Utilization of the Ugi Four-Component Reaction for the Synthesis of Lipophilic Peptidomimetics as Potential Antimicrobials

Dissertation

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„Die Wissenschaft ist der auserlesenste Weg, um das Menschengemüt heroisch zu gestalten."

Giordano Bruno

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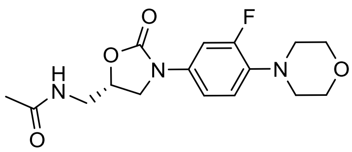
1 Introduction

In the last decades the use of antimicrobials has evolved to the standard therapy of a large number of infectious diseases caused by bacteria, fungi and other parasites. Since the release of arsphenamine, better known under the trademark Salvarsan®, as the first antibacterial agent on the market in 1910[1], there has been an extensive evolution in the field of antimicrobials. Nowadays more than 10 classes of antibiotics are used for the treatment of bacterial infections (Table 1)[2].

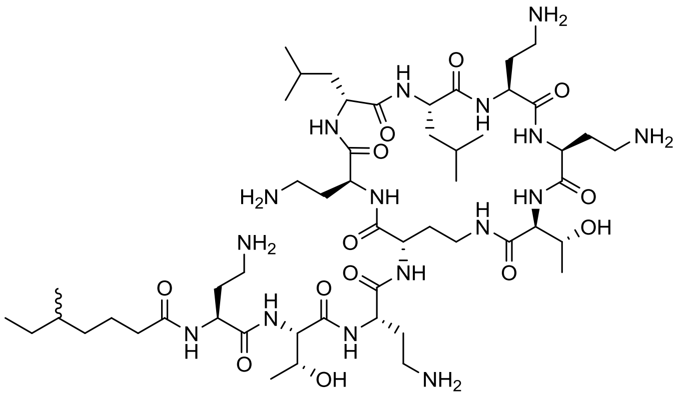
Table 1. Selection of classes of antibiotics, which are used for the treatment of infectious diseases in order of the year of introduction[2]

Antibiotic class; example compound	Year of discovery	Year of release	Year of first resistance	Mechanism of action	Activity or target species
Sulfadrugs; prontosil	1932	1936	1942	Inhibition of dihydropteroate synthetase	Gram-positive bacteria
β-lactams; penicillin	1928	1938	1945	Inhibition of cell wall biosynthesis	Broad spectrum activity
Aminoglycosides; streptomycin	1943	1946	1946	Binding of 30S ribosomal subunit	Broad spectrum activity
Chloramphenicols; chloramphenicol	1946	1948	1950	Binding of 50S ribosomal subunit	Broad spectrum activity
Macrolides; erythromycin	1948	1951	1955	Binding of 50S ribosomal subunit	Broad spectrum activity
Tetracyclins; chlortetracycline	1944	1952	1950	Binding of 30S ribosoma l subunit	Broad spectrum activity
Rifamycins; rifampicin	1957	1958	1962	Binding of RNA polymerase β-subunit	Gram-positive bacteria
Glycopeptides; vancomycin	1953	1958	1960	Inhibition of cell wall biosynthesis	Gram-positive bacteria
Quinolones; ciprofloxacin	1961	1968	1968	Inhibition of DNA synthesis	Broad spectrum activity
Streptogramins; streptogramin B	1963	1998	1964	Binding of 50S ribosomal subunit	Gram-positive bacteria
Oxazolidinones; linezolid (**1**)	1955	2000	2001	Binding of 50S ribosomal subunit	Gram-positive bacteria
Lipopeptides; daptomycin	1986	2003	1987	Depolarization of cell membrane	Gram-positive bacteria
Fidaxomicin	1948	2011	1977	Inhibition of RNA polymerase	Gram-positive bacteria
Diarylquinolines; bedaquiline	1997	2012	2006	Inhibiion of F_1F_o-ATPase	Narrow spectrum activity (Mycobacterium tuberculosis)

Due to the exhaustive application of those compounds – not only to cure diseases, but also in factory farming and other industrial sectors[3], combined with a lax use of antibiotics by physicians, treating infections of unknown kind with standard broad spectrum antibiotics as the first line of defense, a lot of bacterial strains have become resistant against many formerly active substances[4]. Those multi-resistant germs need to be treated with intensive care and the use of “drugs of the last resort” like linezolid (**1**)[5] or colistin (**2**)[6] (Figure 1), but there are already bacterial strains, which are insensitive against any available antibiotic[7].



linezolid (1)



colistin (2)

Figure 1. Structural formulas of linezolid and colistin (polymyxin E)

This permanently worsening situation may only be improved by screening for new classes of compounds, which could be possible candidates for the treatment of infectious diseases.

1.1 Natural Polypeptide Antibiotics

Polypeptide antibiotics are an interesting subclass of antimicrobial agents for the treatment of resistant bacteria due to their mode of action, which mostly leads to a destruction of the bacterial membrane e.g. by pore formation, interaction with cell wall building enzymes or even intracellular regulatory systems. These antimicrobials are mostly bactericides, which can be an advantage in preventing the development of resistance, because the selection of resistant forms is exacerbated[8].

1.1.1 Ribosomal Peptide Antibiotics

Among the huge group of peptide antibiotics, ribosomally synthesized peptides have a long history in science, since they are known for decades. Yet their structural diversity, which isn't limited to the pure amino acid encoding during the translation in the ribosome, is only slowly being revealed[9]. Due to their more or less direct footprint in the genetic code without the detour of huge enzyme clusters necessary for their synthesis, ribosomally synthesized peptides are promising subjects to be screened for by bio-informational methods. A direct search within the genetic code can be applied to identify precursor peptides, which might be modified after their synthesis. Antibiotic peptides themselves can be found in all kingdoms of life, which might give rise to a huge pool of promising lead structures.

1.1.1.1 Bacteriocins

Within the field of ribosomal peptide antibiotics the bacteriocins play a major role due to their early discovery. In 1925 the first bacteriocin was described by GRATIA as a substance, which is produced by and kills different strains of *Escherichia coli* bacteria and was therefore named colicin (**2**)[10].

Due to their inhomogeneity different sub-classifications of bacteriocins have been made. The most common way is a subdivision into four classes with possible different sub-classes (Table 2)[11].

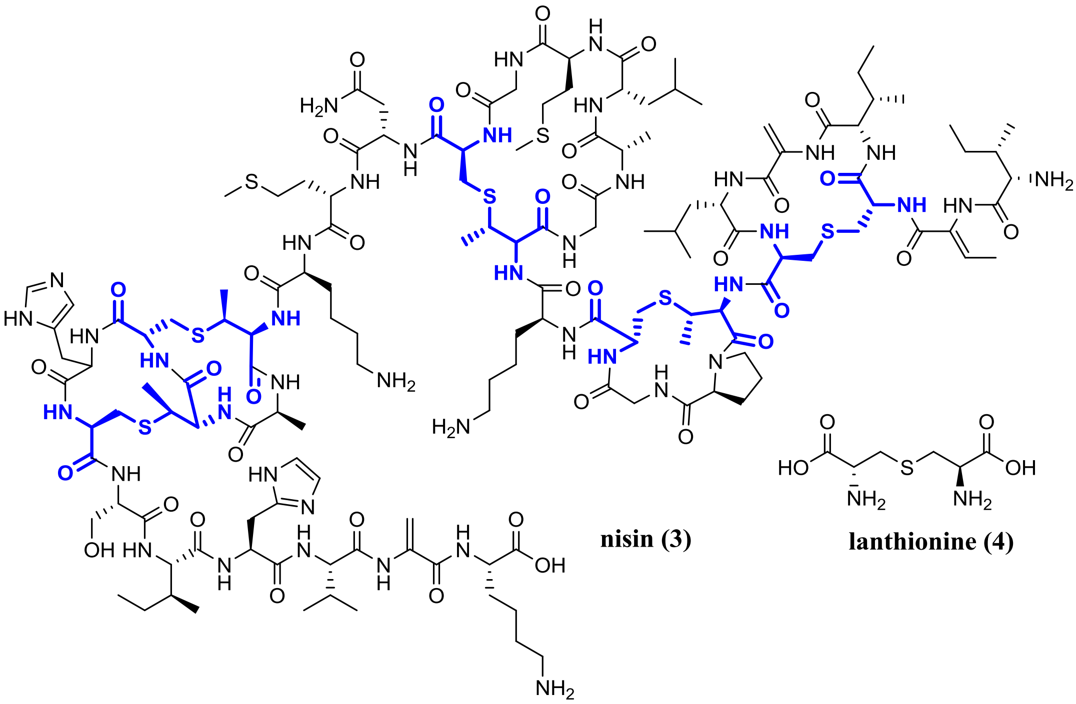
Table 2. Classification of Bacteriocins (modified according to [11][12])

Classification	Remarks	Examples
Class I		
Lanthionine-containing bacteriocins/lantibiotics	Including single- and two-peptide lantibiotics	Nisin, mersacidin, lacticin 3147, cytolysin
Class II		
Non-lanthionine-containing bacteriocins	Small peptides with four subclasses (**IIa**: pediocin-like; **IIb**: two-peptide; **IIc**: cyclic; **IId**: non-pediocin single linear peptides)	**IIa**: pediocin PA1; **IIb**: lactacin F; **IIc**: enterocin AS48; **IId**: lactococcin A
Class III		
Bacteriolysins, non-bacteriocin lytic proteins	Large, heat-labile proteins, often murein hydrolases	Lysostaphin, enterolysin A
Class IV		
Bacteriocins with non-proteinaceous moieties	Containing lipid or carbohydrate moieties	Glycocin F[12]

The inherent task of the bacteriocins is being a chemical weapon in the fight for resources, which also takes place in the very small scale of microbiology. Bacteria need to separate themselves from competing germs, which are mostly other bacterial strains. Hence this long evolved chemical machinery can be a guide to new kinds of antibiotics, which have already been proven by evolution.

Lantibiotics

Lantibiotics are a major class (class I) within the bacteriocins. All these various compounds share a structural motif, which is the amino acid lanthionine (**4**, Figure 2), which might be further modified by additional methyl groups.



nisin (3) **lanthionine (4)**

Figure 2. Structural formulas of nisin (lanthionine-like parts are highlighted in blue) and lanthionine.

Lanthionine (**4**) was first isolated in 1941 from wool[13], whereas the first lantibiotic nisin (**3**) was already discovered in 1933[14]. The thioether-bond in **4** and its derivatives is the inherent structural motif of all lantibiotics.

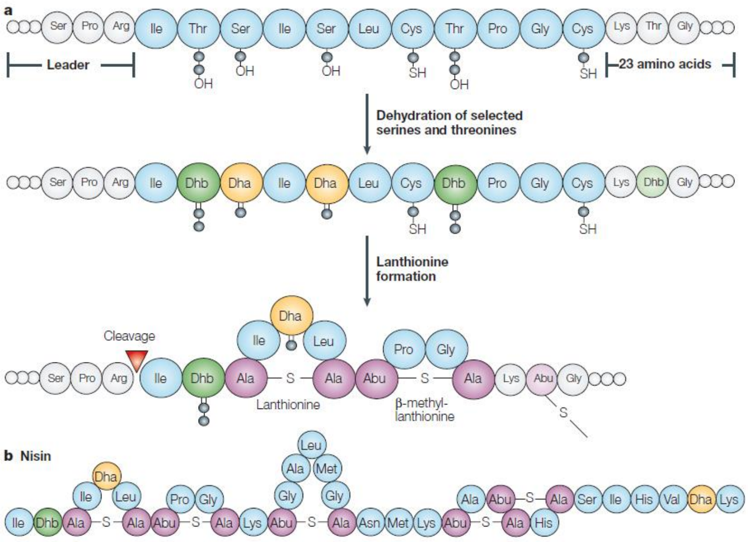


Figure 3. Lanthionine synthesis and lantibiotic structure (from [11]); **a)** lanthionine synthesis by subsequent dehydratation of serine and threonine residues and cyclization with a thiol-group of a cysteine; **b)** structure of the single peptide lantibiotic nisin (**3**).

They are synthesized by classical ribosomal translation and subsequent post-translational modifications, such as dehydratations of serine or threonine residues and 1,4-nucleophilic attack of a neighboring thiol group of a cysteine. Not all dehydro-amino acids might be transformed into thioethers, so that these might be present in the finally released compound. In nearly all cases the primary amino acid sequence contains a kind of leading motif, which is cleaved off after the lanthionine forming reactions (Figure 3)[11].

Lantibiotics are secreted to the environment of the producing germ and can then attack concurring microorganisms. They mostly show a dual mode of action like pore-formation in the cytoplasmic membrane and inhibition of the cell wall synthesis[15,16]. Predominantly gram-positive bacteria and only a few gram-negative germs are inhibited in their growth or even killed by lantibiotics. This selectivity is caused by the outer membrane of gram-negative bacteria, which isn't permeable by these compounds[17,18]. For an inhibitory effect it is crucial for a lantibiotic like **3** to reach the inner membrane to form stable complexes with lipid I and lipid II[15]. These constructs penetrate the inner membrane and form short-lived pores, which lead to an efflux of small molecules and cations (especially potassium ions and amino acids)[19–22]. The disturbed membrane potential then leads to a decreased vitality of the cell or even causes the exitus of the microorganism[23,24]. Nisin (**3**, Figure 2, Figure 3), which is industrially produced by *Lactococcus lactis*, is widely employed as a food additive for dairy products, because it is highly active against pathogens and their spores occurring in these groceries, but is also quickly decomposed in the human stomach, so that it doesn't influence the intestinal flora[25]. The inhibitory activity of nisin against *Micrococcus flavus* is described to be in the nanomolar range, which illustrates the potential of this compound class[26].

To gain information about the structural diversity of the bacteriocins a web-database, named BACTIBASE has been installed in 2007 incorporating details of 123 bacteriocins[27]. Two years later, in 2009, the second version of this database was released, now containing information about 177 different bacteriocins[28]. Based on this knowledge a detailed investigation about the structure of bacteriocins can be made. An interesting fact, which can be distilled out of this bunch of data is that the majority of bacteriocins carry a positive net charge (Figure 4).

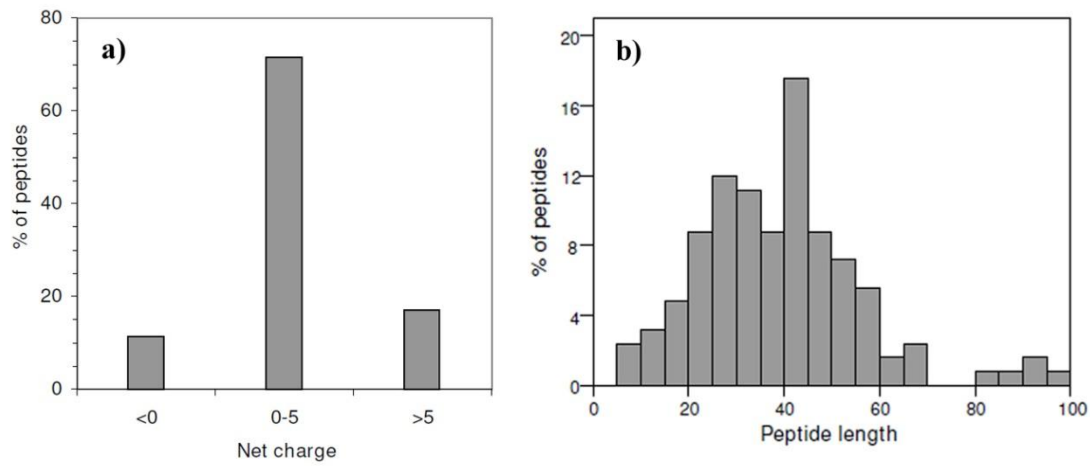


Figure 4. Histograms of the distribution of **a)** the net charge and **b)** the peptide length in BACTIBASE database in 2007(from [27]).

There are only a few representatives of negatively charged compounds. The amino acid distribution shows that glycine, alanine, lysine and serine are the most prominent residues capping more than 40% of the occurring amino acids[27]. This reflects the positive net charge, which seems to be an important structural element for their mode of action. Due to the negative charge of a bacterial membrane, it is obvious that interfering molecules might have easier access if they carry the opposite charge. Also the chain length distribution is an interesting finding, showing that the most prominent size of the peptide is about 40 amino acid residues (Figure 4).

1.1.1.2 Eucaryocines

The production of small peptides as defense molecules is not unique to bacteria. Actually nearly all vertebrates and invertebrates as well as plants and fungi produce this kind of defensive peptides[29,30]. In contrast to the broad range of small molecule secondary metabolites, which are well known as potent toxins or are even used as pharmaceuticals, these peptides or small proteins haven't been extensively investigated to date. However, a lot of them are already described and are under further investigation. The mode of action is dual type like for the bacteriocins, which share structural similarities. Eucaryocins may interact with the bacterial or cellular membrane to form pores and disturb the membrane potential or they penetrate the cell to from complexes with DNA or RNA, which are targets as well due to their negative charge. Some peptides may also disturb the bacterial cell wall synthesis, but this mode of action plays only a minor role. The sub-classification of eucaryocins acting as antimicrobial peptides (AMPs) is rather difficult due to their inhomogeneity. The most common way to subdivide euracyocins is shown in Table 3[31].

Table 3. Types and examples of Eucaryocins (modified according to [8,32])

Type	Characteristics	Examples (genus)
Anionic Peptides	Rich in glutamic and aspartic acid	Maximin H5 (*Bombina*)[33] Dermcidin (*Homo*)[34]
Linear cationic α-helical peptides	Lack in cysteine	Moricin (*Bombyx*)[35] Melittin (*Apis*)[36] Magainin (*Xenopus*)[37] Dermaseptin (*Phyllomedusa*)[38] Bombinin (*Bombina*)[39] CAP18 (*Oryctolagus*)[40] LL37 (*Homo*)[41–43]
Cationic peptides enriched for a specific amino acid	Rich in proline, arginine, phenylalanine, glycine, tryptophan	Abaecin (*Apis*)[44] Prophenin (*Sus*)[45] Indolicidin (*Bos*)[46]
Anionic and cationic peptides that contain cysteine and form disulfide bonds	Contain at least one disulfide bridge	***1 Bond:*** Brevinins (Neobatrachia)[47] ***2 Bonds:*** Protegrin (*Sus*)[48] Tachyplesins (*Tachypleus*)[49] ***3 or more Bonds:*** Defensins (animals, plants)[29,30,50] Drosomycin (*Drosophila*)[51]

Comparing the diversity of the AMPs produced by eucaryots it is obvious that most of them are small (op to 50 amino acids) basic peptides. This architecture seems to be incomparably successful in tackling microorganisms, especially pathogens like bacteria or fungi. The concept of small peptides employed as chemical weapons for innate immunity is effective in such a way that the basic composition is conserved from bacteria to mammals.

Defensins

A special type of cationic peptides, which play a disproportionate role in innate immunity or defense of animals, plants and fungi are the defensins[50]. They are also small, basic peptides, which differ from their relatives just by the number of disulfide bridges. Most of the defensins contain six cysteine residues, which form three intramolecular disulfide bonds. According to the connectivity of the disulfide bridges the defensins are sub-classified into α-, β- and θ-defensins (Figure 5).

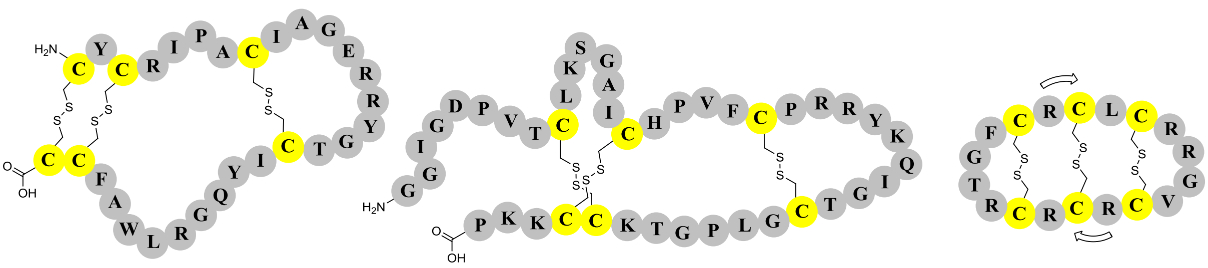


Figure 5. Sequences and the disulfide pairing of cysteines of α-, β- and θ-defensins (modified after [50]). In α-defensins **(a)** the six cysteines are linked in a 1-6, 2-4, 3-5 pattern[52], in β-defensins **(b)** the pattern is 1-5, 2-4, 3-6[53] and the cyclic θ-defensins **(c)** are formed from two hemi-α-defensins[54]. The arrows mark the beginning and the sequence direction of the two hemi-α-defensins.

This structural motif leads to an enhanced stability of the secondary structure adopted by the peptide and therefore increases the specificity and activity against certain target organisms. To simplify the structure and to facilitate the chemical synthesis of defensin-like compounds, there have been investigations whether cysteine-depletion variants with the same sequence still show antibacterial activity. For the model peptide HNP-1 it is indeed possible to abandon the rigidifying cysteins from certain sequences without losing too much of the antibacterial activity (Table 4). The loss of activity is in the range of one order of magnitude. This shows that the pre-formation of secondary structure by cystine unites within the peptide can lead to the fixation of the most active secondary structure. Without losing the whole activity a flexible, analogous sequence is able to kill the respective bacteria as well, although the free concentration of the most active foldamer is lower than that for the native peptide[55].

Table 4. Primary structure of HNP-1 and analogues and their activity against different bacteria stains (from [55]). For the natural defensin HNP-1 the linked cysteins each have the same color.

Peptide	Sequence	Lethal concentration of the peptide [µM]		
		Escherichia coli W 160-37	*Pseudomonas aeruginosa NCTC 6750*	*Staphylococcus aureus NCTC 8530*
HNP-1	ACYCRIPACIAGER-RYGTCIYQGRLWAFCC	1.0 ± 0.2	1.0 ± 0.2	0.8 ± 0.2
HNP1-ΔC	AYRIPAIAGER-RYGTIYQGRLWAF-$CONH_2$	14.0 ± 2.0	14.0 ± 2.0	7.0 ± 2.0
HNP1-ΔC18	IAGER-RYGTIYQGRLWAF-$CONH_2$	19.0 ± 3.0	23.0 ± 3.0	10.0 ± 2.0
HNP1-ΔC18A	IAAER-RYATIYQARLWAF-$CONH_2$	14.0 ± 2.0	23.0 ± 3.0	9.0 ± 2.0

It is an interesting fact that defensins like many other AMPs act on cytoplasmic membranes of pathogens without a severe effect on the host organism. The selectivity of the host originated peptides to just disrupt membranes of invading germs is a fact, which is hardly understood by now. The possibility to generate billions and billions of different small peptides leads to the question of how to gain any structure-activity relationship (SAR) in order to predict and synthesize promising antibiotics[56]. It is very complex to understand the interaction of certain molecules with membrane constructs, but herein might be an advantage in chemotherapy of infectious diseases. Due to differences in the composition of pathogen membranes a directed attack by antibiotic peptides seems to be possible[57]. And this is what nature does since millions of years. Taking the huge scope of cationic ribosomally synthesized peptides as an example, one can distill out important and promising structural motifs.

1.1.2 Non-Ribosomal Peptide Antibiotics (NRPs)

In nature not only ribosoms are used to synthesize peptides or peptide-like structures. For the synthesis of modified or substituted peptides, whole enzyme complexes called nonribosomal peptide synthetases (NRPSs) can be found exclusively in microorganisms like bacteria or fungi[58]. There are some examples for the occurrence of NRPs in higher organisms, but it is supposed that they are produced by incorporated microorganisms as well[59].

In contrast to the ribosoms and due to the fixed modular architecture of the NRPSs, each one can only synthesize a single defined compound. Additionally, other reactions than peptide bond formation are catalyzed. This enables these enzyme clusters (Figure 6) to synthesize highly sophisticated molecules with lipids, carbohydrates, terpenes or other moieties connected to a more or less modified peptide backbone. The scope of accessible structures is therefore even broader than for a combination of ribosomal synthesis and post-translational modifications[60,61].

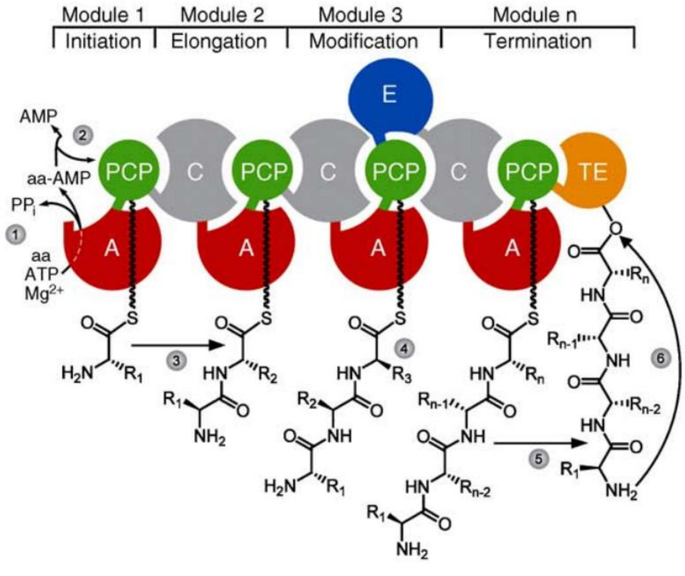


Figure 6. Simplified mechanism of nonribosomal peptide (NRP) synthesis (from [58]). (1) The amino acid is activated as aminoacyl-adenosine monophosphate (aa-AMP) by the adenylation domain. (2) Transfer of the amino acid onto the PCP domain. (3) Condensation of PCP-bound amino acids. (4) Possibility of amino acid modifications, for example by epimerization domains. (5) Transesterification of the peptide chain from the terminal PCP onto the TE domain. (6) TE catalyzed product release by either hydrolysis or macrocyclization. The number of modification domains and modules is very variable[58].

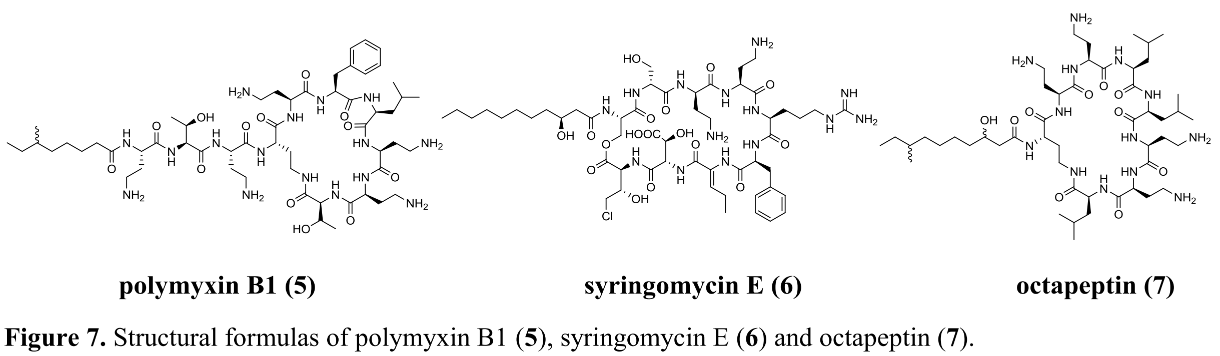
The main disadvantage is that the huge enzyme complexes need to be optimized for the certain target molecule and it is therefore more difficult to gain information regarding new peptide compounds in organisms directly from their genetic code. The encryption goes always detour via the modular enzyme complexes, which might be conserved in major parts or also vary from species to species. Due to the broader range of possible modifications the mode of action of non-ribosomally synthesized peptide antibiotics is wide-ranging. The interference with membrane compounds can still be a target tackled by these compounds. Common other points of attack are the murein biosynthesis, the ribosomal translation, bacterial cell wall synthesis in general – they can also have cytotoxic effects. The prediction of the activity of NRPs with computational methods has been shown by ABBO ET AL. in 2012[62]. This chemoinformatic tool has been proven by the application on NORINE, an open-source database of more than 1000 NRPs accessible in the internet. The pure number of registered sequences is ten-fold higher than for the bacteriocins in BACTIBASE[27,28,61,63].

1.1.2.1 Lipopeptides

A very common concept of antimicrobial peptides is their membrane activity. Targeting a membrane, a compound is believed to be cationic and lipophilic in the vast majority of examples. Whilst hydrophobic amino acids arrange the hydrophobicity of certain parts of a ribosomally synthesized peptide, the NRPS machinery can attach lipophilic moieties directly, just like linear or branched fatty acids. The amphiphilic nature of lipopeptides enables these compounds to be potent antimicrobials. Surprisingly the nature of the polar part of the NRP isn't too important – the trick is the secondary structure, which is adopted by the lipopeptides within the membrane. These folding properties are very often supported by a cyclic architecture.

Cationic Lipopeptides

A subclass of lipopeptides is taken by cationic compounds, which are mostly very small peptides (8 – 20 amino acid residues) that show a high affinity to bacterial membranes. In contrast to their ribosomally synthesized relatives they exhibit a high activity against gram-negative germs. This is due to the complex formation of cationic lipopeptides with negatively charged lipopolysaccharides in the bacterial membrane. Additionaly they are cyclopeptides and consist of a mixture of D- and L-amino acids and sometimes non-proteinogenic amino acids like 2,4-diaminobutyric acid (DAB), which makes them more resistant against hydrolytic enzymes. Prominent representatives include polymyxin B (**5**), syringomycin E (**6**) and octapeptin (**7**, Figure 7)[64,65].



polymyxin B1 (5) **syringomycin E (6)** **octapeptin (7)**

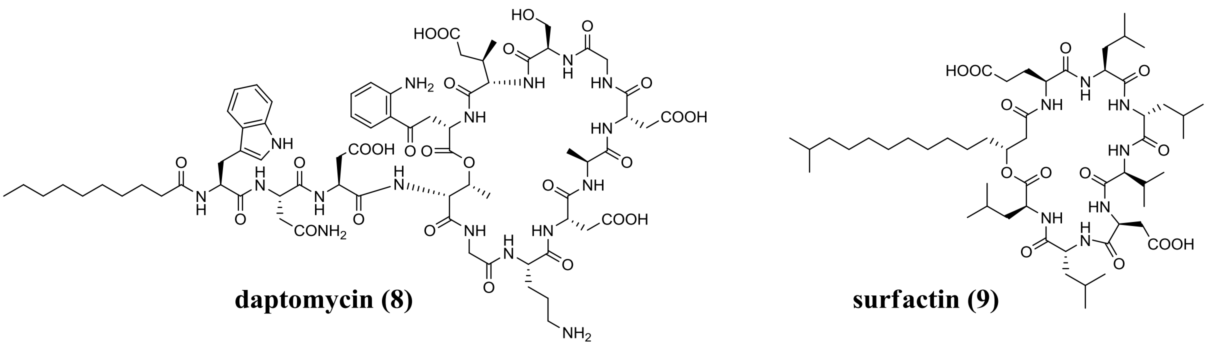
Figure 7. Structural formulas of polymyxin B1 (**5**), syringomycin E (**6**) and octapeptin (**7**).

The fatty acid component is indispensable for the activity. It has been shown that the withdrawal of the acyl moiety of **2** or **5** leads to a nearly complete loss of antibacterial activity[64,66–70].

Anionic Lipopeptides

In contrast to the attraction of the opposite charges of cationic lipopeptides and the bacterial membrane the second class of lipopeptides show a negative net charge. This seems to be a contradiction to the mode of action these molecules demonstrate. They also interfere with the bacterial membrane, but they incorporate as some kind of phosphatidylglycerol mimics possibly masked by bivalent calcium ions, which has been shown for daptomycin (**8**)[71–73]. Once located in the membrane, they tend to aggregate forming holes that enable ions to leak out of the cell. The caused depolarization leads to a fast death of the treated cell. Due to the outer membrane of gram-negative bacteria, which cannot be penetrated by anionic lipopeptides, they only show activity against gram-positive germs.

Two prominent representatives of anionic lipopeptides are **8** and surfactin (**9**, Figure 8).



daptomycin (8) **surfactin (9)**

Figure 8. Structural formulas of daptomycin (**8**) and surfactin (**9**).

Although **8** is already employed as a therapeutic antibiotic as Cubicin®, **9** is even more potent in killing bacteria, but also disrupts eukaryotic cell membranes and is therefore hard to apply in the treatment of diseases[74][75]. This is due to the detergent-like mode of action of surfactin in biological membranes[76]. This behavior and the ability to form micelles in solution lead to a hardly controlable activity of surfactin. Efforts have been made to tune the molecule synthetically to generate compounds, which show a more distinct activity profile and leave eukaryotic membranes intact even in higher application doses.

The main advantage of the application of lipopeptides is also their most important drawback – the mostly uncontrolled membrane-activity. Like it has already been shown for the cationic lipopeptides, the fatty acid component also plays a major role in the activity of their anionic counterparts. This working point makes a big variety of synthetic modifications possible – just by exchanging the fatty acid part. It seems to be possible to fine-tune the lipopeptides to a certain target organism by adjusting the fatty acid component or even cutting the cyclic structure to a linear shape[74,77].

1.2 Peptaibols

A unique group amongst the naturally occurring antibiotic compounds with peptide-like structures are the so called peptaibols. Their name is composed of ***pep****tide*, the incorporated *α*-aminoisobutyric acid (***Aib***) and the ending for *alcoh****ol***. Peptaibols are therefore ac(et)ylated polypeptides containing the powerful helical inductor Aib and a reduced *C*-terminus. This architecture makes them more resistant against proteases and enables them to be strongly membrane-active against potential pathogens. They can be found in fungi exclusively and exhibit strong antibiotic properties. It is assumed that they are produced by the host organisms to fight against bacterial and other microbial invaders[78].

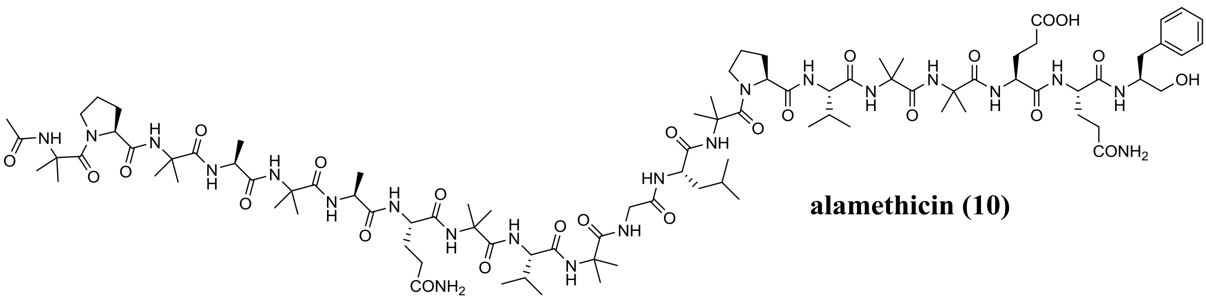
1.2.1 Classification of Peptaibols

Peptaibols are generally small “peptides” (less than 20 amino acids) and contain substantial amounts of unnatural amino acid residues like isovaline (Iva), hydroxyproline (Hyp) and ethylnorvaline (Etnor). A chemoinformatical analysis led to the sub-classification into nine classes according to sequence-identity parameters (Table 5)[78].

Table 5. Sub-classification of peptaibols. The one-letter code refers to natural amino acids and the following unnatural ones: U = Aib; H = Hyp; X = Iva and Z = Etnor (modified according to [78]).

Subfamily	Examples	Sequence
SF1	Alamethicin_F30 (**10**)	UPUAUAQUVUGLUPVUUEQF
	Longhibrachin_LGAIV	UAUAUUQUVUGLUPVUXQQF
	Trichorzin_PA_IV	USAUXQXVUGLUPLUUQW
	Chrysospermin_C	FUSUXLQGUUAAUPUUUQW
SF2	Antiamoebin_I	FUUUXGLUUHQXHUPF
	Emerimicin_IV	FUUUVGLUUHQXHAF
	Bergofungin_D	VUUVGLUUHQXHUF
SF3	L1_zervamicin	LIQXITULUHQUHUPF
	Emiricin_IIA	WIQUITULUHQUHUPF
	XR586	WXQUITULUPQUHXPFG
SF4	Harzianin_HC_I	UNLUPSVUPULUPL
	Harzianin_HC_XV	UQLUPAIUPXLUPL
	Trichorovin_TV_XIIa	UNIIUPLLUPI
	Hypomurocin_HMA2	XQVVUPLLUPL
SF5	Trichogin_A_IV	UGLUGGLUGIL
	Trikoningin_KBI	UGVUGGVUGIL
	Trikoningin_KB_II	XGVUGGVUGIL
SF6	Ampullosporin	WAUULUQUUUQLUQL
	Tylopeptin_A	WVUXAQAUSUALUQL
	Tylopeptin_B	WVUUAQAUSUALUQL
SF7	LP237_F5	UPYUQQUZQAL
	LP237_F7	UPFUQQUUQAL
	LP237_F8	UPFUQQUZQAL
SF8	Clonostachin	UHLXHLXHUXUHXI
SF9	Peptaibolin	LULUF

The most prominent peptaibol alamethicin (**10**, Figure 9) contains 20 amino acids and can be isolated from the fungus *Trichoderma viride*[79,80].



alamethicin (10)

Figure 9. Structural formula of alamethicin.

Like the databases available for bacteriocins and non-ribosomal peptides a similar one for the peptaibols has been created and is accessible in the internet[81]. Currently 317 sequences are registered, which is a high number for this kind of unusual compounds[82].

1.2.2 Mode of action of peptaibols

Peptaibols are membrane-active compounds, which form pores within a cellular membrane. Due to the different size of peptaibols at least two mechanisms of action are proposed.

For the bigger peptaibols like **10** (Figure 9, Table 5) their pure chain length is assumed to be enough to span a membrane. Although this might be an important factor – their secondary structure is not less important. The hydrophobic *N*-terminus penetrates the membrane and pulls the molecule into the membrane. Most of the longer peptaibols contain (hydroxy-)proline(s) in their middle-part. These residues stop the penetration process due to the kink in the secondary structure they cause. Once the penetration process has stopped it can only be reanimated again by a voltage applied to the membrane. This external energy thrust leads to a re-folding of the peptaibol, which enables the molecule to insert completely into the membrane. Several of already incorporated molecules self-assemble afterwards and form pores that allow ions to pass through the membrane according to their concentration gradient. This mostly leads to the death of the penetrated cell. It strongly depends on the sequence of the respective peptaibol, how many monomers assemble. Due to the architecture of the formed pore, this mode of action is called the “barrel-stave model” (Figure 10)[83].

If the secondary structure of the long-chain peptaibols is too curved like in antiamoebin they cannot be re-folded completely by external voltage application. For this kind of peptaibols a carrier-like mode of action is discussed. They can only penetrate special kinds of lipid membranes[84,85].

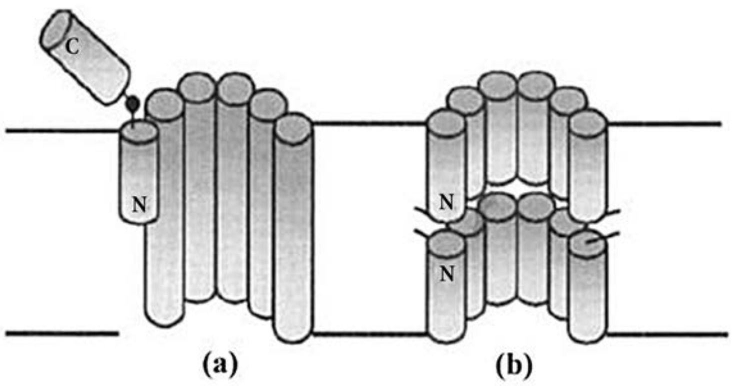


Figure 10. Models for the insertion of (a) long and (b) short peptaibols into membranes. (a) “Barrel-stave-model” - one molecule of the bundle shows a proline (represented as a black ball) forming a kink in the structure between the *N*- and *C*-terminal helices. After application of a voltage, the entire molecule inserts into the membrane, as shown by the other molecules. (b) The *N*-terminus-to-*N*-terminus association of two short monomers in the centre of the bilayer is shown (from [78]).

The shorter peptaibols cannot penetrate the membrane completely. Therefore another mode of action (“carpet mechanism”) for their activity is proposed. They are supposed to accumulate on the outside of the membrane in a kind of molecular carpet. Once the critical local concentration has been reached they also penetrate the membrane, but they reach just to the middle of the bilayer. Another “half-pore”, formed analogously on the other surface of the membrane can pair with the existing ones and create a full pore that leads to ion leakage[86]. For trichogin it is assumed that the *N*-terminal acylation (octanoyl) leads to an intra-membrane association of the *N*-termini of two half-pores. In different experiments the chain length of the *N*-terminal acylating fatty acid has been modified. There were clear incidences that a shorter and therefore more hydrophilic alkyl chain leads to a decrease or even complete loss of the membrane modifying activity[87,88].

Although there are different modes of action described for the peptaibols, especially the shorter ones are strongly dependent on the nature of the *N*-terminal acylation. The amphiphilic architecture allows for strong membrane activity like it is described for other antimicrobial peptides. Furthermore their unique structure incorporating unnatural amino acids makes them more resistant to proteases and other cellular defense mechanisms. Some structural motifs of peptaibols might be used to design tailor-made compounds with distinct activity against special microbial targets.

1.3 Cell-Penetrating Peptides (CPPs)

The quite heterogeneous class of cell-penetrating peptides (CPPs) is composed of natural as well as artificial sequences. Since in 1988 the first natural CPP, the trans-activating transcriptional activator (Tat)[89], was discovered, more than 100 different compounds have been described. These peptides can pass membranes of eukaryotic cells without harming the cells viability. Therefore they can be used as transporters for molecular cargo of different kinds. Typical representatives consist of 5-30 amino acids and might also carry the intracellular active motif within their own sequence instead of just transporting a second molecule through the membrane.

1.3.1 Classification of CPPs

Three major classes are described for CPPs – cationic, amphipathic and hydrophobic. In contrast to the lipopeptides there is no subclass for anionic CPPs – they are assigned to either amphipathic or hydrophobic classes[90]. The classification is not as clear as for other peptides, because the pure net-charge does not lead to a classification as a cationic CPP (Figure 11).

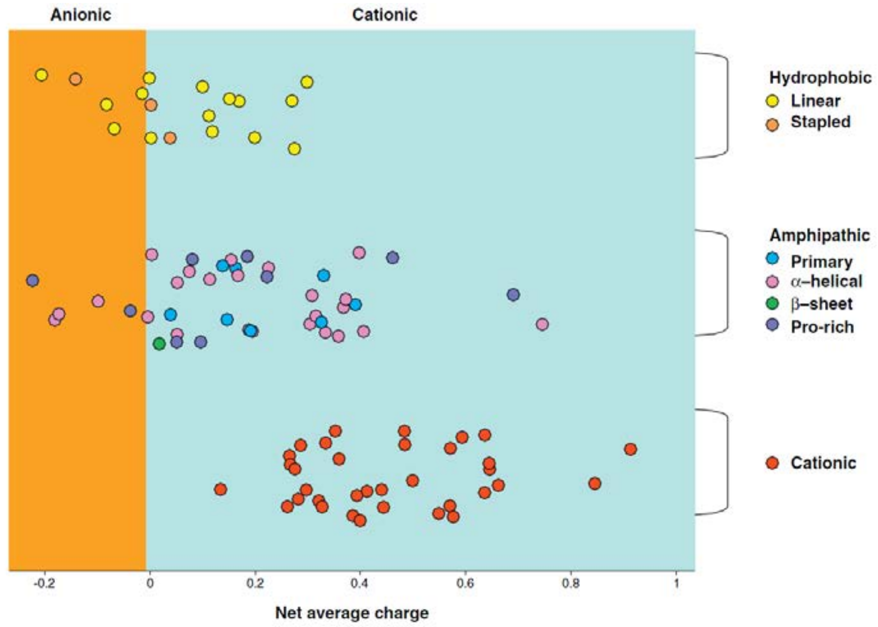


Figure 11. Distribution of CPPs by net average charge and class. Anionic CPPs can be classified as hydrophobic or amphipathic CPPs. By contrast, many cationic CPPs are highly charged peptides, without any amphipathic arrangement or hydrophobic character (from [90]).

Cationic CPPs

The first natural CPP to be discovered (Tat) is cationic and efforts have been made to determine the minimal length of a polycationic peptide consisting of only arginine or lysine. Even before the discovery of natural representatives artificial CPPs have been used already in the 1960s and 70s to accelerate the cellular uptake of small molecules, albumin or other proteins[91,92]. It has been shown that at least eight residues of arginine are needed for cellular uptake, but a longer sequence would be beneficial. Additionally the difference between oligo-arginine and oligo-lysine is impressive. The ability of cell penetration is much weaker for a pure lysine peptide[93]. Experiments showed that at least eight positively charged residues, most likely arginines, also in a hydrophobic or amphiphilic CPP, are beneficial or even needed for a good cellular uptake[94].

Amphiphilic CPPs

For the amphiphatic CPPs two differentiations can be made. On the one hand it is often possible to fuse a single cationic CPP to a hydrophobic peptide chain via a linker sequence and assume that the fusion peptide will be taken up by a cell due to the cationic lead sequence. In some cases this is enough to facilitate the cellular uptake, but in some cases the signal peptide had a big influence on the penetration mechanism and the pure cationic tail didn't lead to a sufficient membrane penetration[90,95]. Another structural motif can be seen analogously to the peptaibols – the formation of mostly helical structures, although β-sheets or rigid proline domains have also been described[96–98]. Amphipathic CPPs do not necessarily carry a positive charge. Like described for anionic lipopeptides a negative or a net charge of zero in combination with the appropriate sequence can lead to very active CPPs as well[99,100].

Hydrophobic CPPs

The vaguest class containing the most heterogeneous members is the hydrophobic CPP subclass. An assumption has to be made, because a pure hydrophobic sequence alone would hardly be able to attack membranes. Therefore a certain ratio of hydrophobic to polar residues in the CPP is used as limit to assign a peptide into this class. For some very short CPPs with just five amino acid residues it could be shown that the sequence isn't responsible for an enhanced activity. Even undirected scrambling of sequences does not lead to a loss in activity – contradictory to what is observed for cationic and amphiphilic CPPs[101,102].

1.3.2 Mode of action of CPPs

The cellular uptake or the penetration of CPPs through membranes into the inner lumen of a eukaryotic cell can be mediated by different factors. Three pathways can be discussed how a CPP finds its way into the cell – direct penetration, macropinocytosis and receptor-mediated endocytosis (Figure 12)[103].

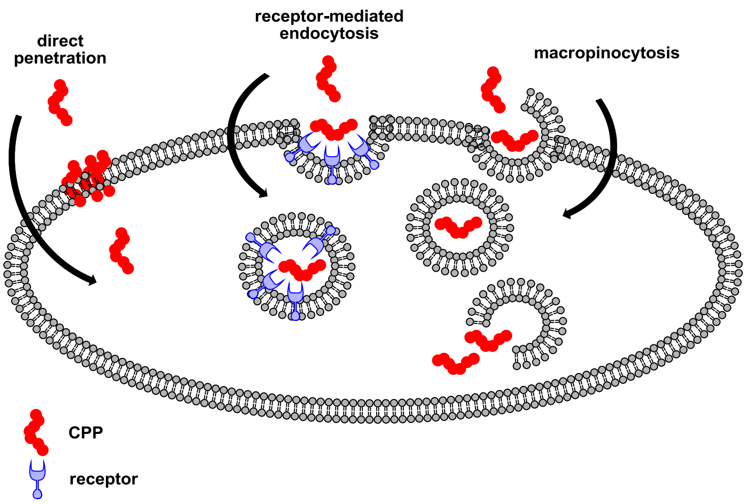


Figure 12. Scheme for different suggested uptake pathways for CPPs (modified after [103])

1.3.2.1 Direct penetration

Depending on the architecture of the CPP in principle four different mechanisms seem to be possible for direct penetration of a cell membrane – micelle formation[104], pore formation[105], the carpet-like model[106] and the membrane thinning model[107]. They all share the fact that they work without any energy-consuming processes.

For most of the different CPPs an attraction of the cationic peptide and the negatively charged membrane compounds like heparin sulfate (HS) or the phospholipids is assumed. Once located on the membrane surface the CPPs cause different effects according their structural features. For penetratin it is described that an inverted micelle is formed, which is incorporated by the cell[108]. For highly cationic CPPs, which lack of hydrophobic amino acids, this is not the most likely mechanism. As described for the peptaibols, CPPs can adopt the barrel-stave and additionally a modified toroid mechanism. The toroid model involves an arrangement of the CPP close to the head groups of the phospholipids and both – lipids and CPPs- tend to form the pore et the end[105]. Both pore-formation mechanisms are strongly concentration-dependant and the secondary structure of the CPP has a great influence on the effective concentration. Finally the carpet-like model seems to be the same as for peptaibols described. A variation of it is the thinning of the cellular membrane by certain CPPs, which is caused by high local concentrations and might lead to a CPP diffusion into the cell[107].

1.3.2.2 Cellular uptake by endocytosis

Endocytosis mediated uptake mechanisms require an energy input by the host cell, most likely the activation of membrane folding by NTP consumption. This membrane folding can be triggered by several pathways. The uptake of solutes in form of pinocytosis can be subdivided into a clathrin or calveolin dependant and a clathrin or calveolin independent endocytosis. Macropinocytosis is the receptor independent pathway and works through the inward folding of the outer membrane surface. The formed vesicles, the macropinosomes, are surrounded by a membrane that has the same composition like the cellular membrane. For the cellular uptake dynamin protein is required, which consumes GTP as energy source[103,109].

In the receptor-mediated uptake, the CPP binds to an external receptor that transfers the information to the clathrin or calveolin molecules, which are located on the inner surface of the cellular membrane. The vesicles formed are still covered with the respective protein and are directed to a certain cell target, depending on the type of receptor/protein. There is an inherent size difference of the particles detectable - due to the nature of the protein covering the vesicular surface. Generally clathrin-coated vesicles show an average diameter of a few hundred nanometers whereas calveolinic vesicles are only 50-80 nm in diameter[103,110,111].

A generalization for the most likely uptake mechanism for CPPs cannot be made. The assumption that most peptides pass the membrane by an energy-independent direct penetration mechanism turned out to be wrong in recent years. Therefore it is supposed that most of the CPPs make use of the cellular transporter systems that form vesicles as vectors for cellular uptake[112,113].

1.4 Peptidomimetics

The overwhelming natural diversity of peptides that show promising biological activities already incorporates modifications on the peptide backbone that lead far from the original proteinogenic amino acid sequence. Examples for those modifications are the already mentioned lantibiotics, peptaibols, D-amino acid containing peptides or cyclic peptides. These variances lead to an enhanced stability of those compounds towards proteases or other cellular defense mechanisms. This strategy was adopted and enhanced during the search for new bio-active compounds. Three important ways to generate compounds with a peptide-like structure and activity, but enhanced stability are β-peptides, D-peptides and peptoids (Figure 13).

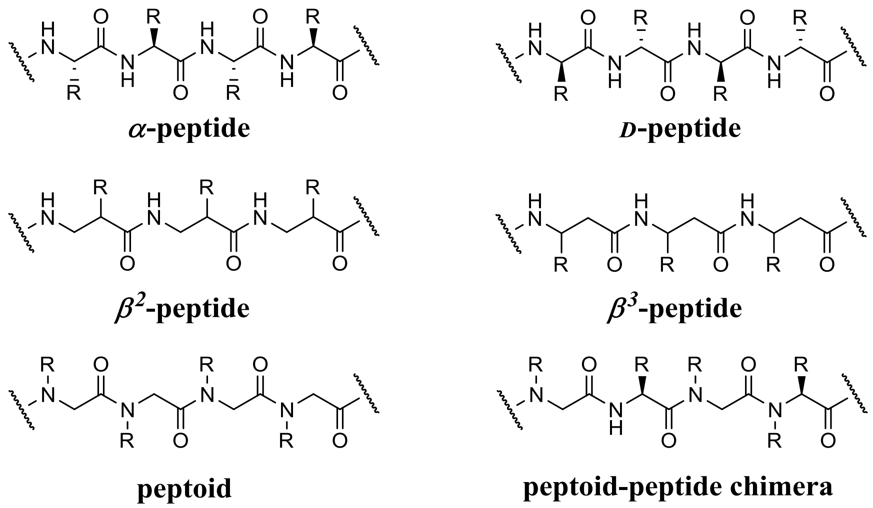


Figure 13. Generalized structural formulas of different peptidomimetics in comparison to the natural peptide structure. *D*-peptides are composed of amino acids, having an inverse stereo center, but can still be declared as *α*-peptides. *β*-peptides can be sub-divided into two classes, depending on the position of the side chain residue on the peptide backbone. Peptoids are *N*-alkylated oligo-glycins that can be further subdivided into chimeric peptoid-peptides if not every peptide bond is alkylated.

The development of the three different compound classes was premised on the assumption that proteases or hydrolases in general are motif-dependant. Due to the lack of the classical peptide bond distance, the occurrence of the wrong enantiomeric amino acid or even the lack of the amide proton, enzymes will hardly cleave the connecting amide bonds. Efforts have been made to mimic even natural antibiotic peptides in a *β*-peptide fashion to fix the promising activity and make the potential drug more resistant towards degradation by enzymes[114].

1.4.1 Peptoids

Like described above peptoids are *N*-alkylated oligo-glycins. Due to the lack of amide protons the tertiary amide bond is more flexible with respect to (*S*)-trans versus (*S*)-cis conformation as the energy barrier for the rotation of the amide bond is much lower than for classical peptides. This is the reason for the occurrence of (*S*)-cis species next to (*S*)-trans configured peptoids in solution at room temperature (Figure 14)[115,116]. However, peptoids are not more flexible in general, they occupy just different favored conformations than peptides as can be seen from Ramachandran plots of peptoids. Some are less flexible than peptides[117,118].

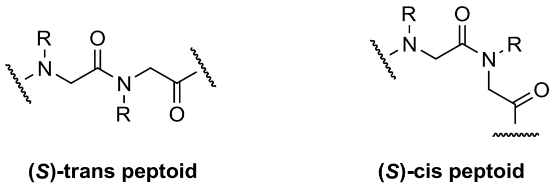


Figure 14. Structural motifs of a (*S*)-cis and a (*S*)-trans peptoid.

There is always an equilibrium between both forms detectable. The missing hydrogen-bond donor functionality at the peptoid backbone is closely connected to the more flexible structure of peptoids, which can arrange in many different folding states[119–121].

1.4.1.1 Naturally occurring peptoids

In the vast pool of secondary metabolites that can be extracted from different kinds of organisms, also peptoid-like compounds can be found. These substances aren't pure peptoids, because they would carry an achiral backbone, but they incorporate chiral *N*-methylated amino acid residues in their backbone. A prominent representative of this natural compound class is cyclosporine (**11**, Figure 15) that is used as an immunosuppressive agent since the early 1970s[122]. Also small compounds like viridic acid (**12**)[123] or the very potent cytotoxin tubulysin (**13**)[124–126] with a prominent peptoid moiety and unusual aromatic residues can be found in nature (Figure 15).

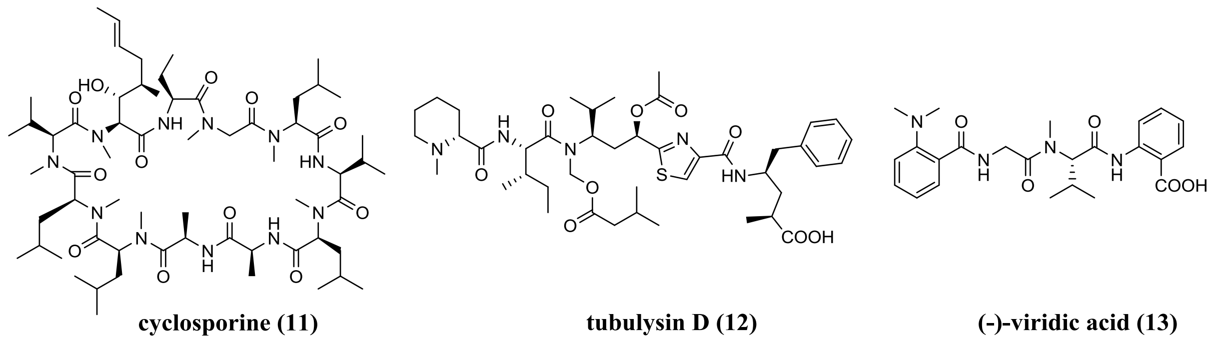
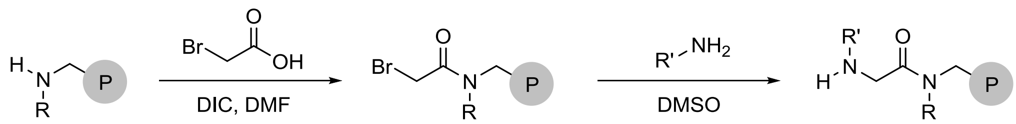


Figure 15. Naturally occurring peptoid-peptide chimeras. Structural formulas of cyclosporine (**11**), tubulysin D (**12**) and (–)-viridic acid (**13**).

11 itself combines several strategies of defense against hydrolysis by enzymes. It incorporates non-proteinogenic amino acids, D-amino acids, *N*-alkylation and is furthermore a cyclic molecule and it is synthesized by a non-ribosomal peptide synthase. Hence this complicated peptide-peptoid structure makes the chemical synthesis of natural peptoid-like compounds rather challenging.

1.4.1.2 Synthesis of peptoids

Pure classical peptoids (i.e. glycine-based) can be synthesized following the robust ZUCKERMANN submonomer protocol. In this synthesis, a classical peptide coupling step of bromoacetic acid to a resin-bound *N*-alkylated amino acid is followed by a nucleophilic substitution of the bromine against an amine. Long oligomeric peptoids are accessible following this method (Scheme 1)[127].



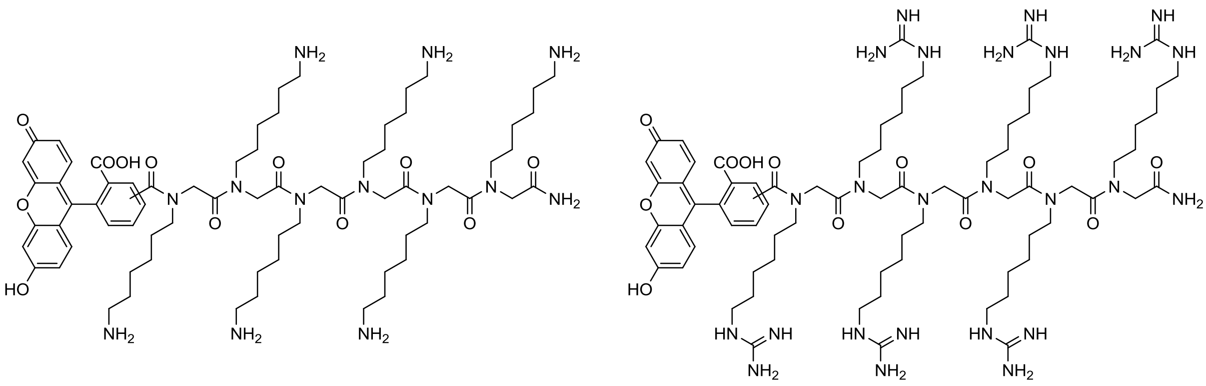
Scheme 1. Solid-phase synthesis of *N*-substituted glycins via the submonomer approach (P = polymer)[127].

Applying this method in automatic synthesizers, a broad variety of compounds can be synthesized. In contrast to ribosomally made peptides, the alkyl side chains are freely selectable, because nearly every primary amine can be used to alkylate the resin bound bromoacetic acid residue in high yield[128].

Peptoids with cell-penetrating properties

Combining the structural features of cell-penetrating peptides (CPPs) and peptoids a lot of effort has been made to create compounds that can act as molecular cargo transporters into host cells, but may be freely designable by the submonomer approach. Due to the peptoidic structure of those compounds they share a high stability against hydrolytic enzymes or cellular degradation and might therefore be monitored longer during their cellular uptake and the subsequent distribution in the host cell. The formation of stable helical structures for very short peptoids (four or five glycine residues) with chiral centers in their side chains could be shown[129]. This leads to the assumption that even much shorter sequences compared to analogous peptides could lead to tailor-made membrane-active compounds.

A lot of membrane-active peptoids carry cationic charges, which has already been shown to be beneficial in different classes of antimicrobial peptides. These kinds of compounds can exhibit a high activity against very resistant pathogens like *Mycobacterium tuberculosis* or multiresistant germs[130]. An advantage of this compound class is their lower hemolytic activity in comparison to some prominent polycationic peptides like melittin, which lead to cell lysis instantly[131].



14 **15**

Figure 16. Structural formulas of fluorescence-labelled cell-penetrating peptoids with poly-amine (**14**) or poly-guanidinium (**15**) side chains (from [132]).

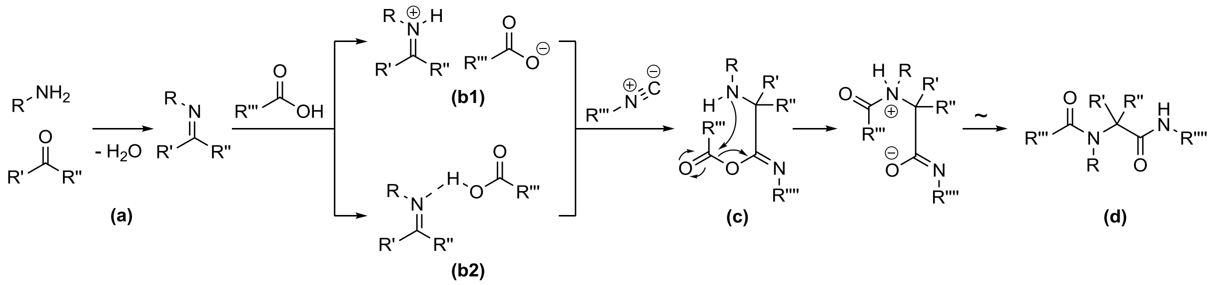
The feature to leave eukaryotic cells intact led to the development of a huge library of transporter peptoids, which can be employed as cargo transporters into the cell or can even accumulate within certain cellular structures, depending on the nature of the cationic side chains. A much better uptake can be reached if the simple amino group in **14** is exchanged against a guanidinium residue like in **15** (Figure 16). This exchange also leads to an enhanced affinity of the peptoid to cell organelles like the nucleus. By coupling fluorescent dyes to the cell-penetrating peptoids the distribution could be determined in detail[132,133].

1.4.1.3 Synthesis of peptoid-peptide chimeras

In contrast to pure peptoids, chimeric compounds do not carry alkyl chains on every glycine residue. The synthesis of alternating peptoid-peptide connections in a chimeric backbone can be achieved by classical coupling reactions or by applying a multicomponent-reaction (MCR) approach. In MCRs more than two components react with each other to form a distinct product.

Ugi four-component reaction

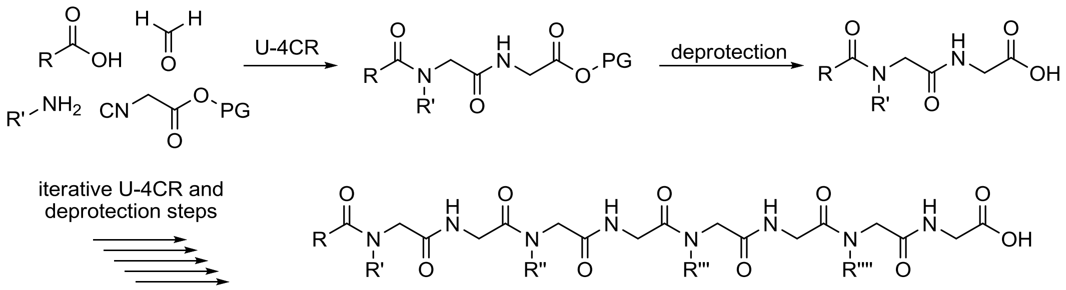
For the synthesis of peptoid-like structures, the isonitrile based Ugi four-component reaction (U-4CR) is used[134,135]. The biggest advantage in comparison to the submonomer approach is the formation of two amide bonds within one reaction, whereas for the generation of a single peptoid bond in the ZUCKERMANN protocol two steps are needed[127].



Scheme 2. Mechanism of the Ugi-four-component reaction (U-4CR). (a) Imine formation via a condensation of an amine and a ketone/aldehyde. (b1) Protonation of the imine by the carboxylic acid, forming an ion pair. (b2) Formation of a hydrogen-bond bridged complex between the imine and the carboxylic acid. (c) Primary Ugi product (α-adduct) undergoing the Mumm rearrangement. (d) Final Ugi product.

For the U-4CR, an amine, a ketone or aldehyde, an acid (or other nucleophile) and an isonitrile (isocyanide) are needed. The initial step is the condensation of the amine with the keto-component to an imine under the loss of water. This single released water molecule is the only by-product generated in the U-4CR, which makes it therefore very atom-efficient. The next step was for decades believed to be a protonation of the imine by the acid. However, recent computational studies showed that a protonation is not as likely as a kind of complex formation between the imine and the acid – yet the real mechanism remains to be unsolved until now[136]. The amine complex or the iminium ion as well as the carboxylate afterwards react with the isonitrile to the primary Ugi product or α-adduct, which needs to rearrange through an acyl-shift that runs in a similar fashion like a classical Mumm rearrangement (Scheme 2)[137]. In the final product the amine has been coupled to the carboxylic acid and is furthermore aklylated with the former keto-component carrying a primary amide with the former isonitrile residue. The so formed peptoid-peptide chimera can be isolated in high to very high yields in most of the cases. It could be shown that also crowded products with an Aib moiety could be readily accessed by using acetone one of the Ugi-reactive groups (URGs)[138].

The U-4CR can be used to synthesize long peptide-peptoid sequences, if one of the components carries a protected Ugi-reactive group (PURGs), which is deprotected successively and the obtained, activated U-4CR product itself is employed as one of the starting materials of another U-4CR. To generate longer peptoid-peptide sequences it is necessary to install the respective protecting group on a carboxylic acid, which is part of the isonitrile used in the first U-4CR[139,140]. This URG can be seen as a glycine derivative, where the amine functionality is transformed into an isocyanide moiety and the carboxylic acid is protected as an ester. After the deprotection step of the U-4CR product a carboxylic acid is obtained and can be used in the next step (Scheme 3). The peptidic pattern is not mixed in this approach, whereas a protected, bifuntional keto-, acid- or amine-building block would lead to branched oligo peptides, if it is used in a consecutive fashion. For the generation of an alternating peptoid-peptide sequence containing classical peptoid parts (*N*-substituted glycins) the use of formaldehyde is inevitable.



Scheme 3. Sequential U-4CRs and deprotection steps in the synthesis of a peptide-peptoid chimera.

In this sequential approach it is possible to use fatty acids to generate analogs of lipopeptides that are known for a promising bioactivity. In first approaches, it could be shown that chimeric lipopeptide-peptoids (LPPs) can be effective against some different kinds of fungi. Nevertheless, only two iterative U-4CRs have been implemented and no structure-activity correlation analysis has been applied[141]. For reasons of directed tackling special pathogens, this analysis would be highly beneficial.

1.5 Synopsis and objective

Most of the natural peptidic compounds that show antibiotic activity do act on the cellular membrane. This mode of action is especially applied by amphiphilic compounds with a hydrophobic and a hydrophilic part like the lipopeptides[142–145]. The hydrophobicity of the fatty acid component allows for a membrane attachment or the formation of supramolecular structures within it. Unlike classic detergents the membrane is not completely ruptured, rather pores or artificial channels are formed. The architecture of membrane-active (lipo-)peptides has already been adapted by approaches based on peptoids that are more stable against hydrolytic enzymes[146,147]. To facilitate the synthesis of long peptide/peptoid sequences with a broad variety of side chains, the Ugi four-component reaction has been applied successfully[141]. With this facile setup the most promising features of membrane active compounds can easily be combined and it could be shown that also mixed peptidic-peptoidic compounds with a hydrophobic tail can be active against pathogens.

To get a broader knowledge about the influence of the length of the fatty acid attached to the hydrophilic part and the number of side chain residues needed for an antimicrobial activity it was planned to synthesize a library of chimeric polycationic lipopeptide-peptoids. After the successful synthesis, this compound library should be activity screened against the gram-negative model organism *Aliivibrio fischeri*, different fungal strains, human cancer cells and their hemolytic activity was determined. Based on earlier work on consecutive Ugi four-component reactions some promising sequences were re-synthesized with a fatty acid attached as activity trigger. Also fluorescence-labeled model compounds were planned to be generated to get an insight into the cellular distribution by fluorescence-microscopy.

2 Polycationic Lipophilic Peptoid-Peptide Chimeras

2.1 General setup and synthesis of 4-azidobutylamine

For the synthesis of a library of polycationic lipophilic peptoid-peptide chimeras (LPPs) lipopolylysins[148] and polycationic peptoids[133] were used as blueprints (Figure 17). The length of the side chain is therefore fixed at four carbon atoms with a terminal amino moiety. Regarding the Ugi-reactivity of a free amino group on the side chain it needs to be protected or masked during the synthesis.

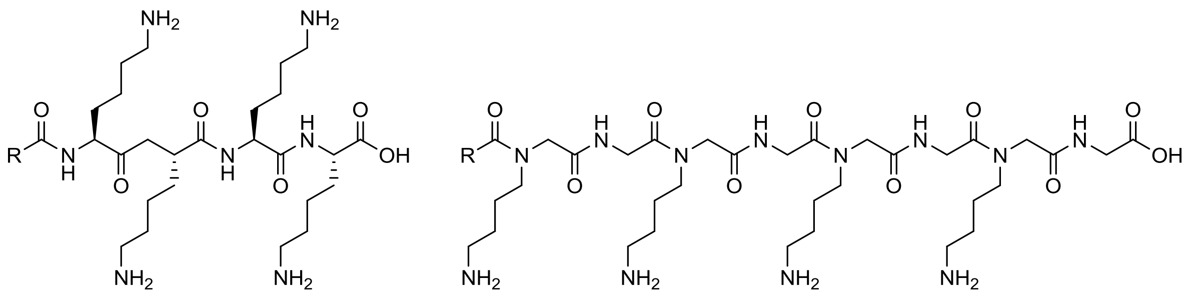
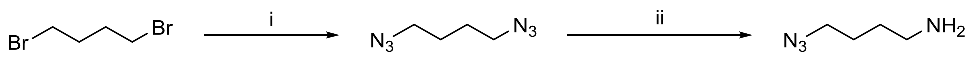


Figure 17. Structural formulas of a lipotetra(*L*-lysine) and a tetrameric LPP. Although both compounds carry the same number of amino groups the peptidic lysine derivative is much shorter than the LPP, which consists in principle of eight glycine residues in contrast to only four lysins in the lipopeptide.

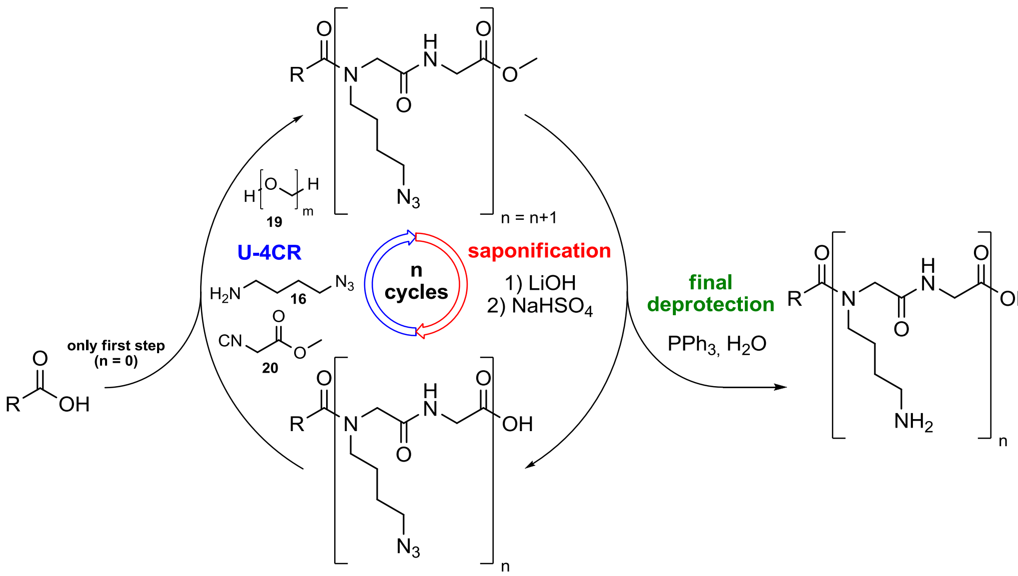
Due to the low polarity, the easy introduction and the chemical versatility allowing different kinds of transformations, the azide group was chosen[149]. Therefore 4-azidobutylamine (**16**) needed to be synthesized as a building block for the sequential U-4CR. The synthesis started with commercially available 1,4-dibromobutane (**17**), which was reacted with sodium azide to generate the symmetrical 1,4-diazidobutane (**18**).



Scheme 4. Synthesis of 4-azidobutylamine (**16**) starting from 1,4-diaminobutane (**17**). *Reagents and conditions*: i) 2.1 eqv. NaN_3, DMF/H_2O, RT → 80 °C, 20 h, no purification; ii) 0.86 eqv. PPh_3, 1M HCl, EtOAc, 0 °C → RT, 16 h, 84% over two steps.

This intermediate was used without purification due to its high nitrogen content of 60 w% in order to avoid explosive decomposition. A selective reduction of only one of the azide moieties in an acidic biphasic system of ethyl acetate and water employing triphenylphosphine as the reducing agent gave access to the desired building block **16** in high yield (Scheme 4)[150].

The LPP synthesis strategy itself follows a cyclic pathway. A U-4CR is followed by alkaline hydrolysis of the terminal methyl ester of the Ugi product and a subsequent U-4CR[139]. This methodology is accomplished four times and after the fourth saponification of the ester moiety to generate the free acid, the protected amino groups are deprotected by a multiple Staudinger reduction using triphenylphosphine. The final compounds are chimeric octapeptoids carrying an *N*-terminal acylation (Scheme 5).



Scheme 5. Synthesis cycle for the generation of LPPs. The first cycle starts with an U-4CR of a fatty acid, 4-azidobutylamine (**16**), paraformaldehyde (**19**) and methyl isocyanoacetate (**20**). Subsequent hydrolysis is accomplished by alkaline saponification with lithiumhydroxide. The free LPP acid is then used as the carboxylic acid component in another U-4CR. After the desired number of cycles the azide moieties of the LPP acid are transformed into amino groups by Staudinger reduction. (R = H, CH_3 → $C_{19}H_{39}$).

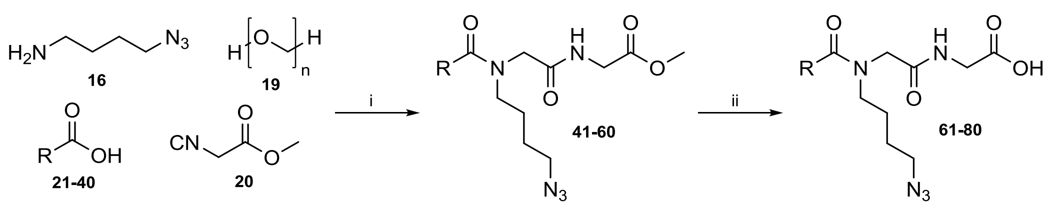
The fatty acids, which were used in the initial step, are linear ones with a carbon atom number ranging from 3 to 20. Additionally formic and acetic acid were used to cover also the range of very polar cationic LPPs (Table 6). This broad range of hydrophobicity was believed to include most of the chain lengths of fatty acids that are found in biological membranes. Therefore an activity maximum for a certain fatty acid chain length in the final LPP is expected.

Table 6. List of carboxylic (fatty) acids used for the synthesis of the LPP library.

C-atoms	Systematic name	Trivial name	Code	Molecular weight
1	Methanoic acid	Formic acid	**21**	46.03 g/mol
2	Ethanoic acid	Acetic acid	**22**	60.05 g/mol
3	Propanoic acid	Propionic acid	**23**	74.08 g/mol
4	Butanoic acid	Butyric acid	**24**	88.11 g/mol
5	Pentanoic acid	Valeric acid	**25**	102.13 g/mol
6	Hexanoic acid	Caproic acid	**26**	116.16 g/mol
7	Heptanoic acid	Enanthic acid	**27**	130.18 g/mol
8	Octanoic acid	Caprylic acid	**28**	144.21 g/mol
9	Nonanoic acid	Pelargonic acid	**29**	158.24 g/mol
10	Decanoic acid	Capric acid	**30**	172.26 g/mol
11	Undecanoic acid	Undecylic acid	**31**	186.29 g/mol
12	Dodecanoic acid	Lauric acid	**32**	200.32 g/mol
13	Tridecanoic acid	Tridecylic acid	**33**	214.34 g/mol
14	Tetradecanoic acid	Myristic acid	**34**	228.37 g/mol
15	Pentadecanoic acid	Pentadecylic acid	**35**	242.40 g/mol
16	Hexadecanoic acid	Palmitic acid	**36**	256.42 g/mol
17	Heptadecanoic acid	Margaric acid	**37**	270.45 g/mol
18	Octadecanoic acid	Stearic acid	**38**	284.48 g/mol
19	Nonadecanoic acid	Nonadecylic acid	**39**	298.50 g/mol
20	Eicosanoic acid	Arachidic acid	**40**	312.53 g/mol

2.2 Synthesis of the first generation of azido-LPPs

For the synthesis of the first generation (U-4CR and saponification) of azido-LPPs a general U-4CR approach was used. To pre-form the imine a 100 mM solution of amine **16** in methanol was treated with 1.67 equivalents of polymeric aldehyde **19** and the heterogenous mixture was stirred for two hours at room temperature, whereupon it got almost clear. For an easier execution the imine solution was prepared for respectively ten U-4CR at once. It was afterwards split into ten parts and the respective carboxylic acids were added equimolarly to the solutions. Successively an equimolar amount of isonitrile **20** was added and the reaction mixtures were stirred at least over night. After flash chromatography on silica gel with mixtures of ethyl acetate and *n*-hexane as eluents all desired compounds were obtained as sticky oils or amorphous solids, depending on the chain length of the fatty acid.



Scheme 6. Synthesis of the first generation azido-LPPs **61-80**. (R = H, CH_3 → $C_{19}H_{39}$). *Reagents and conditions*: i) MeOH, RT, 16 h; ii) LiOH · H_2O, THF/H_2O, RT, 2 h.

The Ugi-products **41** – **60** were then saponified with lithiumhydroxide in a mixture of tetrahydrofurane and water. The *C*-terminal methyl ester is very reactive towards alkaline saponification and therefore the reactions proceeded quickly. After acidification with saturated sodium hydrogensulfate solution the LPP acids could be extracted with ethyl acetate. Compounds **61** – **80**, which were obtained after evaporation of the solvent showed already high purity and were therefore not further purified (Scheme 6).

Table 7. Isolated yields for the first generation azido-LPPs of the U-4CR and subsequent saponification.

C-atoms (fatty acid)	Code		Molecular weight		Yield		
	Ester	*Acid*	*Ester*	*Acid*	*U-4CR*	*Sap.*	*2 steps*
1	**41**	**61**	257.25 g/mol	243.22 g/mol	75%	53%	40%
2	**42**	**62**	271.27 g/mol	257.25 g/mol	82%	98%	80%
3	**43**	**63**	285.30 g/mol	271.27 g/mol	77%	93%	72%
4	**44**	**64**	299.33 g/mol	285.30 g/mol	78%	99%	78%
5	**45**	**65**	313.35 g/mol	299.33 g/mol	79%	99%	78%
6	**46**	**66**	327.38 g/mol	313.35 g/mol	77%	99%	76%
7	**47**	**67**	341.41 g/mol	327.38 g/mol	78%	99%	78%
8	**48**	**68**	355.43 g/mol	341.41 g/mol	79%	99%	79%
9	**49**	**69**	369.46 g/mol	355.43 g/mol	79%	99%	79%
10	**50**	**70**	383.49 g/mol	369.46 g/mol	77%	99%	76%
11	**51**	**71**	397.51 g/mol	383.49 g/mol	75%	99%	78%
12	**52**	**72**	411.54 g/mol	397.51 g/mol	80%	99%	79%
13	**53**	**73**	425.57 g/mol	411.54 g/mol	77%	99%	77%
14	**54**	**74**	439.59 g/mol	425.57 g/mol	77%	99%	77%
15	**55**	**75**	453.62 g/mol	439.59 g/mol	76%	99%	76%
16	**56**	**76**	467.65 g/mol	453.62 g/mol	77%	99%	76%
17	**57**	**77**	481.67 g/mol	467.65 g/mol	77%	99%	76%
18	**58**	**78**	495.70 g/mol	481.67 g/mol	73%	99%	72%
19	**59**	**79**	509.72 g/mol	495.70 g/mol	72%	quant.	72%
20	**60**	**80**	523.75 g/mol	509.72 g/mol	68%	99%	67%

The yields of the U-4CR were generally in the range of 75% or higher for most of the carboxylic acids. In case of the fatty acids **38**, **39** and **40** with chain lengths of 18 or more carbon atoms the yields of the U-4CR started to decrease with increasing alkyl chain length – maybe due to limited solubility of the free fatty acids in methanol during the reaction. The saponification step worked in high yields, except for compound **61**, the formic acid derivative (Table 7). This might be due to the very high polarity of this compound, which leads to a better solubility in the acidified aqueous phase after the saponification step and therefore an unfavorable partition coefficient between the aqueous phase and ethyl acetate.

All compounds were investigated among others by NMR analysis. The U-4CR products were freely soluble in deuterochloroform, whereas the free acids showed a much lower solubility. Therefore the spectra of the free acids needed to be recorded in deuteromethanol. The spectra of all compounds showed the existence of the (*S*)-cis as well as the (*S*)-trans rotamer. The ratio is, besides the nature of the *C*-terminus, strongly solvent-dependant. For the methyl esters **41–60**, dissolved in deuterochloroform, the observed ratio between both rotamers is approximately 1:3. Most likely the transoid species is higher populated than the cisoid one. The free acids **61–80**, dissolved in deuteromethanol, showed a different behavior. Both rotamers were equally populated for nearly all compounds. Only the LPP acids with formyl and acetyl moieties (**61**, **62**) showed one slightly favoured rotamer.

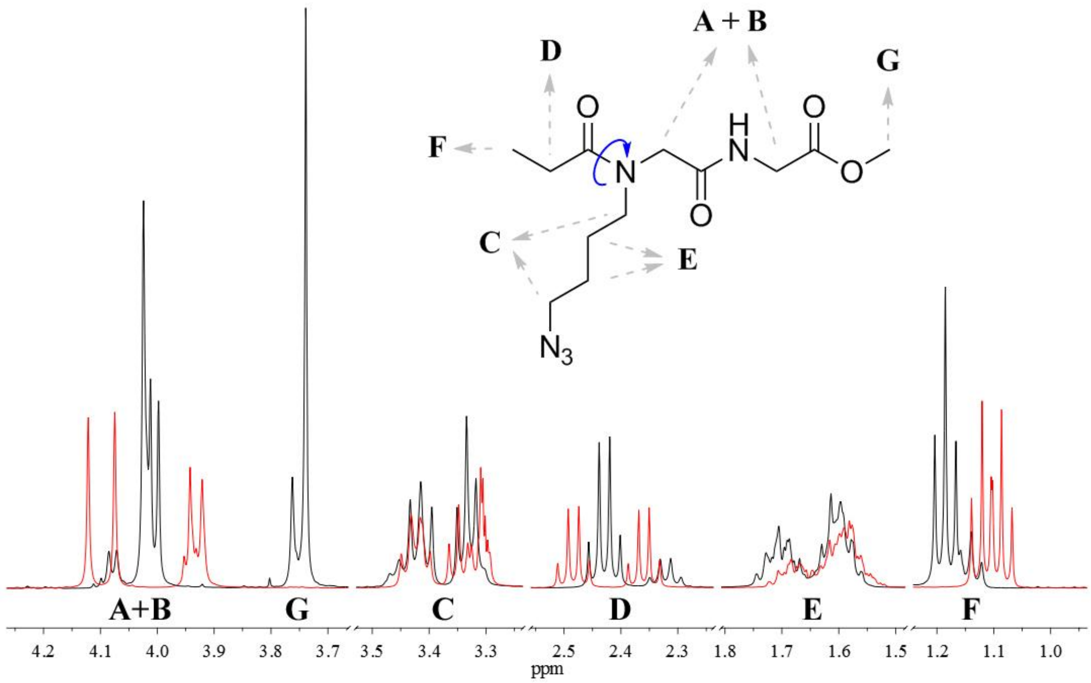
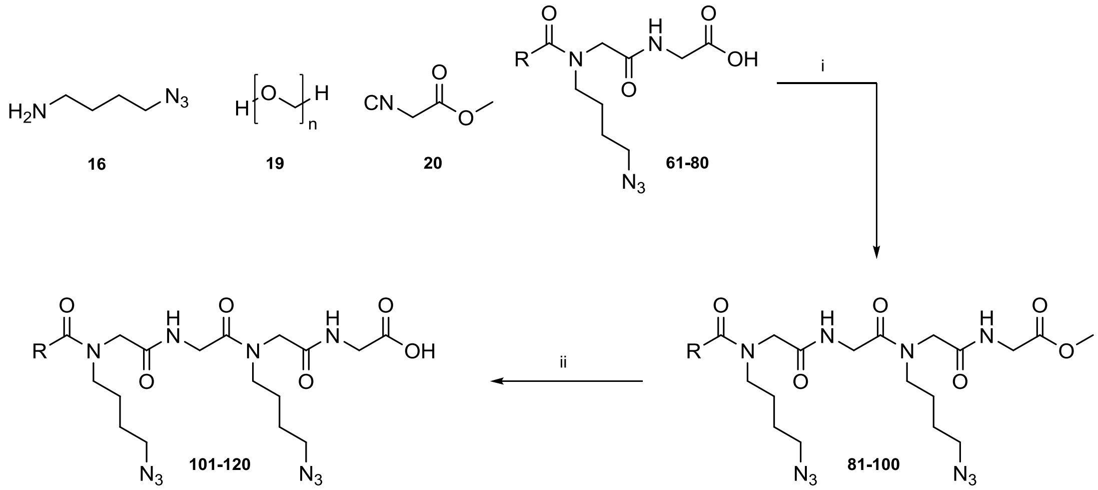


Figure 18. Segmented and superimposed [1]H-NMR spectra at 400 MHz of **43** in $CDCl_3$ (**black** line) and **63** in CD_3OD (**red** line). The **blue** arrow marks the tertiary amide bond, which is the cause for the appearance of rotamers. *Assignment:* (A+B) 4 methylene protons of the glycine moieties; (C) 4 methylene protons of the side chain directly connected to nitrogen atoms of the amide or the azide; (D) 2 *α*-methylene protons of the propioic acid moiety; (E) 4 inner methylene protons of the side chain; (F) 3 terminal methyl protons of the propionic acid residue; (G) 3 methyl protons of the methyl ester (only present in **43**).

In direct comparison of the spectra of a protected in contrast to a deprotected *C*-terminus the major difference besides the occurrence of rotamers is the appearance of the backbone methylene protons. In the methyl ester LPPs the chemical shifts of the protons is quite similar, whereas in the free acid a clear separation into two segments can be observed. This observation might be due to solvent effects in combination with the modified structure (Figure 18).

2.3 Synthesis of the second generation of azido-LPPs

For the synthesis of the second generation of azido-LPPs the same U-4CR approach was used. After the imine formation of amine **16** and 1.67 equivalents of paraformaldehyde **19** in methanol (100 mM) equimolar amounts of the respective carboxylic acids **61–80** were dissolved in the solution and afterwards the isonitrile **20** was added equimolarily. The reaction mixtures were stirred at least over night at room temperature before the crude compounds were purified by flash chromatography employing mixtures of ethyl acetate and methanol as eluents. Compounds **81–100** were obtained as oils or amorphous solids in slightly higher yields (>80%) than for the respective first generation U-4CR.



Scheme 7. Synthesis of the second generation azido-LPPs **101-120**. (R = H, CH_3 → $C_{19}H_{39}$). *Reagents and conditions*: i) MeOH, RT, 16 h; ii) LiOH · H_2O, THF/H_2O, RT, 2 h.

The saponification reactions proceeded analogously like for the first generation (Scheme 7). After extraction with ethyl acetate, the free acids **101** – **120** could be obtained as amorphous solids, except **101** and **102**, which appeared to be sticky oils. Due to the longer peptoid-peptide sequence and the vanishing influence of the short, hydrophilic formyl residue in compound **101** the yield for the saponification after the extraction with ethyl acetate is with 99.6% much higher in this step than the yield for the first step of 53% for precursor compound **61** (Table 8).

Table 8. Isolated yields for the second generation azido-LPPs of the U-4CR and subsequent saponification. [a] Lower yield due to loss of parts of the product during purification.

C-atoms (fatty acid)	Code		Molecular weight		Yield		
	Ester	*Acid*	*Ester*	*Acid*	*U-4CR*	*Sap.*	*2 steps*
1	**81**	**101**	482.49 g/mol	468.47 g/mol	78%	99%	78%
2	**82**	**102**	496.52 g/mol	482.49 g/mol	82%	95%	77%
3	**83**	**103**	510.55 g/mol	496.52 g/mol	85%	93%	79%
4	**84**	**104**	524.57 g/mol	510.55 g/mol	80%	99%	80%
5	**85**	**105**	538.60 g/mol	524.57 g/mol	81%	99%	81%
6	**86**	**106**	552.63 g/mol	538.60 g/mol	81%	97%	78%
7	**87**	**107**	566.65 g/mol	552.63 g/mol	78%	99%	78%
8	**88**	**108**	580.68 g/mol	566.65 g/mol	81%	97%	78%
9	**89**	**109**	594.71 g/mol	580.68 g/mol	69%[a]	99%	69%[a]
10	**90**	**110**	608.73 g/mol	594.71 g/mol	79%	99%	78%
11	**91**	**111**	622.76 g/mol	608.73 g/mol	84%	96%	80%
12	**92**	**112**	636.79 g/mol	622.76 g/mol	80%	99%	79%
13	**93**	**113**	650.81 g/mol	636.79 g/mol	83%	94%	78%
14	**94**	**114**	664.84 g/mol	650.81 g/mol	86%	95%	82%
15	**95**	**115**	678.87 g/mol	664.84 g/mol	81%	97%	79%
16	**96**	**116**	692.89 g/mol	678.87 g/mol	82%	97%	80%
17	**97**	**117**	706.92 g/mol	692.89 g/mol	83%	95%	78%
18	**98**	**118**	720.95 g/mol	706.92 g/mol	86%	95%	81%
19	**99**	**119**	734.97 g/mol	720.95 g/mol	81%	99%	80%
20	**100**	**120**	749.00 g/mol	734.97 g/mol	77%	97%	74%

NMR spectroscopical investigations revealed that due to the existence of two peptoid bonds the number of possible isomers increased. This leads to spectra with smoothed signals in contrast to the respective spectra of compounds of the first generation. In case of a direct comparison of the propionyl derivatives **63** and **103**, which are both carboxylic acids, dissolved in deuteromethanol, it is clearly visible that the first generation of free azido-LPP acids give distinct signals for both rotamers, whereas a differentiation of single rotameric species is not longer possible for the second generation products (Figure 19).

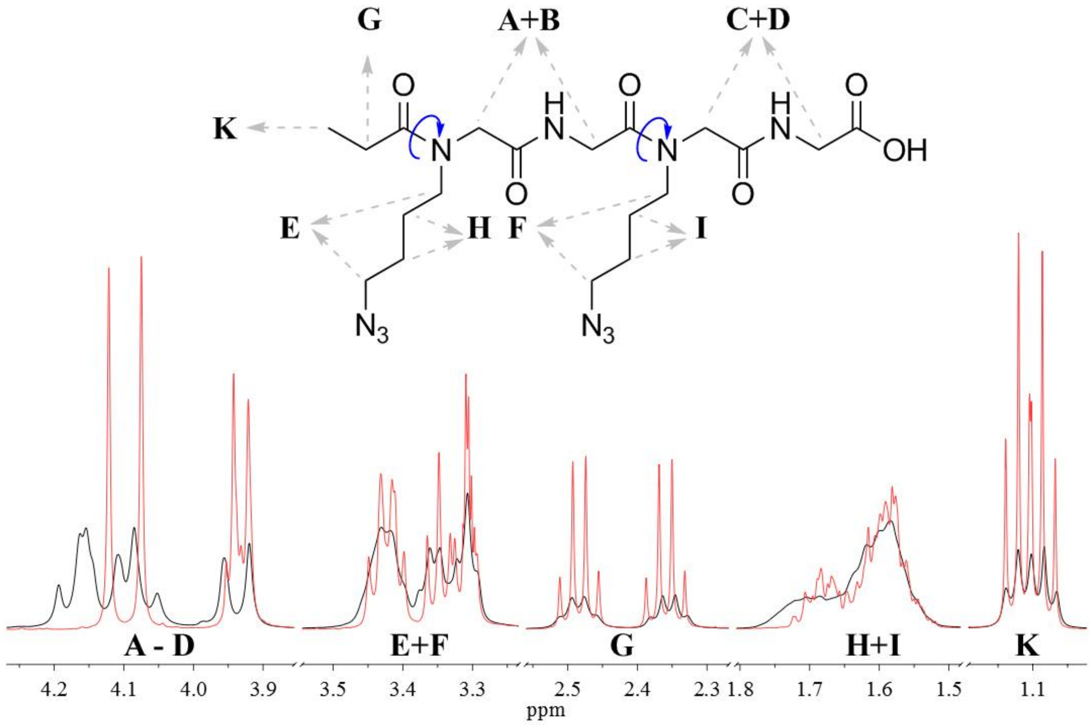
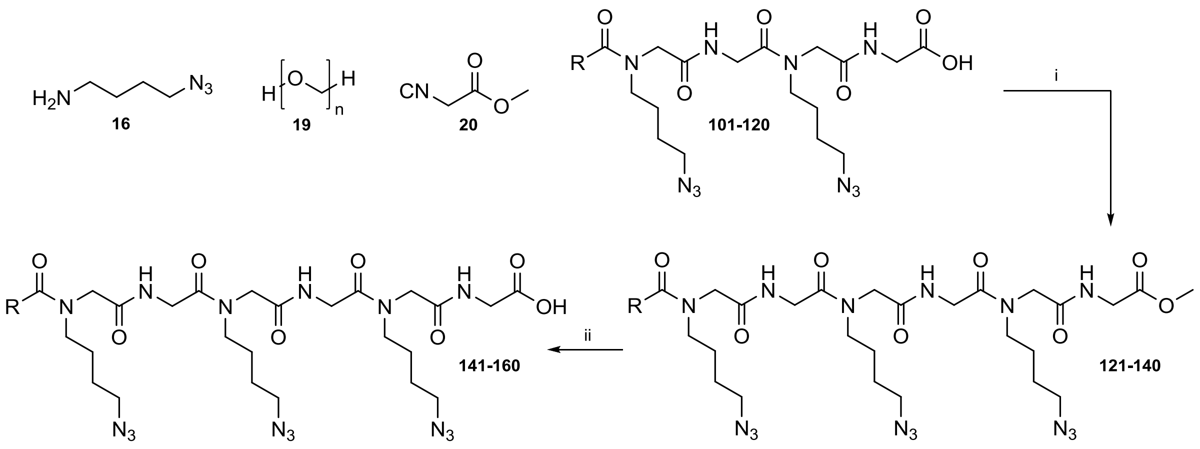


Figure 19. Segmented and superimposed ^{1}H-NMR spectra at 400 MHz of **103** in CD_3OD (**black** line) and **63** in CD_3OD (**red** line). The **blue** arrow marks the tertiary amide bond, which is the cause for the appearance of rotamers. *Assignment (for* ***103****):* (A-D) 8 methylene protons of the glycin moieties; (E+F) 8 methylene protons of the side chains directly connected to nitrogen atoms of the amide or the azide; (G) 2 α-methylene protons of the propioic acid moiety; (H+I) 8 inner methylene protons of the side chains; (K) 3 terminal methyl protons of the propionic acid residue. The assignment for compound **63** is the same in shift regions, but lacking C, D, F and I.

The observation that the ratio of rotamers is solvent-depending like described for the first generation LPPs is also true for the second generation. In aprotic deuterochloroform at least one of the possible rotamers seems to be higher populated. Maybe the missing hydrogen-bonds between the LPP and the solvent lead to the formation of a kind of secondary structure with internal stabilization via hydrogen-bonding.

2.4 Synthesis of the third generation of azido-LPPs

The third generation of azido-LPPs was synthesized analogously to the first two generations. Each second generation azido-LPP acid **101–120** was dissolved equimolarily in a preformed imine solution (100 mM) of amine **16** and polymeric aldehyde **19** in methanol in a 1:1.67 molar ratio. Successively the isonitrile **20** was added and the mixtures were stirred at room temperature. All reactions proceeded over night and the compounds **137–140** with the longest alkyl chains of the fatty acid precipitated partially from the reaction solution. All other compounds **121–136** stayed dissolved and were purified by flash chromatography like the respective generations before.



Scheme 8. Synthesis of the third generation azido-LPPs **141-160**. (R = H, CH_3 → $C_{19}H_{39}$). *Reagents and conditions*: i) MeOH, RT, 16 h; ii) LiOH · H_2O, THF/H_2O, RT, 2 h.

The precipitated products were filtrated and the filtrate was purified analogously to the non-precipitated ones. After investgating the filter residues with NMR spectroscopical methods it turned out that the precipitate was consisting of very pure product. No differences in the distribution of distinct rotamers were observable in direct comparison of the spectra of the precipitated to the conventionally purified compounds. Therefore, both batches were combined and submitted to the saponification reaction like the rest of the compounds. The average yield of the U-4CR for the third step was much lower (60% - 70 %) in comparison to the two U-4CR before (Table 9) for the short chain compounds **121–130**. For the compounds **131–140** with a more hydrophobic fatty acid the yields increased and reached a level comparable to the U-4CR steps before.

During flash chromatographic purification of compound **121** it could be observed that in the region where the product was expected at least two different compounds eluated. In mass spectrometrical investigations both compounds showed the same *m*/*z* ratio and the same fragmentation pattern. This leads to the assumption that both compounds are semi-stable rotamers of **121**. After short incubation of a pure fraction of one of the compounds and the development of a second TLC both spots were visible again. This is a strong hint that both isoforms are in a kind of kinetic equilibrium. However, the transformation of the two isomers is slower than the time scale of a normal flash chromatographic purification and they are therefore separable, but not stable as single isomers. Hence, both fractions were combined and further determined as compound **121**. With the appearance of two detectable isomers in this reaction it cannot be excluded that there are even more non-detectable rotamers (due to low concentrations) separable by chromatography, which lower the isolated yield, because they were not spotted properly. Nevertheless, the combined batches of **121** showed consistent NMR spectra in comparison to the expected pattern – only the variety of rotamers was visible in the spectra.

Table 9. Isolated yields for the third generation azido-LPPs of the U-4CR and subsequent saponification. [a] yield of the precipitate; [b] yield derived from the purified filtrate.

C-atoms (fatty acid)	Code		Molecular weight		Yield		
	Ester	*Acid*	*Ester*	*Acid*	*U-4CR*	*Sap.*	*2 steps*
1	**121**	**141**	693.72 g/mol	679.69 g/mol	58%	quant.	58%
2	**122**	**142**	707.74 g/mol	693.72 g/mol	71%	quant.	71%
3	**123**	**143**	721.77 g/mol	707.74 g/mol	68%	quant.	68%
4	**124**	**144**	735.80 g/mol	721.77 g/mol	56%	quant.	56%
5	**125**	**145**	749.82 g/mol	735.80 g/mol	57%	quant.	57%
6	**126**	**146**	763.85 g/mol	749.82 g/mol	72%	quant.	72%
7	**127**	**147**	777.87 g/mol	763.85 g/mol	66%	quant.	66%
8	**128**	**148**	791.90 g/mol	777.87 g/mol	62%	quant.	62%
9	**129**	**149**	805.93 g/mol	791.90 g/mol	66%	quant.	66%
10	**130**	**150**	819.95 g/mol	805.93 g/mol	72%	quant.	72%
11	**131**	**151**	833.98 g/mol	819.95 g/mol	73%	quant.	73%
12	**132**	**152**	848.01 g/mol	833.98 g/mol	74%	quant.	74%
13	**133**	**153**	862.03 g/mol	848.01 g/mol	73%	quant.	73%
14	**134**	**154**	876.06 g/mol	862.03 g/mol	73%	quant.	73%
15	**135**	**155**	890.09 g/mol	876.06 g/mol	78%	99%	78%
16	**136**	**156**	904.11 g/mol	890.09 g/mol	78%	99%	78%
17	**137**	**157**	918.14 g/mol	904.11 g/mol	20%[a] 57%[b]	99%	76%
18	**138**	**158**	932.17 g/mol	918.14 g/mol	13%[a] 62%[b]	98%	74%
19	**139**	**159**	946.19 g/mol	932.17 g/mol	10%[a] 65%[b]	98%	73%
20	**140**	**160**	960.22 g/mol	946.19 g/mol	23%[a] 50%[b]	99%	73%

Saponification of compounds **121–140** in a mixture of THF and water with 2.5 equivalents of lithium hydroxide afforted the third generation azido-LPP acids **141–160** in quantitative yields as amorphous solids (Scheme 8). All compounds contained variable amounts (up to 2%) of non-removable water from the extraction process. This impurity could not be removed – even by extensive drying in high vacuum. Since this kind of trace impurity does not interfere with the next reaction step, all compounds were used without further purification.

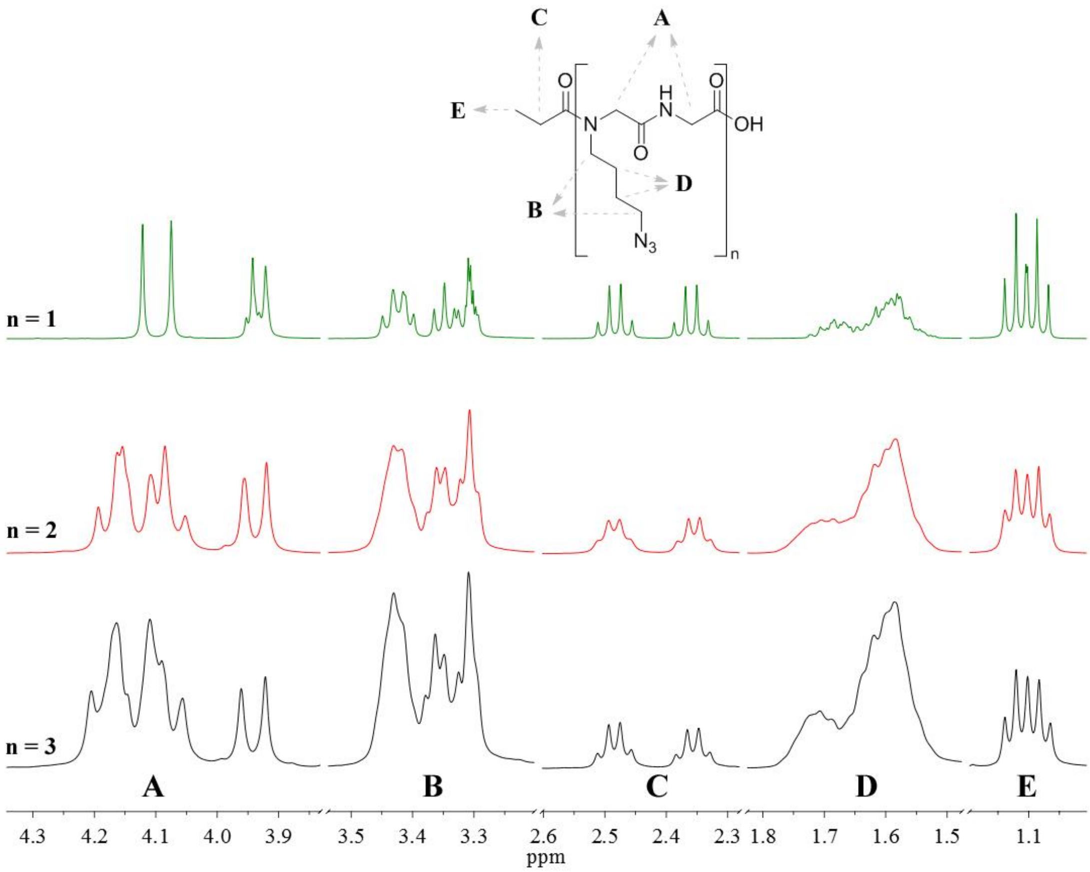
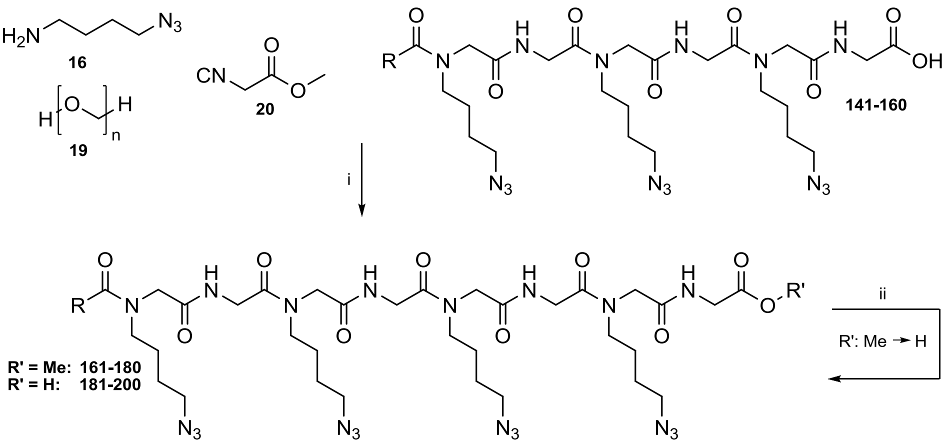


Figure 20. Segmented and superimposed ^{1}H-NMR spectra at 400 MHz in CD_3OD of **63** (green line), **103** (**red** line) and **143** (**black** line). *General assignment:* (A) methylene protons of the glycine residues in the backbone; (B) methylene protons of the side chains directly connected to nitrogen atoms of the amide or the azide; (C) 2 *α*-methylene protons of the propioic acid moiety; (D) inner methylene protons of the side chains; (E) 3 terminal methyl protons of the propionic acid residue.

NMR spectroscopic analysis revealed no relevant structural differences in comparison to the proton spectrum of generation two. There are only slight changes in the signal shape of the glycine backbone protons visible (Figure 20). Even the ratio of the two main isomers, generated by the rotation around the *N*-terminal peptoid bond, remains constant for the three compounds **63**, **103** and **143** (signal C, Figure 20). This observation is true for all other third generation compounds of the library and their respective earlier generation relatives.

2.5 Synthesis of the fourth generation of azido-LPPs

The synthesis of the fourth generation of azido-LPPs was accomplished like the syntheses before. The respective third generation azido-LPP acids **141–160** were dissolved equimolarily in the preformed imine solution of amine **16** and 1.67 equivalents of paraformaldehyde **19** in methanol (100 mM). Successively isonitrile **20** was added and the mixtures were stirred at least over night. In contrast to the third generation reaction all reactions with fatty acid chain lengths of five or more carbon atoms produced a precipitate consisting of pure U-4CR products **165–180**. The precipitates were collected by filtration and the filtrates were additionally purified by means of flash chromatography.



Scheme 9. Synthesis of the fourth generation azido-LPPs **181-200**. (R = H, CH_3 → $C_{19}H_{39}$). *Reagents and conditions*: i) MeOH, RT, 16 h; ii) LiOH · H_2O, THF/H_2O, RT, 2 h.

The four short chain representatives **161–164** did not show any precipitation and were therefore purified directly by flash chromatography. The precipitated compounds appeared to be white to slightly yellow powders in contrast to the chromatographed batches, which were mostly colorless to slightly coloured amorphous, glassy solids. Both batches of each compound showed up to be identical in NMR and MS analyses and were therefore combined like it was done already for the third generation U-4CR products. The average yields of the U-4CR were again lower (40% - 60%) than for the generation before. Only the two compounds **176** and **177** could be isolated in yields slightly higher than 70%. This drastical difference between the short- and the long-chain derivatives might be caused by folding effects, which are strongly influenced by the length of the fatty acid residue. Maybe back-folding effects are occurring for the short-chain compounds, which lead to a sterical shielding of the carboxylic acid moiety at the *C*-terminus (Table 10).

Saponification of compounds **161–180** with 2.5 equivalents of lithium hydroxide in a mixture of THF and water at room temperature afforded the free acids of fourth generation azido-LPPs **181–200** (Scheme 9). All compounds were purified by precipitation. The solvent combinations were quite variable for the separate compounds. Although the purity of the compounds was already high after the precipitation step a subsequent purification procedure was accomplished to remove inorganic and polymeric by-products as well as coloured impurities. Therefore all compounds were dissolved in mixtures of methanol and ethanol depending on their chain length. All solutions formed a mucous, light brown precipitate after incubation at room temperature over night, which was removed by filtration over a 0.22 µm PTFE syringe filter. Evaporation of the alcoholic solutions afforded pure azide-LPP acids as colorless, amorphous solids in moderate to high yields for the higher representatives of the compound library (Table 10).

Table 10. Isolated yields for the fourth generation azido-LPPs of the U-4CR and subsequent saponification. [a] yield of the precipitate; [b] yield derived from the purified filtrate; [c] a part of the product was lost.

C-atoms (fatty acid)	Code		Molecular weight		Yield		
	Ester	*Acid*	*Ester*	*Acid*	*U-4CR*	*Sap.*	*2 steps*
1	**161**	**181**	904.94 g/mol	890.91 g/mol	41%	61%	25%
2	**162**	**182**	918.96 g/mol	904.94 g/mol	52%	66%	35%
3	**163**	**183**	932.99 g/mol	918.96 g/mol	54%	91%	49%
4	**164**	**184**	947.02 g/mol	932.99 g/mol	46%	59%	27%
5	**165**	**185**	961.04 g/mol	947.02 g/mol	40%[a] 12%[b]	44%	23%
6	**166**	**186**	975.07 g/mol	961.04 g/mol	23%[a] 39%[b]	65%	40%
7	**167**	**187**	989.10 g/mol	975.07 g/mol	35%[a] 23%[b]	63%	36%
8	**168**	**188**	1003.12 g/mol	989.10 g/mol	20%[a] 37%[b]	75%	43%
9	**169**	**189**	1017.15 g/mol	1003.12 g/mol	29%[a] 38%[b]	81%	54%
10	**170**	**190**	1031.18 g/mol	1017.15 g/mol	27%[a] 19%[b]	73%	34%
11	**171**	**191**	1045.20 g/mol	1031.18 g/mol	26%[a] 36%[b]	98%	60%
12	**172**	**192**	1059.23 g/mol	1045.20 g/mol	27%[a] 31%[b]	99%	58%
13	**173**	**193**	1073.26 g/mol	1059.23 g/mol	26%[a] 28%[b]	94%	51%
14	**174**	**194**	1087.28 g/mol	1073.26 g/mol	42%[a] 17%[b]	61%[c]	36%[c]
15	**175**	**195**	1101.31 g/mol	1087.28 g/mol	19%[a] 36%[b]	99%	55%
16	**176**	**196**	1115.34 g/mol	1101.31 g/mol	41%[a] 30%[b]	quant.	71%
17	**177**	**197**	1129.36 g/mol	1115.34 g/mol	31%[a] 41%[b]	99%	71%
18	**178**	**198**	1143.39 g/mol	1129.36 g/mol	42%[a] 19%[b]	quant.	60%
19	**179**	**199**	1157.41 g/mol	1143.39 g/mol	32%[a] 33%[b]	quant.	65%
20	**180**	**200**	1171.44 g/mol	1157.41 g/mol	45%[a] 16%[b]	96%	59%

NMR spectroscopical analyses did not show big changes in comparison to the former generation. In an example spectrum of **163** (Figure 21) it is clearly visible that this compound consists of more than one isomer. The signal of the methyl group is a singulett, but it can be found three times in the spectrum and appears as a non-symmetrical triplett (signal C, Figure 21). The same is true for the ester carbonyl signal in the respective ^{13}C-NMR spectrum. At least three clear separated signals can be determined, belonging to this carbon atom (signal 2, Figure 21).

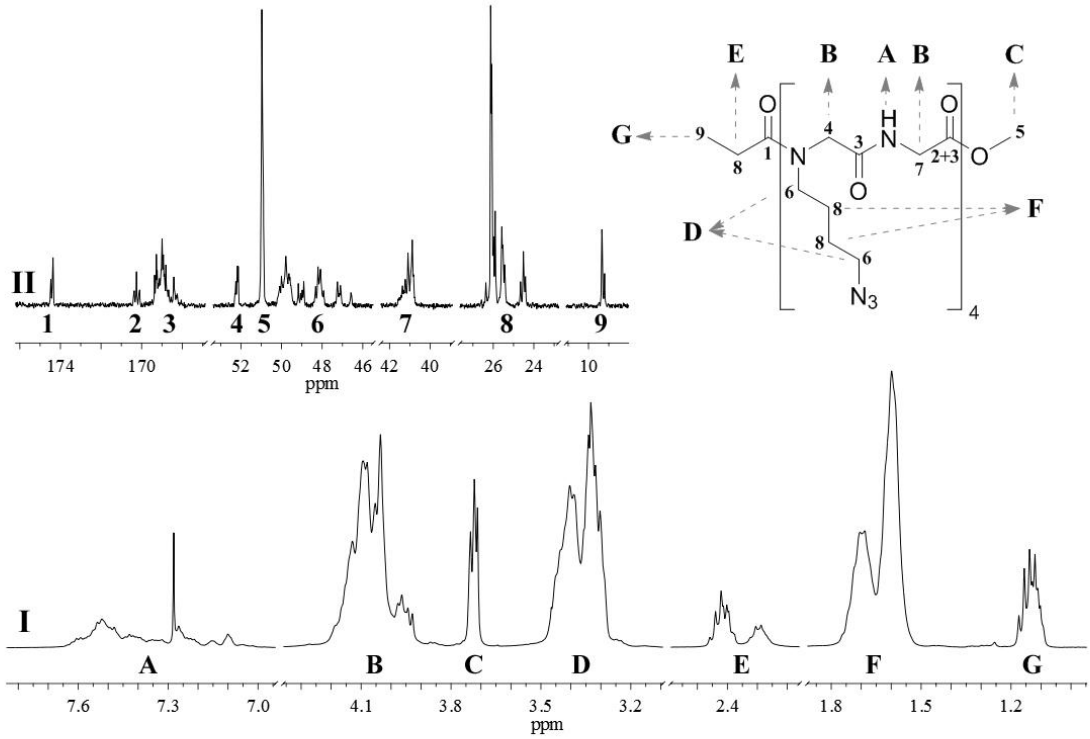


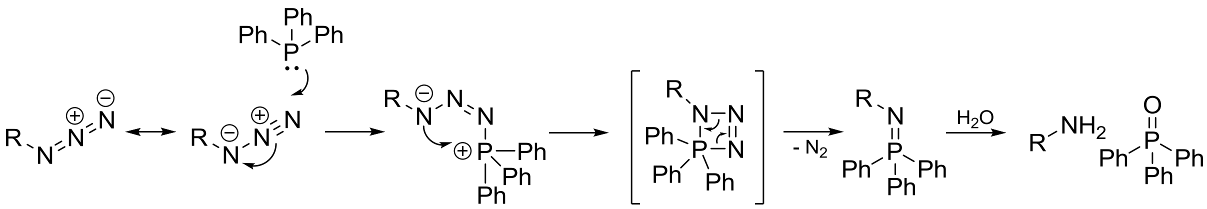
Figure 21. Segmented 400 MHz-^{1}H-NMR (I) and 100 MHz-^{13}C-NMR (II) spectra of compound **163** in $CDCl_3$. *Assignments*: ^{1}H-NMR: (A) 4 amide protons; (B) 16 methylene protons of the glycine backbone; (C) 3 protons of the methyl ester; (D) 16 methylene protons of the side chains directly connected to nitrogen atoms of the amide or the azide; (E) 2 α-methylene protons of the propioic acid moiety; (F) inner methylene protons of the side chains; (G) 3 terminal methyl protons of the propionic acid residue. ^{13}C-NMR: (1) propionyl carbonyl; (2) ester carbonyl; (3) backbone amide carbonyls; (4) glycine α-carbon of alkylated glycine; (5) methyl ester; (6) methylene carbons of the side chains attached to nitrogen; (7) glycine α-carbon of non-alkylated glycine; (8) methylene carbons at the inner side chains; (9) terminal methyl group of the propionyl moiety.

After the fourth reaction step no further prolongation reactions were done. The azido-LPP acids were used for bioactivity screening and the non-saponified azido-LPP methyl esters were submitted to azide reduction.

2.6 Staudinger reduction of the azido-LPPs

2.6.1 Staudinger reduction

Among the multiple options of reducing azide moieties to amines the Staudinger reduction is a robust method using non-expensive triphenylphosphine as the reducing agent[149,151,152]. The only drawback of this reaction is the formation of equimolar amounts of triphenylphoshine oxide as side product, which is often hardly removable from the desired product.

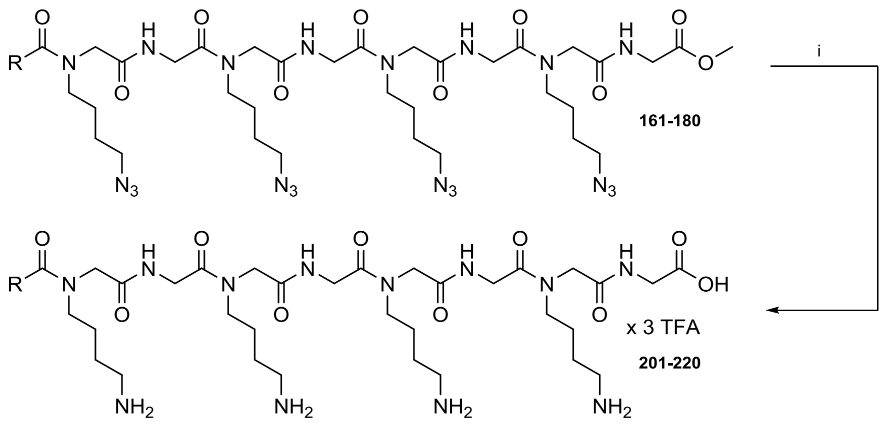


Scheme 10. Reaction mechanism of the Staudinger reduction with triphenylphosphine.

The key step of this reaction is the formation of a phosphazene by the addition of the phosphine to the azide under subsequent loss of molecular nitrogen. The strongly basic phosphazene needs to be hydrolyzed by the attack of water to form the free amine and triphenylphosphine oxide as the reduction side product (Scheme 10).

2.6.2 Synthesis of fourth generation amino-LPP acids

Through the strongly basic phosphazene intermediate and the basic amines generated during the reaction of an azido-LPP methyl ester two transformations could be accomplished at once. Besides the formation of the free amines in the side chain the alkaline environment facilitated the saponification of the terminal methyl ester moiety to the carboxylic acid (Scheme 11). All present protecting groups could therefore be removed in a single reaction step. Whereas the first step of the reduction, the phosphazene formation proceeds very quickly, the hydrolysis takes more time to be complete. Due to this fact and the four azide moieties present in each azido-LPP the single reactions needed to be stirred for more than three days at room temperature.



Scheme 11. Synthesis of the fourth generation amino-LPP acid TFA salts **201-220**. (R = H, CH_3 → $C_{19}H_{39}$). *Reagents and conditions*: i) PPh_3, THF/H_2O, RT, 84 h, then H_2O/TFA, RT, 2 h.

The respective azido-LPP methyl esters **161** – **180** were reacted with 6 molar equivalents of triphenylphosphine in a mixture of THF and water for 84 hours under an inert gas atmosphere. After removal of the THF *in vacuo* the suspension was acidified with TFA to complete phosphazene hydrolysis and stabilize the free amines as ammonium salts. The dried, crude material was afterwards purified by repeated precipitation from a methanolic solution by diethyl ether.

Table 11. Isolated yields for the Staudinger reduction of fourth generation azido-LPP methyl esters to amino LPP acid TFA salts. a) determined by ^{1}H-/^{19}F-NMR measurements with **221** as internal reference (see chapter 2.6.3); b) a part of the product was lost; c) yield lowered due to loss of product in one of the synthetic steps.

C-atoms (fatty acid)	Code		Molecular weight		Yield		TFA content[a)]
	Ester	*Amino Acid*	*Ester*	*Amino Acid (x 3 TFA)*	*Reduction*	*total (8 steps)*	
1	**161**	**201**	904.94 g/mol	1128.99 g/mol	80%	5.9%	3.4
2	**162**	**202**	918.96 g/mol	1143.02 g/mol	91%	20.9%	3.6
3	**163**	**203**	932.99 g/mol	1157.04 g/mol	52%[b)]	10.7%[c)]	4.1
4	**164**	**204**	947.02 g/mol	1171.07 g/mol	73%	11.5%	3.4
5	**165**	**205**	961.04 g/mol	1185.10 g/mol	81%	15.2%	2.9
6	**166**	**206**	975.07 g/mol	1199.12 g/mol	93%	24.8%	3.4
7	**167**	**207**	989.10 g/mol	1213.15 g/mol	9%	21.7%	3.5
8	**168**	**208**	1003.12 g/mol	1227.18 g/mol	81%	17.4%	3.0
9	**169**	**209**	1017.15 g/mol	1241.20 g/mol	92%	21.7%[c)]	2.9
10	**170**	**210**	1031.18 g/mol	1255.23 g/mol	92%	18.0%	2.8
11	**171**	**211**	1045.20 g/mol	1269.26 g/mol	90%	24.5%	3.1
12	**172**	**212**	1059.23 g/mol	1283.28 g/mol	92%	24.9%	3.0
13	**173**	**213**	1073.26 g/mol	1297.31 g/mol	87%	20.3%	3.0
14	**174**	**214**	1087.28 g/mol	1311.34 g/mol	93%	25.1%	3.4
15	**175**	**215**	1101.31 g/mol	1325.36 g/mol	54%[b)]	13.8%[c)]	3.9
16	**176**	**216**	1115.34 g/mol	1339.39 g/mol	94%	31.5%	2.9
17	**177**	**217**	1129.36 g/mol	1353.42 g/mol	92%	29.8%	3.1
18	**178**	**218**	1143.39 g/mol	1367.44 g/mol	82%	21.2%	3.3
19	**179**	**219**	1157.41 g/mol	1381.47 g/mol	83%	22.3%	3.1
20	**180**	**220**	1171.44 g/mol	1395.49 g/mol	81%	18.0%	3.0

This procedure allowed for the complete removal of remaining triphenylphosphine and its oxide due to the solubility of both compounds in diethyl ether. The amino-LPP acid TFA salts **201** – **220** are insoluble in ether and could therefore be obtained as white to off-white, non hygroscopic powders with no triphenylphosphine or its oxide as contaminants detectable. The isolated yields for the combined reduction/saponification were high to excellent with >80% for most of the derivatives and >90% for half of the compounds (Table 11).

2.6.3 Determination of the TFA content of the amino-LPP acids

The final products **201** – **220** contain four amino groups as well as a carboxylic acid moiety (Figure 22). Due to the acidic workup with excess TFA the final acid content in the compounds needed to be determined.

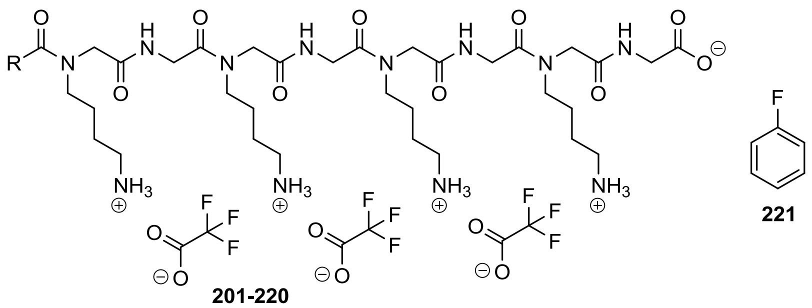


Figure 22. Structural formulas of the final amino-LPP acids **201** – **220** and fluorobenzene (**221**). (R = H, CH_3 → $C_{19}H_{39}$).

After the several precipitation steps the additional acid should be removed and three molar equivalents should remain in the final products due to one amino group forming a betain with the terminal carboxylic acid. For determination of the TFA content NMR samples were prepared in deuteromethanol and a small amount of fluorobenzene (**221**) was added as internal reference (Figure 22). The signals of **221** in the ^{1}H-NMR spectrum appear in a region where the LPP doesn't show a resonance (7.00 – 7.40 ppm). The same is true for the signal of the fluorine atom in **221** compared to TFA in the ^{19}F-NMR spectrum (–74.9 and –113.5 ppm).

$$\frac{2I_{F,TFA}I_{H,FB}}{9I_{F,FB}I_{H,LPP}} = Q_{TFA/LPP} \quad (1)$$

$$\frac{16I_{F,TFA}I_{H,FB}}{9I_{F,FB}I_{H,LPP}} = Q_{TFA/LPP} \quad (2)$$

Therefore it was possible to achieve quantitative measurements and after integration of the signals a double relation could be calculated to give the final molar relation between TFA and the respective amino-LPP acid (Figure 23). The integrals of the respective signals of the two α-protons ($I_{H,LPP}$), three protons of fluorobenzene ($I_{H,FB}$), the fluorine atom of fluorobenzene ($I_{F,FB}$) and the three fluorine atoms of TFA ($I_{F,TFA}$) were used for the calculations of the TFA/LPP ratios ($Q_{TFA/LPP}$) of compounds **204** – **220** (formula 1). For the three short chain compounds **201** – **203** another integral for the LPP (16 inner side chain protons) and a modified formula was used due to the shift or non-existence of the two α-protons (formula 2).

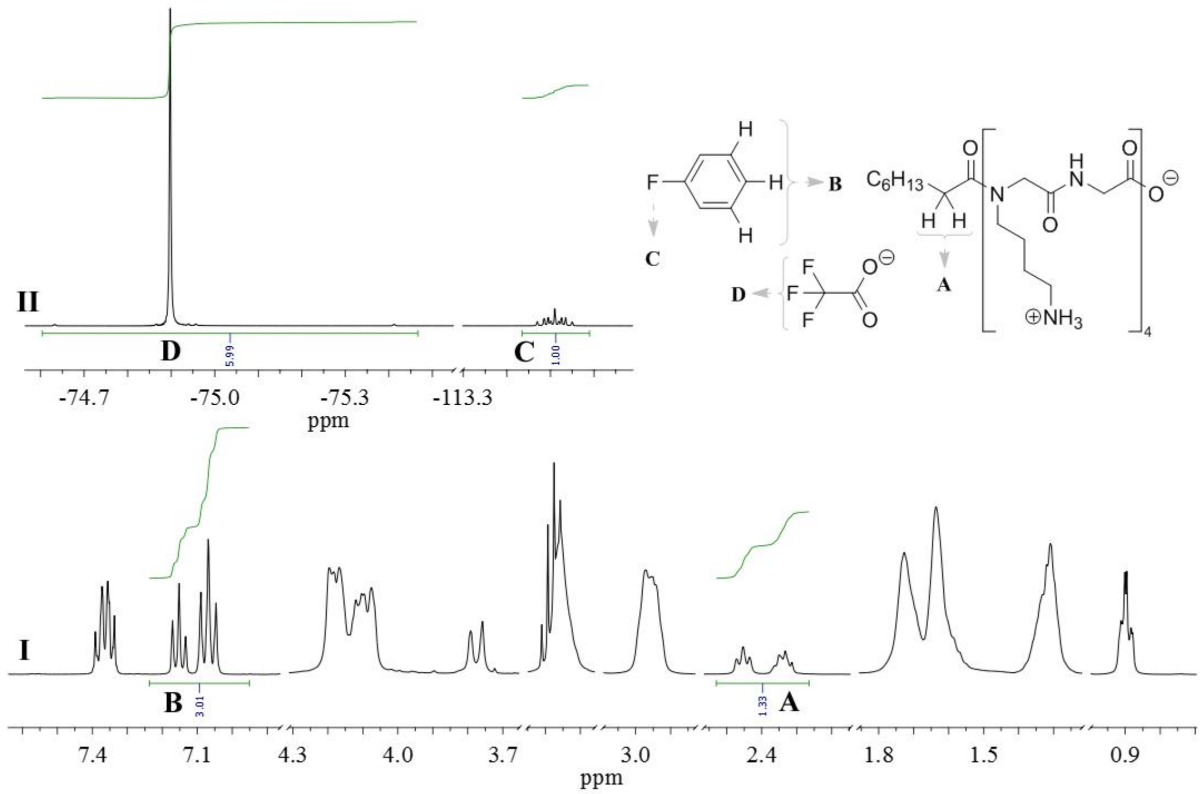


Figure 23. Segmented 400 MHz-^{1}H-NMR (I) and 376 MHz-^{19}F-NMR (II) spectra of compound **208** spiked with **221** in CD_3OD. *Assignments*: ^{1}H-NMR: (A) 2 α-protons of the fatty acid; (B) 3 *m*- and *p*-protons of fluorobenzene. ^{19}F-NMR: (C) 1 fluorine atom of fluorobenzene; (D) 3 fluorine atoms of TFA. The respective TFA content was calculated from the integrals of this quantitative measurement.

