Syntheses and Evaluation of Peptoids as Bioinert Coatings for Nanoparticles

CHRISTOFFER WEBER

Master thesis project

Department of Organic Chemistry, Faculty of Engineering (LTH), Lund University

June 2018

Company supervisor: Rikard Larsson

Principal supervisor & examiner: Peter Somfai

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NANOMEDICAL

Acknowledgments

First, I would like to thank my supervisor at Spago Nanomedical, Rikard Larsson, for taking the time to teach me the laboratory work needed to accomplish this thesis, for motivating me and staying positive during this whole process. I would also like to thank Oskar Axelsson for giving me the opportunity to do my thesis in Spago Nanomedical's facilities and his input on my work. I am very grateful for the access to laboratory, equipment, and chemicals I have been provided with there. Thank you to Atia Arif for helping me with mass spectrometry and to Petter Lauritzon for his help with NMR and purchasing of equipment and chemicals.

I am also thankful to all the other colleagues at Spago Nanomedical: Rodrigo Petoral, Sania Bäckström, Gabriel Cabrera, Olof Björnberg, Jonas Nehlin, Andreas Gram, Yi-Chi Liu, Louise Egeblad, Tove Sivik, Mats Hansen and Anders Olsson for welcoming me into a very nice workplace. I wish you all the best for the future.

I would like to thank my supervisor at Lund University and examiner Peter Somfai for his feedback on this report.

Finally, I cannot believe that five years of studies have come to an end. I thank my family and loved ones, who have always supported me, and my classmates that have made these years memorable. Thank you!

Lund, June 2018

Christoffer Weber

Abstract

Polyethylene glycol is commonly used as a coating material for nanoparticles but issues with immunogenicity and fouling, i.e., non-specific adsorption of microorganisms, proteins, and cell attachment have led to research pursuing alternative coating materials. Polymers of *N*-substituted glycine (NSG) or as they are also called, peptoids, have shown potential in biomedical applications due to proteolytic resistance, among other interesting properties. These peptidomimetic polymers are based on an *N*-substituted glycine backbone, carrying a side chain on the amino acids nitrogen instead of the α-carbons. The hydrogen bonding sites on the amine in peptides have thus been removed and this gives rise to peptoids bioinert properties and conformational differences to peptides.

In this thesis project, two synthetic routes have been performed and evaluated to achieve a better understanding of large-scale syntheses of peptoids as a bioinert coating for nanoparticles. The primary focus has been on a solution-phase synthesis where the monomer, *N*-(3,6,9-trioxadecyl)glycine, has been synthesized via an oxidation of a primary alcohol to aldehyde followed by reductive amination. A "submonomer" approach, where the peptoid is grown on a resin in an iterative process of nucleophilic displacements and acylations, has also been performed but with a 3-methoxypropyl side chain. Peptoids containing 6 repeating units have been successfully synthesized via the submonomer approach while only the monomer has been synthesized via the solution-phase strategy. The number of synthetic steps and purifications in the solution-phase synthesis shown in this work implies that a submonomer approach is a better alternative than a solution-phase synthesis of peptoids in an early stage of development of a new polymer coating.

Sammanfattning

Polyetylenglykol (PEG) används bland annat som bioinert ytbeläggning av nanopartiklar och mediciner på grund av dess biokompatibilitet, tillgänglighet samt ”anti-fouling”-egenskaper. Fouling innebär ospecifik adsorption av mikroorganismer, proteiner samt cellbindning på nanopartiklars eller proteiners yta. Studier har på senare år visat på immunologiska reaktioner mot PEG samt fouling vilket har lett till ökade forskningsansträngningar för att finna alternativ. Polymerer av *N*-substituerad glycin (NSG), eller som de även kallas, peptoider, har visat potential i biomedicinska tillämpningar. I motsats till peptider så är de motståndskraftiga mot proteolys. Detta är ett resultat av att peptoider är substituerade på aminosyrans kväve istället för på α-kolet som i peptider. De vätebindningar mellan väteatomen, som är bunden till peptidbindningens kväve och karboxylgruppens dubbelbundna syre, som är orsak till peptiders sekundära struktur, är därmed inte möjliga för peptoider.

I detta arbetet har peptoider för användning som bioinert ytbeläggning av nanopartiklar framställts genom fastfas-syntes samt att monomeren har framställts genom syntes i lösningsmedel. De båda tillvägagångssätten har utvärderats för att finna det bäst lämpade att framställa peptoider under en utvecklingsfas i större skala. Fokus har legat på den sistnämnda där monomeren, *N*-(3,6,9-trioxadecyl)glycin, har syntetiserats genom att oxidera en primär alkohol till aldehyd följt av en reduktiv aminering. Sidoreaktioner och uppreningssteg har resulterat i låga utbyten. Fastfas-syntesen har genomförts och utvärderats parallellt, där peptoider med 6 repeterande enheter har syntetiserats på ett resin genom en iterativ process av nukleofil attack med 3-metoxypropylamin följt av acylering med bromättiksyra. Detta arbete har visat att en fastfas-syntes av peptoider är att föredra framför en syntes i lösningsmedel under en utvecklingsfas av en ny polymer ytbeläggning.

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Abbreviations

Abs	Antibodies
AcOH	Acetic acid
Bn	Benzyl group
BOC_2O	Di-*tert*-butyl dicarbonate
CA	Contrast agent
CBZ	Carboxybenzyl group
CBZ-OSu	*N*-(benzyloxycarbonyloxy)succinimide
DCM	Dichloromethane
DIC	*N,N'*-diisopropylcarbodiimide
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMP	Dess-Martin Periodinane
DMF	*N,N*-Dimethylformamide
DNP	Dinitrophenylhydrazine
EtOAc	Ethyl acetate
FDA	Food & Drug Administration
h	Hour
HOMO	Highest occupied molecular orbital
HPLC	High-performance liquid chromatography
IBX	2-Iodoxybenzoic acid
LC-MS	Liquid chromatography-mass spectrometry
LUMO	Lowest unoccupied molecular orbital
mPEG	Methoxypoly(ethylene glycol)
MRI	Magnetic Resonance Imaging
MS	Mass spectrometry
NMR	Nuclear Magnetic Resonance
NSG	*N*-substituted glycine
PEG	Polyethylene glycol
ROP	Ring-opening polymerization
rt	Room temperature
SPPS	Solid-Phase Peptide Synthesis
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TIPS	Triisopropylsilane
TLC	Thin layer chromatography

1 Introduction

1.1 General Background

Methoxypoly(ethylene glycol)-coated (mPEG) nanoparticles with chelated manganese ions have shown potential as a contrast agent (CA) for magnetic resonance imaging (MRI). This novel material has been developed by the Swedish company Spago Nanomedical which has shown signal enhancement capabilities of many times greater values than competing CAs.[1] Most CAs today are based on gadolinium but findings in recent studies indicate deposition of gadolinium in the brain after the use of CA in MRI.[2] This is one of the driving forces to develop manganese-based CAs. Nanoparticles with an application in biological tissue or fluid environment need to be protected against the surroundings. PEGylation of nanomedical products is for this purpose widely used because of its ability to prevent non-specific adsorption of proteins onto the nanoparticles surface. However, it is not a flawless method due to autoxidation of PEG and the temperature dependence of solutions of PEG.[3] In addition, in the recent decade animal studies have shown immunogenetic limitations for PEGylated proteins and nanoparticles.[4]

As an alternative to mPEG, peptoids were investigated in this thesis. Peptoids are polymers of *N*-substituted glycines (NSG), cheap and biocompatible because of their resemblance to peptides. Whereas in a peptide a side chain is attached to the α-carbon, it is attached to the amide N^{α} in a peptoid. The hydrogen on the secondary amide is substituted with a side chain R. This will create a comb polymer structure with high resistance against proteases because the peptoid has a tertiary- instead of a secondary amide. The misalignment of the side chains and the carbonyl groups with the nucleophilic catalyst at the active sites on proteases causes the C-N peptide bond to be out of range and thus not susceptible to cleavage.[5]

The submonomer solid-phase synthesis approach introduced by Ron Zuckermann et al.[6] is one possible strategy for the synthesis of peptoids. Another possibility that was investigated is the synthesis of peptoids in solution. The latter is less mentioned in the literature, but from a commercial point of view it is not clear which approach is superior

when considering the scalability of peptoids synthesis. In this work, the two approaches were compared.

The polypeptoids need to be activated on the C-terminus. The activation allows for an allyl moiety to be added to the C-terminus followed by hydrosylilation. The silane moiety can then be anchored to the nanoparticle surface. It is important to achieve a good polymer surface coverage on the nanoparticles so that properties such as viscosity and water solubility are suitable for the application. The polymer coating also acts as a physical barrier between the nanoparticle and the environment. Fouling is a common phenomenon for surfaces where the accumulation of material from the environment on to the particle surface occurs. For this biological application, the mentioned fouling material would primarily include proteins, cells, and bacteria. Non-specific adsorption could alter the functionality of the nanoparticles and it is, therefore, crucial that the polymers are non-fouling. To minimize fouling, some polymer- and polymer coating properties such as steric hindrance effects, chain length, surface coverage, chain conformation and hydrophilicity needs to be optimized.[7]

Apart from the mentioned reasons for coating the nanoparticles, other issues are also addressed such as stabilization of the nanoparticles and prevention of aggregation. Without the coating, the body will produce an immune response against the nanoparticles. To summarize, the polymer coating on the nanoparticle surface is an essential part of this application both from a technical- and biological perspective.

1.2 Specific Aims

- Develop a synthesis route for the monomer.
- Develop a protocol for the synthesis of peptoids in solution.
- Synthesize peptoids via solid-phase synthesis.

See *Figure 1* for structures of the target compounds.

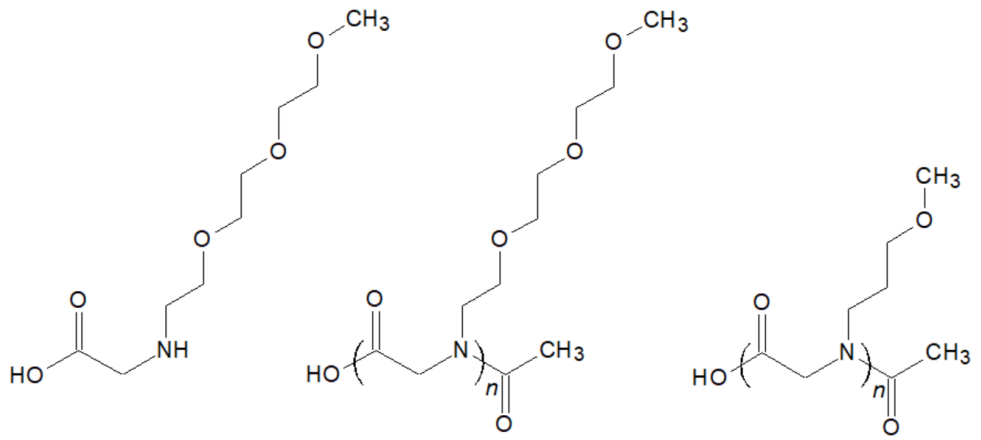


Figure 1. Molecular structure of the target monomer (left) and peptoid (middle) via solution-phase synthesis and the target peptoid (right) via solid-phase synthesis.

1.3 Bioinert Polymer Coatings

The nature of a physiological environment such as body fluid or surrounding tissue limits the type of polymers that can be used as a bioinert coating of nanomedical products. One of the major issues with introducing nanoparticles into the body is fouling: non-specific adsorption of proteins and cell attachement onto the nanoparticles.

Hydrophobic and hydration forces are believed to be one of the major reasons for protein adsorption onto surfaces. Depending on the contact angle θ between a surface and a water droplet, the surface is either hydrophobic ($\theta > 65°$), or hydrophilic ($\theta < 65°$). A hydrophobic surface does not adsorb proteins like a hydrophilic surface. Unfortunately, this is only one of the parameters that are considered to play a role in protein adsorption and theories are continually being updated or investigated.[8] Nevertheless, the polymer-water interfacial energy is expected to play an important role in the antifouling properties of the coating material. Hydrophobic coatings have a high

interfacial energy between the surface and water, leading to thermodynamically favorable interactions between the surface and surrounding organisms. For these surfaces, the focus is on fouling release instead of adhesion prevention. Poly(dimethylsiloxane) (PDMS) is an example of a hydrophobic elastomeric polymer (interfacial energy with water is about 52 mJ m^{-2}) with good fouling release properties due to its low modulus and low surface energy. Even though amphiphilic molecules such as proteins adhere to the hydrophobic surface to minimize the interfacial energy, the adhered organisms easily detach from the surface. Most attention has been given to hydrophilic polymer coatings such as PEG that has interfacial energy with water below 5 mJ m^{-2}. Fouling is not thermodynamically favorable in this case because the interfacial energy is already low enough and resistance to protein adsorption is therefore high (see *Figure 2*).[9] The antifouling properties of PEG have been attributed to steric repulsion of the PEG chains as macromolecules are approaching. Furthermore, the hydration barrier associated with PEG is also believed to play a part in the mechanism of PEG's antifouling properties.[10]

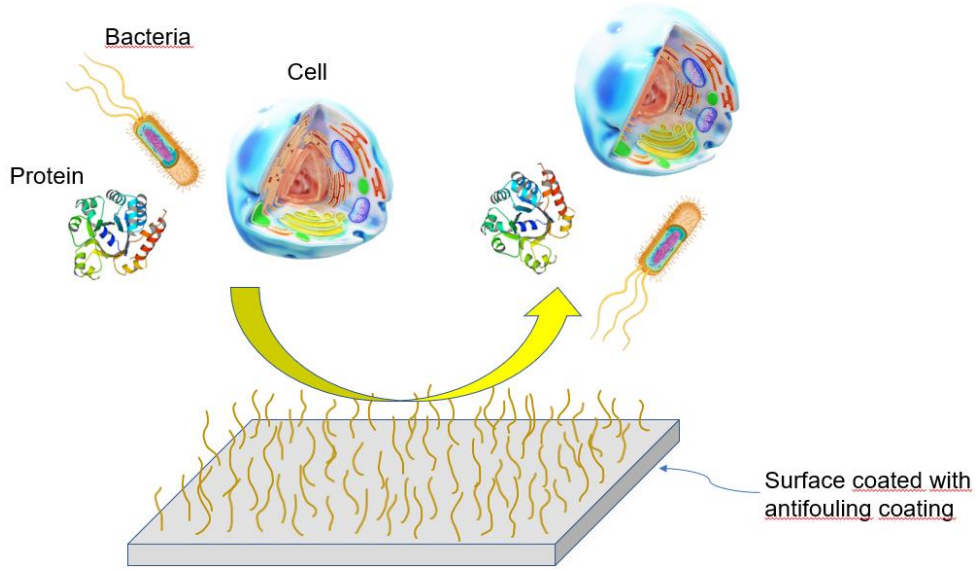


Figure 2. Grafted polymers (orange) anchored onto the underlying surface (grey). The coating provides a physical barrier to the surrounding proteins, cells and bacteria. The yellow arrow indicates the adsorption resistance of the grafted polymer coating.

1.3.1 Issues with Polyethylene Glycol

In 2016, Zhang et al. reported that at least 10 PEGylated drugs had been approved by the Food & Drug Administration (FDA) and >20 were in clinical trials at the time. Early immunogenicity and antigenicity studies had considered PEG as a bioinert material, but more recent studies have shown PEGylated proteins and PEG-modified nanoparticles stimulating the generation of anti-PEG Abs in animals.[4]

PEG is a polyether that is susceptible to autoxidation by O_2 or transition-metal ions. Oxidation of the terminal hydroxyl group to aldehydes by alcohol dehydrogenase and further to an acid by aldehyde dehydrogenase is another possibility.[11] These terminal groups are sensitive to cell attachment or to attack by molecules with amine groups such as proteins. To avoid oxidation of PEG one can use mPEG instead, where the hydroxyl end has been substituted with a methoxy group. However, this does not solve potential issues with PEG's upper- and lower critical solution temperature as well as the slow degradation and accumulation of PEG in the body.[12]

Abovementioned limitations of PEG have led to extensive research for alternative bioinert coating polymers (*Figure 3*).[13]

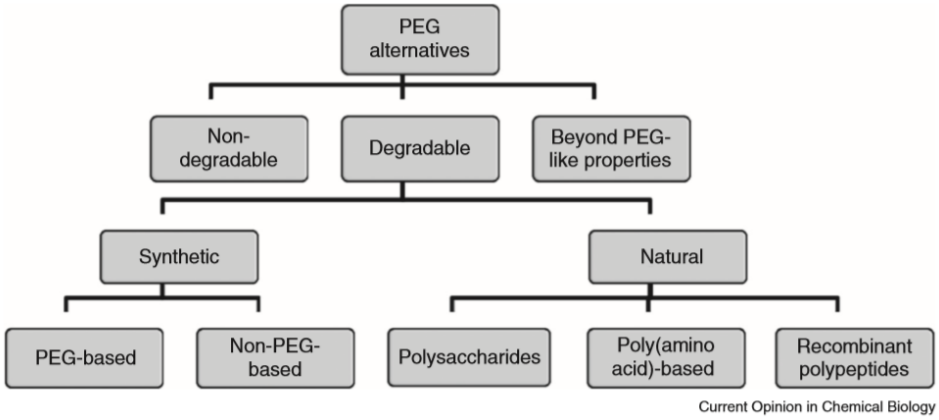


Figure 3. Different types of polymers that are reported in the literature to have been investigated as an alternative to PEG.[13]

1.4 Peptoids

Peptoids are polymers with a backbone of *N*-substituted glycine which have gained increased popularity in research as polymer coatings with anti-fouling properties. The similarities with peptides can be seen in *Figure 4*. Peptoids lack the two major contributors to the secondary structure of proteins: hydrogen bond donors in the backbone as well as C^{α} substituents. Instead, they have a side chain appended to the amide N^{α} in the glycine backbone. Therefore, it is more challenging to predict the secondary structure of peptoids compared to that of peptides.[14] On the other hand, it results in a higher flexibility of the main chain and less susceptibility to proteolytic degradation.[15]

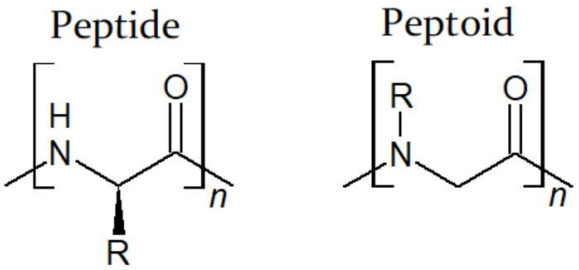


Figure 4. A comparison between a peptide and a peptoid with a side chain R.

Two fundamentally different approaches, solid-phase synthesis and solution-phase polymerization, are two possibilities for the synthesis of peptoids. The solid-phase synthesis is commonly referred to as a submonomer approach because the peptoid is not considered as a homopolymer of *N*-substituted glycine units, but a copolymer of acetate and amine units. Because the acetate and amine units are incorporated in different steps, these are referred to as submonomers. The submonomer approach enables the syntheses of highly monodisperse polymers with chain lengths of up to 50 monomers.[16] The solution polymerization can aid larger-scale syntheses of high molecular weight peptoids but with less chemical control resulting in polydispersity.[17]

For use as a bioinert coating, the peptoids have to be anchored to the nanoparticle surface. This can be done by capping the N-terminus and activating the C-terminus followed by nucleophilic attack on the carbonyl carbon by an allyl amine. After hydrosylilation of the unsaturated end, the peptoid is ready for grafting onto a silica layer on the nanoparticle.

1.5 Peptoid Side Chains

The coating needs to be hydrophilic to work in the biological environment it is intended for. The fact that there is a wide range of side chains that can be used for the peptoid is a great advantage. Properties such as water solubility and structure of the coating can with just the change of the peptoid's side chains be regulated to suit the application of the coating.

