Development of Methods for Solid Phase Synthesis of Polyamine Conjugates and Initial Biological Evaluation of Their Potential to Enhance Cellular Uptake

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1 Summary

Cationic peptides have shown their potency to efficiently enhance cellular uptake of covalently coupled molecules. In order to avoid their rapid degradation by peptidases, diverse classes of peptide mimics like peptoids, ß-peptides, and oligocarbamates were exploited. Polyamines, although bearing high density of positive charges at physiological pH, found only less regard for the purpose to act as covalently coupled drug delivery agents.

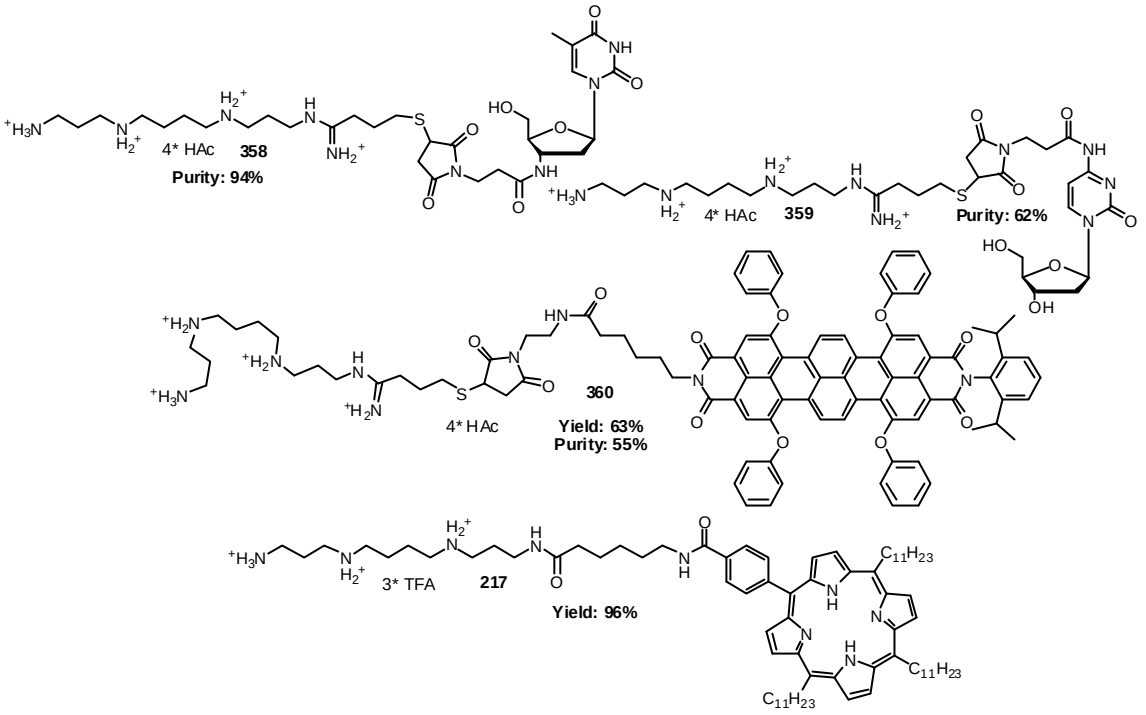
To solve problems, which occurred during the synthesis of polyamines, a solid phase strategy was developed, which especially allows the generation of acid sensitive polyamine-conjugates. On the strongly acid labile trityl linkers, which allow the mild cleavage from the resin, the Aloc group was introduced into polyamine chemistry, to serve for the permanent protection of the backbone amines. *Fukuyama alkylation* was identified as the method of choice for backbone elongation. Compatibility with the protection group strategy was demonstrated and the reaction conditions were optimised. Finally, *Fukuyama alkylation* allowed the introduction of a variety of functional groups, which could not be used with other frequently used alkylation methods. The combination of trityl linker and Aloc-, *o*-Nosyl-, and Dde-protection groups allowed branching by introduction of side chains on particular amines of the backbone. For this, *Reductive amination* turned out to be the appropriate alkylation method. For the bioconjugation of polyamines, the *Michael addition* of thiols to maleimides was exploited, which already showed its utility to couple large molecules like oligonucleotides and peptides. After introduction of a maleimide to an immobilised polyamine, its reaction with different sulfides was investigated. It showed suitability to efficiently couple diverse sulfides of medium polarity on polystyrene resins. The *vice versa* strategy, integration of a thiol on the immobilised polyamine and coupling with maleimides was also investigated. The coupling with 2-iminothiolane showed to be superior to all other examined methods. An alkoxytrityl resins with even higher acid sensitivity than most commercially available resins, was prepared. On this resin, coupling with 2-iminothiolane allows the quick generation of polyamine conjugates under very mild conditions. The selectivity of 2-iminothiolane for primary amines supersedes the use of complex protection schemes. The synthetic methods were used to generate a variety of polyamine conjugates with nucleosides, fluorophores, porphyrin, Indomethacin, and a radical scavenger. Evaluation of some of these conjugates by fluorescence microscopy showed enhanced cellular uptake. Intensive investigation on the amphiphilic polyamine-porphyrin conjugate showed a mitochondrial localisation. Irradiation caused severe cell damage. Co-cultures of mammalian cells with tumour cells or bacteria, respectively, showed a beneficial differentiation between those cell types. As the uptake into tumour cells was faster than into bacteria, which was again faster than that into mammalian cells, the compound is a candidate for application in *photodynamic therapy*. Toxicological data for most of the tested compounds were determined by the MTT assay.

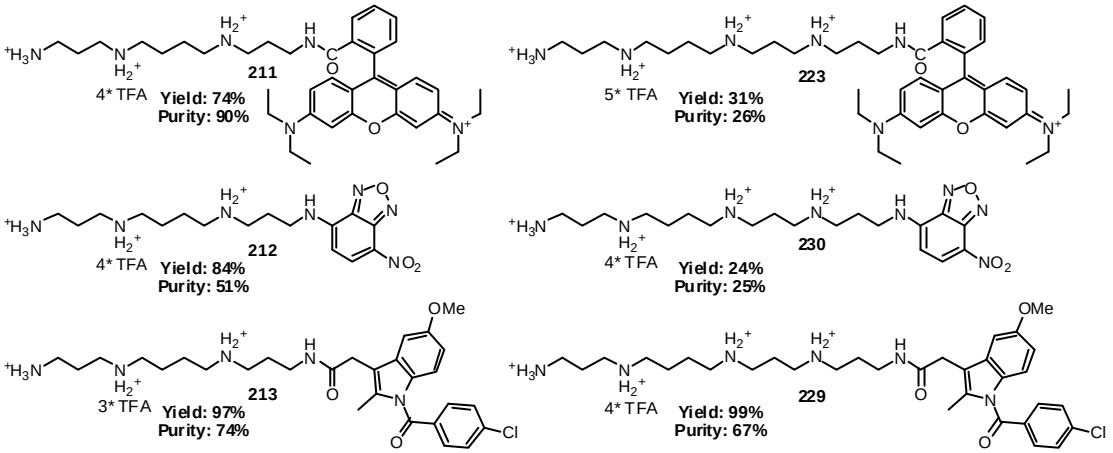
Zusammenfassung

Kationische Peptide haben ihre Potenzial bewiesen, die zelluläre Aufnahmen kovalent gebundener Moleküle zu verbessern. Um ihren schnellen Abbau durch Peptidasen zu verhindern wurden unterschiedliche Klassen von Peptidmimetika, wie Peptoide, ß-Peptide, and Oligocarbamate untersucht. Polyamine, welcher ebenfalls eine hohe Dichte an positiven Ladungen bei physiologischem pH-Wert tragen, wurden bereits für die Komplexierung negativ geladener Moleküle wie beispielsweise DNA verwendet. Allerdings fanden sie zum jetzigen Zeitpunkt kaum Beachtung als kovalent gebundener Transporter für die zelluläre Aufnahme von Wirkstoffen.

Um die Probleme zu lösen, die im Laufe der Polyaminsynthese gewöhnlich auftreten, wurde eine festphasengebundene Synthesestrategie entwickelt, die vor allem der Darstellung empfindlicher Polyaminkonjugate dienen soll. Während stark säurelabile Trityllinker eine milde Abspaltung erlauben, wurde die Alloc-Gruppe, welche als permanente Schutzgruppe für die Amine des Rückgrades fungiert, in die Polyaminchemie eingeführt. *Fukuyama-Alkylierung* wurde als Methode der Wahl für die Verlängerung des Rückgrades identifiziert. Die Kompatibilität mit der gewählten Schutzgruppenstrategie wurde gezeigt und die Reaktionsbedingungen optimiert. Zusätzlich wurde gezeigt, dass die *Fukuyama-Alkylierung* die Einführung unterschiedlicher funktioneller Gruppen erlaubt, deren Verwendung bei anderen Alkylierungsmethoden nicht möglich wäre. Die Kombination von orthogonalem Trityl-Linker, Aloc-, *o*-Nosyl- und Dde-Schutzgruppe erlaubte Verzweigung durch selektive Einführung von Seitenketten an bestimmten Aminen des Rückgrades. Hierbei zeigte sich *Reduktive Aminierung* als probate Alkylierungsmethode.

Für die *Biokonjugation* von Polyaminen wurde die *Michael-Addition* von Thiolen an Maleimide gewählt, welche bereits ihre Verwendbarkeit für die Kupplung großer Moleküle wie Oligonucleotide und Peptide gezeigt hat. Nach Bindung eines Maleimides an das immobilisierte Polyamin wurde dessen Reaktion mit Sulfiden untersucht. Es zeigte sich, dass die Reaktion geeignet ist, Sulfide mittlerer Polarität an Polystorenharzen zu kuppeln. Die umgekehrte Strategie, Einführung eines Thiols an ein immobilisiertes Polyamin und Bindung an Maleimide wurde ebenfalls untersucht. Kupplung mit 2-Iminothiolan zeigte sich gegenüber allen anderen untersuchten Methoden als überlegen. Ein Alkoxytritylharz mit hoher Säurelabilität wurde hergestellt. An diesem Harz erlaubte die Kupplung mit 2-Iminothiolan eine schnelle Darstellung von Polyaminkonjugaten unter sehr milden Bedingungen durchzuführen. Die Selektivität von 2-Iminothiolan für primäre Amine macht die Verwendung von komplexen Schutzgruppen-Schemata überflüssig. Diese synthetischen Methoden wurden verwendet um Polyaminkonjugate mit Nucleosiden, Fluorophoren, Porphyrin, Indomethacin und einem großen aromatischen Radikalfänger darzustellen. Untersuchung einiger dieser Konjugate durch Fluoreszenzmikroskopie zeigte verstärkte zelluläre Aufnahme. Intensive Untersuchung des amphiphilen Polyamin-Porphyrinkonjugates in Fibroblasten, HeLa-, HepG2- und COS7-Zellen zeigte dessen mitochondriale Lokalisierung. Bestrahlung verursachte schwerewiegende Zerstörung der Zellen. Co-Kulturen von Säugetierzellen und Tumorzellen bzw. Bakterien zeigten eine vorteilhafte Differenzierung zwischen diesen Zelltypen. Da die Aufnahme in Tumorzellen stärker war als die in Bakterien und diese wiederum stärker als die in Säugerzellen, ist die Verbindung ein Kandidat für den Einsatz in der photodynamischen Therapie. Die toxokologischen Daten der meisten dargestellten Polyaminkonjugate wurden mit dem MTT-Test bestimmt.


4* HAc
358
Purity: 94%
4* HAc
359
Purity: 62%
360
4* HAc
Yield: 63%
Purity: 55%
3* TFA
217
Yield: 96%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Yield: 74%
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5* TFA
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Yield: 31%
Purity: 26%
4* TFA
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Yield: 84%
Purity: 51%
4* TFA
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Yield: 24%
Purity: 25%
3* TFA
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Yield: 97%
Purity: 74%
4* TFA
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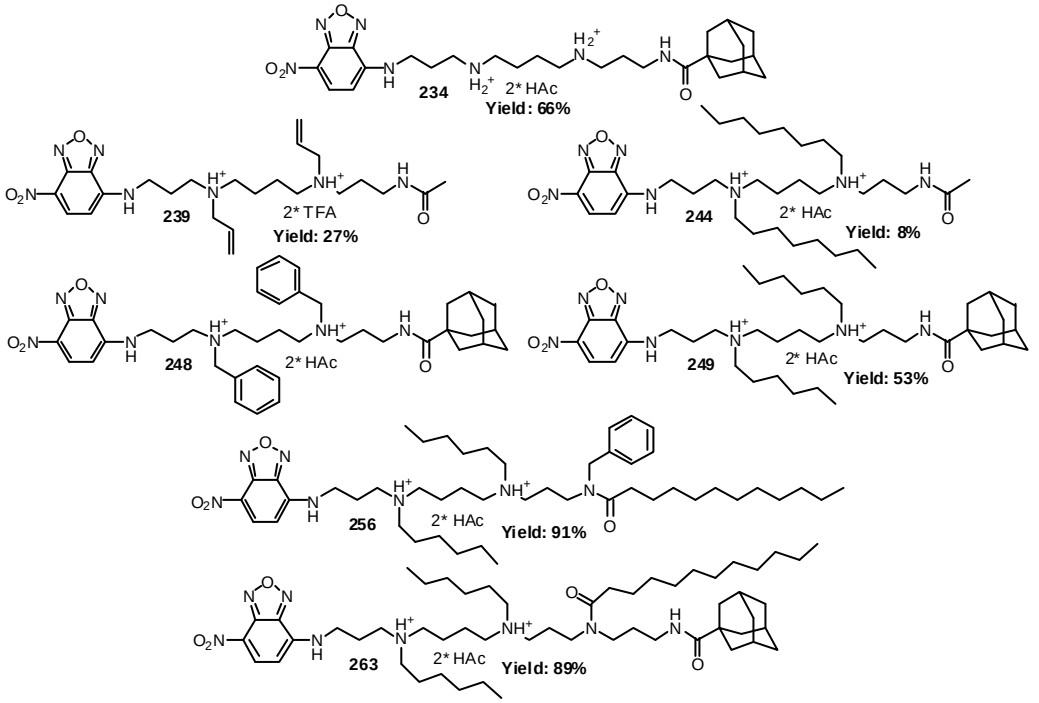
Yield: 99%
Purity: 67%


234
2* HAc
Yield: 66%
239
2* TFA
Yield: 27%
244
2* HAc
Yield: 8%
248
2* HAc
249
2* HAc
Yield: 53%
256
2* HAc
Yield: 91%
263
2* HAc
Yield: 89%

2 Introduction

2.1 Drug delivery

2.1.1 The problem of restricted bioavailability

A limiting factor in the application of drugs and biologically active target molecules is often their low uptake efficiency. Many drugs, which have shown their potency in *in vitro* assays, are restricted in their *in vivo* application by their low biodistribution after oral application. The efficiency of an *in vivo* treatment largely depends on the targeting of active compounds into specific organs, cell types, and even the right cellular compartments. Based on empirical data, the ´*Lipinski rule of five*´ points towards the bioavailability of potential drug molecules.[1, 2] These rules are:

- Five or less hydrogen bond donators (for example OH- or NH groups)
- Ten or less hydrogen bond acceptors (for example oxygen or nitrogen)
- A molecular mass below 500 g / mol
- A partition coefficient (*log P*) of five or lower

The number of potential drug molecules is strongly limited by such restrictions. In order to evolve the full potential of modern high throughput drug delivery strategies, it is of great interest to find ways to enhance the bioavailability of drug candidates. Especially the transfection of DNA and RNA into cells is crucial in the establishment of gene therapy. To reduce uptake problems, several invasive methods have been developed, for example electroporation[3-7], microinjection[8], and particle guns[9-18]. Encapsulation into liposomes[19-24], micro- and nanoparticles, and supramolecular structures (cyclodextrins[29] and dendrimers) showed good results especially for the delivery of DNA or RNA-oligomers. Viral delivery is also an efficient method, but is restricted in its use, and clinical applications also showed severe side effects.[30] A relatively novel field in research is the covalent coupling of drugs to carrier molecules, which facilitate cellular uptake. Naturally occurring members are the so-called ´cell penetrating peptides´ (CPPs). They are virtually ´pre-optimized´ by evolution and can thereby act as helpful templates for synthetic analogues with improved properties. A detailed introduction into all above-mentioned classes of delivery agents would go beyond the scope of this thesis. Thus, dendrimers, covalently bound carriers, and polyamines are discussed in more detail, as they all share one common motif: Their positive net charge seems to be of major importance for their ability to enhance cellular uptake. In the course of this work, ´cellular uptake´ will be used as a collective term to describe uptake into any cellular compartment. The term ´molecular transporter´ will not stand for intrinsic cellular system like the polyamine transport system. ´Molecular transporter´ will be used for molecules, which enhance cellular uptake after covalent attachment.

2.1.2 Dendrimers

In contrast to linear polymers, dendrimers optimally have a perfectly branched structure. Unlike the classical polymerization process, which results in linear polymers of different sizes, the mode of dendrimers-preparation gives precise control over the molecular size, structure, and physical properties.[31-38] This is especially important for triggering of the water-solubility of dendrimers using different building blocks. Dendrimers of lower generations (0 – 2) have a highly asymmetric shape and consist of a more open structure compared to their higher generation relatives. As the chains grow longer and the branching increases, the structure of higher generation dendrimers becomes globular and more densely packed. After reaching a critical branch state, further growing is inhibited by the lack of space, a phenomenon known as the *starburst effect*.[39] In contrast to initial expectations, it became obvious in recent years that with growing density of the outer shells, the dendrimers reduce the tension in their outer shells. This is accomplished by backfolding of the outer dendrons into their interior to fill the gaps, which are existent in the core generations.[40]

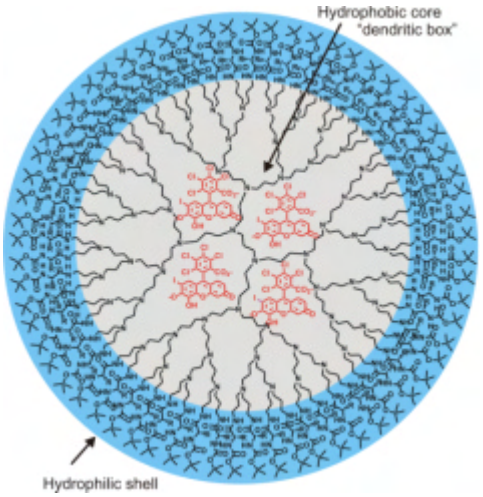


Figure 1 Molecular encapsulation of drugs by a polypropyleneimine-dendrimer. A globular dendrimer with a dense amino acid shell can hold four molecules of Rose Bengal (shown in red) and many smaller molecules (not shown) inside the dendrimer's flexible cavities. R = benzyl.[8]

While the hydrophilic shell of the dendrimers ensures water solubility, the interior can be hydrophobic[40-45], enabling dendrimers to incorporate hydrophobic drug molecules and release them slowly (Figure 1).[42] The core of the dendrimer can also be hydrophilic and carry even large, highly polar molecules like DNA. Acidic drugs such as the cytostatics methotrexate[41] and adriamycin can be incorporated by dendrimers with a basic interior. Prominent examples of basic dendrimers, which are generated starting from polyamine building blocks, are polyamidoamines (PAMAM). Drug release systems with a hydrophobic shell cause problems in native systems due to aggregation and low solubility.[46] In addition, surface modification with poly(ethylene glycol) (PEG) yielded dendrimers with a biocompatible surface, since the polymer coating enhances circulation half-life by two orders of magnitude. After some of the widely used dendrimers had been shown to be toxic, biodegradable and biocompatible dendrimers were developed.[47] Cationic dendrimers displaying amino groups on the outer shell, which are protonated at lower pH values, are generally hemolytic and cytotoxic. Their toxicity is generation-dependent and increases with the number of protonated amines on the surface. The putative pathway for cellular entry of dendrimer-DNA complexes seems to be endocytosis.[48] The complexes are entrapped into

endocytic vesicles that originate from the invagination of the cell surface membrane. However, it is still unknown whether the complexes interact with the membrane surface or are simply captured with the extra-cellular fluid that is taken up by pinocytosis.[8] Once inside the cell, the endocytic vesicle fuses with the acid endosomal compartments.[49, 50] It is obvious that positive net charge is important for this uptake mechanism.

2.1.3 Covalently bound carriers

In contrast to dendrimers, liposomes, and other core shell architectures, covalent attachment of transport molecules to drugs can enhance the stability of coupled cargo.[8]

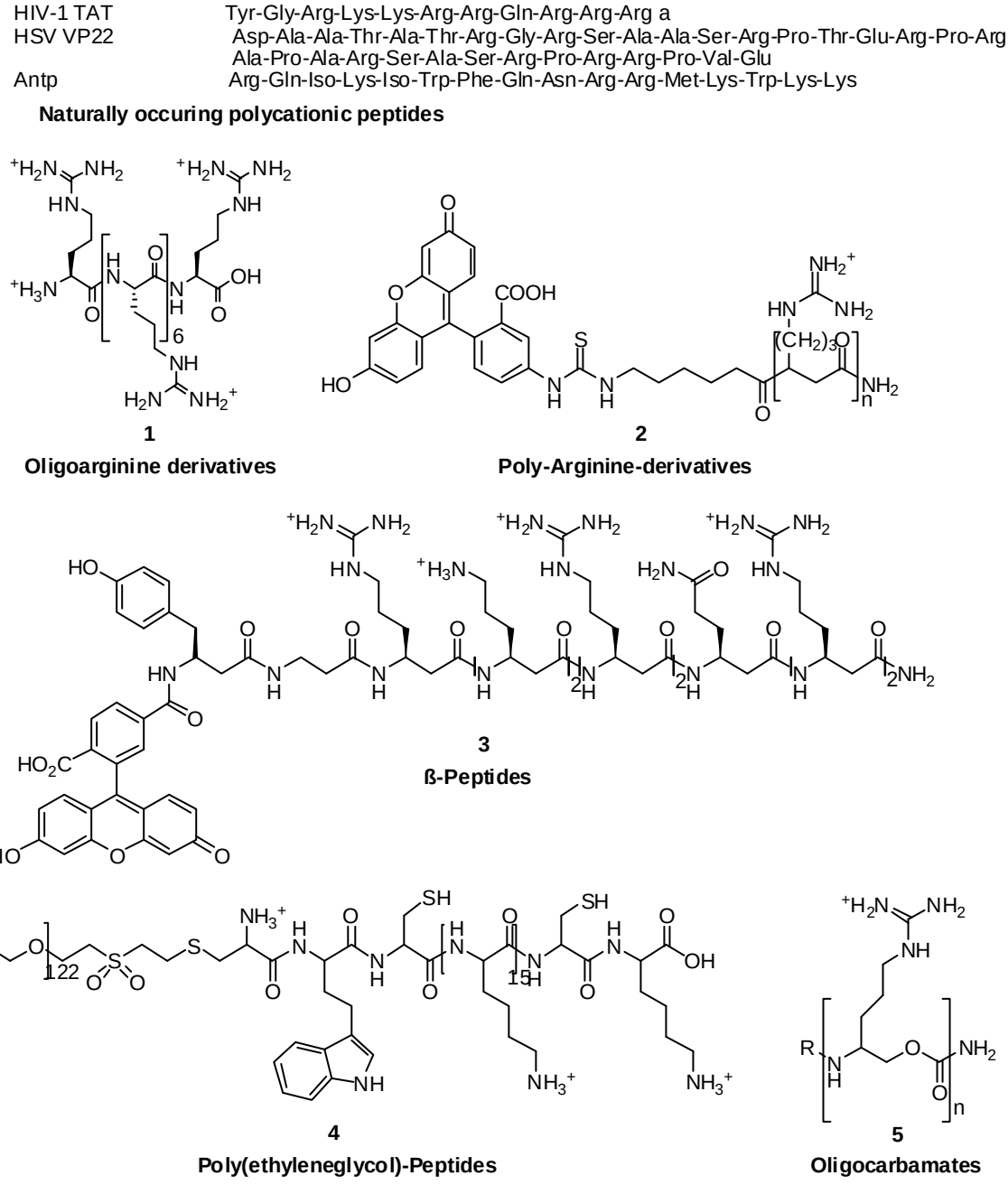


Figure 2 Known naturally and synthetic polycationic molecules, which can enhance cellular uptake of covalent coupled cargo.[51]

By covalent attachment, distinct structures with exact ratios of functional units are formed. This is especially beneficial in terms of identifying the pharmacokinetics of such conjugates.[52] Non-covalently bound delivering agents have to be formulated with the respective drug molecules and it his hard to predict the exact composition of such formulations. The procedures can cause inhomogeneities, whose effect on the outcome cannot be determined. A summary of some covalently bound uptake enhancers is shown in Figure 2.

2.1.3.1 Cell penetrating peptides

Cell penetrating peptides (CPPs), are structures provided by nature, which can serve as templates for the development of other peptido mimetica with improved cellular uptake. In the late 80^{ies}, a novel class of protein domains was discovered that could enter the cells in a receptor and, as it was then believed, in an energy-independent fashion carrying the rest of the protein into the interior of the cells.[8, 53] In the past, this novel way of intracellular delivery was termed “protein transduction”, and the responsible peptide sequences from native proteins were referred to as *protein transduction domains* (PTD). In the following, the term CPPs will be used to describe both, *protein transduction domains* and *cell penetrating peptides*.

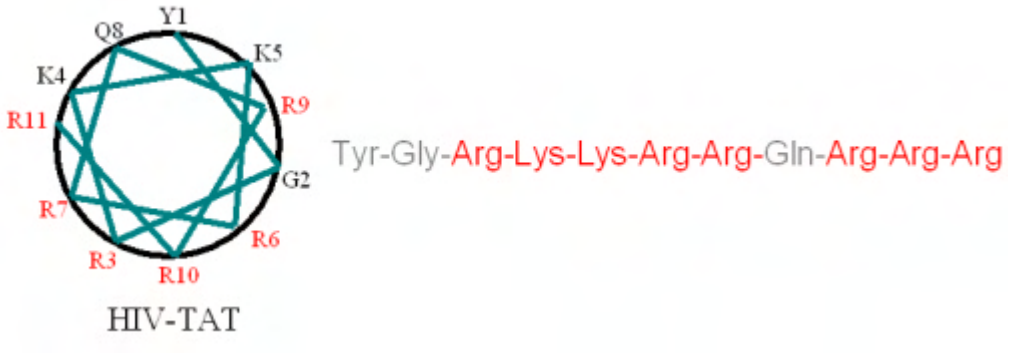


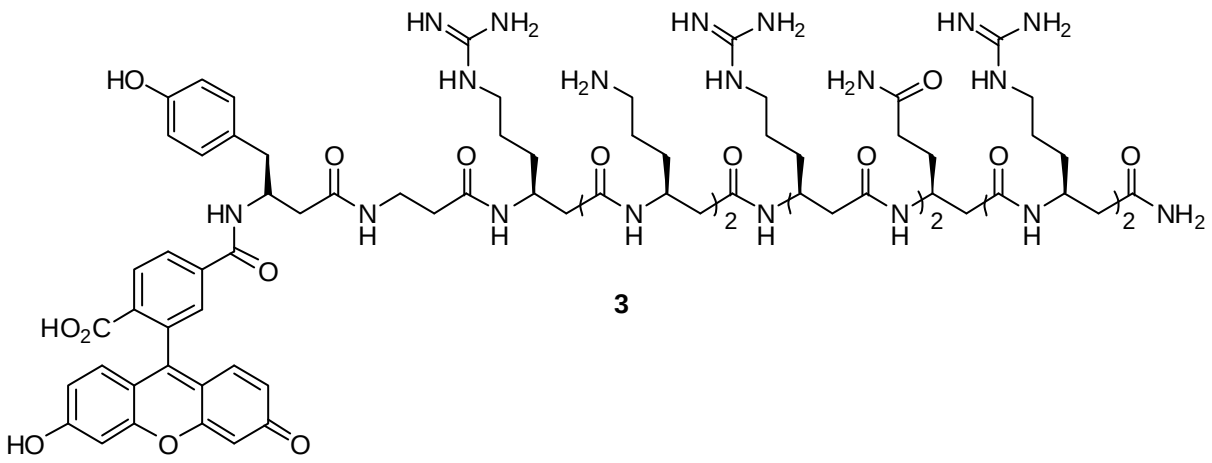
Figure 3 Sequence of the active domain of the HIV-TAT protein. It mainly consists of the basic amino acids lysine **(17)** and arginine **(20)**. Uncharged residues give HIV-TAT an amphiphilic overall structure.[8]

It has been shown that isolated CPPs are able to introduce a variety of covalently bound cargo into almost all cell types.[54] As described for a variety of proteins, CPPs are well suited as fusion tags, which transport the rest of the protein through membranes. Prominent members of this family are the *HIV-TAT* domain, *HSV VP22*, *penetratin*, *antennapedia*, and *transportan*. It was shown that the enhancement of cellular uptake mainly depends on the integrity of short sequences of these CPPs, which mainly consist of basic residues (Figure 3). These short domains delivered several cargos into cells with nearly the same efficiency as the full CPPs. Consequently, short homooligomers of lysine **(17)**,[55] ornithine **(18)**[56], and arginine **(20)**[56] were also shown to efficiently enhance cellular uptake of cargos. Development of optimized CPPs resulted in candidates, which are even more efficient than the naturally occurring CPPs. The amphipathic KLAL (HO-KLALKLALKALKAAKLA-NH_2) was one of the first synthetic peptides derived from naturally occurring peptides. In a comparison study with natural CPPs it showed to be more efficient in cargo transport than transportan, $TAT_{48\text{-}60}$ and, penetratin. In the beginning of CPP research it was believed that these cationic amphipathic peptides enter the cells either in inverse micelles or by just flipping through the membrane after inserting the hydrophobic part into the membrane while bound to

negatively charged molecules on the surface of the cells. This theory has recently been proven wrong.[57-60] Due to a paraformaldehyde fixation of the cells after treatment, the CPPs are mislocalised to the nucleus of the cells leading to a misinterpretation of the mechanism of cellular entry. Revised experiments in live cells revealed that endocytosis is involved in the uptake of most of the CPPs.[57, 61-63] However, complete interruption of the endocytic pathways mainly inhibits but does not exclude the cellular uptake of CPPs. To date, it is believed that most of the CPPs bearing cationic residues enter the cells either by clathrin-mediated endocytosis, macropinocytosis or caveolin-mediated endocytosis dependent on their charge, their lipophilicity, and their cargo, while binding with their cationic moiety to the negatively charged proteoglycans on the extracellular surface of the cellular membrane.[8] Proteoglycans contain sugar groups, which are modified with sialic acids and sulfates and are differently distributed on different cell types, which probably explains their different cellular uptake.[64-67] Mutant cells defective for glycosaminoglycan synthesis showed dramatically reduced penetratin- or TAT-mediated cellular uptake confirming the role of these complex polysaccharides in CPP-mediated cellular uptake.[8] Moreover, it has been shown that the cellular uptake efficiency is dependent on the cellular differentiation status. After endocytosis, most of the CPPs are entrapped and accumulated in macropinosomes or endosomal vesicles, while being slowly released into the cytosol or transported to other organelles. However, there are still debates on the exact mechanisms of endosomal escape. Albeit their improved cellular uptake efficiency, CPPs just have limited potential for the application in whole organisms, since they are rapidly degraded in the bloodstream by proteases, before they can be taken up by the specified tissue. This leads to high doses and subsequent treatments. Furthermore, besides several successful treatment reports largely cationic CPPs such as the HIV-TAT peptide or octa-arginines have to be shown to exhibit strong toxicity. Therefore, peptide analogues have been examined to overcome those problems. The best-investigated analogues so far are ß-peptides, peptoids, and oligocarbamates bearing basic side chains.

2.1.3.2 b-Peptides

β-Amino acid oligomers ("β-peptides") have extensively been studied in recent years[68]. They can adopt a broader range of well-defined protein-like secondary structures than α-peptides [69-72], and several β-peptides with interesting biological activities have been reported. For example, synthetic β–peptides were found to perform functions, which are naturally carried out by a-peptides. They can act as antagonists for somatostatin receptor $hsst_4$ and as inhibitors of cholesterol transport through Caco-2-cells.[73, 74] Metabolic stability was observed in most mammalian, insect, and plant-cell cultures. Slow biodegradation was only observed in human kidney and in a sample of microorganisms. In this case, decomposition was not accomplished by one organism, but symbiotically by a colony of microorganisms. None of them was able to grow with the offered β-tripeptide as only C- or N-source.[75, 76] Since β-peptides are resistant to degradation by proteases[76], and their secondary structure can be controlled, they are useful analogues to peptidic molecular transporters and may help to elucidate the mechanism of translocation.[77]



3

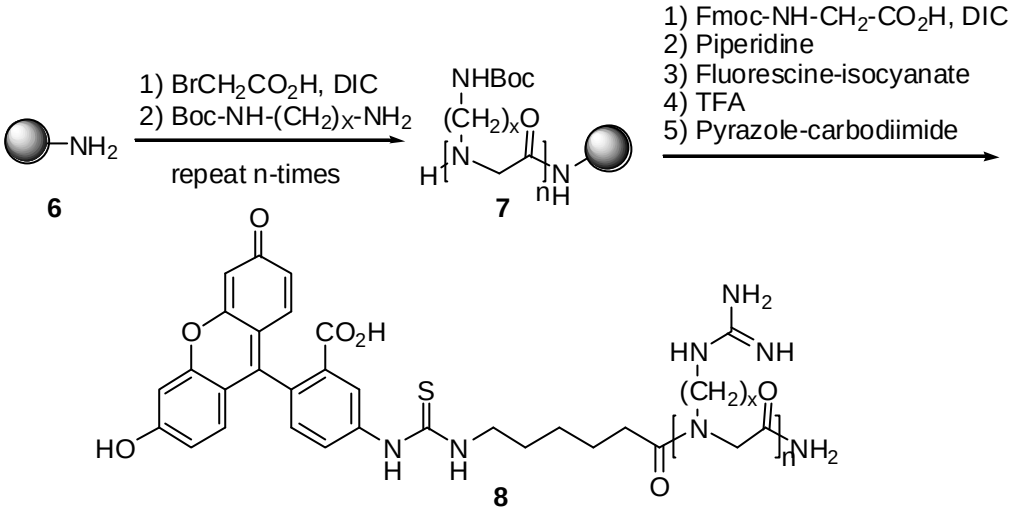
Fl-β^{3}hTyr-β^{3}hGly-β^{3}hArg-β^{3}hLys-β^{3}hLys-β^{3}hArg-β^{3}hArg-β^{3}hGln-β^{3}hArg-β^{3}hArg-β^{3}hArg-NH_2

Figure 4 Optimized β-peptide transporter.[78]

Successful uptake experiments were carried out with β-homoarginine[79], β-homolysine[80], and β-TAT[81]. Peptide uptake was not completely suppressed in experiments at low temperature, in the presence of endocytosis inhibitors, under cell-energy-depletion conditions, or with microorganisms (*E. coli* and *B.megaterium*).[78] This is consistent with prior reports about β-peptide $TAT_{47-59,}$ suggesting that cellular uptake does not exclusively depend on endocytosis. The uptake of β-oligopeptides strongly depends on their chain length. As β-tetraarginines only adsorb to the cell surface, β-decaarginines migrate rapidly to the *nucleoli*.[73, 74] The membrane translocation activity is preserved in β-peptides despite a drastic alteration in the oligomer backbone (amide group spacing) in comparison to the α-peptides. Since β-peptides are easily programmed to adopt specific conformations, they represent a platform that can be used to explore the effects of conformational constraints on cellular uptake activity.

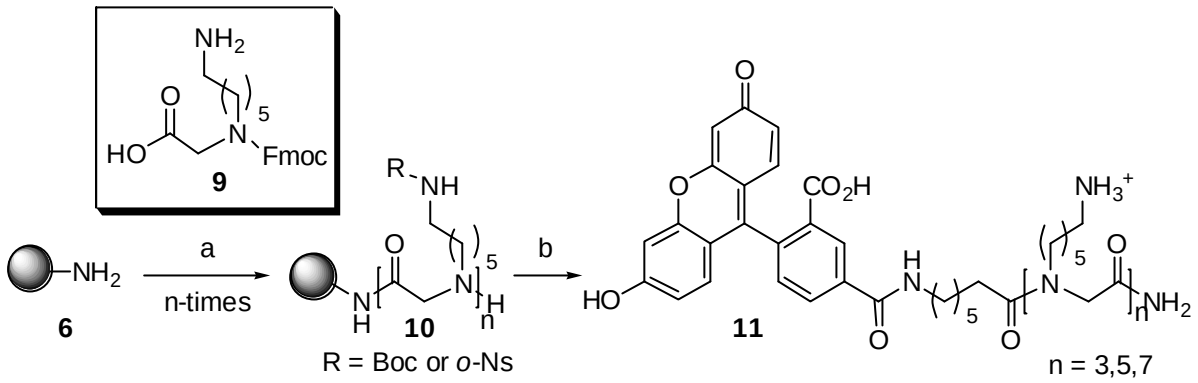
2.1.3.3 Peptoids

"Peptoids" (poly-*N*-substituted glycines) are a diverse family of non-natural oligomers with close similarity to natural polypeptides. Sequence-specific peptoid oligomers are easily assembled from various primary amines with different functionalities using the solid-phase submonomer method and offer particular advantages for the design of biomimetic materials.[82, 83] These polymers, pioneered by Zuckermann, are protease resistant, which makes them suitable transporters for *in vivo* applications. The achiral backbone of oligo-*N*-substituted glycines or "peptoids" lacks hydrogen-bond donors, which effectively prevent formation of the regular, intrachain hydrogen bonds that stabilize peptide R-helical structures.



Scheme 1 Optimized peptoid transporter and its synthesis.[51]

Since the guanidinium groups of $TAT_{49–57}$ play a greater role in facilitating cellular uptake than rather charge or backbone structure, Wender al. designed and synthesized a class of polyguanidine peptoid derivatives (Scheme 1).[84] Remarkably, the subset of peptoid analogues containing a six-methylene spacer between the guanidine head group and backbone exhibited significantly enhanced cellular uptake compared to $TAT_{49–57}$ and even to polyarginine. These studies led to a transporter, which is superior to $TAT_{49–57}$, protease resistant, and more easily prepared. Several revisions of the submonomer method were developed that allow efficient incorporation of unprotected imidazoles, pyridines, pyrazines, indoles, quinolines, and thiol containing functionalities into oligomers of up to 15 monomers[85-87]. The incorporation of novel side chains should enable the synthesis of peptoids with entirely novel properties. In general, cationic peptoid polymers and cationic lipitoid polymers (peptoid-phospholipid conjugates) are efficient gene transfer molecules. Measurements revealed that the biophysical properties of DNA-polymer complexes (size, ζ-potential, ethidium bromide exclusion) varied with polymer structure and complex (+ / -) charge ratio, but were not directly predictive of transfection efficiency.[88] A versatile and efficient solid phase approach has been developed for the synthesis of these cell permeable peptoid oligomers. These peptoids consisted of monomer units derived from ethyl bromoacetate and protected 1,6-hexanediamine. A series of fluorescein conjugates were constructed (Scheme 2). Fluorescence microscopy and FACS analysis indicated that these materials possessed the ability to be successfully taken up the cells. The uptake efficiency of the peptoids **11** was dependent on the number of monomer units of the oligomer. The heptamer exhibited maximum internalisation (> 99% of cells labelled). Cellular uptake was concentration-dependent, although cellular enrichment was evident in all cases.[51]

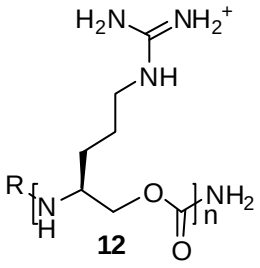


Scheme 2 Optimized peptoid transporter and its synthesis. Reagents and conditions: (a) **9**, PyBrOP then piperidine; (b) 1. *N*-Fmoc-6-aminocaproic acid, PyBrOP, 2. piperidine, 3. carboxyfluorescine, PyBrOP, 4. TFA or ß-mercaptoethanol then TFA.[51]

These findings complement reports by other groups, who have also indicated an enhanced uptake rate for the larger oligomers.[82] First studies in cells showed that in contrast to CPPs, the uptake of peptoid transporters is ATP-dependent and can be completely inhibited by the addition of sodium azide to the cultured cells prior to exposure to the peptoids. The uptake rate was also decreased at 20 °C, whereas no internalization was observed at 4 °C. These temperature-dependent results suggest the involvement of an endocytic pathway, which has been implicated in the transport of small organic molecules by poly-*L*-lysine, which shares some structural similarities with the peptoids. None of the peptoids were found to be toxic at concentrations up to 400 µM of the concentrations tested, which qualifies the peptoids as delivery agents for in vivo studies.[8, 51]

2.1.3.4 Oligocarbamates

Since guanidine-rich peptides and peptoids with 1,4-spaced side chains are superior to TAT_{49-57} in cellular uptake[8], Wender et al. explored whether the amide backbone common to peptide and peptoid transporters could be replaced with a carbamate, and whether the resulting increased spacing and enhanced flexibility (1,4 to 1,6) would enhance uptake into cells and tissues.[84]



a: R = Fluorescinyl; n = 5
b: R = Fluorescinyl; n = 7
c: R = Fluorescinyl; n = 9
d: R = Biotinyl; n = 5

Figure 5 Optimized oligocarbamate transporters.[89]

A series of novel guanidine-rich oligocarbamates was prepared. The synthesis was designed to produce an oligoamine, which upon perguanidinylation would be converted to the desired oligoguanidine (Figure 5). The oligocarbamate 5-, 7-, and 9-mers of **12** were synthesized on solid phase with an automated peptide synthesizer. The ability of the oligocarbamates to enable cellular uptake was determined by FACS analysis of Jurkat cells that had been incubated with fluoresceinated oligomers. The pentacarbamate and pentaarginine conjugates displayed very limited cellular uptake. In contrast, the hepta- and nonacarbamate conjugates had entered virtually all cells after 3 minutes. The rate of uptake increased with guanidine content (8 > 7) and concentration, which corresponds to the behaviour of other TAT-related transporters.[84, 89] Carbamate 9-mer translocated into cells 2.3 times faster than a-Arg_9, which was taken up into cells 100 times faster than TAT, making it one of the best guanidine-based transporters studied to date.

2.2 Polyamines

2.2.1 Distribution and function inside the cell

Polyamines are aliphatic molecules, carrying two or more amino functions.[90] Although the pK_a-values of the several amino functions differ, they are usually all protonated at physiological pH-values. Consequently, polyamines appear in organisms as highly positively charged molecules and their function mainly relies on those polycationic properties. Some prominent aliphatic polyamines are shown in Figure 6.

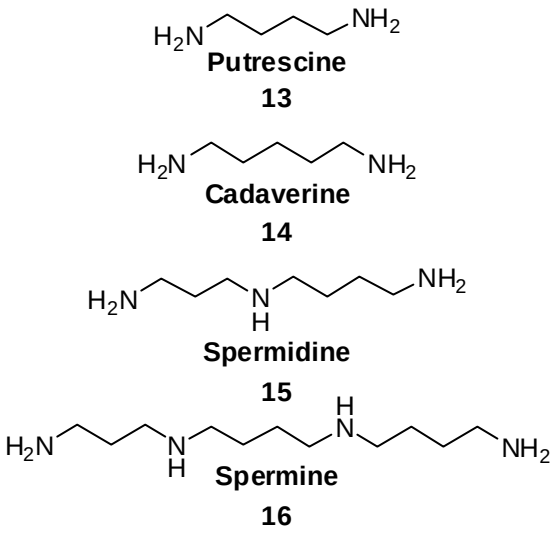
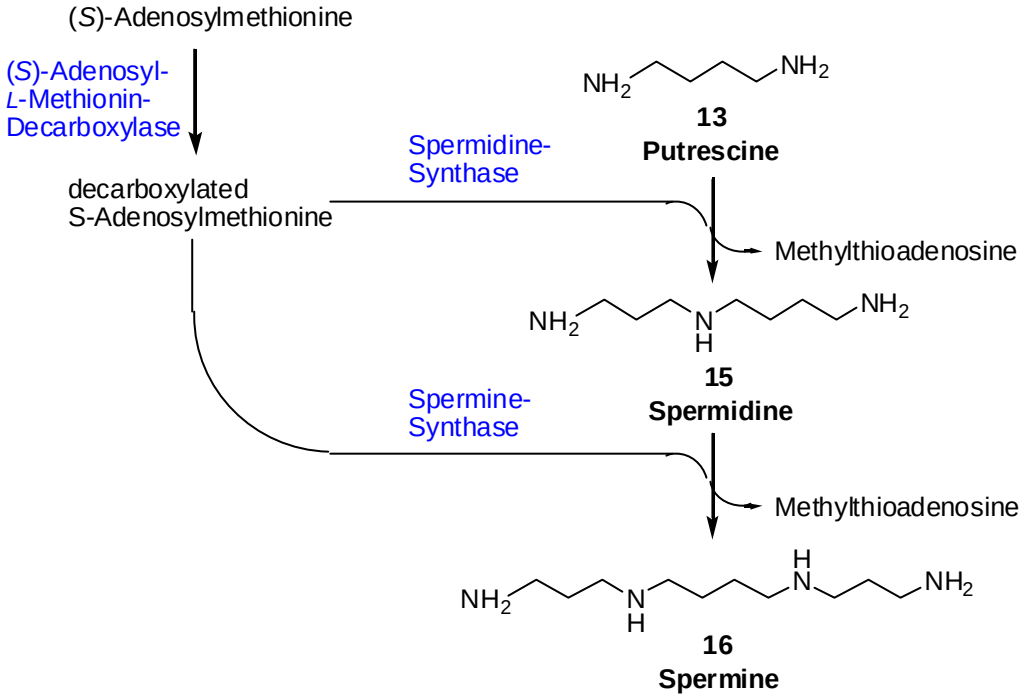


Figure 6 Four of the most abundant polyamines in nature. Usually, they are protonated at physiological pH.[91]

Besides their biosynthesis, a high amount of polyamines are obtained from diet and bacteria residing in the gut.[91] These polyamines are transported through the mucosa, presumably by passive diffusion through the paracellular pathway, and reach the systemic circulation. Eventually, they are distributed throughout the body and are widely implicated in cell growth, development of tissues and tissue repair. They have been involved in the growth not only of normal tissue but also of malignant tumours.[91] Furthermore, it has been implied that they play an important role in the development of the gut and the repair of gastric and duodenal injuries, as well as in other wound healing processes. Increase in polyamine levels in several other tissues has been associated with the normal growth and hypertrophy of several tissues such as skin, breast, kidney, and heart.[91] Since polyamines are protonated under physiological conditions, they can interact with many negatively charged or zwitterionic biomolecules such as DNA, RNA, proteoglycans, and phosphate groups of phospholipids, and negatively charged amino acids of proteins such as aspartate and glutamate.[92] Especially, spermine **(16)** has been shown to condense the DNA and chromatin, thus stabilizing DNA conformations such as triplexes, allowing either modulation of protein-DNA interactions or protection of DNA from degradation. The hydrophilicity / hydrophobicity of most polyamines is balanced, allowing them to perform physiological functions by interacting with some of these anionic structures, without impairing the functionality of others.[92] In addition to the genuine aliphatic polyamines, numerous naturally occurring polyamine conjugates have been isolated and characterized, displaying a broad range of biological functions and activities. These range from potent immunosuppressive agents such as deoxyspergualin, over

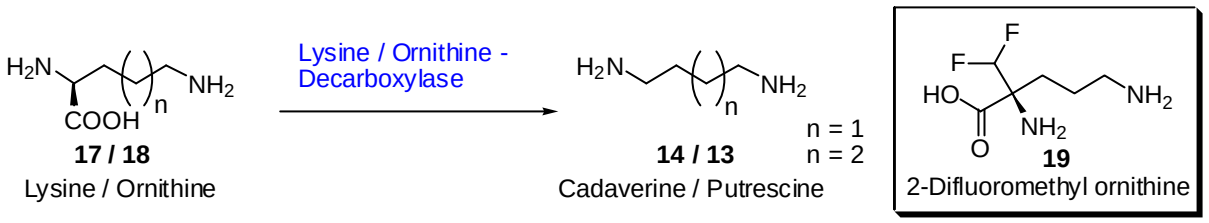
antibacterial and broad-spectrum antibiotics such as squalamine **(35)**, Edeine A, guanidospermidine, and glycinamoylspermidine, to alkaloids such as wasp toxins and spider venoms.[92] Due to their cationic properties, polyamine moieties were found to enhance water solubility and cellular uptake of many therapeutic molecules, making them potential drug delivery agents. For most of the properties of polyamines, the biological mechanisms are not well understood or still in debate. Therefore, current research on natural and synthetic polyamines requires the synthesis of those compounds, as well as many derivatives in order to investigate their function and to obtain novel mimics for therapeutic applications.

2.2.2 Biosynthesis of Polyamines



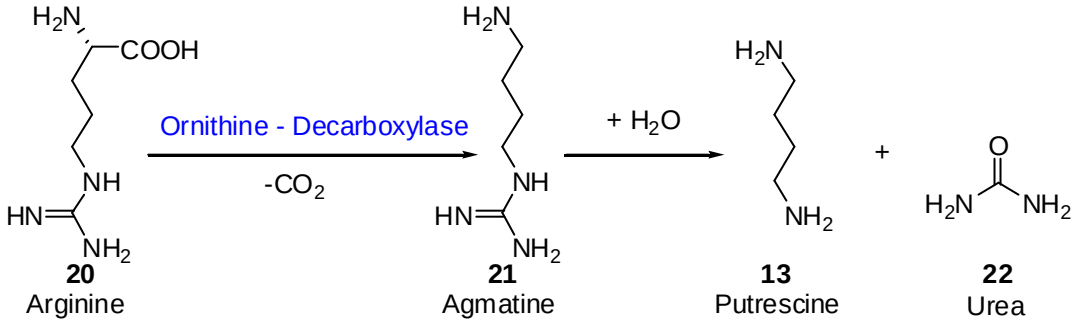
Scheme 3 Biosynthesis of the three most often appearing polyamines putrescine **(13)**, spermidine **(15)**, and spermine **(16)**.[90, 91]

The common biosynthetic pathway of spermine (Spm) **(16)** and spermidine (Spd) **(15)** starts with putrescine (Put) **(13)** (Scheme 3). Putrescine **(13)** is alkylated with a 3-aminopropyl building block by spermidine synthase, leading to spermidine **(15)**, which is again alkylated by the spermine synthase, yielding spermine **(16)**. The exclusive 3-aminopropylation on the second amino group derived from cadaverine illustrates that polyamine-forming enzymes are highly regiospecific. The 3-aminopropyl group is transferred from *S*-adenosylmethionine, which was previously decarboxylated by the *S*-adenosylmethionine decarboxylase. This alkylation is rather uncommon, since *S*-adenosylmethionine usually acts as a methylating agent. Finally, all the building blocks for the polyamine biosynthesis derive from amino acids, which are decarboxylated and activated for alkylation. Thus, the polyamine biosynthesis has common points with the citric acid cycle.



Scheme 4 Biosynthesis of cadaverine / putrescine **(13)** starting from lysine **(17)** / ornithine **(18)** in mammalian cells.[90, 91]

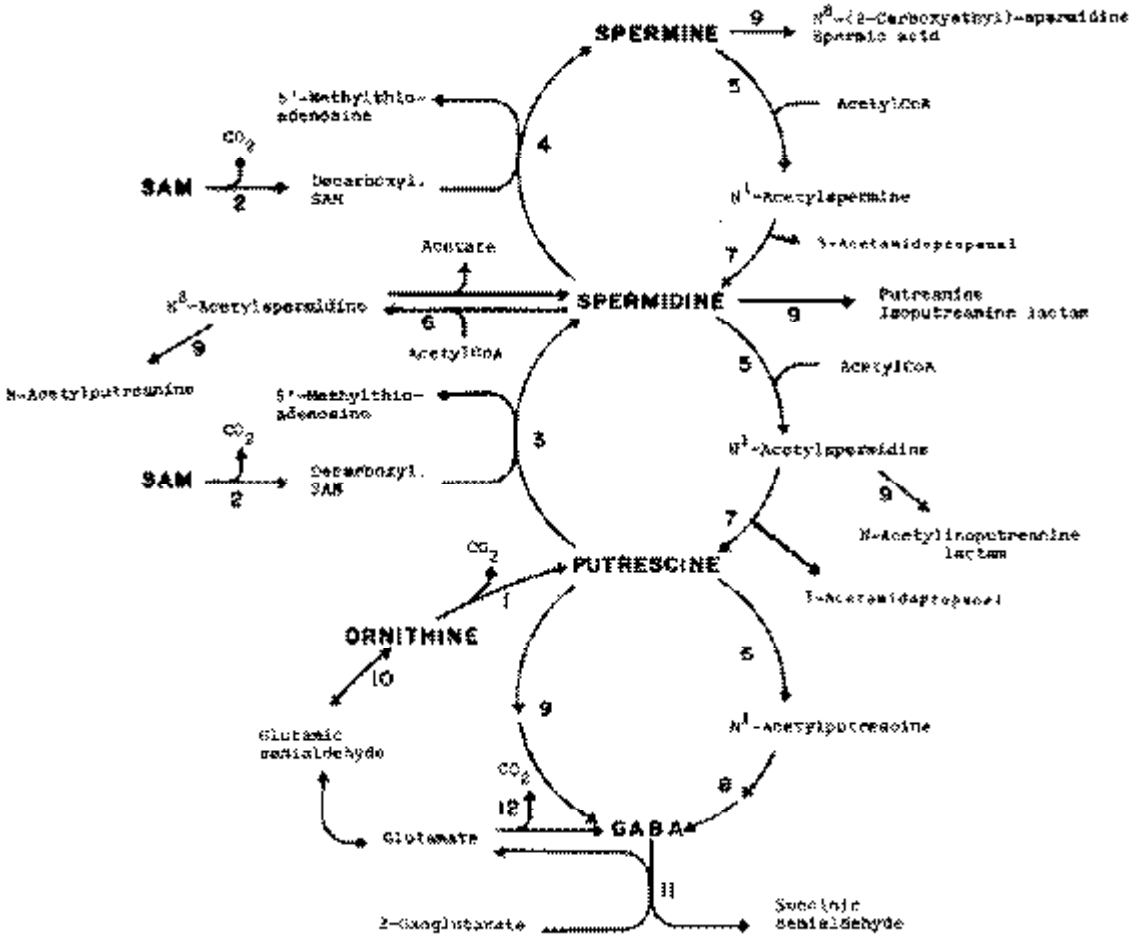
The diamines putrescine **(13)** and cadaverine **(14)** are derived by decarboxylation of ornithine **(18)** and lysine **(17)**, respectively (Scheme 4). Putrescine **(13)** has a special regulatory function in the spermine **(16)** / spermidine **(15)** biosynthetic pathway. It inhibits the *ornithine decarboxylase* (ODC), acts as Co-factor for the *S*-adenosylmethionine decarboxylase, and as substrate for the spermidine synthase. Consequently, the inhibition of ODC is a major target for the down-regulation of polyamine biosynthesis. Difluoromethyl ornithine **(19)** (DFMO) was developed as a potent inhibitor of ODC with strong effect on global cellular polyamine levels. Further contributing factors are discussed in chapters 2.2.2 and 2.2.3.



Scheme 5 Biosynthesis of putrescine **(13)** starting from arginine **(20)** in plant cells.[90, 91]

Plants lack ODC and derive putrescine **(13)** from decarboxylation of arginine and subsequent hydrolysis (Scheme 5). The unstable multifunctional enzyme *putrescine synthase* that combines the activities of agmatine iminohydrolase, putrescine transcarbamylase, ornithine transcarbamylase, and carbamate kinase, was purified from *Lathyrus sativus*. The catabolism of aliphatic polyamines is dominated by acetylation and oxidative deamination reactions (Scheme 6). The degradation of spermine **(16)** leads back to putrescine **(13)** *via* spermidine **(15)**. The N^1-position of either spermine **(16)** or spermidine **(15)** is acetylated by the acetyl-CoA dependent spermidine-N^1-acetyltransferase. The ubiquitous FAD-containing *polyamine oxidase* (PAO) is capable of degrading the acetyl-derivative to 3-acetamidopropanal and spermidine **(15)** or putrescine **(13)** respectively. N^8-acetylspermidine is not oxidized by *polyamine oxidase*, also showing the regioselectivity of catabolic enzymes. It is noteworthy that the *polyamine oxidase* also degrades non-acetylated polyamines, but with a significantly lower activity. The oxidative degradation of putrescine **(13)** represents an alternative route for the biosynthesis of χ-butyric acid (GABA), which can replace the decarboxylation of *L*-glutamate by the pyridoxal phosphate (PLP)-depending glutamic acid decarboxylase (GAD). Consequently, the inhibition of GAD

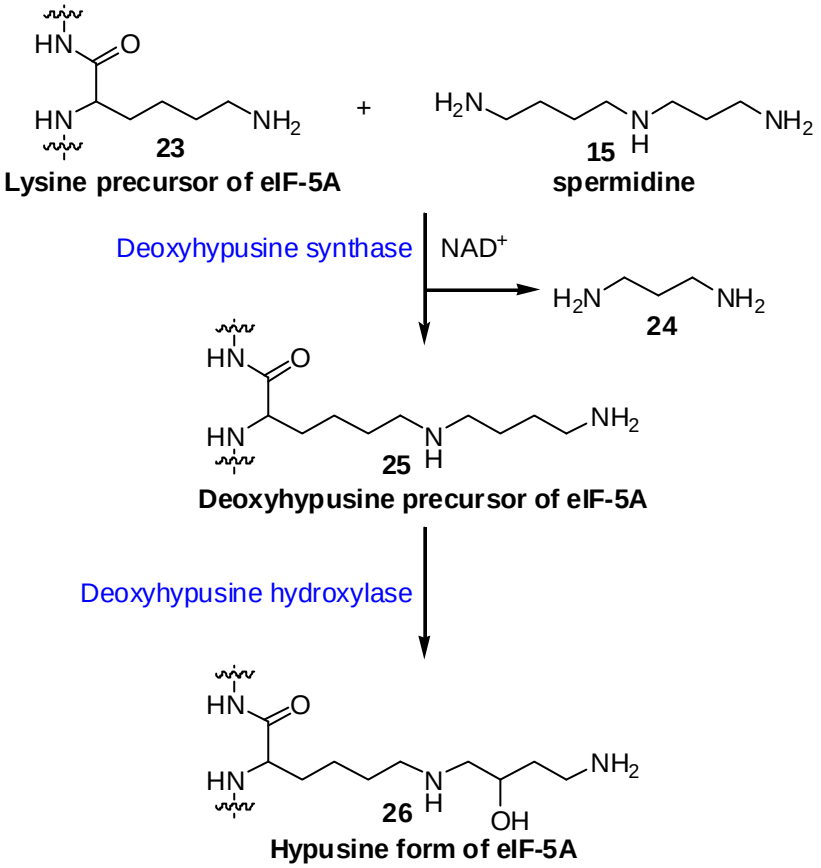
markedly increases ODC-activity, while decreasing *S*-adenosylmethionine decarboxylase, despite a rise in putrescine concentration.[93]



1 = Ornithine decarboxylase (ODC)
2 = S-Adenosylmethione decarboxylase (SAMDC)
3 = Spermidine synthase
4 = Spermine synthase
5 = AcetyI-CoA: polyamine acetyltransferase (cytoplasmic)
6 = Acetyl-CoA: spermidine NS-acetyltransferase (nuclear)
7 = Polyamine oxidase (PAO)
8 = Monoamine oxidase
9 = Diamine oxidase (or other copper-containing amine oxidases)
10 = Ornithine-2-oxoacid aminotransferase
11 = 4-Aminobutyrate: 2-oxoacid aminotransferase (GABA-T)
12 = Glutamate decarboxylase (GAD)

Scheme 6 The polyamine metabolism in human. (Image taken from Cohen, A guide to the polyamines, 1998)[94]

Putrescine **(13)** is accepted by the acetyl CoA-dependent spermidine-N^8-acetyltransferase for acetylation to N^1-acetylputrescine, which is then oxidized to GABA by the monoamine oxidase. By systematic fractionation of rat urinary metabolites of radiolabelled putrescine **(13)**, spermidine **(15)**, and spermine **(16)**, a lot of other metabolites were obtained.[95] Prominent members are 2-pyrrolidone, GABA, 2-hydroxyputrescine (from putrescine **(13)**), putreanine, isoputreanine, monacetylated polyamines (from spermidine **(15)**), spermic acid, and N^8-(2-carboxyethyl)spermidine (from spermine **(16)**). Several catabolic intermediates deriving from the action of acetylases, oxidases and lactam forming cyclases were identified in mammalian tissues and sera.



Scheme 7 Biosynthesis of hypusine from lysine in eIF-5A.[91]

Interestingly, the synthesis of the amino acid hypusine directly links the spermidine metabolism to the expression of the Eukaryotic translation initiation factor 5A (eIF-5A, see Scheme 7). The modification, consisting in the alkylation of the e-amino function of lysine by a 4-aminobutyl moiety and their subsequent hydroxylation in the ß-position appears to be posttranslational. Simultaneously, spermidine **(15)** is degraded to 1,3-diaminopropane **(24)**.[91]

2.2.3 Polyamine transport system

Besides the rate of biosynthesis, the action of the polyamine transport system (PTS) has a great influence on the cellular concentration of polyamines. Although the rate of biosynthesis is high to maintain the level of polyamines in cells, it can be largely substituted by active transport into cells. This is supported by the failure of DFMO **(19)** to solely inhibit the compensatory growth of organs and tumours *in vivo*.[96-100] Likewise, the lifetime of mice incubated with mutant L1210 leukemia cell line deficient in polyamine transport was extended by 60 – 70% compared to mice incubated with the parental cells.[101] Nearly all cells are able to take up and secret polyamines. Tissues with a high demand, like prostate glands, tumours, and normal but rapidly proliferating cells take up polyamines in large amounts from the environment.[102-106] Polyamine uptake characteristics can vary, depending on the state of cellular growth and maturation of the organism. Nevertheless, several general features of the polyamine transport are common. Transport is

- energy and temperature dependent
- saturable,
- activated by Na^+.

The saturation suggests a carrier-mediated transport of the polyamines. Experiments in the absence of sodium suggest that in some cell lines, also a Na^+ independent transport exists.[107] However, most cells seem to have only one polyamine transporter with growing affinity for increasing number of positive charges.[107] As it is least selective for putrescine **(13)**, some cells also have an additional transport system for putrescine **(13)**. Finally, little is known about the biochemical components, which are involved in the polyamine transport and several models exist for uptake mechanism.[52, 108, 109] Three important aspects of the polyamine transport system are of special interest to understand polyamine analogue effects on mammalian cells. The transporter has high affinity for spermidine **(15)** and spermine **(16)**, the velocity of uptake is quite substantial and the transport system is feedback-regulated. The specificity of the transporter is not stringent.[112, 113], as it tolerates multiple analogues with methyl and ethyl substituents on the amines or in a-position.[114-117] In contrast, benzyl modifications on the amines appear to be too bulky to be transported by the uptake system of naturally occurring polyamines. However, experimental evidence exists that anti malaria active bis(benzyl)polyamine analogues are transported by a different mechanism.[118, 119] Especially the putrescine moiety of all polyamines seems to be tolerant as it can be replaced by cyclohexyl rings or unsaturations and bear free amino groups.[120-122] Aminooxy analogues are also taken up as well as fluorinated analogues.[123, 124] The uptake system is also shared by structurally only weakly related compounds like *N,N´*-dimethyl-4,4´-bipyridylium (Paraquat) **(27)** [125], mimicking 1,6-diaminohexane, and methylglyoxal-bis(guanylhydrazone) (MGBG) **(28)** [126-128], mimicking spermidine **(15)** (Figure 7). MGBG **(28)** also acts potent inhibitor of the *S*-adenosylmethionine decarboxylase.[129]

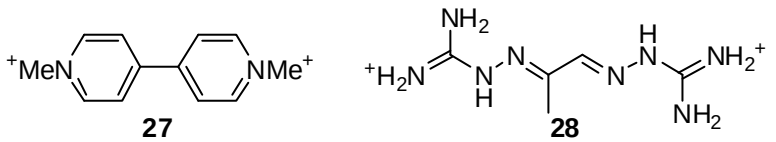


Figure 7 Compounds sharing the polyamine uptake system.

Enhanced polyamine uptake is often coupled to increased *de novo* biosynthesis of polyamines. Factors, enhancing the formation of polyamines often also increase their uptake from environment. In *E. coli*, the genes coding for ODC and the putrescine transporter protein coexist on 16 min of the same chromosome.[130] Cells overexpressing ODC are unable to downregulate uptake and thus accumulate exogenous polyamines to toxic concentrations.[131-133] Both, uptake and *de novo* biosynthesis of the polyamines are feedback-regulated by the cellular concentrations of the polyamines. The arginine **(20)** rather than the ornithine **(18)** concentration seems to have a strong influence on putrescine uptake.[134-136] At supra-normal concentrations of polyamines, antizyme proteins are expressed, which inactivate ODC complex formation and thus block *de novo* biosynthesis of polyamines at the biosynthetical starting point.[137-140]

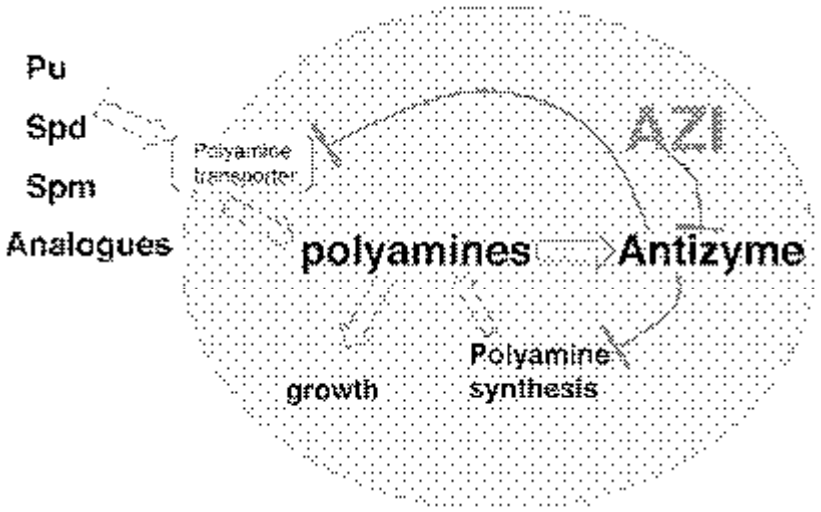


Figure 8 Control of cellular polyamine levels by antizyme.(Image taken from Mitchell, Biochem. Soc. Transac., 2007)[109]

Antizyme also showed to have influence on the feedback control of polyamine uptake, unless the mechanism is not yet understood (Figure 8). Especially this feedback control of uptake is important, as the polyamine transporter is highly active and would otherwise over-accumulate polyamines inside the cell. Cells of higher eukaryotes posses an antizyme-binding protein, termed antizyme inhibitor (AzI) that appears to be involved in lowering antizyme activity and closely interacts with polyamine levels.[143] Consequently, some tumour cell lines over-express antizyme inhibitor to enable accumulation of polyamines required for their aggressive growth.[144] Thus, exposure of cancer cells lines to antizyme stimulating polyamine analogues can efficiently suppress polyamine transport.[145] Besides lowering *de novo* biosynthesis and restricting uptake of polyamines, cells can also react by enhanced release of polyamines and acetylpolyamines. This export appears to be carrier mediated.[146] Uptake and export of polyamines are mediated by different transport systems, for which spermine **(16)** as well as spermidine **(15)** rather than putrescine **(13)** or other diamines seem to act as signalling compounds. However, spermine **(16)** is usually only released in minimal amounts.[147-149] It is either degraded to spermidine **(15)** or exported as its N^1-acetyl derivative (Figure 9). Likewise, spermidine **(15)** is largely converted to putrescine **(13)** or its acetyl derivatives before excretion. The preferentially excreted polyamine derivative strongly depends on the environment. As the polyamine metabolism is linked to the amino acid metabolism, conversion into their oxidative deamination products displays another possible route for their degradation. However, since the required enzymes are not ubiquitously present, this way is restricted to selected tissues.[150] A coherence of polyamine transport and Ca^{2+} concentration is also described.[52, 151, 152] Exposure of cultured cells to putrescine **(13)** and spermidine **(15)** increased intracellular Ca^{2+}. On the other hand, calmodulin antagonists inhibit ODC and polyamine uptake, suggesting a role of polyamines in the regulation of transmembrane signalling pathways as well as participation of Ca^{2+} in polyamine uptake mechanism.[152-154] Besides calcium ions, Mn^{2+} is an activator of polyamine uptake. Other divalent cations like Ba^{2+}, Sr^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+} also have either a weaker or opposite effect.

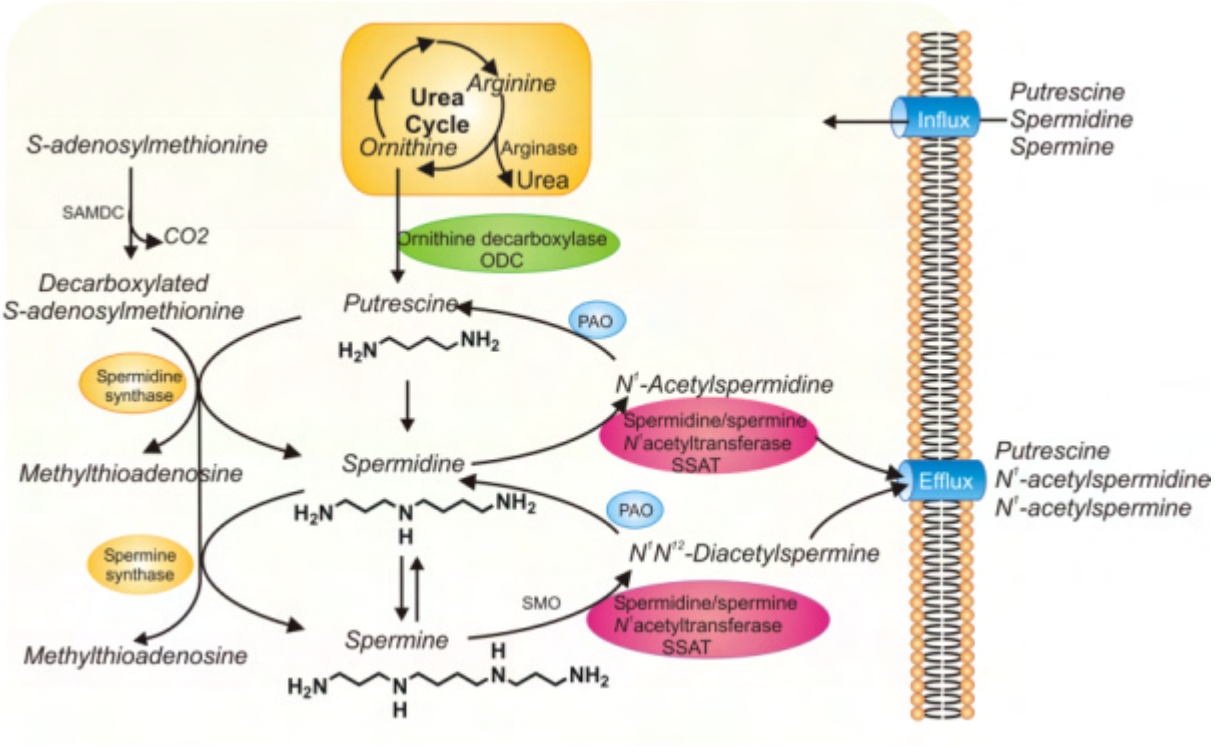


Figure 9 Major polyamine transport ways through the cell membrane their interconnection to the mayor biosynthetic pathways. The polyamines are mainly taken up in their native form. However, only putrescine **(13)** is released in significant amounts. Spermidine **(15)** and spermine **(16)** are converted to their acetyl-derivatives by SSAT prior excretion. (Image taken from Hahn, Schepers, in Combinatorial Chemistry on Solid Supports, 2007)[92]

2.2.4 Therapeutic applications of polyamines

The described properties of polyamines make them suitable candidates for therapeutic use. The applications can be classified into five major groups:

- DNA recognition and binding
- Natural products of toxins and venoms
- Polyamine conjugates with antibiotic, bactericidal, or antiparasitic properties
- Cancer therapy
- Drug delivery

In all cases the efficacy of polyamines is either based on their high charge density, which enables strong interaction with negatively charged DNA, RNA, proteoglycans, and phospholipids, or it is based on the highly amphiphilic nature of polyamine conjugates with hydrophobic moieties.

2.2.4.1 DNA recognition and binding

A number of studies have suggested that DNA is a major target of polyamines. As already described above, aliphatic polyamines have shown to either condense or bend DNA by binding to the nucleic acid via strong salt bridges, or disturb the DNA structure by introducing intercalating conjugates. Polyamine-DNA interactions play a pivotal role in DNA conformational transitions, condensation / decondensation, and stabilisation.[155, 156] Stabilisation of specific DNA conformations may be important for nucleosome formation, chromatin condensation, and gene expression. The binding of the polyamines alters the DNA structure from the common B-helix to the A and Z forms.[157] It also leads to aggregation at higher concentration. Such an effect might be important for the DNA physiology, as a tight correlation exists between the acquisition of the Z-Form and the transcriptional activity on DNA. The stability of the double helix increases with the ratio of the positively charged amino groups to the negatively charged phosphate groups.[158] Direct binding of polyamines to DNA and their ability to modulate DNA-protein interactions appear to be important in the molecular mechanisms of polyamine action in cell proliferation. Therefore, DNA-binding polyamines have the potential to exert anticancer activity. Interactions of polyamines with DNA were thoroughly studied in order to allow for the selective design of anticancer drugs by the synthesis of a series of analogues. Recent studies indicated that programmed cell death might be a common characteristic for polyamine analogue-induced cytotoxicity in cancer cells.[159-161] Interesting DNA-interacting polyamine analogues are naphthalimides and bis-naphthalimides, N^1-(anthracene-9-carbonyl)-spermine and N^1,N^{12}-bisaziridinyl-spermine, which have been shown to intercalate into the DNA backbone.[162-167]

2.2.4.2 Natural products of toxins and venoms

In the last couple of years research was focused on the synthesis of polyamines present in spider and wasp toxins such as Nephilatoxin-643 (NPhTX-643), philanthotoxin-433 (PhTX-433) **(33)** and many other analogues. These natural products are mainly present in the venom glands of the spider or wasp and serve as a paralyzing toxin (Figure 10).[168, 169] Most of the polyamine toxins show a high degree of structural similarity, implying that the polyamine part of the toxin has an important function. The interest in these acylpolyamines arises from their ability to act as potent antagonists of the mammalian neuroexcitatory or ionotropic glutamic acid receptors (iGluR), or as inhibitors of the nicotinic acetylcholine receptors. The family of the iGluR comprises the *N*-methyl-*D*-aspartate (NMDA), the 2-amino-3-(3-hydroxy-5-methyl-4-isooxazolyl)-propionic acid (AMPA), and the kainaite receptor. These are partially blocked by the polyamine toxins in an open cationic channel conformation.[169] Attempts have been made to exploit this interaction for the therapy or prevention of neurotoxicity, epilepsy, and neurodegenerative diseases.[170, 171] As described, for many polyamine toxins, the polyamine moiety is coupled via an amide bond to an aromatic moiety, mostly consisting of 2,4-dihydroxyphenylacetyl, *N*-butoryl-tyrosine, or indol-3-acetyl moieties.

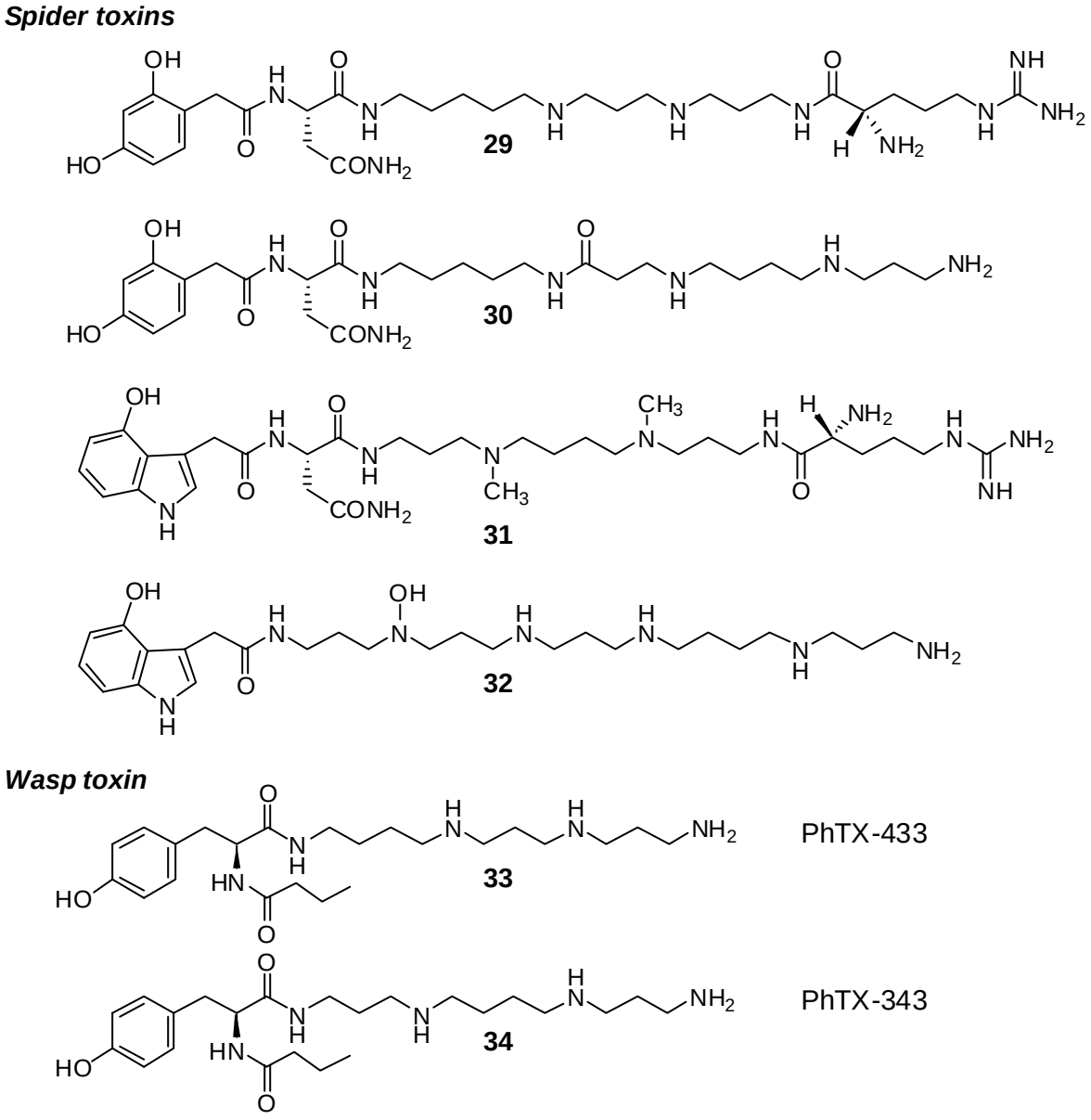
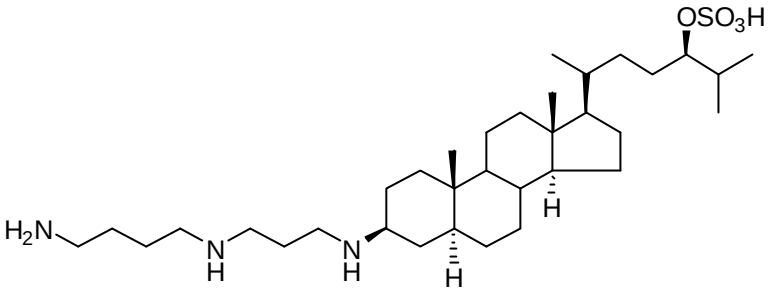


Figure 10 Naturally occurring spider and wasp toxins containing polyamine backbones.[169]

2.2.4.3 Polyamine conjugates with antibiotic, bactericidal, or antiparasitic properties

The emergence of multidrug resistant microorganisms such as the methicillin-resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus*, and drug resistant *Mycobacterium tuberculosis*, pushes the pharmaceutical industry to expand their research on novel classes of antibiotics.[172-174] Lipopolyamines are of particular interest. They are characterized by the presence of long-chain acyl- or alkyl substituents on polyamine scaffolds. Members of the lipopolyamine class bind lipo-polysaccharide (LPS), are effective in preventing endotoxic shock in animal models, and yet appear to be non-toxic both *in vitro* and *in vivo*.[175] Recent reports indicate a correlation between antiendotoxic and antibacterial activity in lipoamines with stable amide-linked acyl groups and in cationic amphipathic peptides[176-178]. Recent reports proved the interactions of a series of *N*-acylated homologated spermine compounds with LPS.[179] Importantly, they are also non-toxic due to their

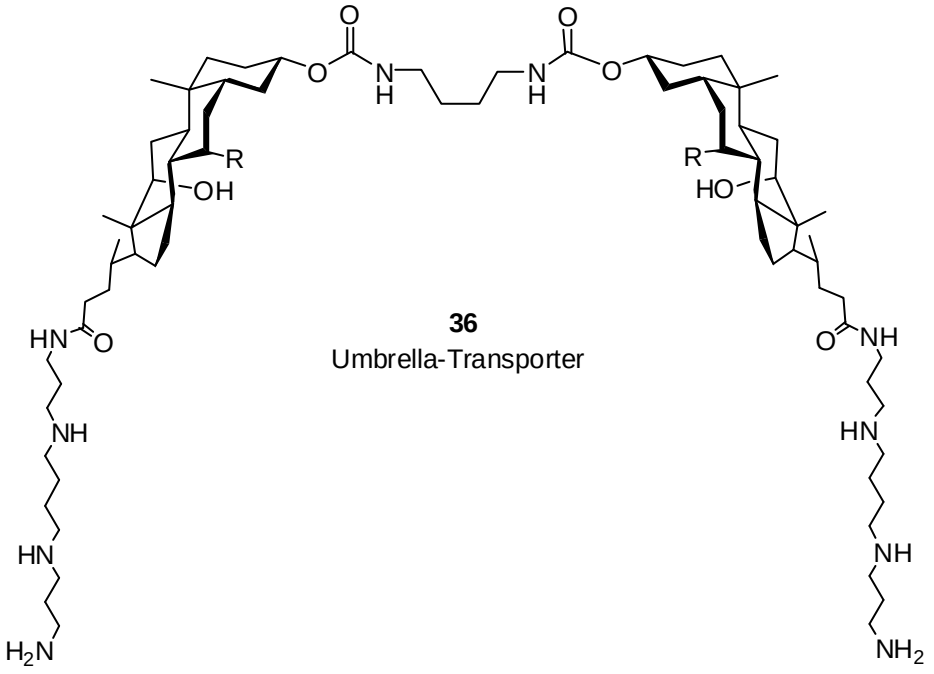
biodegradation to physiological substituents (polyamine and CoA-fatty acid esters).[175, 180] In recent years, an interesting broadband antibiotic, squalamine **(35)** (3-β-*N*-1-(*N*-[3-(4-aminobutyl)]-1,3-diaminopropane)-7-α-24-ζ-dihydroxy-5-α-cholestane-24-sulfate), has been isolated from the dogfish shark Squalus acanthias (Figure 11). Squalamine **(35)** is a cationic steroid characterized by a condensation of an anionic bile salt intermediate with spermidine **(15)** and exhibits potent antibiotic and microbiotic activity against both gram-negative and gram-positive bacteria, as well as against fungi.[174, 181]



35
Squalamine

Figure 11 Structure of Squalamine **(35)**.[181]

In addition, squalamine **(35)** induces osmotic lysis of protozoa and show antiangiogenic activity, making it useful for the treatment of different diseases such as lung, ovarian, brain and other cancers, as well as age-related macular degeneration (AMD) and the control of body weight in human.[181, 182] Its amphiphilic structure, derived from the hydrophilic spermidine chain and the hydrophobic sterol scaffold, enables squalamine **(35)** to interfere with membrane lipids, thus enhancing cellular uptake. Moreover, some of the squalamine mimics, as well as related compounds based on the squalamine structure, effectively permeabilise the outer membranes of gram-negative bacteria, thus sensitizing these organisms to hydrophobic antibiotics.[183]

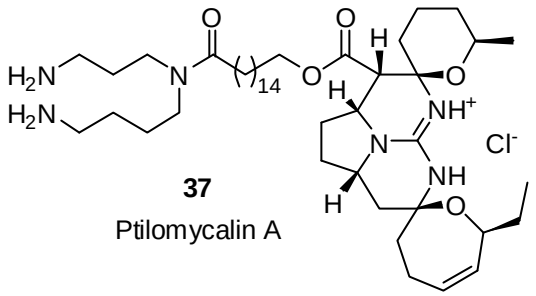


36
Umbrella-Transporter

Figure 12 Squalamine-based umbrella transporter.[184]

A kinetic analysis of the membrane activity of squalamine analogues in discharging pH gradients across liposomal membranes supported a model in which a monomer and a non-covalently-linked dimer coexist in equilibrium. Based on this model, molecular umbrella molecules were generated, in which two molecules of squalamine **(35)** or its derivatives are covalently linked to form dimers (Figure 12). These dimers can be coupled with a variety of cargo and drug molecules, for which cellular uptake is enhanced. Coupled molecules range from small molecules such as amino acids up to ATP, AMP, glutathione, anti HIV drugs, and even oligonucleotides.[184-187]

Other polyamines such as pseudokeratidine[188-190], which is used in antifouling paints are secondary metabolites from marine sponges with antimicrobial activity. The majority of those alkaloid polyamines contains a spermidine moiety such as Ptilomycalin A **(37)** (Figure 13).[191]



37
Ptilomycalin A

Figure 13 Structure of Ptilomycalin A **(37)**. The molecule contains a spermidine- and a guanidinium moiety, which are separated by an ester linked long chain aliphatic, hydrophobic region, and a polycyclic non-aromatic region.[191, 192]

It was long known that cytotoxic properties of many polyamine analogues depend on the number of nitrogen atoms in the chain, and therefore the charge distribution of these molecules at physiological pH, the distance between the nitrogen atoms, and the nature of the terminal alkyl substituents. Anticancer derivatives of oxa-polyamines and bis-oxy-naphtalimides are examples for cytotoxic polyamines with strong effects. While the parent oxa-spermine and spermidine do not show biological activity, their sulfonamide derivatives exhibit cytotoxicity against a broad panel of malignant and proliferating cells.[193-199] In recent years, many synthetic polyamine conjugates have been synthesized in which the polyamine moiety is covalently coupled to cytotoxic compounds such as chlorambucil or porphyrins.[200] The polyamine enhances the solubility and the cellular uptake of the cytotoxic compounds.

2.2.4.4 Polyamines in cancer therapy

A large body of data indicates that the polyamine pathway can be a molecular target for therapeutic intervention in several cancer types (see chapter 2.2.1).[201] The last two decades have seen an increasing interest in the cytotoxic response to agents that do not directly inhibit the metabolic enzymes in tumour cells, but rather the regulation of the polyamine metabolism.[202] This interest resulted in the generation of a variety of polyamine analogues and conjugates displaying effective cytotoxicity (see chapter 2.2.3). Having a similar structure to natural polyamines, these analogues efficiently enter the cells via the polyamine transport and uptake machinery. However, they also have a better specificity for cancer cells exhibiting, thus lower toxicity to normal cells, as well as a better metabolic stability. The cytotoxicity of these analogues for cancer cells has been attributed to various mechanisms such as the

induction of the catabolic enzymes or the reduction in the activity of the anabolic enzymes. Both mechanisms serve to reduce the polyamine content of the cell. Once in the cell, they can mimic intracellular polyamines that serve as anti-metabolites, displacing the natural polyamines from their binding sites but not substituting their growth promoting properties.[202] They lower the polyamine level below the level necessary for cell survival. Some of these polyamine analogues are also able to interfere with the polyamine uptake from nutrition, as well as to accelerate the degradation and the export of the natural polyamines.[203-206]

2.2.4.5 Polyamines in drug delivery

Since polyamines are highly positively charged at physiological pH, they can interact with biological membranes that mainly contain zwitterionically- and anionically charged phospholipids, proteins, and proteoglycans.[91] Electrostatic interaction with membranes often leads to internalization of the bound molecules via endocytic mechanisms. Additionally, many organisms have an active, but largely unknown polyamine uptake system.[52, 108] When covalently bound to a therapeutically active drug, polyamines and polyamine conjugates with lipophilic anchors can be used as drug delivery moieties. The polyamine moiety often serves as a solubilising agent for very hydrophobic drugs in the aqueous environment, additionally increasing the electrostatic interaction with the biological membrane. Further, the polyamine moiety can serve as a complexion agent to neutralize negatively charged drugs, which would otherwise not interact with a mainly negatively charged membrane, and therefore be excluded from endocytic uptake. To date, polycationic moieties are used for a variety of drugs to enhance their cellular uptake (Figure 14).

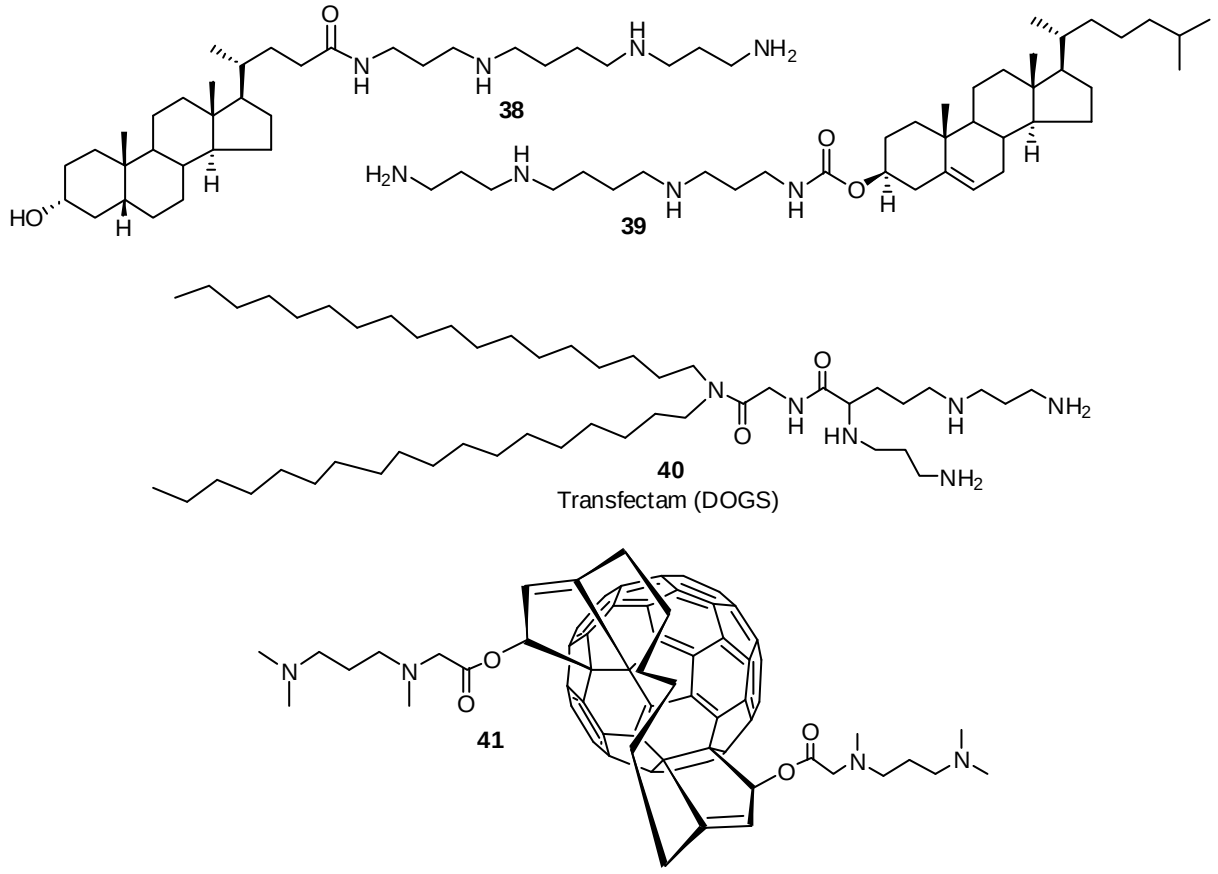
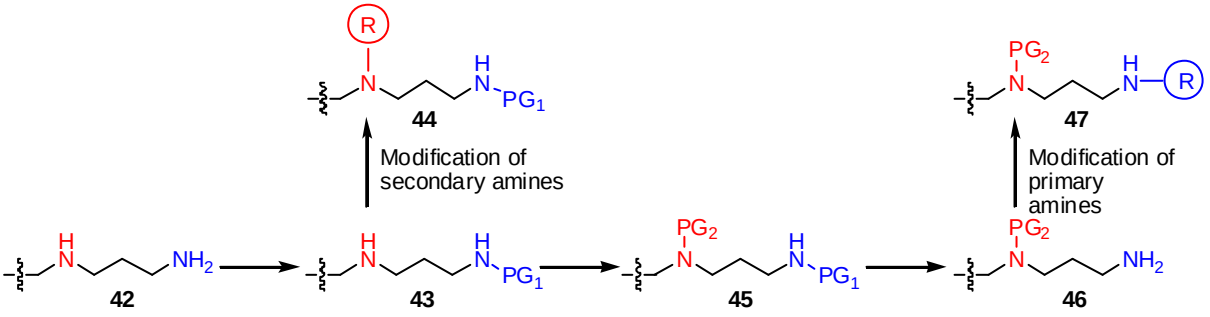


Figure 14 Polyamines used for transfection of DNA and nucleotides.[92]

Polyamine scaffolds have been chosen as precursors for DNA delivering agents since they display some important features. Prerequisites for delivery of DNA across the cellular membrane are the condensation of the DNA and the masking of the negative charges of the nucleotide backbone as described by polyamines.[207] Conjugates of polyamines with long aliphatic chains[208], bile acids[208], cholesterol and steroids[209-212], as well as other lipid moieties[180, 213], have been recently developed as gene delivery agents (Figure 14).The attachment of hydrophobic residues, such as a lipid moiety, further enhances the condensation and the crossing of the hydrophobic membrane part.[214-216] The mechanism by which these compounds mediate cellular uptake is still poorly understood. It was proposed that the polyamines still interact with the phospholipids head groups of the membrane lipids, leading to close neighbourhood of the DNA and the membrane. The complex eventually will be taken up by endocytosis and delivery into the endosomes. There are many proposed mechanisms such as the proton sponge theory or the retrograde transport of DNA via the Golgi apparatus.[217-219] Combinatorial approaches on solid phase have been used to synthesize amphiphilic polyamine conjugates as non-viral gene delivery agents. The highest activity was detected with compounds, which contain two guanidinium head-groups and only one aliphatic or hydrophobic chain. While nearly the entire steroid conjugates showed poor DNA binding and transfection efficiency, some of the straight aliphatic chain conjugates displayed good transfection activities.

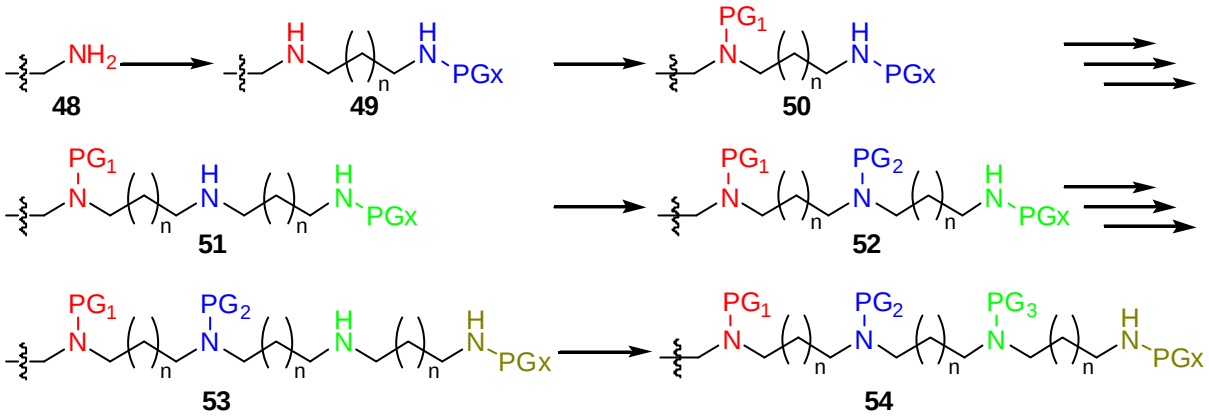
2.2.5 Polyamine synthesis

Polyamine chemistry often encounters problems regarding the selective protection or the directed reaction of the various secondary and primary amino functionalities. However, only few possibilities for the direct derivatization of particular amines in a polyamine are known. Selectivity is mostly achieved by the use of complex protection group schemes, which differentiate between primary amines and secondary amines (see chapter 2.3.4.1). For a modification of the primary amine it is necessary to orthogonally protect the secondary amine and re-liberate the primary amine (Scheme 8).[220, 221]



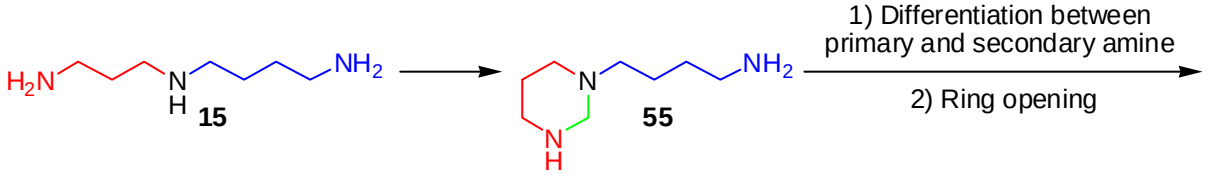
Scheme 8 Schematic description of the differentiation between primary and secondary amines in **42** by the use of a protection group, which is selective for primary amines.[92]

To accomplish derivatization between the secondary amines, a modular construction of the polyamine backbone allows the introduction of orthogonal protection groups for each secondary amine (Scheme 9).[225] This strategy results in complex synthetic schemes and is limited by the number of orthogonal amino protection groups, which are compatible with the alkylation method. Introduction of the branching before finishing generation of the backbone allows the reuse of the respective protection group.



Scheme 9 Schematic description of the orthogonal protection of secondary amines. Primary amine **48** is alkylated with a building block that contains a protected primary amine. The resulting secondary amines in **49** are protected with an orthogonal protection group. The number of orthogonal sets of amino protection groups, which can be applied, limits this strategy.[92, 225]

The reversible reaction of 1,3-diaminopropylgroups and formaldehyde in the presence of other diamine fragments with different methylene group spacers leads to selective formation of an aminal. This reaction is a valuable tool for the diversification of unsymmetrical polyamines such as spermidine **(15)** (Scheme 10).[228, 229] As it is a stoichiometric reaction, it is suitable for the solution phase preparation of building blocks. However, it is not appropriate for a solid phase methodology.



Scheme 10 Schematic description of the differentiation between 3-aminopropyl and other amino-alkylgroups with different length of the methylene group spacer. 1,3-Diaminopropyl groups form an aminal **55** and increase the alkylation state of the involved amines. This can be used for differentiation from unmodified amines. The ring closure is reversible and therefore serves as quasi-selective protection for the three carbon spacer.[228, 229]

2.2.6 Work-up of polyamine conjugates

The tediousness of the purification procedures is also an important issue in polyamine syntheses and analytics. Work-up is complicated by the high polarities of even the protected polyamines. For elution on silica gel, high amounts of polar solvents such as water and methanol are necessary to shorten elution times. This increases the amount of impurities resulting from disintegration of the silica gel. The use of vacuum liquid chromatography (*VLC*) can improve separation, however it does not principally solve this problem.[220] The protected polyamines often appear oily and therefore purification methods such as recrystallisation and precipitation are excluded. On the other hand, the fact that partially protected polyamines can change their solubility behaviour depending on their protonation state, is advantageous.[220] If several free amines are present, the protonated building blocks are often soluble in water, while at basic pH, they are non-protonated, less polar, and can thus be extracted with organic solvents. Unprotected polyamines are highly polar and reversed

phase instead of silica gel materials are generally suitable for their separation. For those reasons, it is highly desirable to immobilize the substrate to a resin. This allows the removal of excess-reagents by simple washing steps after each reaction. After cleavage from the resin, the final products can then be separated by chromatography, if necessary in multiple runs.[220] As mentioned above, many polyamine conjugates have rather non-polar regions that confer a high amphiphilicity to the overall structure. This attribute can drastically complicate the purification procedure.[200] If the final conjugates have more than two amines, their migration on silica gel is often too slow to allow separation. On the other hand, the non-polar parts of the molecule can interact too tightly with standard C_8 / C_{18} RP-material. This is not crucial with small aromatic systems, aliphatic rings, and short aliphatic chains. Philanthotoxins for example have an amphiphilic structure but can be separated by HPLC on C_{18}-columns because the hydrophobic parts are below the size limit. Larger aromatic systems as well as longer aliphatic chains avoid migration, even if the positively charged parts of the molecule show nearly no interaction with the RP-column material.[200] In the context of this work, a procedure was applied, which solves problems during work-up of amphiphilic conjugates by the use of solid phase synthesis (work-up of compound **217** in chapter 4.2.1). For these reasons, it is highly desirable to reduce the number of polyamine synthesis steps and transfer as many as possible to the solid phase.

2.3 Polyamine solid phase synthesis

2.3.1 Solid phase synthesis

Merrifield's pioneering work on the synthesis of the tetrapeptide *L*-leucyl-*L*-alanylglycyl-*L*-valine, which he published in 1963 can be viewed as origin of solid phase synthesis.[233] He used the substrate immobilization onto a cross-linked polystyrene polymer (*Merrifield resin* **(56),** Figure 15), to synthesise a peptide backbone. The revolutionary development of this method was the minimisation of the purification steps and the acceleration of the whole synthetic procedure. Simple washing steps, which remove the reagents from the resin after every reaction cycle substitute recrystallisation, column chromatography and other tedious purification steps. The diffusion-inhibition of the reactions that was caused by the shift of the reaction to the inside of the resin beads was efficiently overcome by the use of high excess of the reagents. After esterification of a protected monomer to the solid support, the procedure consists of the following, repetitive steps:

1. Deprotection of the amino group
2. Peptide bond formation with the next protected monomer
3. ´End-capping´ of the remaining free amines

After final deprotection, the peptide is removed from the polymer. Although the synthetic scheme of *solid phase peptide synthesis* (SPPS) was optimized in the course of time to enable the synthesis of longer polypeptides with biological activity, which are often sensitive to the harsh deprotection and cleavage conditions or necessitate further modifications. As solid phase synthesis uses the reaction of immobilised substrates, some modifications are important compared to solution phase chemistry. As usually more than 90% of the reaction sites are located inside the resin beads, only solvents can be applied, which swell the resin. For polystyrene resins, the most commonly used swelling solvents are *N*,*N*-dimethylformamide, *N*-methylpyrrolidone, tetrahydrofuran, dichloromethane, and toluene. Cycloalkanes, alkanes, diethylether, ethylacetate, acetonitrile, dimethylsulfoxide, and water collapse the resin and reactions in those solvents usually only proceed to a minor degree. Due to the sensitivity of the resin to mechanical stress, some reaction conditions like high temperatures or aggressive reagents, which destroy the matrix are excluded. The monitoring of a reaction is more difficult than in solution phase chemistry, as the substrate is immobilized and techniques like thin layer chromatography (TLC) are not directly accessible. There are some methods for the qualitative or quantitative detection of the reaction progress on the immobilized compound, for example IR-spectroscopy, ´on bead´-mass spectrometry, solid state NMR, or colorimetric assays. A rather beneficial effect of the immobilization is the distinctly higher quasi-molecular mass of immobilized substrates. Thus it is possible to handle even minimal amounts of substance. The possibility to automatise all working steps makes solid phase synthesis an ideal technique for combinatorial approaches. Polyamine chemistry uses some of the established methods of peptide solid phase synthesis. Both share the use of some linkers (see chapter 2.3.2), protection groups (see chapter 2.3.3), and peptide bond forming methods.[92] In the last couple of years, solid phase chemistry of polyamines afforded methods, which take more advantage on their special needs.

