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# New Broad-Spectrum Antibacterial Amphiphilic Aminoglycosides Active against Resistant Bacteria: From Neamine Derivatives to Smaller Neosamine Analogues

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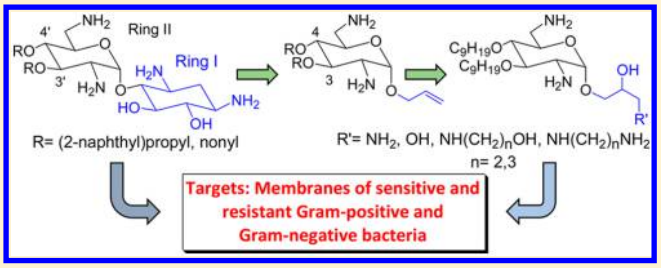
Supporting Information

**ABSTRACT:** Aminoglycosides (AGs) constitute a major family of potent and broad-spectrum antibiotics disturbing protein synthesis through binding to the A site of 16S rRNA. Decades of widespread clinical use of AGs strongly reduced their clinical efficacy through the selection of resistant bacteria. Recently, conjugation of lipophilic groups to AGs generated a novel class of potent antibacterial amphiphilic aminoglycosides (AAGs) with significant improved activities against various sensitive and resistant bacterial strains. We have identified amphiphilic 3′,6-dialkyl derivatives of the small aminoglycoside neamine as broad spectrum antibacterial agents targeting bacterial membranes. Here, we report on the synthesis and the activity against sensitive and resistant Gram-negative and/or Gram-positive bacteria of new amphiphilic 3′,4′-dialkyl neamine derivatives and of their smaller analogues in the 6-aminoglucosamine (neosamine) series prepared from *N*-acetylglucosamine.




## INTRODUCTION

Neomycin B **1** (Figure 1) and its fragment neamine **2** are members of the major family of potent and broad-spectrum antibiotic drugs aminoglycosides (AGs) that are polycationic at physiological pH and act on bacteria through binding to the A site of 16S rRNA causing *in fine* protein synthesis alteration.[1−10] Decades of widespread clinical use of AGs strongly reduced their clinical efficacy through the selection of resistant bacteria.[8−19]

In the search for new antibiotics less toxic and susceptible to resistance than conventional AGs, Hanessian, Westhof, and co-workers reported the first example of ether-modified amino-glycoside paromomycin with in vivo antibacterial activities.[20,21] Structural data with bacterial RNA have provided evidence of a novel binding mode, rendering these ether-substituted analogues less susceptible to inactivating enzymes.[22,23] Subsequent studies from our laboratories[24−29] and else-where[11,30−45] have extended these results to other AGs named antibacterial amphiphilic AGs (antibacterial AAGs) resulting from the introduction of one to four lipophilic groups on AG cores. Such an increase in the AG lipophilicity results in a bacterial target shifting from rRNA to membranes and significantly improves activity against bacterial strains resistant to the parent AG drugs and to other classes of antibiotic drugs. Antifungal derivatives were also obtained in such an approach.[44−46]

Targeting bacterial membrane functions remains an under-exploited mechanism of action in the fight against resistant bacteria.[47] Persistent infections involving slow- and nongrowing bacteria are difficult to treat with antibiotics that target biosynthetic processes. In the fight against resistant bacteria, there is currently a growing interest for antibacterial cationic amphiphilic drugs acting on bacterial membranes through binding to their negatively charged constituents.[24,48−56] For instance, the polycationic lipopeptide colistin, that is, one of the drug of last resort to combat *multidrug-resistant* (MDR) *Pseudomonas aeruginosa* infections, acts through binding to lipopolysaccharides (LPS) with displacement of divalent cations that stabilize LPS.[57−63] Insertion of the hydrophobic moiety of the lipopeptide in the membrane causes fusion of the inner leaflets of the outer membrane and the outer leaflet of the cytoplasmic membrane, causing osmotic imbalance and driving the antibiotic into the cytoplasmic membrane through polar and nonpolar channels.

Membrane-targeting antibacterials may use differences in the membrane composition to interact selectively with bacterial cells.[51,64−69] Unlike most mammalian cell membranes, bacterial membranes are rich in negatively charged lipids (cardiolipin, phosphatidylglycerol, LPS, lipoteichoic acids) that could be

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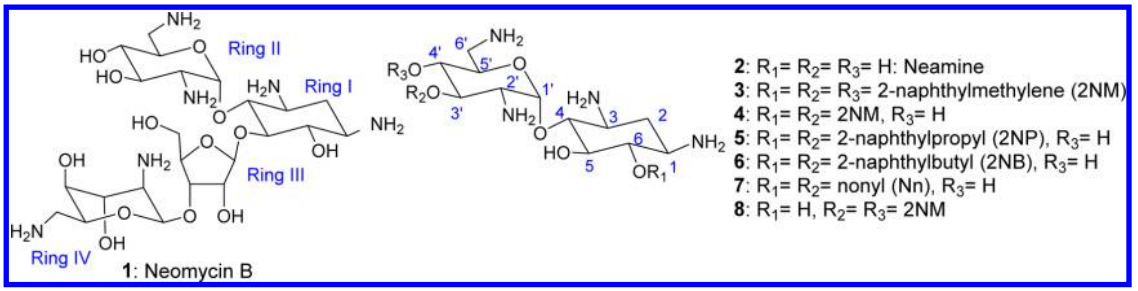


**Figure 1.** Structure of the natural antibiotic aminoglycoside neomycin B, of neamine and its amphiphilic derivatives previously prepared.[24−29]

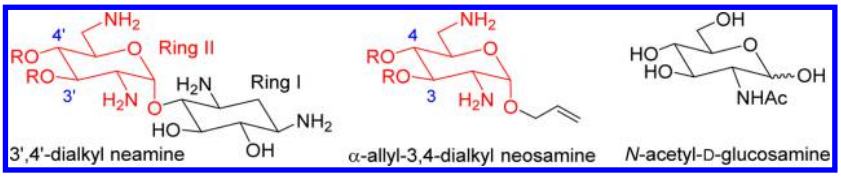


**Figure 2.** Comparison of the structures of amphiphilic 3′,4′-dialkyl neamine derivatives and 1-$\alpha$-allyl-3,4-dialkyl neosamine derivatives (allyl 3,4-dialkyl-6-amino-$\alpha$-D-glucosaminides) prepared from *N*-acetyl-D-glucosamine.

selectively recognized by cationic amphiphiles (CAs) through ionic interactions and hydrophobic effects.

Membrane-active agents can interact with many targets in the bacterial membranes and inhibit the corresponding functions. Through binding to anionic lipids, they could modify the activity of membrane proteins such as efflux pumps and/or proteins involved in cell division for which a dependence upon negatively charged lipids has been demonstrated. For example, in the regulation of bacterial cell division, proteins MinD/MinE bind tightly to anionic lipids such as cardiolipin positioned at the cell pole, and thus anionic lipids can be useful targets for antimicrobial development.[29,70,71]

Limited in vitro resistances to these amphiphiles have been observed due to their multiple modes of action.[48,49,56] Membrane-active agents cannot be inactivated by intracellular bacterial enzymes and flushed out by efflux pumps. In addition, due to their interactions with many key membrane targets present in a great number of copies, biochemical modifications of such multiple targets should have a high cost for the bacteria and should result in a high sensitivity to other antibiotic drugs.

However, the clinical use of CA drugs such as antimicrobial or host defense peptides is limited due to protease susceptibility and toxicity.[48−50] Over the years, the therapeutic potential of these amphiphiles has been improved by (i) reducing their ability to lyse red blood cells, (ii) increasing selectivity toward bacteria,[51−55] (iii) reducing nonspecific binding to human serum proteins, and (iv) improving serum stability.[72]

AAGs are expected to possess improved metabolic stability in regard to peptide-based CA antibiotics and are more difficult to modify by bacterial resistance-causing enzymes than AGs in regard to their expected mode of action. AAGs can also boost the innate immune response, specifically the recruitment of immune cells such as neutrophils required for the resolution of infections and can selectively control inflammatory responses induced in the presence of endotoxins to prevent septic shock.[41]

Several strategies for obtaining antibacterial AAGs have been developed including complete or partial conversion of the AG amine and hydroxyl functions into alkyl- or aryl-amide and -ether groups, respectively.[24]

In our approach in the field of antibacterial AAGs, we assumed that the presence of a large number of amine functions in AG derivatives like in neomycin **1**, which carries six amine functions, can be a source of toxicity through nonspecific binding to the target.[24−29] Neamine **2** carrying four amine functions is less toxic than neomycin,[73−76] and the neamine core corresponds to the minimum scaffold necessary for binding to 16S rRNA.[5,6] This core was used in the synthesis of new AGs in the search for antibacterial and antiviral agents targeting RNA, gene therapy vectors, and for the treatment of the Méniere's disease of the inner ear characterized by recurring attacks of disabling vertigo, hearing loss, and tinnitus.[10,77−98] Therefore, for obtaining amphiphilic AGs targeting rRNA, we have modified selectively the small AG neamine **2** on one, two, three, and the four hydroxyl functions in order to keep unchanged the four amine functions potentially protonated at physiological pH, at least partially, in regard to their major role in the binding to anionic targets in bacterial membranes and rRNA. We have identified a first antibacterial amphiphilic neamine derivative, namely 3′,4′,6-tri2-naphthylmethylene (3′,4′,6-tri2NM) neamine **3** (Figure 1), having a broad spectrum of activity[25] and targeting LPS in the outer membrane of *P. aeruginosa*.[26] Structure−activity and structure−cytotoxicity relationships were delineated from various amphiphilic 3′,6-dialkyl neamine derivatives for obtaining compounds **4**−**7** (Figure 1) more active than **3** against sensitive and resistant Gram-positive and/or Gram-negative bacteria and strongly less toxic against eukaryotic cells.[27] A critical window of lipophilicity appeared to be necessary for optimal antibacterial effects. The study of the mode of action of compounds **5**−**7** confirmed a strong binding to LPS of *P. aeruginosa* as well as membrane depolarization.[28] Compound **7** has been found the most efficient neamine derivative against Gram-negative bacteria. It appeared to also be able to inhibit growth of *P. aeruginosa* biofilms and be active against *P. aeruginosa* strains resistant to colistin, suggesting a different mode of action from the one of colistin.[28]

In our first report on the identification of the tri-2NM neamine derivative **3**, we have described the 3′,6- and 3′,4′-di2NM neamine derivatives **4** and **8** showing similar antibacterial effects against sensitive and resistant strains of *Staphylococcus aureus* and weak activity against Gram-negative bacteria.[25] Herein, we report on the synthesis and the antibacterial activities of new 3′,4′-dialkyl neamine derivatives


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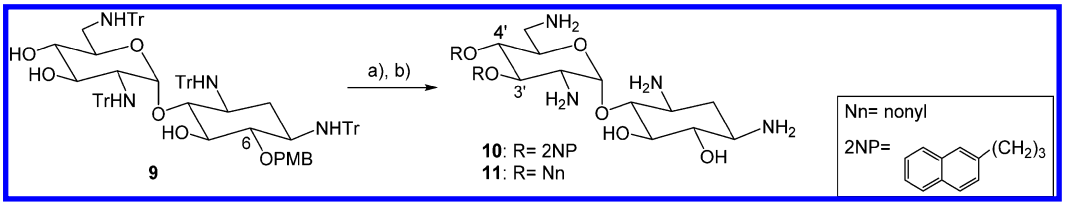
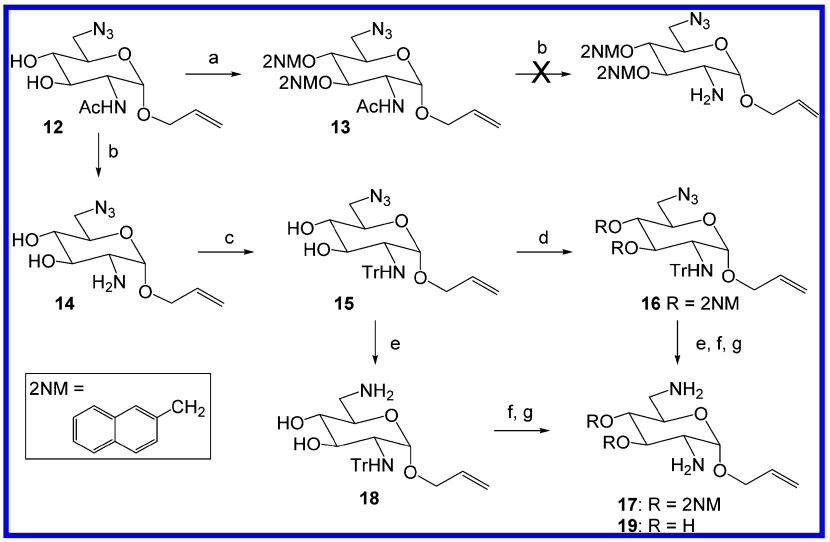
**Scheme 1. Synthesis of the 3′,4′-Dialkylneamine Derivatives from the *p*-Methoxybenzyl Neamine Derivative 9[a96]**




[a]Reagents and conditions: (a) R = 2NP: NaH/DMF, 3-(2′-naphthyl)propyl bromide (2NPBr),[27] rt, 5 h, 44%; R = Nn: TBAF (2 equiv), 50% aq NaOH/toluene, 1-nonyl bromide (1NnBr), rt, 43%. (b) TFA/$CH_2Cl_2$, anisole, 0 °C; **10**, 45%; **11**, 55%.

**Scheme 2. Preparation of the α-Allyl-3′,4′-di2NM Neosamine Derivative 17 and the Corresponding Reference Compound α-Allylneosamine 19[a]**




[a]Reagents and conditions: (a) 2NMBr, BEMP, DMF, rt, 48 h, 28%. (b) KOH, EtOH, reflux or $Ba(OH)_2{\cdot}8H_2O$, $H_2O$, reflux, 8 h, 78%. (c) TrCl, DMF, $Et_3N$, rt, 8 h, 82%. (d) 2NMBr, NaH, DMF, rt, 10 h, 79%. (e) $Ph_3P$, THF/$H_2O$ (19/1), 80 °C, 6 h, 91%. (f) TFA/anisole (1/1), 0 °C, 3 h. (g) Dowex resin ($Cl^-$ ion exchange); **17**, 62%; **19**, 84% (3 steps).

active against sensitive and resistant Gram-positive and Gram-negative bacteria in comparison to their active 3′,6-dialkyl neamine isomers **5** and **7** previously described.

In the previously identified antibacterial 3′,4′-di2NM neamine derivative **8** (Figure 1), both lipophilic groups are attached on the glucosamine ring II. Therefore, small AAGs in which ring II carries at the 3- and 4-positions two lipophilic groups and at the 1-position acyclic side chains generated from an allyl group (Figure 2) could have attractive antibacterial properties. Such an approach has been previously developed from ring I or ring II of neamine in the search for antibiotic aminosugars targeting 16S rRNA.[97−100]

We have also previously conjugated ring II of neamine to a peptide nucleic acid targeting transactivation response element of HIV-1 RNA genome that shows a high bioavailability in human cells and strongly inhibits Tat-mediated transactivation of HIV-1 transcription.[101]

Amphiphilic 3,4-dialkyl derivatives of 6-amino-6-deoxyglucosamine named neosamine were synthesized from *N*-acetylglucosamine. First, derivatives carrying an allyl group introduced in α-configuration at the anomeric position corresponding to the location of ring I on ring II in neamine were prepared (Figure 2). Second, the reactive allyl group of the 3,4-dinonyl neosamine derivative was chemically modified in order to adjust the lipophilicity/hydrophily balance of the resulting AAGs, balance previously identified in the neamine series as a key parameter for obtaining a broad spectrum antibacterial activity. This group was converted to an epoxide ring (oxirane) in order to introduce by ring-opening hydroxyl and/or amine functions like those found in ring I of the corresponding 3′,4′-dialkyl neamine derivatives. The route using epoxides as intermediates was selected for the resulting obtention of diasteroisomers that extends the molecular diversity in the antibacterial evaluation. Herein, we compared the antibacterial effects of the prepared 3,4-dialkyl neosamine derivatives to those of the corresponding novel 3′,4′-dialkyl neamines described here and to those of the previously described 3′,6-dialkyl neamines. *P. aeruginosa* inner membrane permeabilization assays and MIC changes against *P. aeruginosa* induced upon exposure to some of the most active AAGs identified are also reported.

## SYNTHESIS

**Synthesis of New 3′,4′-Dialkyl Neamine Derivatives.** In regard to the structure of the 3′,6-dialkyl neamine derivatives previously identified as interesting antibacterials, the 3′,4′-di-2-naphthylpropyl (2NP) and 3′,4′-dinonyl (Nn) neamine derivatives were prepared for evaluation of their antibacterial effects (Scheme 1). The *N*-tetratrityl neamine derivative **9**[90] selectively protected at the 6-position by the *p*-methoxybenzyl group was first prepared in good yield under phase transfer conditions with TBAF as a phase transfer agent from *N*-tetratritylneamine.[96] Then, the 3′,4′-di2NP (**10**) and 3′,4′-diNn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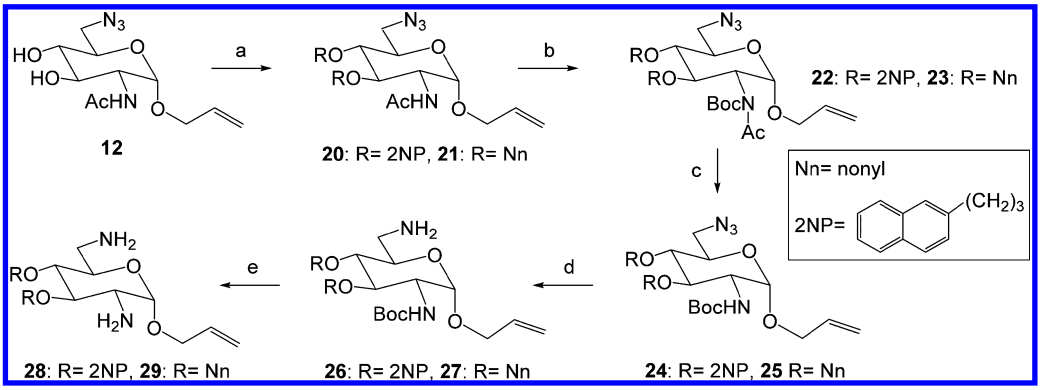
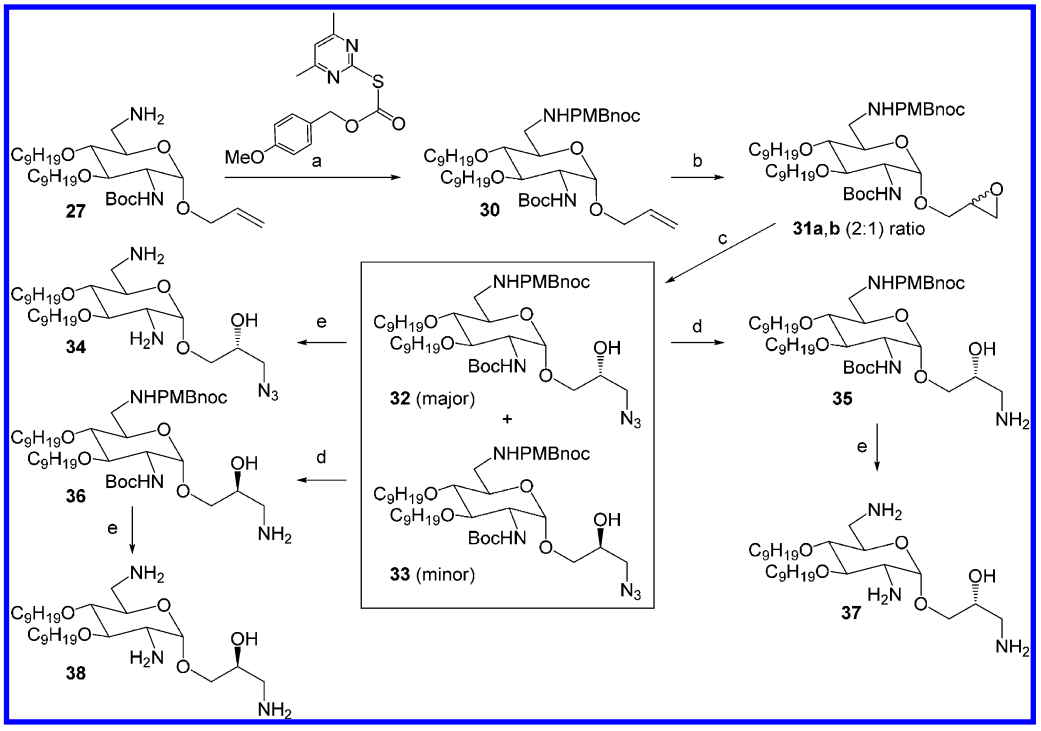
**Scheme 3. Preparation of α-Allyl-3,4-di2NP (28) and -DiNn (29) Neosamine Derivatives**[a]




[a]Reagents and conditions: (a) TBAF (2 equiv), 50% aq NaOH/toluene or NaH/DMF, $C_9H_{19}Br$ (3 equiv), rt, 5 h, 57%; NaH, DMF, 2NPBr,[32] 50 °C, 20 h, 28%. (b) $Boc_2O$, DMAP, THF, 45 °C, 6 h; **22**, 86%; **23**, 77%. (c) ($CH_3O^-$, $Na^+$)/$CH_3OH$, rt, 6 h. (d) $PPh_3$, THF/$H_2O$, rt, 4 h; **26**, 83%; **27**, 81% (2 steps). (e) TFA, DCM, rt, 4 h; **28**, 98%; **29**, 97%.

**Scheme 4. Synthesis of Azido and Amino Derivatives of the α-Allyl-3,4-diNn Neosamine 27 through Epoxidation and Ring-Opening**[a]




[a]Reagents and conditions, PMBnoc = *p*-methoxybenzyloxycarbonyl. (a) *p*-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (1.2 equiv), DCM, rt, 14 h, 90%. (b) mCPBA (2.5 equiv), DCM, rt, 14 h, 98%. (c) $NaN_3$ (excess), DMF, 70 °C, 14 h; **32**, 57%; **33**, 29%. (d) $PPh_3$ (3 equiv), THF/$H_2O$, rt, 14 h; **35**, 87%; **36**, 83%. (e) TFA, DCM, rt, 4 h; **34**, 98%; **37**, 94%; **38**, 92%.

(**11**) neamine derivatives were obtained with moderate yields in two steps: (i) alkylation of **9** with the corresponding bromoalkane RBr under phase transfer conditions (R = Nn) or in the presence of NaH in DMF (R = 2NP) and (ii) deprotection with TFA/anisole.

**Synthesis in the Neosamine Series.** The neosamine core corresponding to ring II in neamine has been previously used in the search for antibacterial agents targeting 16S ribosomal A-site RNA.[82,97−99] For instance, a library of compounds based upon this core has been synthesized through a combinatorial approach and screened for binding specifically to 16S rRNA by the Wong group to lead to effective binders to a model of A-site 16S RNA in the micromolar range.[97] One of the key intermediates used in the different reported approaches has been compound **12**, which was one of our key intermediates in the preparation of amphiphilic neosamine derivatives.

*Synthesis of α-Allyl-3,4-di(2′-naphthylmethylene) Neosamine Derivative **17** and of the Corresponding Reference Compound α-Allylneosamine **19**.* *N*-Acetylglucosamine was converted to compound **12** (Scheme 2), possessing an allyl group at the anomeric position in order to allow further modifications, using a three steps sequence adapted from the method described by Wong et al.:[97] (i) conversion of *N*-acetylglucosamine to the corresponding α-allyl glycoside, (ii) selective tosylation of the primary alcohol function, and (iii) displacement of the tosyl group with sodium azide. Then the



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alkylation of **12** with 2-bromomethylnaphthalene (2NMBr) was somewhat troublesome (Scheme 2), and under various basic conditions (NaH or $K_2CO_3$ or lutidine) was ineffective; using an hindered organic base (BEMP: 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine), compound **13** was obtained in a low 28% yield. The deprotection of the 2-amine function in **13** could not be achieved using either potassium hydroxide in ethanol or barium hydroxide in water at reflux. Thus, compound **12** was first deprotected by refluxing with aqueous barium hydroxide to provide **14** in 78% yield,[97] and then the free amino group was protected by reaction with trityl chloride to give compound **15** (82%). Then, **15** was alkylated with 2NMBr in DMF and sodium hydride to obtain **16** in 79% yield. The 6-azido group was reduced with triphenylphosphine in a THF/water mixture, and then the trityl group was removed under acidic conditions at 0 °C. A final treatment with an ion-exchange resin afforded the 3,4-di2NM neosamine derivative **17** as a dichlorohydrate (62%, 3 steps). To evaluate the role of the 3,4-dialkyl groups in the antibacterial activity of the prepared amphiphilic neosamine derivatives, the parent compound **19**[99] was prepared from **15** using the same three-step sequence (76% from **15**) through preparation of the intermediate amine **18**.

*Synthesis of α-Allyl-3,4-di(2′-naphthylpropyl) and -Dinonyl Neosamine Derivatives **28** and **29** (Allyl 3,4-dialkyl-6-amino-α-D-glucosaminides).* α-Allyl-3,4-dialkyl neosamine derivatives carrying the same alkyl groups as the prepared 3′,4′-dialkyl neamines were synthesized for antibacterial evaluation. Two 2-naphthylpropyl and two nonyl groups were introduced at the 3 and 4 positions of α-allyl neosamine starting from compound **12** (Scheme 3). Previously, the removal of the *N*-acetyl group in the 3,4-di2NM intermediate **13** has failed under different drastic basic and nucleophilic conditions and also with other prepared *N*-acetyl-*O*-3,4-dialkylneosamines. Thus, a Boc group was introduced on the *N*-acetyl group in order to facilitate the final deprotection of the 2-amine function according to the method developed by Chapleur et al. (Scheme 3).[102]

Compound **12** was dialkylated with 2-(3′-bromopropyl)-naphthalene[27] and 1-bromononane under phase transfer conditions[96] or in the presence of NaH in DMF, and then the Boc group was introduced to lead to compound **22** and **23**, respectively. These compounds were deacetylated in the presence of sodium methoxide to lead to compounds **24** and **25** carrying a 2-NHBoc group. Reduction of the azido group afforded compounds **26** and **27**, which were deprotected in the presence of TFA to lead to the AAGs **28** and **29** incorporating two 2NP and two nonyl groups, respectively.

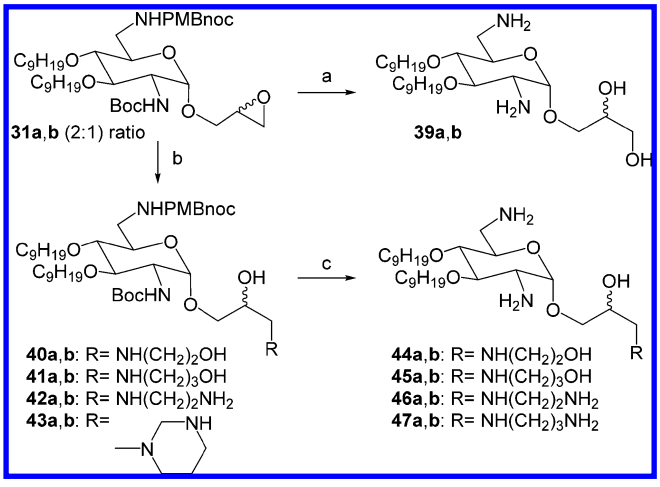
*Synthesis of Hydrophilic 3,4-Dialkyl Neosamine Derivatives through Epoxidation of the Allyl Group and Opening of the Obtained Oxiranes with Nucleophiles.* According to the higher antibacterial activity against *P. aeruginosa* strains of the 3′,6-dinonyl neamine derivative **7** in comparison to the 3′,6-di2-naphthylpropyl (**5**) and 3′,6-di2-naphthylbutyl (**6**) derivatives (Figure 1),[27] the allyl group found in 3,4-diNn neosamine **29** was modified from compound **27** (Scheme 4) in which the 2-*N*-acetyl group was replaced by a Boc group in order to avoid the *N*-deacetylation difficulty encountered previously with compound **13**, for example (Scheme 2, step b).

In a first step (Scheme 4), the 6-amine function present in **27** was protected in good yield by reaction with a stoichiometric amount of *p*-methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate.

In a second step, the double bond of the allylic group was oxidized with mCPBA to lead in high yield to the 2:1 diasteroisomeric mixture of (*R*)- and (*S*)-α-epoxypropyl derivatives **31a,b** characterized by NMR. The configuration of these isomers could not be determined by NOESY experiments at 500 and 600 MHz, and the next steps were performed with the isolated mixture. Such an asymmetric induction has been reported in the epoxidation by mCPBA of β-alkenyl glycosides of *O*-acetyl- and/or *O*-benzyl-*N*-acetyl-β-D-glucosamines.[103] In this study, the *R* configuration has been assigned to the major isomers through correlation by $^1$H NMR with (*R*)-glycerinaldehyde. In the mixture of (*R*)- and (*S*)-α-epoxides **31a,b**, we observed, by $^1$H NMR, for one proton of the methylene group in the epoxide ring (C3′-H), a signal more shielded in the minor isomer than in the major isomer ($CDCl_3$, $\Delta\delta$ = 0.07 ppm). Previously, a similar difference in the corresponding chemical shifts ($CDCl_3$) has been observed after the selective synthesis of (*R*)- and (*S*)-(2′,3′-epoxy)propyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosides by glycosylation with the corresponding chiral 2,3-epoxide alcohols ($CDCl_3$, $\Delta\delta \approx 0.1$ ppm).[104] Therefore, we assigned the (*R*) configuration to the major epoxide **31a**.

Both (*R*)- and (*S*)-epoxides in mixture were opened with sodium azide, 1,2-aminoethanol, 1,3-aminopropanol, 1,2-diaminoethane, and 1,3-diaminopropane, respectively, in order to introduce hydrophilic side chains positively charged at physiological pH attached to the anomeric position (Schemes 4 and 5). After opening of the epoxide mixture with sodium azide, it was possible to isolate by chromatography on silica gel the two main products in good yields (57 and 29%). Those compounds were characterized by NMR and mass spectrometry as being two diasteroisomers **32** (major) and **33** (minor) (Scheme 4). However, it was not possible to determine by NMR their configuration. The 2:1 ratio in isolated diasteroisomers was similar to the ratio of both starting epoxides determined by NMR, and thus the (*R*) configuration was assigned to the major isomer **32** according to the epoxide

**Scheme 5. Hydrolysis and Opening of the Isolated 2:1 Epoxide Mixture 31a,b to Lead to Hydrophilic Neosamine Derivatives of AAG 44−47**[a]




[a]Reagents and conditions: (a) TFA/$H_2O$, rt, 14 h; **39a,b**, 93%. (b) Amine in excess, DCM, 35 °C, 6 h; **40a,b**, 91%; **41a,b**, 99%; **42a,b**, 86%; **43a,b**, 96%. (c) TFA, DCM, rt, 4 h; **44a,b**, 91%; **45a,b**, 93%; **46a,b**, 89%; **47a,b**. 72%.



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**Table 1. clogP Values Calculated Using MarvinSketch Software 5.11.4[105] for the Tetraprotonated Amphiphilic Neamine and Neosamine Derivatives Used in This Study in Comparison to Reference Compounds**

| antibacterials | | | | | |
|---|---|---|---|---|---|
| reference antibacterials selected as comparators and 3′,6- and 3′,4′-dialkyl neamines | | neosamine derivatives | | | |
| compd | clogP | neosamines | clogP | 1-($CH_2$-CHOH-$CH_2$R)-3,4-dinonyl neosamines R = | clogP |
| neamine **2** | −19.4 | α-allyl-3,4-di2NP **28** | −0.92 | $N_3$ **34** | −2.83 |
| colistin | −29.7 | α-allyl-3,4-diNn **29** | −1.39 | OH **39a,b** | −3.55 |
| neomycin B **1** | −29.9 | α-allyl-3,4-di2NM **17** | −2.21 | $NH_2$ **37**, **38** | −7.04 |
| | | α-allyl neosamine **19** | −8.93 | $NH(CH_2)_3OH$ **45a,b** | −7.49 |
| Di2NP **5**[27] and **10** | −11.4 | | | $NH(CH_2)_2OH$ **44a,b** | −7.54 |
| DiNn **7**[27] and **11** | −11.9 | | | $NH(CH_2)_3NH_2$ **47a,b** | −10.98 |
| Di2NM **4** and **8**[25] | −12.7 | | | $NH(CH_2)_2NH_2$ **46a,b** | −11.03 |

**Table 2. Minimum Inhibitory Concentrations (MIC) of the Neamine and Neosamine Derivatives Synthesized and Some Representative AG against Sensitive and Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* Strains[d]**

| Aminoglycosides | MIC μg/mL | | | | | | |
|---|---|---|---|---|---|---|---|
| | *S. aureus* | | | *P. aeruginosa* | | | |
| | ATCC 25923 | SA-1 Pump NorA | ATCC 33592 HA-MRSA | ATCC 27853 | Psa. FO3[a] | PA22[b] | PA406[c] |
| Amikacin | 4 | 2 | 8-16 | 2-4 | 8 | 8 | 1 |
| Gentamicin | 0.25-0.5 | 0.5 | 1 | 1 | >128 | 4 | < 0.25 |
| Tobramycin | 0.25 | 0.5 | 64 | < 0.25 | 128 | 0.5 | < 0.25 |
| Neomycin B **1** | 1-2 | 0.5 | >128 | 64 | 128 | 32-64 | 2 |
| Neamine **2** | 16-32 | 8 | >128 | >128 | >128 | >128 | 64 |
| Dialkyl neamines | | | | | | | |
| 3',6-di2NM **4**[25] | 8 | 4-8 | 8-16 | 64-128 | 64-128 | >128 | 2-4 |
| 3',4'-di2NM **8**[25] | 2-4 | 2-4 | 4-8 | 16 | 128 | 128 | 2-4 |
| 3',6-di2NP **5**[27] | 2 | 2 | 2 | 8-16 | 16-32 | 16-32 | 2 |
| 3',4'-di2NP **10** | 2-4 | 0.5-1 | 1-2 | 4 | 8 | 16 | 4 |
| 3',6-diNn **7**[27] | 1-2 | 2 | 2-4 | 2-4 | 4-8 | 4 | 2-4 |
| 3',4'-diNn **11** | 4 | 2 | 4 | 8-16 | 8-16 | 8-16 | 8-16 |
| Allyl neosamines | | | | | | | |
| allyl neosamine **19** | >128 | >128 | >128 | >128 | >128 | >128 | >128 |
| 3,4-di2NM **17** | 8 | 8 | 8 | >128 | >128 | >128 | 32 |
| 3,4-di2NP **28** | 4-8 | 4 | 4-8 | 128 | >128 | >128 | 32 |
| 3,4-diNn **29** | 4 | 4 | 4 | >128 | >128 | >128 | >128 |
| 1- ($CH_2$-CHOH-$CH_2$R)-3,4-Dinonyl neosamines, R= | | | | | | | |
| $N_3$ (major) **34** | 2 | 2 | 2 | >128 | >128 | 128 | >128 |
| $NH_2$ (major) **37** | 2 | 2 | 2 | 8 | 8 | 8 | 8 |
| $NH_2$ (minor) **38** | 2 | 1 | 2 | 4-8 | 8 | 8 | 4-8 |
| OH **39a,b** | 2 | 2 | 2 | 64 | 64 | 64 | 64 |
| $NH(CH_2)_2OH$ **44a,b** | 4 | 1-2 | 1-2 | 8 | 8 | 8 | 8 |
| $NH(CH_2)_3OH$ **45a,b** | 2 | 1-2 | 1-2 | 8 | 8 | 8 | 8 |
| $NH(CH_2)_2NH_2$ **46a,b** | 2 | 2 | 2 | 8 | 4-8 | 4-8 | 8 |
| $NH(CH_2)_3NH_2$ **47a,b** | 2 | 2 | 2 | 8 | 8 | 8 | 4 |

[a]Psa.F03 AAC6′-IIA. [b]Surexp MexXY. [c]PAO509.5 ΔtriABC. [d]MIC values of AAGs strains lower than or equal to 2−4 μg/mL against *S. aureus* are highlighted in blue and MIC values equal or lower than 4-8 μg/mL against *P. aeruginosa* strains are highlighted in yellow.

opening from a nucleophilic attack on the methylene group that was confirmed by NMR (for example DEPTQ experiments). For biological evaluation, the azide **32** was deprotected by treatment with TFA to give the azido AAGs **34** (major *R*). The reduction of azides **32** and **33** with $PPh_3/H_2O$ led to the corresponding amine **35** and **36**, which were deprotected in the presence of TFA to afford AAGs **37** and **38**, respectively.