In general the calculations led to the result that three molecules of TFA are part of the LPP salts **201 – 220**. With respect to the manual integration and the individual processing of each spectrum a tolerance of 15% is acceptable. This tolerance is true for 16 out of 20 compounds (TFA/LPP range from 2.55 to 3.45). Only four compounds show a higher deviation. Nevertheless, all yields were calculated with three molecules of TFA due to the average result of the NMR measurements (Table 11).

2.6.4 pH stability of the amino-LPP acids

The routine NMR spectra of the amino-LPP acid TFA salts **201 – 220** were measured in deuteromethanol. In order to get an insight into the behavior of the compounds in water and at different pH values, compound **216** was picked and several experiments were conducted. For the ^{1}H-NMR measurements it was necessary to use deuterium oxide instead of normal water. This leads to a problem in maintaining the pD instead of the pH value of those solutions. It is known that a glass electrode calibrated in a protic system and then transferred to a system with deuterium oxide gives pH* values that differ about 0.40 units from the values measured in an appropriate protic system[153]. As long as the measurements are conducted quickly and the electrode isn't in contact with the deuterium oxide solutions for too long the observable shift in the electrode potential can be neglected[154]. For the pH experiments with the amino-LPP acid TFA salts a standard glass electrode was calibrated in a protic system, kept in deuterium oxide for 15 min to reach the equilibrium and afterwards the single measurements were done.

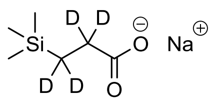


Figure 24. Structural formula of TMPA.

As internal standard for the NMR measurements the sodium salt of trimethylsilyldeuteropropionic acid (TMPA) was used in a concentration of 10 µM and the shift of the methyl protons was set as 0.00 ppm in the ^{1}H-NMR spectra (Figure 24). The pH value of a 4.48 mM solution of **216** in water was determined to be 3.71, which fits to the appearance as a TFA salt. For adjusting the pH* values of the deuterated solutions NaOD and DCl in D_2O were used and the pH* values were shortly set before the NMR measurements. In this set of different spectra three major observations can be made (Figure 25). The first obvious finding is the shift of the *C*-terminal *α*-protons of the glycin from about 4.05 ppm to 3.80 ppm in the pH* region of 1.78 to 3.77 (green region in Figure 25). This shift might be explained by the deprotonation of the *C*-terminus at pH* values higher than 1.78 and a higher shielding of the respective *α*-protons by a carboxylate in contrast to a protonated carboxylic acid moiety. The same is true for the signals of the protons next to the side chain amino groups (red region in Figure 25). The protonated amino groups do not shield the neighbored protons as much as non-protonated amino groups do in very alkaline media. Due to the four side chains the pH* range of the shift from 3.05 ppm to 2.65 ppm is broad (pH* 8.83 to 12.75) and a signal split can be observed in the pH* region from 9.97 to 12.17. This might be due to the different state of protonation of the separate side-chain amino groups and a therefore resulting different chemical environment for the neighbored protons, which is equalized at very high and very low pH* values. The third observation is the signal shape of the slightly acidic α-protons of the fatty acid (black region in Figure 25) and the protons of the peptoidic/peptidic backbone (blue region in Figure 25). For those protons no strong shift in the spectra could be observed, but a loss in signal shape at higher pH* values of more than pH* 9.41. This finding can be explained by the accelerated exchange of protons to deuterium atoms in strongly alkaline media. This might be the reason for the apparent disappearance of those two signal groups at high pH* values.

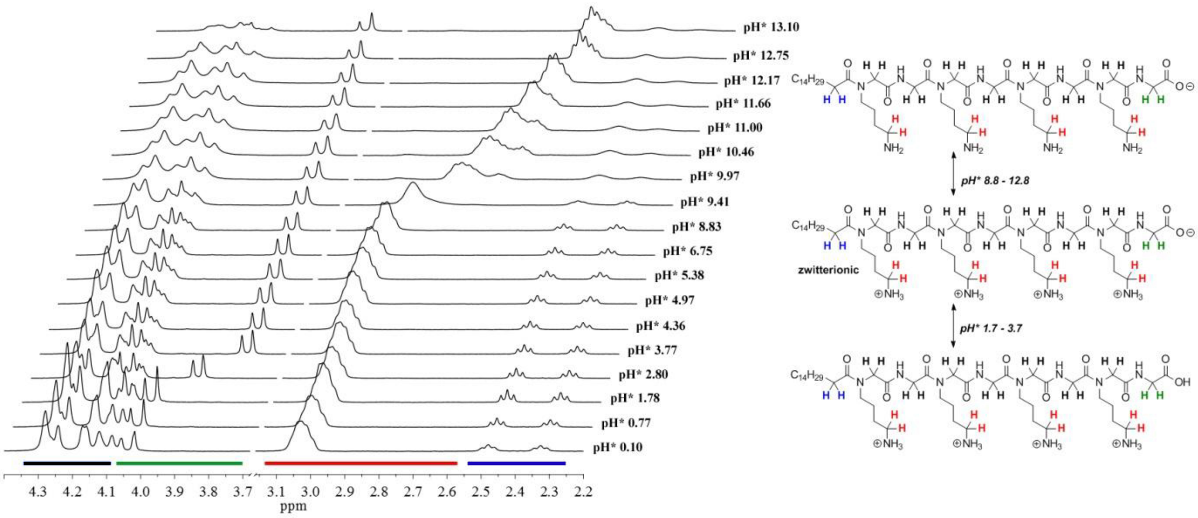


Figure 25. Segmented and stacked 400 MHz-^{1}H-NMR spectra of a ~1 mM solution of **216** in D_2O at different pH* values measured at 25 °C with ~10 µM TMPA as internal reference. The pH* values were set by apropriate addition of DCl or NaOD solutions to a 1 mM LPP solution in D_2O. The spectra are segmented for the better visualization of the interesting field regions from 2.20 – 4.40 ppm. *Assignments*: (**black** protons) protons of the backbone; (**green** protons) α-protons of LPP *C*-terminus; (**red** protons) protons directly next to the amino/ammonium moieties; (**blue** protons) α-protons of the fatty acid.

To verify this assumption and simultaneously check the long-term stability of **216** towards hydrolysis at extreme pH values the respective samples with a pH* value of 0.77, 6.75 and 12.75 were kept at room temperature and were measured again after 44 h. After this time only the spectra of the solutions with a pH* value of 12.75 differ. The signals of the backbone protons (red region frame in Figure 26) disappeared nearly completely, whereas the α-protons of the fatty acid remain smooth, but do not disappear. This leads to the assumption that only the real backbone protons in the amino-LPP acids are acidic enough to be exchanged against deuterium at elevated pH* values. Besides this finding no decomposition of the LPPs could be observed, neither at high nor at low pH* values after 44 h at room temperature. The architecture of this peptide-peptoid chimera makes these compounds obviously very resistant towards hydrolysis.

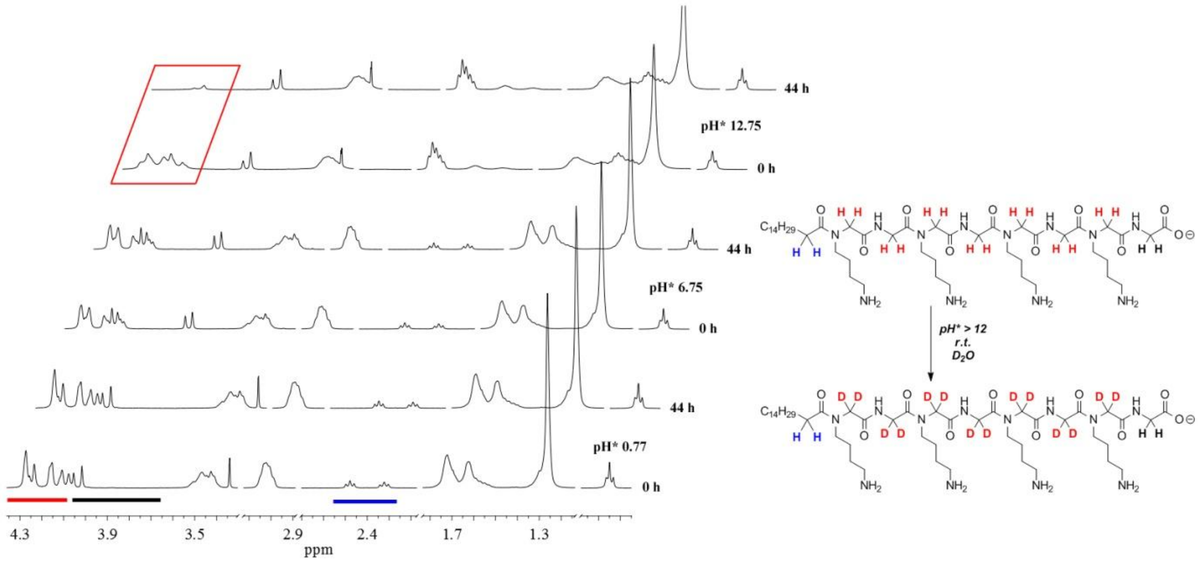
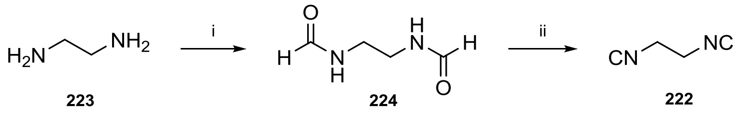


Figure 26. Segmented and stacked 400 MHz-^{1}H-NMR spectra of a ~1 mM solution of **216** in D_2O with three different pH* values measured at 25 °C with ~10 µM TMPA as internal reference at 0 h and after 44 h. *Assignments*: (**red** protons) protons of the backbone; (**black** protons) *α*-protons of LPP *C*-terminus; (**blue** protons) *α*-protons of the fatty acid. The **red** frame marks the shift region of the disappearing signals for the backbone protons at pH* 12.75 over 44 h.

2.7 Ugi-4CR dimerization of an azido-LPP acid with 1,2-diisocyanoethane

2.7.1 Synthesis of 1,2-diisocyanoethane

During the stepwise prolongation of the azido-LPPs with an U-4CR and a subsequent saponification (Scheme 5) the yields for the MCR step dropped significantly from the third generation on. To generate even longer cationic molecules with a peptoid-peptide-like structure another approach has to be done. The use of bifunctional MCR reactive building blocks is one option and was employed in the past for different purposes, especially macrocyclizations[155]. Until date the use of the smallest reasonably stable diisocyanide diisocyanoethane (**222**)[156,157] in a MCR approach has not been reported[158]. Therefore a synthesis of **222** starting from ethylene diamine **223** via the di-formamide **224** and subsequent dehydratisation with phosphoryl chloride was conducted (Scheme 12)[159].

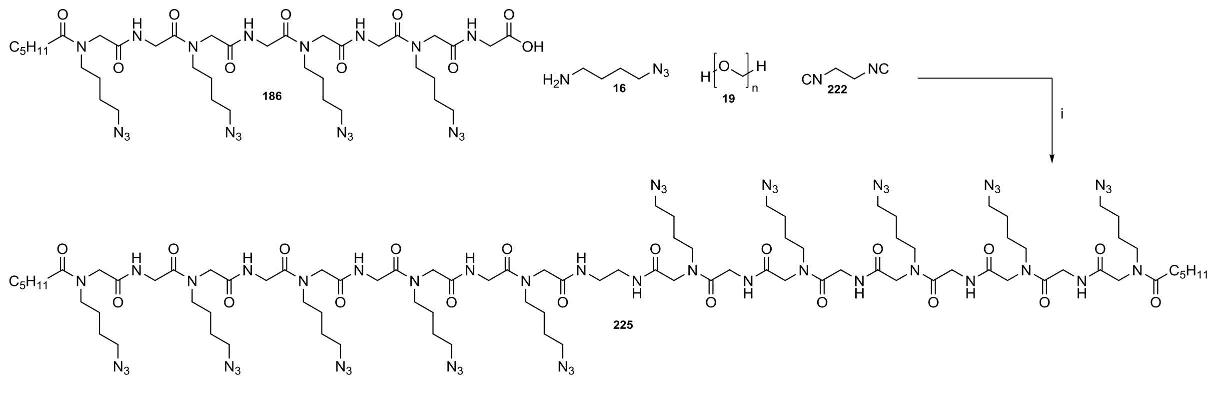


Scheme 12. Synthesis of diisocyanoethane **222** from ethylene diamine **223**. *Reagents and conditions*: i) EtOCHO, reflux, 6 h, 91%; ii) DCM, $POCl_3$, TEA, -60 °C → RT, 15 h, 61%.

The overall yield of 55% over two steps was reasonable and could possibly be further optimized. Yet the generated diisonitrile **222** is a liquid at room temperature, which solidifies under 0 °C. The most exceptional property of this compound is its missing pungent, isonitrile smell, although it contains two isocyanide moieties. In fact it is, freshly prepared, completely odorless.

2.7.2 Dimerization of LPP 166 with 1,2-diisocyanoethane

As a model compound for the dimerization of azido-LPP acids with **222** the hexanoic acid derivative **186** was used. The putative dimerization would lead to a deca-cationic molecule (after Staudinger reduction) and two acylated *N*-termini due to a sequence shift by the use of the symmetric diisocyanide **222**. The short fatty acid chain derivative **186** was chosen due to the investigation whether the pure number of cationic moieties would have an effect in the LPP interaction with bacterial membranes or the combination of a long apolar residue is essential.



Scheme 13. Synthesis of LPP dimer **225** in an U-4CR with diisonitrile **222** and azido-LPP acid **186**. *Reagents and conditions*: i) MeOH, RT, 15 h, 30%.

The reaction in methanol was accomplished like a normal U-4CR, but only 0.5 molar equivalents of **222** were used to achieve complete dimerization of the acid (Scheme 13). Directly after the addition of the respective acid **186** and isonitrile **222** to the preformed imine solution the intermediate **226** with an isocyanide moiety attached to the peptoid-peptide backbone could be detected by ESI-MS analysis (Figure 27).

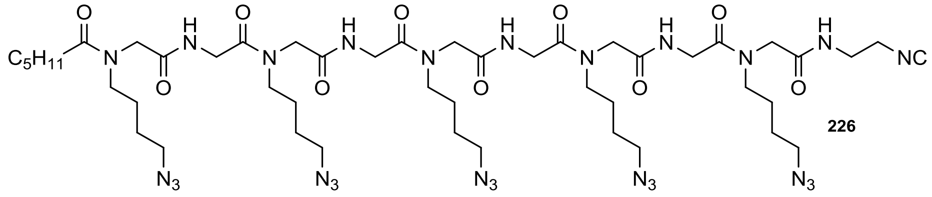
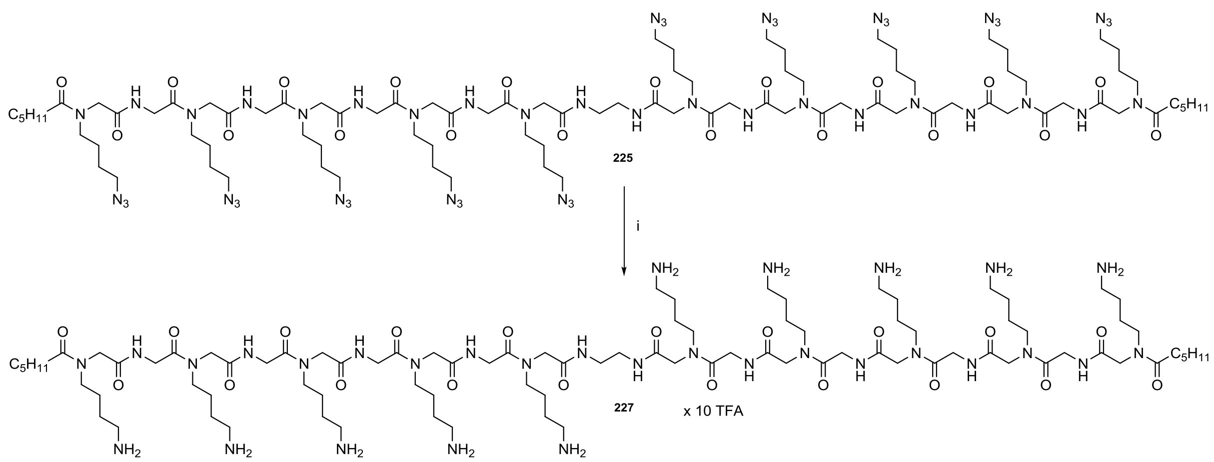


Figure 27. Structural formula of the isonitrile intermediate **226**.

This intermediate obviously reacts significantly slower with acid **186** and the imine than the small diisonitrile **222**. After incubation over night and subsequent pruifictaion by flash chromatography it was possible to isolate compound **225** in a yield of 30% and a 50 mg scale.

2.7.3 Staudinger reduction of deca-peptoidic azido-LPP 225

The generated compound **225** with its ten azido side-chain moieties is lacking a *C*-terminal end due to the dimerization with **222**, which led to a sequence shift. Therefore no terminal ester moieties needed to be saponified. Only the multiple azido groups had to be reduced to amines by the already employed Staudinger reduction[149,151,152]. The reduction itself was conducted analogously to the smaller azido-LPP methyl esters **161 – 180** (Scheme 14).



Scheme 14. Staudinger reduction of **225**. *Reagents and conditions*: i) PPh_3, THF/H_2O, RT, 84 h, then H_2O/TFA, RT, 2 h, 83%.

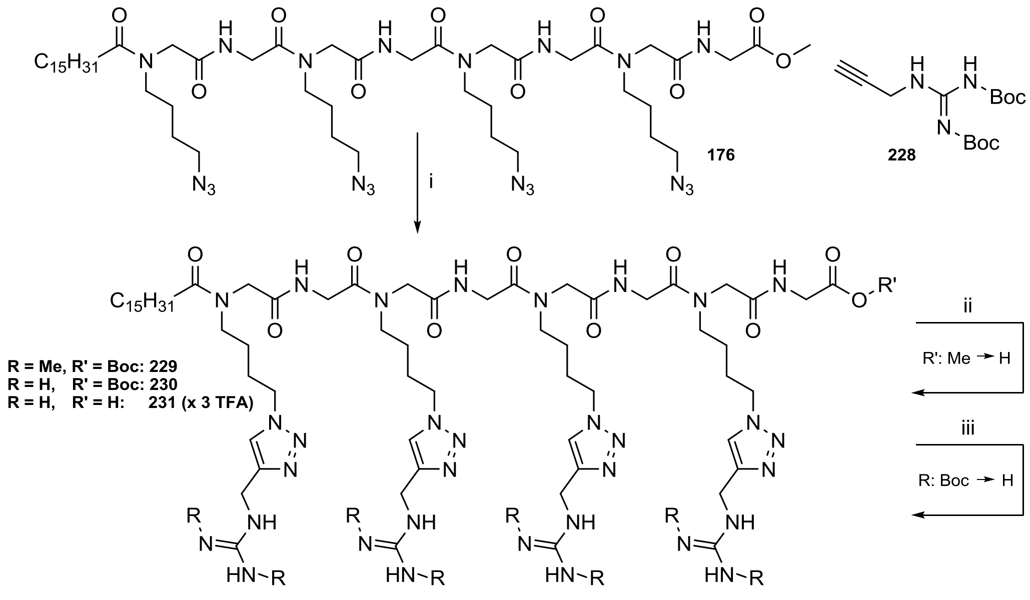
The TFA salt **227** was obtained as white powder in high yield after several precipitation steps to remove excess triphenylphosphine and residual triphenylphosphine oxide. The TFA content was determined to be 8.97 by NMR measurements like described for compounds **201 – 220** in chapter 2.6.3 and calculated with formula 3 and the use of the peptoid integral from the signal of the four fatty acid α-protons.

$$\frac{4I_{F,TFA}I_{H,FB}}{9I_{F,FB}I_{H,LPP}} = Q_{TFA/LPP} \quad (3)$$

The theoretical value of 10 molecules TFA per LPP lies within the acceptable range of 15% for this calculation. Due to its strongly cationic character compound **227** is freely soluble in water and methanol and its membrane activity should therefore easily be determinable in bacterial assays.

2.8 Azide-alkyne Huisgen cycloaddition of an azido-LPP methyl ester

The use of azides as masked amino groups in the synthesis of amino-LPPs allows for a subsequent derivatization of the azido intermediates. The prominent azide-alkyne Huisgen cycloaddition can be used to attach different kinds of residues to the side chains in a click type fashion under the generation of 1,2,3-triazole connecting moieties[160,161]. By employing an alkyne attached to a protected guanidinium group within this cycloaddition it is possible to transform the azido side chains, which are precursors for oligolysine-like LPPs, into LPPs that are decorated with protected arginine-like side chains.



Scheme 15. Azide-alkyne Huisgen cycloaddition of **176** and **228** and subsequent deprotection of the methyl ester and the Boc groups. *Reagents and conditions*: i) sodium ascorbate, copper(II) acetate, *tert*-butanol/H_2O, RT, 12 h, 66%; ii) LiOH, THF/H_2O, RT, 2h, 97%; iii) TFA, DCM, RT, 2 h, quant.

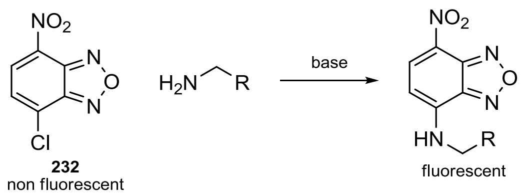
After deprotection the difference between the two cationic side chains towards membrane and biological activity can be determined. As model compound for this transformation azido-LPP methyl ester **176** was chosen due to the promising bioactivity of the structural combination of a long chain fatty acid and the cationic side chain part. In a copper(I) catalyzed Huisgen cycloaddition with alkyne **228** the Boc-protected tetraguanidinium derivative **229** could be obtained in moderate yield (Scheme 15). The alkaline saponification with lithium hydroxide and subsequent deprotection of the Boc groups with TFA in dichloromethane proceeded with excellent yield over two steps. The deprotected amine **231** was not further purified, but used directly for bioactivity screening. Therefore the exact TFA content was not determined by NMR measurements.

2.9 Synthesis of fluorescently labeled LPPs

For the localization of bioactive compounds within an organism or even a single cell, fluorescence labeling is a widely employed method. An intrinsic problem of this method is the influence of the fluorescent group itself on the bioactivity of the investigated compound. To minimize this effect, it is widely accepted that a rather small fluorescence label that does not contain cytotoxic moieties is inevitable. A wide scope of compounds is available for this purpose, but for the stepwise synthesis within the U-4CR protocol most of the fluorescence lables are useless due to the presence of Ugi reactive groups[162]. Also the polarity of the label is important, because one aim of this protocol was an isolation procedure for the U-4CR products by normal phase silica chromatography avoiding HPLC purification, which is only easily possible with uncharged fluorescence labels.

2.9.1 Probes based on 7-nitrobenzo[c][1,2,5]oxadiazole (NBD)

A dye that was used in lipid staining already[163] is based on 4-chloro-7-nitrobenzo[c][1,2,5]oxadiazole (NBD, **232**) that itself is non-fluorescent and has to be reacted with nucleophiles like thiols or amines to install an electron-donating moiety on the ring-system in an aromatic nucleophilic substitution reaction (Scheme 16).



Scheme 16. Synthesis scheme for the generation of fluorescently labeled amines with NBD-Cl **232**.

The fluorescence properties of the amino substituted dyes are comparable to the ones of widely used fluoresceine except for the quantum yield (Table 12)[162].

Table 12. Fluorescence properties of fluoresceine and NBD.

Dye	Excitation maximum	Emission maximum	Quantum yield in water
Fluoresceine	494 nm	518 nm	> 0.90[164]
NBD-amines	465 nm	535 nm	< 0.01[165]

Although the quantum yield of NBD-amines in water is very low, they show some unique properties. They are quite sensitive towards environmental effects like ion composition or solvent polarity and their fluorescence is strongly increased in an apolar surrounding like a lipid membrane. Furthermore they have been employed in staining living cells already and did not show any toxic effects or indications to be degradable in biological systems[165]. Therefore NBD is well suited for the application in LPP staining for biolocalization experiments.

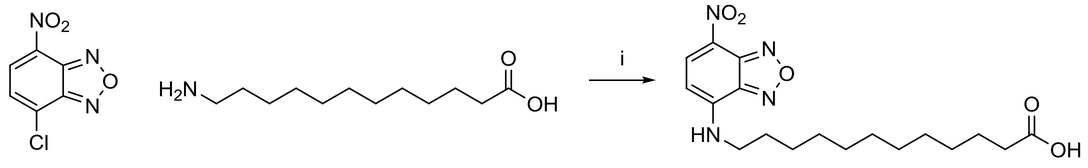
2.9.2 Synthesis of NBD labeled polycationic peptide/peptoid chimeras

2.9.2.1 Consecutive U-4CRs for the generation of fluorescent azido-LPPs

The synthesis of NBD labeled azido-LPPs was conducted like described for the respective non-fluorescent LPPs. The attachment of the fluorescent dye was chosen to be the *N*-terminus of the peptoid with a fatty acid spacer. This spacer should provide the hydrophobicity that is needed for the fluorescent compounds to be directly comparable with the non-fluorescent LPPs in biological experiments.

Synthesis of the fluorescent carboxylic acid building block

As spacer length, an alkyl chain of 12 *C*-atoms was chosen, because this would install an intermediate polarity in the LPP molecule, which makes the resulting compound putatively better comparable to non-fluorescent ones. Furthermore 12-aminolauric acid **233** used as starting material is easily commercially available. The initial step of this building block synthesis is the nucleophilic aromatic substitution of the chlorine in **232** by this respective amino acid, which could be used directly and did not have to be protected in advance (Scheme 17).



232 **233** **234**

Scheme 17. Synthesis of NBD labeled U-4CR starting material **234** based on 12-aminolauric acid **233**. *Reagents and conditions*: i) HCl, then $NaHCO_3$, $MeOH/H_2O$, reflux → RT, 59%.

The synthesis of this building block could be accomplished in moderate yield and the obtained acid was used for the synthesis of the first U-4CR product **235**.

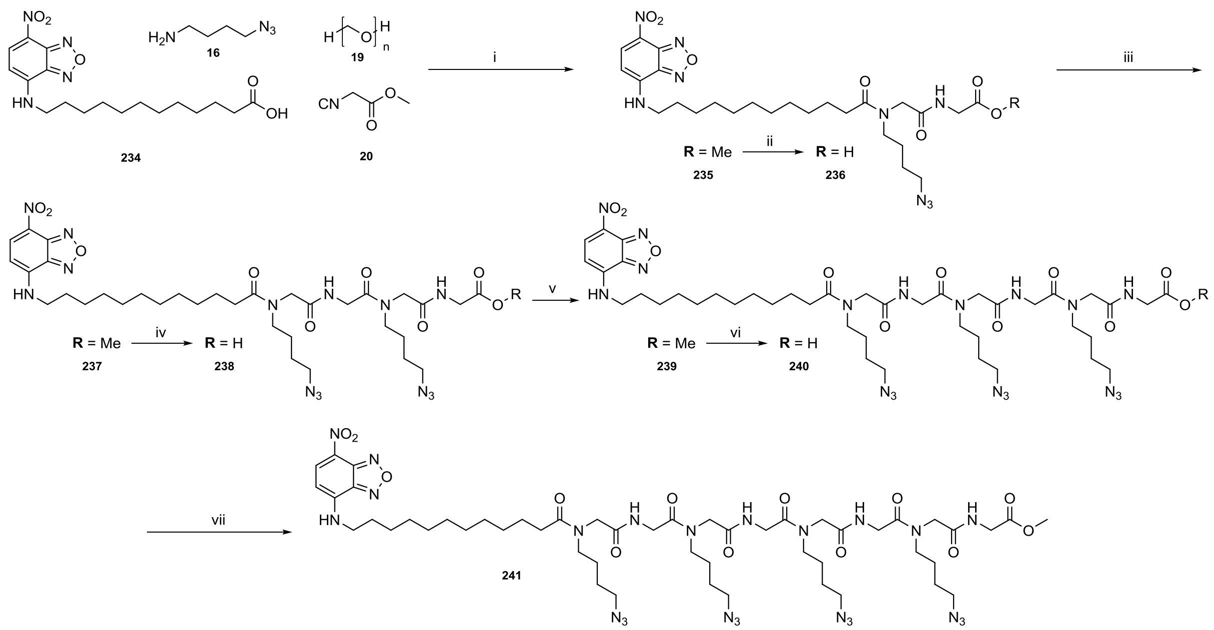
Synthetic cycle for the generation of a fourth generation fluorescent azido LPP

The U-4CR steps were conducted without modification by the reaction of the preformed imine from amine **16** and 1.67 molar equivalents of polymeric aldehyde **19** with the respective carboxylic acids and isonitrile **20** each in a 1:1 molar ratio calculated in relation to the amine (Scheme 18). The yield of 70% for the first U-4CR reaction was in the range that could be observed for the non-fluorescent derivative **52**. By following the synthetic cycle (Scheme 5) including subsequent U-4CRs and alkaline saponification steps with lithium hydroxide until reaching the fourth generation the desired NBD labeled azido-LPP **241** could be obtained in an overall yield of 16% (Table 13).

Table 13. Isolated yields for the U-4CR and saponification steps in the generation of NBD labeled azido-LPP **241** and all its intermediates. [a] yield of the precipitate; [b] yield derived from the purified filtrate.

Prolongation step	**Compound (Molecular Mass)**		**Yield**	
	Methyl ester	***Acid***	***U-4CR***	***Saponification***
1	**235** (603.67 g/mol)	**236** (589.64 g/mol)	70%	quant.
2	**237** (814.89 g/mol)	**238** (800.87 g/mol)	73%[a] 15%[b]	98%
3	**239** (1026.11 g/mol)	**239** (1012.09 g/mol)	54%	quant.
4	**241** (1237.33 g/mol)	/	50%	/
Total yield (7 steps)			**16.0%**	

The single U-4CR reactions produced precipitates, which were consisting of the pure U-4CR, from the second prolongation step on, which is in contrast to the syntheses of the non-fluorescent compounds, which showed precipitates within the U-4CR step starting only in generation three for the most unpolar derivatives.



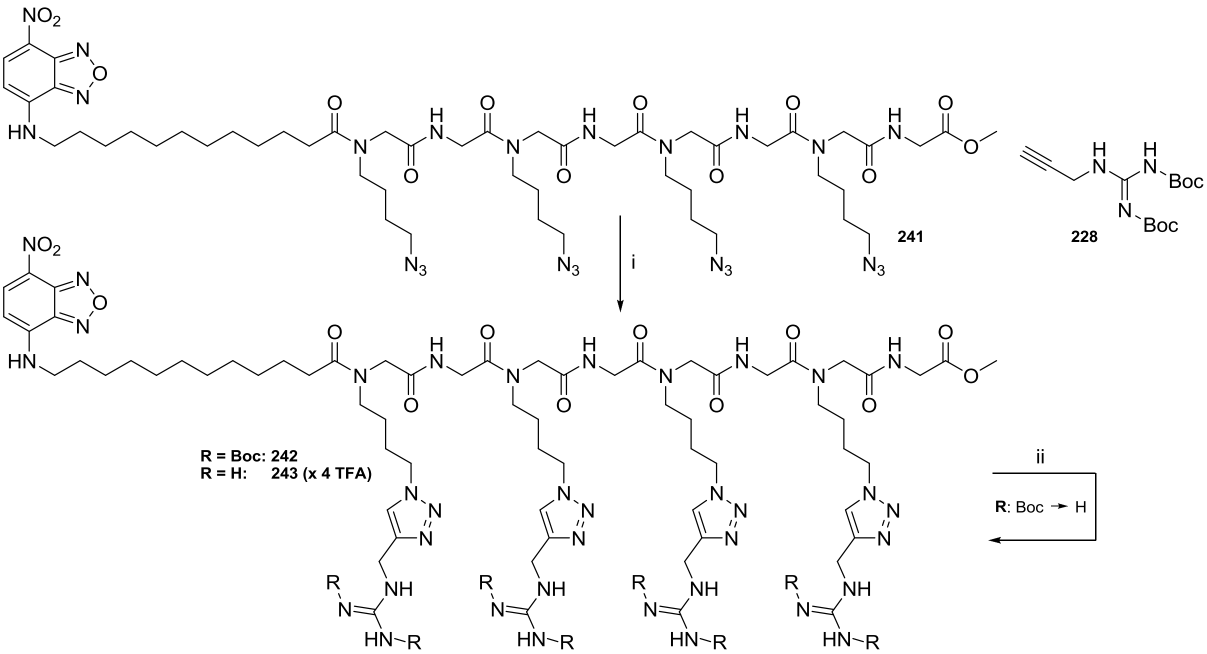
Scheme 18. Synthesis of NBD-tetraazido-LPP **241**. *Reagents and conditions*: i) MeOH, RT, 18 h, 70%; ii) LiOH · H_2O, THF/H_2O, RT, 2 h, quant.; iii) **16**, **19**, **20**, MeOH, RT, 18 h, 88%; iv) LiOH · H_2O, THF/H_2O, RT, 2 h, 98%; v) **16**, **19**, **20**, MeOH, RT, 18 h, 54%; vi) LiOH · H_2O, THF/H_2O, RT, 2 h, quant.; vii) **16**, **19**, **20**, MeOH, RT, 18 h, 50%.

The reason for this fact might be the lower solubility of the fluorescently labeled derivatives in methanol due to the aromatic NBD moiety. The precipitate was separated from the reaction mixture only in step two. For U-4CR number three and four the precipitate was purified together with the whole reaction mixture. The yield of the second MCR was remarkably high with >87%, but the following reactions proceeded only in moderate to almost poor yields of ~50%. The fourth generation U-4CR product **241** was only used for the azide transformation steps and no separate methyl ester saponification was conducted.

2.9.2.2 Side chain modifications of the fourth generation NBD labeled azido-LPP

Azide-alkyne Huisgen cycloaddition

For the investigation of a putatively different cellular distribution of LPPs with amino and guanidine side chains the fluorescent derivative **241** was used for the copper(I) catalyzed azide-alkyne Huisgen cycloaddition to generate the protected, fluorescent guanidine derivative **242**. This click-reaction proceeded with a yield of 51% (Scheme 19), but the purification of **242** was rather difficult due to the high affinity of this compound to the polar silica surface.

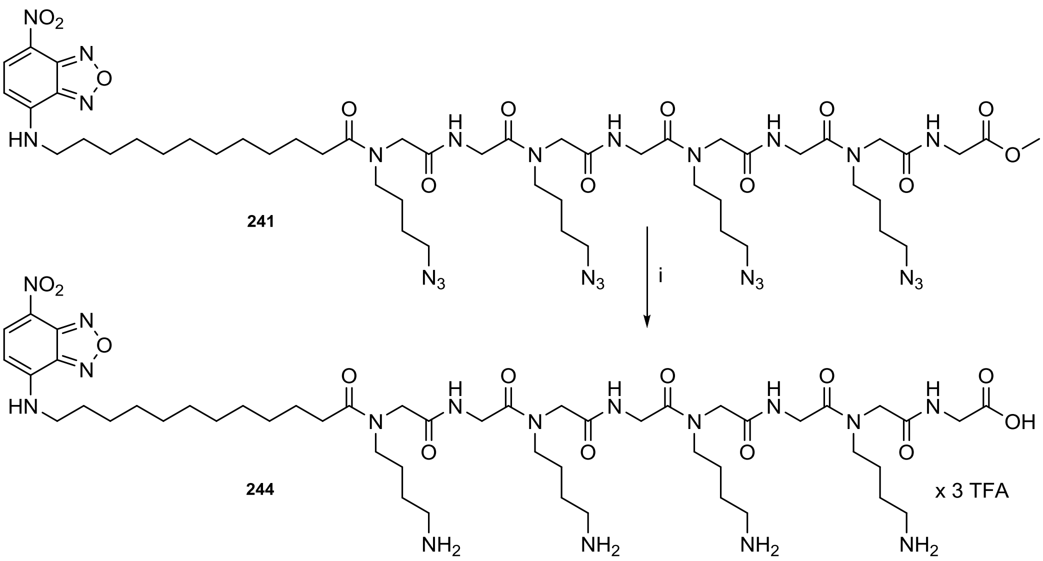


Scheme 19. Synthesis of the guanidine derivatives **242** and **243** from the NBD labeled tetraazido-LPP methyl ester **241**. *Reagents and conditions*: i) sodium ascorbate, copper(II) acetate, *tert*-butanol/H_2O, RT, 12 h, 51%; ii) TFA, DCM, RT, 2 h, 74%.

Although this compound could be detected on a TLC plate and revealed a R_f of 0.20 in a system of dichloromethane/ethyl acetate/methanol 2:2:1 it was impossible to get this compound eluted with a suitable solvent system from a flash column that was applied as purification method. The silica based column had to be flushed with pure methanol to elute the substance. A successively conducted preparative TLC finally allowed for the purification of **242**. In the following deprotection step with TFA the Boc protecting groups could be removed from the guanidine side chains and compound **243** could be obtained as TFA salt in high purity after precipitation from 56iethylether.

Staudinger reduction

The final reduction of the four azido groups in LPP derivative **241** by triphenylphosphine was conducted like described for the non-fluorescent derivatives (2.7.3). A six molar excess of reducing agent was applied to achieve complete transformation of all azides into amino moieties.



Scheme 20. Staudinger reduction of compound **241**. *Reagents and conditions*: i) PPh_3, THF/H_2O, RT, 24 h, then H_2O/TFA, RT, 2 h, 65%.

The final purification of very polar **244** was accomplished by two precipitation steps from a methanolic solution by 57iethylether and the fluorescent tetraamine TFA salt was obtained in a moderate yield of 65%. The spectroscopical properties of compound **243** and **244** were measured in water (Figure 28).

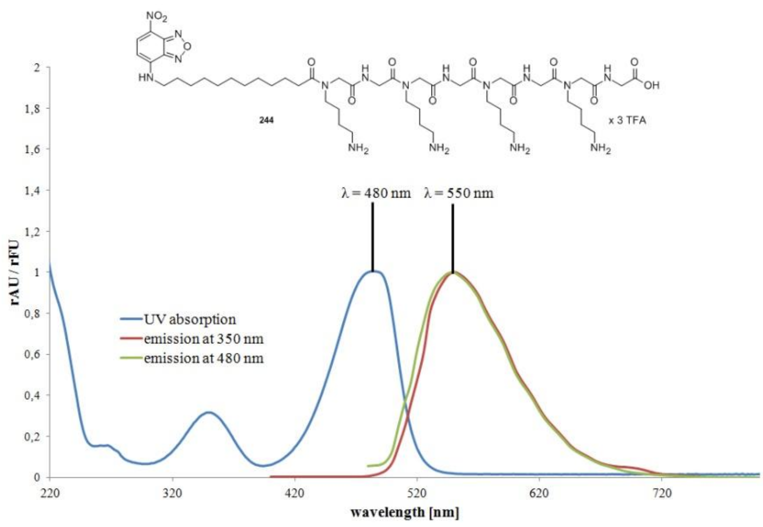


Figure 28. Normalized UV absorption and fluorescence spectra (excitation λ = 350 nm and 480 nm) of **244** in water.

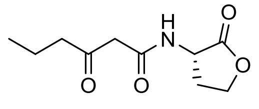
The UV and the fluorescence spectra do not differ for both compounds. The respectice spectra for two different excitation wavelengths are shown for compound **244** in Figure 28. The spectra recorded for the guanidine derivative **243** in water are not shown, but look completely similar with an emission maximum of λ = 550 nm for an excitation wavelength of λ = 480 nm. Hence the spectroscopical properties of the NBD moiety do not seem to be influenced by the side chain properties of the LPPs, at least in direct comparison of amino and guanidine moieties.

3 Biological Evaluation of Selected Peptidomimetics

The synthesized compounds of the LPP library were designed to show effects on biological membranes. The most important question was the influence of the fatty acid chain length on the biological activity. Due to the different composition and polarity of membranes in different species a kind of activity distribution of the applied compounds was expected. For the screening assays different kinds of model organisms representing some of the most important targets in medicinal and agricultural chemistry were used, *i.e.* gram-negative and gram-positive bacteria, pathogenic fungi and human cancer cells.

3.1 *Aliivibrio fischeri* luminescence assay

For determination of the general toxicity of pure compounds, mixtures or waste water on biological systems a bacterial assay employing luminescent *Aliivibrio fischeri* (syn. *Vibrio fischeri*) is a widely used method[166]. This standardized assay is commercially available and facilitates the fast screening for water pollutants or acute toxic substances[167]. Due to the sensitivity of the bioluminescence of this bacterial strain, many toxic substances show an influence on this highly regulated system and lead to a decrease in luminescence within 30 minutes after application. This quick response is also a drawback if not only the primary effects of the respective compounds are of interest in a biological screening, but also long-term and secondary effects on protein synthesis or other metabolomic pathways are the subject of investigation. Therefore efforts have been made to compare the acute and the 24 h toxicity of compounds employing the same test system[168].



245

Figure 29. Structural formula of *N*-(Ketocaproyl)-L-homoserin lactone (**245**), an acylated *α*-homoserine lactone emitted by *A. fischeri* as quorum sensing molecule.

A. fischeri is a marine bacterium and needs a certain minimum cell density within the test solution for luminescence. This is due to the bacterial luminescence being a biological response on environmental properties. Every distinct bacterial cell emits certain acyl *α*-homoserine lactones like *N*-(Ketocaproyl)-*L*-homoserin lactone (**245**) as quorum sensing signalling molecules, which can be detected by other cells of *A. fischeri* present in the surrounding (Figure 29). Once this messenger substance reaches a certain minimum concentration the luminescence cascade is triggered in all bacteria at once and the bacterial colony starts to glow[169]. But the emission of light is not the only effect of quorum sensing. Furthtermore it can trigger the formation of biofilms that increase the resistance of the bacterial colony against environmental influences and toxins.

3.1.1 General setup of the luminescence assay

To investigate the short as well as the long term influences of interesting compounds on the luminescence of *A. fischeri* a special bioassay had to be developed. The respective test strain DSM507 (batch no. 1209) was ordered from "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (DSMZ) as lyophylized pellet[170]. After recultivation and separation of a single colony on BOSS medium agar plates[171] a stock culture was inoculated. Of this stock culture glycerol stock aliquots were prepared that were stored at -80 °C and were freshly used for the inoculation of a pre-culture for each test run. The test culture was incubated at 23 °C for 16 h and was afterwards diluted with fresh BOSS medium to an appropriate cell number. The assay itself was conducted on black flat bottom 96 well plates in a volume of 200 µl of medium in each well. To enhance the solubility of hardly water soluble compounds the test medium for bacterial growth contained 1% (v/v) DMSO, which was tested not to interfere with the bacterial growth behavior and vitality. The luminescence of the distinct compounds was measured and an inhibition value relative to a non-treated control was calculated. Measurements were done for eight points in time and for four concentrations. In sum, 32 data points were taken for each compound. As a kind of positive control and for the validation of this assay, the antibiotic chloramphenicol **253** was used, because it is known to show a strong inhibition of gram-negative bacterial growth[168].

3.1.2 Evaluation of the luminescence assay with reference compounds

The sensitivity and the general scope of this assay were determined by the application of twelve reference compounds that can be subdivided into two classes depending on their expected influence on the luminescence of *A. fischeri* – toxic and indifferent compounds

(Table 14, Figure 30). The short-term as well as the long-term effects of the toxic compounds should be determined to gain information about the different inhibition kinetics of various compound classes.

Table 14. Reference compounds for the evaluation of the *A. fischeri* luminescence assay.

Compound	Class	Putative mode of action	Code
Barium chloride	toxic	Ion channel blocking[172]	**246**
Benzethonium chloride		Membrane disruption[173]	**247**
Copper(II) sulfate		Oligodynamic effect[174]	**248**
Digitonin		Membrane interaction	**249**
Sodium azide		Respiratory chain[175]	**250**
Sodium cholate		Membrane effects[176]	**251**
Sodium dodecylsulfate		Membrane effects[177,178]	**252**
Surfactin		Membrane pores[77]	**9**
Chloramphenicol		Protein biosynthesis[179]	**253**
D-glucose	indifferent	No or beneficial effects	**254**
Saccharose			**255**
Sodium trifluoroacetate			**256**

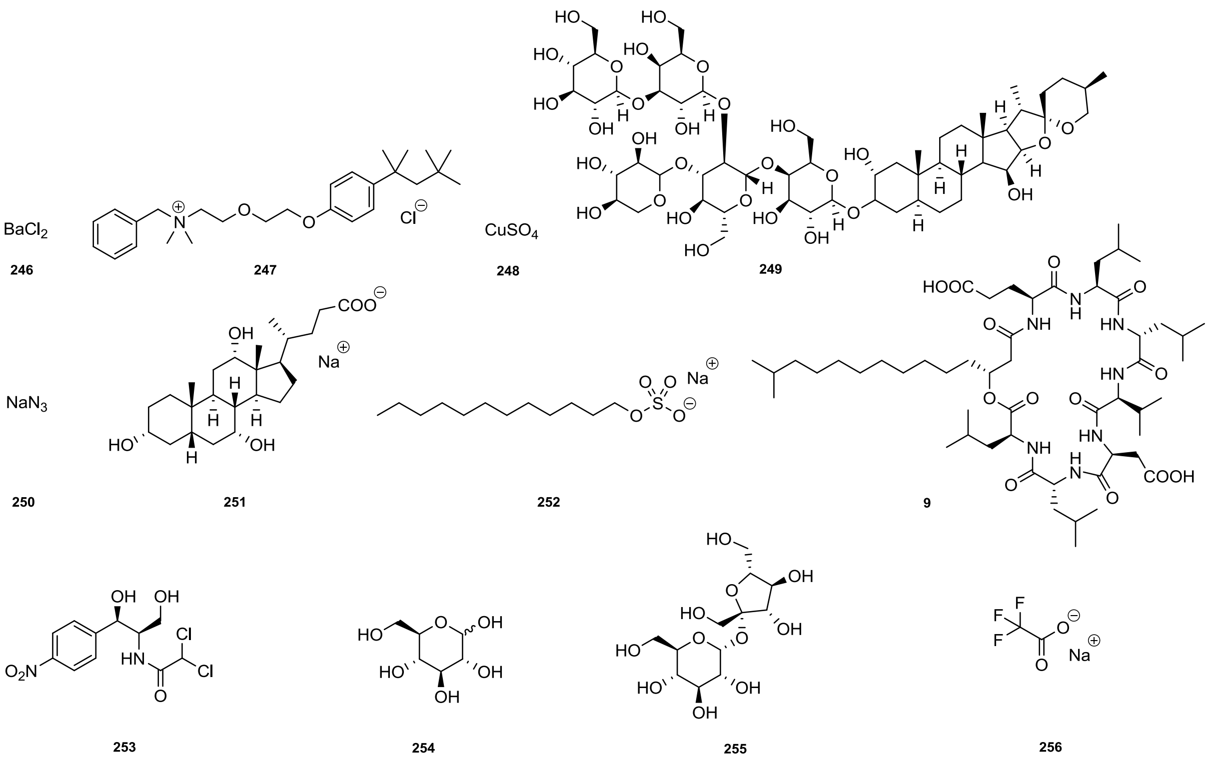


Figure 30. Structural formulas of the reference compounds used for the *A. fischeri* luminescence assay.

3.1.2.1 Chloramphenicol

After the application of the reference compound chloramphenicol (**253**), a strong reaction of the bacteria expressed by their luminescence could be observed. Due to the expected long-term effect of this antibiotic and the protein biosynthesis being its target, the influence on the bacterial viability shows an unusual progression within the first four hours. For the two extreme concentrations of 1 and 1000 µM almost no change of the luminescence in comparison to the control could be detected, whereas the intermediate concentrations of 10 and 100 µM show a strong beneficial effect. The bacteria treated with 10 µM of **253** increased their relative luminescence to almost 250% within the first four hours. Almost the same is true for the second highest concentration of 100 µM, which leads to a maximum in relative luminescence of ~200% after four hours. After incubation for six hours, the effect drastically changes into a strong inhibition of the bacterial luminescence, but still showing an unusual concentration dependency of the relative luminescence according to the presence of compound **253**. After eight hours, almost no differences between the single concentrations are observable anymore. The relative luminescence decreased to 5–10% for all applied concentrations. Finally the last measurement after 24 h showed the expected outcome of a concentration of 1 µM leading to a relative luminescence of ~20% and all higher concentrations leading to an almost complete depletion of the bacterial glowing, which can be equalized with the death of almost all of the bacteria (Figure 31, page 63). Due to this impressive activity profile of **253** on the bacterial luminescence it was chosen to be the reference compound for all conducted assays as a kind of positive control.

3.1.2.2 Barium chloride, copper(II) sulfate and sodium azide

The two metal salts of barium (**246**) and copper (**248**) did not show a huge effect on the bacterial luminescence. For barium, the toxicity on *A. fischeri* can be considered as very low, because no effects can be detected over the whole incubation time and even after 24 h. For the copper salt **248** only the two higher concentrations of 100 µM and 1000 µM showed an effect after 24 h. While the highest concentration led to some kind of periodical changes in the bacterial luminescence with maxima of ~150% relative to the control after four and six hours, the 100 µM setup showed an increase in the luminescence only after 24 h of incubation (Figure 31, page 63). Barium and copper salts turned out to be non toxic to the bacterium, whereby barium did not show any and copper induced only a mild response in the luminescence profile.

A different outcome was determined for compound **250**, which is widely used as supplement in biochemistry to prevent microbial contamination in protein solutions, water baths and other sensitive, aqueous media. The application of the tree lowest concentrations did not show any effect on the bacterial viability. Only the highest concentration of 1000 µM induced a luminescence profile quite similar to that caused by reference compound **253**.

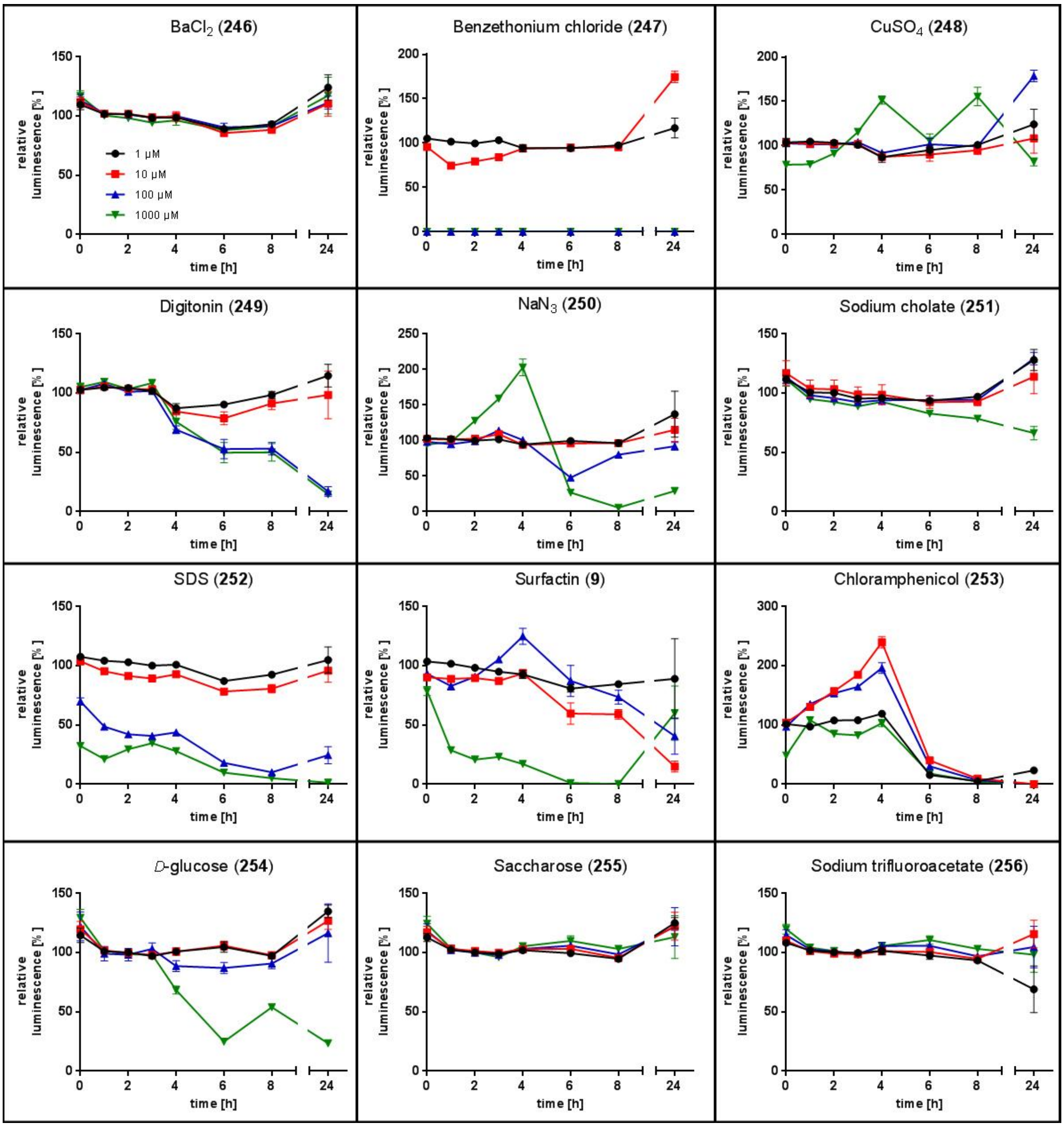


Figure 31. Activity of different reference compounds in the *A. fischeri* luminescence assay. The respective compounds **9** and **246 – 256** were applied in concentrations of 1 µM (**black** line), 10 µM (**red** line), 100 µM (**blue** line) and 1000 µM (**green** line) and measurements of the luminescence were conducted for eight different points in time.

After a strong increase of the light emission to ~200% relative to the control within the first four hours, the viability decreased drastically to end up at ~25% after 24 h (Figure 31, page 63). The effective concentration equals a ~65 ppm solution, which lies in the range the compound is usually applied as antimicrobial in the laboratory.

3.1.2.3 Detergents

To determine the effect of detergents on the bacterial membrane of *A. fischeri* different kinds of surface active compounds were applied. Besides the widely used disinfectant **247** as a member of cationic detergents[180], three anionic surfactants **251**, **252** and **9**, amongst them surfactin (**9**) as one of the most potent ones[77], were chosen. As a member of the non-ionic detergents family, digitonin (**249**) was applied.

Benzethonium chloride (247)

Of all tested reference compounds, benzethonium chloride (**247**) as cationic surfactant was the only substance that instantly killed the bacteria, when it was applied in the two highest concentrations of 100 µM and 1000 µM. Nevertheless, the two lower concentrations did not show any effect in reducing the viability of the bacteria. Only after 24 h the bacteria treated with 10 µM of **247** showed a positive response to the compound, meaning an increase of the luminescence to ~175% relative to the control. The lowest concentration of 1 µM did not change the relative luminescence of the bacteria over the whole incubation time (Figure 31, page 63). The observed effects may be due to the detergent being active as micells towards the bacterial membrane. Below the critical micelle forming concentration (CMC) the substance cannot interfere with the membrane. The CMC of **247**, which is ~2.8 mM in aqueous solution shifts into the range between 10 – 100 µM in this medium[181]. The already published effect of a lower CMCs in saline solution is caused by the culture medium, which contains 3% w/w of NaCl[180,181].

Anionic detergents

The anionic surfactants that were used as reference compounds contained SDS (**252**) with a sulfuric acid monoester moiety carrying the anionic charge and the two members **9** and **251**, which contain carboxylic acid moieties. The effect of SDS (**252**) is comparable to that of the powerful cationic compound **247**, although it does not kill the bacteria instantly. In fact, the two highest concentrations of **252** decrease the luminescence constantly, already from the beginning to a value of ~10% after eight hours, but only the highest concentration of 1000 µM leads to an effective inhibition of the bacterial luminescence, whereas the bacteria in the 100 µM SDS solution slightly recovered to a ~25% luminescence in comparison to the control after 24 h. The two lowest concentrations did not show any effect on the bacteria, which might be due to the same CMC effect like described for benzethonium chloride **247** (page 64).

In contrast to these results compound **251** does not show any strong effects of inhibiting or forcing bacterial luminescence within the first eight hours of incubation. Only after 24 h the highest concentration of 1000 µM shows a slight decrease of the relative luminescence to ~75%. Due to the steroidal architecture of the compound and the absence of this kind of compounds in bacterial membranes it might be hard for this surfactant to integrate into and disrupt the membrane. It is known that cationic steroids can exhibit strong antibacterial properties[182], but the positive charge is urgently needed for membrane interaction and therefore the anionic compound **251** cannot show an effect agains *A. fischeri*.

Surfactin (**9**) as third anionic detergent exhibits a special kind of activity due to pore formation within the phospholipid bilayer membranes of bacteria. Because of the existence of a second membrane in gram-negative bacteria, compound **9** cannot easily reach the inner membrane and act bactericidally by pore formation. This effect is observable in the bioassay, because only the highest concentration of 1000 µM leads to a fast decrease of the relative luminescence within six hours of incubation. The lower concentrations noteworthy don't inhibit the luminescence within the first eight hours. This might be due to the penetration effect of the compound through the outer membrane. Only in the highest concentrated solution enough surfactin (**9**) is present to saturate the outer membrane and enable further material to find the way to the inner, crucial membrane. Although the luminescence vanished almost completely (<0.2%) for the 1000 µM control after eight hours, the bacteria recovered to show a high viability after 24 h again. This effect is hard to understand, because the concentration of 10 µM leads to a decrease in relative luminescence to ~15% after 24 h. In fact, the concentration dependency of the luminescence upon treatment with surfactin is completely the opposite to what can be expected. Apart from the lowest concentration of 1 µM, which does not show any effect, all other concentrations led to a higher viability of *A. fischeri* the higher surfactin (**9**) was concentrated (Figure 31, page 63). This result may be caused by the special mode of action and an obvious cellular response that might lead to a partial detoxification of **9** by efflux or degradation. The cellular answer may be effected by a certain concentration limit of **9**, which is not yet reached in a 10 µM solution, but triggers the response for higher concentrations.

Digitonin (249)

In contrast to the anionic detergents, which are non-preferable compounds to interact with the also negatively charged bacterial membrane, the non-ionic **249** cannot be repelled by electrostatic interactions. Therefore the observed behavior in the bioassay is in correlation to the expected. The two lower concentrations of 1 µM and 10 µM do not show any effect on the relative luminescence, whereas the two higher concentrations slowly decrease bacterial viability four hours after start of the incubation. This might be due to the CMC of digitonin **249** being in the range of 10 – 100 µM in the nutrient used for this assay. No difference between the two highest concentrations, even after 24 h, can be observed. The final inhibition after 24 h resulted in a relative luminescence of ~ 20% for 100 µM and 1000 µM of **249** (Figure 31, page 63). The main difference of the effect of **249** on the bacteria in contrast to the antibiotic **253** and sodium azide **250** is the lack of the boost phase before the luminescence decreases. Digitonin **249** reduces the bacterial viability constantly without the induction of a cellular response in form of increasing relative luminescence before the cells are killed or harmed.

3.1.2.4 Indifferent compounds

The application of indifferent compounds in this assay should lead to no change in the relative fluorescence of the bacteria. Trifluoroacetate ions are supposed not to influence bacterial growth or viability in contrast to highly toxic monofluoroacetates, which are transformed into enzyme inhibitors in the citric acid cycle[183]. Due to the LPP TFA salts that were synthesized for this research the non-toxic properties of this respective anion should be determined. To determine if TFA is inactive at relevant concentrations, sodium trifluoroacetate (**256**) was applied in this assay. In fact, no inhibition or beneficial effects of **256** could be observed, even after 24 h of incubation up to a concentration of 1000 µM. Only the lowest concentration showed a slight decrease in the relative luminescence, but the single measured values scattered strongly for this concentration, whereby the mean value decreased slightly. In general, it can be assumed that trifluoroacetates are non-toxic to *A. fischeri* and their co-application with the synthesized LPPs shouldn't have an influence on the activity profile of the main compound.

An interesting behavior of *A. fischeri* was observed for the actually non-toxic sugars **254** and **255**. Whereas disaccharide **255** did not show any effect on the relative luminescence up to eight hours of incubation and only a slight increase after 24 h, *D*-glucose (**254**) inhibited the bacterial luminescence steadily after four hours of incubation. This effect is only observable for the highest concentration of 1000 µM, but the inhibition led to a decreased relative luminescence to ~20% after 24 h. This kind of catabolic repression is known for **254** and *A. fischeri*. The inhibition of the bacterial luminescence is *c*AMP triggered and does not lead to a reduced viability of the cell[184]. Therefore caution should be used in the interpretation of luminescence assay results according to the bacterial viability, when mixtures of compounds that could contain *D*-glucose (**254**) are tested.

3.1.3 Heat maps as fingerprints of the antibacterial activity

Due to the number of 32 datapoints that were taken for every compound applied in the luminescence assay the plotting of the results as heatmap became possible. Heatmaps are two-dimensional graphs with a third, color-coded dimension. Therefore they can be used to illustrate the luminescence inhibition as time as well as concentration dependency of a certain compound in this assay.

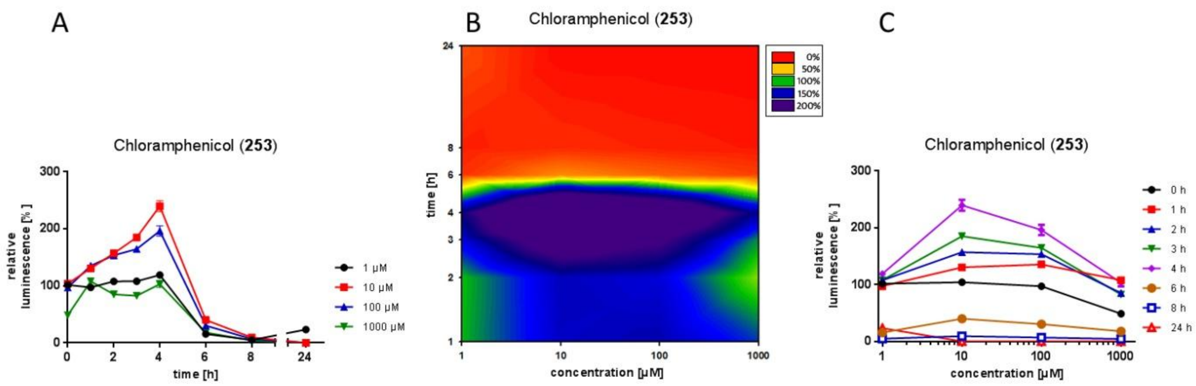


Figure 32. Activity profile of compound **253** in the *A. fischeri* luminescence assay. *Assignments*: A) Graph of the activity data – axes: time and relative luminescence; B) Heatmap of the activity data; C) Graph of the activity data – axes: concentration and relative luminescence.

The color code and the resulting pattern allows for a quick estimation of the potency as well as the putative mode of action of a compound with respect to the acute or long-term toxicity. The heatmap used for this bioassay consists of two logarithmic axes. The concentration of the applied compound is plotted on the horizontal one and the incubation time is represented by the vertical axis. Due to the nature of the logarithmic scale the respective values for an incubation time of 0 h could not be plotted. The heatmap itself was therefore generated from 28 datapoints. For chloramphenicol (**253**) the heatmap clearly shows a horizontal color change from dark purple (>200% relative luminescence) to red (~0% relative luminescence) in the time frame from four to six hours over the whole concentration range (Figure 32). The whole heatmap does not contain a big area of green color, which means that the compound interferes strongly with the bacteria over the whole range of the applied concentrations. Beginning with a luminescence increase (symbolized by the blue and purple areas) for the intermediate concentrations all luminescence is quenched after six hours and for all concentrations.

The pattern that was found for compound **253** is typical for a potent, long-term toxic compound that decreases the bacterial viability only after a certain incubation time, but inhibits their growth effectively. All these information can be taken out of the heatmap plot within a short time without the need of absolute inhibition values. Therefore this kind of plotting can be used as fingerprint for the different compounds. The evaluated reference compounds led to the finding that all substances can be subdivided into three activity classes, like it was assumed in the beginning of this chapter. A classification can be made into acute toxic, long-term toxic and indifferent (non-toxic) compounds. The three respective extreme fingerprints for **247**, **253** and **256** are shown in Figure 33.

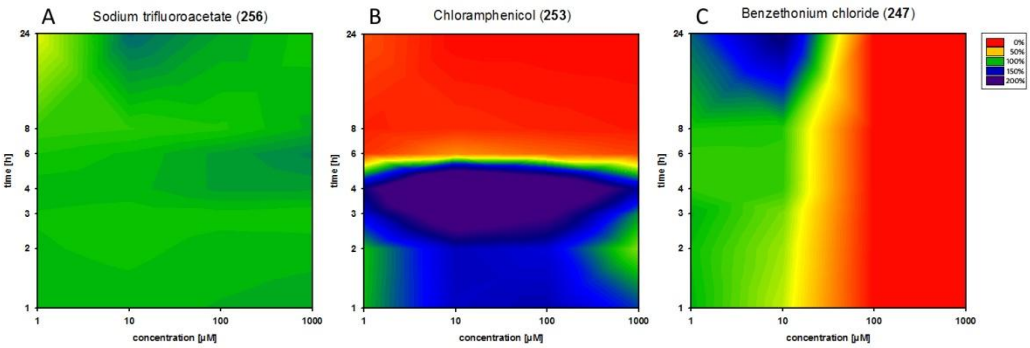


Figure 33. Exemplary heatmaps of **247**, **253** and **256** as fingerprints for the three classes of compounds regarding their activity in the *A. fischeri* luminescence assay. *Assignment*: A) Heatmap of the activity of sodium trifluoroacetate **256** as a an example for an inactive compound; B) Heatmap of chloramphenicol **253** as an example for a long-term toxic compound with initial burst; C) Heatmap of benzethonium chloride **247** as an example for an acute toxic compound.

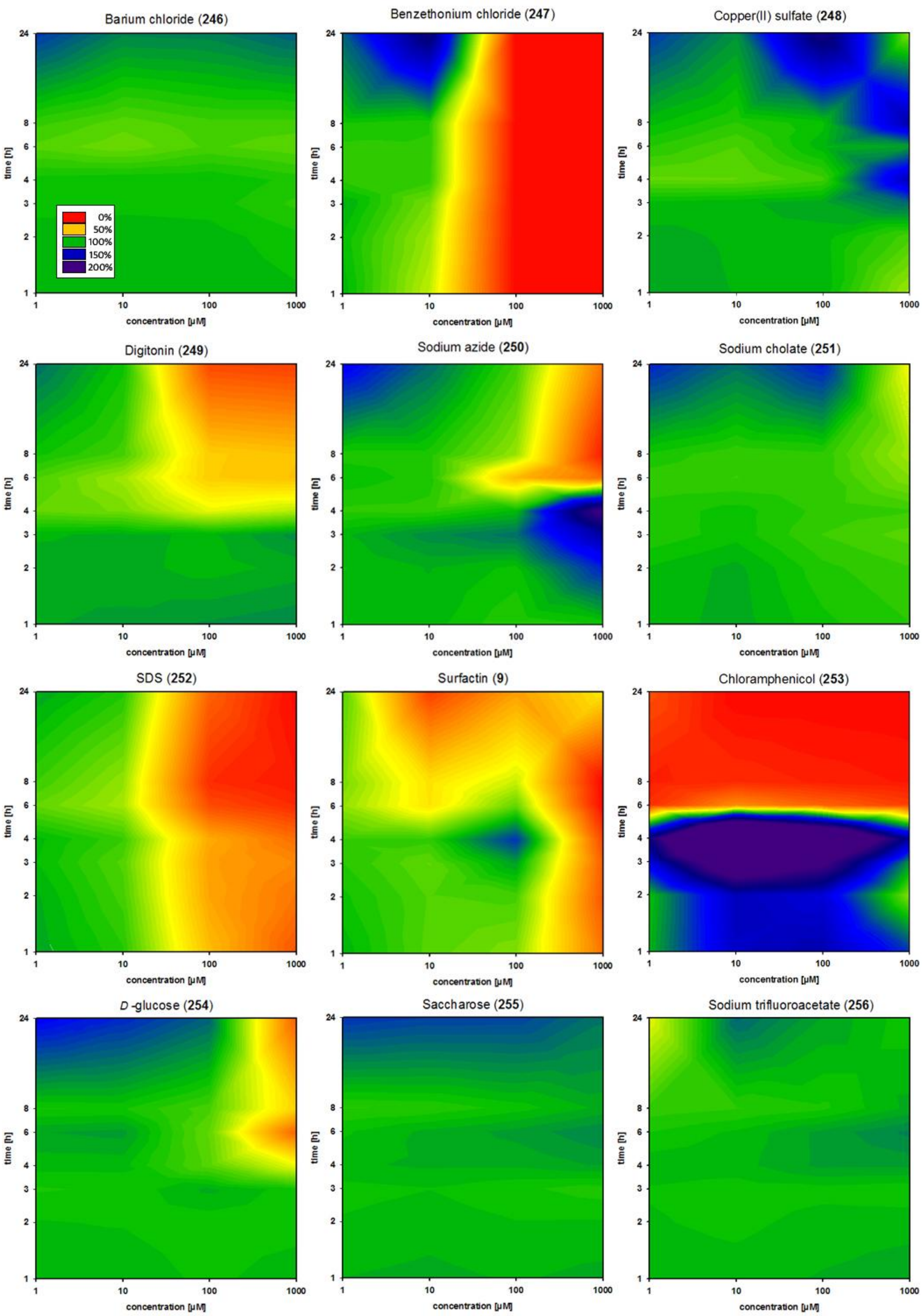


Figure 34. Heatmaps of all reference compounds applied in the luminescence assay. See Figure 31 on page 63 for the direct comparison of the activity data plotted as standard two-dimensional graphs.

An inactive compound leads to an almost completely green fingerprint, whereas an acute toxic compound shows a red area confined by a vertical border zone to lower concentrations and beginning directly from the first point in time (1 h). In contrast to this, the red area is twisted by 90 ° for substances with a long-term toxicity and the confinement line to lower activities is now a horizontal one, caused by the effect of a late inhibition within the incubation period. A conceivable fourth class of active compounds would lead to a mostly blue or purple fingerprint without red and only small green areas. This would be caused by compounds that induce bacterial luminescence or enhance the bacterial viability. Due to the fact that none of this kind of compounds was identified during this thesis, no fingerprint can be shown and this hypothetic class is skipped from the discussion. Obviously, combinations of the three extrema are possible and can be found within the scope of reference compounds (Figure 34, page 70). Another interesting finding is the fact that long-term toxic compounds do not act via the same pathway – differing due to the compound class they are belonging to. For the antibiotic **9** and sodium azide **250** below the red area a dark blue to purple area appears in the heatmap, caused by an initial increase of the bacterial luminescence before the bacteria are inhibited. In contrast to that the long-term active compounds **249** and **254** do not show an increase in luminescence before their inhibitory effect begins. This macroscopically visible difference between a variety of activity mechanisms can be determined very quickly from the heatmaps – even for a bigger number of compounds that were tested. Therefore the heatmap tool is a useful addition to the standard graphs and will be used to visualize the differences in the activity profiles of the applied substances exclusively, whereas the classical charts including the IC50 calculations for the *A. fischeri* luminescence assay can be found in the appendix (8.2).

3.1.4 Activity of the amino LPP acids 201 – 220 in the luminescence assay

The library of oligo-cationic LPPs **201 – 220** was investigated with the luminescence assay. Regarding their general structure it could be supposed that an initial attraction between the polycationic charge of the compounds and the negatively charged bacterial membrane would play an important role in their antimicrobial activity. The influence of the chain length of the fatty acid connected to the *N*-terminus was the issue of this assay setup. Due to unique lipid composition of the bacterial membrane different effects of interaction between the apolar part of the LPPs and the cellular bilayer could be expected. For the application of the LPPs **201 –**

220 no solubility problems could be observed. Due to their cationic net charge they all exposed a high water solubility, even in the saline assay medium.

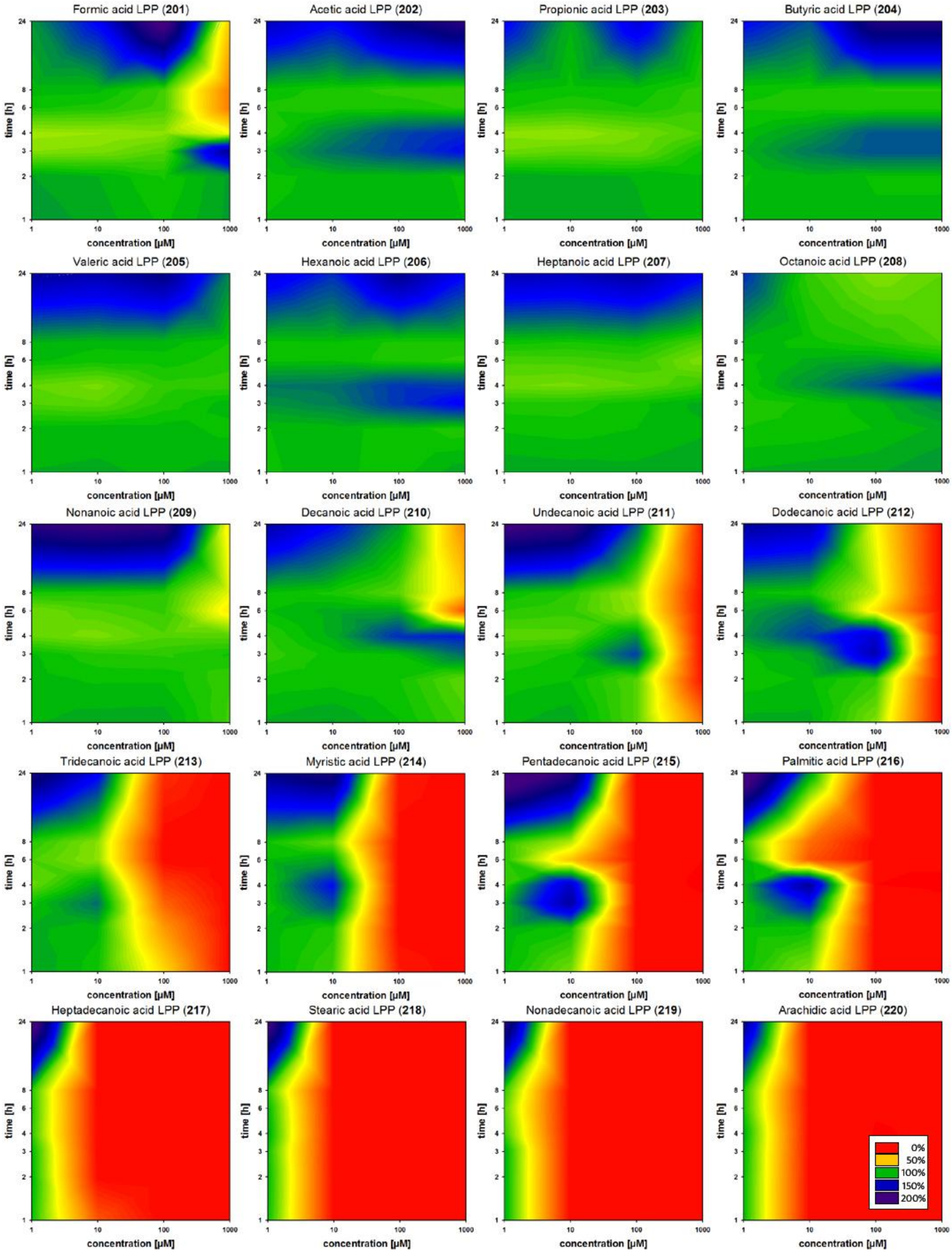


Figure 35. Heatmaps of the luminescence assay data of tetra-Lys-like acyl peptoid-peptides **201 – 220**.

In direct comparison of the inhibitory potential of the twenty compounds a clear dependency of the antimicrobial activity on the chain length of the fatty acid can be observed (Figure 35, page 72). The nine shortest fatty acids in compounds **201** – **209** do not show significant effects on the bacterial luminescence, except the formylated compound **201**, which reduces the luminescence after four hours at the highest concentration of 1000 µM. For the eight substances **202** – **209** an interesting effect in the time frame from two to four hours can be observed for almost all concentrations. While the fatty acid derivatives with an even number of carbon atoms in their alkyl chain induce luminescence in the bacteria during this period, the ones with an odd number do not show such an effect or induce just a slight decrease in the bacterial viability.

From compound **210** – **220** the potency of inhibiting the bacteria rises with every added carbon atom. The intermediate length decanoyl derivative **210** shows a similar profile to the formic acid compound **201** with an inhibitory effect after six hours just for the highest concentration with a luminescence peak upstream between two and four hours. Treating the bacteria with the undecanoic and lauric acid derivatives **211** and **212** the highest concentration of 1000 µM of both substances leads to an instant depletion of the luminescence like it was observed for benzethonium chloride **247** displaying an acute toxicity of those derivatives at high concentrations.

An increase of the chain length to 13 up to 16 leads to a boost in activity of the LPPs by one order of magnitude. For compounds **213** – **216** a concentration of 100 µM is already enough to lead to an instant death of the bacteria even directly after the first contact with the substances. The heatmaps of benzethonium chloride **247** and **213** show the same shape and activity profile. For compounds **214** – **216** an increase of the luminescence between four and six hours to more than 150% can be observed for the concentration of 10 µM. This increase is interrupted by an indifferent or even an inhibition phase (**215**, **216**) from six to eight hours. Interestingly most of the substances (except **201**) lead to an increase of the bacterial luminescence relative to the control ranging from 150–200% after 24 h at the lowest concentration of 1 µM. This cellular response might be due to the membrane effect of the compounds leading to a signalling cascade in the cell, but the inhibitory concentration not being high enough to severely damage the membrane and lead to cell death.

Anyhow, the installation of very long fatty acids to the LPPs like in compounds **217 – 220** with alkyl chains ranging from 17 to 20, leads to another activity boost by again one order of magnitude. Bacteria treated with a 10 µM solution of those LPPs instantly loose their luminescence strongly suggesting that they are directly killed by the substances.

In summary a longer fatty acid attached to the *N*-terminus of the LPPs leads to a higher acitivity towards *A. fischeri* with a maximum from 17 carbon atoms that cannot be increased further by the installation of even longer chains up to 20 carbon atoms. All applied compounds **201 – 220** did not show any promising long-term effects, but acted in an acute manner comparable to the membrane active benzethonium chloride **247**, which is consistent with their supposed membrane activity. Nevertheless, an active concentration of 10 µM (~13.5 mg/l) for compound **217** makes the higher derivatives interesting candidates for new kinds of disinfectants that are very fast in killing even gram-negative bacteria and can be applied in concentrations as low as 20 ppm.

3.1.5 Activity of the azido LPP acids 181 – 200 in the luminescence assay

The promising results of the polycationic LPPs **201 – 220** in the luminescence assay led to the assumption that the general architecture of the peptoid-peptide chimeras with cationic side chains and an *N*-terminal fatty acid is an important structural feature to induce antimicrobial activity. Due to the inherent mode of action of the completely deprotected amino LPP acids **201 – 220** an acute toxicity of those compounds could be determined. For the treatment of infectious diseases, aside from pure disinfection purposes, it is mostly not wanted to kill all microorganisms instantly at the same time lysing their cells and releasing the toxic volume, which might lead to severe reactions in the infected organism (septic shock). Therefore, a kind of delayed toxicity like it was determined for chloramphenicol **253** would be desirable. For this purpose the still azide protected final compounds **181 – 200** were also applied in the luminescence assay as a kind of prodrug, assuming that the azido moieties could possibly be reduced to amine by intracellular reductases and thereby provide the active compound at a later stage of the treatment. Due to the more hydrophobic structure of the azides, their solubility in the saline medium was not as good as it has been observed for their polycationic relatives, but it was still high enough to cover the whole range relevant test concentrations from 1–1000 µM.

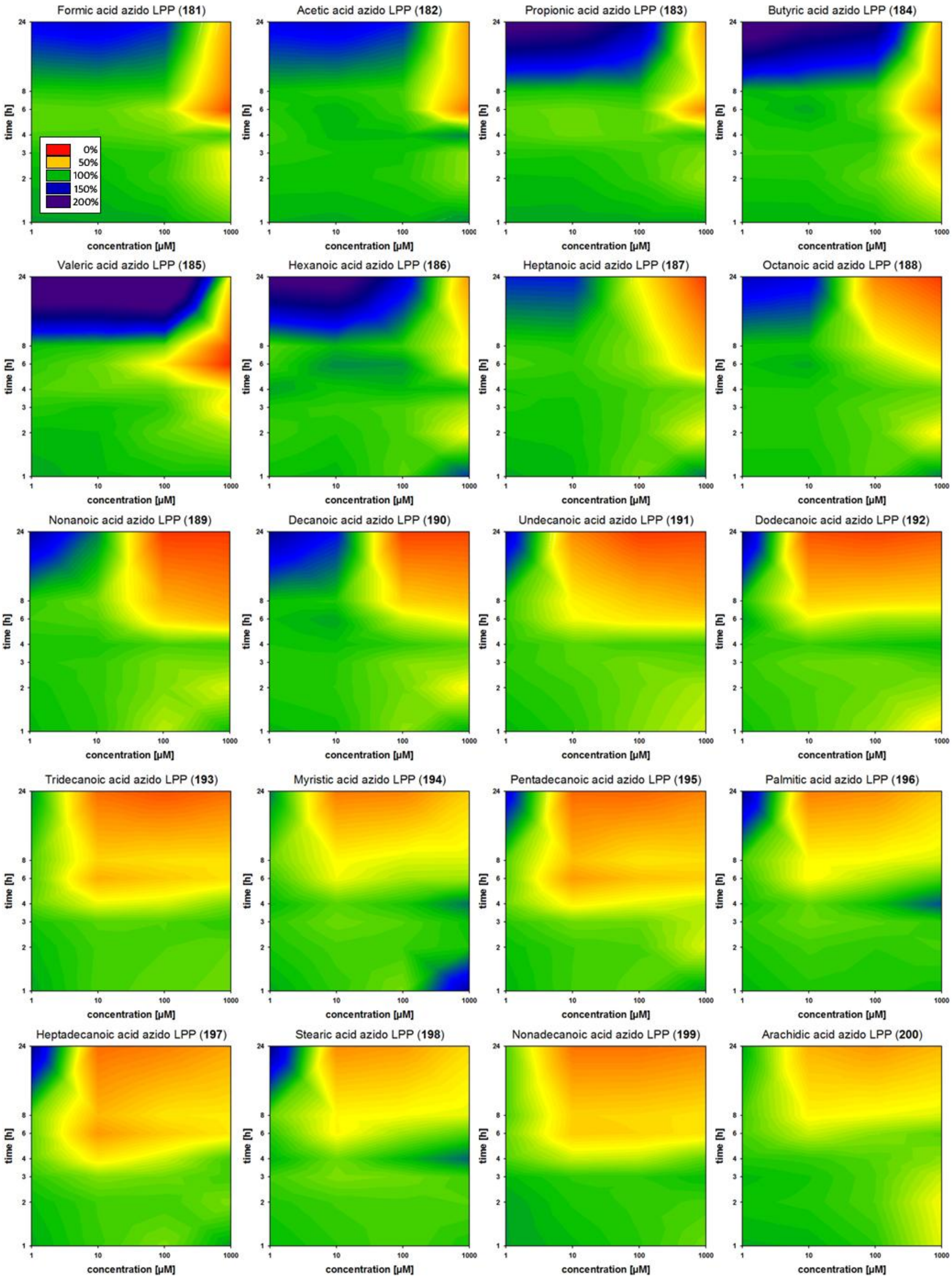


Figure 36. Heatmaps of the luminescence assay data of tetra-azido-like acyl peptoid-peptides **181 – 200**.

All compounds showed an effect in the luminescence assay (Figure 36), but the detected reaction of the bacteria can be used to subdivide the compounds into three classes. The first class containing compounds **181 – 186** showed to an increase of the luminescence to 150 – 200 % after 24 h at the three lowest concentrations of 1 µM, 10 µM and 100 µM, whereas the highest concentration of 1000 µM led to a decrease of the bacterial viability after 24 h to ~25%. This decreasing effect starts already after four to six hours post application, whereas the inducing effect can be observed only after 24 h. This luminescence boosting effect is most distinct for the valeric acid derivative **185**, which leads to a bacterial luminescence of ~200% for the three lowest concentrations after 24 h relative to the control.

With the chain length of the fatty acid increasing from seven to twelve carbon atoms in compounds **187 – 192** the induction effect of the lower concentrations nearly completely vanishes and can only be observed for the lowest concentration of 1 µM, mostly. In contrast to this, the inhibitory potential of the compounds is increasing figuratively spoken with every carbon atom that is attached to the alkyl chain reaching a maximum for the undecanoic and dodecanoic acid derivatives **191** and **192**. But even those two compounds do not kill the bacteria completely, the luminescence of *A. fischeri* is reduced by ~80% after 24 h relative to the control for the three highest concentrations of 10 µM, 100 µM and 1000 µM.

The azido LPP derivatives **193 – 200** with the longest fatty acids show similarly shaped heatmaps like the compounds of the subclass before with two exceptions. The inhibitory potential of these compounds stays constant or even slightly decreases with increasing chain length. The arachidic acid derivative **200** shows the same shape of the heatmap, but the inhibition of the bacterial luminescence just reaches values of <50% after 24 h for the three highest concentrations. An additional effect of this compound class can be observed in the time frame from four to six hours of incubation. Whereas the fatty acids with an odd number of carbon atoms (**193, 195, 197** and **199**) show a decrease of the luminescence for the three highest concentrations in this period the ones with an even number (**194**, **196**, **198** and **200**) do not show an effect in this time-frame for those concentrations or just slightly induced luminescence. This effect was observed for the amino LPP acids as well, but for the shorter chain length derivatives **202 – 209**. Obviously the type of fatty acid has an effect on the bacterial metabolism, even if it is attached to a peptoid-peptide scaffold like in the LPPs. Not only the pure chain length of the fatty acid shows an effect, but also the type of alkyl chain, whether the number of carbon atoms is odd or even. This might be due to the fact that fatty acids with an odd number of carbon atoms are rarely found in nature and derivatives containing them lead to an increased stress level of the treated cell due to unknown membrane interactions and catabolism of a remaining *C*1-compound of the unnatural fatty acid containing compounds during the membranous incorporation process.

3.1.6 Activity of the dimeric, the guanidine and the dye labeled LPP derivatives 227, 231, 243 and 244 in the luminescence assay

Not only the completely deprotected amino derivatives **201 – 220** and the azido LPP acids **181 – 200** were applied in this bacterial assay. To get a deeper insight into the qualitative reaction of *A. fischeri* as a model organism for gram-negative bacteria also the further derivatized or dimerized LPPs were tested. On the one hand this was necessary to investigate the influence of the nature of the cationic side chain and if a guanidinium moiety would have a greater effect than simple amines. On the other hand the fluorescently labeled compounds should be tested to verify that their activity equals the activity of the non-fluorescent relatives.

3.1.6.1 Hexanoic acid amino LPP dimer 227

The library LPP amino acids **201 – 220** consist of an *N*-terminal fatty acid, a *C*-terminal carboxyl moiety and four amino groups attached to the side chains, equaling a net charge of +3 for the whole LPP molecule. The effect of the fatty acid chain length could clearly be shown in chapter 713.1.4 with the longer chain derivatives being the most active ones. For the hexanoic acid derivative **206** (Figure 37) no distinct activity could be determined.

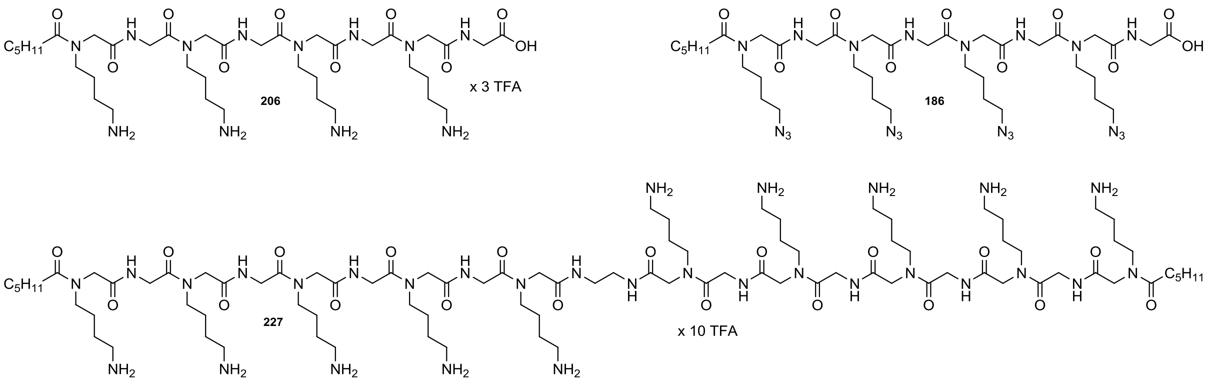


Figure 37. Structural formulas of compounds **186**, **206** and **227**.

Therefore it was interesting to check, whether a second fatty acid on the opposite *N*-terminus and the lack of a free carboxyl group in combination with a net charge of +10, due to the presence of ten amino groups in the side chains, in compound **227** would lead to a different effect in the luminescence assay (Figure 37).

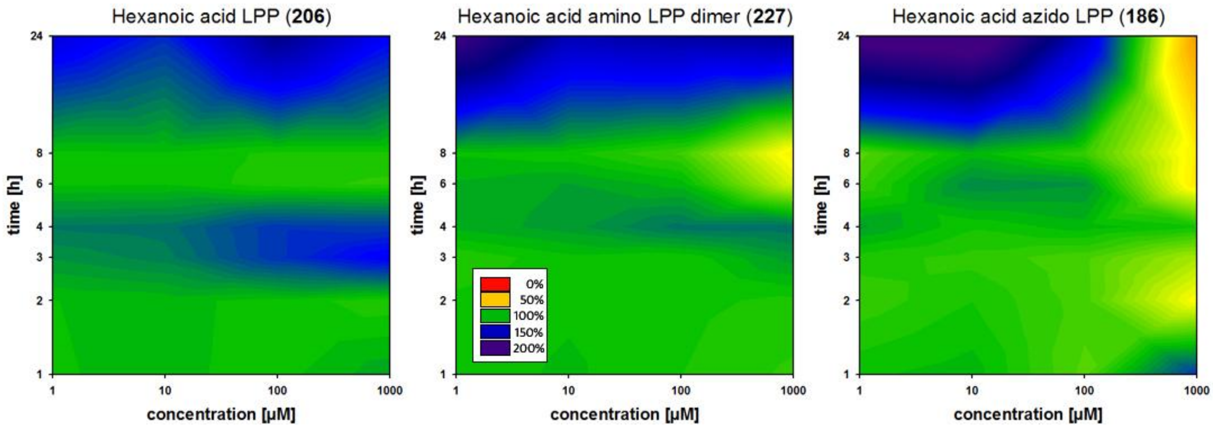


Figure 38. Heatmaps of the luminescence assay data of compounds **186**, **206** and **227**.

The direct comparison of the activity profile of compounds **186** and **227** does not show a significant difference. Over the whole scope of concentrations both substances do not change the luminescence relative to the control within the first two hours of incubation. After a moderate increase of the bacterial light emission between two and four hours post application that is detectable for both compounds, but which is more distinct for the monomeric **206**, an intermediate phase from six to eight hours follows. Within this time-frame compound **206** shows no difference in the bacterial luminescence relative to the control. For the dimeric LPP **227** only for the highest concentration of 1000 µM a moderate decrease of the light emission to ~50% could be observed. After 24 h both hexanoic acid derivatives show an increase of the bacterial luminescence over the whole range of concentrations. In contrast to that result the prodrug-like azido compound **186** shows a slightly different behavior. Two maxima of luminescence decrease can be determined for the highest concentration. The first peak is detectable two hours post application and the second one starts moderately at six hours of incubation and increases slowly to reach its maximum after 24 h.

The installation of a second fatty acid moiety and even the more than doubled number of cationic residues does not lead to a huge effect in the activity profile of the LPP amino acids. Therefore it can be assumed that even the relatively small number of four amino groups and the even lower net charge of +3 is enough to interfere with the bacterial membrane, as long as the terminal fatty acid has a minimum chain length. Carrying a short chain fatty acid leads to a loss of activity, which cannot be compensated by the installation of a second hydrophobic residue of the same length or the enlargement of the number of cationic residues.

3.1.6.2 Palmitic acid guanidine LPP derivative 231

The amino LPP derivative **216** with a palmitic acid residue attached to the *N*-terminus showed an intermediate activity against *A. fischeri* in the luminescence assay (chapter 3.1.4). Therefore it was interesting to investigate the effect of changing the cationic moieties from amino to guanidine groups in compound **231** (Figure 39, A).

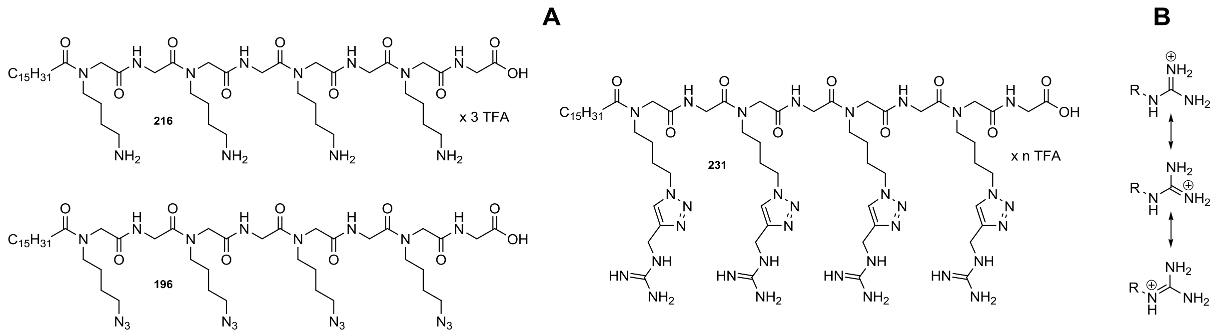


Figure 39. A) Structural formulas of compounds **196**, **216** and **231**. B) Tautomerism of the guanidinium moiety.

The cationic charge is distributed between three nitrogen atoms in a guanidinium residue (Figure 39, B) in comparison to a single nitrogen atom carrying the charge in ammonium group leading to a bigger cation with a lower charge density and maybe other effects on the bacterial membrane.

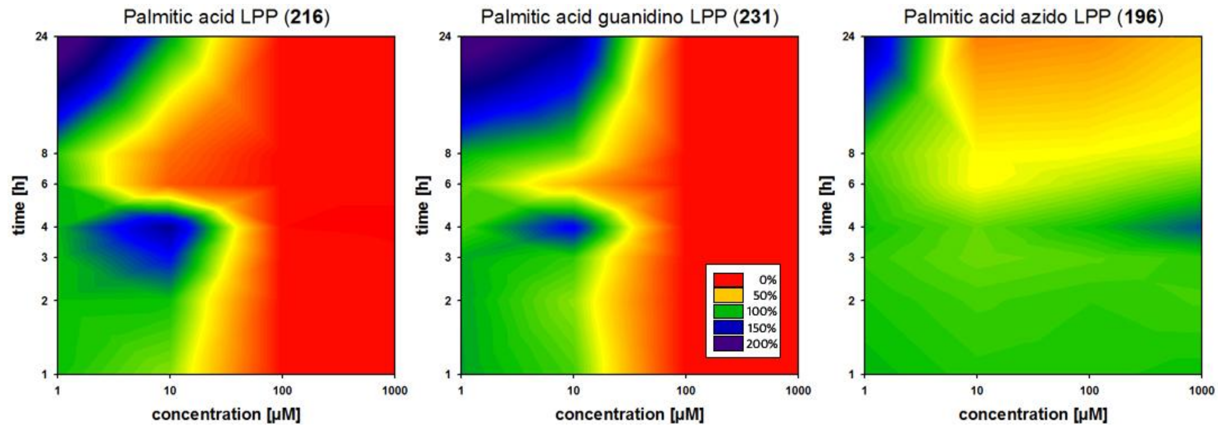


Figure 40. Heatmaps of the luminescence assay data of compounds **196**, **216** and **231**.

The heatmaps of the amino and the guanidine LPPs **216** and **231** reveal that there is no big difference in the activity profile of both compounds. They essentially have the same shape with the minima and maxima of the luminescence being located at the same positions. Therefore it can be assumed that the nature of the cationic charge, being located at an ammonium or a guanidinium moiety does not have an effect on the membrane activity of the investigated LPPs. The pure cationic properties in combination with the lipophilic terminus are enough to kill the bacteria. For the palmitic acid derivatives **216** and **231** an initial concentration of 100 µM needs to be used to instantly kill all bacteria in the assay setup. In contrast to that, the application of the non-cationic compound **196** with the same fatty acid moiety does not effect an instant killing of the microorganisms, but furthermore leads to a delayed luminescence decrease that is not as strong as for the cationic relatives, because it just diminishes the luminescence to ~25% after 24 h relative to the control. These results support the assumption that only a combination of only a few cationic residues with a lipophilic tail of effective size would lead to a strong effect on the bacterial membrane.

3.1.6.3 Fluorescently labeled derivatives 243 and 244

The NBD labelled derivatives **243** and **244** share the same architecture, just differing in the nature of the cationic moieties attached to the side chains. The fluorescence labeling should allow for a localization of the compounds by fluorescence microscopy, when applied to eucaryotic cells that are big enough to give a good resolution of the cellular compartiments.

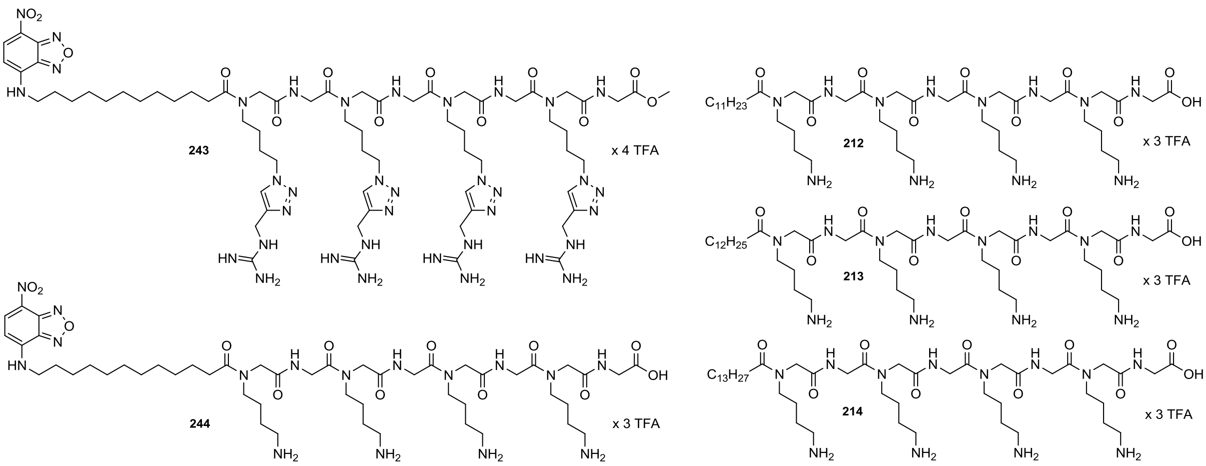


Figure 41. Structural formulas of compounds **212 – 214**, **243** and **244**.

For this application the activity of the labeled compounds needed to be determined and compared to the activity of the non-fluorescent relatives at least in the bacterial luminescence assay. Due to the light emission of *A. fischeri* being the range of the local UV absorption maximum of NBD amines an interference of the assay setup with the testing of NBD labeled compounds was apprehended. Due to the very low concentrations of the compounds the luminescence output was not decreased (directly after the addition of the compounds) by the application of the substances and the assay could therefore being used to investigate the activity of **243** and **244** (Figure 42).

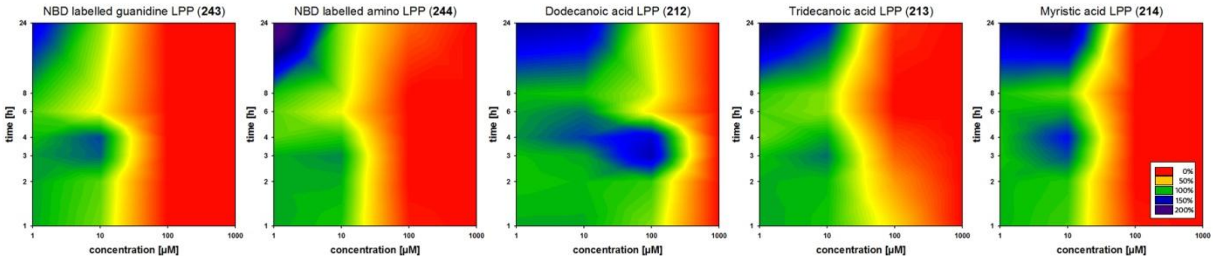


Figure 42. Heatmaps of the luminescence assay data of compounds **212 – 214**, **243** and **244**.

Due to the spacer length of twelve carbon atoms with the terminal NBD substitution the activity of compounds **243** and **244** was supposed to be in the range of the appropriate amino LPP derivatives with twelve to fourteen carbon atoms (Figure 41). This assumption could be verified by the luminescence measurements. The heatmaps of the NBD derivatives are quite similar to the ones from the non-fluorescent derivatives **213** and **214**. The dye labeling does not have an influence on the activity of the compounds towards *A. fischeri*. Even the long-term profile looks the same for both compounds and they are able to kill the bacteria instantly at concentrations higher than 100 µM like it was observed for the fatty acid derivatives **213** and **214**.

3.1.7 Comparison between amino and azido LPPs

The application of amino/azido LPPs to luminescent cultures of *A. fischeri* leads to strong effects on the luminescence relative to a control culture. The luminescence inducing or decreasing effects are strongly dependent on the fatty acid chain length of the compounds. An inhibitory effect could be found down to an applied concentration of 10 µM for the most active compounds. Focussing on this value as a kind of activity barrier, a direct plotting of all measured luminescence data after 24 h for the 10 µM approaches of the amino LPP acids **201 – 220** and the azido LPP acids **181 – 200**, respectively, leads to the finding that there is a second activity barrier visible, depending on the chain length of the fatty acid. Interestingly this barrier is different for both kinds of derivatives.

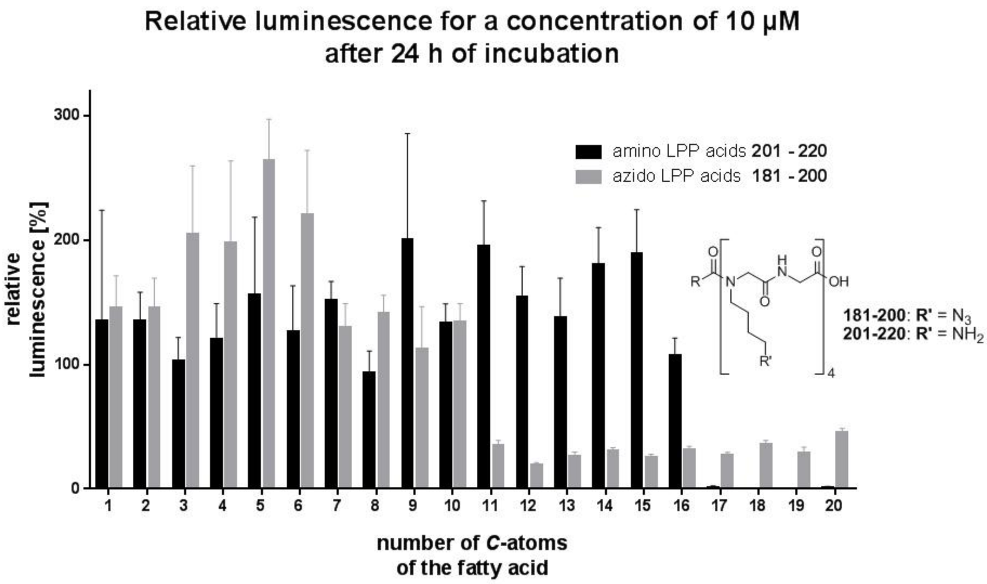


Figure 43. Relative luminescence data for the application of compounds **181** – **220** in the *A. fischeri* assay at a concentration of 10 µM and after an incubation time of 24 h. The mean value of six biological replicates with the standard deviation is plotted.

Whereas the polycationic ones show a kind of random induction of luminescence up to compounds with a fatty acid length of 16 carbon atoms and a complete vanishing of the light emission for the higer derivatives, the azido compounds show this activity boost between two and eleven carbon atoms in the fatty acid chain. Furthermore, the azido derivatives do not completely kill the bacteria so that for all higher derivatives a rest luminescence of ~30% is still detectable after 24 h (Figure 43). The reason for this different effect could not further be determined.

3.2 Bacillus subtilis fluorescence assay

The antibacterial activity of the synthesized LPPs should not only be evaluated towards their potency to inhibit gram-negative germs. Amongst nosocomial infections methicillin-resistant *Staphylococcus aureus*, a gram-positive bacterium with a high ability of developing resistances against any kind of antibiotic compound, causes some of the most severe forms[185,186]. Due to the fact that an antibacterial assay with this pathogen is complicated, because of the numerous precautions to avoid infections, a model assay with a non-pathogenic strain of *Bacillus subtilis* was employed. The employed fluorescent bacterial strain served as a model organism in a harmless assay system for evaluation of the antibacterial activity of the LPPs towards gram-positive germs. The bacterial assay was conducted by fluorescence measurements relative to a control after an incubation of the bacteria over night like described by MICHELS in 2011[187].

3.2.1 Activity of amino LPP acids 201 – 220 in the fluorescence assay

Due to the promising results of the antibacterial activity of compounds **201 – 220** against gram-negative *A. fischeri* in the concentration of 10 µM, this concentration was used as a marker for activity. The whole compound library was applied in concentrations of 1 µM and 10 µM.

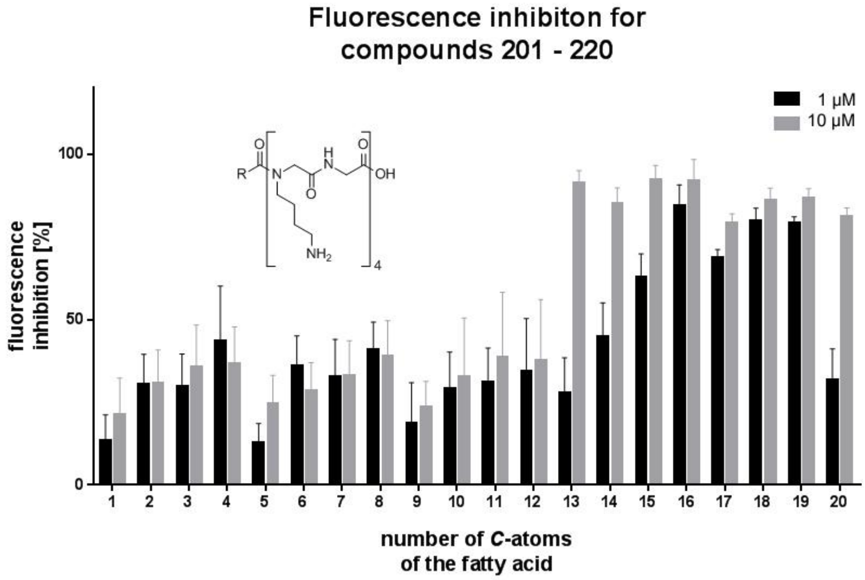


Figure 44. Fluorescence inhibition data for the application of compounds **201 – 220** in the *B. subtilis* assay at concentrations of 1 µM and 10 µM. The mean value is plotted with the standard deviation.

The experimental data show a general fluorescence inhibition of the polycationic compounds of 20% to 50% and a fatty acid chain length of one to twelve for both applied concentrations (Figure 44). Like the results that were obtained from the gram-negative assay (3.1.4, page 71ff) the higher homologues were able to inhibit the fluorescence of the bacteria and thus deteriorate the bacterial viability almost completely. This effect can be observed for compounds **213 – 220** increasing drastically from twelve to thirteen carbon atoms in the fatty acid for a concentration of 10 µM. The lower concentration of 1 µM shows similar effects only for compounds **216 – 219**. The longest fatty acid derivative applied (i.e. arachidic acid (**220**)) is only able to inhibit the bacterial fluorescence by less than 50% at a concentration of 1 µM.

The assay results show clearly that the lipophilic, polycationic compounds **216 – 219** with chain lengths of the fatty acid in the area of 16 to 19 carbon atoms are highly potent inhibitors of gram-positive bacterial growth. Their general activity can be assumed to be one order of magnitude higher against gram-positive *B. subtilis* than against gram-negative *A. fischeri*. This result is in accordance with the architecture of the bacterial membrane and the assumed membrane tackling mode of action of the compounds. Due to the missing outer membrane the LPPs can attack the inner bacterial membrane directly and are therefore more effective at lower concentrations, although the do not seem to kill the bacteria completely like it was observed for the gram-negative germs. A residual fluorescence can still be detected, but the inhibition reaches values of more than 84% for the C16 derivative **216** and a concentration of 1 μM.

3.2.2 Activity of the first generation azide LPP acids 61 – 80 in the fluorescence assay

The first generation azido LPP acids **61 – 80** were also applied in the fluorescence assay. Unlike the final compounds **201 – 220** they only consist of one peptoidic side chain with a terminal azide moiety. The free carboxylate on the *C*-terminus was necessary for water solubility. Any attempts to dissolve the respective methyl esters **41 – 60** in the assay solution failed. The testing of the first generation azido LPP acids should give a better insight into the fatty acid dependency of the putative inhibition of the compounds. Due to the side chain azide being only present once and the whole backbone being much shorter than in the fourth generation compounds, the influence of the *N*-terminal fatty acid becomes greater and should have a bigger effect on the bacterial inhibition.

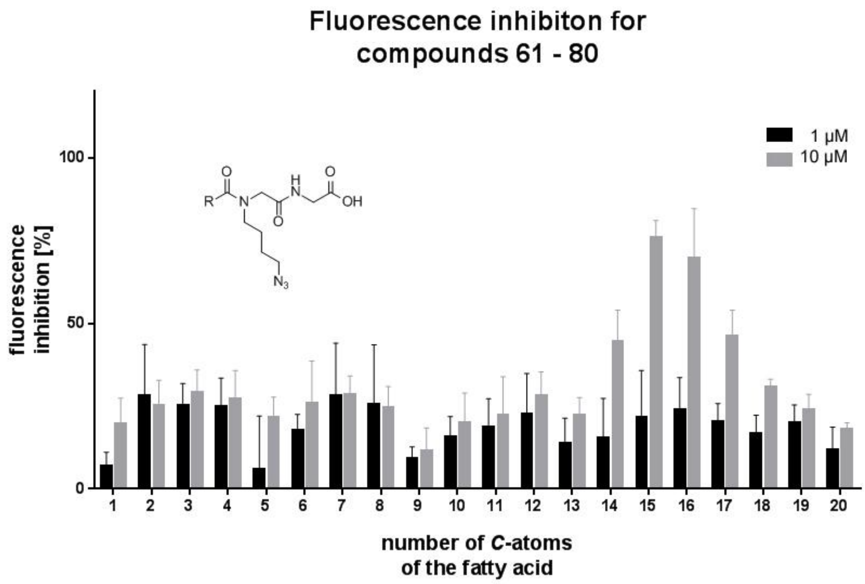


Figure 45. Fluorescence inhibition data for the application of first generation compounds **61 – 80** in the *B. subtilis* assay at concentrations of 1 µM and 10 µM. The mean value is plotted with the standard deviation.

The effect of the first generation azido LPP acids **61 – 80** on the bacterial fluorescence lays in the range of 10% to 25% inhibition for concentrations of 1 µM and 10 µM (Figure 45). There are only four compounds that show a higher activity against the bacteria in a concentration of 10 µM. The active compounds contain fatty acids with the chain length of 14 to 17 carbon atoms and show an inhibition maximum of ~70% for the C15 and C16 derivatives **75** and **76** at 10 µM. The longer chain derivatives do not differ in their activity profile from the short chain compounds, i.e. there is a chain length maximum as is known from other antibiotics.

The effect of the fatty acid chain length could be impressively shown for the first generation azido LPP acids. *B. subtilis* cells are obviously most sensitive towards lipopeptoids containing alkyl chains of 15 or 16 carbon atoms. The combination of this alkyl chain length attached to the *N*-terminus of the molecule with several cationic residues in the side chains enhances the antibacterial activity by a whole order of magnitude to result in highly active compounds that are able to inhibit bacterial growth by 80% and more at a concentration of 1 µM. The antibacterial properties of the LPPs are therefore significantly more distinct towards gram-positive germs than towards gram-negative bacteria. Although *A. fischeri* was killed by compounds **201 – 220**, whereas the viability of *B. subtilis* just decreased to ~15 % for the most active compounds resulting in growth deficient, but still viable colonies of bacteria.

3.3 Plant pathogenic fungi assay

Not only human pathogens are interesting targets of antibacterials. In agriculture some of the most problematic pathogens belong to the kingdom of fungi. To tackle the more and more evolving resistances of the major plant pathogens new kinds of compounds should be evaluated. Due to the fact that fungal cultures are sensitive towards fatty acids of a certain chain length the effect of the amino LPP acids with the most promising activities towards bacteria were applied[188,189]. The two fungi *Septoria tritici*, *Botrytis cinerea* and the oomycete *Phytophtora infestans* were investigated towards their sensitivity against the amino LPP acids **214 – 220**. The microorganisms were grown on adequate media and the density of the treated cultures in comparison to the non-treated ones was measured photometrically at 405 nm after seven days of incubation and a percentual growth inhibition was calculated[190].

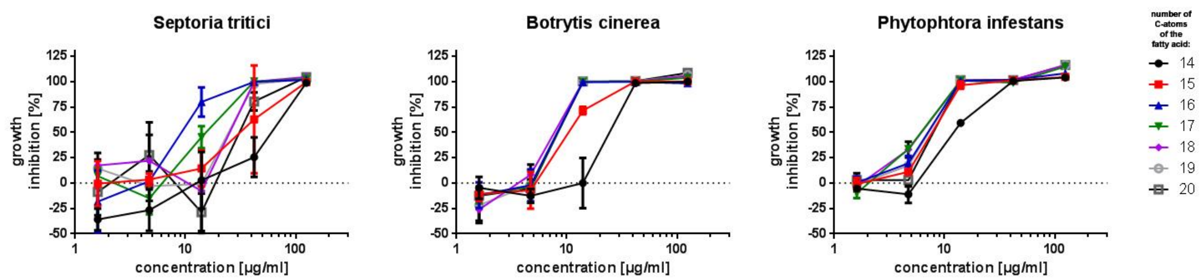


Figure 46. Inhibition data of compounds **214 – 220** in the anti-fungal assay. The activity of the compounds towards *S. tritici*, *B. cinerea* and *P. infestans* is plotted together with the calculated standard deviation.

The interesting result of this assay is that the activity profile of the LPP amino acids looks essentially the same for *B. cinerea* and *P. infestans*. Both species are inhibited at concentrations of higher than 14 µg/ml almost completely. Only compound **214** containing myristic acid showed a slightly lower activity in this assay against those two plant pathogens. The third species, *S. tritici* does not show such a high sensitivity towards the compounds. Only the highest concentration of 125 µg/ml leads to a total inhibition caused by all compounds, independent on their fatty acid. The only derivative that is comparable in its potency to the activity towards the other two species is **216** containing palmitic acid. All other compounds show a significantly lower ability to inhibit *S. tritici*.

The amino LPP acids are potent inhibitors of fungal growth in the conducted assay. The most active derivative is the palmitic acid compound **216**, which inhibits the growth of all three fungi at concentrations higher than 14 µg/ml, which equals a molar concentration of ~10 µM.

The results obtained in the anti-fungal assay regarding the potency of the polycationic substances in inhibiting microorganisms are therefore consistent with the observed activities of the LPP amino acids towards bacteria. Not only the chain length of the fatty acid in the most active derivative lies in the same range like it was observed for the antibacterial activity, also the minimum concentration of a total inhibition of the respective microorganism is essentially the same. The effect of the LPPs on the fungal cell membrane hence might be caused by the same mode of action as was assumed for the activity towards bacterial membranes.

3.4 Hemolysis assay

For the putative application of an antibacterial compound in the treatment of bacterial diseases the activity of this substance towards human cells needs to be determined. Due to the membrane destroying properties of the LPPs the hemolytic potency of the compounds are of interest. The hemolytic properties of the compounds were determined by a modified photometrical method after KAHN[191] and HARBOE[192]. As a positive control, completely lysed erythrocytes were used. The total lysis was conducted by treatment with distilled water. The acute as well as the long-term hemolytic activity (90 min and 18 or 20 h) were determined.

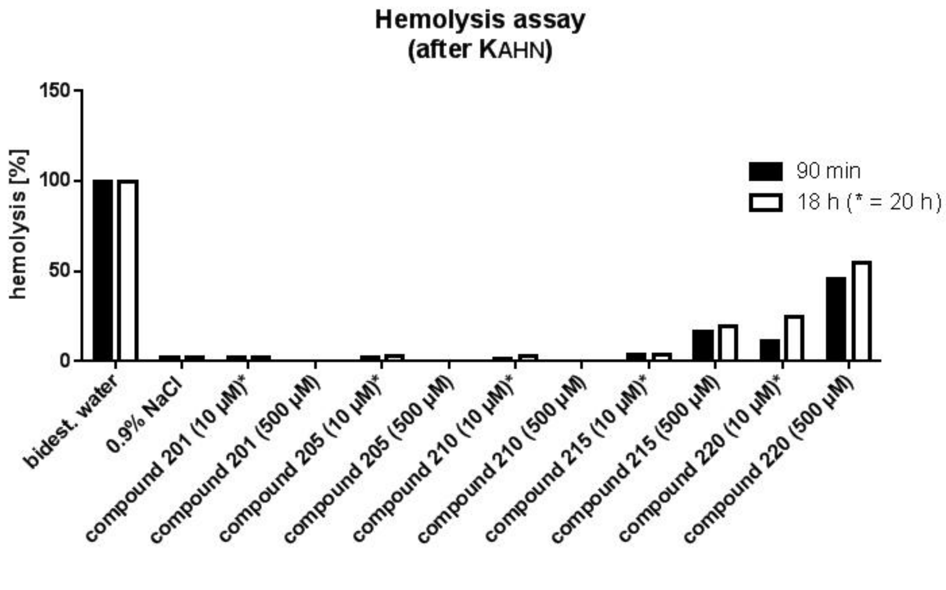


Figure 47. Hemolysis data of compounds **201**, **205**, **210**, **215** and **220** (after KAHN[191]).

The results of the hemolysis assay are comparable to the results from the bacterial as well as the fungal assays. The LPPs with short or intermediate chain lengths of the fatty acids do not show a hemolytic effect. Even the highest concentration of 500 µM does not lead to hemolysis even after a prolonged incubation time of 18 h. This effect is true for compounds **201**, **205** and **210**. A slight effect can be observed for compound **215** at a concentration of 500 µM, whereas the same substance does not lyse the erythrocytes at a concentration of 10 µM. The highest homologue **220** is capable of lysing the erythrocytes at both applied concentrations of 10 µM and 500 µM. Interestingly, only a slight difference between the acute and the long-term hemolysis can be observed for the active compounds **215** and **220**. The incubation time does not make a big difference in the hemolytic progress. The most potent compound **220** lyses ~50% of the erythrocytes at a concentration of 500 µM after 90 minutes as well as after 18 h. This very quick response effect is the same that was observed for the compounds when they were applied in the *A. fischeri* luminescence assay.

The amino LPP acids are capable of lysing erythrocytes, even in concentrations of 10 µM for the higher homologues. The therapeutic index of the LPPs seems to be rather small taking this result into consideration. Nevertheless the use of the LPPs is not necessarily limited to an intraveneous application. Instead of this, a topic application or the use as general disinfectants might be possible due to their rapid mode of action towards gram-negative bacteria. For those purposes the hemolysis would not be a disadvantage, because the contact with erythrocytes would be prevented.

3.5 Fluorescence microscopy of LPP treated human cancer cells

The activity of the higher homologues of the synthesized amino LPP acids **201 – 220** against microorganisms could be shown as well as their hemolytic acitivity. To determine the distribution of this compound class within a eukaryotic cell the NBD-labeled derivatives **243** and **244** were used.

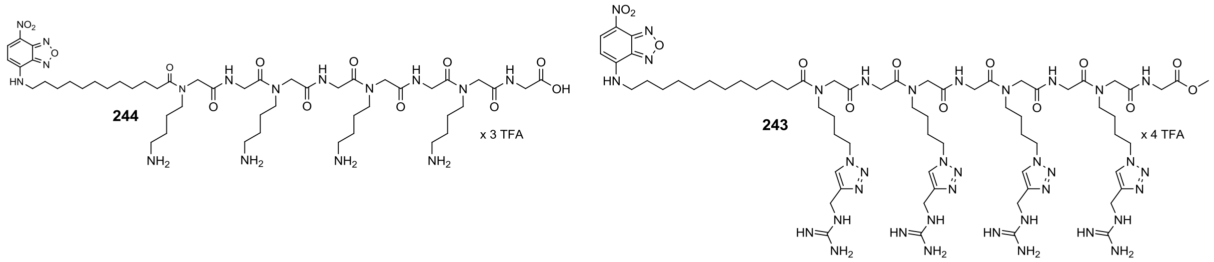


Figure 48. Structural formulas of NBD-labeled compounds **243** and **244**.

The dye labeling allowed for the recording of a fluorescence image with a confocal laser fluorescence microscope. As a cellular model, PC3 cells were grown adhesively on transparent deep-well plates. Compounds **243** and **244** were dissolved in RPMI medium and the precultivated cells were incubated with the dye solution for 60 minutes before the excess staining solution was washed away with buffer. For a better contrast and the generation of a kind of fix point the nuclei were additionally stained with DAPI. Images were taken in confocal mode to generate 3D images of the dye distribution within the cancer cells.

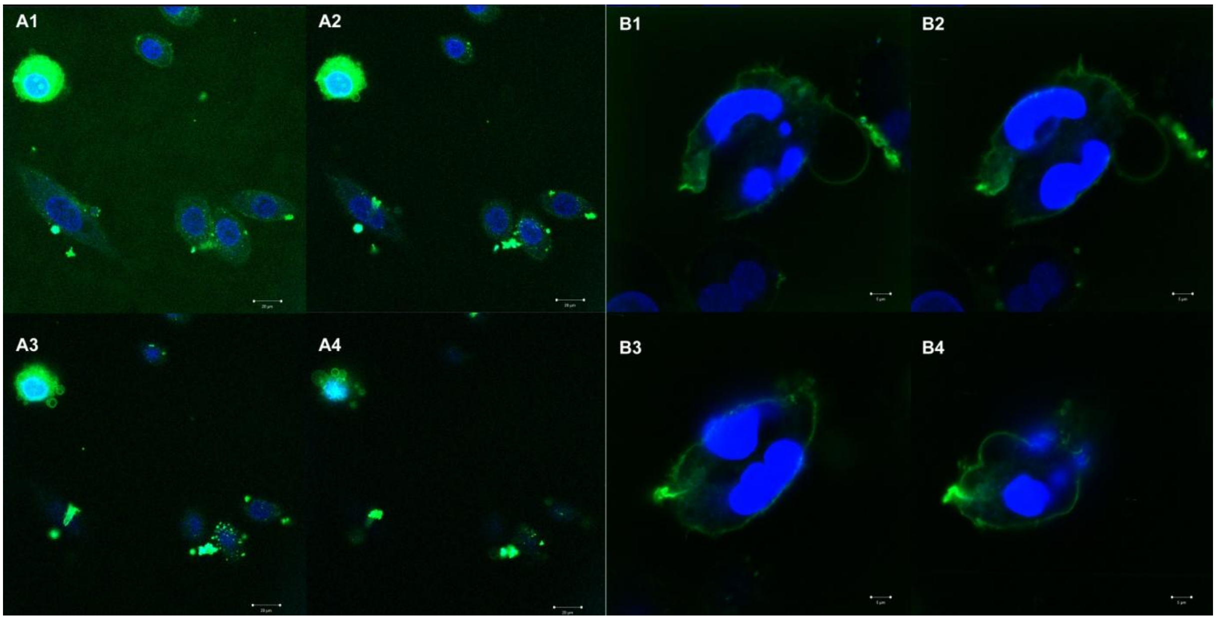


Figure 49. Confocal laserscan microscopic images of PC3 cells treated with NBD-labeled lysine-like-compounds **244** (A series) and arginine-like compounds **243** (B series). Cellular nuclei are stained with DAPI in both series. The overlays are calculated from two separate images generated from an excitation wavelength of λ = 360 nm and an emission wavelength of λ = 460 nm for the DAPI stain and = 480 nm; λ = 550 nm for compounds **243** and **244**, respectively. The vertical distance of the single images of each series is 2 µm (lowest = 1). The white bar eqals 20 µm in the A series and 5 µm in the B series.

The confocal images (Figure 49) clearly show a distinct distribution of both compounds within the PC3 cells, but differences between the two differently substituted compounds. The amino derivative **244** is located in intracellular vesicles that seem to be formed by the stressed cell and are a putative sign for apoptosis. In the early stage a staining is hardly visible and the compound is not detectable in the intact outer membrane. Instead of this, it seems that the polycationic **244** passes the intact membrane and accumulates in the intracellular vesicles of unknown origin. Some cells are completely filled up with fluorescent vesicles that seem to detach from the cell. This observation could be due to induced apoptosis in the stressed cancer cell. Interestingly the vesicular membrane seems to be more affine towards the fluorescent compound than the outer membrane is (Figure 49, A series).

The guanidinium derivatized compound **243** shows a different behavior (Figure 49, B series). In all observed cells the compound remains in the outer membrane and no intracellular vesicle staining becomes observable. The shown cell is also stressed and shows an abnormal membrane shape, but no inner fluorescence is visible – in contrast to the staining with compound **244**. The differences between the two cationic compounds maybe due to the changed side chain substitution and the residues carrying the positive charge. Obviously the amino derivatives can diffuse or are transported into the cell, whereas the guanidinium compound remains in the outer membrane. This effect shows that even small changes in the architecture of a compound can lead to different cellular distribution and also a different mode of action. The results from this eukaryotic incubation experiment cannot directly be compared to the qualitative findings in the prokaryotic assays, but they may give a hint that the interaction of LPP derivatives with cellular membranes is a complex process and the target of those compounds is not always as clear as it seems to be at first sight.

