: C, 58.62; H, 6.81; N, 10.52. Found: C, 58.37; H, 6.78; N, 10.44.

Method 2: 2-(3,3-Diethylthioureido)-*N*,*N*-diethylbenzamide (**133**, 307 mg, 1.00 mmol) was heated to reflux in anhydrous methanolic HCl (0.25 M, 5 mL) for 3 h. The mixture was allowed to cool to rt and kept at −15 °C. The precipitate was removed by suction filtration to give **70** (169 mg, 63%) as white needles.

Methyl 2-(3-Cyclohexyl-3-methylthioureido)benzoate (**71**)



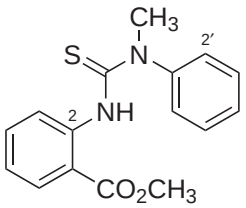
M = 306.42 g/mol

According to the preparation of **70** (Method 1), **71** (1.50 g, 98%) was obtained from methyl 2-isothiocyanatobenzoate and *N*-methylcyclohexylamine as a semisolid crude material.

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05–1.95 (m, 10H, 2′–6′-H), 3.20 (s, 3H, NCH_3), 3.88 (s, 3H, CO_2CH_3), 5.04 (br s, 1H, 1′-H), 7.03 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 5-H), 7.48 (ddd, J = 8.8, 7.1, 1.6 Hz, 1H, 4-H), 7.93 (dd, J = 8.2, 1.6 Hz, 1H, 6-H), 8.71(d, J = 8.2 Hz, 1H, 3-H), 10.70 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 25.48 (C-4′), 25.54 (C-3′/5′), 30.01 (C-2′/6′), 32.57 (NCH_3), 52.29 ($CO_2\underline{C}H_3$), 59.23 (C-1′), 116.74 (C-1), 122.16 (C-5), 123.35 (C-3), 130.23 (C-6), 132.86 (C-4), 143.27 (C-2), 168.99 ($\underline{C}O_2CH_3$), 179.90 (NHCS).

Methyl 2-(3-Methyl-3-phenylthioureido)benzoate (**72**)



M = 300.38 g/mol

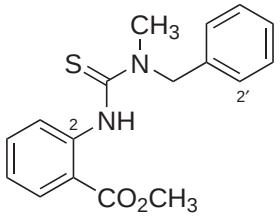
According to the preparation of **70** (Method 1), **72** (1.28 g, 84%) was obtained from methyl 2-isothiocyanatobenzoate and *N*-methylaniline as colorless needles, mp 70–71 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.61 (s, 3H, NCH_3), 3.70 (s, 3H, CO_2CH_3), 7.15–7.17 (m, 1H, 5-H), 7.39–7.45 (m, 3H, 2′/4′/6′-H), 7.50–7.55 (m, 3H, 4/3′/5′-H), 7.77 (dd, J = 8.2, 1.6 Hz, 1H, 6-H), 8.24 (dd, J = 8.4, 1.0 Hz, 1H, 3-H), 9.67 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 43.28 (NCH_3), 52.44 ($CO_2\underline{C}H_3$), 120.31 (C-1), 123.65 (C-5), 125.06 (C-3), 127.01 (C-2′/6′), 128.27 (C-4′), 130.01 (C-6), 130.26 (C-3′/5′), 132.46 (C-4), 141.32 (C-2), 143.71 (C-1′), 167.08 ($\underline{C}O_2CH_3$), 180.48 (NHCS).

Anal. Calcd for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33. Found: C, 63.71; H, 5.39; N, 9.30.

Methyl 2-(3-Benzyl-3-methylthioureido)benzoate (**73**)



M = 314.40 g/mol

According to the preparation of **70** (Method 1), **73** (1.45 g, 92%) was obtained from methyl 2-isothiocyanatobenzoate and *N*-benzylmethylamine as white plates, mp 88–92 °C (EtOH).

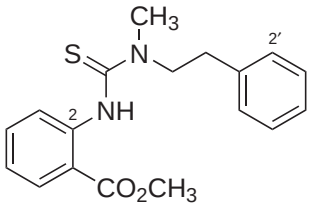
^{1}H NMR (500 MHz, $CDCl_3$) δ 3.30 (s, 3H, NCH_3), 3.87 (s, 3H, CO_2CH_3), 5.25 (s, 2H, $C\underline{H}_2Ph$), 7.08 (ddd, J = 8.4, 7.1, 1.3 Hz, 1H, 5-H), 7.26–7.28 (m, 1H, 4′-H), 7.29–7.34 (m,

4H, 2′/3′/5′/6′-H), 7.53 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 4-H), 7.96 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.87 (d, J = 8.6 Hz, 1H, 3-H), 10.93 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 37.63 (NCH_3), 52.36 ($CO_2\underline{C}H_3$), 56.78 ($\underline{C}H_2Ph$), 117.06 (C-1), 122.64 (C-5), 123.42 (C-3), 127.52 (C-2′/6′), 127.58 (C-4′), 128.72 (C-3′/5′), 130.31 (C-6), 133.01 (C-4), 136.43 (C-1′), 142.98 (C-2), 168.95 ($\underline{C}O_2CH_3$), 180.94 (NHCS).

Anal. Calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91. Found: C, 64.88; H, 6.05; N, 8.89.

Methyl 2-[3-Methyl-3-(2-phenylethyl)thioureido]benzoate (**74**)



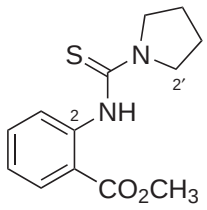
M = 328.43 g/mol

According to the preparation of **70** (Method 1), **74** (1.47 g, 90%) was obtained from methyl 2-isothiocyanatobenzoate and *N*-methylphenethylamine as a semisolid crude material.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.07 (t, J = 7.9 Hz, 2H, $CH_2\underline{C}H_2Ph$), 3.28 (s, 3H, NCH_3), 3.90 (s, 3H, CO_2CH_3), 4.05–4.14 (m, 2H, $C\underline{H}_2CH_2Ph$), 7.06 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 5-H), 7.19–7.23 (m, 1H, 4′-H), 7.27–7.31 (m, 4H, 2′/3′/5′/6′-H), 7.51 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H, 4-H), 7.96 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.80 (d, J = 8.6 Hz, 1H, 3-H), 10.84 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 33.38 ($CH_2\underline{C}H_2Ph$), 39.22 (NCH_3), 52.35 ($CO_2\underline{C}H_3$), 55.82 ($\underline{C}H_2CH_2Ph$), 116.80 (C-1) 122.41 (C-5), 123.27 (C-3), 126.52 (C-4′), 128.60 (C-2′/6′), 128.91 (C-3′/5′), 130.27 (C-6), 132.99 (C-4), 138.55 (C-1′), 143.05 (C-2), 169.02 ($\underline{C}O_2CH_3$), 179.87 (NHCS).

Methyl 2-[(1-Pyrrolidinylthiocarbonyl)amino]benzoate (**75**)



M = 364.34 g/mol

Method 1: According to the preparation of **70** (Method 1), **75** (1.32 g, 82%) was obtained from methyl 2-isothiocyanatobenzoate and pyrrolidine as colorless needles, mp 124–127 °C (EtOH).

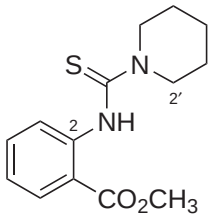
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.87–2.16 (m, 4H, 3′/4′-H), 3.65–3.96 (m, 7H, CO_2CH_3, 2′/5′-H), 7.04 (ddd, J = 7.9, 7.3, 1.3 Hz, 1H, 5-H), 7.51 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 4-H), 7.95 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 9.00 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.82 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 24.59 (C-3′/4′), 26.20 (C-3′/4′), 48.30 (C-2′/5′), 52.13 (C-2′/5′), 52.33 ($CO_2\underline{C}H_3$), 116.40 (C-1), 122.26 (C-3/5), 122.65 (C-3/5), 130.35 (C-6), 133.16 (C-4), 142.88 (C-2), 169.05 ($\underline{C}O_2CH_3$), 176.47 (NHCS).

Anal. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60. Found: C, 59.09; H, 6.35; N, 10.49.

Method 2: According to the preparation of **70** (Method 2), **75** (222 mg, 84%) was obtained from **134** as colorless needles.

Methyl 2-[(1-Piperidinylthiocarbonyl)amino]benzoate (**76**)



M = 278.37 g/mol

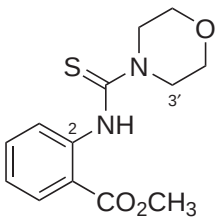
According to the preparation of **70** (Method 1), **76** (1.11 g, 80%) was obtained from methyl 2-isothiocyanatobenzoate and piperidine as colorless plates, mp 116–117 °C (EtOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.68–1.73 (m, 6H, 3′–5′-H), 3.88 (s, 3H, CO_2CH_3), 3.95–4.01 (m, 4H, 2′/6′-H), 7.02 (ddd, J = 8.2, 7.3, 1.3 Hz, 1H, 5-H), 7.48 (ddd, J = 8.8, 7.3, 1.6 Hz, 1H, 4-H), 7.93 (dd, J = 8.1, 1.8 Hz, 1H, 6-H), 8.53 (dd, J = 8.5, 1.3 Hz, 1H, 3-H), 10.75 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 24.40 (C-4′), 25.67 (C-3′/5′), 49.68 (C-2′/6′), 52.33 ($CO_2\underline{C}H_3$), 116.41 (C-1), 122.04 (C-5), 123.03 (C-3), 130.33 (C-6), 132.97 (C-4), 143.40 (C-2), 169.06 ($\underline{C}O_2CH_3$), 179.60 (NHCS).

Anal. Calcd for $C_{14}H_{18}N_2O_2S$: C, 60.41; H, 6.52; N, 10.06. Found: C, 60.57; H, 6.55; N, 10.10.

Methyl 2-[(4-Morpholinylthiocarbonyl)amino]benzoate (**77**)



M = 280.34 g/mol

Method 1: According to the preparation of **70** (Method 1), **77** (1.11 g, 80%) was obtained from methyl 2-isothiocyanatobenzoate and morpholine as a white solid, mp 103–107 °C (EtOH; lit.[243] 106–110 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.79 (t, J = 4.9 Hz, 4H, 2′/6′-H), 3.89 (s, 3H, CO_2CH_3), 4.04 (t, J = 4.9 Hz, 4H, 3′/5′-H), 7.06 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 5-H), 7.51 (ddd, J = 8.6, 7.3, 1.6 Hz, 1H, 4-H), 7.96 (dd, J = 8.1, 1.6 Hz, 1H, 6-H), 8.67 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.97 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 48.25 (C-3′/5′), 52.48 ($CO_2\underline{C}H_3$), 66.29 (C-2′/6′), 116.57 (C-1), 122.58 (C-3/5), 122.93 (C-3/5), 130.43 (C-6), 133.19 (C-4), 142.95 (C-2), 169.19 ($\underline{C}O_2CH_3$), 180.72 (NHCS).

Anal. Calcd for $C_{13}H_{16}N_2O_3S$: C, 55.70; H, 5.75; N, 9.99. Found: C, 55.96; H, 5.94; N, 9.84.

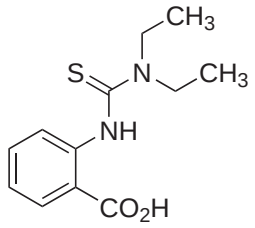
Method 2: According to the preparation of **70** (Method 2), **77** (229 mg, 82%) was obtained from **135** as a light yellow solid.

Method 3: 2-(Morpholin-4-yl)-4*H*-3,1-benzothiazin-4-one (**130**; 160 mg, 640 μmol) was heated to reflux in anhydrous methanolic HCl (0.25 M, 3 mL) for 3 h. The mixture was allowed to cool to rt and kept at −15 °C. The precipitate was removed by suction filtration to give **77** (151 mg, 84%) as a light yellow solid.

2-Thioureidobenzoic Acids **78–85**; General Procedure

A mixture of the appropriate methyl 2-thioureidobenzoate (**70**–**77**, 2.00 mmol), aqueous NaOH (1 M, 10 mL) and EtOH (10 mL) was heated to reflux for 1 h. The reaction was allowed to cool to rt and H_2O (30 mL) was added. After filtration and cooling to 0 °C, the solution was acidified with 2 M HCl. The precipitate was removed by suction filtration and washed with H_2O (50 mL).

2-(3,3-Diethylthioureido)benzoic Acid (**78**)



M = 252.33 g/mol

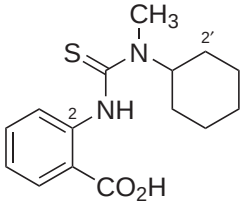
White solid; yield: 394 mg (78%); mp 114–116 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.23 (t, J = 7.1 Hz, 6H, $CH_2C\underline{H}_3$), 3.76 (q, J = 7.0 Hz, 4H, $C\underline{H}_2CH_3$), 7.11 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 5-H), 7.50 (ddd, J = 8.5, 7.1, 1.6 Hz, 1H, 4-H), 7.90 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.49 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.62 (s, 1H, NH), 13.53 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.50 ($CH_2\underline{C}H_3$), 45.03 ($\underline{C}H_2CH_3$), 119.10 (C-1), 122.65 (C-5), 123.93 (C-3), 130.49 (C-6), 132.40 (C-4), 142.97 (C-2), 169.83 (CO_2H), 178.41 (NHCS).

Anal. Calcd for $C_{12}H_{16}N_2O_2S$: C, 57.12; H, 6.39; N, 11.10. Found: C, 56.55; H, 6.47; N, 11.00.

2-(3-Cyclohexyl-3-methylthioureido)benzoic Acid (**79**)



$M = 292.40$ g/mol

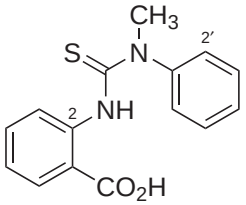
White solid; yield: 525 mg (90%); mp 130–132 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.07–1.35 (m, 10H, 2′–6′-H), 3.13 (s, 3H, NCH_3), 4.88 (br s, 1H, 1′-H), 7.11 (ddd, J = 7.9, 7.3, 1.3 Hz, 1H, 5-H), 7.50 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 4-H), 7.90 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.42 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.66 (s, 1H, NH), 13.48 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 25.01 (C-4′), 25.41 (C-3′/5′), 29.45 (C-2′/6′), 32.60 (NCH_3), 58.71 (C-1′), 119.31 (C-1), 122.69 (C-5), 123.84 (C-3), 130.51 (C-6), 132.39 (C-4), 142.88 (C-2), 169.74 (CO_2H), 179.37 (NHCS).

Anal. Calcd for $C_{15}H_{20}N_2O_2S$: C, 61.62; H, 6.89; N, 9.58. Found: C, 61.46; H, 7.18; N, 9.59.

2-(3-Methyl-3-phenylthioureido)benzoic (**80**)



$M = 286.35$ g/mol

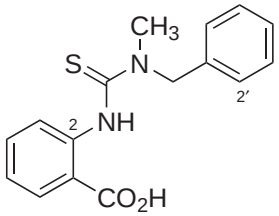
White solid; yield: 378 mg (66%); mp 135–138 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.61 (s, 3H, NCH_3), 7.08 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 5-H), 7.38–7.41 (m, 3H, 2′/4′/6′-H), 7.48–7.51 (m, 3H, 4/3′/5′-H), 7.80 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.68 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.54 (s, 1H, NH), 13.33 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 43.23 (NCH_3), 118.36 (C-1), 122.75 (C-3/5), 122.98 (C-3/5), 127.12 (C-2′/6′), 128.45 (C-4′), 130.32 (C-3′/5′), 130.40 (C-6), 132.43 (C-4), 142.13 (C-2/1′), 143.53 (C-2/1′), 169.33 (CO_2H), 179.74 (NHCS).

Anal. Calcd for $C_{15}H_{14}N_2O_2S$: C, 62.92; H, 4.93; N, 9.78. Found: C, 62.72; H, 5.07; N, 9.69.

2-(3-Benzyl-3-methylthioureido)benzoic Acid (**81**)



$M = 300.38$ g/mol

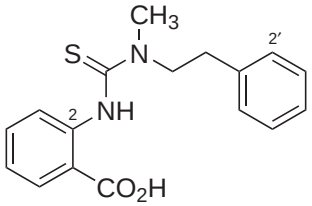
White solid; yield: 365 mg, (61%); mp 117–119 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.24 (s, 3H, NCH_3), 5.19 (s, 2H, $C\underline{H}_2Ph$), 7.17 (ddd, J = 8.2, 7.3, 1.0 Hz, 1H, 5-H), 7.25–7.29 (m, 5H, 2′–6′-H), 7.54 (ddd, J = 8.5, 7.3, 1.3 Hz, 1H, 4-H), 7.92 (dd, J = 7.9, 1.3 Hz, 1H, 6-H), 8.44 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.75 (s, 1H, NH), 13.46 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 37.78 (NCH_3), 55.92 ($\underline{C}H_2Ph$), 120.41 (C-1), 123.34 (C-5), 124.50 (C-3), 127.26 (C-2′/6′), 127.32 (C-4′), 128.66 (C-3′/5′), 130.54 (C-6), 132.42 (C-4), 137.11 (C-1′), 142.55 (C-2), 169.42 (CO_2H), 180.53 (NHCS).

Anal. Calcd for $C_{16}H_{16}N_2O_2S$: C, 63.98; H, 5.37; N, 9.33. Found: C, 63.82; H, 5.53; N, 9.46.

2-[3-Methyl-3-(2-phenylethyl)thioureido]benzoic Acid (**82**)



$M = 314.40$ g/mol

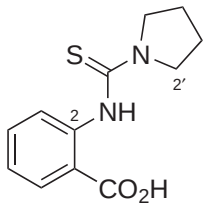
Light yellow solid; yield: 509 mg (81%); mp 130–133 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.97 (t, J = 7.9 Hz, 2H, $CH_2C\underline{H}_2Ph$), 3.25 (s, 3H, NCH_3), 4.02 (t, J = 7.6 Hz, 2H, $C\underline{H}_2CH_2Ph$), 7.12–7.16 (m, 1H, 5-H), 7.19–7.23 (m, 1H, 4-H′), 7.28–7.31 (m, 4H, 2′/3′/5′/6′-H), 7.53 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 4-H), 7.92 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.46 (d, J = 7.9 Hz, 1H, 3-H), 10.71 (s, 1H, NH), 13.52 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 32.69 ($CH_2\underline{C}H_2Ph$), 54.78 ($\underline{C}H_2CH_2Ph$), 119.45 (C-1), 122.91 (C-5), 123.95 (C-3), 126.42 (C-4′), 128.54 (C-2′/3′/5′/6′), 128.91 (C-2′/3′/5′/6′), 130.52 (C-6), 132.45 (C-4), 138.83 (C-1′), 142.69 (C-2), 169.69 (CO_2H), 179.32 (NHCS).

Anal. Calcd for $C_{17}H_{18}N_2O_2S$: C, 64.94; H, 5.77; N, 8.91. Found: C, 64.59; H, 6.10; N, 8.74.

2-[(1-Pyrrolidinylthiocarbonyl)amino]benzoic Acid (**83**)



$M = 250.32$ g/mol

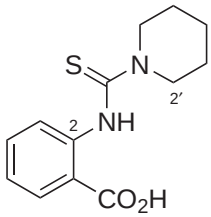
White solid; yield: 425 mg (85%); mp 164–166 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.85–2.18 (m, 4H, 3′/4′-H), 3.55–3.75 (m, 4H, 2′/5′-H), 7.11 (ddd, J = 7.9, 7.3, 1.3 Hz, 1H, 5-H), 7.53 (ddd, J = 8.5, 7.1, 1.6 Hz, 1H, 4-H), 7.93 (dd, J = 7.9, 1.6 Hz, 1H, 6-H), 8.80 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.86 (s, 1H, NH), 13.52 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.25 (C-3′/4′), 25.61 (C-3′/4′), 48.24 (C-2′/5′), 51.94 (C-2′/5′), 118.14 (C-1), 122.49 (C-3/5), 122.59 (C-3/5), 130.67 (C-6), 132.66 (C-4), 142.64 (C-2), 169.87 (CO_2H), 175.83 (NHCS).

Anal. Calcd for $C_{12}H_{14}N_2O_2S$: C, 57.58; H, 5.64; N, 11.19. Found: C, 57.21; H, 5.91; N, 10.93.

2-[(1-Piperidinylthiocarbonyl)amino]benzoic Acid (**84**)



$M = 264.34$ g/mol

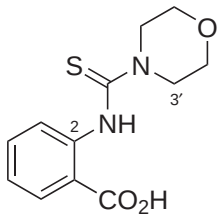
White solid; yield: 444 mg (84%); mp 124–127 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.55–1.69 (m, 6H, 3′–5′-H), 3.89–3.93 (m, 4H, 2′/6′-H), 7.11 (ddd, J = 7.9, 7.3, 1.3 Hz, 1H, 5-H), 7.50 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 4-H), 7.89 (dd, J = 8.0, 1.6 Hz, 1H, 6-H), 8.17 (dd, J = 8.5, 1.0 Hz, 1H, 3-H), 10.60 (s, 1H, NH), 13.44 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.00 (C-4′), 25.52 (C-3′/5′), 49.23 (C-2′/6′), 119.58 (C-1), 122.73 (C-5), 123.89 (C-3), 130.55 (C-6), 132.47 (C-4), 142.85 (C-2), 169.60 (CO_2H), 179.07 (NHCS).

Anal. Calcd for $C_{13}H_{16}N_2O_2S$: C, 59.07; H, 6.10; N, 10.60. Found: C, 58.70; H, 6.14; N, 10.68.

2-[(4-Morpholinylthiocarbonyl)amino]benzoic Acid (**85**)



$M = 266.32$ g/mol

White solid; yield: 399 mg (75%); mp 132–135 °C.

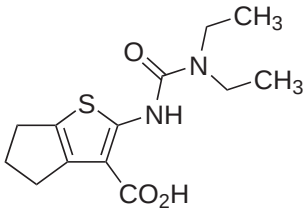
^{1}H NMR (500 MHz, DMSO-d_6) δ 3.67 (t, $J = 4.9$ Hz, 4H, 2′/6′-H), 3.93 (t, $J = 4.9$ Hz, 4H, 3′/5′-H), 7.16 (ddd, $J = 7.9$, 7.0, 1.3 Hz, 1H, 5-H), 7.52 (ddd, $J = 8.4$, 7.3, 1.6 Hz, 1H, 4-H), 7.89 (dd, $J = 7.9$, 1.6 Hz, 1H, 6-H), 8.11 (dd, $J = 8.2$, 1.0 Hz, 1H, 3-H), 10.53 (s, 1H, NH), 13.37 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 48.31 (C-3′/5′), 65.82 (C-2′/6′), 120.95 (C-1), 123.44 (C-3/5), 124.68 (C-3/5), 130.58 (C-6), 132.47 (C-4), 142.31 (C-2), 169.23 (CO_2H), 180.47 (NHCS).

Anal. Calcd for $C_{12}H_{14}N_2O_3S$: C, 54.12; H, 5.30; N, 10.52. Found: C, 54.04; H, 5.47; N, 10.40.

8.8 2-Ureidothiophene-3-carboxylic Acids

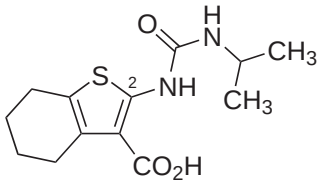
2-(3,3-Diethylureido)-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylic Acid (**86**)



$M = 282.36$ g/mol

Compound was taken from the substance library.

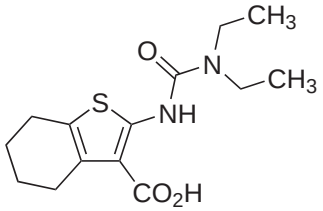
2-(3-Isopropylureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**87**)



$M = 282.36$ g/mol

Compound was taken from the substance library.

2-(3,3-Diethylureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**88**)



$M = 296.39$ g/mol

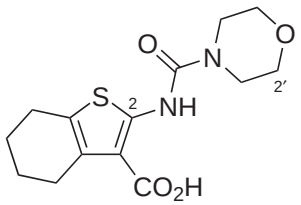
This compound was prepared according to Gütschow *et al.*[129] A mixture of 2-(diethylamino)-5,6,7,8-tetrahydro-4*H*-[1]benzothieno[2,3-d][1,3]oxazin-4-one (100 mg, 0.36 mmol), aqueous NaOH (1.5 M, 10 mL), and acetone (5 mL) was refluxed for 5 min. The reaction mixture was allowed to cool to rt and filtrated into HCl (3 M, 10 mL). The precipitate was collected by suction filtration to obtain **88** as a white solid; 77 mg (72%); mp 168–170 °C (lit.[129] 168–169 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.13 (t, J = 7.1 Hz, 6H, $CH_2C\underline{H}_3$), 1.64–1.73 (m, 4H, 5/6-H), 2.50–2.62 (m, 2H, 4/7-H), 2.64–2.74 (m, 2H, 4/7-H), 3.32 (q, J = 7.0 Hz, 4H, $C\underline{H}_2CH_3$), 11.17 (s, 1H, NH), 12.80 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 13.69 ($CH_2\underline{C}H_3$), 22.51 (C-4–7), 22.80 (C-4–7), 23.79 (C-4–7), 25.99 (C-4–7), 41.25 ($\underline{C}H_2CH_3$), 109.04 (C-3), 123.65 (C-7a), 130.49 (C-3a), 150.96 (C-2), 152.30 (NHCO), 168.26 (CO_2H).

Anal. Calcd for $C_{14}H_{20}N_2O_3S$: C, 56.73; H, 6.80; N, 9.45. Found: C, 56.36; H, 6.92; N, 9.09.

2-[(4-Morpholinylcarbonyl)amino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**89**)



$M = 310.37$ g/mol

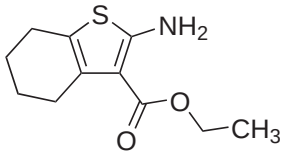
Compound was taken from the substance library.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.65–1.74 (m, 4H, 5/6-H), 2.51–2.62 (m, 2H, 4/7-H), 2.64–2.75 (m, 2H, 4/7-H), 3.38 (t, $J = 4.8$ Hz, 4H, 3′/5′-H), 3.64 (t, $J = 4.8$ Hz, 4H, 2′/6′-H), 11.18 (s, 1H, NH), 12.79 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 22.47 (C-4–7), 22.76 (C-4–7), 23.79 (C-4–7), 26.01 (C-4–7), 43.57 (C-3′/5′), 65.73 (C-2′/6′), 109.63 (C-3), 124.11 (C-7a), 130.68 (C-3a), 150.37 (C-2), 152.69 (NHCO), 168.06 (CO_2H).

8.9 2-Substituted Ethyl Benzo[*b*]thiophene-3-carboxylates

Ethyl 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**90**)



M = 225.31 g/mol

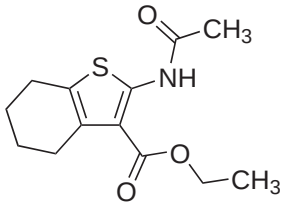
Compound **90** was prepared by a procedure described by Gewald *et al.*[81] Diethylamine (21.3 g, 30.0 mL, 291 mmol) was slowly added to a stirred mixture of cyclohexanone (29.4 g, 31.1 mL, 300 mmol), ethyl cyanoacetate (33.9 g, 32.0 mL, 300 mmol), and sulfur (9.62 g, 300 mmol) in EtOH (100 mL), so that the temperature did not exceed 50 °C. After 1 h the reaction mixture was cooled to 5 °C. The precipitate was removed by suction filtration and recrystallized from EtOH to obtain **90** as colorless needles; yield: 56.5 g (84%); mp 114–115 °C (EtOH; lit.[81] 115 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.23 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.61–1.71 (m, 4H, 5/6-H), 2.37–2.42 (m, 2H, 4/7-H), 2.57–2.60 (m, 2H, 4/7-H), 4.13 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 7.17 (br s, 2H, NH_2).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.48 ($CH_2\underline{C}H_3$), 22.56 (C-4–7), 22.96 (C-4–7), 24.06 (C-4–7), 26.63 (C-4–7), 58.71 ($\underline{C}H_2CH_3$), 102.82 (C-3), 115.60 (C-7a), 131.44 (C-3a), 162.99 (C-2), 165.18 ($\underline{C}O_2CH_2$).

Anal. Calcd for $C_{11}H_{15}NO_2S$: C, 58.64; H, 6.71; N, 6.22. Found: C, 58.42; H, 6.77; N, 6.20.

Ethyl 2-(Acetylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**91**)



M = 267.34 g/mol

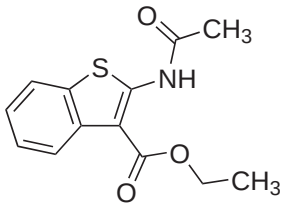
Compounds **91**–**93** were synthesized according to ref.[134] Ethyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**90**; 113 g, 500 mmol) was refluxed in Ac_2O (250 mL) for 10 min (*caution: boiling delay*). After cooling to 5 °C, the precipitate was removed by suction filtration and washed with hot water (100 mL) to recover **91** as white needles; yield: 117 g (88%); mp 120–121 °C (lit.[134] 123 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 1.67–1.74 (m, 4H, 5/6-H), 2.20 (s, 3H, $COC\underline{H}_3$), 2.54–2.58 (m, 2H, 4/7-H), 2.66–2.70 (m, 2H, 4/7-H), 4.27 (q, J = 7.1 Hz, 2H, $C\underline{H}_2CH_3$), 10.92 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.20 ($CH_2\underline{C}H_3$), 22.45 (C-4–7/$CO\underline{C}H_3$), 22.61 (C-4–7/$CO\underline{C}H_3$), 23.36 (C-4–7/$CO\underline{C}H_3$), 23.81 (C-4–7/$CO\underline{C}H_3$), 25.93 (C-4–7/$CO\underline{C}H_3$), 60.38 ($\underline{C}H_2CH_3$), 111.17 (C-3), 125.99 (C-7a), 130.37 (C-3a), 146.31 (C-2), 165.12 ($\underline{C}O_2CH_2$), 167.26 ($\underline{C}OCH_3$).

Anal. Calcd for $C_{13}H_{17}NO_3S$: C, 58.40; H, 6.41; N, 5.24. Found: C, 58.65; H, 6.33; N, 5.13.

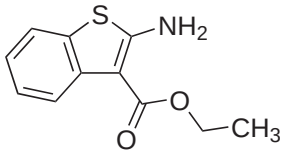
Ethyl 2-(Acetylamino)benzo[*b*]thiophene-3-carboxylate (**92**)



M = 263.31 g/mol

A mixture of ethyl 2-(acetylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**91**; 107 g, 400 mmol), sulfur (25.7 g, 800 mmol), and dimethyl phthalate (150 mL) was heated to 220 ℃. After 6 h, the reaction mixture was poured into EtOH (100 mL) and kept at 5 ℃. The precipitate was removed by suction filtration and washed with EtOH (300 mL, −15 ℃) to give **92** as a light yellow solid; yield (crude product): 85 g (81%). The material was used without further purification.

Ethyl 2-Aminobenzo[*b*]thiophene-3-carboxylate (**93**)



M = 221.28 g/mol

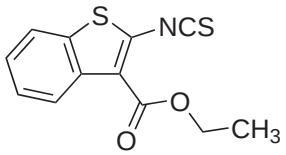
A solution of KOH (2.40 g, 42.7 mmol) in H_2O (6.0 mL) was added dropwise to a refluxing mixture of ethyl 2-(acetylamino)benzo[*b*]thiophene-3-carboxylate (**92**; 10.5 g, 40.0 mmol) in EtOH (40 mL). After complete addition of the base, the resulting solution was poured into ice water (300 mL). The precipitate was removed and recrystallized from PhMe to obtain **93** as a yellow solid; yield: 6.10 g (69%); mp 106–107 ℃ (PhMe; lit.[134] 107–108 ℃).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 4.30 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 7.03–7.07 (m, 1H, 5/6-H), 7.22–7.26 (m, 1H, 5/6-H), 7.59 (dt, J = 7.9, 1.0 Hz, 1H, 4/7-H), 7.95 (br s, 2H, NH_2), 7.96–7.99 (m, 1H, 4/7-H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.61 ($CH_2\underline{C}H_3$), 59.21 ($\underline{C}H_2CH_3$), 96.57 (C-3), 121.37 (C-4–7), 121.60 (C-4–7), 121.75 (C-4–7), 125.28 (C-4–7), 128.52 (C-3a/7a), 137.52 (C-3a/7a), 165.16 (C-2/$\underline{C}O_2CH_2$), 165.58 (C-2/$\underline{C}O_2CH_2$).

Anal. Calcd for $C_{11}H_{11}NO_2S$: C, 59.71; H, 5.01; N, 6.33. Found: C, 59.15; H, 5.11; N, 6.17.

Ethyl 2-Isothiocyanatobenzo[*b*]thiophene-3-carboxylate (**94**)



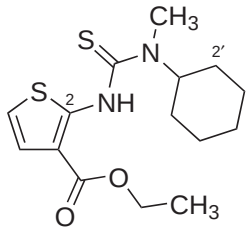
M = 263.34 g/mol

Compound **94** was prepared by a method according to Gütschow *et al.*[131] A mixture prepared from thiophosgene (5.75 g, 3.81 mL, 50.0 mmol), $CaCO_3$ (5.00 g, 50.0 mmol), CH_2Cl_2 (25 mL), and H_2O (50 mL) was stirred at 0 °C. A solution of ethyl 2-aminobenzo[*b*]thiophene-3-carboxylate (**93**; 11.1 g, 50.0 mmol) in CH_2Cl_2 (90 mL) was added dropwise over a period of 45 min. The mixture was stirred overnight at rt. The organic layer was washed with H_2O (2 × 30 mL) and dried over Na_2SO_4. After removal of the solvent, the crude product was purified by column chromatography (petroleum ether–EtOAc, 4:1) to give **94** as a yellowish solid; yield: 5.52 g (42%); mp 72–73 °C (lit.[131] 70–71 °C).

Anal. Calcd for $C_{12}H_9NO_2S_2$: C, 54.73; H, 3.44; N, 5.32. Found: C, 54.52; H, 3.53; N, 5.33.

8.10 Ethyl 2-Thioureidothiophene-3-carboxylates

Ethyl 2-(3-Cyclohexyl-3-methylthioureido)thiophene-3-carboxylate (**95**)



M = 326.48 g/mol

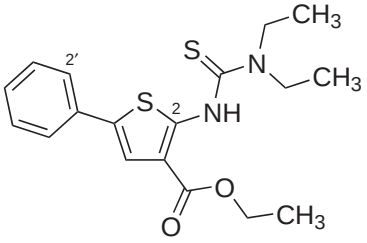
N-Methylcyclohexylamine (736 mg, 856 µL, 6.50 mmol) was added dropwise to a stirring solution of ethyl 2-(isothiocyanato)thiophene-3-carboxylate[131] (1.07 g, 5.00 mmol) in CH_2Cl_2 (20 mL). The reaction mixture was stirred at rt for 3 h. The organic layer was washed with HCl (0.5 M, 2 × 5 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. Recrystallization from EtOH gave **95** as orange needles; yield: 1.26 g (77%); mp 143–145 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.11–1.82 (m, 13H, $CH_2C\underline{H}_3$/2′–6′-H), 3.16 (s, 3H, NCH_3), 4.32 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 4.49 (br s, 1H, 1′-H), 6.83 (d, J = 5.7 Hz, 1H, 4/5-H), 7.18 (d, J = 5.7 Hz, 1H, 4/5-H), 11.70 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.30 ($CH_2\underline{C}H_3$), 24.92 (C-4′), 25.38 (C-3′/5′), 29.22 (C-2′/6′), 31.75 (NCH_3), 59.32 (C-1′), 60.80 ($\underline{C}H_2CH_3$), 112.46 (C-3), 115.90 (C-4/5), 123.12 (C-4/5), 152.85 (C-2), 165.91 ($\underline{C}O_2CH_2$), 175.17 (NHCS).

Anal. Calcd for $C_{15}H_{22}N_2O_2S_2$: C, 55.18; H, 6.79; N, 8.58. Found: C, 54.82; H, 6.71; N, 8.55.

Ethyl 2-(3,3-Diethylthioureido)-5-phenylthiophene-3-carboxylate (**96**)



M = 362.51 g/mol

Diethylamine (219 mg, 308 µL, 3.00 mmol) was added dropwise to a stirring solution of ethyl 2-isothiocyanato-5-phenylthiophene-3-carboxylate[244] (578 mg, 2.00 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was stirred at rt for 2 h. The organic layer was washed with HCl (0.5 M, 2 × 5 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. Recrystallization from EtOH gave **96** as white needles; yield: 560 mg (77%); mp 122–123 °C (EtOH).

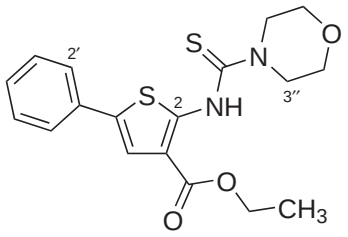
^{1}H NMR (500 MHz, DMSO-d_6) δ 1.25 (t, J = 7.1 Hz, 6H, $CH_2C\underline{H}_3$), 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.74–3.82 (m, 4H, $C\underline{H}_2CH_3$), 4.35 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 7.26–7.30

(m, 1H, 4′-H), 7.37–7.41 (m, 2H, 3′/5′-H), 7.51 (s, 1H, 4-H), 7.60–7.64 (m, 2H, 2′/6′-H), 11.78 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.28 ($CH_2\underline{C}H_3$), 14.30 ($CH_2\underline{C}H_3$), 45.77 ($\underline{C}H_2CH_3$), 61.01 ($\underline{C}H_2CH_3$), 113.17 (C-3), 118.86 (C-4), 125.18 (C-2′/6′), 127.44 (C-4′), 129.25 (C-3′/5′), 131.11 (C-5/1′), 133.46 (C-5/1′), 152.14 (C-2), 165.90 ($\underline{C}O_2CH_2$), 174.12 (NHCS).

Anal. Calcd for $C_{18}H_{22}N_2O_2S_2$: C, 59.64; H, 6.12; N, 7.73. Found: C, 58.95; H, 6.08; N, 7.50.

Ethyl 2-[(4-Morpholinylthiocarbonyl)amino]-5-phenylthiophene-3-carboxylate (**97**)



M = 376.49 g/mol

Morpholine (261 mg, 264 µL, 3.00 mmol) was added dropwise to a stirring solution of ethyl 2-isothiocyanato-5-phenylthiophene-3-carboxylate[244] (578 mg, 2.00 mmol) in CH_2Cl_2 (25 mL). The reaction mixture was stirred at rt for 2 h. The organic layer was washed with HCl (0.5 M, 2 × 5 mL), dried over Na_2SO_4, filtered, and evaporated to dryness. Recrystallization from EtOAc gave **97** as yellow needles; yield: 708 mg (94%); mp 186–188 °C (EtOAc).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.34 (t, J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$), 3.73 (t, J = 4.9 Hz, 4H, 3″/5″-H), 3.92 (t, J = 4.9 Hz, 4H, 2″/6″-H), 4.35 (q, J = 7.0 Hz, 2H, $C\underline{H}_2CH_3$), 7.27–7.30 (m, 1H, 4′-H), 7.38–7.42 (m, 2H, 3′/5′-H), 7.54 (s, 1H, 4-H), 7.62–7.65 (m, 2H, 2′/6′-H), 11.82 (s, 1H, NH).

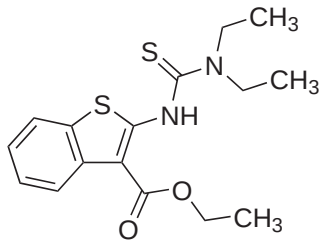
^{13}C NMR (125 MHz, DMSO-d_6) δ 14.32 ($CH_2\underline{C}H_3$), 47.89 (C-3″/5″), 61.10 ($\underline{C}H_2CH_3$), 65.45 (C-2″/6″), 113.80 (C-3), 119.07 (C-4), 125.21 (C-2′/6′), 127.56 (C-4′), 129.28 (C-3′/5′), 131.51 (C-5/1′), 133.23 (C-5/1′), 151.65 (C-2), 165.70 ($\underline{C}O_2CH_2$), 175.63 (NHCS).

Anal. Calcd for $C_{18}H_{20}N_2O_3S_2$: C, 57.42; H, 5.35; N, 7.44. Found: C, 56.94; H, 5.55; N, 7.38.

Ethyl 2-Thioureidobenzo[*b*]thiophene-3-carboxylates **98–100**; General Procedure[131]

The appropriate secondary amine (7.50 mmol) was added dropwise to a solution of ethyl 2-(isothiocyanato)benzo[*b*]thiophene-3-carboxylate (**94**; 1.30 g, 5.00 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was allowed to warm to ambient temperature, stirred for further 2 h, and acidified with 1M HCl/EtOH. The precipitate was collected by suction filtration and recrystallized from EtOH.

Ethyl 2-(3,3-Diethylthioureido)benzo[*b*]thiophene-3-carboxylate (**98**)



$M = 336.47$ g/mol

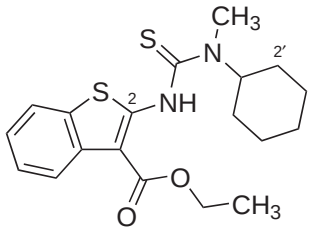
White needles; yield: 1.05 g (62%); mp 136–138 °C (EtOH; lit.[131] 137–138 °C).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.26 (t, $J = 6.8$ Hz, 6H, $CH_2C\underline{H}_3$), 1.42 (t, $J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$), 3.79–3.86 (m, 4H, $C\underline{H}_2CH_3$), 4.46 (q, $J = 7.1$ Hz, 2H, $C\underline{H}_2CH_3$), 7.29 (ddd, $J = 8.0$, 7.0, 1.1 Hz, 1H, 5/6-H), 7.41 (ddd, $J = 8.2$, 7.3, 1.3 Hz, 1H, 5/6-H), 7.88 (d, $J = 7.9$ Hz, 1H, 4/7-H), 8.20 (dt, $J = 8.2$, 1.0 Hz, 1H, 4/7-H), 12.56 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.25 ($CH_2\underline{C}H_3$), 14.28 ($CH_2\underline{C}H_3$), 46.06 ($\underline{C}H_2CH_3$), 61.27 ($\underline{C}H_2CH_3$), 105.21 (C-3), 122.00 (C-4–7), 122.49 (C-4–7), 123.80 (C-4–7), 125.72 (C-4–7), 132.70 (C-3a/7a), 133.18 (C-3a/7a), 156.96 (C-2), 167.18 ($\underline{C}O_2CH_2$), 174.77 (NHCS).

Anal. Calcd for $C_{16}H_{20}N_2O_2S_2$: C, 57.11; H, 5.99; N, 8.33. Found: C, 57.00; H, 6.10; N, 8.10.

Ethyl 2-(3-Cyclohexyl-3-methylthioureido)benzo[*b*]thiophene-3-carboxylate (**99**)



$M = 376.54$ g/mol

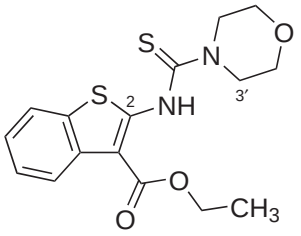
Yellowish plates; yield: 1.30 g (69%); mp 177–178 °C (EtOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.10–1.87 (m, 13H, $CH_2C\underline{H}_3$/2′–6′-H), 3.05 (s, 3H, NCH_3), 4.47 (q, $J = 7.2$ Hz, 2H, $C\underline{H}_2CH_3$), 5.17 (br s, 1H, 1′-H), 7.25 (ddd, $J = 8.2$, 7.1, 1.3 Hz, 1H, 5/6-H), 7.36 (ddd, $J = 8.2$, 7.1, 1.3 Hz, 1H, 5/6-H), 7.70 (ddd, $J = 7.9$, 1.3, 1.0 Hz, 1H, 4/7-H), 8.23 (ddd, $J = 8.2$, 1.3, 1.0 Hz, 1H, 4/7-H), 12.74 (s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 14.38 ($CH_2\underline{C}H_3$), 25.41 (C-4′), 25.48 (C-3′/5′), 29.93 (C-2′/6′), 59.71 (C-1′), 61.01 ($\underline{C}H_2CH_3$), 105.89 (C-3), 121.57 (C-4–7), 122.70 (C-4–7), 123.49 (C-4–7), 125.26 (C-4–7), 133.40 (C-3a/7a), 133.61 (C-3a/7a), 157.90 (C-2), 167.99 ($\underline{C}O_2CH_2$), 176.61 (NHCS).

Anal. Calcd for $C_{19}H_{24}N_2O_2S_2$: C, 60.61; H, 6.42; N, 7.44. Found: C, 60.30; H, 6.67; N, 7.40.

Ethyl 2-[(4-Morpholinylthiocarbonyl)amino]benzo[*b*]thiophene-3-carboxylate (**100**)



$M = 350.46$ g/mol

Yellow needles; yield: 1.14 g (65%); mp 175–176 °C (EtOH).

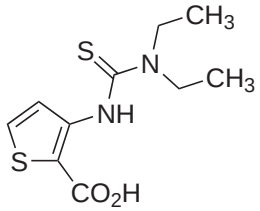
^{1}H NMR (500 MHz, DMSO-d_6) δ 1.42 (t, $J = 7.1$ Hz, 3H, $CH_2C\underline{H}_3$), 3.75 (t, $J = 4.9$ Hz, 4H, 3′/5′-H), 3.96 (t, $J = 4.9$ Hz, 4H, 2′/6′-H), 4.45 (q, $J = 7.0$ Hz, 2H, $C\underline{H}_2CH_3$), 7.31 (ddd, $J = 8.0, 7.1, 1.0$ Hz, 1H, 5/6-H), 7.42 (ddd, $J = 8.2, 7.3, 1.0$ Hz, 1H, 5/6-H), 7.90 (d, $J = 7.9$ Hz, 1H, 4/7-H), 8.20 (d, $J = 8.2$ Hz, 1H, 4/7-H), 12.58 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 14.30 ($CH_2\underline{C}H_3$) 48.10 (C-3′/5′), 61.37 ($\underline{C}H_2CH_3$), 65.45 (C-2′/6′), 105.70 (C-3), 122.05 (C-4–7), 122.58 (C-4–7), 123.96 (C-4–7), 125.79 (C-4–7), 132.72 (C-3a/7a), 133.23 (C-3a/7a), 156.59 (C-2), 166.99 ($\underline{C}O_2CH_2$), 176.17 (NHCS).

Anal. Calcd for $C_{16}H_{18}N_2O_3S_2$: C, 54.83; H, 5.18; N, 7.99. Found: C, 55.01; H, 5.26; N, 7.96.

8.11 *o*-Thioureidothiophenecarboxylic Acids

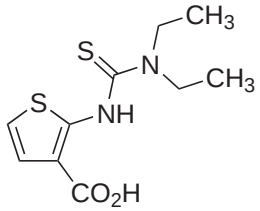
3-(3,3-Diethylthioureido)thiophene-2-carboxylic Acid (**101**)[131]



$M = 258.36$ g/mol

Compound was taken from the substance library.

2-(3,3-Diethylthioureido)thiophene-3-carboxylic Acid (**102**)[131]



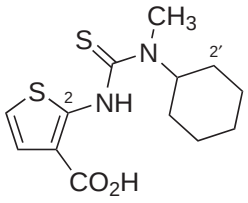
$M = 258.36$ g/mol

Compound was taken from the substance library.

2-Thioureidothiophene-3-carboxylic Acids **103** and **105–107**; General Procedure

A mixture of the appropriate ethyl 2-thioureidothiophene-3-carboxylate (2.00 mmol), NaOH (1 M, 10 mL), and EtOH (10 mL) was refluxed for 1 h. The reaction was allowed to cool to rt, and H_2O (30 mL) was added. The mixture was filtered, cooled to 0 °C, and acidified with 2 M HCl. The precipitate was removed by suction filtration and washed with H_2O (50 mL).

2-(3-Cyclohexyl-3-methylthioureido)thiophene-3-carboxylic Acid (**103**)



$M = 298.42$ g/mol

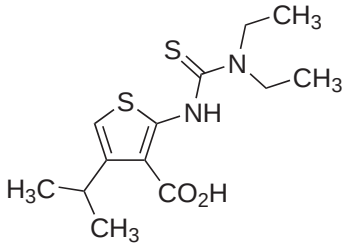
Saponification of ethyl 2-(3-cyclohexyl-3-methylthioureido)thiophene-3-carboxylate (**95**) according to the aforementioned procedure gave **103** as a white solid; yield: 480 mg (78%); mp 174–175 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.06–1.87 (m, 10H, 2′–6′-H), 3.15 (s, 3H, NCH_3), 4.83 (br s, 1H, 1′-H), 6.79 (d, J = 5.7 Hz, 1H, 4/5-H), 7.15 (d, J = 6.0 Hz, 1H, 4/5-H), 12.08 (s, 1H, NH), 13.20 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.91 (C-4′), 25.40 (C-3′/5′), 29.32 (C-2′/6′), 32.29 (NCH_3), 59.18 (C-1′), 113.21 (C-3), 115.40 (C-4/5), 123.69 (C-4/5), 152.73 (C-2), 167.88 (CO_2H), 175.20 (NHCS).

Anal. Calcd for $C_{13}H_{18}N_2O_2S_2$: C, 52.32; H, 6.08; N, 9.39. Found: C, 51.95; H, 6.29; N, 9.28.

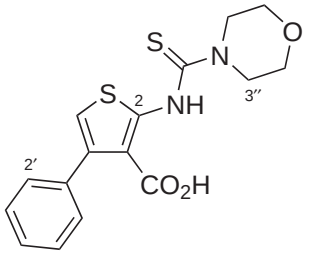
2-(3,3-Diethylthioureido)-4-isopropylthiophene-3-carboxylic Acid (**104**)[131]



M = 300.44 g/mol

Compound was taken from the substance library.

2-[(4-Morpholinylthiocarbonyl)amino]-4-phenylthiophene-3-carboxylic Acid (**105**)



M = 348.44 g/mol

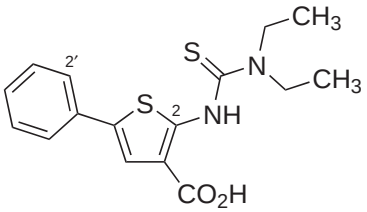
Saponification of ethyl 2-[(4-morpholinylthiocarbonyl)amino]-4-phenylthiophene-3-carboxylate[166] gave **105** as a white solid; yield: 585 mg (84%); mp 142–144 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.71 (t, J = 4.9 Hz, 4H, 3″/5″-H), 3.91 (t, J = 4.7 Hz, 4H, 2″/6″-H), 6.70 (s, 1H, 5-H), 7.26–7.34 (m, 5H, 2′–6′-H), 12.55 (s, 1H, NH), 13.05 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 47.89 (C-3″/5″), 65.53 (C-2″/6″), 112.27 (C-3), 114.79 (C-5), 126.88 (C-4′), 127.49 (C-2′/6′), 129.06 (C-3′/5′), 137.76 (C-4/1′), 139.39 (C-4/1′), 153.73 (C-2), 168.23 (CO_2H), 176.11 (NHCS).

Anal. Calcd for $C_{16}H_{16}N_2O_3S_2$: C, 55.15; H, 4.63; N, 8.04. Found: C, 55.13; H, 4.91; N, 8.08.

2-(3,3-Diethylthioureido)-5-phenylthiophene-3-carboxylic Acid (**106**)



$M = 334.46$ g/mol

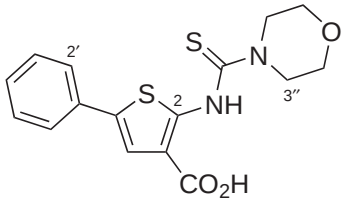
Saponification of ethyl 2-(3,3-diethylthioureido)-5-phenylthiophene-3-carboxylate (**96**) gave **106** as a white solid; yield: 600 mg (90%); mp 181–182 °C. Material for X-ray crystallography was recrystallized from MeOH.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.24 (t, $J = 7.1$ Hz, 6H, $CH_2C\underline{H}_3$), 3.74–3.83 (m, 4H, $C\underline{H}_2CH_3$), 7.24–7.28 (m, 1H, 4′-H), 7.36–7.40 (m, 2H, 3′/5′-H), 7.50 (s, 1H, 4-H), 7.59–7.64 (m, 2H, 2′/6′-H), 12.24 (s, 1H, NH), 13.47 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.30 ($CH_2\underline{C}H_3$), 45.52 ($\underline{C}H_2CH_3$), 114.06 (C-3), 119.50 (C-4), 125.10 (C-2′/6′), 127.28 (C-4′), 129.23 (C-3′/5′), 130.68 (C-5), 133.68 (C-1′), 152.12 (C-2), 167.91 (CO_2H), 174.15 (NHCS).

Anal. Calcd for $C_{16}H_{18}N_2O_2S_2$: C, 57.46; H, 5.42; N, 8.38. Found: C, 57.44; H, 5.47; N, 8.45.

2-[(4-Morpholinylthiocarbonyl)amino]-5-phenylthiophene-3-carboxylic Acid (**107**)



$M = 348.44$ g/mol

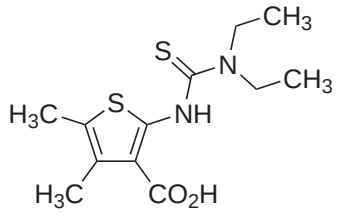
Saponification of ethyl 2-[(4-morpholinylthiocarbonyl)amino]-5-phenylthiophene-3-carboxylate (**97**) gave **107** as a white solid; yield: 511 mg (73%); mp 199–200 °C.

^{1}H NMR (500 MHz, DMSO-d_6) δ 3.72 (t, $J = 4.9$ Hz, 4H, 3″/5″-H), 3.90 (t, $J = 4.9$ Hz, 4H, 2″/6″-H), 7.22–7.30 (m, 1H, 4′-H), 7.35–7.41 (m, 2H, 3′/5′-H), 7.52 (s, 1H, 4-H), 7.60–7.64 (m, 2H, 2′/6′-H), 12.25 (s, 1H, NH), 13.42 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 47.88 (C-3″/5″), 65.49 (C-2″/6″), 114.61 (C-3), 119.69 (C-4), 125.14 (C-2′/6′), 127.39 (C-4′), 129.26 (C-3′/5′), 131.08 (C-5), 133.55 (C-1′), 151.63 (C-2), 167.64 (CO_2H), 175.63 (NHCS).

Anal. Calcd for $C_{16}H_{16}N_2O_3S_2$: C, 55.15; H, 4.63; N, 8.04. Found: C, 54.69; H, 4.94; N, 7.83.

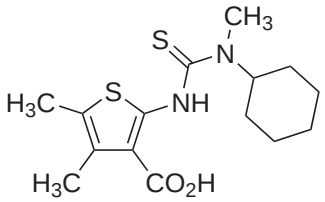
2-(3,3-Diethylthioureido)-4,5-dimethylthiophene-3-carboxylic Acid (**108**)[129]



$M = 286.41$ g/mol

Compound was taken from the substance library.

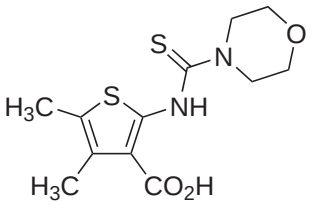
2-(3-Cyclohexyl-3-methylthioureido)-4,5-dimethylthiophene-3-carboxylic Acid (**109**)[129]



$M = 326.48$ g/mol

Compound was taken from the substance library.

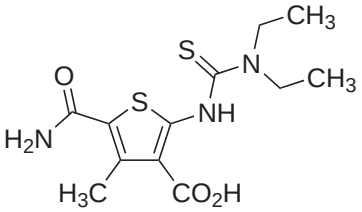
4,5-Dimethyl-2-[(4-morpholinylthiocarbonyl)amino]thiophene-3-carboxylic Acid (**110**)[129]



$M = 300.40$ g/mol

Compound was taken from the substance library.

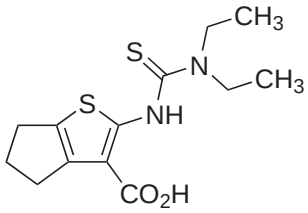
5-Carbamoyl-2-(3,3-diethylthioureido)-4-methylthiophene-3-carboxylic Acid (**111**)[131]



$M = 315.41$ g/mol

Compound was taken from the substance library.

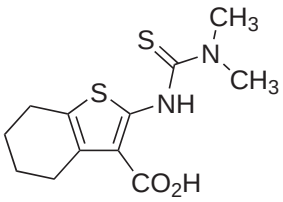
2-(3,3-Diethylthioureido)-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carboxylic Acid (**112**)[131]



$M = 298.42$ g/mol

Compound was taken from the substance library.

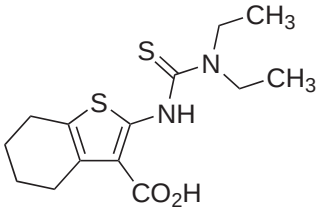
2-(3,3-Dimethylthioureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**113**)[166]



$M = 284.40$ g/mol

Compound was taken from the substance library.

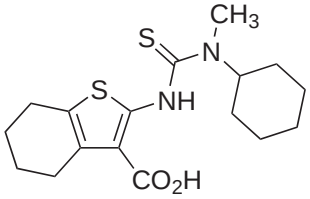
2-(3,3-Diethylthioureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**114**)[129]



$M = 312.45$ g/mol

Compound was taken from the substance library.

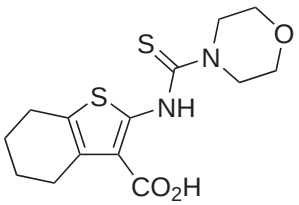
2-(3-Cyclohexyl-3-methylthioureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**115**)[129]



$M = 352.51$ g/mol

Compound was taken from the substance library.

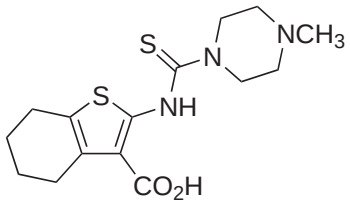
2-[(4-Morpholinylthiocarbonyl)amino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**116**)[129]



$M = 326.43$ g/mol

Compound was taken from the substance library.

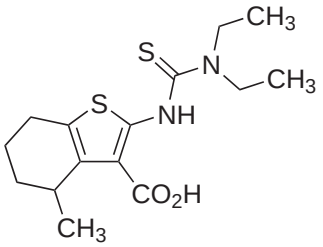
2-{[(4-Methyl-1-piperazinyl)thiocarbonyl]amino}-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**117**)[166]



$M = 339.48$ g/mol

Compound was taken from the substance library.

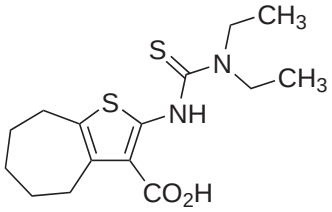
(*R*,*S*)-2-(3,3-Diethylthioureido)-4-methyl-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**118**)[131]



$M = 326.48$ g/mol

Compound was taken from the substance library.

2-(3,3-Diethylthioureido)-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylic Acid (**119**)[131]



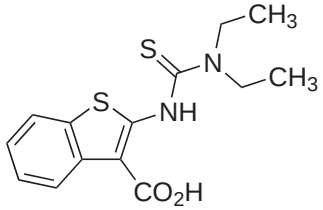
$M = 326.48$ g/mol

Compound was taken from the substance library.

2-Thioureidobenzo[*b*]thiophene-3-carboxylic Acids **120–122**; General Procedure

A mixture of the appropriate ethyl 2-thioureidobenzo[*b*]thiophene-3-carboxylate (**98**–**100**, 2.00 mmol), NaOH (1 M, 10 mL), and EtOH (10 mL) was refluxed for 1 h. The reaction was allowed to cool to rt, and H_2O (30 mL) was added. The solution was cooled to 0 ℃ and acidified with 2 M HCl. The precipitate was removed by suction filtration, washed with H_2O (35 mL), and recrystallized from EtOH.

2-(3,3-Diethylthioureido)benzo[*b*]thiophene-3-carboxylic Acid (**120**)



$M = 308.42$ g/mol

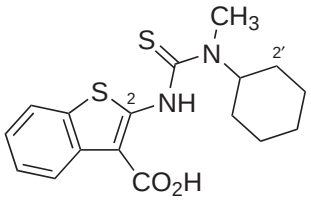
Yellowish needles; yield: 250 mg (41%); mp 153–155 ℃ (EtOH; lit.[131] 142–144 ℃).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.26 (t, J = 6.8 Hz, 6H, $CH_2C\underline{H}_3$), 3.70–3.90 (m, 4H, $C\underline{H}_2CH_3$), 7.27 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 5/6-H), 7.38 (ddd, J = 8.4, 7.2, 1.3 Hz, 1H, 5/6-H), 7.86 (ddd, J = 7.9, 1.3, 0.6 Hz, 1H, 4/7-H), 8.23 (dt, J = 8.2, 1.0 Hz, 1H), 13.02 (s, 1H, NH), 13.78 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.28 ($CH_2\underline{C}H_3$), 46.13 ($\underline{C}H_2CH_3$), 105.85 (C-3), 121.88 (C-4–7), 122.54 (C-4–7), 123.58 (C-4–7), 125.53 (C-4–7), 132.67 (C-3a/7a), 133.84 (C-3a/7a), 156.85 (C-2), 169.09 (CO_2H), 174.86 (NHCS).

Anal. Calcd for $C_{14}H_{16}N_2O_2S_2$: C, 54.52; H, 5.23; N, 9.08. Found: C, 54.14; H, 5.47; N, 8.93.

2-(3-Cyclohexyl-3-methylthioureido)benzo[*b*]thiophene-3-carboxylic (**121**)



$M = 348.48$ g/mol

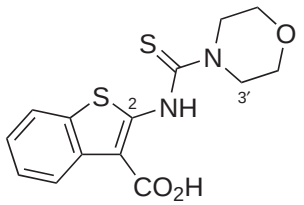
Yellow needles; yield: 563 mg (81%); mp 167–170 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.14–1.82 (m, 10H, 2′–6′-H), 3.21 (s, 3H, NCH_3), 5.03 (br s, 1H, 1′-H), 7.27 (ddd, J = 8.0, 6.9, 1.1 Hz, 1H, 5/6-H), 7.38 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H, 5/6-H), 7.86 (d, J = 7.9 Hz, 1H, 4/7-H), 8.24 (d, J = 8.2 Hz, 1H, 4/7-H), 12.98 (s, 1H, NH), 13.71 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.90 (C-4′), 25.38 (C-3′/5′), 29.24 (C-2′/6′), 31.81 (NCH_3), 59.49 (C-1′), 106.08 (C-3), 121.86 (C-4–7), 122.58 (C-4–7), 123.59 (C-4–7), 125.49 (C-4–7), 132.73 (C-3a/7a), 133.89 (C-3a/7a), 156.83 (C-2), 168.93 (CO_2H), 175.65 (NHCS).

Anal. Calcd for $C_{17}H_{20}N_2O_2S_2$: C, 58.59; H, 5.78; N, 8.04. Found: C, 58.20; H, 5.63; N, 7.66.

2-[(4-Morpholinylthiocarbonyl)amino]benzo[*b*]thiophene-3-carboxylic Acid (**122**)



$M = 322.40$ g/mol

Orange needles; yield: 418 mg (65%); mp 136–139 °C (EtOH).

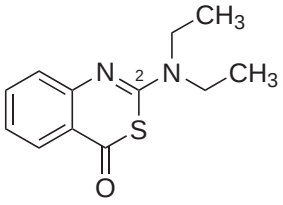
^{1}H NMR (500 MHz, DMSO-d_6) δ 3.74 (t, J = 4.9 Hz, 4H, 3′/5′-H), 3.96 (t, J = 4.7 Hz, 4H, 2′/6′-H), 7.28 (ddd, J = 8.0, 7.1, 1.0 Hz, 1H, 5/6-H), 7.39 (ddd, J = 8.2, 7.1, 1.1 Hz, 1H, 5/6-H), 7.87 (d, J = 7.6 Hz, 1H, 4/7-H), 8.24 (d, J = 8.2 Hz, 1H, 4/7-H), 12.05 (s, 1H, NH), 13.68 (br s, 1H, CO_2H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 48.11 (C-3′/5′), 65.49 (C-2′/6′), 106.37 (C-3), 121.93 (C-4–7), 122.67 (C-4–7), 123.76 (C-4–7), 125.60 (C-4–7), 132.70 (C-3a/7a), 133.87 (C-3a/7a), 156.45 (C-2), 168.84 (CO_2H), 176.22 (NHCS).

Anal. Calcd for $C_{14}H_{14}N_2O_3S_2$: C, 52.16; H, 4.38; N, 8.69. Found: C, 52.41; H, 4.43; N, 8.33.

8.12 4*H*-3,1-Benzothiazin-4-ones

2-(Diethylamino)-4*H*-3,1-benzothiazin-4-one (**123**)



M = 234.30 g/mol

Method 1: Methyl 2-(3,3-diethylthioureido)benzoate (**70**; 0.799 g, 3.00 mmol) was kept in concd H_2SO_4 (12 mL) at rt for 24 h. The solution was poured into a mixture of ice–water (100 mL) and EtOAc (100 mL). After neutralization, the aqueous layer was further extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated to dryness. Recrystallisation from MeOH yielded **123** (0.505 g, 72%) as colorless needles, mp 74–75 °C (MeOH; lit.[189] 72–74 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.24 (t, J = 7.3 Hz, 6H, $CH_2C\underline{H}_3$), 3.59 (q, J = 7.3 Hz, 4H, $C\underline{H}_2CH_3$), 7.10 (ddd, J = 8.2, 7.6, 1.3 Hz, 1H, 6-H), 7.37 (dd, J = 7.9, 1.3 Hz, 1H, 8-H), 7.55 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H, 7-H), 8.00 (dd, J = 8.0, 1.6 Hz, 1H, 5-H).

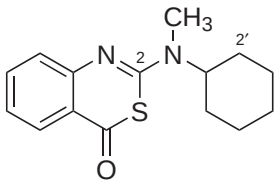
^{13}C NMR (125 MHz, $CDCl_3$) δ 13.03 ($CH_2\underline{C}H_3$), 43.35 ($\underline{C}H_2CH_3$), 116.29 (C-4a), 122.93 (C-6), 124.71 (C-5), 128.21 (C-8), 135.61 (C-7), 151.50 (C-8a), 155.43 (C-2), 184.52 (C-4).

Anal. Calcd for $C_{12}H_{14}N_2OS$: C, 61.51; H, 6.02; N, 11.96. Found: C, 61.50; H, 5.99; N, 11.96.

Method 2: 2-(3,3-Diethylthioureido)-*N*,*N*-diethylbenzamide (**133**; 0.615 g, 2.00 mmol) was treated with concd H_2SO_4 (8 mL) as described under Method 1 obtaining **123** (0.449 g, 96%) as a white solid.

Method 3: 2-(3,3-Diethylthioureido)benzoic acid (**78**; 0.450 g, 1.78 mmol) and TFAA (4.5 mL) were kept at rt for 12 h. After removal of the solvent, the resulting crude material was purified by column chromatography on silica gel using petroleum ether–EtOAc (2:1) as eluent to give **123** (0.107 g, 26%) as a white solid.

2-(*N*-Cyclohexyl-*N*-methylamino)-4*H*-3,1-benzothiazin-4-one (**124**)



M = 274.38 g/mol

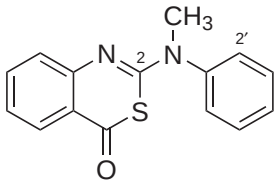
According to the preparation of **123** (Method 1), **124** (0.607 g, 74%) was obtained from methyl 2-(3-cyclohexyl-3-methylthioureido)benzoate (**71**) as colorless plates, mp 111–114 °C (EtOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.05–1.88 (m, 10H, 2′/3′/4′/5′/6′-H), 3.05 (s, 3H, NCH_3), 4.22 (br s, 1H, 1′-H), 7.10 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 6-H), 7.38 (dd, J = 8.2, 1.3 Hz, 1H, 8-H), 7.56 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H, 7-H), 8.00 (dd, J = 8.2, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 25.40 (C-4′), 25.73 (C-3′/5′), 30.14 (C-2′/6′), 30.45 (NCH_3), 56.82 (C-1′), 116.50 (C-4a), 123.01 (C-6), 124.75 (C-5), 128.18 (C-8), 135.64 (C-7), 151.36 (C-8a), 156.68 (C-2), 184.48 (C-4).

Anal. Calcd for $C_{15}H_{18}N_2OS$: C, 65.66; H, 6.61; N, 10.21. Found: C, 65.41; H, 6.63; N, 10.11.

2-(*N*-Methyl-*N*-phenylamino)-4*H*-3,1-benzothiazin-4-one (**125**)



M = 268.33 g/mol

Method 1: According to the preparation of **123** (Method 1), **125** (0.160 g, 20%) was obtained from methyl 2-(3-methyl-3-phenylthioureido)benzoate (**72**) as colorless needles, mp 78–79 °C (EtOH).

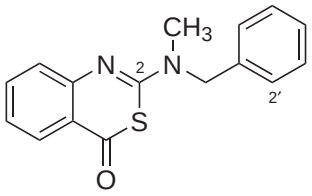
^{1}H NMR (500 MHz, $CDCl_3$) δ 3.59 (s, 3H, NCH_3), 7.18 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 6-H), 7.25–7.29 (m, 2H, 2′/6′-H), 7.37–7.42 (m, 1H, 4′-H), 7.42–7.47 (m, 2H, 3′/5′-H), 7.51 (dd, J = 8.2, 1.0 Hz, 1H, 8-H), 7.62 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H, 7-H), 8.02 (dd, J = 8.0, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 39.93 (NCH_3), 117.07 (C-4a), 123.93 (C-6), 125.00 (C-5), 128.26 (C-2′/6′), 128.29 (C-8), 128.76 (C-4′), 130.16 (C-3′/5′), 135.72 (C-7), 142.20 (C-1′), 150.50 (C-8a), 156.97 (C-2), 184.20 (C-4).

Anal. Calcd for $C_{15}H_{12}N_2OS$: C, 67.14; H, 4.51; N, 10.44. Found: C, 67.02; H, 4.61; N, 10.40.

Method 2: 2-(3-Methyl-3-phenylthioureido)benzoic acid (**80**; 0.859 g, 3.00 mmol) and Ac_2O (7.0 mL) were kept at rt for 12 h. The solvent was removed under reduced pressure. Recrystallization from EtOH gave **125** (0.346 g, 43%).

2-(*N*-Benzyl-*N*-methylamino)-4*H*-3,1-benzothiazin-4-one (**126**)



M = 282.36 g/mol

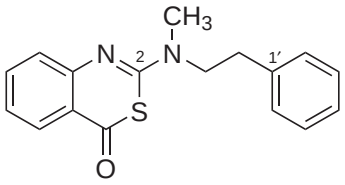
2-(3-Benzyl-3-methylthioureido)benzoic acid (**81**; 0.150 g, 0.500 mmol) and Ac_2O (1.0 mL) were kept at rt for 8 h. The resulting crystals were removed by suction filtration to obtain **126** (0.109 g, 77%) as colorless needles, mp 70–71 °C.

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.13 (s, 3H, NCH_3), 4.87 (s, 2H, $C\underline{H}_2Ph$), 7.15 (ddd, J = 8.0, 6.9, 1.0 Hz, 1H, 6-H), 7.26–7.36 (m, 5H, 2′/3′/4′/5′/6′-H), 7.42 (dd, J = 8.2, 1.0 Hz, 1H, 8-H), 7.59 (ddd, J = 8.4, 6.9, 1.6 Hz, 1H, 7-H), 8.03 (dd, J = 8.0, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 35.95 (NCH_3), 53.56 ($\underline{C}H_2Ph$), 116.34 (C-4a), 123.42 (C-6), 124.84 (C-5), 127.58 (C-2′/6′), 127.73 (C-4′), 128.30 (C-8), 128.79 (C-3′/5′), 135.76 (C-7), 136.31 (C-1′), 151.04 (C-8a), 157.07 (C-2), 183.95 (C-4).

Anal. Calcd for $C_{16}H_{14}N_2OS$: C, 68.06; H, 5.00; N, 9.92. Found: C, 67.74; H, 5.18; N, 9.83.

2-[*N*-Methyl-*N*-(2-phenylethyl)amino]-4*H*-3,1-benzothiazin-4-one (**127**)



M = 296.39 g/mol

Method 1: According to the preparation of **125** (Method 2), **127** (0.578 g, 65%) was obtained from 2-[3-methyl-3-(2-phenylethyl)thioureido]benzoic acid (**82**) as a white solid, mp 72–75 °C (EtOH).

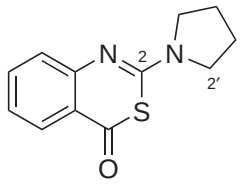
^{1}H NMR (500 MHz, $CDCl_3$) δ 2.96 (t, J = 7.6 Hz, 2H, $CH_2C\underline{H}_2Ph$), 3.08 (s, 3H, NCH_3), 3.81 (t, J = 7.6 Hz, 2H, $C\underline{H}_2CH_2Ph$), 7.14 (ddd, J = 8.2, 7.1, 1.3 Hz, 1H, 6-H), 7.20–7.33 (m, 5H, 2′/3′/4′/5′/6′-H), 7.44 (dd, J = 8.2, 1.0 Hz, 1H, 8-H), 7.59 (ddd, J = 8.2, 6.9, 1.6 Hz, 1H, 7-H), 8.03 (dd, J = 7.9, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 33.76 ($CH_2\underline{C}H_2Ph$), 37.07 (NCH_3), 53.03 ($\underline{C}H_2CH_2Ph$), 116.23 (C-4a), 123.38 (C-6), 124.83 (C-5), 126.68 (C-4′), 128.18 (C-8), 128.70 (C-2′/6′), 128.84 (C-3′/5′), 135.77 (C-7), 138.32 (C-1′), 150.90 (C-8a), 156.35 (C-2), 183.91 (C-4).

Anal. Calcd for $C_{17}H_{16}N_2OS$: C, 68.89; H, 5.44; N, 9.45. Found: C, 68.93; H, 5.44; N, 9.50.

Method 2: 2-[3-Methyl-3-(2-phenylethyl)thioureido]benzoic acid (**82**; 0.940 g, 3.00 mmol) and TFAA (7.0 mL) were kept at rt for 12 h. After removal of the solvent, the resulting crude material was purified by column chromatography on silica gel using petroleum ether–EtOAc (8:1) as eluent to give **127** (0.249 g, 28%) as a yellowish solid.

2-(Pyrrolidin-1-yl)-4*H*-3,1-benzothiazin-4-one (**128**)



M = 232.30 g/mol

Method 1: According to the preparation of **123** (Method 1), **128** (0.514 g, 74%) was obtained from methyl 2-[(1-pyrrolidinylthiocarbonyl)amino]benzoate (**75**) as white needles, mp 105–107 °C (EtOH).

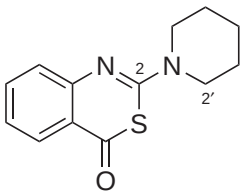
^{1}H NMR (500 MHz, $CDCl_3$) δ 1.97–2.04 (m, 4H, 3′/4′-H), 3.58 (br s, 4H, 2′/5′-H), 7.10 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 6-H), 7.39 (dd, J = 8.2, 1.0 Hz, 1H, 8-H), 7.56 (ddd, J = 8.5, 7.3, 1.6 Hz, 1H, 7-H), 8.01 (dd, J = 8.4, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 24.97 (C-3′/4′), 47.67 (C-2′/5′), 116.58 (C-4a), 122.87 (C-6), 124.86 (C-5), 128.06 (C-8), 135.65 (C-7), 151.57 (C-8a), 154.62 (C-2), 184.22 (C-4).

Anal. Calcd for $C_{12}H_{12}N_2OS$: C, 62.04; H, 5.21; N, 12.06. Found: C, 61.88; H, 5.28; N, 11.87.

Method 2: According to the preparation of **123** (Method 2), **128** (0.435 g, 94%) was obtained from *N*-[2-(pyrrolidin-1-ylcarbonyl)phenyl]pyrrolidine-1-carbothioamide (**134**) as a light yellow solid.

2-(Piperidin-1-yl)-4*H*-3,1-benzothiazin-4-one (**129**)



M = 246.36 g/mol

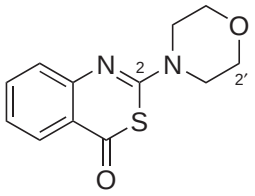
According to the preparation of **123** (Method 1), **129** (0.594 g, 80%) was obtained from methyl 2-[(1-piperidinylthiocarbonyl)amino]benzoate (**76**) as white needles, mp 87–88 °C (EtOH).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.60–1.72 (m, 6H, 3′/4′/5′-H), 3.67–3.72 (m, 4H, 2′/6′-H), 7.12 (ddd, J = 8.2, 6.9, 1.3 Hz, 1H, 6-H), 7.36 (dd, J = 8.2, 1.0 Hz, 1H, 8-H), 7.56 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H, 7-H), 8.00 (dd, J = 7.9, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 24.64 (C-4′), 25.62 (C-3′/5′), 46.84 (C-2′/6′), 116.36 (C-4a), 123.28 (C-6), 124.81 (C-5), 128.13 (C-8), 135.69 (C-7), 151.22 (C-8a), 156.27 (C-2), 184.29 (C-4).

Anal. Calcd for $C_{13}H_{14}N_2OS$: C, 63.39; H, 5.73; N, 11.37. Found: C, 63.35; H, 5.87; N, 11.30.

2-(Morpholin-4-yl)-4*H*-3,1-benzothiazin-4-one (**130**)



M = 248.30 g/mol

Method 1: According to the preparation of **123** (Method 1), **130** (0.395 g, 53%) was obtained from methyl 2-[(4-morpholinylthiocarbonyl)amino]benzoate (**77**) as colorless needles, mp 137–138 °C (EtOH; lit.[245] 136–137 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 3.71–3.79 (m, 8H, 2′/3′/5′/6′-H), 7.18 (ddd, J = 8.0, 6.9, 1.3 Hz, 1H, 6-H), 7.38 (dd, J = 8.2, 1.3 Hz, 1H, 8-H), 7.60 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H, 7-H), 8.02 (dd, J = 8.1, 1.7 Hz, 1H, 5-H).

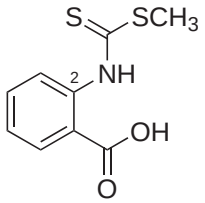
^{13}C NMR (125 MHz, $CDCl_3$) δ 45.90 (C-3′/5′), 66.40 (C-2′/6′), 116.74 (C-4a), 124.08 (C-6), 124.95 (C-5), 128.32 (C-8), 135.88 (C-7), 150.45 (C-8a), 156.75 (C-2), 183.44 (C-4).

Anal. Calcd for $C_{12}H_{12}N_2O_2S$: C, 58.05; H, 4.87; N, 11.28. Found: C, 58.08; H, 4.90; N, 11.22.

Method 2: According to the preparation of **123** (Method 2), **130** (0.453 g, 92%) was obtained from *N*-[2-(morpholin-4-ylcarbonyl)phenyl]morpholine-4-carbothioamide (**135**) as light yellow needles.

Method 3: *N*-[2-(Morpholin-4-ylcarbonyl)phenyl]morpholine-4-carbothioamide (**135**; 0.711 g, 2.00 mmol) was heated under reflux in anhydrous methanolic HCl (0.25 M, 10 mL) for 2 min. After cooling to rt, the precipitate was removed by suction filtration, washed with H_2O (30 mL), and dried *in vacuo* to give **130** (0.380 g, 77%) as light yellow needles.

2-[(Methylthio)thiocarbonylamino]benzoic Acid (**131**)



M = 227.30 g/mol

Triethylamine (1.70 g, 2.34 mL, 16.8 mmol) was added dropwise to an ice-cooled solution of anthranilic acid (0.960 g, 7.00 mmol) and carbon disulfide (1.07 g, 0.845 mL, 14.0 mmol) in 1,4-dioxane (30 mL). The cooled mixture was stirred for 5.5 h, followed by a dropwise addition of methyl iodide (1.09 g, 0.479 mL, 7.70 mmol) in 1,4-dioxane (20 mL). After stirring for further 1.5 h in the ice bath, the reaction mixture was allowed to warm to rt and stirred for 21 h under light protection. The solvent was removed under reduced pressure, and the crude material was partionated between EtOAc (100 mL) and HCl (0.2 M, 100 mL). The aqueous phase was further extracted with EtOAc (2 × 200 mL). The combined organic layers

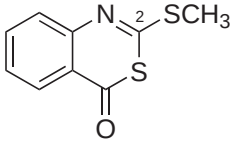
were dried (Na_2SO_4), filtered and evaporated to dryness. Recrystallization from PhMe gave **131** (1.22 g, 77%) as light yellow needles, mp 148–150 °C (PhMe).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.67 (s, 3H, SCH_3), 7.22 (td, J = 7.8, 1.3 Hz, 1H, 5-H), 7.62 (ddd, J = 8.5, 7.1, 1.6 Hz, 1H, 4-H), 8.15 (dd, J = 8.0, 1.7 Hz, 1H, 6-H), 9.16 (d, J = 8.5 Hz, 1H, 3-H), 11.91 (br s, 1H, NH).

^{13}C NMR (125 MHz, $CDCl_3$) δ 18.52 (SCH_3), 115.88 (C-1), 122.17 (C-3), 124.43 (C-5), 131.95 (C-6), 134.90 (C-4), 142.15 (C-2), 171.44 (CO_2H), 198.34 (NHCS).

Anal. Calcd for $C_9H_9NO_2S_2$: C, 47.56; H, 3.99; N, 6.16. Found: C, 47.32; H, 4.30; N, 6.18.

2-(Methylthio)-4*H*-3,1-benzothiazin-4-one (**132**)



M = 209.29 g/mol

Method 1: 2-[(Methylthio)thiocarbonylamino]benzoic acid (**131**; 0.909 g, 4.00 mmol) was heated to reflux in Ac_2O (10 mL) for 30 min. The solvent was removed under reduced pressure, and the crude material was recrystallized from MeOH to obtain **132** (0.782 g, 93%) as colorless needles, mp 54–56 °C (MeOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 2.72 (s, 3H, SCH_3), 7.58 (ddd, J = 7.9, 7.3, 1.3 Hz, 1H, 6-H), 7.72 (dd, J = 8.0, 1.2 Hz, 1H, 8-H), 7.92 (ddd, J = 8.5, 7.3, 1.9 Hz, 1H, 7-H), 8.06 (dd, J = 8.2, 1.9 Hz, 1H, 5-H).

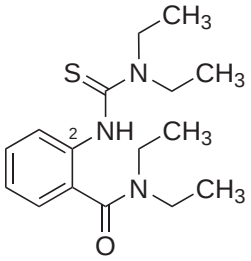
^{13}C NMR (125 MHz, DMSO-d_6) δ 13.92 (SCH_3), 118.65 (C-4a), 124.68 (C-5), 128.33 (C-6), 129.86 (C-8), 136.84 (C-7), 147.50 (C-8a), 163.47 (C-2), 182.33 (C-4).

Anal. Calcd for $C_9H_7NOS_2$: C, 51.65; H, 3.37; N, 6.69. Found: C, 51.72; H, 3.36; N, 6.68.

Method 2: 2-[(Methylthio)thiocarbonylamino]benzoic acid (**131**; 0.682 g, 3.00 mmol) and TFAA (7.0 mL) were kept at rt for 12 h. After removal of the solvent, the resulting crude material was purified by column chromatography on silica gel using petroleum ether–EtOAc (8:1) as eluent to give **132** (0.571 g, 91%) as yellowish needles.

8.13 2-Thioureidobenzamides

2-(3,3-Diethylthioureido)-*N*,*N*-diethylbenzamide (**133**)



M = 307.45 g/mol

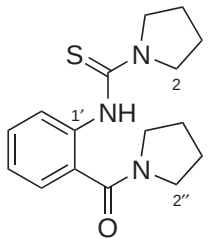
Diethylamine (0.914 g, 1.29 mL, 12.5 mmol) was added dropwise to a solution of 2-(methylthio)-4*H*-3,1-benzothiazin-4-one (**132**; 1.05 g, 5.00 mmol) in acetone (15 mL). After stirring for 1 h, the mixture was heated to reflux for 1 h, and allowed to cool to ambient temperature. The solvent was removed under reduced pressure. Recrystallization from EtOH gave **133** (0.998 g, 65%) as colorless prisms, mp 116–117 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.01–1.08 (m, 6H, 2 × $CH_2C\underline{H}_3$), 1.14 (t, J = 7.1 Hz, 6H, 2 × $CH_2C\underline{H}_3$), 3.20 (q, J = 6.9 Hz, 2H, $C\underline{H}_2CH_3$), 3.37 (q, J = 6.9 Hz, 2H, $C\underline{H}_2CH_3$), 3.67 (q, J = 6.9 Hz, 4H, 2 × $C\underline{H}_2CH_3$), 7.20–7.26 (m, 2H, 5/6-H), 7.33–7.38 (m, 1H, 4-H), 7.41 (d, J = 7.9 Hz, 1H, 3-H), 8.67 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 12.44 ($CH_2\underline{C}H_3$), 12.64 ($CH_2\underline{C}H_3$), 13.91 ($CH_2\underline{C}H_3$), 38.20 ($\underline{C}H_2CH_3$), 43.09 ($\underline{C}H_2CH_3$), 44.88 ($\underline{C}H_2CH_3$), 125.37 (C-5/6), 125.84 (C-5/6), 128.58 (C-4), 130.00 (C-3), 134.59 (C-1), 138.17 (C-2), 168.26 (CON), 179.34 (NHCS).

Anal. Calcd for $C_{16}H_{25}N_3OS$: C, 62.50; H, 8.20; N, 13.67. Found: C, 62.78; H, 8.14; N, 13.78.

N-[2-(Pyrrolidin-1-ylcarbonyl)phenyl]pyrrolidine-1-carbothioamide (**134**)



M = 303.42 g/mol

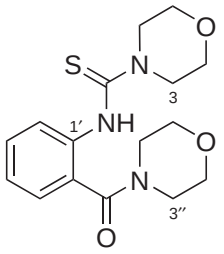
According to the preparation of **133**, compound **134** (1.13 g, 74%) was obtained from **132** and pyrrolidine as light yellow prisms, mp 160–162 °C (EtOH).

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.71–2.08 (m, 8H, 3/4/3″/4″-H), 3.38–3.70 (m, 8H, 2/5/2″/5″-H), 7.16 (td, J = 7.6, 1.3 Hz, 1H, 4′-H), 7.38 (td, J = 7.9, 1.6 Hz, 1H, 5′-H), 7.42 (dd, J = 7.7, 1.4 Hz, 1H, 3′-H), 7.88 (d, J = 7.9 Hz, 1H, 6′-H), 9.35 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 24.02 (C-3/4/3″/4″), 25.91 (C-3/4/3″/4″), 46.03 (C-2/5/2″/5″), 49.13 (C-2/5/2″/5″), 124.03 (C-4′), 126.53 (C-6′), 127.20 (C-3′), 129.26 (C-5′), 130.52 (C-2′), 138.44 (C-1′), 167.39 (CON), 176.74 (NHCS).

Anal. Calcd for $C_{16}H_{21}N_3OS$: C, 63.33; H, 6.98; N, 13.85. Found: C, 63.49; H, 7.09; N, 13.82.

N-[2-(Morpholin-4-ylcarbonyl)phenyl]morpholine-4-carbothioamide (**135**)



M = 335.42 g/mol

Method 1: According to the preparation of **133**, compound **135** (1.51 g, 90%) was obtained from **132** and morpholine as colorless prisms, mp 170–173 °C (EtOH).

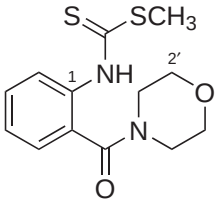
^{1}H NMR (500 MHz, DMSO-d_6) δ 3.48–3.63 (m, 12H, 2/6/2′/3′/5′/6′-H) 3.85 (t, J = 4.6 Hz, 4H, 3/5-H), 7.23–7.29 (m, 3H, 3′/4′/6′-H), 7.39 (ddd, J = 7.8, 6.9, 1.9 Hz, 1H, 5′-H), 9.31 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 41.70 (C-2/3/5/6/2″/3″/5″/6″), 47.48 (C-2/3/5/6/2″/3″/5″/6″), 48.85 (C-2/3/5/6/2″/3″/5″/6″), 66.06 (C-2/3/5/6/2″/3″/5″/6″), 66.12 (C-2/3/5/6/2″/3″/5″/6″), 66.30 (C-2/3/5/6/2″/3″/5″/6″), 125.75 (C-3′), 127.26 (C-4′), 129.29 (C-5′), 129.35 (C-6′), 133.38 (C-2′), 138.38 (C-1′), 167.06 (CON), 181.93 (NHCS).

Anal. Calcd for $C_{16}H_{21}N_3O_3S$: C, 57.29; H, 6.31; N, 12.53. Found: C, 57.52; H, 6.35; N, 12.35.

Method 2: Morpholine (0.392 g, 0.396 mL, 4.50 mmol) was added dropwise to a solution of 2-(morpholin-4-yl)-4*H*-3,1-benzothiazin-4-one (**130**; 0.497 g, 2.00 mmol) in acetone (6.0 mL). After stirring for 1 h, the mixture was heated to reflux for 1 h, and allowed to cool to rt. The formed precipitate was removed by suction filtration and washed with cold acetone (5.0 mL) to give **135** (0.490 g, 73%) as a white solid.

Methyl 2-(Morpholin-4-ylcarbonyl)phenyldithiocarbamate (**136**)



$M = 296.41$ g/mol

Morpholine (0.392 g, 0.396 mL, 4.50 mmol) was added dropwise to a solution of 2-(methylthio)-4*H*-3,1-benzothiazin-4-one (**132**; 0.418 g, 2.00 mmol) in acetone (6.0 mL). After stirring for 1 h, the resulting precipitate was removed by suction filtration and washed with cold acetone (5.0 mL) to obtain **136** (0.299 g, 50%) as a white solid, mp 143–145 °C.

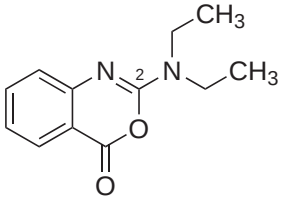
^{1}H NMR (500 MHz, DMSO-d_6) δ 2.55 (s, 3H, SCH_3), 3.15–3.64 (m, 8H, 2′/3′/5′/6′-H), 7.35–7.49 (m, 4H, 3/4/5/6-H), 11.52 (s, 1H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 18.24 (SCH_3), 41.90 (C-3′/5′), 47.39 (C-3′/5′), 66.09 (C-2′/6′), 66.13 (C-2′/6′), 127.50 (C-3/4), 127.98 (C-3/4), 128.65 (C-5/6), 129.77 (C-5/6), 133.22 (C-2), 136.57 (C-1), 166.40 (CO), 199.94 (NHCS).

Anal. Calcd for $C_{13}H_{16}N_2O_2S_2$: C, 52.68; H, 5.44; N, 9.45. Found: C, 52.81; H, 5.45; N, 9.49.

8.14 4*H*-3,1-Benzoxazin-4-ones

2-(Diethylamino)-4*H*-3,1-benzoxazin-4-one (**137**)



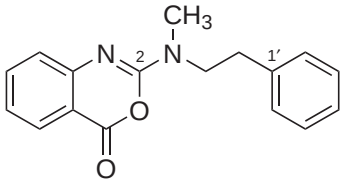
M = 218.25 g/mol

In the course of the preparation of 2-(diethylamino)-4*H*-3,1-benzothiazin-4-one (**123**) using TFAA (Method 3), purification of the crude material by column chromatography on silica gave **137** (0.253 g, 65%) as colorless semisolid material (lit.[149] 46–47 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.22 (t, J = 7.1 Hz, 6H, $CH_2C\underline{H}_3$), 3.53 (q, J = 7.0 Hz, 4H, $C\underline{H}_2CH_3$), 7.06 (ddd, J = 8.2, 6.9, 1.0 Hz, 1H, 6-H), 7.20 (d, J = 8.2 Hz, 1H, 8-H), 7.55 (ddd, J = 8.5, 6.9, 1.6 Hz, 1H, 7-H), 7.96 (dd, J = 7.9, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 13.30 ($CH_2\underline{C}H_3$), 42.19 ($\underline{C}H_2CH_3$), 112.02 (C-4a), 122.70 (C-5/6/8), 123.96 (C-5/6/8), 138.53 (C-5/6/8), 136.49 (C-7), 151.13 (C-8a), 153.43 (C-2), 160.15 (C-4).

2-[*N*-Methyl-*N*-(2-phenylethyl)amino]-4*H*-3,1-benzoxazin-4-one (**138**)



M = 280.32 g/mol

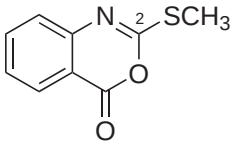
In the course of the preparation of 2-[*N*-methyl-*N*-(2-phenylethyl)amino]-4*H*-3,1-benzothiazin-4-one (**127**) using TFAA (Method 2), purification of the crude material by column chromatography on silica gave **138** (0.570 g, 68%) as a white solid, mp 68–70 °C (lit.[154] 68.5–69 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.94 (t, J = 7.3 Hz, 2H, $CH_2C\underline{H}_2Ph$), 3.05 (s, 3H, NCH_3), 3.75 (t, J = 7.3 Hz, 2H, $C\underline{H}_2CH_2Ph$), 7.07–7.12 (m, 1H, 6-H), 7.18–7.30 (m, 6H, 8/2′–6′-H), 7.58 (ddd, J = 7.8, 7.4, 1.8 Hz, 1H, 7-H), 7.97 (dd, J = 7.9, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 34.01 ($CH_2\underline{C}H_2Ph$), 35.68 (NCH_3), 51.35 ($\underline{C}H_2CH_2Ph$), 112.06 (C-4a), 123.00 (C-5/6/8), 124.15 (C-5/6/8), 128.61 (C-5/6/8), 126.54 (C-4′), 128.57 (C-2′/6′), 128.82 (C-3′/5′), 136.56 (C-7), 138.42 (C-1′), 150.93 (C-8a), 153.84 (C-2), 159.90 (C-4).

Anal. Calcd for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.44; H, 6.05; N, 9.70.

2-(Methylthio)-4*H*-3,1-benzoxazin-4-one (**139**)



$M = 193.22$ g/mol

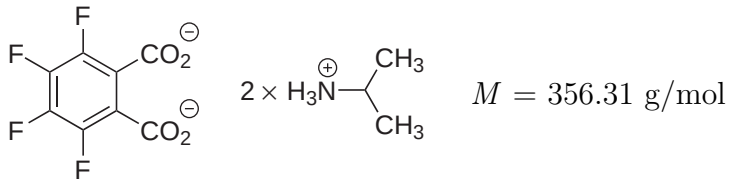
In the course of the preparation of 2-(methylthio)-4*H*-3,1-benzothiazin-4-one (**132**) using TFAA (Method 2), purification of the crude material by column chromatography on silica yielded **139** (0.010 g, 2%) as a white solid, mp 103–105 °C (lit.[149] 108–109 °C).

^{1}H NMR (500 MHz, $CDCl_3$) δ 2.58 (s, 3H, SCH_3), 7.40 (ddd, J = 8.2, 7.2, 1.3 Hz, 1H, 6-H), 7.45 (d, J = 7.9 Hz, 1H, 8-H), 7.74 (ddd, J = 7.9, 7.3, 1.6 Hz, 1H, 7-H), 8.11 (dd, J = 8.0, 1.6 Hz, 1H, 5-H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 14.16 (SCH_3), 115.59 (C-4a), 125.59 (C-5/6/8), 127.22 (C-5/6/8), 128.77 (C-5/6/8), 136.76 (C-7), 146.89 (C-8a), 158.80 (C-4), 164.00 (C-2).

8.15 Isopropylammonium Tetrafluoro(hydrogen)phthalates

Bis(isopropylammonium) Tetrafluorophthalate (**140**)



$M = 356.31$ g/mol

This substance was prepared according to an unpublished procedure.[246] A mixture of tetrafluorophthalic acid (357 mg, 1.50 mmol), isopropylamine (177 mg, 257 µL, 3.00 mmol) and anhydrous PhMe (100 mL) was refluxed for 3 h and kept at rt for additional 2 h. The precipitate was isolated by suction filtrations and dried to give pure **140** as a white solid; yield: 475 mg (89%); mp 197–200 °C. IR (KBr): 3205, 2988, 2952, 2548, 1651, 1570, 1528, 1464, 1376, 1216, 1171, 1109, 1061, 1008 cm^{-1}. Material for X-ray crystallography was obtained as follows: Compound **140** was dissolved in 1-propanol/water (500:1 v/v). Slow evaporation of this solution at rt afforded colorless single crystals.

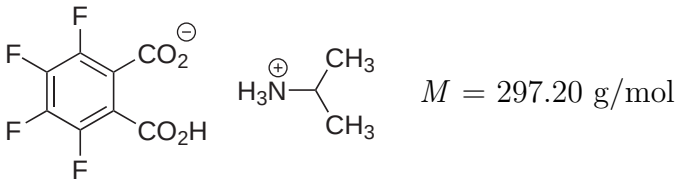
^{1}H NMR (500 MHz, DMSO-d_6) δ 1.12 (d, ^3J = 6.6 Hz, 12H, CH_3), 3.20 (sept, ^3J = 6.5 Hz, 2H, CH), 7.93 (br s, 6H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 20.76 (CH_3), 42.69 (CH), 125.85 (d, $^2J_{C–F}$ = 16 Hz, C-1/2), 137.63 (d, $^1J_{C–F}$ = 244 Hz, C-4/5), 142.43 (d, $^1J_{C–F}$ = 236 Hz, C-3/6), 164.46 (CO).

Solid-state ^{13}C NMR δ 20.9 (CH_3), 43.1 (CH), 125.7 (C-1/2), 136–149 (C-3–6), 168.1 (CO).

Anal. Calcd for $C_{14}H_{20}F_4N_2O_4$: C, 47.19; H, 5.66; N, 7.86. Found: C, 47.39; H, 6.02; N, 7.72.

Isopropylammonium Tetrafluorohydrogenphthalate (**141**)



$M = 297.20$ g/mol

A solution of isopropylamine (120 mg, 173 µL, 2.00 mmol) in water (2.0 mL) was added to tetrafluorophthalic acid (714 mg, 2.00 mmol). After the mixture was stirred at rt for 10 min, the solvent was evaporated under reduced pressure. The crude product was recrystallized from CH_3CN to give **141** as colorless rhombs; yield: 502 mg (84%); mp 154–156 °C (CH_3CN). IR (KBr): 3161, 2942, 2570, 1917, 1718, 1628, 1592, 1475, 1376, 1124, 1068 cm^{-1}.

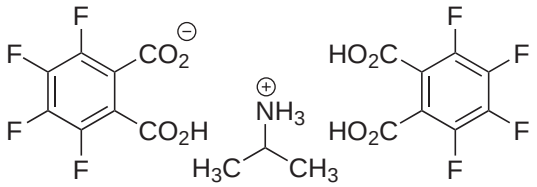
^{1}H NMR (500 MHz, DMSO-d_6) δ 1.16 (d, ^3J = 6.6 Hz, 6H, CH_3), 3.26 (sept, ^3J = 6.6 Hz, 1H, CH), 7.90 (br s, 3H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 20.53 (CH_3), 43.00 (CH), 122.19 (d, $^2J_{C–F}$ = 14 Hz, C-1/2), 139.60 (d, $^1J_{C–F}$ = 253 Hz, C-4/5), 143.78 (d, $^1J_{C–F}$ = 248 Hz, C-3/6), 163.13 (CO).

Solid-state ^{13}C NMR δ 20.1 (CH_3), 46.6 (CH), 118.0 (C-1/2), 122.9 (C-1/2), 134–149 (C-3–6), 165.4 (CO).

Anal. Calcd for $C_{11}H_{11}F_4NO_4$: C, 44.45; H, 3.73; N, 4.71. Found: C, 44.44; H, 3.79; N, 4.65.

Isopropylammonium Tetrafluorohydrogenphthalate × Tetrafluorophthalic Acid (**142**)



M = 535.30 g/mol

This substance was prepared according to an unpublished procedure.[246] Compound **140** (356 mg, 1.00 mmol) was dissolved in water (5 mL). After addition of HCl (2 M, 2.5 mL) the mixture was kept at rt in an open vessel. Evaporation gave a reduced volume of the solution, and colorless needles were formed; yield: 139 mg (52%); mp 152–155 °C. IR (KBr): 3226, 3161, 2870, 2550, 1900, 1717, 1628, 1575, 1527, 1475, 1425, 1376, 1322, 1295, 1247, 1123, 1067 cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 1.17 (d, ^3J = 6.6 Hz, 6H, CH_3), 3.27 (sept, ^3J = 6.0 Hz, 1H, CH), 7.87 (br s, 3H, NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ 20.54 (CH_3), 43.07 (CH), 120.34 (d, $^2J_{C–F}$ = 14 Hz, C-1/2), 140.41 (d, $^1J_{C–F}$ = 253 Hz, C-4/5), 144.23 (d, $^1J_{C–F}$ = 244 Hz, C-3/6), 162.90 (CO).

Solid-state ^{13}C NMR δ 21.3 (CH_3), 47.1 (CH), 114.2 (C-1/2), 120.3 (C-1/2), 122.3 (C-1′/2′), 138–151 (C-3–6/3′–6′), 167.1 (CO).

Anal. Calcd for $C_{19}H_{13}F_8NO_8$: C, 42.63; H, 2.45; N, 2.62. Found: C, 42.77; H, 2.82; N, 3.29.

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Bibliography

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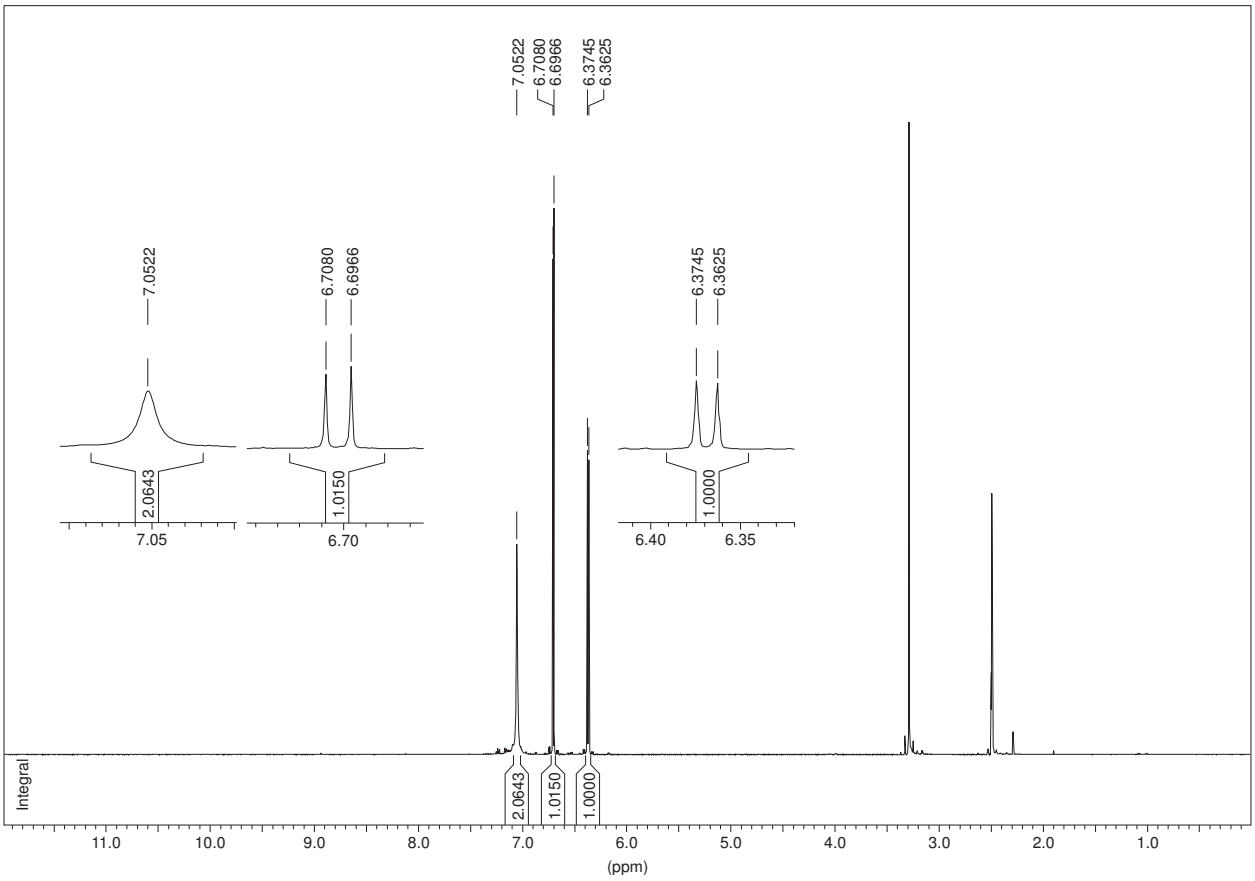
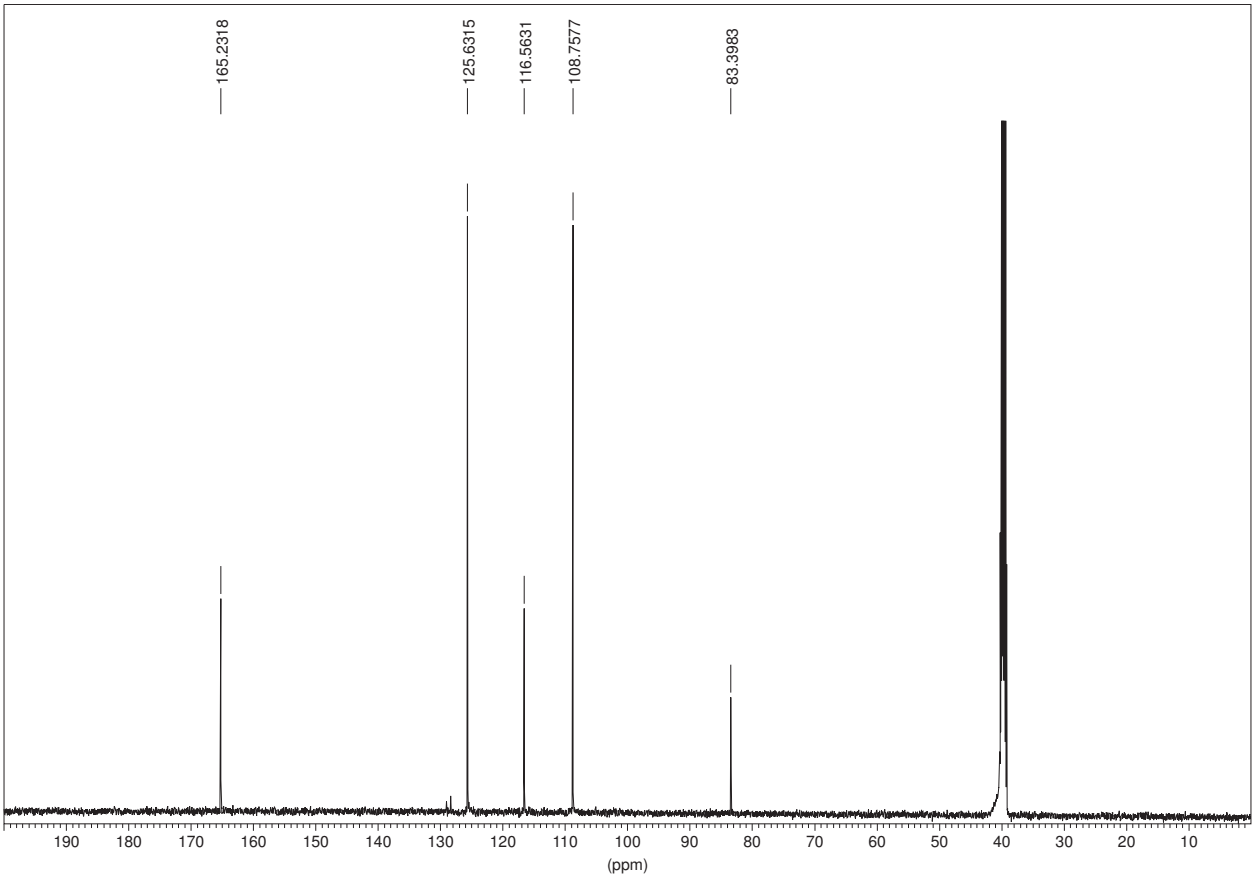
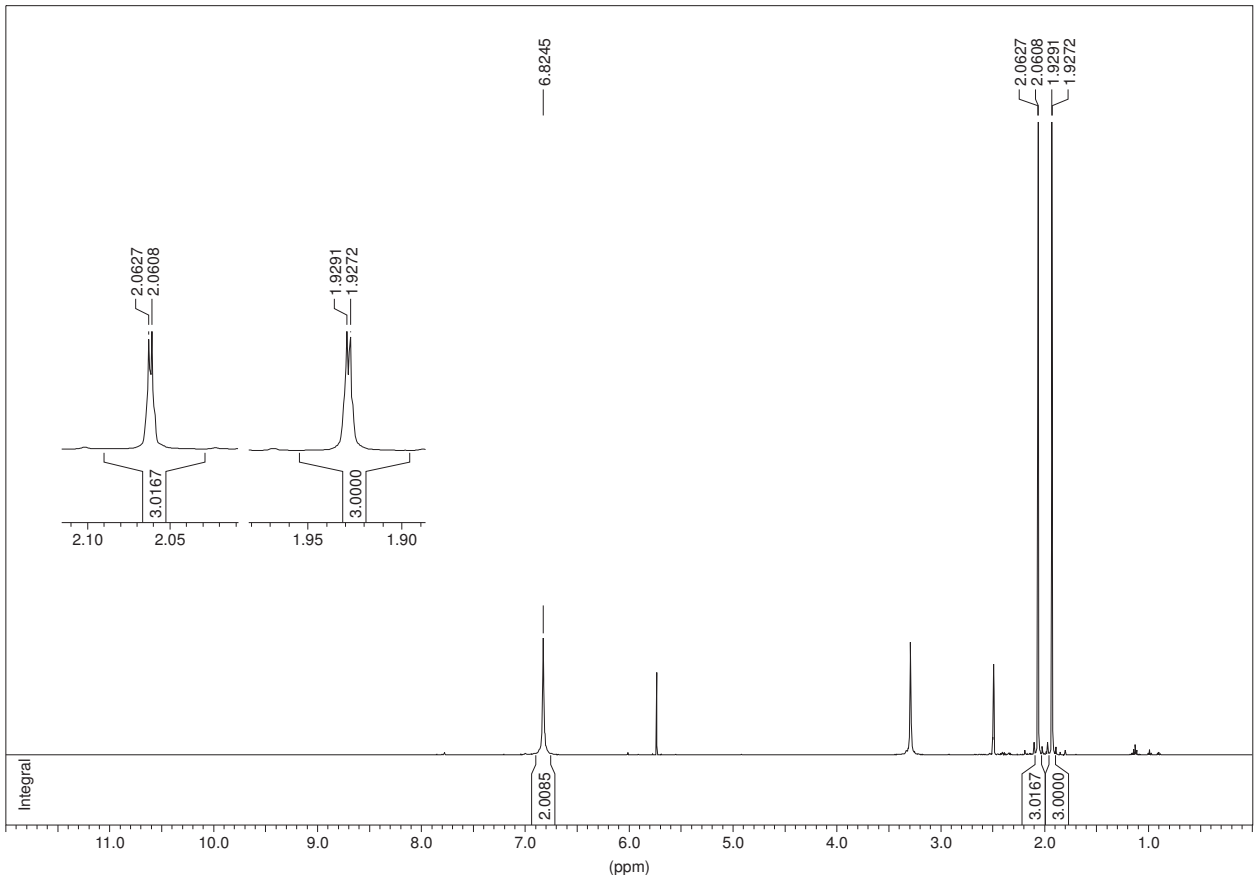
NMR Spectra

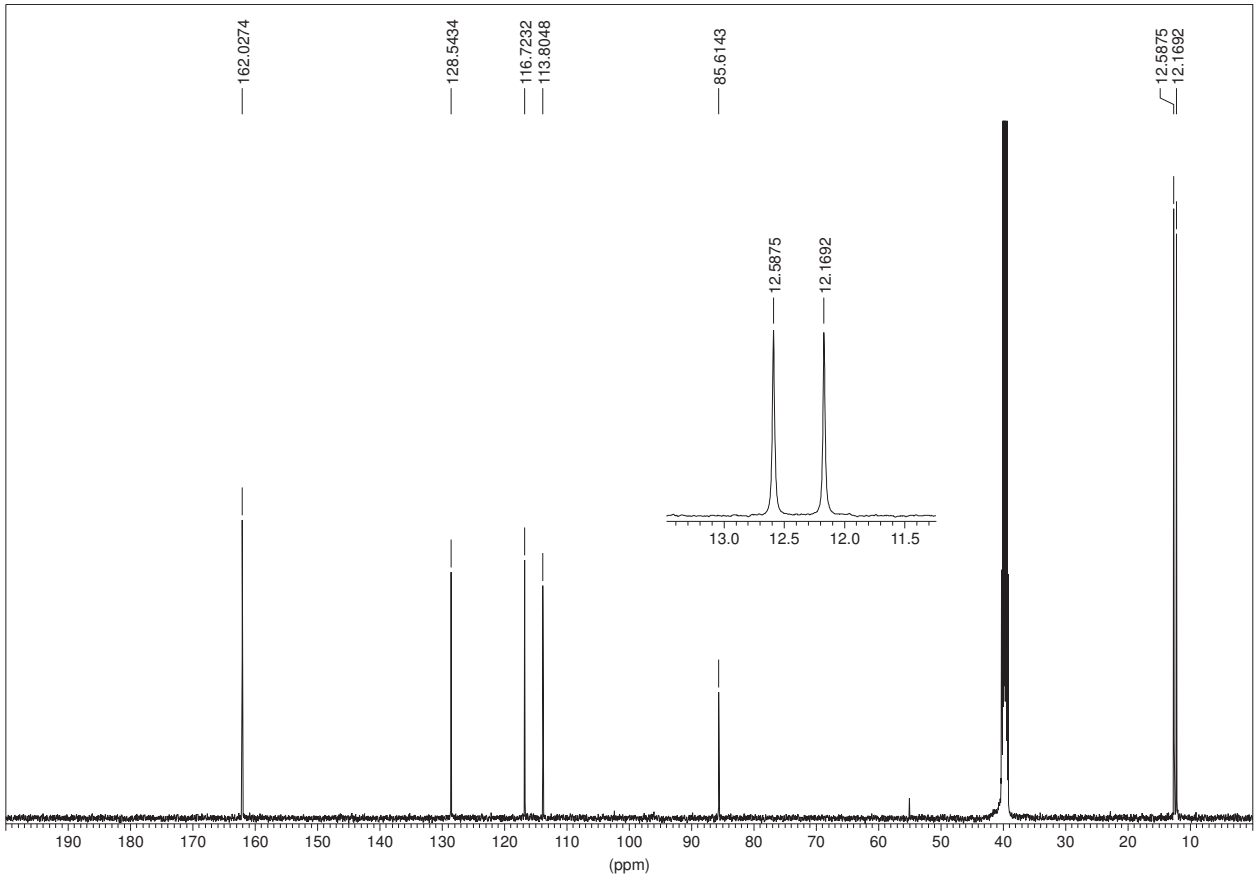
2-Aminothiophene-3-carbonitrile (**1**)



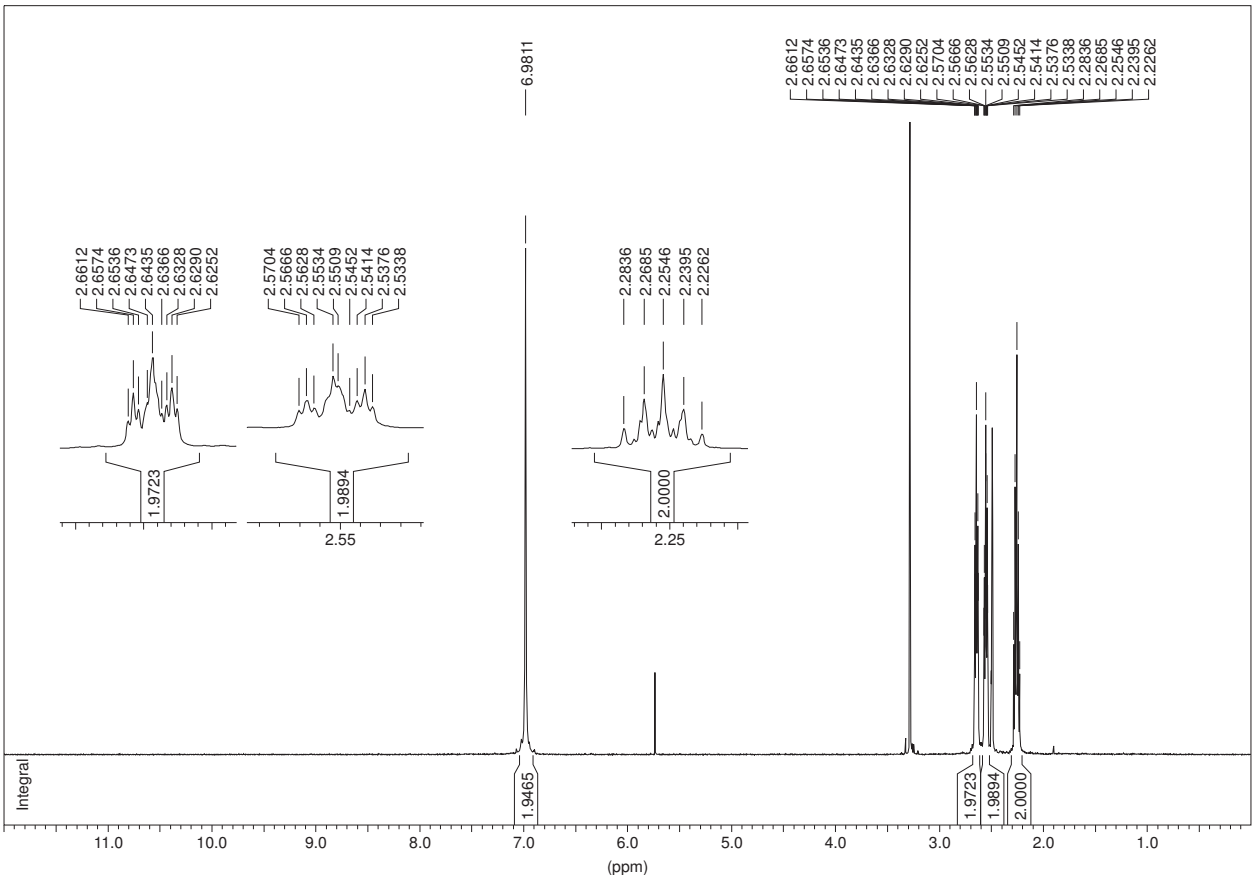


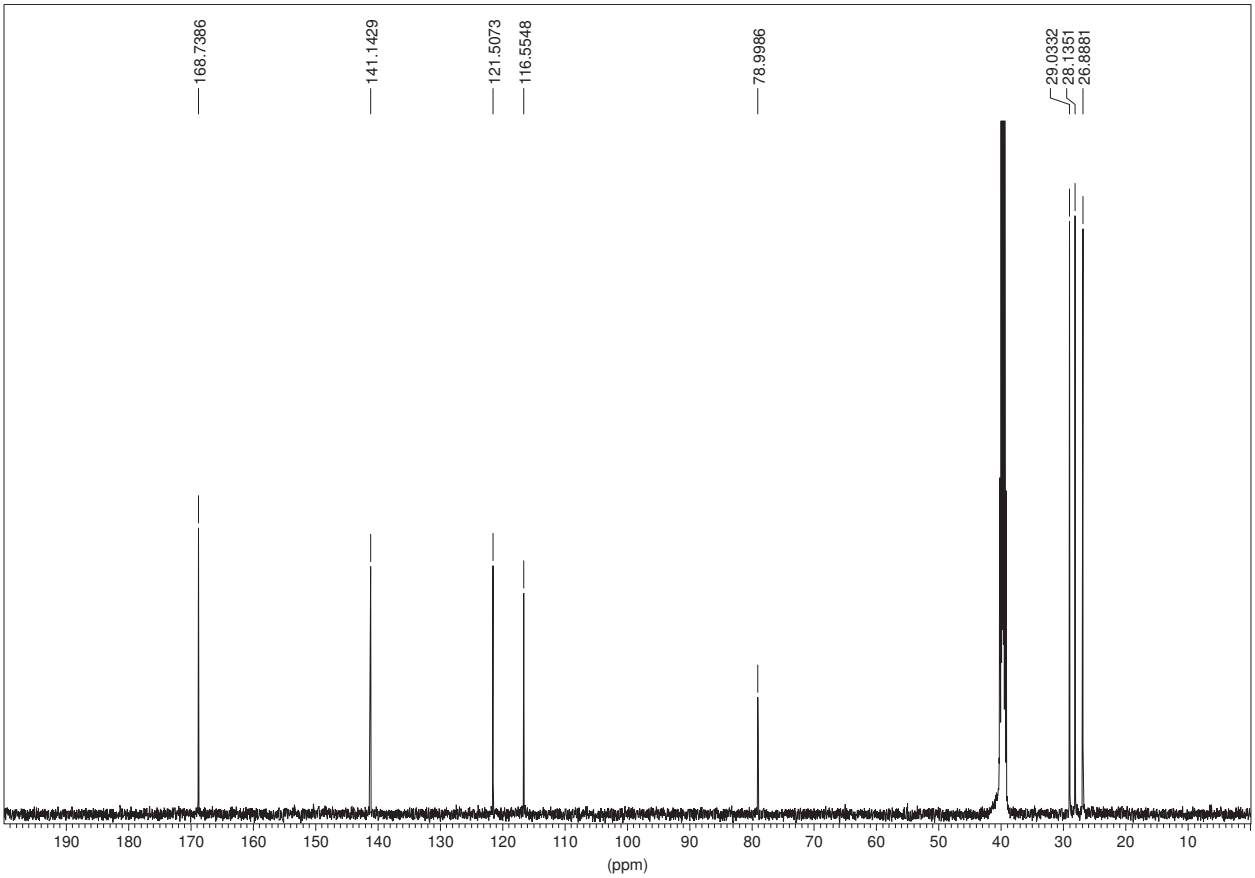
2-Amino-4,5-dimethylthiophene-3-carbonitrile (**2**)



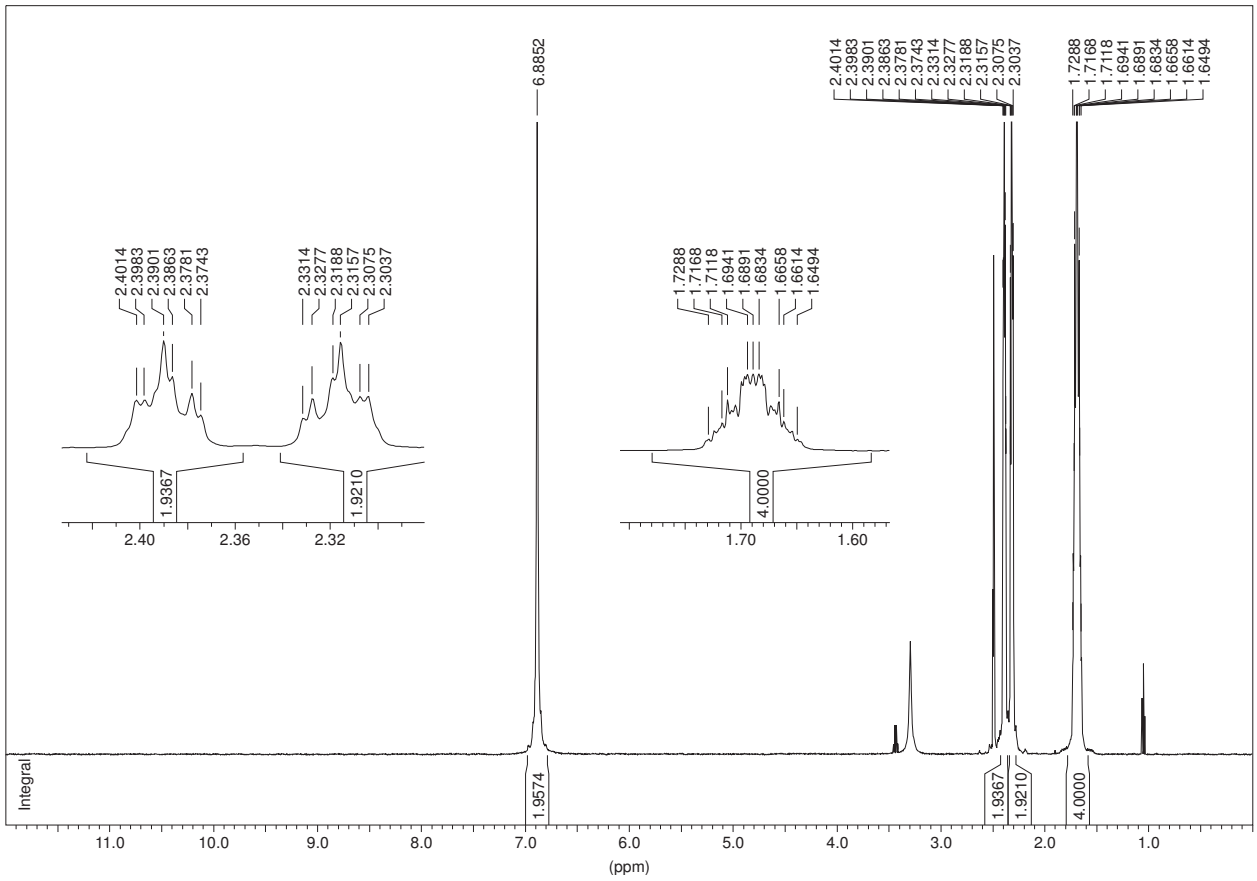


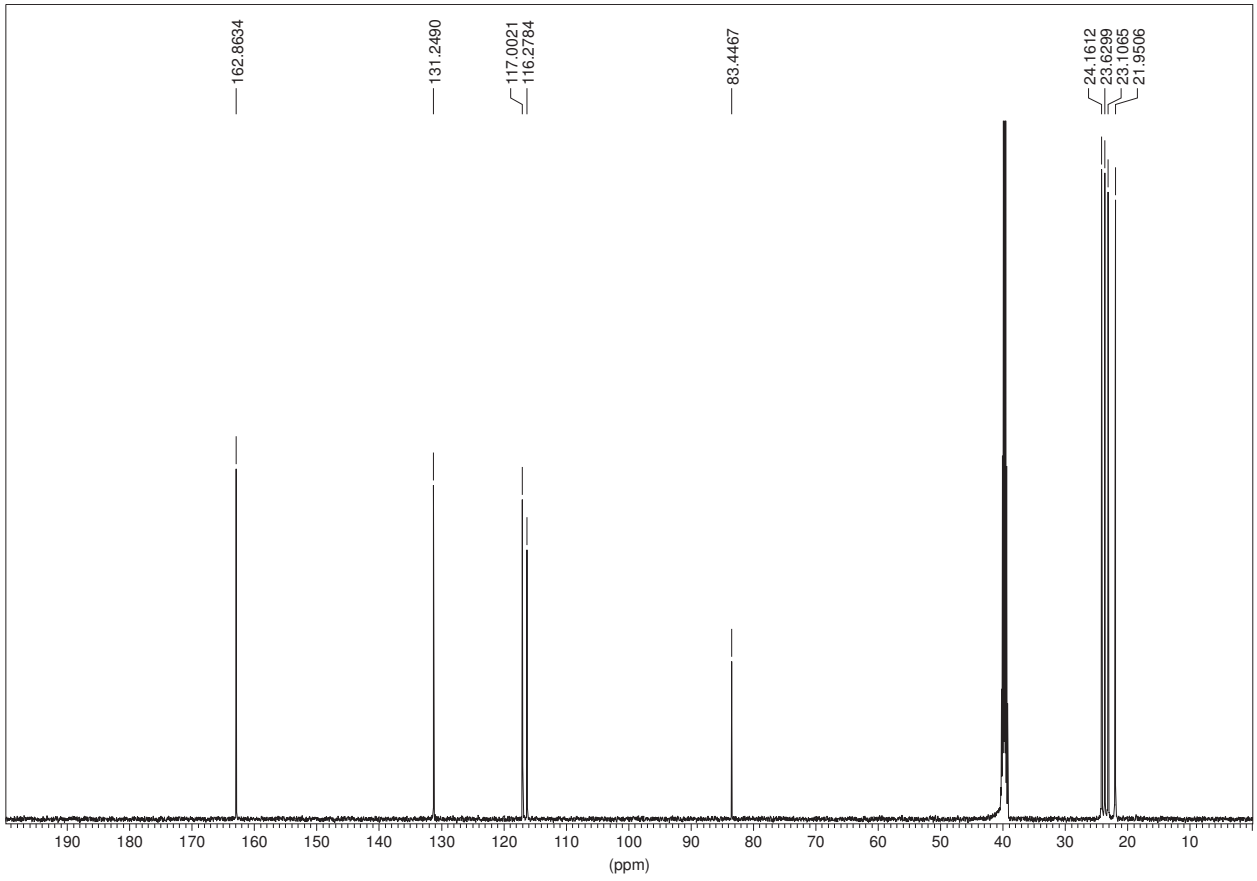
2-Amino-5,6-dihydro-4*H*-cyclopenta[*b*]thiophene-3-carbonitrile (**3**)