NOE experiments were performed at 500 and 600 MHz with **37** and **38** in $CD_3OD$. The NOESY spectra revealed only for the minor isomer **38** a correlation between the anomeric C1-proton and the proton carried by the asymmetric 2′-carbon



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Table 3. Minimum Inhibitory Concentrations (MIC) of the Neamine and Neosamine Derivatives Synthesized and Some Representative AG against Selected Bacterial Sensitive and Resistant *Acinetobacter lwoffi*, *Escherichia coli*, and *Klebsiella pneumonia* Strains[d]

| Aminoglycosides | MIC μg/mL | | | | | |
|---|---|---|---|---|---|---|
| | *A. lwoffi* | | *E. coli* | | | *K. pneumonia* |
| | ATCC 17925 | AI.88-483[a] | ATCC 25922 | PAZ505H8101[b] | L8058.1[c] | ATCC 700603 |
| Amikacin | < 0.25 | 128 | 4 | 64 | 2 | 1 |
| Gentamicin | < 0.25 | 2 | 0.5 | 1 | 64 | 4-8 |
| Tobramycin | < 0.25 | 0.5-1 | 1 | 32 | 64 | 4-8 |
| Neomycin B **1** | < 0.25 | 64-128 | 2 | 4 | 1 | 4-8 |
| Neamine **2** | 0.5 | >128 | 32 | >128 | 32 | 32 |
| | Dialkyl neamines | | | | | |
| 3',6-di2NM **4** [25] | 4-8 | 128 | 32-64 | 32 | 64-128 | 128 |
| 3',4'-di2NM **8** [25] | 4 | >128 | 16-32 | 8-16 | 16-32 | >128 |
| 3',6-di2NP **5** [27] | 2 | 32-64 | 16 | 8 | 16 | 32 |
| 3',4'-di2NP **10** | 2-4 | 32-64 | 16-32 | 2 | 4-8 | 64 |
| 3',6-diNn **7** [27] | 1-2 | 32 | 2-4 | 2-4 | 2-4 | 2-4 |
| 3',4'-diNn **11** | 2-4 | 32 | 4-8 | 2-4 | 4-8 | 16-32 |
| | Allyl neosamines | | | | | |
| allyl neosamine **19** | >128 | >128 | >128 | >128 | >128 | >128 |
| 3,4-di2NM **17** | 8 | 64 | 64 | 64 | 128 | >128 |
| 3,4-di2NP **28** | 4-8 | 64 | 32-64 | 64 | 64 | 128 |
| 3,4-diNn **29** | 4-8 | 32 | 32-64 | >128 | >128 | 128 |
| | 1- ($CH_2$-CHOH-$CH_2$R)-3,4-Dinonyl neosamines, R= | | | | | |
| $N_3$ (major) **34** | 4 | >128 | 32 | >128 | >128 | >128 |
| $NH_2$ (major) **37** | 2 | 8 | 4 | 4 | 4 | 8-16 |
| $NH_2$ (minor) **38** | 2 | 4 | 4 | 4 | 4-8 | 8 |
| OH **39a,b** | 1 | 8 | 8 | 16 | 8 | 64 |
| $NH(CH_2)_2OH$ **44a,b** | 2 | 4-8 | 4-8 | 8 | 4-8 | 8 |
| $NH(CH_2)_3OH$ **45a,b** | 1 | 8 | 4-8 | 4 | 4 | 8-16 |
| $NH(CH_2)_2NH_2$ **46a,b** | 2 | 16-32 | 8 | 4-8 | 4-8 | 8-16 |
| $NH(CH_2)_3NH_2$ **47a,b** | 2 | 8 | 8 | 4 | 8 | 32 |

[a]APH3′-VIA. [b]AAC6′-IB. [c]ANT2″-IA. [d]MIC values of AAGs lower than or equal to 4 μg/mL are highlighted in green and MIC values equal to 4−8 or 8 μg/mL highlighted in purple.

atom in the side chain, whereas correlations between the H-1 and H-1′ were detected for both isomers. Such an additional correlation should be optimal in conformations in which the C1, H1, O1, C1′, C2′, and H2′ atoms are nearly coplanar. In such conformations, the sugar intracyclic oxygen atom and the 2′-oxygen atom of the 2′-hydroxyl group are in a relative trans position in the (*S*)-isomer minimizing the electronic repulsive effects induced by the oxygen atoms, whereas their relative cis position in the (*R*)-isomer should disadvantage a NOE correlation. Thus, the observed NOE effect confirmed the (*S*)-configuration assigned to the minor isomer from the proposed asymmetric induction observed in the epoxidation.

Because **37** and **38** could be used to evaluate the role of the stereochemistry of the side chain in the biological properties, the next synthesis were performed without separation of the diasteroisomers formed in the opening of the mixture of epoxides (Scheme 5). Hydrolysis of the epoxide mixture **31a,b** and deprotection were performed in the presence of TFA to lead to the mixture of AAGs **39a,b**. The opening of **31a,b** with different amines was performed by heating at 35 °C in DCM in the presence of an excess of amine. The reactions with 1,2-aminoethanol, 1,3-aminopropanol, and with 1,2-diaminoethane led in excellent yields under mild conditions to the mixtures of isomers **40a,b**, **41a,b**, and **42a,b**, respectively, which cannot be separated on TLC. They were deprotected in TFA to afford AAGs **44a,b**, **45a,b**, and **46a,b**, respectively. The opening of the epoxides **31a,b** with 1,3-diaminopropane in DCM led surprisingly to the isomers **43a,b** in which an additional methylene group was observed by $^1$H and $^{13}$C NMR and mass spectrometry. The presence of this methylene group results from cyclization of 1,3-diamino group with the solvent DCM (Scheme 5). It was removed concomitantly with the other carbamate protecting groups by treatment with TFA to give AAG **47a,b**. In the mixtures **40a,b**−**47a,b**, it was not possible to detect the presence of the diasteroisomers by NMR.

## LIPOPHILICITY OF THE SYNTHESIZED DERIVATIVES

In our previous report on the antibacterial activity of amphiphilic neamine derivatives, their lipophilicity expressed by clogP values has been identified to be a key parameter in the


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antibacterial activity as well as in their cytotoxicity. The clogP values of the completely protonated derivatives used in this study were calculated using the MarvinSketch software 5.11.4 and are compared in Table 1.[105]

## ANTIBACTERIAL ACTIVITY, RESULTS AND DISCUSSION

Three modes of bacterial resistance to AGs have been identified: (i) reduction in the intracellular concentration of the antibiotics by efflux pump proteins or through reduced membrane permeability, (ii) deactivation by AG-modifying enzymes, and (iii) structural modifications of the 16S rRNA binding site that lead to reduced target affinity.[8−19] Many AG-inactiving enzymes that modify the hydroxyl and/or amine functions have been identified and are classified in three families: AG nucleotidyltransferases (ANTs), AG phosphotransferases (APHs), and AG acetyltransferases (AACs).[8−16] Regarding the modifications of 16S rRNA, the methylation of specific nucleotides within the A-site hampers binding of AGs and appeared more and more to be a serious threat to the aminoglycoside antibiotics through the action of plasmid-mediated methyltransferases (r-methylases).[16−19] These enzymes that are spreading to different species confer high levels of resistance to clinically useful AG such as amikacin, tobramycin, and gentamicin. In many cases, AG-resistant bacteria have selected combinations of resistance mechanisms that render them very difficult to eradicate.

The synthesized amphiphilic aminoglycosides were evaluated against a large panel of Gram-positive and Gram-negative bacteria. In the former class of bacteria, the mimimum inhibitory concentrations (MIC) were measured against sensitive *Staphylococcus aureus* ATCC 25923 and two resistant strains, the SA-1 strain surexpressing resistance pump (NorA) and the methicillin-resistant strains ATCC 33592 HA-MRSA (Table 2). In the Gram-negative class of bacteria, the effects were evaluated against sensitive and resistant strains of *P. aeruginosa* (Table 2), *Acinetobacter lwoffi*, and *Escherichia coli* surexpressing aminoglycoside-modifying enzymes or efflux pumps and against the sensitive ATCC 700603 *Klebsiella pneumoniae* strain (Table 3).

**Antibacterial Activities of the 3′,4-Dialkyl Neamine Derivatives 8, 10, and 11.** Against the selected *S. aureus* strains (Table 2), the 3′,6-2NM neamine **4** showed the highest MIC values (4−16 μg/mL) and the 3′,4′-2NM neamine **8** exhibited lower MIC values (2−8 μg/mL). The 3,4′-di2NP (**10**) and -diNn (**11**) neamine derivatives showed MIC values slightly lower than those measured with the 3′,4′-di2NM derivative **8** (MIC = 0.5−4 and 2−8 μg/mL, respectively). No significant difference in the MIC values were observed between **10** and **11** and their 3′,6-isomers **5** and **7**, respectively. Among the six evaluated dialkyl neamine derivatives, the best derivatives appeared to be the 3′,4′-di2NP derivative **10** reported here.

Against *P. aeruginosa* strains (Table 2), in contrast to the 3′,4′-di2NM (**8**) and 3′,6-di2NM (**4**) derivatives which are inactive, the 3,4′-di2NP **10** showed low MIC values slightly lower than those observed with the 3′,4′-diNn derivative **11** (MIC= 4−16 and 8−16 μg/mL, respectively). As previously observed in the 3′,6-dialkyl series, increases of the clogP values from the 2NM to the Nn and 2NP derivatives (Table 1) strongly enhance the antibacterial activity against Gram-negative bacteria. In the 3′,4′-series, the most active derivative is the 2NP derivative (**10**), whereas, in the 3′,6-series, the diNn derivative **7** appeared to be the best anti-*P. aeruginosa* agent. These Nn and 2NP derivatives have close lipophilicities (Table 1). Interestingly, all derivatives showed an activity against *P. aeruginosa* PAO509.5 ΔtriABC (MIC = 2−16 μg/mL).

As shown in Table 3, low MIC values against the sensitive strain of *A. lwoffi* were obtained with the 3′,4′-dialkyl derivatives evaluated (MIC = 2−4 μg/mL) as well as with the 3′,6-dialkyl derivatives. Higher MIC values against the resistant strain AI.88−483 (MIC = 32 to >128 μg/mL) were measured and the di2NM derivatives **4** and **8** were found to be inactive (MIC = 128 and >128 μg/mL, respectively). For the same lipophilic group introduced, the measured MIC values in the 3′,4′- and 3′,6-series were close. The difference of activity against sensitive and resistant *A. lwoffi* observed for the 3′,4′- and 3′,6-dialkyl derivatives is surprising. This difference cannot be related to the overexpression of the APH3′-VIA enzyme in the AI.88-483 strain and the corresponding AAG 3′-modification because an alkyl group is present at the 3′-position. It could be related to difference in the membrane structure of sensitive and resistant *A. lwoffi*.

Against the sensitive and resistant *E. coli* strains (Table 3), among the three 3′,4′-dialkyl neamines evaluated, **8**, **10**, and **11**, the 3′,4′-diNn derivative **11** appeared to be the most active (MIC = 2−8 μg/mL). Similarly, in the 3′,6-series, the diNn derivative **7** showed the lowest MIC values (MIC = 2−4 μg/mL) as well as against the selected *P. aeruginosa* strains.

Against sensitive *K. pneumonia*, the 3′,4′-di2NM compound **8** and its 3′,6-isomer **4** were inactive as well as against the resistant strains of *A. lwoffi* (MIC > 128 μg/mL). The MIC values measured with the 3′,4′-di2NP (**10**), 3′,4′-diNn (**11**), and 3′,6-di2NP (**5**) neamines appeared to be high (MIC = 16−64 μg/mL for) in comparison to the MIC obtained with the 3′,6-diNn derivative **7** showing low 2−4 μg/mL MIC.

**Antibacterial Activities of α-Allyl-3,4-dialkyl Neosamines 17, 28, and 29 and Hydrophilic 3,4-Dialkyl Neosamines 34, 37−39, and 44−47.** The allyl derivatives **17**, **28**, and **29** showed only a good activity against *S. aureus* and sensitive *A. lwoffi* strains (MIC = 4−8 μg/mL). The weak or lack of antibacterial activity against the other Gram-negative strains of these compounds should be related to their high lipophilicity (Table 1; clogP = −0.9 to −2.2) as previously observed in the 3′,6-dialkyl neamine series (in this series, clogP value have to be lower than −9 for obtaining a good and broad-spectrum activity).[27] The lack of antibacterial activity of the synthesized hydrophilic parent compound **19** (MIC > 128 μg/mL) confirmed the essential role of the lipophilic groups.

As shown in Table 2, the neosamine derivatives **34**, **37**, **38**, **39a**,**b**, and **44a**,**b**−**47a**,**b**, more hydrophilic than the allyl derivatives **17**, **28**, and **29** (Table 1), showed close and low MIC values against the *S. aureus* strains (MIC = 1−4 μg/mL, mainly 2 μg/mL). The most lipophilic derivatives in this hydrophilic series of neosamine derivatives, the azide **34** (Table 1), appeared to be inactive against the selected Gram-negative bacteria (Tables 2 and 3) except against the sensitive *A. lwoffi* and *E. coli* strains (MIC = 4 and 32 μg/mL, respectively). This azide carries one or two amine function(s) protonated at physiological pH less than the other derivatives in the series and its lipophilicity is probably too high (clogP = −2.8) as previously observed in the neamine series.[27] Among the less lipophilic neosamine derivatives than **34** (Table 3), the diols **39a**,**b** that are, after **34**, the most lipophilic compounds in the series (clogP = −3.6), showed high MIC values against *P. aeruginosa* and *K. pneumonia* strains (Tables 2 and 3; MIC = 64


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μg/mL) and lower good MIC values against all *A. lwoffi* and *E. coli* sensitive and resistant strains (Table 3; MIC = 1−8 and 8−16 μg/mL, respectively).

All other hydrophilic neosamine derivatives **37**, **38** , and **44a,b**−**47a,b** showed low to medium MIC values against *P. aeruginosa*, *A. lwoffi*, *E. coli*, and *K. pneumonia* strains (MIC = 4−8, 1−16, 4−16, and 8−32 μg/mL, respectively). Clearly, the decrease of the lipophilicity (Table 1; clogP = −7 to −11) and the addition of at least one amine function protonated at physiological pH in comparison to the allyl derivatives and the azide **34** strongly increase the antibacterial activity against Gram-negative bacteria. Indeed, at physiological pH, compounds **46** and **47** can bear only three positive charges due to the proximity of the amine functions in their flexible 1-alkyl chain.

Concerning the role in the antibacterial effects observed of the stereochemistry of the hydrophilic side chain attached to the 1-anomeric position (*R* and *S* isomers), the evaluation of the isomeric α-aminoalcohols **37** and **38** led to close MIC values showing weak effect on the antibacterial activity of the configuration of the asymmetric 2′-carbon atom found in the 1-hydrophilic side chain. Moreover, weak variations in the antibacterial activity were observed by replacement in the hydrophilic side chain of a terminal hydroxyl group by an amine function (from compounds **44a,b** to **46a,b** and from **45a,b** to **47a,b**) and by addition a methylene group between the terminal hydroxyl or amine function and the central amino function (from compounds **44a,b** to **45a,b** and from **46a,b** to **47a,b**).

Overall, except for **39a,b**, all derivatives retain antimicrobial activity against all sensitive and resistant strains, even those against which conventional AGs are inactive (for examples, gentamicin against *P. aeruginosa* expressing AAC6′-IIA or *E. coli* expressing ANT2″-IA).

## CYTOTOXICITY

Using the MTT assay, the viability of murine J774 macrophages were evaluated in the presence of 10 and 30 μM of the 3′,4′-dialkyl neamines (**8**, **10**, and **11**) and neosamines (**34**, **37**−**39**, **44**−**47**) derivatives described here. The viability values were compared in Table 4 to those measured under the same conditions in the presence of the antibacterial 3′,6-dialkyl neamines **4**, **5**, and 7 previously described, of conventional AG drugs and the prepared reference AG in the neosamine series, compound **19**, which does not carry lipophilic chains.

At 10 μM, the lipophilic neosamine derivative **34** carrying an azido group showed the lowest viability (62%) whereas all other evaluated derivatives exhibited viability higher than 78%.

Among them, the less lipophilic neosamine derivatives having good and broad-spectrum antibacterial activity **37**−**39** and **44**−**47** showed 85−100% viability.

At 30 μM, the viability decreased for the majority of the evaluated compounds including conventional AGs drugs with the exception of amikacin (92% viability), the 3′,6-di2NP neamine derivative **5** (90% viability), and the neosamine reference compound **19** (82% viability) that has no antibacterial activity.

In the neamine series, the viability strongly decreased from 10 to 30 μM in the presence of the 3′,4′- and 3′,6-di2Nn neamine derivatives **11** and 7 (from 83 to 49% and from 78 to 39%, respectively). It decreased to a lower extent in the presence of the other neamine derivatives, from 88 to 67% in the presence of the active 3′,4′-di2NP neamine **10** whereas unchanged in the presence of the 3′,6-di2NP isomer **5** (91 and 90% from 10 to 30 μM).

**Table 4. Viability (%) of Murine J774 Macrophages Determined Using the MTT Assay in the Presence of 10 and 30 μM of the Prepared Neamine and Neosamine Derivatives**[a]

| Aminoglycosides | Viability | |
|---|---|---|
| | 10 μM | 30 μM |
| Amikacin | 81.2 (3) | 92.0 (2) |
| Gentamicin | 88.2 (9) | 74.2 (2) |
| Tobramycin | 99.7 (4) | 83.2 (2) |
| Neomycin B **1** | 87.3 (10) | 69.8 (2) |
| Neamine **2** | 94.8 (9) | 84.4 (2) |
| Dialkyl neamine | | |
| 3′,6-di2NM **4** [25] | 86.0 (7) | 75.9 (1) |
| 3′,4′-di2NM **8** [25] | 79.1 (2) | / |
| 3′,6-di2NP **5** [27] | 91.1 (13) | 89.5 (2) |
| 3′,4′-di2NP **10** | 87.7 (6) | 66.9 (2) |
| 3′,6-diNn **7** [27] | 78.4 (10) | 38.8 (2) |
| 3′,4′-diNn **11** | 82.7 (4) | 48.6 (2) |
| Allyl neosamines | | |
| allyl neosamine **19** | 82.2 (5) | 82.1 (2) |
| 3,4-di2NM **17** | 86.9 (4) | 28.3 (2) |
| 3,4-di2NP **28** | 105.3 (7) | 7.0 (1) |
| 3,4-diNn **29** | 91.4 (6) | 6.6 (1) |
| 1-($CH_2$-CHOH-$CH_2$R)-3,4-dinonyl neosamines, R= | | |
| $N_3$ (major) **34** | 62.2 (4) | 5.9 (2) |
| $NH_2$ (major) **37** | 88.0 (5) | 14.4 (1) |
| $NH_2$ (minor) **38** | 85.1 (5) | 7.2 (1) |
| OH **39a,b** | 89.7 (4) | 8.8 (2) |
| $NH(CH_2)_2OH$ **44a,b** | 99.1 (7) | 10.1 (2) |
| $NH(CH_2)_3OH$ **45a,b** | 89.9 (5) | 13.7 (2) |
| $NH(CH_2)_2NH_2$ **46a,b** | 87.5 (4) | 53.0 (2) |
| $NH(CH_2)_3NH_2$ **47a,b** | 100.9 (8) | 71.6 (2) |

[a]The numbers of independent experiences are mentioned after the viability values in brackets.

In the neosamine series, the viability strongly decreases for the majority of the derivatives (**17**, **28**, **29**, **34**, **37**, **38**, **39a,b**, and **44a,b**,**45a,b**; viability values from 85 to 100% at 10 μM to 6−28% at 30 μM). For the tetra-amino derivatives **46a,b** and **47a,b**, the viability decreased less strongly, from 88 to 53 in the presence of **46a,b** and from to 100 to 72% for **47a,b**.

Thus, the less cytotoxic AAGs at 30 μM are the 3′,6-di2NM (**4**), 3′,6-di2NP (**5**), and 3′,4′-di2NP (**10**) neamines (76, 90, and 67% viability, respectively) and the diamino neosamine derivatives **47a,b** (72%).

## *P. AERUGINOSA* INNER MEMBRANE PERMEABILIZATION

To investigate if antibacterial activity could be related with a membrane permeabilizing effect, propidium iodide (PI)[29,106,107] was used to investigate *P. aeruginosa* inner membrane permeabilization induced by 3′,6-diNn (**7**) and 3′,4′-diNn (**11**) neamines and 3,4-diNn neosamine **47a,b** (Figure 3). When PI passes through the cell membrane and binds to nucleic acids, fluorescence intensity increases.

The 3′,6-diNn neamine derivative (**7**) induced a dose-dependent permeabilization of sensitive *P. aeruginosa* membranes, reaching a plateau value at 5 μM. This derivative (**7**)



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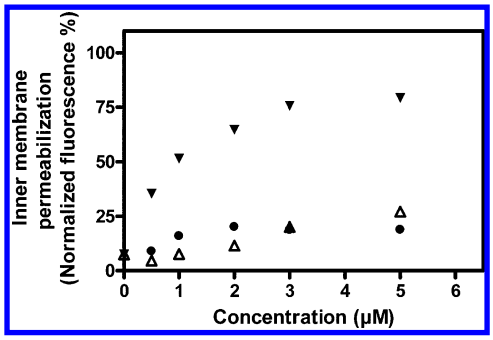


**Figure 3.** Inner membrane permeabilization of *P. aeruginosa* induced by 3′,6-diNn neamine **7** (▼), 3′,4′-diNn neamine **11** (△), and 3,4-diNn neosamine **47a,b** (●) as assessed by enhancing propidium iodide fluorescence.

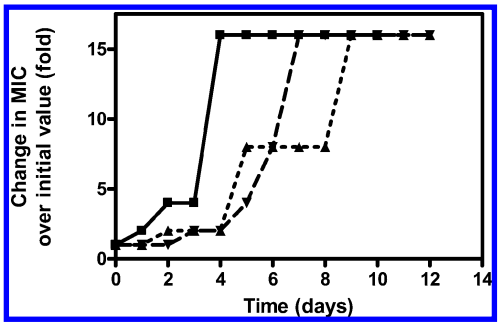


**Figure 4.** Evolution of the MIC against *P. aeruginosa* of 3′,6-di2NP neamine **5** (dotted line) and 3′,4′-di2NP neamine **10** (dots) in comparison with MIC of ciprofloxacin (solid line) after exposure to half-MIC concentrations for the indicated times. The concentration of the antibiotic was readjusted each day to remain equivalent to half the MIC. Results are expressed in changes in MICs over initial value.

induced a higher effect compared to that obtained with 3′,4′-diNn neamine (**11**) or 3,4-diNn neosamine **47a,b** (75% and 25% permeabilization both, respectively).

Interestingly, these results demonstrated the critical role of the position of the hydrophobic substituents (3′,6 versus 3′,4′), with a higher effect on inner membrane permeabilization induced when the substituents are more distant. When the hydrophobic substituent were adjacent (3′,4′-diNn neamine **11** and neosamine derivatives **47a,b**), no major difference was observed upon modification of ring I. The small effect on inner membrane permeabilization induced by neamine **11** and neosamines **47a,b** is probably sufficient to provoke membrane depolarization and bacterial cell death. A similar behavior has been described for some ceragenins,[108] which showed membrane depolarization and bactericidal effect without effect on inner membrane permeabilization to probes such as *O*-nitrophenyl-β-D-galactoside. Elucidation of the respective roles of membrane permeabilization and depolarization is therefore critical for understanding the mechanisms of action of antimicrobial agents and for providing evidence of the role of membrane integrity in bacterial viability. The absence of correlation between bacterial inner membrane permeabilisation and low values of MIC could be explained by differences in binding to bacterial outer membranes or to other mechanisms like changes in lipid environment required for proper activities of proteins inserted within lipid bilayers.

## MIC CHANGES AGAINST *P. AERUGINOSA* UPON LONG EXPOSURE TO AAGS

To study MIC changes upon exposure to 3′,6-di2NP (**5**) and 3′,4′-di2NP (**10**) neamines in comparison to the fluoroquinolone ciprofloxacin, *P. aeruginosa* ATCC 27853 were grown in the continuous presence of a drug concentration corresponding to half of the MIC.[109]

The changes in MIC were observed during the 12 days of exposure to half-MIC concentrations of 3′,6-di2NP (**5**) and 3′,4′-di2NP (**10**) neamines in comparison to ciprofloxacin (Figure 4). Ciprofloxacin induced a marked increase of MIC (∼15-fold) at day 4. In comparison, the effect afforded by both di2NP neamines **5** and **10** appeared slower with a 15-fold increase observed after days 7 and 9, respectively.

These results show that exposure of *P. aeruginosa* to subinhibitory concentrations of ciprofloxacin, 3′,6-di2NP (**5**), and 3′,4′-di2NP (**10**) neamines caused a decrease in susceptibility which appears later for new amphiphilic neamine derivatives as compared to ciprofloxacin. The resistance mechanisms will be investigated in another study.

## DISCUSSION AND CONCLUSION

**Comparison of the Antibacterial Activity along the Bacteria Strains and Structure−Activity Relationships.** Among the AAGs compared here for their antibacterial activity, the 3′,6-diNn neamine **7** previously described is the most active against all selected bacteria strains excepted the resistant *A. lwoffi* strain (MIC = 1−8 μg/mL against sensitive and resistant *S. aureus*, *P. aeruginosa*, *E. coli* and sensitive *A. lwoffi* and *K. pneumonia* and MIC= 32 μg/mL against the resistant *A. lwoffi* strain). Its 3′,4′-diNn isomer **11** showed similar good activities against sensitive and resistant *S. aureus* and *E. coli* strains and sensitive *A. lwoffi* and lower activities against sensitive and resistant *P. aeruginosa* strains and sensitive *K. pneumonia*. The 3′,4′-di2NP derivative **10** having close lipophilicity to **7** and **11** has good activities similar to the ones of **7** against sensitive and resistant *S. aureus* and *P. aeruginosa*, resistant *E. coli* and sensitive *A. lwoffi*, activities stronger than those of its 3,′6-di2NP isomer **5**.

Concerning the neosamine derivatives prepared, the more hydrophilic **37**, **38**, **39a,b**, **44a,b**−**47a,b** having clogP values between −7 to −11 (Table 1) showed similar good antibacterial activity against all selected sensitive and resistant strains (MIC= 1−16 μg/mL). Their antibacterial activities are weaker than the ones of **7** mainly against sensitive *K. pneumonia* (MIC= 8−16 and 2−4 μg/mL, respectively) but better against resistant *A. lwoffi* (MIC= 8−16 and 32 μg/mL, respectively). They are the most active derivative against this resistant strain.

These results point out the existence of different structure−activity relationships against the selected Gram-negative bacteria strains in regard to the role of ring I and its acyclic scaffold mimics. On the contrary, good and close activities were obtained with neamine and neosamine derivatives against *S. aureus* strains in the three series of compounds studied (3′,4′- and 3′,6-dialkyl neamines and 3,4-dialkyl neosamine derivatives).

Regarding Gram-negative bacteria and compounds having similar lipophilicity near to −11 (Table 1), the results obtained are not very different in the neamine and neosamine series against *P. aeruginosa* and *E. coli* strains. Against *A. lwoffi* and *K. pneumonia* strains, flexibility appears to increase the antibacterial activity.



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In the neamine series, the di2NP and diNn derivatives have close clogP values (Table 1). Among them, the 3′,4′-di2NP (**10**) and 3′,6-di2NP (**5**) derivatives show the best activity against *S. aureus* strains. Against *P. aeruginosa* strains, in 3′,4′-series, the 2NP and Nn substituents led to similar results whereas, in the 3′,6-series, the diNn substituent produces better antibacterial effects than the di2NP group. Better activities of the 3′,4′-di2NP (**10**) and 3′,6-diNn (**7**) neamine derivatives are also observed against *E. coli* strains. A comparative study of the modes of action of these derivatives on bacterial membranes could explain this result that merits attention for future lead selection.

In the neamine and neosamine series, the viability of murine J774 macrophages appeared to be mainly higher or close to 80% at 10 $\mu$M. It decreases significantly at 30 $\mu$M in the neamine series in the presence of the strongly active 3′,4′- and 3′,6-di2Nn neamine derivatives **11** and **7** and decreases even more drastically in the presence of the active neosamine derivatives **37**, **38**, **39a,b** and **44a,b**−**47a,b**. The viability remains close to 90% at 30 $\mu$M in the presence of the active 3′,6-di2NP neamine **5** and decreased to be near to 70% in the presence of the 3′,4′-di2NP **10** and the tetra-amino derivatives **47a,b**. These three compounds have strong antibacterial activity against the sensitive and resistant *S. aureus* strains and showed viability near or higher than 90% at 5 to 15-fold the corresponding MIC, respectively.

The amphiphilic hydrophilic neosamine derivatives synthesized extend the spectrum of action of the antibacterial AAGs in the class of Gram-negative bacteria to the resistant *A. lwoffi*. Focusing on *A. lwoffi*, all dialkyl neamine derivatives are weakly active or inactive against the selected resistant strain. The molecular basis of this result probably related to the composition and structure of the membranes of the resistant strain merits to be studied because multidrug-resistant *A. lwoffi* clinical isolates are increasingly reported worldwide.

Concerning the chemical modifications performed in the acyclic scaffolds of the neosamine derivatives, the results point out that (i) the lipophilicity of the derivatives and/or the presence of at least three amine functions are parameters especially critical for a good activity against *P. aeruginosa* strains, (ii) the clogP values of the antibacterial neosamine derivatives have to be lower than −3.5 for exhibiting a large spectrum of action, and (iii) the modifications made in the acyclic scaffold weakly affect the antibacterial activity in the −7.5 to −11.0 clogP range for compounds having at least at physiological pH one protonated amine function in the side chain. Thus, we can conclude that ring I is the main pharmacophoric element in the antibacterial activity of amphiphilic neamine and neosamine derivatives and that the presence at physiological pH of at least three protonated amine functions and not more are necessary for a good antibacterial activity. This conclusion completes the structure−activity relationships delineated in our previous report, leading to the conclusion that the lipophilicy of dialkyl neamine derivatives have to be included in the window −12.5 to −9 for obtaining good antibacterial activities.[27] We are not far from this range in the neosamine series.

In conclusion, we show here that the broad spectrum of antibacterial activity observed previously in the 3′,6′-dialkyl and 3′,4′,6-trialkyl neamine series can be extended to the 3′,4′-dialkyl neamine and 3,4-dialkyl neosamine series. Membrane permeabilization assays performed with the most active dinonyl derivative in each series (**7**, **11**, and **47a,b**) showed permeabilization of the *P. aeruginosa* inner membrane with emphasis for the 3′,6-dinonyl neamine derivatives. Regarding the development of resistance, first measurements of MIC changes against *P. aeruginosa* upon long exposure to the 3′,6- and 3′,4′-di2NP neamine derivatives showed a slower increase of MICs in comparison to ciprofloxacin. The mechanisms of action and resistance will be studied further.

## EXPERIMENTAL SECTION

**Calculation of clogP Values.** The lipophilicity character of the neamine derivatives prepared was estimated through the calculation of clogP values (octanol/water partition coefficients) using the MarvinSketch software [Marvin 5.11.4, 2012, ChemAxon (http://www.chemaxon.com)]. The clogP plug-in in this software calculates the octanol/water partition coefficient, which is used in QSAR analysis and rational drug design as a measure of molecular hydrophobicity. The calculation method used here is based on a modification of the method published by Viswanadhan and Ghose et al. (VG method).[105] The clogP of the substituents were determined through calculation with the same method from the structure of the corresponding alkanes.

**Synthesis.** *General Procedures. Procedure I.* General procedure for the deprotection of the alkylated tetra-*N*-tritylated neamine and Boc-*N*-neosamine derivatives. The protected compound was dissolved at 0 °C or at room temperature in $CH_2Cl_2$/TFA (4/1, v/v). For the tetra-*N*-tritylated neamines, anisole (0.1 mL/mL) was added. After 2 h stirring at rt, the solvents were evaporated under reduced pressure. $H_2O$ and $Et_2O$ were added and the aqueous phase was washed twice with $Et_2O$ before being evaporated to dryness and then the residue was chromatographed on C18 reversed phase eluting with a $H_2O$/MeOH gradient and obtained pure as a TFA salt.

*Procedure II.* General procedure for opening of epoxides **31a,b** by amine substrates. To a solution of epoxides **31a,b** dissolved in $CH_2Cl_2$ were added the amines in excess. After 16 h at 35 °C, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (×3). The dried organic layer was evaporated to dryness. The residue was chromatographed on silica gel with ethyl acetate/methanol (5−10%) to give the *β*-aminoalcohol derivatives.

*Purification.* The aminosugar purity of the evaluated compounds was ≥95%. Before the final deprotection step under acidic conditions, careful purifications of tritylated or Boc derivatives allowing minor isomers or impurities removal were performed by chromatography on silica gel.

The purities were measured by HPLC for the derivatives carrying chromophores and was controlled by $^1$H NMR spectrometry and TLC on silica gel (eluent, EtOH/$H_2O$/($NH_3$, $H_2O$) (20%) 80:10:10; TLC visualizations, sulfuric acid spray (5 mL in 100 mL of EtOH) and ninhydrin spray (0.3 g, 3 mL AcOH, 100 mL of EtOH)). For example, under these TLC conditions, the retardation factors of the 3′,6-dinonyl derivative 7 and its 3′,4′-isomer **11** were 0.5 and 0.3, respectively. It was not possible to detect by NMR at 400 MHz the presence of two diastereoisomers in compounds **39a,b** and **44a,b**−**47a,b**.

**3′,4′-Di-*O*-alkylneamines.** *3′,4′-Di-O-(3″-(2‴-naphthyl)propyl)-neamine (**10**).* To a solution of compound **9**[96] (0.75 g, 0.53 mmol) in dry DMF under argon were added NaH (60%, 213 mg, 5.31 mmol), and then, after 30 min at rt, 2-(3′-bromopropyl)naphthalene (0.53 g, 2.13 mmol). After 1 h stirring at rt, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (×3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with toluene/ethyl acetate (95:5) to give the *N*-tetratrityl 3′,4′-*O*-di(2-naphthylpropyl)-6-*O*-*para*-methoxybenzyl neamine and 3′,4′,6-*O*-alkyl derivatives with 46% yield (0.41 g, 0.24 mmol). HRMS (ESI$^+$) *m*/*z*: [M + K]$^+$ calcd, 1785.8319; found, 1785.8327. HRMS (ESI$^+$) *m*/*z*: [M + Na]$^+$ calcd, 1769.85797; found, 1769.8570. The deprotection of 0.30 g of this product was achieved following procedure II. **10**: 97% yield (0.17g, 0.17 mmol white solid). $^1$H NMR (400 MHz, $CD_3OD$) *δ* 7.74−7.16 (m, 14H, H ar), 5.89 (d, 1H, *J* = 3.7 Hz, H-1′), 4.07 (td, 1H, *J* = 2.5,6.2 Hz, H-5′), 3.95 (t, 1H, *J* = 6.5 Hz, H-4), 3.88 (dd, 1H, *J*


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= 8.6, 10.4 Hz, H-3′), 3.81−3.75 (m, 1H, $CH_2O$), 3.72−3.61 (m, 2H, $CH_2O$), 3.56 (t, 1H, *J* = 9,1 Hz, H-5), 3.52−3.47 (m, 1H, $CH_2O$), 3.43−3.36 (m, 2H, *H* = 6.3), 3.31−3.26 (m, 2H, H-6′, H-2), 3.19−3.11 (m, 2H, H-4, H-1), 3.06 (dd, 1H, *J* = 9.5, 13.1 Hz, H-6′), 2.68 (t, 2H, *J* = 7.4 Hz, $CH_2$ np), 2.61 (t, 2H, *J* = 7.6 Hz, H-$CH_2$ np), 2.40 (td, 1H, *J* = 4.1, 12.5 Hz, H-2), 2.02−1.87 (m, 3H, H-2, $CH_2$), 1.77−1.67 (m, 2H, $CH_2$). $^{13}$C NMR (100 MHz, $CD_3OD$) δ 133.6−135.1 (6C np), 126.3−129.0 (14 CH np), 96.8 (C1′), 81.4 (C4′), 79.2 (C4), 77.8 (C3′), 77.3 (C5), 74.4 (C6), 73.7, 74.1 ($2CH_2O$), 70.9 (C5′), 54.7 (C2′), 51.5 (C1), 50.1 (C3), 41.6 (C6′), 33.1−33.2 ($2CH_2$ np), 32.3, 32.6 ($2CH_2$), 30.0 (C2). HRMS ($ESI^+$) *m*/*z*: $[M + H]^+$ calcd 659.3803, found 659.3803. HRMS ($ESI^+$) *m*/*z*: $[M + Na]^+$ calcd 681.3622, found 681.3631.