Regarding the water solubility, approximations could be a useful tool in predicting the possible outcome of different side chains influence on the coating solubility. The partition-coefficient (P) is a measure of the difference in solubility of a compound between two immiscible solvents (water and 1-octanol are common) and is often expressed as Log P. This gives a concentration ratio of the compound between two immiscible solvents as can be seen in *Figure* 5.

$$\log P = \log \frac{[\mathrm{X}_{oct}]}{[\mathrm{X}_{Water}]}$$

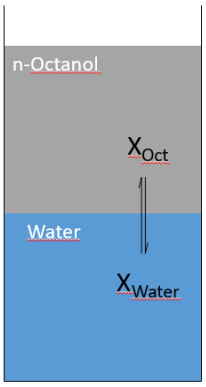


Figure 5. An illustration of the partitioning of compound X in two immiscible solvents at equilibrium. To the left is the equation for the partition-coefficient as a ratio between the concentration of the compound in the two phases.

The addition of hydrophobic groups such as methyl- or ethyl groups can make the compound more practical to work with due to decreased water solubility, however, the loss of oxygen in the side chain will decrease the number of hydrogen-bond accepting sites thus making it less suitable as a bioinert hydrophilic coating.

Short mPEG residue side chains have shown good anti-fouling properties. These are hydrophilic molecules with hydrogen-bond acceptors (no hydrogen-bond donors), and they have an overall neutral electrical charge.[18]

For measurement of the hydrophilicity of peptoids with varying side chains, one can use reverse-phase high-performance liquid chromatography (RP-HPLC) or UV spectroscopy to measure the distribution of the compound in the two-phase system to obtain Log P values.

1.6 Solution-Phase Synthesis

1.6.1 Protection of Carboxylic Group on Glycine

In peptide synthesis, protecting groups are used to avoid side reactions. The use of protecting groups makes the peptide synthesis into a reliable and predictable process that can be done with machines (automated peptide synthesizers). The zwitterionic nature of amino acids makes unprotected coupling inefficient. The same goes for amino acids in other reactions than peptide synthesis, such as reductive amination. Without the use of protecting groups, the reaction is likely to result in low yields due to side reactions.

Figure 6 shows a comparison of possible outcomes when unprotected- and protected glycine reacts with an aldehyde. Without the protecting group, the carboxylic acid of glycine can react with the aldehyde instead of the amino group. On the right side of *Figure 6*, the CO_2H has been protected with a benzyl group which removes the reactivity of the CO_2H.

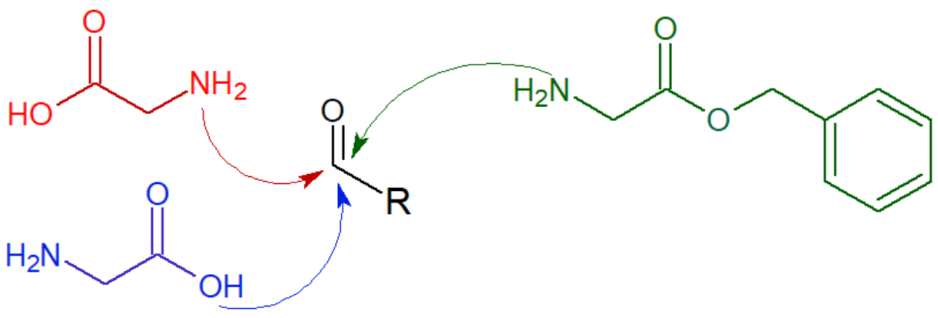


Figure 6. Nucleophilic attack on aldehyde with side chain R by unprotected glycine (left) and protected glycine benzyl ester (right).

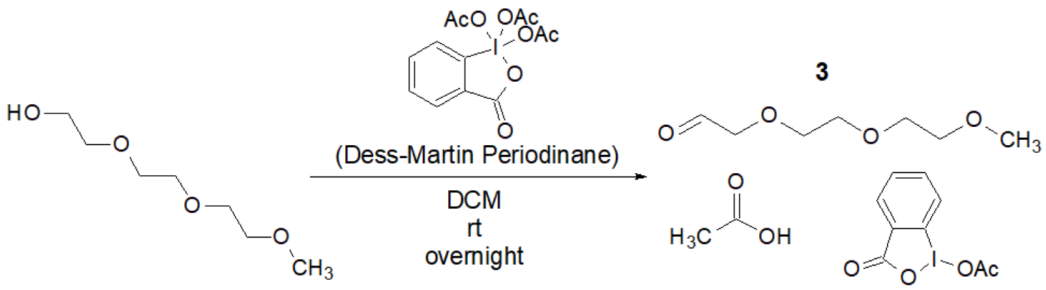
The choice of protecting group for the carboxylic acid comes down to the subsequent synthetic use of the protected glycine, which deprotection step is preferred, as well as

improving the handling of the compound. In comparison, a benzyl group would give a more lipophilic product than would a methyl group.

For deprotection by hydrogenolysis with Pd/C in H_2 atmosphere, benzyl ester is a good alternative. Other alternatives include methyl ester and t-butyl ester, but these are often deprotected by basic- and acidic hydrolysis, respectively. One should also consider the analytical methods that will be used. For gas chromatography, a more volatile molecule would be preferred. Analysis by NMR can be simplified if the protecting group does not interfere with the chemical shift of other parts of the molecule.[19]

1.6.2 Oxidation of a Primary Alcohol

1.6.2.1 Dess-Martin Oxidation



Scheme 1. Synthesis of the target aldehyde **3** via DMP oxidation of triethylene glycol monomethyl ether.

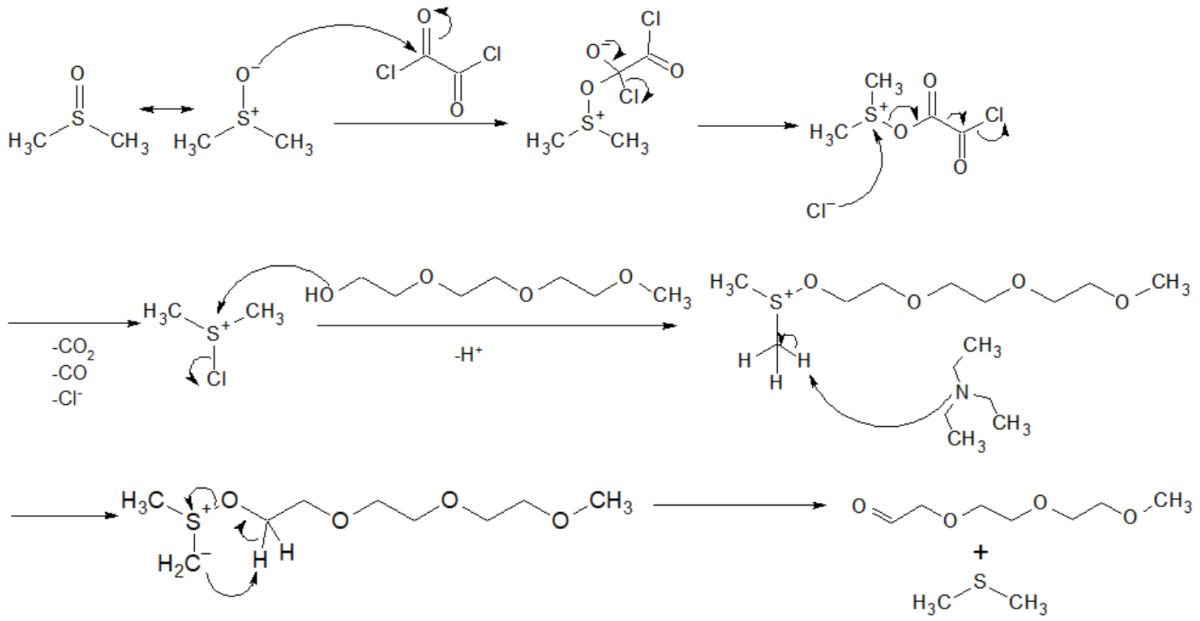
Dess-Martin periodinane is a hypervalent iodine oxidizing reagent used for oxidation of primary alcohols and secondary alcohols to aldehydes and ketones respectively. It has some advantages over other commonly used oxidation methods (chromium- or DMSO-based), such as mild reagent, simple work-up, and mild reaction conditions (room temperature).[20] On the other hand, it is a high molecular weight oxidizing agent. Atom economy is a concept in green chemistry philosophy where, ideally, the amount of starting material equals the amount of all desired products. The high molecular weight of DMP, and more importantly the high molecular weight of the iodo-compound byproduct (see *Scheme 1*), is a concern from this point of view. The high cost of DMP is of course also an issue for large-scale oxidation reactions.

1.6.2.2 Activated DMSO Oxidations

Over the last half-century, a number of DMSO activators have been proposed and used for the oxidation of either primary or secondary alcohols into aldehydes and ketones respectively. The most common of these is oxalyl chloride.

Dimethyl sulfoxide [sulfur(IV)] is the oxidizing agent in the Swern oxidation of primary or secondary alcohols (see *Scheme 2*). Oxalyl chloride is used as the activator of DMSO to form a reactive intermediate that will be attacked by the alcohol in the following step. The first step, the addition of the DMSO solution to the oxalyl chloride solution, is very exothermic and needs to be done at below -60 °C to avoid side reactions. The DMSO will react with the oxalyl chloride in a nucleophilic attack on the carbonyl carbon which ultimately leads to the entropically favored formation of the intermediate chloro(dimethyl)sulfonium chloride, CO, CO_2, and a chloride ion. This is followed by the addition of the alcohol (triethylene glycol monomethyl ether in this work) which reacts with the activated DMSO to form an alkoxysulfonium ion intermediate. The addition of a base such as triethylamine leads to a sulfur ylide intermediate that decomposes to the aldehyde under the release of dimethyl sulfide [sulfur(II)]. The reaction is carried out at low temperatures to avoid side reactions such as a Pummerer rearrangement which is an elimination reaction that is favored by high temperatures (> -60 °C).

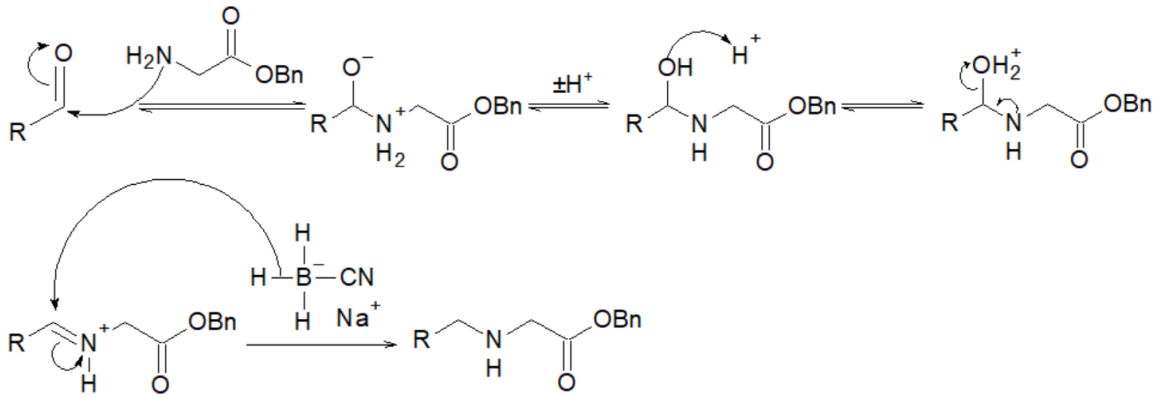
Even though this procedure involves unpractical experimental conditions for large-scale syntheses and the formation of highly toxic byproducts, it is one of the most popular oxidation methods due to oxalyl chloride being readily available but also the high yields that are in general possible with this reagent as a DMSO activator.[20]



Scheme 2. Mechanism of Swern oxidation for the synthesis of the target aldehyde.

1.6.3 Reductive Amination

Syntheses of secondary amines are often troubled by the overalkylation to tertiary amines or ammonium salts. Reductive amination is one of the few successful ways of making secondary amines by a reaction between an aldehyde and a primary amine. This forms an imine (or iminium ion) that is subsequently reduced by a reducing agent like $Na(CN)BH_3$ (see *Scheme 3*).



Scheme 3. Mechanism of a reductive amination using $Na(CN)BH_3$ and R is a side chain.

Direct reductive amination refers to when aldehyde, amine, and reducing agents are mixed without prior formation of the imine. The risk is that the reducing agent reduces

the aldehyde instead of the imine. In contrast, *indirect* or *stepwise* reductive amination allows the formation of imine before adding the reducing agent.

With the proper choice of reducing agent, one can avoid the possible reduction of aldehyde. The choice of reducing agent is important so that the aldehyde is not reduced to an alcohol before the imine is formed.

An overview of different reducing agent's strengths is shown in *Table 1*. The carbonyl groups are ordered in decreasing reactivity from left to right. With imines being highly reactive, the choice of reducing agent in reactions like the one shown in *Scheme 3* is both a matter of propensity of the reducing agent to reduce aldehydes (should be avoided), as well as toxicity.

Table 1. Summary of common reducing agents and their ability to reduce (green), reduce slowly (yellow), or not usually reduce (red) the carbonyl groups. Figure adapted from reference 21.

$Na(CN)BH_3$ is a mild reducing agent from a reactivity point of view. In comparison to $NaBH_4$, the electron-withdrawing nitrile group greatly stabilizes the anion thus decreasing its reducing strength. As far as toxicity goes, the product may be contaminated with residual cyanide. A further limitation is the release of deadly HCN during workup.[22]

Even though $Na(CN)BH_3$ is a good candidate as a mild reducing agent to successfully reduce imines while minimizing side-reactions, the toxicity that comes along with it have led to gained popularity for $Na(OAc)_3BH$ (sodium triacetoxyborohydride) as a

hydride source. The three acetoxy groups both add sterical hindrance and electronically stabilize the boron-hydrogen bond, making it a milder reducing agent than $NaBH_4$ and $Na(CN)BH_3$. A limitation of this reagent is its water-sensitivity. Water and methanol are thus not suitable solvents together with a triacetoxyborohydride.

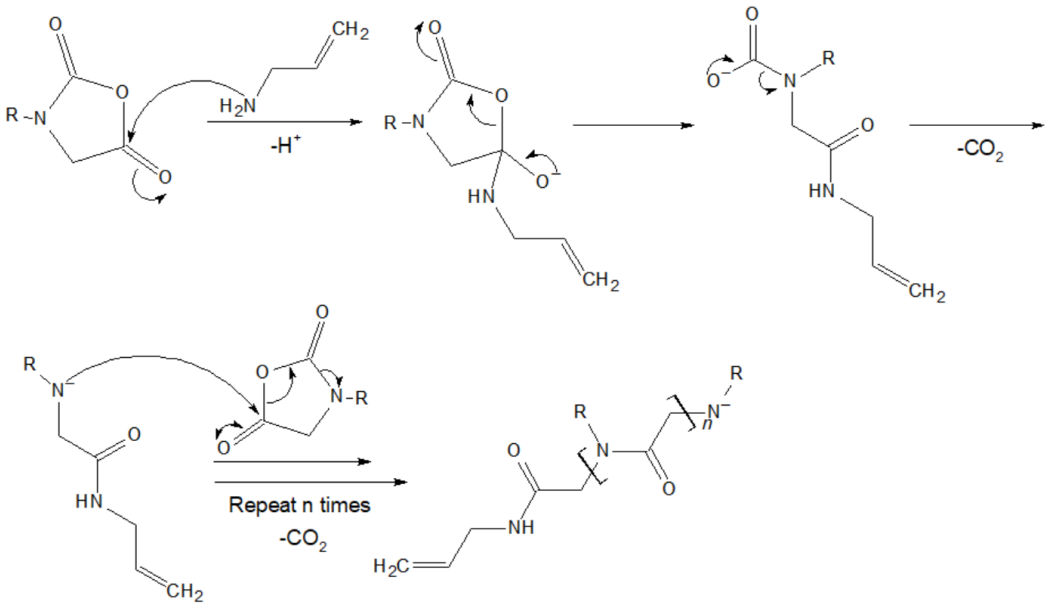
For a large-scale synthesis, catalytic hydrogenation with palladium, nickel or platinum should be considered as an economical alternative as a reducing agent in reductive amination.

1.6.4 Hydrogenolysis

Hydrogenolysis is a straightforward way of removing protective groups like benzyl esters. Uncatalyzed hydrogenolysis requires high temperatures, so usually, a metal catalyst like palladium, nickel or platinum is used. On a laboratory scale, it is common to use balloons filled with hydrogen gas that is either attached to a needle and inserted to the reaction flask via a rubber septum or a valve with ground glass joint adapter.

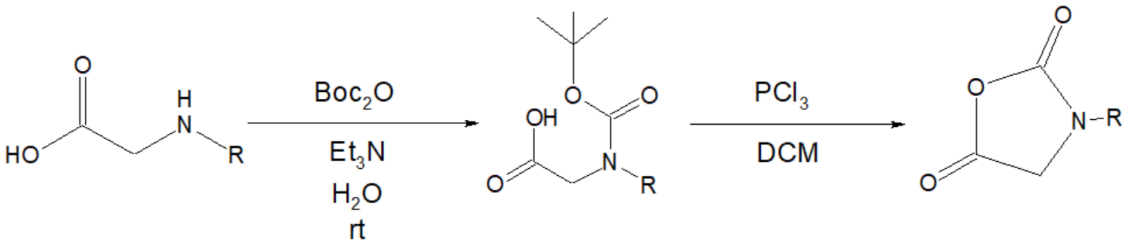
1.6.5 Polymerization

Among the alternatives for polymerization in this work, an amine-initiated ring-opening polymerization (ROP) was chosen as the best option (see *Scheme 4*).



Scheme 4. Allyl amine-initiated ROP where R represents a side chain.

The advantage of using an allyl amine initiator is that it facilitates for the subsequent coupling to the nanoparticles. The ring monomer can be synthesized as shown in *Scheme 5* which is a modified version of a procedure reported by Xuan et al.[18]

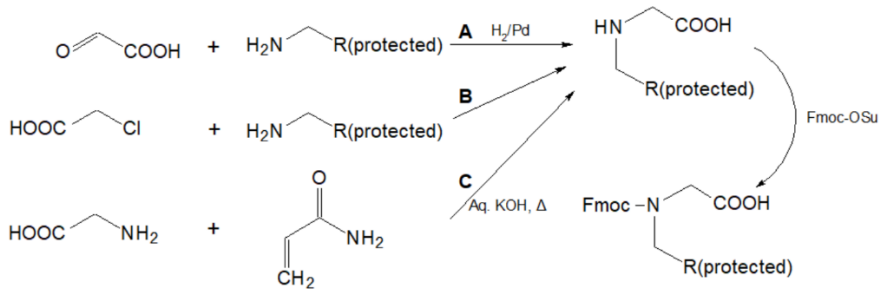


Scheme 5. Reaction scheme for the monomer ring formation. R = -$(CH_2CH_2O)_3CH_3$.

As an alternative to ROP, one could use coupling reagents to facilitate polymerization, but we had concerns over an increased risk of more complex side reactions for both the polymerization and post-polymerization modifications compared to the chosen synthesis. In addition, byproducts from coupling reagents can be challenging to remove and might be toxic.