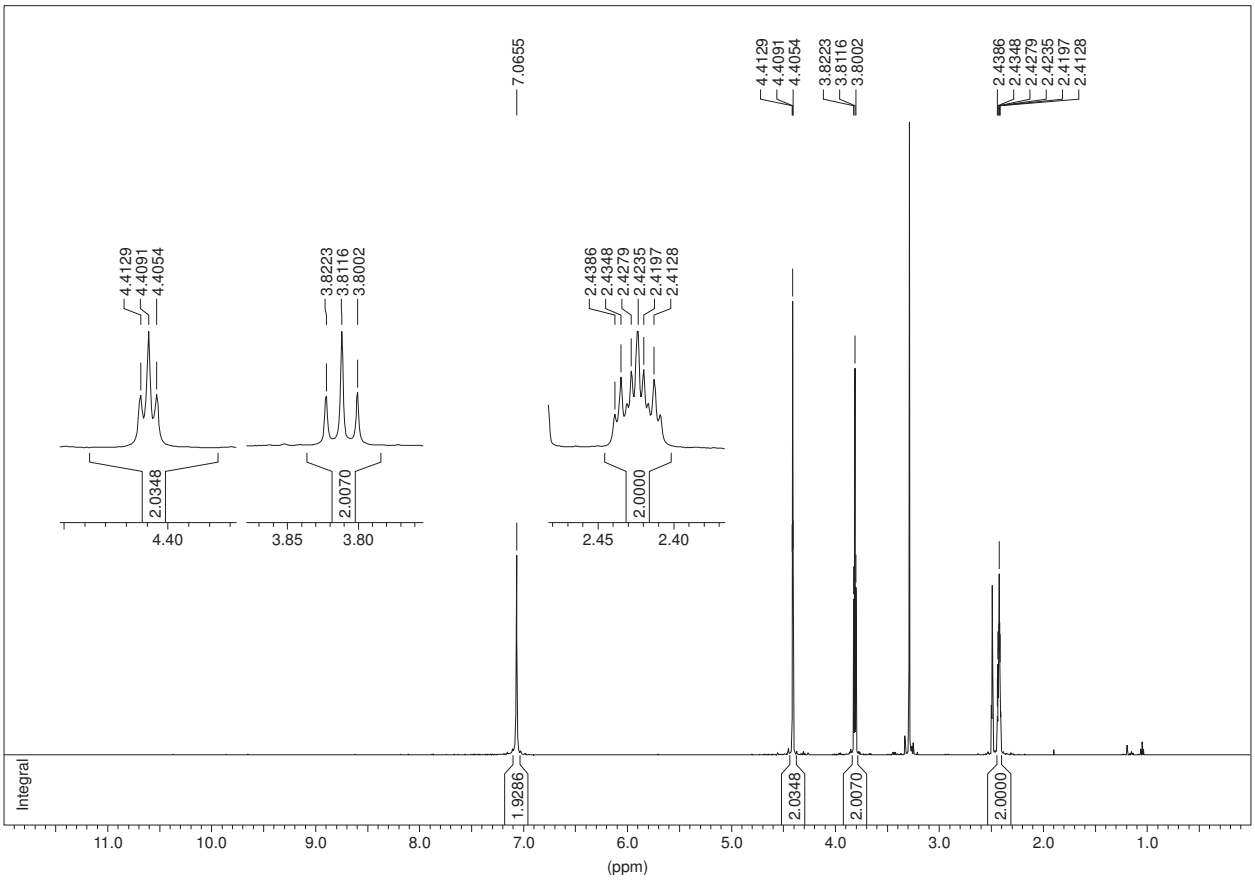


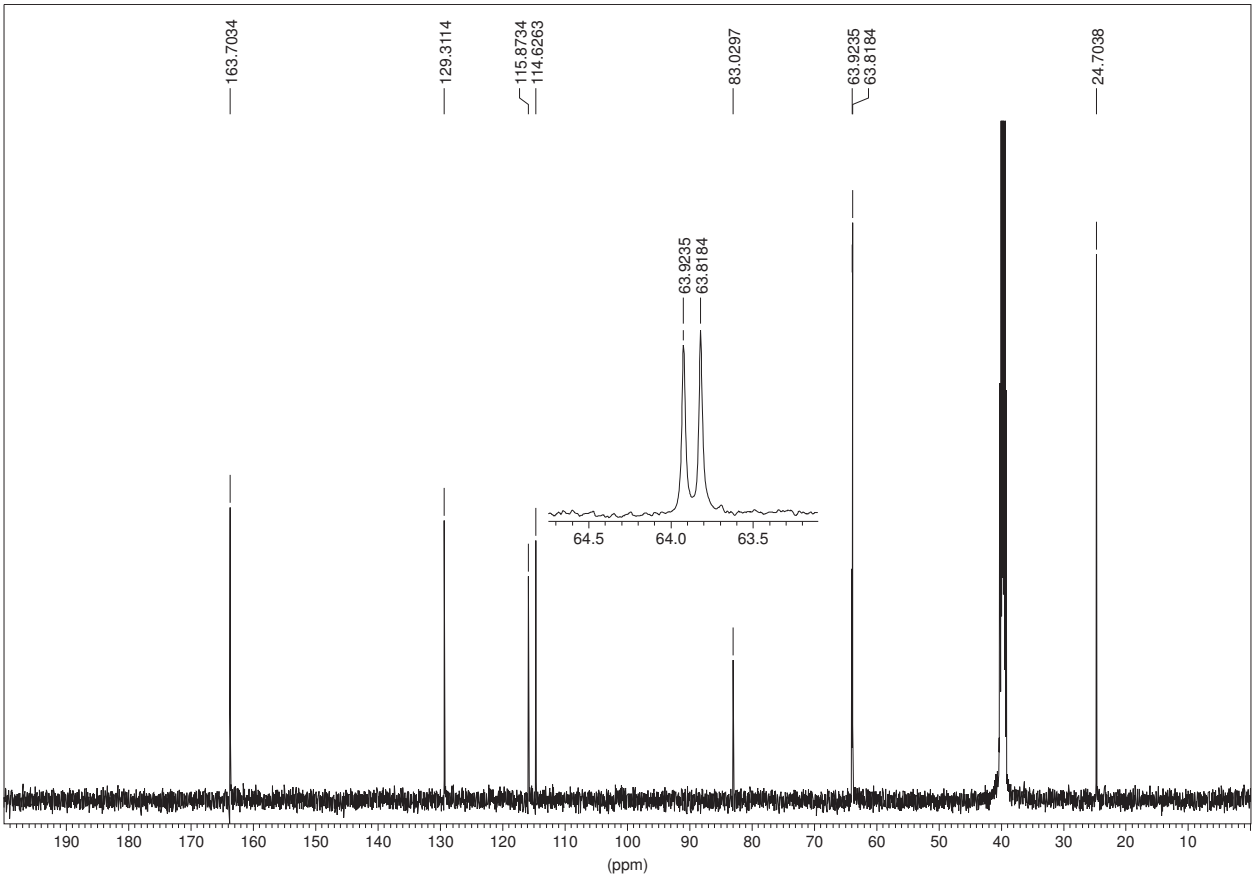
2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (**4**)



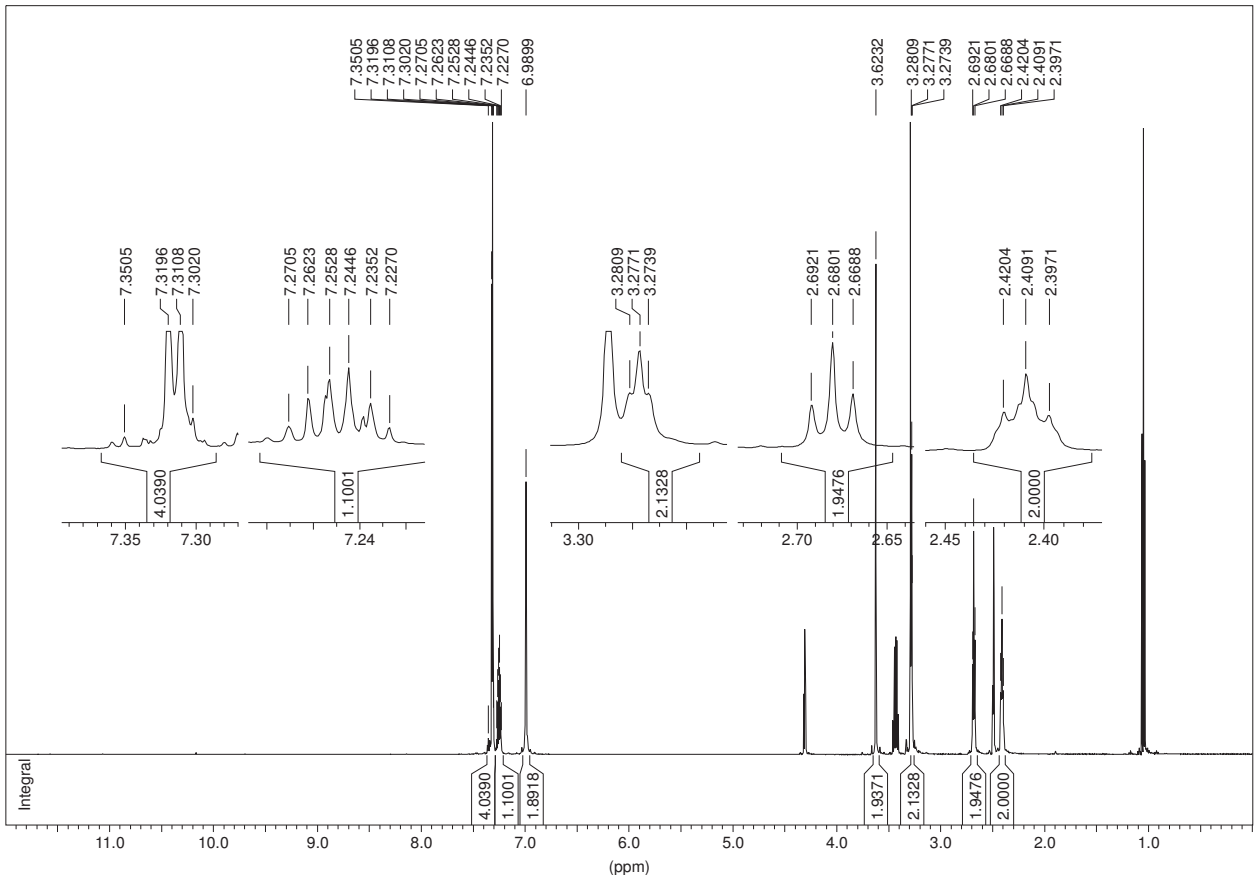


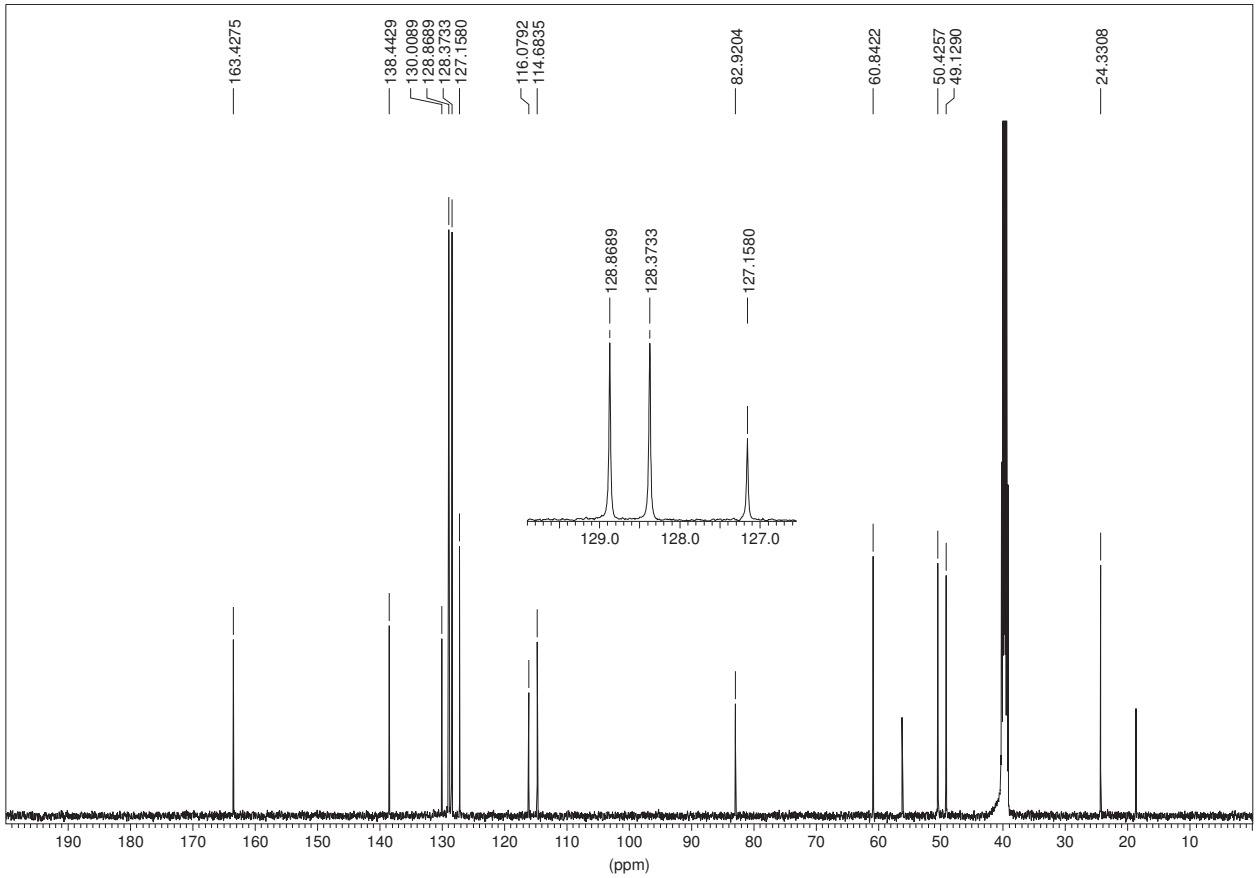
2-Amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carbonitrile (**5**)



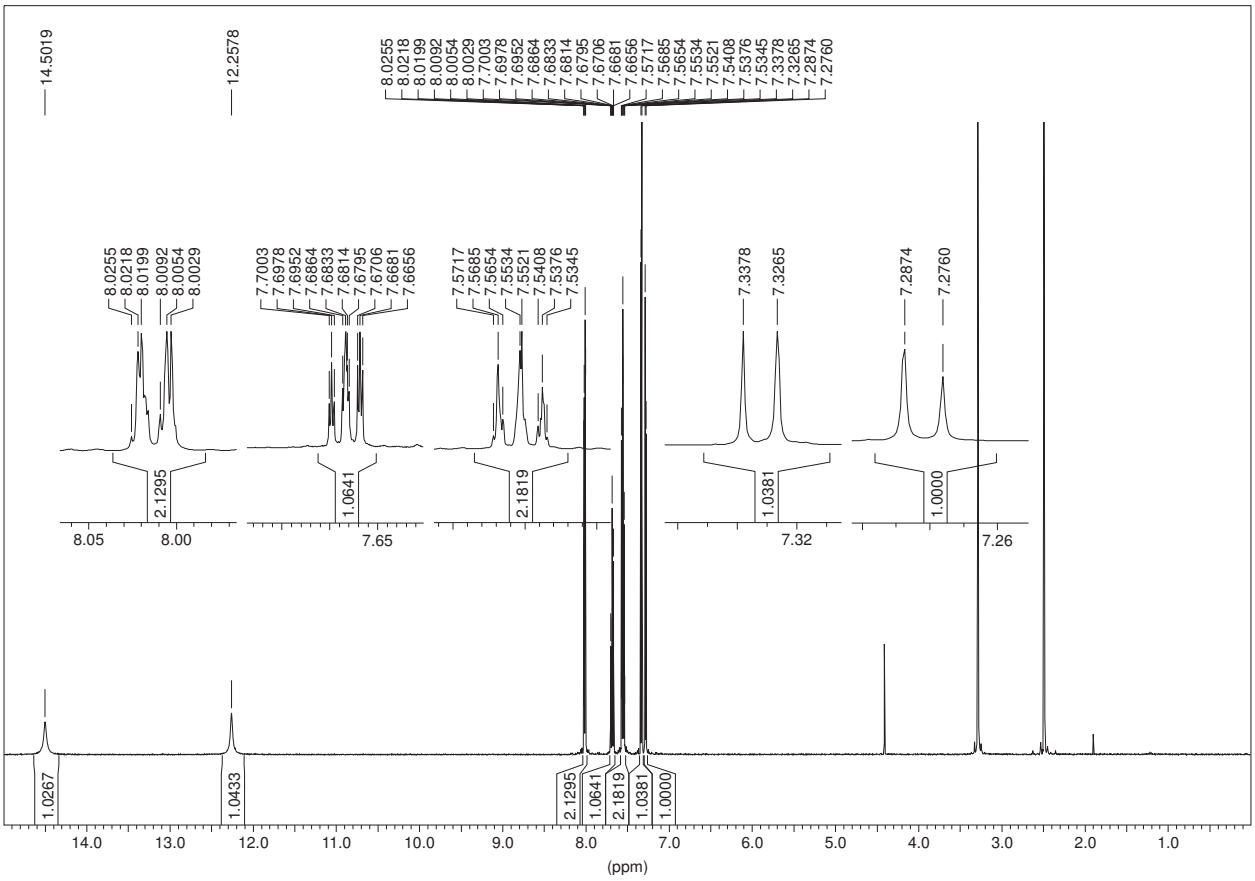


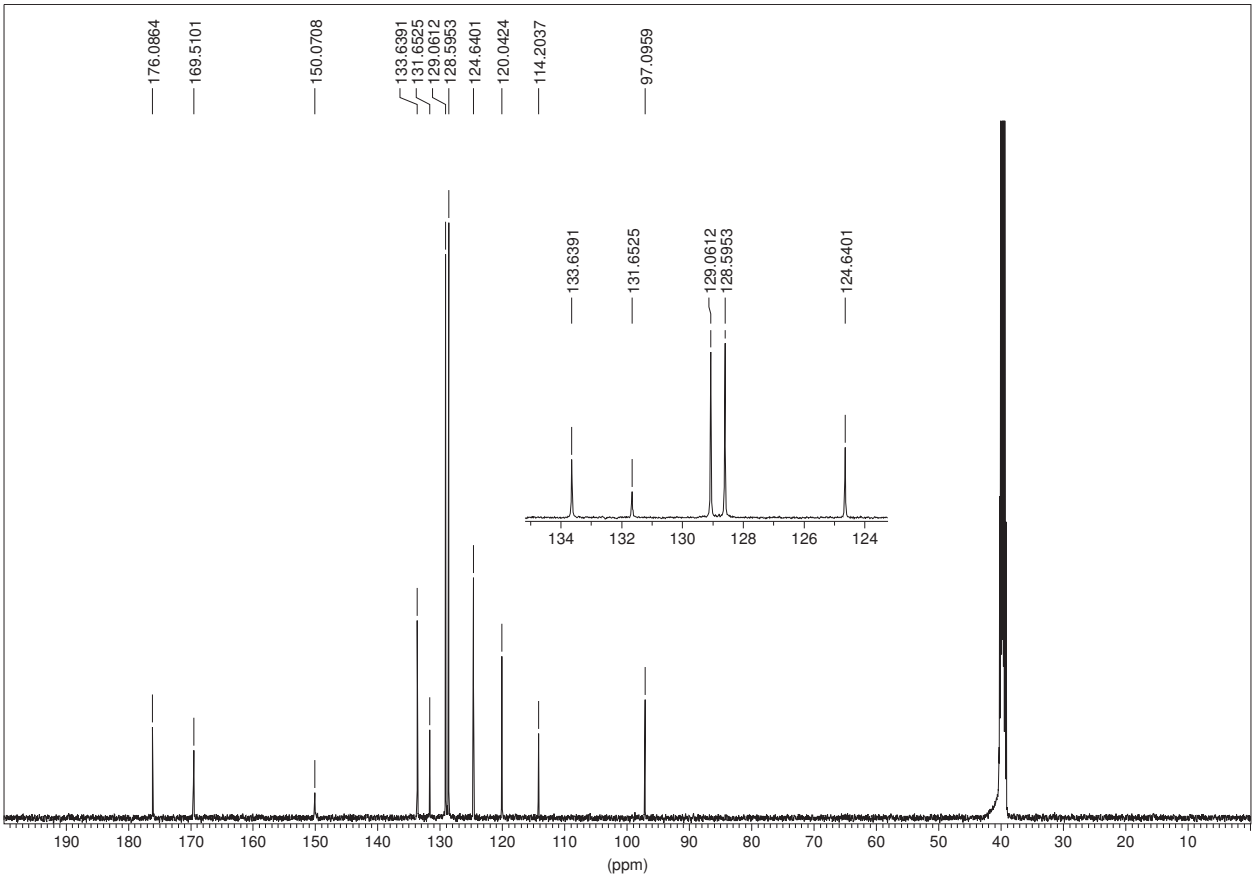
2-Amino-6-benzyl-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine-3-carbonitrile (**6**)



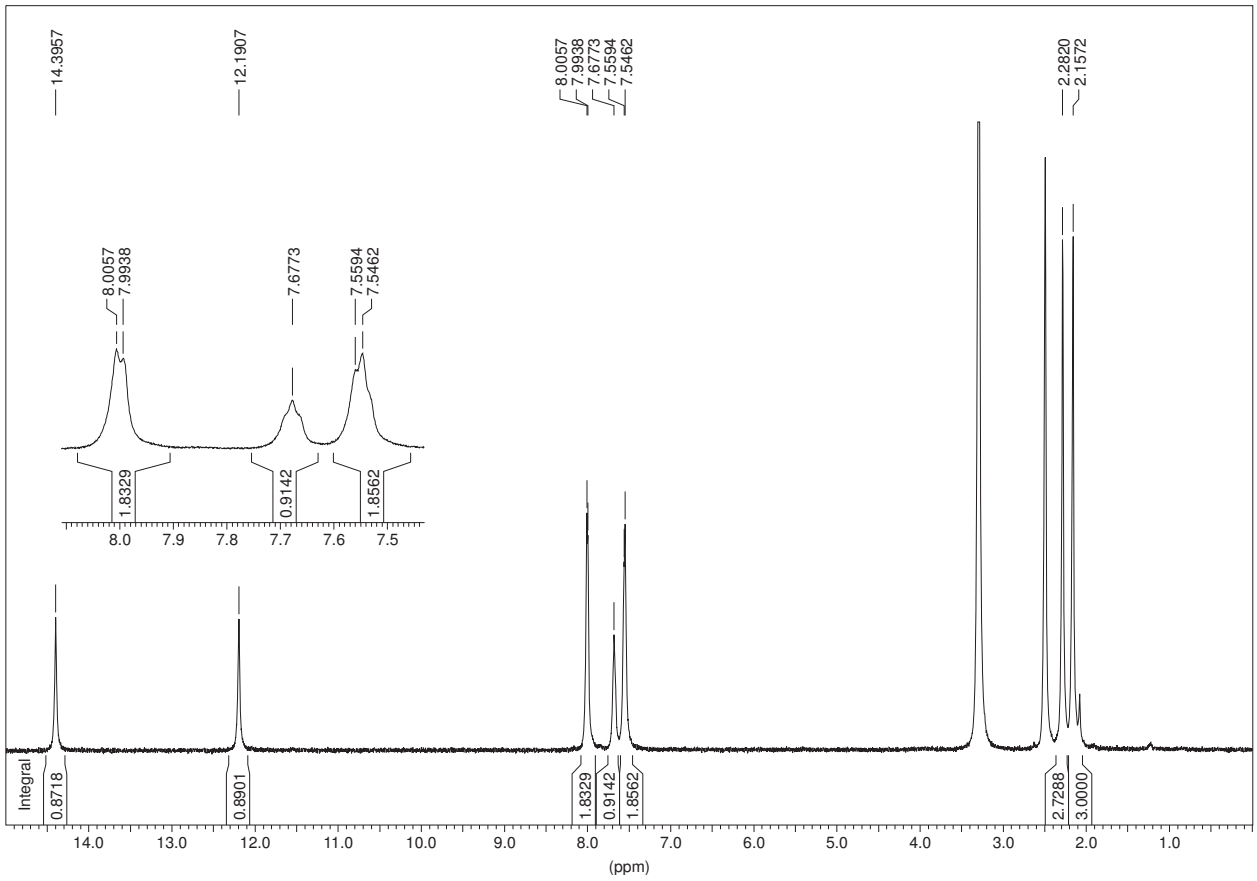


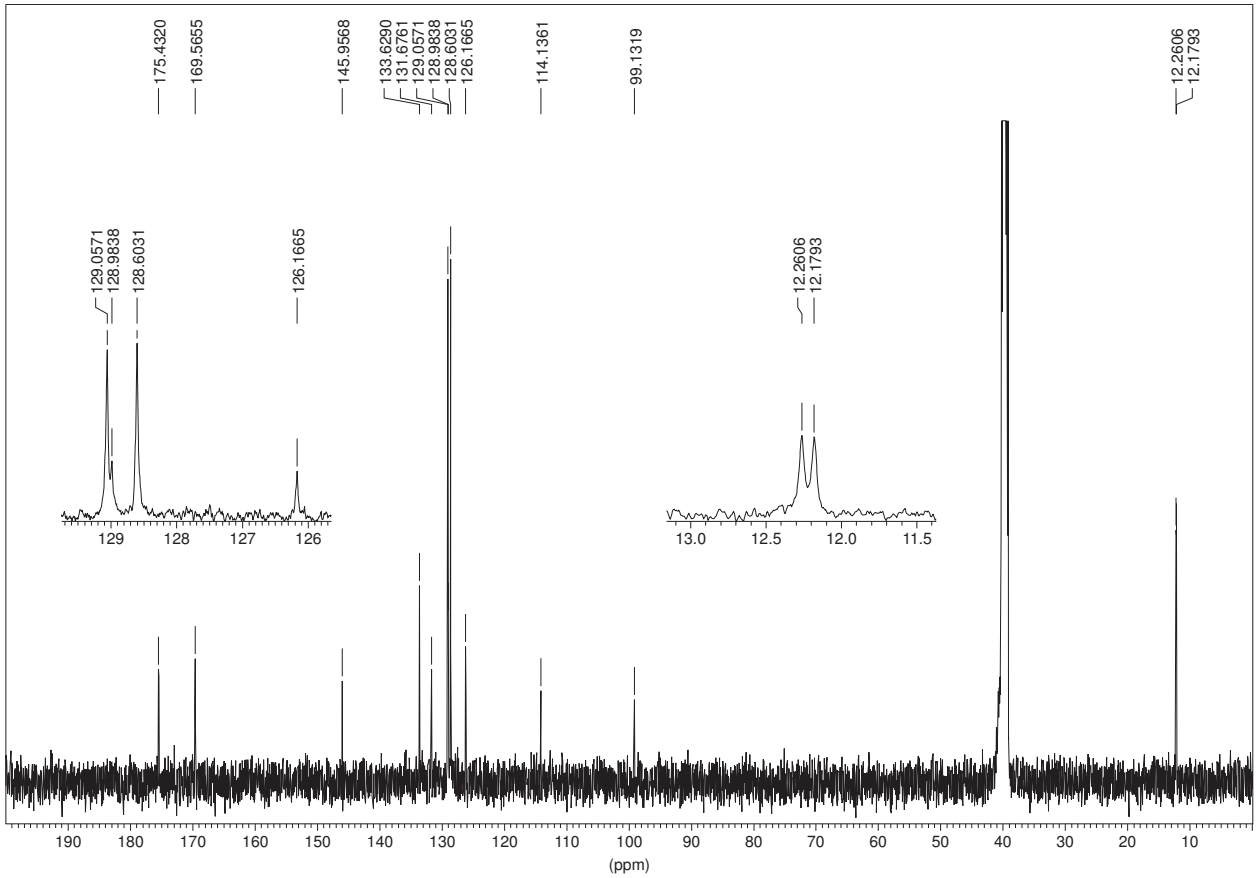
N-Benzoyl-*N*′-(3-cyano-2-thienyl)thiourea (**9**)



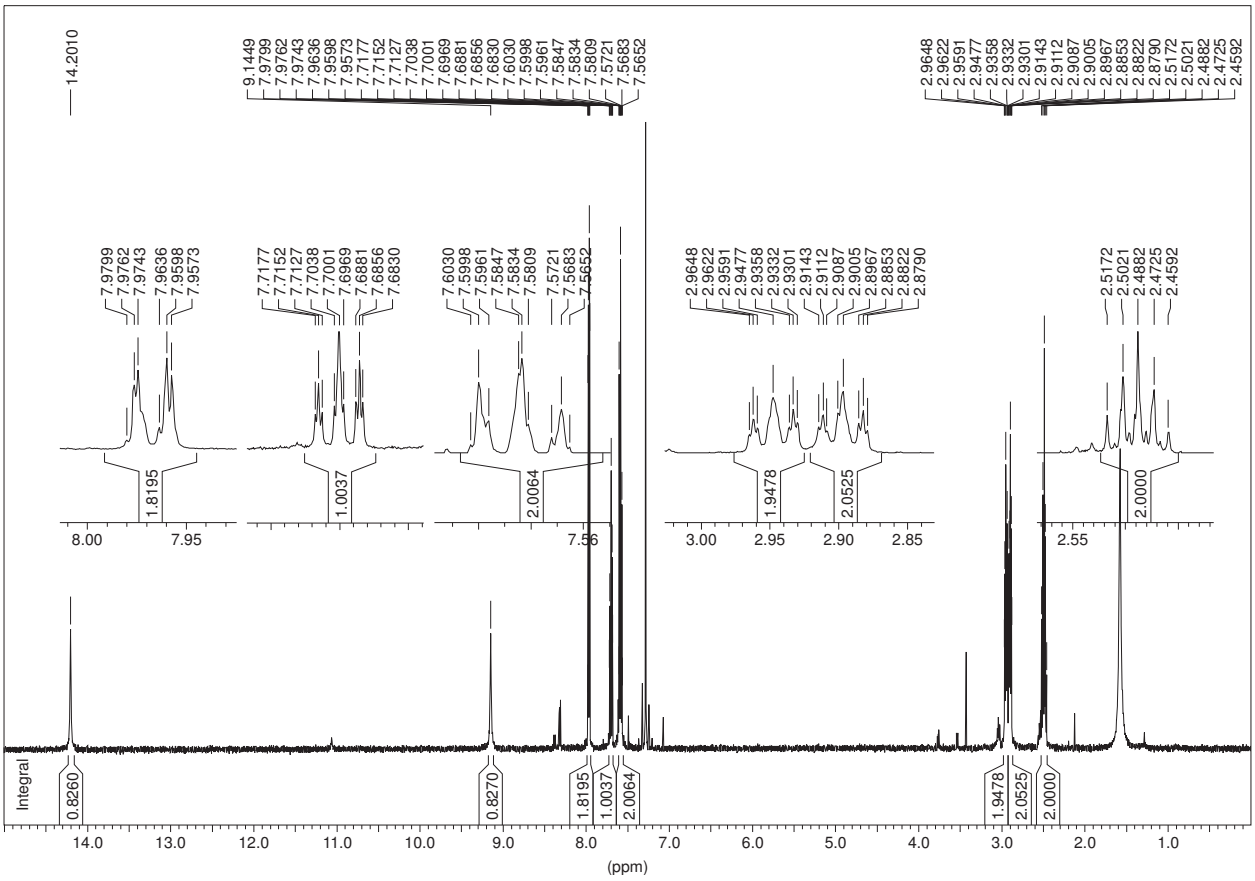


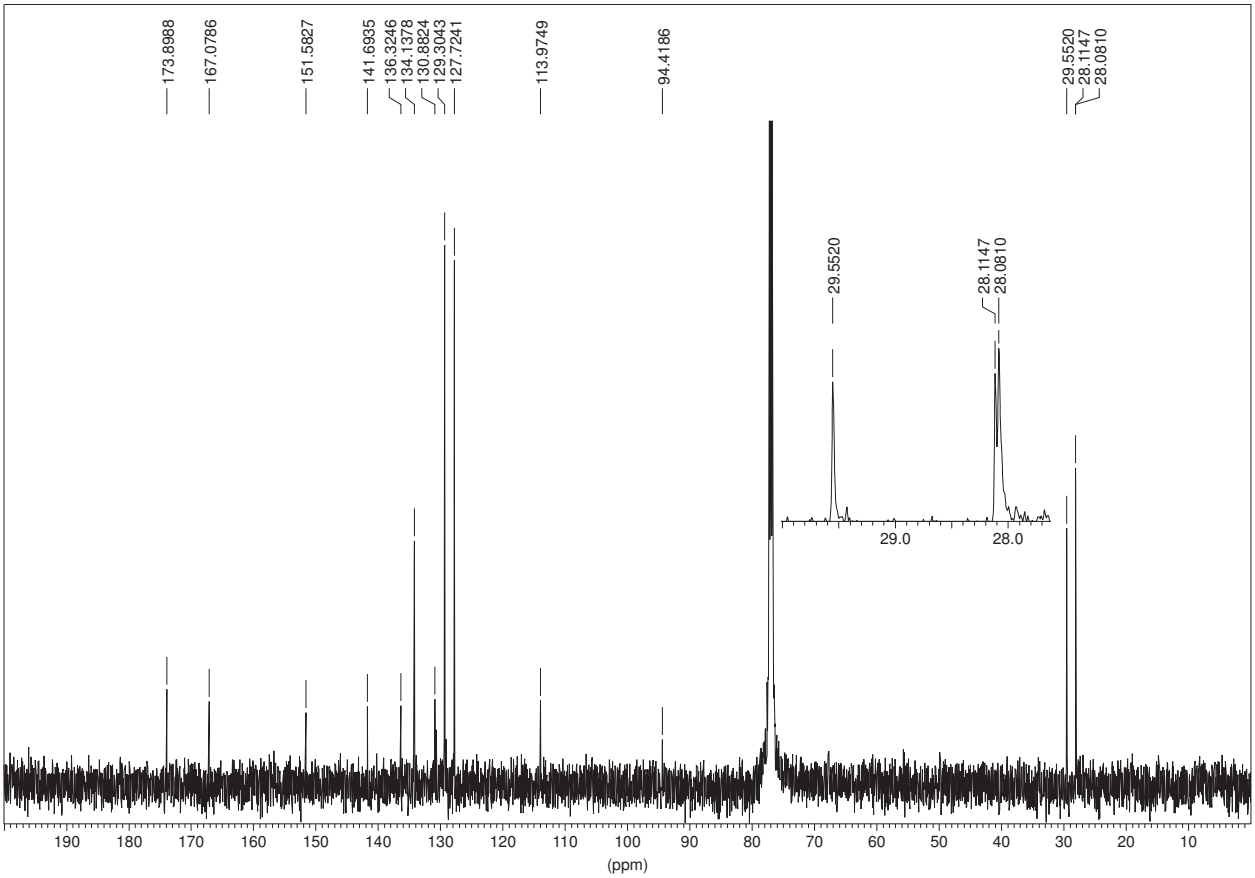
N-Benzoyl-*N*′-(3-cyano-4,5-dimethyl-2-thienyl)thiourea (**10**)



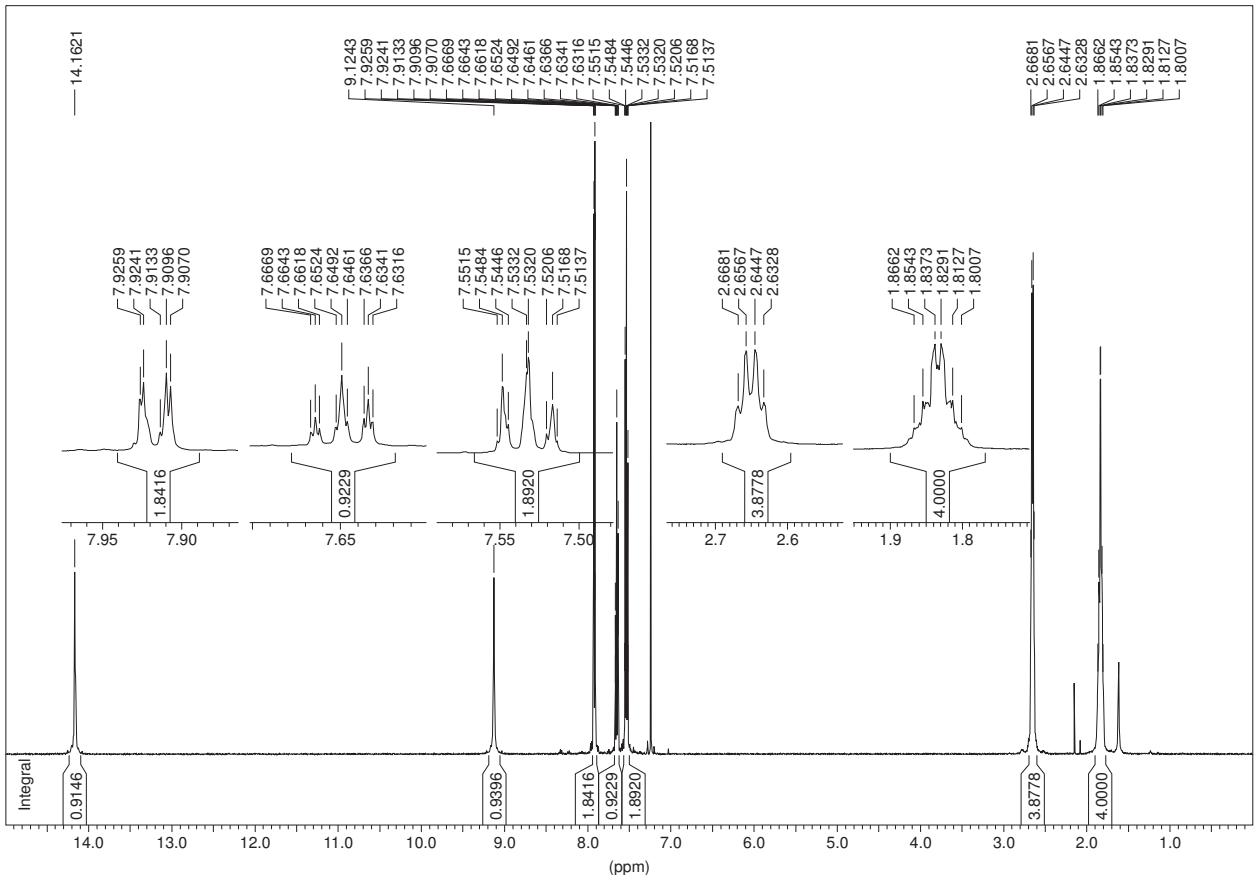


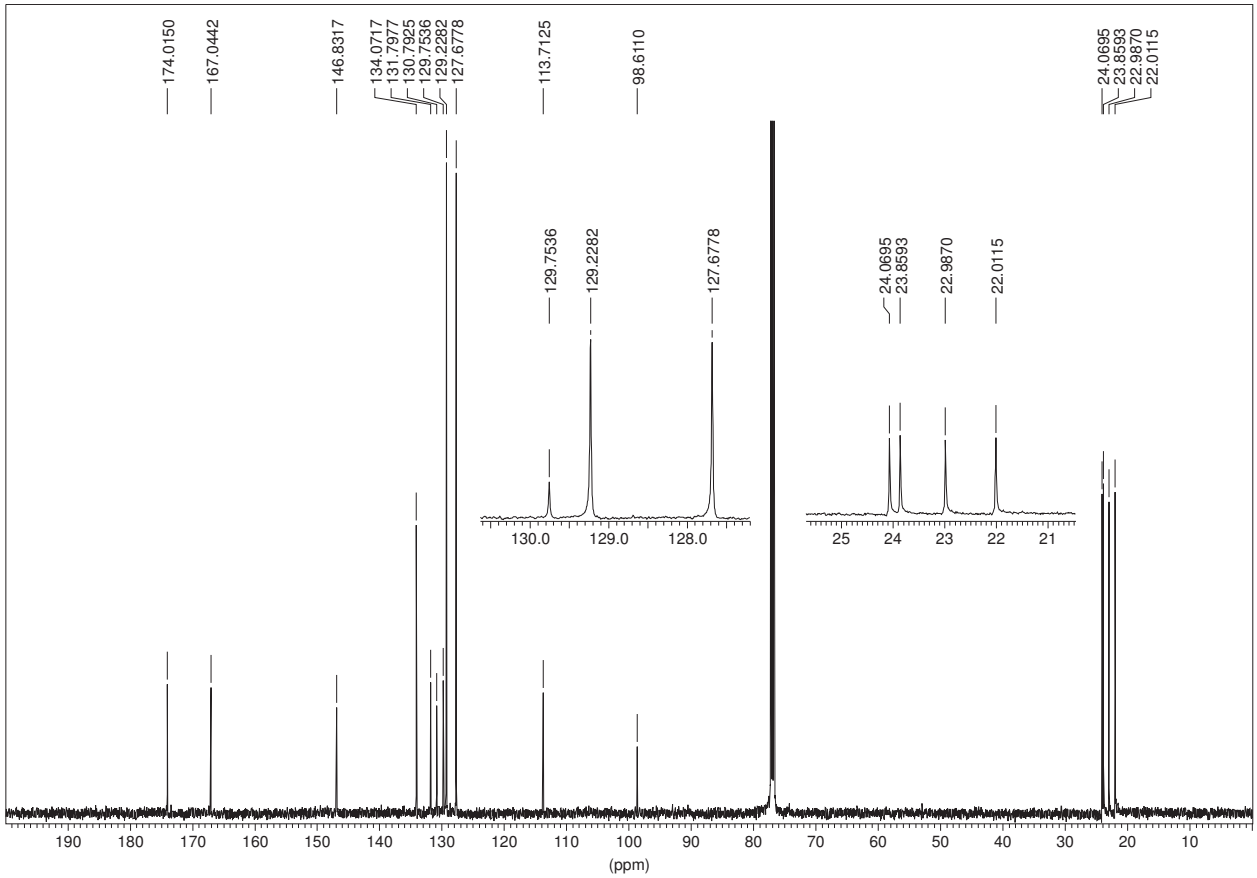
N-Benzoyl-*N*′-(3-cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)thiourea (**11**)



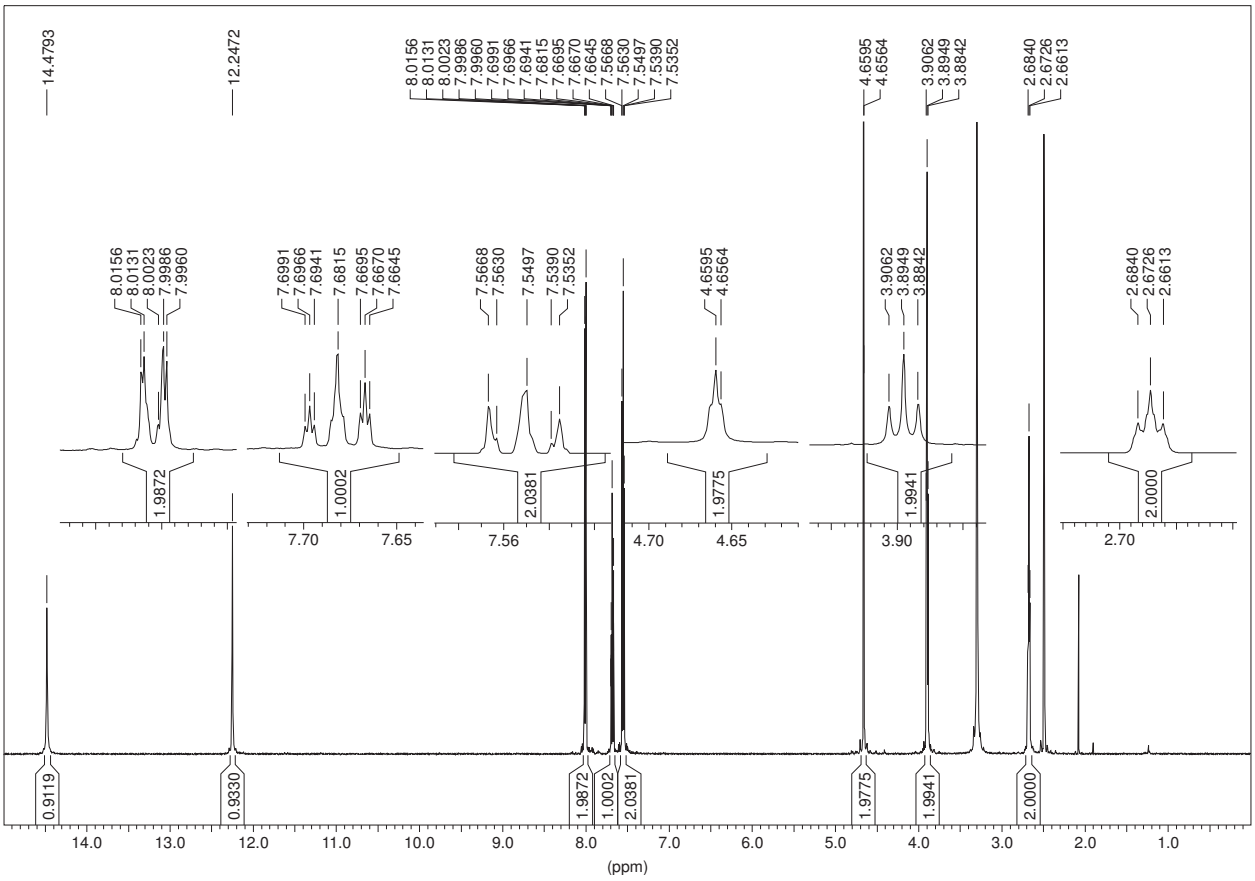


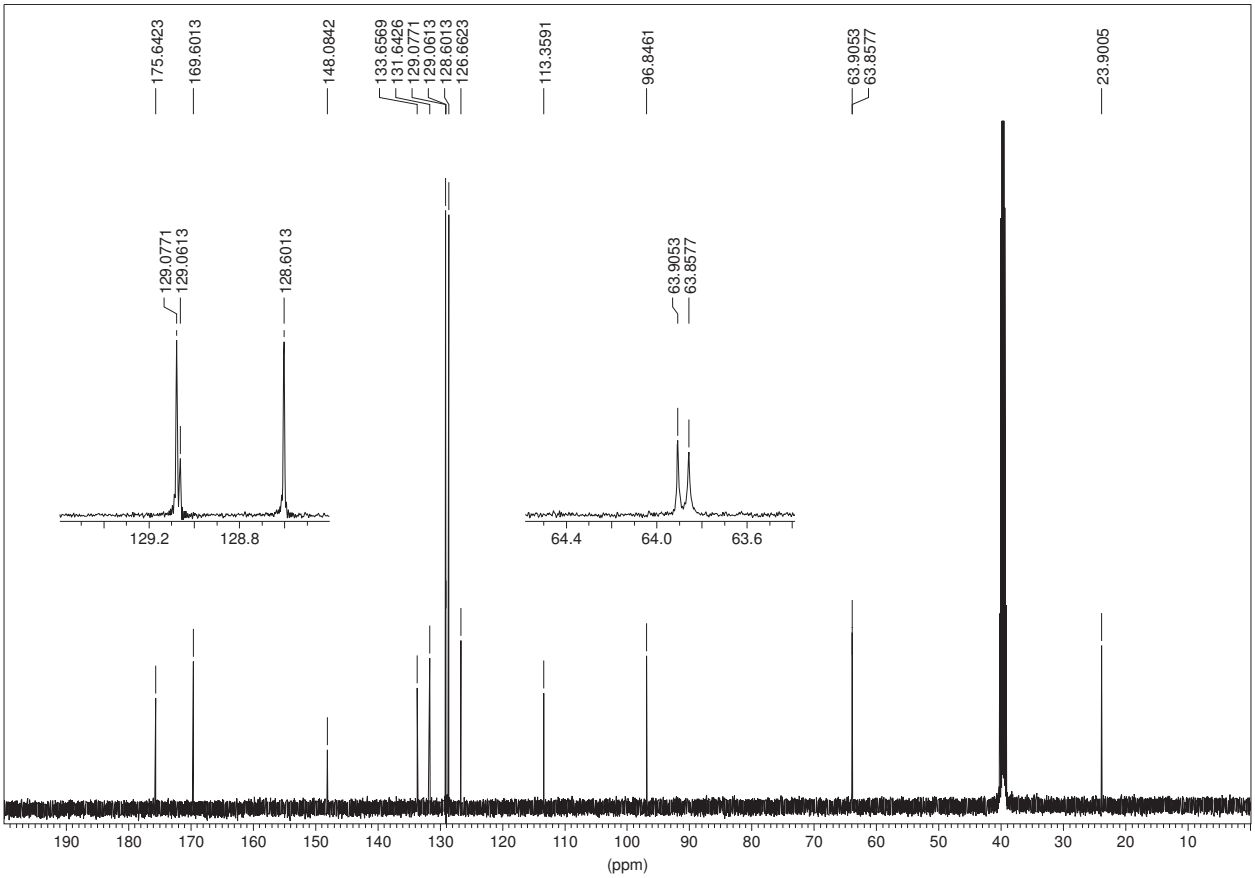
N-Benzoyl-*N*′-(3-cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)thiourea (**12**)





N-Benzoyl-*N*′-(3-cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)thiourea (**13**)





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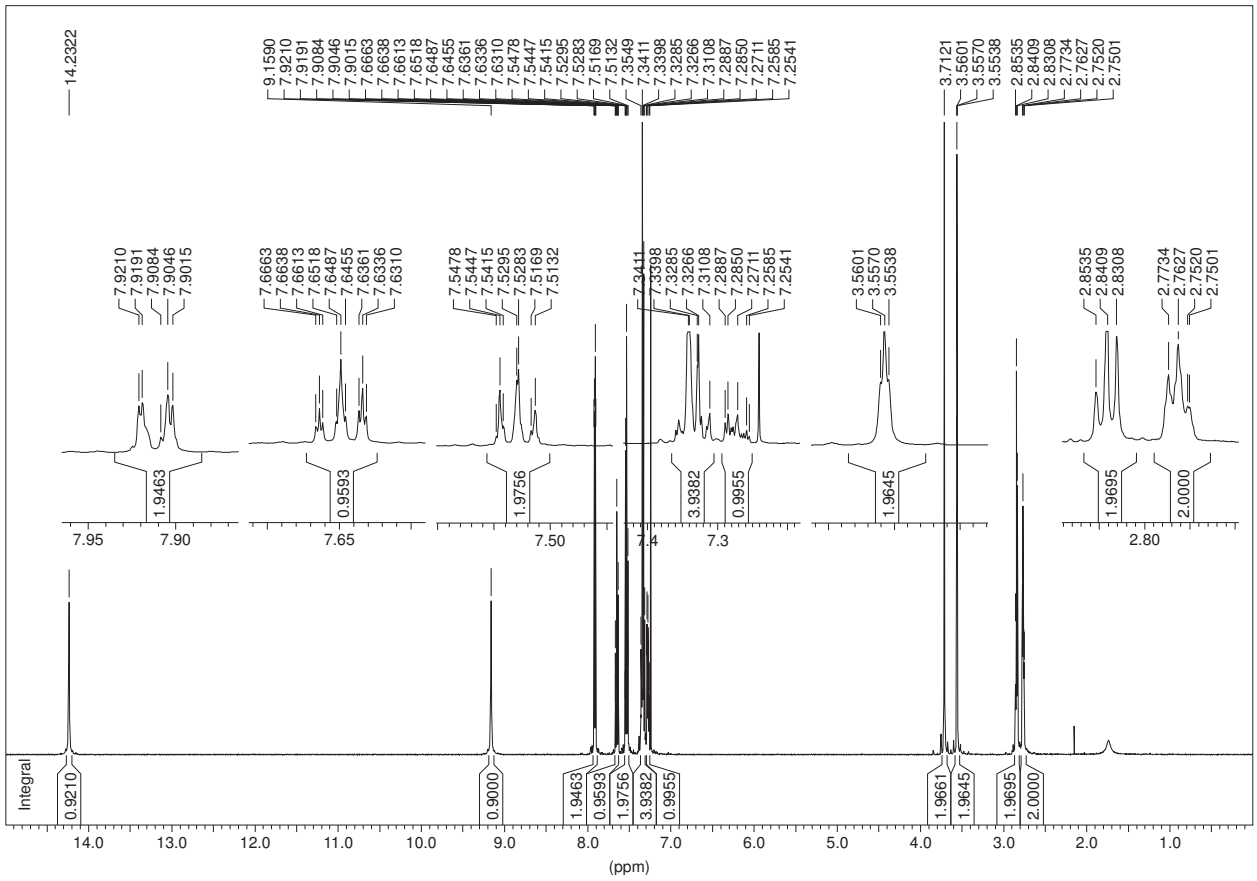

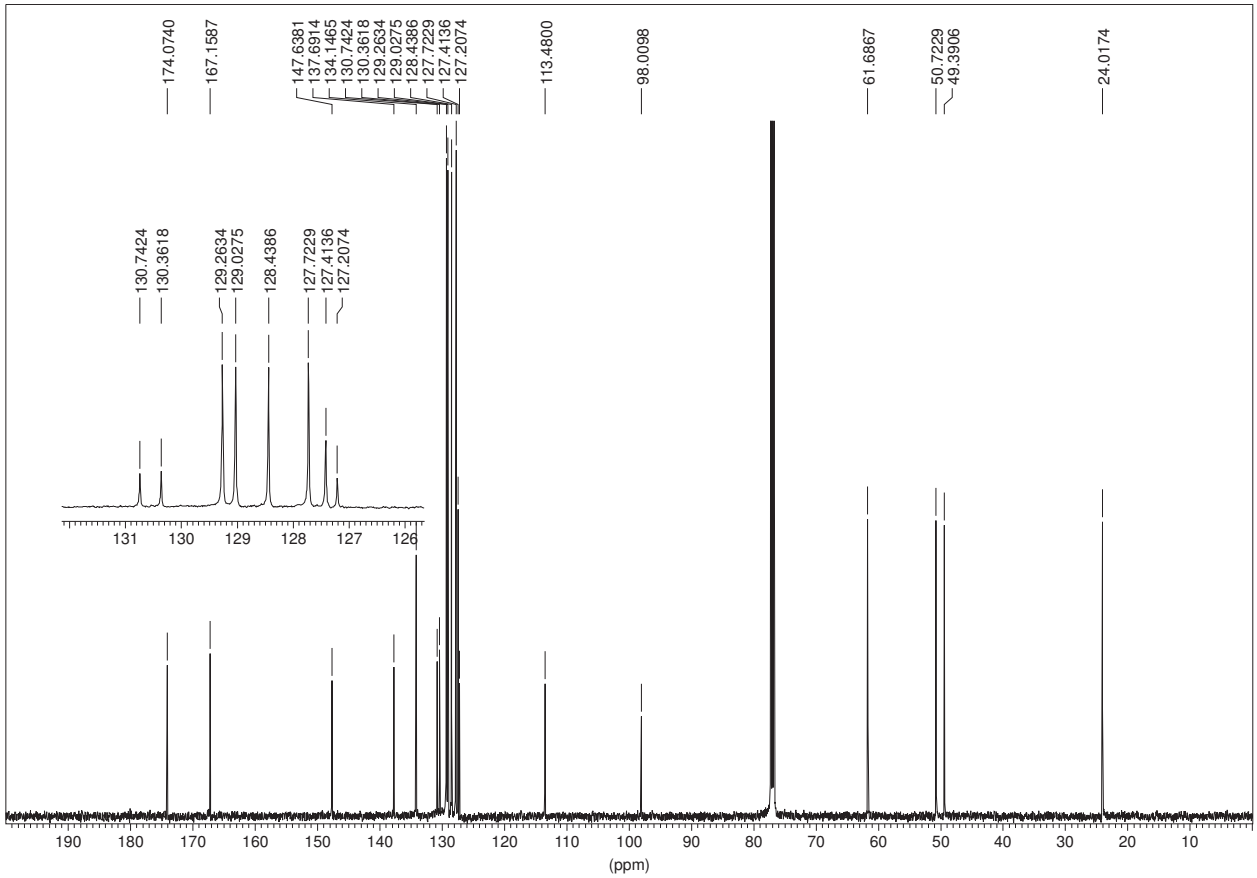

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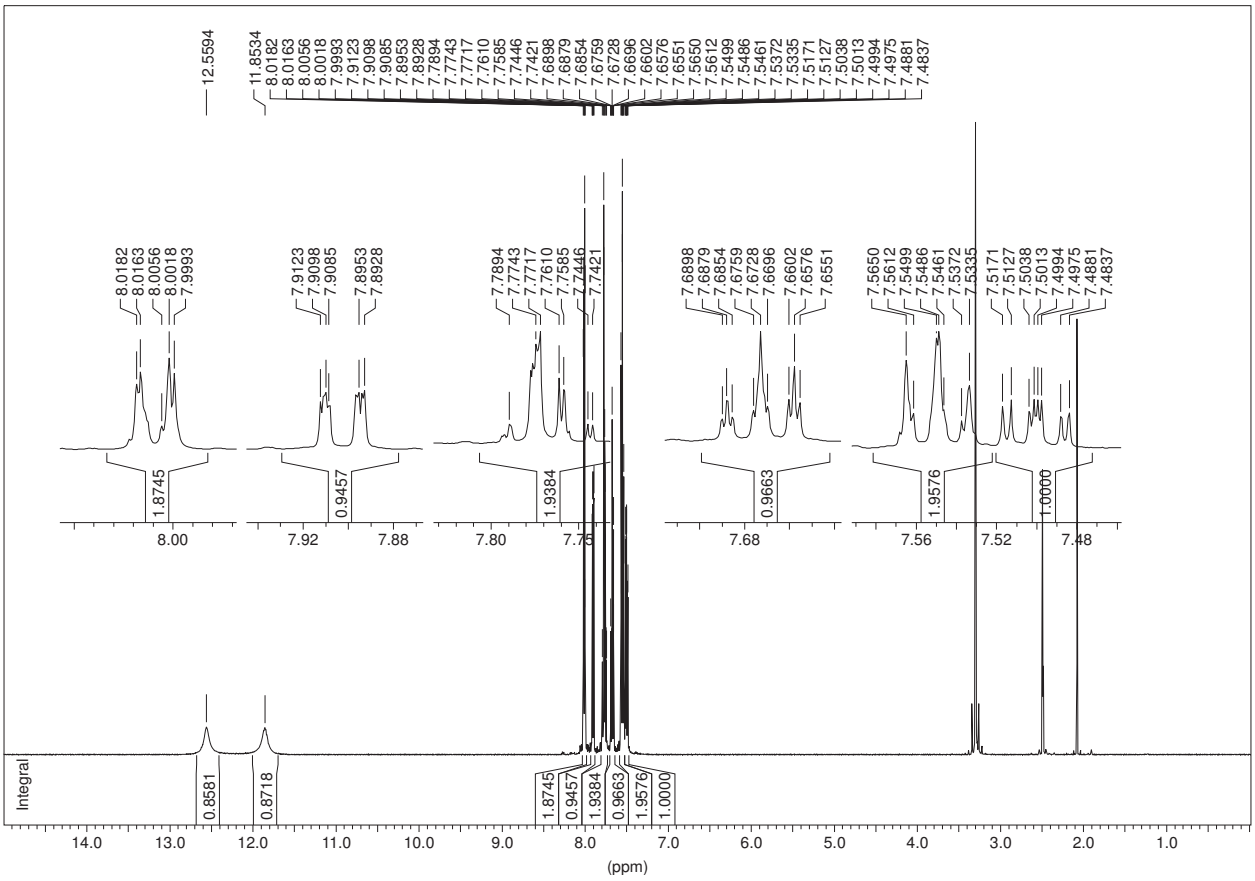
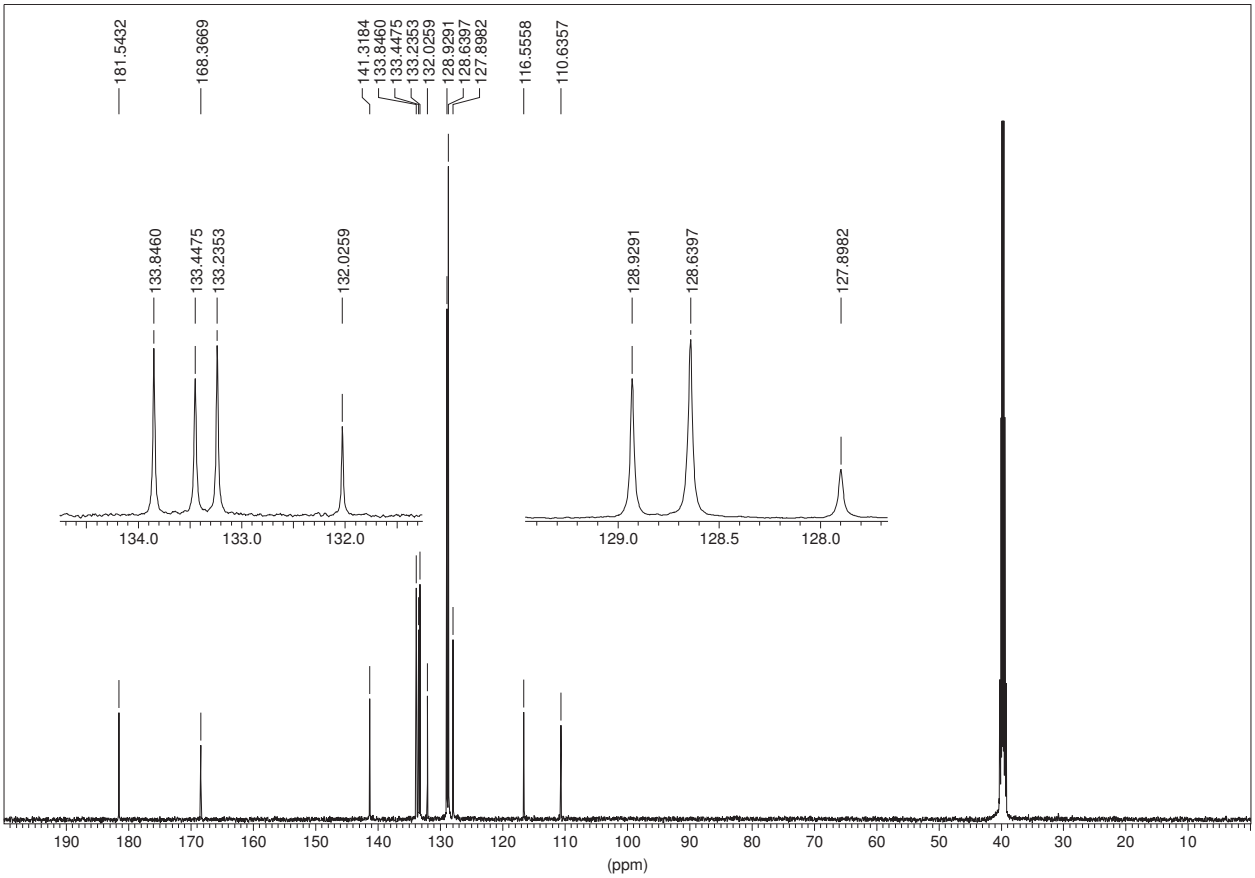
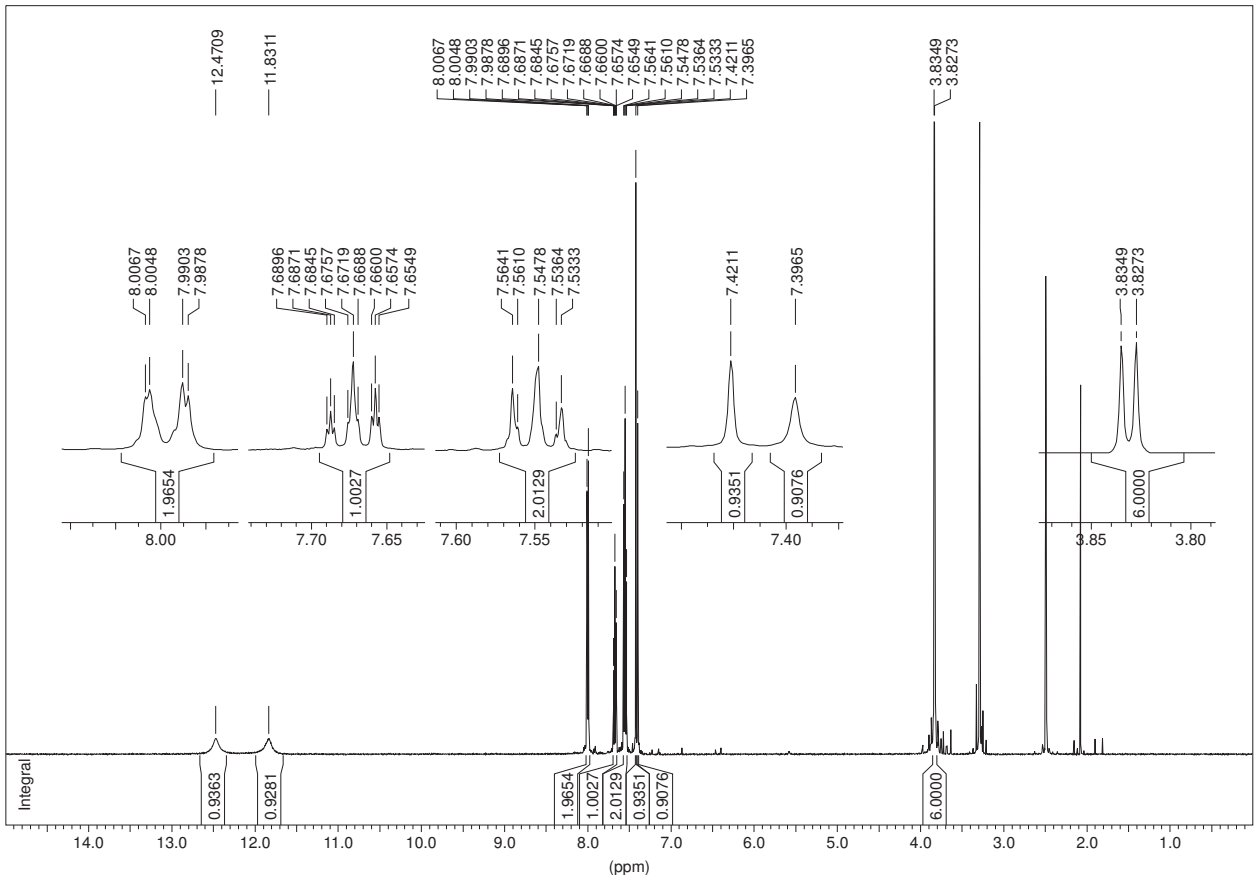
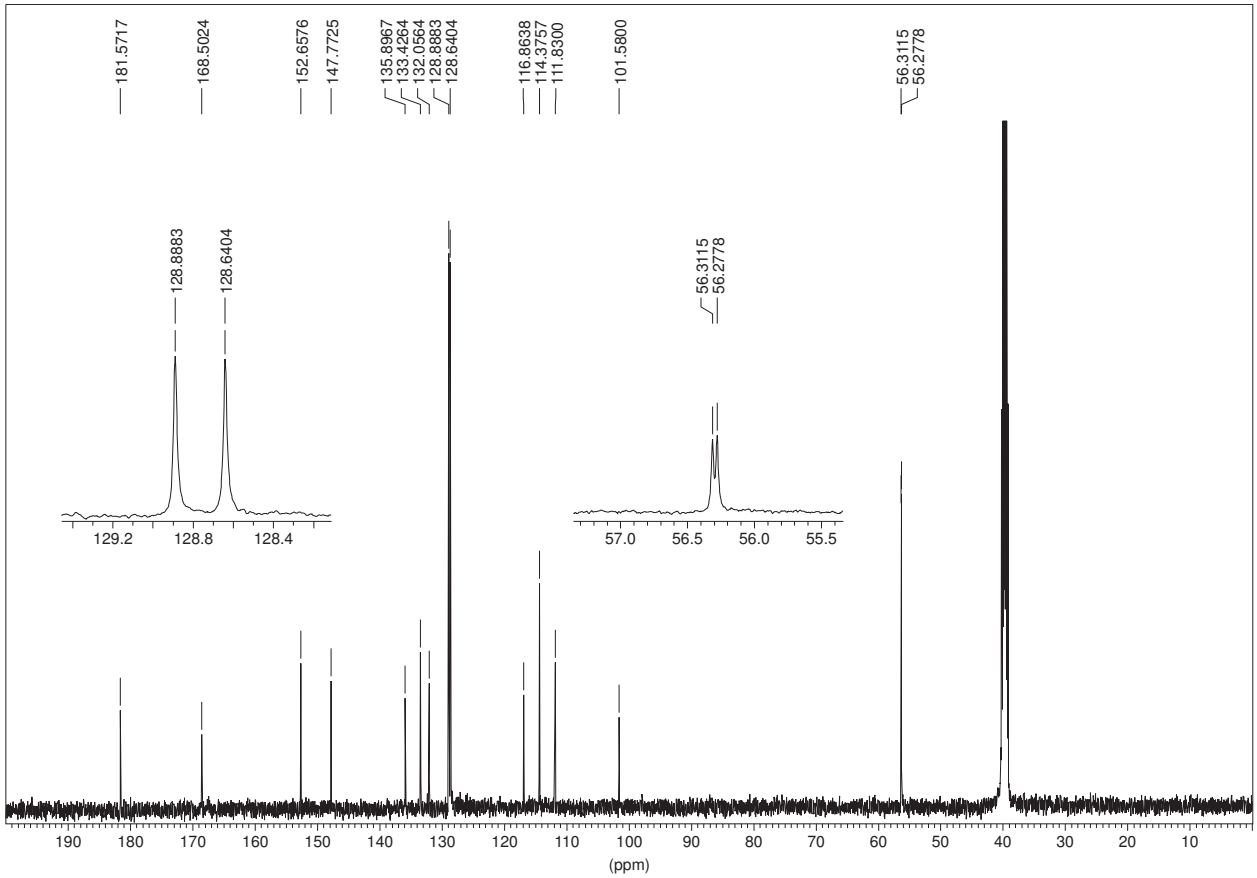


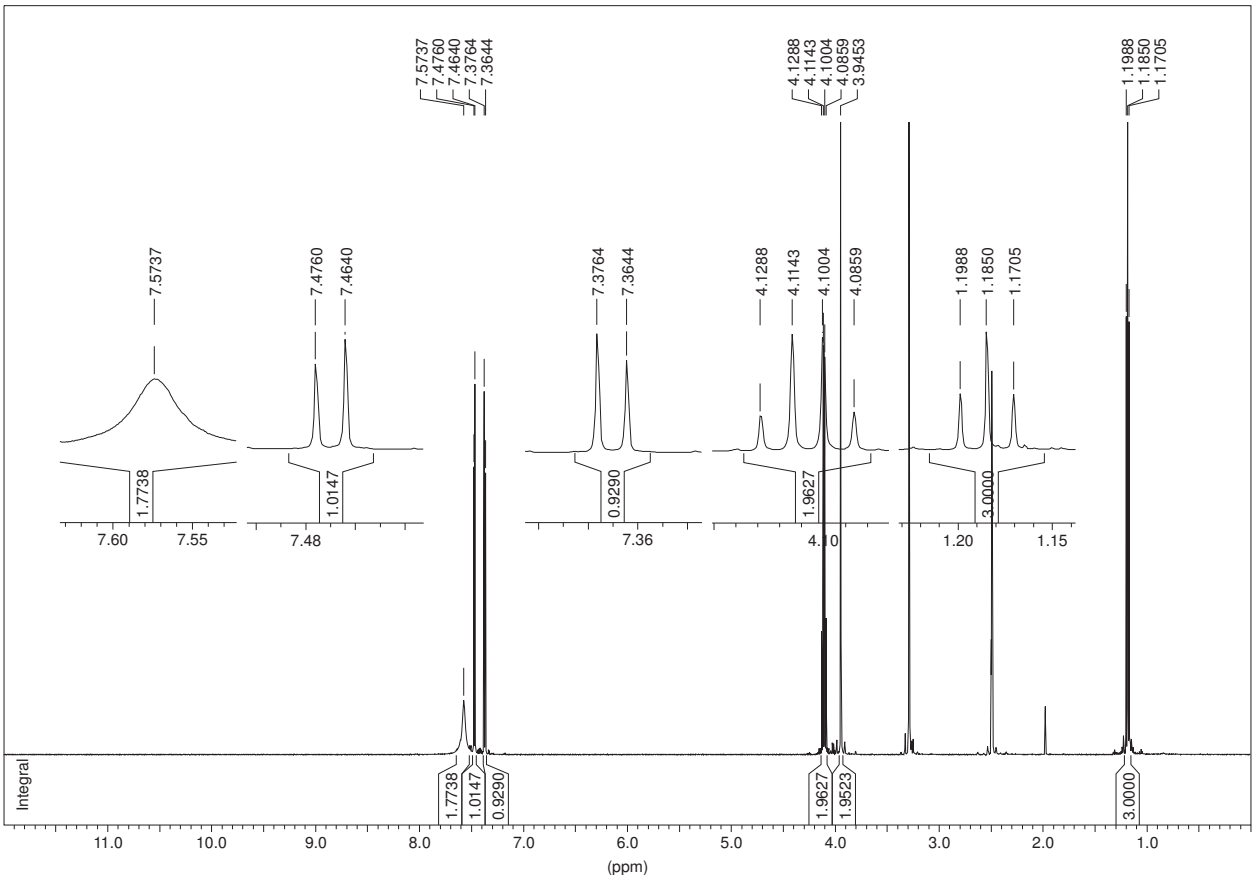


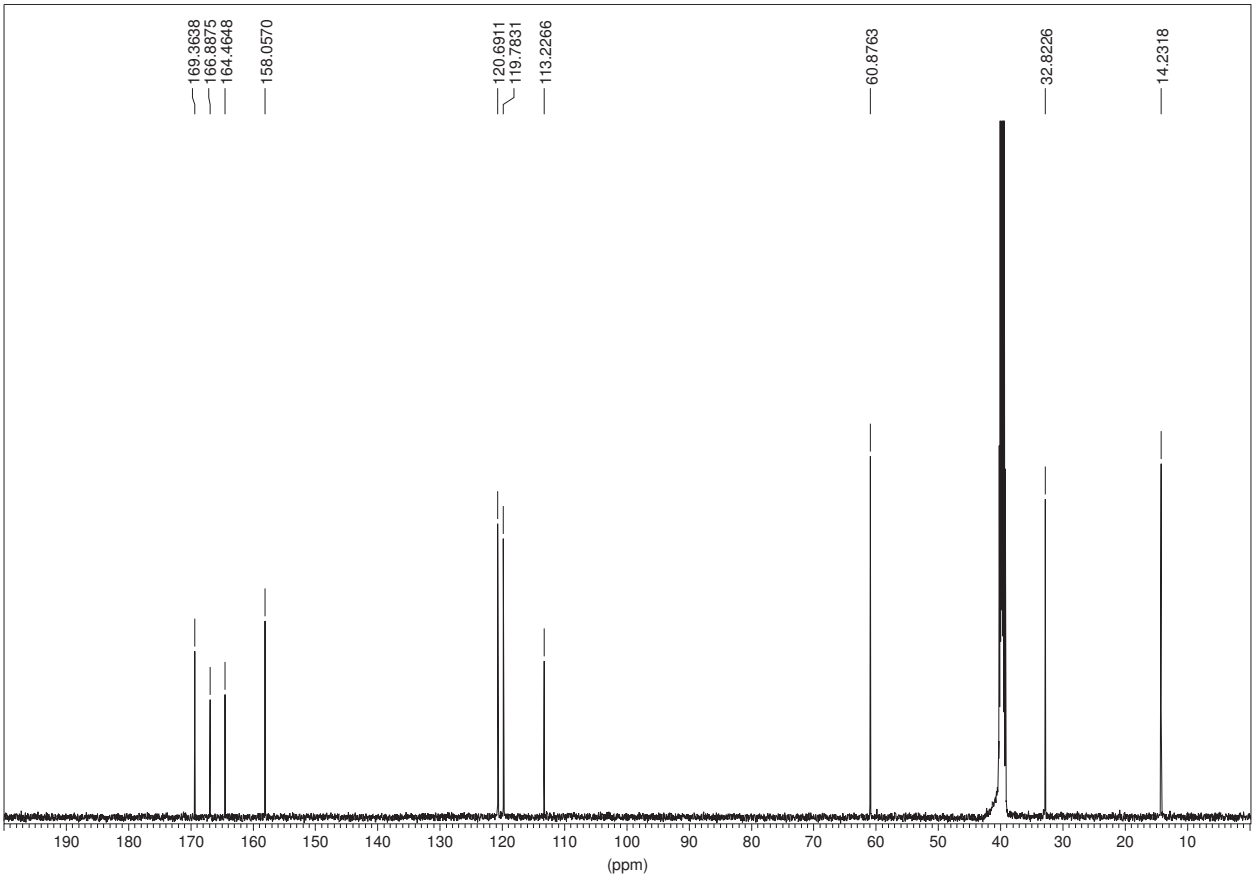
N-Benzoyl-*N*′-(2-cyano-4,5-dimethoxyphenyl)thiourea (**16**)





Ethyl [(4-Aminothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]acetate (**17**)





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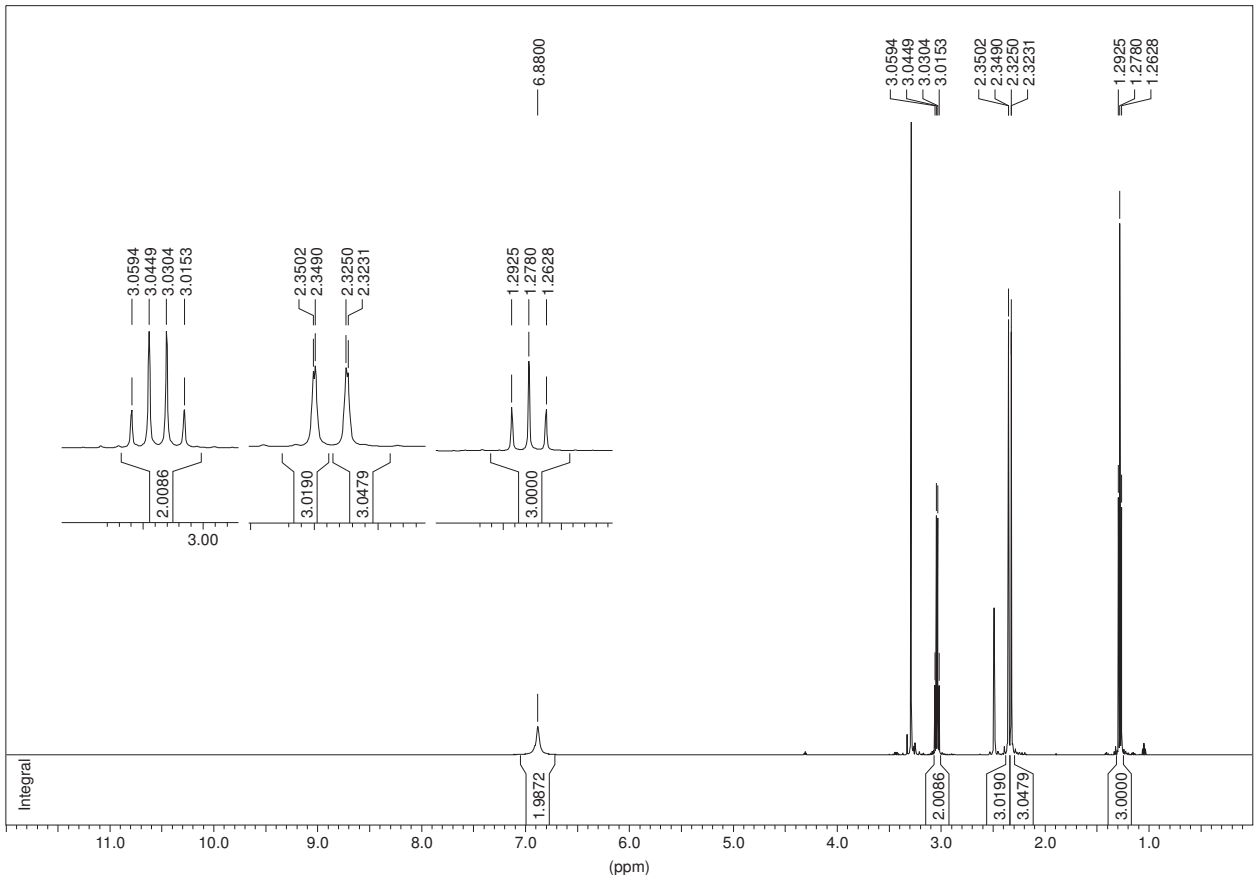
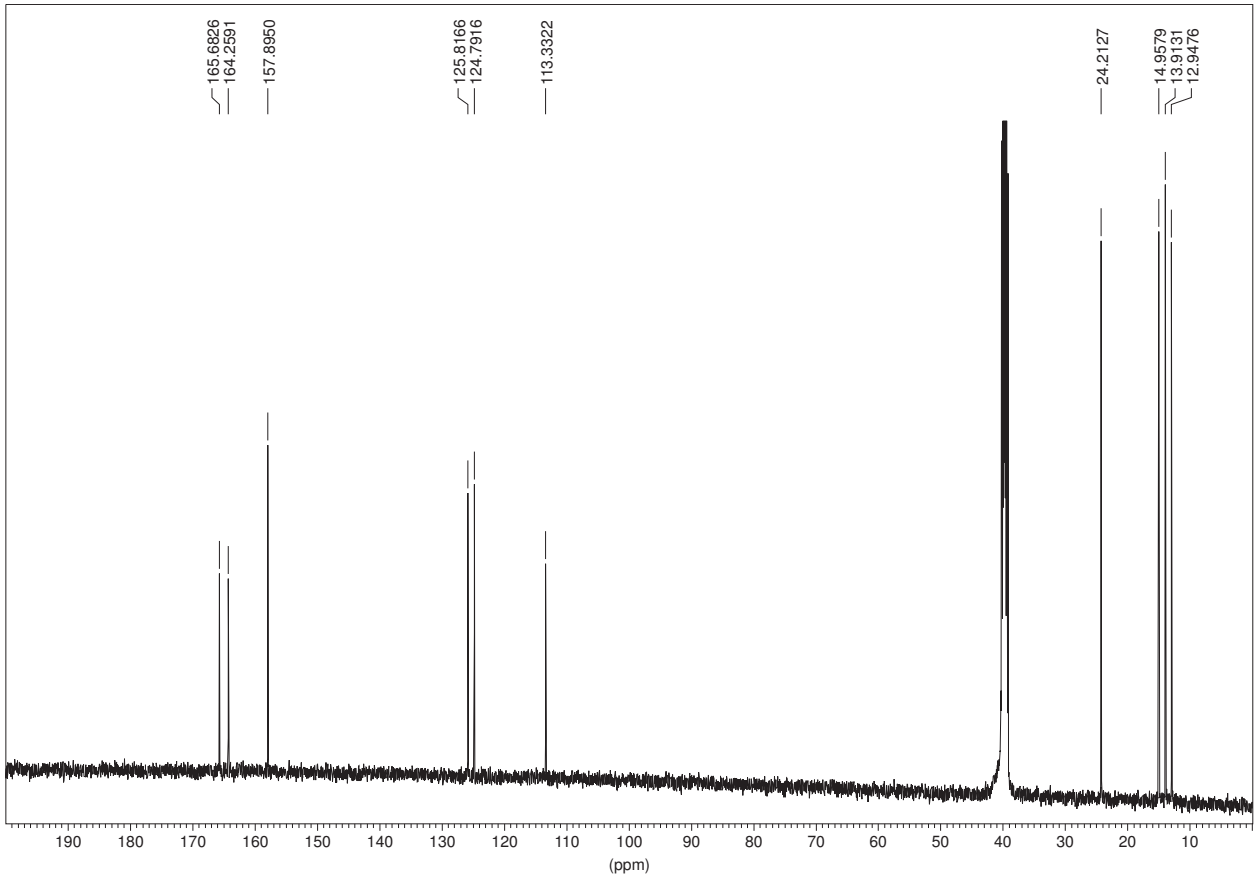
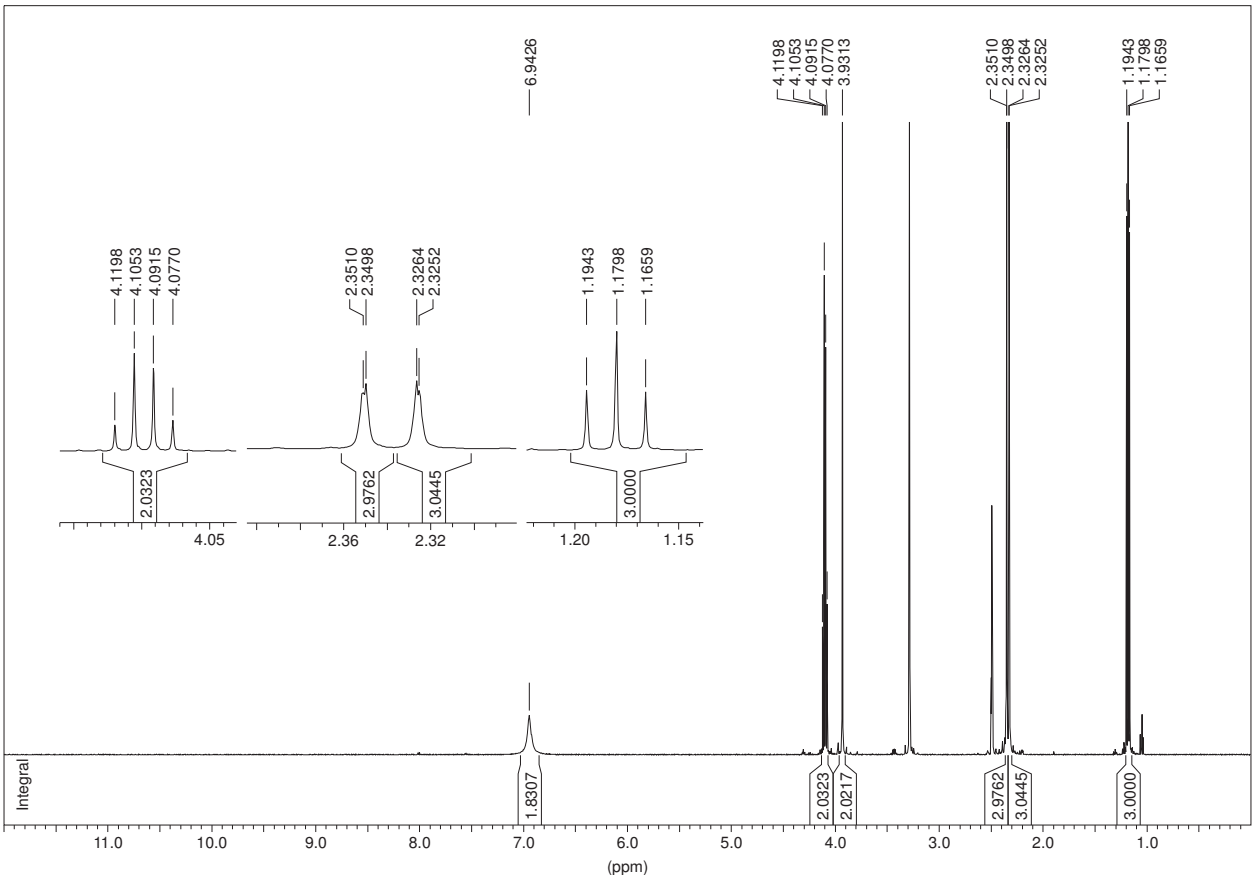
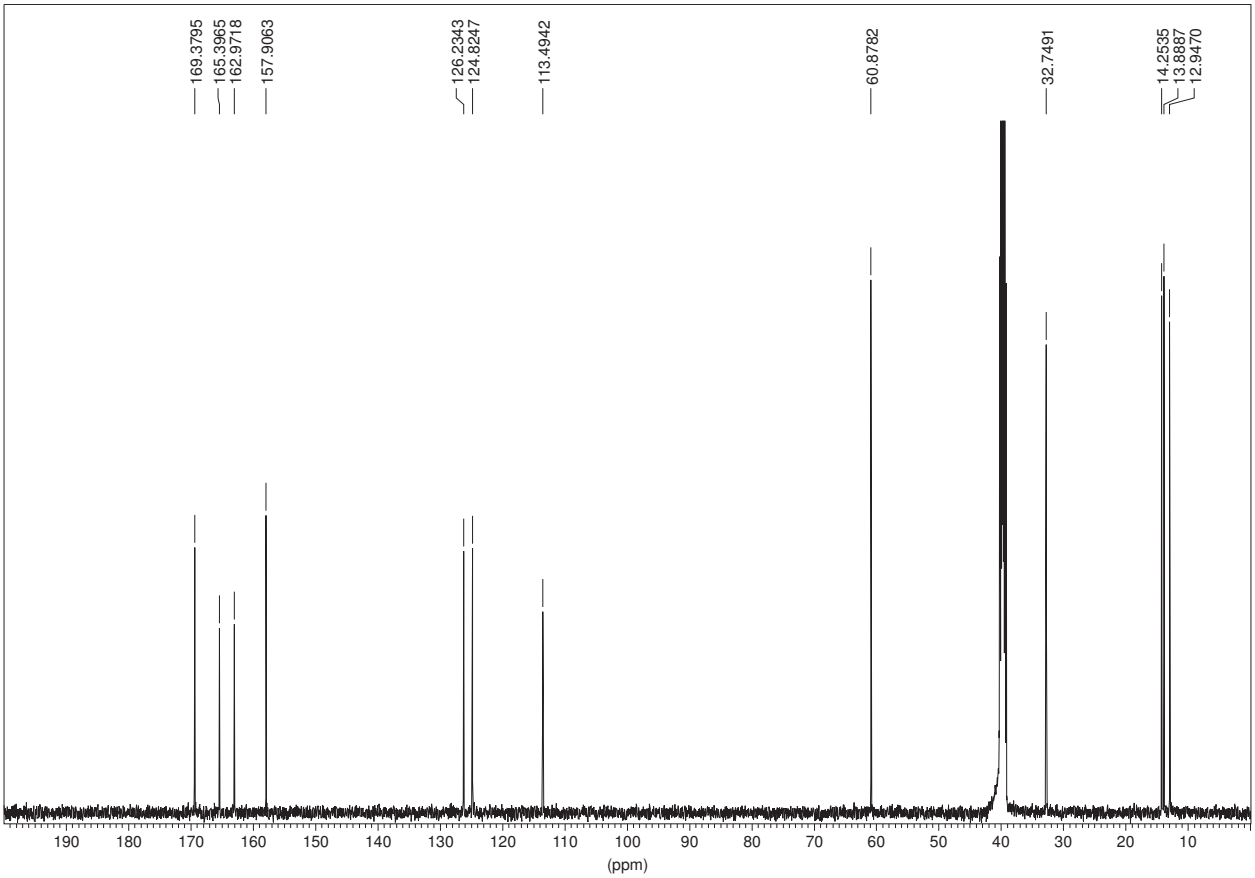


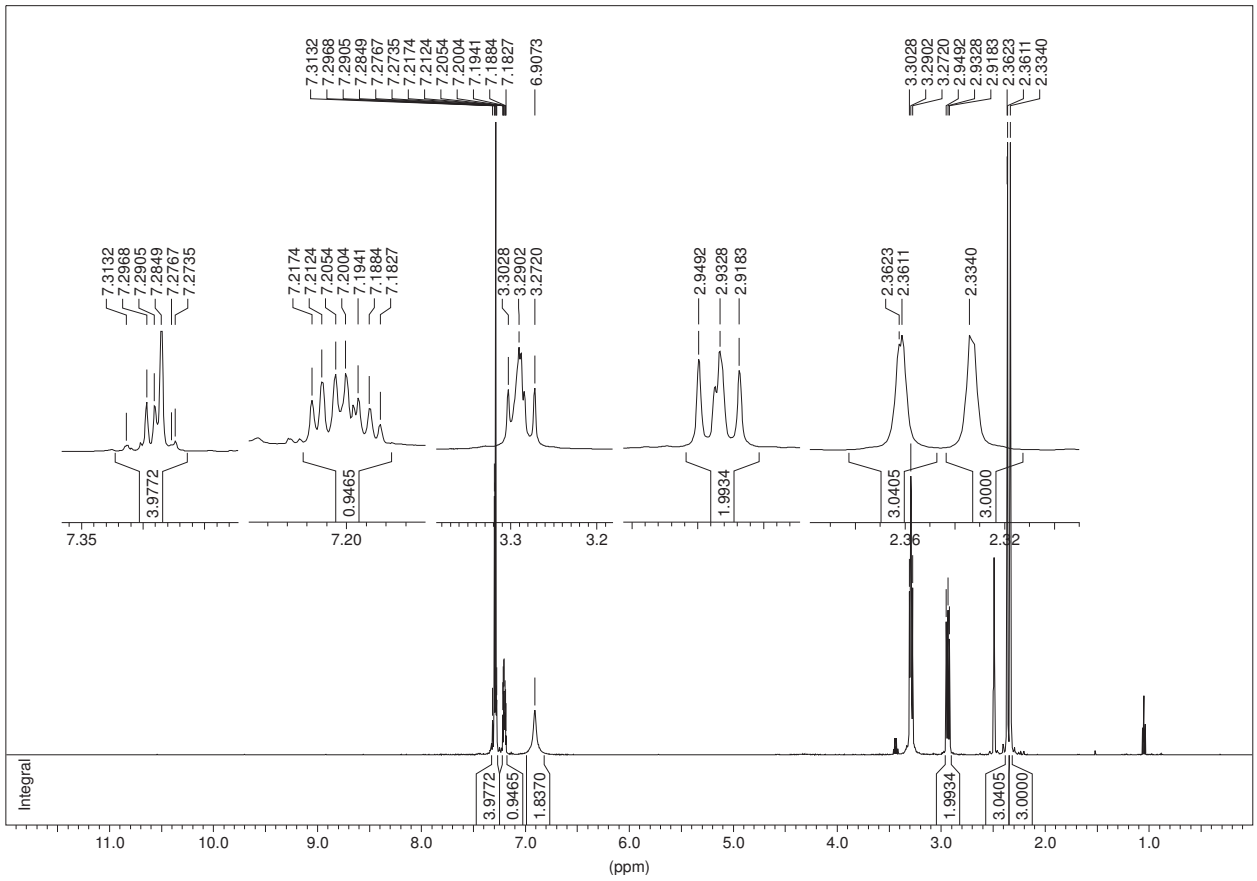


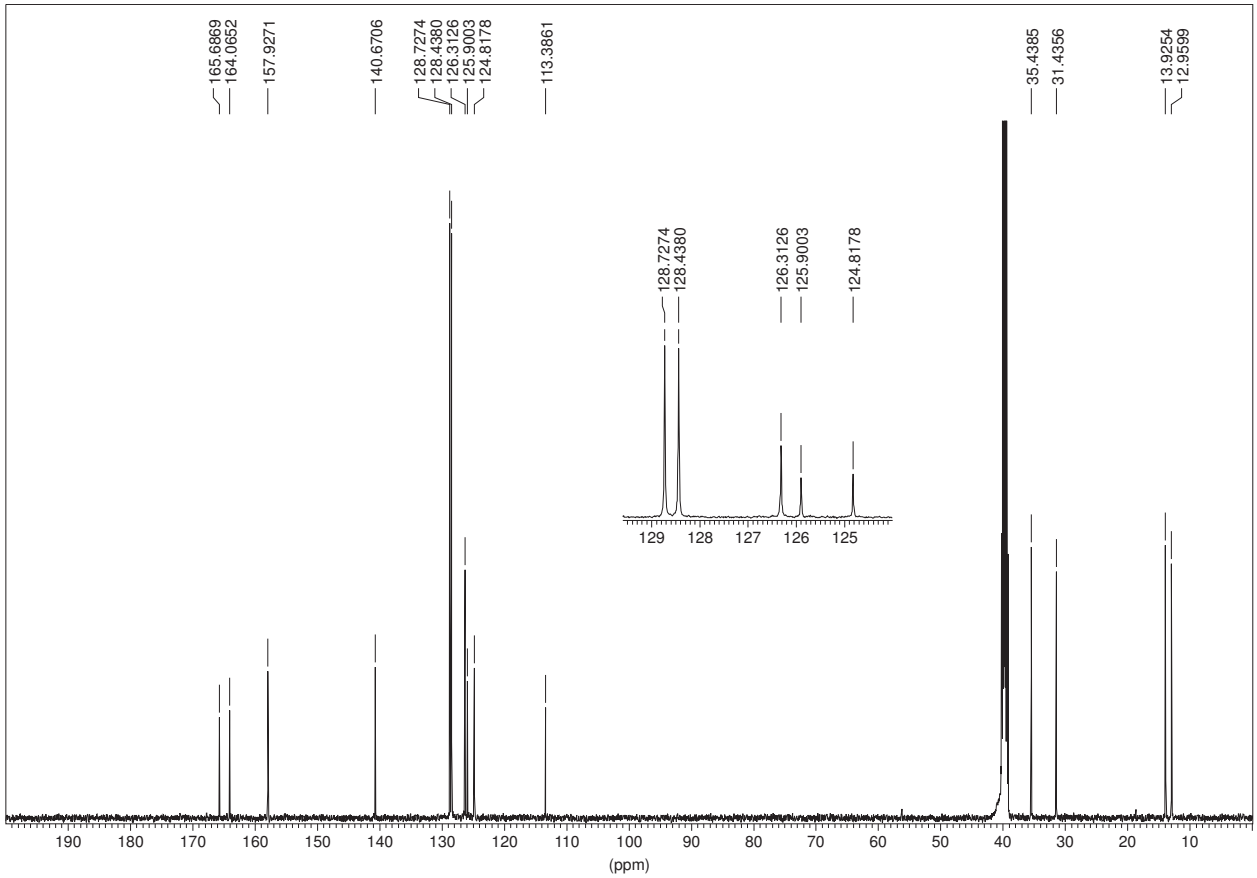
Ethyl [(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]acetate (**21**)



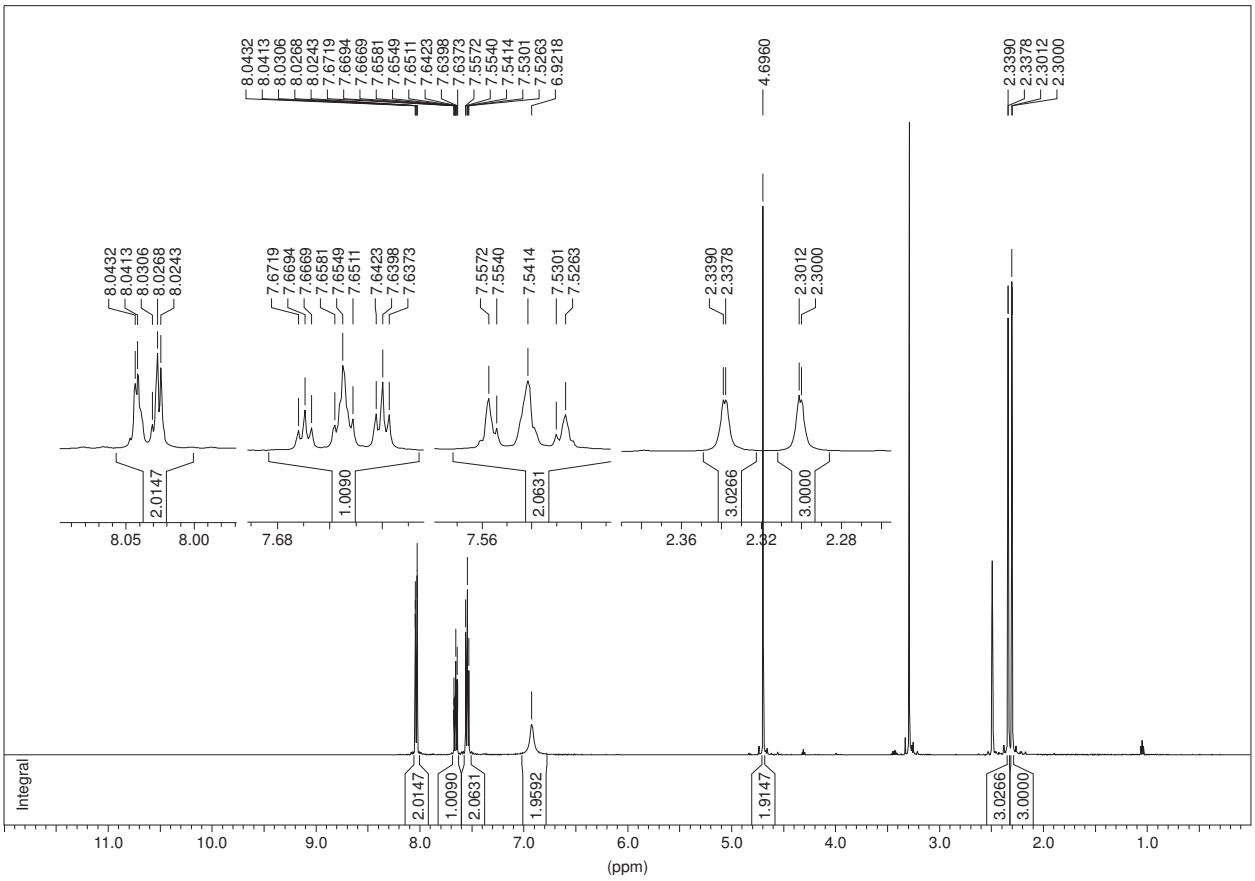


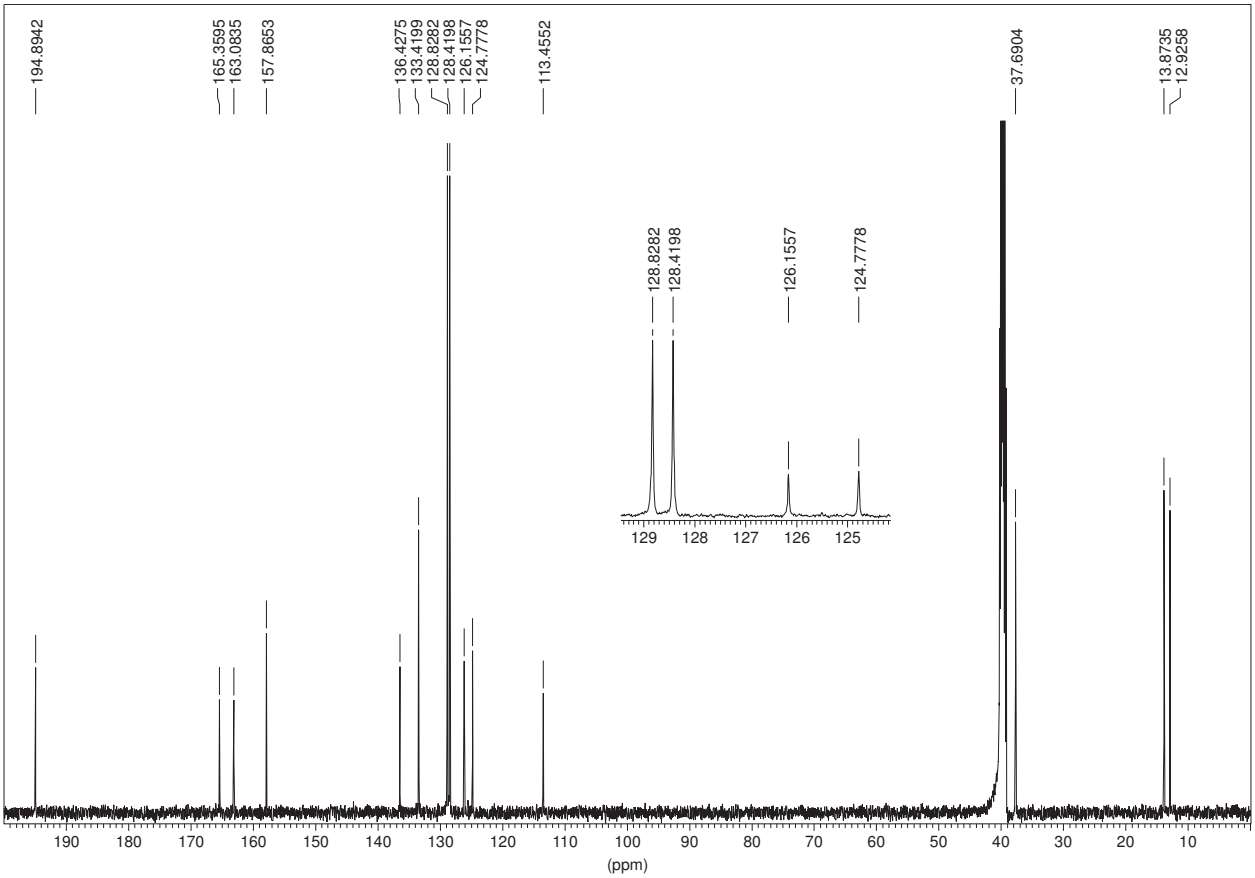
5,6-Dimethyl-2-[(2-phenylethyl)sulfanyl]thieno[2,3-*d*]pyrimidin-4-amine (**22**)



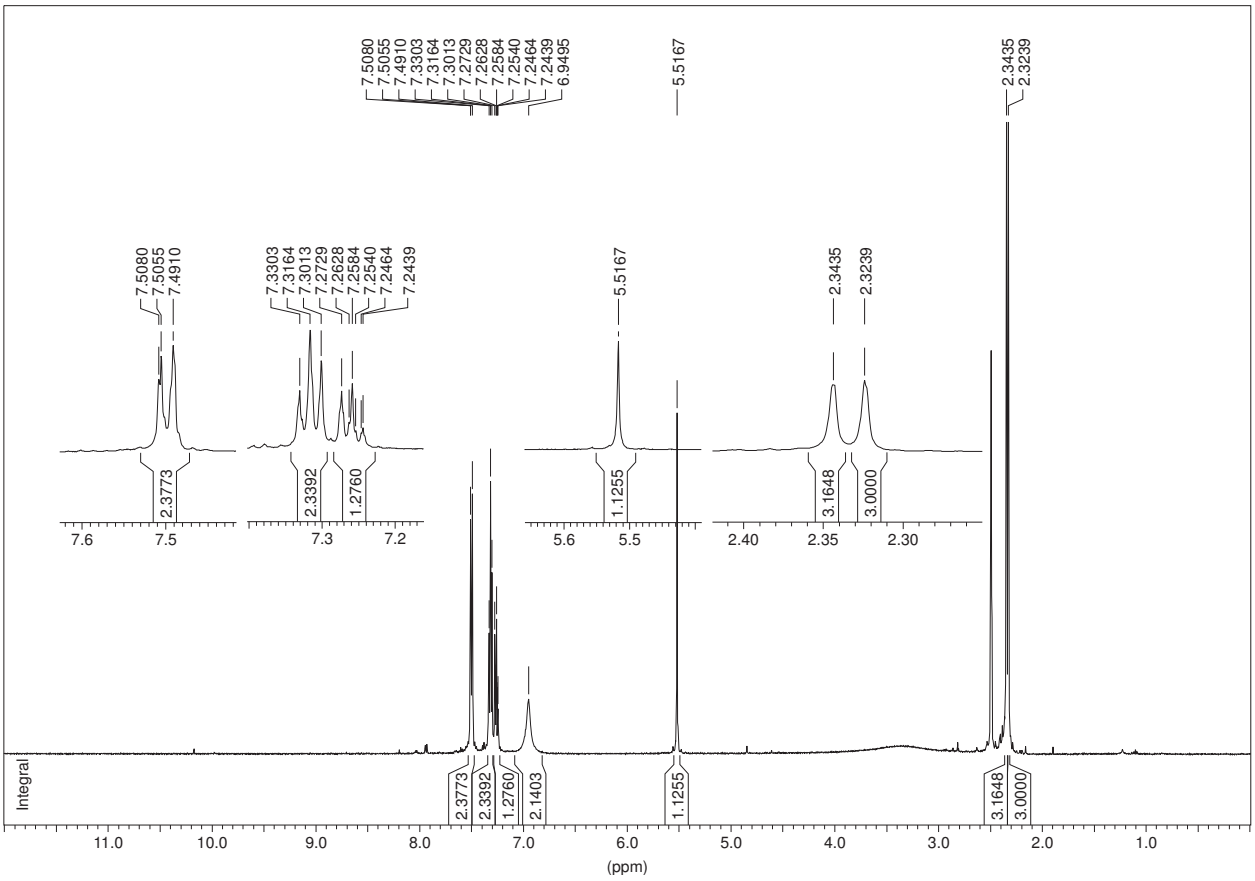


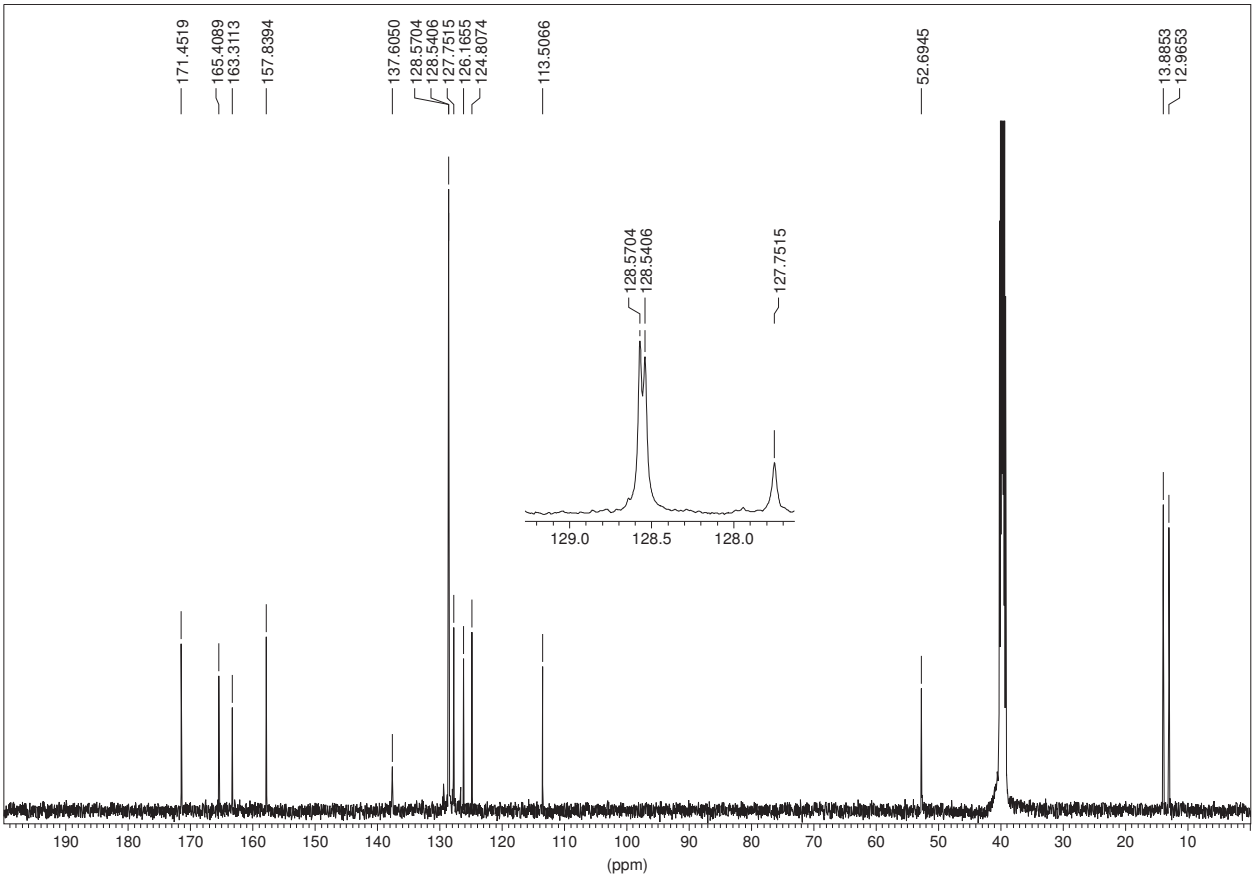
2-[(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-1-phenylethanone (**23**)



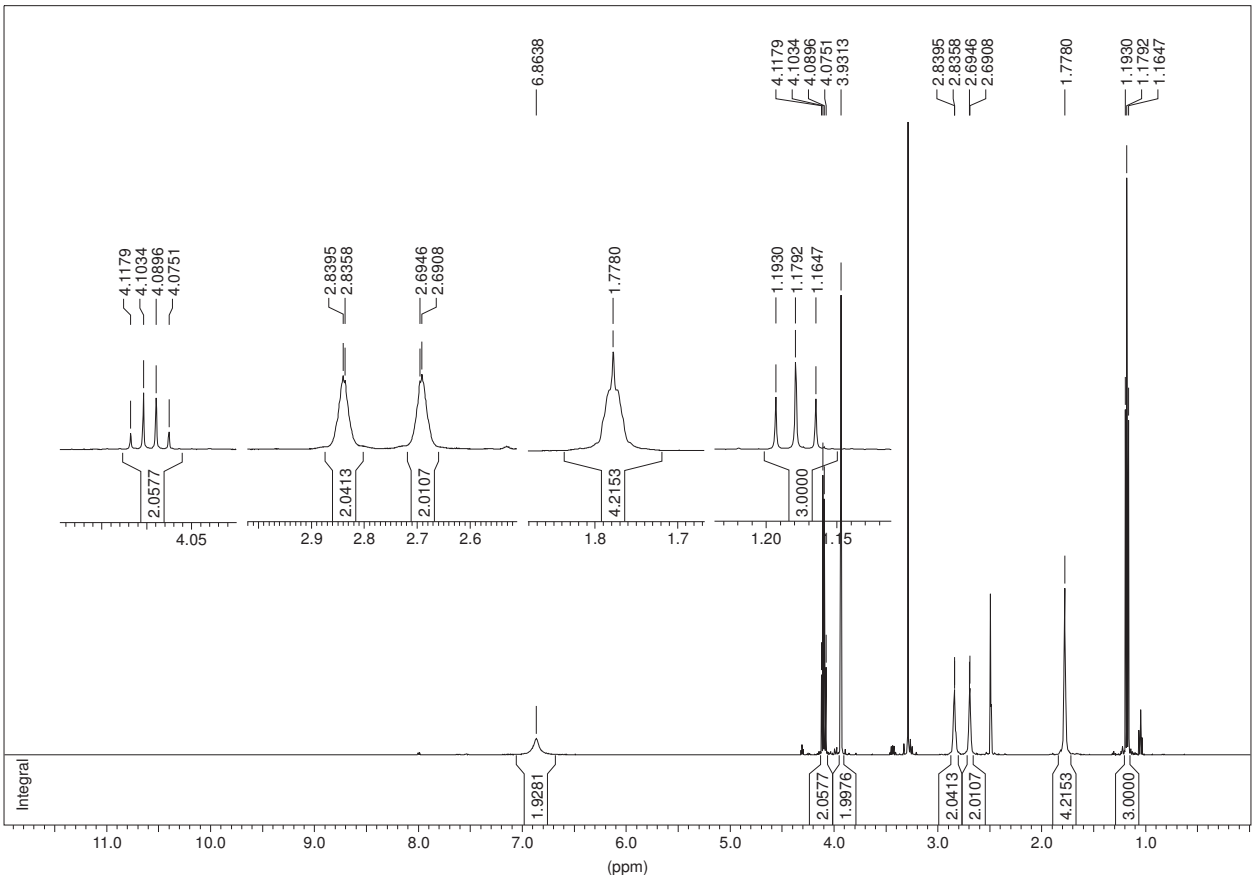


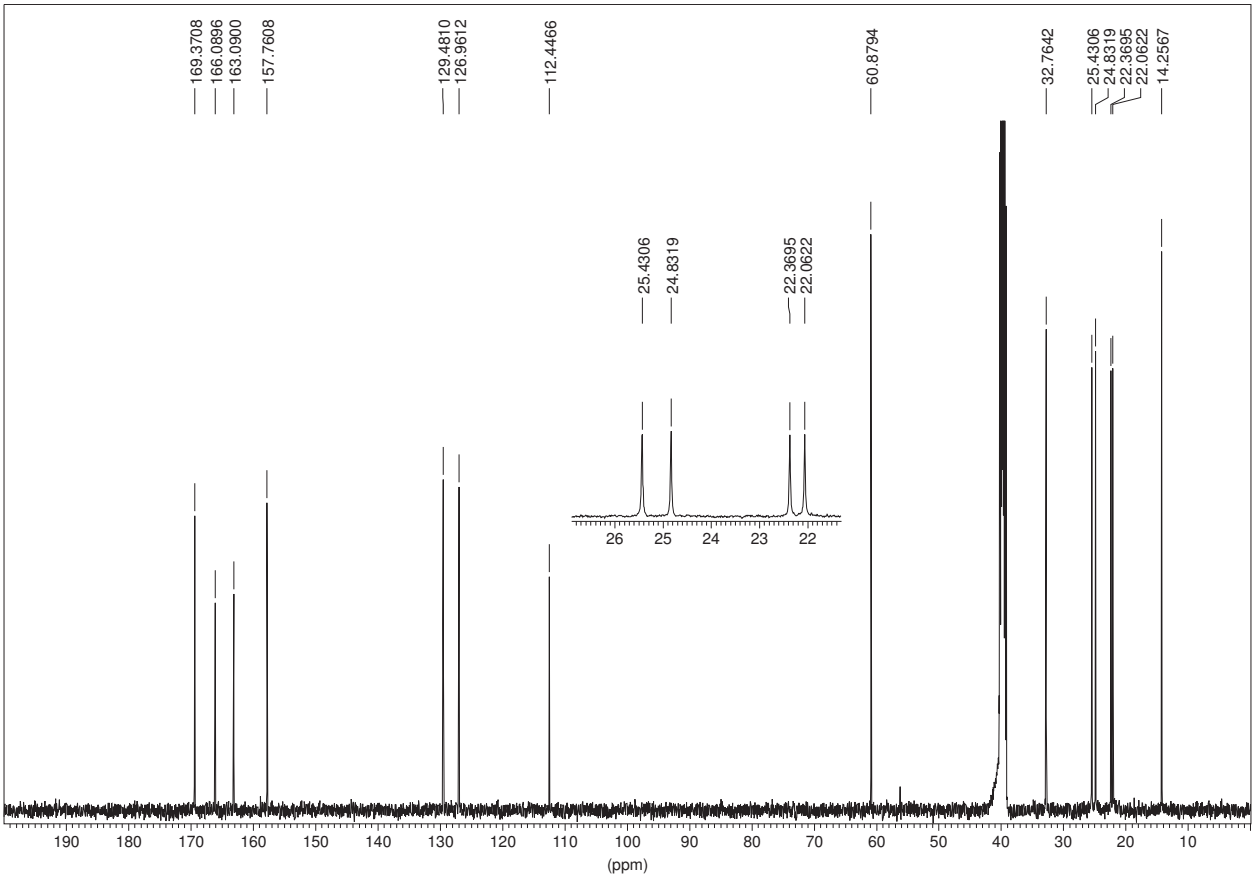
[(4-Amino-5,6-dimethylthieno[2,3-*d*]pyrimidin-2-yl)sulfanyl](phenyl)acetic Acid (**24**)



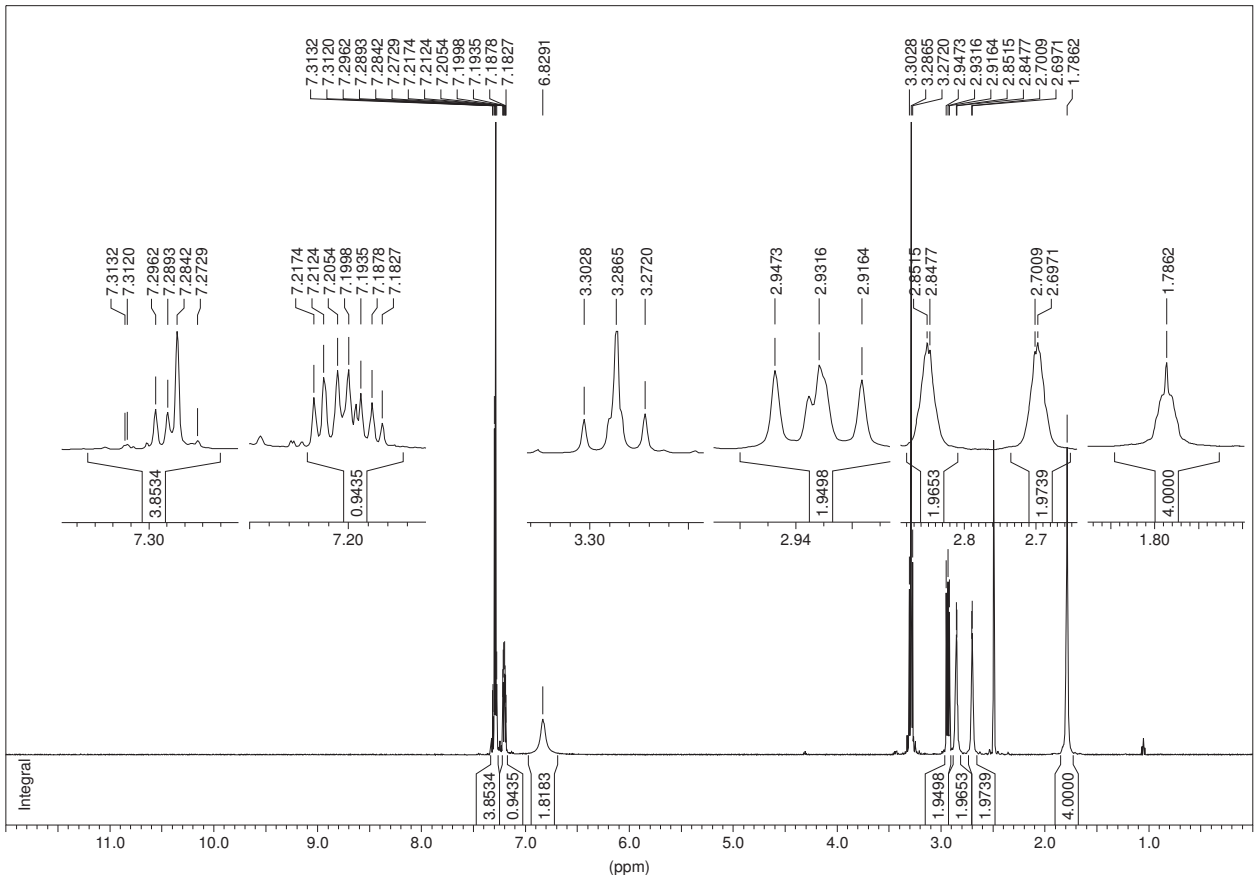


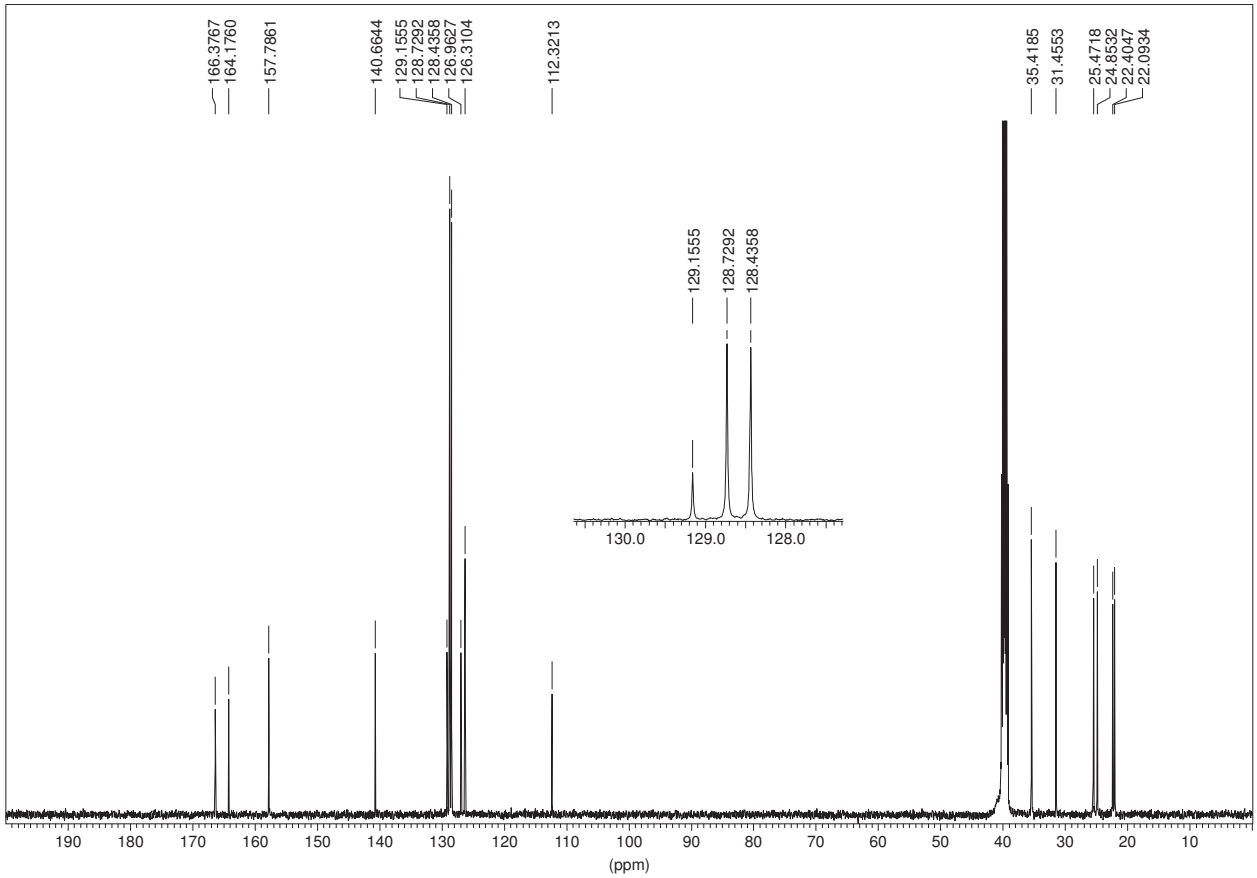
Ethyl [(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]acetate (**28**)