*3′,4′-Di-O-(1″-nonyl)neamine (11).* To a solution of compound **9**[96] (1.73 g, 1.23 mmol) in toluene (85 mL) were added TBAF·$3H_2O$ (1.55 g, 4.92 mmol), 1-bromononane (1.04 mL, 5.55 mmol), and an aqueous solution of NaOH (50% w/w, 42.5 mL). The resulting mixture was stirred vigorously for 24 h at rt. The organic solution was diluted with ethyl acetate and then washed twice with an aqueous saturated ammonium chloride solution before being dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with toluene/ethyl acetate (95:5) to give the tetratrityl 3′,4′-*O*-dinonyl-6-*O*-*para*-methoxybenzyl neamine with 43% yield (0.88 g, 0.53 mmol). HRMS ($ESI^+$) *m*/*z*: $[M + K]^+$calcd, 1701.9259; found, 1701.9297. The deprotection of 0.50 g of this product was achieved following procedure II. **11**: 98% yield (0.30 g, 0.29 mmol, white solid). $^1$H NMR (400 MHz, $CD_3OD$) δ 5.88 (d, 1H, *J* = 3.7 Hz, H-1′), 4.06 (td, 1H, *J* = 2.5, 6.2 Hz, H-5′), 3.96 (t, 1H, *J* = 6.5 Hz, H-4), 3.88 (dd, 1H, *J* = 8.6, 10.4 Hz, H-3′), 3.80−3.73 (m, 1H, $CH_2O$), 3.72−3.60 (m, 2H, $CH_2O$), 3.57 (t, 1H, *J* = 9.1 Hz, H-5), 3.53−3.47 (m, 1H, $CH_2O$), 3.44−3.35 (m, 2H, H-6, H-3), 3.32−3.25 (m, 2H, H-6a′, H-2′), 3.19−3.10 (m, 2H, H-4, H-1), 3.06 (dd, 1H, *J* = 9.5, 13.1 Hz, H-6′b) 2.41 (td, 1H, *J* = 4.1, 12.5 Hz, H-2a), 2.04−1.86 (m, 1H, H-2b) 1.79−1.60 (m, 4H, $2CH_2$), 1.47−1.28 (m, 24H, $12CH_2$), 0.94 (m, 6H, $2CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) δ 96.7 (C1′), 82.3 (C4′), 79.0 (C4), 77.7 (C5), 77.5 (C3′), 75.1 ($CH_2O$), 74.7 ($CH_2O$), 73.5 (C6), 71.7 (C5′), 54.7 (C2′), 50.6 (C1), 50.3 (C3), 41.9 (C6′), 33.1 ($2CH_2$), 31.2 ($CH_2$), 31.1 ($CH_2$), 30.7 ($4CH_2$), 30.4 ($2CH_2$), 30.1 (C2), 27.1 ($CH_2$), 26.9 ($CH_2$), 23.7 ($2CH_2$), 14.5 (2CH3). HRMS ($ESI^+$) *m*/*z*: $[M + K]^+$ calcd 597.4562, found 597.4573. HRMS ($ESI^+$) *m*/*z*: $[M + H]^+$ calcd 575.4742, found, 575.4740.

**3,4-Di-*O*-alkylneosamines.** *Allyl 6-Azido-2,6-dideoxy-2-tritylamino-α-D-glucopyranoside (15).* To a stirred solution of **14**[97] (0.3 g, 1.23 mmol) in DMF (8 mL) and $Et_3N$ (0.5 mL) were added $Et_3N$ (0.5 mL) and trityl chloride (1.03 g, 3.68 mmol, 3 equiv) in DMF (3 mL), and the mixture was stirred at room temperature for 8 h under argon atmosphere. A saturated $NH_4Cl$ solution (10 mL) was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and filtered, and the filtrate was concentrated under reduced pressure. The residue obtained was purified by chromatography on silica gel in ethyl acetate/cyclohexane (1:3) with a few drops of $Et_3N$ to give compound **15** (0.49 g, 82%) as a white crystalline solid; mp 128−130 °C. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.54−7.56 (m, 5H ar), 7.23−7.32 (m, 10H ar), 5.81 (m, 1H, CH all), 5.21 (dd, 1H, *J* = 4.0, 16.0 Hz, $CH_2$ all), 5.13 (dd, 1H, *J* = 4.0, 12.0 Hz, $CH_2$ all), 3.75−3.80 (m, 2H, H-3, $OCH_2$), 3.60 (m, 1H, H-5), 3.43−3.48 (m, 2H, H-4, H-6), 3.27−3.38 (m, 2H, H-6′, $OCH_2$), 3.07 (d, 1H, *J* = 4.0 Hz, H-1), 2.96 (dd, 1H, *J* = 4.0, 12.0 Hz, H-2). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 146.7, 134.0 (CH all), 129.0, 128.2, 126.9, 117.1 ($CH_2$ all), 97.5 (C-1), 73.9 (C-3), 71.6 (C-4), 70.4 (C-5, $CPh_3$), 68.7 ($OCH_2$), 57.6 (C-2), 51.7 (C-6). HRMS ($ESI^+$) *m*/*z*: $[M+K]^+$ calcd 525.1941, found 525.1931; $[M + Na]^+$ calcd 509.2165, found 509.2164.

*Allyl 6-Azido-2,6-dideoxy-3,4-di-O-[(2-naphthyl)methyl]-2-tritylamino-α-D-glucopyranoside (16).* To a stirred solution of **15** (1.22 g, 2.51 mmol) in DMF (10 mL) were added NaH (0.4 g, 10.04 mmol, 4 equiv) and 2-bromomethylnaphthalene (2.22 g, 10.04 mmol, 4 equiv). The resulting mixture was stirred at ambient temperature for 10 h under argon atmosphere. A saturated $NH_4Cl$ solution was added, and the mixture was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried over $MgSO_4$ and filtered, and the filtrate was concentrated under reduced pressure. The residue obtained was purified by chromatography on silica gel in ethyl acetate/cyclohexane (1:45) with a few drops of $Et_3N$ to afford compound **16** (1.52 g, 79%) as a white crystalline solid; mp 118−120 °C. $^1$H NMR (400 MHz, $CDCl_3$) δ 7.17−8.01 (m, 29H ar), 5.80 (m, 1H, CH all), 5.70 (m, 1H, $CH_2$ np), 5.18−5.23 (m, 2H, $CH_2$ all, $CH_2$ np), 5.11−5.14 (m, 2H, $CH_2$ all, $CH_2$), 4.76 (m, 1H, $CH_2$ np), 3.94 (t, 1H, *J* = 8.0 Hz, H-3), 3.65−3.72 (m, 2H, H-5, $OCH_2$ all), 3.50 (dd, 1H, *J* = 8.0, 12.0 Hz, H-4), 3.42 (m, 1H, H-6), 3.14−3.32 (m, 3H, H-2, H-6′, $OCH_2$ all), 2.71 (d, 1H, *J* = 4.0 Hz, H-1), 2.46 (d, *J* = 10.7 Hz, 1H). $^{13}$C NMR (100 MHz, $CDCl_3$) δ 147.4, 136.6, 135.7, 134.2 (CH all), 133.6, 133.4, 133.1, 133.0, 129.2, 128.4, 128.3, 128.1, 127.9, 127.8, 126.7, 126.6, 126.3, 126.2, 126.1, 126.0, 125.9, 117.0 ($CH_2$ all), 97.9 (C-1), 83.4 (C-3), 79.7 (C-4), 77.2 ($CH_2$ np), 75.5 ($CH_2$ np), 70.8 ($CPh_3$), 70.0 (C-5), 68.8 ($OCH_2$ all), 58.3 (C-2), 51.6 (C-6). HRMS ($ESI^+$) *m*/*z*: $[M + Na]^+$ calcd 789.3417, found 789.3434; $[M - N2 + Na]^+$ calculated 761.3353, found 761.3339.

*Allyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-[(2-naphthyl)methyl]-α-D-glucopyranoside (17).* To a stirred solution of **16** (0.41 g, 0.53 mmol) in a 19:1 THF/$H_2O$ mixture was added $Ph_3P$ (0.21 g, 0.80 mmol, 1.5 equiv), and the mixture was heated at 80 °C for 8 h. After evaporation to dryness, the resulting residue was purified by chromatography on silica gel in ethyl acetate/cyclohexane (1:4) with a few drops of $Et_3N$ to afford the detritylated compound. This compound was treated with a 1:1 TFA/anisole mixture for 3 h at 0 °C under argon atmosphere. The mixture was co-evaporated twice with toluene. The residue obtained was washed with dry $Et_2O$ (3 × 5 mL) to obtain a yellow compound. This compound was chromatographed on an ion-exchange resin (Dowex resin $Cl^-$ form) in methanol to afford the hydrochloride salt of compound **17** (0.19 g, 62% for the 3 steps) as a yellowish white crystalline solid; mp 183−185 °C. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.34−7.81 (m, 14H ar), 6.03 (m, 1H, CH all), 5.43 (m, 1H, $CH_2$ all), 5.32 (m, 1H, $CH_2$ all), 5.21 (d, 1H, *J* = 4.0 Hz, H-1), 5.07 (dd, 2H, $CH_2$ np), 4.91 (m, 2H, $CH_2$ np), 4.37 (dd, 1H, *J* = 8.0, 12.0 Hz, $OCH_2$ all), 4.20 (dd, 1H, *J* = 8.0, 12.0 Hz, $OCH_2$ all), 3.99−4.15 (m, 2H, H-3, H-5), 3.68 (t, 1H, *J* = 8.0 Hz, H-4), 3.53 (dd, 1H, *J* = 4.0, 12.0 Hz, H-2), 3.35−3.40 (m, 1H, H-6), 3.04 (m, 1H, H-6′). $^{13}$C NMR (100 MHz, $D_2O$) δ 136.7, 136.3, 134.8, 134.7, 134.6 (CH all), 134.5, 129.4, 129.2, 129.1, 128.8, 127.7, 127.4, 127.3, 127.2, 126.8, 126.7, 119.5 ($CH_2$ all), 96.4 (C-1), 81.5 (C-4), 79.5 (C-3), 76.5 ($OCH_2$ np), 76.4 ($OCH_2$ np), 70.8 ($OCH_2$ all), 69.8 (C-5), 54.9 (C-2), 41.8 (C-6). HRMS ($ESI^+$) *m*/*z*: $[M + Na]^+$ calcd 521.2416, found 521.2404; $[M + H]^+$ calcd 499.2597, found 499.2594. Elemental Analysis for $C_{31}H_{36}N_2O_4$ + 1.5$H_2O$ Calcd: C, 62.20; H, 6.57; N, 4.68. Found: C, 62.44; H, 6.30; N, 4.88.

*Allyl 6-Amino-2,6-dideoxy-2-N-tritylamino-α-D-glucopyranoside (18).* To a stirred solution of **15** (0.22 g, 0.45 mmol) in a 19:1 THF/$H_2O$ mixture was added $Ph_3P$ (0.15 g, 0.59 mmol, 1.3 equiv), and the mixture was heated at 80 °C for 6 h. The solvent was evaporated to dryness, and the resulting residue was purified by chromatography on silica gel in methanol/ethyl acetate (1:9 to 1:1) with a few drops of $Et_3N$ to afford compound **18** (0.19 g, 91%) as a white crystalline solid; mp 139−141 °C. $^1$H NMR (400 MHz, $CD_3OD$) δ 7.66−7.69 (m, 5H ar), 7.15−7.27 (m, 10H ar), 5.85 (m, 1H, CH all), 5.25 (dd, 1H, *J* = 4.0, 16.0 Hz, $CH_2$ all), 5.12 (m, 1H, $CH_2$ all), 3.84 (dd, 1H, *J* = 4.0, 12.0 Hz, $OCH_2$), 3.74 (t, 1H, *J* = 8.0, 12.0 Hz, H-3), 3.58 (m, 1H, H-5), 3.24 (m, 2H, H-6, $OCH_2$), 3.15 (m, 1H, H-4), 2.90 (dd, 1H, *J* = 4.0, 12.0 Hz, H-6′), 2.87 (d, 1H, *J* = 4.0 Hz, H-1), 2.75 (dd, 1H, *J* = 4.0, 12.0 Hz, H-2). $^{13}$C NMR (100 MHz, $CD_3OD$) δ 148.7, 135.5 (CH all), 130.3, 129.0, 127.6, 117.0 ($CH_2$ all), 99.4 (C-1), 74.5 (C-3), 73.8 (C-4), 71.7 ($CPh_3$), 69.8 ($OCH_2$), 69.3 (C-5), 59.2 (C-2), 42.4 (C-6). HRMS ($ESI^+$) *m*/*z*: $[M + Na]^+$ calcd 483.2260, found 483.2260; $[M + H]^+$ calcd 461.2440, found 461.2442.

*Allyl 2,6-Diamino-2,6-dideoxy-α-D-glucopyranoside (19).*[99] A solution of **18** (0.11 g, 0.24 mmol) in a 1:1 TFA/anisole mixture was stirred for 6 h at 0 °C under argon atmosphere. The mixture was coevaporated twice with toluene. The residue was washed with dry $Et_2O$ (3 × 5 mL) to obtain a gummy liquid, which was chromatographed on an ion-exchange resin (Dowex resin $Cl^-$ form)



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in $H_2O$ to afford the hydrochloride salt of compound **19** (0.06 g, 84% for both steps) as a yellow gummy liquid. $^1$H NMR (400 MHz, $D_2O$) $\delta$ 5.96 (m, 1H, CH all), 5.36 (dd, 1H, $J$ = 4.0, 16.0 Hz, $CH_2$ all), 5.28 (dd, 1H, $J$ = 4.0, 12.0 Hz, $CH_2$ all), 5.21 (d, 1H, $J$ = 4.0 Hz, H-1), 4.26 (m, 1H, $OCH_2$), 4.05 (m, 1H, $OCH_2$), 3.85−3.93 (m, 2H, H-4, H-5), 3.36−3.46 (m, 3H, H-2, H-3, H-6), 3.12 (dd, 1H, $J$ = 4.0, 12.0 Hz, H-6′). $^{13}$C NMR (100 MHz, $D_2O$) $\delta$ 133.0 (CH all), 118.7 ($CH_2$ all), 94.3 (C-1), 71.1 (C-3), 69.4 (C-4), 69.0 ($OCH_{2\ all}$), 68.0 (C-5), 53.6 (C-2), 40.2 (C-6). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd for $C_9H_{18}N_2O_4$ 219.1345, found 219.1353.

*Allyl 2-Acetamido-6-azido-2,6-dideoxy-3,4-di-O-[(2-naphthyl)-propyl]-α-D-glucopyranoside (**20**).* To a solution of compound **12** (1.0 g, 3.49 mmol) in dry DMF (20 mL) under argon were added NaH (60%, 0.42 g, 10.5 mol) and, then, after 30 min at rt the 2-naphthylpropyl bromide (1.74 g, 6.99 mmol). After 6 h stirring at rt, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (x3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with toluene/ethyl acetate (80:20) to give compound **20** with 29% yield (0.64 g, 1.02 mmol, white solid). **20**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.75−7.18 (m, 14H, H-ar), 5.93−5.78 (m, 1H, H-2′), 5.46 (d, 1H, $J$ = 4.2 Hz, NH), 5.23 (dd, 1H, $J$ = 1.2, 17.5 Hz, H-3a′), 5.20 (dd, 1H, $J$ = 1.1, 10.9 Hz, H-3b′), 4,70 (d, 1H, $J$ = 6.5 Hz, H-1), 4.23 (dd, 1H, $J$ = 5.3, 12.8 Hz, H-1a′), 4.03 (dd, 1H, $J$ = 6.0, 12.8 Hz, H-1b′), 3.88 (dd, 1H, $J$ = 3.5, 10.1 Hz, H-2), 3.75−3.69 (m, 1H, H-5), 3.63−3.56 (m, 3H, $CH_2O$, H-6), 3.50−3.48 (m, 2H, $CH_2O$), 3.44−3.23 (m, 3H, H-3, H-4, H-6), 2.70−2.55 (m, 4H, $CH_2$ ar), 1.93−1.76 (m, 7H, $2CH_2$, $CH_3$).$^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 168.7 (C═O), 139.5−135.0 (6C ar), 132.1 (C2′), 126.1−128.7 (14CH ar), 118.3 (C3′), 96.8 (C1), 81.2 (C3), 79.02 (C4), 72.5 (C5), 71.1, 68.4 ($2CH_2O$), 62.3 (C1′), 52.4 (C2), 51.4 (C6), 34.1, 32.5, 32.2, 31.8 ($4CH_2$), 23.5 ($CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd, 623.3233; found, 623.3229.

*Allyl 2-Acetamido-6-azido-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**21**).* To a solution of compound **12** (1.0 g, 3.49 mmol) in toluene (50 mL) were added TBAF.$3H_2O$ (2.75 g, 8.72 mmol), the 1-bromononane (2.66 mL, 13.9 mmol) and an aqueous solution of NaOH (50% w/w, 25 mL). The resulting mixture was stirred vigorously for 16 h at rt. The organic solution was diluted with ethyl acetate and then washed twice with an aqueous saturated ammonium chloride solution before being dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with toluene/ethyl acetate (80:20) to give compound **21** with 57% yield (1.069 g, 1.98 mmol white solid). **21**: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$5.92−5.82 (m, 1H, H-2′), 5.61 (d, 1H, NH), 5.28 (dd, 1H, $J$ = 1.5, 17.2 Hz, H-3a′), 5.21 (dd, 1H, $J$ = 1.4, 10.4 Hz, H3-b′), 4.78 (d, 1H, $J$ = 3.7 Hz, H-1), 4.22−4.14 (m, 2H, H-2, H-1a′), 3.97 (ddt, 1H, $J$ = 1.2, 6.3, 12,8 Hz, H1b′), 3.81−3,67 (m, 3H, $CH_2$), 3.54−3,33 (m, 5H, H-3, H-4, H-5, H-6, $CH_2$), 3.27−3.23 (m, 1H, H-6), 2.00 (s, 3H, $CH_3$), 1.57−1.46 (m, 4H, $CH_2$), 1.35−1.19 (m, 24H, $CH_2$), 0.86 (dt, 6H, $J$ = 3.3, 7.0 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.8 (C═O), 133.6 (C2′), 118.3 (C3′), 97.0 (C1), 81.6 (C3), 79.1 (C4), 73.5, 73.4 ($2CH_2O$), 71.4 (C5), 68.6 (C1′), 52.7 (C2), 51.6 (C6), 32.1−23.7 ($CH_3$, $14CH_2$),22.9 ($CH_3$), 14.3 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + Na]^+$ calcd 561.3992, found 561.3990. HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 539.4167, found 539.4167.

*Compounds **22** and **23**.* To a solution of compound **20** or **21** (0.10 g, 0.159 or 0.186 mmol) in THF (6 mL) were added $Boc_2O$ (0.30 g, 1.33 mmol) and DMAP (63 mg, 0.52 mmol). After 6 h at 70 °Cthe solvent was evaporated under reduced pressure.The crude product was diluted with ethyl acetate and washed with water (×3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with cyclohexane/ethyl acetate (90:10) to give compound **22** with 86% yield (0.10 g, 0.14 mmol colorless oil) or **23** with 88% yield (0.10 g, 0.16 mmol colorless oil).

*Allyl 2-[N-(tert-Butoxycarbonyl)acetamido]-6-azido-2,6-dideoxy-3,4-di-O-[(2-naphthyl)propyl]-α-D-glucopyranoside (**22**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.84−7.30 (m, 14H, H ar), 5.94−5.84 (m, 1H, H-2′), 5.34 (dd, 1H, $J$ = 1.3, 17.2 Hz, H-3a′), 5.26 (dd, 1H, $J$ = 1.0, 10.4 Hz, H-3b′), 4,90 (d, 1H, $J$ = 3.6 Hz, H-1), 5.70 (s, 1H, H-2), 4.52 (t, 1H, $J$ = 9.3 Hz, H-3), 4.20 (dd, 1H, $J$ = 4.9, 13.1 Hz, H-1a′), 4.07 (dd, 1H, $J$ = 6.8, 13.1 Hz, H-1b′), 3.92−3.78 (m, 4H, H-5, $CH_2$), 3.67−3.61 (m, 1H, $CH_2$), 3.53 (dd, 1H, $J$ = 2.2, 12.9 Hz, H-6), 3.45 (dd, 1H, $J$ = 6.0, 13.0 Hz, H-6), 3.31−3.27 (m, 1H, H-4), 2.86−2.71 (m, 4H, $CH_2$), 2.31 (s, 3H, $CH_3$), 2.01−1.85 (m, 4H, $CH_2$), 1.54 (s, 9H, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.5 (C═O), 154.9 (COO), 139.3−131.9 (6C ar), 133.7 (C2′), 128.0−125.1 (14CH ar), 118.3 (C3′), 97.3 (C1), 81.6 (C3), 79.6 (C−O), 79.3 (C4), 72.6 (C5), 71.1, 68.5 ($2CH_2O$), 54.2 (C1′), 54.1 (C2), 51.4 (C6), 32.7, 32.5, 32.0, 31.7 ($4CH_2$), 23.2 ($CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 723.3758, found 723.3760.

*Allyl 2-[N-(tert-Butoxycarbonyl)acetamido]-6-azido-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**23**).* $^1$H NMR (400 MHz, $CDCl_3$)$\delta$ 5.92−5.82 (m, 1H, H-2′), 5.31 (dd, 1H, $J$ = 1.5, 17.2 Hz, H-3a′), 5.23 (dd, 1H, $J$ = 1.4, 10.4 Hz, H3-b′), 4.86 (d, 1H, $J$ = 3.7 Hz, H-1), 4.63 (d, 1H, $J$ = 9.2 Hz, H-2), 4.48−4.39 (m, 1H, H-3), 4.17 (ddd, 1H, $J$ = 3.2, 5.0, 7.3 Hz, H-1a′), 4.05 (dd, 1H, $J$ = 6.8, 13.1 Hz, H-1b′), 3.87−3,69 (m, 4H, H-5, $CH_2$), 3.58−3,49 (m, 2H, H-6, $CH_2$), 3.43 (dd, 1H, $J$ = 6.1, 12.9 Hz, H-6), 3.22 (dd, 1H, $J$ = 8.6, 9.9 Hz, H-4), 2.35 (s, 3H, $CH_3$), 1.64−1.51 (m, 13H, $CH_2$, $CH_3$), 1.37−1.25 (m, 24H, $CH_2$), 0.93 (dt, 6H, $J$ = 3.2, 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 172.9 (C═O), 153.9 (COO), 133.5 (C2′), 118.3 (C3′), 96.9 (C1), 83.6 (C−O), 81.0 (C3), 78.4 (C4), 73.2, 71.9 ($2CH_2O$), 71.0 (C5), 68.3 (C1′), 57.4 (C2), 51.5 (C6), 31,9−22.7 ($4CH_3$, $14CH_2$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 639.4691, found 639.4687. HRMS (ESI$^+$) $m/z$: $[M + Na]^+$ calcd 661.4512, found 661.4507.

*Compounds **24** and **25**.* To a solution of **22** (138 mg, 0.19 mmol) or **23** (89 mg, 0.14 mmol) in anhydrous MeOH (14 or 9 mL) was added MeONa (15.6 mg, 0.29 mmol or 11.4 mg, 0.21 mmol). After 6 h at rt, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (×3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with cyclohexane/ethyl acetate (90:10) to give compound **24** with 96% yield (125 mg, 0.18 mmol, white solid) or **25** with 99% yield (82.3 mg, 0.14 mmol, white solid).

*Allyl 6-Azido-2-(tert-butoxycarbonylamino)-2,6-dideoxy-3,4-di-O-[(2-naphthyl)propyl]-α-D-glucopyranoside (**24**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.85−7.31 (m, 14H, H-ar), 6.03−5.93 (m, 1H, H-2′), 5.38 (dd, 1H, $J$ = 1.3, 17.2 Hz, H-3a′), 5.30 (dd, 1H, $J$ = 1.2, 10.4 Hz, H-3b′), 4,89 (d, 1H, $J$ = 3.3 Hz, H-1), 4.83 (d, 1H, $J$ = 10.1 Hz, NH), 4.26 (dd, 1H, $J$ = 5.3, 12.8 Hz, H-1a′), 4.07 (dd, 1H, $J$ = 6.1, 12.8 Hz, H-1b′), 3.98−3.91 (m, 2H, H-6, $CH_2$), 3.85−3.62 (m, 4H, $CH_2O$, H-5), 3.58−3.40 (m, 3H, H-3, H-6), 3.35 (t, 1H, $J$ = 9.3 Hz, H-4), 2.89−2.77 (m, 4H, $CH_2$ ar), 2.04−1.94 (m, 4H, $2CH_2$), 1.48(s, 9H, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 155.4 (COO), 139.6−132.0 (6C ar), 133.7 (C2′), 128.0−125.2 (14CH ar), 118.3 (C3′), 97.3 (C1), 81.6 (C3), 79.7 (C−O), 79.3 (C4), 72.6 (C5), 71.1, 68.4 ($2CH_2O$), 54.2 (C1′), 53.5 (C2), 51.4 (C6), 32.6, 32.5, 32.0, 31.8 ($4CH_2$), 28.4 ($3CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 681.3652, found 681.3649.

*Allyl 6-Azido-2-(tert-butoxycarbonylamino)-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**25**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.99−5.89 (m, 1H, H-2′), 5.34 (dd, 1H, $J$ = 1.5, 17.2 Hz, H-3a′), 5.26 (dd, 1H, $J$ = 1.2, 11.6 Hz, H3-b′), 4.84 (d, 1H, $J$ = 3.4 Hz, H-1), 4.73 (d, 1H, $J$ = 10.8 Hz, NH), 4.21 (dd, 1H, $J$ = 5.3, 12,8 Hz, H-1a′), 4.03 (dd, 1H, $J$ = 6.1, 12,8 Hz, H-1b′), 3.91−3,82 (m, 3H, H-2, $CH_2$), 3.80−3,70 (m, 2H, H-5, $CH_2$), 3.63 (dd, 1H, $J$ = 7.3, 15.1 Hz, $CH_2$), 3.57−3.51 (m, 2H, H-6, $CH_2$), 3.45−3.38 (m, 2H, H-3, H-6),3.26 (t, 1H, $J$ = 9.4 Hz, H-4), 1.62−1.54 (m, 4H, $CH_2$), 1.51 (s, 9H, $CH_3$), 1.38−1.25 (m, 24H, $CH_2$), 0.93 (dd, 6H, $J$ = 6.4, 7.1 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 155.3 (COO), 133.6 (C2′), 117.7 (C3′), 97.3 (C1), 81.5 (C3), 79.5 (C−O), 79.1 (C4), 73.6, 73.4 ($2CH_2O$), 71.1 (C5), 68.4 (C1′), 54.2 (C2), 51.4 (C6), 31.9−26.1 ($14CH_2$), 22.7 ($3CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 597.4586, found 597.4582. HRMS (ESI$^+$) $m/z$: $[M + Na]^+$ calcd 619.4405, found 619.4400.

*Compounds **26** and **27**.* To a solution of **24** (100 mg, 0.15 mmol) or **23** (93 mg, 0.16 mmol) in THF (10 mL) were added water (3.3



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mL) and $PPh_3$ (116 mg, 0.44 mmol or 123 mg, 0.47 mmol). After 4 h at rt, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (×3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with ethyl acetate/MeOH (90:10) to give compound **26** with 86% yield (82.7 mg, 0.13 mmol, white solid) or **27** with 96% yield (85.4 mg, 0.15 mmol, white solid).

*Allyl 6-Amino-2-(tert-butoxycarbonylamino)-2,6-dideoxy-3,4-di-O-[(2-naphthyl)propyl]-α-D-glucopyranoside (**26**).* $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.72−7.17 (m, 14H, H-ar), 5.97−5.87 (m, 1H, H-2′), 5.32 (dd, 1H, $J$ = 1.5, 17.3 Hz, H-3a′), 5.17 (dd, 1H, $J$ = 1.2, 10.4 Hz, H-3b′), 4,72 (d, 1H, $J$ = 3.4 Hz, H-1), 4.17 (dd, 1H, $J$ = 5.1, 13.1 Hz, H-1a′), 3.97 (dd, 1H, $J$ = 6.1, 13.1 Hz, H-1b′), 3.75 (dt, 1H, $J$ = 6.7, 15.5 Hz, $CH_2O$), 3.66−3.43 (m, 6H, H-2, H-3, H-5, $CH_2O$), 3.06−3.00 (m, 2H, H-4, H-6), 2.78−2.60 (m, 5H, H-6, $CH_2$ ar), 1.86−1.71 (m, 4H, $CH_2$), 1.34 (s, 9H, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 158.2 (COO), 141.0−133.5 (6C ar), 135.4 (C2′), 129.0−126.1 (14CH ar), 117.9 (C3′), 98.4 (C1), 82.1 (C3), 82.0 (C4), 80.45 (C−O), 73.7, 73.4 ($2CH_2O$), 72.2 (C5), 54.2 (C1′), 69.4 (C2), 43.4 (C6), 33.6, 33.4, 33.3, 32.9 ($4CH_2$), 28.9 ($3CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 655.3747, found 655.3749.

*Allyl 6-Amino-2-(tert-butoxycarbonylamino)-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**27**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 5.92−5.82 (m, 1H, H-2′), 5.25 (dd, 1H, $J$ = 1.5, 17.2 Hz, H-3a′), 5.17 (dd, 1H, $J$ = 1.3, 10.4 Hz, H-3b′), 4.74 (d, 1H, $J$ = 3.4 Hz, H-1), 4.70 (d, 1H, $J$ = 10.0 Hz, NH), 4.13 (dd, 1H, $J$ = 5.3, 12,8 Hz, H-1a′), 3.92 (dd, 1H, $J$ = 6.0, 12,6 Hz, H-1b′), 3.80−3.45 (m, 6H, H-2, H-5, $CH_2O$), 3.36 (t, 1H, $J$ = 9.6 Hz, H-3), 3.09 (t, 1H, $J$ = 9.3 Hz, H-4), 3.00 (d, 1H, $J$ = 12.4 Hz, H-6), 2.77 (dd, 6H, $J$ = 6.6, 12.6 Hz, H-6), 2.05−1.94 (m, 2H, NH), 1.59−1.48 (m, 4H, $CH_2$), 1.43 (s, 9H, $CH_3$), 1.34−1.15 (m, 24H, $CH_2$), 0.85 (t, 6H, $J$ = 6.7 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 155.4 (COO), 133.9 (C2′), 117.4 (C3′), 97.3 (C1), 81.6 (C3), 79.9 (C4), 79.4 (C−O), 73.5, 73.3 ($2CH_2O$), 72.6 (C5), 68.1 (C1′), 54.4 (C2), 42.9 (C6), 31.9−26.1 ($14CH_2$), 22.7 ($3CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 571.4686, found 571.4684.

*Compounds **28** and **29**.* The deprotection of **26** (73 mg, 0.11 mmol) or **27** (80 mg, 0.14 mmol) was achieved following procedure I.

*Allyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-[(2-naphthyl)propyl]-α-D-glucopyranoside (**28**).* Yield 98% (85.6 mg, 0.11 mmol, white solid). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 7.77−7.20 (m, 14H, H-ar), 6.06−5.96 (m, 1H, H-2′), 5.40 (dd, 1H, $J$ = 1.4, 17.2 Hz, H-3a′), 5.30 (dd, 1H, $J$ = 1.3, 10.4 Hz, H-3b′), 5.15 (d, 1H, $J$ = 3.2 Hz, H-1), 4.33 (dd, 1H, $J$ = 5.5, 12.5 Hz, H-1a′), 3.97 (dd, 1H, $J$ = 6.0, 13.0 Hz, H-1b′), 3.91−3.79 (m, 2H, H-5, $CH_2O$), 3.75−3.64 (m, 3H, H-3, $CH_2O$), 3.61−3.52 (m, 1H, $CH_2O$), 3.39−3.28 (m, 2H, H-2, H-6), 3.24 (t, 1H, $J$ = 9.3 Hz, H-4), 3.10 (dd, 1H, $J$ = 9.5, 13.0 Hz, H-6), 2.73 (t, 2H, $J$ = 7.4 Hz, $CH_2$ ar), 2.65 (t, 2H, $J$ = 7.6 Hz, $CH_2$ ar), 2.02−1.93 (m, 2H, $CH_2$), 1.85−1.71 (m, 2H, $CH_2$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 140.4−133.6 (6C ar), 135.1 (C2′), 129.1−126.3 (14CH ar), 119.4 (C3′), 96.0 (C1), 81.7 (C4), 79.4 (C3), 74.2, 73.8 ($2CH_2O$), 70.4 (C1′), 69.7 (C5), 54.6 (C2), 41.6 (C6), 33.1, 33.1, 32.6, 32.3 ($4CH_2$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$calcd 555.3223, found 555.3217.

*Allyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**29**).* Yield 97% (0.95 g, 0.14 mmol, white solid). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.93−5.83 (m, 1H, H-2′), 5.27 (dd, 1H, $J$ = 1.4, 17.2 Hz, H-3a′), 5.15 (dd, 1H, $J$ = 1.1, 10.4 Hz, H-3b′), 5.03 (d, 1H, $J$ = 3.5 Hz, H-1), 4.20 (dd, 1H, $J$ = 5.5, 12.5 Hz, H-1a′), 3.98 (dd, 1H, $J$ = 6.5, 12.5 Hz, H-1b′), 3.79−3.63 (m, 3H, H-5, $CH_2O$), 3.53−3.44 (m, 3H, H-3, $CH_2O$), 3.26−3.15 (m, 2H, H-2, H-6), 3.11 (t, 1H, $J$ = 9.3 Hz, H-4), 3.00 (dd, 1H, $J$ = 9.5, 12.9 Hz, H-6), 1.60−1.43 (m, 4H, $CH_2$), 1.33−1.10 (m, 24H, $CH_2$), 0.79 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 135.2 (C2′), 119.3 (C3′), 96.0 (C1), 81.7 (C4), 79.6 (C3), 74.1, 73.8 ($2CH_2O$), 70.3 (C1′), 69.5 (C5), 54.7 (C2), 40.9 (C6), 32.1−26.0 ($14CH_2$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + Na]^+$ calcd 577.3941, found 577.3939.

*Allyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**30**).* Compound **27** (2.5 g, 4.4 mmol) was dissolved in $CH_2Cl_2$ (15 mL). *p*-Methoxybenzyl-*S*-(4,6-dimethylpyrimidin-2-yl) thiocarbonate (1.47 g, 4.8 mmol) was added, and the reaction was stirred for 16 h at rt. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on silica gel with toluene/ethyl acetate (95:5) to give compound **30** with 90% yield (2.89 g, 3.95 mmol, white paste). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.29 (d, 2H, $J$ = 8.5 Hz, H ar), 6.87 (d, 2H, $J$ = 8.6 Hz, H ar), 5.89−5.79 (m, 1H, H-2′), 5.23 (dd, 1H, $J$ = 1.5, 17.2 Hz, H-3a′), 5.17 (dd, 1H, $J$ = 1.3, 10.4 Hz, H-3b′), 5.09−4.95 (m, 3H, $CH_2$ ar, NH), 4.71 (d, 1H, $J$ = 3.0 Hz, H-1), 4.67 (d, 1H, $J$ = 10.0 Hz, NH), 4.05 (dd, 1H, $J$ = 5.4, 12,8 Hz, H-1a′), 3.90 (dd, 1H, $J$ = 6.0, 12,7 Hz, H-1b′), 3.80 (s, 3H, $CH_3$), 3.78−3,63 (m, 3H, H-2, $CH_2O$), 3.61−3.45 (m, 4H, H-5, H-6, $CH_2O$), 3.43−3.34 (m, 2 H, H-3, H-6), 3.07 (t, 1H, $J$ = 9.2 Hz, H-4), 1.61−1.48 (m, 4H, $CH_2$), 1.44 (s, 9H, $CH_3$), 1.34−1.20 (m, 24H, $CH_2$), 0.87 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.6 (C ar), 156.4 (COO), 155.4 (COO), 133.7(C2′),129.9 (CH ar), 128.8 (C ar), 117.7 (C3′), 113.9 (CH ar), 97.4 (C1), 81.3 (C3), 79.5 (C4, C−O), 73.7, 73.5 ($2CH_2O$), 70.3 (C5), 68.4 (C1′), 66.7 ($CH_2$ ar), 55.3 ($CH_3$), 54.2 (C2), 41.6 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 735.5154, found 735.5153.

*(2′R/S)-2′,3′-Epoxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**31a,b**).* To a solution of **30** (2.88 g, 3.92 mmol) in $CH_2Cl_2$ (28 mL) was added mCPBA (2.33 g, 9.8 mmol). After one night of stirring at 75 °C, $CH_2Cl_2$ (42 mL) was added and the solution was washed with $NaOH_{aq}$ (5%, 50 mL) and twice with water (50 mL). The organic layer was dried over $MgSO_4$ and evaporated to dryness to give mixture **31a,b** with 98% yield (2.89 g, 3.85 mmol). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.29 (d, 2H, $J$ = 8.5 Hz, H ar), 6.87 (d, 2H, $J$ = 8.6 Hz, H ar), 5.13−4.99 (m, 3H, $CH_2$ ar, NH), 4.73−4.67 (m, 2H, H-1, NH), 3.80 (s, 3H, CH3), 3.78−3,48 (m, 8H, H-2, H-5, H-6, H-1′, $CH_2O$) 3.43−3.29 (m, 3H, H-3, H-6, H-1′), 3.12−3.05 (m, 2H, H-4, H-2′), 2.89−2.81 (m, 1H, H-3′), 2.66 (dd, 0.66H, $J$ = 2.7, 4.9 Hz, H-3a′), 2.59 (dd, 0.33H, $J$ = 2.6, 4.8 Hz, H-3b′), 1.59−1.49 (m, 4H, $CH_2$), 1.45 (s, 9H, $CH_3$), 1.32−1.23 (m, 24H, $CH_2$), 0.88 (dd, 6H, $J$ = 6.3, 7.0 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.6 (C ar), 156.5 (COO), 155.4 (COO), 130.0 (CH ar), 128.8 (C ar), 113.9 (CH ar), 98.6 (C1a), 98.3 (C1b), 81.2 (C3), 79.7, 79.5 (C4, C−O), 73.8, 73.5 ($2CH_2O$), 70.5 (C5), 69.0 (C2, C1′), 66.5 ($CH_2$ ar), 55.3 ($CH_3$), 50.5 (C2′b), 50.3 (C2′a), 44.5 (C3′a), 44.4 (C3′b), 41.7 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 751.5103, found 751.5101.