2.3.2 Resins and Linkers

Some of the linkers, which were used in solid phase peptide synthesis as well as solid phase organic synthesis are shown in Figure 15.[92]

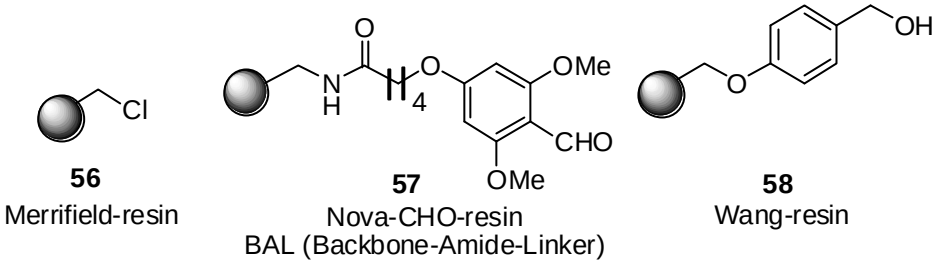


Figure 15 Some linker groups, which were used for solid phase synthesis.

The original *Merrifield resin* **56** liberates its substrate after treatment with strong acids (> 90% TFA in CH_2Cl_2, HBr in HOAc) or under basic saponification conditions.[233] As these conditions are not suitable with most substrates, many linkers were developed, which can be cleaved under milder and more selective conditions (Figure 15). For example, substituted benzyl-linker (30 – 80%), trityl-linker (0.1 – 10%) and triazene-linker (< 10%) can be cleaved with distinctly lower concentrations of TFA in CH_2Cl_2.[234, 235] For polyamine chemistry, more recently linkers were used, which take care of the attributes of polyamines. A variety of polyamine linkers were developed by Bradley et al.[234, 236, 237] They mainly used an aminomethylated polystyrene resin. The polyamine was coupled *via* a urethane linkage, which was cleavable using 10 equiv. of trifluoromethanesulfonic acid-trifluoroacetic acid (TFMSA-TFA). However, the cleavage of many polyamine-coupled biomolecules such as DNA, which are hydrolyzed when treated with high concentrations of TFA requires mild conditions.

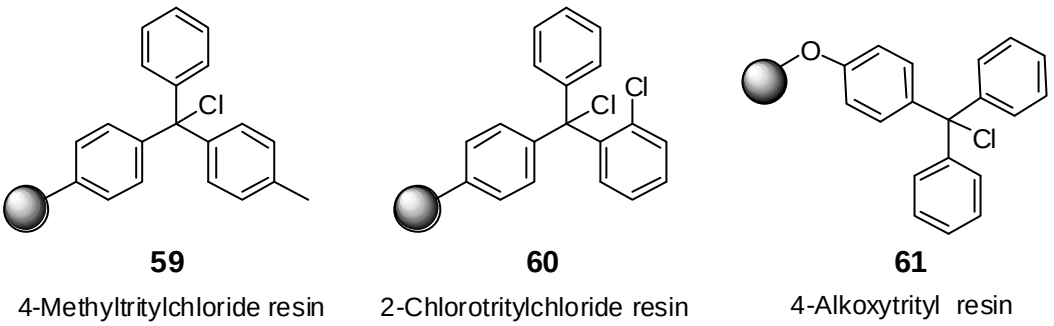


Figure 16 Trityl linkers, applied for the synthesis of polyamine on solid support.[238-240]

Byk et al. have developed a solid phase methodology, which allows a quick and easy access to a high number of mono-functionalized geometrically varied polyamines. They used mild cleavage methods of a trityl tethered resin **60**, cleavable using low concentrations of TFA (see chapter 2.3.4.1).[238] The family of trityl-derived linkers has since then frequently been used for polyamine synthesis on solid phase (Figure 16). It unites several advantages as selectivity for primary amines and mild cleavage conditions. It is important to note that trityl linkers are stable against all alkylation procedures, which were developed for polyamine synthesis. Especially the reductive methods like BH_3·THF-reduction of amides and imine reduction with borohydrides were successfully executed on solid phase.[231, 241, 242] *Fukuyama alkylation* has also been shown to be unproblematic.[243] A mentionable improve for the synthesis of acid

sensitive conjugates is the alkoxytrityl resin **61**, developed by Fukuyama (Figure 16). It is a cheap alternative to obtain large amounts of a resin (Scheme 53), which is even more acid labile than the usually applied trityl- or 2-chlorotrityl resins.[239] The enhanced acid lability is caused by the +M-effect of the ether-bridge that couples resin and linker. Most of the solid phase syntheses that are performed to date take advantage of these types of resin with derivatised trityl linkers. In this work the trityl-based linkers are used for the same reasons described above (4.3.6.1). Various other linker systems, e.g. FMOP (4-formyl-3-methoxy-phenoxymethyl-polystyrol) linker, immobilize amines by *Reductive amination* in benzylic position (Scheme 27). The cleavage conditions are milder than that for the *Merrifield resin*, but generally harsher than those of trityl resins. They additionally lack the beneficial selectivity for primary amines and hence their use in the ´conventional´ synthesis of polyamines is rather seldom.

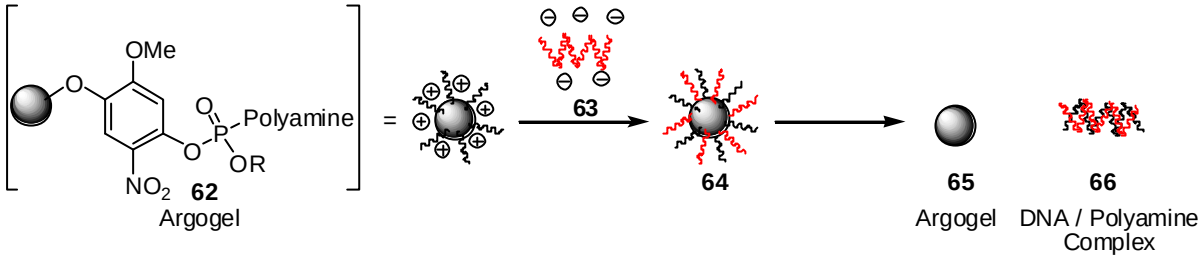


Figure 17 DNA conjugation to polyamines on Argo*Gel*-NH_2 as reported by Kim and Diamond.[244, 245]

Nevertheless, they found application on the externally controlled cleavage of covalently linked prodrugs, proteins, or solid-phase formulation vehicles, which offers potential advantages for controlled drug or gene delivery. Diamond and co-workers tested a series of *o*-nitrobenzyl ester compounds that allow a systematic study of resins with linkers for polyamine synthesis expressing a photolability for mild cleavage of the polyamine conjugate (Figure 17).[244, 245] With this linker system, DNA-loaded solid phase **64** is assumed to be exploited for spatially, temporally, or dose-controlled release of DNA-polyamine complexes, at extracellular or intracellular sites. To avoid acidic cleaving conditions, Bycroft and colleagues developed a linker, which bases on the same reactivity as the Dde protecting group (cleavage with hydrazine hydrate or primary amines).[246] It is fully orthogonal with Boc and Fmoc protection groups. So, it enabled discrimination between primary and secondary amines during the loading step and served as linker for polyamine synthesis on solid supports in several examples (see Scheme 20 in 2.3.4.4).

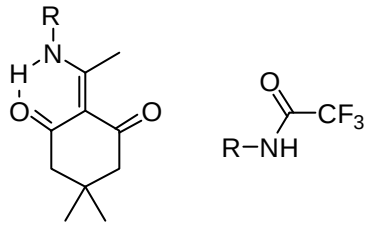
2.3.3 Protection groups

The discrimination between the respective amino-functionalities is highly desired to allow a directed synthesis of polyamine. This requires an extensive and well-planned protection group strategy.[92, 247] The polyamine synthesis uses the pool of common amino-protection groups from peptide synthesis, like the butyloxycarbonyl group (Boc), the allyloxycarbonyl group (Aloc), the triphenylmethyl group (trityl, Tr), and with restrictions the 9*H*-fluoren-9-ylmethoxycarbonyl group (Fmoc).[248] Special needs of polyamine chemistry force the expansion of the set of orthogonal protection groups in polyamine synthesis (Table 1).

Table 1 Prominent protection groups, which were used for polyamine syntheses.[248]

Protection group	Deprotection conditions
Z, Boc, Trt, MMTr, DMTr	acidic
Tfa, Fmoc	basic
Aloc	Pd^0
o-Nosyl, *p*-Nosyl, d-Nosyl	thiolates
Dde, Pht	$N_2H_4 \cdot H_2O$
Teoc	F^-

The Boc-group, Trt-groups, the *p*-Nosyl-group and the Phthaloyl group (Pht) allow differentiation between primary and secondary amines if the protection group is introduced in stoichiometric amounts or in low excess.[249-253] Their selectivity arises from sterical demands of the protection group or by the much quicker reaction of primary amines with the introducing agent compared to secondary amines. An exclusive selectivity is observed for the Dde-group **67** (Figure 18) and the Tfa-group **68**.[220, 222-224, 230, 254-256]



67 **68**

Figure 18 The Dde-protected primary amine is stabilized by a hydrogen bond that forms a six membered ring.[248]

In case of Dde, the readily protected primary amines **67** are stabilized by hydrogen bonds, which were not present in the protected secondary amines. The Tfa group **68** can be selectively introduced at primary amines, if ethyl trifluoroacetate is used instead of the anhydride. Selectivity for α-monosubstituted primary amines in the presence of α-di- or trisubstituted primary amines is also observed. Alkylation methods, which include the use of specific protection groups make use of the Z-group, the Pht-group and the Nosyl-groups.[257-260] Due to the bulky phenyl moiety, primary amines are selectively mono-protected by benzylbromide in the presence of weak bases. The resulting secondary amine can be alkylated with electrophiles in a simple S_N2-reaction. After deprotection, secondary amines are selectively obtained. The harsh acidic deprotection conditions of the Z-group are rarely compatible with the commonly used resins in polyamine SPS. Pht-group and the Nosyl-groups play a key role in the selective formation of primary / secondary amines. In both, the pK_S-value of the activated N-H is distinctly lowered and in the presence of bases the hydrogen can be abstracted to obtain much higher rates in halogen displacement-reactions or *Mitsunobu-reactions*.[225, 239, 261-263] After their relatively mild deprotection, the liberated

amines are selectively obtained. Since they play an important role in the present work some protection groups are specified.

2.3.3.1 The Dde group

The Dde-group is introduced by reaction of primary amines with 2-acetyldimedone **191** and weak bases in polar solvents.[223, 224] Unlike during the introduction of most other protection groups, the mechanism contains an *aza-Michael-addition* followed by elimination of water. The protected state is stabilized by hydrogen bonding and can be disturbed by other primary amines. Thus, selective protection of only one of several primary amines is not possible as *transamination* to all present primary amines would take place and quickly decompose the monoprotected compound.[92] On the other hand this mechanism is used for the mild deprotection with primary amines. Since Fmoc is also affected by the primary amine the use of hydrazine-hydrate is more popular. The reaction occurs quickly without any affection of the Fmoc-group (Figure 19).[264, 265]

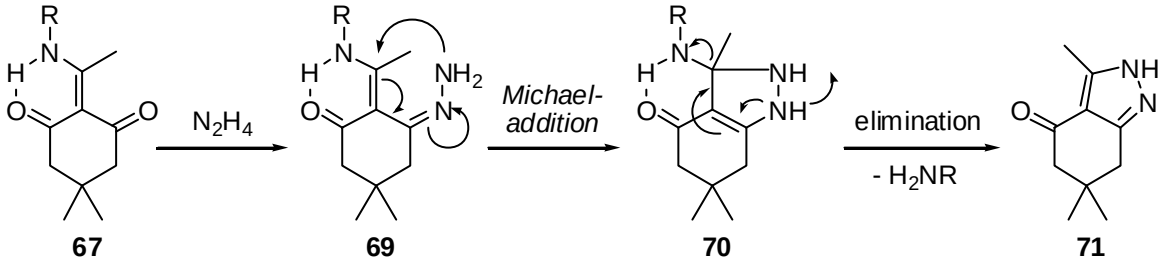


Figure 19 Deprotection of the Dde group.[248]

The formation of the 3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1*H*-indazole **(71)** is irreversible. Its strong UV-absorption at 270 or 290 nm can be used for quantitative monitoring of the deprotection. If carried out with hydrazine hydrate, the orthogonality between Dde- and Aloc groups is not fully given as hydrazine partially reduces the Aloc group to the corresponding propyl carbamate. Addition of 100 equiv. of allyl alcohol avoids this side reaction without the necessity of higher excess of hydrazine or elongating the reaction time.[266]

2.3.3.2 The Aloc group

Allyl type protection groups have extensively been used for the protection of amines, carboxylic acids, phosphates and ethers. [267, 268] Aloc is introduced by *N*-acylation with allyl-chloroformate under *Schotten-Baumann conditions* or with diallyl dicarbonate in the presence of bases.[248] It features a remarkable stability during removal of other protection groups. Likewise, Aloc deprotection occurs under extremely mild conditions, which make it orthogonal to nearly all other amino protection groups. During deprotection, the allyl moiety is transferred to a Pd^0 species, $Pd(PPh_3)_4$ or Pd(II) that is reduced *in situ.* After protonation, the carbamic acid decomposes under loss of carbon dioxide to give the free amine (Figure 20).[268]

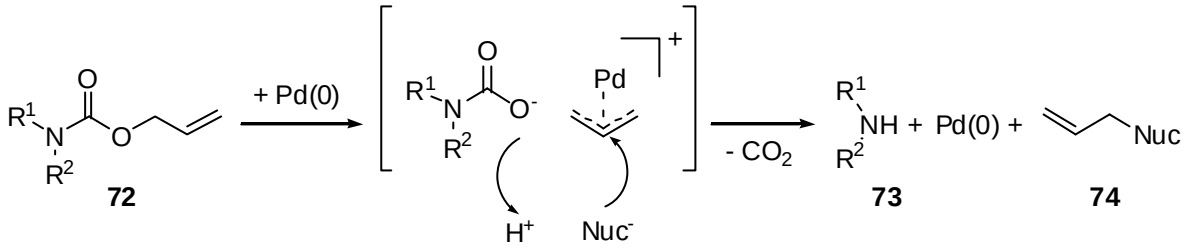


Figure 20 Deprotection of an Aloc-protected amine **72**.[268]

The destruction of the Pd-allyl complex (step two in Figure 20) is crucial. This has to appear quickly by nucleophilic attack of a scavenger, as the liberated amine will act itself as a scavenger for the allyl cation. The variety of scavengers is broad and enables flexible use, depending on the presence of susceptible functions in the molecule.[248, 269-275]

Table 2 Classes of scavengers, which were previously applied for Aloc deprotection.[268]

Class of scavenger	Substitute	Allylamine formation
Oxygen nucleophiles	Carboxylic acids	No
	Carboxylic acid salts	Yes
	HOBt	No
	N-Hydroxysuccinimide	No
Nitrogen nucleophiles	Morpholine	Yes
	Pyrrolidine	Yes
	Piperidine	Yes
	N-Methylaniline	Yes
	Me_2NH	Yes
Carbon nucleophiles	Dimedone	No
	N,*N´*-Dimethyl barbituric acid	No
	Dimethyl malonate	No
Sulfur nucleophiles	2-Thiobenzoic acid	No
Silylating derivatives	R_2NSiMe_3	No
	Me_3SiN_3 / Bu_4NF	No
Hydride donors	Formic acid	No
	$HSnBu_3$	No
	Borohydrides	No
	Silanes	No

Nevertheless, some scavengers only suppress the formation of ally amines but do not completely avoid it. For carboxylic acid salts, it is crucial that the formed allyl-carboxylate is less stable than the allyamines. Thus, the trapping process is irreversible and both, the amine and the carboxylic acid salt compete for the allyl cation.[248] Acidic scavengers reprotonate the amine to such a far extend that trapping of the allyl cation is excluded. Tin hydrides, silanes,

boron hydrides, and silylated derivates of nucleophiles inactivate the amine by converting the carbamate to the corresponding (semi-)metalated carbamates **75** (Figure 21). The (semi)-metal can be exchanged under weakly acidic conditions and give the deprotected amine **73** after subsequent decarboxylation of the carbamic acid **76**. The use of dry solvents during the allyl transfer is highly recommended as traces of water can prematurely destroy the silylated carbamate.[276, 277]

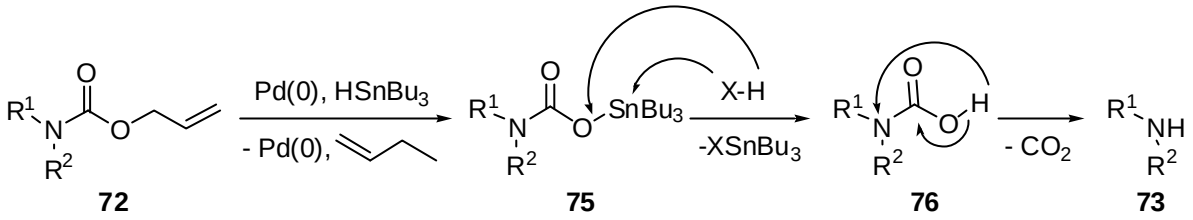
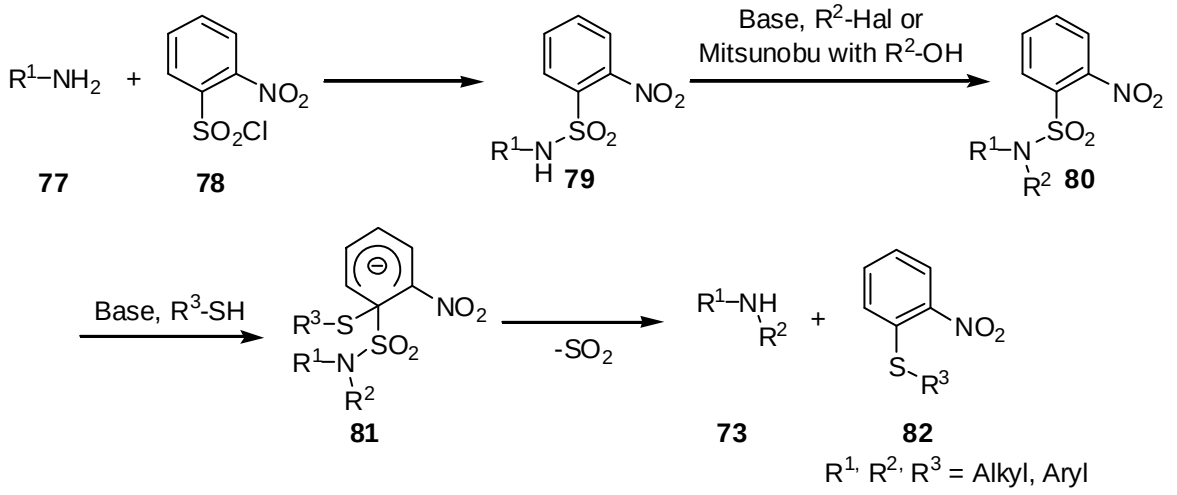


Figure 21 Pd-mediated allyl-transfer in the presence of (semi)-metal hydrides or silyl donors results in the formation of the respective carbamates **75**. After protonation and decarboxylation, the deprotected amine **73** is obtained. Representatively, the reaction with $HSnBu_3$ is shown.[248, 268]

The Pd-catalyzed rearrangement of allyl carbamates to allylamines is a common method for the selective mono-allylation of amines.[268]

2.3.3.3 The Nosyl group

The Nosyl group is introduced by reaction of amines with the respective sulfonyl chloride in the presence of weak nitrogen bases. 2-mono- (*o*-Nosyl), 4-mono- (*p*-Nosyl), and 2,4-dinitrophenylsulfonyl (*d*-Nosyl) groups are reported. Due to the much lower costs of the respective sulfonyl chloride, the *o*-Nosyl group is usually applied.[248, 278, 279] The formed sulfonamide **79** is stable and can be smoothly removed under mild conditions. Due to its electron deficiency, the phenyl ring can be readily attacked by thiolates. Those are usually generated *in situ* by reaction of a thiol and a base (Scheme 11).[261]



Scheme 11 General mechanism of *Fukuyama alkylation* and subsequent removal of the *o*-Nosyl group. After introduction of the Nitrobenzenesulfonyl-protecting group, an *N*-alkylsulfonamide **79** is obtained in which the acidity of the NH-proton is significantly enhanced. After deprotonation of the sulfonamide, it can displace a halide or an alcohol in a *Mitsunobu reaction*. Thereby, the former activating group changes its function to a protecting group. Thiolates form a *Meisenheimer complex* **81** with the electron deficient ring of the Nosyl-group, which decomposes under loss of SO_2 and nitrophenyl thioether **82** to release the secondary amine **73**.[261]

The choice of the thiol seems to be of low importance. Different examples such as β-mercaptoethanol, thiophenol and 2-mercaptoacetic acid are reported.[239, 279] Usually, bases are carbonate and DBU, whereas DBU significantly accelerates the reaction compared to carbonate. The use of readily prepared thiol salts, for example sodium thiomethylate, is also reported. Compared to other sulfonamides, which are usually removed under strongly basic aqueous alkaline, these deprotection conditions are very mild and predestine the Nosyl group for use in polyamine solid phase chemistry. In analogy to other sulfonamides, Nosyl does not only function as a protection group for primary and secondary amines. It can also act as an activation group for primary amines (see Scheme 11 and chapter 2.3.4.3). This possibility to discriminate between primary and secondary amines and to selectively alkylate masked primary amines makes it one of the most frequently used protection groups in polyamine chemistry.[261]

2.3.4 Strategies for Polyamine Solid Phase Synthesis

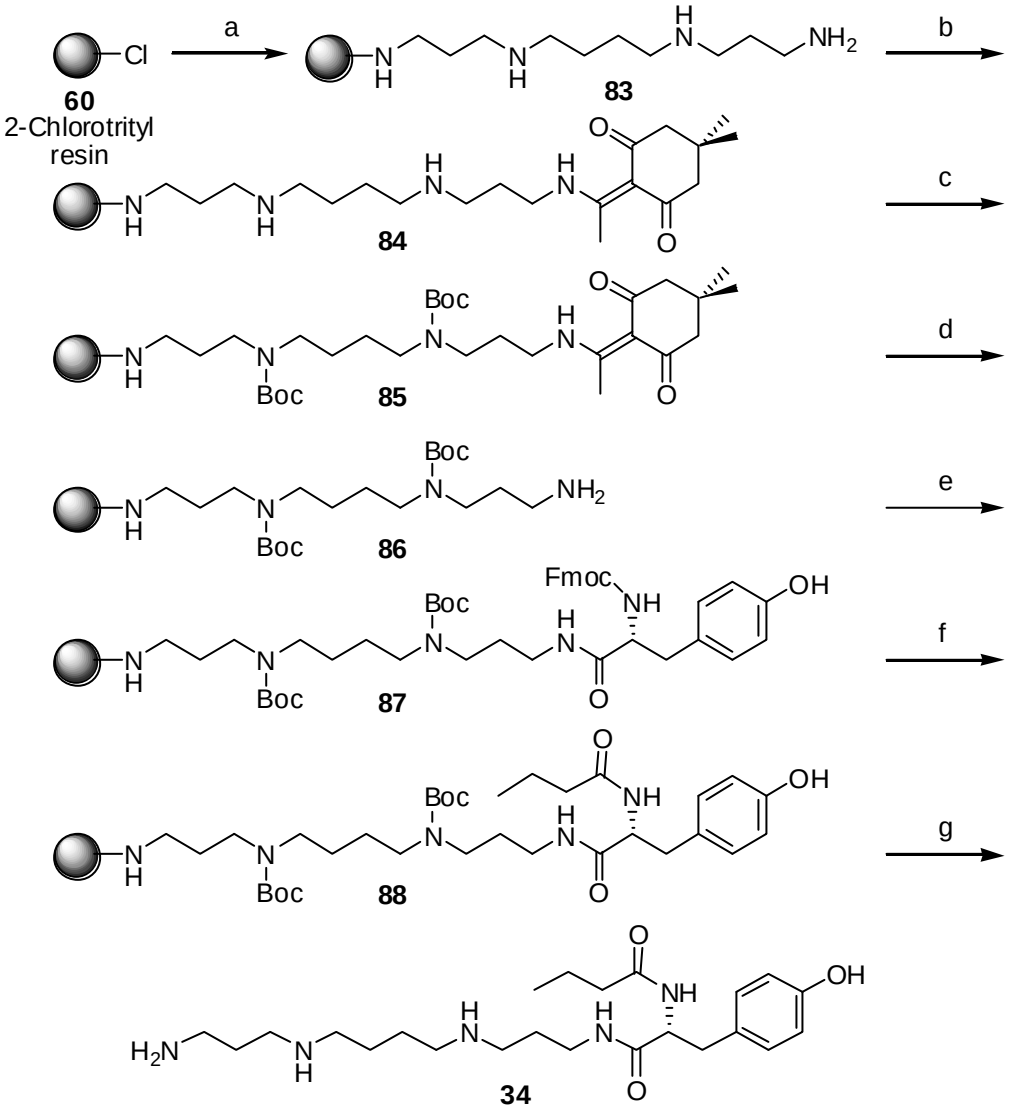
In the last ten years, many efforts were undertaken to generate reliable methods for polyamine solid phase synthesis. In general, solid phase synthesis of polyamines and their conjugates can be pursued by two strategies.[247, 280] The first is based on the stepwise or modular elongation of the backbone with small building blocks. The second consists in the coupling of larger partially protected polyamine building blocks in order to rapidly elongate the backbone. The preference for one of these methods depends on the functional properties of the building blocks. Finally, both require a combination of solution and solid phase methods. Although the first strategy allows the introduction of more diversity in the aliphatic backbone between two amino groups and in the side chains, it is limited by the fact that every elongation step decreases the yield. Furthermore, the smaller building blocks have to be prepared in at least one solution phase step, making the modular approach less favourable for the generation of longer backbones. For the synthesis of longer backbones it is more economical to prepare building blocks, which can be coupled to the support in one step. This method is especially advantageous for commercially available backbones such as spermine **(16)**. However, this requires an efficient protection of the primary and secondary amino groups in a solution phase synthesis step.[232, 236, 247, 259, 260, 280, 281] For high throughput library productions and large-scale SPS large quantities of highly stable and partially or fully protected polyamine building blocks are required. They should be asymmetrically protected to exclude cross-linking reactions that would severely decrease yields and purities of the solid phase synthesis. The substitution should also allow direct access to a variety of different polyamine structures without laborious conversions after coupling to the solid support. The necessity of building up a polyamine backbone by alkylation instead of acylation is an additional concern. Usually, alkylation conditions are harsher than those for peptide bond formation. They therefore restrict the range of tolerated functional- and protection groups. The transfer of such alkylation procedures to the solid phase and the compatibility to several protection groups has been extensively studied. Ultimately, there is no generally applicable alkylation and protection scheme, and the final choice of the methods strongly depends on the desired structure of the product.

First solid phase syntheses of polyamines were reported by Sergheraert and co-workers in 1994.[282] The synthesis of trypanothione disulfide involved the selective protection of the primary amines of spermidine **(15)** with tertbutyldiphenylsilyl chloride followed by coupling the disilyl derivative to a methylchloroformylated Merrifield polystyrene resin. After

deprotection and assembly of the peptidic chain the product was cleaved from the resin with HF. Oxidation and purification afforded the product in 38% yield. In the following years, many efforts were undertaken to improve polyamine solid phase synthesis, especially in terms of linkers, alkylation methods, and protection groups, which allow diversification between the particular amines. Development of milder reaction conditions than those mentioned in the example of Sergheraert is also an important point.

2.3.4.1 Direct attachment of protected polyamines

An early example is the first mention of a solid phase synthesis of PhTX-343 published by Bycroft et al in 1996 (Scheme 12).[240] They attached unmodified spermine **(16)** to a 2-chlorotrityl resin **(60)** and used Dde- and Boc-group to differentiate between primary and secondary amines. Investigation of the crude product after Dde-protection showed no bis-Dde-spermine **(89f)**, proving the selectivity of trityl linkers for primary amines under these conditions.



Scheme 12 Synthesis of PhTX-343 using Dde- and Boc-protecting group. Reagents and conditions: (a) Spermine, CH_2Cl_2; (b) 2-acetyldimedon, DMF; (c) Boc_2O, DiPEA; (d) 2% N_2H_4 in DMF; (e) Fmoc-*L*-Tyr(*O*tBu)-OH, HBTU, HOBt, DiPEA, DMF; (f) 20% piperidine in DMF; (g) Fmoc-*L*-Tyr(*O*tBu)-OH, HBTU, HOBt, DiPEA, DMF.[240]

Protecting groups, which enable the primary-secondary amine-differentiation were described in chapter 2.3.3. They were previously used to prepare some valuable, protected polyamine derivates, shown in Figure 22.[92, 247]

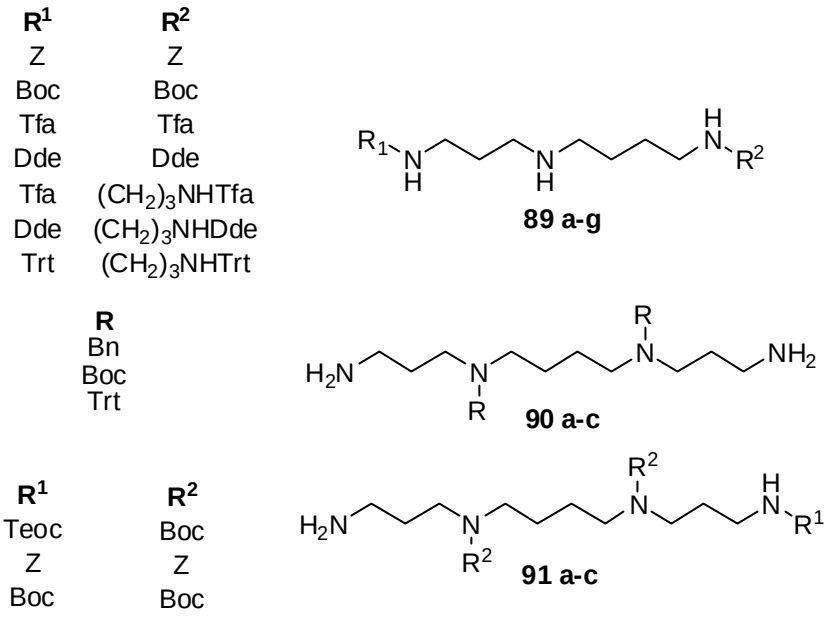
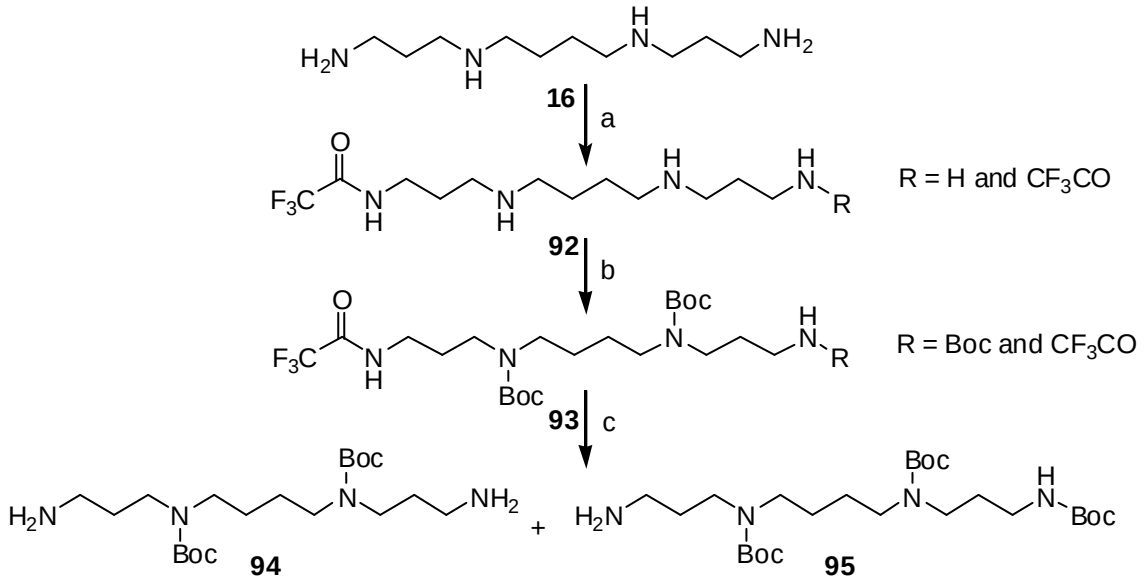


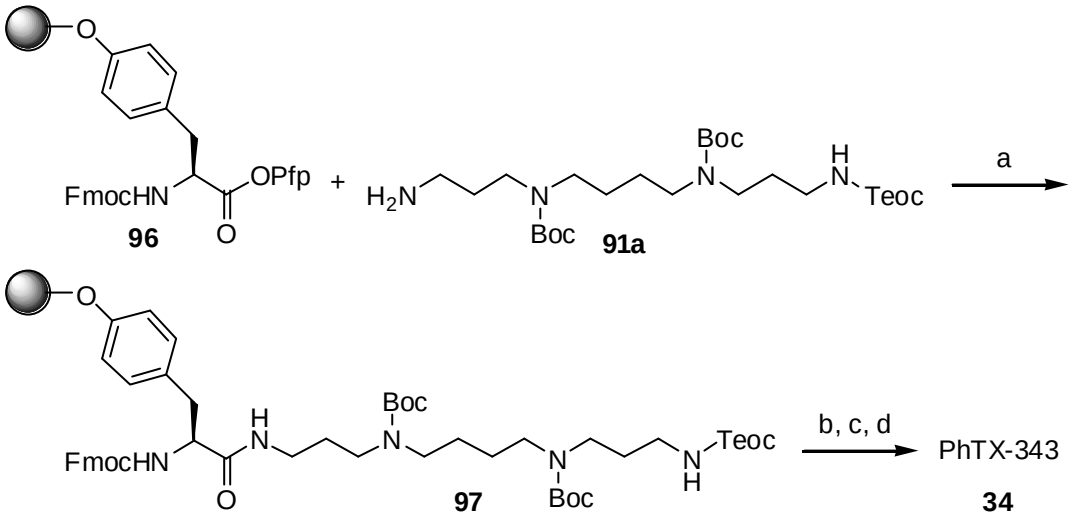
Figure 22 Some synthetically useful polyamine building blocks.[92]

One example for the selective protection of secondary amines, which served as a template for the reactions in chapter 4.1.1.1, is shown in Scheme 13.[220, 230, 283] Differentiation is accomplished by an indirect method after blocking of the primary amines. Therein, the Tfa group partially or fully protects spermine **(16)** at its primary amines and the remaining amines are saturated with Boc-groups. After cleavage of the Tfa group with aqueous NaOH, different Boc-spermines are obtained.



Scheme 13 Exemplary synthesis of di- and tri-Boc protected spermine. The Tfa-group is used for temporary blocking of the primary amines. Di- and tri-Boc-spermine are obtained in 18% and 43% yield after chromatographic workup by *vacuum liquid chromatography* (VLC); Reagents and conditions: (a) 1.2 Equiv. TfaOEt, MeOH, -50 °C; (b) Boc_2O, MeOH, 0 °C; (c) $NaOH_{conc}$ / H_2O.[230]

By choosing the excess of ethyl-trifluoroacetate, the amounts of di- and tri-Boc-spermines **94** and **95** can be controlled. Both products are useful building blocks for solution as well as for solid phase chemistry. An asymmetrically protected building block was applied in the synthesis of PhTX-343 (Scheme 14).[221] After immobilisation of the Fmoc-protected and Pfp-activated tyrosine moiety on a trityl bromide resin, amide generation with an asymmetrically protected spermine building block **91a** resulted in PhTX-343 **(34)** in 43% overall yield.

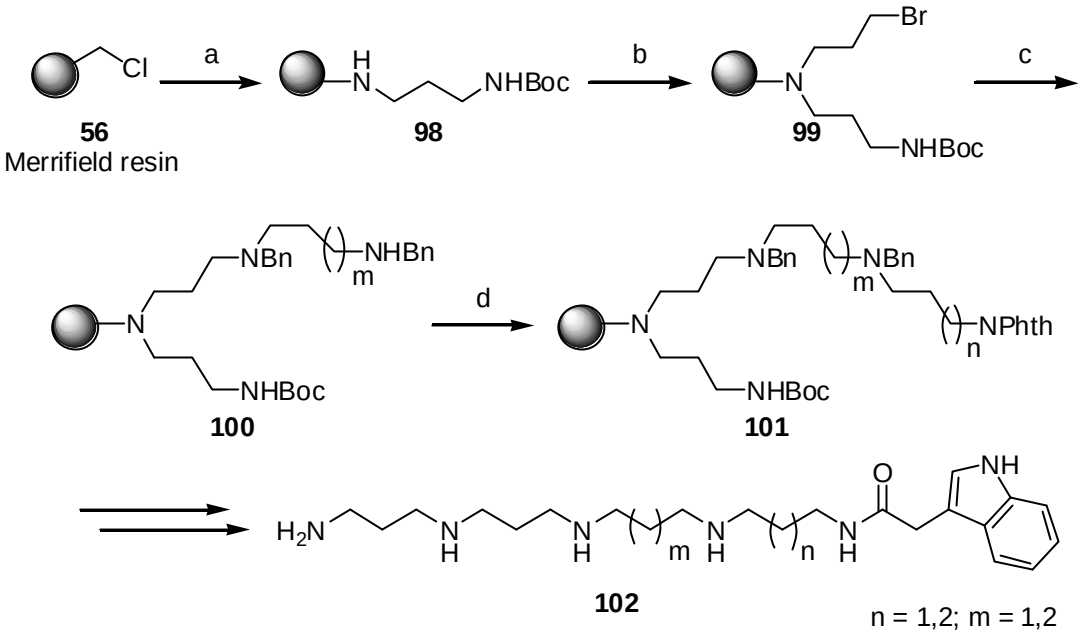


Scheme 14 Synthesis of PhTX-343. Reagents and conditions: (a) DiPEA, HODhbt; (b) 20% piperidine in DMF; (c) $C_3H_7COOPfp$, DiPEA, HODhbt; (d) TFA in CH_2Cl_2.[221]

2.3.4.2 S_N2-Alkylation

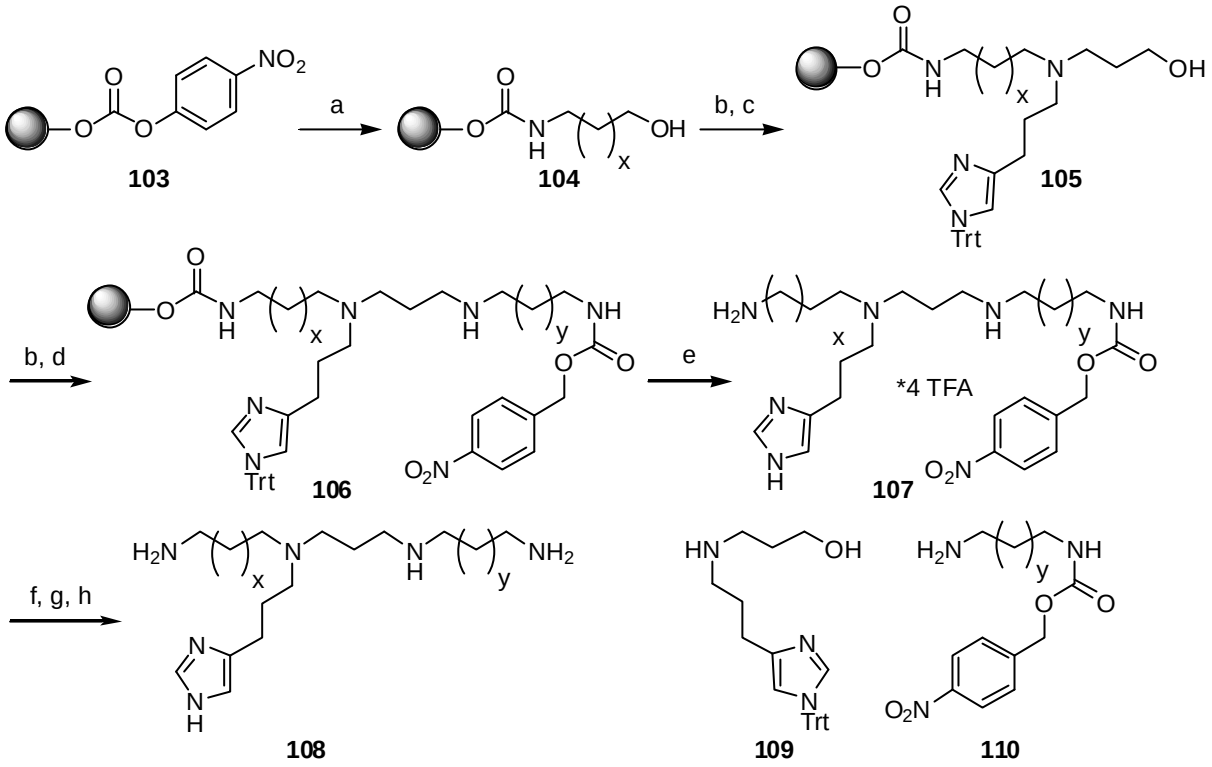
SPS approaches for both, amine and electrophile bound to the resin have been reported. The latter one is superior, due direct alkylation of primary amines can easily lead to overalkylation.[284] However, this strategy is also not sufficient in all cases. It has to be taken care of cross linkage between solid phase bound electrophiles and primary amines, which leads to the formation of tertiary amines and significantly lowers yield and purity. Thereby common *S_N2-alkylation* is mostly limited to the homo-dialkylation of primary amines and monoalkylation of secondary amines. Appropriate electrophiles are halides and sulfonates (mesylates, tosylates and nosylates).[284]

An example that starts the synthesis of polyamine toxins from a central amino group immobilized on a *Merrifield resin* **(55)** is shown in Scheme 15. The backbone is elongated by alternate coupling of benzyl-protected amines and bromides.[257, 258] Due to the protection group strategy, consisting of orthogonal Boc-, benzyl- and phthalimides it is possible to synthesize polyamines from centre to tail with high control over the amino substitution. Unfortunately the overall yield after three alkylation steps is only 30%, which drops the overall yield to 10 – 30%. Besides the incompleteness of the bromine displacement caused by the use of hindered *N*-benzylamines, this probably arises from the two steps in which symmetrical building blocks serve for the elongation of the polyamine in steps **b** and **c**. Both steps probably suffered from cross-linking.



Scheme 15 Asymmetric synthesis of polyamine starting from an internal amino functionality. Reagents and conditions: (a) *N*-Boc-diaminopropane, DiPEA; (b) dibromopropane, DiPEA; (c) N^1,N^5-dibenzyldiaminopropane, DiPEA; (d) N^1-benzyl-N^5-phthaloyldiaminopropane.[257, 258]

In a detailed study on S_N2 alkylation by Jaroszewski et al, similar results were obtained for the alkylation of immobilized bromides and iodides generated from alcohols if the reaction is carried out on a trityl resin.[285] Interestingly, the change to a *p*-nitrophenylcarbonate-activated Argopore *Wang resin* resulted in significantly higher overall yields for both steps. Again, the formation of side products by cross-linking is problematic. This obstacle could not be solved by the use of higher excess of amine or electrophile. Use of only partially loaded resins with loadings about 0.5 mmol / g was suggested to largely suppress the cross-linking. The 2-nitrophenylsulfonate leaving group was examined in the alkylation of cyclic secondary amines.[286] However, the overall yield (26%) of this synthesis containing two alkylation steps is low, as the cyclic amines are sterically demanding. If only one piperidine moiety is introduced, the overall yield rose up to 60%, confirming that the alkylation step is crucial. Nevertheless, the *O*-*o*-Nosyl-group seems to be the leaving group of choice, because the use of the appropriate mesylate revealed no product. Finally, mesylates found an application in the solid phase synthesis of polyamine conjugates with imidazole and usnic acid, both published by Uriac et al. (Scheme 16).[287, 288] They showed that the use of longer reaction times (32 h) and higher temperatures (70 °C) produces good yields for the alkylation of secondary amines. Primary amines are alkylated in much shorter time (6 h) and at lower temperatures (50 °C).



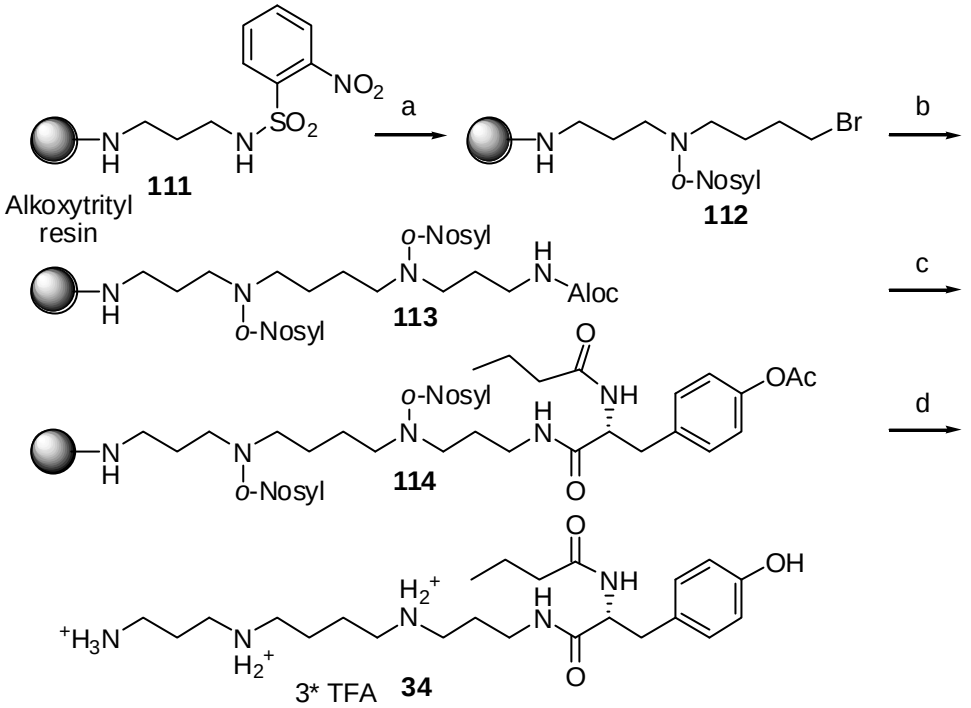
Scheme 16 Syntheses of Polyamine-Imidazole Conjugates. Reagents and conditions: (a) Aminoalcohol, CH_2Cl_2, 2 h, r.t.; (b) Methanesulfonyl chloride, pyridine, 30 min, r.t.; (c) **109**, DMSO, 32 h, 70 °C; (d) **110**, DMSO, 5 h, 50 °C; (e) 50% TFA in CH_2Cl_2, 1 h, r.t.; (f) NaOH then CH_2Cl_2, chromatography; (g) 20% $Pd(OH)_2$ / C, H_2, MeOH, 4 h; (h) water, HCl / EtOH, 3 h, 0 °C then r.t.[288]

2.3.4.3 Fukuyama alkylation

The *Fukuyama alkylation* is a special case of S_N2-reaction with activated amines. It was developed by Fukuyama et al. in 1995 for solution phase synthesis and found its first mention for solid phase chemistry in 1997 by Nguyen et al.[279, 289] It is particularly useful for the selective monoalkylation of primary amines under mild conditions. This is a major advantage over other alkylation methods used for the generation of polyamine backbones. Several Nosyl groups with different nitro substitution patterns can be applied (see chapter 2.3.3.3).[278, 279] One advantage of *d*-Nosyl over mono-Nosyl is the greater rate in sulfonamide formation. By the enhanced electron withdrawing effect of the second nitro-group the amide-proton of the d-Nosyl amide has an additionally lowered pK_a-value leading to higher rates in alkylation reactions.[278] On the other hand this makes it even more susceptible to the basic deprotection conditions used for *o*-Nosyl. This enables *o*-Nosyl and *d*-Nosyl to be used as fully orthogonal protecting groups. Otherwise, this also limits the scope of the *d*-Nosyl group. Weak bases like *n*-propylamine, and higher temperatures partially deprotect the amine, which makes the use of *d*-Nosyl problematic. By all means it is not possible to keep the protecting group on the resin for multiple alkylation steps. Alkylation with tosyl- and trifluoroacetamides has also been reported but due to their harsh deprotection conditions, they were not suitable for solid phase chemistry of polyamines.[290]

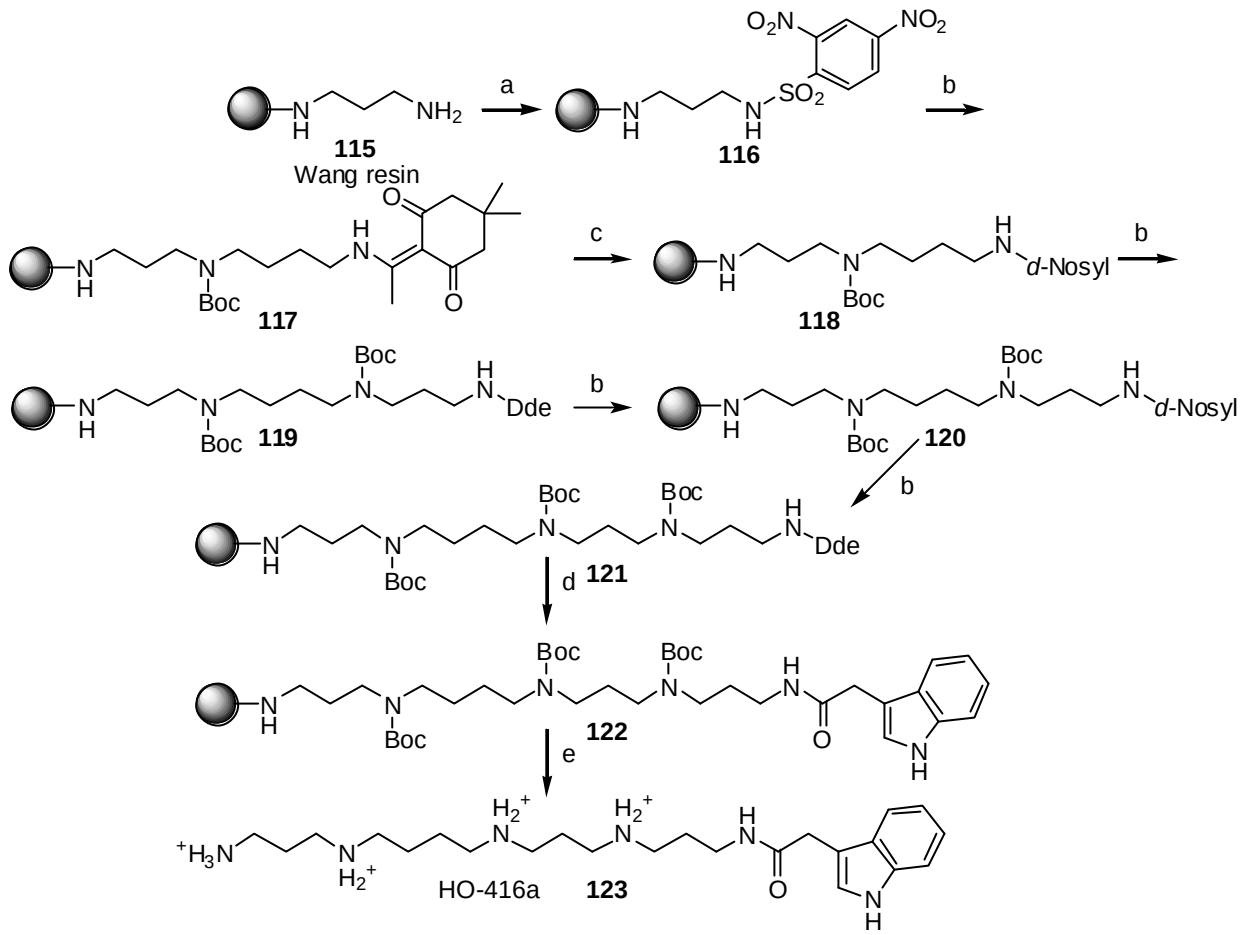
After publishing a mixed solution-solid phase synthesis of polyamine toxin HO-416b in 1999, Fukuyama et al. demonstrated the versatility of the Ns-strategy for the complete

synthesis of longer polyamines on solid phase in 2002 by the synthesis of PhTX-343 **(34)** (Scheme 17).[239] The product was isolated in 75% yield. A possible source of loss in this synthesis is the expectable cross-linking in the attachment of unprotected diaminopropane to the resin and in step **a**.



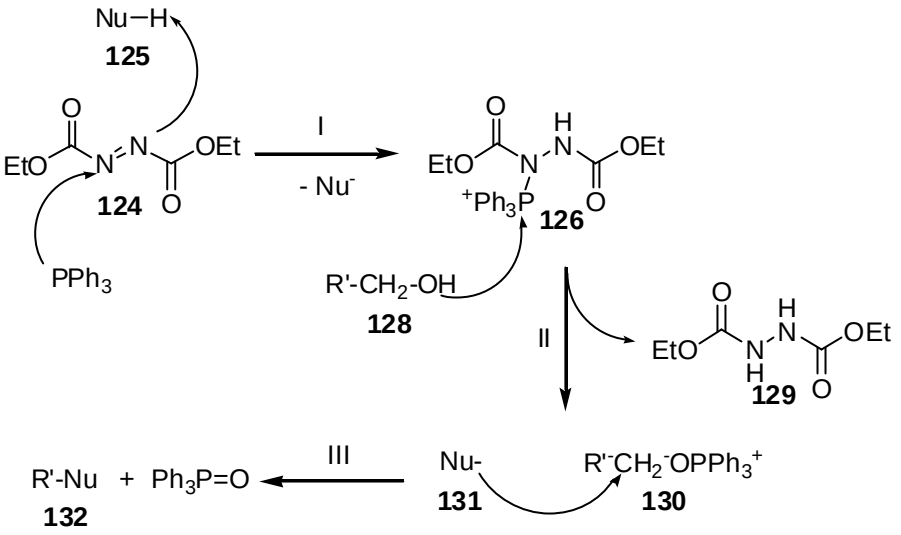
Scheme 17 Synthesis of PhTX-343 using *o*-Nosyl group and primary bromides for synthesis of the polyamine backbone. Reagents and conditions: (a) 1,4-Dibromobutane, K_2CO_3, DMF, 60 °C; (b) N^1-Nosyl-N^3-Aloc-1,3-diaminopropane, K_2CO_3, DMF, 60 °C; (c) $Pd(PPh_3)_4$, pyrrolidine, DMF, r.t.; *N*-Butyryl-*O*-acetyl-*L*-tyrosine-*p*-nitrophenol ester, CH_2Cl_2, r.t.; (d) K_2CO_3, MeOH; mercaptoethanol, DBU, DMF, r.t.; TFA in CH_2Cl_2, r.t.[239]

This problem is circumvented by the use of monoprotected diamine building blocks or amino alcohols that can be alkylated under *Mitsunobu conditions*. Hone et al. showed the utility of this method in the synthesis of spider toxin HO-416a **(123)** in 2000 (Scheme 18).[225] The whole 16-step reaction sequence revealed HO-416a **(123)** in 88% yield and 94% purity. Herein, the *d*-Nosyl group is used and it is obvious that the instability of *d*-Nosyl requires multiple transprotection steps during each alkylation step.



Scheme 18 Synthesis of Agel 416 using *d*-Nosyl-amide alkylation as central tool. Reagents and conditions: (a) 2,4-Dinitrobenzenesulfonyl chloride, 2,6-lutidine, CH_2Cl_2, 3 h; (b) 1. *N*-Dde-butanolamine or *N*-Dde-propanolamine, PPh_3, DEAD, THF, 3h; 2. mercaptoacetic acid, DiPEA, CH_2Cl_2, 3 h; 3. Boc_2O, DiPEA, CH_2Cl_2, 3h; (c) hydrazine-hydrate (2% in DMF), 30 min; 2,4-dinitrobenzenesulfonyl chloride, 2,6-lutidine, CH_2Cl_2, 3h; (d) hydrazine-hydrate (2% in DMF), 30 min; indole-3-acetic acid, HOBt, DIC, CH_2Cl_2, 1h; (e) 5% TFA in CH_2Cl_2, 5 min; 4M HCl in dioxane, 3 h.[225]

The alkylation of amines using *Mitsunobu-Fukuyama conditions* has since then extensively been studied and applied in the synthesis of polyamines (Scheme 19). Stromgaard et al. investigated reagent concentration, excess, reaction time, number of reaction cycles and order of reagent addition. It appeared that a reagent-concentration of 200 mM, reagent addition in the order tributyl phosphine (TBP) followed by 1,1'-(azodicarbonyl)dipiperidine (ADDP), and reaction times of three times three h yielded 100% conversion for the first alkylation step.[291] After one further alkylation and acylation, PhTX-433 analogues are obtained in overall yields of 23 – 40%. This suggests a distinct loss of conversion in either the second alkylation or the acylation steps.

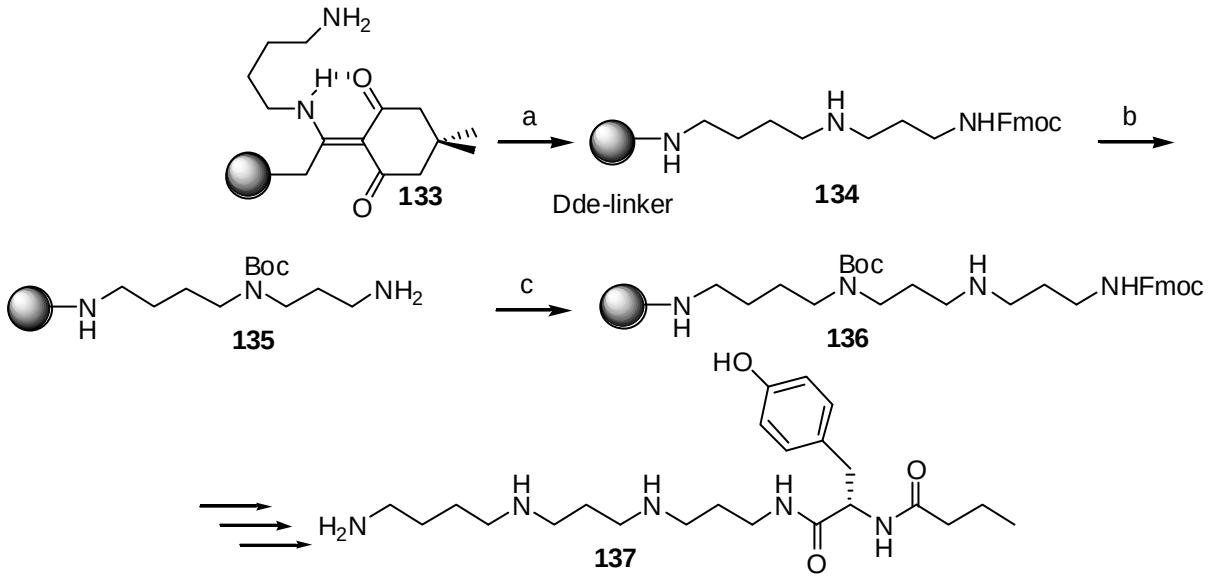


Scheme 19 Mechanism of the *Mitsunobu reaction*. The essential compounds are a phosphane and a diazocompound, which are able to generate the oxophosphonium intermediate **130**. The S_N2 reaction in step III is the rate determinant step. The outcome of the reaction strongly depends on the sterical environment of the alcohol **128** and the acidity of **125**. In this case the traditional Mitsunobu reagents diethylazodicarboxylate **(124)** (DEAD) and triphenylphosphane are shown as participants.[92]

A further study of the reaction by Olsen et al. tested the influence of base addition, different solvent systems and Mitsunobu reagent pairs.[263] The use of diethylazodicarboxylate (124) in combination with PPh_3 or PEt_3 seems favourable, however with an obvious dependence on the substrate type. Efforts to synthesize curtatoxins with more than 5 amino groups did not give the desired product. This loss of yield is probable due to the necessity of four components to act together during *Mitsunobu-reaction*, which is rather disadvantageous for a solid phase reaction, as the concentration of the activated species inside the resin beads remains low. The results for the alkylation with secondary alcohols were even worse. In agreement with the work of Stromgaard this shows that the scope of *Fukuyama-Mitsunobu alkylation* with protected amino alcohols is limited to less steps, if acceptable yields are demanded. A study on the optimization of the reaction conditions for conventional *Fukuyama alkylation* with electrophiles comes again from Stromgaard et al.[243] They showed that halides are best leaving groups, that DMF is the solvent of choice and that the outcome of the reaction strongly depends on the applied base, more than on the temperature. The reaction is carried out in three cycles of 3 h applying 6 equiv. of both, base and halide. HBTU gave slightly better results than DBU, while carbonate and tertiary nitrogen bases gave low or no conversion under these conditions. Those results were the background for the study on conventional *Fukuyama alkylation* with halides in chapter 4.1.2.4 in which it was emphasized to suggest conditions that allow the more economic incorporation of more diverse building blocks than the simple iodoalkanes or protected amino iodides in Stroomgaard's example.

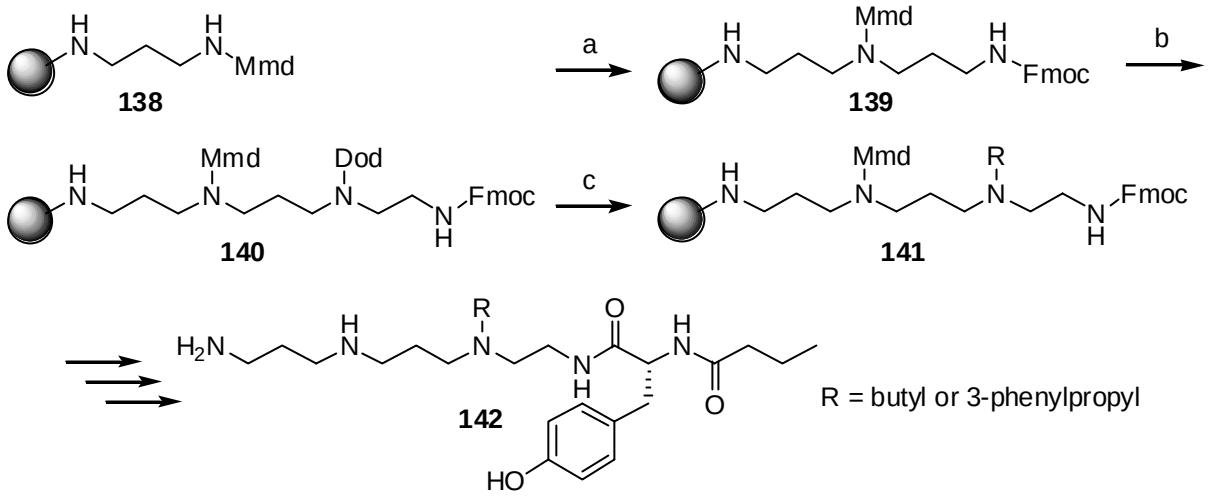
2.3.4.4 Reductive amination

Reductive amination was the earliest of the here described amine forming methods for solid phase.[292, 293] Then, it was used for the alkylation of *Somatostatin Octapeptide* analogues. Due to the early development of this alkylation method it found entry into the synthesis of diverse substance classes like *N*-terminal modified peptides, carbohydrate mimetics[294], glycopeptides[295, 296], oligonucleotides[297] and of course polyamines. For the same reasons as the *Amide reduction* approach, it is suitable to be used in library formations.



Scheme 20 First use of *Reductive amination* in the solid phase preparation of a PhTX-334 by Chhabra et al. Reagents and conditions: (a) 1. *N*-Fmoc-3-aminopropanal, 1% AcOH / DMF, 1.5 h; 2. $Na(CN)BH_3$, 1% AcOH / DMF, 10 min; (b) 1. Boc_2O, DiPEA, DMF, 20 h; 2. 20% piperidine in DMF; (c) 1. *N*-Fmoc-3-aminopropanal, 1% AcOH / DMF, 1.5 h; 2. $Na(CN)BH_3$, 1% AcOH / DMF, 10 min.[246]

The first time that this approach was used for the synthesis of a polyamine backbone was in 2000 by Bycroft and co-workers during the synthesis of PhTX-334 **(137)** (Scheme 20).[246, 298] After attachment of 1,4-butanediamine to a Dde resin, the asymmetric backbone of the polyamine moiety was established stepwise. *N*-Fmoc-3-aminopropanal building blocks were used for elongation, with $Na(CN)BH_3$ / HOAc as reducing agent. Protection group conversion and repetitive chain elongation gave immobilised tetraamine **136**. Finally, PhTX-334 **(137)** was obtained in 65% overall yield and in 90% purity. A drawback for the general use of *Reductive amination* in polyamine synthesis is that to date all attempts to generate *N*-protected C_4- and C_5-aminoaldehydes failed. This limits the application of *Reductive amination* to the elongation with less than four or more than five methylene group spacers. Remarkable syntheses were published by Jönsson. A complex protection strategy, which makes us of the slightly varying acid labilities of 4-methoxy dityl- (Mmd) and 4,4´-dimethoxy dityl (Dod) groups, in combination with *Reductive amination* enabled the directed synthesis of branched polyamines (Scheme 21). The staggered acid lability of Mmd and Dod arises from their different number of methoxy substituents and enables the selective removal of the Dod group in the presence of the Mmd group. The intermediate protection with these two alkyl-type protection groups avoids overalkylation during *Reductive amination*. However, this strategy is not compatible with the use on highly acid labile trityl linkers. Finally, the products were obtained in > 80% purity and 75 – 80% overall yield.

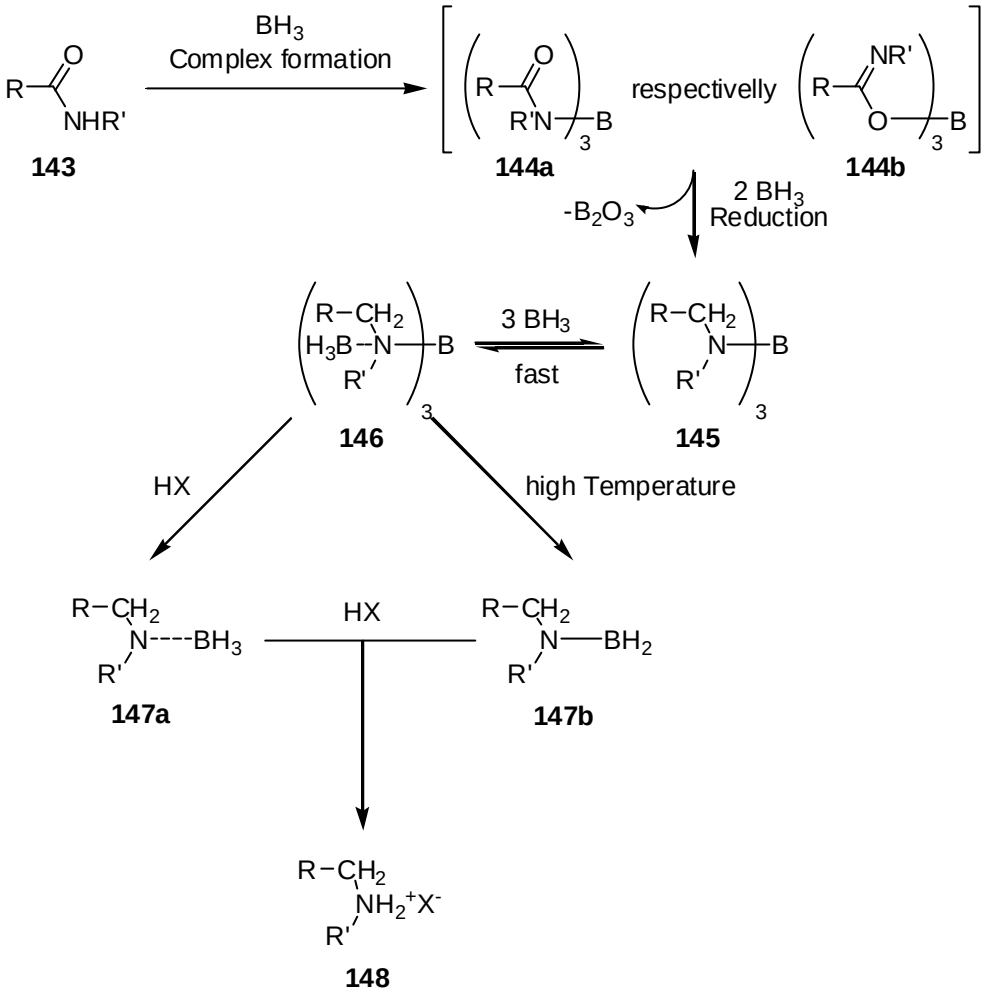


Scheme 21 Synthesis of PhTX-233 by Jönsson. Reagents and conditions: (a) 1. *N*-Fmoc-3-amino-aminopropanal; 2. $Na(CN)BH_3$, 3% AcOH, NMP, 40 °C; (b) 1. piperidine in DMF; 2. Dod-Cl, DiPEA, CH_2Cl_2; 3. 5% TFA; 4. *N*-Fmoc-2-aminoacetaldehyde; 5. $Na(CN)BH_3$, 3% AcOH, NMP; (c) 1. 5% TFA in CH_2Cl_2; 2. *n*-butyraldehyde or 3-phenylpropionaldehyde; 3. $Na(CN)BH_3$, 3% AcOH, NMP, 40 °C.

In another example, overalkylation during *Reductive amination* was circumvented by the choice of appropriate excess of aldehyde.[299]

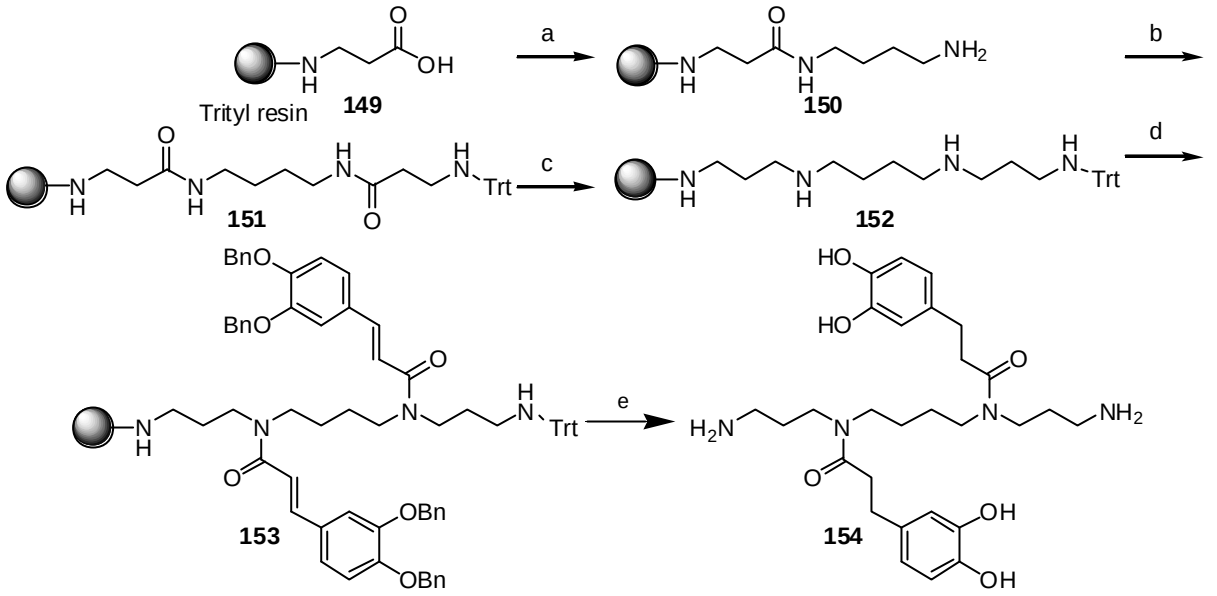
2.3.4.5 Reduction of Amides

The first works on solid phase dealing with the reduction of amides were published by Schultz et al. for the synthesis of *N*,*N*-dialkylated oligocarbamates.[300] They used diborane, a milder reducing agent compared to $LiAlH_4$, which had been used in solution phase. Due to its harshness against several other reducible groups and linker systems, BH_3*THF is favoured in the recent literature.[301-303] The method itself is high yielding for the formation of secondary amines. In combination with the large number of commercially available acylation reagents it opens access to a broad range of structurally diverse polyamines. Scheme 22 shows the supposed mechanism for the reduction of amides by BH_3.[304] The reaction proceeds well *via* exhaustive complexation of the boron by three amides and subsequent elimination of B_2O_3, giving tris-aminoborane **145**. Unfortunately its complexation with three additional equivalents of borane to **146** occurs faster than the reduction of further amides, which requires extra excess of BH_3·THF for complete reduction. **146** is stable and can only be transformed to **147b** at high temperatures (110 °C in toluene). The aminoborane is converted to the secondary amine **148** by acidic, aqueous workup. As these conditions are not suitable for solid phase chemistry on the most acid labile resins, a different work-up procedure is necessary. If reduction is the terminal step of a synthesis, complex **146** can be destroyed during the cleavage from the resin with strong acids. Alternative decomplexation conditions are the use of DBU in NMP : MeOH (9 : 1)[300], piperidine[241, 305] and ones known from solution phase like e.g. exposure to refluxing methanol[306] and basic hydrolysis with hydrochloric acid or saturated ammonium chloride[307]. However, those conditions are often not suitable with either resin requirements, commonly used linker systems and amino-protection groups.



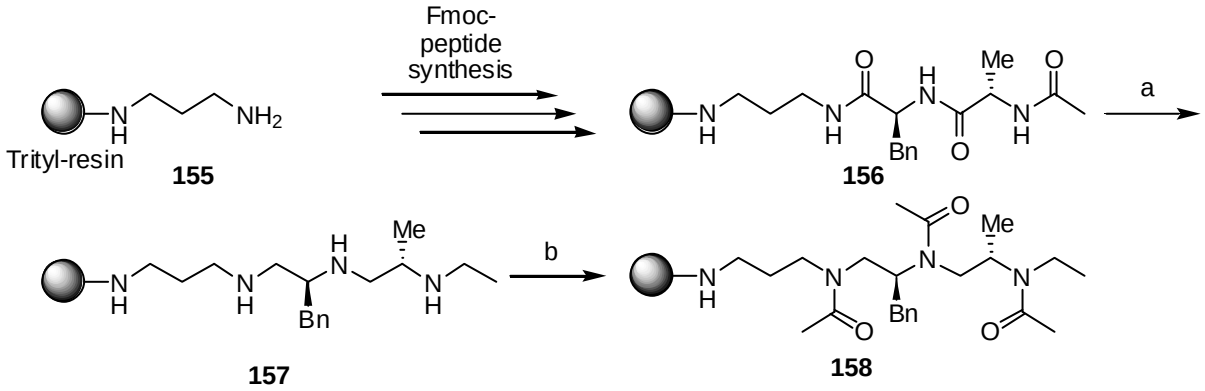
Scheme 22 Supposed mechanism of *Amide reduction* by BH_3 and subsequent liberation of the resulting amino-borane complex.[304]

Hall et al. developed a milder method employing iodine in an *i*-Pr_2NH / HOAc buffered solution for polyamine synthesis on a trityl linker.[304] In this approach it is believed that the acetate anion is able to rapidly cleave the covalently bound aminoborane unit in **146** (Scheme 22). The hydrides in **147a** can successively be removed by iodine, which can be displaced by another acetate ion in a simple S_N2-reaction. Back bonding of the oxygen weakens the boron-nitrogen bond and the complex decomposes irreversible after protonation of the resulting amine. Karigiannis et al. used *Amide reduction* in the synthesis of Kukoamine isomers (Scheme 23).[301] The spermine backbone is established by amide formation of diaminobutane **(13)** and trityl protected respectively trityl-resin bound β-alanine. The amide groups are reduced with BH_3 and kukoamine C **(154)** is obtained in 65% overall yield after further modification. There was no mention about, how the amine-borane complex was destroyed.



Scheme 23 Synthesis of Kukoamine C **(154)**. Reagents and conditions: (a) DIC / HOBt, diaminobutane; (b) *N*-Trt-3-aminopropionyl-succinate, Et_3N; (c) BH_3, THF; (d) *O,O´*-benzylcaffeyl chloride, Et_3N; (e) 1. TFA, Et_3SiH, 2. H_2 / Pd-C.[301]

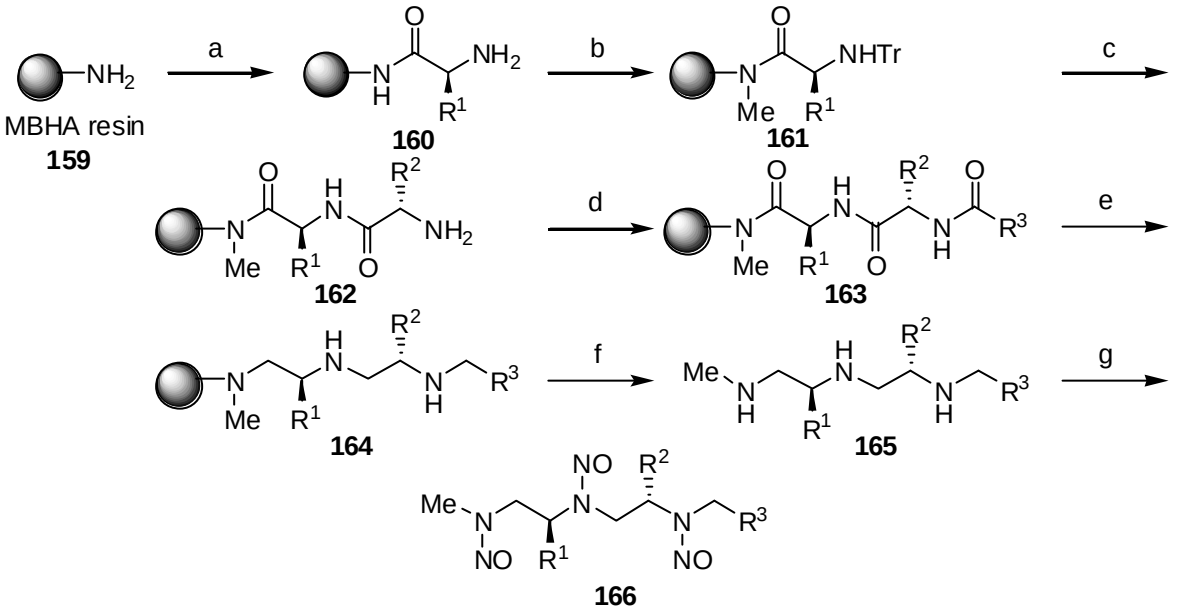
The synthesis of chiral polyamines by reduction of the precursor amides was also shown to work without loss of optical activity (Scheme 24).[304] Stepwise coupling of *L*-amino acids and following reduction of the triamide **156** gives the polyamine backbone. The aminoborane complex is destroyed by the above mentioned iodine procedure and the resulting secondary amines were again acylated. The products were obtained in nearly quantitative yield and with high purity. A comparison study with the piperidine workup showed that the iodine workup is superior. Analysis of the products showed no racemisation at the stereogenic centres.



Scheme 24 Synthesis of chiral polyamine by coupling of amino acids and subsequent reduction. Reagents and conditions: (a) 1. BH_3, THF, 24 h, 65 °C; 2. I_2, (1 : 2 : 7) i-Pr_2EtN / AcOH / THF, 4 h, r.t.; 3. neutralisation; (b) 1. Ac_2O, Et_3N, DMF; 2. 5% TFA / CH_2Cl_2.[304]

The possibility to introduce branching was also investigated. It showed that yields dropped drastically, if the secondary amines were acylated and the resulting amides were reduced with BH_3·THF. Due to the sluggish reduction of the tertiary amide, this led to many only partially reduced side products, even after 5 d of reaction at 65 °C. The problem was satisfactorily solved by *Reductive amination* with aldehydes.

Finally, the advantages of the amide-formation / BH_3-reduction strategy are numerous. The well-investigated methods for amide formation known from peptide synthesis facilitate the generation of polyamine backbones in high overall yields. The great amount of commercially available acyl-building blocks enables the generation of large compound libraries, if the relatively harsh reduction conditions are tolerated.[92, 284] Examples for such libraries come again from Hall et al. and especially from Houghten et al., who based their ´Libraries from libraries´ concept on *Amide reduction*.[308-320] In this concept, the polyamines are only intermediates *en route* to other functional groups. The number of their published examples is extraordinary and so one should be mentioned as representative for the whole concept (Scheme 25).[320] *N*-alkylated tripeptide **163** was synthesised and reduced. After cleavage from the solid support, the free amines were converted to nitrosamines.



Scheme 25 Amide reduction in ´Libraries from libraries´. Polyamines are intermediates in the conversion to nitrosamines. Reagents and conditions: (a) 1. *N*-Boc-AA-OH, peptide coupling; 2. 5% TFA in CH_2Cl_2; (b) 1. TrtCl, DiPEA, CH_2Cl_2; 2. CH_3I, *t*BuOLi, DMSO; (c) 1. 2% TFA in CH_2Cl_2; 2. 5% DiPEA in CH_2Cl_2; 3. *N*-Fmoc-AA-OH, peptide coupling; 4. 20% piperidine in DMF; (d) R_3COOH, DIC, HOBt, DMF; (e) BH_3?THF, 65 °C; (f) HF / anisole, 0 °C; (g) EtONO, CH_2Cl_2.[320]

3 Aims

As displayed above, there is a need for improved methods of cellular delivery. Especially the covalently bound CPPs and their mimics are of interest (see 2.1.3). Polyamines previously found entry into the field of drug delivery as complexing-agents, but they were rather unattended as covalently bound carriers.[209, 218, 321] Their backbones are positively charged at physiological pH, making them potential candidates for drug delivery (see chapter 2.1.1 and chapter 2.2.4.5). As polyamines are also abundantly present in nearly all organisms, it can be expected that the toxicity of their conjugates is rather low compared to non-natural compounds.[52, 108]

One probable reason for the less-regard of polyamines as covalently bound carriers is the limited scope of their synthesis. Polyamine syntheses are ideally carried out on solid phase. While rational solid phase strategies for the synthesis of α- and β-peptides, peptoids, and oligocarbamates are already established and can be automatised for combinatorial use, polyamine syntheses are much more difficult to unitise.[92, 169, 284] The necessity to use alkylation instead of acylation reactions for the generation of the backbone is disadvantageous as alkylation conditions are usually harsher than the conditions for peptide bond formation. Consequently, *Amide reduction* of short oligopeptides is the only established alkylation method for polyamine synthesis that has ever been applied in the formation of larger polyamine libraries (see chapter 2.3.4.5).[316, 322] However, the harsh conditions of the reduction make it unsuitable in the presence of sensitive groups, requiring a milder method for backbone elongation. The choice of an appropriate protection group strategy is also important. It should tolerate the method of backbone elongation, coupling to other molecules, and allow the introduction of branches into the backbone.

To address the depicted points, the following aims were selected for of this thesis:

Aim 1: Development of a suitable protection strategy for the mild solid phase synthesis of sensitive polyamine conjugates

Aim 2: Development of coupling methods for the formation of sensitive polyamine-drug conjugates

Aim 3: Synthesis of conjugates, which can be tested for their biologic activity

Aim 4: Initial biological testing of the derived polyamine-conjugates concerning their potential to enhance cellular uptake

3.1 Aim 1: Development of a suitable protection strategy for the mild solid phase synthesis of acid sensitive polyamine conjugates

As a prerequisite, trityl resins were chosen as solid support for the syntheses. It allows the liberation of the conjugates by such a mild acidic treatment, which is compatible with nearly all protection- and functional groups (see chapter 2.3.3.2). Otherwise, it is known that trityl resins are stable during the usual conditions of polyamine syntheses.[92] According to previous experiments in which fluorescence labelled derivatives of diverse polyamines were compared in terms of their uptake efficiency, spermine **(16)** was chosen as the scaffold for the polyamine backbones.[323] Thus, partially protected building blocks derived from spermine **(16)** should be prepared and loaded onto the trityl resin. The choice of the protection groups should allow a direct access to backbone elongation and side chain modification of the polyamine. An alkylation method, which converges with the protection groups strategy and allows efficient elongation of the backbone under mild conditions, should be identified.

3.1.1 Protection strategy and synthesis of the building blocks

Since the final conjugates in this work should derive from spermine **(16)**, it is obvious to prepare protected entities based on the 3-4-3 polyamine skeleton. One pot procedures for the selective N^4,N^9-protection of spermine **(16)** with Boc-, Bn- and Tr-groups were reported, but the use of these protection groups was not suitable reach the aims of this thesis.[230, 251] Since Bn- and Boc-groups are removed with high concentrations of TFA in CH_2Cl_2, they cannot be used with highly acid labile trityl resins. After attachment to the resin the secondary amines would be blocked and their modification would no longer be possible. Additionally, the deprotection conditions are too harsh for the synthesis of highly acid labile polyamine conjugates. On the other hand, the synthesis of N^4,N^9-bis-tritylspermine **90c** suffers from low yields and the huge sterical demand of the trityl groups prohibits the attachment to a bulky trityl linker.[251] Application of base labile protection groups such as Fmoc is excluded, as other amino functionalities, even if only temporarily liberated, can effect partial Fmoc-deprotection. Indeed, the fluoride sensitive Teoc group fulfils the constraints in terms of orthogonality and mild removal. However, the reagents for its introduction (usually 4-nitrophenyl 2-(trimethylsilyl)ethyl carbonate or the respective chloride) are too expensive for a large scale synthesis.[248] The Aloc group circumvents the above-depicted drawbacks. It can be introduced under modified *Schotten-Baumann* conditions using allyl chloroformiate.[248] Aloc protection is stable during cleavage of most other amino protection groups and linkers and it can gently be removed with Pd^0 species.[268] The range of reported allyl-scavengers is broad and can be adjusted to different substrate susceptibilities.

3.1.2 Alkylation procedure for backbone elongation

There are four established methods for the elongation of polyamine backbones, *S_N2-displacement*, *Fukuyama alkylation*, *reduction of amides* and *Reductive amination*. All of these methods display certain limits for their applicability on solid phase (see chapter 2.3.4.3 and chapter 2.3.4). The use of Aloc protection and the above-depicted drawbacks exclude the use of *S_N2-alkylation* and reductive methods and thus, the *Fukuyama alkylation* is chosen. It is

selective for the generation of secondary amines and compared to the reductive methods all steps can be carried out under milder conditions.[92, 284, 324] Although particular efforts have been made to optimize the *Fukuyama-Mitsunobu alkylation*, it showed to be unsuitable for the generation of longer backbones.[263, 291] As *Mitsunobu-conditions* are also highly sensitive to air and humidity, the more robust reaction with halides should be applied.[243] A broad range of halides is commercially available or can easily be obtained from the corresponding alcohols by reaction with PPh_3, iodine and imidazole.[243] There were only few examples of a conventional *Fukuyama alkylation* with halides on solid phase.[239] The above-mentioned study of Stromgaard et al. gave first information on the reaction and should be further investigated and optimised to allow the introduction of diverse functionalities and building blocks.[243]

3.1.3 Introduction of side chain modifications

Besides the generation of a polyamine backbone, it is important to achieve modifications of the backbone in order to introduce side chains. Efficient alkylation procedures for secondary amines were necessary. *Fukuyama alkylation* is not suitable, as it is restricted to the formation of secondary amines. Remaining approaches from the set of methods were *S_N2-alkylation*, *Reductive amination* and *Amide reduction* after acylation of the secondary amines. These methods shall be explored for their potential to introduce side chain modifications.

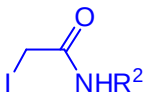
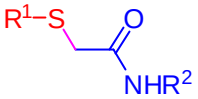
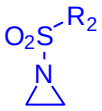
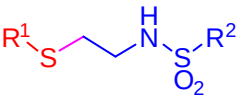
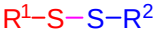
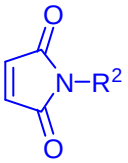
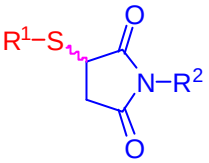
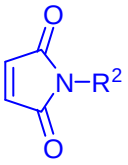
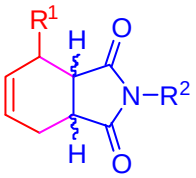
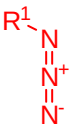
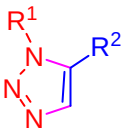
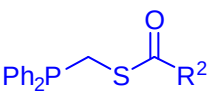
3.2 Aim 2: Development of coupling methods for the formation of acid sensitive polyamine-drug conjugates

For the coupling of polyamines to acid sensitive molecules it is important to have methods, which allow a mild, quick, selective, and high yielding reaction. In the last years, some reactions for the chemoselective *Bioconjugation* emerged, which exhibit high selectivity and are sufficiently mild for the use with sensitive biomacromolecules (Table 3).[325-327] Such reactions enable the site-specific labelling and cross-linking of biomolecules.

Sulfides and maleimides are frequently involved in such *bioconjugation* reactions (Table 3). This often origins in the fact that sulphur is a soft centre. It preferentially reacts with other soft centres like iodides, sulfenyls, or the β-position of α,β-unsaturated carbonyls (Entries 1, 3, and 4 in Table 3).[328] Maleimides are also common; they specifically react with sulfides in a *Michael addition* or with 1,3-dienes in a *Diels-Alder reaction*. In both cases, the driving force is the reduction of the strong ring strain by conversion of the double bond forming carbons from sp^2-hybridisation to sp^3-hybridisation.[327] More recently, azides received emerged attention. Their reaction with alkynes is probably one of the most cited examples of the concept of *Click chemistry*, introduced by Sharpless in 2002.[325, 329, 330] This is due it perfectly suits the terms of this concept, namely a high driving force (*1,3-dipolar cycloaddition*), high selectivity, non-air and non-water sensitive educts, and simple reaction conditions. The traceless *Staudinger ligation* also found increasing attention for the same reason as the *1,3-dipolar cycloaddition* between azides and alkynes.[331, 332] *Bioconjugation* via the *Michael addition* of a thiolate, often from the side chain of a cysteine residue, to a maleimide to form a succinimidyl thioether is especially common. The addition proceeds rapidly and in high yield in neutral aqueous solutions at room temperature, making it ideal for biological applications. It has been frequently used for the coupling of oligonucleotides,

peptides, and other large molecules.[333-337] Its advantages are the formation of a stable, non-reducible thioester bond and the quick reaction in high yield. The reaction does not need any catalyst and is traceless. For the generation of a polyamine with a maleimido-binding site, a compound that allows the introduction of the maleimide under avoidance of cross-linking on the resin should be prepared. Finally, the coupling reaction should be investigated with some model compounds and integrated into the established synthetic scheme (Aim 1, see chapter 3.1).

Table 3 Some common bioconjugation reactions.

	Reactant 1	Reactant 2	Linkage
1			
2			
3			
4			
5			
6			
7			

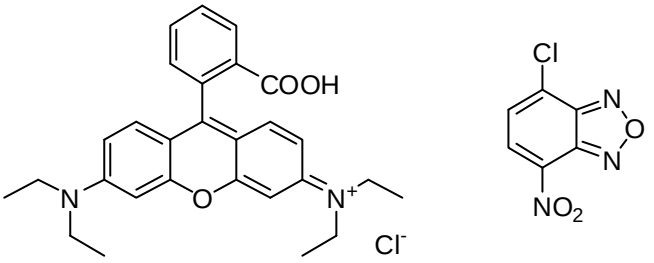
3.3 Aim 3: Synthesis of conjugates and tests of their biologic activity

After development of the synthetic methods, the derived knowledge should be used to synthesise polyamine conjugates, which can be tested for their biological activity. Cargo molecules are:

1. Fluorophores
2. Porphyrins and radical scavenger
3. Indomethacin
4. Oligonucleotides

3.3.1 Fluorophores

Incubation with fluorophore-labelled compounds is one possible approach to determine the uptake of polyamines and their conjugates into cells.[78, 82, 84] If cells are incubated with polyamino-fluorophores, the migration and accumulation of the latter can be investigated by fluorescence microscopy of the living cells. This method is very demonstrative for the temporal and spatial resolution of the uptake process. Co-localisation experiments with fluorescent sub-cellular markers will enable the assignment compartments, reached by the fluorophore.



167 Rhodamine B **168** NBDCl

Figure 23 Structures of the fluorophoric compounds rhodamine B and NBDCl.

In similar experiments with peptoids, which were executed in the group, rhodamine B **167** was used for the labelling and showed good results in cell tests (Figure 23).[82] Another prominent fluorescent marker is 7-nitro-1,2,3-benzoxadiazole-4-yl (NBD) **168**.[338-341] Due to the electron-deficient ring system of NBDCl the chlorine can be displaced in an S_NAr reaction with an amine. In both cases the amine used for the coupling is no longer charged at physiological pH. In case of NBD, aniline with three electron-withdrawing substitutions on the ring is generated by this reaction. The pK_b value of aniline is 9.34 and as basicity is distinctly decreased by the substitutions of the phenyl ring, it is not protonated under physiological conditions.[342] As it is known that enhanced lipophilicity improves the uptake of cationic conjugates by tightening them to the membrane, polyamine with lipophilic parts should be synthesised. A schematic illustration of such a lipophilic conjugate is shown in Figure 24.

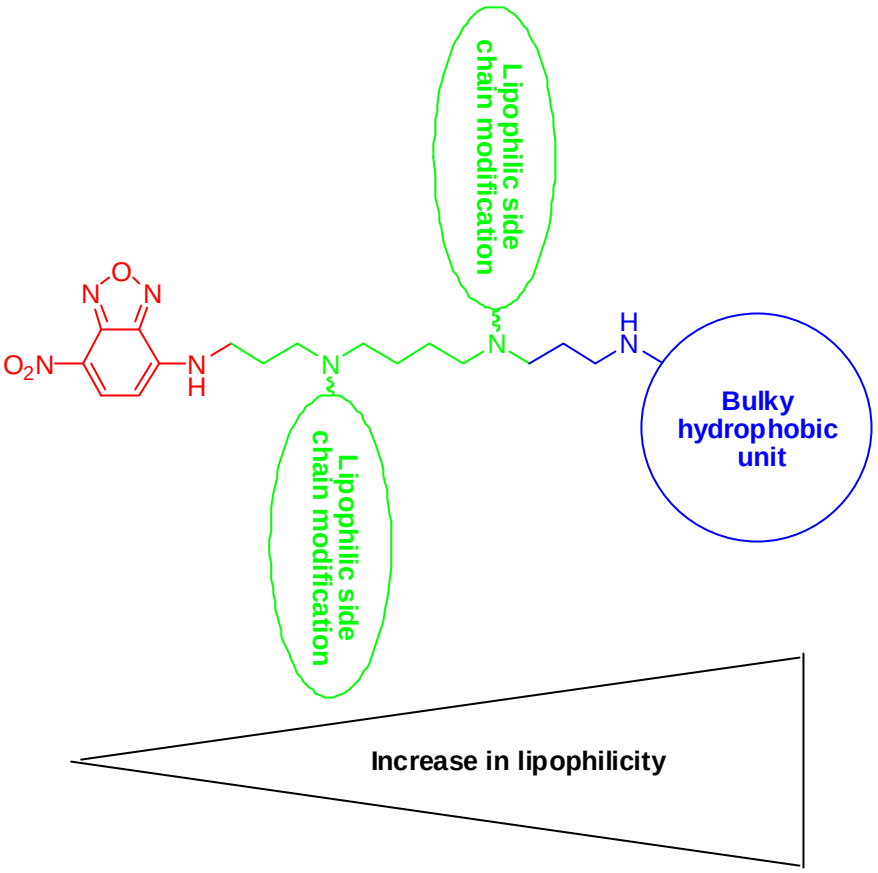


Figure 24 Schematic description of the NBD-labelled polyamines with lipophilic modifications.

Although NBD allows transversal diffusion, it is a polar moiety and would therefore be pushed out of the bilayer to the aqueous media. Thus, it should be attached to the polar part of the molecules.

3.3.2 Porphyrins and radical scavenger

Photodynamic therapy (PDT) is an important application in the treatment of age-related macular degeneration, various skin disorders, and an increasing number of cancers.[343-347] Photosensitizers are administered to initiate a light-activated destruction of a variety of tissue.[348-352] The porphyrin is excited by light of a certain wavelength. In the presence of oxygen, this excited state of the porphyrin is relaxed by excitation of triplet- into singlet oxygen. This singlet oxygen is a highly reactive di-radical species, which readily reacts with unsaturated molecules.[349, 351] DNA, RNA, aromatic amino acids, and especially unsaturated fatty acids are target for these oxidation- and peroxidation reactions.[351] It is known that peroxidised fatty acids play a role as mediators of apoptotic cell death. If delivered into the right cellular compartments, cell death can specifically induce apoptosis and not necrosis.[353-355]

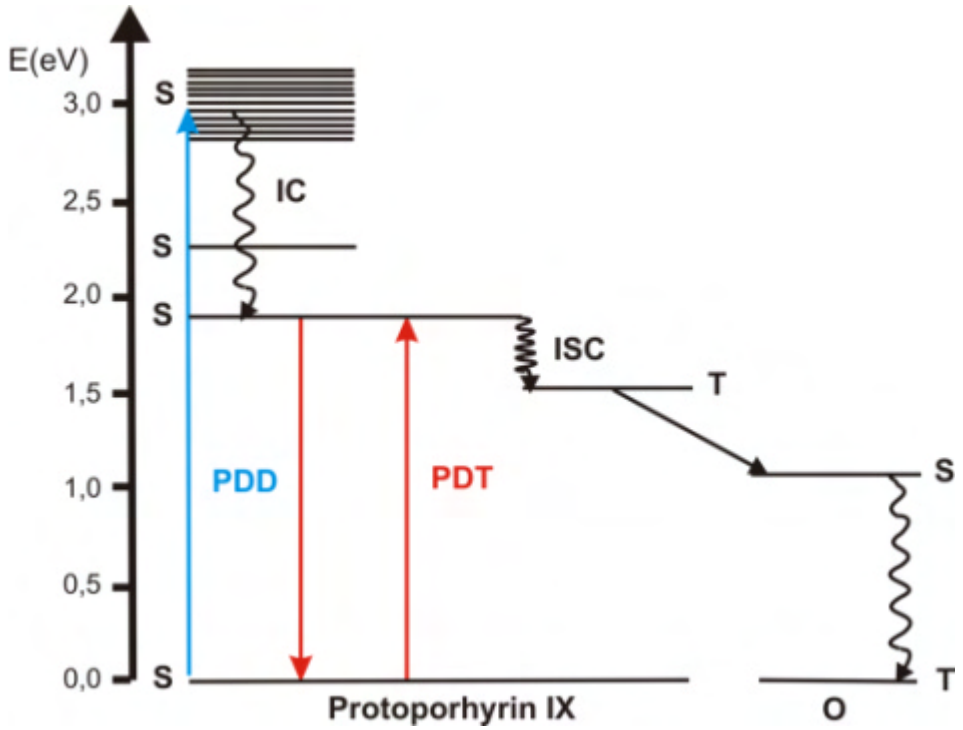
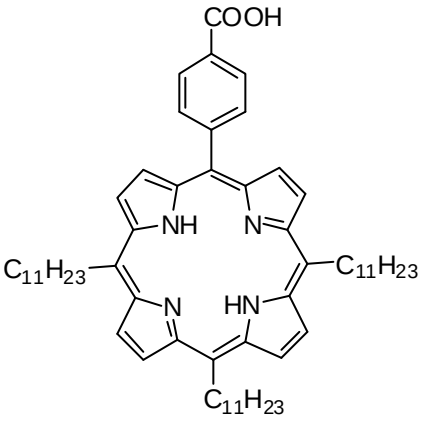


Figure 25 Schematic description of excitation and relaxation of a porphyrin. Two processes can be initiated by irradiation with particular wavelengths. Either leads to red fluorescence, while excitation at longer wavelength promotes the porphyrin to a level from which it can return by *inter system crossing* followed by transfer of its energy to an oxygen molecule (if present), or fluorescence at a longer wavelength.

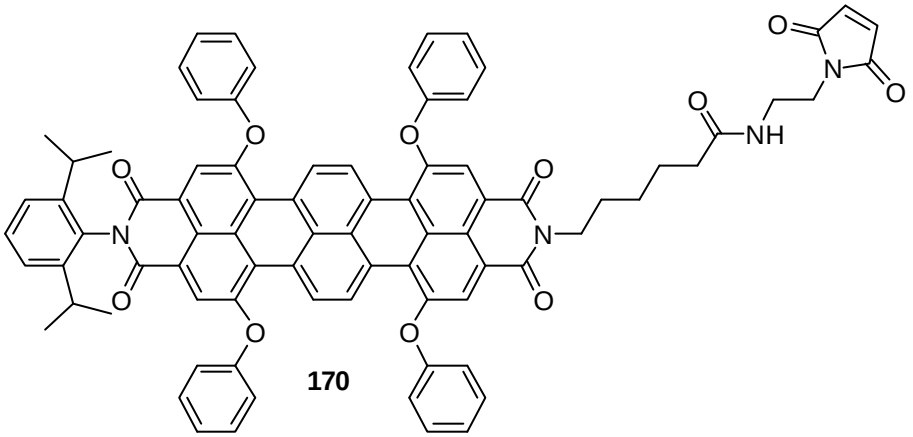
A highly lipophilic porphyrin should be coupled to the polyamine. This lipophilicity is especially important, as the efficiency of porphyrins in PDT mainly depends on the environment of the tetrapyrrole ring. Lipophilic porphyrins are able to penetrate deeply into the membrane in order to generate cytotoxic singlet oxygen in direct neighbourhood to unsaturated fatty acids and to cause membrane destabilisation. This localisation inside the membrane strongly enhances the phototoxic efficiency of porphyrins.



169
6,11,16-Tris-undecanyl-*p*-carboxyphenyl porphyrin

Figure 26 Structure of a lipophilic porphyrin, kindly provided by the group of Prof. Dr. T.S. Balaban. This porphyrin should be coupled to a polyamine. The conjugates can be visualised for live microscopy by the fluorescence of the porphyrin at 420 nm. Depending on the wavelength, the porphyrin can also be excited to generate singlet-oxygen, which will cause local cell damage. This could finally be used for therapeutic applications.

Water solubility and a potential site for interaction with the outer membrane should be provided by the polyamine modification. The polyamine should be coupled to only one side of the porphyrin *via* an appropriate spacer, assigning it a highly amphiphilic structure. The introduction of a linker should allow the porphyrin to maintain flexibility once the polyamine part has attached to the membrane. Polar porphyrins are not able to penetrate into membranes and there, they only evolve minimal parts of their photodynamic potential. However, unpolar porphyrins are only poorly soluble in water and they are not easy to apply to aqueous media. The described polyamine-porphyrin conjugate should supersede these drawbacks of both, polar and unpolar agents. Besides the knowledge about the temporal and spatial effects of porphyrins in PDT, it is interesting to enable its deactivation. This could be achieved by delivery of a compound, which aborts its efficacy. The polycyclic compound **170** shown in Figure 27 is able to quench singlet oxygen by transforming it into the less detrimental triplet oxygen.[356]



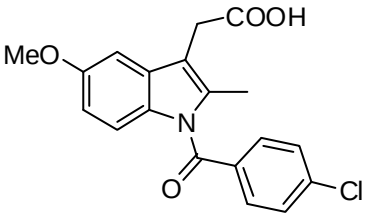
170

Figure 27 Structure of a radical scavenger, kindly provided by the group of Prof. Dr. K. Müllen. The molecule is able to quench singlet oxygen and transform it into the less detrimental triplet oxygen.