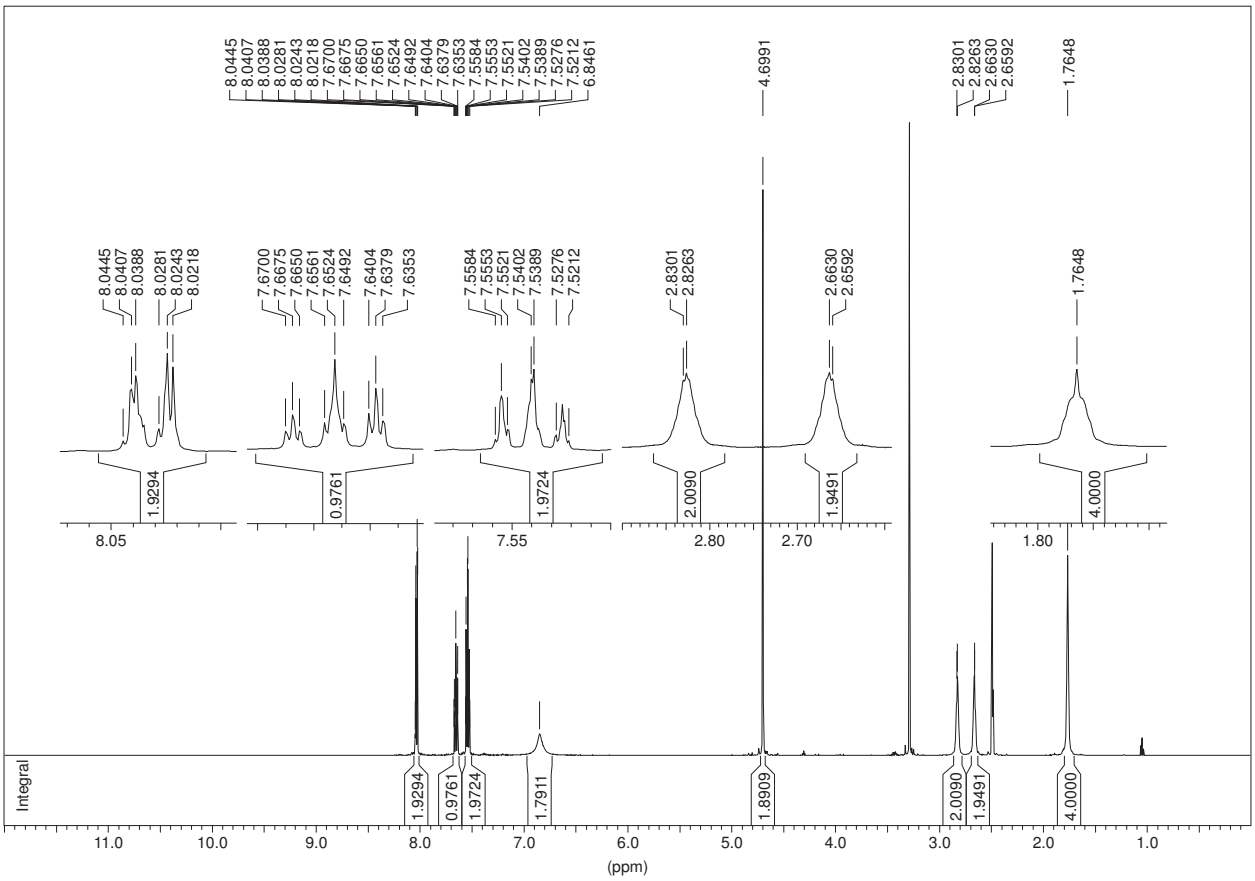


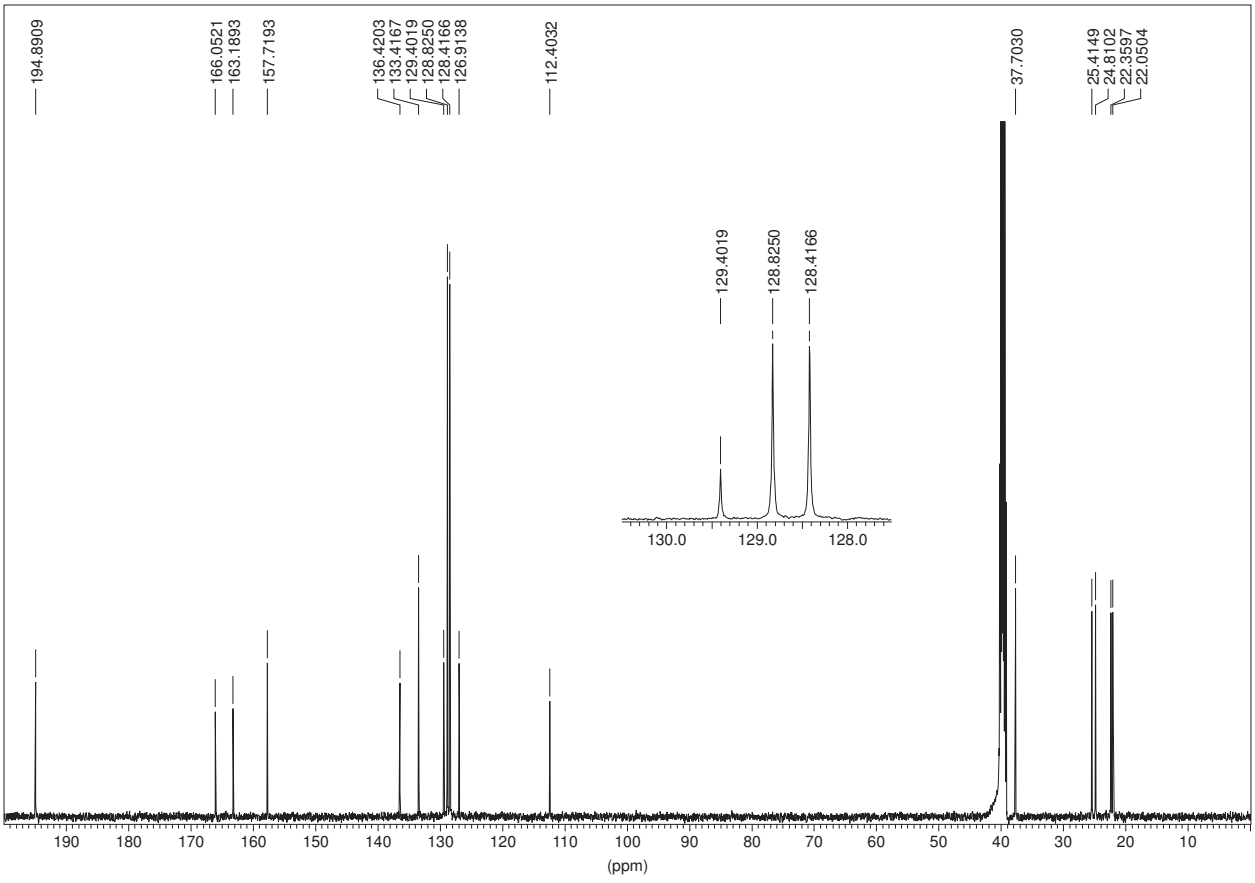
2-[(2-Phenylethyl)sulfanyl]-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-4-amine (**29**)



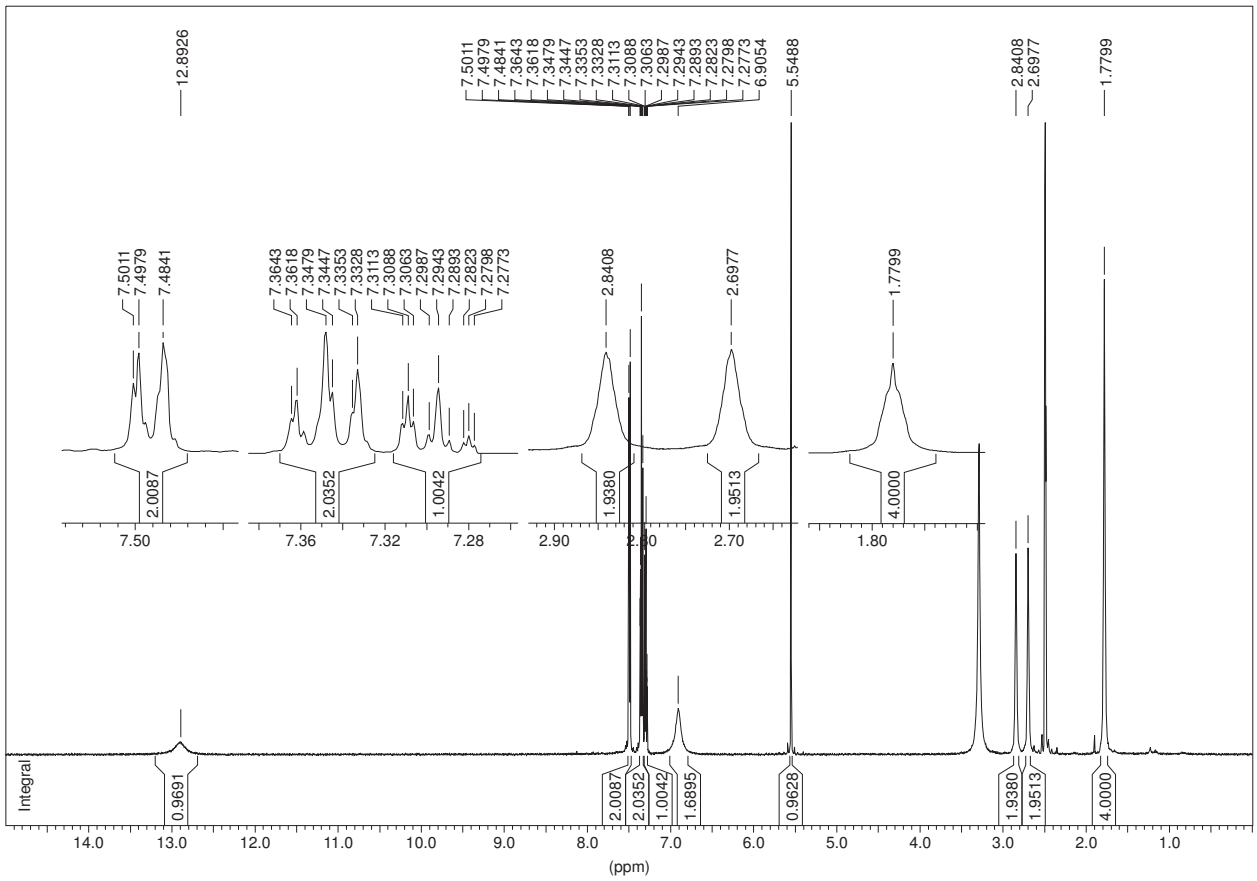


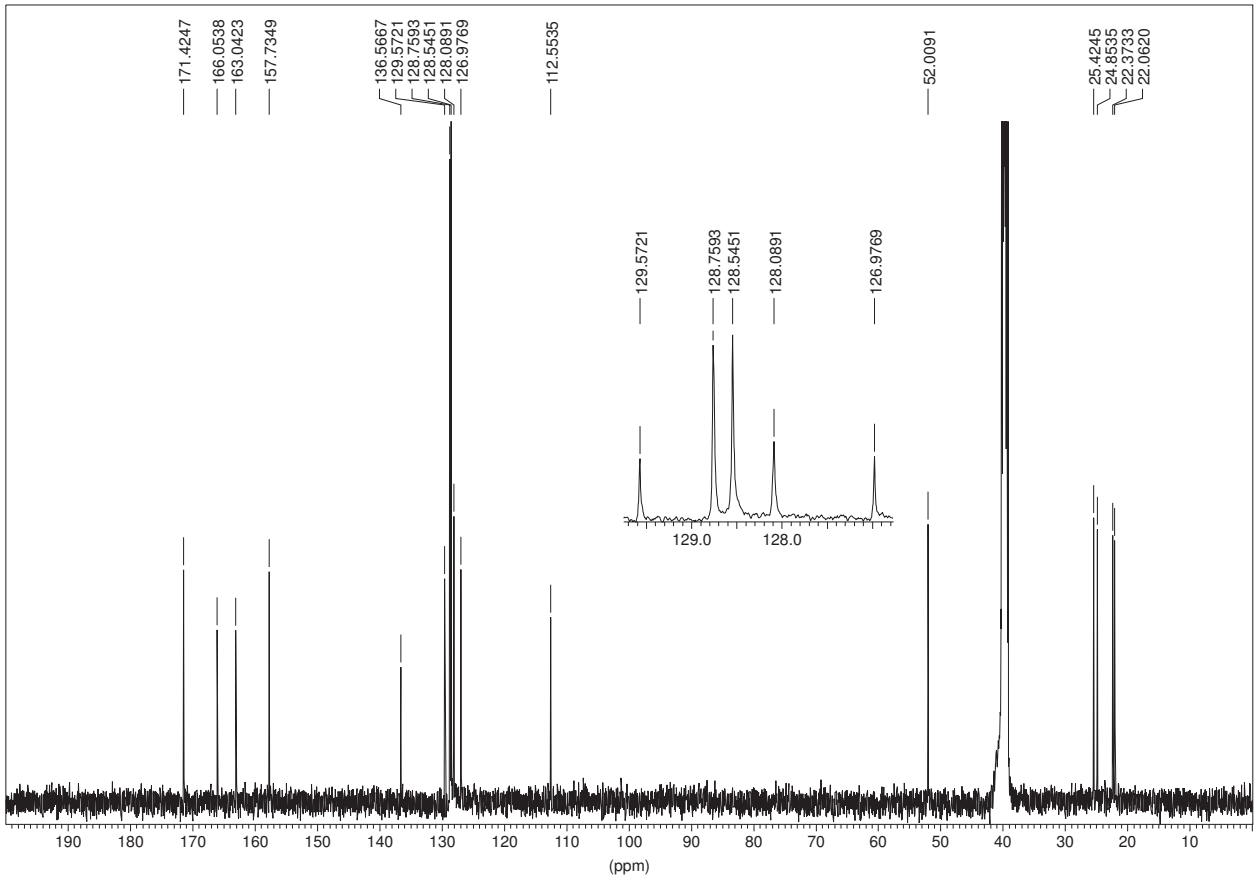
2-[(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-1-phenylethanone (**30**)



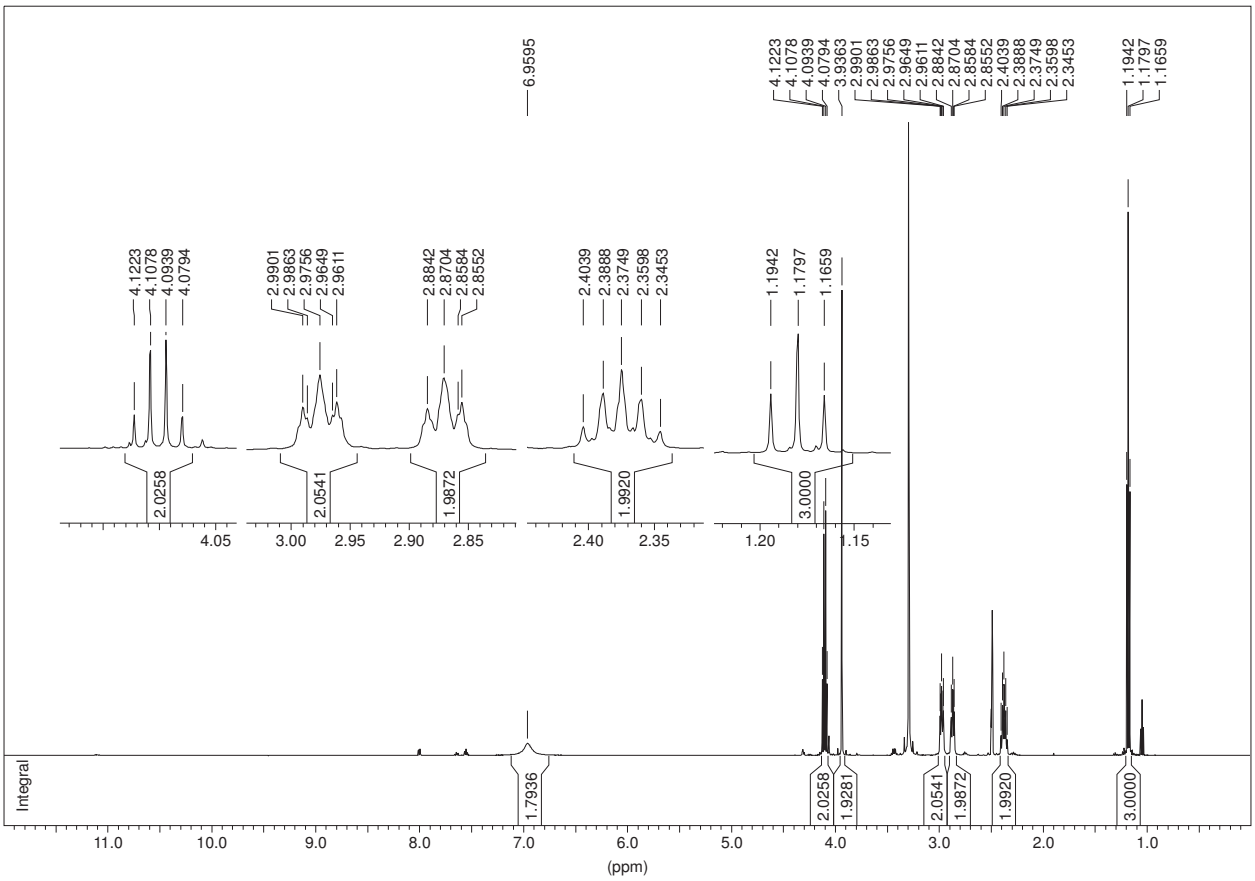


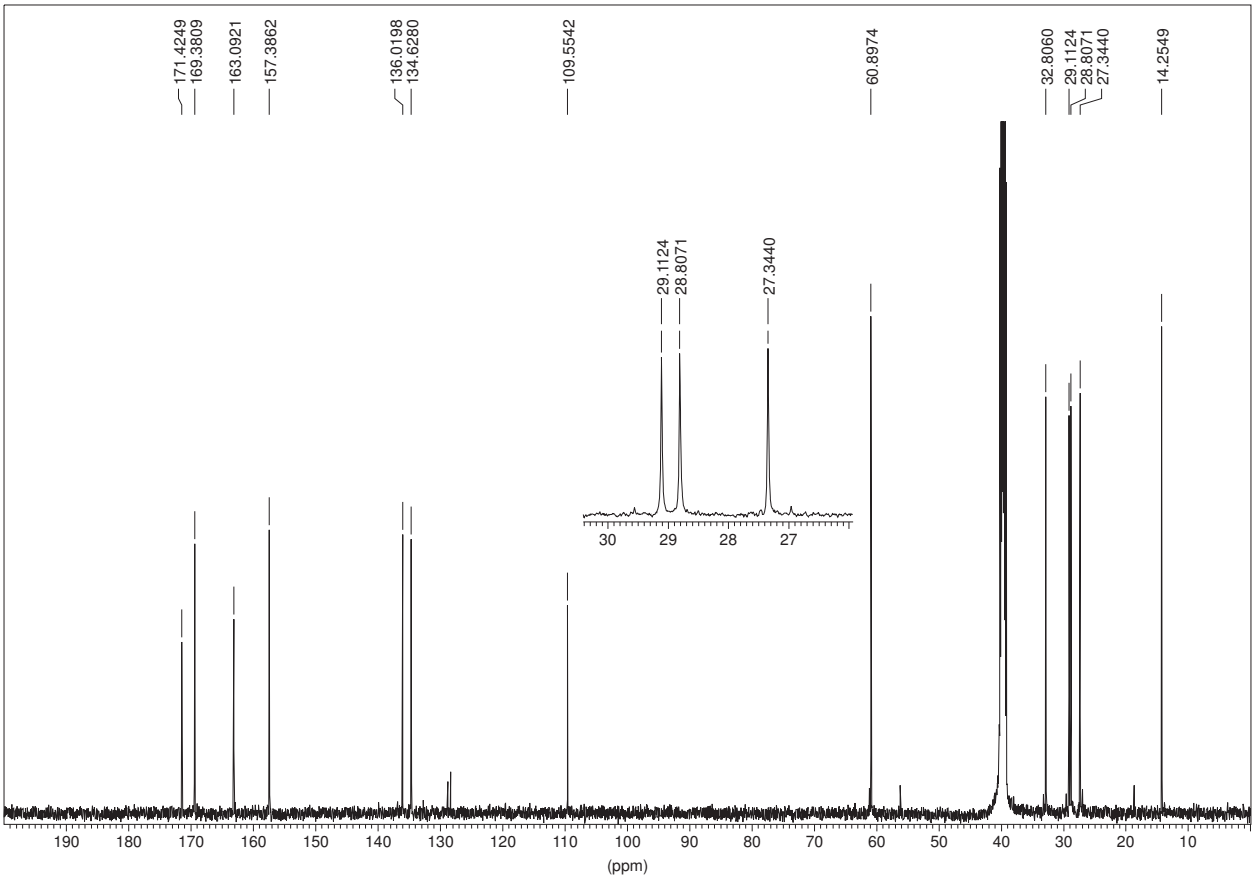
[(4-Amino-5,6,7,8-tetrahydrobenzothieno[2,3-*d*]pyrimidin-2-yl)sulfanyl](phenyl)acetic Acid (**31**)



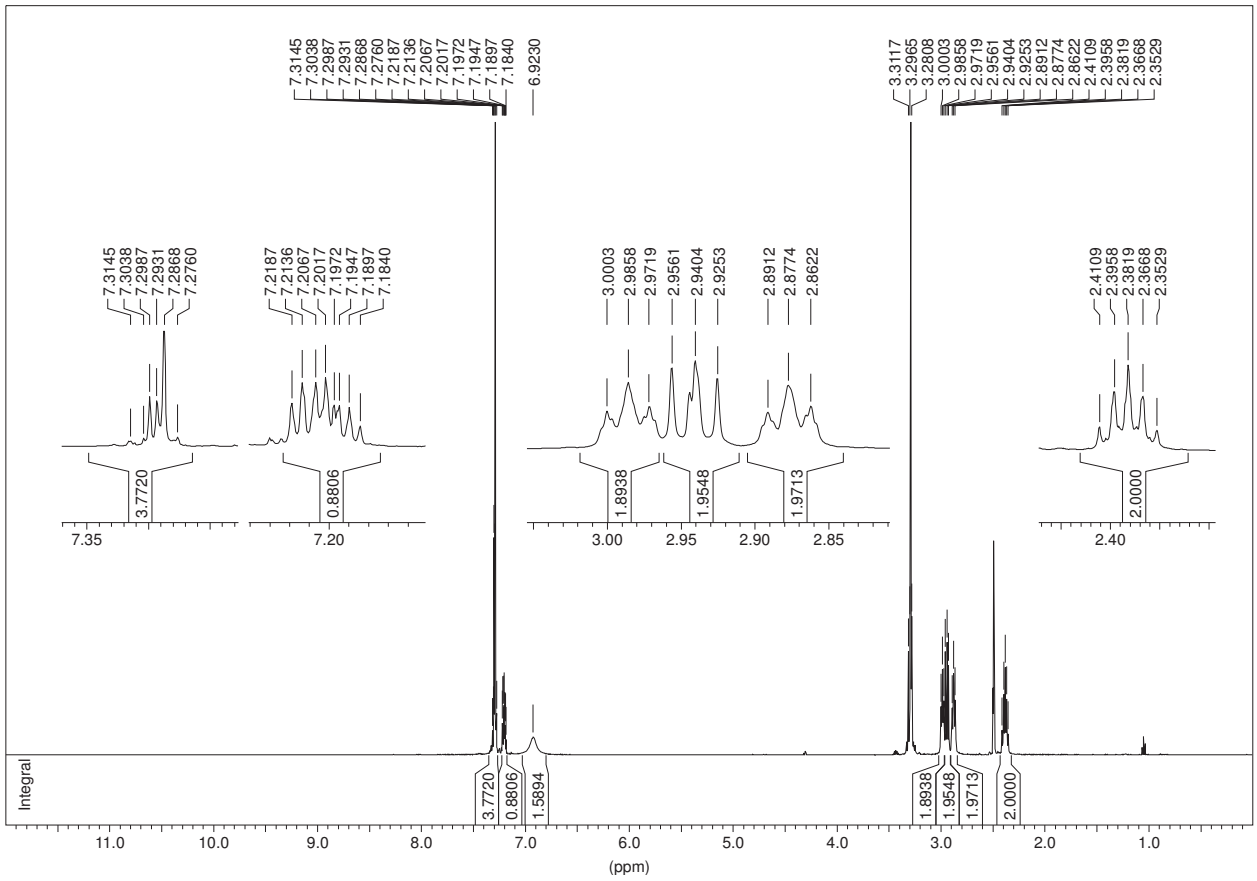


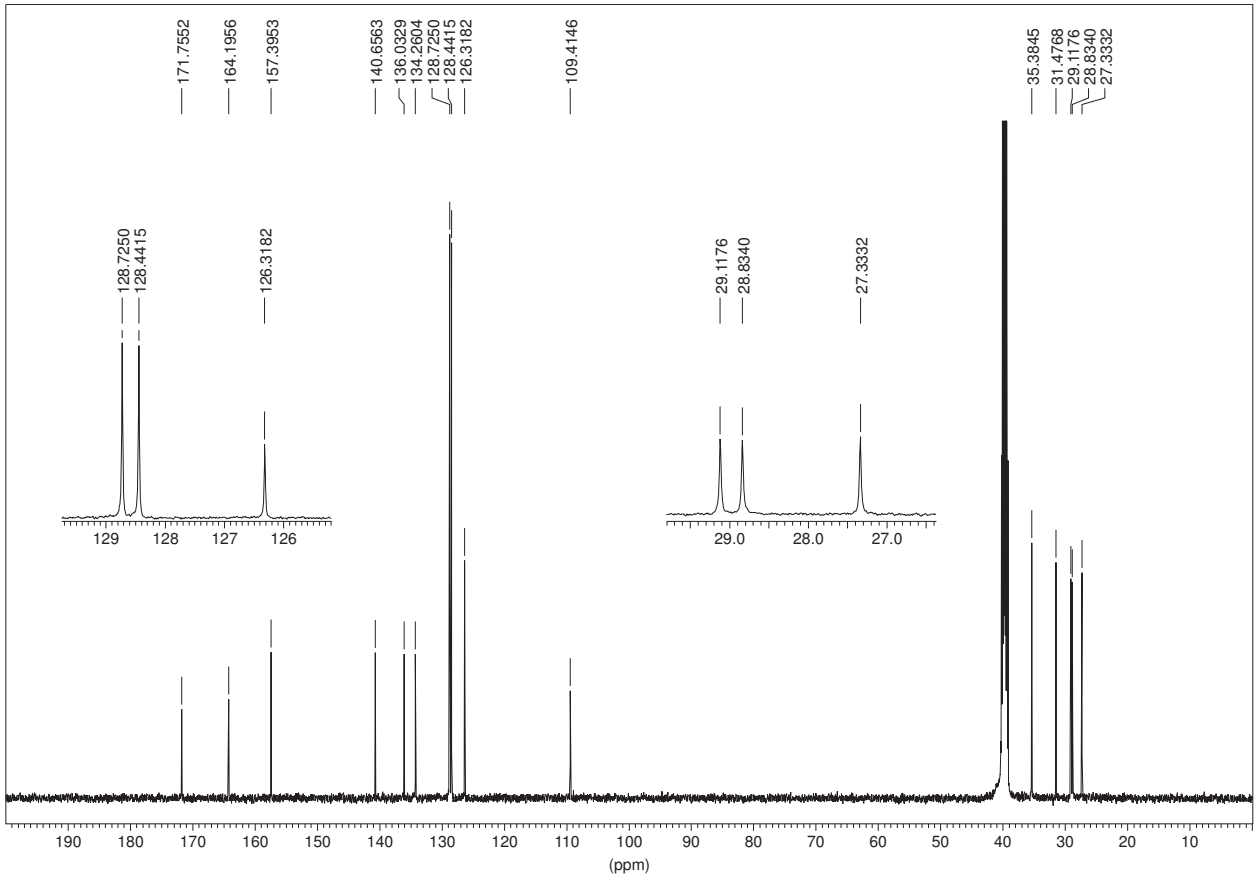
Ethyl [(4-Amino-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-acetate (**32**)



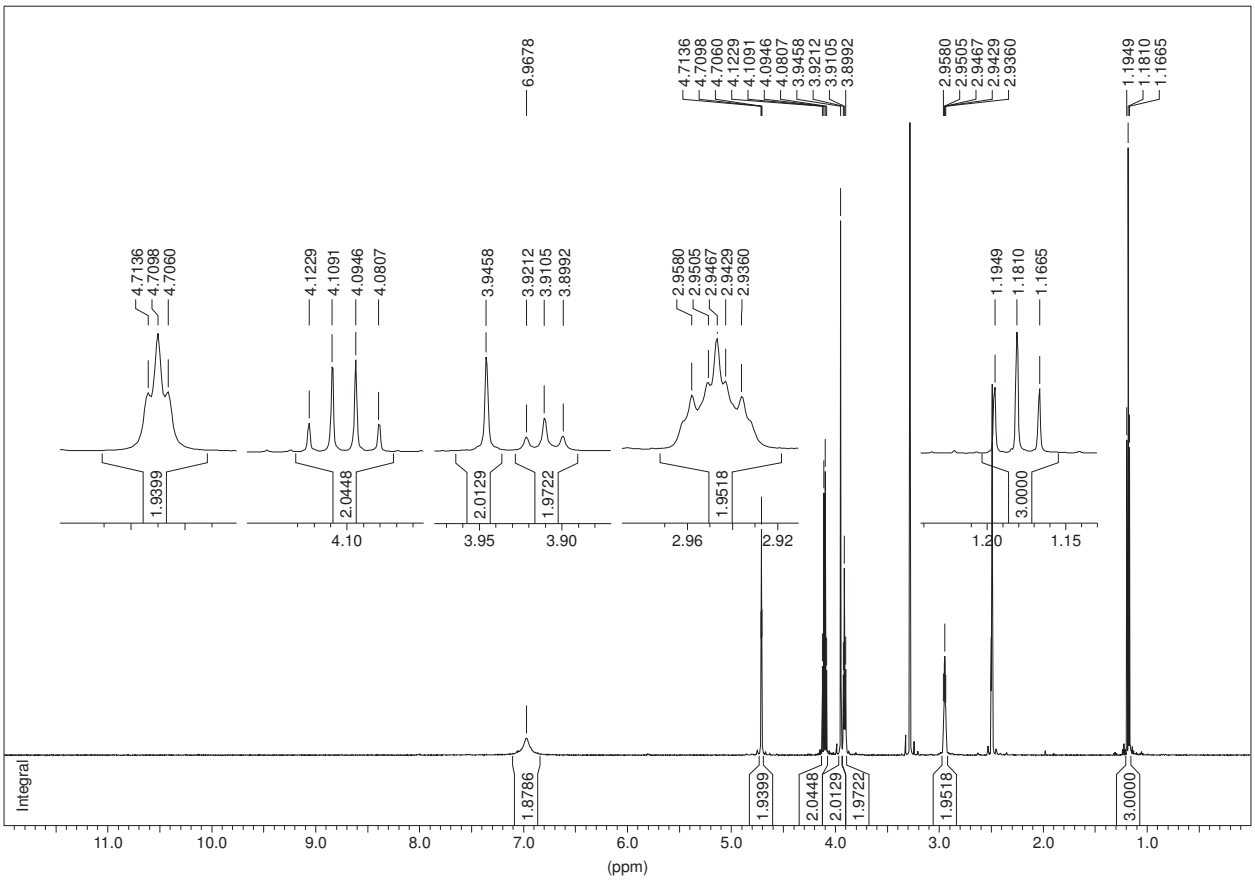


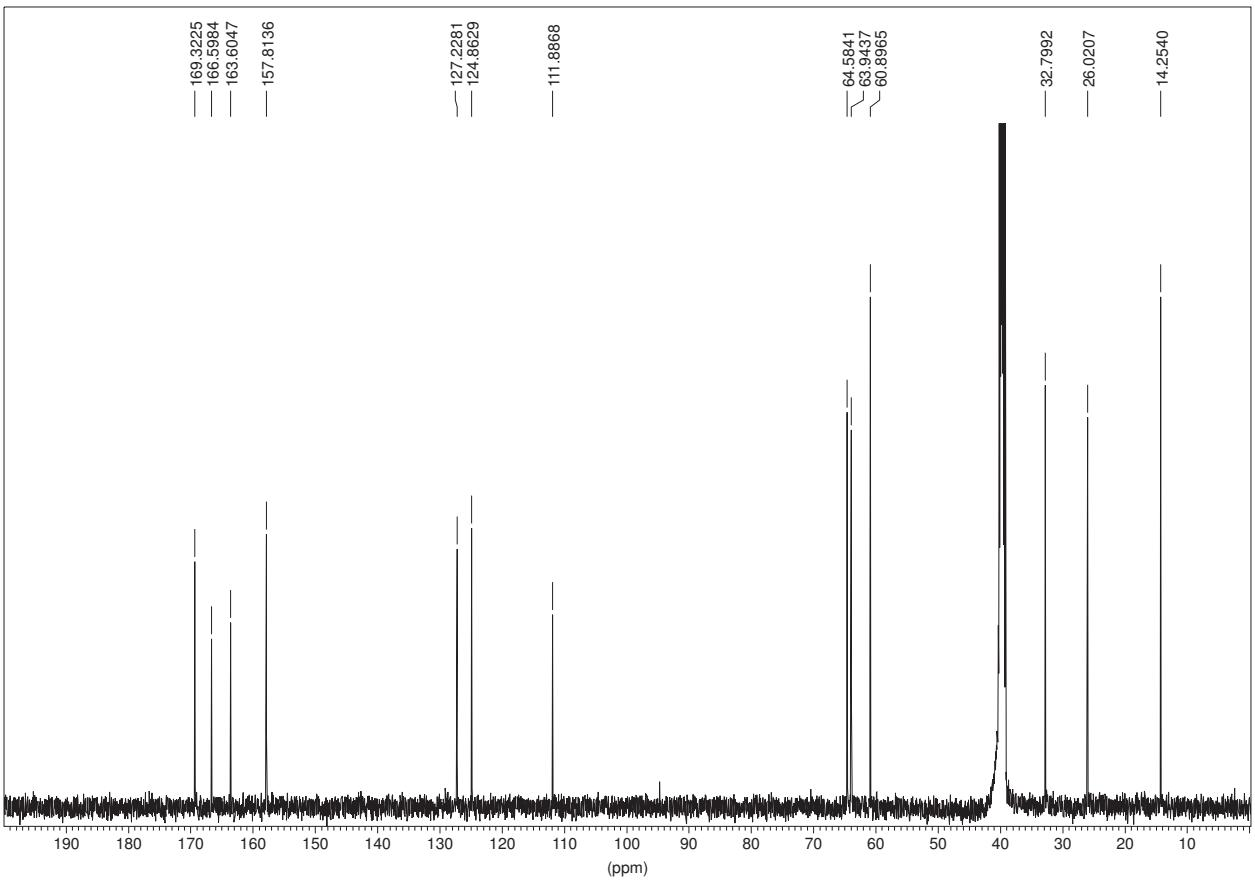
2-[(2-Phenylethyl)sulfanyl]-6,7-dihydro-5*H*-cyclopenta[4,5]thieno[2,3-*d*]pyrimidin-4-amine (**33**)



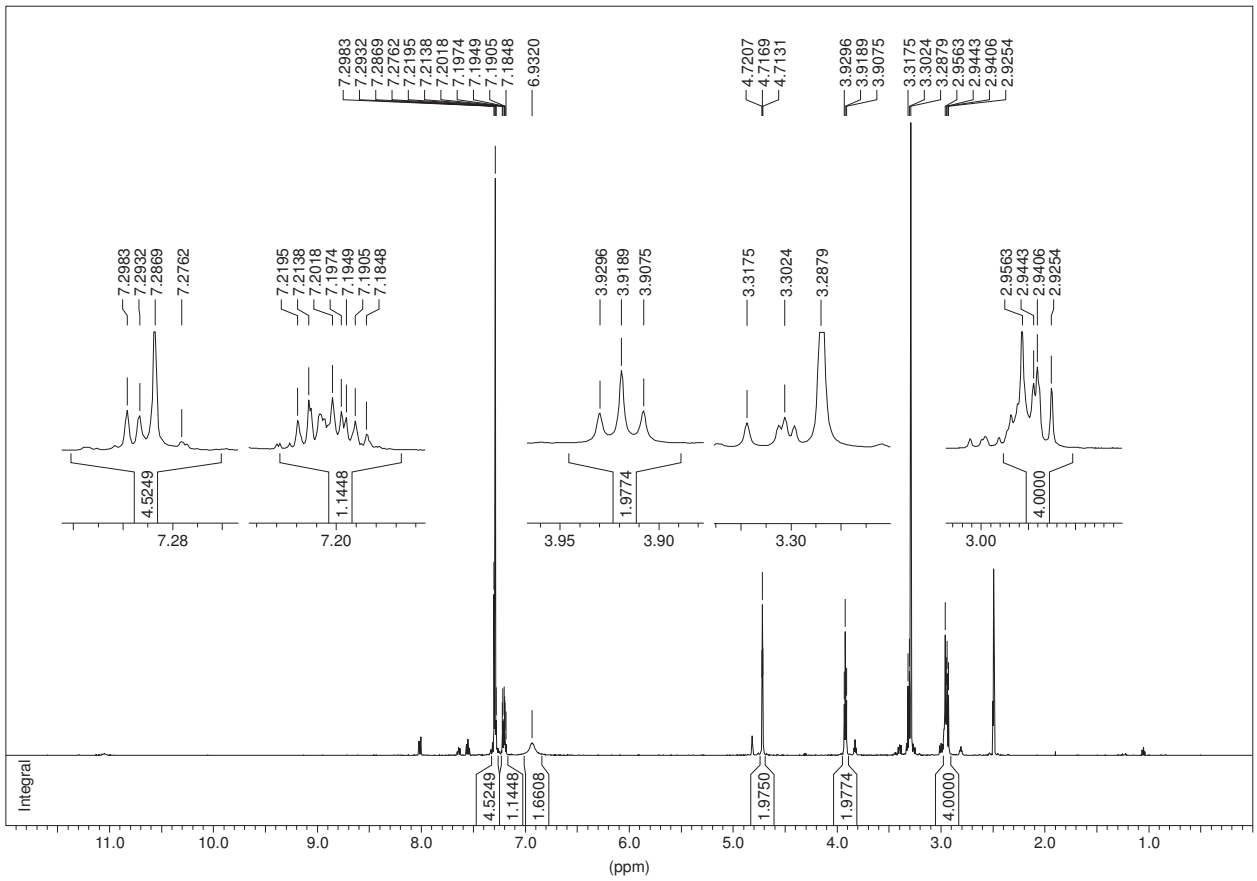


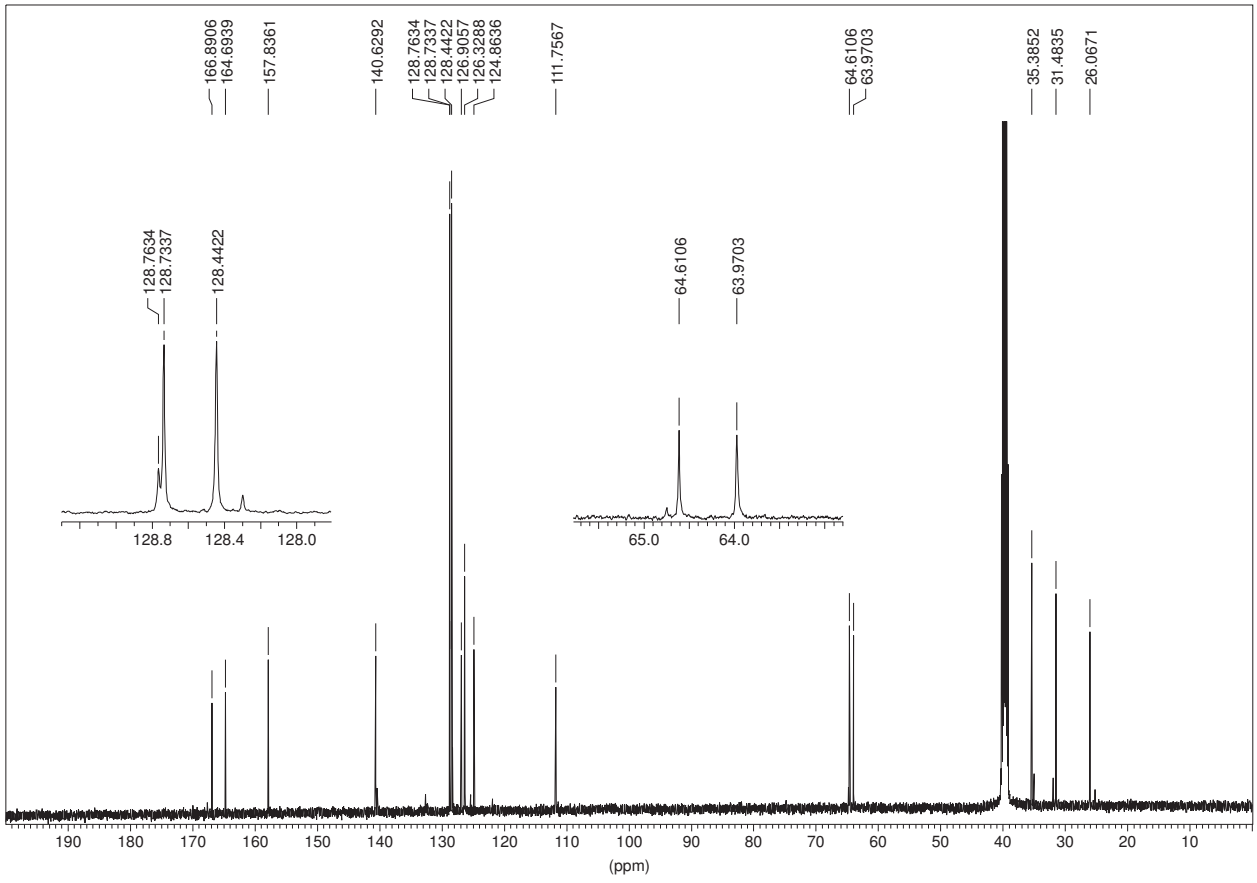
Ethyl [(4-Amino-5,8-dihydro-6*H*-pyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-2-yl)sulfanyl]-acetate (**34**)



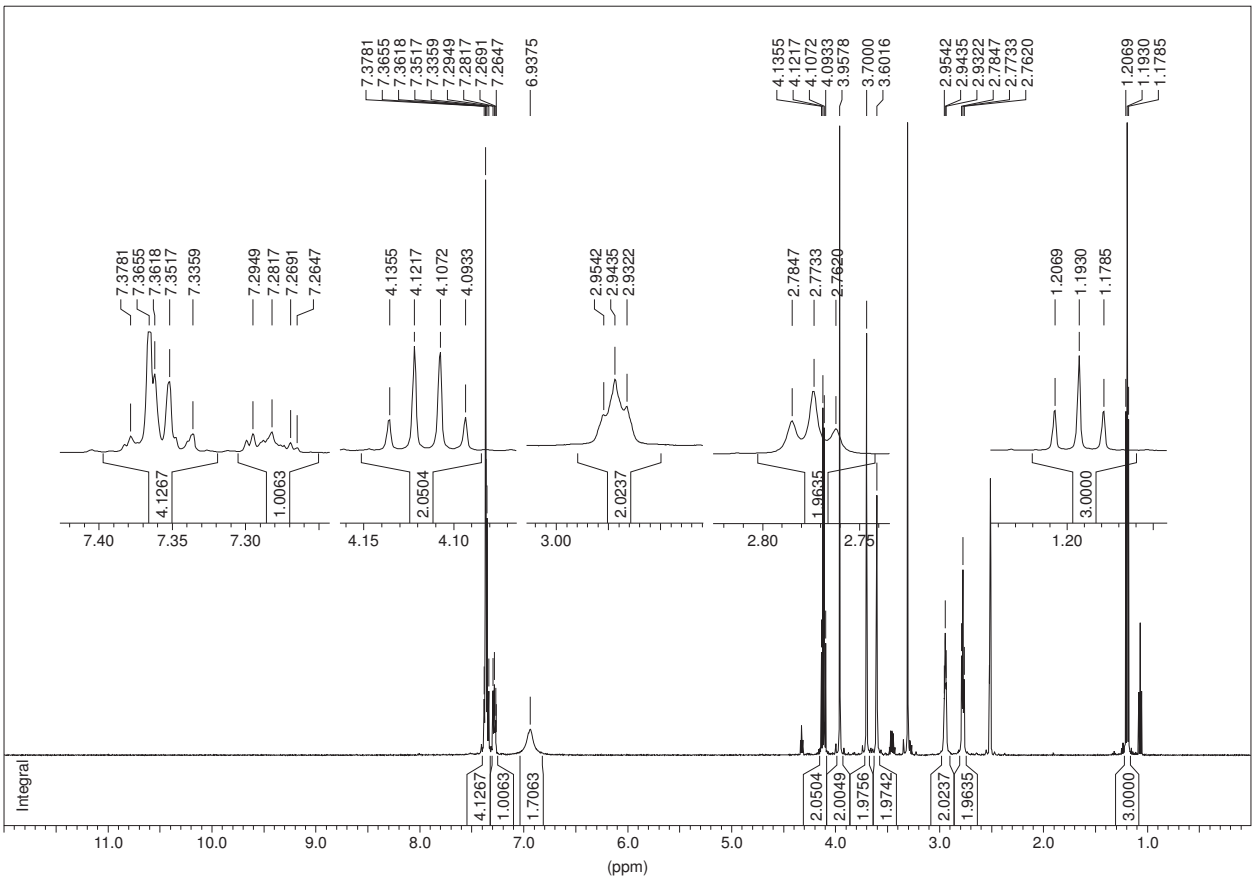


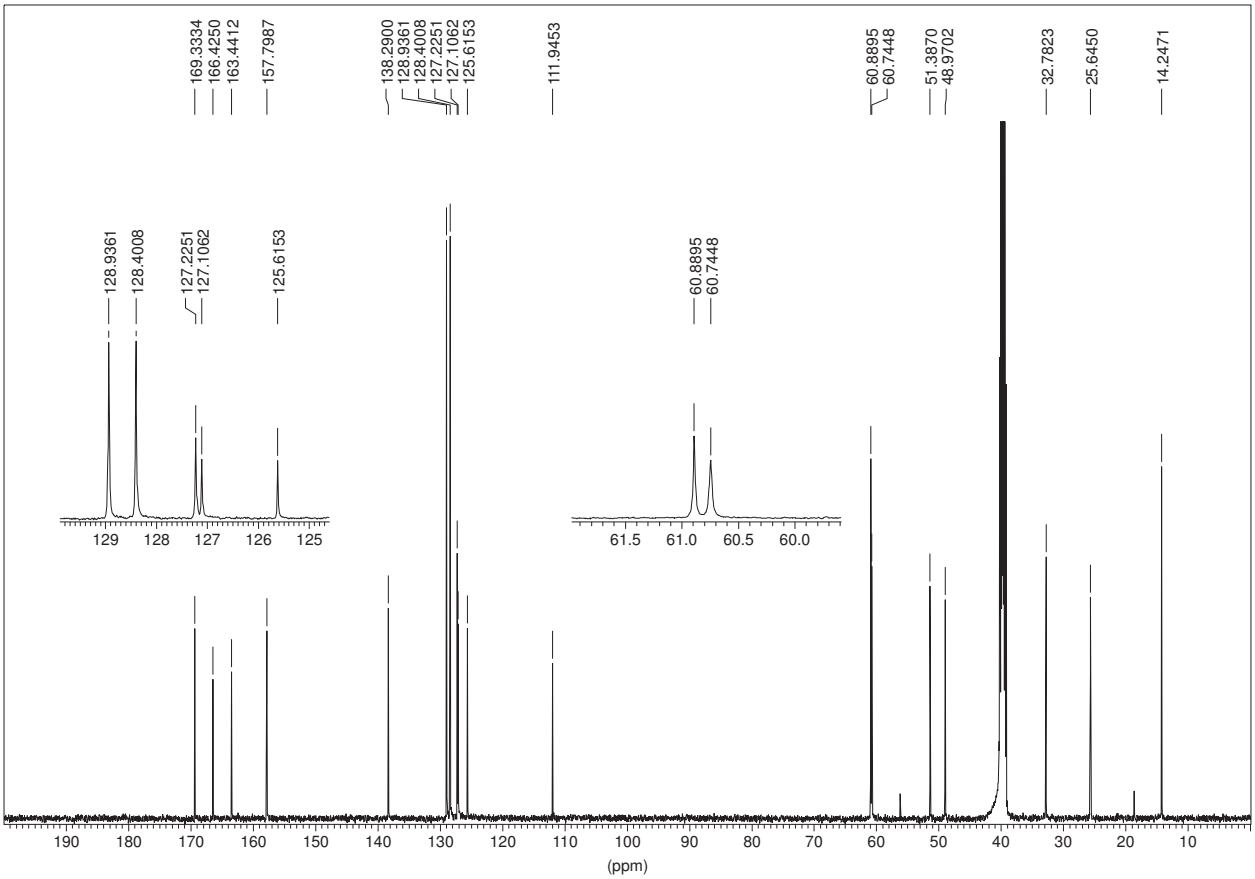
2-[(2-Phenylethyl)sulfanyl]-5,8-dihydro-6*H*-pyrano[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-4-amine (**35**)



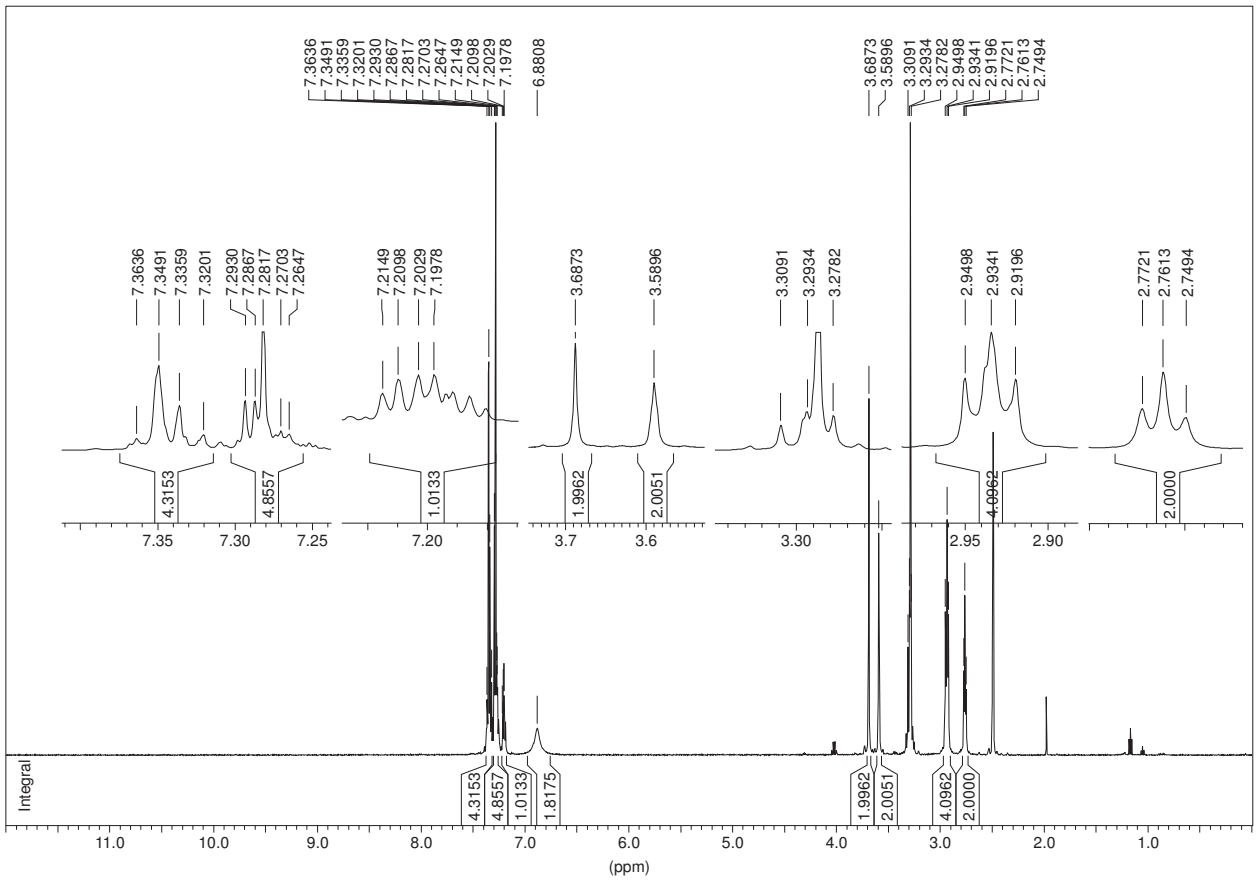


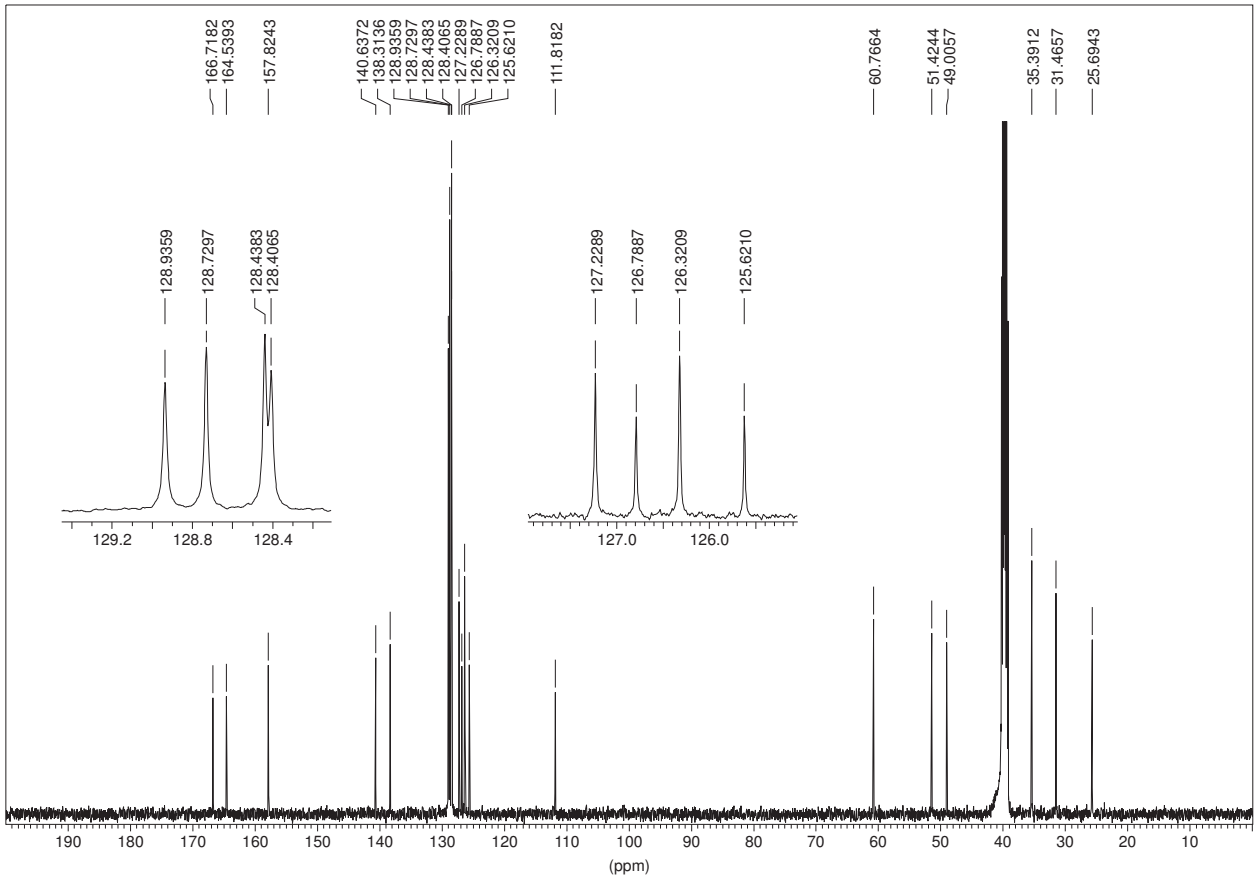
Ethyl [(4-Amino-7-benzyl-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]pyrimidin-2-yl)-sulfanyl]acetate (**36**)



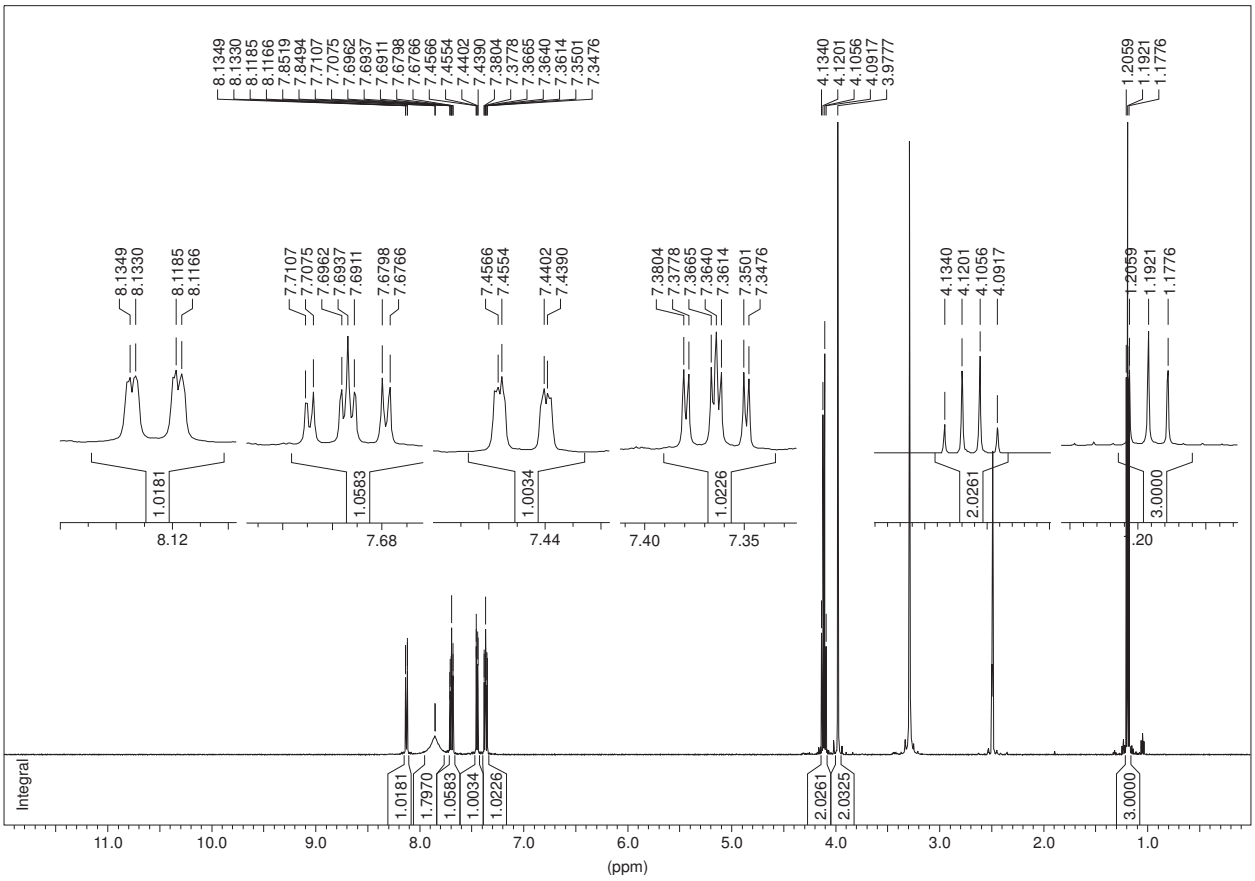


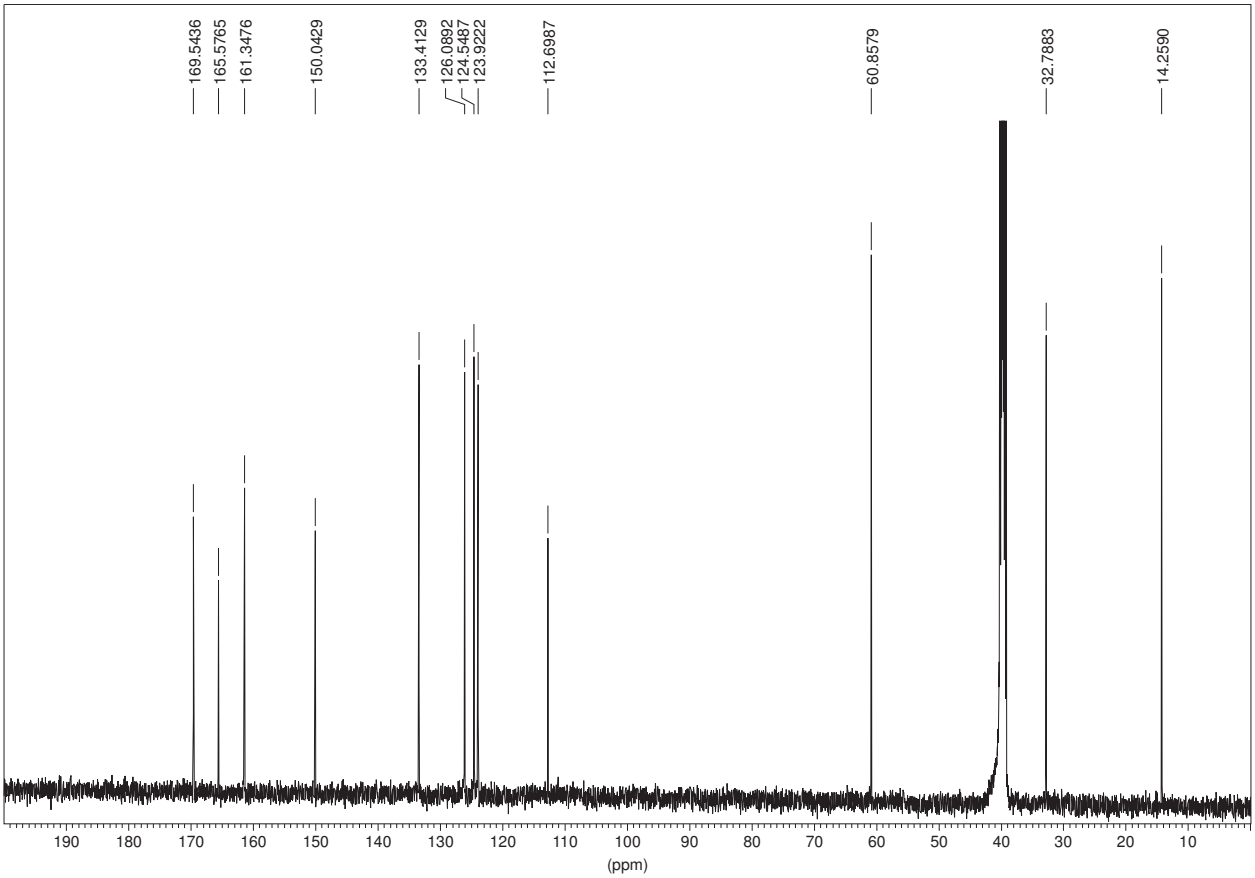
7-Benzyl-2-[(2-phenylethyl)sulfanyl]-5,6,7,8-tetrahydropyrido[4′,3′:4,5]thieno[2,3-*d*]-pyrimidin-4-amine (**37**)



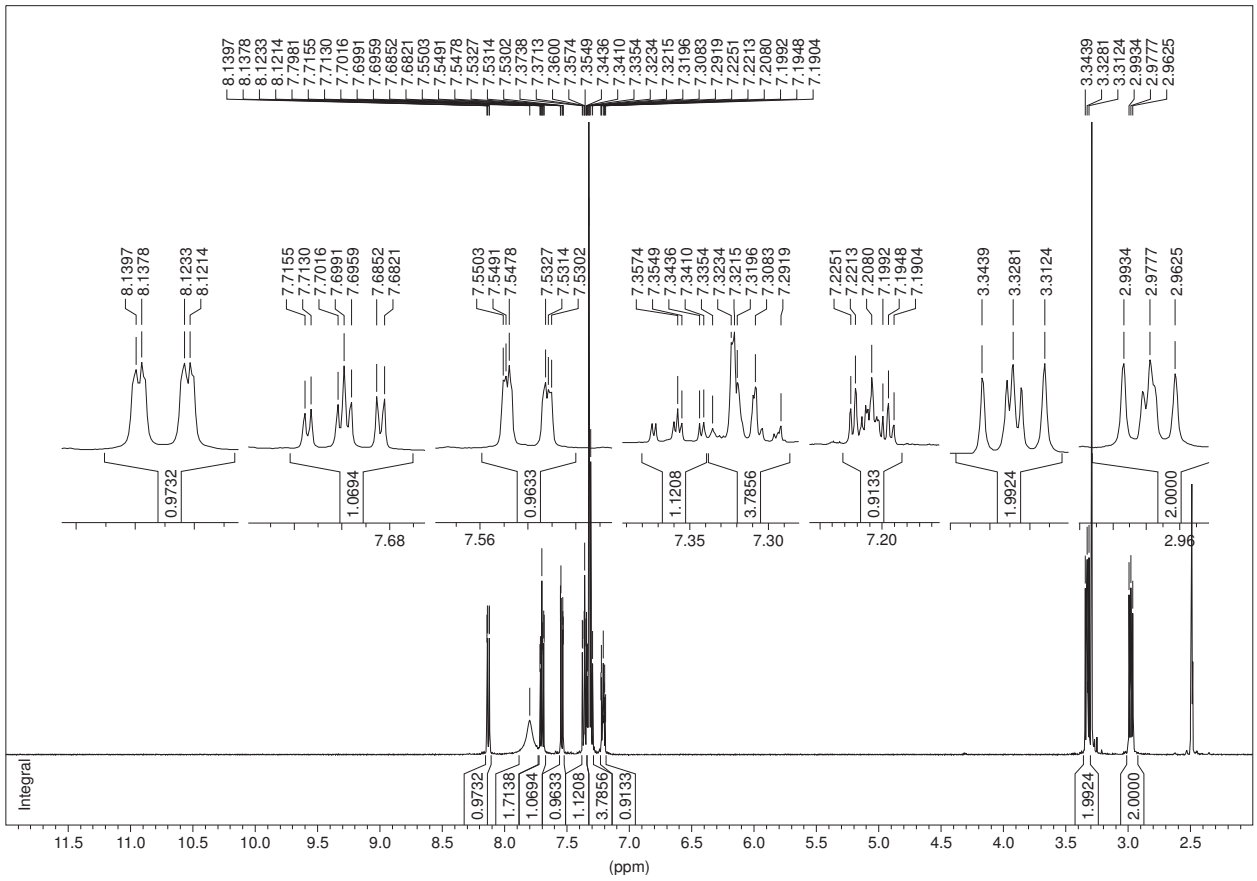


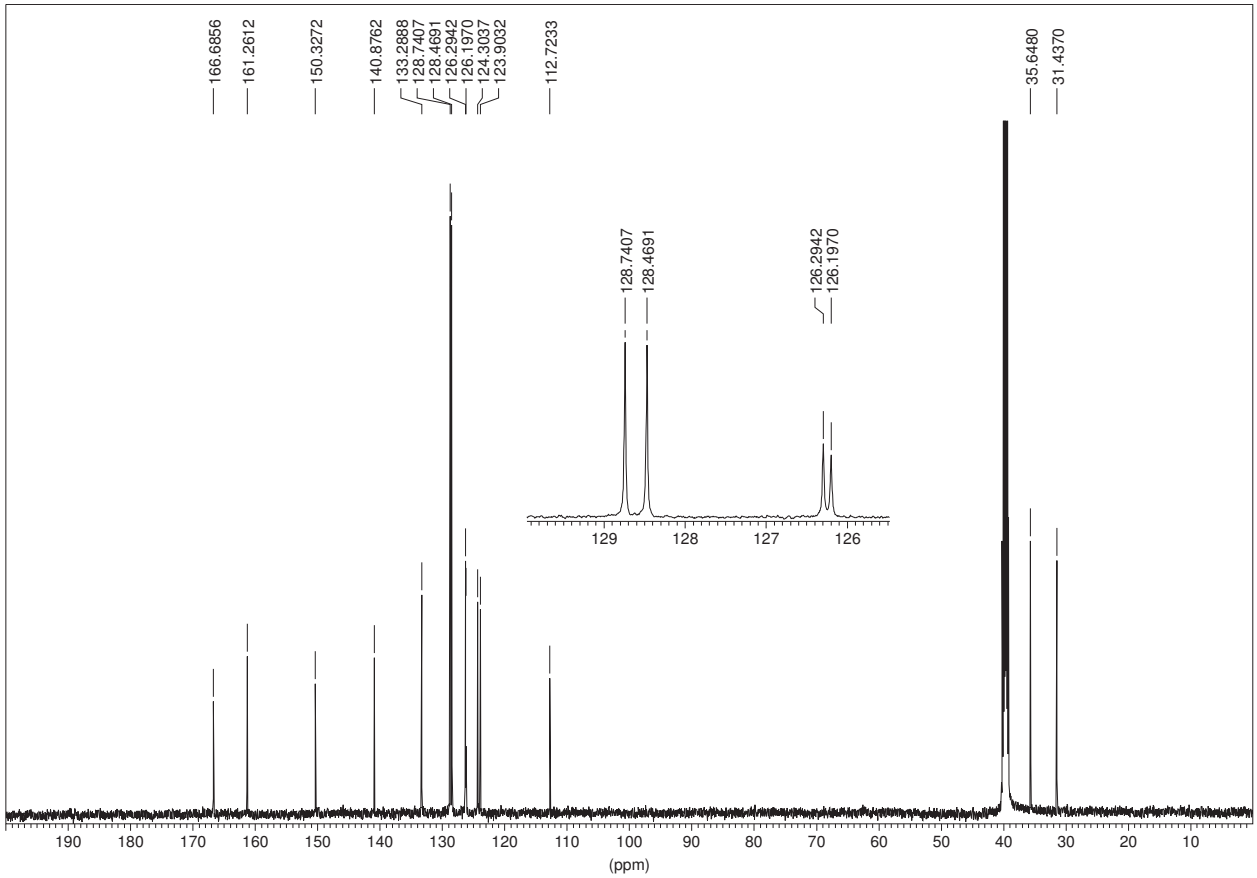
Ethyl [(4-Aminoquinazolin-2-yl)sulfanyl]acetate (**38**)



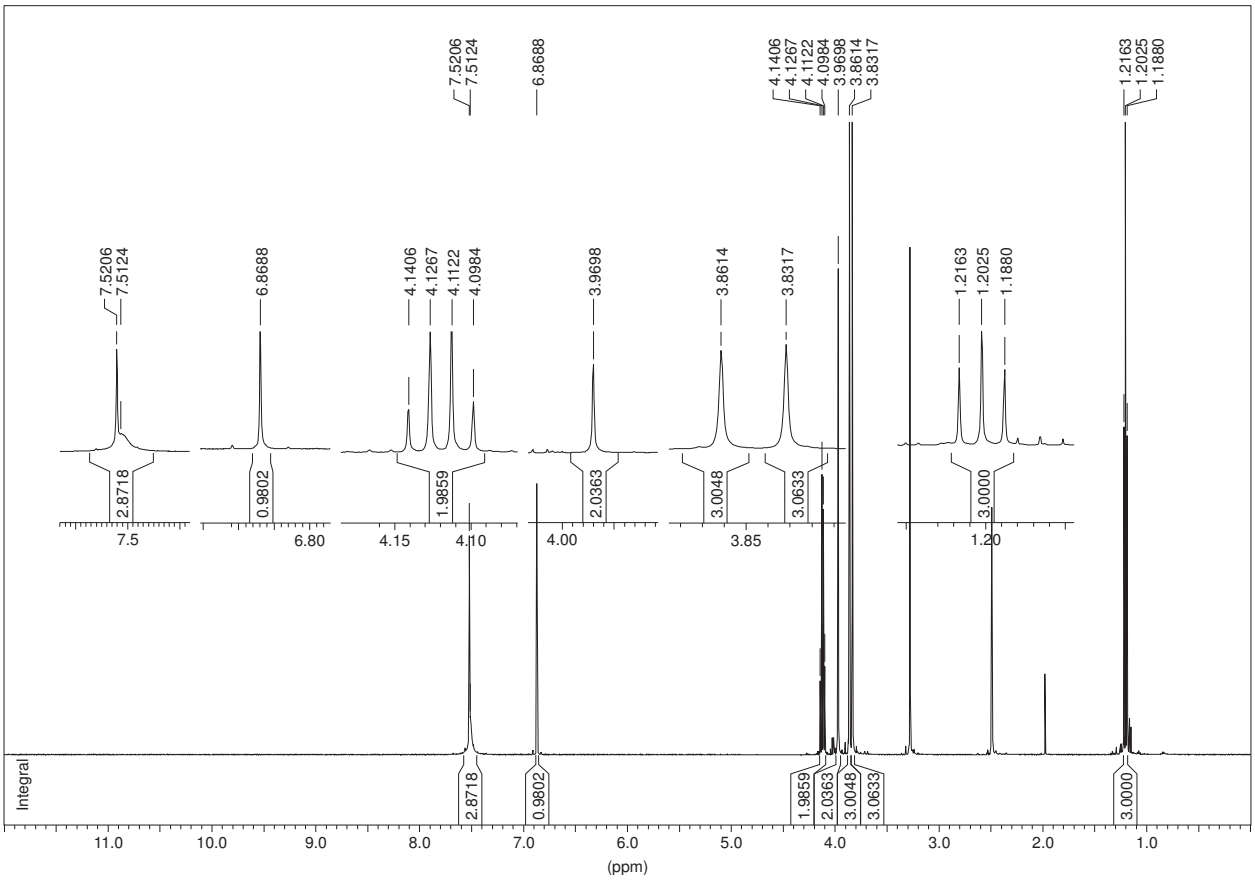


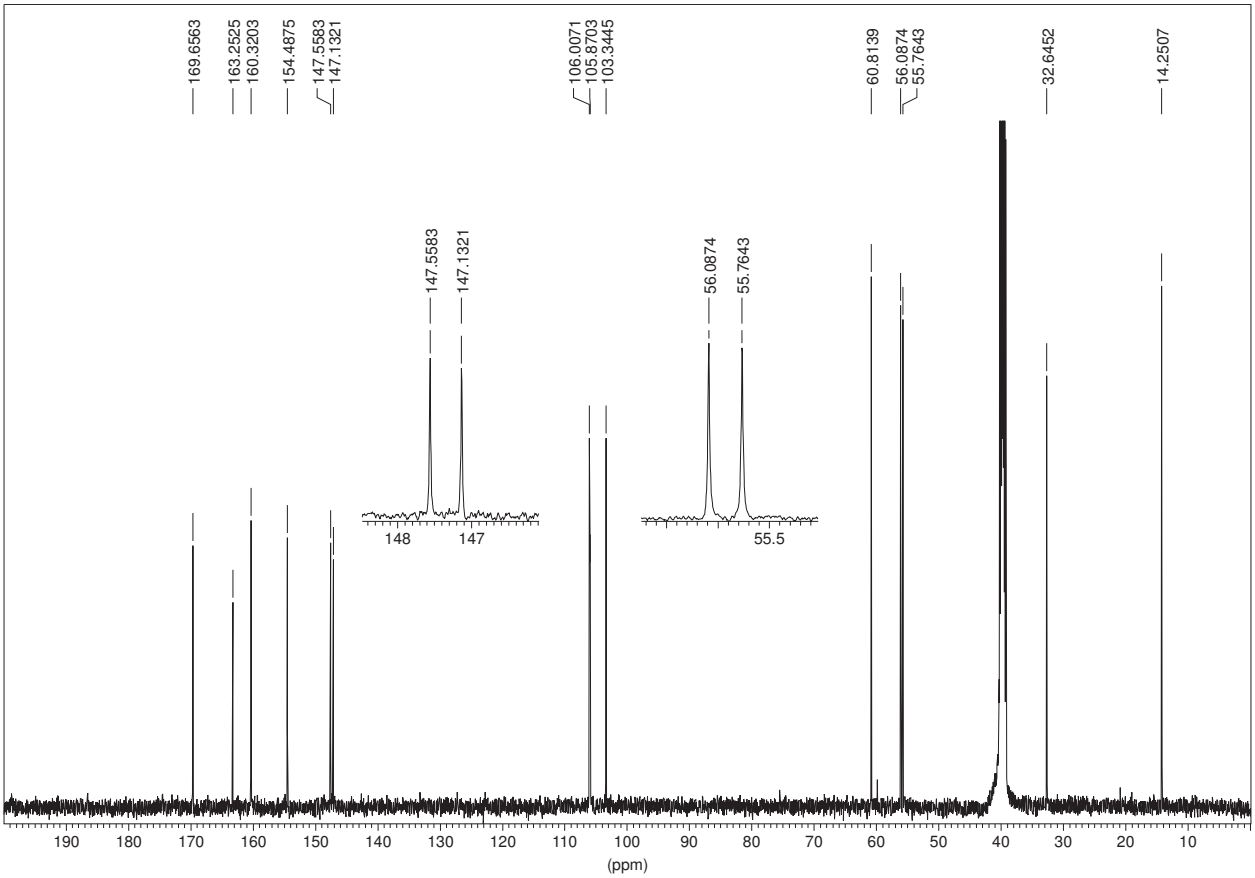
2-[(2-Phenylethyl)sulfanyl]quinazolin-4-amine (**39**)



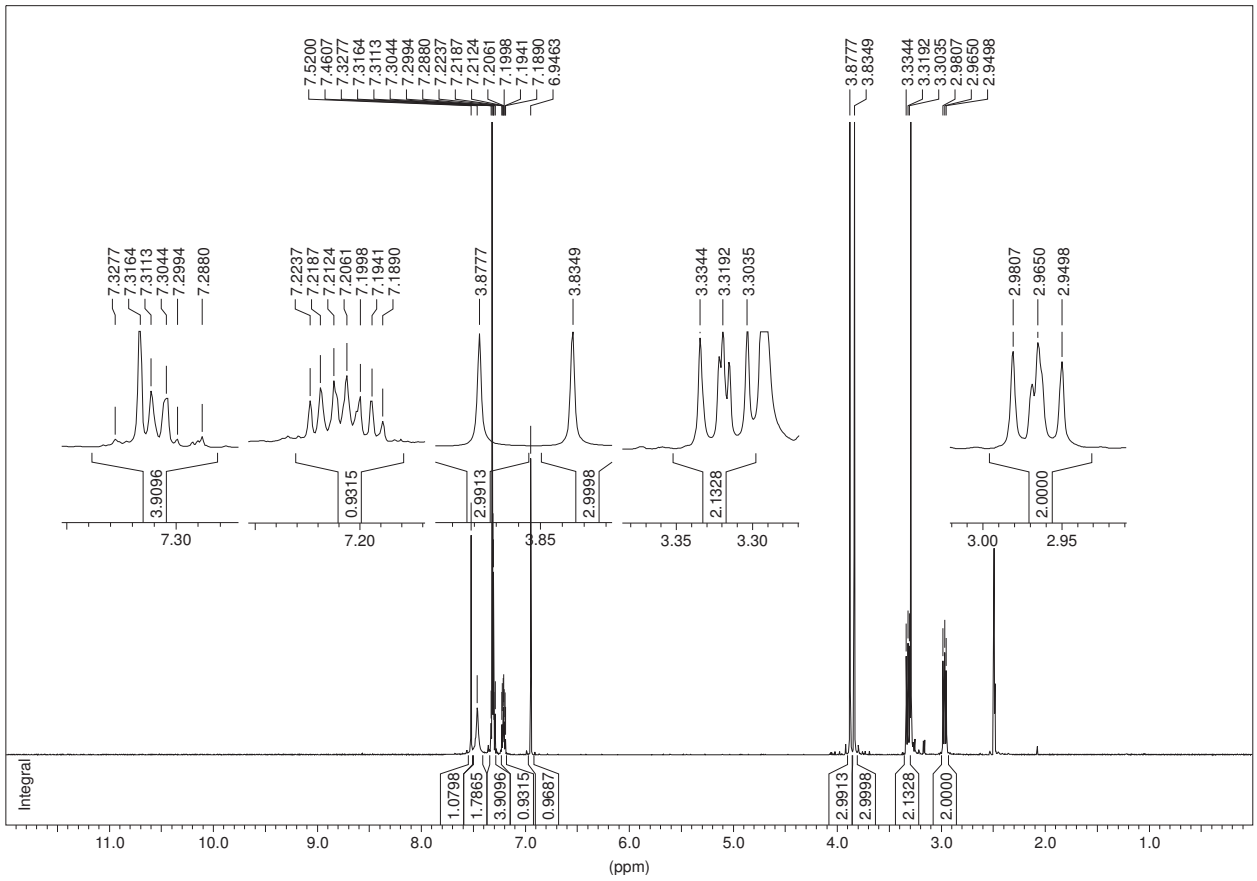


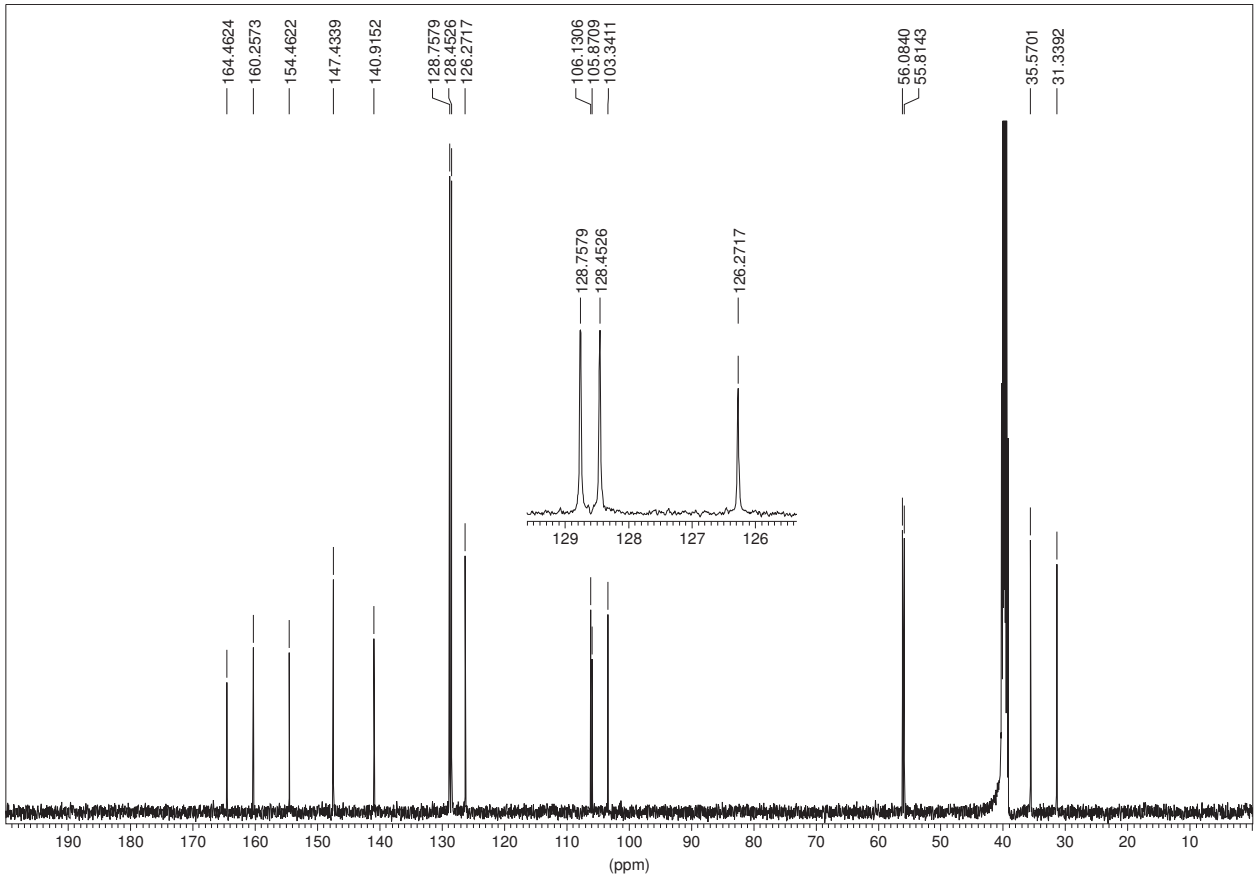
Ethyl [(4-Amino-6,7-dimethoxyquinazolin-2-yl)sulfanyl]acetate (**40**)



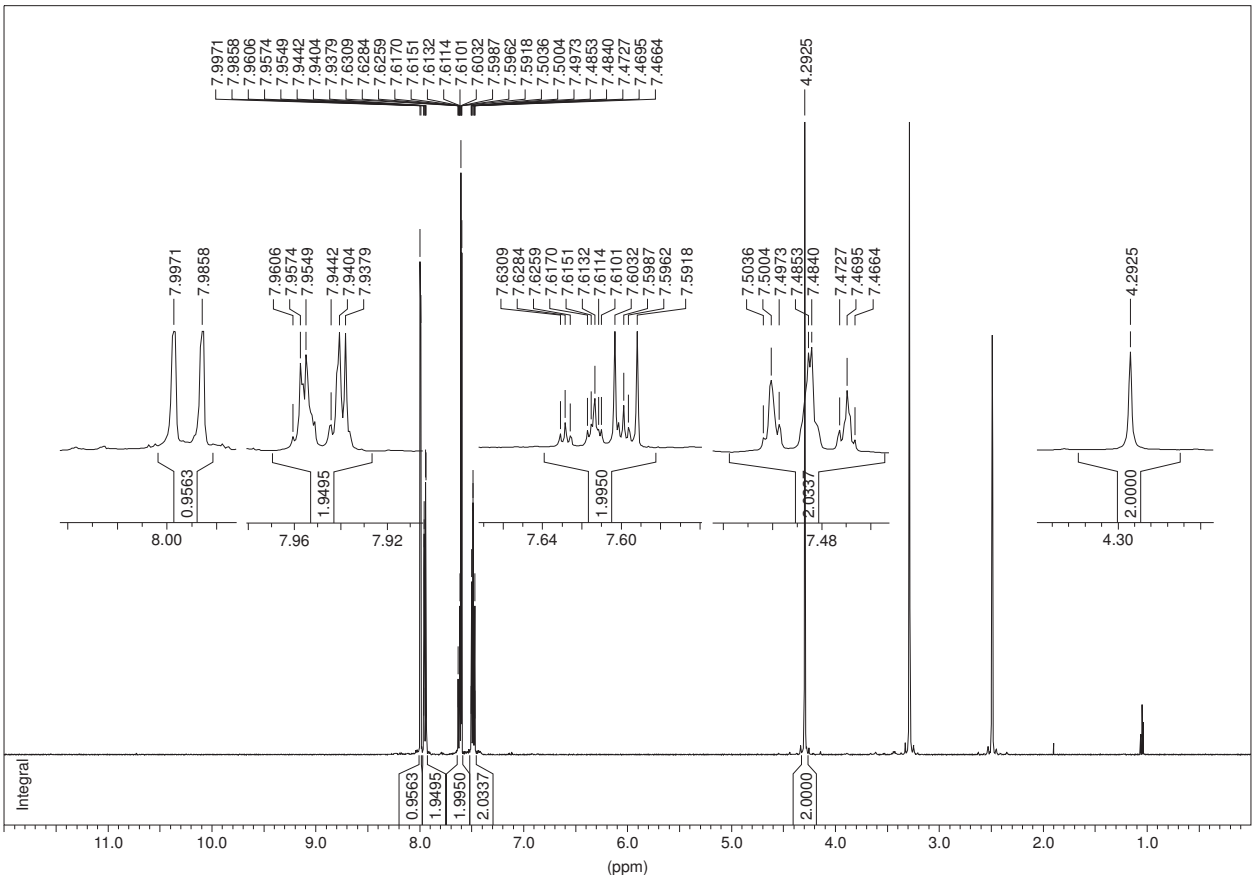


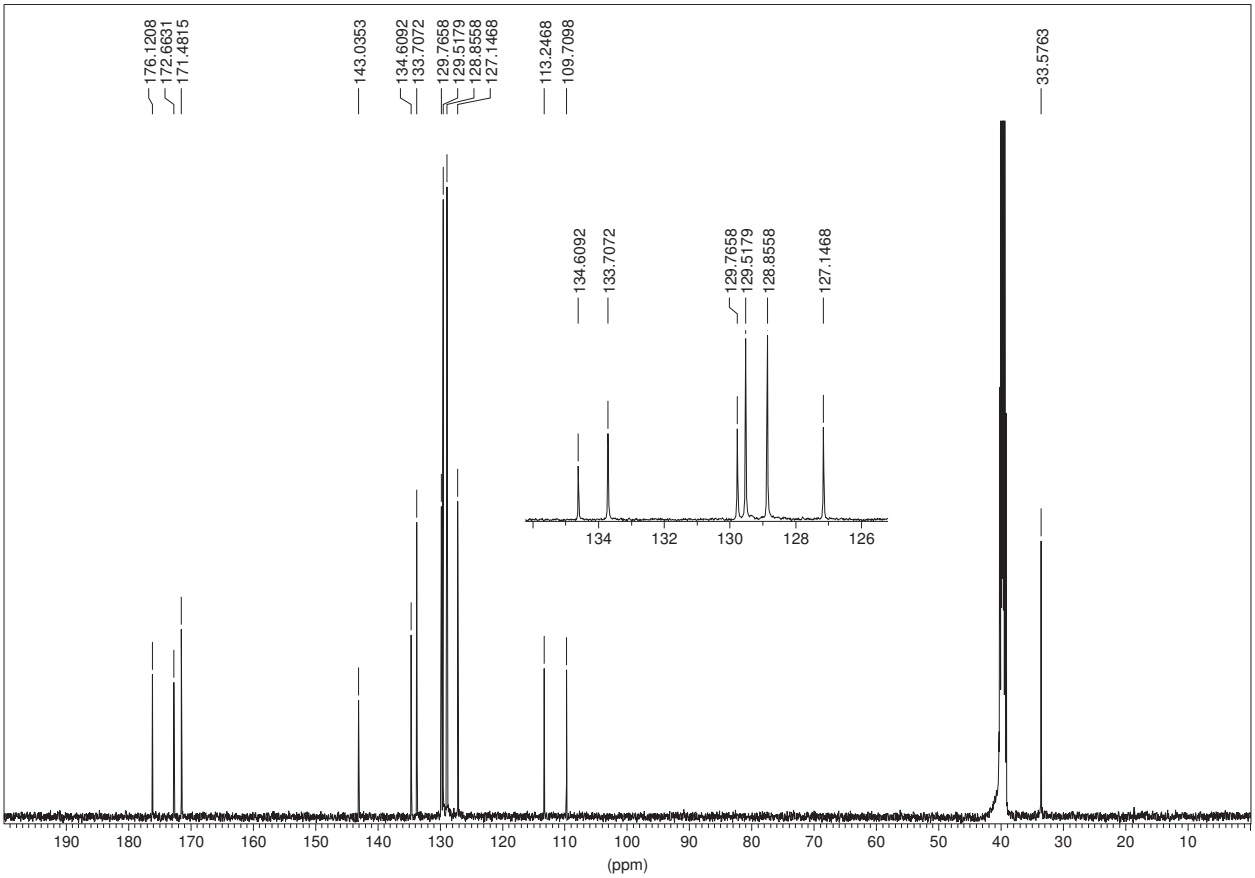
6,7-Dimethoxy-2-[(2-phenylethyl)sulfanyl]quinazolin-4-amine (**41**)



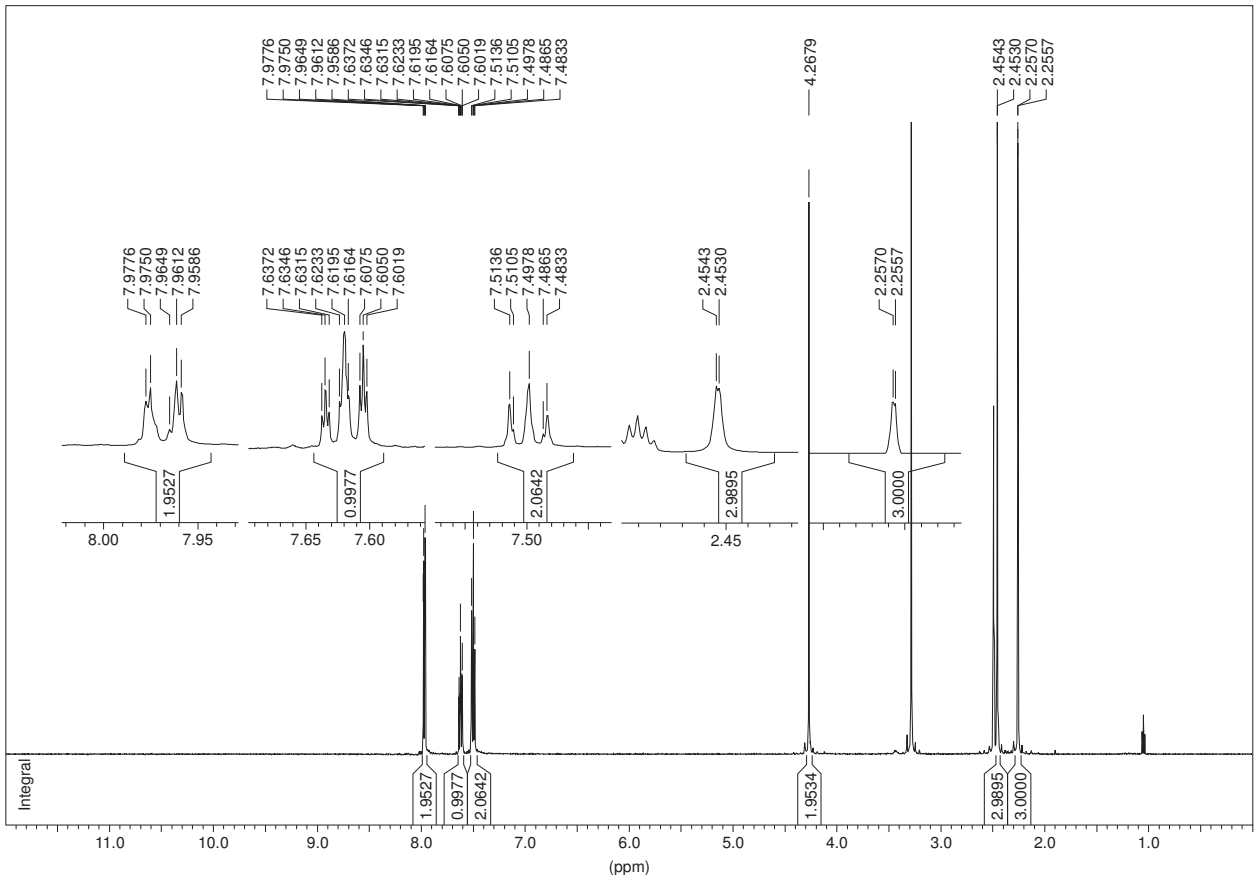


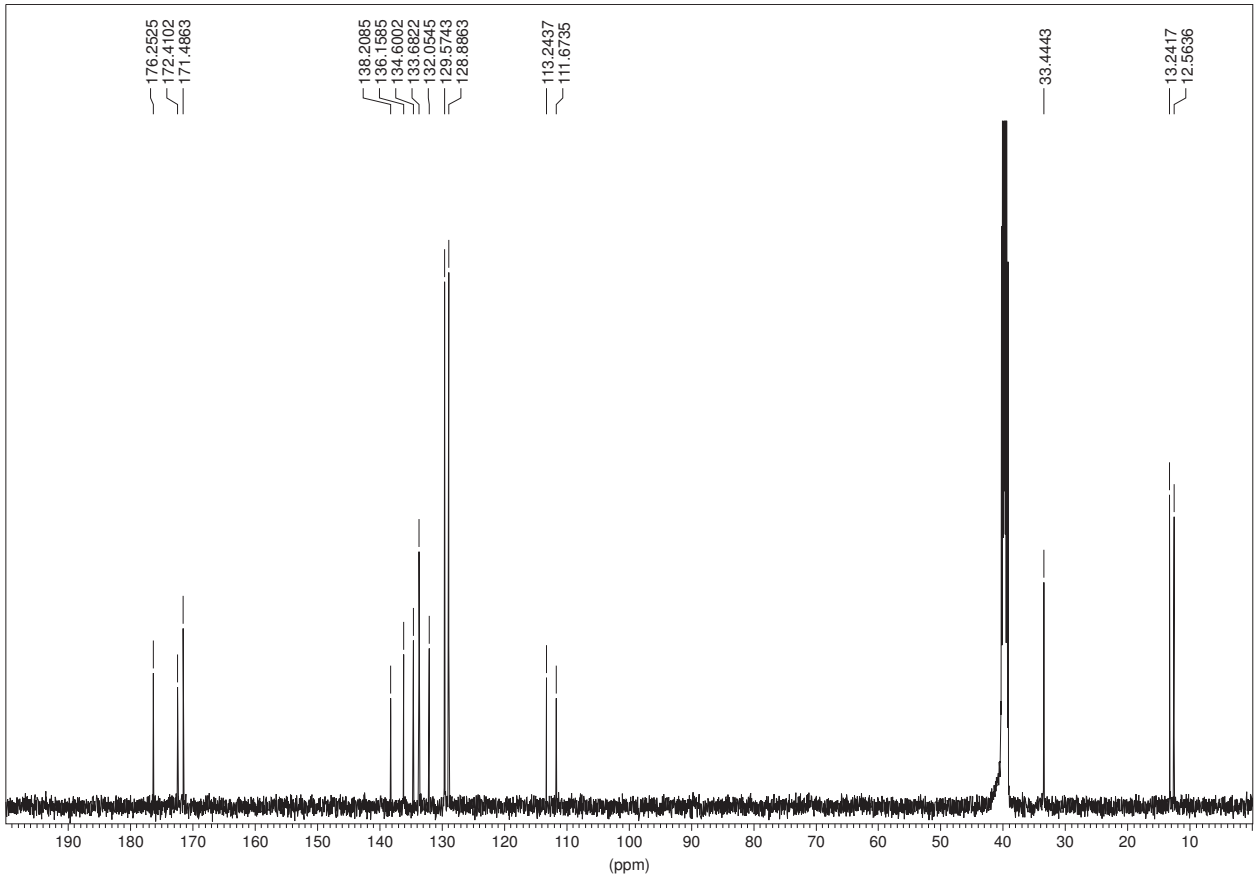
N-[3-(3-Cyano-2-thienyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**42**)



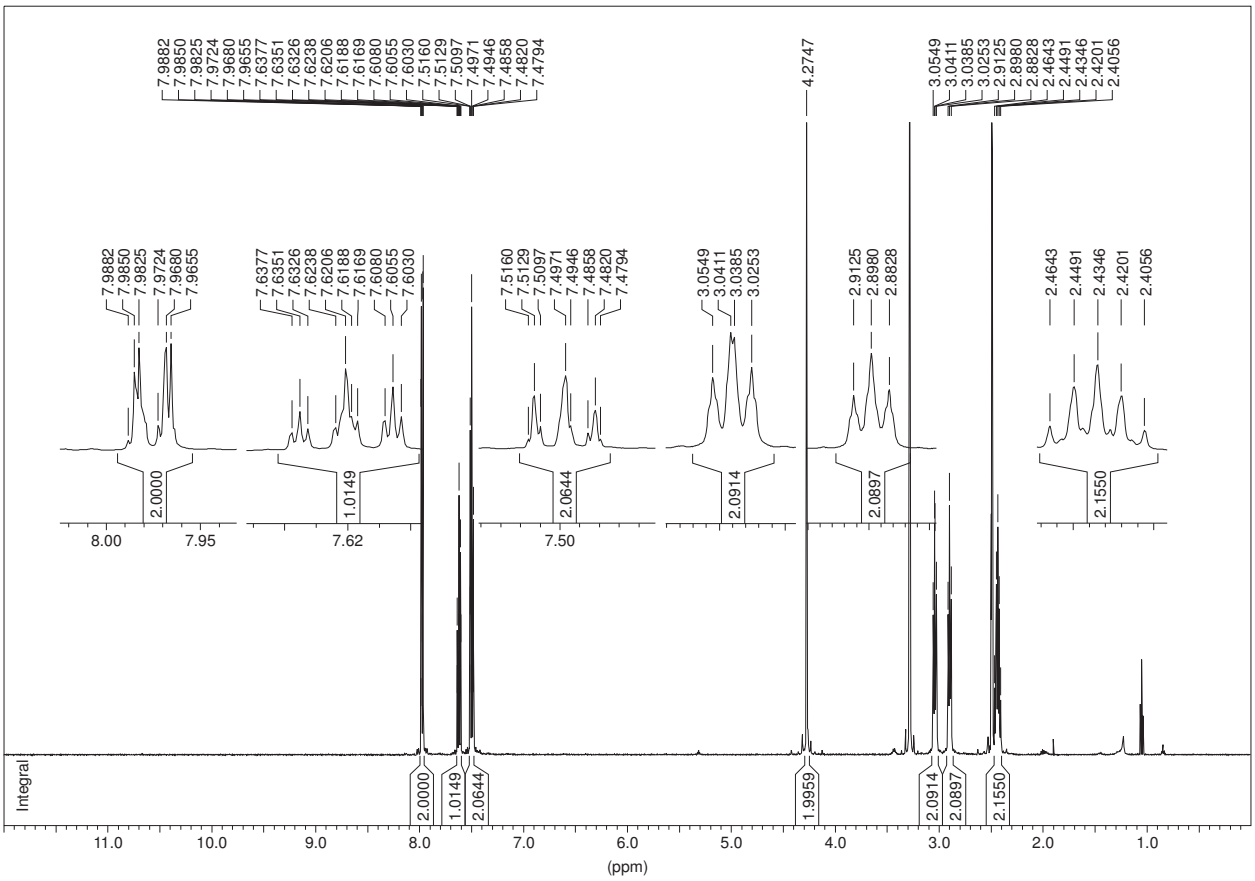


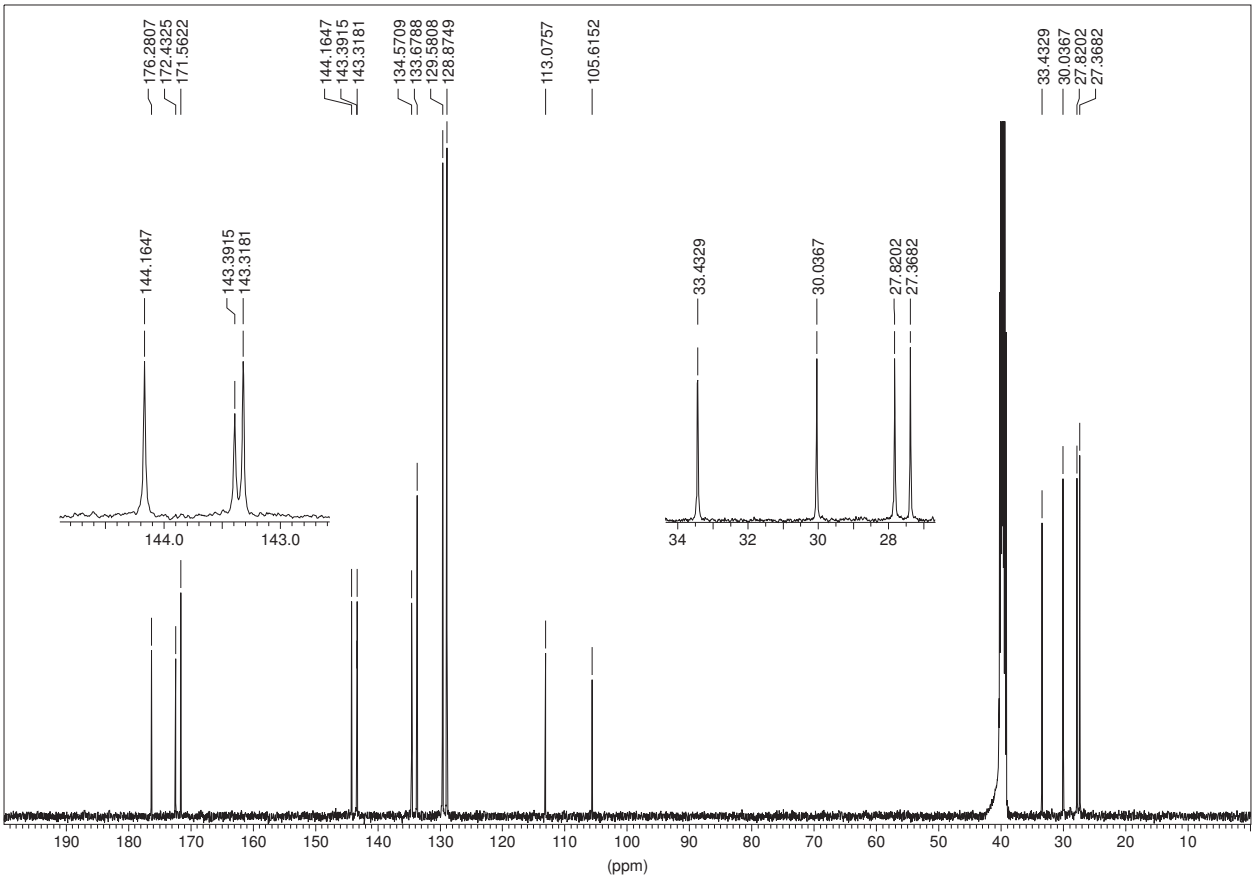
N-[3-(3-Cyano-4,5-dimethyl-2-thienyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**43**)



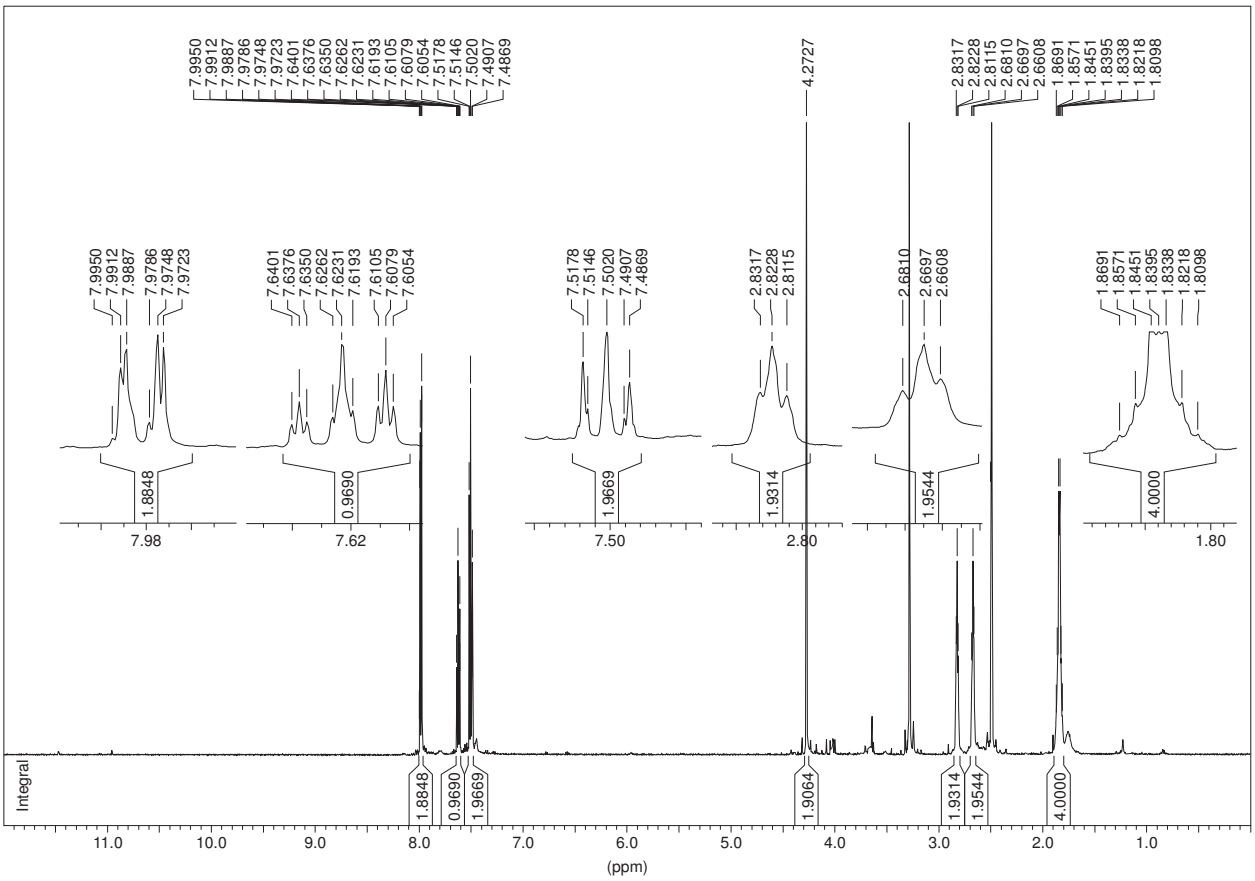


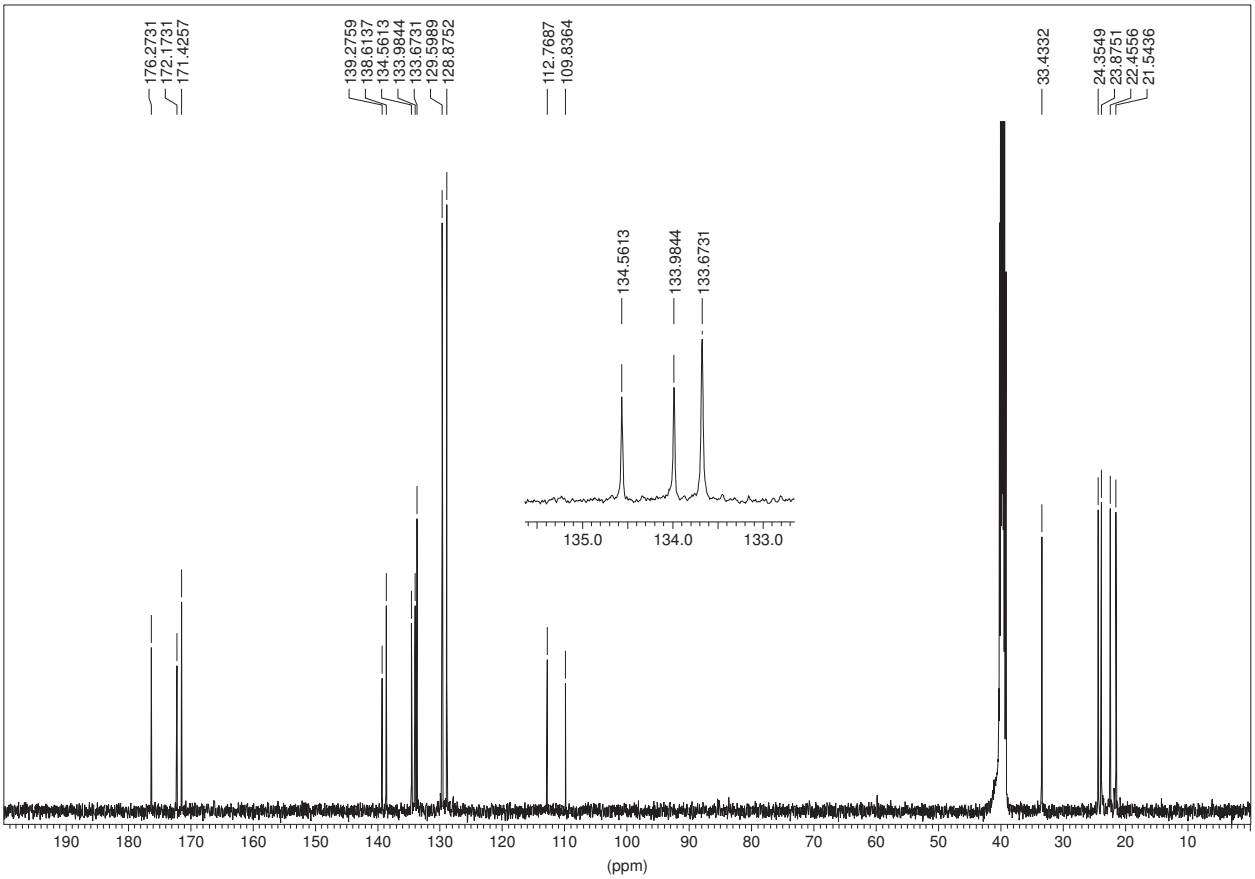
N-[3-(3-Cyano-5,6-dihydro-4*H*-cyclopenta[*b*]thien-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]-benzamide (**44**)



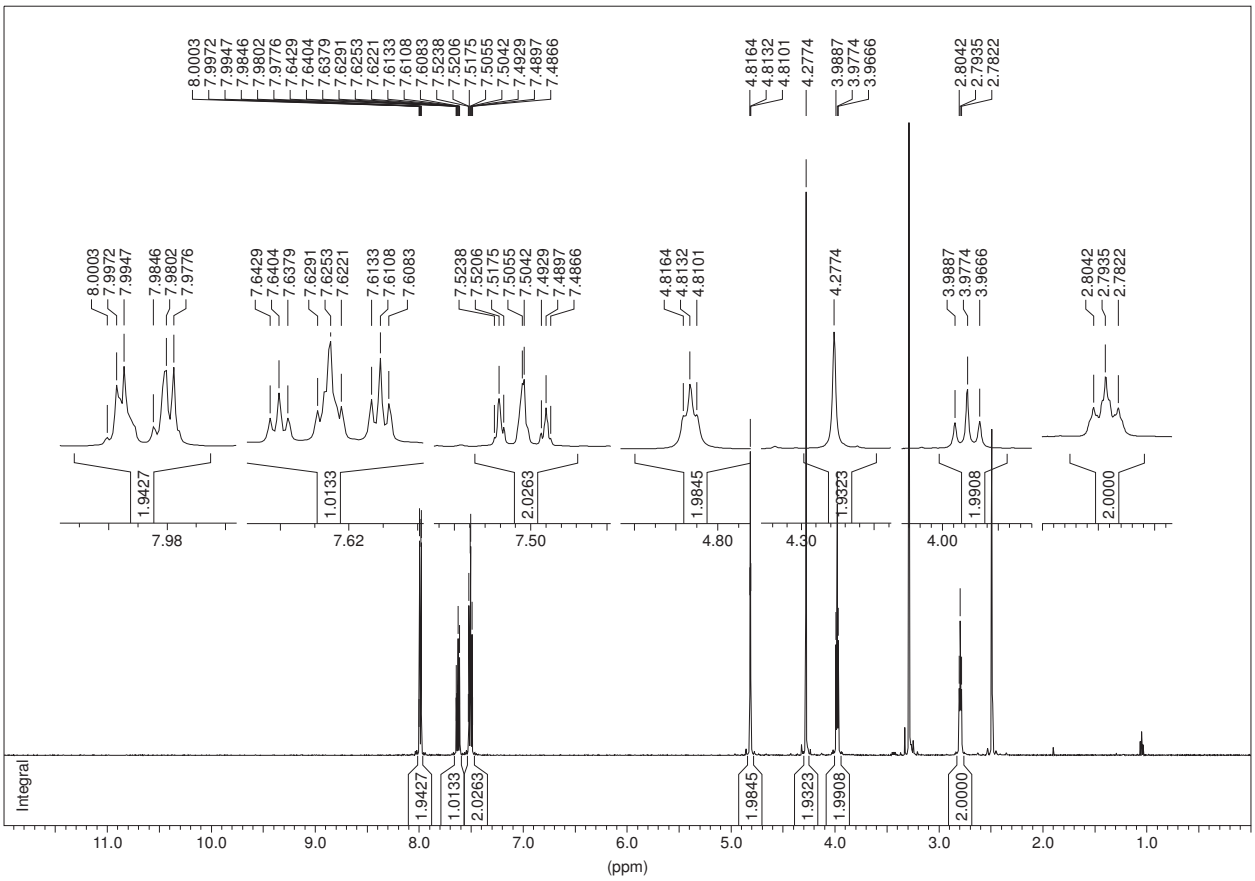


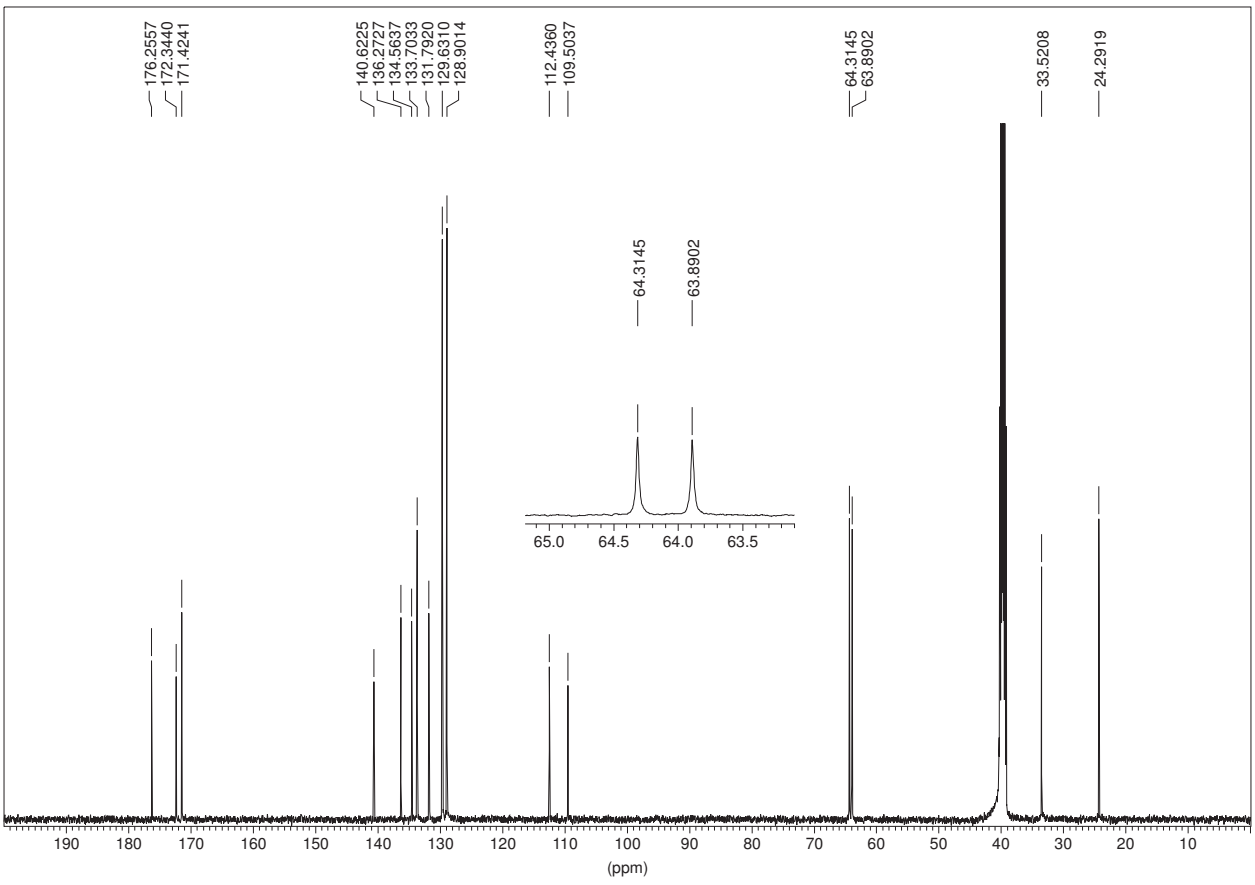
N-[3-(3-Cyano-4,5,6,7-tetrahydrobenzo[*b*]thien-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]-benzamide (**45**)



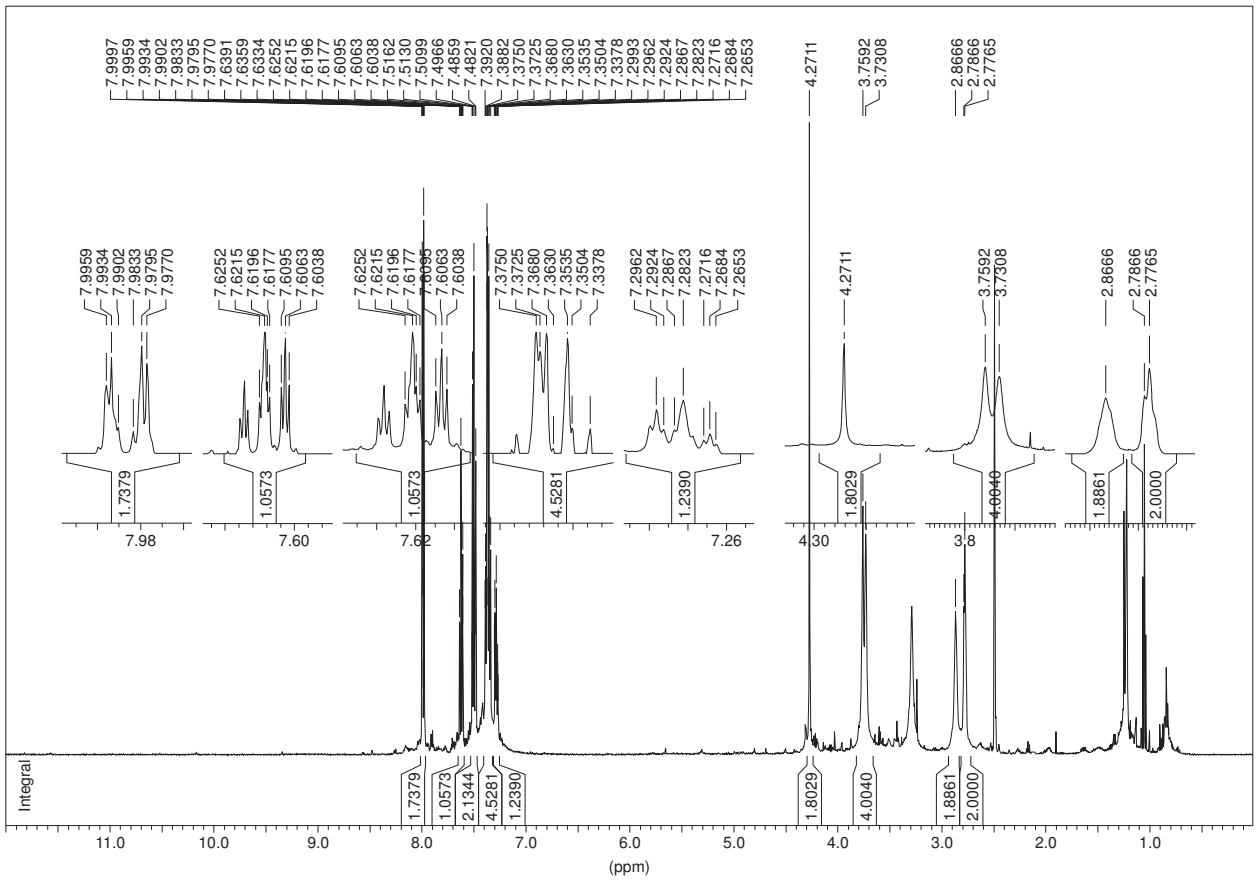


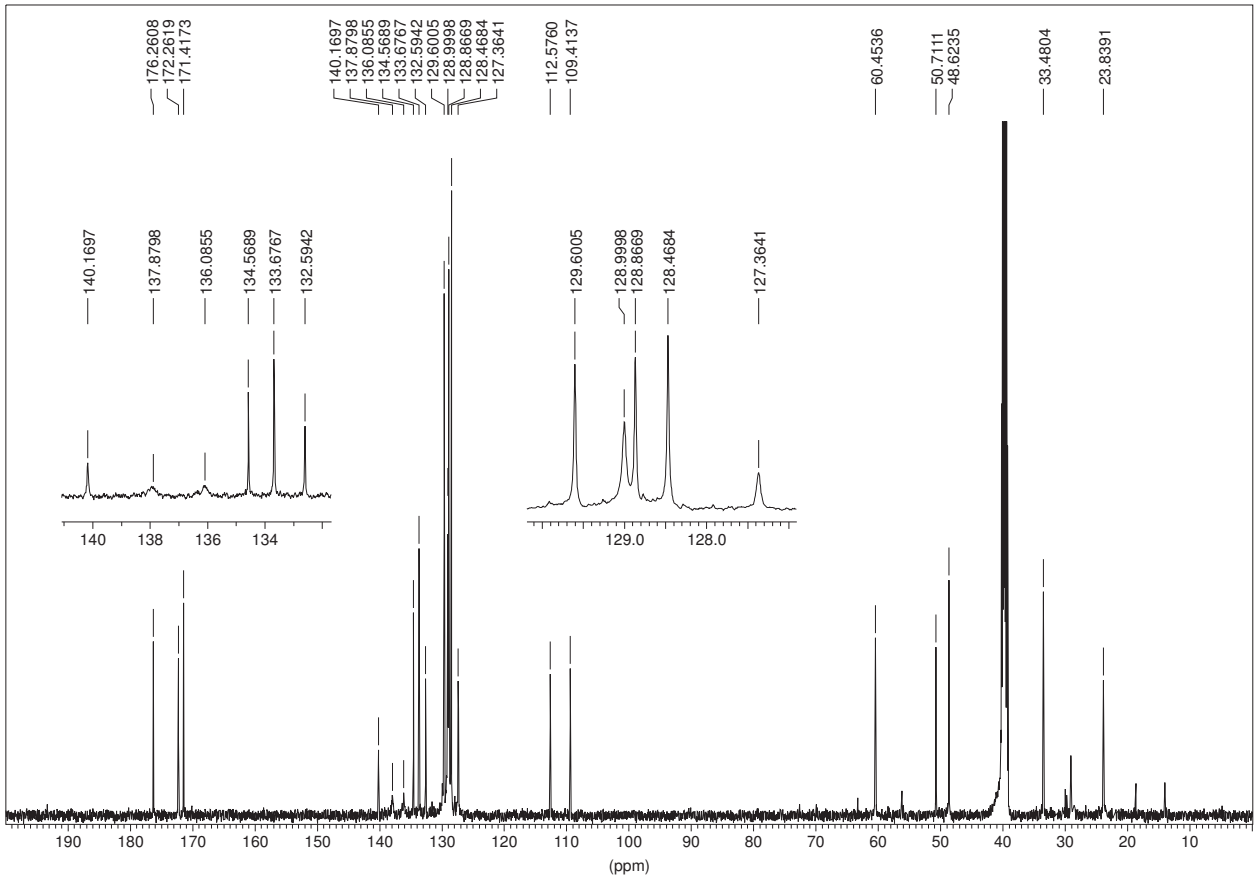
N-[3-(3-Cyano-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]-benzamide (**46**)