*Compounds **32** and **33**.* To a solution of **31a,b** (99 mg, 0.13 mmol) in DMF (5 mL) was added $NaN_3$ (260 mg, 3.98 mmol). After one night of stirring at 75 °C, the solvent was evaporated under reduced pressure.The crude product was diluted with ethyl acetate, filtered, and washed with water (×3). The organic layer was dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel with toluene/ethyl acetate (80:20) to give compound **32** with 57% yield (60 mg, 75 μmol, white solid) and **33** with 29% yield (30 mg, 38 μmol, white solid).

*3′-Azido-(2′R)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**32**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.29 (d, 2H, $J$ = 8.5 Hz, H ar), 6.87 (d, 2H, $J$ = 8.6 Hz, H ar), 5.10−5.02 (m, 3H, $CH_2$ ar, NH), 4.77−4.70 (m, 2H, H-1, NH), 3.93−3.85 (m, 1H, H-2′), 3.80 (s, 3H, $CH_3$), 3.77−3,65 (m, 3H, H-2, $CH_2O$), 3.62−3.26 (m, 10H, H-3, H-1′, H-3′, H-5, H-6, $CH_2O$), 3.07 (t, 1H, $J$ = 9.2 Hz, H-4), 1.60−1.49 (m, 4H, $CH_2$), 1.44 (s, 9H, $CH_3$), 1.35−1.20 (m, 24H, $CH_2$), 0.87 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.6 (C ar), 156.5 (COO), 155.5 (COO), 130.0 (CH ar), 128.7 (C ar), 114.0 (CH ar), 98.5 (C1), 81.0 (C3), 79.8 (C−O), 79.5 (C4), 73.7, 73.5 ($2CH_2O$), 70.5 (C5), 69.7 (C1′), 69.5 (C2′), 66.6 ($CH_2$ ar), 55.3 ($CH_3$), 54.3 (C2), 53.6 (C3′), 41.6 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 794.5274, found 794.5269.

*3′-Azido-(2′S)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**33**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.30 (d, 2H, $J$ = 8.5 Hz, H ar), 6.88 (d, 2H, $J$ = 8.6 Hz, H ar), 5.06−



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5.00 (m, 3H, $CH_2$ ar, NH), 4.79−4.64 (m, 2H, H-1, NH), 3.96−3.87 (m, 1H, H-2′), 3.80 (s, 3H, $CH_3$), 3.77−3.67 (m, 3H, H-2, $CH_2O$), 3.63−3.27 (m, 10H, H-3, H-1′, H-3′, H-5, H-6, $CH_2O$), 3.07 (t, 1H, $J$ = 9.2 Hz, H-4), 1.60−1.50 (m, 4H, $CH_2$), 1.45 (s, 9H, $CH_3$), 1.35−1.25 (m, 24H, $CH_2$), 0.87 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.6 (C ar), 156.5 (COO), 155.5 (COO), 130.0 (CH ar), 128.7 (C ar), 114.0 (CH ar), 98.8 (C1), 81.0 (C3), 79.8 (C−O), 79.5 (C4), 73.7, 73.5 ($2CH_2O$), 70.5 (C5), 69.7 (C1′), 69.6 (C2′), 66.6 ($CH_2$ ar), 55.3 ($CH_3$), 54.3 (C2), 53.5 (C3′), 41.7 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 794.5274, found 794.5272.

*3′-Azido-(2′R)-2′-hydroxypropyl 2,6-diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**34**).* The deprotection of **32** (70 mg, 88.2 μmol) was achieved following procedure II. **34**: 71% yield (52 mg, 68.6 μmol, white solid). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.12 (s, 1H, H-1), 4.07−3.99 (m, 1H, H-2′), 3.96−3.76 (m, 4H, H-5, H-1′, $CH_2O$), 3.75−3.67 (m, 2H, H-3, $CH_2O$), 3.66−3.56 (m, H-1′, $CH_2O$), 3.43 (d, 2H, $J$ = 5.5 Hz, H-3′), 3.38−3.31 (m, 2H, H-2, H-6), 3.25 (t, 1H, $J$ = 9.3 Hz, H-4), 3.20−3.09 (m, 1H, H-6), 1.75−1.57 (m, 4H, $CH_2$), 1.44−1.24 (m, 24H, $CH_2$), 0.92 (t, 6H, $J$ = 6.9 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 95.5 (C1), 80.0 (C4), 77.6 (C3), 73.6, 73.2 ($2CH_2O$), 69.6 (C1′), 68.9 (C2′), 68.4 (C5), 53.4 (C2), 53.2 (C3′), 40.0 (C6), 31.6−22.3 ($14CH_2$), 12.9 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 530.4276, found 530.4277.

*Compounds **35** and **36**.* To a solution of **32** (33 mg, 41.6 μmol) or **33** (69 mg, 86.9 μmol) in THF (4 or 8 mL) were added water (1 or 2 mL) and $PPh_3$ (34 mg, 0.13 mmol or 70 mg, 0.27 mmol). After 4 h at rt, the solvent was evaporated under reduced pressure. The crude product was diluted with ethyl acetate and washed with water (×3). The dried organic layer was evaporated to dryness. The residue was chromatographed on silica gel with ethyl acetate/MeOH (80:20) to give compound **35** with 75% yield (23.9 mg, 31.1 μmol, white paste) or **36** with 96% yield (35 mg, 45.6 μmol, white paste).

*3′-Amino-(2′R)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**35**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.29 (d, 2H, $J$ = 8.5 Hz, H ar), 6.87 (d, 2H, $J$ = 8.6 Hz, H ar), 5.27−4.56 (m, 7H, H-1, $CH_2$ ar, NH, $NH_2$), 4.06−3,20 (m, 15H, H-2, H-3, H-5, H-6, H-1′, H-2′, $CH_2O$, CH3), 3.14−2.69 (m, 3H, H-4, H-3′), 1.89 (s, $NH_2$), 1.66−1.37 (m, 13H, $CH_2$, $CH_3$), 1.36−1.15 (m, 24H, $CH_2$), 0.87 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.6 (C ar), 156.5 (COO), 155.6 (COO), 130.0 (CH ar), 128.7 (C ar), 114.0 (CH ar), 98.7 (C1), 81.1 (C3), 79.5 (C−O), 79.4 (C4), 73.7, 73.5 ($2CH_2O$), 70.3 (C5, C1′), 68.9 (C2′), 66.6 ($CH_2$ ar), 55.3 ($CH_3$), 54.4 (C2), 43.3 (C3′), 41.6 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 768.5369, found 768.5369.

*3′-Amino-(2′S)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**36**).* $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.29 (d, 2H, $J$ = 8.5 Hz, H ar), 6.87 (d, 2H, $J$ = 8.6 Hz, H ar), 5.24−4.96 (m, 4H, $CH_2$ ar, NH), 4.69 (s, 1H, H-1), 4.15−3,24 (m, 17H, H-2, H-3, H-5, H-6, H-1′, H-2′, $CH_2O$, $CH_3$, $NH_2$), 3.10−2.64 (m, 3H, H-4, H-3′), 1.96 (s, $NH_2$), 1.59−1.38 (m, 13H, $CH_2$, $CH_3$), 1.35−1.09 (m, 24H, $CH_2$), 0.87 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.6 (C ar), 156.5 (COO), 155.5 (COO), 130.0 (CH ar), 128.7 (C ar), 114.0 (CH ar), 98.7 (C1), 81.1 (C3), 79.5 (C4, C−O), 73.7, 73.5 ($2CH_2O$), 70.3 (C5, C1′), 69.6 (C2′), 66.6 ($CH_2$ ar), 55.3 ($CH_3$), 54.4 (C2), 43.5 (C3′), 41.7 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 768.5369, found 768.5363.

*Compounds **37** and **38**.* The deprotection of **35** (21 mg, 27.3 μmol) or **36** (28 mg, 36.5 μmol) was achieved following procedure I.

*3′-Amino-(2′R)-2′-hydroxypropyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**37**).* Yield 95% (21.9 mg, 25.9 μmol, colorless paste). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.14 (d, 1H, $J$ = 3.2 Hz, H-1), 4.14−4.05 (m, 1H, H-2′), 3.98−3.68 (m, 6H H-3, H-5, H-1′, $CH_2O$), 3.66−3.51 (m, 2H, H-1′, $CH_2O$), 3.36−3.29 (m, 2H, H-2, H-6), 3.27−3.09 (m, 3H, H-4, H-6, H-3′), 3.02 (dd, 1H, $J$ = 9.5, 12.8 Hz, H-3′), 1.70−1.55 (m, 4H, $CH_2$), 1.42−1.24 (m, 24H, $CH_2$), 0.91 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 97.1 (C1), 81.4 (C4), 78.9 (C3), 75.1, 74.7 ($2CH_2O$), 71.1 (C1′), 69.9 (C5), 67.7 (C2′), 54.9(C2), 43.0 (C3′), 41.5 (C6), 33.1−23.8 ($14CH_2$), 14.4 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 504.4371, found 504.4367.

*3′-Amino-(2′S)-2′-hydroxypropyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**38**).* Yield 93% (28.7 mg, 33.9 μmol, colorless paste). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.10 (d, 1H, $J$ = 3.5 Hz, H-1), 4.10−4.01 (m, 1H, H-2′), 3.89 (td, 1H, $J$ = 2.6, 9.6 Hz, H-5), 3.85−3.72 (m, 3H, H-1′, $CH_2O$), 3.71−3.61 (m, 2H, H-3, $CH_2O$), 3.60−3.47 (m, 2H, H-1′, $CH_2O$), 3.34−3.25 (m, 2H, H-2, H-6), 3.20 (t, 1H, $J$ = 9.3 Hz, H-4), 3.15−3.03 (m, 2H, H-6, H-3′), 2.96 (dd, 1H, $J$ = 9.5, 12.8 Hz, H3′), 1.67−1.51 (m, 4H, $CH_2$), 1.36−1.21 (m, 24H, $CH_2$), 0.87 (t, 6H, $J$ = 6.9 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 97.2 (C1), 81.4 (C4), 79.0 (C3), 75.0, 74.6 ($2CH_2O$), 71.3 (C1′), 69.8 (C5), 67.8 (C2′), 54.8 (C2), 42.8 (C3′), 41.5 (C6), 33.1−23.8 ($14CH_2$), 14.4 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 504.4371, found 504.4365.

*(2′R/S)-2′,3′-Dihydroxypropyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**39a,b**).* The mixture **31a,b** (198 mg, 0.26 mmol) was dissolved in $CH_2Cl_2$/TFA (4/1 mL) in the presence of water (2 mL). After 4 h stirring, the solvents were evaporated under reduced pressure. MeOH (2 × 5 mL) and $H_2O$ (2 × 5 mL) were added and evaporated. Then the residue was chromatographed on C18 reversed phase eluting with a $H_2O$/MeOH (55:45). **39a,b** were obtained with 93% yield as a TFA salt (180 mg, 0.25 mmol, colorless solid). **39a,b**: $\delta$ 5.05 (dd, 1H, $J$ = 3.2, 7.4 Hz, H-1), 3.93−3.76 (m, 5H, H-5, H-1′, $CH_2O$), 3.73−3.11 (m, 6H, H-3, H-2′, H-3′, $CH_2O$), 3.36−3.28 (m, 1H, H-6), 3.23−3.02 (m, 3H, H-2, H-4, H-6), 1.72−1.75 (m, 4H, $CH_2$), 1.42−1.24 (m, 24H, $CH_2$), 0.90 (t, 6H, $J$ = 7.0 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 97.4 (C1), 81.6 (C4), 80.0 (C3), 75.0, 74.6 ($2CH_2O$), 71.7 (C1′), 70.3 (C2′), 69.8 (C5), 63.5 (C3′), 55.3 (C2), 41.5 (C6), 33.1−23.8 ($14CH_2$), 14.4 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 505.4211, found 505.4211.

*3′-(2″-Hydroxyethylamino)-(2′R/S)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**40a,b**).* The opening of **31a,b** (200 mg, 0.27 mmol) by ethanolamine (2 mL) was achieved following procedure II. **40a,b**: 91% (197 mg, 0.24 mmol, colorless paste). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.25 (d, 2H, $J$ = 8.5 Hz, H ar), 6.83 (d, 2H, $J$ = 8.6 Hz, H ar), 5.74−5.17 (m, NH), 5.07−4.92 (m, 2H, $CH_2$ ar, NH), 4.67−4.60 (m, 1H, H-1, NH), 3.87−3.20 (m, 17H, H-2, H-3, H-5, H-6, H-1′, H-2′, H-5′, $CH_2O$, $CH_3$, NH), 3.06−2.98 (m, 1H, H-4), 1.94 (N−H), 2.77−2.52 (m, 4H, H-3′, H-4′), 1.57−1.35 (m, 13H, $CH_2$, $CH_3$), 1.33−1.17 (m, 24H, $CH_2$), 0.84 (t, 6H, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.5 (C ar), 156.6 (COO), 155.7 (COO), 129.9 (CH ar), 128.7 (C ar), 113.9 (CH ar), 98.6, 98.5 (C1), 81.0 (C3), 79.5 (C−O), 79.4 (C4), 73.5 ($2CH_2O$), 70.6(C1′), 70.2 (C5), 68,6 (C2′), 66.5 ($CH_2$ ar), 60.8 (C5′), 55.3 ($CH_3$), 54.4 (C2), 52.0 (C3′), 51.4 (C4′), 41.7 (C6), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 812.5631, found 812.5638

*3′-(3″-Hydroxypropylamino)-(2′R/S)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**41a,b**).* The opening of **31a,b** (200 mg, 0.27 mmol) by propanolamine (2 mL) was achieved following procedure II. **41a,b**: 99% yield (217 mg, 0.26 mmol, colorless paste). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.22 (d, 2H, $J$ = 8.5 Hz, H ar), 6.80 (d, 2H, $J$ = 8.6 Hz, H ar), 5.99−5.24 (m, NH), 4.99−4.91 (m, 2H, $CH_2$ ar, NH), 4.61−4.57 (m, 1H, H-1, NH), 4.06−3.95 (m, 1H, H2′), 3.80−3.25 (m, 15H, H-2, H-3, H-5, H-6, H-1′, $CH_2O$, $CH_3$, NH), 3.10−2.72 (m, 5H, H-4, H-3′, H-4′), 1.89−1.73(m, 2H, H-5′),1.53−1.31 (m, 13H, $CH_2$, $CH_3$), 1.28−1.08 (m, 24H, $CH_2$), 0.80 (dd, 6H, $J$ = 5.4, 6.9 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.5 (C ar), 156.6 (COO), 156.0 (COO), 129.9 (CH ar), 128.7 (C ar), 113.9 (CH ar), 98.9, 98.8 (C1), 81.0 (C3), 79.6 (C−O), 79.2 (C4), 73.6, 73.5 ($2CH_2O$), 70.2 (C5, C1′), 66.7 (C2′), 66.5 ($CH_2$ ar), 61.1 (C6′), 55.3 ($CH_3$), 54.5 (C2), 51.1 (C3′), 47.9 (C4′), 41.7 (C6), 32.1−22.7 (C5′, $14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 826.5787, found 826.5794.



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*3′-(2″-Aminoethylamino)-(2′R/S)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**42a,b**).* The opening of **31a,b** (200 mg, 0.27 mmol) by ethylenediamine (2 mL) was achieved following procedure II. **42a,b**: 86% yield (186 mg, 0.23 mmol, colorless paste). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.26 (d, 2H, $J$ = 8.5 Hz, H ar), 6.85 (d, 2H, $J$ = 8.6 Hz, H ar), 5.34−4.81 (m, 2H, $CH_2$ ar, NH), 4.68 (s, 1H, H-1), 3.86−3.27 (m, 15H, H-2, H-3, H-5, H-6, H-1′, H-2′, $CH_2O$, $CH_3$, NH), 3.09−2.41 (m, 7H, H-4, H-3′, H-4′, H-5′), 1.58−1.38 (m, 13H, $CH_2$, $CH_3$), 1.32−1.15 (m, 24H, $CH_2$), 0.85 (dd, 6H, $J$ = 6.0, 7.0 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.5 (C ar), 156.6 (COO), 155.5 (COO), 130.0 (CH ar), 128.8 (C ar), 113.9 (CH ar), 98.6, 98.5 (C1), 81.3 (C3), 81.2 (C−O), 79.5 (C4), 73.6, 73.5 ($2CH_2O$), 71.0 (C1′), 70.3 (C5), 68,5 (C2′), 66.5 ($CH_2$ ar), 56.4 (C4′),55.3 ($CH_3$), 54.4 (C2), 52.0 (C3′), 41.7 (C6), 41.3 (C5′), 31.9−22.7 ($14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 811.5791, found 811.5788.

*3′-(Tetrahydropyrimidin-1(2H)-yl)-(2′R/S)-2′-hydroxypropyl 2-(tert-Butoxycarbonylamino)-2,6-dideoxy-6-(p-methoxybenzyloxycarbonylamino)-3,4-di-O-nonyl-α-D-glucopyranoside (**43a,b**).* The opening of **31a,b** (195 mg, 0.26 mmol) by 1,3-diaminopropane (2 mL) was achieved following procedure II. **43a,b**: 82% yield (178 mg, 0.21 mmol, colorless paste). $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 7.26 (d, 2H, $J$ = 8.5 Hz, H ar), 6.84 (d, 2H, $J$ = 8.6 Hz, H ar), 5.37−4.92 (m, 2H, $CH_2$ ar, NH), 4.66 (s, 1H, H-1), 4.27−3.85 (m, 1H, H2′, NH), 3.80−3.20 (m, 17H, H-2, H-3, H-5, H-6, H-1′, H-7′, $CH_2O$, $CH_3$, NH), 3.13−2.49 (m, 7H, H-4, H-3′, H-4′, H-6′), 1.94−1.73 (m, 2H, H-5′), 1.57−1.35 (m, 13H, $CH_2$, $CH_3$), 1.33−1.03 (m, 24H, $CH_2$), 0.87−0.82 (m, 6H, $CH_3$). $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 159.5 (C ar), 156.5 (COO), 155.6 (COO), 129.9 (CH ar), 128.8 (C ar), 113.9 (CH ar), 98.7 (C1), 81.1 (C3), 79.5 (C−O), 79.4 (C4), 73.6, 73.5 ($2CH_2O$), 70.2 (C1′), 70.0 (C5), 66.9 (C2′), 66.5 ($CH_2$ ar), 56.7 ($NCH_2N$), 56.6 (C3′), 56.3 (C7′), 55.3 ($CH_3$), 54.4 (C2), 51.6 (C4′), 43.3 (C6′), 41.7 (C6), 31.9−22.7 (C5′, $14CH_2$, $4CH_3$), 14.1 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 825.5947, found 825.5943.

*3′-(2″-Hydroxyethylamino)-(2′R/S)-2′-hydroxypropyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**44a,b**).* The deprotection of **40a,b** (195 mg, 0.24 mmol) was achieved following procedure I. **44a,b**: 91% yield (194 mg, 0.22 mmol, colorless paste). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.13 (d, 1H, $J$ = 3.5 Hz, H-1), 4.23−4.14 (m, 1H, H-2′), 3.97−3.66 (m, 8H, H-3, H-5, H-1′, H-5′, $CH_2O$), 3.62−3.52 (m, 2H, H-1′, $CH_2O$), 3.39−3.28 (m, 3H, H-2, H-6, H-3′), 3.27−3.07 (m, 5H, H-4, H-6, H-3′, H-4′), 1.76−1.53 (m, 4H, $CH_2$), 1.41−1.23 (m, 24H, $CH_2$), 0.90(t, 6H, $J$ = 7.0 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 95.9, 95.8 (C1), 80.1, 80.0 (C4), 77.7, 77.6 (C3), 73.7, 73.2 ($CH_2O$), 70.0, 69.8 (C1′), 68.5 (C5), 65.5, 65.3 (C2′), 56.3 (C5′), 53.5 (C2), 49.3, 49.2, 49.1 (C3′, C4′), 40.1 (C6), 31.7−22.3 ($14CH_2$), 13.0 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 548.4633, found 548.4634.

*3′-(3″-Hydroxypropylamino)-(2′R/S)-2′-hydroxypropyl 2,6-Diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**45a,b**).* The deprotection of **41a,b** (215 mg, 0.29 mmol) was achieved following procedure I. **45a,b**: 93% yield (241 mg, 0.27 mmol, colorless paste). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.14 (d, 1H, $J$ = 3.5 Hz, H-1), 4.19−4.14 (m, 1H, H-2′), 3.97−3.69 (m, 8H, H-3, H-5, H-1′, H-6′, $CH_2O$), 3.64−3.51 (m, 2H, H-1′, $CH_2O$), 3.37−3.09 (m, 8H, H-2, H-4, H-6, H-3′, H-4′), 2.00−1.90 (m, 2H, H-5′), 1.73−1.56 (m, 4H, $CH_2$), 1.42−1.25 (m, 24H, $CH_2$), 0.94−0.87 (m, 6H, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 97.1 (C1), 81.5 (C4), 78.9 (C3), 75.1, 74.7 ($CH_2O$), 71.1 (C1′), 69.9 (C5), 66.8 (C2′), 60.7 (C6′), 54.9 (C2), 50.9 (C3′), 47.8 (C4′), 41.5 (C6), 33.1−23.7 (C5′, $14CH_2$), 14.4 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 562.4790, found 562.4791.

*3′-(2″-Aminoethylamino)-(2′R/S)-2′-hydroxypropyl 2,6-Fiamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**46a,b**).* The deprotection of **42a,b** (181 mg, 0.22 mmol) was achieved following procedure I. **46a,b**: 89% yield (199 mg, 0.20 mmol, colorless paste). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.13 (d, 1H, $J$ = 3.5 Hz, H-1), 4.23−4.14 (m, 1H, H-2′), 3.96−3.66 (m, 6H, H-3, H-5, H-1′, $CH_2O$), 3.63−3.51 (m, 2H, H-1′, $CH_2O$), 3.45−3.27 (m, 7H, H-2, H-6, H-3′, H-4′, H-5′), 3.27−3.16 (m, 2H, H-4, H-3′), 3.11 (dd, $J$ = 9.6, 13.0 Hz, H-6), 1.70−1.56 (m, 4H, $CH_2$), 1.41−1.22 (m, 24H, $CH_2$), 0.90 (t, 6H, $J$ = 6.9 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 97.1 (C1), 81.5 (C4), 78.9 (C3), 75.0, 74.6 ($CH_2O$), 71.0 (C1′), 69.8 (C5), 67.1 (C2′), 54.8 (C2), 51.5 (C3′), 46.0 (C4′), 41.5 (C6), 37.0 (C5′), 33.0−23.7 ($14CH_2$), 14.4 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 547.4793, found 547.4793.

*3′-(3″-Aminopropylamino)-(2′R/S)-2′-hydroxypropyl 2,6-diamino-2,6-dideoxy-3,4-di-O-nonyl-α-D-glucopyranoside (**47a,b**).* The deprotection of **43a,b** (100 mg, 119.5 μmol) was achieved following procedure I. **47a,b**: 72% yield (88 mg, 86.5 μmol, colorless paste). $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 5.13 (d, 1H, $J$ = 3.5 Hz, H-1), 4.21−4.14 (m, 1H, H-2′), 3.97−3.66 (m, 6H, H-3, H-5, H-1′, $CH_2O$), 3.64−3.48 (m, 2H, H-1′, $CH_2O$), 3.40−3.02 (m, 10H, H-2, H-4, H-6, H-3′, H-4′, H-6′), 2.19−1.05 (m, 2H, H-5′), 1.71−1.56 (m, 4H, $CH_2$), 1.42−1.22 (m, 24H, $CH_2$), 0.90 (t, 6H, $J$ = 6.9 Hz, $CH_3$). $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 97.2 (C1), 81.5 (C4), 79.0 (C3), 75.1, 74.6 ($CH_2O$), 71.1 (C1′), 69.8 (C5), 66.9 (C2′), 54.9 (C2), 51.2, 51.1 (C3′), 46.0 (C4′), 41.5 (C6), 37.9 (C6′), 33.1−23.7 (C5′, $14CH_2$), 14.4 ($2CH_3$). HRMS (ESI$^+$) $m/z$: $[M + H]^+$ calcd 562.4790, found 562.4791.

**Biological and Biochemical Assays.** *MIC Determination.* All strains were grown overnight at 37 °C on trypticase soy agar (TSA) petri dishes (BD Diagnostics, BD, Franklin Lakes, NJ). MICs were determined by microdilution using a fresh culture in cation-adjusted Mueller−Hinton broth (CA-MHB) and a starting inoculum of $10^6$ cells, according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI).[110]

*Assessment of Eukaryotic Cell Viability.* Cell viability was assessed by evaluating their metabolic activity using the MTT assay (reduction in mitochondria of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium in purple formazan crystal).[111,112] Briefly, cells exposed for 24 h to 10 or 30 μM of compounds were incubated for 1 h with 0.2 mg/mL MTT. The reaction was stopped by addition of dimethyl sulfoxide (DMSO). The OD was measured at 570 nm.

***P. aeruginosa* Inner Membrane Permeabilization.** *Bacterial Strain and Growth Conditions.* Trypticase soy agar (TSA) medium was used to grow *P. aeruginosa* ATCC 27853 overnight at 37 °C. One colony was suspended in cation-adjusted Müller−Hinton Broth (MHB-CA) and incubated overnight at 37 °C on a rotary shaker (130 rpm). The bacterial suspension was diluted 100-fold in MHB-CA and incubated (130 rpm; 37 °C; 4 h) until it reached the mid logarithmic (mid log) phase ($OD_{620}$ ∼ 0.4−0.5).

*Membrane Permeabilization.*[29] Permeabilization of the inner bacterial membrane was studied with a membrane-impermeable fluorescent dye (propidium iodide). A stock solution of PI (3 mM in pure water) was diluted $10^3$-fold with the bacterial suspension ($OD_{600}$: 0.05). 3′,6-DiNn (**7**) and 3′,4′-diNn (**11**) neamines and 3,4-diNn neosamine **47a,b** in HEPES buffer, at final concentrations ranging from 1 to 10 μM, were added to the propidium iodide-containing bacterial suspension in 96-well microplates. The fluorescence intensity was measured with a SpectraMax M3 microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 25 °C after 15 min of stabilization at excitation and emission wavelengths of 540 and 610 nm, respectively. The data were normalized based on the fluorescence intensity measured in the presence of either quaternary ammonium hexadecyltrimethylammonium bromide (CTAB) 150 μM (positive control, 100%)[107] or imipenem (negative control, 0%).

*Resistance Selection Method.*[109] A serial passage method was used in which *P. aeruginosa* ATCC 27853 were grown in the continuous presence of a drug concentration corresponding to half of the MIC. The bacteria were examined daily for a change in MIC, followed by a corresponding increase of the drug concentration for up to 12 days. For this purpose, an initial inoculum of $2.5 \times 10^6$ cfu/mL of each of the original strain was exposed in broth to a range of antibiotic concentrations from 0.1 to 5−10-fold their original MIC (using arithmetic increases). After 24 h at 37 °C, the tubes were examined to determine the minimal drug concentration preventing bacterial growth (this value was equal to the MIC determined on an agar plate for the corresponding strain). Bacteria growing at a drug concentration of half this value were then readjusted at a density of $2.5 \times 10^6$ cfu/mL and


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again exposed for 24 h to drug concentrations from 0.1 to 5−10-fold the MIC. This process was repeated each day, looking for growth at drug concentrations larger than the original MIC. If this was observed, the new minimal drug concentration preventing bacterial growth was determined based on visual inspection of the cultures (and samples taken for confirmation of the MIC by agar dilution). Bacteria growing at a concentration corresponding to half of this new value were then used for continuation of the experiment, for a total of 12 days.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.6b00818.

General information for the synthesis, $^{1}H$ and $^{13}C$ NMR spectra, and purities of the evaluated amphiphilic derivatives (PDF)

Molecular formula strings (CSV)

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### Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS USED

AAC, aminoglycoside *N*-acetyltransferase; ar, aromatic (NMR); ax, axial (NMR); AG, aminoglycoside; ANT, aminoglycoside *O*-nucleotidyltransferase; APH, aminoglycoside *O*-phosphoryltransferase; CA, cationic amphiphiles; eq, equatorial (NMR); MRSA, methicillin resistant *S. aureus*; 2NB, 2-naphthylbutyl; 2NM, 2-naphthylmethylene; 2NP, 2-naphthylpropyl; np, naphthyl ring (NMR); Nn, nonyl; OM, outer membrane; PMBnoc, *para*-methoxybenzyloxycarbonyl; PI, propidium iodide

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# Supporting information

# New Broad-spectrum Antibacterial Amphiphilic Aminoglycosides Active Against Resistant Bacteria: From Neamine Derivatives to Smaller Neosamine Analogues

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## Purity determination of the evaluated derivatives prepared

The purity was determined by HPLC for the compounds carrying chromophores (also by $^1$H NMR spectrometry and TLC) and by $^1$H NMR spectrometry and TLC for the other derivatives (compounds **12**, **37**, **39**, **40**, **43** described in the manuscript and compounds **27**, **38** previously described).

The AAGs carrying chromophores were analyzed by HPLC on an Agilent 1100 series HPLC machine using C18 reversed phase column (Macherey-Nagel, Nucleodur C18 ISIS, 5 µm particle size, 100 Å pore size, 250 x 4.6 mm) and a diode array detector eluting with mixtures of different solutions:

X: Aqueous solution of trifluroroacetic acid (pH 2.5 at 20°C)

Y: Aqueous solution of ammonium acetate 10 mM (pH 2.9 at 20 °C)

Z: Acetonitrile

Method A: Gradient 100% X to 95:5 Z:X over 30 min, 1 mL/min, detection at 281 nm.

Method B: Gradient 100% X to 95:5 Z:Y over 30 min, 1 mL/min, detection at 281 nm.

Method C: Isocratic 95:5 Z:Y over 30 min, 1 mL/min, detection at 281 nm.

The purity was not determined by elemental analysis since (i) the main difficulty in the purification was the removal of isomers (for example, the 3',6-isomers of the 3',4'-neamine derivatives), (ii) the final compounds are very hygroscopic and (iii) it was not possible to detect by NMR at 400 MHz the presence of two diastereoisomers in compounds **39a**,**b**, **44a**,**b**-**47a**,**b** (expected diastereoisomeric ratio 2:1 from the corresponding epoxides mixture **31a**,**b** detected in the NMR spectrum).

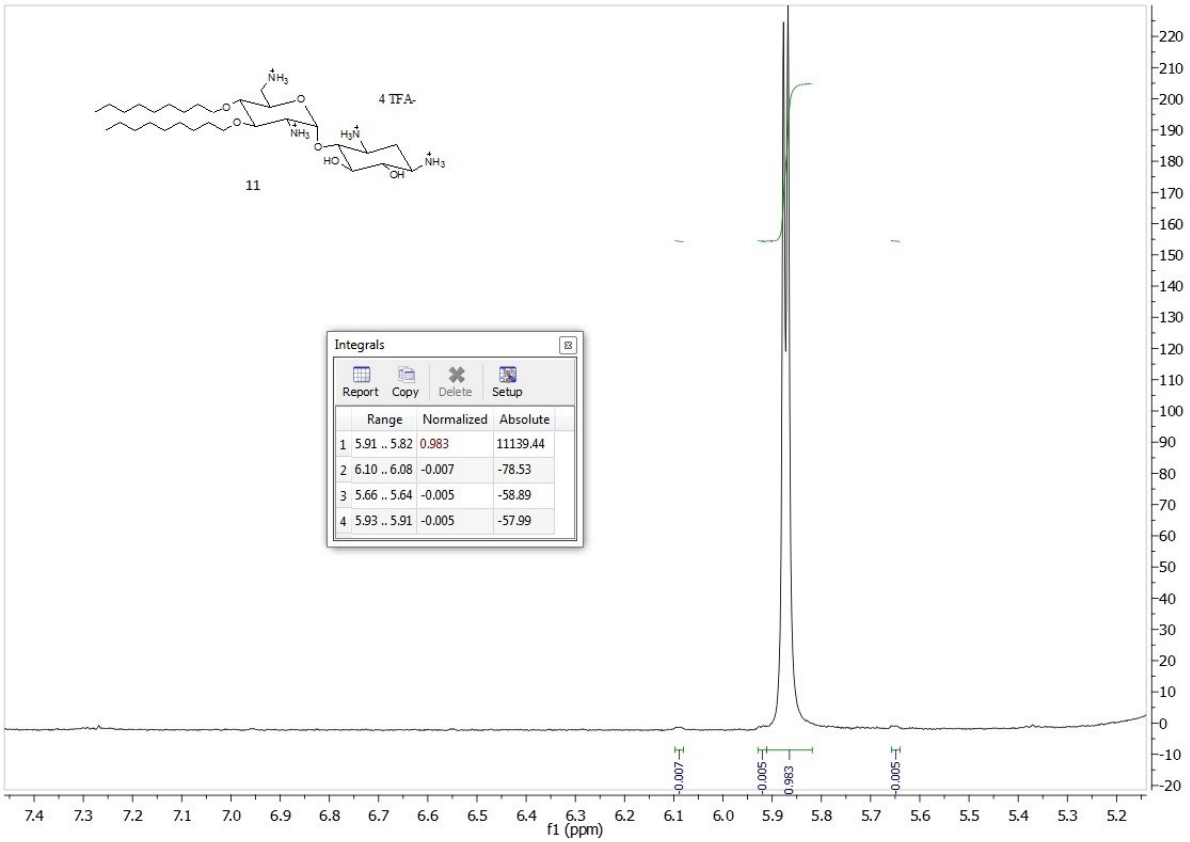
TLC and $^1$H NMR spectrometry allowed to detect the presence of isomers, for instance, the retardation factors of the 3′,6-dinonyl derivative 7 and its 3′,4′-isomer **11** were 0.5 and 0.3, respectively (eluent: EtOH/$H_2O$/($NH_3$, $H_2O$) (20%) 80:10:10; TLC visualizations: sulfuric acid spray (5 mL in 100 mL EtOH) and ninhydrin spray (0.3 g, 3 mL AcOH, 100 mL EtOH).

Therefore, the absence or the presence of aminoglycoside isomers and other aminosugars as minor impurities was checked by $^1$H NMR spectrometry (integration of the peaks corresponding to anomeric protons) and by TLC on silica gel.

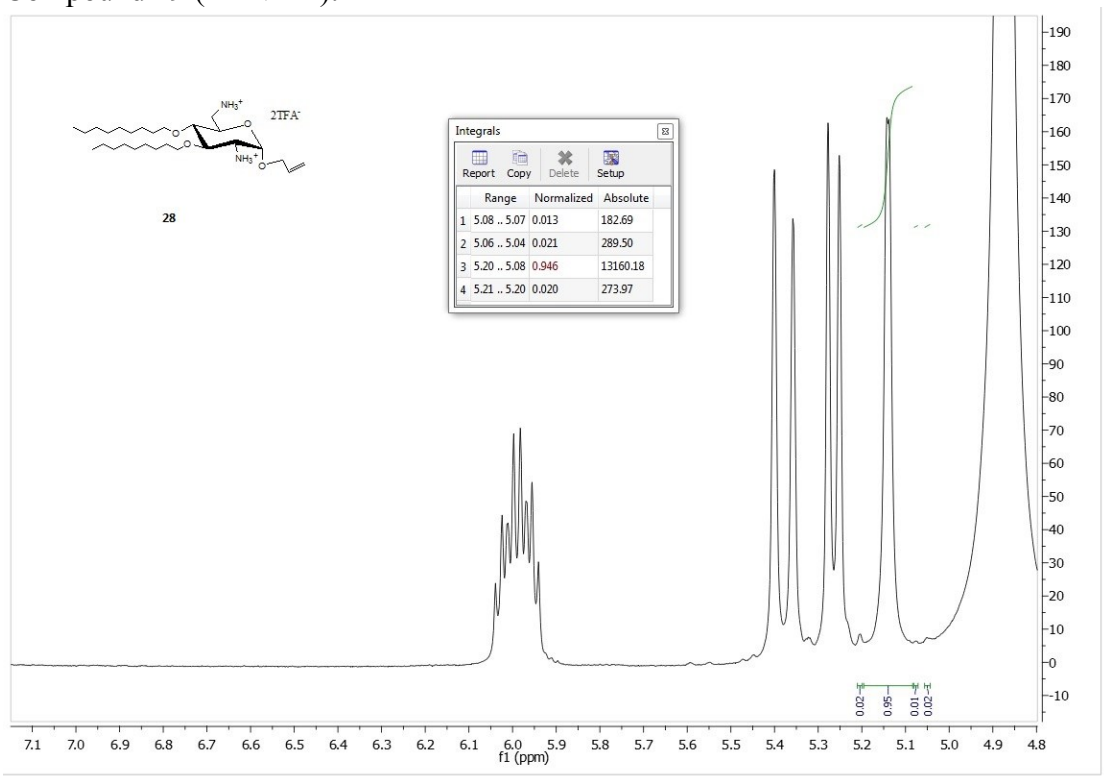
| Compounds | Purity (%) | Method |
|---|---|---|
| **4, 5, 7, 8** | > 95 | HPLC, NMR[a] (previously described) |
| **10** | 98 | HPLC |
| **11** | > 98 | NMR[a] |
| **17** | 96 | HPLC, elemental analysis |
| **28** | 96 | HPLC |
| **29** | > 95 | NMR[a] |
| **34** | > 95 | NMR[a,b] |
| **37** | > 95 | NMR[a,b] |
| **38** | > 95 | NMR[a,b] |
| **39a,b** | > 95 | NMR[a,b] |
| **44a,b** | > 95 | NMR[a,b] |
| **45a,b** | > 95 | NMR[a,b] |
| **46a,b** | > 95 | NMR[a,b] |
| **47a,b** | > 95 | NMR[a,b] |

a: determined by $^{1}$H NMR integration of the observed peaks corresponding to anomeric protons
b: one diastereoisomer
c: 2:1 mixture of two diastereoisomers, the presence of two diastereoisomers cannot be detected by $^{1}$H NMR at 400 MHz.