As it bears a maleimido group, binding to a polyamine *via* the above-mentioned maleimido thiol chemistry should be possible (Aim 2, see chapter 3.2). **170** is unstable to various conditions and thus, the conditions for coupling and liberation of the product should be mild.

3.3.3 Indomethacin

Indomethacin **(171)** is a non-steroidal anti-inflammatory drug, which was discovered in 1963 and first approved for use in the U.S. in 1965 (Figure 28). It is commonly used to reduce fever, pain, stiffness, and swelling. Its mode of action, elucidated in 1971, consists of inhibition of the prostaglandin biosynthesis.[357-360]



171
Indomethacin

Figure 28 Structure of Indomethacin, a methylated indole derivative, which acts as COX-2 inhibitor.

It inhibits the cyclooxygenase, COX-1 and COX-2, which catalyse the formation of prostaglandins from arachidonic acid. In contrast to COX-1, which produces the baseline levels of prostaglandins, COX-2 is responsible for the prostaglandins biosynthesis through stimulation. Especially enhanced COX-2 activity often mediates inflammatory processes. As prostaglandins induce uterine contractions in pregnant women, indomethacin is also an effective tocolytic agent. However, it is highly recommended to prescribe indomethacin only in lowest doses, as it shows several adverse effects. Those are for example peptic ulcers, edema, hyperkalemia, hypernatremis, hypertension (the latter four caused by retention of alkali metal ions), renal damage, and skin reactions. Thus, it is desirable to enhance the efficiency of Indomethacin-uptake in order to reduce the required dose and the adverse effects. Evidence was presented that the carboxylic acid of indomethacin **(171)** is not essential for the activity of indomethacin to act as COX-inhibitor.[361] Esters as well as primary or secondary amides of indomethacin **(171)** have shown to be equally well or superior COX-inhibitors, which beneficially show improved activity against COX-2 compared to COX-1, if related to indomethacin **(171)**.[362] Tertiary amides were less active than carboxylic acid of indomethacin **(171)**. Furthermore, it was shown that the 4-chlorophenyl and the 2-methyl group are essential for the activity of both, indomethacin **(171)** and its carboxylic acid derivatives. Modification on them should hence be avoided. Thus, conjugates of indomethacin **(171)** and polyamines should be prepared. A primary amine should serve for the coupling to the carboxylic acid of Indomethacin. The conditions during the synthesis should be sufficiently mild to avoid side reactions, especially as the 4-chlorobenzamide of indole is sensitive.

3.3.4 Preparation of polyamino-oligonucleotide conjugates

For modern medicinal approaches like gene therapy, reliable methods for the delivery of DNA into cells are important.[8] Complexes of lipo-polyamines and DNA have already shown to be efficient delivery systems for genetic material.[92] However, the lipophilic polyamines showed strong toxicity after longer treatment at higher doses.[91] Covalently coupled polyamines could reduce this problem. In analogy to the promising results that were obtained with conjugates of CPPs and their mimics, one to one-polyamine-oligonucleotide conjugates should be prepared. The strong electrostatic interactions between polyamines and highly negatively charged oligonucleotides complicate the development of such conjugates. As already mentioned in the introduction, the biological function of spermine **(16)** involves complexation of DNA.[91, 92, 247] This is expected to cause difficulties in the selected formation of 1 : 1-conjugates between polyamines and DNA.

Two approaches should be investigated in the course of this work (Figure 29). One is the direct coupling of modified polyamines with oligonucleotides bearing a specific binding site. In this approach, the strategy comprises a complete protection and mild deprotection after coupling. The coupling / deprotection process should be established on solid phase as the terminal step after the synthesis of the polyamine. The second approach is the attachment of a polyamine to nucleotides and the subsequent enzymatic integration of the triphosphate into an oligonucleotide. This method gives more flexibility in terms of the positioning of the modification in the DNA-strand.[363, 364] However, it would either be suitable for modification with smaller polyamines, since the enzymatic integration does not tolerate too bulky substrates. The methods for modification should be mild with respect to the sensitivity of triphosphates.[365]

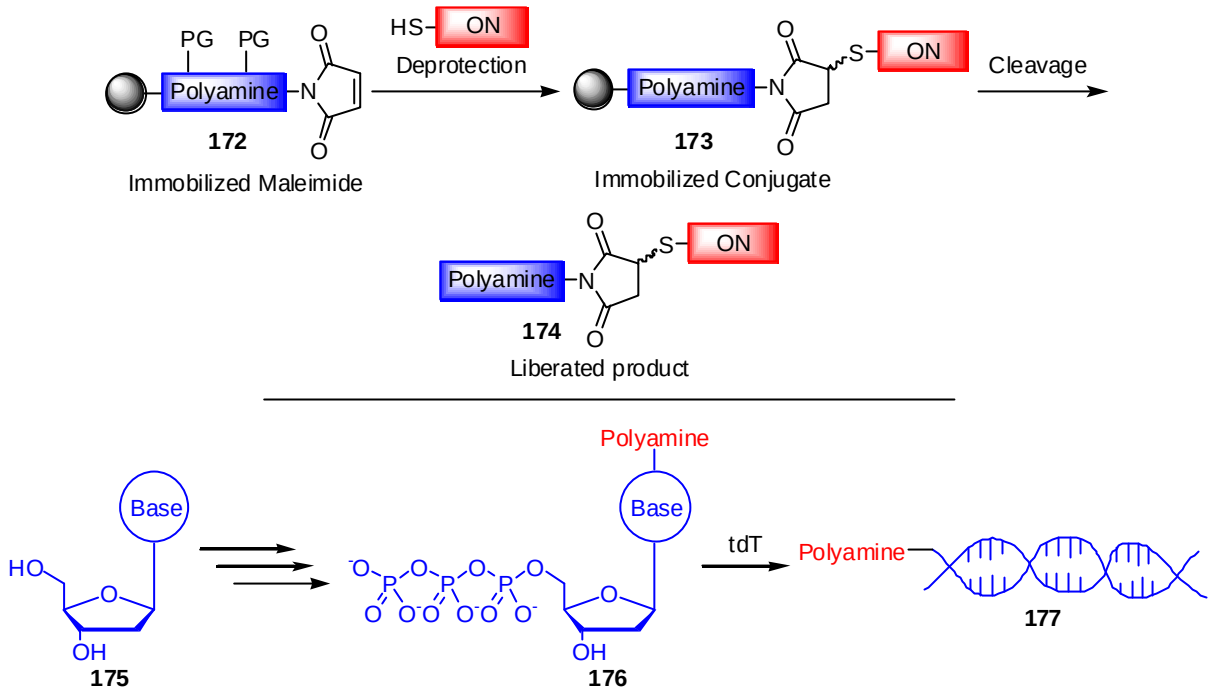


Figure 29 Schematic descriptions of the strategies for the formation of polyamino-oligonucleotide conjugates.

3.3.4.1 The bioconjugation approach

The maleimido-thiol coupling should be tested for its feasibility in the formation of polyamino-oligonucleotides. Thiol-modified oligonucleotides are commercially available and can be readily inserted into the reaction with immobilised polyamino-maleimides.

3.3.4.2 Preparation of polyamino-modified nucleotides and attachment to DNA-oligomers

For the incorporation of polyamine modification to the terminus of a DNA strand it is necessary to synthesize linker-modified nucleoside-triphosphates, which enable an efficient coupling. This approach is based on a previously reported strategy from Taira et al., in which a modified cytidine **331**, bearing a maleimido group linked to the base moiety of the nucleoside, was synthesised.[364] The derivatised nucleoside was then triphosphorylated and attached to the 3'-terminus of a DNA-strand by a terminal desoxy transferase (tdT). Eventually, the oligonucleotide was coupled to the free cysteine of an oligopeptide by a *Michael addition*. Variation of the order of triphosphorylation, introduction of binding site and coupling to the polyamine moiety shall allow the transfer of this concept from peptides to polyamines. Additionally, other nucleosides / nucleotides, also bearing maleimido binding sites, should be prepared and coupled to polyamines.

3.4 Aim 4: Preliminary biological testing of the derived polyamine-conjugates

All conjugates (Aim 3, 3.3) should be tested with regard to the potential of covalently coupled polyamines to act as delivery agents. For the four classes of conjugates, the following testing should be executed:

3.4.1 Fluorophores

The conjugates should be studied by fluorescence microscopy of living cells. Special attention should be paid to the subcellular localisation of the conjugates. Several fluorescent sub-cellular markers are available, which can be used to determine the intracellular fate of the rhodamine B- or NBD-labelled polyamines. The toxicity of the conjugates should also be determined.

3.4.2 Porphyrins and radical scavenger

In analogy to the fluorescent dyes, cells should be incubated with the polyamine-porphyrin conjugate. The localisation of the conjugates inside mammalian cells should be determined by co-localisation with subcellular markers and subsequent fluorescence microscopy of the living cells. The effect of irradiation should be investigated to get at least qualitative information about the phototoxic efficiency. Co-incubation of different cell types and following incubation should reveal information about uptake preference for certain cells. The dark toxicity of the conjugates should be determined. Finally, this should illuminate the suitability of the polyamine-porphyrin conjugate to be used for PDT. The conjugate of polyamine and **170** should also be tested for its ability to enter cells. In order to test the facility to achieve temporal control over the potency of photodynamic treatment, this conjugate should be co-incubated with polyamine-porphyrin conjugate. The influence on the action of **217** should be determined.

3.4.3 Indomethacin

The influence of polyamine homologation should be investigated by comparison of the effects of polyamine-indomethacin conjugates with indomethacin **(171)**. The toxicity of the conjugates should be determined and compared with the respective values of indomethacin **(171)** in order to find out, if effects in toxicity go hand-in-hand with the uptake properties.

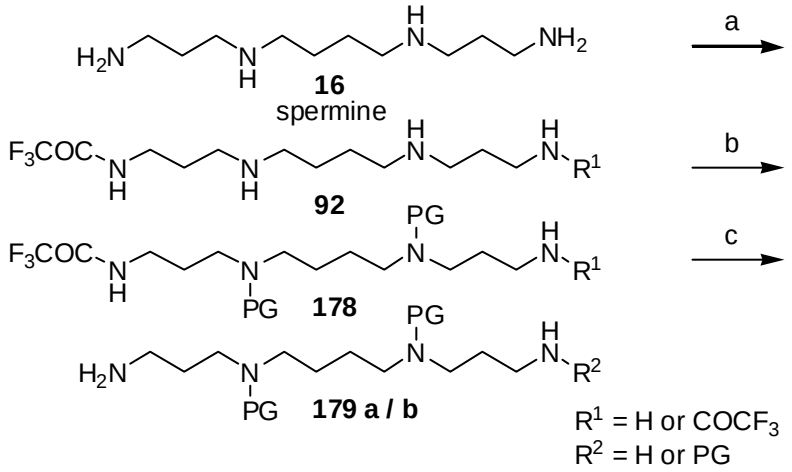
4 Results

4.1 Development of a suitable protection strategy for mild solid phase synthesis of polyamines

4.1.1 Solution phase syntheses of the building blocks

4.1.1.1 Symmetric building blocks

For the introduction of different protection groups onto secondary and primary amines, a procedure with Boc as permanent protection group for the secondary amines and Tfa as temporary protection group for the primary amines was adapted and slightly modified (Scheme 26).[230] Spermine was selectively protected at the primary amines with ethyl trifluoroacetate. The second protection groups were introduced with the respective chloride in the presence of equimolar amounts of a base to remove the released HCl. The Tfa groups were removed by addition of aqueous sodium hydroxide and N^4,N^9-bisprotected building blocks **179a** and **179b** were obtained in good to excellent yield (Scheme 26).



Scheme 26 Synthesis of spermine building blocks. Reagents and conditions: (a) F_3C-COOEt, MeOH, –80 °C; (b) Aloc-Cl and Et_3N or *o*-Nosyl-Cl and DiPEA, 0 °C to r.t.; (c) $NaOH_{conc}$ / H_2O, r.t.

Depending on the initial excess of ethyl trifluoroacetate, the ratio between bis- and tris-protected spermine could be regulated (Table 4). Symmetric (**178** and **180**) or asymmetric (**179** and **181**) building blocks were obtained (Figure 30). The yields for the *o*-Nosyl protected spermine derivates **180** and **181** were lower than those of the Aloc protected building blocks **178** and **179** (Table 4). TLC-monitoring during the reaction showed that this arose from slower reaction of the second and third amine with the *o*-Nosyl chloride, which hinders the overall protection of all amines. This slower reaction of *o*-Nosyl chloride with secondary amines has previously been used for the quasi-selective protection of primary amines in the presence of secondary amines with equimolar amounts of *o*-Nosyl chloride (see chapter 2.3.3).[366] Additionally, the *o*-Nosyl group is not fully stable against the basic conditions of the Tfa deprotection.[248]

Table 4 Control of the ratio of symmetric and asymmetric building blocks by variation of the excess of TfaOEt.

Excess of TfaOEt	Excess of chloride[a]	Reaction time[b]	Yield of bis-protected building block	Yield of tris-protected building block
Aloc protected building blocks				
1.2 equiv.	6 equiv.	17.5 h	44%	23%
1.2 equiv.	14 equiv.	22 h	n.d.	24%
1.1 equiv.	6 equiv.	7.5 h	27%	49%
2 equiv.	6 equiv.	3 h	54%	n.d.
1.9 equiv.	6 equiv.	26 h	21%	n.d.
2.8 equiv.	5 equiv.	8.5 h	85%	n.d.
3.0 equiv.	5 equiv.	26 h	99%	n.d.
3.5 equiv.	5 equiv.	1.5 h	72%	n.d.
o-Nosyl protected building blocks				
1 equiv.	15 equiv.	41.5 h	7%	29%

[a] Equimolar or higher amounts of a tertiary nitrogen base were used.
[b] As reaction control by TLC indicated that the final deprotection with aqueous NaOH was complete in each case, the reaction time is given for the introduction of the protection groups starting from spermine **(16)**. Addition of Tfa-OEt was accomplished during the first 30 min in each case.

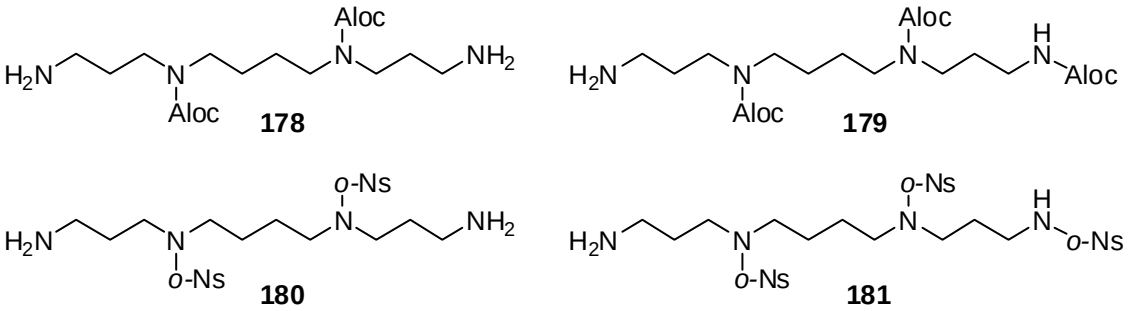


Figure 30 Building blocks obtained from the reaction sequence in Scheme 26.

The symmetric building block **178** was coupled to a conventional 2-chlorotrityl resin (loading: 1.3 mmol/g)[367-369] and an alkoxytrityl resin (loading approximately 0.4 mmol/g)[239, 261] by slowly adding the resin to a large excess (up to 50 equiv) of **178** according to previously published solid phase polyamine syntheses.[230] However, further derivatization and cleavage from the resin revealed a large portion of non-derivatised educt **178**, due to cross-linking of the symmetric spermine building block, which seemed to be independent from the excess, as long as more than 10 equiv. were applied (Figure 31). Those results indicate that once the polyamine is immobilized, the second primary amine reacts with higher rate than the free, mobile diamines. Probably, this is induced by the proximity of multiple trityl linkers to the immobilised primary amine as well as by the hindered diffusion of diamines from the outside of the resin beads.

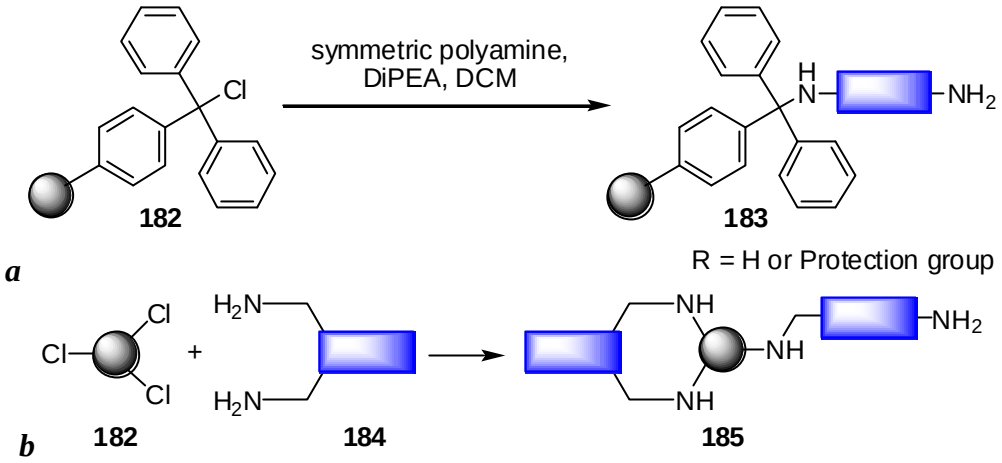
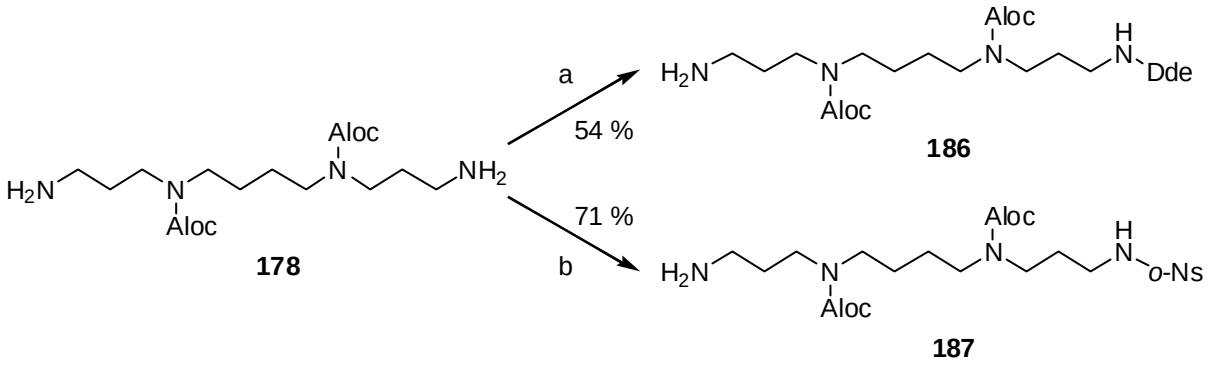


Figure 31 Loading of trityl resins with diamines building blocks. The reaction (**a**) is dimished by the cross-linking of two amines with the resin (**b**).

Cross-linking is a major problem, which frequently occurs during coupling of polyamines with two terminal primary amino groups.[287] It leads to a decrease of the resin loadings and to contamination of the final coupling product with the polyamine after cleavage from the resin. In most cases the impurities are hard to separate and reduce the advantage of solid phase synthesis. Previously, resin cross-linking during further derivation of already-loaded polyamines was prevented by lowering the capacity of the resin. This was done by blocking about 50% of the linker binding sites.[285, 370] However, there are only a few reports on crosslinking during the polyamine loading steps[287], although decreased yields indicate similar problems.[220] To avoid crosslinking, one of the primary amines was protected to ensure an unambiguous course of the reaction, which is more efficient than considerably lowering the resin capacity.

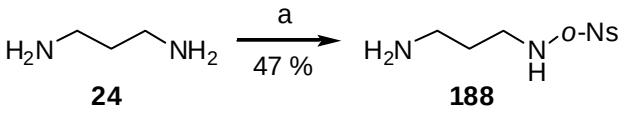
4.1.1.2 Asymmetric building blocks

Symmetric building block **178** was monoprotected with an *o*-Nosyl- or a Dde group, respectively (Scheme 27), by reaction with a substoichiometric amount of acetyldimedone or *o*-Nosyl chloride in the presence of a base. The asymmetric building blocks **186** and **187** were obtained in 54% and 79% yield related to the onset of acetyldimedone or *o*-Nosyl chloride. The best results with respect to the avoidance of the fully protected spermine derivates were obtained, if the acetyldimedone or *o*-Nosyl chloride was slowly added to a solution of **178** and the base. In this case, large portions of the initially applied **178** were recovered and could be reused in further reaction cycles. Unfortunately, TLC showed the appearance of two additional spots already after short agitation of pure mono-Dde-bis-Aloc-spermine **186** in solution. While the ^{1}H NMR remained identical, two signals in the mass spectra emerged, which were consistent with transamination of the Dde group. In contrast, **187** was stable to aqueous solutions of pH-values from 1 to 14, 15 M ammonia solution, and temperatures up to 80°C.



Scheme 27 Protection of one primary amine with orthogonal protection group. Reagents and conditions: (a) 0.6 Equiv. Dde-OH, 0.6 equiv. DiPEA, MeOH, 16 h, r.t.; (b) 0.6 equiv. *o*-Nosyl-Cl, 1 equiv. collidine, CH_2Cl_2, 20 h, r.t.

This stability of Aloc- and *o*-Nosyl group to aqueous acidic conditions greatly facilitates the workup procedure. While the bis-protonated form of **178** and collidine could be extracted with half concentrated HCl, the *mono*-protonated form of **187** completely remained in the organic layer. After removal of collidine from the combined aqueous layers in high vacuum, **178** was recovered in high purity. This acidic treatment is the key-step in the workup-procedure and is not compatible with Boc or Mmt-protection groups, which are sensitive to aqueous solutions with a pH below 5.[248] Hydrolysed *o*-Nosyl chloride was removed by quickly washing the organic layers with NaOH (0.5 M). The resulting crude mixture primarily contains **187** and traces of the bis-nosylated side product, which can easily be separated by filtration over a short bed of silica gel. The bis-nosylated side product is also a useful building block for polyamine synthesis, making the whole procedure very atom-economic. Finally, all of the inserted spermine **(16)** was converted into valuable entities.

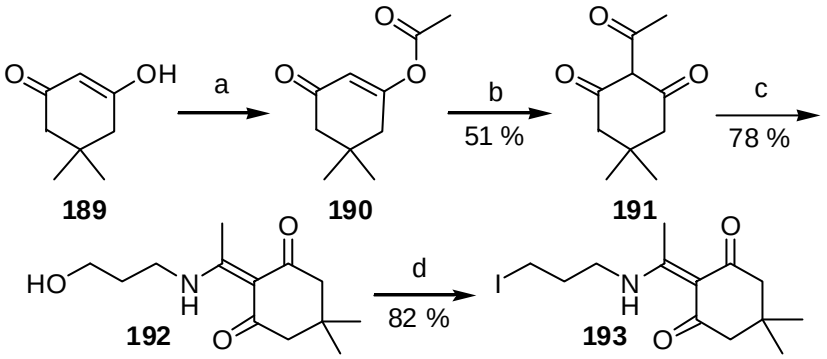


Scheme 28 Synthesis of a small asymmetrically protected building block derived from 1,3-diaminopropane. Reagents and conditions: (a) 0.6 Equiv. *o*-Nosyl chloride, 1 equiv. collidine, CH_2Cl_2, 19.5 h, r.t.

Diaminopropane **(24)** was monoprotected with *o*-Nosyl using the conditions described above (Scheme 27). The stoichiometric reaction revealed **188** in 47% yield related on the onset of *o*-Nosyl chloride. (Scheme 28)

4.1.1.3 Building block for backbone elongation

For the modification and elongation of the backbone by *Fukuyama alkylation* another asymmetric building block was required, which should contain an electrophile and a protected amine. Amino alcohols are the ideal starting material for this purpose as they are less expensive and both functional groups can easily be distinguished for modification of the building block. The amino group in 3-aminopropanol was protected with Dde **191** in analogy to Scheme 27. Dde was chosen as it can quickly be removed with hydrazine hydrate. Likewise it was previously shown that it is compatible with the conditions of *Fukuyama-Mitsunobu* alkylation.

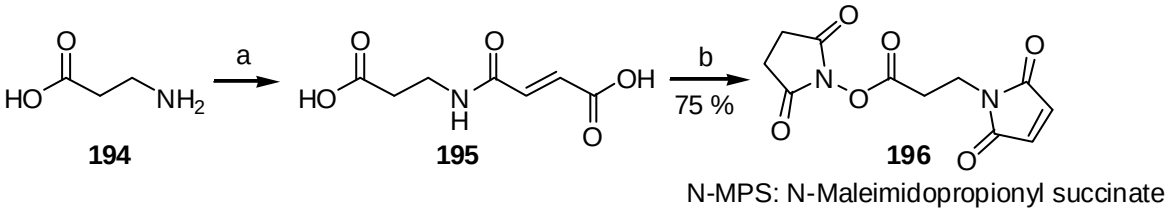


Scheme 29 Synthesis of an *N*-protected aminoiodide starting from dimedone. Reagents and conditions: (a) 1.5 Equiv. acetylchloride, 1.1 equiv. pyridine, $CHCl_3$, 30 min, r.t.; (b) 2 equiv. $AlCl_3$, $CHCl_3$, 1 h, r.t.; (c) 2 equiv. 3-aminopropanol, 1.2 equiv. DiPEA, MeOH, 6.5 h, r.t.; (d) 1.1 equiv. PPh_3, 1.2 equiv. I_2, 1.5 equiv. imidazole, CH_2Cl_2, 30 min, 0°C to r.t.[225]

2-Acetyldimedone **191** was prepared by acetylation of dimedone **189** with acetylchloride and subsequent rearrangement with $AlCl_3$ giving 51% yield over two steps (Scheme 29).[371] The Dde group **191** was reacted with an excess of 3-aminopropanol in presence of DiPEA resulting in protection of the amine of 3-aminopropanol in 78% yield.[225] Conversion of the hydroxyl group into the iodide was accomplished by a modified *Appel reaction* with PPh_3, I_2, and imidazole.[243] Finally, 82% yield in this step led to an overall yield of 33% for a four step synthesis of **193** starting from 2-acetyldimedone.[372]

4.1.1.4 Building block for maleimide introduction

For the introduction of a maleimide onto a polyamine, a building block **196** was prepared, which bears the carboxylic acid function pre-activated as NHS-ester (Scheme 30). This should prevent a potential side reactions between maleimide and amines, which can take place, if the peptide bond formation is slow. The building block is prepared by reaction of 3-aminopropioinic acid **(194)** with 1.2 equiv. of maleic anhydride, followed by 2.4 equiv. of DCC and 1.5 equiv. of NHS.[373, 374]



Scheme 30 Synthesis of the activated building block **196** for the introduction of maleimides. Reagents and conditions: (a) 1.2 Equiv. maleic anhydride, DMF, 120 min, r.t.; (b) 2.4 equiv. DCC, 1.5 equiv. *N*-hydroxysuccinimide, DMF, 150 min, 0 °C then 16 h, r.t.[374]

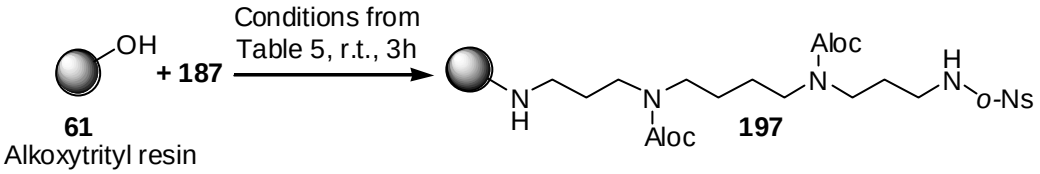
All building blocks were stable during storage at -20°C. In the case of building block **196**, moisture was avoided to avoid slow hydrolysis of the ester linkage. Due to the risk of oxidation and addition of carbon dioxide to the free amines, they were kept under argon during all reactions. The reproducible yields of alkylation reactions with the iodide (Table 7) indicate that it does not significantly suffer from hydrolysis under those conditions.

4.1.2 Reactions on solid phase

4.1.2.1 Optimisation of loading conditions

To find the optimal conditions for the attachment of building block **187** to a trityl resin, with respect to an easy recovery of **187,** the use of different bases, solvents, and excess of building block **187** was investigated. 100 mg of an alkoxytrityl resin **61** were activated with $SOCl_2$ and subsequently reacted under the conditions depicted in Table 5.

Table 5 Attachment of **187** to a Fukuyama-trityl resin.



Entry	Base	Solvent	Excess (equiv.)	Loading (mmol/g)[a]
1	-	CH_2Cl_2	10	0.240
2	Pyridine / CH_2Cl_2 (1:1)		10	-
3	DMAP (0.2 equiv)	Pyridine	10	0.012
4	Et_3N (5 equiv)	CH_2Cl_2	10	0.426
5	DiPEA (2.5 equiv)	CH_2Cl_2	5	0.355
6	DMAP (1 equiv)	CH_2Cl_2	5	0.351
7	Pyridine (2 equiv)	CH_2Cl_2	5	0.354
8	DiPEA (2.5 equiv)	CH_2Cl_2	10	0.424

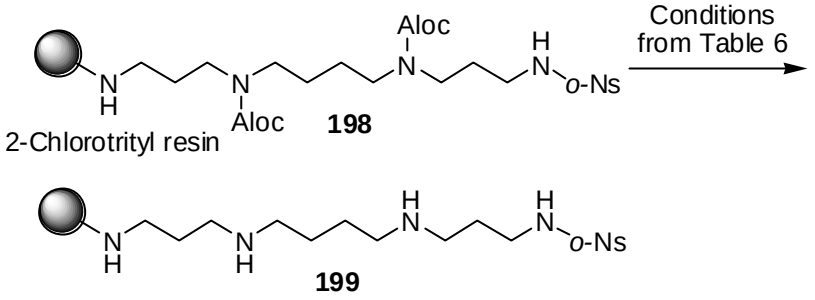
[a] Loadings were calculated by addition of 1,4-dioxane as a quantitative NMR standard.

10 equiv of building block **187** and 2.5 equiv DiPEA in dichloromethane provided the best loadings. The addition of a base seemed to be necessary to get feasible loadings within a short time. The choice of the base was of minor importance, although it should be mentioned that large excess of Et_3N and pyridine partially blocked the linker. Longer reaction times than 3 h did not produce significantly higher loadings. After loading of the resin, excess of building block **187** could easily be recovered by the removal of solvent and Hünig's base in vacuum or by extraction of DiPEA with diluted hydrochloric acid from the filtrate. Recovered building block **187** could be reused in further reactions.

4.1.2.2 Aloc deprotection

During deprotection of the Aloc-groups the formation of the stable allylamines was constantly observed (Table 6). This was probably due to the close proximity of the liberated amines, which could themselves act as allyl-scavenger during deprotection.[248]

Table 6 Aloc deprotection with different scavengers.[a]



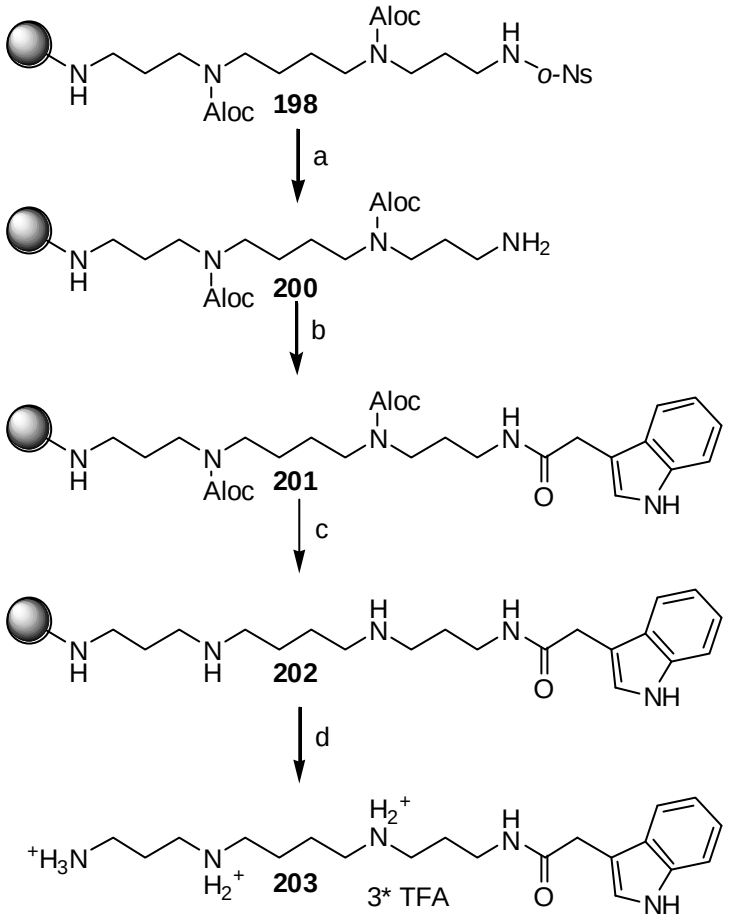
Scavenger	Excess of scavenger (equiv.)	Amount of $Pd(PPh_3)_4$ (mol%)	Reaction time	Deprotection in%[b]	Allylamine formation in%[b]
Pyrrolidine	10	50	20 h	> 99%	33
	20	20	16 h	> 99%	10
	20	5	21 h	27	0
	20	1	21 h	0	0
$PhSiH_3$	9	20	3 h	> 99%	20
	10	5	1.5 h	> 99%	12
	20	1	2 h	0	0
Me_3SiN_3	10	15	17 h	83	10
	8	10	17 h	68	1
Dimedone	10	15	17 h	4	0
	8	10	17 h	29	0
	8	10	0.5 h	0	0
DMBA	10	10	2 h / 35°C	99	< 1

[a] If not indicated, the reactions were carried out at room temperature.
[b] Amounts were calculated from the Aloc-corresponding integrals in the ^1H NMR spectra of the crude product relative to those in **187**. The β-amino / carbamoyl-methylene groups of the spermine backbone between 1.3 and 2.1 ppm were set to be 8.0.

Higher amounts of the Pd-catalyst as well as decreasing levels of scavenger enforced the allylamines formation, while in the *vice versa* situation the deprotection was incomplete even after long reaction times. Basic scavengers, hydride donors, and azides combined with a silylating agent did not sufficiently suppress the allylamine formation. From those results, it can be concluded that some scavengers, which are usually known to prevent allylamines formation during Aloc deprotection, are not efficient during their use with polyamines. Usually, the carbamate, which is liberated after transfer of the allyl moiety to the palladium catalyst, is directly converted to the respective silyl-carbamate in cases of phenylsilane or trimethylsilyl azide and allylation is avoided.[268] The consequent use of anhydrous conditions and application of high excess of scavenger, make it improbable that the side reaction simply arises from partial hydrolysis of the silyl-oxygen bond. It is more likely that allylamines formation derives from a spontaneous elimination of CO_2, which is known to proceed quicker on secondary amines than on primary amines, and a subsequent destruction of the proximate silyl-carbamates by the liberated secondary amines.[268] Indeed, dimedone reprotonated the deprotected amine to some extend and thereby avoided this problem but resulted in only poor deprotection even after 17 h. Usually, Rh- or Ni-catalysts are used to remove allylamines.[248] Due to their costs and the fact that heavy metal complexes are hard to separate from the resin, their use did not display an elegant solution of the problem. In solution phase, it was reported that allylamines can be removed at a slightly raised temperature in presence of Pd-catalyst using *N,N'*-dimethylbarbituric acid as scavenger (Table 6).[375] Finally, reaction with 10 equiv of scavenger and catalyst loading of 10 mol% for 2 h at 35°C resulted in full deprotection without allylamine formation. The *o*-Ns-protection remained unaffected under those conditions.

4.1.2.3 Versatility of the protection group strategy - synthesis of HO359b

To prove the versatility of the Aloc- / *o*-Nosyl protection strategy, resin **198** was used for the solid phase synthesis of philanthotoxin analogue HO359b **(203)** (Scheme 31), which so far has only been synthesized in solution phase.[376, 377] The *o*-Nosyl group was removed from **198** with β-mercaptoethanol / DBU. It was visible by the rapid yellow colouration of the suspension that the *o*-Nosyl deprotection proceeded quickly. 2-Indoleacetic acid was coupled to the free primary amine of **200** by peptide bond formation. Complete removal was accomplished either by triple treatment with 10 equiv. of ß-mercaptoethanol and 5 equiv. of DBU for 15 min or by treatment with 20 equiv. of both over night. Three methods for peptide bond formation were examined. Attempts with pivaloylchloride / DiPEA and PyBrOP / DiPEA for activation did not result in satisfactory coupling. In contrast, the use of DCC in combination with HOBt led to full acylation of all free primary amines, as monitored by the *Kaiser Test*. After Aloc-deprotection as described above (Table 6) and cleavage from the resin, HO359b **(203)** was obtained in 90% yield and 93% purity, demonstrating the suitability of this protection group strategy.

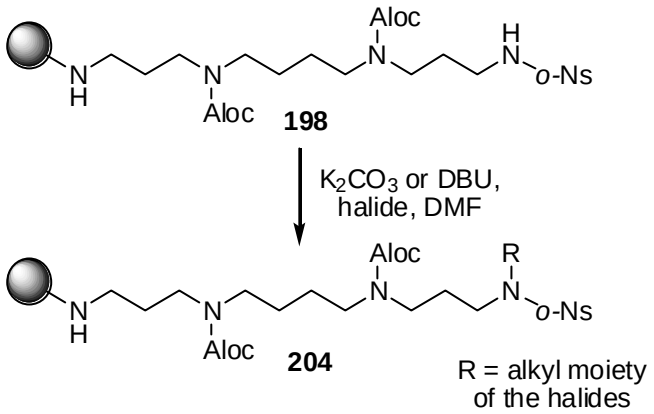


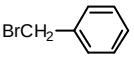
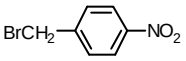
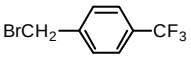
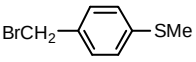
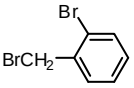
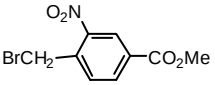
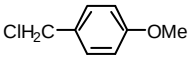
Scheme 31 Synthesis of philanthotoxin analogue HO359b. Reagents and conditions: (a) 10 Equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (b) 5 equiv. indoleacetic acid, 5 equiv. DCC, 5 equiv. HOBt, DMF, 16 h, r.t.; (c) *N,N´*-dimethylbarbituric acid (10 equiv), $Pd(PPh_3)_4$ (0.2 equiv), CH_2Cl_2, 35°C; (d) TFA (5%) / CH_2Cl_2.[324]

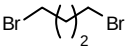
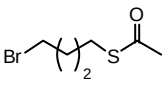
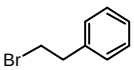
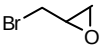
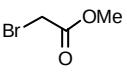
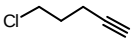
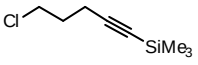
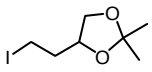
4.1.2.4 Investigation on *Fukuyama alkylation*

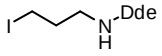
As mentioned above, there was one initial attempt to investigate conventional *Fukuyama alkylation*. The use of different solvents, leaving groups, bases, temperatures, and numbers of reaction cycles was investigated.[243] However, the suggested reaction conditions were only applied to simple alkyliodides and *N*-protected aminoiodides and thus, this gives no information on the tolerance of other functional groups. After all, the method requires 18 equiv. of the respective iodides. To confirm the versatility of the chosen protection group strategy with the conditions of *Fukuyama alkylation* and to explore the possibility to incorporate functional groups and structural elements into the polyamine skeleton, the reaction was investigated with various halides under two sets of conditions (Table 7). The first set was oriented on the original Fukuyama conditions (electrophile (20 equiv.), K_2CO_3 (20 equiv.), 60°C or room temperature, 18 h), which were milder but, due to the weak base, resulted in lower deprotonation and hence lower displacement rates. As the originally applied 20 equiv. could be critical for the use of valuable and expensive halides, the reaction was tested with 13 equiv. of either of them. The second set of conditions (electrophile (6 equiv), DBU (6 equiv), r.t., 3 – 5 h) gives higher deprotonation rates but provokes side reactions due to the stronger basicity of DBU.

Table 7 Conversion of halides in the *Fukuyama alkylation*.



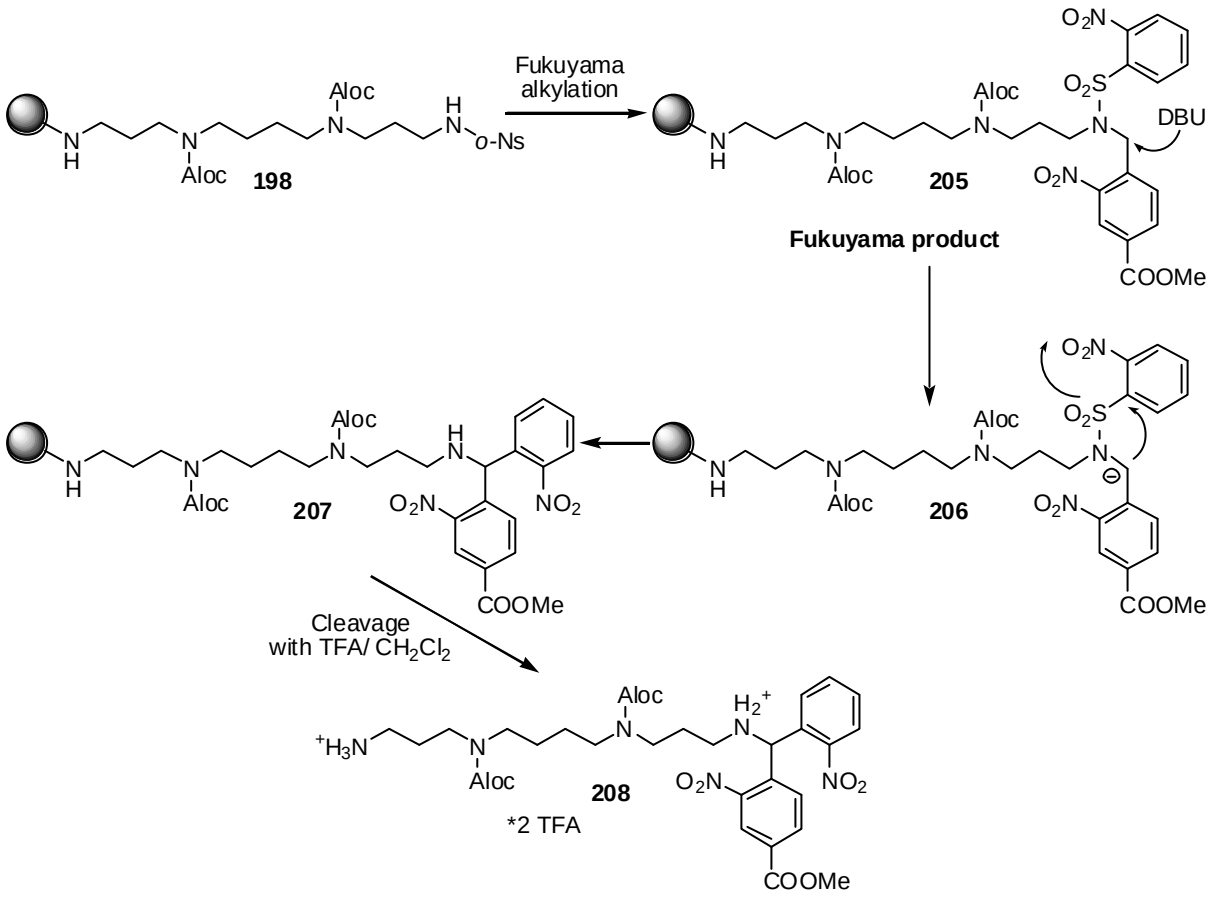
Entry	Halide	Base	Reaction time	Temperature	Conversion in%[a]
1		K_2CO_3	18 h	r.t.	20
		DBU	18 h	r.t.	98
		DBU	8 h	r.t.	100
		DBU	3 h	r.t.	97
2		K_2CO_3	10 h	60°C	30
		DBU	18 h	r.t.	80
		DBU	8 h	r.t.	59
3		DBU	30 h	r.t.	93
		DBU	5 h	r.t.	78
		DBU	3 h	r.t.	66
4		DBU	5 h	r.t.	95
		DBU	3 h	r.t.	84
5		DBU	5 h	r.t.	87
		DBU	3 h	r.t.	95
6		DBU	3 h	r.t.	37
7		DBU	3 h	r.t.	86

8		DBU	3 h	r.t.	97
9		DBU	3 h	r.t.	91
10		DBU	3 h	r.t.	92
11		K_2CO_3 DBU	18 h 3 h	r.t. r.t.	89 38
12		K_2CO_3 K_2CO_3 DBU	18 h 18 h 3 h	60°C r.t. r.t.	57[b] 68[b] 45[b]
13		K_2CO_3	8 h	60°C	93
14		K_2CO_3 K_2CO_3 DBU	8 h 18 h 3 h	60°C r.t. r.t.	97 48 2
15		K_2CO_3 DBU	18 h 3 h	r.t. r.t.	44 20
16		K_2CO_3 DBU	18 h 3 h	r.t. r.t.	75 22
17		K_2CO_3 K_2CO_3 DBU DBU	18 h 18 h 3 h 3 h	60°C r.t. r.t. r.t.	47 6 1 1
18		K_2CO_3 DBU DBU	18 h 3 h 3 h	r.t. r.t. r.t.	< 5 < 5 0
19		K_2CO_3 DBU	18 h 3 h	r.t. r.t.	86 35

20		K_2CO_3 DBU	18 h 3 h	60°C r.t.	98 3

[a] After cleavage from the resin, the conversions were determined by the RP-HPLC peak integration of the product peak in comparison to remaining educt and side products (UV-detection at 254 nm).
[b] The amount of 1,4-dibromobutane was raised to 20 equiv in order to minimize cross-linking of the resin. The yield of the cross-linked product was found in 17% (for K_2CO_3, 18 h, 60 °C), 14% (for K_2CO_3, 18 h, r.t.) and 20% yield (for DBU, 3 h, r.t.).

The results from Table 7 can be summarized as follows:
Benzyl- and allylhalides require DBU as a base. In the presence of K_2CO_3, the conversion was low in each case. Benzyl bromide shows the best result, while electron-rich are superior to electron-deficient benzyl compounds (Entries 1 – 10). In some cases, extension of the reaction time to five hours or more accomplished desirable conversions of 95% or more. In crucial examples (Entries 2 and 3), it should be possible to achieve nearly quantitative conversion by slightly raising the temperature or by repetitive treatment of the resin with 6 equiv. of DBU / halide. Finally, Entry 6 revealed no alkylation product according to Fukuyama. Instead, the formed Fukuyama-product **205** showed deprotonation at the benzyl position of the electron-deficient ring system. Subsequent nucleophilic addition in *ipso*-position of the *o*-Nosyl group and SO_2 elimination resulted in **208** (Scheme 32).



Scheme 32 Isolated product in Entry 6 of Table 7.

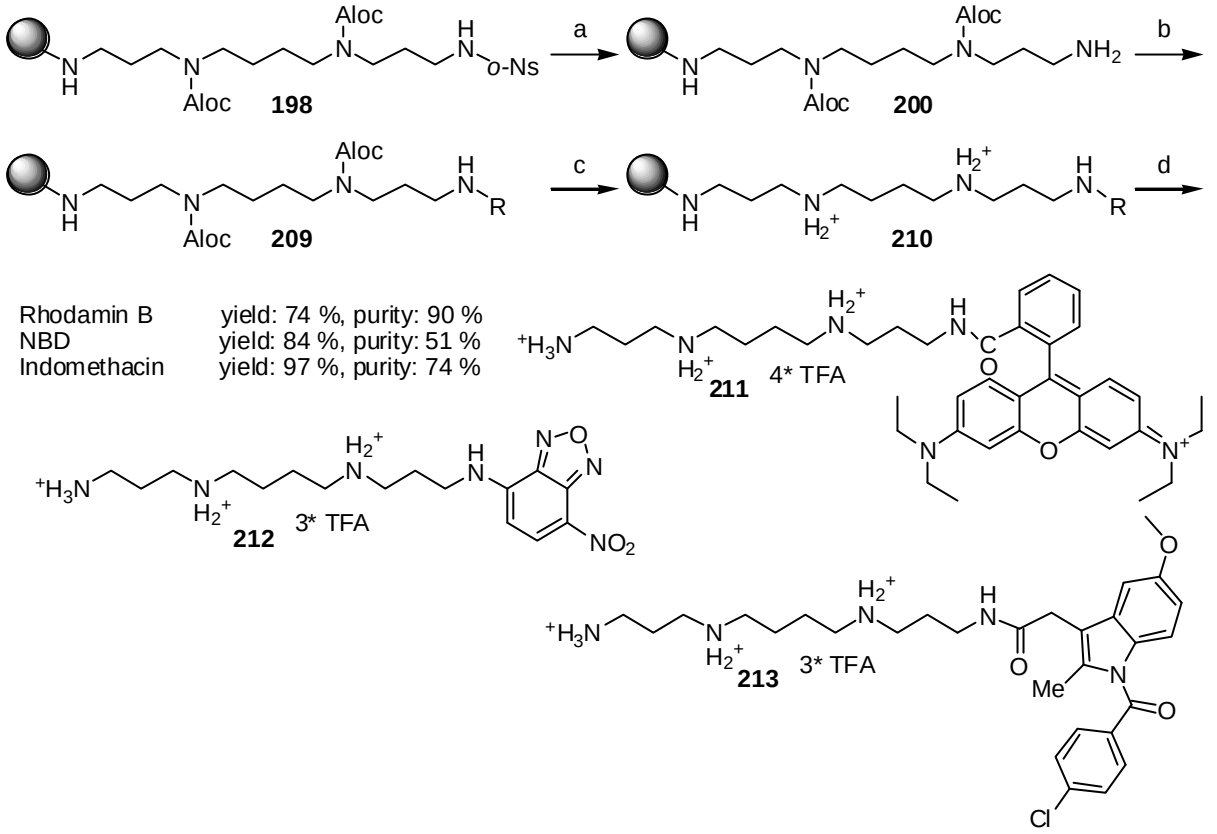
Most aliphatic halides produced low conversion rates with DBU (Entries 11 – 20), especially when the substrates were susceptible to β-elimination (Entry 14). In the presence of carbonate, the conversions were distinctly higher. However, raising the temperature was necessary to achieve higher conversion rates after one reaction cycle. While bromides and iodides reacted equally well (Entries 11 – 16, 19, 20), chlorides showed only poor conversions even after long reaction times or after addition of NaI (1 equiv) (Entries 17 and 18). As expected, the purities in the reaction with the symmetric dibromide (Entry 12) are diminished by the formation of a side product. This is due to the cross-linking reaction of both halides with two immobilized sulfonamides. Although this reaction was carried out with 20 equiv of the bromide, the amount of side product was still significant. In Entries 15 and 19 the products were obtained as the hydrolysed compounds. As no ether-formation was found, it could be concluded that hydrolysis of epoxide and acetal exclusively happened during cleavage from the resin and purification of the crude product. The reaction with building block **193** in Entry 20 proceeded very well showing that elongation of the backbone is efficiently accomplished with conventional *Fukuyama alkylation*. In contrast *Fukuyama-Mitsunobu alkylation* only afforded conversions of lower than 30% during elongation of backbones with comparable length.[263, 291]

4.2 Synthesis of polyamine conjugates

All syntheses started with the universal resin **198**. Variations in the synthetic schemes arose from different numbers of charges and length of the backbones of the final products as well as incompatibility of some deprotection conditions with the cargo molecules.

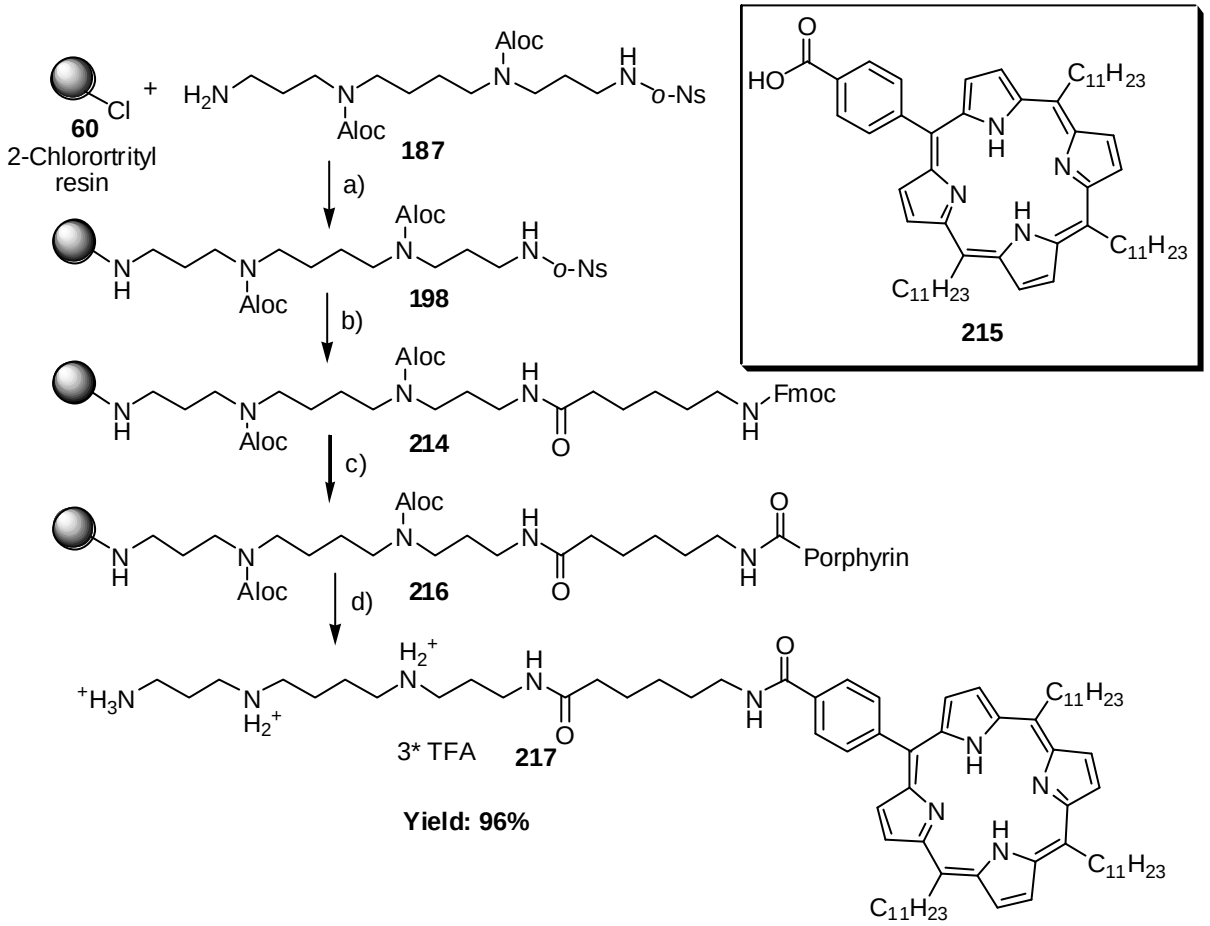
4.2.1 Synthesis of spermidine derivatives

All conjugates use spermine **(16)** as scaffold but some of them can be regarded as spermidine derivates as one terminal charge is depleted (Scheme 33).



Scheme 33 Synthesis of polyamine conjugates derived from spermidine **(15)**. Reagents and conditions: (a) 10 Equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 1 * 30 min + 2 * 15 min, r.t.; (b) 5 equiv. indomethacin or rhodamin B, 5 equiv. DCC, 5 equiv. HOBt, DMF, 16 h, r.t.; or 3.3 equiv. NBDCl, 1.7 equiv. K_2CO_3, DMF, 16 h, r.t.; (c) 10 equiv. *N*,*N*'-dimethylbarbituric acid, 20 mol% $Pd(PPh_3)_4$, CH_2Cl_2, 6- 8 h, 40 °C; (d) 10% TFA in CH_2Cl_2, 3 * 1 min, r.t.

In each case, resin **198** was *o*-Nosyl group deprotected and the respective effector molecule was coupled to the primary amine. After Aloc deprotection, the products were cleaved from the resin and purified by preparative RP-HPLC.



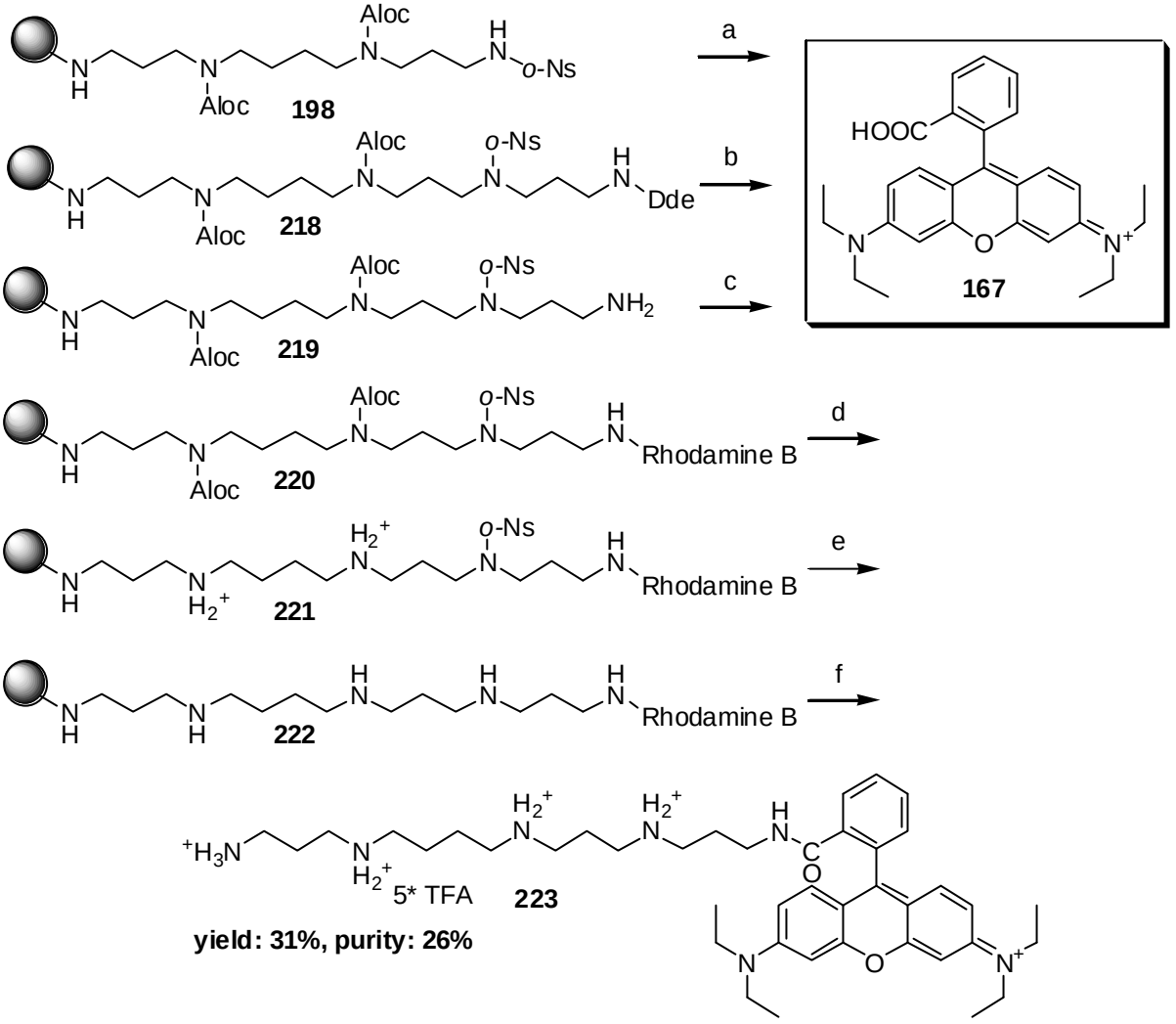
Scheme 34 Synthesis of a polyamino-porphyrin conjugate with C_6-spacer. Compound **215** was kindly provided by the group of Prof. Dr. T.S. Balaban. Reagents and conditions: (a) 20 Equiv. β-mercaptoethanol, 20 equiv. DBU, DMF, 20 h, r.t.; (b) 3 equiv. *N*-Fmoc-6-aminocaproic acid, 2 equiv. PyBrOP, 4 equiv. DiPEA, DMF, 20 h, r.t. (c) pyrrolidine / DMF (1:4 (v / v)), 3 * 2 min, r.t.; (d) 2 equiv. carboxyporphyrin **215**, 3.4 equiv. HOBt, 5 equiv. DCC, CH_2Cl_2 / DMF (1:1, (v / v)), 5d, r.t.; (e) 10 equiv. *N*,*N'*-dimethylbarbituric acid, 20 mol% $Pd(PPh_3)_4$, CH_2Cl_2, 3 h, 40 °C; (f) 10% TFA in CH_2Cl_2, 3 * 1 min, r.t.[200]

For the synthesis of the polyamine-porphyrin conjugate **217**, two additional steps were added. In those caproic acid, which acted as a C_6-spacer between hydrophilic polyamine and hydrophobic porphyrin, was inserted (Scheme 34). The carboxyporphyrin was coupled to the Fmoc deprotected amine by reaction with DCC / HOBt and the resin was extensively washed, until clearance of the filtrate, to remove any remains of the bulky, hydrophobic porphyrin. After Aloc deprotection and an extensive washing of the resin for the complete removal of the Pd-catalyst, the compound was cleaved from the resin and the solvents were removed. As all non-polar compounds were already separated from the resin, the polar truncated fragments were removed by partitioning between an aqueous solution of K_2CO_3 (pH = 10) and $CHCl_3$. Repetitive washings with aqueous solutions removed all polar compounds, while the large hydrophobic moiety guarantees that the product stays in the organic layer (visualized by its strong green fluorescence). After removal of the solvents and drying under HV, a dark solid was obtained in 96% yield and high purity as determined by ^{1}H NMR spectroscopy. The high hydrophobicity of the undecanyl chains of **217** prevented HPLC chromatography. (see chapter 2.2.6)

Taken together, in all four cases the cargo molecules were stable to the mild conditions of the Aloc deprotection. This showed that the Aloc group is suitable for the permanent protection of secondary amines during synthesis of sensitive conjugates.

4.2.2 Synthesis of spermine conjugates

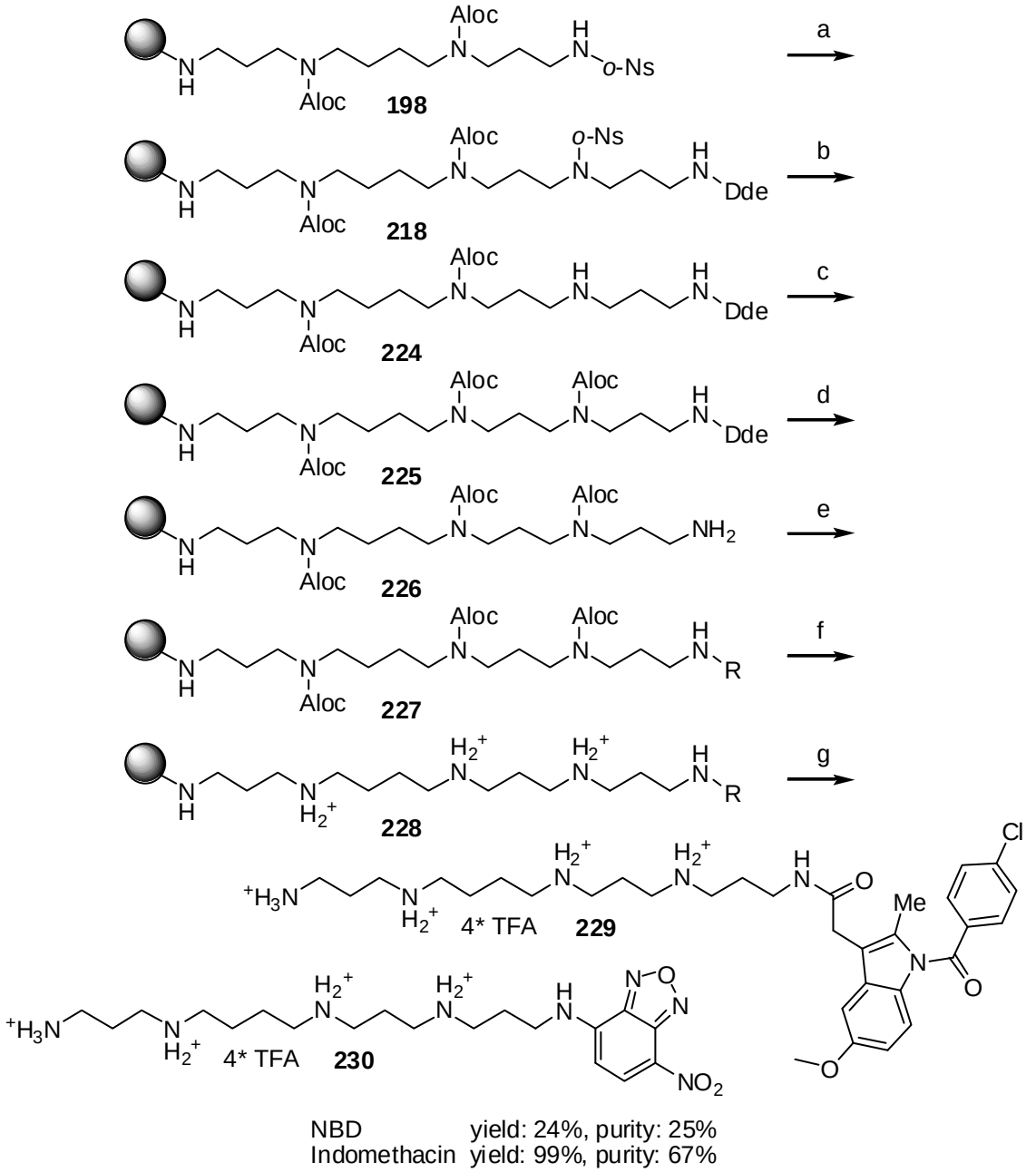
The synthesis of spermine derived conjugates with four positive charges required one elongation step that incorporated one additional amino group into the backbone.



Scheme 35 Synthesis of the spermine-rhodamine conjugate. Reagents and conditions: (a) 13 Equiv. **193**, 13 equiv. K_2CO_3, DMF, 18 h, 60 °C; (b) 10 equiv. $N_2H_4*H_2O$, 100 equiv. allylalcohol, DMF, 3 * 15 min, r.t.; (c) 5 equiv. rhodamine B, 5 equiv. DCC, 5 equiv. HOBt, DMF, 16 h, r.t.; (d) dimethylbarbituric acid (10 equiv), $Pd(PPh_3)_4$ (0.2 equiv), CH_2Cl_2, 35 °C; (d) TFA (5%) / CH_2Cl_2; (e) 10 equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (f) TFA (5%) / CH_2Cl_2.

For the rhodamine derivative, the synthesis started with resin **198**, which is alkylated with building block **193** according to Entry 20 of the study on *Fukuyama alkylation* (Table 7). The resulting resin **218**, bearing three amino protection groups was used for investigation on their orthogonality. After exposure to the respective removal conditions, the products were cleaved from the resin and analyzed by ^{1}H NMR spectroscopy. The results indicated that all three protection groups are orthogonal. Dde was removed and rhodamine B **167** was coupled to the free primary amine, visualized by the strong pink colour of the resin. The remaining protection groups were removed in the order Aloc- and then *o*-Nosyl-group to avoid a potential formation of allylamines. The product was cleaved from the resin and purified by RH-HPLC. The whole synthesis was carried out in 31% yield and with 26% purity after cleavage from the resin. If the synthesis is carried out with NBD- or indomethacin instead of

rhodamine B, no products were obtained. It was concluded that the cargo molecules are unstable to the deprotection conditions of the *o*-Nosyl group. An S_NAr reaction of the sulfide analogously to the *o*-Nosyl removal or in case of NBD a reduction of the nitro function seems to be plausible, as all three molecules contain electron deficient aryl systems.



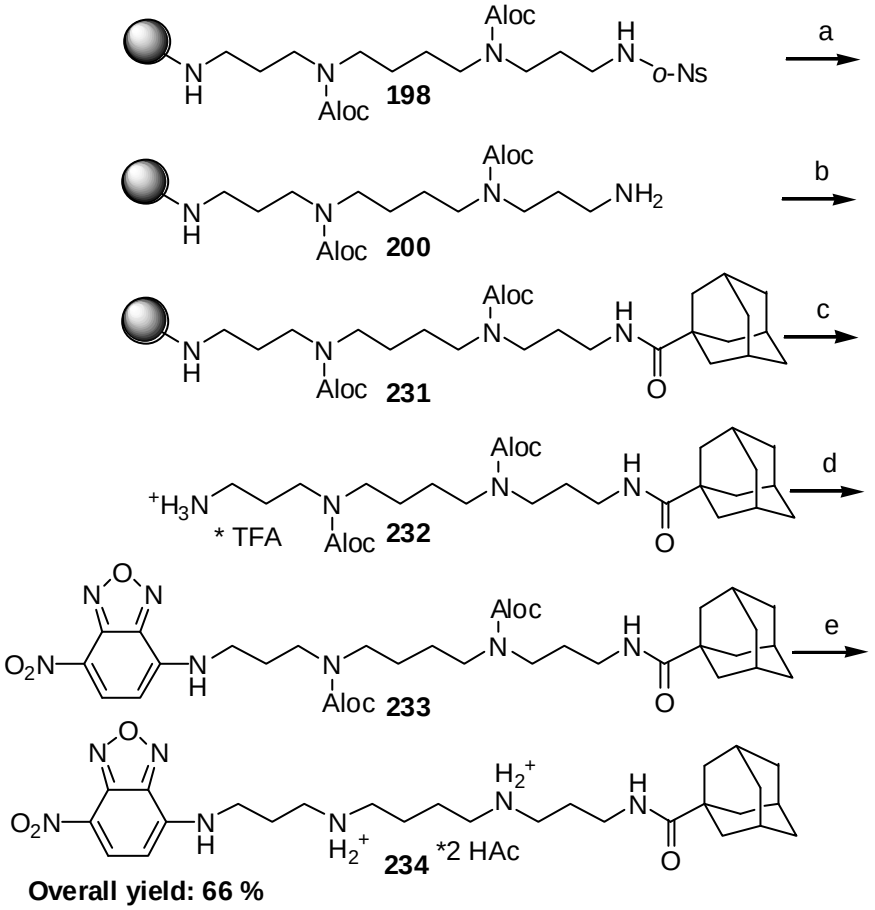
Scheme 36 Synthesis of spermine conjugates with NBD and Indomethacin. Reagents and conditions: (a) 13 Equiv. **193**, 13 equiv. K_2CO_3, DMF, 18 h, 60 °C; (b) 10 equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (c) 5 equiv. allyloxycarbonyl chloride, 5 equiv. Et_3N, CH_2Cl_2, 16 h, r.t.; (d) 10 equiv. N_2H_4*H_2O, 100 equiv. allylalcohol, DMF, 3 * 15 min, r.t.; (e) 5 equiv. Indomethacin, 5 equiv. DCC, 5 equiv. HOBt, DMF, 16 h, r.t.; or 4 equiv. NBDCl, 4 equiv. DiPEA, THF, 16 h, r.t.; (f) dimethylbarbituric acid (10 equiv), $Pd(PPh_3)_4$ (0.2 equiv), CH_2Cl_2, 35 °C; (g) TFA (5%) / CH_2Cl_2.

The syntheses of the NBD- and indomethacin conjugates were therefore modified by the exchange of the *o*-Nosyl- against Aloc-group previous to Dde removal and coupling (Scheme 36). After coupling, the Aloc groups were smoothly removed and the product was cleaved from the resin. Purification by RP-HPLC resulted in 24% yield in case of NBD derivative and in 99% yield in case of the indomethacin derivative. The purities of the crude compounds

after cleavage from the resin were 25% for the NBD derivative and 67% for the indomethacin derivative. After this, conjugates were evaluated in biological tests (see chapter 4.4).

4.2.3 Synthesis of lipophilic putrescine conjugates

As mentioned in the introduction (see 2.2.4.5), it is known that lipophilic modifications on cationic moieties improve cellular uptake.[8, 92] Thus, conjugates with lipophilic building blocks were synthesized. These modifications were thought to improve cellular uptake by tighter anchoring into the membrane.[378-380] As preliminary evaluations of the NBD-conjugates **212** and **230** showed good uptake (see chapter 4.4.1), it was reasoned that the number of positive charges could be reduced to two per molecule. Finally, the products are named putrescine conjugates. In contrast to the conjugates without lipophilic modifications, the syntheses of those higher substituted polyamines could not be entirely executed on solid phase. Due to the instability of NBD to various reaction conditions and light, the polyamines were usually first derivatised with the respective modification and finally labelled with NBD after cleavage from the resin. The higher substitution of the conjugates mostly led to more complex syntheses than in the case of unmodified polyamine backbones.

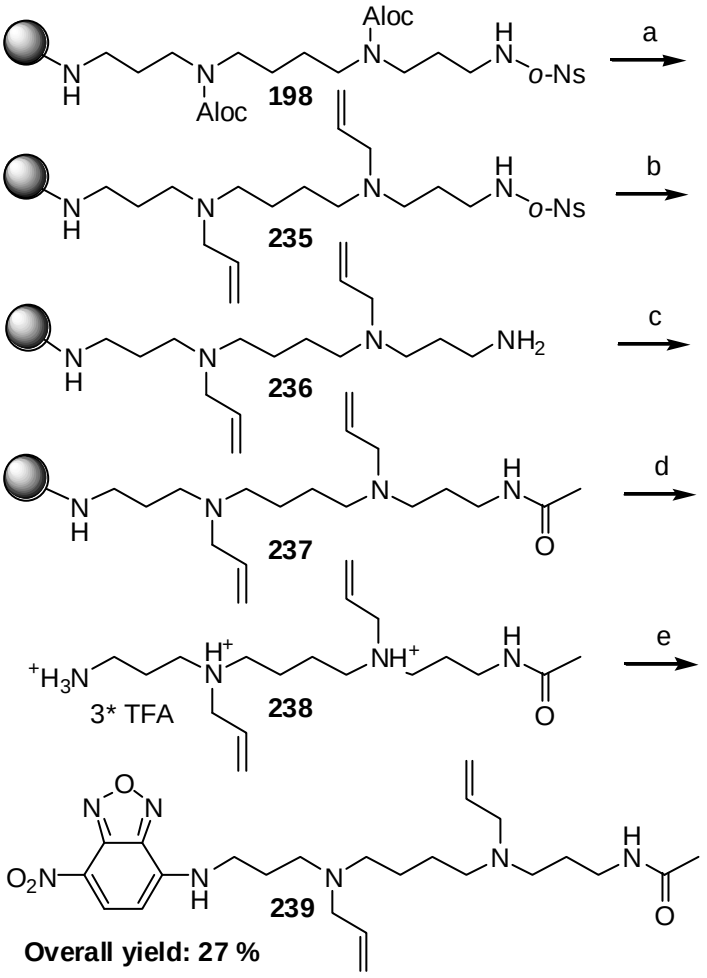


Scheme 37 Synthesis of a conjugate with two charges and terminal lipophilic substitution. Reagents and conditions: (a) 10 Equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (b) 8 equiv. 1-adamantyl carboxylic acid, 8.2 equiv. DCC, 5.1 equiv. NHS, DMF / THF, r.t.; (c) 10% TFA in CH_2Cl_2; (d) 2.9 equiv. NBDCl, 3 equiv. K_2CO_3, THF, 18 h, r.t.; (e) 9.7 equiv. dimethylbarbituric acid, 10 mol% $Pd(PPh_3)_4$, CH_2Cl_2, 2.5 h, 40 °C.

The conjugate from Scheme 37 has an amphiphilic structure in which the hydrophobic and the hydrophilic parts are separated. The synthesis comprises of two solid- and two solution phase steps (Scheme 37). Resin **198** was de-nosylated and an adamantyl-moiety was

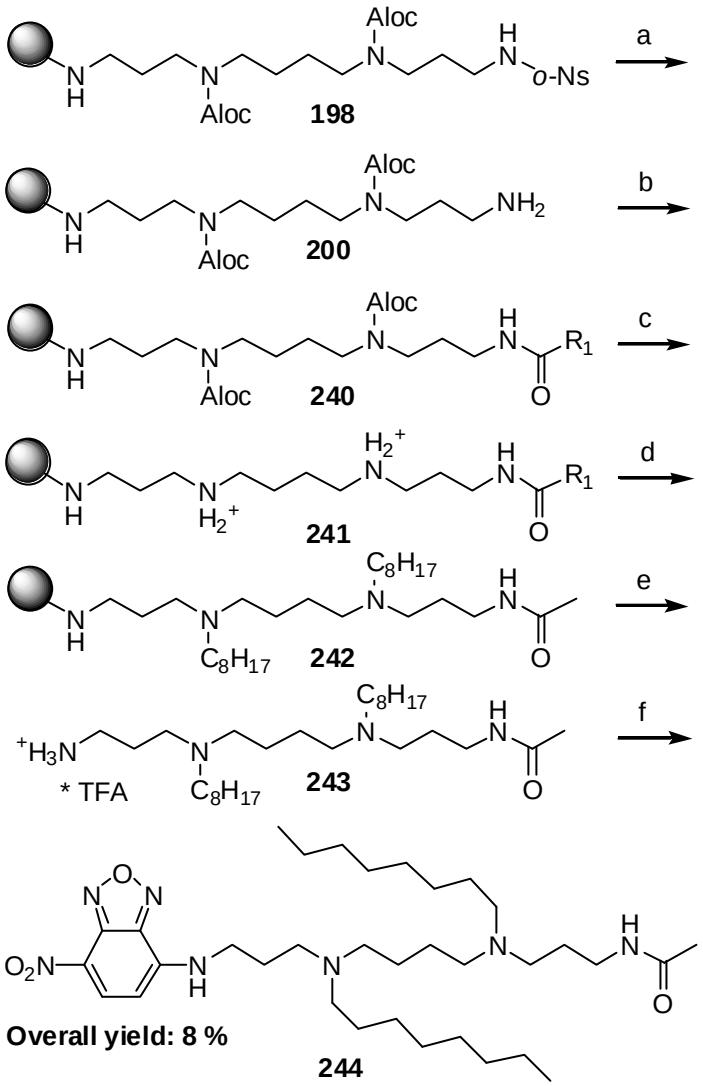
introduced by peptide bond formation using DCC / HOBt / adamantyl carboxylic acid. Qualitative monitoring of the reaction by the *Kaiser Test* indicated completion of the reaction. After cleavage from the resin with 10% TFA in dichloromethane, the liberated amine was labelled with NBD by reaction with excess of NBDCl and K_2CO_3. After workup, Aloc was removed with $Pd(PPh_3)_4$ / *N*,*N´*-DMBA. After purification, the bis-acetic acid salt of (4-{3-[(Adamantane-1-carbonyl)-amino]-propylamino}-butyl)-[3-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-propyl]-amine (**234**) was obtained in 66% yield.

The conjugate **239** shows increased lipophilicity in the backbone, which derived from a substitution of the secondary amines with allyl groups and acetylation of the terminal amino group of **236** (Scheme 38). The introduction of the allyl groups was obtained by transallylation from Aloc-groups to allylamines. It was accomplished by treatment of the resin **198** with 1 equiv. of Pd-catalyst and 0.3 equiv. of allyliodide, in the absence of an allyl-scavenger. The higher amount of $Pd(PPh_3)_4$ was necessary to accelerate the reaction to completion over night. After removal of the *o*-Nosyl group, the free amine was acylated with acetic anhydride and Et_3N. After cleavage from the resin, the primary amine was labelled with NBD. Purification by RP-HPLC afforded the pure bis-TFA salt of N^1-Acetyl-N^5,N^{10}-bis-allyl-N^{12}-NBD-4,9,12-tetraazatetradecane (**239**) in 27% yield.



Scheme 38 Synthesis of a conjugate with two charges and allyl side chain modifications. Reagents and conditions: (a) 1 Equiv. $Pd(PPh_3)_4$, 0.3 equiv. allyliodide, CH_2Cl_2, 16 h, r.t.; (b) 10 equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (c) 4 equiv. Ac_2O, 4 equiv. Et_3N, DMF, 16 h, r.t. (d) 10% TFA in CH_2Cl_2, r.t.; (e) 1.5 equiv. NBDCl, 2 equiv. K_2CO_3, THF, 18 h, r.t.

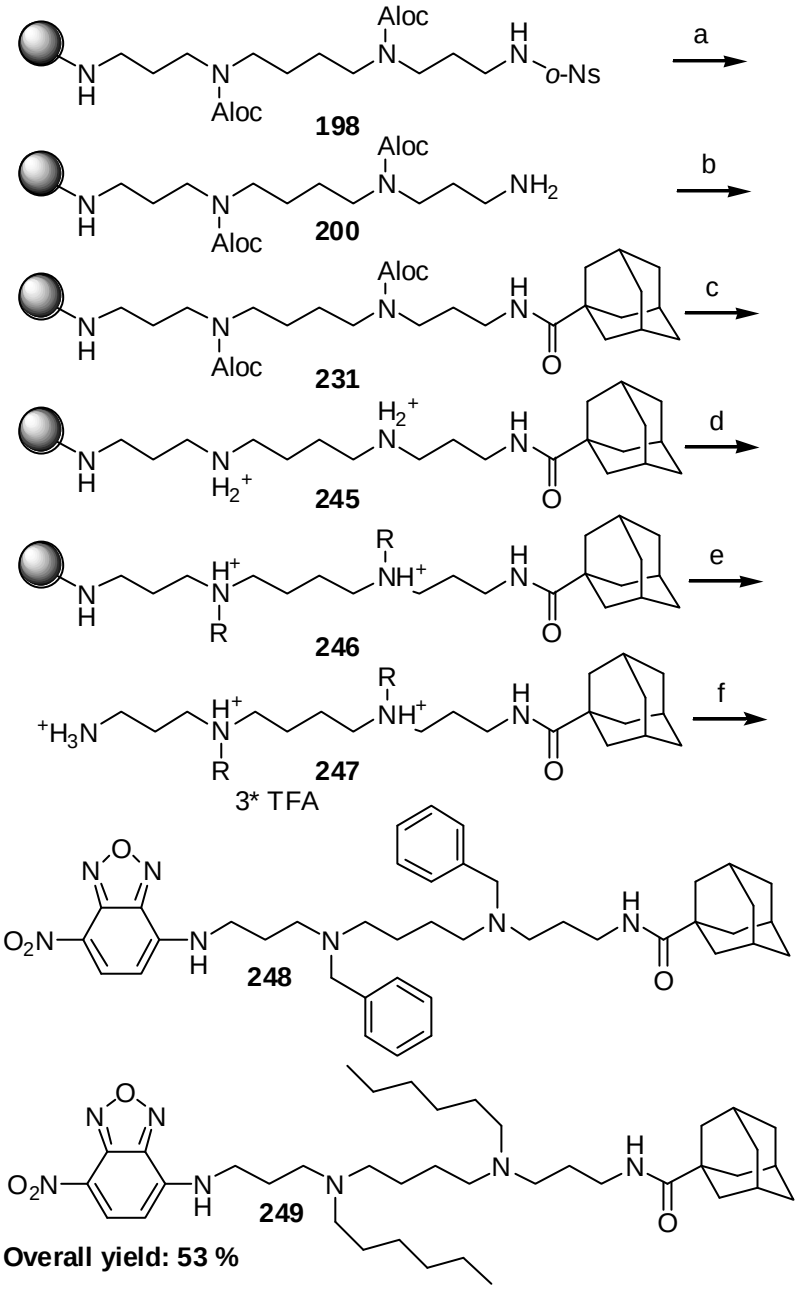
Expansion of the side chain modifications to octyl-chains is shown in Scheme 39. The *o*-Nosyl group was removed from **198** and the amine was acylated. After removal of the Aloc groups, the backbone amines were alkylated by S_N2 reaction with octylbromide and K_2CO_3 at elevated temperature. The reaction was qualitatively followed by the *Kaiser test*. After cleavage from the resin, **243** was labelled with NBD to give N^1-Acetyl-N^5,N^{10}-bis-octyl-N^{12}-NBD-4,9,12-tetraazatetradecane **(244)** in 8 % yield. The low overall yield was referred to the loss during the terminal step (see 4.2.4).



Scheme 39 Synthesis of a conjugate with two charges and octyl side chain modifications. Reagents and conditions: (a) 10 Equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (b) 5 equiv. acetic acid, 5 equiv. DCC, 5 equiv. NHS, DMF, 16 h, r.t.; (c) 10 equiv. dimethylbarbituric acid, 0.2 equiv. $Pd(PPh_3)_4$, CH_2Cl_2, 20 h, 35 °C; (d) 20 equiv. 1-octylbromide, 20 equiv. K_2CO_3, DMF, 72 h, 50 °C; (e) 10% TFA in CH_2Cl_2, r.t.; (f) 4 equiv. NBDCl, 7 equiv. K_2CO_3, THF, 18 h, r.t.

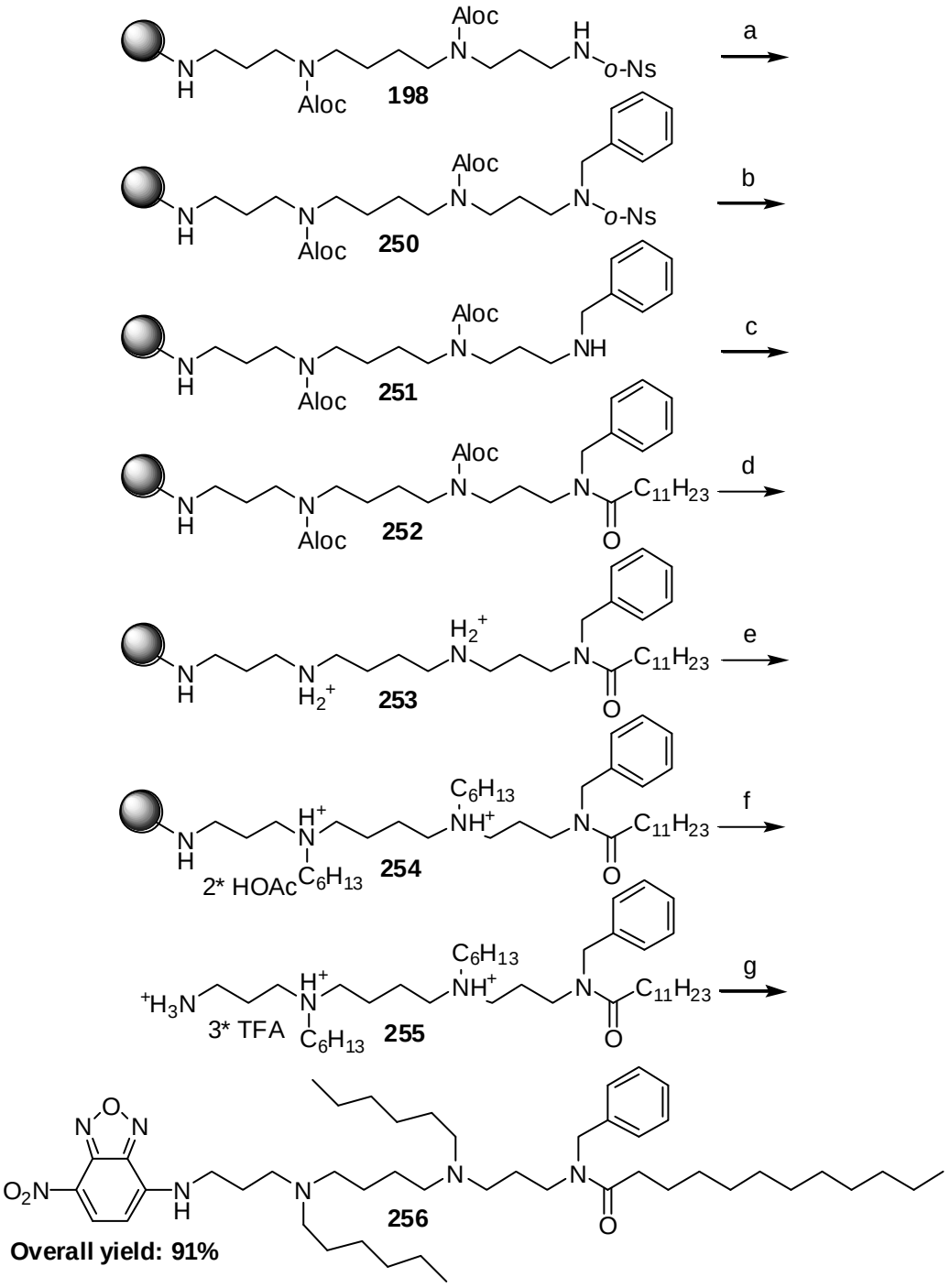
The synthesis of conjugates with both, larger head groups and side chain modifications is shown in Scheme 40. After deprotection of the primary amine, **200** is expediently acylated with adamantyl carboxylic acid / DCC / NHS. The secondary amines in **231** were liberated and alkylated by *Reductive amination* with benzaldehyde / capronaldehyde as described above until the *Kaiser test* was negative. After cleavage from the resin, the free amine was labelled with NBDCl and K_2CO_3 in THF. After purification, N^1-adamantylcarbonyl-N^5,N^{10}-bis-hexyl-

N^{12}-NBD-4,9,12-tetraazatetradecane **(249)** was obtained in 53% yield. Only traces of N^{1}-Acetyl-N^{5},N^{10}-bis-benzyl-N^{12}-NBD-4,9,12-tetraazatetradecane **(248)** were found in the crude mixture.



Scheme 40 Synthesis of conjugates with bulky, terminal adamantyl group and different side chain modifications. Both modifications were introduced by *Reductive amination*. Reagents and conditions: (a) 10 Equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (b) 5 equiv. adamantyl carboxylic acid, 5 equiv. DCC, 5 equiv. NHS, DMF, 16 h, r.t.; (c) 10 equiv. dimethylbarbituric acid, 0.2 equiv. $Pd(PPh_3)_4$, CH_2Cl_2, 20 h, 35 °C; (d) (10 equiv. benzylbromide, 10 equiv. K_2CO_3, DMF, over night, 40 °C then 6 equiv. benzaldehyde, 6 equiv. $Bu_4N(BH_4)$, 3% HOAc in DMF, 2 * 30 min 1 * over night) for **248**, (40 °C; 6 equiv. capronaldehyde, 6 equiv. $Bu_4N(BH_4)$, 3% HOAc in DMF, 2 * 30 min 1 * over night, 40 °C) for **249**; (e) 10% TFA in CH_2Cl_2, r.t.; (f) (4 equiv. NBDCl, 7 equiv. K_2CO_3, THF, 17 h, r.t.) for **248**, (3 equiv. NBDCl, 4 equiv. K_2CO_3, THF, 17 h, r.t.) for **249**.

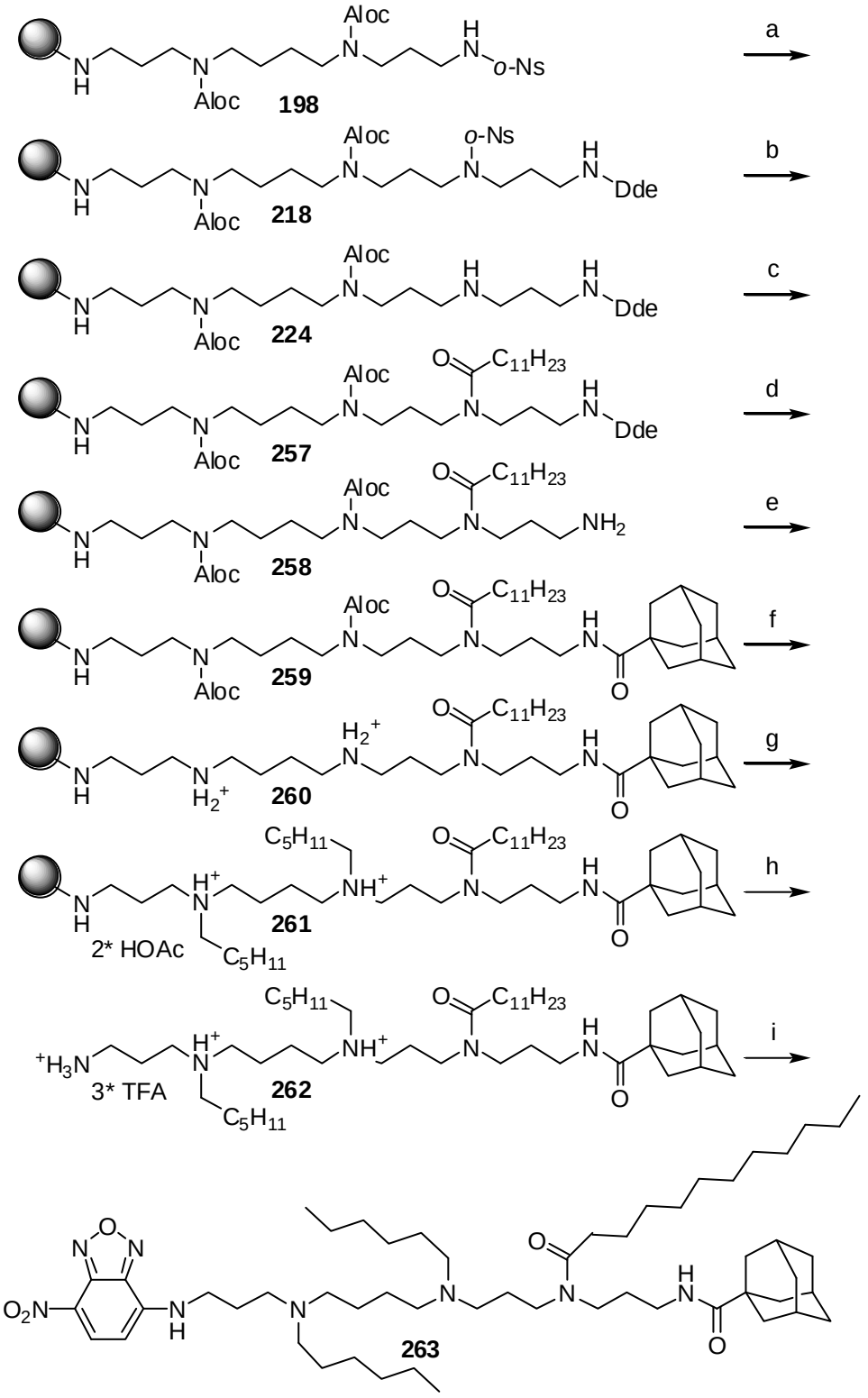
Syntheses of conjugates with expanded lipophilic tail were also accomplished (Scheme 41). According to 4.1.2.4, the resin **198** was exhaustively alkylated by *Fukuyama alkylation* with benzylbromide. After removal of the *o*-Nosyl group, the liberated amine was acylated with lauroyl chloride / pyridine. Monitoring by the *Kaiser Test* showed that the reaction was completed after 18 h, which suggests that the proximity to the bulky trityl linker was the major obstacle for the incomplete acylation of the N^4-amino group in some cases (see chapter 4.2.4).



Scheme 41 Synthesis of a conjugate with expanded lipophilic tail and side chain modifications. Reagents and conditions: (a) 20 Equiv. benzylbromide, 20 equiv. DBU, DMF, 16 h, r.t.; (b) 10 equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (c) 20 equiv. lauroyl chloride, pyridine / CH_2Cl_2 (1:2), 18 h, r.t.; (d) dimethylbarbituric acid (10 equiv), $Pd(PPh_3)_4$ (0.2 equiv), CH_2Cl_2, 20 h, 35 °C; (e) 6 equiv. capronaldehyde, 3% HOAc in DMF, 40 °C, 3 min, then 6 equiv. NBu_4BH_4, 2 * 30 min, 1 * 16 h; (f) 10% TFA in CH_2Cl_2; (g) 3 equiv. NBDCl, 4 equiv. K_2CO_3, THF, 17 h, r.t.

The Aloc groups were removed and the free amines were exhaustively alkylated by *Reductive amination* with capronaldehyde. After cleavage from the resin, the primary amine was labelled with NBD and the product was purified by flash chromatography on silica gel. After treatment with HOAc in $CHCl_3$, pure bis-acetic acid salt of ((*N*-benzyl)-N^1-docecanamidyl)-N^4,N^9-bis-hexyl-12-NBD-4,9-diazadodecane **256** was obtained in 91% yield.

Further expansion of the hydrophobic moiety was accomplished by substitution of the benzyl- by the bulkier adamantyl group, which was connected *via* a short linker to provide some flexibility to the end group (Scheme 42). Resin **198** was alkylated with building block **193**. After *o*-Nosyl deprotection, N^{13} of **224** was selectively acylated with lauroyl chloride in pyridine / dichloromethane. The *Kaiser Test* showed that the branching proceeded quantitatively. The primary amine of **257** was liberated by short treatment with hydrazine hydrate in DMF in the presence of a large excess of allylalcohol. The peptide bond formation with adamantyl carboxylic acid / DCC / NHS in DMF / THF proceeded quantitatively as indicated by the *Kaiser Test*. After Aloc deprotection, the internal secondary amines were alkylated by *Reductive amination* with *n*-hexanal. After cleavage from the resin the remaining primary amine of **262** was labelled with NBDCl and K_2CO_3 in THF. After workup and flash chromatography, N^1-adamantylcarbonyl-N^4-docecanoyl-N^8,N^{13}-bis-hexyl-N^{17}-NBD-1,4,8,13,17-pentaazaoctadecane **(263)** was converted into its bis-acetate salt by treatment with 5% HOAc in $CHCl_3$ for 10 min. The product was obtained in 89% yield.



Scheme 42 Synthesis of a conjugate with expanded hydrophobic tail and unpolar side chain modifications. Reagents and conditions: (a) 13 Equiv. **193**, 13 equiv. K_2CO_3, DMF, 18 h, 60 °C; (b) 10 equiv. β-mercaptoethanol, 5 equiv. DBU, DMF, 3 * 30 min, r.t.; (c) 20 equiv. lauroyl chloride, pyridine / CH_2Cl_2 (1 : 2), 18 h, r.t.; (d) 10 equiv. $N_2H_4*H_2O$, 100 equiv. allylalcohol, DMF, 3 * 15 min, r.t.; (e) 5 equiv. 1-adamantyl carboxylic acid, 5equiv. DCC, 5 equiv. HOBt, DMF / THF (1:1), 20 h, r.t.; (f) dimethylbarbituric acid (10 equiv), $Pd(PPh_3)_4$ (0.2 equiv), CH_2Cl_2, 20 h, 35 °C; (g) 10 equiv. $C_{13}H_{27}CHO$, 3% HOAc in DMF, 40 °C, 3 min, then 10 equiv. NBu_4BH_4, 2 * 30 min, 1 * 16 h; (h) TFA (5%) / CH_2Cl_2; (i) 4.7 equiv. NBDCl, 4.7 equiv. Et_3N, THF, 16 h, r.t.

4.2.4 Experimental findings

Efforts were undertaken to synthesize a large number of conjugates with modifications at the terminus or the backbone amines (Figure 32). In groups **a**, **b**, **c**, and **e** the number of the protonable amines in the backbone was limited to two. The terminal amine was acylated to prevent protonation. The groups **a** to **e** differ in the size of this hydrophobic tail group, opposite to the NBD group. Inside the groups, the conjugates differ in the lipophilicity of their backbone modifications. Group **d (271 – 275)** displays longer backbones. Although these syntheses were not successful, valuable synthetic information, especially about the possibilities of backbone modification, was obtained. Finally, this enabled the synthesis of the lipophilic putrescine conjugates. The sources for the failure of those syntheses are differing.

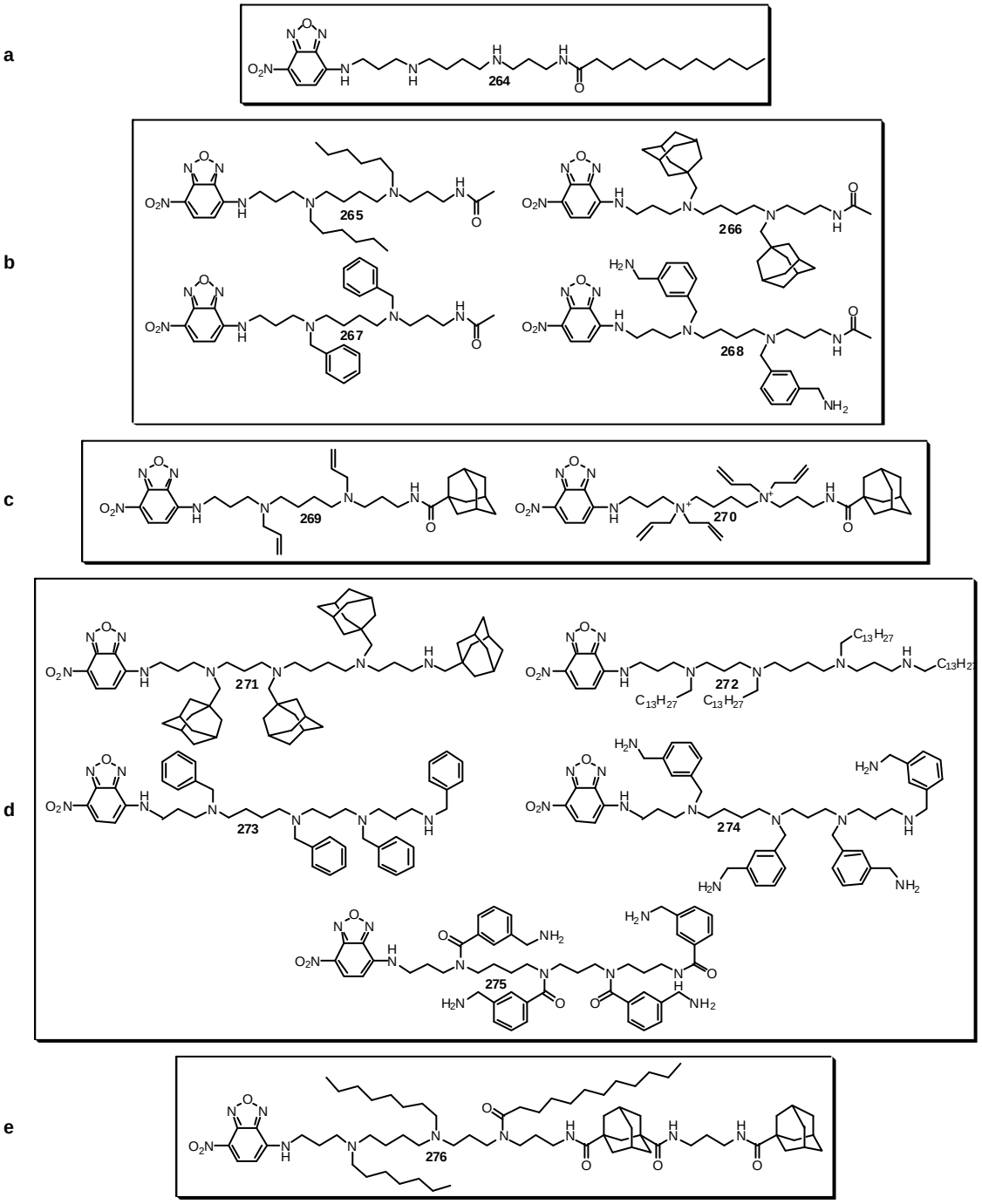


Figure 32 Structures of products of attempted syntheses. The compounds are displayed in groups of similar substitution patterns.