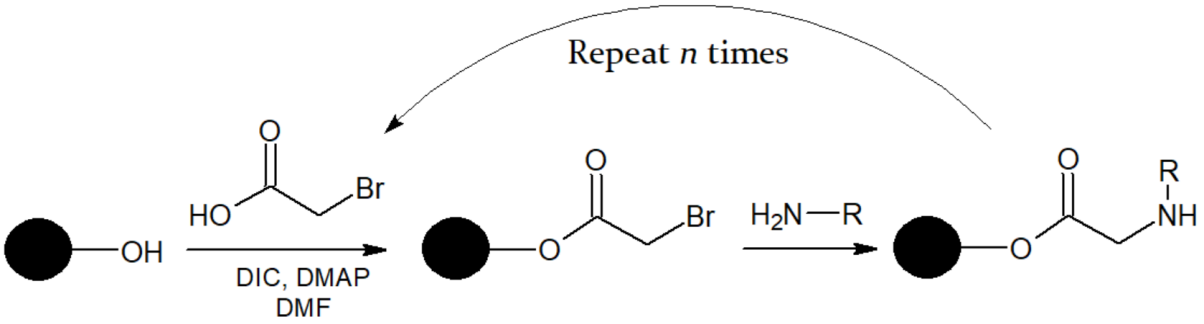
1.7 Submonomer Solid-Phase Synthesis

The submonomer solid-phase synthesis of peptoids was first developed by Zuckermann et al.[15] as a method to create a compound library of peptoids for drug discovery. The modular synthetic nature of peptoids was considered an advantage when a drug candidate had been discovered because it could be rapidly developed. In the original paper, the general procedure reported was similar to that of solid-phase peptide synthesis (SPPS) and reductive amination was concluded, out of three routes (see *Scheme 6*), to be the most useful synthetic method to synthesize *N*-substituted glycine monomers.[15] The carboxylate of the monomer was bound to a resin followed by deprotection of the Fmoc-group. This process was repeated to obtain oligomeric peptoids.



Scheme 6. General routes to the synthesis of peptoid monomers reported by Zuckerman et al.[15] A: Reductive amination. B: Nucleophilic displacement. C: Michael addition of glycine to acrylamide.

The original protocol was limited because of the protection and deprotection steps and not long after, the submonomer approach was reported.[6] In a broad sense, linker molecules are attached to the resin which in a first step is loaded with a haloacetic acid (preferably bromoacetic acid). If the peptoid side chain contains unprotected heteroatoms, chloroacetic acid (chlorine as a leaving group) can be used to avoid undesired side-reactions such as alkylation by the side chains functionalities. This is followed by an iterative process where a primary amine bearing the peptoid side chain adds to the growing peptoid chain by nucleophilic displacement. This concludes the addition of one repeating unit to the growing chain (*Scheme 7*). The acylation and nucleophilic displacement are repeated to reach the desired polymer length.



Scheme 7. Procedure for the synthesis of peptoids using the submonomer approach.

The last step is an acylation of the end amine moiety and cleavage of the peptoid from the resin.

1.8 Nuclear Magnetic Resonance Spectroscopy

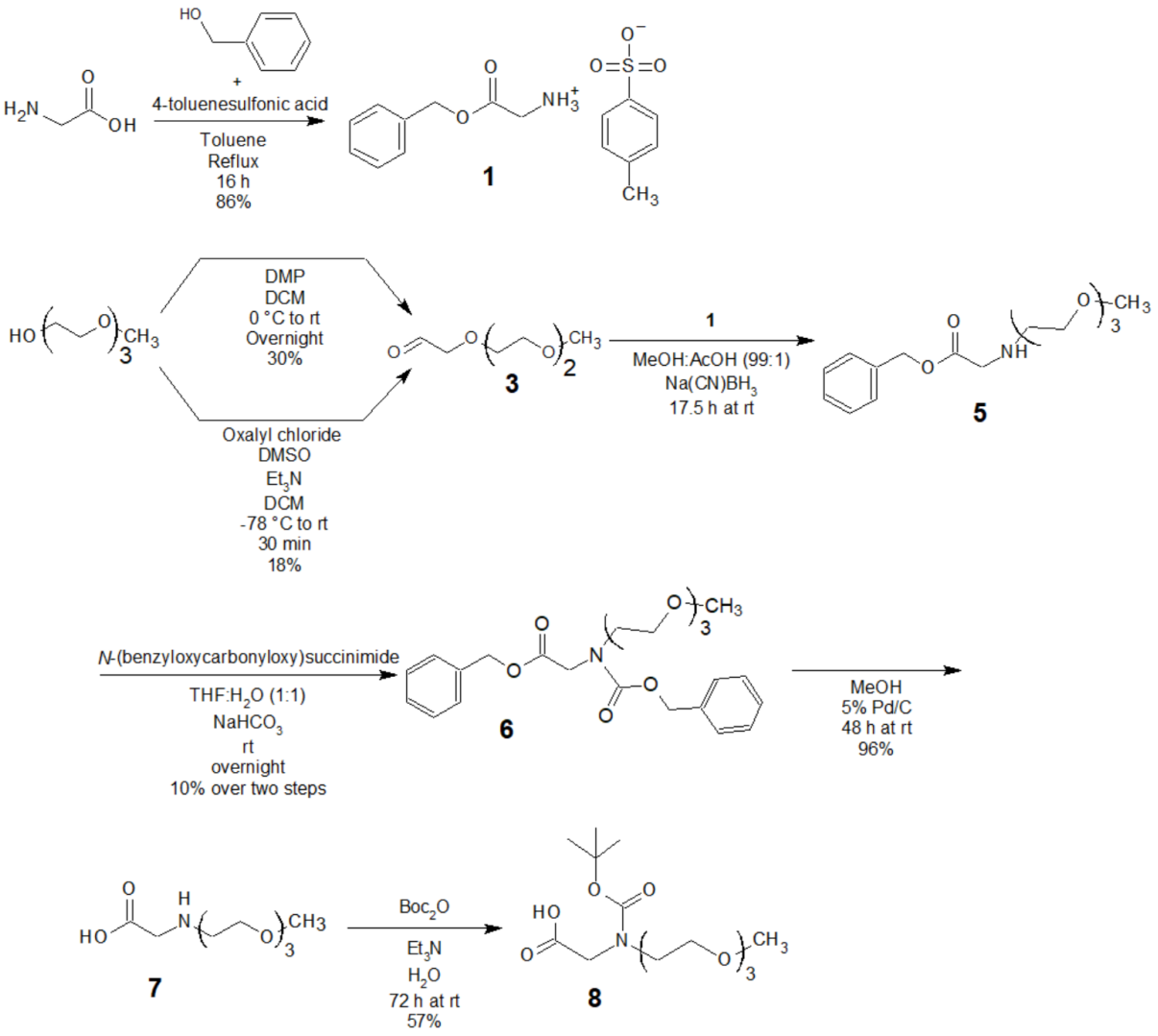
Nuclear magnetic resonance (NMR) spectroscopy is a very useful technique for structure analysis. Isotopes that possess nuclear spin, which are placed in a magnetic field will either have a magnetic spin aligned with (lower in energy) or against (higher in energy) the external field. ^{1}H and ^{13}C, which both have a nuclear spin ($\boldsymbol{I}$) of ½, possess two energy levels (given by $2\boldsymbol{I} + 1$). The energy difference between the two energy levels is in the range of radio-wave frequency. Irradiation of the nuclei with a short pulse of radiofrequency energy excites the nuclei from the more stable energy level to the less stable. The nuclei emit electromagnetic radiation upon relaxation, which is detected. The resulting NMR spectra contain detailed information about the compound's structure and chemical environment. The spectra consist of peaks at different chemical shifts (δ) where each nucleus or symmetry related nucleus is represented by a peak. The chemical shift represents the frequency at which the nucleus resonates, and variations in this are due to varying electron distribution around the nucleus. The electron distribution is dependent on the chemical environment of the nuclei and the local magnetic field experienced by the nucleus.

1.9 Liquid Chromatography-Mass Spectrometry

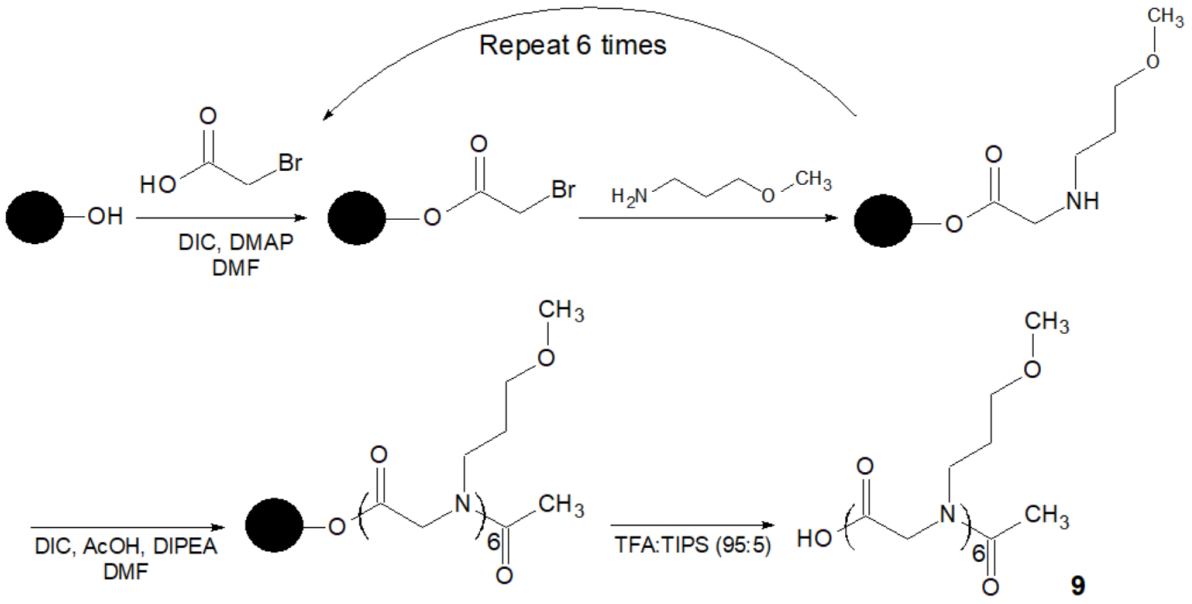
A powerful analytical technique in chemistry is mass spectrometry (MS) which is used to measure the mass of compounds. A common setup is to combine it with liquid chromatography (LC) which first separates the components in the sample based on their affinity for a stationary phase followed by the MS that measure the mass-to-charge ratio of the compounds eluting at different times from the LC column. A reverse-phase column is commonly used in LC which has a hydrophobic stationary phase. The sample is then dissolved in the mobile phase which is a polar solvent that is passed through the stationary phase under high pressure. In contrast, the MS is operated under high vacuum. An interface between the two is therefore necessary. One of the more common interfaces is the electrospray ionization interface which nebulizes the LC eluent into a charged aerosol by passing it through a metal capillary where a voltage has been applied to the tip of the capillary.

2 Results and Discussion

In this section, the results of the solution-phase syntheses (*Scheme 8*) and the solid-phase synthesis (*Scheme 9*) are discussed. Explanations and mechanisms of undesired side reactions, as well as recommendations for future work, are proposed.

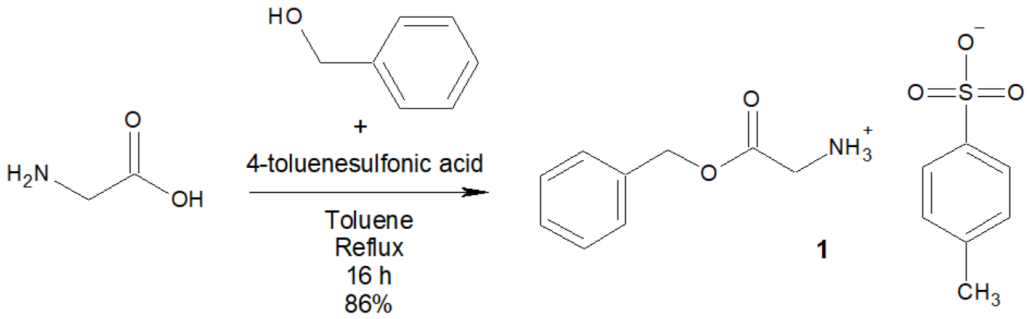


Scheme 8. Synthesis of monomer **7** and the first step of the monomer ring-closure **8**.



Scheme 9. Synthesis of **9** via a submonomer approach.

2.1 Syntheses of Benzyl-Protected Glycine



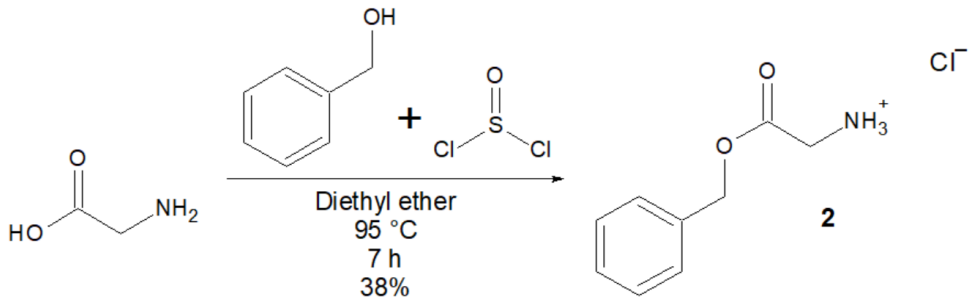
Scheme 10. Synthesis of **1** using glycine and benzyl alcohol.

As covered in section 1.6.1, protection of the CO_2H leaves the amino group as the reactive part of the molecule. The C-terminus of glycine was protected by reacting it with benzyl alcohol to form ester **1** (*Scheme 10*). It was reasoned that this protecting group was in line with our requirements because the aromatic protons on the benzyl ester would not interfere with NMR peaks of the mPEG side chain to be added to the molecule. Additionally, deprotection via hydrogenolysis removes both the benzyl group as well as the CBZ-group used for protection of the amine (see section 2.4).

Different reagents and solvents were investigated for the reaction. The first reaction was catalyzed with *p*-toluenesulfonate acid using toluene as solvent. The acid

protonates the basic amino group and the carbonyl oxygen on glycine followed by a nucleophilic attack on the carbonyl carbon by benzyl alcohol leading to the loss of water. Each step of the acid-catalyzed ester formation is reversible, so the reaction was therefore refluxed in a Dean-Stark trap for azeotropic removal of water to push the reaction towards the ester **1**. After completion, diethyl ether was added to precipitate glycine benzyl ester *p*-toluenesulfonate. The reaction was straightforward with good yields, and the product was used in the reductive amination without further purification.

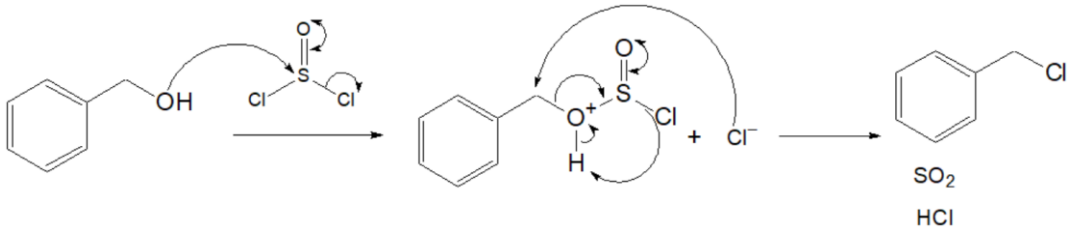
Although, an attempt at removing the *p*-toluenesulfonate counterion prior to the reductive amination was also done to investigate its impact on the reaction (see section 2.3.1 for details). The glycine benzyl ester *p*-toluenesulfonate was dissolved in a saturated solution of sodium carbonate (pH was measured to about 12) to deprotonate the amine followed by extraction with ether.



Scheme 11. Synthesis of **2** using thionyl chloride and benzyl alcohol.

The glycine benzyl ester **2** was also synthesized with thionyl chloride which is attacked by the sp^2 lone pair on the carbonyl oxygen in glycine. This forms an unstable intermediate which reacts with HCl after reprotonation to give a tetrahedral intermediate that collapses to an acyl chloride. This is an irreversible step because SO_2 and HCl are lost as gases from the reaction mixture. The electrophilic carbonyl carbon on the acyl chloride is attacked by the benzyl alcohol. The NMR spectrum showed chemical shifts that could be assigned to unreacted glycine (roughly 20-25% left after 5 h). New attempts were made where the reaction time was increased, and the number of benzyl alcohol equivalents were reduced from 50 to 15 (decrease of excess to avoid that thionyl chloride reacted with benzyl alcohol). None of these actions pushed the reaction

to completion and the synthesis of glycine benzyl ester as described at the beginning of this section was therefore preferred. An explanation for the outcome of the reaction is that the thionyl chloride was added to a suspension of glycine in benzyl alcohol. A possible side reaction is that the benzyl alcohol reacts with thionyl chloride to form benzyl chloride (*Scheme 12*).



Scheme 12. Suggested mechanism for benzyl chloride formation.

2.2 Syntheses of 3,6,9-Trioxadecanaldehyde

The aldehyde **3** was chosen as a suitable aldehyde to be incorporated as the alkyl side chain of the monomer based on the desired hydrophilic properties of the final polymer molecules. Another suggestion was that of 3-methoxypropanal (*Figure 7*) but discussion around the methyl groups effect on the hydrophilicity led to this aldehyde being discarded for the synthesis in solution. The low boiling point which makes it a quite difficult reagent to work with also played a role in this decision. The solid-phase synthesis of peptoids (section 2.6), on the other hand, does not have the same stringent requirements for volatility since the peptoid side chain is added as an amine in a nucleophilic displacement.

3

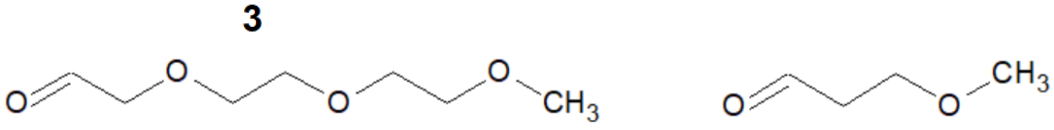
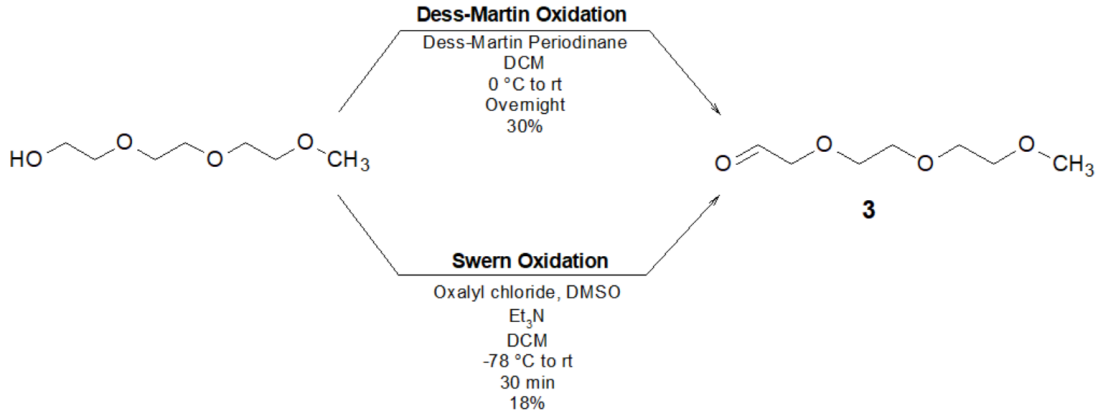


Figure 7. Aldehydes suggested as starting material for the reductive amination: 3,6,9-Trioxadecanaldehyde (left) and 3-methoxypropanal (right).