Compound **11** ($^{1}$H NMR):



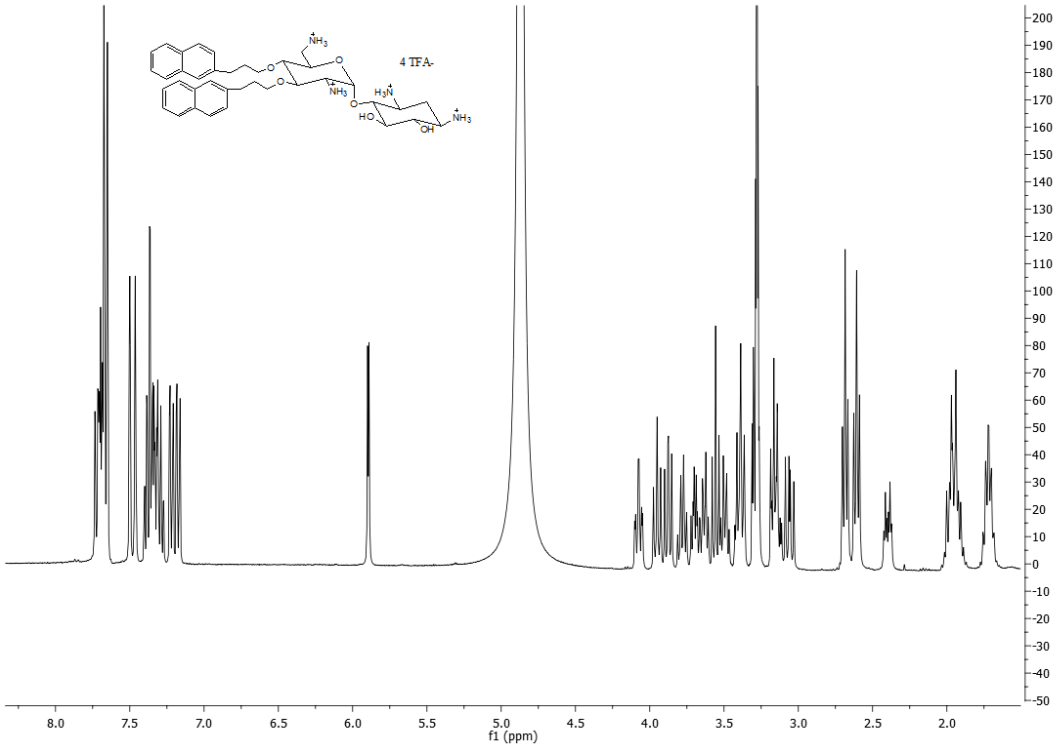
Compound **29** ($^1$H NMR):



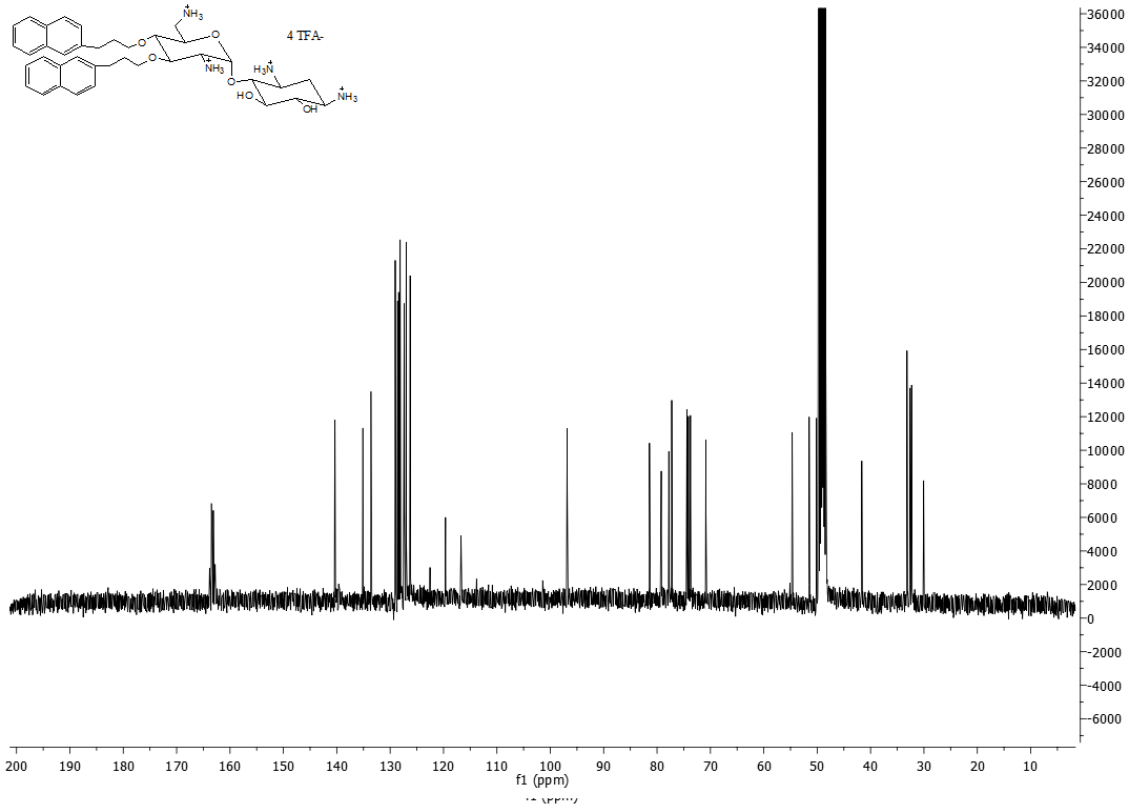
# $^{1}$H and $^{13}$C NMR spectra of compounds

Compound **10**.

$^{1}$H NMR $CD_3OD$

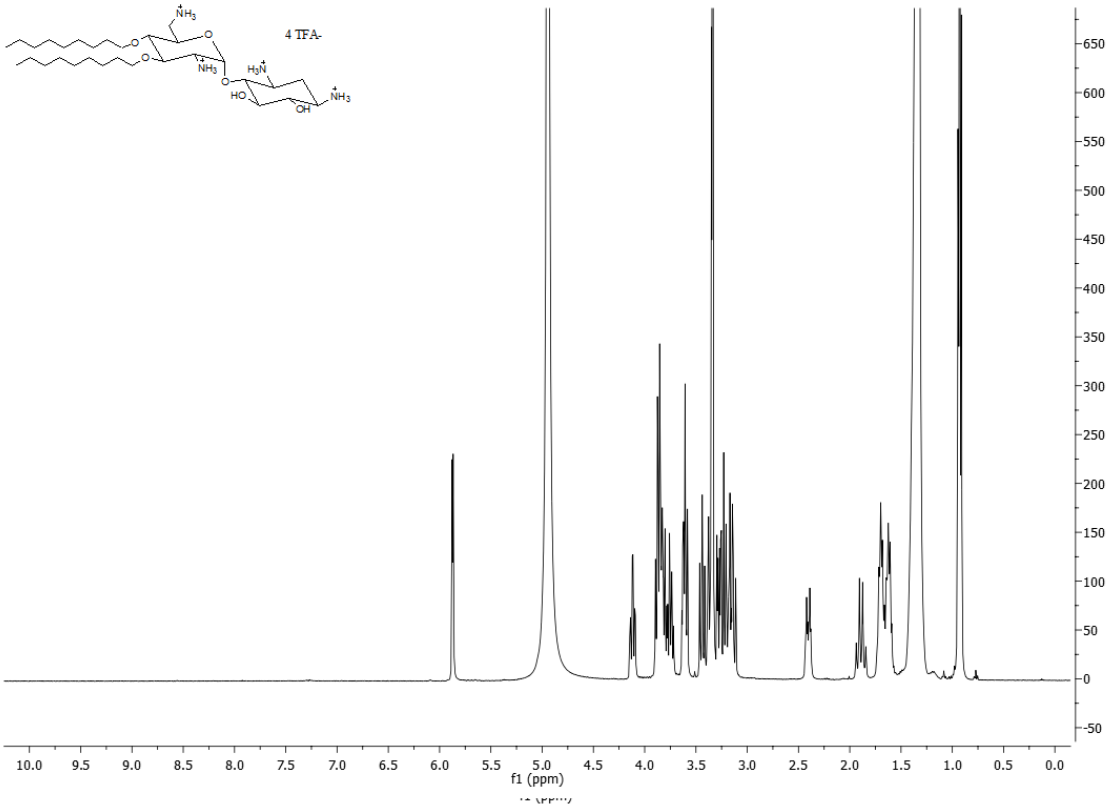



$^{13}$C NMR $CD_3OD$

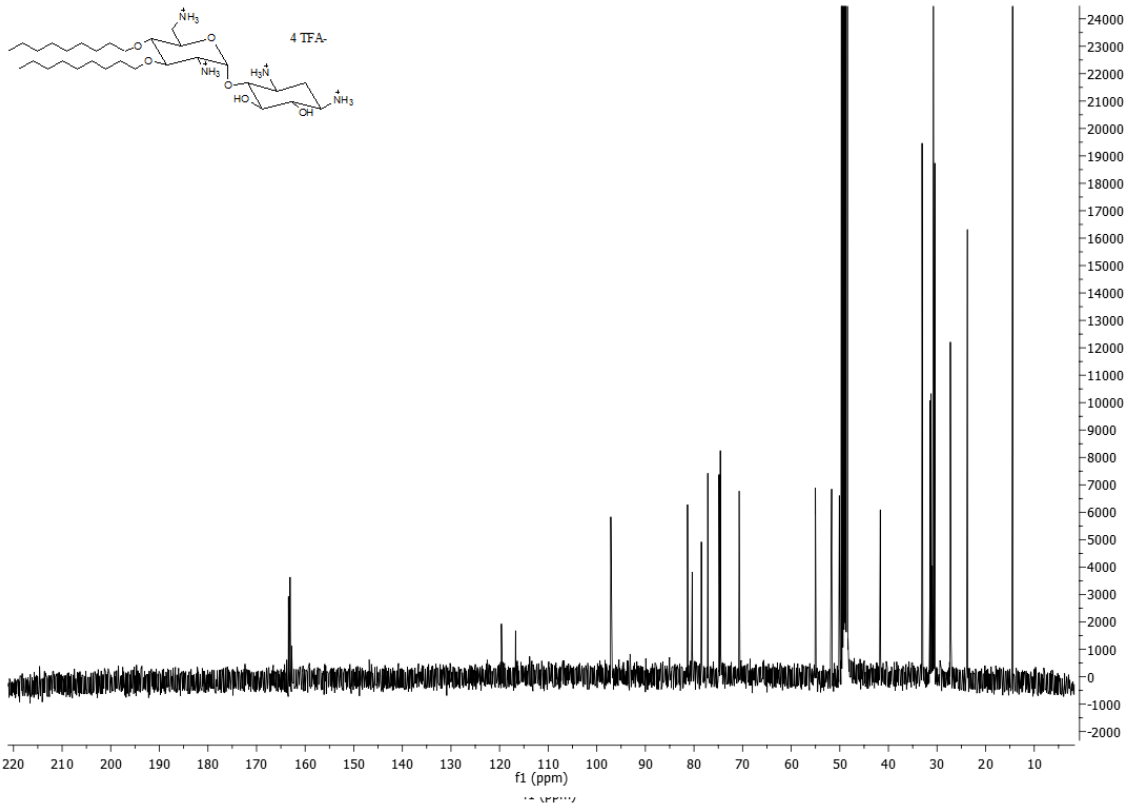


Compound **11**

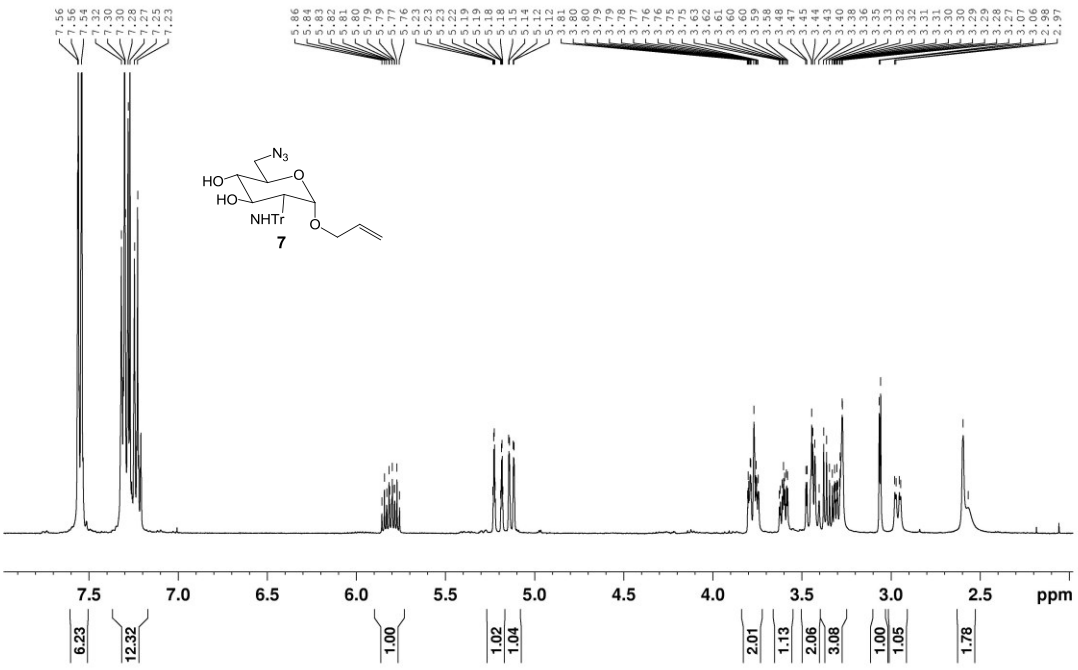
$^{1}$H NMR $CD_3OD$

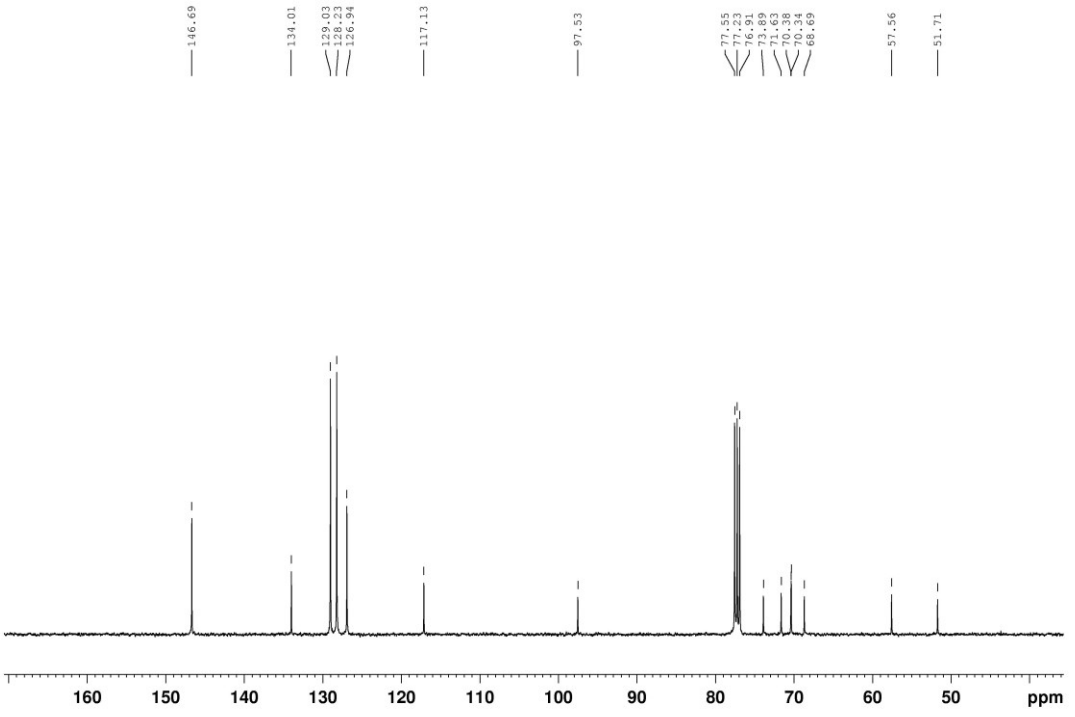



$^{13}$C NMR $CD_3OD$

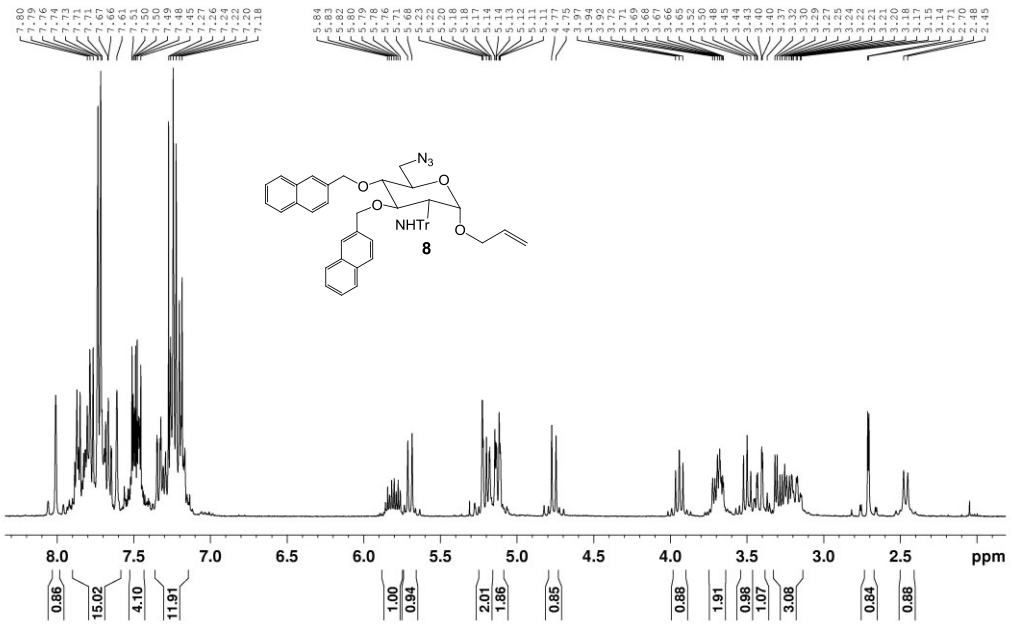


**Allyl 6-azido-2,6-dideoxy-2-tritylamino-α-D-glucopyranoside (15)**
$^{1}$H NMR $CDCl_3$

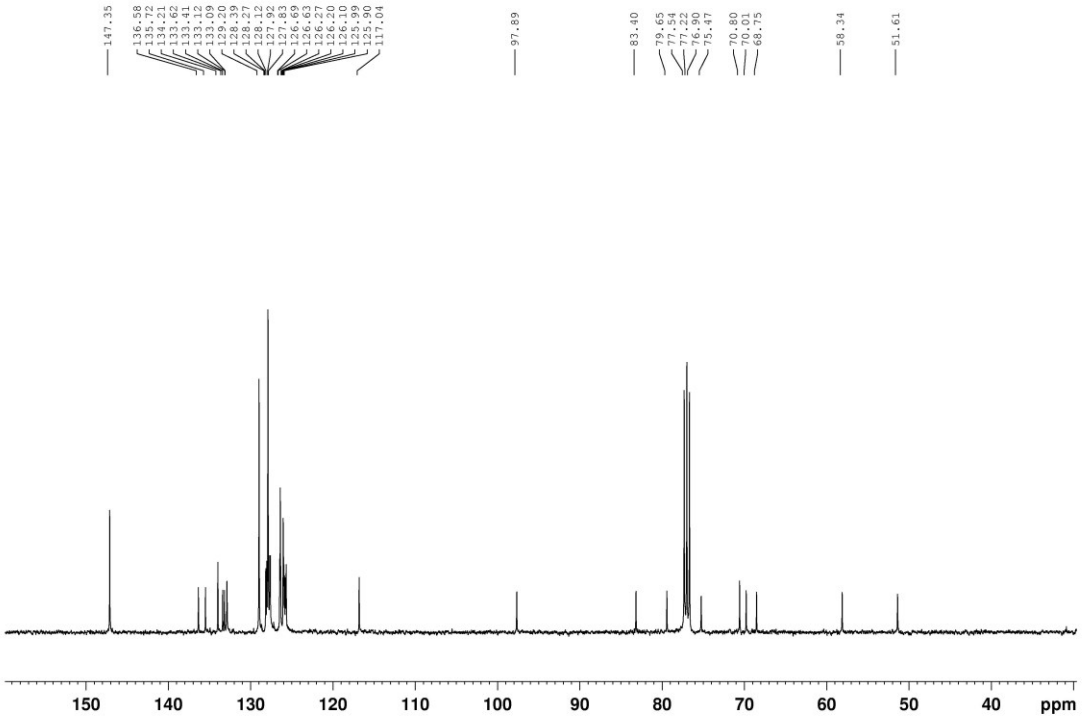


**Allyl 6-azido-2,6-dideoxy-3,4-di-*O*-[(2-naphtyl)methyl]-2-tritylamino-α-D-glucopyranoside (16),** $^{1}$H NMR $CDCl_3$