3.6 Human cancer cell assay

In addition to the fluorescence microscopic images (chapter 3.5), the general toxicity of selected LPPs towards human cancer cell lines (PC-3 and HT-29) was investigated in a pre-screening. No significant activity of the applied compounds could be observed. At a concentration of 10 µM, only azido-LPP **200** and amino-LPP **220** with an arachidic acid chain showed a slight growth inhibition of ~20%.

Table 15. Growth inhibition data of selected compounds in the human cancer cell assay against PC-3 and HT-29 cells. Only compounds **200** and **220** showed a significant, but only slight inhibition of the HT-29 growth (highlighted in dark blue). [a)] A negative growth inhibition was calculated.

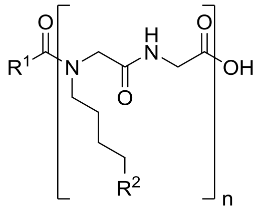
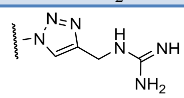
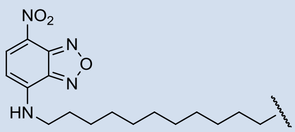
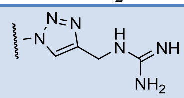
Compound	Description	Concentration	Growth inhibition	
			PC-3 cells	HT-29 cells
181	Formic acid azido LPP	10 µM	8.1% ± 6.6%	2.7% ± 4.6%
		10 nM	9.8% ± 9.2%	12.5% ± 7.1%
196	Palmitic acid azido LPP	10 µM	a)	11.6% ± 4.5%
		10 nM	5.6% ± 6.3%	3.8% ± 5.6%
200	Arachidic acid azido LPP	10 µM	6.9% ± 6.8%	**23.9% ± 4.3%**
		10 nM	a)	a)
201	Formic acid amino LPP	10 µM	6.9% ± 5.2%	a)
		10 nM	9.4% ± 1.3%	0.8% ± 9.6%
206	Hexanoic acid amino LPP	10 µM	0.4% ± 7.0%	3.9% ± 6.6%
		10 nM	10.7% ± 7.5%	10.3% ± 5.1%
215	Pentadecanoic acid amino LPP	10 µM	11.1% ± 7.3%	a)
		10 nM	4.2% ± 2.8%	5.4% ± 9.2%
220	Arachidic acid amino LPP	10 µM	0.3% ± 8.6%	**21.7% ± 2.2%**
		10 nM	8.9% ± 4.3%	9.3% ± 4.4%
243	NDB-guanidino LPP	10 µM	a)	a)
		10 nM	4.7% ± 3.8%	2.9% ± 7.3%
244	NBD-amino LPP	10 µM	a)	a)
		10 nM	2.9% ± 9.1%	12.4% ± 4.6%

In contrast to the results that were obtained in the antimicrobial assays (see chapters 5.2.1 and 5.2.2), compound **220** could not kill the cancer cells at a concentration of 10 µM. Furthermore, only HT-29 cells were slightly sensitive towards the LPPs, whereas the growth of PC-3 cells was not significantly inhibited. This finding elucidates the different membrane architecture and susceptibility of eukaryotic and bacterial cells. Membranes of eukaryotic cells, except erythrocytes (see chapter 3.4) seem to be more resistant against the attack of the amphiphilic LPPs.

4 Summary

In the first chapter of this thesis the U-4CR was introduced for the syntheses of a library of lipophilic peptoid-peptide chimeras. Due to the application of this complex, still easy to be performed synthesis, in a cyclic way, the generation of linear lipophilic peptoid chimeras in high yields became possible.

Table 16. Overview of the synthesized LPP acids. *) Obtained as methyl ester.

 **Compound ID**	**R^1**	**R^2**	**n**
61 – 80	-H, $-CH_3$ → $-C_{19}H_{38}$	$-N_3$	1
101 – 120			2
141 – 160			3
181 – 200			4
201 – 220		$-NH_2$	
231	$-C_{15}H_{31}$		
234		$-N_3$	1
236			2
238			3
244		$-NH_2$	4
243*			

The synthesized compound library is comprised of all homologues from formic acid to arachidic acid, is unique in this widespread form and allows for a broad bioactivity screening of the respective compounds (Table 16). It could be shown that the introduction of four guanidinium moieties can be accomplished by the azide-alkyne Huisgen cycloaddition. A Staudinger reduction finally generated the polycationic amino acid LPPs in high yields and reasonable amounts. In addition to the linear synthesis, even the dimerization of a fourth generation lipophilic tetra-azido acid compound was possible by the use of a small diisocyanide.

Additionally, dye-labeled derivatives were synthesized in the same fashion like the library LPPs. The NBD moiety that was used for labeling, allowed for the application of the compounds in confocal-laserscanning microscopy experiments. The dye labeled LPPs were obtained with amino as well as with guanidino moieties in the side chains and in high yields over the whole synthetic pathway.

In the second chapter, the LPP library was applied to several different bioassays and microscopy experiments. First of all, an easy to conduct luminescence-based gram-negative bacterial cell assay was developed. The use of the self-luminescent *A. fischeri* allowed for a kind of kinetics-screening of the applied compounds. Due to the possibility to measure the treated bacterial colonies photometrically during 24 h at different points in time a 2D-heatmap could be generated for the applied compounds. The application of the lipophilic library compounds in this assay revealetd their high potency to kill gram-negative bacteria. Anyhow, it could be shown that not only the pure number of cationic charges, but also the type of the moieties carrying this charge as well as the combination with a certain lipophilic substitution plays an important role in the antibacterial activity.

The results of the gram-negative luminescence-based assay were proven by the data obtained from several other biological assays employing gram-positive bacteria, fungi, erythrocytes and cancer cells as test organisms. The most active compounds with a clear prokaryote and erythrocyte membrane activity were the ones with four cationic charges located on the terminal amino groups of the side chains in combination with an *N*-terminal acylation with a fatty acid of 15 to 19 carbon atoms.

The compound library and the biological investigation of its members gave insight into the complex field of lipopeptoidic membrane interaction. The nature of the interaction was not part of this thesis, but due to the impressive biological effects that could be semi-quantitatively determined by the conduction of a broad variety of biological assays, the basis of a deeper investigation could be generated. In future experiments the complexity of the side chains and the combination with unsaturated fatty acids or other lipophilic compounds could lead to more distinct tackling of certain microorganisms or even cell organels. The U-4CR proved to be a suitable way to generate this kind of diversity and adds a synthetic tool to the well known sub-monomer approach to synthesize cell-penetrating peptoids[132,133,193–195].

5 Experimental Part

5.1 General remarks

All chemicals and solvents are commercially available and were purchased from Sigma-Aldrich (Switzerland), Acros (Belgium), Fluka (Switzerland), Merck (Germany), Roth (Germany) or Synthon Chemicals (Germany) and were used without further purification. Absoluted solvents were prepared according to standard procedures by refluxing over suitable drying agents and storage under nitrogen. If not otherwise stated, all reactions were conducted at room temperature under a nitrogen gas atmosphere in dry solvents.

Crude compounds were purified by column or flash chromatography on silica 60 (Merck, Germany) with 230 – 400 mesh (0.040 – 0.063 mm). The chromatographic procedure was accomplished by gravity or the application of an overpressure of 0.5 bar.

The analytical thin-layer chromatography (TLC) was conducted with silica coated aluminum plates (silica 60 F_{254}; Merck, Germany). The detection of the compound was accomplished by staining with cerium(IV)-molybdatophorphoric acid, ninhydrin solution and successive heating to ~100 °C or UV light of 254 or 366 nm.

NMR spectra were recorded on spectrometers from Varian (Mercury 300, 400 and 600). The chemicals shifts of the ^{1}H-NMR spectra are referenced on the signal of the internal standard tetramethylsilane (TMS, δ = 0.000 ppm) for spectra in deuterochloroform or deuteromethanol. Spectra in deuterium oxide are referenced on the internal standard trimethylsilyldeuteropropionic acid (TMPA, δ = 0.000 ppm). The ^{13}C-NMR spectra are referenced on the solvent signals of $CDCl_3$ (δ = 77.000 ppm), C_6D_6 (128.000 ppm) and CD_3OD (δ = 49.000 ppm). As external reference for the ^{19}F-NMR spectra trichlorofluoromethane (δ = 0.000 ppm) was used. The signal multiplicities are abbreviated as follows: **s** (singlet), **d** (doublet), **t** (triplet), **q** (quartet), **m** (multiplet), **br** (broad signal).

Standard mass spectra (MS) were measured on an API-150 device (Applied Biosystems) in positive and negative electrospray mode (ESI-/ESI-). The high resolution mass spectra (HR-

MS) were measured on a BioApex 70 eV FT-ICR (Bruker) in positive or negative electrospray mode (ESI+/ESI-).
The luminescence measurements were conducted on the microplate reader Genius Pro from Tecan and wavelengths from 400 nm – 700 nm were measured in an additive mode. The fluorescence measurements were also conducted on the same device. The excitation wavelength was 510 nm and the fluorescence was measured at 535 nm.

The confocal laserscanning microscopy images were recorded with a LSM710 fluorescence microscope (Zeiss, Germany) with a coherent two-photon laser and fluorescence life-time imaging (FLIM, Becker&Hickl).

5.2 Biological assays

5.2.1 Aliivibrio fischeri luminescence assay

The determination of the activity of a substance towards gram-negative bacteria can be achieved in an assay system employing the model organism *Aliivibrio fischeri*. This bacterium evolves a strong luminescence at a certain cell density. This luminescence is proportional to the cell viability and can therefore be used to determine the toxicity of a compound by its ability to inhibit the bacterial luminescence. For the development of this assay the *A. fischeri* strain DSM507 (batch no. 1209) from the "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (DSMZ) was used.

5.2.1.1 Assay medium

The bacteria were grown on Boss medium in liquid culture and on agar plates (Table 17). The saline medium itself was enough to selectively promote the growth of *A. fischeri* and inhibit the growth of putative contaminating germs. No antibiotic for selection of certain colonies was therefore added. The pH value of the medium was adjusted to pH 7.3 after preparation by addition of NaOH solution and was afterwards autoclaved at 121 °C for 20 minutes. The sterilized solutions were stored in glass bottles until usage in the dark at room temperature in 200 ml aliquots. For the preparation of Boss agar plates Agar-Agar Kobe 1 (Roth, Germany) was added to 1.5 % w/V before autoclavation and agar plates were prepared from the hot medium directly after autoclavation. The agar plates were stored in the dark at 4 °C until usage.

Table 17. Composition of Boss medium used for the luminescence assay. *) Only added for the preparation of agar plates.

	amount
NaCl (Fluka)	150 g
glycerin (Sigma-Aldrich)	5 g
peptone (Fluka)	50 g
meat extract (Fluka)	15 g
distilled water	ad 5000 ml
pH value	7.3
Agar-Agar Kobe 1 (Roth)*	1.5 % w/V

The lyophilized pellet, obtained from the DSMZ was used for the inoculation of a Boss agar plate. After incubation at 23 °C over night colonies of *A. fischeri* were visisble. One colony was picked with an inoculation loop and was used to start a liquid culture in 25 ml Boss medium that was incubated at 200 rpm for 18 h and 23 °C in a 100 ml sterile beaker equipped with a cotton plug. The vegetated agar plates were kept at 4 °C for a putative later colony picking.

5.2.1.2 Glycerol stocks

The liquid starter culture was used to producwe glycerol stocks for easier handling of the bacteria. Therefore 50 µl of the liquid culture were used to inoculate another 25 ml of Boss medium that was afterwards incubated for 25 h at 23 °C and 200 rpm. An aliquot of 750 µl of this culture was diluted with 250 µl of glycerin and the resulting bacterial suspension was transferred as 50 µl aliquots into 20 sterilized 1.5 ml eppendorf tubes that were directly closed and deep frozen in liquid nitrogen. The glycerol stocks were kept at -80 °C until usage.

5.2.1.3 Bacterial assay

In a standard screening assay six compounds can be tested for their antibacterial properties. For conducting the assay the inoculation of a liquid culture of 25 ml Boss medium with one 50 µl glycerol stock of *A. fischeri* that was warmed to room temperature within 10 min before application was necessary. The inoculated medium was incubated for 16 h at 23 °C and 23 °C in a 100 ml sterile beaker equipped with a cotton plug.

From the compounds that shall be tested a 100 mM DMSO stock solution needs to be prepared. This stock solution is kept at -20 °C until usage. Before starting the assay the stock solution is warmed to room temperature and a 1:50 dilution of the DMSO stock solution with liquid Boss medium is prepared to result in a 2 mM solution of the compound in Boss medium containing 2% v/v DMSO. Of this solution further three successive 1:10 dilutions were prepared with a previously made solution of 2% v/v DMSO in Boss medium to result in four concentrations of the compound (2 µM, 20 µM, 200 µM and 2000 µM) in Boss medium containing 2% v/v DMSO. This procedure is accomplished for the six test compounds as well as for chloramphenicol (CA, **253**) separately, because this antibiotic is used as a positive control within each assay setup.

Table 18. First pipetting scheme for the preparation of a 96 well-plate with the compound solutions. This scheme is followed for the preparation of two identical plates for each assay setup.

<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>A</td><td colspan="3">empty</td><td colspan="3">100 µl
2 µM
compound 1 solution</td><td colspan="3">100 µl
2 µM
compound 3 solution</td><td colspan="3">100 µl
2 µM
compound 5 solution</td></tr>
<tr><td>B</td><td colspan="3">100 µl BOSS medium
(2% DMSO)</td><td colspan="3">100 µl
20 µM
compound 1 solution</td><td colspan="3">100 µl
20 µM
compound 3 solution</td><td colspan="3">100 µl
20 µM
compound 5 solution</td></tr>
<tr><td>C</td><td colspan="3">100 µl BOSS medium
(2% DMSO)</td><td colspan="3">100 µl
200 µM
compound 1 solution</td><td colspan="3">100 µl
200 µM
compound 3 solution</td><td colspan="3">100 µl
200 µM
compound 5 solution</td></tr>
<tr><td>D</td><td colspan="3">100 µl BOSS medium
(2% DMSO)</td><td colspan="3">100 µl
2000 µM
compound 1 solution</td><td colspan="3">100 µl
2000 µM
compound 3 solution</td><td colspan="3">100 µl
2000 µM
compound 5 solution</td></tr>
<tr><td>E</td><td colspan="3">100 µl
2 µM
CA solution</td><td colspan="3">100 µl
2 µM
compound 2 solution</td><td colspan="3">100 µl
2 µM
compound 4 solution</td><td colspan="3">100 µl
2 µM
compound 6 solution</td></tr>
<tr><td>F</td><td colspan="3">100 µl
20 µM
CA solution</td><td colspan="3">100 µl
20 µM
compound 2 solution</td><td colspan="3">100 µl
20 µM
compound 4 solution</td><td colspan="3">100 µl
20 µM
compound 6 solution</td></tr>
<tr><td>G</td><td colspan="3">100 µl
200 µM
CA solution</td><td colspan="3">100 µl
200 mM
compound 2 solution</td><td colspan="3">100 µl
200 µM
compound 4 solution</td><td colspan="3">100 µl
200 µM
compound 6 solution</td></tr>
<tr><td>H</td><td colspan="3">100 µl
2000 µM
CA solution</td><td colspan="3">100 µl
2000 µM
compound 2 solution</td><td colspan="3">100 µl
2000 µM
compound 4 solution</td><td colspan="3">100 µl
2000 µM
compound 6 solution</td></tr>
</table>

The assay itself is conducted in black 96-well flat-bottom plates (BD Falcon, catalogue number 353376) with a maximum volume of 340 µl in each well. Each compound concentration is measured in six replicates distributed over two identical plates. Three wells on each plate are used as growth control and each concentration is measured in triplicates on each plate. Before the bacterial suspension is prepared from the overnight culture the compound solutions as well as the Boss solutions just containing 2% v/v DMSO are pipetted into two 96-well plates following the scheme in Table 18.

Afterwards 2 ml of the liquid culture were diluted 1:20 with fresh Boss medium to result in 40 ml of a bacterial suspension. To determine the suitability of this dilution twelve aliquots of 100 µl of this suspension were diluted 1:2 with Boss medium containing 2% DMSO w/w in the A row of an empty black 96-well plate. This plate was then measured with the same method that is used for the assay measurements. The obtained luminescence values shall be in the range of 10,000 to 100,000 and should not differ by more than 10% for each well. If the solution is too dense and the obtained luminescence is too high, the bacterial suspension is further diluted with Boss medium and the luminescence test is repeated. Once the luminescence is in the appropriate range the diluted bacterial solution is pipetted to the compound prepared microtiter plates with a multichannel pipette following the scheme in Table 19. The compound solutions are diluted 1:2 by this step to result in test concentrations of 1 µM, 10 µM, 100 µM and 1000 µM with 1% v/v DMSO each.

Table 19. Second pipetting scheme for the addidion of the bacterial suspension to the prepared compound solution plates. Wells A1–A3 and B1–B3 are not inoculated with bacteria to provide a negative control.

	1	2	3	4	5	6	7	8	9	10	11	12
A	/			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
B	/			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
C	100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
D	100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
E	100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
F	100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
G	100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		
H	100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension			100 µl diluted bacterial suspension		

Directly after the addition of the bacterial suspension to the compound solutions in the microtiter plates the plates are measured without lid in the automatic reading device. The whole wavelength range is detected for 1000 ms in each well without preliminary shaking to avoid secondary oxygen effects on the luminescence. After readout the plates are incubated in the dark at 23 °C and 100 % humidity without lid. At certain points in time (1 h, 2 h, 3 h, 4 h, 6 h, 8 h, and 24 h) the plates are transferred into the reader and the luminescence is measured. Luminescence values were obtained in relative luminescence units (RLU).

For each plate the relative luminescence values are calculated by setting each well luminescence with the mean of the control (wells D1–D3) into relation. The six generated luminescence percentages for each compound on the two plates are used for a mean value and a standard deviation calculation using the simplified Formula 4 with L_{pnwm} as the luminescence of well m on plate n and $C_{pnD1\text{-}3}$ as the control luminescence on plate n in wells D1, D2 and D3. The relative luminescence L_{rel} of each compound concentration for a certain point in time is calculated in percent of the non-treated control.

$$L_{rel} = 50\left(\frac{L_{p1w1} + L_{p1w2} + L_{p1w3}}{C_{p1D1} + C_{p1D2} + C_{p1D3}} + \frac{L_{p2w1} + L_{p2w2} + L_{p2w3}}{C_{p2D1} + C_{p2D2} + C_{p2D3}}\right) \tag{4}$$

5.2.2 Bacillus subtilis fluorescence assay

The fluorescence assay with the YFP-producing *Bacillus subtilis* strain was thankfully conducted by MSc Pia Schoene (NWC department, IPB Halle) after a published procedure[187]. The bacteria were incubated with the respective substance concentrations over night and the fluorescence of the colonies was measured. The measured fluorescence in relation to a control gave the growth inhibition values for each applied compound.

5.2.3 Plant pathogenic fungi assay

The fungal assay was thankfully conducted by MSc Alexander Otto (NWC department, IPB Halle) after an unpublished inhouse procedure[190]. The three fungal strains of *Phytophtora infestans*, *Botrytis cinerea* and *Septoria tritici* were grown on suitable media in deep-well plates for one week with and without the respective compounds. The growth inhibition was calculated as the quotient of the optical density measurements of a treated culture to an untreated control.

5.2.4 Hemolysis assay

The hemolysis assay was thankfully conducted by Katrin Vogel (Clinical Pharmacology, MLU Halle-Wittenberg) after literature known procedures[192,196]. The respective compounds were applied to erythrocyte suspensions and the released hemoglobin after 90 min or 18 h (20 h) was photometrically determined. The obtained values in combination with a positive, distilled water lysed control, gave the relative hemolysis data for the applied substances.

5.2.5 Confocal laserscanning microscopy

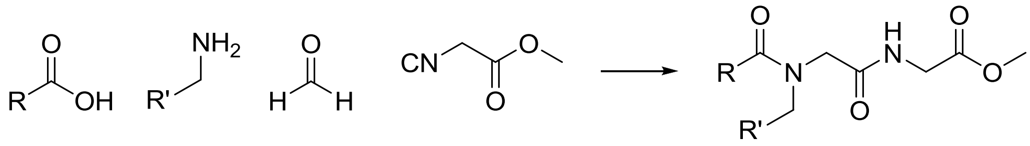
The fluorescence imaging of the dye-labeled compound treated PC3 cells was thankfully conducted by MSc Annika Denkert. The pregrown cells were treated with the respective dye solutions and DAPI for a contrast staining. Images were taken for excitation wavelengths of 360 nm and 480 nm. The fluorescence was measured at 460 nm and 550 nm. Both images were combined to generate an overlay of the two fluorescence pictures. The cells were scanned in slices with 2 µm thickness to get a 3D impression of the dye-labeled compound distribution.

5.2.6 Human cancer cell assay

The human cancer cell assay was thankfully conducted by MSc Annika Denkert (NWC department, IPB Halle) after an unpublished inhouse procedure[197]. The human cancer cells were grown on suitable media in deep-well plates with and without the respective compounds. The growth inhibition was calculated as the quotient of the disctinct absorption of a dye-stained, compound-treated culture at a certain wavelength in relation to the absorption of an untreated control.

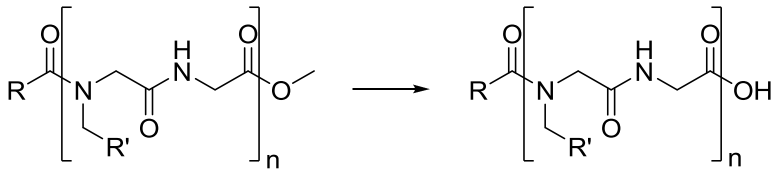
5.3 General chemical procedures

5.3.1 Procedure A – standard-Ugi-4CR



To a stirred solution of a primary amine (1.00 mmol) in methanol (10 ml) paraformaldehyde **19** (50.2 mg, 1.67 mmol) was added. The resulting mixture was stirred at room temperature for two hours. The carboxylic acid (1.00 mmol) and methyl isocyanoacetate **20** (90.7 µl, 99.1 mg, 1.00 mmol) were added successively and the reaction mixture was stirred for at least 18 hours at the same temperature. After checking the completion of the reaction by means of TLC and ESI-MS all volatiles were removed under reduced pressure. The remaining residue was purified by silica column chromatography to afford the pure Ugi-4CR product.

5.3.2 Procedure B – saponification of methyl esters



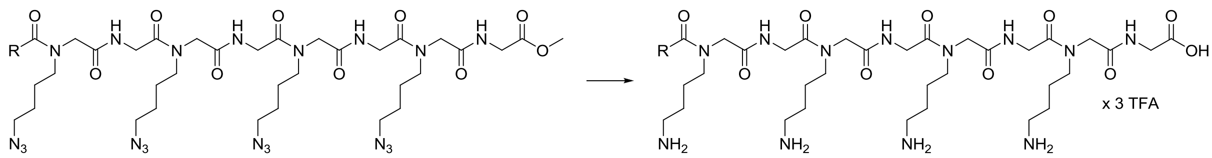
5.3.2.1 Saponification - method 1

To a solution (or suspension) of an appropriate methyl ester (1.00 mmol) in THF/water (2:1, v/v, 20 ml) a solution of lithium hydroxide in water (1.25 ml, 2.50 mmol, 2 M) was added at room temperature and the mixture was stirred until a TLC revealed total consumption of the ester (ca. 2 h). After addition of brine (20 ml) to the reaction mixture the pH value was adjusted to pH 2 by addition of a saturated $NaHSO_4$ solution. The resulting mixture was extracted with ethyl acetate (6 x 100 ml) and the combined organic layers were dried over Na_2SO_4. After filtration the solvent was removed *in vacuo* to afford the acid in high purity. Purification was not necessary in most of the cases. Details for a tentative purification are given for the respective compounds.

5.3.2.2 Saponification - method 2

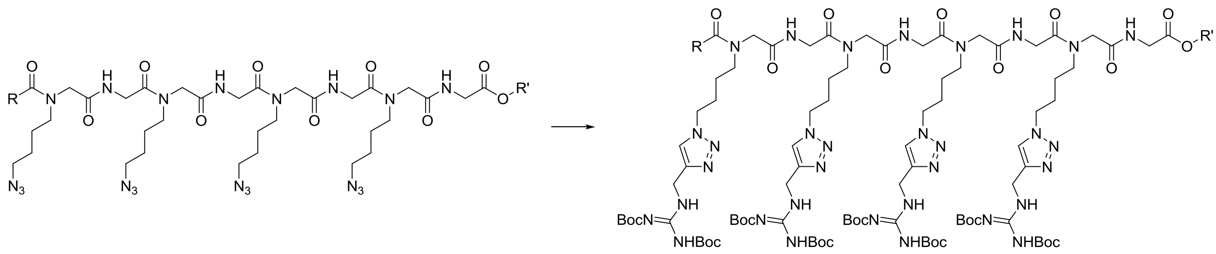
To a suspension of an appropriate methyl ester (0.10 mmol) in THF/water (1:2, v/v, 2.5 ml) a solution of lithium hydroxide in water (125 µl, 0.25 mmol, 2 M) was added at room temperature and the mixture was stirred until a TLC revealed total consumption of the ester (ca. 3.5 h). The reaction mixture and two fractions, gained from flushing the empty reaction vessel with water (2 x 2.5 ml), were then dropped into hydrochloric acid (5.0 ml, 0.2 M) in a centrifuge tube. After centrifugation at 4000 rpm and 0 °C for 30 min the supernatant was removed and the remaining residue was carefully washed with water (2 x 10 ml). Afterwards the product was dissolved in absolute ethanol (1.0 ml) and the solution was kept at room temperature for 60 h. After addition of methanol (2.5 ml) the mixture was filtrated through a 0.22 µm PTFE-syringe filter. All solvents were removed under reduced pressure to afford the pure acid.

5.3.3 Procedure C – Staudinger reduction and saponification of peptoid-tetraazide methyl esters



To a solution of an appropriate peptoid-tetraazide methyl ester (1.00 mmol) in a mixture of THF/water (2:1, v/v, 100 ml) triphenylphosphine (1.57 g, 6.00 mmol) was added and the solution was stirred under a nitrogen gas atmosphere at room temperature for 84 h. Afterwards the THF content of the solution was distilled off at 50 °C with a rotavap. Trifluoroacetic acid (10 ml) was added to the remaining slurry and the mixture was stirred for two hours at room temperature. Then all volatiles were removed *in vacuo* and the remainder was dried at a lyophilization device over night. The crude residue was dissolved in methanol (5 ml) and the resulting solution and two fractions, gained from flushing the empty vessel with methanol (2 x 1 ml), were dropped into a centrifuge tube with diethyl ether (85 ml) without shaking or stirring. The resulting suspension was centrifuged at 4000 rpm and 0 °C for 10 min to give an oily precipitate. The supernatant was discarded and the residue was dissolved in methanol/ethanol (9:1, v/v, 5 ml) again. This described precipitation procedure was accomplished six times overall like described above. The finally formed precipitate was an amorphous powder, which was suspended in diethyl ether (90 ml). After centrifugation of the suspension at 4000 rpm and 0 °C for 60 min the supernatant was separated and the residue was dried *in vacuo*. The trifluoroacetic acid salts of the peptoid-tetraamine acids were obtained as off-white, non-hygroscopic powders.

5.3.4 Procedure D – azide-alkyne Huisgen cycloaddition

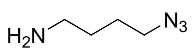


To a solution/suspension of an appropriate peptoid-tetraazide (1.00 mmol) and 2,3-di-(*tert*-butoxycarbonyl)-1-(prop-2-ynyl)guanidine **228** (1.31 g, 4.40 mmol) in *tert*-butyl alcohol (80 ml) solutions of sodium ascorbate (20 ml, 80 mM) and copper(II) acetate (20 ml, 40 mM) in water were successively added at room temperature. The initial turbid solution got clear after 15 min and the mixture was allowed to stir at room temperature for 12 hours. Afterwards all volatiles were removed *in vacuo* and the crude product was purified by silica column chromatography or preparative TLC.

5.4 Detailed chemical syntheses

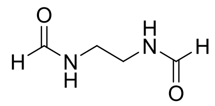
5.4.1 Syntheses of building blocks

4-Azidobutyl-1-amine (**16**)



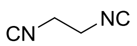
To a solution of 1,4-dibromobutane (**17**, 21.60 g, 100 mmol) in DMF (100 ml) was added a solution of sodium azide (13.65 g, 210 mmol) in water (50 ml). The resulting mixture was stirred in an oil bath at 80 °C for 20 h. After cooling to room temperature brine (200 ml) and *n*-hexane (150 ml) were added. The resulting mixture was then extracted with *n*-hexane (4 x 150 ml) and the combined organic extracts were dried over $NaSO_4$. After filtration the solution was carefully concentrated to approximately 70 ml at a rotavap (T = 30 °C). Ethyl acetate (70 ml) and hydrochloric acid (200 ml, 1 M) were added and the resulting emulsion was cooled to 0 °C. Under vigorous stirring triphenylphosphine (22.54 g, 86 mmol) was added in portions over one hour at the same temperature. After complete addition the mixture was stirred under a nitrogen gas atmosphere at room temperature over night. The reaction mixture was then treated with brine (75 ml) and the emulsion was transferred into a separation funnel, where the multiphasic system separated into three layers. The lowest layer, containing the product dissolved in hydrochlorid acid, was separated from the two upper phases, which were discarded. The isolated aqueous phase was washed with diethyl ether (4 x 100 ml) and its pH value was afterwards adjusted to pH 13 by addition of a solution of NaOH (40%, w/w) in water. The strongly alkaline solution was extracted with dichloromethane (12 x 50 ml) and the combined organic extracts were dried over Na_2SO_4. After filtration the solvent was removed under reduced pressure to afford pure **16** as light yellow oil (9.60 g, 84.0%). R_f 0.41 (DCM/MeOH/TEA 90:10:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.48–1.58 (m, 2H), 1.60–1.70 (m, 2H), 2.73 (t, J = 7.0 Hz, 2H), 3.30 (t, J = 6.8 Hz, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 26.3, 30.8, 41.7, 51.3 ppm.

1,2-Diformamidoethane (**224**) as a mixture of *cis*/*trans*-isomers



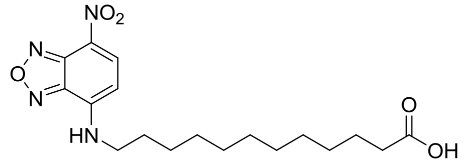
A solution of ethylendiamine **223** (12.02 g, 0.20 mol) in ethyl formate (259 ml, 3.20 mol) was refluxed for six hours. After cooling to room temperature the precipitate was separated by filtration and washed with ethyl acetate (100 ml). The mother liquor formed another crop of material after standing over night at 10 °C. After filtration and washing with ethyl acetate (50 ml) the combined precipitates were dried *in vacuo* to afford **224** (21.07 g, 90.7%) as light yellow powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 3.35 (bs, 4H), 7.97 and 8.07 and 8.09 (3s, 1H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 38.5, 164.2 ppm. MS (ESI+) *m/z* calcd for $C_4H_8N_2O_2$ $[M+Na]^+$ 139.0, found 139.2.

1,2-Diisocyanoethane (**222**)



Triethylamine (103.3 ml, 75.4 g, 745 mmol) and **223** (17.3 g, 149 mmol) were dissolved in dry dichloromethane (250 ml). The solution was cooled to -60 °C under a nitrogen gas atmosphere and phosphoryl chloride (38.0 ml, 63.9 g, 417 mmol) was added in portions (2 ml/min) while stirring at the same temperature. After complete addition the mixture was kept for 30 min at -60 °C before it was allowed to reach room temperature over night. To the reaction mixture was added a solution of KOH (106.0 g, 1.61 mol, 85%) in a mixture of ice/water (1:1, w/w, 1000 ml) at 0 °C. After vigorous stirring for 10 min at this temperature the organic layer was separated and the aqueous phase was extracted with dichloromethane (2 x 150 ml). The combined organic extracts were washed with brine (200 ml) and dried over Na_2SO_4. After filtration the solvents were removed *in vacuo*. The crude residue was purified by silica column chromatography (dichloromethane) to afford **222** (7.28 g, 61.0%) as light yellow oil which solidifies under 0 °C. R_f 0.70 (dichloromethane). ^{1}H-NMR (400 MHz, C_6D_6) δ = 2.42 (brs, 4H) ppm. ^{13}C-NMR (100 MHz, C_6D_6) δ = 40.3 (t, $^{2}J_{C,N}$ = 7.5 Hz), 161.7 (t, $^{1}J_{C,N}$ = 4.5 Hz) ppm.

12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoic acid (**234**)



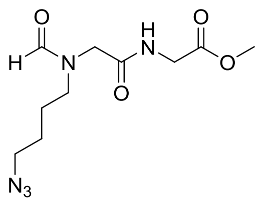
To a solution of 4-chloro-7-nitrobenzo[c][1,2,5]-oxadiazole **232** (2.00 g, 10.0 mmol) in methanol (60 ml) were added 12-aminododecanoic acid **233** (3.23 g, 15.0 mmol) and hydrochloric acid (1.25 ml, 37% w/w). While heating this mixture under reflux a solution of sodium hydrogen carbonate (2.52 g, 30.0 mmol) in water (40 ml) was added dropwise. After complete addition the reaction mixture was heated under reflux for further 30 min and was afterwards kept at room temperature over night. The reaction mixture turned into a suspension to which brine (50 ml) and saturated $NaHSO_4$ solution (20 ml) were added. After extraction with ethyl acetate (4 x 100 ml) the organic phases were combined and dried over Na_2SO_4. After filtration and evaporation of all volatiles *in vacuo* the crude residue was purified by double silica column chromatography [a)dichloromethane/methanol 9:1 and b) ethyl acetate] to afford **234** (2.224 g, 58.8%) as orange-brown, amorphous solid. R_f 0.55 (dichloromethane/methanol 9:1), 0.78 (ethyl acetate). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.22–1.42 (m, 12H, $CH_2(CH_2)_6CH_2$), 1.48 (qnt, ^3J = 7.5 Hz, 2H, CH_2), 1.63 (qnt, ^3J = 7.5 Hz, 2H, CH_2), 1.83 (qnt, ^3J = 7.5 Hz, 2H, CH_2), 2.36 (t, ^3J = 7.5, 2H, CH_2COOH), 3.47–3.56 (m, 2H, NHCH_2), 6.19 (d, ^3J = 8.7 Hz, 1H, NHC=C*H*), 6.43–6.51 (m, 1H, N*H*), 8.49 (d, ^3J = 8.7 Hz, 1H, NO_2C=C*H*), 11.5 (brs, 1H, COO*H*) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.6, 26.9, 28.4, 28.9, 29.1, 29.1, 29.2, 29.3 (8CH_2), 33.9 (CH_2COOH), 44.0 (CH_2NH), 98.5 (*C*H=CNH), 123.6 (C_{quart}), 136.6 (*C*H=CNO_2), 143.9 (C_{quart}), 144.0 (br, C_{quart}), 144.2 (C_{quart}), 180.0 (*C*OOH) ppm. HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{26}N_4O_5$ $[M+Na]^+$ 401.1795, found 401.1792.

5.4.2 Syntheses of the cationic LPP library

5.4.2.1 Syntheses of the first generation azido-LPP acids

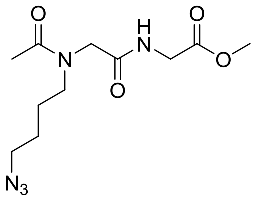
Ugi four-component reactions

N-Formyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**41**) as a mixture of rotamers



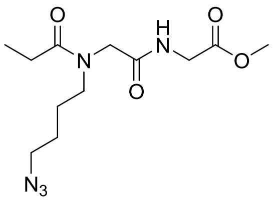
Formic acid (**21**, 189 µl, 230 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) and afforded **41** (1011 mg, 74.6%) as yellow oil. R_f 0.31 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.53–1.76 (m, 4H), 3.28–3.36 and 3.38–3.46 (2m, 4H), 3.75 and 3.76 (2s, 3H), 3.97–4.10 (m, 4H), 6.96–7.05 and 7.50–7.59 (2brm, 1H), 8.11 and 8.15 (2s, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.1, 24.5, 25.6, 25.8, 26.1, 26.3, 40.7, 41.0, 42.9, 47.0, 48.7, 50.4, 50.8, 51.0, 52.3, 52.3, 52.4, 55.2, 58.6, 163.5, 163.7, 168.4, 168.5, 170.0, 170.7, 170.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{10}H_{17}N_5O_4$ $[M+Na]^+$ 294.1173, found 294.1170.

N-Acetyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**42**) as a mixture of rotamers



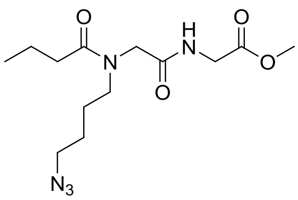
Acetic acid (**22**, 286 µl, 300 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) and afforded **42** (1162 mg, 81.5%) as yellow oil which solidified on standing. R_f 0.27 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.53–1.78 (2m, 4H), 2.11 and 2.18 (2s, 3H), 3.28–3.37 and 3.39–3.47 (2m, 4H), 3.74 and 3.76 (2s, 3H), 3.98–4.05 and 4.06–4.12 (2m, 4H), 6.90 and 7.06 (2brt, *J* = 5.2, 5.2 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 21.2, 21.7, 24.6, 25.8, 26.1, 26.1, 40.9, 46.5, 50.1, 50.5, 50.9, 51.0, 52.0, 52.3, 52.4, 168.6, 169.7, 169.9, 170.0, 171.5, 171.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{11}H_{19}N_5O_4$ $[M+Na]^+$ 308.1329, found 308.1327.

N-Propionyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**43**) as a mixture of rotamers



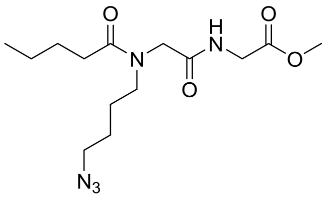
Propionic acid (**23**, 374 µl, 370 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate) to afford **43** (1154 mg, 77.1%) as light yellow oil. R_f 0.27 (ethyl acetate). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.14 and 1.19 (2t, *J* = 7.4, 7.4 Hz, 3H), 1.54–1.76 (m, 4H), 2.32 and 2.43 (2q, *J* = 7.3, 7.4 Hz, 2H), 3.29–3.37 and 3.38–3.49 (2m, 4H), 3.74 and 3.76 (2s, 3H), 3.98–4.05 and 4.06–4.12 (2m, 4H), 6.65–6.75 and 7.00–7.14 (2brs, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 9.1, 9.3, 24.6, 25.9, 26.1, 26.1, 26.5, 40.9, 46.7, 49.2, 50.7, 51.0, 51.1, 52.3, 52.5, 168.8, 169.9, 170.0, 174.5, 174.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{12}H_{21}N_5O_4$ $[M+Na]^+$ 322.1486, found 322.1483.

N-Butyryl-*N*-(4-azidobutyl)glycylglycine methyl ester (**44**) as a mixture of rotamers



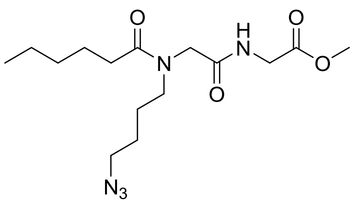
Butyric acid (**24**, 459 µl, 441 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate) to afford **44** (1224 mg, 78.1%) as light yellow oil. R_f 0.37 (ethyl acetate). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.95 and 0.99 (2t, J = 7.4, 7.4 Hz, 3H), 1.54–1.77 (m, 6H), 2.27 and 2.37 (2t, J = 7.4, 7.5 Hz, 2H), 3.28–3.37 and 3.39–3.48 (2brm, 4H), 3.74 and 3.76 (2s, 3H), 3.97–4.11 (m, 4H), 6.70–6.79 and 7.03–7.15 (2m, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 13.8, 18.4, 18.6, 24.6, 25.9, 26.1, 26.1, 34.7, 35.1, 40.9, 46.5, 49.2, 50.7, 50.9, 51.2, 52.2, 52.3, 168.8, 169.9, 170.0, 173.8, 174.1 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{13}H_{23}N_5O_4$ $[M+Na]^+$ 336.1642, found 336.1641.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**45**) as a mixture of rotamers



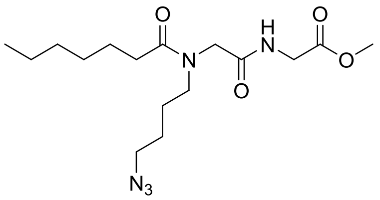
Valeric acid (**25**, 549 µl, 511 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **45** (1287 mg, 78.6%) as light yellow oil which solidified on standing. R_f 0.30 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.91 and 0.94 (2t, J = 7.3, 7.3 Hz, 3H), 1.24–1.44 (m, 2H), 1.54–1.76 (m, 6H), 2.25–2.35 and 2.36–2.43 (2m, 2H), 3.28–3.37 and 3.38–3.48 (2m, 4H), 3.74 and 3.76 (2s, 3H), 3.98–4.04 and 4.06 – 4.10 (2m, 4H), 6.66 and 7.06 (2brt, J = 5.0, 5.3 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 24.4, 22.5, 24.6, 25.9, 26.1, 26.1, 27.1, 27.3, 32.6, 33.0, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.3, 52.5, 168.8, 169.8, 169.9, 170.0, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{14}H_{25}N_5O_4$ $[M+Na]^+$ 350.1799, found 350.1796.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**46**) as a mixture of rotamers



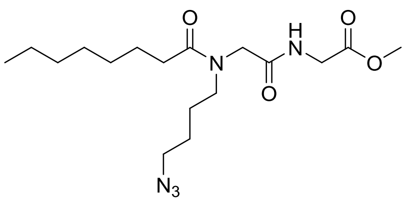
Caproic acid (**26**, 625 µl, 581 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **46** (1312 mg; 76.9%) as light yellow oil, which solidified on standing. R_f 0.33 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.86–0.95 (m, 3H), 1.24–1.41 (m, 4H), 1.54–1.76 (m, 6H), 2.24–2.34 and 2.35–2.43 (2m, 2H), 3.28–3.37 and 3.38–3.48 (2m, 4H), 3.74 and 3.76 (2s, 3H), 3.98–4.04 and 4.06–4.10 (2m, 4H), 6.71 and 7.07 (2brt, J = 5.1, 5.1 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, CDCl$_3$) δ = 13.9, 22.4, 24.5, 24.6, 24.7, 24.9, 25.9, 26.1, 26.1, 31.2, 31.4, 31.5, 32.8, 33.2, 33.7, 40.9, 46.6, 49.3, 50.8, 50.9, 51.2, 52.2, 52.4, 168.8, 169.8, 169.9, 170.0, 174.0, 174.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{15}H_{27}N_5O_4$ $[M+Na]^+$ 364.1955, found 364.1952.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**47**) as a mixture of rotamers



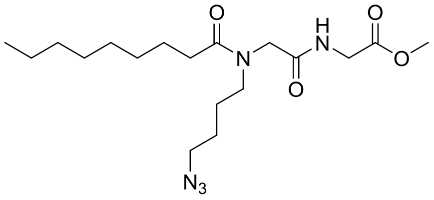
Enanthic acid (**27**, 709 µl, 651 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **47** (1386 mg, 78.0%) as light yellow oil, which solidified on standing. R_f 0.36 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, CDCl$_3$) δ = 0.84–0.94 (m, 3H), 1.23–1.40 (m, 6H), 1.54–1.77 (m, 6H), 2.24–2.34 and 2.35–2.42 (2m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.74 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.75 and 7.08 (2brt, *J* = 5.2, 5.4 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, CDCl$_3$) δ = 14.0, 22.5, 24.6, 24.7, 24.9, 25.1, 25.9, 26.1, 26.1, 28.7, 28.9, 29.0, 31.4, 31.5, 32.9, 33.2, 33.8, 40.9, 46.6, 49.3, 50.7, 50.9, 51.2, 52.2, 52.4, 168.8, 169.8, 169.9, 170.0, 174.1, 174.4, 176.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{16}H_{29}N_5O_4$ $[M+Na]^+$ 378.2112, found 378.2110.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**48**) as a mixture of rotamers



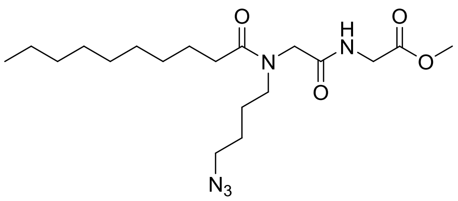
Caprylic acid (**28**, 792 µl, 721 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **48** (1465 mg, 79.3%) as light yellow oil, which solidified on standing. R_f 0.38 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, CDCl$_3$) δ = 0.84–0.92 (m, 3H), 1.22–1.39 (m, 8H), 1.53–1.76 (m, 6H), 2.23–2.34 and 2.35–2.43 (2m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.74 and 3.75 and 3.76 (3s, 3H), 3.98–4.04 and 4.06–4.10 (2m, 4H), 6.66 and 7.06 (2brt, *J* = 5.2, 4.9 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, CDCl$_3$) δ = 14.0, 22.6, 24.6, 24.8, 25.0, 25.2, 25.9, 26.1, 26.1, 28.9, 29.0, 29.2, 29.3, 31.6, 32.9, 33.2, 33.8, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.2, 52.4, 168.7, 169.8, 169.9, 169.9, 174.0, 174.3, 176.2 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{17}H_{31}N_5O_4$ $[M+Na]^+$ 392.2268, found 392.2265.

<u>*N*-Nonanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**49**) as a mixture of rotamers</u>



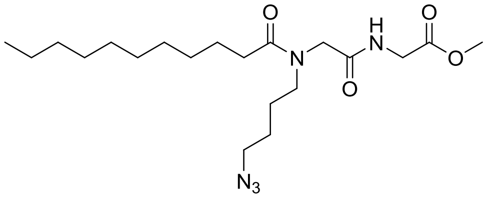
Pelargonic acid (**29**, 879 µl, 791 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **49** (1520 mg, 79.3%) as light yellow oil. R_f 0.43 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.19–1.40 (m, 10H), 1.54–1.76 (m, 6H), 2.24–2.34 and 2.35–2.42 (2m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.74 and 3.76 (2m, 3H), 3.97–4.05 and 4.06–4.10 (2m, 4H), 6.74 and 7.08 (2brt, *J* = 5.3, 5.0 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.6, 24.8, 25.0, 25.2, 25.9, 26.1, 26.1, 29.1, 29.2, 29.3, 29.3, 31.8, 32.9, 33.2, 33.8, 40.9, 46.6, 49.3, 50.8, 50.9, 51.2, 52.2, 52.4, 168.8, 169.8, 169.9, 170.0, 174.1, 174.4, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{33}N_5O_4$ $[M+Na]^+$ 406.2425, found 406.2423.

<u>*N*-Decanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**50**) as a mixture of rotamers</u>



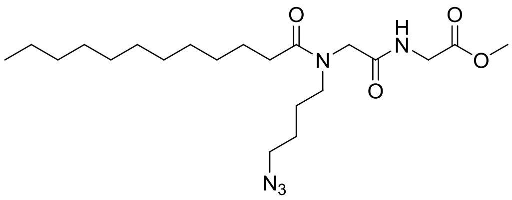
Capric acid (**30**, 861 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **50** (1524 mg, 76.7%) as light yellow oil, which solidified on standing. R_f 0.46 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.19–1.39 (m, 12H), 1.54–1.76 (m, 6H), 2.23–2.34 and 2.35–2.43 (2m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.73 and 3.75 and 3.76 (3s, 3H), 3.97–4.05 and 4.06–4.10 (2brm, 4H), 6.57–6.67 and 7.00–7.14 (2m, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 24.8, 25.0, 25.2, 25.9, 26.1, 26.1, 29.1, 29.2, 29.4, 29.4, 31.8, 32.9, 33.3, 33.8, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.2, 52.5, 168.7, 169.8, 169.9, 169.9, 174.0, 174.4, 176.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{35}N_5O_4$ $[M+Na]^+$ 420.2581, found 420.2577.

N-Undecanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**51**) as a mixture of rotamers



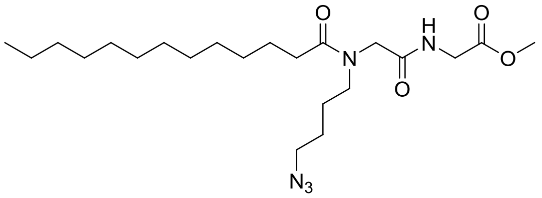
Undecylic acid (**31**, 931 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **51** (1542 mg, 74.9%) as colorless oil, which solidified on standing. R_f 0.19 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.20–1.40 (m, 14H), 1.54–1.76 (m, 6H), 2.19–2.44 (m, 2H), 3.28–3.37 and 3.38–3.48 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.04 and 4.06–4.11 (2m, 4H), 6.58 and 7.05 (2brt, J = 5.3, 4.9 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 24.8, 25.0, 25.2, 25.9, 26.1, 26.1, 29.3, 29.4, 29.4, 29.5, 29.5, 31.8, 32.9, 33.3, 40.9, 46.6, 49.3, 50.9, 51.0, 51.3, 52.2, 52.5, 168.7, 169.8, 169.9, 170.0, 173.9, 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{37}N_5O_4$ $[M+Na]^+$ 434.2738, found 434.2734.

N-Lauroyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**52**) as a mixture of rotamers



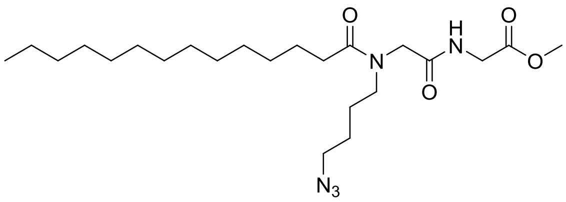
Lauric acid (**32**, 1002 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **52** (1691 mg, 79.5%) as colorless oil, which solidified on standing. R_f 0.21 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.20–1.39 (m, 16H), 1.54–1.78 (m, 6H), 2.21–2.43 (m, 2H), 2.28–3.37 and 3.38–3.50 (2m, 4H), 3.74 and 3.77 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.50 and 7.03 (2brt, J = 5.2, 4.8 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.7, 24.7, 25.0, 25.2, 26.0, 26.1, 26.2, 29.3, 29.4, 29.4, 29.5, 29.6, 30.9, 31.9, 32.9, 33.3, 40.9, 46.6, 49.3, 50.9, 51.0, 51.3, 52.3, 52.5, 168.7, 169.7, 169.9, 170.0, 173.9, 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{39}N_5O_4$ $[M+Na]^+$ 448.2894, found 448.2893.

N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**53**) as a mixture of rotamers



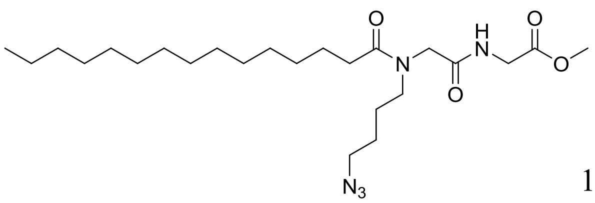
Tridecylic acid (**33**, 1072 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **53** (1690 mg, 76.9%) as light yellow, amorphous solid. R_f 0.21 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.18–1.40 (m, 18H), 1.53–1.76 (m, 6H), 2.19–1.44 (m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.63 and 7.06 (2brt, J = 5.3, 5.0 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 25.0, 25.2, 25.9, 26.1, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.9, 32.9, 33.3, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.2, 52.4, 168.7, 169.8, 169.9, 169.9, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{22}H_{41}N_5O_4$ $[M+Na]^+$ 462.3051, found 462.3046.

N-Myristoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**54**) as a mixture of rotamers



Myristic acid (**34**, 1142 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **54** (1755 mg, 77.4%) as white, amorphous solid. R_f 0.21 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.83–0.93 (m, 3H), 1.20–1.39 (m, 20H), 1.54–1.76 (m, 6H), 2.19–2.46 (m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.66 and 7.06 (2brt, J = 5.3, 5.1 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 24.8, 25.0, 25.2, 25.9, 26.1, 26.1, 29.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.6, 31.9, 32.9, 33.3, 33.8, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.2, 52.4, 168.7, 169.8, 169.9, 169.9, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{23}H_{43}N_5O_4$ $[M+Na]^+$ 476.3207, found 476.3203.

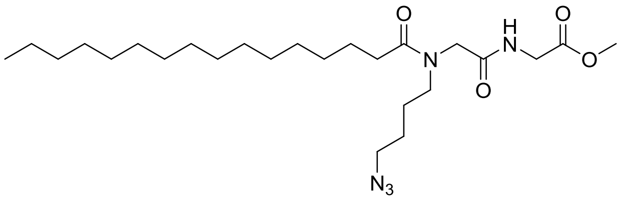
N-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**55**) as a mixture of rotamers



Pentadecylic acid (**35**, 1212 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00

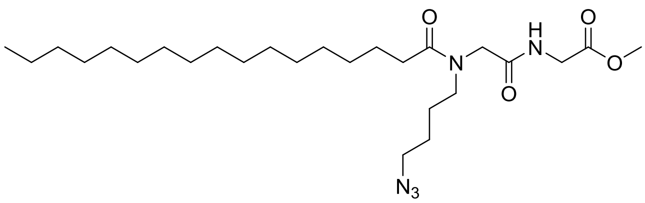
mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **55** (1774 mg, 75.9%) as light yellow, amorphous solid. R_f 0.23 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.16–1.42 (m, 22H), 1.53–1.76 (m, 6H), 2.19–2.43 (m, 2H), 3.28–3.37 and 3.38–3.48 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.66 and 7.06 (2brt, J = 5.4, 5.2 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 24.8, 25.0, 25.2, 25.2, 25.9, 26.1, 26.1, 29.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.6, 31.9, 32.9, 33.3, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.2, 52.4, 168.7, 169.8, 169.9, 169.9, 173.7, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{45}N_5O_4$ $[M+Na]^+$ 490.3364, found 490.3360.

<u>*N*-Palmitoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**56**) as a mixture of rotamers</u>



Palmitic acid (**36**, 1282 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **56** (1845 mg, 76.6%) as white, amorphous solid. R_f 0.24 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.18–1.42 (m, 24H), 1.53–1.76 (m, 6H), 2.19–2.43 (m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.61 and 7.05 (2brt, J = 5.4, 5.2 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 25.0, 25.2, 25.9, 26.1, 26.2, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 31.9, 32.9, 33.3, 40.9, 46.6, 49.3, 50.8, 51.0, 51.3, 52.2, 52.5, 168.7, 169.8, 169.9, 169.9, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{25}H_{47}N_5O_4$ $[M+Na]^+$ 504.3520, found 504.3518.

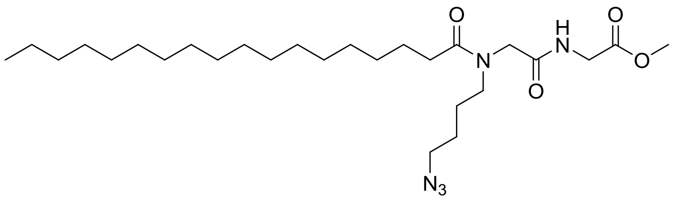
<u>*N*-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**57**) as a mixture of rotamers</u>



Margaric acid (**37**, 1352 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-

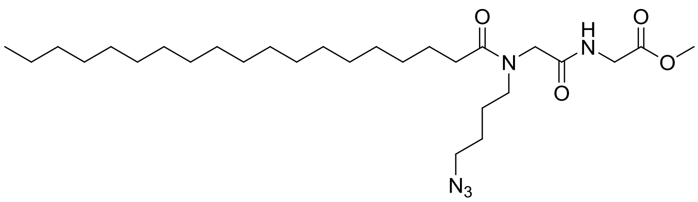
hexane/ethyl acetate 2:3) to afford **57** (1901 mg, 76.7%) as white, amorphous solid. R_f 0.24 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.18–1.40 (m, 26H), 1.53–1.76 (m, 6H), 2.19–2.43 (m, 2H), 3.28–3.37 and 3.38–3.48 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.04 and 4.06–4.11 (2m, 4H), 6.54 and 7.04 (2brt, *J* = 5.3, 5.1 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.7, 24.7, 25.0, 25.2, 26.0, 26.1, 26.2, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 32.9, 33.3, 40.9, 46.6, 49.3, 50.9, 51.0, 51.3, 52.2, 52.5, 168.7, 169.8, 169.9, 169.9, 173.9, 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{26}H_{49}N_5O_4$ $[M+Na]^+$ 518.3677, found 518.3673.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**58**) as a mixture of rotamers



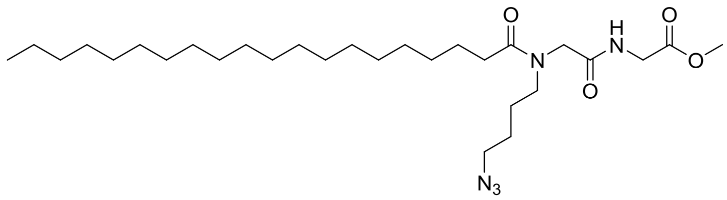
Stearic acid (**38**, 1422 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **58** (1851 mg, 72.6%) as white, amorphous solid. R_f 0.25 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.18–1.42 (m, 28H), 1.53–1.76 (m, 6H), 2.19–2.43 (m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.59 and 7.05 (2brt, *J* = 5.3, 5.1 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.7, 24.7, 25.0, 25.2, 26.0, 26.1, 26.2, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 32.9, 33.3, 40.9, 46.6, 49.3, 50.9, 51.0, 51.3, 52.2, 52.5, 168.7, 169.8, 169.9, 169.9, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{51}N_5O_4$ $[M+Na]^+$ 532.3833, found 532.3827.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**59**) as a mixture of rotamers



Nonadecylic acid (**39**, 1360 mg, 4.56 mmol), **19** (228 mg, 7.60 mmol), **16** (521 mg, 4.56 mmol) and **20** (414 µl, 452 mg, 4.56 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **59** (1714 mg, 71.8%) as white, amorphous solid. R_f 0.26 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.19–1.42 (m, 30H), 1.53–1.77 (m, 6H), 2.19–2.44 (m, 2H), 3.28–3.37 and 3.38–3.50 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.11 (2m, 4H), 6.72 and 7.07 (2brt, J = 5.4, 5.2 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 25.0, 25.2, 25.9, 26.1, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 31.9, 32.9, 33.2, 40.9, 46.5, 49.3, 50.8, 50.9, 51.2, 52.2, 52.4, 168.7, 169.8, 169.9, 169.9, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{53}N_5O_4$ $[M+Na]^+$ 546.3990, found 546.3984.

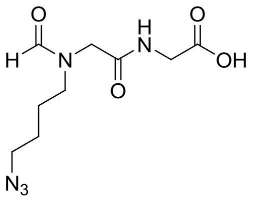
<u>*N*-Arachidoyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**60**) as a mixture of rotamers</u>



Arachidic acid (**40**, 1563 mg, 5.00 mmol), **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 2:3) to afford **60** (1814 mg, 67.5%) as white, amorphous solid. R_f 0.31 (*n*-hexane/ethyl acetate 2:3). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.16–1.40 (m, 32H), 1.53–1.76 (m, 6H), 2.19–2.43 (m, 2H), 3.28–3.37 and 3.38–3.49 (2m, 4H), 3.73 and 3.76 (2s, 3H), 3.97–4.05 and 4.06–4.10 (2m, 4H), 6.70 and 7.07 (2brt, J = 5.4, 5.3 Hz, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.6, 24.8, 25.0, 25.2, 25.9, 26.1, 26.1, 29.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 31.9, 32.9, 33.2, 40.9, 46.5, 49.3, 50.8, 50.9, 51.2, 52.2, 52.4, 168.7, 169.8, 169.9, 169.9, 173.9, 174.3 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{29}H_{55}N_5O_4$ $[M+Na]^+$ 560.4146, found 560.4141.

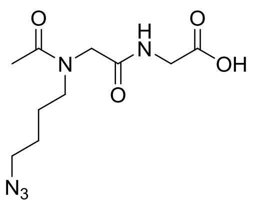
Saponifications

<u>*N*-Formyl-*N*-(4-azidobutyl)glycylglycine (**61**) as a mixture of rotamers</u>



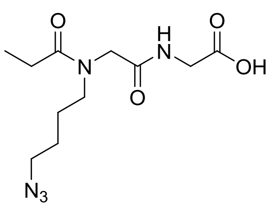
The saponification of **41** (971 mg, 3.58 mmol) with lithium hydroxide solution (4.48 ml, 8.95 mmol, 2 M) following general procedure B (method 1) afforded **61** (490 mg, 53.2%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.51–1.72 (m, 4H), 3.28–3.46 (m, 4H), 3.93 and 3.95 (2s, 2H), 4.07 and 4.08 (2s, 2H), 8.08 and 8.17 (2s, 1H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.2, 26.5, 26.9, 27.2, 41.7, 41.8, 44.0, 46.0, 49.3, 51.0, 52.0, 52.0, 165.8, 166.4, 170.7, 171.4, 172.7, 172.7 ppm. HRMS (ESI+) *m*/*z* calcd for $C_9H_{15}N_5O_4$ $[M+Na]^+$ 280.1016, found 280.1014.

<u>*N*-Acetyl-*N*-(4-azidobutyl)glycylglycine (**62**) as a mixture of rotamers</u>



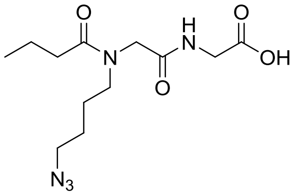
The saponification of **42** (1115 mg, 3.91 mmol) with lithium hydroxide solution (4.89 ml, 9.78 mmol, 2 M) following general procedure B (method 1) afforded **62** (1037 mg, 97.9%) as light yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.51–1.75 (m, 4H), 2.08 and 2.17 (2s, 3H), 3.28–3.48 (m, 4H), 3.92 and 3.95 (2s, 2H), 4.08 and 4.13 (2s, 2H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 21.1, 21.7, 25.6, 26.6, 27.1, 27.2, 41.7, 41.8. 47.8, 49.6, 50.8, 52.1, 52.1, 52.3, 171.4, 171.7, 172.7, 172.8, 173.9, 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{10}H_{17}N_5O_4$ $[M+Na]^+$ 294.1173, found 294.1170.

<u>*N*-Propionyl-*N*-(4-azidobutyl)glycylglycine (**63**) as a mixture of rotamers</u>



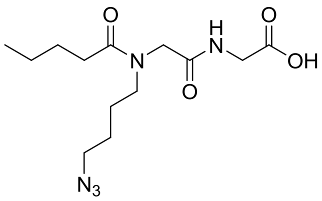
The saponification of **43** (1103 mg, 3.68 mmol) with lithium hydroxide solution (4.61 ml, 9.20 mmol, 2 M) following general procedure B (method 1) afforded **63** (979 mg, 93.1%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.09 and 1.12 (2t, J = 7.4, 7.4 Hz, 3H), 1.51–1.74 (m, 4H), 2.36 and 2.48 (2q, J = 7.4 Hz, 2H), 3.28–3.38 and 3.39–3.47 (2m, 4H), 3.90–3.97 (m, 2H), 4.07 and 4.12 (2s, 2H), 8.18 and 8.38 (2brt, J = 5.4, 5.5 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 9.7, 9.7, 25.7, 26.7, 26.9, 27.1, 27.2, 27.3, 41.7, 41.8, 48.0, 49.9, 51.5, 52.1, 52.2, 171.6, 171.9, 172.7, 172.9, 176.8, 177.2 ppm. HRMS (ESI+) *m/z* calcd for $C_{11}H_{19}N_5O_4$ $[M+Na]^+$ 308.1329, found 308.1328.

N-Butyryl-*N*-(4-azidobutyl)glycylglycine (**64**) as a mixture of rotamers



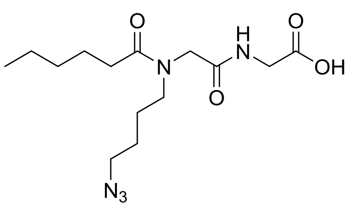
The saponification of **44** (1177 mg, 3.76 mmol) with lithium hydroxide solution (4.70 ml, 9.40 mmol, 2 M) following general procedure B (method 1) afforded **64** (1122 mg, 99.7%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.94 and 0.98 (2t, *J* = 7.4, 7.4 Hz, 3H), 1.51–1.74 (m, 6H), 2.32 and 2.43 (2t, *J* = 7.5, 7.5 Hz, 2H), 3.27–3.38 and 3.39–3.48 (2m, 4H), 3.90–3.98 (m, 2H), 4.08 and 4.13 (2s, 2H), 8.15 and 8.37 (2t, *J* = 5.5, 5.7 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.1, 14.2, 19.6, 19.7, 25.6, 26.8, 27.1, 27.2, 35.5, 36.0, 41.7, 41.8, 47.9, 49.8, 50.0, 51.6, 52.1, 52.1, 171.5, 171.8, 172.7, 172.8, 176.0, 176.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{12}H_{21}N_5O_4$ $[M+Na]^+$ 322.1486, found 322.1483.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycine (**65**) as a mixture of rotamers



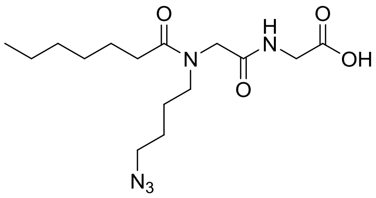
The saponification of **45** (1216 mg, 3.71 mmol) with lithium hydroxide solution (4.64 ml, 9.28 mmol, 2 M) following general procedure B (method 1) afforded **65** (1159 mg, 99.7%) as light brown oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.92 and 0.94 (2t, *J* = 7.4, 7.3 Hz, 3H), 1.28–1.45 (m, 2H), 1.51–1.74 (m, 6H), 2.34 and 2.46 (2t, *J* = 7.6, 7.6 Hz, 2H), 3.28–3.38 and 3.39–3.49 (2m, 4H), 3.91–3.97 (m, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, *J* = 5.5, 5.7 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.2, 14.3, 23.4, 23.5, 25.6, 26.8, 27.1, 27.2, 28.4, 28.5, 33.4, 33.8, 41.7, 41.8, 47.9, 49.8, 50.0, 51.6, 52.1, 52.1, 171.5, 171.8, 172.7, 172.8, 176.2, 176.5 ppm. HRMS (ESI+) *m/z* calcd for $C_{13}H_{23}N_5O_4$ $[M+Na]^+$ 336.1642, found 336.1640.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycine (**66**) as a mixture of rotamers



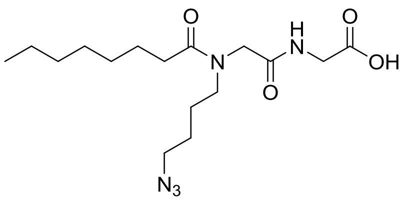
The saponification of **46** (1272 mg, 3.72 mmol) with lithium hydroxide solution (4.66 ml, 9.30 mmol, 2 M) following general procedure B (method 1) afforded **66** (1210 mg, 99.4%) as light yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.97 (m, 3H), 1.26–1.41 (m, 4H), 1.51–1.74 (m, 6H), 2.33 and 2.45 (2t, *J* = 7.6, 7.6 Hz, 2H), 3.28–3.38 and 3.39–3.48 (2m, 4H), 3.92 and 3.95 (2s, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, *J* = 5.8, 5.9 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 23.5, 23.6, 25.6, 26.0, 26.1, 26.8, 27.1, 27.2, 32.6, 32.6, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.1, 171.5, 171.8, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{14}H_{25}N_5O_4$ $[M+Na]^+$ 350.1799, found 350.1797.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycine (**67**) as a mixture of rotamers



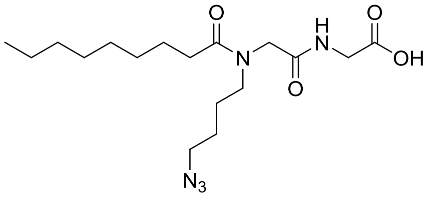
The saponification of **47** (1327 mg, 3.73 mmol) with lithium hydroxide solution (4.67 ml, 9.33 mmol, 2 M) following general procedure B (method 1) afforded **67** (1270 mg, 99.7%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.85–0.96 (m, 3H), 1.25–1.43 (m, 6H), 1.51–1.74 (m, 6H), 2.24–2.37 and 2.45 (m, t, *J* = 7.6 Hz, 2H), 3.27–3.49 (m, 4H), 3.92 and 3.94 (2s, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, *J* = 5.2, 5.5 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 14.4, 23.6, 25.6, 26.0, 26.3, 26.3, 26.8, 27.1, 27.2, 29.9, 30.0, 30.1, 32.7, 32.8, 32.8, 33.6, 34.1, 34.9, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{15}H_{27}N_5O_4$ $[M+Na]^+$ 364.1955, found 364.1952.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycine (**68**) as a mixture of rotamers



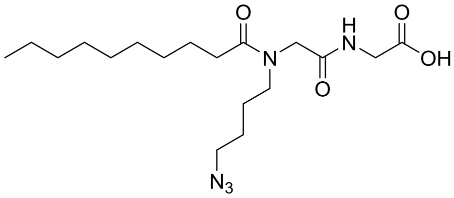
The saponification of **48** (1421 mg, 3.85 mmol) with lithium hydroxide solution (4.81 ml, 9.63 mmol, 2 M) following general procedure B (method 1) afforded **68** (1362 mg, 99.5%), as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.21–1.41 (m, 8H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.45 (m, t, *J* = 7.6 Hz, 2H), 3.27–3.48 (m, 4H), 3.92 and 3.94 (2s, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, *J* = 5.5, 5.7 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 23.7, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.3, 30.3, 30.3, 30.4, 32.9, 32.9, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{16}H_{29}N_5O_4$ $[M+Na]^+$ 378.2112, found 378.2108.

N-Nonanoyl-*N*-(4-azidobutyl)glycylglycine (**69**) as a mixture of rotamers



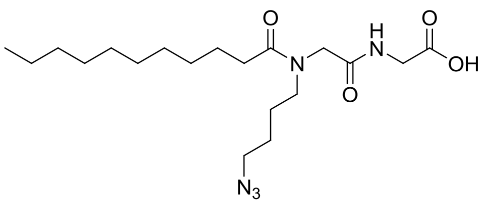
The saponification of **49** (1474 mg, 3.84 mmol) with lithium hydroxide solution (4.80 ml, 9.60 mmol, 2 M) following general procedure B (method 1) afforded **69** (1415 mg, 99.7%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.85–0.95 (m, 3H), 1.22–1.41 (m, 10H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.45 (m, t, J = 7.6 Hz, 2H), 3.28–3.48 (m, 4H), 3.92 and 3.95 (2s, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, J = 5.6, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.1, 26.3, 26.4, 26.8, 27.1, 27.2, 30.2, 30.3, 30.3, 30.3, 30.4, 30.4, 30.5, 33.0, 33.0, 33.0, 33.6, 34.1, 34.9, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.1, 171.5, 171.8, 172.7, 172.8, 176.2, 176,6 ppm. HRMS (ESI+) m/z calcd for $C_{17}H_{31}N_5O_4$ $[M+Na]^+$ 392.2268, found 392.2267.

N-Decanoyl-*N*-(4-azidobutyl)glycylglycine (**70**) as a mixture of rotamers



The saponification of **50** (1479 mg, 3.72 mmol) with lithium hydroxide solution (4.65 ml, 9.30 mmol, 2 M) following general procedure B (method 1) afforded **70** (1421 mg, 99.6%) as light brown oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.21–1.41 (m, 12H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.45 (m, t, J = 7.6 Hz, 2H), 3.28–3.48 (m, 4H), 3,92 and 3.94 (2s, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, J = 5.6, 5.7 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.4, 30.6, 30.6, 30.6, 33.0, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI+) m/z calcd for $C_{18}H_{33}N_5O_4$ $[M+Na]^+$ 406.2425, found 406.2425.

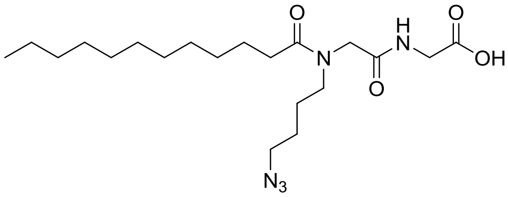
N-Undecanoyl-*N*-(4-azidobutyl)glycylglycine (**71**) as a mixture of rotamers



The saponification of **51** (1488 mg, 3.62 mmol) with lithium hydroxide solution (4.52 ml, 9.05 mmol, 2 M) following general procedure B (method 1) afforded **71** (1436 mg, 99.8%), as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.22–1.41 (m, 14H), 1.51–1.74 (m, 6H), 2.24–2.35 and 2.45 (m, t, J = 7.6 Hz, 2H), 3.28–3.48 (m, 4H), 3.90–3.98 (m, 2H), 4.07 and 4.13 (2s, 2H), 8.16

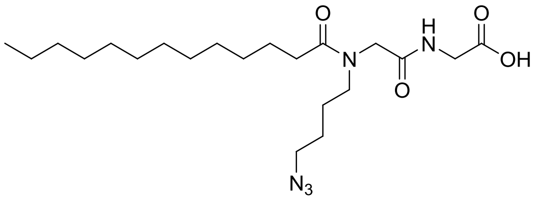
and 8.37 (2brt, J = 5.5, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.4, 30.5, 30.6, 30.7, 30.7, 30.7, 33.1, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI+) m/z calcd for $C_{19}H_{35}N_5O_4$ $[M+Na]^+$ 420.2581, found 420.2579.

N-Lauroyl-*N*-(4-azidobutyl)glycylglycine (**72**) as a mixture of rotamers



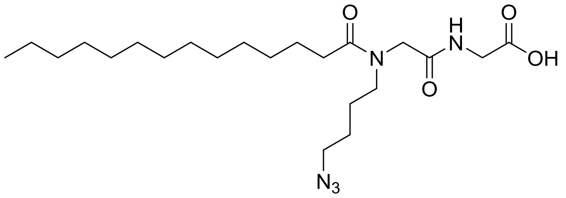
The saponification of **52** (1659 mg, 3.90 mmol) with lithium hydroxide solution (4.87 ml, 9.75 mmol, 2 M) following general procedure B (method 1) afforded **72** (1600 mg, 99.7%) as brown oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.22–1.41 (m, 16H), 1.51–1.74 (m, 6H), 2.24–2.37 and 2.45 (m, t, J = 7.6 Hz, 2H), 3.28–3.49 (m, 4H), 3.90–3.98 (m, 2H), 4.07 and 4.13 (2s, 2H), 8.16 and 8.38 (2brt, J = 5.6, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.4, 30.5, 30.6, 30.7, 30.7, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI+) m/z calcd for $C_{20}H_{37}N_5O_4$ $[M+Na]^+$ 434.2738, found 434.2735.

N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycine (**73**) as a mixture of rotamers



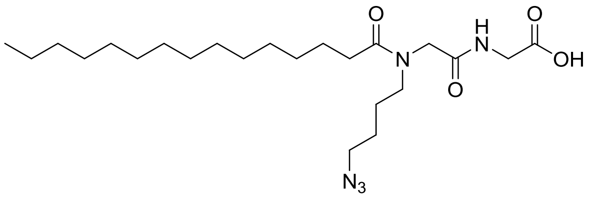
The saponification of **53** (1644 mg, 3.74 mmol) with lithium hydroxide solution (4.68 ml, 9.35 mmol, 2 M) following general procedure B (method 1) afforded **73** (1589 mg, 99.8%) as light brown, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.21–1.41 (m, 18H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.45 (m, t, J = 7.4 Hz, 2H), 3.28–3.48 (m, 4H), 3.90–3.97 (m, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, J = 5.6, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.4, 30.5, 30.6, 30.7, 30.7, 30.8, 30.8, 30.8, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.5 ppm. HRMS (ESI+) m/z calcd for $C_{21}H_{39}N_5O_4$ $[M+Na]^+$ 448.2894, found 448.2891.

N-Myristoyl-*N*-(4-azidobutyl)glycylglycine (**74**) as a mixture of rotamers



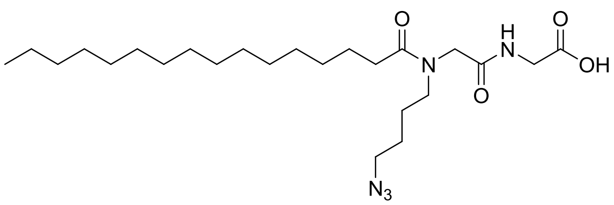
The saponification of **54** (1702 mg, 3.75 mmol) with lithium hydroxide solution (4.69 ml, 9.38 mmol, 2 M) following general procedure B (method 1) afforded **74** (1645 mg, 99.8%) as white, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.22–1.41 (m, 20H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.45 (m, t, J = 7.6 Hz, 2H), 3.28–3.48 (m, 4H), 3.90–3.97 (m, 2H), 4.07 and 4.13 (2s, 2H), 8.16 and 8.38 (2brt, J = 5.6, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.1, 26.3, 26.4, 26.8, 27.1, 27.2, 30.2, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 30.8, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.9, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{22}H_{41}N_5O_4$ $[M+Na]^+$ 462.3051, found 462.3045.

N-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycine (**75**) as a mixture of rotamers



The saponification of **55** (1720 mg, 3.68 mmol) with lithium hydroxide solution (4.60 ml, 9.20 mmol, 2 M) following general procedure B (method 1) afforded **75** (1665 mg, 99.7%) as off-white, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.22–1.41 (m, 22H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.44 (m, t, J = 7.6, 2H), 3.28–3.48 (m, 4H), 3.92 and 3.94 (2s, 2H), 4.07 and 4.12 (2s, 2H), 8.15 and 8.37 (2brt, J = 5.5, 5.6 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 30.8, 30.8, 30.8, 30.8, 41.7, 41.8, 47.9, 49.8, 50.0, 51.6, 52.1, 52.1, 171.5, 171.8, 172.7, 172.8, 176.1, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{23}H_{43}N_5O_4$ $[M+Na]^+$ 476.3205, found 476.3203.

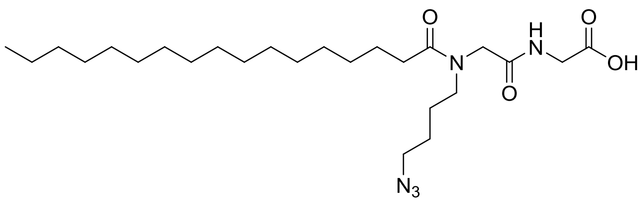
N-Palmitoyl-*N*-(4-azidobutyl)glycylglycine (**76**) as a mixture of rotamers



The saponification of **56** (1800 mg, 3.74 mmol) with lithium hydroxide solution (4.67 ml, 9.35 mmol, 2 M) following general procedure B (method 1) afforded **76** (1740 mg, 99.5%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–

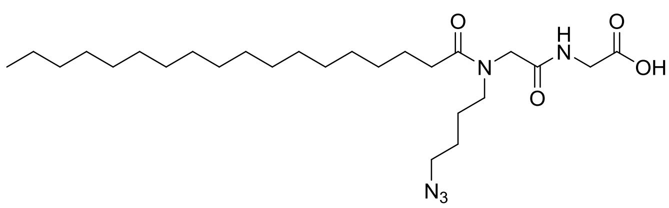
0.94 (m, 3H), 1.20–1.42 (m, 24H), 1.51–1.74 (m, 6H), 2.24–2.35 and 2.45 (m, t, J = 7.6 Hz, 2H), 3.27–3.49 (m, 4H), 3.90–3.98 (m, 2H), 4.07 and 4.13 (2s, 2H), 8.15 and 8.37 (2brt, J = 5.7, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.4, 30.5, 30.6, 30.7, 30.7, 30.8, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.5 ppm. HRMS (ESI+) m/z calcd for $C_{24}H_{45}N_5O_4$ $[M+Na]^+$ 490.3364, found 490.3356.

N-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycine (**77**) as a mixture of rotamers



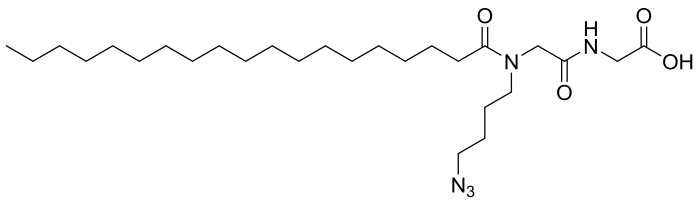
The saponification of **57** (1871 mg, 3.78 mmol) with lithium hydroxide solution (4.72 ml, 9.45 mmol, 2 M) following general procedure B (method 1) afforded **77** (1810 mg, 99.4%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.20–1.42 (m, 26H), 1.51–1.74 (m, 6H), 2.24–2.35 and 2.44 (m, t, J = 7.6 Hz, 2H), 3.27–3.48 (m, 4H), 3.90–3.98 (m, 2H), 4.07 and 4.12 (2s, 2H), 8.15 and 8.36 (2brt, J = 5.6, 5.8 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.5, 30.5, 30.6, 30.6, 30.7, 30.7, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 47.9, 49.8, 50.0, 51.6, 52.1, 52.1, 171.5, 171.8, 172.6, 172.8, 176.1, 176.5 ppm. HRMS (ESI+) m/z calcd for $C_{25}H_{47}N_5O_4$ $[M+Na]^+$ 504.3520, found 504.3511.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycine (**78**) as a mixture of rotamers



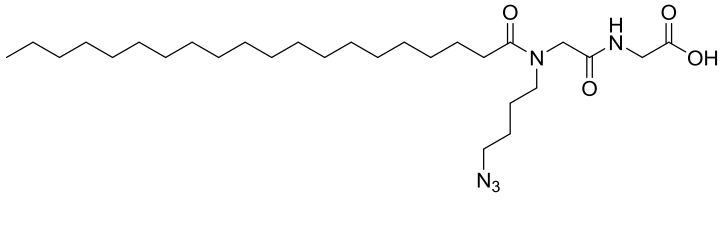
The saponification of **58** (1811 mg, 3.55 mmol) with lithium hydroxide solution (4.44 ml, 8.88 mmol, 2 M) following general procedure B (method 1) afforded **78** (1750 mg, 99.4%) as white, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.20–1.42 (m, 28H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.45 (m, t, J = 7.4 Hz, 2H), 3.28–3.48 (m, 4H), 3.92 and 3.94 (2s, 2H), 4.07 and 4.12 (2s, 2H), 8.16 and 8.37 (2brt, J = 5.4, 5.9 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 26.3, 26.4, 26.9, 27.1, 27.2, 30.4, 30.5, 30.5, 30.6, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{26}H_{49}N_5O_4$ $[M+Na]^+$ 518.3677, found 518.3671.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycine (**79**) as a mixture of rotamers



The saponification of **59** (1641 mg, 3.13 mmol) with lithium hydroxide solution (3.92 ml, 7.83 mmol, 2 M) following general procedure B (method 1) afforded **79** (1595 mg, quant.) as white, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.20–1.42 (m, 30H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.44 (m, t, *J* = 7.6 Hz, 2H), 3.28–3.48 (m, 4H), 3.92 and 3.94 (2s, 2H), 4.07 and 4.12 (2s, 2H), 8.15 and 8.37 (2brt, *J* = 5.6, 5.7 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.5, 30.5, 30.6, 30.7, 30.8, 33.1, 33.7, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.2, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{51}N_5O_4$ $[M+Na]^+$ 532.3833, found 532.3828.

N-Arachidoyl-*N*-(4-azidobutyl)glycylglycine (**80**) as a mixture of rotamers

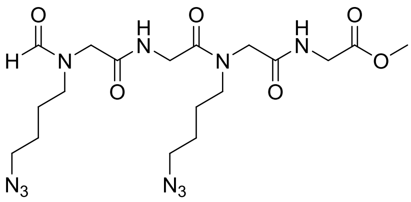


The saponification of **60** (1743 mg, 3.24 mmol) with lithium hydroxide solution (4.05 ml, 8.10 mmol, 2 M) following general procedure B (method 1) afforded **80** (1690 mg, 99.6%), as white, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.20–1.42 (m, 32H), 1.51–1.74 (m, 6H), 2.24–2.36 and 2.44 (m, t, *J* = 7.6 Hz, 2H), 3.27–3.49 (m, 4H), 3.90–3.99 (m, 2H), 4.07 and 4.12 (2s, 2H), 8.15 and 8.37 (2brt, *J* = 5.7, 5.7 Hz, 1H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 26.3, 26.4, 26.8, 27.1, 27.2, 30.4, 30.5, 30.5, 30.6, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.1, 41.7, 41.8, 47.9, 49.9, 50.0, 51.6, 52.1, 52.2, 171.5, 171.8, 172.7, 172.8, 176.1, 176.5 ppm. HRMS (ESI+) *m/z* calcd for $C_{28}H_{53}N_5O_4$ $[M+Na]^+$ 546.3990, found 546.3986.

5.4.2.2 Syntheses of the second generation azido-LPP acids

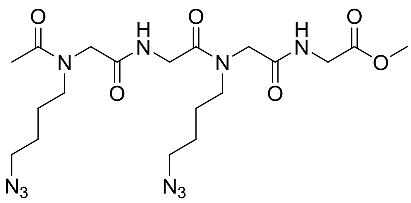
Ugi four-component reactions

N-Formyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**81**) as a mixture of rotamers



Aldehyde **19** (86 mg, 2.85 mmol), **16** (195 mg, 1.71 mmol), **61** (440 mg, 1.71 mmol), and **20** (155 µl, 169 mg, 1.71 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 9:1) and afforded **81** (643 mg, 77.9%) as yellow oil. R_f 0.17 (ethyl acetate/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.53–1.78 (m, 8H), 3.20 –3.50 (m, 8H), 3.74 and 3.74 and 3.75 (3s, 3H), 3.95–4.20 (m, 8H), 7.12–7.48 (m, 2H), 8.10 and 8.12 and 8.13 and 8.15 (4s, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.1, 24.4, 25.5, 25.5, 25.6, 25.8, 26.0, 26.0, 26.1, 40.9, 41.0, 41.0, 41.3, 42.9, 42.9, 46.2, 46.4, 47.2, 47.3, 48.1, 48.2, 48.4, 48.5, 49.8, 49.9, 50.2, 50.2, 50.8, 50.9, 50.8, 52.3, 52.3, 52.4, 52.4, 163.4, 163.9, 164.0, 168.1, 168.2, 168.2, 168.5, 168.6, 168.7, 168.7, 168.8, 168.8, 168.9, 168.9, 170.1, 170.2, 170.2 ppm. HRMS (ESI+) *m/z* calcd for $C_{18}H_{30}N_{10}O_6$ $[M+Na]^+$ 505.2242, found 505.2235.

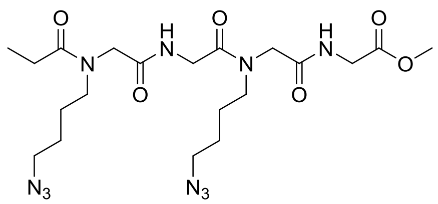
N-Acetyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**82**) as a mixture of rotamers



Aldehyde **19** (182 mg, 6.07 mmol), **16** (416 mg, 3.64 mmol), **62** (987 mg, 3.64 mmol), and **20** (331 µl, 361 mg, 3.64 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 9:1) to afford **82** (1474 mg, 81.6%) as yellow oil. R_f 0.15 (ethyl acetate/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.52–1.78 (m, 8H), 2.09 and 2.17 and 2.18 (3s, 3H), 3.24–3.50 (m, 8H), 3.73 and 3.74 (2s, 3H), 3.96–

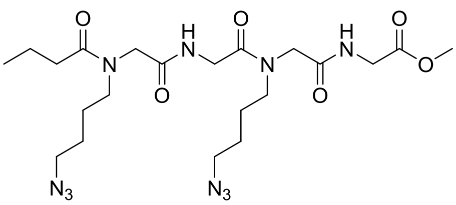
4.22 (m, 8H), 6.95–7.50 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 21.0, 21.1. 21.7, 24.4, 24.5, 24.6, 25.5, 25.8, 26.1, 40.9, 40.9, 41.0, 41.0, 41.2, 46.4, 46.5, 47.2, 48.1, 48.2, 49.7, 49.9, 50.0, 50.7, 50.9, 51.0, 51.8, 51.8, 52.2, 52.3, 168.2, 268.6, 168.6, 168.7, 168.7, 168.8, 169.0, 169.1, 169.2, 170.0, 170.1, 171.4, 171.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{19}H_{32}N_{10}O_6$ $[M+Na]^+$ 519.2398, found 519.2393.

N-Propionyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**83**) as a mixture of rotamers



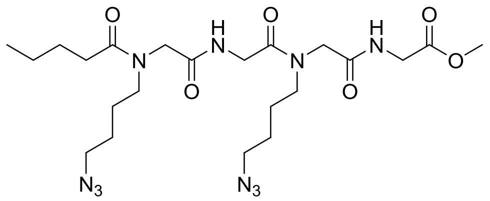
Aldehyde **19** (163 mg, 5.43 mmol), **16** (372 mg, 3.26 mmol), **63** (929mg, 3.26 mmol), and **20** (296 µl, 323 mg, 3.26 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **83** (1414 mg, 85.0%) as yellow oil. R_f 0.14 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.09–1.20 (m, 3H), 1.53–1.78 (m, 8H), 2.25–2.34 and 2.38–2.48 (2m, 2H), 3.24–3.50 (m, 8H), 3.73 and 3.74 (2s, 3H), 3.96–4.20 (m, 8H), 6.95–7.50 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 9.2. 9.3, 24.4, 24.6, 25.5, 25.9, 26.0, 26.0, 26.1, 26.4, 40.9, 41.0, 41.2, 46.6, 46.7, 47.2, 47.3, 48.1, 48.2, 48.9, 49.1, 49.9, 50.0, 50.1, 50.9, 50.9, 51.0, 52.2, 52.3, 168.2, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.1, 174.4, 174.5, 174.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{20}H_{34}N_{10}O_6$ $[M+Na]^+$ 533.2555, found 533.2548.

N-Butyryl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**84**) as a mixture of rotamers



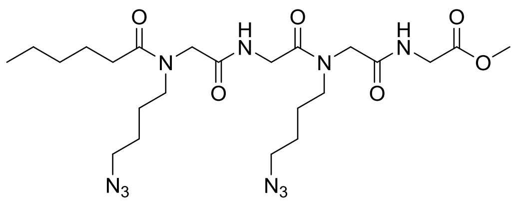
Aldehyde **19** (180 mg, 5.98 mmol), **16** (410 mg, 3.59 mmol), **64** (1075 mg, 3.59 mmol), and **20** (326 µl, 356 mg, 3.59 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **84** (1510 mg, 80.2%) as yellow oil. R_f 0.17 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.90–1-01 (m, 3H), 1.52–1.78 (m, 10H), 2.20–2.28 and 2.33–2.41 (2m, 2H), 3.24–3.50 (m, 8H), 3.73 and 3.74 (2s, 3H), 3.96–4.21 (m, 8H), 6.95–7.48 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 13.8, 18.4, 18.6, 24.4, 24.6, 25.5, 26.0, 26.0, 26.1, 34.7, 35.0, 40.9, 41.0, 41.3, 46.5, 47.8, 48.1, 48.2, 49.0, 49.1, 49.9, 50.1, 50.9, 50.9, 51.0, 52.2, 52.3, 168.2, 168.6, 168.6, 168.8, 168.8, 169.0, 169.3, 169.4, 170.1, 173.7, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{36}N_{10}O_6$ $[M+Na]^+$ 547.2712, found 547.2709.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**85**) as a mixture of rotamers



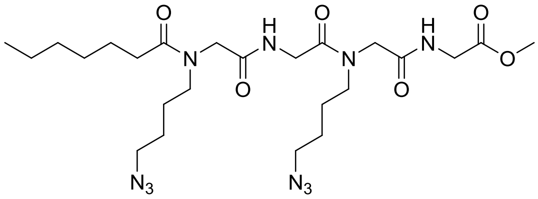
Aldehyde **19** (176 mg, 5.85 mmol), **16** (401 mg, 3.51 mmol), **65** (1100 mg, 3.51 mmol), and **20** (319 µl, 348 mg, 3.51 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **85** (1531 mg, 81.0%) as yellow oil, which solidified on standing. R_f 0.18 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.87–0.98 (m, 3H), 1.24–1.44 (m, 2H), 1.52–1.78 (m, 10H), 2.22–2.30 and 2.34–2.44 (2m, 2H), 3.22–3.50 (m, 8H), 3.71–3.80 (m, 3H), 3.96–4.20 (m, 8H), 6.87–7.37 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 22.3, 22.4, 24.4, 24.7, 25.5, 26.0, 26.1, 27.1, 27.2, 32.5, 32.9, 40.9, 41.1, 41.3, 45.6, 47.2, 48.1, 48.2, 49.0, 49.2, 50.0, 50.1, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.2, 168.5, 168.6, 168.7, 168.8, 169.0, 169.3, 169.3, 170.0, 173.7, 173.9, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{22}H_{38}N_{10}O_6$ $[M+Na]^+$ 561.2868, found 561.2863.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**86**) as a mixture of rotamers



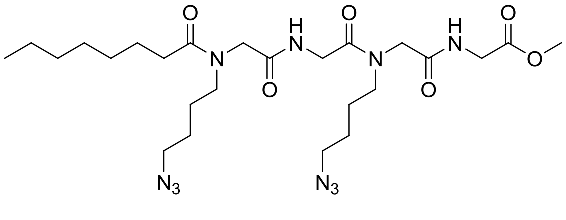
Aldehyde **19** (178 mg, 5.93 mmol), **16** (406 mg, 3.56 mmol), **66** (1167 mg, 3.56 mmol), and **20** (323 µl, 353 mg, 3.56 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **86** (1593 mg, 81.0%) as yellow oil, which solidified on standing. R_f 0.21 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.96 (m, 3H), 1.24–1.40 (m, 4H), 1.53–1.78 (m, 10H), 2.20–2.32 and 2.34–2.42 (2m, 2H), 3.22–3.50 (m, 8H), 3.73 and 3.74 and 3.75 (3s, 3H), 3.96–4.20 (m, 8H), 6.95–7.45 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.9, 22.4, 24.4, 24.6, 24.7, 24.8, 25.5, 25.9, 26.0, 26.1, 31.4, 31.5, 32.8, 33.1, 40.9, 41.0, 41.2, 46.5, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.2, 49.9, 50.0, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.0, 168.2, 168.5, 168.6, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.1, 170.1, 173.8, 173.8, 174.0, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{23}H_{40}N_{10}O_6$ $[M+Na]^+$ 575.3025, found 575.3018.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**87**) as a mixture of rotamers



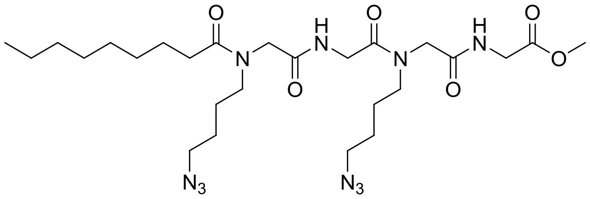
Aldehyde **19** (180 mg, 5.98 mmol), **16** (410 mg, 3.59 mmol), **67** (1225 mg, 3.59 mmol), and **20** (326 µl, 356 mg, 3.59 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **87** (1590 mg, 78.2%) as yellow oil, which solidified on standing. R_f 0.22 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.94 (m, 3H), 1.22–1.40 (m, 6H), 1.52–1.78 (m, 10H), 2.22–2.29 and 2.34–2.42 (2m, 2H), 3.22–3.52 (m, 8H), 3.73 and 3.74 (2s, 3H), 3.96–4.20 (m, 8H), 6.88–7.37 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.6, 25.0, 25.1, 25.5, 26.0, 26.1, 26.1, 28.9, 29.0, 31.6, 32.8, 33.2, 40.9, 41.0, 41.3, 46.5, 46.6, 47.2, 47.3, 48.1, 49.0, 49.2, 50.0, 50.1, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.1, 168.5, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 173.8, 173.8, 174.0, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{42}N_{10}O_6$ $[M+Na]^+$ 589.3181, found 589.3176.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**88**) as a mixture of rotamers



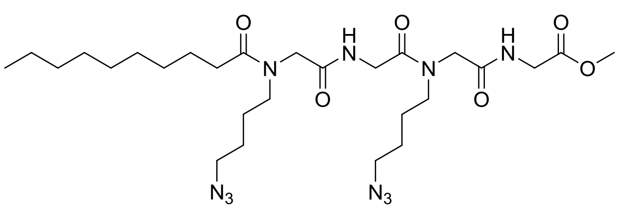
Aldehyde **19** (186 mg, 6.18 mmol), **16** (423 mg, 3.71 mmol), **68** (1317 mg, 3.71 mmol), and **20** (336 µl, 367 mg, 3.71 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **88** (1734 mg, 80.5%) as yellow oil. R_f 0.26 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.20–1.39 (m, 8H), 1.52–1.78 m, 10H), 2.22–2.29 and 2.34–2.43 (2m, 2H), 3.22–3.52 (m, 8H), 3.73 and 3.74 and 3.75 (3s, 3H), 3.95–4.22 (m, 8H), 7.00–7.52 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.6, 25.0, 25.1, 25.5, 25.9, 26.0, 26.0, 29.0, 29.2, 29.3, 31.6, 32.8, 33.1, 40.9, 41.0, 41.2, 41.2, 46.4, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.1, 49.8, 50.0, 50.8, 50.9, 51.0, 51.0, 52.2, 52.3, 168.2, 168.6, 168.6, 168.8, 168.8, 169.0, 169.3, 169.4, 169.6, 169.6, 170.1, 173.8, 174.0, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{25}H_{44}N_{10}O_6$ $[M+Na]^+$ 603.3338, found 603.3329.

N-Nonanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**89**) as a mixture of rotamers



Aldehyde **19** (185 mg, 6.15 mmol), **16** (421 mg, 3.69 mmol), **69** (1365 mg, 3.69 mmol), and **20** (335 µl, 366 mg, 3.69 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **89** (1524 mg, 69.1%) as yellow oil. R_f 0.28 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.20–1.41 (m, 10H), 1.52–1.78 (m, 10H), 2.20–2.29 and 2.33–2.42 (2m, 2H), 3.22–3.50 (m, 8H), 3.73 and 3.74 and 3.75 (3s, 3H), 3.96–4.20 (m, 8H), 6.94–7.45 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 25.9, 26.0, 26.1, 29.1, 29.3, 29.3, 31.7, 32.8, 33.2, 40.9, 41.0, 41.2, 46.4, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.1, 49.7, 49.9, 50.0, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.2, 168.6, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{26}H_{46}N_{10}O_6$ $[M+Na]^+$ 617.3494, found 617.3487.

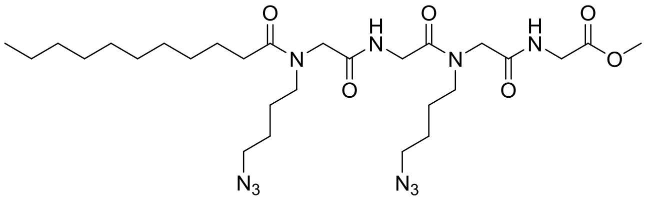
N-Decanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**90**) as a mixture of rotamers



Aldehyde **19** (179 mg, 5.95 mmol), **16** (408 mg, 3.57 mmol), **70** (1370 mg, 3.57 mmol), and **20** (324 µl, 354 mg, 3.57 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **90** (1721 mg, 78.8%) as yellow oil, which solidified on standing. R_f 0.29 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.20–1.39 (m, 12H), 1.53–1.77 (m, 10H), 2.22–2.29 and 2.34–2.42 (2m, 2H), 3.22–3.49 (m, 8H), 3.73 and 3.74 and 3.75 (3s, 3H), 3.96–4.20 (m, 8H), 6.90–7.40 (m, 2H) ppm.

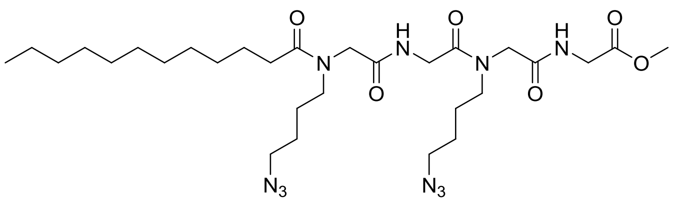
^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 26.0, 26.0, 26.1, 29.2, 29.3, 29.3, 29.4, 31.8, 32.8, 33.2, 40.9, 41.0, 41.2, 46.5, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.2, 49.9, 50.1, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 52.3, 168.0, 168.2, 168.5, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 170.0, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{27}H_{48}N_{10}O_6$ $[M+Na]^+$ 631.3651, found 631.3647.

N-Undecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**91**) as a mixture of rotamers



Aldehyde **19** (180 mg, 6.00 mmol), **16** (411 mg, 3.60 mmol), **71** (1431 mg, 3.60 mmol), and **20** (327 μl, 357 mg, 3.60 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **91** (1876 mg, 83.7%) as yellow oil. R_f 0.32 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.20–1.38 (m, 14H), 1.53–1.78 (m, 10H), 2.20–2.29 and 2.33–2.42 (2m, 2H), 3.22–3.50 (m, 8H), 3.73 and 3.74 and 3.75 (3s, 3H), 3.86–4.26 (m, 8H), 6.95–7.55 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 25.9, 26.0, 26.1, 29.2, 29.3, 29.3, 29.4, 29.4, 29.5, 31.8, 32.8, 33.2, 40.9, 41.0, 41.2, 46.4, 46.5, 47.2, 48.1, 48.2, 49.0, 49.2, 49.9, 50.0, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.0, 168.2, 168.5, 168.6, 168.7, 168.7, 168.8, 169.0, 169.2, 169.3, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{28}H_{50}N_{10}O_6$ $[M+Na]^+$ 645.3807, found 645.3800.

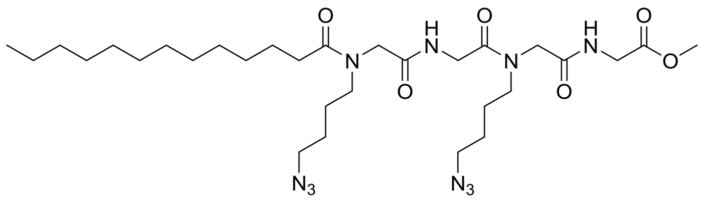
N-Lauroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**92**) as a mixture of rotamers



Aldehyde **19** (199 mg, 6.62 mmol), **16** (453 mg, 3.97 mmol), **72** (1635 mg, 3.97 mmol), and **20** (360 μl, 393 mg, 3.97 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol

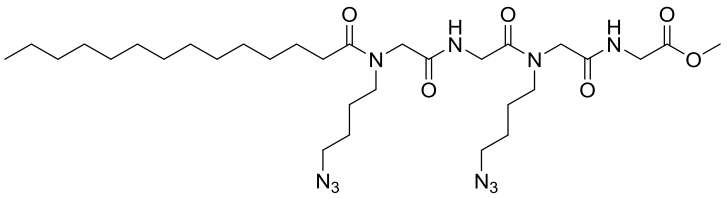
95:5) to afford **92** (2017 mg, 79.8%) as light brown, amorphous solid. R_f 0.33 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.20–1.39 (m, 16H), 1.53–1.78 (m, 10H), 2.21–2.27 and 2.33–2.42 (2m, 2H), 3.22–3.50 (m, 8H), 3.71–3.80 (m, 3H), 3.87–4.26 (m, 8H), 6.87–7.53 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.4, 24.5, 24.6, 25.0, 25.2, 25.5, 26.0, 26.1, 26.1, 29.3, 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 31.8, 32.8, 33.2, 40.9, 41.0, 41.3, 46.5, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.2, 50.0, 50.1, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 52.4, 168.2, 168.5, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{29}H_{52}N_{10}O_6$ $[M+Na]^+$ 659.3964, found 659.3960.

N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**93**) as a mixture of rotamers



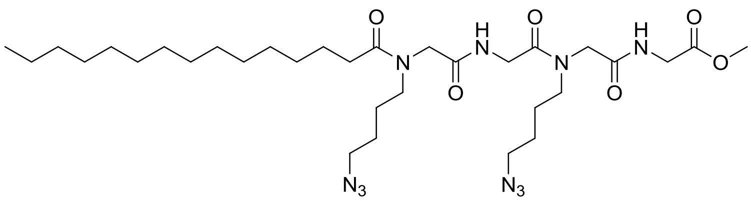
Aldehyde **19** (189 mg, 6.30 mmol), **16** (431 mg, 3.78 mmol), **73** (1609 mg, 3.78 mmol), and **20** (343 µl, 375 mg, 3.78 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **93** (2047 mg, 83.2%) as yellow oil, which solidified on standing. R_f 0.33 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.20–1.38 (m, 18H), 1.52–1.78 (m, 10H), 2.22–2.29 and 2.32–2.42 (2m, 2H), 3.20–3.50 (m, 8H), 3.70–3.79 (m, 3H), 3.87–4.27 (m, 8H), 6.87–7.60 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.6, 25.0, 25.1, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.4, 29.5, 29.5, 31.8, 32.8, 33.1, 40.7, 40.8, 41.0, 41.2, 46.4, 46.5, 47.1, 47.2, 48.0, 48.1, 49.0, 49.1, 49.8, 49.8, 50.0, 50.8, 50.9, 50.9, 52.1, 52.2, 168.1, 168.2, 168.5, 168.6, 168.7, 168.8, 169.0, 169.2, 169.3, 169.5, 169.6, 170.0, 173.7, 173.9, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{30}H_{54}N_{10}O_6$ $[M+Na]^+$ 673.4120, found 673.4116.

N-Myristoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**94**) as a mixture of rotamers



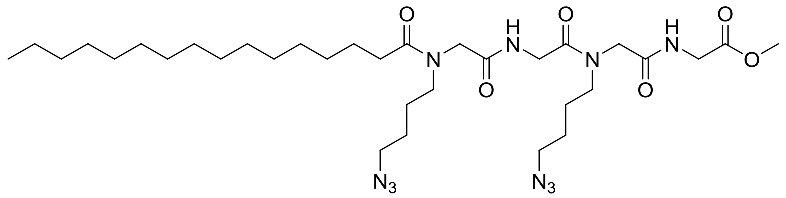
Aldehyde **19** (188 mg, 6.25 mmol), **16** (428 mg, 3.75 mmol), **74** (1650 mg, 3.75 mmol), and **20** (341 µl, 372 mg, 3.75 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **94** (2139 mg, 85.8%) as yellow oil, which solidified on standing. R_f 0.33 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.19–1.38 (m, 20H), 1.52–1.77 (m, 10H), 2.21–2.29 and 2.33–2.42 (2m, 2H), 3.22–3.50 (m, 8H), 3.71–3.81 (m, 3H), 3.86–4.26 (m, 8H), 6.85–7.52 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 26.0, 26.1, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 31.8, 32.8, 33.2, 40.9, 41.0, 41.3, 46.5, 46.6, 47.2, 47.3, 48.2, 48.2, 49.0, 49.2, 50.0, 50.1, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.1, 168.5, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 173.8, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{31}H_{56}N_{10}O_6$ $[M+Na]^+$ 687.4277, found 687.4272.

<u>*N*-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**95**) as a mixture of rotamers</u>



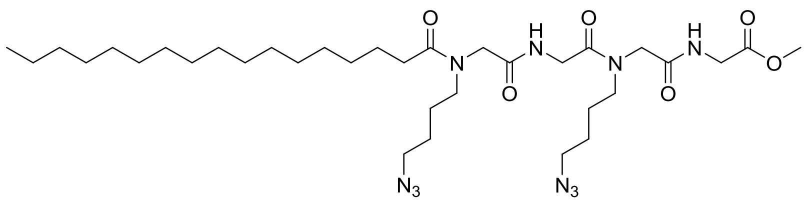
Aldehyde **19** (178 mg, 5.93 mmol), **16** (406 mg, 3.56 mmol), **75** (1614 mg, 3.56 mmol), and **20** (323 µl, 353 mg, 3.56 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **95** (1953 mg, 80.8%) as yellow oil, which solidified on standing. R_f 0.33 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.19-1.38 (m, 22H), 1.52–1.77 (m, 10H), 2.21–2.29 and 2.33–2.42 (2m, 2H), 3.20–3.50 (m, 8H), 3.70–3.81 (m, 3H), 3.87–4.26 (m, 8H), 6.97–7.60 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 25.9, 26.0, 26.1, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 31.8, 32.8, 33.2, 40.9, 41.0, 41.2, 46.4, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.2, 49.8, 50.0, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.2, 168.6, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 170.1, 173.8, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{32}H_{58}N_{10}O_6$ $[M+Na]^+$ 701.4433, found 701.4422.

N-Palmitoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**96**) as a mixture of rotamers



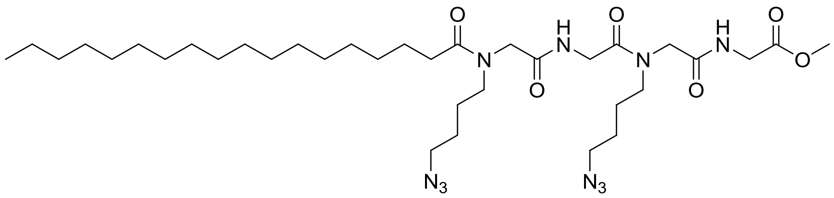
Aldehyde **19** (184 mg, 6.13 mmol), **16** (420 mg, 3.68 mmol), **76** (1719 mg, 3.68 mmol), and **20** (334 µl, 365 mg, 3.68 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **96** (2099 mg, 82.3%) as yellow oil. R_f 0.34 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.18–1.46(m, 24H), 1.52–1.78 (m, 10H), 2.22–2.42 (m, 2H), 3.22–3.50 (m, 8H), 3.71–3.79 (m, 3H), 3.87–4.27 (m, 8H), 7.03–7.60 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.1, 25.5, 25.9, 26.0, 26.0, 29.2, 29.3, 29.4, 29.4, 29.5, 29.6, 31.8, 32.8, 33.2, 40.7, 40.9, 41.0, 41.2, 46.4, 46.5, 47.1, 47.2, 48.1, 48.2, 49.0, 49.1, 49.8, 49.8, 50.0, 50.8, 50.9, 51.0, 52.1, 52.2, 168.1, 168.3, 168.6, 168.6, 168.7, 168.8, 168.8, 169.0, 169.2, 169.4, 169.6, 170.1, 173.8, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{33}H_{60}N_{10}O_6$ $[M+Na]^+$ 715.4590, found 715.4586.

N-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**97**) as a mixture of rotamers



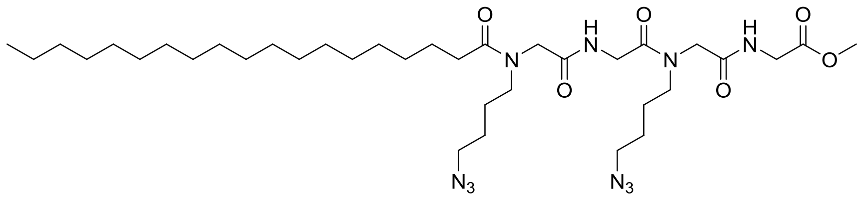
Aldehyde **19** (182 mg, 6.05 mmol), **16** (414 mg, 3.63 mmol), **77** (1748 mg, 3.63 mmol), and **20** (330 µl, 360 mg, 3.63 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **97** (2129 mg, 83.0%) as light yellow oil. R_f 0.34 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.14–1.46 (m, 26H), 1.52–1.78 (m, 10H), 2.20–2.29 and 2.33–2.42 (2m, 2H), 3.21–3.52 (m, 8H), 3.70–3.80 (m, 3H), 3.86–4.27 (m, 8H), 6.95–7.55 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 25.9, 26.0, 26.1, 29.3, 29.3, 29.4, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.7, 40.9, 41.0, 41.2, 46.4, 46.5, 47.2, 47.2, 48.1, 48.2, 49.0, 49.2, 49.9, 50.0, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.0, 168.2, 168.5, 168.6, 168.7, 168.8, 169.0, 169.2, 169.3, 170.0, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{62}N_{10}O_6$ $[M+Na]^+$ 729.4746, found 729.4732.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**98**) as a mixture of rotamers



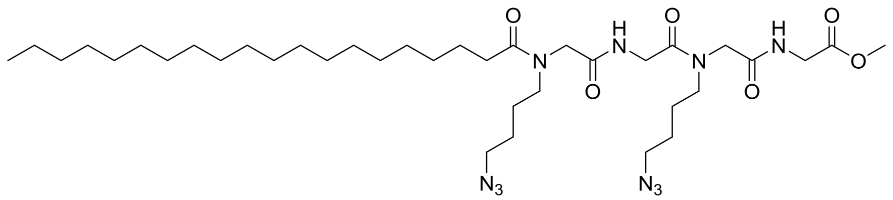
Aldehyde **19** (175 mg, 5.82 mmol), **16** (398 mg, 3.49 mmol), **78** (1729 mg, 3.49 mmol), and **20** (317 µl, 346 mg, 3.49 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **98** (2151 mg, 85.5%) as yellow oil, which solidified on standing. R_f 0.35 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.06–1.46 (m, 28H), 1.52–1.79 (m, 10H), 2.20–2.29 and 2.31–2.42 (2m, 2H), 3.22–3.52 (m, 8H), 3.70–3.80 (m, 3H), 3.86–4.26 (m, 8H), 7.02–7.57 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.0, 25.1, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 31.8, 32.8, 33.1, 40.7, 40.8, 41.0, 41.2, 46.4, 46.5, 47.1, 47.2, 48.0, 48.1, 49.0, 49.1, 49.8, 49.8, 50.0, 50.8, 50.8, 50.9, 52.1, 52.2, 52.3, 168.1, 168.2, 168.6, 168.6, 168.7, 168.7, 168.8, 169.0, 169.2, 169.3, 170.0, 170.0, 173.7, 173.8, 173.9, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{64}N_{10}O_6$ $[M+Na]^+$ 743.4903, found 743.4899.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**99**) as a mixture of rotamers



Aldehyde **19** (152 mg, 5.05 mmol), **16** (346 mg, 3.03 mmol), **79** (1546 mg, 3.03 mmol), and **20** (275 µl, 300 mg, 3.03 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **99** (1792 mg, 80.5%) as light yellow, amorphous solid. R_f 0.35 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.06–1.46 (m, 30H), 1.52–1.77 (m, 10H), 2.20–2.29 and 2.33–2.42 (2m, 2H), 3.22–3.52 (m, 8H), 3.70–3.80 (m, 3H), 3.86–4.26 (m, 8H), 6.95–7.57 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 26.0, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 31.8, 32.8, 33.2, 40.7, 40.9, 41-0 41.2, 46.4, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.2, 49.9, 50.0, 50.9, 50.9, 51.0, 51.1, 52.2, 52.3, 168.0, 168.2, 168.6, 168.6, 168.7, 168.8, 169.0, 169.3, 169.4, 170.0, 173.8, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{36}H_{66}N_{10}O_6$ $[M+Na]^+$ 757.5059, found 757.5060.

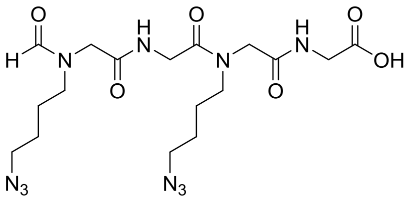
N-Arachidoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**100**) as a mixture of rotamers



Aldehyde **19** (162 mg, 5.38 mmol), **16** (369 mg, 3.23 mmol), **80** (1690 mg, 3.23 mmol), and **20** (293 µl, 320 mg, 3.23 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (ethyl acetate/methanol 95:5) to afford **100** (1851 mg, 76.5%) as light yellow, amorphous solid. R_f 0.35 (ethyl acetate/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.06–1.46 (m, 32H), 1.52–1.77 (m, 10H), 2.21–2.30 and 2.33–2.42 (2m, 2H), 3.22–3.52 (m, 8H), 3.70–3.80 (m, 3H), 3.86–4.26 (m, 8H), 6.95–7.57 (m, 2H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.6, 25.0, 25.2, 25.5, 25.9, 26.0, 26.1, 29.3, 29.3, 29.4, 29.4, 29.5, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.7, 40.9, 41.0, 41.2, 46.4, 46.5, 47.2, 47.3, 48.1, 48.2, 49.0, 49.2, 49.9, 50.0, 50.9, 50.9, 51.0, 52.2, 52.3, 52.3, 168.0, 168.2, 168.6, 168.6, 168.7, 168.7, 168.8, 169.0, 169.2, 169.3, 169.5, 170.0, 173.8, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{37}H_{68}N_{10}O_6$ $[M+Na]^+$ 771.5216, found 771.5208.

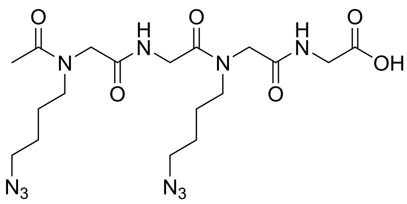
Saponifications

<u>*N*-Formyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**101**) as a mixture of rotamers</u>



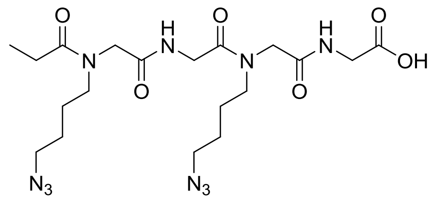
The saponification of **81** (595 mg, 1.23 mmol) with lithium hydroxide solution (1.54 ml, 3.08 mmol, 2 M) following general procedure B (method 1) afforded **101** (574 mg, 99.6%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.51–1.80 (m, 8H), 3.26–3.50 (m, 8H), 3.89–3.99 (m, 2H), 4.03–4.23 (m, 6H), 8.09 and 8.18 (2brs, 1H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.2, 25.6, 26.5, 26.9, 27.1, 27.2, 30.9, 41.7, 41.8, 41.9, 42.1, 42.1, 44.1, 46.1, 46.2, 48.3, 49.1, 49.3, 49.9, 50.8, 51.0, 52.1, 52.1, 52.1, 165.9 (br), 166.5, 170.5, 170.7, 170.8, 170.9, 171.1, 171.2, 171.4, 171.5, 172.7, 172.8 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{17}H_{28}N_{10}O_6$ $[M+Na]^+$ 491.2085, found 491.2080.

<u>*N*-Acetyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**102**) as a mixture of rotamers</u>



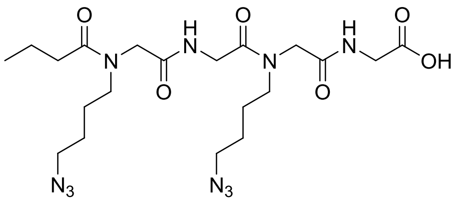
The saponification of **82** (1427 mg, 2.87 mmol) with lithium hydroxide solution (3.59 ml, 7.18 mmol, 2 M) following general procedure B (method 1) afforded **102** (1309 mg, 94.5%) as yellow oil. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.49–1.80 (m, 8H), 2.08 and 2.17 (2brs, 3H), 3.24–3.52 (m, 8H), 3.88–4.26 (m, 8H), 8.04 and 8.22 and 8.44 (3brs, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 21.2, 21.8, 25.5, 25.6, 26.5, 26.6, 27.1, 27.2, 27.2, 41.7, 41.9, 42.1, 47.8, 48.3, 48.3, 49.0, 49.8, 49.9, 50.8, 52.1, 52.1, 52.1, 52.3, 170.8, 170.9, 171.0, 171.1, 171.2, 171.3, 171.4, 172.6, 172.7, 173.9 (br), 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{18}H_{30}N_{10}O_6$ $[M+Na]^+$ 505.2242, found 505.2236.

<u>*N*-Propionyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**103**) as a mixture of rotamers</u>



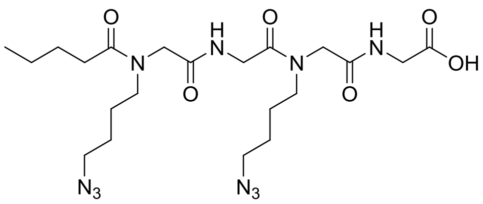
The saponification of **83** (1370 mg, 2.68 mmol) with lithium hydroxide solution (3.35 ml, 6.70 mmol, 2 M) following general procedure B (method 1) afforded **103** (1237 mg, 93.0%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.04–1.17 (m, 3H), 1.49–1.80 (m, 8H), 2.30–2.40 and 2.44–2.55 (2m, 2H), 3.25–3.50 (m, 8H), 3.88–4.24 (m, 8H), 8.02 and 8.22 and8.44 (3brs, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 9.7, 9.8, 25.6, 25.7, 26.5, 26.8, 26.9, 27.1, 27.1, 27.2, 27.2, 27.3, 41.7, 41.8, 41.9, 42.0, 42.0, 47.9, 48.3, 48.4, 49.0, 49.9, 50.1, 50.8, 51.5, 52.1 52.1, 52.2, 170.9, 171.0, 171.1, 171.1, 171.3, 171.3, 171.5, 171.6, 171.7, 171.8, 172.7, 172.7, 176.8, 177.2 ppm. HRMS (ESI+) *m/z* calcd for $C_{19}H_{32}N_{10}O_6$ $[M+Na]^+$ 519.2398, found 519.2396.

N-Butyryl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**104**) as a mixture of rotamers



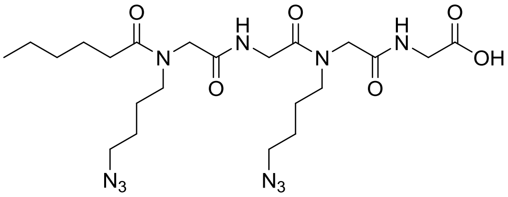
The saponification of **84** (1463 mg, 2.79 mmol) with lithium hydroxide solution (3.49 ml, 6.98 mmol, 2 M) following general procedure B (method 1) afforded **104** (1416 mg, 99.4%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.89–1.02 (m, 3H), 1.50 –1.78 (m, 10H), 2.27–2.35 and 2.39–2.48 (2m, 2H), 3.20–3.50 (m, 8H), 3.80–4.28 (m, 8H), 8.00 and 8.21 and 8.40–8.48 (2brs, brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.1, 14.2, 19.6, 19.7, 25.5, 26.6, 26.5, 26.9, 27.1, 27.2, 27.2, 35.5, 35.9, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 51.6, 52.1, 52.1, 52.1, 170.9, 171.0, 171.1, 171.3, 171.5, 171.6, 171.7, 172.7, 172.7, 176.0, 176.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{20}H_{34}N_{10}O_6$ $[M+Na]^+$ 533.2555, found 533.2542.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**105**) as a mixture of rotamers



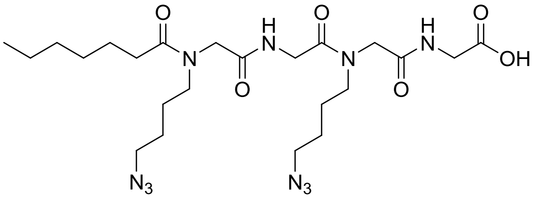
The saponification of **85** (1487 mg, 2.76 mmol) with lithium hydroxide solution (3.45 ml, 6.90 mmol, 2 M) following general procedure B (method 1) afforded **105** (1440 mg, 99.5%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.88–0.98 (m, 3H), 1.27–1.45 (m, 2H), 1.50–1.79 (m, 10H), 2.29–2.37 and 2.41–2.50 (2m, 2H), 3.21–3.51 (m, 8H), 3.85–4.35 (m, 8H), 7.99 and 8.21 and 8.40–8.47 (2brs, brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 23.4, 23.5, 25.5, 25.6, 26.5, 26.9, 27.1, 27.2, 27.2, 28.4, 28.5, 30.9, 33.7, 33.8, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.1, 180.8, 170.9, 171.0, 171.2, 171.3, 171.4, 171.5, 171.6, 171.7, 172.6, 172.7, 176.2, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{21}H_{36}N_{10}O_6$ $[M+Na]^+$ 547.2712, found 547.2706.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**106**) as a mixture of rotamers



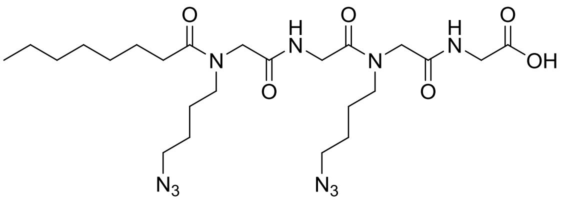
The saponification of **86** (1537 mg, 2.78 mmol) with lithium hydroxide solution (3.48 ml, 6.95 mmol, 2 M) following general procedure B (method 1) afforded **106** (1450 mg, 96.8%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.96 (m, 3H), 1.26–1.43 (m, 4H), 1.51–1.79 (m, 10H), 2.29–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.51 (m, 8H), 3.80–4.35 (m, 8H), 8.01 and 8.22 and 8.42–8.48 (2brs, brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 14.3, 23.6, 25.6, 25.7, 26.0, 26.1, 26.5, 26.9, 27.1, 27.2, 27.2, 32.6, 32.6, 33.6, 34.0, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.4, 49.0, 49.9, 50.0, 50.1, 51.6, 52.1, 52.1, 52.2, 170.9, 171.0, 171.1, 171.1, 171.2, 171.3, 171.5, 171.6, 171.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{22}H_{38}N_{10}O_6$ $[M+Na]^+$ 561.2868, found 561.2863.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**107**) as a mixture of rotamers



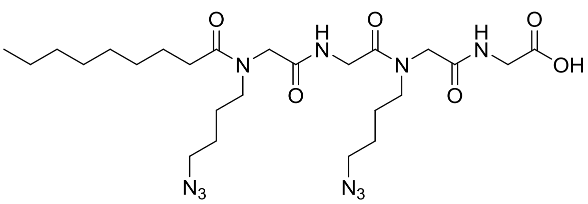
The saponification of **87** (1544 mg, 2.72 mmol) with lithium hydroxide solution (3.41 ml, 6.80 mmol, 2 M) following general procedure B (method 1) afforded **107** (1500 mg, 99.8%), as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.95 (m, 3H), 1.25–1.43 (m, 6H), 1.50–1.79 (m, 10H), 2.29–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.50 (m, 8H), 3.80–4.35 (m, 8H), 8.22 and 7.95–8.05 and 8.41–8.48 (brs, 2brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.6, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.0, 30.1, 32.8, 33.6, 34.1, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.1, 50.8, 50.8, 51.6, 52.1, 52.1, 52.1, 52.2, 170.8, 171.0, 171.1, 171.1, 171.2, 171.3, 171.5, 171.6, 171.7, 172.6, 172.7, 172.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{23}H_{40}N_{10}O_6$ $[M+Na]^+$ 575.3025, found 575.3020.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**108**) as a mixture of rotamers



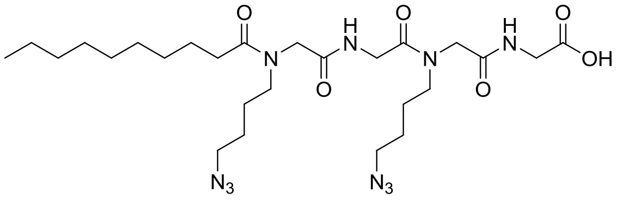
The saponification of **88** (1659 mg, 2.86 mmol) with lithium hydroxide solution (3.57 ml, 7.15 mmol, 2 M) following general procedure B (method 1) afforded **108** (1575 mg, 97.2%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.22–1.42 (m, 8H), 1.50–1.79 (m, 10H), 2.28–2.36 and 2.41–2.50 (2m, 2H), 3.20–3.55 (m, 8H), 3.80–4.35 (m, 8H), 7.95–8.05 and 8.17–8.27 and 8.41–8.57 (3brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.3, 30.3, 30.4, 32.9, 32.9, 33.6, 34.1, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.1, 52.2, 170.8, 170.9, 171.0, 171.1, 171.2, 171.3, 171.4, 171.4, 171.5, 171.7, 172.6, 172.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{24}H_{42}N_{10}O_6$ $[M+Na]^+$ 589.3181, found 589.3173.