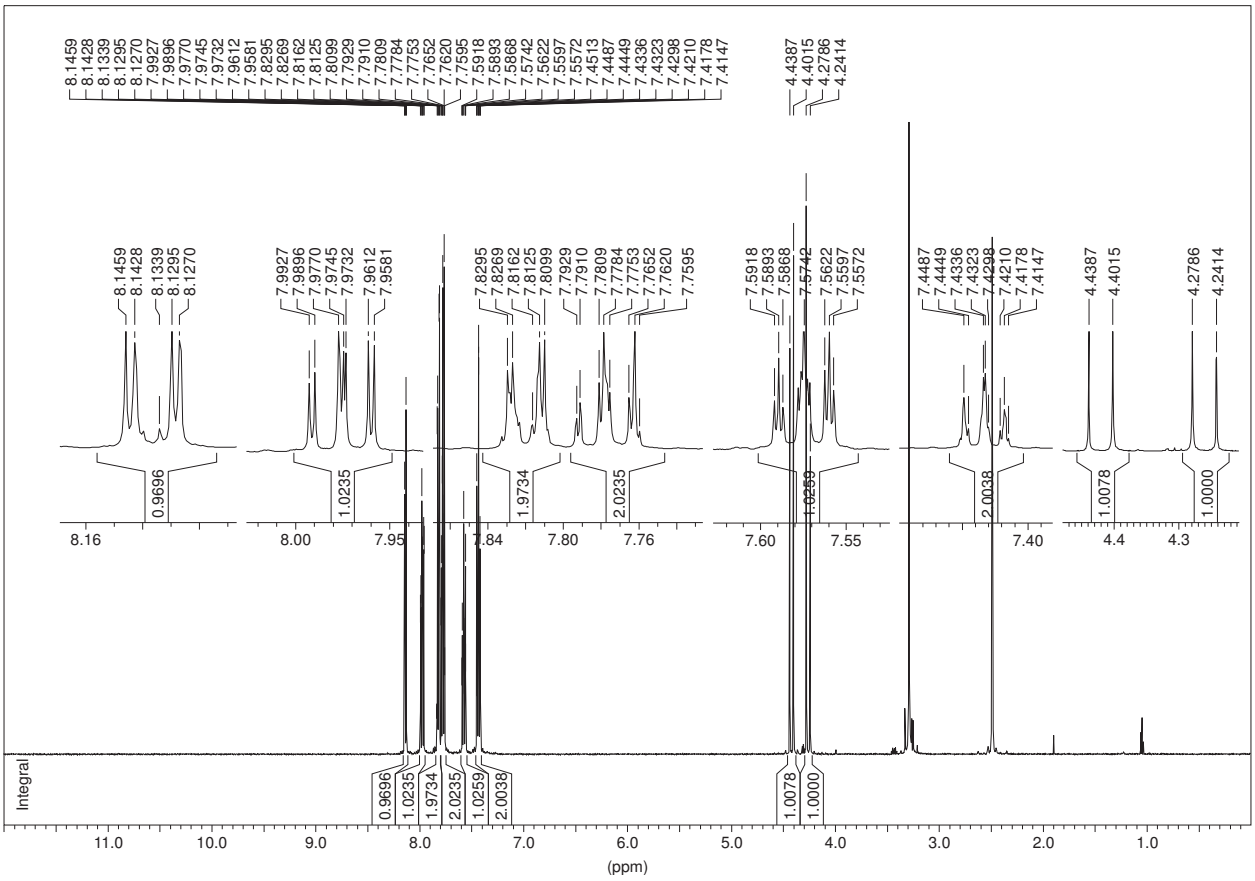


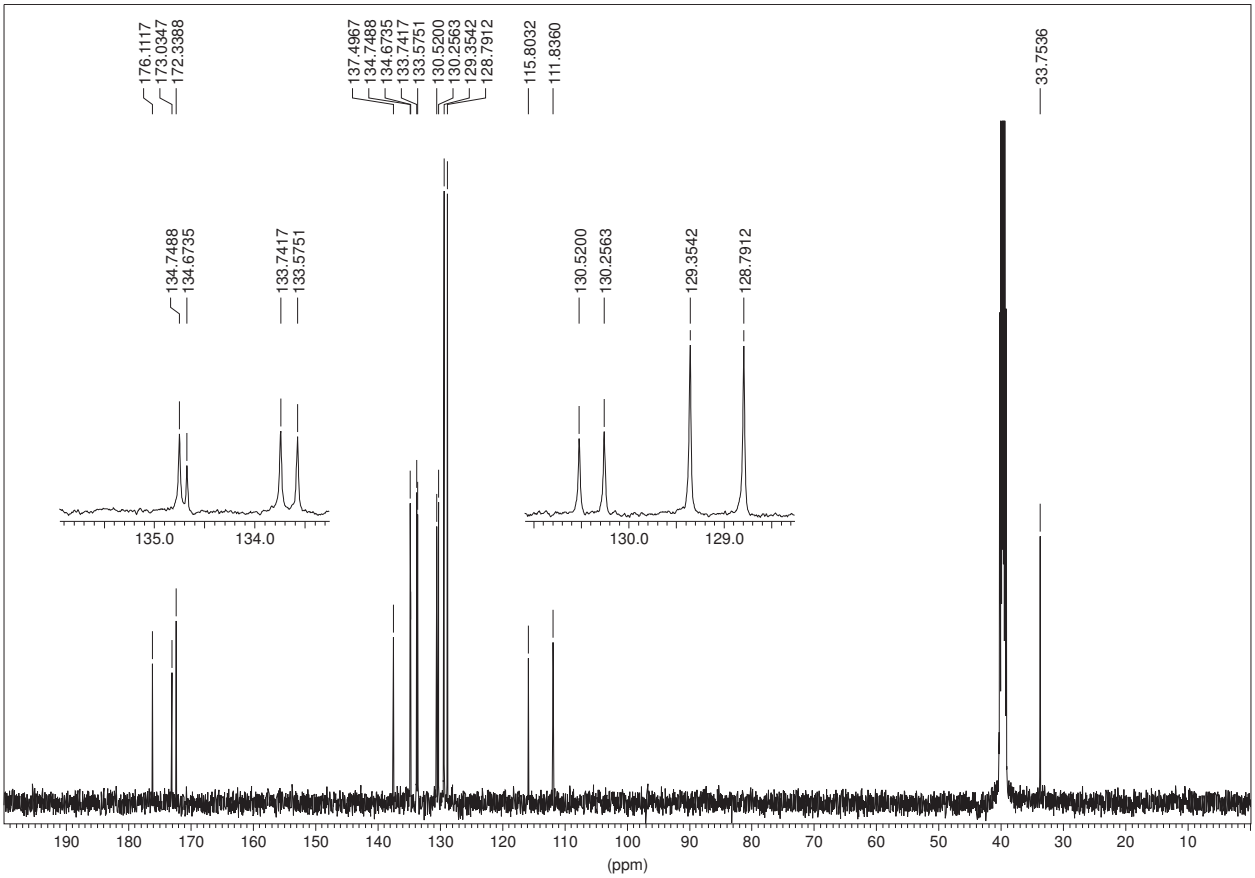
N-[3-(6-Benzyl-3-cyano-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridin-2-yl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**47**)



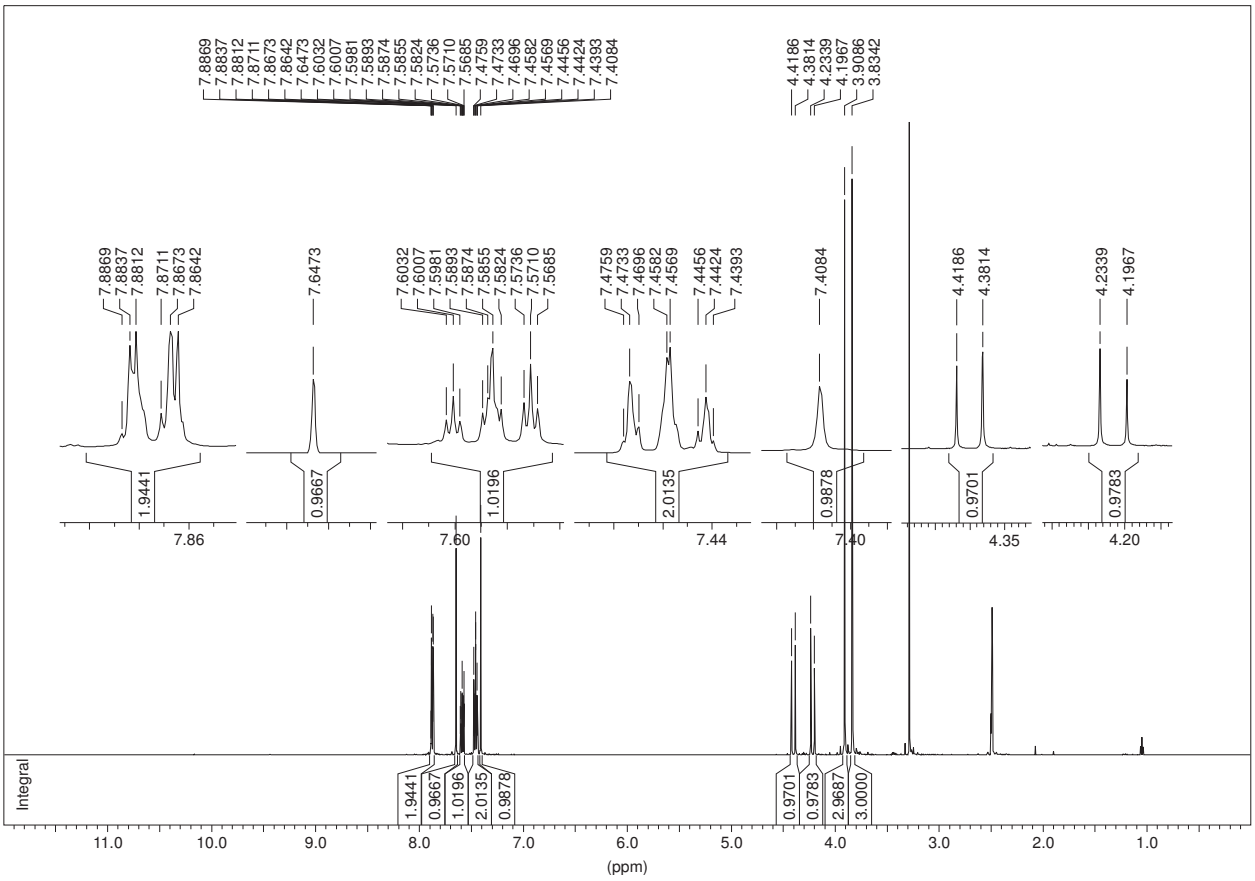


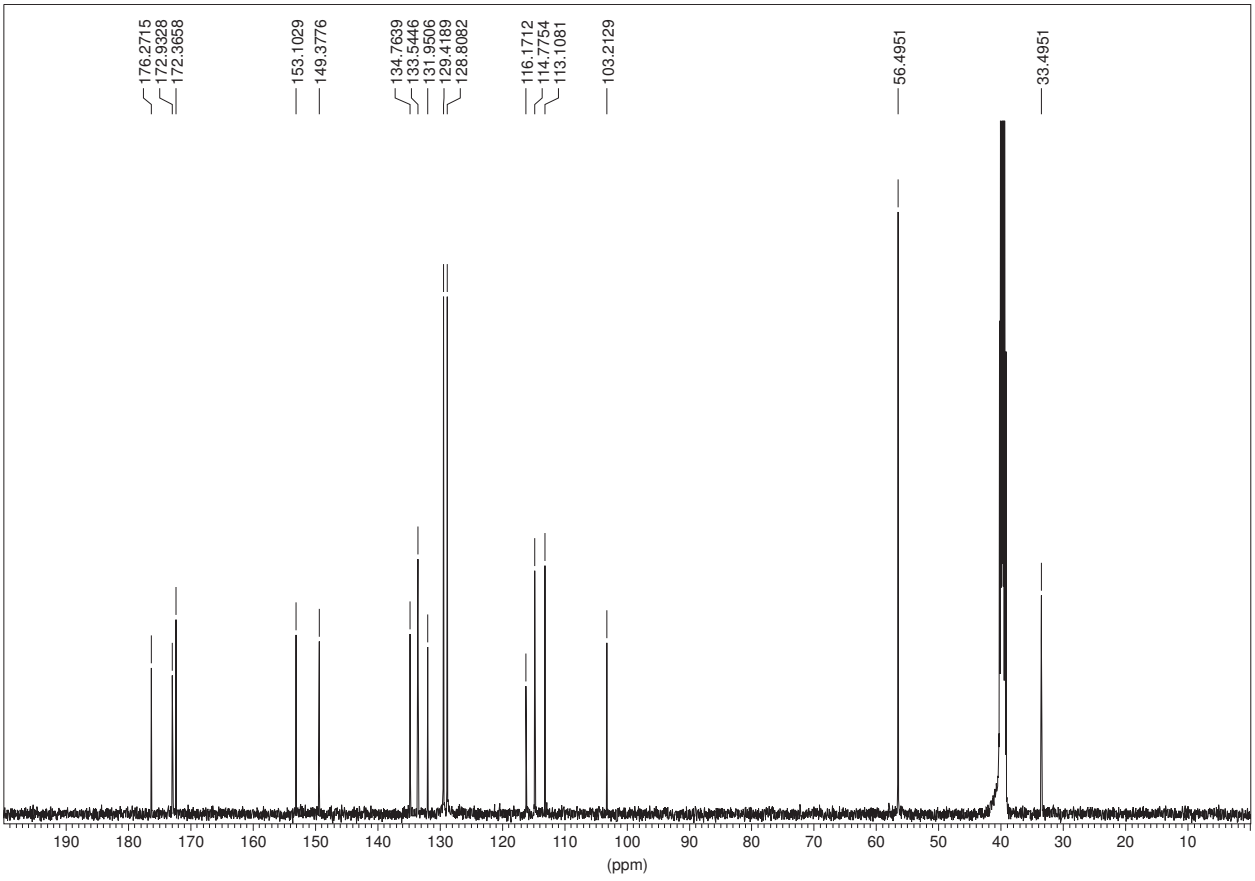
N-[3-(2-Cyanophenyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**48**)



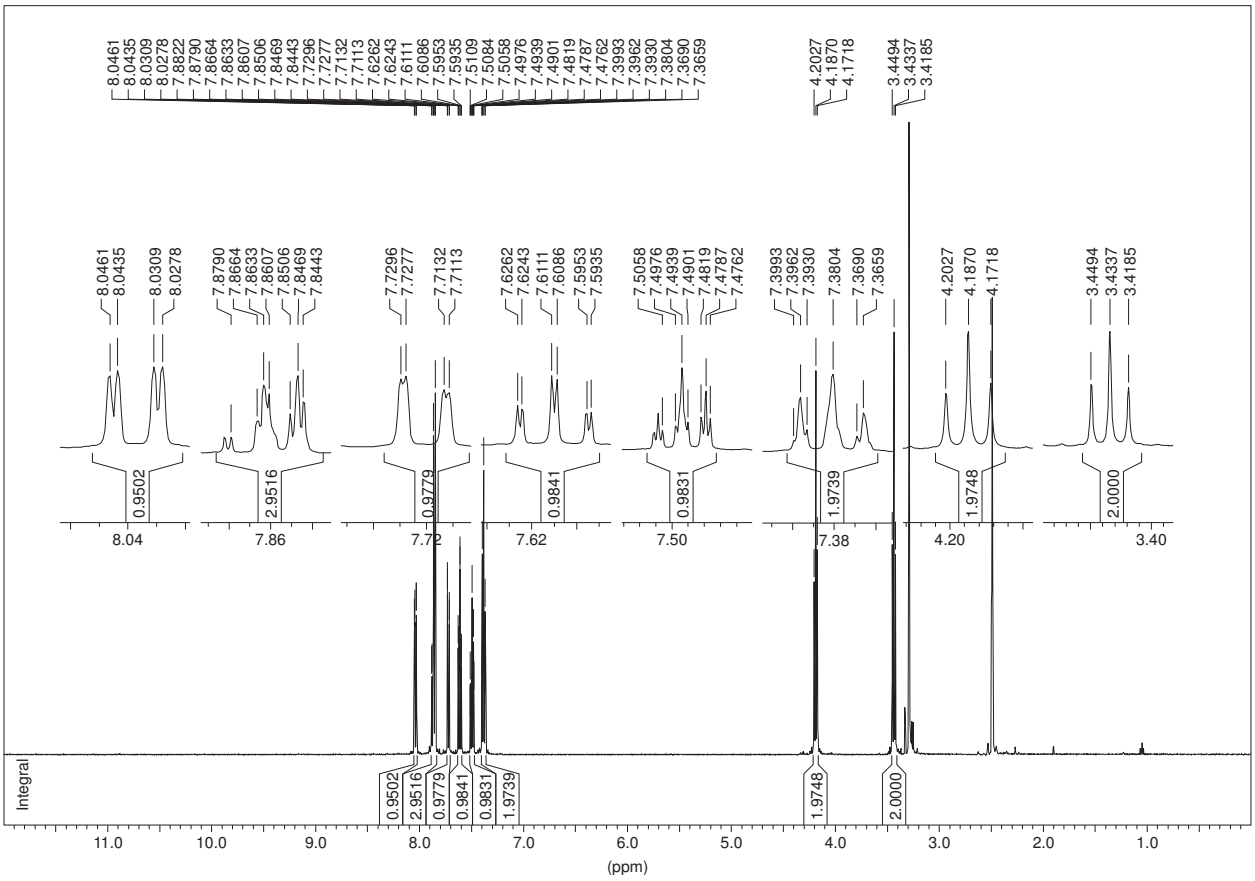


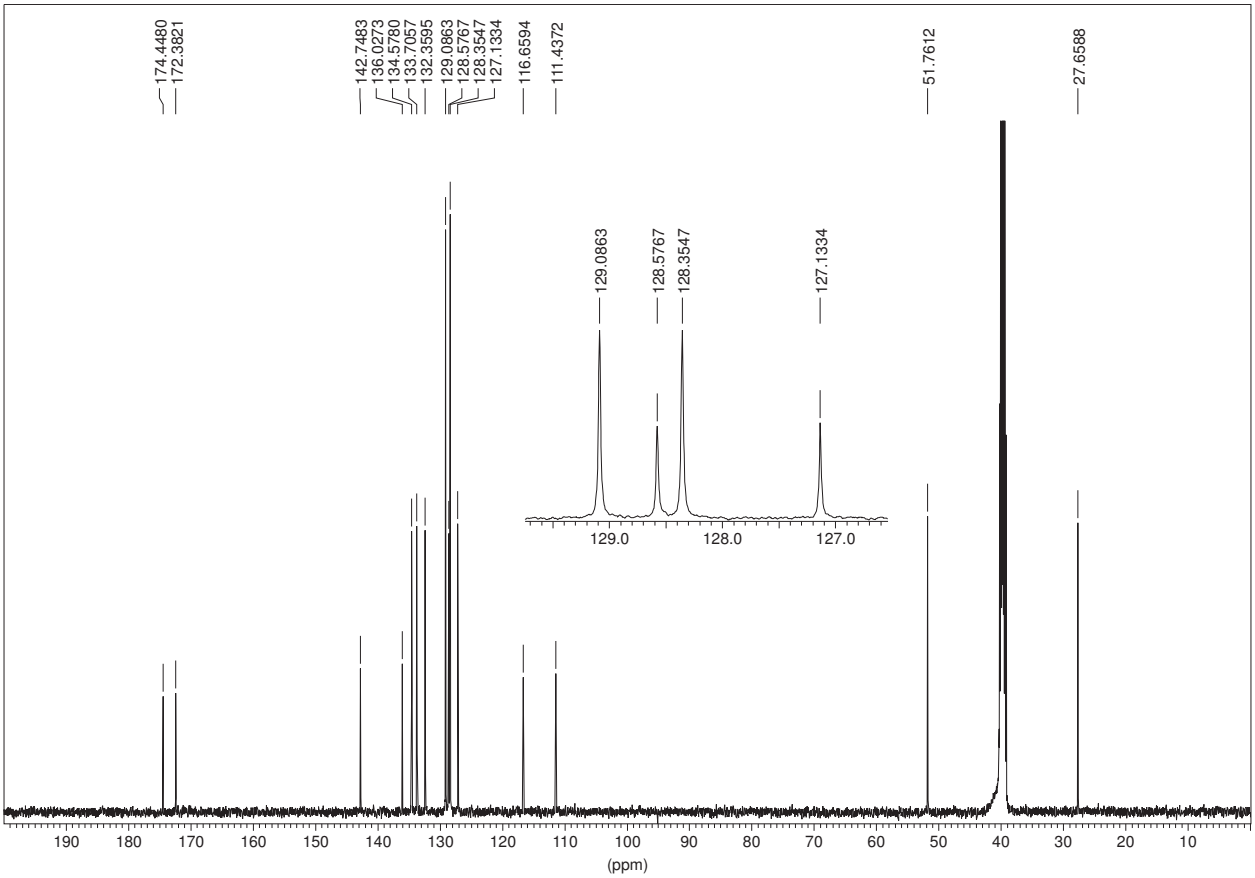
N-[3-(2-Cyano-4,5-dimethoxyphenyl)-4-oxo-1,3-thiazolidin-2-ylidene]benzamide (**49**)



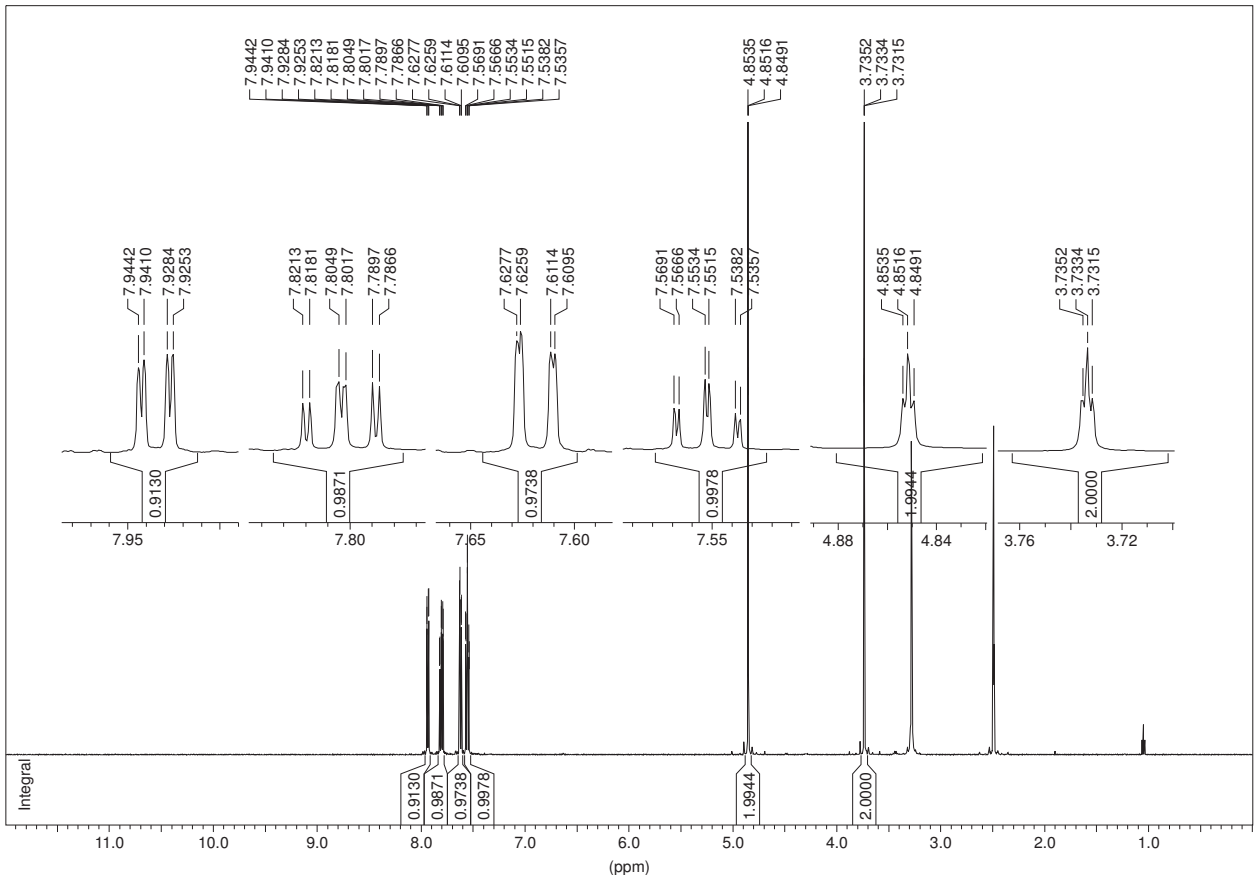


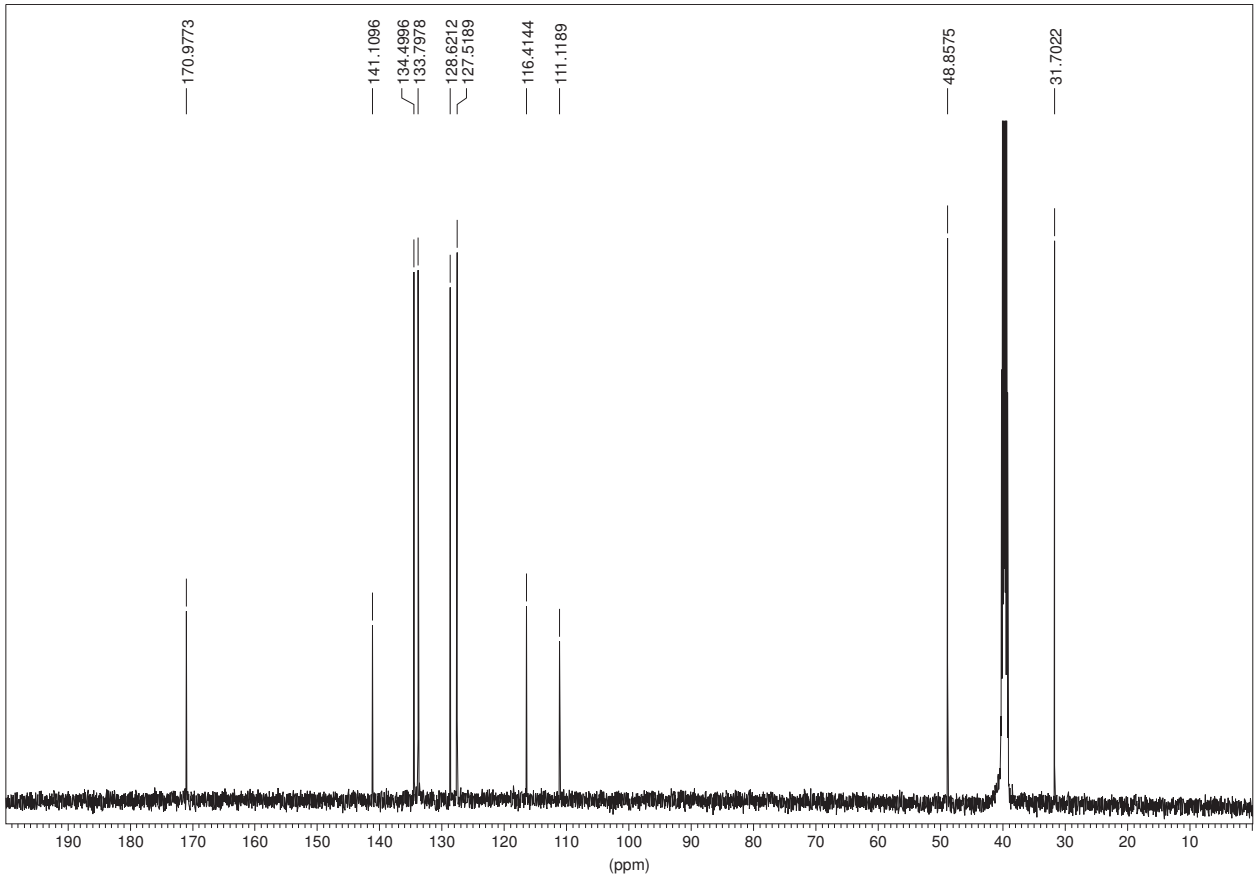
N-[3-(2-Cyanophenyl)-1,3-thiazolidin-2-ylidene]benzamide (**50**)



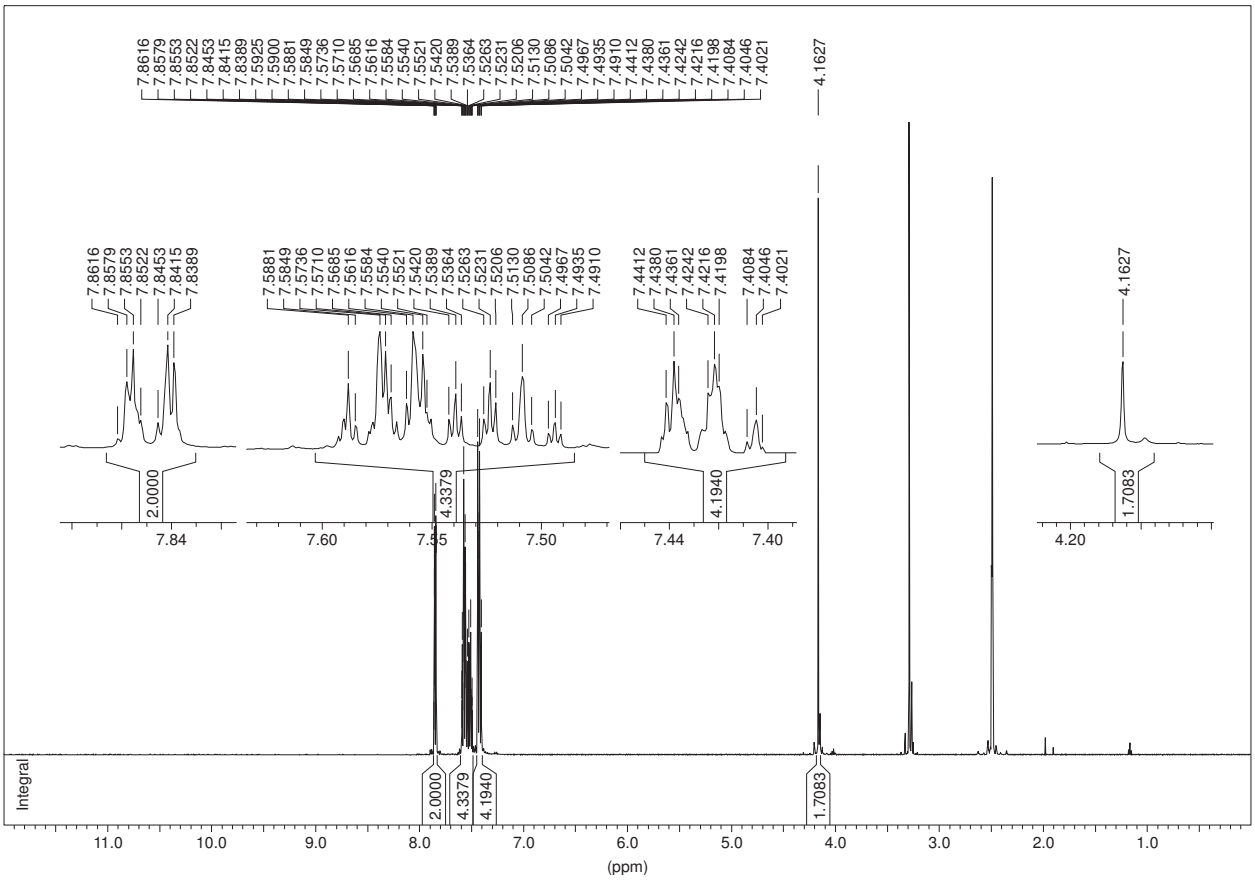


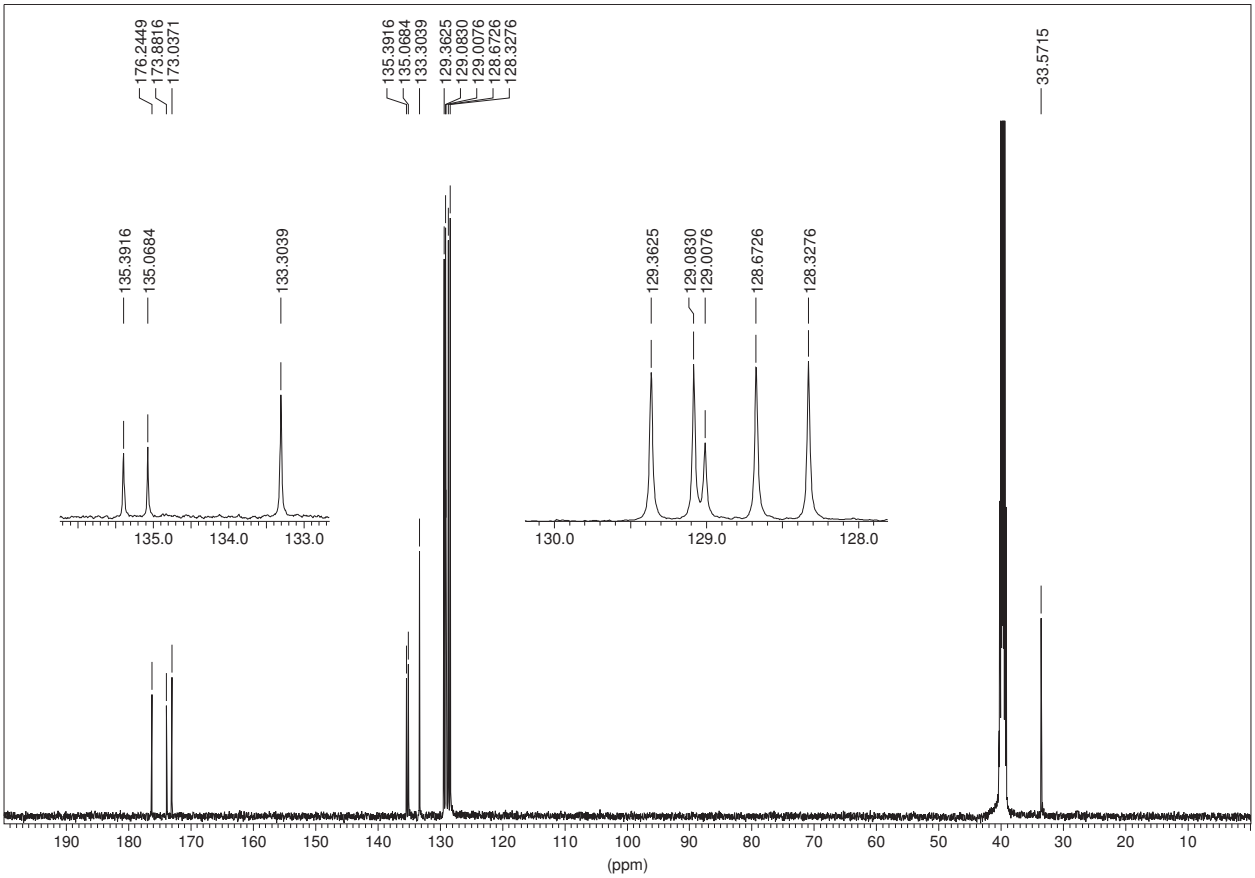
2-(4-Oxo-1,3-thiazolidin-3-yl)benzonitrile (**51**)



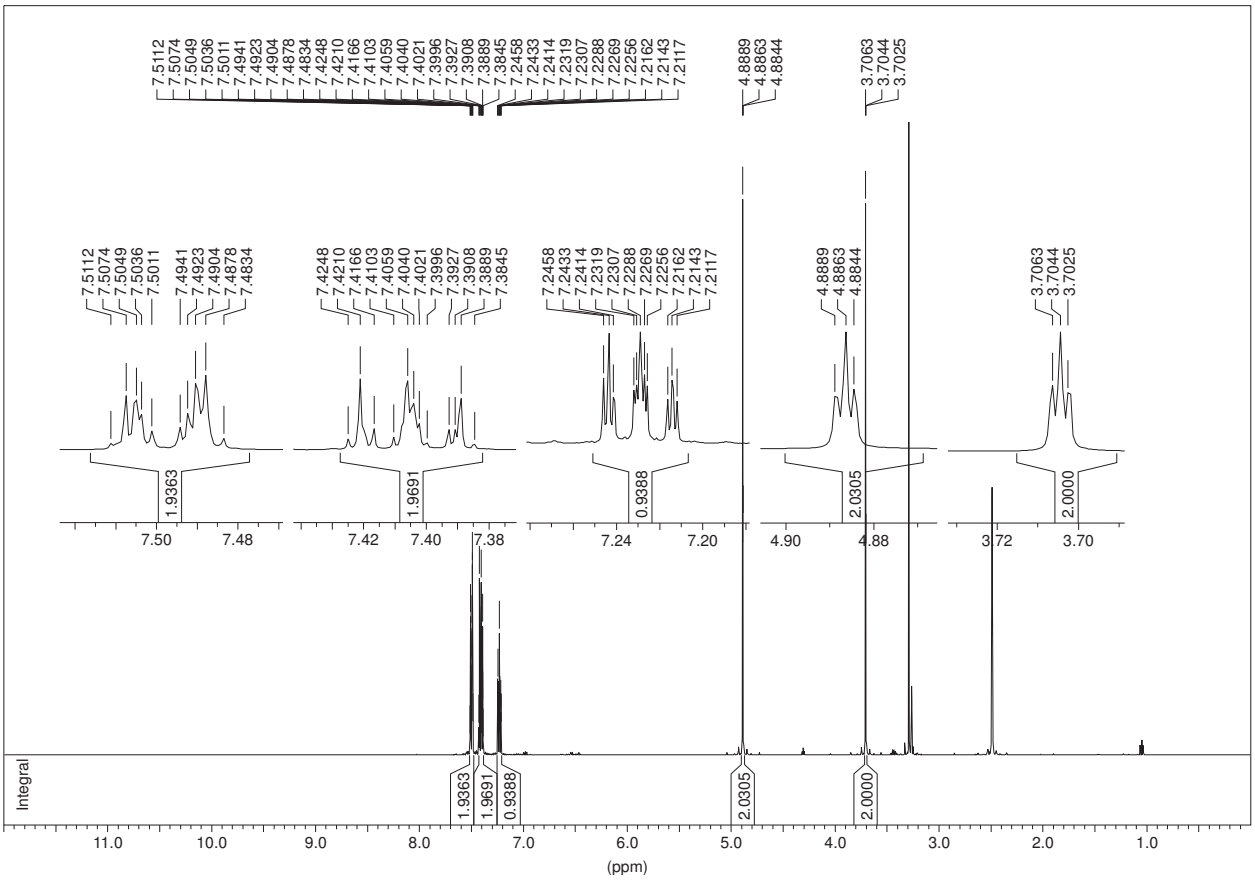


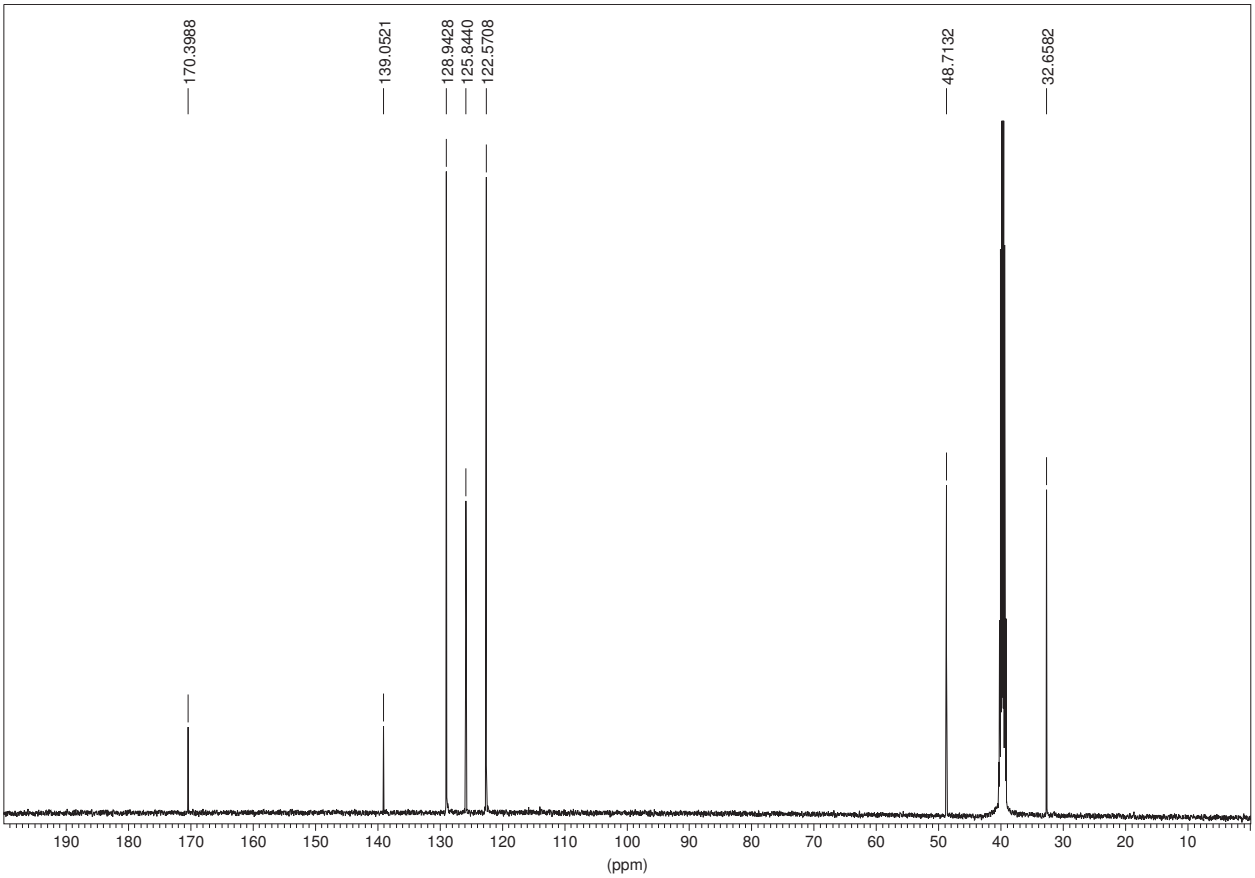
N-(4-Oxo-3-phenyl-1,3-thiazolidin-2-ylidene)benzamide (**52**)



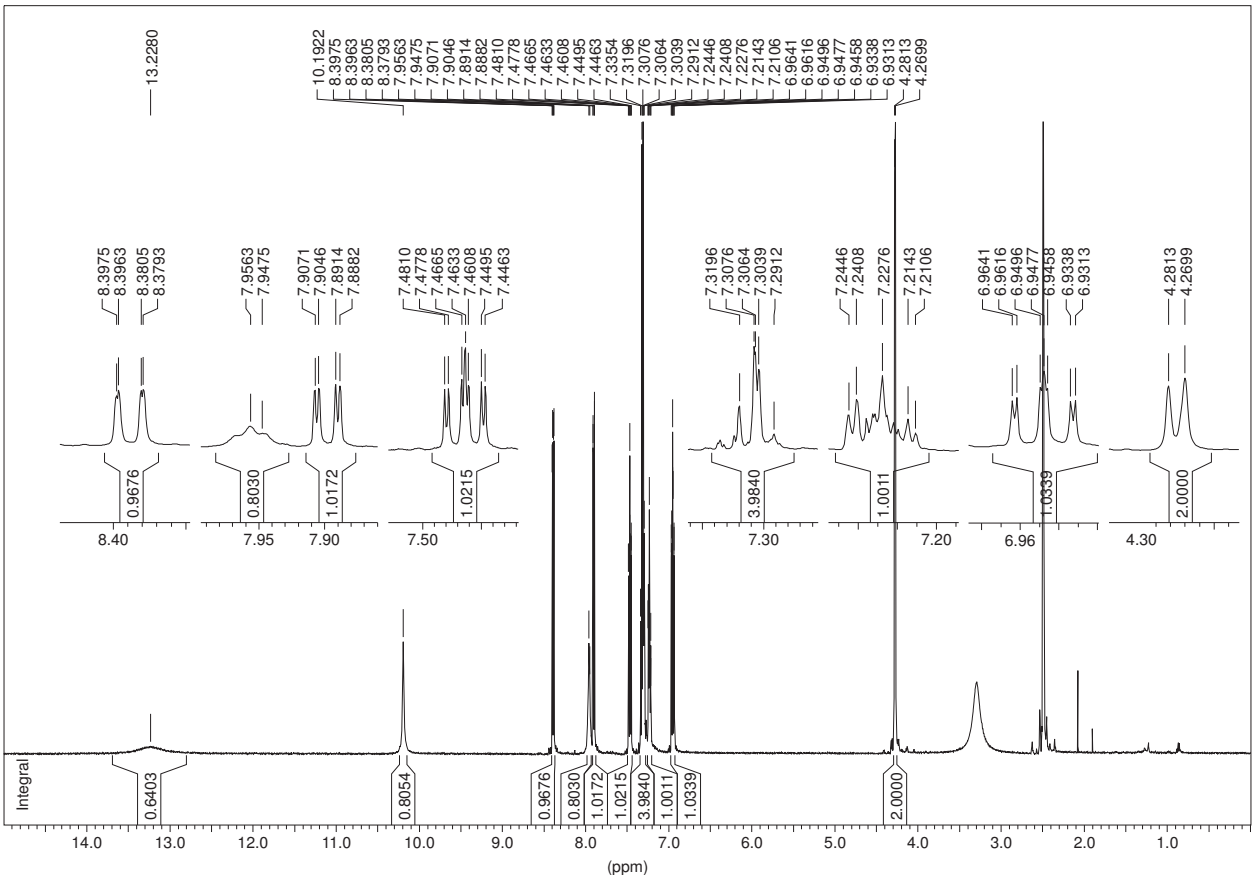


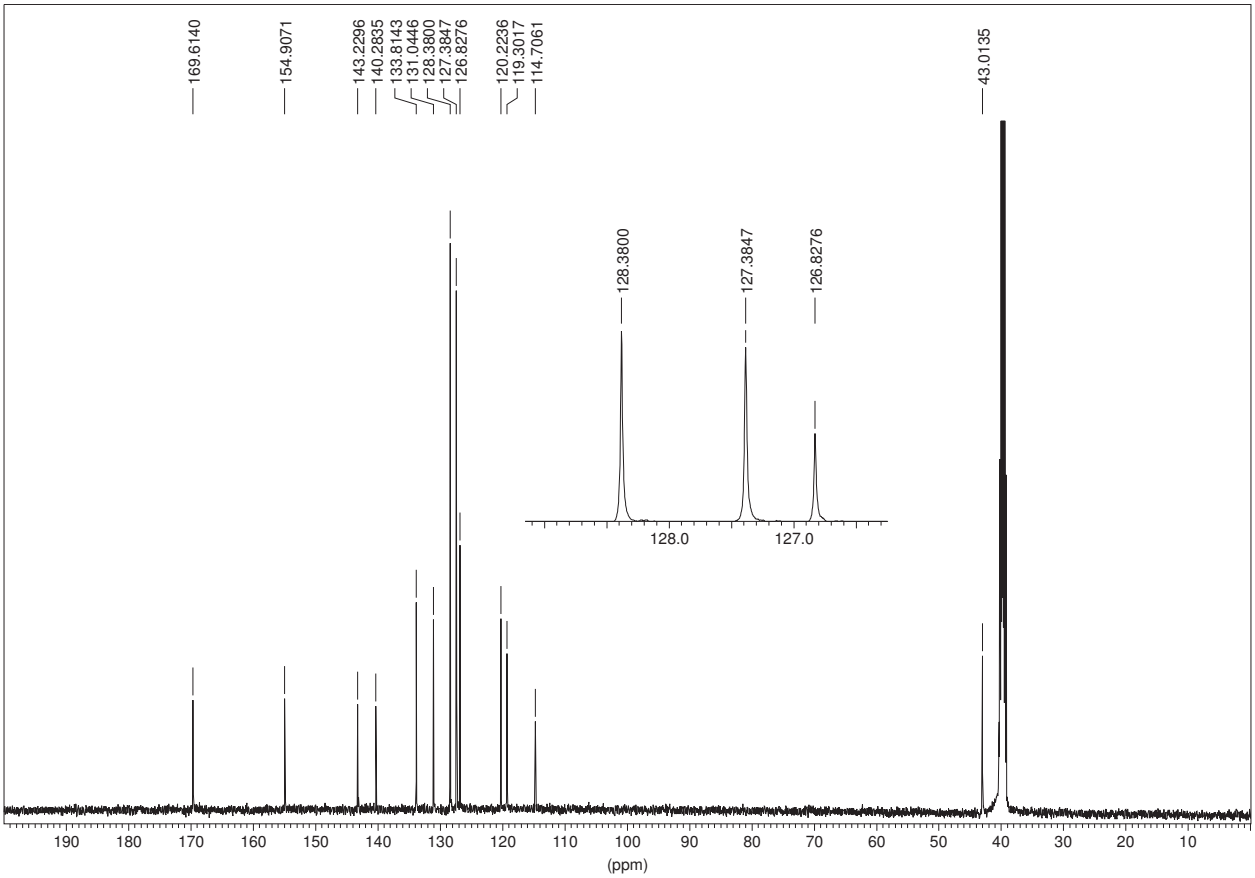
3-Phenyl-1,3-thiazolidin-4-one (**53**)



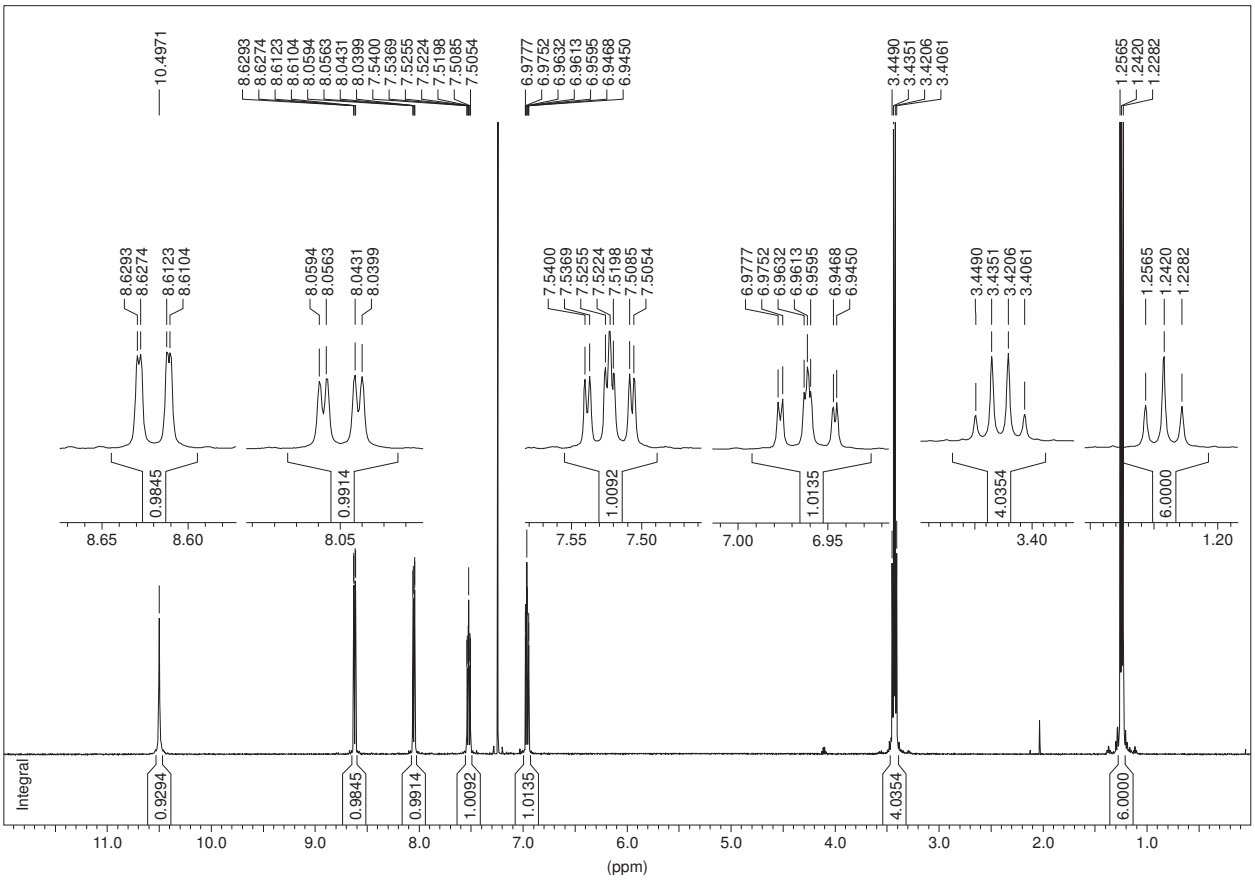


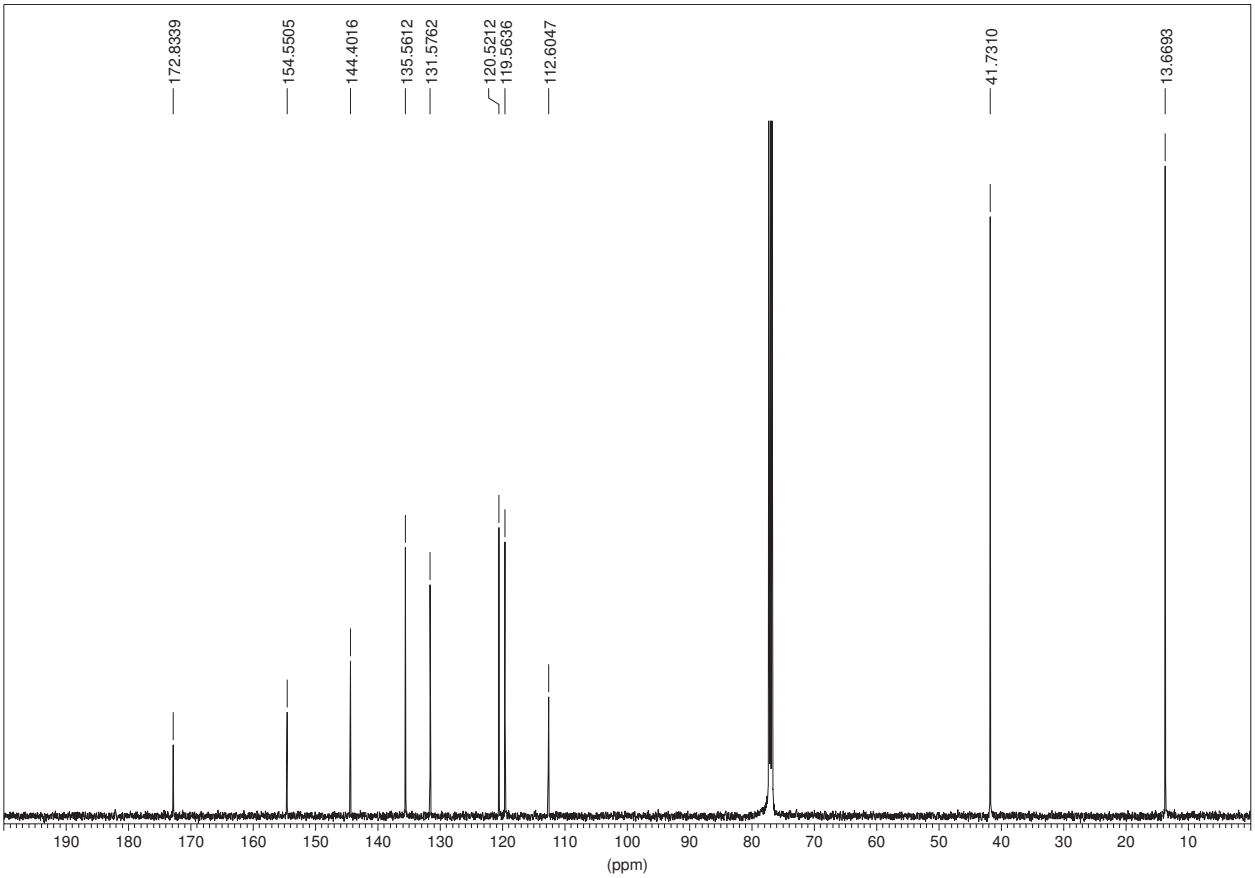
2-(3-Benzylureido)benzoic Acid (**54**)



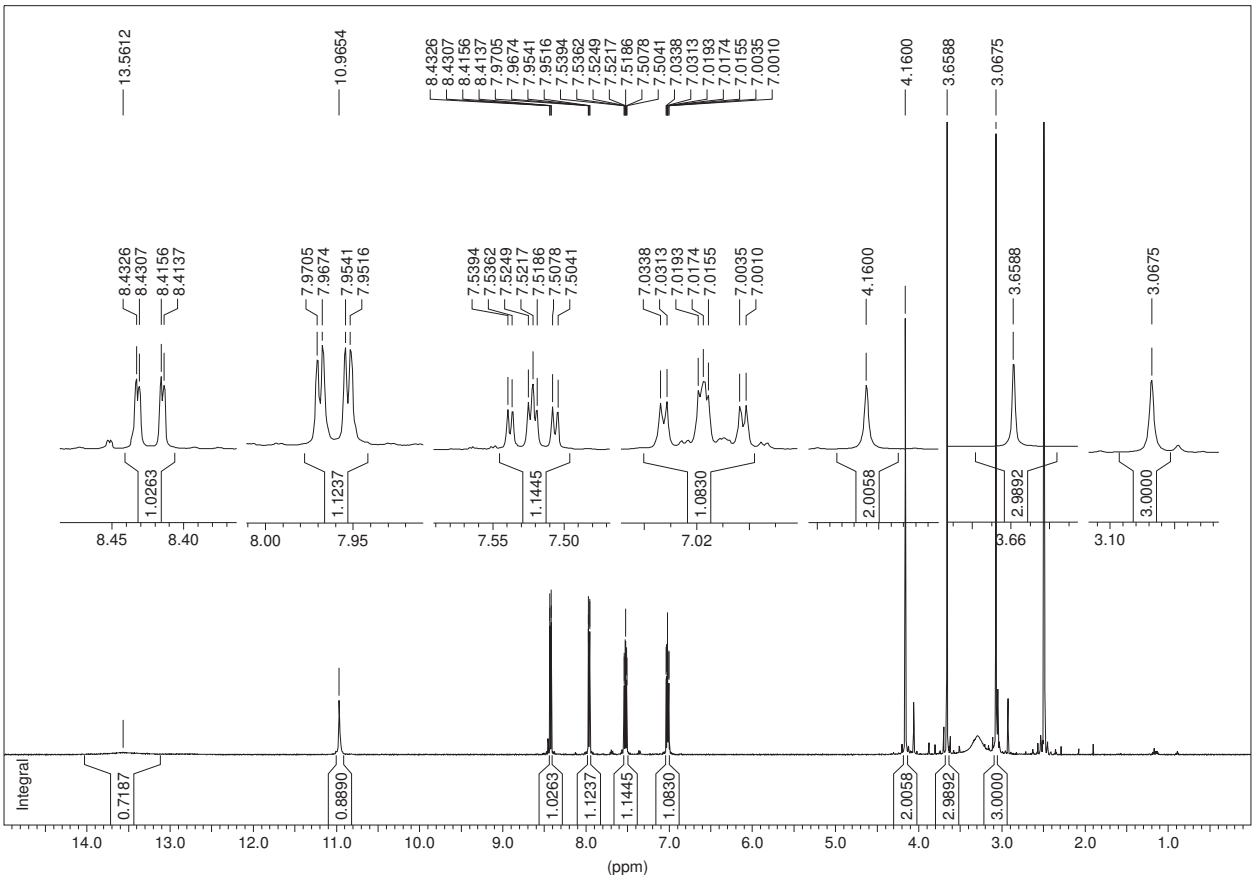


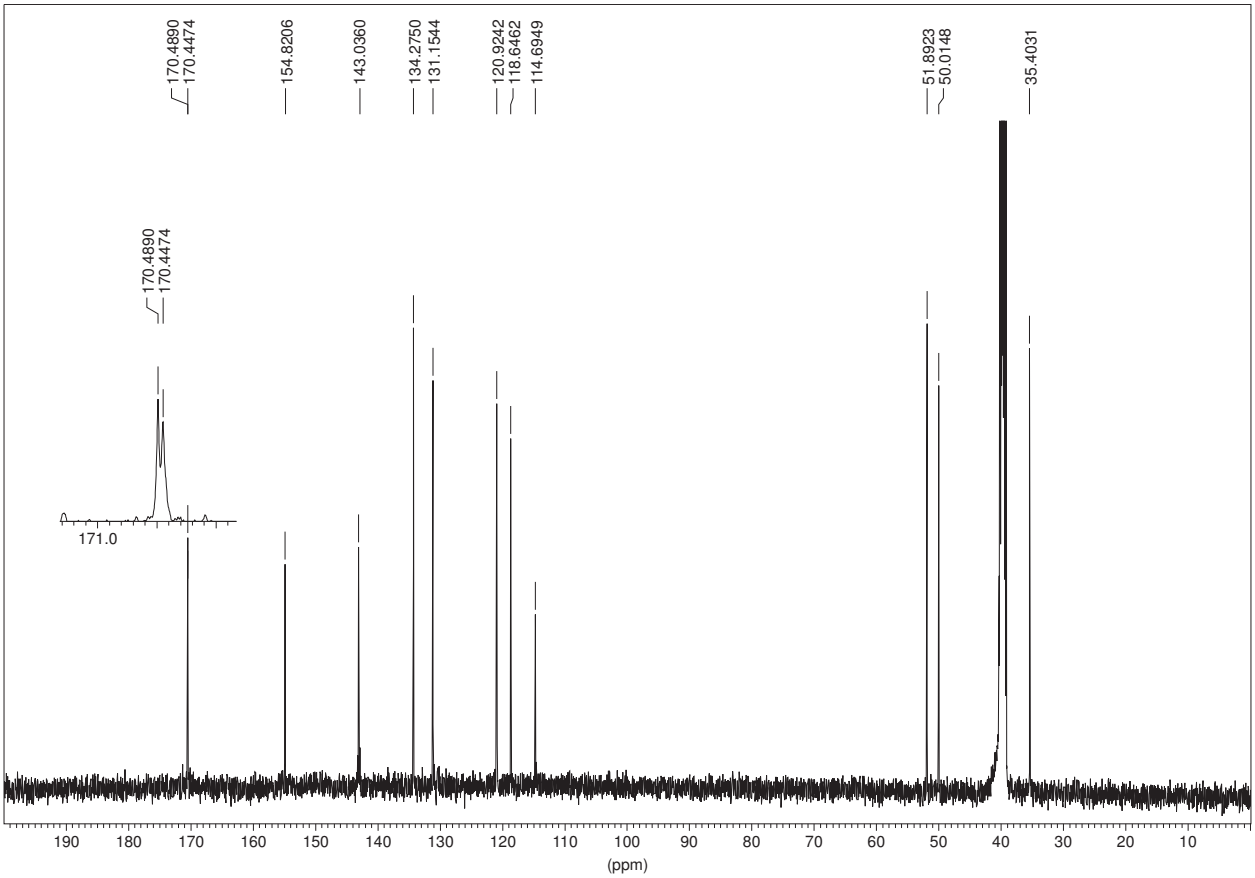
2-(3,3-Diethylureido)benzoic Acid (**55**)



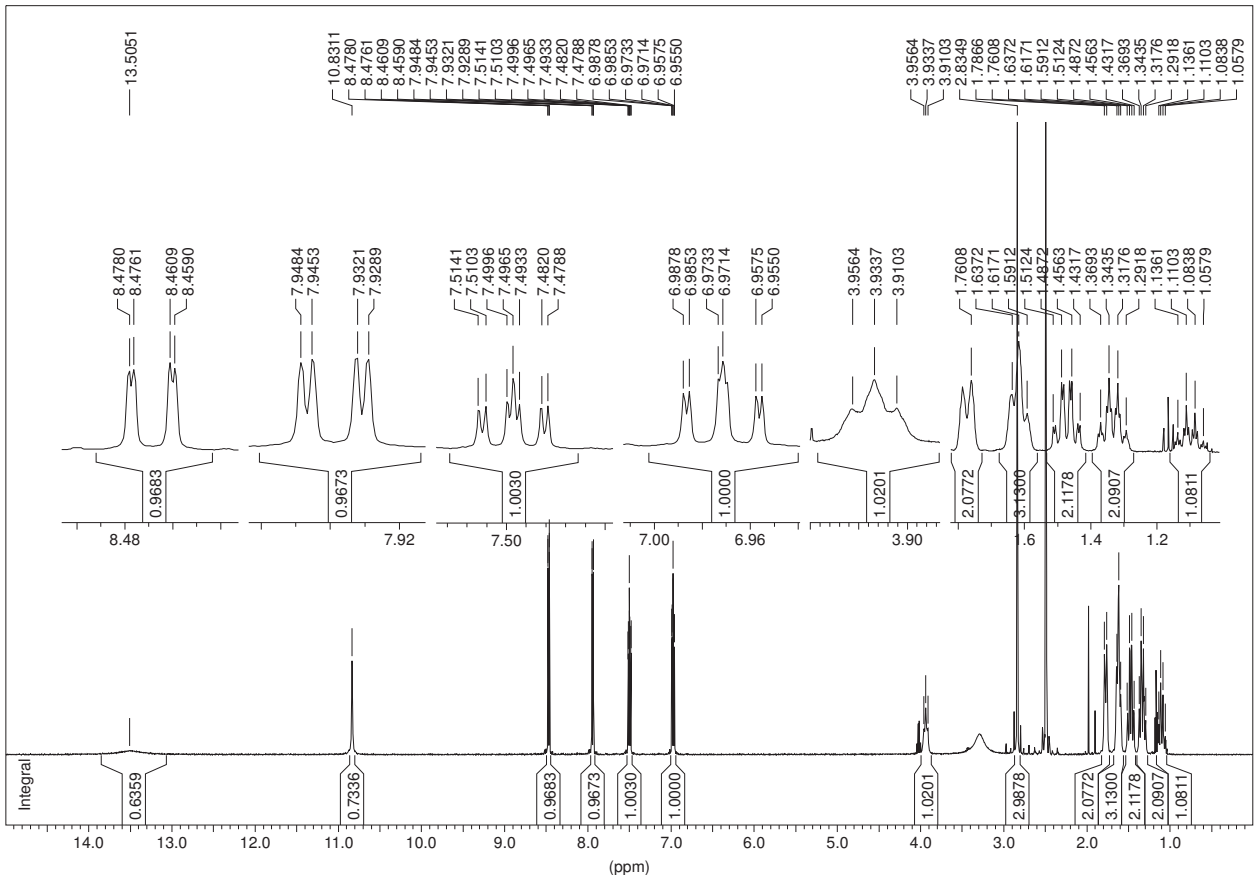


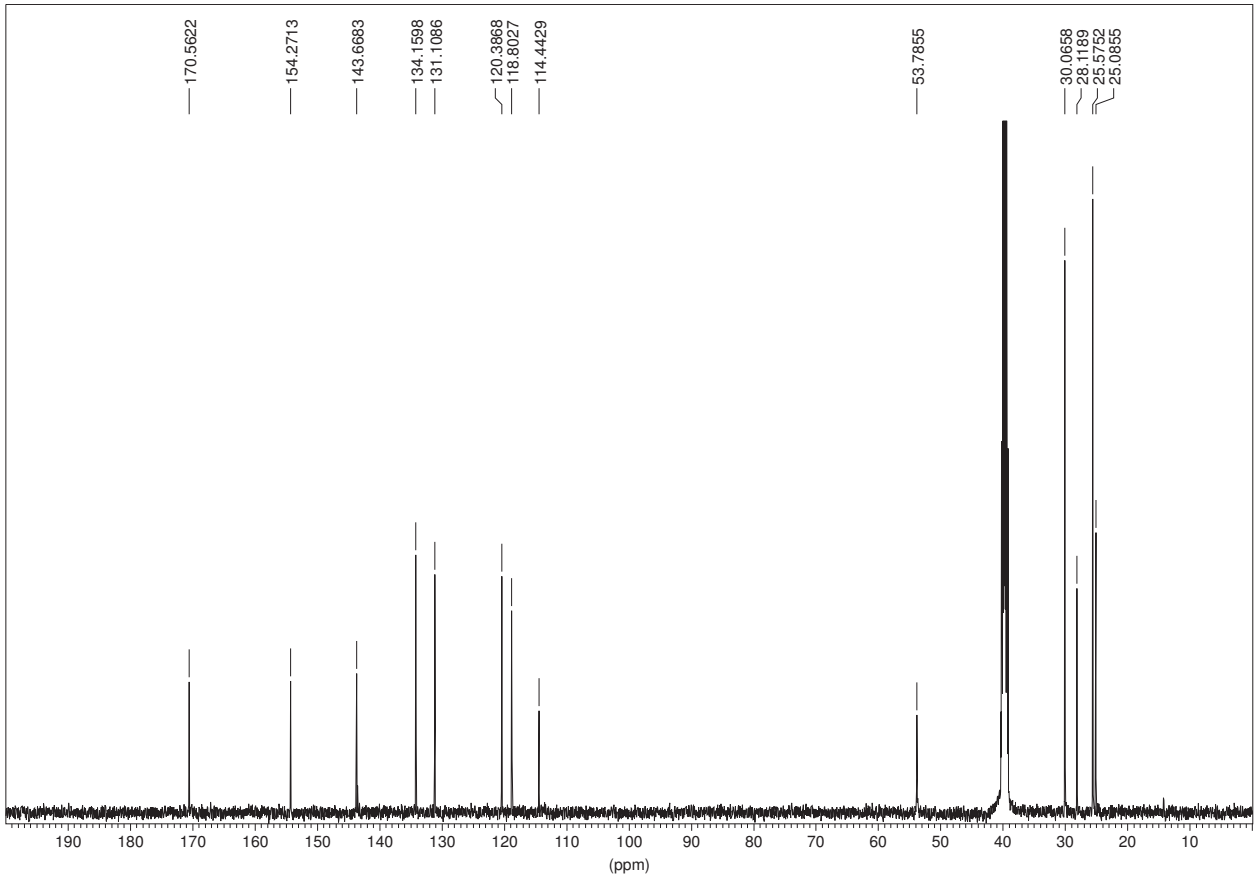
2-[3-(Methoxycarbonylmethyl)-3-methylureido]benzoic Acid (**56**)



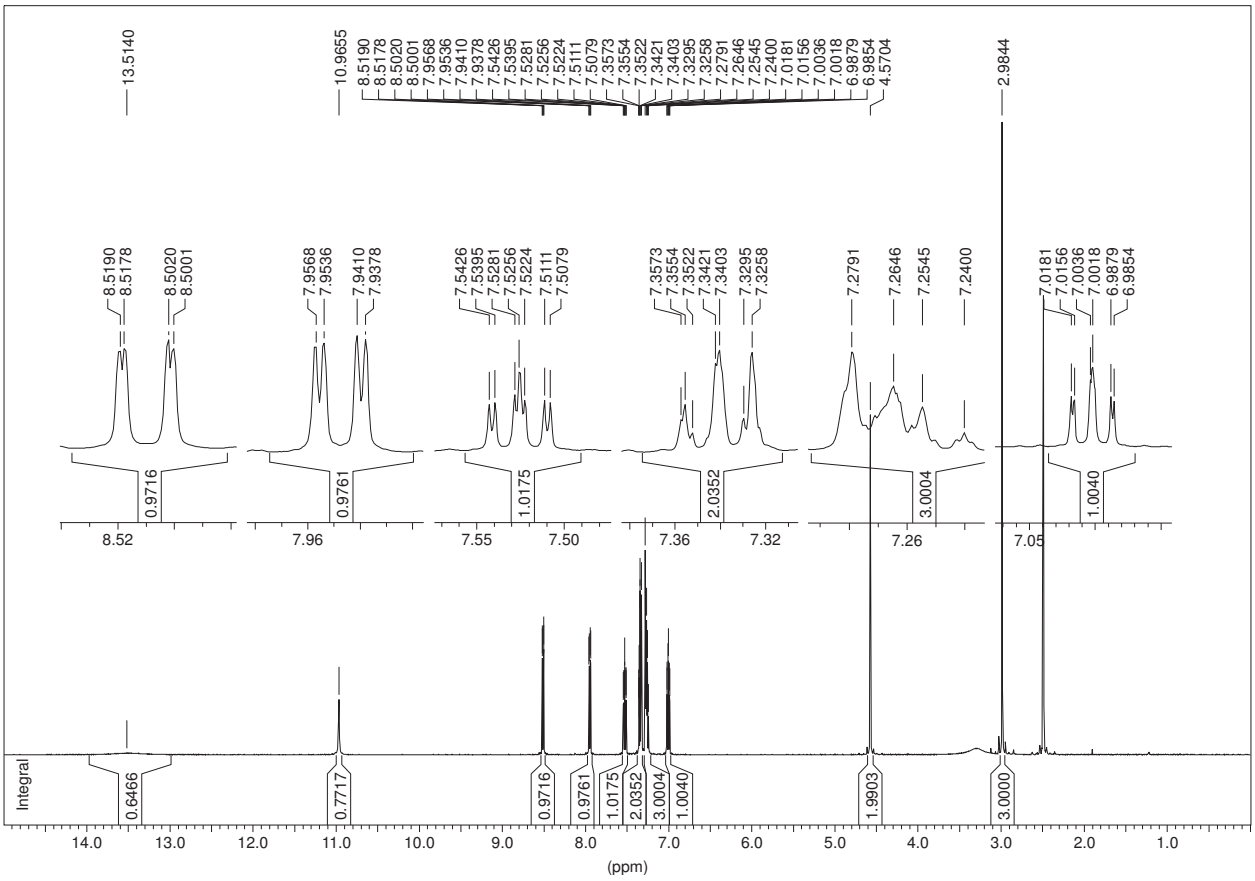


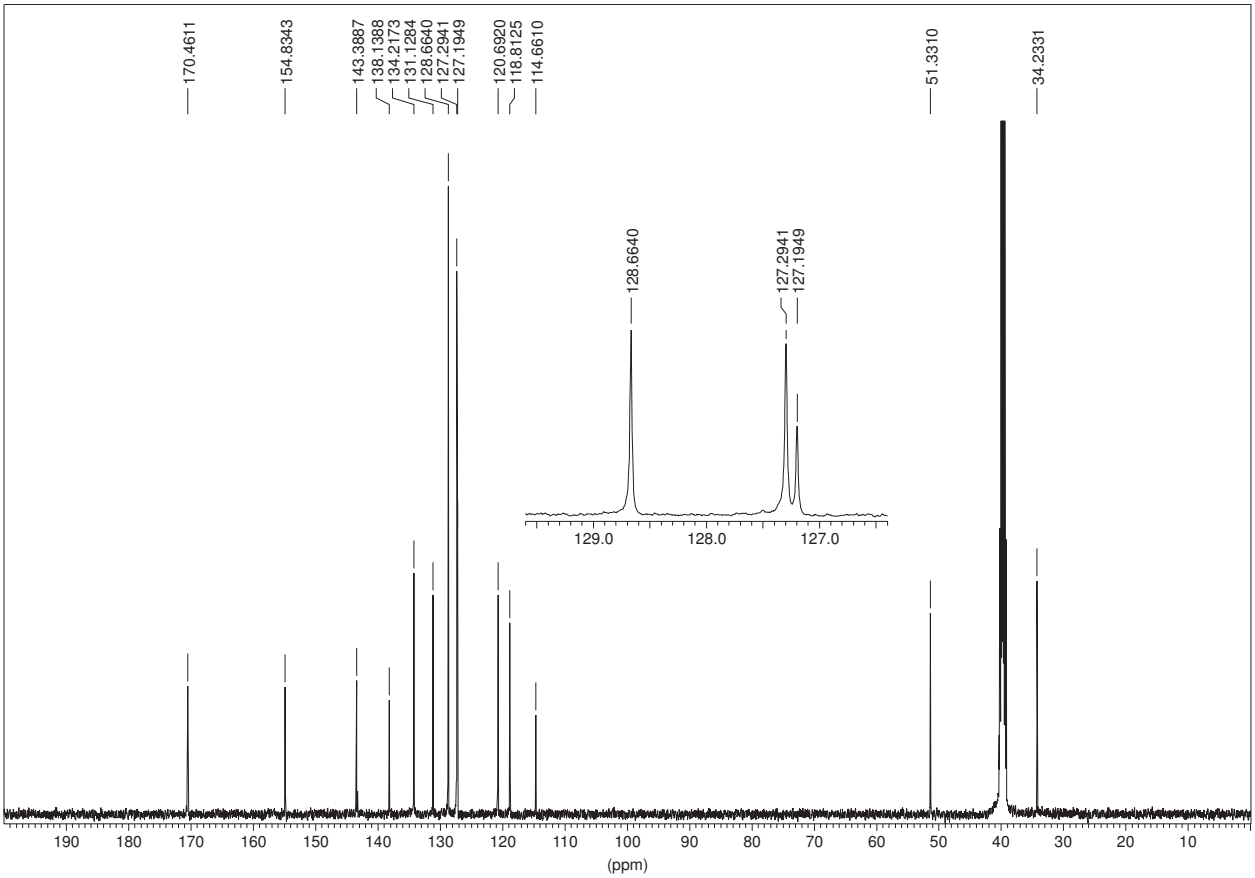
2-(3-Cyclohexyl-3-methylureido)benzoic Acid (**57**)



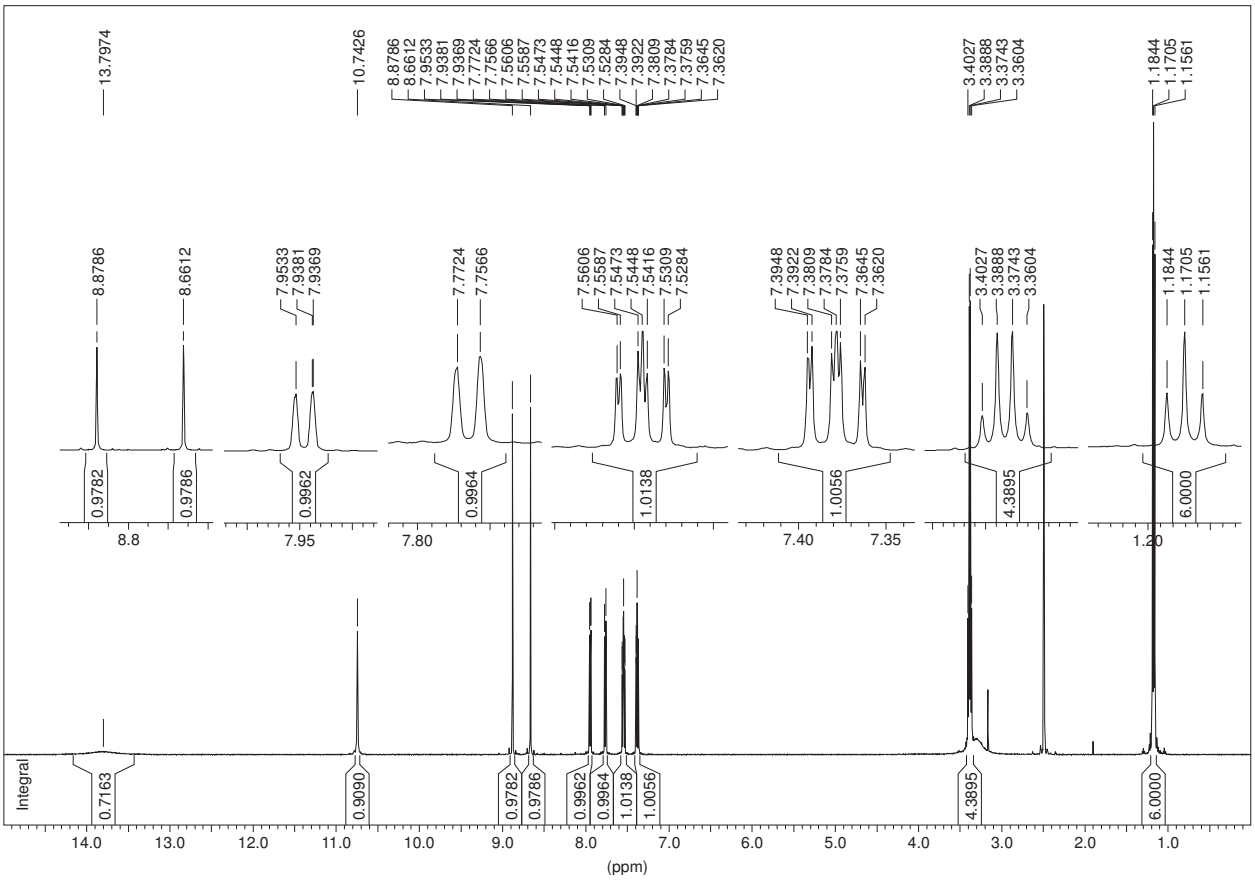


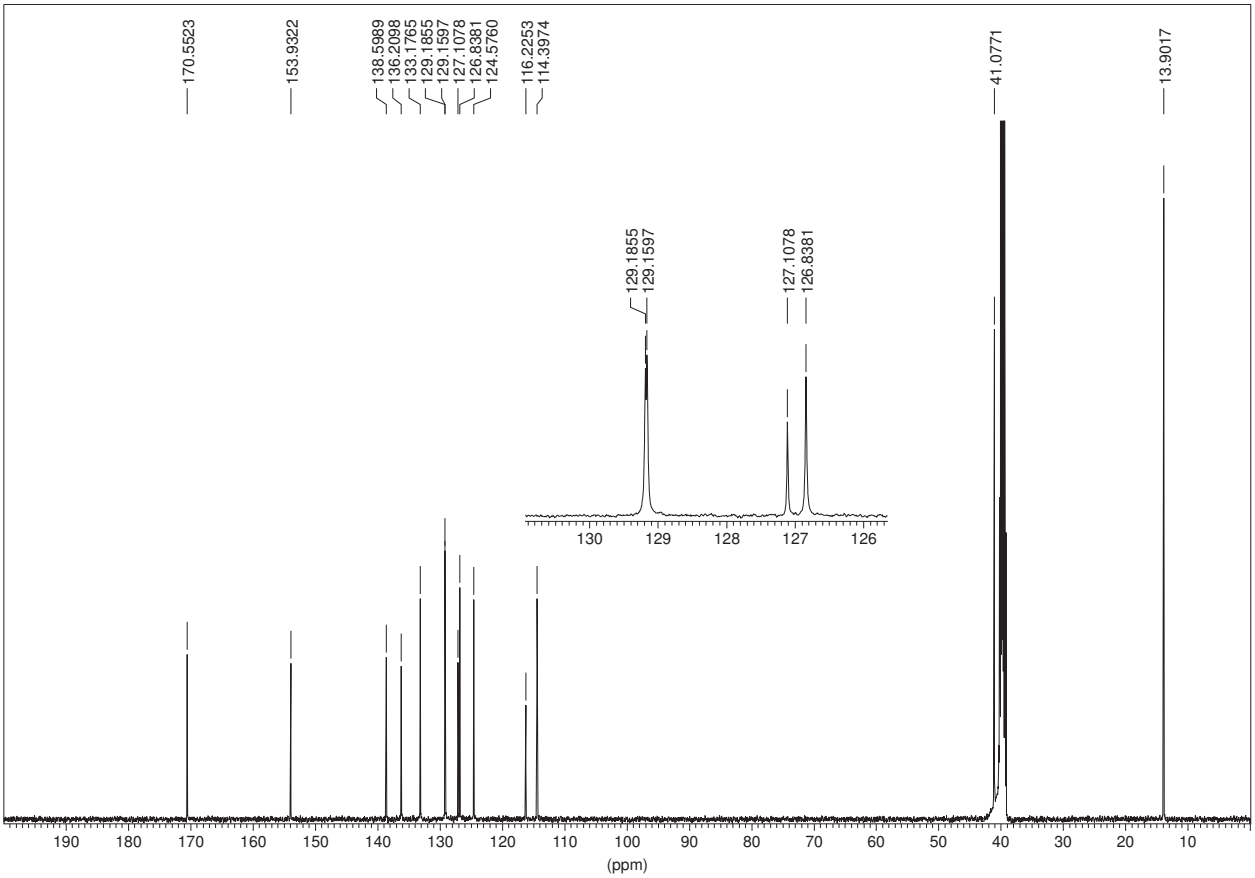
2-(3-Benzyl-3-methylureido)benzoic Acid (**59**)



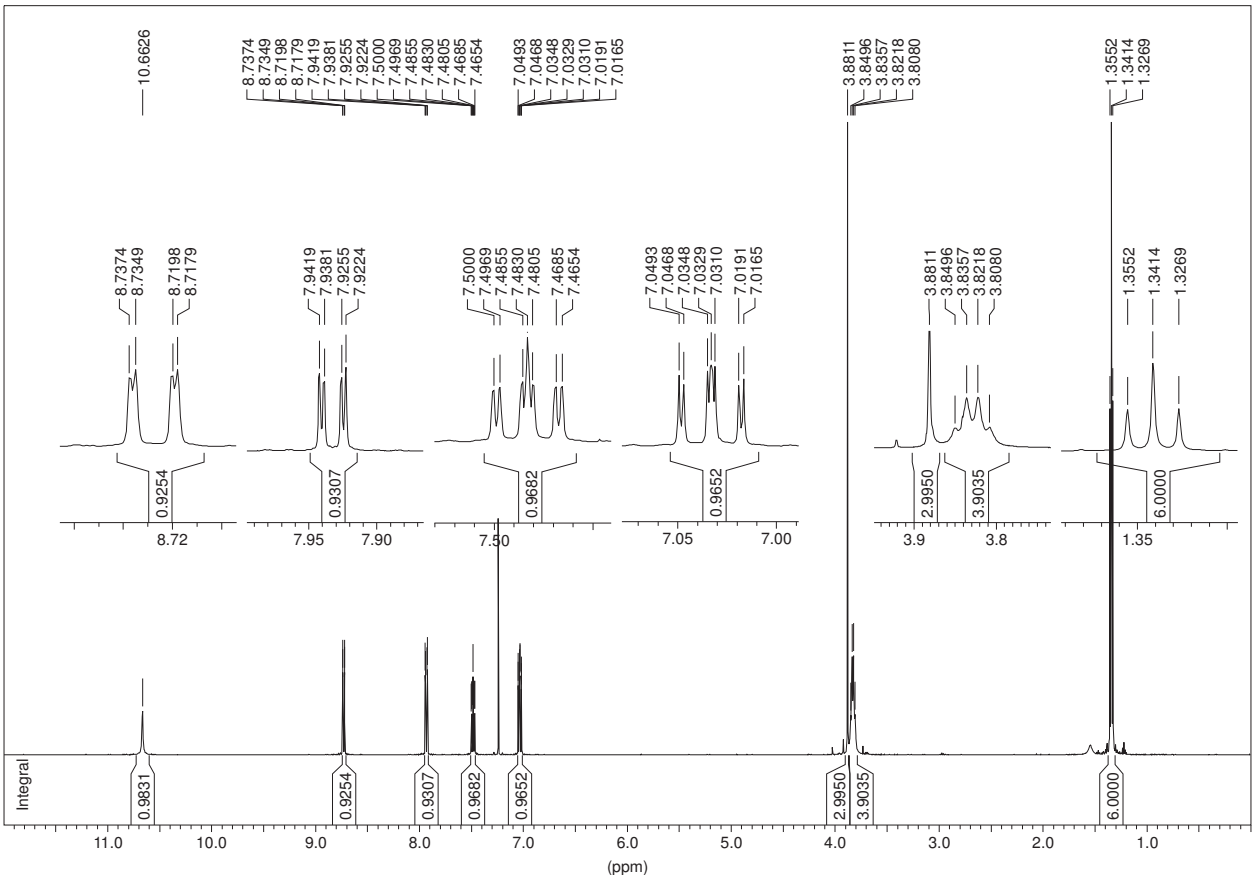


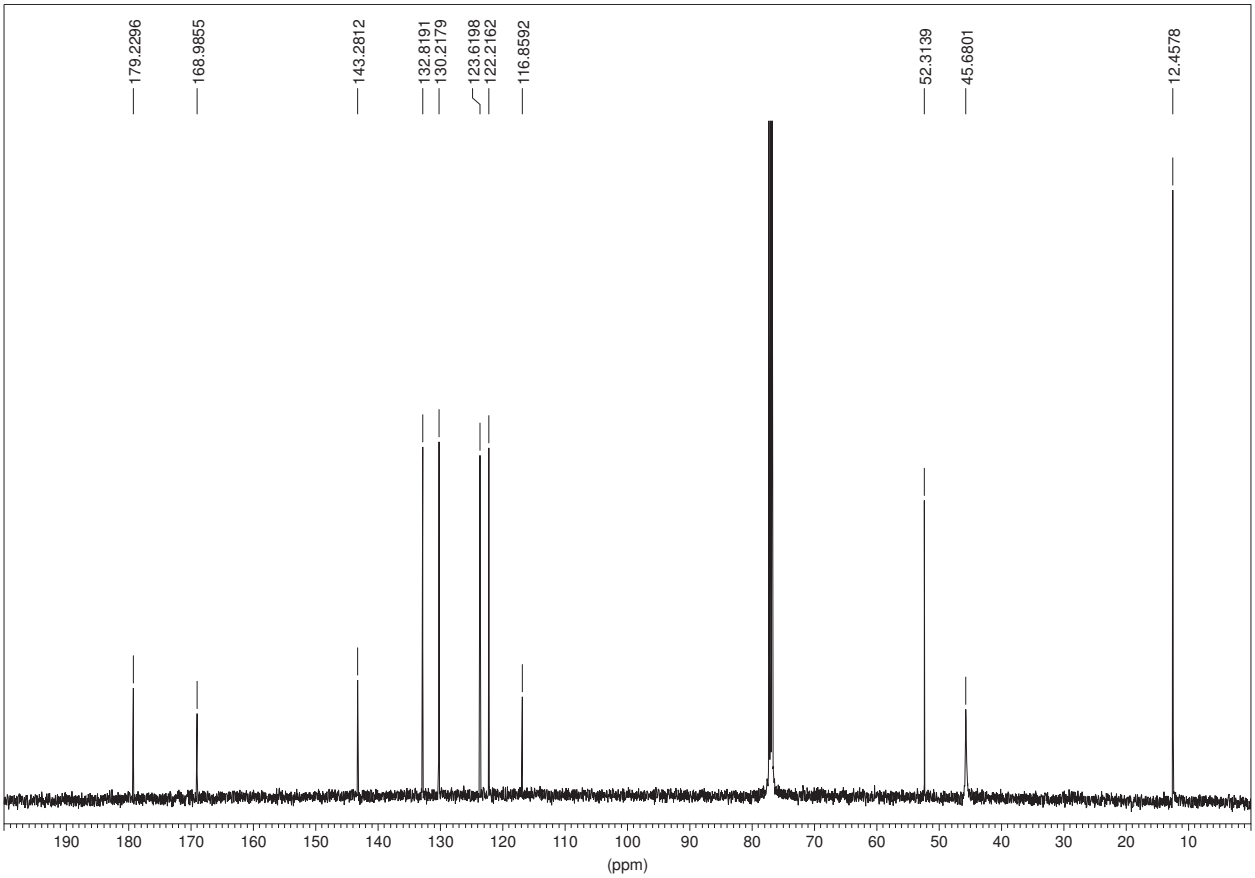
3-(3,3-Diethylureido)-2-naphthoic Acid (**69**)



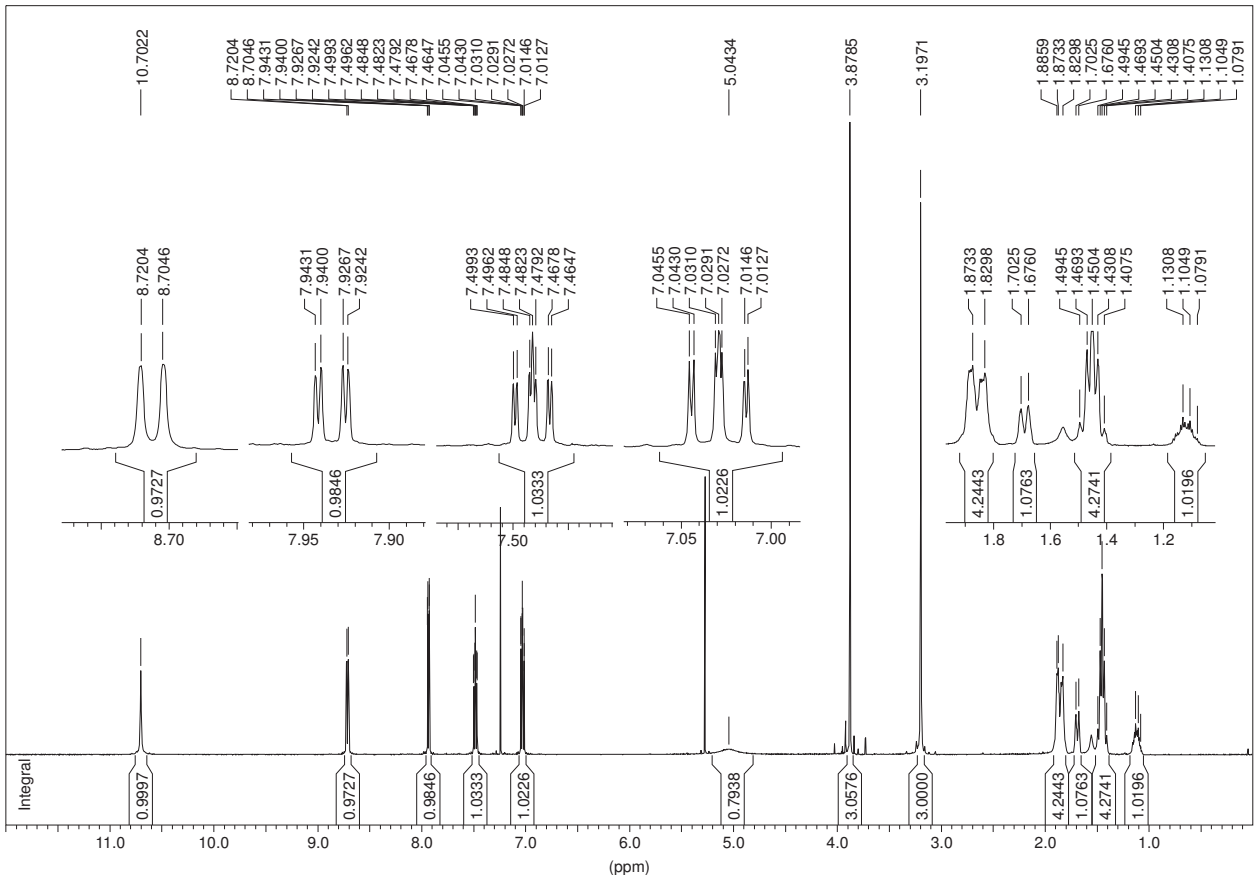


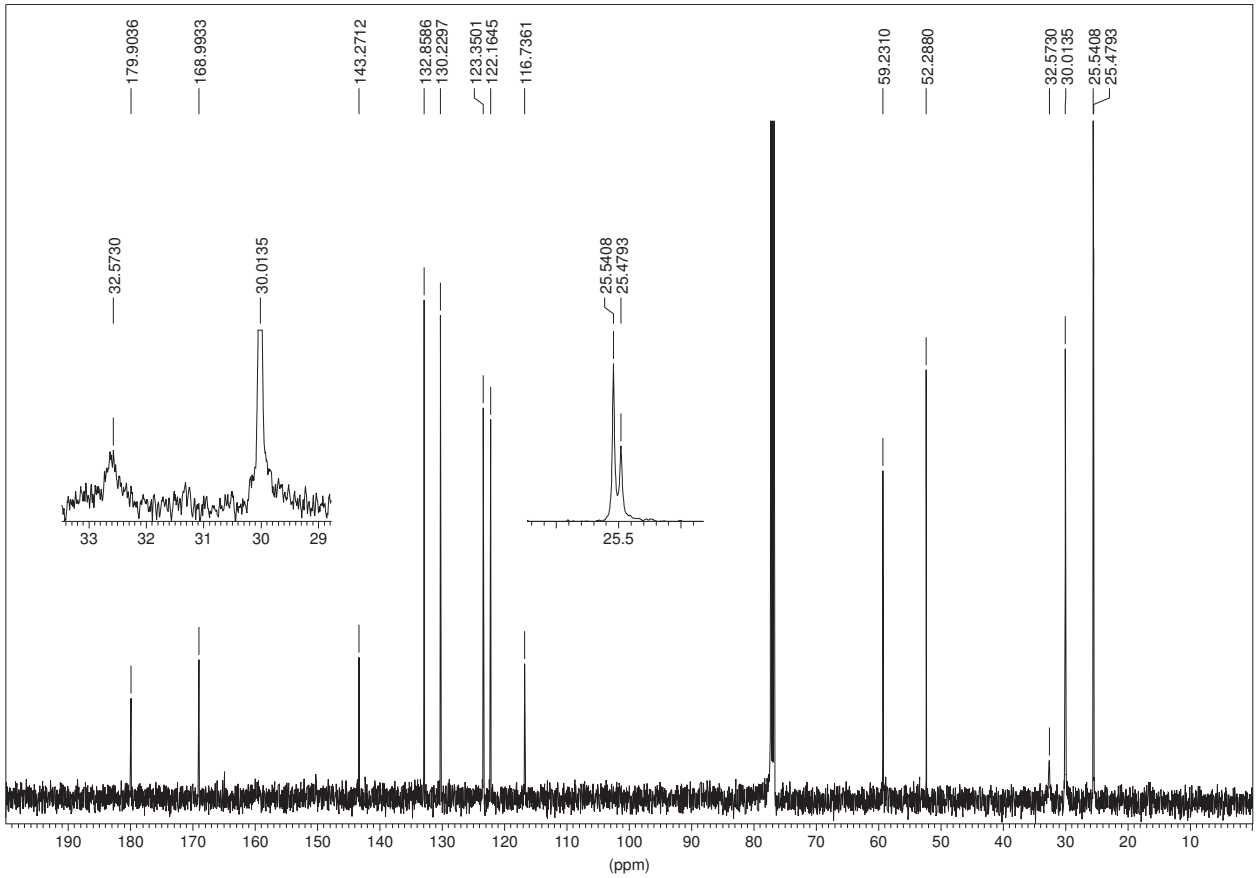
Methyl 2-(3,3-Diethylthioureido)benzoate (**70**)



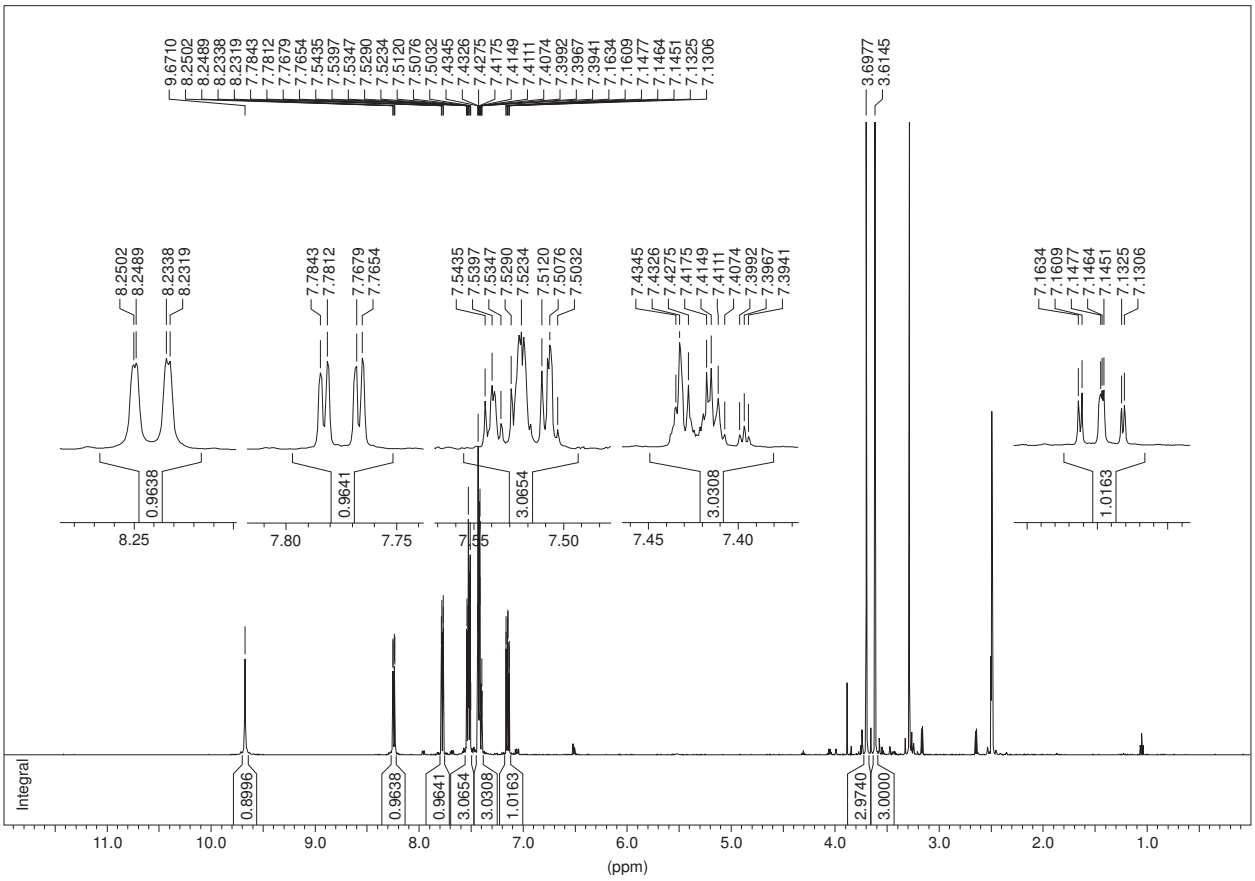


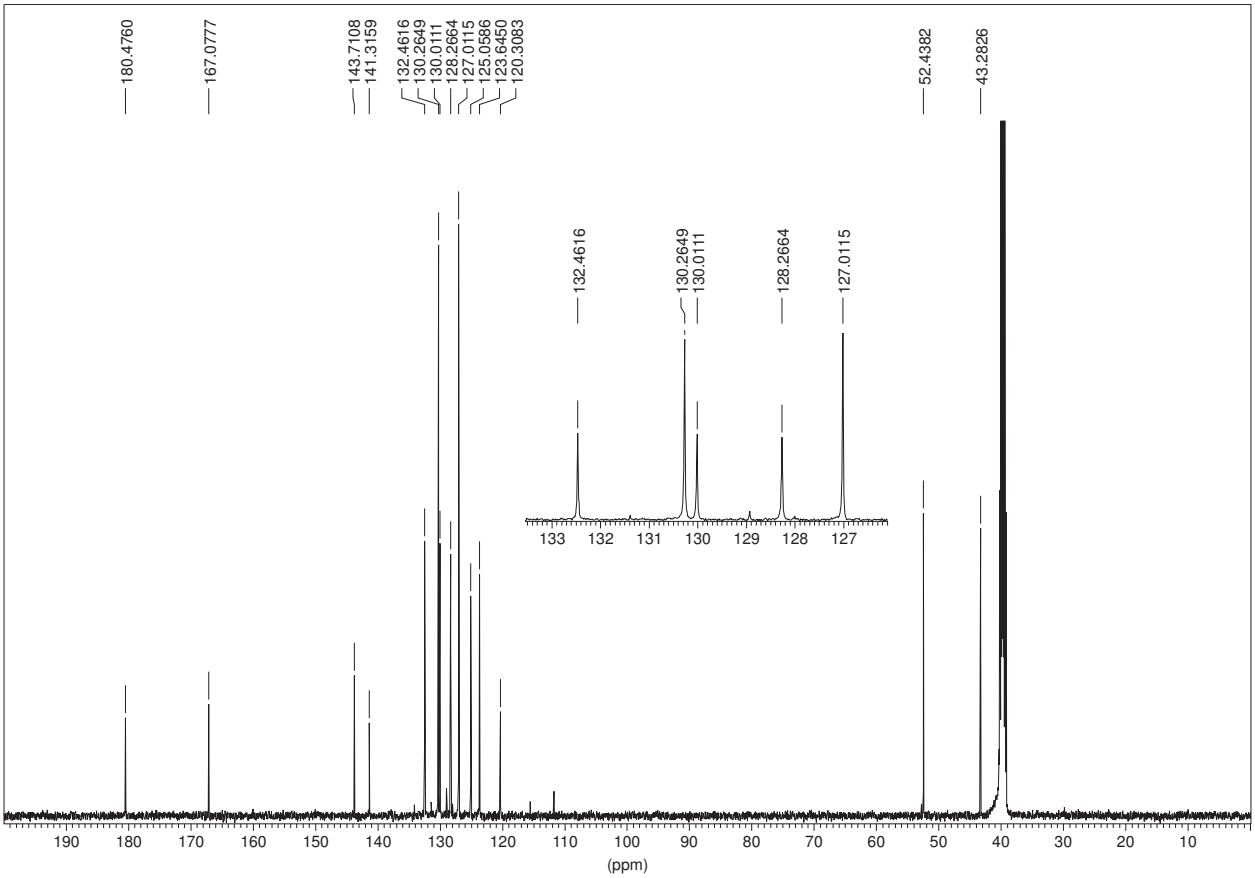
Methyl 2-(3-Cyclohexyl-3-methylthioureido)benzoate (**71**)



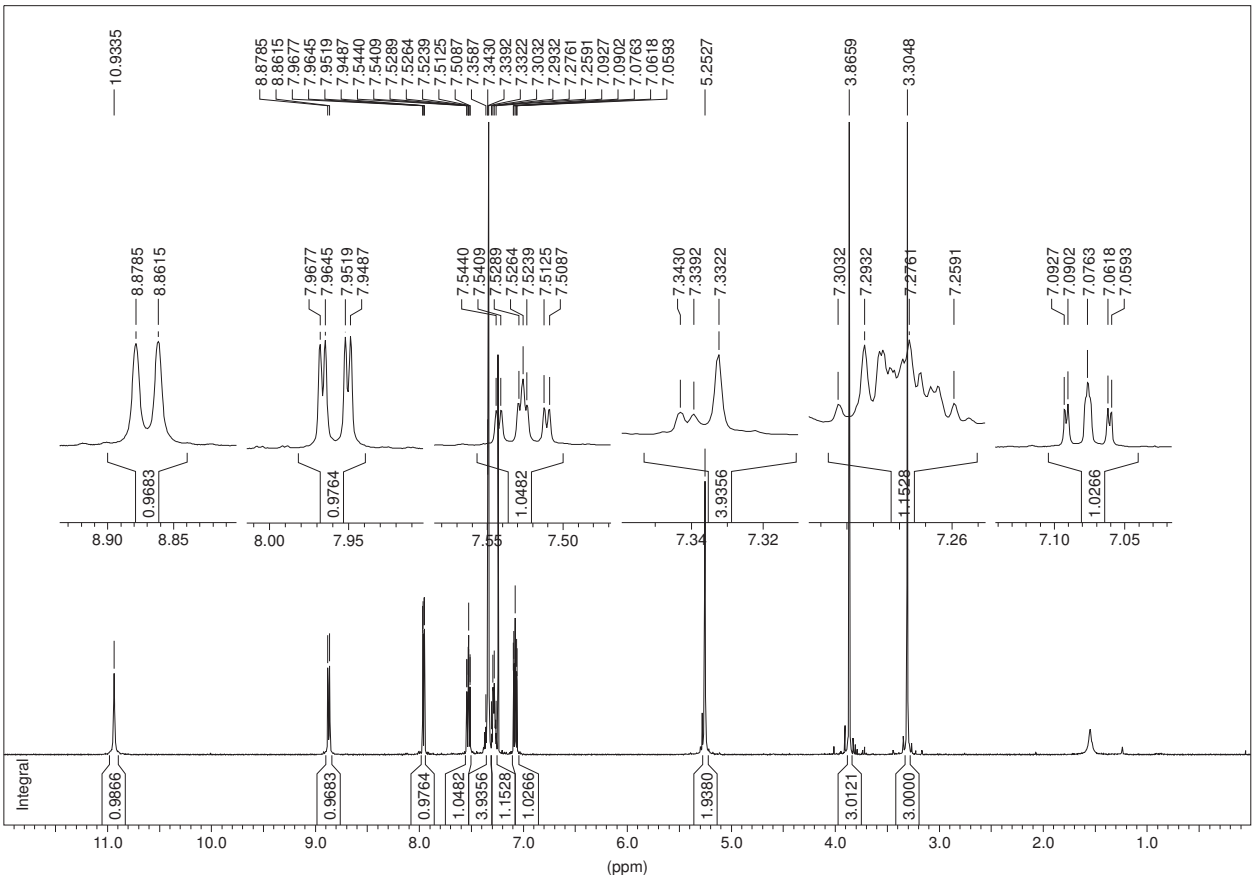


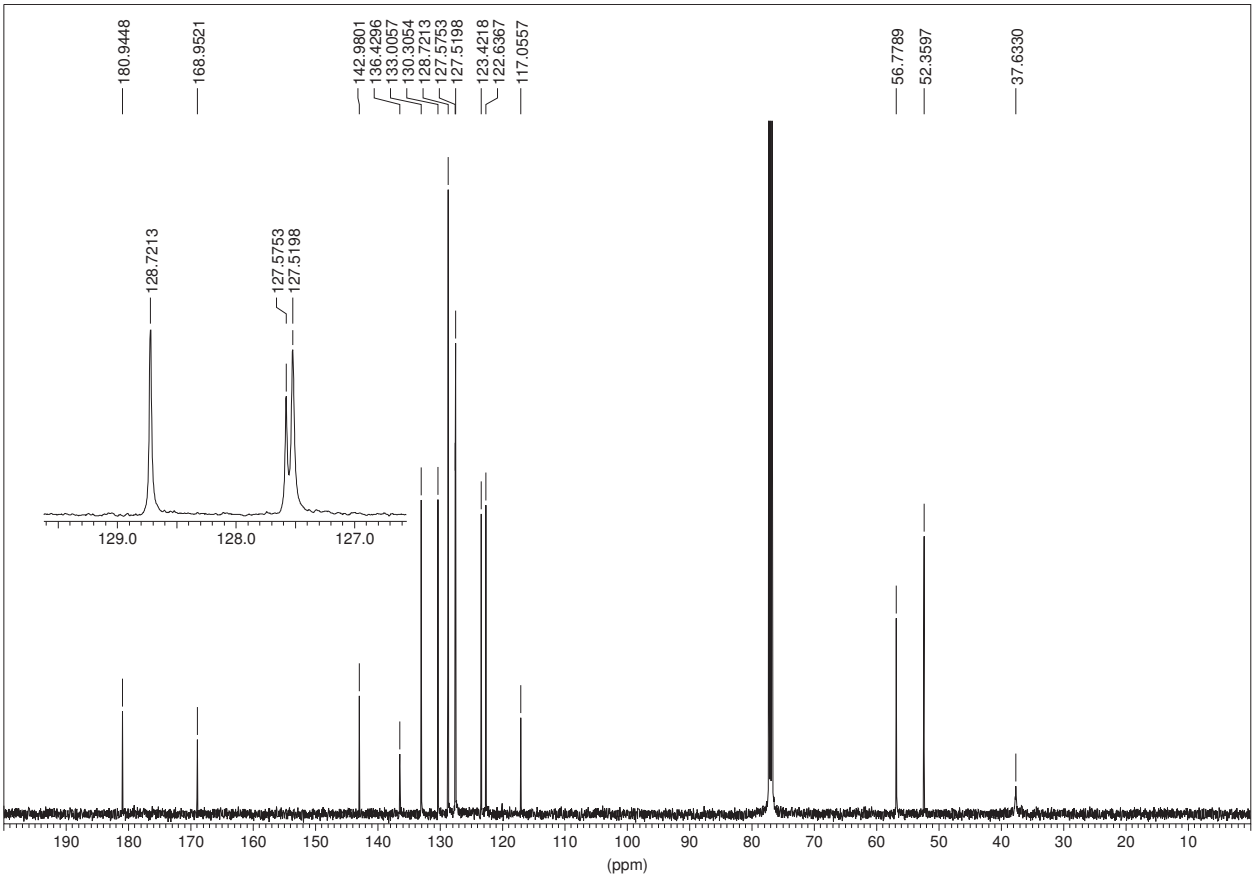
Methyl 2-(3-Methyl-3-phenylthioureido)benzoate (**72**)



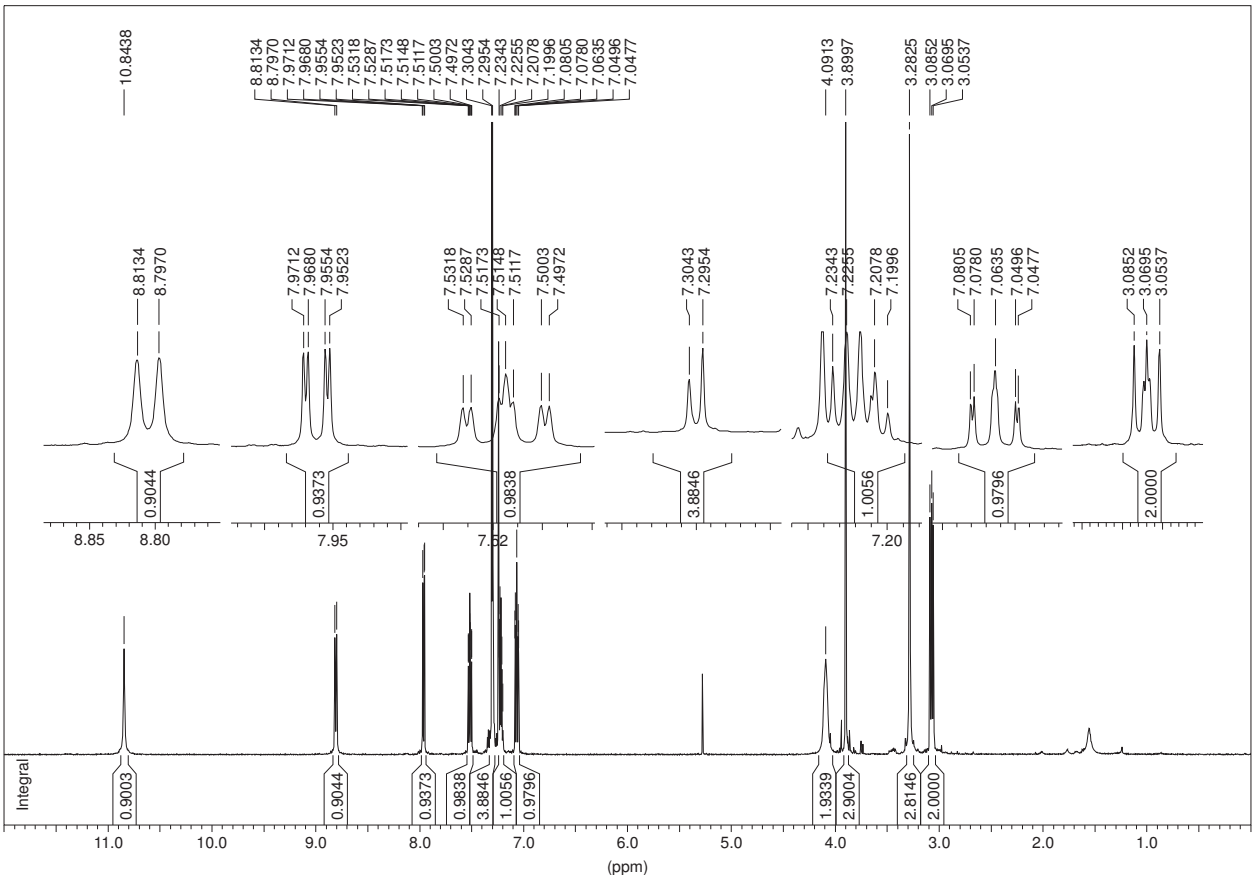


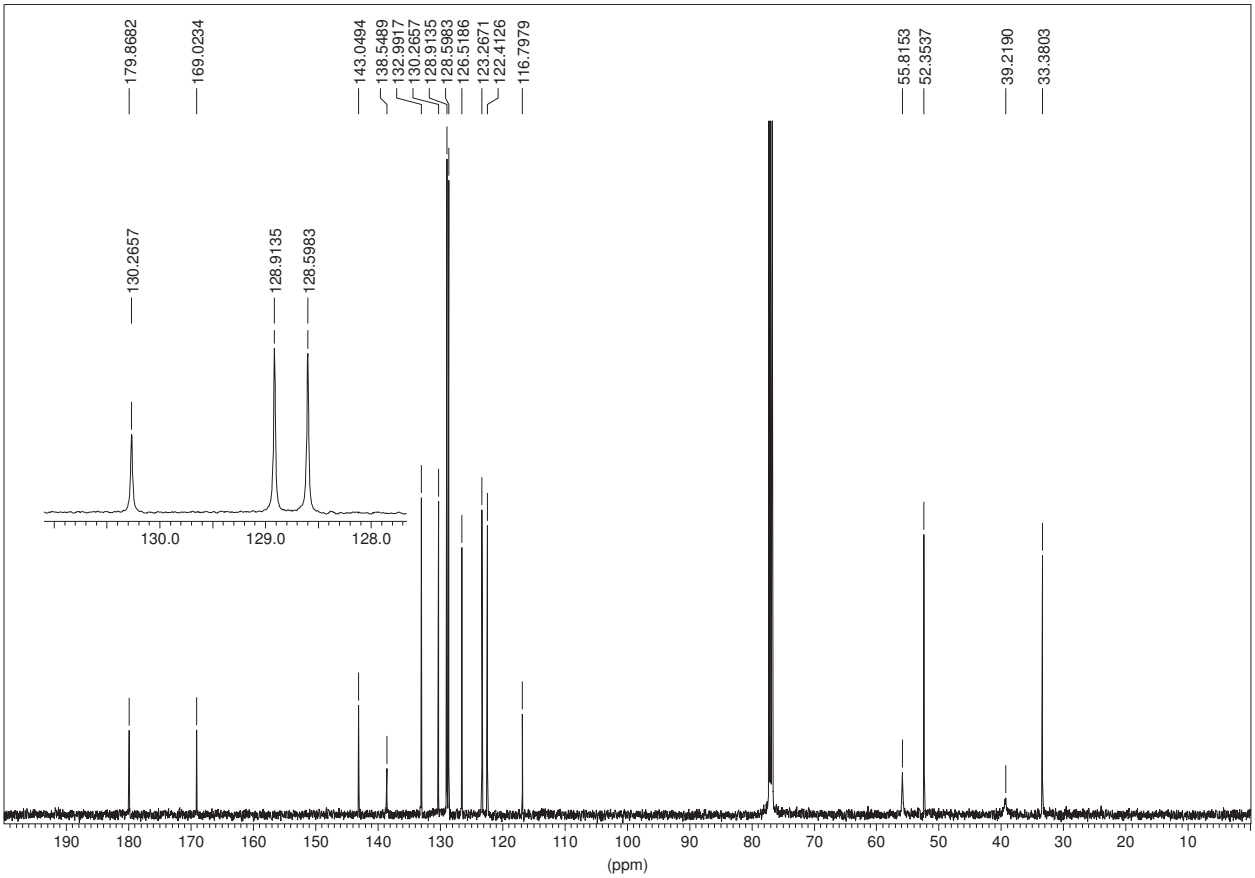
Methyl 2-(3-Benzyl-3-methylthioureido)benzoate (**73**)



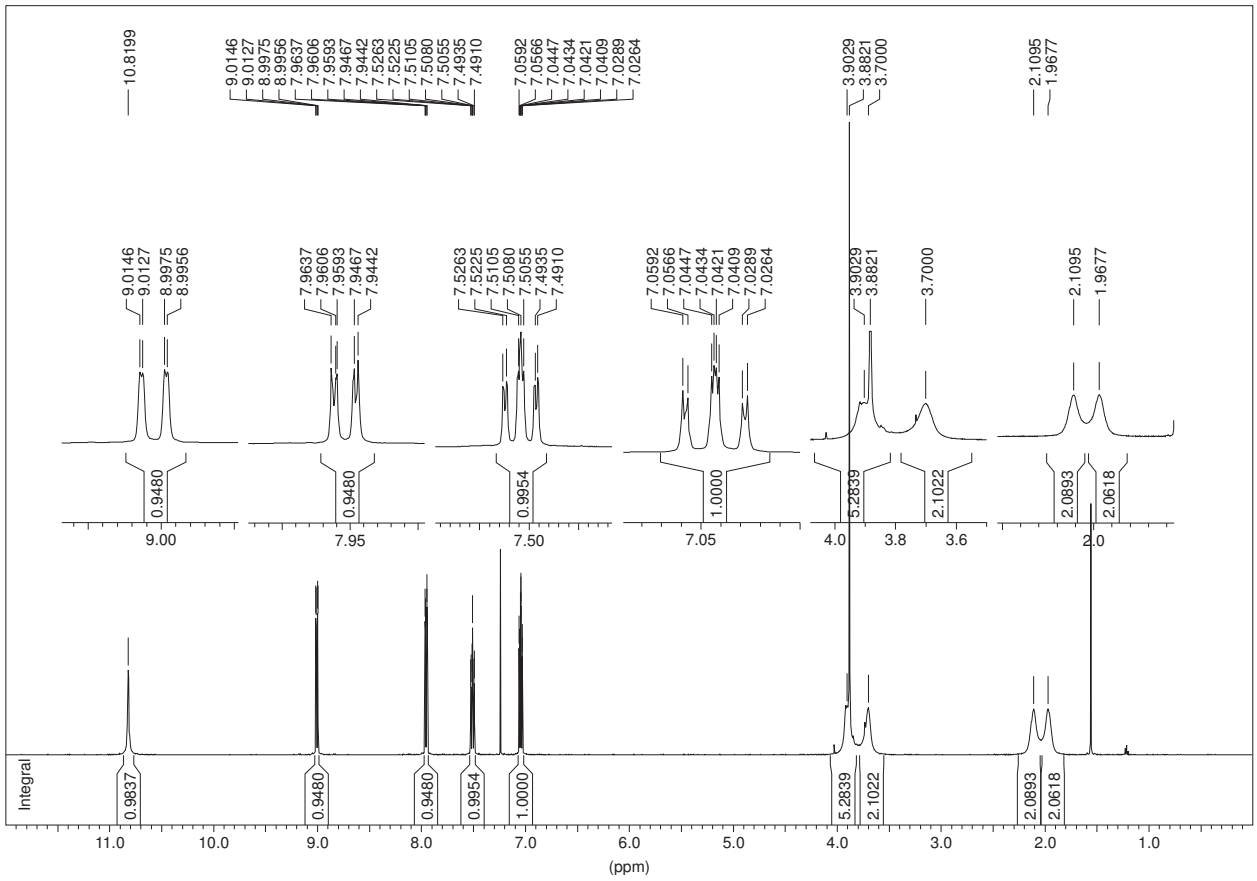


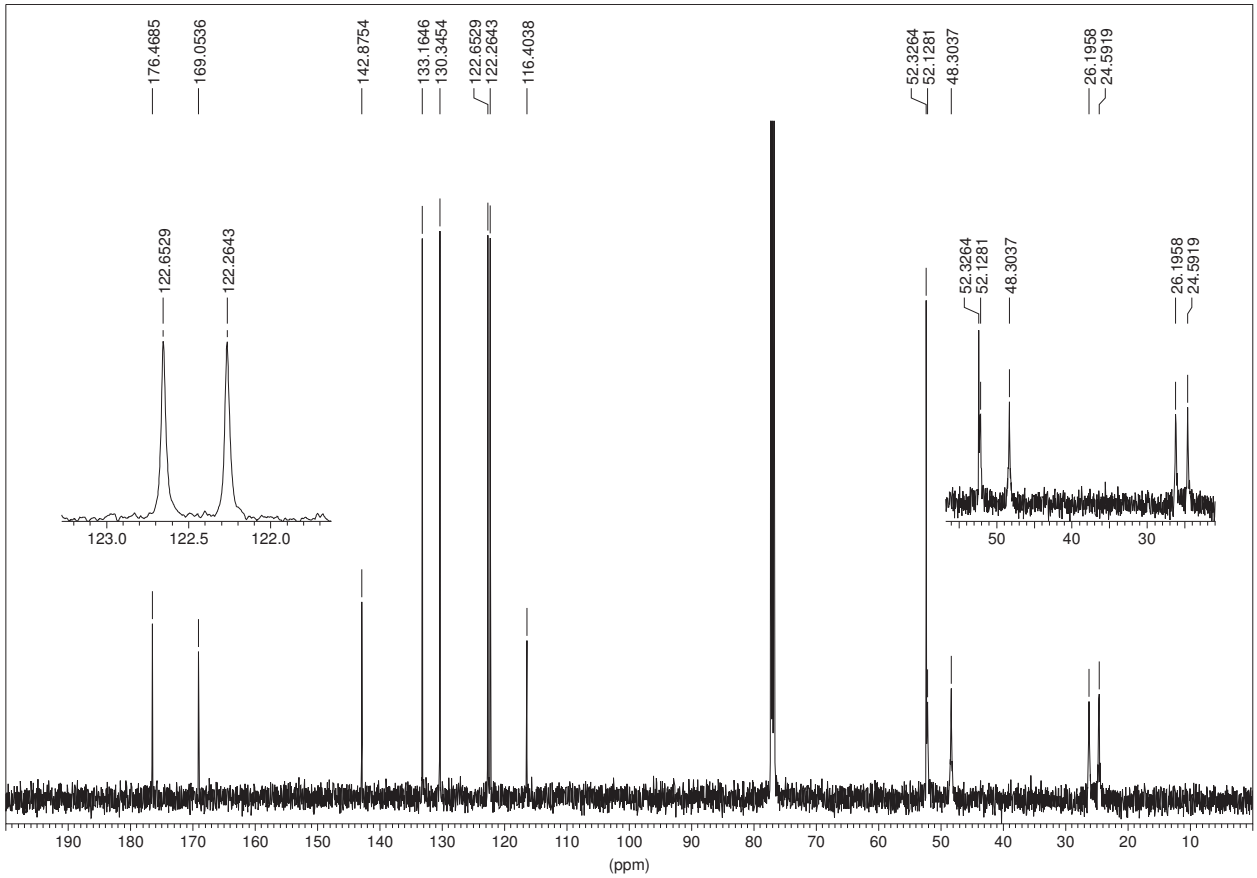
Methyl 2-[3-Methyl-3-(2-phenylethyl)thioureido]benzoate (**74**)