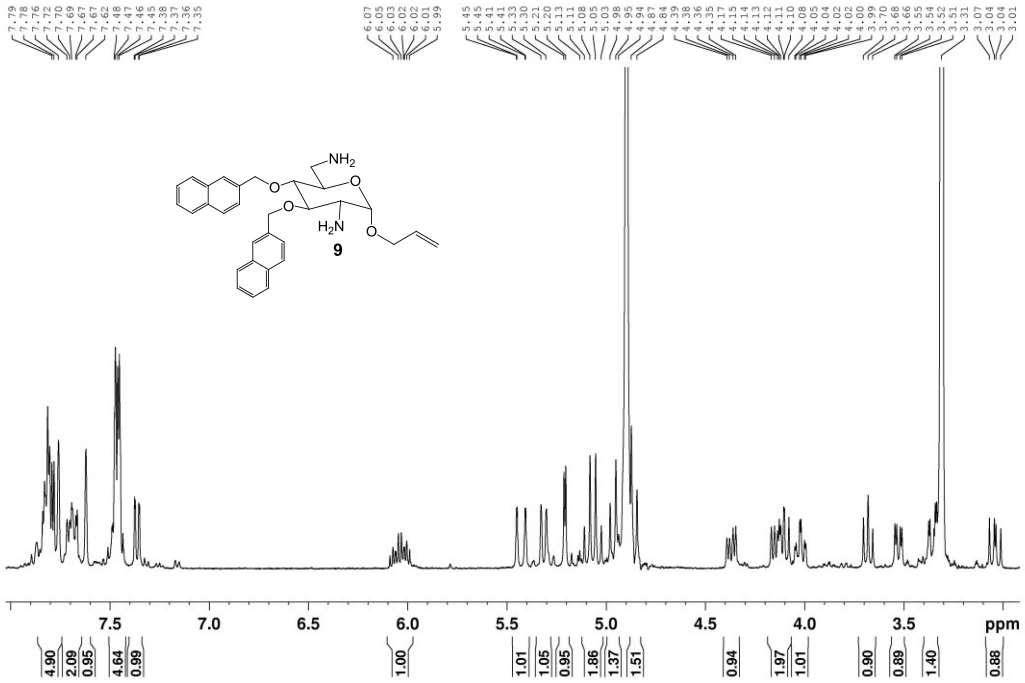



$^{13}$C NMR $CD_3OD$

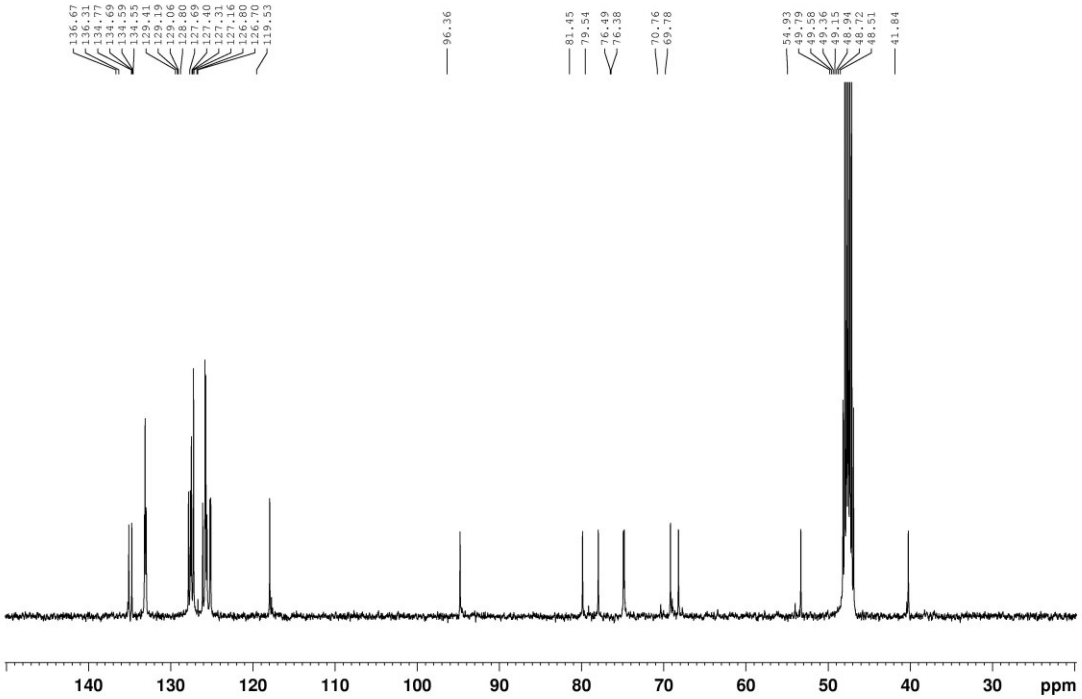


**Allyl 2,6-diamino-2,6-dideoxy-3,4-di-*O*-[(2-naphtyl)methyl]-α-D-glucopyranoside (17)**

$^{1}$H NMR $CDCl_3$

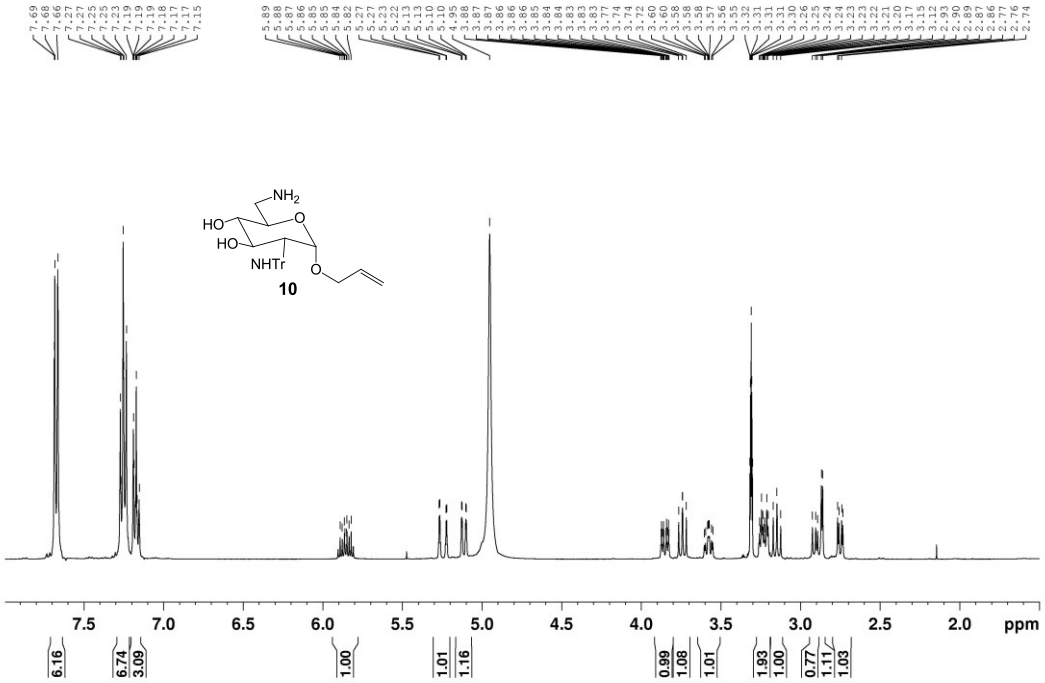



$^{13}$C NMR $CD_3OD$

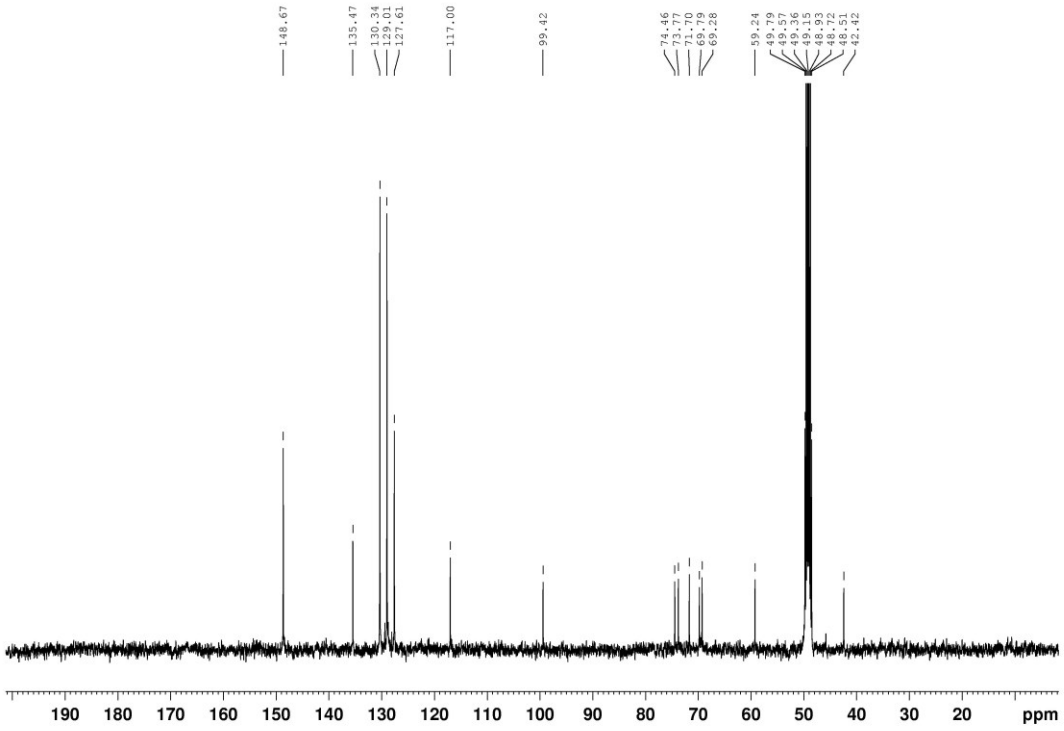


**Allyl 6-amino-2,6-dideoxy-2-tritylamino-α-D-glucopyranoside (18)**

$^{1}$H NMR $CDCl_3$

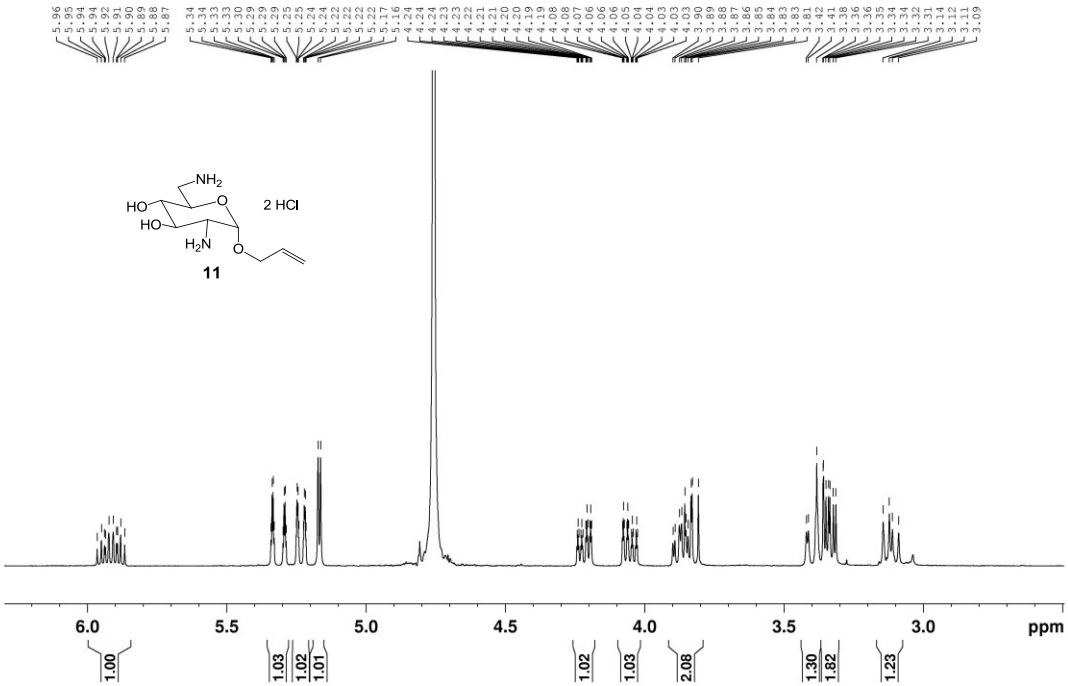



$^{13}$C NMR $CD_3OD$

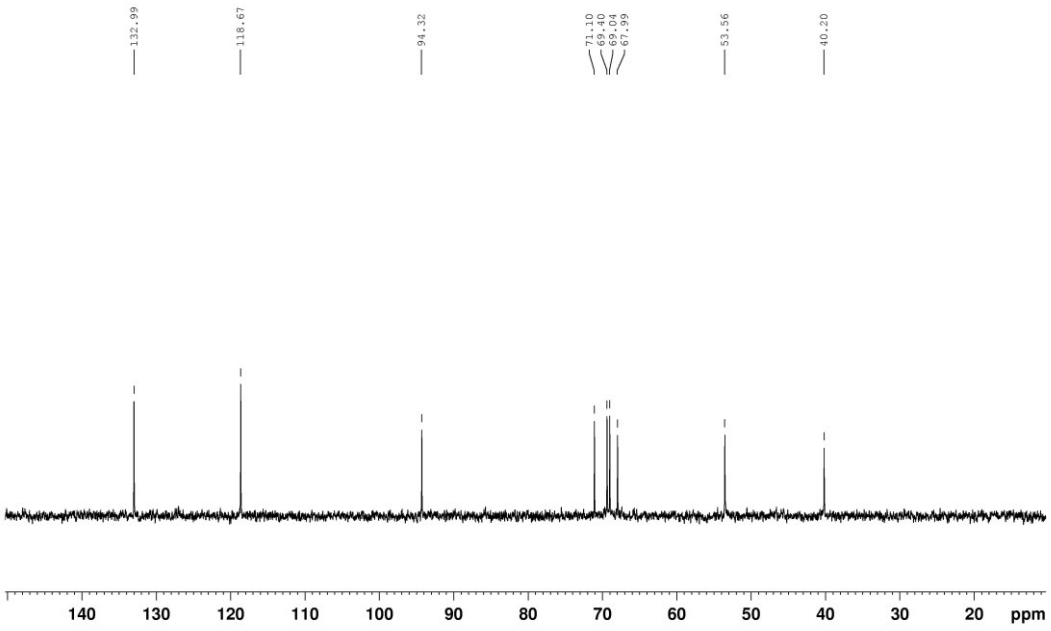


**Allyl 2,6-diamino-2,6-dideoxy-α-D-glucopyranoside (19)**

$^{1}$H NMR $CDCl_3$

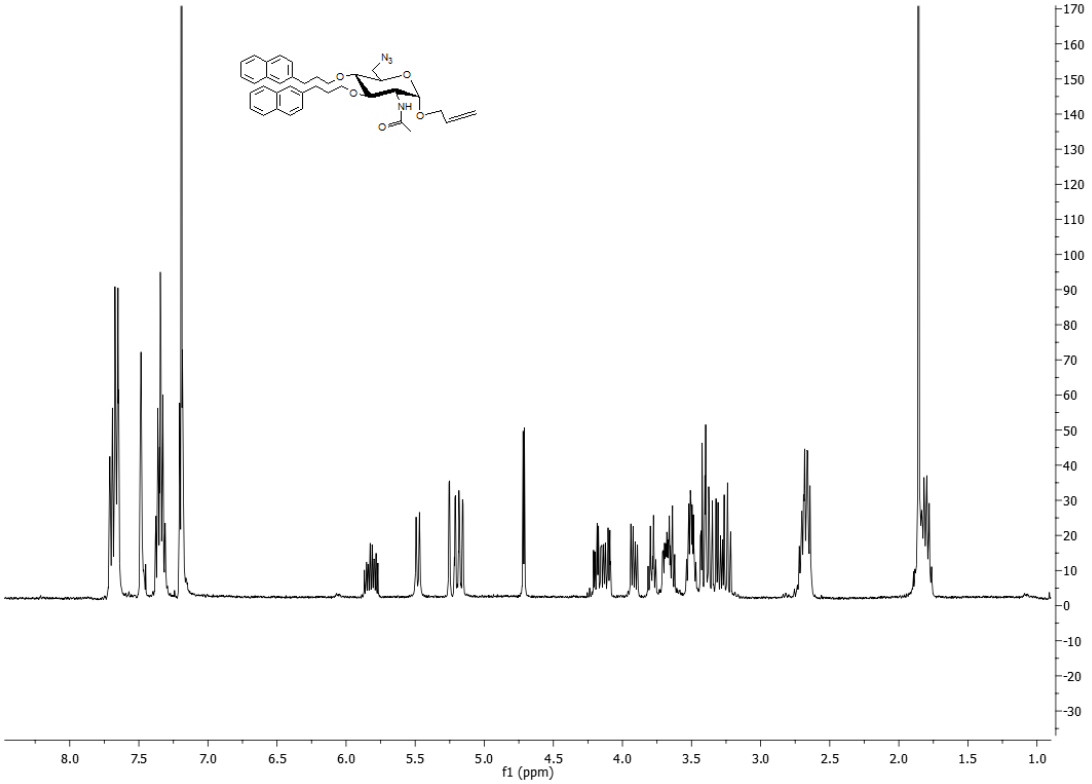



$^{13}$C NMR $CD_3OD$

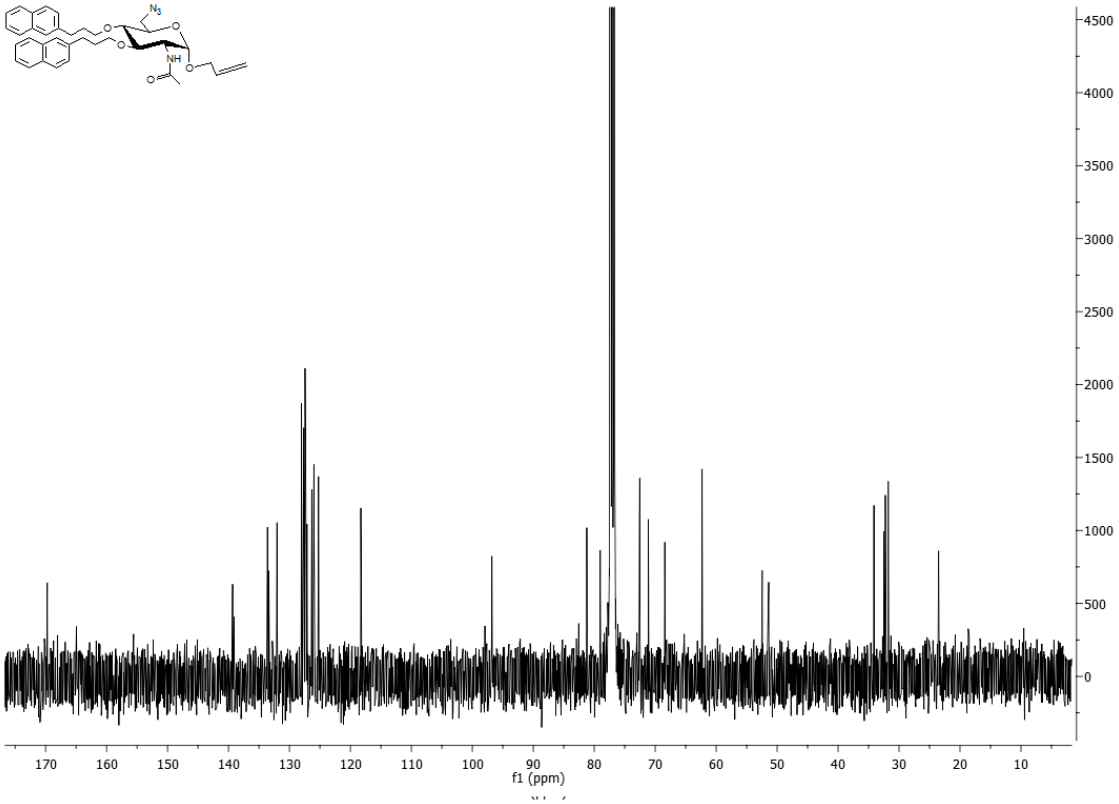


Compound **20**

$^{1}H$ NMR $CDCl_3$

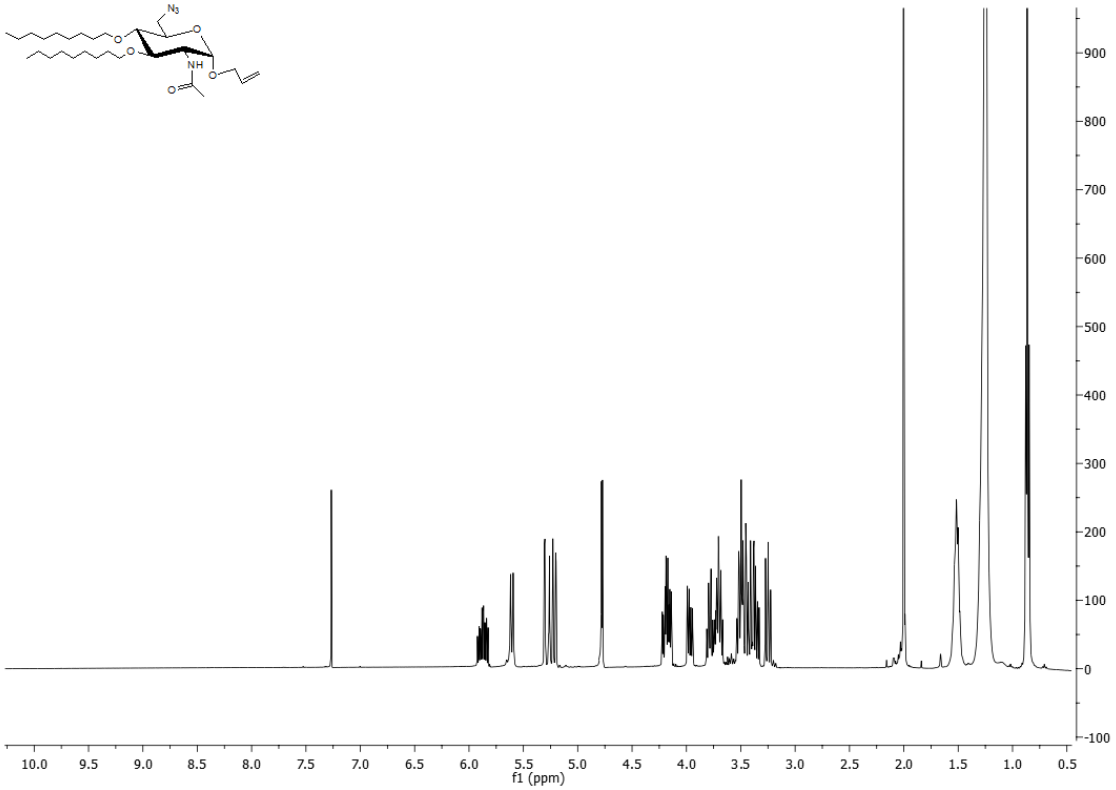



$^{13}C$ NMR $CDCl_3$

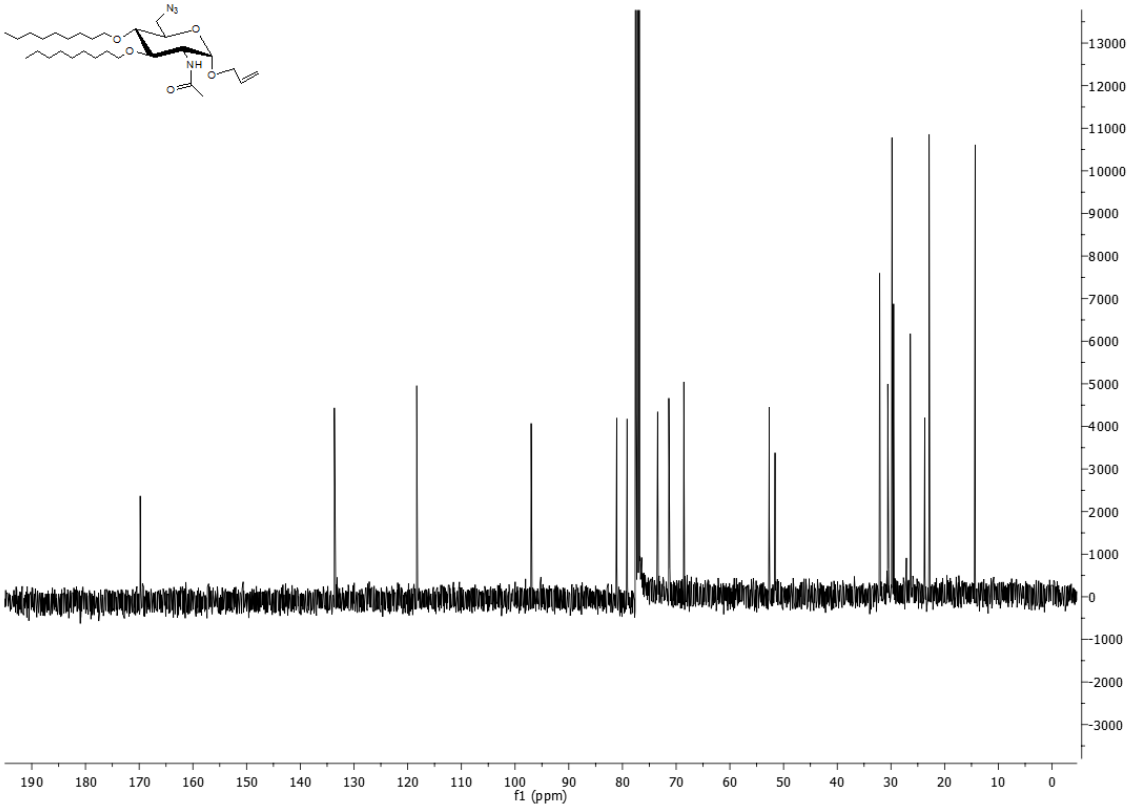



Compound **21**

$^{1}H$ NMR $CDCl_3$

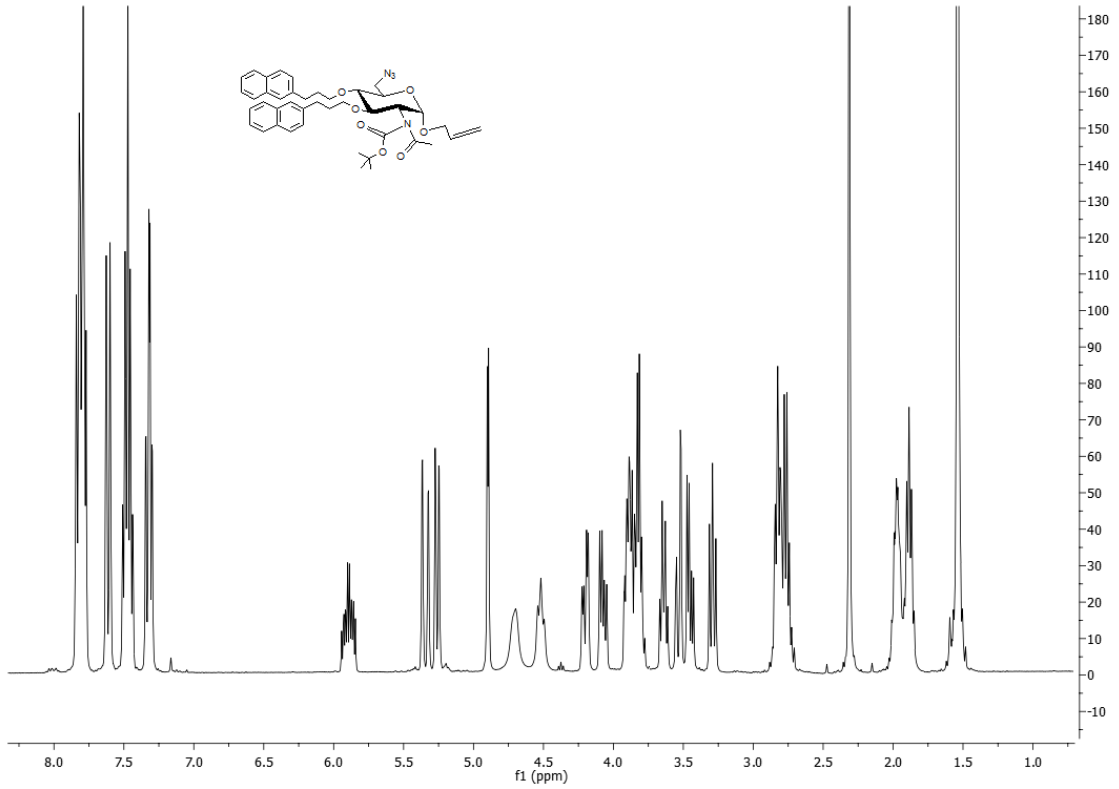



$^{13}C$ NMR $CDCl_3$

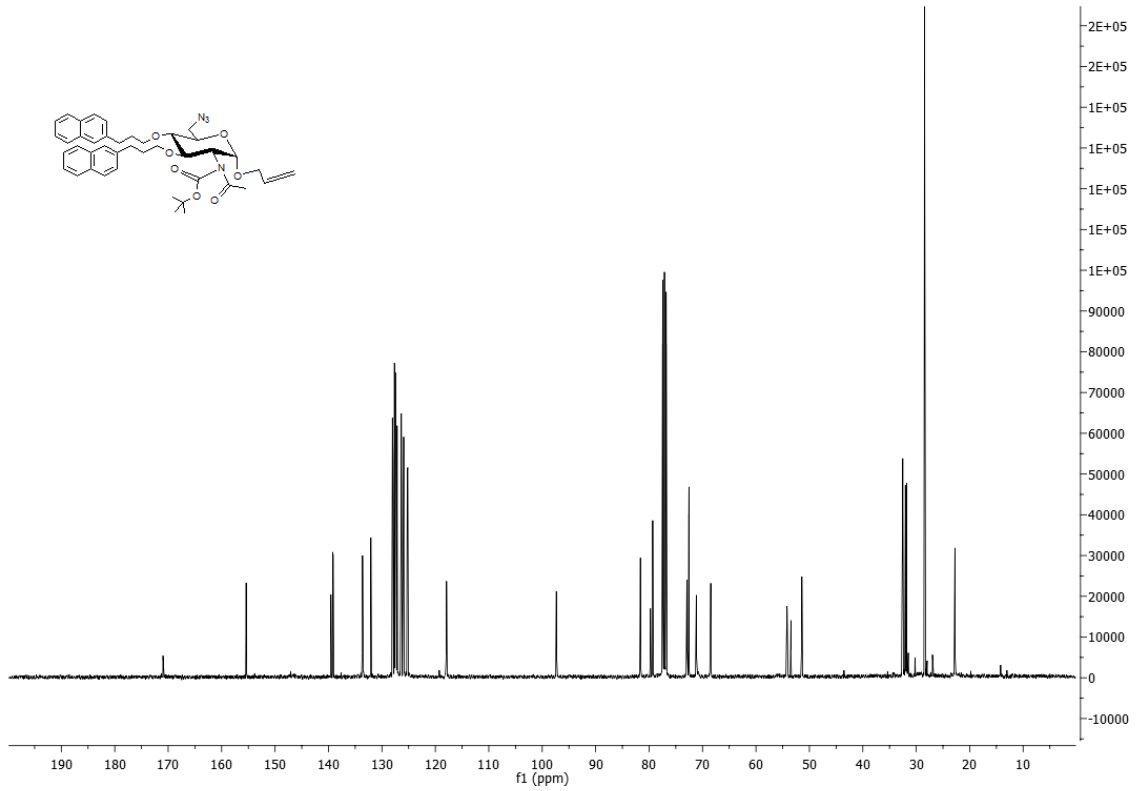


Compound **22**

$^{1}H$ NMR $CDCl_3$

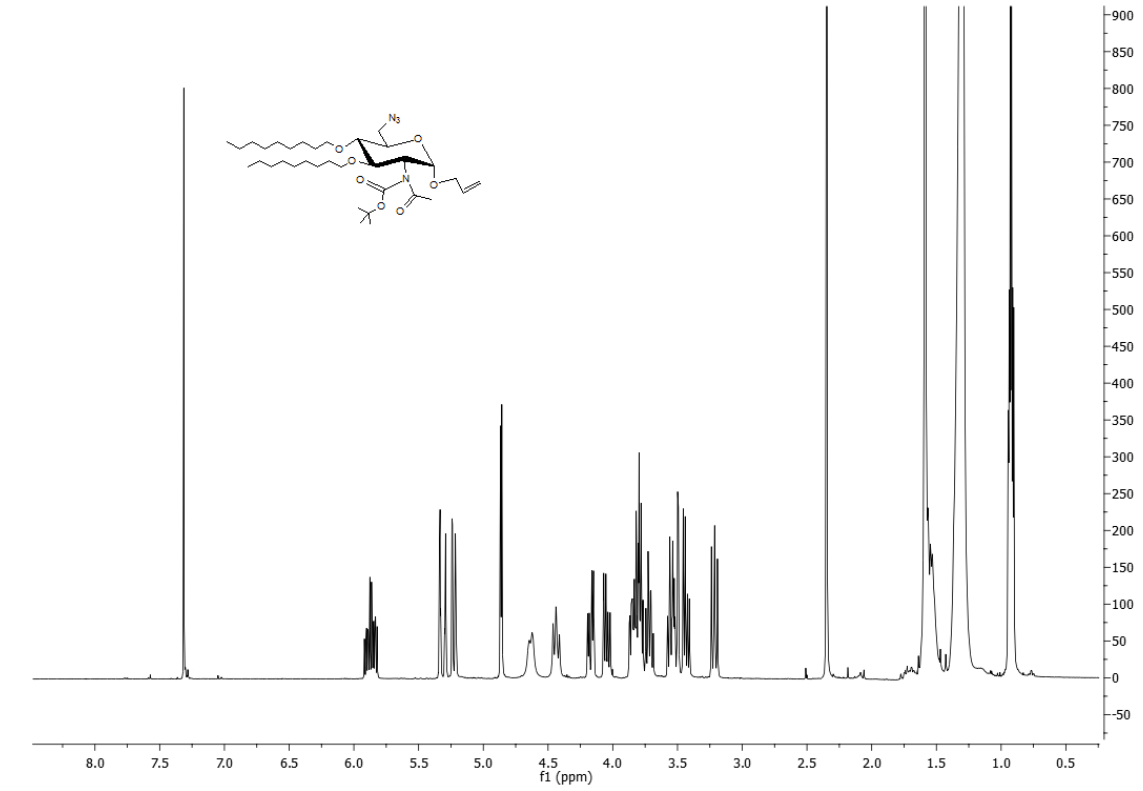



$^{13}C$ NMR $CDCl_3$