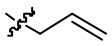
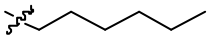
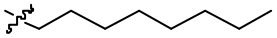
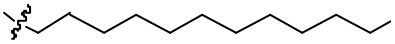
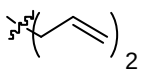
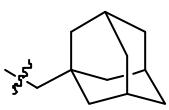
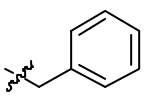
4.2.4.1 NBD-labelling

A problem, which could be easily prevented during further syntheses, was the unreliability of the NBD labelling. This was crucial in the syntheses of conjugates **264, 265**, **269**, **270**, and **273**. Therein, the identity of the precursors was correct. However, no final product and only traces of the educt could be isolated after the labelling step. Recovery of the precursor was also not possible. The initial protocol used NBDCl **(168)** together with K_2CO_3 in THF. However, this procedure did only work during some the syntheses. Often a compound was isolated, which showed only two aromatic protons in the ^1H NMR spectra. Unlike NBDCl **(168)**, it was insoluble in $CDCl_3$ and soluble in polar solvents like MeOH or water. It was assumed that the NBDCl **(168)** is either largely hydrolyzed or reacted with the high excess of K_2CO_3 to form this polar compound. Alternatively, the use of Et_3N in DMF showed to be more reliable.

4.2.4.2 Alkylation of secondary amines

A general synthetic problem was the modification of the secondary amines in the backbone. The established method of *Fukuyama alkylation* was not suitable as it is restricted to the formation of secondary amines. Remaining approaches from the set of alkylation methods were *S_N2-alkylation*, *Reductive amination* and *Amide reduction*. All three methods were applied, but the outcome differed strongly. Table 8 shows some representative moieties and the investigated methods for their introduction.

Table 8 Methods and conditions for the modification of secondary amines

Modification	Alkylation Method
	10 Equiv. allyliodide, 10 equiv. K_2CO_3, DMF, 16 h, r.t.
	6 / 10 Equiv. capronaldehyde, 3% HOAc in DMF, 40 °C, 3 min, then 6 / 10 equiv. NBu_4BH_4; 2 * 30 min, 1 * over night
	20 Equiv. bromooctane, 20 equiv. K_2CO_3, DMF, 3 d, 50 °C
	20 Equiv. lauroyl chloride, pyridine / CH_2Cl_2 (1 : 2), r.t.; 1 M BH_3·THF, 16 h, 60 °C, then pyrrolidine, 16 h, 60 °C
	20 Equiv. allyliodide, 20 equiv. K_2CO_3, DMF, 16 h, 40 °C
	4 Equiv. 1-adamantyl carboxylic acid, 4 equiv. PyBrOP, 4 equiv. DiPEA, DMF / THF (1 : 1), 20 h, r.t.; 1 M BH_3·THF, 16 h, 60 °C, then pyrrolidine, 16 h, 60 °C
	20 Equiv. benzylbromide, 20 equiv. K_2CO_3, DMF, 16 h, 40 °C; 10 Equiv. benzaldehyde, 3% HOAc in DMF, 40 °C, 3 min,

then 10 equiv. NBu_4BH_4, 2 * 30 min, 1 * 16 h;

4.2.4.2.1 S_N2 alkylation

The S_N2 alkylation was efficient for the monoalkylation to tertiary amines, if sterically less demanding moieties were exploited. Allyl groups were easily introduced by simple decarboxylation of the Aloc groups with $Pd(PPh_3)_4$ in the absence of an allyl-scavenger at room temperature. The S_N2-reaction with the bulkier *n*-octyl group needed activation by raising the temperature to 50 °C and elongation of the reaction time to 72 h.[257, 258] The introduction of benzyl groups using benzylbromide / K_2CO_3 was not quantitative, even after reaction at higher temperatures not all secondary amines were saturated, indicated by the *Kaiser test*.[238] Attempts to overalkylate the tertiary amines to generate permanent charges with more unpolar neighbourhood were not successful. The introduction of octyl groups stopped at the level of tertiary amines and bis-alkyl-bis-allylammonium ions could not exhaustively be introduced even after prolonged reaction at elevated temperatures. The resulting crude product contained a mixture of various compounds with different degrees of alkylation and therefore this approach was discarded.

4.2.4.2.2 Amide formation / amide reduction

The attempts using amide formation with subsequent reduction were even less successful. Yet, the peptide bond formation was crucial. Although acid chlorides are one of the strongest activated forms of carboxylic acids, the reaction with lauroylchloride / pyridine resulted in insufficient amide formation. Similar to the results of *S_N2-alkylation*, this indicates that the secondary amines are much less reactive and would need even higher activation to complete the reaction. A possible solution would be to raise temperature. However, resins suffer strong mechanical damage at temperatures above 60°C. For the introduction of 1-adamantyl carboxylic acid, the use of different activation methods was investigated. As expected, the degree of acylation increased from carboxylic acid / DCC / HOBt over carboxylic acid / DCC / NHS to carboxylic acid chloride / pyridine and carboxylic acid / PyBrOP / DiPEA, qualitatively and quantitatively monitored by TLC and RP-HPLC. However, ^{1}H NMR of the final products revealed that peptide bond formation stopped at the level of monoacylation. This additional decrease can be explained by the high steric demand of the adamantyl group, which presumably prevents a sufficient approximation to the N^4-amine. The trityl linker probably shields this secondary amine. Similar results are reported for solution phase syntheses, in which trityl protected amines also shielded the entry site, drastically decreased reactivity, and lowered the yield of modification on secondary amines with a distance of only three methylene groups from the trityl group.[253] It is likely that this effect is even more prominent for immobilized substrates, in which the diffusion of reactants is additionally hindered. On the other hand, this differentiation between the backbone amines could be used to distinguish them, if a smaller entity should be introduced at the N^4-position. After finishing the reaction sequence, it was also obvious that the subsequent reduction of the amides was incomplete and would also require very long reaction times. This finding is confirmed in the literature and seems to be a general phenomenon.[241, 304] Examples in which *Amide reduction* was used for the generation of tertiary amines suffered from low yields. Finally, the amide formation / -reduction approach is limited to the generation of tertiary amines with sterically less demanding rests.[304]

4.2.4.2.3 Reductive amination

Finally, *Reductive amination* solved the problem of incomplete reaction. Monitoring of the reaction progress by the *Kaiser Test* during treatment with capronaldehyde in 3% HOAc / DMF at 40 °C and subsequent reduction with NBu_4BH_4 showed that the reaction was complete after cycles of 2 * 30 min and 1 * over night. This was confirmed by the absence of products that would derive from incomplete alkylation of the secondary amines after labelling with NBD. In the synthesis of conjugate **263**, the excess of capronaldehyde and hydride had to be elevated to 10 equiv. to achieve complete substitution of all secondary amines. The proximity of both, trityl linker and adamantyl group hindered the reaction. For the introduction of benzyl groups, a mixed approach consisting of previous partial benzylation with benzylbromide / K_2CO_3 and subsequent *Reductive amination* with benzaldehyde / NBu_4BH_4 was successfully applied. The pretreatment with benzylbromide was necessary because it was previously reported that in similar systems the reaction with benzaldehyde led to formation of cyclic aminals from two neighboured amines and benzaldehyde, which were not released during the repetitive reaction cycles.[304]

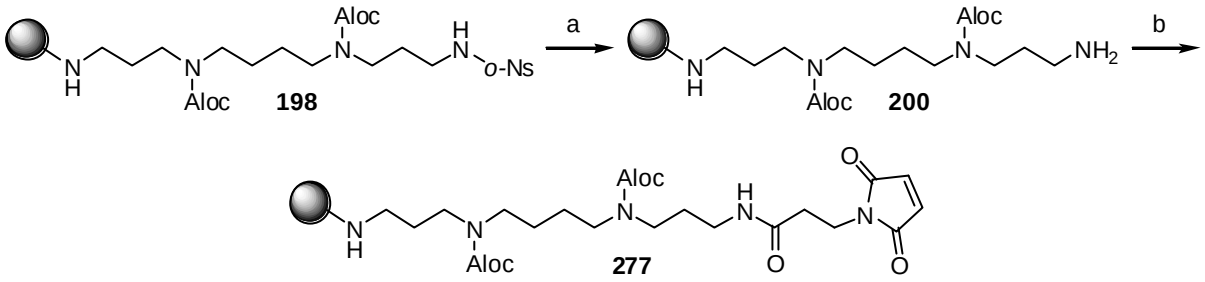
4.2.4.3 Conclusion

In conclusion, it was shown that *Reductive amination* is the method of choice for the selective alkylation of secondary amines in the presence of trityl linkers. *S_N2-alkylation* is only suitable, if the respective building block and the other substituents have low sterical demands. An exhaustive overalkylation could not be achieved by using this approach. Amide formation / reduction is not suitable due to sometimes insufficient amide bond formation and generally incomplete reduction.[304] The problem of incomplete acylation could be reduced by the choice of a different linker with less sterical demand.

4.3 Development of a coupling method for bioconjugation

4.3.1 Introduction of a maleimide

For the introduction of a maleimide onto a polyamine, building block **196** was applied (Scheme 30).[373, 374, 381] Resin **198** was de-nosylated and the liberated amine was acylated with MPS / DiPEA (Scheme 43). Unfortunately, the loading of the resin dropped from about 0.4 mmol / g to values of about 0.2 mmol / g during this step. As reported, activated acyl compounds like carboxylic acid chlorides can effect partial detachment from trityl-linkers.[243] It is likely that the activated carboxylic acid of the relatively small and motile *N*-MPS acted accordingly.



Scheme 43 Introduction of a maleimido-binding site onto immobilized spermine **(200)**. Reagents and conditions: (a) 20 Equiv. β-mercaptoethanol, 20 equiv. DBU, DMF, 16 h, r.t.; (b) 6 equiv. **196**, 6 equiv. DiPEA, DMF, 16 h, r.t.[381]

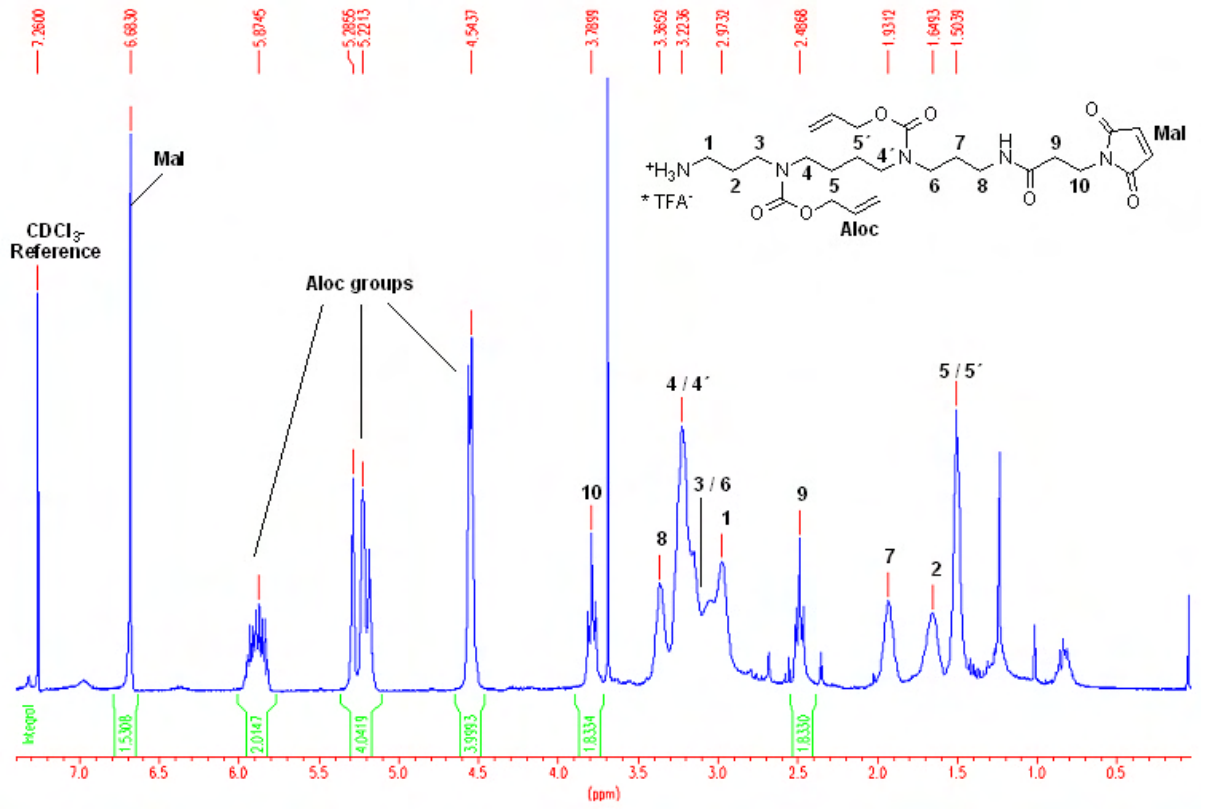


Figure 33 ^{1}H NMR spectrum of cleaved **277**.

The ^{1}H NMR spectrum of the compound directly after cleavage from the resin is shown in Figure 33. Besides the various a- and ß-azamethylene protons, especially the singlet at 6.68 ppm, displaying the aromatic protons of the maleimido double bond, is characteristic.

4.3.2 Conditions for the *Michael addition*

To investigate the suitability of the *Michael addition* for solid phase synthesis, ethylsulfide was chosen as small model compound (Table 9). Reaction of **277** with an excess of 1.2 equiv. of ethylsulfide in DMF in absence of any catalyst showed complete disappearance of the characteristic maleimido-singlet after 6 h.

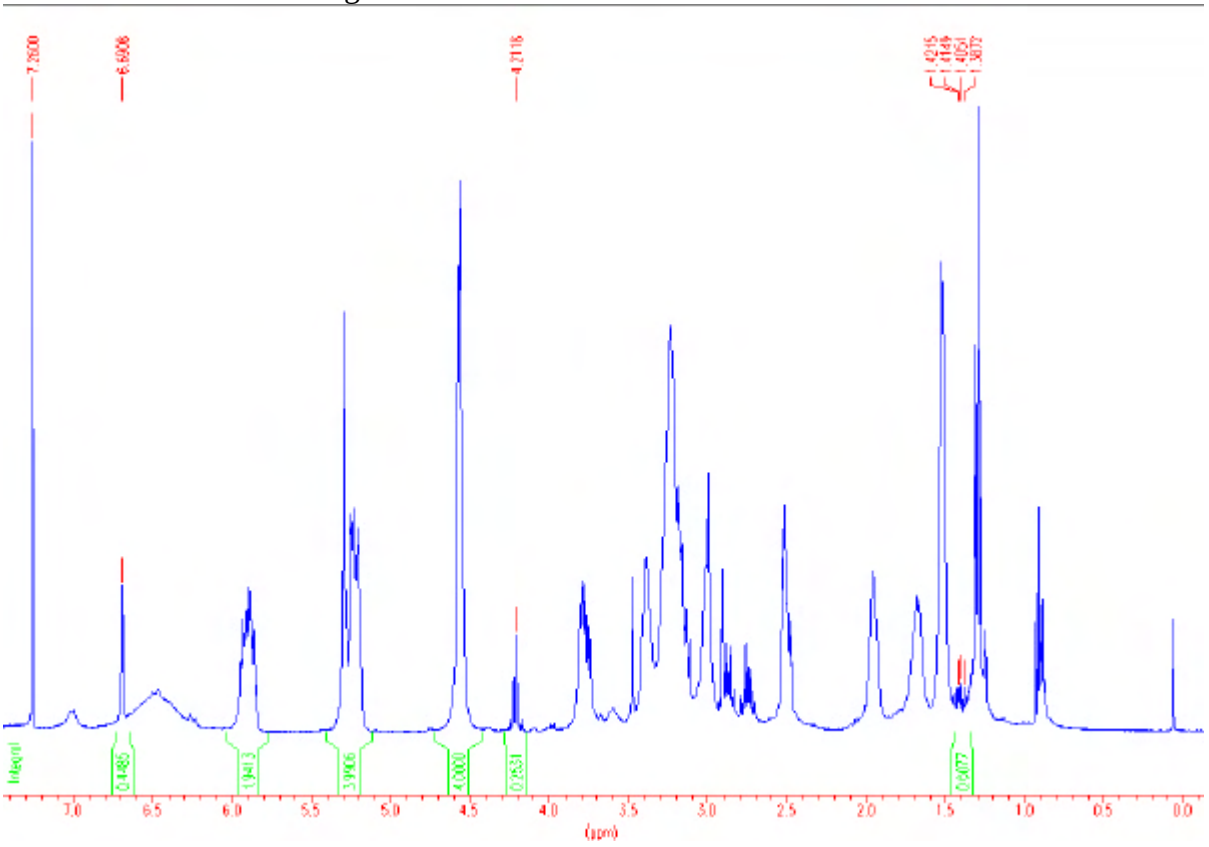
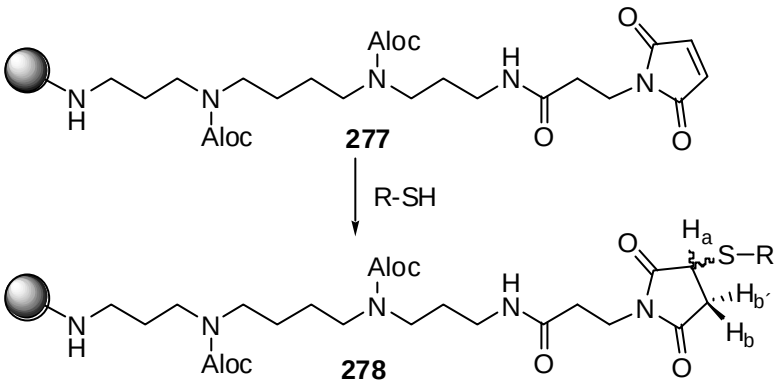
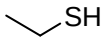
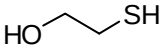
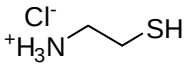
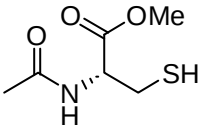
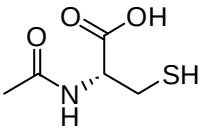
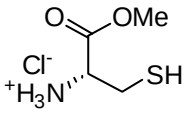
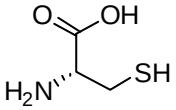
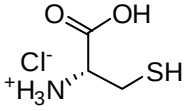


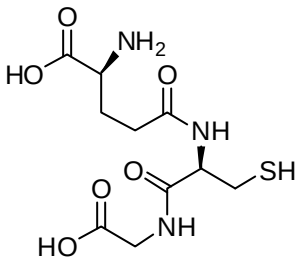
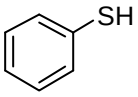
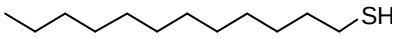
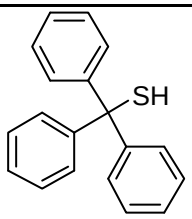
Figure 34 ^{1}H NMR spectrum of the *Michael addition* product of 1.2 equiv. ethylsulfide after 3 h. The spectrum is measured in $CDCl_3$. The singlet of the maleimide is visible at 6.69 ppm and is overlaying with an unidentified impurity that was only present in this spectrum. The signals of the arising side product are found at 4.21 ppm and 1.41 ppm.

After 3 h, a no further determined side product was generated. Therefore, the excess of sulfide was slightly increased to 1.5 equiv (Figure 34). After 3 h, 94% conversion was detected without formation of any side products, demonstrating that the reaction is suitable for its use on solid phase. An investigation with a small library of sulfides is shown in Table 9.

Table 9 Tested sulfides in the addition of thiol to immobilized maleimide [a]

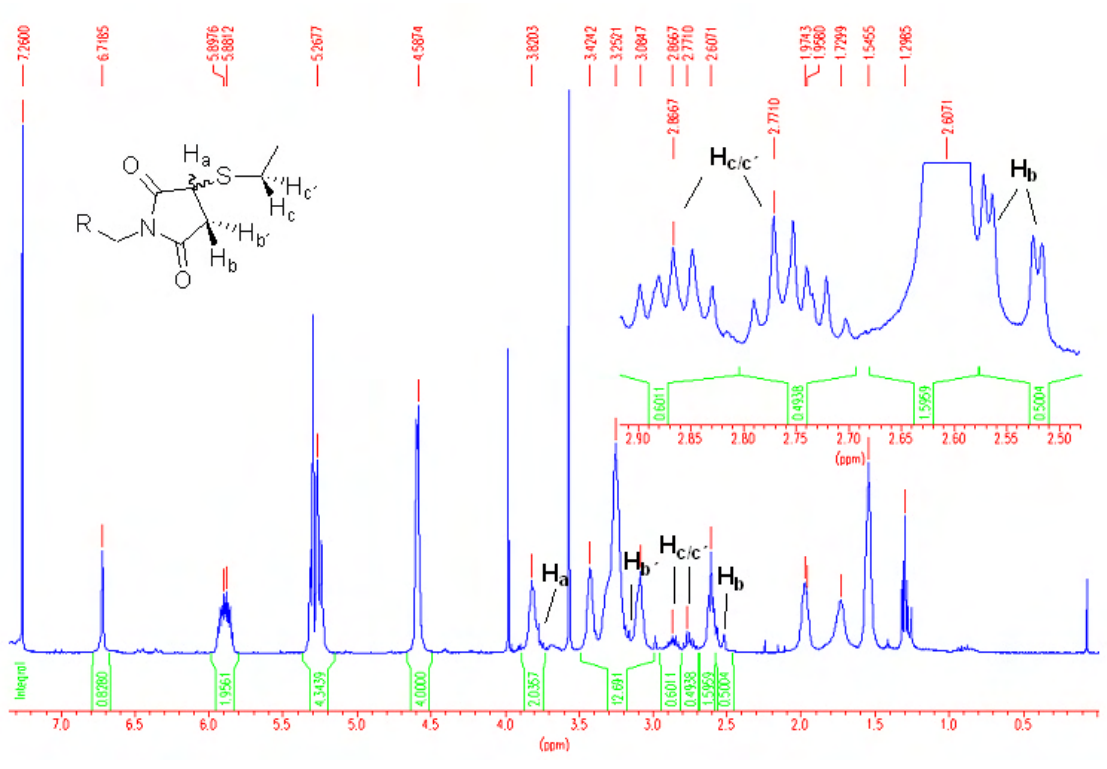


Entry	Sulfide	Excess	Solvent	Reaction time	Conversion in %
1		1.5	DMF	3 h	94
2		1.5	DMF	3 h	>99
3		1.5	DMF	3 h	>99
4		1.5	DMF	3 h	93
5		1.5	DMF	3 h	>99
		5	DMF	3 h	99
6		1.5	DMF	3 h	>99
		5	DMF	3 h	>99
7		1.5	DMF	3 h	89
		5	DMF	3 h	63
8		1.5	DMF / buffer-pH 4.0	3 h	96
		5	DMF / buffer-pH 4.0	3 h	>99

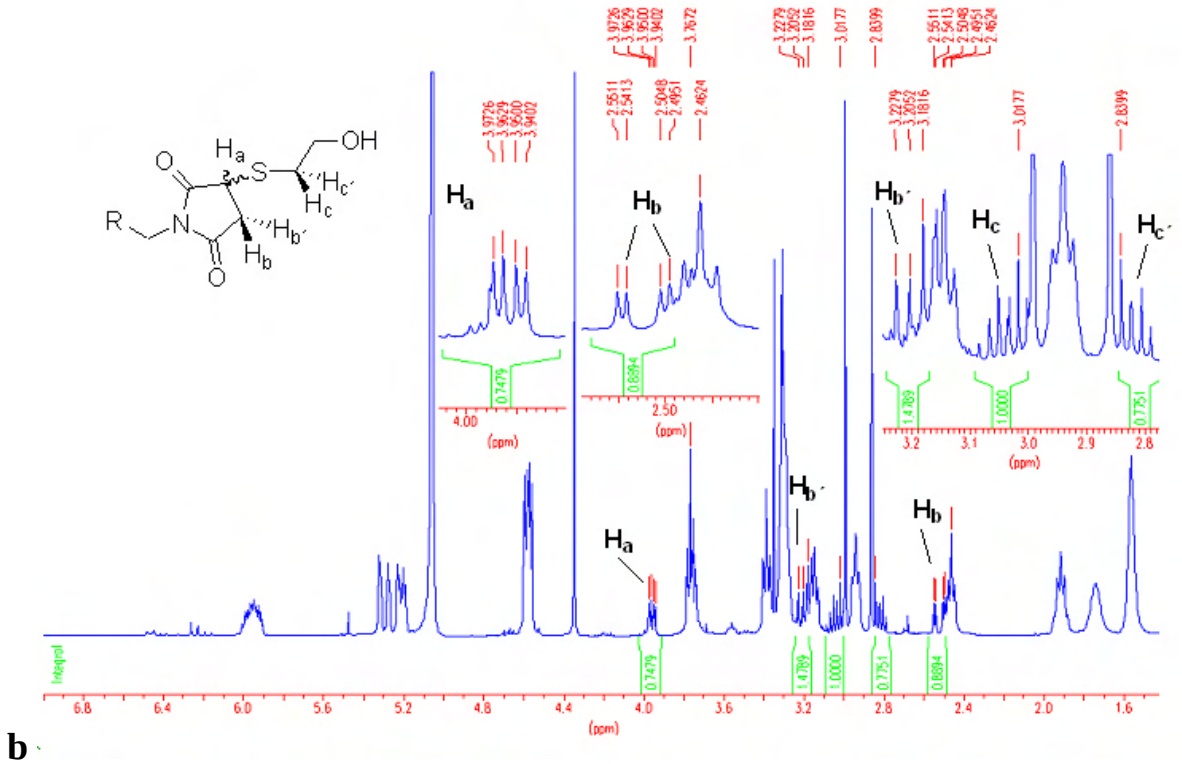
9		1.5	DMF	3 h	< 5
		5	DMF	3 h	28
		1.5	DMF / H_2O	3 h	30
		1.5	DMF / buffer-pH 4.0	3 h	34
		1.5	DMF / H_2O	18 h	>99
10		1.5	DMF	3 h	93
11		1.5	DMF	3 h	0
		1.5	THF	3 h	0
12		1.5	DMF	3 h	0

[a] The conversion was calculated by the disappearance of the maleimido corresponding singlet at 6.8 ppm.

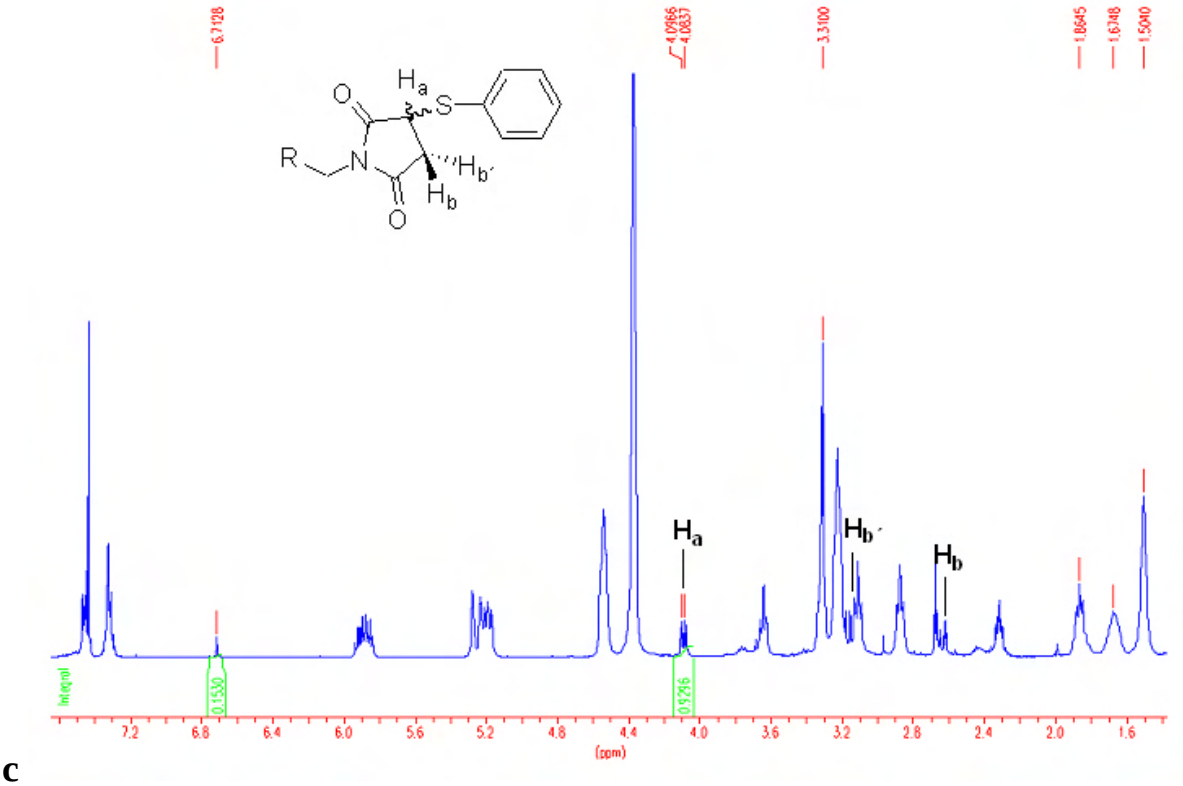
With respect to the instability of some of the products during purification on HPLC and the fact that the *thio-Michael product* is hard to separate from the side product of the *aza-Michael-Addition* the conversions in the crude mixture were determined by ^{1}H NMR.



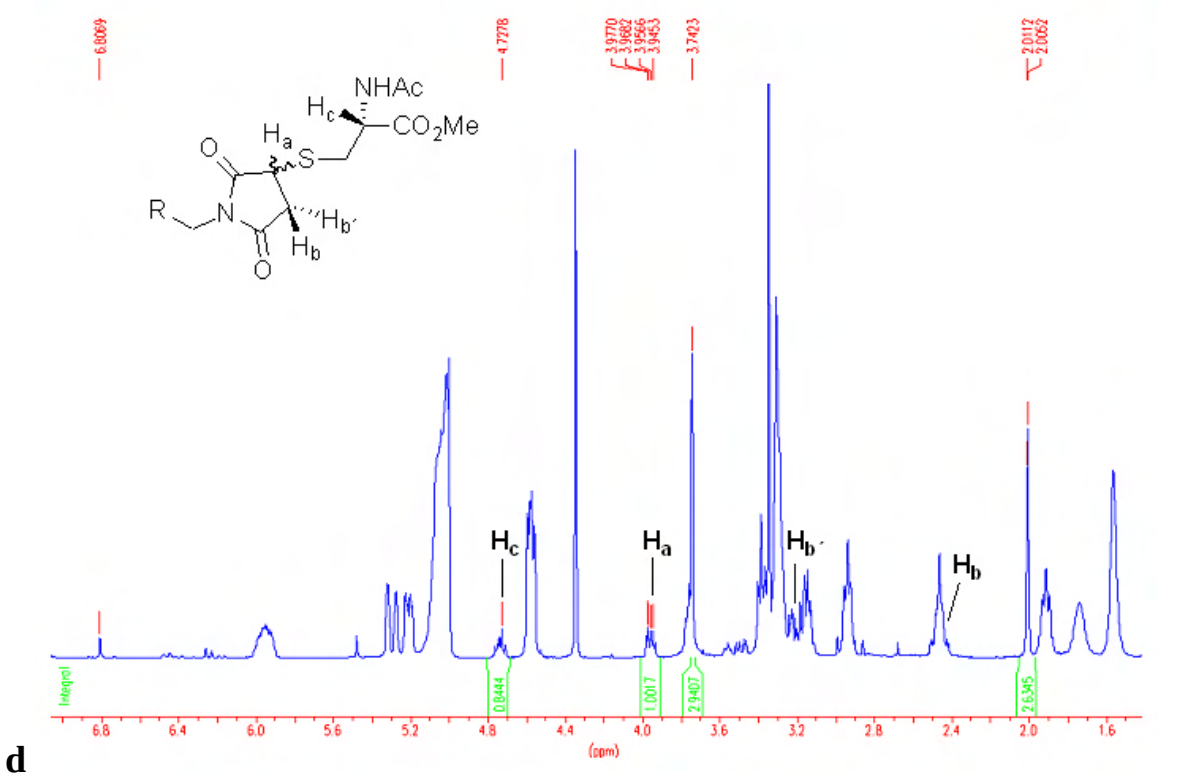
a



b



c



d

Figure 35 Spectra of some addition products. (a) Addition of ethylsulfide after 1 h. The spectrum was measured in $CDCl_3$ and the dd of the maleimido-H_a collapsed with the t of the α-maleimido methylene group. The maleimido singlet at 6.71 was decreasing while the t of the terminal methyl group was occurring. (b) Addition of β-mercaptoethanol after 3 h reaction. The characteristic protons of the 3-*S*-succimidyl moiety are indicated. The dd of H_a was switched to 3.96 ppm in CD_3OD and did not collapse with the t of the α-maleimido methylene group. The shifts of H_b and $H_{b'}$ differed by 0.7 ppm and they could be identified by their coupling constants, which were in accordance with the expected values for AB_2 systems along a bond with fixed confirmation. (c) Addition of phenylsulfide after 3 h. The shifts of the AB_2 system were nearly similar and the coupling constants were almost identical. (d) Addition of *N*-acetyl-*L*-cysteine methyl ester after 3 h.

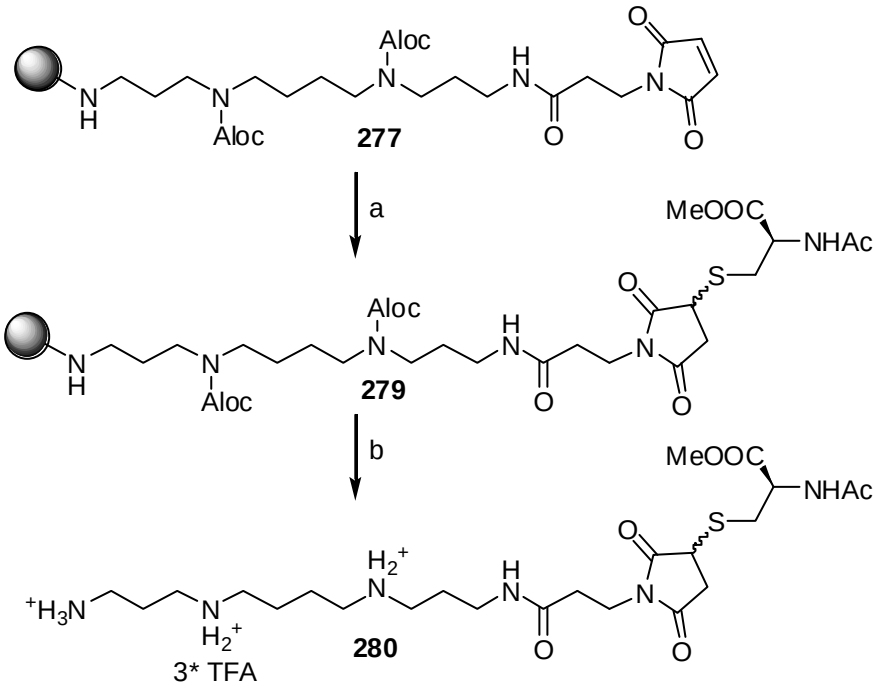
The NMR-spectra of **278** showed a very characteristic singlet at 6.70 ppm in $CDCl_3$ (6.81 ppm in MeOD) (Figure 33), which decreased with proceeding addition of the thiol to the double bond and therefore indicated the progress of the reaction (Figure 35). Meanwhile a dd occurred at 3.96 ppm (in MeOD; in $CDCl_3$, the dd collapses with the t of the α-maleimido methylene group) that corresponds to H_a in **278** and is characteristic for the addition of a sulfide to the maleimide while addition of an amine would give a dd at approximately 4.5 ppm. The other signals in the spectrum were in accordance to the structure. The particular characteristic signals in the ^{1}H-NMR are less anisochronic to measure their integrals. Thus, the ratios of the diastereomers, which were formed in the reaction with chiral cysteine- and glutathione derivatives, could not be determined. However, it could be expected that racemic mixtures were obtained. The identity of the compounds was additionally confirmed by ESI-HRMS. The reaction with the small molecules in Entries 1 – 6 and Entry 10 proceeded well, leading to conversions of > 90% after short incubation. Surprisingly, the hydrophobic thiols in Entries 12 and 13 did not react. A change to better swelling, less polar solvents such as THF did not improve the reaction. While it is reasonable that the bulky trityl group could prevent the approximation of thiol and maleimide, the source of the low reactivity of dodecanethiol is not clear. As ethylsulfide reacts well, it must be caused by the elongated alkyl chain. The polar substrates in Entries 7 – 9 were only poorly soluble in DMF and required addition of

10% water to allow sufficient conversion. Analysis of the products from Entries 3 and Entries 7 - 10 showed that the *aza-Michael-Addition* of the primary amine was competing with the desired addition of the sulfide, once the amine was unprotonated. This was also visible, if acidic buffer was added instead of water to minimize the amount of free amines. Only if ammonium salts were applied in pure DMF, the side reaction was avoided (Entry 3). This side reaction led to complex product mixtures arising from cross-linking and concomitant addition of the primary amine. The markedly quicker reaction with cysteine compared to glutathione suggests that the size of the polar thiols has a strong influence on the rate of the reaction carried out on polystyrene resins. Standard conditions (1.5 equiv.; 3 h) resulted in low conversion rates. Carrying out the reaction over night resulted in full conversion, while increasing the excess of thiol to 5 equiv. provided conversion of only 28% after 3 h. Elongation of the reaction time is thus more efficient for improving the conversion than insertion of additional thiol building block.

4.3.3 Compatibility of maleimido-thiol coupling and Aloc protection strategy

4.3.3.1 Aloc removal after maleimido-thiol coupling

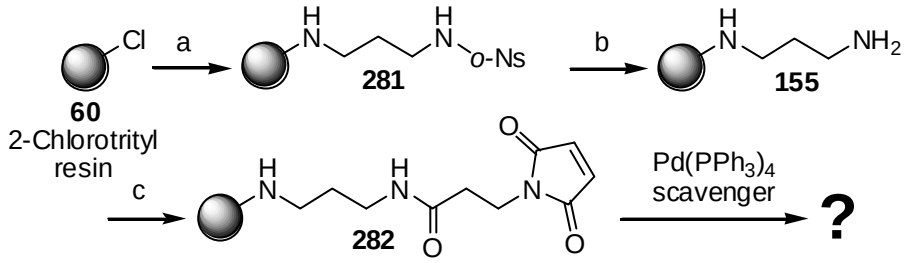
The stability of the 3-thiosuccimidyl linkage during Aloc deprotection was proven by the sequence shown in Scheme 44. *N*-Acetyl-*L*-cysteine methyl ester was coupled to the maleimide of **277** to generate the 3-thiosuccimidyl moiety and subsequently the Aloc groups were removed with $Pd(PPh_3)_4$ and *N,N´*-DMBA under the conditions established above (see 4.1.2.2). ^{1}H NMRs were recorded after cleavage from the resin and subsequent purification by RP-HPLC. It was apparent that the Aloc groups were completely removed and the thioether is still intact indicating that the thioether does not interfere with the Pd catalyst. The ESI-HRMS spectrum was also fully consistent with the structure of **280**.



Scheme 44 Deprotection of Aloc groups in the presence of the 3-thiosuccinimide. Reagents and conditions: (a) 5 Equiv. *N*-acetyl-*L*-cysteine methyl ester, DMF, 6 h, r.t.; (b) 10 mol% $Pd(PPh_3)_4$, 10 equiv. *N,N´*-dimethylbarbituric acid, CH_2Cl_2, 15 h, 40 °C then 10% TFA in CH_2Cl_2.

4.3.3.2 Deprotection of Aloc in the presence of maleimides

The *vice versa* strategy for coupling-deprotection was also explored. Aloc removal in the presence of the maleimides is especially challenging as the double bond could either interact with the palladium catalyst or could be attacked by the mainly nucleophilic or reductive allyl-scavenger.[248, 268] In a first approach, the stability of the maleimide against several representatives of different classes of allyl scavenger was examined (Scheme 45; Table 10).



Scheme 45 Synthesis of a small model system for the examination of maleimide stability against several Aloc deprotection conditions. Reagents and conditions: (a) 10 Equiv. *N*-*o*-nosyl-diaminopropane, 2.5 equiv. DiPEA, CH_2Cl_2, 16 h, r.t.; (b) 20 equiv. β-mercaptoethanol, 20 equiv. DBU, DMF, 12 h, r.t.; (c) 6 equiv. MPS, 4 equiv. DiPEA, DMF, 17 h, r.t.; For conditions of the last step see Table 10.

In summary, the presence of Pd-catalyst was not harmful, if the reaction time is short or if the amount of the catalyst is low. All nitrogen nucleophiles are generally incompatible scavengers, as well as *N*,*N*´-DMBA and hydride donors in the presence of HOAc. Previously, it had been shown that the addition of HOAc drastically enhances Aloc deprotection rate of peptides with $Pd(PPh_3)_4$ / $HSnBu_4$[268], however, it is not necessary to get a protection at all. The maleimide remained largely unreactive against dimedone, oxygen nucleophiles, and hydride donors (in the absence of proton sources). Surprisingly, dimedone and *N*,*N*´-DMBA showed different behaviour, although they are structurally closely related.

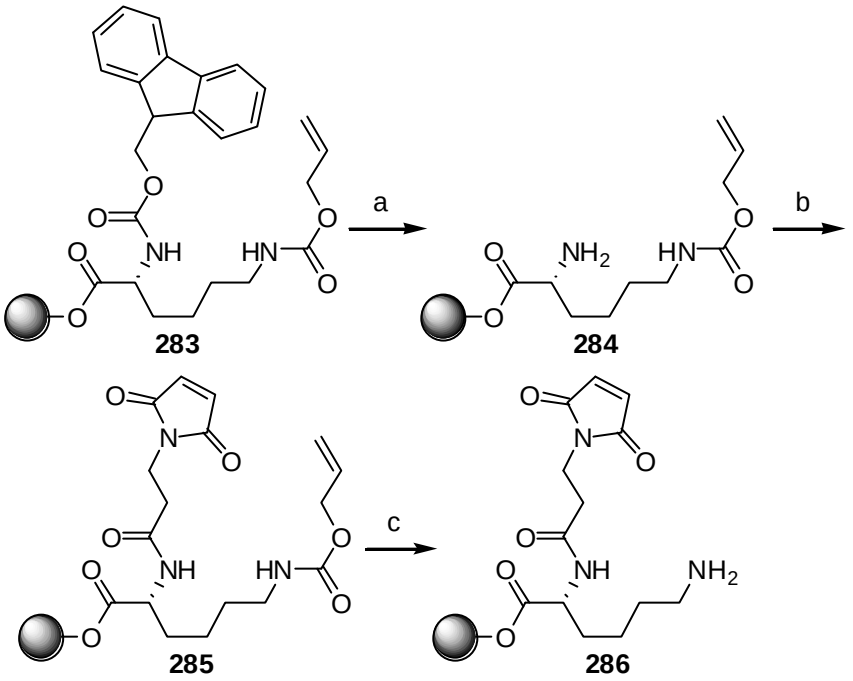
Table 10 Conditions for the removal of Aloc groups applied to resin **282**.[a]

Scavenger	Excess of scavenger	Amount of $Pd(PPh_3)_4$ in mol%	Solvent	Reaction time in min	Stability of the maleimide
		Catalyst			
-	-	4 / 10 / 40	THF	30	Yes
-	-	40	THF	120	No
		Nitrogen nucleophiles			
Pyrrolidine[239]	10 equiv.	20	THF	30	No[b]
NaN_3[274, 382]	5 equiv.	10	DMF	30	No[c]
		Carbon nucleophiles			
Dimedone[248]	8 equiv.	10	THF	30	Yes
N,N'-DMBA[248]	10 equiv.	15	CH_2Cl_2	60	No[d]
		Hydride donors			
$PhSiH_3$[264]	6 equiv.	10	THF	30	Yes
Bu_3SnH[275]	7 equiv.	20	CH_2Cl_2	30	Yes
Bu_3SnH / HOAc[268]	3 / 3 equiv.	8	CH_2Cl_2	5	No
		Oxygen nucleophiles			
HOBt[273]	6 equiv.	10	DMF	30	Yes
NHS[383]	8 equiv.	20	THF	30	Yes
HOAc[269]	20 equiv.	20	THF	30	Yes
Water[270]	50 equiv.	20	THF	30	Yes

[a] The integrity of then maleimide was determined from the 1H NMR spectra of the crude product directly after cleavage from the resin.
[b] *Aza-Michael addition*
[c] Cleavage of the 3-*N*-maleimidopropionate. The mechanism was not further investigated.
[d] Ring opening of the maleimide.
[e] Complete reduction of the maleimide to the succinimide.

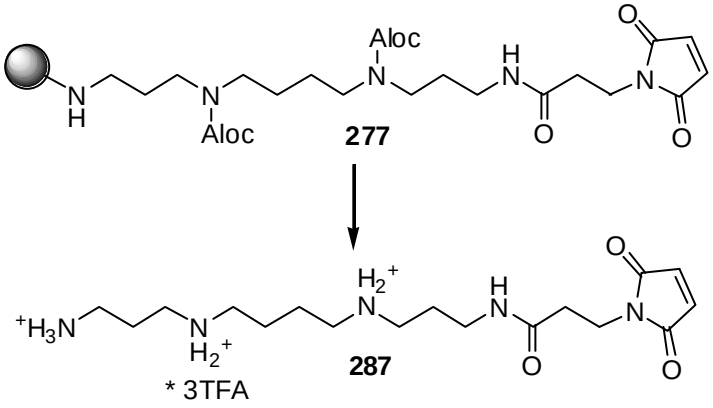
Further, the ability to remove Aloc groups in the presence of a maleimide was investigated on immobilized lysine bearing both functionalities as a model system (Scheme 46). Commercially available *N*-(a)-Fmoc-*N*-(*e*)-*L*-lysine was loaded onto a chlorotrityl resin **60** as described by Barlos et al.[368, 384] The Fmoc group was removed with pyrrolidine in DMF and the maleimidopropionate was coupled to the liberated amine of **284** (see chapter 4.3.1).[381] The functional scavengers from Table 10 were applied to resin **286** (Scheme 46). Finally, only hydride donors showed suitability. The removal with oxygen nucleophiles and dimedone was very sluggish requiring high amounts of Pd-catalyst. Even though, the deprotection was

mostly incomplete. If the Aloc group was removed with $PhSiH_3$-scavenger, some allylamines formation was observed. However, $HSnBu_3$ tracelessly remove the Aloc group without formation of side products. After cleavage from the resin, the product was obtained in 92% purity. The relatively high amounts of required catalyst indicate, that the maleimide interacts with the catalyst and reduces its efficiency. This interaction seems to be not necessarily detrimental as the maleimide is recovered intactly after cleavage from the resin.



Scheme 46 Exchange of Fmoc-group against a maleimidopropionyl group and subsequent removal of the Aloc group. Reagents and conditions: (a) Pyrrolidine / DMF (1 : 3), 3 * 15 min, r.t.; (b) 7.2 equiv. MPS, 4 equiv. DiPEA, DMF, 17 h, r.t.;(c) 120 mol% $Pd(PPh_3)_4$, 8 equiv. $HSnBu_3$, THF, r.t.

If the conditions were transmitted to resin **277** it turned out that although the maleimide stayed intact and the Aloc groups were disintegrated, the formation of allylamines was significant (Scheme 47). Increasing the excess of tributyltin hydride did not improve this result. This is in accordance with the findings from chapter 4.1.2.2: If scavengers, which usually avoid or largely suppress the formation of allylamines are used in the deprotection of Aloc-protected polyamines, they can still be inefficient avoiding this side reaction.



Scheme 47 Removal of Aloc from **277** and subsequent cleavage from the resin.

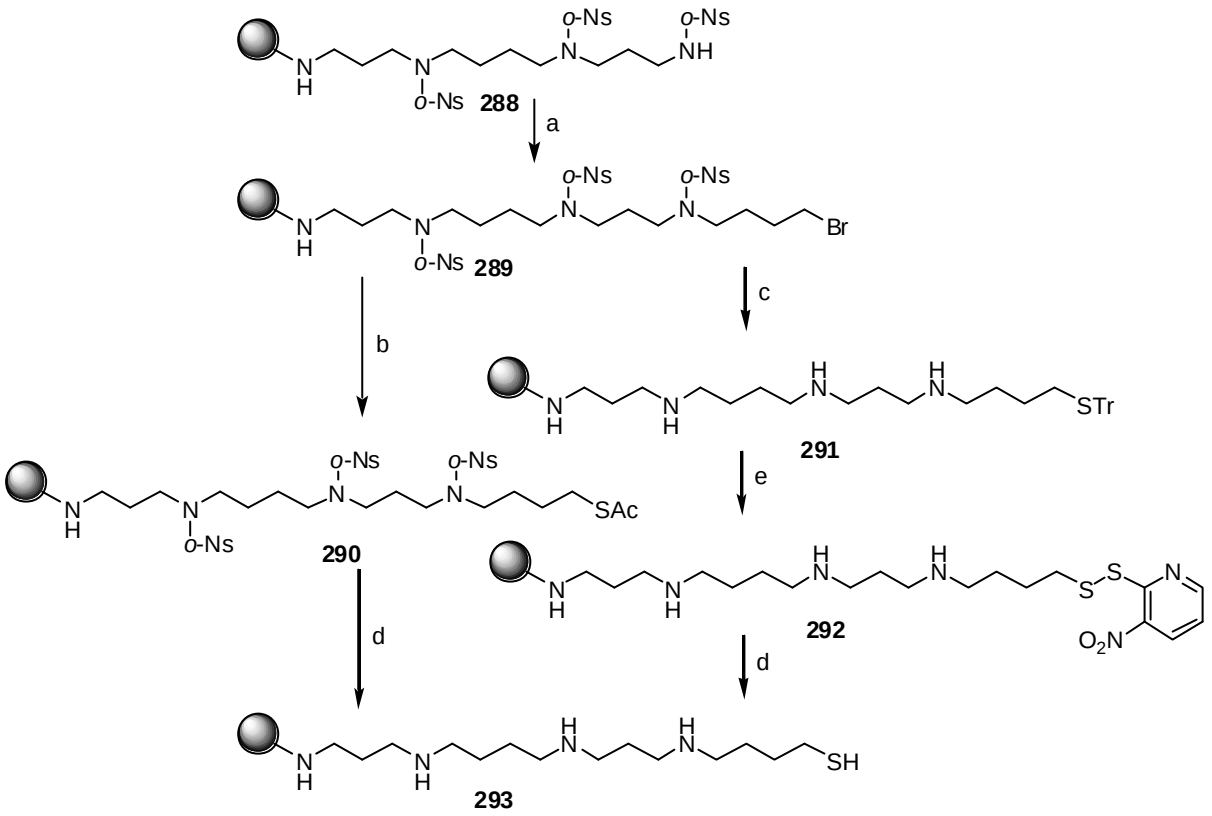
Previous reports showed that besides the excluded carbon nucleophiles the scavenger-combination of formic acid / nitrogen base / NHS, $PhSiH_3$ / silylating agent, and 2-mercaptobenzoic acid efficiently avoid allylamine formation.[268] As sulfides are the binding partners of the maleimides, the latter was not used. Alternatively, tritylsulfide, which does not add to the maleimide (Table 9), was used as trapping agent. However, its use inactivated the Pd-catalyst. Similar results occurred with the use of both other combinations of scavengers: The maleimide was stable, but no deprotection was observed. If a combination of $HSnBu_3$ and *N,N´*-DMBA was used, the results were similar. Finally, it can be concluded that the Aloc deprotection in the presence of maleimides is possible for protected primary amines, if $HSnBu_3$ is used together with high amounts of Pd catalyst. Secondary (poly-)amines are prone to allylamines formation, which could neither be efficiently suppressed by the use of known scavengers nor by the use of rather unusual combinations of scavengers and carbamoyl-trapping agents. The necessity to use wasteful amounts of catalyst (>1 equiv.) to achieve a deprotection, makes the whole approach unsuitable. However, the above demonstrated approach to remove Aloc groups after the conjugation allowed the combined use of the Aloc-based strategy for the synthesis of the polyamine and the maleimido-thiol coupling for the (*bio-*)*conjugation*.

4.3.4 Application of the reaction for bioconjugation

The results of Table 9 showed that the reaction is suitable for the coupling of thiols and immobilized maleimides. The thiol building block can be completely recovered for a further reaction cycles. The reaction excellently proceeded for substrates of medium and well for substrates of higher polarity. In the latter case it was necessary to elongate the reaction time and to add small amounts of water to achieve full conversion. The reaction is selective for thiols in the presence of several functional groups; only amines are competing. It is already known that the addition of amines appeared if the coupling is carried out on solution phase to a minor degree.[333-337] In case of the solid phase reaction, it is likely that the side reaction is enforced by a similar mechanism that caused the cross-linking during loading of the resin (see chapter 4.1.1.1). A solution for this problem would be to use thiol building blocks with a similar amino protection group as the polyamine or with a protection group that is prone to the cleavage conditions of the linker. In both cases, the deprotection of the building blocks and cleavage from the resin could take place simultaneous, avoiding a further step. This is especially compatible with the chosen protection strategy for the polyamine synthesis, which comprises a permanent protection of the amines by Aloc groups and attachment to the resin via trityl linker. Both, Trt- and Aloc-group are common protection groups for the basic side chains in automated peptide synthesis and a prepared maleimido-peptide could directly be inserted, superseding its separate deprotection.[248] In case of oligonucleotides, the combination of trityl- and allyl type groups for permanent protection of phosphonates and amino groups during the synthesis is also reported, but is not typically used.[271] In both cases, the lowered polarity compared to the unprotected building blocks would be advantageous for the reaction on polystyrol resins. While the use of amino-deprotected oligopeptides is excluded, it should be possible to use unprotected, thiomodified oligonucleotides. Due to their lower nucleophilicity the amino groups of the bases are relatively unreactive against maleimides.[364] However, oligonucleotides are very poorly soluble in DMF and also on mixtures of minor degrees of water in DMF. Polystyrol resins usually show decreased swelling if the degree of water in the solvent exceeds a certain concentration. A test series in

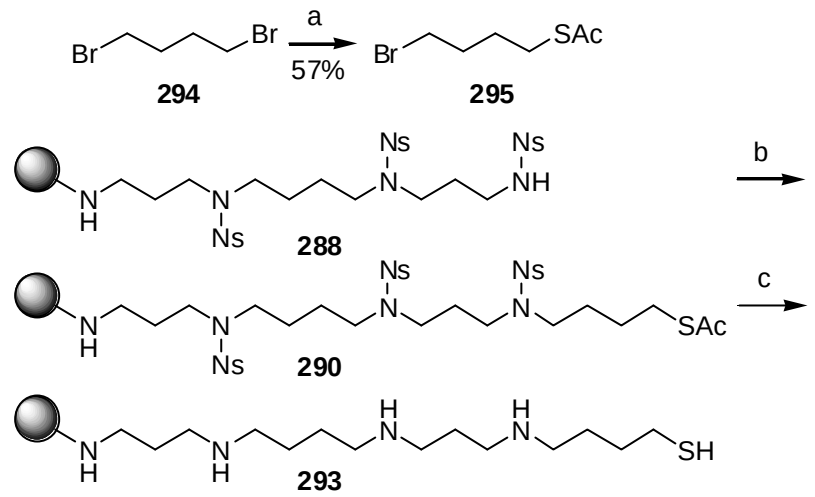
which resin **282** was reacted with glutathione, as model for a water soluble, polar substrate in increasing proportions of water in DMF, showed that the reaction did not take place if the amount of water was higher than 20% and had its maximum at 10% water. The solution for this problem was the use of resins, which exhibit good swelling properties in water or solvent mixtures with a high water content. Tentagels and other resins with scaffold modifications were developed (see chapter 6.2). The synthesis of the polyamine-maleimide, shown in Scheme 43, was carried out on such a Tentagel resin. Unfortunately, analysis of the crude mixture after cleavage from the resin showed only minor degrees of the demanded product. The ESI-MS spectrum indeed showed the product peak, but NMR analysis revealed the occurrence of many side products. Those probably arose from oxidative cleavage of the PEG-linker of the resin scaffold. This degradation is a well-known problem during the use of Tentagel resins and in further studies, special care should be taken avoiding air contact.

4.3.5 ´On resin´-introduction of a thiol



Scheme 48 Introduction of a single thiol to a polyamine avoiding a loss of charge. Reagents and conditions: (a) 20 Equiv. dibromobutane, 20 equiv. K_2CO_3, DMF, 18 h, r.t.; (b) 10 equiv. KSAc, DMF, 3 d, r.t.;(c) 10 equiv. HSTr, 10 equiv. DBU, DMF, 17 h, r.t.; (d) 20 equiv. β-mercaptoethanol, 20 equiv. DBU, DMF, 3 d, r.t.; (e) 6 equiv. 3-nitropyridinyl-2-sulfenyl chloride, HOAc / CH_2Cl_2, 15 h, r.t.

To avoid a loss of charge, the thiol function should be introduced by alkylation to the terminal amine of spermine **(16)**. Therefore, the above-mentioned *Fukuyama alkylation* was used to introduce an electrophile (Scheme 48). The bromide of **288** was displaced by reaction with either potassium thioacetate or tritylsulfide and the protection groups were removed.[85] It was apparent that the *o*-Nosyl groups were stable during treatment with KSAc and that the bromine displacement proceeded well. Analysis of the crude product by ^{1}H NMR and mass spectroscopy showed that the concomitant deprotection of acetyl- and *o*-Nosyl groups led to a removal of both. Displacement of the bromine by tritylsulfide also proceeded well, while simultaneously the *o*-Nosyl groups were removed. This is in accordance with reports, which describe that removal of the *o*-Nosyl group is extremely tolerant against many functional groups or bulky substituents of the thiolate.[261, 279] Removal of the trityl group in **290** with 3-nitropyridinyl-2-sulfenyl chloride and subsequent cleavage of the disulfide bridge with β-mercaptoethanol / DBU showed the successful removal of the trityl group.[85] However, both approaches resulted in impure products. To shorten the reaction sequence and to minimize possible cross-linking, the synthesis was modified (Scheme 49).



Scheme 49 Attempt to introduce a thiol onto a polyamine. Reagents and conditions: (a) 0.6 Equiv. KSAc, DMF, 21 h, r.t.; (b) 13 equiv. **294**, 13 equiv. K_2CO_3, DMF, 8 h; 60 °C; (c) 10 equiv. NaSMe, DMF, 18 h, r.t.

An asymmetric building block bearing a protected sulfide and bromine was prepared by reaction of dibromobutane and KSAc in DMF in 57% yield. Coupling of the halide to resin **287** proceeded well (see chapter 4.1.2.4). While the removal of acetyl- and *o*-Nosyl group with sodium thiomethylate failed, it was successful with mercaptoethanol / DBU (Scheme 48). Finally, there were still relevant amounts of impurities.

2-Iminothiolane **(296)** (*Traut's reagent*) is a popular cross-linking agent for peptides and it has frequently been used for the conversion of the lysine side chain amino groups into thiols **297**.[385-389] Since it discriminates between primary and secondary amines and preserves the charge of the primary amine by transformation to an amidite, it is interesting for polyamine chemistry (Figure 36). It is commercially available or can easily be generated in large portions.

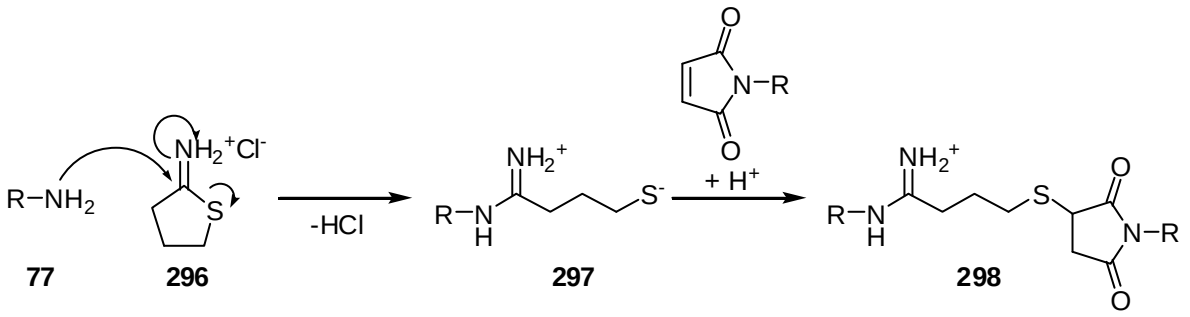
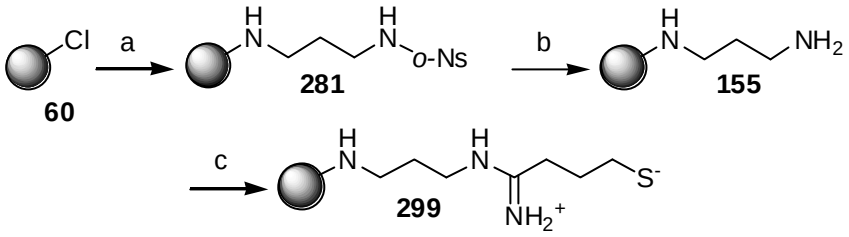


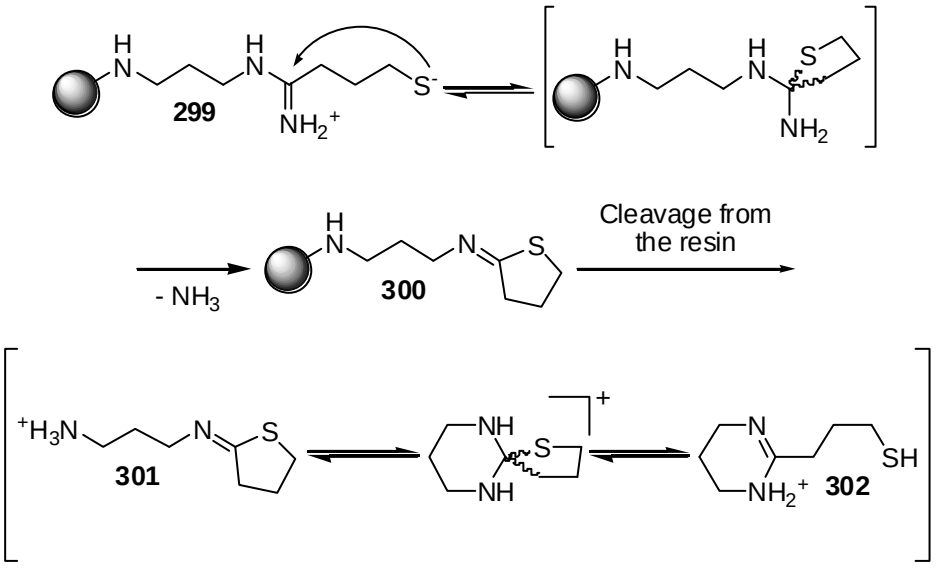
Figure 36 Reaction of 2-iminothiolane with primary amines and subsequent coupling to a maleimide.

The reaction between 2-iminothiolane **(296)** and a primary amine consists of a nucleophilic attack of the amine on the electrophilic carbon (Figure 36). The tetrahedral transition state is disintegrated by ring opening, results in an amidite and liberates a thiolate that can react with a maleimide. Since a previous experiment with immobilized spermine and phenylmaleimide provided good conversion the reaction was optimized for the use on solid phase.



Scheme 50 Loading of the resin, subsequent deprotection, and reaction with **296**. Reagents and conditions: (a) 10 Equiv. *N*-*o*-nosyl-diaminopropane, 2.5 equiv. DiPEA, CH_2Cl_2, 16 h, r.t.; (b) 20 equiv. 2 mercaptoethanol, 20 equiv. DBU, DMF, 12 h, r.t.; (c) 2 equiv. 2-iminothiolane, varying solvents and additives, see text below.[390]

To efficiently convert the reaction to the solid phase, a model system with a short diamine was prepared (Scheme 50). 2-chlorotrityl resin (1.3 mmol/g) was loaded with building block **188** in order to avoid cross-linking of the symmetrical diamine. The *o*-Nosyl group was removed with β-mercaptoethanol / DBU in DMF and the free primary amine was reacted with an excess of 2-iminothiolane hydrochloride. The reaction was usually carried out in DMSO or in aqueous buffers at pH values above 7.0.[391-394] However, both solvents are less suitable for reactions on polystyrene resins. Therefore, some other solvent systems were additionally investigated. 2-iminothiolane was added to the pre-swollen resin in the respective solvent. After 15 h the resin was washed and the cleaved crude product was analyzed by ^{1}H NMR spectroscopy and mass spectrometry. As expected, the resins showed only minimal swelling in phosphate buffer (pH 8.0) and DMSO and the reaction between the 2-iminothiolane **(296)** and the primary amine was prevented. Likewise, the reaction in THF was very poor, probably due to the low solubility of 2-iminothiolane **(296)**. Although the solubility in DMF was higher, even after 15 h not all primary amines were reacted. The addition of 2 equivalents of DiPEA in order to accelerate the reaction did not markedly improve the conversion. However, complete reaction was observed if 2-iminothiolane **(296)** was dissolved in water and was added to a suspension of the resin in THF, giving a solvent mixture of water / THF (1 : 9). The liquid phase was clean indicating that the 2-iminothiolane was entirely solvated, while the resin was sufficiently swollen.

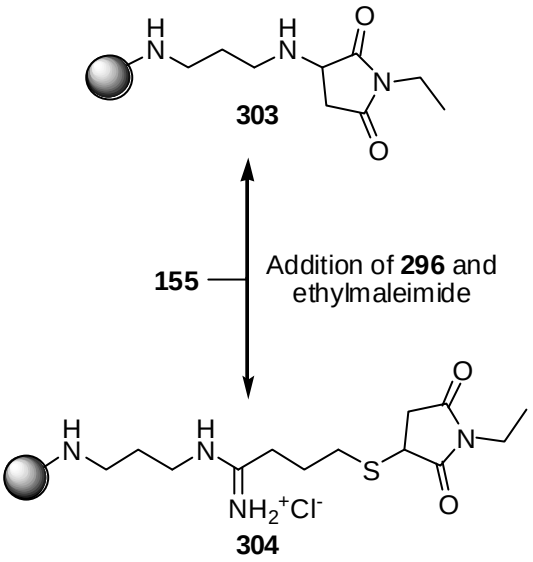


Scheme 51 Side reaction of **299** leading to restitution of a *N*-substituted iminothiolane **300**.[390]

Analysis of the crude mixture indeed showed the absence of diaminopropane but also the presence of a second compound. The mass spectra showed a peak at $[M - 16]^+$ (159.1 u)

additionally to that of the expected product (Scheme 51). This fragment of 17 u is due to the loss of ammonia as already reported previously. The formation of this side product arises from the nucleophilic attack of the liberated thiolate onto the amidite giving the *N*-substituted iminothiolane **300**.

Table 11 Optimization of conditions for the reaction with three components.[390]

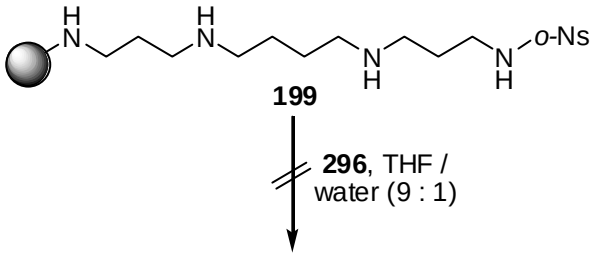


Reaction time	Amount and times of addition of 2-iminothiolane	Amount and times of addition of ethylmaleimide[a]	Ratio		
			5	**8**	**9**
15 h	2 / 0	5 / 780	1.69	0	1
15 h	2 / 0	5 / 2	0.13	0.47	1
15 h	1 / 0 1 / 5	1 / 0 1 / 5	0.10	0.10	1
15 h	1 / 0 1.5 / 1	1 / 1 0.3 / 2.5	0.08	0.10	1
15 h	0.5 / 0 1.5 / 2	1.3 / 2	0.10	0.31	1
15 h	3 / 0	2 / 1	0.06	0.04	1

[a] Amount in equiv., Times of addition in min, 1 / 5 means: Addition of 1 equiv. after 5 min
[b] The ration between **300** and **304** was determined by peak integration of the characteristic triplets at 3.7 and 3.4 ppm.
[c] The ratio of **303** and **304** was determined by integration of the characteristic dd at 4.5 and 3.9 ppm in MeOD.

If the reaction was carried out over night in the presence of a weak base, only this side product could be detected by ^{1}H NMR. After addition of excess (5 equiv.) of ethylmaleimide 2 h prior cleavage from the resin, no coupling could be observed indicating the absence of free thiols and also the irreversibility of the ring closure. After standing in a protic solvent and purification on preparative HPLC, the compound further showed severe decomposition arising from attack of primary amines to the *N*-substituted iminothiolane **301**. This attack seems to occur intra- as well as intermolecular, indicated by ^{1}H NMR and ESI-MS. To avoid the formation of this side product and to optimize the protocol for the maleimido-thiol coupling, the respective maleimide and 2-iminothiolane were simultaneously added to resin **155**. Although this mainly prevented the formation of the cyclisation product, the simultaneous coupling reaction of the amine to the maleimide resulted a second side product **303**. The reaction time, excess and times of addition were varied to both side reactions (Table 11).

Finally, the desired product after cleavage of resin **304** could be obtained in 95% purity after short reaction with 3 equiv. of **296** and 2 equiv. of ethylmaleimide. The formation of both side products could be suppressed by adding the whole maleimide two minutes after the addition of 2-iminothiolane, since the recyclization proceeded slower then the coupling reaction.



Scheme 52 Immobilized secondary amines do not react with **296**.

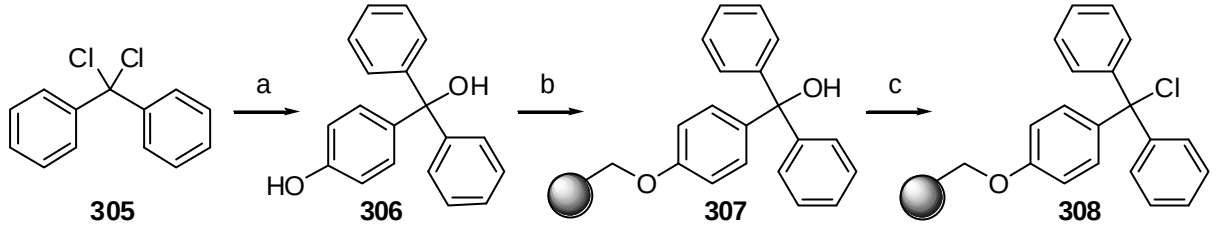
After establishing the conditions, the reaction was carried out using immobilized, mono-protected spermine **199**. Treating **199** with 4 equiv. of **296** for 4 h and subsequent analysis confirmed the selectivity for primary amines (Scheme 52).

4.3.6 Application of 2-iminothiolane protocol for the mild coupling

After optimisation of the conditions, which should allow the mild solid phase coupling of maleimides and polyamines *via* 2-iminothiolane, the reaction should be applied to some maleimides.

4.3.6.1 Synthesis of a suitable resin

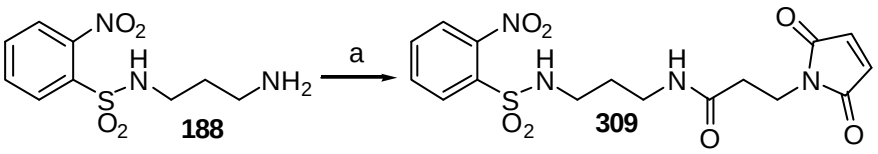
In order to allow the synthesis of very acid sensitive conjugates, the syntheses should be carried out on a very acid labile trityl resin. This was prepared as described in Scheme 53.[239]



Scheme 53 Synthesis of a 4hydroxytrityl alcohol and subsequent attachment to a *Merrifield resin*. After activation with thionyl chloride, the resin can be used for SPS. Reagents and conditions: (a) 1 Equiv. $AlCl_3$, 1 equiv. phenol, CS_2, 3 h, 50 °C; (b) 1 equiv. K_2CO_3, 5 mol% *Merrifield resin*, DMF, 22 h, 60 °C; (c) 2 equiv. $SOCl_2$, CH_2Cl_2, 2 h, r.t.[239]

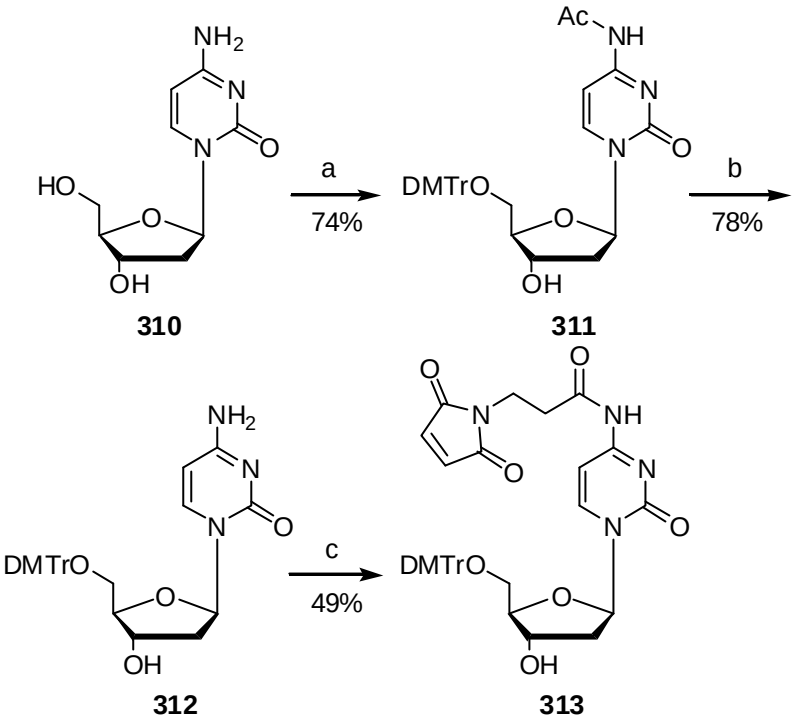
Starting from α,α-diphenyldichlormethane **(305)**, 4-hydroxytrityl alcohol **(306)** was prepared by a *Friedel-Crafts alkylation* giving 87% yield. The trityl alcohol **(306)** was coupled to a *Merrifield resin* by reaction with K_2CO_3 in DMF for 22 h. After thoroughly washing the resin **307** to remove all remains of the reagents, the resin was activated by chlorination with thionyl chloride. The final loading of the resin **308** was in the range of 0.6 – 0.8 mmol / g.[239]

4.3.6.2 Synthesis of maleimides



Scheme 54 Synthesis of a maleimido building block. Reagents and conditions: (a) 3 Equiv. **196**, 3 equiv. DiPEA, THF / DMF (1 : 1), 2 d, r.t.

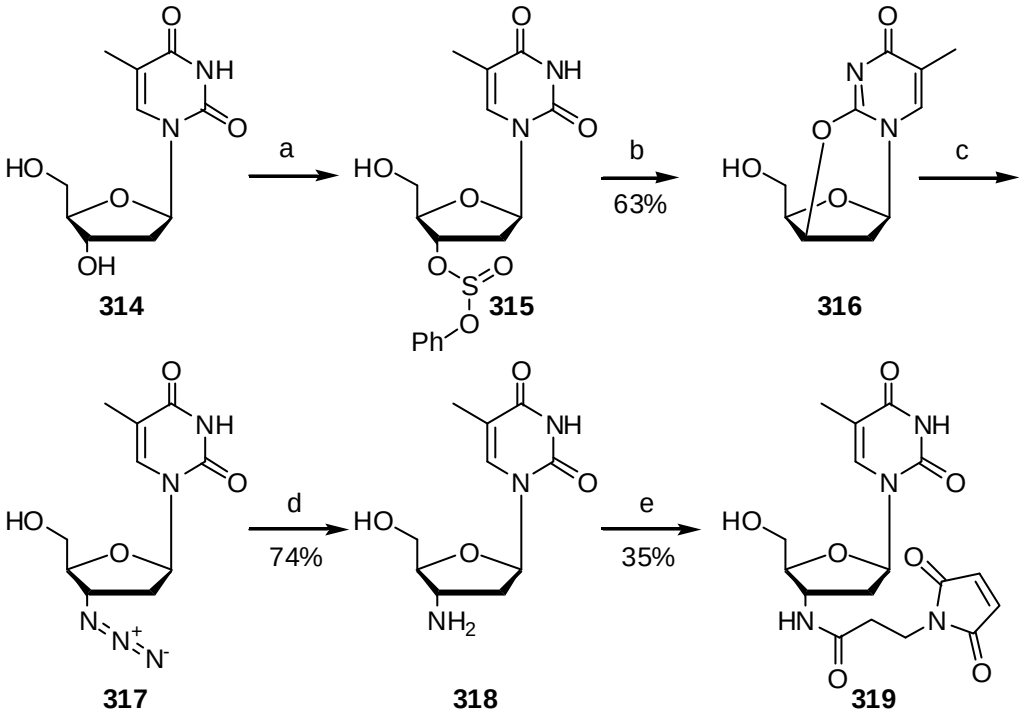
One maleimido building block was prepared, starting from *N*-*o*-Nosyl-monoprotected diaminopropane **(188)**. Reaction with 3 equiv. of **196** in the presence of 3 equiv. of DiPEA gave product **309** in 47% yield.



Scheme 55 Synthesis of a maleimido-modified 2´-desoxycytidine. Reagents and conditions: (a) 1 Equiv. Ac_2O, 2 equiv. Et_3N, DMF, 6 h, r.t. then 1.3 equiv. DMTrCl, pyridine, 6 h, r.t.; (b) 0.7 equiv. K_2CO_3, MeOH, 24 h, r.t.; (c) 1.7 equiv. *N*-maleimidopropionic acid, 1.7 equiv. DCC, 1.7 equiv. HOBt, 0.17 equiv. DMAP, 1.7 equiv. Et_3N, CH_2Cl_2, 240 min, r.t.[364]

A 2´-desoxycytidine was maleimido-modified at the 4-amino group of the base (Scheme 55).[364] Commercially available 2´-desoxycytidine **(310)** was *N*-acylated with Ac_2O / Et_3N in DMF and 5´- protected with DMTrCl in pyridine without isolating the intermediate monoprotected nucleoside. The bis-protected 2´-desoxycytidine **311** was isolated in 74% yield. The acetyl protection group was selectively removed by treatment with a mild base in 78% yield. The liberated amine of the nucleobase was coupled to 3-*N*-maleimidopropionic acid. Due to the low reactivity of the amine, the reaction required the use of DCC / HOBt / DMAP / Et_3N to strongly activate the carboxylate and accelerate the reaction. The last step resulted in 49% yield giving an overall yield of 28% five steps.

Likewise, maleimido-modified thymidine **319** was synthesised (Scheme 56). Starting from thymidine, the 3´-hydroxy group was transferred into a leaving group that could be attacked by the 2´-oxygen of the base leading to a six membered ring in 63% yield. The 3´-position could be attacked by an azide, which was eventually reduced to an amine with palladium on carbon under hydrogen atmosphere in 74% yield.[395] Peptide bond formation with building block **196** resulted in the 3´-(3-*N*-maleimidopropionyl) modified thymidine **319** in 16% overall yield.

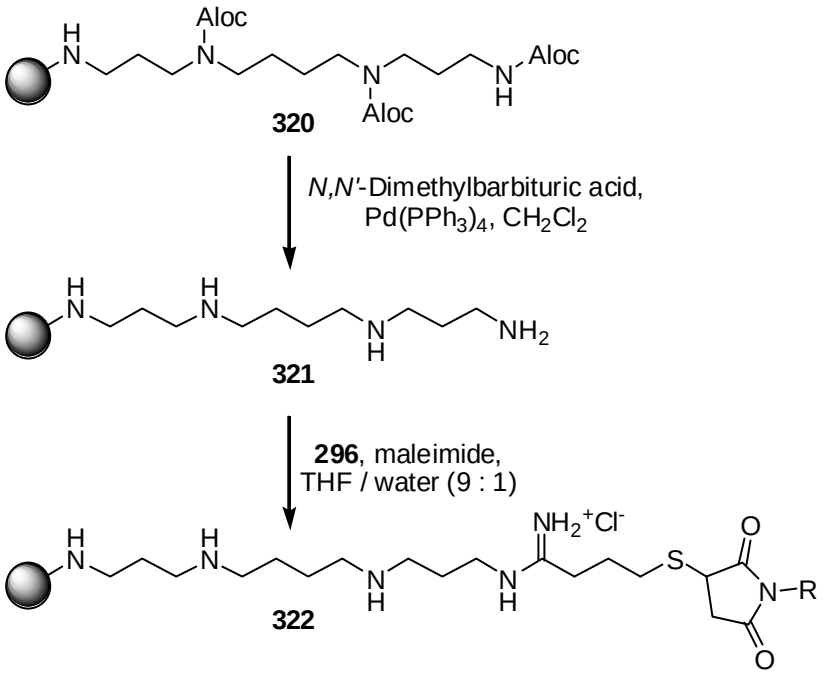


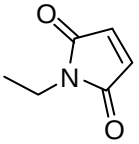
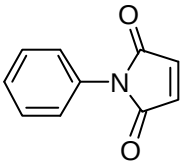
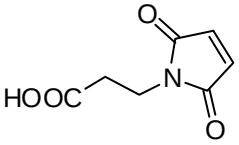
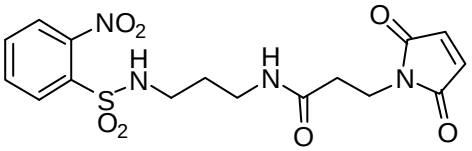
Scheme 56 Synthesis of a maleimido modified thymidine. The first four steps were performed together with Henning Breyhan during his diploma thesis. Reagents and conditions: (a) 4 Equiv. diphenylsulfite, 0.2 equiv. 1-methylimidazole, DMAP, 60 min at 150 °C; (b) Et_3N / water (1 : 2), 40 min, 0 °C to r.t.; (c) 2 equiv. NaN_3, 0.3 equiv. NH_4Cl, DMF, 18 h, reflux; (d) 2 mol% Pd / C, H_2-atmosphere, MeOH, 3 h, r.t.; (e) 2.2 equiv. MPS, 2.2 equiv. DiPEA, DMF, 16 h, r.t.[395]

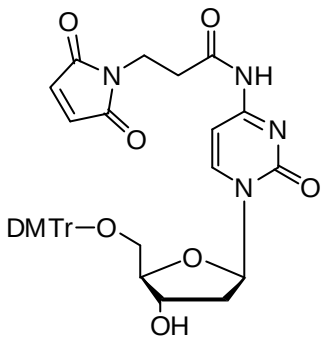
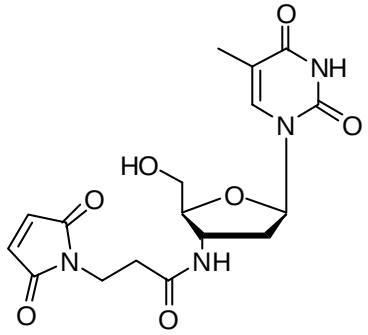
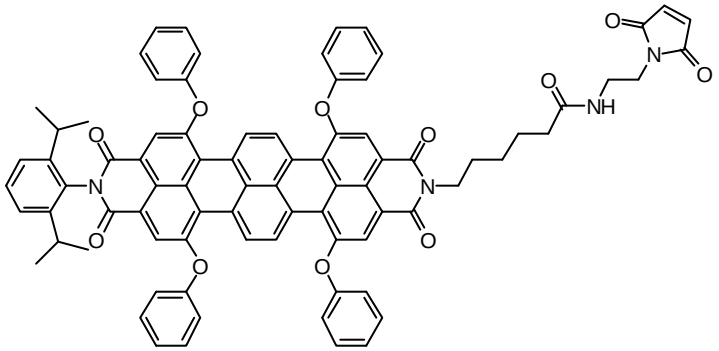
4.3.6.3 Reaction of maleimides and 2-iminothiolane on immobilised, non cross-linked spermine

The alkoxytrityl resin was loaded with tris-Aloc-spermine **179** and cleanly deprotected with $Pd(PPh_3)_4$ / *N*,*N*´-DMBA giving immobilized, non cross linked spermine. Subsequently, a variety of maleimides was coupled using the protocol developed from the results of Table 11. The alkoxytrityl resin was chosen due to its extremely mild cleavage conditions that allow the solid phase synthesis of acid and base labile conjugates. After one hour, the resin was washed and the product was cleaved from the resin with 1% TFA in dichloromethane and purified by preparative HPLC.

Table 12 Regioselective synthesis of spermine conjugates.[390]



Entry	Maleimide: (Mal-R)	Yield (%)	Purity (%)[a]
1		75	93
2		82	94
3		72	98
4		76	99

5		n.d.	62
6		n.d.	94
7[b]		63	55[c]

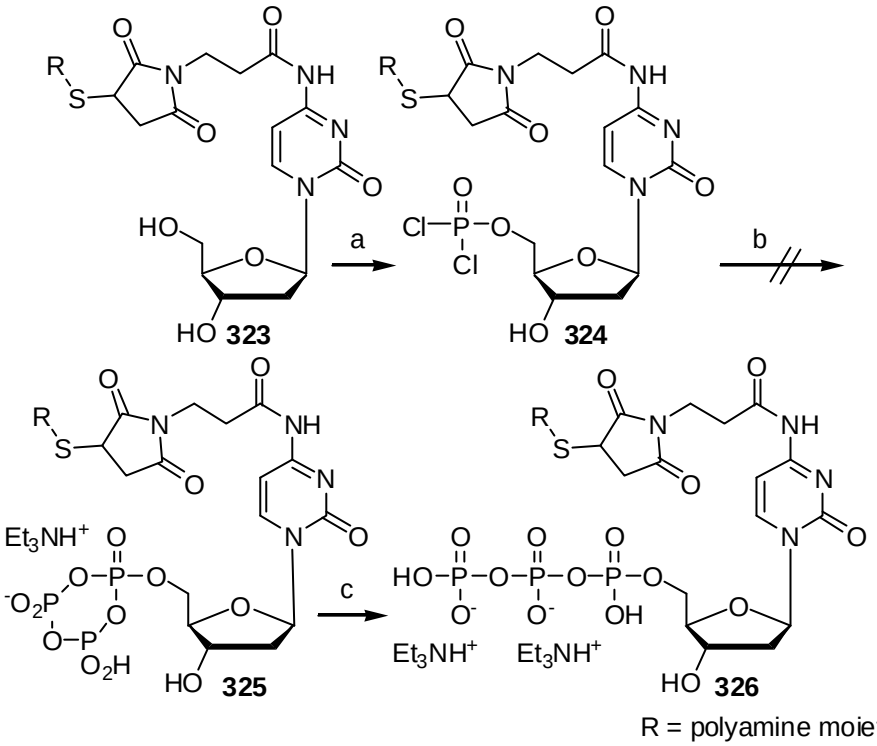
[a] The purities were calculated by integration of the relative peak area of the HPLC with UV-detection at 280 nm.
[b] Only 1.7 equiv. of the maleimide were applied.
[c] The high amphipilicity of the conjugate prevents purification on RP-C_{18} as well as on silica gel. The conversion was calculated from ^{1}H NMR spectra of the crude product.

As shown in Table 12, the coupling efficiently proceeds with a variety of structurally diverse maleimides. The small maleimides from Entries 1 – 3 reacted smoothly and the products were obtained in high purity. Entries 4 – 7 showed that also bulkier maleimido building blocks with a broad variance in polarity are tolerated. More polar substrates seem to react better than less polar. The lower excess of the maleimide **170** can explain the poorer conversion rate in Entry 7. Entries 5 and 6 of Table 12 show that coupling of spermine **(16)** to the maleimido-modified nucleosides *via* 2-iminothiolane **(296)** worked successful. In summary, the introduction of a thiol and coupling to maleimides is most efficiently carried out using 2-iminothiolane. Although the reaction is also principally possible by the more conventional route from Scheme 49, the mild conditions, the broader tolerance for functional groups and the higher flexibility in the synthetic strategy favour the use of 2-iminothiolane **(296)**.

4.3.7 Preparation of polyamino-nucleotides

4.3.7.1 Triphosphorylation of coupling product

The next step after successful coupling between polyamine and nucleoside was the triphosphorylation. A standard procedure was described from Kovacs and Ötvös (Scheme 57).[396]

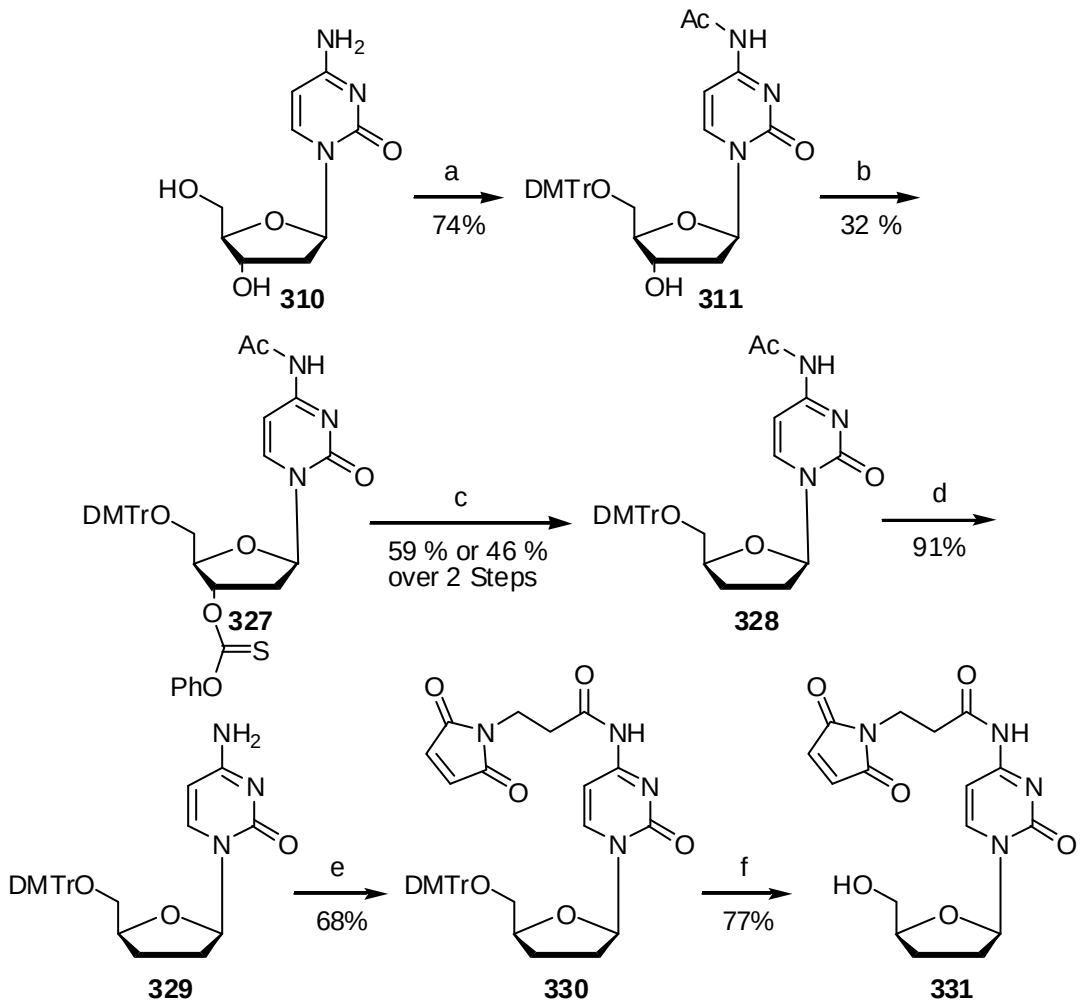


Scheme 57 Triphosphorylation of **323**. The reaction only proceeded to the formation of the monophosphate. Reagents and conditions: (a) 2.3 Equiv. $POCl_3$, 3 equiv. ´proton sponge´, Me_3PO_4, 2 h, 0 °C; (b) 4.3 equiv. $(Bu_3N)_{1.6}H_4PO_4$, kat. Bu_3N, DMF, 1.5 min to 2 h, 0 °C; (c) $(Et_3N)H_2CO_3$, 20 min, r.t.[396]

The nucleoside was first reacted with phosphoryl chloride in trimethylphosphate in the presence of *´proton sponge´*, which led to the 5´-dichloromonophosphate **324** of the respective nucleoside as the main intermediate. After addition of tributylammonium pyrophosphate and tributylamine in DMF a cyclic metaphosphate **325** was supposed to form, which is hydrolysed to the triphosphate by addition of aqueous buffer. However, reaction with substrate **323** did not produce the desired triphosphate. Analysis of the crude product by ESI-(-)-MS, ^{1}H NMR and ^{31}P NMR revealed that the reaction stopped on the level of the monophosphate. Elongation of the reaction time in the second step (from 1.5 min to 120 min) did not improve this result. It is known that the addition of the pyrophosphate is crucial during the Kovacs / Ötvös procedure, because the transition state is fragile and sensitive to disruption by sterically demanding substituents.[397] Usually modifications of the base are less affecting than modifications of the ribose, but in the case of the polyamino-nucleoside it can be assumed that the polyamine interferes with the transition state.

4.3.7.2 Triphosphorylation of 2´,3´-Dideoxy- N^4-(3-*N*-maleimidopropionyl)-cytidine

To avoid disturbance by both, the base substituent and the 3´-hydroxyl group, the 2´-deoxycytidine **310** was additionally deoxygenated at the 3´-position. The coupling to the polyamine should be carried out after the triphosphorylation (Scheme 58). 2´-Desoxycytidine **310** is protected as described in Scheme 55 and the 3´-hydroxyl group was removed by a *Barton-McCombie reaction*. Therefore the 3´-hydroxyl group was transferred into a thiocarbonate **327** by reaction with phenylchlorothioformiate / DMAP. Short reaction at 120°C with $HSnBu_3$ and catalytical amounts of AIBN resulted in 46% yield over two steps. The best results were obtained, if the intermediate **327** was not isolated but the crude product was directly inserted to the next step after removal of the solvent. If the thiocarbonate **327** was purified by column chromatography, the yield for both steps were 32% and 59% giving an overall yield of only 19%. The crucial step was the formation of the thiocarbonate, which was instable during purification on silica gel. The following steps were carried out as previously presented in Scheme 58, resulting in improved yields. This could be explained by the absence of interactions with the 3´-OH group. Removal of the DMTr group gave 77% yield after quick reaction of **330** with 1% TFA in dichloromethane.[364]



Scheme 58 Synthesis of a maleimido-modified 2´,3´-didesoxycytidine. Reagents and conditions: (a) 1 Equiv. Ac_2O, 2 equiv. Et_3N, DMF, 6 h, r.t. then 1.3 equiv. DMTrCl, pyridine, 6 h, r.t.; (b) 1.2 equiv. phenylchlorothioformiate, 2 equiv. DMAP, MeCN, 20 h, r.t.; (c) 3 equiv. $HSnBu_3$, 0.38 equiv. AIBN, toluene, 50 min, 120 °C; (d) 0.7 equiv. K_2CO_3, MeOH, r.t.; (e) 1.7 equiv. *N*-maleimidopropionic acid, 1.7 equiv. DCC, 1.7 equiv. HOBt, 0.17 equiv. DMAP, 1.7 equiv. Et_3N, CH_2Cl_2, 120 min, r.t.; (f) 1% TFA in CH_2Cl_2, 2 min, r.t. [364]

The ^{1}H NMR spectrum of the product recorded immediately after purification is shown in Figure 37.

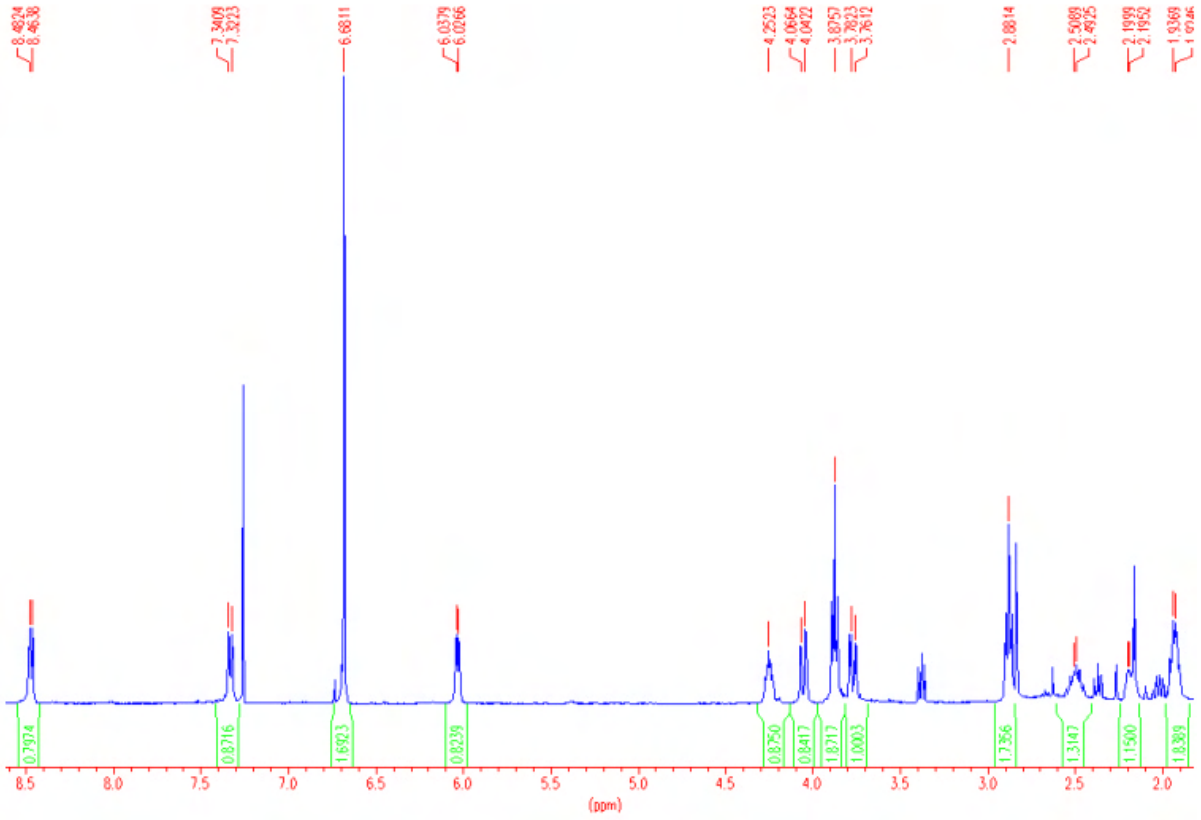
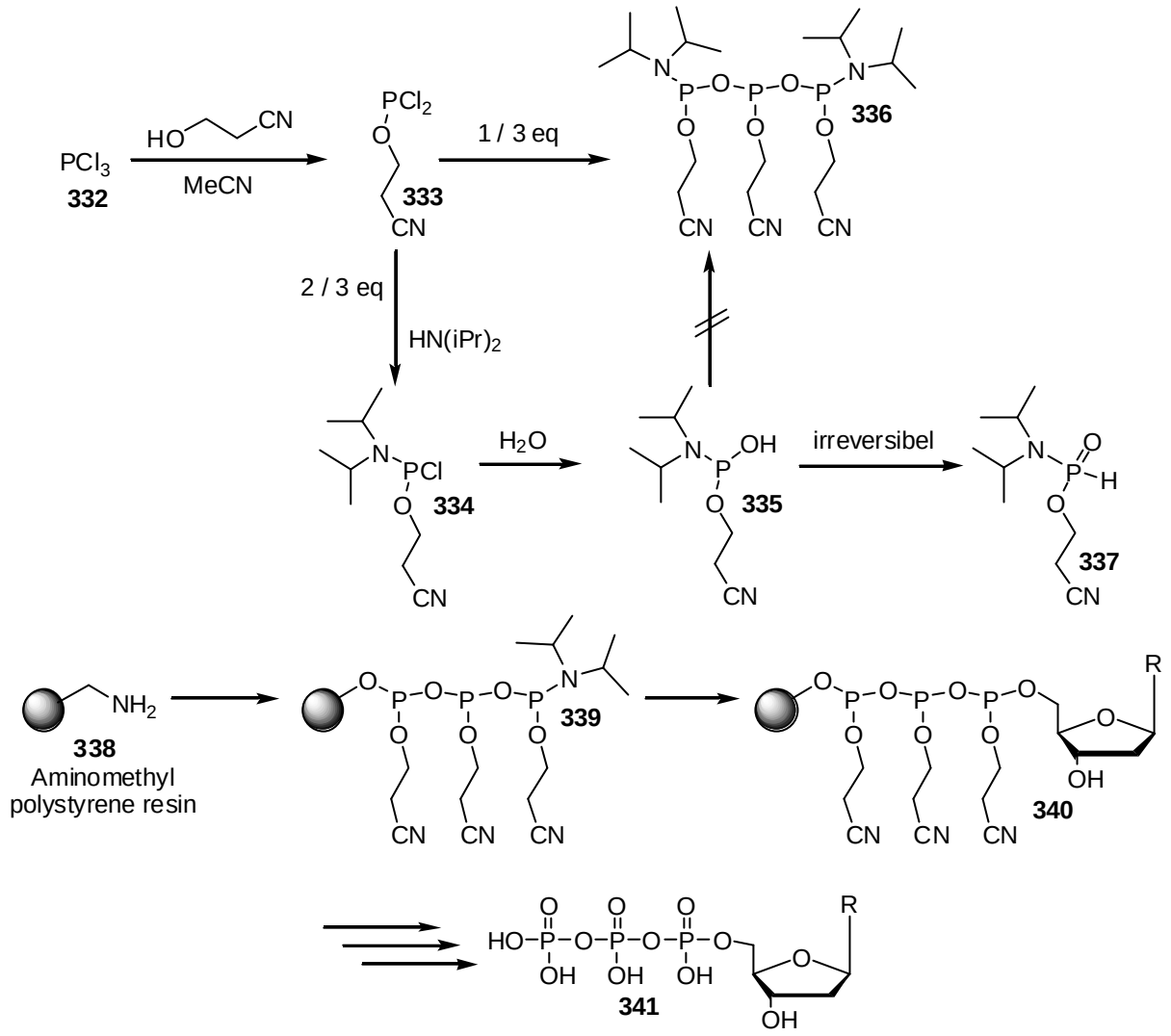


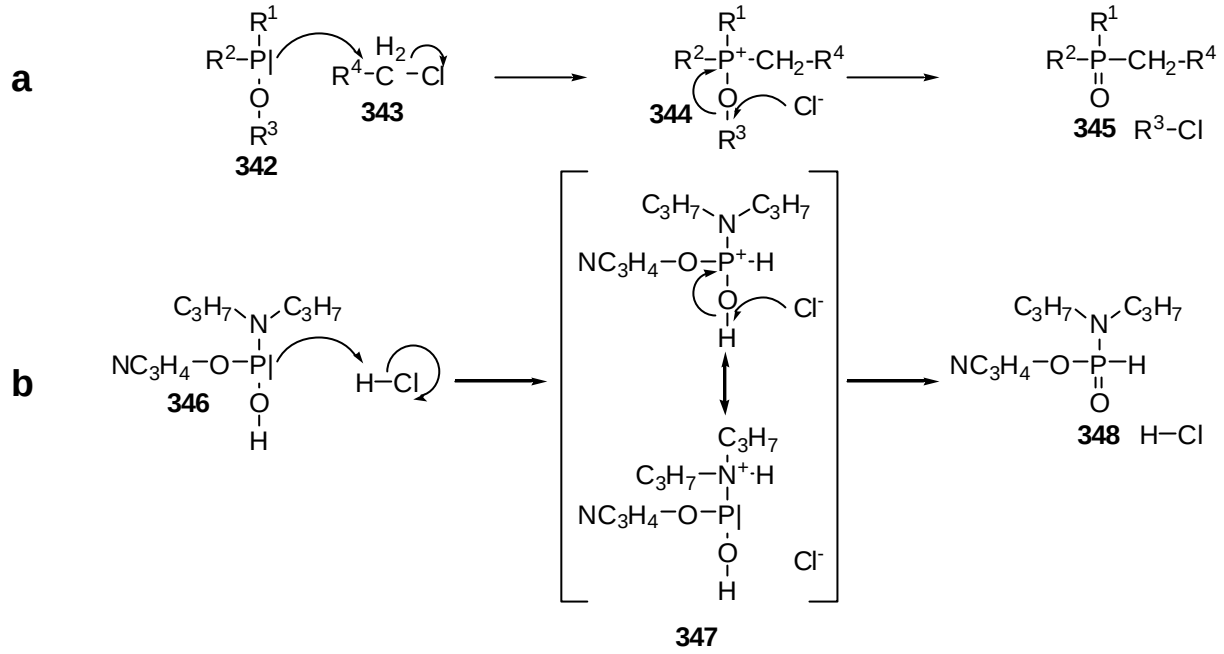
Figure 37 ^{1}H NMR spectrum of **331** in MeOD. The spectrum was measured directly after purification, because the compound decomposed quickly.