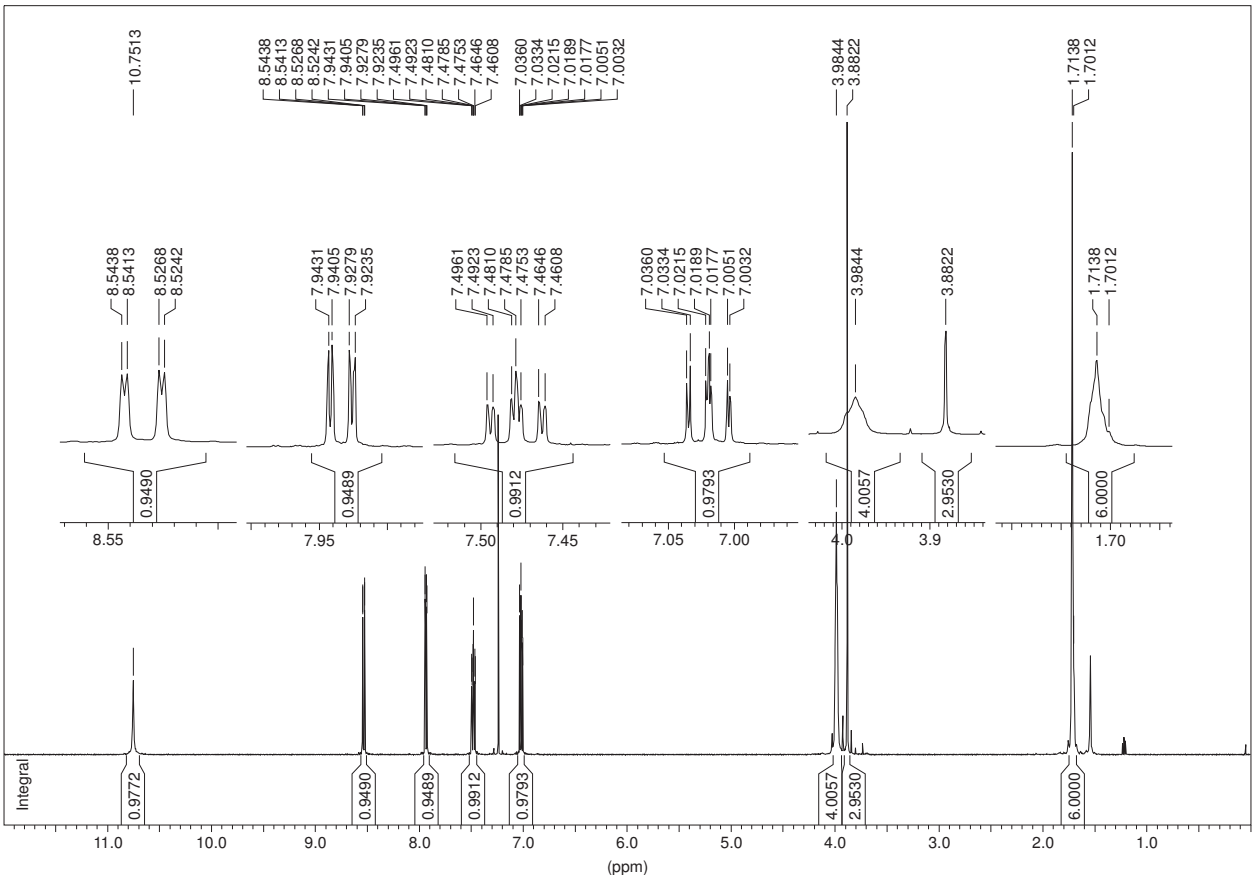


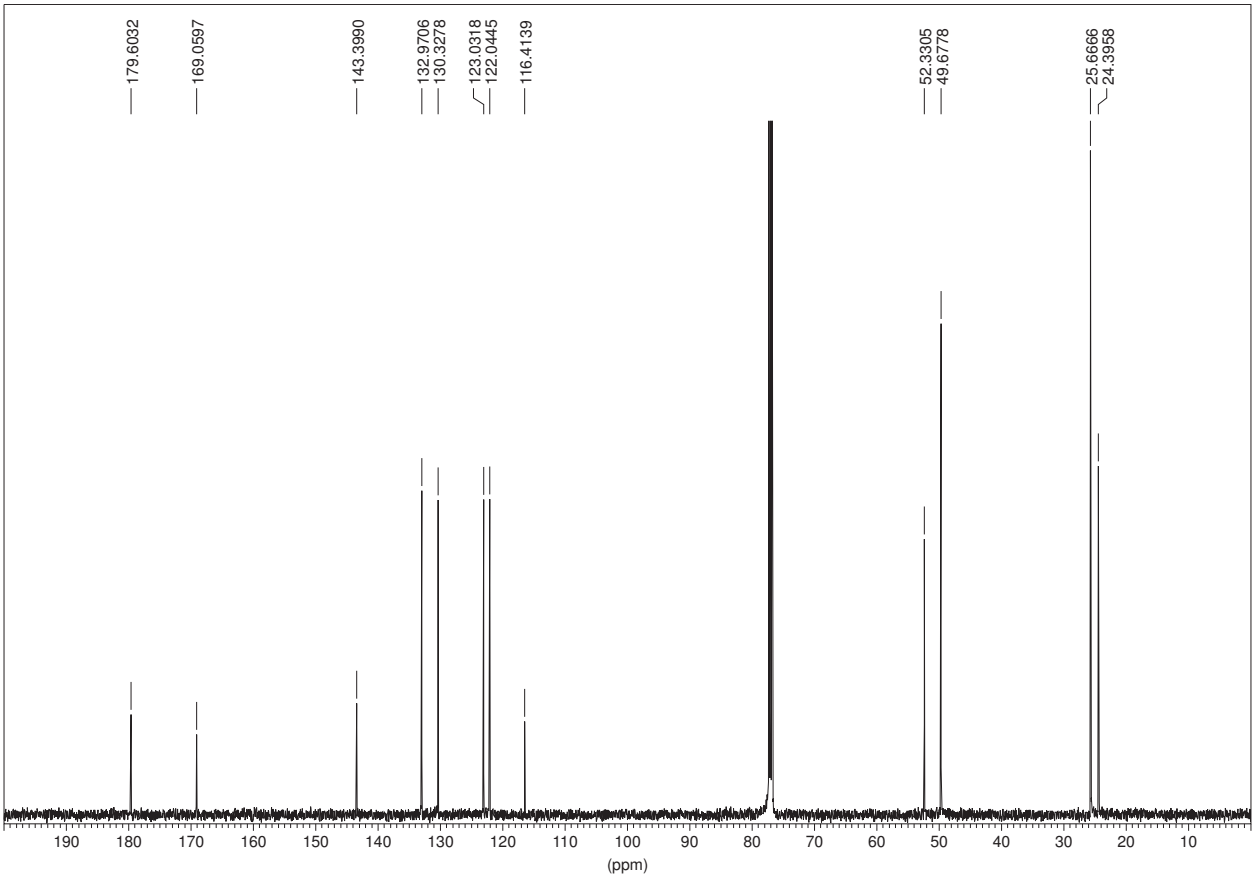
Methyl 2-[(1-Pyrrolidinylthiocarbonyl)amino]benzoate (**75**)



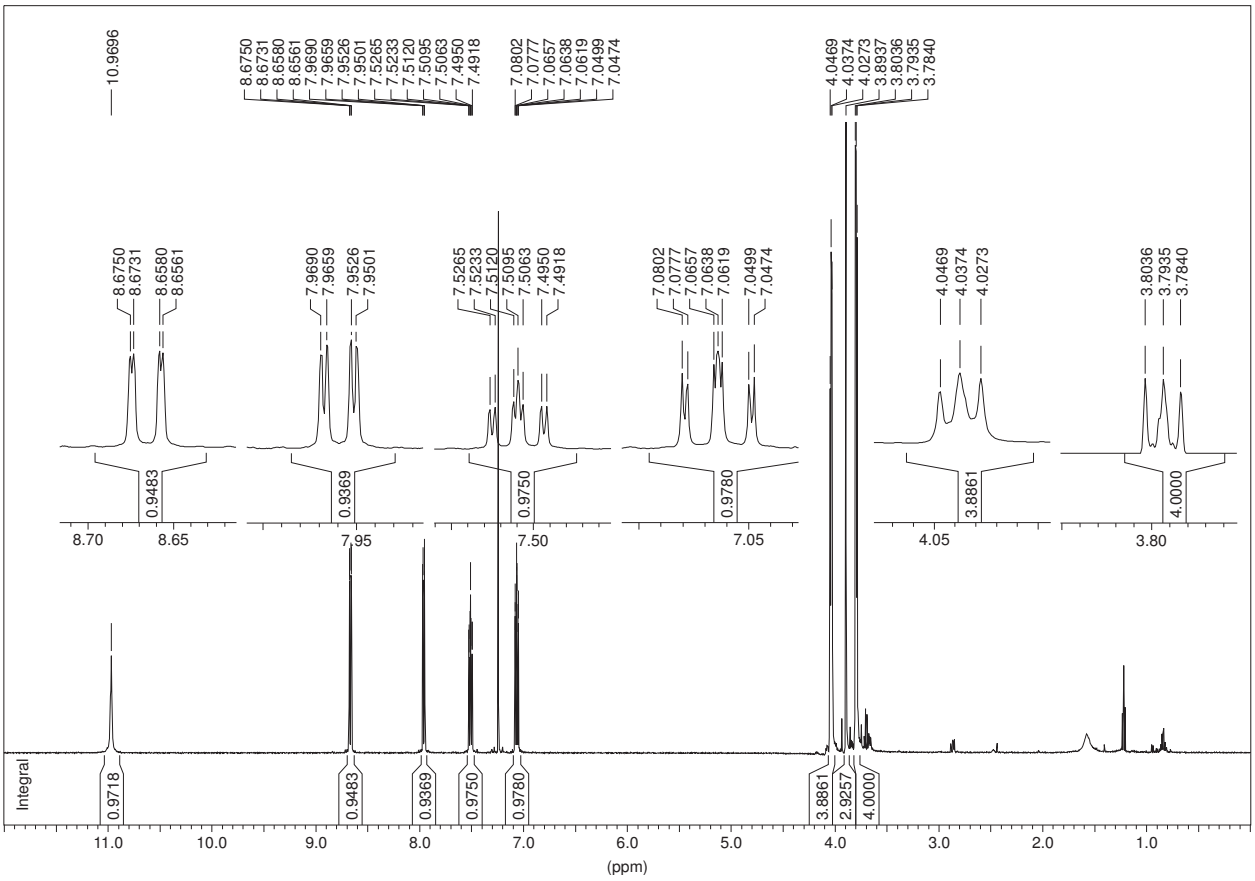


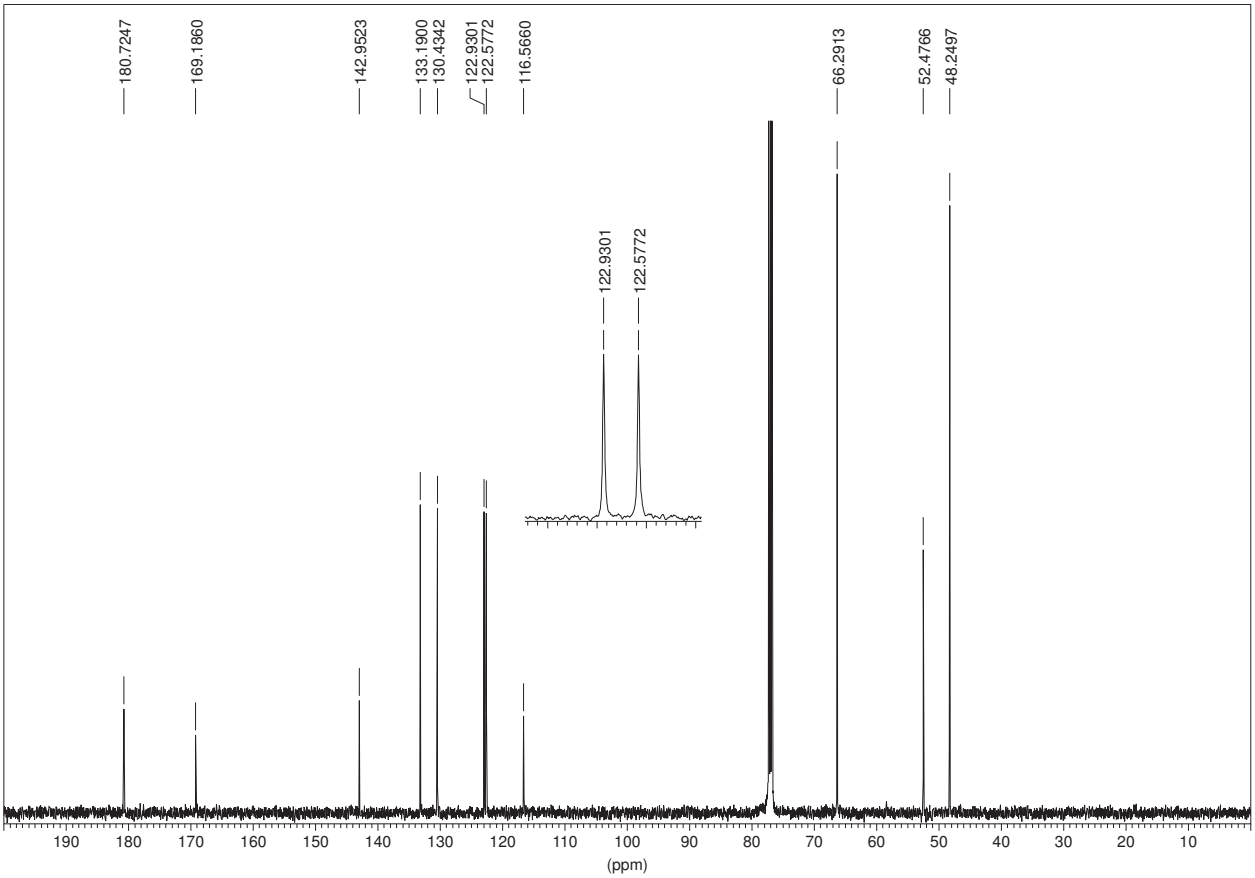
Methyl 2-[(1-Piperidinylthiocarbonyl)amino]benzoate (**76**)



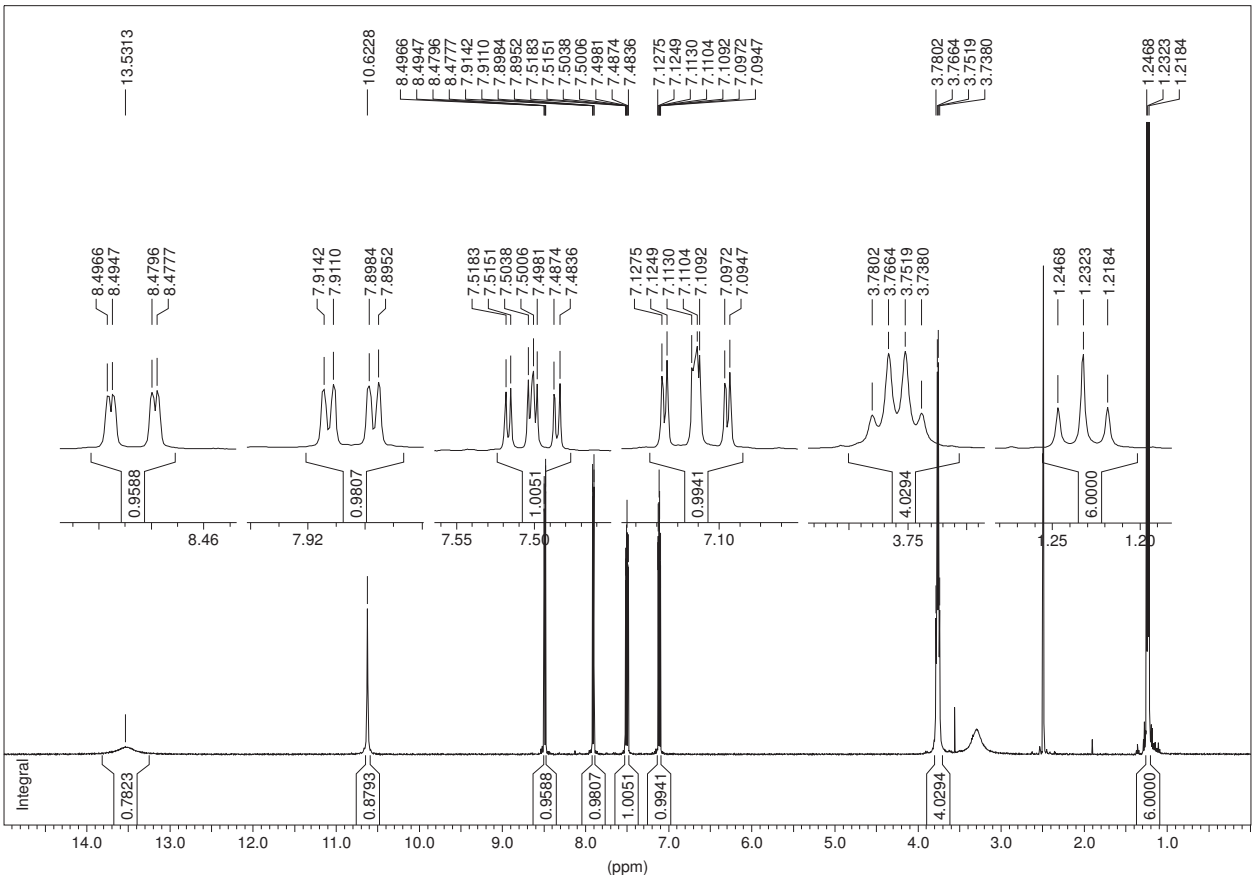


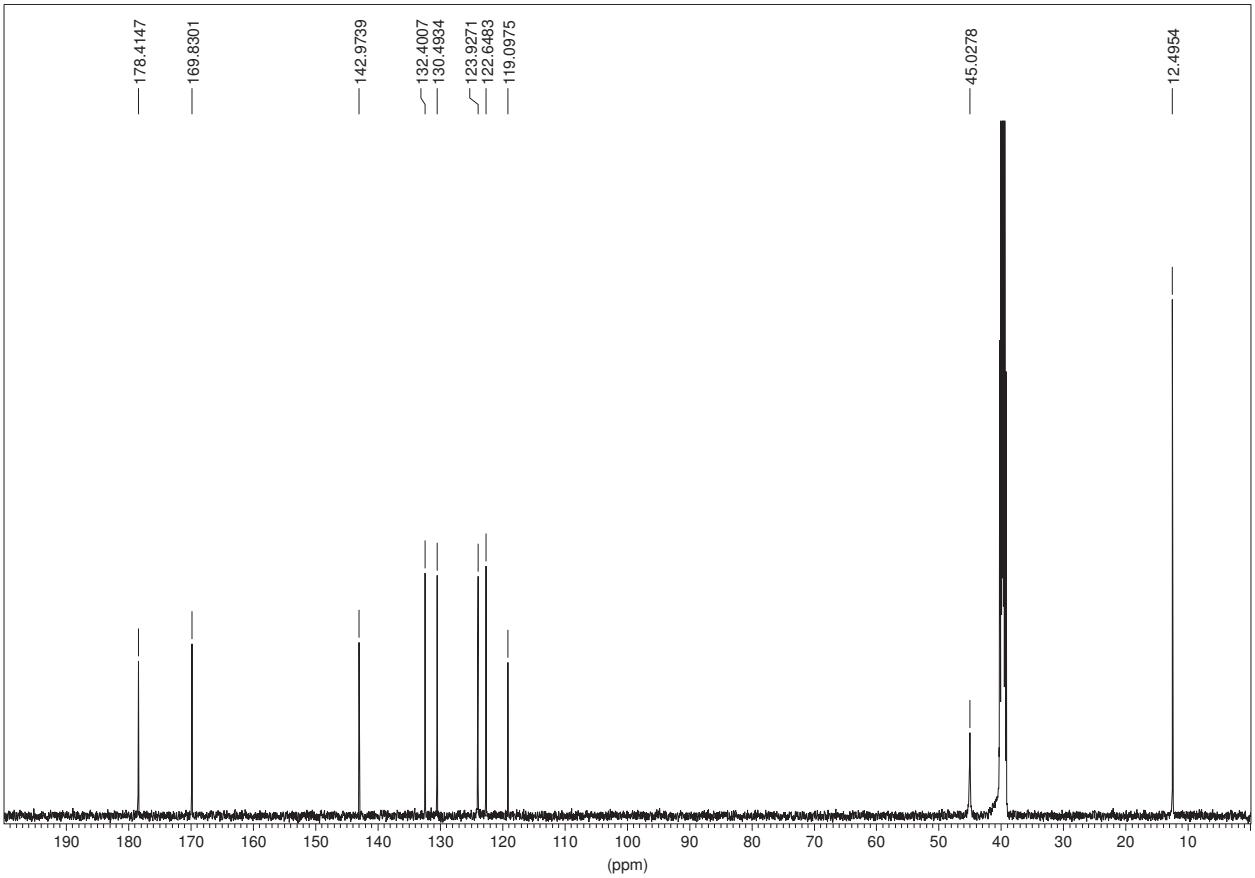
Methyl 2-[(4-Morpholinylthiocarbonyl)amino]benzoate (**77**)



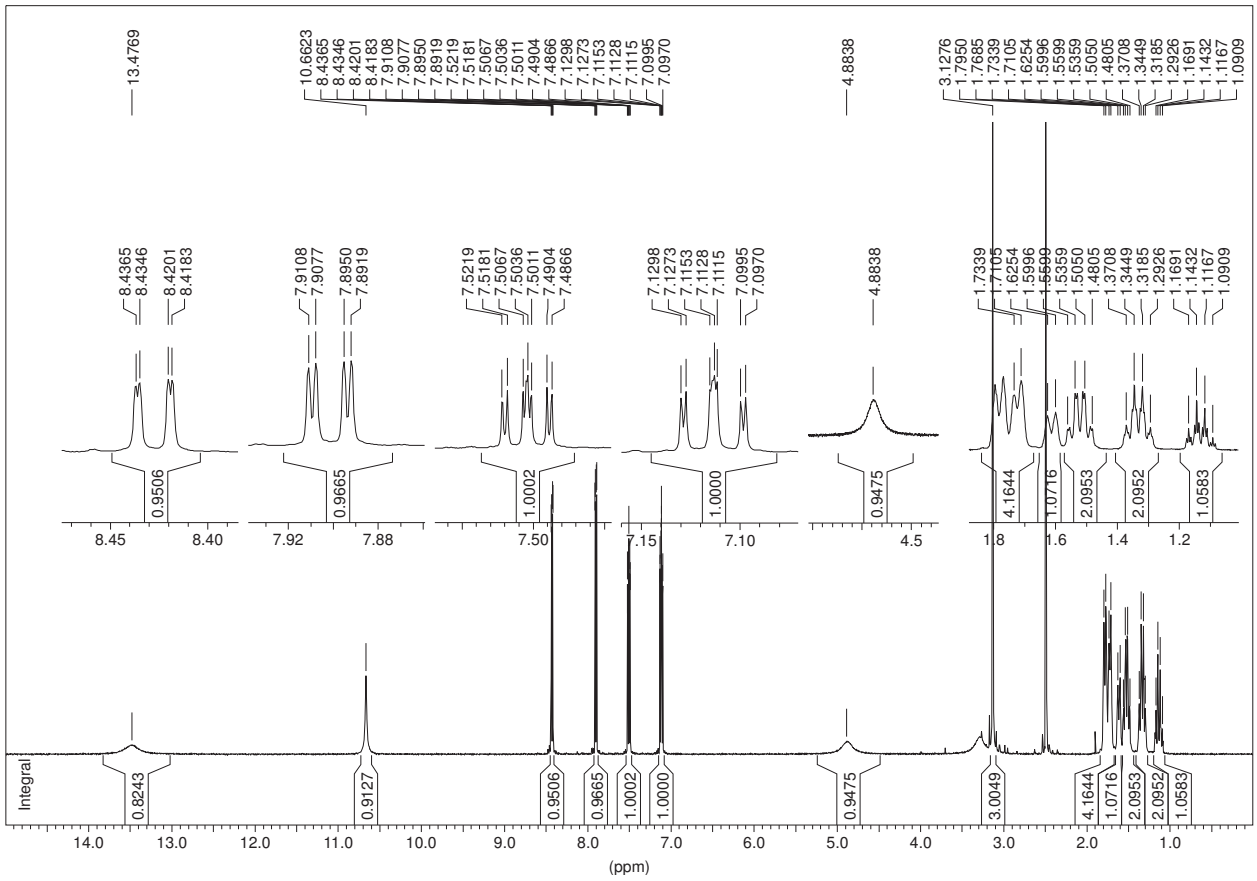


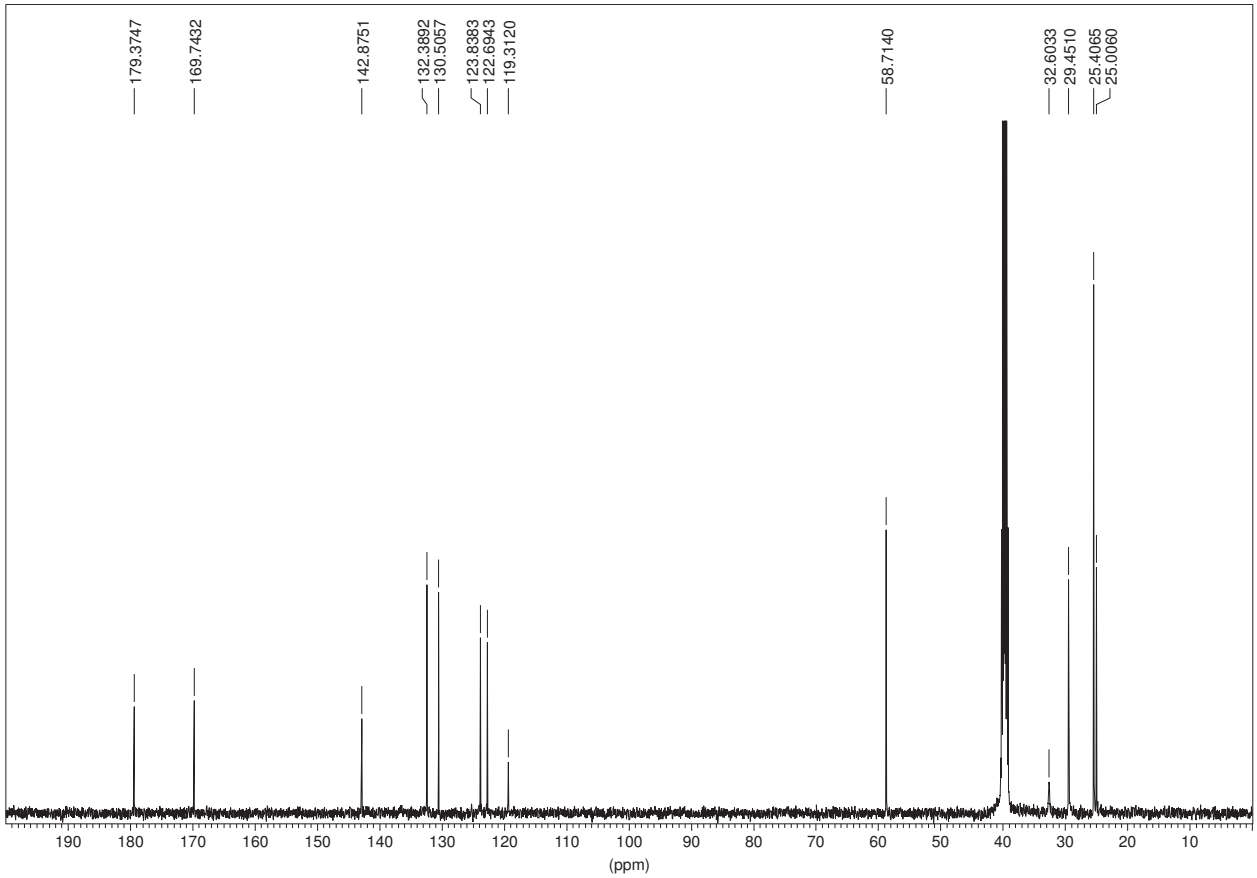
2-(3,3-Diethylthioureido)benzoic Acid (**78**)



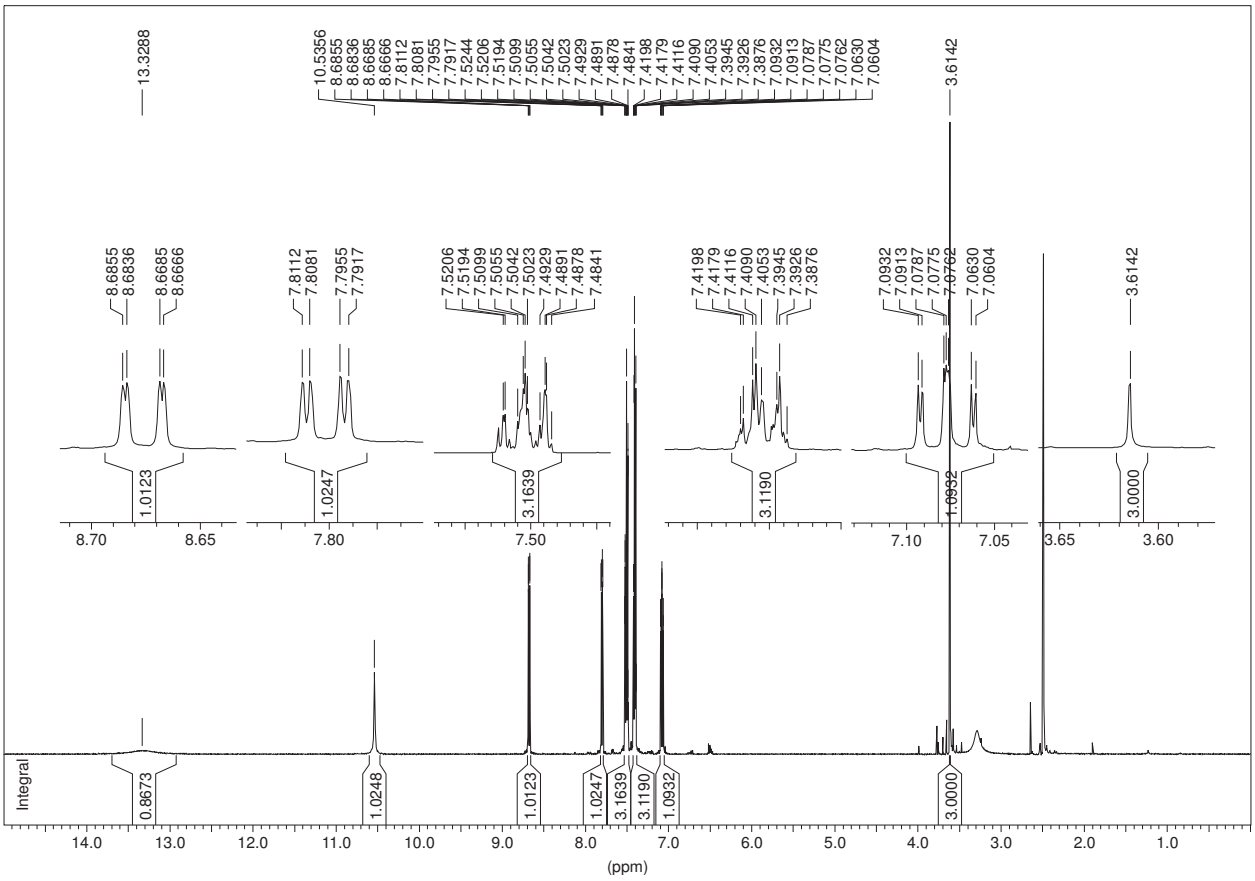


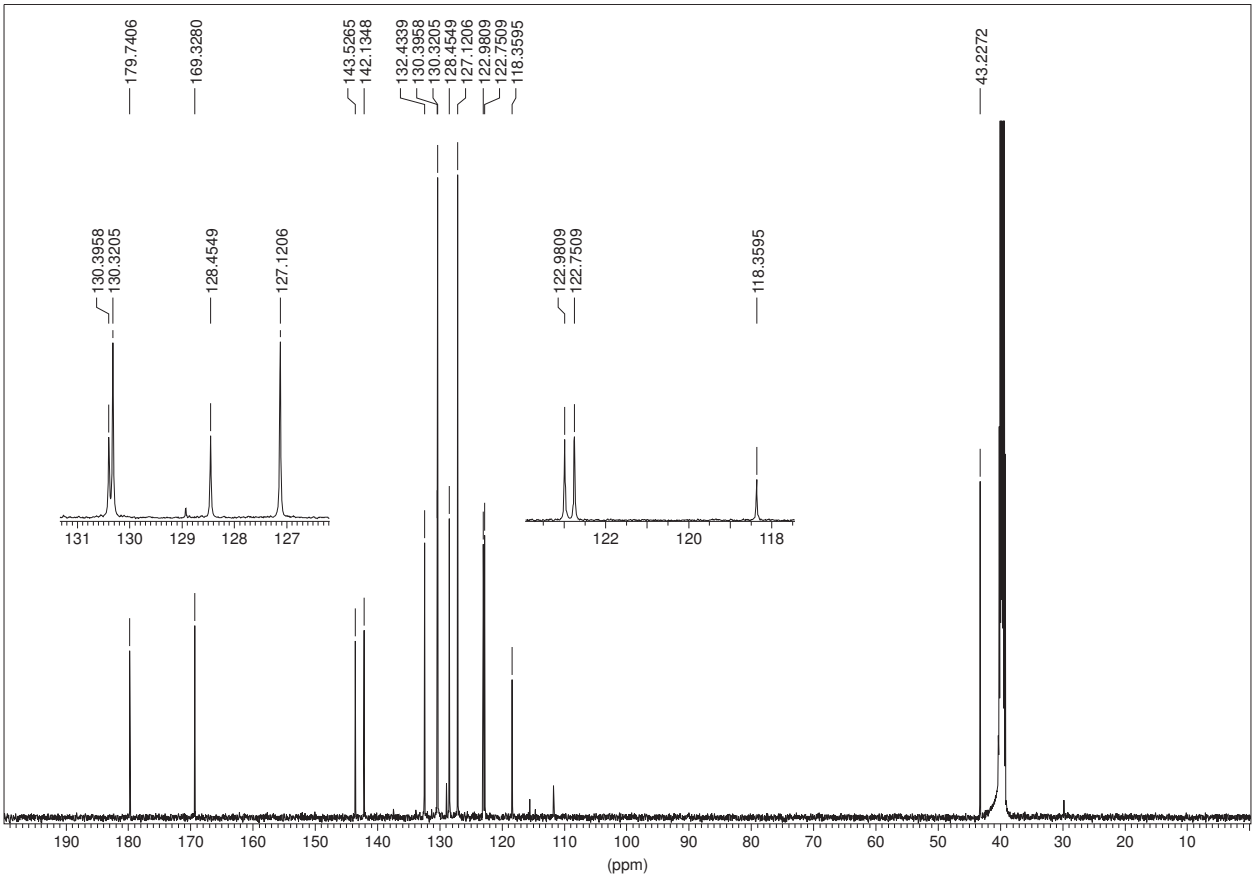
2-(3-Cyclohexyl-3-methylthioureido)benzoic Acid (**79**)



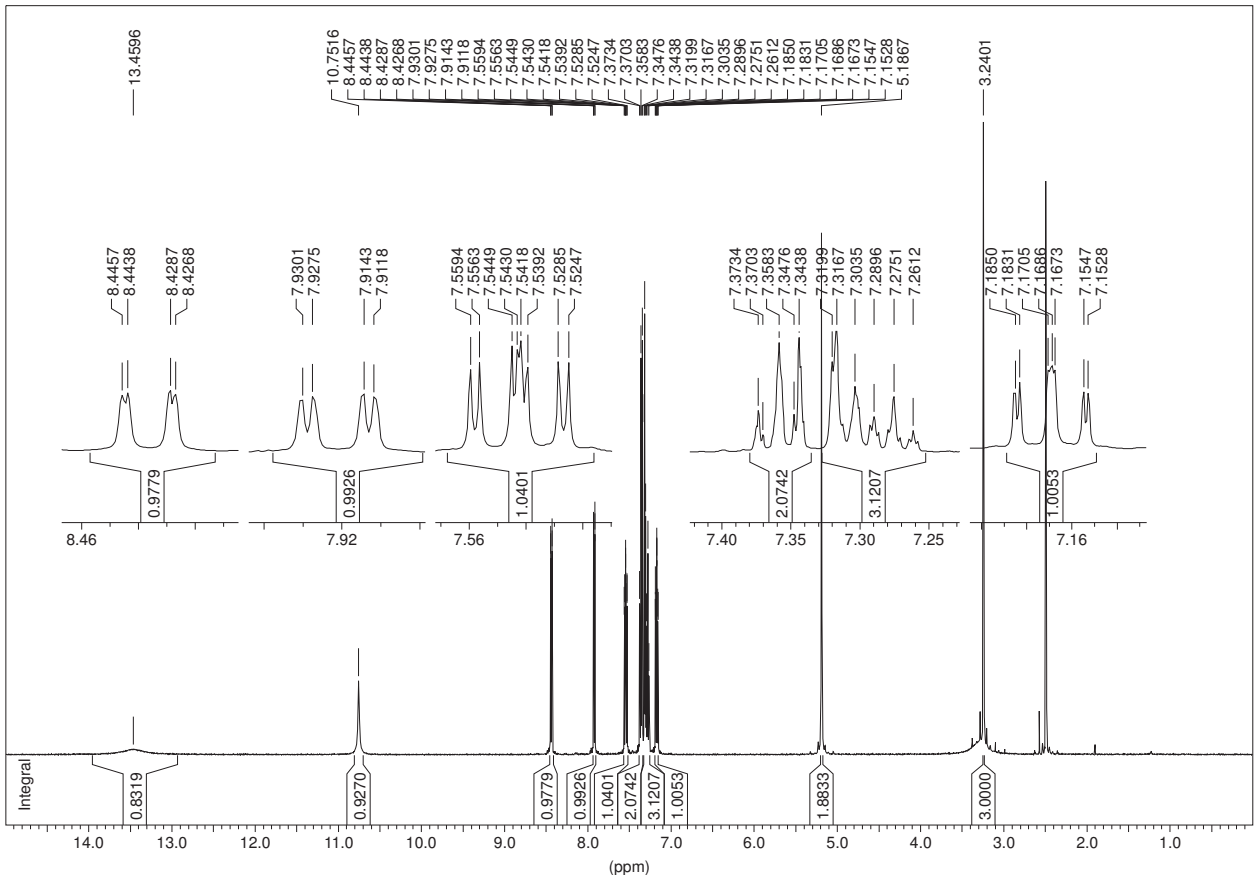


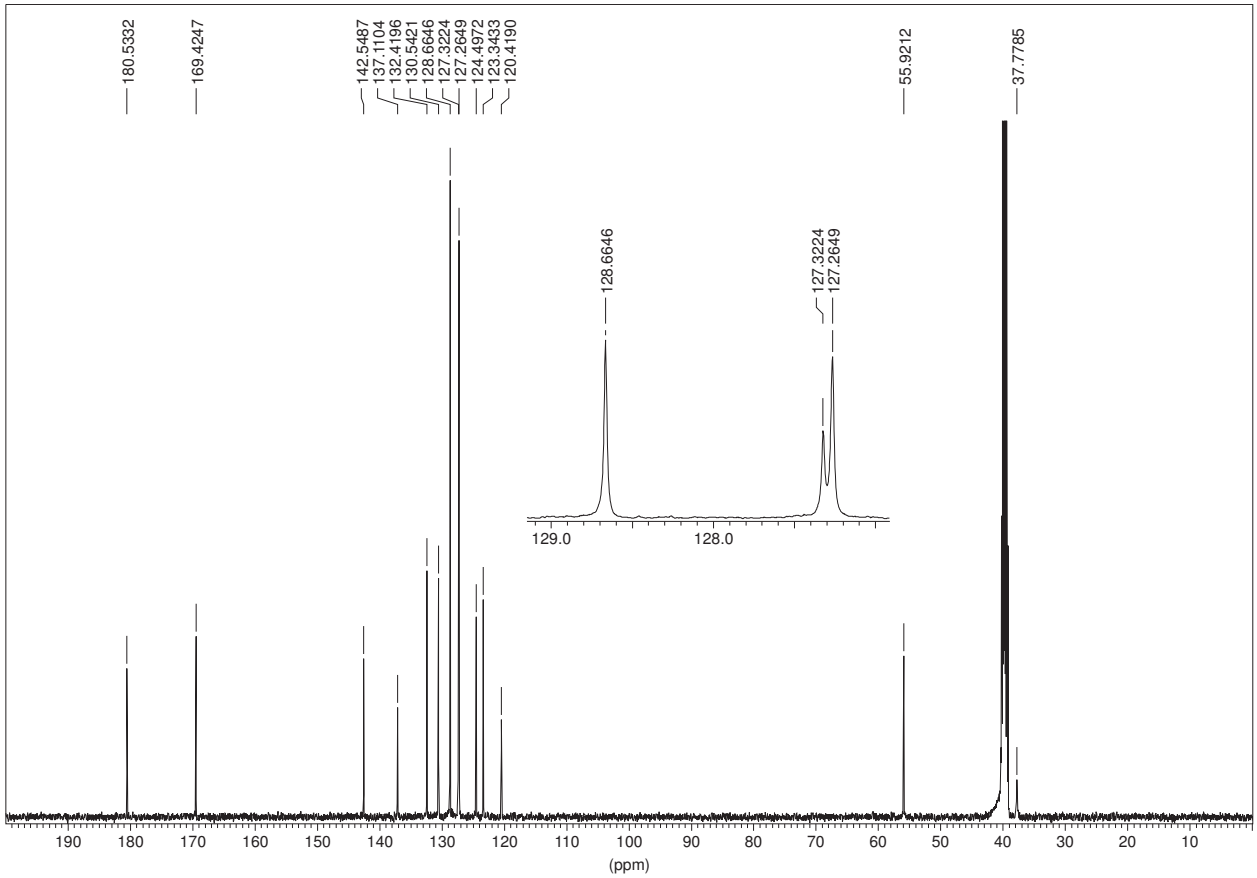
2-(3-Methyl-3-phenylthioureido)benzoic (**80**)



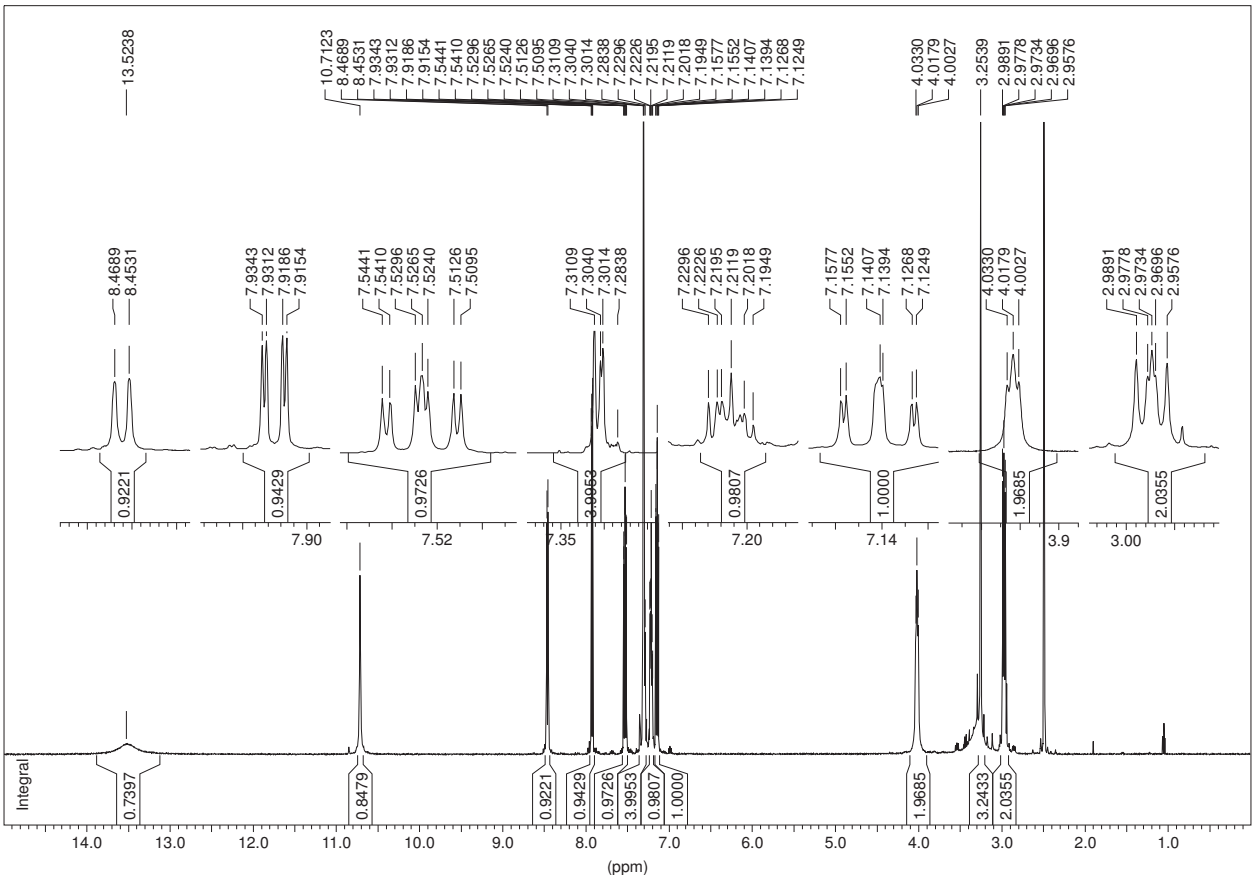


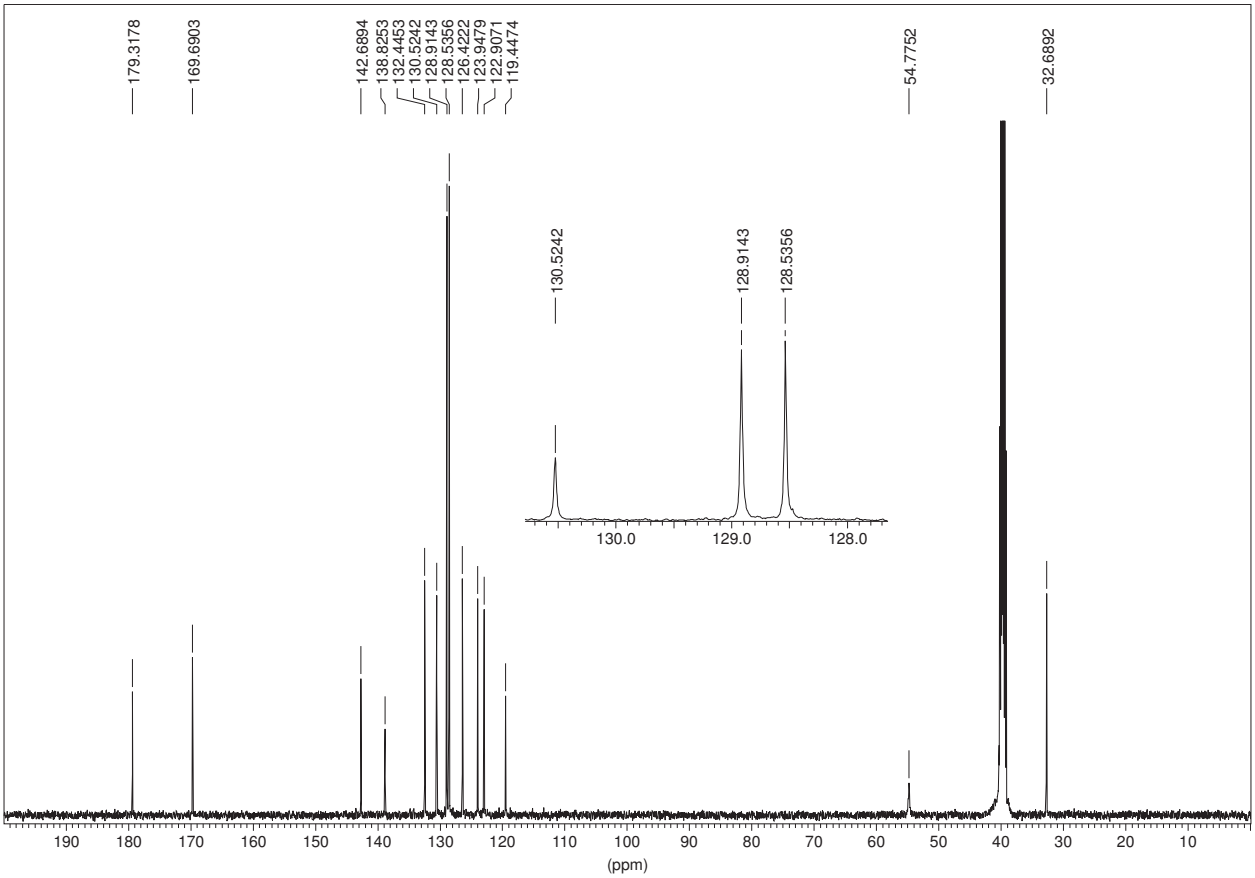
2-(3-Benzyl-3-methylthioureido)benzoic Acid (**81**)



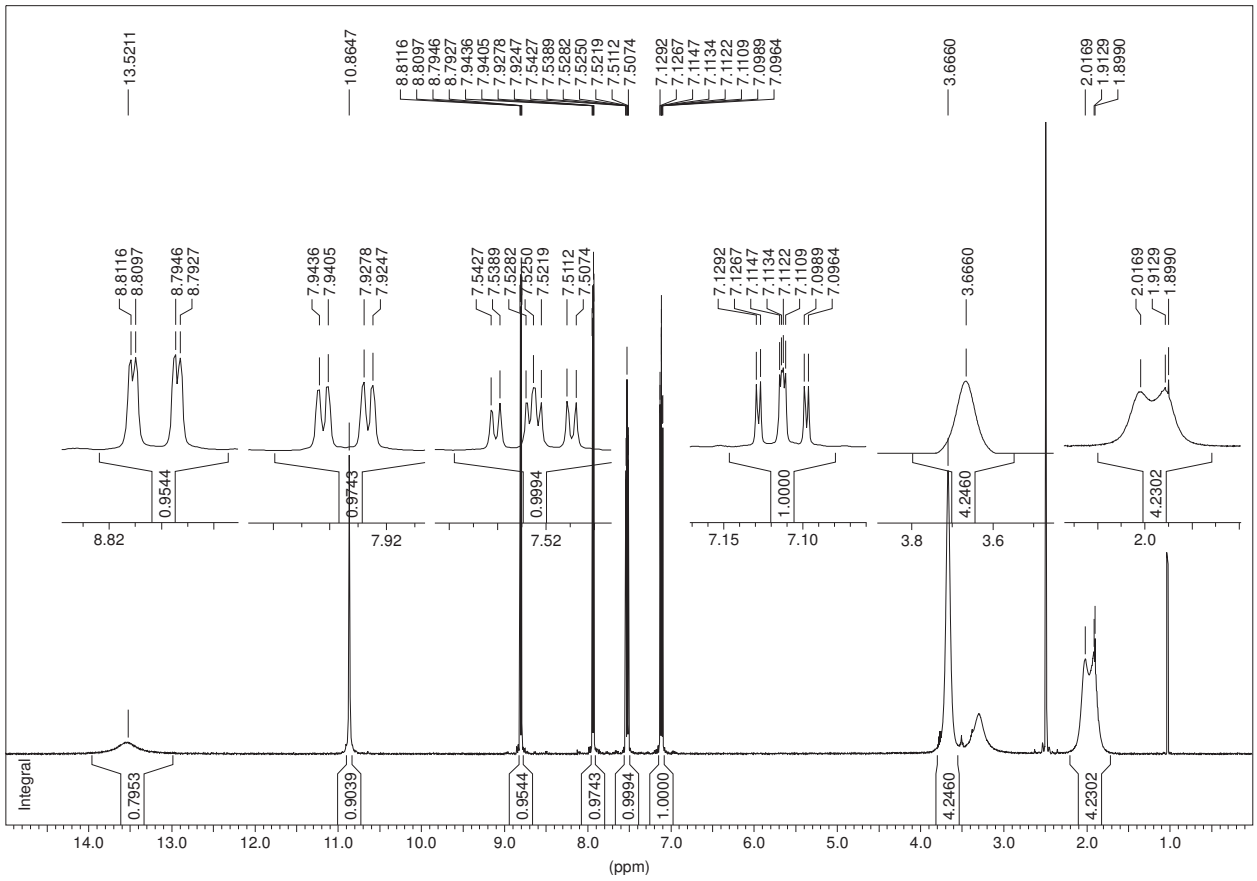


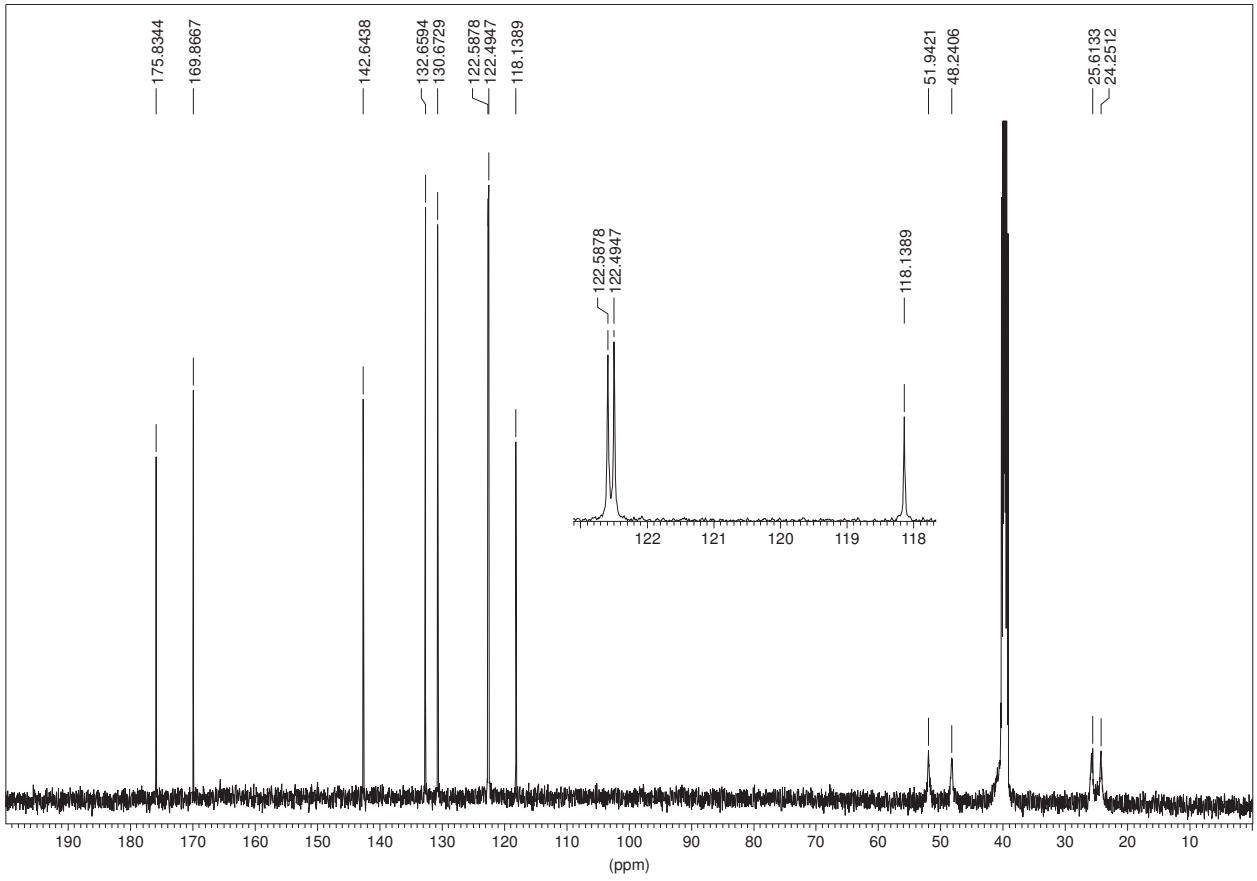
2-[3-Methyl-3-(2-phenylethyl)thioureido]benzoic Acid (**82**)



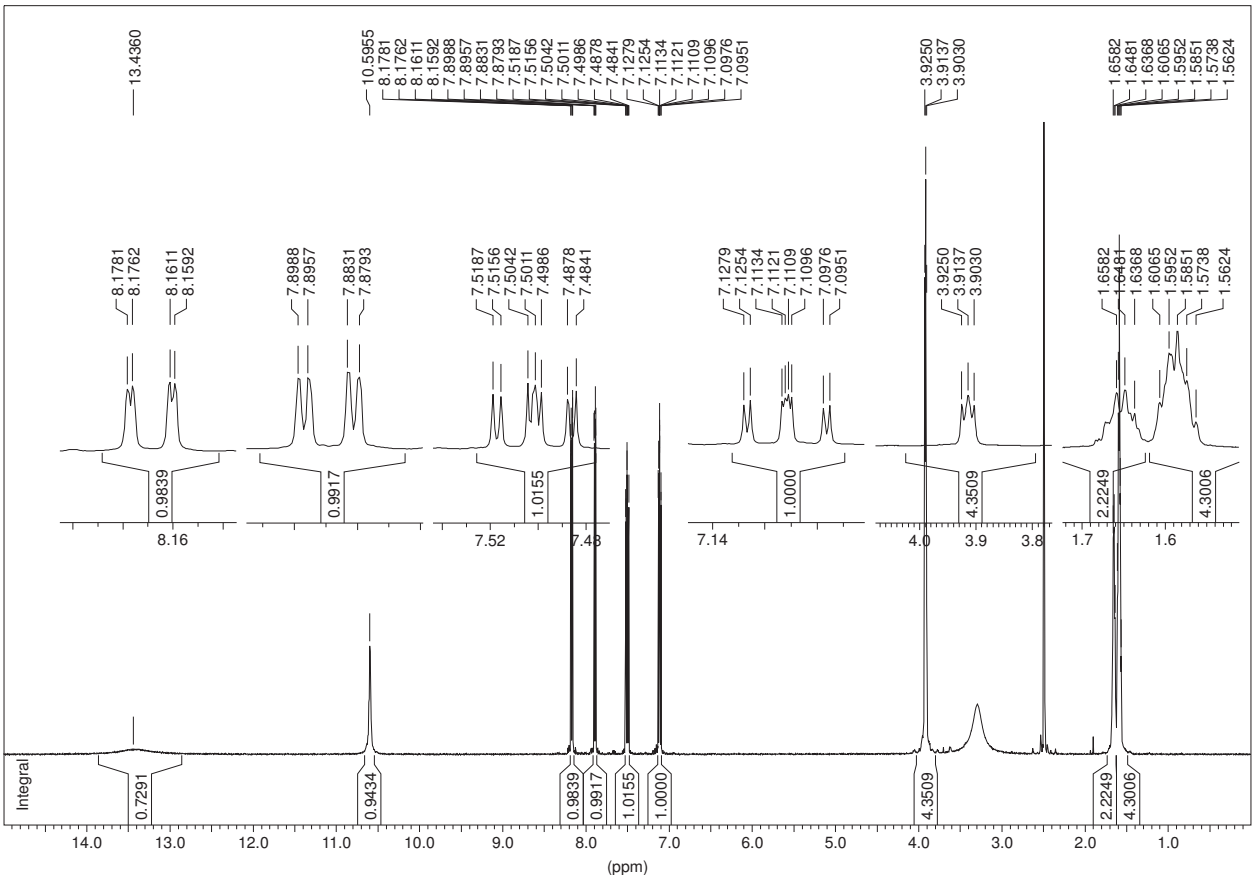


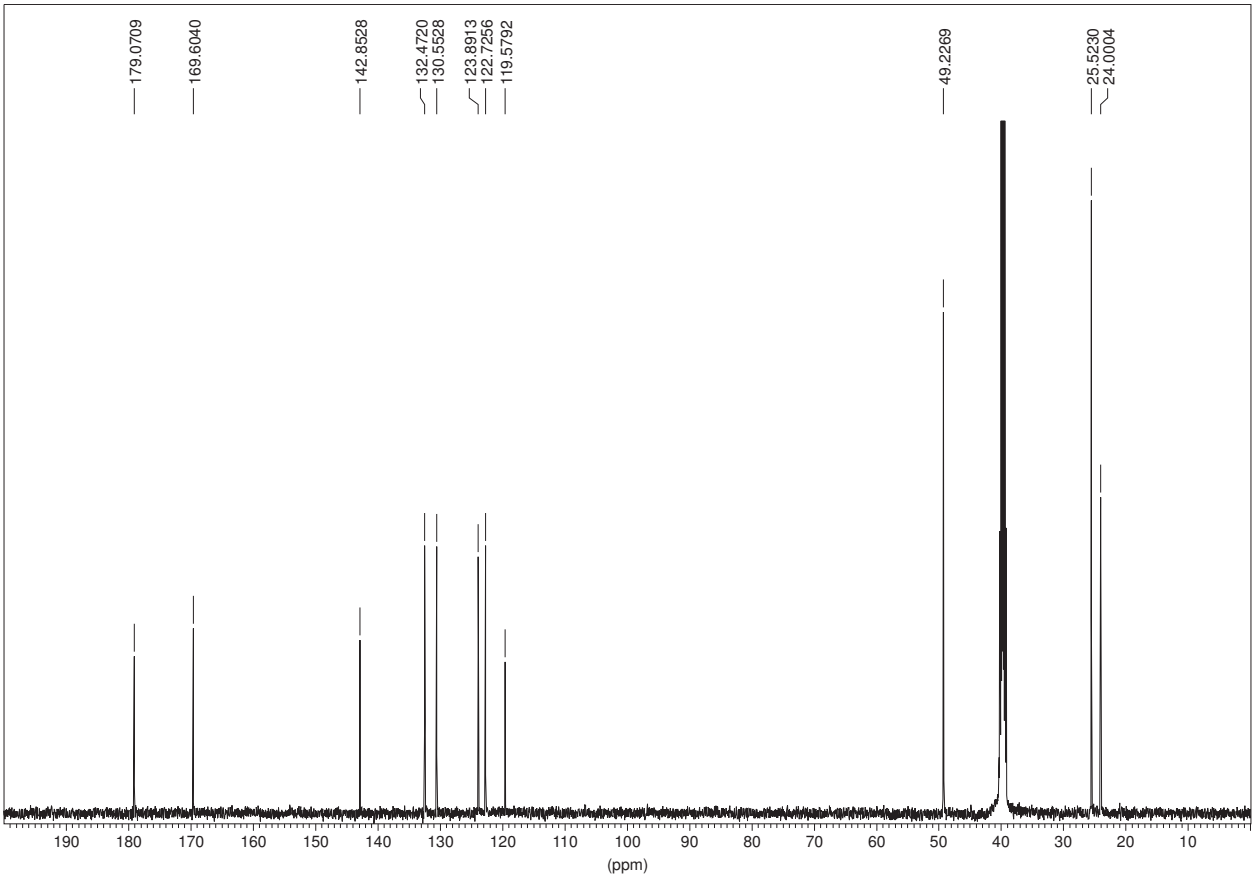
2-[(1-Pyrrolidinylthiocarbonyl)amino]benzoic Acid (**83**)



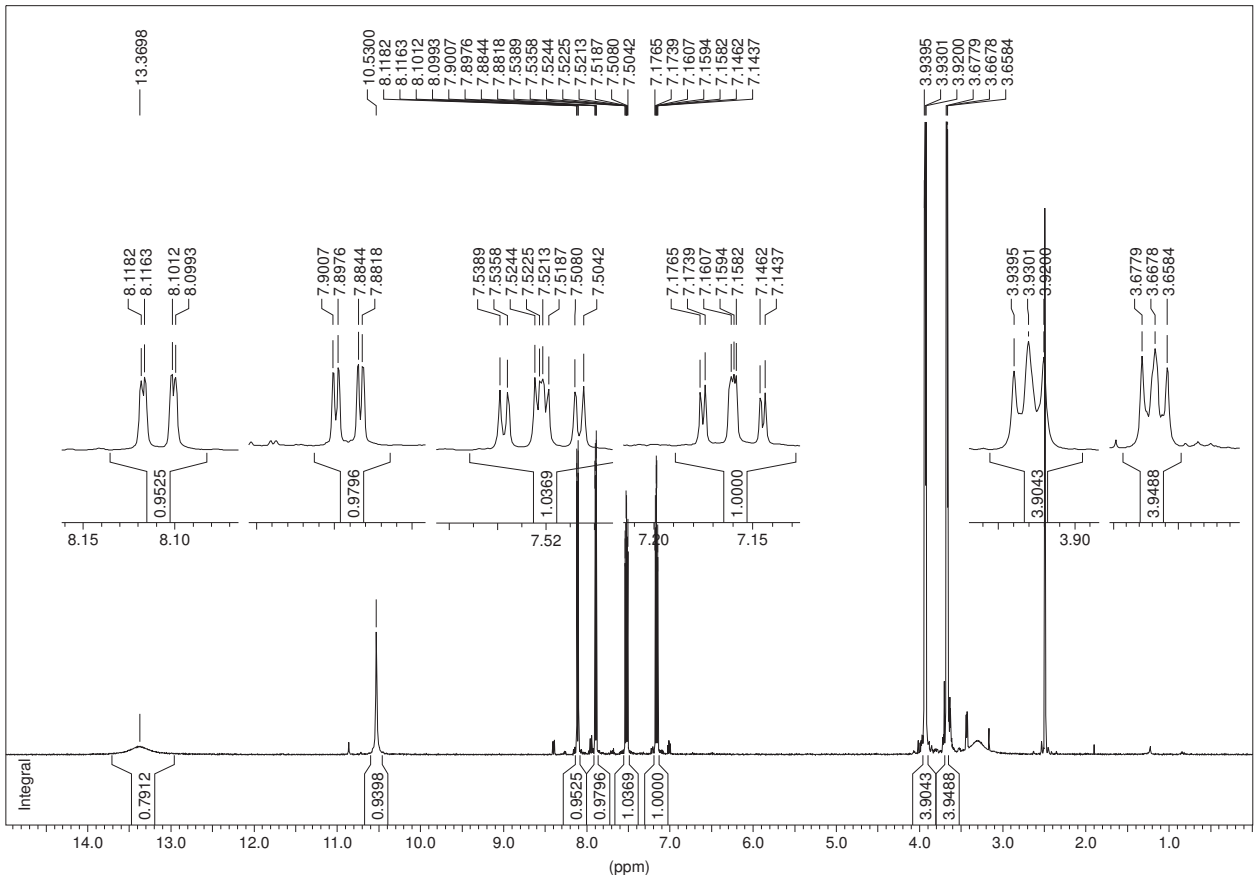


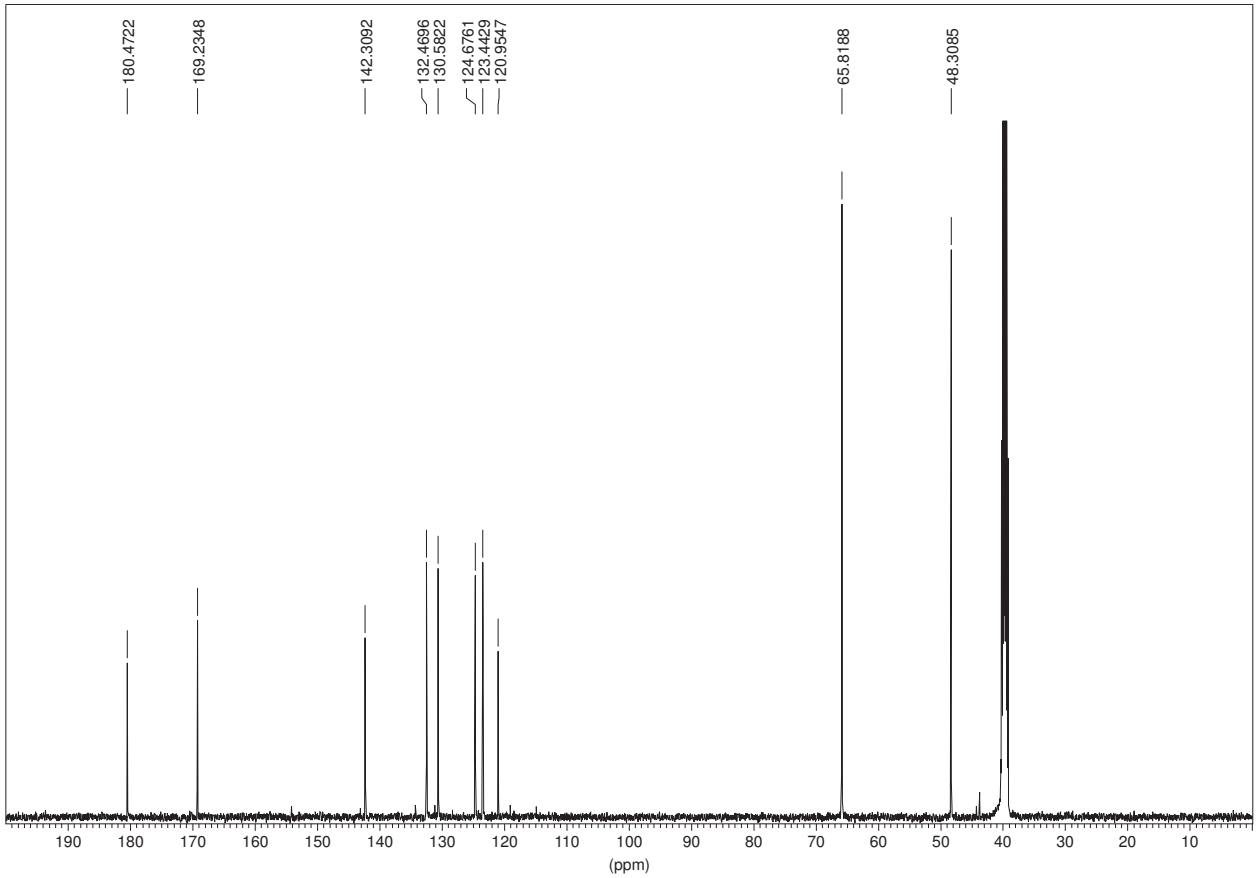
2-[(1-Piperidinylthiocarbonyl)amino]benzoic Acid (**84**)



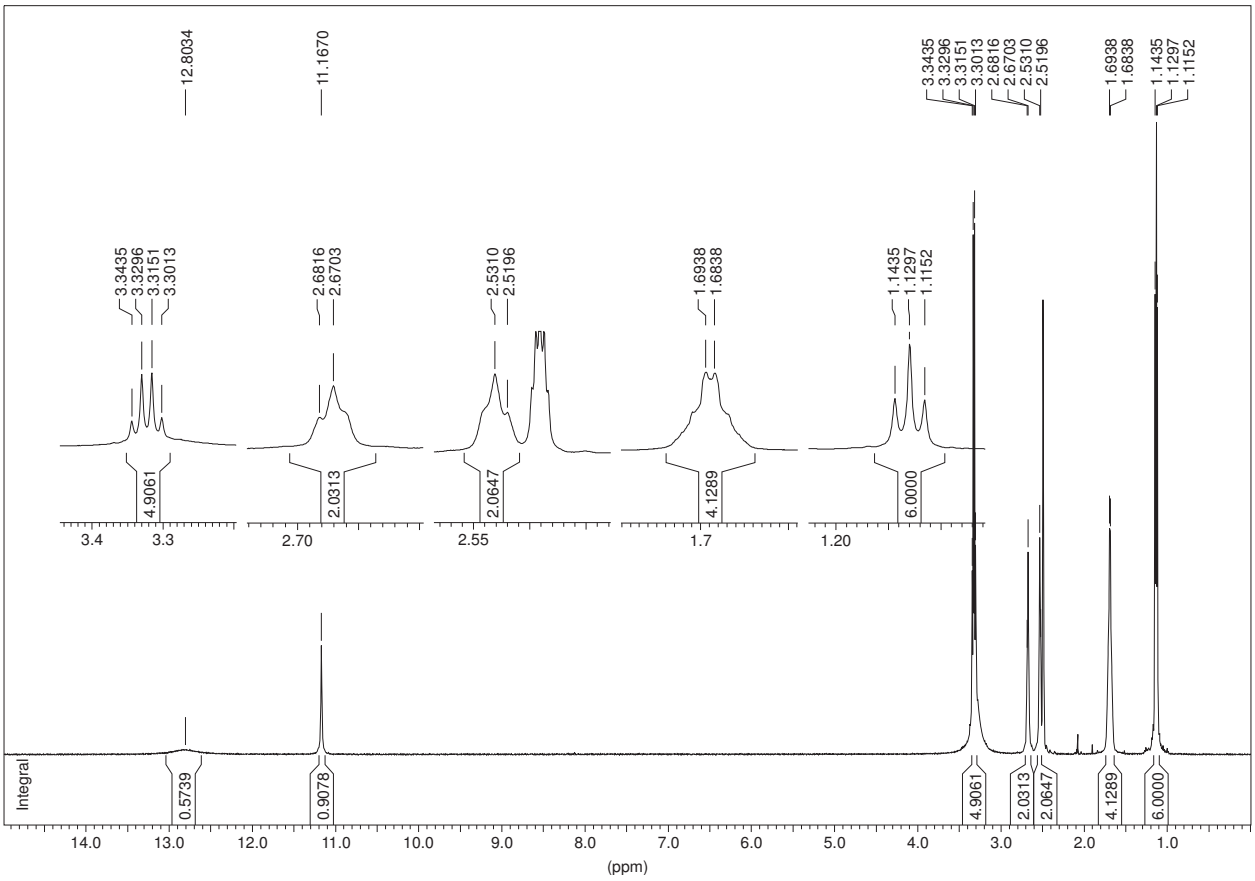


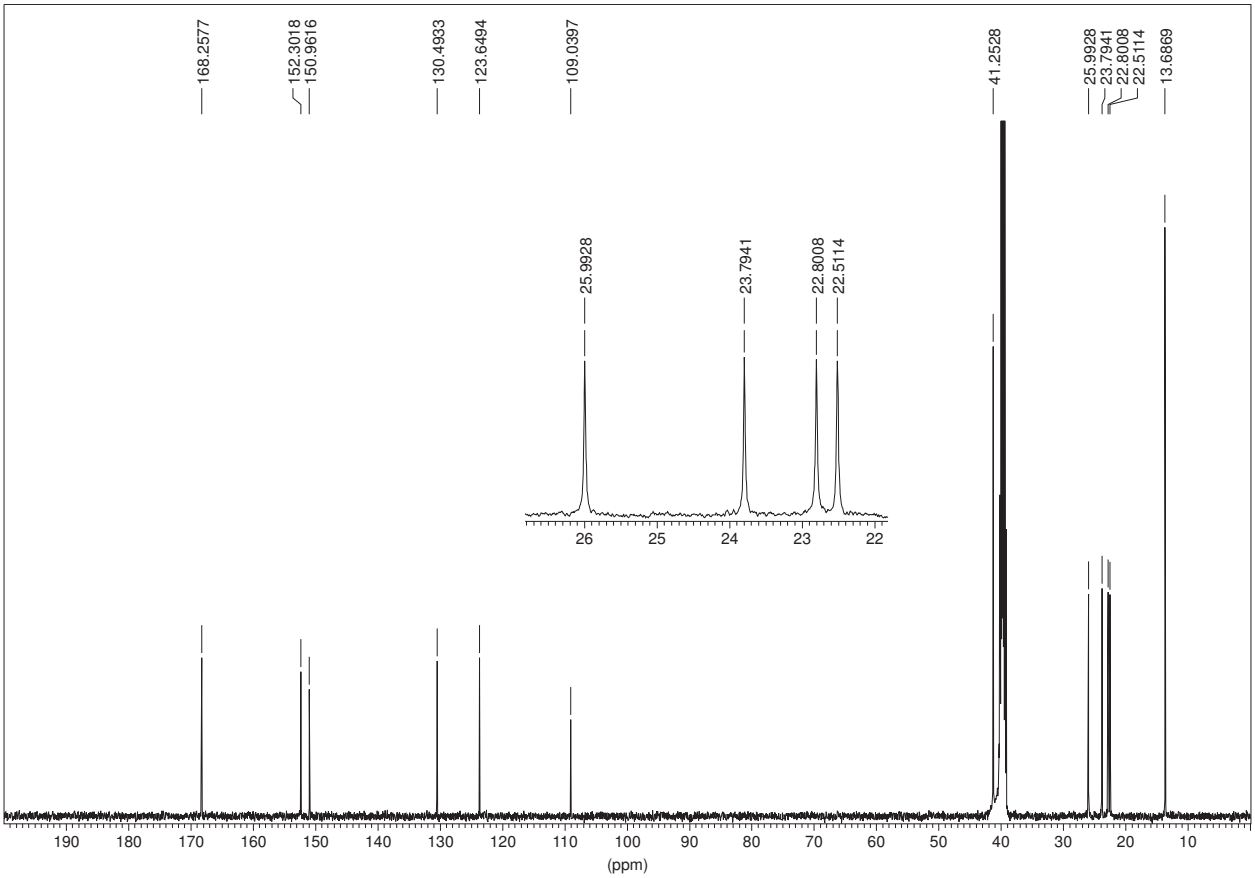
2-[(4-Morpholinylthiocarbonyl)amino]benzoic Acid (**85**)



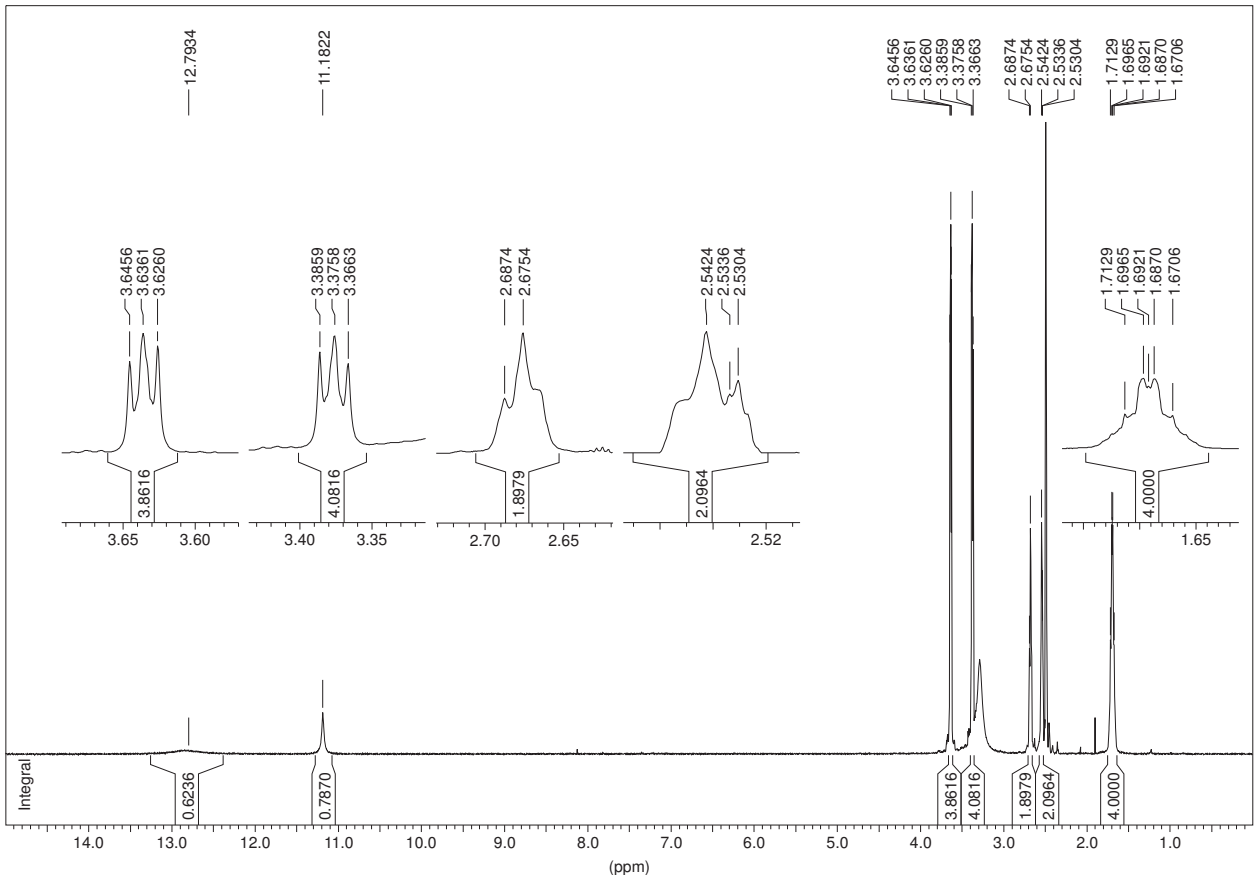


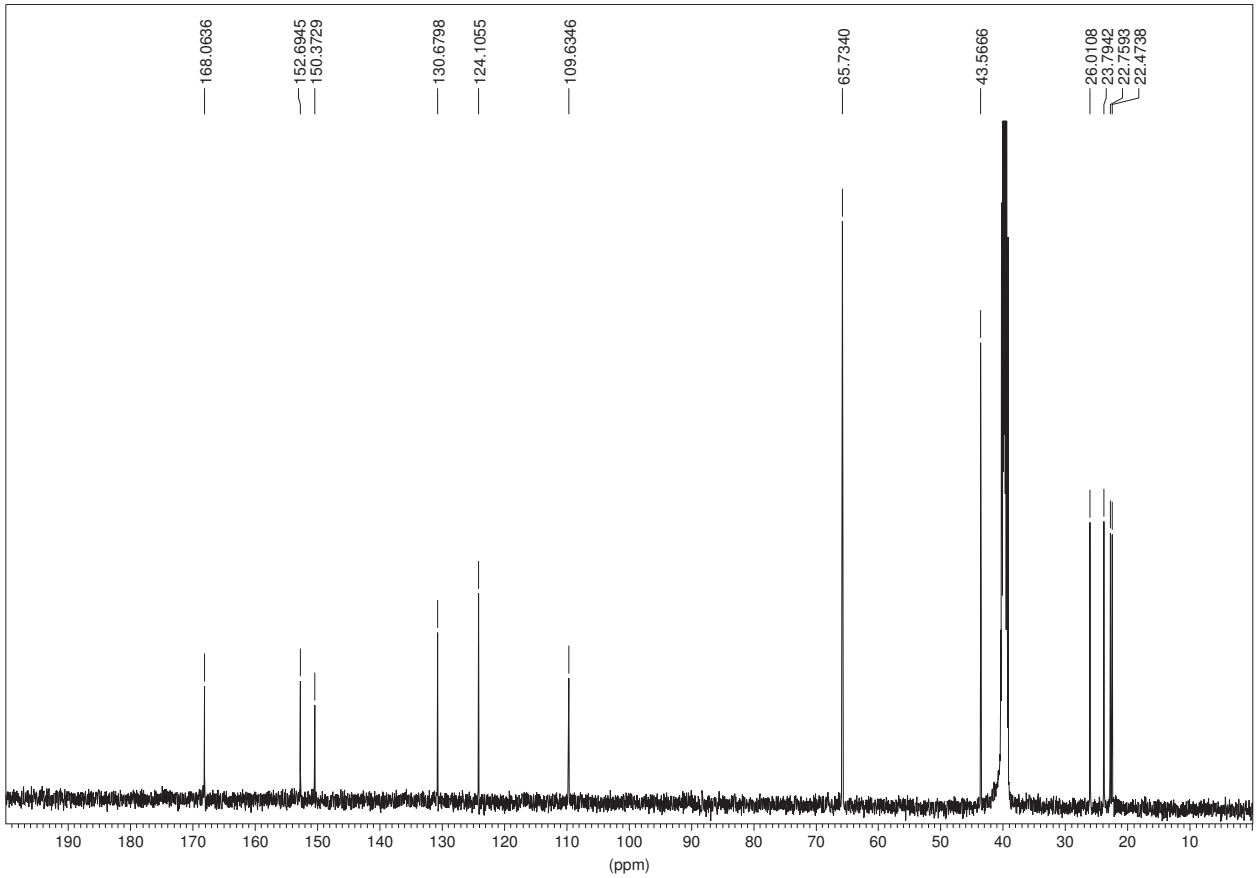
2-(3,3-Diethylureido)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**88**)



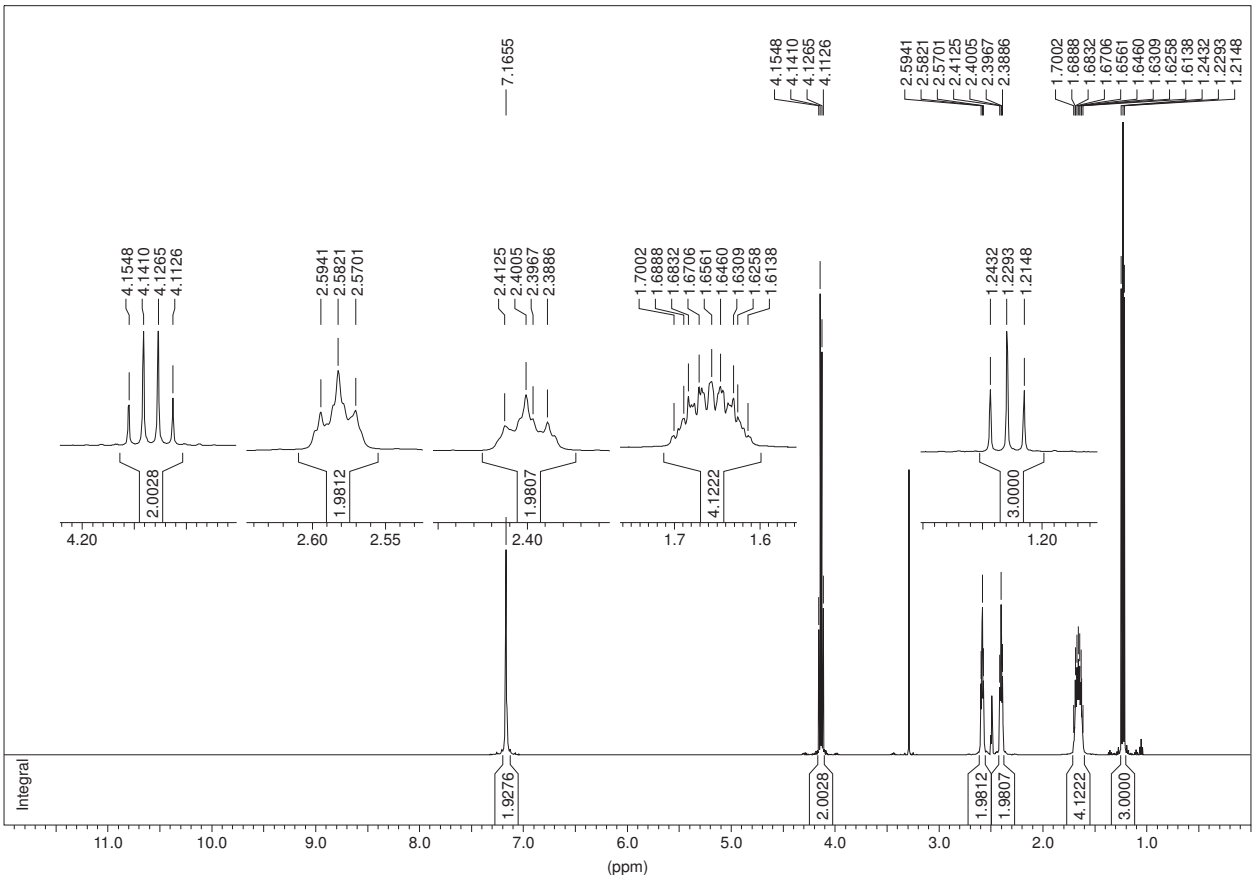


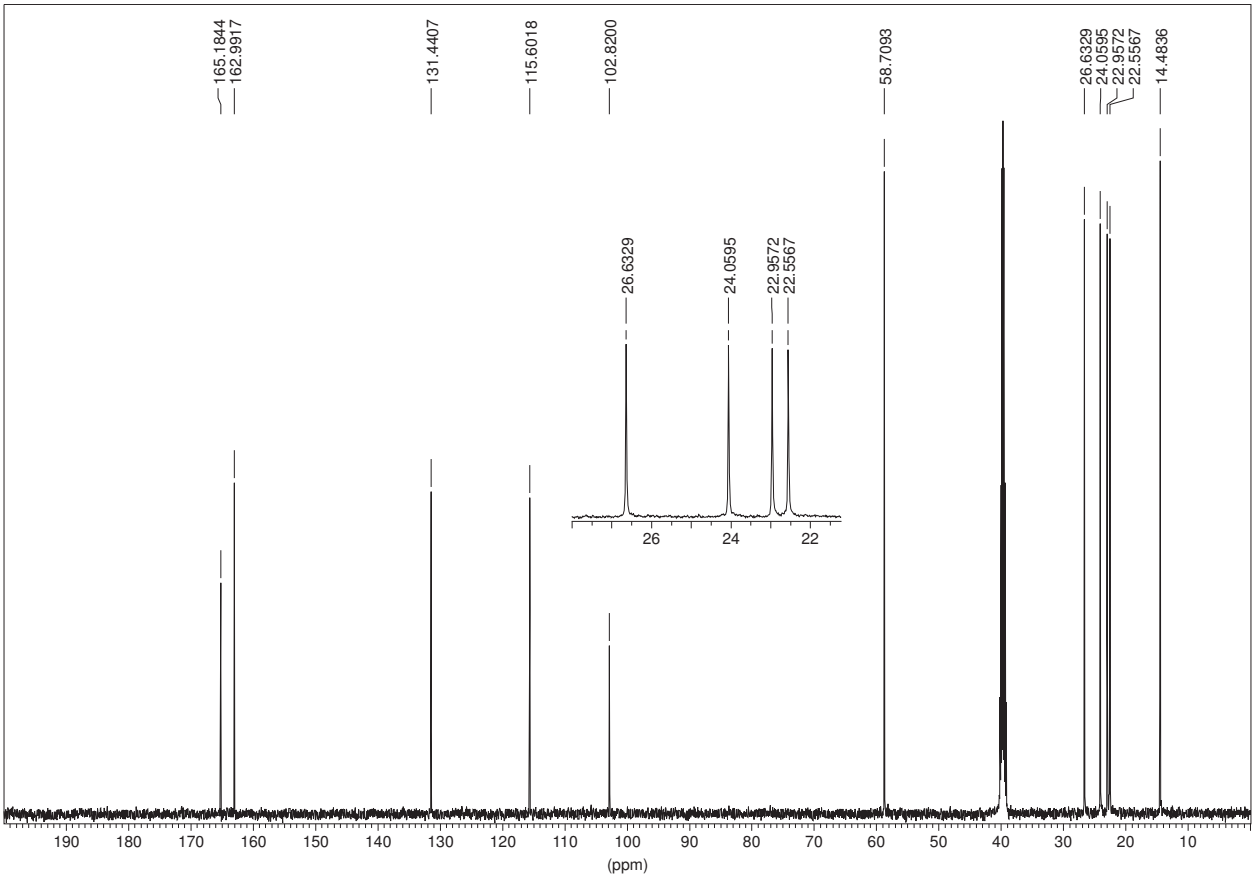
2-[(4-Morpholinylcarbonyl)amino]-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylic Acid (**89**)



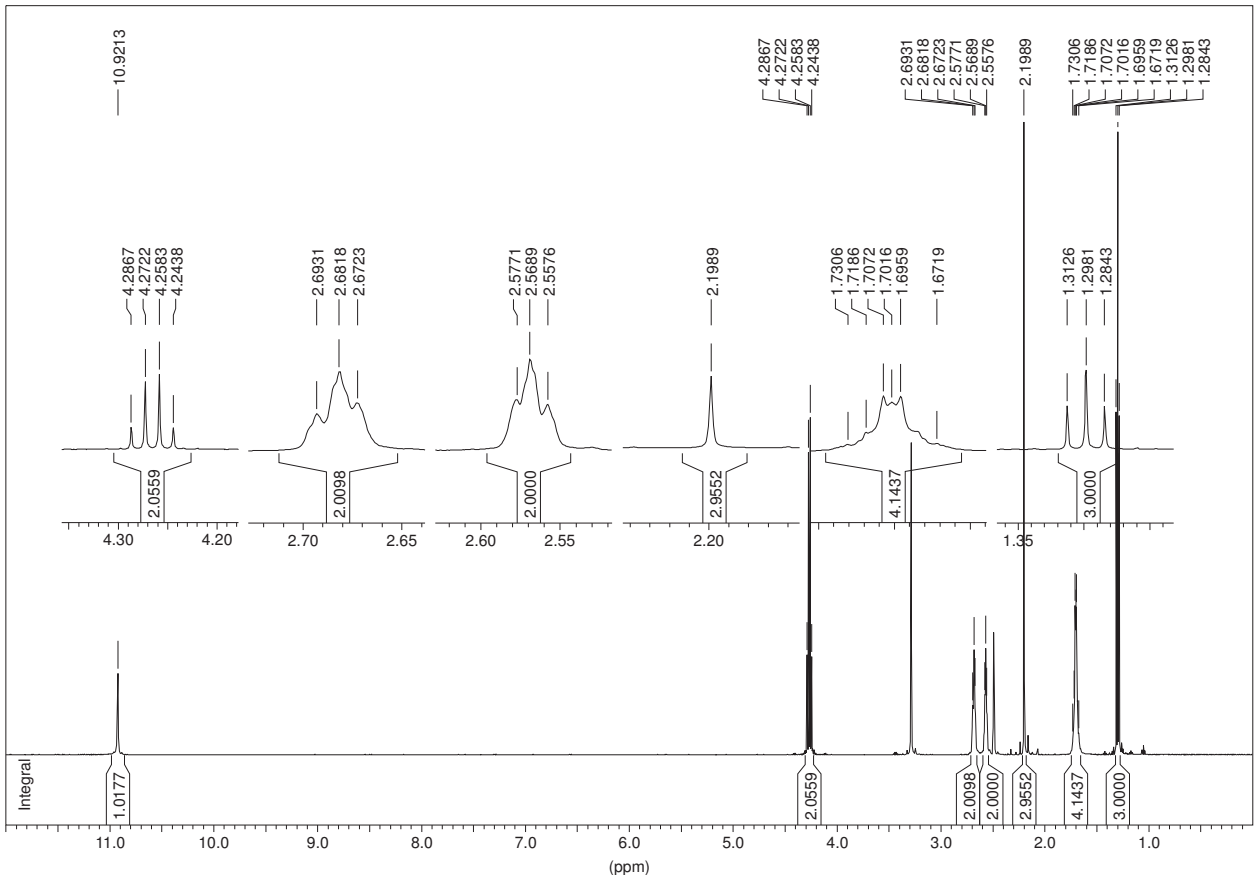


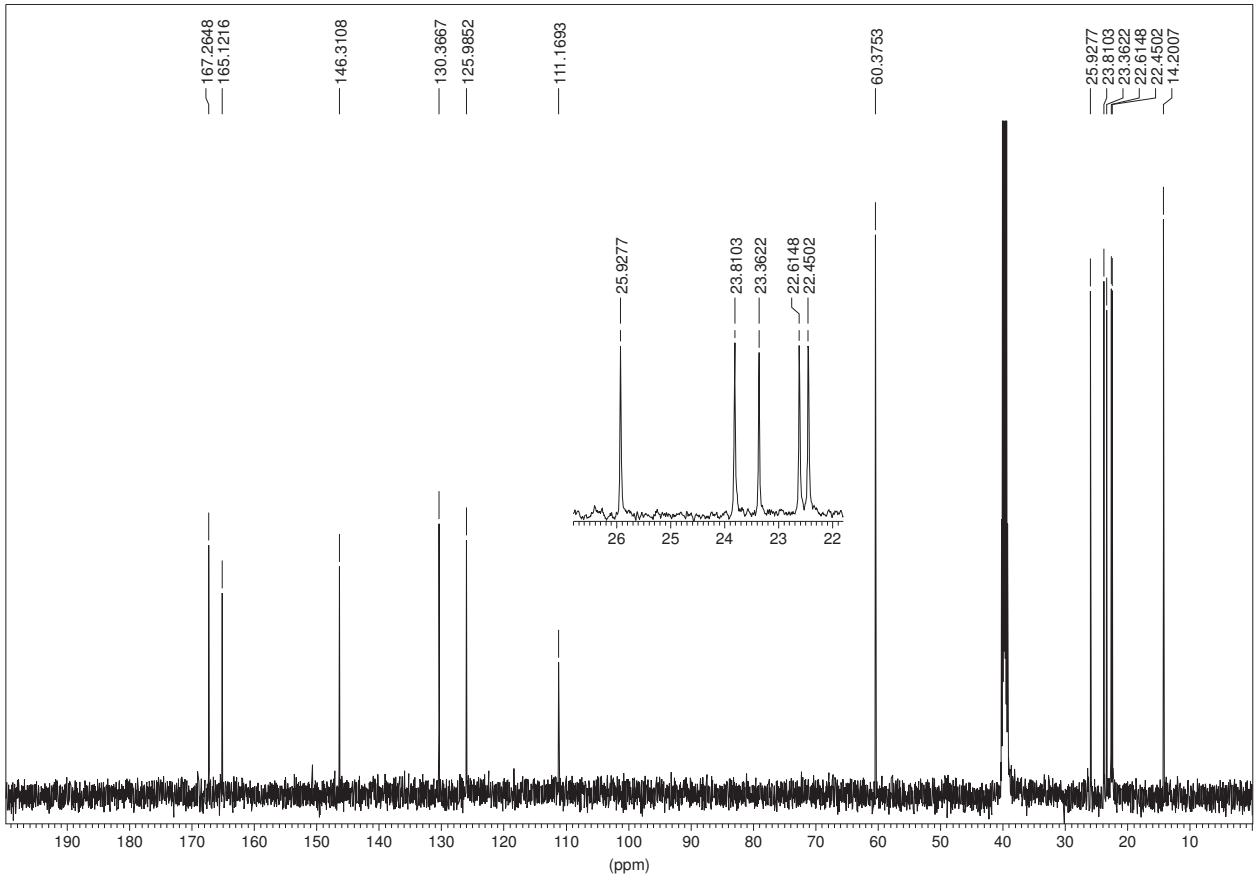
Ethyl 2-Amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**90**)



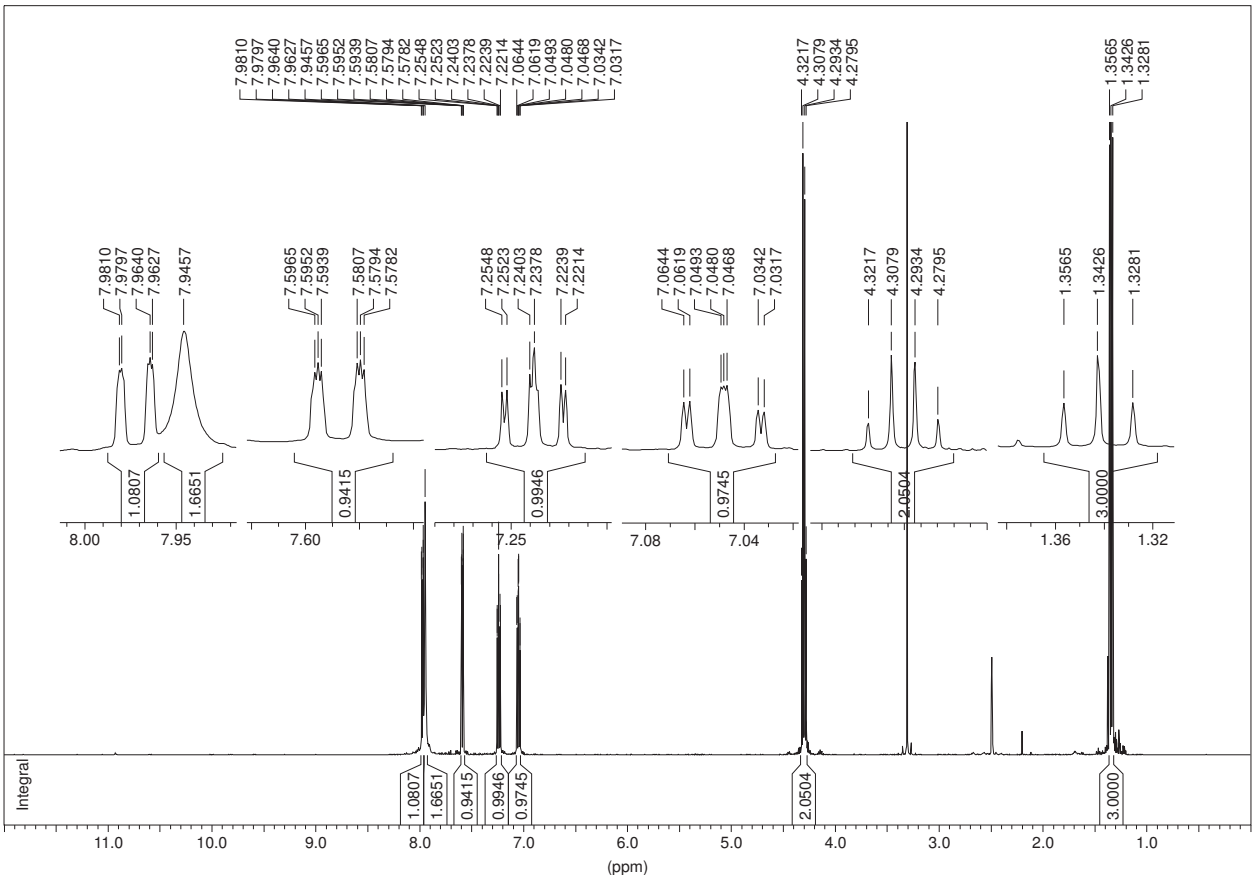


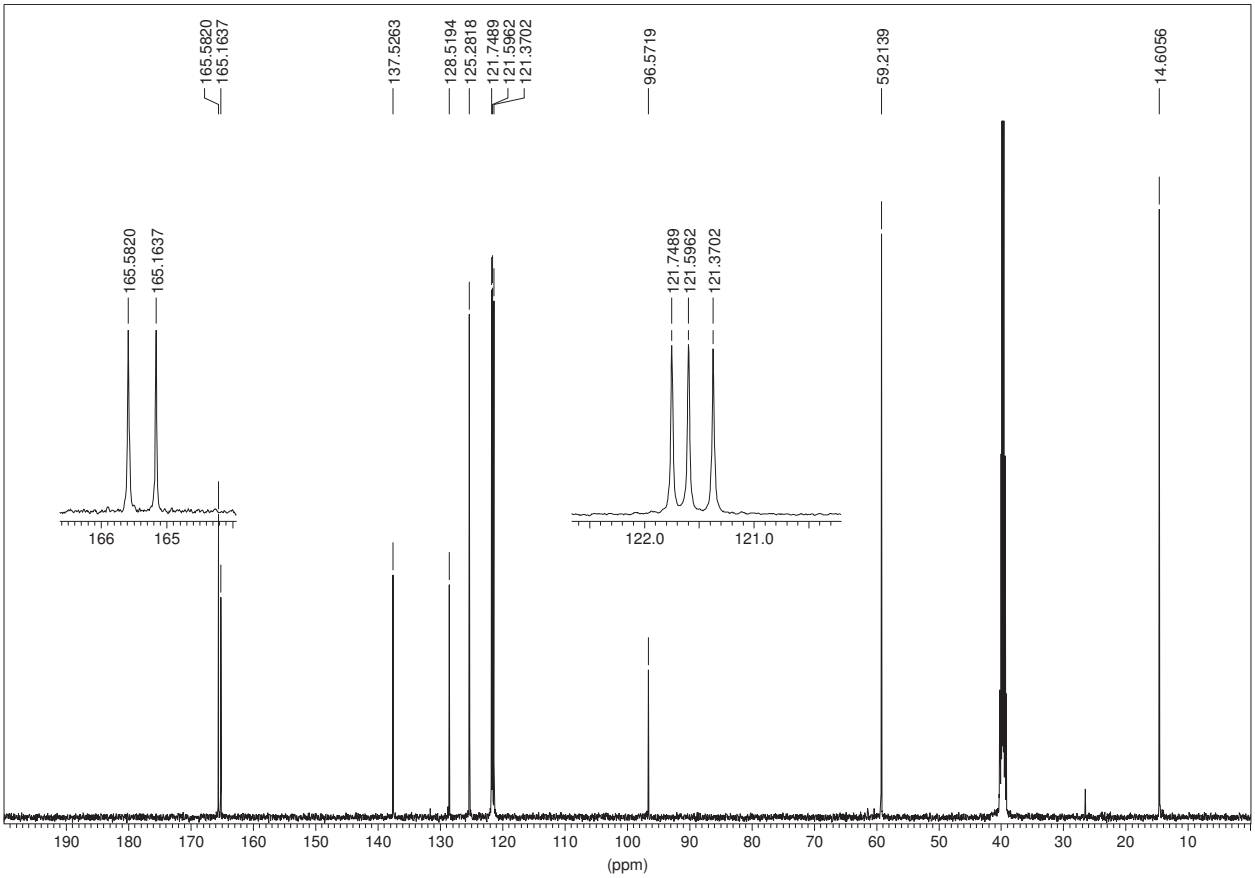
Ethyl 2-(Acetylamino)-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carboxylate (**91**)



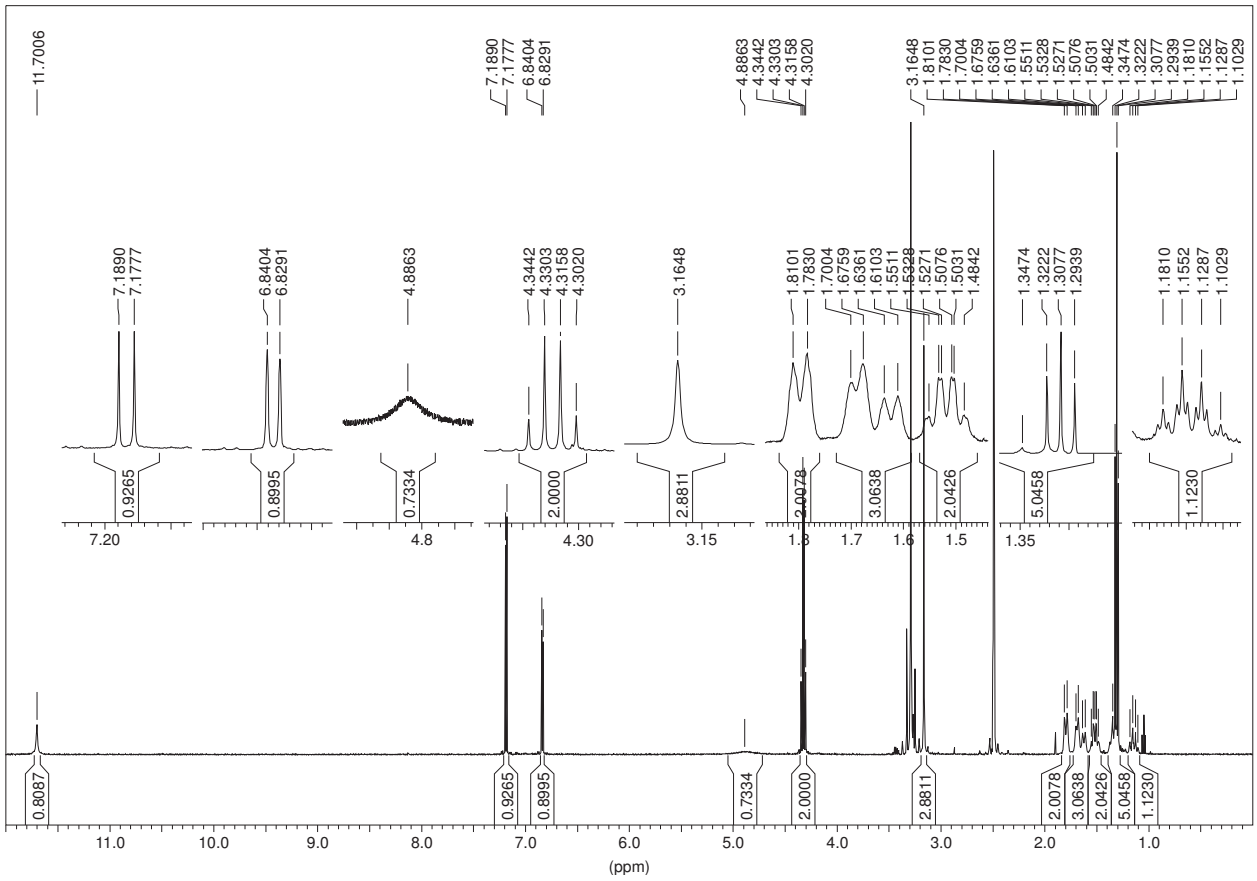


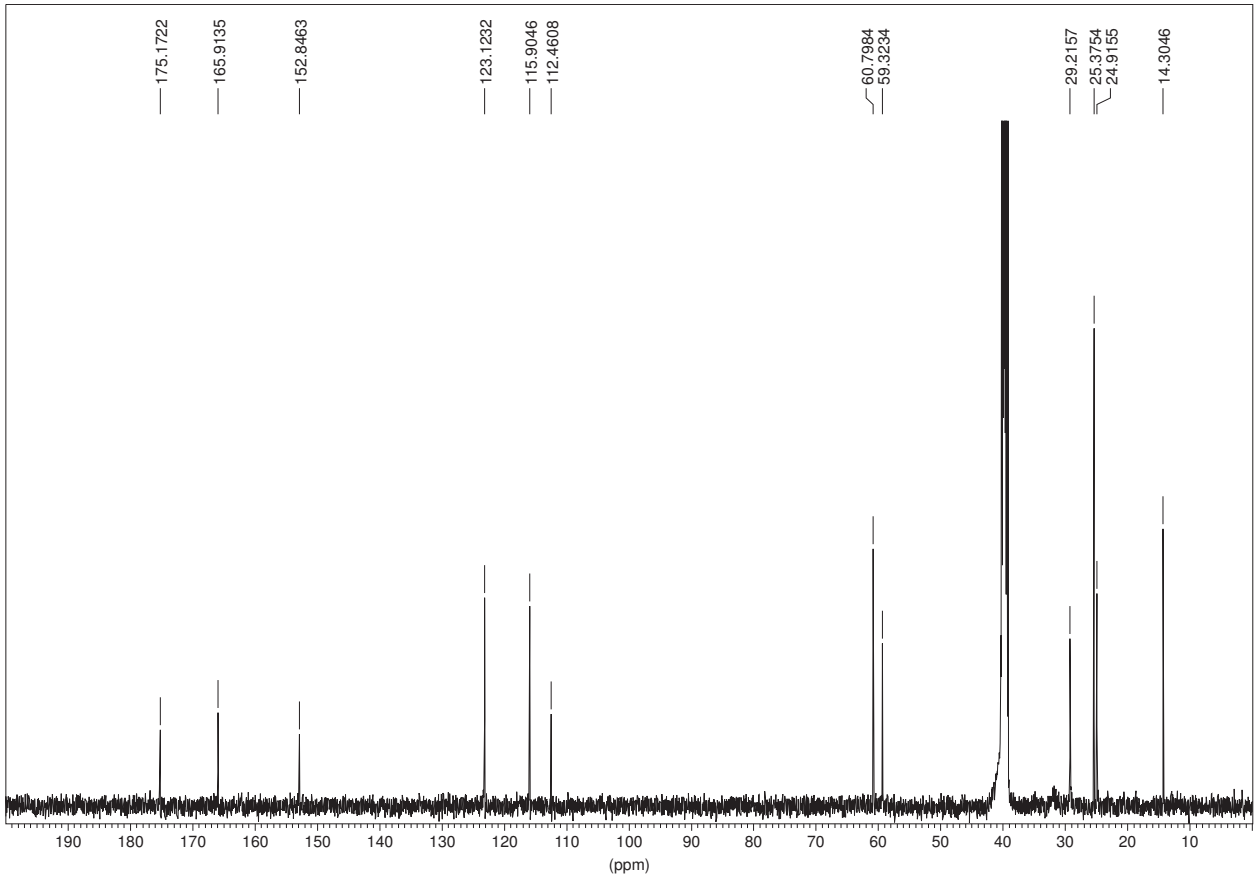
Ethyl 2-Aminobenzo[*b*]thiophene-3-carboxylate (**93**)



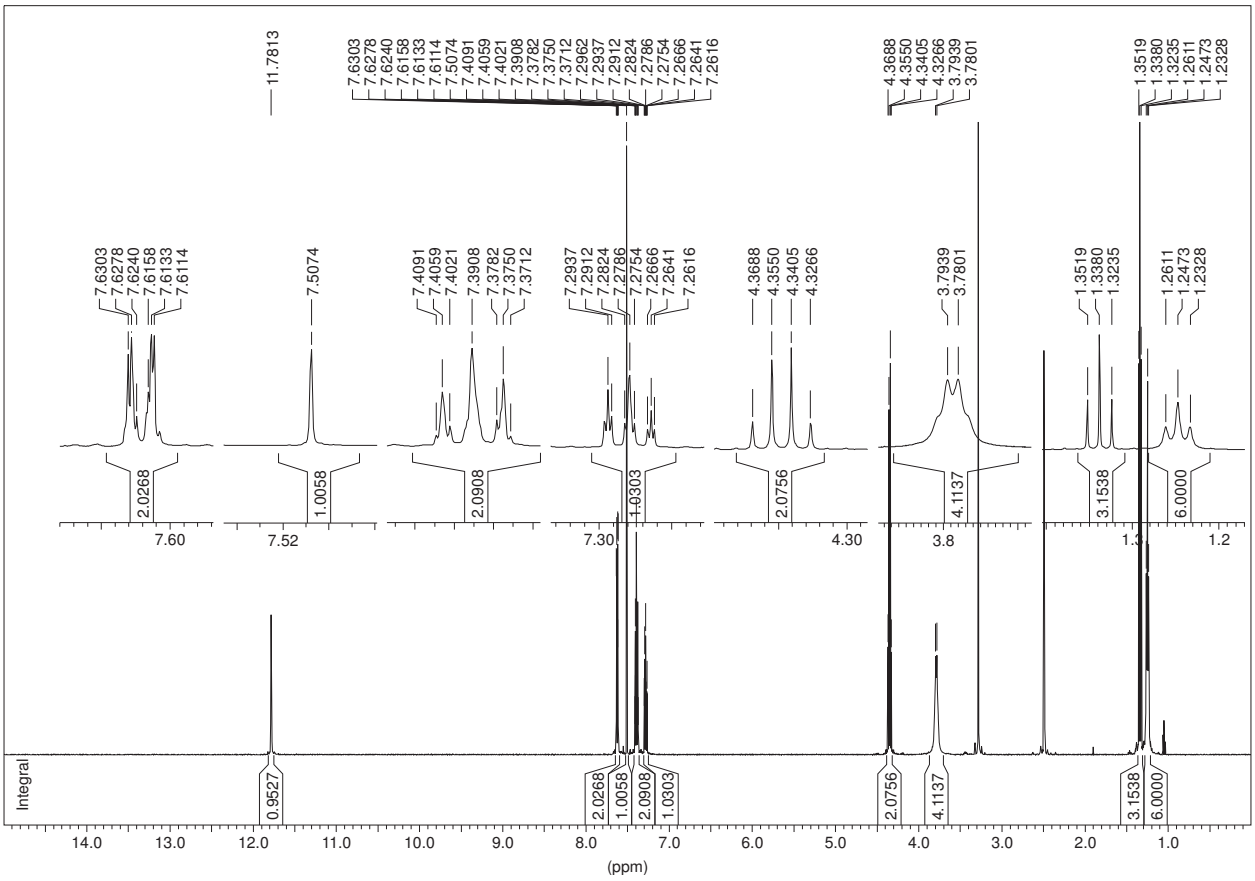


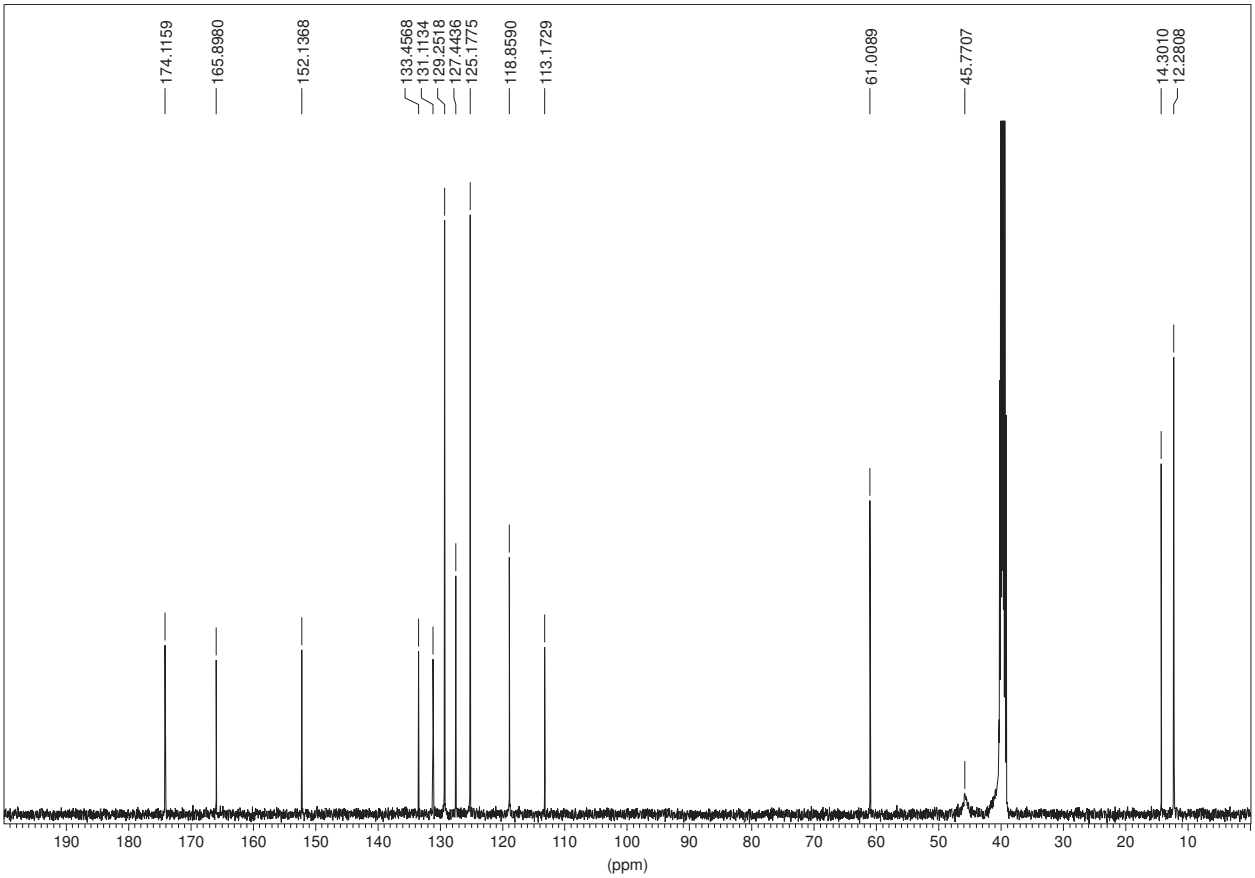
Ethyl 2-(3-Cyclohexyl-3-methylthioureido)thiophene-3-carboxylate (**95**)



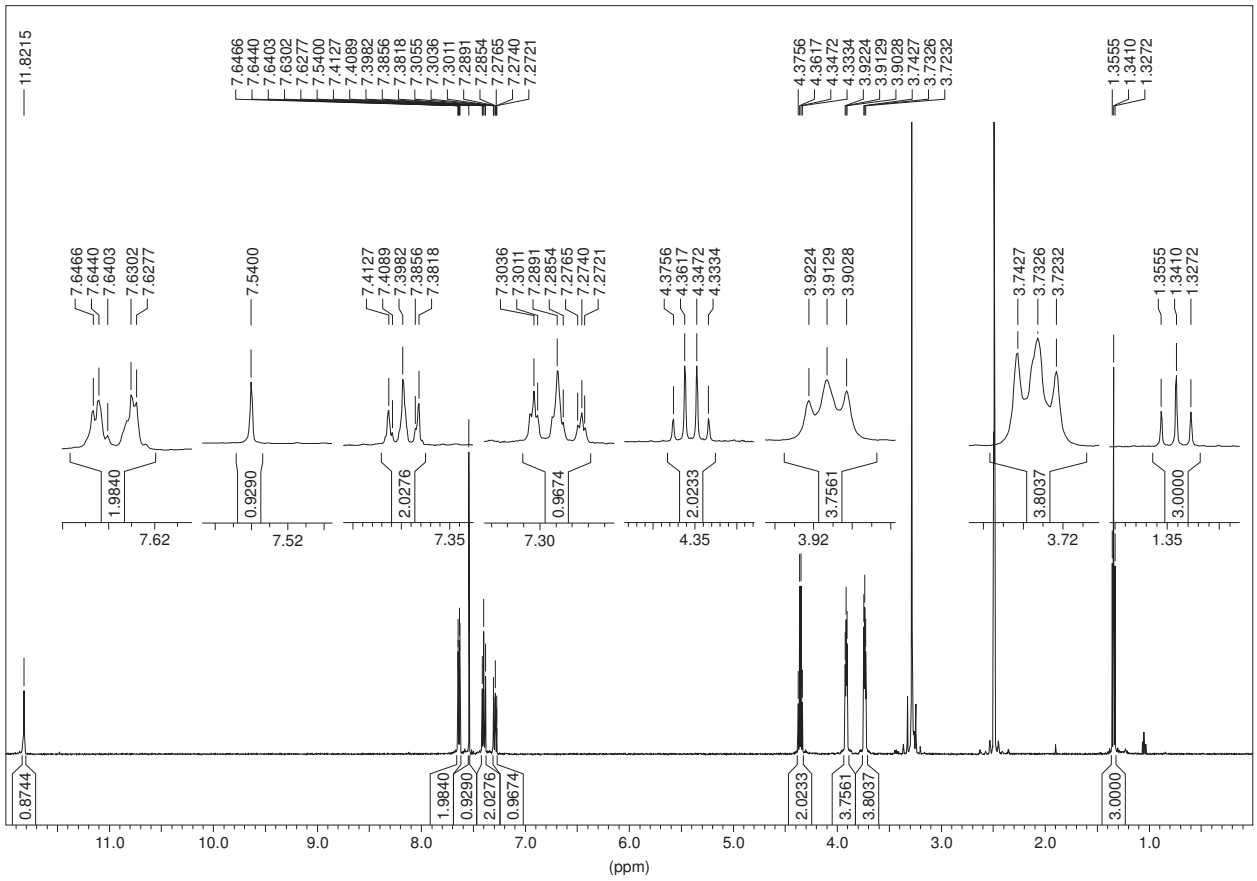


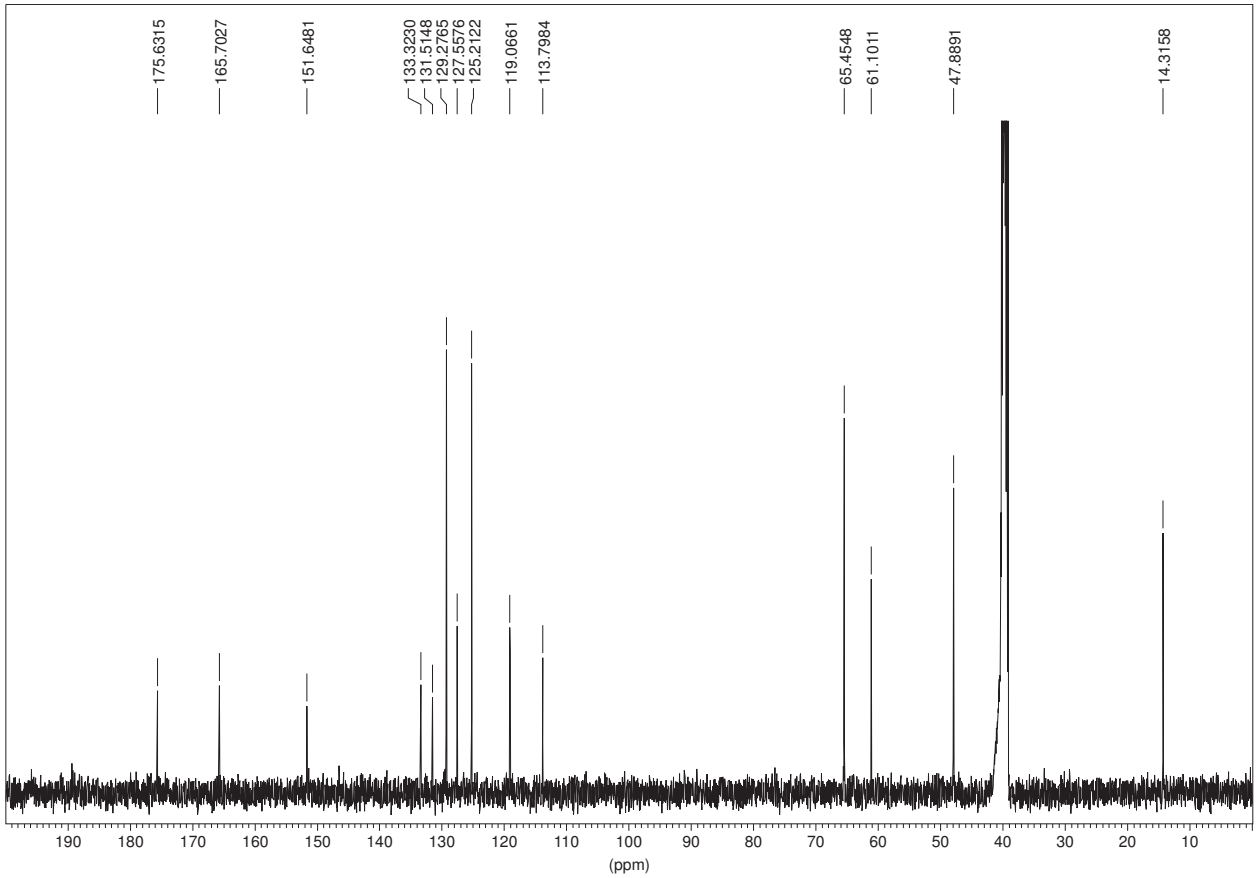
Ethyl 2-(3,3-Diethylthioureido)-5-phenylthiophene-3-carboxylate (**96**)