N-Nonanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**109**) as a mixture of rotamers



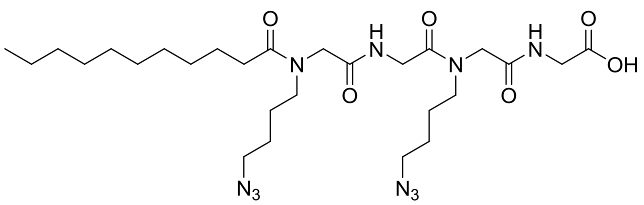
The saponification of **89** (1454 mg, 2.45 mmol) with lithium hydroxide solution (3.06 ml, 6.13 mmol, 2 M) following general procedure B (method 1) afforded **109** (1414 mg, 99.4%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.22–1.42 (m, 10H), 1.50–1.79 (m, 10H), 2.24–2.37 and 2.40–2.50 (2m, 2H), 3.20–3.55 (m, 8H), 3.80–4.35 (m, 8H), 8.00 and 8.22 and 8.45 (3brs, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.7, 27.1, 27.2, 27.2, 30.3, 30.4, 30.4, 30.5, 33.0, 33.6, 34.1, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 171.0, 171.1, 171.2, 171.3, 171.4, 171.5, 171.6, 171.7, 172.6, 172.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{25}H_{44}N_{10}O_6$ $[M+Na]^+$ 603.3338, found 603.3333.

N-Decanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**110**) as a mixture of rotamers



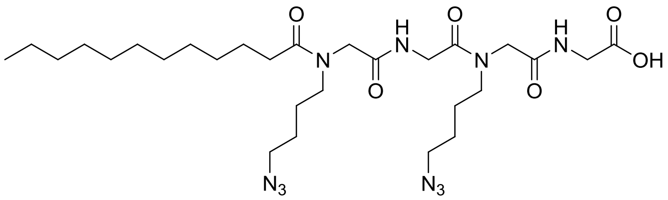
The saponification of **90** (1667 mg, 2.74 mmol) with lithium hydroxide solution (3.42 ml, 6.85 mmol, 2 M) following general procedure B (method 1) afforded **110** (1612 mg, 98.9%) as a light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.20–1.42 (m, 12H), 1.51–1.80 (m, 10H), 2.28–2.37 and 2.40–2.51 (2m, 2H), 3.20–3.54 (m, 8H), 3.80–4.35 (m, 8H), 8.00 and 8.22 and 8.41–8.48 (2brs, brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.6, 33.0, 33.6, 34.1, 41.7, 41.8, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.1, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 171.0, 171.1, 171.2, 171.3, 171.4, 171.5, 171.7, 172.6, 172.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{26}H_{46}N_{10}O_6$ $[M+Na]^+$ 617.3494, found 617.3480.

N-Undecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**111**) as a mixture of rotamers



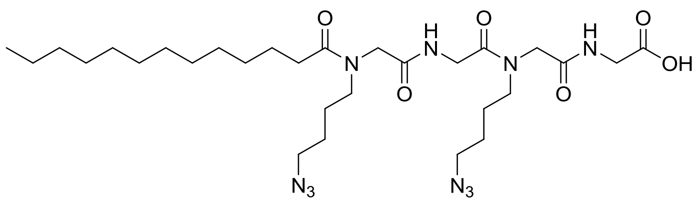
The saponification of **91** (1801 mg, 2.89 mmol) with lithium hydroxide solution (3.61 ml, 7.23 mmol, 2 M) following general procedure B (method 1) afforded **111** (1691 mg, 96.1%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.22–1.42 (m, 14H), 1.51–1.79 (m, 10H), 2.28–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.52 (m, 8H), 3.80–4.35 (m, 8H), 8.00 and 8.22 and 8.41–8.48 (2brs, brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.4, 30.6, 30.6, 30.6, 30.7, 33.0, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.1, 52.2, 170.8, 171.0, 171.0, 171.1, 171.2, 171.3, 171.4, 171.5, 171.7, 172.7, 172.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{48}N_{10}O_6$ $[M+Na]^+$ 631.3651, found 631.3636.

<u>*N*-Lauroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**112**) as a mixture of rotamers</u>



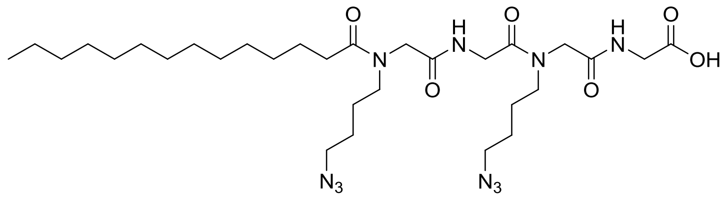
The saponification of **92** (1966 mg, 3.09 mmol) with lithium hydroxide solution (3.86 ml, 7.73 mmol, 2 M) following general procedure B (method 1) afforded **112** (1902 mg, 98.8%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.22–1.42 (m, 16H), 1.51–1.79 (m, 10H), 2.28–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.54 (m, 8H), 3.80–4.35 (m, 8H), 7.96–8.05 and 8.23 and 8.42–8.49 (brm, brs, brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.5, 30.6, 30.6, 30.6, 30.7, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.4, 49.0, 49.9, 50.0, 50.1, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 52.2, 170.9, 171.0, 171.1, 171.1, 171.2, 171.3, 171.3, 171.5, 171.6, 171.7, 172.7, 172.7, 172.7, 176.2, 276.6, 276.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{50}N_{10}O_6$ $[M+Na]^+$ 645.3807, found 645.3790.

<u>*N*-Tridecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**113**) as a mixture of rotamers</u>



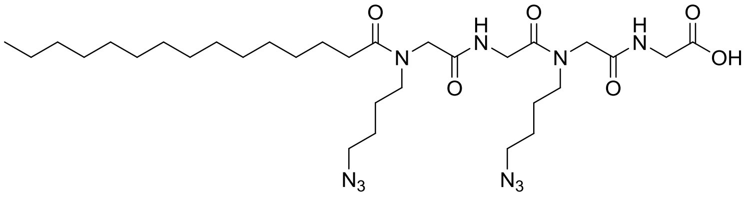
The saponification of **93** (1947 mg, 2.99 mmol) with lithium hydroxide solution (3.74 ml, 7.48 mmol, 2 M) following general procedure B (method 1) afforded **113** (1783 mg, 93.6%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.08–1.48 (m, 18H), 1.51–1.79 (m, 10H), 2.28–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.54 (m, 8H), 3.80–4.35 (m, 8H), 7.95–8.05 and 8.17–8.27 and 8.41–8.48 (3brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.5, 30.6, 30.6, 30.6, 30.7, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.1, 52.2, 170.8, 171.0, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.7, 172.6, 172.7, 172.7, 176.2, 176.6, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{29}H_{52}N_{10}O_6$ $[M+Na]^+$ 659.3964, found 659.3949.

N-Myristoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**114**) as a mixture of rotamers



The saponification of **94** (2059 mg, 3.10 mmol) with lithium hydroxide solution (3.87 ml, 7.75 mmol, 2 M) following general procedure B (method 1) afforded **114** (1919 mg, 95.1%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.10–1.48 (m, 20H), 1.51–1.79 (m, 10H), 2.24–2.36 and 2.40–2.49 (2m, 2H), 3.10–3.54 (m, 8H), 3.75–4.35 (m, 8H), 7.96–8.05 and 8.19–8.26 and 8.43–8.49 (3brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.4, 30.5, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.4, 49.0, 49.9, 50.0, 50.1, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 52.2, 170.9, 171.0, 171.1, 171.1, 171.3, 171.3, 171.3, 171.5, 171.6, 171.7, 172.7, 172.7, 172.7, 176.2, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{54}N_{10}O_6$ $[M+Na]^+$ 673.4120, found 673.4102.

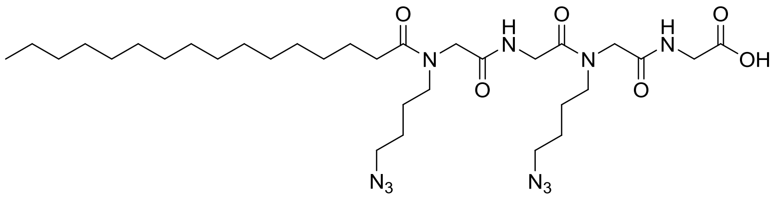
N-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**115**) as a mixture of rotamers



The saponification of **95** (1866 mg, 2.75 mmol) with lithium hydroxide solution (3.43 ml, 6.88 mmol, 2 M) following general procedure B (method 1) afforded **115** (1779 mg, 97.3%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.10–1.48 (m, 22H), 1.51–1.79 (m, 10H), 2.28–2.36 and 2.41–2.50 (2m, 2H), 3.20–3.55 (m, 8H), 3.80–4.35 (m, 8H), 7.95–8.04 and 8.17–8.27 and 8.42–8.48 (3brm, 2H, weak) ppm. ^{13}C-NMR

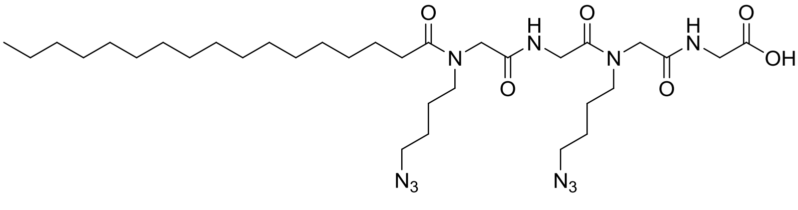
(100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 171.0, 171.1, 171.2, 171.3, 171.4, 171.5, 171.7, 172.6, 172.6, 172.7, 176.1, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{31}H_{56}N_{10}O_6$ $[M+Na]^+$ 687.4277, found 687.4263.

N-Palmitoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**116**) as a mixture of rotamers



The saponification of **96** (1992 mg, 2.87 mmol) with lithium hydroxide solution (3.59 ml, 7.18 mmol, 2 M) following general procedure B (method 1) afforded **116** (1894 mg, 97.2%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.10–1.48 (m, 24H), 1.51–1.79 (m, 10H), 2.24–2.36 and 2.41–2.48 (2m, 2H), 3.20–3.54 (m, 8H), 3.80–4.35 (m, 8H), 7.96–8.05 and 8.17–8.25 and 8.42–8.48 (3brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.4, 30.5, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.1, 51.6, 52.1, 52.1, 52.2, 52.2, 170.9, 171.0, 171.1, 171.1, 171.2, 171.3, 171.3, 171.5, 171.5, 171.7, 172.6, 172.7, 172.7, 176.2, 176.6, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{32}H_{58}N_{10}O_6$ $[M+Na]^+$ 701.4433, found 701.4416.

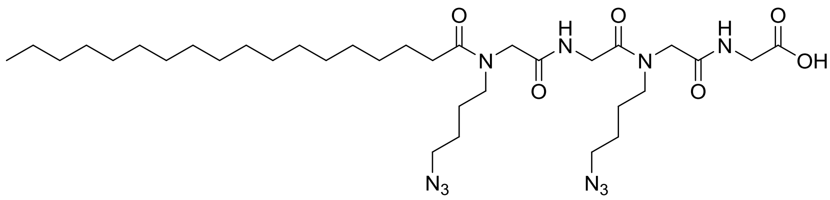
N-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**117**) as a mixture of rotamers



The saponification of **97** (2044 mg, 2.89 mmol) with lithium hydroxide solution (3.61 ml, 7.23 mmol, 2 M) following general procedure B (method 1) afforded **117** (1893 mg, 94.5%)

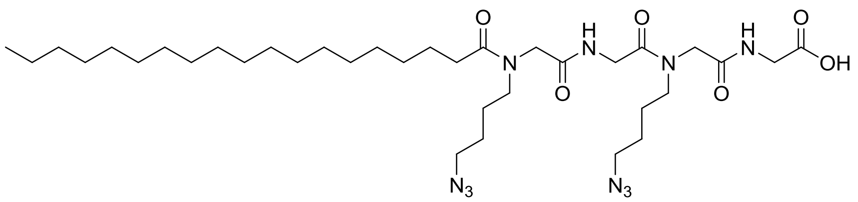
as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.10–1.48 (m, 26H), 1.51–1.79 (m, 10H), 2.24–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.54 (m, 8H), 3.80–4.35 (m, 8H), 7.96–8.05 and 8.17–8.26 and 8.41–8.47 (3brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.1, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 52.2, 170.8, 171.0, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.7, 172.6, 172.7, 172.7, 176.1, 176.5, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{60}N_{10}O_6$ $[M+Na]^+$ 715.4590, found 715.4573.

<u>*N*-Stearoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**118**) as a mixture of rotamers</u>



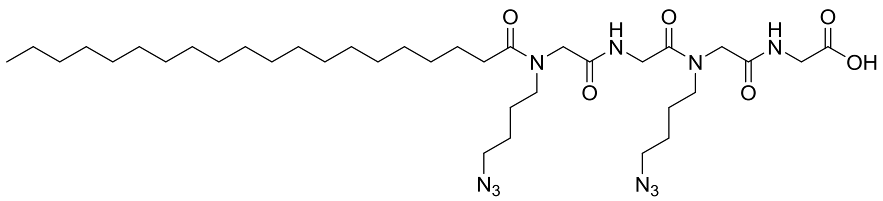
The saponification of **98** (2029 mg, 2.81 mmol) with lithium hydroxide solution (3.52 ml, 7.03 mmol, 2 M) following general procedure B (method 1) afforded **118** (1884 mg, 94.8%) as yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.10–1.49 (m, 28H), 1.51–1.79 (m, 10H), 2.24–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.54 (m, 8H), 3.80–4.35 (m, 8H), 7.82–7.90 and 7.96–8.05 and 8.17–8.27 and 8.41–8.49 (4brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.5, 30.5, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.7, 172.6, 172.7, 172.7, 176.1, 176.5, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{62}N_{10}O_6$ $[M+Na]^+$ 729.4746, found 729.4738.

<u>*N*-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**119**) as a mixture of rotamers</u>



The saponification of **99** (1704 mg, 2.32 mmol) with lithium hydroxide solution (2.90 ml, 5.80 mmol, 2 M) following general procedure B (method 1) afforded **119** (1655 mg, 98.9%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.09–1.49 (m, 30H), 1.51–1.79 (m, 10H), 2.24–2.36 and 2,41–2.49 (2m, 2H), 3.20–3.50 (m, 8H), 3.80–4.32 (m, 8H), 7.96–8.05 and 8.17–8.27 and 8.42–8.47 (3brm, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.8, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.5, 30.5, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.1, 41.7, 71.8, 41.9, 41.9, 42.0, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.1, 50.8, 50.8, 51.6, 52.1, 52.2, 52.2, 170.9, 171.0, 171.1, 171.1, 171.2, 171.3, 171.3, 171.5, 171.6, 171.7, 172.7, 172.7, 172.7, 176.2, 176.6, 176.6 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{64}N_{10}O_6$ $[M+Na]^+$ 743.4903, found 743.4890.

N-Arachidyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**120**) as a mixture of rotamers

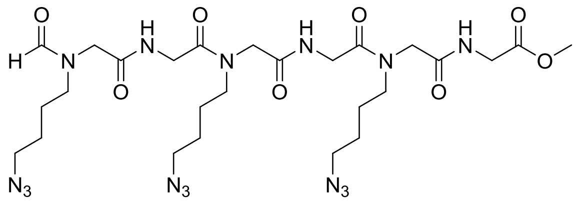


The saponification of **100** (1755 mg, 2.34 mmol) with lithium hydroxide solution (2.93 ml, 5.85 mmol, 2 M) following general procedure B (method 1) afforded **120** (1660 mg, 96.5%) as light yellow, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.95 (m, 3H), 1.09–1.80 (m, 42H), 2.25–2.52 (m, 2H), 3.15–3.54 (m, 8H), 3.80–4.35 (m, 8H), 7.98 and 8.20 and 8.42 (3brs, 2H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.5, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.5, 30.5, 30.6, 30.6, 30.7, 30.8, 30.8, 33.1, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 47.8, 48.3, 48.3, 49.0, 49.9, 50.0, 50.0, 50.7, 50.8, 51.6, 52.1, 52.1, 52.1, 170.8, 171.0, 171.0, 171.0, 171.2, 171.3, 171.4, 171.5, 171.7, 172.6, 172.7, 176.0, 176.5, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{36}H_{66}N_{10}O_6$ $[M+Na]^+$ 757.5059, found 757.5048.

5.4.2.3 Syntheses of the third generation azido-LPP acids

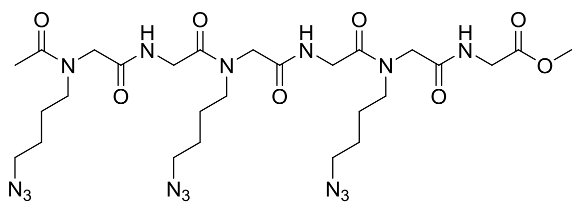
Ugi four-component reactions

N-Formyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**121**) as a mixture of rotamers



Aldehyde **19** (56 mg, 1.87 mmol), **16** (128 mg, 1.12 mmol), **101** (525 mg, 1.12 mmol), and **20** (102 µl, 111 mg, 1.12 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **121** (448 mg, 57.7%) as light yellow, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.52–1.78 (m, 12H), 3.20–3.52 (m, 12H), 3.73 and 3.74 (2s, 3H), 3.83–4.22 (m, 12H), 7.14–7.64 (m, 3H), 8.10–8.20 (m, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.1, 24.4, 24.5, 25.4, 25.5, 25.6, 25.6, 25.8, 26.0, 40.8, 40.9, 41.1, 41.2, 41.6, 42.8, 46.2, 46.3, 47.1, 47.2, 47.9, 48.1, 48.4, 48.5, 49.5, 49.6, 49.7, 49.7, 49.9, 50.0, 50.1, 50.9, 50.9, 52.2, 52.3, 52.3, 163.3, 163.4, 164.0, 164.1, 164.1, 167.9, 167.9, 168.1, 168.1, 168.3, 168.3, 168.4, 168.6, 168.7, 168.8, 168.8, 168.9, 168.9, 169.0, 169.0, 169.2, 169.3, 170.1, 170.3, 170.3, 170.4, 170.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{26}H_{43}N_{15}O_8$ $[M+Na]^+$ 716.3311, found 716.3297.

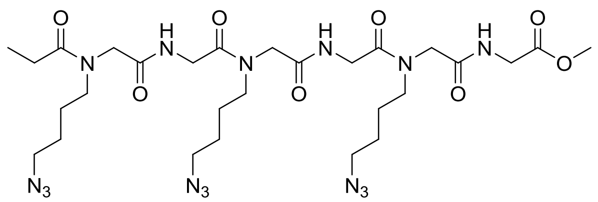
N-Acetyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**122**) as a mixture of rotamers



Aldehyde **19** (125 mg, 4.15 mmol), **16** (284 mg, 2.49 mmol), **102** (1200 mg, 2.49 mmol), and **20** (226 µl, 247 mg, 2.49 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **122** (1258 mg, 71.4%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.52–1.78 (m, 12H), 2.08 and 2.09 and 2.15 and 2.17 (4s, 3H), 3.22–3.50 (m, 12H), 3.73 and 3.74 and 3.74 (3s, 3H), 3.94–4.22 (m, 12H), 7.05–7.67 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ =

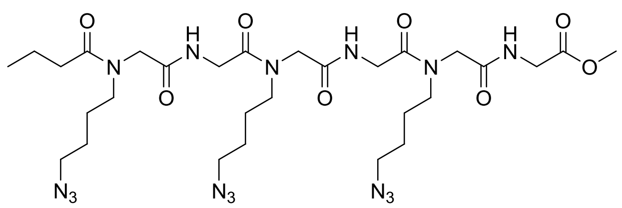
21.1, 21.7, 24.4, 24.5, 24.6, 25.4, 25.5, 25.5, 25.6, 25.8, 26.1, 26.1, 40.9, 41.1, 46.4, 46.4, 47.1, 47.3, 47.9, 48.1, 48.2, 49.6, 49.6, 49.7, 49.8, 49.8, 49.9 50.0, 50.0, 50.9, 51.0, 51.0, 51.8, 52.2, 52.3, 52.3, 168.3, 168.7, 168.8, 168.8, 168.9, 168.9, 168.9, 169.0, 169.0, 169.2, 169.2, 169.2, 170.1, 170.3, 170.4, 171.3, 171.3, 171.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{45}N_{15}O_8$ $[M+Na]^+$ 730.3468, found 730.3460.

N-Propionyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**123**) as a mixture of rotamers



Aldehyde **19** (117 mg, 3.90 mmol), **16** (267 mg, 2.34 mmol), **103** (1163 mg, 2.34 mmol), and **20** (212 µl, 232 mg, 2.34 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **123** (1140 mg, 67.5%) as colorless, amorphous solid. R_f 0.20 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.08–1.19 (m, 3H), 1.52–1.78 (m, 12H), 2.24–2.34 and 2.36–2.50 (2m, 2H), 3.20–3.52 (m, 12H), 3.72 and 3.73 and 3.74 (3s, 3H), 3.84–4.23 (m, 12H), 6.85–7.75 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 9.2, 9.3, 24.4, 24.4, 24.6, 25.3, 25.4, 25.5, 25.9, 25.9, 26.0, 26.1, 26.3, 40.9, 41.0, 41.3, 41.5, 46.6, 47.1, 47.2, 47.8, 48.0, 48.1, 49.1, 49.4, 49.6, 49.7, 49.9, 50.9, 50.9, 51.0, 52.1, 52.2, 168.1, 168.3, 168.3, 168.5, 168.6, 168.6, 168.6, 168.7, 168.7, 168.8, 168.9, 169.0, 169.0, 169.1, 169.2, 169.3, 170.1, 170.2, 170.3, 174.3, 174.4, 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{47}N_{15}O_8$ $[M+Na]^+$ 744.3624, found 744.3610.

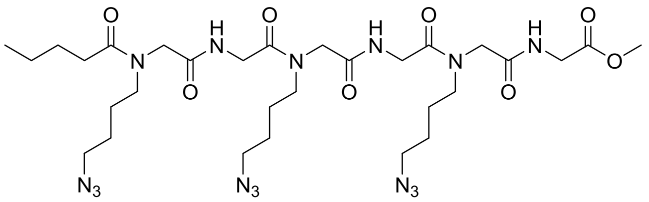
N-Butyryl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**124**) as a mixture of rotamers



Aldehyde **19** (139 mg, 4.63 mmol), **16** (317 mg, 2.78 mmol), **104** (1419 mg, 2.78 mmol), and **20** (252 µl, 275 mg, 2.78 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **124** (1135 mg, 55.5%) as colorless, amorphous solid. R_f 0.20 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.90–1.01

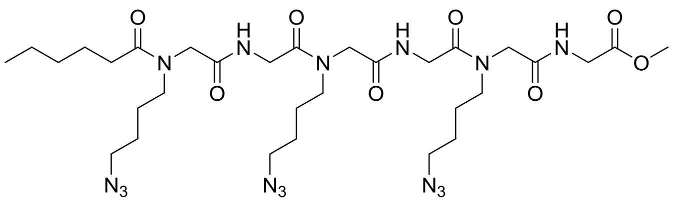
(m, 3H), 1.51–1.78 (m, 14H), 2.20–2.28 and 2.30–2.43 (2m, 2H), 3.22–3.52 (m, 12H), 3.69–3.76 (m, 3H), 3.84–4.22 (m, 12H), 6.82–7.70 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 18.4, 18.6, 24.4, 24.5, 24.6, 25.3, 25.5, 25.5, 25.9, 26.1, 34.6, 35.0, 40.8, 40.9, 41.1, 41.1, 41.2, 41.3, 41.4, 41.5, 46.4, 47.1, 47.2, 47.9, 48.0, 48.1, 49.0, 49.2, 49.5, 49.5, 49.6, 49.7, 49.8, 49.9, 50.0, 50.9, 52.2, 52.2, 52.3, 168.3, 168.3, 168.6, 168.7, 168.7, 168.8, 168.9, 168.9, 169.0, 169.1, 169.2, 169.2, 170.1, 170.2, 170.3, 173.7, 173.7 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{29}H_{49}N_{15}O_8$ $[M+Na]^+$ 758.3781, found 758.3765.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**125**) as a mixture of rotamers



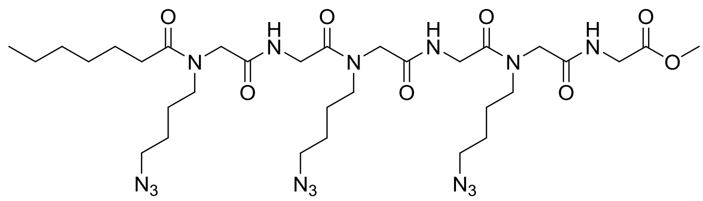
Aldehyde **19** (134 mg, 4.45 mmol), **16** (305 mg, 2.67 mmol), **105** (1402 mg, 2.67 mmol), and **20** (243 µl, 265 mg, 2.67 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **125** (1140 mg, 56.9%) as white, amorphous solid. R_f 0.20 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.87–0.98 (m, 3H), 1.24–1.44 (m, 2H), 1.51–1.78 (m, 14H), 2.22–2.30 and 2.32–2.43 (2m, 2H), 3.20–3.52 (m, 12H), 3.69–3.76 (m, 3H), 3.84–4.22 (m, 12H), 6.85–7.74 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 22.3, 22.4, 24.4, 24.4, 24.6, 25.3, 25.4, 25.5, 25.9, 26.0, 27.1, 27.2, 32.4, 32.8, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.4, 46.4, 47.1, 47.1, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.4, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 52.1, 52.2, 52.2, 168.3, 168.3, 168.5, 168.6, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.2, 169.2, 170.0, 170.2, 170.3, 170.4, 173.7, 173.8 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{51}N_{15}O_8$ $[M+Na]^+$ 772.3937, found 772.3916.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**126**) as a mixture of rotamers



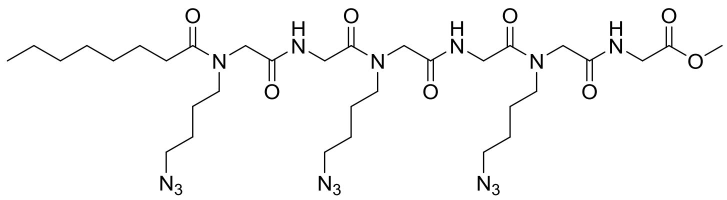
Aldehyde **19** (128 mg, 4.25 mmol), **16** (291 mg, 2.55 mmol), **106** (1372 mg, 2.55 mmol), and **20** (232 µl, 253 mg, 2.55 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **126** (1395 mg, 71.6%) as white, amorphous solid. R_f 0.21 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.95 (m, 3H), 1.23–1.39 (m, 4H), 1.52–1.77 (m, 14H), 2.21–2.29 and 2.32–2.42 (2m, 2H), 3.20–3.52 (m, 12H), 3.68–3.78 (m, 3H), 3.83–4.30 (m, 12H), 6.87–7.74 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.8, 22.4, 24.3, 24.4, 24.6 24.7, 24.8, 25.3, 25.4, 25.5, 25.9, 26.0, 31.4, 32.7, 33.0, 40.7, 40.8, 40.8, 41.0, 41.1, 41.3, 41.4, 46.4, 47.0, 47.1, 47.8, 47.9, 48.1, 48.9, 49.2, 49.3, 49.4, 49.4, 49.5, 49.6, 49.8, 49.8, 50.8, 50.9, 52.1, 52.1, 52.2, 168.1, 168.2, 168.3, 168.3, 168.4, 168.5, 168.5, 168.6, 168.8, 168.9, 169.0, 169.0, 169.1, 169.2, 170.0, 170.2, 170.3, 170.3, 173.7, 173.8 ppm. HRMS (ESI+) *m/z* calcd for $C_{31}H_{53}N_{15}O_8$ $[M+Na]^+$ 786.4094, found 786.4072.

<u>*N*-Heptanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**127**) as a mixture of rotamers</u>



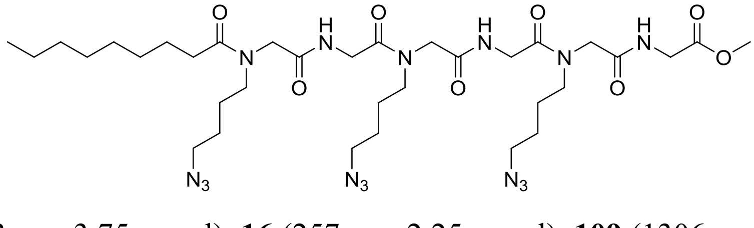
Aldehyde **19** (130 mg, 4.32 mmol), **16** (296 mg, 2.59 mmol), **107** (1432 mg, 2.59 mmol), and **20** (235 µl, 257 mg, 2.59 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **127** (1323 mg, 65.7%) as white, amorphous solid. R_f 0.22 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.94 (m, 3H), 1.22–1.39 (m, 6H), 1.52–1.77 (m, 14H), 2.21–2.29 and 2.32–2.43 (2m, 2H), 3.10–3.56 (m, 12H), 3.69–3.79 (m, 3H), 3.85–4.30 (m, 12H), 6.87–7.72 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.9, 22.4, 24.4, 24.5, 24.6, 25.0, 25.1, 25.3, 25.4, 25.5, 25.9, 26.0, 28.9, 29.0, 31.5, 32.8, 32.8, 33.1, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.5, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.5, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 50.9, 52.1, 52.2, 52.2, 168.1, 168.2, 168.3, 168.4, 168.5, 168.6, 168.7, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 170.0, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{32}H_{55}N_{15}O_8$ $[M+Na]^+$ 800.4250, found 800.4237.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**128**) as a mixture of rotamers



Aldehyde **19** (131 mg, 4.37 mmol), **16** (299 mg, 2.62 mmol), **108** (1486 mg, 2.62 mmol), and **20** (238 µl, 260 mg, 2.62 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **128** (1289 mg, 62.1%) as white, amorphous solid. R_f 0.23 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, C_6D_6/CD_3OD 9:1) δ = 0.88–0.96 (m, 3H), 1.13–1.54 (m, 20H), 1.65–1.77 (m, 2H), 2.22–2.34 (m, 2H), 2.82–2.98 and 3.02–3.19 (2m, 10H), 3.32–3.47 (m, 5H), 3.87–4.23 (m, 12H), 7.55–8.30 (m, 3H, weak) ppm. ^{13}C-NMR (100 MHz, C_6D_6/CD_3OD 9:1) δ = 14.5, 23.3, 24.9, 25.0, 25.0, 25.1, 25.2, 25.8, 25.8, 25.9, 26.2, 26.2, 26.3, 26.5, 29.8, 29.9, 30.0, 32.4, 33.2, 33.7, 41.3, 41.4, 41.5, 41.6, 41.7, 41.8, 47.2, 47.2, 47.8, 47.8, 48.3, 48.4, 49.3, 49.4, 49.7, 49.7, 49.8, 49.9, 50.2, 50.3, 51.2, 51.4, 51.4, 52.0, 52.1, 169.7, 169.8, 169.9, 169.9, 170.0, 170.0, 170.1, 170.2, 170.2, 170.2, 170.3, 170.3, 170.4, 170.5, 170.6, 170.7, 170.8, 174.8, 174.8, 175.2 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{57}N_{15}O_8$ $[M+Na]^+$ 814.4407, found 814.4392.

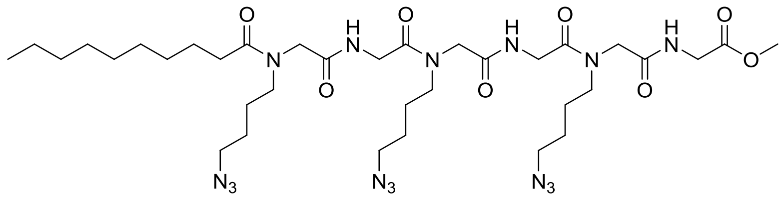
N-Nonanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**129**) as a mixture of rotamers



Aldehyde **19** (113 mg, 3.75 mmol), **16** (257 mg, 2.25 mmol), **109** (1306 mg, 2.25 mmol), and **20** (204 µl, 223 mg, 2.25 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **129** (1187 mg, 65.5%) as white, amorphous solid. R_f 0.24 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.20–

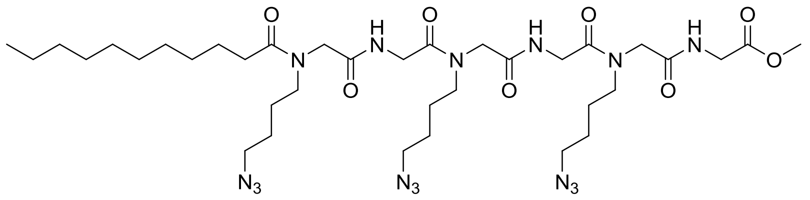
1.39 (m, 10H), 1.52–1.78 (m, 14H), 2.21–2.42 (m, 2H), 3.20–3.54 (m, 12H), 3.70–3.80 (m, 3H), 3.85–4.30 (m, 12H), 6.97–7.66 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.5, 25.6, 26.0, 26.1, 29.1, 29.4, 31.7, 32.8, 33.2, 40.8, 40.9, 40.9, 41.1, 41.1, 41.2, 41.4, 41.5, 46.4, 47.1, 47.2, 47.9, 48.0, 48.2, 49.0, 49.2, 49.5, 49.6, 49.6, 49.7, 49.8, 49.9, 49.9, 50.9, 52.2, 52.2, 52.3, 168.1, 168.3, 168.3, 168.5, 168.7, 168.9, 169.0, 169.0, 169.0, 169.1, 169.3, 170.1, 170.2, 170.3, 173.8, 173.8, 173.9, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{34}H_{59}N_{15}O_8$ $[M+Na]^+$ 828.4563, found 828.4549.

<u>*N*-Decanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**130**) as a mixture of rotamers</u>



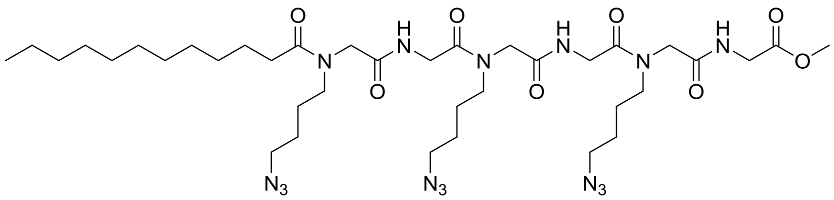
Aldehyde **19** (127 mg, 4.22 mmol), **16** (289 mg, 2.53 mmol), **110** (1506 mg, 2.53 mmol), and **20** (230 µl, 251 mg, 2.53 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **130** (1486 mg, 71.6%) as light yellow, amorphous solid. R_f 0.28 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.08–1.48 (m, 12H), 1.51–1.78 (m, 14H), 2.21–2.42 (m, 2H), 3.10–3.56 (m, 12H), 3.69–3.80 (m, 3H), 3.84–4.32 (m, 12H), 6.85–7.80 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.5, 24.6, 25.0, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 31.7, 32.8, 33.1, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.4, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.4, 49.6, 49.7, 49.8, 49.9, 50.0, 50.9, 50.9, 52.1, 52.2, 52.2, 168.3, 168.4, 168.5, 168.6, 168.7, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.2, 170.0, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{35}H_{61}N_{15}O_8$ $[M+Na]^+$ 842.4720, found 842.4703.

<u>*N*-Undecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**131**) as a mixture of rotamers</u>



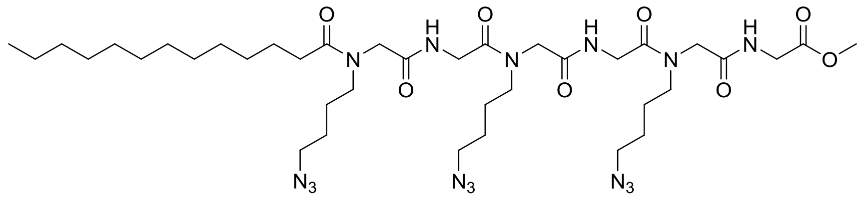
Aldehyde **19** (132 mg, 4.38 mmol), **16** (300 mg, 2.63 mmol), **111** (1602 mg, 2.63 mmol), and **20** (239 µl, 261 mg, 2.63 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **131** (1600 mg, 72.9%) as white, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.18–1.38 (m, 14H), 1.50–1.79 (m, 14H), 2.14–2.46 (m, 2H), 3.10–3.56 (m, 12H), 3.69–3.80 (m, 3H), 3.85–4.30 (m, 12H), 6.85–7.73 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.4, 24.5, 24.6, 25.0, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.4, 29.5, 31.8, 32.8, 33.2, 40.8, 40.8, 40.9, 41.0, 41.1, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.1, 49.2, 49.4, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 52.1, 52.2, 52.2, 168.3, 168.3, 168.4, 168.5, 168.6, 168.8, 168.9, 169.0, 169.1, 169.2, 169.2, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{36}H_{63}N_{15}O_8$ $[M+Na]^+$ 856.4876, found 856.4862.

N-Lauroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**132**) as a mixture of rotamers



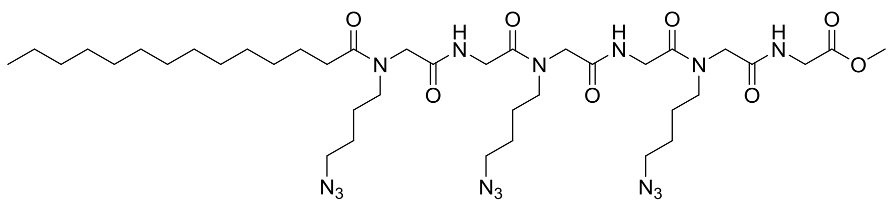
Aldehyde **19** (148 mg, 4.92 mmol), **16** (337 mg, 2.95 mmol), **112** (1835 mg, 2.95 mmol), and **20** (267 µl, 292 mg, 2.95 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **132** (1856 mg, 74.2%) as white, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.18–1.38 (m, 16H), 1.52–1.78 (m, 14H), 2.06–2.42 (m, 2H), 3.10–3.56 (m, 12H), 3.69–3.79 (m, 3H), 3.85–4.31 (m, 12H), 7.00–7.74 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.4, 24.6, 25.0, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.4, 29.5, 29.5, 31.8, 32.8, 33.1, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.5, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 50.9, 52.1, 52.2, 52.2, 168.3, 168.5, 168.6, 168.8, 168.9, 168.9, 169.0, 169.0, 169.2, 169.2, 170.0, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{37}H_{65}N_{15}O_8$ $[M+Na]^+$ 870.5033, found 870.5003.

N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**133**) as a mixture of rotamers



Aldehyde **19** (133 mg, 4.43 mmol), **16** (304 mg, 2.66 mmol), **113** (1694 mg, 2.66 mmol), and **20** (242 µl, 264 mg, 2.66 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **133** (1662 mg, 72.5%) as off-white, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.48 (m, 18H), 1.51–1.78 (m, 14H), 2.05–2.42 (m, 2H), 3.10–3.55 (m, 12H), 3.69–3.79 (m, 3H), 3.84–4.30 (m, 12H), 7.00–7.73 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.4, 24.6, 25.0, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.4, 29.5, 29.5, 31.8, 32.8, 33.1, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.5, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.1, 49.2, 49.4, 49.5, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 52.1, 52.2. 52.2, 168.3, 168.3, 168.4, 168.5, 168.6, 168.8, 168.9, 168.9, 169.0, 169.0, 169.1, 169.2, 170.0, 170.2, 170.3, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{38}H_{67}N_{15}O_8$ $[M+Na]^+$ 884.5189, found 884.5164.

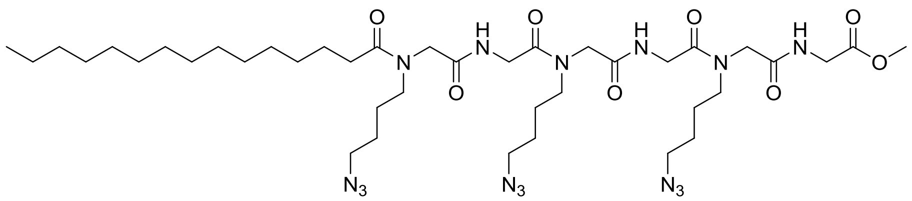
N-Myristoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**134**) as a mixture of rotamers



Aldehyde **19** (142 mg, 4.73 mmol), **16** (324 mg, 2.84 mmol), **114** (1850 mg, 2.84 mmol), and **20** (257 µl, 281 mg, 2.84 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol

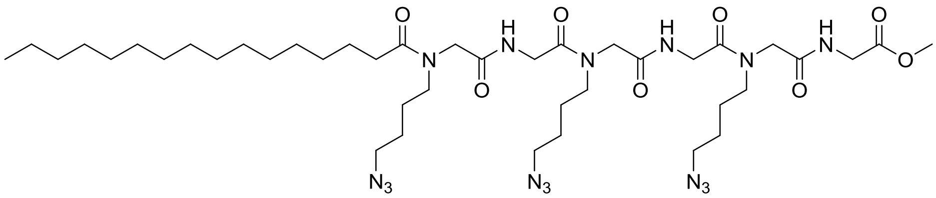
95:5) to afford **134** (1806 mg, 72.6%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.06–1.47 (m, 20H), 1.51–1.78 (m, 14H), 2.16–2.42 (m, 2H), 3.10–3.57 (m, 12H), 3.69–3.79 (m, 3H), 3.85–4.30 (m, 12H), 7.00–7.74 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.5, 29.5, 29.5, 29.6, 31.8, 32.8, 33.2, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.4, 46.3, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 52.1, 52.2, 52.3, 168.3, 168.3, 168.4, 168.5, 168.7, 168.9, 169.0, 169.0, 169.1, 169.2, 169.3, 170.1, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{39}H_{69}N_{15}O_8$ $[M+Na]^+$ 898.5346, found 898.5321.

<u>*N*-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**135**) as a mixture of rotamers</u>



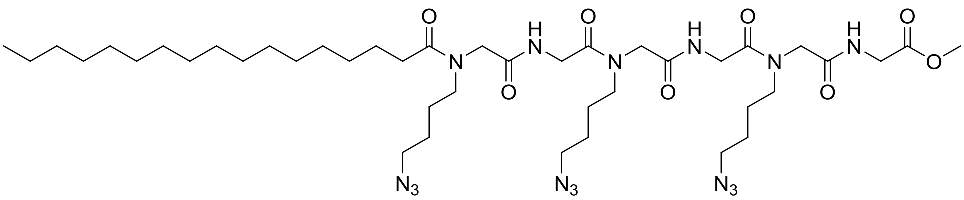
Aldehyde **19** (126 mg, 4.20 mmol), **16** (288 mg, 2.52 mmol), **115** (1672 mg, 2.52 mmol), and **20** (229 µl, 250 mg, 2.52 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **135** (1755 mg, 78.2%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.46 (m, 22H), 1.51–1.79 (m, 14H), 2.20–2.42 (m, 2H), 3.10–3.58 (m, 12H), 3.69–3.80 (m, 3H), 3.84–4.30 (m, 12H), 6.98–7.70 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 24.6, 25.1, 25.2, 25.3, 25.5, 25.5, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.8, 40.9, 40.9, 41.1 41.1, 41.3, 41.5, 47.1, 47.2, 47.9, 48.0, 48.2, 49.0, 49.1, 49.2, 49.5, 49.6, 49.8, 49.8, 49.9, 50.9, 52.1, 52.2, 52.3, 168.3, 168.5, 168.7, 168.8, 168.9, 168.9, 169.0, 169.0, 169.0, 169.2, 169.3, 170.1, 170.2, 170.3, 173.8, 173.9, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{40}H_{71}N_{15}O_8$ $[M+Na]^+$ 912.5502, found 912.5475.

<u>*N*-Palmitoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**136**) as a mixture of rotamers</u>



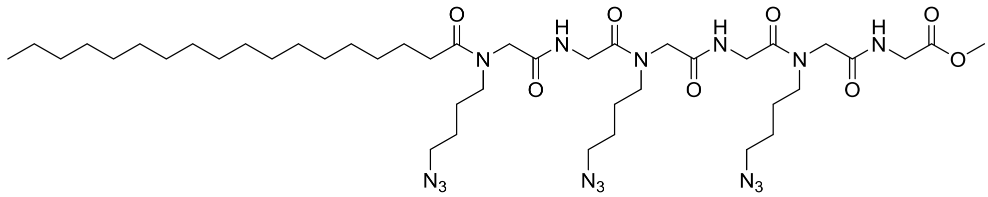
Aldehyde **19** (134 mg, 4.45 mmol), **16** (305 mg, 2.67 mmol), **116** (1815 mg, 2.67 mmol), and **20** (243 µl, 265 mg, 2.67 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 95:5) to afford **136** (1893 mg, 78.4%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.47 (m, 24H), 1.51–1.78 (m, 14H), 2.20–2.42 (m, 2H), 3.10–3.57 (m, 12H), 3.69–3.80 (m, 3H), 3.84–4.30 (m, 12H), 7.03–7.75 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.4, 24.6, 25.1, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.5, 29.5, 29.5, 29.6, 31.8, 32.8, 33.1, 40.9, 41.0, 41.1, 41.3, 41.5, 46.3, 46.4, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.4, 49.6, 49.7, 49.8, 50.0, 50.9, 50.9, 52.1, 52.2, 52.2, 168.3, 168.3, 168.4, 168.6, 168.7, 168.8, 168.9, 168.9, 169.0, 169.0, 169.2, 169.2, 170.1, 170.2, 170.3, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{41}H_{73}N_{15}O_8$ $[M+Na]^+$ 926.5659, found 926.5635.

<u>*N*-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**137**) as a mixture of rotamers</u>



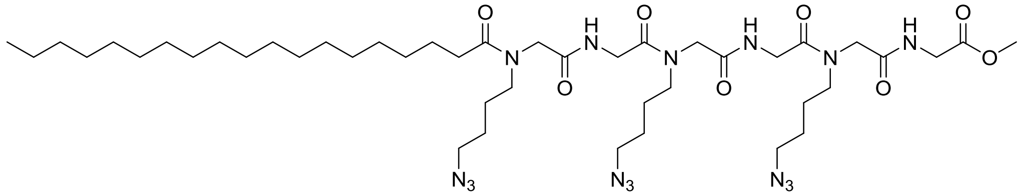
Aldehyde **19** (130 mg, 4.32 mmol), **16** (296 mg, 2.59 mmol), **117** (1796 mg, 2.59 mmol), and **20** (235 µl, 257 mg, 2.59 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **137** (485 mg, 20.4%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 95:5) to afford another crop of **137** (1351 mg, 56.8%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.06–1.47 (m, 26H), 1.50–1.79 (m, 14H), 2.16–2.42 (m, 2H), 3.10–3.58 (m, 12H), 3.69–3.79 (m, 3H), 3.85–4.30 (m, 12H), 7.00–7.72 (m, 3H) ppm. [13]C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.3, 25.4, 25.5, 25.9, 26.1, 29.2, 29.4, 29.4, 29.5, 29.5, 29.6, 31.8, 32.8, 40.8, 40.8, 40.9, 41.1, 41.1, 41.2, 41.4, 41.5, 46.4, 47.1, 47.2, 47.9, 48.0, 48.2, 49.0, 49.1, 49.2, 49.5, 49.6, 49.7, 49.9, 50.9, 52.1, 52.2, 168.3, 168.4, 168.6, 168.7, 168.8, 168.9, 168.9, 169.0, 169.2, 169.3, 170.1, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{42}H_{75}N_{15}O_8$ $[M+Na]^+$ 940.5815, found 940.5787.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**138**) as a mixture of rotamers



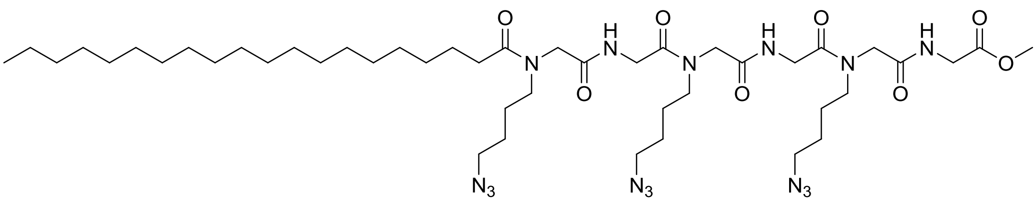
Aldehyde **19** (127 mg, 4.22 mmol), **16** (289 mg, 2.53 mmol), **118** (1786 mg, 2.53 mmol), and **20** (230 µl, 251 mg, 2.53 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **138** (311 mg, 13.2%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 95:5) to afford another crop of **138** (1469 mg, 62.3%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). [1]H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.46 (m, 28H), 1.52–1.78 (m, 14H), 2.20–2.42 (m, 2H), 3.10–3.56 (m, 12H), 3.69–3.80 (m, 3H), 3.84–4.30 (m, 12H), 7.02–7.73 (m, 3H). [13]C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.3, 25.4, 25.5, 25.9, 26.0, 29.2, 29.3, 29.4, 29.5, 29.5, 29.6, 31.8, 32.8, 33.2, 40.8, 40.8, 40.9, 41.0, 41.1, 41.3, 41.5, 46.3, 47.1, 47.2, 47.8, 48.0, 48.1, 49.0, 49.2, 49.4, 49.5, 49.5, 49.6, 49.7, 49.8, 49.9, 50.9, 50.9, 52.1, 52.2, 52.2, 168.3, 168.3, 168.4, 168.6, 168.7, 168.8, 168.9, 168.9, 169.0, 169.0, 169.2, 169.3, 170.1, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{43}H_{77}N_{15}O_8$ $[M+Na]^+$ 954.5972, found 954.5953.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**139**) as a mixture of rotamers



Aldehyde **19** (110 mg, 3.65 mmol), **16** (250 mg, 2.19 mmol), **119** (1576 mg, 2.19 mmol), and **20** (199 µl, 217 mg, 2.19 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **139** (198 mg, 9.6%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 95:5) to afford another crop of **139** (1342 mg, 64.8%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.92 (m, 3H), 1.06–1.47 (m, 30H), 1.51–1.78 (m, 14H), 2.20–2.42 (m, 2H), 3.10–3.58 (m, 12H), 3.69–3.80 (m, 3H), 3.85–4.31 (m, 12H), 6.99–7.70 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.3, 25.5, 25.5, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.8, 40.9, 40.9, 41.1, 41.1, 41.4, 41.5, 46.4, 47.1, 47.2, 47.8, 48.0, 48.2, 49.0, 49.2, 49.5, 49.6, 49.8, 49.8, 49.9, 50.9, 52.1, 52.2, 52.3, 168.3, 168.4, 168.6, 168.7, 168.8, 168.9, 169.0, 169.1, 169.1, 169.2, 169.3, 170.1, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{44}H_{79}N_{15}O_8$ $[M+Na]^+$ 968.6128, found 968.6097.

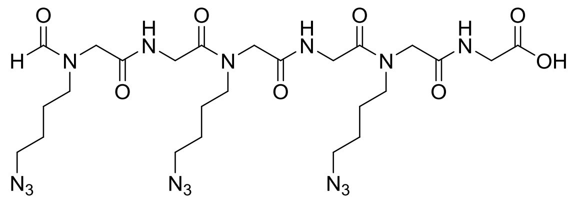
<u>*N*-Arachidoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**140**) as a mixture of rotamers</u>



Aldehyde **19** (104 mg, 3.45 mmol), **16** (236 mg, 2.07 mmol), **120** (1519 mg, 2.07 mmol), and **20** (188 µl, 205 mg, 2.07 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **140** (465 mg, 23.4%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 95:5) to afford another crop of **140** (996 mg, 50.1%) as colorless, amorphous solid. R_f 0.18 (dichloromethane/methanol 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.06–1.46 (m, 32H), 1.52–1.79 (m, 14H), 2.20–2.42 (m, 2H), 3.10–3.58 (m, 12H), 3.69–3.80 (m, 3H), 3.85–4.30 (m, 12H), 6.98–7.80 (m, 3H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.5, 24.6, 25.1, 25.2, 25.4, 25.5, 25.6, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.8, 40.9, 40.9, 41.1, 41.1, 41.3, 41.5, 47.1, 47.2, 47.9, 48.0, 48.2, 49.0, 49.1., 49.2, 49.5, 49.7, 49.8, 49.9, 50.1, 50.9, 52.2, 52.2, 52.3, 168.3, 168.4, 168.9, 168.9, 169.0, 169.0, 169.2, 169.3, 169.3, 170.1, 170.2, 170.4, 173.9, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{45}H_{81}N_{15}O_8$ $[M+Na]^+$ 982.6285, found 982.6263.

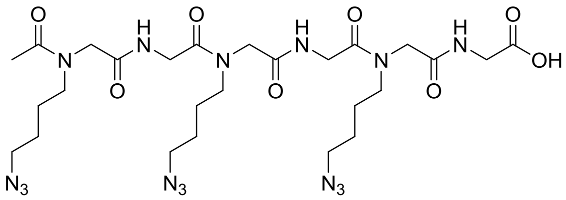
Saponifications

N-Formyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**141**) as a mixture of rotamers



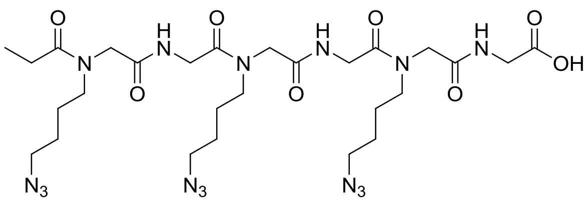
The saponification of **121** (367 mg, 0.53 mmol) with lithium hydroxide solution (0.66 ml, 1.33 mmol, 2 M) following general procedure B (method 1) afforded **141** (364 mg, quant.) as a light brown, amorphous solid. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.48–1.85 (m, 12H), 3.15–3.55 (m, 12H), 3.80–4.35 (m, 12H), 8.09 and 8.17 (2brs, 1H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.3, 25.6, 26.5, 26.6, 26.9, 27.1, 27.2, 48.4, 49.1, 49.3, 49.4, 50.0, 50.1, 50.8, 51.0, 52.1, 52.1, 52.2, 165.8, 166.4, 170.5, 170.6, 170.9, 171.1, 171.2, 171.3, 171.4, 173.2 ppm. HRMS (ESI+) *m/z* calcd for $C_{25}H_{41}N_{15}O_8$ $[M+Na]^+$ 702.3155, found 702.3139.

N-Acetyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**142**) as a mixture of rotamers



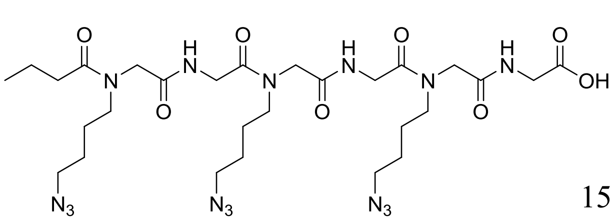
The saponification of **122** (1228 mg, 1.74 mmol) with lithium hydroxide solution (2.18 ml, 4.35 mmol, 2 M) following general procedure B (method 1) afforded **142** (1303 mg, quant.) as a light brown, amorphous solid. The crude product, containing a small, non-removable amount of acetic acid, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.48–1.80 (m, 12H), 2.08 and 2.17 (2brs, 3H), 3.16–3.56 (m, 12H), 3.85–4.32 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 21.2, 21.8, 25.6, 25.6, 26.5, 26.7, 27.1, 27.2, 41.7, 41.9, 41.9, 42.0, 42.1, 47.8, 48.3, 49.1, 49.8, 49.9, 50.0, 50.1, 50.1, 50.8, 50.8, 52.1, 52.2, 52.3, 170.9, 170.9, 171.0, 171.0, 171.0, 171.1, 171.1, 171.1, 171.3, 171.3, 171.4, 171.4, 171.6, 173.8, 174.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{26}H_{43}N_{15}O_8$ $[M+Na]^+$ 716.3311, found 716.3296.

N-Propionyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**143**) as a mixture of rotamers



The saponification of **123** (1074 mg, 1.49 mmol) with lithium hydroxide solution (1.86 ml, 3.73 mmol, 2 M) following general procedure B (method 1) afforded **143** (1132 mg, quant.) as a light brown, amorphous solid. The crude product, containing a small, non-removable amount of acetic acid, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.04–1.16 (m, 3H), 1.48–1.79 (m, 12H), 2.31–2.40 and 2.44–2.53 (2m, 2H), 3.10–3.56 (m, 12H), 3.82–4.26 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 9.7, 9.8, 25.6, 25.7, 26.5, 26.8, 26.9, 27.1, 27.1, 27.2, 27.3, 41.7, 41.9, 42.0, 42.1, 42.1, 47.9, 48.3, 49.0, 49.9, 50.0, 50.0, 50.1, 50.8, 51.5, 52.1, 52.2, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.4, 171.4, 171.4, 171.6, 171.7, 172.7, 172.8, 176.8, 177.2 ppm. HRMS (ESI+) *m/z* calcd for $C_{27}H_{45}N_{15}O_8$ $[M+Na]^+$ 730.3468, found 730.3455.

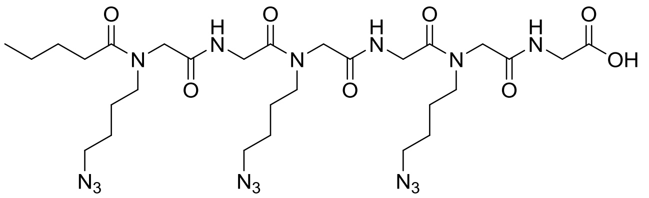
N-Butyryl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**144**) as a mixture of rotamers



The saponification of **124** (1080 mg, 1.47 mmol) with lithium hydroxide solution

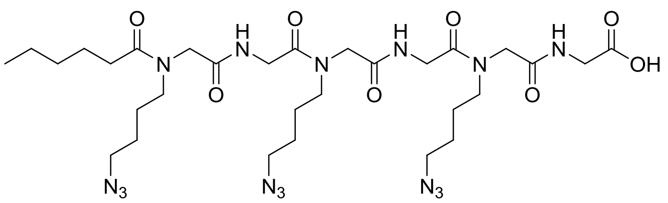
(1.84 ml, 3.68 mmol, 2 M) following general procedure B (method 1) afforded **144** (1122 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of acetic acid, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.89–1.02 (m, 3H), 1.48–1.79 (m, 14H), 2.26–2.36 and 2.39–2.49 (2m, 2H), 3.16–3.56 (m, 12H), 3.80–4.40 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.2, 14.2, 19.6, 19.7, 25.6, 25.6, 26.5, 26.9, 27.1, 27.2, 27.2, 35.5, 35.9, 41.9, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 49.1, 50.0, 50.0, 50.8, 51.6, 52.1, 52.1, 170.9, 170.9, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.9 (br), 176.0, 176.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{28}H_{47}N_{15}O_8$ $[M+Na]^+$ 744.3624, found 744.3616.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**145**) as a mixture of rotamers



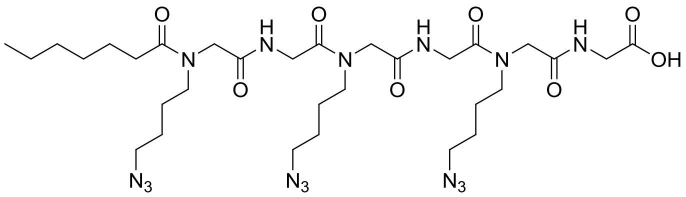
The saponification of **125** (1060 mg, 1.41 mmol) with lithium hydroxide solution (1.76 ml, 3.53 mmol, 2 M) following general procedure B (method 1) afforded **145** (1119 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of acetic acid, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.83–1.02 (m, 3H), 1.15–1.85 (m, 16H), 2.25–2.53 (m, 2H), 3.10–3.56 (m, 12H), 3.80–4.35 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 23.4, 23.5, 25.6, 25.6, 26.5, 26.9, 27.1, 27.2, 28.4, 28.5, 33.4, 33.8, 41.7, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.1, 171.0, 171.0, 171.0, 171.3, 171.3, 171.3, 171.4, 171.5, 171.6, 171.6, 172.7, 172.8, 176.1, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{29}H_{49}N_{15}O_8$ $[M+Na]^+$ 758.3781, found 758.3757.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**146**) as a mixture of rotamers



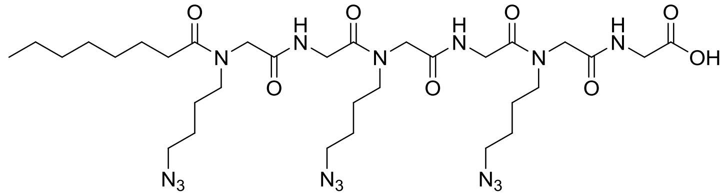
The saponification of **126** (1306 mg, 1.71 mmol) with lithium hydroxide solution (2.14 ml, 4.28 mmol, 2 M) following general procedure B (method 1) afforded **146** (1307 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.85–0.98 (m, 3H), 1.24–1.42 (m, 4H), 1.48–1.82 (m, 14H), 2.28–2.37 and 2.40–2.50 (2m, 2H), 3.10–3.56 (m, 12H), 3.80–4.40 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.5, 25.6, 25.6, 26.0, 26.1, 26.5, 26.9, 27.1, 27.2, 32.6, 32.6, 33.6, 34.0, 41.8, 41.9, 41.9, 42.0, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 49.1, 50.0, 50.0, 50.8, 51.6, 52.1, 52.1, 52.1, 170.8, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.8 (br), 176.1, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{30}H_{51}N_{15}O_8$ $[M+Na]^+$ 772.3937, found 772.3919.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**147**) as a mixture of rotamers



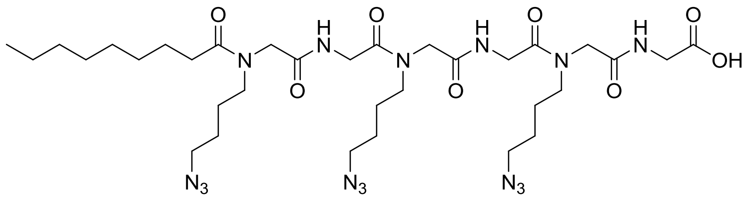
The saponification of **127** (1256 mg, 1.61 mmol) with lithium hydroxide solution (2.01 ml, 4.03 mmol, 2 M) following general procedure B (method 1) afforded **147** (1261 mg, quant.) as a light brown foam. The crude product, containing small, non-removable amounts of water and acetic acid, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.85–0.96 (m, 3H), 1.24–1.43 (m, 6H), 1.50–1.80 (m, 14H), 2.28–2.37 and 2.40–2.50 (2m, 2H), 3.10–3.65 (m, 12H), 3.80–4.37 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.6, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.0, 30.1, 32.8, 33.6, 34.1, 41.9, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.0, 50.8, 51.6, 52.1, 52.1, 52.1, 170.8, 170.9, 170.9, 171.0, 171.1, 171.2, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.8 (br), 176.1, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{31}H_{53}N_{15}O_8$ $[M+Na]^+$ 786.4094, found 786.4071.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**148**) as a mixture of rotamers



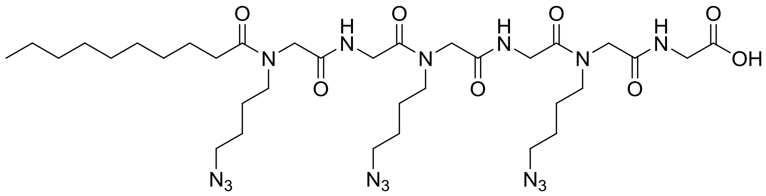
The saponification of **128** (1207 mg, 1.52 mmol) with lithium hydroxide solution (1.90 ml, 3.80 mmol, 2 M) following general procedure B (method 1) afforded **148** (1208 mg, quant.) as a light brown foam. The crude product, containing small, non-removable amounts of water and acetic acid, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.85–0.96 (m, 3H), 1.22–1.43 (m, 8H), 1.49–1.81 (m, 14H), 2.28–2.37 and 2.41–2.50 (2m, 2H), 3.16–3.56 (m, 12H), 3.80–4.35 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.2, 30.3, 30.4, 32.9, 33.6, 34.1, 41.8, 41.9, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.1, 170.9, 170.9, 170.9, 171.0, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.8, 172.9, 176.1, 176.5 ppm. HRMS (ESI+) *m/z* calcd for $C_{32}H_{55}N_{15}O_8$ $[M+Na]^+$ 800.4250, found 800.4233.

<u>*N*-Nonanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**149**) as a mixture of rotamers</u>



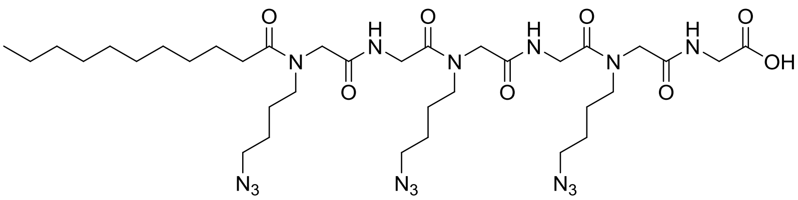
The saponification of **129** (1134 mg, 1.41 mmol) with lithium hydroxide solution (1.76 ml, 3.53 mmol, 2 M) following general procedure B (method 1) afforded **149** (1124 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.85–0.96 (m, 3H), 1.21–1.47 (m, 10H), 1.49–1.82 (m, 14H), 2.28–2.37 and 2.40–2.50 (2m, 2H), 3.14–3.56 (m, 12H), 3.80–4.37 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.3, 30.4, 30.4, 30.5, 33.0, 33.6, 34.1, 41.9, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.0, 50.8, 51.6, 52.1, 52.1, 52.1, 170.9, 170.9, 170.9, 171.0, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.8 (br), 176.1, 176.5 ppm. HRMS (ESI+) *m/z* calcd for $C_{33}H_{57}N_{15}O_8$ $[M+Na]^+$ 814.4407, found 814.4376.

N-Decanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**150**) as a mixture of rotamers



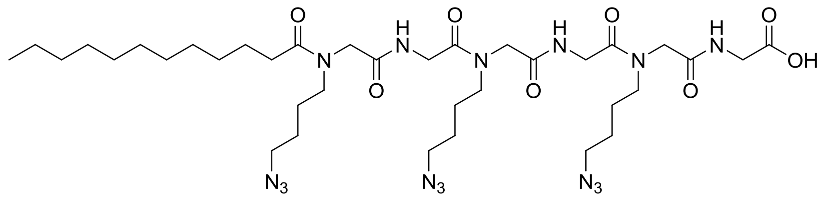
The saponification of **130** (1418 mg, 1.73 mmol) with lithium hydroxide solution (2.16 ml, 4.33 mmol, 2 M) following general procedure B (method 1) afforded **150** (1407 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.23–1.42 (m, 12H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.16–3.56 (m, 12H), 3.80–4.36 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.6, 30.6, 33.0, 33.6, 34.1, 41.8, 41.9, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.9, 170.9, 170.9, 171.0, 171.1, 171.2, 171.3, 171.3, 171.3, 171.4, 171.5, 171.6, 172.8, 172.8, 176.1, 176.5, 176.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{59}N_{15}O_8$ $[M+Na]^+$ 828.4563, found 828.4536.

N-Undecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**151**) as a mixture of rotamers



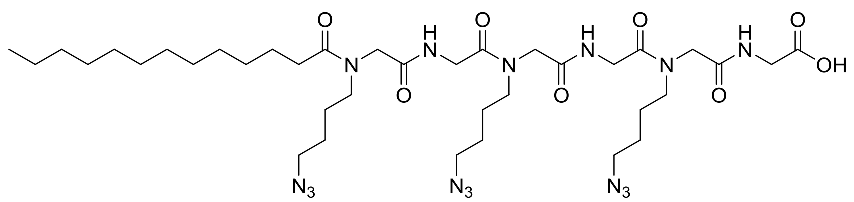
The saponification of **131** (1530 mg, 1.83 mmol) with lithium hydroxide solution (2.29 ml, 4.58 mmol, 2 M) following general procedure B (method 1) afforded **151** (1503 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.22–1.41 (m, 14H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.20–3.54 (m, 12H), 3.86–4.32 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 33.0, 33.6, 34.1, 34.1, 41.7, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.1, 170.8, 170.8, 170.9, 170.9, 171.0, 171.0, 171.2, 171.2, 171.3, 171.4, 171.5, 171.6, 172.6, 172.7, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{35}H_{61}N_{15}O_8$ $[M-H]^-$ 818.4755, found 818.4735.

N-Lauroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**152**) as a mixture of rotamers



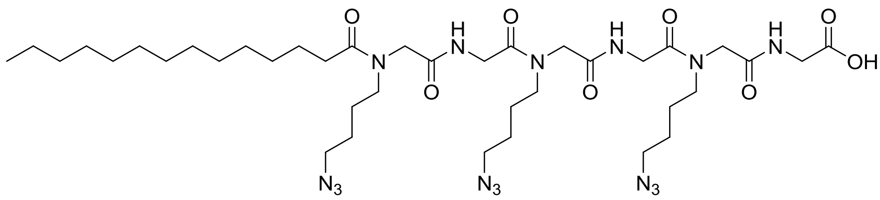
The saponification of **132** (1770 mg, 2.09 mmol) with lithium hydroxide solution (2.62 ml, 5.23 mmol, 2 M) following general procedure B (method 1) afforded **152** (1748 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.22–1.41 (m, 16H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.41–2.49 (2m, 2H), 3.20–3.56 (m, 12H), 3.85–4.35 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.6, 30.6, 30.6, 30.7, 33.0, 33.6, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.8, 170.9, 170.9, 171.0, 171.0, 171.2, 171.2, 171.2, 171.3, 171.4, 171.5, 171.6, 172.8, 172.8, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{36}H_{63}N_{15}O_8$ $[M-H]^-$ 832.4911, found 832.4905.

N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**153**) as a mixture of rotamers



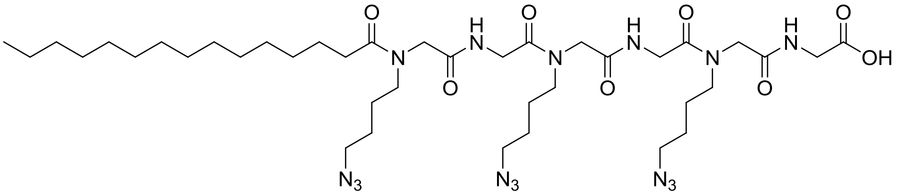
The saponification of **133** (1580 mg, 1.83 mmol) with lithium hydroxide solution (2.29 ml, 4.58 mmol, 2 M) following general procedure B (method 1) afforded **153** (1578 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. [1]H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.22–1.40 (m, 18H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.20–3.56 (m, 12H), 3.81–4.36 (m, 12H) ppm. [13]C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 33.0, 33.6, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.8, 170.9, 170.9, 171.0, 171.0, 171.2, 171.2, 171.3, 171.4, 171.5, 171.6, 172.7, 172.8, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m/z* calcd for $C_{37}H_{65}N_{15}O_8$ $[M-H]^-$ 846.5068, found 846.5071.

<u>*N*-Myristoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**154**) as a mixture of rotamers</u>



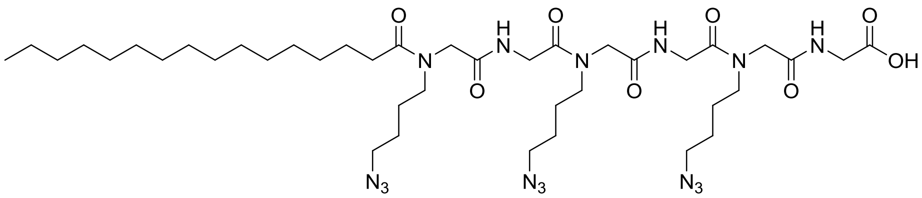
The saponification of **134** (1730 mg, 1.97 mmol) with lithium hydroxide solution (2.46 ml, 4.93 mmol, 2 M) following general procedure B (method 1) afforded **154** (1700 mg, quant.) as a light brown foam. The crude product, containing a small, non-removable amount of water, was used directly in the next step without further purification. [1]H-NMR (400 MHz, CD_3OD) δ = 0.86–0.92 (m, 3H), 1.21–1.40 (m, 20H), 1.51–1.78 (m, 14H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.20–3.56 (m, 12H), 3.81–4.35 (m, 12H) ppm. [13]C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 33.0, 33.6, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.0, 50.8, 51.6, 52.1, 52.1, 52.1, 170.8, 170.8, 170.9, 170.9, 170.9, 171.0, 171.2, 171.2, 171.2, 171.3, 171.3, 171.4, 171.6, 172.7, 172.7, 176.0, 176.5, 176.5 ppm. HRMS (ESI-) *m/z* calcd for $C_{38}H_{67}N_{15}O_8$ $[M-H]^-$ 860.5224, found 860.5236.

<u>*N*-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**155**) as a mixture of rotamers</u>



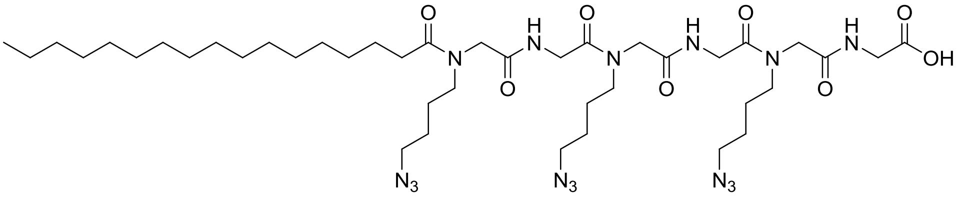
The saponification of **135** (1690 mg, 1.90 mmol) with lithium hydroxide solution (2.38 ml, 4.75 mmol, 2 M) following general procedure B (method 1) afforded **155** (1658 mg, 99.6%) as a light brown foam. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.20–1.41 (m, 22H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.20–3.56 (m, 12H), 3,81–4.35 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.6, 30.6, 30.6, 30.7, 30.7, 30.8, 33.0, 33.6, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.1, 47.8, 48.3, 49.0, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.8, 170.9, 170.9, 171.0, 171.0, 171.2, 171.2, 171.2, 171.3, 171.4, 171.4, 171.6, 172.7, 172.8, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{39}H_{69}N_{15}O_8$ $[M-H]^-$ 874.5381, found 874.5379.

<u>*N*-Palmitoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**156**) as a mixture of rotamers</u>



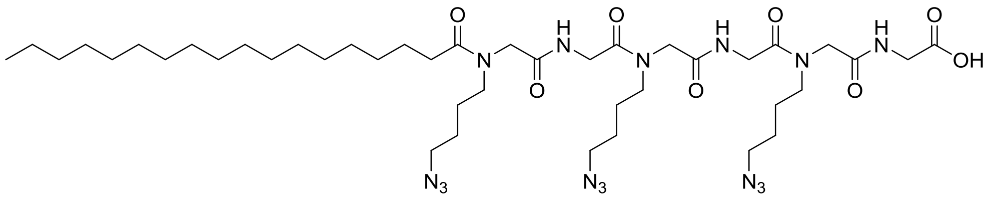
The saponification of **136** (1700 mg, 1.88 mmol) with lithium hydroxide solution (2.35 ml, 4.70 mmol, 2 M) following general procedure B (method 1) afforded **136** (1666 mg, 99.6%) as a light brown foam. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.92 (m, 3H), 1.21–1.41 (m, 24H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.40–2.48 (2m, 2H), 3.20–3.56 (m, 12H), 3.81–4.34 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.5, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 32.8, 33.0, 33.6, 34.1, 34.1, 41.7, 41.9, 42.0, 42.1, 42.1, 47.7, 48.3, 49.0, 50.0, 50.0, 50.8, 51.6, 52.1, 52.1, 170.7, 170.8, 170.8, 170.9, 170.9, 171.1, 171.2, 171.2, 171.3, 171.4, 171.5, 172.6, 172.7, 176.0, 176.4, 176.4 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{40}H_{71}N_{15}O_8$ $[M-H]^-$ 888.5537, found 888.5542.

N-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**157**) as a mixture of rotamers



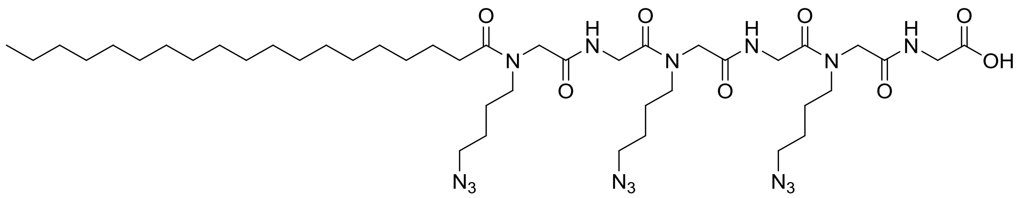
The saponification of **137** (1760 mg, 1.92 mmol) with lithium hydroxide solution (2.40 ml, 4.80 mmol, 2 M) following general procedure B (method 1) afforded **157** (1710 mg, 98.5%) as a light brown foam. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.10–1.48 (m, 26H), 1.51–1.79 (m, 14H), 2.28–2.36 and 2.40–2.48 (2m, 2H), 3.16–3.56 (m, 12H), 3.81–4.36 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.5, 25.6, 26.3, 26.3, 26.4, 26.9, 27.0, 27.1, 27.1, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 33.0, 33.6, 34.0, 34.1, 41.7, 41.9, 42.0, 42.0, 42.1, 47.7, 48.2, 49.0, 50.0, 50.7, 51.5, 52.1, 52.1, 170.7, 170.7, 170.9, 171.0, 171.2, 171.3, 171.3, 171.5, 172.6, 172.6, 175.9, 176.3, 176.4 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{41}H_{73}N_{15}O_8$ $[M-H]^-$ 902.5694, found 902.5702.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**158**) as a mixture of rotamers



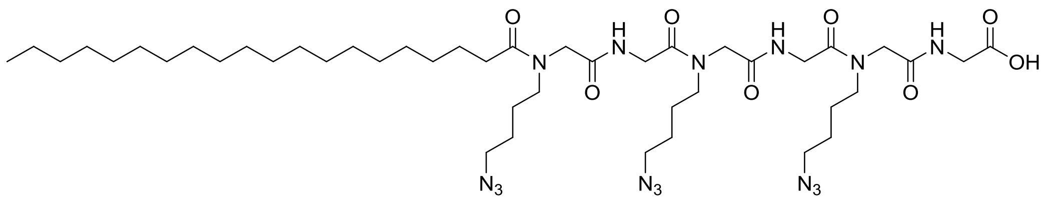
The saponification of **138** (1680 mg, 1.80 mmol) with lithium hydroxide solution (2.25 ml, 4.50 mmol, 2 M) following general procedure B (method 1) afforded **158** (1620 mg, 98.0%) as a light brown foam. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.10–1.47 (m, 28H), 1.51–1.79 (m, 14H), 2.29–2.36 and 2.40–2.48 (2m, 2H), 3.15–3.56 (m, 12H), 3.80–4.40 8m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.5, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.4, 30.4, 30.6, 30.6, 30.7, 30.7, 30.8, 33.0, 33.6, 34.1, 34.1, 41.7, 41.9, 42.0, 42.1, 42.1, 47.7, 48.3, 49.0, 50.0, 50.8, 51.6, 52.1, 52.1, 170.7, 170.8, 170.9, 171.1, 171.2, 171.3, 171.4, 171.5, 172.6, 172.7, 175.9, 176.4, 176.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{42}H_{75}N_{15}O_8$ $[M-H]^-$ 916.5850, found 916.5860.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**159**) as a mixture of rotamers



The saponification of **139** (1440 mg, 1.52 mmol) with lithium hydroxide solution (1.90 ml, 3.80 mmol, 2 M) following general procedure B (method 1) afforded **159** (1381 mg, 97.5%) as a light brown foam. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.08–1.47 (m, 30H), 1.51–1.80 (m, 14H), 2.28–2.36 and 2.39–2.49 (2m, 2H), 3.16–3.56 (m, 12H), 3.80–4.40 (m, 12H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.6, 23.7, 25.5, 25.6, 26.3, 26.4, 26.4, 26.9, 27.0, 27.1, 30.4, 30.6, 30.6, 30.7, 30.7, 30.8, 33.0, 33.6, 34.1, 41.8, 41.9, 42.0, 47.7, 48.2, 49.0, 50.0, 50.7, 51.5, 52.0, 52.1, 170.8, 170.8, 171.0, 171.2, 171.2, 171.3, 171.5, 172.6, 172.7, 175.8, 176.3, 176.3 ppm. HRMS (ESI-) *m/z* calcd for $C_{43}H_{77}N_{15}O_8$ $[M-H]^-$ 930.6007, found 930.6011.

N-Arachidoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**160**) as a mixture of rotamers

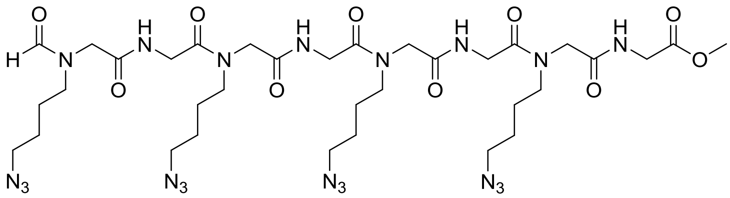


The saponification of **140** (1380 mg, 1.44 mmol) with lithium hydroxide solution (1.80 ml, 3.60 mmol, 2 M) following general procedure B (method 1) afforded **160** (1353 mg, 99.3%) as a light brown foam. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.09–1.48 (m, 32H), 1.50–1.81 (m, 14H), 2.28–2.37 and 2.40–2.50 (2m, 2H), 3.16–3.56 (m, 12H), 3.80–4.37 (m, 12H), ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.5, 25.6, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 30.4, 30.4, 30.6, 30.6, 30.7, 30.7, 30.8, 33.0, 33.7, 34.1, 41.8, 41.9, 41.9, 42.0, 42.1, 47.7, 48.3, 49.0, 50.0, 50.8, 51.6, 52.1, 52.1, 170.8, 170.9, 171.0, 171.2, 171.4, 171.4, 171.5, 171.6, 172.6, 172.7, 176.0, 176.4 ppm. HRMS (ESI-) *m/z* calcd for $C_{44}H_{79}N_{15}O_8$ $[M-H]^-$ 944.6163, found 944.6177.

5.4.2.4 Syntheses of the fourth generation azido-LPP acids

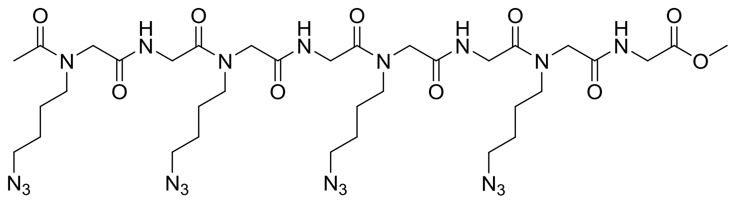
Ugi four-component reaction

N-Formyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**161**) as a mixture of rotamers



Paraformaldehyde (**19**, 23 mg, 0.77 mmol), **16** (53 mg, 0.46 mmol), **141** (310 mg, 0.46 mmol), and **20** (42 µl, 46 mg, 0.46 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 93:7) to afford **161** (171 mg, 41.1%) as colorless, amorphous solid. R_f 0.54 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.50–1.78 (m, 16H), 3.25–3.50 (m, 16H), 3.72 and 3.73 (2s, 3H), 3.80–4.30 (m, 16H), 6.85–7.80 (m, 4H), 8.13 (brs, 1H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.1, 24.3, 24.4, 25.4, 25.4, 25.5, 25.5, 26.0, 40.8, 41.0, 41.3, 41.4, 42.8, 45.7, 46.0, 46.0, 46.1, 47.1, 47.2, 47.9, 48.1, 48.3, 48.4, 48.4, 49.4, 49.6, 49.8, 50.1, 50.8, 50.8, 50.9, 52.1, 52.2, 163.4, (br), 164.1 (br), 167.9 (br), 168.1, 168.5, 168.9 (br), 169.2 (br), 170.2, 170.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{56}N_{20}O_{10}$ $[M+Na]^+$ 927.4380, found 927.4353.

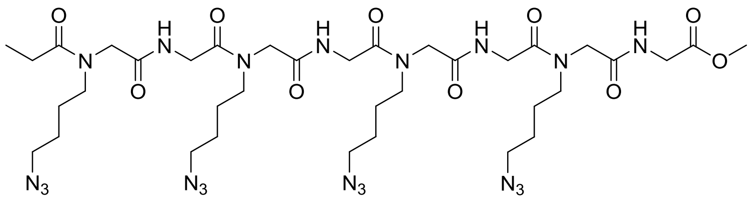
N-Acetyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**162**) as a mixture of rotamers



Paraformaldehyde (**19**, 87 mg, 2.90 mmol), **16** (199 mg, 1.74 mmol), **142** (1205 mg, 1.74 mmol), and **20** (158 µl, 172 mg, 1.74 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 93:7) to afford **162** (838 mg, 52.4%) as yellow, amorphous solid.

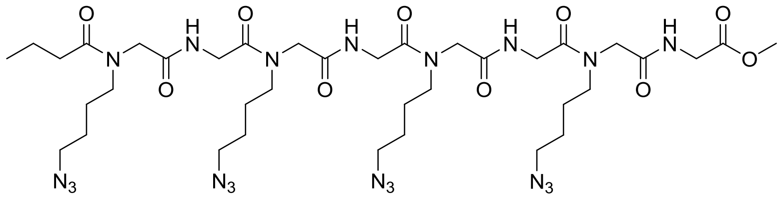
R_f 0.50 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.52–1.78 (m, 16H), 2.08 and 2.14 and 2.15 and 2.17 (4s, 3H), 3.19–3.56 (m, 16H), 3.72 and 3.73 and 3.74 (3s, 3H), 3.82–4.30 (m, 16H), 6.80–7.75 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 21.1, 21.1, 21.7, 24.4, 24.5, 24.6, 25.4, 25.5, 25.5, 25.6, 25.8, 26.1, 26.1, 40.9, 41.1, 41.3, 41.4, 41.4, 46.4, 47.1, 47.2, 47.9, 48.2, 48.3, 49.6, 49.7, 49.8, 49.9, 49.9, 50.0, 50.0, 50.9, 50.9, 51.0, 51.8, 52.1, 52.2, 52.3, 168.1, 168.3, 168.4, 168.4, 168.5, 168.6, 168.7, 168.8, 168.9, 168.9, 169.0, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 170.1, 170.2, 170.3, 170.4, 170.4, 171.3, 171.3, 171.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{35}H_{58}N_{20}O_{10}$ $[M+Na]^+$ 941.4537, found 941.4508.

N-Propionyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**163**) as a mixture of rotamers



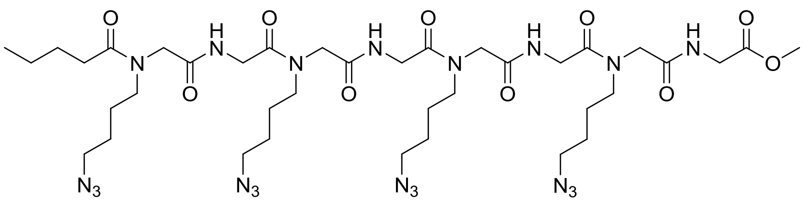
Paraformaldehyde (**19**, 73 mg, 2.42 mmol), **16** (166 mg, 1.45 mmol), **143** (1030 mg, 1.45 mmol), and **20** (132 μl, 144 mg, 1.45 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 93:7) to afford **163** (730 mg, 54.0%) as yellow, amorphous solid. R_f 0.50 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.08–1.19 (m, 3H), 1.52–1.79 (m, 16H), 2.23–2.34 and 2.37–2.48 (2m, 2H), 3.20–3.56 (m, 16H), 3.71 and 3.72 and 3.74 (3s, 3H), 3.82–4.30 (m, 16H), 6.97–7.70 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 9.2, 9.4, 24.4, 24.5, 24.7, 25.5, 25.5, 25.6, 25.9, 26.0, 26.1, 26.1, 26.4, 40.8, 40.9, 41.0, 41.1, 41.2, 41.4, 46.6, 47.1, 47.3, 48.1, 48.2, 48.9, 49.2, 49.6, 49.6, 49.8, 49.9, 50.0, 50.1, 51.0, 52.1, 52.2, 52.3, 168.3, 168.3, 168.4, 168.7, 168.8, 168.8, 168.9, 168.9, 169.1, 169.2, 169.2, 169.3, 169.4, 170.1, 170.1, 170.3, 170.4, 170.4, 174.4, 174.5 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{36}H_{60}N_{20}O_{10}$ $[M+Na]^+$ 955.4693, found 955.4671.

N-Butyryl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**164**) as a mixture of rotamers



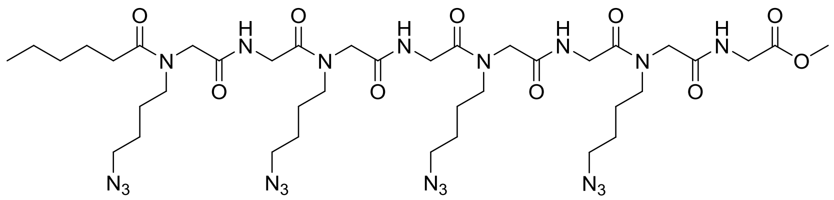
Paraformaldehyde (**19**, 68 mg, 2.28 mmol), **16** (156 mg, 1.37 mmol), **144** (992 mg, 1.37 mmol), and **20** (125 µl, 136 mg, 1.37 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 93:7) to afford **164** (593 mg, 45.7%) as yellow, amorphous solid. R_f 0.50 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.90–1.00 (m, 3H), 1.51–1.78 (m, 18H), 2.21–2.28 and 2.31–2.41 (2m, 2H), 3.10–3.60 (m, 16H), 3.68–3.78 (m, 3H), 3.80–4.35 (m, 16H), 7.00–7.85 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.5, 13.6, 18.1, 18.3, 24.1, 24.2, 24.3, 25.2 (br), 25.7, 25.8, 34.3, 34.7, 40.6, 40.8, 40.9, 41.1, 46.1, 46.8, 46.8, 47.6, 47.7, 47.8, 47.9, 48.7, 48.7, 48.9, 49.0, 49.2, 49.4, 49.5, 49.7, 50.6, 50.7, 51.8, 51.8, 51.9, 167.9, 168.0, 168.1, 168.2, 168.2, 168.3, 168.4, 168.6, 168.7, 168.8, 168.8, 168.9, 168.9, 169.0, 169.0, 169.1, 169.9, 169.9, 170.0, 170.1, 170.1, 173.3, 173.4 ppm. HRMS (ESI+) *m/z* calcd for $C_{37}H_{62}N_{20}O_{10}$ $[M+Na]^+$ 969.4850, found 969.4809.

<u>*N*-Valeroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**165**) as a mixture of rotamers</u>



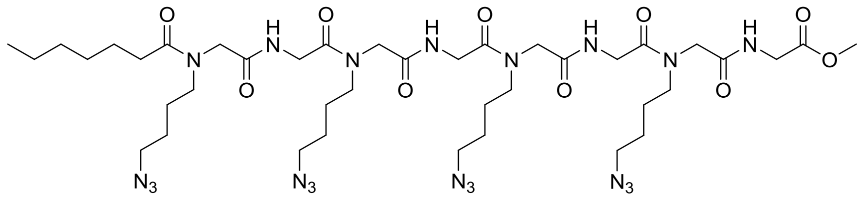
Paraformaldehyde (**19**, 68 mg, 2.28 mmol), **16** (156 mg, 1.37 mmol), **145** (1008 mg, 1.37 mmol), and **20** (125 µl, 136 mg, 1.37 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **165** (532 mg, 40.4%) as light brown, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **165** (153 mg, 11.6%) as colorless, amorphous solid. R_f 0.50 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.87–0.96 (m, 3H), 1.23–1.43 (m, 2H), 1.51–1.78 (m, 18H), 2.22–2.29 and 2.33–2.42 (2m, 2H), 3.10–3.56 (m, 16H), 3.70–3.76 (m, 3H), 3.85–4.30 (m, 16H), 6.95–7.60 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.9, 22.4, 24.4, 24.5, 24.7, 25.4, 25.5, 25.6, 26.0, 26.1, 27.2, 27.3, 32.5, 32.6, 32.9, 40.9, 40.9, 41.0, 41.1, 41.3, 41.4, 46.5, 47.1, 47.2, 47.3, 48.1, 48.2, 48.3, 48.4, 49.1, 49.1, 49.3, 49.7, 49.8, 50.0, 50.2, 51.0, 52.2, 52.3, 52.3, 52.4, 52.4, 168.4, 168.8, 168.9, 169.0, 169.0, 169.1, 169.2, 169.2, 169.3, 169.4, 170.1, 170.3, 170.4, 170.5, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{38}H_{64}N_{20}O_{10}$ $[M+Na]^+$ 983.5006, found 983.4992.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**166**) as a mixture of rotamers



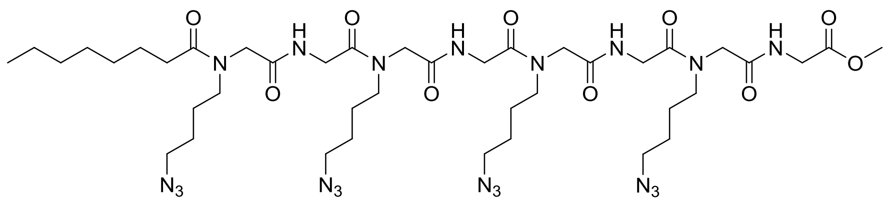
Paraformaldehyde (**19**, 80 mg, 2.65 mmol), **16** (181 mg, 1.59 mmol), **146** (1189 mg, 1.59 mmol), and **20** (145 µl, 158 mg, 1.59 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **166** (361 mg, 23.3%) as light brown, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **166** (604 mg, 39.0%) as light yellow, amorphous solid. R_f 0.51 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.95 (m, 3H), 1.24–1.39 (m, 4H), 1.50–1.80 (m, 18H), 2.21–2.42 (m, 2H), 3.10–3.61 (m, 16H), 3.69–3.76 (m, 3H), 3.85–4.30 (m, 16H), 6.96–7.67 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 13.9, 22.4, 24.4, 24.5, 24.6, 24.7, 24.9, 25.5, 25.5, 25.6, 26.0, 26.1, 31.4, 31.5, 32.8, 33.1, 40.8, 40.9, 41.0, 41.1, 41.2, 41.3, 41.4, 41.5, 46.5, 47.1, 47.2, 47.9, 48.1, 48.2, 48.3, 49.0, 49.1, 49.2, 49.3, 49.6, 49.8, 49.9, 50.1, 51.0, 52.1, 52.3, 168.3, 168.4, 168.6, 168.7, 168.8, 168.8, 168.9, 168.9, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{39}H_{66}N_{20}O_{10}$ $[M+Na]^+$ 997.5163, found 997.5127.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**167**) as a mixture of rotamers



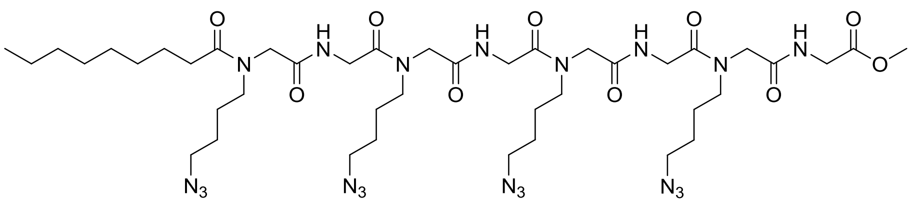
Paraformaldehyde (**19**, 74 mg, 2.45 mmol), **16** (168 mg, 1.47 mmol), **147** (1124 mg, 1.47 mmol), and **20** (134 µl, 146 mg, 1.47 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **167** (502 mg, 34.5%) as off-white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **167** (331 mg, 22.8%) as light yellow, amorphous solid. R_f 0.51 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.22–1.40 (m, 6H), 1.51–1.80 (m, 18H), 2.22–2.29 and 2.32–2.43 (2m, 2H), 3.10–3.60 (m, 16H), 3.69–3.76 (m, 3H), 3.87–4.22 (m, 16H), 6.96–7.70 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.4, 24.4, 24.5, 24.6, 25.0, 25.2, 25.4, 25.5, 25.6, 26.0, 26.1, 28.9, 29.0, 31.5, 32.8, 33.2, 40.8, 40.9, 40.9, 41.1, 41.2, 41.3, 41.5, 46.4, 47.1, 47.2, 47.9, 48.0, 48.2, 48.3, 49.0, 49.1, 49.2, 49.3, 49.5, 49.6, 49.7, 49.9, 50.0, 50.9, 50.9, 52.1, 52.1, 52.2, 168.3, 168.4, 168.5, 168.6, 168.6, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.1, 170.2, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{40}H_{68}N_{20}O_{10}$ $[M+Na]^+$ 1011.5319, found 1011.5288.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**168**) as a mixture of rotamers



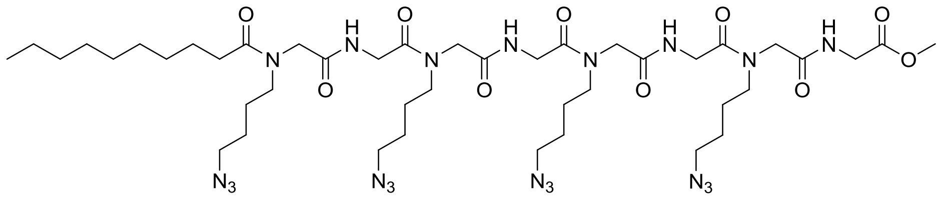
Paraformaldehyde (**19**, 70 mg, 2.33 mmol), **16** (160 mg, 1.40 mmol), **148** (1090 mg, 1.40 mmol), and **20** (127 µl, 139 mg, 1.40 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **168** (279 mg, 19.9%) as off-white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **168** (524 mg, 37.3%) as light yellow, amorphous solid. R_f 0.53 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84–0.93 (m, 3H), 1.21–1.39 (m, 8H), 1.51–1.80 (m, 18H), 2.20–2.44 (m, 2H), 3.10–3.60 (m, 16H), 3.69–3.77 (m, 3H), 3.87–4.32 (m, 16H), 6.95–7.67 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.5, 24.7, 25.1, 25.2, 25.5, 25.5, 25.6, 26.0, 26.1, 29.1, 29.3, 31.7, 32.8, 33.2, 40.9, 41.0, 41.1, 41.2, 41.4, 41.5, 46.4, 47.1, 47.3, 47.3, 47.9, 48.1, 48.2, 48.3, 49.0, 49.1, 49.2, 49.3, 49.6, 49.8, 49.9, 49.9, 50.1, 51.0, 52.2, 52.3, 168.3, 168.4, 168.7, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{41}H_{70}N_{20}O_{10}$ $[M+Na]^+$ 1025.5476, found 1025.5451.

N-Nonanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**169**) as a mixture of rotamers



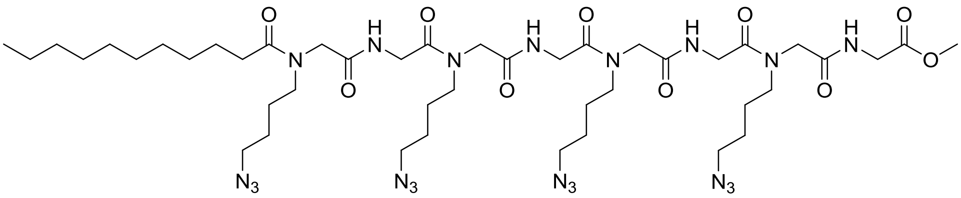
Paraformaldehyde (**19**, 62 mg, 2.08 mmol), **16** (143 mg, 1.25 mmol), **149** (989 mg, 1.25 mmol), and **20** (114 µl, 124 mg, 1.25 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **169** (363 mg, 28.6%) as off-white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **169** (481 mg, 37.8%) as light yellow, amorphous solid. R_f 0.55 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.21–1.38 (m, 10H), 1.52–1.78 (m, 18H), 2.22–2.29 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.69–3.79 (m, 3H), 3.82–4.32 (m, 16H), 7.00–7.71 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.4, 25.5, 25.5, 25.9, 26.1, 29.1, 29.3, 31.7, 32.8, 33.1, 40.8, 40.8, 40.9, 41.1, 41.2, 41.3, 41.3, 41.5, 46.4, 47.1, 47.2, 47.9, 48.0, 48.1, 48.3, 49.0, 49.1, 49.1, 49.2, 49.6, 49.7, 50.0, 50.9, 50.9, 50.9, 52.1, 52.1, 52.2, 168.4, 168.4, 168.4, 168.5, 168.6, 168.8, 168.8, 168.9, 168.9, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.2, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{42}H_{72}N_{20}O_{10}$ $[M+Na]^+$ 1039.5632, found 1039.5616.

N-Decanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**170**) as a mixture of rotamers



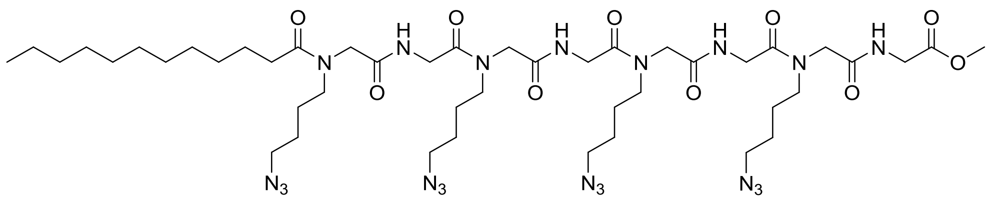
Paraformaldehyde (**19**, 80 mg, 2.67 mmol), **16** (183 mg, 1.60 mmol), **150** (1291 mg, 1.60 mmol), and **20** (146 µl, 159 mg, 1.60 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **170** (448 mg, 27.2%) as light yellow, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **170** (311 mg, 18.8%) as light yellow, amorphous solid. R_f 0.59 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.19–1.38 (m, 12H), 1.52–1.78 (m, 18H), 2.22–2.29 and 2.32–2.42 (2m, 2H), 3.10–3.56 (m, 16H), 3.68–3.79 (m, 3H), 3.82–4.34 (m, 16H), 7.00–7.73 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.5, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.4, 25.5, 25.5, 25.9, 26.1, 29.2, 29.3, 29.3, 29.4, 29.4, 31.7, 32.8, 33.1, 40.8, 40.8, 40.9, 41.0, 41.2, 41.3, 41.3, 41.4, 46.4, 47.0, 47.2, 47.2, 47.9, 48.0, 48.1, 48.3, 49.0, 49.1, 49.1, 49.2, 49.4, 49.6, 49.7, 49.9, 50.9, 50.9, 50.9, 52.1, 52.1, 52.2, 168.1, 168.3, 168.4, 168.4, 168.6, 168.7, 168.7, 168.8, 168.8, 168.9, 168.9, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.1, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{43}H_{74}N_{20}O_{10}$ $[M+Na]^+$ 1053.5789, found 1053.5762.

N-Undecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**171**) as a mixture of rotamers



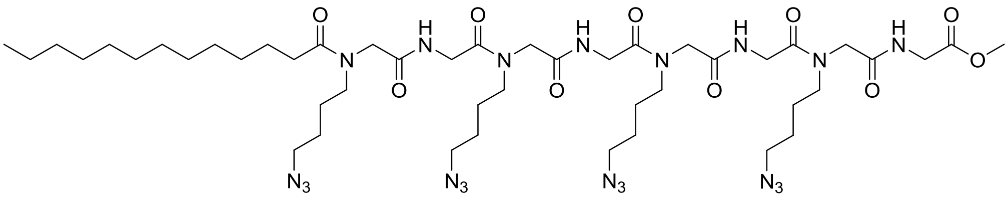
Paraformaldehyde (**19**, 86 mg, 2.85 mmol), **16** (195 mg, 1.71 mmol), **151** (1403 mg, 1.71 mmol), and **20** (155 µl, 169 mg, 1.71 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **171** (460mg, 25.7%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **171** (646 mg, 36.1%) as light yellow, amorphous solid. R_f 0.60 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.20–1.38 (m, 14H), 1.52–1.78 (m, 18H), 2.21–2.28 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.69–3.79 (m, 3H), 3.83–4.34 (m, 16H), 6.94–7.62 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.5, 24.6, 24.7, 25.1, 25.3, 25.5, 25.6, 25.6, 26.0, 26.2, 29.3, 29.4, 29.4, 29.4, 29.5, 29.5, 31.8, 32.9, 33.2, 40.9, 40.9, 41.0, 41.1, 41.3, 41.3, 41.4, 46.5, 47.1, 47.3, 48.1, 48.2, 48.4, 49.0, 49.1, 49.2, 49.3, 49.6, 49.8, 49.9, 50.2, 50.9, 51.0, 52.2, 52.3, 168.2, 168.2, 168.2, 168.3, 168.4, 168.4, 168.6, 168.6, 168.7, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 169.4, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{44}H_{76}N_{20}O_{10}$ $[M+Na]^+$ 1067.5945, found 1067.5923.

N-Lauroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**172**) as a mixture of rotamers



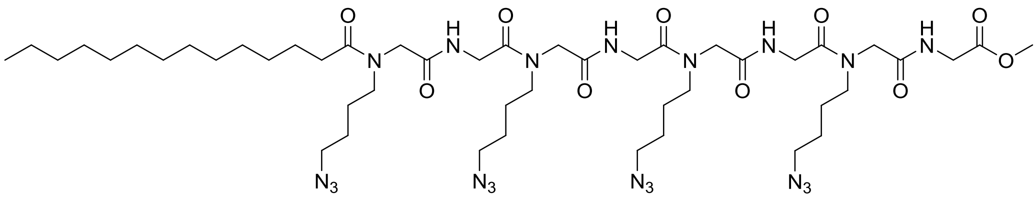
Paraformaldehyde (**19**, 97 mg, 3.23 mmol), **16** (221 mg, 1.94 mmol), **152** (1620 mg, 1.94 mmol), and **20** (176 µl, 192 mg, 1.94 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **172** (562 mg, 27.3%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **172** (642 mg, 31.2%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.20–1.38 (m, 16H), 1.52–1.78 (m, 18H), 2.21–2.29 and 2.32–2.43 (2m, 2H), 3.10–3.60 (m, 16H), 3.68–3.80 (m, 3H), 3.84–4.32 (m, 16H), 6.97–7.70 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.4, 25.5, 25.6, 26.0, 26.1, 29.2, 29.4, 29.4, 29.5, 29.5, 29.6, 31.8, 32.8, 33.2, 40.8, 40.9, 40.9, 41.1, 41.2, 41.3, 41.4, 41.5, 46.4, 47.1, 47.2, 47.9, 48.1, 48.2, 48.3, 49.0, 49.1, 49.3, 49.6, 49.7, 49.8, 49.9, 50.0, 50.9, 50.9, 51.0, 52.1, 52.2, 52.2, 168.1, 168.4, 168.4, 168.6, 168.7, 168.8, 168.9, 169.0, 169.0, 169.0, 169.1, 169.2, 169.2, 169.3, 169.3, 170.1, 170.2, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{45}H_{78}N_{20}O_{10}$ $[M+Na]^+$ 1081.6102, found 1081.6059.

N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**173**) as a mixture of rotamers



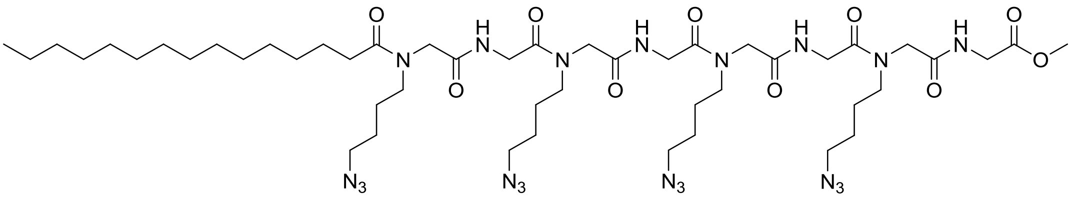
Paraformaldehyde (**19**, 85 mg, 2.83 mmol), **16** (194 mg, 1.70 mmol), **153** (1440 mg, 1.70 mmol), and **20** (154 µl, 168 mg, 1.70 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **173** (475 mg, 26.0%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **173** (510 mg, 28.0%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.19–1.38 (m, 18H), 1.52–1.79 (m, 18H), 2.21–2.29 and 2.31–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.69–3.76 (m, 3H), 3.88–4.24 (m, 16H), 6.97–7.70 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.5, 25.6, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.9, 41.1, 41.3, 41.4, 41.6, 46.4, 47.1, 47.2, 48.1, 48.2, 48.2, 48.3, 49.0, 49.1, 49.2, 49.3, 49.6, 49.8, 49.9, 50.1, 51.0, 52.1, 52.3, 168.2, 168.3, 168.3, 168.4, 168.6, 168.6, 168.6, 168.7, 168.9, 169.9, 169.0, 169.0, 169.1, 169.2, 169.3, 169.3, 169.3, 170.1, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{46}H_{80}N_{20}O_{10}$ $[M+Na]^+$ 1095.6259, found 1095.6235.

N-Myristoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**174**) as a mixture of rotamers



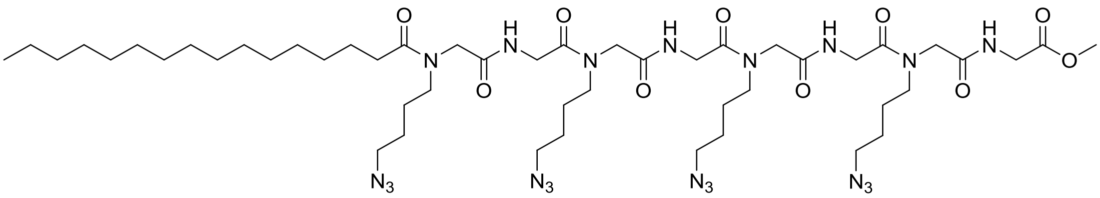
Paraformaldehyde (**19**, 89 mg, 2.98 mmol), **16** (204 mg, 1.79 mmol), **154** (1542 mg, 1.79 mmol), and **20** (162 µl, 177 mg, 1.79 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **174** (824 mg, 42.3%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **174** (331 mg, 17.0%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.18–1.38 (m, 20H), 1.52–1.78 (m, 18H), 2.22–2.29 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.68–3.79 (m, 3H), 3.85–4.32 (m, 16H), 6.98–7.71 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.5, 25.5, 25.9, 26.1, 29.2, 29.3, 29.4, 29.5, 29.5, 29.5, 29.6, 31.8, 32.8, 33.2, 40.8, 40.8, 40.9, 41.0, 41.2, 41.3, 41.5, 46.4, 47.0, 47.2, 47.9, 48.0, 48.1, 48.3, 49.0, 49.1, 49.2, 49.4, 49.6, 49.7, 49.8, 50.0, 50.9, 50.9, 52.1, 52.1, 52.2, 168.1, 168.3, 168.4, 168.4, 168.5, 168.6, 168.7, 168.9, 168.9, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.1, 170.2, 170.3, 173.8, 173.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{47}H_{82}N_{20}O_{10}$ $[M+Na]^+$ 1109.6415, found 1109.6482.

N-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azido-butyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**175**) as a mixture of rotamers



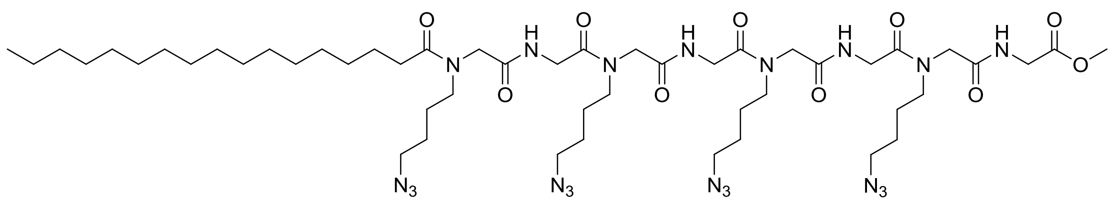
Paraformaldehyde (**19**, 87 mg, 2.90 mmol), **16** (199 mg, 1.74 mmol), **155** (1521 mg, 1.74 mmol), and **20** (158 µl, 172 mg, 1.74 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **175** (363 mg, 18.9%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **175** (694 mg, 36.2%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.19–1.37 (m, 22H), 1.52–1.78 (m, 18H), 2.21–2.28 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.68–3.79 (m, 3H), 3.83–4.33 (m, 16H), 6.96–7.68 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.0, 22.6, 24.4, 24.5, 24.6, 25.1, 25.2, 25.4, 25.5, 25.6, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.6, 31.8, 32.8, 33.2, 40.8, 40.9, 41.0, 41.1, 41.2, 41.3, 41.5, 41.5, 46.4, 47.1, 47.2, 47.2, 47.9, 48.1, 48.2, 48.3, 49.0, 49.3, 49.5, 49.5, 49.6, 49.8, 49.8, 49.9, 50.0, 50.9, 51.0, 52.1, 52.1, 52.2, 168.0, 168.2, 168.3, 168.3, 168.4, 168.6, 168.7, 168.8, 168.9, 168.9, 169.1, 169.2, 169.2, 169.3, 169.3, 170.1, 170.1, 170.2, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{48}H_{84}N_{20}O_{10}$ $[M+Na]^+$ 1123.6572, found 1123.6540.

<u>*N*-Palmitoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**176**) as a mixture of rotamers</u>



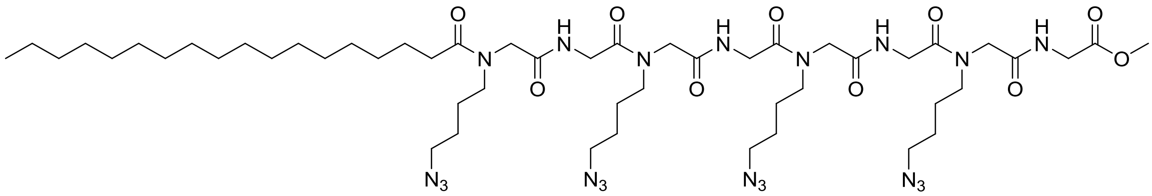
Paraformaldehyde (**19**, 82 mg, 2.73 mmol), **16** (187 mg, 1.64 mmol), **156** (1460 mg, 1.64 mmol), and **20** (149 µl, 163 mg, 1.64 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **176** (740 mg, 40.5%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **176** (553 mg, 30.2%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.46 (m, 24H), 1.52–1.78 (m, 18H), 2.21–2.28 and 2.32–2.42 (2m, 2H), 3.11–3.58 (m, 16H), 3.69–3.79 (m, 3H), 3.83–4.32 (m, 16H), 6.95–7.63 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.5, 24.5, 24.7, 25.1, 25.3, 25.5, 25.5, 25.6, 25.6, 26.0, 26.1, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 29.6, 31.9, 32.9, 33.2, 40.9, 41.1, 41.3, 41.4, 41.6, 46.4, 47.1, 47.3, 48.0, 48.1, 48.2, 48.2, 48.4, 49.0, 49.1, 49.2, 49.3, 49.7, 49.8, 50.0, 50.2, 50.9, 51.0, 52.2, 52.3, 168.0, 168.0, 168.3, 168.3, 168.4, 168.5, 168.6, 168.6, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.3, 169.3, 169.4, 170.1, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{49}H_{86}N_{20}O_{10}$ $[M+Na]^+$ 1137.6728, found 1137.6688.

N-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azido-butyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**177**) as a mixture of rotamers



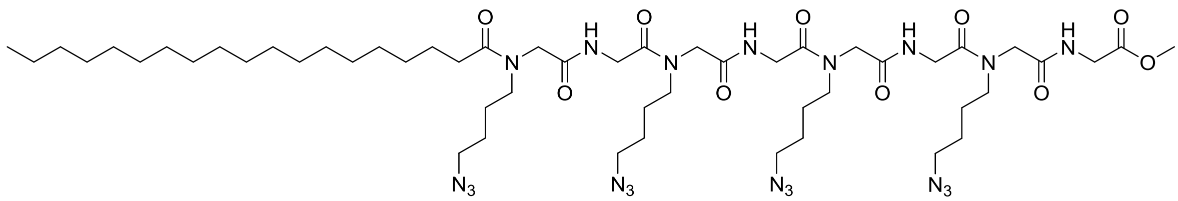
Paraformaldehyde (**19**, 78 mg, 2.60 mmol), **16** (178 mg, 1.56 mmol), **157** (1411 mg, 1.56 mmol), and **20** (142 µl, 155 mg, 1.56 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **177** (542 mg, 30.8%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **177** (718 mg, 40.8%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.47 (m, 26H), 1.52–1.78 (m, 18H), 2.22–2.28 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.69–3.79 (m, 3H), 3.83–4.33 (m, 16H), 6.95–7.62 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.5, 24.5, 24.7, 25.1, 25.3, 25.4, 25.5, 25.6, 25.6, 26.0, 26.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.6, 29.7, 31.9, 32.9, 33.2, 40.8, 40.9, 41.0, 41.1, 41.3, 41.4, 41.5, 41.6, 46.6, 47.1, 47.3, 48.0, 48.1, 48.2, 48.4, 49.0, 49.1, 49.3, 49.7, 49.8, 49.9, 50.1, 50.9, 51.0, 52.2, 52.2, 52.3, 168.1, 168.2, 168.3, 168.4, 168.6, 168.7, 168.8, 168.9, 169.0, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 170.1, 170.3, 170.4, 170.4, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{50}H_{88}N_{20}O_{10}$ $[M+Na]^+$ 1151.6885, found 1151.6831.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**178**) as a mixture of rotamers



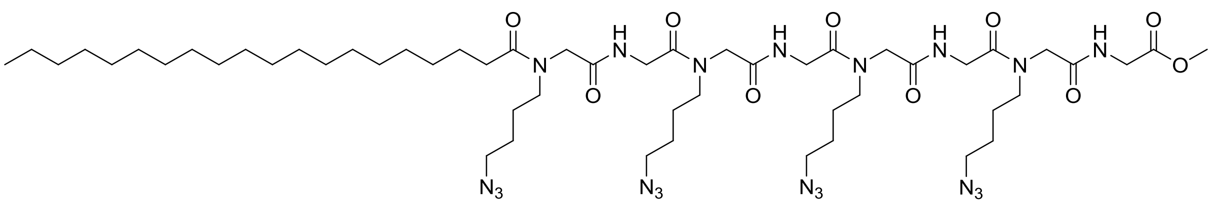
Paraformaldehyde (**19**, 75 mg, 2.50 mmol), **16** (171 mg, 1.50 mmol), **158** (1373 mg, 1.50 mmol), and **20** (136 µl, 149 mg, 1.50 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **178** (711 mg, 41.5%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **178** (317 mg, 18.5%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.46 (m, 28H), 1.52–1.78 (m, 18H), 2.21–2.28 and 2.32–2.41 (2m, 2H), 3.20–3.56 (m, 16H), 3.69–3.79 (m, 3H), 3.83–4.32 (m, 16H), 6.94–7.62 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.5, 24.5, 24.7, 25.1, 25.3, 25.5, 25.5, 25.6, 25.6, 26.0, 26.1, 29.3, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 32.9, 33.2, 40.8, 40.9, 41.0, 41.1, 41.2, 41.4, 41.6, 46.5, 47.1, 47.3, 48.1, 48.2, 48.4, 49.0, 49.1, 49.2, 49.3, 49.7, 49.8, 49.9, 50.0, 50.1, 50.9, 51.0, 52.2, 52.2, 52.3, 168.1, 168.2, 168.3, 168.3, 168.4, 168.4, 168.5, 168.6, 168.6, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 169.3, 170.1, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9, 174.0 ppm. HRMS (ESI+) *m/z* calcd for $C_{51}H_{90}N_{20}O_{10}$ $[M+Na]^+$ 1165.7041, found 1165.7004.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azido-butyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**179**) as a mixture of rotamers



Paraformaldehyde (**19**, 58 mg, 1.92 mmol), **16** (131 mg, 1.15 mmol), **159** (1072 mg, 1.15 mmol), and **20** (104 µl, 114 mg, 1.15 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **179** (421 mg, 31.6%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **179** (441 mg, 33.1%) as light yellow, amorphous solid. R_f 0.61 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.92 (m, 3H), 1.06–1.46 (m, 30H), 1.52–1.79 (m, 18H), 2.21–2.29 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.68–3.78 (m, 3H), 3.84–4.32 (m, 16H), 6.95–7.65 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.4, 24.5, 24.7, 25.2, 25.3, 25.5, 25.5, 25.6, 26.0, 26.1, 29.3, 29.4, 29.5, 29.5, 29.6, 29.6, 31.8, 32.8, 33.2, 40.8, 40.9, 41.0, 41.1, 41.2, 41.3, 41.4, 41.6, 46.4, 47.1, 47.2, 47.9, 48.1, 48.2, 48.3, 49.0, 49.1, 49.2, 49.3, 49.7, 49.8, 49.8, 49.9, 50.1, 51.0, 52.1, 52.3, 168.0, 168.2, 168.3, 168.3, 168.4, 168.5, 168.6, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 169.3, 170.1, 170.1, 170.3, 170.4, 170.4, 173.8, 173.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{52}H_{92}N_{20}O_{10}$ $[M+Na]^+$ 1179.7198, found 1179.7167.

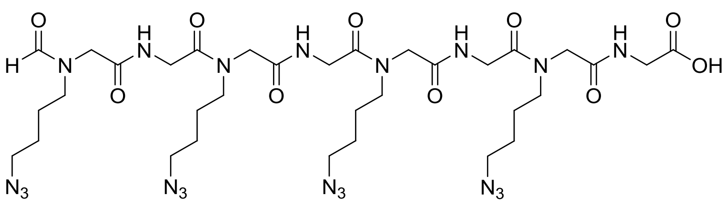
<u>*N*-Arachidoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**180**) as a mixture of rotamers</u>



Paraformaldehyde (**19**, 62 mg, 2.07 mmol), **16** (142 mg, 1.24 mmol), **160** (1178 mg, 1.24 mmol), and **20** (113 µl, 123 mg, 1.24 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml). The filtration residue was dried *in vacuo* to give pure **180** (655 mg, 45.1%) as white, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (dichloromethane/methanol 93:7) to afford another crop of **180** (234 mg, 16.1%) as light yellow, amorphous solid. R_f 0.62 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.85–0.91 (m, 3H), 1.06–1.46 (m, 32H), 1.52–1.78 (m, 18H), 2.22–2.28 and 2.32–2.42 (2m, 2H), 3.10–3.60 (m, 16H), 3.69–3.79 (m, 3H), 3.83–4.30 (m, 16H), 6.95–7.63 (m, 4H) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 14.1, 22.6, 24.4, 24.5, 24.7, 25.1, 25.3, 25.4, 25.5, 25.6, 25.6, 26.0, 26.1, 29.3, 29.4, 29.4, 29.5, 29.6, 29.6, 29.7, 31.9, 32.9, 33.2, 40.8, 40.9, 41.0, 41.1, 41.2, 41.3, 41.4, 46.4, 47.1, 47.3, 47.3, 48.0, 48.1, 48.2, 48.4, 49.0, 49.1, 49.3, 49.6, 49.6, 49.8, 49.9, 50.1, 50.9, 51.0, 52.2, 52.2, 52.3, 168.1, 168.2, 168.3, 168.3, 168.4, 168.4, 168.5, 168.6, 168.7, 168.8, 168.8, 168.9, 169.0, 169.0, 169.1, 169.1, 169.2, 169.3, 169.3, 169.3, 170.1, 170.1, 170.3, 170.4, 170.4, 173.9, 174.0 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{53}H_{94}N_{20}O_{10}$ $[M+Na]^+$ 1193.7354, found 1193.7315.

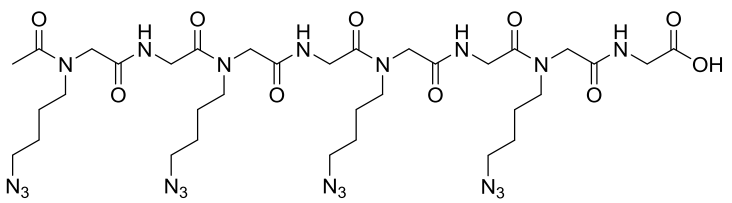
Saponifications

N-Formyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**181**) as a mixture of rotamers



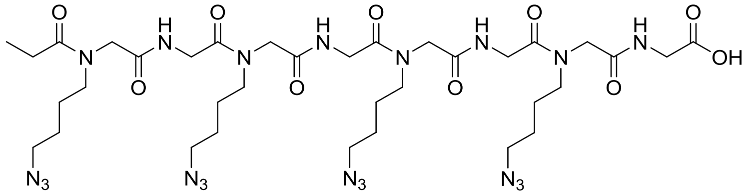
The saponification of **161** (92 mg, 101.7 µmol) with lithium hydroxide solution (127 µl, 254.3 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **181** as crude product, which was dissolved in methanol (1.0 ml) and precipitated by addition of 2-propanol (8.0 ml). After centrifugation at 4000 rpm and 0 °C for 10 min the supernatant was discarded and the residuum was carefully washed with 2-propanol (20 ml). The residue was dissolved in absolute EtOH (5 ml) and the solution was filtrated over a 0.22 µm PTFE-syringe filter. The solvent was removed *in vacuo* to yield **181** (55 mg, 60.7%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.52–1.78 (m, 16H), 3.28–3.48 (m, 16H), 3.93 and 3.97 (2s, 2H), 3.99–4.30 (m, 14H), 8.09 and 8.18 2s, 1H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.3, 25.6, 26.5 (br), 26.9, 27.1, 27.2, 41.8, 41.9, 42.0, 42.0, 42.2 (br), 44.1, 46.2, 46.3, 46.1, 48.4, 49.1, 49.2, 49.3, 49.4, 50.0, 50.1, 50.2, 50.8, 51.0, 52.1, 52.1, 52.2, 165.8, 166.5, 170.5, 170.6, 170.9, 170.9, 171.0, 171.1, 171.1, 171.3, 171.4, 171.5, 172.7, 172.8 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{33}H_{54}N_{20}O_{10}$ $[M+Na]^+$ 913.4224, found 913.4222.

N-Acetyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**182**) as a mixture of rotamers



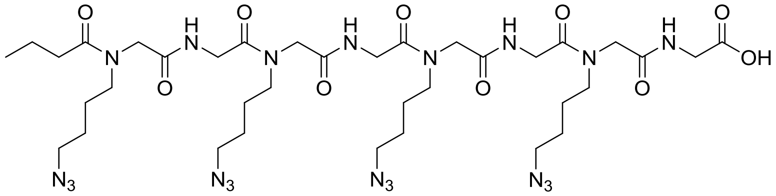
The saponification of **162** (89 mg, 96.8 µmol) with lithium hydroxide solution (121 µl, 242.0 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **182** as crude product, which was dissolved in a mixture of methanol/2-propanol (1:5, v/v, 3.0 ml) and precipitated by addition of *n*-hexane (4.0 ml). After centrifugation at 4000 rpm and 0 °C for 10 min the supernatant was discarded and the residuum was carefully washed with *n*-hexane (20 ml). The residue was dissolved in methanol (5 ml) and the solution was filtrated over a 0.22 µm PTFE-syringe filter after standing over night. The solvent was removed *in vacuo* to yield **182** (58 mg, 66.2%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.52–1.78 (m, 16H), 2.08 and 2.17 (2s, 3H), 3.20–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.02–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 21.2, 21.8, 25.6, 26.5, 26.6, 26.6, 26.7, 27.1, 27.2, 41.8, 41.9, 42.0, 42.0, 42.1, 42.2, 47.8, 47.8, 48.4, 49.1, 49.2, 49.8, 49.9, 50.0, 50.1, 50.1, 50.2, 50.2, 50.8, 52.1, 52.1, 52.2, 52.3, 170.9, 170.9, 171.0, 171.0, 171.1, 171.1, 171.3, 171.4, 171.4, 171.5, 171.6, 171.6, 172.8, 172.9, 173.9, 174.4 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{34}H_{56}N_{20}O_{10}$ $[M+Na]^+$ 927.4380, found 927.4349.