The overall yield starting from 2´-desoxycytidine was 18%, which is improved if compared to the literature result (11% overall yield). As the final product is unstable, even if stored at temperatures of –20°C, it was in each case deprotected in appropriate amounts shortly before insertion into the next step. The triphosphorylation of **331** was executed by the Kovacs / Ötvös procedure as also described by Taira et al. Although the steric hindrance was considerably minimized compared to substrate **323**, the results were similar to those previously obtained. No higher phosphorylated product than the monophosphate could be achieved, even if the default conditions were varied like described for the 2´-monodeoxygenated cytidine-spermine construct. Another approach that was investigated came from Ahmadibeni and Parang (Scheme 59).[398] The method based on the preparation of a triphosphorylation agent that could be used to couple nucleosides after attachment to the solid phase. Eventually this leads to the respective triphosphates after oxidation, deprotection and cleavage from the resin. The preparation of the triphosphorylating agent started from phosphorous trichloride **(332)**, which was transferred into a dichloride **333** by reaction with 3-hydroxypropionitril. After partition, two third were reacted with diisopropylamine and were hydrolyzed with equimolar amounts of water. After reuniting the condensation should yield a triphosphane **336**, which can be attached to a hydroxyl-resin.



Scheme 59 Synthesis of triphosphates on solid phase.[398]

Unfortunately it was not possible to reproduce the synthesis of the triphosphorylating agent. The ^{31}P-NMR spectra did not show any signals corresponding to P(III) or higher phosphate species than monophosphates. This probably resulted from a quick rearrangement reaction of **335** to the pentavalent **337**. This reaction can be classified as an analogue of an *Arbuzov rearrangement* (**a** in Scheme 60).[399, 400] In both depicted mechanisms, the P(+III) is transferred into a P(+V). The driving force is the formation of the phosphorous-oxygen double bond and the oxidation of the phosphorous to +V. Isolated hydroxyl-P(+III) species like **346** are only rarely found in literature. Mostly, their formation leads to the further reaction to analogous products as described in row **b** of Scheme 60. Moreover, this rearrangement is sometimes suggested as optimal route to such hydrido-phosphorazanes like **348**, starting from phosphodiazanes or chlorophosphazanes.[401, 402] There is only one example in which a molecule like **348** could be isolated.[403] However, in this case the amino group was substituted by a sulfonamide, which lowers the basicity of both, phosphorous and nitrogen. This could avoid or decelerates the proposed mechanism.

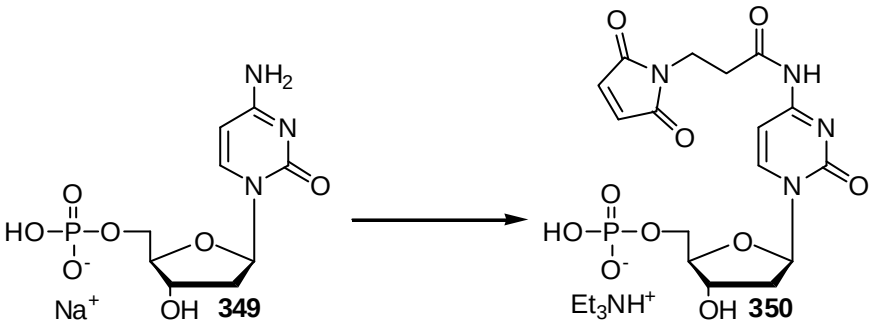


Scheme 60 Mechanism of the *Arbuzov rearrangement* **(a)** and the putative mechanism of the side reaction **(b)**. In both cases, the P(+III) is transferred into a P(+V).[400]

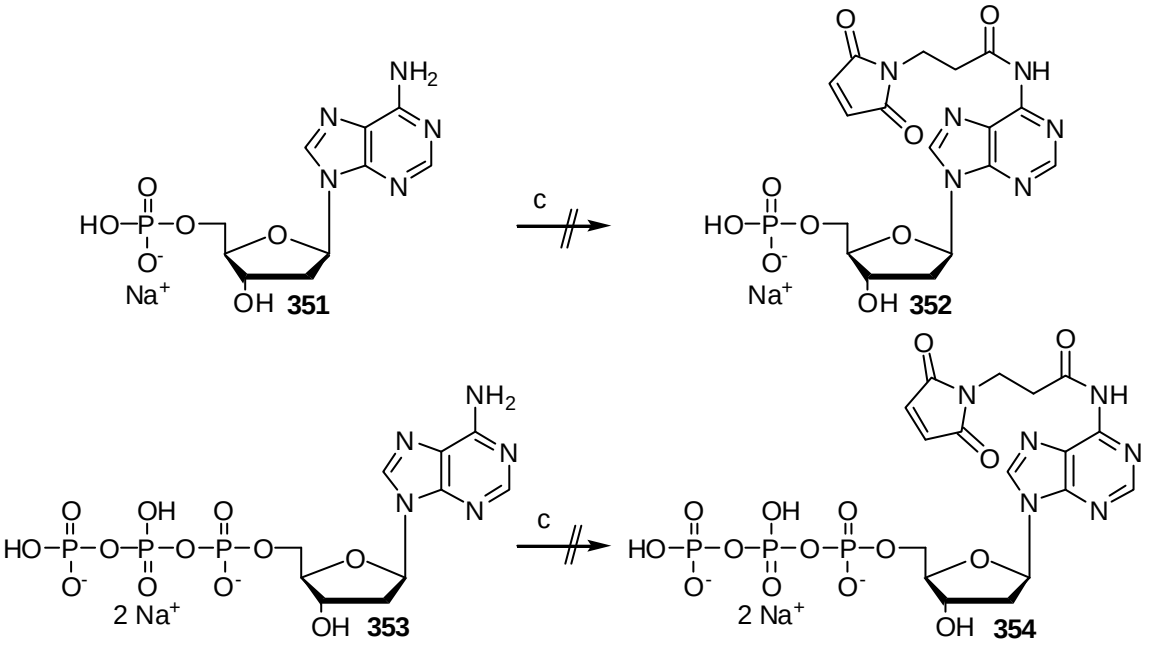
Finally, these facts question the whole procedure. A personal communication from the authors of the publication suggested carrying out the reaction in the absence of humidity, what was adhered strictly. However, as the key intermediate is prepared by reaction with water, this did not lead to an improved result. To minimize the side reaction, the original protocol was then modified. Diisopropylamine was added directly to the undivided solution of **233**. After stirring, water was added in small portions to keep the concentration of **335** low and favour the further reaction with **333** against the rearrangement. However, the outcome of this approach was identical to the original experiment. Further efforts to directly phosphorylate the nucleoside were not undertaken.

4.3.7.3 Introduction of a maleimide onto triphosphates

As the triphosphorylation seemed to be crucial, it was tried to avoid this step. Phosphorylated analogues were applied in the reaction with maleimido building block **196**. 2´-Desoxycytydine monophosphate, 2´-desoxyadenosine monophosphate and 2´-desoxyadenosine triphosphate were reacted with 3-*N*-maleimidopropionyl succinimide under various conditions (Scheme 61). Like for nucleoside **329**, it was necessary to strongly activate the carboxyl compound to enable considerable peptide bond formation (Scheme 61). It was reported that imidazole ester are sufficiently activated to be used for this reaction. In this case the succimidyl ester was applied and the imidazole ester was formed *in situ* by addition of 3.2 equiv. of imidazole to the reaction mixture. While neither the conditions **a** nor **b** gave any detectable amounts of product **350**, reaction under condition **c** yielded a mass peak that could correspond to the product and was present before and after purification on RP-HPLC. Unfortunately the ^{1}H NMR could not confirm the proposed structure, but showed a crude mixture of compounds.



a: 3 equiv. MPS, 3 equiv. DiPEA, DMF, 5 h, r.t.
b: 3 equiv. MPS, 1 equiv. DMAP, 3 equiv. DiPEA, DMF, 6 h, r.t.
c: 5 equiv. MPS, 2 equiv. DMAP, 3.2 equiv. imidazole, DMF, 60 h, 50°C



Scheme 61 Attempts for the introduction of a maleimide to phosphorylated nucleosides. The conditions are depicted in the scheme.

It could only be speculated that the product is unstable, keeping in mind that compound **319** is also prone to quick degradation. Analysis of the crude product showed that large amounts of the monophosphorylated educt were recovered showing that it remains unchanged during the reaction. However, the activated maleimido-propionate was not recovered and instead the respective acid was present after reaction. It is probable that the activated ester was quickly hydrolysed and the peptide bond formation did not take place. For the other nucleotides, the result was identical.

4.4 Biological evaluation of the compounds

To evaluate the potential of the different polyamine structures to serve as molecular transporters, they were applied in *in vivo* tests. Therefore, the polyamine moiety was labelled with fluorophores to allow a monitoring of their temporal and spatial uptake and cell specificity by live imaging and fluorescence detection. Common fluorophores such as rhodamine B, NBD, and also a porphyrin moiety, which can also serve as a phototoxic drug in Photodynamic Therapy (PDT) were chosen, since they all were suitable for most of the microscope filter sets and could be coupled to the terminal primary amino group. Co-incubation with subcellular markers and subsequent live imaging should also reveal a more detailed picture of the intracellular localization and migration of the fluorescent molecules.

4.4.1 Fluorophore conjugates

Initially, rhodamine B with excitation / emission wavelength at 562 / 573 nm was chosen to label the polyamine moiety (Figure 38).

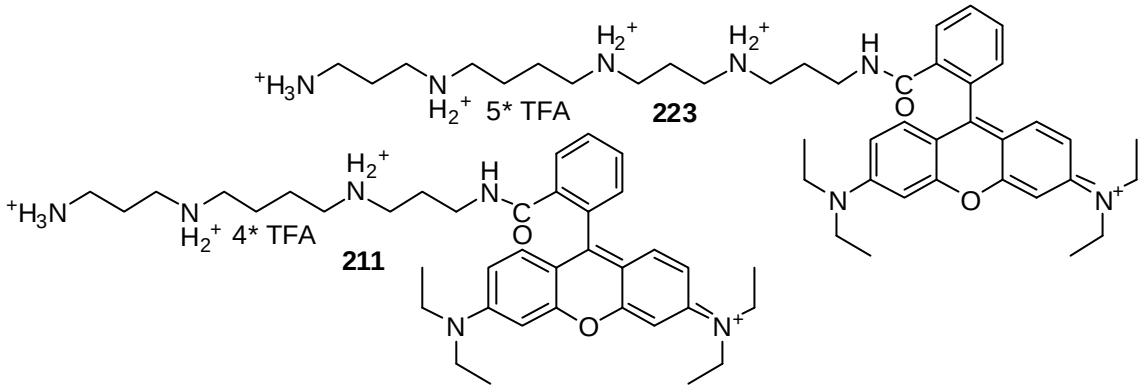


Figure 38 Structures of spermidine-rhodamine **211** and spermine-rhodamine **223**.

However, only minimal fluorescence was observed in live imaging experiments suggesting an interference with the polyamine moiety.

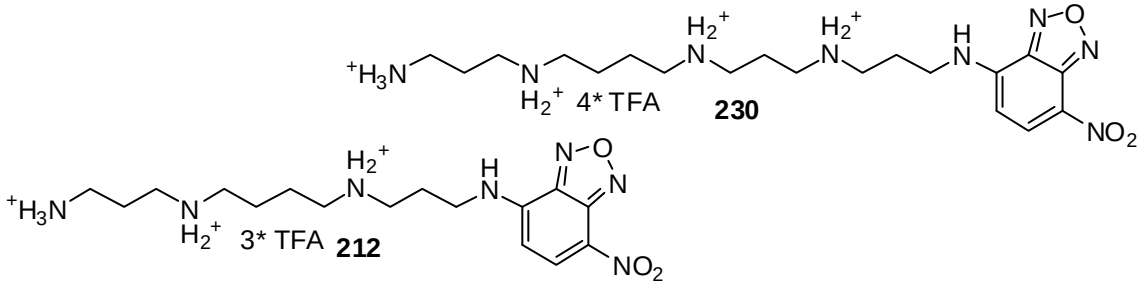


Figure 39 Structures of spermidine-NBD **212** and spermine-NBD **230**.

The NBD conjugates **212** and **230** are soluble in water, facilitating their application in cell culture (Figure 39). Calculations of the behaviour of molecule **230** in water showed that the polyamine chain is mostly existent in a stretched form. Only the NBD moiety adjacent to

NBD is bended, probably to optimise the hydrophobic interactions of NBD and the non-charged part of the chain. Finally, all charges of the backbone are accessible for interaction with negatively charged groups on the cellular membrane (Figure 40).

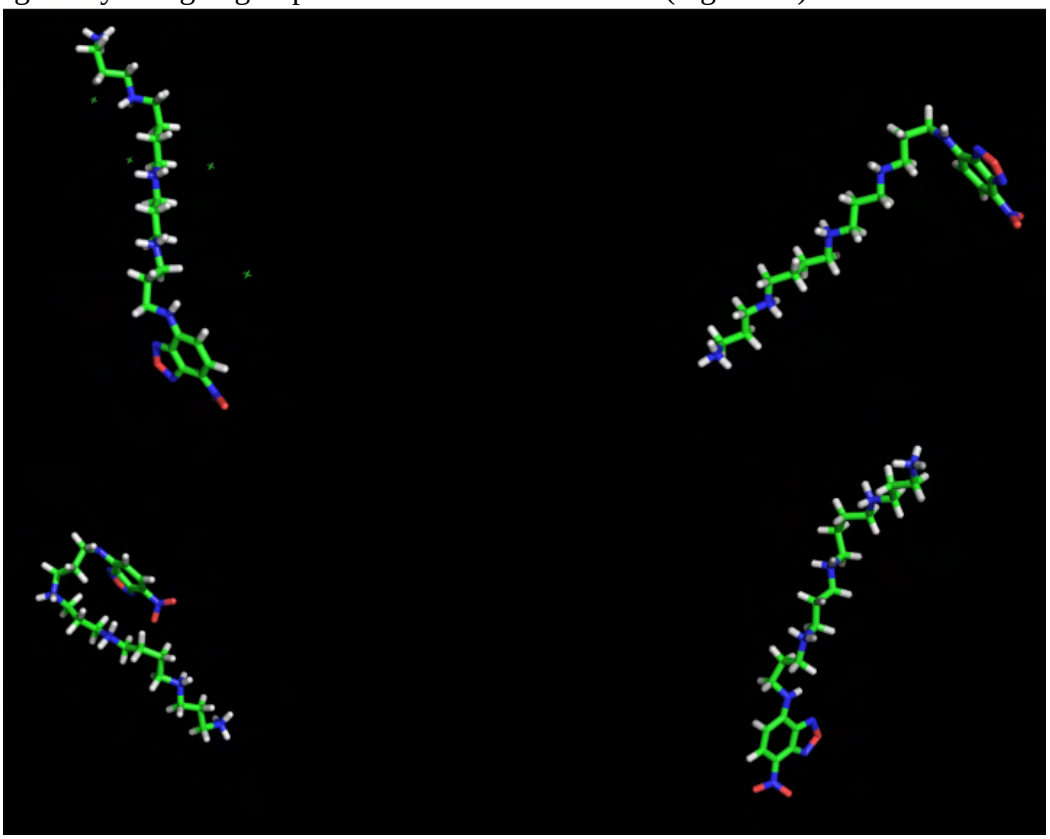


Figure 40 Calculation of the arrangement of spermine-NBD conjugate in water. (Molecular dynamics calculations using AMBER-software; in collaboration with J. Setzler, W. Wenzel, KIT Karlsruhe)

Incubation of human primary fibroblasts with both spermidine-NBD **212** and spermine-NBD **230** revealed a strong punctuate fluorescence inside the cells (Figure 41), suggesting an accumulation in vesicular structures.

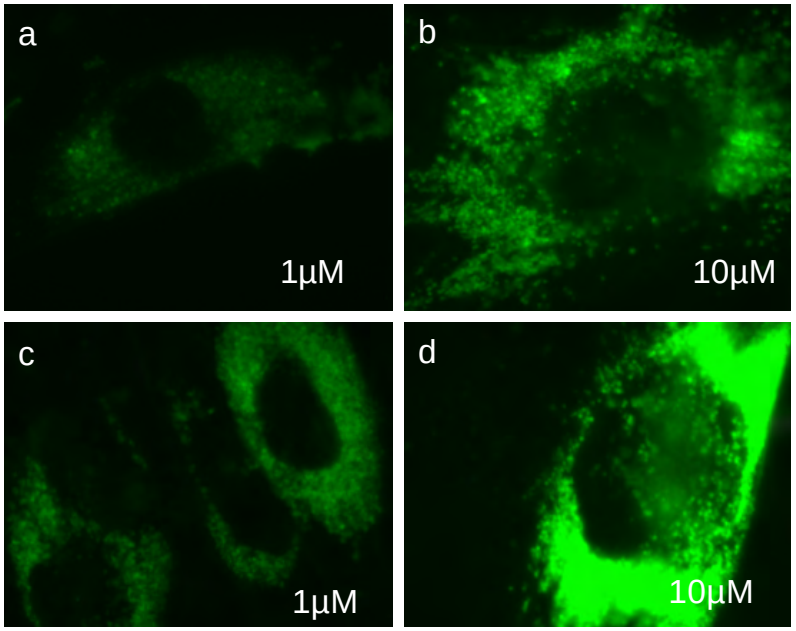


Figure 41 Live imaging of human primary fibroblasts incubated with spermidine- (**a, b**) and spermine-NBD (**c, d**). The cells were incubated with 1 µM or 10 µM of conjugates **212** and **230** (**a, b**) and y (**c, d**) for 24 h.[404]

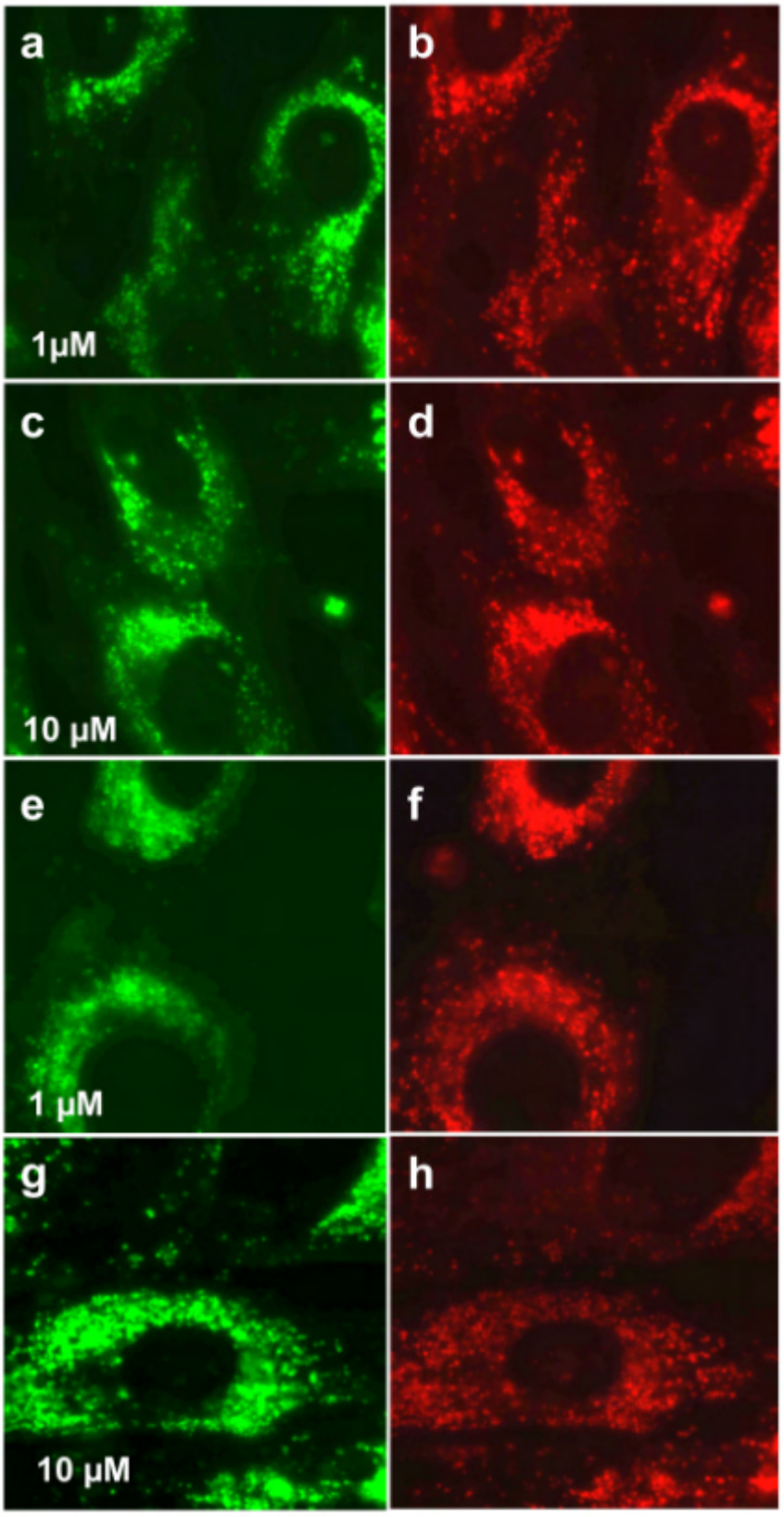


Figure 42 Fluorescence confocal microscopy of living human primary fibroblasts, incubated with spermidine-NBD conjugate **212** (**a-d**) or spermine-NBD conjugate **230** (**e-h**). **a / b** and **e / f** were incubated with 1 µM, **c / d** and **g / h** were incubated with 10 µM of the compound. The cells were co-incubated for 1 h with 150 nM *LysoTracker Red* to label the endosomal-lysosomal compartment (**b, d, f,** and **h**).[404]

The live images showed a pronounced uptake of the spermine conjugate **230** compared to the spermidine conjugate **212**, while the latter is still considerably taken up. It was concluded that the efficiency of intracellular accumulation depends on the number of positive charges as already observed for the CPPs.[8] The intracellular fate of the NBD derivatives **212** and **230** was determined by co-localisation studies with organellar markers. Co-incubation with *LysoTracker Red* for staining of the endosomal / lysosomal compartments and *MitoTracker Red* for the staining of mitochondria revealed a clear overlap with the endosomal / lysosomal compartment and therefore the uptake via the endocytic pathway for both polyamine moieties (Figure 42).

In order to test, whether either the cellular uptake can be increased or the intracellular routing of the polyamine can be directed to different organelles, the lipophilicity of the polyamine moiety was increased as described above (see chapter 4.2.3). After labelling with NBD, the lipophilic polyamines shown in Figure 43 were tested in cell culture.

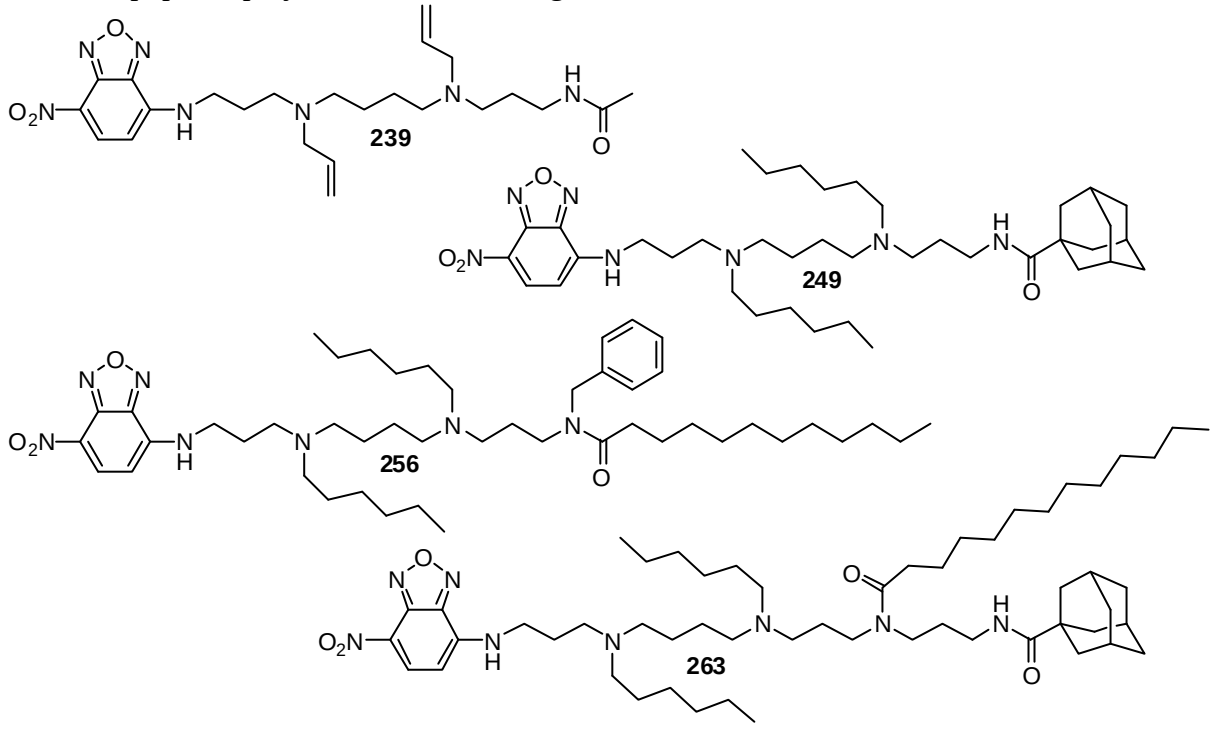


Figure 43 Structures of four lipophilic polyamines, which were labelled with NBD. These were also investigated by live-imaging.

Molecular dynamics calculations in collaboration with W. Wenzel (KIT, Karlsruhe) revealed that in contrast to the spermine conjugate **230** (Figure 40), the backbone of **249** is curved to minimise the steric interaction between the large hydrophobic substituents (Figure 44). The aliphatic C_6-side chains are mostly orientated to the same side of the backbone.

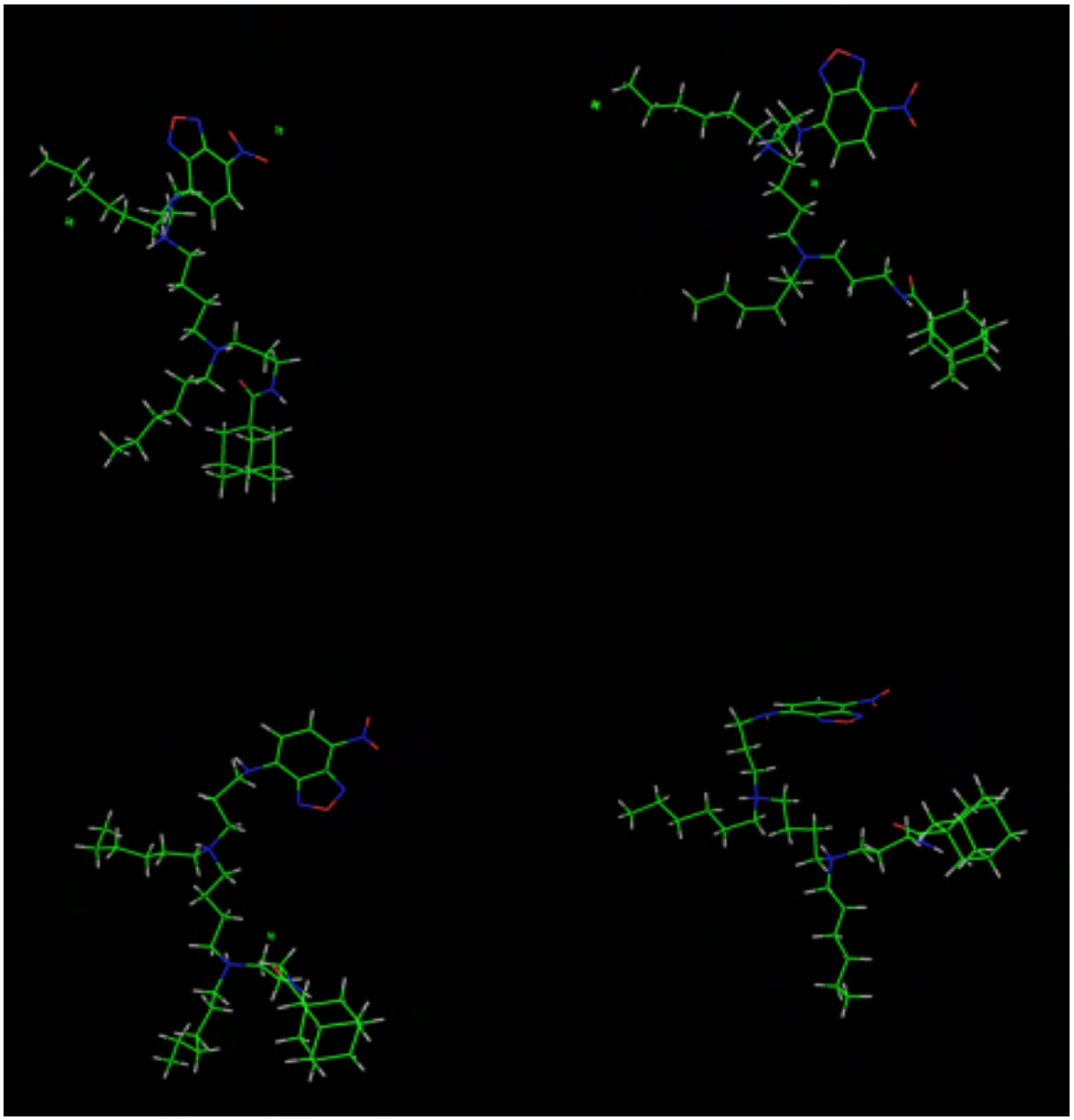


Figure 44 Calculations of lipophilic putrescine conjugate **249** arrangement in water. (Molecular dynamics calculations using AMBER-software; in collaboration with J. Setzler, W. Wenzel, KIT Karlsruhe)

HepG2 cells (human hepatic tumour), HeLa cells (human cervix tumour), and human primary fibroblasts (Figure 45) were incubated with these conjugates and evaluated by live imaging as described for the spermine- and spermidine conjugates. The intracellular distribution was determined by co-localisation studies using *LysoTracker Red* and others. All conjugates were efficiently taken up in human primary fibroblasts (Figure 45). The accumulation appeared to be mainly in vesicular structures, which are distributed in the entire cytosol. Co-incubation with *LysoTracker Red* for endosome / lysosome staining resulted in an overlap with the NBD fluorescence, indicating an endosomal distribution as observed for the more hydrophilic NBD-polyamines **212** and **230**.

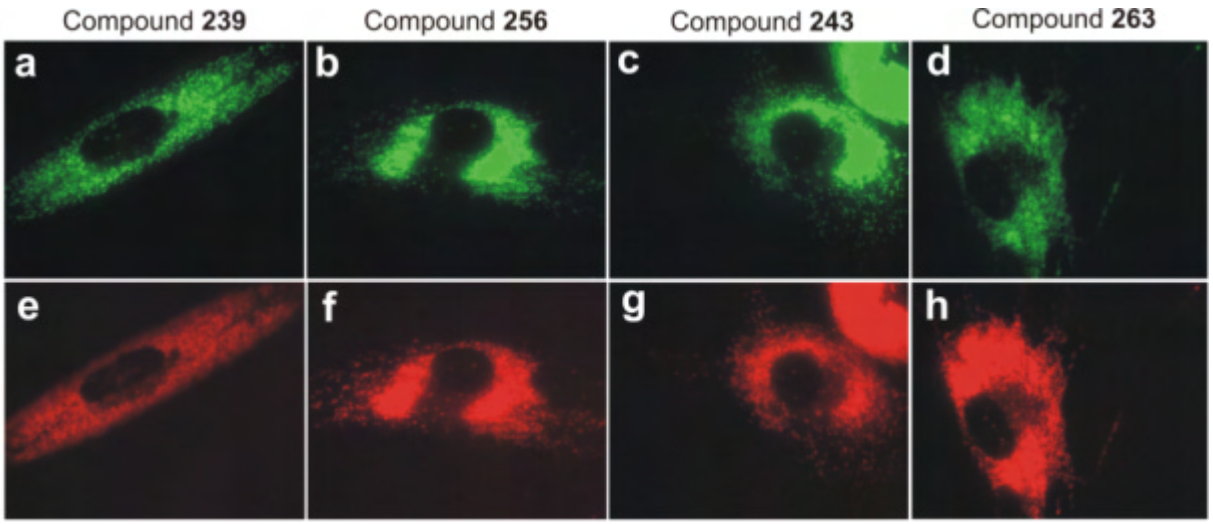


Figure 45 Fluorescence microscopy of living human primary fibroblasts. **a** to **d** show the cells after treatment with **239** (**a**), **256** (**b**), **249** (**c**), and **263** (**d**) (1 µM). One hour prior microscopy, the cells were co-incubated with 150 nM *LysoTracker Red* to label the endosomal-lysosomal compartment (e to h).[404]

However, the intracellular routing of the polyamines in tumour cells is different than in the non-tumourous fibroblasts. HepG2 and HeLa cells showed good uptake of the conjugates (Figure 46). While the localisation in the HeLa cells seems to be mainly vesicular in the perinuclear region, the accumulation in HepG2 cells occurred in larger compartments (Figure 47). The identity of those compartments is currently under investigation. First experiments in HepG2 cells pointed towards a colocalization with lipid droplets due to lipophilicity of the polyamines.

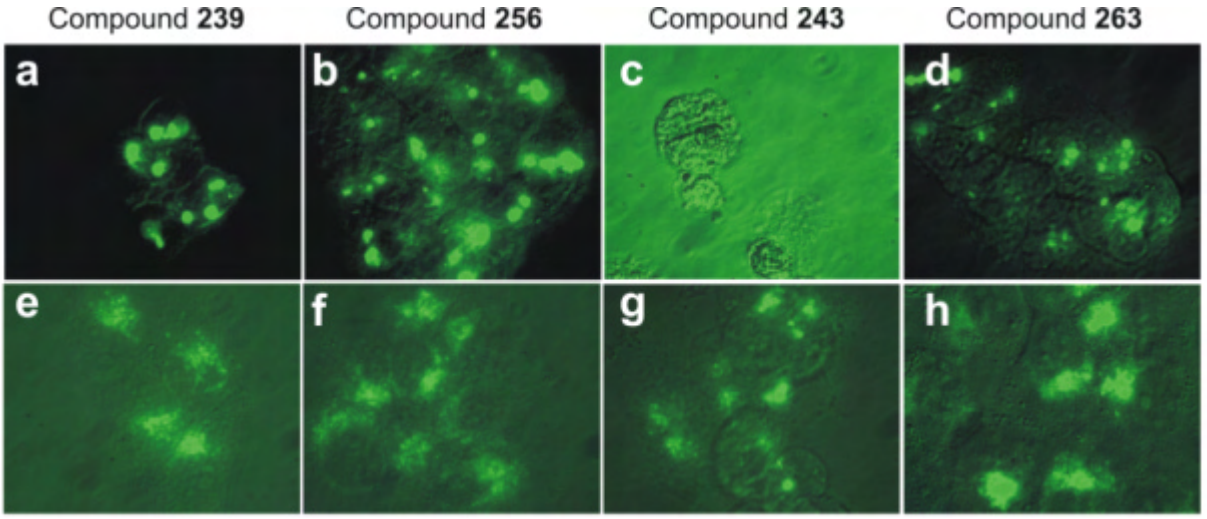


Figure 46 Fluorescence microscopy of HepG2 cells (**a** to **d**) and HeLa cells (**e** to **h**). The cells were incubated with 1 µM of **239** (a and e), **256** (b and f), **249** (c and g), and **263** (d and h) for 24 h.[404]

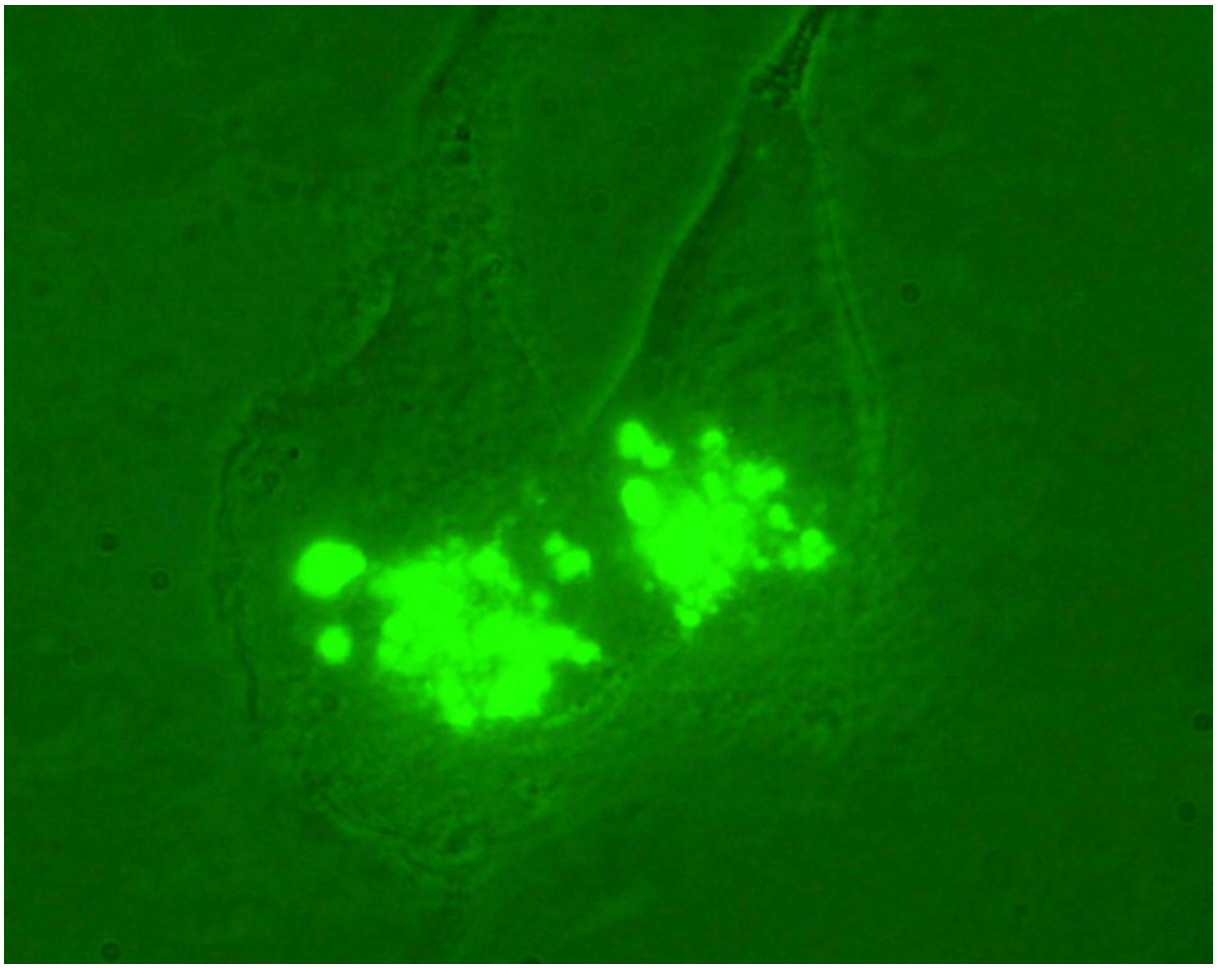


Figure 47 Accumulation of **239** in large compartments of the HepG2 cells.[404]

To quantify the cellular uptake of all polyamine moieties, the intracellular content of the NBD-polyamine conjugates was determined by a fluorescence-based assay.[404] HepG2 and HeLa-cells were incubated with 0.1, 1, 10, 20, and 100 µM of the respective conjugates. After 2, 24, 48, and 72 h the cellular surface bound polyamines were removed by trypsination and high salt washes and the fluorescence was determined and normalized to the number of living cells. The uptake of conjugates **239, 256, 249,** and **263** into HeLa cells is shown in Figure 48. The assays showed a concentration (**a – f**) and time dependent (**e, f)** uptake (Figure 48).

At higher concentrations up to 100 µM, the uptake is also markedly higher. Although the fluorescence is easily detectable by fluorescence microscopy in almost all cases, the uptake is not very high. HPLC determination of the intracellular content revealed that only up to 2 – 3% of spermine-NBD were taken up after 24 h.[404] However, the assays suggested a saturation of the cells, which has been also shown for other polyamine conjugates such as the spermidine-porphyrin conjugate **217** (see chapter 4.4.2).

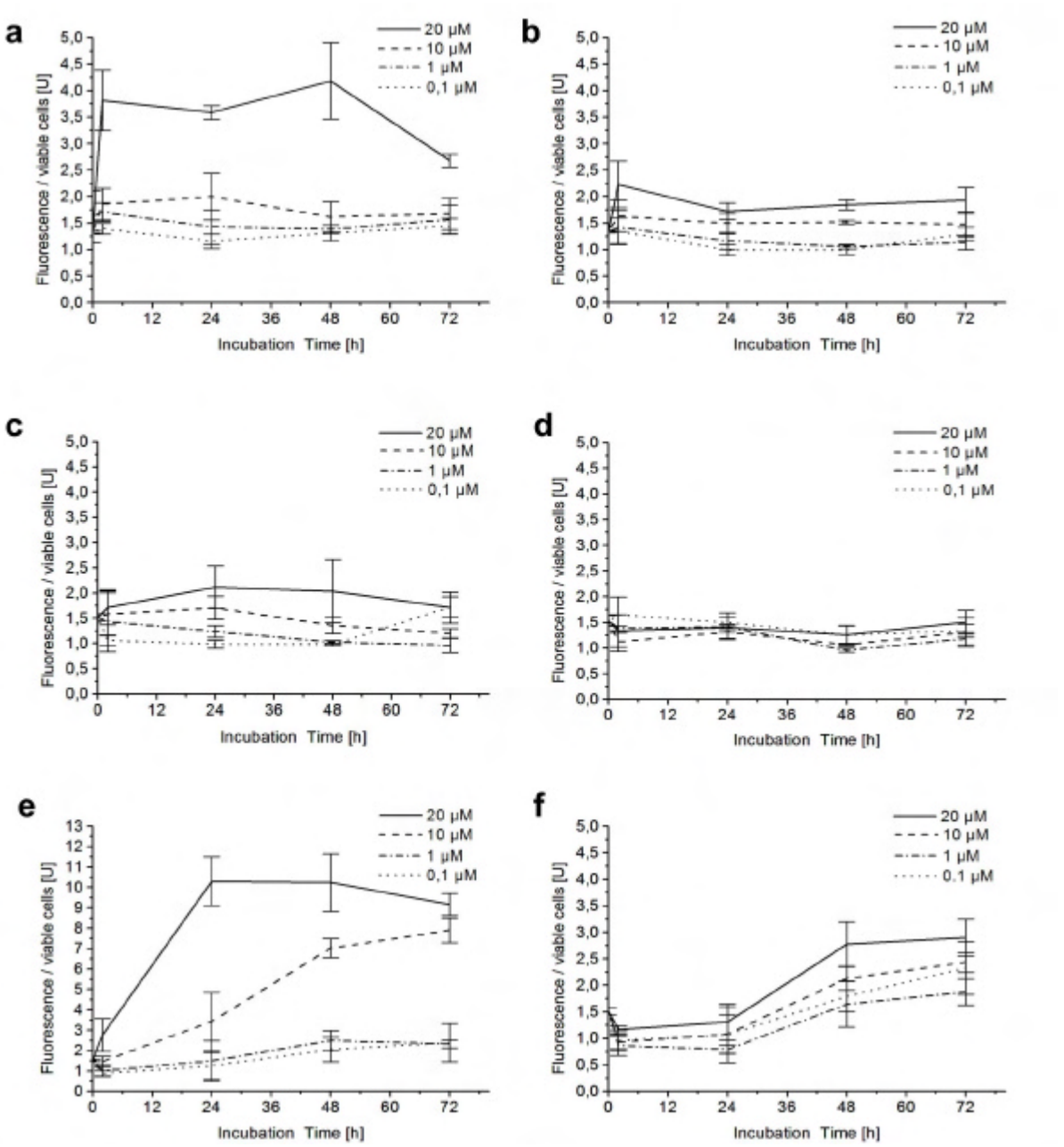
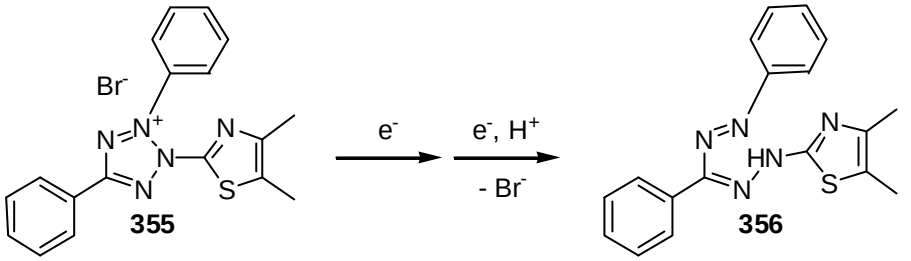


Figure 48 Uptake of conjugates **212** (**a**), **230** (**b**), **239** (**c**), **256** (**d**) , **249** (**e**), and **263** (**f**) into HeLa cells. To determine the NBD-polyamine concentration, fluorescence emission was read at 464 / 512 nm (excitation / emission) after 2, 24, 48, and 72 h by using a Thermo Scientific Varioskan plate reader. The emissions of n = 3 experiments were quantified and normalized to the viability of cells, previously determined by the MTT assay and the SD was calculated.[404]

For the application of the polyamine-conjugates as `molecular transporters´ it was additionally important to determine the toxicology of the polyamine moiety and the respective conjugates with potential drugs. Therefore, a classical colorimetric viability assay, the MTT assay was used. The MTT assay is based on the reduction of the tetrazoliumsalt MTT **(355)** to the purple, water insoluble formazan **(356)** in the mitochondria of living cells (Figure 49).[405] The reduction occurs by the mitochondrial succinate-dehydrogenase, which belongs to the respiratory chain of the mitochondria and is active only in metabolically active cells.



3-(4,5-Dimethylthiazol-2-yl)-
2,5-diphenyltetrazoliumbromid

(2*Z*,4*E*)-(4,5-dimethylthiazol-
2-yl)-3,5-diphenylformazan

Figure 49 Reduction of MTT **(355)** to formazan **(356)**.[405]

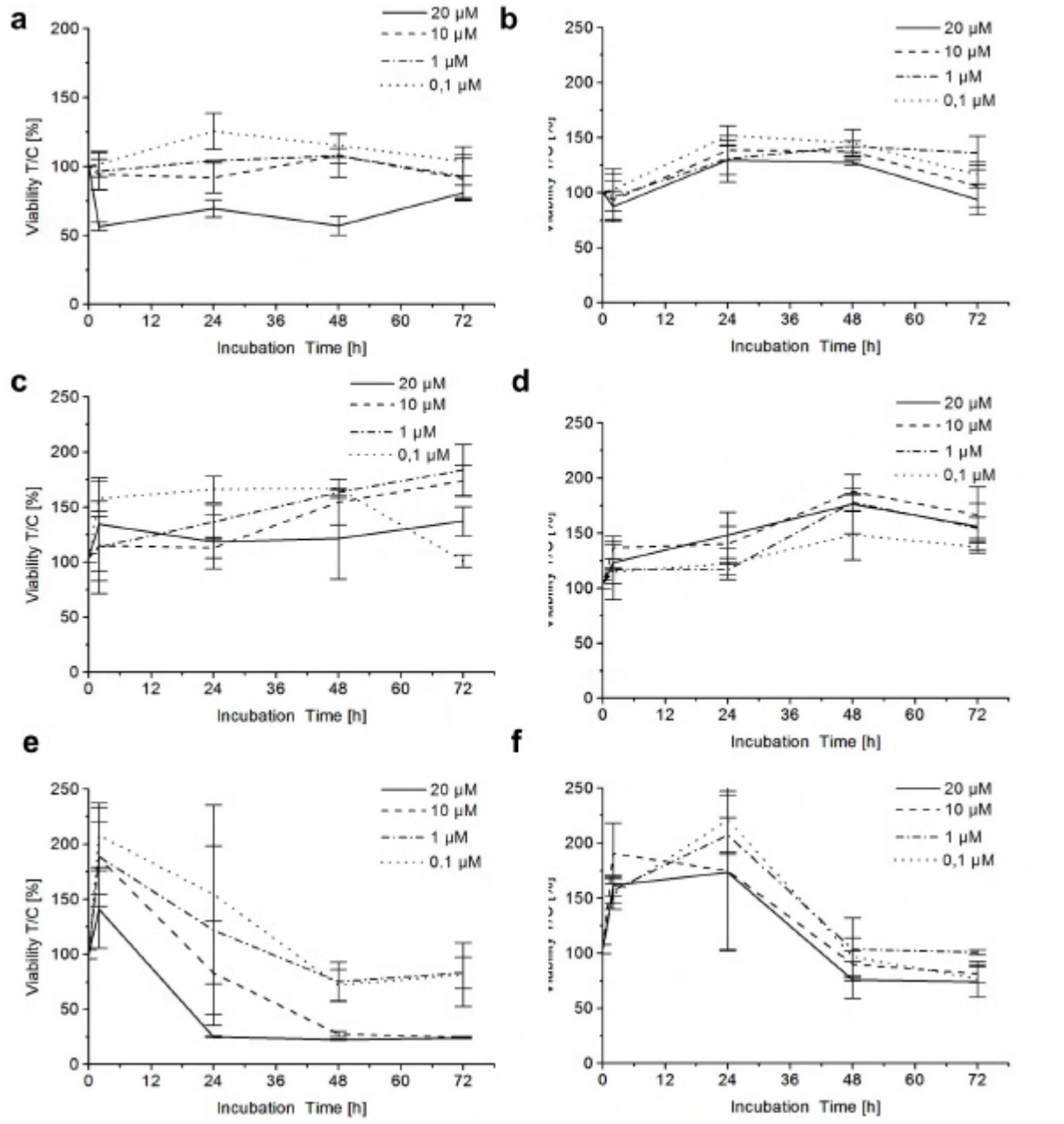


Figure 50 Time-and concentration dependent toxicity of conjugates **212** (**a**), **230** (**b**), **239** (**c**), **256** (**d**), **243** (**e**), and **263** (**f**). The toxicity was measured after 2, 24, 48, and 72 h upon incubation with varying concentrations of the compounds. The viability of the cells was measured using the Cell Titer 96 assay from Promega. T / C [%] = test over control (value for the viability of cells). SD was calculated from the n = 3 experiments.[404]

In analogy to the uptake assays, the cellular viability was measured in a time (2, 24, 48, 72 h) and concentration (0.1, 1, 10, 20, 100 µM) dependent manner (Figure 50). Most compounds showed a proliferative effect after 2 – 24 h in accordance with the proliferative effects reported for many other polyamines and their analogues.[52, 91, 92, 108] After longer treatments, the more lipophilic compounds bearing the adamantyl modification (compounds **249** and **263**) showed increased toxicity compared to the hydrophilic spermine and spermidine-NBDs **212** and **230** even in lower concentrations (Figure 50). The IC_{50} (Tox) values after 72 h treatment were ranging from 4 µM to 80 µM as depicted in Figure 51. Again, compound **243** showed the highest toxicity, while compounds **212**, **230**, and **263** were in a similar range as well as compound **239** and **256**.

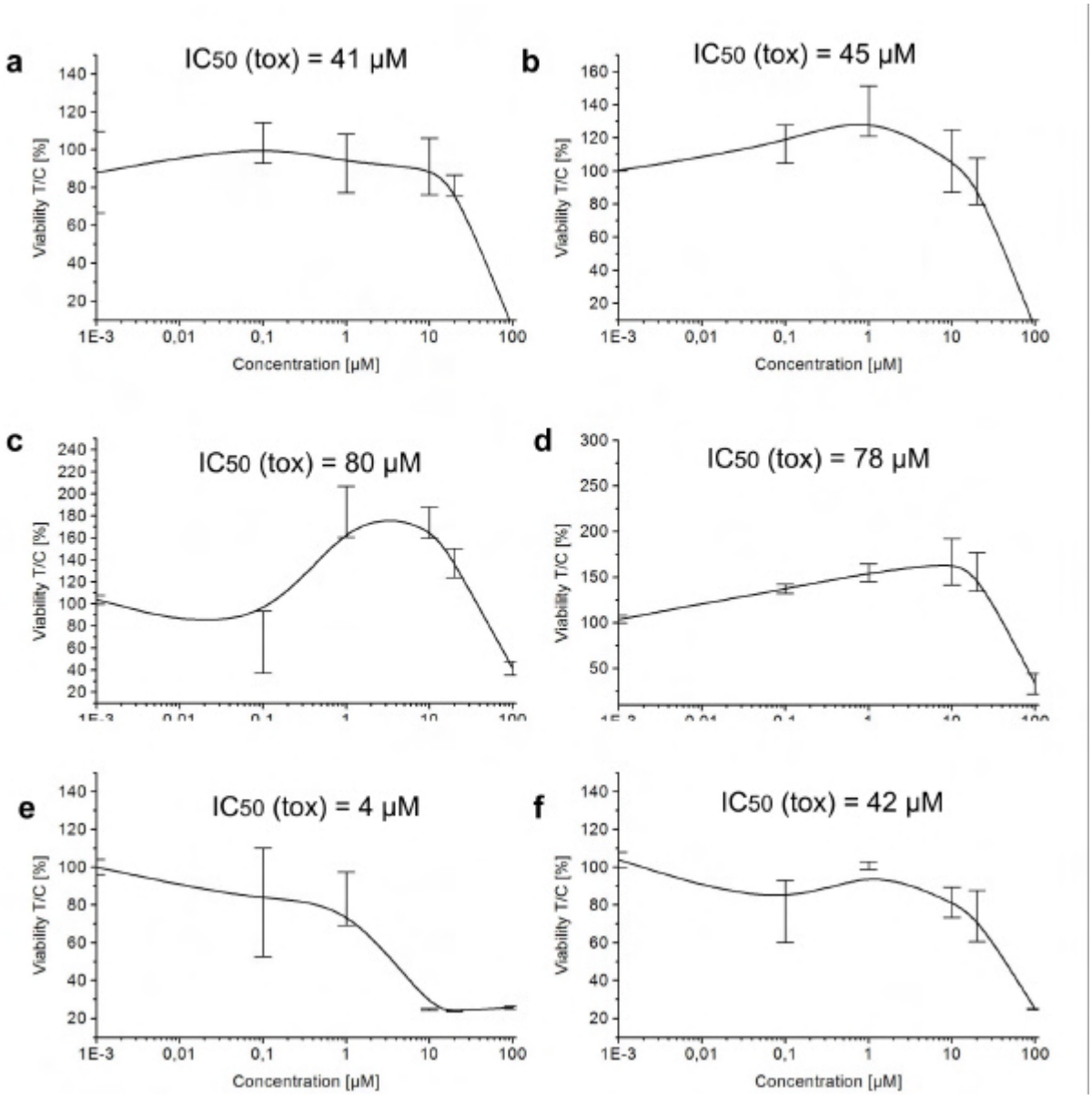


Figure 51 Concentration-dependent application of the toxicity of conjugates **212** (**a**), **230** (**b**), **239** (**c**), **256** (**d**), **243** (**e**), and **263** (**f**). The toxicity was measured after 72 h upon incubation with varying concentrations of the compounds. The viability of the cells was measured using the Cell Titer 96 assay from Promega. T / C [%] = test over control (value for the viability of cells). SD was calculated from the n = 3 experiments.[404]

At 100 µM, all conjugates showed a severe toxicity, which was also correlated with the amount of cellular uptake as depicted in Figure 48. It could be concluded that the toxicity of the conjugates is related to the amount of uptake. The stronger toxicity of conjugate **243** is again apparently, while it is visible that the increased toxicity goes along with stronger uptake of the compound from the medium. Further studies on the uptake and toxicity in other cell types are ongoing.

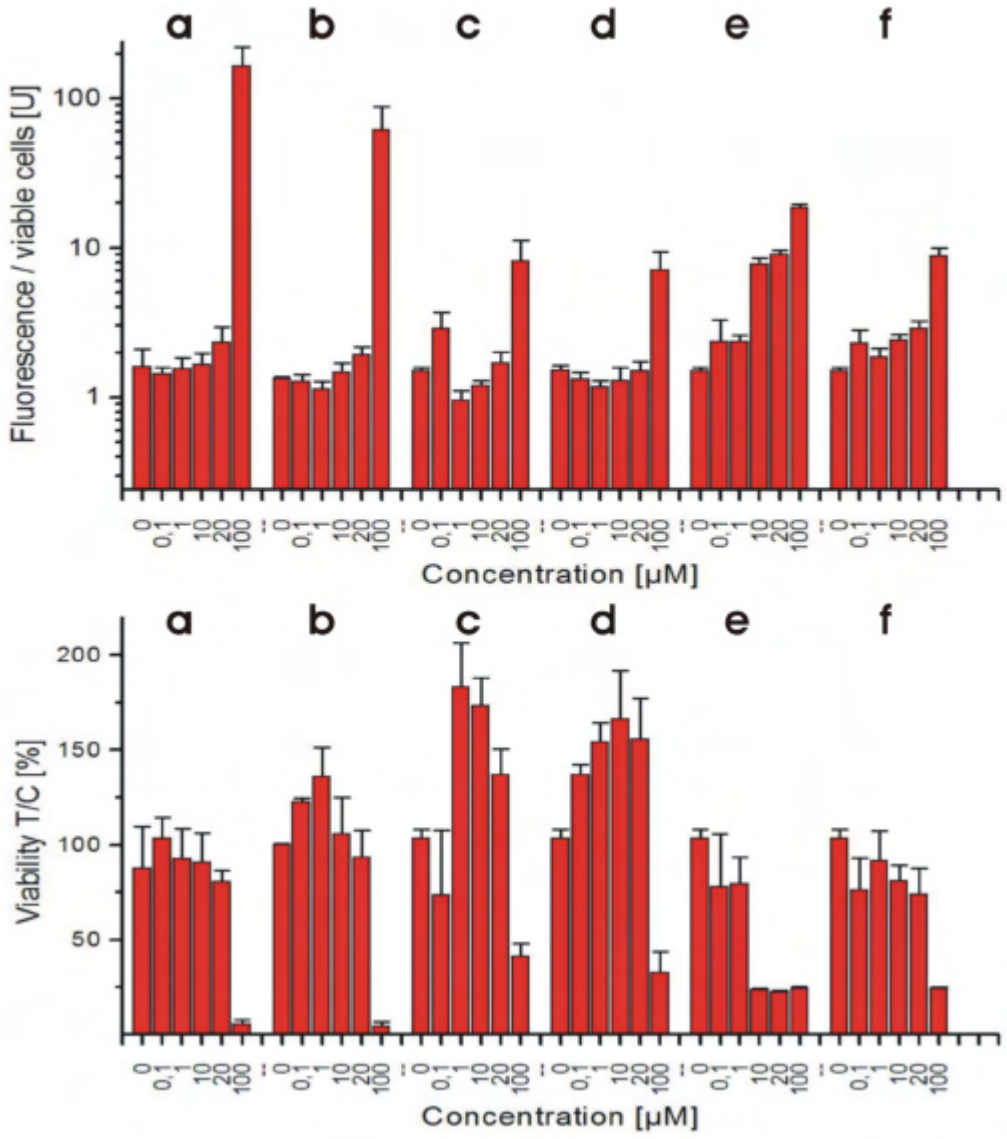


Figure 52 Compilation of the uptake and the toxicity of all NBD labelled conjugates. The toxicity of the conjugates is related to the amount of uptake (**212** (**a**), **230** (**b**), **239** (**c**), **256** (**d**), **243** (**e**), and **263** (**f**)). To determine the NBD-polyamine concentration, fluorescence emission was read at 464 / 512 nm (excitation / emission) by using a Thermo Scientific Varioskan plate reader. The emissions of n = 3 experiments were quantified and normalized to the viability of cells, previously determined by the MTT assay and the SD was calculated. The toxicity was measured after 72 h upon incubation with varying concentrations of the compounds. The viability of the cells was measured using the Cell Titer 96 assay from Promega. T / C [%] = test over control (value for the viability of cells). SD was calculated from the n = 3 experiments.[200]

4.4.2 Porphyrin-conjugate 217

To test, whether the polyamine moieties are capable to enhance uptake of therapeutic drugs into cells and tissues, the cellular uptake and biophysiological properties of the polyamine conjugates with porphyrin and Indomethacin **(171)** were further determined.

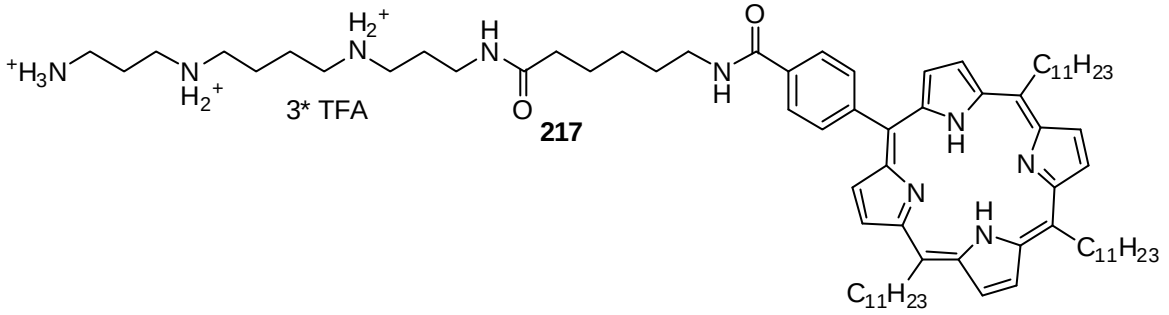


Figure 53 Structure of the spermidine porphyrin-conjugate.[200]

The polyamino-porphyrin conjugate **217**, which is shown in Figure 53, was well investigated during this work. The polyamine is separated from the porphyrin by a C_6-spacer that should allow a separation between the hydrophilic and the hydrophobic parts of the molecule (see chapter 4.2.1). Solubility studies with **217** showed that the protonated form is well soluble in polar alcohols such as methanol, ethanol and 50% methanol as well as in polar solvents like DMF or DMSO. Furthermore, it is poorly soluble in water, dichloromethane, and chloroform and insoluble in unpolar solvents like diethylether or cyclohexane. Therefore, the conjugate was solved in 50% methanol to reveal a stock solution of 20 mM allowing a low methanol content of the final incubation media. The UV spectra of solutions of **217** in water, containing low amounts of alcohol, showed the integrity of the typical porphyrin bands. This shows, that the conjugate is really solvated and that no unspecific aggregation occurs on the molecular level (see chapter 6.7 in the experimental section). On the other hand, carboxyporphyrin x was insoluble in water, poorly soluble in methanol and well soluble in chlorinated solvents. The highest concentration, which could be observed in methanol was below 40 µM. Finally, this required high percentages of methanol (more than 10%) in the media to achieve a final concentration of 5 µM after dilution. Such high methanol contents make it unsuitable for the use in PDT under physiological conditions.

COS-7 cells, HeLa cells, and fibroblasts were incubated for 24 h in the absence of light with concentrations of **217** ranging from 0.01 to 100 µM (Figure 54). Live imaging, which avoids artefactual diffusion due to membrane perforation during fixation[57, 61-63], showed that the conjugate is taken up into vesicle like structures in the perinuclear region. A nuclear or cytosolic accumulation was not observed. Removal of the incubation media and washing of the cell-surface with trypsine ensured that the conjugate is no longer attached to the outside of the plasma membrane.

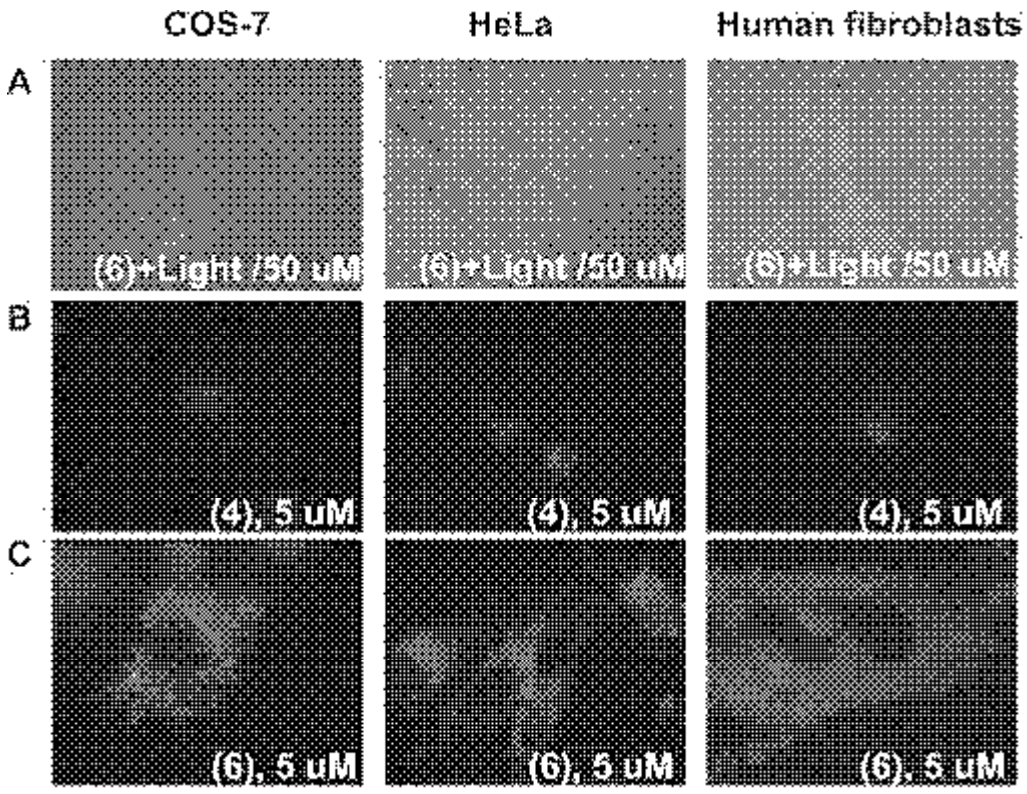


Figure 54 Treatment of COS-7, HeLa-cells and human fibroblasts with spermidine-porphyrin **217**: **A**, after a 4 h treatment with high doses (50 µM) of spermidine-porphyrin **217**, the majority of cells was killed in the presence of light. **B**, 24 h treatment with carboxy porphyrin **215** in the absence of light. **C**, 24 h treatment with concentration of 5 µM of spermidine-porphyrin **217** in the absence of light.[200]

As opposed to **215** that could not be detected inside the cells at a concentration of 5 µM, **217** exhibited a highly improved cellular uptake at the same concentration (Figure 54) as well as an increased uptake with the incubation time. Equivalent treatment of cells with **217** and 10% methanol concentration still showed efficient uptake but also decreased the cell viability to lower than 50%. Cellular uptake of **217** could be observed by fluorescence microscopy and fluorometric determination for concentrations as low as 0.5 µM. In almost all cells, uptake increased with time and concentration, implying a slow uptake of the membrane-attached compound. Studies on the dose and time dependent cellular uptake were performed in HeLa cells (Figure 55).

Figure 55 shows the time and concentration dependent quantification of the uptake. The amount of internalized spermidine-porphyrin was related to the amount of cellular protein after removal of the incubation media and cell-lysis. The treatment of cells with **215** at 5 µM did not show any significant uptake or accumulation. Although **217** is already rapidly taken up by the cells in low concentrations, the uptake reaches a saturation after 16 – 24 h depending on the initial concentration. At higher concentrations, the fluorescence seemed to saturate in the cells at 13 nmol / mg protein, and no further increase was found after longer exposure times. It is not clear, whether this saturation is due concentration quenching or real saturation of the compound. However, this result is similar to these of some NBD-conjugates presented in chapter 4.4.1. The proliferative effect that was obtained after feeding of some polyamine conjugates might be responsible for the dilution and the following quasi-stationary state (see Figure 60 and chapter 4.4.1).

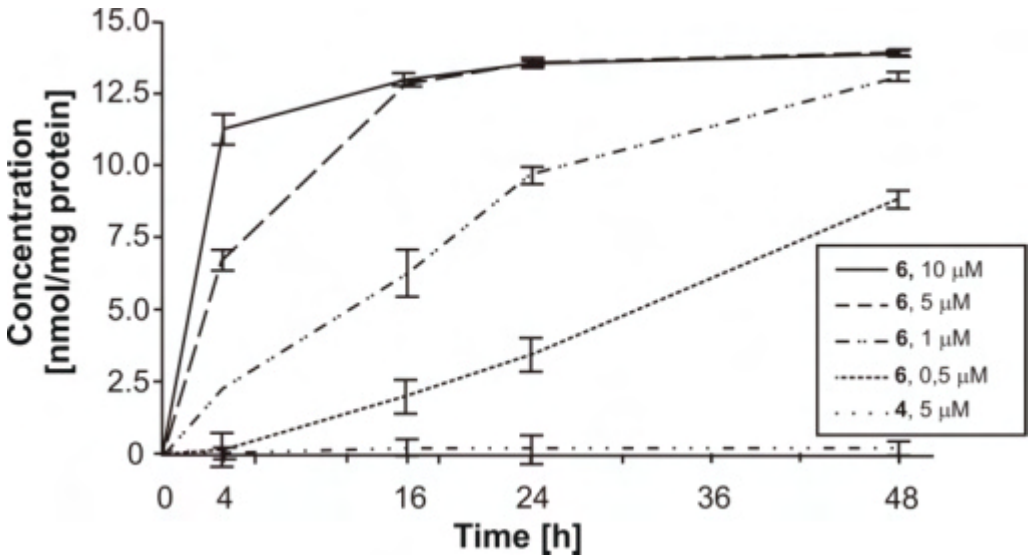


Figure 55 Time and concentration dependent uptake of polyamine-coupled porphyrin **217** in HeLa cells. HeLa cells were treated with different concentrations (0.5, 1, 5, and 10 µM) of **217** and 5 µM of **215** and harvested at different time points as described (see chapter 6.8 in the experimental section). To determine the polyamine-porphyrin concentration, fluorescence emission was read at 420 / 650 nm (excitation / emission) by using a Thermo Scientific Varioskan plate reader. The emissions of n = 4 experiments were quantified and normalized to the amount of the total protein and the SD was calculated.[200]

Co-localisation studies with cellular markers such as *LysoTracker Green*, *MitoTracker Green*, NBD-C_6-ceramide for Golgi staining and DAPI for nuclear staining were performed to investigate the cellular fate of **217** (Figure 56). Staining with Lysotracker did not reveal any co-localisation, even though the punctuate pattern is indicating vesicular structures. Co-incubation experiments with *MitoTracker Green*, however, showed a mitochondrial localisation of the amphiphilic porphyrin and often a strong vesicular accumulation in the perinuclear region especially in tumour cells (**e-h** in Figure 56). At higher doses or after prolonged incubation time, the vesicular structures in perinuclear region became more pronounced. However, these structures could not be co-labelled with *LysoTracker green* or *MitoTracker green* and their identity has to be determined in further studies. In hepatic tumour cells (HepG2), there are preliminary indications that the perinuclear vesicles are derived from lipid droplet fusion.

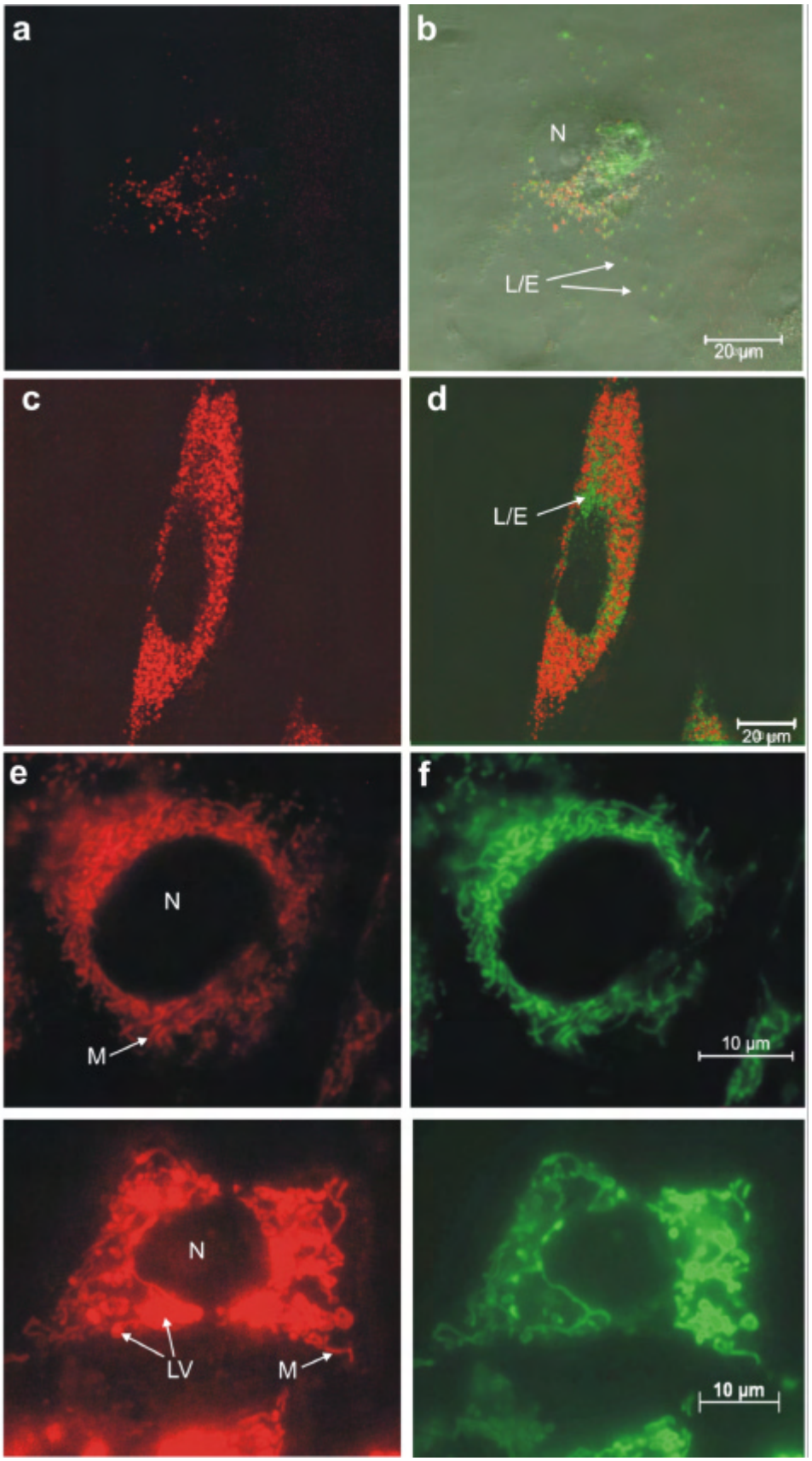


Figure 56 Fluorescence confocal microscopy of living COS-7 cells **(a, b)**, human primary fibroblasts **(c, d),** HeLa cells **(e, f),** and HepG2 cells **(g, h)**. The images show cells after co-incubation with compound **217** (**a, c, e:** 5 µM**, g**: 1 µM) and *MitoTracker Green* (**b, d**: 150 nM) or *LysoTracker Green* (**f, h**: 150 nm).

For PDT, a mitochondrial localisation is desired to exhibit a stronger phototoxic effect and to induce not only necrosis but also apoptosis. It has recently been shown that amphiphilic cations including guanidyl-poprphyrins[378, 380, 406] are rapidly delivered to mitochondria due to their highly negative membrane potential. The hydrophobic moiety is believed to help with the integration into the lipid bilayer.[378, 380]Previously, a photodynamic approach has been reported for the killing of bacteria on the skin analogous to PDT for cancer.[407] A variety of compounds with photoactive properties have been tested against gram-negative and gram-positive bacteria.[408, 409] Anionic porphyrins showed no activity against either gram-positive or gram- negative bacteria due to their impaired uptake or interaction with the bacterial cell wall. As it is well known that both *E. coli* and gram- positive bacteria interact with positively charged peptides, the positively charged spermine–porphyrin **217** was tested for its phototoxic effect on *E. coli* and gram-positive lactobacillus. The aim of this study was to find in vitro conditions for the killing of bacteria in presence of eukaryotic cells as a model for a later treatment of bacterial infection in vivo. Polyamine-porphyrin **217** showed efficient uptake in all tested organisms such as tumour and primary cells, gram-positive and gram-negative bacteria, and yeast (**a-c, f** in Figure 57). However, in a co-culture of bacteria and tumour cells, tumour cells preferentially bound and took up the conjugate, while in a co-culture of bacteria and primary cells, the uptake into bacteria was preferred after 2 h. After 15 min irradiation, all of the bacteria were found dead and no re-growth could be detected even after 72 h cultivation.

Likewise, a co-culture of tumour cells and primary cells revealed a preferential uptake of the **217** in tumour cells when the cells are treated with low concentrations and for short incubation times (**i** in Figure 57) This phenomenon is probably due to the density of negative charges on the outer membranes, which is increasing from primary cells over bacteria to tumour cells. It is not clear, whether the different uptake efficiency will rely on the interaction of the spermine moiety with the proteoglycans or negatively charged lipids on the surface of the different cells and organisms or whether it depends on an active transport system such as the polyamine transporter. Nevertheless, this assumes that **217** might be a potent candidate for PDT in vivo with less risk for the damage of healthy tissue. Further studies with multiresistant *Staphylococcus aureus* strains are ongoing.

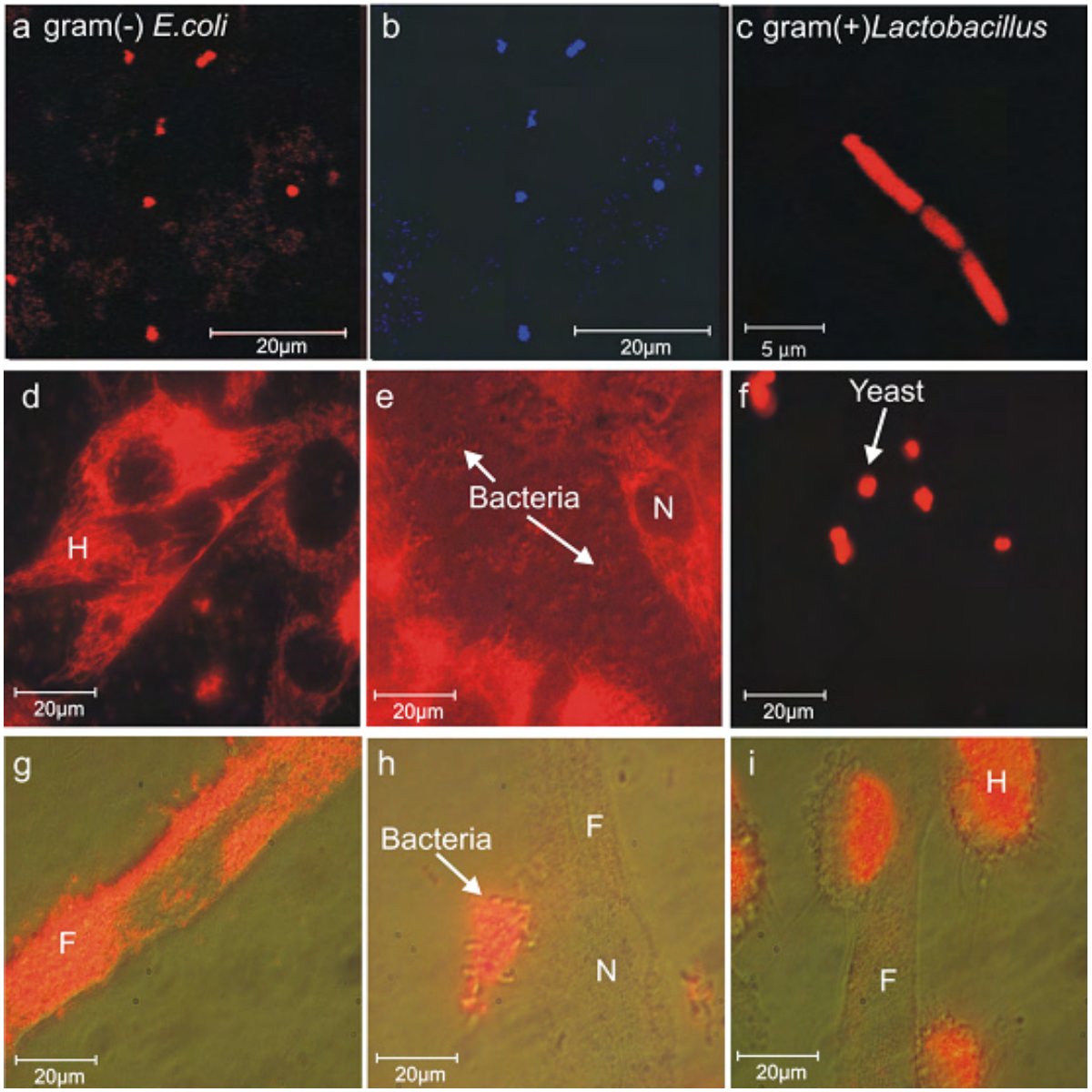


Figure 57 Gram-(-) (*E. coli*) (**a, b** = DAPI counterstain) and gram-(+) (lactobacillus) (**c**) bacteria as well as yeast (**f**) revealed excellent uptake of **217** (1µM) after 2 h while **215** was not taken up. Irradiation of 15 min and 24 - 72 h incubation at 37 °C did not show any bacterial regrowth in a colony-forming assay. Simultaneous treatment of a co-culture of tumour cells (HeLa) and gram-(+) bacteria (**e**) or primary cells (human fibroblasts) and gram-(+) bacteria (**h**) showed a competition between the cells and the bacteria compared to non infected cells (**d** for HeLa; **g** for fibroblasts). Likewise, HeLa cells take up **217** faster than fibroblasts when incubated for 1 h After 24 h both cell lines show equal uptake. H= HeLa cell, F= fibroblast. **a-c**, **f** confocal images. **d,e g-i**, Nomarski images were merged with the fluorescent images of **217** (red).[200]

In vivo studies in mouse model showed an accumulation of conjugate **217** in the liver while a peptoid-conjugate bearing the same porphyrin moiety is targeted to lung and heart (Figure 58).[410] In comparison with the peptoid-derivative, the emission of both conjugates differed when excited at the same wavelength. The peptoid conjugate is still detected at 680 nm, while the polyamine-porphyrin is detected at 800 nm. A similar effect was observed for the rhodamine derivatives (see chapter 4.4.1).

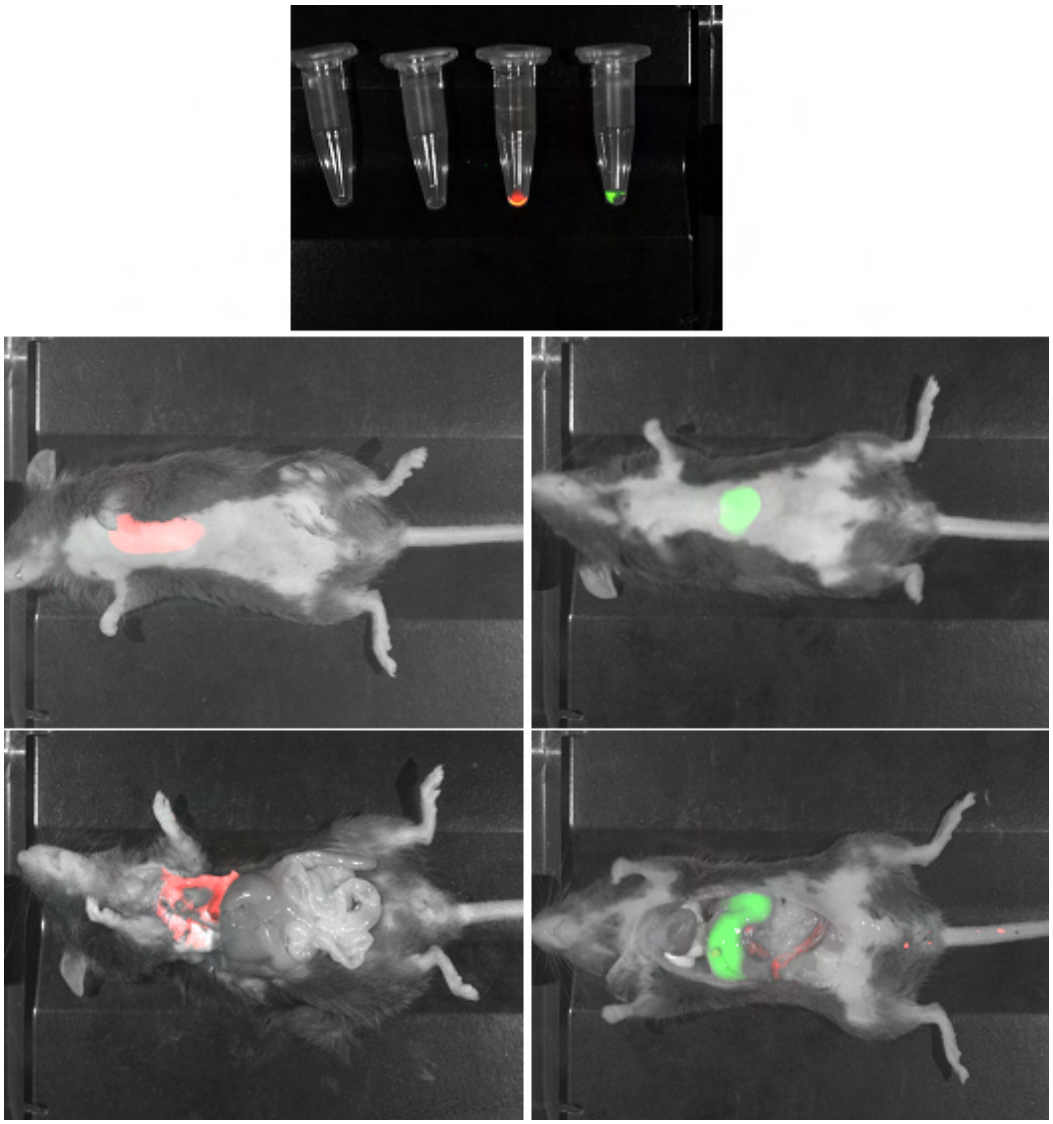


Figure 58 Comparison of a peptoid-porphyrin conjugate[82] and polyamine-porphyrin conjugate **217**.[200] Although they bear the same lipophilic porphyrin, their emission after excitation at the same wavelength is different (first row). After injection into mice, the conjugates accumulate in different organs (second and third row). While the peptoid conjugate appears mainly in the lungs, the polyamine conjugate was mainly found in the liver.[410]

As already mentioned, many porphyrins exhibit a strong phototoxicity and are suitable for Photodynamic Therapy (PDT). Their phototoxic efficacy depends on the chemical modification and their intracellular localisation. As shown in Figure 59, spermidine-porphyrin treated cells could be destroyed by irradiation with visible light. The mitochondrial accumulation of **217** suggested a very efficient phototoxicity, which would only require low concentrations of conjugate **217** to induce apoptosis and necrosis. The cells show severe necrosis already 2 min after visualization in the combined fluorescence and transmission light mode of the microscope. The membrane structure of the cells seemed to be disrupted and a release of the fluorescent material is observed indicating necrosis (**A**). After a 24 h incubation post photo-irradiation, many cells display nuclear fragmentation indicating apoptotic cell death (**B**). In both cases, the Nomarski images were merged with the fluorescent images of the porphyrin **217** labelling

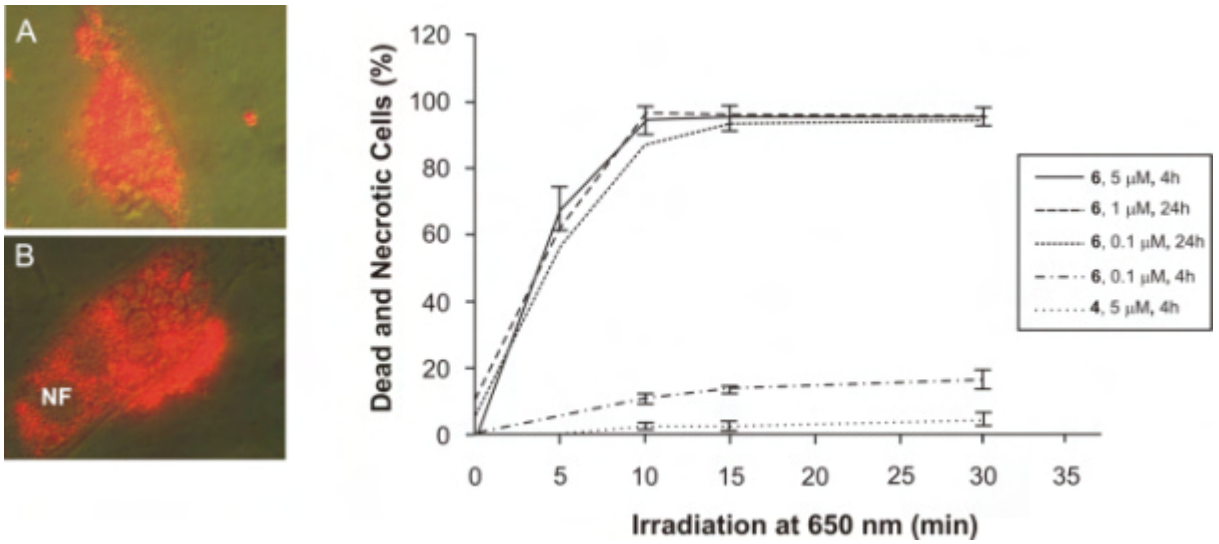


Figure 59 Live imaging of primary fibroblasts treated with 1 µM of **217** after photo-irradiation with visible light (**A, B**).[200] Quantification of the phototoxic effect of **217** on HeLa cells. Cells were incubated with 0.1, 1, and 5 µM of **217** and 5 µM of **215** for 4 h and 24 h. Eventually, the cells were irradiated with 650 nm laser light for various times. Quantification of dead and necrotic cells was performed after staining with propidium iodide and trypan blue. The irradiation of cells in absence of photosensitisers did not trigger cell death. The cells were counted in 3 x 4 random fields framed with the Axiovision software and the readout was evaluated by the student's test. SD was calculated from the n = 5 experiments for the following conditions: **217**, 5 µM, 4 h; **217**, 0.1 µM, 4 h; **215**, 5 µM, 4 h. The data (**217**, 0.1 µM, 24 h; **217**, 1 µM, 24 h) were taken from single experiments.[200]

A more detailed investigation on the course of cell death is shown in Figure 59. It is visible that carboxyporphyrin **215** has only minor effects. The minimal cell death, even after 30 min irradiation, indicates a very constricted uptake. For **217**, which was incubated for 4 h and 24 h in concentrations of 0.1, 1, and 5 µM, the experiment shows that severe phototoxicity was already visible after 5 min of irradiation with 650 nm light. When cells are treated with 5 µM of **217** for 4 h, about 70% of the cells were killed, while no cells survived the treatment for 15 min. In lower concentrations, the compound has to be enriched for a longer incubation period to induce efficient cell death. Only in case of very low dose (0.1 µM) and short incubation time of 4 h, the incubation did not suffice to kill all the cells within 15 min. Comparison with the results from Figure 55 yield that once an inner-cellular concentration of 6.5 nmol / mg protein was reached, the cells could be entirely killed after 10 min irradiation. Thereby, cell death correlated with intracellular saturation of the compound and irradiation time, while non-irradiated cells were not affected. The effective dose (ED_{50}) for irradiation seems to be dependent on the total amount of the internalized or membrane bound compound. The dark toxicity of **217** was measured 72 h after addition (Figure 60). While exhibiting a strong phototoxic effect, the dark toxicity of **217** was low but not as low as reported for porphyrin derivatives coupled to polycationic peptides, which accumulate in the endosomal compartment.[406] It is assumed that the mitochondrial localization of **217** is responsible for the higher toxicity, as it has been shown for other mitochondria localized porphyrin derivatives.[406] The IC_{50} values of the dark toxicity were estimated as 81,2 µM for HeLa cells and 98,4 µM for primary fibroblasts (**c** in Figure 60) making the compound well suited for the application as a photosensitiser.

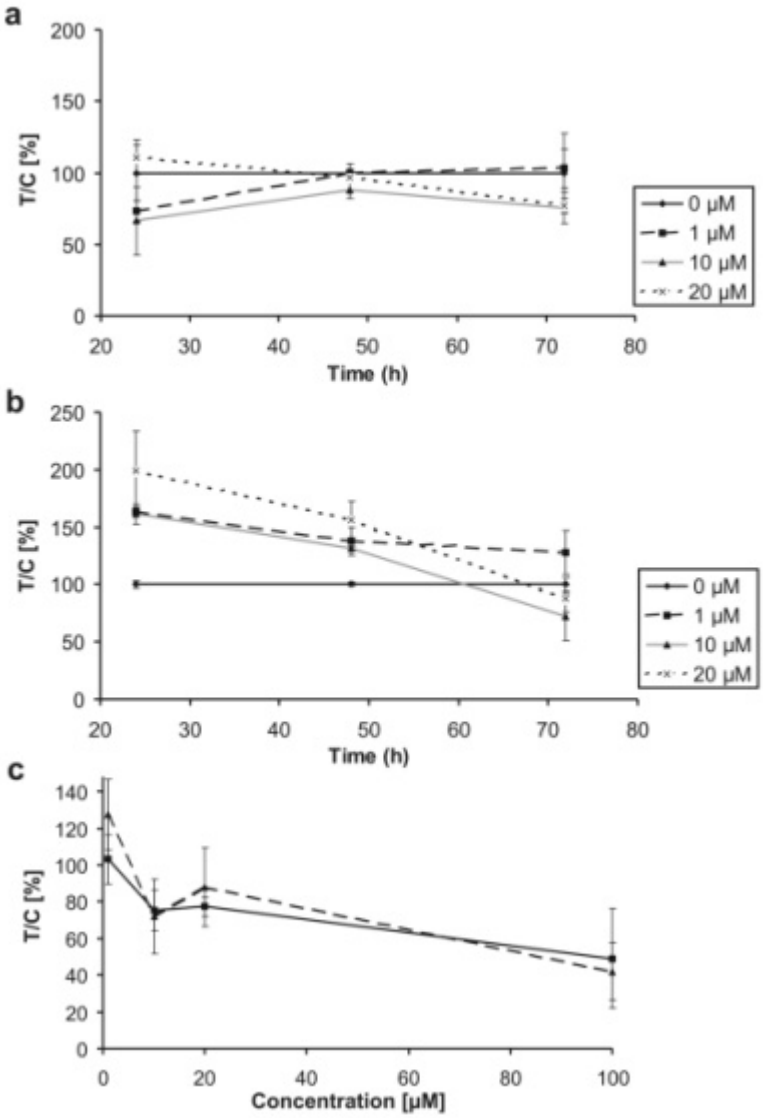


Figure 60 Dark toxicity of spermine-porphyrin **217** in primary human fibroblasts (**a**) and HeLa cells (**b**). The dark toxicity was measured after 24, 48, and 72 h upon incubation with varying concentrations of **217**. The viability of the cells was measured using the Cell Titer 96 assay from Promega. (**c**). Concentration dependent dark toxicity after 72 h in HeLa cells (dashed line) and primary human fibroblasts (full line). T / C [%] = test over control (value for the viability of cells). SD was calculated from n = 3 experiments.[200]

4.4.3 Indomethacin conjugate

It can be expected that Indomethacin-polyamine conjugates bear high toxicity, as even the intrinsic indomethacin is toxic and an improved uptake would enhance its effect. This can be tolerated, if the refinement of the impact is equal or higher than that of the toxicity.

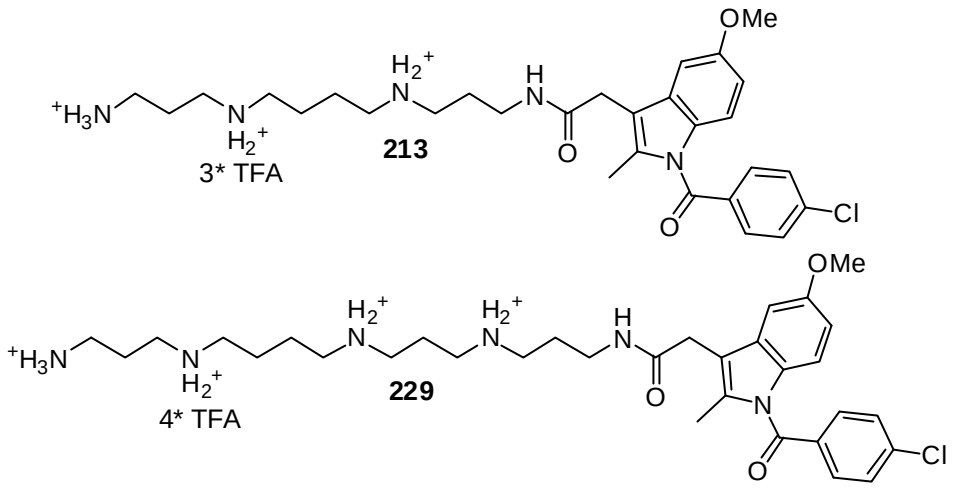


Figure 61 Structures of spermidine-indomethacin **213** and spermine-indomethacin **229**.

The toxicity was determined by the SRB assay. The sulforhodamine B test was used to stain cellular proteins can be stained by sulforhodamine B in weakly acidic media as all basic side chains are sufficiently protonated to be complexed by the sulfonate groups. The dye can be removed from the proteins under basic conditions. Finally, it can be measured photometrically at 570 nm.

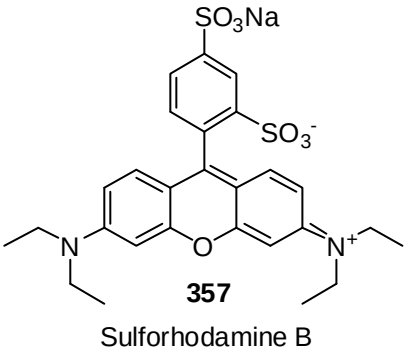


Figure 62 In slightly acidic medium, Sulforhodamine B **357** stains the basic side chains of proteins by complexation with its sulfonate groups.[411,412]

The results from Figure 63 show that spermidine-indomethacin conjugate **213** and spermine-indomethacin conjugate **229** show strong toxicity for concentrations above 1 µM. The toxicity for both follows the trends of spermidine **(15)** and spermine **(16)**, with the conjugates being slightly more toxic than the particular polyamines. Indomethacin **(171)** is less toxic than both, the conjugates and the polyamines. Compared to spermidine-indomethacin **213,** the toxicity of spermine-indomethacin conjugate **229** is slightly increased. The same correlation exists between the unmodified polyamines.

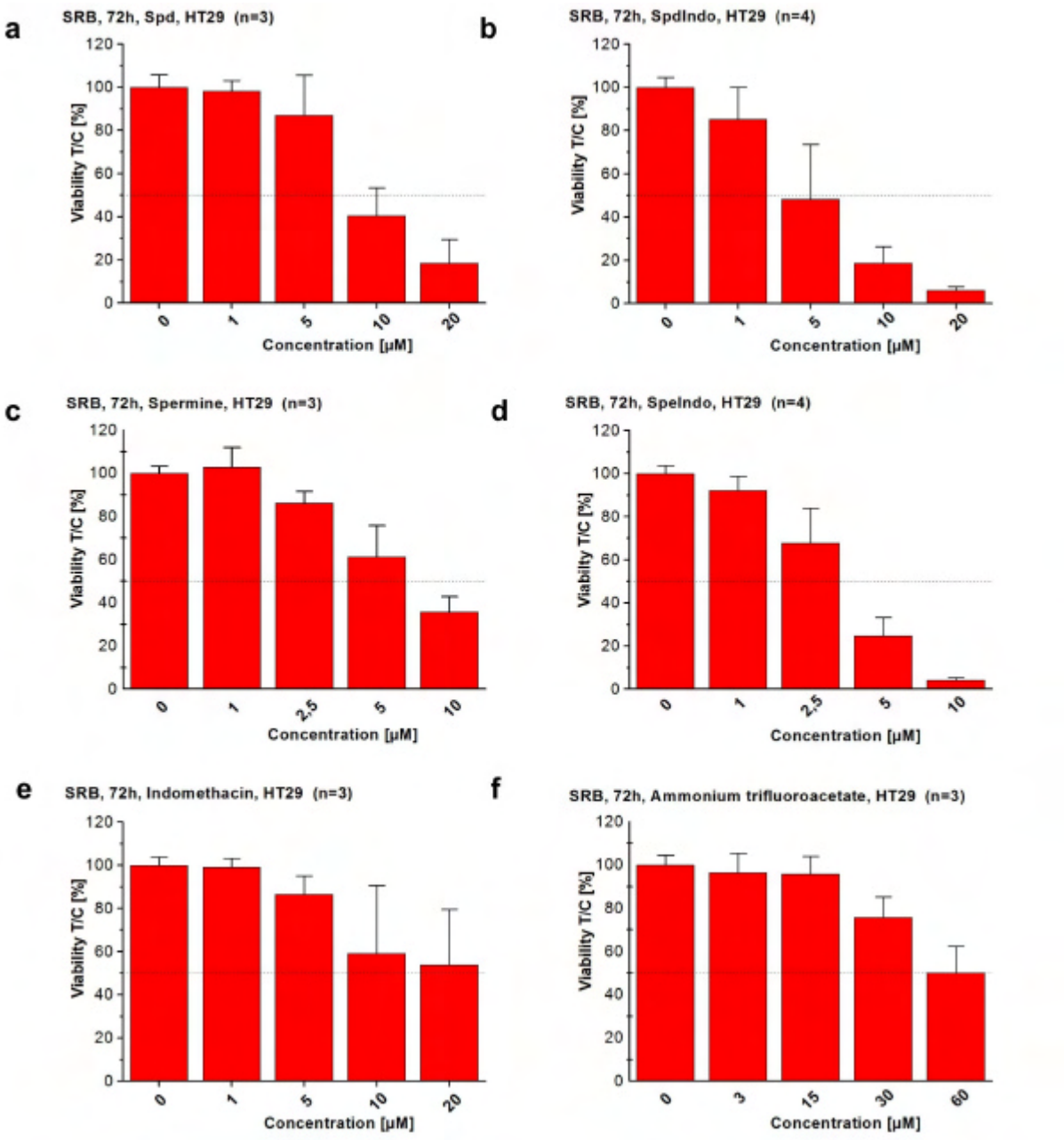


Figure 63 SRB assay of spermidine **(15) (a)**, spermidine-indomethacin conjugate **213 (b)**, spermine **(16) (c)**, spermine-indomethacin conjugate **229 (d)**, indomethacin **(171) (e)**, and $NH_4(F_3COO)$ **(f)**. The test was performed with HT29 cells and the incubation time was 72 h. The average values and standard deviations were related to the control value of cell growth in medium spiked with 1% DMSO. (in collaboration with J. Pelka, D. Marko, KIT Karlsruhe)

5 Conclusion and outlook

A variety of symmetric and asymmetric spermine building blocks were prepared. The combination of *o*-Nosyl- and Aloc group was advantageous, because it greatly facilitates the work-up and gives stable entities. The asymmetric building blocks **179, 181**, and **187** were applied for solid phase chemistry as they afford loading of resins under avoidance of cross-linking. Especially building block **187** was useful as it additionally allows the differentiation of primary and secondary amines. Furthermore, it also permits the direct elongation of the backbone by *Fukuyama alkylation* without requiring trans-protection. Thus, 2-chlorotrityl resin **(60)** loaded with **187** was chosen to act as universal starting point for most of the following solid phase syntheses. The versatility of the novel protection group system for polyamine solid phase chemistry was consistently demonstrated. Problems occurring during the Aloc deprotection were successfully solved. Finally, the first solid phase synthesis of the spider toxin HO359b **(203)** proved that the protection group combination is equal to previously established combinations. *Fukuyama alkylation* with halides was identified as the method of choice for backbone elongation and modification. A detailed study revealed optimised reaction conditions for different substrate types. Those allow the introduction of several functional groups and are more economical in terms of inserted halide than previously published examples.[289] The combination of *o*-Nosyl / Aloc protection and *Fukuyama alkylation* for backbone elongation is selective and milder than many previously reported examples. Thus, it allows the synthesis of structurally more diverse molecules on trityl linkers. In contrast to examples, in which Boc- or Nosyl groups served for the permanent protection of backbone-secondary amines, Aloc groups can be selectively removed and allow further modification of the liberated amines. It showed that *Reductive amination* is able to mono-alkylate the backbone amines, allowing their substitution with aldehyde-building blocks. *S_N2-alkylation* is restricted to sterically less hindered building blocks. The use of other resins, which do not bear a rigid trityl linker, could improve this result and allow the introduction of such bulky groups as for example adamantyl groups.