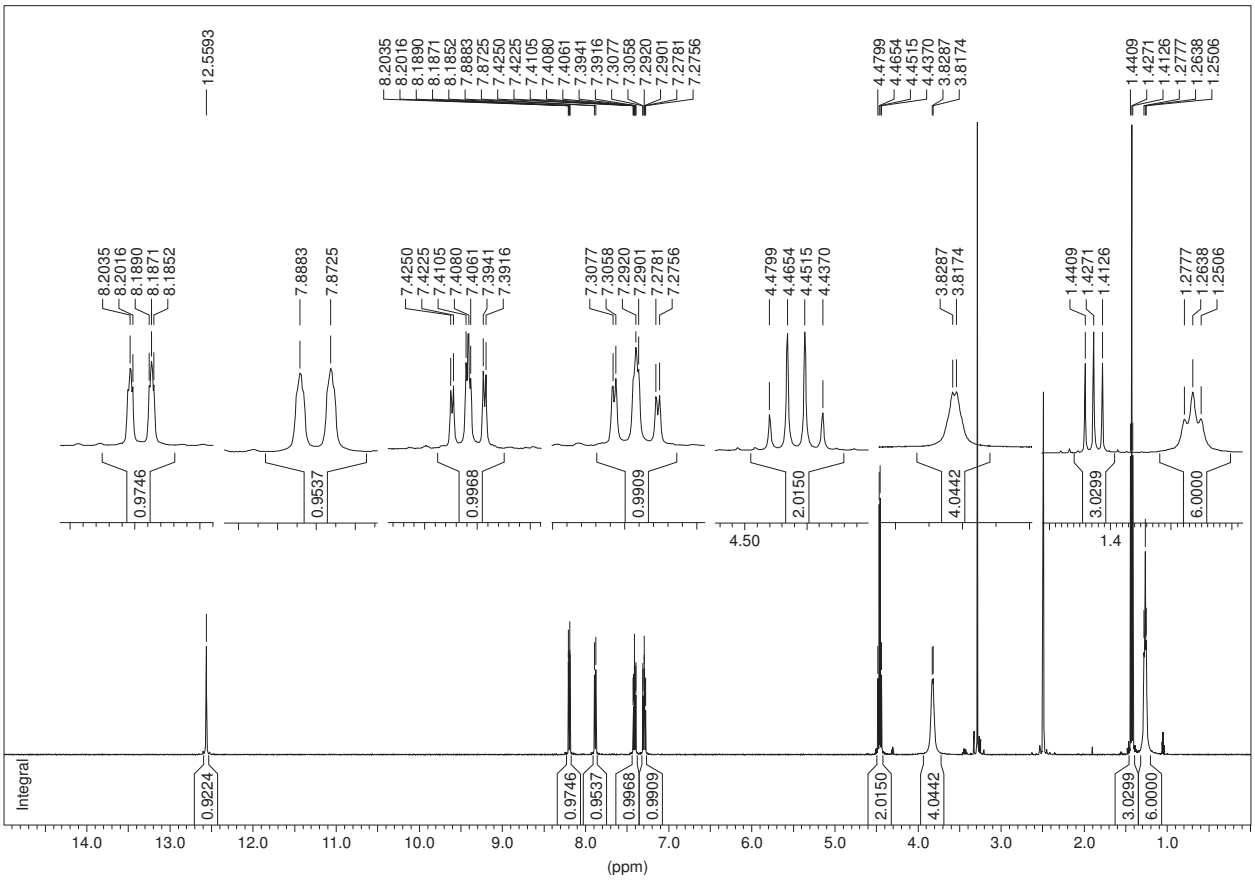


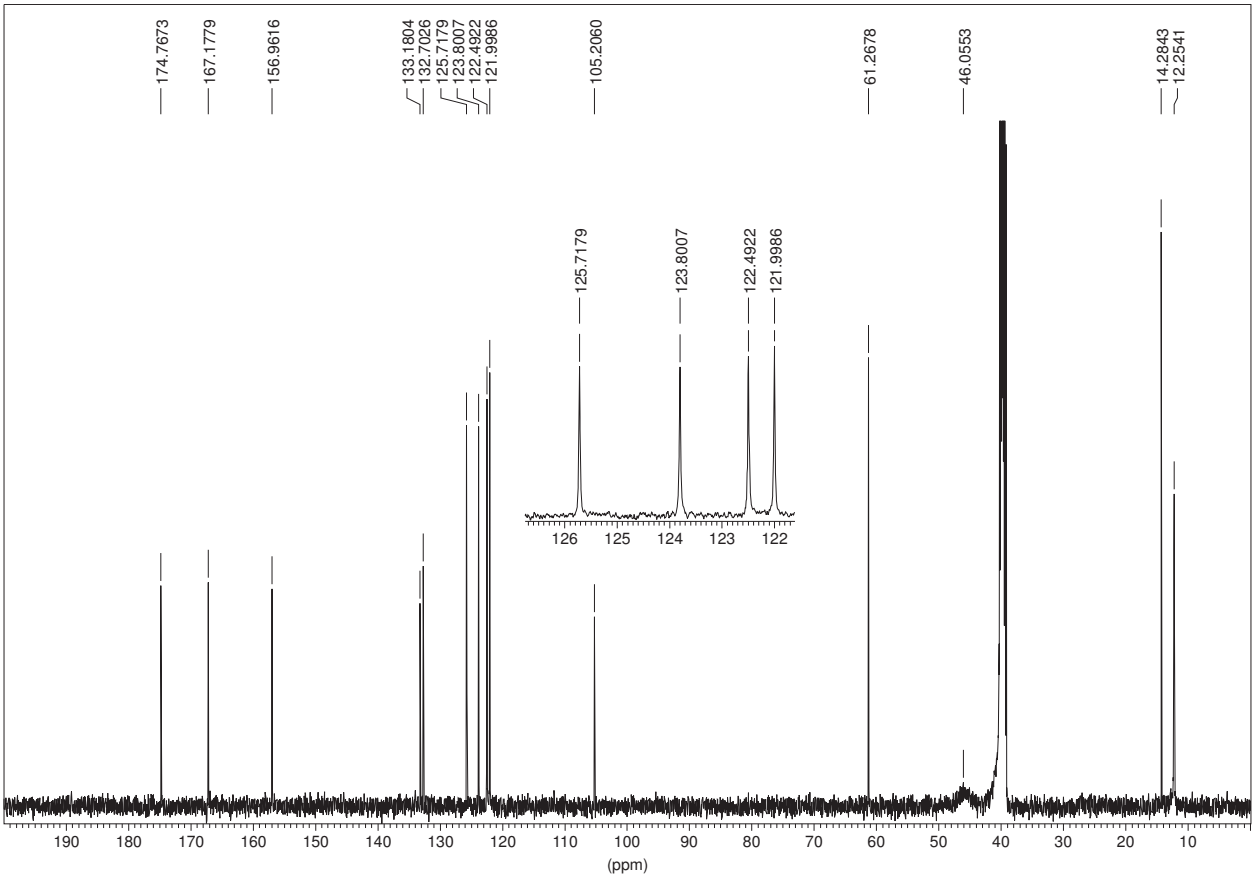
Ethyl 2-[(4-Morpholinylthiocarbonyl)amino]-5-phenylthiophene-3-carboxylate (**97**)



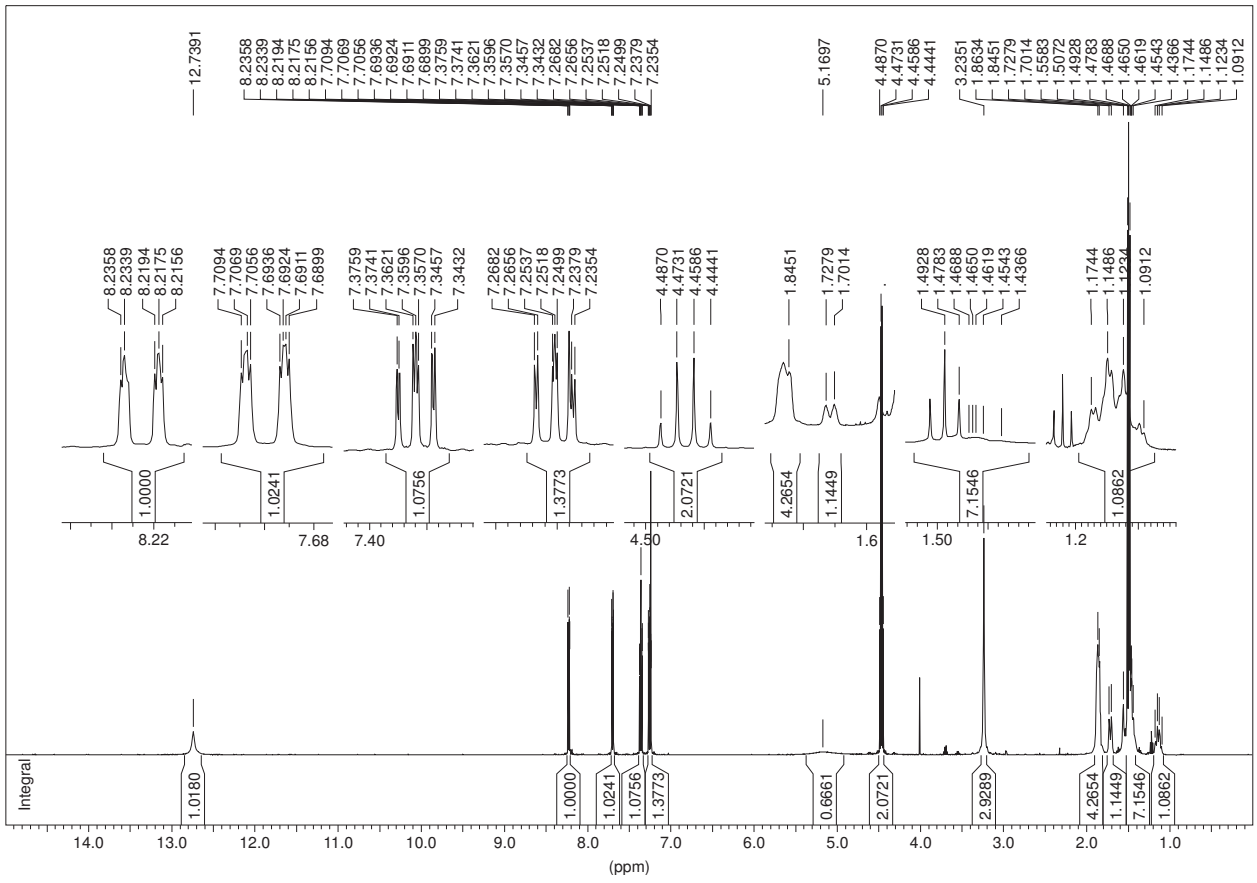


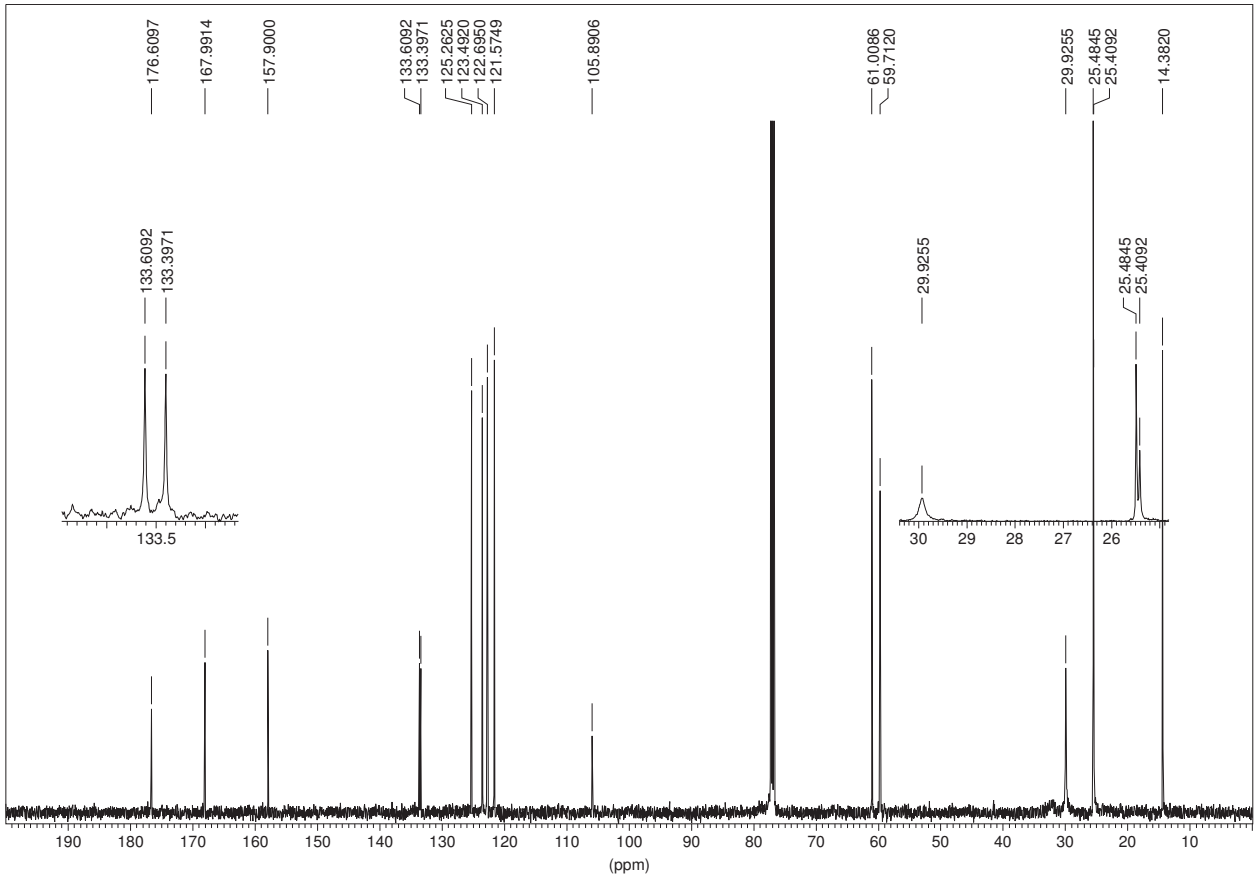
Ethyl 2-(3,3-Diethylthioureido)benzo[*b*]thiophene-3-carboxylate (**98**)



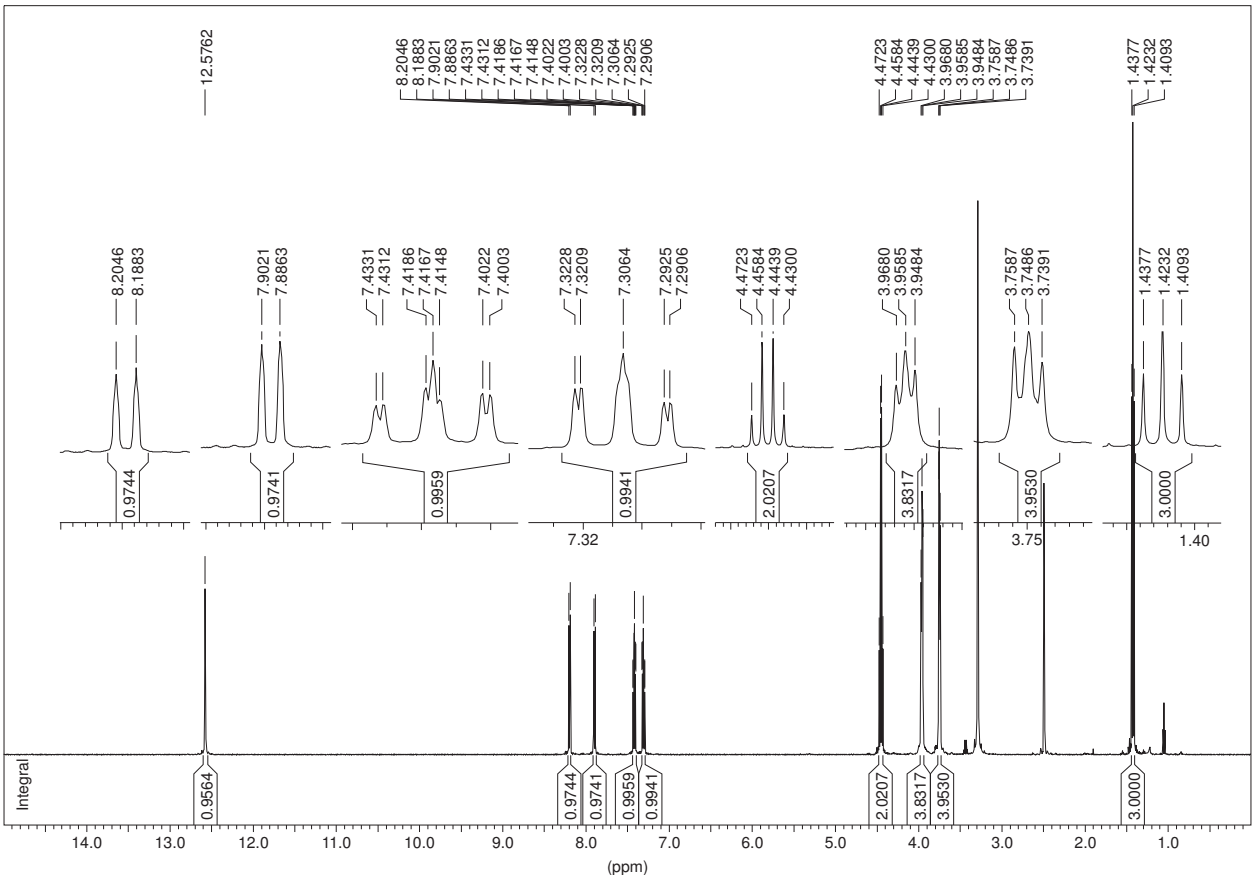


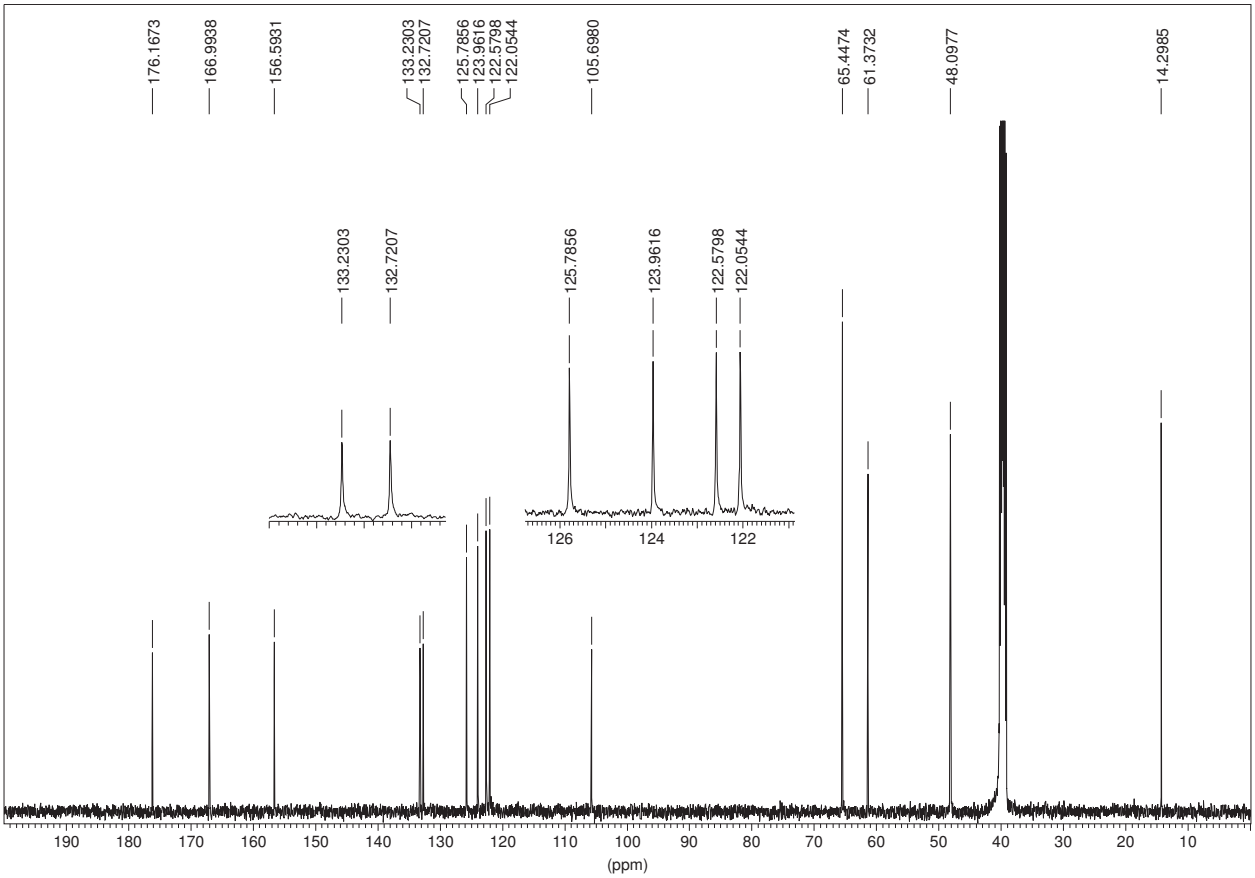
Ethyl 2-(3-Cyclohexyl-3-methylthioureido)benzo[*b*]thiophene-3-carboxylate (**99**)



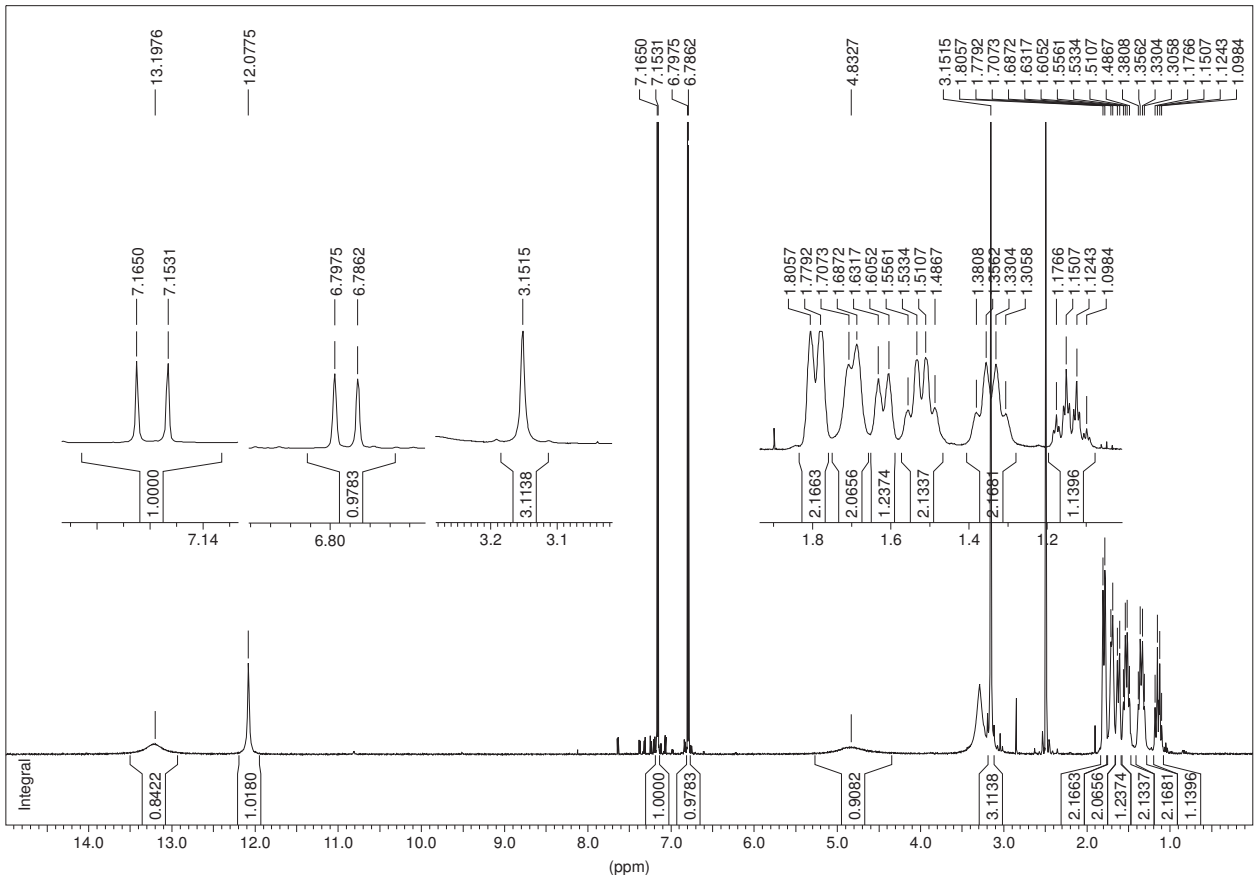


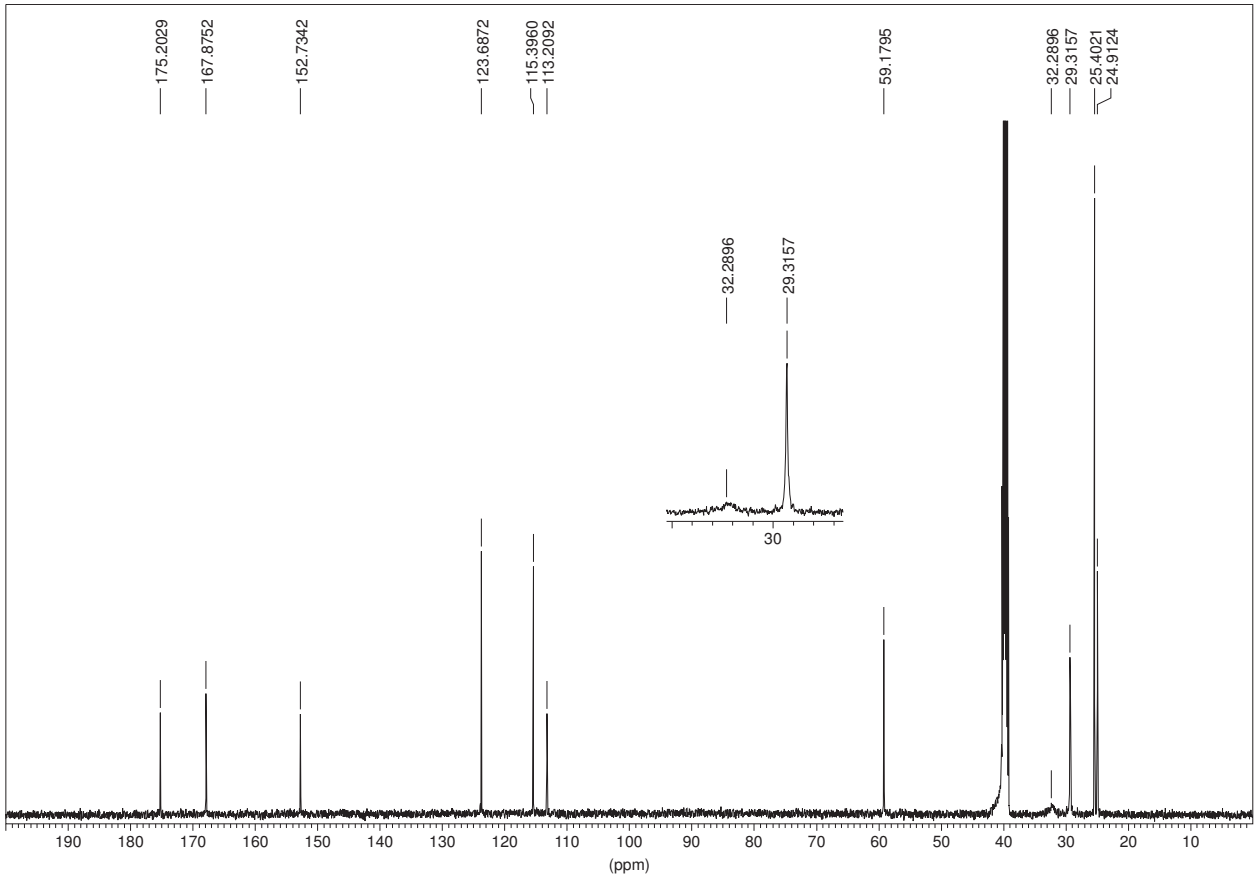
Ethyl 2-[(4-Morpholinylthiocarbonyl)amino]benzo[*b*]thiophene-3-carboxylate (**100**)



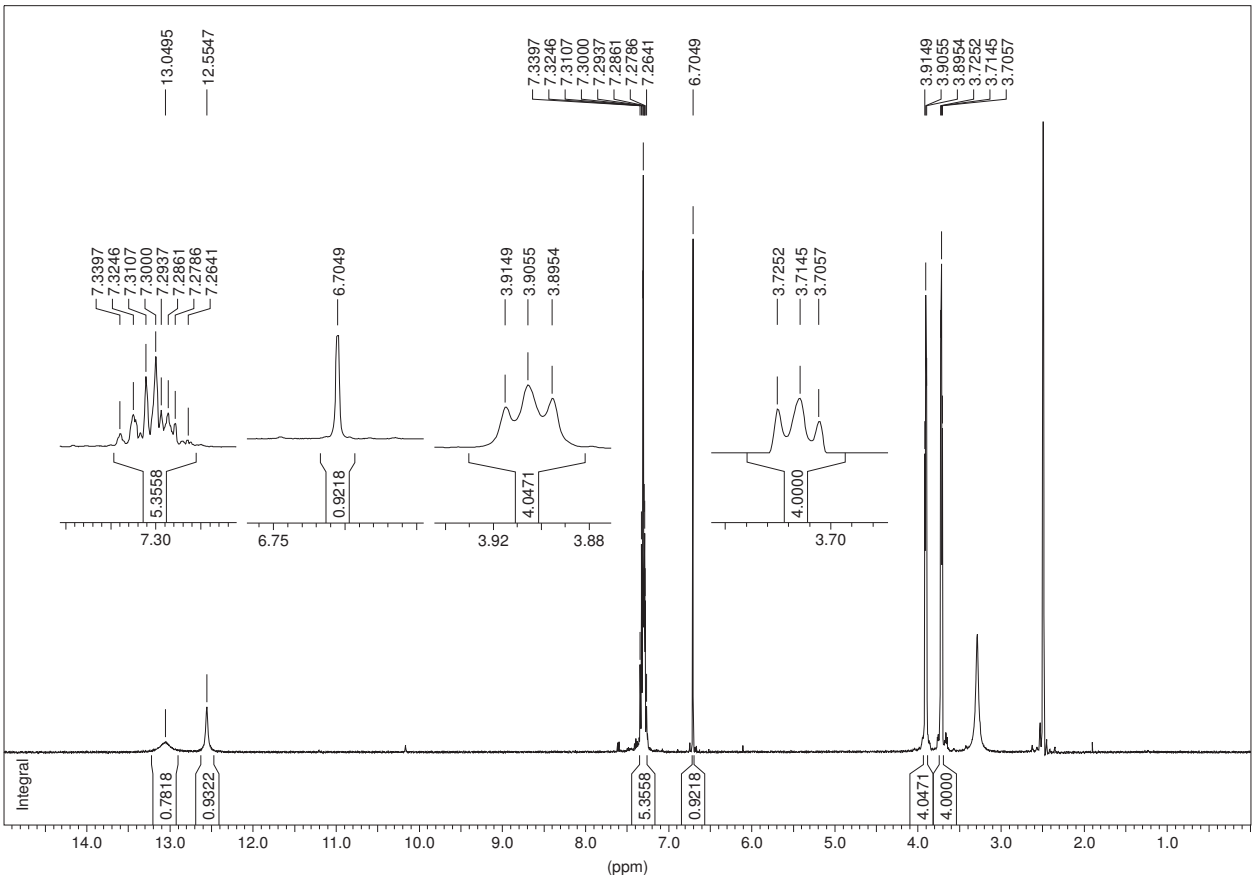


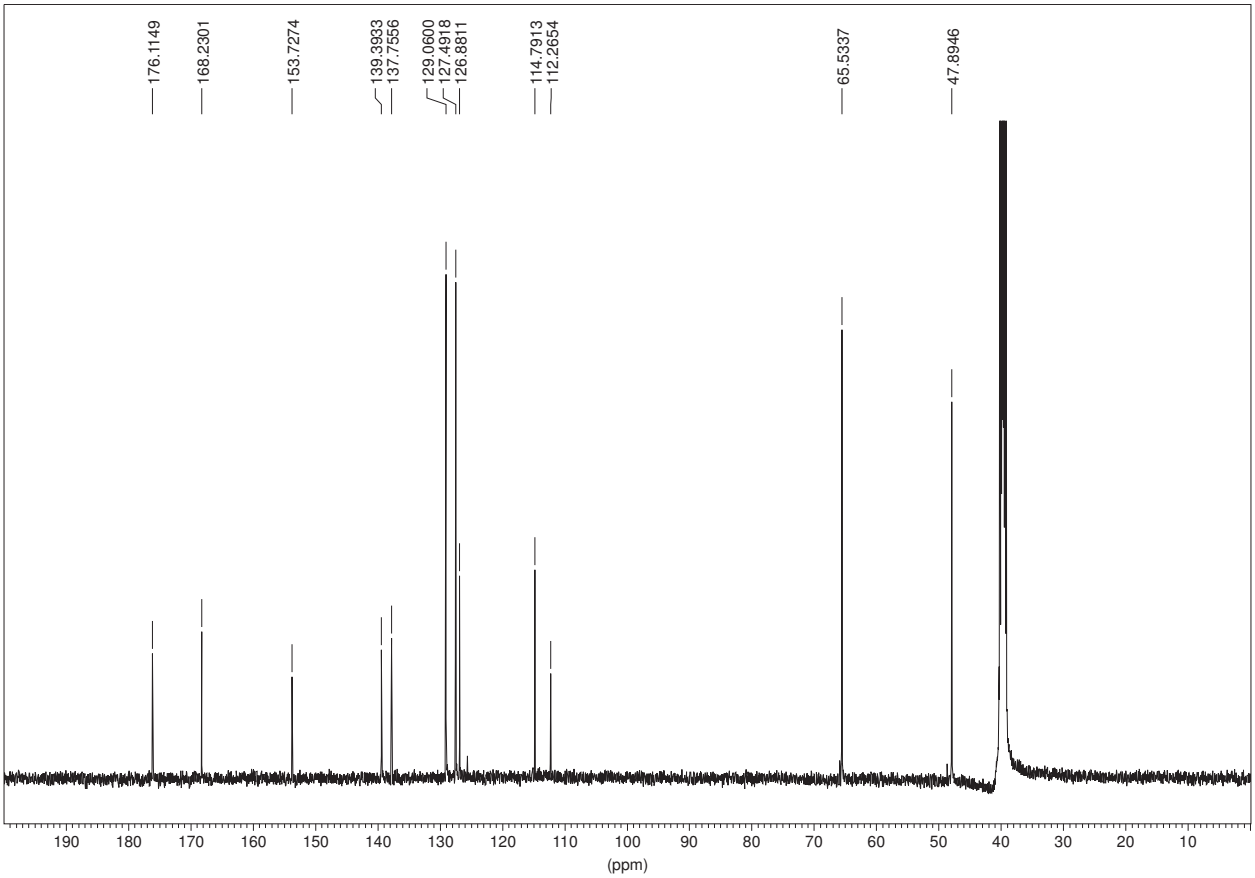
2-(3-Cyclohexyl-3-methylthioureido)thiophene-3-carboxylic Acid (**103**)



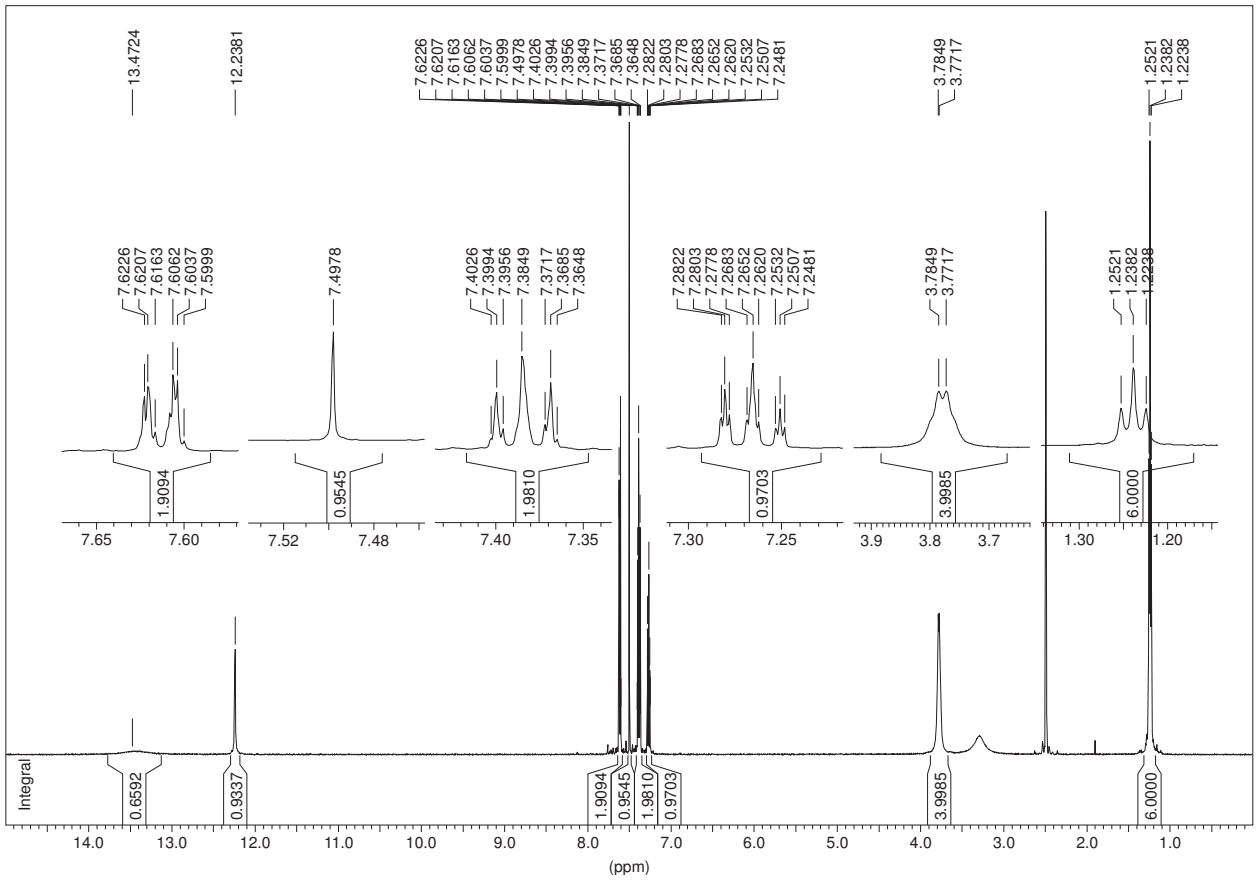


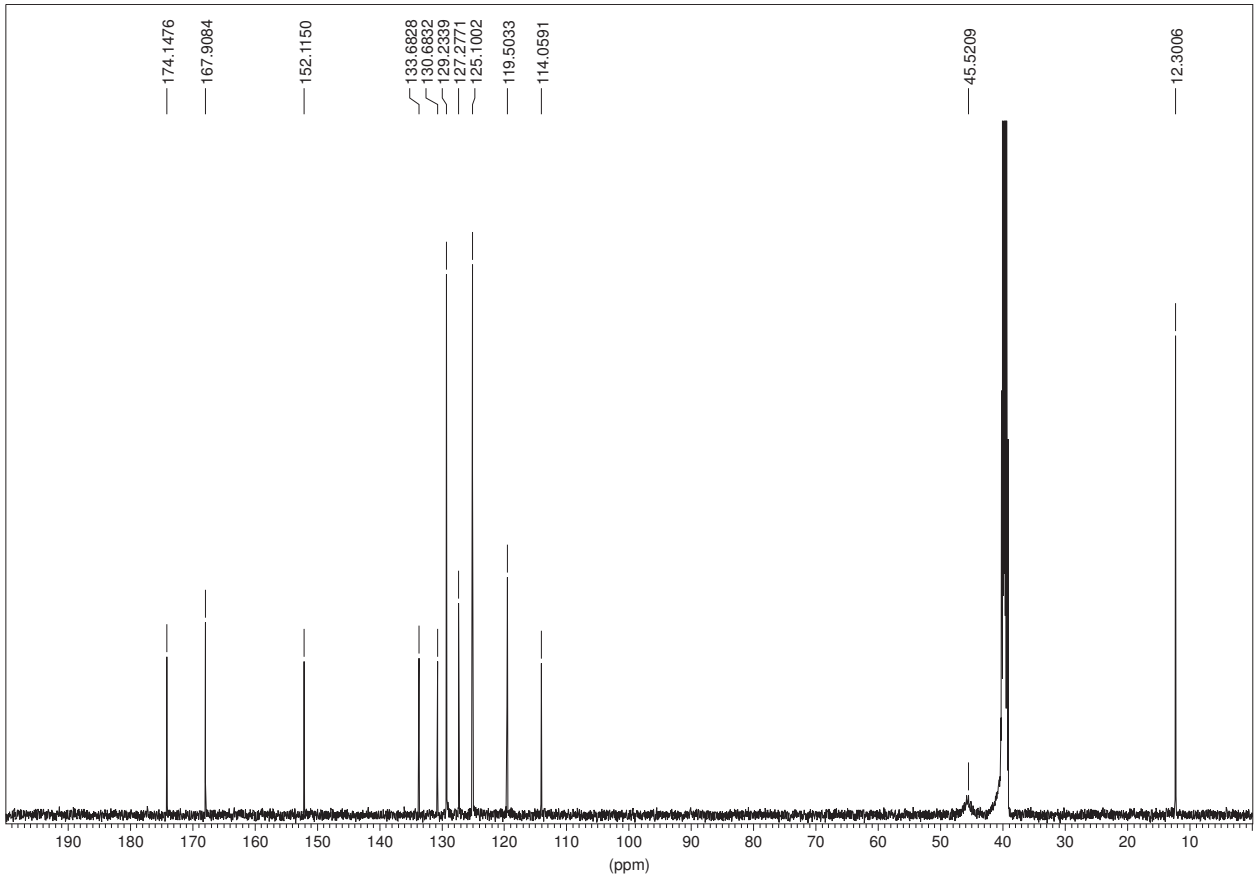
2-[(4-Morpholinylthiocarbonyl)amino]-4-phenylthiophene-3-carboxylic Acid (**105**)



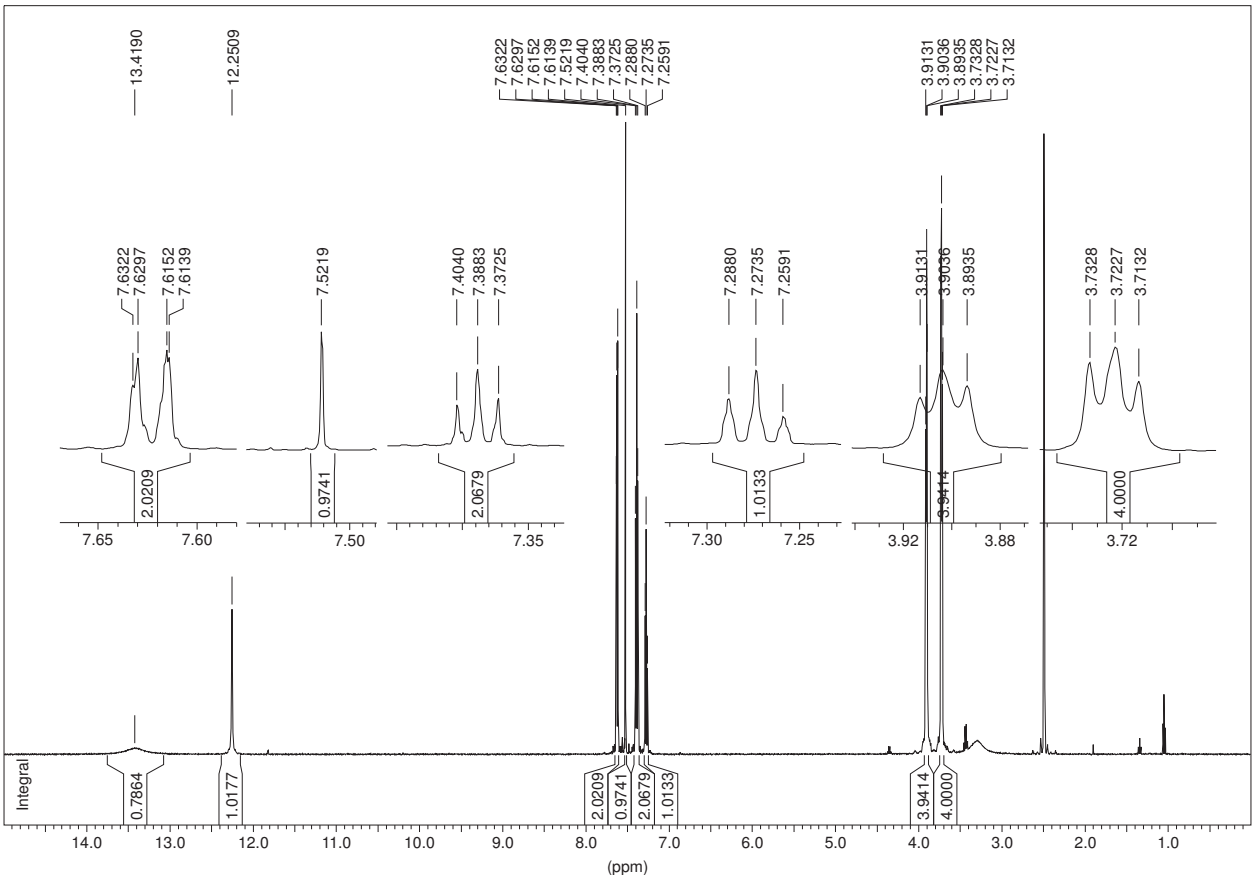


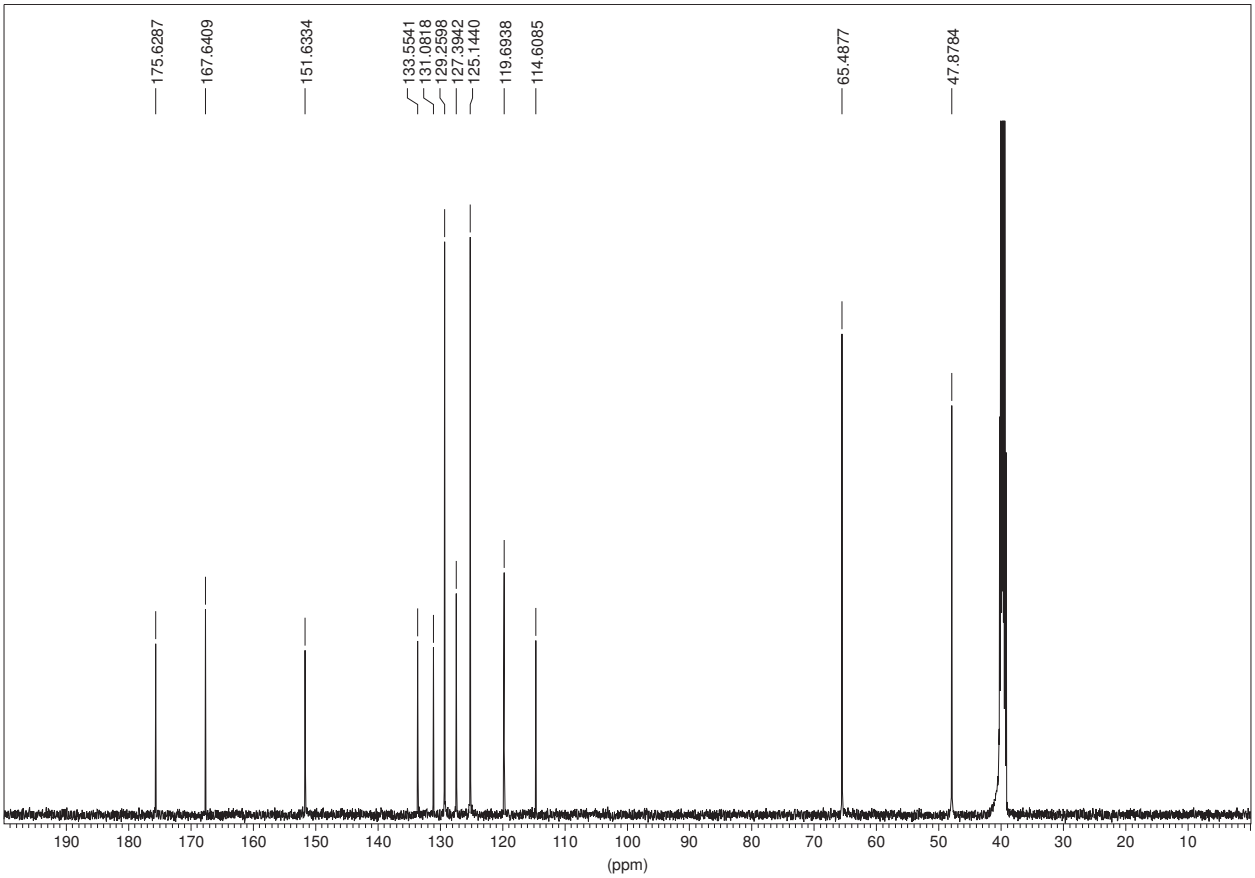
2-(3,3-Diethylthioureido)-5-phenylthiophene-3-carboxylic Acid (**106**)



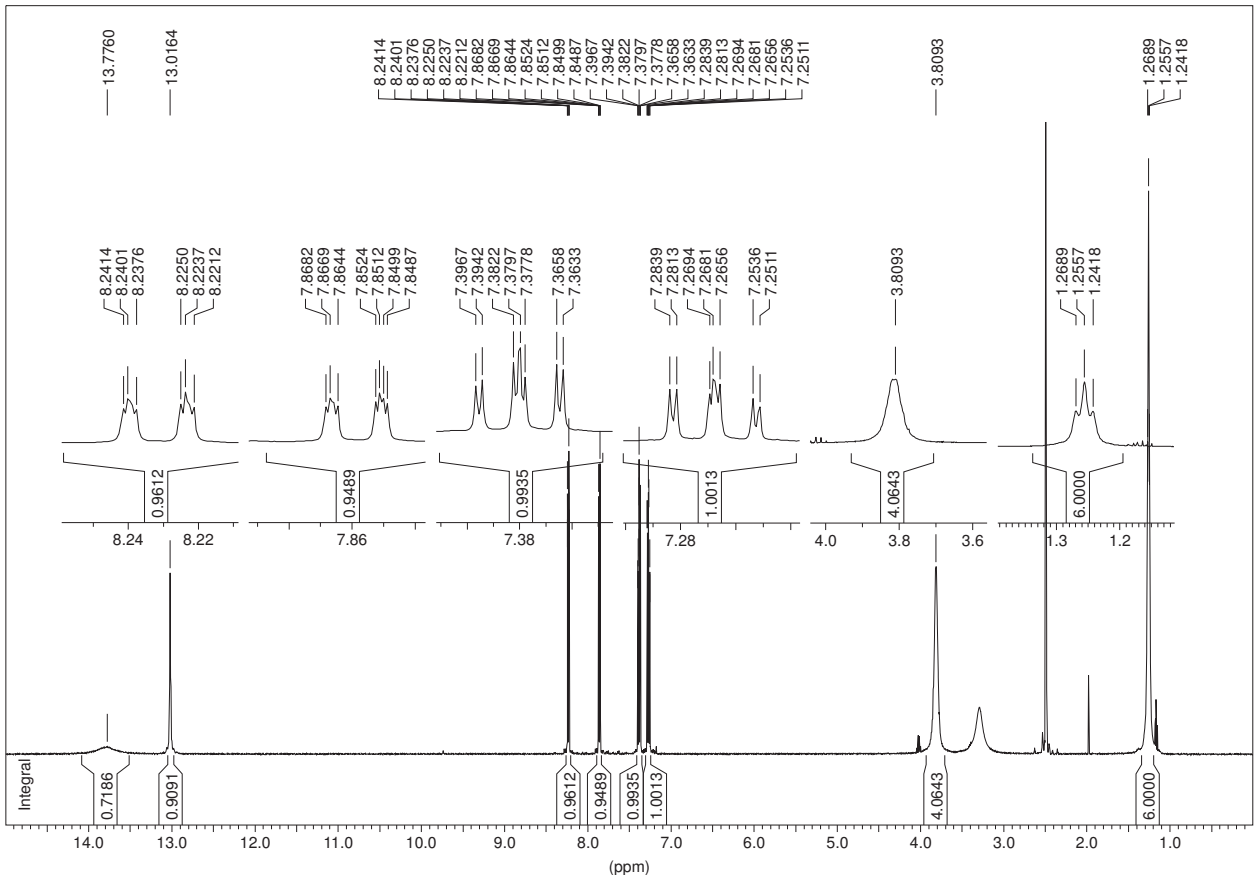


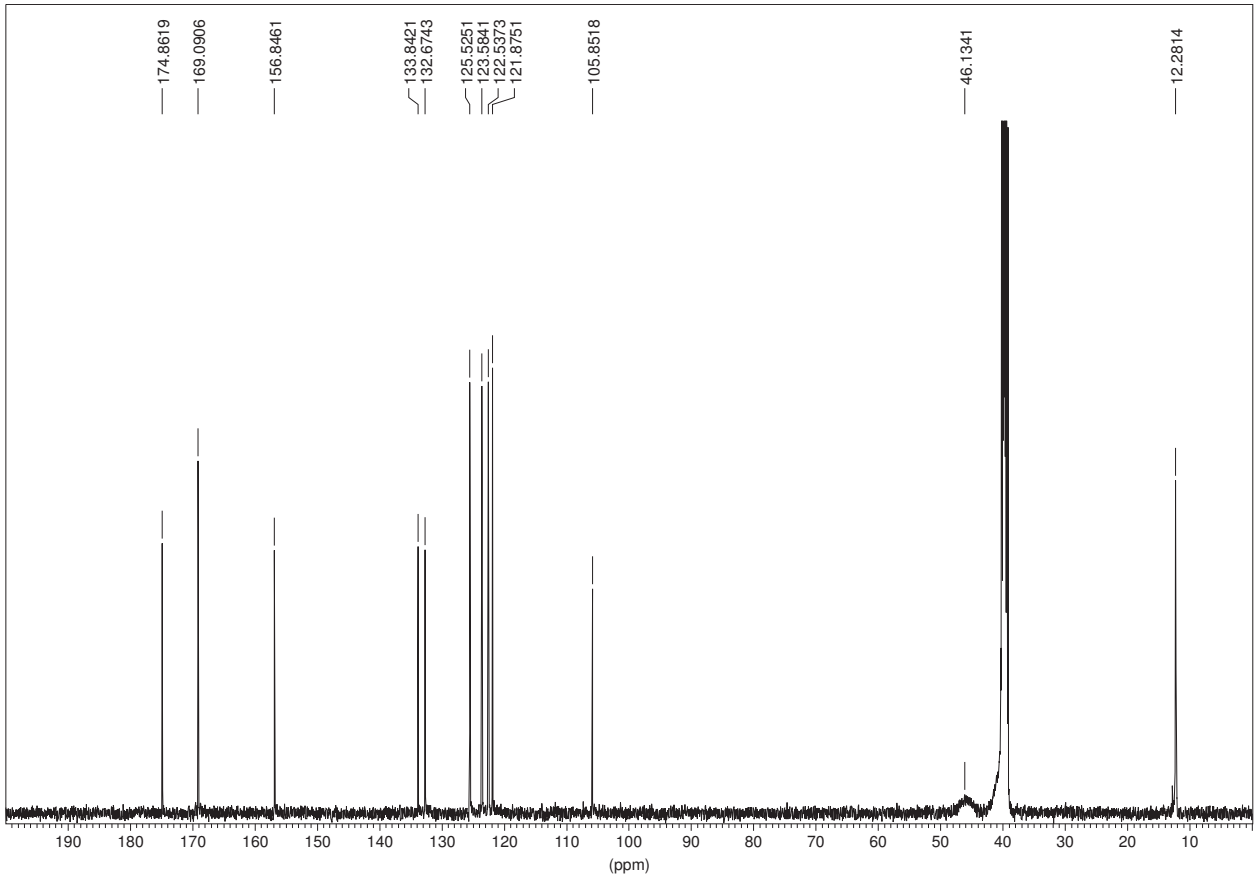
2-[(4-Morpholinylthiocarbonyl)amino]-5-phenylthiophene-3-carboxylic Acid (**107**)



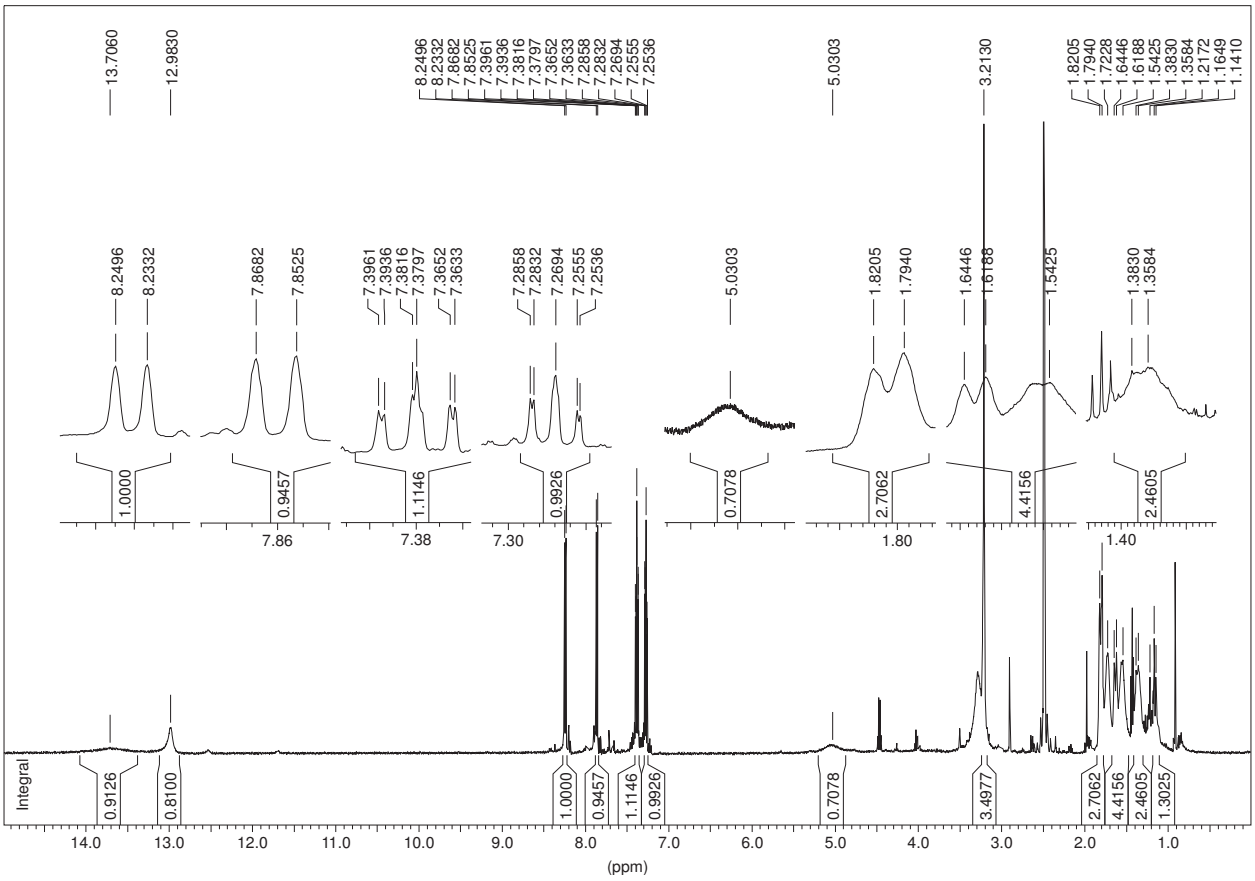


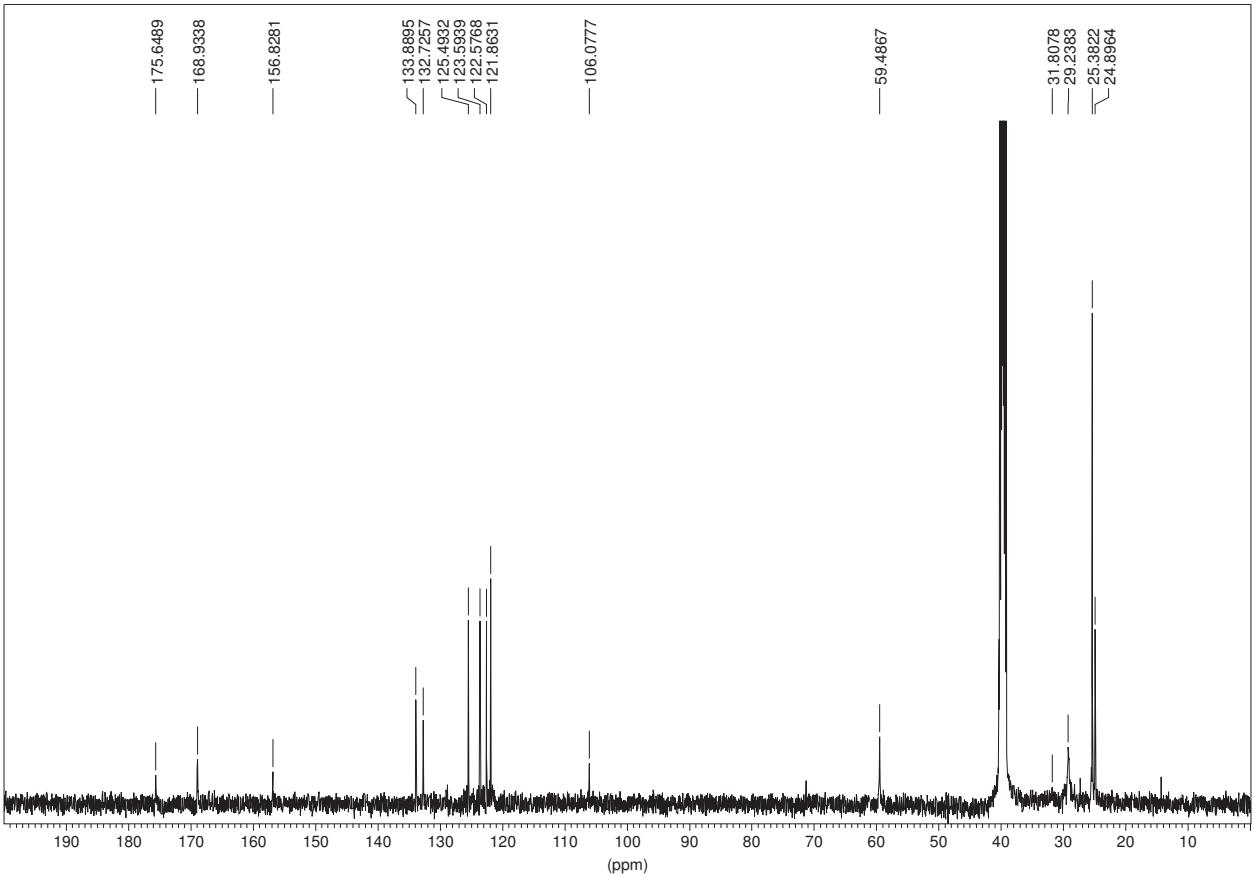
2-(3,3-Diethylthioureido)benzo[*b*]thiophene-3-carboxylic Acid (**120**)



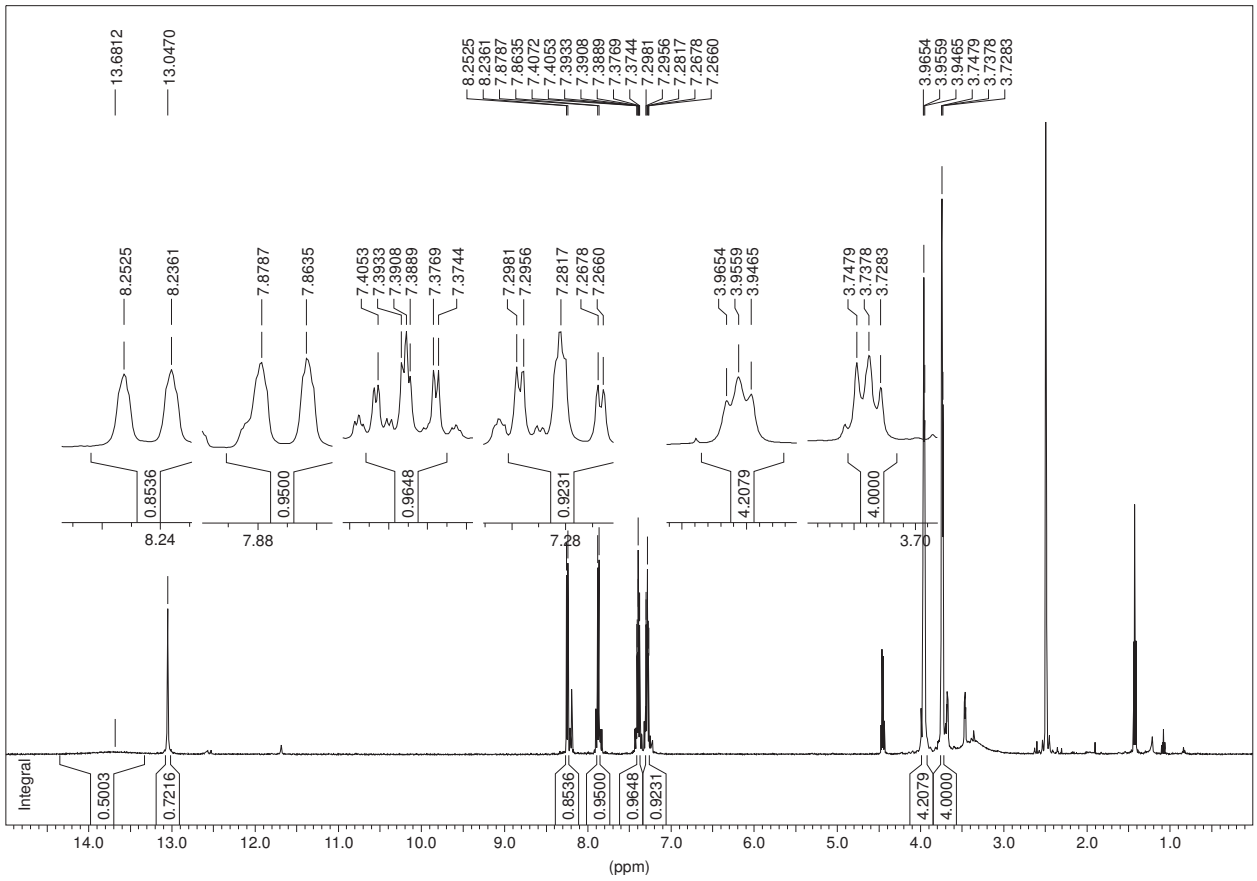


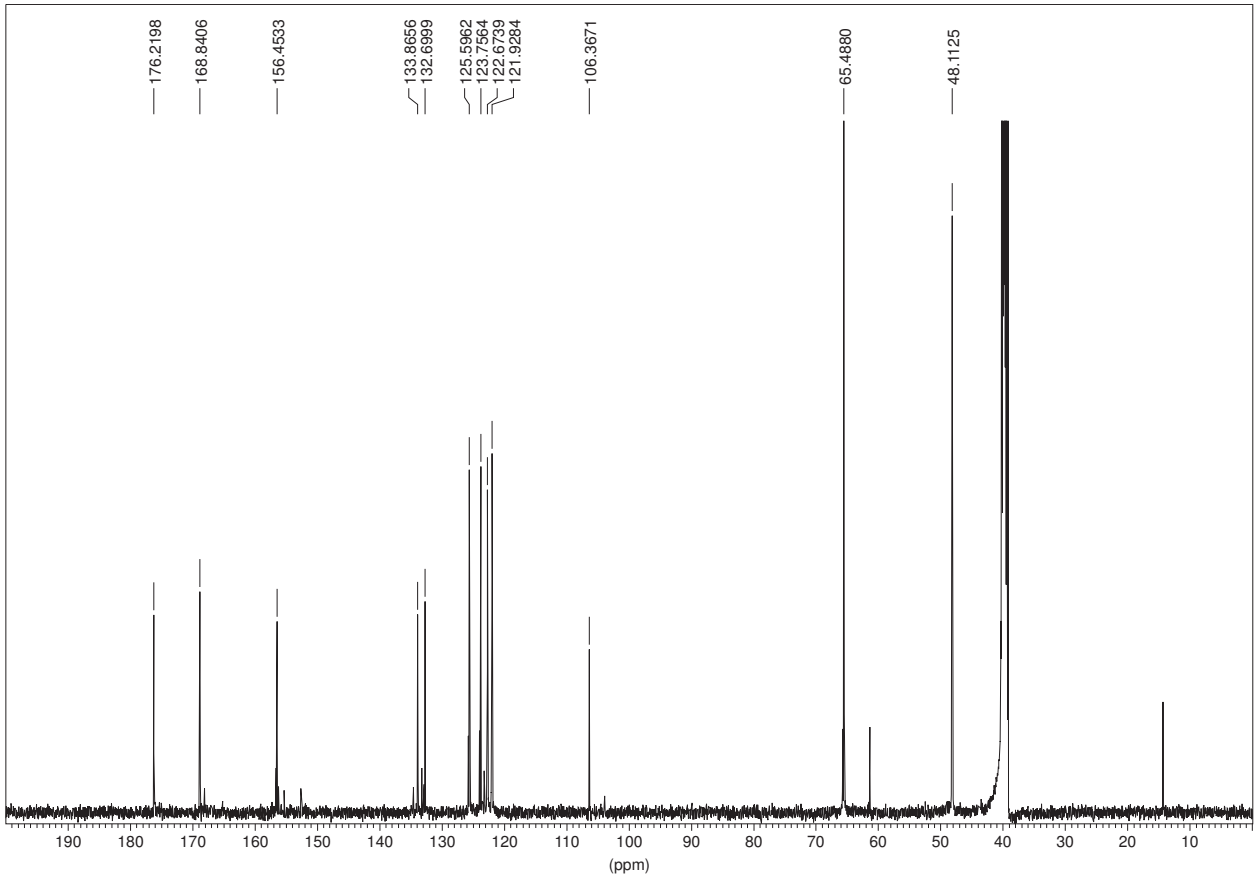
2-(3-Cyclohexyl-3-methylthioureido)benzo[*b*]thiophene-3-carboxylic (**121**)



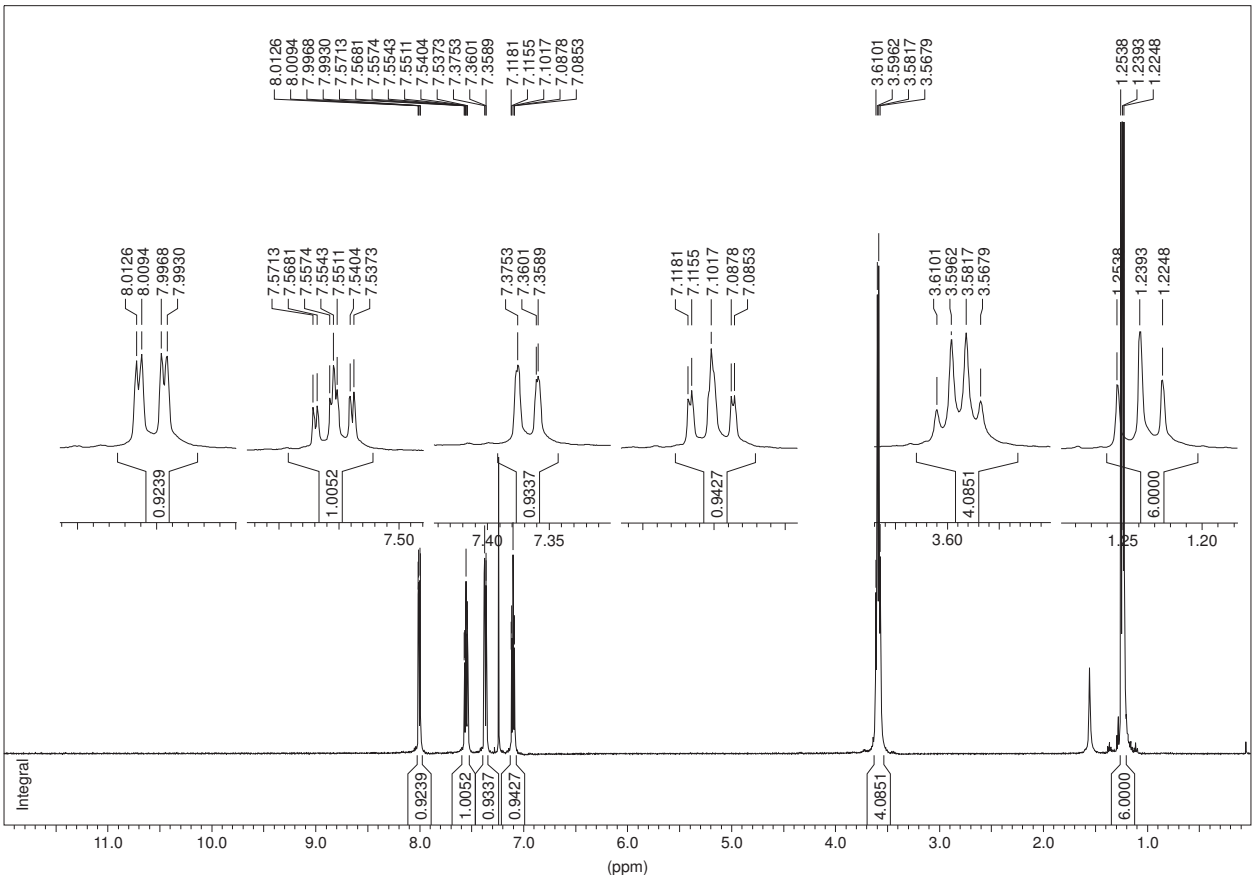


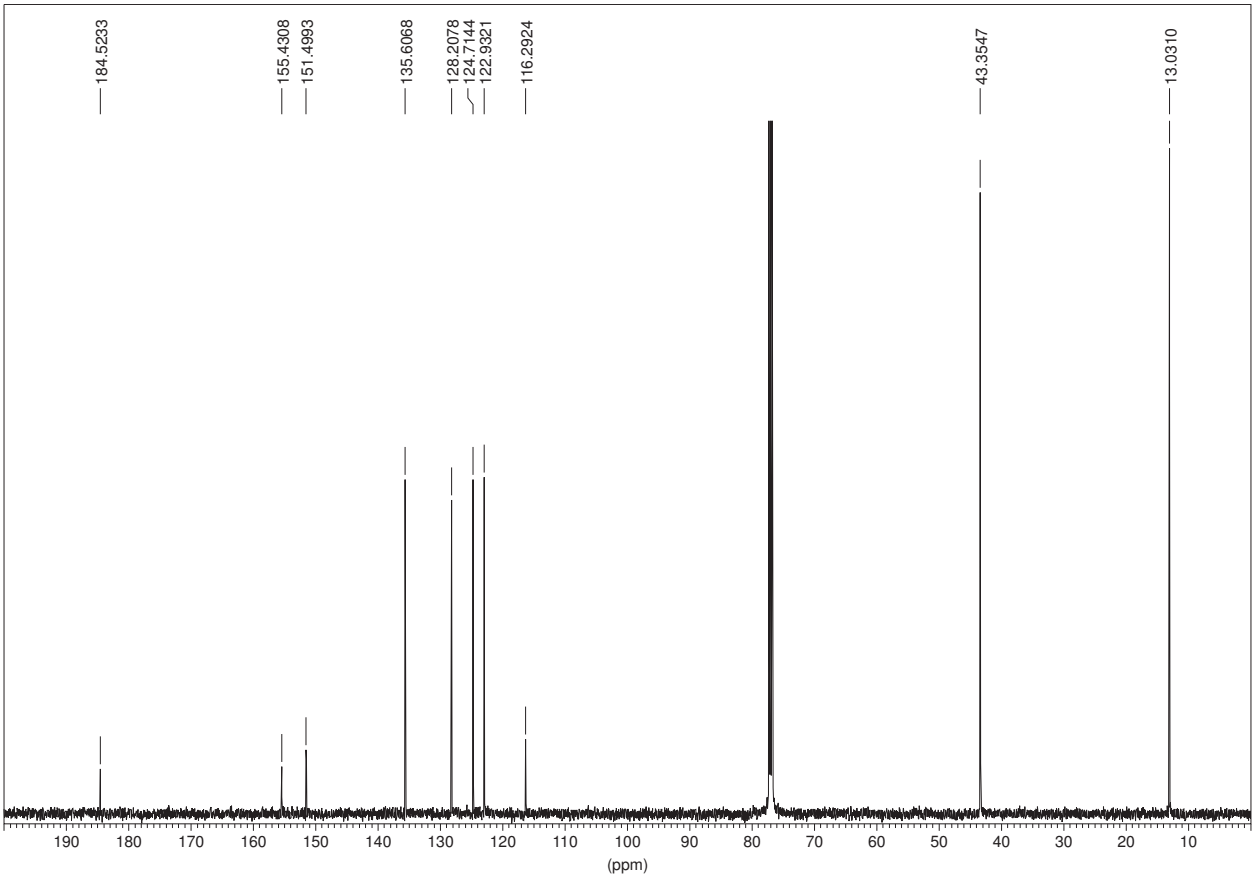
2-[(4-Morpholinylthiocarbonyl)amino]benzo[*b*]thiophene-3-carboxylic Acid (**122**)



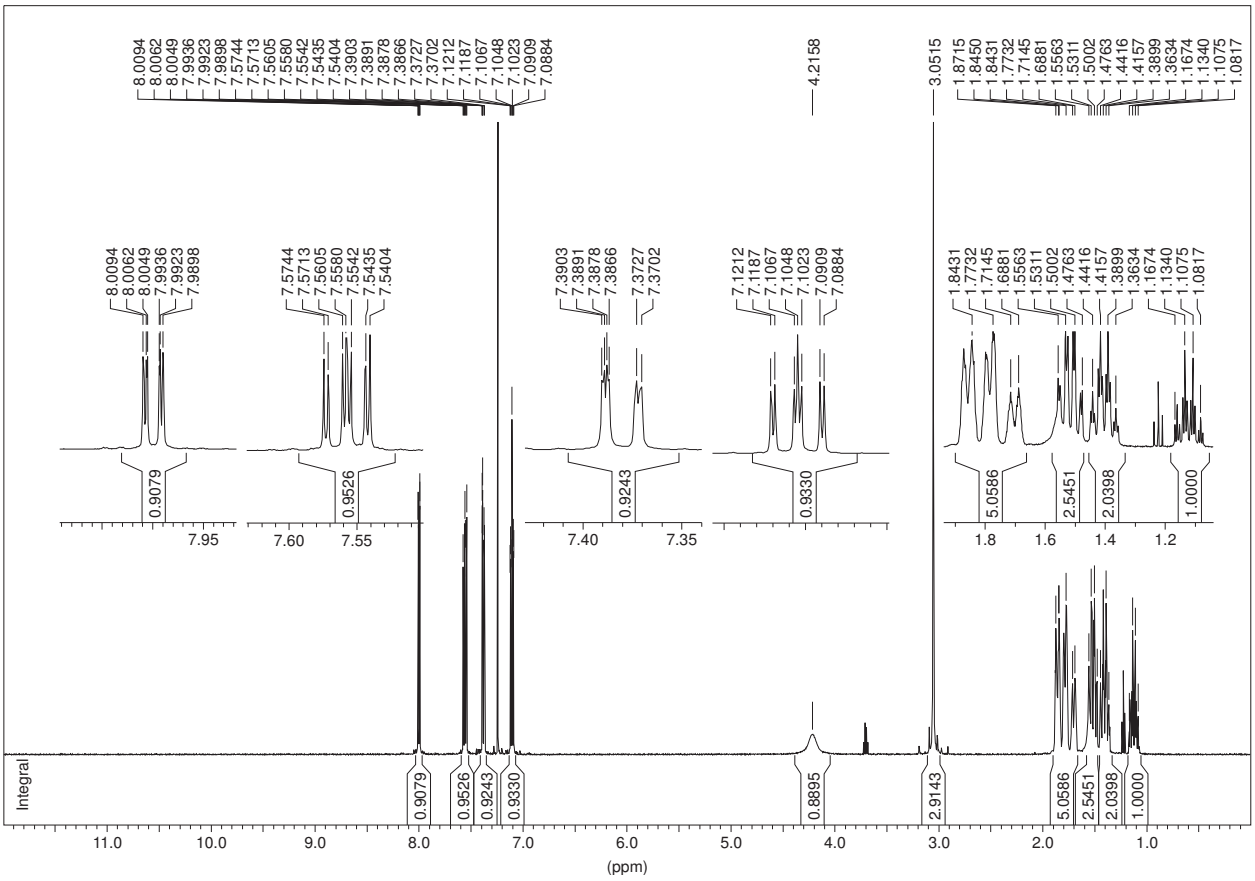


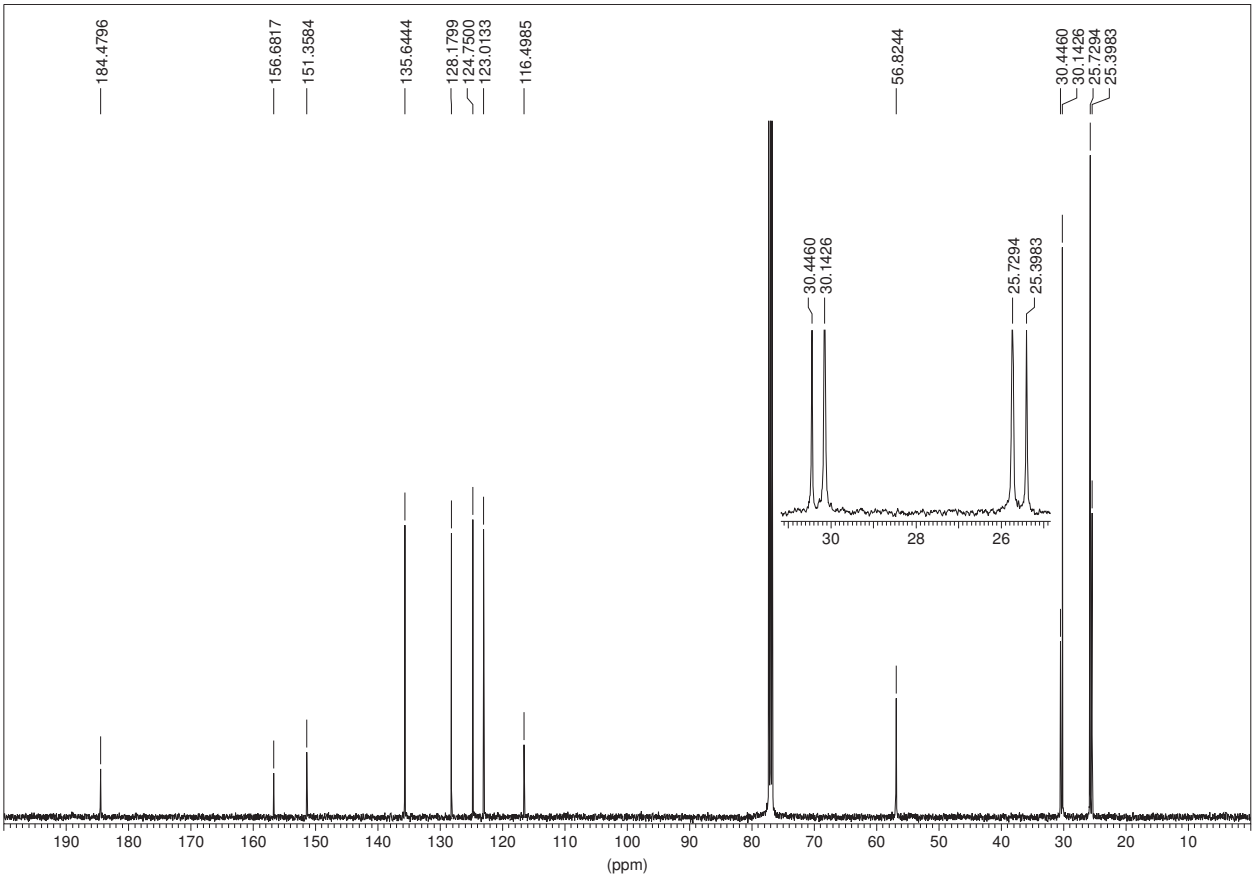
2-(Diethylamino)-4*H*-3,1-benzothiazin-4-one (**123**)



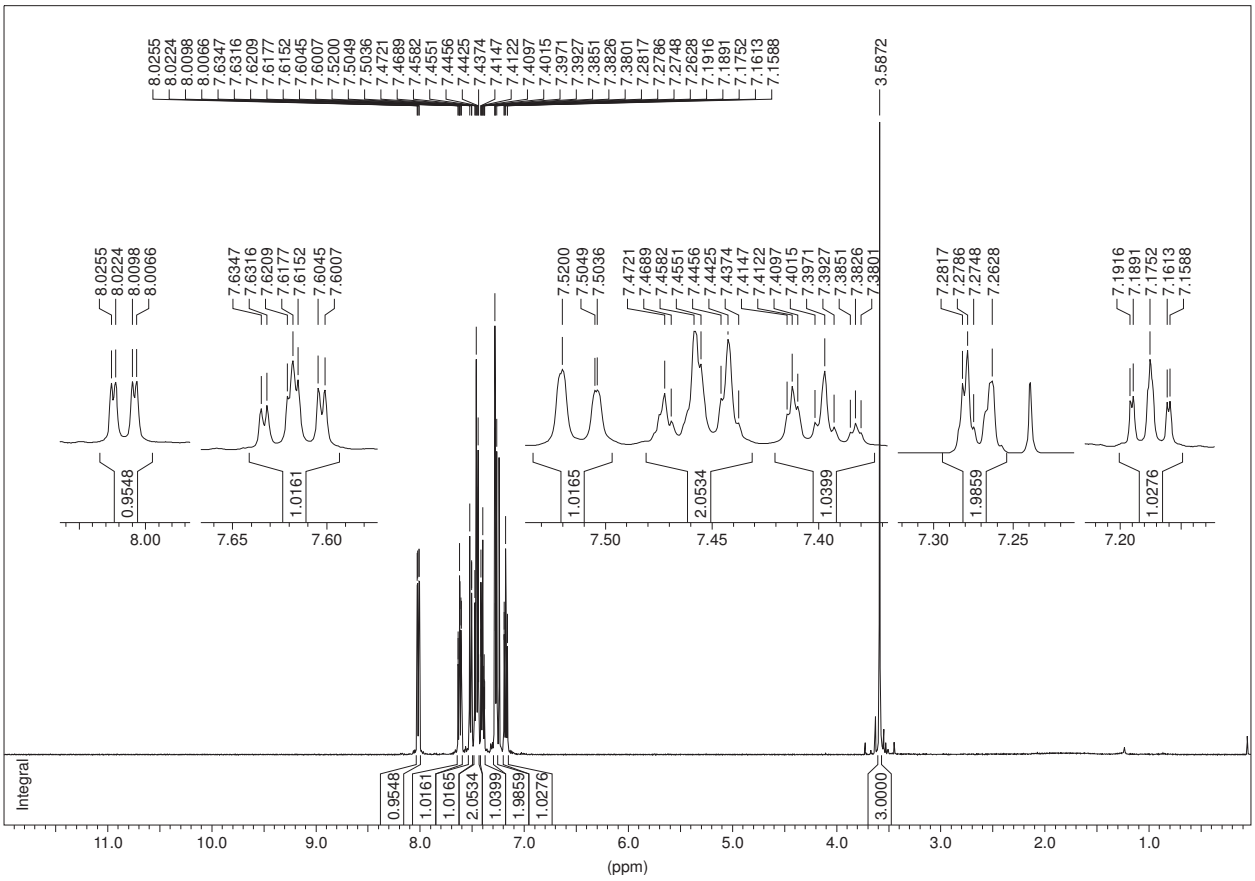


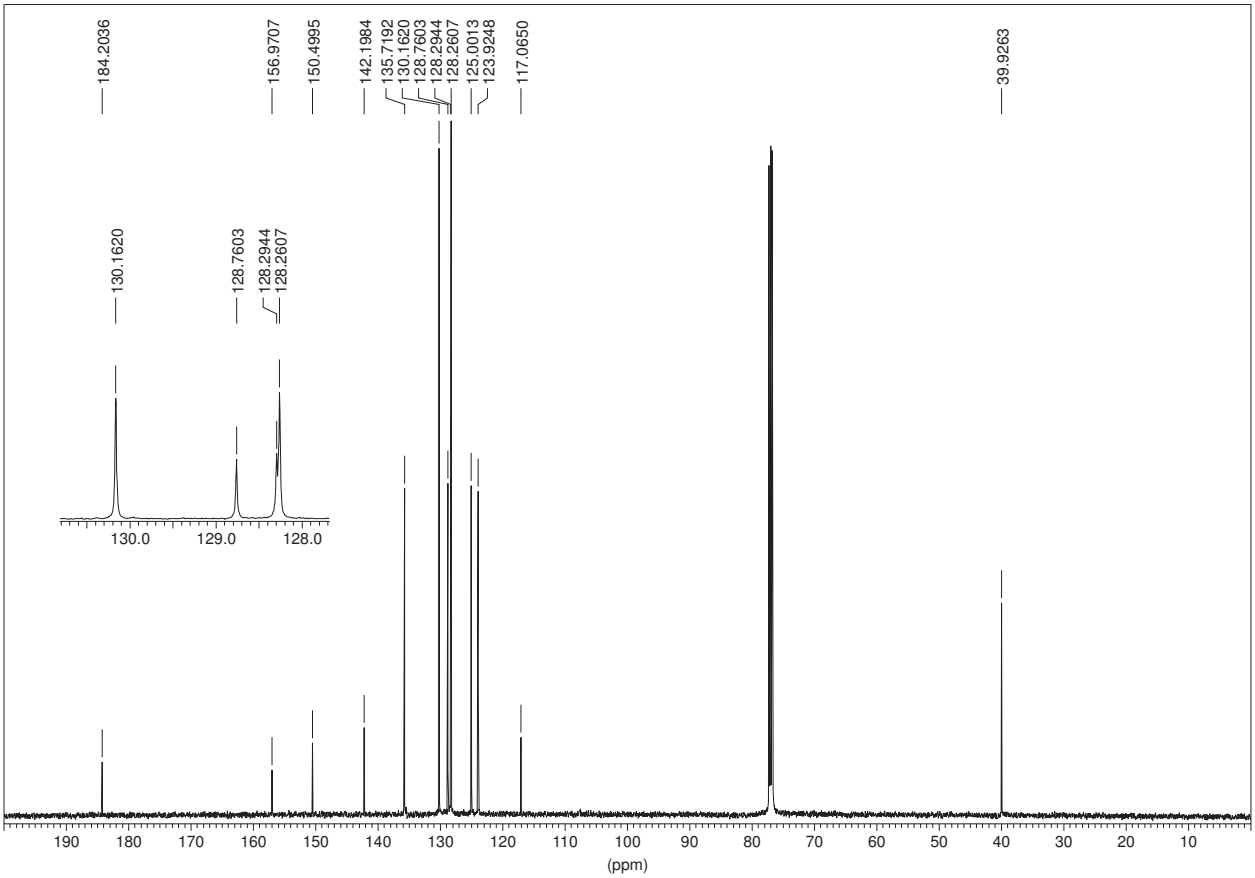
2-(*N*-Cyclohexyl-*N*-methylamino)-4*H*-3,1-benzothiazin-4-one (**124**)



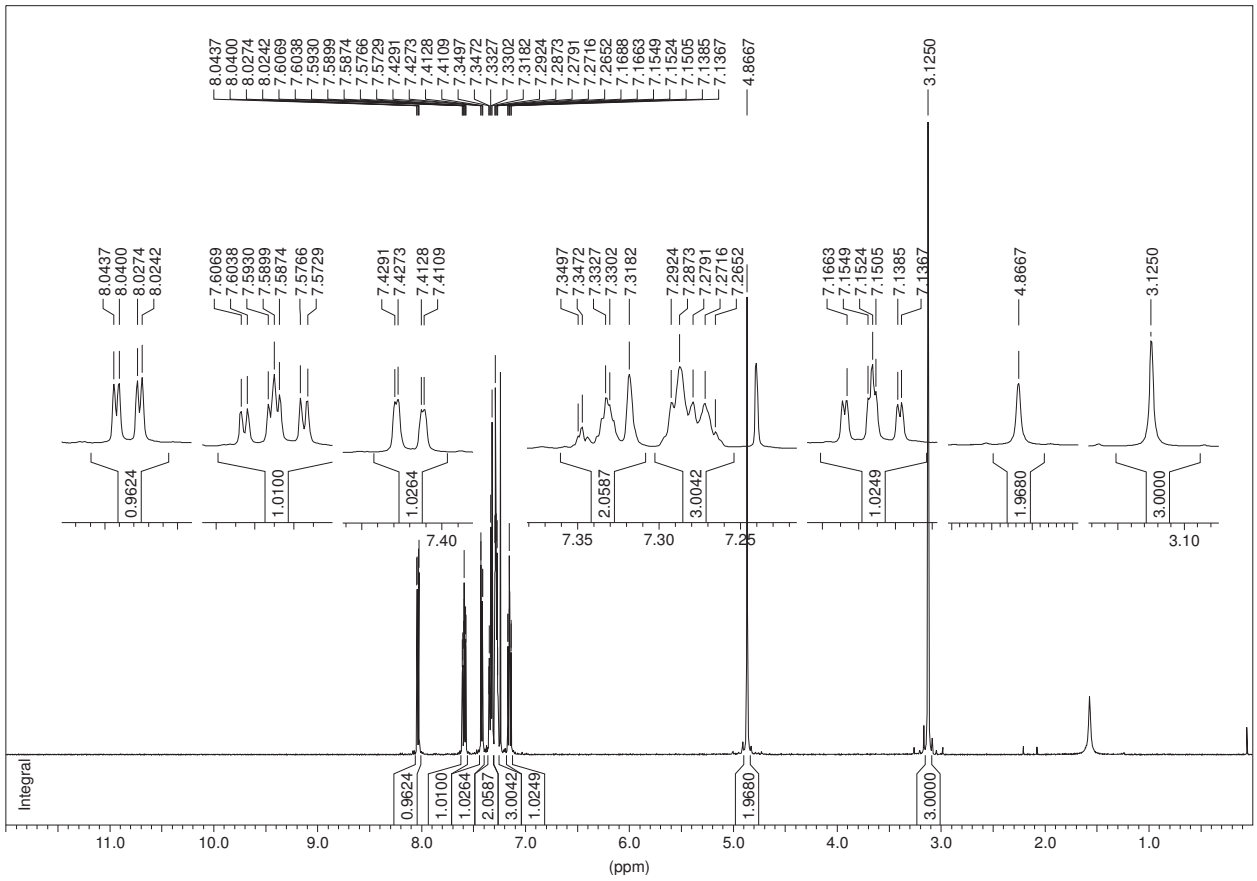


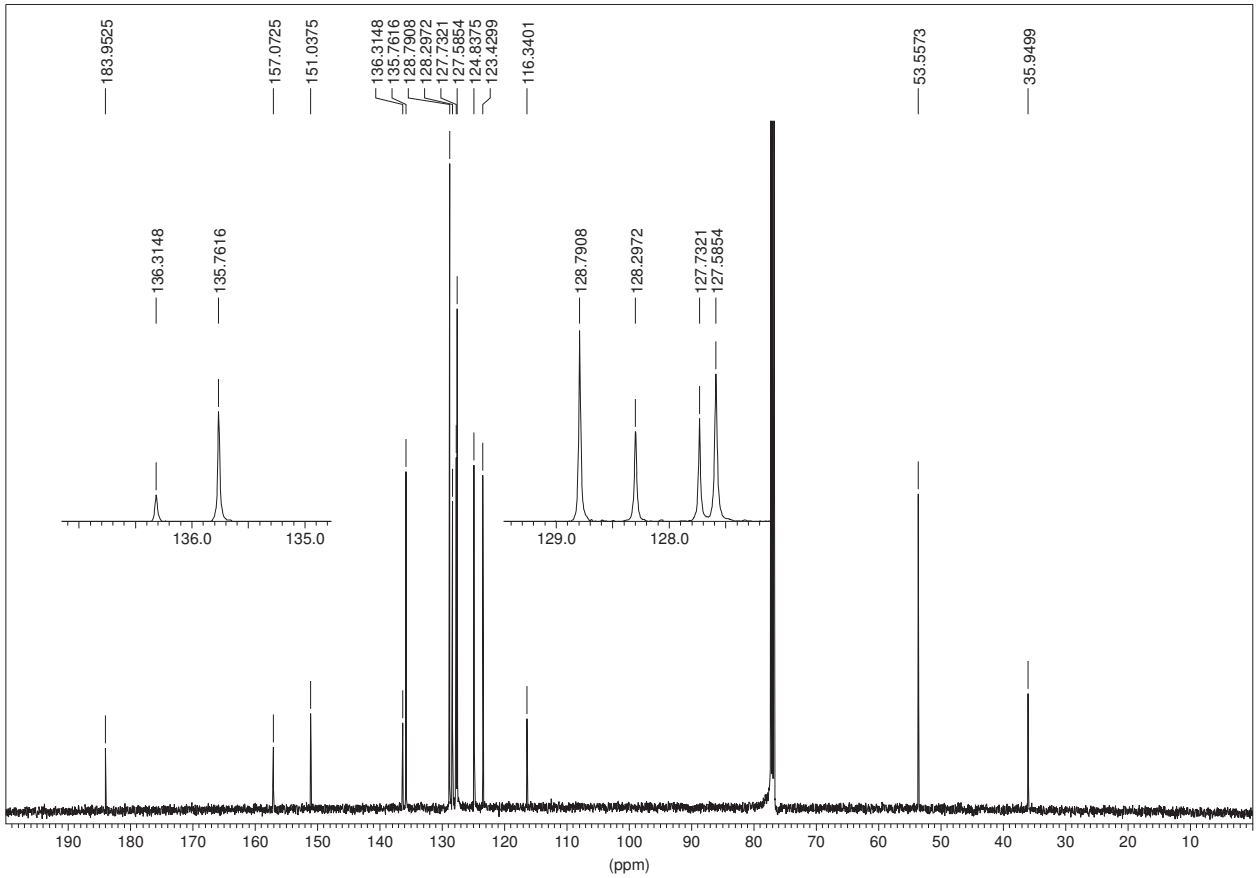
2-(*N*-Methyl-*N*-phenylamino)-4*H*-3,1-benzothiazin-4-one (**125**)



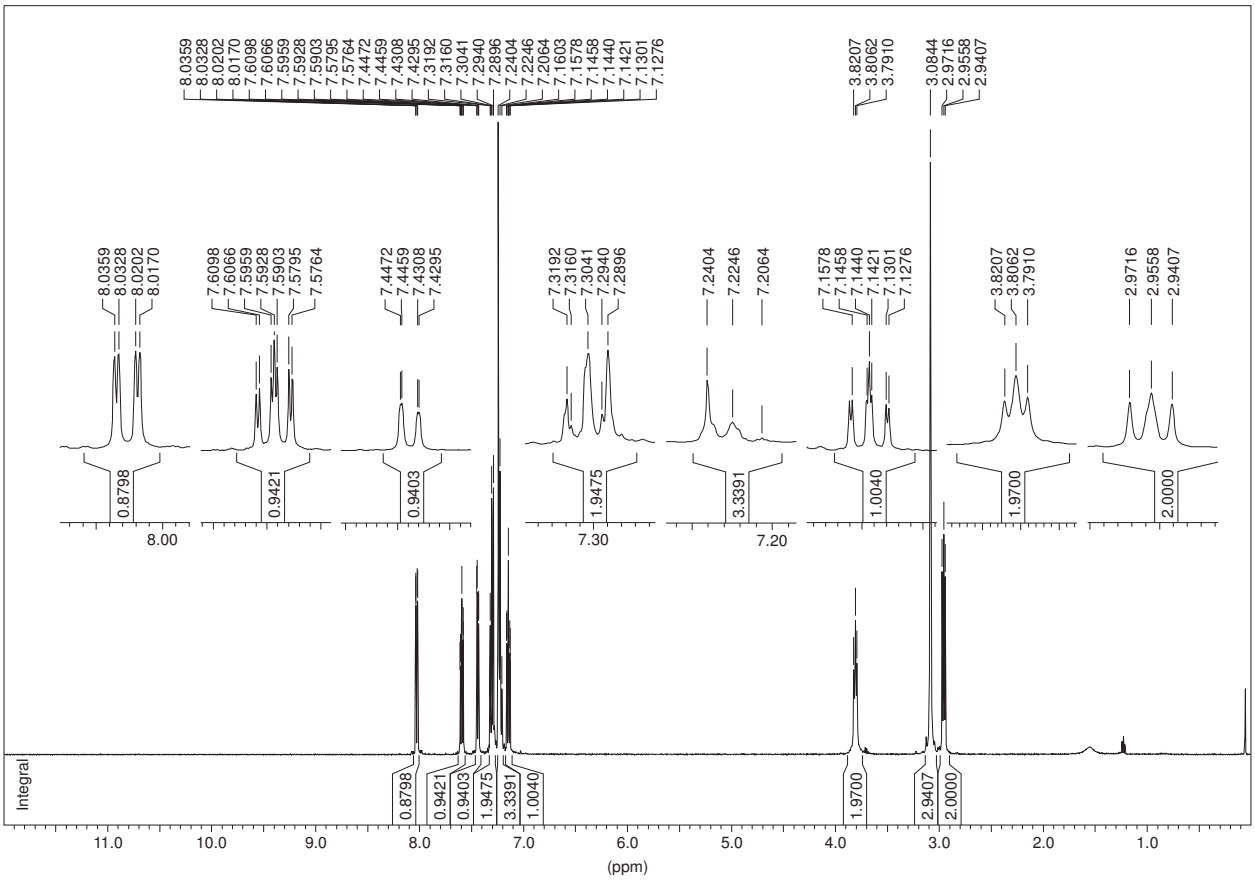


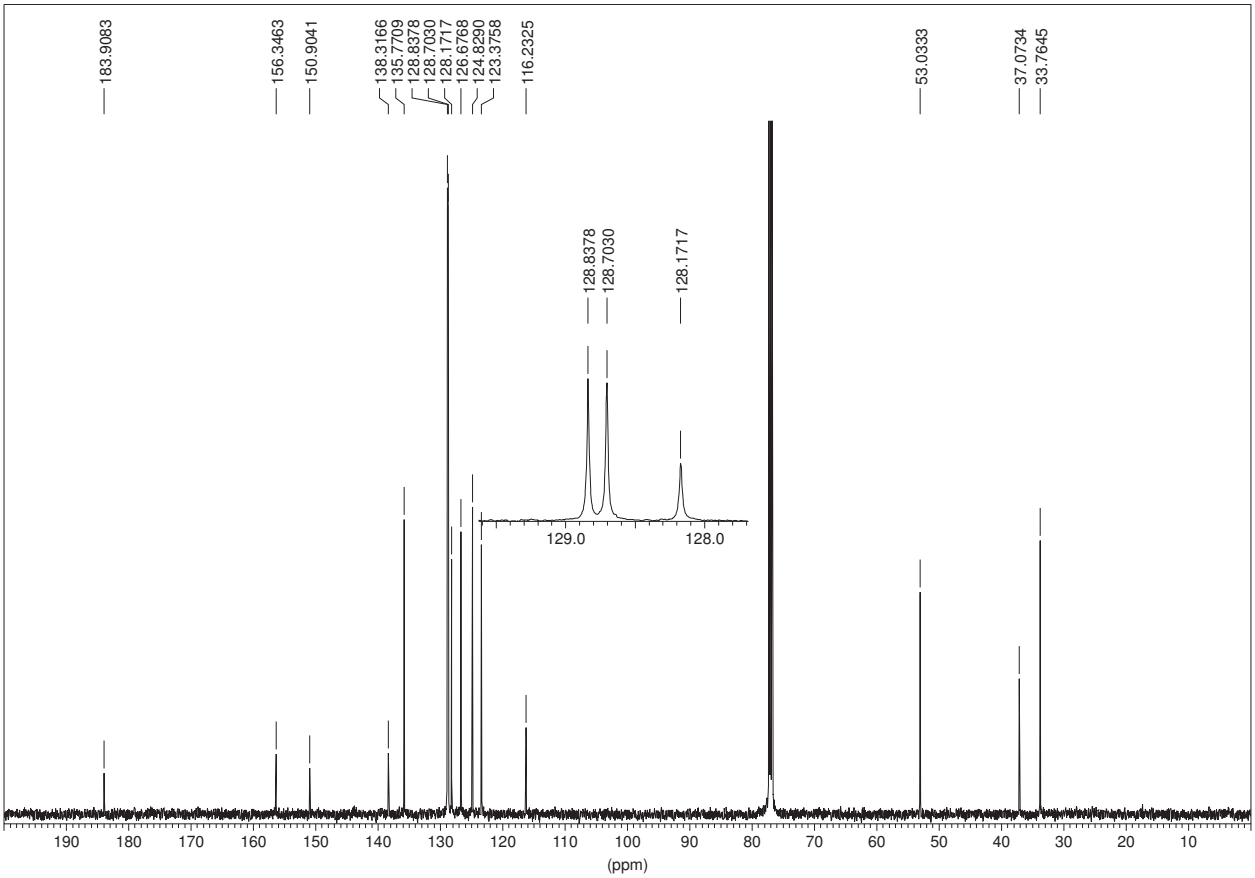
2-(*N*-Benzyl-*N*-methylamino)-4*H*-3,1-benzothiazin-4-one (**126**)



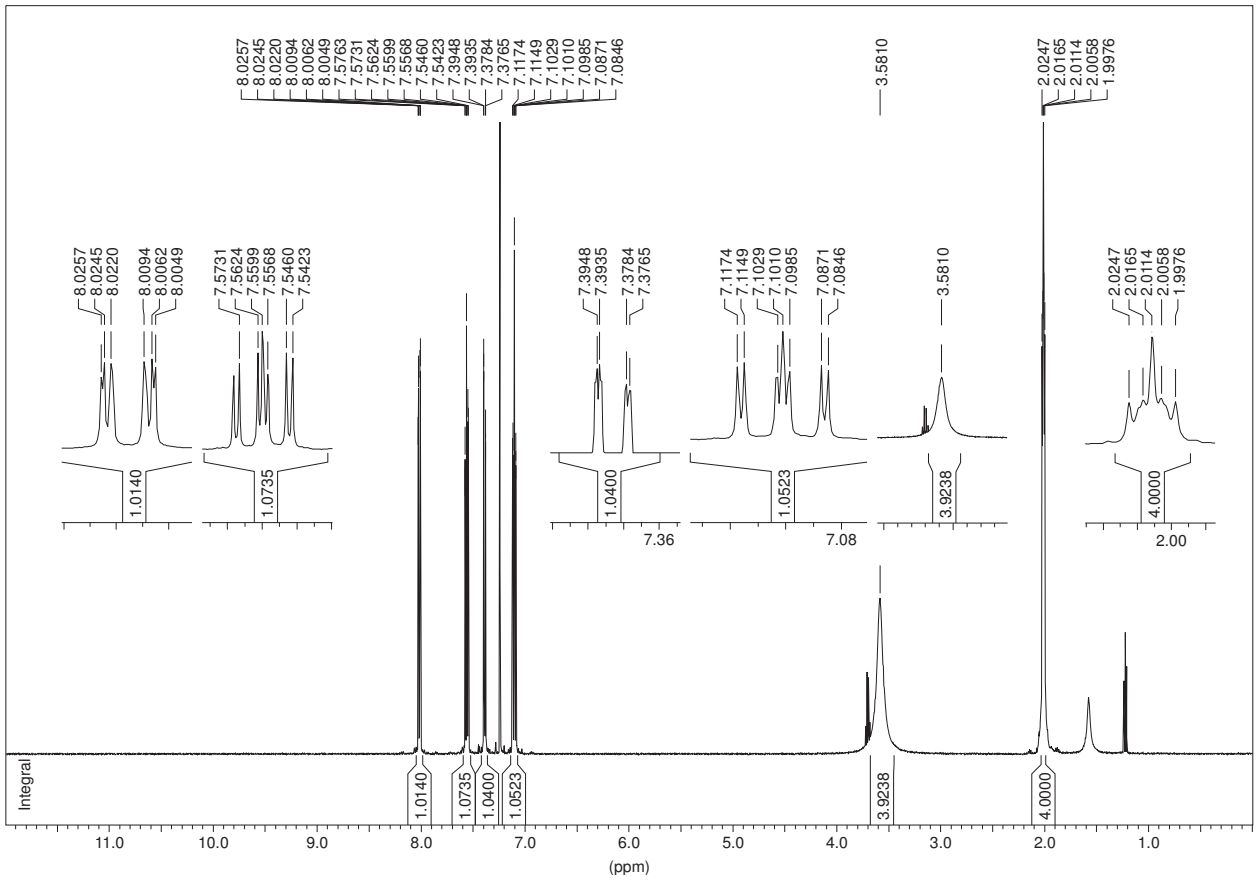


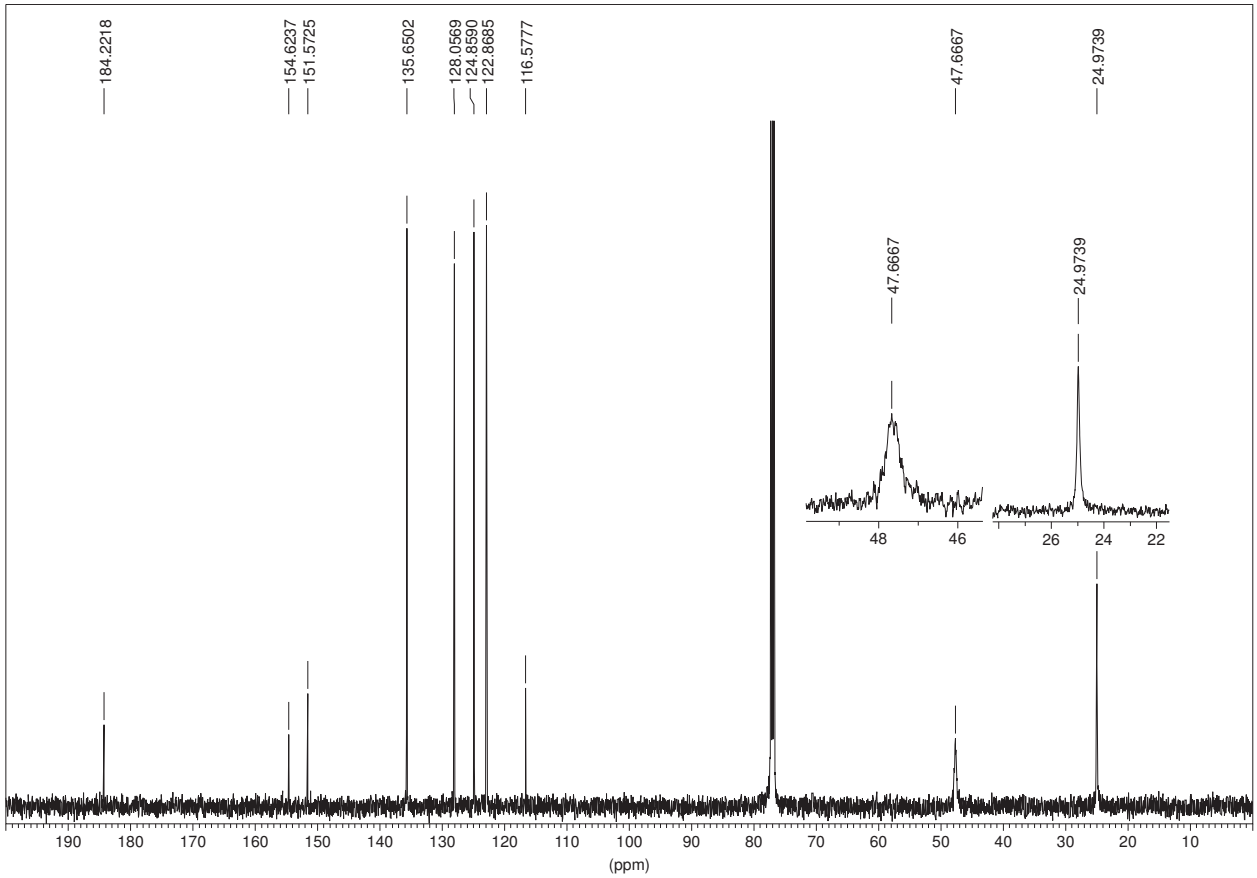
2-[*N*-Methyl-*N*-(2-phenylethyl)amino]-4*H*-3,1-benzothiazin-4-one (**127**)



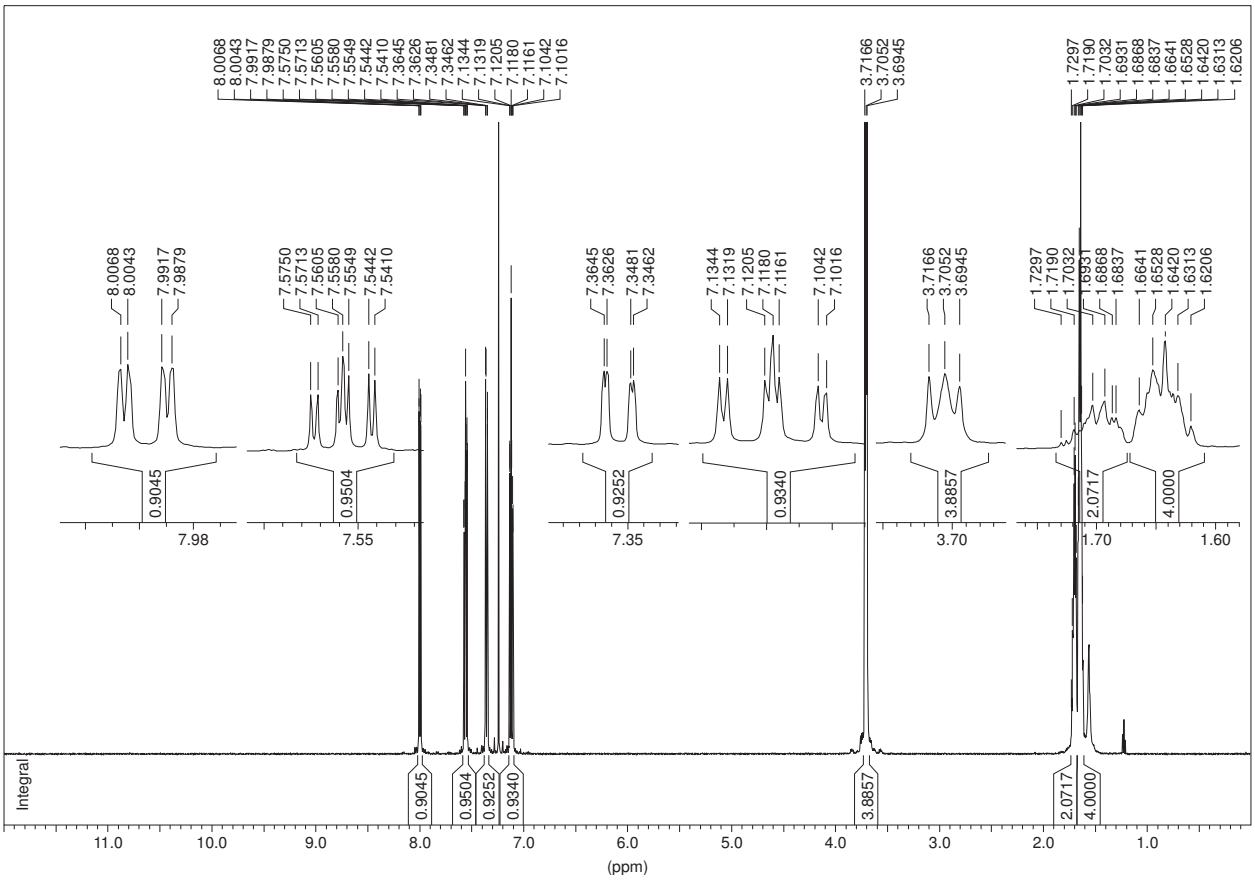


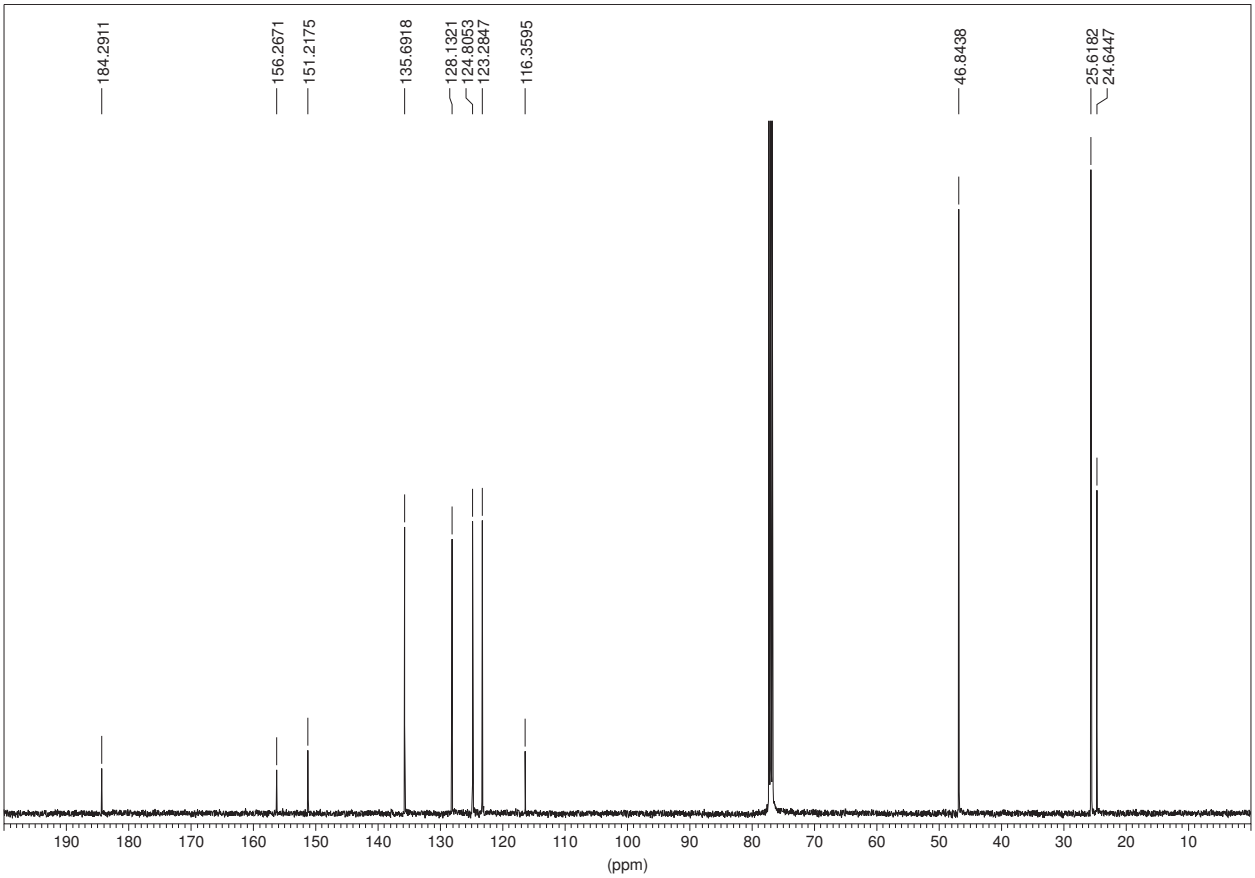
2-(Pyrrolidin-1-yl)-4*H*-3,1-benzothiazin-4-one (**128**)



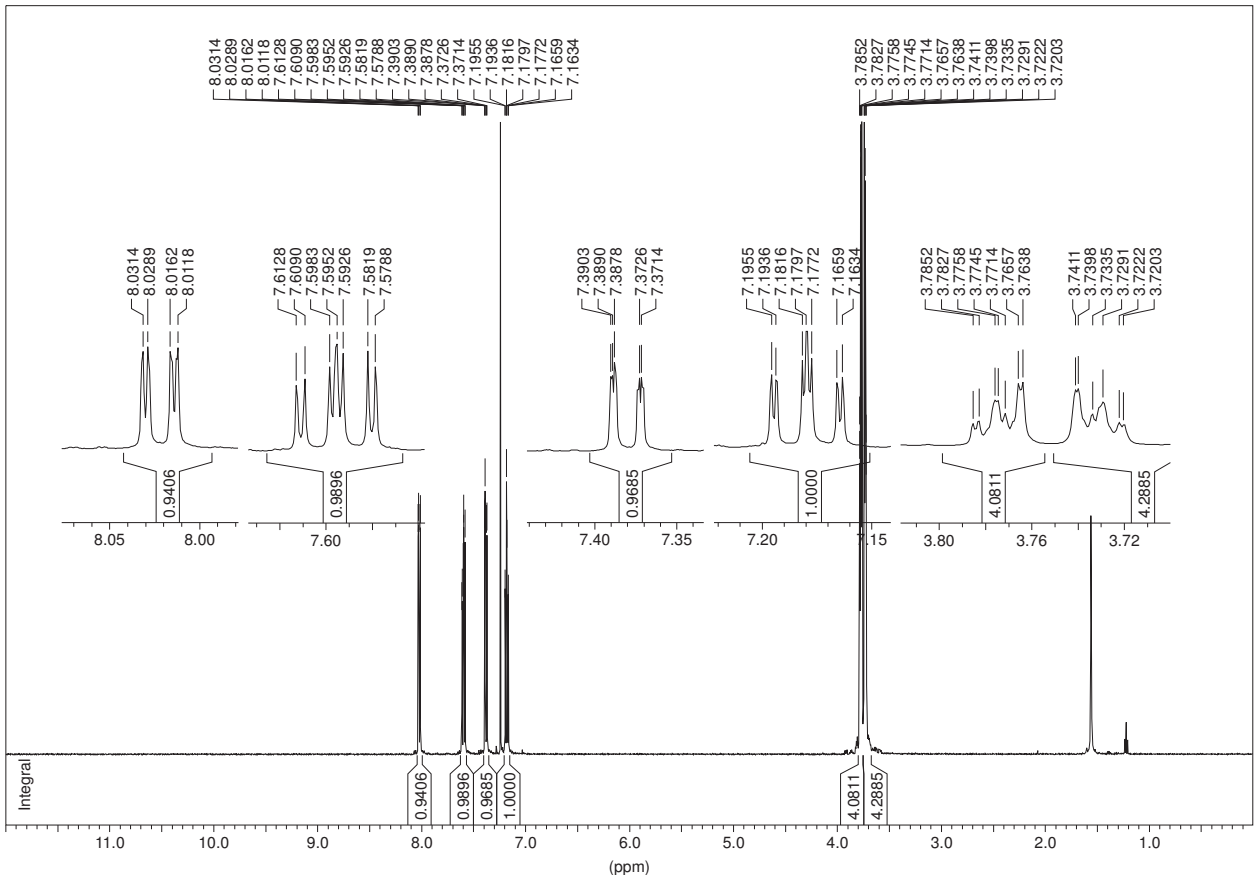


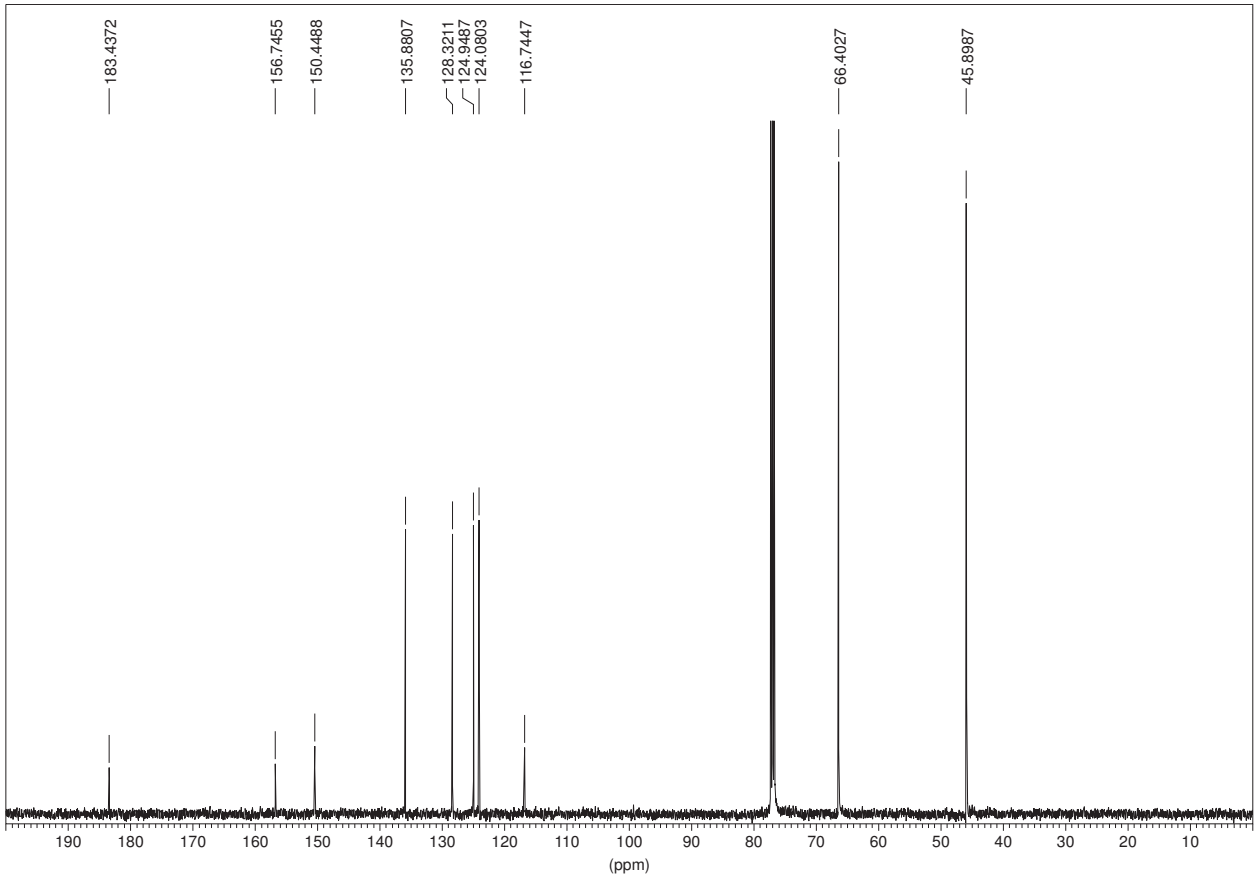
2-(Piperidin-1-yl)-4*H*-3,1-benzothiazin-4-one (**129**)