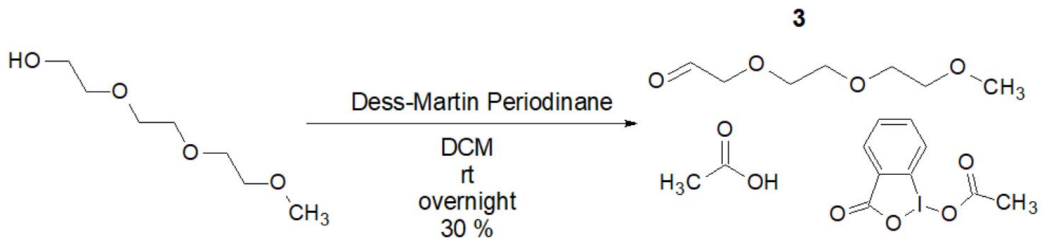


Scheme 13. Synthetic routes for the oxidation of triethylene glycol monomethyl ether to 3,6,9-Trioxadecanaldehyde **3**.

The aldehyde **3** was synthesized via oxidation of triethylene glycol monomethyl ether by the two different oxidations shown in *Scheme 13*.

2.2.1 Dess-Martin Oxidation

Using mild reagents such as Dess-Martin Periodinane (DMP) was preferred over more toxic options such as chromium-based oxidizing agents. Additionally, DMP oxidation is a very practical reaction that is run at rt after addition of the alcohol to a solution of DMP in DCM (*Scheme 14*). The alcohol was added at 0 °C because the first step in the reaction is fast and exothermic.



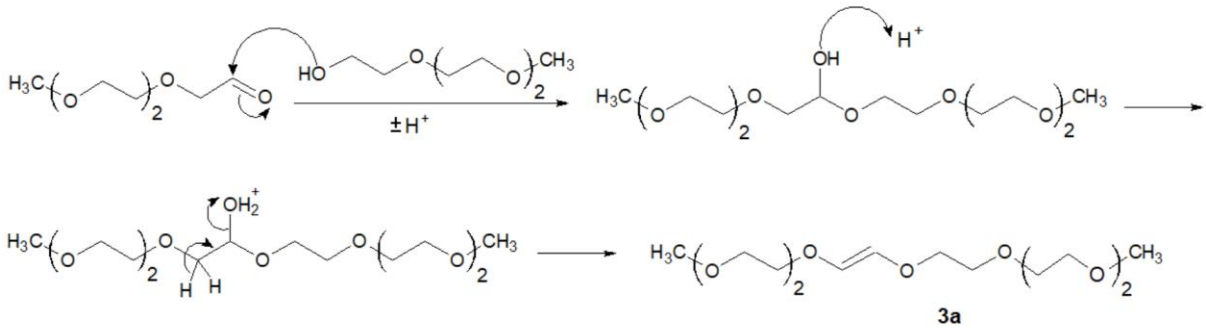
Scheme 14. Oxidation of triethylene glycol monomethyl ether using DMP.

Two different experiments were performed, using the same protocol but with slight changes in the workup. In the first experiment, the reaction mixture had to be filtered three times because of solid material passing through the filter. This probably decreased the yield. After the removal of solvent, the crude product contained a mixture of clear

liquid and white solid. It was suggested that the mixture contained periodinane byproducts and heptane was added in an attempt of dissolving the aldehyde. The aldehyde was obtained in 19% yield.

The second experiment was done in a similar fashion although cyclohexane was used instead of heptane and it was added prior to the filtration. DCM was removed *in vacuo* leaving the crude product dissolved in cyclohexane and the aldehyde could be isolated in 30% yield.

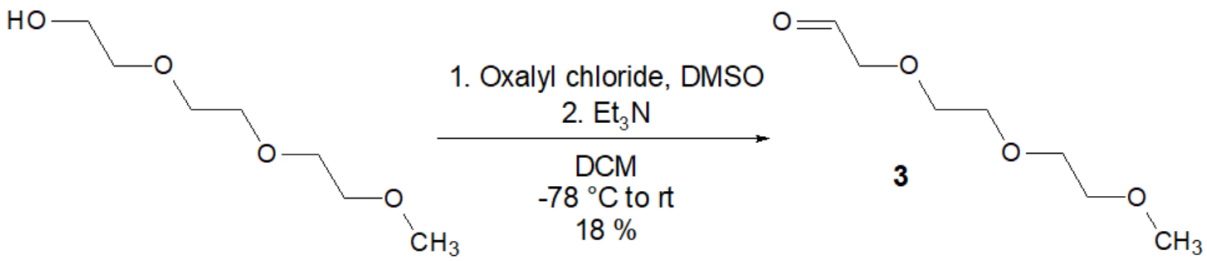
LC-MS of the first attempt showed both the desired product and heavier side products, one of which were assigned to be the alcohol dimer **3a** (*Scheme 15*). Periodinane byproducts and derivates are expected to be some of the heavier constituents of the mixture, but specific structures of these could not convincingly be assigned to the MS data.



Scheme 15. Suggested mechanism for the formation of the alcohol dimer **3a**.

The main drawback we considered for DMP-oxidation was the high molecular weight of DMP (424.14 g/mole). This requires quite high mass of DMP being used and taking its high cost into account, it is less likely to be the reagent of choice for large-scale synthesis. DMP byproducts were present in high amounts based on LC-MS data and, of course, such high molecular weight waste is preferably avoided.

2.2.2 Swern Oxidation



Scheme 16. Swern oxidation of triethylene glycol monomethyl ether to give **3**.

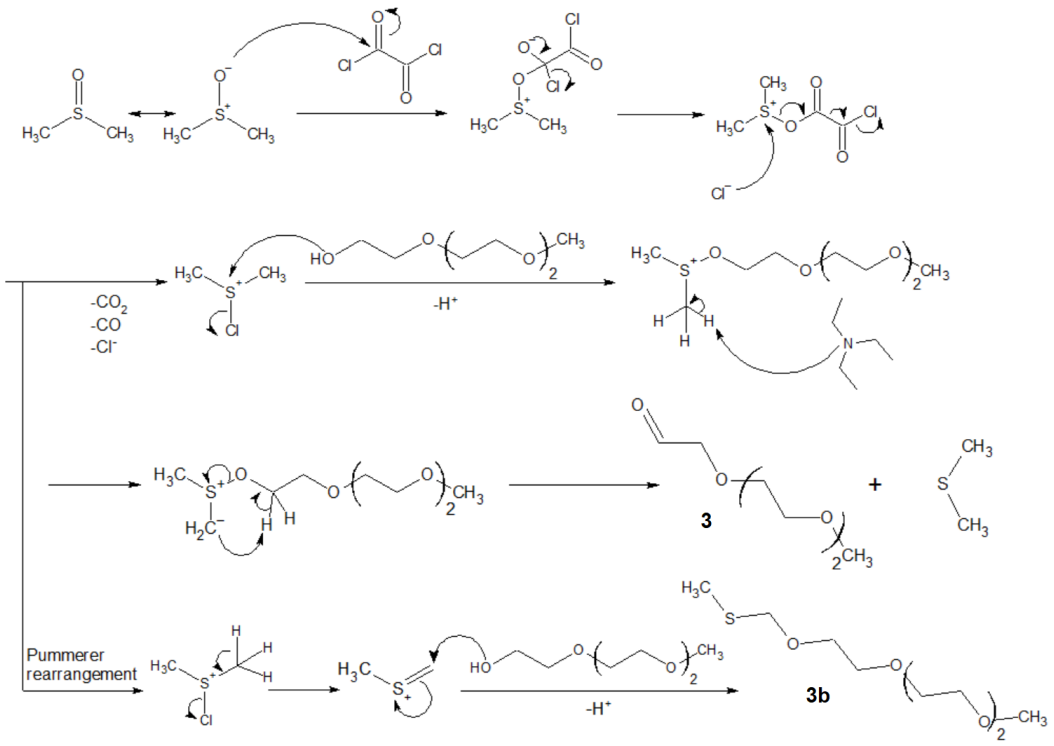
Parallel to the DMP oxidation, a Swern oxidation was performed (*Scheme 16*). The yield obtained for the aldehyde **3** is in general low according to the literature, and we obtained even lower than the 56% obtained by Da Ros et al. (see section 5.1) for the Swern oxidation. The workup procedure was difficult because of the high water solubility of the aldehyde (mPEG chain with n = 3) leading to low isolated yields. In addition, compared to the original protocol by Da Ros et al., we did the reaction on a larger scale and it was clear that the low temperatures required became an issue in this case.

A standard workup procedure, such as separation of aqueous and organic layers followed by extraction of the aqueous layer with an organic solvent, is often performed after quenching the reaction. An excess of base (4.8 equivalents) is typically used in the reaction. Two different workup procedures were examined after a standard workup did not suffice. Initially, a 0.1 M HCl solution saturated with NaCl was added to protonate excess triethylamine and the aqueous phase was extracted with EtOAc. It became evident that only small amounts of aldehyde entered the organic phase in each extraction.

The problem was to some extent solved by increasing the polarity of both the aqueous phase and the extract. Calcium chloride was added to the aqueous phase and further extractions with EtOAc did not lead to an improvement. Changing the extract to acetonitrile and adding calcium chloride gave a better result with more product being extracted. This “salting-out” method is used for polar solvents to achieve a two-phase system.[23]

NMR and LC-MS of the product showed not only the desired aldehyde but also typical side-products of the Swern oxidation. Issues with thioacetal formation can arise

in the Swern oxidation if the temperature is not kept below -60 °C according to the literature. The intermediate chloro(dimethyl)sulfonium chloride has a good leaving group (Cl^- with a pK_a of -7 for the conjugate acid). Temperatures above -60 °C can, therefore, lead to elimination of a chloride ion with the formation of a double bond to sulfur that still has a positive charge (Pummerer rearrangement, see *Scheme 17*). The temperature was controlled during the reaction and it did not rise above -66 °C, but thioacetal formation did still occur. This is most likely due to variations in the temperature throughout the mixture because of cooling difficulties in a large-scale synthesis.



Scheme 17. Mechanism of the Swern oxidation to obtain the aldehyde **3** and Pummerer rearrangement leading to the thioacetal side product **3b**.

One of the two free electron pairs in a non-bonding sp^3 orbital on the alcohol (HOMO) attack the electrophilic sp^2 hybridized carbon by donating electron density into the empty π^* orbital in the sulfur-carbon double bond (LUMO) thus forming a thioacetal side product (*Figure 8*).

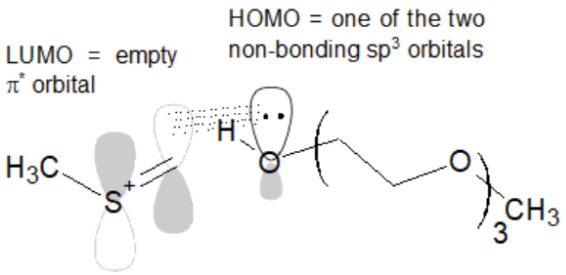


Figure 8. HOMO of the nucleophilic alcohol interacts with LUMO of the charged sulfur compound. Only one of the two free electron pairs on the alcohol is shown for convenience.

For a large-scale production, the low reaction temperatures necessary for the Swern oxidation becomes unpractical. The toxic volatile byproducts (CO and dimethyl sulfide) is another argument against a large-scale production with this method.

2.3 Reductive Amination

2.3.1 Attempts with *p*-Anisaldehyde

Initial experiments were done with readily available starting materials to get an appreciation of the necessary reducing strength of the reducing agents. Reductive amination with both glycine and glycine benzyl ester *p*-toluenesulfonate were done and different solvents were also investigated, such as DCM, 1,2-dichloroethane, methanol:acetic acid (99:1).

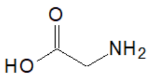
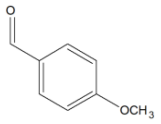
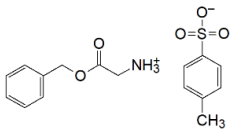
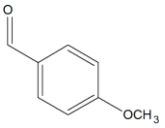
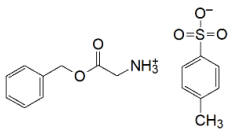
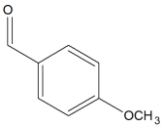
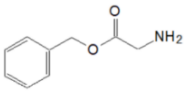
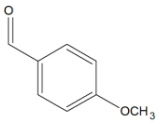
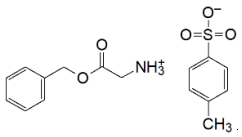
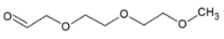
In all initial cases, *p*-anisaldehyde was used for the sake of simplicity to test the reaction. It was reasoned to be easier to follow the reaction with a UV-visible reagent than the aldehyde **3** to be used in the peptoid monomer synthesis and was, therefore, a good starting material for determining which of the reductive amination protocols that was more likely to succeed.

The counterion, *p*-toluenesulfonate, to the glycine benzyl ester was not believed to pose a problem in the reaction. Mass spectrometry showed low relative intensities of the product for all initial reactions with the counterion, so attempts, where the counterion was removed to see if it had an impact on the reaction, were also performed (see entry **4** in *Table 2*). This led entirely to formation of dialkylated product compared to a 30:70 mixture of mono- and dialkylated product when the amine was added as the

glycine benzyl ester salt. The experiment with the free amine was done with the addition of AcOH to increase the reactivity of the aldehyde. In any case, indications that it would be advantageous to remove the counterion were not observed so this strategy was abandoned.

Many studies in the literature endorse the use of sodium triacetoxyborohydride as a borohydride source. This was tried initially and would have been preferred due to toxic byproducts of sodium cyanoborohydride. Low yields, even though it was unclear if this was due to the choice of reducing agent, led to $Na(CN)BH_3$ being used instead to increase the likelihood of all imines to be reduced.

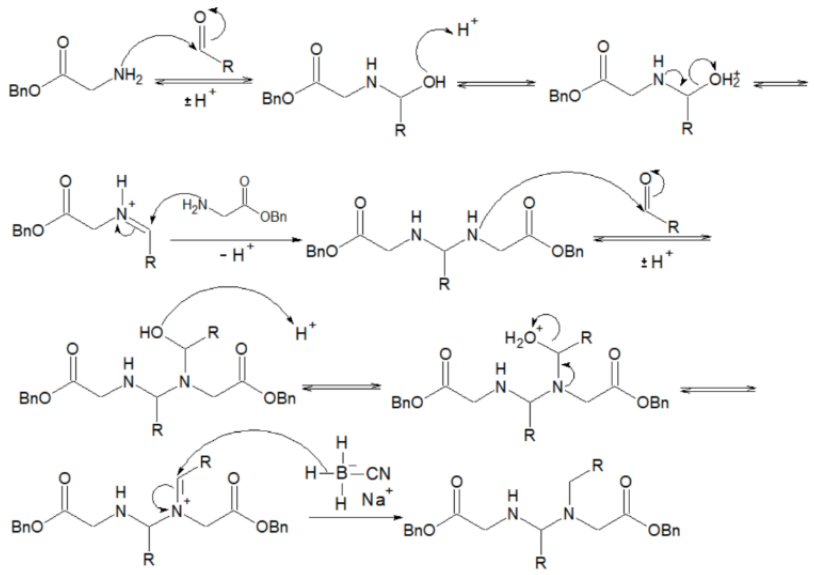
Table 2. Summary of the performed reductive aminations. *The ratio obtained between monoalkylated, dialkylated and aminal formation.

Entry	Amine	Solvent	Catalyst	Hydride source	Aldehyde/ketone	Ratio*
1		DCM	Et_3N	$Na(OAc)_3BH$		0:100:0
2		DCM	Et_3N	$Na(OAc)_3BH$		10:90:0
3		1,2-dichloroethane	-	$Na(OAc)_3BH$		30:70:0
4		1,2-dichloroethane	AcOH	$Na(OAc)_3BH$		0:100:0
5		Methanol:AcOH (99:1)	AcOH	$Na(CN)BH_3$		42:23:35

The reaction conditions used are presented in *Table 2*. Entry **1** led almost exclusively to the formation of the dialkylated product. The basic solution even led to the carboxylic acid being alkylated. The relative intensities of the products in the MS were very low which led us to believe that the efficiency of reductive amination with unprotected glycine is inferior. Mass spectra of entry **2** showed a 10:90 mixture of mono- and

dialkylated product. In both entry **1** and **2**, Et_3N was added to neutralize the primary amine. Changing the solvent from DCM to 1,2-dichloroethane (entry **3**), and without addition of Et_3N, improved the result to a 30:70 mixture. When AcOH was added to the same reaction (entry **4**), only dialkylated amine was observed in MS ($C_{25}H_{27}NO_4$ $[M+H]^+$: 406.6). Addition of acid protonates the carbonyl oxygen to give it a positive charge which increases the electrophilicity of the carbonyl group. The rate of imine formation is therefore increased.

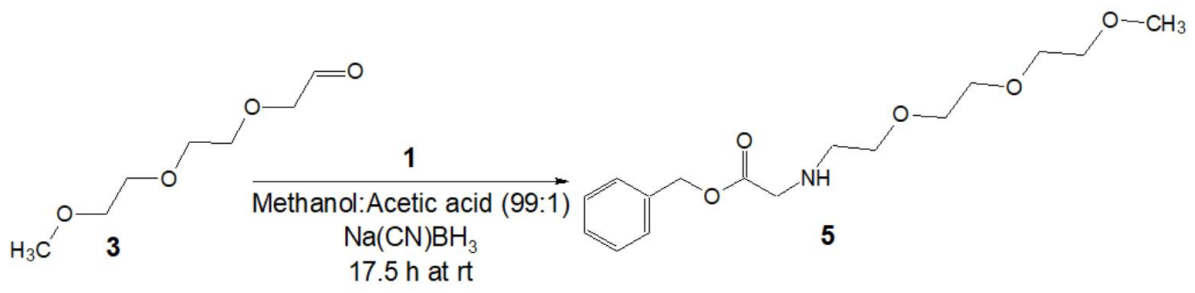
Even higher molecular weight side products than the dialkylated product were observed in MS. Aminal formation (suggested mechanism in *Scheme 18*), is a possible side reaction and the product corresponds well to the heavy mass in the MS spectra. Aminals are known to be unstable moieties so it was surprising that it did not hydrolyze in the acidic reaction mixture. Most likely, some aminal did hydrolyze but MS data suggest that a significant amount (up to 25%) survived decomposition. Entry **5** was performed with methanol and 1% AcOH as the solvent as previously reported by Da Ros et al.[24] Stronger reducing agents than $Na(OAc)_3BH$ such as $Na(CN)BH_3$ was considered a better choice because it might reduce the formed imine faster. The result was a 41:35:24 mixture of monoalkylated, dialkylated and aminal product in direct reductive amination. Using a weaker reducing agent could have been beneficial in the case of direct reductive amination to allow imine formation. Instead, entry **5** was done as an indirect reductive amination which did decrease dialkylation but increased aminal formation (42:23:35). In this case, $Na(CN)BH_3$ was added after 4.5 h. Imine formation was clearly successful, but the time should be optimized to minimize aminal formation.



Scheme 18. Suggested mechanism of aminal formation. R = -$CH_2OCH_2CH_2OCH_2CH_2OCH_3$.

In all of the reductive aminations, 1-11 equivalents of aldehyde were used. In hindsight, a direct reductive amination (to avoid aminal formation) with an excess of amine could have solved the problem since it would then be more likely that aldehyde would collide with a primary- rather than a secondary amine. It should be noted that the quantification with MS is not completely reliable due to variations in the response factor of different compounds. In section 2.3.2, the choice of reaction conditions and another suggestion on how to solve the challenge with overalkylation is described.