For the development of coupling methods, the *Michael addition* between maleimides and thiols was chosen. Starting from resin **198**, the *o*-Nosyl group was removed and a maleimidopropionyl group was introduced avoiding cross-linking, by reaction of the free amine with pre-activated building block 3-*N*-maleimidopropionyl succinate **(196)**. The reaction conditions for addition of ethylsulfide to the maleimide were optimised and the reaction was applied to a small library of sulfides. The reaction showed to be suitable for the coupling of thiols of medium polarity, but not suitable for unpolar thiols. For coupling of polar thiols, it is necessary to add raising amounts of water into the solvent, in order to achieve sufficient solubility of the thiol. This collides with the swelling properties of polystyrene resins, which only tolerate low doses of protic solvents before they collapse. It was assumed that the solution would be the use of resins with better swelling properties in polar solvents. This is suited by PEG-modified resins, which are commercially available. Transfer of the reaction conditions of the synthesis of resin **277** to a Tentagel resin gave the product in only poor yield and purity. As it is known that Tentagel resins are difficult to handle, oxidation of the PEG scaffold upon air contact during synthesis could be responsible for this. For further studies, it would be necessary to optimise the preparative conditions. Once established, the coupling of polar thiols, particularly thiol-modified oligonucleotides, avoiding undesired electrostatic interactions, should be possible. It was shown that Aloc deprotection takes place and the formed thioether is not detrimental to the Pd-catalyst. The

chosen protection strategy would also converge with the coupling of peptides or DNA, because both are stable during Aloc deprotection.

The *vice versa* strategy, introduction of a thiol on solid phase and subsequent coupling to maleimides was also examined. For this, the introduction of 2-iminothiolane proved to be especially promising. After successful suppression of diverse side reactions, the developed protocol showed to work excellently for the cross-linking reaction. If used with extremely acid labile alkoxytrityl resins, sensitive conjugates like polyamino nucleosides and spermine conjugate **360** could be successfully synthesised (see end of this chapter). The selectivity of 2-iminothiolane for primary amines supersedes the use of complex protection schemes to allow regioselective introduction. The precursor maleimides **313** and **319** were prepared starting from the 2´-deoxynucleosides in four or five steps giving 28% or 16% yield, respectively. A triphosphorylation of the conjugate **323** was not successful following the standard protocol of Kovacs and Ötvös. To exclude that a negative interaction with the 3´-hydroxy group or the huge polyamine substituents was the reason for this, 2´,3´-dideoxy-N^4-3-maleimidopropionylcytidine **(331)** was synthesised. Reaction over seven steps gave an overall yield of 18%. As the triphosphorylation in analogy to Kovacs / Ötvös was again not successful, a more recent protocol from Ahmadibeni / Parang was applied on modified dideoxycytidine **331**. These attempts also did not result in the desired triphosphate. Taken together, the synthesis of a maleimido-modified triphosphate was not successful. The most promising point of contact for further studies could be the isolation of the monophosphorylated nucleoside from the method according to Kovacs and Ötvös and use of another method for its conversion into the triphosphate. Alternatively another coupling method could be used, which does not require the synthesis of maleimido-modified nucleotides, which are susceptible to quick degradation. Finally, two methods were afforded for the introduction of a thiol to a polyamine from which one was further elaborated and showed to be useful for the synthesis of sensitive conjugates between maleimides and polyamines.

Several conjugates of polyamines with fluorescent dyes, porphyrins and indomethacin were successfully synthesised. The above described protection system (see chapter 4.1.2) was extended in the way that it finally consists of the orthogonal use of trityl linker and Aloc-, o-Nosyl-, and Dde-protection group, and enabled the synthesis of natural product HO359b, conjugates with porphyrins, fluorescent dyes, and Indomethacin. Most of the conjugates found entry into biological evaluation (see chapter 4.4). The yields of the syntheses ranged from 8% to 99%. Problems during the labelling with NBDCl were the main reason for the low yields in some cases.

Several of the synthesised conjugates were evaluated for their biological activity. Toxicological data were collected for all compounds. Fluorescence live imaging of incubated cells was used to gain information on their uptake into mammalian cells. The cellular uptake of polar NBD-derivatives **212** and **230** as well as lipophilic NBD conjugates **239**, **249**, **256**, and **263** into HeLa cells, HepG2 cells, and fibroblasts was investigated by live imaging. It showed that the cells efficiently took up the conjugates. Two positive charges seem to be sufficient to achieve this uptake. The NBD-spermidine **212** and NBD-spermine **230** were taken up into endosomal and lysosomal compartments. The lipophilic NBD conjugates were also found in vesicular compartments in fibroblasts. In HeLa cells and even more distinct in HepG2 cells, the conjugates also appeared in larger compartments. The experiments for the exact determination of these intracellular compartments are ongoing. The rhodamine

derivatives could not be detected after excitation with the fixed wavelength of the fluorescence microscope.

Porphyrin conjugate **217** was the most intensively studied conjugate. Fluorescence microscopy of incubated COS-7 cells, HeLa cells, HepG2 cells, and human fibroblasts showed a strong uptake compared to the non-modified, anionic carboxyporphyrin **215** (Figure 54). Co-incubation with vesicular cellular markers in fibroblasts, HeLa cells, HepG2 cells, and COS-7 cells suggested a final accumulation in mitochondria (Figure 56 and chapter 4.4.2). A time dependent determination of the uptake showed that **217** is readily internalised and after longer incubation, the concentration of the conjugate inside the cell ends in a saturation. The time, when this plateau is reached depends on the concentration of **217** in the incubation medium. The reason for this saturation is not clear. Possibly, the cells take up huge amounts of the conjugate during their growth state and after this, the uptake is slowed down. This would be in accordance with the finding that most of the polyamine conjugates showed a slight proliferative effect in the first 24 h of the incubation. Another possibility is that cells actively regulate the content of porphyrin conjugate **217**. Finally, a fluorescence quenching effect is also imaginable. However, the latter is rather improbable as the effective concentration in the cell lysate during fluorescence measurement is low. Consequently, illumination of HeLa cells showed strong phototoxicity already after short irradiation for 10 min after previous incubation with 0.1 µM of **217** for 24 h. Incubation with higher concentrations up to 5 µM showed the same result, however already after 4 h. This strong effect is in accordance with the fact that oxidative stress in the mitochondria is especially harmful for the cell.[406, 413] In contrast, the dark toxicity of porphyrin conjugate **217** is low in fibroblasts, and in HeLa cells even a slight proliferative effect is observed during the first 48 h. Co-cultures of different cell types with spermidine-porphyrin **217** showed very beneficial results. The rate of uptake increased in the line from fibroblasts over bacteria to tumour cells. After short irradiation of a pre-incubated culture of bacteria, no re-growth of the bacteria could be observed even after 72 h of further incubation. This assumes the use of **217** to act in photodynamic therapy of dermal disorders, skin-deep infections, and skin cancer. Further studies with multi-resistant *Staphylococcus aureus* strains and mouse models are ongoing.

The toxicology of the indomethacin conjugates **213** and **229** was tested by an SRB assay. Both compounds showed increased toxicity compared to indomethacin and the particular polyamines. The toxicity of the conjugates is in both cases more similar to the polyamine toxicity than to indomethacin. The increased toxicity gets significant for values above 5 µM for the spermidine-indomethacin conjugate **213** and for values above 2.5 µM for the spermine-indomethacin conjugate **229**, indicating a higher toxicity of the spermine conjugate. The next steps would be to determine the uptake efficiency of the conjugates and determine the effective dose ED_{50}. Comparison with the toxicity will show, if polyamine homologation is of benefit for application of Indomethacin.

6 Experimental section

6.1 General

Materials

Starting materials were obtained from commercial suppliers and were used without further purification (Acros, Aldrich, Fluka, Merck, Riedel de Haen, Sigma). 2-Chlorotrityl chloride resin (100 – 200 mesh, cross-linked with 1% divinylbenzene; loading: 1.3 mmol / g) and NovaSyn® TGT alcohol resin (100 – 200 mesh, cross-linked with 1% divinylbenzene; 90µm beads, loading: 0.27 mmol / g) were obtained from Novabiochem (Switzerland), *Merrifield resin* (75 – 150 mesh, 0.97 mmol / g) was obtained from Polymer Laboratories. Solvents were purchased dry and stored over molecular sieve (0.3 nm pore). Et_3N, diisopropylamine, and acetanhydride were distilled prior to use. *VLC* and flash chromatography were performed using Merck silica gel 60, 40 – 63 µm, preparative RP-flash chromatography was performed on LiChroPrep-C_{18}.

HPLC

Analytical HPLC was performed on a Pharmacia SMART-HPLC-system, using a CGE Bioscience C18 (2) 3⌠ column (150 · 4.6 mm) eluted at a rate of 0.1 mL / min and evaluated using SMART-Manager software. Preparative HPLC was performed on an Amersham-Pharmacia-Biotech ETTAN-LC-system, using a VarioPrep 250 / 10 NUCLEODUR 100 – 5 C18 ec, eluted at a rate of 2 mL / min and evaluated using UNICORN 4.0 software. Mobile phases were gradients of eluant A (MeCN - H_2O - TFA 10 : 90 : 0.1) and eluant B (MeCN - H_2O - TFA 80 : 20 : 0.1), eluant A (MeCN - TEAA (0.05 mmol, pH 6.5) 5 : 95) and eluant B (MeCN - TEAA(0.05 mmol, pH 7.3) 95 : 5), and eluant A (MeCN - H_2O - HOAc 5 : 95 : 0.01) and eluant B (MeCN - H_2O - HOAc 95 : 5 : 0.01). The purities determined from HPLC were calculated from the values of the peak integrals.

Reactions

If not otherwise stated, all reactions were carried out at room temperature, 1.013 bar, and under argon atmosphere. Reactions in flasks were heated using an oil bath. Vials were heated with a Reacti-Therm (Pearce). All solid-phase reactions were performed in glas frits or glas vials. Suspensions of the resins were agitated on a Janke and Kunkel KS 501 D shaker. Solution phase syntheses were, depending on the reaction volume, carried out on glass flasks or in glass vials. Dry solvents were transferred by plastic coatings and steel cannulae. The washing steps were usually carried out on air. For the reactions, dry solvents were used and the first washing steps were usually carried out with solvents of technical grade, while the terminal washing was carried out with absolute, puriss.-grade solvent. The reactions were carried out in sealed glass frits or sealed glass tubes. Cooling mixtures:

- 0 °C: ice in water
- –80 °C: dry ice in acetone

Large volumes of solvents were removed in a rotary evaporator. Compounds were dried at possibly low temperature in high vacuum. Compounds were dried with a vacuubrand 6 oilpump. Column chromatography was doen in analogy to the procedure from Still.[414] The yields were given for products with purities above 95%. Otherwise the purities were stated.

Thin layer chromatography

To control the completion of a reaction or to monitor column chromatography samples of the reaction mixture, chromatography fractions are analyzed by thin layer chromatography (TLC) on silicagel-aluminium plates (2.5 x 6.5 cm or 7 x 5 cm respectively). Prior to each run, the liquid phase was allowed to equilibrate in the TLC chamber to obtain an atmosphere saturated with the applied solvents. TLC plates were dried and evaluated either under UV-light or by staining with Seebach reagent or 10% ninhydrin in EtOH.

Analysis of the compounds

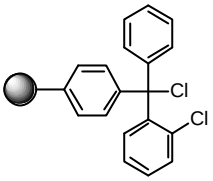
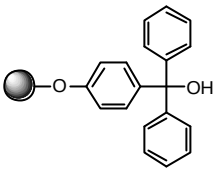
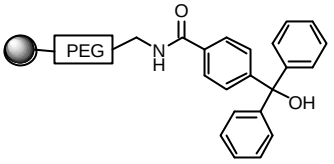
In solid phase reactions, the substances were cleaved from the resin and are either directly analyzed by HRMS and ^{1}H NMR spectroscopy or first purified by RP-HPLC or flash chromatography. If sufficient amounts of substances were available, also ^{13}C NMR spectra were also recorded. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400.14 MHz and at 100.6 MHz on a Bruker DP 400 using $CDCl_3$ or CD_3OD as solvents and TMS as internal standard. The solvent residual peaks were chosen as 3.31 ppm for CD_3OD and 7.26 for $CDCl_3$. Coupling constants (*J* values) are given in hertz (Hz). Multiplicities of ^{1}H NMR signals are reported as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; b, broad signal. ESI mass spectra and high-resolution mass spectra (HRMS-ESI) for exact mass determination were recorded with a Bruker ESI-Micro-Q-TOF (70 eV). FAB (fast atom bombardment) mass spectra were recorded in meta-nitrobenzoic acid (mNBA) matrix on a Kratos MS 50 (70 eV) instrument, Thermo Quest Finnigan MAT 95 XL. EI (electron impact ionization) spectra were recorded on a Kratos MS50 (I) instrument, ThermoQuest Finnigan MAT 95 XL (II). MALDI (Matrix assisted laser desorption ionization) spectra were obtained with a HiResMALDI FT-ICR (Ionspec, Lake Forrest, CA; 7 T magnet, pulsed laser 337 nm).

6.2 Resins

Applied resins

Three resins were used for the solid phase syntheses, which all bear trityl linker. Those are 2-chlorotrityl resin, alkoxytrityl resin (*Fukuyama resin*), and TGT alcohol resin (Table 13).[239] They differ in swelling properties and cleavage conditions. 2-Chlorotritylchloride resin and TGT alcohol resin were purchased from commercial suppliers.

Table 13 All three applied resins bear a trityl type linker.

	2-Chlorotrityl chloride resin	p-Alkoxytrityl resin	NovaSyn® TGT resin
Structure			
Cleavage	AcOH / TFE / CH_2Cl_2, > 1% TFA or HFIP in CH_2Cl_2	< 1% TFA in CH_2Cl_2	AcOH / MeOH / CH_2Cl_2, 0.5% TFA or HFIP in CH_2Cl_2
Activation	Available in the activated form, recyclable without loss of loading	Activation with 1.5 equiv. $SOCl_2$, recyclable under slight loss of loading	Activation with 1.5 equiv. $SOCl_2$, recyclable without loss of loading
Loading in mmol / g	1.3	0.8	0.2

Preactivation of TGT alcohol resin

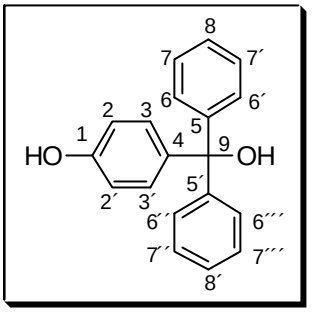
TGT alcohol resin required pre-activation with $SOCl_2$. Therefore, the resin was swollen and three times washed with CH_2Cl_2. Two equiv. of $SOCl_2$ in CH_2Cl_2 were added and the resin was agitated for 60 min. After three times washing with CH_2Cl_2, the resin was instantly loaded with the respective building block.

Preparation of alkoxytrityl resin 308

(4-hydroxyphenyl)-diphenylmethanol

Alkoxytrityl resin was prepared in accordance with the reported procedure from Fukuyama et al.[239] To a solution of 13.44 g (100.79 mmol; 1 equiv.) $AlCl_3$ suspended in 30 mL carbon disulfide was added under stirring 19 mL (100.08 mmol; 1 equiv.) of diphenyldichloromethane in 10 mL carbon disulfide. The colour of the solution changed to red and 9.47 g (100.74 mmol; 1 equiv.) of phenol in 40 mL carbon disulfide were slowly added. After addition of further 10 mL of carbon disulfide, the suspension was refluxed at 50 °C for 3 h and then the reaction was quenched with 200 mL ice water. The reaction mixture was extracted with 200 mL Et_2O and the solvent was removed from the united organic layers. The remaining yellow solid was dissolved in 350 mL of a 1 N aqueous solution of KOH. The product was precipitated by liberation of CO_2, achieved by addition of $KHCO_3$ and slow addition of HCl_{conc} followed by the addition of dry ice. The solid was extracted with Et_2O. After drying of the organic layer on Na_2SO_4, the solvent was removed

and the remaining oil was dried on HV. 24.15 g of the pure product are obtained as a yellow solid giving 87%.



$C_{19}H_{16}O_2$

R_f = 0.63 ($CHCl_3$: MeOH - 10 : 1 (v/v)); ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 6.69 (d, *J* = 8.47 Hz, 2 H, 3-H 3´-H); 7.02 (d, *J* = 8.47 Hz, 2 H, 2-H 2´-H); 7.18 – 7.23 (m, 10 H, 6-H 6´-H, 6´´-H, 6´´´-H, 7-H, 7´-H, 7´´-H, 7´´´-H, 8-H, 8´-H); ^{13}C NMR (75 MHz, CD_3OD), δ (ppm): 155.96 (C_{quart}, C-1) , 147.68 (C_{quart}, C-5 C-5´), 138.37 (C_{quart}, C-4), 129.13 (+, C-3 C-3´), 127.74 (+, C-7 C-7´ C-7´´ C-7´´´), 127.06 (+, C-6 C-6´ C-6´´ C-6´´´), 126.34 (+, C-8 C-8´), 113.81 (+, C-2 C-2´), 81.27 (C_{quart}, C-9); MS (ESI) (10 eV/z) [m / z (%)]: 575.18 (100) $[2 M + Na]^+$; 299.06 (95) $[M + Na]^+$; 259.08 (37) $[M – OH]^+$; 245.06 (15) $[M – 2 OH]^+$.

To a suspension of 23.2 g (84.20 mmol) (4-hydroxyphenyl)-diphenylmethanol **306** and 4.41 g (4.27 mmol) *Merrifield resin* in 80 ml DMF, 5.80 g (420 mmol) K_2CO_3 were added. The suspension was stirred and heated to 60 °C for 22 h. After cooling to room temperature, the solvent was removed and the resin was washed with H_2O, H_2O / THF (1:10), CH_2Cl_2 / MeOH, and CH_2Cl_2. After drying in HV, the pink resin **306** was obtained. The loading was calculated by the weight difference before and after binding of the linker. Usually, values of 0.6 – 0.8 mmol / g were obtained. For activation, the resin was treated with a solution of 2 equiv. of $SOCl_2$ in CH_2Cl_2 and agitated for 2 h. After washing with CH_2Cl_2 and drying in HV, a yellow resin **308** is obtained.

Recycling of the resins

The 2-chlorotrityl resin was recycled by the following procedure:

1. The resin was treated with 10% TFA / CH_2Cl_2 and agitated for 30 min.
2. The solvent was removed and the resin was agitated with 10% 0.001 M aqueous NaOH / THF (3 * 5 min)
3. The resin was washed with dry THF (3 * 2 min) and dry CH_2Cl_2 (3 * 2 min)
4. The resin was activated as described above.

Kaiser Test

For the qualitative determination of amino functionalities on immobilized substrates the *Kaiser Test* was applied.[238, 415] It is basically an adoption of the *Ninhydrin Assay* for the determination of amines on TLC plates. It provides the possibility to distinguish between primary and secondary amines, because in contrast to primary amines, secondary amines only show a positive reaction if the test is carried out at elevated temperatures.[238] A small portion

of the resin is placed in a small tube and is washed three times with MeOH. Subsequently, few drops of the following solution are added:

1. 0.5 g ninhydrine in 10 mL EtOH
2. 80 g phenol in 20 mL EtOH
3. 0.4 mL 0.001 M aqueous KCN in 20 mL pyridine

In the presence of primary amines the resin beads quickly adopt an intensive dark blue colour. The detection of secondary amines requires heating with a blow drier for 30 seconds. In the absence of amino groups the resin keeps its initial colour or turns to slight brown.

Swelling of the resins

The resin is placed in the reaction vessel, the swelling solvent is added in a way that disperses the solid and it is shortly shaken until the entire polymer is suspended. The resin is abandoned for at least five minutes and the solvent is removed.

Washing of the resins

The solvent is added to the resin and the suspension is agitated for two minutes. The solvent is removed by suction. Washing with one solvent consists of three repetitive of those steps. Swelling and collapsing solvents are used alternatingly after every of those steps. The solvents are applied in the order of decreasing polarity and the washing sequence is usually terminated with CH_2Cl_2. The removal of DMF is sluggish and thus one washing step with water and addition of 10% water to the following solvents is necessary to achieve complete removal.

Cleavage from the resins

Alkoxytrityl resin: 100 mg of the resin are treated with a solution of 1% TFA in CH_2Cl_2 (3 * 3 min). The drained cleavage mixture is combined with washings of CH_2Cl_2 and MeOH and the solvent is removed in vacuum.

2-Chlorotrityl resin: 50 mg of the resin are treated with a solution of 5% TFA in CH_2Cl_2 (3 * 3 min). The drained cleavage mixture is combined with washings of CH_2Cl_2 and MeOH and the solvent is removed in vacuum.

PEG-trityl resin: 100 mg of the resin are treated with a solution of 2% TFA in CH_2Cl_2 (3 * 3 min). The drained cleavage mixture is combined with washings of CH_2Cl_2 and MeOH and the solvent is removed in vacuum.

Determination of resin loading

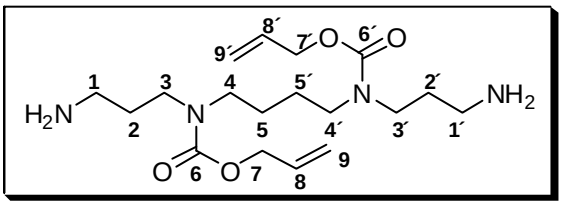
After all impurities were washed from the resin it is dried until all solvent is removed. The compound is cleaved from the resin, the solvents are removed, and the compound is dried on high vacuum in a 25 mL flask. The dry compound is etched with 0.1 mL of $CDCl_3$ and 0.5 mL of a reference solution of 1,4-dioxane in $CDCl_3$ are added (86 µmol dioxane / ml $CDCl_3$). The solution is transferred into a NMR tube and the remaining compound in the flask is transferred into the tube with $CDCl_3$. For polar compounds CD_3OD is used instead of $CDCl_3$ for etching and transferring to ensure solubility of the compound in the reference

solution. For determination of the amount of compound the integral of the singlet from dioxane appearing at 3.8 ppm in the ^{1}H NMR is set to 8. The quantity of material is calculated by the intensity of the protons of the internal methylene groups 6 and 7 appearing at 1.5 ppm in the ^{1}H NMR spectra. The conversion factor was obtained by measuring four defined amounts of spermine **(16)** in the dioxane / $CDCl_3$ NMR solvent.

6.3 Synthesis of the building blocks

1,12-diamino-4,9-bis-(allyloxycarbonyl)-4,9-diazadodecane (178)

4.91 g (24.3 mmol; 1 equiv.) of spermine **(16)** were dissolved in 400 mL MeOH, cooled to – 80 °C and stirred under argon. 10.0 mL (84.0 mmol; 3.5 equiv.) of TfaOEt in 20 mL MeOH were slowly added through a syringe during 90 min, whereas a white solid precipitates. Stirring was continued for further 50 min. The suspension was warmed to 0 °C and 16.8 mL (121 mmol; 5 equiv.) of Et_3N and 12.9 mL (121 mmol; 5 equiv.) of allylchloroformate were added in two portions. Stirring of the clear solution was continued for 5 h at room temperature. After addition of 62 mL of a mixture of $NaOH_{conc}$ / H_2O (4 : 3) a white solid precipitated and stirring was continued for 1 d. The MeOH was removed under reduced pressure. The resulting clear aqueous solution was diluted to a five-fold volume with H_2O_{dist} and was ten times extracted with $CHCl_3$. After removal of the solvent, Et_3N was removed from the mixture by heating on high vacuum. The product **178** was obtained as colourless, hygroscopic syrup (8.89 g; 24.0 mmol) in 99% yield.

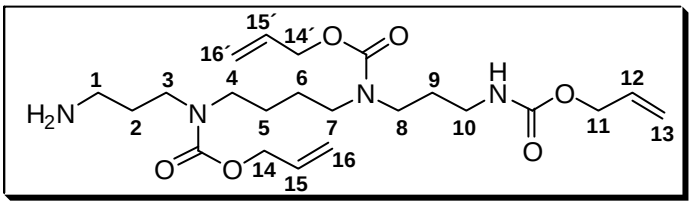


$C_{18}H_{34}N_4O_4$

R_f = 0.39 (CH_2Cl_2 : MeOH : $NH_{3(aq)}$ (25%) - 6 : 1 : 0.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.90 (ddt, J_1 = 17.2 Hz, J_2 = 10.5 Hz, J_3 = 5.5 Hz, 2 H, 8-H 8´-H), 5.27 (ddt, J_1 = 17.2 Hz, J_2 = 1.5 Hz, J_3 = 1.5 Hz, 2 H, 9-Ha 9´-Ha), 5.19 (ddt, J_1 = 10.5 Hz, J_2 = 1.5 Hz, J_3 = 1.5 Hz, 2 H, 9-Hb 9´-Hb), 4.57 (ddd, J_1 = 5.5 Hz, J_2 = 1.5 Hz, J_3 = 1.5 Hz, 4 H, 7-H 7´-H), 3.15 – 3.34 (m, 8 H, 3-H 3´-H 4-H 4´-H), 2.68 (t, J = 6.9 Hz, 4 H, 1-H 1´-H), 1.65 (tt, J_1 = 7.0 Hz, J_2 = 6.9 Hz, 4 H, 2-H 2´-H), 1.51 (m, 4 H, 5-H 5´-H), 1.29 (bs, 4 H, N-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.2, 156.1 (C_{quart}, C-5), 133.2 (+, C-8), 117.4, 117.2 (–, C-9), 65.9 (–, C-7), 47.0, 46.5 (–, C-4 C-4´), 44.6, 44.4 (–, C-3 C-3´), 39.4, 39.2 (–, C-1 C-1´), 32.5, 31.8 (–, C-2 C-2´), 25.9, 25.4 (–, C-5 C-5´); HRMS (EI) $[M + H]^+$ calc. 370.2580, found 370.2582; FAB-MS: 371.2 (100) $[M + H]^+$, 393.2 (43) $[M + Na]^+$.

1,12-diamino-N^4,N^9-bis-(allyloxycarbonyl)-4,9-diazadodecane (178) / 12-amino-1-allylcarbamyl-N^4,N^9-bis-(allyloxycarbonyl)-5,10-diazadodecane (179)

3.00 g (14.8 mmol; 1 equiv.) of spermine **(16)** were dissolved in 150 mL MeOH, cooled to – 80 °C and stirred under argon. 1.94 mL (16.3 mmol; 1.1 equiv.) of TfaOEt in 18 mL MeOH were slowly added through a syringe during 30 min, whereas a white solid precipitates. The suspension was warmed to 0 °C and stirring was continued for 60 min. 5.14 mL (74.1 mmol; 5 equiv.) of Et_3N and 9.45 mL (88.96 mmol; 6 equiv.) of allylchloroformate were added in one portions. Stirring of the clear solution was continued for 6 h at room temperature. After addition of 38 mL of a mixture of $NaOH_{conc}$ / H_2O (11 : 8) a white solid precipitated and stirring was continued for 5 d at 0 °C. The MeOH was removed under reduced pressure. The resulting precipitate was between H_2O and $CHCl_3$ and the aqueous layer was eight times extracted with $CHCl_3$. The organic layers were dried over Na_2SO_4. After flash chromatography on silica gel ($CHCl_3$: MeOH : NH_{3aq}) (10 : 1 : 0.1) to ($CHCl_3$: MeOH : NH_{3aq}) (2.5 : 1 : 0.1) 3.32 g (7.31 mmol) of 13-amino-1,5,10-tri-(allyloxycarbonyl)-1,5,10-triazatridecane **179** (49% yield) and 1.49 g (4.01 mmol) of 1,12-diamino-4,9-bis-(allyloxycarbonyl)-4,9-diazadodecane **178** (27% yield), both as highly viscose syrups were obtained.



$C_{22}H_{38}N_4O_6$

R_f = 0.30 (CH_2Cl_2 : MeOH : $NH_{3(aq)}$ (25%) - 10 : 1 : 0.1); ^{1}H NMR (400 MHz, CD_3OD) δ 5.96 (ddt, J_1 = 17.3 Hz, J_2 = 10.6 Hz, J_3 = 5.3 Hz, 2 H, 15-H 15´-H), 5.92 (ddt, J_1 = 17.3 Hz, J_2 = 9.9 Hz, J_3 = 5.4 Hz, 1 H, 12-H), 5.30 (ddt, J_1 = 17.3 Hz, J_2 = 1.7 Hz, J_3 = 1.4 Hz, 3 H, 13a-Ha 16-Ha 16´-Ha), 5.21 (ddt, J_1 = 10.6 Hz, J_2 = 1.7 Hz, J_3 = 1.4 Hz, 2 H, 16-Hb 16´-Hb), 5.18 (d, J = 9.9 Hz, 1 H, 13-Hb), 4.57 (ddd, J_1 = 5.3 Hz, J_2 = 1.7 Hz, J_3 = 1.4 Hz, 4 H, 14-H 14´-H), 4.52 (d, J = 5.4 Hz, 2 H, 11-H), 3.34 (t, J = 6.8 Hz, 2 H, 3-H), 3.33 – 3.26 (m, 6 H, 4-H 7-H 8-H), 3.11 (t, J = 6.9 Hz, 2 H, 10-H), 2.68 (t, J = 6.8 Hz, 2 H, 1-H), 1.74 (tt, J_1 = 6.9 Hz, J_2 = 6.8 Hz, 4 H, 2-H 9-H), 1.59 – 1.52 (m, 4 H, 5-H 6-H); FAB-MS: 455.2 (100) $[M + H]^+$, 495.2 (63) $[M + K]^+$.

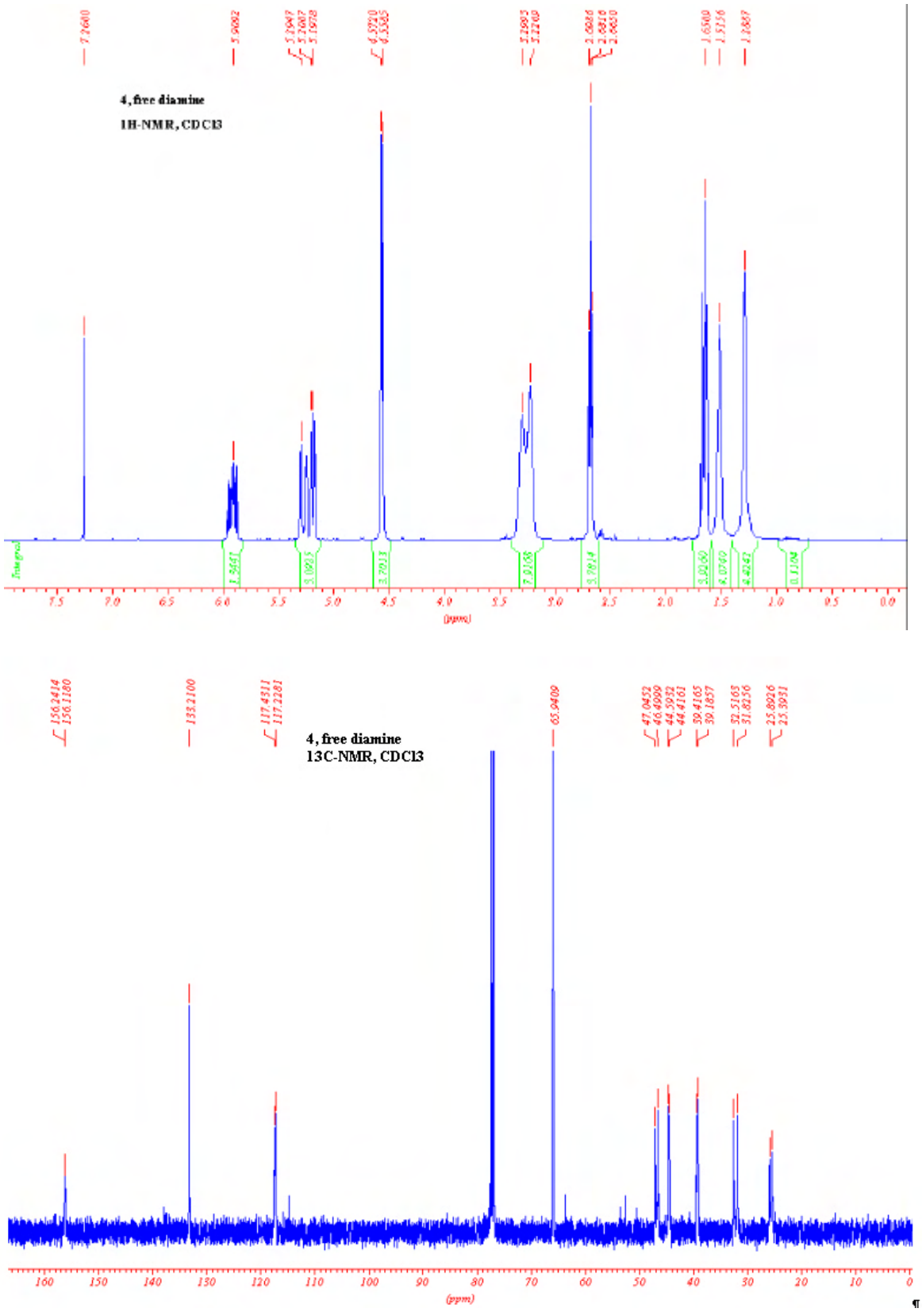


Figure 64 ^{1}H NMR and ^{13}C NMR of **178**, both measured in $CDCl_3$. Some of the signals corresponding to the backbone methylene groups are splitted due to rotational isomerism of the tertiary carbamates.

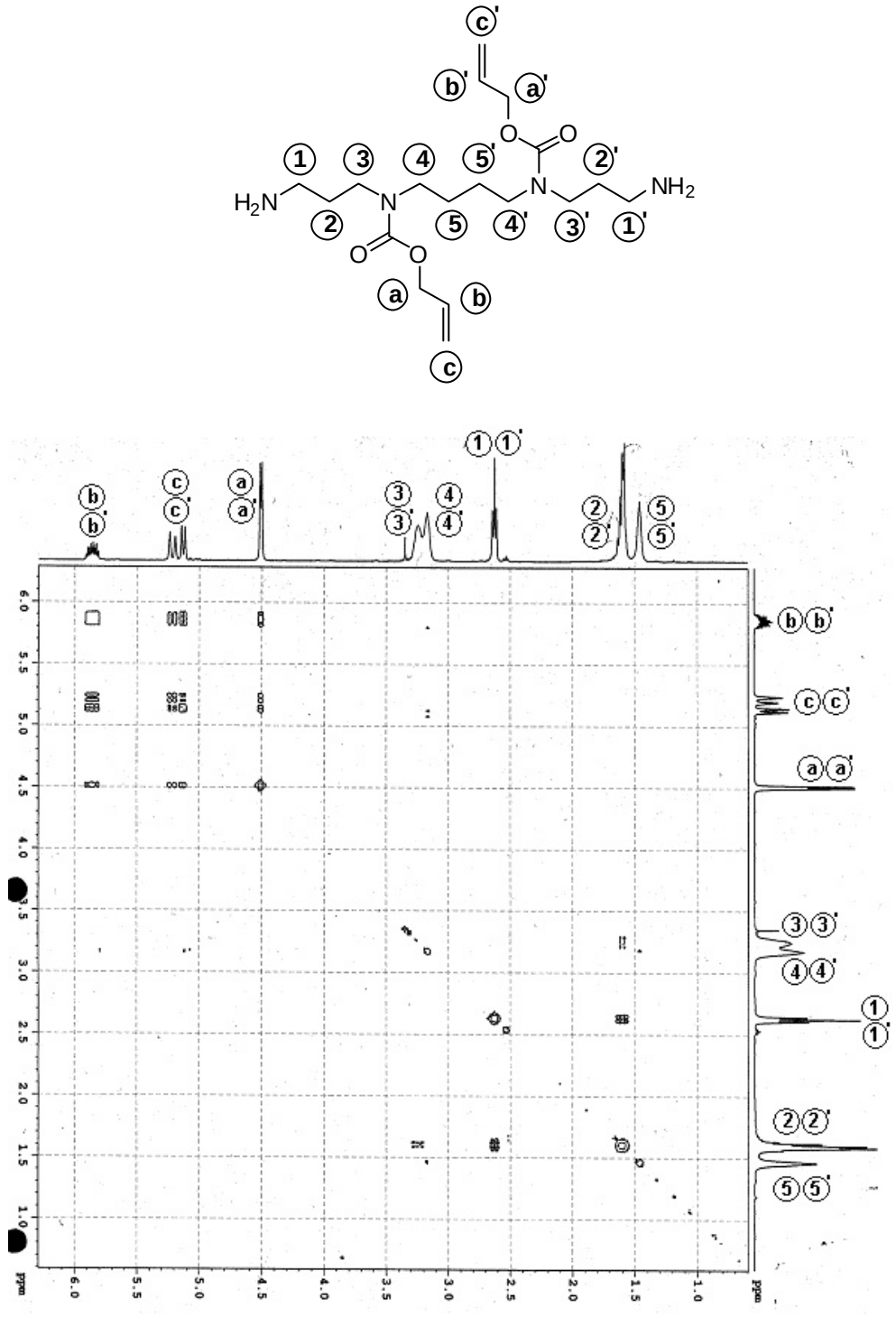


Figure 65 ^{1}H-^{1}H-COSY-Spectrum of **178**, measured in $CDCl_3$. The signal at 2.7 ppm corresponds to the methylene group 1. This was confirmed by the fact that solely this multiplet changes distinctly to lower field after protonation of the amino group in its trifluoroacetic acid salt. 1 and 3 show a correlation with multiplet 2 indicating that those three conform to the terminal methylene group spacer. The correlation between methylene groups 4 and 5 is also slightly visible. The shift of the NH-protons is depending on then concentration. It is 1.6 ppm in this spectrum and 1.2 ppm in a lower concentrated probe.

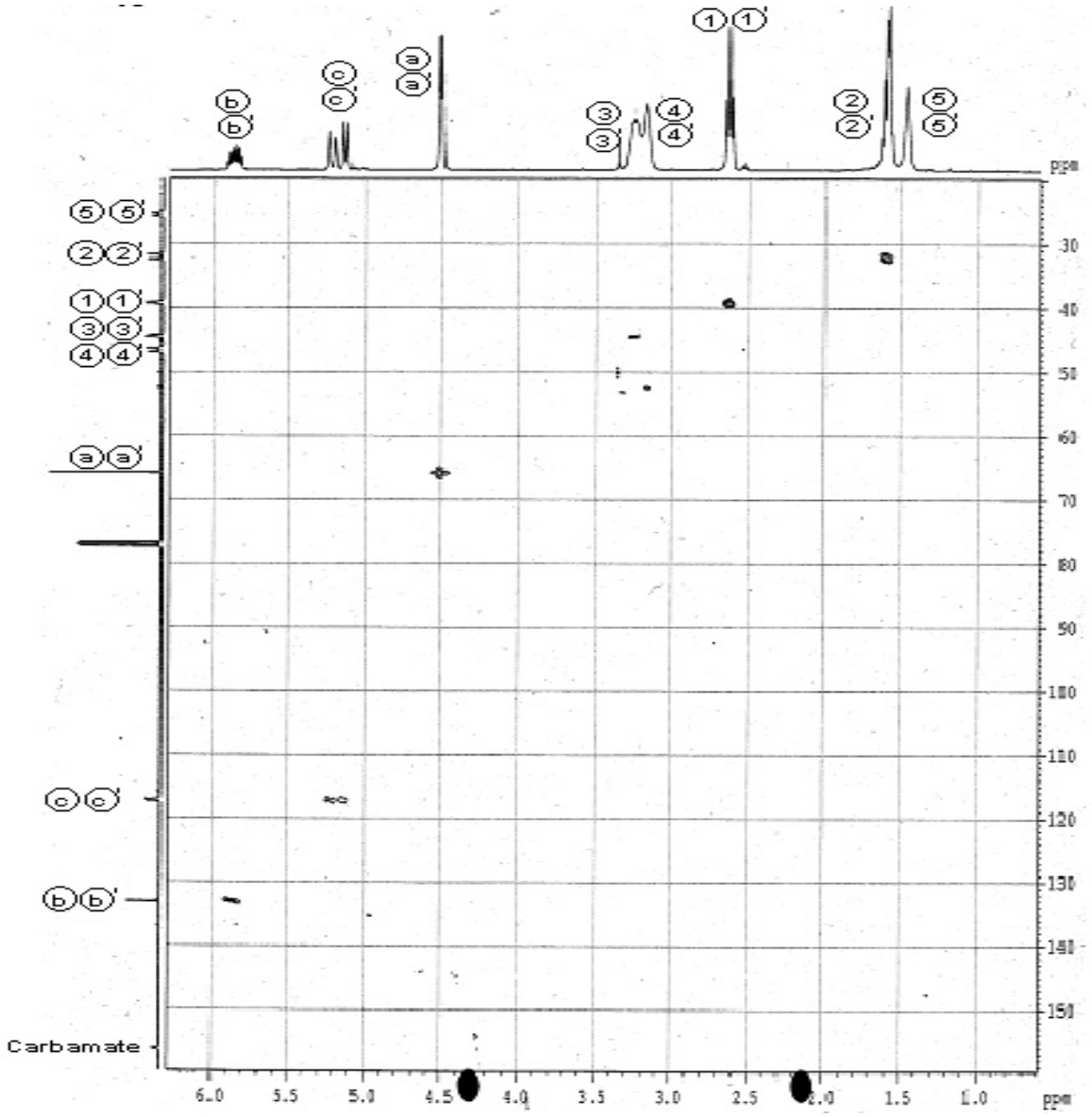


Figure 66 2D-^{1}H-^{13}C HMQC-Spectrum of **178,** measured in $CDCl_3$. The signals of the ^{13}C NMR spectra are broadened / splitted. This is due to dynamic effects that result from hindered rotation along the bonds of the backbone.

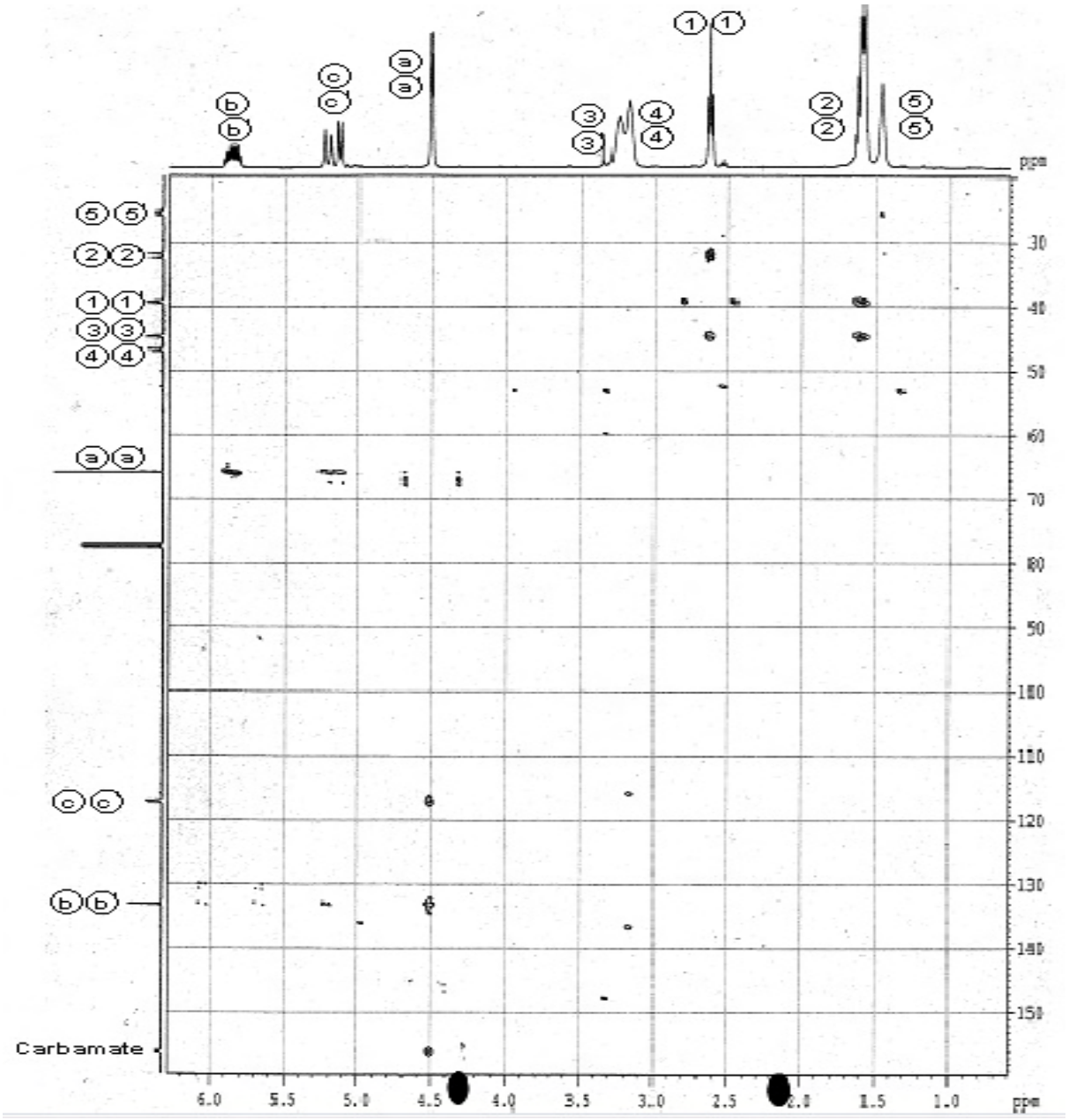
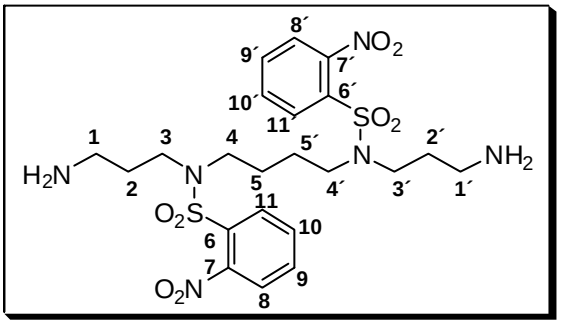


Figure 67 2D-^{1}H-^{13}C HMBC-Spectrum of **178**, measured in $CDCl_3$.

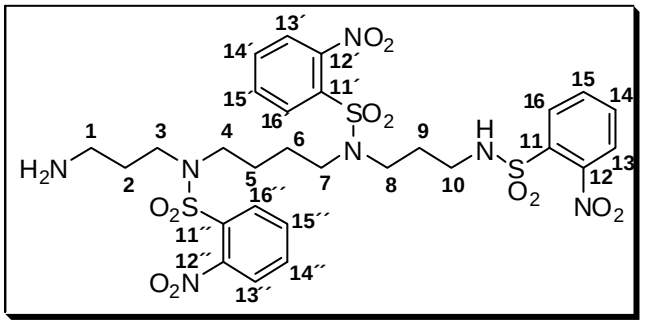
1,12-diamino-4,9-bis-(2-nitrophenylsulfonyl)-4,9-diazadodecane (180) / 12-amino-5,10-bis-(2-nitrophenylsulfonyl)-1-(2-nitrophenylsulfonamido)-5,10-diazadodecane (181)

500 mg (2.47 mmol; 1 equiv.) of spermine **(16)** were dissolved in 25 mL MeOH, cooled to –78 °C and stirred under argon. 0.29 mL (2.47 mmol; 1.0 equiv.) of TfaOEt in 4 mL MeOH were slowly added through a syringe during 30 min, whereas a white solid precipitates. The suspension was warmed to 0 °C and stirring was continued for 60 min. 6.35 mL (37.1 mmol; 15 equiv.) of DiPEA and 9.860 g (44.5 mmol; 18.0 equiv.) of 2-nitrophenylsulfonyl chloride were added in one portions. Stirring of the clear solution was continued for 40 h at room temperature. A mixture of $NaOH_{conc}$ / H_2O (1 : 1) was added in several portions until the pH was constantly adjusted above 12 and stirring was continued for 16 h. The MeOH was removed under reduced pressure. The resulting precipitate was separated between 5% aqueous $NaHCO_3$ and CH_2Cl_2 and the aqueous layer was three times extracted with CH_2Cl_2. The solvents were removed and after flash chromatography on silica gel (($CHCl_3$: MeOH : $NH_{3(aq)}$(25%)) (20 : 1 : 0.1) to ($CHCl_3$: MeOH : $NH_{3(aq)}$(25%)) (2 : 1 : 0.1)), 94 mg (160 µmol) of 1,12-diamino-4,9-bis-(2-nitrophenylsulfonyl)-4,9-diazadodecane **(180)** (7% yield) and 535 mg (712 µmol) of 12-amino-5,10-bis-(2-nitrophenylsulfonyl)-1-(2-nitrophenylsulfonamido)-5,10-diazadodecane **(181)** (29% yield), both as yellow solids were obtained.



$C_{22}H_{32}N_6O_8S_2$

R_f = 0.60 (CH_2Cl_2 : MeOH : $NH_{3(aq)}$ (25%) - 3 : 2 : 0.5); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.03 – 7.97 (m, 2 H, 8-H 8´-H), 7.83 – 7.69 (m, 6 H, 9-H 9´-H 10-H 10´-H 11-H 11´-H), 3.35 (t, *J* = 7.2 Hz, 4 H, 3-H 3´-H), 3.32 – 3.28 (m, 4 H, 4-H 4, ´-H), 2.67 (t, *J* = 7.1 Hz, 4 H, 1-H 1´-H), 1.72 (tt, J_1 = 7.2 Hz, J_2 = 7.1 Hz, 4 H, 2-H 2´-H), 1.55 – 1.49 (m, 4 H, 5-H 5´-H); FAB-MS: 573.1 (78) $[M + H]^+$.



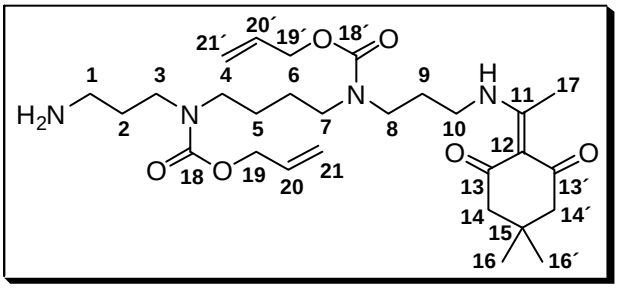
$C_{28}H_{35}N_7O_{12}S_3$

R_f = 0.87 (CH_2Cl_2 : MeOH : $NH_{3(aq)}$ (25%) - 3 : 2 : 0.5); ^{1}H NMR (400 MHz, CD_3OD / $CDCl_3$) δ 8.07 – 7.95 (m, 3 H, 13-H 13´-H 13´´-H), 7.86 – 7.68 (m, 9 H, 14-H 14´-H 14´´-H

15-H 15´-H 15´´-H 16-H 16´-H 16´´-H), 3.35 (t, *J* = 7.3 Hz, 2 H, 3-H), 3.32 – 3.24 (m, 6 H, 4-H 7-H 8-H), 3.02 (t, *J* = 6.8 Hz, 2 H, 10-H), 2.66 (t, *J* = 7.0 Hz, 2 H, 1-H), 1.79 – 1.67 (m, 4 H, 2-H 9-H), 1.54 – 1.44 (m, 4 H, 5-H 6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.2, 149.1 (C_{quart}, C-12 C-12´ C-12´´), 135.0, 134.8 (+, C-15 C-15´ C-15´´), 133.7, 133.5 (C_{quart}, C-11 C-11´ C-11´´), 133.0 (+, C-14 C-14´ C-14´´), 131.4, 131.2 (+, C-16 C-16´ C-16´´), 125.8, 125.2 (+, C-13 C-13´ C-13´´), 48.1, 47.9 (–, C-4 C-7), 46.1, 45.9 (–, C-3 C-8), 41.5 (–, C-10), 39.2 (–, C-1), 30.5 (–, C-2), 29.6 (–, C-9), 25.9 (–, C-5 C-6); FAB-MS: 758.1 (51) $[M + H]^+$, 742.1 (6) $[M – NH]^+$, 573.1 (4) $[M – o\text{-Nosyl}]^+$.

1-Amino-12-(1-(4,4-dimethyl-2,6-dioxocyclohexyliden)-ethylamino)-4,9-bis-(allyloxycarbonyl)-4,9-diazadodecane (186)

200 mg (0.54 mmol; 1 equiv.) of **178** were dissolved in 10 mL MeOH and 55 µl (0.32 mmol; 0.6 equiv.) of DiPEA were added. A solution of 60 mg (0.32 mmol; 0.6 equiv.) of Dde-OH **191** in 5 mL MeOH was slowly added during 30 min and stirring was continued over night. The solvent was removed and the slightly yellow solution was separated between 5% aqueous $NaHCO_3$ and CH_2Cl_2 and the aqueous layer was extracted two times with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and the solvent was removed. The product was purified by flash chromatography on silica gel ($CHCl_3$: MeOH : $NH_{3(aq)}$ (25%) (10 : 1 : 0.1) and $CHCl_3$: MeOH : $NH_{3(aq)}$ (25%) (5 : 1 : 0.1)). After drying on HV, 89 mg (0.18 mmol) of **186** were obtained giving 54% yield related to the insert of Dde-OH **191** respectively 32% related to the input of **178**. 100 mg (0.27 mmol; 50%) of **178** were recovered and could be reused for further reactions.

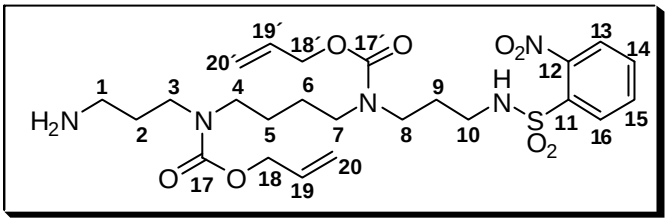


$C_{28}H_{46}N_4O_6$

R_f = 0.46 (10 : 1 : 0.1 - $CHCl_3$: MeOH : $NH_{3(aq)}$ (25%)); ^{1}H NMR (400 MHz, CD_3OD) δ 5.94 (ddt, J_1 = 17.3 Hz, J_2 = 10.5 Hz, J_3 = 5.4 Hz, 2 H, 20-H 20´-H), 5.29 (ddt, J_1 = 17.3 Hz, J_2 = 1.5 Hz, J_3 = 1.4 Hz, 2 H, 21-Ha 21´-Ha), 5.20 (ddt, J_1 = 10.5 Hz, J_2 = 1.5 Hz, J_3 = 1.4 Hz, 2 H, 21-Hb 21´-Hb), 4.57 (dt, J_1 = 5.4 Hz, J_2 = 1.4 Hz, 4 H, 19-H 19´-H), 3.52 (t, *J* = 6.8 Hz, 2 H, 10-H), 3.41 (t, *J* = 6.7 Hz, 2 H, 8-H), 3.37 – 3.25 (m, 6 H, 3-H 4-H 7-H), 2.65 (t, *J* = 7.0 Hz, 2 H, 1-H), 2.55 (s, 3 H, 17-H), 2.37 (s, 4 H, 14-H 14´-H), 1.94 (tt, J_1 = 6.8 Hz, J_2 = 6.7 Hz, 2 H, 9-H), 1.72 (tt, J_1 = 7.1 Hz, J_2 = 7.0 Hz, 2 H, 2-H), 1.57 (m, 4 H, 5-H 6-H), 0.91 (s, 6 H, 16-H 16´-H); ^{13}C NMR (75 MHz, CD_3OD) δ 199.7 (C_{quart}, C-13 C-13´), 175.4 (C_{quart}, C-11), 157.7, 157.6 (C_{quart}, C-18 C-18´), 134.3 (+, C-20, C-20´), 117.7 (–, C-21, C-21´, splitted and broadened signal), 108.7 (C_{quart}, C-12), 67.0 (–, C-19, C-19´), 53.4 (–, C-14 C-14´), 48.0 (–, C-4 C-7), 46.1, 45.3 (–, C-3 C-8), 42.1, 39.5 (–, C-1 C-10, splitted and broadened signal), 31.0 (C_{quart}, C-15), 29.0, 28.7 (–, C-2 C-9, splitted and broadened signal), 28.4 (+, C-16 C-16´), 26.7, 26.2 (–, C-5 C-6, splitted and broadened signal), 18.2 (+, C-17); FAB-MS: 699.3 (62) $[M + Dde]^+$, 535.2 (100) $[M + H]^+$, 371.2 (21) $[M – Dde]^+$.

1-Amino-12-(2-nitrophenylsulfonamido)-4,9-bis-(allyloxycarbonyl)-4,9-diazadodecane (187)

5,71 g (15.4 mmol; 1 equiv.) of **178** together with 2.06 mL (15.4 mmol; 1 equiv.) of collidine were dissolved in 100 mL CH_2Cl_2. 2.05 g (9.26 mmol; 0.6 equiv.) of 2-nitrophenylsulfonylchloride in 50 mL CH_2Cl_2 were slowly added during 4 h and stirring was continued over night. The slightly yellow solution was washed three times with half concentrated hydrochloric acid to separate collidine and **178**. The organic layer was quickly washed with 0.5 M NaOH and then dried over Na_2SO_4. The product was separated from its bis-nosylated by-product by filtration over a short pad of silica gel ($CHCl_3$ and $CHCl_3$: MeOH : $NH_{3(aq)}$ (25%) (10 : 1 : 0.1)). The silicagel was removed by washing with 10 mL of water, the solvent was removed under reduced pressure, and the product was dried on HV. 3.62 g (6.53 mmol) of **187** were obtained to give 71% yield related to the input of 2-nitrophenylsulfonylchloride respectively 43% related to the insert of **178**. Collidine and **178** were recovered by increasing the pH of the acidic aqueous layer to 14 and extraction with $CHCl_3$. Collidine was removed by heating under HV and 2.32 g (6.25 mmol; 41%) of **178** were recovered for further reaction cycles.



$C_{24}H_{37}N_5O_8S$

R_f = 0.48 (CH_2Cl_2 : MeOH : $NH_{3(aq)}$ (25%) - 10 : 1 : 0.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 – 8.25 (m, 1 H, 13-H), 7.81 (m, 1 H, 16-H), 7.73 (m, 2 H, 14-H 15-H), 5.90 (ddt, J_1 = 17.2 Hz, J_2 = 10.5 Hz, J_3 = 5.5 Hz, 2 H, 19-H 19´-H), 5.27 (ddt, J_1 = 17.2 Hz, J_2 = 1.4 Hz, J_3 = 1.5 Hz, 2 H, 20-Ha 20´-Ha), 5.19 (ddt, J_1 = 10.5 Hz, J_2 = 1.4 Hz, J_3 = 1.5 Hz, 2 H, 20-Hb 20´-Hb), 4.57 (ddd, J_1 = 5.5 Hz, J_2 = 1.5 Hz, J_3 = 1.5 Hz, 4 H, 18-H 18´-H), 3.37 (m, 2 H, 3-H), 3.30 (m, 2 H, 8-H), 3.20 (m, 4 H, 4-H 7-H), 3.08 (t, J = 6.7 Hz, 2 H, 10-H), 2.98 (m, 2 H, 1-H), 1.93 (m, 2 H, 2-H), 1.74 (m, 2 H, 9-H), 1.50 (m, 4 H, 5-H 6-H); ^{1}H NMR (400 MHz, CD_3OD / $CDCl_3$) δ 8.04 – 8.04 (m, 1 H, 13-H), 7.68 – 7.86 (m, 3 H, 14-H 15-H 16-H), 5.90 (ddt, J_1 = 17.2 Hz, J_2 = 10.4 Hz, J_3 = 5.3 Hz, 2 H), 5.28 (ddt, J_1 = 17.2 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, 1 H, 20-Ha), 5.25 (ddt, J_1 = 17.2 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, 1 H, 20´-Ha), 5.21 (ddt, J_1 = 10.4 Hz, J_2 = 1.4 Hz, J_3 = 1.3 Hz, 1 H, 20-Hb), 5.17 (ddt, J_1 = 10.4 Hz, J_2 = 1.4 Hz, J_3 = 1.4 Hz, 1 H, 20´-Hb), 4.57 (ddd, J_1 = 5.3 Hz, J_2 = 1.5 Hz, J_3 = 1.3 Hz, 2 H, 18-H), 4.53 (d, J_1 = 5.3 Hz, 2 H, 18´-H), 3.35 (t, J = 6.9 Hz, 2 H, 3-H), 3.18 – 3.30 (m, 6 H, 4-H 7-H 8-H), 3.06 (t, J = 6.7 Hz, 2 H, 10-H), 2.87 (t, J = 6.4 Hz, 2 H, 1-H), 1.89 (tt, J_1 = 6.9 Hz, J_2 = 6.4 Hz, 2 H, 2-H), 1.75 (m, 2 H, 9-H), 1.52 (m, 4 H, 5-H 6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157.1, 156.5 (C_{quart}, C-17 C-17´), 147.9 (C_{quart}, C-12), 133.6 (C_{quart}, C-11), 132.5, 130.5, 124.9 (+, 4 C, C-13 C-14 C-15 C-16)), 132.5, 132.6 (+, C-19, C-19´), 117.6 (–, C-20, C-20´), 66.3, 66.0 (–, C-18, C-18´), 46.7 (–, C-4 C-7), 43.7, 44.2 (–, C-3 C-8), 40.6 (–, C-10), 36.8 (–, C-1), 28.6 (–, C-9), 25.4, 25.2, 24.9 (–, C-2 C-5 C-6, splitted and broadened signal); HRMS (EI) $[M]^+$ calc. 555.2363, found 555.2354; FAB-MS: 556.3 (100) $[M + H]^+$, 540.2 (5) $[M – NH]^+$, 391.3 (49) $[M – o\text{-Nosyl} + Na]^+$.

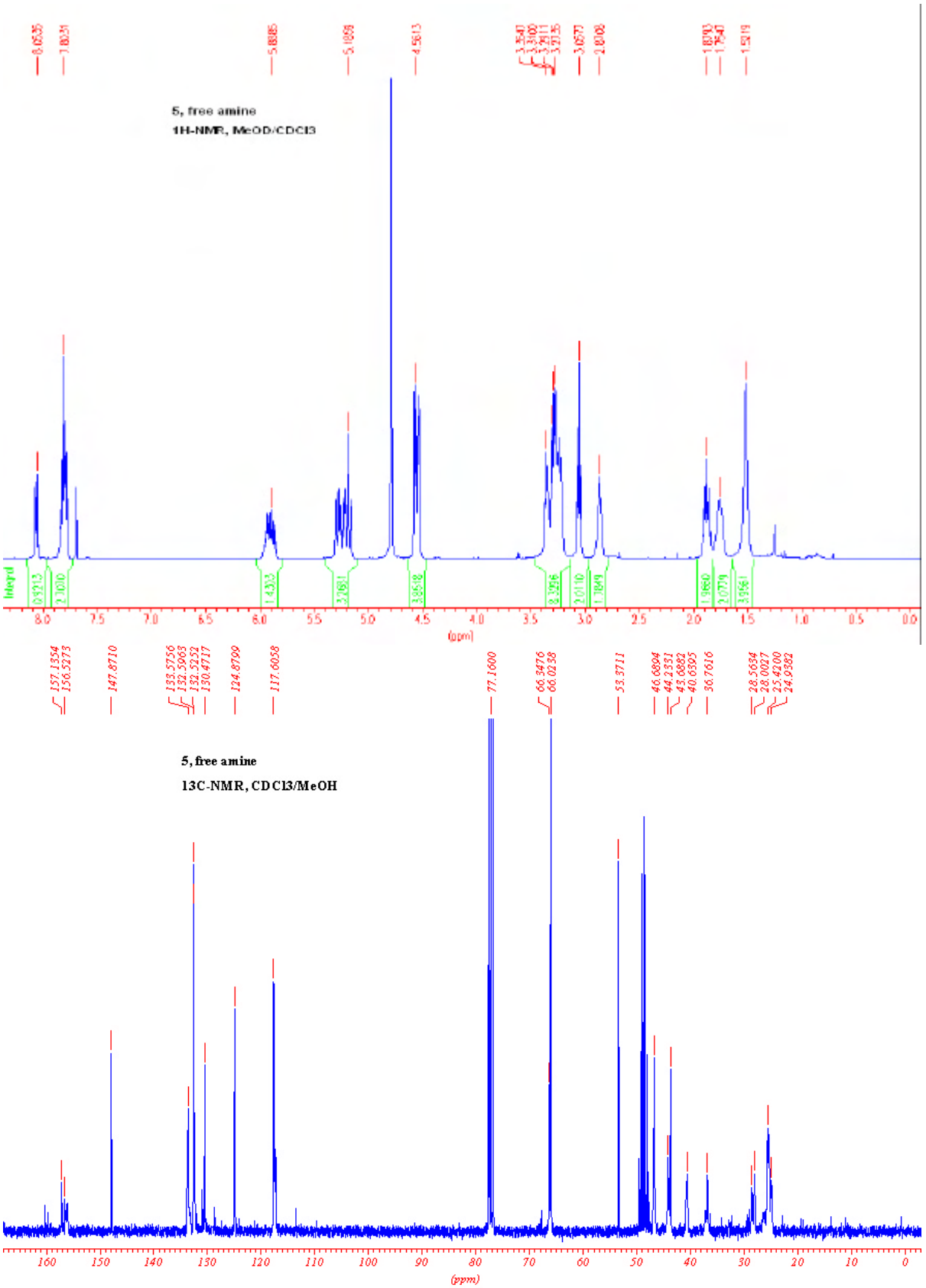


Figure 68 ^{1}H NMR and ^{13}C NMR of **187**, measured in $CDCl_3$ / CD_3OD. Some of the signals corresponding to the backbone methylene groups are splitted due to rotational isomerism of the tertiary carbamates.

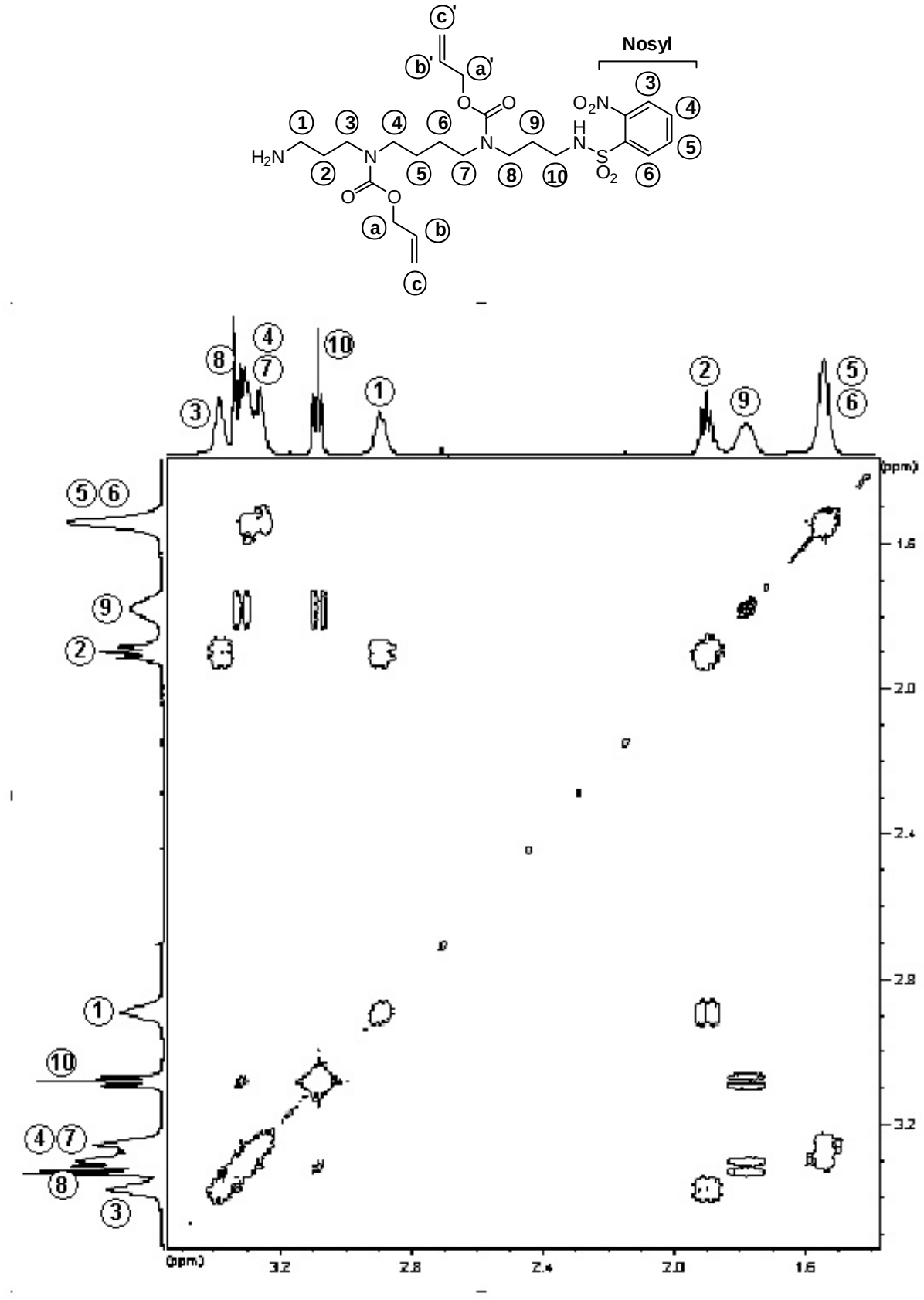


Figure 69 ^{1}H-^{1}H-COSY-Spectrum of **187**, measured in $CDCl_3$ / MeOH (1 : 5). The signal at 2.9 ppm corresponds to the methylene group 1. This is confirmed by the fact that solely this multiplet changes distinctly to lower field after protonation of the amino group in its trifluoroacetic acid salt. The identity of methylene groups 8 and 10 was allocated by comparison with the values of 3 / 3´ in **178**.

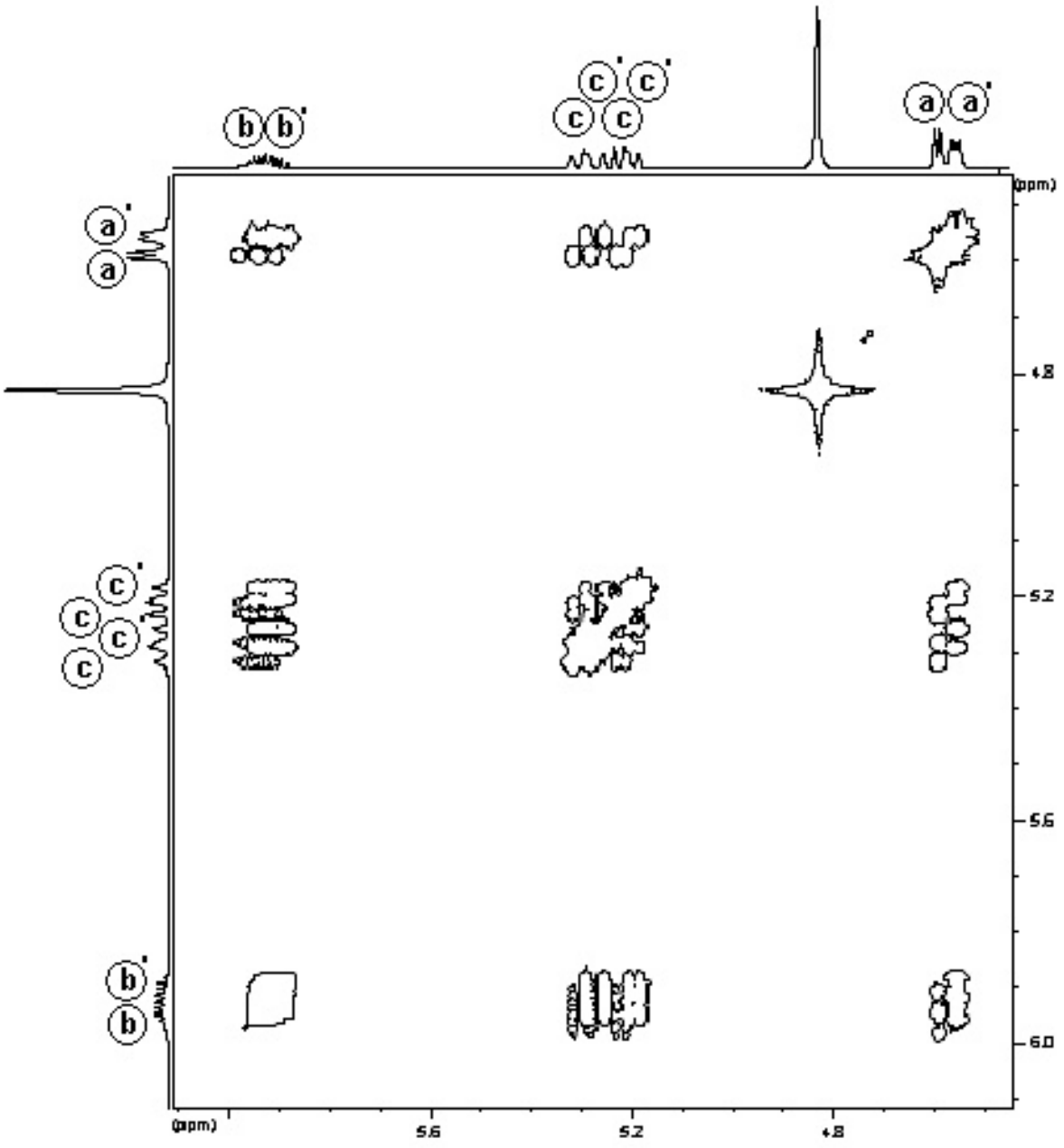


Figure 70 Section of the ^{1}H-^{1}H-COSY-Spectrum of **187**, measured in $CDCl_3$ / MeOH (1 : 5). The Aloc-groups show slightly different patterns. This differentiation is not present in the symmetrical molecule **178** and indicates affection by the *o*-Nosyl group.

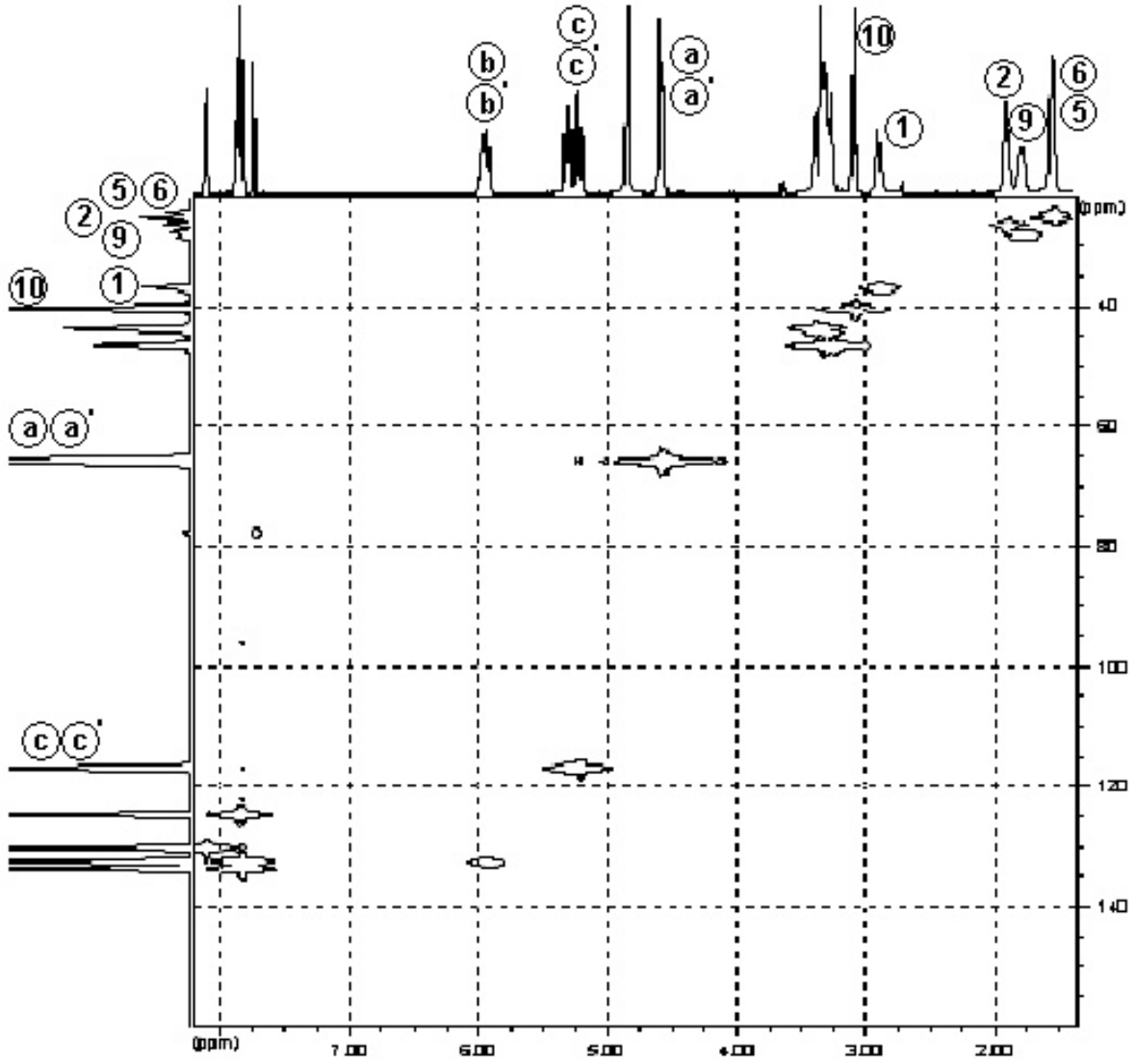
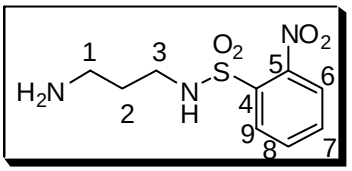


Figure 71 2D-^{1}H-^{13}C HMQC-Spectrum of **187**, measured in $CDCl_3$ / MeOH (1 : 5). The signals of the ^{13}C NMR spectra are broadened / splitted. This is due to dynamic effects that result from hindered rotation along the bonds of the backbone. The Aloc groups show additional splitting, probably due to a long-range influence of the *o*-Nosyl group.

1-(2-Nitrophenylsulfonamido)-3-aminopropane

5.63 mL (67.5 mmol; 1 equiv.) of 1,3-diaminopropane **(24)** were dissolved in 100 mL CH_2Cl_2 and 8.92 mL (67.5 mmol; 1 equiv.) of collidine were added in one portion. A solution of 8.86 g (40.0 mmol; 0.6 equiv.) of 2-nitrophenylsulfonylchloride in 50 mL CH_2Cl_2 was slowly added during 90 min and the solution was stirred for 18 h. The solution was three times washed with NaOH (1 M) and dried over Na_2SO_4. The solvent was removed and the product was purified by flash chromatography on silica gel ($CHCl_3$: MeOH (20 : 1) to $CHCl_3$: MeOH : NH_3 (25%) (1 : 1 : 0.1)). The silicagel was removed by washing with 10 mL of water, the solvent was removed under reduced pressure, and the product was dried on HV. 4.86 g (18.8 mmol) of **188** were obtained to give 47% yield related to the insert of 2-nitrophenylsulfonylchloride respectively 28% related to the input of diaminopropane **(24)**.



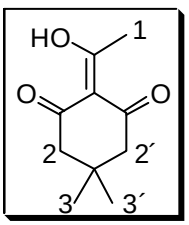
$C_9H_{13}N_3O_4S$

R_f = 0.08 ($CHCl_3$: MeOH - 10 : 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (dd, J_1 = 5.9 Hz, J_2 = 3.3 Hz, 1 H, 6-H), 7.78 – 7.88 (m, 3 H, 7-H 8-H 9-H), 3.10 (t, J = 6.9 Hz, 2 H, 3-H), 2.71 (t, J = 7.0 Hz, 2 H, 1-H), 1.68 (tt, J_1 = 7.0 Hz, J_2 = 6.9 Hz, 2 H, 2-H); HRMS (ESI) $[M + H]^+$ calc. 260.0700, found 260.0704.

2-(1-(3-Iodopropylamino)ethylidene)-5,5-dimethylcyclohexan-1,3-dione (193)

2-(1-Hydroxyethylidene)-5,5-dimethylcyclohexan-1,3-dione (191)

12.0 g (85.0 mmol; 1 equiv.) of dimedone **(189)** and 7.60 mL (94.2 mmol; 1.1 equiv.) of pyridine were dissolved in 250 mL $CHCl_3$. The solution was cooled to 0 °C and 9.20 mL (128 mmol; 1.5 equiv.) of acetylchloride were added. The solution was stirred for 30 min and the organic layer was then washed with water, solutions of half-concentrated HCl, concentrated $NaHCO_3$ and water. The organic layer was dried over Na_2SO_4 and 22.9 g (171 mmol; 2 equiv.) of $AlCl_3$ were added. After stirring for 60 min the reaction mixture was given into 300 mL of a mixture of ice and concentrated hydrochloric acid (1 : 1). The aqueous layer was three times extracted with $CHCl_3$. The combined organic layers were dried over Na_2SO_4 and the solvent was removed. A yellow solid was obtained. The product **191** was purified by flash chromatography on silica gel (cyclohexane : EtOAc (2 : 1)). A slightly yellow and highly viscose syrup was obtained that turns into a solid after drying on HV for 2 d and storage for 10 h at –20 °C. 7.88 g (43.1 mmol) were achieved giving 51% yield over two steps.

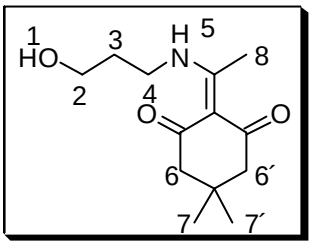


$C_{10}H_{14}O_3$

R_f = 0.32 (CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.54 (s, 2 H, 2-Ha 2´-Ha); 2.50 (s, 2 H, 2-Hb 2´-Hb); 2.32 (s, 3 H, 1-H); 1.04 (s, 6 H, 3-H 3´-H).

2-(1-(3-Hydroxypropylamino)ethylidene)-5,5-dimethylcyclohexan-1,3-dione (192)

7.88 g (43.3 mmol; 1 equiv.) of **191** were dissolved in 200 mL of MeOH. 9.06 mL (51.9 mmol; 1.2 equiv.) of DiPEA and 6.62 mL (86.5 mmol; 2.0 equiv.) of 3-amino-1-propanol were added and the clear yellow solution was stirred for 6.5 h. The solvent was removed and the residue was dissolved in 150 mL of CH_2Cl_2. The organic layer was three times washed with half concentrated HCl and dried over Na_2SO_4. The solvent was removed and the remaining solid was dried on HV. 8.07 g (33.8 mmol) of a yellow solid **192** were obtained giving 78% yield.

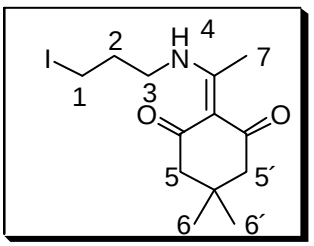


$C_{13}H_{21}NO_3$

R_f = 0.16 (cyclohexane : EtOAc - 1 : 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.40 (s, 1 H, 5-H, 1-H); 3.74 (t, J_1 = 5.9, 2 H, 2-H); 3.53 (dt, J_1 = 5,7, J_2 = 6.8, 2 H, 4-H); 2.53 (s, 4 H, 6-H 6´-H); 2.32 (s, 3 H, 8-H); 1.89 (tt, J_1 = 6.8, J_2 = 5.9, 2 H, 3-H); 0.99 (s, 6 H, 7-H 7´-H); MS (ESI): 262.1 (100) $[M + Na]^+$, 240.0 (100) $[M + H]^+$.

2-(1-(3-Iodopropylamino)ethylidene)-5,5-dimethylcyclohexan-1,3-dione (193)

3.02 g (11.5 mmol; 1.1 equiv.) of PPh_3 were dissolved in 5 mL CH_2Cl_2, cooled to 0 °C and stirred. Solutions of 999 mg (14.7 mmol; 1.4 equiv.) of imidazole in 9 mL CH_2Cl_2, 3.19 g (12.6 mmol; 1.2 equiv.) of iodine in 8 mL CH_2Cl_2 and 2.51 g (10.5 mmol; 1 equiv.) of **192** were added and stirring was continued for 30 min. The reaction mixture was warmed to room temperature and the solvent was removed. The residue was purified by flash chromatography on silica gel (cyclohexane : EtOAc (2 : 1) to cyclohexane : EtOAc (1 : 1)). After drying on HV, 3.02 g (8.64 mmol) of a slightly yellow solid **193** were obtained giving 82% yield.

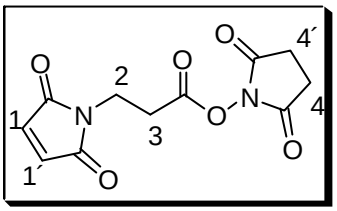


$C_{13}H_{20}NO_2I$

R_f = 0.39 (cyclohexane : EtOAc - 1 : 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.47 (dt, J_1 = 5.5 Hz, J_2 = 6.7 Hz, 2 H, 3-H); 3.19 (t, J = 6.6 Hz, 2 H, 1-H); 2.52 (s, 3 H, 7-H); 2.29 (s, 4 H, 5-H 5´-H); 2.09 (tt, J_1 = 6.7 Hz, J_2 = 6.6 Hz, 2 H, 2-H); 0.96 (s, 6 H, 6-H 6´-H).

***N*-Maleimidopropionylsuccinate (196)**

14 g (135 mmol; 1.2 equiv) of maleic anhydride were dissolved in 120 mL DMF. 10 g (112 mmol; 1 equiv.) of ß-alanine **194** in 20 mL DMF were added and stirred for 120 min. The reaction mixture was cooled to 0 °C and 55.5 g (269 mmol; 2.4 equiv.) of DCC and 19.4 g (168 mmol; 1.5 equiv.) of HNS were added contemporaneously in two equimolar portions. The colour of the suspension turned to purple during further stirring at 0 °C for 150 min. The suspension was filtered to remove residues of dicyclohexyl urea. The filtrate was partitioned between CH_2Cl_2 and water and the organic layer was washed with water (3 times), 5% $NaHCO_3$ solution (three times) and concentrated NaCl solution (two times). The combined aqueous layers were re-extracted with a small amount of CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. 19.0 g (84.0 mmol) of a slightly purple solid **196** were obtained giving 75% yield.



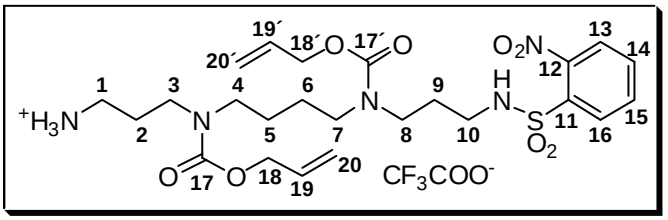
$C_{11}H_{10}N_2O_6$

R_f = 0.47 (cyclohexane : EtOAc - 1 : 1); ^{1}H NMR (400 MHz, d_6-DMSO) δ 6.92 (s, 2 H, H-1, 1-H 1´-H), 3.74 (t, *J* = 6.9 Hz, 2 H, 2-H), 3.32 (s, 4 H, 4-H 4´-H), 3.04 (t, *J* = 6.9 Hz, 2 H, 3-H); FAB-MS: 289.2 (51) [M^+ + Na]; 267.1 (100) [M^+ + H]; 225.2 (31) [M^+ + H – ($COCH_2$)]Establishment of the solid phase methods

6.4 Development of the solid phase methods

6.4.1 Optimisation of loading conditions with building block 187

100 mg (720 µmol; 1 equiv.) of inactivated alkoxytrityl resin were swollen in CH_2Cl_2 and 5 µl (290 µmol; 4 equiv.) of $SOCl_2$ were added. The suspension was agitated for 1 h and the resin was washed with CH_2Cl_2. A solution of base and building block **187** in CH_2Cl_2 was added and the suspension was agitated for 3 h. The resin was washed with CH_2Cl_2 / MeOH and CH_2Cl_2. The product was cleaved from the resin. The amount of attached material was calculated by the addition of dioxane as a quantitative NMR-standard.



$C_{26}H_{38}F_3N_5O_{10}S$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (m, 1 H, 13-H), 7.82 (bs, 3 H, N-H), 7.73 (m, 3 H, 14-H 15-H 16-H), 5.87 (ddt, J_1 = 17.2 Hz, J_2 = 10.5 Hz, J_3 = 5.4 Hz, 2 H, 19-H 19´-H), 5.24 (m, 2 H, 20-H), 5.18 (m, 2 H, 20´-H), 4.55 (m, 4 H, 18-H 18´-H), 3.37 (m, 2 H, 3-H), 3.30 (m,

2 H, 8-H), 3.21 (m, 4 H, 4-H 7-H), 3.08 (t, *J* = 6.7 Hz, 2 H, 10-H), 3.01 (m, 2 H, 1-H), 1.93 (m, 2 H, 2-H), 1.74 (m, 2 H, 9-H), 1.50 (m, 4 H, 5-H 6-H).

General loading conditions

The activated resin is swollen in CH_2Cl_2 and after removal of the solvent a solution of 2.5 equiv. of DiPEA and 10 equiv. of the respective building block in CH_2Cl_2 is added. The suspension is agitated over night. 1 mL MeOH per 100 mg resin is added to the suspension to block the remaining binding sites and it is agitated for further 15 minutes. The resin is washed with CH_2Cl_2 / MeOH and CH_2Cl_2. After removal of the solvent and removal of DiPEA in HV the respective building block could be recovered for further reaction cycles.

Introduction of Aloc group

The resin is swollen in CH_2Cl_2 and after removal of the solvent a solution of 10 equiv. of allylchloroformate and 10 equiv. of Et_3N in CH_2Cl_2 is added. The suspension is agitated over night, the solvent is removed, and the resin is washed with CH_2Cl_2 / MeOH and CH_2Cl_2.

6.4.2 Exhaustive Aloc deprotection

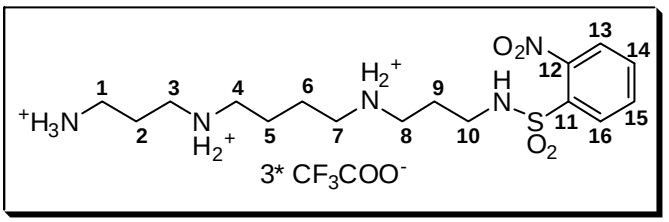
The following procedures were used for Entries of Table 6:

(a) The resin **198** is swollen in THF and after removal of the solvent a solution of 10 – 20 equiv. of pyrrolidine / 8 – 10 equiv. of dimedone and 1 – 50 mol% of $Pd(PPh_3)_4$ in 1.5 mL THF are added. The suspension is agitated for 0.5 – 21 h in the absence of light. The resin is washed with a solution of sodium-diethylaminodithiocarbamate in THF / MeOH, THF and CH_2Cl_2. The crude is cleaved from the resin. The drained cleavage mixture is combined with washings of CH_2Cl_2 and MeOH respectively.

(b) The resin **198** is swollen in CH_2Cl_2 and after removal of the solvent a solution of 9 – 20 equiv. phenylsilane / 8 – 10 equiv. of trimethylsillylazide and 1 – 20 mol% of $Pd(PPh_3)_4$ in 1.5 mL CH_2Cl_2 are added. The suspension is agitated for 1.5 – 17 h in the absence of light. The resin is washed with a solution of sodium-diethylaminodithiocarbamate in THF / MeOH, THF and CH_2Cl_2.

The successful procedure with *N,N'*-dimethylbarbituric acid was carried out as follows:

The resin **198** was swollen in CH_2Cl_2 in a lock-up vial and 8 equiv. of *N,N'*-dimethylbarbituric acid and 10 mol% of $Pd(PPh_3)_4$ in 1.5 mL CH_2Cl_2 are added. The suspension was agitated for 2 h at 35 °C in the absence of light. The resin is transferred to a glas frit, washed with a solution of sodium-diethylaminodithiocarbamate in CH_2Cl_2 / MeOH and CH_2Cl_2. After cleavage, **199** is obtained in high purity.



$C_{16}H_{38}N_5O_{10}F_9S$

^{1}H NMR (400 MHz, CD_3OD) δ 7.80 – 8.10 (m, 4 H, 13-H 14-H 15-H 16-H), 3.16 (t, J = 6.8 Hz, 2 H, 3-H), 3.00 – 3.15 (m, 10 H, 1-H 4-H 7-H 8-H 10-H), 2.09 (tt, J_1 = 7.5, J_2 = 7.7, 2 H, 9-H), 1.94 (tt, J_1 = 7.4, J_2 = 6.8, 2 H, 2-H), 1.79 (m, 4 H, 5-H 6-H); HRMS (EI) calc. 388.2013, found 388.2011.

Introduction of *o*-Nosyl group

The resin is swollen in CH_2Cl_2 and after removal of the solvent a solution of 10 equiv. of *o*-Nosyl chloride and 10 equiv. of collidine in CH_2Cl_2 is added. The suspension is agitated over night, the solvent is removed, and the resin is washed with CH_2Cl_2 / MeOH and CH_2Cl_2.

o-Nosyl removal

The resin is swollen in DMF and after removal of the solvent a solution 10 equiv. of β–mercaptoethanol and 5 equiv. of DBU in DMF are added. The suspension is agitated for 1 h and the colour of the liquid turns to intensive yellow. After removal of solvent the resin is washed with DMF until the filtrate is colourless. The procedure is repeated in steps of 30 min until the supernatant stays colourless after the reaction. Usually the reaction is complete after 2 h overall reaction time. Alternatively the resin is treated with 20 equiv. of mercaptoethanol and 20 equiv. of DBU overnight and subsequently washed with DMF until the filtrate is colourless.

Dde removal

The resin is swollen in DMF and after removal of the solvent a solution of 10 equiv. N_2H_4*H_2O (80% in H_2O) in DMF is added. In the presence of Aloc groups 100 equiv. of allyl alcohol are added. The suspension is agitated (1 * 30 min, 2 * 15 min) and after removal of the solvent the resin is washed with DMF in each case.

Fmoc removal

The resin is swollen in DMF and after removal of the solvent a solution of pyrrolidine / DMF (1 : 3) is added. The suspension is agitated (1 * 30 min, 2 * 15 min) and after removal of the solvent the resin is washed with DMF in each case.

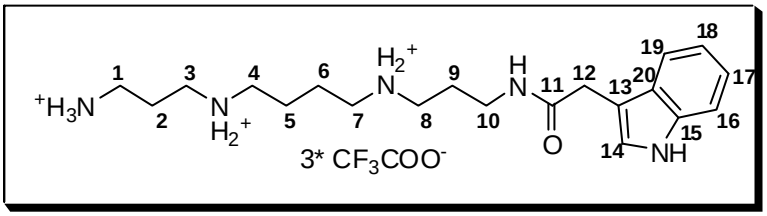
6.4.3 Versatility of the protection group strategy - synthesis of HO359b

SP-bound-bis-Aloc-protected HO359b (201)

510 mg of 2-chlorotrityl resin were loaded with **187** under the conditions described above. Fifteen minutes prior termination of the reaction, 4 mL of MeOH were added to block the remaining binding sites on the resin. After drying 150 mg (82 µmol; 1 equiv.) of the resin **198** were *o*-Nosyl deprotected. The cold *Kaiser Test* was positive. A solution of 55 mg (0.41 mmol; 5 equiv.) hydroxybenzotriazole and 72 mg (0.41 mmol; 5 equiv.) 3-indoleacetic acid in 1 mL DMF was added to resin **7** and the suspension was shortly agitated. 84 mg (0.41 mmol; 5 equiv.) dicyclohexylcarbodiimide in 1.7 mL were added and the suspension agitated for 4 h in the absence of light. The resin was washed with DMF, CH_2Cl_2 / MeOH and then with CH_2Cl_2. The *Kaiser Test* was negative. The product was cleaved from a small portion of the resin **201** and the crude product was analyzed by FAB-MS: 528.3 (100) $[M + H]^+$.

HO359b (203)

Aloc-deprotection of 56 mg of resin **201** was carried out following the above described procedure with *N,N'*-dimethylbarbituric acid. The product was cleaved from the resin and after removal of the solvents, the crude product was purified by preparative HPLC (RP-18, H_2O - MeCN - TFA (10 : 90 : 0.1 to 80 : 20 : 0.1)). 19.3 mg (27.5 µmol) of a yellow solid **203**, which changes into white foam after 4 d drying on HV were obtained. Yield: 90% calculated on the loading of resin **198**.



$C_{26}H_{36}F_9N_5O_7$

^{1}H NMR (400 MHz, CD_3OD) δ 7.55 (d, *J* = 7.7 Hz, 1 H, 19-H), 7.37 (d, *J* = 7.9 Hz, 1 H, 16-H), 7.21 (s, 1 H, 14-H), 7.11 (dt, J_1 = 7.9 Hz, J_2 = 0.7 Hz, 1 H, 17-H), 7.03 (dt, J_1 = 7.7 Hz, J_2 = 0.7 Hz, 1 H, 18-H), 3.68 (s, 2 H, 12-H), 3.30 (t, *J* = 6.7 Hz, 2 H, 10-H), 3.08 (t, *J* = 7.7 Hz, 2 H, 4-H), 3.03 (t, *J* = 7.7 Hz, 2 H, 7-H), 2.96 (t, *J* = 7.9 Hz, 2 H, 3-H), 2.80 (t, *J* = 7.0 Hz, 2 H, 8-H), 2.78 (t, *J* = 7.7 Hz, 2 H, 1-H), 2.06 (tt, J_1 = 7.9 Hz, J_2 = 7.7 Hz, 2 H, 2-H), 1.80 (tt, J_1 = 7.0 Hz, J_2 = 6.7 Hz, 2 H, 9-H), 1.70 (m, 2 H, 5-H), 1.61 (m, 2 H, 6-H); ^{13}C NMR (75 MHz, CD_3OD) δ 176.3 (C_{quart}, C-11), 138.1 (C_{quart}, C-15), 128.4 (C_{quart}, C-20), 125.2, 122.7 (+, C-14 C-18), 120.1 (+, C-17), 119.3 (+, C-19), 112.6 (+, C-16), 109.4 (C_{quart}, C-13), 48.1, 47.9 (–, C-4 C-7), 46.0, 45.8 (–, C-3 C-8), 37.7, 36.8 (–, C-1 C-10), 33.9 (–, C-12), 27.4, 25.2 (–, C-2 C-9), 24.0 (–, C-5 C-6); HRMS (ESI) $[M + H]^+$ calc. 360.2758, found 360.2755.

6.4.4 Investigation on *Fukuyama alkylation*

General procedure with K_2CO_3

1 equiv. of **198** and 13 equiv. of K_2CO_3 were suspended in DMF and after 5 min 13 equiv. of halide were added. The suspension is agitated at room temperature / 60°C for 18 h. The resin is washed with water, THF : MeOH (2 : 1) / MeOH and CH_2Cl_2.

General procedure with DBU

1 equiv. of **198** is swollen in DMF for 5 min and 6 equiv. of DBU and 6 equiv. of halide were added. The suspension is agitated at room temperature for 3, 5, 8, 18 or 30 h. The resin is washed with DMF, THF : MeOH (2 : 1) / MeOH and CH_2Cl_2.

Purity determination

Conversions were calculated by the purity of the crude products, which was determined by integration of the relative areas of RP-HPLC with UV-detection at 254 nm. The identity of the peaks was confirmed by HRMS. The crude product was purified by preparative HPLC and subsequently the ^{1}H NMR spectra were measured in $CDCl_3$ or $CDCl_3$ / CD_3OD (1 : 1).

Analytical data of alkylation products[a]

Entry 1: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 8.20 (m, 4 H, 13-H N*H*$_3$), 7.60 – 7.80 (m, 3 H, 14-H 15-H 16-H), 7.20 – 7.40 (m, 5 H, Ar$_l$-H$_{benzy}$), 5.90 (ddt, J_1 = 17.2 Hz, J_2 = 10.5 Hz, J_3 = 5.6 Hz, 2 H, 19-H 19´-H), 5.27 (dd, J_1 = 17.2 Hz, J_2 = 1.3 Hz, 2 H, 20-Ha 20´-Ha), 5.18 (d, J = 10.5 Hz, 2 H, 20-Hb 20´-Hb), 4.40 – 4.80 (m, 6 H, 18-H 18´-H (C*H*$_2$)$_{benzyl}$), 3.39 (m, 2 H, 3-H), 3.26 (m, J = 7.3 Hz, 8-H), 3.20 (m, 2 H, 10-H), 3.07 (m, 4 H, 4-H 7-H), 2.99 (m, 2 H, 1-H), 1.93 (m, 2 H, 2-H), 1.63 (m, 2 H, 9-H), 1.43 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 646.2905, found 646.2905.

Entry 2: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.17 (d, J = 8.6 Hz, 2 H, Ar-H$_{meta\text{-}benzyl}$), 8.16 (m, 3 H, N*H*$_3$), 8.00 (d, J = 7.3 Hz, 1 H, 13-H), 7.60 – 7.80 (m, 3 H, 14-H 15-H 16-H), 7.47 (d, J = 8.6 Hz, 2 H, Ar -H$_{para\text{-}benzyl}$), 5.90 (ddt, J_1 = 17.2 Hz, J_2 = 10.7 Hz, J_3 = 5.5 Hz, 2 H, 19-H 19´-H), 5.27 (dd, J_1 = 17.2 Hz, J_2 = 1.4 Hz, 2 H, 20-Ha 20´-Ha), 5.18 (d, J = 10.7, 2 H, 20-Hb 20´-Hb), 4.40 – 4.70 (s, 6 H, 18-H 18´-H (C*H*$_2$)$_{benzyl}$), 3.38 (m, 2 H, 3-H), 3.28 (t, J = 7.0 Hz, 2 H, 8-H), 3.19 (m, 2 H, 10-H), 3.09 (m, 4 H, 4-H 7-H), 2.97 (m, 2 H, 1-H), 1.93 (m, 2 H, 2-H), 1.64 (m, 2 H, 9-H), 1.43 (m, 4 H, 5-H 6-H); FAB-MS: 691.2 (100) $[M + H]^+$, 713.2 (6) $[M + Na]^+$; HRMS (ESI) $[M + H]^+$ calc. 691.2756, found 691.2753.