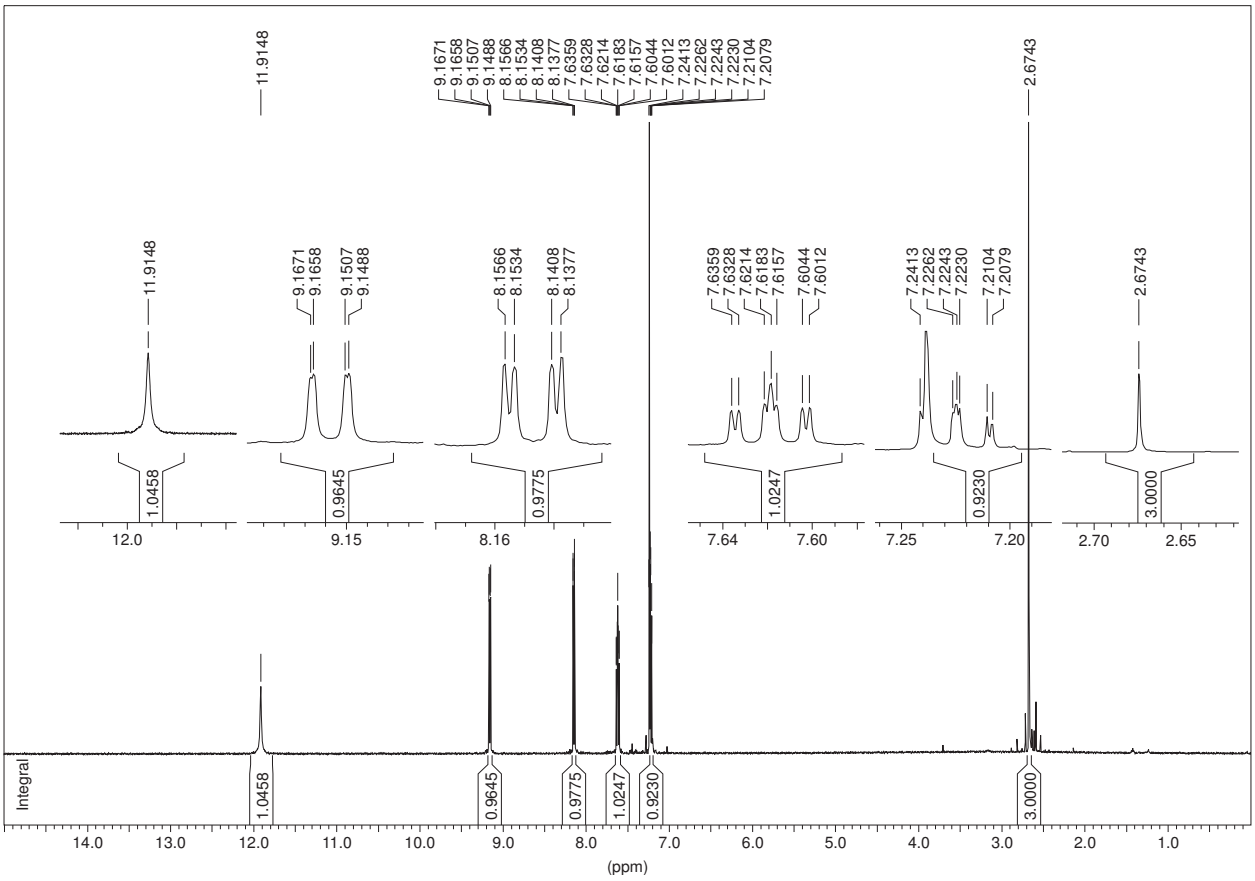


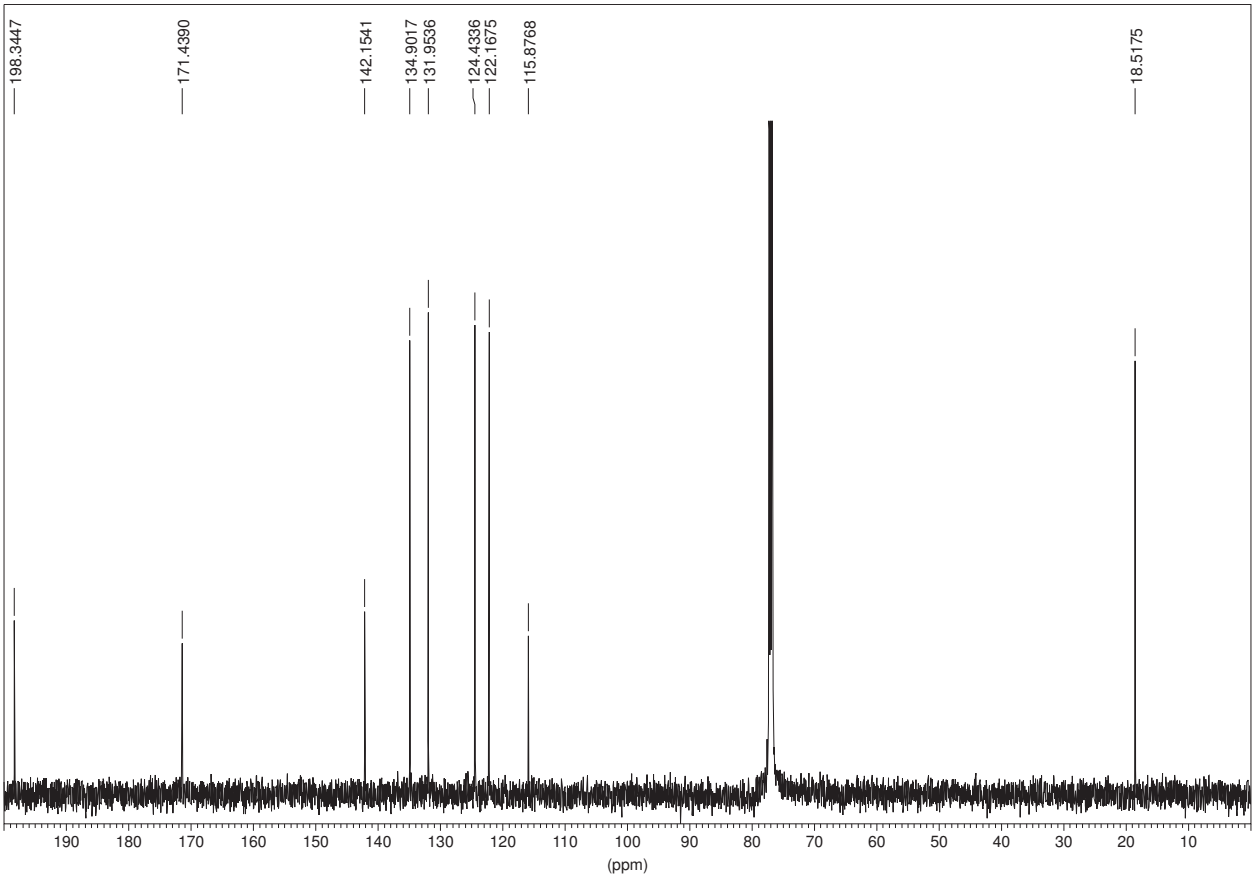
2-(Morpholin-4-yl)-4*H*-3,1-benzothiazin-4-one (**130**)



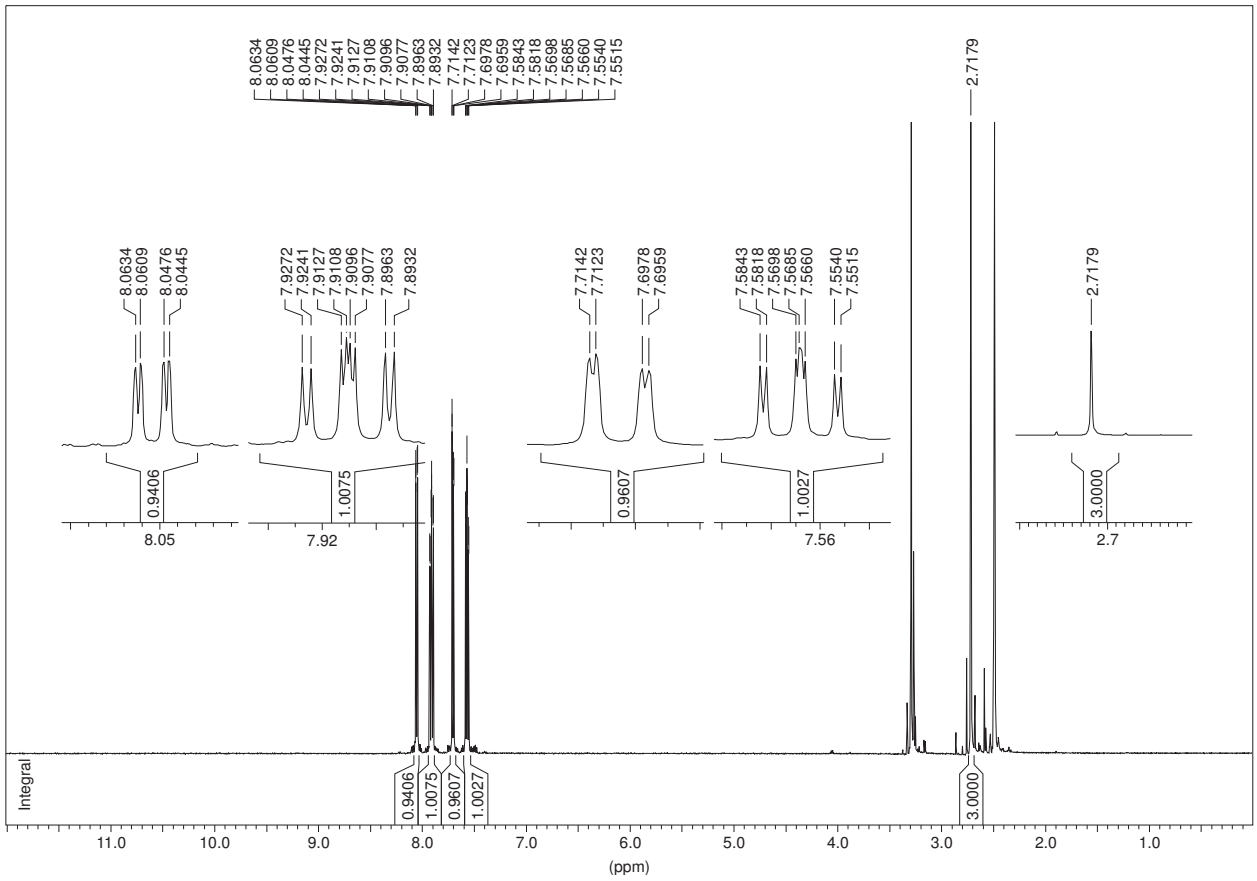


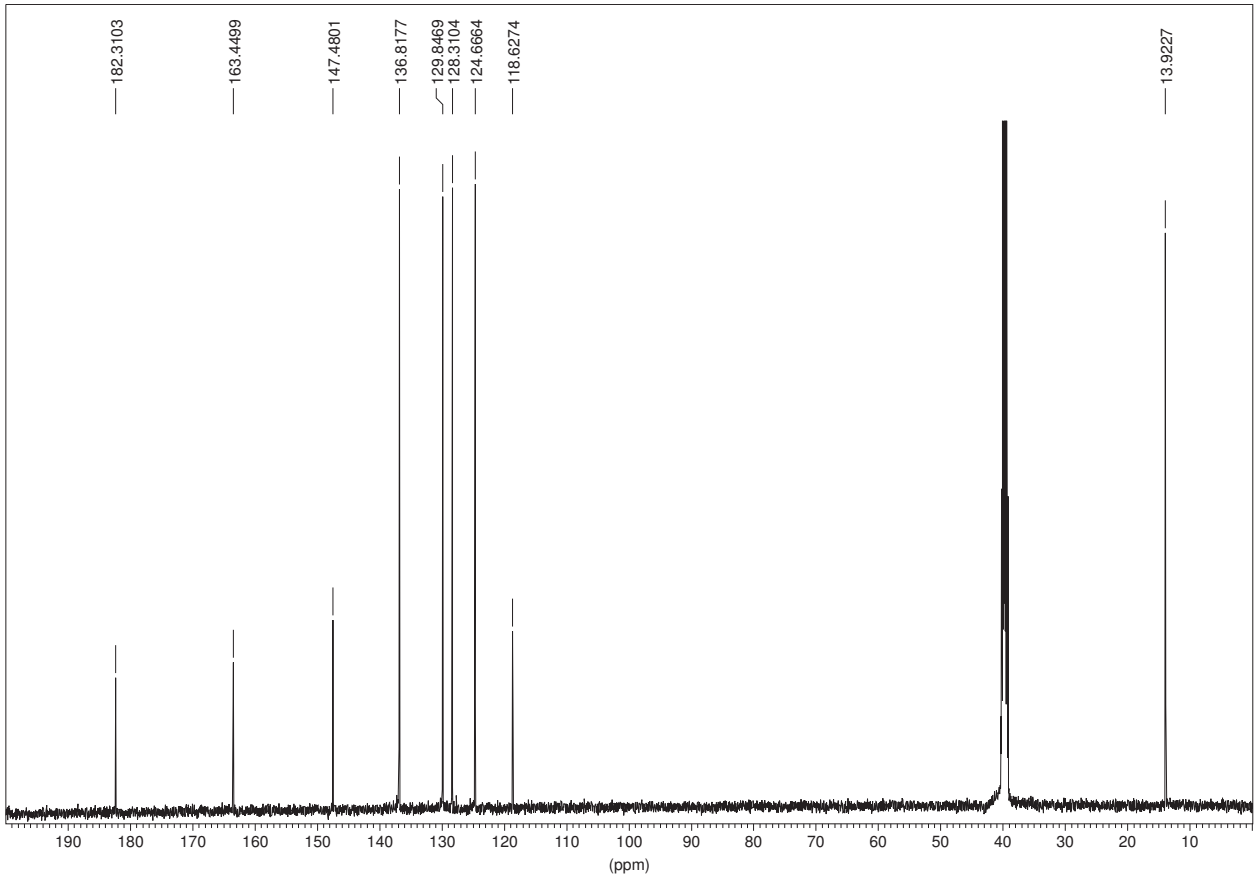
2-[(Methylthio)thiocarbonylamino]benzoic Acid (**131**)



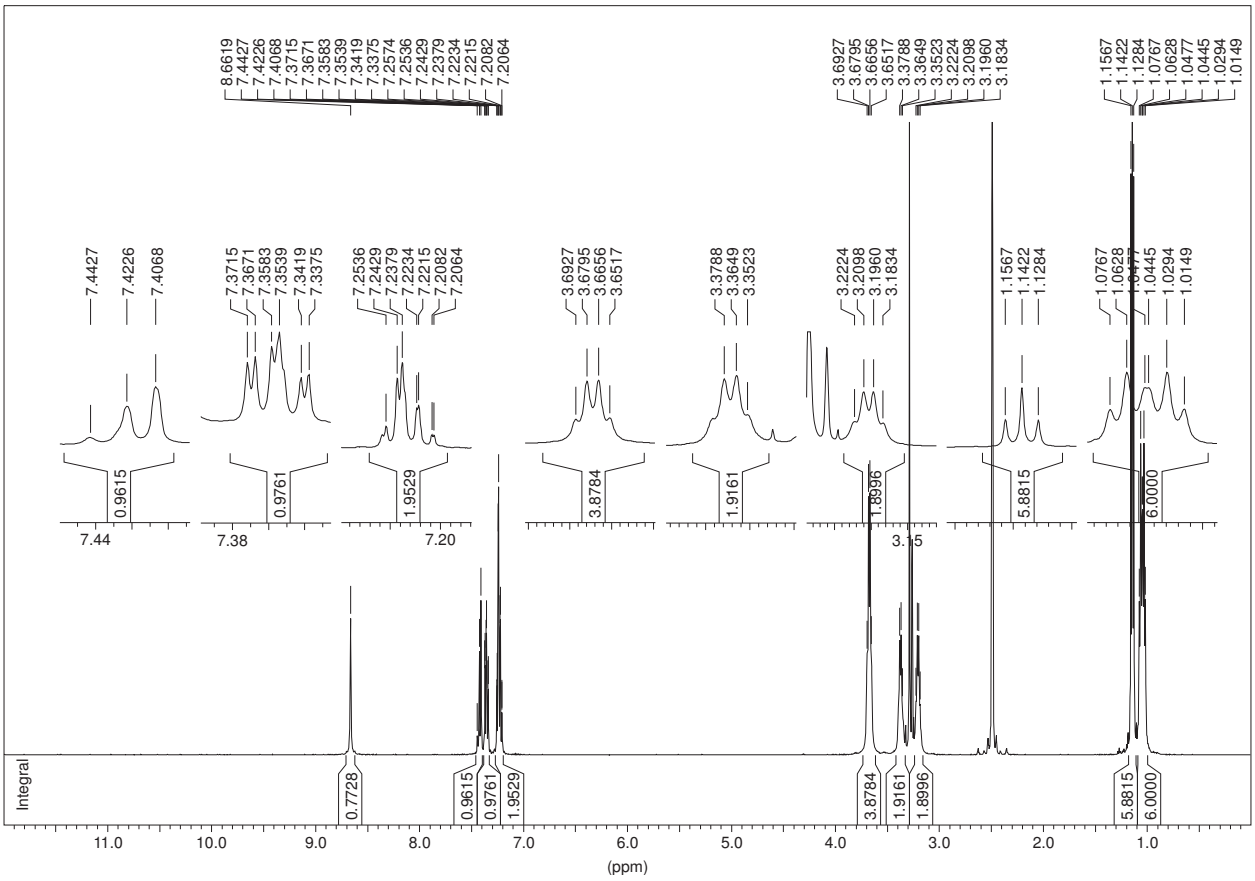


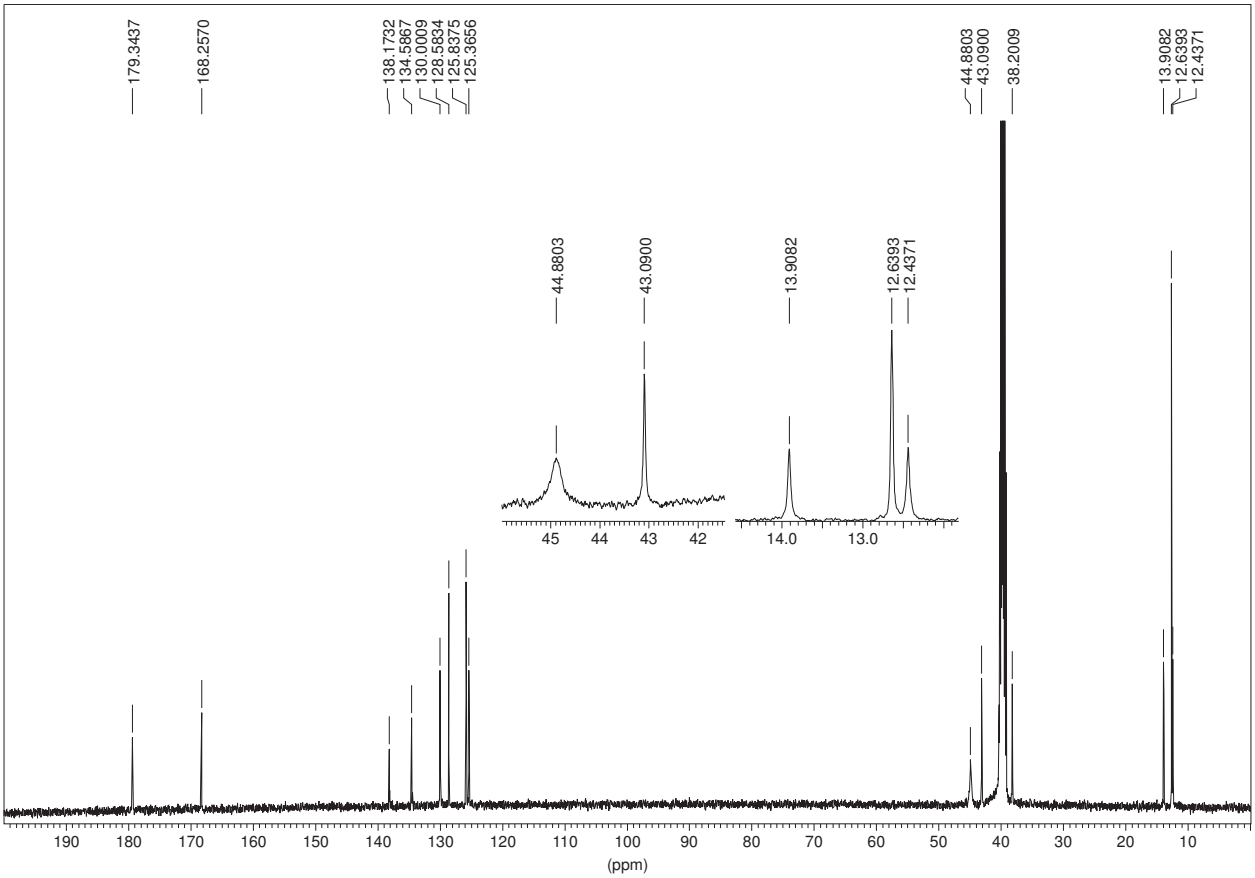
2-(Methylthio)-4*H*-3,1-benzothiazin-4-one (**132**)



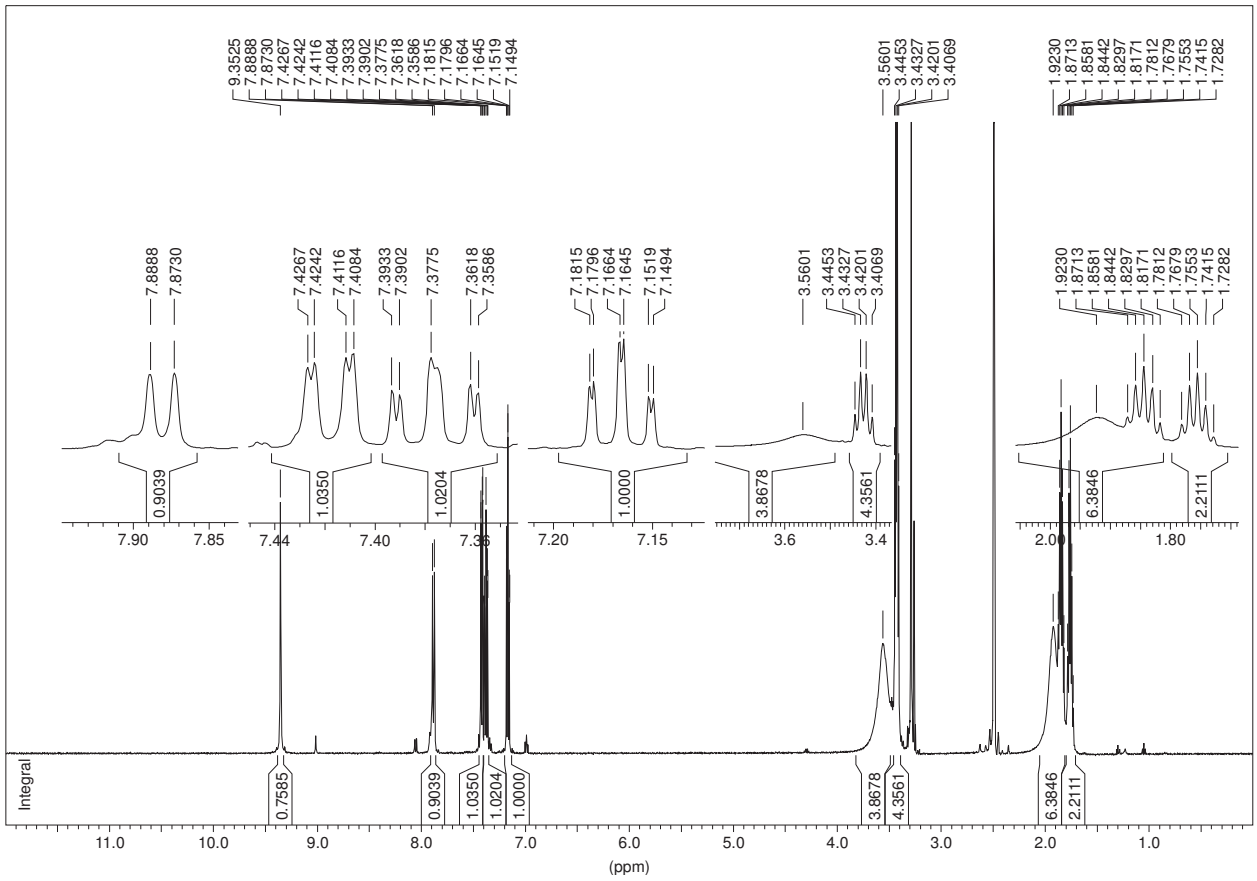


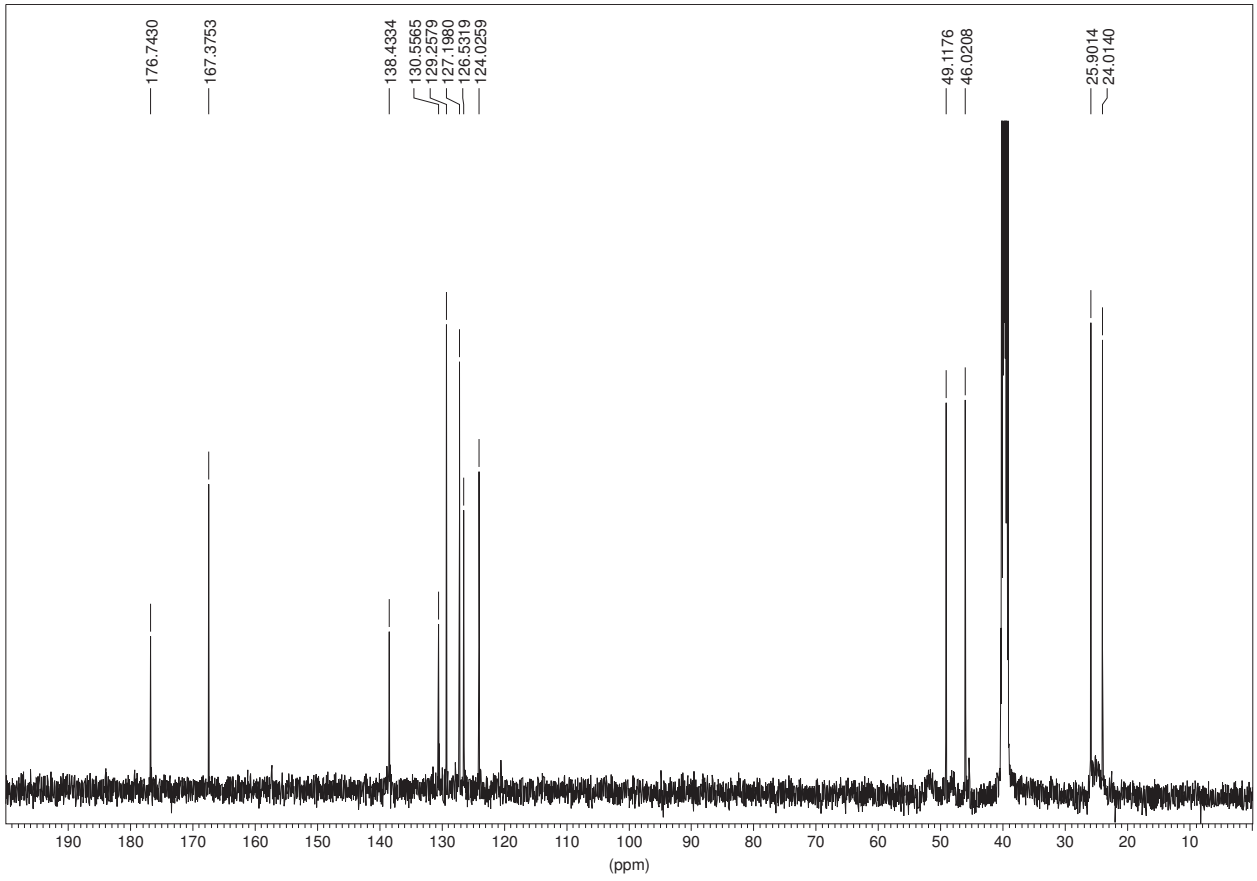
2-(3,3-Diethylthioureido)-*N*,*N*-diethylbenzamide (**133**)



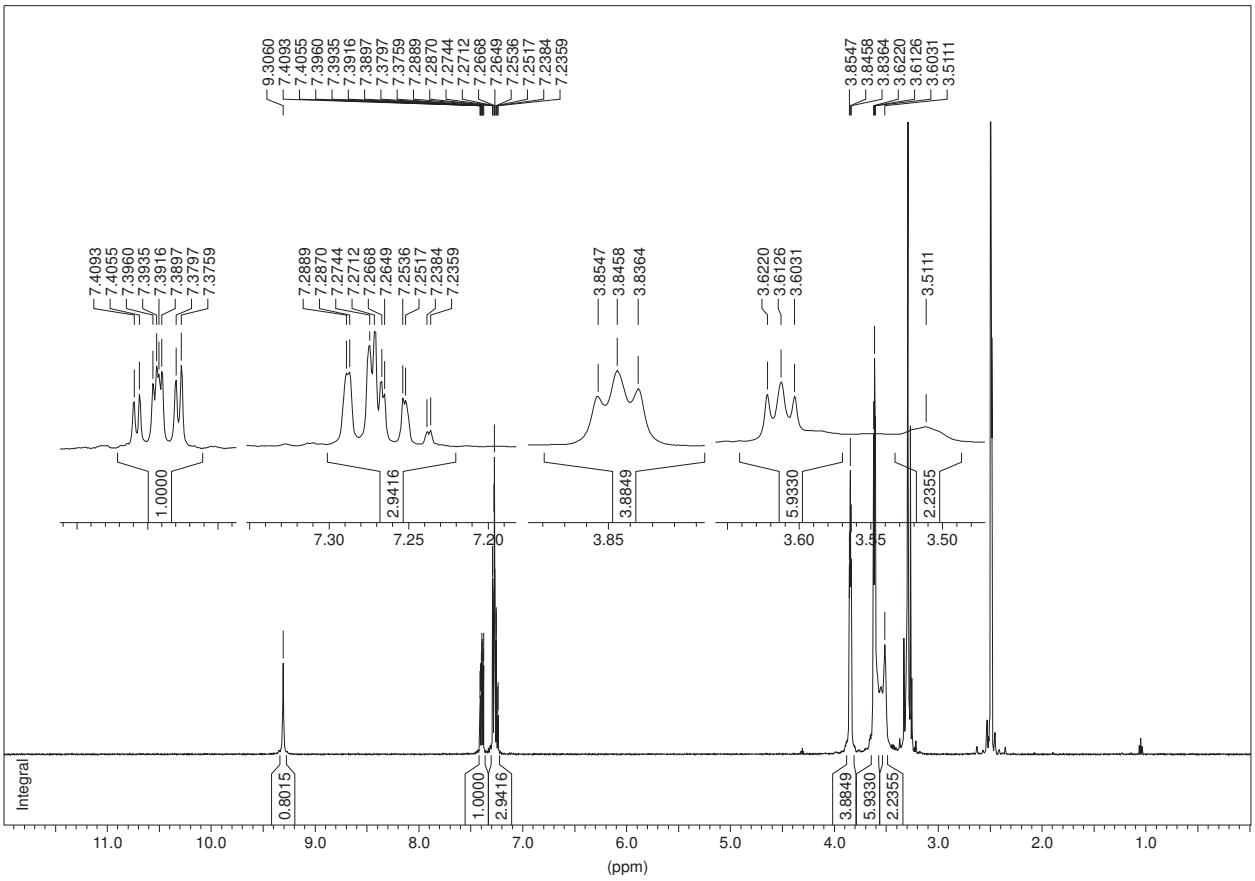


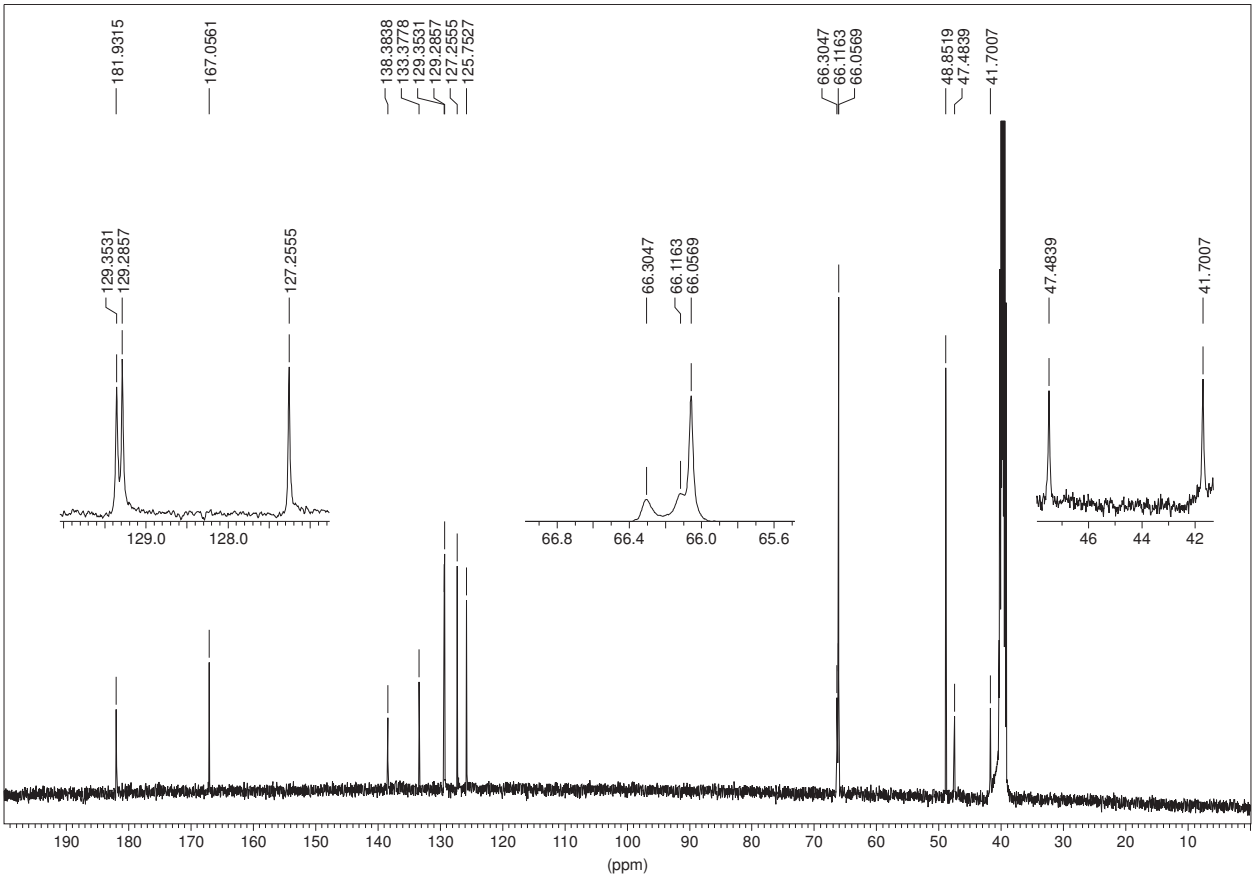
N-[2-(Pyrrolidin-1-ylcarbonyl)phenyl]pyrrolidine-1-carbothioamide (**134**)



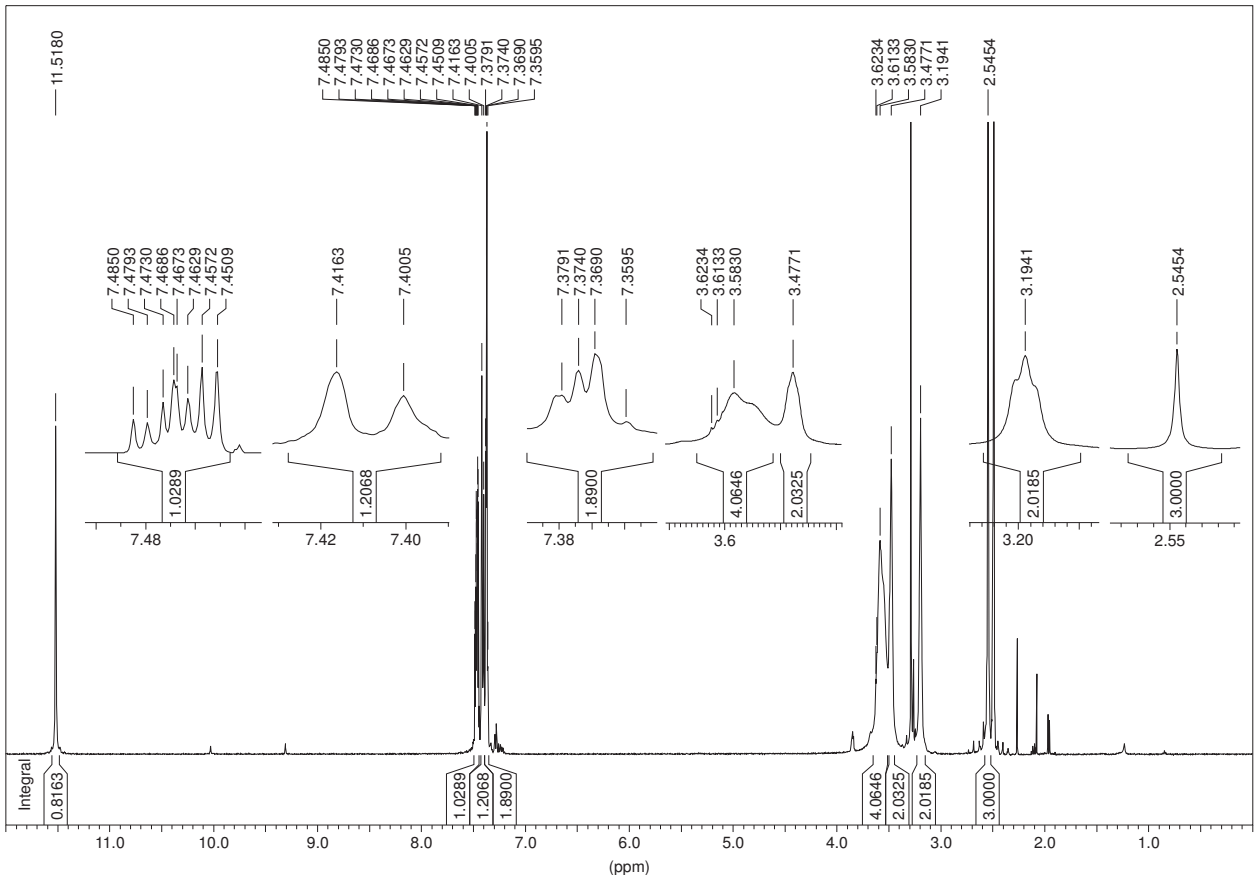


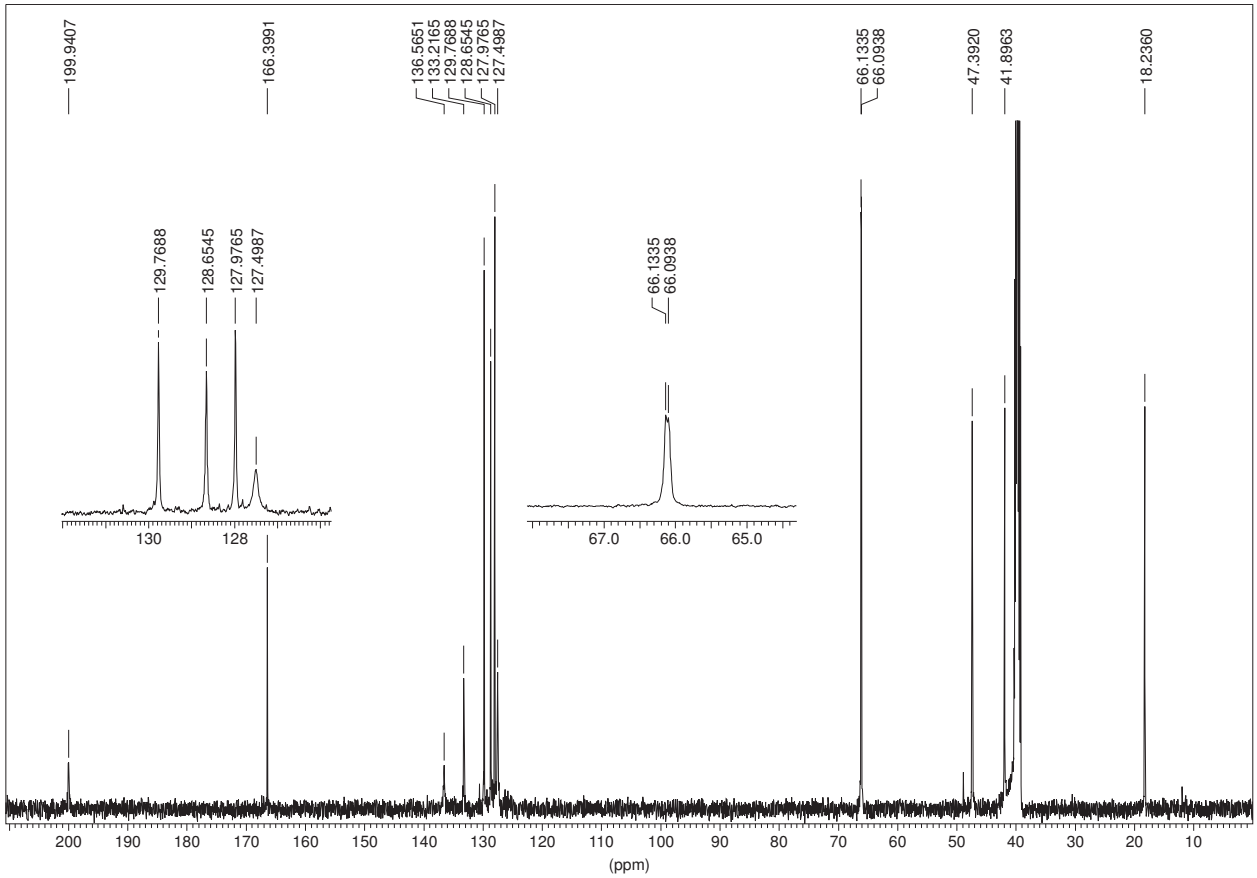
N-[2-(Morpholin-4-ylcarbonyl)phenyl]morpholine-4-carbothioamide (**135**)



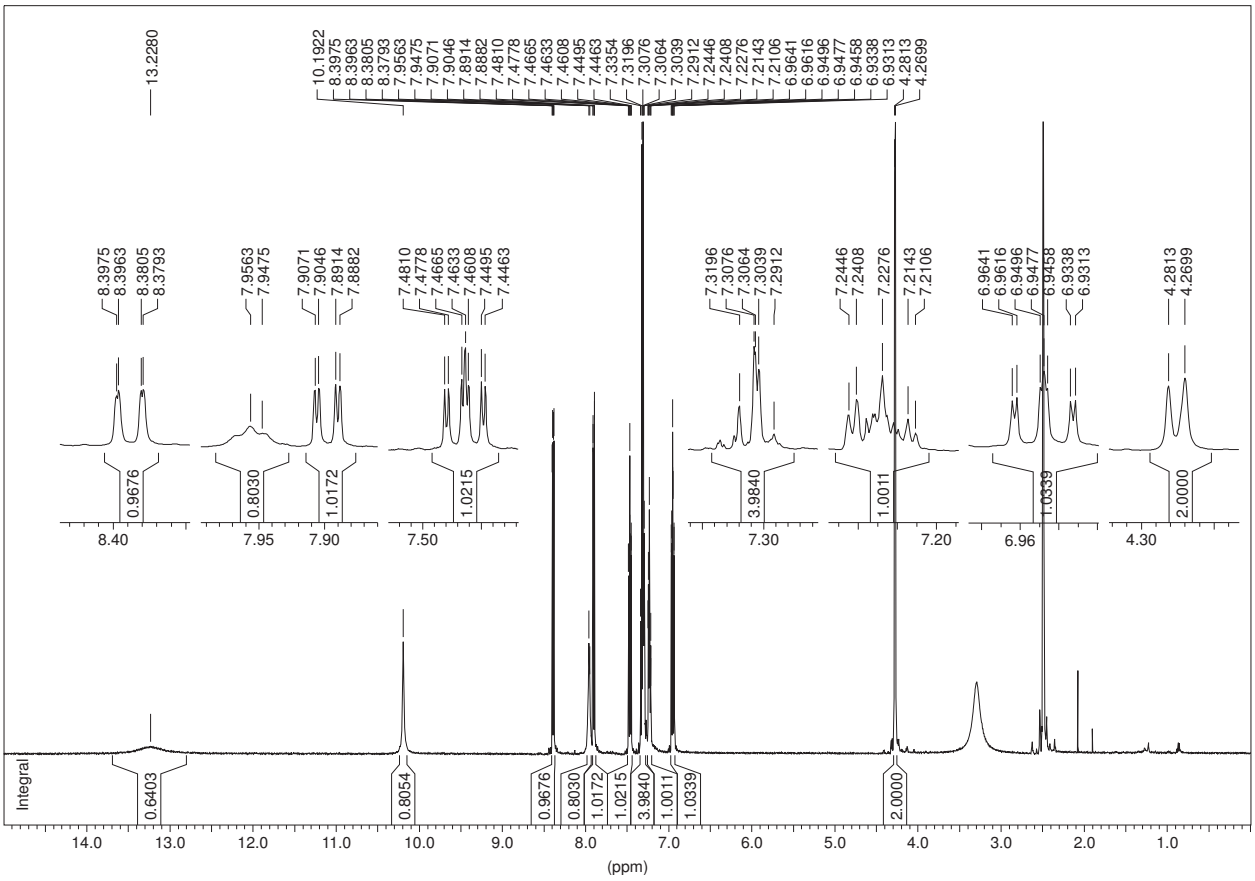


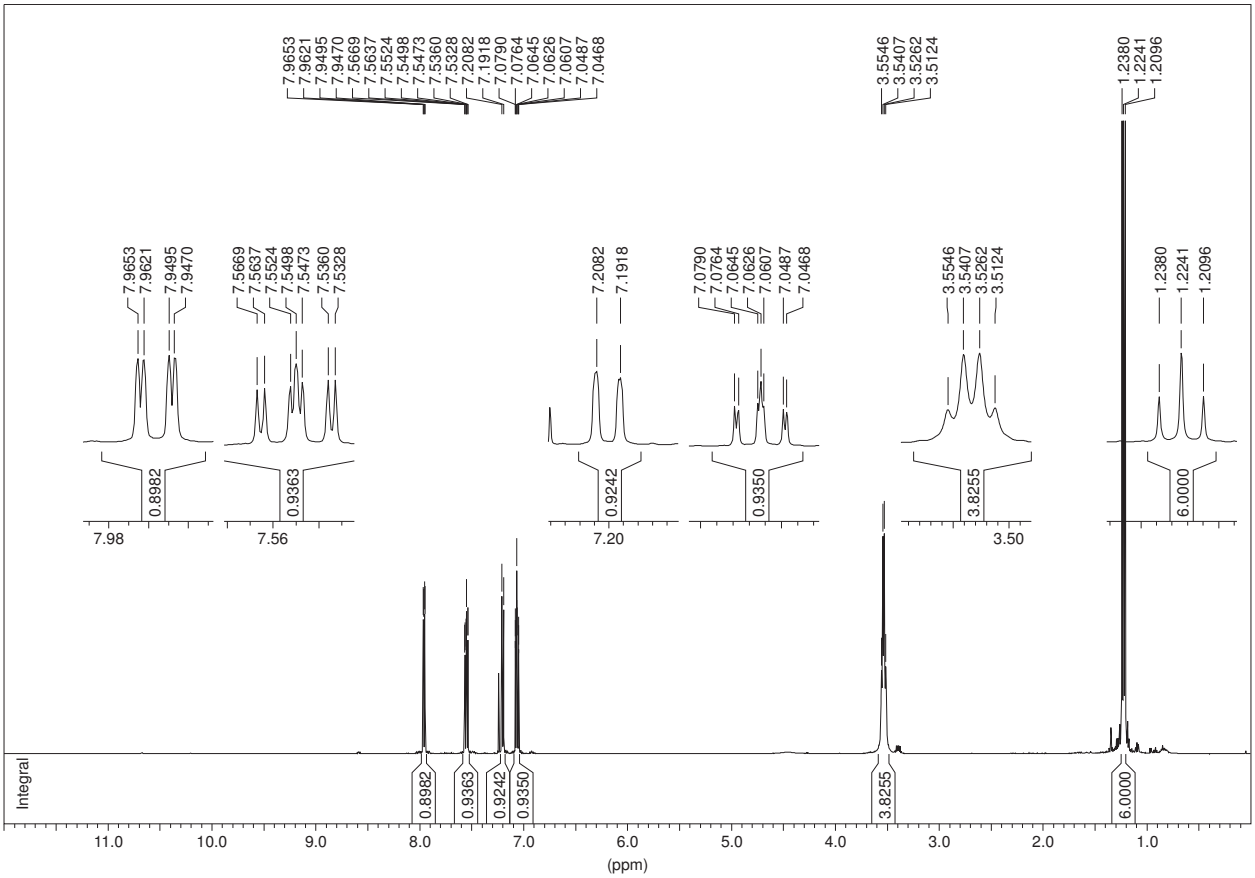
Methyl 2-(Morpholin-4-ylcarbonyl)phenyldithiocarbamate (**136**)



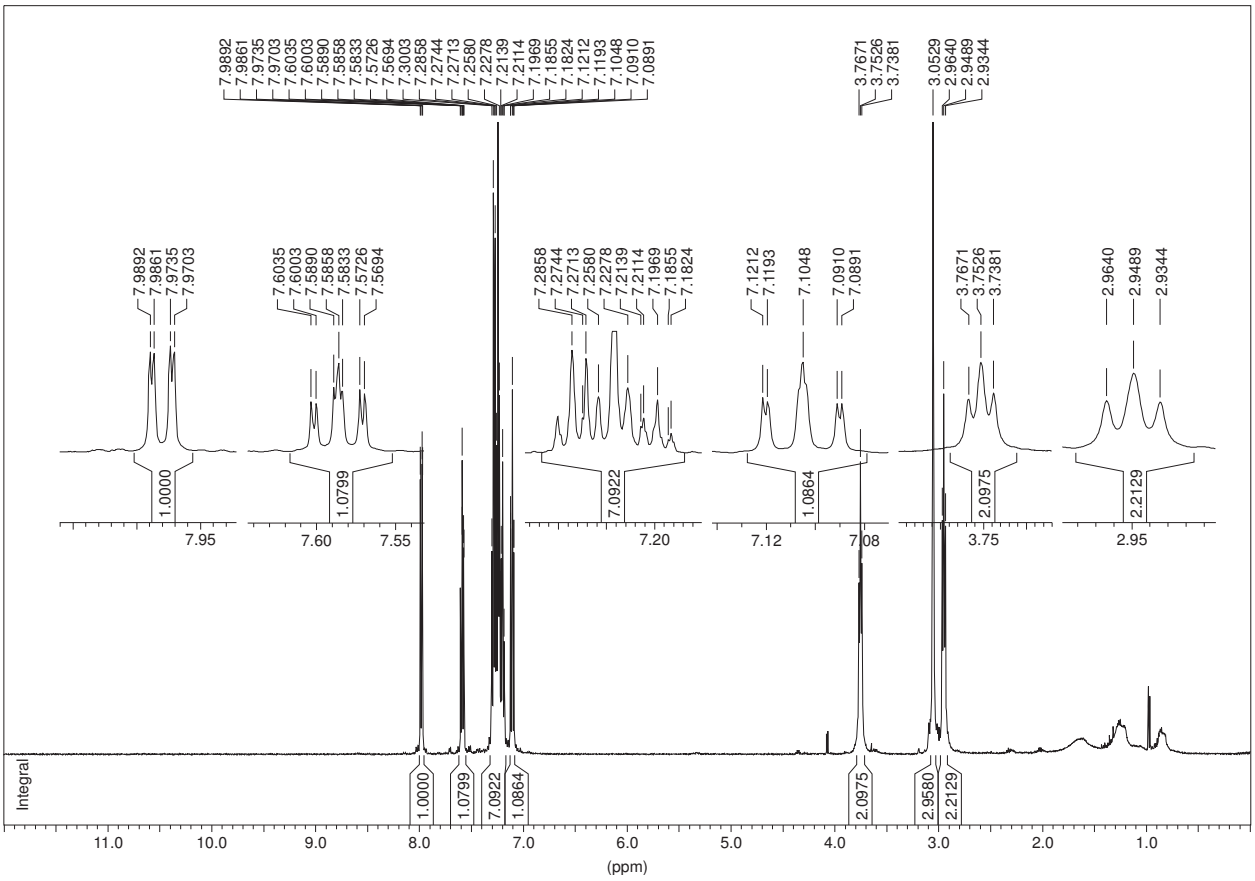


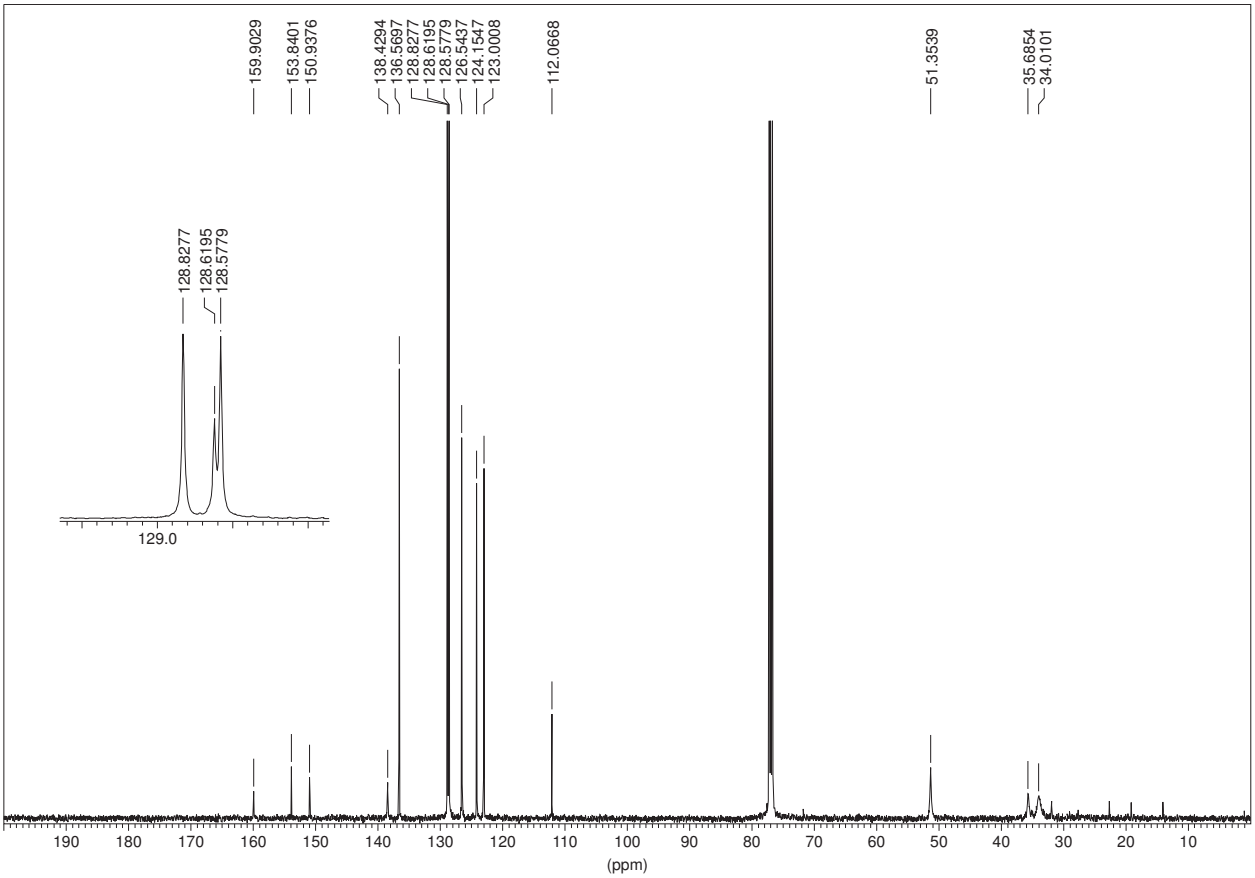
2-(Diethylamino)-4*H*-3,1-benzoxazin-4-one (**137**)



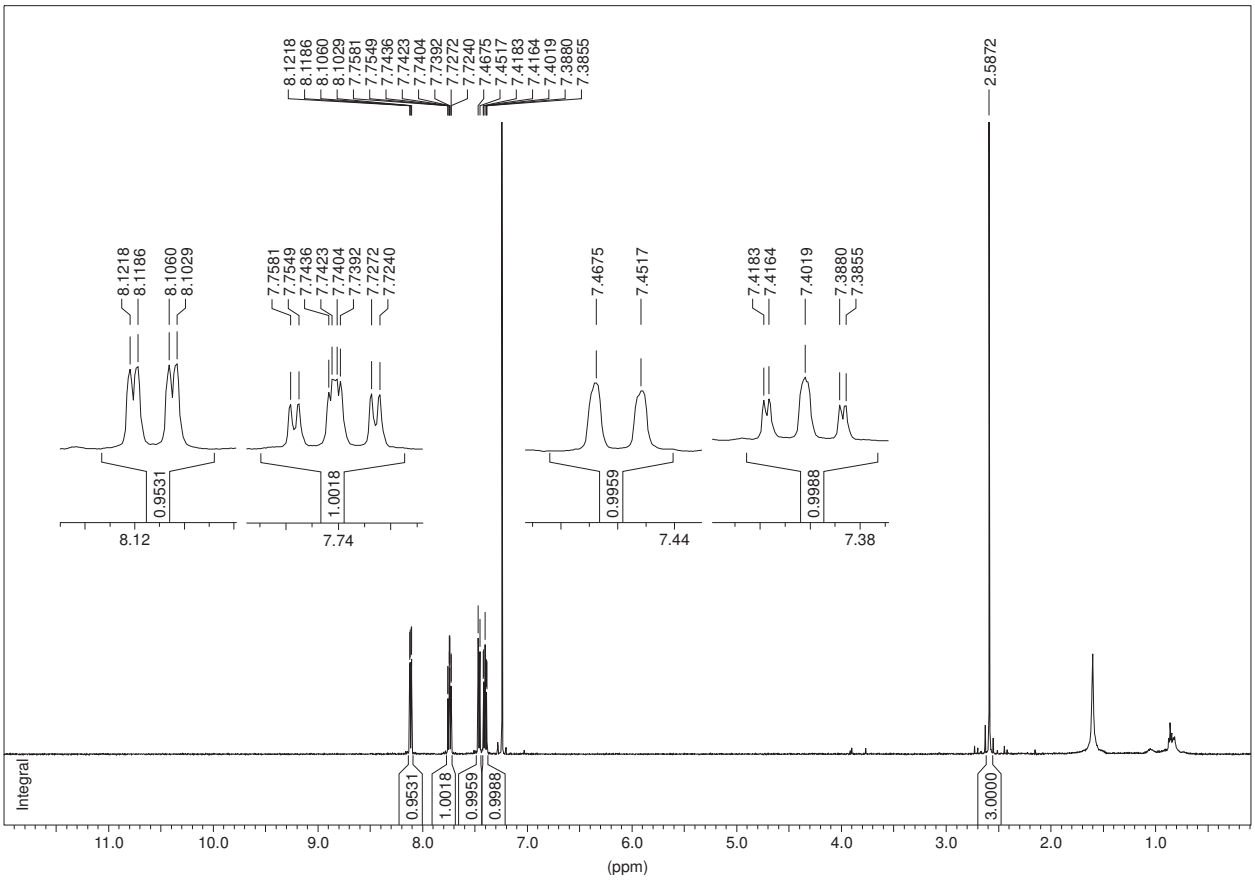


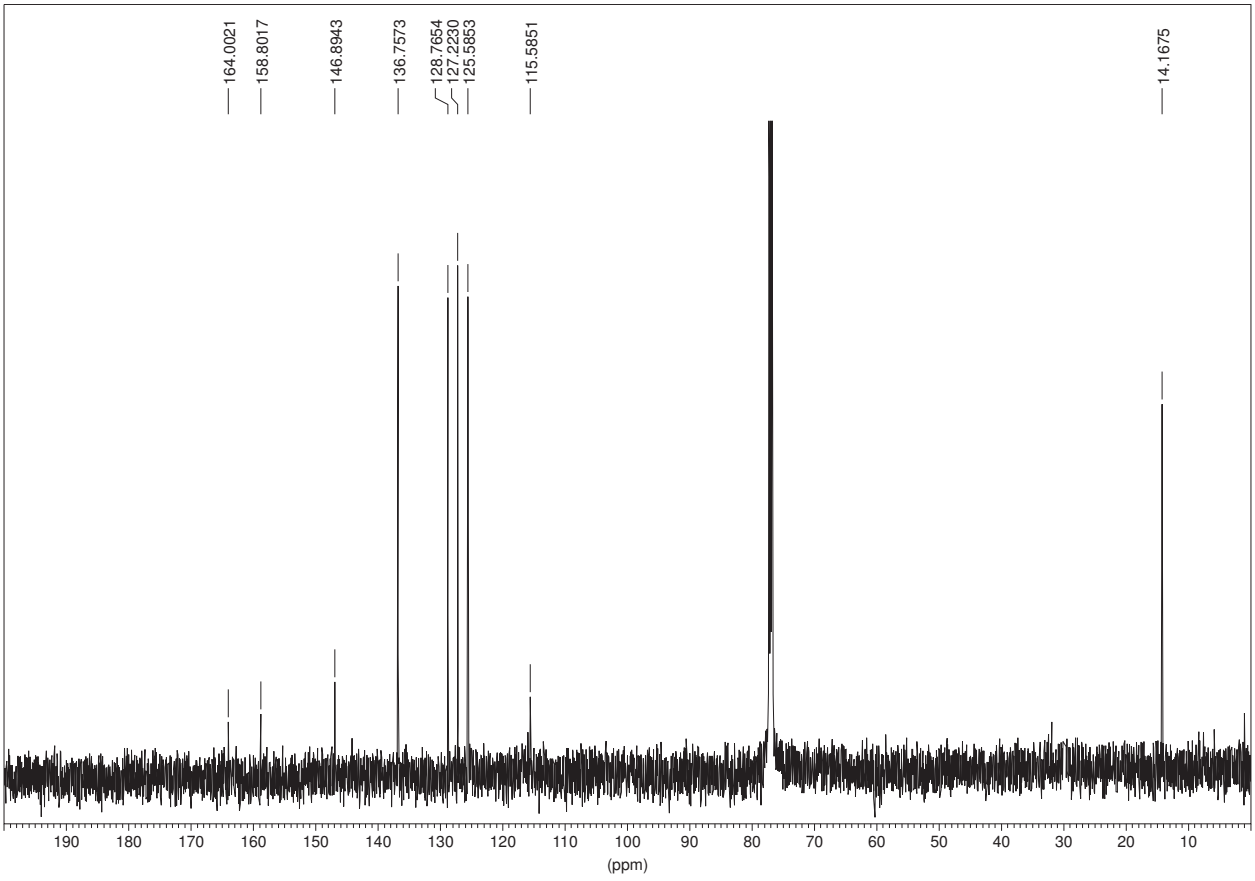
2-[*N*-Methyl-*N*-(2-phenylethyl)amino]-4*H*-3,1-benzoxazin-4-one (**138**)



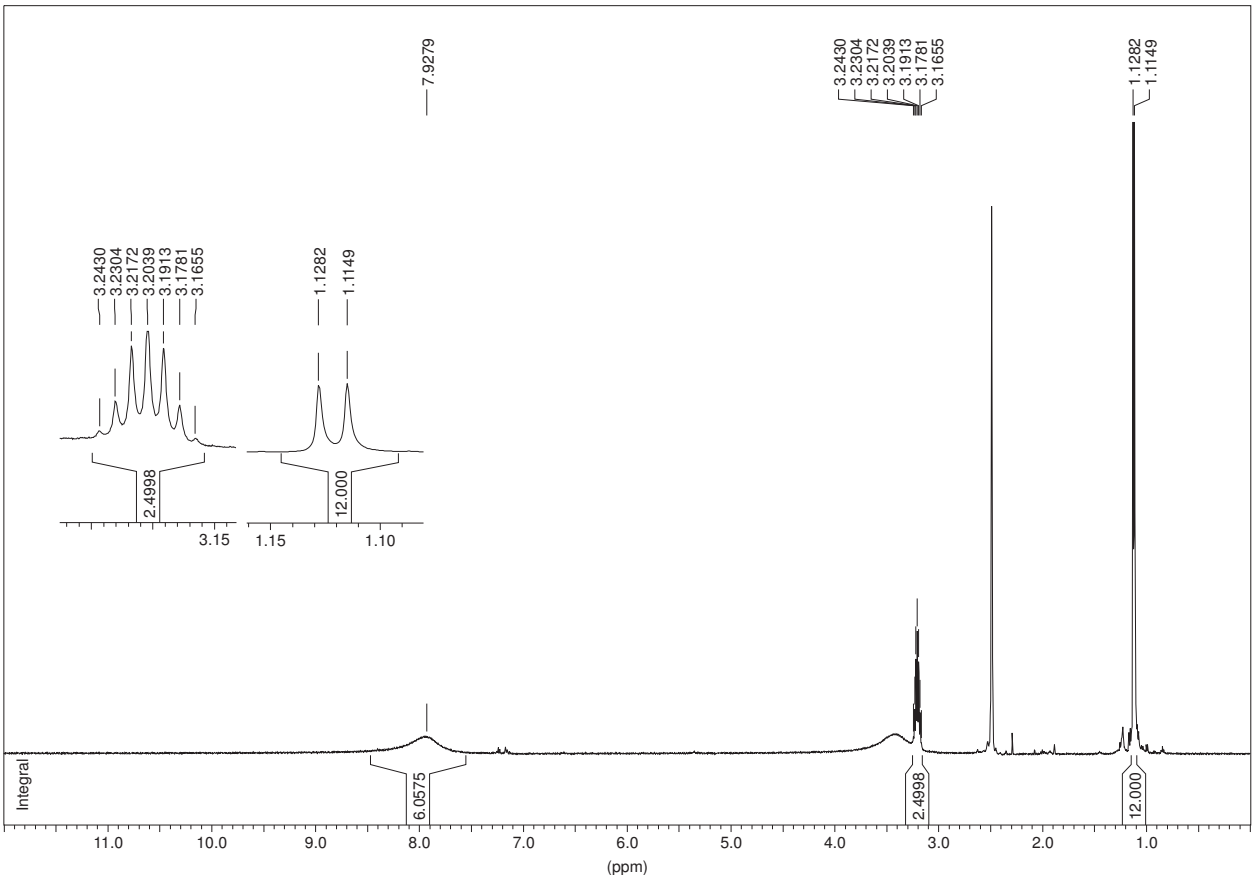


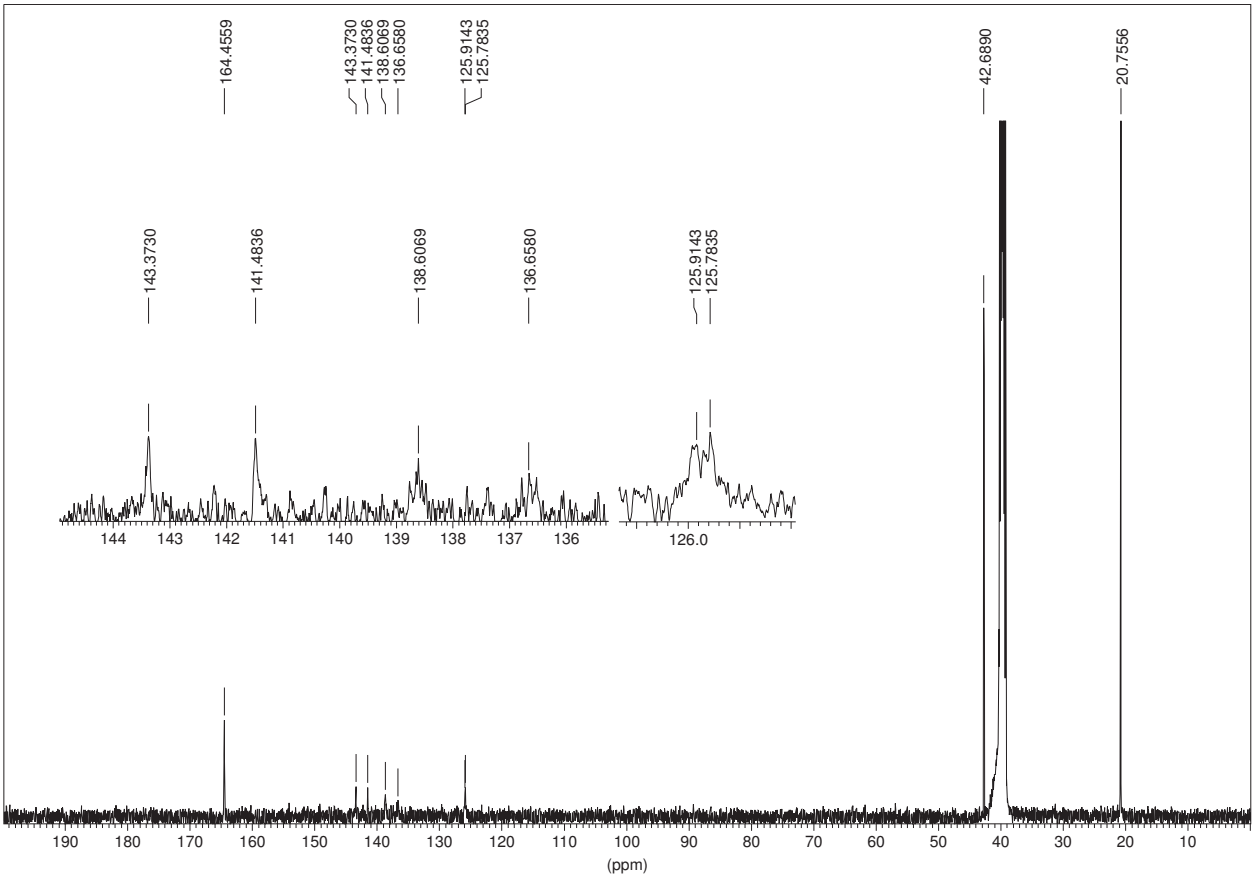
2-(Methylthio)-4*H*-3,1-benzoxazin-4-one (**139**)



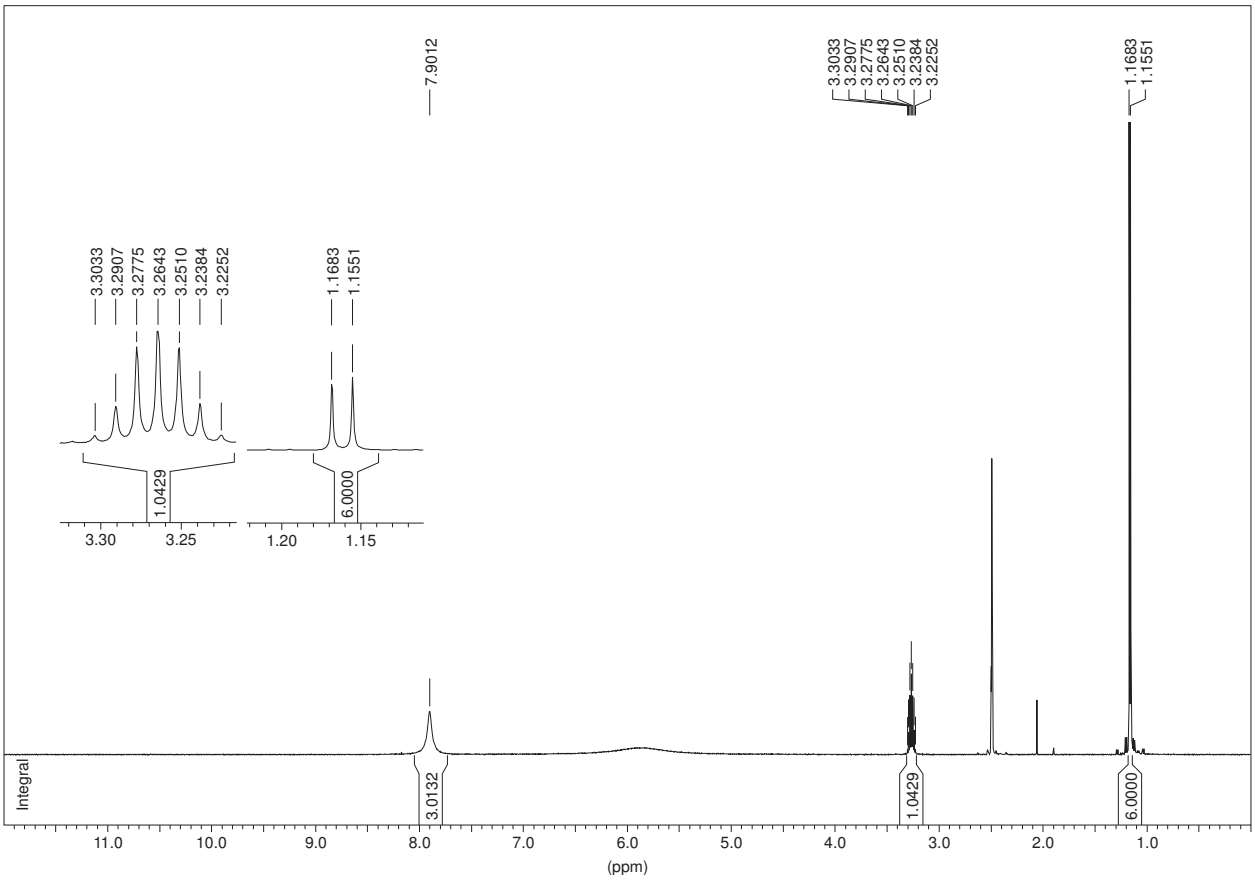


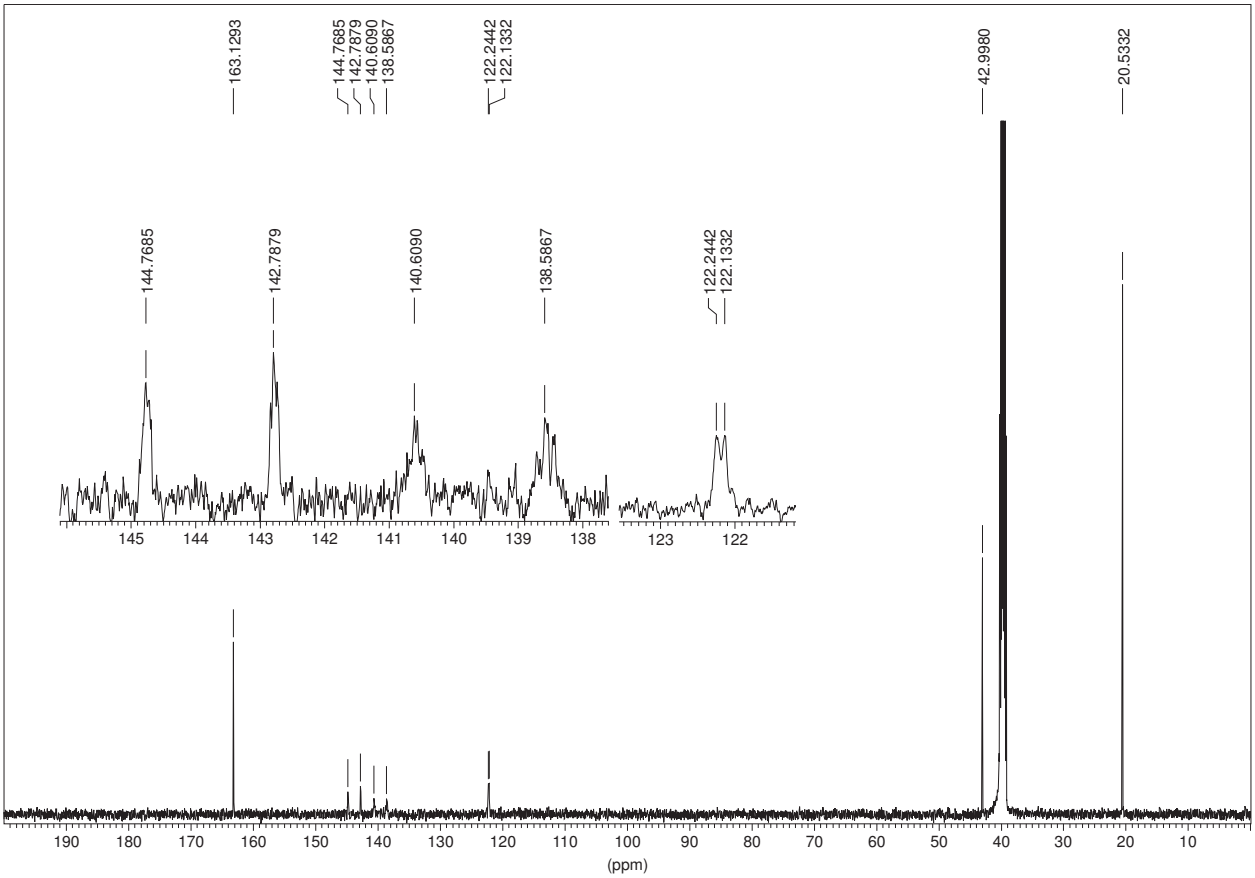
Bis(isopropylammonium) Tetrafluorophthalate (**140**)



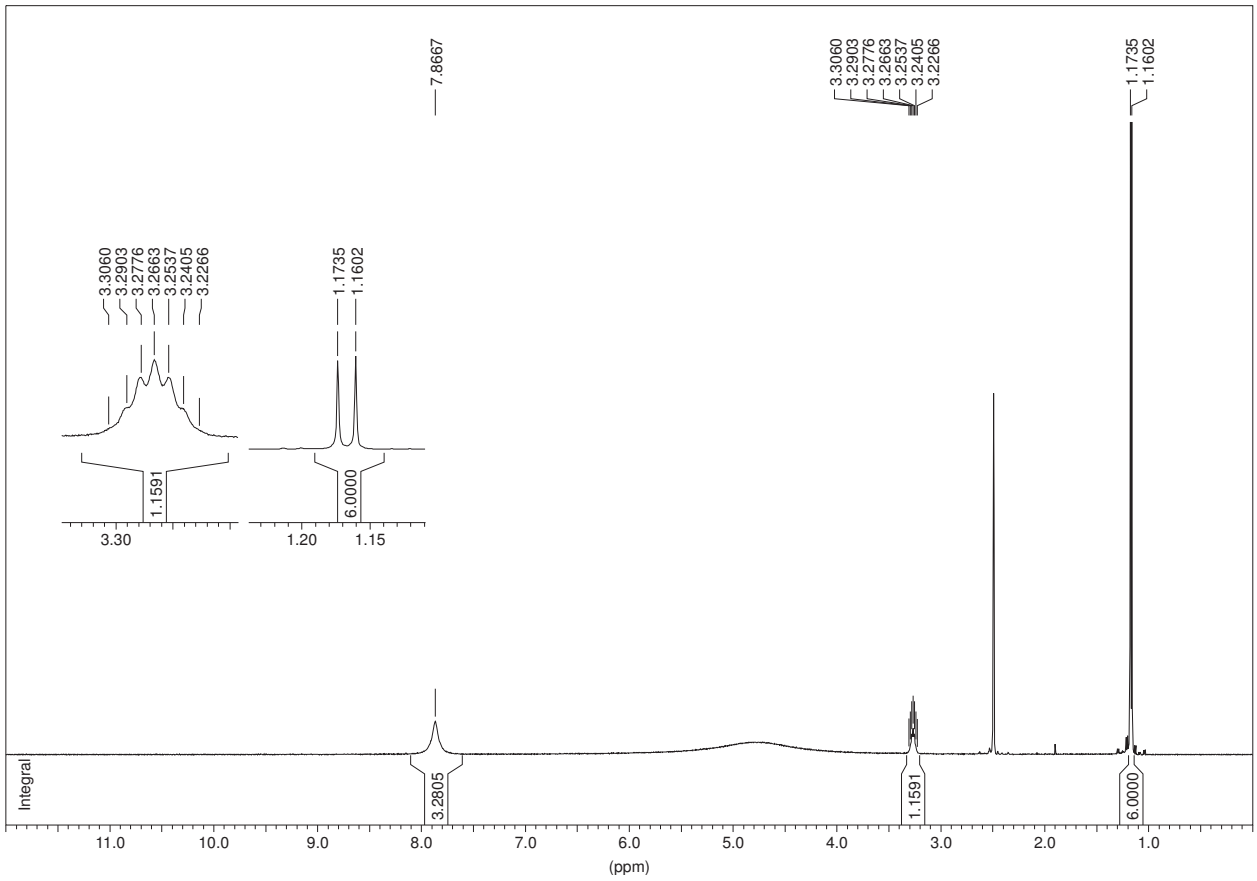


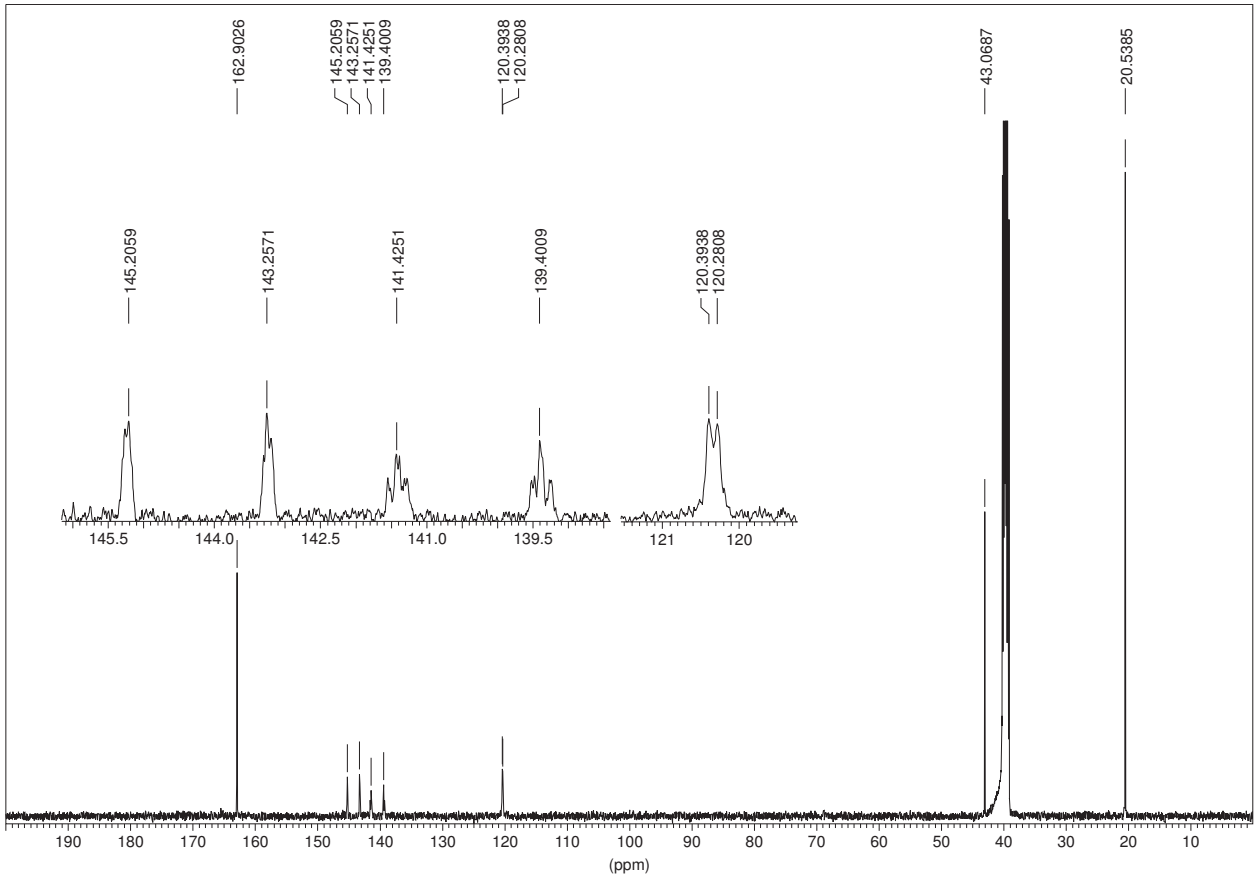
Isopropylammonium Tetrafluorohydrogenphthalate (**141**)





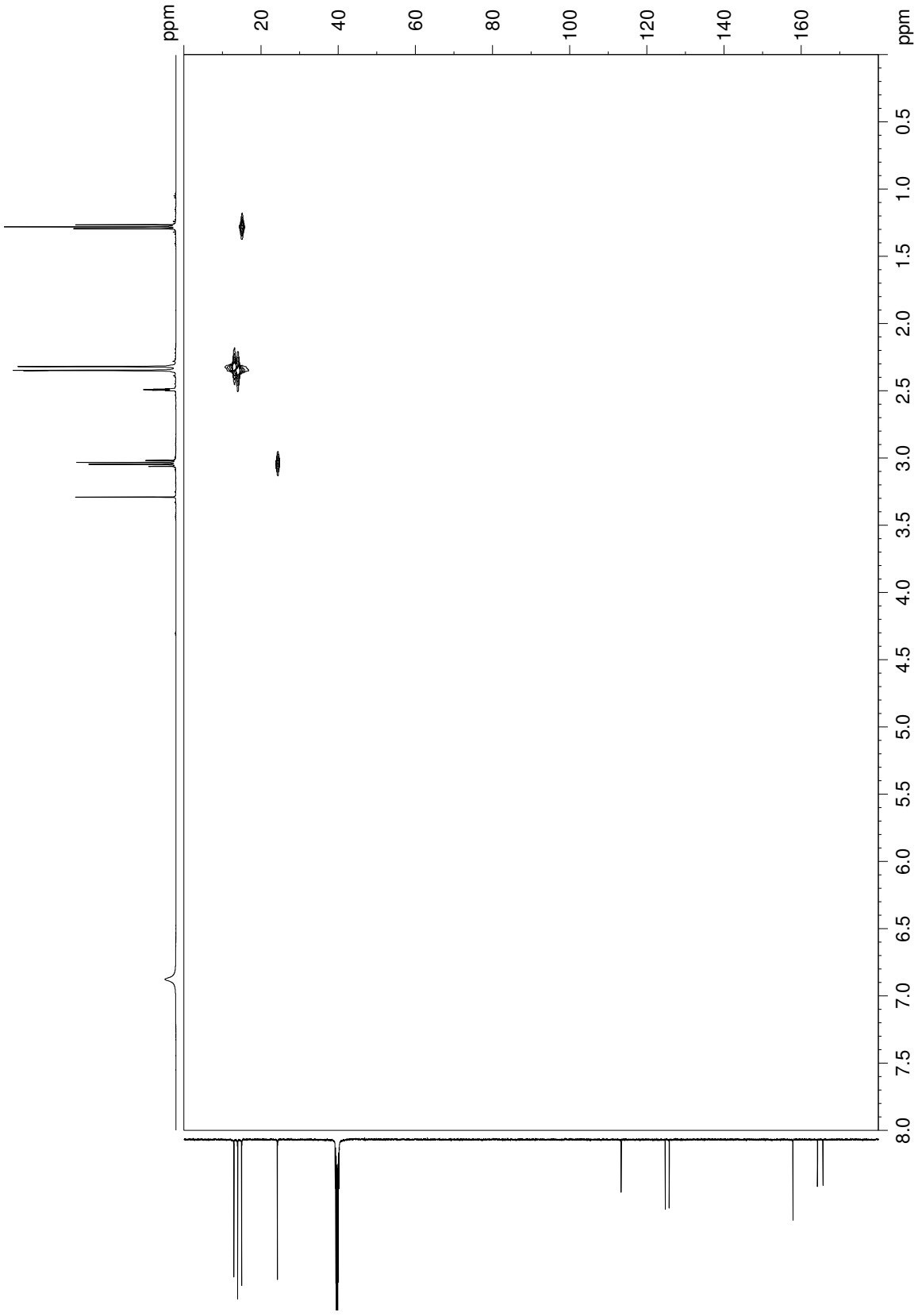
Isopropylammonium Tetrafluorohydrogenphthalate × Tetrafluorophthalic Acid (**142**)





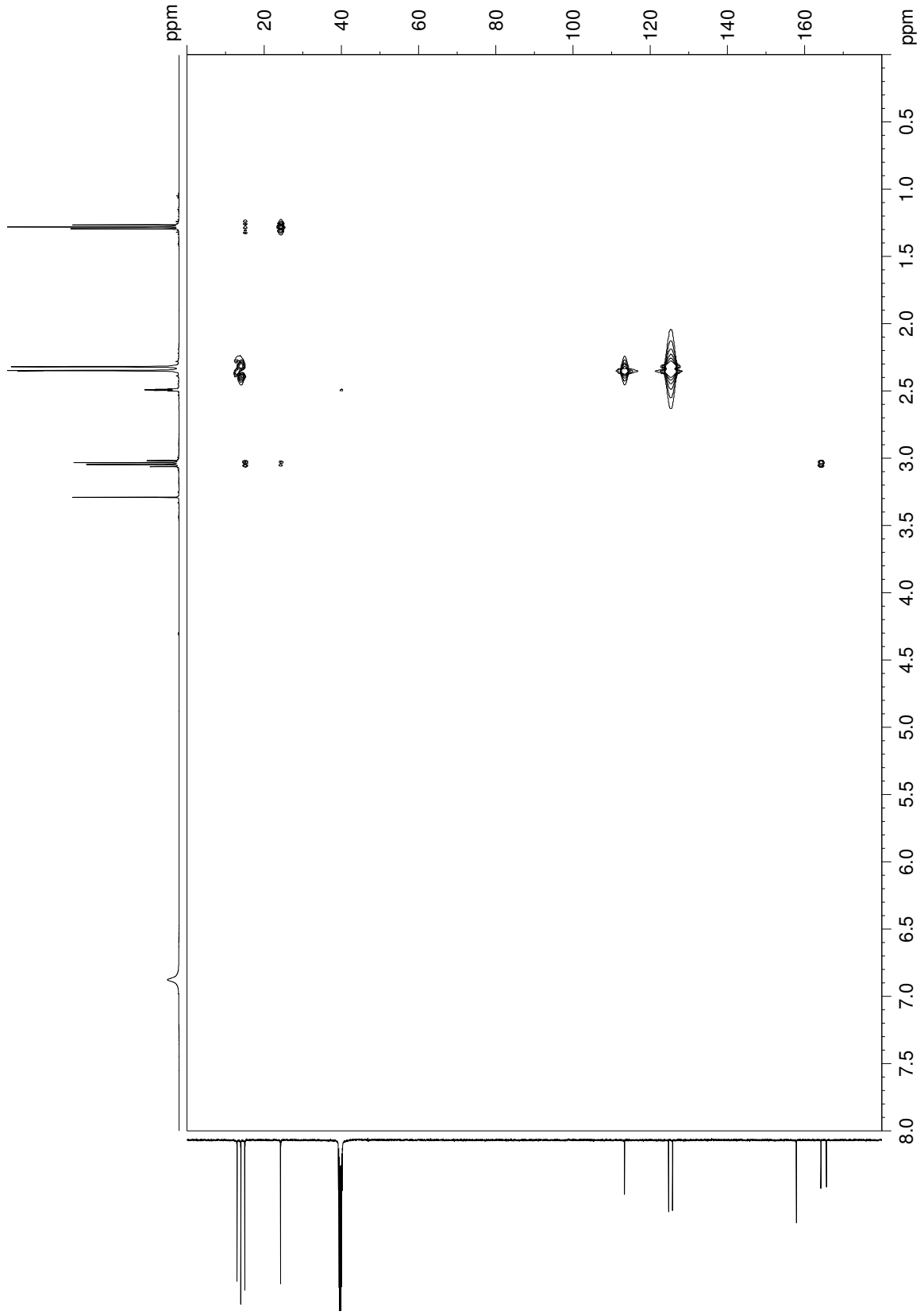
Two-Dimensional NMR Spectra

HMQC spectrum of **19**



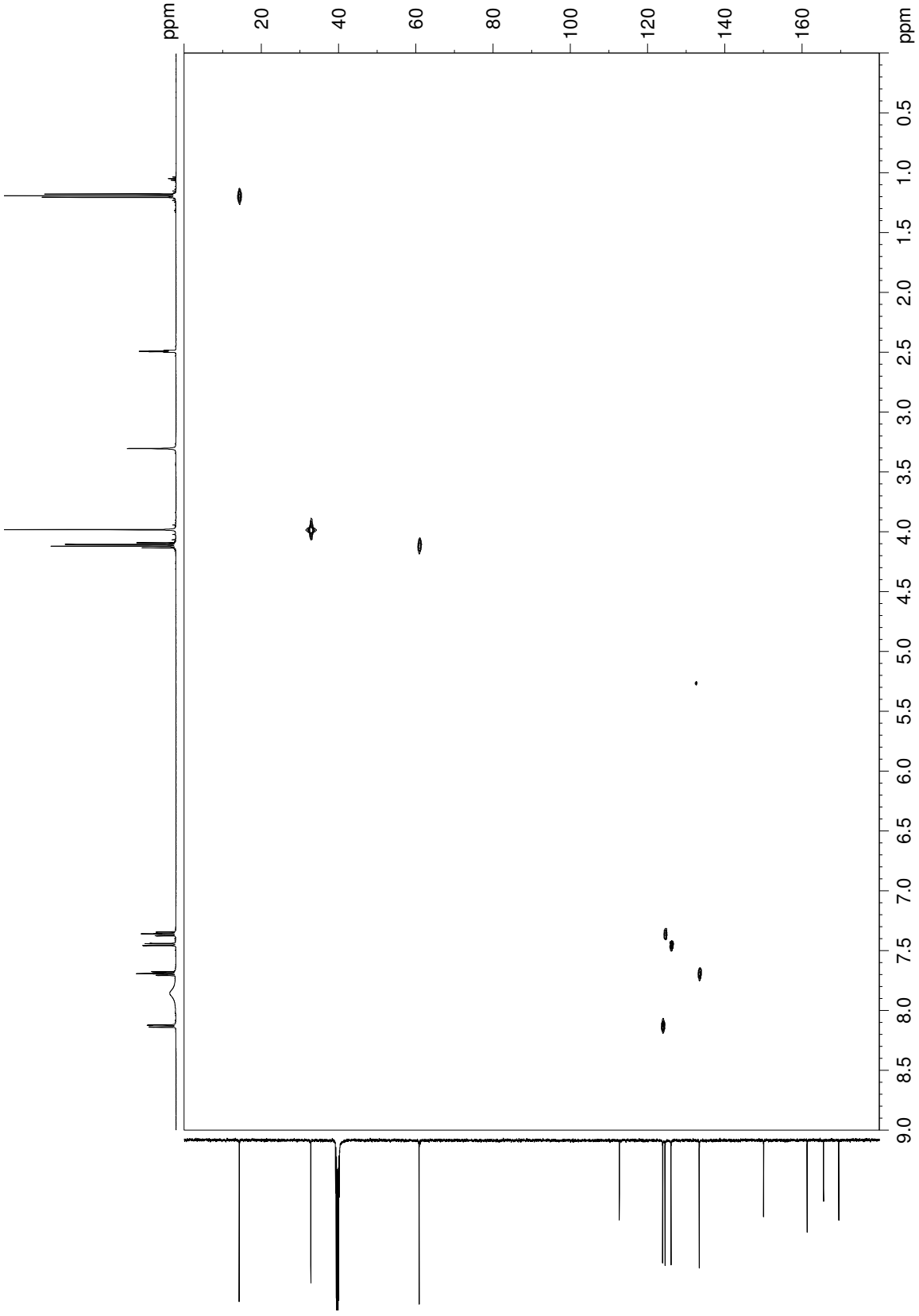
For details see Supplementary Information of ref.[87]

HMBC spectrum of **19**



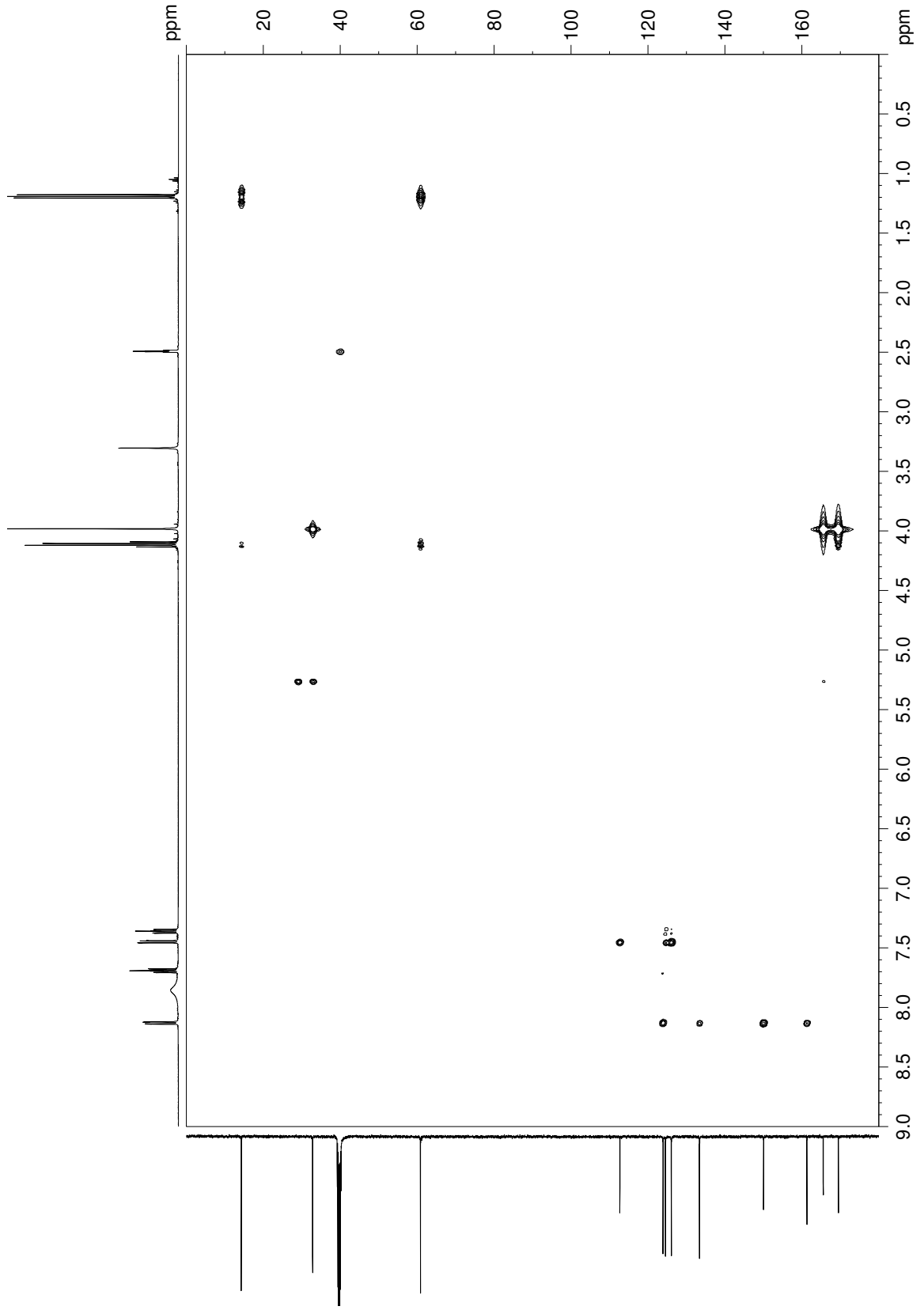
For details see Supplementary Information of ref.[87]

HMQC spectrum of **38**



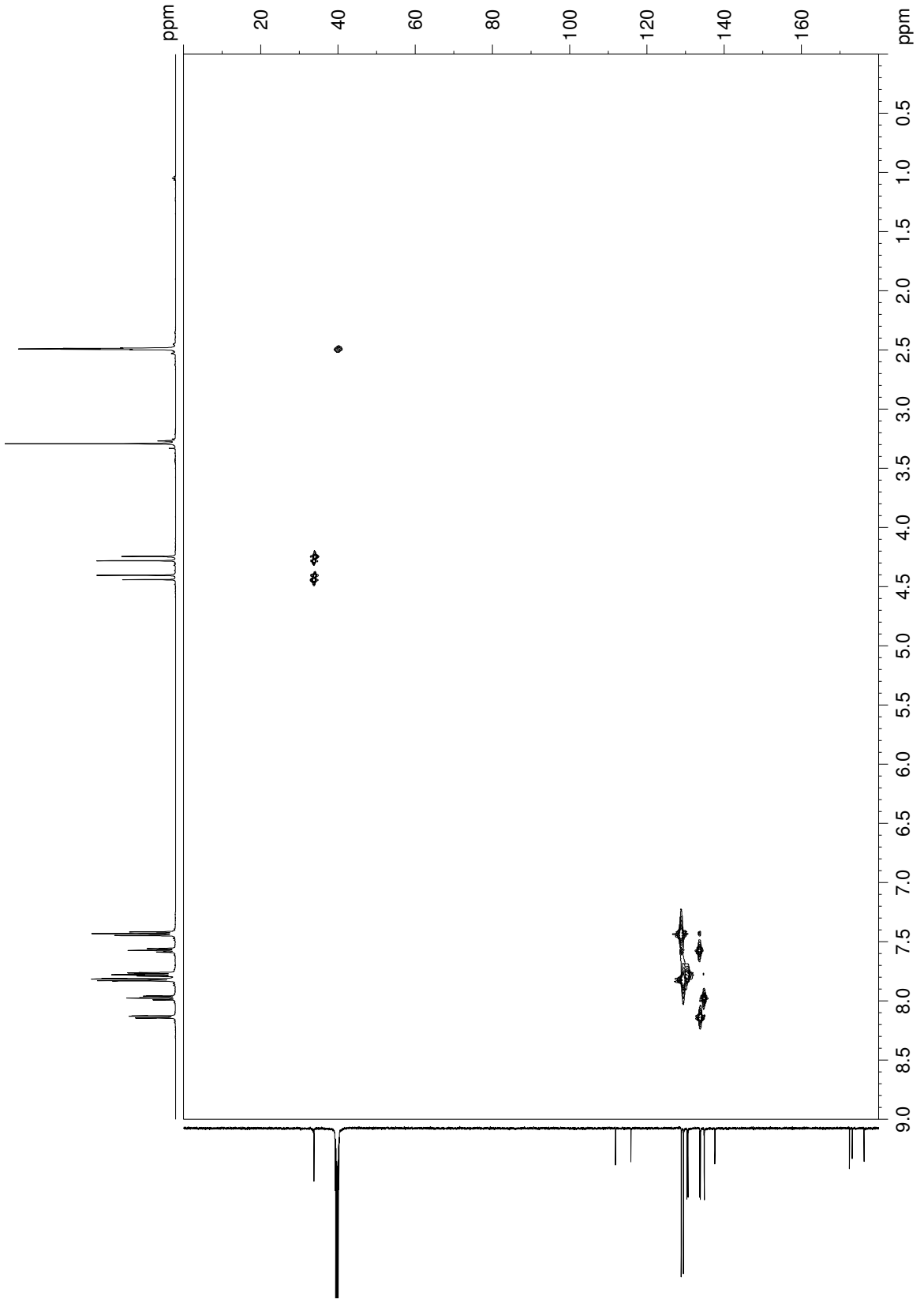
For details see Supplementary Information of ref.[87]

HMBC spectrum of **38**

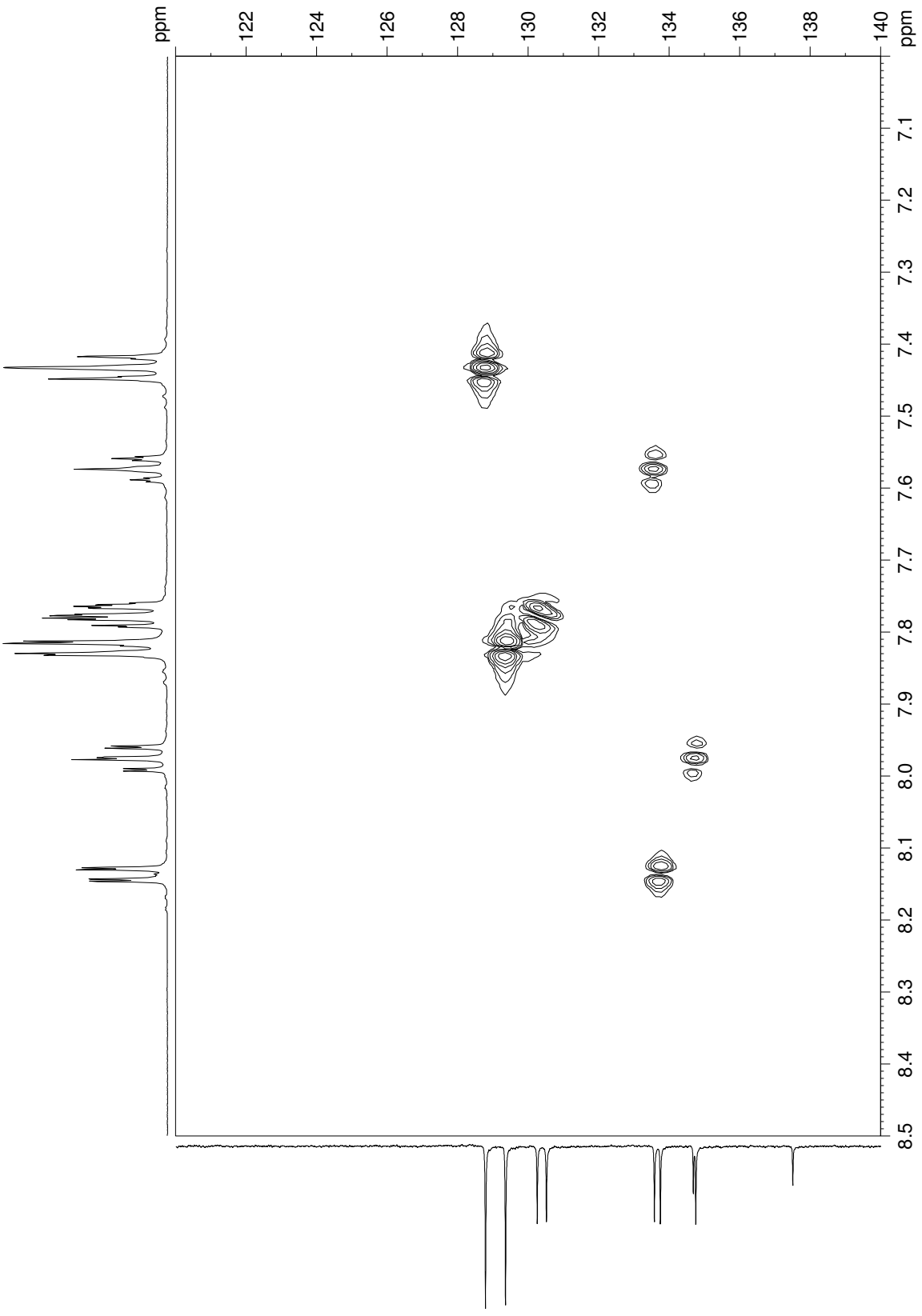


For details see Supplementary Information of ref.[87]