N-Propionyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**183**) as a mixture of rotamers



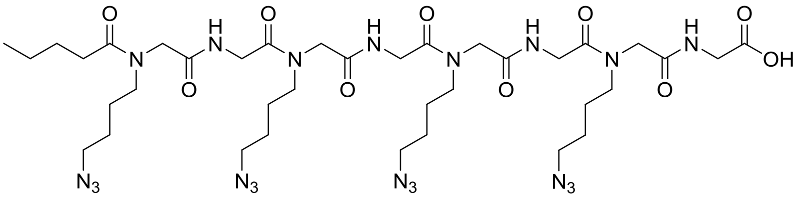
The saponification of **163** (109 mg, 116.8 µmol) with lithium hydroxide solution (146 µl, 292.0 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **183** as crude product, which was dissolved in a mixture of methanol/2-propanol (1:5, v/v, 3.0 ml) and precipitated by addition of *n*-hexane (4.0 ml). After centrifugation at 4000 rpm and 0 °C for 10 min the supernatant was discarded and the residuum was carefully washed with *n*-hexane (20 ml). The residue was dissolved in methanol (5 ml) and the solution was filtrated over a 0.22 µm PTFE-syringe filter after standing over night. The solvent was removed *in vacuo* to yield **183** (98 mg, 91.3%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.05–1.15 (m, 3H), 1.52–1.78 (m, 16H), 2.32–2.40 and 2.44–2.52 (2m, 2H), 3.18–3.54 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.01–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 9.7, 9.8, 25.6, 25.7, 26.5, 26.9, 27.1, 27.2, 27.3, 41.8, 41.9, 41.9, 42.0, 42.1, 42.2, 47.9, 48.4, 48.4, 49.1, 49.9, 50.0, 50.1, 50.8, 51.5, 52.1, 52.2, 170.9, 171.0, 171.0, 171.0, 171.1, 171.3, 171.4, 171.5, 171.6, 171.8, 172.9, 172.9, 176.8, 177.2 ppm. HRMS (ESI-) *m/z* calcd for $C_{35}H_{58}N_{20}O_{10}$ $[M-H]^-$ 917.4572, found 917.4543.

N-Butyryl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**184**) as a mixture of rotamers



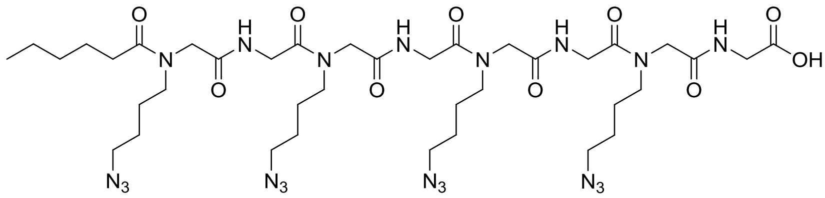
The saponification of **164** (104 mg, 109.5 µmol) with lithium hydroxide solution (137 µl, 273.8 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **184** as crude product, which was dissolved in methanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 µm PTFE-syringe filter. After removing the solvent *in vacuo*, **184** (60 mg, 58.7%) was obtained as a colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.91–1.01 (m, 3H), 1.51–1.78 (m, 18H), 2.28–2.34 and 2.40–2.47 (2m, 2H), 3.18–3.50 (m, 16H), 3.90–3.98 (m, 2H), 4.00–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.2 14.2, 19.7, 19.7, 25.6, 25.7, 26.5, 26.6, 26.9, 27.1, 27.2, 27.3, 35.5, 36.0, 41.8, 41.9, 42.0, 42.1, 42.1, 42.2, 47.8, 48.4, 49.1, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.2, 170.9, 170.9, 170.9, 171.0, 171.1, 171.1, 171.3, 171.3, 171.4, 171.5, 171.6, 171.7, 172.8, 172.8, 176.0, 176.4 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{36}H_{60}N_{20}O_{10}$ $[M-H]^-$ 931.4729, found 931.4707.

N-Valeroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**185**) as a mixture of rotamers



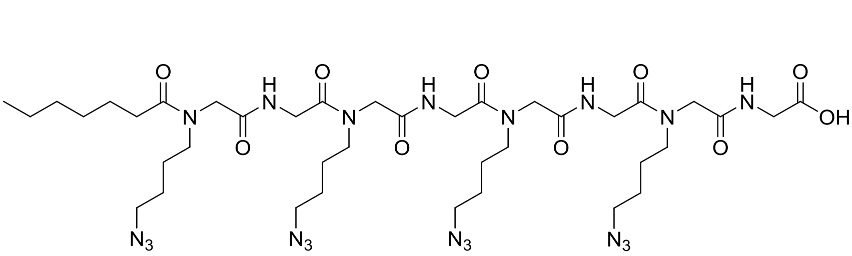
The saponification of **165** (100 mg, 104.1 µmol) with lithium hydroxide solution (130 µl, 260.3 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **185** as crude product, which was dissolved in methanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 µm PTFE-syringe filter. After removing the solvent *in vacuo*, **185** (43 mg, 43.6%) was obtained as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.98 (m, 3H), 1.29–1.45 (m, 2H), 1.51–1.78 (m, 18H), 2.30–2.36 and 2.42–2.51 (2m, 2H), 3.22–3.56 (m, 16H), 3.90–3.98 (m, 2H), 4.02–4.24 (m, 14H), 7.92–8.50 (m, 4H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 23.5, 23.5, 25.6, 25.7, 26.5, 26.9, 27.1, 27.2, 28.5, 28.6, 33.4, 33.9, 41.8, 41.9, 42.0, 42.0, 42.2, 47.9, 48.4, 49.1, 50.0, 50.1, 51.6, 52.1, 52.2, 52.2, 170.9, 170.9, 171.0, 171.0, 171.0, 171.1, 171.1, 171.3, 171.3, 171.4, 171.5, 171.5, 171.7, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{37}H_{62}N_{20}O_{10}$ $[M-H]^-$ 945.4885, found 945.4874.

N-Hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**186**) as a mixture of rotamers



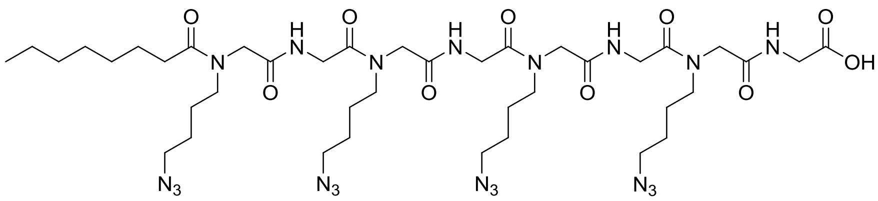
The saponification of **166** (100 mg, 102.6 µmol) with lithium hydroxide solution (128 µl, 256.6 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **186** as crude product, which was dissolved in absolute ethanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 µm PTFE-syringe filter. After removing the solvent *in vacuo*, **186** (64 mg, 64.9%) was obtained as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.96 (m, 3H), 1.22–1.42 (m, 4H), 1.51–1.80 (m, 18H), 2.30–2.36 and 2.42–2.48 (2m, 2H), 3.24–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.02–4.24 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 14.4, 23.6, 25.6, 25.7, 26.0, 26.1, 26.5, 26.6, 26.9, 27.1, 27.2, 27.2, 32.6, 32.7, 33.6, 34.1, 41.8, 41.9, 42.0, 42.1, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.1, 171.2, 171.3, 171.4, 171.4, 171.4, 171.5, 171.6, 172.7, 172.8, 176.2, 176.6 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{38}H_{64}N_{20}O_{10}$ $[M-H]^-$ 959.5042, found 959.5015.

N-Heptanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**187**) as a mixture of rotamers



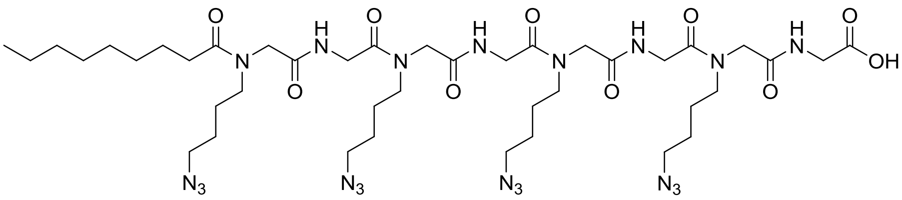
The saponification of **167** (100 mg, 101.1 µmol) with lithium hydroxide solution (126 µl, 252.8 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **187** as crude product, which was dissolved in absolute ethanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 µm PTFE-syringe filter. After removing the solvent *in vacuo*, **187** (62 mg, 62.9%) was obtained as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.94 (m, 3H), 1.21–1.42 (m, 6H), 1.51–1.79 (m, 18H), 2.29–2.36 and 2.42–2.48 (2m, 2H), 3.27–3.54 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.02–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.6, 25.6, 25.7, 26.3, 26.4, 26.5, 27.1, 27.2, 27.2, 30.1, 30.1, 32.8, 33.7, 34.1, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.1, 171.2, 171.3, 171.4, 171.4, 171.4, 171.5, 171.6, 172.7, 172.7, 176.2, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{39}H_{66}N_{20}O_{10}$ $[M-H]^-$ 973.5198, found 973.5205.

N-Octanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**188**) as a mixture of rotamers



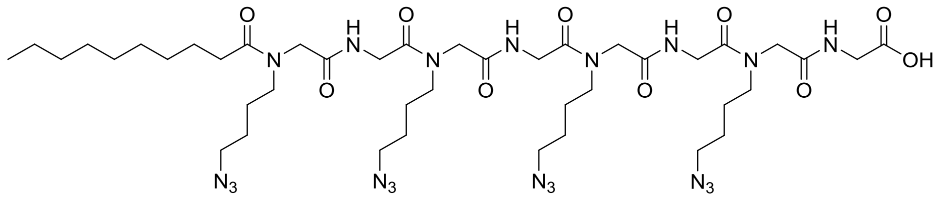
The saponification of **168** (100 mg, 99.7 µmol) with lithium hydroxide solution (125 µl, 249.3 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **188** as crude product, which was dissolved in absolute ethanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 µm PTFE-syringe filter. After removing the solvent *in vacuo*, **188** (74 mg, 75.0%) was obtained as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.94 (m, 3H), 1.22–1.42 (m, 8H), 1.51–1.79 (m, 18H), 2.29–2.36 and 2.42–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.02–4.24 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 14.5, 23.7, 25.6, 25.7, 26.4, 26.5, 26.5, 26.9, 27.1, 27.2, 27.2, 30.2, 30.3, 30.4, 30.4, 32.9, 33.7, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.3, 171.4, 171.5, 171.6, 172.7, 172.7, 176.1, 176.6, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{40}H_{68}N_{20}O_{10}$ [M-H]$^-$ 987.5355, found 987.5329.

N-Nonanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**189**) as a mixture of rotamers



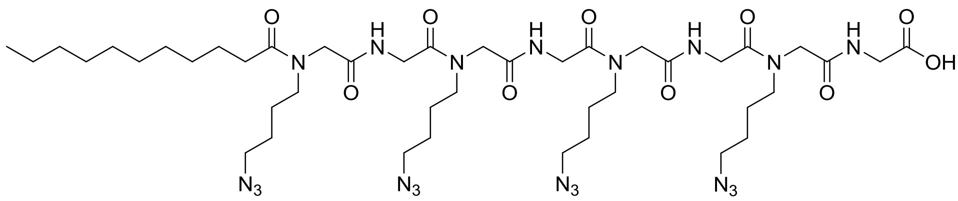
The saponification of **169** (100 mg, 98.3 µmol) with lithium hydroxide solution (123 µl, 245.8 µmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **189** as crude product, which was dissolved in absolute ethanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 µm PTFE-syringe filter. After removing the solvent *in vacuo*, **189** (80 mg, 81.1%) was obtained as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.93 (m, 3H), 1.20–1.42 (m, 10H), 1.52–1.78 (m, 18H), 2.29–2.36 and 2.42–2.48 (2m, 2H), 3.24–3.56 (m, 16H), 3.86–4.00 and 4.01–4.30 (2m, 16H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.3, 30.4, 30.4, 30.5, 30.7, 33.0, 33.7, 34.1, 41.8, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.3, 171.4, 171.5, 171.6, 172.7, 172.7, 176.1, 176.6, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{41}H_{70}N_{20}O_{10}$ [M-H]$^-$ 1001.5511, found 1001.5507.

N-Decanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**190**) as a mixture of rotamers



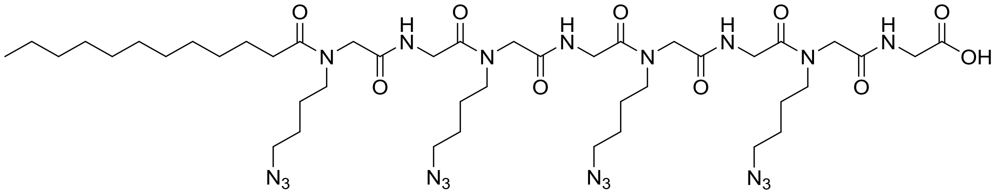
The saponification of **170** (100 mg, 97.0 μmol) with lithium hydroxide solution (121 μl, 242.5 μmol, 2 M) following general procedure B (method 1) using hydrochloric acid (2 M) for acidification instead of saturated $NaHSO_4$ solution afforded **190** as crude product, which was dissolved in absolute ethanol (3 ml). The resulting solution was dropped into water (20 ml) in a centrifuge tube. The formed emulsion was centrifuged at 4000 rpm and 0 °C for 20 min and the supernatant was removed. The residuum was washed with water (20 ml) and was afterwards dissolved in methanol (10 ml) again. After standing over night the solution was filtrated over a 0.22 μm PTFE-syringe filter. After removing the solvent *in vacuo*, **190** (72 mg, 73.0%) was obtained as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.21–1.41 (m, 12H), 1.51–1.78 (m, 18H), 2.29–2.36 and 2.42–2.48 (2m, 2H), 3.23–3.55 (m, 16H), 4.86–4.00 and 4.01–4.30 (2m, 16H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.3, 30.4, 30.4, 30.6, 30.6, 33.0, 33.7, 34.1, 34.2, 41.8, 41.9, 42.0, 42.1, 42.1, 42.2, 47.8, 48.4, 49.1, 49.1, 49.1, 50.0, 50.1, 50.1, 50.8, 51.6, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.4, 171.4, 171.5, 171.6, 172.7, 172.7, 176.2, 176.6, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{42}H_{72}N_{20}O_{10}$ $[M-H]^-$ 1015.5668, found 1015.5688.

N-Undecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**191**) as a mixture of rotamers



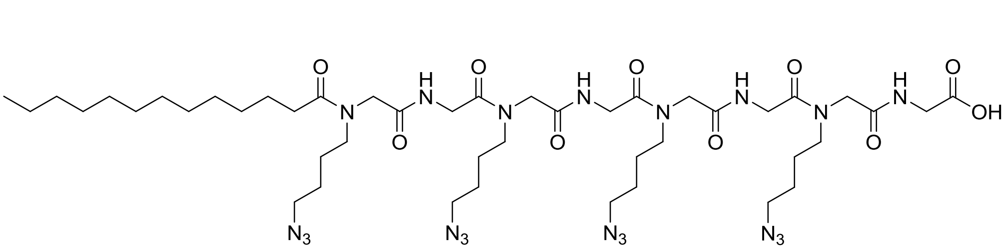
The saponification of **171** (200 mg, 191 μmol) with lithium hydroxide solution (239 μl, 478 μmol, 2 M) following general procedure B (method 2) afforded **191** (192 mg, 97.5%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.22–1.40 (m, 14H), 1.51–1.78 (m, 18H), 2.29–2.36 and 2.42–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.86–4.00 and 4.01–4.36 (2m, 16H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 26.9, 27.1, 27.2, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 33.1, 33.7, 34.1, 34.2, 41.7, 41.9, 42.0, 42.0, 42.2, 42.3, 47.8, 48.4, 48.4, 49.1, 49.1, 50.0, 50.0, 50.1, 50.1, 50.8, 51.6, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 171.0, 171.0, 171.1, 171.1, 171.3, 171.3, 171.3, 171.4, 171.4, 171.5, 171.5, 171.6, 171.7, 171.8, 176.2, 176.6, 176.6 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{43}H_{74}N_{20}O_{10}$ [M-H]$^-$ 1029.5824, found 1029.5828.

N-Lauroyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**192**) as a mixture of rotamers



The saponification of **172** (200 mg, 189 µmol) with lithium hydroxide solution (237 µl, 473 µmol, 2 M) following general procedure B (method 2) afforded **192** (197 mg, 99.7%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.22–1.42 (m, 16H), 1.50–1.78 (m, 18H), 2.29–2.36 and 2.42–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.89–3.99 and 4.02–4.30 (2m, 16H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 30.8, 33.1, 33.7, 34.1, 34.1, 41.7, 41.7, 41.9, 42.0, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 170.9, 170.9, 170.9, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.4, 171.5, 171.6, 172.7, 172.8, 176.1, 176.5, 176.6 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{44}H_{76}N_{20}O_{10}$ [M-H]$^-$ 1043.5981, found 1043.5992.

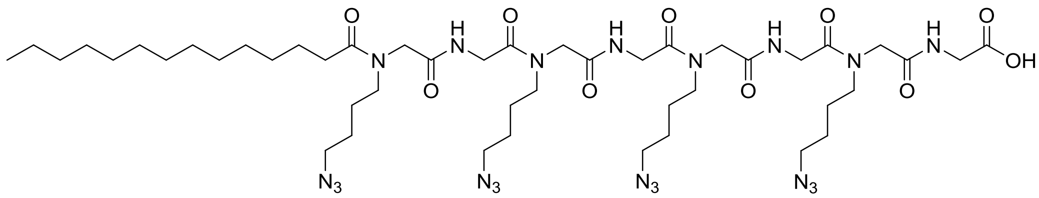
N-Tridecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**193**) as a mixture of rotamers



The saponification of **173** (200 mg, 186 µmol) with lithium hydroxide solution (237 µl, 473 µmol, 2 M) following general procedure B (method 2) afforded **193** (192 mg, 94.4%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.23–1.41 (m, 18H), 1.51–1.78 (m, 18H), 2.30–2.36 and 2.42–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.89–

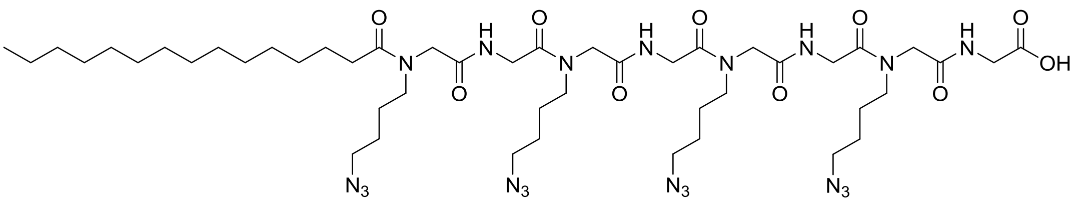
3.99 and 4.01–4.26 (2m, 16H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.6, 26.9, 27.1, 27.2, 27.3, 30.4, 30.4, 30.5, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 30.8, 33.1, 33.7, 34.1, 34.2, 41.8, 41.9, 42.0, 42.1, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.7, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 171.0, 171.0, 171.1, 171.2, 171.3, 171.4, 171.4, 171.5, 171.6, 172.7, 172.8, 176.2, 176.6, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{45}H_{78}N_{20}O_{10}$ [M-H]$^-$ 1057.6137, found 1057.6145.

N-Myristoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**194**) as a mixture of rotamers



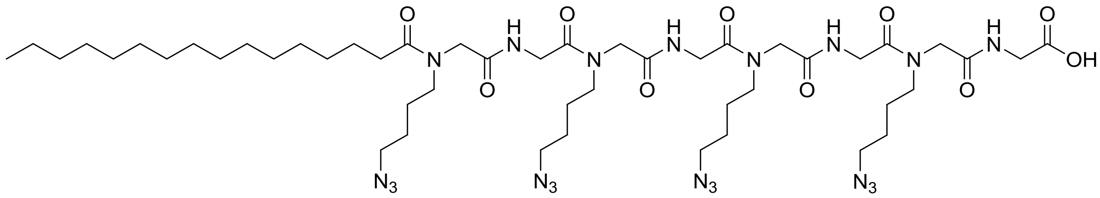
The saponification of **174** (200 mg, 184 µmol) with lithium hydroxide solution (230 µl, 460 µmol, 2 M) following general procedure B (method 2) afforded **194** (121 mg, 61.3%) as colorless, amorphous solid. A part of the product was lost by a broken centrifuge tube. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.22–1.41 (m, 20H), 1.51–1.78 (m, 18H), 2.30–2.36 and 2.41–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.88–3.99 and 4.02–4.30 (2m, 16H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 26.9, 27.1, 27.2, 30.4, 30.4, 30.5, 30.6, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.0, 33.7, 34.1, 34.2, 41.8, 41.9, 41.9, 42.0, 42.0, 42.1, 42.2, 47.8, 48.4, 49.1, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.9, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.4, 171.5, 171.6, 172.7, 172.8, 176.1, 176.5, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{46}H_{80}N_{20}O_{10}$ [M-H]$^-$ 1071.6294, found 1071.6309.

N-Pentadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azido-butyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**195**) as a mixture of rotamers



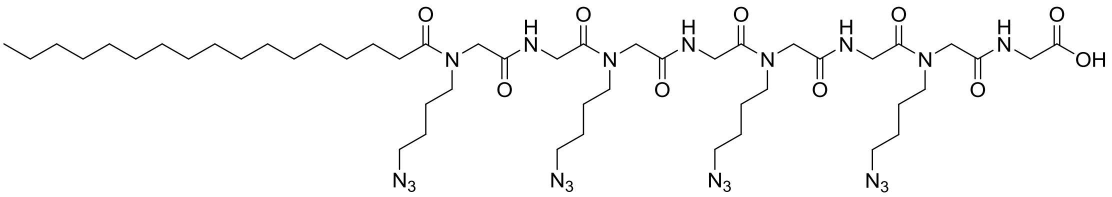
The saponification of **175** (200 mg, 182 µmol) with lithium hydroxide solution (228 µl, 455 µmol, 2 M) following general procedure B (method 2) afforded **195** (196 mg, 99.0%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.22–1.41 (m, 22H), 1.51–1.79 (m, 18H), 2.29–2.36 and 2.41–2.49 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.01–4.24 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 30.4, 30.6, 30.6, 30.6, 30.7, 30.7, 30.8, 33.0, 33.7, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.3, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.7, 172.7, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m/z* calcd for $C_{47}H_{82}N_{20}O_{10}$ $[M-H]^-$ 1085.6450, found 1085.6466.

N-Palmitoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**196**) as a mixture of rotamers



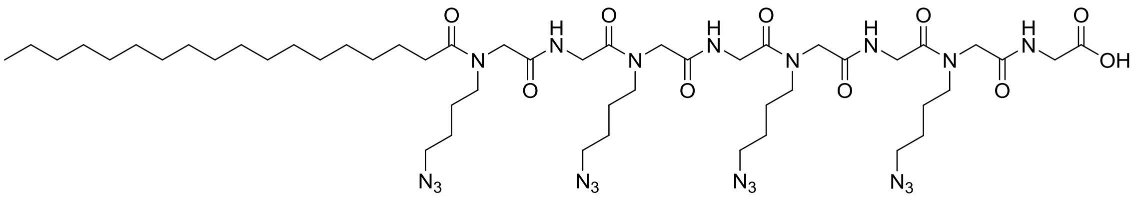
The saponification of **176** (200 mg, 179 µmol) with lithium hydroxide solution (224 µl, 448 µmol, 2 M) following general procedure B (method 2) afforded **196** (204 mg, quant.) as colorless, amorphous solid, containing a small, non-removable amount of water. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.22–1.40 (m, 24H), 1.51–1.79 (m, 18H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.02–4.27 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.5, 26.9, 27.1, 27.2, 30.4, 30.4, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 33.0, 33.7, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.3, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.8, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.4, 171.6, 172.7, 172.7, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m/z* calcd for $C_{48}H_{84}N_{20}O_{10}$ $[M-H]^-$ 1099.6607, found 1099.6616.

N-Heptadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azido-butyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**197**) as a mixture of rotamers



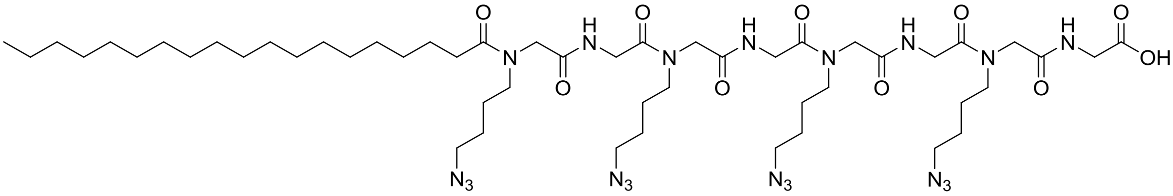
The saponification of **177** (200 mg, 177 μmol) with lithium hydroxide solution (221 μl, 443 μmol, 2 M) following general procedure B (method 2) afforded **197** (196 mg, 99.3%) as colorless, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.21–1.40 (m, 26H), 1.51–1.79 (m, 18H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.01–4.24 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 30.4, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 33.0, 33.7, 34.1, 34.1, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.3, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.1, 52.2, 170.7, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.4, 171.6, 172.7, 172.7, 176.1, 176.5, 176.5 ppm. HRMS (ESI-) *m/z* calcd for $C_{49}H_{86}N_{20}O_{10}$ $[M-H]^-$ 1113.6763, found 1113.6754.

N-Stearoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**198**) as a mixture of rotamers



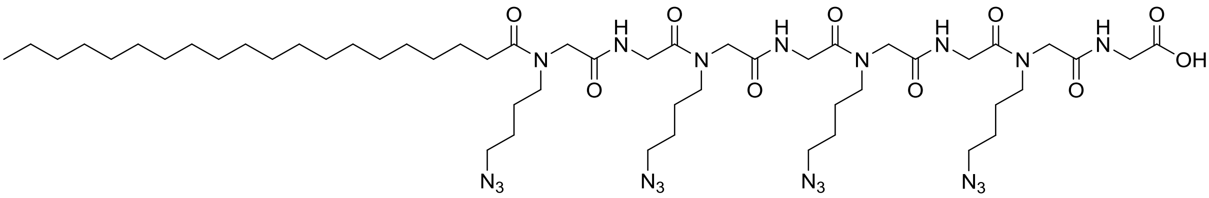
The saponification of **178** (200 mg, 175 μmol) with lithium hydroxide solution (219 μl, 438 μmol, 2 M) following general procedure B (method 2) afforded **198** (198 mg, quant.) as colorless, amorphous solid, containing a small, non-removable amount of ethanol. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.22–1.41 (m, 28H), 1.51–1.78 (m, 18H), 2.29–2.36 and 2,41–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.01–4.26 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.6, 26.9, 27.1, 27.2, 27.3, 30.4, 30.4, 30.5, 30.6, 30.6, 30.7, 30.7, 30.7, 30.8, 30.8, 30.8, 33.1, 33.7, 34.1, 34.2, 41.8, 41.9, 42.0, 42.1, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.7, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.4, 171.5, 171.6, 172.7, 172.8, 176.1, 176.6, 176.6 ppm. HRMS (ESI-) *m/z* calcd for $C_{50}H_{88}N_{20}O_{10}$ $[M-H]^-$ 1127.6920, found 1127.6925.

N-Nonadecanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azido-butyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**199**) as a mixture of rotamers



The saponification of **179** (200 mg, 173 μmol) with lithium hydroxide solution (217 μl, 433 μmol, 2 M) following general procedure B (method 2) afforded **199** (198 mg, quant.) as colorless, amorphous solid, containing a small, non-removable amount of ethanol. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.21–1.41 (m, 30H), 1.51–1.80 (m, 18H), 2.29–2.36 and 2.41–2.49 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.02–4.26 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 30.5, 30.6, 30.6, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.1, 34.2, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.4, 49.1, 50.0, 50.1, 50.8, 51.6, 52.2, 52.2, 170.8, 170.9, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.5, 171.6, 172.7, 172.7, 176.1, 176.5, 176.6 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{51}H_{90}N_{20}O_{10}$ $[M-H]^-$ 1141.7076, found 1141.7085.

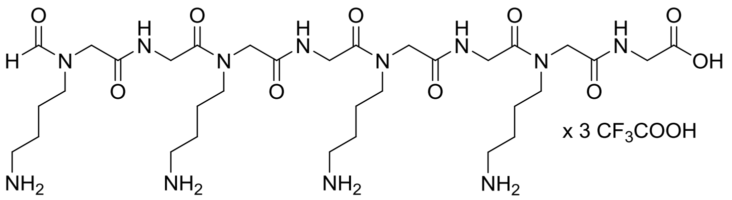
N-Arachidoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)-glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**200**) as a mixture of rotamers



The saponification of **180** (200 mg, 171 μmol) with lithium hydroxide solution (214 μl, 428 μmol, 2 M) following general procedure B (method 2) afforded **200** (190 mg, 96.0%) as colorless, amorphous solid, containing a small, non-removable amount of ethanol. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.22–1.40 (m, 32H), 1.51–1.79 (m, 18H), 2.29–2.36 and 2.41–2.48 (2m, 2H), 3.22–3.56 (m, 16H), 3.92 and 3.96 (2s, 2H), 4.01–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5, 23.7, 25.6, 25.7, 26.4, 26.4, 26.5, 26.9, 27.1, 27.2, 30.5, 30.6, 30.6, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.1, 34.2, 41.8, 41.9, 42.0, 42.1, 42.2, 47.8, 48.3, 49.1, 50.0, 50.1, 50.8, 51.6, 52.1, 52.2, 170.7, 170.8, 170.9, 170.9, 170.9, 171.0, 171.0, 171.2, 171.3, 171.3, 171.4, 171.4, 171.6 172.7, 172.7, 176.0, 176.5, 176.5 ppm. HRMS (ESI-) *m/z* calcd for $C_{52}H_{92}N_{20}O_{10}$ $[M\text{-}H]^-$ 1155.7233, found 1155.7243.

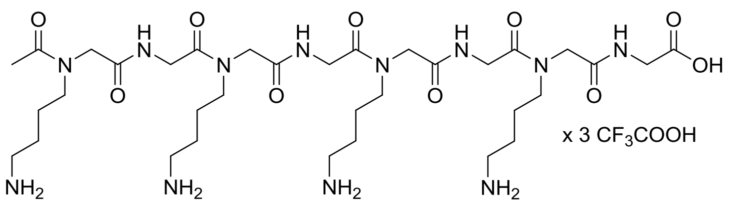
5.4.2.5 Staudinger reduction of the fourth generation azido-LPP methyl esters

N-Formyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**201**) as a mixture of rotamers



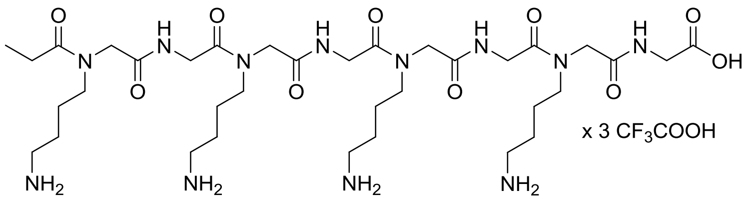
The deprotection of **160** (78 mg, 86.2 µmol) with triphenylphosphine (136 mg, 517 µmol) following general procedure C afforded **201** (78 mg, 80.1%) as white powder, containing small, non-removable amounts of ethanol and diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.55–1.80 (m, 16H), 2.90–3.02 (m, 8H), 3.37–3.51 (m, 8H), 3.80–3.92 (m, 2H), 3.97–4.27 (m, 14H), 8.06–8.11 and 8.14–8.20 (2m, 1H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.0, 25.0, 25.3, 25.5, 25.7, 25.7, 26.2, 26.3, 26.3, 26.4, 27.1, 27.2, 40.4, 41.8, 42.0, 42.2, 43.1, 48.3, (br), 49.4, 50.8, (br), 51.1, 52.1, 118.3 (q, $^1J_{C,F}$ = 292 Hz), 163.0 (q, $^2J_{C,F}$ = 34.2 Hz), 165.8, 166.6, 170.6, 170.8, 171.0, 171.0, 171.1, 171.2, 171.3, 171.4, 171.7, 171.7, 171.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI-) *m/z* calcd for $C_{33}H_{62}N_{12}O_{10}$ $[M\text{-}H]^-$ 785.4639, found 785.4643; (ESI+) $[M+H]^+$ 787.4785, found 787.4773.

N-Acetyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**202**) as a mixture of rotamers



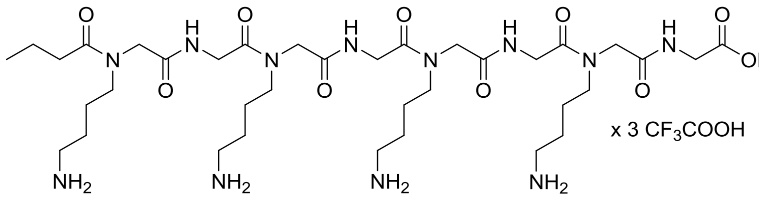
The deprotection of **161** (700 mg, 762 µmol) with triphenylphosphine (1199 mg, 4.57 mmol) following general procedure C afforded **202** (791 mg, 90.8%) as white powder, containing small, non-removable amounts of ethanol and diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.56–1.80 (m, 16H), 2.07–2.11 and 2.16–2.19 (m, brs, 3H), 2.89–3.03 (m, 8H), 3.38–3.52 (m, 8H), 3.75–3.91 (m, 2H), 3.94–4.28 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 21.2, 21.3, 21.3, 21.8, 25.3, 25.7, 26.3, 26.4, 26.5, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 43.5, 43.6, 47.6, 47.8, 48.2, (br), 48.3, 48.4, 48.5, 49.1, 49.2, 49.3, 49.4, 49.5, 50.1, 50.3, 50.4, 50.5, 50.6, 50.8, 50.8, 51.0, 51.2, 52.4, 52.6, 118.2 (q, $^1J_{C,F}$ = 293.2 Hz), 163.0 (q, $^2J_{C,F}$ = 34.3 Hz), 170.7, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.4, 171.6, 171.7, 171.7, 171.8, 171.8, 171.9, 173.9, 173.9, 174.6, 175.2 (br), 175.4 (br) ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI-) *m/z* calcd for $C_{34}H_{64}N_{12}O_{10}$ [M-H]$^-$ 799.4796, found 799.4797.

<u>*N*-Propionyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**203**) as a mixture of rotamers</u>



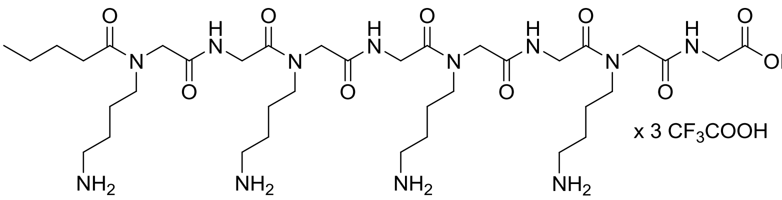
The deprotection of **163** (576 mg, 617 µmol) with triphenylphosphine (971 mg, 3.70 mmol) following general procedure C afforded **203** (371 mg, 51.7%) as white powder, containing small, non-removable amounts of ethanol and diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.06–1.15 (m, 3H), 1.55–1.82 (m, 16H), 2.30–2.42 and 2.45–2.53 (2m, 2H), 2.88–3.02 (m, 8H), 3.39–3.52 (m, 8H), 3.74–3.86 (m, 2H), 3.96–4.29 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 9.7, 9.8, 25.3, 25.4, 25.7, 25.8, 26.3, 26.5, 26.9, 27.3, 40.0, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 43.8 (br), 47.7, 47.9 (br), 48.2 (br), 48.4, 49.2 (br), 49.3, 49.4, 49.9, 50.0, 50.3, 50.4, (br), 50.6, 50.7, 50.9 (br), 51.1, 51.1, 51.5, 51.6, 51.7 (br), 118.2 (q, $^1J_{C,F}$ = 293.2 Hz), 163.0 (q, $^2J_{C,F}$ = 34.3 Hz), 170.7, 171.0, 171.0, 171.1, 171.2, 171.3, 171.3, 171.3, 171.4, 171.4, 171.6, 171.6, 171.7, 171.7, 171.8, 171.8, 172.0, 172.0, 172.0, 175.4, 175.4, 175.5, 175.6, 175.7, 176.9, 177.4 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI-) *m/z* calcd for $C_{35}H_{66}N_{12}O_{10}$ [M-H]$^-$ 813.4952, found 813.4948.

<u>*N*-Butyryl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**204**) as a mixture of rotamers</u>



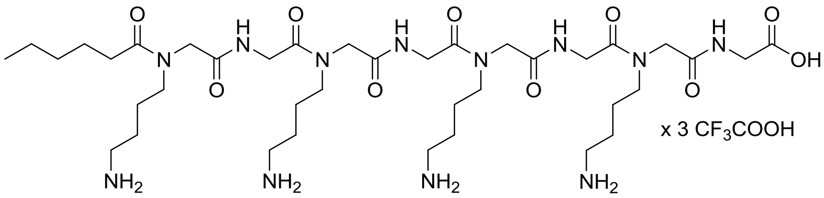
The deprotection of **164** (257 mg, 271 µmol) with triphenylphosphine (428 mg, 1.63 mmol) following general procedure C afforded **204** (234 mg, 73.7%) as off-white powder, containing a small, non-removable amount of diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.92–1.01 (m, 3H), 1.50–1.82 (m, 18H), 2.28–2.36 and 2.41–2.48 (2m, 2H), 2.88–3.02 (m, 8H), 3.38–3.52 (m, 8H), 3.75–3.86 (m, 2H), 3.96–4.28 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.2, 19.6, 19.7, 25.3, 25.4, 25.7, 25.8, 26.3, 26.6, 35.6, 36.0, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 43.8 (br), 47.7, 47.7, 47.8, 48.1, 48.1, 48.2, 48.2, 48.3, 48.4, 48.5, 49.2, 49.3, 49.4, 50.0, 50.2, 50.2, 50.4, 50.5, 50.6, 50.7, 50.8, 50.8, 51.1, 51.2, 51.7, 51.8, 51.8, 118.2 (q, $^1J_{C,F}$ = 293.2 Hz), 163.0 (q, $^2J_{C,F}$ = 34.4 Hz), 170.7, 170.9, 171.0, 171.0, 171.1, 171.1, 171.2, 171.3, 171.3, 171.4, 171.4, 171.6, 171.6, 171.7, 171.7, 171.7, 171.8, 171.9, 172.0, 175.5, 175.5, 175.7, 175.7, 176.1, 176.1, 176.6 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI-) *m/z* calcd for $C_{36}H_{68}N_{12}O_{10}$ $[M-H]^-$ 827.5109, found 827.5122.

N-Valeroyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**205**) as a mixture of rotamers



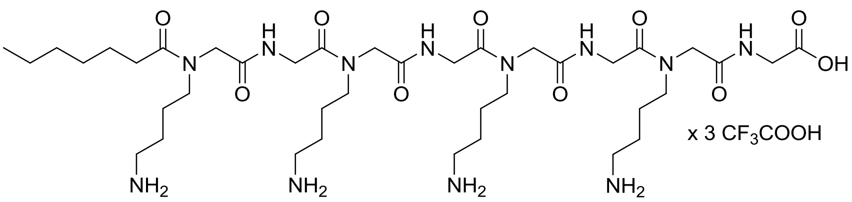
The deprotection of **165** (414 mg, 431 µmol) with triphenylphosphine (679 mg, 2.59 mmol) following general procedure C afforded **205** (415 mg, 81.2%) as off-white powder, containing a small, non-removable amount of diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.89–0.98 (m, 3H), 1.27–1.44 and 1.52–1.82 (2m, 20H), 2.30–2.38 and 2.42–2.50 (2m, 2H), 2.89–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.75–3.86 (m, 2H), 3.96–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 14.3, 23.5, 23.5, 25.3, 25.4, 25.7, 25.8, 26.3, 26.3, 26.4, 26.6, 28.4, 28.5, 33.5, 33.5, 33.9, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 43.6, 43.7, 47.7, 47.8, 47.9, 48.2, 48.3 (br), 48,4 48.5, 48.7, 49.2, 49.2, 49.2, 49.3, 49.4, 49.4, 50.1, 50.2, 50.3, 50.4, 50.4, 50.5, 50.6, 50.7, 50.9, 51.0, 51.2, 51.7, 51.9 (br), 118.2 (q, $^1J_{C,F}$ = 293.5 Hz), 163.0 (q, $^2J_{C,F}$ = 34.3 Hz), 170.7, 170.7, 171.0, 171.1, 171.1, 171.2, 171.3, 171.3, 171.4, 171.4, 171.5, 171.6, 171.6, 171.7, 171.7, 171.7, 171.7, 171.8, 171.9, 171.9, 172.0, 175.2 (br), 175.4 (br), 176.2, 176.2, 176.8 ppm. [19]F-NMR (376 MHz, CD_3OD) *δ* = -74.9 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{37}H_{70}N_{12}O_{10}$ $[M-H]^-$ 841.5265, found 841.5262.

N-Hexanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**206**) as a mixture of rotamers



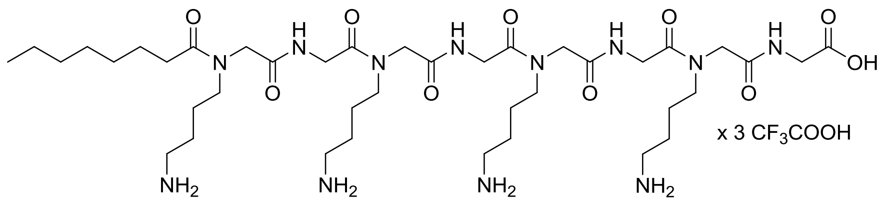
The deprotection of **166** (152 mg, 156 µmol) with triphenylphosphine (145 mg, 935 µmol) following general procedure C afforded **206** (174 mg, 93.0%) as off-white powder, containing small, non-removable amounts of ethanol and diethylether. [1]H-NMR (400 MHz, CD_3OD) *δ* = 0.88–0.95 (m, 3H), 1.27–1.40 (m, 4H), 1.54–1.81 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.89–3.03 (m, 8H), 3.37–3.54 (m, 8H), 3.74–3.84 (m, 2H), 3.96–4.30 (m, 14H) ppm. [13]C-NMR (100 MHz, CD_3OD) *δ* = 14.3, 23.6, 23.6, 25.3, 25.7, 25.8, 26.0, 26.1, 26.3, 26.4, 26.6, 32.6, 32.6, 33.7 (br), 34.1, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 43.9 (br), 47.7, 47.9, 48.1, 48.2 (br), 48.3 (br), 48.4, 49.2, 49.3, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.7, 50.8, 50.9, 50.9, 51.0, 51.1, 51.2, 51.7, 51.9 (br), 118.2 (q, $^1J_{C,F}$ = 293.1 Hz), 163.0 (q, $^2J_{C,F}$ = 34.5 Hz), 170.6, 170.9, 171.0, 171.0, 171.1, 171.2, 171.2, 171.3, 171.4, 171.5, 171.7, 171.7, 171.7, 171.8, 171.9, 175.5 8 (br), 175.7 (br), 176.2, 176.2, 176.8 ppm. [19]F-NMR (376 MHz, CD_3OD) *δ* = -74.9 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{38}H_{72}N_{12}O_{10}$ $[M-H]^-$ 855.5422, found 855.5419; (ESI+) $[M+H]^+$ 857.5567, found 857.5563.

N-Heptanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**207**) as a mixture of rotamers



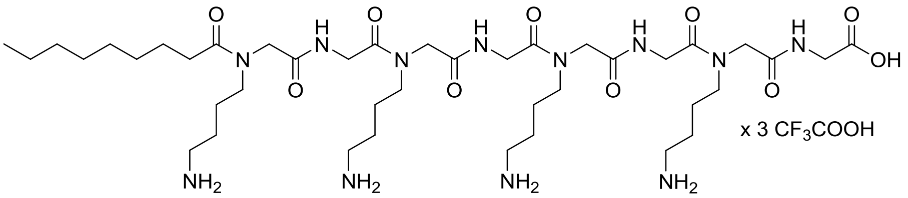
The deprotection of **167** (217 mg, 219 µmol) with triphenylphosphine (345 mg, 1.32 mmol) following general procedure C afforded **207** (251 mg, 94.5%) as off-white powder, containing small, non-removable amounts of ethanol and diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.94 (m, 3H), 1.25–1.42 (m, 6H), 1.52–1.81 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.87–3.03 (m, 8H), 3.37–3.53 (m, 8H), 3.74–3.85 (m, 2H), 4.02–4.28 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.6, 25.3, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.1, 30.1, 32.8, 32.9, 33.8, 34.1, 40.4, 40.4, 41.9, 42.0, 42.0, 42.2, 42.3, 42.4, 43.8, 47.7, 47.8, 48.1, 48.2, 48.3, 48.4, 48.5, 49.1, 49.2, 49.3, 49.4, 50.1, 50.2, 50.3, 50.4, 50.6, 50.7, 50.9, 51.2, 51.7, 51.8, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.3 Hz), 170.7, 171.0, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.7, 171.7, 171.7, 171.8, 171.9, 171.9, 172.0, 172.1, 175.4 (br), 175.7 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{39}H_{74}N_{12}O_{10}$ $[M+H]^+$ 871.5724, found 871.5712; $[M+2H]^{2+}$ 436.2898, found 436.2897.

<u>*N*-Octanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**208**) as a mixture of rotamers</u>



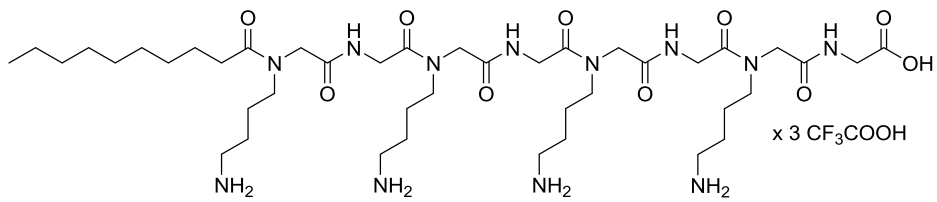
The deprotection of **168** (103 mg, 103 μmol) with triphenylphosphine (162 mg, 616 μmol) following general procedure C afforded **208** (102 mg, 80.7%) as off-white powder, containing small, non-removable amounts of ethanol and diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.25–1.39 (m, 8H), 1.53–1.81 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.03 (m, 8H), 3.36–3.54 (m, 8H), 3.71–3.84 (m, 2H), 4.02–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.3, 30.3, 30.4, 30.4, 32.9, 33.8, 34.1, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 44.0, 44.1, 47.7, 47.9, 48.1, 48.2, 48.3, 48.4, 48.4, 48.5, 49.3, 49.4, 49.6, 50.1, 50.2, 50.3, 50.4, 50.6, 50.6, 50.9, 50.9, 51.0, 51.1, 51.2, 51.7, 51.7, 51.9, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.1 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.6 Hz), 170.6, 170.9, 170.9, 171.0, 171.1, 171.2, 171.2, 171.3, 171.4, 171.4, 171.5, 171.6, 171.7, 171.7, 171.7, 171.9, 175.8, 176.0, 176.2, 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{40}H_{76}N_{12}O_{10}$ $[M+H]^+$ 885.5880, found 885.5894; $[M+2H]^{2+}$ 443.2976, found 443.2976.

N-Nonanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**209**) as a mixture of rotamers



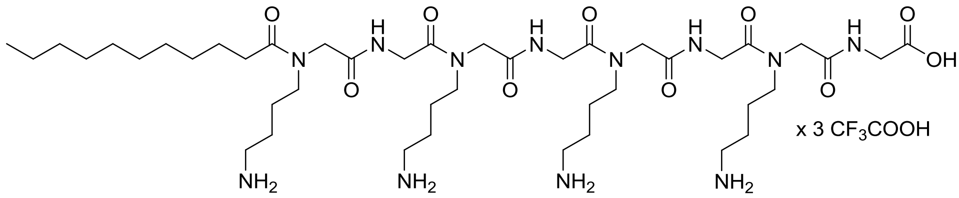
The deprotection of **169** (130 mg, 128 µmol) with triphenylphosphine (201 mg, 767 µmol) following general procedure C afforded **209** (146 mg, 91.9%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.23–1.40 (m, 10H), 1.53–1.81 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.72–3.84 (m, 2H), 3.96–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.4, 25.7, 25.8, 26.3, 26.4, 26.6, 30.3, 30.4 30.5, 30.6, 30.6, 33.0, 33.8, 34.2, 40.4, 40.4, 41.8, 42.0, 42.1, 42.2, 42.3, 44.0, 47.7, 47.9, 48.2, 48.4, 48.5, 48.6, 49.4, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.7, 50.8, 50.9, 51.2, 51.3, 51.7, 51.9, 118.3 (q, $^{1}J_{C,F}$ = 293.0 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.5 Hz), 170.6, 170.6, 170.9, 171.1, 171.1, 171.2, 171.3, 171.3, 171.4, 171.5, 171.5, 171.7, 171.7, 171.9, 175.7 (br), 175.9 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{41}H_{78}N_{12}O_{10}$ $[M+H]^+$ 899.6037, found 899.6043; $[M+2H]^{2+}$ 450.3055, found 450.3054.

N-Decanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**210**) as a mixture of rotamers



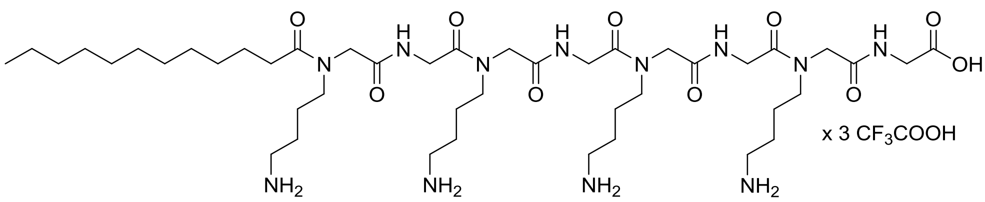
The deprotection of **170** (192 mg, 186 µmol) with triphenylphosphine (293 mg, 1.12 mmol) following general procedure C afforded **210** (214 mg, 91.7%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.93 (m, 3H), 1.23–1.41 (m, 12H), 1.53–1.82 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.77 and 3.81 (2brs, 2H), 4.02–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.4, 30.5, 30.6, 30.7, 33.0, 33.8, 34.2, 34.2, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 43.9, 47.7, 47.8, 48.1, 48.2, 48.3, 48.4, 49.3, 49.5, 50.1, 50.2, 50.4, 50.6, 50.9 (br), 51.1, 51.2, 51.7, 51.9 (br), 118.2 (q, ${}^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, ${}^{2}J_{C,F}$ = 34.3 Hz), 170.6, 170.6, 170.9, 171.0, 171.0, 171.1, 171.2, 171.3, 171.4, 171.4, 171.5, 171.6, 171.7, 171.7, 171.8, 171.9, 171.9, 171.9, 172.0, 175.6 (br), 175.8 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{42}H_{80}N_{12}O_{10}$ $[M+H]^+$ 913.6193, found 913.6179; $[M+2H]^{2+}$ 457.3133, found 457.3134.

N-Undecanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-amino-butyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**211**) as a mixture of rotamers



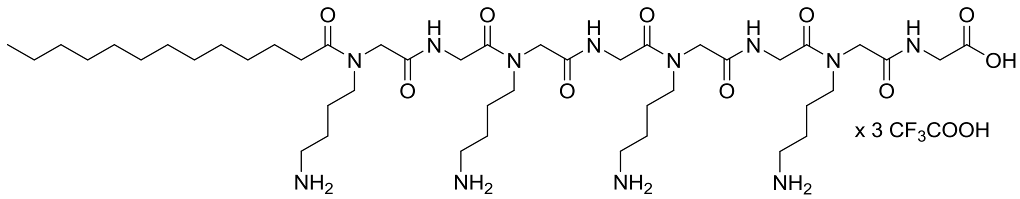
The deprotection of **171** (205 mg, 196 μmol) with triphenylphosphine (309 mg, 1.18 mmol) following general procedure C afforded **211** (225 mg, 90.4%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.22–1.39 (m, 14H), 1.52–1.80 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.86–3.03 (m, 8H), 3.34–3.54 (m, 8H), 3.77 and 3.81 (2brs, 2H), 4.00–4.28 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.5, 30.5, 30.6, 30.7, 30.7, 33.0, 33.8, 33.8, 34.2, 40.4, 40.4, 40.4, 40.4, 41.9, 42.0, 42.1, 42.2, 42.3, 42.4, 43.9, 47.7, 47.8, 48.1, 48.2, 48.3, 48.4, 48.5, 49.3, 49.4, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.8, 50.9, 50.9, 51.1, 51.2, 51.7, 51.9, 51.9, 118.2 (q, ${}^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, ${}^{2}J_{C,F}$ = 34.3 Hz), 170.6, 171.0, 171.1, 171.1, 171.2, 171.3, 171.4, 171.4, 171.5, 171.6, 171.7, 171.9, 171.9, 175.6 (br), 175.8 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{43}H_{82}N_{12}O_{10}$ $[M+H]^+$ 927.6350, found 927.6360; $[M+2H]^{2+}$ 464.3211, found 464.3211.

N-Lauroyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**212**) as a mixture of rotamers



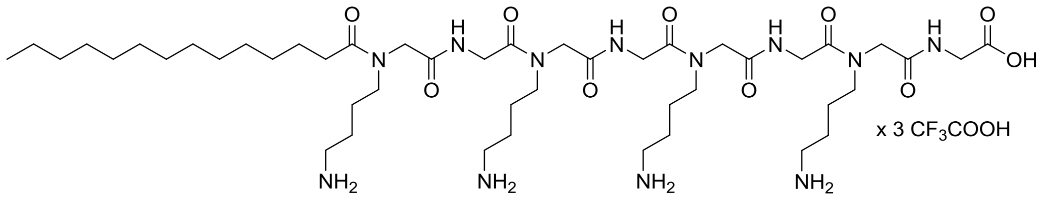
The deprotection of **172** (252 mg, 238 μmol) with triphenylphosphine (374 mg, 1.43 mmol) following general procedure C afforded **212** (280 mg, 91.7%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.23–1.40 (m, 16H), 1.53–1.80 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.03 (m, 8H), 3.36–3.56 (m, 8H), 3.78 and 3.81 (2brs, 2H), 3.96–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.4, 30.5, 30.6, 30.7, 30.7, 33.0, 33.8 (br), 34.2, 40.4, 40.4, 41.8, 41.9, 41.9, 42.0, 42.1, 42.2, 42.3, 42.4, 43.8, 43.9, 47.7, 47.9, 48.1, 48.1, 48.3, 48.4, 49.3, 49.4, 49.4, 50.0, 50.2, 50.3, 50.4, 50.6, 50.7, 50.8, 50.9, 50.9, 51.1, 51.2, 51.7, 51.8, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.4 Hz), 170.6, 171.0, 171.0, 171.0, 171.1, 171.2, 171.2, 171.3, 171.3, 171.4, 171.5, 171.7, 171.7, 171.8, 171.9, 172.0, 175.5 (br), 175.7 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{44}H_{84}N_{12}O_{10}$ $[M+H]^+$ 941.6506, found 941.6484; $[M+2H]^{2+}$ 471.3289, found 471.3288.

N-Tridecanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-amino-butyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**213**) as a mixture of rotamers



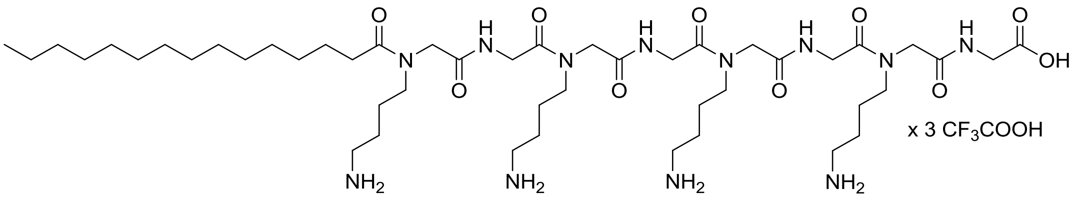
The deprotection of **173** (210 mg, 196 μmol) with triphenylphosphine (308 mg, 1.17 mmol) following general procedure C afforded **213** (221 mg, 86.9%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.22–1.40 (m, 18H), 1.50–1.81 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 3.87–3.05 (m, 8H), 3.34–3.54 (m, 8H), 3.79 and 3.82 (2brs, 2H), 3.94–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.5, 30.5, 30.6, 30.7, 30.7, 30.8, 33.1, 33.8, 34.2, 40.4, 40.4, 41.9, 42.0, 42.0, 42.2, 42.3, 42.4, 43.8, 47.7, 47.9, 48.1, 48.2, 48.2, 48.4, 49.2, 49.3, 49.4, 49.4, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.8, 50.9, 50.9, 51.2, 51.7, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.2 Hz), 170.7, 171.0, 171.1, 171.2, 171.3, 171.4, 171.4, 171.5, 171.7, 171.7, 171.9, 172.0, 172.0, 175.4 (br), 175.6 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m/z* calcd for $C_{45}H_{86}N_{12}O_{10}$ $[M+H]^+$ 955.6663, found 955.6684; $[M+2H]^{2+}$ 478.3368, found 478.3374.

N-Myristoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**214**) as a mixture of rotamers



The deprotection of **174** (342 mg, 315 µmol) with triphenylphosphine (495 mg, 1.89 mmol) following general procedure C afforded **214** (383 mg, 92.7%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.24–1.39 (m, 20H), 1.53–1.80 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.79 and 3.82 (2brs, 2H), 3.98–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 26.3, 26.3, 26.4, 26.6, 26.6, 30.5, 30.5, 30.6, 30.7, 30.8, 30.8, 33.1, 33.8 (br), 34.2, 40.4, 40.4, 41.9, 42.0, 42.0, 42.2, 42.3, 42.4, 43.7 (br), 47.7, 47.9, 48.3 (br), 49.3, 49.5, 50.1, 50.2, 50.4, 50.6, 50.8, 50.9, 50.9, 51.2, 51.7, 51.9 (br), 118.2 (q, $^1J_{C,F}$ = 293.1 Hz), 163.0 (q, $^2J_{C,F}$ = 34.4 Hz), 170.7, 171.0, 171.0, 171.0, 171.1, 171.3, 171.4, 171.4, 171.5, 171.7, 171.8, 171.8, 172.0, 175.3 (br), 175.5 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{46}H_{88}N_{12}O_{10}$ $[M+H]^+$ 969.6819, found 969.6812; $[M+2H]^{2+}$ 485.3446, found 485.3445.

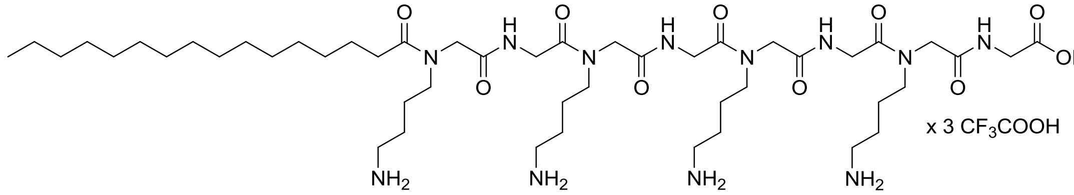
N-Pentadecanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-amino-butyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**215**) as a mixture of rotamers



The deprotection of **175** (693 mg, 630 µmol) with triphenylphosphine (992 mg, 3.78 mmol) following general procedure C afforded **215** (452 mg, 54.1%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.23–1.40 (m, 22H), 1.54–1.80 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.87–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.76–3.92 (m, 2H), 3.96–4.32 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.5, 30.7, 30.7, 30.8, 30.8, 30.8, 33.8, 34.2, 40.4, 40.4, 42.0, 42.2, 42.3, 43.7, 48.4, 49.1, 49.3, 49.5, 49.7, 50.1, 50.3, 50.6, 50.8, 51.2, 118.2 (q, $^1J_{C,F}$ = 293.1 Hz), 163.0 (q,

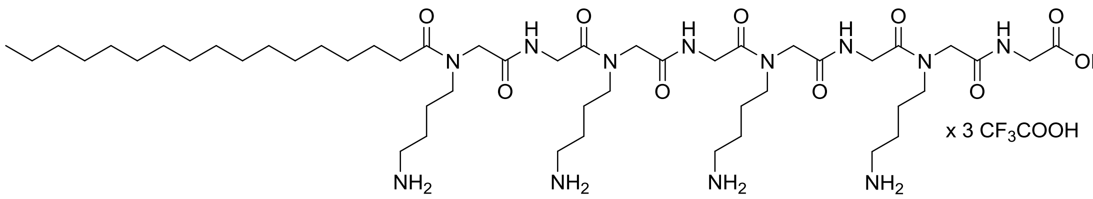
$^{2}J_{C,F}$ = 34.4 Hz), 170.7, 171.0, 171.2, 171.3, 171.3, 171.4, 171.6, 171.7, 171.9, 175.3 (br), 175.5 (br), 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) *δ* = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{47}H_{90}N_{12}O_{10}$ $[M+H]^+$ 983.6976, found 983.6977; $[M+2H]^{2+}$ 492.3524, found 492.3517; $[M+3H]^{3+}$ 328.5707, found 328.5707; (ESI-) $[M-H]^-$ 981.6830, found 981.6826.

N-Palmitoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**216**) as a mixture of rotamers



The deprotection of **176** (201 mg, 180 μmol) with triphenylphosphine (284 mg, 1.08 mmol) following general procedure C afforded **216** (226 mg, 93.7%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) *δ* = 0.87–0.92 (m, 3H), 1.23–1.40 (m, 24H), 1.54–1.80 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.77 and 3.80 (2brs, 2H), 3.96–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) *δ* = 14.4, 23.7, 25.3, 25.7, 26.3, 26.3, 26.4, 26.6, 30.5, 30.5, 30.7, 30.7, 30.7, 30.8, 33.1, 33.8, 34.2, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 44.0 (br), 47.7, 47.9, 48.1, 48.2, 48.3, 48.5, 48.5, 49.2, 49.3, 49.5, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.8, 50.9, 50.9, 51.1, 51.2, 51.7, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.1 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.4 Hz), 170.6, 170.9, 171.1, 171.1, 171.2, 171.3, 171.3, 171.3, 171.4, 171.5, 171.6, 171.7, 171.7, 171.9, 171.9, 172.0, 175.7 (br), 175.9 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) *δ* = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{48}H_{92}N_{12}O_{10}$ $[M+H]^+$ 997.7132, found 997.7130; $[M+2H]^{2+}$ 499.3602, found 499.3598.

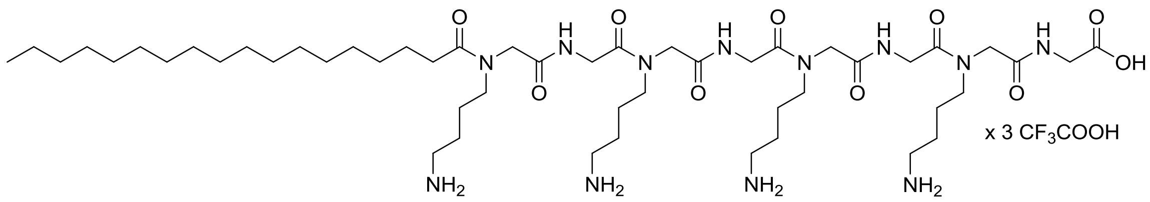
N-Heptadecanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-amino-butyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**217**) as a mixture of rotamers



The deprotection of **177** (200 mg, 177 μmol) with triphenylphosphine (279 mg, 1.06 mmol) following general procedure C afforded **217** (220 mg, 91.8%) as white powder. ^{1}H-NMR (400

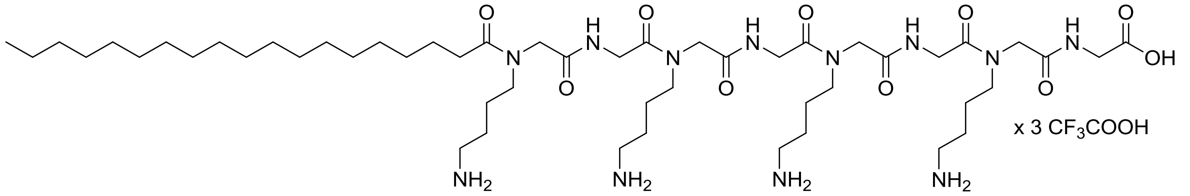
MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.23–1.39 (m, 26H), 1.53–1.80 (m, 18H), 2.30–2.38 and 2.42–2.48 (2m, 2H), 2.88–3.03 (m, 8H), 3.36–3.55 (m, 8H), 3.73–3.84 (m, 2H), 3.96–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.5, 30.5, 30.7, 30.7, 30.7, 30.8, 33.1, 33.8, 34.2, 40.4, 40.4, 41.8, 42.0, 42.2, 42.3, 42.4, 47.7, 47.9, 48.1, 48.2, 48.3, 48.4, 48.5, 49.4, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.6, 50.8, 50.8, 50.9, 51.1, 51.2, 51.7, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.2 Hz), 170.6, 171.0, 171.0, 171.0, 171.1, 171.2, 171.2, 171.3, 171.4, 171.5, 171.5, 171.6, 171.7, 171.7, 171.7, 171.9, 171.9, 175.6 (br), 175.8 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{49}H_{94}N_{12}O_{10}$ $[M+H]^+$ 1011.7289, found 1011.7303; $[M+2H]^{2+}$ 506.3681, found 506.3674.

N-Stearoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**218**) as a mixture of rotamers



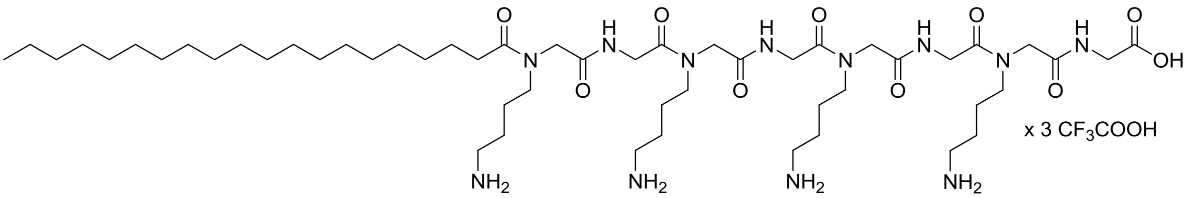
The deprotection of **178** (207 mg, 181 μmol) with triphenylphosphine (285 mg, 1.09 mmol) following general procedure C afforded **218** (202 mg, 81.6%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.23–1.40 (m, 28H), 1.53–1.81 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 2.88–3.04 (m, 8H), 3.36–3.56 (m, 8H), 3.73–3.84 (m, 2H), 3.96–4.32 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 26.3, 26.4, 26.6, 30.5, 30.5, 30.7, 30.7, 30.7, 30.8, 33.1, 33.8, 34.2, 40.4, 40.4, 41.9, 42.0, 42.2, 42.3, 42.4, 44.0, 47.7, 47.9, 48.1, 48.2, 48.3, 48.5, 48.5, 49.2, 49.2, 49.3, 49.5, 50.1, 50.2, 50.3, 50.4, 50.6, 50.6, 50.8, 50.9, 51.0, 51.1, 51.2, 51.7, 51.9, 51.9, 118.2 (q, $^{1}J_{C,F}$ = 293.0 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.4 Hz), 170.6, 170.9, 170.9, 171.0, 171.1, 171.2, 171.3, 171.3, 171.3, 171.4, 171.5, 171.5, 171.6, 171.7, 171.7, 171.7, 171.8, 171.8, 171.9, 171.9, 171.9, 175.7 (br), 175.9, 175.9, 176.2, 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{50}H_{96}N_{12}O_{10}$ $[M+H]^+$ 1025.7445, found 1025.7468; $[M+2H]^{2+}$ 513.3759, found 513.3750.

N-Nonadecanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-amino-butyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**219**) as a mixture of rotamers



The deprotection of **179** (161 mg, 139 μmol) with triphenylphosphine (219 mg, 0.83 mmol) following general procedure C afforded **219** (160 mg, 83.3%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.22–1.40 (m, 30H), 1.54–1.80 (m, 18H), 2.30–2.38 and 2.42–2.48 (2m, 2H), 2.88–3.03 (m, 8H), 3.36–3.56 (m, 8H), 3.72–3.85 (m, 2H), 3.96–4.30 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 26.6, 30.5, 30.5, 30.7, 30.7, 30.7, 30.8, 33.1, 33.8, 34.2, 40.4, 40.4, 41.8, 41.9, 42.0, 42.2, 42.3, 42.4, 44.0, 47.7, 47.9, 48.1, 48.2, 48.4, 48.5, 48.6, 49.1, 49.4, 50.1, 50.2, 50.4, 50.6, 50.7, 50.9, 51.0, 51.0, 51.1, 51.3, 51.7, 51.9, 118.3 (q, $^1J_{C,F}$ = 292.8 Hz), 163.0 (q, $^2J_{C,F}$ = 34.2 Hz), 170.6, 170.7, 170.9, 171.0, 171.1, 171.1, 171.2, 171.3, 171.3, 171.4, 171.4, 171.5, 171.6, 171.6, 171.7, 171.7, 171.8, 171.9, 172.0, 175.7 (br), 175.8 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{51}H_{98}N_{12}O_{10}$ $[M+H]^+$ 1039.7602, found 1039.7632; $[M+2H]^{2+}$ 520.3837, found 520.3822.

N-Arachidoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)-glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**220**) as a mixture of rotamers

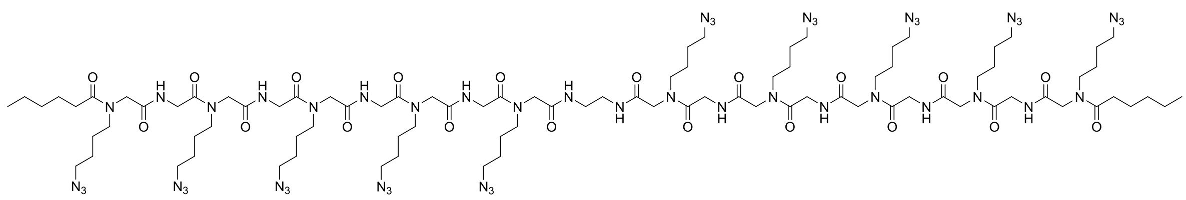


The deprotection of **180** (206 mg, 176 μmol) with triphenylphosphine (277 mg, 1.06 mmol) following general procedure C afforded **220** (200 mg, 81.4%) as white powder. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H), 1.24–1.38 (m, 32H), 1.53–1.80 (m, 18H), 2.30–2.38 and 2.42–2.49 (2m, 2H), 3.87–3.04 (m, 8H), 3.36–3.54 (m, 8H), 3.74–3.84 (m, 2H), 3.96–4.28 (m, 14H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.7, 25.3, 25.7, 25.8, 26.3, 26.4, 26.6, 30.5, 30.5, 30.7, 30.7, 30.7, 30.8, 33.1, 33.8, 34.2, 40.4, 40.4, 41.8, 42.0, 42.2, 42.3, 42.4, 43.9, 47.7, 47.9, 47.9, 48.2, 48.4, 48.5, 48.6, 49.2, 49.4, 50.1, 50.2, 50.3, 50.4, 50.7, 50.8, 50.9, 50.9, 51.0, 51.3, 51.7, 51.9, 118.3 (q, $^{1}J_{C,F}$ = 292.8 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.2 Hz), 170.6, 170.9, 171.0, 171.0, 171.0, 171.1, 171.2, 171.2, 171.3, 171.4, 171.4, 171.4, 171.5, 171.6, 171.6, 171.7, 171.7, 171.8, 171.8, 171.9, 171.9, 171.9, 175.6 (br), 175.8 (br), 176.2, 176.2, 176.8 ppm. ^{19}F-NMR (376 MHz, CD_3OD) δ = -74.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{52}H_{100}N_{12}O_{10}$ $[M+H]^+$ 1053.7758, found 1053.7784; $[M+2H]^{2+}$ 527.3915, found 527.3908; $[M+3H]^{3+}$ 351.9301, found 351.9306.

5.4.2.6 Synthesis of a deca-cationic amino-LPP

Ugi four-component reaction

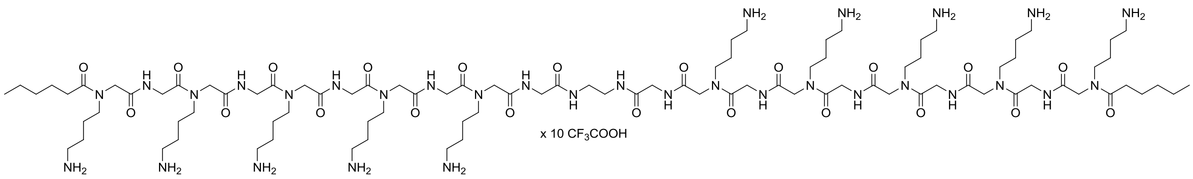
N,*N*'-Bis(*N*-hexanoyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycyl)ethylene diamine (**225**) as a mixture of rotamers



Paraformaldehyde (15.6 mg, 520 µmol), **16** (35.6 mg, 312 µmol), **186** (150 mg, 156 µmol) and 0.5 equivalents of **222** (6.3 mg, 78 µmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (dichloromethane/methanol 9:1) to afford **225** (52 mg, 29.6%) as colorless, amorphous solid. R_f 0.40 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.88–0.96 (m, 6H), 1.25–1.42 and 1.52–1.79 (2m, 52H), 2.30–2.36 and 2.42–2.48 (2m, 4H), 3.26–3.50 (m, 44H), 3.87–4.30 (m, 36H), 7.70–8.30 (m, 10H, weak) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.4, 23.6, 25.7, 26.2, 26.2, 26.6, 26.8, 27.0, 27.1, 27.2, 27.3, 32.6, 32.7, 33.7, 34.1, 40.2 (br), 40.4 (br), 42.0, 42.1, 42.3, 47.9, 48.4, 49.1, 49.5, 50.1, 50.8, 51.0, 61.7, 52.1, 52.2, 52.2, 170.8, 170.9, 170.9, 170.9, 171.1, 171.2, 171.2, 171.3, 171.4, 171.5, 171.6, 176.1, 176.5, 176.6 ppm. HRMS (ESI-) *m*/*z* calcd for $C_{90}H_{152}N_{50}O_{20}$ $[M-2H]^{2-}$ 1125.6134, found 1125.6168.

Staudinger reduction

N,*N*'-Bis(*N*-hexanoyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycyl)ethylene diamine deca(trifluoroacetate) (**227**) as a mixture of rotamers

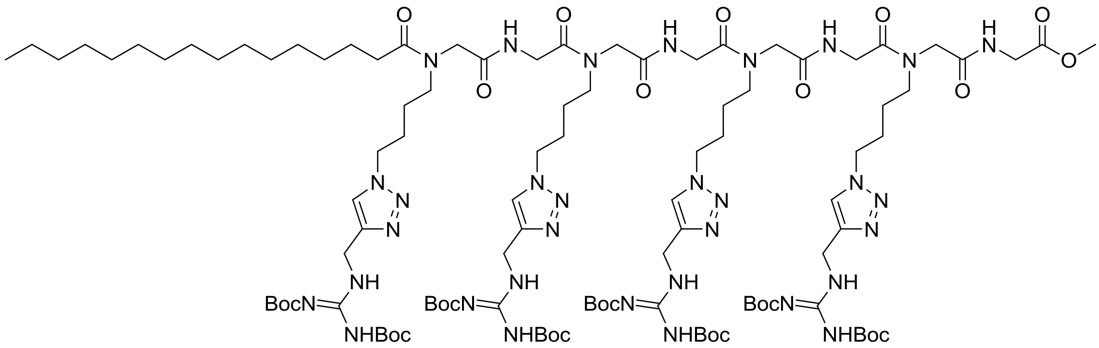


The deprotection of **225** (52 mg, 23.1 μmol) with 15 equivalents of triphenylphosphine (91 mg, 346 mmol) following general procedure C afforded **227** (62 mg, 82.6%) as white powder, containing a small, non-removable amount of diethylether. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.88–0.95 (m, 6H), 1.26–1.41 and 1.53–1.81 (2m, 52H), 2.30–2.37 and 2.42–2.48 (2m, 4H), 2.89–3.04 (m, 20H), 3.30–3.52 (m, 24H), 3.97–4.28 (m, 36H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.3, 23.6, 23.6, 25.2, 25.4, 25.7, 25.8, 26.0, 26.1, 26.3, 26.3, 26.7, 32.6, 32.7, 33.7, 34.1, 40.4, 41.9, 41.9, 42.0, 42.0, 42.1, 42.3, 47.6, 47.8, 48.1, 48.2, 48.3, 49.2, 49.2, 50.1, 50.1, 50.2, 50.3, 50.4, 50.8, 50.9, 51.0, 51.6, 51.7, 51.8, 118.3 (q, $^{1}J_{C,F}$ = 293.2 Hz), 163.0 (q, $^{2}J_{C,F}$ = 34.3 Hz), 170.5, 170.6, 170.8, 171.0, 171.1, 171.2, 171.3, 171.4, 171.5, 171.6, 171.6, 171.8, 171.9, 176.2, 176.8 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{90}H_{172}N_{30}O_{20}$ $[M+2H]^{2+}$ 997.6755, found 997.6758; $[M+3H]^{3+}$ 665.4527, found 665.4531; $[M+4H]^{4+}$ 499.3414, found 499.3422; $[M+5H]^{5+}$ 399.6746, found 399.6750.

5.4.2.7 Synthesis of guanidine derivatives of a azido-LPP methyl ester

Azide-alkyne Huisgen cycloaddition

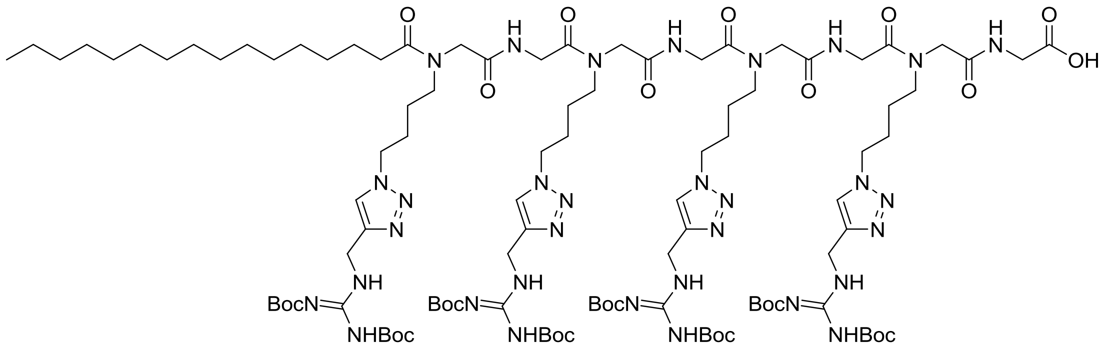
N-Palmitoyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycine methyl ester (**229**) as a mixture of rotamers



176 (112 mg, 100 µmol) and **228** (131 mg, 440 µmol) were reacted together following general procedure D. Purification was accomplished by silica column chromatography (dichloromethane/methanol 10:1) to afford **229** (152 mg, 66.0%) as colorless, amorphous solid. R_f 0.63 (dichloromethane/methanol 9:1). ^{1}H-NMR (400 MHz, C_6D_6) δ = 0.90–0.96 (m, 3H, C*H*$_3$CH$_2$), 1.05–1.95 (m, 114H, 8(C*H*$_3$)$_3$C, 4C*H*$_2$C*H*$_2$CH$_2$N, CH$_3$(C*H*$_2$)$_{13}$), 2.27–2.60 (m, 2H, CH$_2$C*H*$_2$CO), 3.36 and 3.55 (2brs, 13H, C*H*$_2$COOC*H*$_3$, 4triazole-C*H*$_2$CH$_2$), 3.75–4.60 (m, 26H, 4CON(C*H*$_2$)$_2$, 3CONHC*H*$_2$CONR$_2$, 4N*H*CH$_2$triazole), 4.82 (brs, 8H, 4NHC*H*$_2$triazole), 7.32–8.63 (m, 4H, 4CON*H*), 9.05 (brs, 4H, 4C$_2$N$_3$*H*), 12.13 (brs, 4H, 4(CH$_3$)$_3$COCON*H*) ppm. ^{13}C-NMR (100 MHz, C_6D_6) δ = 14.4 (*C*H$_3$(CH$_2$)$_{14}$), 23.1 (CH$_3$*C*H$_2$), 24.6 (br), 24.7, (br), 25.3 (br), 25.5 (br), 25.9 (br), 27.8 ((*C*H$_3$)$_3$C), 28.4 ((*C*H$_3$)$_3$C), 29.8, 30.0, 30.1, 30.2, 30.2, 30.2, 30.3, 32.3, 33.2, 33.5 36.8, 41.4 and 41.6 (2br, CO*C*H$_2$NH), 46.4 (br), 47.2 (br), 48.1 (br), 48.9 (br), 49.8, 49.8, 50.1, 51.2, 52.0 and 52.1 (COO*C*H$_3$), 78.8 (3x, (CH$_3$)$_3$*C*), 82.6 and 82.7 ((CH$_3$)$_3$*C*), 122.7 and 122.9 (triazole-*C*H), 144.2 (triazole-*C*$_{\text{quart}}$), 153.3 and 156.5 ((CH$_3$)$_3$CO*C*O), 164.2 and 164.3 (guanidine-*C*), 169.7, 169.8, 170.3, 170.2, 170.8, 171.0, 173.9, 173.9, 174.0, 174.1, 176.9 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{105}H_{178}N_{32}O_{26}$ $[M+H+Na]^{2+}$ 1163.6778, found 1163.6773; $[M+2Na]^{2+}$ 1174.6687, found 1174.6633; $[M+Na+K]^{2+}$ 1182.6557, found 1182.6595.

Saponification

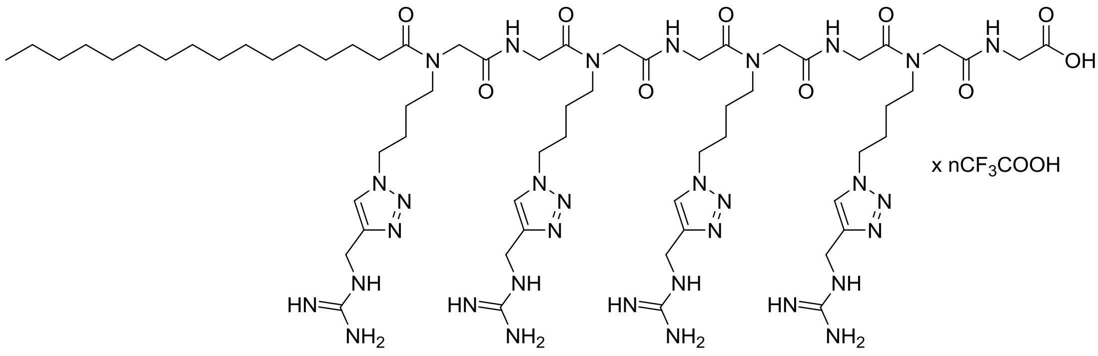
N-Palmitoyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)-glycylglycine (**230**) as a mixture of rotamers



The saponification of **229** (143 mg, 62.1 µmol) with lithium hydroxide solution (77.7 µl, 155.3 µmol, 2 M) following general procedure B (method 1) afforded **230** (138 mg, 97.0%) as white, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.86–0.92 (m, 3H, CH_3CH_2), 1.20–1.36 and 1.38–1.73 and 1.82–2.02 (3m, 114H, 8$(CH_3)_3C$, 4$CH_2CH_2CH_2N$, $CH_3(CH_2)_{13}$), 2.28–2.43 (m, 2H, CH_2CH_2CO), 3.36–3.52 (m, 8H, 4triazole-CH_2CH_2), 3.84–3.98 and 4.00–4.24 (2m, 16H, 7NCH_2CO, NHCH_2COOH), 4.35–4.51 (m, 8H, 4NCH_2CH_2), 4.69 (brs, 8H, 4NHCH_2triazole), 7.96–8.06 (m, 4H, 4C_2N_3H) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5 (2x, $CH_3(CH_2)_{14}$), 23.7 (CH_3CH_2), 25.3, 25.3, 26.2, 26.2, 26.4, 26.4, 26.5, 28.2 and 28.3 ($(CH_3)_3C$), 28.4, and 28.5 ($(CH_3)_3C$), 30.5, 30.7, 30.7, 30.8, 30.8, 33.1, 33.7, 34.2, 37.3, 41.8 and 41.9–42.4 (br, COCH_2NH), 47.5, 48.0, 50.1, 50.9, 51.0, 51.1, 51.7, 71.5 ($(CH_3)_3C$), 81.8 and 81.9 ($(CH_3)_3C$), 85.1 ($(CH_3)_3C$), 124.7 (triazole-CH), 144.7 and 144.9 (2br, triazole-C_{quart}), 153.7 and 157.0 ($(CH_3)_3COCO$), 162.4 (guanidine-C), 170.9, 171.0, 171.0, 171.2, 171.4, 171.4, 171.5, 171.6, 172.7, 172.8, 176.1, 176.6, 176.6 ppm. HRMS (ESI+) *m/z* calcd for $C_{104}H_{176}N_{32}O_{26}$ $[M+2H]^{2+}$ 1145.6801, found 1145.6808; $[M+H+Na]^{2+}$ 1156.6710, found 1156.6689; $[M+2Na]^{2+}$ 1167.6620, found 1167.6597; $[M+2H+Na]^{3+}$ 771.4490, found 771.4492.

Boc-deprotection

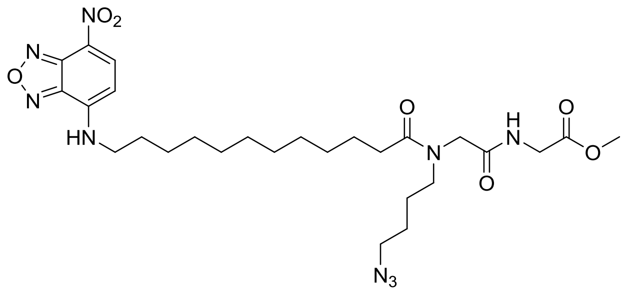
N-Palmitoyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)-butyl)glycylglycyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycine trifluoroacetic acid salt (**231**) as a mixture of rotamers



To a solution of **230** (102 mg, 44.5 µmol) in dichloromethane (3 ml) was added trifluoroacetic acid (1.37 ml, 2.03 g, 17.80 mmol) and the mixture was stirred at room temperature for two hours. After addition of toluene (10 ml) all volatiles were removed at a rotavap at 50 °C to yield **231** (91 mg, quant.) as light yellow foam. The crude product was used without further purification. ^{1}H-NMR (400 MHz, CD_3OD) δ = 0.87–0.92 (m, 3H, C$H_3$$CH_2$), 1.21–1.37 and 1.46–1.68 and 1.82–2.03 (3m, 42H, 4CH_2C$H_2$$CH_2$N, CH_3(CH_2)$_{13}$), 2.28–2.35 and 2.38–2.46 (2m, 2H, CH_2CH_2CO), 3.34–3.50 (m, 8H, 4triazole-C$H_2$$CH_2$), 3.83–4.22 (m, 16H, 7NCH_2CO, NHCH_2COOH), 4.39–4.52 (m, 16H, 4NC$H_2$$CH_2$, 4NHC$H_2$triazole), 7.97–8.06 (m, 4H, 4C_2N_3*H*) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 14.5 (*C*H_3(CH_2)$_{14}$), 23.7 (CH_3*C*H_2), 25.2, 26.0, 26.4, 26.4, 28.2, 28.2, 28.3, 28.4, 28.5, 30.5, 30.7, 30.7, 30.8, 30.8, 33.1, 33.8, 34.2, 35.8, 37.6, 41.8–42.4 (br, CO*C*H_2NH), 47.6, 48.0, 48.9, 49.9, 50.2, 50.2, 50.8, 50.9, 51.0, 51.0, 51.7, 124. (triazole-*C*H), 144.3 (triazole-C_{quart}), 158.8 (2x, guanidine-*C*), 162.8 (br), 171.1, 171.1, 171.1, 171.2, 171.4, 171.5, 171.5, 171.6, 171.2, 171.8, 172.9, 172.9, 176.3, 176.7, 176.8 ppm. HRMS (ESI+) *m/z* calcd for $C_{64}H_{112}N_{32}O_{10}$ $[M+2H]^{2+}$ 745.4692, found 745.4691; $[M+3H]^{3+}$ 497.3152, found 497.3151; $[M+4H]^{4+}$ 373.2383, found 373.2385 ppm.

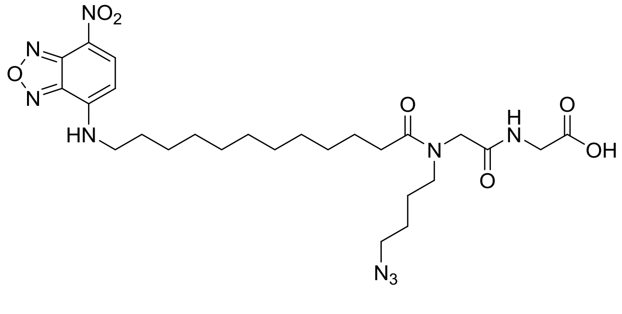
5.4.2.8 Synthesis of NDB labeled LPP derivatives

N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycine methyl ester (**235**) as a mixture of rotamers



Paraformaldehyde **19** (250 mg, 8.33 mmol), **16** (571 mg, 5.00 mmol), **234** (1892 mg, 5.00 mmol) and **20** (454 µl, 495 mg, 5.00 mmol) were reacted together following general procedure A. Purification was accomplished by silica column chromatography (*n*-hexane/ethyl acetate 1:4) to afford **235** (2108 mg, 69.8%) as dark red oil, which solidified on standing. R_f 0.23 (*n*-hexane/ethyl acetate 1:4). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.20–1.52 and 1.54–1.87 (2m, 22H, $(CH_2)_9CH_2COOMe$, $(CH_2)_2CH_2N_3$), 2.30 and 2.40 (2t, ^3J = 7.5, 7.6 Hz, 2H, CH_2CH_2CON), 3.28–3.37 (m, 2H, aromatNHCH_2), 3.40–3.58 (m, 4H, $NCH_2(CH_2)_2CH_2N_3$), 3.73 and 3.75 (2s, 3H, $COOCH_3$), 4.00 and 40.1 and 4.05 and 4.06 and 4.10 and 4.12 (6s, 4H, 2NCH_2CO), 6.19 (d, ^3J = 8.7 Hz, 1H, NHC=C*H*), 6.73–6.96 and 7.00–7.10 (2m, 2H, 2N*H*), 8.49 (d, ^3J = 8.7 Hz, 1H, NO_2C=C*H*) ppm. ^{13}C-NMR (100 and Hz, $CDCl_3$) δ = 24.6, 24.9, 25.1, 25.9, 26.0, 26.1, 28.3, 29.0, 29.0, 29.1, 29.2, 29.2, 29.3, 32.8, 33.2, 40.9, 44.0, 46.6, 49.2, 50.6, 50.9, 50.9, 51.1, 52.2 and 52.4 (COO*C*H_3), 98.4 (*C*H=CNH), 123.2 (C_{quart}), 136.7 (*C*H=CNO_2), 143.9 (C_{quart}), 144.2 (C_{quart}), 168.8 (*C*ONH), 169.8 and 169.9 (*C*OOMe), 173.9 and 174.2 (*C*ONR_2) ppm. HRMS (ESI+) *m*/*z* calcd for $C_{27}H_{41}N_9O_7$ $[M+Na]^+$ 626.3021, found 626.3015.

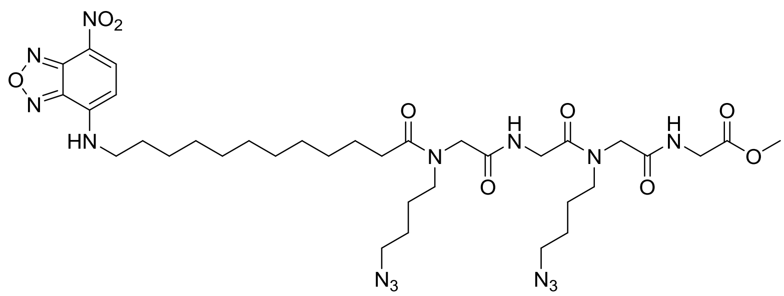
N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycine (**236**) as a mixture of rotamers



The saponification of **235** (2034 mg, 3.37 mmol) with lithium hydroxide solution (4.21 ml, 8.43 mmol, 2 M) following general procedure B (method 1) afforded **236** (2002 mg, quant.) as orange, amorphous solid, containg small, non-removable amounts of acetic acid and ethyl acetate. ^{1}H-NMR (400 MHz, $CD_3OD/CDCl_3$ 1:1)

δ = 1.21–1.82 (m, 22H, (CH_2)$_9$CH$_2$CON, (CH_2)$_2$CH$_2$N$_3$), 2.25–2.33 and 2.36–2.45 (2m, 2H, CH$_2$CH_2CON), 3.26–3.58 (m, 6H, aromatNHCH_2, NCH_2(CH$_2$)$_2$CH_2N$_3$), 3.92–4.16 (m, 4H, 2NCH_2CO), 6.20 (d, ^3J = 8.1 Hz, 1H, NHC=CH), 8.50 (d, ^3J = 8.1 Hz, 1H, NO$_2$C=CH) ppm. ^{13}C-NMR (100 MHz, CD$_3$OD/CDCl$_3$ 1:1) δ = 24.3, 24.8, 24.9, 25.6, 25.9, 25.9, 26.7, 28.0 (br), 28.9, 28.9, 29.0, 29.1, 29.1, 30.0, 32.6, 33.0, 40.7, 43.7 (br), 46.5, 48.9, 49.5, 50.8, 50.8, 98.2 (*C*H=CNH), 122.2 (C_{quart}), 136.9 (*C*H=CNO$_2$), 143.9 (C_{quart}), 144.1 (C_{quart}), 144.6 (C_{quart}), 169.0 and 169.6 (*C*ONH), 171.0 and 171.1 (*C*ONR$_2$), 174.5 (br, *C*OOH) ppm. HRMS (ESI-) *m/z* calcd for $C_{26}H_{39}N_9O_7$ [M-H]$^-$ 588.2900, found 588.2907.

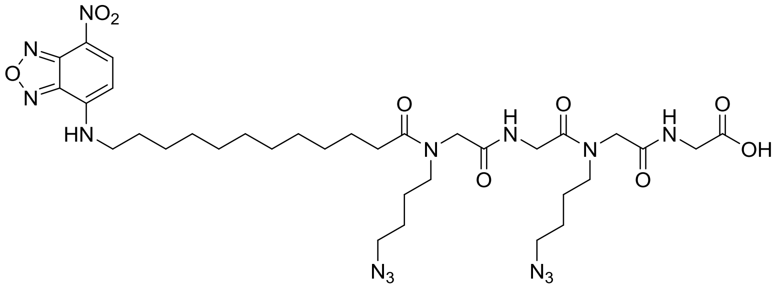
N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**237**) as a mixture of rotamers



Paraformaldehyde **19** (170 mg, 5.67 mmol), **16** (388 mg, 3.40 mmol), **236** (2002 mg, 3.40 mmol) and **20** (309 µl, 337 mg, 3.40 mmol) were reacted together following general procedure A. The reaction produced a precipitate, which was removed by filtration and washed with ice-cold methanol (10 ml) and acetone (10 ml). The filtration residue was dried *in vacuo* to give pure **237** (2016 mg, 72.8%) as orange, amorphous powder. The mother liquor was combined with the washing fraction and the solvent was removed under reduced pressure. The remainders were purified by silica column chromatography (ethyl acetate/methanol 9:1) to afford another crop of **237** (410 mg, 14.8%) as orange, amorphous solid. R_f 0.43 (ethyl acetate/methanol 9:1). ^{1}H-NMR (400 MHz, CDCl$_3$) δ = 1.22–1.87 (m, 26H, (CH_2)$_9$CH$_2$COOMe, 2(CH_2)$_2$CH$_2$N$_3$), 2.23–2.30 and 2.35–2.43 (2m, 2H, CH$_2$CH_2CON), 3.24–3.56 (m, 10H, aromatNHCH_2, 2NCH_2(CH$_2$)$_2$CH_2N$_3$), 3.72–3.77 (m, 3H, COOCH_3), 3.97–4.25 (m, 8H, 4NCH_2CO), 6.19 (d, ^3J = 8.7 Hz, 1H, NHC=CH), 6.60–7.17 (m, 3H, 3NH), 8.50 (d, ^3J = 8.7 Hz, 1H, NO$_2$C=CH) ppm. ^{13}C-NMR (100 MHz, CDCl$_3$) δ = 24.5, 24.7, 24.9, 25.1, 25.6, 26.0, 26.1, 26.2, 26.8, 28.4, 29.0, 29.2, 29.3, 32.9, 33.2, 41.0, 41.6, 44.0, 48.2, 49.1, 49.1, 50.0, 50.1, 50.2, 50.9, 51.0, 51.0, 52.3 and 52.4 and 52.4 (COO*C*H$_3$), 98.5 (*C*H=CNH), 123.6 (C_{quart}), 136.6 and 136.8 (*C*H=CNO$_2$), 143.9 (C_{quart}), 144.1 (C_{quart}), 144.3 (C_{quart}), 168.0, 168.6, 168.6, 168.7, 168.8, 168.9, 169.4, 170.0, 170.0, 173.9 and 174.0

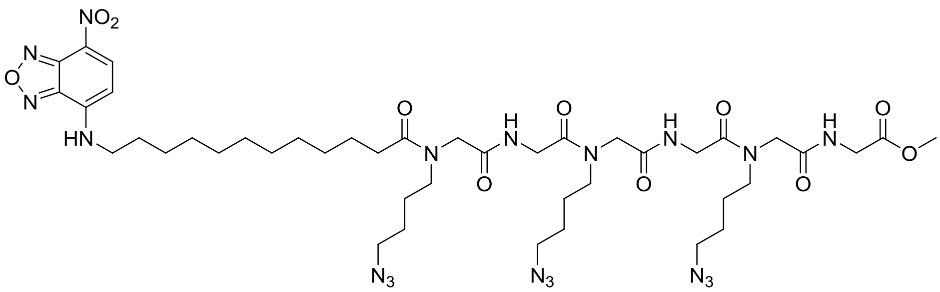
and 174.0 ($CH_2CH_2CONR_2$) ppm. HRMS (ESI+) *m/z* calcd for $C_{35}H_{54}N_{14}O_9$ $[M+Na]^+$ 837.4090, found 837.4078.

<u>*N*-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycyl-*N*-(4-azidobutyl)glycylglycine (**238**) as a mixture of rotamers</u>



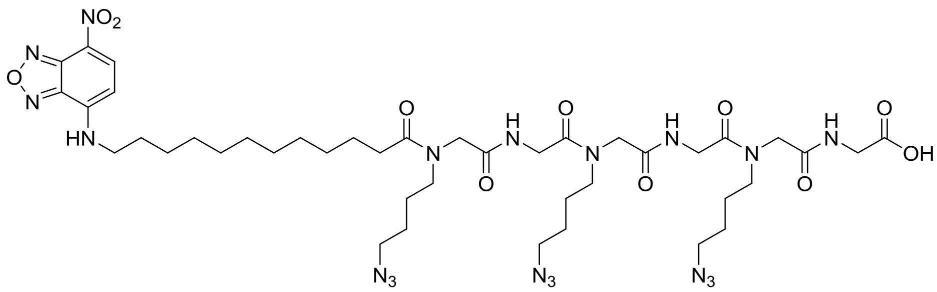
The saponification of **237** (2330 mg, 2.86 mmol) with lithium hydroxide solution (3.58 ml, 7.15 mmol, 2 M) following general procedure B (method 1) afforded **238** (2240 mg, 97.8%) as dark orange, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.22–1.82 (m, 26H, $(CH_2)_9CH_2CON$, $2(CH_2)_2CH_2N_3$), 2.29–2.36 and 2.40–2.48 (2m, 2H, CH_2CH_2CON), 3.20–3.56 (m, 10H, aromatNHCH_2, 2N$CH_2(CH_2)_2CH_2N_3$), 3.88–4.00 (m, 2H, NHCH_2COOH), 4.03–4.04 (m, 6H, 3NCH_2CON), 6.28 (d, ^3J = 8.8 Hz, 1H, NHC=C*H*), 8.45 (d, ^3J = 8.8 Hz, 1H, NO_2C=C*H*) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.6, 25.7, 26.3, 26.4, 26.5, 26.9, 27.1, 27.2, 27.2, 28.0, 29.2, 30.3, 30.6, 30.5, 30.6, 30.9, 33.6, 34.1, 41.7, 41.9, 41.9, 42.0, 42.0, 42.1, 44.8, 47.8, 48.3, 48.3, 49.0, 50.0, 50.1, 50.8, 50.8, 51.6, 52.1, 52.1, 52.2, 99.6 (*C*H=CNH), 122.7 (C_{quart}), 138.6 (*C*H=CNO_2), 145.4 (C_{quart}), 145.7 (C_{quart}), 146.5 (C_{quart}), 170.9, 170.9, 171.0, 171.0, 171.2, 171.2, 171.3, 171.4, 171.4, 171.5, 171.7, 171.7 (12x, *C*ONH, *C*ONR$_2$), 172.6 and 172.7 (CH_2CH_2*C*ONR$_2$), 176.1 and 176.5 (*C*OOH) ppm. HRMS (ESI+) *m/z* calcd for $C_{34}H_{52}N_{14}O_9$ $[M+Na]^+$ 823.3934, found 823.3940; (ESI-) $[M-H]^-$ 799.3969, found 799.3969.

<u>*N*-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine methyl ester (**239**) as a mixture of rotamers</u>



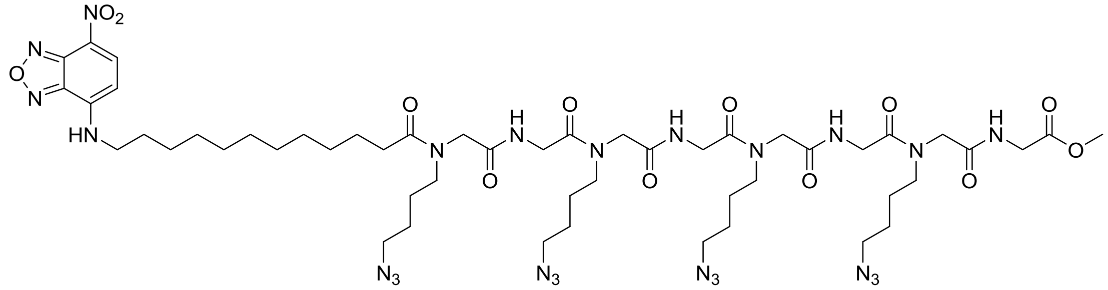
Paraformaldehyde **19** (134 mg, 4.45 mmol), **16** (305 mg, 2.67 mmol), **238** (2140 mg, 2.67 mmol) and **20** (243 µl, 265 mg, 2.67 mmol) were reacted together following general procedure A. The formed precipitate was purified together with the whole reaction mixture by gradient silica column chromatography (ethyl acetate/methanol 9:1 to 7:3) to afford **239** (1466 mg, 53.5%) as orange, amorphous solid. R_f 0.26 (ethyl acetate/methanol 9:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.23–1.87 (m, 30H, $(CH_2)_9CH_2CON$, $3(CH_2)_2CH_2N_3$), 2.24–2.30 and 2.34–2.42 (2m, 2H, CH_2CH_2CON), 3.22–3.58 (m, 14H, aromatNHCH_2, $3NCH_2(CH_2)_2CH_2N_3$), 3.69–3.75 (m, 3H, $COOCH_3$), 3.87–4.31 (m, 12H, $6NCH_2CO$), 6.19 (d, ^3J = 8.8 Hz, 1H, NHC=C*H*), 6.95–7.64 (m, 4H, 4N*H*), 8.48 (d, ^3J = 8.8 Hz, 1H, NO_2C=C*H*) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.4, 24.4, 24.6, 24.9, 25.1, 25.3, 25.5, 25.5, 25.9, 26.0, 26.8, 28.2, 29.0, 29.2, 32.7, 33.1, 40.8, 40.9, 40.9, 41.0, 41.1, 41.2, 41.3, 41.4, 44.0, 46.4, 47.1, 47.1, 47.9, 48.0, 48.1, 48.2, 48.9, 49.0, 49.1, 49.5, 49.7, 49.8, 49.9, 50.9, 50.9, 52.1, and 52.2 and 52.3 ($COOCH_3$), 98.4 (*C*H=CNH), 123.0 (C_{quart}), 136.8 (*C*H=CNO_2), 143.9 (C_{quart}), 144.2 (C_{quart}), 168.2, 168.3, 168.4, 168.5, 168.8, 168.9, 168.9, 169.0, 169.0, 169.2, 169.3, 170.0, 170.2, 170.3, 173.8, and 173.9 ($CH_2CH_2CONR_2$) ppm. HRMS (ESI+) *m*/*z* calcd for $C_{43}H_{67}N_{19}O_{11}$ $[M+Na]^+$ 1048.5160, found 1048.5156.

N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycine (**240**) as a mixture of rotamers



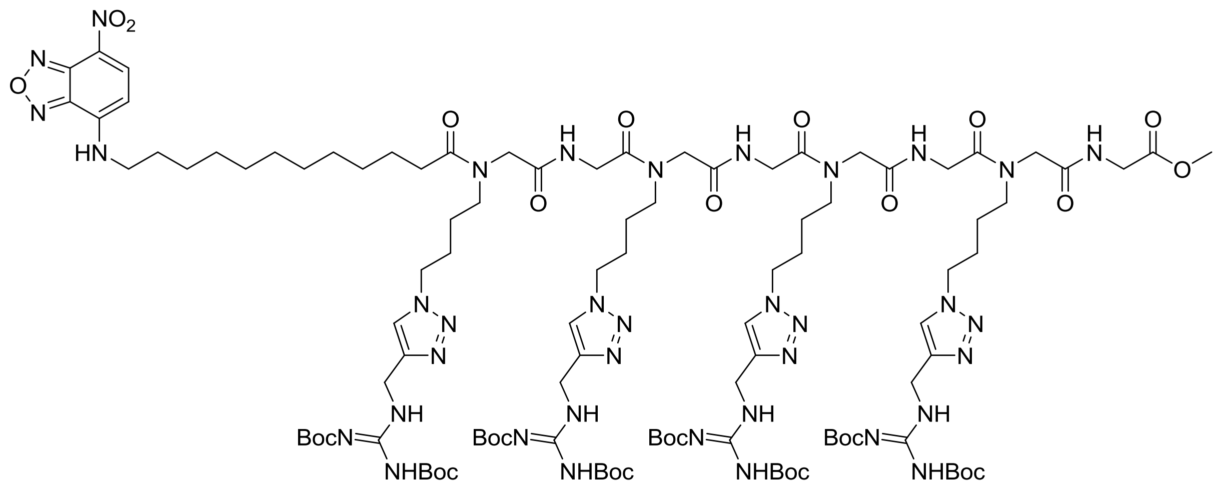
The saponification of **239** (1378 mg, 1.34 mmol) with lithium hydroxide solution (1.68 ml, 3.35 mmol, 2 M) following general procedure B (method 1) afforded **240** (1410 mg, quant.) as orange, amorphous solid, containing small, non-removable amounts of ethyl acetate and acetic acid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.20–1.83 (m, 30H, $(CH_2)_9CH_2CON$, $3(CH_2)_2CH_2N_3$), 2.32 and 2.44 (2brs, 2H, CH_2CH_2CON), 3.20–3.56 (m, 14H, aromatNHCH_2, $3NCH_2(CH_2)_2CH_2N_3$), 3.87–4.30 (m, 12H, $6NCH_2CO$), 6.27–6.36 (m, 1H, NHC=C*H*), 8.43–8.54 (m, 1H, NO_2C=C*H*) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.6, 25.7, 26.3, 26.3, 26.5, 26.9, 27.1, 27.2, 27.2, 28.0, 29.2, 30.3, 30.3, 30.5, 33.6, 34.1, 41.7, 41.8, 41.9, 41.9, 42.0, 42.1, 44.8, 47.8, 48.3, 49.1, 50.0, 50.1, 50.8, 51.7, 52.7, 99.6 (*C*H=CNH), 122.7 (C_{quart}), 138.6 (*C*H=CNO_2), 145.5 (C_{quart}), 145.8 (C_{quart}), 146.6 (C_{quart}), 170.9, 171.0, 171.2, 171.3, 171.4, 171.5, 171.6, 172.6, 172.7, 173.3 (10x, *C*ONH, *C*ONR_2), 176.2 and 176.6 (*C*OOH) ppm. HRMS (ESI-) *m*/*z* calcd for $C_{42}H_{65}N_{19}O_{11}$ $[M+Na]^+$ 1034.5003, found 1034.5021.

N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-azidobutyl)glycyl-glycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycylglycyl-*N*-(4-azidobutyl)glycyl-glycine methyl ester (**241**) as a mixture of rotamers



Paraformaldehyde **19** (65 mg, 2.15 mmol), **16** (147 mg, 1.29 mmol), **240** (1308 mg, 1.29 mmol) and **20** (117 µl, 128 mg, 1.29 mmol) were reacted together following general procedure A. The formed precipitate was purified together with the whole reaction mixture by silica column chromatography (dichloromethane/ethyl acetate/methanol 10:8:3). The purified product was dissolved in dichloromethane (5 ml) and the solution was filtrated over a 0.22 µm-syringe filter to remove remaining silica gel. After evaporation of the solvent *in vacuo* **241** (800 mg, 50.1%) was obtained as orange foam. R_f 0.11 (dichloromethane/ethyl acetate/methanol 5:4:1). ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 1.22–1.86 (m, 34H, $(CH_2)_9CH_2CON$, $4(CH_2)_2CH_2N_3$), 2.22–2.29 and 2.32–2.42 (2m, 2H, CH_2CH_2CON), 3.20–3.58 (m, 18H, aromatNHCH_2, 4N$CH_2(CH_2)_2CH_2N_3$), 3.66–3.80 (m, 3H, COOCH_3), 3.83–4.32 (m, 16H, 8NCH_2CO), 6.19 (d, ^3J = 8.6 Hz, 1H, NHC=C*H*), 6.82–7.90 (m, 5H, 5N*H*), 8.49 (d, ^3J = 8.6 Hz, 1H, NO_2C=C*H*) ppm. ^{13}C-NMR (100 MHz, $CDCl_3$) δ = 24.3, 24.4, 24.5, 24.6, 25.0, 25.0, 25.1, 25.5, 25.6, 25.6, 25.9, 26.1, 26.8, 28.2, 29.0, 29.0, 29.2, 29.6, 32.8, 33.1, 40.8, 40.9, 41.0, 41.3, 41.5, 44.0, 46.4, 46.5, 47.1, 47.1, 47.2, 47.9, 48.2, 48.3, 48.5, 48.6, 48.9, 49.1, 49.6, 49.8, 50.1, 50.2, 50.9, 50.9, 52.1 and 52.2 and 52.3 (COO*C*H_3), 98.5 (*C*H=CNH), 123.2 (C_{quart}), 136.7 (*C*H=CNO_2), 143.9 (C_{quart}), 144.2 (C_{quart}), 168.4, 168.4, 168.6, 168.6, 168.7, 168.8, 168.9, 168.9, 168.9, 169.0, 169.0, 169.1, 169.2, 169.3, 169.4, 169.5, 169.5, 169.6, 169.7, 169.7, 169.8, 169.8, 169.9, 170.0, 170.1, 170.3, 170.4, 170.4, 170.4, 170.4, 170.5, 173.9 and 174.0 (CH_2CH_2*C*ONR_2) ppm. HRMS (ESI+) *m*/*z* calcd for $C_{51}H_{80}N_{24}O_{13}$ $[M+Na]^+$ 1259.6229, found 1259.6222.

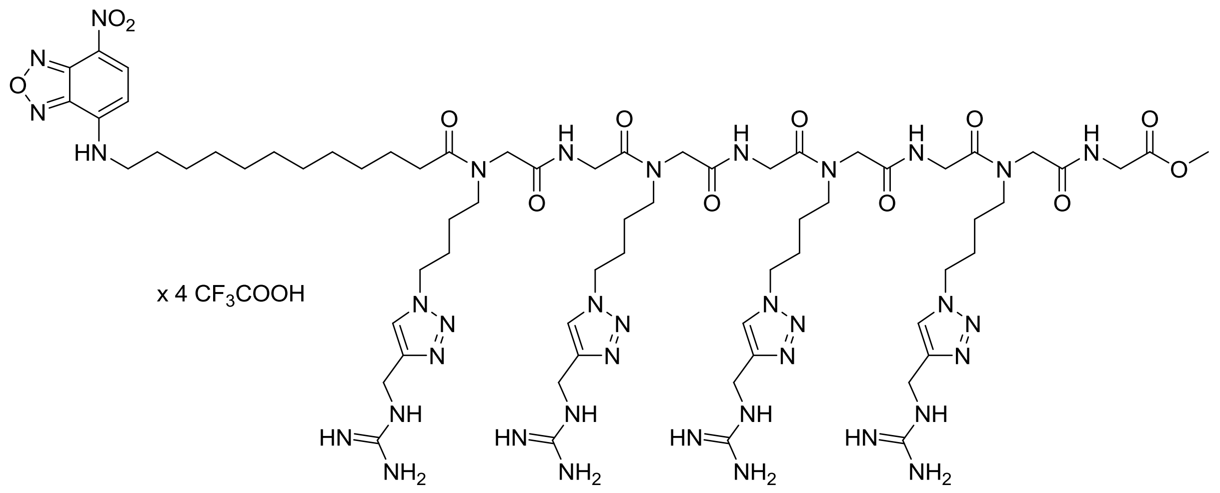
N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(2,3-di-(*tert*-butoxycarbonyl)-guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycine methyl ester (**242**) as a mixture of rotamers



241 (50 mg, 40.4 µmol) and **228** (53 mg, 177.8 µmol) were reacted together following general procedure D. The crude residue was pre-purified by gradient column chromatography (dichloromethane/ethyl acetate/methanol 2:2:1, 4:3:4; 0:0:1). All product containing fractions were combined and the solvents were removed *in vacuo*. The final purification was accomplished by preparative thin layer chromatography (100 µl dichloromethane, 9 cm x 9 cm, dichloromethane/ethyl acetate/methanol 2:2:1). The product zone was cut off the plate and was extracted with methanol (75 ml, 50 °C). The solvent was evaporated from the extract under reduced pressure without filtration. The silica containing product was dissolved in dichloromethane (5 ml) and was filtrated through a 0.22 µm-syringe filter to afford **242** (50 mg, 51.0%) as orange, amorphous solid after distilling off the solvent *in vacuo*. R_f 0.20 (dichloromethane/ethyl acetate/methanol 2:2:1). ^{1}H-NMR (400 MHz, C_6D_6) δ = 1.18–1.92 (m, 106H, $(CH_2)_9CH_2CON$, $4(CH_2)_2CH_2N_3$), $8(CH_3)_3C$), 2.27–2.57 (m, 2H, CH_2CH_2CON), 3.10–3.75 and 3.95–4.55 (m, 41H, 4triazoleCH_2CH_2, aromatNHCH_2, COOCH_3, 8NCH_2CO, 4CONRCH_2CH_2, 4N*H*CH_2triazole), 4.83 (brs, 8H, 4NHCH_2triazole), 5.74 (brs, 1H, NHC=C*H*), 7.33–8.60 (m, 6H, 5N*H*, NO_2C=C*H*), 9.03 (brs, 4H, $4C_2N_3H$), 12.13 (brs, 4H, $(CH_3)_3$COCON*H*) ppm. ^{13}C-NMR (100 MHz, C_6D_6) δ = 23.1, 24.8 (br), 25.6, 25.9, 27.1, 27.8 ($(CH_3)_3C$), 28.4 ($(CH_3)_3C$), 29.4, 29.6, 29.7, 30.2, 32.3, 33.0, 36.8, 41.6 (br), 49.8 (br), 52.1

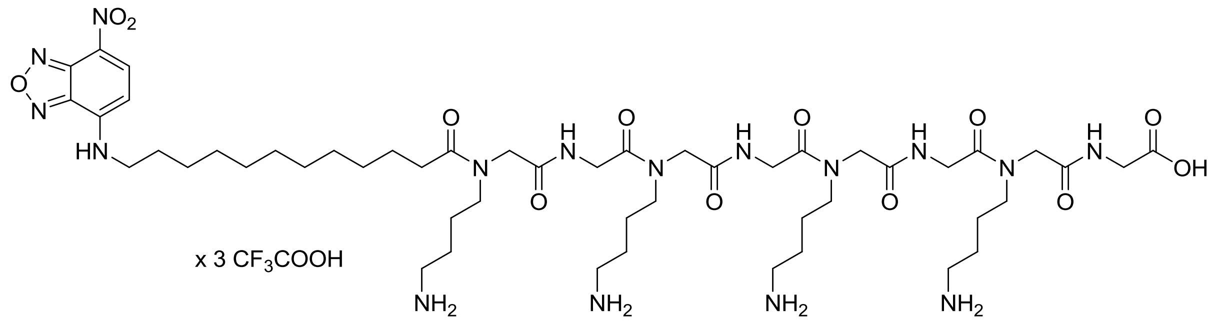
(2x, COOCH_3), 78.8 ((CH_3)$_3$*C*), 82.8 ((CH_3)$_3$*C*), 98.4 (weak, *C*H=CNH), 122.9 (br, triazole-*C*H), 136.8 (*C*H=CNO_2), 144.1, 144.3, 144.7, 144.9, 153.5 ((CH_3)$_3$COCO), 156.5 ((CH_3)$_3$CO*C*O), 164.3 (guanidine-*C*), 169.7 (br), 170.1 (br), 170.8, 171.0, 174.0, 174.1, 174.2 ppm. ESI-MS (ESI+) *m*/*z* calcd for $C_{107}H_{172}N_{36}O_{29}$ $[M+2Na]^{2+}$ 1235.7, found 1236.2.

N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycyl-*N*-(4-(4-(guanidinomethyl)-1,2,3-triazolyl)butyl)glycylglycine methyl ester tetra(trifluoroacetate) (**243**) as a mixture of rotamers



To a solution of **242** (46.0 mg, 18.96 µmol) in dichloromethane (1.5 ml) was added trifluoacetic acid (582 µl, 867 mg, 7.6 mmol). After stirring at room temperature for three hours the mixture was concentrated to a volume of 200 µl at a rotavap and methanol (2 ml) was added. The methanolic solution was then added dropwise to diethylether (15 ml) in a centrifuge tube. After centrifugation at 4000 rpm and 0 °C for 10 min the supernatant was discarded. The precipitate was washed with diethylether (15 ml) and was centrifuged again like described before. After separation of the etheric phase the residue was dried *in vacuo* to afford **243** (29 mg, 73.5%) as orange, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.22–2.03 (m, 34H, (CH_2)$_9$CH_2CON, 4(CH_2)$_2$CH_2triazole), 2.25–2.33 and 2.36–2.44 (2m, 2H, CH_2CH_2CON), 3.35–3.57 (m, 10H, 4triazole-CH_2CH_2, NHCH_2CH_2), 3.70 and 3.71 (2s, 3H, COOCH_3), 3.90–4.20 (m, 16H, 7NCH_2CO, NHCH_2COOH), 4.37–4.53 (m, 16H, 4NCH_2CH_2, 4NHCH_2triazole), 6.36 (d, ^3J = 8.8 Hz, 1H, NHC=C*H*), 7.83–8.63 (m, ca. 8H, 4C_2N_3*H*, NO_2C=C*H*, xN*H*) ppm. ^{13}C-NMR (100 MHz, CD_3OD) δ = 25.2, 26.1, 26.3, 26.4, 26.5, 27.9, 27.9, 28.2, 28.3, 28.4, 28.5, 28.5, 29.2 (br), 30.2, 30.2, 30.4, 30.4, 30.5, 30.5, 30.5, 33.7, 34.1, 41.8, 42.0, 42.0, 44.7 (br), 47.6, 47.9, 48.0, 48.1, 48.9, 48.9, 49.9, 50.1, 50.2, 50.8, 50.8, 50.9, 51.0, 51.6, 51.6, 52.7 and 52.8 (COO*C*H_3), 99.6 (*C*H=CNH), 124.5 (triazole-*C*H), 138.7 (*C*H=CNO_2), 144.2, 144.5, 144.4, 145.6, 145.9, 146.8, 158.8 (guanidine-*C*), 163.0 (q, $^2J_{C,F}$ = 34.6 Hz), 170.9, 170.9, 171.0, 171.0, 171.0, 171.1, 171.2, 171.2, 171.3, 171.3, 171.4, 171.5, 171.5, 171.6, 171.7, 171.7, 176.3, 176.3, 176.7, 176.7 ppm. HRMS (ESI+) *m*/*z* calcd for $C_{67}H_{108}N_{36}O_{13}$ $[M+2H]^{2+}$ 813.4521, found 813.4517.

N-(12-(7-Nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)dodecanoyl)-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycyl-*N*-(4-aminobutyl)glycylglycine tri(trifluoroacetate) (**244**) as a mixture of rotamers



To a solution of **241** (40.0 mg, 32.3 µmol) in a mixture of THF and water (2:1, v/v, 3 ml) was added triphenylphosphine (51 mg, 194 µmol) and the solution was stirred under nitrogen atmosphere at room temperature for 24 h. Afterwards trifluoroacetic acid (200 µl, 296 mg, 2.60 mmol) was added and the mixture was concentrated to a volume of 1 ml under reduced pressure. After the addition of methanol (2 ml) the solution was added dropwise to diethylether (15 ml) in a centrifuge tube. After centrifugation at 4000 rpm and 0 °C for 10 min the supernatant was discarded. The residue was dissolved in methanol (1 ml) and the resulting solution was added dropwise to methyl-*tert*-butylether (15 ml) in a centrifuge tube. After centrifugation at 4000 rpm and 0 °C for 10 min the supernatant was discarded and the residue was dried *in vacuo* to afford **244** (33 mg, 64.9%) as orange, amorphous solid. ^{1}H-NMR (400 MHz, CD_3OD) δ = 1.22–1.83 (m, 34H, (C*H*$_2$)$_9$CH_2CON, 4(C*H*$_2$)$_2$CH_2triazole), 2.27–2.36 and 2.39–1.48 (2m, 2H, CH_2C*H*$_2$CON), 2.87–3.02 (m, 8H, 4C*H*$_2$NH_2), 3.37–3.60 (m, 10H, aromatNHC*H*$_2$, 4CONRC*H*$_2$CH_2), 3.76–3.89 (m, 2H, NHC*H*$_2$COOH), 3.94–4.24 (m, 14H, NC*H*$_2$CON), 6.36 (d, ^3J = 8.8 Hz, 1H, NHC=C*H*), 7.71–7.92 (m, 4H, 4N*H*), 8.53 (d, ^3J = 8.8

Hz, 1H, NO_2C=C*H*) ppm. HRMS (ESI+) *m*/*z* calcd for $C_{50}H_{86}N_{16}O_{13}$ $[M+H]^+$ 1119.6633, found 1119.6603; $[M+2H]^{2+}$ 560.3353, found 560.3365.