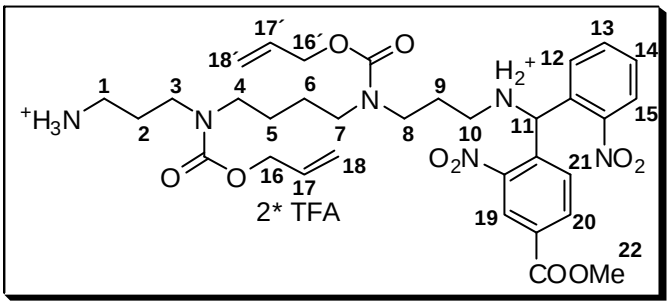
Entry 3: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (bs, 3 H, N*H*$_3$); 7.96 (m, 1 H, 13-H), 7.60 – 7.80 (m, 3 H, 14-H 15-H 16-H), 7.56 (d, J = 7.9 Hz, 2 H, Ar-H$_{meta\text{-}benzyl}$), 7.39 (d, J = 7.9 Hz, 2 H, Ar-H$_{ortho\text{-}benzyl}$), 5.90 (ddt, J_1 = 17.3 Hz, J_2 = 10.8 Hz, J_3 = 5.6 Hz, 2 H, 19-H 19´-H), 5.27 (dd, J_1 = 17.3 Hz, J_2 = 1.6 Hz, 2 H, 20-Ha 20´-Ha), 5.22 (d, J = 10.8 Hz, 2 H, 20-Hb 20´-Hb), 4.55 (m, 4 H, 18-H 18´-H), 4.54 (s, 2 H, (C*H*$_2$)$_{benzyl}$), 3.39 (m, 2 H, 3-H), 3.28 (t, J = 7.1 Hz, 2 H, 8-H), 3.20 (m, 2 H, 10-H), 3.08 (m, 4 H, 4-H 7-H), 2.98 (m, 2 H, 1-H), 1.93 (m, 2 H, 2-H), 1.65 (m, 2 H, 9-H), 1.43 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 714.2779, found 714.2769.

Entry 4: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (m, 3 H, N*H*$_3$), 7.95 (m, 1 H, 13-H), 7.69 (m, 3 H, 14-H 15-H 16-H), 7.55 (d, J = 8.0 Hz, 2 H, Ar-H$_{meta\text{-}benzyl}$), 7.39 (d, J = 8.0 Hz, 2 H, Ar-H$_{ortho\text{-}benzyl}$), 5.88 (ddt, J_1 = 17.3 Hz, J_2 = 10.5 Hz, J_3 = 5.5 Hz, 2 H, 19-H 19´-H), 5.27 (dd, J_1 = 17.3 Hz, J_2 = 1.5 Hz, 2 H, 20-Ha 20´-Ha), 5.22 (m, 2 H, 20-Hb 20´-Hb), 4.40 – 4.70 (m, 6 H, 18-H 18´-H (C*H*$_2$)$_{benzyl}$), 3.38 (m, 2 H, 3-H), 3.27 (m, 2 H, 8-H), 3.20 (m, 2 H, 10-H),

3.07 (m, 4 H, 4-H 7-H), 2.97 (m, 2 H, 1-H), 1.92 (m, 2 H, 2-H), 1.64 (m, 2 H, 9-H), 1.43 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 692.2782, found 692.2799.

Entry 5: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (bs, 3 H, N*H*$_3$), 7.93 (d, *J* = 7.6 Hz, 1 H, 13-H), 7.64 (m, 3 H, 14-H 15-H 16-H), 7.51 (d, *J* = 7.5 Hz, 1 H, Ar-H$_{benzyl(3)}$), 7.42 (d, *J* = 7.5 Hz, 1 H, Ar-H$_{benzyl(6)}$), 7.25 (dd, J_1 = 7.5 Hz, J_2 = 7.4 Hz, 1 H, Ar-H$_{benzyl(5)}$), 7.13 (dd, J_1 = 7.5 Hz, J_2 = 7.4 Hz, 1 H, Ar-H$_{benzyl(4)}$), 5.90 (ddt, J_1 = 17.3 Hz, J_2 = 10.8 Hz, J_3 = 5.4 Hz, 2 H, 19-H 19´-H), 5.27 (dd, J_1 = 17.3 Hz, J_2 = 1.5 Hz, 2 H, 20-Ha 20´-Ha), 5.22 (d, *J* = 10.8, 2 H, 20-Hb 20´-Hb), 4.40 – 4.70 (m, 6 H, 18-H 18´-H (C*H*$_2$)$_{benzyl}$), 3.37 (m, 2 H, 3-H), 3.33 (t, *J* = 7.5 Hz, 2 H, 8-H), 3.20 (m, 2 H, 10-H), 3.09 (m, 4 H, 4-H 7-H), 2.98 (m, 2 H, 1-H), 1.91 (tt, J_1 = 6.3 Hz, J_2 = 6.2 Hz, 2 H, 2-H), 1.67 (m, 2 H, 9-H), 1.44 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 724.2010, found 724.1997.

Entry 6:



$C_{37}H_{46}N_6O_{14}F_6$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.72 (s, 1 H, 19-H), 8.37 (d, *J* = 7.8, 1 H, 20-H), 8.14 (d, *J* = 7.9, 2 H, 21-H), 7.80 – 8.10 (bs, 3 H, N*H*$_3$), 8.02 (d, *J* = 7.5, 1 H, 15-H), 7.79 (dd, J_1 = 7.6, J_2 = 7.6, 1 H, 13-H), 7.63 (dd, J_1 = 7.6, J_2 = 7.5, 1 H, 14-H), 6.91 (m, 1 H, 12-H), 5.88 (ddt, J_1 = 17.2 Hz, J_2 = 10.5 Hz, J_3 = 5.4 Hz, 2 H, 17-H 17´-H), 5.80 – 5.00 (bs, 3 H, 11-H N*H*$_2$), 5.26 (dd, J_1 = 17.2 Hz, J_2 = 1.4 Hz, 2 H, 18-Ha 18´-Ha), 5.20 (d, *J* = 10.5 Hz, 2 H, 18-Hb 18´-Hb), 4.40 – 4.60 (m, 4 H, 16-H 16´-H), 3.97 (s, 3 H, 22-H), 3.44 (m, 2 H, 8-H), 3.35 (m, 2 H, 3-H), 3.23 (m, 6 H, 4-H 7-H 10-H), 2.96 (m, 2 H, 1-H), 2.09 (tt, J_1 = 6.0, J_2 = 6.4, 2 H, 9-H), 1.91 (tt, J_1 = 6.3, J_2 = 6.3, 2 H, 2-H), 1.50 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 685.3192, found 685.3188.

Entry 7: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (m, 4 H, 13-H N*H*$_3$), 7.60 – 7.80 (m, 3 H, 14-H 15-H 16-H), 7.15 (d, *J* = 8.4 Hz, 2 H, Ar-H$_{meta\text{-}benzyl}$), 6.81 (d, *J* = 8.4 Hz, 2 H, Ar-H$_{ortho\text{-}benzyl}$), 5.90 (ddt, J_1 = 16.9 Hz, J_2 = 10.7 Hz, J_3 = 5.6 Hz, 2 H, 19-H 19´-H), 5.28 (dd, J_1 = 16.9 Hz, J_2 = 1.7 Hz, 2 H, 20-Ha 20´-Ha), 5.19 (d, *J* = 10.7 Hz, 2 H, 20-Hb 20´-Hb), 4.48 – 4.70 (m, 4 H, 18-H 18´-H), 4.41 (s, 2 H, (C*H*$_2$)$_{benzyl}$), 3.78 (s, 3 H, C*H*$_3$), 3.40 (m, 2 H, 3-H), 3.24 (m, 4 H, 8-H 10-H), 3.08 (m, 4 H, 4-H 7-H), 3.01 (m, 2 H, 1-H), 1.94 (m, 2 H, 2-H), 1.64 (m, 2 H, 9-H), 1.45 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 676.3011, found 676.2997.

Entry 8 / 9: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 8.20 (m, 7 H, 13-H 14-H 15-H 16-H N*H*$_3$), 5.90 (ddt, J_1 = 16.7 Hz, J_2 = 10.9 Hz, J_3 = 5.7 Hz, 2 H, 19-H 19´-H), 5.66 (ddt, J_1 = 17.0 Hz, J_2 = 10.2 Hz, J_3 = 6.5 Hz, 1 H, C*H*$_{allyl(2)}$), 5.12 – 5.35 (m, 6 H, 20-H 20´-H (C*H*$_2$)$_{allyl(3)}$), 4.58 (d, *J* = 5.7 Hz, 2 H, 18-H), 4.56 (d, *J* = 5.7 Hz, 2 H, 18´-H), 3.91 (m, 2 H(C*H*$_2$)$_{allyl(1)}$), 3.40 (m, 2 H, 3-H), 3.30 (t, *J* = 7.3 Hz, 2 H, 10-H), 3.22 (m, 6 H, 4-H 7-H 8-H), 3.01 (m, 2 H, 1-H), 1.94 (m, 2 H, 2-H), 1.81 (m, 2 H, 9-H), 1.51 (m, 4 H, 5-H 6-H); MS (ESI): 596.3 (100) $[M + H]^+$, 618.3 (2) $[M + Na]^+$; HRMS (ESI) $[M + H]^+$ calc. 596.2749, found 596.2749.

Entry 10: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 8.20 (m, 7 H, 13-H 14-H 15-H 16-H NH_3), 5.90 (ddt, J_1 = 17.1 Hz, J_2 = 11.3 Hz, J_3 = 5.7 Hz, 2 H, 19-H 19´-H), 5.85 (m, 1 H, (CH_2)$_{allyl(3)}$), 5.59 (d, J = 17.2 Hz, 1 H, (CH_2)$_{allyl(3)}$), 5.28 (d, J = 17.1 Hz, 2 H, 20-Ha 20´-Ha), 5.20 (m, 2 H, 20-Hb 20´-Hb), 4.58 (d, J = 5.7 Hz, 2 H, 18-H), 4.56 (d, J = 5.7 Hz, 2 H, 18´-H), 4.20 (s, 2 H, (CH_2)$_{allyl(1)}$), 3.37 (d, J = 5.8 Hz, 2 H, 3-H), 3.10 – 3.40 (m, 8 H, 4-H 7-H, 8-H 10-H), 3.03 (m, 2 H, 1-H), 1.94 (m, 2 H, 2-H), 1.81 (m, 2 H, 9-H), 1.51 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 674.1854, found 674.1860.

Entry 11: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 – 8.20 (m, 7 H, 13-H 14-H 15-H 16-H NH_3), 5.91 (ddt, J_1 = 17.1 Hz, J_2 = 10.8 Hz, J_3 = 5.8 Hz, 2 H, 19-H 19´-H), 5.28 (dd, J_1 = 17.1 Hz, J_2 = 6.2 Hz, 2 H, 20-Ha 20´-Ha), 5.22 (d, J = 10.8 Hz, 2 H, 20-Hb 20´-Hb), 4.59 (d, J = 5.8 Hz, 2 H, 18-H), 4.57 (d, J = 5.8 Hz, 2 H, 18´-H), 3.41 (m, 2 H, 3-H), 3.31 (t, J = 7.7 Hz, 2 H, 10-H), 3.24 (m, 8 H, 4-H 7-H 8-H CH_2(2)), 3.01 (m, 2 H, 1-H), 1.94 (m, 2 H, 2-H), 1.82 (m, 2 H, 9-H), 1.52 (m, 6 H, 5-H 6-H CH_2(2)), 1.10 – 1.40 (m, 22 H, CH_2(3-13)), 0.88 (t, J = 7.0 Hz, 3 H, CH_3); HRMS (ESI) $[M + H]^+$ calc. 752.4627, found 752.4624.

Entry 12: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 – 8.10 (m, 7 H, 13-H 14-H 15-H 16-H NH_3), 5.90 (ddt, J_1 = 16.7 Hz, J_2 = 10.7 Hz, J_3 = 5.7 Hz, 2 H, 19-H 19´-H), 5.20 – 5.40 (m, 4 H, 20-H 20´-H), 4.60 (m, 4 H, 18-H 18´-H), 3.42 (m, 2 H, 3-H), 3.37 (t, J = 6.3 Hz, 2 H, CH_2(4)), 3.31 (m, 4 H, 10-H CH_2(1)), 3.26 (m, 6 H, 4-H 7-H 8-H), 3.07 (m, 2 H, 1-H), 1.95 (m, 2 H, 2-H), 1.81 (tt, J_1 = 7.5 Hz, J_2 = 6.3 Hz, 4 H, 9-H CH_2(3)), 1.68 (tt, J_1 = 7.5 Hz, J_2 = 7.1 Hz, 2 H, CH_2(2)), 1.54 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 690.2167, found 690.2167.

Entry 13: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.25 (bs, 3 H, NH_3), 7.78 (m, 1 H, 13-H), 7.60 – 7.80 (m, 3 H, 14-H 15-H 16-H), 5.91 (ddt, J_1 = 17.1 Hz, J_2 = 10.6 Hz, J_3 = 5.6 Hz, 2 H, 19-H 19´-H), 5.15 – 5.30 (m, 4 H, 20-H 20´-H), 4.50 – 4.70 (m, 4 H, 18-H 18´-H), 3.42 (m, 2 H, 3-H), 3.30 (t, J = 7.4 Hz, 2 H, CH_2(1)), 3.20 – 3.35 (m, 8 H, 4-H 7-H 8-H 10-H), 2.99 (m, 2 H, 1-H), 2.82 (t, J = 6.9, 2 H, CH_2(4)), 2.31 (s, 3 H, CH_3), 1.95 (m, 2 H, 2-H), 1.81 (m, 2 H, 9-H), 1.45 – 1.64 (m, 8 H, 5-H 6-H CH_2(2) CH_2(3)); HRMS (ESI) $[M + H]^+$ calc. 686.2881, found 686.2888.

Entry 14: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (m, 3 H, NH_3), 7.93 (m, 1 H, 13-H), 7.60 – 7.75 (m, 3 H, 14-H 15-H 16-H), 7.10 – 7.35 (m, 5 H, Ar-H$_{phenyl}$), 5.90 (ddt, J_1 = 17.1 Hz, J_2 = 10.7 Hz, J_3 = 5.5 Hz, 2 H, 19-H 19´-H), 5.10 – 5.40 (m, 4 H, 20-H 20´-H), 4.57 (m, 4 H, 18-H 18´-H), 3.50 (t, J = 7.8 Hz, 2 H CH_2(1)), 3.36 (t, J = 7.4 Hz, 2 H, 3-H), 3.30 – 3.45 (m, 2 H, 10-H), 3.22 (m, 6 H, 4-H 7-H 8-H), 2.98 (m, 2 H, 1-H), 2.83 (t, J = 7.8 Hz, 2 H, CH_2(2)), 1.93 (m, 2 H, 2-H), 1.84 (m, 2 H, 9-H), 1.51 (m, 4 H, 5-H 6-H); FAB-MS: 660.3 (100) $[M + H]^+$, 682.2 (22) $[M + Na]^+$; HRMS (ESI) $[M + H]^+$ calc. 660.3062, found 660.3041.

Entry 15: ^{1}H NMR (400 MHz, $CDCl_3$ / CD_3OD) δ 7.78 (m, 1 H, 13-H), 7.40 – 7.60 (m, 3 H, 14-H 15-H 16-H), 5.68 (ddt, J_1 = 17.1 Hz, J_2 = 11.4 Hz, J_3 = 5.7 Hz, 2 H, 19-H 19´-H), 4.90 – 5.15 (m, 4 H, 20-H 20´-H), 4.20 – 4.40 (m, 4 H, 18-H 18´-H), 3.59 (m, 1 H, CH_2(3)a), 3.31 (m, 1 H, CH_2(3)b), 3.27 (m, 2 H, 3-H), 3.23 (m, 1 H, CH), 2.90 – 3.20 (m, 10 H, 1-H 4-H 7-H 8-H CH_2(1)), 2.67 (t, J = 7.1 Hz, 2 H, 10-H), 1.66 (tt, J_1 = 6.9 Hz, J_2 = 6.9 Hz, 4 H, 2-H 9-H), 1.30 (m, 4 H, 5-H 6-H); MS (ESI): 630.26 (100) $[M + H]^+$.

Entry 16: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 – 8.20 (m, 4 H, 13-H NH_3), 7.70 (m, 2 H, 14-H 16-H), 7.63 (m, 1 H, 15-H), 5.90 (ddt, J_1 = 17.0, J_2 = 10.5, J_3 = 5.4, 2 H, 19-H 19´-H), 5.27 (dd, J_1 = 17.0, J_2 = 6.3, 2 H, 20-Ha 20´-Ha), 5.20 (d, J = 10.5, 2 H, 20-Hb 20´-Hb), 4.57 (m, 4 H, 18-H 18´-H), 4.18 (s, 2 H, CH_2), 3.65 (s, 3 H, CH_3), 3.41 (m, 4 H, 3-H 10-H), 3.24 (m, 6 H, 4-H 7-H 8-H), 3.01 (m, 2 H, 1-H), 1.95 (m, 2 H, 2-H), 1.82 (m, 2 H, 9-H), 1.52 (m,

4 H, 5-H 6-H); MS (ESI): 628.3 (100) $[M + H]^+$, 650.2 (14) $[M + Na]^+$; HRMS (ESI) $[M + H]^+$ calc. 628.2647, found 628.2622.

Entry 17: HRMS (ESI) calc. $[M + H]^+$ 622.2905, found 622.2913. The amount of product from Entry 17 was too low for ^{1}H NMR.

Entry 19: ^{1}H NMR (400 MHz, $CDCl_3$ / CD_3OD) δ 7.85 (m, 1 H, 13-H), 7.50 – 7.70 (m, 3 H, 14-H 15-H 16-H), 5.70 – 5.90 (m, 2 H, 19-H 19´-H), 5.04 – 5.20 (m, 4 H, 20-H 20´-H), 4.40 – 4.50 (m, 4 H, 18-H 18´-H), 3.53 (m, 1 H, C*H*$_2$(4)a), 3.10 – 3.40 (m, 14 H, 1-H 3-H, 4-H 7-H 8-H C*H*$_2$(1) C*H*(3) C*H*$_2$(4)b), 2.78 (m, 2 H, 10-H), 1.77 (m, 4 H, 2-H 9-H), 1.61 (m, 1 H, C*H*$_2$(2)a), 1.50 (m, 1 H, C*H*$_2$(2)b), 1.40 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 644.2960, found 644.2956.

Entry 20: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 – 8.20 (m, 7 H, 13-H 14-H 15-H 16-H N*H*$_3$), 5.89 (ddt, J_1 = 16.9 Hz, J_2 = 10.8 Hz, J_3 = 5.6 Hz, 2 H, 19-H 19´-H), 5.28 (d, J = 16.9 Hz, 2 H, 20-Ha 20´-Ha), 5.21 (d, J = 10.8 Hz, 2 H, 20-Hb 20´-Hb), 4.59 (d, J = 5.6 Hz, 4 H, 18-H 18´-H), 3.54 (m, 2 H, C*H*$_2$(3)), 3.42 (m, 4 H, 3-H 10-H), 3.32 (t, J = 7.1 Hz, 2 H, C*H*$_2$(1)), 3.25 (m, 6 H, 4-H 7-H 8-H), 3.09 (m, 2 H, 1-H), 2.54 (s, 3 H, C*H*$_3$), 2.46 (s, 4 H, (C*H*$_2$)$_{cyclohexyl}$), 1.96 (m, 4 H, 2-H C*H*$_2$(2)), 1.85 (m, 2 H, 9-H), 1.53 (m, 4 H, 5-H 6-H), 1.05 (s, 6 H, 2 * C*H*$_3$); HRMS (ESI) $[M + H]^+$ calc. 777.3851, found 777.3847.

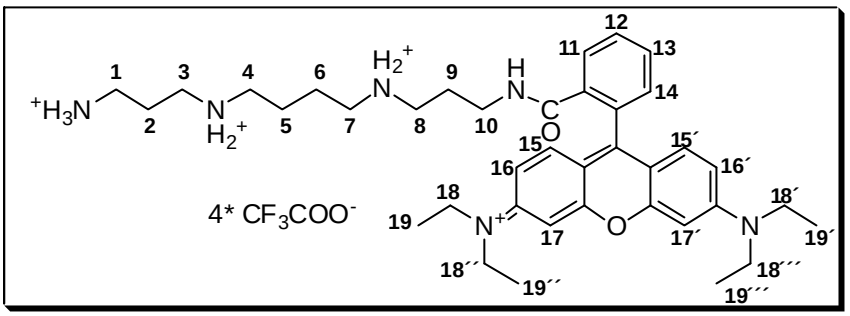
[a] Numeral correlation of the protons is in analogy to compound **187**. The substituents are described in the style C*H*$_x$(y).

6.5 Synthesis of polyamine conjugates

6.5.1 Synthesis of spermidine derivatives

[9-(2-{3-[4-(3-Amino-propylamino)-butylamino]-propylcarbamoyl}-phenyl)-6-diethylamino-xanthen-3-ylidene]-diethyl-ammonium tetrakis-trifuoracetate (211)

100 mg (45 µmol) of resin **198** were *o*-Nosyl deprotected. A solution of 110 mg rhodamine B **167** (230 µmol; 5 equiv.), 47 mg (230 µmol; 5 equiv.) of DCC, and 31 mg (230 µmol; 5 equiv.) of HOBt in DMF was shortly agitated and added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with DMF, THF / H_2O (10 : 1) / MeOH, and CH_2Cl_2. The colour of the resin turned to intensive pink and the *Kaiser Test* was negative. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA, treated with 10% TFA in CH_2Cl_2 and the crude product was purified by preparative HPLC (90% purity after cleavage from the resin, determined by UV detection at 254 nm). After removal of the solvent, the solid was dried on HV. 36 mg (34 µmol) of a pink solid were obtained giving 74% yield calculated on the loading of resin **198**.

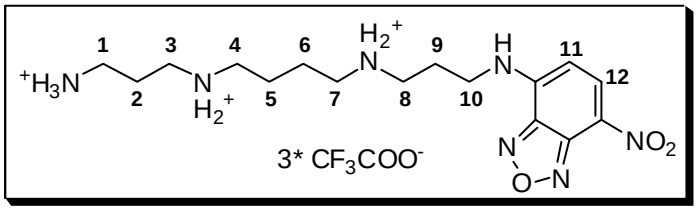


$C_{46}H_{58}N_6O_{10}F_{12}$

^{1}H NMR (400 MHz, CD_3OD) δ 8.00 – 7.95 (m, 1 H, 11-H), 7.67 – 7.59 (m, 2 H, 13-H 14-H), 7.52 – 7.46 (m, 2 H, 15-H 15´-H), 7.25 – 7.11 (m, 3 H, 12-H 16-H 16´-H), 6.99 – 6.91 (m, 2 H, 17-H 17´-H), 3.64 (q, *J* = 7.0 Hz, 8 H, 18-H 18´-H 18´´-H 18´´´-H), 3.35 (t, *J* = 6.4 Hz, 2 H, 3-H), 3.15 (t, *J* = 7.7 Hz, 2 H, 10-H), 3.12 – 2.98 (m, 6 H, 4-H 7-H 8-H), 2.92 (t, *J* = 6.5 Hz, 2 H, 1-H), 2.12 (tt, J_1 = 7.7 Hz, J_2 = 7.3 Hz, 2 H, 9-H), 1.88 – 1.78 (m, 4 H, 5-H 6-H), 1.54 (tt, J_1 = 6.8 Hz, J_2 = 6.5 Hz, 2 H, 2-H), 1.30 and 1.16 (t, *J* = 7.0 Hz, 12 H, 19-H 19´-H 19´´-H 19´´´-H); HRMS (ESI) $[M + H]^+$ calc. 627.4381, found 627.4362.

[4-(3-Amino-propylamino)-butyl]-[3-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-propyl]-ammonium tris-trifluoroacetate (212)

100 mg (50 µmol) of resin **198** were *o*-Nosyl deprotected. The resin was washed with THF and a solution of 33 mg (170 µmol; 3.3 equiv.) of NBDCl **168** and 15 µl (84 µmol; 1.7 equiv.) of DiPEA in DMF was added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with THF / MeOH, and CH_2Cl_2. The colour of the resin turned to black. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA, treated with 10% TFA in CH_2Cl_2 and the crude product was purified by preparative HPLC (51% purity by UV detection). After removal of the solvent, the solid was dried on HV. 29.7 mg (42 µmol) of a dark solid **212** were obtained giving 84% yield calculated on the loading of resin **198**.

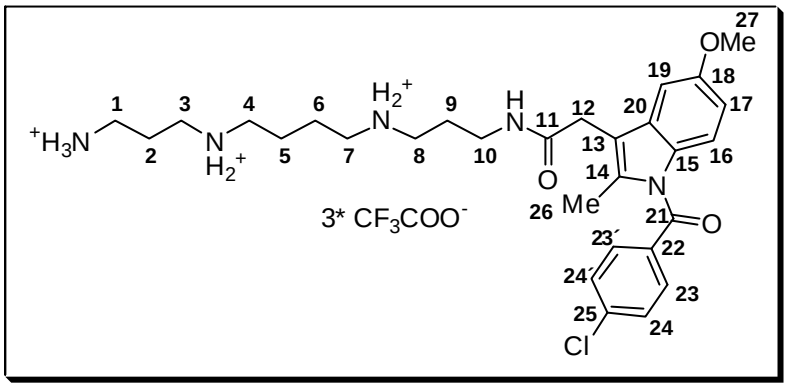


$C_{22}H_{30}N_7O_9F_9$

^{1}H NMR (400 MHz, CD_3OD) δ 8.50 (d, *J* = 8.8 Hz, 1 H, 12-H), 6.39 (d, *J* = 8.8 Hz, 1 H, 11-H), 3.74 – 3.64 (m, 2 H, 10-H), 3.20 (t, *J* = 7.9 Hz, 2 H, 3-H), 3.17 – 3.03 (m, 8 H, 1-H 4-H 7-H 8-H), 2.18 (tt, J_1 = 7.8 Hz, J_2 = 7.4 Hz, 2 H, 9-H), 2.08 (tt, J_1 = 7.8 Hz, J_2 = 7.7 Hz, 2 H, 2-H), 1.84 – 1.78 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 366.2248, found 366.2252.

[4-(3-Amino-propylamino)-butyl]-(3-{2-[1-(4-chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-propyl)-ammonium tris-trifluoroacetate (213)

100 mg (51 µmol) of resin **198** were *o*-Nosyl deprotected. A solution of 89 mg (250 µmol; 5 equiv.) indomethacin **171**, 51 mg (250 µmol; 5 equiv.) DCC, and 34 mg (250 µmol; 5 equiv.) HOBt in DMF was shortly agitated and added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with DMF, THF : H_2O (10 : 1) / MeOH, and CH_2Cl_2. The colour of the resin turned to orange and the *Kaiser Test* was negative. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA, treated with 10% TFA in CH_2Cl_2 and the crude product was purified by preparative HPLC (74% purity by peak integration at 254 nm UV detection). After removal of the solvent, the solid was dried on HV. 43.3 mg (49 µmol) of a white solid **213** were obtained giving 97% yield calculated on the loading of resin **198**.



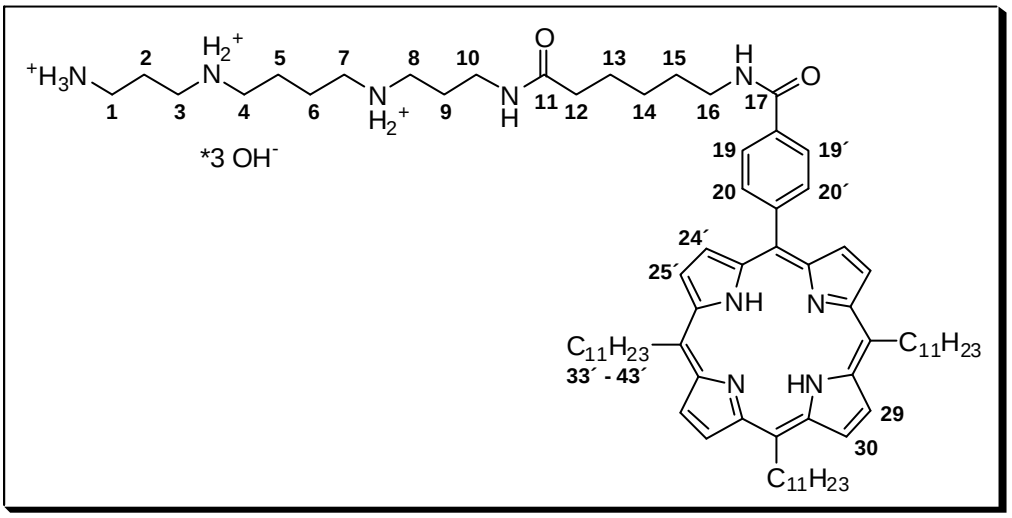
$C_{35}H_{43}N_5O_9ClF_9$

^{1}H NMR (400 MHz, CD_3OD) δ 7.69 (d, *J* = 8.4 Hz, 2 H, 23-H 23´-H), 7.55 (d, *J* = 8.4 Hz, 2 H, 24-H 24-´H), 7.02 (d, *J* = 2.5 Hz, 1 H, 19-H), 6.94 (d, *J* = 9.0 Hz, 1 H, 16-H), 6.69 (dd, J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1 H, 17-H), 3.80 (s, 3 H, 27-H), 3.65 (s, 2 H, 12-H), 3.32 (t, *J* = 6.7 Hz, 2 H, 3-H), 3.12 (t, *J* = 7.7 Hz, 2 H, 10-H), 3.06 (t, *J* = 7.8 Hz, 2 H, 8-H), 3.07 – 3.00 (m, 4 H, 4-H 7-H), 2.95 (t, *J* = 7.1 Hz, 2 H, 1-H), 2.32 (s, 3 H, 26-H), 2.09 (tt, J_1 = 7.8 Hz, J_2 = 7.7 Hz, 2 H, 9-H), 1.88 (tt, J_1 = 7.1 Hz, J_2 = 6.7 Hz, 2 H, 2-H), 1.81 – 1.67 (m, 4 H, 5-H 6-H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.4 (C_{quart}, C-11), 170.0 (C_{quart}, C-21), 157.6 (C_{quart}, C-18), 140.3 (C_{quart}, C-25), 137.3 (C_{quart}, C-14), 135.6 (C_{quart}, C-15), 132.3 (+, C-23a,b), 132.1 (C_{quart}, C-20), 130.2 (+, C-24a,b), 116.0 (1 C, C-17), 114.6 (1 C, C-16), 112.5 (C_{quart}, C-13), 102.6 (+, C-19), 56.2 (+, C-27), 48.2, 48.0 (–, C-4 C-7), 46.3 (–, C-8), 45.8 (–, C-3), 37.8, 37.1 (–, 2 C, C-1 C-10), 32.3 (–, C-12), 27.5 (–, C-2), 25.3 (–, C-9), 24.2 (–, 3 C, C-5 C-6 C-9), 13.6 (+, C-26); HRMS (ESI) $[M + H]^+$ calc. 542.2892, found 542.2890.

[4-(3-Amino-propylamino)-butyl]-(3-{6-[4-(10,15,20-triundecyl-porphyrin-5-yl)-benzoylamino]-hexanoylamino}-propyl)-ammonium tris-trifluoroacetate (217)

123 mg (58 µmol) of resin **198** were *o*-Nosyl deprotected. A solution of 51 mg (140 µmol; 3 equiv.) of *N*-Fmoc-aminocaproic acid, 67 mg (140 µmol; 3 equiv.) of PyBrOP, and 33 µl (190 µmol; 4 equiv.) of DiPEA in DMF / CH_2Cl_2 (1:1 (v/v)) was added to the resin. The suspension was agitated for 28 h. After removal of the solvent the resin was washed with DMF, THF : H_2O (10 : 1) / MeOH, and CH_2Cl_2. The *Kaiser Test* was negative. The resin was

Fmoc deprotected. A solution of 81 mg (9.6 µmol; 2 equiv.) of carboxyporphyrin **215**, 50 mg (240 µmol; 5 equiv.) of DCC, and 22 mg (190 µmol; 4 equiv.) of NHS in 3 mL DMF was shortly agitated and added to the resin. The resin was agitated for 3 d and after removal of the solvent the resin was washed with THF : H_2O (10 : 1) / MeOH and with CH_2Cl_2 until the filtrate was clear. The colour of the resin had turned to black. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA and treated with 10% TFA in CH_2Cl_2. After removal of the solvent the solid was partitioned between an aqueous solution of K_2CO_3 (pH = 10) and $CHCl_3$. The organic layer was successively washed with an aqueous solution of K_2CO_3 (pH = 10) and water. The combined aqueous layers were re-extracted with a small amount of $CHCl_3$. The organic layers were dried over Na_2SO_4, the solvent was removed and the remaining solid was dried in HV. 70 mg (56 µmol) of a green solid were obtained giving 96% yield calculated on the loading of resin **198**. The ^{1}H NMR spectrum shows that the compound was pure.



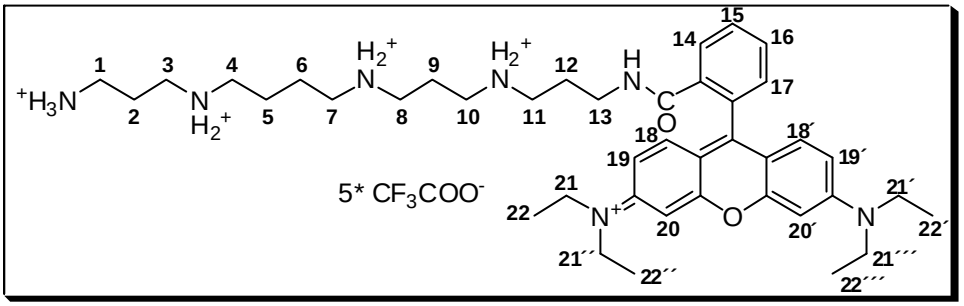
$C_{79}H_{122}N_9O_8F_6$

^{1}H NMR (400 MHz, CD_3OD) δ 9.64 (s, 4 H, 29-H 29´-H 30-H 30´-H), 9.56 (d, *J* = 4.9 Hz, 2 H, 19-H 19´-H), 8.80 (d, *J* = 4.9 Hz, 2 H, 20-H 20´-H), 8.60 (d, *J* = 8.2 Hz, 2 H, 25-H 25´-H), 8.44 (d, *J* = 8.2 Hz, 2 H, 24-H 24´-H), 5.10 (m, 6 H, 33-H 33´-H 33´´-H), 3.57 (t, *J* = 7.1 Hz, 2 H, 16-H), 3.34 (t, *J* = 6.4 Hz, 2 H, 3-H), 3.16 (t, *J* = 7.7 Hz, 2 H, 10-H), 2.97 – 3.11 (m, 8 H, 1-H 4-H 7-H 8-H), 2.50 – 2.60 (m, 6 H, 34-H 34´-H 34´´-H), 2.34 (t, *J* = 6.5 Hz, 2 H, 12-H), 2.10 (tt, J_1 = 9.0 Hz, J_2 = 6.4 Hz, 2 H, 2-H), 1.93 (tt, J_1 = 6.9 Hz, J_2 = 6.8 Hz, 2 H, 9-H), 1.73 – 1.86 (m, 10 H, 5-H 6-H 35-H 35´-H 35´´-H), 1.47 – 1.58 (m, 8 H, 15-H 36-H 36´-H 36´´-H), 1.20 – 1.38 (m, 40 H, 13-H 14-H (37-H to 42´´-H)), 0.87 (t, *J* = 7.0 Hz, 9 H, 43-H 43´-H 43´´-H); ^{13}C NMR (75 MHz, CD_3OD) δ 177.3 (C_{quart}, C-11), 169.7 (C_{quart}, C-17), 146.4, 146.1, 146.1, 145.6, 139.2, 137.1, 128.5, 128.3, 128.2, 121.0, 115.9, 113.6 (C_{quart} and +, C-22 to C-32), 143.4 (C_{quart}, C-21), 130.5 (C_{quart}, C-18), 124.6, 124.5 (+, C-19a C-19b C-20a C-20b), 48.3, 48.2 (–, C-4 C-7), 46.5, 45.9 (–, 2 C, C-3 C-8), 41.2 (–, C-16), 37.8, 37.0 (–, 3 C, C-1 C-10 C-12), 33.1 (–, C-41 C-41´ C-41´´), 31.4, 31.3 (–, C-41 C-41´ C-41´´), 30.9, 30.8, 30.5, 30.4 (–, C-13 C-15 C-35 to C-44´´), 27.9 (–, C-34 C-34´ C-34´´), 26.8 (–, C33 C-33´ C-33´´), 27.8, 25.4 (–, 2 C, C-2 C-9), 24.4, 24.3 (–, C-5 C-6), 23.8 (–, C-14 C-42 C-42´ C-42´´), 14.5 (+, C-43 C-43´ C-43´´); HRMS (ESI) $[M + 2 H]^{2+}$ calc. 595.9816, found 595.9816.

6.5.2 Synthesis of spermine conjugates

{9-[2-(3-{3-[4-(3-Amino-propylamino)-butylamino]-propylamino}-propylcarbamoyl)-phenyl]-6-diethylamino-xanthen-3-ylidene}-diethyl-ammonium pentakis-trifluoroacetate (223)

Resin **198** was alkylated with (*N*-Dde)-3-aminopropyliodide **193** as described in Entry 20 of the study on *Fukuyama alkylation* and then 72 mg (36 µmol) of the resin were Dde-deprotected. A solution of 86 mg rhodamine B (180 µmol, equiv.), 24 mg HOBt (180 µmol, 5 equiv.), and 66 mg DCC (320 µmol, 8.9 equiv.) in DMF was added to the resin and agitated for 16 h. After removal of the solvent the resin was washed with DMF, H_2O / THF (1:10) / MeOH and CH_2Cl_2. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA, *o*-Nosyl-deprotected treated with 10% TFA in CH_2Cl_2 and the crude product was purified by preparative HPLC (26% purity by UV detection). After removal of the solvent the solid was dried on HV. 13.5 mg (11 µmol) of a pink solid were obtained giving 31% yield calculated on the loading of resin **198**.



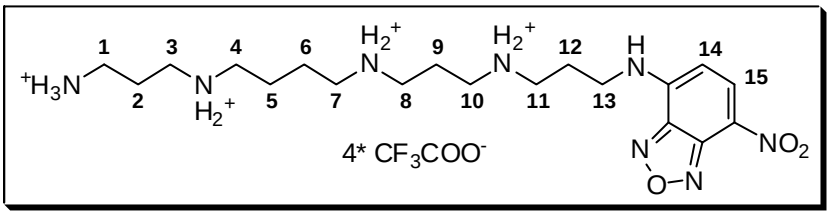
$C_{51}H_{66}N_7O_{12}F_{15}$

^{1}H NMR (400 MHz, CD_3OD) δ 7.97 – 7.92 (m, 1 H, 14-H), 7.65 – 7.59 (m, 2 H, 16-H 17-H), 7.15 – 7.10 (s, 1 H, 15-H), 7.06 – 6.96 (m, 2 H, 18-H 18´-H), 6.83 – 6.76 (m, 2 H, 19-H 19´-H), 6.71 – 6.65 (m, 2 H, 20-H 20´-H), 3.64 and 3.46 (q, *J* = 7.1 Hz, 8 H, 21-H 21´-H 21´´-H 22´´´-H), 3.33 – 3.28 (m, 2 H, 3-H), 3.22 – 3.03 (m, 12 H, 4-H 7-H 8-H 10-H 11-H 13-H), 2.89 (t, *J* = 6.7 Hz, 2 H, 1-H), 2.18 – 2.04 (m, 4 H, 9-H 12-H), 1.86 – 1.77 (m, 4 H, 5-H 6-H), 1.54 – 1.45 (m, 2 H, 2-H), 1.31 and 1.17 (t, *J* = 7.2 Hz, 12 H, 22-H 22´-H 22´´-H 22´´´-H); HRMS (ESI) $[M + 2 H]^{2+}$ calc. 343.2553, found 343.2490.

{3-[4-(3-Amino-propylamino)-butyl]-propylamino}-[3-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-propyl]-ammonium tetrakis-trifluoroacetate (230)

Resin **198** was alkylated with (*N*-Dde)-3-aminopropyliodide **193** as described in Entry 20 of the study on *Fukuyama alkylation*. On 100 mg (42 µmol) of the resin the *o*-Nosyl group was removed and Aloc was introduced instead. 100 mg of the resin (42 µmol) were Dde deprotected, washed with THF, and a solution of 33.5 mg NBDCl (168 µmol; 4 equiv.) and 29 µl DiPEA (168 µmol; 4 equiv.) in THF was added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with THF / MeOH, and CH_2Cl_2. The colour of the resin turned to black. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA, *o*-Nosyl deprotected, treated with 10% TFA in CH_2Cl_2 and the crude product was purified by preparative HPLC (25% purity by UV detection). After removal of

the solvent the solid was dried on HV. 8.8 mg (21 µmol) of a black solid were obtained giving 24% yield calculated on the loading of resin **198**.

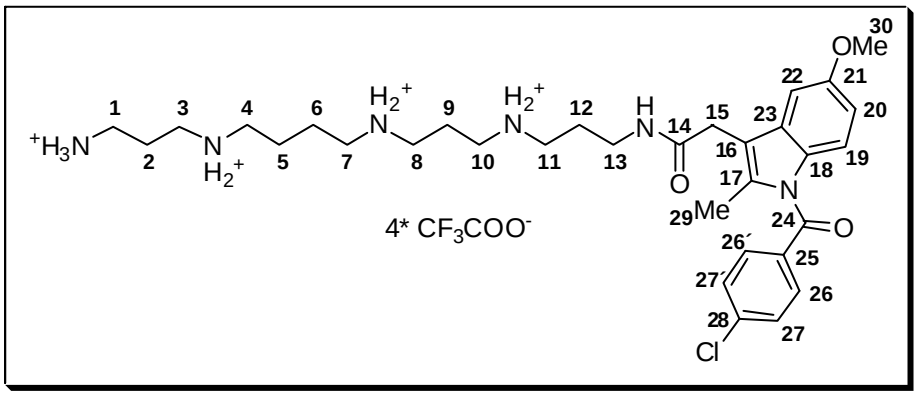


$C_{27}H_{38}N_8O_{11}F_8$

^{1}H NMR (400 MHz, CD_3OD) δ 8.53 (d, *J* = 8.7 Hz, 1 H, 15-H), 6.40 (d, *J* = 8.7 Hz, 1 H, 14-H), 3.75 – 3.65 (m, 2 H, 13-H), 3.23 – 3.03 (m, 14 H), 2.17 (tt, J_1 = 8.1 Hz, J_2 = 6.9 Hz, , 12-H), 2.19 – 2.11 (m, 2 H, 9-H), 2.08 (tt, J_1 = 7.8 Hz, J_2 = 7.8 Hz, 2 H, 2-H), 1.83 – 1.77 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 423.2827, found 423.2820.

{3-[4-(3-Amino-propylamino)-butyl]-propylamino}-(3-{2-[1-(4-chloro-benzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]-acetylamino}-propyl)-ammonium tetrakis-trifluoroacetate (229)

The resin **198** was alkylated with (*N*-Dde)-3-aminopropyliodide **193** as described in Entry 20 of the study on *Fukuyama alkylation*. On 100 mg (43 µmol) of the resin the *o*-Nosyl group was removed and Aloc was introduced instead. After this the resin was washed with Dde deprotected and a solution of 75 mg indomethacin (210 µmol; 4.9 equiv.), 43 mg DCC (209 µmol; 4.9 equiv.), and 28 mg HOBt (207 µmol; 4.8 equiv.) in DMF was shortly agitated and added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with THF / MeOH, and CH_2Cl_2. The colour of the resin turned to orange. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA, treated with 10% TFA in CH_2Cl_2 and the crude product was purified by preparative HPLC (67% purity by peak integration at 254 nm UV detection). After removal of the solvent the solid was dried on HV. 43.9 mg (42 µmol) were obtained giving 99% yield calculated on the loading of resin **198**.



$C_{40}H_{51}ClN_6O_{11}F_{12}$

^{1}H NMR (400 MHz, CD_3OD) δ 7.67 (d, *J* = 8.4 Hz, 2 H, 26-H 26´-H), 7.54 (d, *J* = 8.4 Hz, 2 H, 27-H 27´-H), 7.02 (d, *J* = 2.5 Hz, 1 H, 22-H), 6.92 (d, *J* = 9.0 Hz, 1 H, 19-H), 6.68 (dd,

J_1 = 9.0 Hz, J_2 = 2.5 Hz, 1 H, 20-H), 3.80 (s, 3 H, 30-H), 3.65 (s, 2 H, 15-H), 3.32 (t, J = 6.9 Hz, 2 H, 3-H), 3.14 (t, J = 7.7 Hz, 2 H, 13-H), 3.14 – 3.02 (m, 10 H, 4-H 7-H 8-H 10-H 11-H), 2.98 (t, J = 7.1 Hz, 2 H, 1-H), 2.31 (s, 3 H, 29-H), 2.09 (tt, J_1 = 7.7 Hz, J_2 = 7.6 Hz, 4 H, 9-H 12-H), 1.89 (tt, J_1 = 7.1 Hz, J_2 = 6.9 Hz, 2 H, 2-H), 1.83 – 1.77 (m, 4 H, 5-H 6-H); ^{13}C NMR (75 MHz, CD_3OD) δ 174.5 (C_{quart}, C-14), 170.0 (C_{quart}, C-24), 157.7 (C_{quart}, C-21), 140.4 (C_{quart}, C-28), 137.4 (C_{quart}, C-17), 135.6 (C_{quart}, C-18), 132.4 (+, C-26a,b), 132.2 (C_{quart}, C-23), 130.3 (+, C-27a,b), 116.1 (+, C-20), 114.7 (+, C-19), 112.6 (C_{quart}, C-16), 102.7 (+, C-22), 56.3 (+, C-30), 48.3 (–, C-4 C-7), 46.6 (–, C-11), 45.9 (–, 2 C, C-8 C-10), 45.8 (–, C-3), 37.9, 37.2 (–, 2 C, C-1 C-13), 32.3 (–, C-15), 27.6 (–, C-2), 25.4 (–, C-12), 24.3, 24.2 (–, 3 C, C-5 C-6 C-9), 13.6 (+, C-29); HRMS (ESI) $[M + H]^+$ calc. 599.3471, found 599.3465.

6.5.3 Synthesis of lipophilic putrescine conjugates

6.5.3.1 General procedures

Alkylation with alkyliodide / K_2CO_3

The resin is placed in a closed vial together with 13 equiv. of K_2CO_3. 3 mL of DMF are added and the suspension is shortly agitated. 13 equiv. of allyliodide are added, the vial is closed and the suspension is agitated at 40°C for xx h: The resin is washed with water, water / THF (1 : 10) and CH_2Cl_2.

Alkylation with octylbromide / K_2CO_3

The resin is placed in a closed vial together with 20 equiv. of K_2CO_3. 3 mL of DMF are added and the suspension is shortly agitated. 20 equiv. of octylbromide are added, the vial is closed and the suspension is agitated at 50°C for 72 h: The resin is washed with water, water / THF (1:10) and CH_2Cl_2.

Alkylation with benzylbromide / K_2CO_3

The resin is placed in a closed vial together with 10 equiv. of K_2CO_3. 3 mL of DMF are added and the suspension is shortly agitated. 10 equiv. of benzylbromide are added, the vial is closed and the suspension is agitated at 40°C for over night. The resin is washed with water, water / THF (1:10) and CH_2Cl_2.

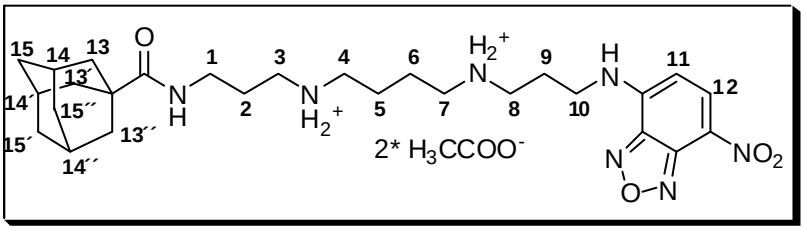
Alkylation with capronaldehyde / tetrabutylammonium borohydride

The resin is placed in a frit with a plastic cap and 3 mL of 3% HOAc in DMF are added. The suspension is shortly agitated, heated to 40°C and 6 equiv. of capronaldehyde are added. The suspension is left for 30 min under occasional stirring and then 6 equiv. of tetrabutylammonium borohydride are added. The suspension is again left for 30 min under occasional stirring and is then cooled to room temperature. The resin is washed with DMF. The alkylation is twice repeated but in the last cycle reaction was left over night after the addition of tetrabutylammonium borohydride. The resin is washed with DMF, THF / MeOH and CH_2Cl_2.

6.5.3.2 Synthesis of the conjugates

(4-{3-[(Adamantane-1-carbonyl)-amino]-propylamino}-butyl)-[3-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-propyl]-ammonium bis-acetate (234)

100 mg (41 µmol) of resin **198** were *o*-Nosyl deprotected. The resin was washed with DMF and a suspension of 59 mg (328 µmol; 8 equiv.) of adamantylcarboxylic acid, 69 mg (334 µmol; 8.2 equiv.) of DCC, and 24 mg (209 µmol; 5.1 equiv.) of NHS in 3 mL DMF : THF (1:1) was shortly agitated and added to the resin. The resin was washed with THF / MeOH and CH_2Cl_2 and the resin was treated with 10% TFA in CH_2Cl_2. After removal of the solvents and drying on HV, 24 mg (120 µmol; 2.9 equiv.) of NBDCl, 17 mg (123 µmol; 3 equiv.) of K_2CO_3, and 3 mL THF were added. The suspension was agitated for 18 h. The THF was removed in constant flow of N_2 and the residue was partitioned between $CHCl_3$ and water. The organic layer was three times washed with water. After removal of the solvent and drying on HV the solid was dissolved in CH_2Cl_2 and 62 mg (397 µmol; 9.7 equiv.) of *N,N´*-dimethylbarbituric acid and 4.6 mg (4 µmol; 0.1 equiv.) of $Pd(PPh_3)_4$ were added. The solution was stirred for 150 min at 40 °C under avoidance of light. 400 µl of acetic acid were added. The solvents were removed and the remaining solid was extracted with Et_2O until the extract was colourless. The solid was dissolved in a solution of 3% HOAc in water and extracted three times with a solution of CH_2Cl_2 : Et_2O (1:1). The water was removed and the solid was dried on HV. 14.3 mg (27 µmol) of a dark solid were obtained giving 66% yield calculated on the loading of resin **198**.



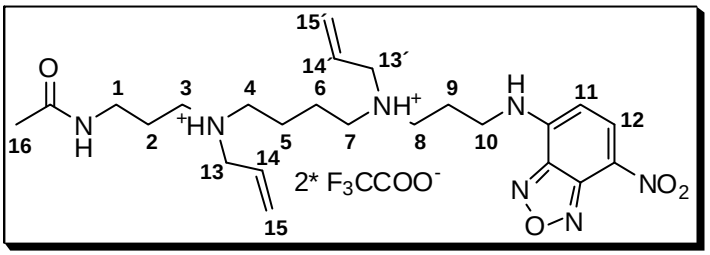
$C_{31}H_{49}N_7O_8$

^{1}H NMR (400 MHz, CD_3OD) δ 8.46 (d, *J* = 8.8 Hz, 1 H, 12-H), 6.38 (d, *J* = 8.8 Hz, 1 H, 11-H), 3.74 – 3.63 (m, 2 H, 10-H), 3.30 (t, *J* = 6.6 Hz, 2 H, 3-H), 3.22 (t, *J* = 7.2 Hz, 2 H, 1-H), (3.12 (t, *J* = 7.1 Hz, 2 H) and 2.95 (t, *J* = 7.1 Hz, 2 H) 4-H and 7-H), 3.04 (t, *J* = 6.8 Hz, 2 H, 8-H), 2.20 (tt, J_1 = 7.8 Hz, J_2 = 6.8 Hz, 2 H, 9-H), 2.03 – 1.98 (m, 3 H, 14-H 14´-H 14´´-H), 1.88 (tt, J_1 = 7.2 Hz, J_2 = 6.6 Hz, 2 H, 2-H), 1.87 – 1.67 (m, 16 H, 5-H 6-H (13-H 13´-H 13´´-H 15-H 15´-H 15´´-H)); HRMS (ESI) $[M + H]^+$ calc. 528.3293, found 528.3304.

***N*^{1}-Acetyl-*N*^{5},*N*^{10}-bis-allyl-*N*^{12}-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-4,9,12-tetraazatetradecane bis-trifluoroacetate (239)**

50 mg (25 µmol) of resin **198** were swollen in CH_2Cl_2. After removal of the solvent a solution of 30 mg (0.025 mmol; 1 equiv.) of $Pd(PPh_3)_4$ and 0.7 µl (7.5 µmol; 0.3 equiv.) of allyliodide in CH_2Cl_2 were added. The suspension was agitated for 16 h and was then washed analogously to the procedure of Aloc protection. The resin was *o*-Nosyl deprotected and then

a solution of 9.5 µl (100 µmol; 4 equiv.) of acetic anhydride and 14 µl (100 µmol, 4 equiv.) of Et_3N in DMF was added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with H_2O / THF (1:10) / MeOH, and CH_2Cl_2. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. 6.9 mg (50 µmol, 2 equiv.) of K_2CO_3 were added and it was suspended with a solution of 7.5 mg NBDCl (38 µmol, 1.5 equiv.) in 2 mL THF. The suspension was agitated for 18 h and THF was removed in a constant flow of N_2. The remaining solid was purified by preparative HPLC (30% purity by UV detection at 254 nm). After removal of the solvent the solid was dried on HV. 4.7 mg (7 µmol) of a dark brown solid were obtained giving 27% yield calculated on the loading of resin **198**.



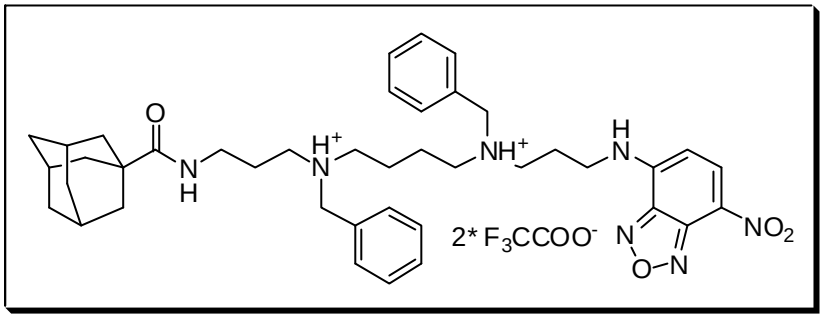
$C_{28}H_{39}N_7O_8F_6$

^{1}H NMR (400 MHz, CD_3OD) δ 8.56 – 8.50 (m, 1 H, 12-H), 6.41 (d, *J* = 8.6 Hz, 1 H, 11-H), 5.97 (ddt, J_1 = 17.1 Hz, J_2 = 10.1 Hz, J_3 = 7.2 Hz, 2 H, 14-H 14´-H), 5.68 – 5.58 (m, 4 H, 15-H 15´-H), 3.87 (d, *J* = 7.2 Hz, 2 H, 13-H), 3.83 (d, *J* = 7.2 Hz, 2 H, 13´-H), 3.73 – 3.66 (m, 2 H, 10-H), 3.35 – 3.30 (m, 2 H, 3-H), (3.29 – 3.23 (m, 2 H) and 3.24 (t, *J* = 7.3 Hz, 2 H) 4-H and 7-H), 3.17 (t, *J* = 7.6 Hz, 2 H, 1-H), 3.15 (t, *J* = 8.3 Hz, 2 H, 8-H), 2.22 (tt, J_1 = 8.3 Hz, J_2 = 7.1 Hz, 2 H, 9-H), 1.97 (s, 3 H, 16-H), 1.93 (tt, J_1 = 7.6 Hz, J_2 = 6.8 Hz, 2 H, 2-H), 1.87 – 1.78 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 488,2980, found 488,2972.

N^1-Acetyl-*N*^{5},*N*^{10}-bis-benzyl-*N*^{12}-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-4,9,12-tetraazatetradecane bis-trifluoroacetate (248)

100 mg (40 µmol) of resin **198** were *o*-Nosyl deprotected. The resin was washed with DMF and a solution of 36 mg (200 µmol; 5 equiv.) adamantylcarboxylic acid, 23 mg (200 µmol, 5 equiv.) NHS, and 41 mg (200 µmol; 5 equiv.) DCC in DMF was added to the resin. The suspension was agitated for 18 h. After removal of the solvent the resin was washed with H_2O / THF (1:10) / MeOH, and CH_2Cl_2. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA and the free secondary amines were partially alkylated with benzylbromide / K_2CO_3 in DMF. The *Kaiser Test* was still positive. The remaining secondary amines were expediently alkylated with benzaldehyde / tetrabutylammonium borohydride in 3% HOAc in DMF. Now the *Kaiser Test* was negative. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. 39 mg (0.280 mmol, 7 equiv.) of K_2CO_3 were added and it was suspended with a solution of 32 mg (0.160 mmol, 4 equiv.) of NBDCl in THF. The suspension was agitated for 17 h and THF was removed in a constant flow of N_2. The remaining solid was partitioned between CHCl3 and water and the

organic layer was twice washed with a solution of K_2CO_3 (pH 8.5). The combined aqueous layers were re-extracted with a small amount of $CHCl_3$. The organic layers were combined and dried over Na_2SO_4. After removal of the solvent the solid was dried on HV. The ESI-MS showed the presence of minorities of a compound that had a molecular mass, which was consistent with the product. The product was purified by flash chromatography on silica gel. ($CHCl_3$: MeOH (20 : 1) to $CHCl_3$: MeOH (5 : 1). After removal of the solvent and drying on HV, no remains of product were obtained.

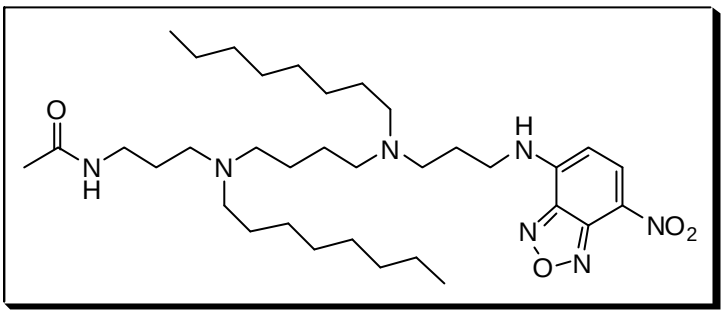


$C_{45}H_{55}N_7O_8F_6$

MS (ESI): 708.43 (10) $[M + H]^+$**.**

***N*^{1}-Acetyl-*N*^{5},*N*^{10}-bis-octyl-*N*^{12}-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-4,9,12-tetraazatetradecane (244)**

100 mg (40 µmol) of resin **198** were *o*-Nosyl deprotected. The resin was washed with DMF and a solution of 11.4 µl (200 µmol; 5 equiv.) of acetic acid, 23 mg (200 µmol, 5 equiv.) of NHS, and 41 mg (200 µmol; 5 equiv.) of DCC in DMF was added to the resin. The suspension was agitated for 16 h. After removal of the solvent the resin was washed with H_2O / THF (1:10) / MeOH, and CH_2Cl_2. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA and the free secondary amines were alkylated with octylbromide / K_2CO_3 in DMF. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. 39 mg (280 µmol, 7 equiv.) of K_2CO_3 were added and it was suspended with a solution of 32 mg (160 µmol, 4 equiv.) of NBDCl in THF. The suspension was agitated for 17 h and THF was removed in a constant flow of N_2. The remaining solid was partitioned between CHCl3 and water and the organic layer was twice washed with a solution of K_2CO_3 (pH 8.5). The combined aqueous layers were re-extracted with a small amount of CHCl3. The organic layers were combined and dried over Na_2SO_4. After removal of the solvent the solid was dried on HV. The product was purified by flash chromatography on silica gel. ($CHCl_3$: MeOH (19:1) to $CHCl_3$: MeOH (2:1)). 2.2 mg (3 µmol) of a dark brown solid were obtained giving 8% yield calculated on the loading of resin **198**.

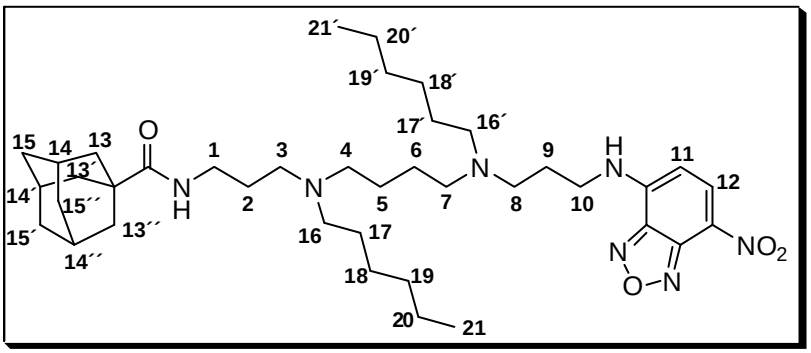


$C_{34}H_{61}N_7O_4$

MS (ESI): 632.50 $[M+H]^+$.

N^1-adamantylcarbonyl-N^5,N^{10}-bis-hexyl-N^{12}-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-4,9,12-tetraazatetradecane (249)

100 mg (40 µmol) of resin **198** were *o*-Nosyl deprotected. The resin was washed with THF and a solution of 56 mg 1-adamantylcarboxylic acid (312 µmol; 8 equiv.), 22 mg NHS (312 µmol, 8 equiv.), and 65 mg DCC (195 µmol; 5 equiv.) in DMF / THF (1:1) was added to the resin. The suspension was agitated for 24 h. After removal of the solvent the resin was washed with H_2O : THF (1:10) / MeOH, and CH_2Cl_2. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA and the free secondary amines were alkylated with capronaldehyde / tetrabutylammonium borohydride in 3% HOAc in DMF. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. 22 mg (160 µmol, 4 equiv.) of K_2CO_3 were added and it was suspended with a solution of 24 mg NBDCl (120 µmol, 3 equiv.) in THF. The suspension was agitated for 17 h and THF was removed in a constant flow of N_2. The remaining solid was partitioned between CHCl3 and water and the organic layer was twice washed with a solution of K_2CO_3 (pH 8.5). The combined aqueous layers were re-extracted with a small amount of CHCl3. The organic layers were combined and dried over Na_2SO_4. After removal of the solvent the solid was dried on HV. The product was purified by flash chromatography on silica gel. ($CHCl_3$: MeOH (10 : 1) to $CHCl_3$: MeOH (5 : 1)). 14.6 mg (21 µmol) of a dark brown solid were obtained giving 53% yield calculated on the loading of resin **198**.

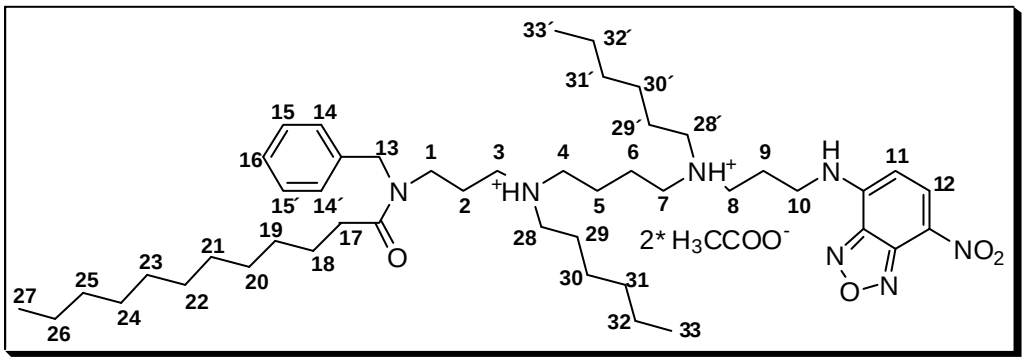


$C_{39}H_{65}N_7O_4$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 – 8.30 (m, 1 H, 12-H), 6.71 (m, 1 H, 11-H), 3.75 – 3.62 (m, 2 H, 10-H), 3.38 – 3.30 (m, 2 H, 1-H), 3.27 – 3.22 (m, 4 H, 16-H 16´-H), 3.16 – 2.94 (m, 8 H, 3-H 4-H 7-H 8-H), 2.32 – 2.21 (m, 2 H, 9-H), 2.04 – 1.97 (m, 5 H, 2-H 14-H 14´-H 14´´-H), 1.90 – 1.86 (m, 4 H, 17-H 17´-H), 1.85 – 1.81 (m, 4 H, 5-H 6-H), 1.76 – 1.60 (m, 12 H, 13-H 13´-H 13´´-H 15-H 15´-H 15´´-H), 1.45 – 1.22 (m, 12 H, (18-H to 20´´-H)), 0.90 – 0.81 (m, 6 H, 21-H 21´-H); HRMS (ESI) $[M + H]^+$ calc. 696.5171, found 696.5167.

((*N*-benzyl)-*N*^{1}-docecanamidyl)-*N*^{4},*N*^{9}-bis-hexyl-12-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-4,9-diazadodecane bis-acetate (256)

200 mg of resin **198** (71 µmol) were alkylated with benzylbromide / DBU according to Entry 1 of the study on *Fukuyama alkylation*. The resin was *o*-Nosyl deprotected. A solution of 254 µl (1.42 mmol, 20 equiv.) lauroyl chloride in 3 mL pyridine / CH_2Cl_2 (1:2) was added and the resin was agitated for 18 h. After removal of the solvent the resin was washed with CH_2Cl_2 / MeOH and CH_2Cl_2. The *Kaiser Test* was negative and the TLC showed only one spot. One third of the resin was treated with 10% TFA in CH_2Cl_2. The filtrate was collected and after removal of the solvents the remaining solid was analysed by ^{1}H NMR spectroscopy. The spectrum showed that the lauroyl- and the benzyl moiety were expediently bound. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA and the free secondary amines were alkylated with capronaldehyde / tetrabutylammonium borohydride in 3% HOAc in DMF. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. 26 mg (0.188 mmol, 4 equiv.) of K_2CO_3 were added and it was suspended with a solution of 28 mg NBDCl (0.141 mmol, 3 equiv.) in THF. The suspension was agitated for 17 h and THF was removed in a constant flow of N_2. The remaining solid was partitioned between $CHCl_3$ and water and the organic layer was twice washed with water. The combined aqueous layers were re-extracted with a small amount of $CHCl_3$. The organic layers were combined and dried over Na_2SO_4. After removal of the solvent the solid was dried on HV. The product was purified by flash chromatography on silica gel. ($CHCl_3$: MeOH (15 : 1) to $CHCl_3$: MeOH (10 : 1). The product was converted to its bis-acetate salt by stirring it for 10 min in 5% HOAc in $CHCl_3$. After removal of the solvent and drying on HV 40.0 mg (43.3 µmol) of a dark brown solid were obtained giving 91% yield calculated on the loading of resin **198**.



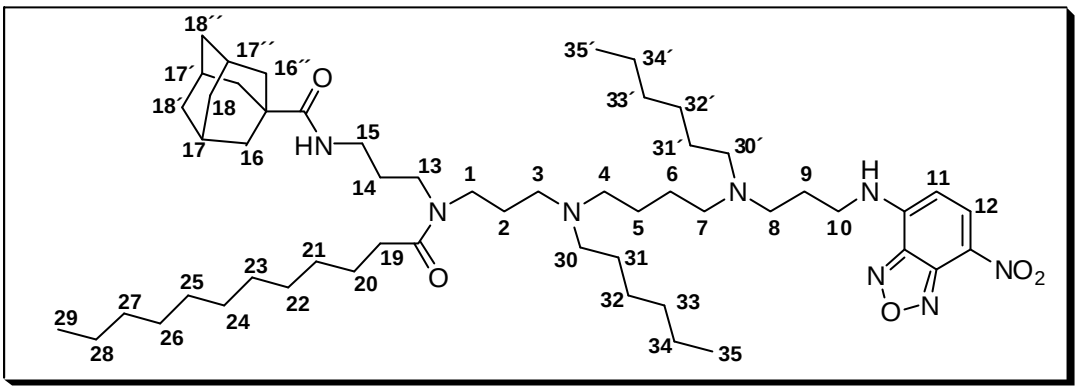
$C_{51}H_{87}N_7O_8$

^{1}H NMR (400 MHz, CD_3OD) δ 8.42 (d, *J* = 8.7 Hz, 1 H, 12-H), 7.40 – 7.16 (m, 5 H, 14-H 14´-H 15-H 15´-H 16-H), 6.29 (d, *J* = 8.7 Hz, 1 H, 11-H), 4.62 (s, 2 H, 13-H), 3.68 – 3.58 (m,

2 H, 10-H), 3.42 (t, J = 7.0 Hz, 2 H, 1-H), 3.12 – 2.84 (m, 12 H, 3-H 4-H 7-H 8-H 28-H 28´-H), 2.40 (t, J = 7.6 Hz, 2 H, 17-H), 2.11 (tt, J_1 = 7.1 Hz, J_2 = 7.1 Hz, 2 H, 9-H), 1.89 (tt, J_1 = 7.3 Hz, J_2 = 7.0 Hz, 2 H, 2-H), 1.77 – 1.69 (m, 4 H, 5-H 6-H), 1.69 – 1.52 (m, 6 H, 18-H 29-H 29´-H), 1.35 – 1.19 (m, 28 H, (19-H to 26-H) (30-H to 32´-H)), 0.91 – 0.83 (m, 9 H, 27-H 33-H 33´-H); HRMS (ESI) $[M + H]^+$ calc. 806.6266, found 806.6249.

N^1-adamantylcarbonyl-N^4-docecanoyl-N^8,N^{13}-bis-hexyl-N^{17}-(7-nitro-benzo[1,2,5]oxadiazol-4-ylamino)-1,4,8,13,17-pentaazaoctadecane bis-acetate (263)

The resin **198** was alkylated with (*N*-Dde)-3-aminopropyliodid **193** as described in Entry 20 of the study on *Fukuyama alkylation*. On 100 mg (43 µmol) of the resin the *o*-Nosyl group was removed. A solution of 203 µl (860 µmol; 20 equiv.) lauroyl chloride in pyridine / CH_2Cl_2 (1 : 2) was added and the resin was agitated for 51 h. The resin was washed with CH_2Cl_2 / MeOH and CH_2Cl_2. The *Kaiser Test* was negative. The resin was Dde deprotected and a solution of 56 mg adamantylcarboxylic acid (344 µmol; 8 equiv.), 65 mg DCC (215 µmol; 5 equiv.), and 22 mg NHS (344 µmol; 8 equiv.) in 2 mL DMF / THF (1 : 1) was shortly agitated and added to the resin. The suspension was agitated for 24 h. The *Kaiser Test* was negative. After removal of the solvent the resin was washed with H_2O / THF (1 : 10) / MeOH, and CH_2Cl_2. The *Kaiser Test* was negative. The resin was Aloc-deprotected with $Pd(PPh_3)_4$ / DMBA and the free secondary amines were alkylated with capronaldehyde / tetrabutylammonium borohydride in 3% HOAc in DMF. Excess of 10 equiv. instead of 6 equiv. of both, aldehyde and hydride were necessary to achieve saturation of all secondary amines. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. A solution of 40 mg (200 µmol, 4.7 equiv.) of NBDCl and 28 µl Et_3N (201 µmol, 4.7 equiv.) in DMF was added. The dark brown solution was agitated for 19 h, partitioned between $CHCl_3$ and water, and the organic layer was three times washed with water. The combined aqueous layers were re-extracted with a small amount of CHCl3. The organic layers were combined and dried over Na_2SO_4. After removal of the solvent the solid was dried on HV until the last remains of DMF were removed. The product was purified by flash chromatography on silica gel. ($CHCl_3$: MeOH (15 : 1) to $CHCl_3$: MeOH : $NH_{3(aq)}$(25%) (3 : 1 : 0.1)). 36 mg (38 µmol) of a dark brown solid were obtained giving 89% yield calculated on the loading of resin **198**. The product was converted into its bis-acetate salt by treatment with 5% HOAc in $CHCl_3$ for 10 min, subsequent removal of the solvent, and drying on HV. The spectra confirmed the identity of the compound but the ESI-MS spectra also showed a second compound with $[M + 20]^+$ which could neither be separated nor be assigned to a suggestive structure consistent with the synthetic scheme.



$C_{55}H_{96}N_8O_5$

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 – 8.33 (m, 1 H, 12-H), 6.31 – 6.26 (m, 1 H, 11-H), 6.09 – 6.01 (m, 1 H), 3.66 – 3.54 (m, 2 H, 10-H), 3.42 – 3.34 (m, 2 H, 15-H), 3.34 – 3.22 (m, 4 H, 1-H 13-H), 3.16 – 2.84 (m, 8 H, 3-H 4-H 7-H 8-H), 2.78 – 2.60 (m, 4 H, 30-H 30´-H), 2.30 (t, *J* = 7.1 Hz, 2 H, 19-H), 2.22 – 2.15 (m, 2 H, 9-H), 2.08 – 1.96 (m, 7 H, 14-H 17-H 17´-H 17´´-H), 1.88 – 1.81 (m, 6 H, 2-H 5-H 6-H), 1.72 – 1.66 (m, 6 H, 16-H 16´-H 16´´-H), 1.66 – 1.50 (m, 12 H, 18-H 18´-H 18´´-H 20-H 31-H 31´-H), 1.34 – 1.16 (m, 28 H, (21-H to 28-H) (32-H to 34´-H)), 0.90 – 0.79 (m, 9 H, 29-H 35-H 35´-H); HRMS (ESI) $[M + H]^+$ calc. 935,7420, found 936.7415.

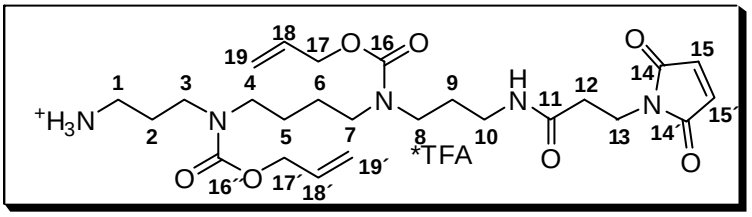
6.6 Development of coupling methods for the formation of sensitive polyamine-drug conjugates

6.6.1 Establishment of the conditions for the *Michael addition*

Introduction of a maleimide

N^{13}-(*N*-Maleimidopropionyl)-N^4,N^9-bis-allyloxycarbonyl-N^1-amino-4,9,13-triazatridecane trifluoroacetate (277)

100 mg (42 µmol) of resin **198** were *o*-Nosyl deprotected. After this a solution of 94 mg (333 µmol, 8 equiv.) of **196** and 58 µl (333 µmol, 8 equiv.) of DiPEA in 2 mL DMF were added to the resin. The suspension was agitated for 16 h and the resin was washed with DMF, THF / MeOH and CH_2Cl_2. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA.



$C_{27}H_{40}N_5O_9F_3$

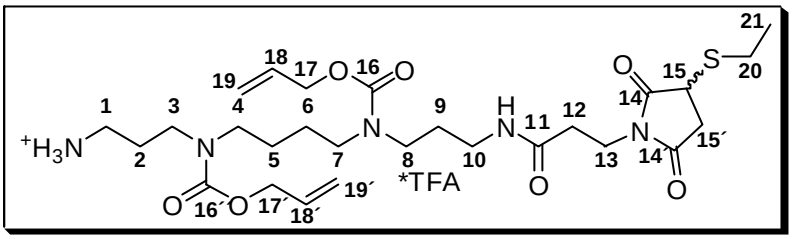
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.69 (s, 2 H, 15-H 15´-H), 5.89 (ddt, J_1 = 17.1 Hz, J_2 = 10.3 Hz, J_3 = 5.5 Hz, 2 H, 18-H 18´-H), 5.28 (d, J = 17.1 Hz, 2 H, 19-Ha 19´-Ha), 5.23 (d, J = 10.3 Hz, 2 H, 19-Hb 19´-Hb), 4.60 – 4.54 (m, 4 H, 17-H 17´-H), 3.82 (t, J = 6.8 Hz, 2 H, 13-H), 3.44 – 3.37 (m, 2 H, 3-H), 3.32 – 3.16 (m, 8 H, 4-H 7-H 8-H 10-H), 3.08 – 3.01 (m, 2 H, 1-H), 2.56 (t, J = 6.8 Hz, 2 H, 12-H), 1.99 – 1.91 (m, 2 H, 2-H), 1.75 – 1.64 (m, 2 H, 9-H), 1.56 – 1.49 (m, 4 H, 5-H 6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5 (C_{quart}, C-14 C-14´), 157.5 (C_{quart}, C-16 C-16´), 134.2 (+, C-15 C-15´), 132.8 (+, C-18 C-18´), 117.9 (–, C-19 C-19´), 67.0, 66.1 (–, C-17 C-17´), 46.9 (–, C-4 C-7), 44.4 (–, 2 C, C-3 C-8), 34.7 (–, C-1), 34.3 (–, C-10), 29.6 (–, C-9), 25.7 (–, 3 C, C-2 C-5 C-6); HRMS (ESI) $[M + H]^+$ calc. 522.2922, found 522.2916; FAB: (70 eV) (m / z) (%) = 560.2 (10) $[M + K]^+$, 544.3 (52) $[M + Na]^+$; FAB-MS: 522.3 (13) $[M + H]^+$, 459.3 (100) $[M – 63]^+$.

General procedure

1 equiv. of resin **277** was swollen in DMF and the solvent was removed. In case of solid thiols, 1.5 or 5 equiv. of the respective thiol were weight in to the resin and 2 mL of DMF were added. In case of liquid thiols, the resin was suspended in 1 mL DMF, 1.5 equiv. of the thiol were added as 10% solution in DMF and the volume of the liquid phase was filled up to 2 mL. The suspension was added for 3 h or 18 h and the resin was washed with DMF, THF : water (10 : 1) / MeOH, and CH_2Cl_2. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. Due to the

instability of some adducts during separation on RP-HPLC with gradients of water / MeCN / TFA, water / MeCN / HOAc, and water / MeCN, the crude products were interpreted from their ^{1}H NMR spectra and the identity was additionally confirmed by ESI-HRMS.

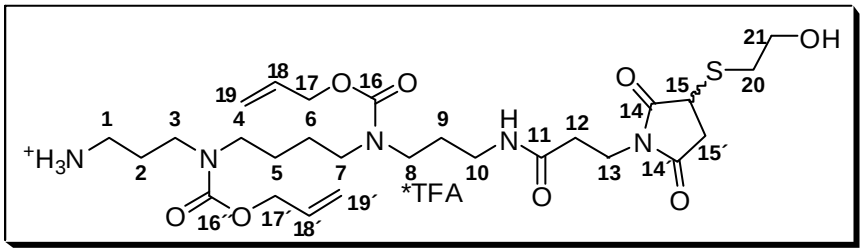
N^1-{3-[3-(ethylthio)-2,5-dioxopyrrolidin-1-yl]propanoyl}-N^4,N^9-bis-allyloxycarbonyl-spermine trifluoroacetate



$C_{29}H_{46}N_5O_9F_3S$

^{1}H NMR (400 MHz, CD_3OD) δ 5.89 (ddt, J_1 = 17.3 Hz, J_2 = 10.6 Hz, J_3 = 5.3 Hz, 2 H, 18-H 18´-H), 5.23 (d, J = 17.3 Hz, 2 H, 19-Ha 19´-Ha), 5.23 (d, J = 10.6 Hz, 2 H, 19-Hb 19´-Hb), 4.57 (d, J = 5.3 Hz, 4 H, 17-H 17´-H), 3.79 (m, 2 H, 13-H), 3.76 (dd, J_1 = 9.1 Hz, J_2 = 3.7 Hz, 1 H, 15-H), 3.39 (m, 2 H, 3-H), 3.15 – 3.30 (m, 8 H, 4-H 7-H 8-H 10-H), 3.15 (dd, J_1 = 18.6 Hz, J_2 = 9.1 Hz, 2 H), 3.01 (m, 2 H, 1-H, 15´-Ha), 2.90 and 2.87 (q, J = 7.3 Hz, 1 H, 20-Ha), 2.77 and 2.73 (q, J = 7.3 Hz, 1 H, 20-Hb), 2.48 – 2.56 (m, 2 H, 12-H), 2.50 (dd, J_1 = 18.6 Hz, J_2 = 3.7 Hz, 1 H, 15´-Hb), 1.91 – 2.00 (m, 2 H, 2-H), 1.68 (tt, J_1 = 6.0 Hz, J_2 = 5.8 Hz, 2 H, 9-H), 1.48 – 1.58 (m, 4 H, 5-H 6-H), 1.29 (t, J = 7.3 Hz, 2 H, 21-H); HRMS (ESI) $[M + H]^+$ calc. 584.3112, found 584.3100; FAB: 606.2 (13) $[M^+ + Na]$, 584.3 (100) $[M^+ + H]$.

N^1-{3-[3-(2-hydroxyethylthio)-2,5-dioxopyrrolidin-1-yl]propanoyl}-N^4,N^9-bis-allyloxycarbonyl-spermine trifluoroacetate

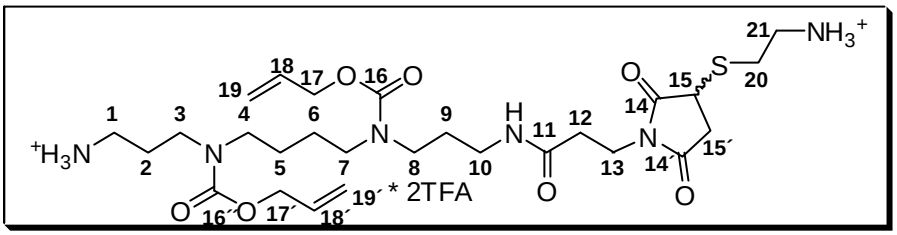


$C_{29}H_{46}N_5O_{10}SF_3$

^{1}H NMR (400 MHz, $CDCl_3$ / CD_3OD) δ 5.84 (ddt, J_1 = 17.2 Hz, J_2 = 10.6 Hz, J_3 = 5.8 Hz, 2 H, 18-H 18´-H), 5.21 (d, J = 17.2 Hz, 2 H, 19-Ha 19´-Ha), 5.15 (d, J = 10.6 Hz, 2 H, 19-Hb 19´-Hb), 4.50 (m, 4 H, 5-H 6-H), 3.81 (dd, J_1 = 9.2 Hz, J_2 = 3.8 Hz, 1 H, 15-H), 3.73 (t, J = 6.2 Hz, 2 H, 13-H), 3.62 – 3.70 (m, 2 H, 21-H), 3.32 – 3.24 (m, 2 H, 3-H), 3.22 – 3.13 (m, 8 H, 4-H 7-H 8-H 10-H), 3.10 (dd, J_1 = 18.8 Hz, J_2 = 9.2 Hz, 1 H, 15´-Ha), 2.95 (dt, J_1 = 14.1 Hz, J_2 = 5.8 Hz, 1 H, 20-Ha), 2.83 (t, J = 6.4 Hz, 2 H, 1-H), 2.77 (dt, J_1 = 14.1 Hz, J_2 = 5.8 Hz, 1 H, 20-Hb), 2.50 (dd, J_1 = 18.8 Hz, J_2 = 3.8 Hz, 1 H, 15´-Hb), 2.40 (t, J = 6.2 Hz, 2 H, 12-H), 1.84 (tt, J_1 = 6.7 Hz, J_2 = 6.8 Hz, 2 H, 2-H), 1.57 – 1.68 (m, 2 H, 9-H),

1.42 – 1.48 (m, 4 H, 5-H 6-H); ^{1}H NMR (400 MHz, CD_3OD) δ 5.94 (ddt, J_1 = 17.2 Hz, J_2 = 10.5 Hz, J_3 = 5.4 Hz, 2 H, 18-H 18´-H), 5.30 (ddt, J_1 = 17.2 Hz, J_2 = 5.0 Hz, J_3 = 1.5 Hz, 2 H, 19-Ha 19´-Ha), 5.21 (ddt, J_1 = 10.5 Hz, J_2 = 4.5 Hz, J_3 = 1.5 Hz, 2 H, 19-Hb 19´-Hb), 4.58 (d, J = 5.4 Hz, 2 H, 17-H), 4.56 (d, J = 5.4 Hz, 2 H, 17´-H), 3.96 (dd, J_1 = 9.1 Hz, J_2 = 4.0 Hz, 1 H, 15-H), 3.76 (t, J = 6.7 Hz, 2 H, 13-H), 3.76 (m, 2 H, 21-H), 3.38 (t, J = 6.8 Hz, 2 H, 3-H), 3.34 – 3.25 (m, 6 H, 4-H 7-H 10-H), 3.19 (dd, J_1 = 18.6 Hz, J_2 = 9.1 Hz, 1 H, 15´-Ha), 3.14 (t, J = 7.1 Hz, 2 H, 8-H), 3.04 (dt, J_1 = 13.6 Hz, J_2 = 6.2 Hz, 1 H, 16-Ha), 2.94 (t, J = 7.2 Hz, 2 H, 1-H), 2.82 (dt, J_1 = 13.6 Hz, J_2 = 6.4 Hz, 1 H, 16-Hb), 2.52 (dd, J_1 = 18.6 Hz, J_2 = 4.0 Hz, 1 H, 15´-Hb), 2.46 (t, J = 6.7 Hz, 2 H, 12-H), 1.92 (tt, J_1 = 7.2 Hz, J_2 = 6.8 Hz, 2 H, 2-H), 1.68 – 1.78 (m, 2 H, 9-H), 1.52 – 1.59 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 600.3062, found 600.3040; FAB-MS: 622.4 (10) $[M + Na]^+$, 600.4 (100) $[M + H]^+$.

N^1-{3-[3-(2-aminoethylthio)-2,5-dioxopyrrolidin-1-yl]propanoyl}-N^4,N^9-bis-allyloxycarbonyl-spermine bis-trifluoroacetate



$C_{31}H_{48}N_6O_{13}F_6S$

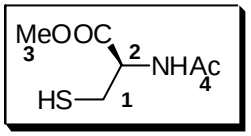
^{1}H NMR (400 MHz, CD_3OD) δ 5.96 (ddt, J_1 = 17.1 Hz, J_2 = 10.7 Hz, J_3 = 5.5 Hz, 2 H, 18-H 18´-H), 5.30 (ddt, J_1 = 17.1 Hz, J_2 = 5.0 Hz, J_3 = 1.5 Hz, 2 H, 19-Ha 19´-Ha), 5.21 (ddt, J_1 = 10.7 Hz, J_2 = 5.3 Hz, J_3 = 1.5 Hz, 2 H, 19-Hb 19´-Hb), 4.59 (d, J = 5.5 Hz, 2 H, 17-H), 4.57 (dt, J_1 = 5.5 Hz, J_2 = 1.5 Hz, 2 H, 17´-H), 3.97 (dd, J_1 = 9.3 Hz, J_2 = 4.3 Hz, 1 H, 15-H), 3.72 – 3.80 (m, 2 H, 13-H), 3.38 (t, J = 7.0 Hz, 2 H, 3-H), 3.26 – 3.34 (m, 6 H, 4-H 7-H 10-H), 3.23 – 3.14 (m, 1 H, 21-H), 3.22 (dt, J_1 = 18.5 Hz, J_2 = 9.3 Hz, 1 H, 15´-Ha), 3.14 (t, J = 6.9 Hz, 2 H, 8-H), 3.04 (dt, J_1 = 14.6 Hz, J_2 = 6.5 Hz, 1 H, 20-Hb), 2.93 (t, J = 7.4 Hz, 2 H, 1-H), 2.52 (dd, J_1 = 18.5 Hz, J_2 = 4.3 Hz, 1 H, 15´-Hb), 2.45 – 2.50 (m, 2 H, 12-H), 1.91 (tt, J_1 = 7.4 Hz, J_2 = 7.0 Hz, 2 H, 2-H), 1.70 – 1.78 (m, 2 H, 9-H), 1.53 – 1.59 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 599.3221, found 599.3215; FAB-MS: 621.4 (8) $[M + Na]^+$, 599.4 (100) $[M + H]^+$.

N^1-(3-{3-[(*R*)-2-acetamido-3-methylpropanoyl-thio]-2,5-dioxopyrrolidin-1-yl}propanoyl)- N^4,N^9-bis-allyloxycarbonyl-spermine trifluoroacetate

(*R*)-methyl 2-acetamido-3-mercaptopropanoate

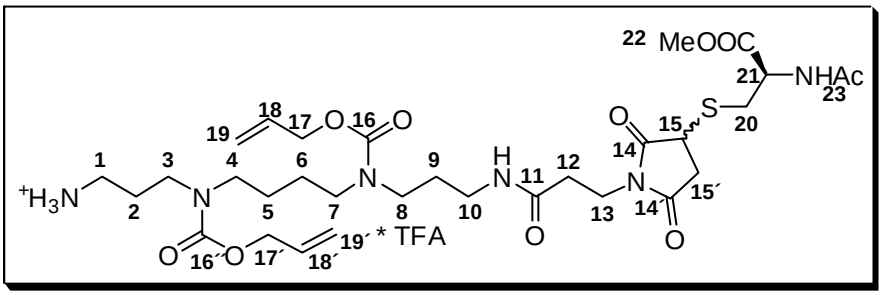
300 mg (1.37 mmol) of *N*,*S*-diacetyl-L-cysteine methyl ester was dissolved in 14 ml MeOH and 96 mg (1.37 mmol, 1.0 equiv.) of sodium thiomethylate were added. The solution was stirred for 30 min and the reaction was terminated by addition of 20 mL of 0.1 M HCl. The aqueous solution was extracted with CH2C12. The combined organic layers are washed with

brine, dried over $MgSO_4$, and filtered. After removal of the solvent, 238 mg (1.34 mmol) of the pure compound were obtained giving 98% yield.



$C_6H_{11}NO_3S$

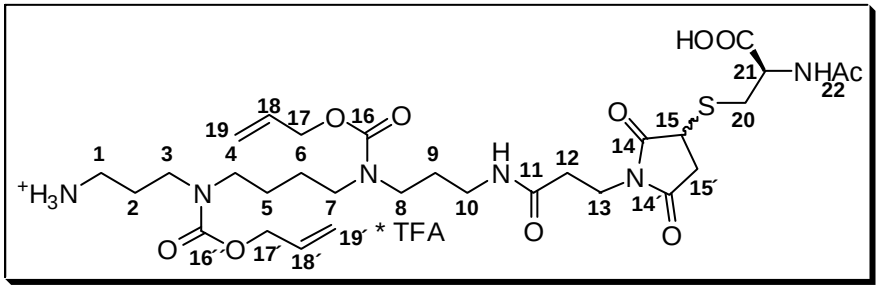
^{1}H NMR (400 MHz, CD_3OD) δ 4.63 (dd, J_1 = 6.8 Hz, J_2 = 4.8 Hz, 1 H, 2-H), 3.75 (s, 3 H, 3-H), 2.93 (dd, J_1 = 13.9 Hz, J_1 = 4.8 Hz, 1 H, 1-Ha), 2.85 (dd, J_1 = 13.9 Hz, J_1 = 6.8 Hz, 1 H, 1-Hb), 2.02 (s, 3 H, 4-H); MS (EI): 177.0 (12) $[M]^+$, 144.0 (4) $[M - (SH)]^+$, 118.0 (38) $[M - (COOMe)]^+$.



$C_{33}H_{51}N_6O_{12}F_3S$

^{1}H NMR (400 MHz, CD_3OD) δ 5.95 (ddt, J_1 = 17.1 Hz, J_2 = 10.5 Hz, J_3 = 5.4 Hz, 2 H, 18-H 18´-H), 5.30 (d, J = 17.1 Hz, 2 H, 19-Ha 19´-Ha), 5.21 (d, J = 10.5 Hz, 2 H, 19-Hb 19´-Hb), 4.74 (m, 1 H, 21-H), 4.59 (d, J = 5.4 Hz, 2 H, 17-H), 4.57 (d, J_1 = 5.4 Hz, , 17´-H), 3.95 (dd, J_1 = 9.1 Hz, J_2 = 4.5 Hz, 1 H, 15-H), 3.76 (t, J = 7.1 Hz, 2 H, 13-H), 3.74 (s, 3 H, 22-H), 3.39 (t, J = 6.8 Hz, 2 H, 3-H), 3.33 – 3.26 (m, 6 H, 4-H 7-H 10-H), 3.25 – 3.12 (m, 3 H, 15-Ha 20-H), 2.94 (t, J = 7.2 Hz, 2 H, 1-H), 2.46 (t, J = 7.1 Hz, 2 H, 12-H), 2.41 – 2.51 (m, 1 H, 15´-Hb), 2.00 (s, 3 H, 23-H), 1.91 (tt, J_1 = 6.9 Hz, J_2 = 6.8 Hz, 2 H, 2-H), 1.78 – 1.70 (m, 2 H, 9-H), 1.60 – 1.52 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 699.3382, found 699.3377; FAB-MS: 721.5 (15) $[M + Na]^+$, 699.5 (100) $[M + H]^+$.

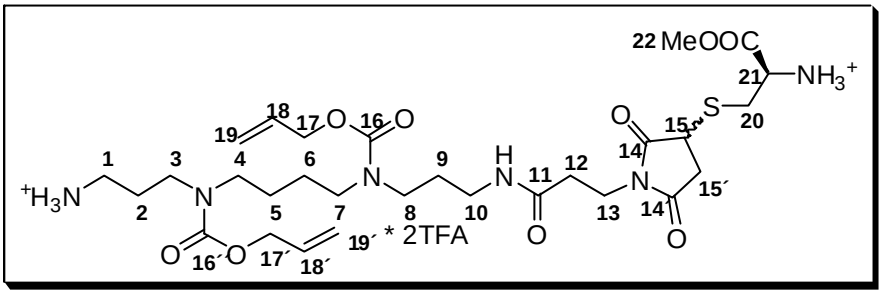
N^1-(3-{3-[(*R*)-2-acetamido-3-propanoyl-thio]-2,5-dioxopyrrolidin-1-yl}propanoyl)-N^4,N^9-bis-allyloxycarbonyl-spermine



$C_{32}H_{49}N_6O_{12}F_3S$

^{1}H NMR (400 MHz, $CDCl_3/CD_3OD$) δ 5.88 (ddt, J_1 = 17.1 Hz, J_2 = 10.5 Hz, J_3 = 5.5 Hz, 2 H, 18-H 18´-H), 5.25 (d, J = 17.1 Hz, 2 H, 19-Ha 19´-H), 5.19 (d, J = 10.5 Hz, 2 H, 19-Hb 19´-Hb), 4.74 – 4.69 (m, 1 H, 21-H), 4.57 – 4.51 (m, 4 H, 17-H 17´-H), (3.90 (dd, J_1 = 9.2 Hz, J_2 = 3.9 Hz) and 3.85 (dd, J_1 = 9.2 Hz, J_2 = 3.9 Hz) together 1 H, 15-H), 3.74 (t, J = 6.8 Hz, 2 H, 13-H), 3.35 – 3.30 (m, 2 H, 3-H), 3.26 – 3.19 (m, 6 H, 4-H 7-H 10-H), 3.19 – 3.09 (m, 4 H, 8-H 20-H), 2.91 – 2.84 (m, 1H, 15´-Ha), 2.88 (t, J = 7.1 Hz, 2 H, 1-H), 2.47 – 2.40 (m, 2 H, 12-H), 2.44 (dd, J_1 = 18.6 Hz, J_2 = 3.9 Hz, 1 H, 15´-Hb), 2.00 (s, 3 H, 22-H), 1.87 (tt, J_1 = 7.1 Hz, J_2 = 6.8 Hz, 2 H, 2-H), 1.73 – 1.63 (m, 2 H, 9-H), 1.54 – 1.47 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 685.3225, found 685.3224.

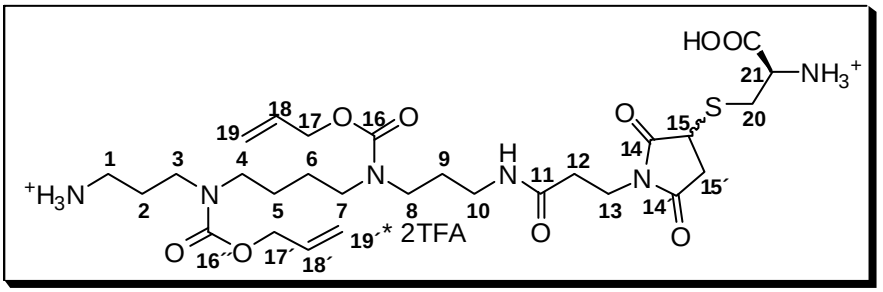
***N*^{1}-(3-{3-[(*R*)-2-amino-3-methylpropanoyl-thio]-2,5-dioxopyrrolidin-1-yl}propanoyl)-*N*^{4},*N*^{9}-bis-allyloxycarbonyl-spermine bis-trifluoroacetate**



$C_{33}H_{50}N_6O_{13}F_6S$

^{1}H NMR (400 MHz, CD_3OD) δ 5.96 (ddt, J_1 = 17.1 Hz, J_2 = 10.3 Hz, J_3 = 5.7 Hz, 2 H, 18-H 18´-H), 5.31 (ddt, J_1 = 17.1 Hz, J_2 = 5.3 Hz, J_3 = 1.4 Hz, 2 H, 19-Ha 19´-Hb), 5.21 (ddt, J_1 = 10.3 Hz, J_2 = 5.3 Hz, J_3 = 1.4 Hz, 2 H, 19-Hb 19´-Hb), 4.59 (d, J = 5.7 Hz, 2 H, 17-H), 4.57 (d, J_1 = 5.5 Hz, 2 H, 17´-H), (4.55 (dd, J_1 = 8.6 Hz, J_2 = 4.5 Hz) and 4.43 (dd, J_1 = 8.6 Hz, J_2 = 4.5 Hz) together 1 H, 21-H), 4.02 (dd, J_1 = 9.3 Hz, J_2 = 4.4 Hz, 1 H, 15-H), 3.87 (s, 3 H, 22-H), 3.76 (t, J = 6.9 Hz, 2 H, 13-H), 3.38 (t, J = 7.0 Hz, 2 H, 3-H), 3.34 – 3.27 (m, 8 H, 4-H 7-H 10-H 20-H), 3.21 (dd, J_1 = 18.5 Hz, J_2 = 9.3 Hz, 1 H, 15´-Ha), 3.14 (m, 2 H, 8-H), 2.93 (t, J = 7.3 Hz, 2 H, 1-H), 2.52 (dd, J_1 = 18.5 Hz, J_2 = 4.4 Hz, 1 H, 15´-Hb), 2.52 – 2.45 (m, 2 H, 12-H), 1.91 (tt, J_1 = 7.2 Hz, J_2 = 7.0 Hz, 2 H, 2-H), 1.79 – 1.70 (m, 2 H, 9-H), 1.60 – 1.52 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 657.3276, found 657.3265.

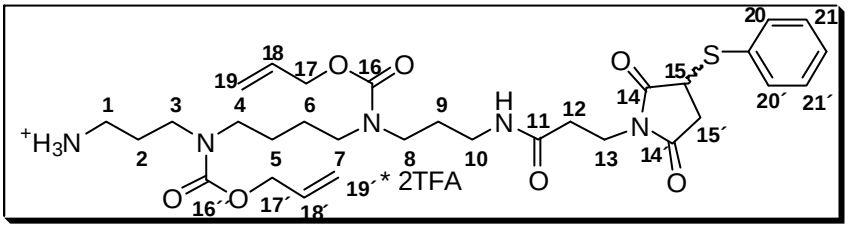
***N*^{1}-(3-{3-[(*R*)-2-amino-3-propanoyl-thio]-2,5-dioxopyrrolidin-1-yl}propanoyl)-*N*^{4},*N*^{9}-bis-allyloxycarbonyl-spermine bis-trifluoroacetate**



$C_{32}H_{48}N_6O_{13}F_6S$

^{1}H NMR (400 MHz, CD_3OD) δ 5.96 (ddt, J_1 = 17.3 Hz, J_2 = 10.6 Hz, J_3 = 5.5 Hz, 2 H, 18-H 18´-H), 5.30 (ddt, J_1 = 17.3 Hz, J_2 = 4.8 Hz, J_3 = 1.4 Hz, 2 H, 19-Ha 19´-Ha), 5.22 (ddt, J_1 = 10.6 Hz, J_2 = 4.8 Hz, J_3 = 1.4 Hz, 2 H, 19-Hb 19´-Hb), 4.59 (d, J = 5.5 Hz, 2 H, 17-H), 4.57 (d, J_1 = 5.3 Hz, 2 H, 17´-H), (4.45 (dd, J_1 = 8.7 Hz, J_2 = 4.3 Hz) and 4.33 (dd, J_1 = 8.7 Hz, J_2 = 4.3 Hz) together 1 H, 21-H), 4.03 (dd, J_1 = 9.1 Hz, J_2 = 4.5 Hz, 1 H, 15-H), 3.83 – 3.69 (m, 2 H, 13-H), 3.38 (t, J = 6.9 Hz, 2 H, 3-H), 3.34 – 3.26 (m, 8 H, 4-H 7-H 10-H 20-H), 3.22 (dd, J_1 = 18.6 Hz, J_2 = 9.1 Hz, 1 H, 15´-Ha), 3.14 (t, J = 6.8 Hz, 2 H, 8-H), 2.93 (t, J = 7.3 Hz, 2 H, 1-H), 2.53 (dd, J_1 = 18.4 Hz, J_2 = 4.5 Hz, 1 H, 15´-Hb), 2.52 – 2.45 (m, 2 H, 12-H), 1.91 (tt, J_1 = 7.3 Hz, J_2 = 6.9 Hz, 2 H, 2-H), 1.78 – 1.70 (m, 2 H, 9-H), 1.60 – 1.53 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 643.3120 found 643.3117.

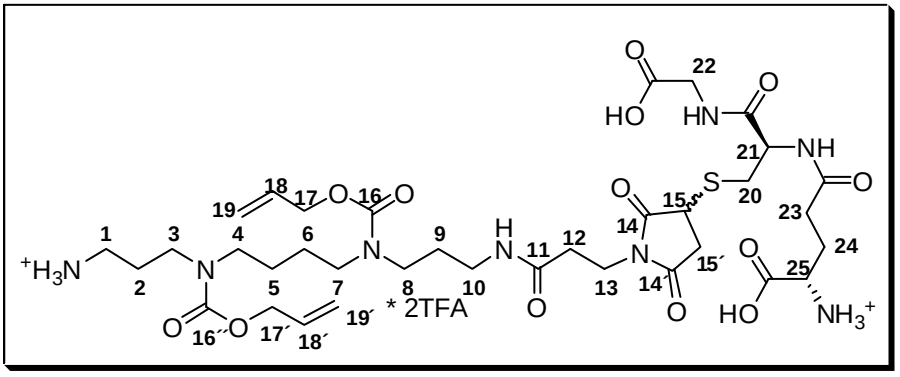
N^1-{3-[3-(phenylthio)-2,5-dioxopyrrolidin-1-yl]propanoyl}-N^4,N^9-bis-allyloxycarbonyl-spermine trifluoroacetate



$C_{33}H_{46}N_5O_9F_3S$

^{1}H NMR (400 MHz, $CDCl_3$ / CD_3OD) δ 7.50 – 7.46 (m, 2 H, 20-H 20´-H), 7.36 – 7.32 (m, 3 H, 21-H 21´-H 22-H), 5.89 (ddt, J_1 = 17.1 Hz, J_2 = 10.6 Hz, J_3 = 5.5 Hz, 2 H, 18-H 18´-H), 5.28 (d, J = 17.1 Hz, 2 H, 19-Ha 19´-Hb), 5.23 (d, J = 10.6 Hz, 2 H, 19-Hb 19´-Hb), 4.60 – 4.53 (m, 4 H, 17-H 17´-H), 4.07 (dd, J_1 = 9.1 Hz, J_2 = 3.8 Hz, 1 H), 3.69 (t, J = 6.8 Hz, 2 H, 13-H), 3.43 – 3.35 (m, 2 H, 3-H), 3.32 – 3.15 (m, 8 H, 4-H 7-H 8-H 10-H), 3.17 (dd, J_1 = 18.9 Hz, J_2 = 9.1 Hz, 1 H, 15´-Ha), 3.06 – 2.99 (m, 2 H, 1-H), 2.68 (dd, J_1 = 18.9 Hz, J_2 = 3.8 Hz, 1 H, 15´-Hb), 2.43 (t, J = 6.8 Hz, 2 H, 12-H), 1.98 – 1.89 (m, 2 H, 2-H), 1.74 – 1.62 (m, 2 H, 9-H), 1.55 – 1.48 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 632.3112, found 632.3110.