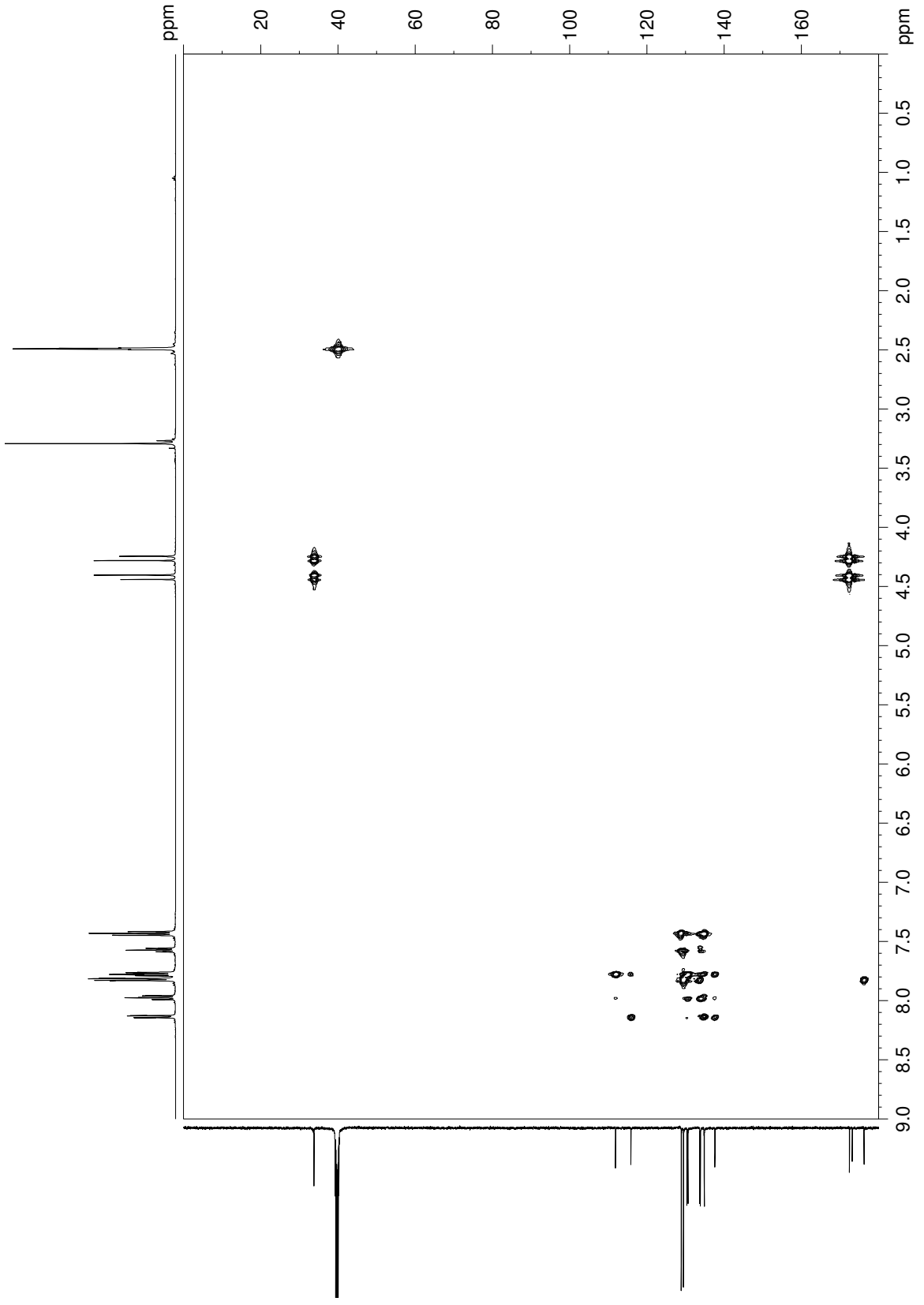
HMQC spectrum of **48**



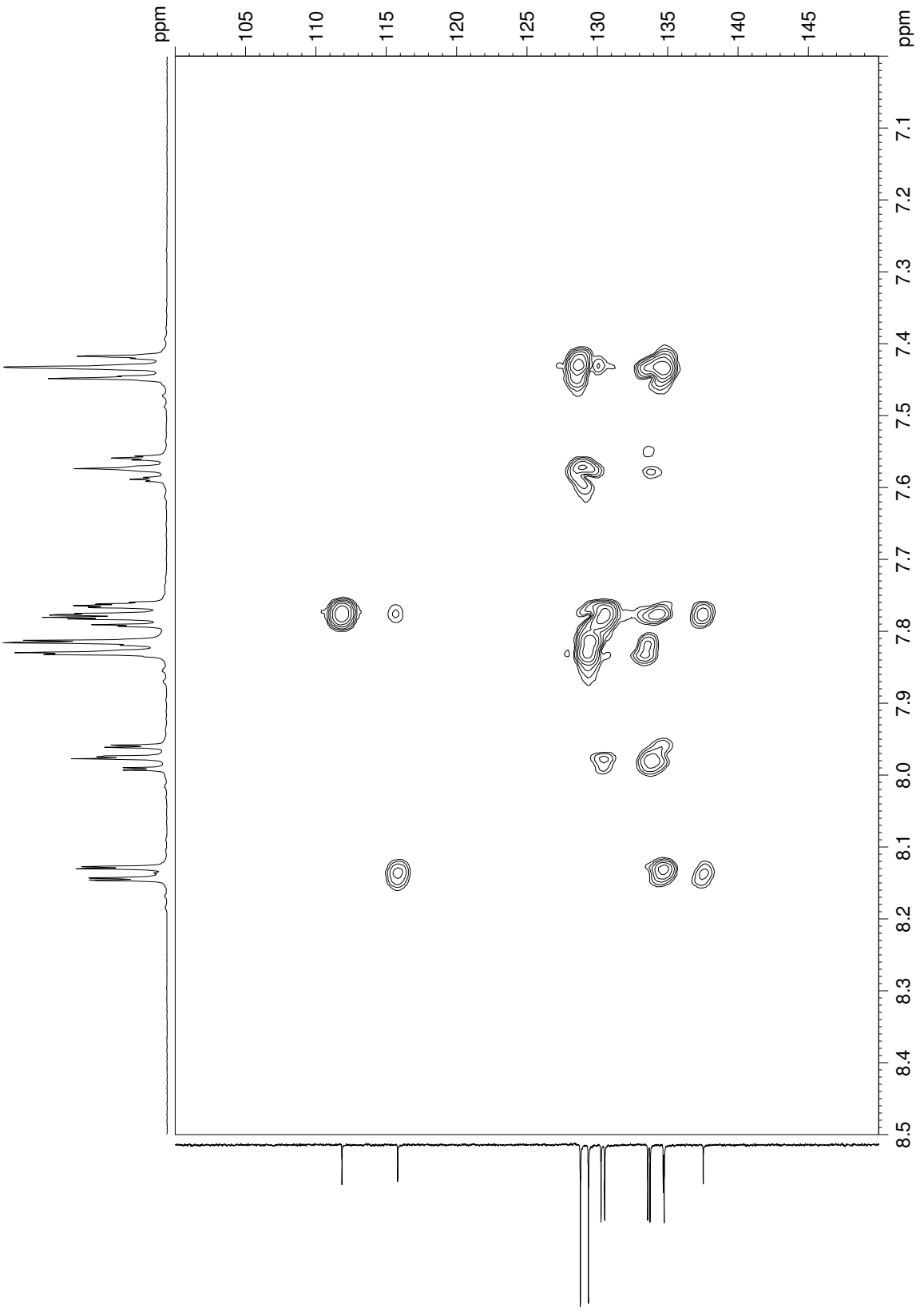
HMQC spectrum (extract) of **48**



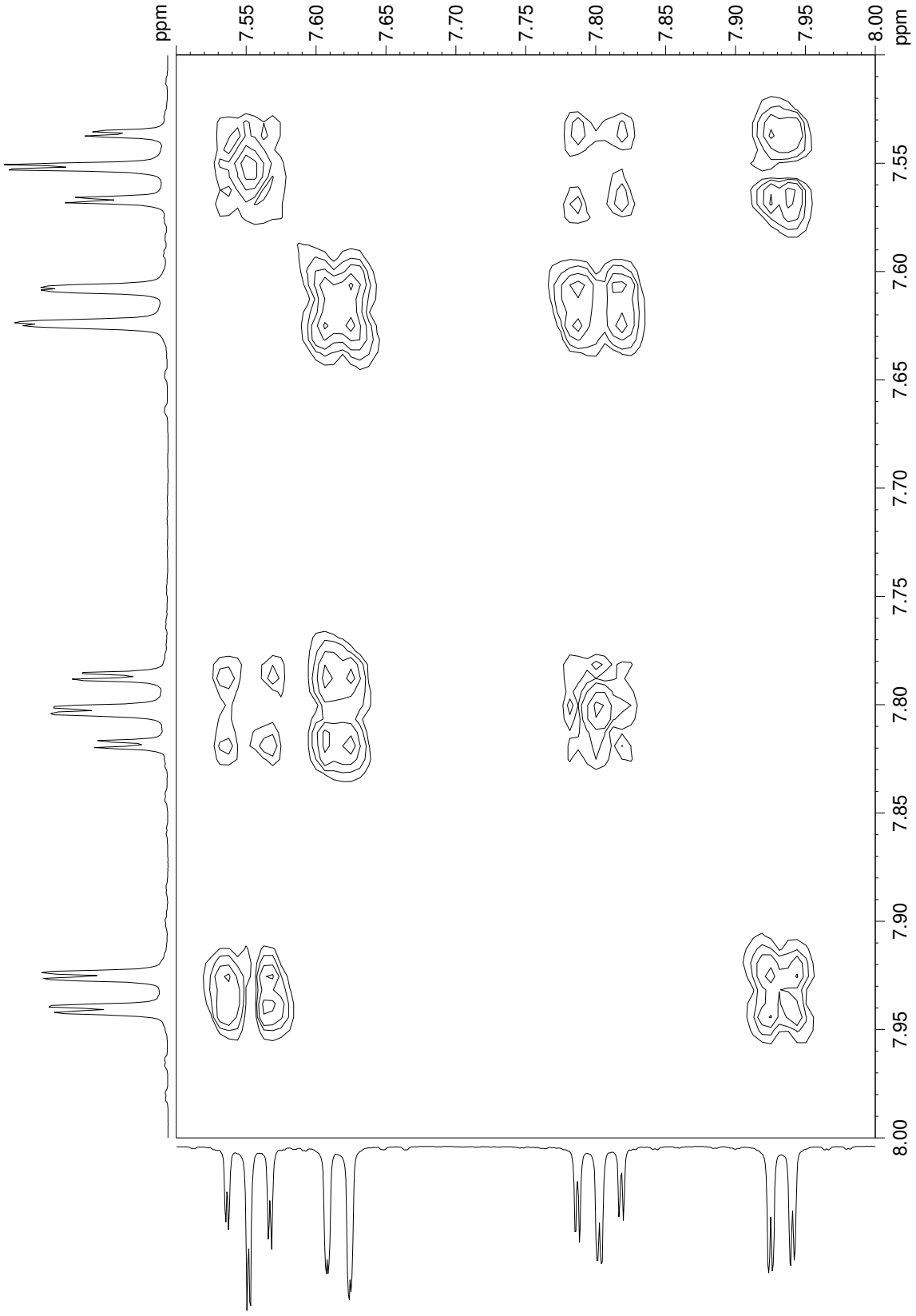
HMBC spectrum of **48**



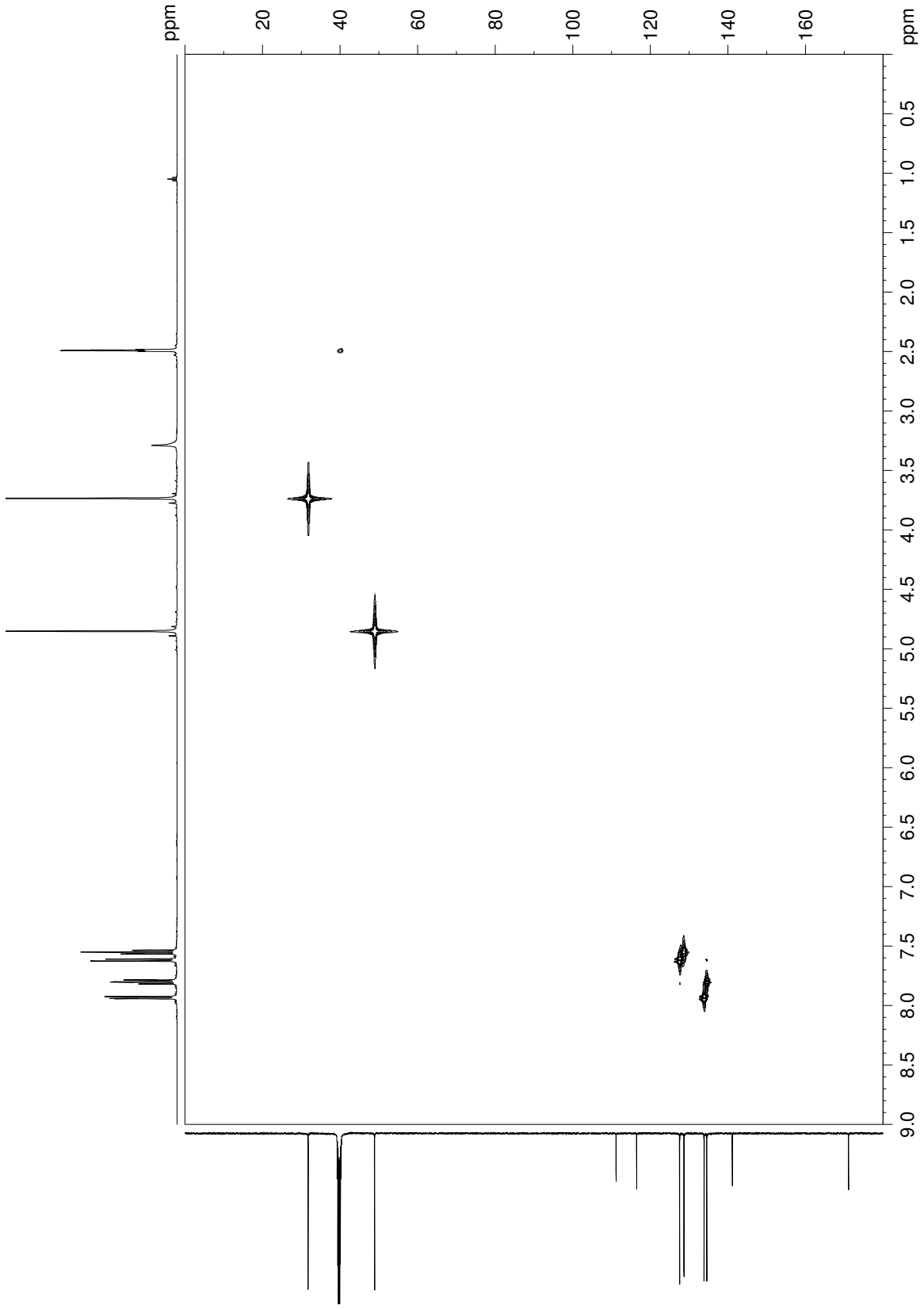
HMBC spectrum (extract) of **48**



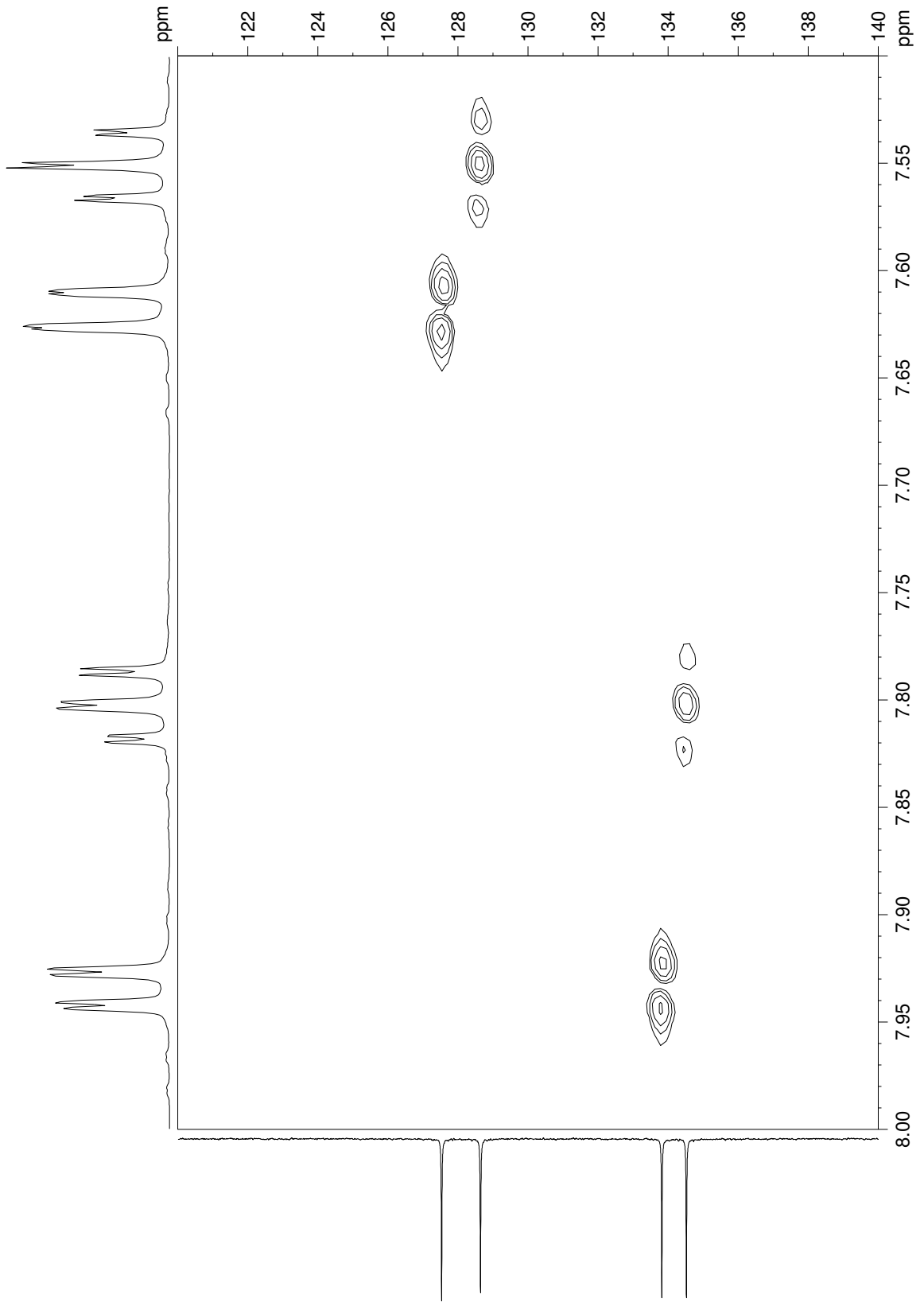
COSY spectrum of **51**



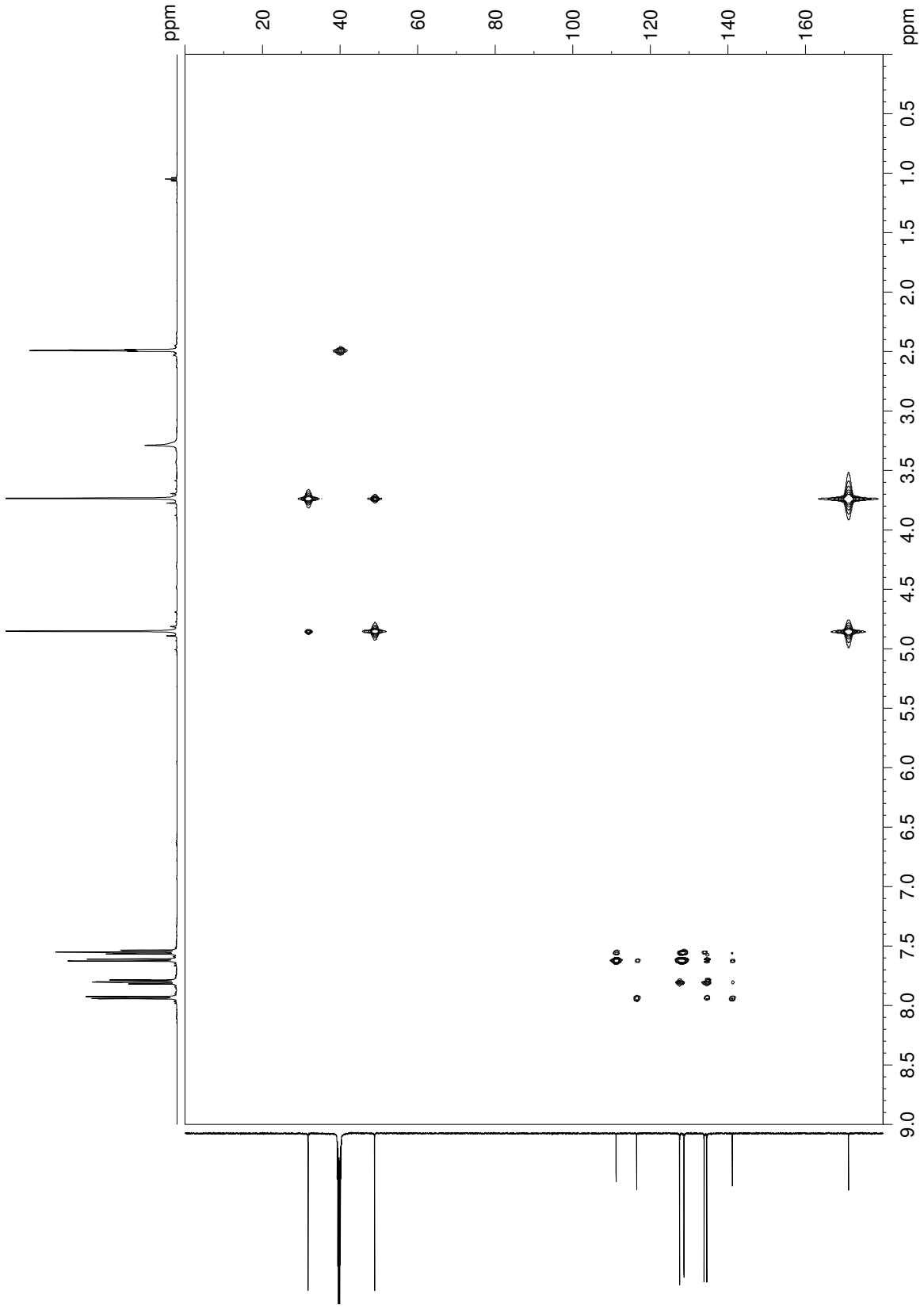
HMQC spectrum of **51**



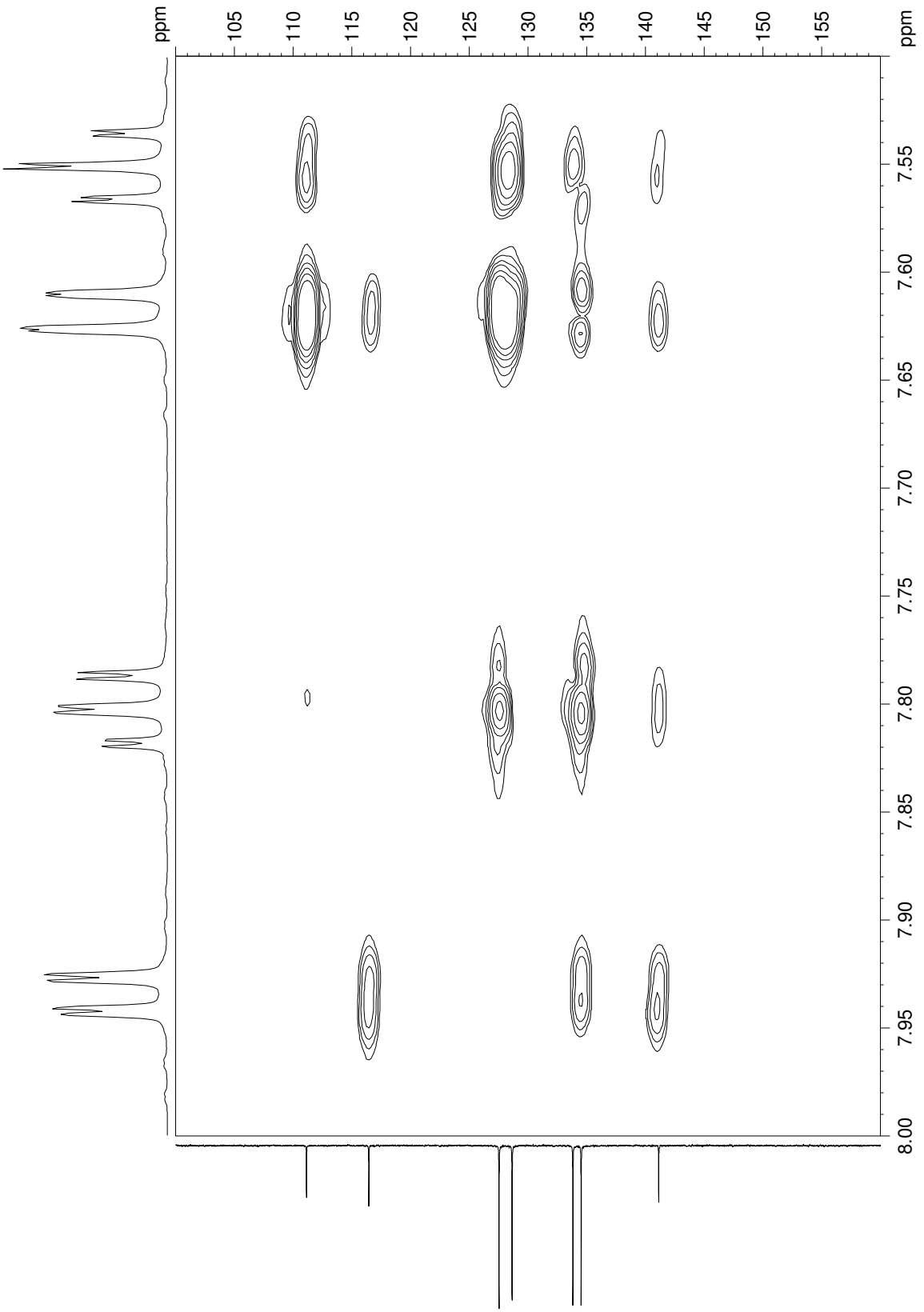
HMQC (extract) spectrum of **51**



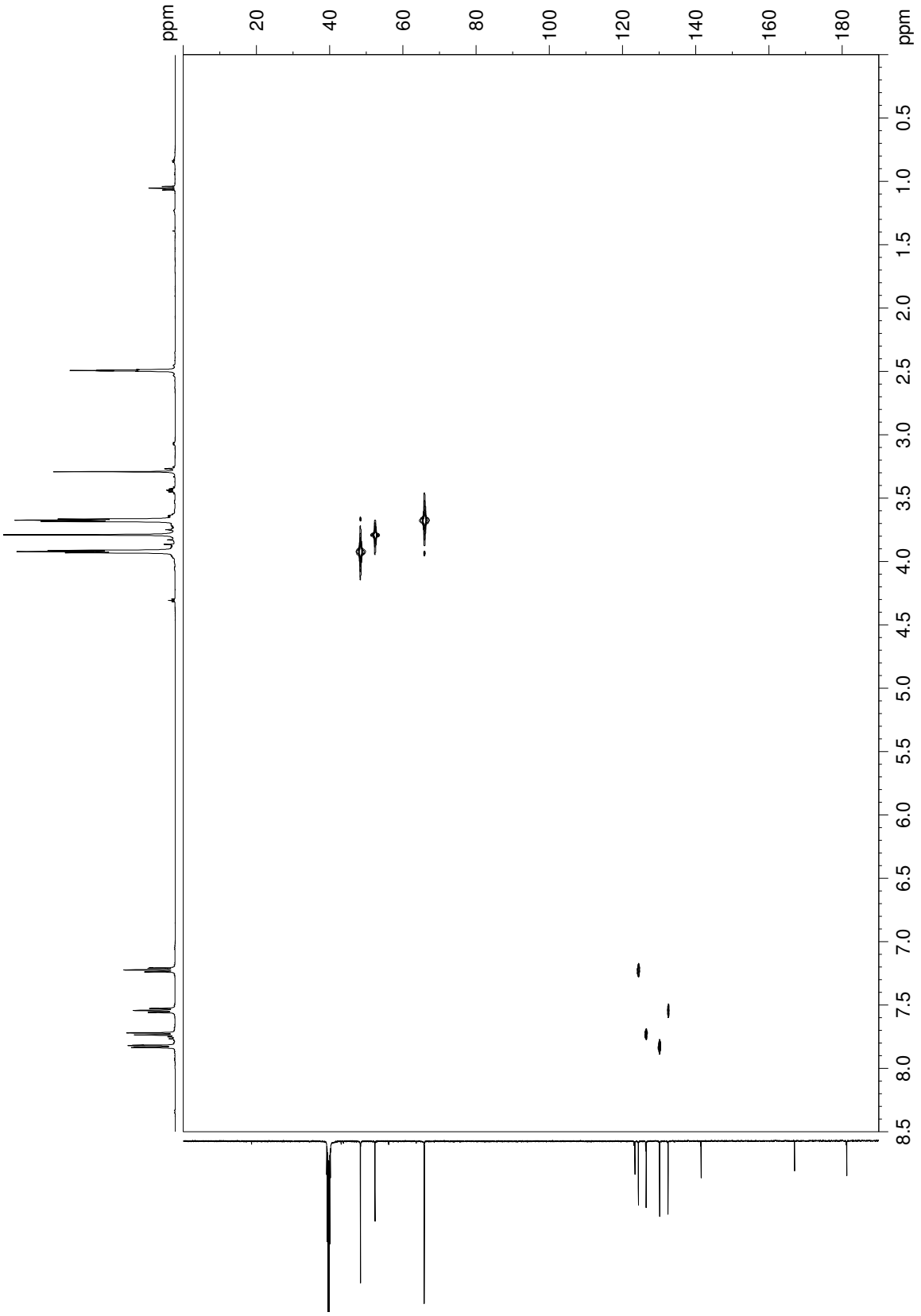
HMBC spectrum of **51**



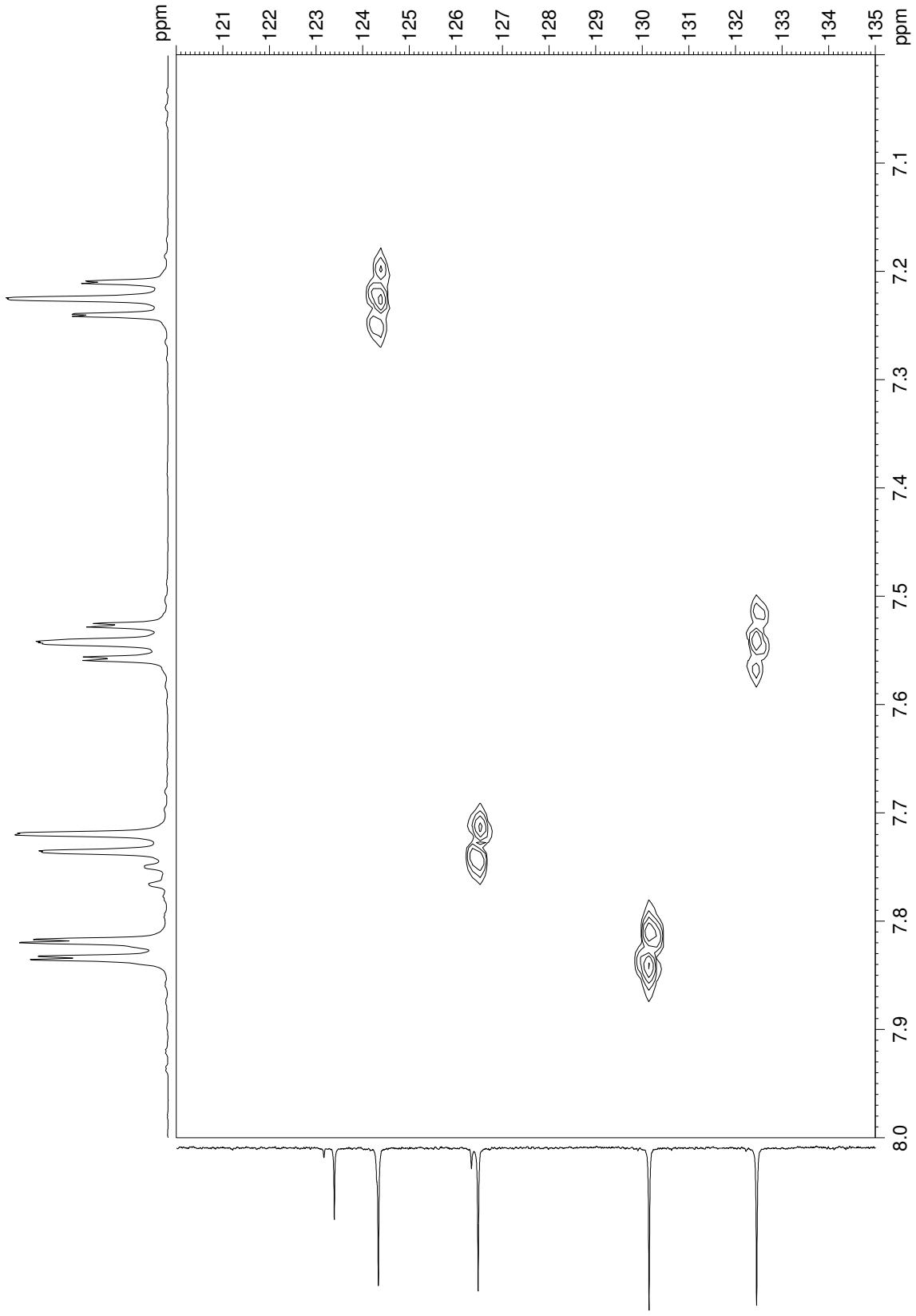
HMBC spectrum (extract) of **51**



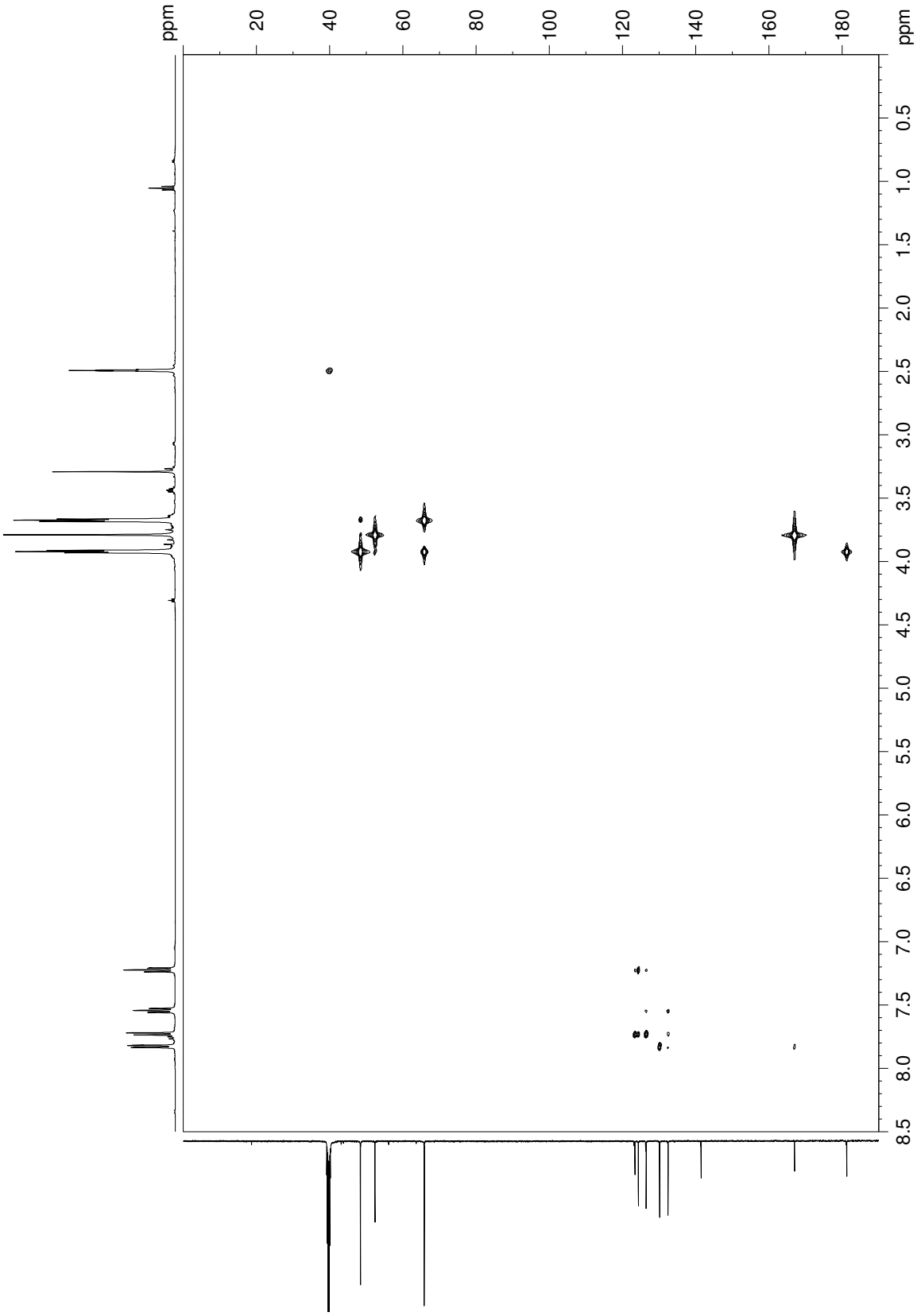
HMQC spectrum of **77**



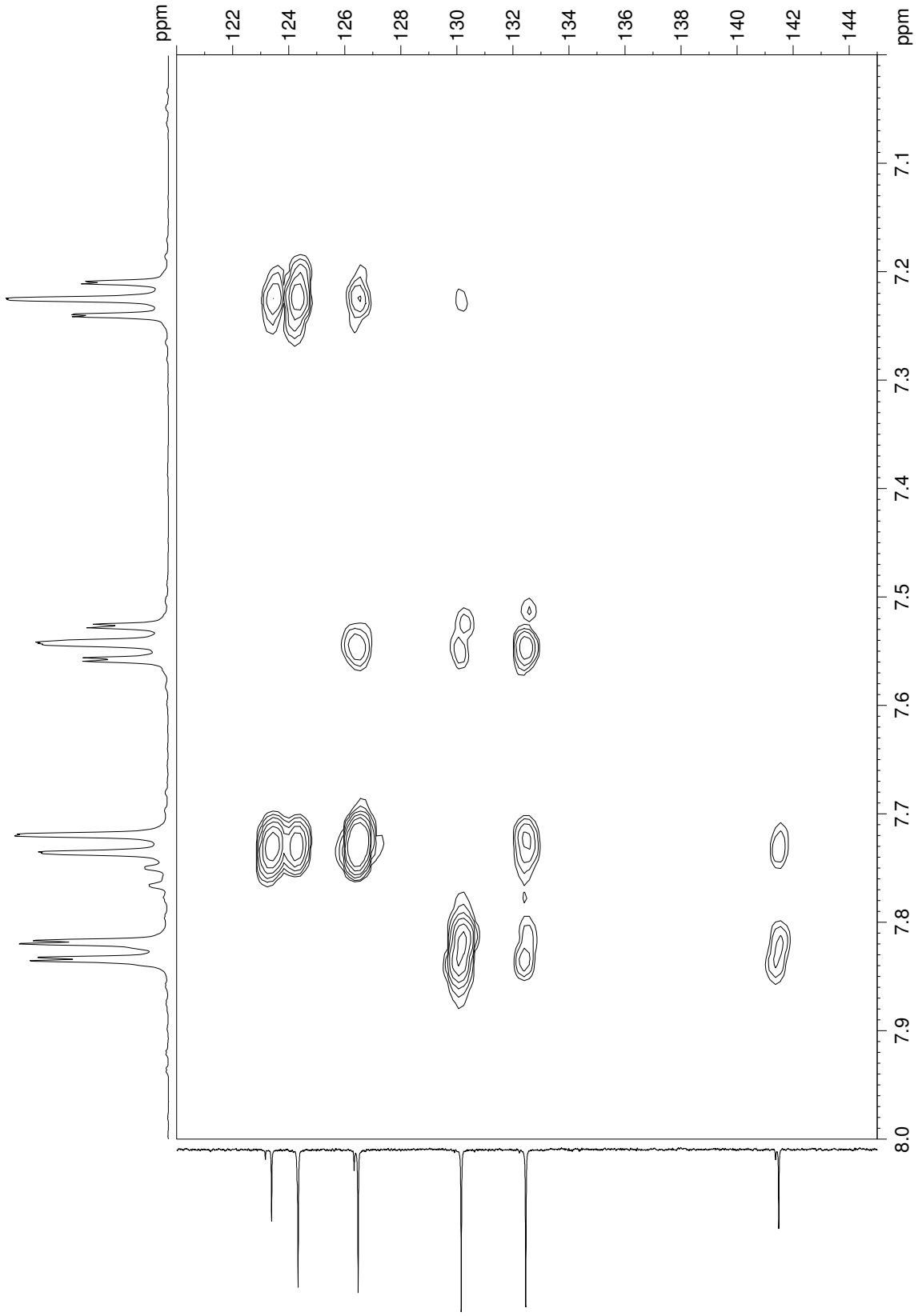
HMQC (extract) spectrum of **77**



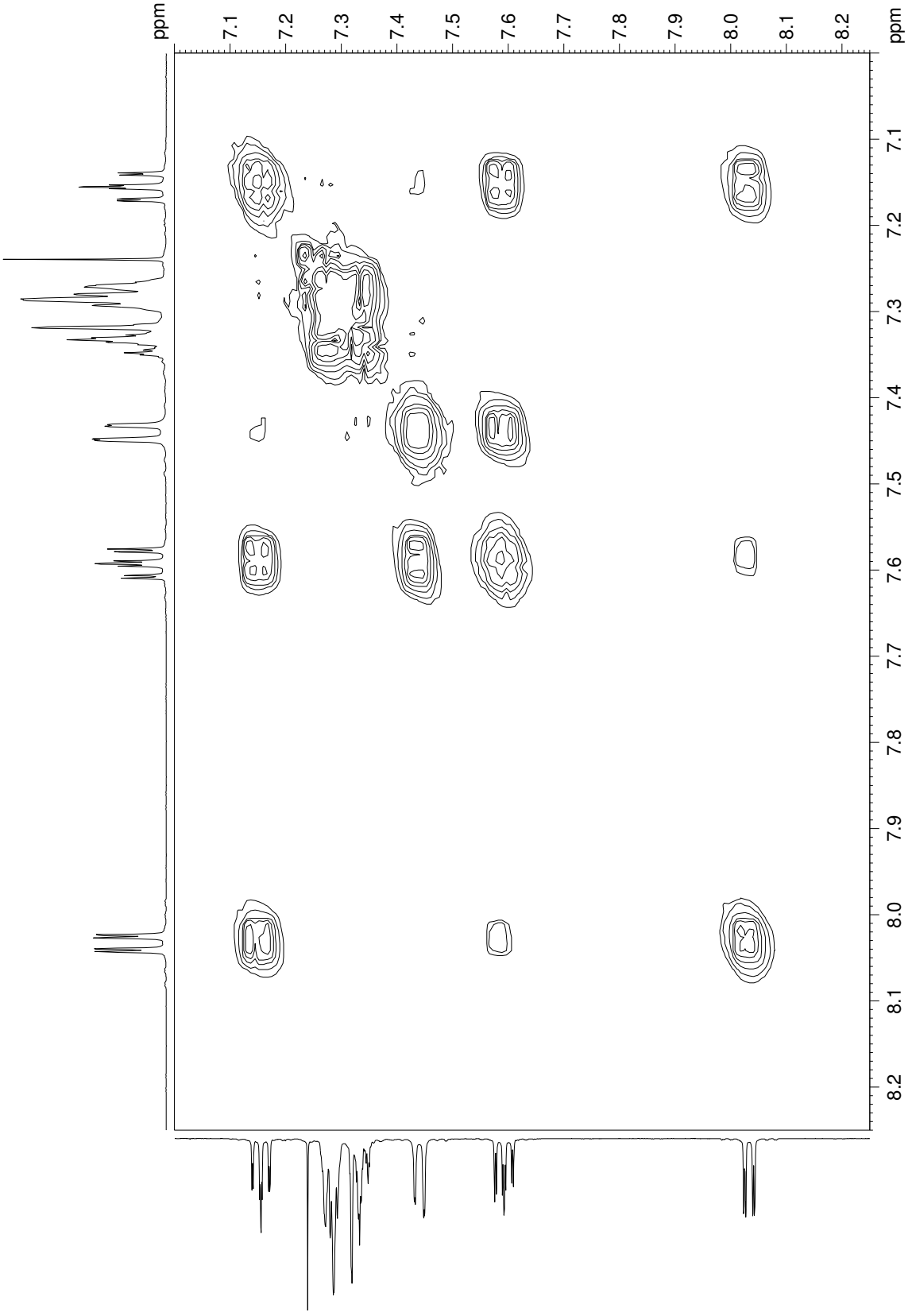
HMBC spectrum of **77**



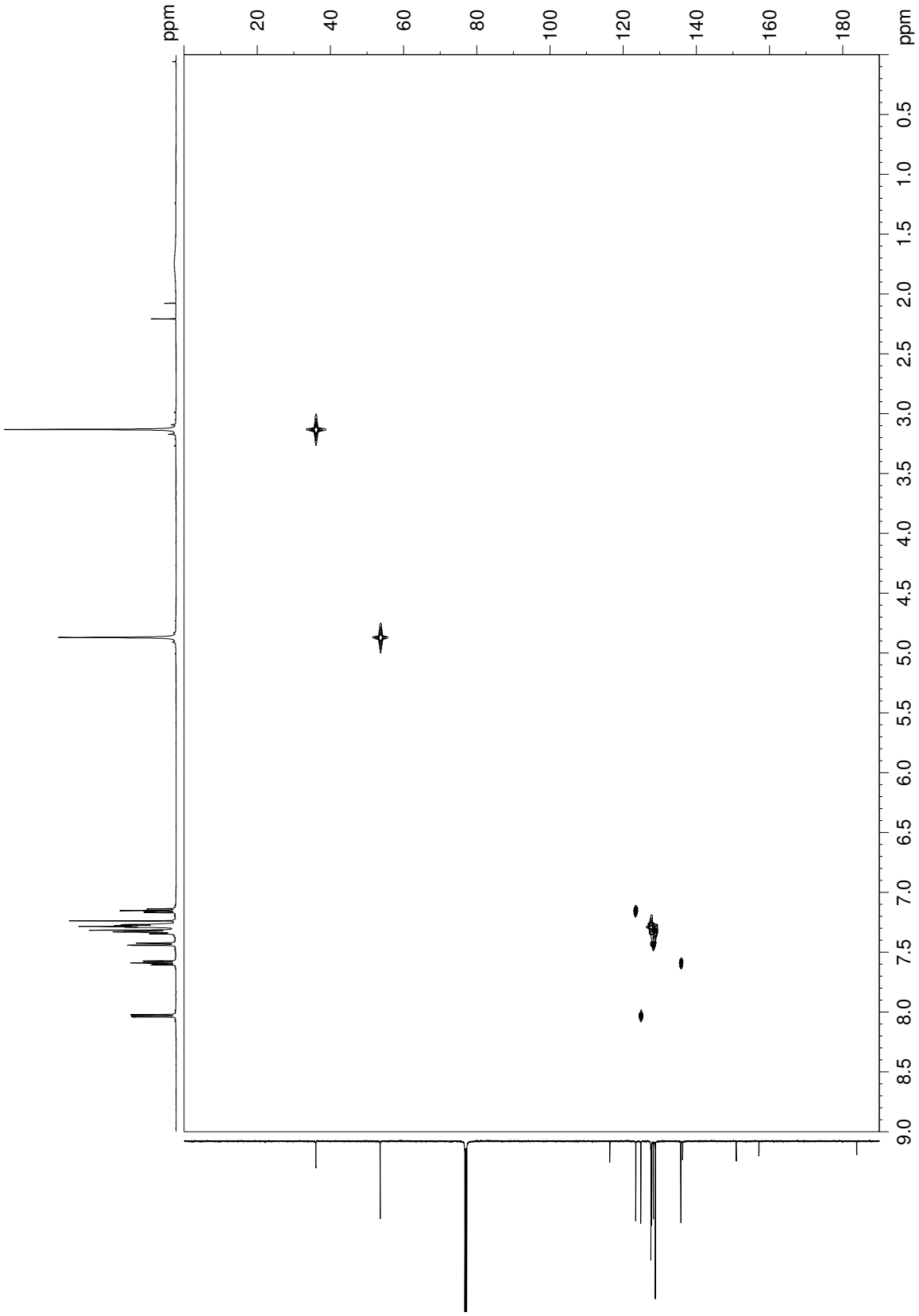
HMBC spectrum (extract) of **77**



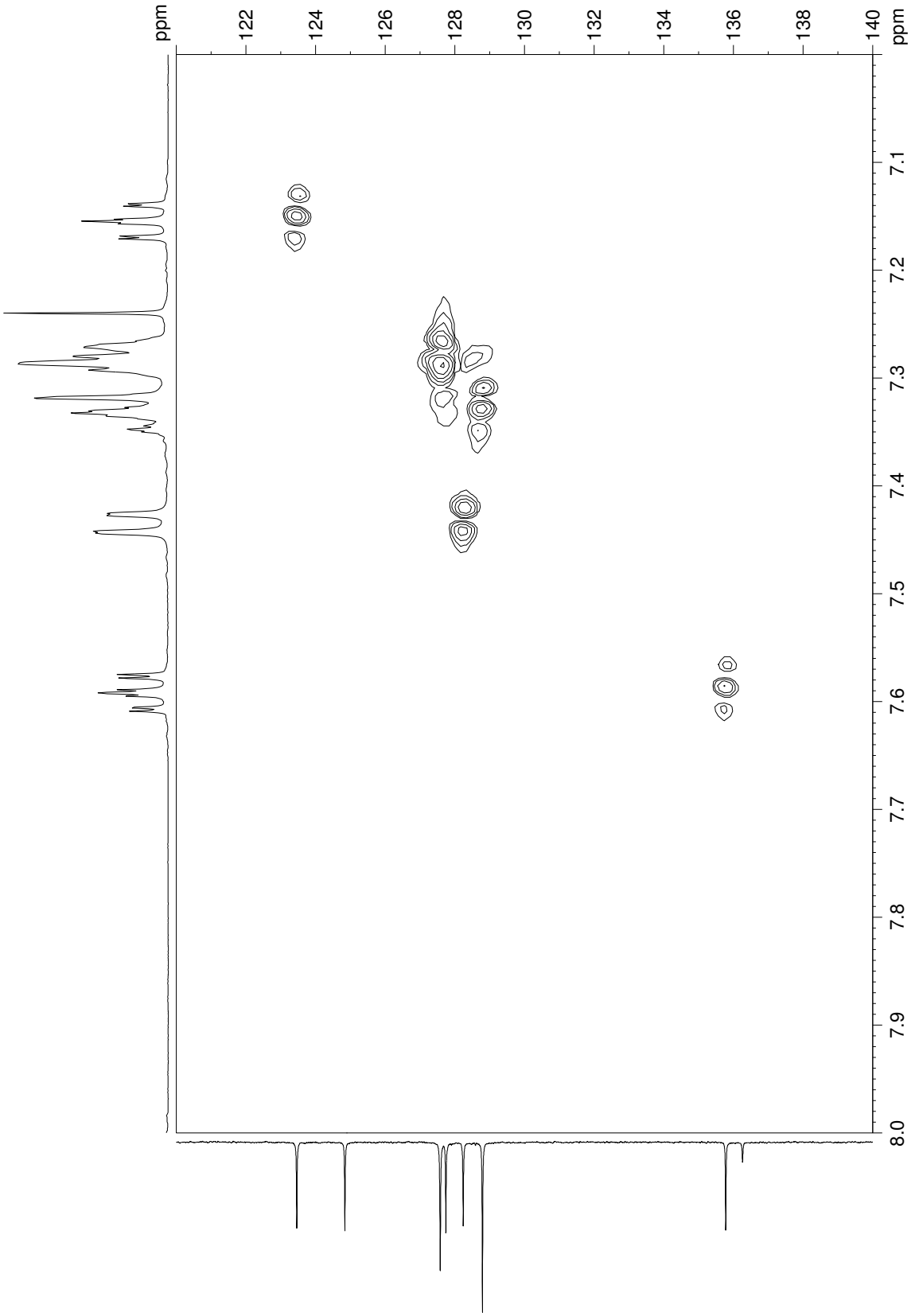
COSY spectrum of **126**



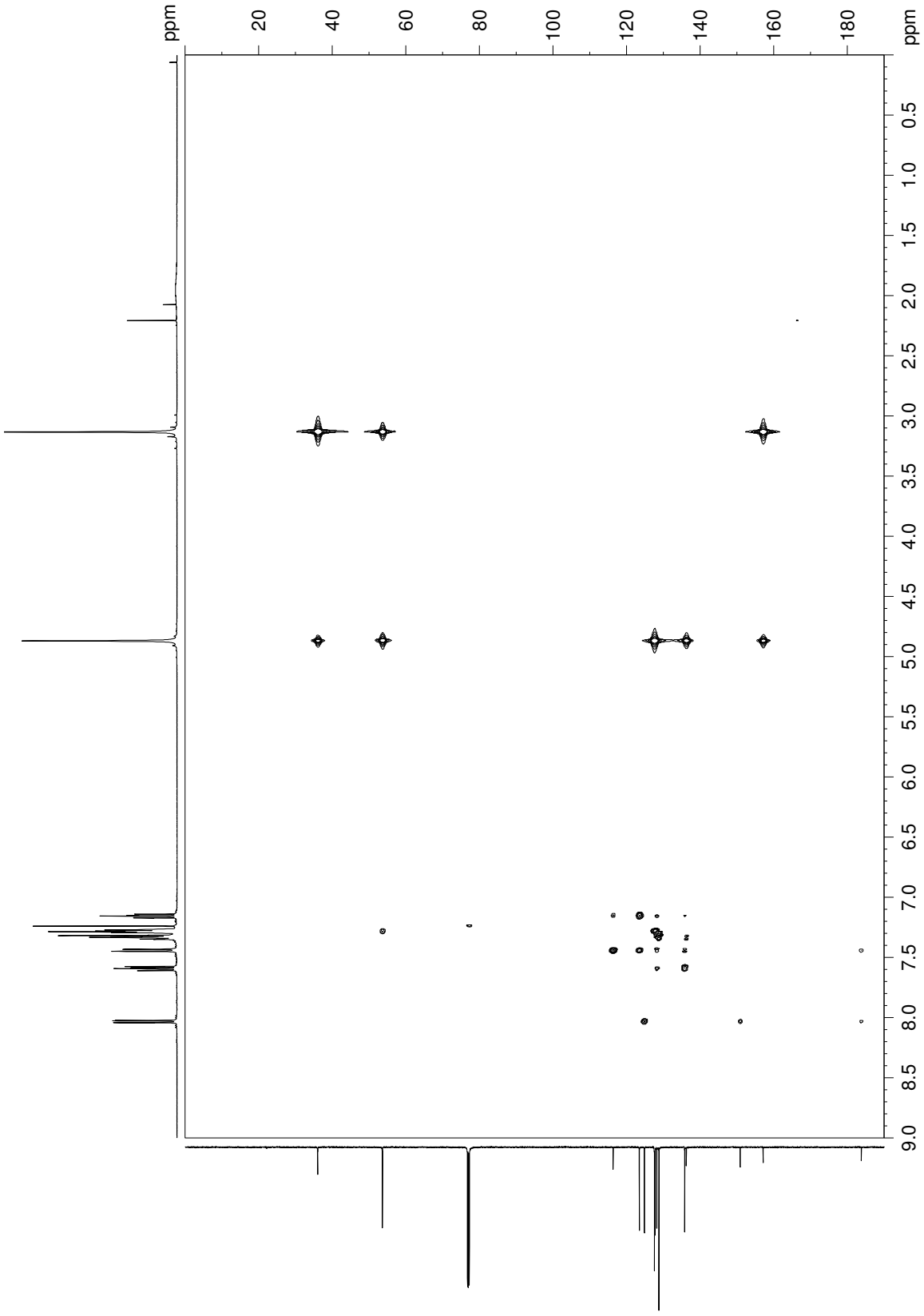
HMQC spectrum of **126**



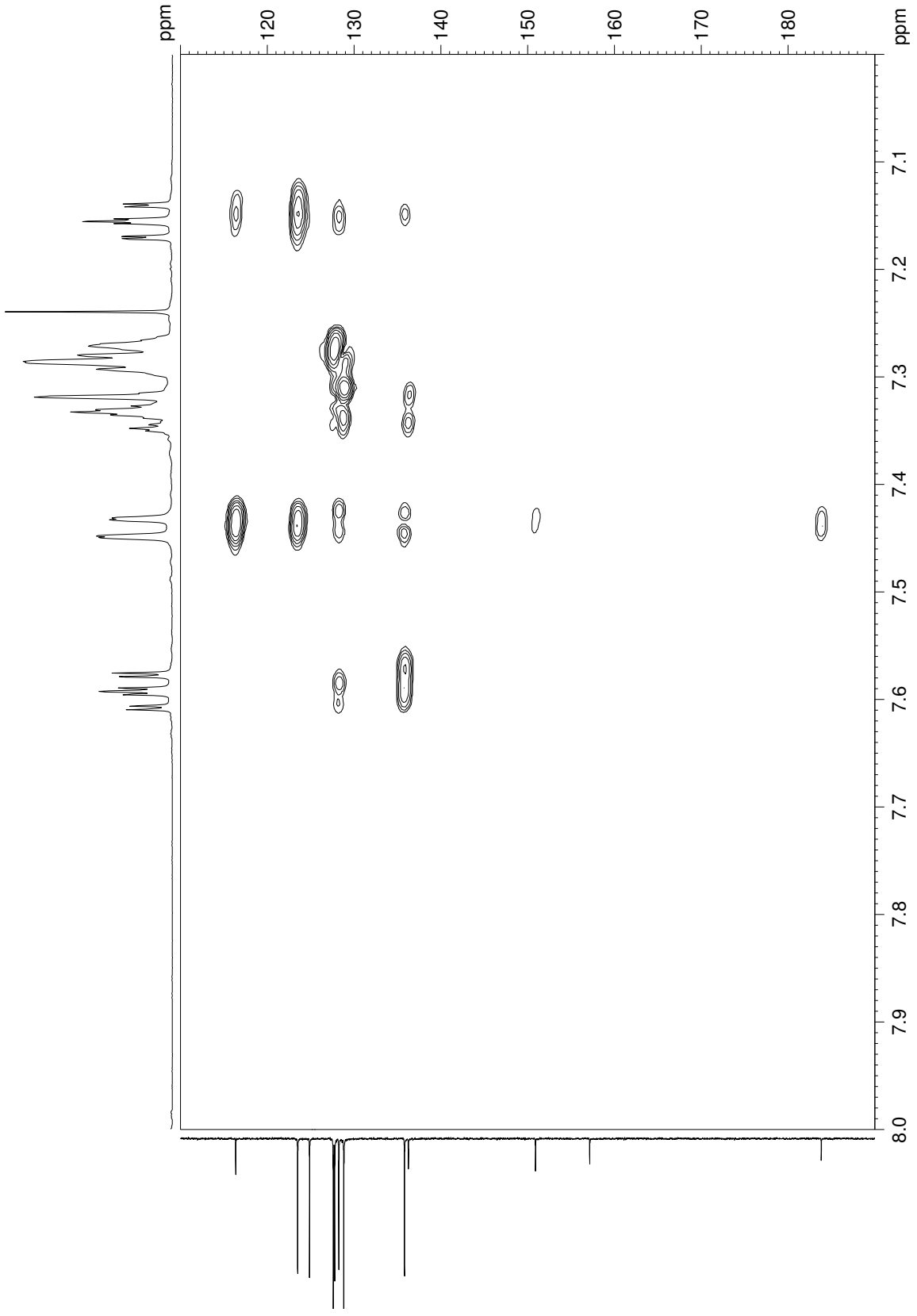
HMQC (extract) spectrum of **126**



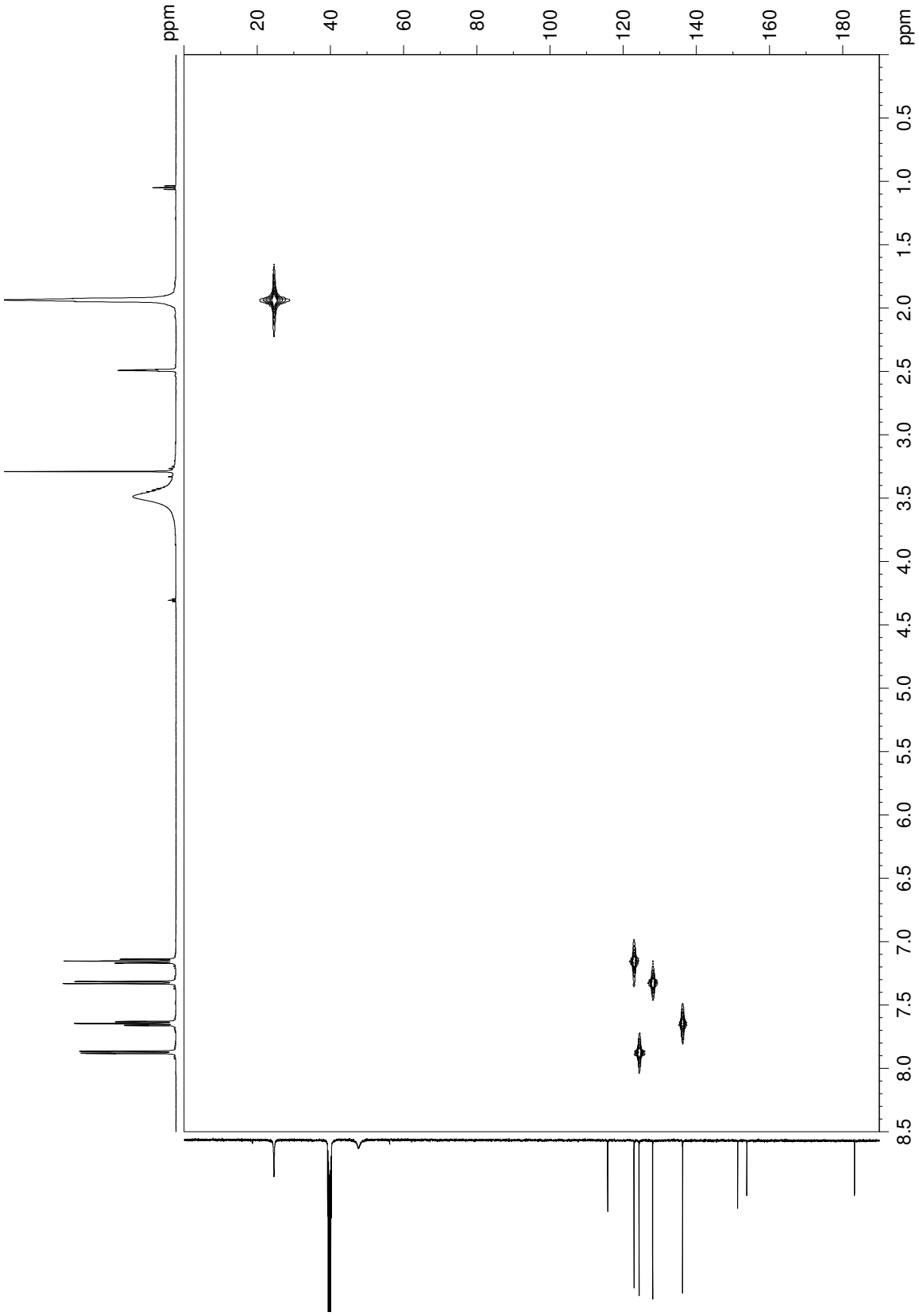
HMBC spectrum of **126**



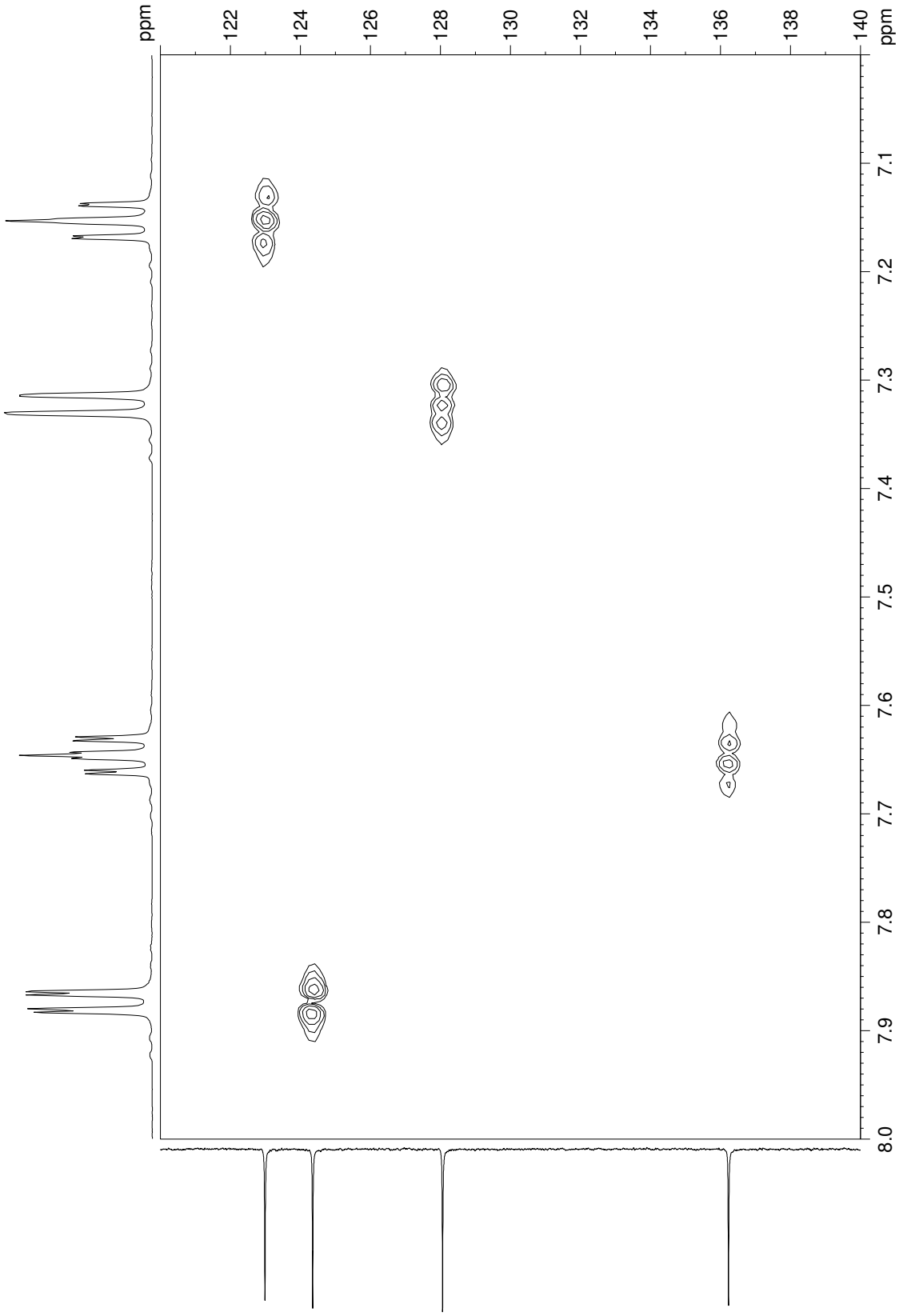
HMBC spectrum (extract) of **126**



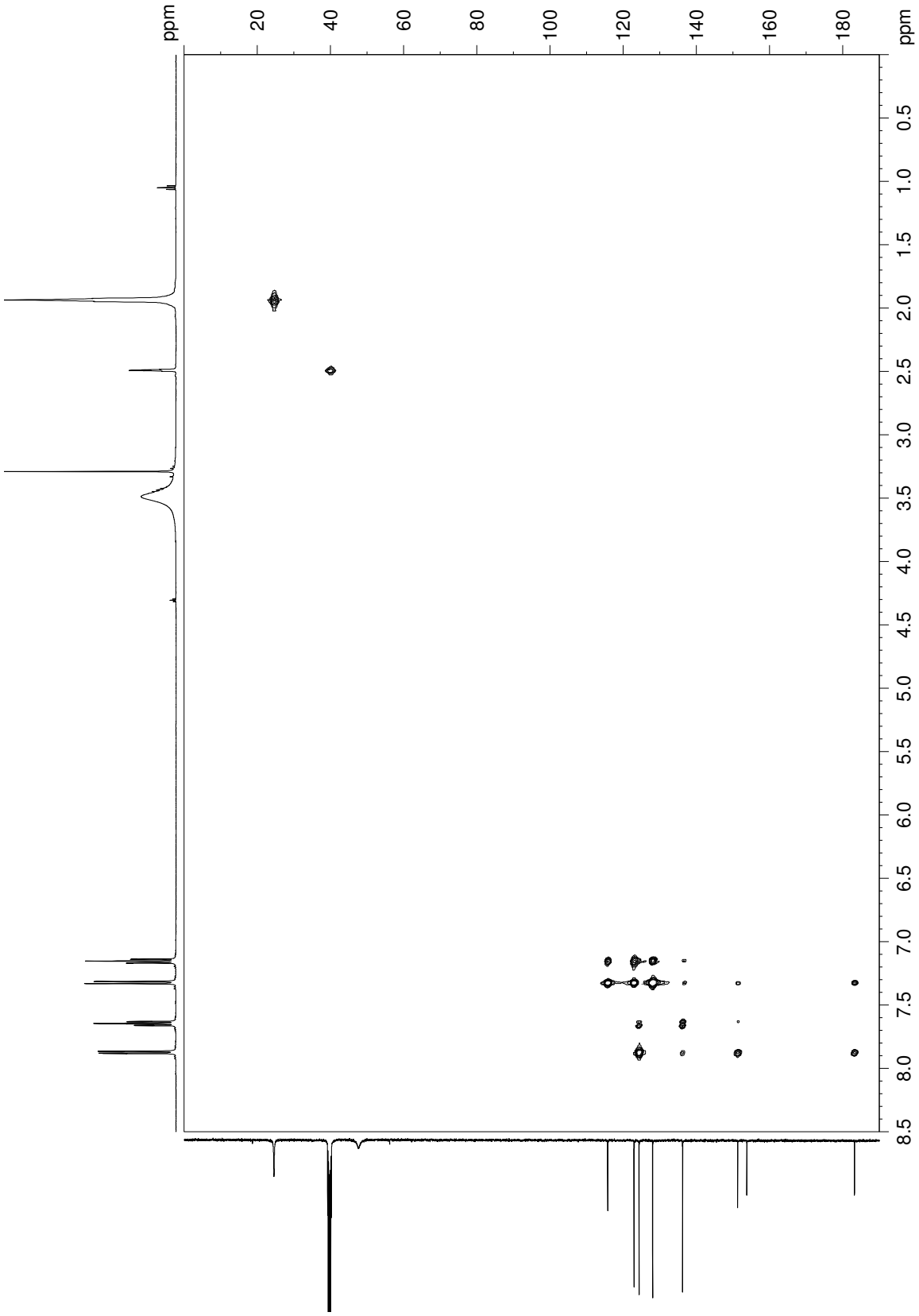
HMQC spectrum of **128**



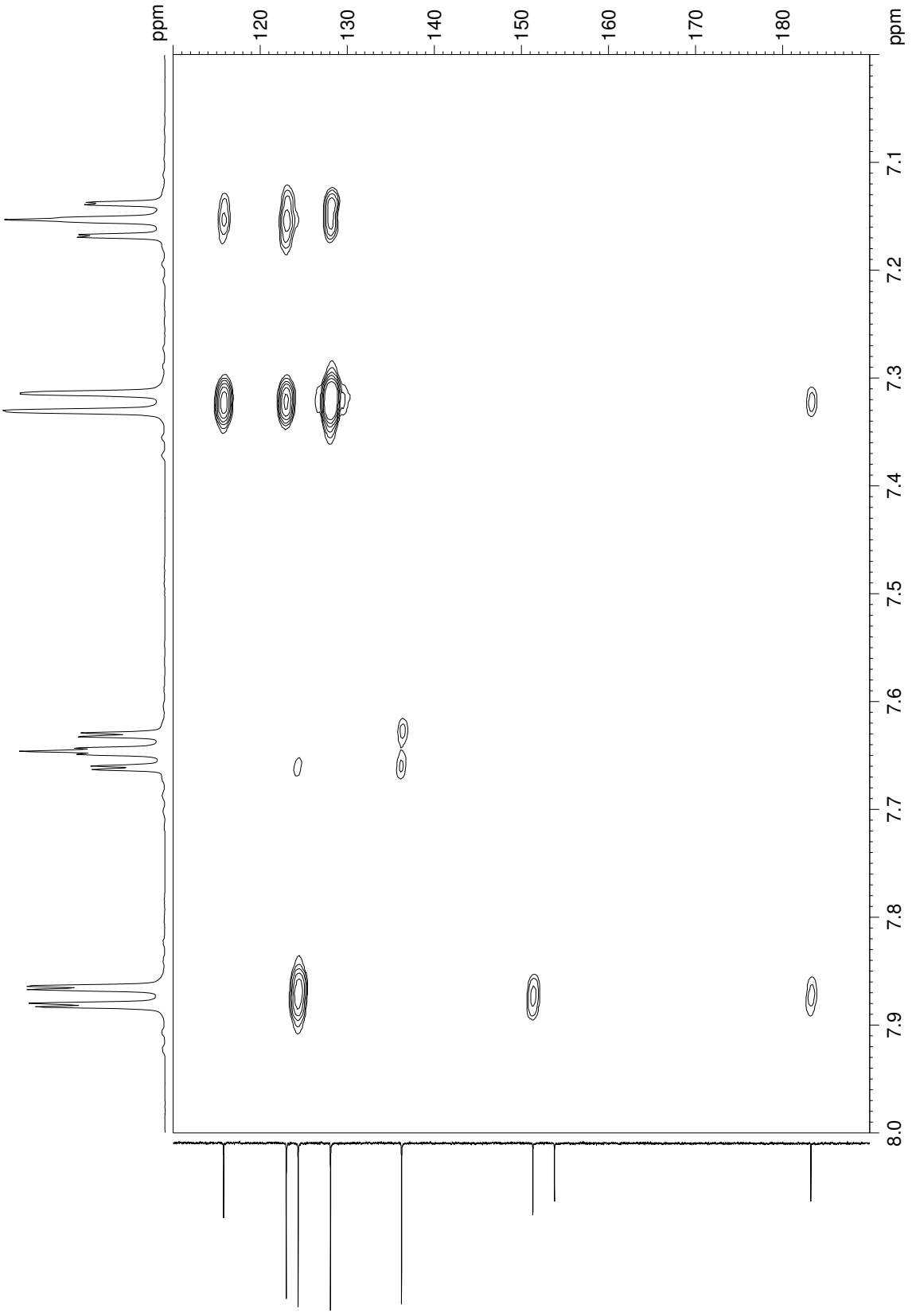
HMQC (extract) spectrum of **128**



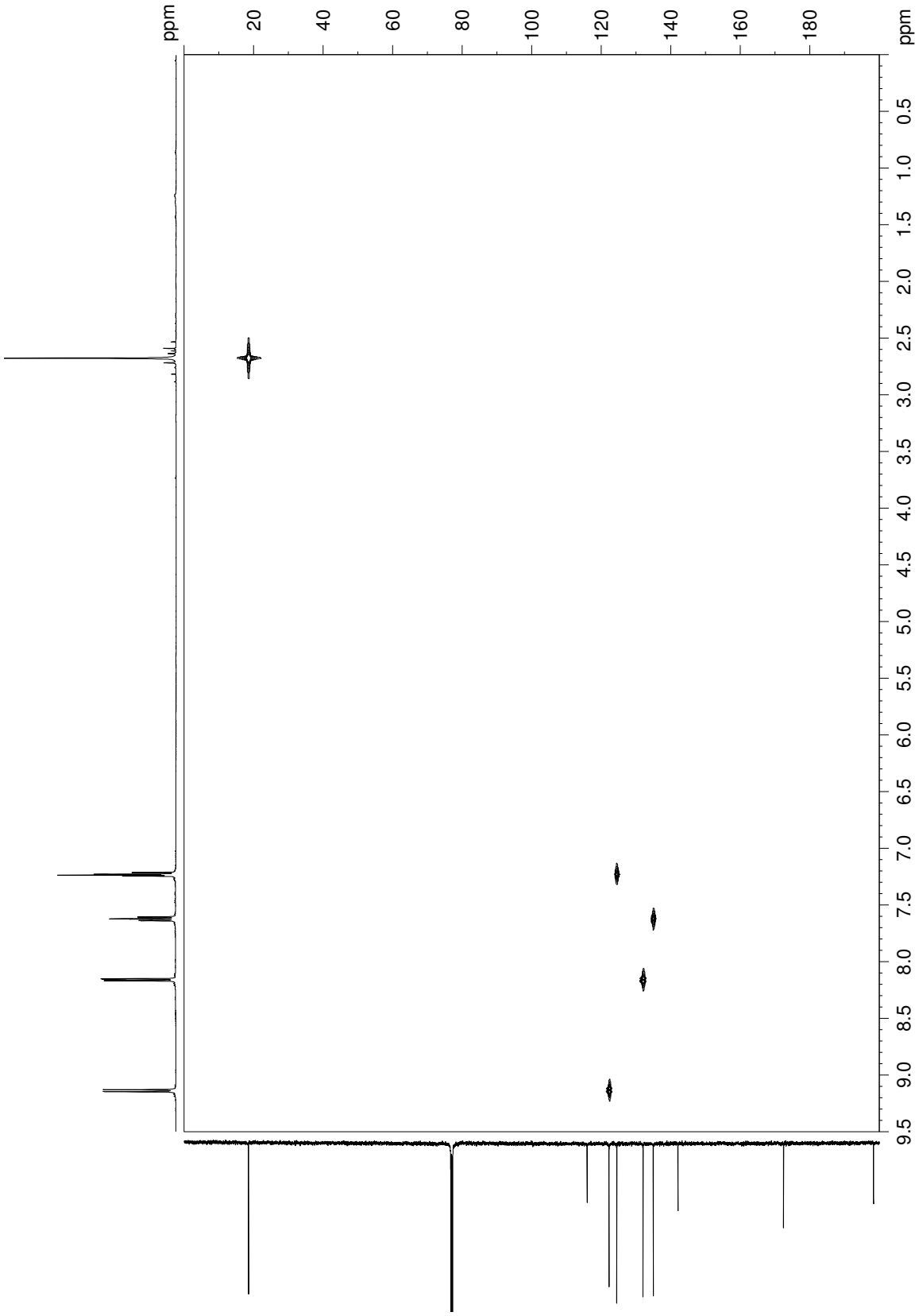
HMBC spectrum of **128**



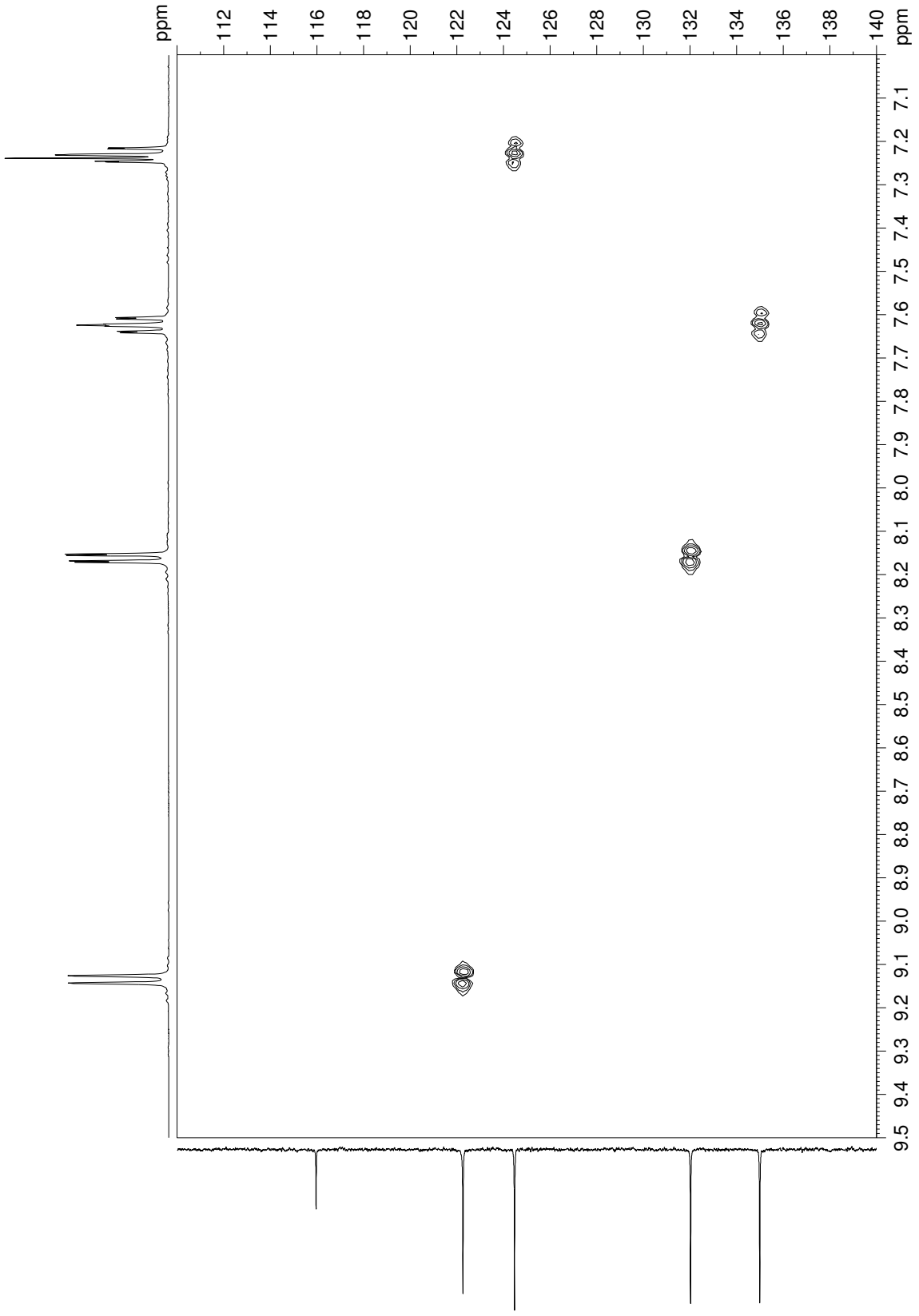
HMBC spectrum (extract) of **128**



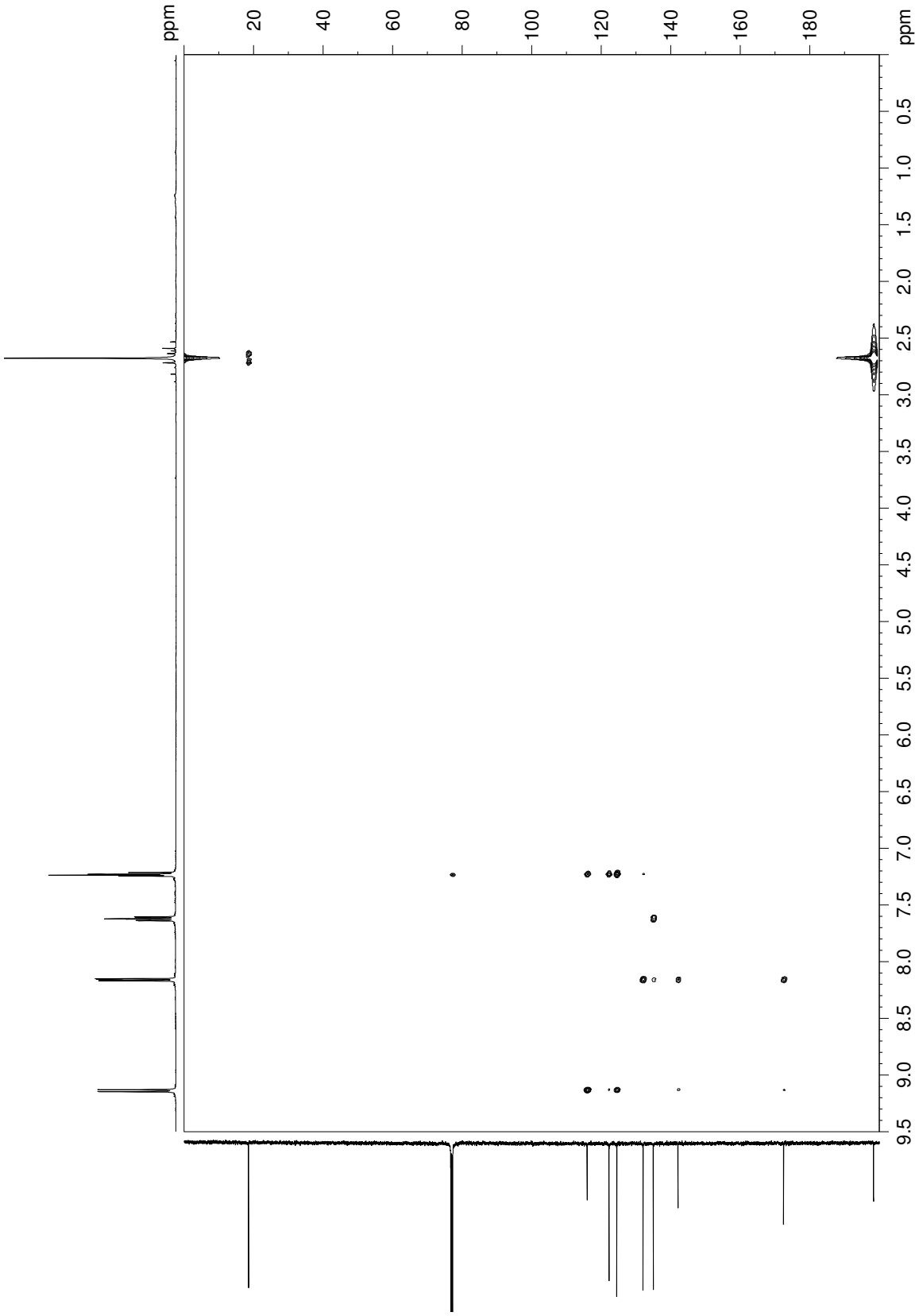
HMQC spectrum of **131**



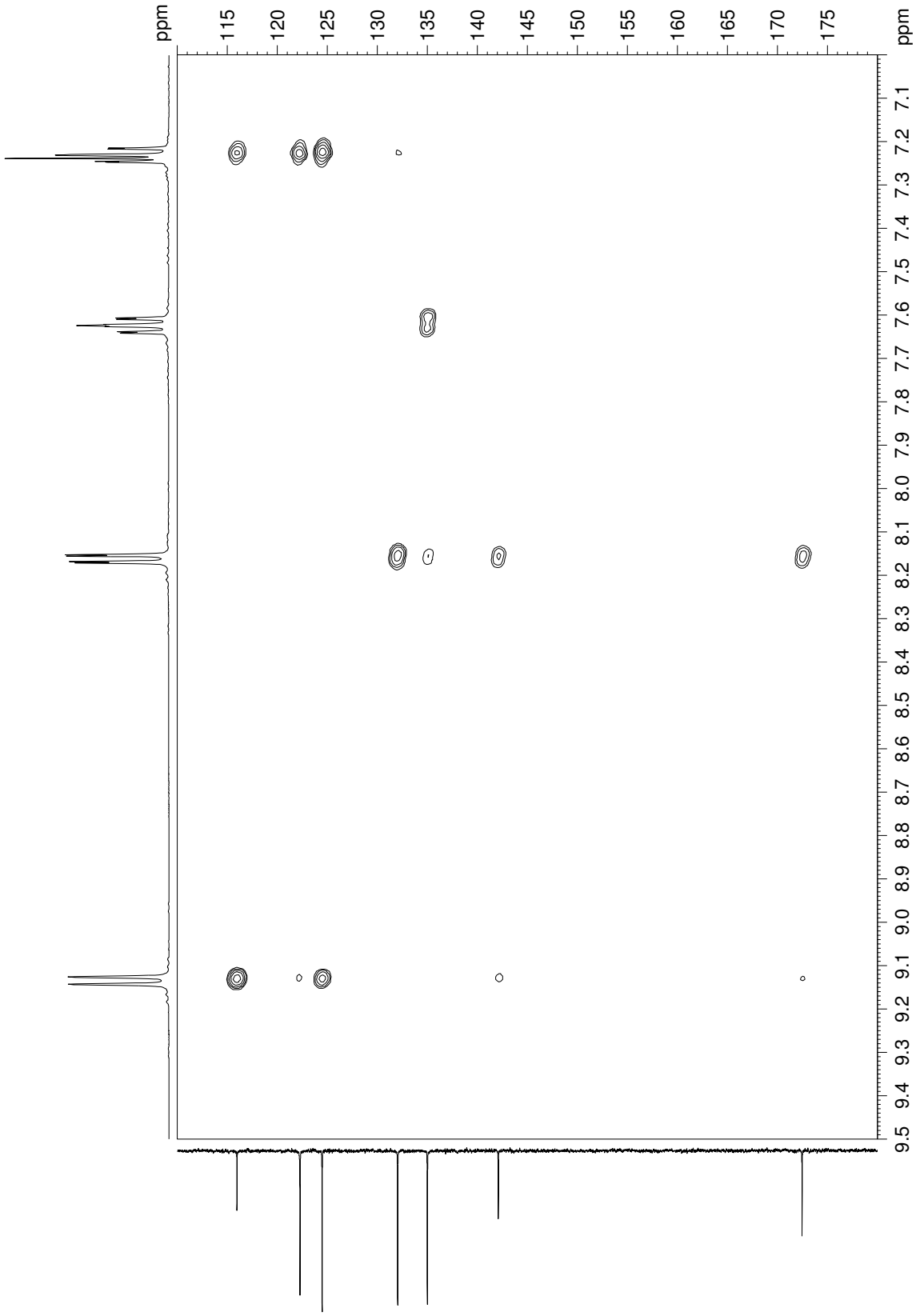
HMQC (extract) spectrum of **131**



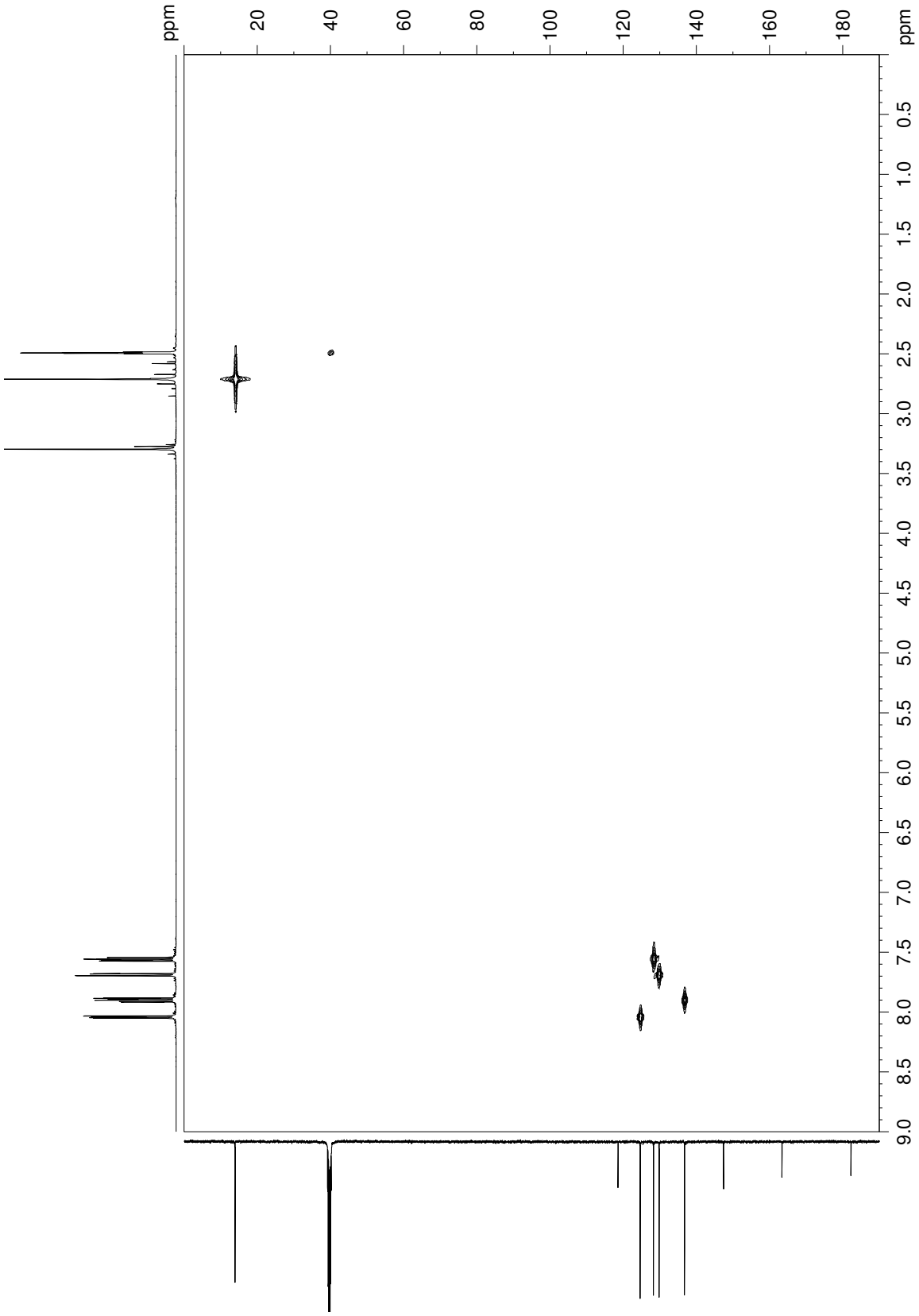
HMBC spectrum of **131**



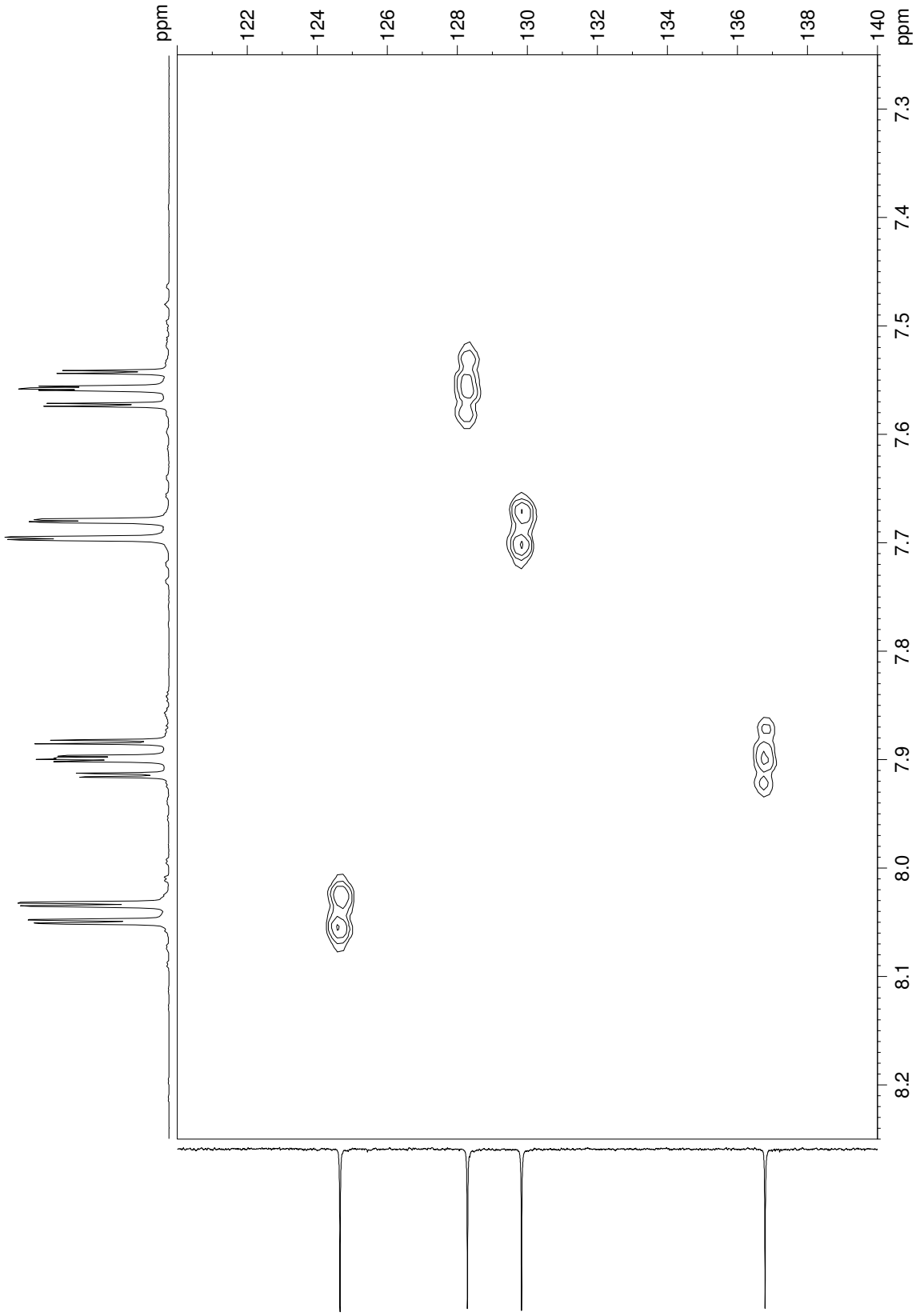
HMBC spectrum (extract) of **131**



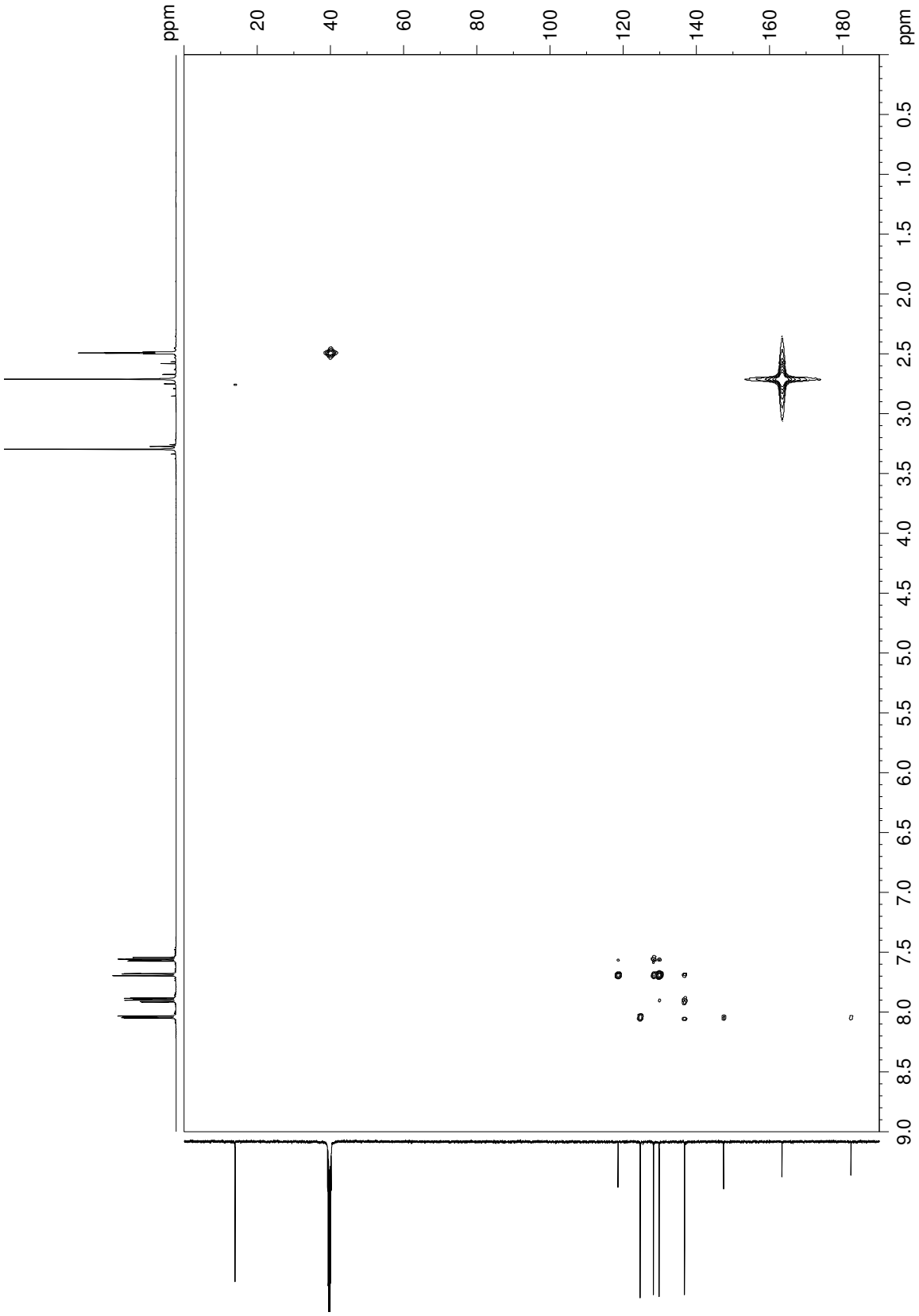
HMQC spectrum of **132**



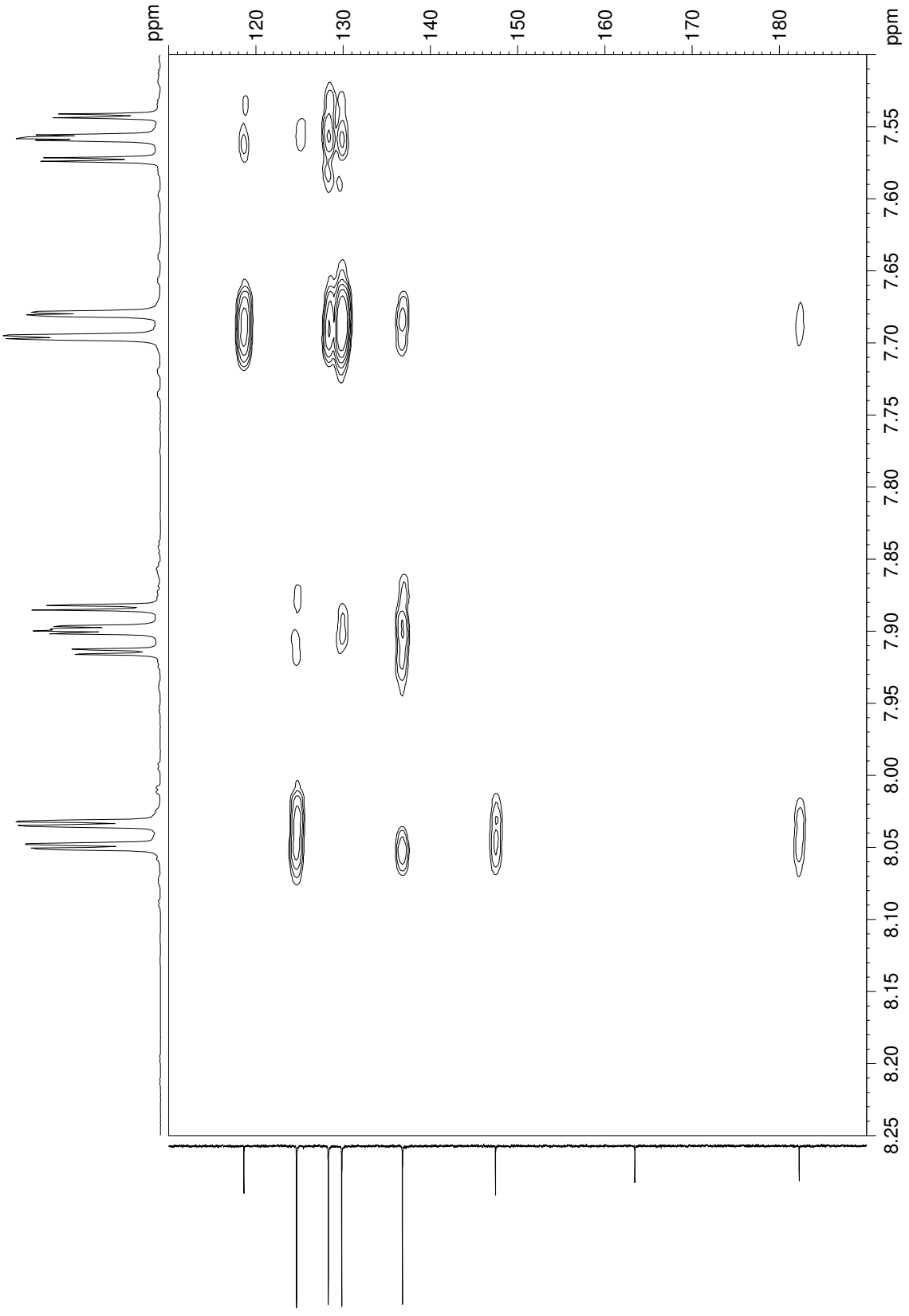
HMQC (extract) spectrum of **132**



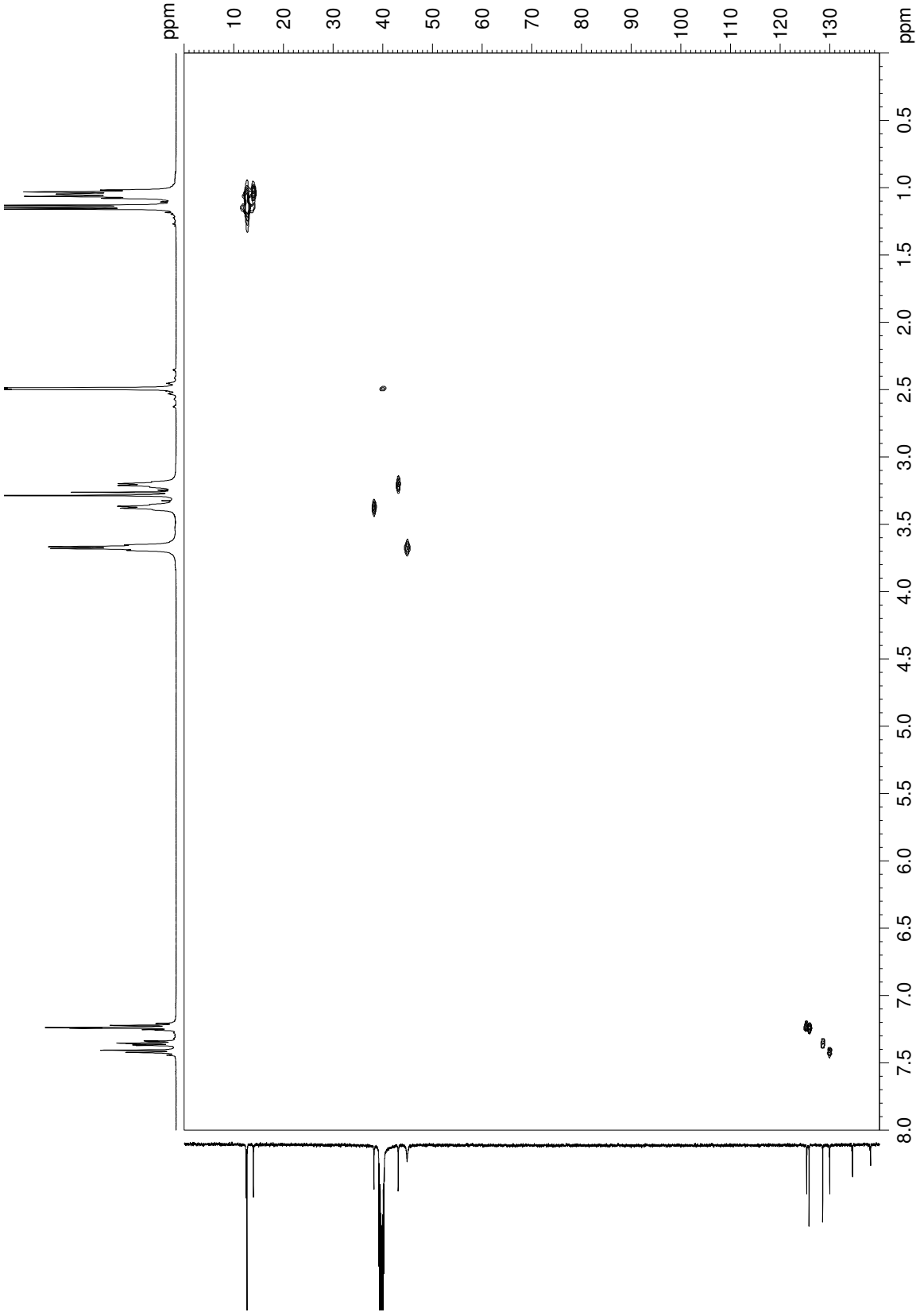
HMBC spectrum of **132**



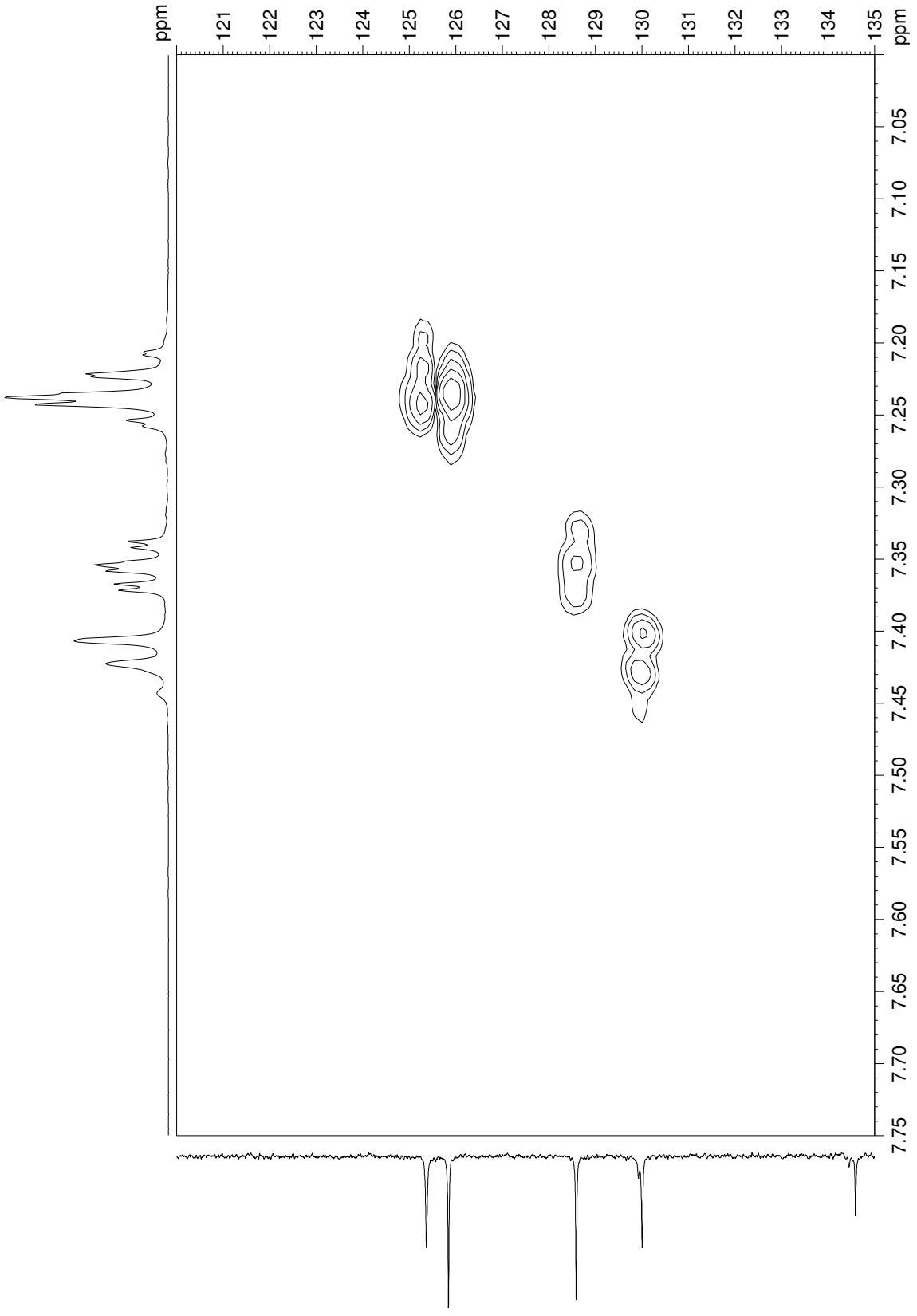
HMBC spectrum (extract) of **132**



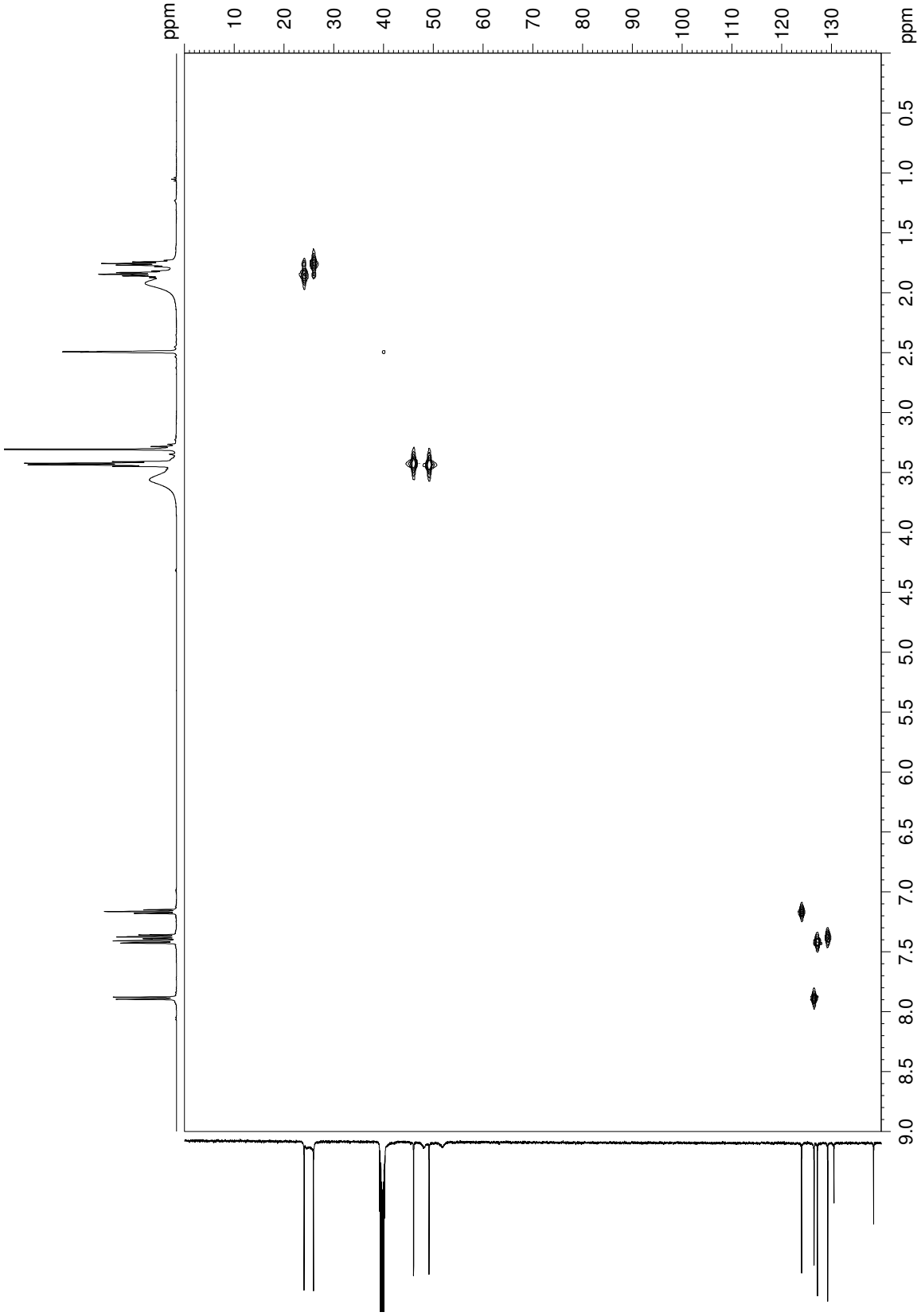
HMQC spectrum of **133**



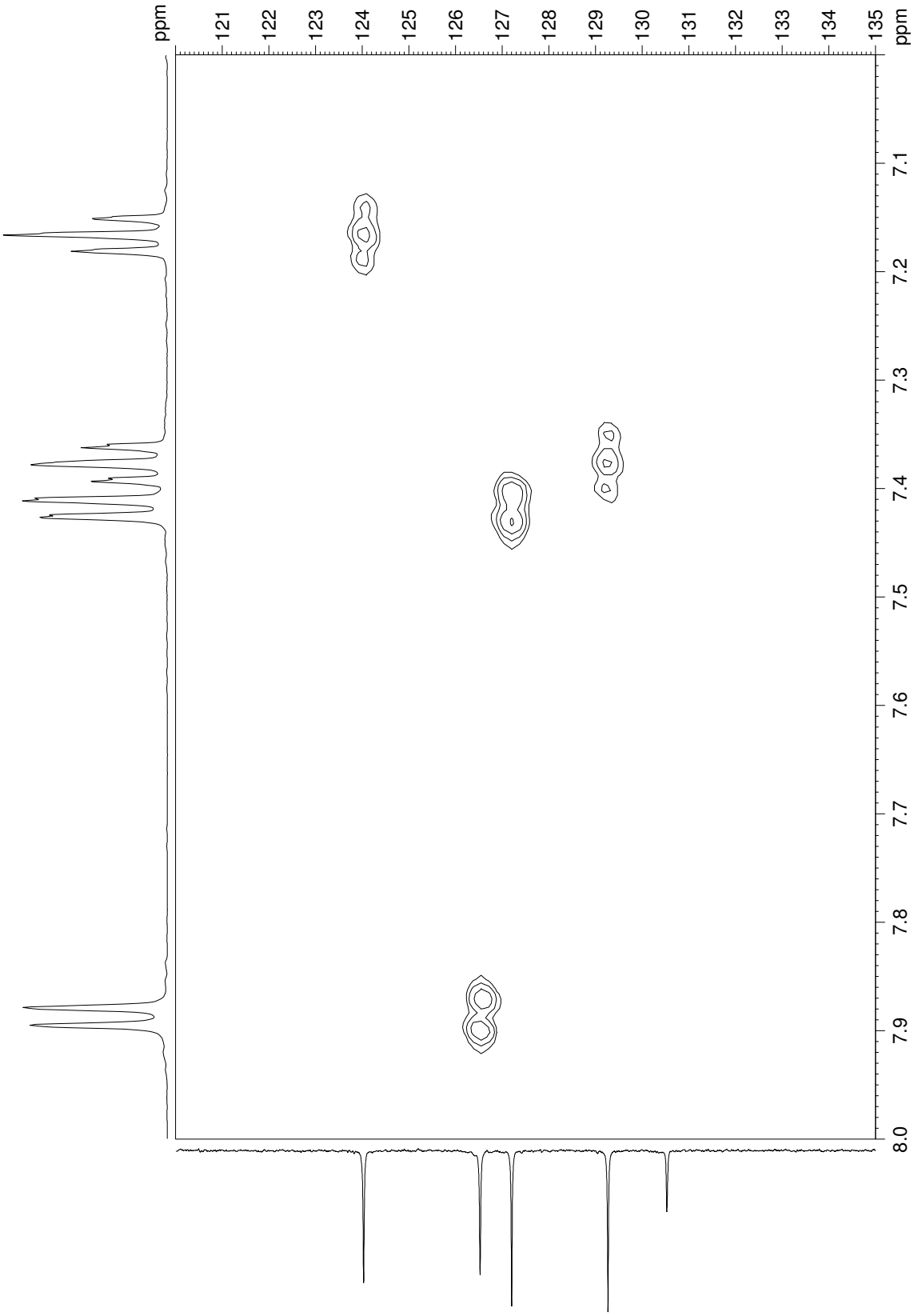
HMQC (extract) spectrum of **133**



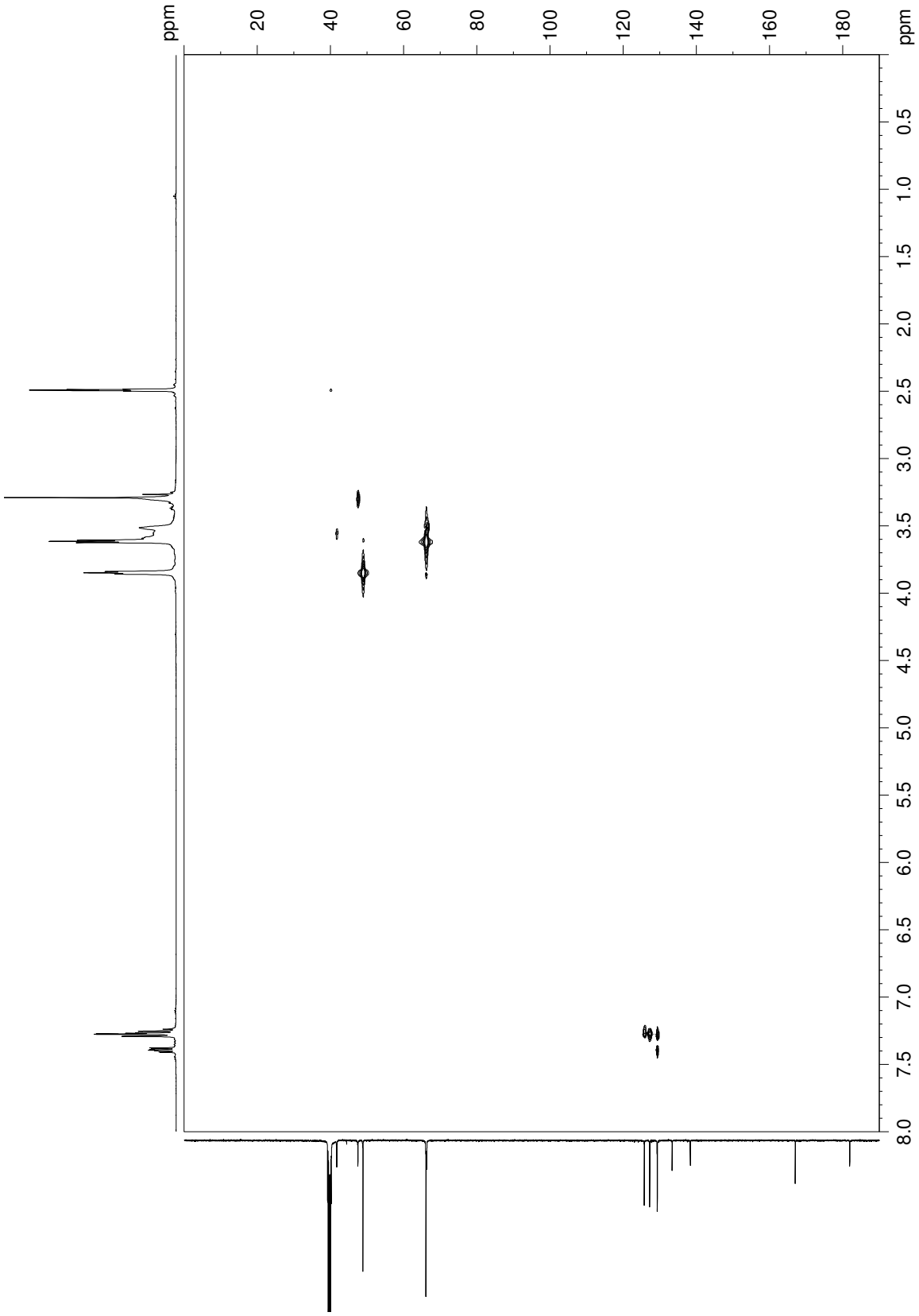
HMQC spectrum of **134**



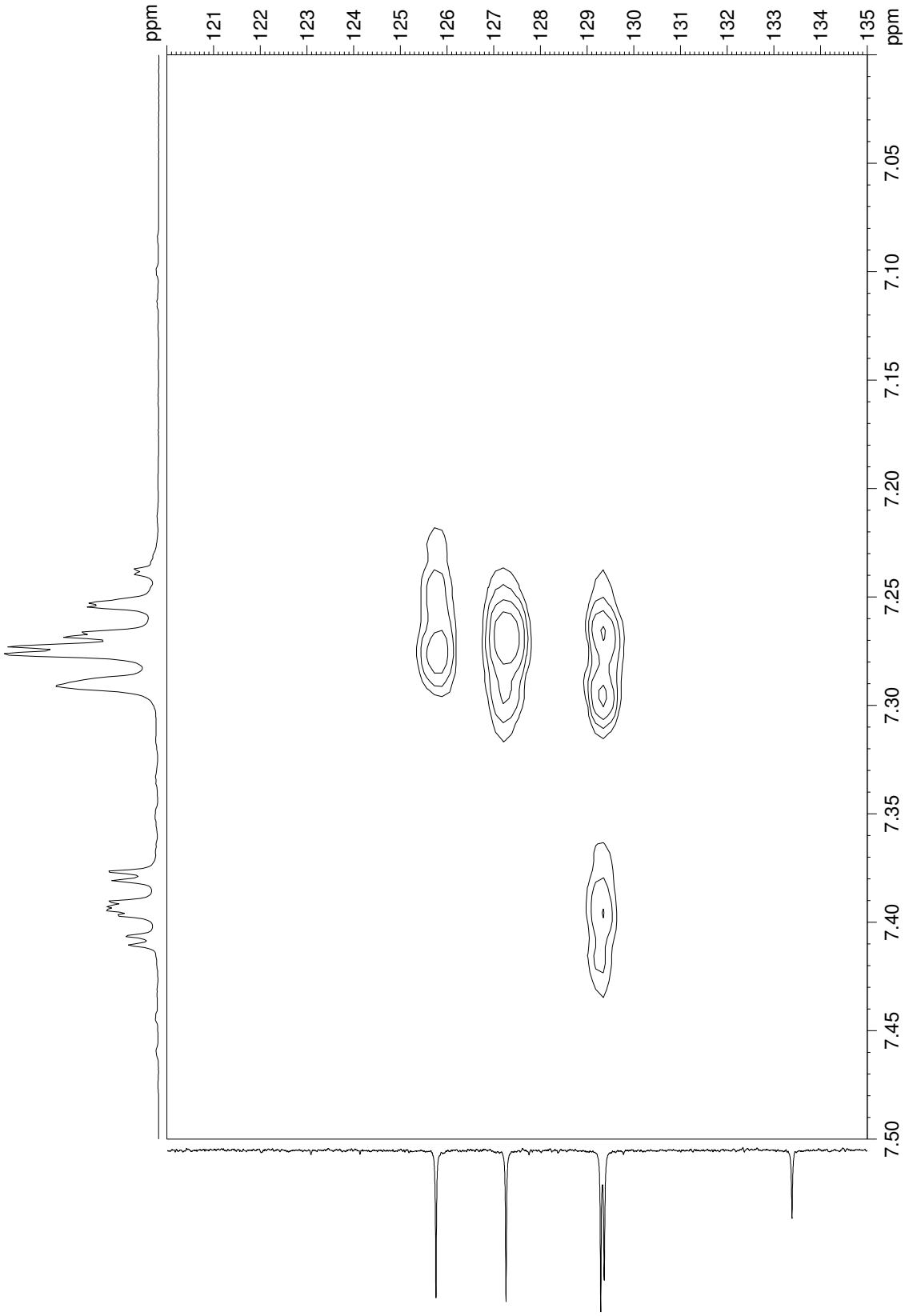
HMQC (extract) spectrum of **134**



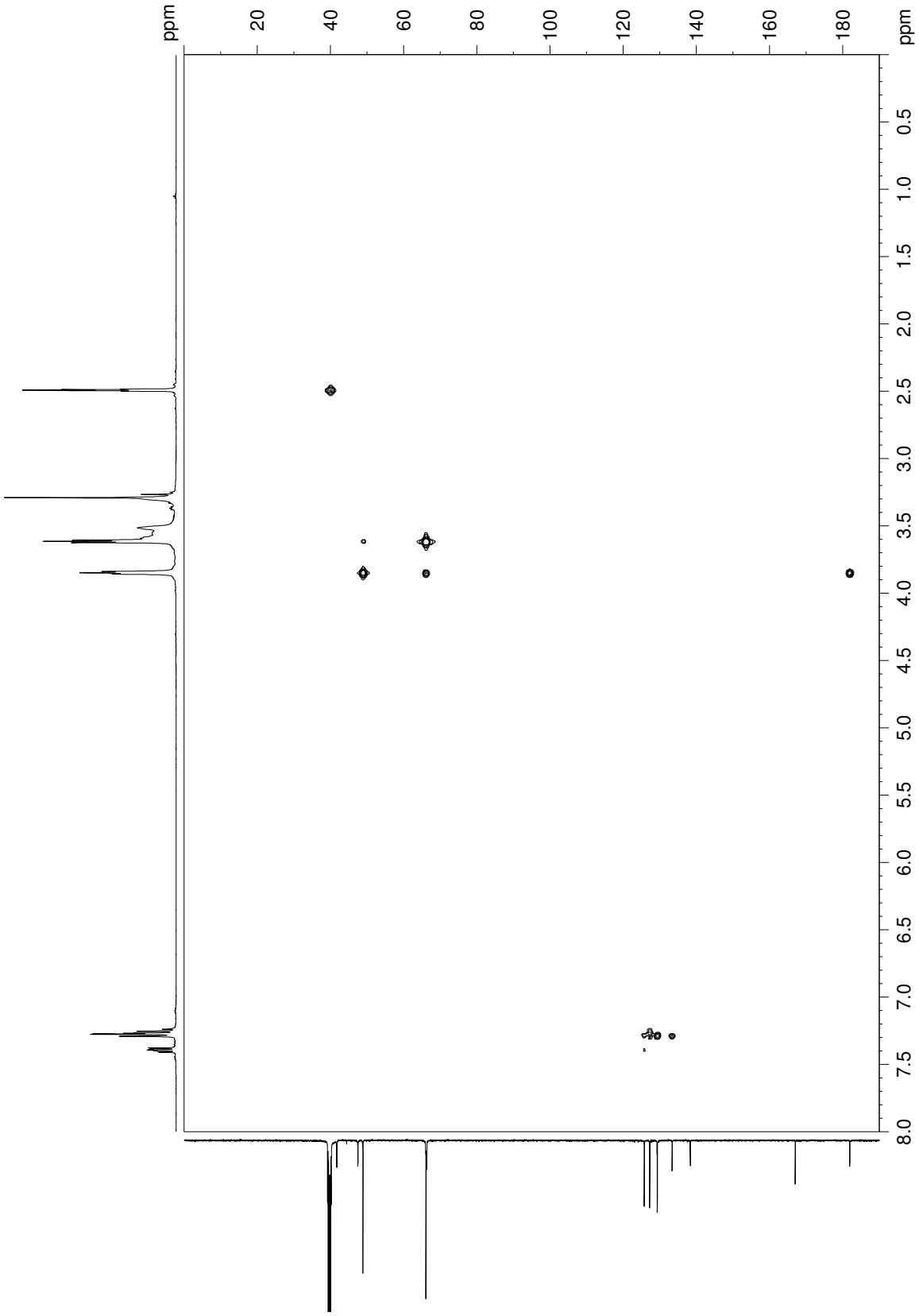
HMQC spectrum of **135**



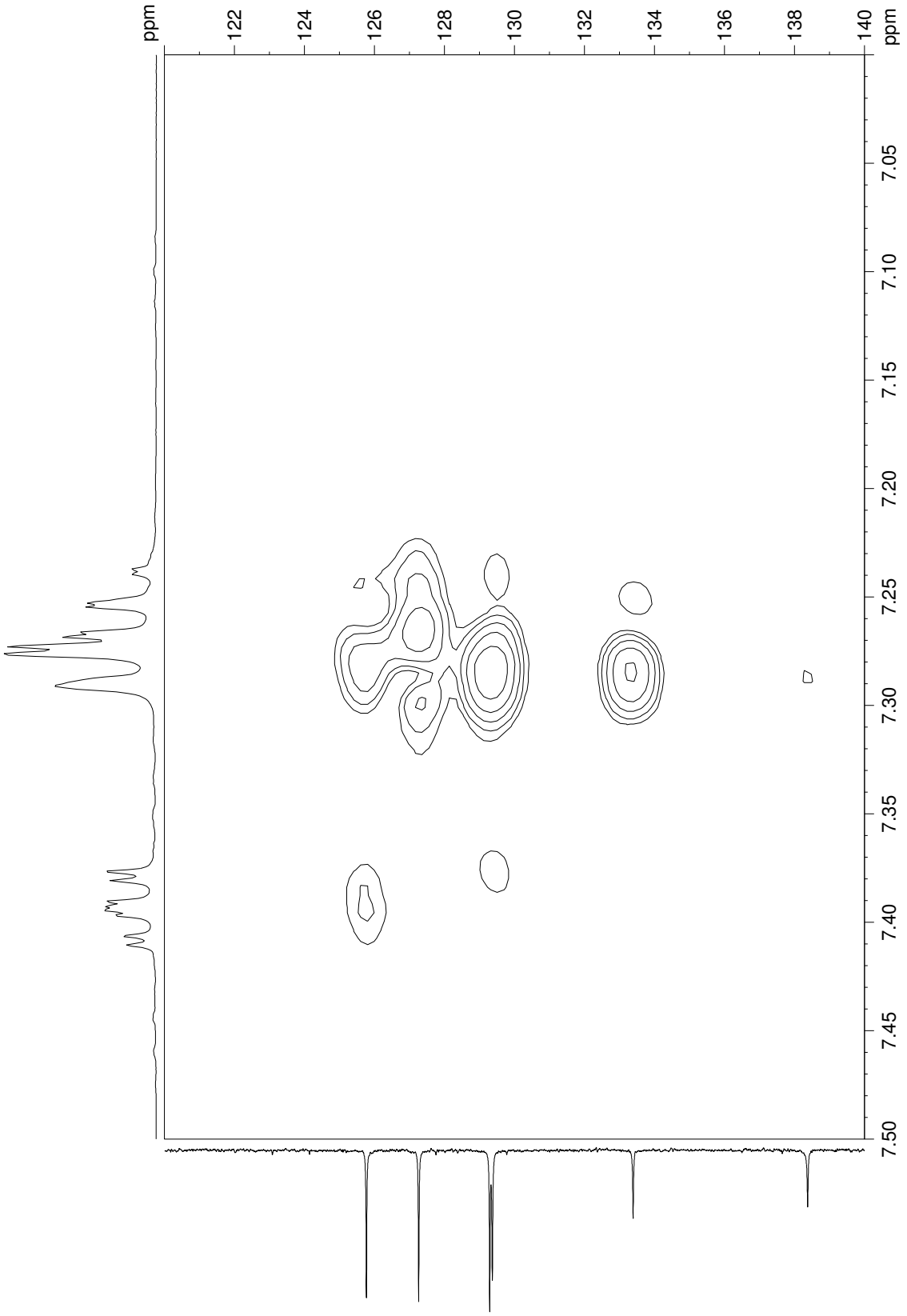
HMQC (extract) spectrum of **135**



HMBC spectrum of **135**



HMBC spectrum (extract) of **135**



Crystallographic Data

Table 1: Crystallographic data for **17** and **36**

Crystal data	**17**	**36**
CCDC number	706312	743232
Empirical formula	$C_{10}H_{11}N_3O_2S_2$	$C_{20}H_{22}N_4O_2S_2$
Formula weight	269.34	414.54
Temperature (K)	100(2)	123(2)
Wavelength (Å)	0.71073	0.71073
Crystal size (mm)	0.34 × 0.24 × 0.10	0.60 × 0.36 × 0.24
Crystal system	Triclinic	Triclinic
Space group	$P-1$	$P-1$
a (Å)	6.9565(3)	8.9281(3)
b (Å)	7.6288(2)	9.0083(5)
c (Å)	11.0688(4)	14.0333(6)
α (°)	98.289(2)	89.437(2)
β (°)	97.257(2)	77.579(3)
γ (°)	91.730(2)	65.822(3)
V (Å^3)	575.92(4)	1001.66(8)
Z	2	2
Calculated density (g/cm^3)	1.553	1.374
Absorption coefficient (mm^{-1})	0.455	0.290
$F(000)$	280	436
θ Range for data collection (°)	3.05–27.47	2.57–32.04
Completeness to θ (%)	98.7 ($\theta_{max} = 27.47°$)	96.4 ($\theta_{max} = 32.04°$)
Range of h,k,l	−9/9, −9/9, −14/14	−12/13, −13/13, −20/20
Reflections collected/unique	10420/2598 [R(int) = 0.0383]	16369/6737 [R(int) = 0.0461]
Absorption correction	Analytical	None
Max. and min. transmission	0.9559 and 0.8607	0.9337 and 0.8453
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	2598/0/198	6737/0/342
Goodness-of-fit on F^2	1.078	0.909
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0331$, $\omega R_2 = 0.0824$	$R_1 = 0.0395$, $\omega R_2 = 0.0753$
R indices (all data)	$R_1 = 0.0418$, $\omega R_2 = 0.0865$	$R_1 = 0.0841$, $\omega R_2 = 0.0851$
Largest diff. peak and hole (e/Å^3)	0.311 and −0.520	0.325 and −0.296

Table 2: Crystallographic data for **42** and **43**

Crystal data	**42**	**43**
CCDC number	706313	706314
Empirical formula	$C_{17}H_{13}N_3O_3S_2$	$C_{17}H_{13}N_3O_2S_2$
Formula weight	371.42	355.42
Temperature (K)	100(2)	123(2)
Wavelength (Å)	0.71073	0.71073
Crystal size (mm)	0.52 × 0.09 × 0.04	0.40 × 0.12 × 0.01
Crystal system	Triclinic	Monoclinic
Space group	$P-1$	$P2_1/c$
a (Å)	7.9203(7)	8.2696(6)
b (Å)	9.5787(8)	18.7233(17)
c (Å)	11.8410(10)	10.6990(6)
α (°)	75.019(4)	90
β (°)	77.427(4)	101.037(4)
γ (°)	78.011(5)	90
V (Å^3)	835.99(12)	1625.9(2)
Z	2	4
Calculated density (g/cm^3)	1.476	1.452
Absorption coefficient (mm^{-1})	0.341	0.342
F(000)	384	736
θ Range for data collection (°)	2.54–27.51	2.91–28.99
Completeness to θ (%)	97.9 ($\theta_{max} = 27.51°$)	95.1 ($\theta_{max} = 28.99°$)
Range of h,k,l	−9/10, −12/12, −15/15	−11/7, −25/23, −14/14
Reflections collected/unique	14557/3777 [R(int) = 0.0486]	9914/4112 [R(int) = 0.0832]
Absorption correction	Analytical	Semi-empirical from equivalents
Max. and min. transmission	0.9865 and 0.8428	0.98525 and 0.93160
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3777/0/291	4112/0/219
Goodness-of-fit on F^2	0.948	0.921
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0367$, $\omega R_2 = 0.0783$	$R_1 = 0.0551$, $\omega R_2 = 0.1016$
R indices (all data)	$R_1 = 0.0644$, $\omega R_2 = 0.0860$	$R_1 = 0.1477$, $\omega R_2 = 0.1305$
Largest diff. peak and hole (e/Å^3)	0.276 and −0.314	0.334 and −0.445

Table 3: Crystallographic data for **48** and **106**

Crystal data	**48**	**106**
CCDC number	706315	749825
Empirical formula	$C_{17}H_{11}N_3O_2S$	$C_{16}H_{18}N_2O_2S_2$
Formula weight	321.36	334.44
Temperature (K)	293(2)	100(2)
Wavelength (Å)	0.71073	0.71073
Crystal size (mm)	0.231 × 0.15 × 0.08	0.62 × 0.16 × 0.05
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/a$	$P2_1/c$
a (Å)	10.3434(4)	15.5096(6)
b (Å)	9.6504(5)	9.1662(3)
c (Å)	15.0913(7)	23.1287(9)
α (°)	90	90
β (°)	102.649(3)	100.876(2)
γ (°)	90	90
V (Å^3)	1469.82(12)	3229.0(2)
Z	4	8
Calculated density (g/cm^3)	1.452	1.376
Absorption coefficient (mm^{-1})	0.234	0.338
$F(000)$	664	1408
θ Range for data collection (°)	2.92–30.03	2.59–27.47
Completeness to θ (%)	96.3 (θ_{max} = 30.03°)	97.1 (θ_{max} = 27.47°)
Range of h,k,l	−14/13, −13/12, −21/21	−18/20, −11/10, −30/24
Reflections collected/unique	17540/4139 [R(int) = 0.0651]	21578/7171 [R(int) = 0.0721]
Absorption correction	Analytical	Analytical
Max. and min. transmission	0.9823 and 0.9559	0.9833 and 0.8179
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	4139/0/252	7171/0/541
Goodness-of-fit on F^2	0.904	0.876
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0417, ωR_2 = 0.0861	R_1 = 0.0398, ωR_2 = 0.0646
R indices (all data)	R_1 = 0.1092, ωR_2 = 0.0994	R_1 = 0.0937, ωR_2 = 0.0740
Largest diff. peak and hole (e/Å^3)	0.326 and −0.315	0.316 and −0.317

Table 4: Crystallographic data for **126** and **140**

Crystal data	**126**	**140**
CCDC number	704615	647436
Empirical formula	$C_{16}H_{14}N_2OS$	$C_{14}H_{20}F_4N_2O_4$
Formula weight	282.35	356.32
Temperature (K)	123(2)	123(2)
Wavelength (Å)	0.71073	0.71073
Crystal size (mm)	0.60 × 0.24 × 0.20	0.48 × 0.06 × 0.06
Crystal system	Triclinic	Monoclinic
Space group	$P-1$	$C2/c$
a (Å)	5.2288(3)	12.196(1)
b (Å)	9.5012(5)	19.608(2)
c (Å)	13.6203(9)	7.3393(6)
α (°)	95.276(3)	90
β (°)	92.293(3)	103.175(7)
γ (°)	94.088(4)	90
V (Å^3)	671.33(7)	1709.0(3)
Z	2	4
Calculated density (g/cm^3)	1.397	1.385
Absorption coefficient (mm^{-1})	0.237	0.128
F(000)	296	744
θ Range for data collection (°)	2.51–27.88	3.43–27.49
Completeness to θ (%)	95.0 ($\theta_{max} = 27.88°$)	98.0 ($\theta_{max} = 27.49°$)
Range of h,k,l	−6/6, −12/11, −16/17	−13/15, −21/25, −7/9
Reflections collected/unique	7138/3040 [R(int) = 0.0569]	5383/1926 [R(int) = 0.0516]
Absorption correction	Semi-empirical from equivalents	None
Max. and min. transmission	0.9799 and 0.8704	0.9923 and 0.9409
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3040/0/182	1926/0/150
Goodness-of-fit on F^2	1.067	0.859
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0398$, $\omega R_2 = 0.1018$	$R_1 = 0.0345$, $\omega R_2 = 0.0640$
R indices (all data)	$R_1 = 0.0507$, $\omega R_2 = 0.1074$	$R_1 = 0.0797$, $\omega R_2 = 0.0724$
Largest diff. peak and hole (e/Å^3)	0.339 and −0.485	0.192 and −0.150

Table 5: Crystallographic data for **141** and **142**

Crystal data	**141**	**142**
CCDC number	730532	646562
Empirical formula	$C_{11}H_{11}F_4NO_4$	$C_{19}H_{13}F_8NO_8$
Formula weight	297.20	535.30
Temperature (K)	123(2)	123(2)
Wavelength (Å)	0.71073	0.71073
Crystal size (mm)	0.34 × 0.20 × 0.10	0.35 × 0.15 × 0.10
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/n$
a (Å)	7.8520(2)	9.4897(1)
b (Å)	16.2123(5)	10.6655(1)
c (Å)	9.2330(3)	19.8758(3)
α (°)	90	90
β (°)	98.026(2)	90.874(1)
γ (°)	90	90
V (Å^3)	1163.84(6)	2011.44(4)
Z	4	4
Calculated density (g/cm^3)	1.696	1.768
Absorption coefficient (mm^{-1})	0.168	0.183
$F(000)$	608	1080
θ Range for data collection (°)	2.91–30.04	3.04–25.03
Completeness to θ (%)	98.6 (θ_{max} = 30.04°)	99.2 (θ_{max} = 25.03°)
Range of h,k,l	−10/11, −22/18, −13/12	−11/11, −12/12, −23/23
Reflections collected/unique	15278/3345 [R(int) = 0.0479]	32213/3517 [R(int) = 0.0452]
Absorption correction	Analytical	None
Max. and min. transmission	0.9840 and 0.9616	
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data/restraints/parameters	3345/0/225	3517/18/343
Goodness-of-fit on F^2	0.943	1.102
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0373, ωR_2 = 0.0816	R_1 = 0.0541, ωR_2 = 0.1421
R indices (all data)	R_1 = 0.0660, ωR_2 = 0.0904	R_1 = 0.0630, ωR_2 = 0.1456
Largest diff. peak and hole (e/Å^3)	0.250 and −0.352	0.351 and −0.351