6 Abbreviations

2D/3D	two-dimensional/three-dimensional
aa	amino acid
a/o	and/or
a.s.o	and so on
Aib	aminoisobutyric acid
AMP	antimicrobial peptide
Boc	*tert*-butyloxycarbonyl
CA	chloramphenicol
*c*AMP	cyclic adenosine monophosphate
CMC	critical micelle forming concentration
CPP	cell-penetrating peptide
DAB	2,4-diaminobutyric acid
DAPI	4',6-diamidino-2-phenylindole
DCM	dichloromethane
DMF	*N,N*-dimethylformamide
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen (germ.) – German collection of microorganisms and cell cultures
e.g.	*exempli gratia* (lat.) – for example
ESI	electrospray
Etnor	ethylnorvaline
FLIM	fluorescence life-time imaging
FT-ICR	fourier transform ion cyclotron resonance
GTP	guanosine triphosphate
HNP	human neutrophile peptide
HPLC	high pressure/performance liquid chromatography
HR	high resolution
HS	heparin sulfate
Hyp	hydroxyproline
i.e.	*id est* (lat.) – it is

Iva	isovaline
LPP	(chimeric) lipopeptide-peptoids
MCR	multicomponent reaction
MS	mass spectrometry
NBD	7-nitrobenzo[c][1,2,5]oxadiazole
NMR	Nuclear magnetic resonance (spectroscopy)
NRP	non-ribosomal peptide antibiotic
NRPS	non-ribosomal peptide synthetases
NTP	nucleoside triphosphate
PC3	prostate cancer cell line
PCP	peptide carrier protein
pH	*pondus Hydrogenii* (lat.) – negative, decadic logarithm of the activity of the (solvated) hydronium ion
ppm	parts per million
PTFE	polytetrafluoroethylene
PURG	protected Ugi-reactive group
quant.	quantitative
R_f	retardation factor
RNA	ribonucleic acid
RPMI	Roswell Park Memorial Institute
RLU	relative luminescence unit
RT	room temperature
SAR	structure-activity relationship
SDS	sodium dodecylsulfate
SF	subfamily
Tat	transkriptional activator
TE	termination (domain)
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofurane
TLC	thin layer chromatography
TMPA	trimethylsilyldeuteropropionic acid
TMS	tetrasilylmethane
U-4CR	Ugi four-component reaction

URG	Ugi-reactive group
UV	ultraviolet

7 Literature

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8 Appendix

8.1 Selected NMR spectra

8.1.1 Compound 181

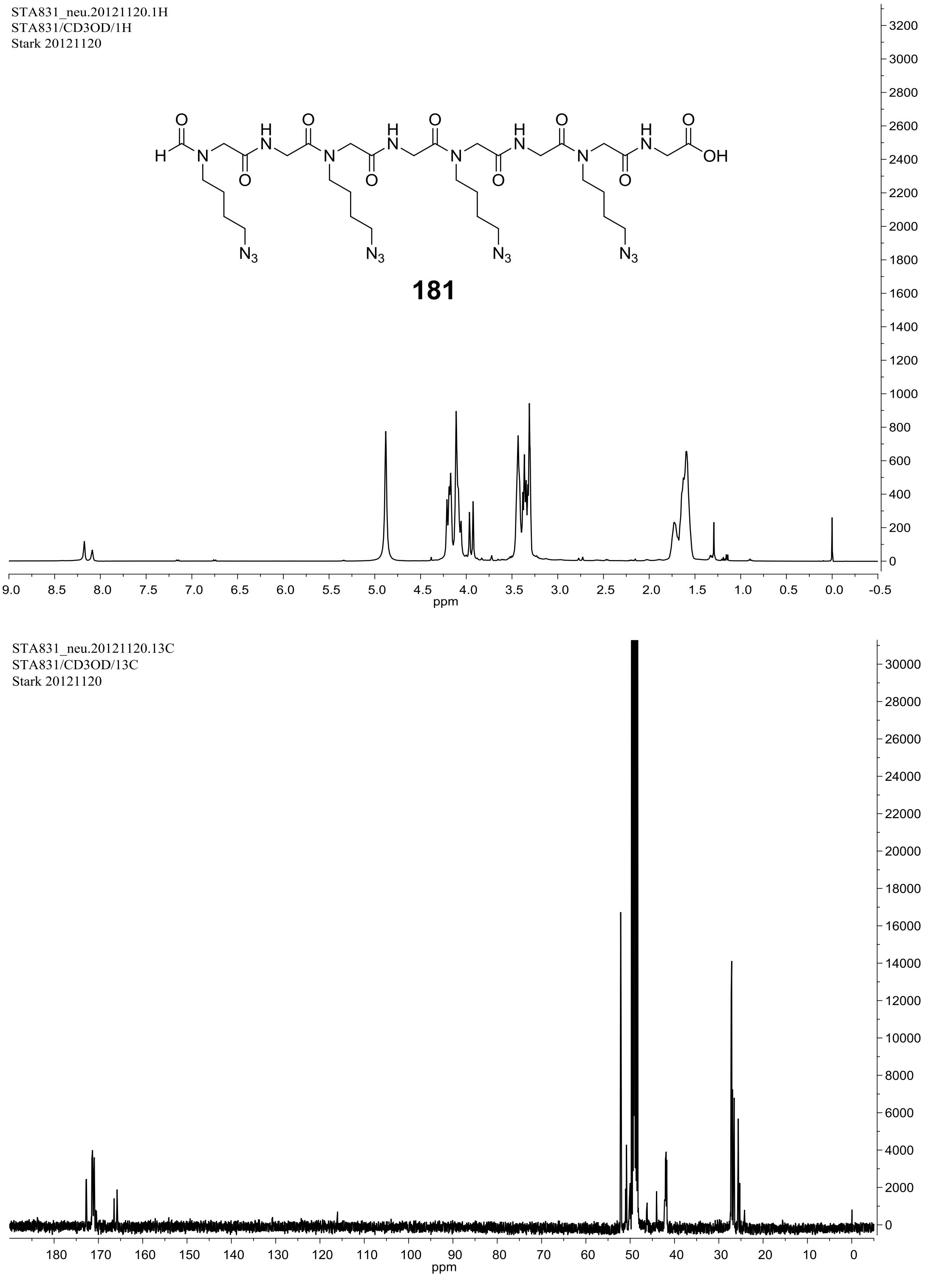
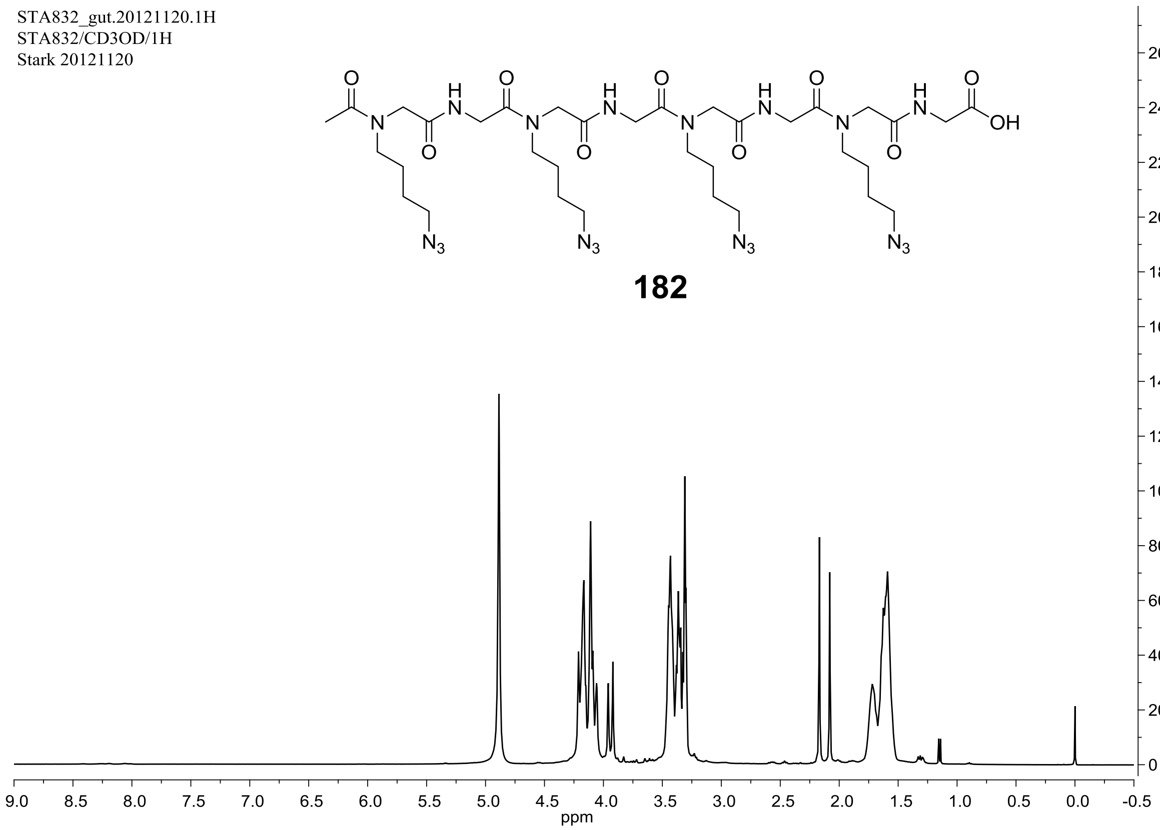


Figure 50. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Formic acid azido LPP **181** in CD_3OD.

8.1.2 Compound 182



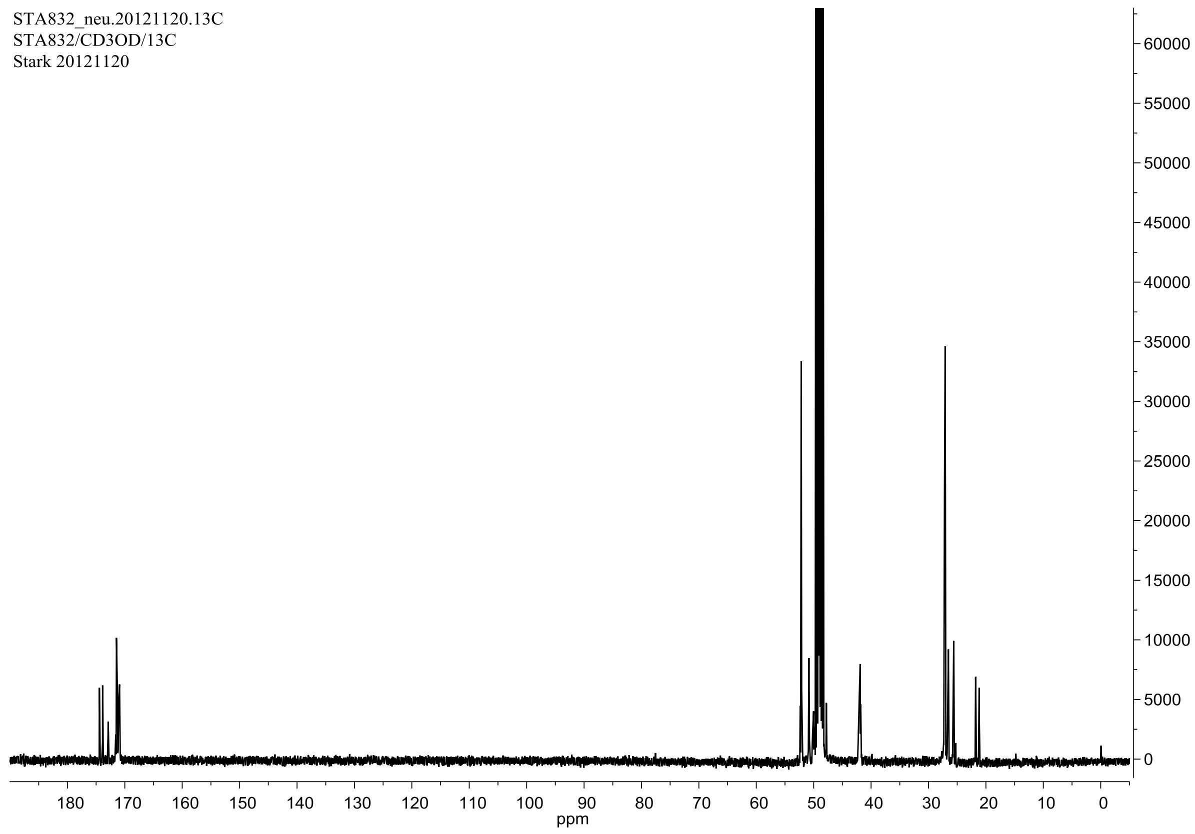
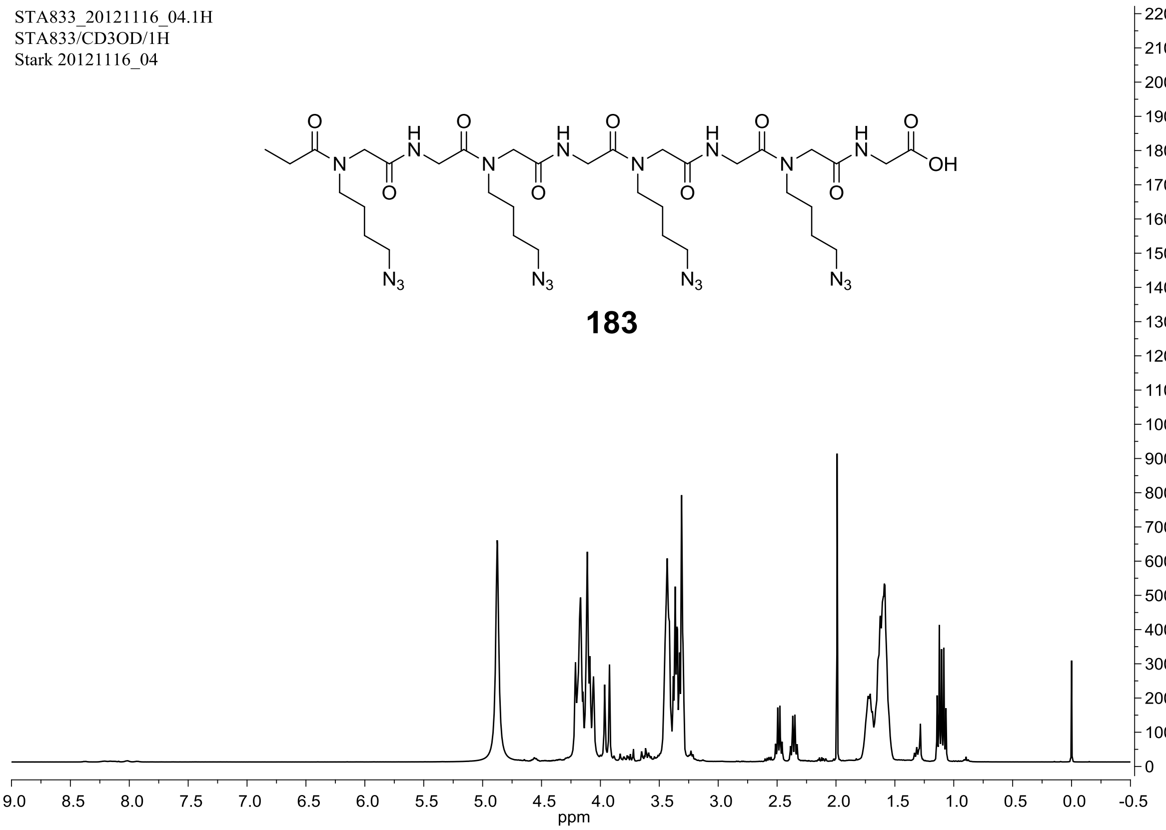


Figure 51. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Acetic acid azido LPP **182** in CD_3OD.

8.1.3 Compound 183



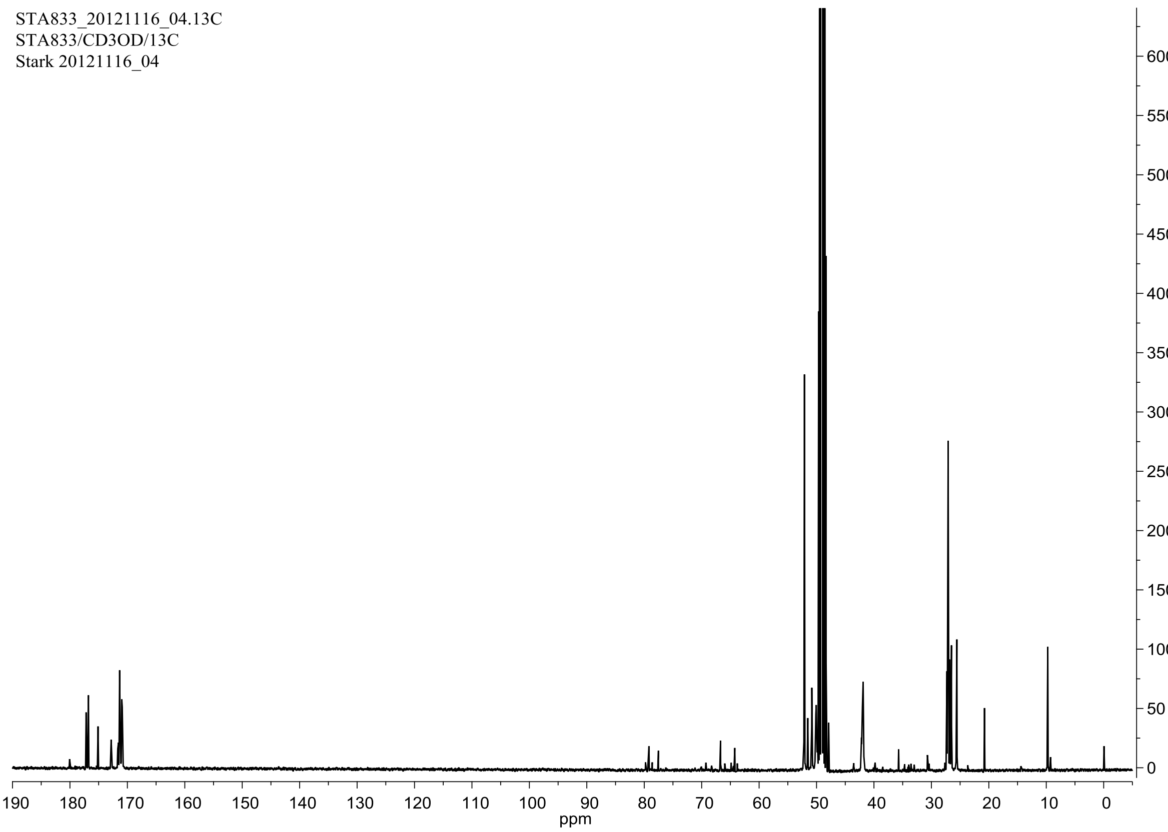


Figure 52. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Propionic acid azido LPP **183** in CD_3OD.

8.1.4 Compound 184

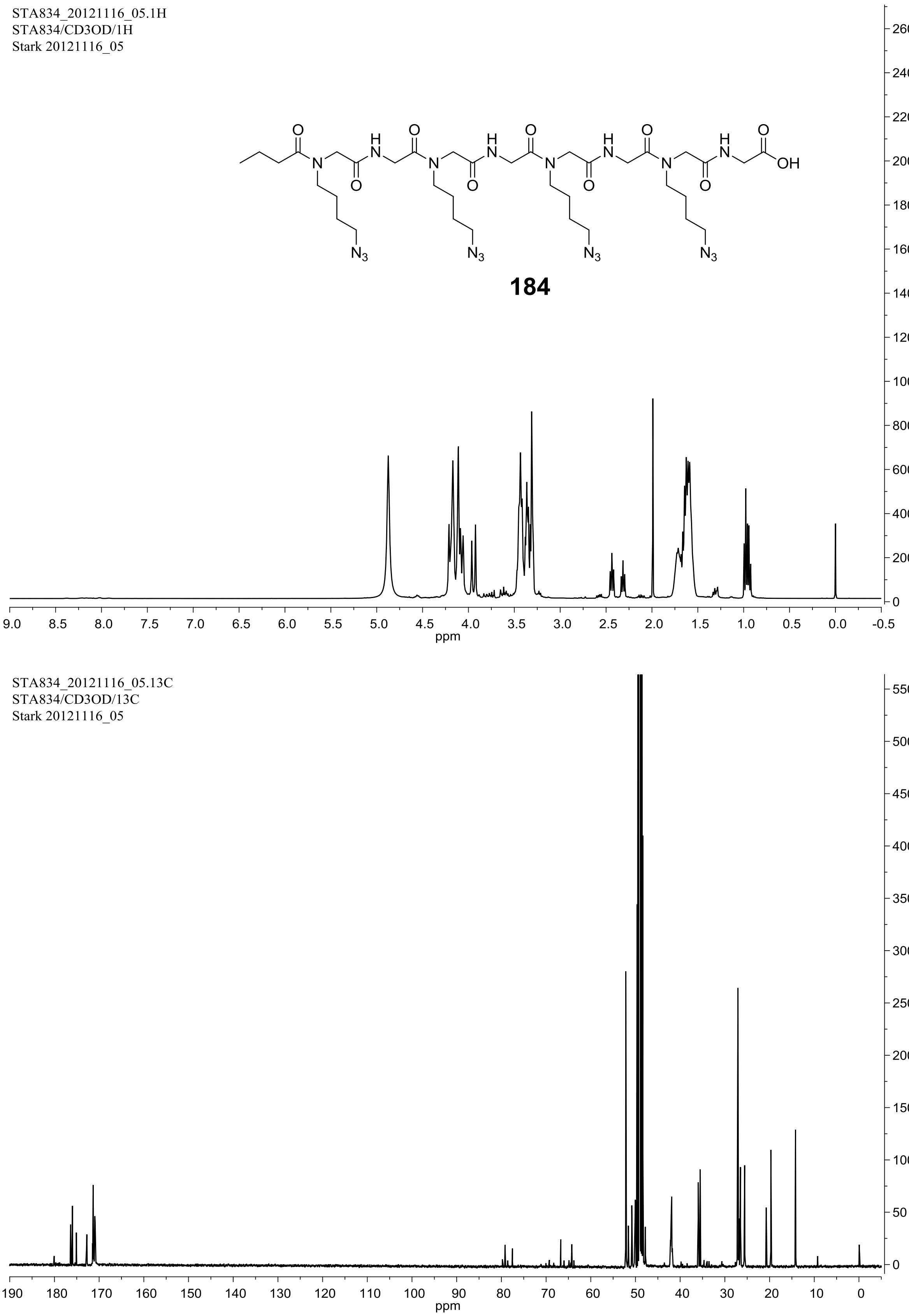
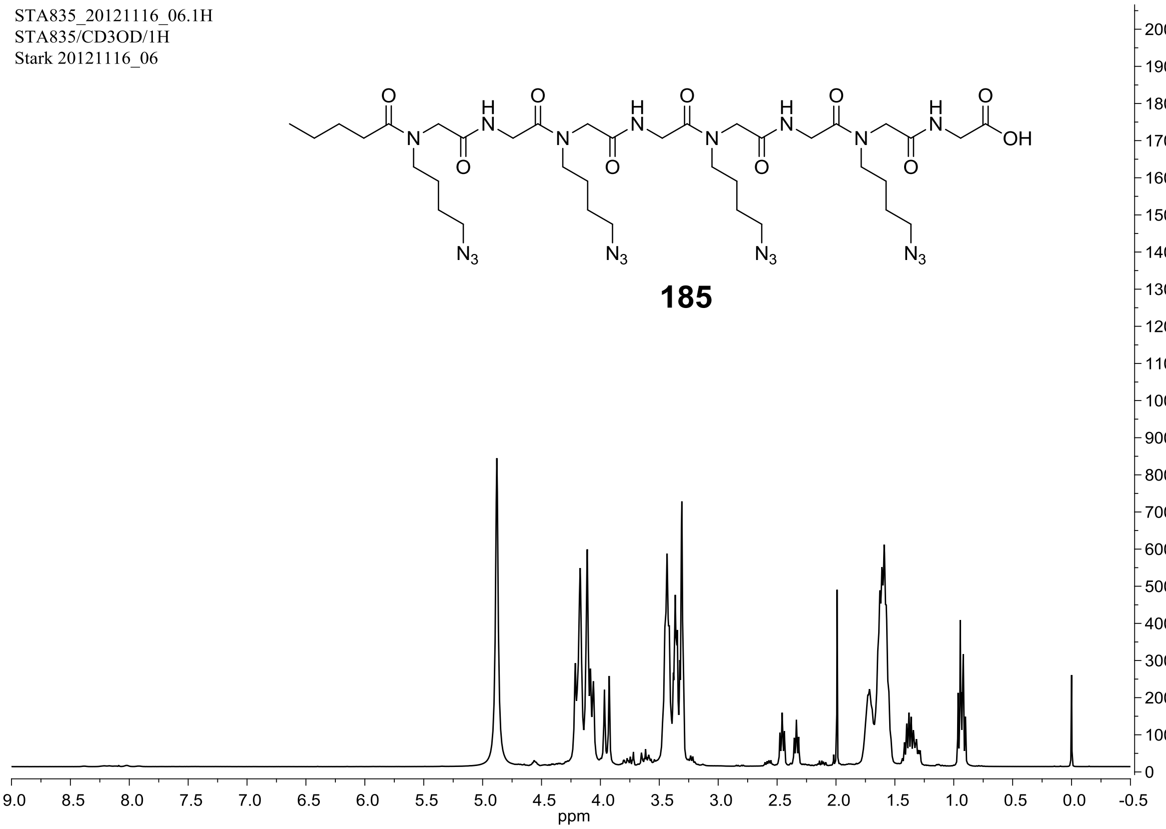


Figure 53. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Butyric acid azido LPP **184** in CD_3OD.

8.1.5 Compound 185



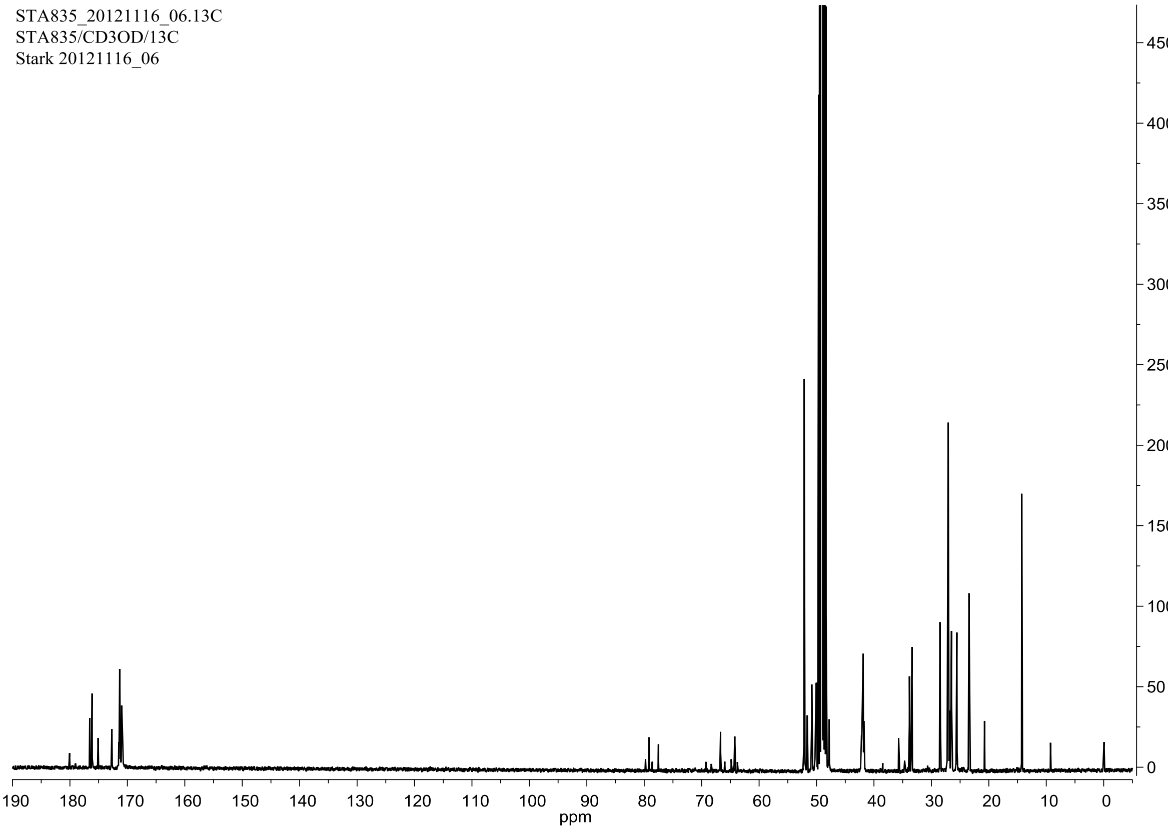
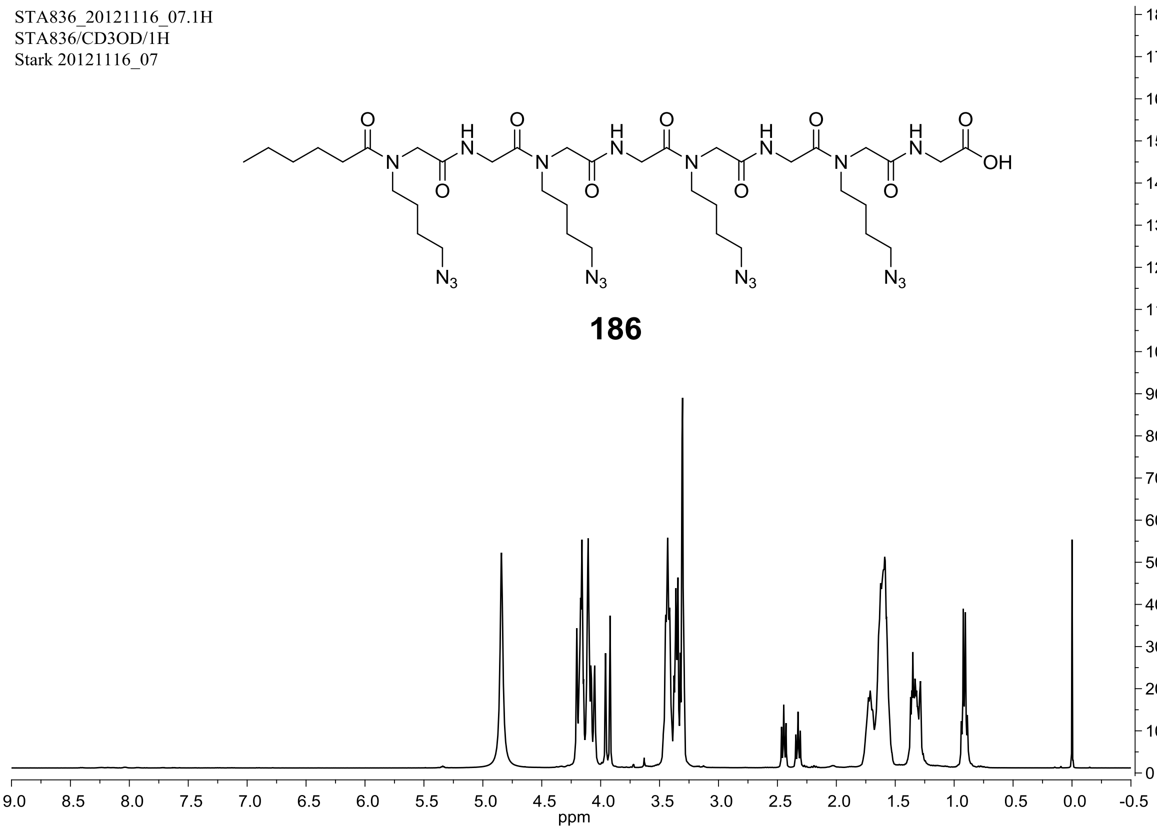


Figure 54. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Valeric acid azido LPP **185** in CD_3OD.

8.1.6 Compound 186



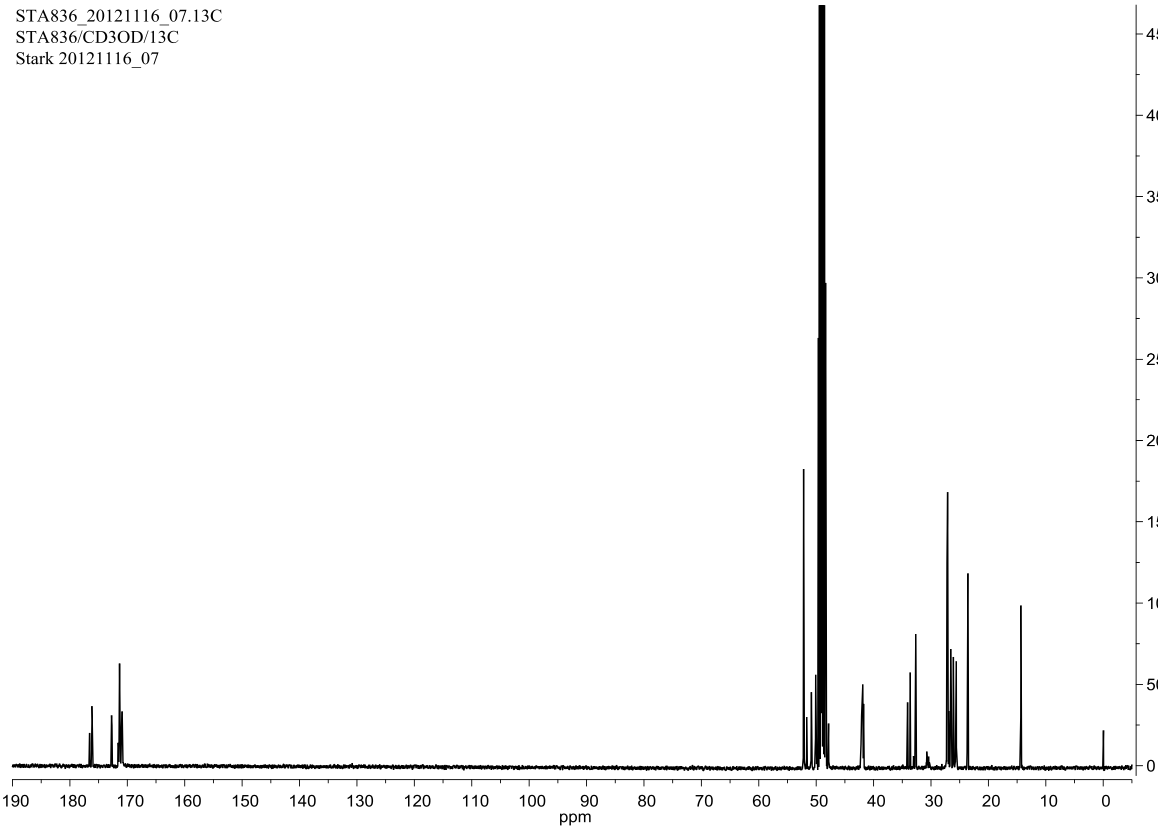


Figure 55. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Hexanoic acid azido LPP **186** in CD_3OD.

8.1.7 Compound 187

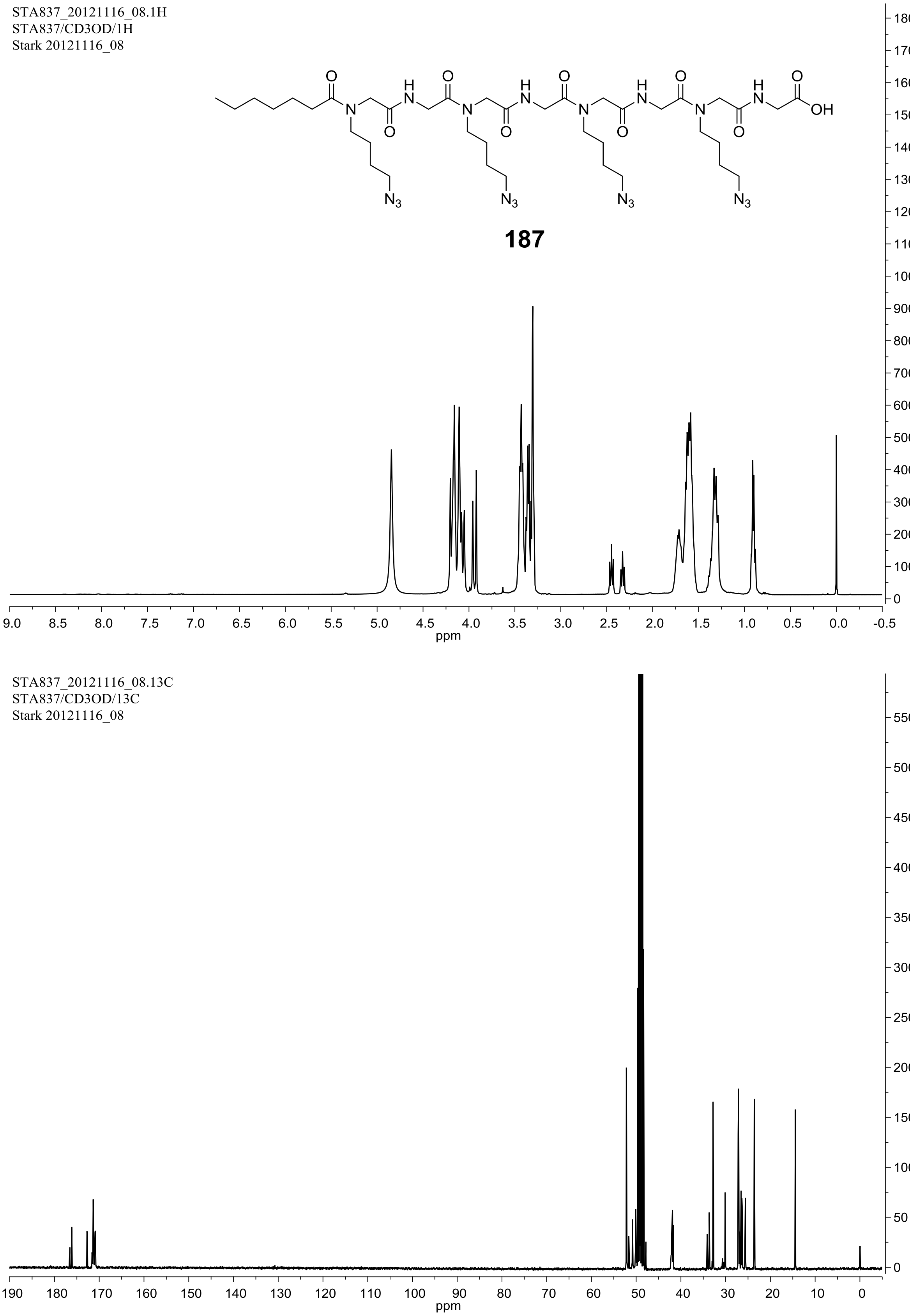


Figure 56. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Heptanoic acid azido LPP **187** in CD_3OD.

8.1.8 Compound 188

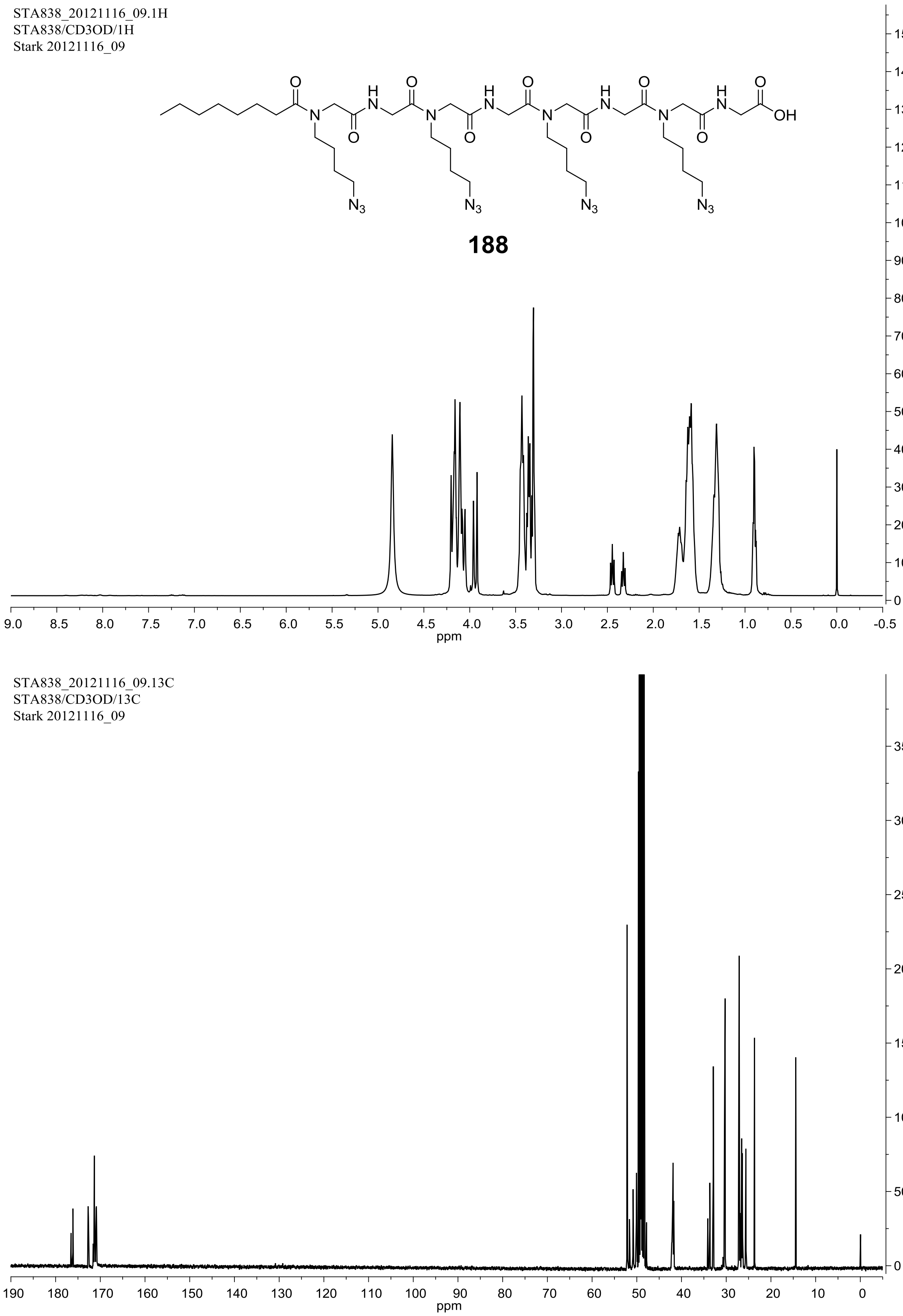


Figure 57. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Octanoic acid azido LPP **188** in CD_3OD.

8.1.9 Compound 189

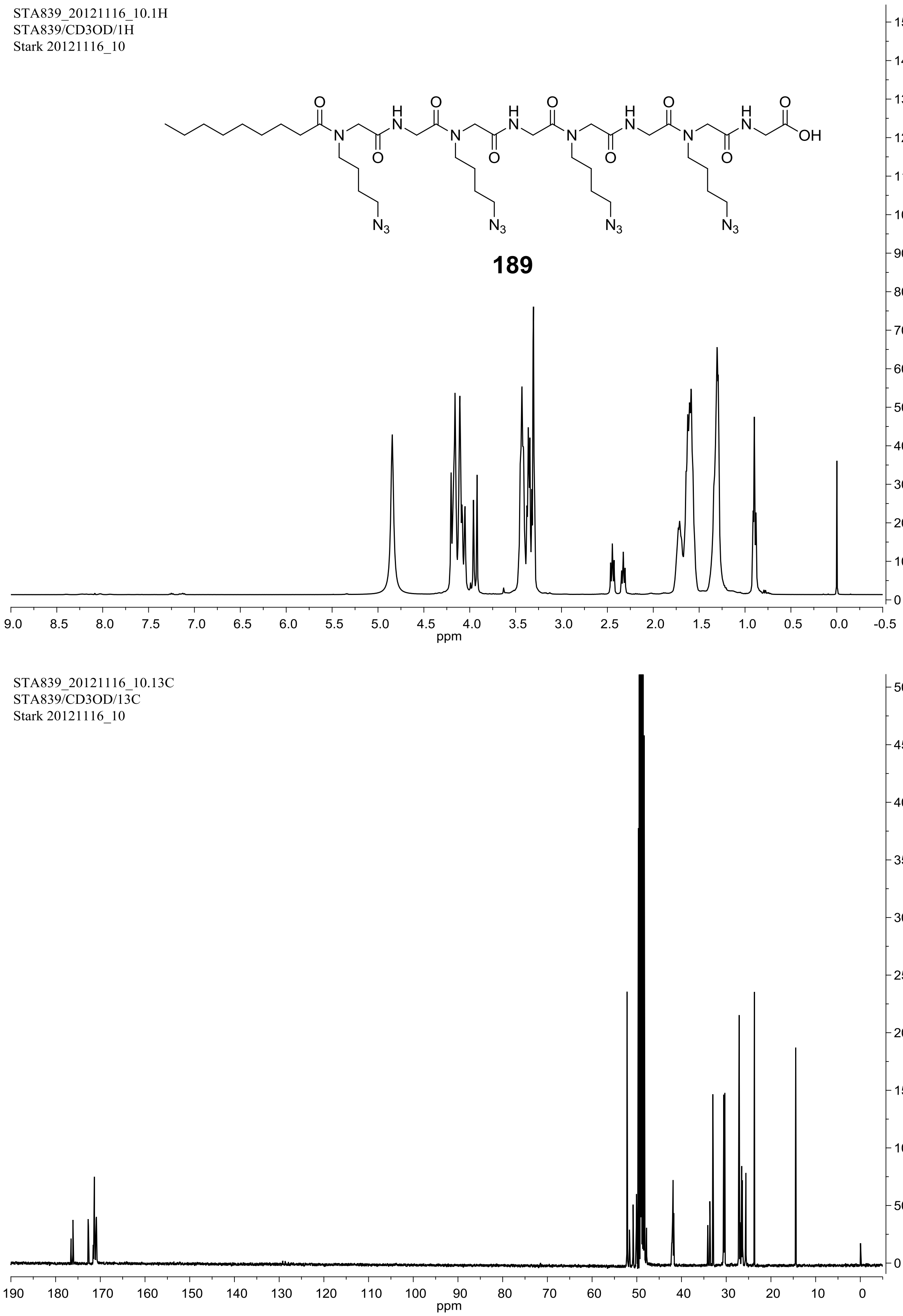


Figure 58. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Nonanoic acid azido LPP **189** in CD_3OD.

8.1.10 Compound 190

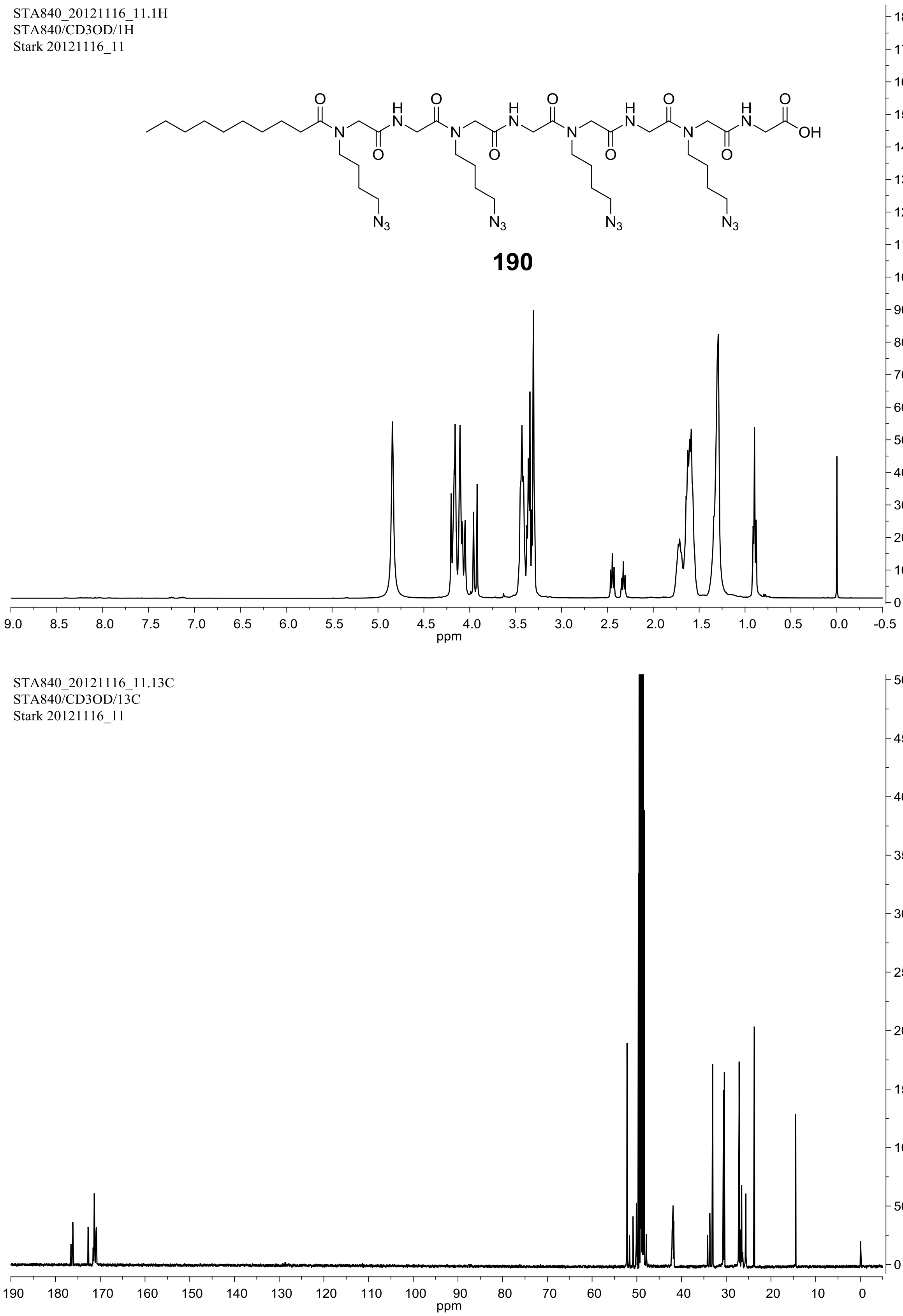
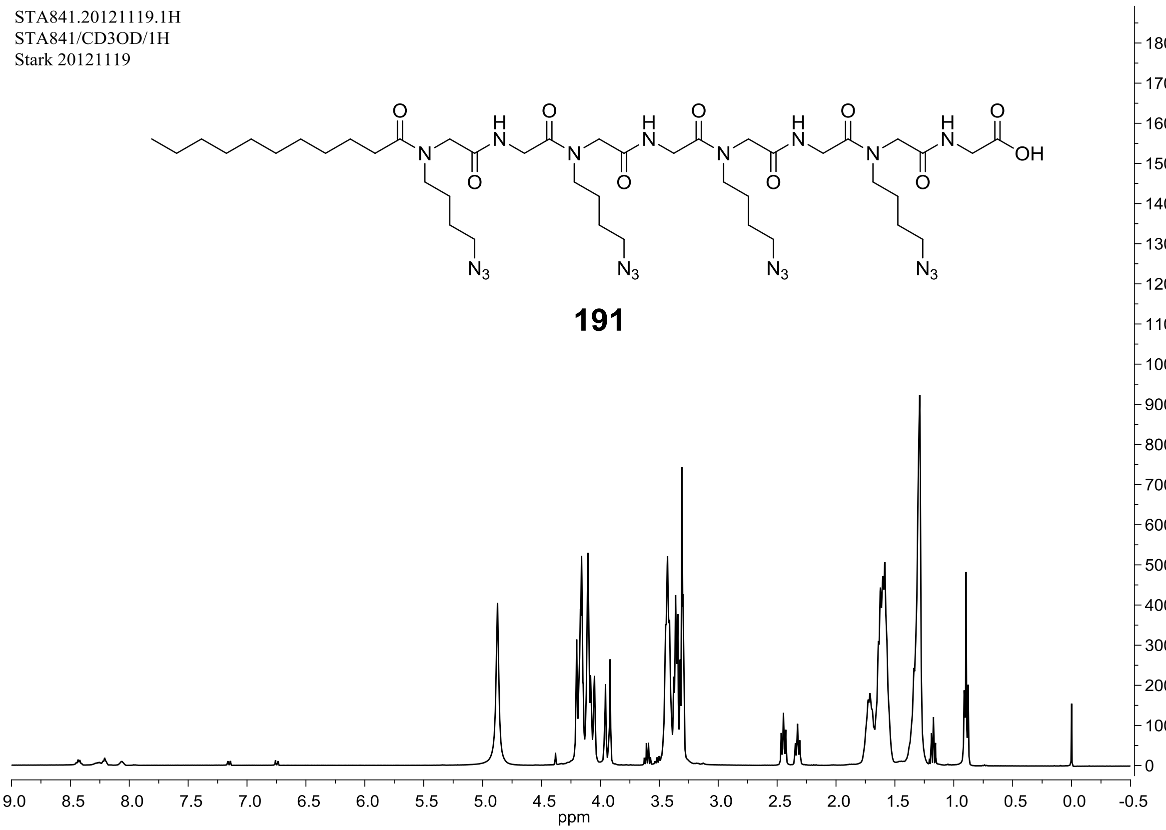


Figure 59. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Decanoic acid azido LPP **190** in CD_3OD.

8.1.11 Compound 191



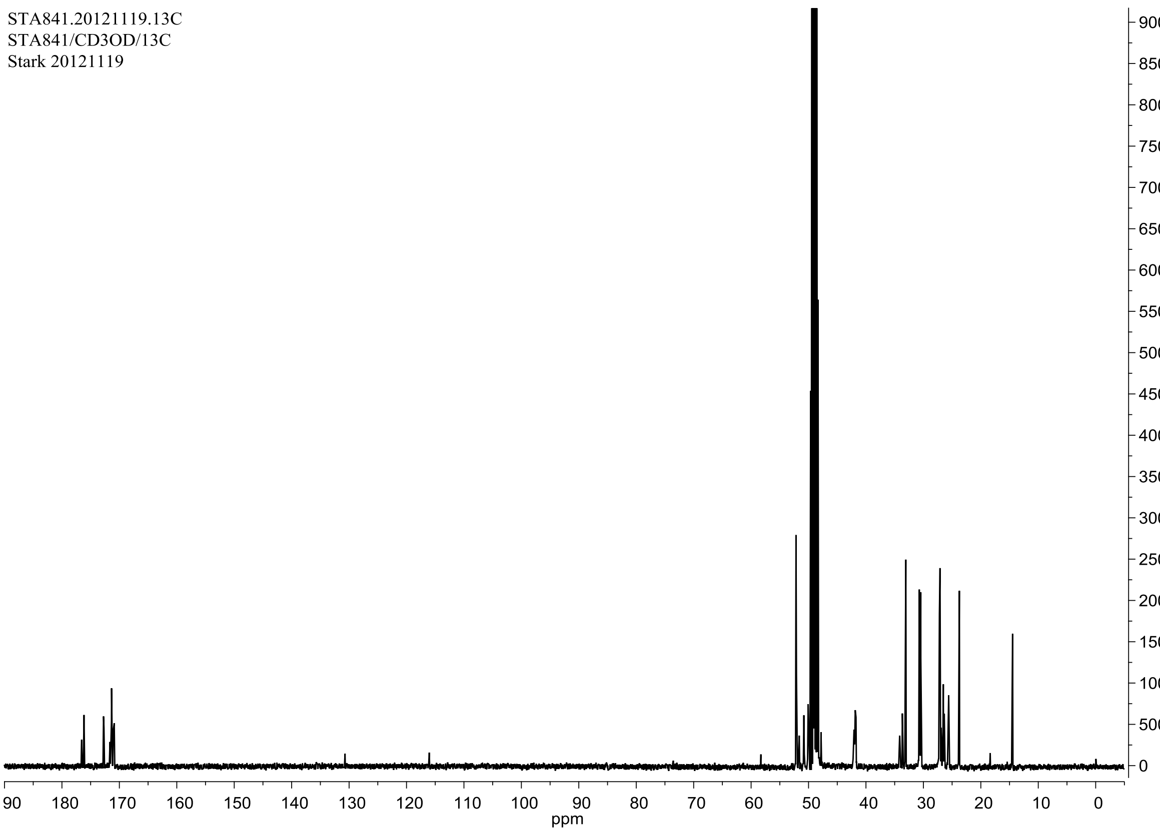
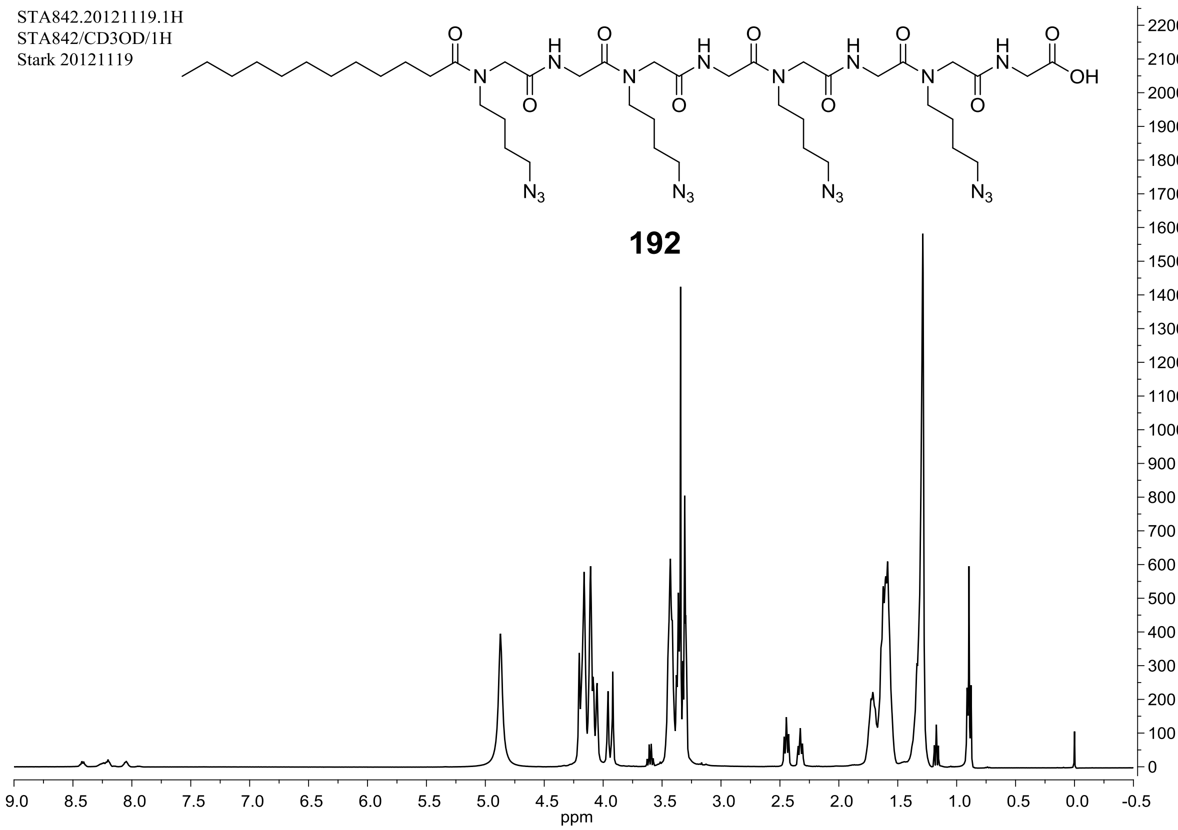


Figure 60. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Undecanoic acid azido LPP **191** in CD_3OD.

8.1.12 Compound 192



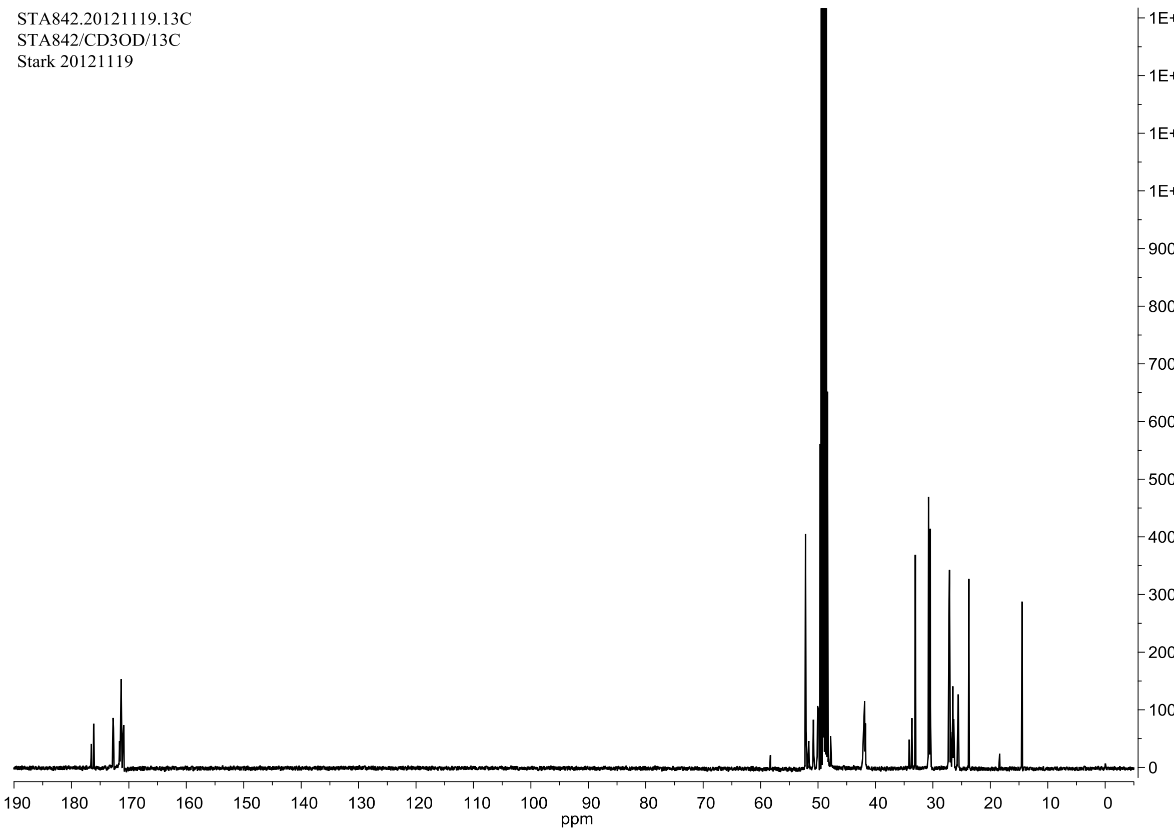
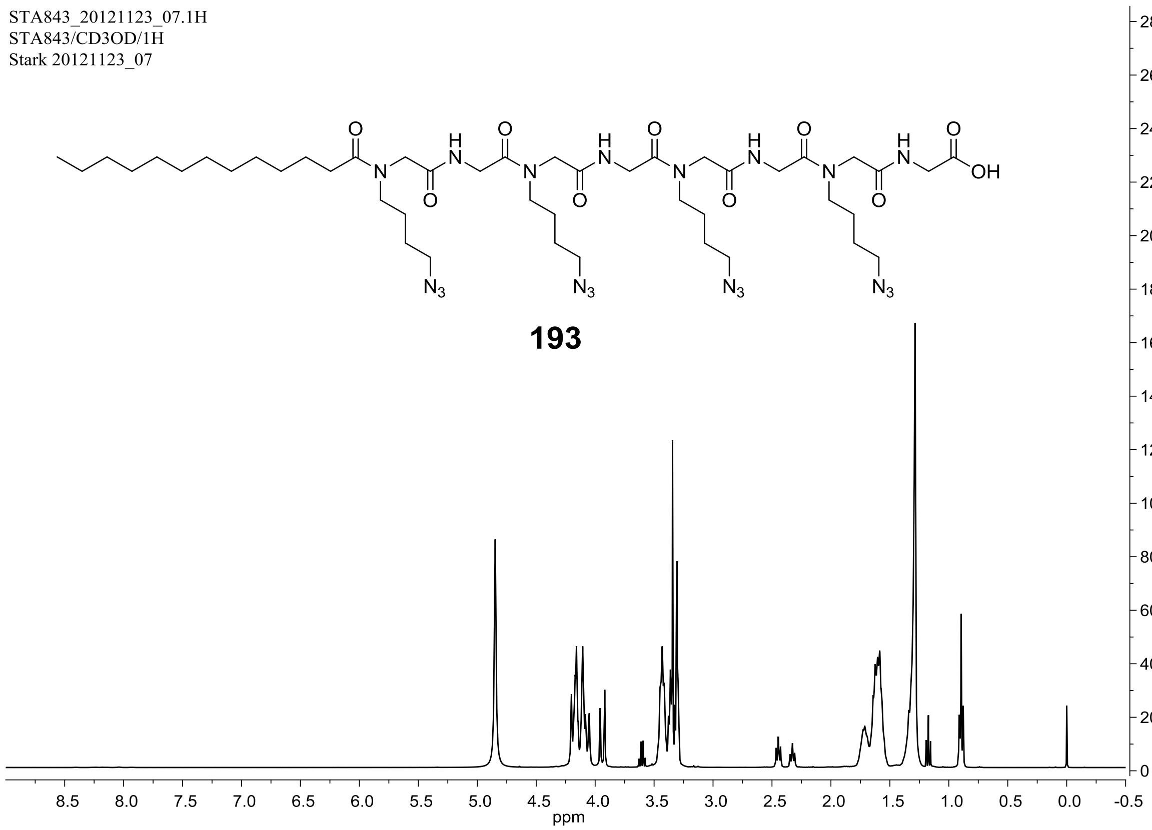


Figure 61. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Dodecanoic acid azido LPP **192** in CD_3OD.

8.1.13 Compound 193



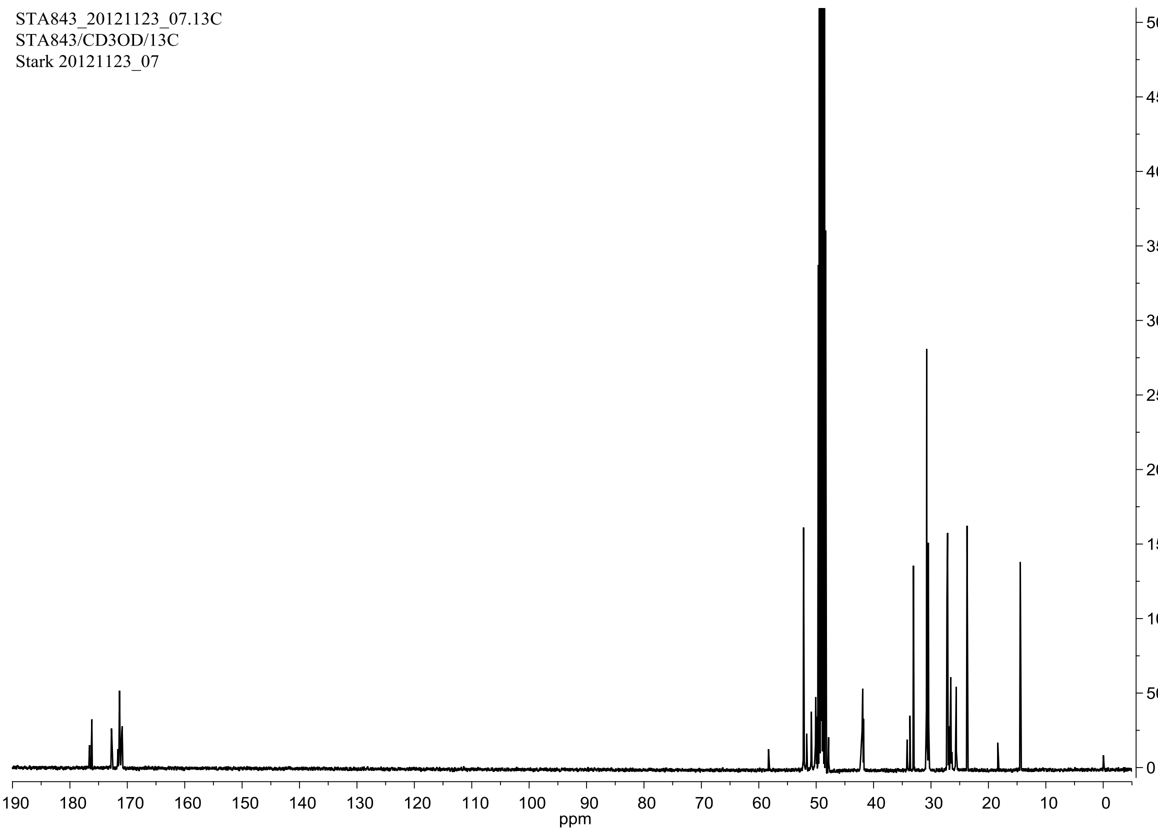
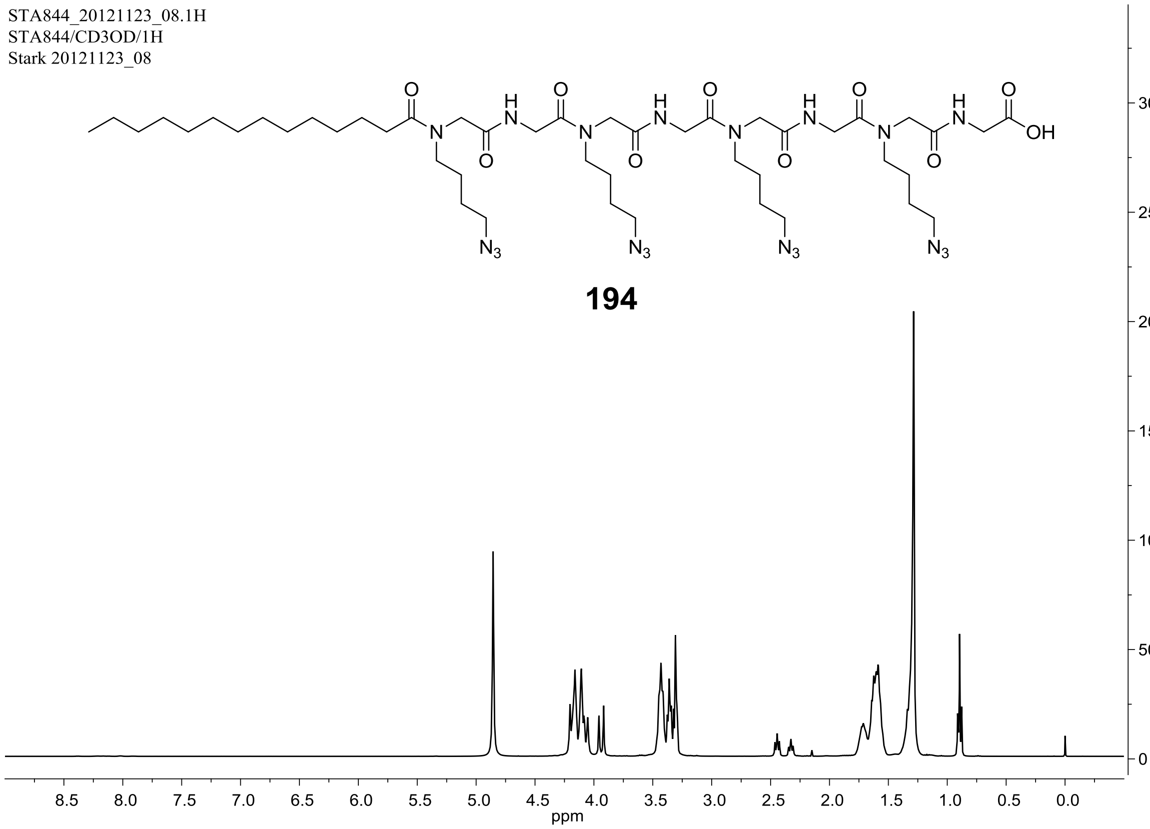


Figure 62. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Tridecanoic acid azido LPP **193** in CD_3OD.

8.1.14 Compound 194



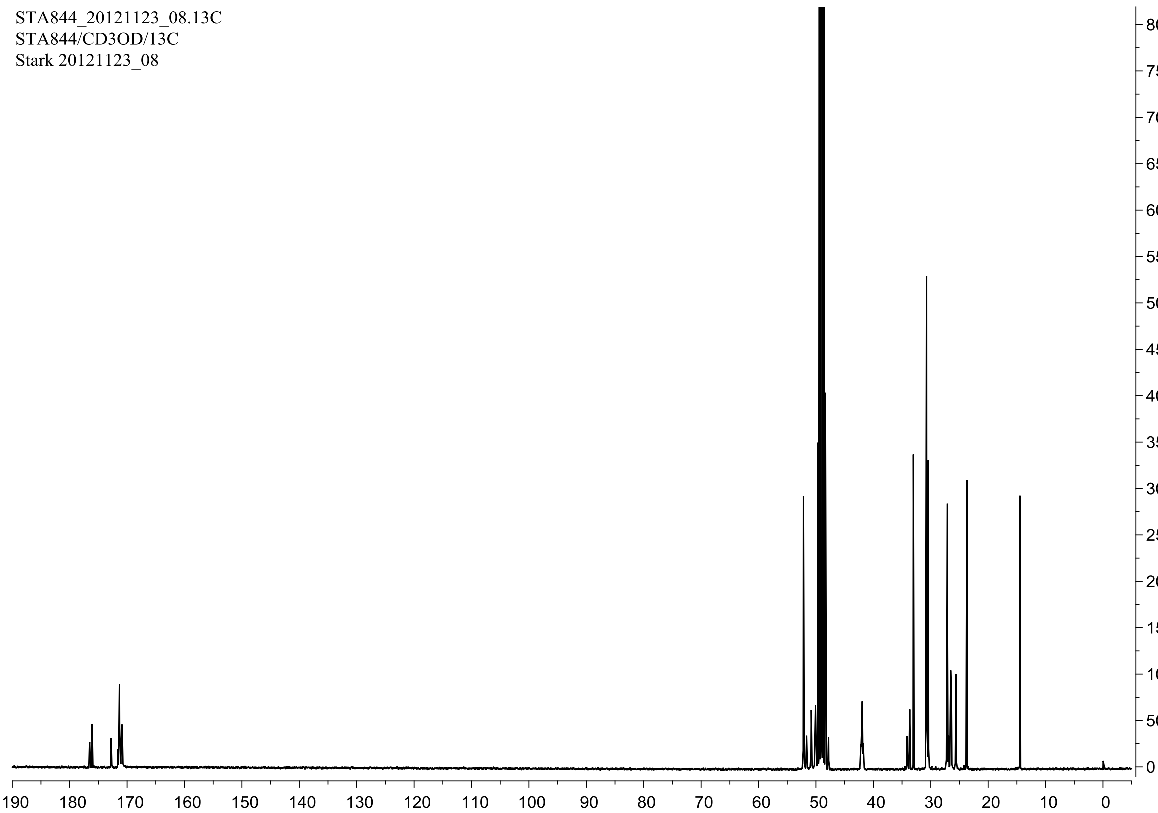
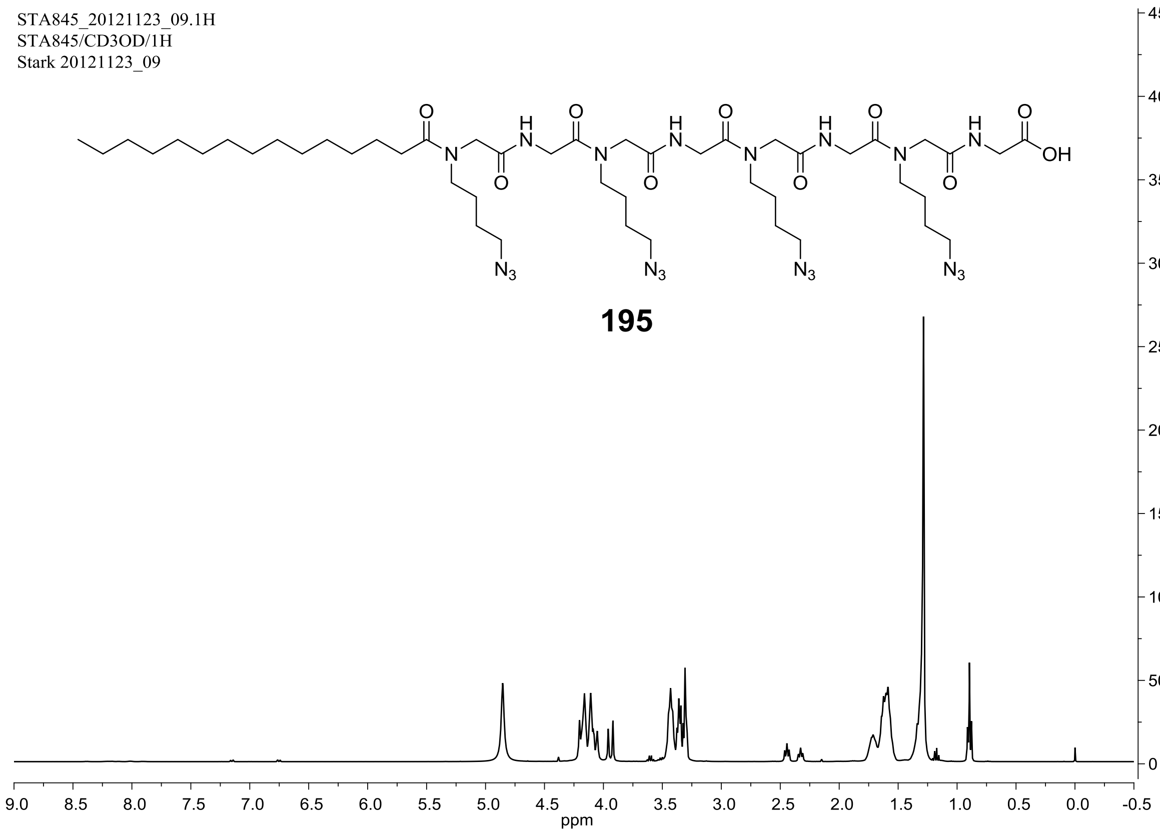


Figure 63. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Myristic acid azido LPP **194** in CD_3OD.

8.1.15 Compound 195



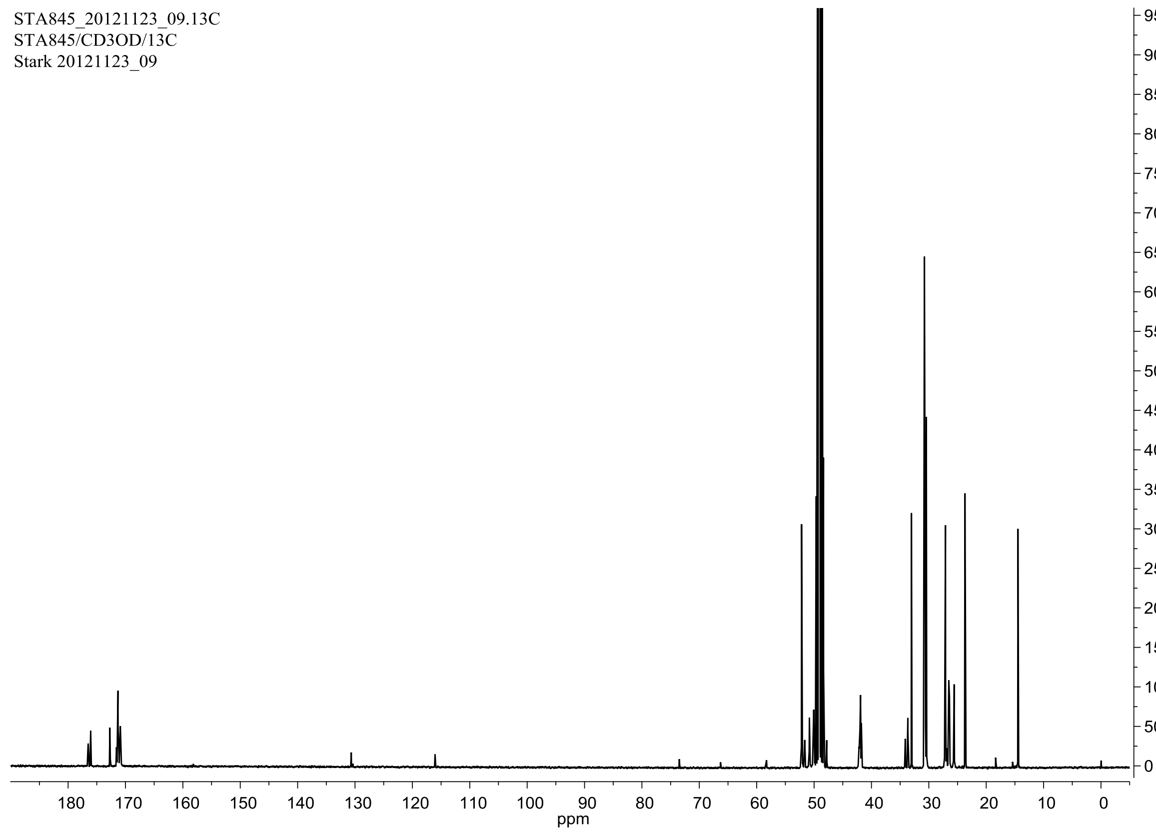
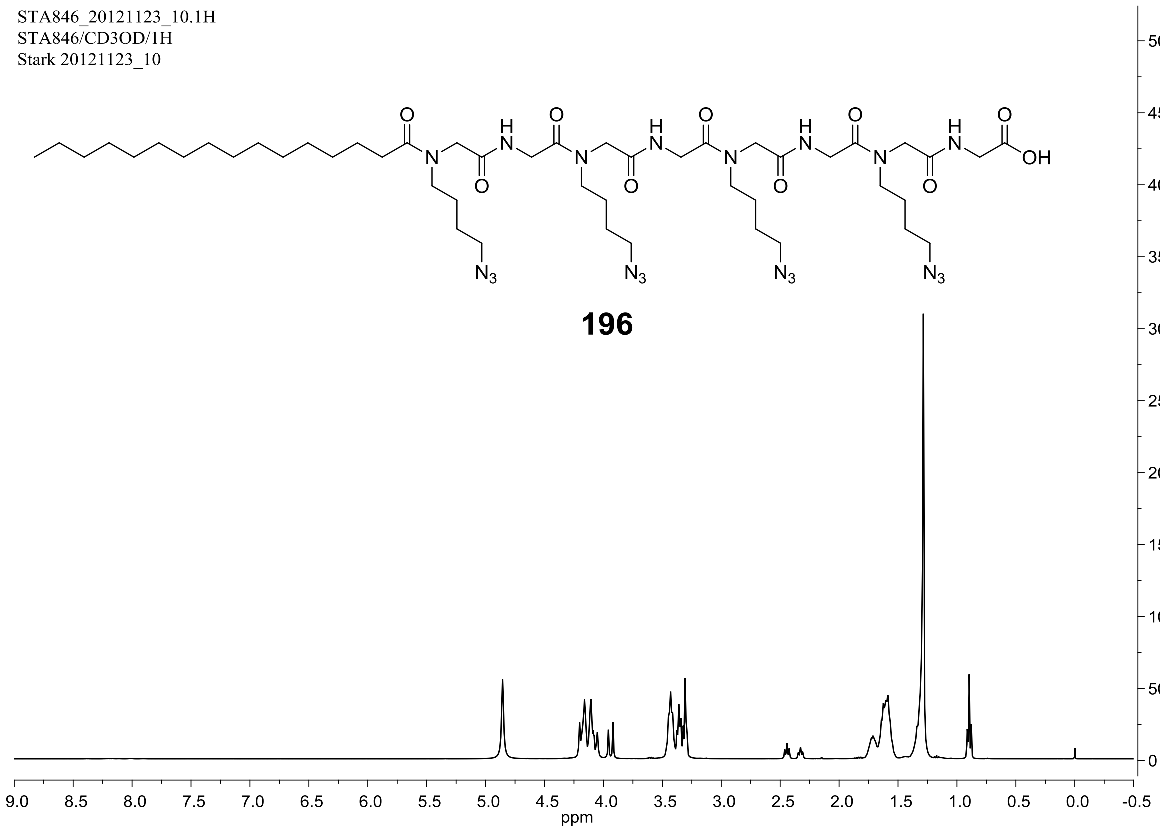


Figure 64. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Pentadecanoic acid azido LPP **195** in CD_3OD.

8.1.16 Compound 196



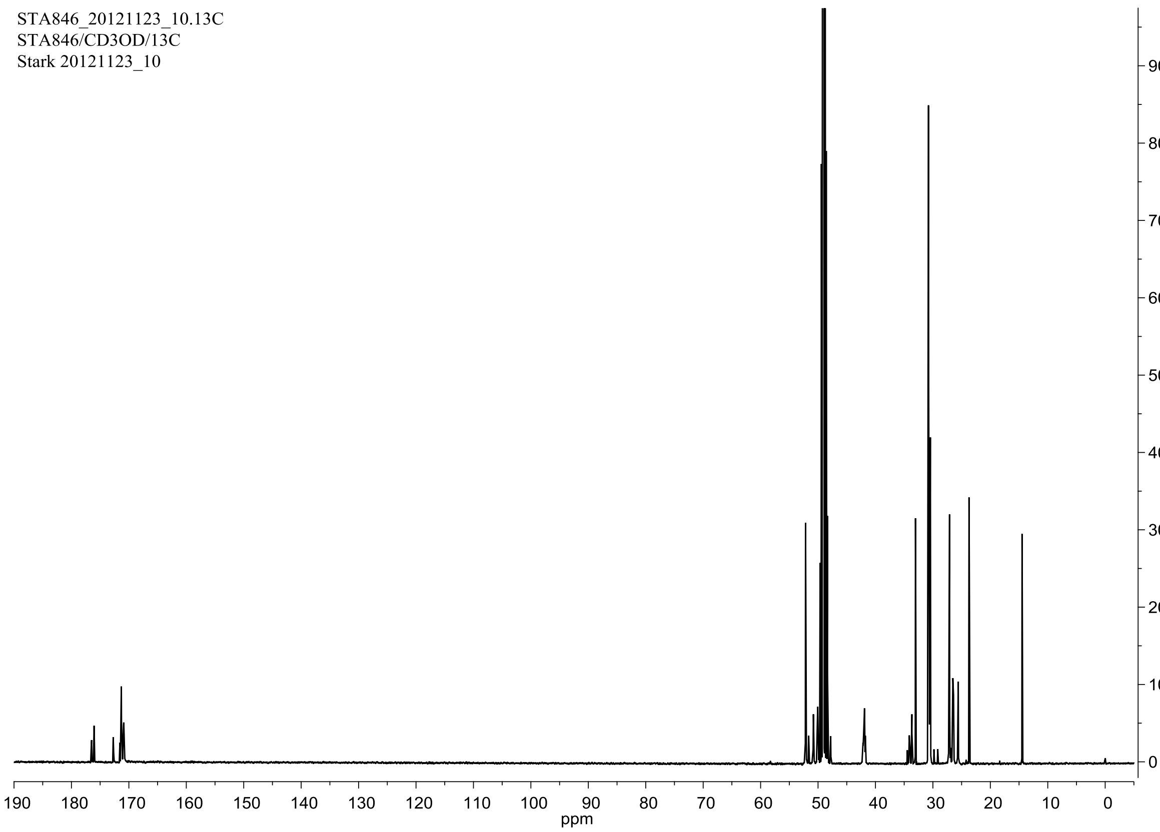
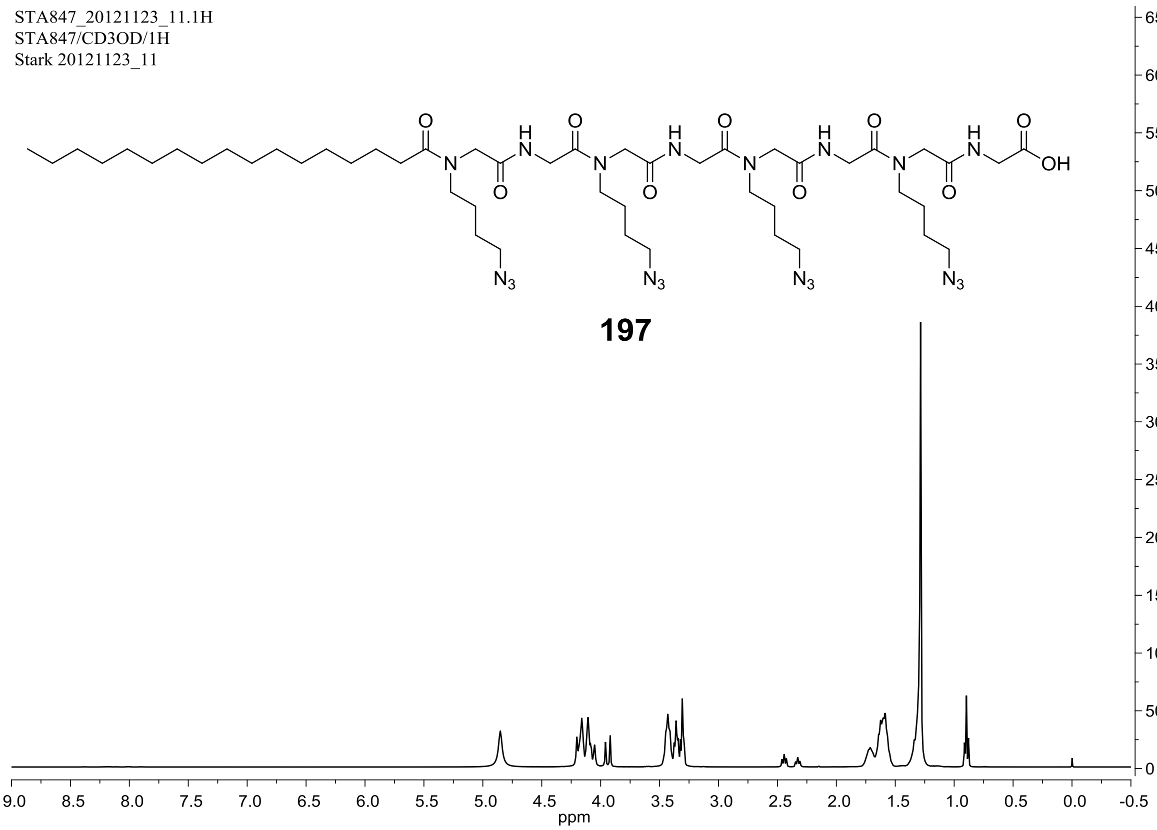


Figure 65. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Palmitic acid azido LPP **196** in CD_3OD.

8.1.17 Compound 197



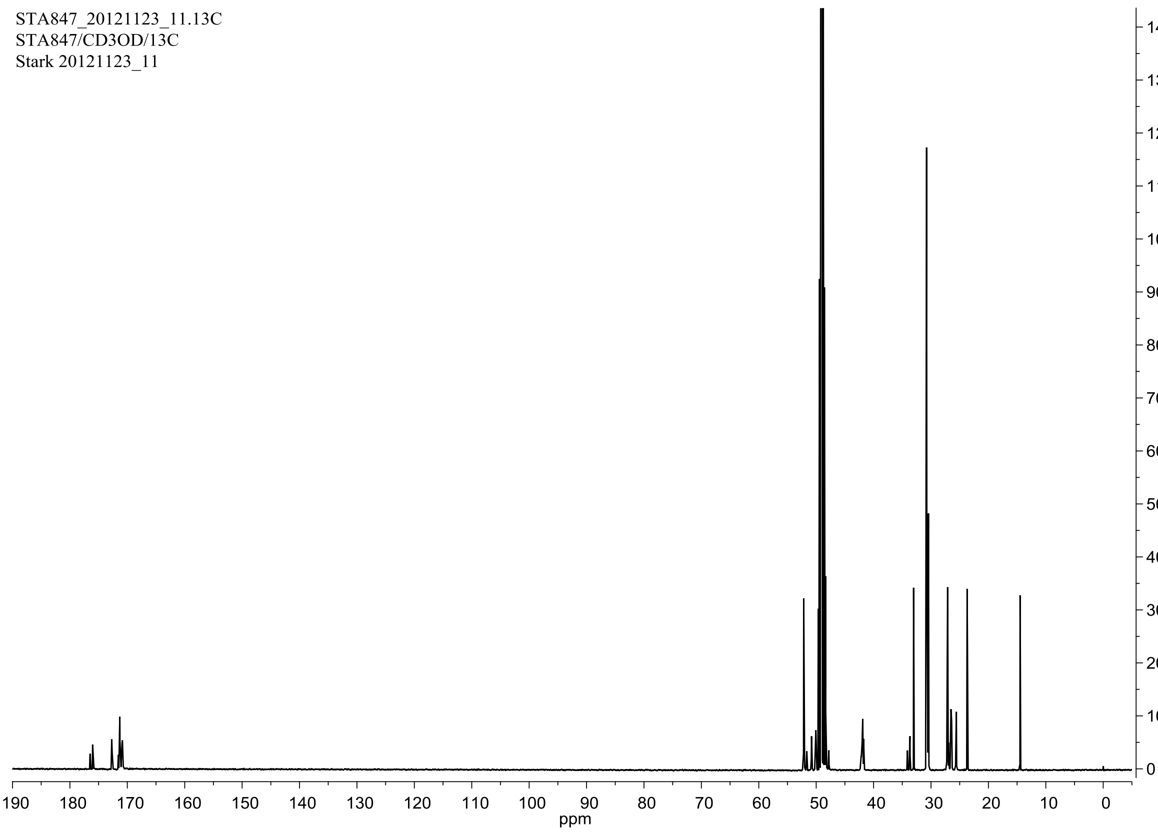
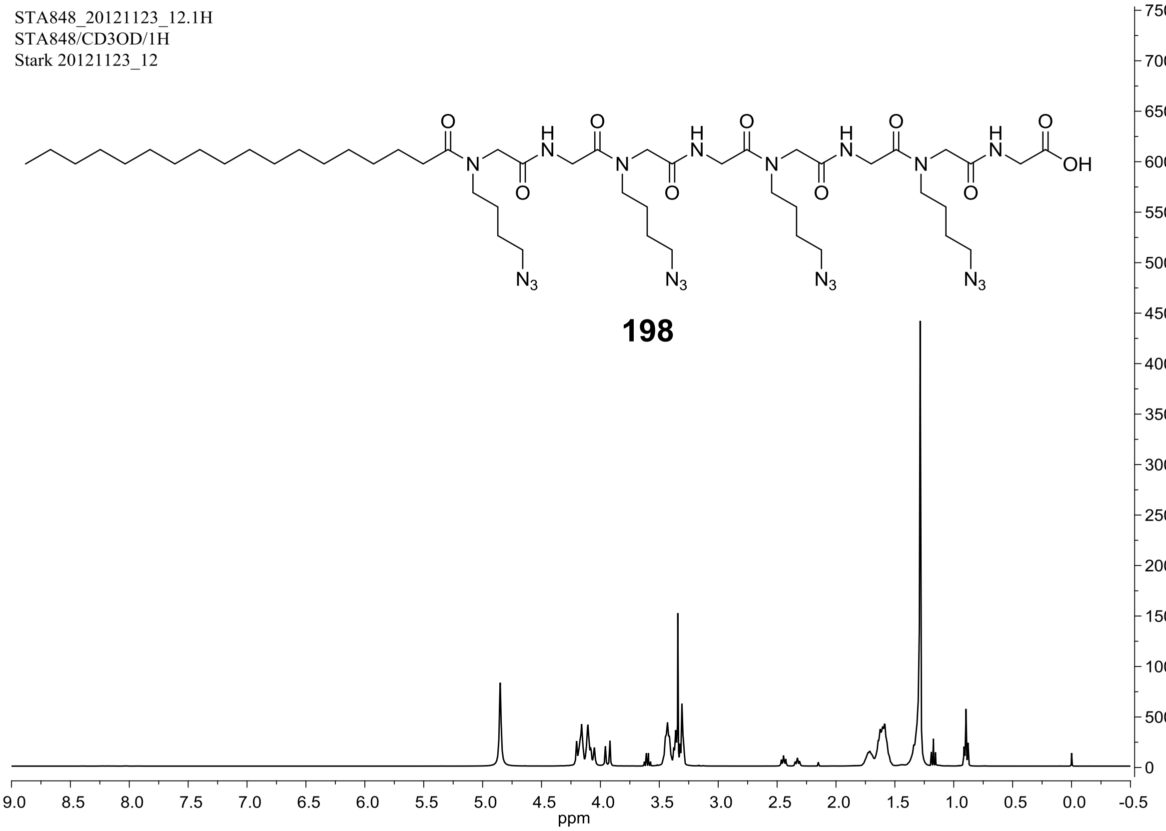


Figure 66. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Heptadecanoic acid azido LPP **197** in CD_3OD.

8.1.18 Compound 198



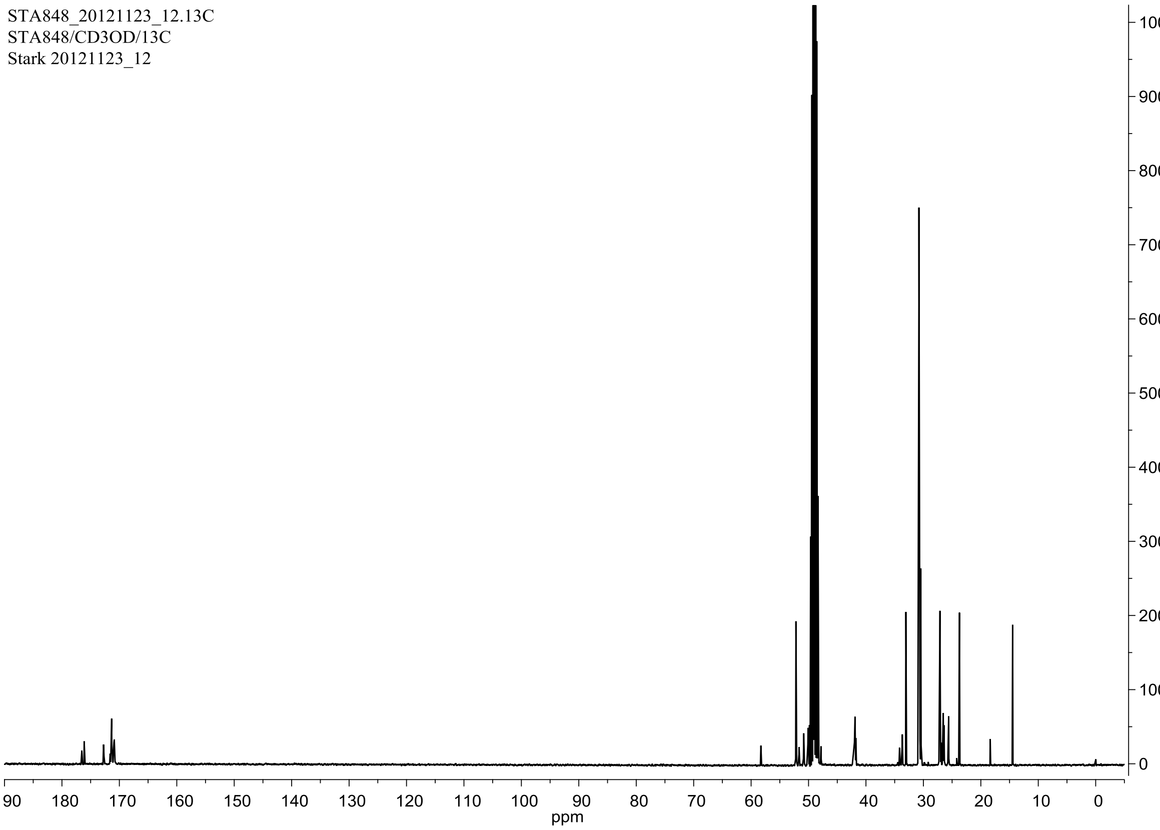


Figure 67. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Stearic acid azido LPP **198** in CD_3OD.

8.1.19 Compound 199

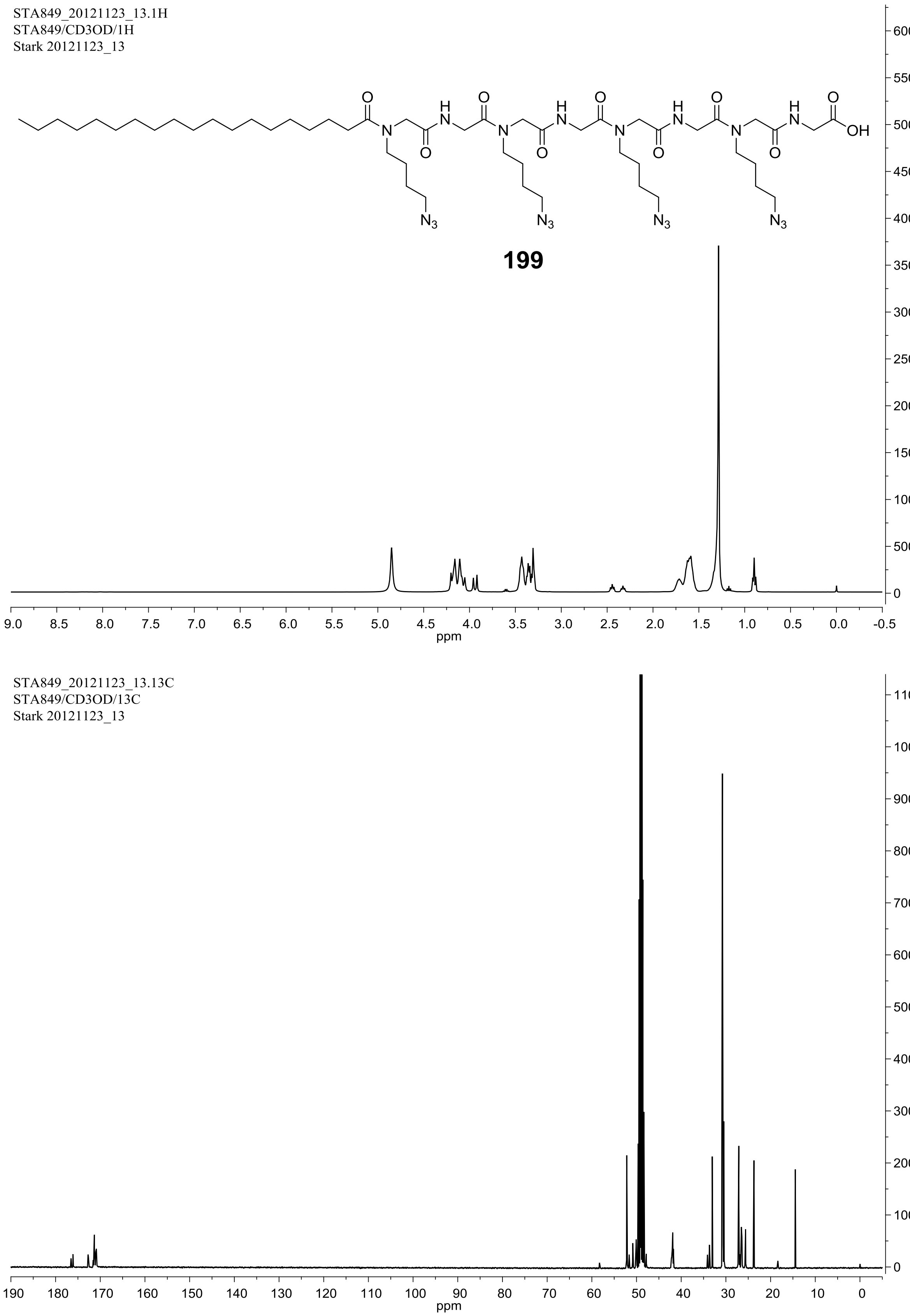


Figure 68. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Nonadecanoic acid azido LPP **199** in CD_3OD.

8.1.20 Compound 200

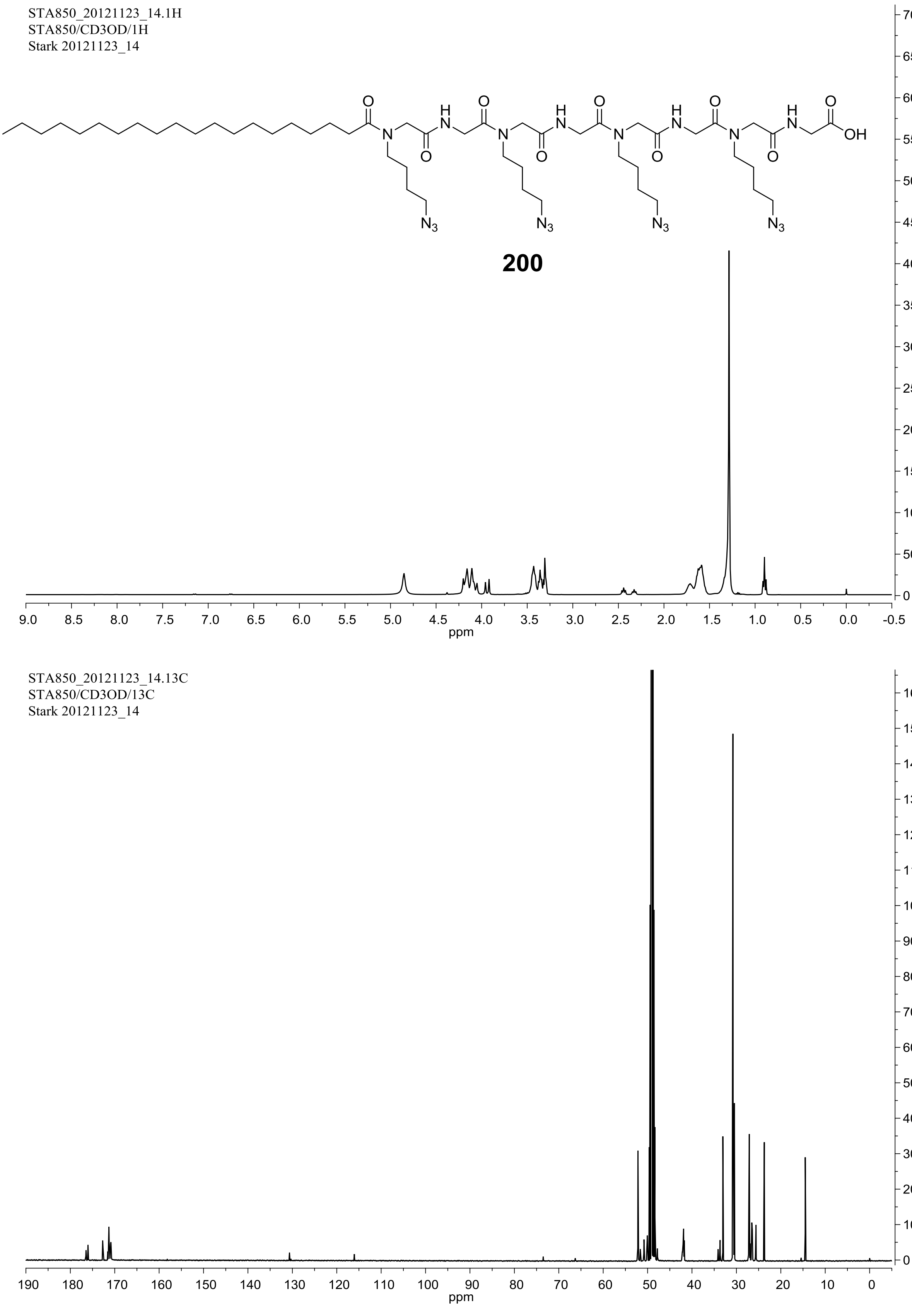
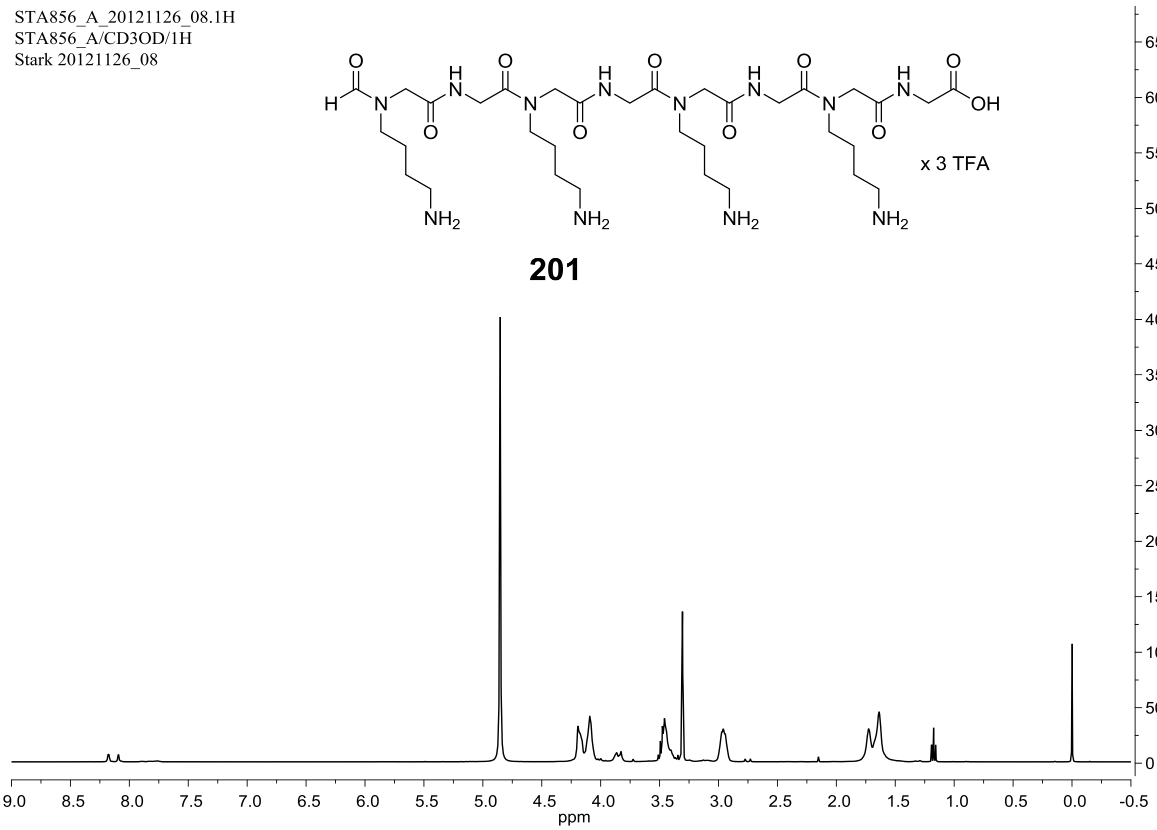


Figure 69. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Arachidic acid azido LPP **200** in CD_3OD.

8.1.21 Compound 201



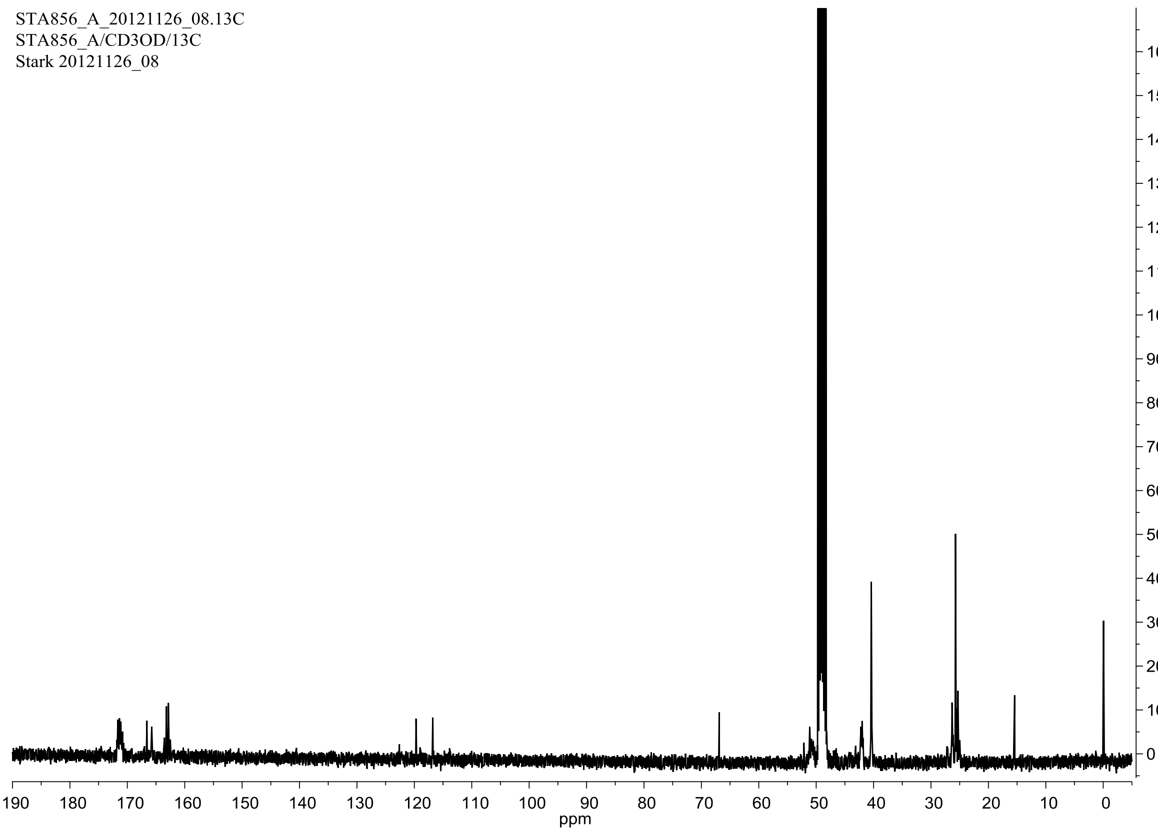
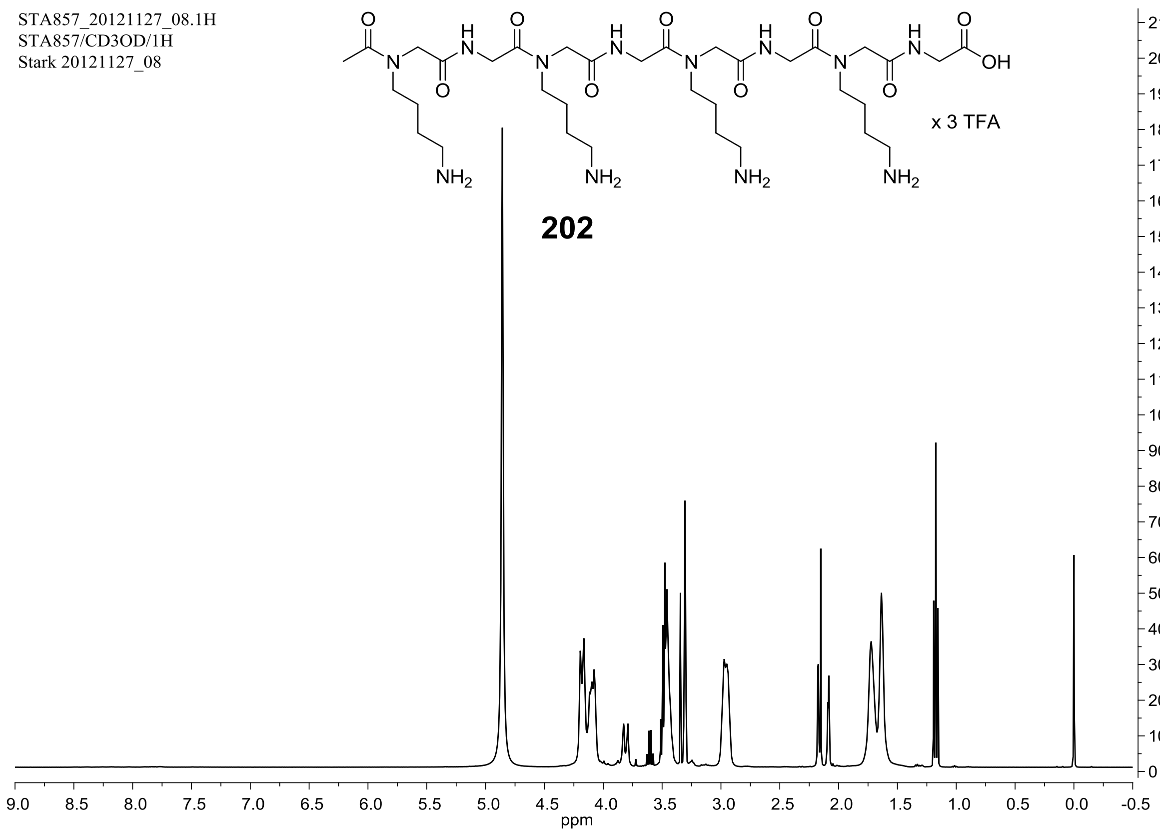


Figure 70. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Formic acid LPP **201** in CD_3OD.

8.1.22 Compound 202



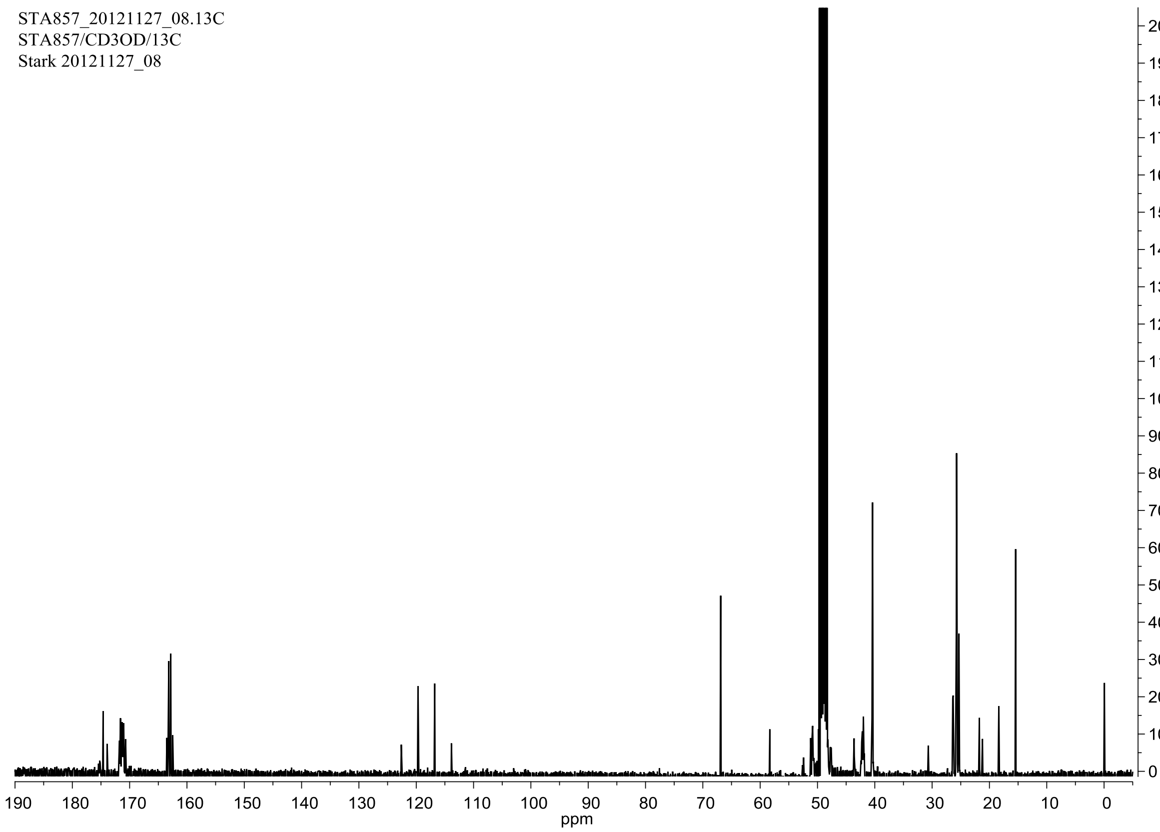
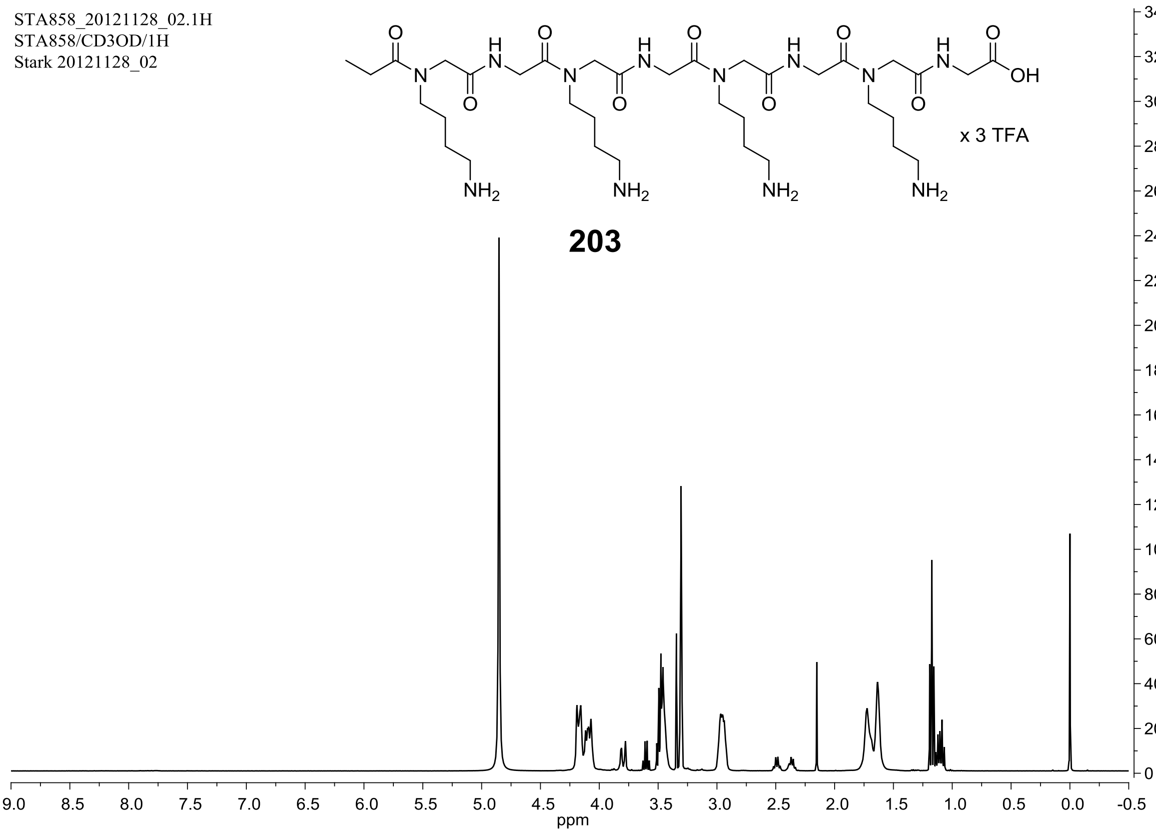


Figure 71. 400 MHz-[1]H- and 100 MHz-[13]C-NMR spectra of Acetic acid LPP **202** in CD_3OD.

8.1.23 Compound 203



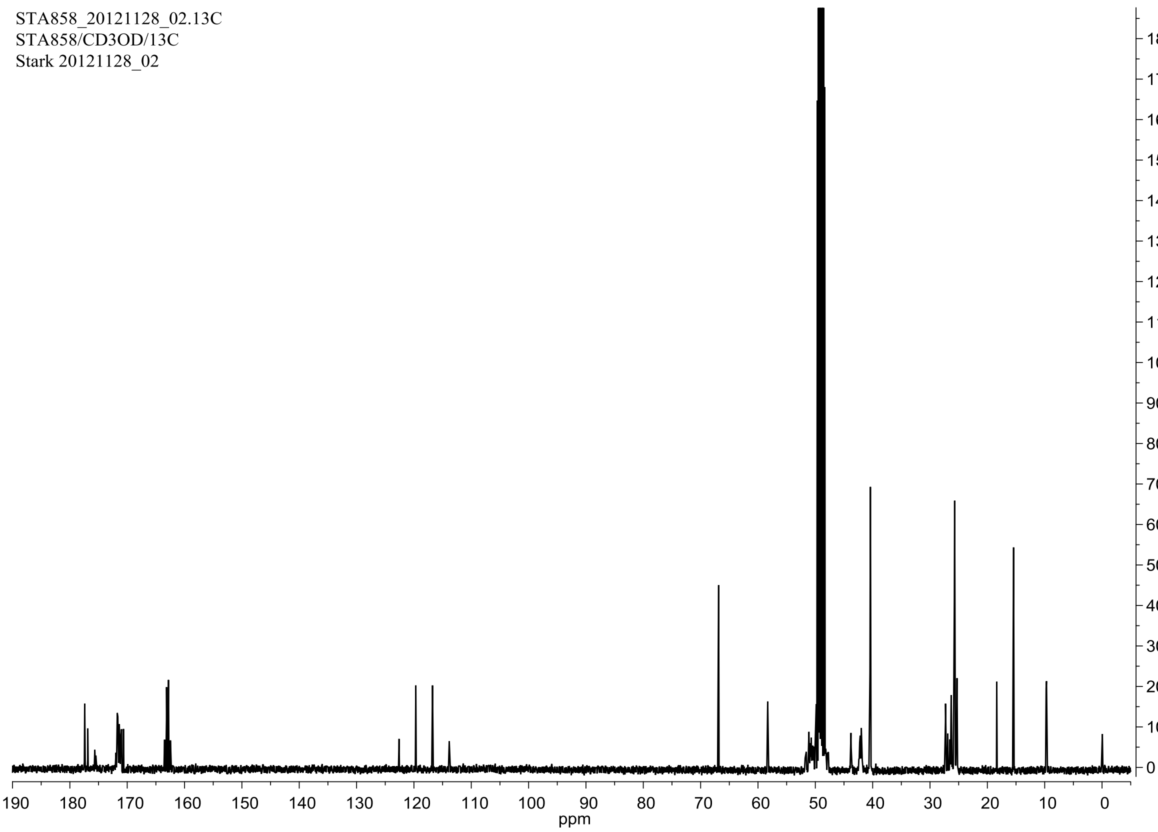


Figure 72. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Propionic acid LPP **203** in CD_3OD.

8.1.24 Compound 204

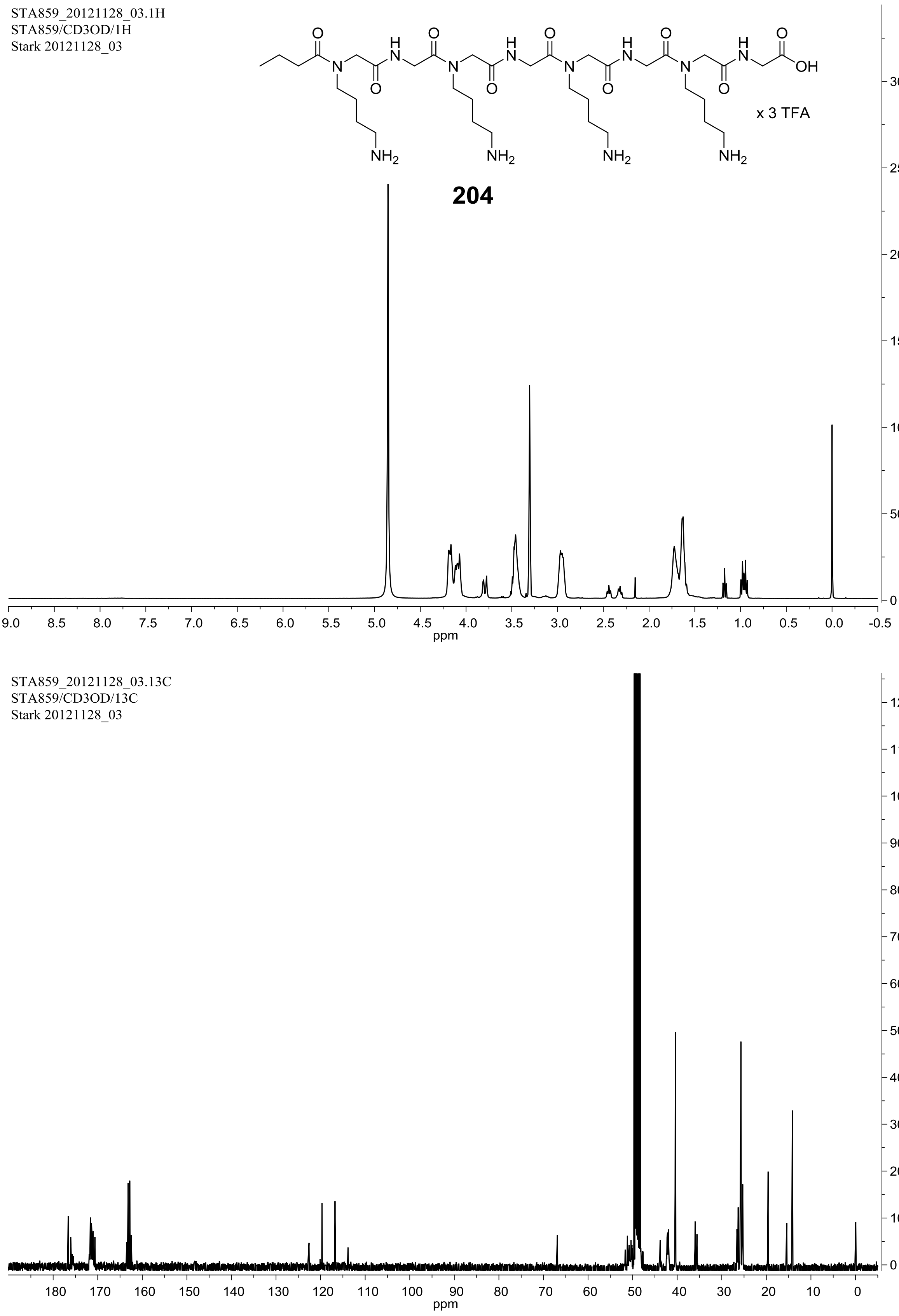
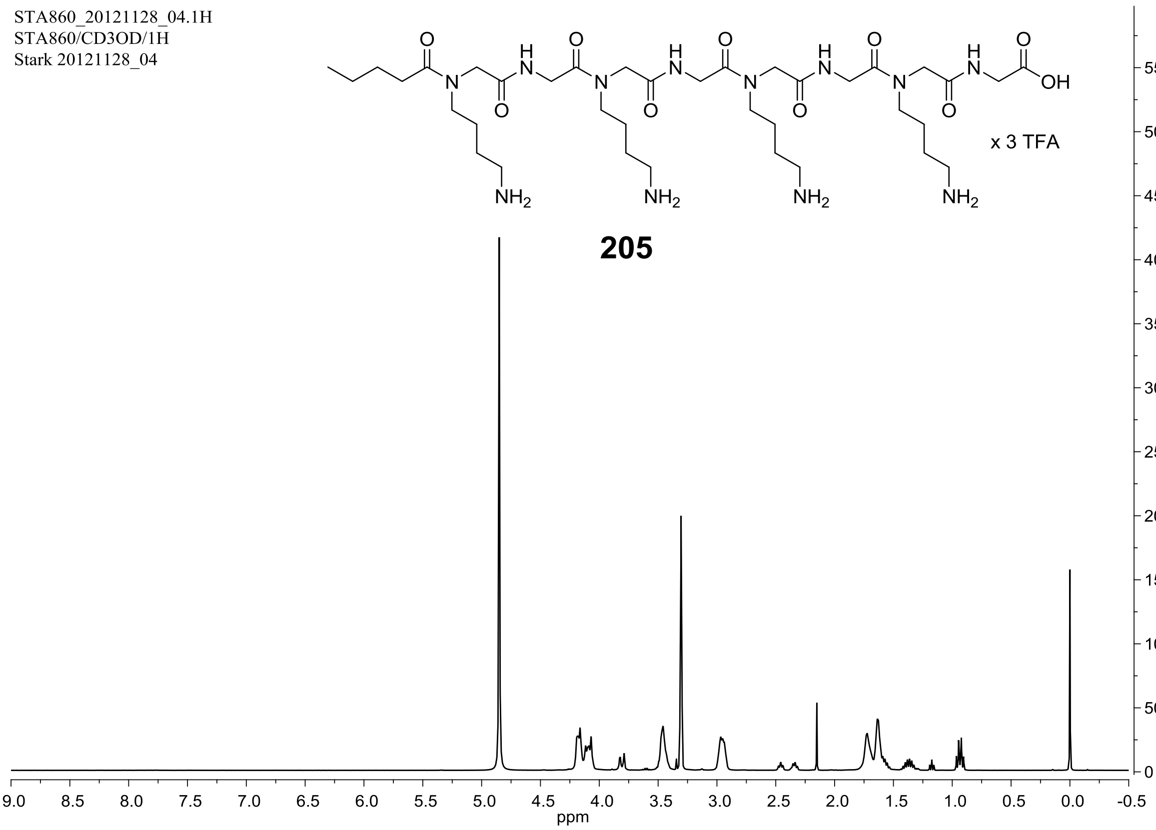


Figure 73. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Butyric acid LPP **204** in CD_3OD.