2.3.2 Synthesis of *N*-(3,6,9-Trioxadecyl)glycine Benzyl Ester



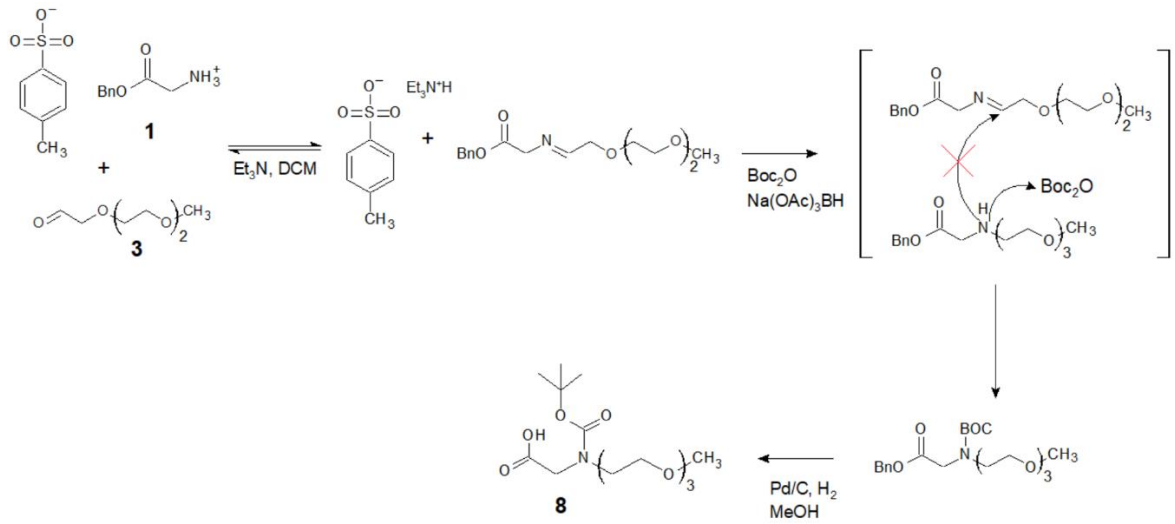
Scheme 19. Reductive amination to obtain the protected monomer. The yield was not calculated due to the product being an inseparable mixture of mono- and dialkylated monomers.

Aldehyde **3** and amine **1** were used in a reductive amination using sodium cyanoborohydride as the reducing agent (*Scheme 19*). Direct reductive amination led unsurprisingly to the formation of *N,N*-disubstituted glycine monomer. The desired product, a monoalkylated *N*-substituted glycine benzyl ester **5**, is a secondary amine that has an increased nucleophilicity compared to the starting primary amine. The alkyl chain on the secondary amine donates electron density to the nitrogen rendering it more reactive. One could argue that the sterical hindrance on the amine due to the alkyl side chain should offset the increased reactivity, but the tetrahedral bond angles of the amine keeps the lone pair on distance from the side chain and available for another nucleophilic attack on excess aldehyde. Any residual aldehyde is therefore likely to react with the monoalkylated product leading to dialkylation. Inseparable mixtures of up to 54% monoalkylated and 46% dialkylated product were noted in MS spectra.

Indirect reductive amination, as described in section 1.6.3 was used to solve the problem where the formation of the Schiff base was allowed prior to the addition of the reducing agent. Delays of 30 minutes and 4.5 h before addition of reducing agent were tried, and the latter showed less dialkylation but more aminal formation.

All protons of the product **5** were assigned in the NMR spectrum (see Appendix). Two peaks at 2.67 and 2.78 ppm, respectively, with an integration sum of 2H were not credibly assigned but might be associated with **5**.

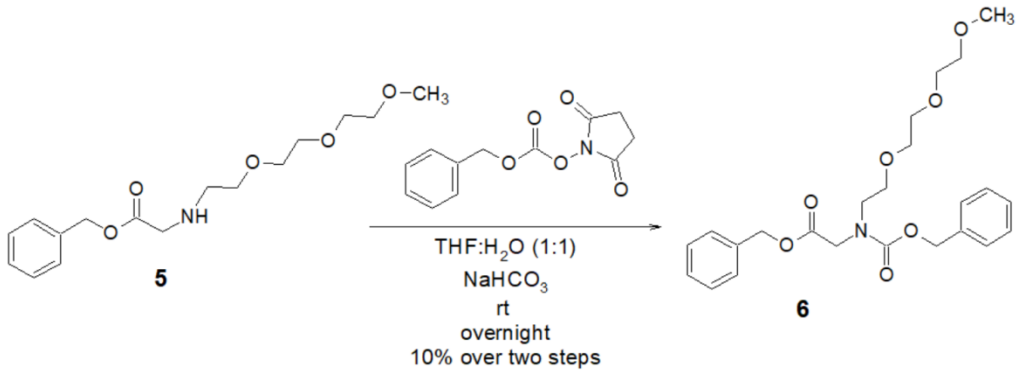
The experiments have shown that imine formation was not the issue, on the contrary it is the rate-determining step and AcOH could, therefore, have been omitted or even changed to Et_3N. An unverified way to avoid overalkylation is to add an electrophile such as Boc_2O together with the borohydride in an indirect reductive amination. The secondary amine formed upon imine reduction would attack the BOC anhydride instead of proceeding with overalkylation (*Scheme 20*).[25] Attack on the BOC anhydride by the primary amine is of course also likely to occur, but the secondary amine will be BOC-protected to a larger extent because it is more nucleophilic. If successful, this would reduce the synthetic route presented in *Scheme 8* with one step and potentially increase yields considerably.



Scheme 20. Mechanism of suggested solution to overalkylation issues in the reductive amination and subsequent deprotection to obtain **8** with one less synthetic step than presented in this report.

Impurities from the starting material (aldehyde) could be isolated with flash chromatography and analysis with MS and ^{1}H NMR revealed the thioacetal **3b** (*Scheme 17*). This can be explained as a side-product in the Swern oxidation if the temperature is not kept low enough as described in section 2.2.2.

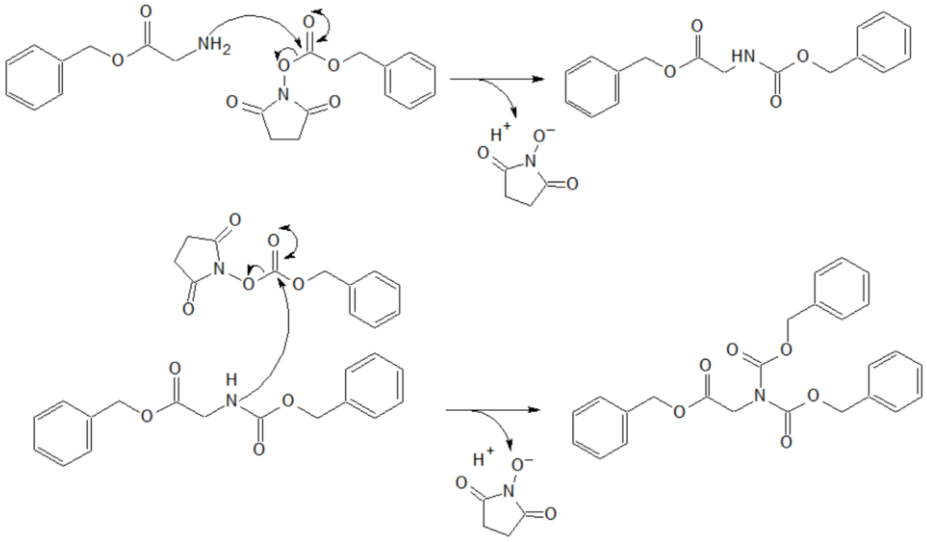
2.4 Synthesis of CBZ-Protected Monomer and Deprotection



Scheme 21. CBZ-protection of the secondary amine to facilitate separation from dialkylated **6**.

Due to the reductive amination resulting in an inseparable mixture of mono- and dialkylated product, an attempt of a protection reaction using *N*-(benzyloxycarbonyloxy)succinimide (CBZ-OSu) was performed (*Scheme 21*) to enable

separation of the mixtures constituents. The CBZ-protected monoalkylated compound could be isolated via flash chromatography. The first attempts were made with an excess of 1.18 equivalents of the succinimide but this was observed via NMR analysis to be insufficient. Because the monoalkylated product from the reductive amination continues to consume more aldehyde to form the dialkylated product and aminal as discussed in section 2.3.2, unreacted glycine benzyl ester will also be one of the constituents of the mixture. The glycine can react two times with the succinimide (see *Scheme 22*), although compounds like that are likely to be very short lived during workup. The peaks in the MS were too small to confirm the occurrence of this side product after workup. The equivalents of succinimide were still increased to 3.5 to give a 10% yield from the aldehyde.

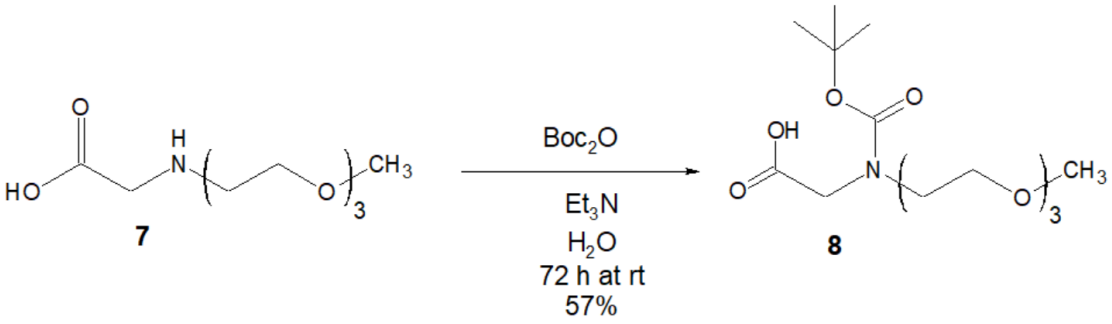


Scheme 22. Suggested mechanism for the CBZ dialkylation of glycine benzyl ester.

NMR spectra of the purified product **6** showed peaks at similar chemical shifts and integration values as reported in the literature.[24] Chemical shifts at δ 4.13 and 4.09 ppm were assigned to the carbonyl alfa position. There is a high rotational barrier between the nitrogen and the adjacent carbonyl group due to resonance between the nitrogen's lone pair of electrons and the carbonyl group. The slow rotation at room temperature, therefore, leads to the protons adjacent to the nitrogen being magnetically different. The peaks could be assigned to the alfa-protons convincingly, so no further investigations were made. For further study, one could do a 2D NMR experiment or variable-temperature NMR to confirm the peak assignment.

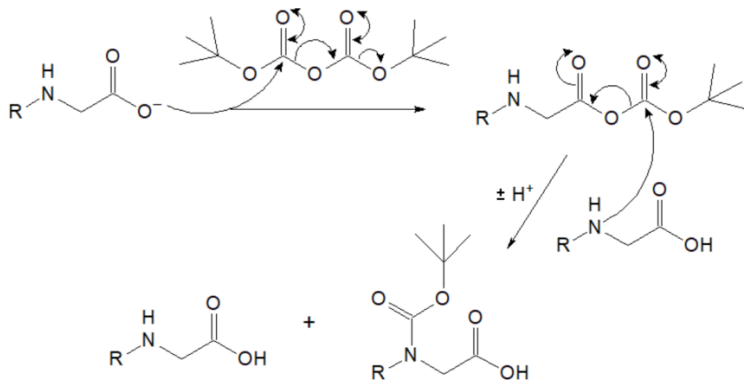
Deprotection of the monomer in one step to remove both the benzyl ester and the CBZ-group was done via catalytic hydrogenolysis using 5% Pd/C in H_2 atmosphere. There was no access to hydrogen tubes, Parr shaker or similar in the laboratory. Hydrogen gas was obtained by reacting granular zinc in water and carefully adding concentrated HCl. The reaction worked well, and the desired monomer could be obtained in 96% yield.

2.5 Synthesis of BOC-Protected Monomer



Scheme 23. BOC-protection of the amine **8**.

The first plan was to polymerize the *N*-substituted glycine with coupling reagents. The removal of byproducts from coupling reagents is a known challenge[26], therefore, this idea was set aside. The chosen strategy was instead to ring-close the monomer followed by a ring-opening polymerization. The first step of the ring-closure was done by protecting the amine with BOC (*Scheme 23*). Initially, 2.56 equivalents of BOC_2O were used but up to one equivalent could have been consumed by deprotonated carboxylic acid on the monomer. Even though the BOC-protected carboxylic acid can still function as a BOC reagent for protection of the amine (see *Scheme 24*), additional Et_3N and di-*tert*-butyl dicarbonate was added after 48 h. The final step would have been to allow the BOC-protected amine to react with phosphorous trichloride to achieve a ring-closure but this was not achieved due to limited time of the thesis work.



Scheme 24. Mechanism of BOC-protected side product functioning as BOC reagent for the starting material.

2.6 Submonomer Approach

The iterative process of synthesizing peptoids via the submonomer approach is one of its advantages. Apart from the washing steps, there is no purification during the synthesis. Protection steps, as in the solution-phase synthesis, are also not necessary.

For the application as a bioinert coating, the polymers should have a chain length of 10-20 repeating units. The submonomer approach in this work was a pilot project with the objective to synthesize short polymer chains. Therefore, the reaction was not monitored but NMR analysis was used after capping and cleaving the peptoids from the resin for investigation of the product. For synthesis of longer peptoids, interactions between the chains need to be considered. In order to monitor the reaction, one could use a Kaiser[27]-, chloranil[28]-, bromophenol blue[29]- or deClercq[28] test for the acylation step and chloranil[27] or deClercq[27] test for the nucleophilic displacement.

Two different ways of fluidizing the resin were performed. The first was by passing N_2 through the reaction mixture. This way proved to be difficult to control when the N_2 from the Schlenk line was used in other reactions at the same time. The superior way was to use a shaking table to get a controlled fluidization.

N-methoxypropyl glycine peptoid with six repeating units was synthesized and identified via NMR but DMF was also present in large proportions as can be seen by three distinct peaks in the NMR spectrum. With DMF present in the mixture, cleavage of the peptoids from the resin by TFA is suppressed and it was presumed that this was the reason for the low yield. Before the peptoids were cleaved from the resin it was washed three times with DMF followed by three times with DCM (20 minutes for each

wash) and shortly dried under reduced pressure. It was believed that the final washes with DCM would remove the DMF but these results suggest that there are longer equilibration times than 20 minutes for the solvent to penetrate the beads. The final wash should, therefore, include more than three washes (> 20 minutes for each wash) with DCM as well as a thorough drying (> 2 h). The yields were low but since this was believed to be due to the residual DMF the reaction can easily be optimized to obtain better yields.

3 Conclusions

The unpractical experimental conditions for large-scale synthesis of aldehyde via Swern oxidation gave rise to a thioacetal side product via Pummerer rearrangement side reaction. In comparison, Dess-Martin oxidation showed fewer side reactions and easier purification steps although the starting materials are more expensive and not in line with green chemistry philosophy. DMSO-activated oxidations should not be discarded and a Parikh-Doering oxidation[20] that might circumvent the thioacetal formation and low reaction temperatures of the Swern oxidation is recommended for future work.

Dialkylation in reductive amination resulted in low yields of the final material. In most cases, an inseparable 50:50 mixture of mono- and dialkylated product was observed leading to the necessity of CBZ-protection to isolate the desired monoalkylated product. Additionally, aminal formation of up to 35% of the product was observed.

In summary, the synthetic route in solution that has been performed in this work is doable, but for a large-scale synthesis of coating material, it is essential to reach higher yields or optimized reaction conditions. The desired monomer has been synthesized and a ring-opening polymerization procedure has been proposed for the synthesis of the peptoid coating material.

Parallel to the synthesis in solution, peptoids with six repeating units were synthesized via a submonomer approach. The submonomer approach offers an iterative process with a large variety of possible peptoid side chains, and when compared to the synthesis in solution, many purification and protection steps are avoided.

For the development of a new peptoid coating material, the submonomer approach is recommended both due to fewer synthetic steps that need purification as well as the diversity of side chains that are available and more easily incorporated into the submonomer approach.

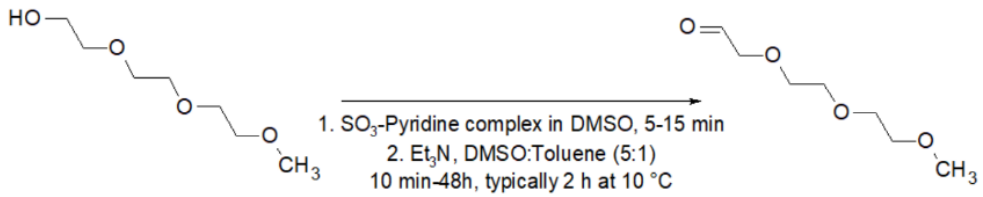
4 Future work

To circumvent the issues described in previous sections with the Swern oxidation, a Parikh-Doering oxidation[20] should be considered.

The next step in this work would be to coat nanoparticles with the peptoid. One can also study the properties of other side chains and optimize the coating material for the application. In this case, log P measurements can be used for determination of hydrophilicity.

Although a heavy initial investment, peptoids can be synthesized with an automated peptide synthesizer if one wishes to proceed with the submonomer approach to produce larger batches of coating material. This would especially facilitate in the development phase of a new coating material when a variety of different side chains might be of interest.

4.1.1 Parikh-Doering Oxidation



Scheme 25. Recommended protocol[20] of a Parikh-Doering oxidation for future work.

The Parikh-Doering oxidation (see *Scheme 25*) is an alternative to the Swern oxidation which might be more suitable if the reaction is to be used on a larger scale. The main difference between the two is the DMSO activator. Oxalyl chloride is used in a Swern oxidation but for the Parikh-Doering oxidation, an SO_3-pyridine complex is used. Reaction conditions are more suitable for a large-scale production with reaction temperatures between 0 °C – rt, compared to the Swern oxidation (see section 2.2.2) that required temperatures below -66 °C and even then, a large amount of thioacetal side product was formed. Additionally, reagents are easier to handle and the reaction conditions allow for higher flexibility to add more reagents if the reaction does not proceed to completion.[30] This oxidation also avoids the formation of the toxic volatile

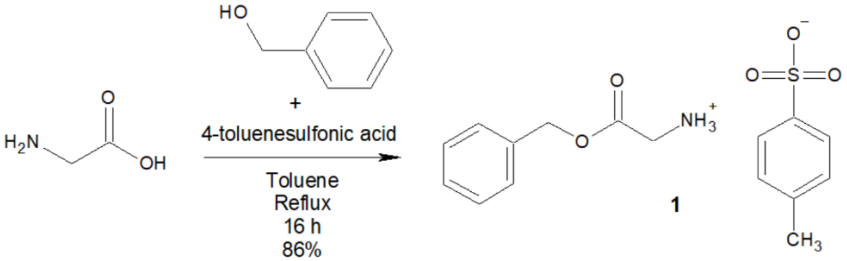
byproducts formed in the Swern oxidation. The complex SO_3 · pyridine is first reacted with DMSO before adding it to the alcohol. If it is not completely consumed it can attack nucleophilic sites such as alcohols.

5 Experimental Section

All chemicals used were purchased from Sigma-Aldrich and used without further purification. A Synthware peptide synthesis vessel with fritted disc and T-bore PTFE stopcock from Sigma-Aldrich was used for the submonomer approach. ^{1}H NMR spectra were recorded on a Varian Unity INOVA 400 MHz in deuterated solvents ($CDCl_3$ and DMSO-d_6) and chemical shifts are reported as ppm relative to the residual peak of the deuterated solvent. NMR spectra with structure assignments are included in the appendix. Mass spectra (ESI) were recorded on a SCIEX API 3200 triple quadrupole MS using MeOH + 0.1% formic acid as solvent. All flash chromatography was performed on silica gel (60 Å, 200-425 µm mesh particle size). TLC was performed on silica gel 60 F_{254}(Merck) sheets. UV-light or TLC stains ($KMnO_4$, ninhydrin, and DNP) followed by heating was used for visualization.