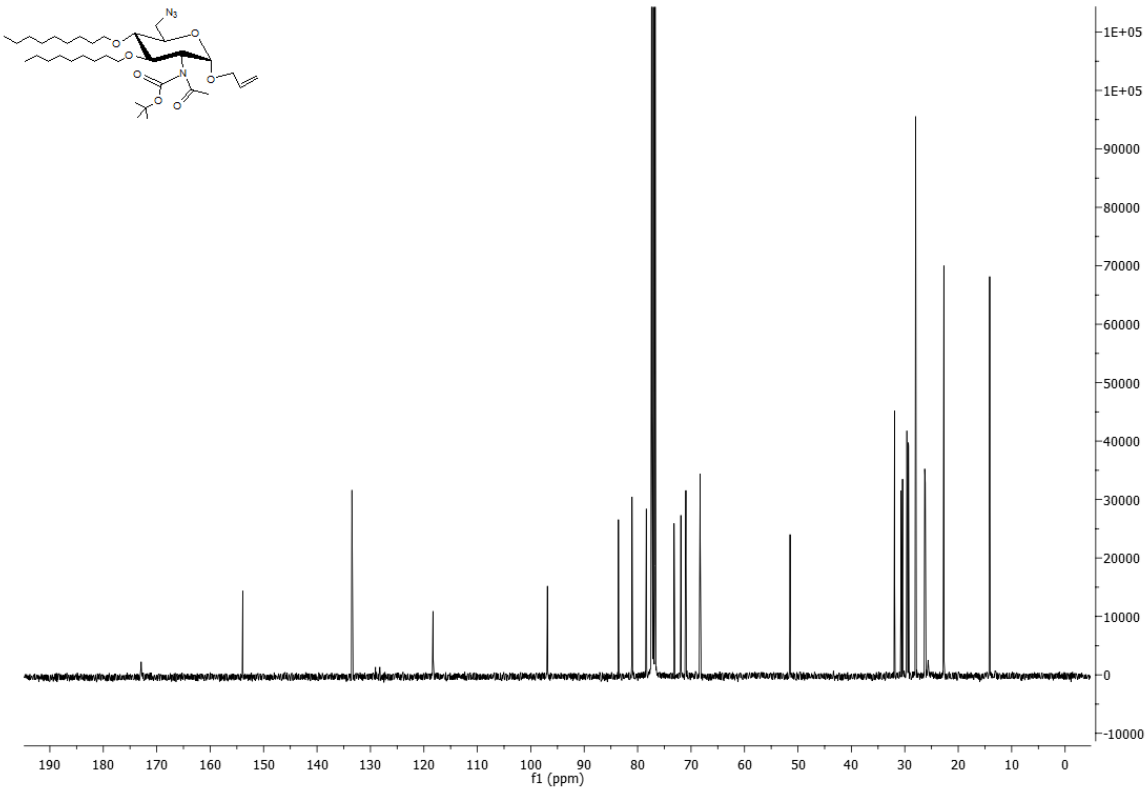


Compound **23**

$^{1}H$ NMR $CDCl_3$

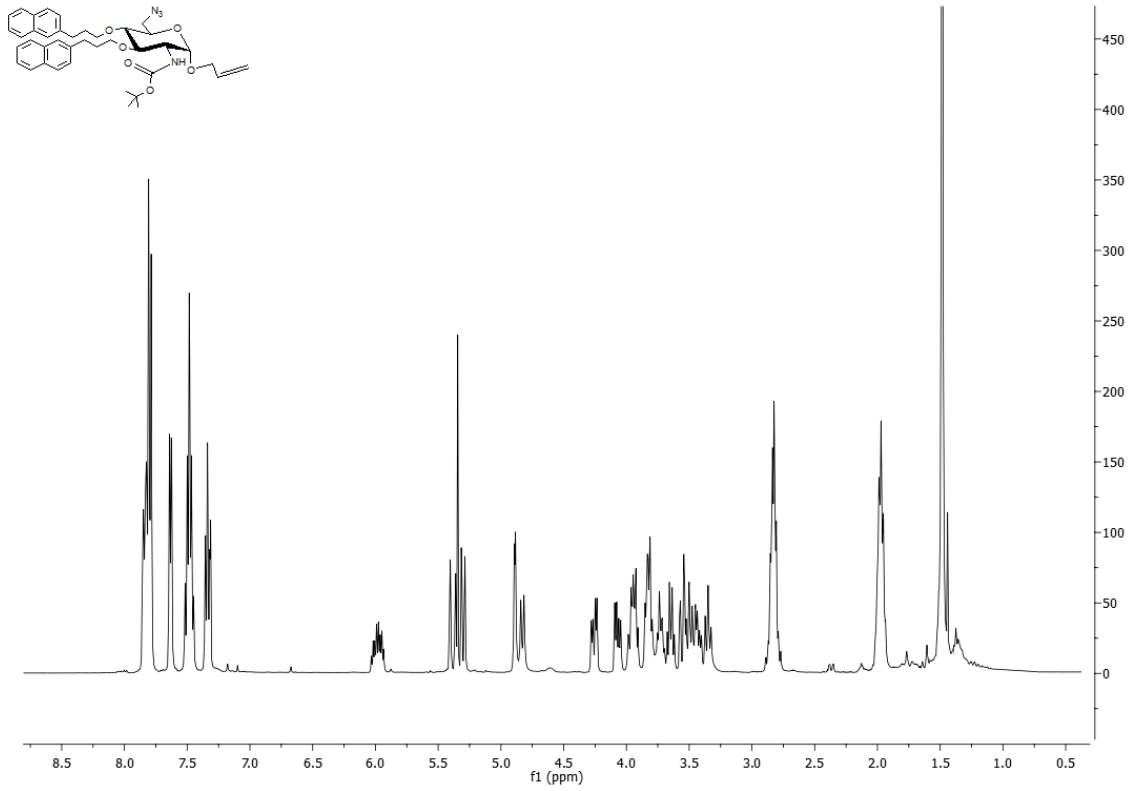



$^{13}C$ NMR $CDCl_3$

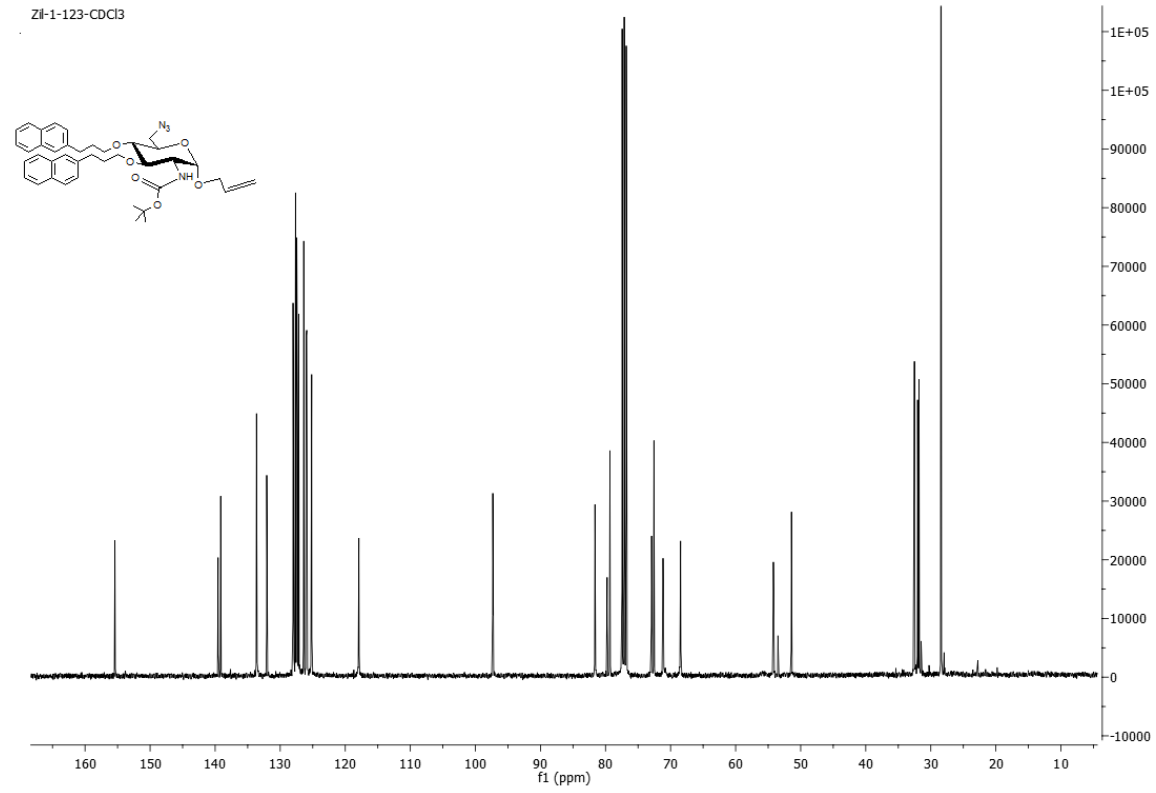


Compound **24**

$^{1}H$ NMR $CDCl_3$

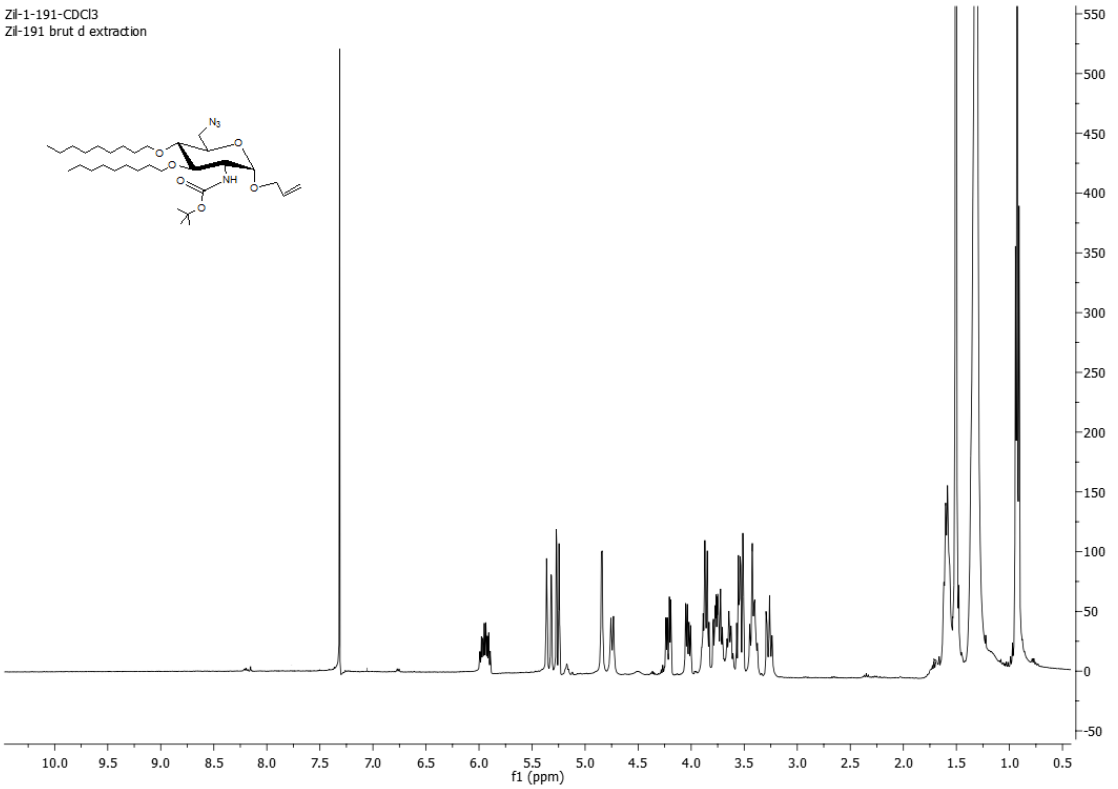



$^{13}C$ NMR $CDCl_3$

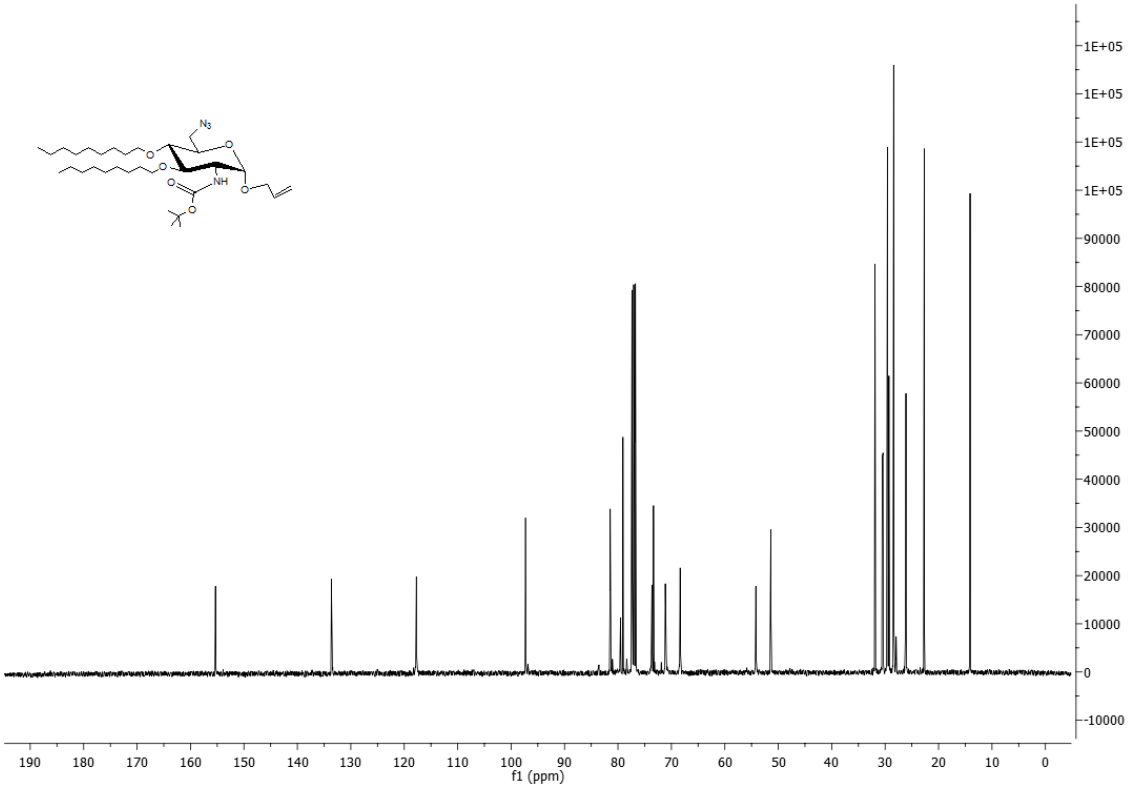


Compound **25**

$^{1}H$ NMR $CDCl_3$

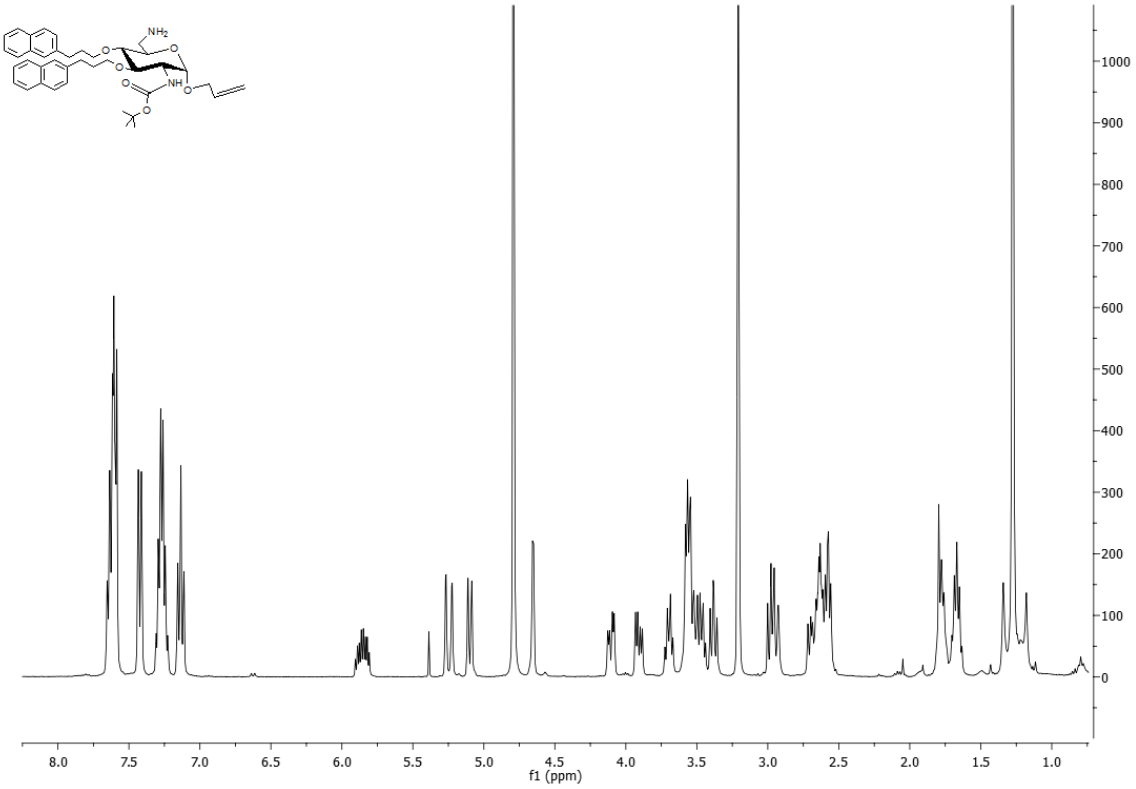



$^{13}C$ NMR $CDCl_3$

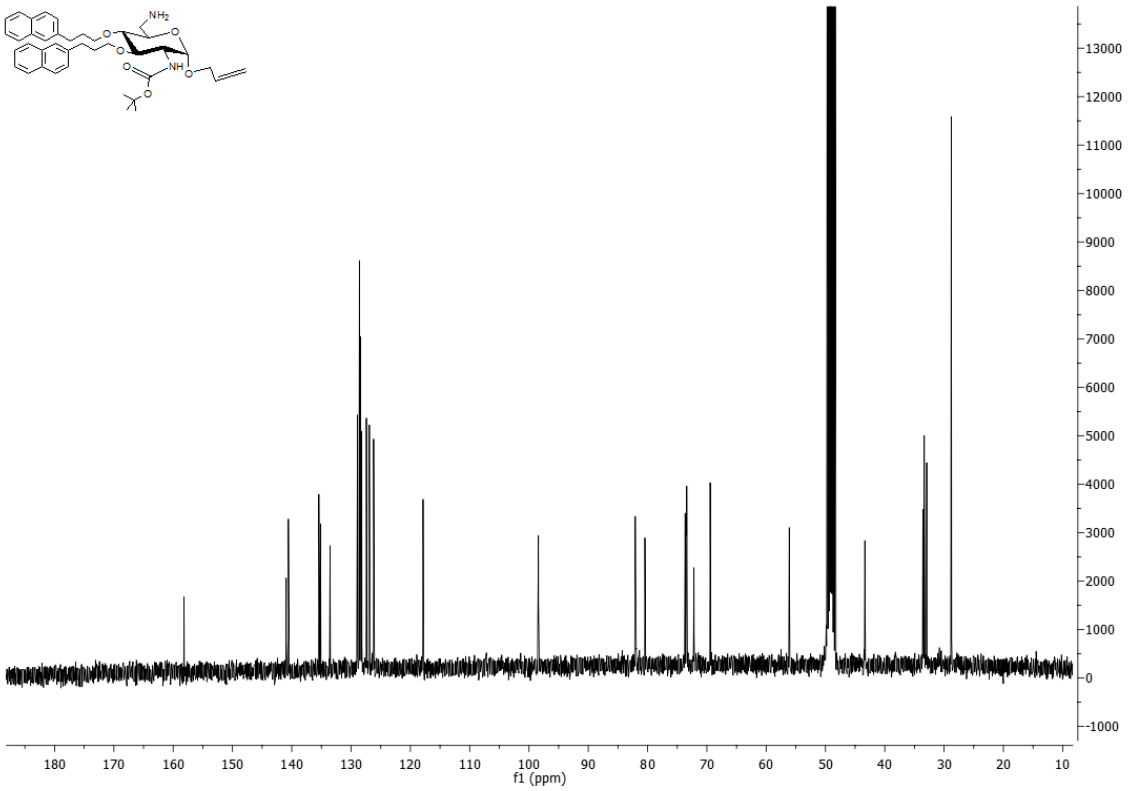


Compound **26**

$^{1}$H NMR $CD_3OD$

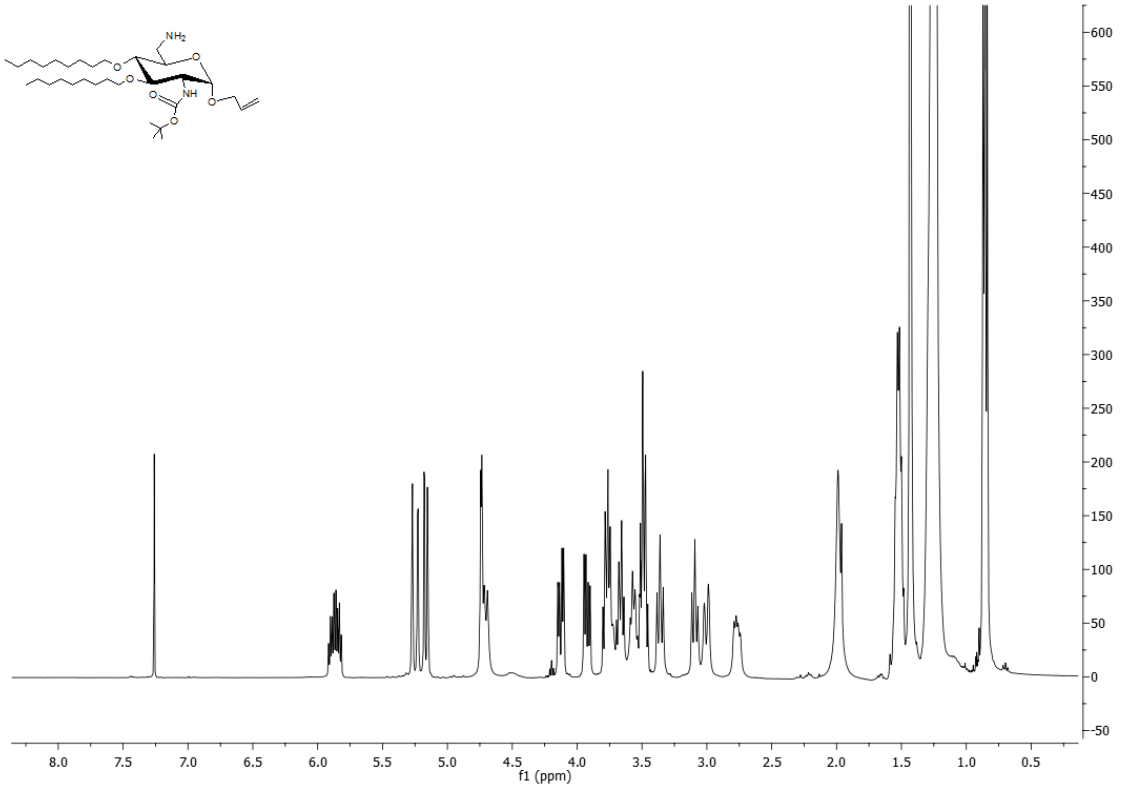



$^{13}$C NMR $CD_3OD$

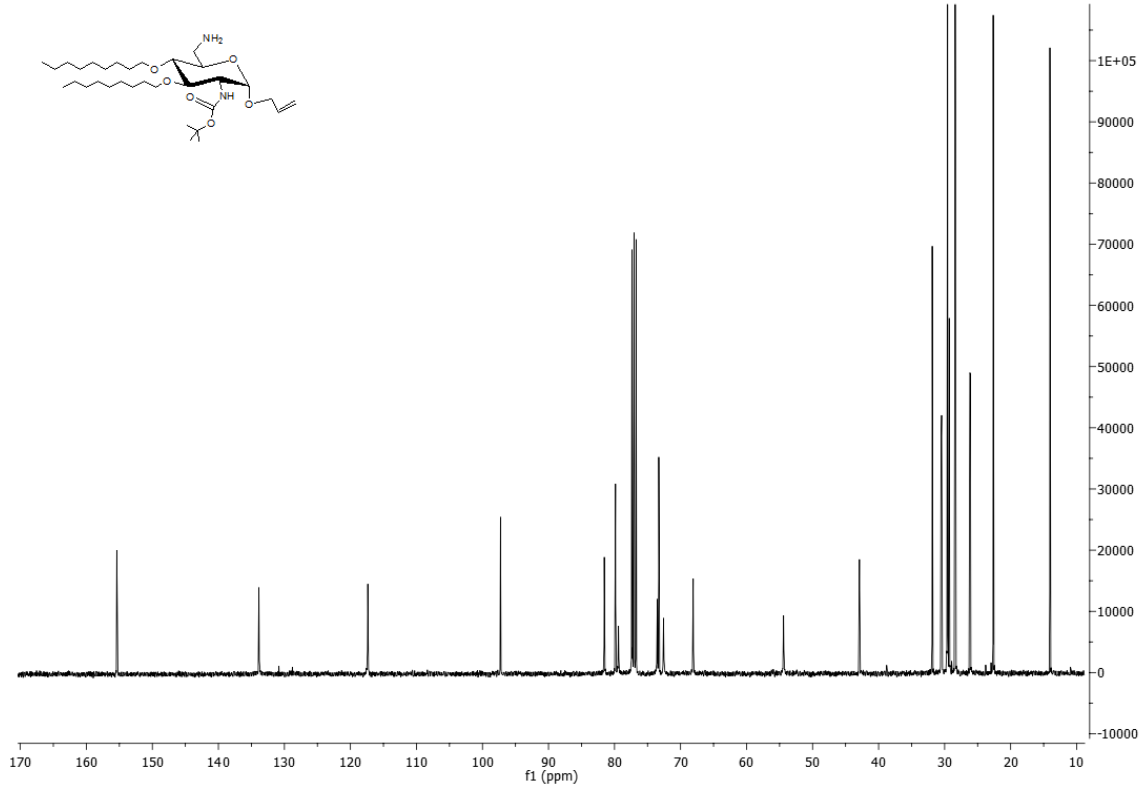


Compound **27**

$^{1}H$ NMR $CDCl_3$

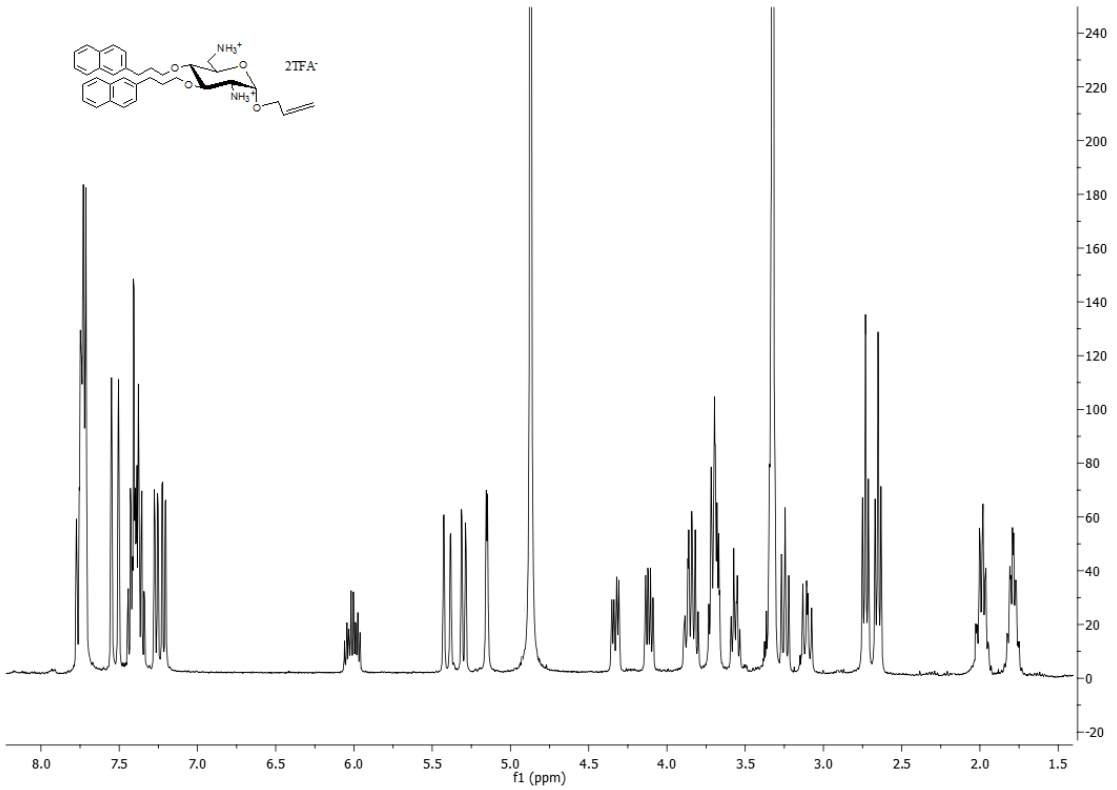



$^{13}C$ NMR $CDCl_3$

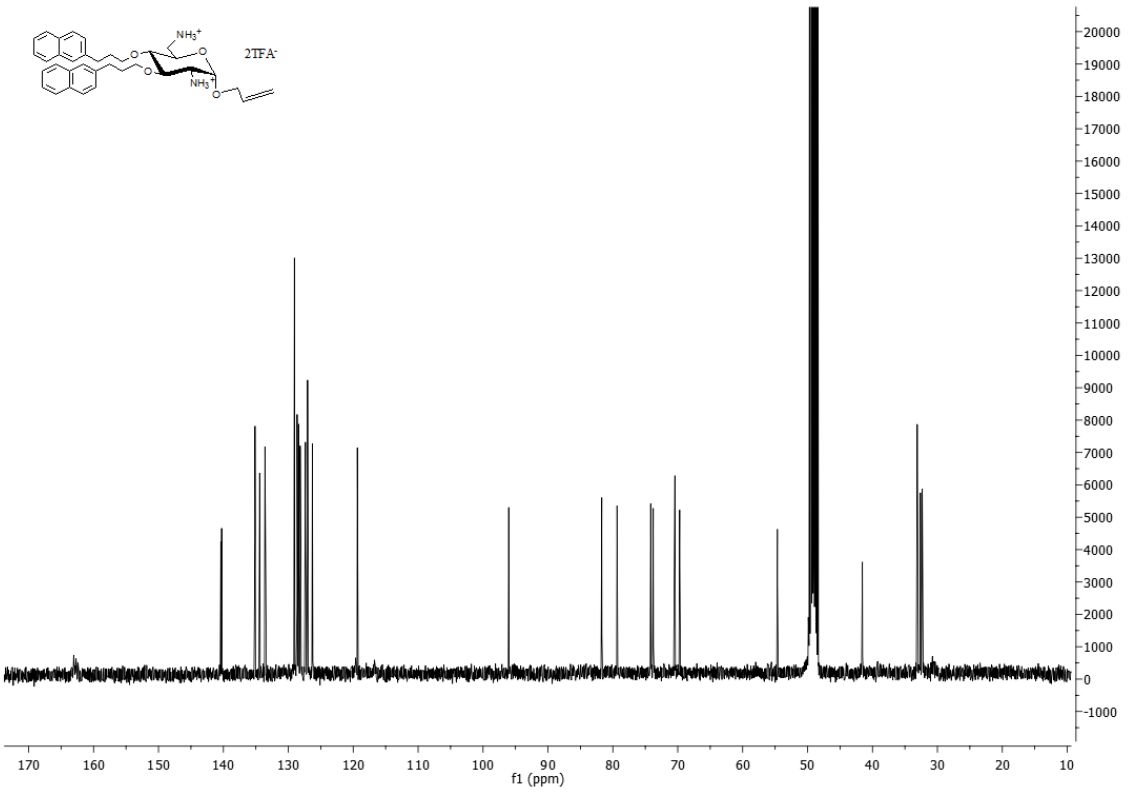


Compound **28**

$^{1}$H NMR $CD_3OD$

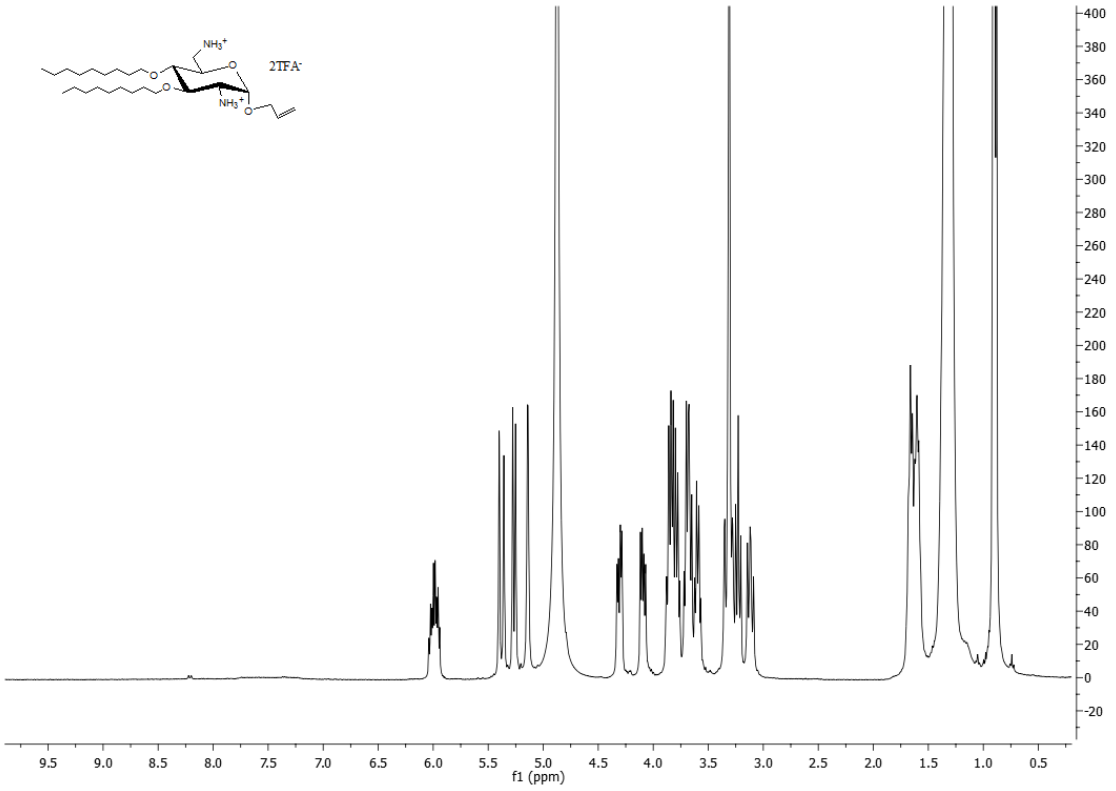



$^{13}$C NMR $CD_3OD$

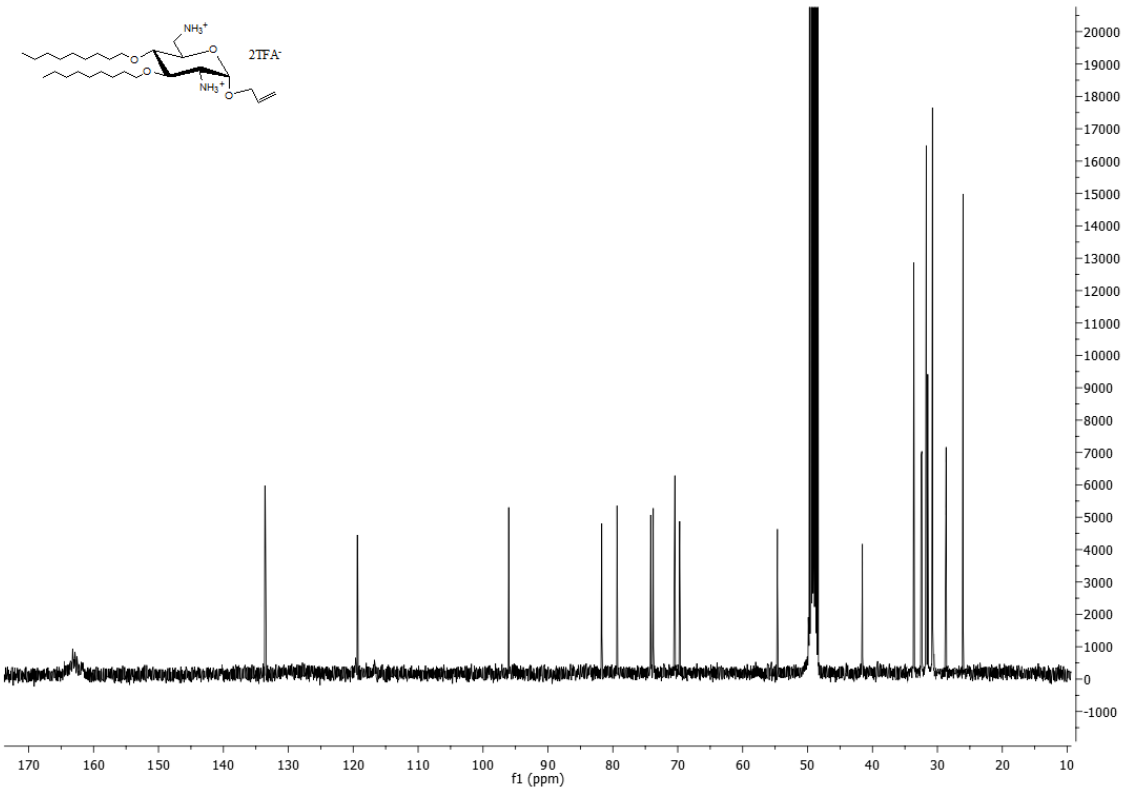


Compound **29**

$^{1}$H NMR $CD_3OD$

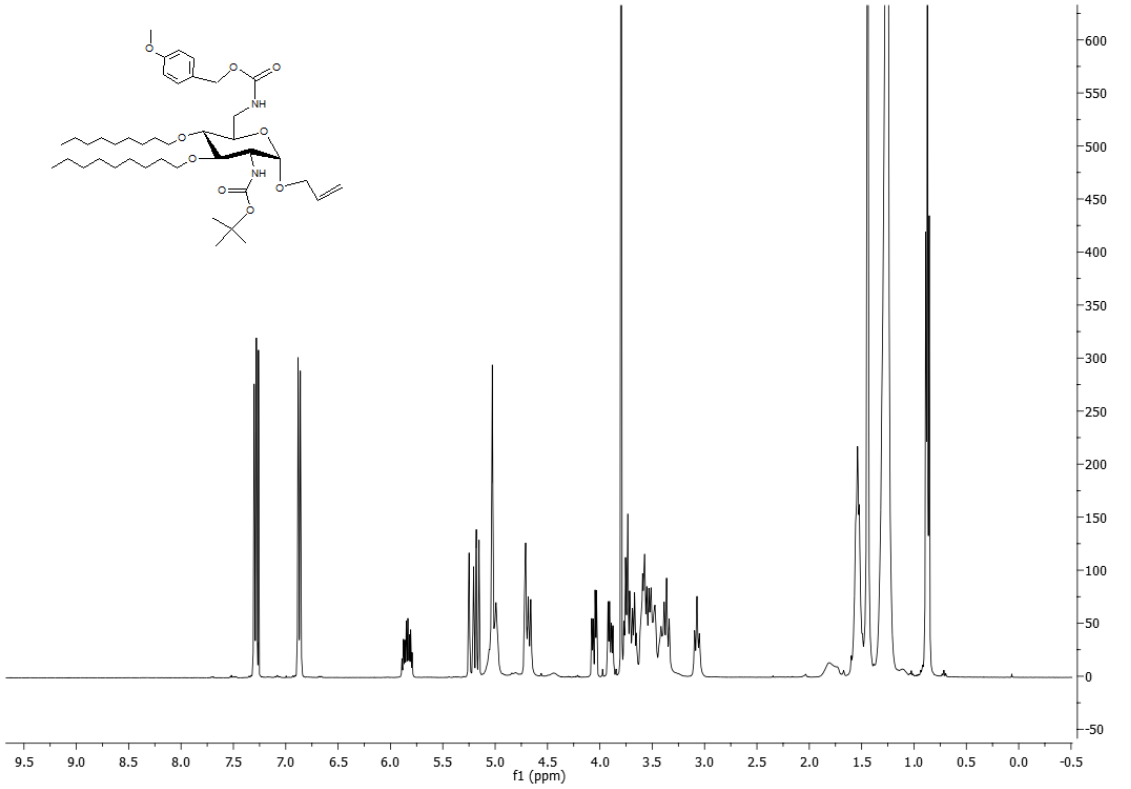



$^{13}$C NMR $CD_3OD$

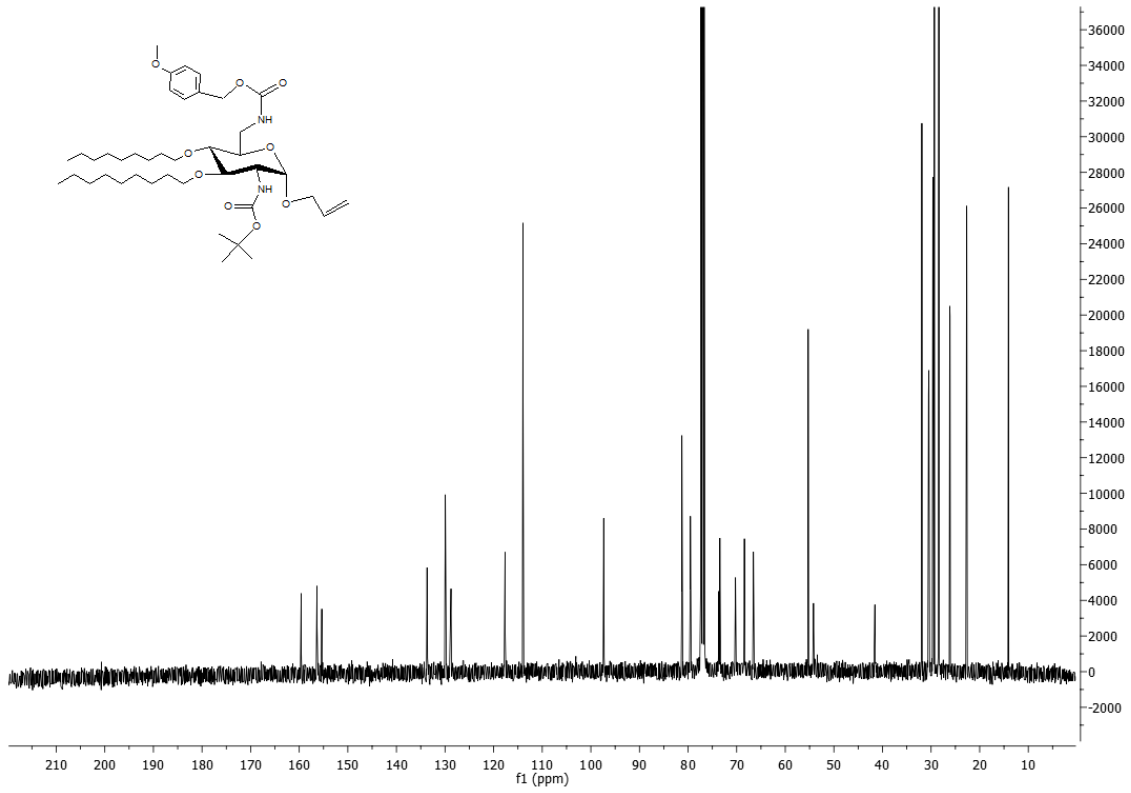


Compound **30**

$^{1}H$ NMR $CDCl_3$

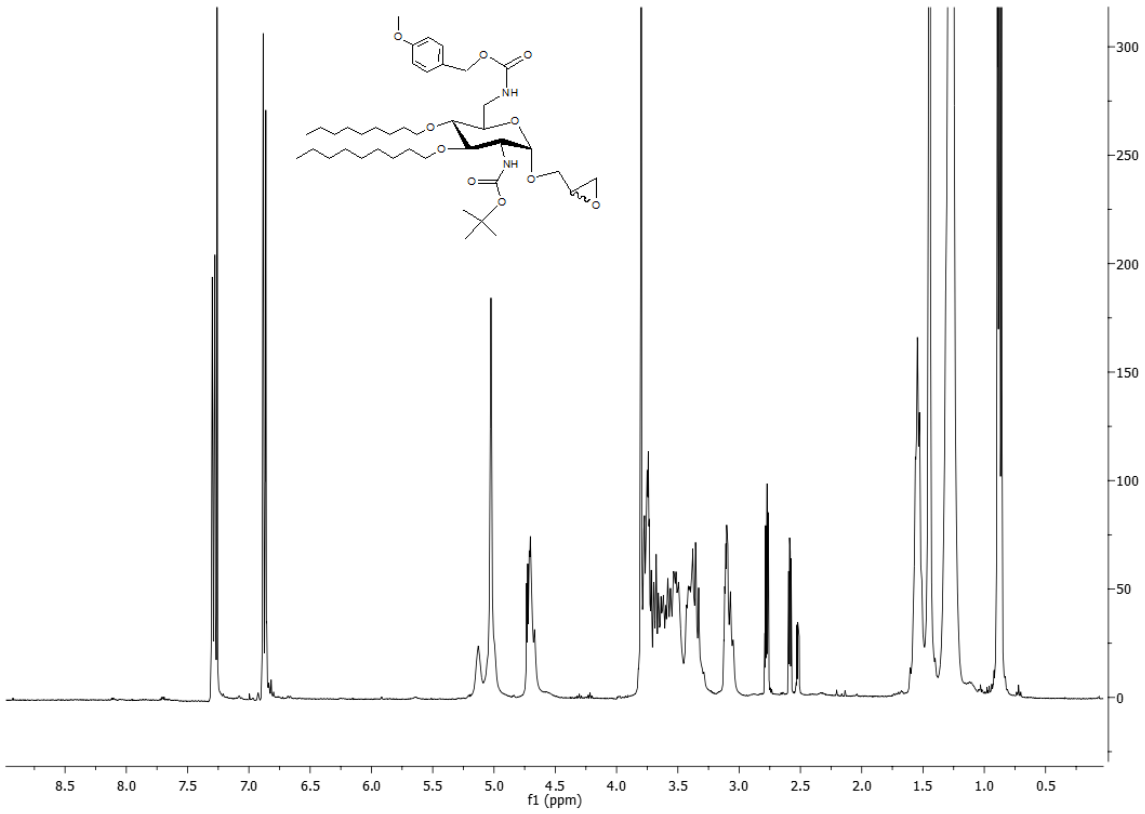