8.1.25 Compound 205



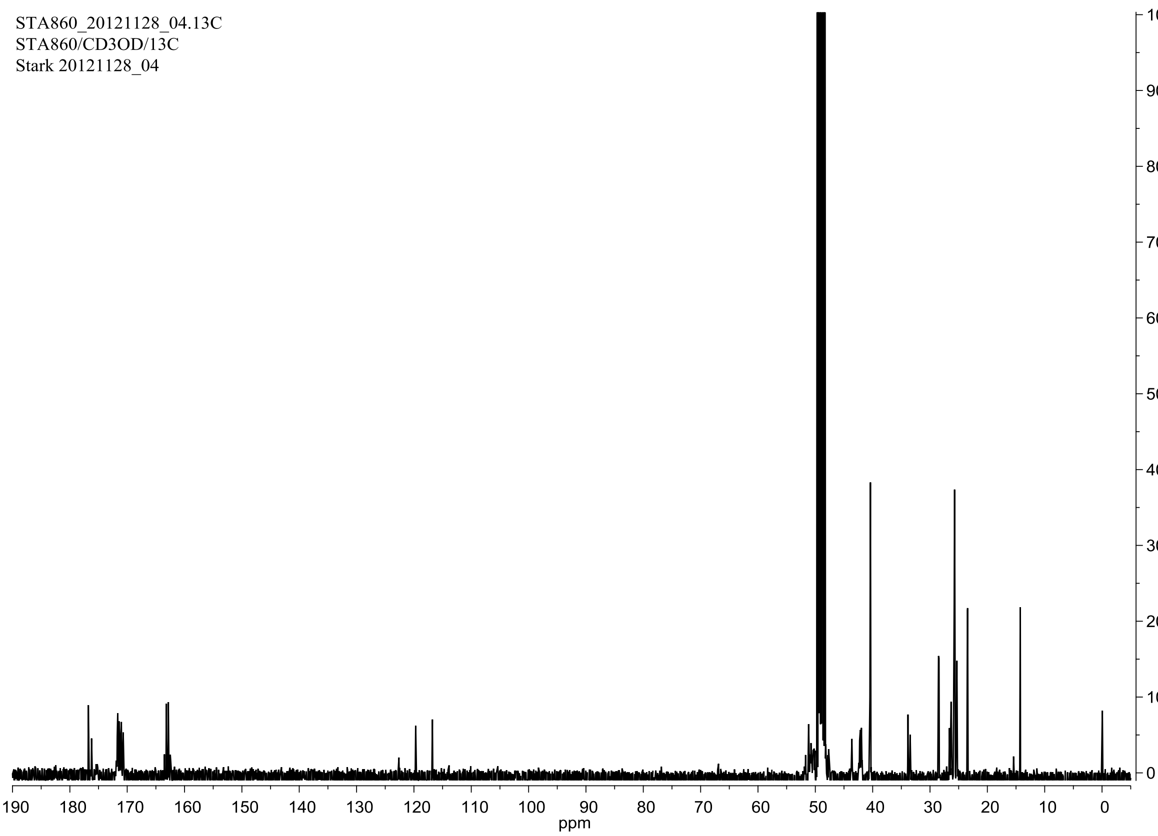
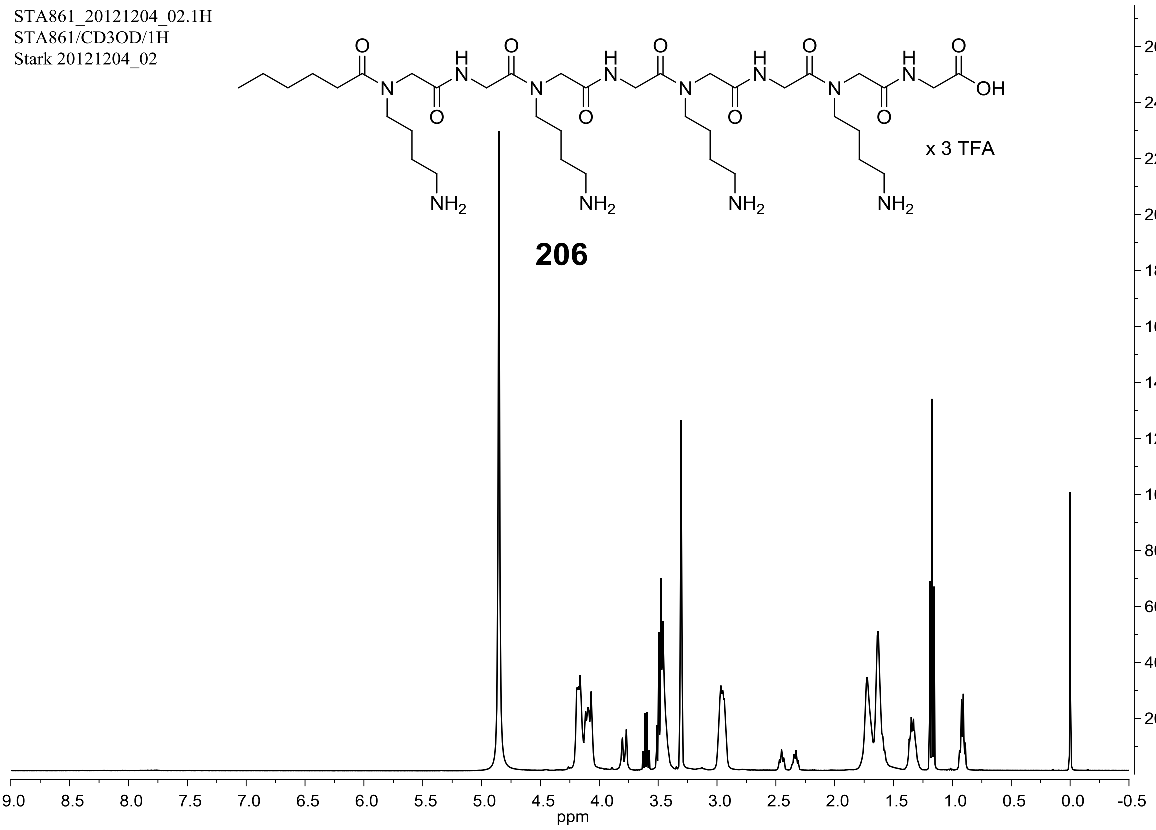


Figure 74. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Valeric acid LPP **205** in CD_3OD.

8.1.26 Compound 206



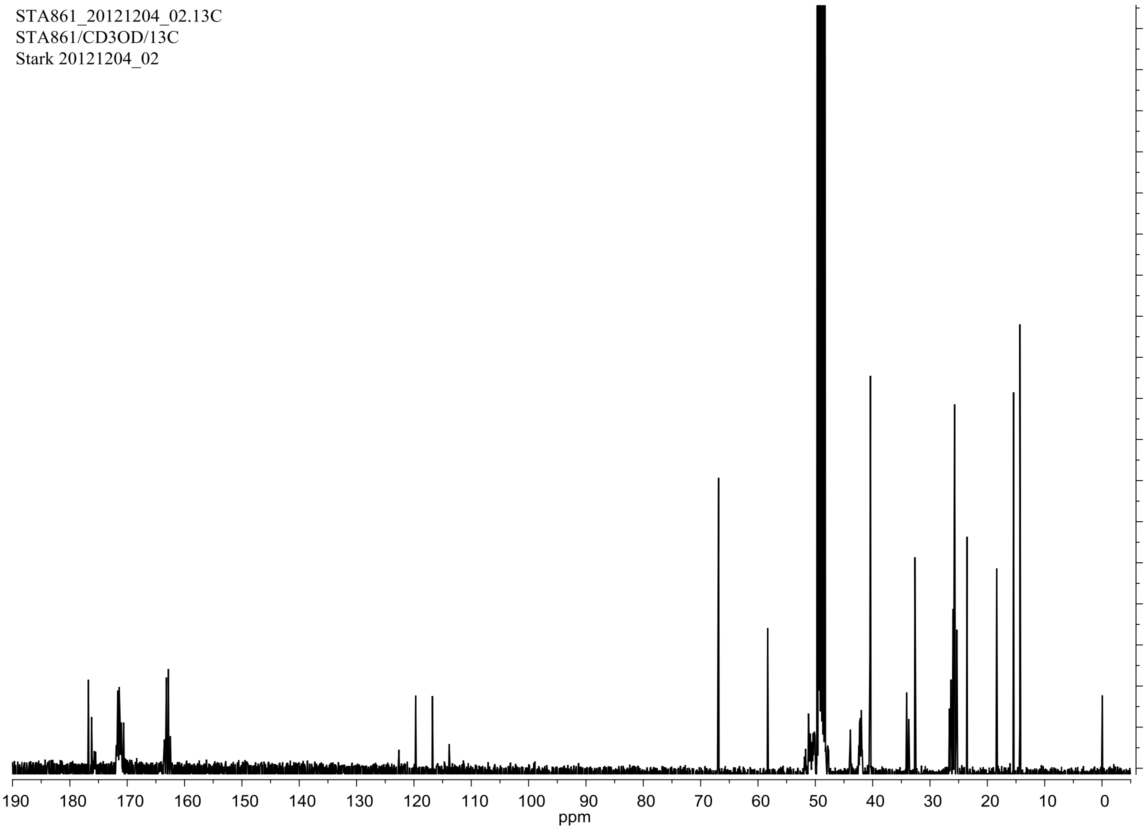
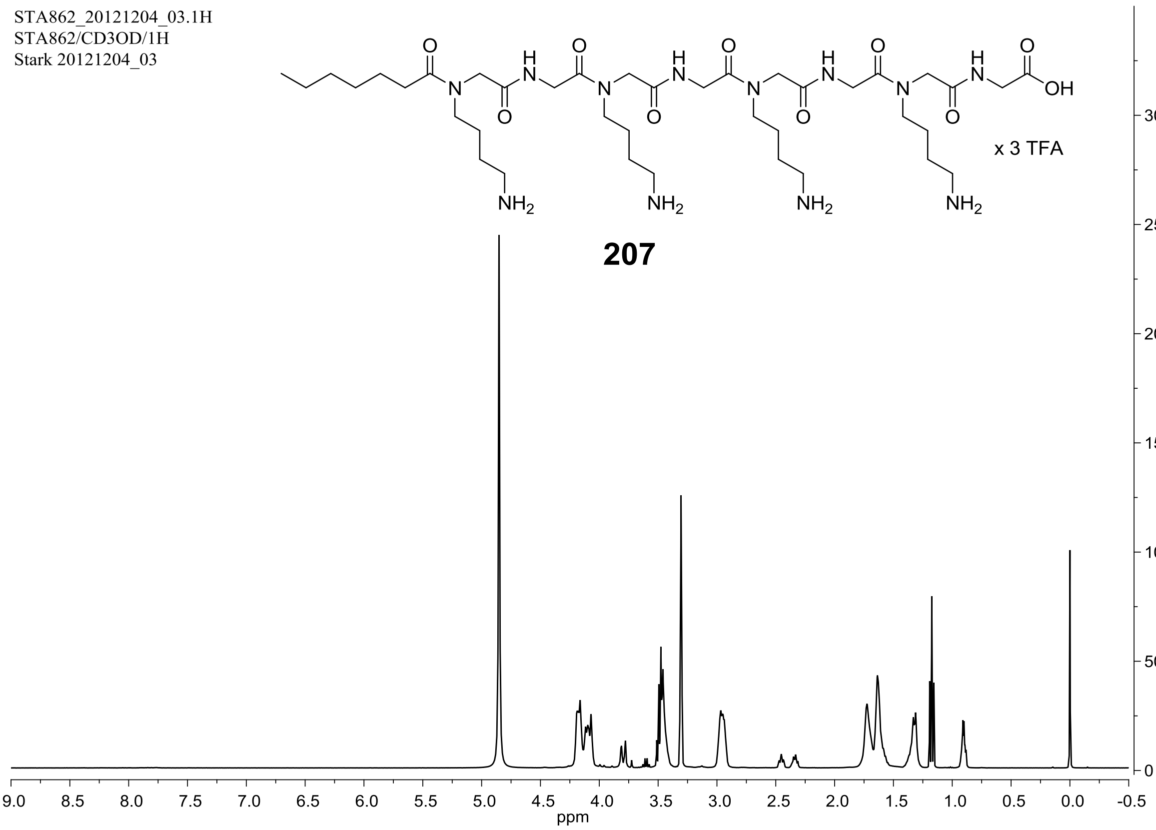


Figure 75. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Hexanoic acid LPP **206** in CD_3OD.

8.1.27 Compound 207



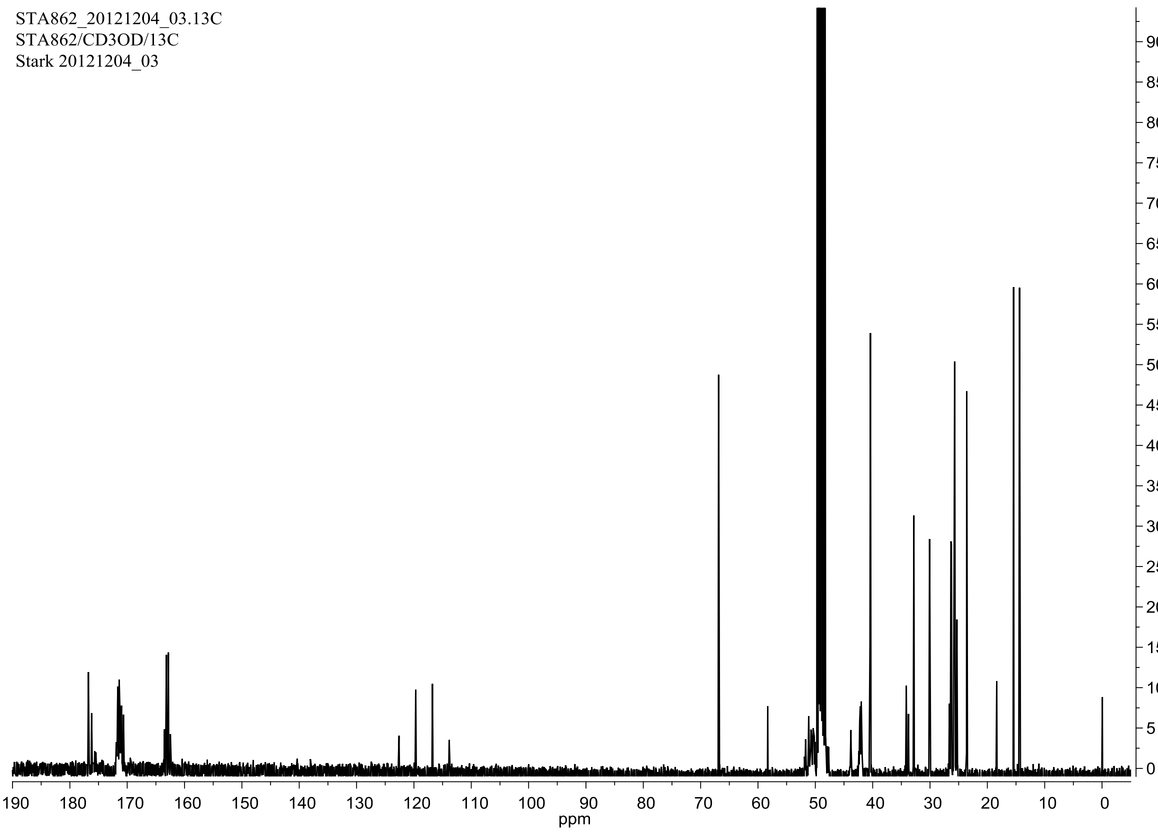
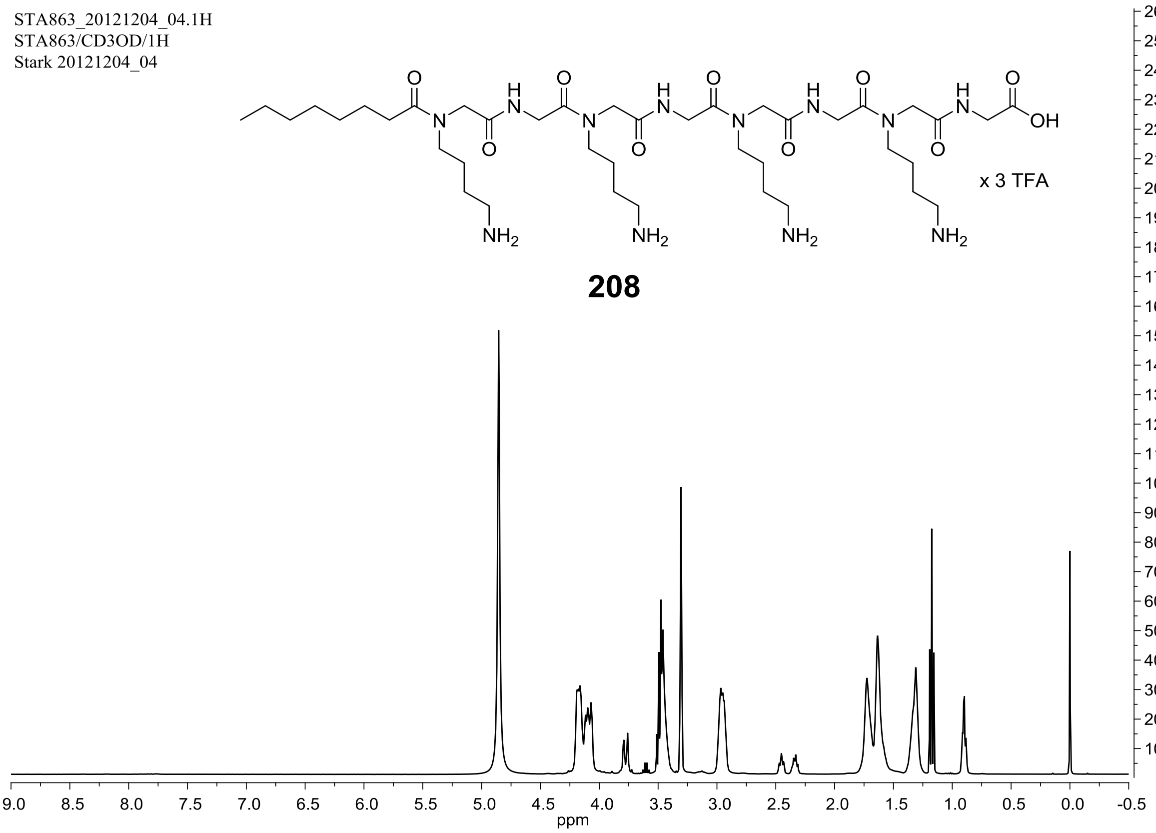


Figure 76. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Heptanoic acid LPP **207** in CD_3OD.

8.1.28 Compound 208



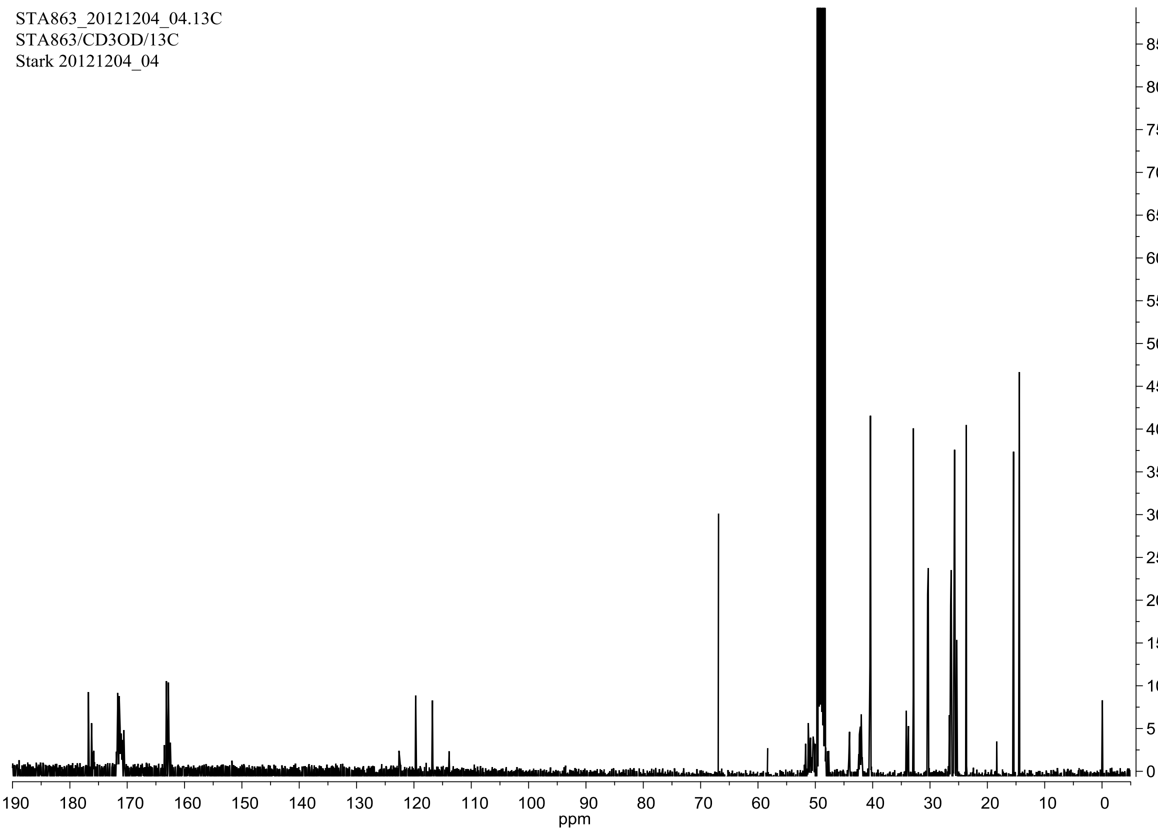


Figure 77. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Octanoic acid LPP **208** in CD_3OD.

8.1.29 Compound 209

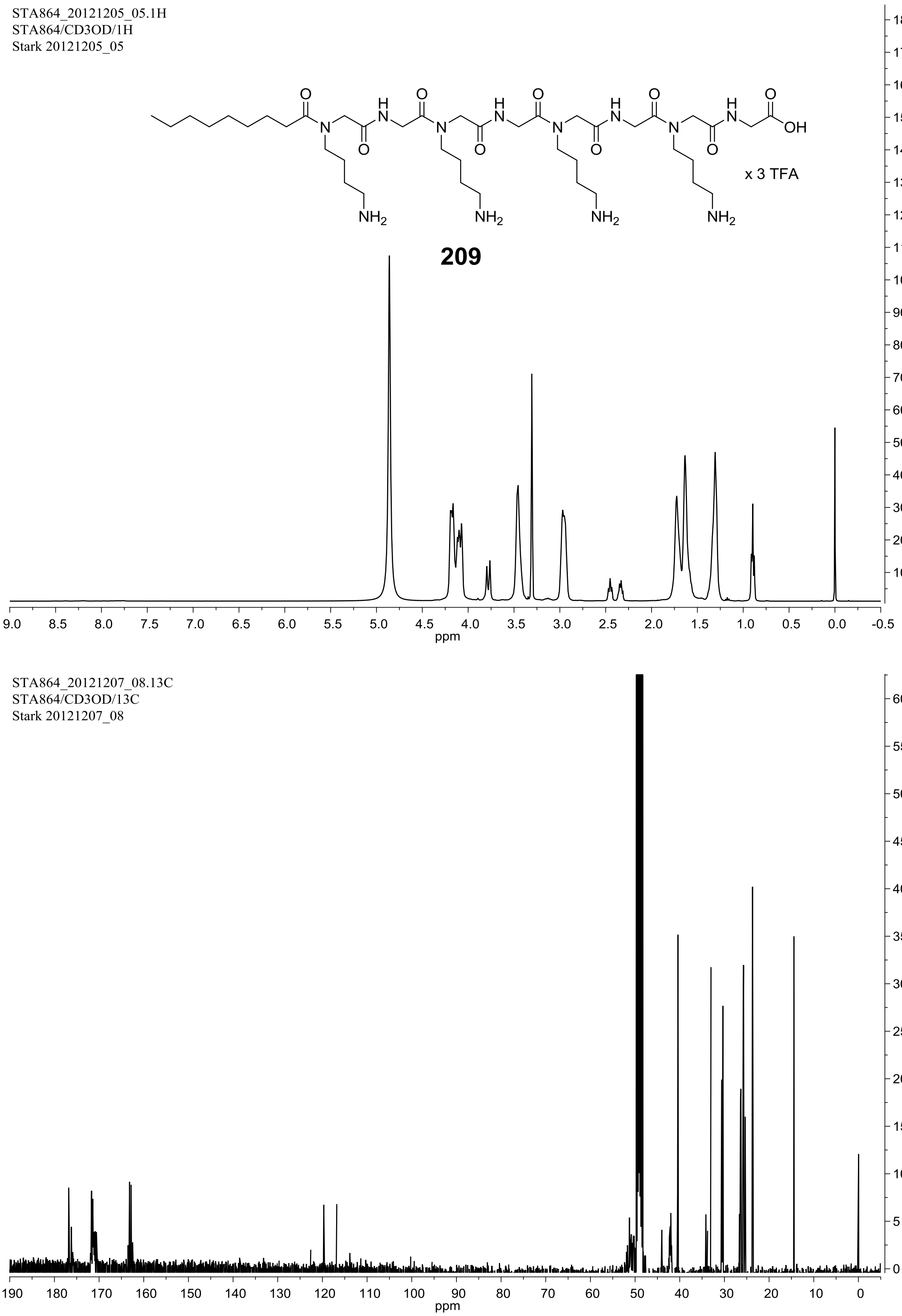
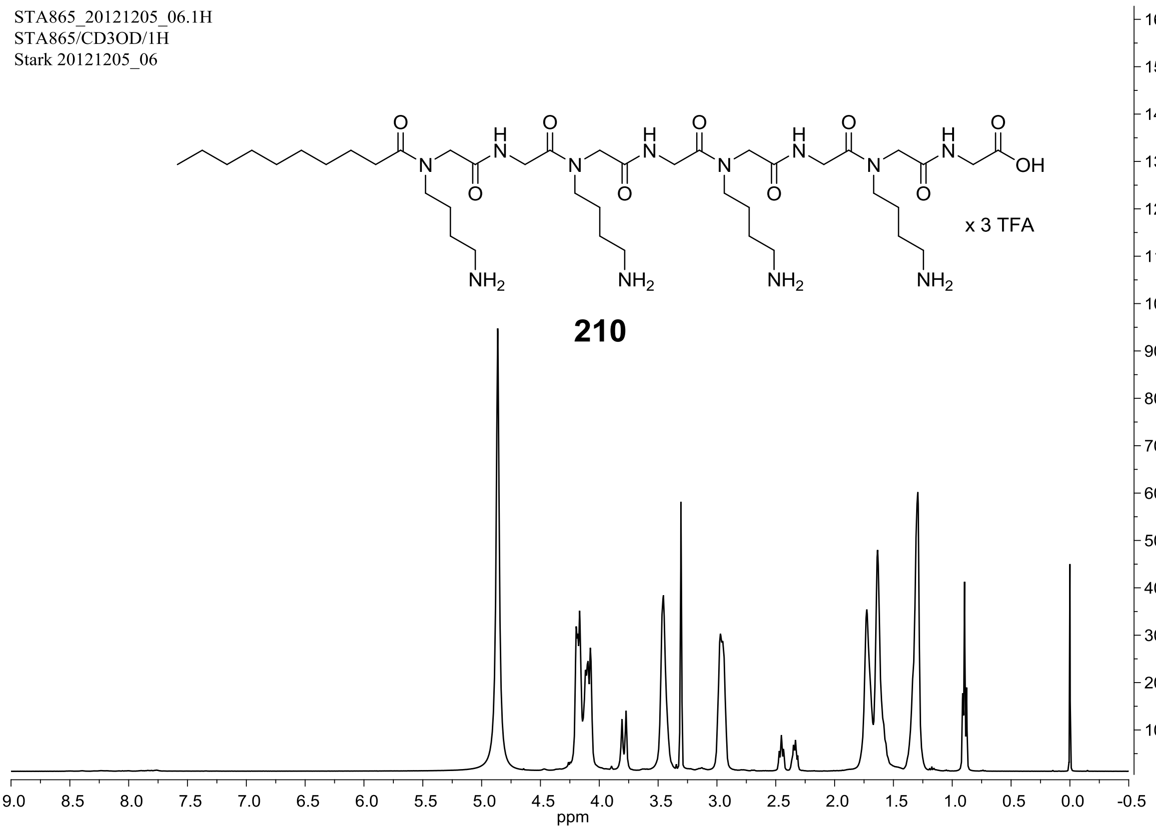


Figure 78. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Nonanoic acid LPP **209** in CD_3OD.

8.1.30 Compound 210



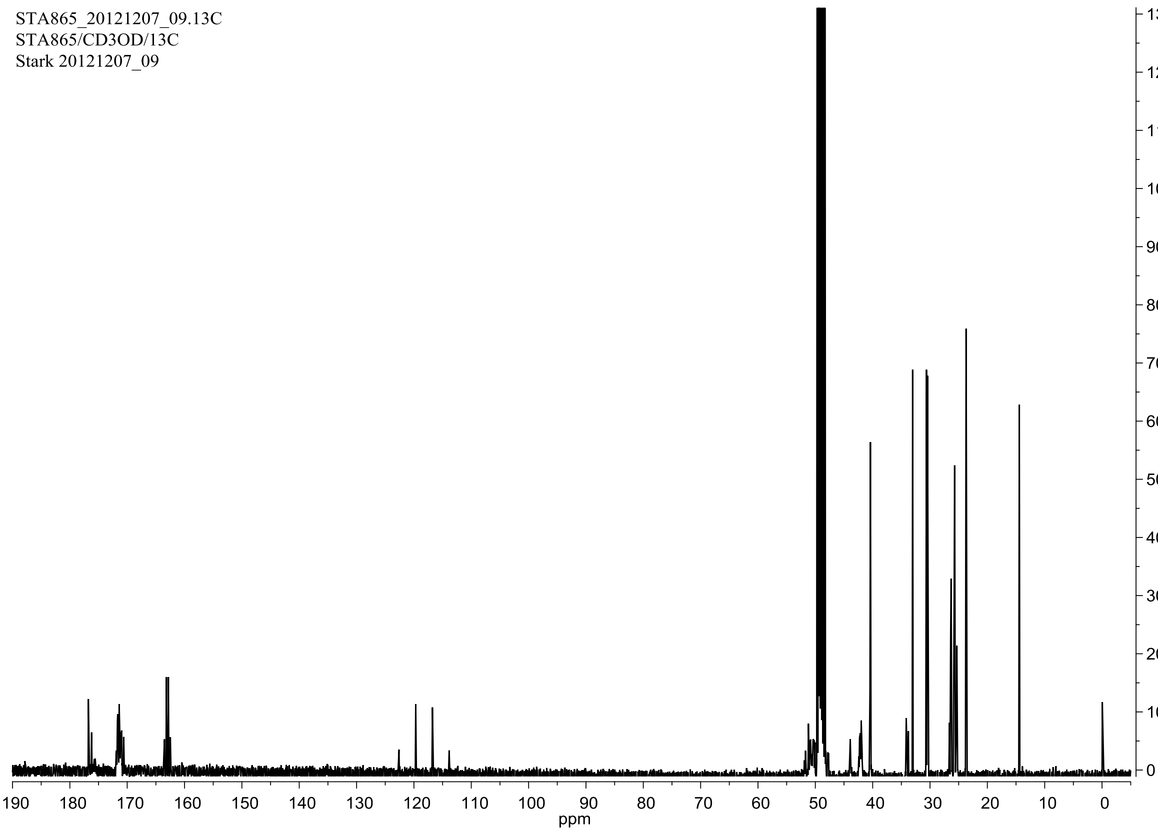
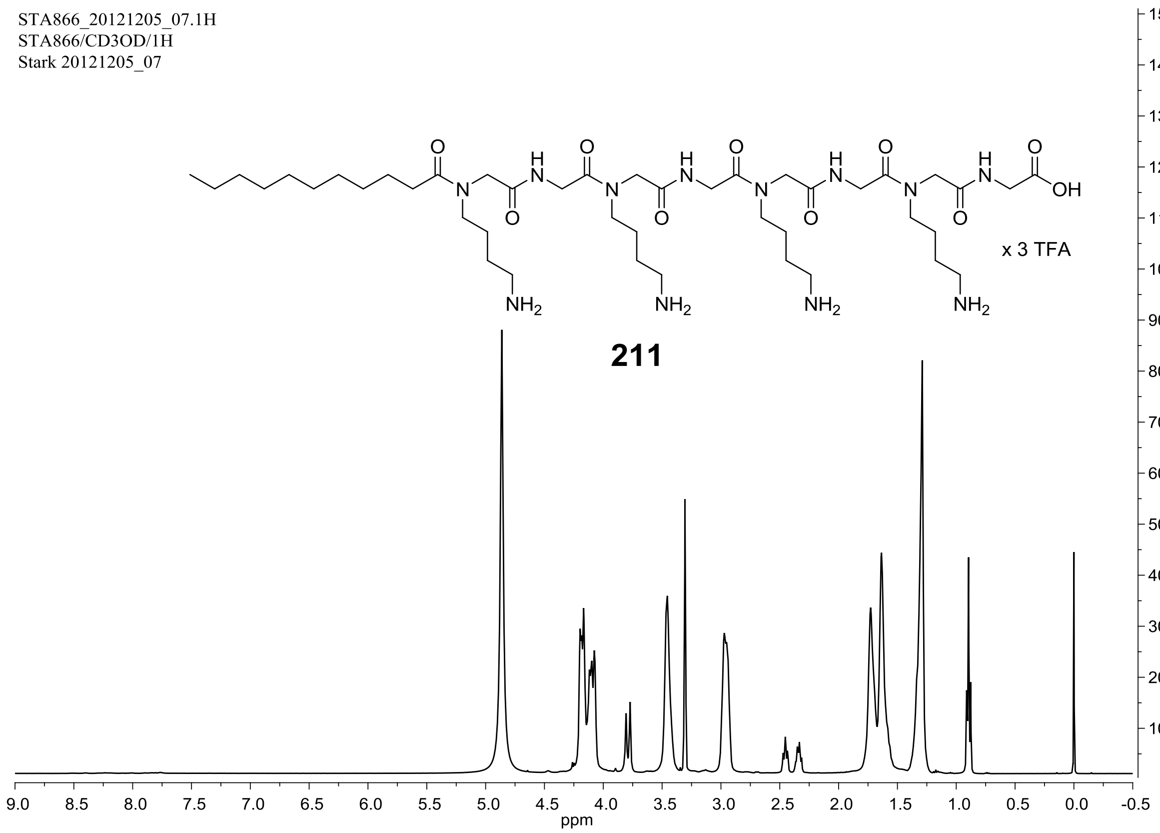


Figure 79. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Decanoic acid LPP **210** in CD_3OD.

8.1.31 Compound 211



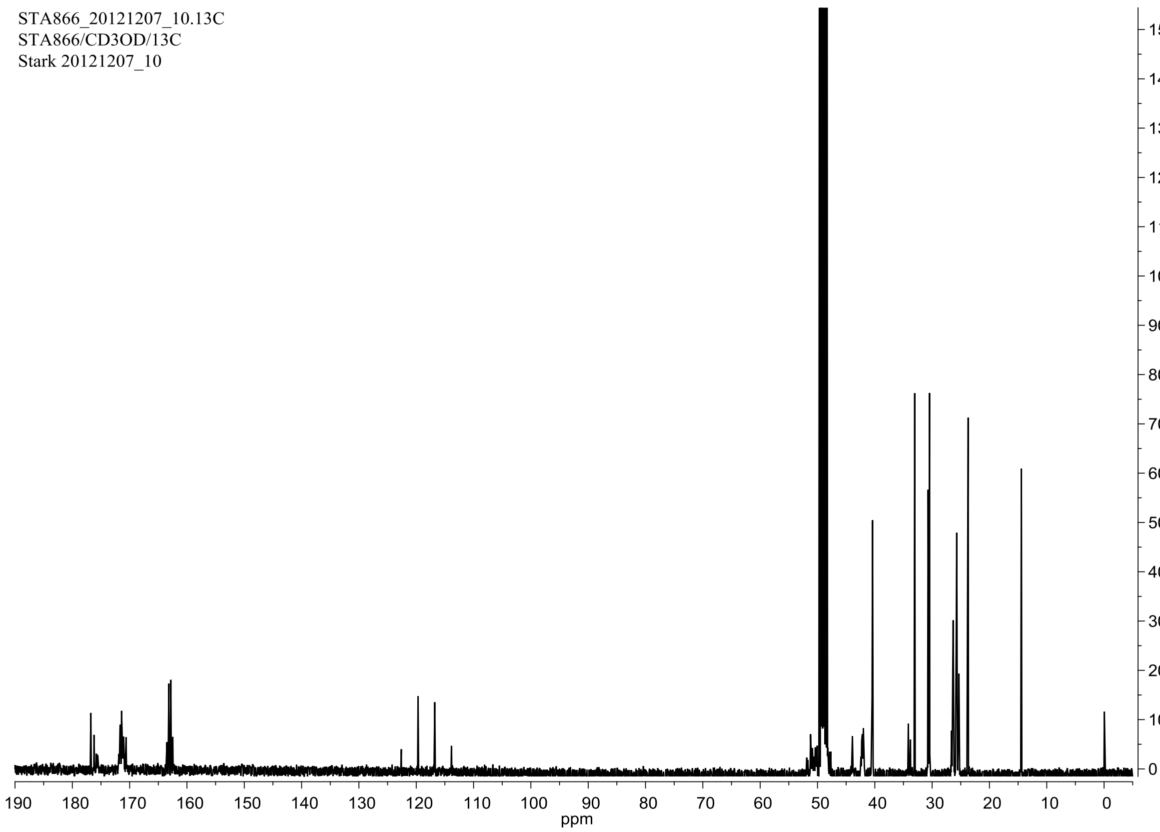


Figure 80. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Undecanoic acid LPP **211** in CD_3OD.

8.1.32 Compound 212

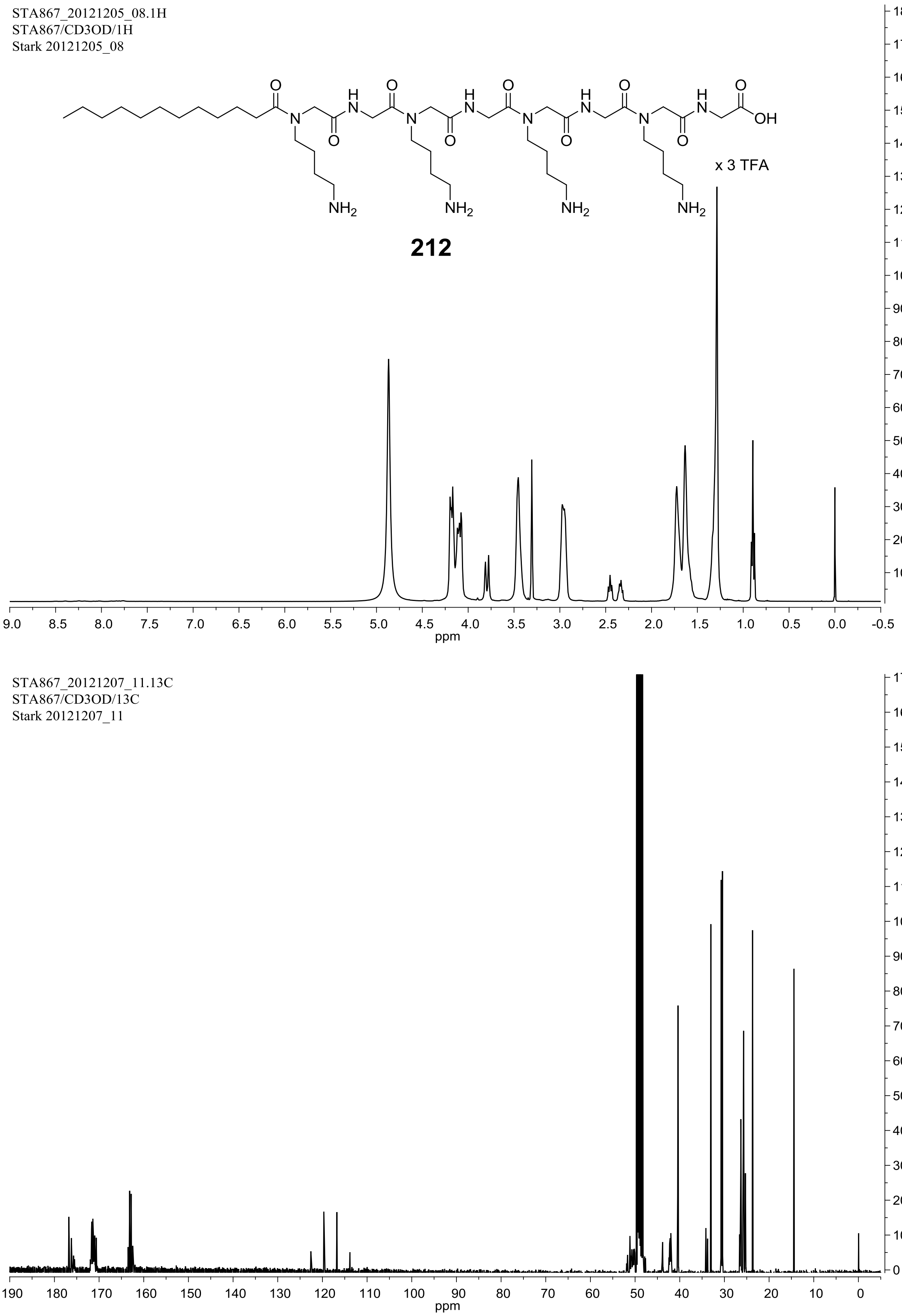


Figure 81. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Dodecanoic acid LPP **212** in CD_3OD.

8.1.33 Compound 213

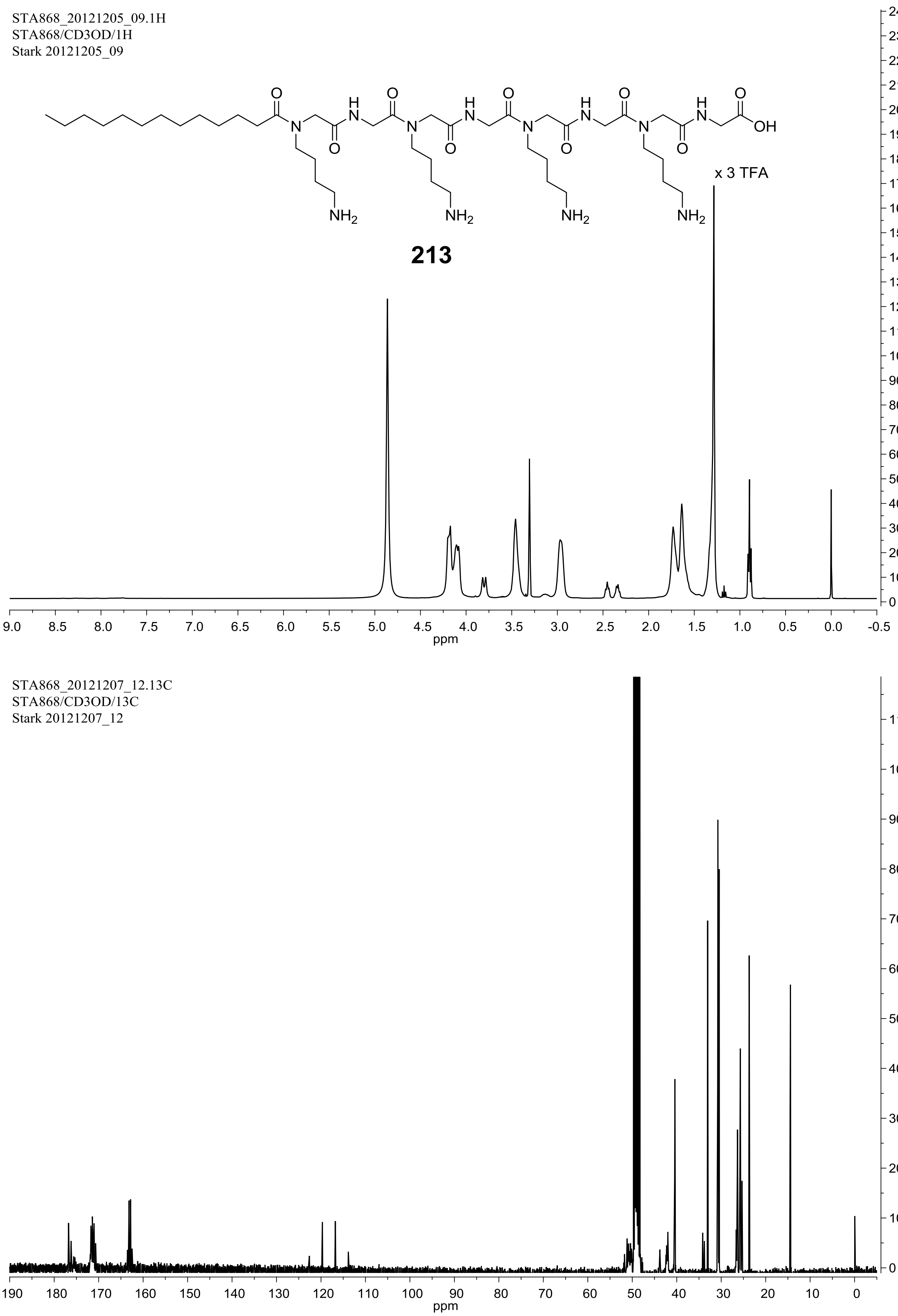


Figure 82. 400 MHz-[1]H- and 100 MHz-[13]C-NMR spectra of Tridecanoic acid LPP **213** in CD_3OD.

8.1.34 Compound 214

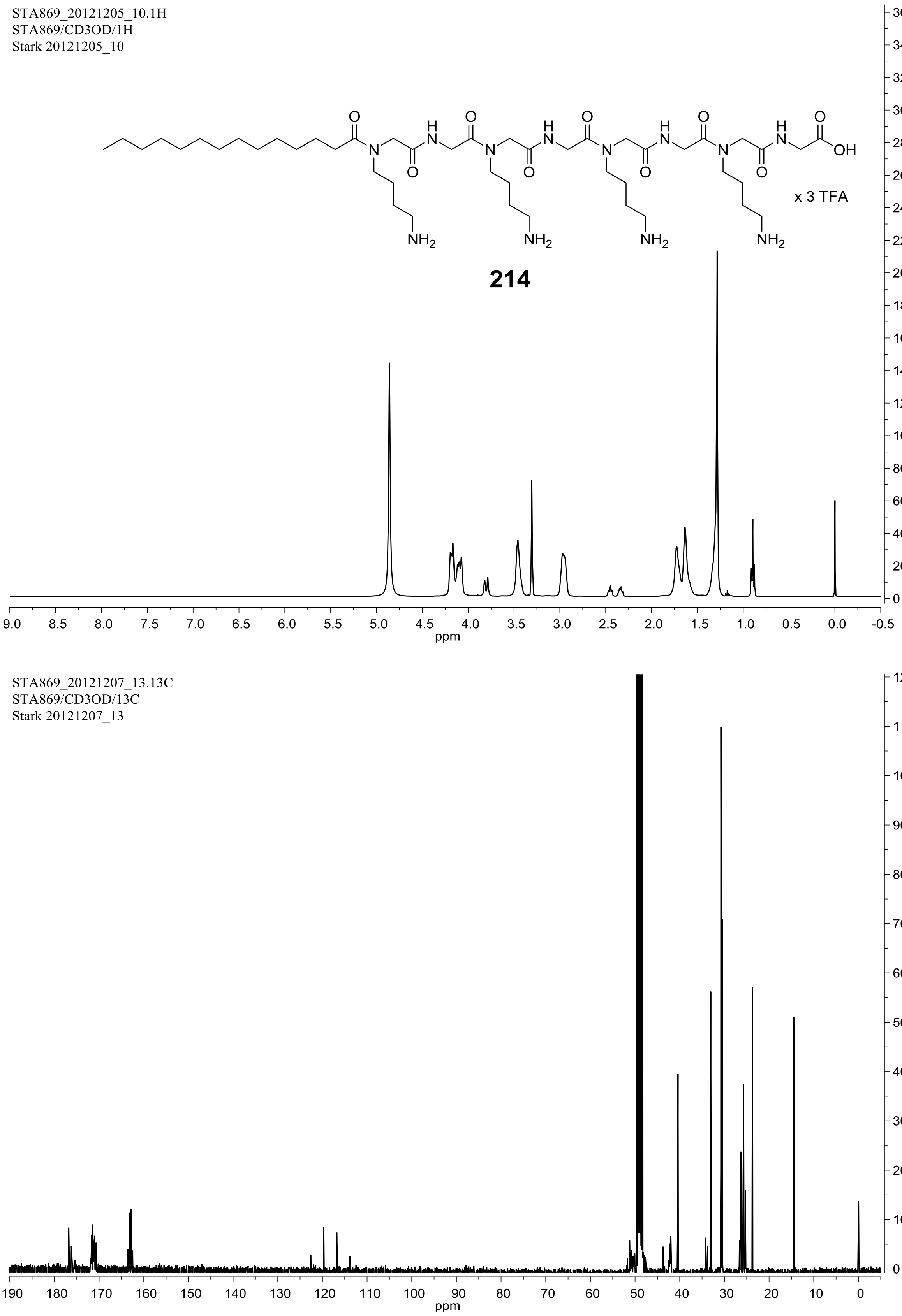


Figure 83. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Myristic acid LPP **214** in CD_3OD.

8.1.35 Compound 215

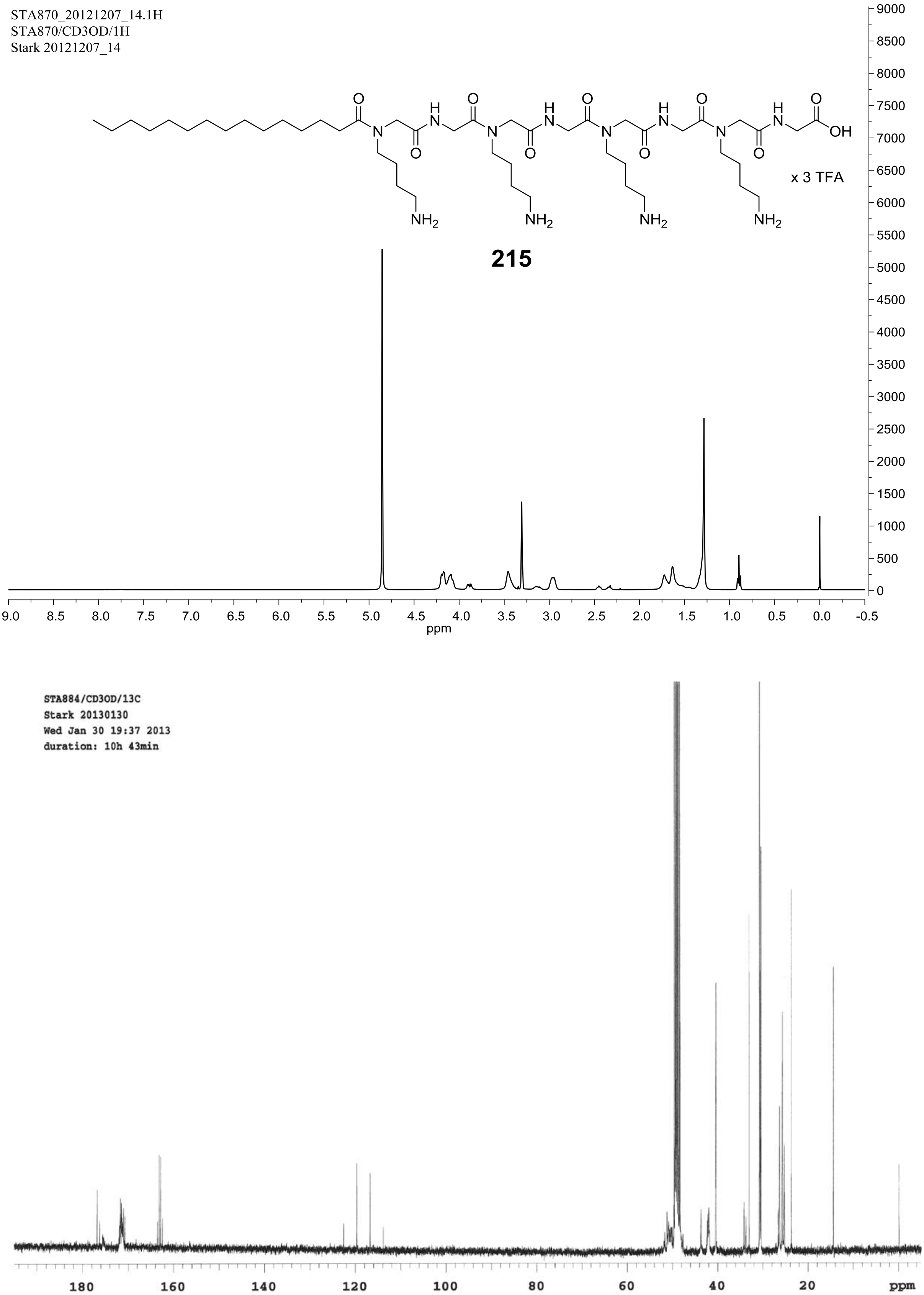
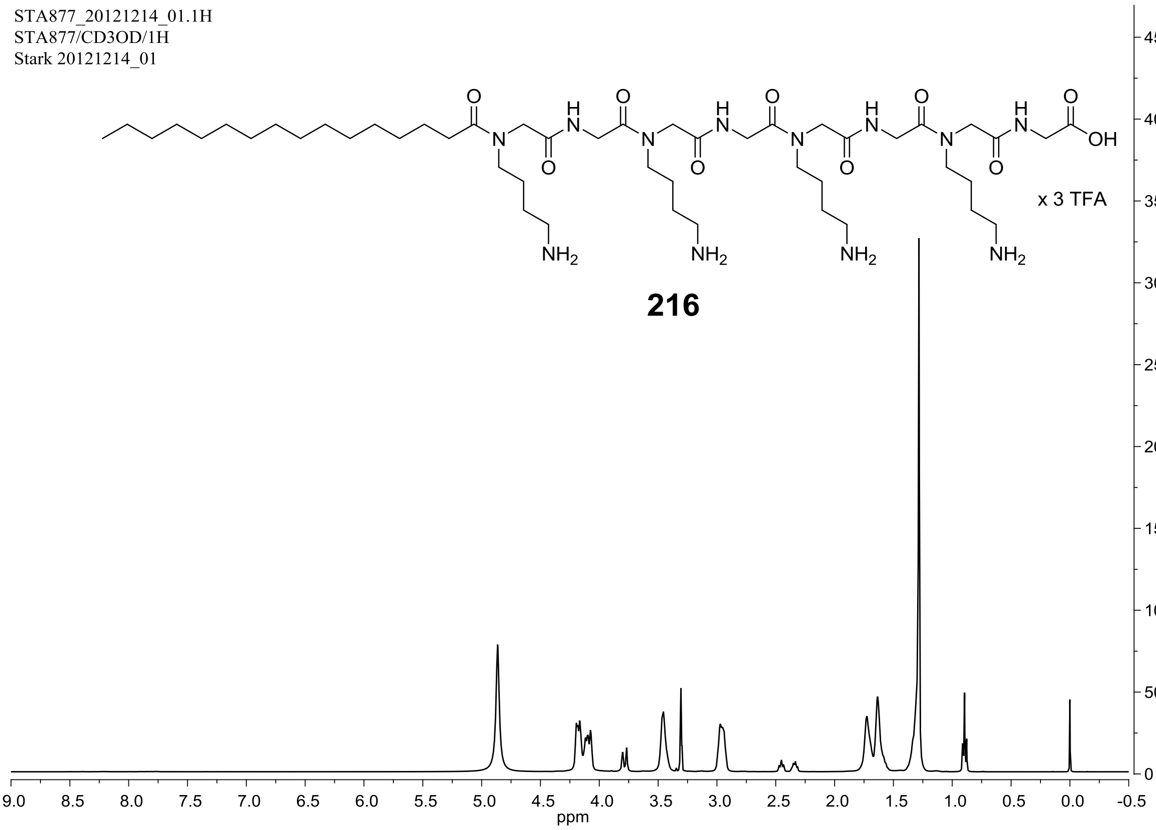


Figure 84. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Pentadecanoic acid LPP **215** in CD_3OD.

8.1.36 Compound 216



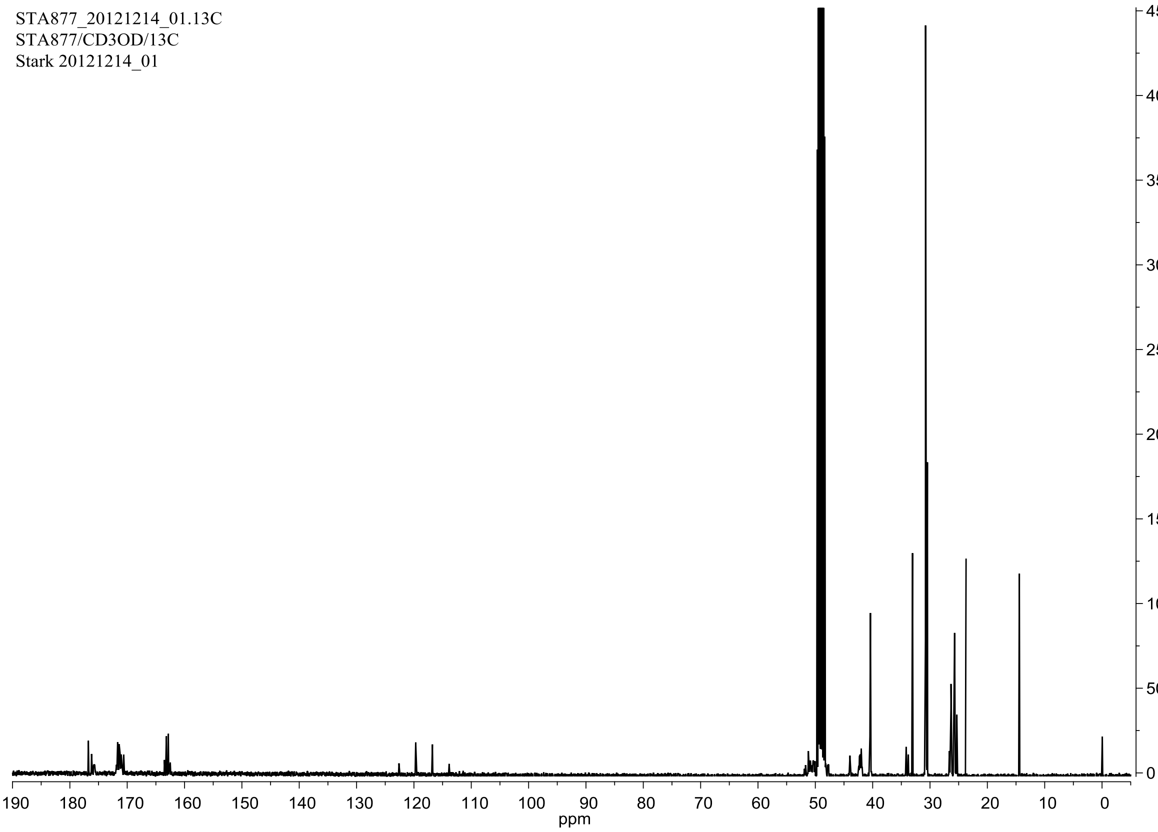
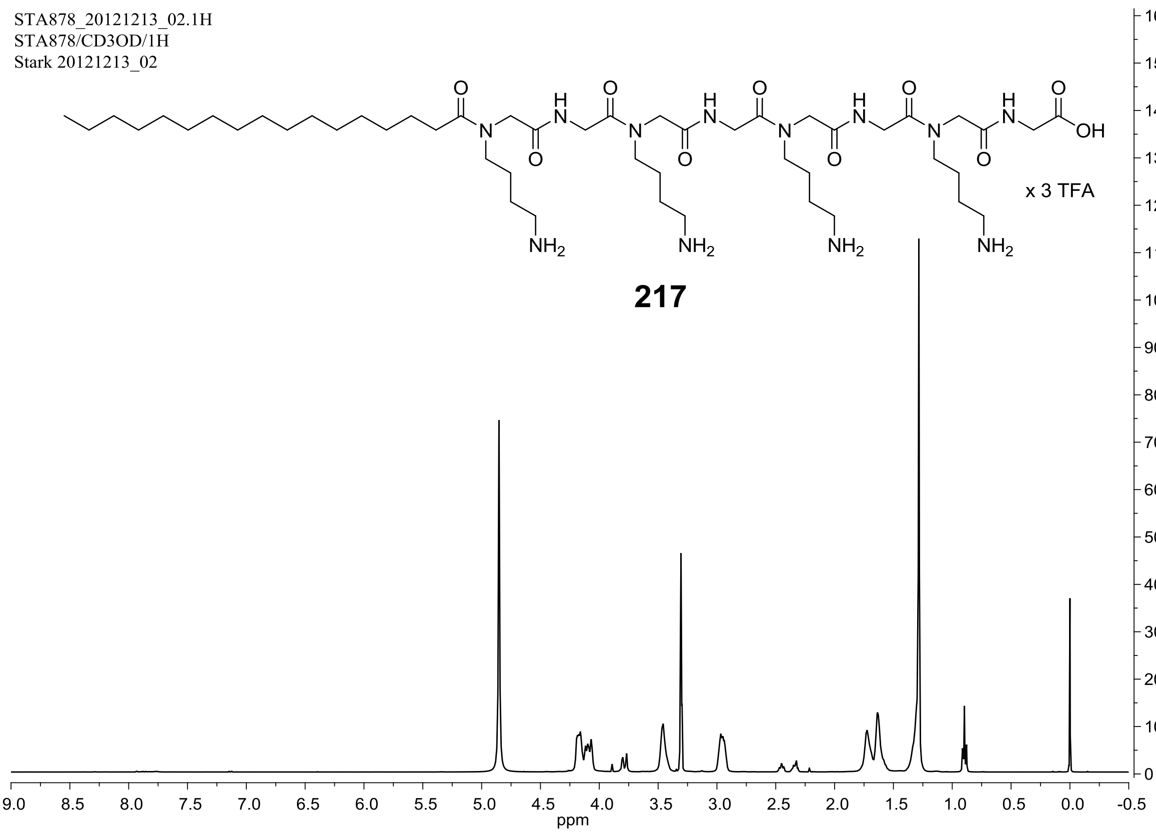


Figure 85. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Palmitic acid LPP **216** in CD_3OD.

8.1.37 Compound 217



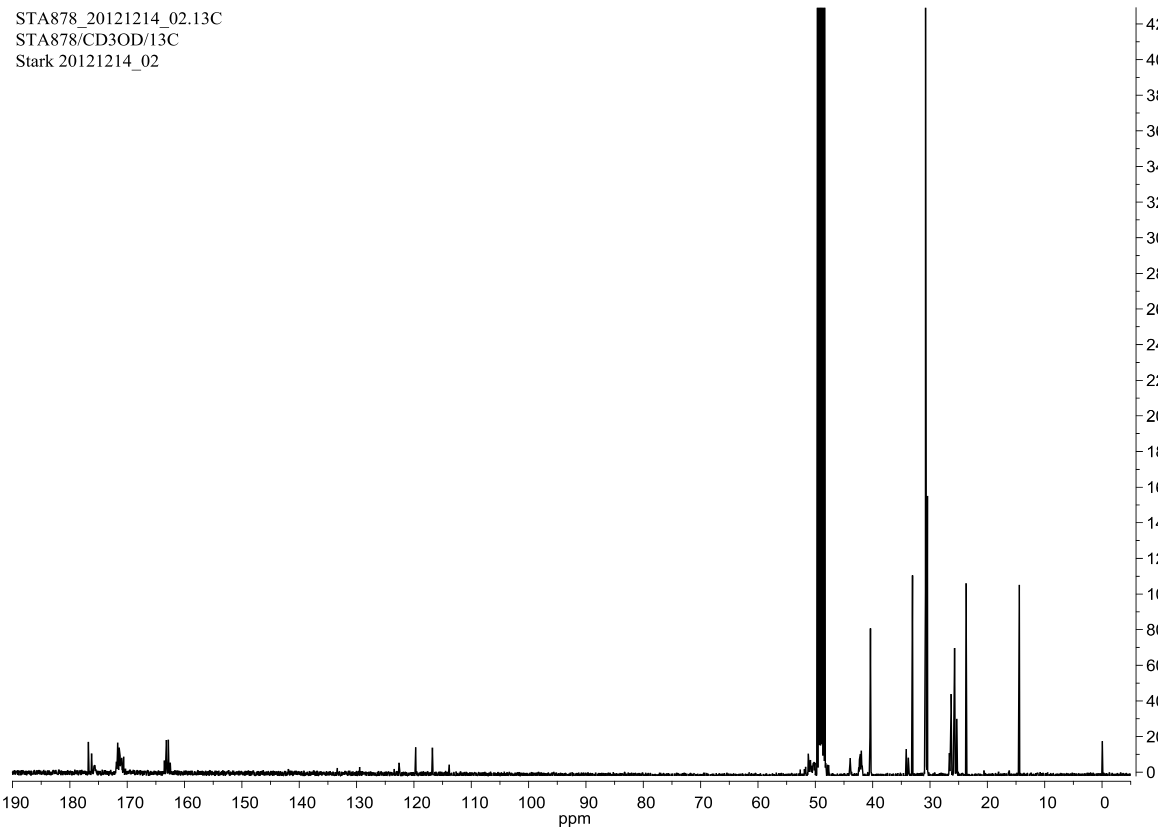
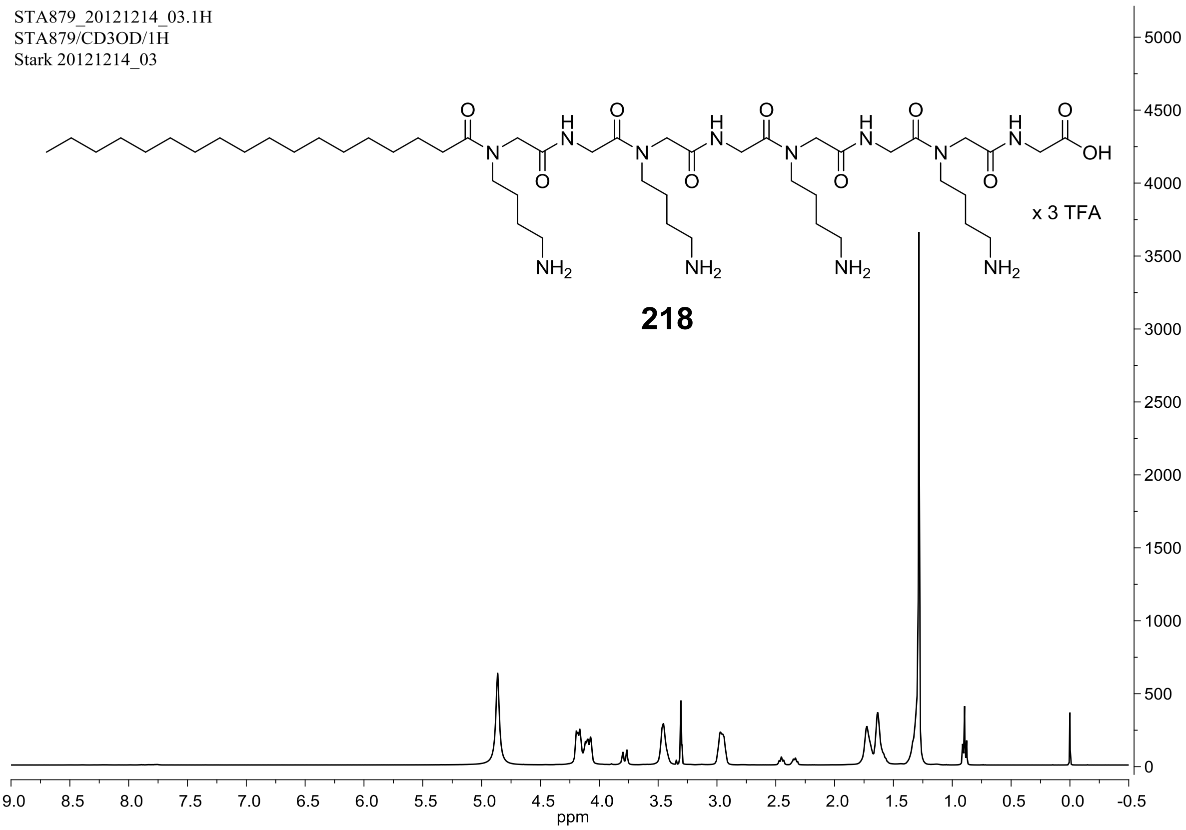


Figure 86. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Heptadecanoic acid LPP **217** in CD_3OD.

8.1.38 Compound 218



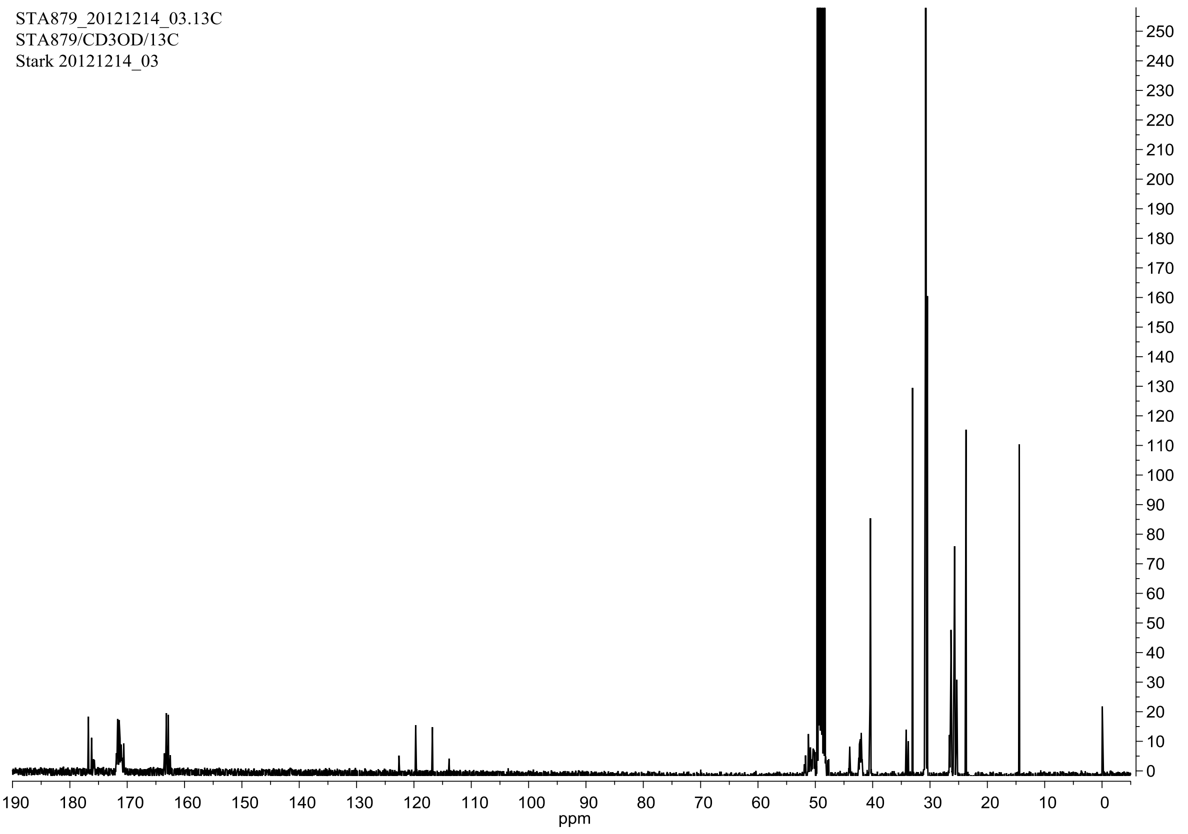
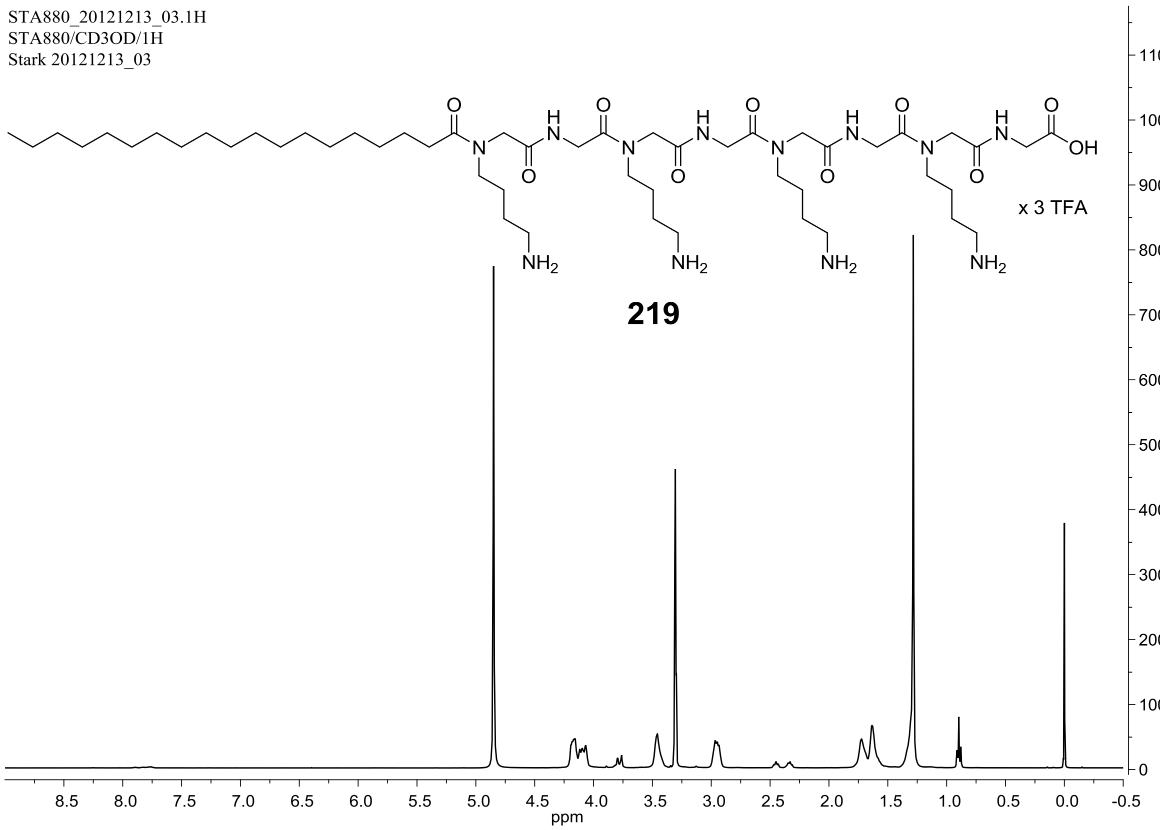


Figure 87. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Stearic acid LPP **218** in CD_3OD.

8.1.39 Compound 219



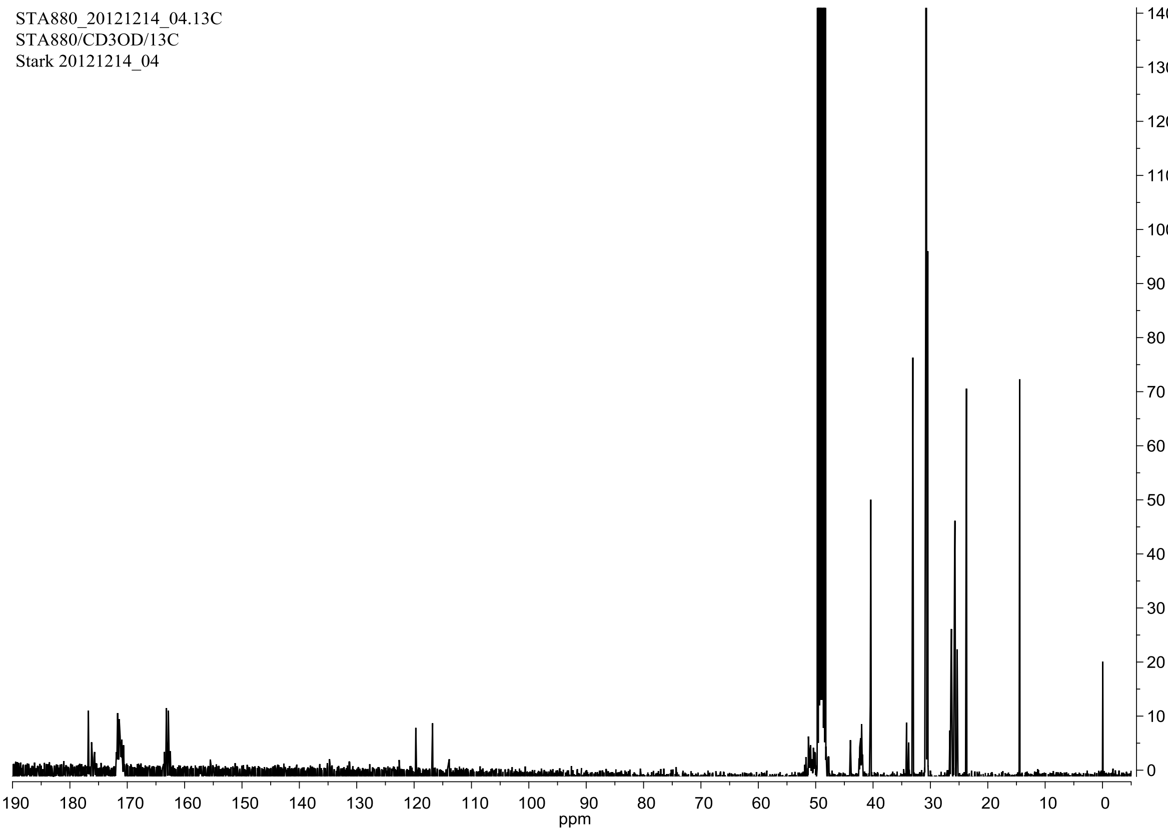
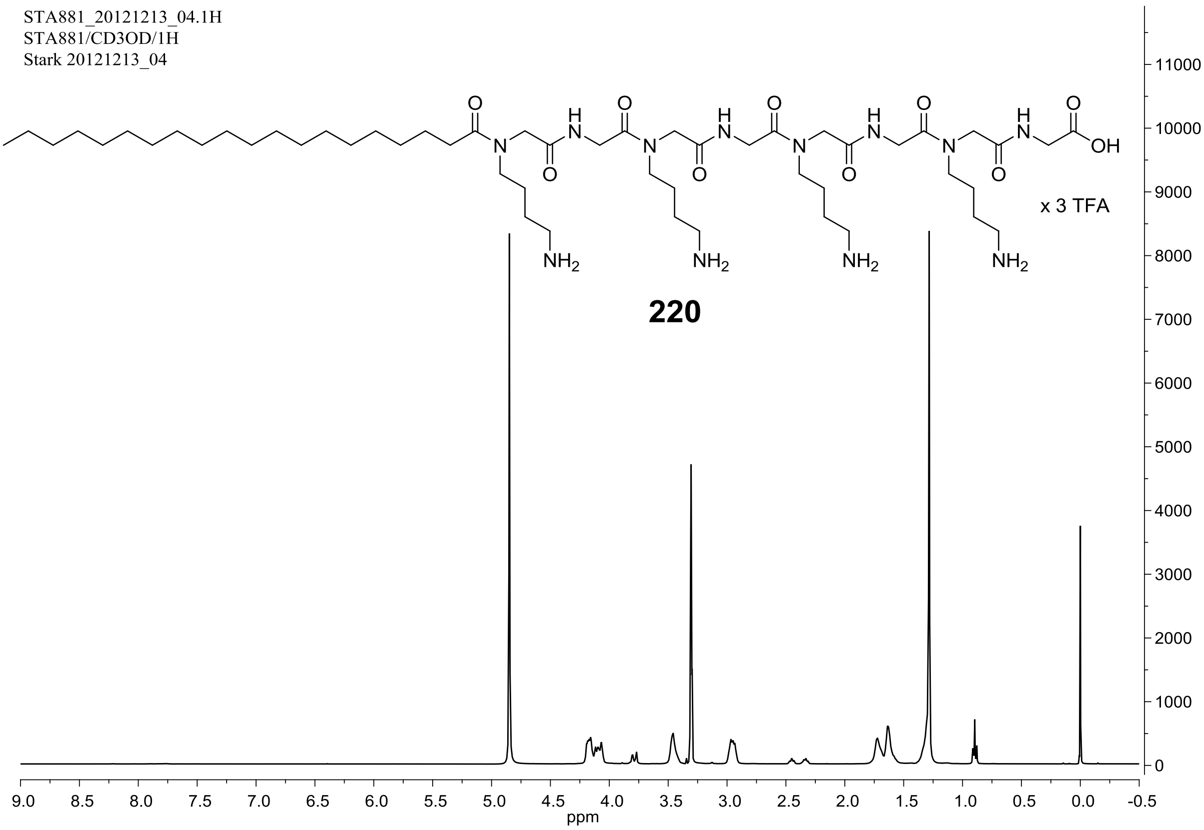


Figure 88. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Nonadecanoic acid LPP **219** in CD_3OD.

8.1.40 Compound 220



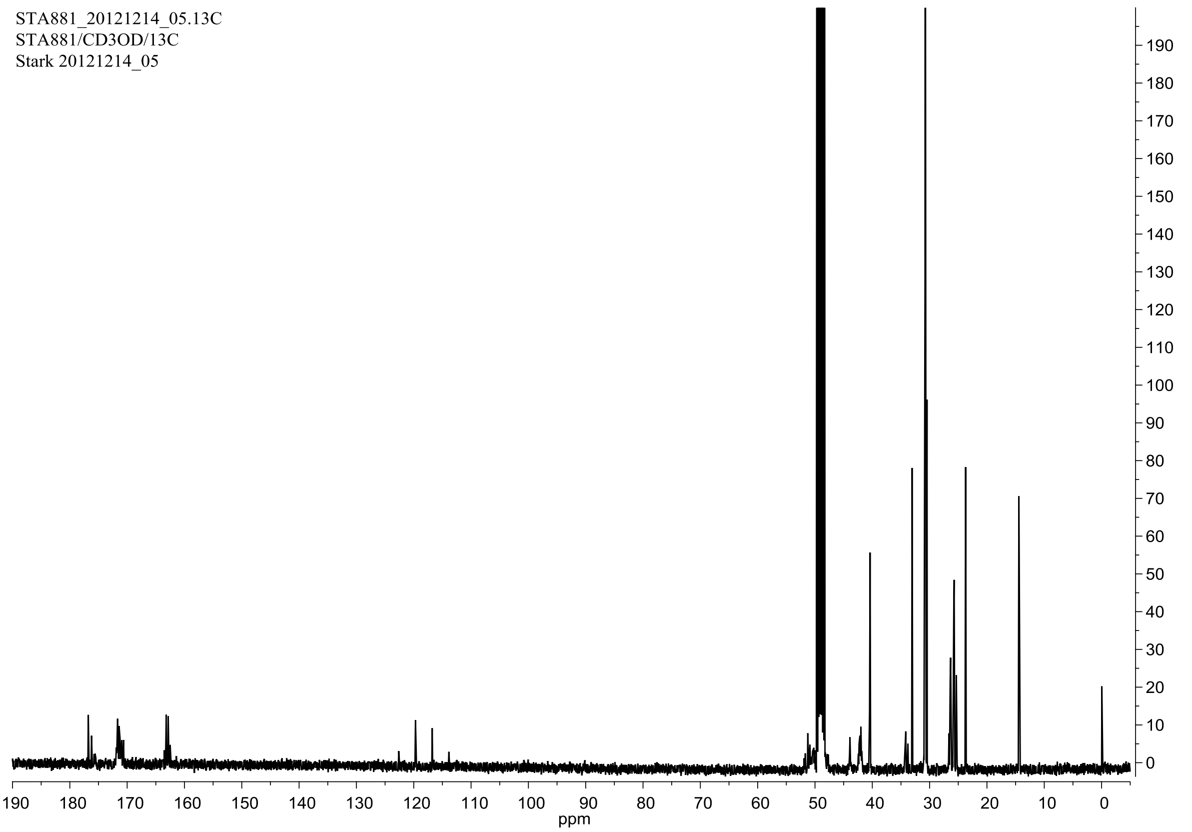
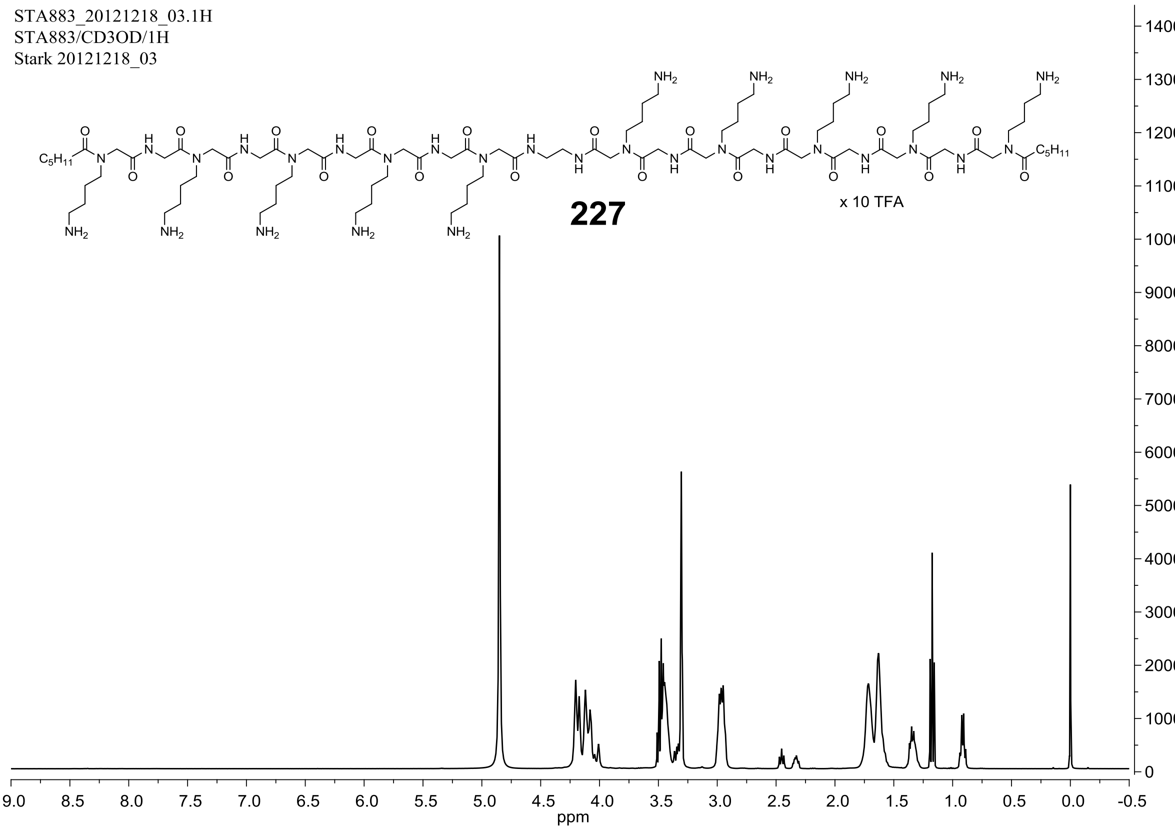


Figure 89. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of Arachidic acid LPP **220** in CD_3OD.

8.1.41 Compound 227



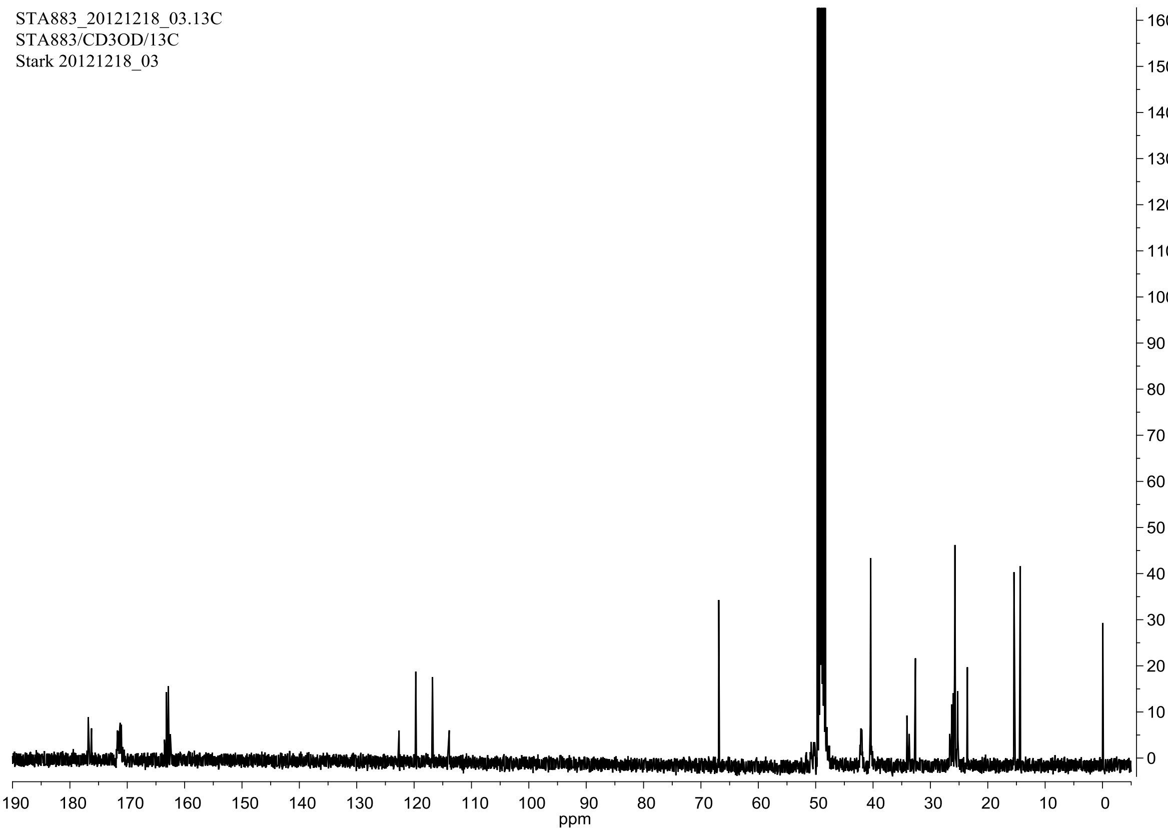


Figure 90. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of dimeric LPP **227** in CD_3OD.

8.1.42 Compound 231

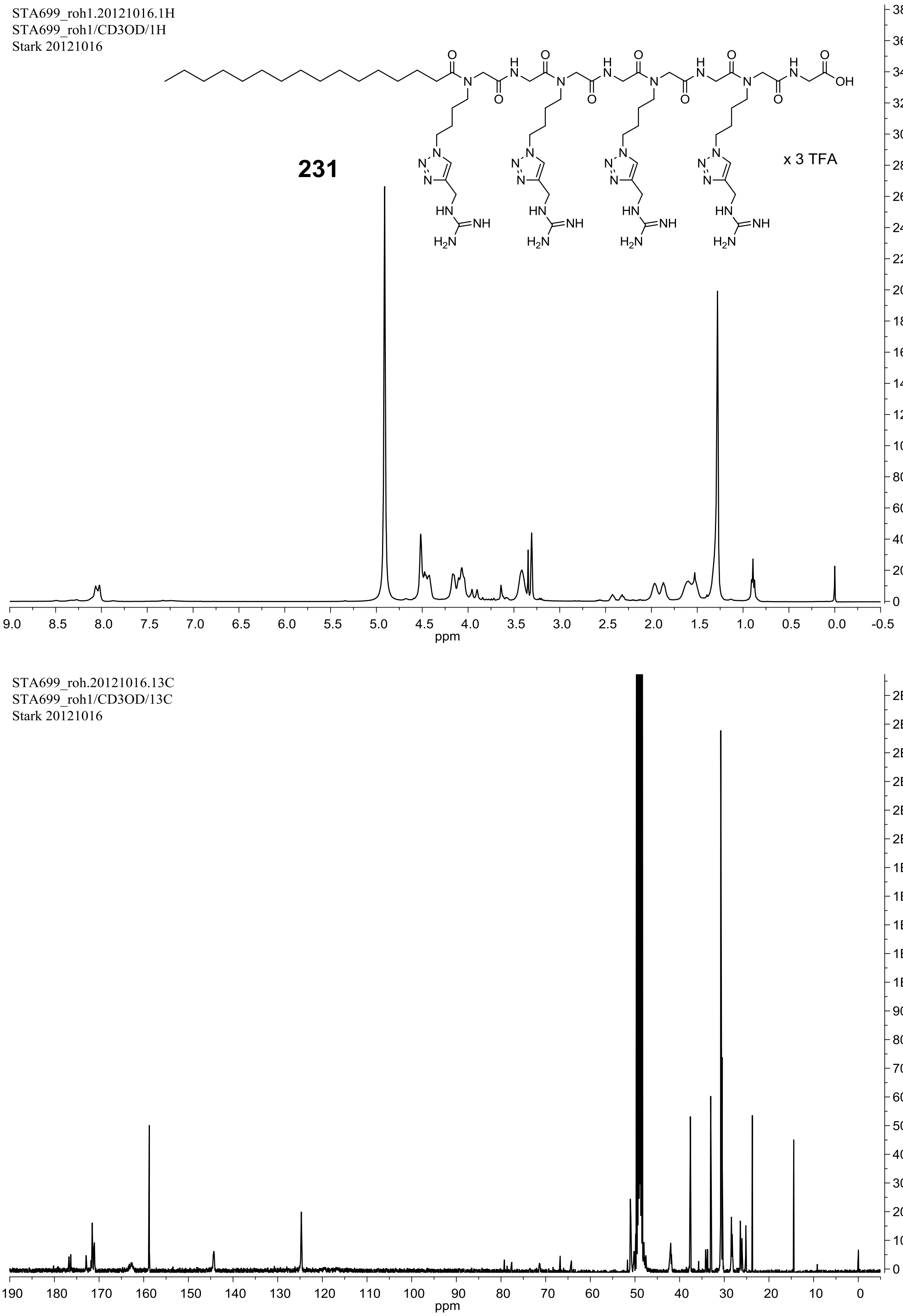
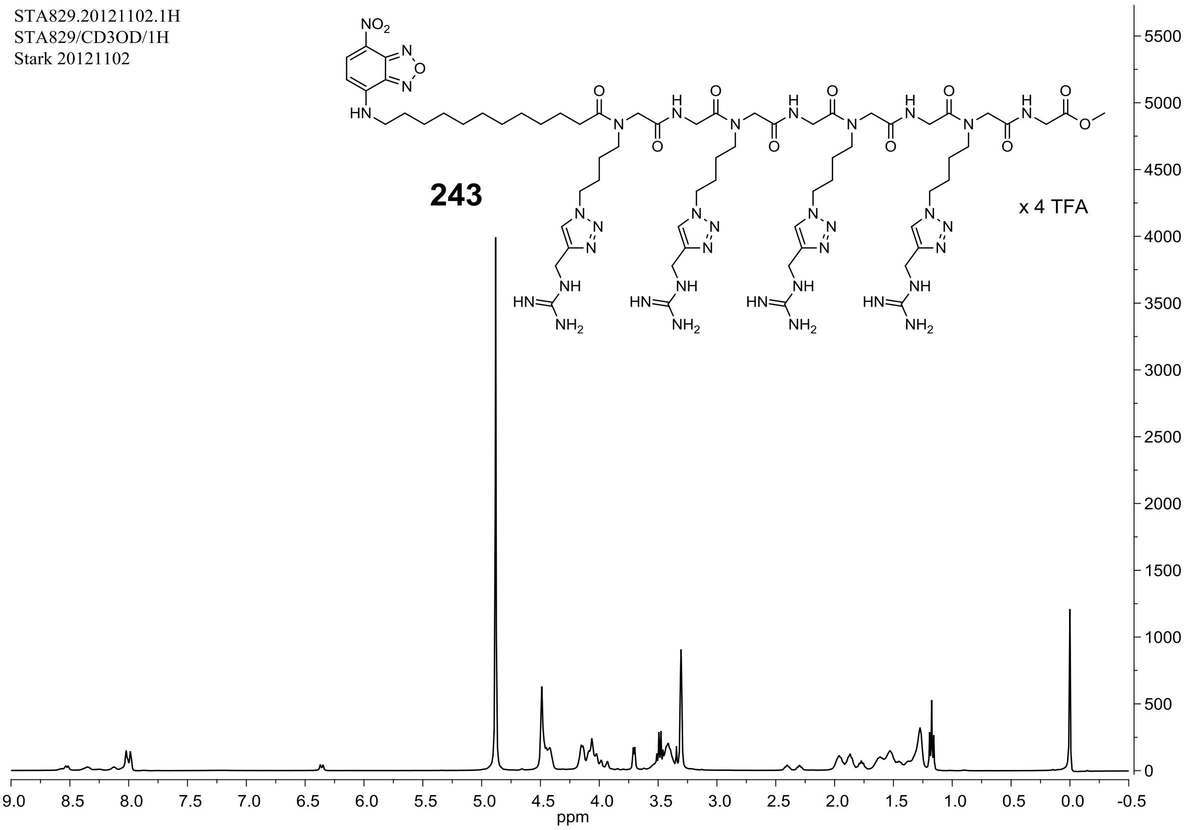


Figure 91. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of guanidino LPP **231** in CD_3OD.

8.1.43 Compound 243



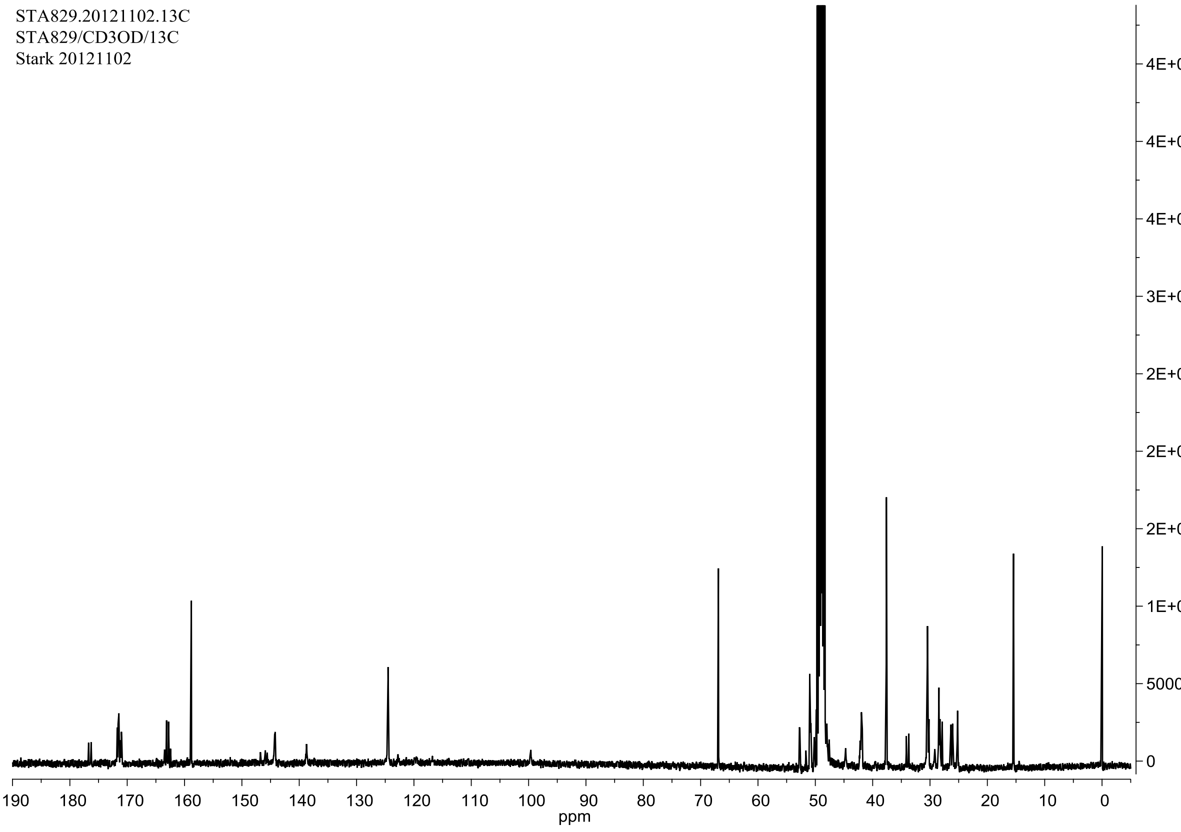


Figure 92. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of NBD guanidino LPP **243** in CD_3OD.

8.1.44 Compound 244

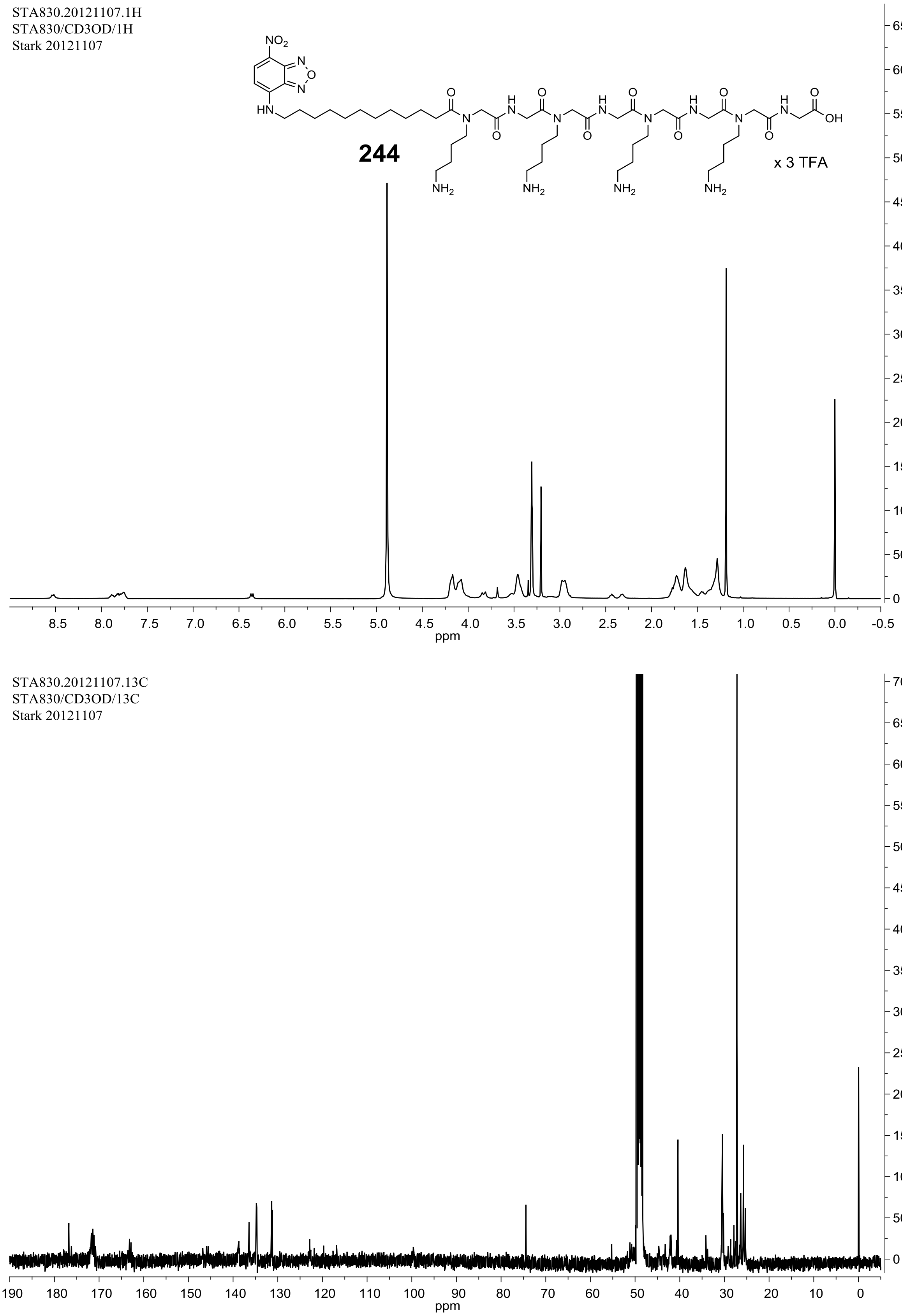


Figure 93. 400 MHz-^{1}H- and 100 MHz-^{13}C-NMR spectra of NBD amino LPP **244** in CD_3OD.

8.2 IC50 values of compounds in the luminescence assay

8.2.1 Reference Compounds 9, 246–256

Table 20. IC50 values/ranges of the reference compounds **9**, **246–256** in the bacterial luminescence assay.

Compound	Code	IC50		remarks
		8 h	24 h	
Surfactin	**9**	100 – 1000 µM	1 – 10 µM	only ranges could be estimated
$BaCl_2$	**246**	/	/	no inhibition observed
Benzethonium chloride	**247**	10–100 µM	10–100 µM	only ranges could be estimated
$CuSO_4$	**248**	/	/	no inhibition observed
Digitonin	**249**	25 ± 1 µM	20 ± 2 µM	/
NaN_3	**250**	183 ± 12 µM	100–1000 µM	24 h value not reliable
Sodium cholate	**251**	> 1000 µM	> 1000 µM	slight inhibition at 1000 µM
SDS	**252**	25 ± 1 µM	46 ± 1 µM	/
Chloramphenicol	**253**	< 1 µM	< 1 µM	value could only be estimated
D-Glucose	**254**	~1000 µM	100–1000 µM	only ranges could be estimated
Saccharose	**255**	/	/	no inhibition observed
Sodium trifluoroacetate	**256**	/	/	no inhibition observed

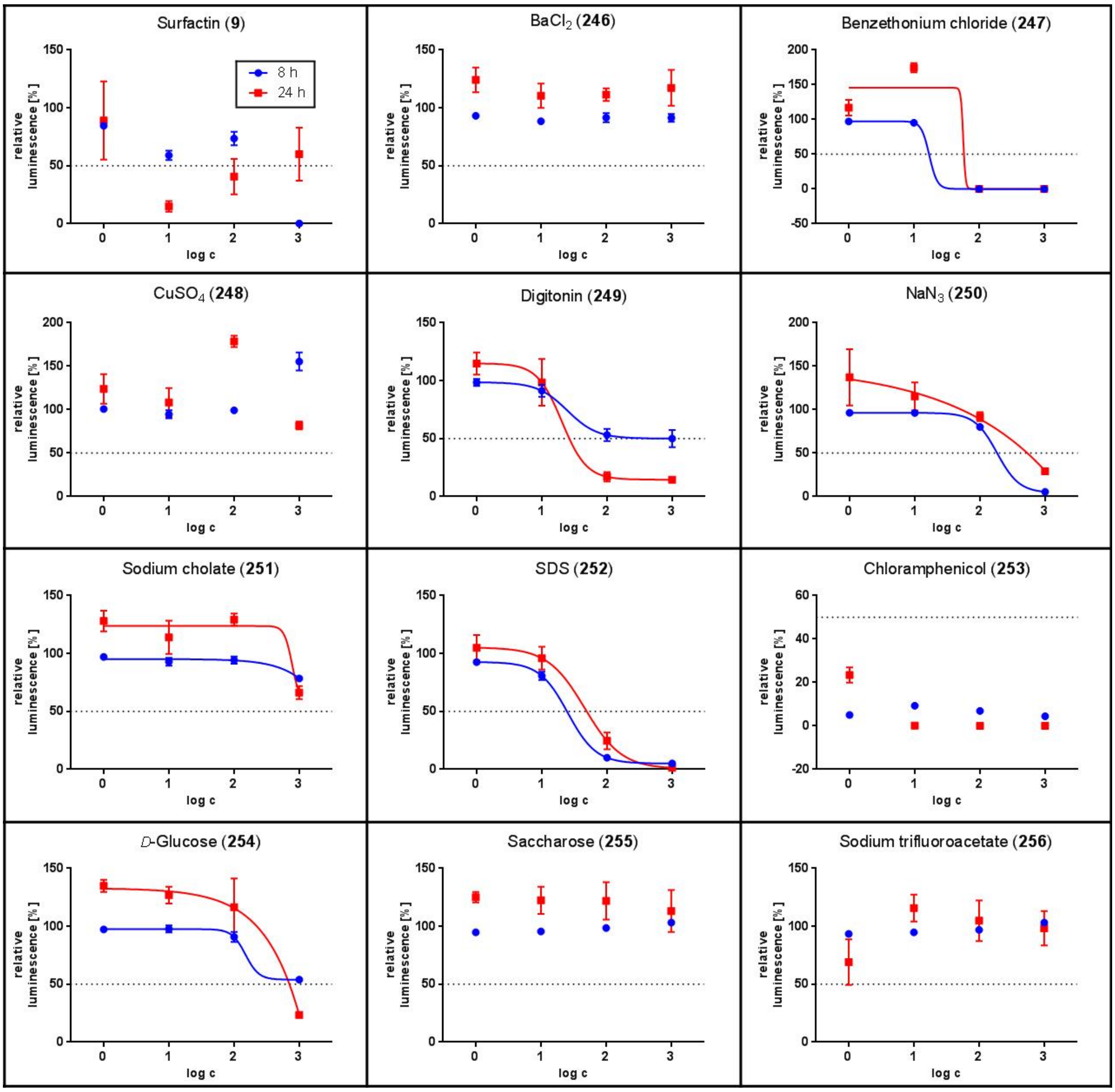


Figure 94. IC50 plots of the reference compounds **9**, **246–256** in the bacterial luminescence assay.

8.2.2 Azido LPPs 181–200

Table 21. IC50 values/ranges of the azido LPPs **181**–**200** in the bacterial luminescence assay.

Compound	Code	IC50		remarks
		8 h	24 h	
Formic acid azido LPP	**181**	10 – 100 µM	10 – 100 µM	only ranges could be estimated
Acetic acid azido LPP	**182**	~1000 µM	> 1000 µM	only ranges could be estimated
Propionic acid azido LPP	**183**	~1000 µM	~1000 µM	only ranges could be estimated
Butyric acid azido LPP	**184**	~1000 µM	~1000 µM	only ranges could be estimated
Valeric acid azido LPP	**185**	100 – 1000 µM	~1000 µM	only ranges could be estimated
Hexanoic acid azido LPP	**186**	~1000 µM	~1000 µM	only ranges could be estimated
Heptanoic acid azido LPP	**187**	~1000 µM	~100 µM	only ranges could be estimated
Octanoic acid azido LPP	**188**	~100 µM	40 ± 1 µM	/
Nonanoic acid azido LPP	**189**	29 ± 1 µM	13 ± 3 µM	/
Decanoic acid azido LPP	**190**	38 ± 1 µM	13 ± 3 µM	/
Undecanoic acid azido LPP	**191**	~100 µM	1 – 10 µM	only ranges could be estimated
Dodecanoic acid azido LPP	**192**	~10 µM	1 – 10 µM	only ranges could be estimated
Tridecanoic acid azido LPP	**193**	~10 µM	1 – 10 µM	only ranges could be estimated
Myristic acid azido LPP	**194**	> 1000 µM	1 – 10 µM	only ranges could be estimated
Pentadecanoic acid azido LPP	**195**	>10 µM	1 – 10 µM	only ranges could be estimated
Palmitic acid azido LPP	**196**	> 1000 µM	1 – 10 µM	only ranges could be estimated
Heptadecanoic acid azido LPP	**197**	>10 µM	1 – 10 µM	only ranges could be estimated
Stearic acid azido LPP	**198**	> 1000 µM	1 – 10 µM	only ranges could be estimated
Nonadecanoic acid azido LPP	**199**	> 1000 µM	1 – 10 µM	only ranges could be estimated
Arachidic acid azido LPP	**200**	> 1000 µM	~10 µM	only ranges could be estimated

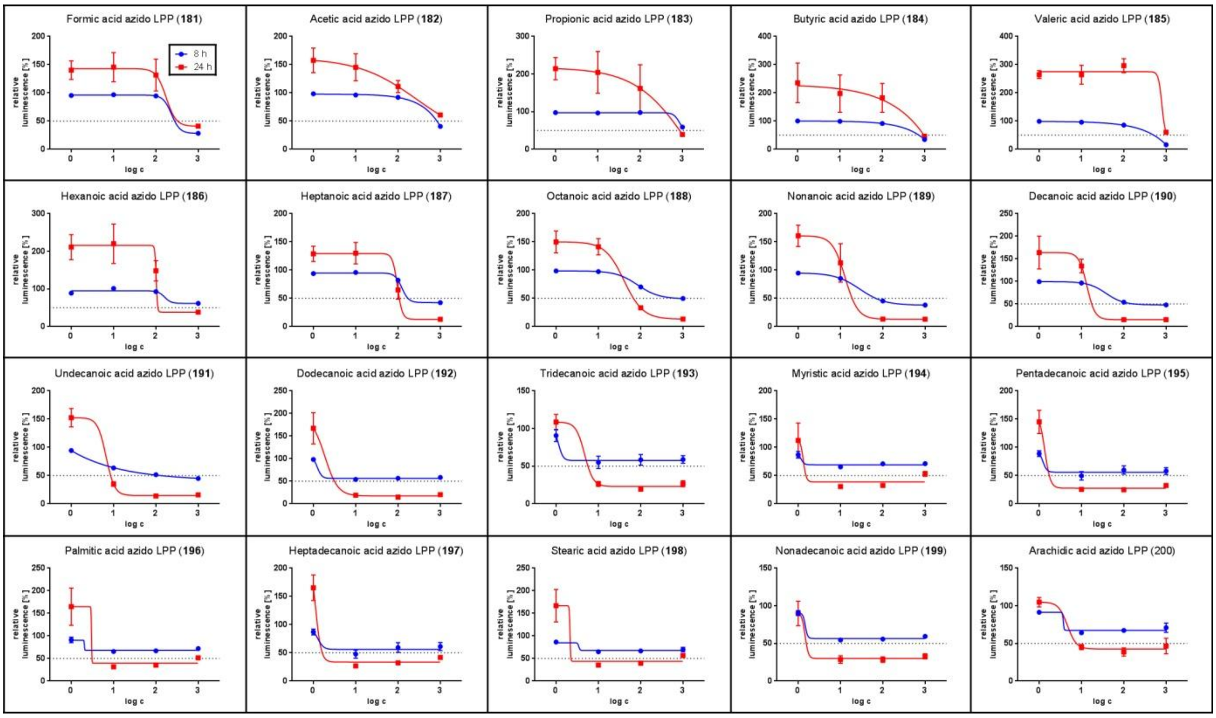


Figure 95. IC50 plots of the azido LPPs **181**–**200** in the bacterial luminescence assay.

8.2.3 Amino LPPs 201–220

Table 22. IC50 values/ranges of the amino LPPs **201**–**220** in the bacterial luminescence assay.

Compound	Code	IC50		remarks
		8 h	24 h	
Formic acid LPP	**201**	100–1000 µM	~1000 µM	only ranges could be estimated
Acetic acid LPP	**202**	/	/	no inhibition observed
Propionic acid LPP	**203**	/	/	no inhibition observed
Butyric acid LPP	**204**	/	/	no inhibition observed
Valeric acid LPP	**205**	/	/	no inhibition observed
Hexanoic acid LPP	**206**	/	/	no inhibition observed
Heptanoic acid LPP	**207**	/	/	no inhibition observed
Octanoic acid LPP	**208**	/	/	no inhibition observed
Nonanoic acid LPP	**209**	> 1000 µM	~1000 µM	slight inhibition at 1000 µM
Decanoic acid LPP	**210**	~1000 µM	~1000 µM	slight inhibition at 1000 µM
Undecanoic acid LPP	**211**	100–1000 µM	100–1000 µM	only ranges could be estimated
Dodecanoic acid LPP	**212**	362 ± 2 µM	82 ± 1 µM	/
Tridecanoic acid LPP	**213**	15 ± 15 µM	19 ± 1 µM	/
Myristic acid LPP	**214**	20 ± 1 µM	10 – 100 µM	only a range could be estimated (24 h)
Pentadecanoic acid LPP	**215**	14 ± 6 µM	16 ± 2 µM	/
Palmitic acid LPP	**216**	8 ± 7 µM	10 – 100 µM	only a range could be estimated (24 h)
Heptadecanoic acid LPP	**217**	1 – 10 µM	1 – 10 µM	only ranges could be estimated
Stearic acid LPP	**218**	1 – 10 µM	1 – 10 µM	only ranges could be estimated
Nonadecanoic acid LPP	**219**	1 – 10 µM	1 – 10 µM	only ranges could be estimated
Arachidic acid LPP	**220**	1 – 10 µM	1 – 10 µM	only ranges could be estimated

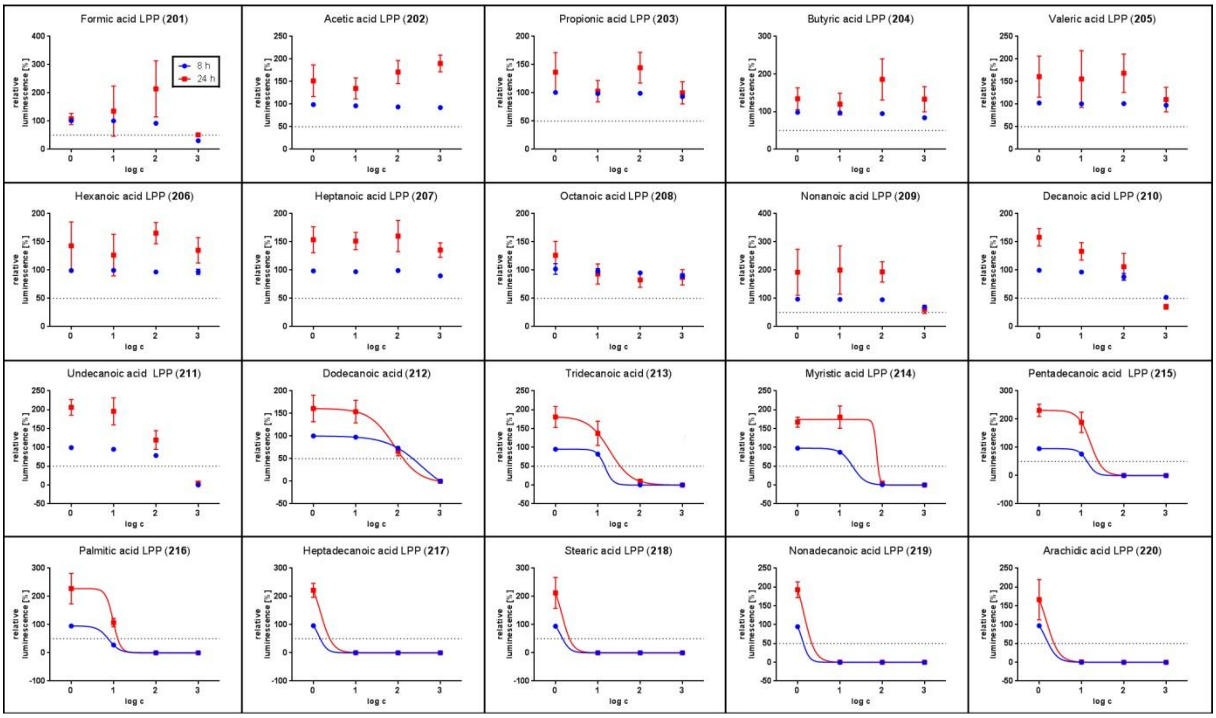


Figure 96. IC50 plots of the azido LPPs **201**–**220** in the bacterial luminescence assay.

8.2.4 Compounds 227, 231, 243 and 244

Table 23. IC50 values/ranges of the LPPs **227**, **231**, **243** and **244** in the bacterial luminescence assay.

Compound	Code	IC50		remarks
		8 h	24 h	
Dimeric valeric acid LPP	**227**	> 1000 µM	/	only slight inhibition (8 h)
Palmitic acid guanidino LPP	**231**	10–100 µM	10–100 µM	only ranges could be estimated
NBD guanidino LPP	**243**	10–100 µM	10–100 µM	only ranges could be estimated
NBD amino LPP	**244**	10–100 µM	10–100 µM	only ranges could be estimated

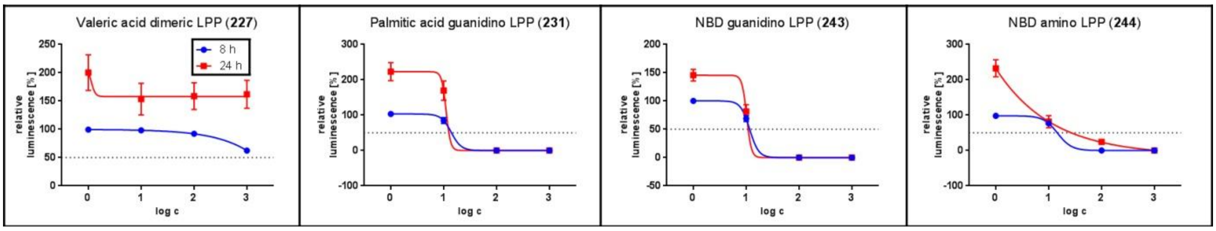


Figure 97. IC50 plots of the LPPs **227**, **231**, **243** and **244** in the bacterial luminescence assay.

8.3 Compound code assignment

Table 24. List of compounds that were either synthesized a/o tested in the bacterial luminescence assay in this work. The respective compound code, the experiment code, its notebook number and page, the MOL-ID and the experiment number of the luminescence assay are listed. Compounds that were not synthesized a/o not tested in the luminescence assay are not listed.

Code	Experiment/s (synthesis)	MOL-ID	Notebook # and page	Biotest (*A. fischeri*)
9	/	/	/	M0035/STA740
16	STA448	10,000	3-133	/
41	STA438	11,693	3-130	/
42	STA439	11,694	3-130	/
43	STA440	11,695	3-130	/
44	STA441	11,696	3-130	/
45	STA442	11,697	3-130	/
46	STA443	11,698	3-130	/
47	STA444	11,699	3-130	/
48	STA445	11,700	3-130	/
49	STA446	11,701	3-130	/
50	STA447	11,702	3-130	/
51	STA449	11,703	3-136	/
52	STA450	11,704	3-136	/
53	STA451	11,705	3-136	/
54	STA452	11,706	3-136	/
55	STA453	11,707	3-136	/
56	STA454	10,002	3-136	/
57	STA455	11,708	3-136	/

Code	Experiment/s (synthesis)	MOL-ID	Notebook # and page	Biotest (*A. fischeri*)
58	STA456	11,709	3-136	/
59	STA457	11,710	3-136	/
60	STA458	11,711	3-136	/
61	STA468	11,717	3-150	/
62	STA469	11,718	3-150	/
63	STA470	11,719	3-150	/
64	STA471	11,720	3-150	/
65	STA472	11,721	3-150	/
66	STA473	11,722	3-150	/
67	STA474	11,723	3-150	/
68	STA475	11,724	3-150	/
69	STA476	11,725	3-150	/
70	STA477	11,726	3-150	/
71	STA478	11,727	3-150	/
72	STA479	11,728	3-150	/
73	STA480	11,729	3-150	/
74	STA481	11,730	3-150	/
75	STA482	11,731	3-150	/
76	STA483	10,011	3-150	/
77	STA484	11,732	3-150	/
78	STA485	11,733	3-150	/
79	STA486	11,734	3-150	/
80	STA487	11,735	3-150	/
81	STA488	11,736	3-156	/
82	STA489	11,737	3-156	/
83	STA490	11,738	3-156	/
84	STA491	11,739	3-156	/
85	STA492	11,740	3-156	/
86	STA493	11,741	3-156	/
87	STA494	11,742	3-156	/
88	STA495	11,743	3-156	/
89	STA496	11,744	3-156	/
90	STA497	11,745	3-156	/
91	STA498	11,746	3-159	/
92	STA499	11,747	3-159	/
93	STA500	11,748	3-159	/
94	STA501	11,749	3-159	/
95	STA502	11,750	3-159	/
96	STA503	10,017	3-159	/
97	STA504	11,751	3-159	/
98	STA505	11,752	3-159	/
99	STA506	11,753	3-159	/
100	STA507	11,754	3-159	/
101	STA509	11,756	3-164	/
102	STA510	11,757	3-164	/
103	STA511	11,758	3-164	/
104	STA512	11,759	3-164	/
105	STA513	11,760	3-164	/
106	STA514	11,761	3-164	/
107	STA515	11,762	3-164	/
108	STA516	11,763	3-164	/
109	STA517	11,764	3-164	/
110	STA518	11,765	3-164	/
111	STA519	11,766	3-164	/
112	STA520	11,767	3-164	/
113	STA521	11,768	3-164	/
114	STA522	11,769	3-164	/
115	STA523	11,770	3-164	/
116	STA524	10,039	3-164	/
117	STA525	11,771	3-164	/

Code	Experiment/s (synthesis)	MOL-ID	Notebook # and page	Biotest (*A. fischeri*)
118	STA526	11,772	3-164	/
119	STA527	11,773	3-164	/
120	STA528	11,774	3-164	/
121	STA529	11,775	3-169	/
122	STA530	11,776	3-169	/
123	STA531	11,777	3-169	/
124	STA532	11,778	3-169	/
125	STA533	11,779	3-169	/
126	STA534	11,780	3-169	/
127	STA535	11,781	3-169	/
128	STA536	11,782	3-169	/
129	STA537	11,783	3-169	/
130	STA538	11,784	3-169	/
131	STA539	11,785	3-174	/
132	STA540	11,786	3-174	/
133	STA541	11,787	3-174	/
134	STA542	11,788	3-174	/
135	STA543	11,789	3-174	/
136	STA544	11,790	3-174	/
137	STA545	11,791	3-174	/
138	STA546	11,792	3-174	/
139	STA547	11,793	3-174	/
140	STA548	11,794	3-174	/
141	STA589	10,187	5-029	/
142	STA590	10,188	5-029	/
143	STA591	10,189	5-029	/
144	STA592	10,190	5-029	/
145	STA593	10,191	5-029	/
146	STA594	10,192	5-029	/
147	STA595	10,193	5-029	/
148	STA596	10,194	5-029	/
149	STA597	10,195	5-029	/
150	STA598	10,196	5-029	/
151	STA644	11,833	5-081	/
152	STA645	11,834	5-081	/
153	STA646	11,835	5-081	/
154	STA647	11,836	5-081	/
155	STA648	11,837	5-081	/
156	STA649	11,838	5-081	/
157	STA650	11,839	5-081	/
158	STA651	11,840	5-081	/
159	STA652	11,841	5-081	/
160	STA653	11,842	5-081	/
161	STA800	11,877	5-125	/
162	STA801	11,878	5-125	/
163	STA802	11,879	5-125	/
164	STA803	11,880	5-125	/
165	STA804	11,881	5-125	/
166	STA805	11,882	5-125	/
167	STA806	11,883	5-125	/
168	STA807	11,884	5-125	/
169	STA808	11,885	5-125	/
170	STA809	11,886	5-125	/
171	STA815	11,889	5-137	/
172	STA816	11,890	5-137	/
173	STA817	11,891	5-137	/
174	STA818	11,892	5-137	/
175	STA819	11,893	5-137	/
176	STA820	11,894	5-137	/
177	STA821	11,895	5-137	/

Code	Experiment/s (synthesis)	MOL-ID	Notebook # and page	Biotest (*A. fischeri*)
178	STA822	11,896	5-137	/
179	STA823	11,897	5-137	/
180	STA824	11,898	5-137	/
181	STA831	11,905	5-157	M0032/STA739
182	STA832	11,906	5-157	M0032/STA739
183	STA833	11,907	5-157	M0032/STA739
184	STA834	11,908	5-157	M0032/STA739
185	STA835	11,909	5-157	M0032/STA739
186	STA836/STA871	11,910	5-157/5-174	M0032/STA739
187	STA837/STA872	11,911	5-157/5-174	M0033/STA739
188	STA838/STA873	11,912	5-157/5-174	M0033/STA739
189	STA839/STA874	11,913	5-157/5-174	M0033/STA739
190	STA840/STA875	11,914	5-157/5-174	M0033/STA739
191	STA841	11,915	5-160	M0033/STA739
192	STA842	11,916	5-160	M0033/STA739
193	STA843	11,917	5-160	M0034/STA740
194	STA844	11,918	5-160	M0034/STA740
195	STA845	11,919	5-160	M0034/STA740
196	STA846	11,920	5-160	M0034/STA740
197	STA847	11,921	5-160	M0034/STA740
198	STA848	11,922	5-160	M0034/STA740
199	STA849	11,923	5-160	M0038/STA742
200	STA850	11,924	5-160	M0038/STA742
201	STA856	11,930	5-165	M0022/STA734
202	STA857	11,931	5-165	M0022/STA734
203	STA858	11,932	5-165	M0022/STA734
204	STA859	11,933	5-165	M0022/STA734
205	STA860	11,934	5-165	M0022/STA734
206	STA861	11,935	5-172	M0022/STA734
207	STA862	11,936	5-172	M0024/STA735
208	STA863	11,937	5-172	M0024/STA735
209	STA864	11,938	5-172	M0024/STA735
210	STA865	11,939	5-172	M0024/STA735
211	STA866	11,940	5-172	M0024/STA735
212	STA867	11,941	5-172	M0024/STA735
213	STA868	11,942	5-172	M0026/STA736
214	STA869	11,943	5-172	M0026/STA736
215	STA870/STA884	11,944	5-172/5-181	M0026/STA736
216	STA877	11,946	5-177	M0026/STA736
217	STA878	11,947	5-177	M0026/STA736
218	STA879	11,948	5-177	M0026/STA736
219	STA880	11,949	5-177	M0028/STA737
220	STA881	11,950	5-177	M0028/STA737
222	STA341	9,816	3-019	/
224	STA340	9,914	3-018	/
225	STA882	11,951	5-178	/
227	STA883	11,952	5-180	M0028/STA737
229	STA697	11,874	5-146	/
230	STA698	11,875	5-147	/
231	STA699	11,876	5-148	M0028/STA737
234	STA601	10,199	5-033	/
235	STA602	10,200	5-035	/
236	STA603	11,800	5-036	/
237	STA604	11,801	5-037	/
238	STA638	11,827	5-075	/
239	STA641	11,830	5-078	/
240	STA642	11,831	5-079	/
241	STA643	11,832	5-080	/
242	STA827	11,901	5-151	/
243	STA829	11,903	5-154	M0028/STA737

Code	**Experiment/s (synthesis)**	**MOL-ID**	**Notebook # and page**	**Biotest (*A. fischeri*)**
244	STA830	11,904	5-156	M0028/STA737
246	/	/	/	M0039/STA742
247	/	/	/	M0038/STA742
248	/	/	/	M0038/STA742
249	/	/	/	M0038/STA742
250	/	/	/	M0038/STA742
251	/	/	/	M0039/STA742
252	/	/	/	M0039/STA742
253	/	/	/	reference antibiotic
254	/	/	/	M0039/STA742
255	/	/	/	M0039/STA742
256	/	/	/	M0039/STA742

8.4 Raw data of the *A. fischeri* assay

Hereinafter, uncorrected, raw luminescence data in RLU of the *A. fischeri* assay are shown (for a detailed description see chapter 5.2.1).