5.1 Solution-Phase Synthesis

Glycine benzyl ester *p*-toluenesulfonate (1)



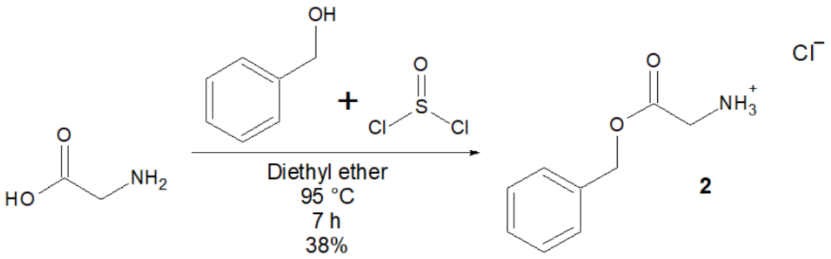
To a round-bottom flask was added glycine (8.003 g, 106.6 mmol), toluene (107 mL), *p*-toluenesulfonic acid monohydrate (21.284 g, 111.9 mmol) followed by benzyl alcohol (22.06 mL, 213.1 mmol). The reaction mixture was heated at reflux in a Dean-Stark set-up for azeotropic removal of water overnight. After 16 h the heating was removed and the reaction mixture was left to cool down to rt under slight mixing. White crystals

precipitated upon cooling. The precipitate was washed with Et_2O and **(1)** (yield: 30.847 g, 91.4 mmol, 86%) was collected by filtration.

^{1}H NMR (400 MHz, DMSO) δ 8.20 (s, 3H, -NH_3^+), 7.48 (d, *J* = 7.7, 2H, TS CH-2,6), 7.35-7.43 (m, overlapping signals, 5H, Ph CH-13,14,15,16,17), 7.11 (d, *J* = 7.9 Hz, 2H, TS CH-3,5), 5.24 (s, 2H, -OCH_2Ph), 3.90 (s, 2H, -$OOCCH_2$-), 2.29 (s, 3H, Ts -CH_3)

m/z calc. for $C_9H_{12}NO_2$+ $[M+]^+$: 166.0857; found: 166.2

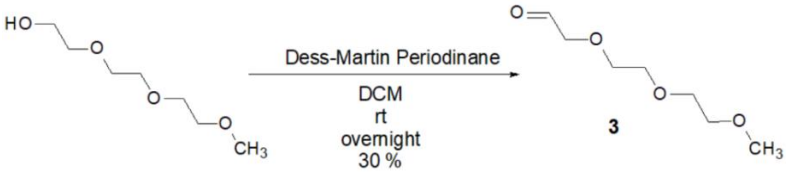
Glycine benzyl ester, hydrochloride (2)



Glycine (0.501 g, 6.66 mmol) was suspended in benzyl alcohol (34.5 mL, 333.4 mmol) under dry conditions. Thionyl chloride (2.4 mL, 32.9 mmol) was added to the reaction mixture which was then immersed in an oil bath and heated at 95 °C under an atmosphere of N_2. After 7 h the reaction mixture was allowed to cool down to rt and placed in an ice bath. Diethyl ether (5 × volume of benzyl alcohol) was added until the solution turned turbid. The solids were filtered, washed with Et_2O, then recrystallized from EtOH and Et_2O to give **(2)** (yield: 0.5162 g, 2.56 mmol, 38%).

^{1}H NMR (400 MHz, DMSO) δ 8.40 (br s, 3H, -NH_3^+), 7.35-7.44 (m, overlapping signals, 5H, -CCHCHCHCHCH), 5.24 (s, 2H, -OCH_2Ph), 3.87 (m, overlapping signals, 2H, -$OOCCH_2$-)

3,6,9-Trioxadecanaldehyde (3)



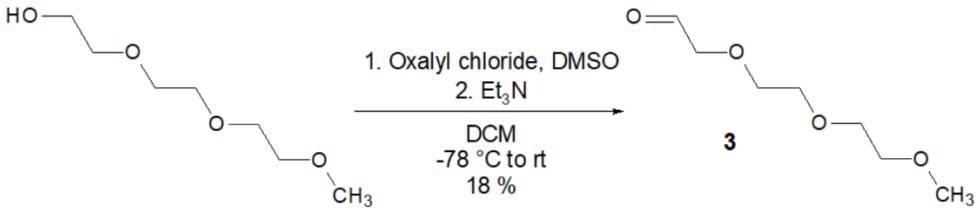
To a solution of Dess-Martin Periodinane (15.505 g, 36.5 mmol) in DCM (37 mL) was added triethylene glycol monomethyl ether (4.8 mL, 30.6 mmol) dropwise at 0 °C under

an N_2 atmosphere. The reaction mixture was allowed to warm to rt and left to stir overnight. The reaction mixture was diluted with cyclohexane and filtered. The solvent was removed *in vacuo* followed by flash chromatography (DCM:MeOH, 15:1) to give pure **(3)** (yield: 1.473 g, 9.08 mmol, 30%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H, OHC-), 4.16 (s, 1H, $OHCCH_2$-), 3.55-3.75 (m, overlapping signals, 9H, $-OCH_2CH_2OCH_2CH_2O$-), 3.39 (s, 3H, $-OCH_3$)

m/z calc. for $C_7H_{14}O_4$ $[M+H]^+$: 163.0964; found: 163.0

3,6,9-Trioxadecanaldehyde (3)

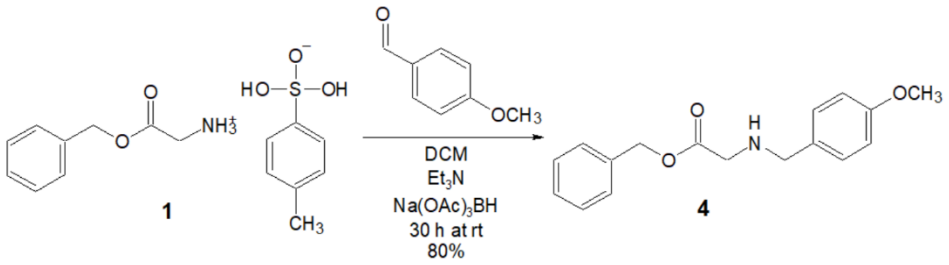


To a solution of oxalyl chloride (7.55 g, 59.5 mmol) in DCM (126 mL) under an N_2 atmosphere and cooled with a dry ice-acetone bath, was carefully added DMSO (9.26 g, 118.5 mmol) in DCM (25 mL). The temperature was not allowed to reach above -66 °C at any time. The mixture was stirred for 10 minutes. A solution of triethylene glycol monomethyl ether (7.8 mL, 50.0 mmol) in DCM (51 mL) was added dropwise to the reaction mixture. After 15 minutes, triethylamine (33.7 mL, 241.5 mmol) was added dropwise over a period of 35 minutes. The reaction mixture was left to stir at -70 °C for 30 minutes before allowed to reach rt. Sat. (aq) $NaCl/CaCl_2$ 0.1 M HCl (150 mL) was added to the reaction mixture. The aqueous phase was extracted with acetonitrile (7 × 35 mL). The organic phases were combined, washed with sat. (aq) $NaCl/CaCl_2$ (3 × 50 mL) and dried over $MgSO_4$. The solvent was removed *in vacuo*. Flash chromatography (EtOAc:MeOH, 9:1) gave pure **(3)** (yield: 1.4335 g, 8.84 mmol, 18%). The fractions were treated separately by removing the solvent *in vacuo* followed by analysis with LC-MS and NMR.

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.74 (s, 1H, OHC-), 4.16 (s, 1H, $OHCCH_2$-), 3.55-3.75 (m, overlapping signals, 9H, $-OCH_2CH_2OCH_2CH_2O$-), 3.39 (s, 3H, $-OCH_3$)

m/z calc. for $C_7H_{14}O_4$ $[M+H]^+$: 163.0964; found: 163.2

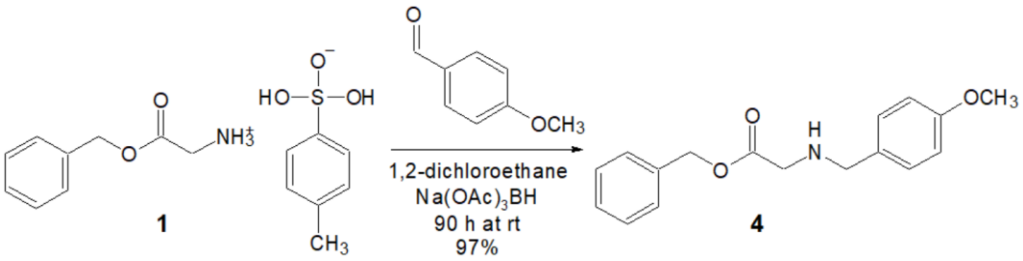
Benzyl {[(4-methoxyphenyl)methyl]amino}acetate (4)



To a solution of **(1)** (0.5011 g, 1.48 mmol) in DCM (14 mL) was added triethylamine (0.45 mL, 3.23 mmol), *p*-anisaldehyde (0.20 mL, 1.64 mmol) followed by $Na(OAc)_3BH$ (0.4764 g, 2.25 mmol). The reaction mixture was left to stir under N_2 atmosphere for 30 hours and then quenched with distilled water. The reaction mixture was diluted with EtOAc (50 mL) followed by addition of 1 M HCl (18 mL). The aqueous and organic layers were separated. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed *in vacuo* to obtain **(4)** as a yellow oil (yield: 0.3464 g, 1.21 mmol, 80%).

m/z calc. for $C_{17}H_{19}NO_3$ $[M+H]^+$: 286.1437; found: 286.0

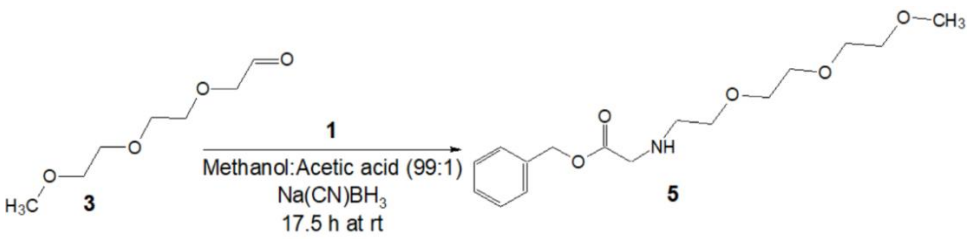
Benzyl {[(4-methoxyphenyl)methyl]amino}acetate (4)



To a solution of **(1)** (0.4983 g, 1.39 mmol) in 1,2-dichloroethane (4.8 mL) was added *p*-anisaldehyde (0.17 mL, 1.39 mmol). The reaction mixture was left to stir for 15 minutes under an atmosphere of N_2 followed by the addition of $Na(OAc)_3BH$ (0.4113 g, 1.94 mmol). After 90 h, the reaction mixture was quenched by the addition of sat. (aq) $NaHCO_3$ (1 mL). The mixture was diluted with EtOAc (50 mL). Brine (20 mL) was added and the aqueous and organic layers were separated. The organic layer was washed with brine (1 × 20 mL) and dried over Na_2SO_4. The solvent was removed *in vacuo* to obtain **(4)** as a yellow oil (yield: 0.3869 g, 1.36 mmol, 97%). No further purification was made.

m/z calc. for $C_{17}H_{19}NO_3$ $[M+H]^+$: 286.1437; found: 286.3

***N*-(3,6,9-trioxadecyl)glycine benzyl ester (5)**

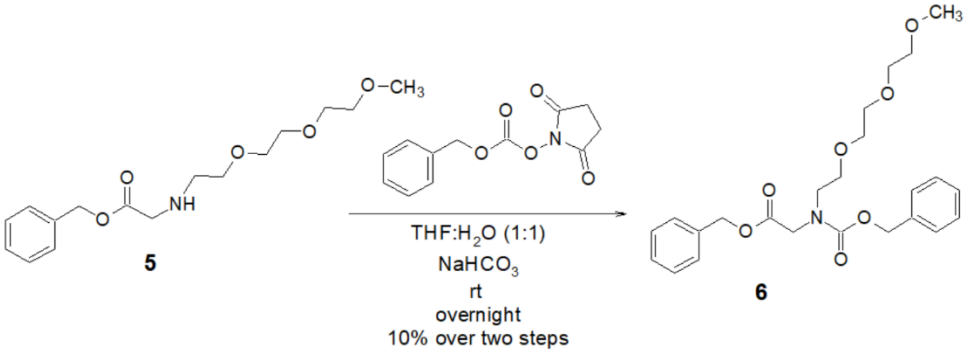


To a solution of (**1**) (1.754 g, 5.20 mmol) in MeOH:AcOH (99:1) (40 mL), was added (**3**) (0.843 g, 5.198 mmol) and left to stir for 30 minutes. Sodium cyanoborohydride (0.407 g, 6.48 mmol) was added to the reaction mixture. The solution was stirred for 17.5 hours at rt after which it was poured into sat. (aq) $NaHCO_3$ (95 mL). The aqueous layer was extracted with ethyl acetate (6 × 30 mL). The organic layers 1 and 5 were proceeded with separately and organic layers 2-4 and 6 were combined. The organic layers were washed with brine and dried over $MgSO_4$. The solvent was removed *in vacuo* to obtain the crude product (organic layer 1; 0.4715 g, organic layer 2-4 and 6; 0.607 g, organic layer 5; 0.607 g) which was an inseparable mixture of *N*-(3,6,9-trioxadecyl)glycine benzyl ester (**5**) and *N,N*-bis(3,6,9-trioxadecyl)glycine benzyl ester.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30-7.40 (m, overlapping signals, -Ph), 5.12 (s, 2H, -OCH_2Ph), 3.32-3.53 (m, overlapping signals, 17H, -$CH_2CH_2OCH_2CH_2OCH_2CH_2O$-, -$OOCCH_2$N-), 3.23 (s, 3H, -$OCH_3$)

m/z calc. for $C_{16}H_{25}NO_5$ $[M+H]^+$: 312.1805; found: 312.3

_N_-CBZ-_N_-(3,6,9-trioxadecyl)glycine benzyl ester (6)

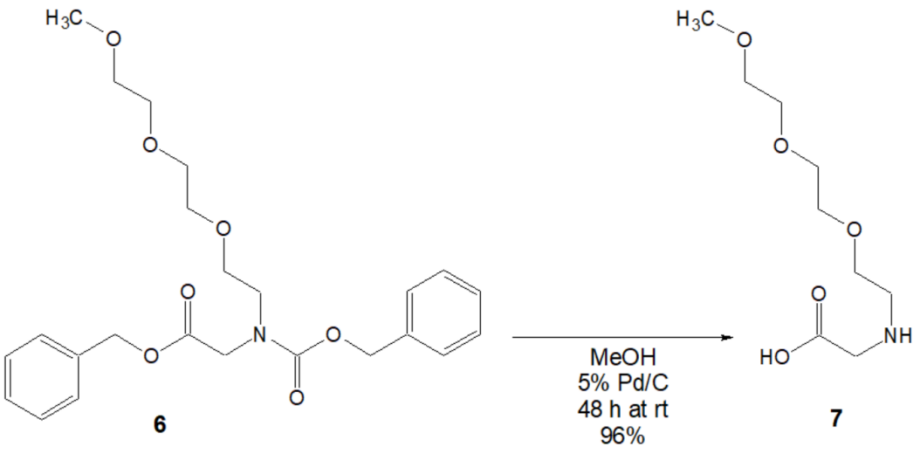


To a mixture of monoalkylated *N*-(3,6,9-trioxadecyl)glycine **(5)** and dialkylated *N,N*-bis(3,6,9-trioxadecyl)glycine (0.8325 g, 2.67 mmol) in THF:H_2O (1:1) (12 mL) was added $NaHCO_3$ (0.4453 g, 5.30 mmol) followed by *N*-(benzyloxycarbonyloxy)succinimide (0.787 g, 3.16 mmol). The reaction mixture was left to stir overnight and then diluted with EtOAc (10 mL). The two layers were separated, and the aqueous layer was extracted with EtOAc (2 × 10 mL). The organic layers were pooled, washed with brine (1 × 10 mL) and dried over $MgSO_4$. The crude product was purified with flash chromatography (EtOAc:heptane (3:2) followed by pentane:EtOAc (3:1), Rf=0.17) to give pure **(6)** (yield: 0.2403 g, 0.54 mmol, 10% from the aldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18-7.27 (m, overlapping signals, 10H, Gly -CCHCHCHCHCH, CBZ -CCHCHCHCHCH), 5.09 (s, 2H, Gly -OCH_2Ph), 5.01 (d, *J* = 4.16 Hz, 2H, CBZ -OCH_2Ph), 4.13 (s, 1H, -$OOCCH_2$N-), 4.09 (s, 1H, -$OOCCH_2$N-), 3.43-3.56 (m, 14H, -$CH_2CH_2OCH_2CH_2OCH_2CH_2O$-), 3.27 (s, 3H, -$OCH_3$)

m/z calc. for $C_{24}H_{31}NO_7$ $[M+Na]^+$: 468.1987; found: 468.4

***N*-(3,6,9-trioxadecyl)glycine (7)**

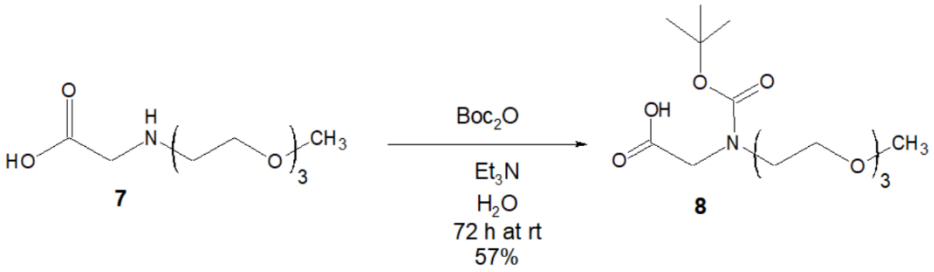


6 **7**

To 5% Pd/C (0.3565 g) was added **(6)** (0.9599 g, 2.08 mmol) in methanol (25 mL). A balloon was connected to a three-necked round bottom flask in which H_2 was produced by reacting granular Zn with conc. HCl. After vacuum/N_2 cycles (× 3) followed by vacuum/H_2 cycles (× 3), the suspension was stirred under an atmosphere of H_2 overnight. The reaction was monitored by TLC (n-butanol:H_2O:AcOH, 3:1:1, R_f=0.22) and 5% Pd/C (0.100 g) was added to the mixture. After 48 h the suspension was filtered on celite and the solvent was removed *in vacuo* to obtain **(7)** (yield: 0.4423 g, 2.00 mmol, 96%).