N^1-(3-{3-[(*R*)-2-(e-glutamylamido)-3-glycinylpropanoyl-thio]-2,5-dioxopyrrolidin-1-yl}propanoyl)-N^4,N^9-bis-allyloxycarbonyl-spermine bis-trifluoroacetate



$C_{95}H_{58}N_8O_{17}F_6S$

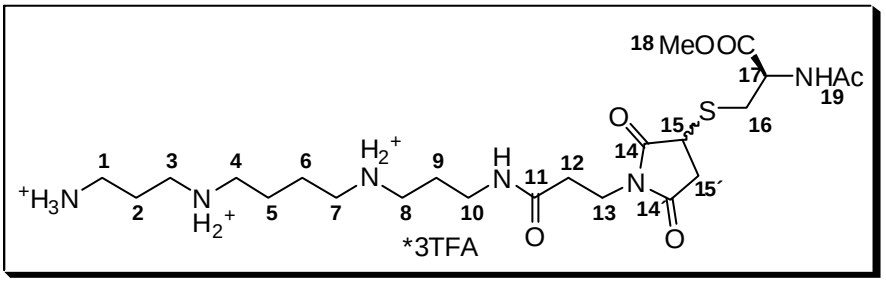
^{1}H NMR (400 MHz, CD_3OD) δ 5.96 (ddt, J_1 = 17.3 Hz, J_2 = 10.6 Hz, J_3 = 5.4 Hz, 2 H, 18-H 18´-H), 5.30 (ddt, J_1 = 17.3 Hz, J_2 = 5.0 Hz, J_3 = 1.7 Hz, 2 H, 19-Ha 19´-Hb), 5.22 (ddt, J_1 = 10.6 Hz, J_2 = 5.0 Hz, J_3 = 1.7 Hz, 2 H, 19-Hb 19´-Hb), 4.67 – 4.75 (m, 1 H, 21-H), 4.59 (d, J = 5.4 Hz, 2 H, 17-H), 4.57 (dt, J_1 = 5.4 Hz, J_2 = 1.5 Hz, 2 H, 17´-H), 4.09 – 4.04 (m, 1 H, 25-H), 4.01 (dd, J_1 = 9.1 Hz, J_2 = 3.9 Hz, 1 H, 15´-H), 3.94 (s, 2 H, 22-H), 3.76 (t, J = 6.8 Hz, 2 H, 13-H), 3.39 (t, J = 6.8 Hz, 2 H, 3-H), 3.33 – 3.26 (m, 6 H, 4-H 7-H 10-H), 3.22 – 3.17 (m, 1 H, 20-Ha), 3.21 (dd, J_1 = 18.5 Hz, J_2 = 9.1 Hz, 1 H, 15´-Ha), 3.14 (t, J = 6.8 Hz, 2 H, 8-H), 2.94 (t, J = 7.4 Hz, 2 H, 1-H), 2.94 – 2.88 (m, 1 H, 20-Hb), 2.60 (t, J = 7.1 Hz, 2 H, 12-H), 2.53 (dd, J_1 = 18.5 Hz, J_2 = 3.9 Hz, 1 H, 15´-Hb), 2.47 (t, J = 6.8 Hz, 2 H, 24-H), 2.30 – 2.14 (m, 2 H, 23-H), 1.91 (tt, J_1 = 7.4 Hz, J_2 = 6.8 Hz, 2 H, 2-H), 1.79 – 1.68 (m, 2 H, 9-H), 1.60 – 1.52 (m, 4 H, 5-H 6-H).

6.6.2 Compatibility of maleimido-thiol coupling and Aloc protection strategy

6.6.2.1 Removal of Aloc groups after coupling of maleimide and thiol

***N*^{1}-(3-{3-[(*R*)-2-acetamido-3-methylpropanoyl-thio]-2,5-dioxopyrrolidin-1-yl}propanoyl)-spermine tris-trifluoroacetate (280)**

1 equiv. of resin **277** was swollen in DMF and the solvent was removed. 1.5 equiv. of *N*-acetyl-L-cysteine methyl ester were added and the solids were suspended by 2 mL of DMF. The suspension was added for 3 h and the resin was washed with DMF, THF : water (10 : 1) / MeOH, and CH_2Cl_2. The resin **279** was Aloc-deprotected with *N*,*N*´-DMBA as described above. The resin was treated with 10% TFA in CH_2Cl_2 and the cleavage mixture was combined with washings of CH_2Cl_2 and MeOH. The solvents were removed and the solid was dried on HV to remove remains of TFA. The crude product was purified by RP-HPLC with a gradient of water / MeCN / HOAc.



$C_{29}H_{45}N_6O_{12}F_9S$

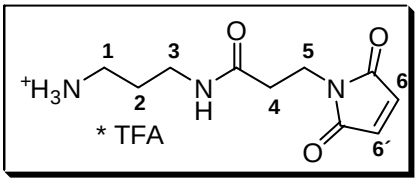
^{1}H NMR (400 MHz, CD_3OD) δ 4.77 – 4.70 (m, 1 H, 17-H), 3.98 (dd, J_1 = 9.1 Hz, J_2 = 3.7 Hz) and 3.97 (dd, J_1 = 9.1 Hz, J_2 = 3.7 Hz) together 1 H, 15-H), 3.78 (t, J = 6.9 Hz, 2 H, 13-H), 3.75 (s, 3 H, 18-H), 3.31 – 2.90 (m, 15 H, 1-H 3-H 4-H 7-H 8-H 10-H 15´-Ha 16-H), 2.50 (t, J = 6.9 Hz, 2 H, 12-H), 2.48 (dd, J_1 = 18.4 Hz, J_2 = 3.7 Hz, 1 H, 15´-Hb), 2.14 – 2.04 (m, 2 H, 2-H), 2.00 (s, 3 H, 19-H), 1.92 – 1.78 (m, 2 H, 9-H), 1.82 – 1.71 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 531,2959, found 531.2943.

6.6.2.2 Deprotection of Aloc in the presence of maleimides

Investigation of maleimide stability

***N^3*-(3-*N*-maleimidopropionyl)-1,3-diaminopropane (281)**

900 mg (1.17 mmol) of 2-chlorotrityl resin **(60)** were swollen in CH_2Cl_2 and after removal of the solvent a solution of 4.50 g (17.4 mmol; 14.8 equiv.) of N^3-(2-nitrophenylsulfonyl)-1,3-diaminopropane **(188)** and 408 µl (2.34 mmol; 2 equiv.) of DiPEA in 30 mL CH_2Cl_2 was added. The suspension was agitated for 27 h and 10 mL of MeOH were added 15 min prior termination. The resin was washed with CH_2Cl_2 / MeOH and CH_2Cl_2. The resin was *o*-Nosyl deprotected. To 630 µmol of the resin was added a solution of 1.01 g (3.78 mmol; 6 equiv.) of 3-*N*-maleimidopropionyl succinate **196** and 440 µl (2.53 mmol; 4 equiv.) of DiPEA in 12 mL DMF. The suspension was agitated for 17 h and was then washed with water : THF (1 : 10), THF / MeOH and CH_2Cl_2.



$C_{12}H_{16}N_3O_5F_3$

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.65 (s, 2 H, 6-H 6´-H), 3.75 (t, *J* = 6.8 Hz, 2 H, 5-H), 3.21 (t, *J* = 6.3 Hz, 2 H, 3-H), 2.90 (t, *J* = 6.8 Hz, 2 H, 1-H), 2.45 (t, *J* = 6.8 Hz, 2 H, 4-H), 1.79 (tt, J_1 = 6.8 Hz, J_2 = 6.3 Hz, 2 H, 2-H)

General procedure

After swelling, 50 mg of the resin were treated with a solution of scavenger and Pd catalyst in the respective solvent. The suspension was agitated under avoidance of light and then the resin was washed with CH_2Cl_2 / MeOH and CH_2Cl_2. After cleavage from the resin, the product was analyzed by ^{1}H NMR without further purification.

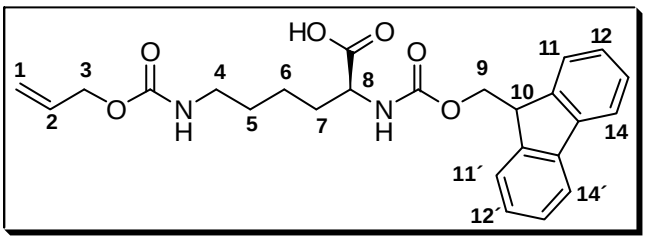
Table 14 Reaction conditions applied to resin **281**.

Scavenger	Excess of scavenger in equiv.	Amount of $Pd(PPh_3)_4$ in mol%	Solvent	Reaction time in min
-	-	4 / 10 / 40	THF	30
-	-	40	THF	120
Pyrrolidine	10	20	THF	30
Dimedone	8	10	THF	30
N,*N'*-Dimethylbarbituric acid	10	15	CH_2Cl_2	60
$PhSiH_3$	6	10	THF	30
Bu_3SnH / HOAc	3 / 3	8	CH_2Cl_2	5
Bu_3SnH	7	20	CH_2Cl_2	30
HOBt	6	10	DMF	30
N-Hydroxysuccinimide	8	20	THF	30
NaN_3	5	10	DMF	30
HOAc	20	20	THF	30
Water	50	20	THF	30

Synthesis of resin 285

N^a-Fluorenylmethyloxycarbonyl-N^e-allyloxycarbonyl-L-lysine resin 282

340 mg (0.44 mmol) of 2-chlorotrityl resin **(60)** were swollen in CH_2Cl_2 and after removal of the solvent a solution of 1.00 g (2.20 mmol; 5 equiv.) of N^a-fluorenylmethyloxycarbonyl-N^e-allyloxycarbonyl-L-lysine and 116 µl (663 µmol; 1.5 equiv.) of DiPEA in 12 mL CH_2Cl_2 was added. The suspension was agitated for 26 h and 4 mL of MeOH were added 15 min prior termination. The resin was washed with CH_2Cl_2 / MeOH and CH_2Cl_2. After drying on high vacuum, the product was cleaved from 50 mg of the resin **282** to confirm the identity of the immobilized substrate. The loading was determined to be approximately 0.4 mmol / g resin.

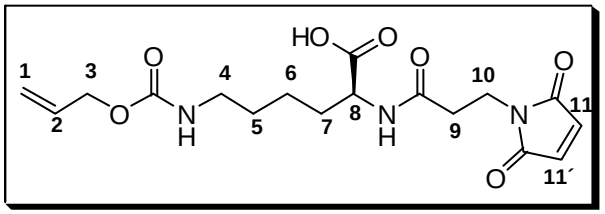


$C_{25}H_{28}N_2O_6$

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.3 Hz, 2 H, 14-H 14´-H), 7.62 – 7.55 (m, 2 H, 11-H 11´-H), 7.39 (ddd, J_1 = 7.3, J_2 = 7.0, J_3 = 5.8, 2 H, 13-H 13´-H), 7.30 (ddd, J_1 = 7.3, J_2 = 7.0, J_3 = 6.3, 2 H, 12-H 12´-H), 6.09 – 5.81 (m, 1 H, 2-H), 5.34 – 5.14 (m, 2 H, 1-H), 4.66 – 4.59 (m, 1 H, 10-H), 4.59 – 4.48 (m, 2 H, 9-H), 4.40 (d, *J* = 5.8 Hz, 2 H, 3-H), 4.20 (t, *J* = 5.9 Hz, 1 H, 8-H), 3.24 – 3.10 (m, 2 H, 4-H), 1.96 – 1.81 (m, 1 H, 7-Ha), 1.81 – 1.69 (m, 1 H, 7-Hb), 1.58 – 1.47 (m, 2 H, 5-H), 1.47 – 1.35 (m, 2 H, 6-H).

N^a-(3-*N*-Maleimidopropionl)-N^e-allyloxycarbonyl-L-lysine resin 284

Resin **282** was Fmoc-deprotected. A solution 327 mg (1.02 mmol; 7.2 equiv.) of 3-*N*-maleimido-propionyl succinate **196** and 118 µl (680 µmol; 4 equiv.) of DiPEA in 8 mL DMF were added to the resin and the suspension was agitated for 20 h. After washing with THF : MeOH (4 : 1) / MeOH and CH_2Cl_2 and drying in HV, a white resin was obtained. The product was cleaved from 50 mg of the resin to confirm the identity of the immobilized substrate. The loading was determined to be 0.41 mmol / g resin.



$C_{17}H_{23}N_3O_7$

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.71 (s, 2 H, 11-H, 11´-H), 5.97 – 5.83 (m, 1 H, 2-H), 5.29 (d, *J* = 17.1 Hz, 1 H, 1-Ha), 5.20 (d, *J* = 10.3 Hz, 1 H, 1-Hb), 4.63 – 4.57 (m, 1 H, 8-H), 4.57 – 4.51 (m, 2 H, 3-H), 3.87 – 3.80 (m, 2 H, 10-H), 3.24 – 3.13 (m, 2 H, 7-H), 2.60 (t, *J* = 6.8 Hz, 2 H, 9-H), 1.92 – 1.80 (m, 1 H, 6-Ha), 1.80 – 1.68 (m, 1 H, 6-Hb), 1.52 (tt, J_1 = 6.5 Hz, J_2 = 6.5 Hz, 2 H, 5-H), 1.38 (tt, J_1 = 6.8 Hz, J_2 = 6.8 Hz, 2 H, 6-H); HRMS (ESI) $[M + H]^+$ calc. 404,1428, found 404,1428.

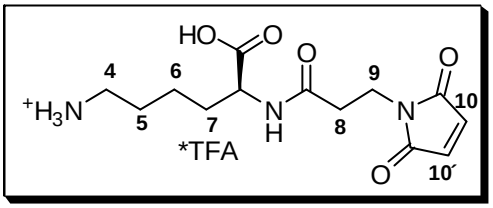
Aloc removal from resin 284

General procedure

The deprotection was carried out under the same conditions as described for the stability tests of the maleimide. However, due to the interaction of maleimide and catalyst, the amount of $Pd(PPh_3)_4$ was raised to 1.2 equiv. The successful experiment is described below.

***N*^{a}-(3-*N*-maleimidopropiopnyl)-lysine 285**

50 mg (20 µmol) of the resin were swollen in THF and after removal of the solvent a solution of 43 µl (160 µmol; 8 equiv.) of tributyltinhydride and 28 mg (24 µmol; 1.2 equiv.) of $Pd(PPh_3)_4$ in 2 mL THF were added. The suspension was agitated for 5 h in the absence of light. The resin was washed with 10% HOAc in CH_2Cl_2 and CH_2Cl_2 / MeOH and the crude was cleaved from the resin with 10% TFA in CH_2Cl_2. The drained cleavage mixture was combined with washings of CH_2Cl_2 and MeOH and after removal of the solvent and drying on high vacuum the substance was purified by RP-HPLC (92% purity, detection at 214 nm).



$C_{15}H_{20}N_3O_7F_3$

HRMS (ESI) $[M + H]^+$ calc. 298,1397, found 298,1397.

Aloc removal from resin 277

General procedure

The good procedure for the removal of the Aloc group from resin **284** was applied. Additional experiments were carried out as described below:

Table 15 Conditions applied to resin **277**.

Scavenger	Excess of scavenger in equiv.	Amount of $Pd(PPh_3)_4$ in mol%	Solvent	Reaction time in min
Formic acid / pyridine / *N*-hydroxysuccinimide	8 / 8 / 8	80	THF	240
$HsnBu_3$ / DMBA	8 / 2	40	THF	240
$PhSiH_3$ / TmsCl	8 / 8	40	THF	240
Dimedone	8	50	THF	960
HOAc	40	4 * 110	CH_2Cl_2	300
HOAc / *N*-ethylmorpholine	40 / 10	4 * 50	CH_2Cl_2	240
$HSnBu_3$	8	120	THF	180
Tritylsulfide	4	120	THF	240

6.6.3 Application of 2-Iminothiolane for Polyamine-SPS

6.6.3.1 Optimisation of the reaction

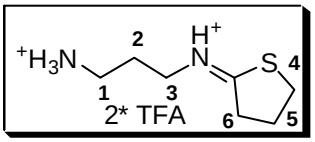
Optimisation of solvent system

500 mg of chlorotrityl resin **(60)** were loaded with 1-(2-nitrophenylsulfonamido)-1,3-diaminopropane **188** and *o*-Nosyl deprotected. 50 mg (27 µmol) of the resin were swollen in the respective solvent. The solvent was removed and 7.5 mg (55 µmol; 2 equiv.) of 2-iminothiolane **(296)** were added to the resin. 2 mL of the solvent or the solvent mixture were added and the suspension was agitated for 13 h. The solvent was removed and the resin was washed with water, THF / MeOH and CH_2Cl_2. The product was cleaved from the resin. After removal of the solvents and drying on HV the products were analyzed by ^{1}H NMR.

Optimisation of reaction protocol

50 mg (34 µmol) of **155** were suspended in 1 mL THF. Ethylmaleimide and 2-iminothiolane **(296)** were dissolved in 0,5 mL THF or 200 µl water respectively and added to the suspension at the particular times and in the particular amounts. The volume was filled up to 2 mL solvent and the suspension was agitated for 15 h. After removal of the solvent, the resin was washed with water, THF / MeOH and CH_2Cl_2. The product was cleaved from the resin. After removal of the solvents and drying on HV the products were analyzed by ^{1}H NMR and ESI-MS.

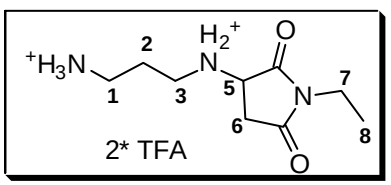
(*E*)-*N*^{1}-(dihydrothiophen-2(3*H*)-ylidene)propane-1,3-diamine bis-trifluoroacetate 301



$C_{11}H_{16}N_2O_2F_6S$

^{1}H NMR (400 MHz, CD_3OD): δ = 3.69 (t, *J* = 7.1 Hz, 2 H, 4-H), 3.65 (t, 2 H, *J* = 6.7 Hz, 6-H), 3.26 (t, 2 H, *J* = 7.3 Hz, 3-H), 3.09 (t, 2 H, *J* = 7.5 Hz, 1-H), 2.44 (tt, 2 H, J_1 = 7.1 Hz, J_2 = 6.7 Hz, 5-H), 2.18 (tt, 2 H, J_1 = 7.5 Hz, J_2 = 7.3 Hz, 2-H); MS (ESI): 159.1 $[M + H]^+$.

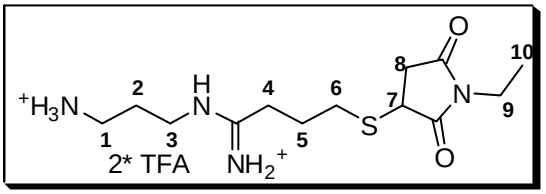
3-(3-aminopropylamino)-1-ethylpyrrolidine-2,5-dione *bis*-trifluoroacetate 303



$C_{13}H_{19}N_3O_6F_6$

^{1}H NMR (400 MHz, CD_3OD): δ = 4.45 (dd, J_1 =9.2 Hz, J_2 = 5.7 Hz, 1 H, 5-H), 3.57 (q, *J* = 7.3 Hz, 7-H), 3.16 (dd, J_1 = 17.9 Hz, J_2 = 9.2 Hz, 1 H, 6-Hb), 3.08 (t, *J* = 7.6 Hz, 2 H, 3-H), 3.05 (t, *J* = 7.7 Hz, 2 H, 1-H), 2.91 (dd, J_1 =17.9 Hz, J_2 = 5.7 Hz, 1 H, 6-Hb), 2.13 (m, 2 H, 2-H), 1.17 (t, *J* = 7.3 Hz, 8-H); HRMS (ESI) $[M + H]^+$ calc. 200.1399, found 200.1406.

4-(1-ethyl-2,5-dioxopyrrolidin-3-ylthio)-*N*-(3-aminopropyl)butanamidine *bis*-trifluoroacetate 304



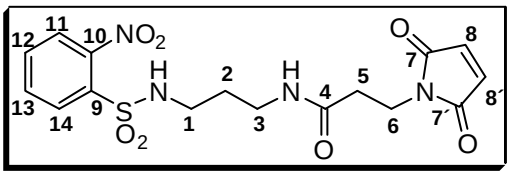
$C_{17}H_{26}N_4O_6F_6S$

^{1}H NMR (400 MHz, CD_3OD): δ = 3.89 (dd, J_1 = 9.1 Hz, J_2 = 3.3 Hz, 1 H, 7-H), 3.52 (q, J = 7.2 Hz, 2 H, 9-H), 3.38 (t, J = 7.1 Hz, 2 H, 3-H), 3.19 (dd, J_1 = 18.5 Hz, J_2 = 9.1 Hz, 1 H, 8-Ha), 3.03 (t, J = 7.4 Hz, 2 H, 1-H), 2.97 (dd, J_1 = 13.5 Hz, J_2 = 7.1 Hz, 1 H, 6-Ha), 2.79 (dd, J_1 = 13.5 Hz, J_2 = 7.1 Hz, 1 H, 6-Hb), 2.61 (t, J = 7.5 Hz, 2 H, 4-H), 2.46 (dd, J_1 = 18.6 Hz, J_2 = 3.3 Hz, 1 H, 8-Hb), 2.08 (tt, J_1 = 7.4 Hz, J_2 = 7.1 Hz, 2 H, 2-H), 2.01 (tt, J_1 = 7.5 Hz, J_2 = 7.1 Hz, 2 H, 5-H), 1.13 (t, J = 7.2 Hz, 3 H, 10-H) – HRMS (ESI) $[M + H]^+$ calc. 301.1693 found 301.1686.

6.6.3.2 Synthesis of maleimides

N^3-(3-*N*-maleimidopropionyl)-N^1-(2-nitrophenylsulfonamido)-1,3-diaminopropane (309)

308 mg (1.16 mmol; 3 equiv.) of 3-maleimidopropionic acid *N*-hydroxysuccinimide ester **196** were dissolved in DMF and 100 mg (386 µmol; 1 equiv.) of 1-(2-nitrophenylsulfonamido)-1,3-diaminopropane **188** and 162 µl (1.16 mmol; 3 equiv.) of Et_3N were added in the same volume of THF. The suspension was stirred for 2 d and THF was removed in vacuum. The remaining suspension was partitioned between $CHCl_3$ and water and the organic layer was three times washed with a solution of 1% HOAc in water. The organic layer was dried over Na_2SO_4 and the product was purified by flash chromatography on silica gel. ($CHCl_3$ to $CHCl_3$: MeOH (5 : 1)). 74 mg of the product **309** were isolated giving 47% yield.



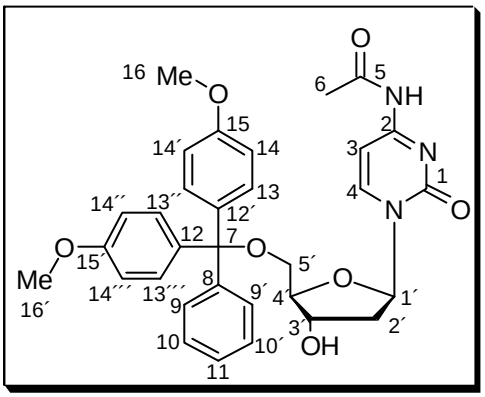
$C_{16}H_{18}N_4O_7S$

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 – 7.12 (m, 1 H, 11-H), 7.70 – 7.84 (m, 3 H, 12-H 13-H 14-H), 6.73 (s, 2 H, 8-H 8´-H), 3.75 (t, J = 7.1 Hz, 2 H, 6-H), 3.17 (t, J = 6.7 Hz, 2 H, 3-H) and 3.05 (t, J = 6.7 Hz, 2 H, 1-H), 2.43 (t, J = 7.1 Hz, 2 H, 5-H), 1.65 (tt, J_1 = 6.7 Hz, J_2 = 6.6 Hz, 2 H, 2-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3 (C_{quart}, C-4), 171.5 (C_{quart}, C-7 C-7´), 148.8 (C_{quart}, C-10), 134.9 (+, C-8 C-8´), 134.4, 134.3, 133.2, 131.1, 125.6 (C_{quart}

and +, C-9 C-11 C-12 C-13 C-14), 41.3 (–, C-1), 36.9 (–, C-6), 35.3 (–, C-3), 29.8 (–, C-5, splitted and broadened signal), 26.1 (–, C-2); HRMS (ESI) $[M + Na]^+$ calc. 433.0788, found 433.0781.

N^4-Actyl-2´-deoxy-$O^{5´}$-di-*p*-methoxytrityl cytidine (311)

To a solution of 5.00 g 2´-deoxycytidine (19.0 mmol) **(310)** in 190 mL DMF was added 1.80 mL acetic anhydride (19.00 mmol; 1 equiv) and 3.4 mL Et_3N (38.0 mmol; 2 equiv.). After the mixture had been stirred at room temperature for 6 h, the solvent was removed under reduced pressure. The resulting residue was dissolved 20 mL pyridine and the solvent was removed by evaporation (two times). The crude product was dissolved in 180 mL pyridine and 8.37 g of 4,4'-dimethoxytritylchlorid (24.7 mmol; 1.3 equiv.) were added. The mixture was stirred at room temperature for 4 h and the solvent was evaporated. The remaining residue was partitioned between 100 mL $CHCl_3$ and 100 mL of 5% aqueous $NaHCO_3$. The aqueous phase was extracted three times with 35 mL $CHCl_3$. The organic phase was dried over Na_2SO_4 and the solvent was removed in vacuum. The product was purified by flash chromatography on silica gel ($CHCl_3$ to $CHCl_3$: MeOH (20 : 1)). 8.03 g of a yellow foam were obtained, giving 74% yield.

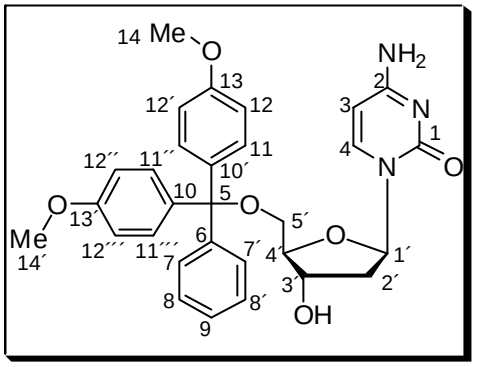


$C_{32}H_{33}N_3O_7$

R_f = 0.31 ($CHCl_3$: MeOH - 11 : 1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (bs, 1 H, O*H*), 8.22 (d, ^3J = 7.4 Hz, 1 H, 4-H), 7.39 (d, ^3J = 7.4 Hz, 1 H, 3-H), 6.80 – 7.33 (m, 13 H, (8-H to 14´´´-H)), 6.27 (t, ^3J = 5.8 Hz, 1 H, 1´-H), 4.50 (t, ^3J = 5.3 Hz, 1 H, 3´-H), 4.13 (dt, ^3J = 3.6 Hz, ^3J = 6.4 Hz, 1H, 4´-H), 3.80 (s, 6H, 16-H 16´-H), 3.49 (dd, ^2J = 10.8 Hz, ^3J = 3.6 Hz, 1 H, 5´-Ha), 3.41 (dd, ^2J = 10.8 Hz, ^3J = 3.6 Hz, 1 H, 5´-Hb), 2.75 (ddd, ^2J = 13.6 Hz, ^3J = 5.8 Hz, ^3J = 5.3 Hz, 1 H, 2´-Ha), 2.23 (m, 1 H, 2´-Hb), 2.20 (s, 3 H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 170.67 (C_{quart}, C-5); 162.58 (+, C-1); 155.69 (C_{quart}, C-2); 158.78, 144.75, 135.58, 135.44, 130.18, 130.15, 128.20, 128.14, 127.22, 113.43 (C_{quart} and +, 18 C, C-8 C-9 C-9´ C-10 C-10´ C-11 C-12 C-12´ C-13 C-13´ C-13´´ C-13´´´ C-14 C-14´ C-14´´ C-14´´´ C-15 C-15´); 144.42 (+, C-4); 96.72 (+, C-3); 87.45 (+, C-4'); 87.03 (C_{quart}, C-7); 86.59 (+, C-1'); 71.02 (+, C 3'); 62.88 (–, C-5'); 55.30, 55.32 (+, C-16 C-16´); 42.16 (–, C-2'); 24.97 (+, C-6); MS (EI): 571.2 $[M]^+$ (5), 528.2 $[M\text{-}C_2H_3O]^+$ (5), 303.1 $[DMTr]^+$ (100); FAB-MS: 572.2 $[M+H]^+$ (61), 303.1 $[DMTr]^+$ (100);

2´-Deoxy-*O*$^{5´}$-di-*p*-methoxytrityl-cytidine (312)

A solution of 1.00 g (1.76 mmol) of *N*^{4}-Actyl-2´-deoxy-*O*$^{5´}$-di-*p*-methoxytrityl cytidine **(311)** and 200 mg (1.45 mmol; 0.8 equiv.) of K_2CO_3 in 22 mL MeOH was stirred for 24 h. The colour of the solution turned to purple. The solvent was removed and the resulting yellow foam was partitioned between 30 mL $CHCl_3$ and 20 mL 5% aqueous $NaHCO_3$. The aqueous layer was extracted three times with 20 mL $CHCl_3$. After removal of the solvent, the crude product was purified by flash chromatography on silica gel ($CHCl_3$: MeOH (25 : 1) to $CHCl_3$: MeOH (10 : 1)). 730 mg of a white foam were obtained, giving 78% yield.

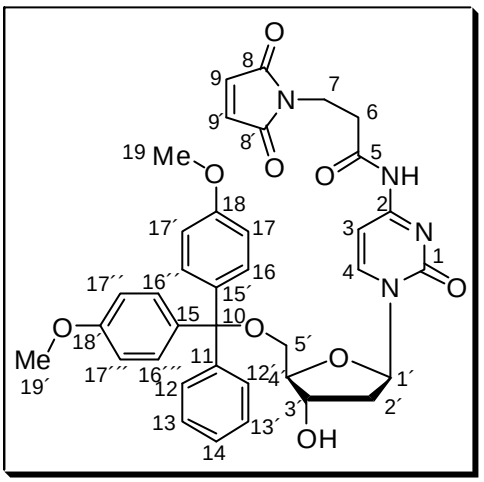


$C_{30}H_{31}N_3O_5$

R_f = 0.30 ($CHCl_3$: MeOH - 10 : 1); FAB-MS: 303.0 [trityl]$^+$(100), 530.1 [M + H]$^+$ (56), 552.1 [M + Na]$^+$ (20), 1059.5 [2 M + H]$^+$ (35).

2´-Deoxy-*O*$^{5´}$-di-*p*-methoxytrityl-*N*^{4}-(3-*N*-maleimidopropionyl)-amino-cytidine (313)

To a solution of 505 mg of 3-*N*-maleimidopropionic acid (2.99 mmol; 1.7 equiv.) in 18 mL CH_2Cl_2 was added 403 mg (2.99 mmol; 1.7 equiv.) of HOBt, 37 mg (299 µmol; 0.1 equiv.) of DMAP, and 616 mg (2.99 mmol; 1.7 equiv.) of 1,3-dicyclohexylcarbodiimide in 2 mL CH_2Cl_2. The solution was stirred for 2 h whereas the colour of the solution turns to red. The suspension was filtrated and the filtrate was directly passed into a solution of 930 mg (1.76 mmol; 1 equiv.) of **312** and 293 µl (2.90 mmol; 1.7 equiv.) of Et_3N in 80 mL CH_2Cl_2. The red solution was stirred for 120 min and was then washed with brine. The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed and the remaining solid was purified by flash chromatography on silica gel ($CHCl_3$: MeOH (15 : 1) to $CHCl_3$: MeOH (10 : 1)). 586 mg of a white foam were obtained, giving 49% yield.

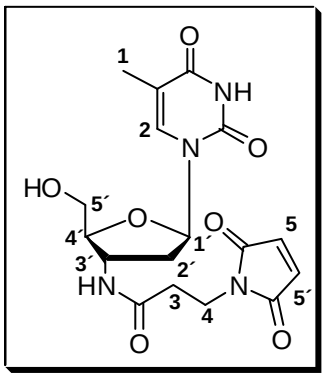


$C_{37}H_{36}N_4O_8$

R_f = 0.48 ($CHCl_3$: MeOH - 10 : 1); ^{1}H NMR (400 MHz, $CDCl_3$), δ 9.70 (bs, 1 H, N*H*), 8.11 (d, ^3J = 7.5 Hz, 1 H, 4-H), 7.34 – 6.72 (m, 14 H, (3-H 11-H to 17´´´-H)), 6.61 (s, 2 H, 9-H 9´-H), 6.17 (t, ^3J = 5.8 Hz, 1 H, 1´-H), 4.42 (dt, ^3J = 4.5 Hz, ^3J = 4.9 Hz, 1 H, 3´-H), 4.08 (dt, J_1 = 4.5 Hz, J_2 = 3.6 Hz,1 H, 4´-H), 3.80 (t, ^3J = 7.1 Hz, 2 H, 7-H), 3.71 (s, 6 H, 19-H 19´-H), 3.38 (dd, ^2J = 10.7 Hz, ^3J = 3.6 Hz, 1 H, 5´-Ha), 3.30 (dd, ^2J = 10.7 Hz, ^3J = 3.6 Hz, 1 H, 5´-Hb), 2.94 – 2.84 (m, 1 H, 2´-Ha), 2.79 – 2.68 (m, 2 H, 6-H), 2.19 – 2.11 (m, 1 H, 2´-Hb); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.6 (C_{quart}, C-8 C-8´); 162.5 (C_{quart}, C-2); 158.8 (C_{quart}, C-18 C-18´); 155.7 (C_{quart}, C-1); 144.7 (C_{quart}, C-11), 144.4 (+, C-4); 134.4 (C_{quart}, C-15 C-15´); 130.1, 130.2, 128.2, 128.1, 127.2 (C_{quart} and +, 9 C, C-12 C-12´ C-13 C-13´ C-14 C-16 C-16´ C-16´´ C-16´´´); 135.7, 135.6 (+, C-9 C-9´); 113.5 (+, C-17 C-17´ C-17´´ C-17´´´); 96.9 (+, C-3); 87.6 (+, C-4'); 87.0 (C_{quart}, C-10); 86.6 (+, C-1'); 71.0 (+, C 3'); 63.0 (–, C-5'); 55.4 (+, C-19 C-19´); 42.1 (–, C-2'); 35.8 (–, C-7); 33.6 (–, C-6); MS (ESI): 703.20 $[M + Na]^+$ (100), 1383.43 $[2 M + Na]^+$ (2).

3´-Deoxy-3´-(3-*N*-maleimidopropylamido)-thymidine (319)

57 mg (236 µmol) of aminothymidine **318** and 138 mg (523 µmol; 2.2 equiv.) of **196** were dissolved in 2 mL DMF and 91 µl of DiPEA were added under stirring. The solution was stirred for 16 h and the solvent was removed in HV. The mixture was purified by flash chromatography on silica gel. ($CHCl_3$: MeOH (10 : 1)) 32 mg of a white solid were obtained, giving 35% yield.



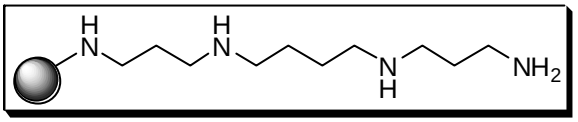
$C_{17}H_{20}N_4O_7$

R_f = 0.31 ($CHCl_3$: MeOH - 10 : 1); ^{1}H NMR (400 MHz, CD_3OD), δ 7.87 (s, 1 H, 2-H), 6.82 (s, 2 H, 5-H 5´-H), 6.19 (t, J = 6.3 Hz, 1 H, 1´-H), 4.42 (ddd, J_1 = 7.6 Hz, J_2 = 6.0 Hz, J_3 = 5.8 Hz, 1 H, 4´-H), 3.87 – 3.69 (m, 3 H, 3´-H 5´-H), 3.79 (t, J = 6.8 Hz, 2 H, 4-H), 2.47 (t, J = 6.8 Hz, 2 H, 3-H), 2.35 – 2.20 (m, 2 H, 2´-H), 1.89 (s, 3 H, 1-H); HRMS (ESI) [M + H]$^+$ calc. 393,1405 found 393,1401.

6.6.3.3 Coupling of spermine and maleimides via 2-iminothiolane (296)

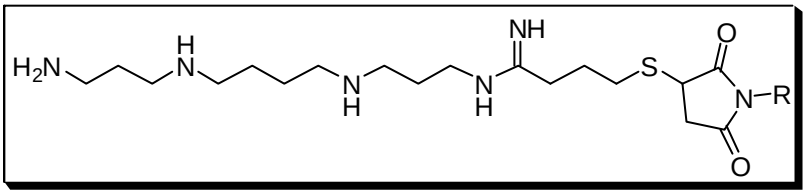
Synthesis of non cross-linked spermine on alkoxytrityl resin 61

1 g (0.7 mmol) of activated alkoxytrityl resin **61** were loaded with tris-Aloc-spermine **179** and 100 mg portions were Aloc-deprotected with $Pd(PPh_3)_4$ and *N*,*N*´-DMBA.



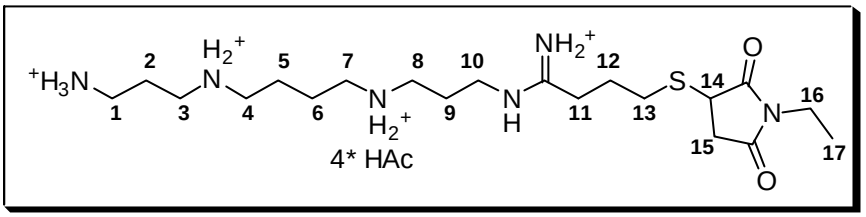
The ^{1}H NMR spectrum of the cleaved product was in accordance with those of protonated samples of commercially purchased spermine **(16)**.

General procedure



27 µmol of the resin **321** were swollen in 1 mL of THF for 10 min and 11 mg (81 µmol, 3 equiv.) of 2-iminothiolane **(296)** in 200 µl of water were added. The suspension was agitated for 2 min. 2 equiv. of the respective maleimide in 800 µl THF were added and it was agitated for 1 h. The resin was washed with water, THF / MeOH and CH_2Cl_2. The crude product was cleaved from the resin with 1% TFA in CH_2Cl_2. The filtrate was combined with the drained washings of CH_2Cl_2 and MeOH. In Entry 5 the 5'-DMTr group was removed by quick treatment of the resin with 1% TFA in dichloromethane and subsequent washing of the polymer with a minimal amount of CH_2Cl_2 / Et_2O (2 : 1) until the filtrate was clear. The product was washed from the resin with CH_2Cl_2 and MeOH. The solvents were removed and the remaining compound was purified by preparative HPLC on C_{18} column with gradients of eluant A (95% TEAA (0.01 M, pH 7.0) / 5% acetonitrile) and eluant B (5% TEAA (0.01 M, pH 7.0) / 95% acetonitrile) or with eluant A (95% water / 5% acetonitrile / 0.1% HOAc) and eluant B (5% water / 95% acetonitrile / 0.1% HOAc).

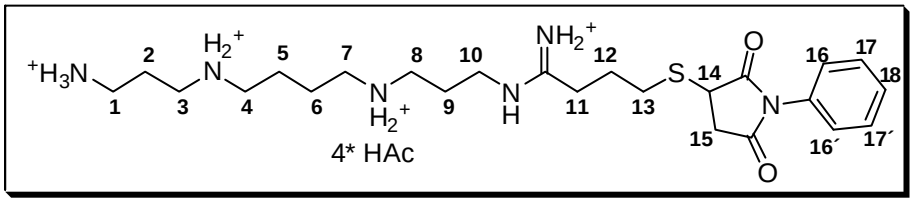
***N*^{1}-(4-thio-*S*-(1-ethylpyrrolidine-2,5-dione-3-yl)-butyramidinyl)-spermine**



$C_{28}H_{56}N_6O_{10}S$

^{1}H NMR (400 MHz, CD_3OD): δ = 3.90 (dd, J_1 = 9.1 Hz, J_2 = 3.9 Hz, 1 H, 14-H) 3.53 (q, J = 7.3 Hz, 2 H, 16-H), 3.40 (t, J = 7.1 Hz, 2 H, 10-H), 3.19 (dd, 1 H, J_1 = 18.5 Hz, J_2 = 9.1 Hz, 15-Hb), 3.00 – 3.15 (m, 10 H, 1-H 3-H 4-H 7-H 8-H), 2.98 (dt, J_1 = 13.6 Hz, J_2 = 7.1 Hz, 1 H, 13-Ha), 2.80 (dt, J_1 = 13.5 Hz, J_2 = 7.1 Hz, 1 H, 13-Hb), 2.62 (t, J = 7.7 Hz, 2 H, 11-H), 2.47 (dd, J_1 = 18.5 Hz, J_2 = 3.9 Hz, 1 H, 15-Hb), (2.13 (m, 2 H) and 2.06 (tt, J_1 = 7.7 Hz, J_2 = 7.1 Hz, 4 H, 12-H) and 2-H 9-H), 1.79 (m, 4 H, 5-H 6-H), 1.14 (t, J = 7.3 Hz, 3 H, 17-H); HRMS (ESI) $[M + H]^+$ calc. 429.3006, found 429.3010.

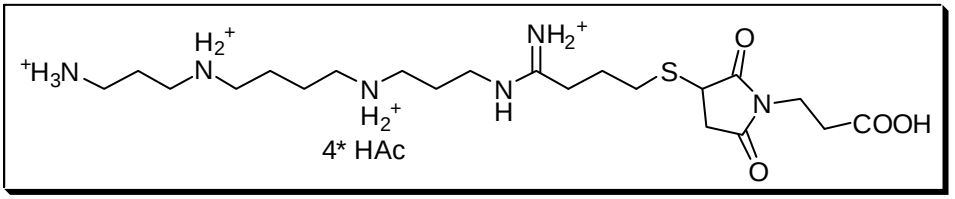
***N*^{1}-(4-thio-*S*-(1-phenylpyrrolidine-2,5-dione-3-yl)-butyramidinyl)-spermine**



$C_{32}H_{56}N_6O_{10}S$

^{1}H NMR (400 MHz, CD_3OD): δ = 7.54 – 7.26 (m, 5 H, 16-H to 18-H), 4.10 (dd, J_1 = 9.1 Hz, J_2 = 3.8 Hz, 1 H, 14-H), 3.40 – 3.32 (m, 1 H, 15-Ha), 3.39 (t, J = 6.5 Hz, 2 H, 10-H), 3.10 – 2.93 (m, 11 H, 1-H 3-H 4-H 7-H 8-H 15-Ha), 2.86 (dt, J_1 = 13.6 Hz, J_2 = 7.1 Hz, 1 H, 13-Hb), 2.70 – 2.60 (m, 3 H, 11-H 15-Hb), 2.14 – 1.96 (m, 6 H, 2-H 9-H 12-H), 1.79 (bm, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 477.3006, found 477.3002.

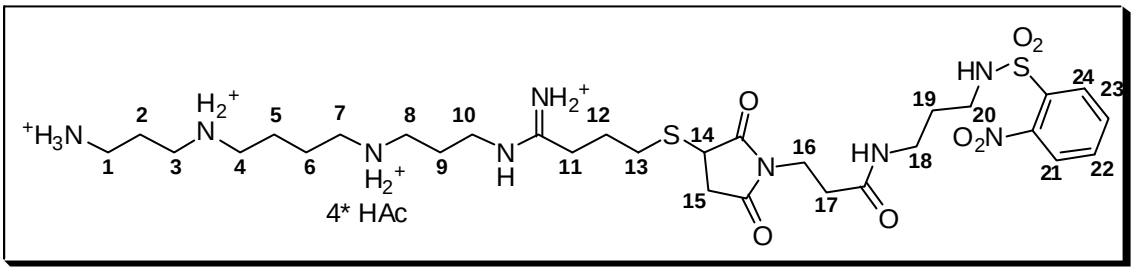
***N*^{1}-(4-thio-*S*-(1-(3-carboxypropyl)-pyrrolidine-2,5-dione-3-yl)-butyramidinyl)-spermine**



$C_{29}H_{56}N_6O_{12}S$

HRMS (ESI) $[M + H]^+$ calc. 473.2905, found 473.2907.

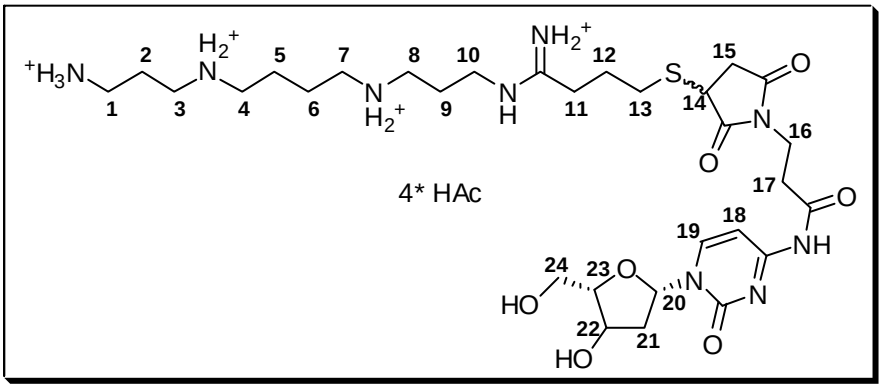
N^1-(4-thio-*S*-(1-(4-aza-3-oxyheptyl-7-*o*-Nosylsulfonamido)-pyrrolidine-2,5-dione-3-yl)-butyramidinyl)-spermine



$C_{38}H_{67}N_9O_{15}S_2$

^{1}H NMR (400 MHz, CD_3OD): δ = 8.11 – 7.80 (m, 4 H, 21-H 22-H 23-H 24-H), 3.90 (dd, J_1 = 9.2 Hz, J_2 = 3.8 Hz, 1 H, 14-H), 3.83 – 3.68 (m, 2 H, 16-H), 3.42 (t, J = 7.1 Hz, 2 H, 10-H), 3.04 – 3.24 (m, 15 H, 1-H 3-H 4-H 7-H 8-H 15-Ha 18-H 20-H), 2.93 (dt, J_1 = 13.9 Hz, J_2 = 6.9 Hz, 1 H, 13-Ha), 2.82 (dt, J_1 = 13.9 Hz, J_2 = 6.5 Hz, 1 H, 13-Hb), 2.70 – 2.44 (m, 4 H, 11-H 17-H), 2.50 (dd, J_1 = 17.9 Hz, J_2 = 3.8 Hz, 1 H, 15-Hb), 2.14 – 2.00 (bum, 6 H, 2-H 9-H 12-H), 1.88 – 1.73 (bum, 4 H, 5-H 6-H), 1.67 (tt; J_1 = 6.7 Hz, J_2 = 6.7 Hz, 2 H, 19-H); HRMS (ESI) $[M + 2 H]^{2+}$ calc. 357.6749, found 357.6736.

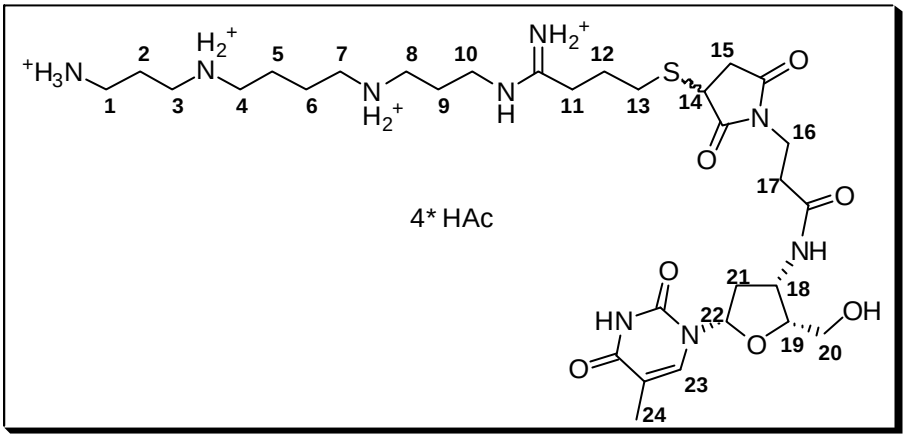
N^1-(4-thio-*S*-(1-(3-oxy-3-(N^4-(2´-deoxycytidine)-propyl)-pyrrolidine-2,5-dione-3-yl)-butyramidinyl)-spermine



$C_{59}H_{85}N_9O_{17}S$

^{1}H NMR (400 MHz, CD_3OD): δ = 8.39 (b, J = 7.8 Hz, 1 H, 19-H), 6.22 (m, 1 H, 20-H), 6.09 (d, J = 7.8 Hz, 1 H, 18-H), 4.39 (dt, J_1 = 6.8 Hz, J_2 = 6.5 Hz, 1 H, 23-H), 4.03 (dt, J_1 = 6.8 Hz, J_2 = 3.5 Hz, 1 H,22-H), 3.93 (dd, J_1 = 9.0 Hz, J_2 = 3.9 Hz, 1 H, 14-H), 3.90 – 3.70 (m, 4 H, 16-H 24-H), 3.42 (t, J = 6.9 Hz, 2 H, 10-H), 3.30 – 3.00 (m, 12 H, 1-H 3-H 4-H 7-H 8-H 13-Ha 15-Ha), 2.95 (dt, J_1 = 13.1 Hz, J_2 = 6.5 Hz, 1 H, 13-Hb), 2.81 (m, 2 H, 11-H), 2.68 – 2.58 (m, 3 H, 15-Hb 17-H), 2.50 (m, 1 H, 21-Ha), 2.21 (m, 1 H, 21-Hb), 2.18 – 2.00 (bum, 6 H, 2-H 9-H 12-H), 1.80 – 1.90 (m, 4 H, 5-H 6-H); MS (ESI): 682.36 $[M + H]^+$, MS (FAB pos.): 682.4 $[M + H]^+$.

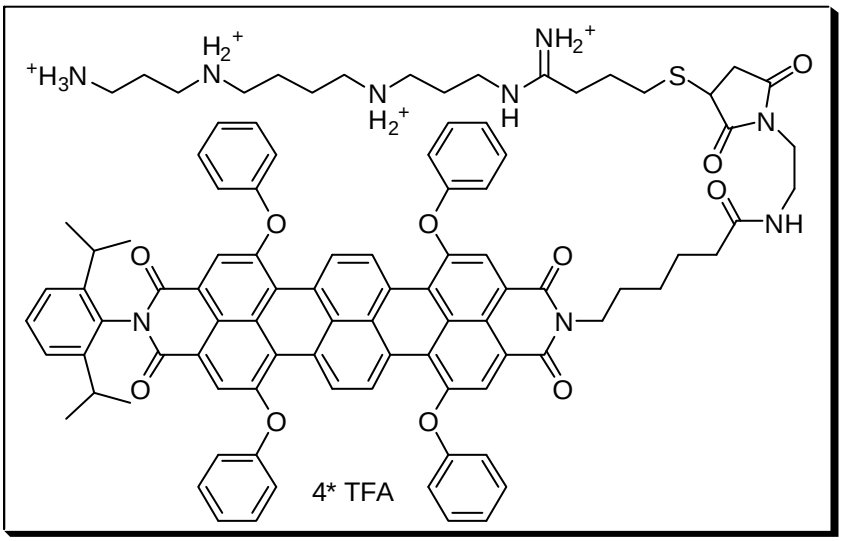
***N*[1]-(4-thio-*S*-(1-(3-oxy-3-(3´-(3´-deoxy-3´-aminothymidine))-propyl)-pyrrolidine-2,5-dione-3-yl)-butyramidinyl)-spermine**



$C_{39}H_{69}N_9O_{15}S$

^{1}H NMR (400 MHz, CD_3OD): δ = 7.87 (s, 1 H, 23-H), 6.21 (m, 1 H, 18-H), 4.42 (dt, J_1 = 7.1 Hz, J_2 = 6.4, 1 H, 19-H), 3.97 – 3.70 (m, 5 H, 16-H 18-H 20-H), 3.45 – 3.37 (m, 2 H, 10-H), 3.28 – 2.91 (m, 12 H, 1-H 3-H 4-H 7-H 8-H 13-Ha 15-Ha), 2.81 (dt, J_1 = 13.3 Hz, J_2 = 7.2 Hz, 1 H, 13-Hb), 2.63 (t, J = 7.6 Hz, 2 H, 11-H), 2.55 – 2.45 (m, 3 H, 15-Hb 17-H), 2.40 – 2.25 (m, 2 H, 21-H), 2.18- 2.00 (m, 6 H, 2-H 9-H 12-H), 1.89 (s, 3 H, 24-H), 1.85 – 1.70 (m, 4 H, 5-H 6-H); HRMS (ESI) $[M + H]^+$ calc. 696.3861, found 696.3859.

´Radical scavenger´-adduct



$C_{104}H_{99}N_9O_{19}F_{12}S$

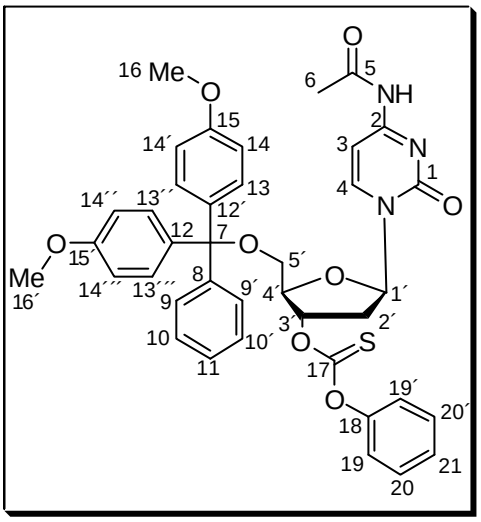
Entry 7: MS (MALDI pos.): 1583.16 $[M + H]^+$; HRMS (ESI) $[M + 2\ H]^{2+}$ calc. 791.8509, found 791.8456.

6.6.4 Preparation of polyamino-nucleotides

6.6.4.1 Synthesis of maleimido modified 2´,3´-deoxycytidine

***N*^{4}-Acetyl-2´deoxy-*O*$^{5´}$-di-*p*-methoxytrityl-*O*$^{3´}$-phenoxythiocarbonyl cytidine (327)**

To a solution of 8.03 g of **311** (14.1 mmol) in 145 mL CH_3CN was added 2.65 g of DMAP (27.8 mmol; 2.0 equiv.) and 2.3 mL *O*-phenyl chlorothionoformiate (17.0 mmol; 1.2 equiv.). The solution was stirred for 20 h and the solvent was evaporated. The residue was partitioned between 90 mL $CHCl_3$ and 100 mL 5% aqueous $NaHCO_3$ and the aqueous layer was extracted three times with 70 mL $CHCl_3$. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure at temperatures lower than 32 °C. The crude product purified by flash chromatography on silica gel ($CHCl_3$ to $CHCl_3$: MeOH (10 : 1)). 3.23 g (4.56 mmol) of a yellow foam were obtained, giving 32% yield.



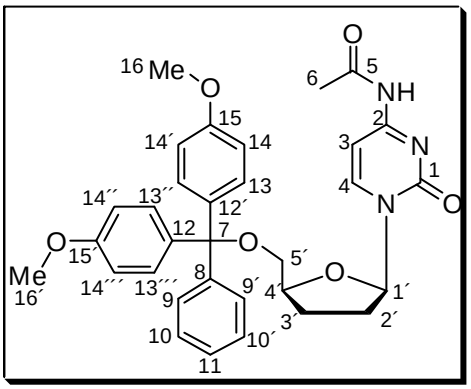
$C_{39}H_{37}N_3O_8S$

R_f = 0.57 ($CHCl_3$: MeOH - 25 : 1); ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm) 8.02 (d, J = 7.6 Hz, 1 H, 4-H), 6.78 – 7.43 (m, 19 H, (3-H 8-H to 14´´´-H) (19-H to 21-H)), 6.32 (dd, ^3J = 7.7 Hz, ^3J = 5.9 Hz, 1 H, 1´-H), 5.86 (d, ^3J = 6.0 Hz, 1 H, 3´-H), 4.51 (m, 1 H, 4´-H), 3.75 (s, 6 H, 16-H 16´-H), 3.57 (dd, ^2J = 10.8 Hz, ^3J = 3.4 Hz, 1 H, 5´-Ha), 3.49 (dd, ^2J = 10.8 Hz, ^3J = 2.6 Hz, 1 H, 5´-Hb), 3.07 (dd, ^2J = 14.7 Hz, ^3J = 5.9 Hz, 1 H, 2´-Ha), 2.42 (m, 1 H, 2´-H), 2.23 (s, 3 H, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 194.31 (C_{quart}, C-17); 170.74 (C_{quart}, C-5); 162.74 (C_{quart}, C-2); 155.23 (C_{quart}, C-1); 158.86, 158.74, 144.40, 135.22, 135.08, 130.17, 130.14, 129.26, 128.21, 128.13, 127.97, 127.90, 127.31, 127.19, 126.94, 113.53, 113.51, 113.29 (C_{quart} and +, 19 C, C-8 C-9 C-9´ C-10 C-10´ C-11 C-12 C-12´ C-13 C-13´ C-13´´ C-13´´´ C-14 C-14´ C-14´´ C-14´´´ C-15 C-15´ C-18); 153.42, 129.78, 129.68, 121.93, 115.56 (C_{quart} and +, 5 C, C-19 C-19´ C-20 C-20´ C-21); 144.14 (1 C, C-4); 96.79 (+, C-3); 87.58 (+, C-4´); 87.40 (C_{quart}, C-7); 84.87 (+, C-1´); 84.07 (+, C-3´); 63.69 (–, C-5´); 55.39 (+, C-16 C-16´); 39.64 (–, C-2´); 25.09 (+, C-6); MS (ESI): 554.24 [M – (Base + Ac)]$^+$ (9), 676.32 [M – OCH_3]$^+$ (100), 730.23 [M + Na]$^+$ (35), 1437.46 [2 M + Na]$^+$ (3).

***N*^{4}-Acetyl-2´,3´-dideoxy-*O*$^{5´}$-di-*p*-methoxytrityl-cytidine (328)**

A solution of 1.00 g of **327** (1.41 mmol) in 190 mL anhydrous toluene was heated to reflux and a mixture of 1.15 mL (4.24 mmol; 3 equiv.) of tributyltinhydride in 2 mL toluene and 89 mg (542 µmol; 0.4 equiv.) of 2,2'-azobisisobutylnitrile in 8 mL toluene was added drop wise during 10 min. After heating to reflux for 40 min, the solution was cooled down to room temperature and the solvent was removed. The resulting residue was partitioned between 30 mL of $CHCl_3$ and 30 mL of 5% aqueous $NaHCO_3$. The aqueous phase was extracted three times with 30 mL $CHCl_3$ and the organic phase was dried over Na_2SO_4. The solvent was removed and the supernatant liquid tinorganyles were removed from the precipitates by decantation. The remaining solid was purified by flash chromatography on silica gel ($CHCl_3$ to $CHCl_3$: MeOH (15 : 1)). 462 mg of a white foam were obtained, giving 59% yield.

If both steps were carried out consecutively starting from 2.9 g of **311**, the overall yield was 46% over two steps. The intermediary thiocarbonate **327** was not purified by flash chromatography. However, the DMAP and the remains of *O*-phenyl chlorothionoformiate were removed by separating the crude product between solutions of dichloromethane and water.

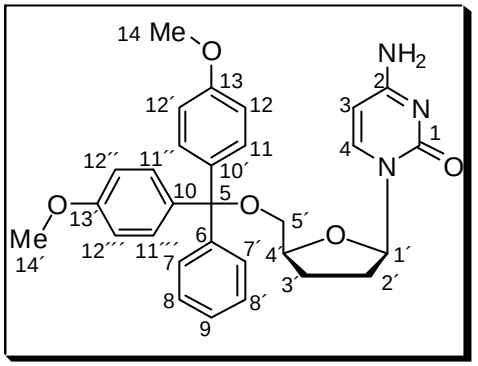


$C_{32}H_{33}N_3O_6$

R_f = 0.27 ($CHCl_3$: MeOH - 15 : 1); ^{1}H NMR (400 MHz, $CDCl_3$), δ 9.59 (bs, 1 H, N*H*), 8.39 (d, ^3J = 7.5 Hz, 1 H, 4-H), 7.06 (d, ^3J = 7.5 Hz, 1 H, 3-H), 6.75 – 7.37 (m, 13 H, (8-H to 14´´´-H)), 6.02 (dd, ^3J = 6.6 Hz, ^3J = 1.6 Hz, 1 H, 1´-H), 4.21 (m, 1 H, 4´-H), 3.74 (s, 6 H, 16-H 16´-H), 3.49 (dd, ^2J = 11.0 Hz, ^3J = 2.6 Hz, 1 H, 5´-Ha), 3.28 (dd, ^2J = 11.0 Hz, ^3J = 3.9 Hz, 1 H, 5´-Hb), 2.43 (m, 1 H, 2´-Ha), 2.19 (s, 3 H, 6-H), 2.11 (m, 1 H, 2´-Hb), 1.87 (m, 2 H, 3´-H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 170.83 (C_{quart}, C-5); 162.61 (C_{quart}, C-2); 155.04 (C_{quart}, C-1); 158.79, 158.73, 144.47, 135.68, 135.53, 130.23, 130.19, 129.25, 128.27, 128.12, 127.94, 127.89, 127.22, 127.16, 113.40, 113.26 (C_{quart} and +, 18 C, C-8 C-9 C-9´ C-10 C-10´ C-11 C-12 C-12´ C-13 C-13´ C-13´´ C-13´´´ C-14 C-14´ C-14´´ C-14´´´ C-15 C-15´); 145.29 (+, C-4); 96.13 (+, C-3); 88.12 (+, C-1´); 86.91 (C_{quart}, C-7); 82.14 (+, C-4´); 63.66 (–, C-5´); 55.37 (+, C-16 C-16´); 33.82 (–, C-2´); 24.99 (–, C-3´); 24.73 (+, C-6); MS (ESI): 425.17 $[M - Base + Na]^+$ (24), 556.25 $[M + H]^+$ (2), 578.21 $[M + Na]^+$ (100), 1133.43 $[2 M + Na]^+$ (31).

2´,3´-Dideoxy-*O*$^{5´}$-di-*p*-methoxytrityl-cytidine (329)

A solution of 462 mg (832 µmol) **328** and 81 mg (582 µmol; 0.7 equiv.) of K_2CO_3 in 10.4 mL MeOH was stirred for 18 h. The solvent was removed in vacuum and the resulting yellow foam was partitioned between $CHCl_3$ and 5% aqueous $NaHCO_3$. The aqueous layer was extracted three times with $CHCl_3$ and the organic phase was dried over Na_2SO_4. After removal of the solvent, the crude product was purified by flash chromatography on silica gel ($CHCl_3$ to $CHCl_3$: MeOH (1 : 1)). 830 mg of a white foam were obtained, giving 91% yield.

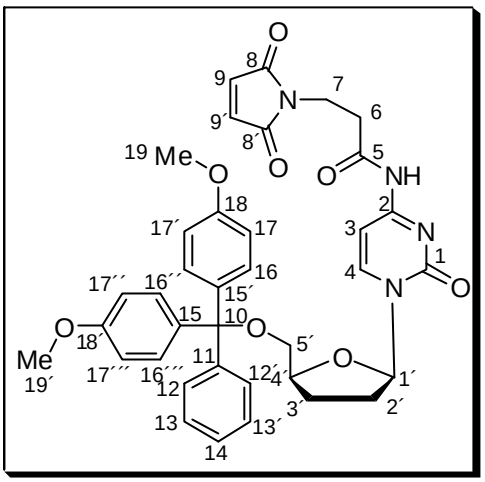


$C_{30}H_{31}N_3O_5$

R_f = 0.36 ($CHCl_3$: MeOH - 15 : 1); ^{1}H NMR (400 MHz, $CDCl_3$), δ 7.91 (bs, 1 H, N*H*), 7.57 (d, ^3J = 7.6 Hz, 1 H, 4-H), 6.64 – 7.34 (m, 13 H, (6-H to 12´´´-H)), 6.09 (t, ^3J = 6.1 Hz, 1 H, 1´-H), 5.44 (d, ^3J = 7.6 Hz, 1 H, 3-H), 4.36 (dt, ^3J = 4.2 Hz, ^3J = 5.6 Hz, 1 H, 3´-H), 3.93 (dt, ^3J = 4.2 Hz, ^3J = 3.1 Hz, 1 H, 4´-H), 3.68 (s, 6 H, 14-H 14´-H), 3.28 (d, ^3J = 3.1 Hz, 2 H, 5´-H), 2.44 (ddd, ^3J = 5.6 Hz, ^3J = 6.1 Hz, ^3J = 13.6 Hz, 1 H, 2´-Ha), 2.12 (ddd, ^3J = 5.6 Hz, ^3J = 6.1 Hz, ^3J = 13.6 Hz, 1 H, 2´-Hb); ^{13}C NMR (100 MHz, $CDCl_3$), δ 165.78 (C_{quart}, C-2); 158.70, 144.68, 135.80, 135.71 (C_{quart}, 5 C, C-13 C-13´ C-6 C-10 C-10´); 156.32 (C_{quart}, C-1); 130.23, 128.30, 128.05, 127.09, 113.38 (+, 13 C, C-7 C-7´ C-8 C-8´ C-9 C-11 C-11´ C-11´´ C-11´´´ C-12 C-12´ C-12´´ C-12´´´); 141.28 (+, C-4); 94.68 (+, C-3); 86.79 (+, C-4´); 86.37 (C_{quart}, C-5); 86.19 (+, C-1´); 71.52 (–, C-3´); 63.43 (–, C-5´); 55.47 (+, C-14 C-14´); 41.94 (–, C-2´); MS (ESI): 530.2 $[M + H]^+$ (100), 552.1 $[M + Na]^+$ (100), 1059.6 $[2 M + H]^+$ (24).

2´,3´-Dideoxy-*O*$^{5´}$-di-*p*-methoxytrityl-*N*^{4}-(3-*N*-maleimidopropionyl)-amino-cytidine (330)

To a solution of 213 mg of 3-*N*-maleimidopropionic acid (1.26 mmol; 1.7 equiv.) in 8 mL CH_2Cl_2 was added 170 mg (1.26 mmol; 1.7 equiv.) of HOBt, 15 mg (75 µmol; 0.2 equiv.) of DMAP, and 260 mg (1.13 mmol; 1.7 equiv.) of 1,3-dicyclohexylcarbodiimide. The solution was stirred for 30 min whereas the colour of the solution turns to pink. The suspension was filtrated and the filtrate was directly given into a solution of 380 mg (743 µmol; 1 equiv.) of **329** and 17 µL (1.26 mmol; 1.7 equiv.) of Et_3N in 12 mL CH_2Cl_2. The red solution was stirred for 90 min and was then washed with brine. The aqueous layer was extracted three times with CH_2Cl_2 and the combined organic layers were dried over Na_2SO_4. The solvent was removed and the remaining solid was purified by flash chromatography on silica gel (cyclohexane : EtOAc (3 : 1) to cyclohexane : EtOAc (1 : 1)). 335 mg of a white foam were obtained, giving 68% yield.

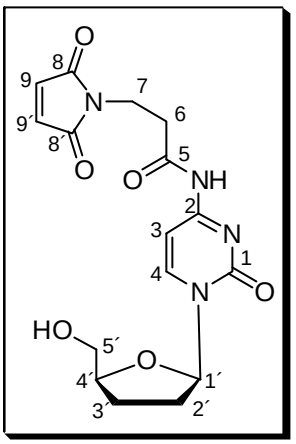


$C_{37}H_{36}N_4O_8$

R_f = 0.13 ($CHCl_3$: MeOH - 20 : 1); ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.19 (d, ^3J = 7.5 Hz, 1 H, 4-H), 6.78 – 7.44 (m, 14 H, 3-H (11-H to 17´´´-H)), 6.69 (s, 2 H, 9-H 9´-H), 5.99 (t, ^3J = 5.8 Hz, 1 H, 1´-H), 4.44 (dt, ^3J = 4.6 Hz, ^3J = 5.1 Hz, 2 H, 3´-H), 4.11 (dt, J_1 = 4.6 Hz, J_2 = 3.6 Hz,1 H, 4´-H), 3.87 (t, ^3J = 7.0 Hz, 2 H, 7-H), 3.79 (s, 6 H, 19-H 19´-H), 3.46 (dd, ^2J = 10.8 Hz, ^3J = 3.6 Hz, 1 H, 5´-Ha), 3.37 (dd, ^2J = 10.8 Hz, ^3J = 3.6 Hz, 1 H, 5´-Hb), 2.77 (m, 2 H, 6-H), 2.65 (ddd, ^3J = 13.6, ^3J = 5.8, ^3J = 5.3, 1 H, 2´-Ha), 2.18 (dt, ^3J = 13.6, ^3J = 5.8, 1 H, 2´-Hb); ^{13}C NMR (100 MHz, $CDCl_3$), δ 162.49 (C_{quart}, C-2); 158.79, 144.39, 135.69, 135.55 (C_{quart}, 5 C, C-18 C-18´ C-15 C-15´ C-11); 155.72 (C_{quart}, C-1); 130.18, 130.13, 129.26, 128.26, 128.12, 127.20, 113.45 (+, 13 C, C-12 C-12´ C-13 C-13´ C-14 C-16 C-16´ C-16´´ C-16´´´ C-17 C-17´ C-17´´ C-17´´´); 144.74 (+, C-4); 134.39 (+, C-9 C-9´); 96.93 (+, C-3); 87.56 (+, C-4´); 87.00 (C_{quart}, C-10); 86.60 (+, C-1´); 71.07 (–, C-3´); 63.01 (–, C-5´); 55.38 (+, C-19 C-19´); 42.12 (–, C-2´); 35.77 (–, C-7); 33.65 (–, C-6); MS (ESI): 703.20 $[M + Na]^+$ (100), 1383.43 $[2 M + Na]^+$ (24).

2´,3´-Dideoxy-*N*^{4}-(3-*N*-maleimidopropionyl)-cytidine (331)

100 mg (152 µmol) of **330** were dissolved in 10 mL CH_2Cl_2 and during the addition of 0.1 mL of TFA, the colour of the solution turns to red. Stirring was continued for 1 min and 4 mL of a 5% aqueous solution of $NaHCO_3$ was added. The aqueous layer was six times extracted with $CHCl_3$ and the solvent was removed in vacuum at temperature lower than 30 °C. The remaining foam was purified by flash chromatography on silica gel. ($CHCl_3$ to $CHCl_3$: MeOH (10 : 1)) 42 mg of a white foam were obtained, giving 77% yield. Due to its instability the compound was only prepared in small amounts and quickly inserted into the next step.



$C_{16}H_{18}N_4O_6$

R_f = 0.16 ($CHCl_3$: MeOH - 12 : 1); ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.47 (d, ^3J = 7.4 Hz, 1 H, 4-H), 7.33 (d, ^3J = 7.4 Hz, 1 H, 3-H), 6.68 (s, 2 H, 9-H 9´-H), 6.03 (dd, $^3J_1$ = 6.7 Hz, $^3J_2$ = 2.0 Hz, 1 H, 1´-H), 4.25 (ddt, $^3J_1$ = 6.4 Hz, $^3J_2$ = 6.4 Hz, $^3J_3$ = 3.1 Hz, 1 H, 4´-H), 4.06 (dd, J_1 = 12.2 Hz, J_2 = 3.1 Hz,1 H, 5´-Ha), 3.88 (t, ^3J = 6.8 Hz, 2H, 7-H), 3.77 (dd, ^2J = 12.2 Hz, ^3J = 3.1 Hz, 1H, 5´-Hb), 2.88 (t, J = 6.8 Hz, 2H, 6-H), 2.56 – 2.44 (m, 1H, 2´-Ha), 2.23 – 2.15 (m, 1 H, 2´-Hb), 1.93 (dt, ^3J = 6.4, ^3J = 3.7, 1 H, 3´-H).

6.7 Solubility of spermine porphyrin 217 in aqueous media

In order to probe whether unspecific aggregation occurs with the transporter, porphyrin solutions of different concentrations were studied both in freshly distilled methylene chloride (from calcium hydride) and in deionised water. The aqueous solution was prepared by dissolving 2.0 mg of construct 6 in 200 ± 10 µL 2-propanol and diluting it into 10 ml water. Short sonication yielded a transparent yellow-orange solution.

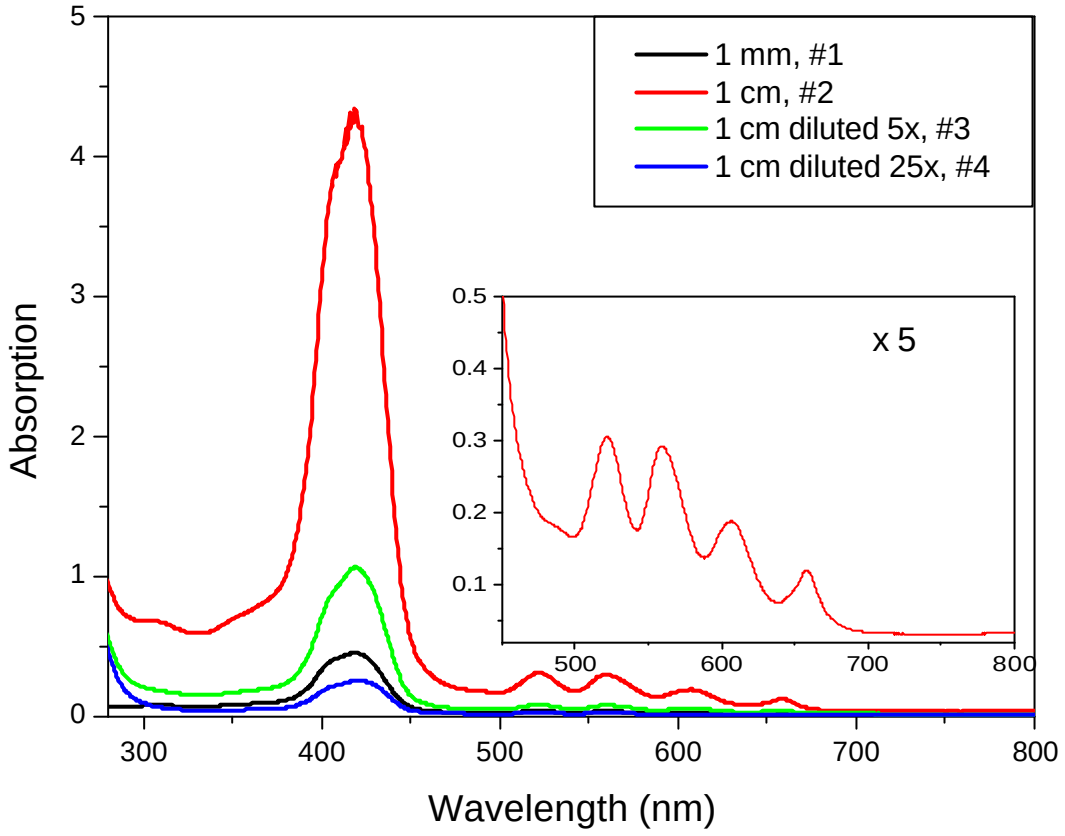


Figure 72 Absorption spectra in 2% (v / v) aqueous 2-propanol. Black trace was recorded in a 1 mm quartz cuvette, while the red traces present the same solution in a 1 cm quartz cuvette. The green and blue traces were obtained after approximately five time dilutions successively, respectively. Note that there are no changes in the blue shoulder of the Soret band or in the Q bands upon dilution indicating absence of aggregation. Absorption spectra were measured on a Cary 500 UV-NIR spectrometer at room temperature.

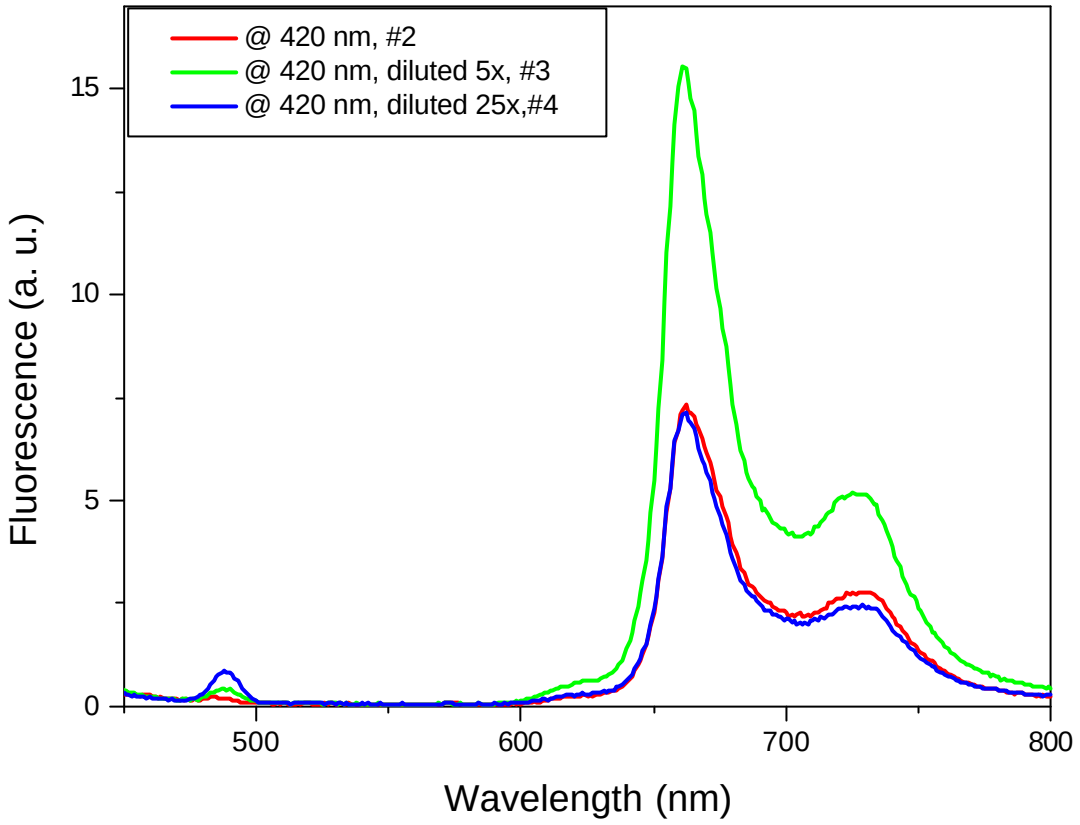


Figure 73 Stationary emission spectra in 2% (v / v) aqueous 2-propanol. The same solutions as in Figure 72 were measured with the excitation wavelength fixed at the Soret maximum at 420 nm. Measurements were performed on a Cary Eclipse fluorimeter in a right angle geometry. Note that some concentration quenching occurs in the more concentrated solution (#2, red trace) in comparison to the more dilute solutions.

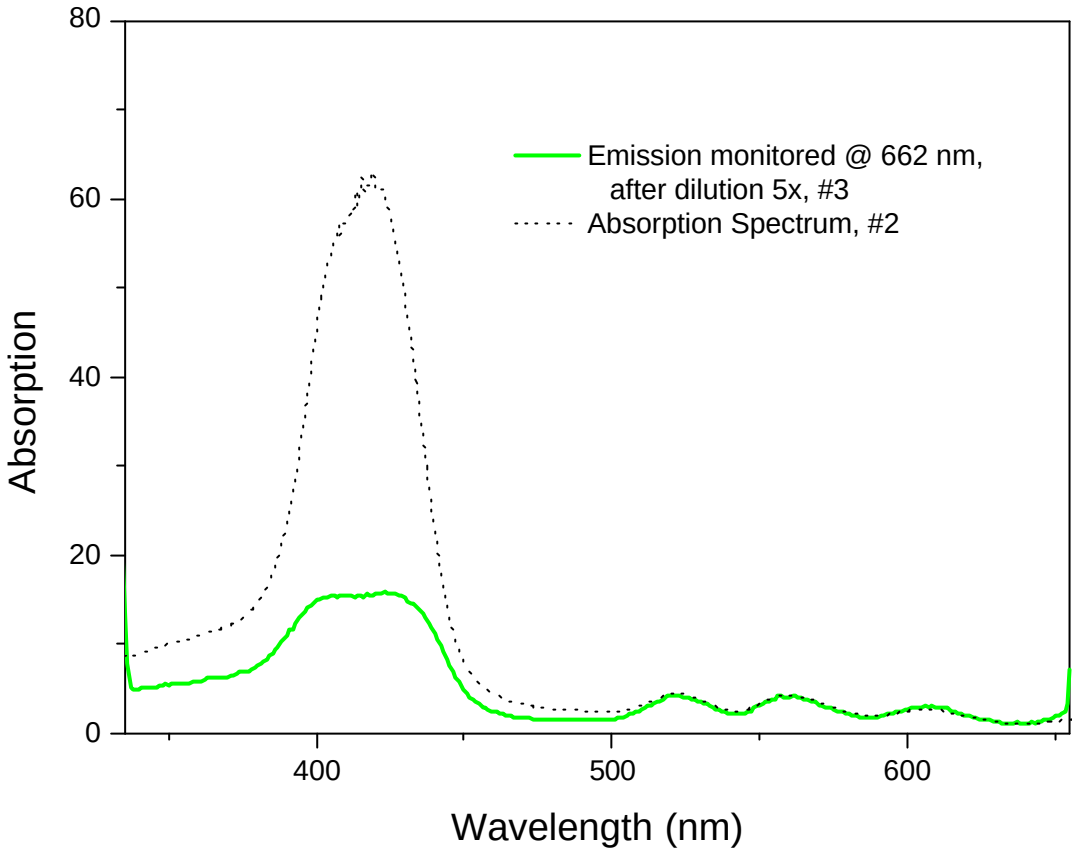


Figure 74 Comparison of the absorption spectrum of solution #2 from Figure 72 and the fluorescence excitation spectrum with emission monitored at 662 nm. Note that due to the absorption in the somewhat split Soret band, very little light passes through the sample to the detector. However in the Q band region, a perfect superposition of the absorption and fluorescence excitation spectrum is observed indicating a normal behaviour of the spermidine-porphyrin **217** in this solution. It was assumed that under physiological conditions and by using methanol instead of 2-propanol, a similar behaviour is encountered.

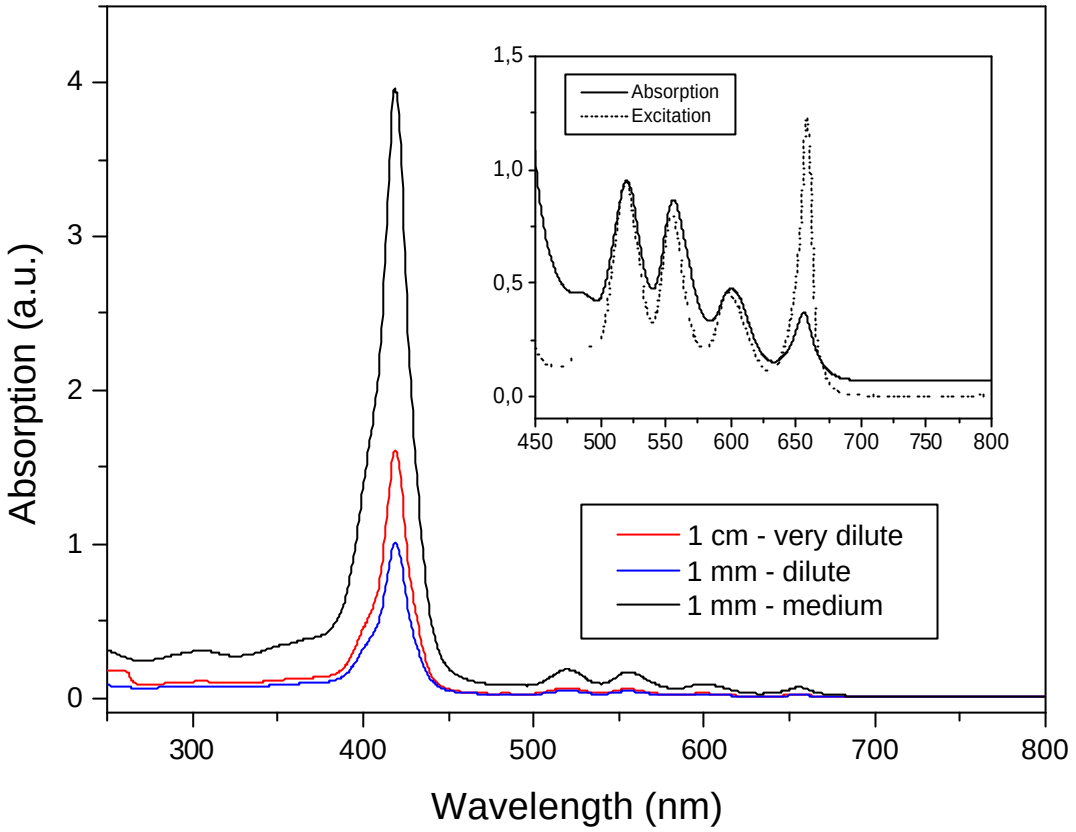


Figure 75 Absorption spectra in dry methylene chloride at three different concentrations. In the inset is presented the comparison between the absorption and the fluorescence excitation spectrum. As the emission was monitored at 652 nm, this explains the very intense last Q band. Note that apart from a slight blue shoulder of the Soret band, which also is less pronounced than in aqueous solution, there are no abnormal behaviours encountered.

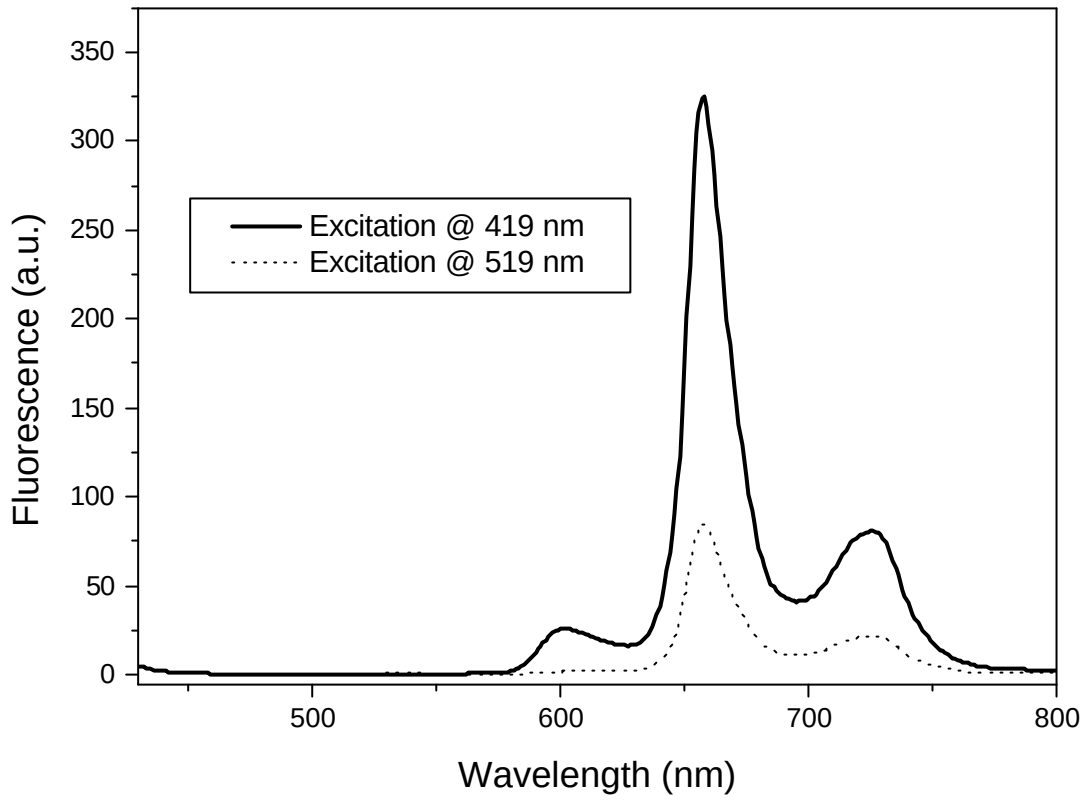


Figure 76 Emission spectra in dry methylene chloride with the excitation wavelength set at the Soret and the Q_x maxima, respectively.

6.8 Fluorescence microscopy

6.8.1 Cell culture techniques for mammalian cells

All procedures with mammalian cells are carried out under sterile conditions. 10^4 – 10^5 HeLa (human cervix carcinoma) cells, COS-7 cells, human primary fibroblast were plated into each well of an 8-well Lab-Tek TM II chambered cover slide (Nalgene, Neerijse, Belgium) or 8-well slide from IBIDI, Germany, and cultured in 200 µl of Dulbecco's modified Eagle's medium, high glucose, (DMEM, Sigma Taufkirchen) supplemented with 10% fetal calf serum (FCS, PAA), and 1 u / ml Penicillin / Streptomycin at 37 °C, 5% CO_2.

6.8.2 Treatment of adherent cells with fluorophore derivatives

The spermine porphyrin **217** and the lipophilic NBD-derivatives were dissolved in methanol to yield a 20 mM / 10 mM stock solution and further diluted with 10% DMEM to yield the respective incubation media. To ensure equal incubation conditions, all dilutions contain equal amounts of methanol. The carboxyporphyrin **215** was dissolved in methanol to yield a stock solution of 18 µM and further diluted with 10% DMEM to yield the respective incubation media. The polar NBD- and rhodamine conjugates were dissolved in 50% EtOH in H_2O to yield 1 mM stock solutions and were further diluted with 10% DMEM. 10^4 – 10^5 HeLa (human cervix carcinoma) cells, HepG2 (human liver carcinoma) cells, COS-7 cells, and human primary fibroblast were plated into each well of an 8-well Lab-Tek TM II chambered cover slide (Nalge Nunc, Neerijse, Belgium) and cultured in 200 µl of Dulbecco's modified Eagle's medium, high glucose, (DMEM, Sigma Taufkirchen) supplemented with 10% fetal calf serum (FCS, PAA), and 1% Penicillin / Streptomycin at 37 °C, 5% CO_2. After the cells reach a confluency of about 80% the culture medium was removed and replaced by 0.2 ml of the respective fluorophore supplemented media. Cellular uptake of all compounds, was measured by live cell imaging, since fixation would alter the intracellular distribution as described for other polycationic species.

6.8.3 Fluorescence and confocal microscopy

Cellular uptake of the conjugates was measured after incubation by live imaging using a fluorescence microscope (Zeiss Axiovert 35, filter: Bp 546 FT 580, LP 590). For confocal microscopy cellular uptake was visualized using a confocal microscope (Zeiss Axiovert 200) fitted with a Zeiss LSM 510 Laser Module (argon, 458 / 488 / 514 nm; HeNe, 543 / 633 nm). Images were collected with the AxioCam MRc and the images were visualized with the AxioVision 3.1 and the LSM imaging software as well as the LSM Image Browser software version 3.1.0.99.

6.8.4 Fluorometric cellular uptake measurements

HeLa cells were plated in black flat bottom 96 well plates at a density of 10^4 cells / well and incubated for 24 h at cell culture conditions. Eventually, the cells were treated with 0.5, 1, 5, and 10 µM of spermine porphyrin **217** and 5 µM of carboxy porphyrin **215** (final concentration in DMEM medium) and incubated for 4, 16, 24, and 48 h. After incubation, the medium was removed and the cells were washed with phosphate buffered saline (PBS). The

cells were lysed with 100 µl of 1 * RIPA buffer (including Complete protease inhibitors, Roche, Mannheim) and the fluorescence was measured using a Thermo Scientific Varioskan plate reader at an excitation / emission wavelength of 420 / 650 nm. After the measurement, the protein concentration was quantified.

6.8.5 Subcellular Localization

For the intracellular localization of labelled conjugates, the cells were co-incubated with fluorescent probes (Molecular Probes, Karlsruhe) for different intracellular compartments: For endosomal / lysosomal labelling: 150 nM Lysotracker®Green DND 26 or Lysotracker®Red for 30 min, 4 h, and 24 h prior to examination; for mitochondria labelling: 1 µM Mitotracker®Green FM for 1 h or 1 µM Mitotracker®Red FM for 30 min prior to examination; For Golgi apparatus: BSA complex of NBD-C_6-ceramide (5 µM), For nucleus labelling: Hoechst 33342 (Invitrogen, Karlsruhe) according to the manufacturers manual.

6.8.6 Colony-forming Assay

The colony-forming assay was performed as it has been previously reported. After photo-irradiation or illumination of human primary fibroblasts, co-incubated with gram (-) and gram (+) bacteria 100 µl, aliquots were removed from the medium for the colony-forming assay, serially diluted, and streaked on agar plates as described in [416, 417]. The plates were incubated in the dark to avoid further photo-irradiation. Controls were bacteria treated with **217** without illumination. The treatment with 1 µM of **217** and illumination for 15 min at 650 nm revealed no colonies after 24 h. Likewise, 100 µl of fresh DMEM supplemented with 10% fetal calf serum in absence of antibiotics were added to the cells to investigate the re-growth of bacteria overnight. After 24 h – 72 h of co-incubation no re-growth of the bacteria could be observed, while the cells were proliferating.

6.9 Toxicological assays

6.9.1 Measurement of cell death rate after illumination at 650 – 660 nm

HeLa (human cervix carcinoma) cells, COS-7 cells, and human primary fibroblast were plated into an 8-well Lab-Tek TM II chambered cover glass (10^4 – 10^5 cells / well) and incubated with 0,01, 0.1, 1, 5, 20, 50, 100 µM spermine porphyrin **217** and 5 µM carboxyporphyrin **215** in 200µl DMEM, high glucose, supplemented with 10% fetal calf serum (FCS, PAA), and 1% Penicillin / Streptomycin at 37 °C, 5% CO_2 for 4 h and 24 h, respectively. Eventually, the cells were washed with PBS and the medium was replaced by DMEM, high glucose without phenol red (Sigma, Taufkirchen), supplemented with 10% fetal calf serum (FCS, PAA). To induce photodynamic destruction the cells were irradiated with light of a 650 + / – 10 nm diode laser at a dose rate of 30 – 42 Mw / cm^2 giving total fluency rate of 10 – 13 J / cm^2. After exposure with light for 5 min, 10 min, 15 min, 30 min, 2 h, 4 h, respectively, the cells were stained with trypan blue and propidium iodide and visualized using a Zeiss Axiovert 35 (filter: Bp 546 FT 580, LP 590) microscope. Necrotic and dead cells were counted in comparison to intact cells.

6.9.2 MTT-assay

The dark toxicity was estimated indirectly by determination of the viability of the cells. HeLa cells and human primary fibroblasts were plated in a Greiner 96 well plate at a density of 3000 cells / well. The cells were incubated with increasing concentrations (1 – 100 µM) of the compounds at 37 °C and the viability was determined after 24, 48, and 72 h using the CellTiter96 viability test (Promega). It is performed by adding the premixed, Dye Solution to culture wells of a 96-well plate, containing various concentrations of test substance. During a 4-hour incubation, living cells convert the MTT tetrazolium component of the Dye Solution into a formazan product. The Stop Solution is then added to the culture wells to solubilise the formazan product, and the absorbance at 570nm is recorded using a 96-well plate reader. The viability was measured by quantification of the absorbance at 570 nm using a Thermofisher Scientific Varioskan plate reader. The signal was normalized to 100% from untreated cells and 0% from lysed cells.

6.9.3 Sulforhodamine B Test

The test was executed in analogy to literature procedures.[411, 412] HT29 cells and A431 cells were plated in a 24 well plate at a density of 5000 HT29 cells / well or 12000 A431 cells / well, respectively. The cells were incubated with **213** and **229** in FKS-containing medium at 37 °C and 5% CO_2. A solution of 1% DMSO in FKS medium served as control experiment. After 72 h, 100 µl of 50% trichloracetic acid were added to stop the reaction. The cells were left for at least 1 h at 4 °C. All wells were washed four times with H_2O_{bidest} to remove cell rests and supernatant medium. The pates were dried and the cells were coloured by 30 min treatment with 250 µl of a 0.4% SRB solution. The cells were left under light exclusion for at least 30 min. The dye was removed from the cells with washings of H_2O_{bidest} and 1% acetic acid in H_2O (two times each). After drying, the dye was dissolved in Tris (pH 10). The photometric measurement was carried out on a microplatereader Victor (3 / TM) Vat at 570 nm wavelength. The absorption values were averaged and correlated to the median of the DMSO-control to give the T / C-value.

7 Abbreviations

AA	amino acid
Ac	acetyl
ADDP	1,1'-(azodicarbonyl)dipiperidine
ADP	adenosine diphosphate
AIBN	2,2'-Azobis(isobutyronitrile)
Ala	alanine
AMD	age-related macular degeneration
AMPA	2-amino-3-(3-hydroxy-5-methyl-4-isooxazolyl)-propionic acid
aq	aqueous
ar	aromatic
Arg	arginine
ATP	adenosine triphosphate
AzI	antizyme inhibitor
BAL	*Backbone Amide Linker*
Bn	benzyl
Boc	tert-butyl oxycarbonyl
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Bu	butyl
Bz	benzoyl
c	*cyclo*
Cbz	benzyloxycarbonyl
Conc.	concentrated
COSY	correlated spectroscopy
COX	cyclooxygenase
CPP	cell penetrating peptide
Cy	cyclohexyl
Cys	cysteine
d	day
DAPI	4',6-diamidino-2-phenylindole
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	*N*,*N´*-dicyclohexylcarbodiimid
Dde	1-(4,4-Dimethyl-2,6-dioxacyclohex-1-yliden)ethyl
DEAD	Diethyl azodicarboxylate
DFMO	2-difluoromethylornithine
DIC	*N*,*N´*-diisopropylcarbodiimid
DiPEA	*N*,*N*-diisopropylethylamine
DMAP	*N*,*N*-dimethylamionopyridine
DMBA	*N*,*N´*-dimethylbarbituric acid
DMF	*N*,*N´*-dimethylformamid
DMSO	dimethyl sulfoxide
DMTr	dimethoxytrityl
DNA	deoxyribonucleic acid
Dod	4,4´-dimethoxydityl
ED_{50}	50% effective dose

EDC	*N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide
eIF-5A	eukaryotic translation initiation factor
equiv.	equivalents
ESI	*Electron Spray Ionisation*
Et	ethyl
FACS	*Fluorescent-Activated Cell Sorting*
FAD	flavin adenine dinucleotide
Fmoc	9*H*-fluoren-9-ylmethoxycarbonyl
FMOP	4-formyl-3-methoxy-phenoxymethyl-polystyrol
g	gram
GABA	γ-aminobutyric acid
GAD	glutamic acid decarboxylase
Gln	glutamine
Glu	glutamic acid
Gly	glycine / glycyl
h	hour
HATU	*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluroniumhexafluorophosphate
HBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate
HeLa	Henrietta Lacks cells
HepG2	human hepatocellular liver carcinoma cell line
HIV	human immunodeficiency virus
HOAc	acetic acid
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole
HODhbt	3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazin
HPLC	*High Pressure (Performance) Liquid Chromatography*
HRMS	*High Resolution Mass Spectrometry*
HV	high vacuum
Hz	Hertz
IC_{50}	50% inhibitory concentration
IgluR	ionotropic glutamate receptor
LPS	lipopolysaccharide
Lys	lysine
m	meta
M	molar
MALDI	*Matrix-Associated Laser Desorption Ionisation*
Me	methyl
Mesyl	methylsulfonyl
Met	methionine
MGBG	methylglyoxal-bis(guanylhydrazone)
MHz	megahertz
min	minute
Mmd	4-methoxydityl
MMt	4-methoxytrityl
MPS	*N*-maleimidopropionyl succinate
MS	*Mass Spectrometry*
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

n.d.	not determined
NBDCl	7-nitro-1,2,3-benzoxadiazole-4-chloride
NHS	*N*-hydroxysuccinimide
NMDA	*N*-methyl-*D*-aspartate
NMP	*N*-methyl-pyrrollidone
NMR	*Nuclear Magnetic Resonance*
Nosyl	nitrophenylsulfonyl
NPhTX	nephilatoxin
o	ortho
ODC	ornithine decarboxylase
PA	polyamine
PAO	polyamine oxidase
p	para
PAMAM	polyamidoamine dendrimers
PAO	polyamine oxidase
Paraquat	*N,N´*-dimethyl-4,4´-bipyridylium
PDT	photodynamic therapy
PEG	polyethylene glycole
Pfp	pentafluorophenyl
ph	phenylene
Ph	phenyl
Phe	phenylalanine
Pht	phthaloyl
PhTX	philanthotoxin
PLP	pyridoxal phosphate
Pro	proline
Prp	propyl
PTD	protein transduction domain
PTS	polyamine transport system
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBrOP	bromotripyrrolidinophosphonium hexafluorophosphate
Put	putrescine
RNA	ribonucleic acid
RP	*Reversed Phase*
SD	standard deviation
Spd	spermidine
Spm	spermine
SPOS	*Solid Phase Organic Synthesis*
SPPS	*Solid Phase Peptide Synthesis*
SPS	*Solid Phase Synthesis*
Succ	succinimide
TAT	transactivator of transcription
TBP	tributyl phosphine
TBAF	tetrabutylammonium fluoride
TEAA	triethylammonium acetate
Teoc	2-(trimethylsilyl)ethyl-oxycarbonyl
Tfa	trifluoroacetyl

TFA	trifluoroacetic acid
TFMSA	trifluoromethylsulfonic acid
THF	tetrahydrofuran
TIS	triisopropyl silane
TLC	*Thin Layer Chromatography*
TMOF	trimethyl orthoformiate
TmsCl	trimethylsilyl chloride
Tox	toxicology
Trp	tryptophane
Trt	trityl
Ts / Tosyl	(4-methyl)-phenylsulfonyl
Tyr	tyrosine / tyrosyl
UV	ultraviolet
Val	valine
VLC	*Vacuum Layer Chromatography*
Z	benzyloxycarbonyl

8 References

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9 Publications

Peer-reviewed articles:

Frank Hahn and Ute Schepers, A versatile procedure for asymmetric and orthogonal protection of symmetric polyamines and its advantages for solid phase synthesis, *Journal of Combinatorial Chemistry,* **2008**, 10 (2), 267–273.

Frank Hahn, Katja Schmitz, Teodor Silviu Balaban, Stefan Bräse, and Ute Schepers; Conjugation of a molecular transporter enhances antitumour and antibiotic properties of highly lipophilic porphyrins, *ChemMedChem*, in press.

Frank Hahn, Klaus Müllen, and Ute Schepers, 2-Iminithiolane as a useful coupling reagent for polyamine solid phase synthesis, *Synlett*, accepted for publication.

Reviews

Frank Hahn and Ute Schepers, Solid phase chemistry for the directed synthesis of biologically active polyamine analogs, Dertivatives, and Conjugates, *Topics in Current Chemistry* (2007), *278*, 135-208