8.4.1 General plate allocation

Table 25. General allocation of a standard luminescence assay plate. For every experiment, two identical plates were prepared, so that every compound concentration was applied and measured in six technical replicates. In a typical luminescence experiment, the biological activity of six different compounds (A – F) was determined.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
		empty		compound A		1 µM	compound C		1 µM	compound E		1 µM	**A**
		empty		compound A		10 µM	compound C		10 µM	compound E		10 µM	**B**
		control		compound A		100 µM	compound C		100 µM	compound E		100 µM	**C**
time		control		compound A		1000 µM	compound C		1000 µM	compound E		1000 µM	**D**
	chloramphenicol		1 µM	compound B		1 µM	compound D		1 µM	compound F		1 µM	**E**
	chloramphenicol		10 µM	compound B		10 µM	compound D		10 µM	compound F		10 µM	**F**
	chloramphenicol		100 µM	compound B		100 µM	compound D		100 µM	compound F		100 µM	**G**
	chloramphenicol		1000 µM	compound B		1000 µM	compound D		1000 µM	compound F		1000 µM	**H**

Table 26. Overview of the plate allocation with test compounds in the different experiments.

Assay number	Experiment number	code of tested compound					
		A	B	C	D	E	F
M0022	STA734	**201**	**202**	**203**	**204**	**205**	**206**
M0024	STA735	**207**	**208**	**209**	**210**	**211**	**212**
M0026	STA736	**213**	**214**	**215**	**216**	**217**	**218**
M0028	STA737	**219**	**220**	**231**	**243**	**244**	**227**
M0032	STA739	**181**	**182**	**183**	**184**	**185**	**186**
M0033	STA739	**187**	**188**	**189**	**190**	**191**	**192**
M0034	STA740	**193**	**194**	**195**	**196**	**197**	**198**
M0035	STA740	**9**			*not listed compounds*		
M0038	STA742	**199**	**200**	**249**	**247**	**248**	**250**
M0039	STA742	**252**	**256**	**246**	**255**	**251**	**254**

8.4.2 Assay data for M0022

Table 27. Raw assay data of M0022/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-5	-2	-5	124848	131264	130857	134825	133757	133980	134611	136580	139524	A
	-2	-1	3	121346	133792	132807	133058	137077	135480	129810	138489	138620	B
	114616	117113	120432	114762	137032	136410	137187	130815	138949	135423	137788	138613	C
	119405	119839	124007	124562	122324	127059	132820	128579	135163	133686	135674	136922	D
	121285	127107	129564	132454	131758	129471	131532	129006	137829	137862	144631	144493	E
	130156	134365	133318	128845	134749	129409	131482	134572	134610	137924	140612	139645	F
	126249	132906	133846	132065	130264	132233	135020	126383	138764	138650	142735	142244	G
	58705	59763	61347	126416	128857	127767	140795	139095	141475	142209	147242	147442	H
60	4	-17	-2	166819	172365	164880	162829	163608	162558	159128	157555	159765	A
	23	15	9	157026	168842	162525	159495	163345	157669	153771	159282	159988	B
	162609	159772	159784	141746	164209	159083	160972	155438	159728	157286	157629	157009	C
	160351	156212	156056	168041	166674	169569	160739	155839	159802	157078	160645	160492	D
	170595	173109	172383	162449	156887	155412	158397	152197	159112	151629	156637	155725	E
	261379	259933	259715	159209	159688	157504	159944	160739	156970	155851	153814	154131	F
	259673	254112	258449	161609	155092	159696	158369	148221	155700	153899	149804	153177	G
	154170	154396	153679	158321	158926	153314	152164	151189	152578	161165	160510	158689	H
120	31	8	0	136642	140199	136880	135841	139648	139507	135516	132298	134229	A
	4	-1	10	135330	133671	137564	135280	137136	132874	130813	134128	134372	B
	134707	134802	136399	119296	136481	131363	137180	129723	135316	132782	130010	131603	C
	133404	132688	132963	138946	137678	141149	133231	130191	131919	128477	131245	130272	D
	144553	148311	147099	135322	121897	131516	134416	117847	135022	130091	133655	131579	E
	238429	236956	238281	133779	133482	131648	136386	133589	134858	132556	132214	130547	F
	218692	219402	221253	137332	129796	133154	132211	124151	131312	131647	128240	128867	G
	118496	121012	120340	134276	133973	130392	120705	120480	119437	125019	124703	123862	H
180	6	2	-1	51282	58764	53595	57558	56732	60457	55666	57523	59617	A
	13	12	-1	50463	53871	55232	54360	58450	54927	53215	59606	61068	B
	57260	58639	60257	51243	58573	58891	58692	57259	60145	60496	61646	70136	C
	66544	65397	66090	121305	122308	122840	66360	64153	64227	65180	70520	68208	D
	129089	132536	131612	68222	63582	62502	65001	64418	69593	66357	74222	74512	E
	202632	202163	205384	77891	70963	71552	72421	73747	69940	74389	72895	78302	F
	180260	181028	183387	83078	77881	80799	81599	70722	82063	84770	82055	90313	G
	105547	106407	108094	97140	88735	92476	99206	94454	88297	91557	91288	92386	H
240	1	-7	-1	63109	72629	70911	72294	77369	73062	75609	78779	85660	A
	-7	-1	14	70054	71163	72751	71767	74981	71217	74991	80306	80896	B
	79778	77843	74044	68861	76623	77269	79141	77864	82757	83791	88726	91278	C
	97677	91464	86130	61621	63359	68922	87395	76448	90537	84445	87575	89969	D
	76896	78992	73461	91362	79302	84973	88868	79609	100927	96852	113665	117882	E
	153569	153331	156323	107889	100248	93660	98008	98339	95032	109866	104681	105716	F
	140239	140978	144224	122578	114476	113105	116070	100297	117627	119268	123184	129273	G
	90564	92994	93487	138388	129105	126678	133546	127582	127780	121476	125552	121167	H
360	-5	5	-2	236202	247997	240476	241285	243369	240550	244208	247885	255262	A
	8	7	11	234725	240404	239865	235758	236849	230274	235279	245567	249847	B
	255267	251398	245071	221009	238497	230148	241887	240915	247504	243977	250997	251152	C
	257886	258507	253765	70948	65223	66974	237075	231384	238804	232118	242068	241574	D
	69380	69801	72084	256151	247679	244099	247151	247084	248567	243416	260136	260663	E
	85020	85348	88103	254919	247785	245154	250413	246649	247123	255576	256707	254127	F
	86399	88049	89695	257145	244345	248913	250120	238839	243818	245725	242068	244995	G
	68229	69132	71299	240596	238138	237841	224473	221564	221908	232973	235844	238294	H
480	22	3	7	363752	373485	366977	368350	369201	366045	366267	373543	385625	A
	4	11	23	365231	367941	363070	353547	358938	350247	356890	363256	375613	B
	375338	371203	373785	335655	342792	332653	353650	357309	357065	357615	363252	361968	C
	366768	367449	362948	112207	105053	107957	335999	334739	337493	337761	349544	353184	D
	101531	100204	101958	357096	370728	359432	346979	371516	350252	348382	362444	363531	E
	45849	47095	47492	345140	346410	357483	346249	344489	350088	352262	355690	360354	F
	55199	55456	56908	342416	329784	336591	341726	342793	336415	340679	340982	351659	G
	49450	50892	51555	328347	323834	339357	295325	294486	292494	335438	339257	343646	H
1440	-2	0	7	856528	611153	693440	1171413	850141	911303	1118066	1225829	1218424	A
	-3	-7	5	1895708	587604	649311	776379	613493	734563	1478260	853384	1316541	B
	808060	580500	492733	2464229	1032976	959017	1103323	1005652	859140	1200668	1121260	1367573	C
	710225	556957	531714	344710	360931	320338	682235	761515	516658	793182	667226	895913	D
	360404	340089	339398	840138	993981	805030	858530	1027411	601902	1222227	699375	1075125	E
	1674	1567	1641	953250	723998	927948	951328	629178	657525	806321	724579	1137348	F
	1582	1566	1566	1161255	1251704	991374	1189680	1737868	893217	1028730	1073571	1115107	G
	1210	1155	1174	1070094	974239	1219304	756185	638825	650158	816580	742488	969509	H

Table 28. Raw assay data of M0022/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-2	-7	0	151666	149511	146395	149512	149600	149265	148305	145263	139915	A
	-18	-13	2	149316	151362	150626	146806	147993	147126	145874	148801	142845	B
	141334	147861	144825	150114	149517	149618	147695	145369	148903	145856	146588	146263	C
	142509	151968	144751	145076	141120	145371	145097	143489	147125	159362	158008	154163	D
	142270	145202	148971	146642	137773	145555	138654	142280	147622	151583	151463	144655	E
	146930	152686	151716	147384	142317	143873	138096	149287	147478	152405	151237	144416	F
	148535	153437	152440	150434	145610	147517	147310	149656	154085	149299	154290	146942	G
	67026	75980	74118	146550	140976	140033	144454	156407	150618	163232	162900	155565	H
60	1	7	0	155181	156407	154578	151032	153555	157415	155238	151491	148781	A
	15	0	2	149245	153268	154030	152377	155763	152563	152158	155053	153873	B
	148298	151914	144522	143686	146527	147248	149939	152767	154772	150675	151726	153173	C
	145424	150267	140828	151291	151398	155467	147725	147564	152385	154116	154104	156102	D
	157515	158894	158823	148042	140235	147779	143928	147499	152232	149969	149150	143232	E
	247104	250207	250663	148379	147002	147834	144907	154047	152454	153485	151341	146469	F
	246643	252516	250584	150887	148907	149842	147329	150793	152028	149429	146454	145018	G
	143070	150592	149589	146538	145140	146112	136951	147150	139635	157781	155050	154609	H
120	-8	-18	9	128641	129578	128467	126728	131085	126663	120088	125838	125148	A
	11	-4	-5	121004	126270	127916	125654	128052	126309	123063	124832	128334	B
	121352	126127	120514	116122	118883	119389	123596	126024	128537	123818	125720	127707	C
	122076	124240	117015	128534	127257	130421	122144	122274	125409	122149	121259	121019	D
	136828	134064	135100	123238	118033	124450	121548	122996	127562	124386	123186	118696	E
	223346	224459	223854	123497	122624	125188	119701	127124	124868	126740	125472	120689	F
	206330	211908	209773	122841	121806	125616	120654	124881	125640	122413	120703	122225	G
	112938	119227	116522	122018	118378	120852	105054	112335	107571	120938	117300	118486	H
180	17	3	10	60203	69218	69259	62698	76576	59414	65183	75240	82636	A
	17	4	-3	72537	69481	67147	69458	80020	67342	67124	71531	74218	B
	81209	71672	67871	76276	73685	74451	62065	65355	74697	68627	74002	86142	C
	85997	79870	75740	113809	113904	115873	73818	80056	80842	76444	82389	82897	D
	122532	122625	122869	83876	84137	85180	77045	84349	91054	87031	80710	91013	E
	189118	192777	192835	100008	97709	85622	89091	97420	92774	94761	86070	81331	F
	168471	174445	171694	104209	95754	97271	96084	99344	97399	97764	93713	96678	G
	96637	102767	101419	102739	97103	100779	91083	106098	97771	110334	105701	105470	H
240	-20	-10	-10	90382	98843	97021	80310	99804	103007	91471	96957	113994	A
	-21	-5	-29	96809	92826	94098	90174	89609	98452	92335	94678	97454	B
	110013	95925	103752	89207	101943	93196	88096	90769	104943	94422	108627	117386	C
	116913	114862	114156	72887	73432	71593	93734	101337	109848	104269	107784	106576	D
	89188	87153	83858	118034	103656	110584	109502	117354	129333	114195	124226	129642	E
	142906	144959	145896	132985	128334	116029	116341	128091	127263	130615	129968	123933	F
	132085	135464	133744	139223	129887	129962	131179	135472	136883	130105	128226	136284	G
	82390	87041	86518	125627	127903	129676	126114	136335	130283	127214	131062	133485	H
360	-4	3	-9	240261	248106	248613	234890	249559	250790	249896	253449	252628	A
	3	-2	15	232388	247393	247395	241558	243884	234668	238699	252262	251156	B
	245888	249432	242795	217333	228488	226977	238955	240473	246534	237813	248324	247400	C
	240091	249887	235217	87933	86508	80920	223101	222358	237639	239081	239786	239578	D
	82167	74499	79213	238723	224431	236703	232244	235499	241929	236346	248511	237216	E
	79552	80393	80892	234194	230403	228811	226485	236978	235829	237255	244307	242843	F
	83419	85573	85061	234411	232436	232828	231681	233782	235772	235312	231760	234697	G
	60770	65301	64204	232382	230022	234543	218828	222334	217608	234455	230804	237812	H
480	24	8	-5	346095	345770	348383	339283	345272	347873	349916	344466	354951	A
	9	9	-4	341205	350050	349179	346143	339711	342289	343462	349569	347372	B
	347013	349912	345714	304400	320465	317685	342084	345698	348984	347547	352401	360574	C
	343867	343343	336759	109146	107745	106942	321155	317882	334359	338709	340184	347861	D
	99579	100312	101264	338975	334793	340339	337791	336642	341675	339273	347074	343424	E
	43063	44277	44457	330359	333264	330499	330497	341227	344407	341114	347177	353789	F
	52599	54472	53648	330412	322482	328678	329112	332518	329352	340225	334561	343276	G
	42427	45891	45753	320306	322922	323592	301469	300340	292722	342632	338866	352614	H
1440	9	3	18	700895	619974	705496	836764	815460	698625	659351	894497	1089357	A
	8	5	20	793458	644345	608925	635667	591421	648084	765873	660399	895766	B
	1101351	613997	635951	1567597	1063882	1230592	959800	924076	767361	896037	941504	976723	C
	849129	595067	690497	326718	322315	322002	682148	757044	510906	579057	577360	744250	D
	357368	317710	322556	769337	1510398	1070151	1038459	1043407	700668	837079	654632	1062557	E
	1621	1535	1584	780631	787879	1095918	1103086	663323	702662	611345	673808	951951	F
	1443	1560	1449	995412	1172404	1095743	1152610	1242346	999592	985377	1045976	1221960	G
	862	946	932	1299117	1487589	1455018	1390196	832525	1034240	785480	826716	1157476	H

8.4.3 Assay data for M0024

Table 29. Raw assay data of M0024/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-19	-15	-14	33054	34132	33749	32369	34312	34380	33824	32745	33771	A
	-20	-10	-2	33685	33581	34426	33524	35253	34090	34416	34548	34078	B
	32983	33293	32713	34098	34342	33965	33472	35269	35088	33131	33327	34683	C
	33021	33150	32636	33854	32637	32676	33087	33813	35174	23529	22742	22225	D
	35179	34761	34790	34372	34130	34310	33477	34868	36091	33388	36329	35733	E
	34863	37026	36156	36210	35637	35245	36072	37143	37404	37080	36720	35879	F
	33387	34299	34410	36917	35457	36583	36742	38050	36677	33908	32226	34158	G
	17316	17541	17708	35932	35138	34526	29513	30591	30186	9331	8105	7275	H
60	-9	1	-1	56976	57213	57030	56733	55719	57376	56797	54753	55431	A
	16	-5	2	55744	55071	58295	57636	58493	57811	58198	56542	57076	B
	55268	54737	55076	57490	56086	56109	57547	58863	57757	48746	48947	48747	C
	55831	54677	53999	58712	57678	56727	50408	51188	52651	17955	17054	17547	D
	56787	56322	56378	55861	56320	56070	55687	57263	56577	55677	56912	56417	E
	82885	82934	83354	57881	57155	56477	58443	59308	58120	58293	57532	54831	F
	83557	85303	85116	58447	56130	57350	55541	55726	54549	47217	44208	46453	G
	69385	69604	67658	59335	59013	58473	50342	50501	53686	364	279	238	H
120	-3	6	-17	48890	50710	50091	49693	49706	50725	50981	49533	49972	A
	-5	9	6	48766	48448	50293	49799	51798	51025	51199	50411	49793	B
	50592	51669	51402	51288	50068	49704	49772	51001	50412	44717	44736	44858	C
	50837	50974	50476	52437	51937	51306	46824	47015	48740	1363	1197	1441	D
	50471	50792	50496	49578	49297	48456	48513	49838	50469	49076	50469	49419	E
	77268	77440	76350	50480	49296	49547	50140	50880	50552	52066	51643	49747	F
	71384	72324	73446	51203	50007	50547	49199	49603	49813	46760	46625	46845	G
	43744	43161	43916	52317	51552	52421	44166	44559	46423	17	23	11	H
180	0	13	-3	24983	28245	26007	24702	26387	26426	25431	25741	26100	A
	-1	2	4	25758	25586	27761	25087	27622	25885	26501	27274	28247	B
	28353	28375	28635	27812	28161	27048	25489	25234	25957	35032	35195	33341	C
	26625	28597	27922	30121	28348	28335	27836	27544	27599	208	205	181	D
	40918	40844	40756	26042	25436	25871	24926	24508	25308	25923	28326	28069	E
	70388	69921	70944	26737	26267	27252	26871	27033	28314	30254	30070	28478	F
	57919	58754	60586	27963	28191	28093	27588	28009	28331	46581	42850	43996	G
	31561	30584	30340	31359	31331	32167	33040	32986	34262	5	-21	4	H
240	1	2	-14	11829	12970	11586	11118	12331	12960	12270	12116	11921	A
	2	-8	-9	12260	11610	12908	12180	13028	11404	12811	13058	13022	B
	14509	13748	13442	13370	13170	12763	12725	12583	12743	16418	15753	18158	C
	15255	14130	14904	14479	12839	12522	13199	12711	12333	57	55	81	D
	26639	27533	27607	14792	13562	13758	13593	12692	12878	14344	16591	14862	E
	59459	60004	60109	16728	15079	15591	15396	14939	14292	17601	17747	16881	F
	43829	45287	45332	17352	17263	16604	18552	17929	18481	21711	21549	23003	G
	25063	24179	24199	21092	21397	21339	20736	20340	20697	-13	10	-17	H
360	9	-4	6	98370	101219	101500	101864	99879	100492	107280	104432	104786	A
	26	-12	38	101878	98295	102092	101613	98810	96848	103241	103007	106066	B
	103540	102956	102902	104105	101959	102349	103419	103674	102085	88254	80067	102273	C
	112324	112440	108744	94253	88763	89311	68232	66145	65069	19	27	20	D
	13353	12344	13043	109641	106491	108140	108216	106537	108328	110269	117632	111195	E
	39997	40311	40242	117142	111335	110229	111753	112442	110798	127337	125587	124130	F
	27134	27687	27975	116577	113761	111299	118862	117502	107498	61527	56417	75937	G
	17907	16693	16771	115297	111232	111863	22283	20963	21649	4	-4	-17	H
480	-5	-16	19	257690	258350	253950	250755	257677	256383	259995	255093	264643	A
	6	14	-9	251224	247087	253017	246102	251240	250558	240594	241208	258959	B
	257394	256935	258075	261540	256019	257546	244690	244936	245453	193864	188373	222123	C
	256448	259743	254504	232413	229787	230219	178132	175400	179966	-5	4	1	D
	17691	16303	15335	253853	250231	312042	254234	254156	251632	258966	263587	251482	E
	25500	26021	26130	271824	244536	257874	243326	249521	248374	250527	245273	257365	F
	16766	17274	17220	248124	249229	234037	230095	238427	202762	171659	189783	198668	G
	12335	11579	11761	223911	219412	222271	130007	131690	138258	-2	3	-2	H
1440	-1	3	5	2466463	3179999	3079249	3875014	3737860	3319637	3626718	3602241	3798667	A
	1	1	2	3309537	2949946	2535515	3484747	2384706	3532087	3600081	2883341	3531169	B
	1449693	1818973	1980219	2572537	2503923	3189208	3553252	3032894	2950448	2346428	1694687	2147976	C
	2023097	1529309	2237744	2226795	2551448	2811871	1083092	1158984	754288	9982	9095	42557	D
	303134	241075	255379	2463706	2637044	1646197	3384326	3214382	3151366	3012961	2384056	2888564	E
	1840	1742	1806	1384544	2287561	1967985	2902724	2377450	2541014	2542021	2515401	3048913	F
	396	368	462	1558130	1704599	1991147	2536525	2484377	1744388	930967	1320394	1254428	G
	123	77	90	1994816	1954812	1787541	535307	638578	673533	412	369	7	H

Table 30. Raw assay data of M0024/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-4	-14	-17	38087	37899	37981	36745	40095	35348	38804	39583	38584	**A**
	-8	-11	3	39105	39102	40076	39650	39628	35139	39093	41098	40101	**B**
	35051	35111	34472	38854	38934	38864	39444	36962	38629	39221	40154	39926	**C**
	35678	35452	36780	36692	35395	37500	38831	37039	37565	22674	23131	22014	**D**
	37736	39757	39489	38849	37948	40286	40001	38405	39617	39859	41282	40615	**E**
	39696	40231	41627	39955	40058	40894	41153	41627	39643	42150	43012	41530	**F**
	38337	38825	39092	41372	40560	41912	42713	41527	41258	37509	38728	36812	**G**
	18287	18809	18730	40127	40301	40558	31965	32297	31062	7127	7179	6478	**H**
60	8	2	-7	57684	57400	57140	55765	53887	55863	54770	56890	55521	**A**
	-6	-6	-16	56804	57701	57449	56967	57759	52156	56933	57497	56128	**B**
	53490	54537	52985	56012	56950	56584	57769	54446	55489	49055	49482	48272	**C**
	54136	53681	55638	57288	55379	58307	50573	48432	47956	15688	15785	15339	**D**
	53778	54360	55574	56590	55248	57388	57729	55841	55327	55868	58132	56009	**E**
	80790	79534	80785	55952	56049	57198	56511	57833	55581	56365	57266	54908	**F**
	85835	84309	83818	57161	55894	58034	55110	54718	53032	44859	45743	44661	**G**
	66783	67471	68160	58800	59118	58772	48764	49112	47455	179	144	129	**H**
120	-26	-15	11	49572	50021	49530	48373	54722	49797	48779	49648	47715	**A**
	-2	8	-11	49168	50006	49760	50000	50131	46422	49388	50737	48552	**B**
	49242	49191	48319	50330	49796	49238	50556	48324	49052	44855	44812	44428	**C**
	49156	49053	49921	50112	49283	51228	46418	45267	43922	987	945	1011	**D**
	48501	49420	48834	48589	48300	49017	49525	48330	48276	48574	50472	47870	**E**
	74718	73512	75477	48358	48071	49210	48032	49492	47941	49806	50038	48240	**F**
	71892	71145	71692	49994	48583	50472	48329	48877	47650	46477	47940	46758	**G**
	38667	39194	38965	51263	51081	51119	42667	43441	41485	1	2	-2	**H**
180	-12	-14	-10	27017	28227	27825	26832	38379	26937	28037	27102	27573	**A**
	0	-7	-5	28277	27947	27153	28443	28104	26005	28087	28042	27225	**B**
	28839	28972	27575	28415	28091	27902	28763	26576	27701	34855	35492	33461	**C**
	28515	28470	28768	29286	28889	29882	28515	26711	27081	163	148	150	**D**
	39198	39328	38810	27929	27776	28891	27960	27649	27592	27973	29289	28155	**E**
	67971	66590	68590	28130	28430	28616	27879	28702	27649	29483	29611	27715	**F**
	58617	57609	59098	29388	28891	28969	28529	28258	28294	43000	42725	41342	**G**
	29774	29694	30047	30780	30277	30155	31814	31376	30583	7	-20	-3	**H**
240	-7	1	13	13329	16182	13158	14313	15237	14617	15425	13772	16086	**A**
	-7	-1	-2	13829	13959	13150	12221	15215	13144	14367	14884	13671	**B**
	15460	15431	13969	13727	13381	14207	14787	14252	15667	16444	16489	16700	**C**
	15871	16299	15900	16231	14331	15763	15063	15000	13874	48	69	47	**D**
	26280	26502	26181	16658	15537	15964	16785	16550	15307	16106	18486	19294	**E**
	57842	56741	58851	17639	18031	16886	16600	17330	16883	20466	21087	20927	**F**
	44817	45128	45157	19540	20335	20382	20593	21115	20073	22833	22775	22721	**G**
	24836	24562	24885	22857	23425	23344	19713	19510	18689	8	-8	-8	**H**
360	6	3	0	103320	101925	102078	100140	66903	99205	106136	104697	107746	**A**
	9	3	10	101845	104772	101762	99372	103751	100227	101852	105206	106259	**B**
	103547	106269	102199	104124	105068	104072	104402	104183	106117	84194	84641	96737	**C**
	111451	113103	111196	95885	91295	95472	70664	70960	67019	2	21	31	**D**
	12901	12796	12417	112371	110119	115543	115099	114578	115764	116771	123288	120287	**E**
	39144	38847	39566	115874	117080	117712	118824	120603	118372	126803	128919	128936	**F**
	27618	27481	27871	120936	117628	122195	120611	121772	113553	66681	65301	72106	**G**
	17066	17141	17488	113011	113334	112234	25253	22723	20798	1	8	-7	**H**
480	-2	7	12	250333	239179	244424	236351	244804	236102	243784	244874	248296	**A**
	-3	8	-2	243094	245304	243892	240574	240144	238859	230439	231998	245026	**B**
	246747	249617	241629	246152	245841	246214	239602	239222	237269	192259	187474	211051	**C**
	251495	251918	251515	226937	226743	226792	170357	175590	175519	18	25	29	**D**
	16205	16714	16960	246186	249518	246948	253055	254391	254450	251017	254557	249868	**E**
	25288	24746	25263	243383	241368	244830	243774	247905	241188	243498	239513	253469	**F**
	17340	17186	17294	239644	238845	240776	225281	239427	208559	177957	193744	186655	**G**
	11797	11452	11690	230794	235924	238939	134627	130895	123073	-5	-9	3	**H**
1440	-2	-2	-2	1145263	1294587	1654538	2432017	418498	2263894	1768212	2010596	2024721	**A**
	6	-3	15	1447239	1219859	1282839	1290437	1737777	3182152	1956743	1561391	2176065	**B**
	780809	666005	1326164	1643773	1303266	1692776	1843650	2156038	1809600	1038812	984749	1425900	**C**
	677644	904258	1027182	1114167	1296328	1245665	543090	732006	559401	67932	67658	90009	**D**
	221839	200905	212969	1125004	1398530	1027807	1146680	1424092	1291160	1672244	1255441	1735371	**E**
	1856	1948	1812	642412	851760	831641	1072335	1022061	1346469	1262508	1401112	1720675	**F**
	350	365	375	561312	644667	731714	687314	1054534	742009	577691	636590	673226	**G**
	110	82	61	617591	672668	683367	315289	345028	341318	297	2	1	**H**

8.4.4 Assay data for M0026

Table 31. Raw assay data of M0026/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-5	8	-7	32583	34133	35161	32416	33155	32084	33466	33881	32848	A
	-7	-3	2	30569	32798	32778	29983	30946	28764	23706	23407	22435	B
	30980	32712	32504	24368	26206	25568	15260	13753	12249	143	109	71	C
	33958	33022	33251	487	455	612	11	21	0	9	-7	-10	D
	33503	33577	34328	33284	33779	34575	35093	34008	34490	34928	34904	34703	E
	35317	34678	35429	33574	34987	35129	26973	27665	27349	23834	23746	23634	F
	33934	33504	33664	23813	23533	23304	1056	556	429	252	236	161	G
	18040	17346	17290	-7	3	48	22	-1	-9	15	-4	15	H
60	17	0	8	55321	56597	56244	53579	52186	51147	51542	51207	51239	A
	-1	7	3	50541	52199	52148	45792	45588	42000	14715	13101	11183	B
	52106	53140	52644	29897	32493	32219	204	56	117	3	16	23	C
	52707	52827	53542	9	10	97	8	9	8	5	15	-3	D
	53870	53412	55384	52863	53982	55678	54390	50548	50216	51339	51209	51998	E
	82624	82533	85008	48735	49944	49586	43190	43277	42540	199	348	181	F
	87819	88993	86868	4003	3961	3322	59	12	14	5	28	10	G
	71977	70612	69673	9	12	0	43	15	18	22	3	3	H
120	-3	-1	-6	50845	52534	52246	50212	50713	48561	50685	49528	49183	A
	-6	4	-12	48980	50639	50054	48830	48992	45898	1010	809	687	B
	49759	51329	50031	21573	22700	22924	103	-2	25	4	0	-1	C
	50362	49449	50157	-2	8	53	8	-7	-6	7	1	-10	D
	50064	50325	50993	49594	50390	52065	50842	48067	48501	50107	49555	50105	E
	77620	77989	80143	46727	48911	48254	48587	49668	49146	34	75	31	F
	77174	78361	78389	776	165	324	-1	4	16	7	2	9	G
	47655	48670	51435	3	-4	-4	15	4	-5	-2	-14	6	H
180	2	5	-7	29635	31063	32545	31899	31496	31145	31158	31453	31525	A
	-2	2	-8	34648	37022	36251	49864	50012	46648	132	103	103	B
	31660	31342	31006	9036	9360	9601	92	4	6	-6	-2	-2	C
	30553	31325	31343	6	-7	22	-2	-1	-10	-3	-4	-6	D
	40333	40708	41372	29711	30303	31311	30910	30660	31233	29601	32243	31055	E
	70554	70807	73033	37504	38621	38578	41542	42153	40961	8	72	8	F
	63480	64364	65182	601	41	268	7	-1	8	3	0	2	G
	33840	33405	33430	-7	0	-9	19	-3	7	-7	0	-5	H
240	7	5	0	15228	15427	16473	16689	16328	16663	16199	16011	16775	A
	-6	6	8	19690	19532	19399	28097	29025	28121	47	23	26	B
	17957	17681	16899	3573	3853	3799	60	10	0	-1	-10	10	C
	18691	18781	18099	-10	-2	14	-5	5	-9	-3	-6	3	D
	28989	29665	30160	18541	18030	18165	19340	18684	19065	18740	20107	18727	E
	60266	61182	62718	24746	25484	25834	32880	32724	31281	3	68	3	F
	49910	51425	51252	557	7	327	11	-2	5	19	11	2	G
	28487	27952	27826	1	9	17	18	4	3	-5	28	11	H
360	24	16	12	88463	87359	90354	94521	92948	92012	93758	90477	94956	A
	24	9	13	80196	80879	76910	51463	48517	48986	13	10	35	B
	96147	94054	88503	340	391	390	160	7	12	17	13	16	C
	105748	99797	97450	10	23	40	14	7	-5	17	16	25	D
	14778	14886	14510	101035	98539	99917	102180	101393	100203	97892	102194	106277	E
	40948	41651	42788	99955	103833	111160	23887	23058	22146	21	187	16	F
	31462	32401	32416	1597	8	1175	26	6	8	17	-2	-8	G
	20894	20771	19981	7	29	9	45	16	8	20	12	5	H
480	-18	-1	-10	219781	223223	226114	224556	224887	218748	220730	221145	229249	A
	-3	-2	8	190542	193655	192197	175531	180068	180345	20	-9	-2	B
	234138	235776	230050	22	74	58	172	-9	13	13	-8	1	C
	245223	238550	233419	-12	-12	28	-2	14	-8	-9	0	-12	D
	16033	14013	13921	232975	226042	229739	229130	222661	220078	215966	223617	220723	E
	25646	26701	27473	188744	198899	214406	70254	61576	57100	-1	380	3	F
	19976	20831	20301	6475	6	4719	-3	-2	-18	-2	-6	-1	G
	13982	13461	13893	9	6	9	27	-4	-7	-1	2	-4	H
1440	1	-1	1	2719515	1933645	2160658	3178685	2554386	2556737	2598653	2645573	2586144	A
	0	-3	4	2105167	1350147	1232476	2009537	1869564	2288832	4865	4	-3	B
	1783126	1132334	1633751	104383	96533	104798	60580	-1	0	8	8	6	C
	1052415	1132136	1305772	12	9	5	13	5	4	0	3	-2	D
	206334	183601	180896	1719301	1923028	1838220	2705216	2466596	2139415	2680619	1817745	2309427	E
	1627	1764	1650	2167440	1714268	1856049	1165616	1054131	1074027	12	16835	9	F
	561	583	531	86819	-4	67492	0	2	15	13	-1	9	G
	221	179	165	4	6	2	7476	-1	10	5	12	1	H

Table 32. Raw assay data of M0026/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-2	-7	-1	36395	35850	37184	36624	36923	38397	38444	39078	40207	A
	3	1	6	34834	38262	37634	34342	36239	35520	24406	24796	24621	B
	34265	35342	35793	24597	24175	24699	12199	11802	11237	81	60	45	C
	35441	36032	36453	278	288	283	-7	-4	3	-2	-2	-8	D
	36383	38456	37735	36419	37940	37336	37205	39496	39902	39798	42088	40662	E
	37459	38839	38617	37564	39473	39563	30085	31667	31828	25331	25257	25151	F
	36070	38354	36600	23464	23751	23550	448	340	235	193	156	145	G
	16779	17529	17304	-8	-9	12	2	1	-4	12	-4	0	H
60	7	-3	7	52684	51470	52888	51043	51039	52112	49973	51724	53341	A
	-4	-12	7	47708	51276	51106	42858	43227	42358	8852	9148	7656	B
	49132	51260	50875	27374	27203	27687	56	33	92	7	-2	-1	C
	50296	49668	50959	3	11	-8	5	-2	-6	-2	5	-10	D
	49561	51746	50825	50028	51573	50834	48371	50276	51925	50803	52909	51782	E
	78813	80349	79679	44676	46201	46263	41832	41383	41648	207	227	102	F
	83641	86253	83060	1999	2003	2016	10	10	9	2	2	0	G
	67084	71509	70163	4	-3	4	-8	1	-9	9	-16	-1	H
120	-9	9	-19	47740	47341	48854	48214	48768	48873	49036	49274	49579	A
	10	-2	9	46327	47926	48146	47198	47612	46510	514	456	337	B
	46548	47348	47966	16080	15654	16621	1	0	61	-1	2	0	C
	47089	45998	47242	-13	-1	-6	8	4	-3	-10	-5	-5	D
	47340	48473	47797	46637	48013	47843	45641	47300	48634	47974	49554	48384	E
	75819	76412	75416	44139	45282	45738	46034	46478	46892	46	66	32	F
	72062	76535	74165	126	73	176	14	4	-11	-8	-7	28	G
	40930	42395	42992	-7	6	17	-12	-14	9	4	1	-2	H
180	11	5	-10	29220	29030	29054	29766	30375	30804	30578	30797	30230	A
	6	-1	3	32679	33605	33935	47810	48214	47490	113	62	52	B
	29771	29831	29334	6193	5684	6110	-8	0	37	-6	2	6	C
	29981	29629	29301	3	16	27	22	17	-5	4	-2	-5	D
	37888	39157	38516	29433	29165	28787	29062	29565	30101	29109	29828	29164	E
	68906	69883	68575	34922	35792	35483	39122	38854	39426	18	24	8	F
	59099	62816	61668	82	1	98	7	0	2	9	-3	13	G
	30480	32062	31519	4	6	2	-2	10	-16	3	-10	-15	H
240	0	0	11	17550	17855	17423	17978	19649	20416	20199	19679	20966	A
	-6	13	11	20323	21897	20065	29334	28175	28984	80	10	10	B
	19099	19569	17542	2217	2166	2326	-13	1	36	3	-5	9	C
	19899	19721	20215	9	-10	4	4	-11	-1	21	2	2	D
	27385	28618	27877	19947	19649	19758	19619	20126	20659	20514	22418	20712	E
	58757	59599	59681	24862	25580	26374	30367	29665	29896	-7	15	-8	F
	47603	50051	48427	56	-2	74	-1	5	-4	11	-6	-6	G
	27065	28030	27309	5	8	16	-6	-11	-12	7	-10	-5	H
360	2	-7	-5	97312	98017	97487	100196	102679	104127	103303	105649	108832	A
	-2	13	11	87476	90209	90585	54316	55006	56623	114	2	23	B
	99818	103749	98499	232	191	231	29	14	119	17	2	11	C
	104445	104964	105546	15	4	4	3	9	-2	-5	-8	9	D
	13528	14191	14050	105462	108945	107166	106103	105503	110249	106022	107921	111626	E
	39340	40810	40530	112930	110094	113612	21857	20964	21126	16	27	11	F
	30103	31936	30956	135	7	183	-1	7	9	1	2	13	G
	18926	19998	19550	26	1	2	8	5	8	8	8	-9	H
480	4	2	1	228569	220158	228831	223295	225788	234037	225858	233890	228785	A
	10	2	-5	195142	191846	199446	182415	177823	187108	244	-10	9	B
	238248	238005	238702	36	21	35	-2	9	133	-6	-2	-8	C
	230443	236195	232003	1	-6	14	3	7	2	-3	-2	-6	D
	14112	15322	14704	233296	232529	230464	224493	224975	227216	222896	226348	229723	E
	25511	25358	25685	212811	208011	210162	72644	70907	62007	11	14	6	F
	19191	20560	19677	181	8	165	16	-1	5	-1	10	4	G
	12406	13293	12980	4	-12	4	-2	-2	6	11	2	18	H
1440	-2	6	-7	1118041	985970	1058678	1419486	1363822	1487540	1179137	1655111	1319951	A
	13	-1	-1	1102328	726696	836055	1074376	1107728	1634447	27751	3	-4	B
	569658	531780	805633	93928	47810	72882	-2	-1	3762	12	4	-2	C
	561162	618316	720888	-2	17	20	1	10	4	4	2	-6	D
	231487	217542	201710	1067709	1139978	1160986	1836056	1835556	1007711	1786980	939386	1648196	E
	1566	1656	1610	1166344	1087897	1479267	757454	738427	777561	321	-1	15	F
	598	635	586	53232	13893	31168	5	0	5	10	5	-4	G
	189	184	198	6	9	-5	8	10	3	6	7	-2	H

8.4.5 Assay data for M0028

Table 33. Raw assay data of M0028/plate1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-12	-10	-12	34689	35188	35757	35935	35758	35772	34694	35132	35905	A
	-10	11	2	34045	33514	33387	29343	27152	27512	30765	30741	31722	B
	33802	35326	34025	30345	28831	27442	2041	1629	1615	18299	18107	18397	C
	34430	36841	34495	28967	27489	26877	17	20	9	215	144	182	D
	34841	37257	36721	36690	36668	36951	35902	36057	35793	36113	37289	36728	E
	36790	37390	37724	37138	35895	36317	33380	33221	33142	37055	38208	38377	F
	33332	35672	35352	35305	34893	35083	14906	13441	13236	36203	37121	37082	G
	17596	17645	17986	38348	36736	36725	-9	5	-8	30153	30987	31340	H
60	-1	-6	-2	54779	52336	54612	55934	54310	54569	52975	54252	55471	A
	9	-6	2	222	60	19	49428	47297	46491	49186	50131	50852	B
	49525	53283	50613	2	-7	8	94	71	46	3401	2999	2796	C
	51132	53058	50368	17	7	17	9	6	6	4	7	2	D
	50870	54088	52972	52633	51967	53086	54973	53919	52470	49241	51256	51150	E
	81055	82684	82933	36	349	279	44194	43257	43735	51050	51675	53113	F
	81970	86443	84289	8	-2	-4	29	4	3	50568	49467	50834	G
	65772	66443	67142	11	-6	7	1	2	14	49474	49570	48530	H
120	5	-16	-15	50464	50075	51237	50436	51874	50724	50103	50487	51556	A
	9	7	-2	15	37	9	41851	37511	37034	48357	49765	50781	B
	47174	49724	47867	1	3	-1	62	29	7	366	187	50	C
	47585	50336	47137	0	1	-8	8	1	-2	-2	2	7	D
	47521	50564	49658	49556	49412	50017	51354	49553	50337	46341	48569	47783	E
	77280	77738	78993	10	318	179	42879	42706	42646	47670	48315	48751	F
	72018	76428	75763	5	7	1	1	-2	7	46685	47672	47601	G
	43252	43317	46153	2	2	2	-2	-2	14	47544	48103	46602	H
180	12	6	9	32976	33278	32693	31059	35113	32651	32625	32146	34099	A
	1	-6	-10	-4	29	14	32991	25290	24042	34363	35360	34071	B
	30528	31782	30998	3	7	7	31	23	-6	305	158	20	C
	30086	32344	30467	2	5	9	-2	6	7	17	15	17	D
	38944	41269	40526	32306	32582	33000	34469	32987	33630	29078	31036	30329	E
	70121	71092	71457	-3	285	128	35931	36322	36748	31151	30681	30945	F
	58662	63029	61648	13	12	-8	11	18	10	30107	30460	31864	G
	30986	31317	31780	5	1	-3	1	23	3	33730	34670	33822	H
240	-15	-6	-2	14851	15443	14548	14833	16322	13497	15205	13546	14547	A
	11	3	-9	-8	9	20	27561	18088	15933	15842	16095	15186	B
	14272	15462	14478	0	-2	5	19	20	6	203	110	6	C
	15251	15983	16292	-7	4	-8	-16	0	3	-11	-10	3	D
	28264	30121	29447	17630	16185	17376	16511	15678	16676	15315	17107	16448	E
	59129	60023	60997	-1	262	95	19897	19469	20075	16946	17314	16208	F
	46536	48957	48738	-5	-7	-4	-7	-14	-5	18367	17977	17830	G
	27020	26666	26602	-3	-3	10	-3	1	-10	19790	18306	18509	H
360	2	-6	-4	89713	87683	88766	91553	91443	85657	88030	85752	88050	A
	22	-3	-8	9	19	8	66407	23870	18827	67324	66693	66638	B
	93698	92734	88522	6	-6	5	37	11	4	783	283	18	C
	96726	102110	93427	14	9	6	11	2	7	-4	17	3	D
	13407	14145	12994	96297	90941	95797	89725	82415	86019	99161	101917	98596	E
	38924	40062	40768	-1	924	485	66084	59865	60116	101401	103696	102765	F
	28923	31163	30443	-3	12	3	19	5	11	98129	98433	102925	G
	19393	19570	19707	4	3	-5	10	16	10	72580	70803	71818	H
480	9	4	9	223441	220114	219160	259347	242430	235380	228482	225992	234106	A
	-2	-6	-16	-6	24	2	210496	171159	175701	178863	176480	178870	B
	228977	230347	228317	9	2	-2	65	24	-11	1595	464	9	C
	229394	239382	230623	-2	-5	6	6	-9	-1	-2	-2	-2	D
	13677	13758	12227	239305	220123	225881	236475	231029	229259	229894	228485	229238	E
	25540	26192	26296	8	2691	723	179516	148743	150349	222820	234019	229753	F
	18319	19598	19641	-8	12	2	-7	-2	9	224379	218018	210031	G
	13073	13040	12995	-2	-18	-9	-7	1	-12	140746	145173	150619	H
1440	10	7	12	2376326	2124936	2371399	2403738	2513378	2526218	2446789	2848933	2622228	A
	2	-2	5	1	17715	10059	1899417	1843102	1882042	1004730	682055	859331	B
	1374352	1117563	1599962	1	6	8	39	2	3	296219	281187	251058	C
	1225068	911398	1583581	5	10	-2	12	6	5	8	-1	27	D
	290927	310925	196730	2133157	1836378	1881398	1969040	1876565	1913673	3130380	2142844	2529373	E
	1568	1473	1595	-2	24978	35254	1009052	1128399	1209972	2333301	1528218	2310116	F
	575	457	515	4	9	5	30	3	10	2098430	1899355	2378056	G
	156	138	107	-1	7	14	-7	4	1	1788348	1853664	1869165	H

Table 34. Raw assay data of M0028/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-2	-4	-7	40144	39833	40154	41228	38465	40960	40203	40317	41002	A
	-10	-9	-12	35165	35234	34350	30422	30725	31240	35375	36021	36162	B
	38704	37966	38237	28998	28688	24984	1332	1097	1095	19529	20222	20449	C
	38942	39247	37774	26414	22211	14747	12	11	11	131	87	94	D
	39676	39417	39838	38817	40660	37667	40117	41065	40902	39904	41059	42032	E
	40862	40129	39893	37325	37184	37532	38175	39470	37454	42202	42145	41926	F
	38798	38958	38542	39550	38932	37735	12567	12158	11359	40913	40572	41067	G
	18544	18507	17837	42575	37715	40073	-6	2	7	32342	32194	32031	H
60	-1	-4	-9	52565	52048	52605	53915	50075	53124	51714	51765	50979	A
	-4	-10	3	384	16	17	45334	44752	44511	48304	48734	48551	B
	50230	49649	51009	-2	-4	0	4	69	19	2340	2397	2250	C
	50055	50310	49461	-13	0	2	3	11	14	-5	-3	15	D
	49811	50042	50489	48898	50648	49047	52344	52806	51560	49152	49350	49556	E
	78714	79006	78355	33	30	22	43093	42831	41894	50173	50018	49999	F
	84606	84035	82509	-14	0	-5	-5	5	-2	49752	47275	49020	G
	65108	67958	66595	-10	13	6	1	-7	3	46797	46405	46594	H
120	2	9	5	48555	48840	48507	48672	48095	48523	47468	47181	46781	A
	5	13	2	252	16	14	37937	38759	38196	46732	48806	47644	B
	46980	46357	46620	9	9	9	2	41	5	197	52	66	C
	46778	46273	45988	6	-2	15	11	5	5	-7	10	7	D
	47543	47378	47320	46273	46743	45379	49042	49742	48804	44478	46386	45332	E
	75722	76096	74765	7	11	5	42040	41350	40574	46167	46106	45621	F
	73227	73723	73151	18	10	11	21	-7	9	45943	45429	45817	G
	39125	41405	40229	-3	7	0	17	7	3	44790	44373	43744	H
180	-3	5	0	30794	30885	29999	30776	29324	29325	30034	29954	28726	A
	-4	12	-2	235	-1	-1	28182	30448	28675	31632	32568	31059	B
	29111	30006	30091	-6	9	-8	8	35	10	109	7	7	C
	29247	29644	29393	-3	7	5	-6	-4	5	-5	-1	11	D
	38700	38358	38834	29292	30313	27764	30872	30820	30333	27299	27715	27556	E
	67401	68936	67767	0	-2	0	35003	34399	34438	28942	29021	28989	F
	59117	60061	59720	6	11	2	7	-3	-3	28800	27471	28606	G
	31042	32503	30926	-3	3	-3	8	-4	4	30691	30488	29403	H
240	13	-2	14	16477	15206	15324	14657	14584	14448	15129	14748	13829	A
	-11	-2	10	257	-8	1	23159	25640	23422	15257	16198	15040	B
	15940	15055	15827	9	3	-3	21	34	9	140	17	12	C
	16792	16909	16342	11	12	11	1	-1	-5	5	-4	6	D
	27590	27828	27847	16673	17114	16801	16431	16068	16250	16553	17835	17341	E
	58089	58588	58009	14	5	-7	19116	20158	19823	18533	17293	17464	F
	46949	47819	47200	7	-3	-8	-3	6	17	18298	19037	19372	G
	26817	29344	27756	5	10	20	19	9	3	19174	17567	18124	H
360	6	4	21	89435	90055	90038	91086	91060	91713	89130	88221	94911	A
	21	12	7	11	-2	24	51920	68156	62887	66794	68565	75590	B
	97952	94630	97864	14	12	12	10	53	10	319	1	2	C
	106410	104456	102537	15	2	20	10	8	3	-1	-1	14	D
	13372	13566	13621	100047	102667	101558	99025	97700	95569	103846	111799	108267	E
	37658	38532	38269	3	7	10	69528	66169	66170	108769	110321	109945	F
	29459	29826	29575	5	31	4	0	20	17	103698	104173	106594	G
	19372	20434	19525	14	-2	13	7	16	23	71276	72139	74449	H
480	-3	-5	3	215998	219868	213026	234333	231881	229422	223857	225684	224288	A
	-8	-10	11	-11	11	-8	199106	208793	209161	176247	175927	178794	B
	226774	221632	225774	16	-8	-10	0	57	8	484	-2	-3	C
	227093	229830	229222	0	-2	-11	-13	-6	-8	5	-7	-2	D
	12818	13200	12485	218263	222154	221528	229749	234701	229130	231310	231395	229173	E
	24184	24726	24254	-3	-6	-5	152560	156870	167703	224727	222353	227761	F
	18932	19322	19477	-14	-14	-3	-5	5	8	206168	204244	213249	G
	12675	13144	12671	-10	6	-4	1	-9	-3	138995	139559	150282	H
1440	-5	10	9	1702138	1516610	1994090	2131345	2168305	2087874	2180318	2138010	2218153	A
	-3	-2	0	261	3	6	1615038	1376808	1878983	761273	808615	880867	B
	806996	1016256	1063611	4	0	5	0	9	9	250845	220873	231021	C
	693247	974328	926389	1	2	4	-6	15	0	1	5	4	D
	193254	202118	199571	1117915	1096937	2341540	1176335	1212565	1150042	1672654	1424352	1849366	E
	1605	1468	1482	-2	6	3	549917	701384	649827	1115822	1223063	1320252	F
	554	525	526	15	6	3	12	1	0	1038579	1333841	1409966	G
	144	125	109	12	3	10	10	8	5	1313467	1418495	1823446	H

8.4.6 Assay data of M0032

Table 35. Raw assay data of M0032/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-9	3	6	33061	34750	33922	32652	34150	34904	33884	35570	34959	A
	-2	1	-2	32151	35232	35430	34230	35356	34038	33817	35901	36463	B
	31849	33603	33149	31922	35212	34741	32205	35415	36390	35029	36569	37310	C
	32491	34201	33609	24228	24881	24891	31218	30701	32475	27448	26855	26943	D
	33794	34286	31629	32283	34322	34204	31689	33881	36171	34883	36092	34658	E
	34095	34850	35356	33982	35459	34372	33771	35194	35988	35172	35933	35857	F
	32320	33536	32543	35932	36676	37153	35831	33643	34947	35130	35382	36170	G
	16601	15761	17571	32409	32878	32684	19913	19914	20114	31067	30605	31473	H
60	-17	-16	-16	56354	56460	55487	54690	52013	54697	53489	55191	55504	A
	-16	-20	-23	54213	56384	56454	54748	55433	53974	51290	52027	53682	B
	50414	53204	52454	52726	56512	55730	51609	55556	55464	51902	53351	54063	C
	51975	53045	52447	47517	48700	48469	55191	54722	57273	52430	51796	51677	D
	54903	54844	52164	51872	54725	55197	51414	53248	54797	52283	52486	51442	E
	69441	70607	70632	54392	56019	55325	52668	53805	54739	52078	53235	54046	F
	72784	75100	71499	53861	54022	54341	56098	52389	54343	47199	46508	47621	G
	61480	57554	62443	59570	58778	58429	44642	44668	45602	65090	64869	66069	H
120	-6	-11	-11	48644	50591	48978	47862	46895	49174	48069	49352	48923	A
	5	-13	-10	48404	49016	49656	48336	48980	47938	47218	48293	48298	B
	47949	48248	48234	43341	45965	45811	43580	45099	46798	41857	42189	43068	C
	48697	49153	48239	33706	33789	33147	38188	37848	39813	38739	39073	38638	D
	50804	50438	48081	46874	48131	48345	45427	46425	48372	44632	46076	43814	E
	66396	67201	66785	47628	48589	47738	45725	47518	48057	46679	47397	47350	F
	65444	66127	65585	47399	47231	47452	44108	41754	43144	43894	44073	45098	G
	41771	44440	44072	39806	39759	38928	29848	30702	30879	31325	31136	31102	H
180	3	9	12	34797	36841	35675	33835	34507	35409	34586	35060	35694	A
	-1	1	5	33780	36297	35588	34771	35725	35256	35225	36757	36168	B
	34400	35682	36918	33714	35383	34845	32004	33556	34540	33778	33747	34192	C
	35935	37021	36072	24231	24242	23907	30425	29832	31882	22298	22293	22005	D
	41426	42362	41248	34306	35258	35819	32912	34113	34639	34170	36257	34275	E
	61556	63341	60999	36029	36262	34854	33265	34827	34976	33666	34671	34208	F
	54117	55455	54569	35955	35651	35983	35091	32773	34051	33689	33723	33824	G
	27157	25198	27007	30307	29883	29269	16095	16515	16610	27387	27517	27780	H
240	-3	-10	-11	14101	13644	14043	13146	15080	13251	13623	13003	13772	A
	-7	-10	-7	14938	13961	13772	13433	13437	13917	14202	13665	13878	B
	15145	15003	15083	14228	14317	14450	13840	14921	13922	14876	14336	14307	C
	16643	16291	15922	15793	15911	15699	15656	16421	16535	11328	10893	11117	D
	29199	29474	28914	14813	14946	14769	14645	14785	15474	15715	17743	16684	E
	52994	53906	53994	16567	16221	16097	15167	15326	15205	15933	15863	15012	F
	42427	43238	43666	16432	16552	16203	16640	15775	16208	17009	16435	16338	G
	20836	18290	19802	18853	18752	18898	9474	9858	9887	16083	15996	16279	H
360	11	21	6	50079	49356	49649	49949	52170	48759	51838	50998	53712	A
	17	-5	-1	53288	48866	50909	49914	47771	47818	48611	46920	48838	B
	55172	53782	51020	43896	43505	44398	50290	51573	51035	34095	32842	36983	C
	57521	58093	54318	8079	8128	7207	19509	17559	16204	4102	3733	3794	D
	13696	14362	13857	56268	54359	52864	54291	53816	56403	50889	52253	53790	E
	39107	39886	39612	60552	58034	58231	59913	57251	60479	64472	63988	61937	F
	27193	28021	27996	56558	53217	50929	55365	49394	49810	61507	62020	65118	G
	13381	11707	12965	17342	15364	15315	14878	13273	13623	31798	29982	30378	H
480	7	-7	1	182634	183971	185101	186274	183772	187462	187043	189769	195583	A
	-21	-4	-12	183928	186134	184666	182018	183370	182420	179623	181123	185259	B
	190869	186329	186266	175377	176886	178106	179459	186708	186673	157584	153122	167777	C
	195373	192936	186845	46282	48865	53332	109753	108522	107821	26766	23836	28735	D
	9370	9115	8525	188470	186984	188481	187035	189981	189503	164982	169347	169582	E
	26551	27087	26923	183608	185215	185527	189204	187323	188076	189496	192288	195419	F
	17454	18002	17780	178546	175909	172874	176616	170823	173724	170938	169934	182865	G
	9338	7681	8908	80130	74226	74050	69942	66203	66999	119510	117793	119428	H
1440	8	-6	3	816259	668528	903774	1574562	1127220	1106259	1620701	1480147	1531024	A
	15	8	0	933182	604386	722231	998736	868501	1348067	1802095	1336861	1521234	B
	722534	533503	514592	945783	520376	694539	1597584	521057	769823	1806016	1484095	1617880	C
	586671	517147	627082	229928	250351	225136	220229	237022	232580	328708	356852	335970	D
	197714	172465	145512	1107967	788085	939568	2113142	1498825	1239308	1400811	1090038	1464709	E
	2024	2138	2037	822403	850373	1102773	1806376	1186778	1276253	1682502	1371603	1477148	F
	447	373	458	688017	661761	725236	1219371	1516839	1160153	1003531	977827	940716	G
	37	17	19	330115	327912	363968	253036	236762	237291	204279	209150	225845	H

Table 36. Raw assay data of M0032/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-5	1	9	34384	34600	34382	35180	35225	35061	35053	35663	36418	A
	7	11	12	32839	34831	34605	35354	35280	34758	36187	36772	36375	B
	33588	32829	33035	32585	34788	33238	35236	35502	35731	36009	38806	38201	C
	32699	34052	33898	22682	23347	22551	31182	30610	31596	26659	26082	27120	D
	32830	34154	34042	33294	32860	33278	34378	34542	35593	34449	35094	36467	E
	33945	34132	34998	33132	33642	33983	35495	35488	35449	35341	35638	36078	F
	33325	32502	33541	34649	34049	35425	33502	33277	33962	34164	33717	36098	G
	17533	19125	19465	30663	29329	30798	18133	18956	18146	31823	30675	30919	H
60	-6	2	2	53765	53178	52826	53727	52770	53499	51920	53388	54041	A
	-21	-2	6	51764	53932	53351	53169	53717	52250	52144	51404	53005	B
	50019	49839	51432	51224	52943	52372	53958	53531	52071	48898	50482	49376	C
	50039	50631	52308	45065	44602	44862	51816	51114	52783	52181	50207	51363	D
	51375	51809	54055	52167	51690	53047	53101	52240	52365	50424	49860	52240	E
	70142	68124	69715	51732	51831	52891	52312	52862	50664	51271	51257	52771	F
	70038	71283	73195	50349	49641	51382	49626	49451	49291	45409	43130	47131	G
	61432	64020	63855	55554	52337	55196	43529	44664	42163	64297	62243	63738	H
120	-5	-2	21	46879	46317	46156	47091	46474	46805	46428	46357	46173	A
	-14	6	-12	44958	46380	46054	46333	45815	45565	46172	46521	46200	B
	45882	45267	46616	42121	43286	42818	43821	44159	42950	39985	42310	40807	C
	44612	45613	46200	31012	31038	30705	36731	35393	37081	36912	36295	36129	D
	48084	48629	49105	45334	43942	45351	44858	44678	45860	42711	43116	42985	E
	65713	64079	65844	44599	44604	45154	45201	45190	43224	44500	44296	44976	F
	63321	63883	66185	44049	42942	44025	39804	39395	39060	41320	40840	42280	G
	35679	37726	38530	37695	35740	38102	27526	27843	26834	30978	30046	30415	H
180	-12	-10	-4	32978	34401	33918	33697	34246	33658	33388	32281	31804	A
	1	-6	1	33500	34585	33912	33912	33559	33430	33908	34795	33289	B
	34517	34211	35022	32760	33581	32823	32676	32672	31574	32040	34185	33337	C
	34107	34459	35031	22647	22645	22156	28642	28107	29527	20922	20173	19904	D
	40363	40540	40612	33970	32724	33430	33743	32935	33175	33177	33487	33231	E
	61860	60992	62068	33647	33192	33487	33127	33309	32212	32599	32649	32213	F
	52748	54906	56532	33984	33493	33593	31784	30900	31396	30243	30674	31115	G
	26741	28197	29585	28407	27294	28891	14964	15095	14882	27670	27195	27331	H
240	-17	-3	-2	15691	15436	15694	15323	16289	15906	15670	16105	16407	A
	-3	14	-11	15277	15289	15501	15069	15260	13663	14812	15465	14268	B
	15975	14971	15905	13981	14283	14027	14212	14729	15333	14135	14304	14698	C
	16022	16956	16285	14413	14177	14047	15411	14661	15734	10822	10349	10599	D
	27682	28049	28579	15240	15417	15802	16228	15944	16108	17323	17969	18131	E
	53387	52910	54869	16843	17119	16419	16143	16519	16354	15859	16191	17208	F
	41586	43592	44943	16865	16199	16444	15298	15439	15405	15940	16651	17072	G
	21954	23065	23769	17543	16509	17945	8485	8545	8271	16151	15446	15990	H
360	-3	-3	3	55588	55028	53230	52979	56605	57081	58559	61418	63101	A
	13	-14	-5	54827	55297	55247	55220	54886	53753	53892	54840	57082	B
	56138	56580	53912	48656	49871	49228	56584	56116	57272	35811	41873	40890	C
	61339	65049	62128	11428	10919	11768	22533	21577	21634	5486	4625	5049	D
	13558	13489	13545	61310	58505	61322	61870	62047	63234	56591	56077	62181	E
	39410	38972	39835	62459	63069	63574	65326	65999	63379	62991	67644	70744	F
	26769	27972	29456	56326	56018	58494	52616	54656	53450	63816	64743	69090	G
	14599	15693	15943	18530	16520	17745	17745	18035	17571	41189	39429	44284	H
480	-2	-11	5	177269	177855	176022	183199	183993	182134	181706	183823	187921	A
	8	3	-5	181262	181381	180864	182040	181343	184330	179400	181159	183875	B
	189313	178892	182552	182989	178942	179486	186827	185282	186306	157355	165465	170122	C
	185184	187163	186821	58814	56223	58116	117716	115220	111983	32991	32342	39223	D
	9811	9682	10231	185752	181825	185305	189390	190371	193041	166260	166743	170347	E
	27232	27241	28304	176385	178974	182320	185012	187897	186540	188343	190193	193223	F
	17738	18349	19047	171285	171312	174087	170412	174114	173133	169943	175952	184014	G
	10219	10709	11456	77600	76309	76993	66141	65763	61169	115708	115949	110500	H
1440	-4	-1	7	680082	715996	834902	1100205	1094522	1074873	1270949	1448661	1383520	A
	-1	-3	-4	903334	775111	837432	921601	991798	1579300	1272440	1236785	1546331	B
	466169	494000	600226	673765	635896	854337	735672	814122	908745	1644755	1514544	1680515	C
	537112	496414	531981	203980	220056	222073	198560	207102	211449	313978	325769	340827	D
	168645	157793	163441	702930	784703	880221	904531	1005211	1079779	1150677	881781	997749	E
	2304	2182	2287	632845	692434	719742	703806	724865	893707	800295	890051	1104011	F
	516	568	636	501803	562574	540499	699291	774496	718186	605671	794534	591456	G
	85	54	59	321833	325315	329179	264581	260040	261507	208046	206380	212309	H

8.4.7 Assay data for M0033

Table 37. Raw assay data for M0033/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	5	-15	4	32275	31446	32160	32789	33643	33032	32709	33785	32511	A
	-3	-1	3	31318	30992	32016	31033	31600	30868	30772	32204	31403	B
	31804	29551	31053	29236	29936	30778	25038	25712	25476	27000	27116	25652	C
	30766	29934	30988	22995	23243	23908	21653	21799	22347	23091	22888	24484	D
	30866	31339	30773	30877	30147	32065	31312	32828	33353	31947	33527	33129	E
	31576	31054	32354	30611	30206	31180	30929	32065	32221	30898	30687	30838	F
	31181	30835	29822	28360	28398	29774	28086	27316	27803	28163	26565	27644	G
	17028	17022	17011	21315	21646	22592	22102	21875	21772	23972	22339	23918	H
60	-7	10	-7	53937	51125	53586	53159	52653	53051	52229	52715	52009	A
	-2	-2	2	51775	50437	52639	49946	49602	48979	48527	49334	49355	B
	51103	49142	52432	43122	44235	44020	38043	39416	38030	42241	41971	40715	C
	52192	49074	50824	58479	58435	59575	50768	52134	52871	36609	36845	37879	D
	50616	49674	49919	50825	50560	52648	51523	53459	52855	50222	51716	51988	E
	69202	66250	68726	51130	49389	52322	50238	51381	50787	47868	45379	46423	F
	76178	73770	72126	43040	43241	45649	42485	42562	40967	43092	37776	39736	G
	61597	63215	62829	54099	55863	56488	54955	53652	52871	29998	28805	29938	H
120	-14	-2	-11	44947	44582	45560	46101	45691	45280	44123	44825	43790	A
	-11	-14	-6	45077	44341	44896	43631	43577	42858	41859	43005	42577	B
	45282	44171	46584	40686	41546	41501	37787	38920	38439	38701	39158	37475	C
	46482	44983	44999	29666	29833	29721	31718	32958	32661	34771	35141	35465	D
	44696	45023	44093	42938	43362	44963	43845	44705	45037	42421	44257	44223	E
	65923	63634	65572	43474	43256	44246	42981	43808	43331	41829	40741	41383	F
	68173	67095	66701	39296	39581	41663	39192	39792	39078	39401	37933	38155	G
	36317	36022	36691	26892	27192	27607	28950	29512	28657	37313	33714	35974	H
180	-5	12	-6	30298	31159	31331	30694	31420	30639	30456	30635	30284	A
	-1	-16	-2	31481	30483	32600	30136	30709	30309	28851	29147	29299	B
	32324	31683	33441	28584	29593	30211	26977	26954	27701	26839	27849	26449	C
	32585	32035	32420	25330	25091	25021	26577	26098	26473	27760	28593	28768	D
	35641	35499	35401	30919	31349	32371	30433	31705	30656	29742	30481	29090	E
	61418	59629	61900	30526	30543	30675	30078	31165	30300	28014	27481	27221	F
	57780	56534	55978	27442	27693	28123	26508	26703	26994	26808	26926	26474	G
	28877	28273	28334	25008	25747	26393	25874	25825	25293	28579	29097	28651	H
240	24	-5	26	18170	16233	17561	17596	18780	17992	17598	17426	17693	A
	12	11	2	18425	16501	17843	18240	17988	17780	16557	16807	15954	B
	18171	17099	18377	16361	17044	15792	16074	16796	16568	15945	16715	16027	C
	19042	17857	18178	15502	14945	15394	16557	16664	16952	16646	17012	16805	D
	24153	24279	24011	17780	17739	18407	18202	18065	18827	17618	16920	17758	E
	54383	53190	54780	18343	17050	17565	18071	18101	17684	16603	16125	16805	F
	45826	45277	45061	16252	16200	16315	17313	16827	16850	18205	16766	16761	G
	24175	23898	23802	15186	15691	15410	16639	15976	15119	18157	17642	17544	H
360	-18	-10	-6	65228	66342	65510	67366	68264	67275	68319	67242	67904	A
	-13	10	-2	69312	67082	66933	65527	64849	65024	48771	48676	46082	B
	68670	66124	68214	61674	55345	57841	42246	38501	39880	41884	40537	44708	C
	70256	69063	68657	35999	32306	33525	32167	31300	32983	45116	40812	43312	D
	11634	11675	11379	72273	68529	70818	72774	68142	71232	72893	72928	75279	E
	40210	39920	41024	73893	68977	69273	72858	72977	69328	50889	47768	49288	F
	30107	30192	29602	56834	53898	53595	53781	53516	51119	56604	54020	55560	G
	16643	15980	15945	44414	44988	44422	50157	43021	43229	56704	54216	55496	H
480	-6	4	4	183894	187604	188212	187069	192560	188697	187349	186572	190007	A
	6	-2	-20	193757	184499	189550	165675	168796	168044	126380	121369	122104	B
	195226	185122	191703	164531	155082	158772	86768	90547	91303	97857	103285	104456	C
	201283	194312	193705	87564	80642	85265	76131	76336	76175	89151	90778	89842	D
	13720	11977	11024	194508	194790	195596	196095	194400	200576	193385	197086	195484	E
	28118	28238	29556	192155	188984	191750	190158	190518	188840	108489	100450	112925	F
	19695	19486	19387	138249	134140	136271	109150	107525	104656	110669	110130	113989	G
	10844	10516	10878	99520	99355	99548	94917	96761	94466	111237	108301	114911	H
1440	6	-5	-1	1748110	1311420	1541746	2225612	1833842	1860449	2051466	2014218	1863019	A
	-2	8	-3	1740447	1274493	1310138	1194271	1047539	1515089	420860	338566	406639	B
	1387753	1365037	1126935	838691	528627	618544	162607	146668	148609	149050	153386	174309	C
	1019383	1357168	1184557	148107	150544	150983	144649	169268	140935	187196	178205	181794	D
	299741	247736	272939	2066657	1879923	1567347	2245843	1588300	1588497	2367075	1523790	2241500	E
	2560	2608	2638	1804232	1719189	1515890	1907731	1504059	1581639	189105	212114	235643	F
	705	641	704	408817	407593	376367	168590	179665	171989	154589	169949	182511	G
	146	109	109	158920	161249	159941	162303	165970	169905	190549	217804	214312	H

Table 38. Raw assay data for M0033/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-4	-5	1	30588	30035	30347	29853	29279	30173	30490	31476	30777	A
	4	6	9	29759	29535	31048	28757	29465	28182	29562	30141	29981	B
	28172	29077	27884	26947	28629	28662	23032	23546	23933	25757	25247	25950	C
	28506	29411	28897	23139	22306	23185	21166	20492	20436	22795	23497	22008	D
	29307	29345	29815	29558	29354	29177	28704	29880	30705	29691	30984	31691	E
	29220	30396	30950	30300	29586	29431	29986	30544	30560	29699	30005	29385	F
	29182	30091	29292	29122	29028	29411	26053	25528	26836	26746	24574	26364	G
	15777	16183	16373	23983	22777	21797	21679	20815	20713	25036	20254	22906	H
60	-10	-3	3	53716	52067	52294	51977	48079	50892	49880	52174	51759	A
	-2	6	-4	51598	50601	52516	49061	48071	47418	48692	48917	48632	B
	49320	51616	49656	43491	44288	43942	37681	38972	38557	43234	42672	43480	C
	51538	51348	51865	57984	56779	57926	52157	49998	49328	37493	38799	35960	D
	49055	49373	48724	51158	50876	50221	49536	50860	51188	48874	49772	50849	E
	66032	66385	67232	51760	50493	51119	50147	50000	50259	46656	47501	45866	F
	75638	75313	74490	46231	46492	46539	42606	40862	41509	42260	44239	41246	G
	58262	58718	60794	60643	58417	54889	53405	52330	51396	32732	29336	30425	H
120	-9	-3	-2	44616	43978	44353	44281	41630	43289	42623	43219	42553	A
	-7	4	-9	44251	42982	44338	41180	41093	40277	40670	41030	40147	B
	43557	45006	43668	39099	39554	39641	36255	36483	36451	38176	37580	37972	C
	43967	44030	44097	29734	29610	29210	32085	30349	31189	34088	35392	33549	D
	43466	43125	43041	42361	42862	41817	41205	42186	42601	40878	41339	41880	E
	62678	63677	64185	43020	42336	42157	42118	41529	41254	39858	40516	39033	F
	66640	67319	66030	40423	40337	39466	36669	36987	37708	37526	41511	37250	G
	34444	35749	35219	30380	29804	27861	28907	28218	27392	37891	37792	35383	H
180	14	-2	-4	28243	28875	28712	28208	27390	27867	27244	27913	27949	A
	-8	-2	-2	28642	28493	29184	27548	27595	26698	26031	26538	24863	B
	28230	28947	29023	25704	26998	26088	24241	24375	24424	25311	24903	24690	C
	28561	29211	29288	24388	23584	23357	26390	25211	25551	26586	26276	24953	D
	33461	33826	33855	28710	28370	27995	27544	27448	28441	27513	27620	27359	E
	58585	59894	60396	28471	27878	27830	28064	27626	28157	25585	25925	25063	F
	56328	56692	55905	26356	26604	25610	24030	24343	24398	24719	26909	24197	G
	28255	29543	28454	24402	23718	23289	24283	23774	24025	28179	27350	26481	H
240	-17	-1	-8	18337	18011	17896	17245	16750	17547	16435	18032	17304	A
	0	18	6	18032	17141	17637	17128	17863	17145	15383	16478	15925	B
	17369	17805	17636	16777	16818	16758	16193	16574	16499	16585	17451	17527	C
	17807	18273	17730	16131	15324	15825	17294	16533	16607	16510	17112	17217	D
	22770	22806	22984	17651	17259	16763	16694	17248	17189	17304	17204	17432	E
	51724	53014	52734	18269	17731	16971	17981	18434	17959	16855	17605	16641	F
	44306	45082	44922	17216	17100	17412	16687	17489	17427	17976	18487	17905	G
	23951	24802	24062	16537	15705	16115	15952	16426	16218	18639	18770	18315	H
360	0	-1	2	61778	61205	61718	63930	62074	59044	60193	64713	64256	A
	-2	-4	2	66686	61506	64541	59594	60172	61340	46704	47028	44982	B
	63956	65237	62941	58475	57230	56449	39366	39619	39812	39537	39398	45009	C
	73611	70554	67862	36177	36483	35833	32327	31385	34593	39131	35931	38605	D
	10919	10648	10923	69273	69294	68525	67955	70423	68251	73324	75646	77043	E
	37611	38859	39783	75822	74913	72698	74514	74751	74598	43352	46767	47554	F
	29390	30014	29514	59228	59329	59035	50293	50130	50089	51887	56152	54118	G
	16192	16615	15762	44364	41356	45343	39969	42912	40918	49511	60119	54397	H
480	-16	-19	7	191515	187772	191818	187835	195406	188369	188805	189650	193750	A
	-2	-13	-7	199275	193580	194080	170221	178127	175752	130165	132982	133577	B
	198989	196134	194134	168712	169332	172396	91692	93416	93113	103607	101677	110447	C
	205296	205191	203953	87590	84427	85860	77916	72851	78862	89345	89115	91611	D
	12424	12359	12150	204559	199374	198615	199249	203243	203672	194175	201923	199910	E
	26781	27773	27761	198308	201331	200312	196295	196993	198604	105973	106755	115838	F
	19403	19518	19658	145524	143052	149100	111095	112363	105707	112607	113999	119376	G
	10751	10969	10600	101047	97492	100878	101945	98688	91483	112225	139272	117876	H
1440	1	-3	7	1558890	1355022	1586496	2030977	1866474	1535439	1567242	1641065	1644183	A
	8	-2	8	1810569	1547301	1499671	1135765	1017861	2047768	426190	400189	478537	B
	1197080	1094654	1445242	1014664	661946	913603	165303	163245	162230	147728	154397	192438	C
	1278199	1027177	1197288	158567	150558	151566	156996	162326	146480	181114	182736	195559	D
	276823	276133	242744	1498392	1624428	1956172	2545515	2067284	1534587	2348404	1463727	1871404	E
	2523	2698	2591	1406631	1710011	1837970	1617039	1505424	1357414	205539	238587	261758	F
	776	686	707	330912	422461	407073	182850	194518	183185	168506	181786	187947	G
	152	131	123	154643	154329	157310	194487	200375	203968	236866	312895	255724	H

8.4.8 Assay data for M0034

Table 39. Raw assay data for M0034/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-4	-9	0	35008	35465	35598	34076	35717	35644	36902	35867	36167	A
	9	-1	1	34330	35004	34125	35710	35898	34718	35070	36023	35493	B
	33809	34953	35062	32964	32767	32552	34777	35865	34768	34412	33655	34038	C
	36229	36312	35749	29732	30700	29298	31151	31171	31578	33427	34245	33622	D
	34061	35331	36254	35965	36730	35400	34642	36950	36189	36106	38873	35874	E
	36805	37745	37545	35269	35801	35475	36243	37334	36757	37625	38326	37174	F
	35013	35855	35273	35934	38710	36334	37637	36459	37720	37596	37877	37359	G
	18635	18373	18417	34253	36261	35602	31844	38338	37581	36611	36137	36677	H
60	22	16	0	54487	54253	54980	54077	53230	52926	54264	55001	54592	A
	10	6	6	48071	49256	48258	48827	48462	47788	47230	49899	49643	B
	50483	53426	53377	47298	45996	47099	47689	46965	45229	44225	42994	44018	C
	53225	53020	53668	46927	47168	45787	57496	57926	56728	57869	59969	57845	D
	51925	51972	54059	53829	54620	54333	53328	54288	51868	52042	54277	52632	E
	79585	79865	80254	48515	50392	50084	50997	51075	50814	50582	50779	50885	F
	86241	84672	83603	43006	47969	43178	54902	48383	49906	48003	45630	47197	G
	70205	70320	69569	82455	87231	85016	53379	56301	53349	52651	52712	52928	H
120	-2	-11	-1	50777	51405	51702	50688	51530	50122	51398	51202	50460	A
	-23	4	-8	46635	47914	47235	47526	47931	46060	45601	48077	46869	B
	49895	52110	50770	46313	46165	45278	46250	46388	44906	46866	45447	46463	C
	51315	51644	51498	43911	43707	43998	35110	34904	35235	41998	42316	43302	D
	50691	50810	51432	48482	49893	48419	48890	49829	48999	47659	51171	47211	E
	76893	75691	76488	45900	46650	46202	46146	47325	46715	47306	47569	47363	F
	72928	73165	74080	46567	48667	45639	51692	46697	47461	45005	45630	46195	G
	47733	48653	46677	50814	53659	50751	49769	46531	46466	45357	45568	46230	H
180	-25	1	-18	32592	32876	32046	31515	32088	31972	32199	31242	31162	A
	-9	8	1	29260	31086	29237	28047	29198	28384	26506	27115	28165	B
	33288	33928	33658	30129	31283	29987	28556	28863	28888	30382	27789	27605	C
	33888	33822	33286	32246	31674	31521	24049	24592	23999	30493	30394	30805	D
	42537	42516	42880	30435	31225	30795	30407	31027	31461	30185	31002	29723	E
	72522	72066	72017	27840	28449	28062	27504	28055	28986	27519	27584	27508	F
	60459	61132	61807	29299	31197	29032	31080	28692	28850	27434	27916	28279	G
	29326	29192	29648	32626	33149	32642	32831	31642	30547	30592	30373	30876	H
240	17	17	-2	15097	15047	14684	14250	14984	16400	15281	14567	15009	A
	8	0	6	10837	11305	10422	10252	11396	10504	10176	9978	10693	B
	15852	16439	16763	11588	11606	11612	11048	11938	12628	12281	11889	11292	C
	17991	17624	17414	13486	12671	12751	12682	12106	12505	15157	15521	16029	D
	31423	31285	30239	17617	18106	17855	17223	18452	18324	18634	19040	18774	E
	62740	62321	62518	15078	15411	15261	14730	15433	15278	14540	14871	15067	F
	47910	48242	48772	17209	16291	16419	16277	17711	16825	17486	17452	18803	G
	24381	24100	24476	21320	21544	21570	23057	22272	22604	20606	21262	21485	H
360	5	-2	17	79801	83693	83475	76590	82703	89295	80082	78308	83990	A
	16	6	-7	36264	32291	32901	32432	34185	31769	30164	31945	38078	B
	85631	87752	92010	40030	38380	40239	39762	41238	42971	38290	41045	46389	C
	98305	96923	95425	45137	43849	43977	44047	42294	44707	47005	48941	53053	D
	15039	15204	15351	87249	87613	91276	87408	91639	96024	90022	94772	94560	E
	44554	45055	45308	58418	58248	59399	55658	54667	60667	52325	58427	62153	F
	30326	31556	31031	71942	69299	75137	73639	70073	71221	70197	77750	81302	G
	17787	17248	17610	78229	75686	79841	92229	92700	94424	88963	92369	93378	H
480	34	-8	9	199833	201872	203655	193291	207985	218907	198954	196629	208340	A
	3	9	4	123800	114051	109561	101964	106652	103232	92203	97772	115046	B
	230492	233781	235103	127883	125260	125197	124177	129378	133653	118812	125869	138242	C
	247104	243793	234918	138053	129501	128035	128127	123856	131155	128102	131402	139706	D
	12191	12191	14039	195347	195688	201954	201572	209234	221531	201771	200763	213721	E
	29858	30209	29856	158488	161216	161385	154388	155747	156778	145064	153275	163329	F
	19936	20212	20208	180381	179333	165112	166279	165943	166700	163613	167244	173605	G
	12124	12001	12350	175427	173860	180424	180266	189193	178404	174662	181351	184599	H
1440	5	2	-8	390086	414678	493642	707678	542036	473847	514692	696967	729974	A
	-9	1	7	115624	102614	109092	105591	104413	108034	106551	113540	112041	B
	342190	344083	448192	83965	77282	83195	107090	99470	102667	120124	128378	137738	C
	374582	377922	398401	121912	114389	109460	133230	131851	119400	152003	160821	171091	D
	230189	220221	253115	399462	419674	661732	842310	783312	504273	670160	499603	743434	E
	2200	2198	2258	122603	125213	130686	135979	130454	123838	133375	136986	160297	F
	543	489	496	134965	137087	135384	143295	145982	147027	152762	151023	177789	G
	100	63	65	219789	217517	217067	210391	215249	196768	228661	212305	244573	H

Table 40. Raw assay data for M0034/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-6	-1	-1	37041	37480	37612	37088	36977	37550	38173	38005	39332	**A**
	16	0	-6	33603	34987	36121	36839	37424	36154	37244	38229	38365	**B**
	35197	35756	35455	32859	34341	33408	36915	37020	37995	36235	36890	37192	**C**
	35738	36406	35885	29704	29213	29391	32553	32522	34339	34487	35031	35041	**D**
	36583	37224	37577	38493	37391	38646	38187	38524	38073	37767	39349	39319	**E**
	36702	38036	38594	37047	37350	36083	37208	38712	38210	39686	38827	39562	**F**
	36457	37279	37432	36858	38543	37910	39337	39203	40606	39968	38667	40925	**G**
	19004	18970	18884	33302	35817	34353	35504	37767	38873	35472	36122	37599	**H**
60	-7	-3	14	54185	54257	54386	52863	52181	54031	52971	53270	54531	**A**
	2	8	-9	45382	47411	47615	47799	48980	47444	47935	48341	48691	**B**
	50599	51460	50953	45141	47509	44325	45857	45551	46609	44284	43158	44230	**C**
	51101	51431	51011	44030	42936	43135	53613	53454	54942	52067	50977	52204	**D**
	49221	50176	51238	54031	54586	54474	53955	53914	51628	50661	52463	52646	**E**
	73571	75911	76905	49090	49196	48018	48076	49888	50253	49492	47729	49104	**F**
	82929	82945	82439	42664	43693	42869	49893	48703	50823	48541	44136	48431	**G**
	70545	70411	70432	79343	81973	82416	47720	49551	50292	48208	47500	50191	**H**
120	1	9	8	48730	49862	49263	47621	48737	48884	48728	48975	49050	**A**
	-3	2	-11	42307	45192	45233	45062	45504	44523	44576	45259	44840	**B**
	48510	48240	47817	42886	45866	42603	43850	44421	44405	45264	45360	45288	**C**
	48100	48366	47858	44089	42541	42912	34858	34837	35873	40806	40687	40888	**D**
	48229	47693	48248	48235	47711	48253	47656	48497	47802	46324	48330	47280	**E**
	73411	74831	75091	45074	44808	43532	43808	45281	44818	44773	43882	44263	**F**
	72925	74340	74294	45527	44663	44953	46037	45329	46402	44674	44107	45509	**G**
	37821	39077	38963	45023	45183	46237	43006	44306	44976	42851	43305	44867	**H**
180	8	-5	-1	30360	30483	30018	29151	29748	29072	29571	29553	28875	**A**
	3	-3	-5	26715	28047	27555	26280	26717	26236	25189	26654	26241	**B**
	31301	30339	30201	27088	28204	26865	26362	27500	26668	26640	26962	26389	**C**
	30816	30645	30426	29812	29103	29159	23586	23890	24005	28708	28598	28333	**D**
	39839	39664	40007	30198	29618	30178	30062	29725	29006	28837	29409	27918	**E**
	67878	69638	70812	27439	27203	26322	26689	27319	27130	26509	25607	26193	**F**
	60444	62158	62542	28071	27370	27889	27454	27580	28409	27079	26724	27210	**G**
	29548	29782	30571	30847	30377	31104	28169	28785	29233	27932	28211	28953	**H**
240	-5	1	-25	17062	19844	19005	16540	19127	17029	18668	18741	18972	**A**
	-7	6	2	15704	16271	15149	14038	13850	12293	12526	13368	13024	**B**
	18046	19491	19593	17039	16491	15098	14914	14324	15226	14900	15835	17657	**C**
	20912	20815	19628	20111	19271	19068	16486	15750	17129	19561	20302	19088	**D**
	27626	27396	27797	20482	21082	20607	21061	19000	18645	19201	21152	20869	**E**
	59771	61397	61780	19489	18744	17844	17970	18227	18212	18071	17129	18637	**F**
	48006	48682	49520	20225	19587	20906	20772	21742	20542	20161	19919	21399	**G**
	25966	26159	26675	21873	21743	21266	22519	22557	23081	21786	22344	25058	**H**
360	-5	-6	-9	100467	103726	101266	94868	100977	97816	95792	95031	102637	**A**
	9	-2	-13	61948	60748	56391	48377	49686	48568	43704	46259	51188	**B**
	104767	102041	98904	66904	69235	66483	59382	64016	59860	60892	63751	72403	**C**
	105950	108948	104167	79432	75694	72657	65281	64163	65635	67619	72442	80129	**D**
	14887	14816	14896	100873	102302	99837	100639	100026	104255	96430	99379	103167	**E**
	41654	43725	43744	80556	80255	76880	75364	75410	76065	71439	71685	78687	**F**
	30783	31618	31924	86659	86535	85734	84357	86376	84668	82005	82628	88480	**G**
	18881	18906	19382	79438	81612	84458	90462	88906	88570	89739	88390	89785	**H**
480	8	-9	1	230507	234074	226436	220447	217108	214593	203656	209896	224217	**A**
	-7	2	0	148229	143450	147827	136298	134090	126143	119679	122375	133689	**B**
	244605	241110	231882	149108	159014	147538	153859	157059	157830	141344	155022	169733	**C**
	236282	235484	232881	150570	148289	148597	154328	143978	149029	155645	152827	166776	**D**
	16499	17347	16390	216535	213598	219085	214678	219387	236418	208068	203539	210810	**E**
	27943	29160	29629	146956	152806	155127	153939	153485	163144	151216	153784	161883	**F**
	19757	21122	20628	156757	173326	158657	157502	152876	148733	147069	147594	157221	**G**
	12876	12618	12969	164837	160730	164074	158834	164766	158916	153213	153021	155402	**H**
1440	2	3	-2	451728	435072	465254	586228	617661	622330	637139	674686	791548	**A**
	-5	-1	-5	121975	98447	95740	95125	106547	103365	102979	100859	119674	**B**
	416983	402909	434251	81467	76113	69119	94894	104030	98573	116408	127855	157130	**C**
	440840	437916	418462	98384	101386	102737	136996	134342	138988	170786	168046	201357	**D**
	257734	281952	265994	385973	393661	467507	560559	548696	763051	661074	564096	951426	**E**
	2104	2223	2148	120696	120264	126130	122768	125339	131003	134861	144425	156228	**F**
	575	582	598	129531	130354	124822	148428	140719	142127	151753	159524	171404	**G**
	160	160	127	220074	212782	207359	204418	227535	209871	229997	222899	244344	**H**

8.4.9 Assay data for M0035

Table 41. Raw assay data for M0035/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-3	-9	-7	33459	34807	34580	34813	35695	35310	36029	35655	36856	A
	-17	-1	-11	29997	29994	30638	35406	36180	34142	33623	34825	35061	B
	32999	32499	33409	31478	30469	30409	34351	35282	35901	31516	31748	32153	C
	33759	33347	32890	25083	25175	26558	21791	21968	22975	34049	34964	35946	D
	32848	33623	33477	34459	34542	35110	35423	37200	36826	34700	36376	36953	E
	33584	33907	34639	35689	36156	36163	30109	30422	31007	35278	36156	37270	F
	32888	33118	32054	34267	34124	33981	16934	16519	16225	26281	24811	25443	G
	17929	17299	17932	24565	24184	24742	4054	4035	4154	4881	4398	4959	H
60	-9	0	-2	51685	51946	53043	53041	52483	53390	52684	52816	53830	A
	-3	3	-20	46894	44254	46354	52992	52990	51515	48727	48520	50793	B
	51692	51186	51984	42287	42071	41078	46975	48205	47662	40643	39817	39609	C
	52320	51464	51726	15727	14911	15404	56509	56845	57938	37974	36720	36096	D
	46949	48209	48742	52408	51667	51894	50899	51128	51087	50718	51800	52666	E
	75626	72679	75055	49916	49359	49655	41940	42945	42907	49337	48833	51377	F
	82906	82915	78361	61307	61801	60556	44301	44638	44294	38628	37541	37428	G
	66411	66721	67800	21404	20302	20370	15909	15881	15779	23172	21727	23813	H
120	3	5	3	45427	46787	46731	47441	47924	47955	47440	47516	48310	A
	-2	-2	-2	42415	42044	42995	47103	47926	45931	44531	44896	45496	B
	47724	47196	47826	42283	42670	41966	45266	46471	45632	42211	42753	42524	C
	47894	46871	47257	10444	9868	9714	26942	27118	27380	12150	11262	10547	D
	44105	44853	44492	43806	46878	46649	44408	46014	46081	45203	47152	47314	E
	74189	71379	73900	45982	46210	46307	38399	38167	38437	44680	45004	45478	F
	73466	74048	70430	36335	35799	35279	26280	26327	25698	35800	34406	34610	G
	37714	36460	37948	4298	3827	3913	20330	20564	20378	16673	16409	17032	H
180	-3	-16	-8	27639	28156	27653	28846	29256	29439	29509	28796	28097	A
	1	20	-5	25427	25823	25936	29455	29388	28320	26123	25888	26266	B
	29551	29217	29583	30485	31409	29854	30570	31016	31540	26633	26478	26730	C
	29598	29284	29204	7101	6923	6723	31158	32704	33819	5250	4782	4638	D
	35065	35868	35396	25329	28694	28358	26745	27460	28319	27727	28518	28224	E
	69172	68275	69457	28630	28352	27991	22693	22759	23440	25683	26909	27142	F
	62002	61086	59058	30294	28452	29209	17893	17248	17025	21932	21231	21105	G
	31449	29948	31082	1060	928	964	16160	16342	16203	14362	13757	14402	H
240	-18	-3	1	20550	21722	20842	22676	22076	23113	22787	22095	21437	A
	-16	-10	9	20463	20521	21854	22654	22117	21531	18626	19589	18975	B
	23062	22336	23921	27236	27936	26684	20894	21086	20957	20232	19935	18618	C
	23890	22390	22516	4360	4156	3934	28409	31025	31625	2365	2272	2099	D
	23151	24053	23171	19480	22159	22076	21019	20523	22497	21656	21724	21484	E
	60831	59589	60438	22362	22089	22849	17821	18941	18274	21044	20842	20758	F
	48875	49390	47685	26472	25175	24535	16450	15790	15786	19248	18325	18581	G
	27232	26169	26973	155	128	96	10434	10289	10320	11314	11069	11011	H
360	-8	2	-18	75282	76796	79133	92729	93670	96132	95420	94498	94043	A
	-5	-12	10	63061	62886	62752	91644	90440	92163	62367	65764	63888	B
	97884	95372	66887	96696	90525	87359	87005	86135	87215	54207	58260	60391	C
	104230	104061	69697	1296	1106	1132	50025	63709	66661	506	418	467	D
	14537	14931	14927	59331	65137	64135	93943	94746	96237	101525	102439	100789	E
	42668	43367	43439	102536	101153	101964	56637	57463	59294	98995	100145	97355	F
	31968	32380	31008	80145	76876	76717	44235	39184	42620	85410	85923	86712	G
	19011	18220	19461	6	19	6	4726	4386	4306	16055	15760	15579	H
480	-6	1	6	172034	167079	172355	209916	212551	212255	215703	213214	215127	A
	4	5	9	129563	122444	118456	214188	208641	211722	139675	139900	147004	B
	233493	224412	134227	163474	153212	150378	224141	223618	225608	113780	119256	131001	C
	232879	232223	129149	462	307	288	174543	299155	299906	153	125	107	D
	21832	22867	20170	121950	124901	129989	204158	201580	201839	217818	221544	221530	E
	29228	28914	29468	138488	215464	216855	111005	112239	106907	211546	212843	218827	F
	20707	21083	20066	191971	190677	193041	73578	66187	70250	199679	203380	208399	G
	12469	11717	12540	13	7	13	7970	7889	7689	94407	75397	91140	H
1440	-2	-1	0	643603	579384	842414	2315422	1995650	1932940	1661404	1617900	1305617	A
	-1	-1	2	106763	114935	123869	547008	1494254	2287723	524291	438204	440232	B
	929584	811184	293736	240837	364690	346388	464232	863699	801284	69813	48873	34346	C
	884757	604525	315565	504258	502739	451506	207298	157563	132360	-1	5	2	D
	309489	306441	208704	222371	335025	407082	666532	1071890	1283965	2303461	1037785	1173761	E
	2469	2581	2301	405909	523573	882145	182442	174975	177777	1487134	1182372	1069438	F
	541	519	503	695714	647545	682214	86995	94921	92335	1635269	1565802	1367635	G
	142	105	91	0	1	11	198908	214991	187808	790669	783514	820174	H

Table 42. Raw assay data for M0035/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-80	-84	-78	30871	31951	32257	34129	31078	33068	33994	34626	34750	A
	-56	-77	-80	26786	26279	28612	34241	34686	32888	30810	33350	32757	B
	30137	29366	30379	27715	28021	29870	33172	33866	33122	29651	30365	29927	C
	29565	30336	31010	24201	23135	25845	21698	21474	22219	32761	33516	33871	D
	31273	31524	32574	32343	32904	32833	34543	33024	34494	33499	35329	34911	E
	31718	31971	32710	32576	33546	33202	28944	30421	29252	36072	35130	35481	F
	31501	31126	31907	31491	32421	31531	16476	16391	16835	25128	24840	25437	G
	16147	16309	16812	22883	23541	23960	3834	4003	4245	4752	4674	4519	H
60	2	-16	2	51157	52701	51346	53304	49887	51073	51620	52601	52644	A
	-10	-4	2	44950	43388	46561	53273	53199	51677	47244	49817	49540	B
	49425	50657	50716	41958	41922	44926	49022	48373	46631	41623	41494	40730	C
	48389	51416	51187	13964	13723	14081	54688	54050	55422	38043	38614	38781	D
	45479	46915	47804	51023	52569	51477	52140	48628	49996	51024	52731	50876	E
	71616	73294	74749	47763	49302	48578	41186	41429	40780	51023	49977	49917	F
	81876	82938	82499	53114	53015	52476	41516	41725	42129	38056	37107	37089	G
	57912	60405	60918	21391	21360	21258	21169	21416	20987	22587	22758	21729	H
120	-6	-10	-2	43576	45271	44472	46877	44837	44750	45204	45933	45983	A
	-2	7	-19	39606	39838	41490	46529	46221	44869	42024	43748	43209	B
	44880	45289	44640	40593	41122	42878	44861	44845	43014	41190	41771	40417	C
	43495	45637	45809	9292	9105	9095	27159	27053	26936	12378	12924	12970	D
	43235	42579	43716	44961	45698	44300	45197	42899	44918	45226	46230	44412	E
	70444	72805	73576	43506	45007	44298	37584	38167	38268	45087	44015	43196	F
	73613	73431	75696	35325	35754	35761	25028	25424	25539	34614	33952	33585	G
	36084	38406	37797	4496	4267	4586	21973	21880	21862	15398	15367	15081	H
180	-9	-5	-12	25563	26845	25981	27450	26358	26873	27327	27166	26629	A
	-18	-16	-17	23600	23099	24769	28065	27652	26270	23977	25589	24542	B
	26855	26965	27299	29232	28872	29640	29530	28695	28256	24792	25277	24261	C
	26650	27829	27688	6389	5941	6105	32309	32509	32468	5520	5797	5563	D
	33283	33349	33929	26844	26197	26344	26164	24684	25864	26098	26920	26264	E
	65218	66942	68423	26175	26414	26071	21745	22108	21895	26074	25887	25462	F
	61067	61202	62916	27323	28061	27151	16414	16674	16726	20720	20625	20259	G
	30446	32619	32140	1069	993	1156	16974	16146	16566	14395	14238	13797	H
240	2	9	-8	20609	22184	20236	21239	21494	20255	23542	21567	21739	A
	-1	8	1	20868	21512	21850	20184	20068	20704	16745	18998	19866	B
	22795	21829	21416	29192	29375	29296	20426	19249	20011	20532	20533	19947	C
	22535	22055	22496	3692	3542	3546	30654	29964	30135	2475	2443	2447	D
	22136	22047	22582	22249	21701	21173	21594	21273	20886	21135	21761	22147	E
	56691	58400	59385	22473	21248	21388	18144	18492	19230	21815	23215	22561	F
	48650	49036	50037	22906	24010	23414	17161	17021	17496	19605	19927	18689	G
	26244	28169	27749	172	189	175	10808	10649	10839	10226	10164	9661	H
360	20	5	9	84647	86633	84920	94643	96052	94905	94863	93188	95207	A
	6	19	-8	56283	56110	55876	94377	93469	96553	61331	67525	69075	B
	104891	98569	97970	83020	78450	86631	90706	89883	89988	51203	58281	56893	C
	112691	110057	104181	1086	1036	999	104316	81555	79499	476	481	459	D
	14909	14785	15193	60287	104364	103667	101730	98331	99641	103302	106143	103918	E
	39491	41773	42925	103920	104711	102995	59116	59308	56734	101373	101977	98695	F
	31043	31944	32808	79734	81011	79721	42830	43224	42419	86970	86687	88927	G
	18166	19645	19334	20	5	26	6285	5782	6036	18303	19977	18819	H
480	6	11	18	193258	195256	195602	221707	225519	233532	223456	221923	227850	A
	33	20	23	129406	129540	133801	218480	224872	222470	157748	164063	169548	B
	238745	230707	225368	160229	161389	161213	236091	235712	241218	123565	128037	135532	C
	244194	231598	228017	339	244	236	347835	315607	310183	168	145	135	D
	23616	24301	24632	124619	228747	223353	210501	208956	212279	223201	224442	232500	E
	27072	28127	29071	219647	221079	223964	112662	113771	110912	214017	217032	219890	F
	20136	20749	21465	206250	201253	200413	72167	72068	71888	203559	203775	216197	G
	12001	12797	12937	6	12	11	9620	10481	10791	102590	105410	103493	H
1440	2	4	-3	630004	639888	1051759	1555559	1789117	913965	973768	931186	669828	A
	4	17	8	119945	134533	140323	692245	1177097	1693879	375615	339533	350465	B
	1804896	948866	1167076	280952	379477	379341	408786	745287	565276	46554	36313	32938	C
	1500054	1056784	1088472	480657	472120	481503	145439	148976	149086	-2	14	9	D
	319516	317091	308982	340083	536189	1009113	1081835	2082580	1042923	1511455	730064	682466	E
	2347	2737	2638	622478	1371749	1889322	214259	181272	177412	921538	854526	613481	F
	581	605	557	548459	794309	834776	106543	84463	74281	1229765	1235925	910264	G
	127	137	85	12	14	0	197907	200780	186764	808720	747568	542110	H

8.4.10 Assay data for M0038

Table 43. Raw assay data for M0038/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-11	-5	-5	29338	28641	28650	28725	29548	29239	27392	29144	29694	A
	1	0	12	28204	28372	29050	29622	29814	27631	28238	29383	29368	B
	27647	27956	28506	29363	30280	29293	28783	29400	28974	28014	29125	29597	C
	28247	28684	28863	30162	30114	30007	29108	29768	29461	22062	22708	22852	D
	28801	28692	28894	29176	28685	28819	29906	29576	29904	27884	29929	30218	E
	29202	28952	30221	29326	28996	30035	27493	27399	26945	29166	29730	28556	F
	27421	27006	28022	30992	30253	30490	7	1	4	27501	28505	29367	G
	14707	14656	14794	25665	24912	25245	0	10	-2	26936	27415	27272	H
60	-19	-2	6	52671	50646	51561	51935	50419	49948	48245	51330	51470	A
	7	-7	-12	49211	47436	49740	52285	52282	49031	49230	50018	48791	B
	47827	49085	49902	46454	47425	45237	52573	52636	52411	48348	50449	49804	C
	48453	48655	48804	42670	42720	41606	52908	53635	52900	38162	37028	39120	D
	46522	46203	47645	51000	49891	49529	50060	49683	47381	48579	50009	50862	E
	63683	60994	64008	49646	49114	50548	36333	35489	33463	49259	50032	49137	F
	64381	63583	66118	47230	46982	46806	22	-13	7	47212	45178	46825	G
	52864	53273	53940	35251	34294	35104	3	8	-5	48308	47006	47493	H
120	0	1	3	41168	40558	41236	40508	40941	39961	38653	40028	40750	A
	-1	5	13	37594	37409	37770	40996	40593	38764	39118	39995	39953	B
	38087	39380	39879	36618	37071	35965	39366	39575	39391	38518	39953	39790	C
	38071	38661	39461	35569	36323	35222	39925	40968	40468	34579	35500	35770	D
	40395	41215	42224	38948	38724	37531	39341	39049	38486	38175	39930	39469	E
	60296	58845	61363	37815	37574	37973	30811	30672	29771	40458	41009	39706	F
	58042	57185	60017	35926	35289	34947	-4	9	-4	38689	39452	39739	G
	32562	33523	34501	26926	26104	26310	-16	-5	6	49761	49429	49948	H
180	-11	-14	-6	29931	31386	30886	30785	31555	31084	29890	31029	30506	A
	0	-6	-11	28670	28470	29009	31682	31510	30782	30547	31037	31372	B
	29604	30702	31816	28711	29372	28276	30349	31042	30664	31056	32330	31106	C
	29014	30739	30847	30429	30771	30436	32659	33049	33057	34217	34913	34818	D
	30669	33040	33330	30653	29985	30553	31164	30994	30725	29876	31636	30999	E
	54589	54542	56834	29018	29376	29690	24790	24966	25285	33731	33756	32685	F
	47698	49048	50753	27088	27254	27373	-15	-19	18	34041	34570	35155	G
	24654	25291	25624	22381	22236	22023	-7	-2	-6	48608	47933	48429	H
240	13	-13	4	17107	16303	16294	15979	16241	16101	15600	15825	15840	A
	-2	-6	1	13166	12437	13353	16678	15811	16051	16239	17019	16295	B
	18864	18355	18090	14153	12834	12900	12956	12246	12823	17666	17262	17401	C
	19515	19185	18392	16654	16231	16208	14244	13306	13890	28557	29954	29912	D
	23109	23628	23709	19073	17606	18436	17269	17299	17335	17540	18081	17742	E
	46863	46224	48220	17713	18083	18375	18220	17508	17207	18160	17992	16446	F
	37163	38493	39929	16977	16626	16378	-17	12	0	19209	19610	20514	G
	20066	20556	20836	16246	15527	15389	-5	5	-8	41239	40314	40366	H
360	-2	-8	-5	69345	61413	64638	67994	66423	70935	69111	67232	71530	A
	-4	4	4	38666	31265	33680	61450	57437	53672	63426	65932	67540	B
	80224	67826	63430	38600	35133	31979	34206	32335	38848	72307	75127	76717	C
	87320	74554	67439	43679	37995	34221	33010	29640	34120	76063	73851	79628	D
	12127	12220	12654	70205	68171	65618	70758	68390	71415	72103	77494	75895	E
	31260	31529	32561	59317	54407	56018	74637	69775	67903	69809	71046	70468	F
	22769	23975	25069	64494	58837	59509	-13	-21	-11	33603	37238	35904	G
	14097	14715	14644	70153	64191	64207	2	-10	-5	21474	21398	21436	H
480	7	10	9	197455	189159	192256	207418	210711	213462	213533	212210	223432	A
	-7	-2	11	125171	111267	112254	194779	191820	185530	197544	201589	203322	B
	226811	212159	207513	123021	120050	116206	103070	101470	118310	208753	212991	218777	C
	234710	214542	209480	134805	127121	126670	99899	89537	99056	329543	314550	336040	D
	10879	9470	9986	198647	196672	198264	210213	212585	215553	204704	208498	218377	E
	19982	19941	21030	148407	139790	140695	211923	203319	201816	200779	210836	220446	F
	14427	15044	15516	152455	149210	153260	1	5	14	170248	177339	180089	G
	9685	10111	10430	147043	140419	144729	-2	2	16	12302	11185	12230	H
1440	11	14	9	1408168	928143	1023680	1672134	1345386	1429033	1595976	1462376	1384508	A
	0	12	6	398709	321958	317736	1148454	1029176	1474256	1346655	1116368	1393445	B
	1766219	1089564	992154	396050	311545	335377	187235	180568	183381	2403019	2307914	2349882	C
	1749213	1119902	1110412	405464	407125	389389	171401	174260	175888	1087521	1075940	1068086	D
	337432	268635	255679	1417849	1257487	1473418	1673741	1485939	1403252	2192976	1322848	1659852	E
	1326	1329	1304	565846	547404	584019	2307526	2219137	2264595	1874774	1360632	1653118	F
	333	339	381	471891	441198	441443	-2	0	2	1189197	1171935	1157338	G
	89	60	36	524716	476513	483884	6	1	6	340060	335702	348953	H

Table 44. Raw assay data for M0038/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
	3	-5	-2	31852	31847	32752	32207	32913	32895	33874	32971	34130	**A**
	-6	10	-10	31526	32014	32724	32568	33440	32427	32065	33101	34831	**B**
	31001	30657	31038	32422	33794	33558	32736	32648	32668	31645	32661	34209	**C**
0	31707	31219	31660	32819	32831	32859	32967	33808	34929	24104	25284	24528	**D**
	31365	33774	32044	31944	32339	32778	33730	32957	33320	31037	33275	33907	**E**
	33403	33672	32554	32705	33119	32586	29561	29954	30871	32328	31718	32763	**F**
	30955	31199	30726	32917	33314	34592	-8	-17	13	30212	29064	32519	**G**
	15123	13604	14772	26484	26886	27396	-15	-7	-13	29348	29207	30127	**H**
	-10	-1	8	49610	49232	50056	49315	49671	50416	49883	49572	50844	**A**
	-15	5	9	46427	47931	49228	50115	50880	49588	47181	49073	50576	**B**
	47646	46970	47456	43954	45144	46443	51520	51249	50653	47211	47780	49454	**C**
60	47319	47126	47661	40273	39855	40383	51427	52069	52752	37285	37938	37933	**D**
	45265	48320	46727	48909	48588	49801	48875	48316	48282	46653	47811	50251	**E**
	62683	63376	61153	48118	48208	48298	36094	36237	36935	47813	47130	48466	**F**
	65725	66109	64195	43921	44319	45588	10	3	-2	45205	42434	46550	**G**
	50952	48316	51946	31736	32020	32405	5	-3	8	46054	46422	47199	**H**
	28	1	6	38028	37884	38416	37681	38262	38054	38111	37466	38069	**A**
	2	21	1	34804	35218	36264	38165	38181	37336	35979	37318	38051	**B**
	37411	36235	36659	33538	33998	35009	36853	36757	36389	36286	37147	37182	**C**
120	37268	35983	36240	33788	32973	32874	37032	37525	37800	32731	33845	33341	**D**
	39188	41070	39000	35750	35538	35741	36294	35651	35921	34645	36306	36955	**E**
	58495	58809	56830	35137	35124	34415	28899	29145	29513	37056	36238	37283	**F**
	56974	58183	55953	32031	32041	32479	3	12	7	34695	34790	36719	**G**
	31053	29191	31510	23099	23477	24054	17	17	12	46187	46961	46569	**H**
	0	1	-13	28246	29434	28749	28730	29165	29154	29536	28710	28851	**A**
	6	-10	-1	26495	26999	27627	29578	29739	29483	28556	29345	29471	**B**
	28441	28205	28865	26571	27109	27669	29774	29200	29253	29591	30084	29963	**C**
180	28251	28963	29112	28351	27909	28828	30758	31277	31231	33013	33980	33445	**D**
	30666	31907	31454	29090	29303	29144	30048	29846	29906	28860	29445	29412	**E**
	53119	54829	53747	28590	27957	28394	24482	24577	24471	31547	31018	30167	**F**
	47041	48849	47916	25509	26507	26034	0	-13	0	32598	32424	32949	**G**
	24215	21933	24611	20872	21001	21084	4	-19	18	45214	45938	45217	**H**
	-2	3	1	18334	17978	18620	17262	18899	17898	17151	19293	18335	**A**
	-6	-12	14	16274	15831	15682	16560	17468	16451	16600	17930	18247	**B**
	20905	19824	18833	16198	15692	16906	14529	14661	13968	18299	17921	19050	**C**
240	21058	19880	19165	18744	18051	17671	15921	17491	14221	29358	29856	29753	**D**
	22104	23699	23125	19009	18757	19439	19131	19855	19794	18095	18958	20313	**E**
	45539	47439	46483	18980	18253	18672	18801	18759	19313	19899	18457	19281	**F**
	36897	39238	38152	18028	18343	18448	-4	-4	-1	20009	19523	19208	**G**
	20403	18483	20497	16066	16486	16099	-13	-2	1	38599	38869	38226	**H**
	0	-1	-2	74422	68619	65495	71304	74363	75935	78586	79260	83667	**A**
	9	-4	-17	50353	47454	50908	66962	64113	68670	68423	77341	82739	**B**
	84714	74747	73189	51429	50517	53005	50067	48328	46385	79620	84402	91420	**C**
360	87968	79804	74784	58853	56123	53101	45013	45659	48311	84852	86628	96560	**D**
	11786	12924	12293	74505	75107	73599	76840	78604	79070	75624	81875	86276	**E**
	30499	31844	31444	58909	60307	59674	76200	77244	81032	80221	79821	83276	**F**
	22978	24523	24190	67007	67547	70126	4	-12	7	36839	39076	42812	**G**
	13961	13119	14477	74391	71607	72489	-2	-12	9	20943	20466	19947	**H**
	20	11	-2	199097	193980	196850	213186	215183	214280	217418	215685	220953	**A**
	6	14	-2	119146	117400	120290	202143	206853	198837	202258	202608	218501	**B**
	226068	209751	207126	118325	122539	120882	123651	118123	121874	204242	217607	220595	**C**
480	220752	209614	204525	131953	124485	124535	121225	111681	125148	328025	332280	368779	**D**
	10805	12756	10960	191816	201498	196194	204494	208703	206914	205080	200234	209398	**E**
	19363	20236	19958	132673	135308	133357	202570	203801	208940	203011	199906	210582	**F**
	14323	15417	14918	140378	138973	136955	13	-16	-7	166205	171450	170099	**G**
	8991	8734	9898	167493	159861	157376	-6	7	-13	11814	11559	11448	**H**
	-2	10	3	1289961	997623	1009413	1433830	1360762	1289975	1379814	1665877	1673579	**A**
	-2	6	2	431661	340847	306322	991970	1127031	1526494	1541133	1222166	1374569	**B**
	1340430	1183705	1308871	390093	328337	343682	271354	230916	201889	2170971	1955222	2095685	**C**
1440	1479142	972486	1011051	436485	414652	386540	192048	177816	186266	959321	865947	1027328	**D**
	318063	305784	248144	1282599	1190682	1174640	1295502	1559681	1271041	2086068	1231436	1679395	**E**
	1338	1341	1396	527019	544707	587031	2058949	2137901	1975655	1141330	1209146	1373816	**F**
	329	345	391	529394	508867	466336	6	5	7	1059008	1172466	1076921	**G**
	68	59	67	667350	641908	624286	10	9	11	375002	369774	377030	**H**

8.4.11 Assay data for M0039

Table 45. Raw assay data for M0039/plate 1.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	0	-7	4	31598	30890	31070	30235	31291	32046	31801	31812	32777	A
	-2	-3	3	29209	29607	30421	31888	32788	31004	30905	32971	32766	B
	28651	27577	28453	19143	19989	20012	31155	32462	32144	31377	31673	33436	C
	29224	28886	29412	8532	9150	9984	32552	32804	34332	31094	30801	31686	D
	29610	30165	30555	30981	30611	31994	31797	31632	33155	31881	31680	32594	E
	30927	30025	31465	30862	31873	31192	32904	32975	32871	32949	33068	33467	F
	29788	29359	28942	31965	32139	33090	33441	32707	33072	33474	33397	34901	G
	13911	13573	13792	33453	34024	34525	35245	34793	34139	35671	35932	36808	H
60	-15	-9	-14	49420	48493	49422	47082	47250	47715	48076	45785	48752	A
	-1	-7	1	44625	44922	45378	48154	47784	47050	46600	48206	47870	B
	47401	44878	46161	22894	23473	23128	47358	47964	48594	46290	45216	47269	C
	47682	45883	47397	9573	10196	10211	46705	46579	48964	44648	45625	44955	D
	44084	44756	45204	47816	48492	48459	48530	47577	49207	46985	47618	47759	E
	62835	60662	62223	47392	47676	47475	48370	49144	48603	46698	48108	48100	F
	67190	66324	65508	47757	47957	48433	47493	47646	48459	46780	44418	46816	G
	48758	49157	50002	48401	48506	50124	48927	47627	47471	47094	47849	48636	H
120	5	-7	-6	38118	37853	38140	36740	37651	38197	37493	35862	37460	A
	6	-17	-6	33548	33481	34167	37486	38114	37120	36645	37852	36907	B
	37370	35952	36996	15605	16038	15808	36661	37280	37636	35427	34656	35728	C
	36913	36914	37161	10962	11076	11453	36281	35648	37703	34176	34509	34273	D
	38091	38607	38759	37133	37444	37610	37298	36040	38291	36516	37046	36922	E
	59019	57667	59367	36575	37136	36931	36912	37579	37623	36034	37227	36600	F
	58941	58503	58409	36924	37006	37697	36247	36093	37267	35983	34917	36448	G
	31010	30743	30867	36868	36918	38331	36198	36497	36200	35847	36682	36454	H
180	8	19	-4	27680	28091	27309	26563	26985	27169	26751	25709	26390	A
	14	10	4	24166	24528	24308	27127	27906	27096	26197	27370	26478	B
	27292	26839	27801	11353	11532	11323	26815	27635	27594	26166	25460	25981	C
	27013	27527	27994	9625	9603	9710	25990	25606	26671	24350	24828	24593	D
	27827	28349	28837	27449	27843	27484	27084	26894	28075	26195	27014	26798	E
	53142	52367	54612	27202	27120	26877	27177	27776	27751	26416	27587	26762	F
	48335	48211	49331	27020	26872	27187	26635	26793	27313	27928	27700	27699	G
	25292	25099	24712	26704	26574	27162	26561	26289	25940	26831	27997	26405	H
240	-2	-1	4	20434	19562	20019	19880	19336	19553	19454	18305	18657	A
	-10	13	-3	18810	17631	18333	20727	19756	18835	18574	19622	18399	B
	19631	18855	19149	8876	8476	8510	19465	19618	19682	18806	18137	18617	C
	20468	19571	19740	5496	5845	5953	18872	18859	18629	18342	18741	18277	D
	20227	20869	20988	20468	19159	19688	20399	19933	20524	19804	19705	19702	E
	45460	45177	46398	20334	20032	19436	20130	20476	20112	19836	19739	19315	F
	37573	38810	38952	21071	20466	20724	20555	19926	20826	16835	17304	17709	G
	21739	21670	21574	21328	20665	21165	21245	20980	19968	14238	14593	13282	H
360	-15	-8	0	75765	70966	69530	72003	72116	74508	78928	76265	78769	A
	-2	-5	-6	68802	64670	63102	69093	70124	70435	74423	75426	75411	B
	81681	71388	71310	18901	15120	16219	70935	75502	75671	74842	75678	80804	C
	87511	83343	78670	8012	8260	8556	75234	70005	74182	65993	68574	70550	D
	11334	11635	11707	84843	78547	81184	81364	81098	82742	81002	88068	85711	E
	30413	30004	30964	84746	83165	81017	87489	83788	85029	84846	86551	85778	F
	23543	24290	24508	86750	87218	87649	85750	85186	86934	69711	69061	70975	G
	15292	15400	15072	91635	87859	90262	92601	88441	85754	18804	20407	21370	H
480	-6	8	-2	205850	198239	193973	193242	198493	199609	202463	204518	215900	A
	-2	-6	5	183294	172191	165545	181676	186191	191465	190661	195632	204196	B
	218255	205059	206594	27172	21706	22127	182870	199568	202753	194221	200493	210990	C
	221864	211036	212877	10702	11281	12653	189234	198674	206029	159697	167549	174758	D
	14876	13521	13918	208666	202865	198384	200837	203021	207699	201027	208149	213210	E
	19248	18967	20094	203362	204121	202731	204360	208925	202815	202137	208152	218557	F
	14833	15208	15240	203463	205651	208976	206257	215089	205864	186492	187744	198698	G
	10173	9751	9916	214869	217427	221710	222157	219630	217209	111259	117493	118960	H
1440	-6	0	-2	1765556	1384982	1385276	2097203	1841618	1679013	1844505	2107382	1777064	A
	7	-1	6	1669732	1375074	1322380	1638649	1474669	1856683	1916986	1554753	1848667	B
	1758153	1419385	1320382	543236	267103	277640	1666356	1720562	1717247	2009511	1921839	1884647	C
	1724050	1521021	1323154	20044	23961	22678	1536004	1891314	2006256	947049	965038	1054311	D
	287337	259870	251771	1001462	1441881	1146303	1879662	2029505	1863470	2178013	1964434	2058227	E
	1306	1280	1241	1958749	1810023	1924143	2169143	1803287	1992069	2063918	1916758	2064092	F
	352	300	338	1817520	1859667	1792609	2041784	2040792	1899690	1958335	2102275	1770920	G
	59	38	34	1657598	1711553	1613953	2130687	1745530	1969869	378782	371686	374190	H

Table 46. Raw assay data for M0039/plate 2.

time [min]	1	2	3	4	5	6	7	8	9	10	11	12	
0	-9	-2	4	28991	28912	28760	28872	30288	30715	30229	29180	31206	**A**
	1	10	1	27550	28060	28693	29363	31868	29941	30182	36256	32036	**B**
	27308	26350	26704	18565	19581	19488	30492	31147	31975	31465	29366	32615	**C**
	27121	26462	26254	8615	8901	8866	30819	32246	32686	31114	29949	31683	**D**
	28283	28111	28530	29123	29262	29160	30105	31178	30731	30973	31203	33229	**E**
	29416	30386	30463	30322	29823	29956	31370	33080	32525	32943	33647	33856	**F**
	28284	27087	27718	31872	31183	31792	32249	32433	33929	34866	29586	38106	**G**
	13552	12225	12632	32253	33170	33082	33907	34384	35368	35354	34742	37605	**H**
60	-10	3	6	47788	47313	46980	45187	47889	46910	46211	45238	45391	**A**
	-2	-6	-10	42878	43705	43381	46620	47649	45931	45051	53923	46453	**B**
	46469	47163	45373	21542	22189	22047	46270	46972	46651	45448	43457	44888	**C**
	45683	45343	45510	9595	9578	9591	44124	46325	46202	43631	42370	42736	**D**
	43052	42307	43540	46050	45835	46160	46124	47416	45751	45605	45977	46710	**E**
	62510	61125	63513	47337	46504	44763	46526	47534	46518	46557	46783	46503	**F**
	67320	64206	65162	48038	46038	47047	46391	46589	47024	47134	41151	48999	**G**
	47641	44110	46088	47836	47264	47406	48009	47544	47584	46752	46187	45994	**H**
120	-7	-1	12	36723	36543	35969	34833	37211	35928	35717	35753	35119	**A**
	2	-10	-1	32252	32108	32594	35820	36617	35741	34986	41936	35545	**B**
	36633	34136	35417	14443	15018	14641	35160	36548	36219	34337	33425	34109	**C**
	35872	34525	35423	10389	10021	10192	33886	34930	34613	32757	32475	32591	**D**
	37251	35968	36264	35423	35084	34870	35405	35682	34697	35410	35627	35654	**E**
	57353	56662	58565	35660	34929	33948	35724	36351	35591	35334	35714	35421	**F**
	57481	56245	55956	36322	35472	35543	35279	35364	36702	35900	32269	37534	**G**
	30767	29252	29673	36227	35563	35986	36404	35731	36138	35598	34709	34644	**H**
180	-15	-5	-3	26079	25896	25580	24964	26750	25624	25617	24417	24130	**A**
	-3	-1	6	23436	23303	23396	25580	26097	25147	24886	28931	24746	**B**
	26556	25528	26113	10597	10548	10139	25368	25435	25507	24048	23183	23441	**C**
	25817	25754	26265	8971	9024	8808	24025	24769	24402	23344	22746	22719	**D**
	26457	26725	26957	25725	25809	25697	25652	25954	25297	25600	25454	24847	**E**
	52365	52082	52873	26164	25986	24835	25930	26047	25598	25552	25898	24825	**F**
	46964	46243	46372	26572	25445	25880	25503	25389	25625	27662	25294	29041	**G**
	25537	24176	24746	26209	25217	25558	25398	25092	24978	25740	25585	25137	**H**
240	-7	8	-6	19808	19291	20559	18651	19950	19740	19563	18844	18858	**A**
	4	-3	8	18329	18115	18830	20485	19419	18895	19061	22574	18712	**B**
	19787	19058	19178	8834	8377	8819	19289	20290	20260	19025	18840	18139	**C**
	19588	19669	19521	5417	5327	5023	18113	19916	19989	18604	18233	17744	**D**
	19386	19380	19556	20130	20339	20659	20292	19880	19904	19761	20274	20344	**E**
	44023	43962	45237	20436	20608	19410	20396	20639	20392	19930	20878	19946	**F**
	37056	36654	36812	21863	20440	20744	20310	20368	20286	17885	16515	18931	**G**
	21842	20854	21385	20997	20070	21074	20935	21164	20659	13350	13197	12552	**H**
360	-21	-17	-22	75656	74767	72263	74492	76751	78559	77829	78245	80741	**A**
	-10	-17	-9	66849	67018	63475	72206	75860	73452	73780	85801	80392	**B**
	85346	78144	76689	13692	13354	13617	75514	76294	81520	79162	79808	84191	**C**
	87569	85034	80976	7861	8487	8297	72904	74332	76638	69534	69052	72832	**D**
	10883	11117	10796	84718	78943	82302	85863	85606	84150	88921	90395	93399	**E**
	29087	29106	30217	87805	85004	83998	88906	89147	84919	89185	94683	92125	**F**
	23244	22852	23127	93614	88904	88272	92290	90552	92113	74750	72862	81017	**G**
	14871	14203	14653	97973	94702	94714	95487	95805	95333	20915	20689	22765	**H**
480	-16	10	-5	200344	205484	197261	202472	208658	208100	209896	209572	218310	**A**
	2	-22	0	183537	175078	167947	191307	201893	195094	196949	209917	208964	**B**
	225277	216769	208975	21172	19306	19746	192839	202521	208502	202657	202868	213012	**C**
	225430	216044	210872	10117	10732	10140	189647	198270	204925	170991	168378	176220	**D**
	13229	14018	13057	207873	194188	200233	206268	206500	206049	210190	210981	219923	**E**
	18591	18382	19127	209709	205300	205321	207169	207677	208870	207798	213427	219694	**F**
	14486	14331	14539	217372	211459	211564	215825	220731	216539	195581	195981	213838	**G**
	9776	8963	9559	230468	229130	226572	228052	223191	229606	114793	117910	119776	**H**
1440	0	0	3	1651410	1633292	1647046	1988870	1706767	1895029	1840562	2050937	1932739	**A**
	9	4	0	1557478	1456558	1277273	1607782	1556552	1849912	1760435	1346302	1861715	**B**
	1507897	1676897	1643740	446452	298773	388878	1587818	1571426	1799788	1844412	2008963	2002161	**C**
	1499172	1559743	1400178	15925	18369	18439	1411896	1810493	1931219	927340	955342	1138645	**D**
	282170	270651	251227	745837	1259664	653219	1805331	1893644	1820494	2049147	1926643	2009505	**E**
	1279	1205	1350	1450310	1604210	1718930	1725087	1665730	1702305	1748047	1808178	1864252	**F**
	305	260	364	1164268	1414481	1431923	1452717	2003118	1577304	1359569	2113299	1228709	**G**
	52	52	29	1075319	1345676	1479537	1502026	1412696	1476473	304149	314587	372303	**H**

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Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die vorliegende Arbeit selbstständig und nur unter der Verwendung der angegebenen Literatur und Hilfsmittel angefertigt habe. Diese Arbeit wurde bisher keiner anderen Institution zur Erlangung eines akademischen Grades vorgelegt.

Sebastian Stark

Halle (Saale), 03.Juli 2015