^{1}H NMR (400 MHz, DMSO) δ 3.39-3.58 (m, overlapping signals, 12H, -$CH_2CH_2OCH_2CH_2OCH_2CH_2O$-), 3.30 (s, 2H, $HOOCCH_3$-), 3.21 (s, 3H, -OCH_3), 2.92 (t, *J* = 5.1 Hz, 1H, HO-), 2.48 (s, overlapping signals, 2H, -NH-)

11-(*Tert*-butoxycarbonyl)-2,5,8-trioxa-11-azatridecan-13-oic acid (8)



7 **8**

To a solution of **(7)** (0.3580 g, 1.62 mmol) in DI H_2O (10 mL) was added Et_3N (0.90 mL, 6.47 mmol) followed by di-*tert*-butyl dicarbonate (0.9041 g, 4.14 mmol). The reaction

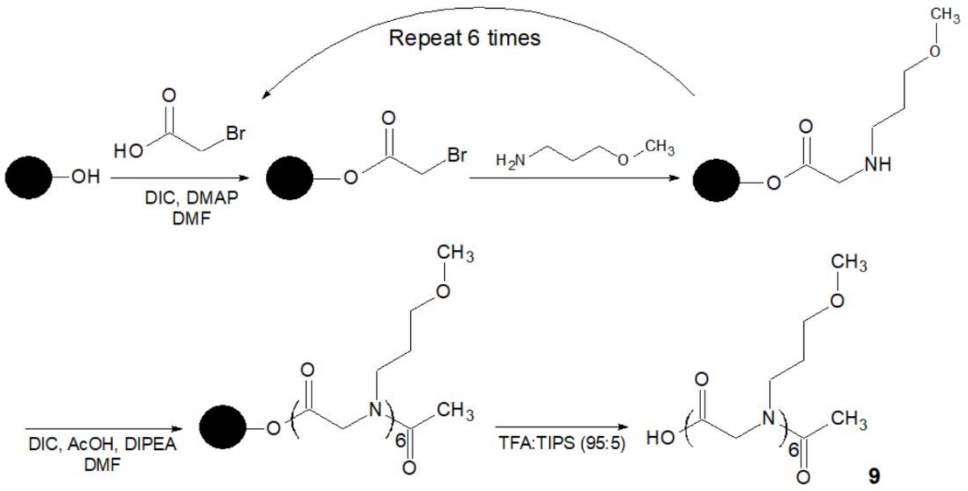
mixture was stirred at rt for 48 h then Et_3N (0.22 mL, 1.58 mmol) and di-*tert*-butyl dicarbonate (0.353 g, 1.62 mmol) was added. After 72 h the reaction mixture was extracted with n-heptane (2 × 10 mL). The aqueous phase was acidified with 4 M HCl and extracted with EtOAc (3 × 10 mL). The solvent was removed *in vacuo* to obtain **(8)** (yield: 0.2979 g, 0.93 mmol, 57%). No further purification was made.

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (s, 2H, $HOOCCH_2$-), 3.47-3.65 (m, overlapping signals, 12H, -$CH_2CH_2OCH_2CH_2OCH_2CH_2O$-), 3.42 (s, 3H, -$OCH_3$), 1.44-1.47 (m, overlapping signals, 9H, -$C(CH_3)_3$)

m/z calc. for $C_{14}H_{27}NO_7$ $[M+H]^+$: 322.1861; found: 322.4

5.2 Submonomer Solid-Phase Synthesis

_N_-methoxypropyl glycine peptoid (9)



Resin loading: In an SPPS reactor Wang resin (1.0008 g, 1.1 mmol/g) was swelled in DMF (10 mL) for 30 minutes on a shaking table. As a pre-activation step, in a separate vial was added bromoacetic acid (0.556 g, 4.00 mmol), dry DCM (10 mL), DMAP (0.025 g, 0.20 mmol), and DIC (0.62 mL, 4.00 mmol). After 5 minutes the solution was added to the SPPS reactor and allowed to mix at rt for an hour. The resin was washed in DMF (3 × 10 mL), DCM (3 × 10 mL), and DMF (3 × 10 mL).

Nucleophilic displacement: 3-Methoxypropyl amine (0.555 mL, 5.44 mmol) in DMF (10 mL) was added to the SPPS reactor and allowed to mix. After 1 h the resin was washed in DMF (3 × 10 mL).

Coupling: In a vial was added bromoacetic acid (0.604 g, 4.35 mmol), dry DCM (10 mL), and DIC (0.68 mL, 4.39 mmol). After 5 minutes the solution was added to the SPPS reactor and allowed to mix at rt for an hour. The resin was washed in DMF (3 × 10 mL). **Capping:** To a vial was added DIC (0.665 mL, 4.30 mmol), DMF (10 mL), acetic acid (0.249 mL, 4.35 mmol), DIPEA (1.51 mL, 8.67 mmol) and was allowed to stir for five minutes. The N-terminus was capped by the addition of the pre-activated solution to the reactor which was allowed to mix for 3 h. The resin was washed in DMF (3 × 10 mL) followed by DCM (3 × 10 mL).

Cleavage: The beads were cleaved in 10 mL TFA:TIPS (95:5) for one hour. The TFA and TIPS residues were removed *in vacuo* to give a yellow oil. Minimal volume of diethyl ether was added to obtain a precipitation of white crystals. The solvent was removed *in vacuo* to yield trace amounts of **9** in DMF.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.10 (s, 12H, -$COCH_2$-), 3.53 (t, J = 7.2 Hz, 12H, -OCH_2-), 3.44 (t, J = 5.9 Hz, 12H, -N**H_2**CH_2-), 3.35 (s, 12H, -OCH_3), 2.14 (s, 3H, -$COCH_3$), 1.85 (m, overlapping signals, 10H, -CH_2**H_2**CH_2-)

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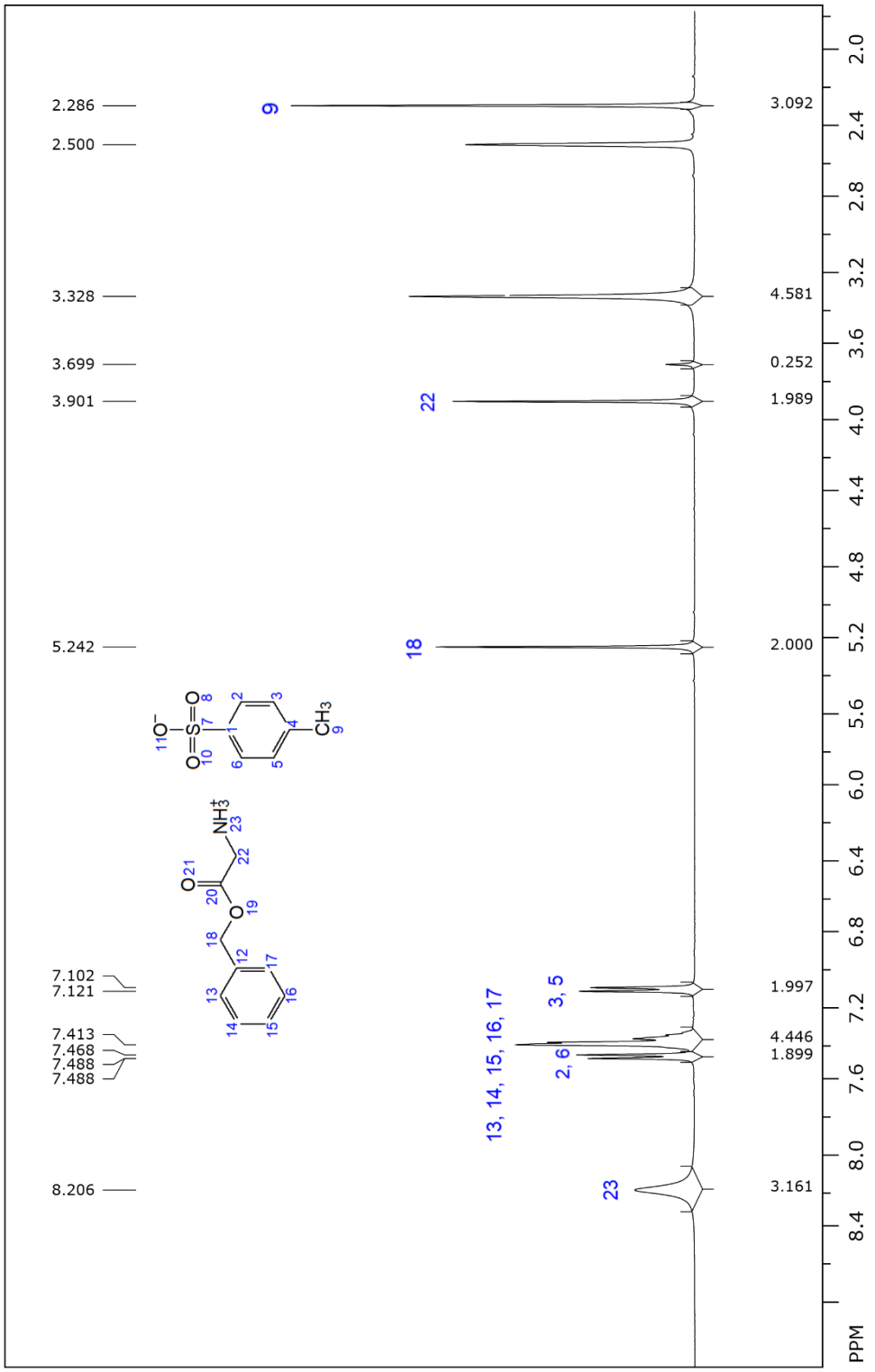
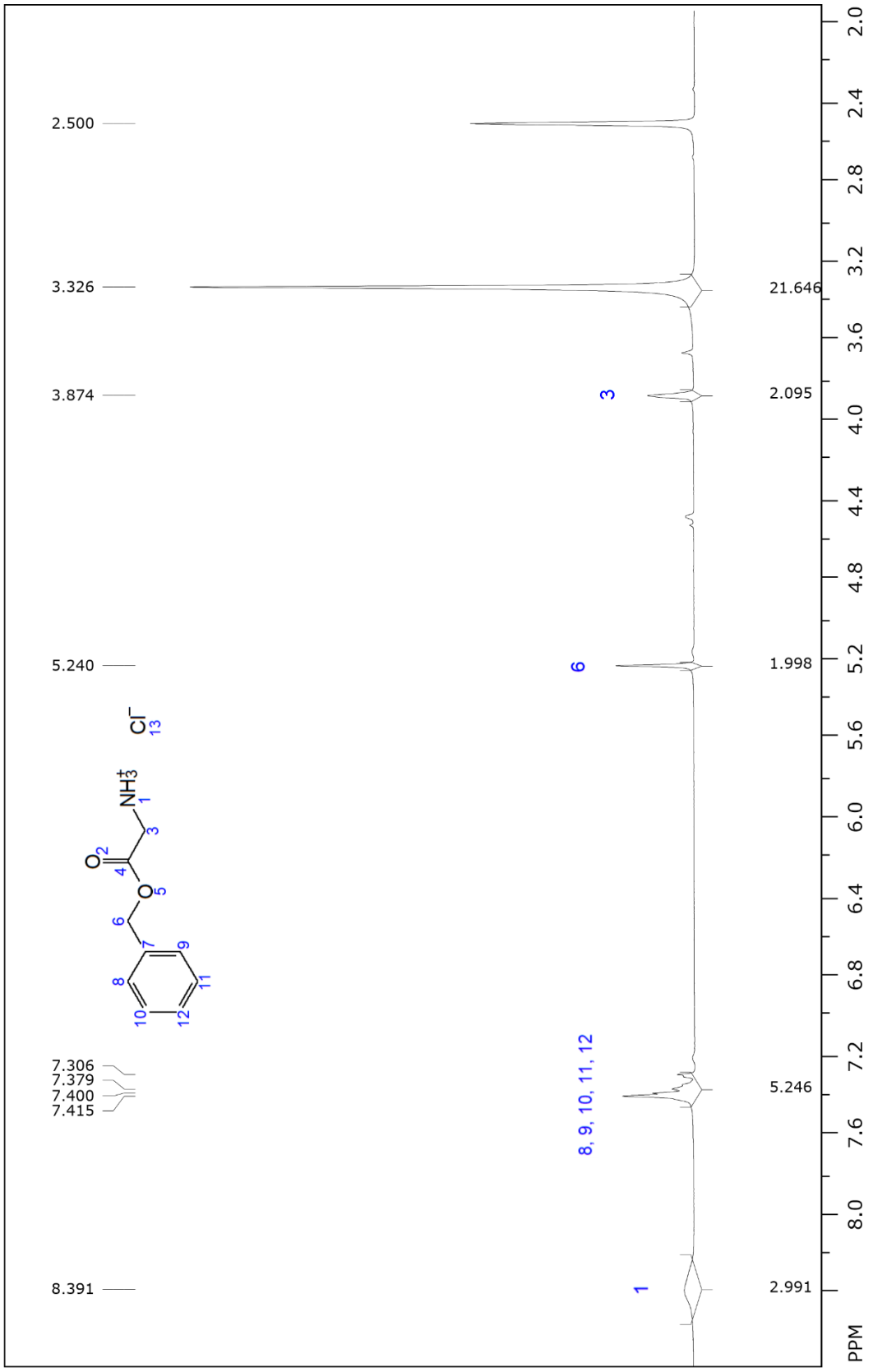
Appendix

NMR spectra:

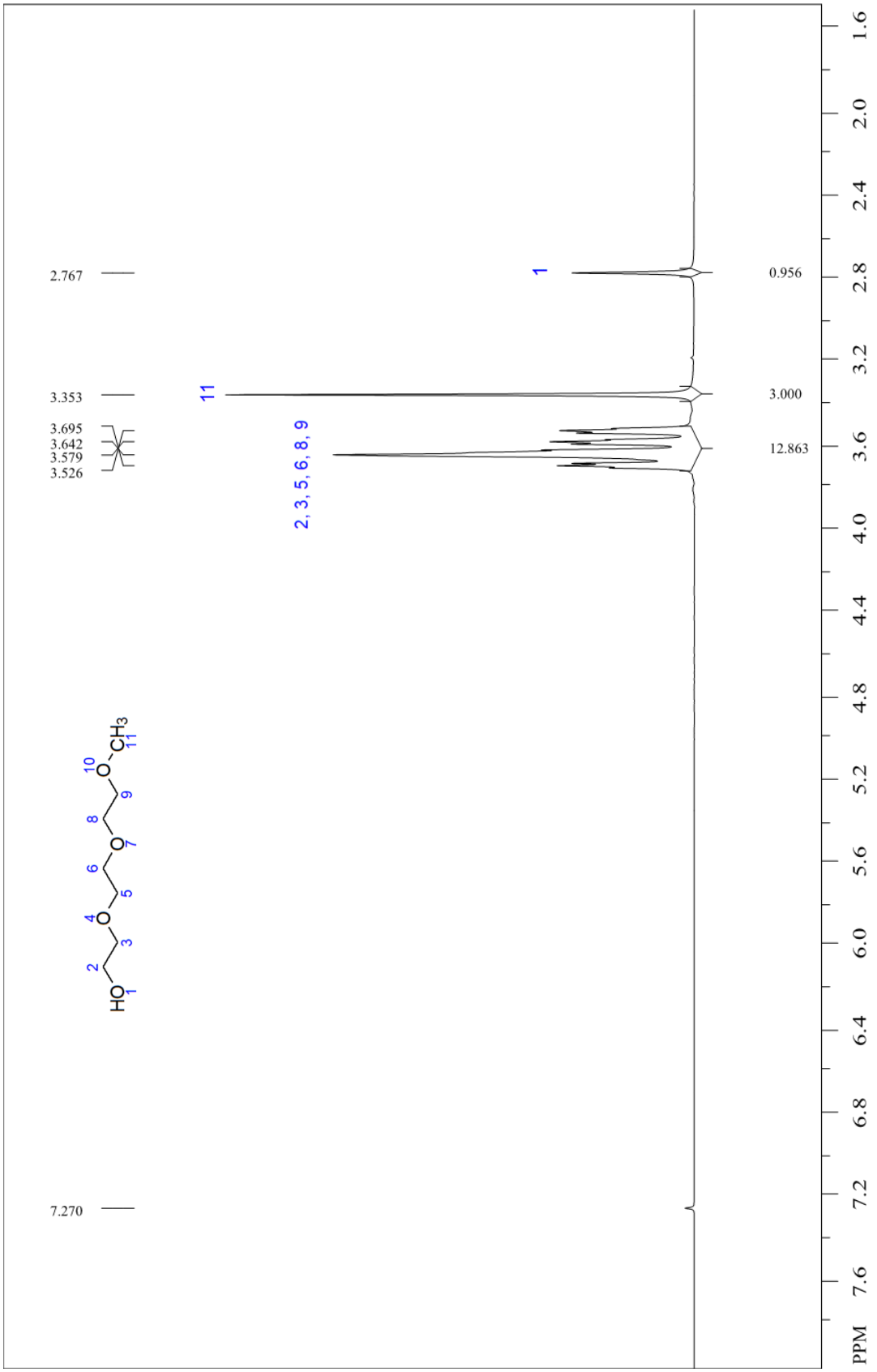
Glycine benzyl ester *p*-toluenesulfonate (**1**)



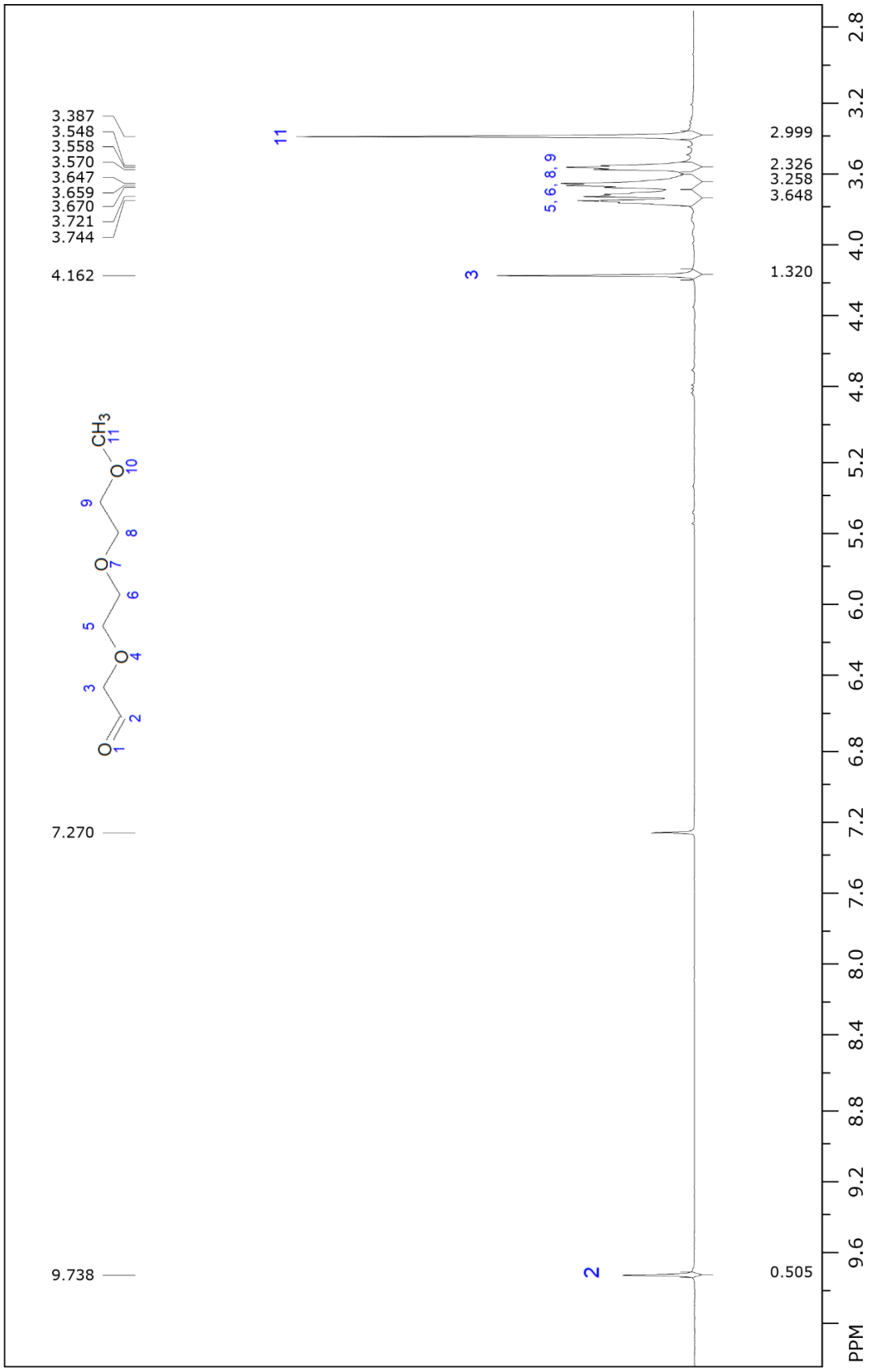
Glycine benzyl ester, hydrochloride (**2**)



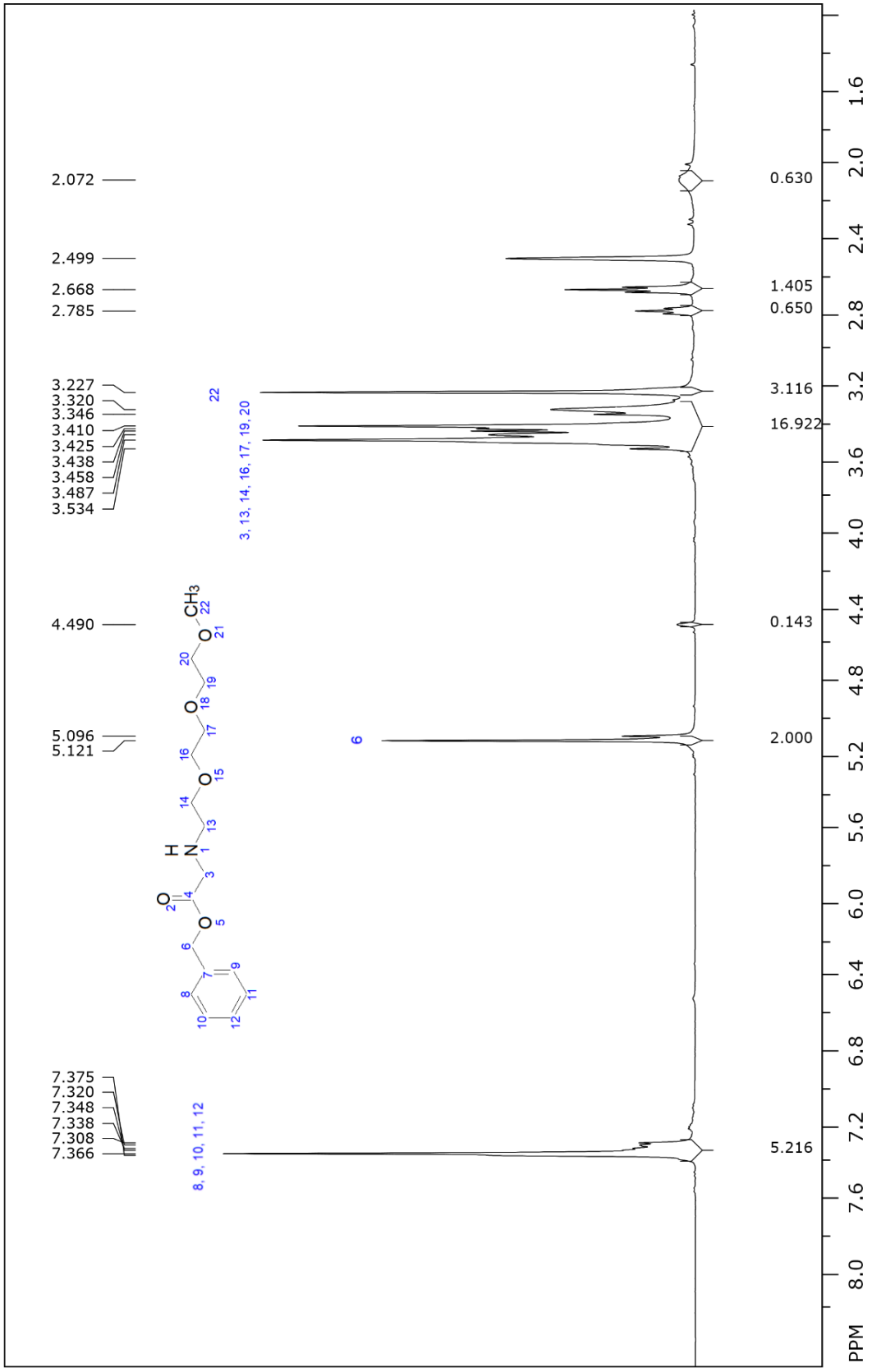
Triethylene glycol monomethyl ether (**reference**)



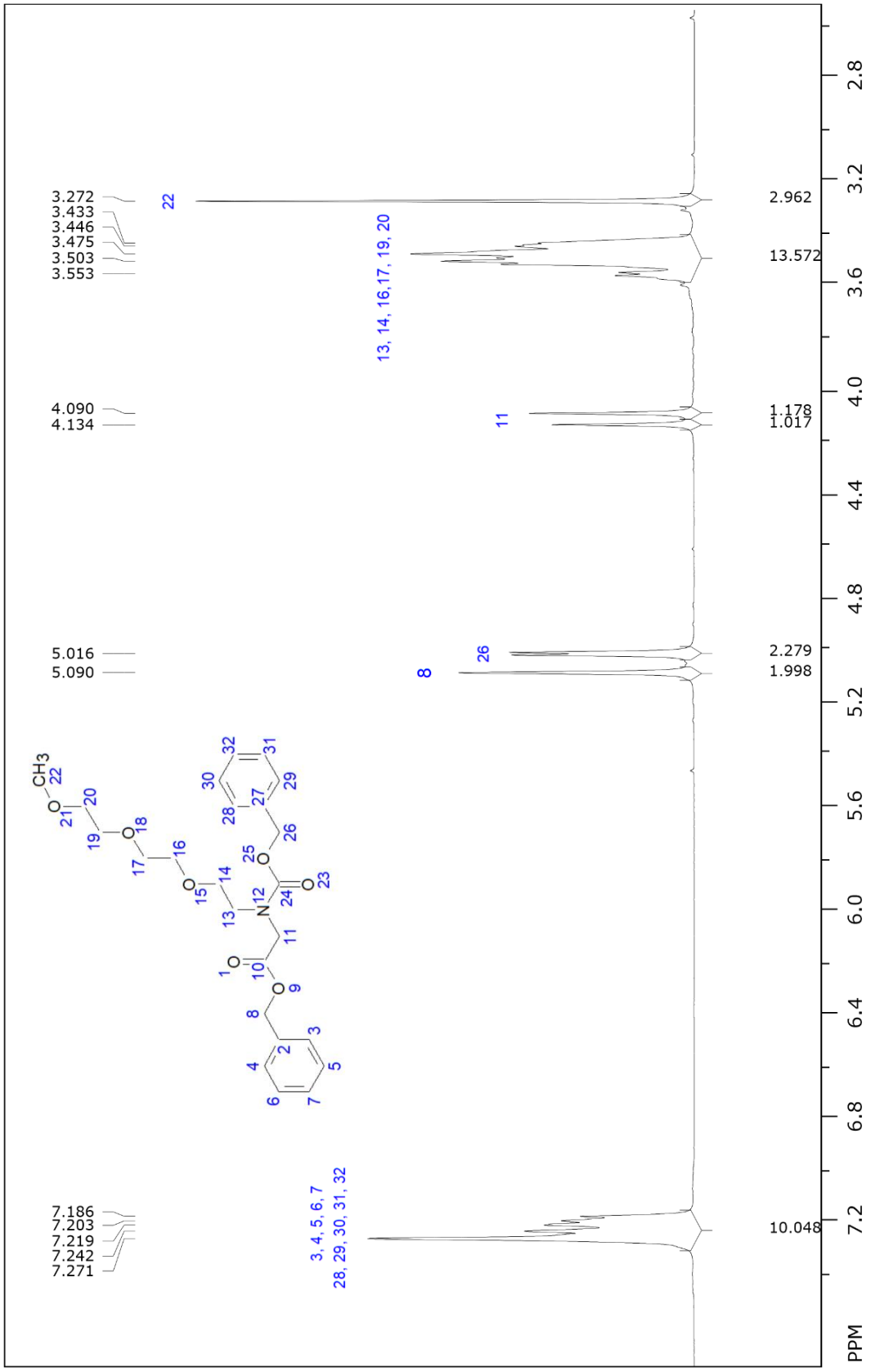
3,6,9-Trioxadecanaldehyde (**3**)



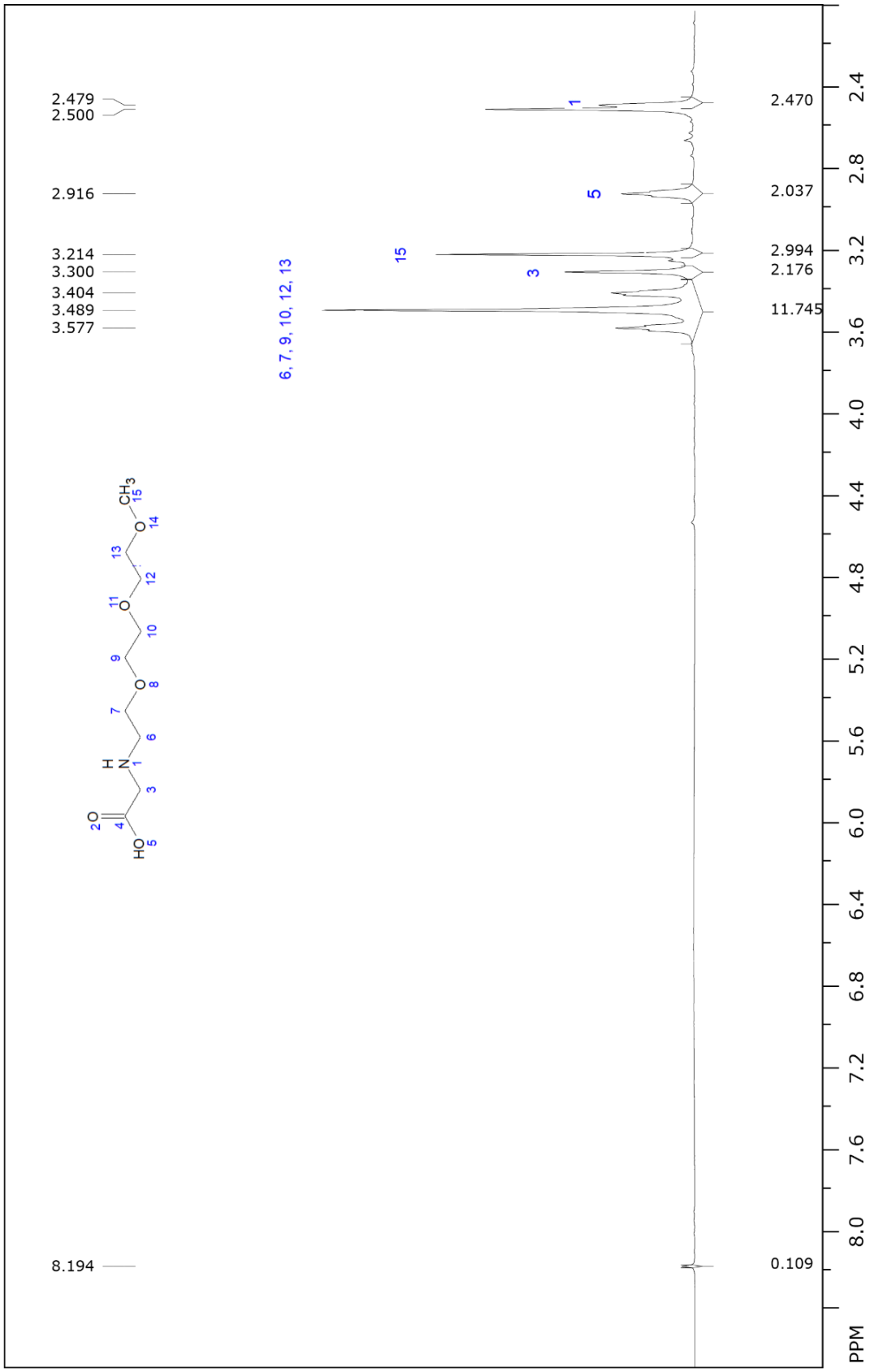
N-(3,6,9-trioxadecyl)glycine benzyl ester (**5**)



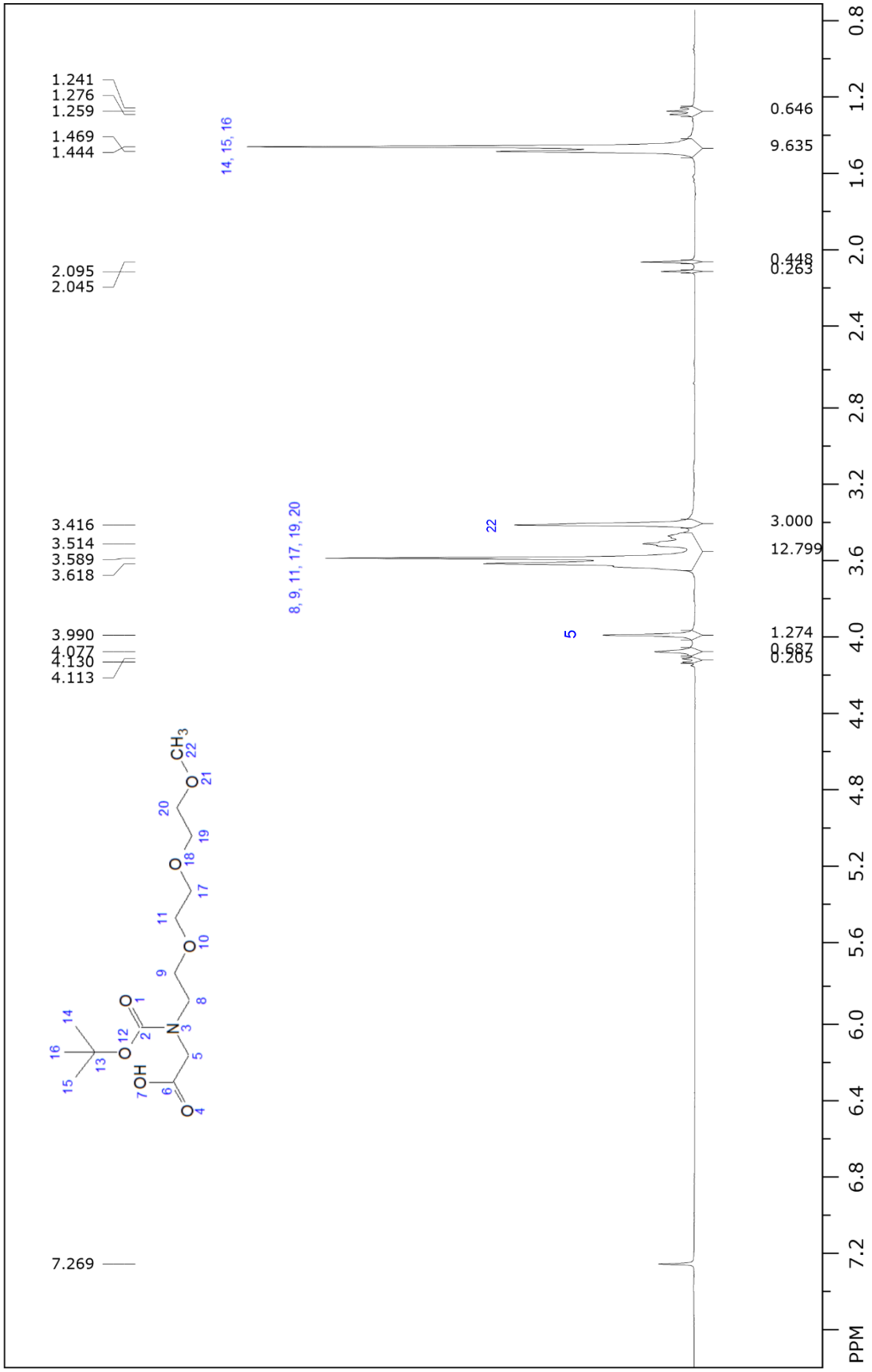
N-CBZ-*N*-(3,6,9-trioxadecyl)glycine benzyl ester (**6**)



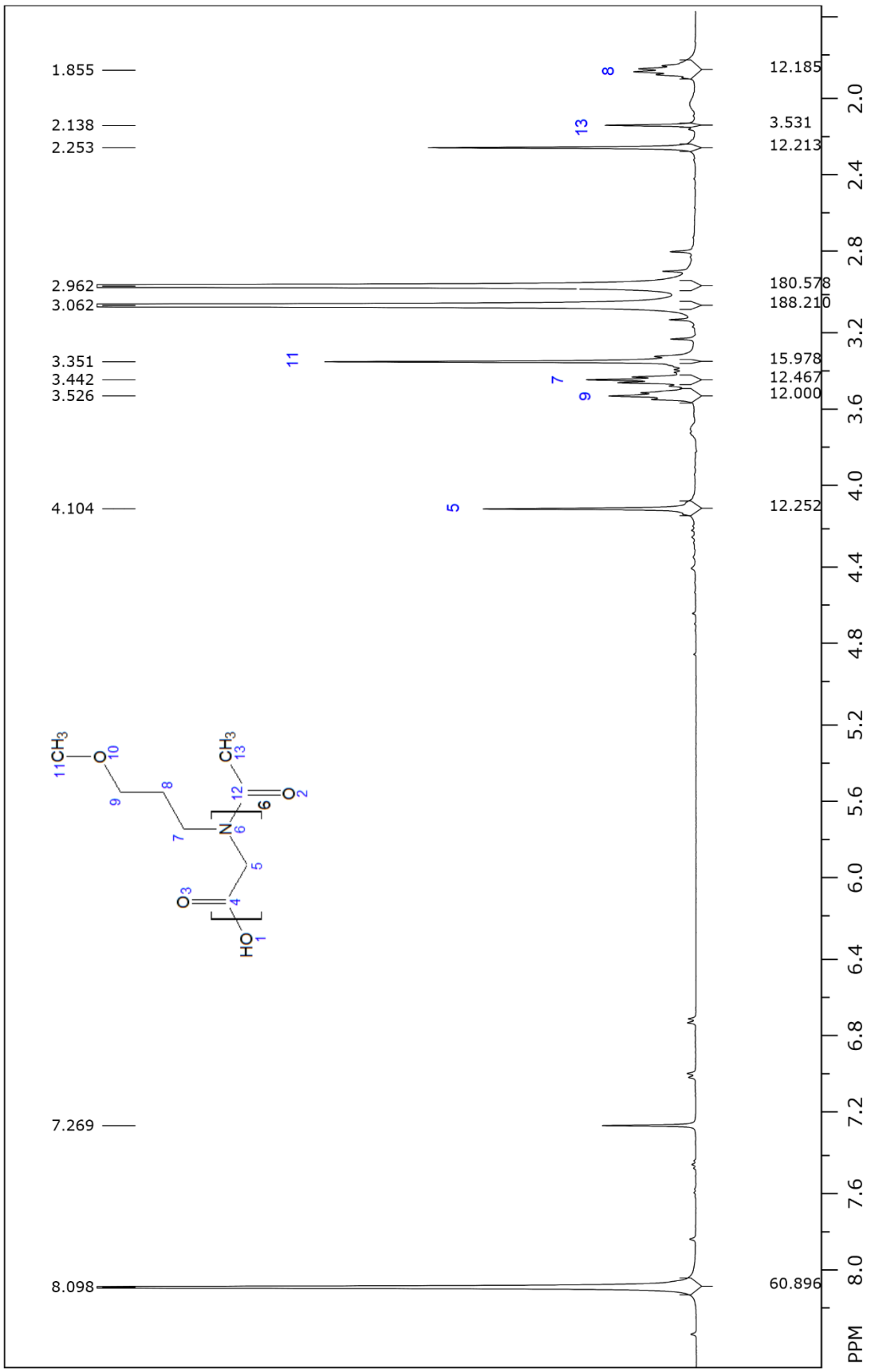
N-(3,6,9-trioxadecyl)glycine (**7**)



11-(*Tert*-butoxycarbonyl)-2,5,8-trioxa-11-azatridecan-13-oic acid **(8)**



N-methoxypropyl glycine peptoid (**9**)



COSY, *N*-methoxypropyl glycine peptoid **(9)**