$^{13}C$ NMR $CDCl_3$

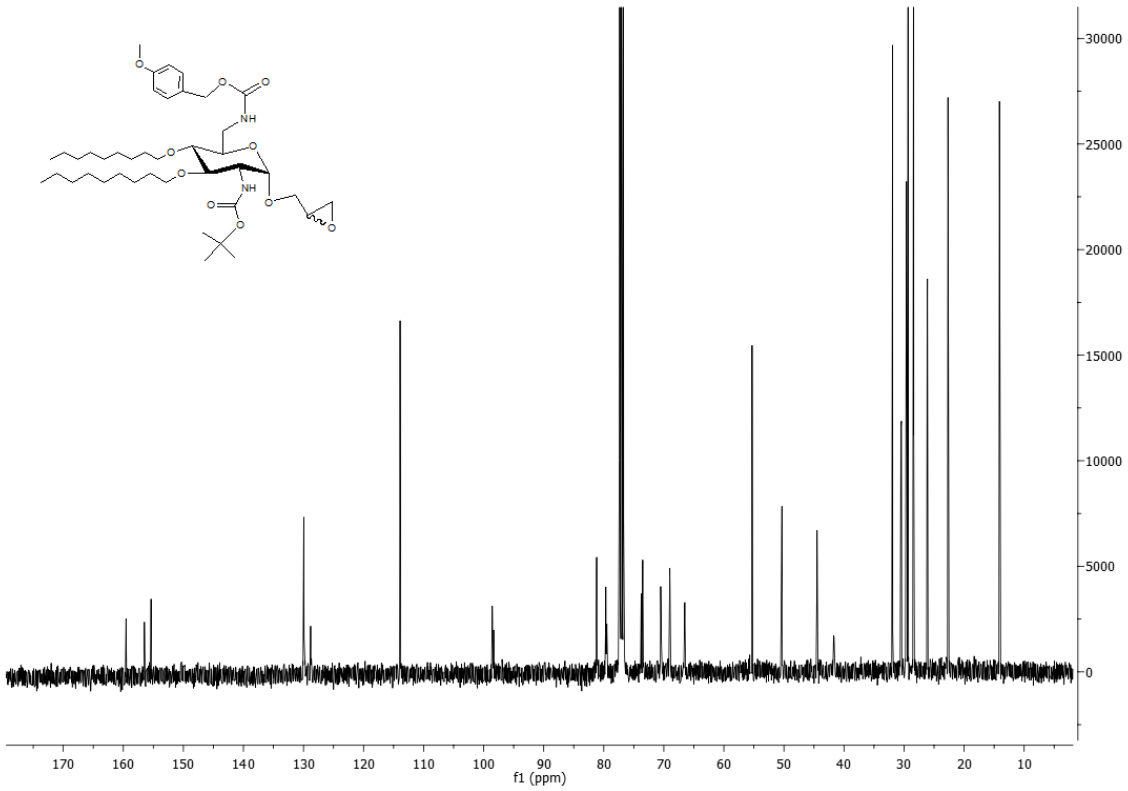


Compound **31a,b**

$^{1}H$ NMR $CDCl_3$

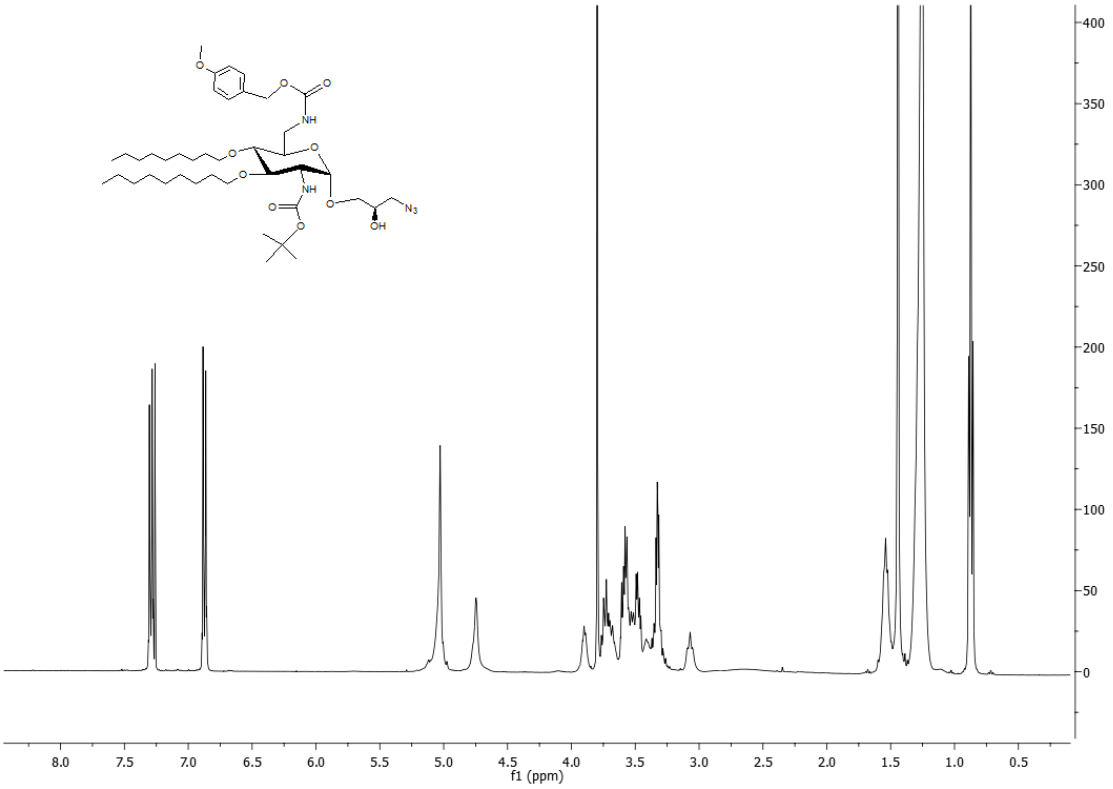



$^{13}C$ NMR $CDCl_3$

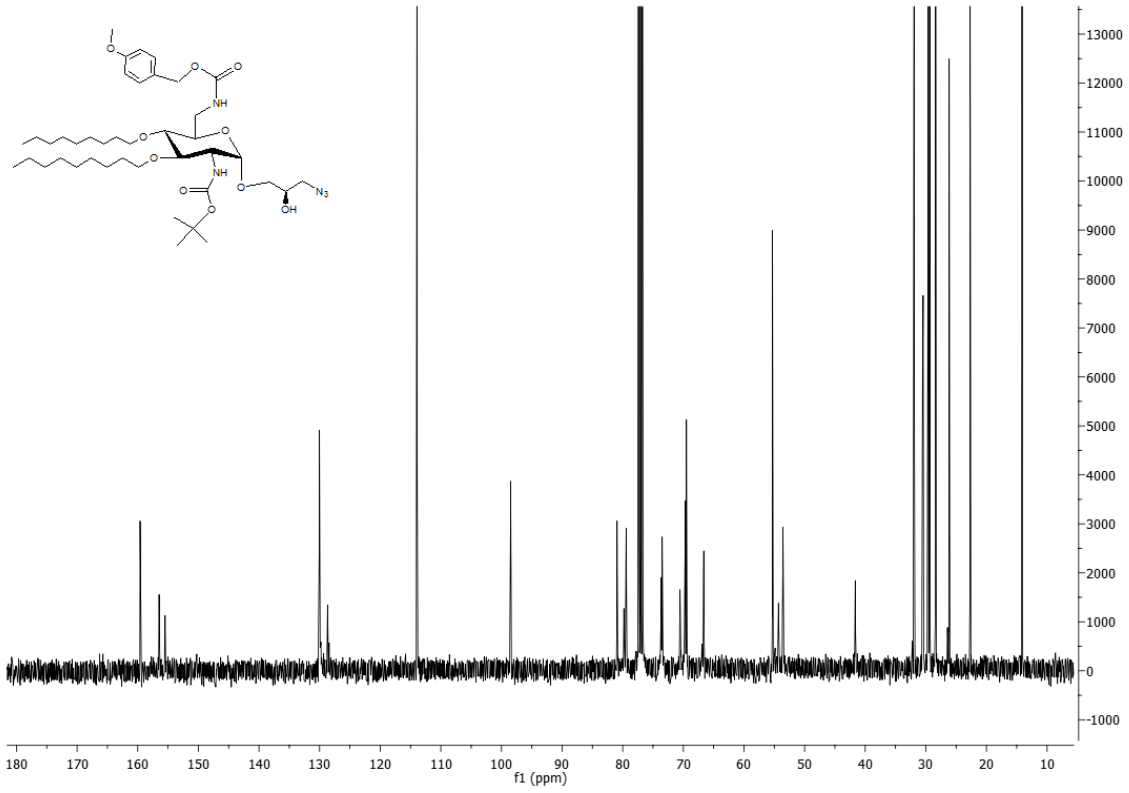


Compound **32**

$^{1}H$ NMR $CDCl_3$

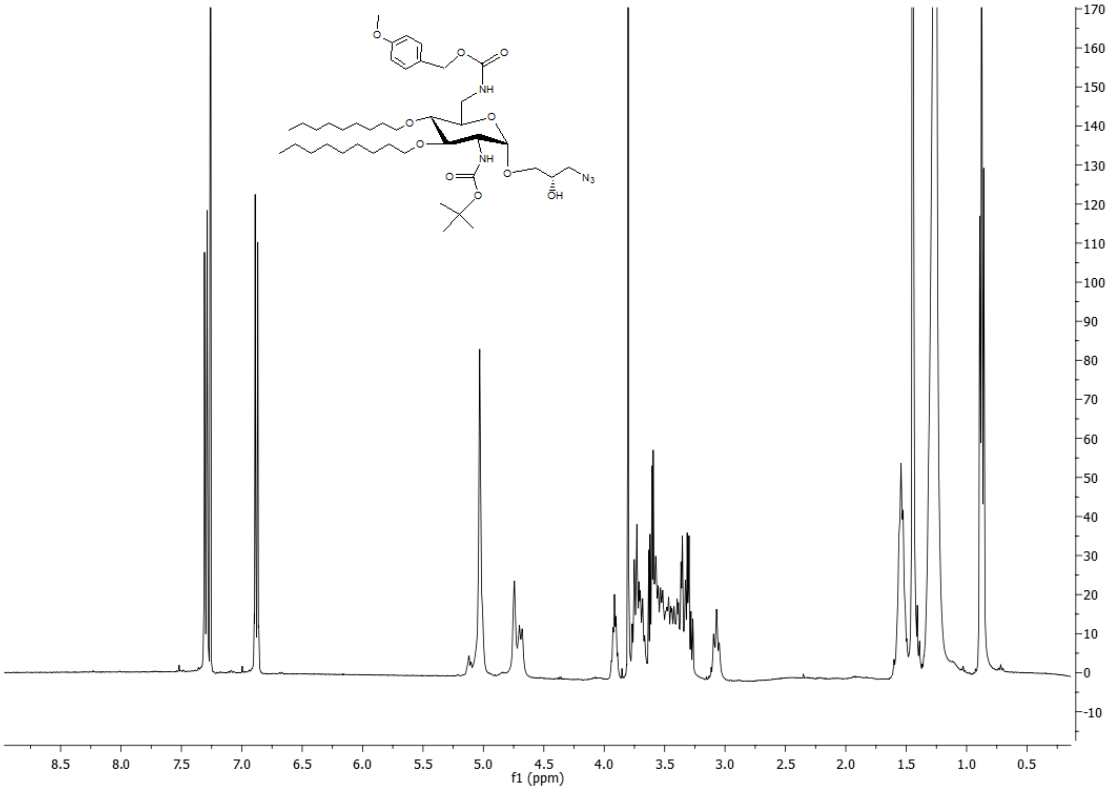



$^{13}C$ NMR $CDCl_3$

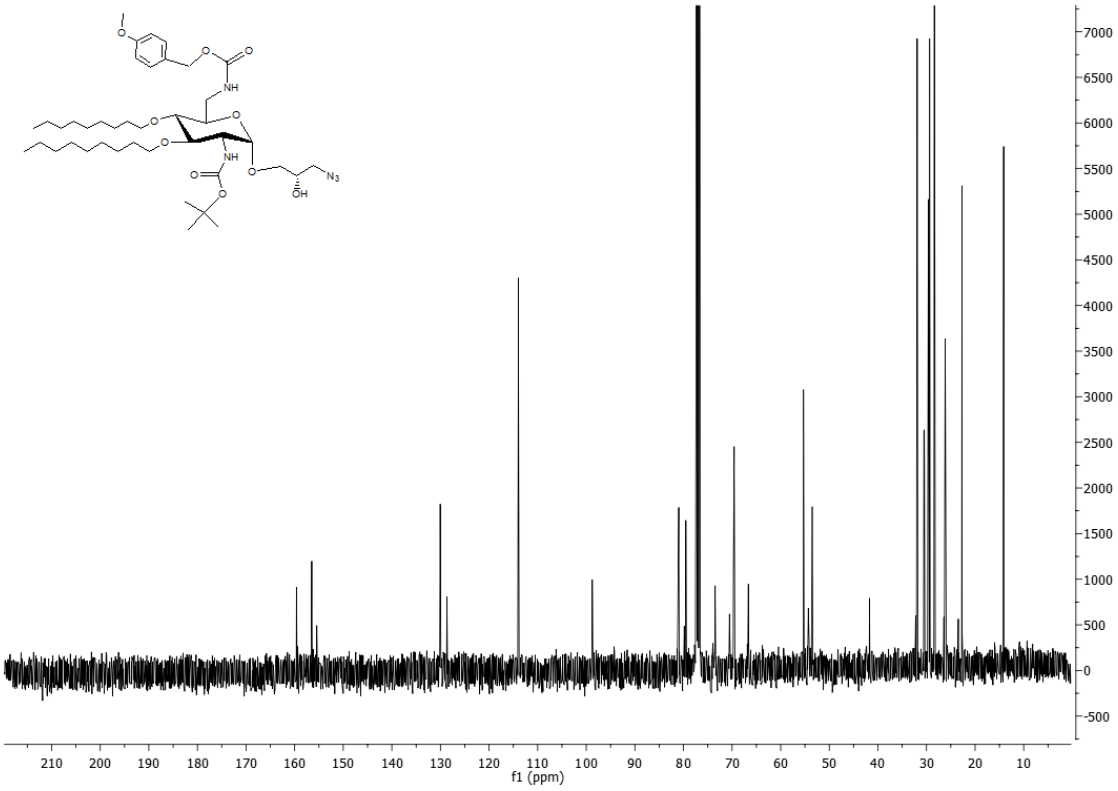


Compound **33**

$^{1}H$ NMR $CDCl_3$

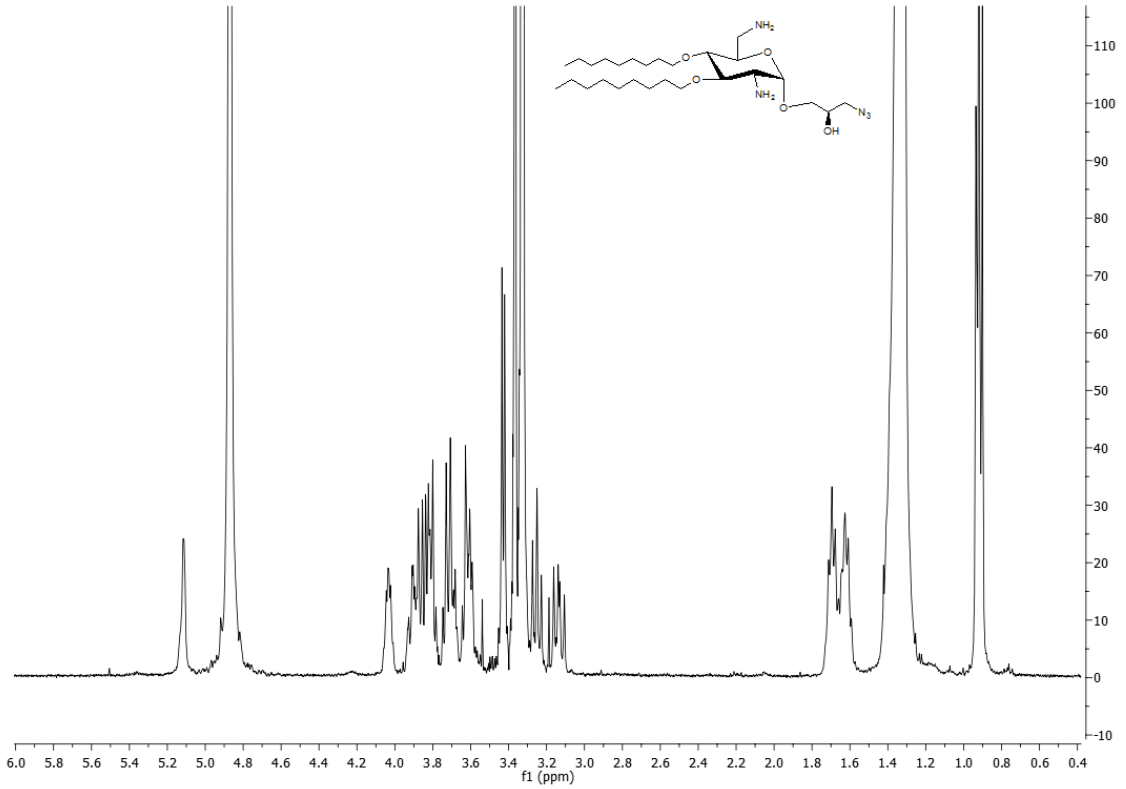



$^{13}C$ NMR $CDCl_3$

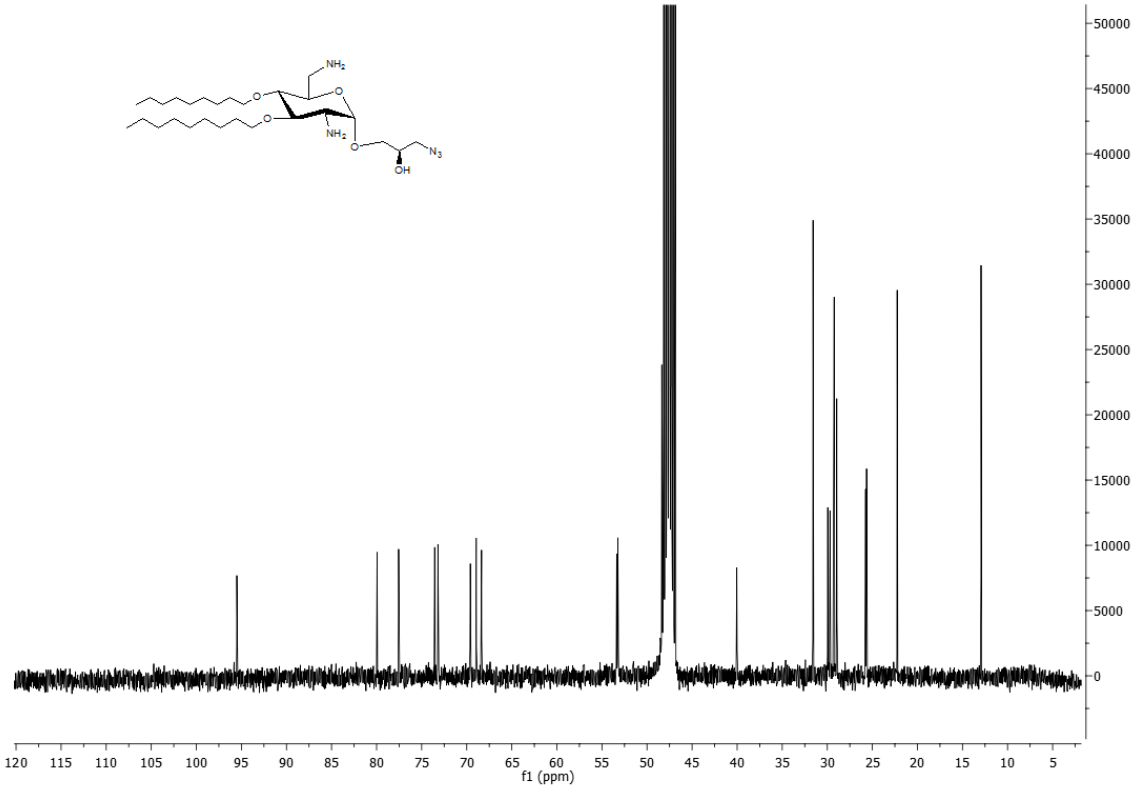


Compound **34**

$^{1}$H NMR $CD_3OD$

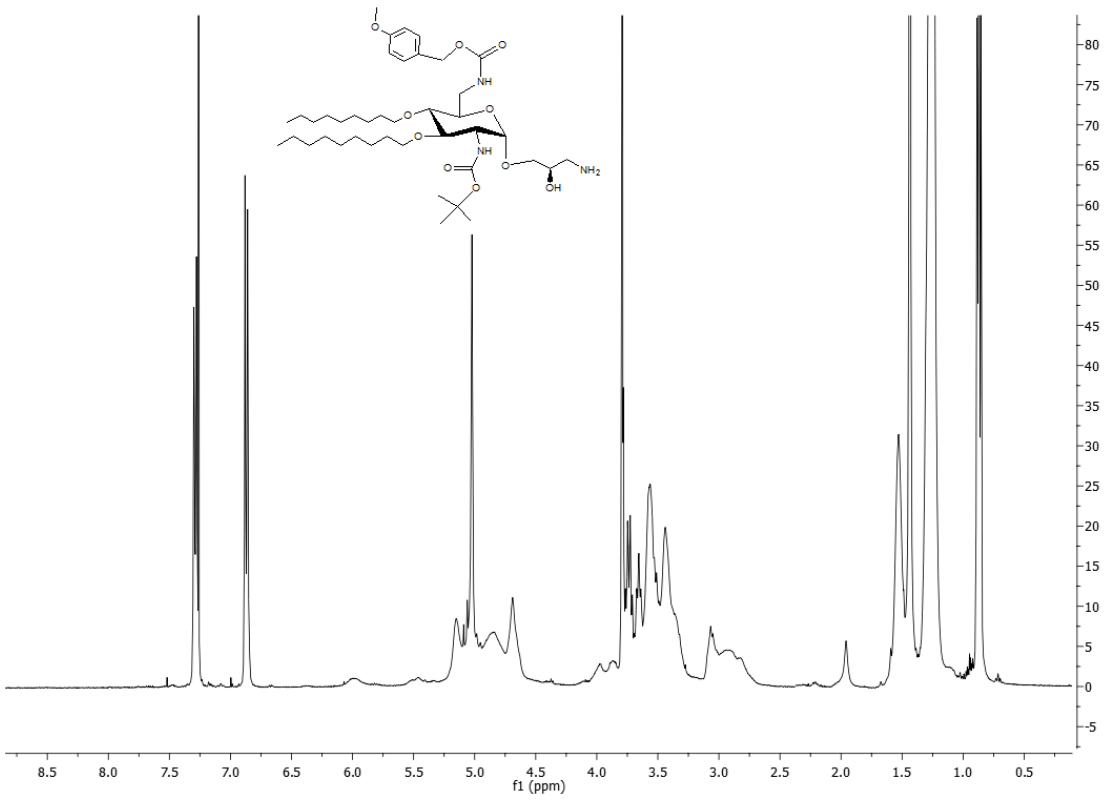



$^{13}$C NMR $CD_3OD$

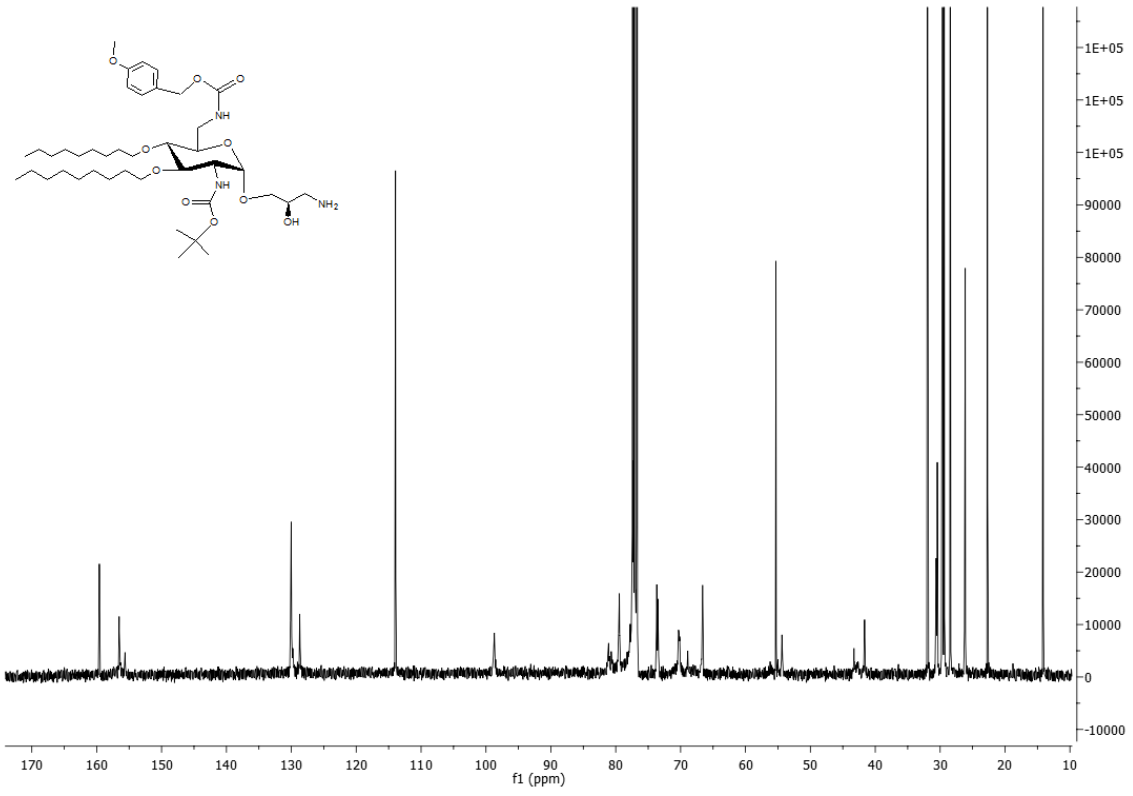


Compound **35**

$^{1}$H NMR $CDCl_3$

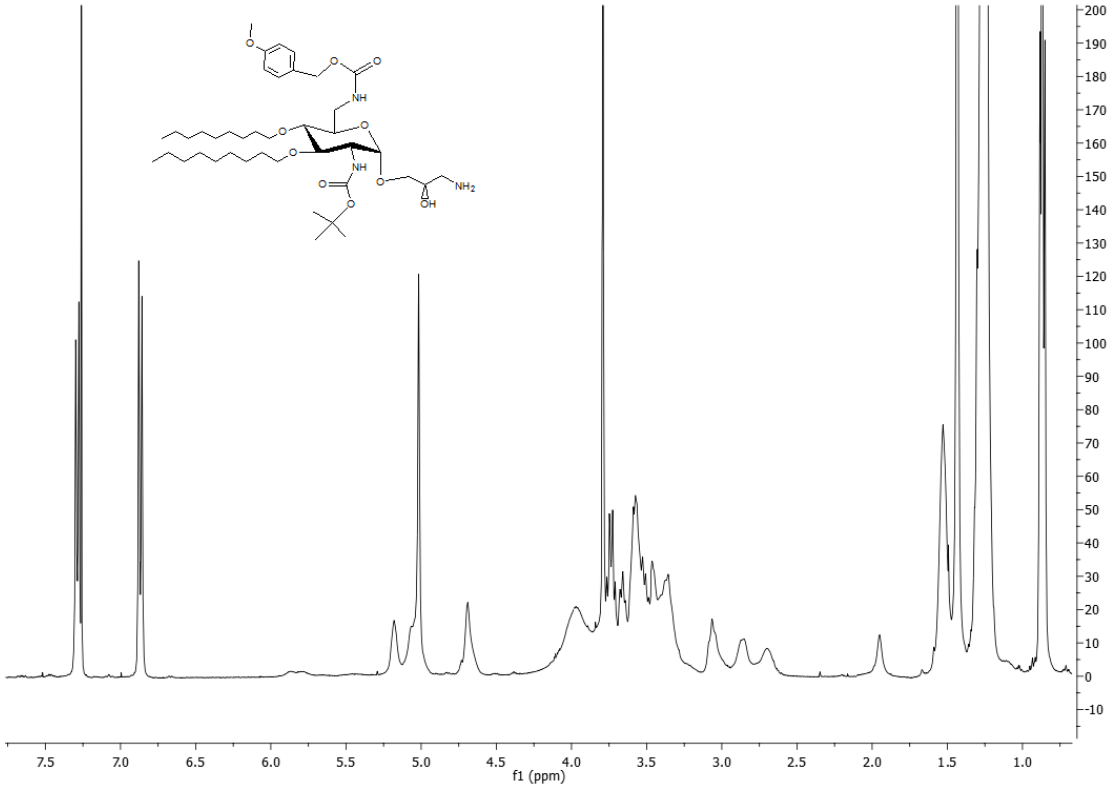



$^{13}$C NMR $CDCl_3$

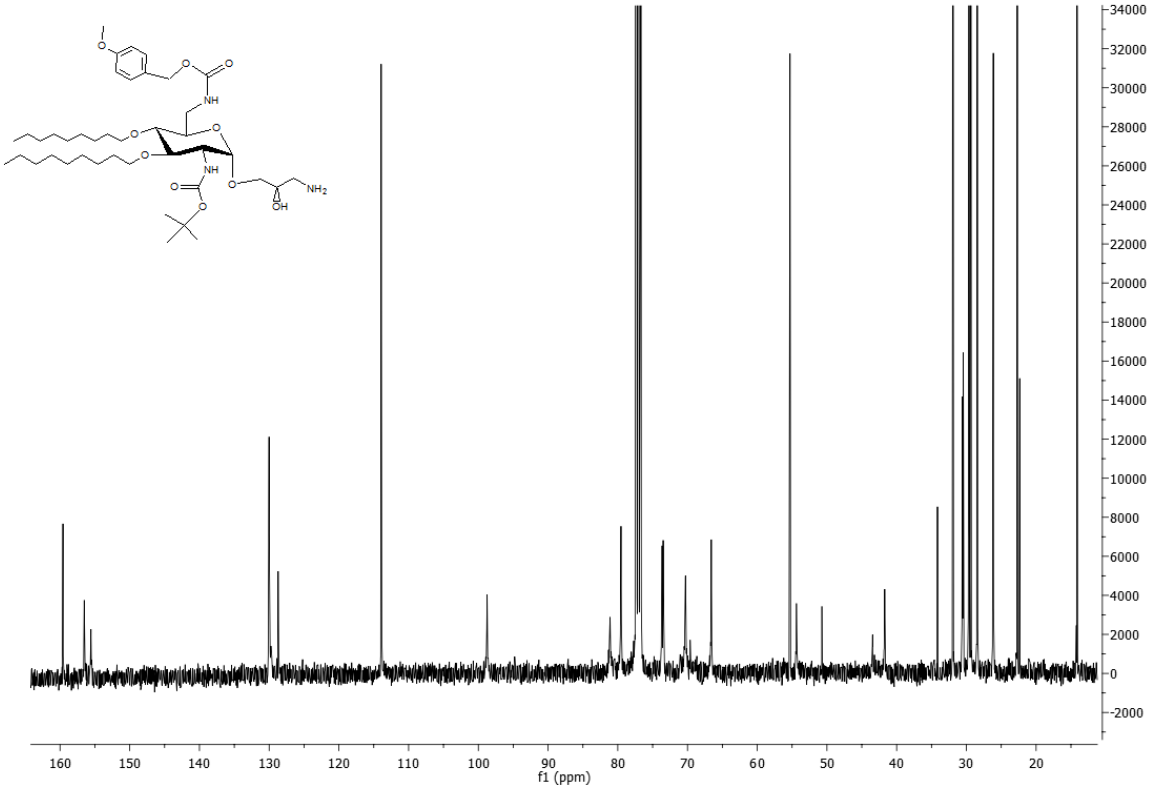


Compound **36**

$^{1}H$ NMR $CDCl_3$

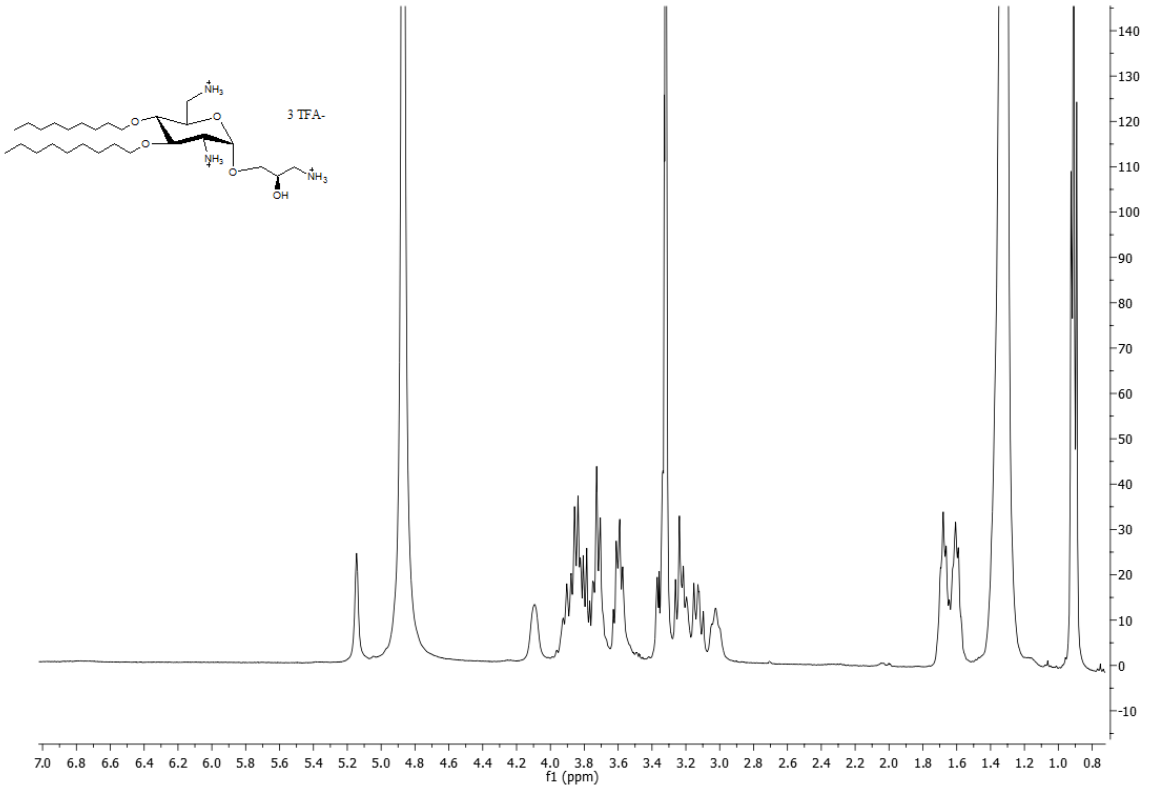



$^{13}C$ NMR $CDCl_3$

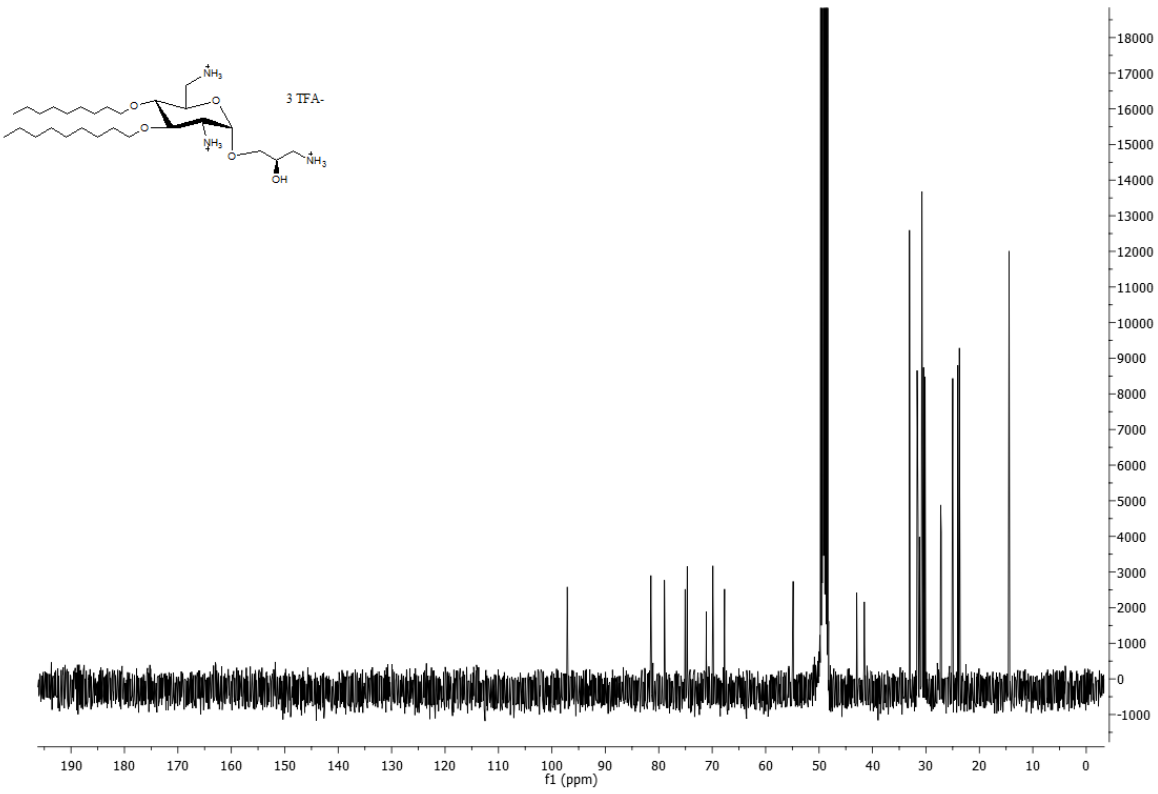


Compound **37**

$^{1}$H NMR $CD_3OD$

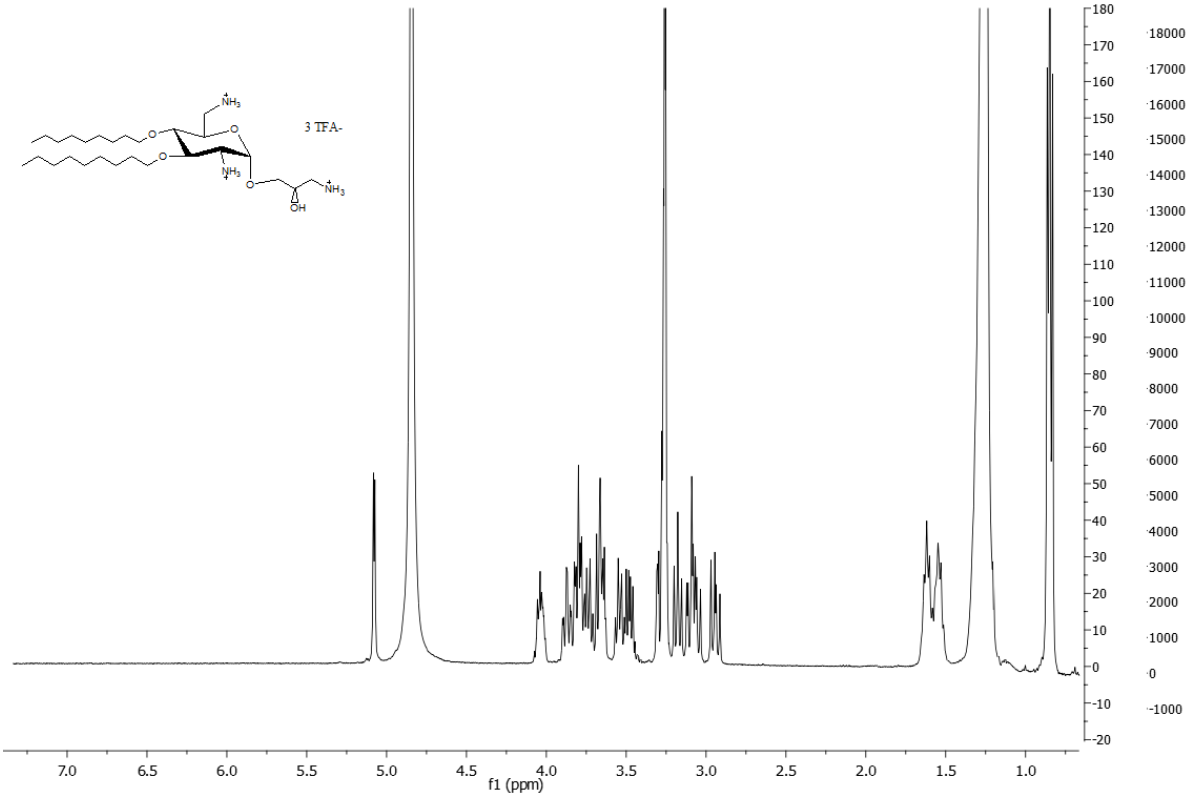



$^{13}$C NMR $CD_3OD$

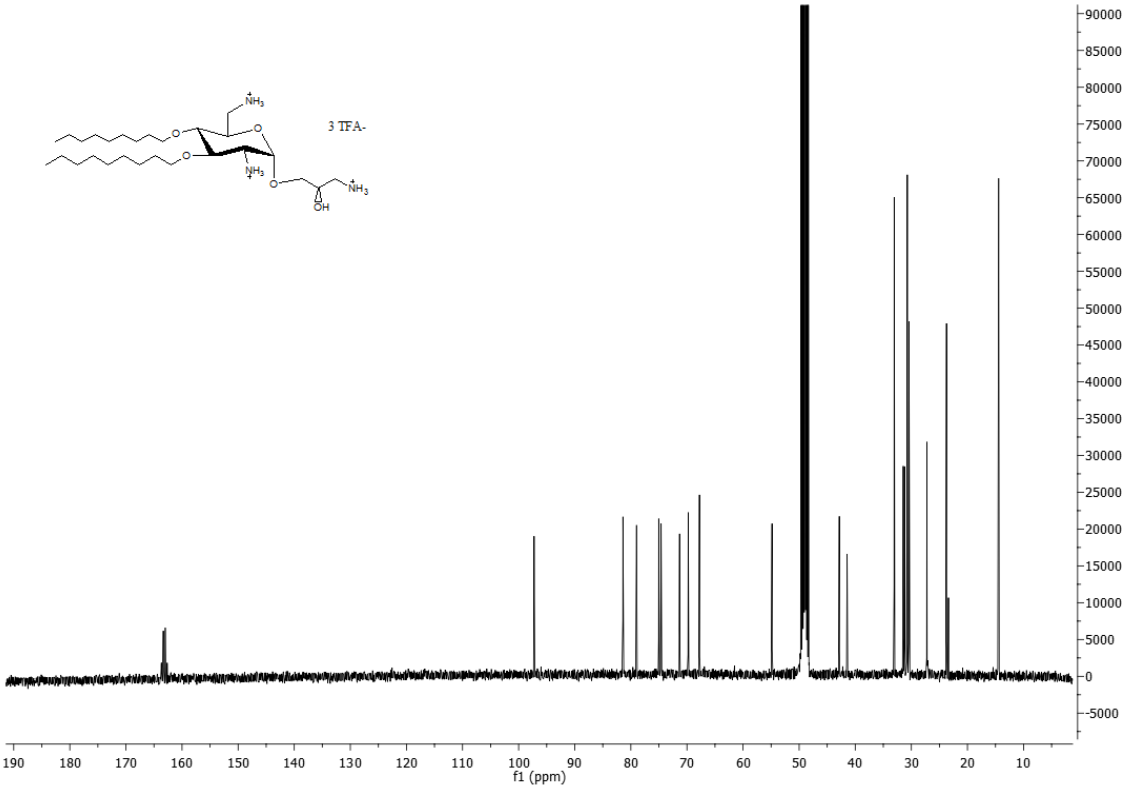


Compound **38**

$^{1}$H NMR $CD_3OD$

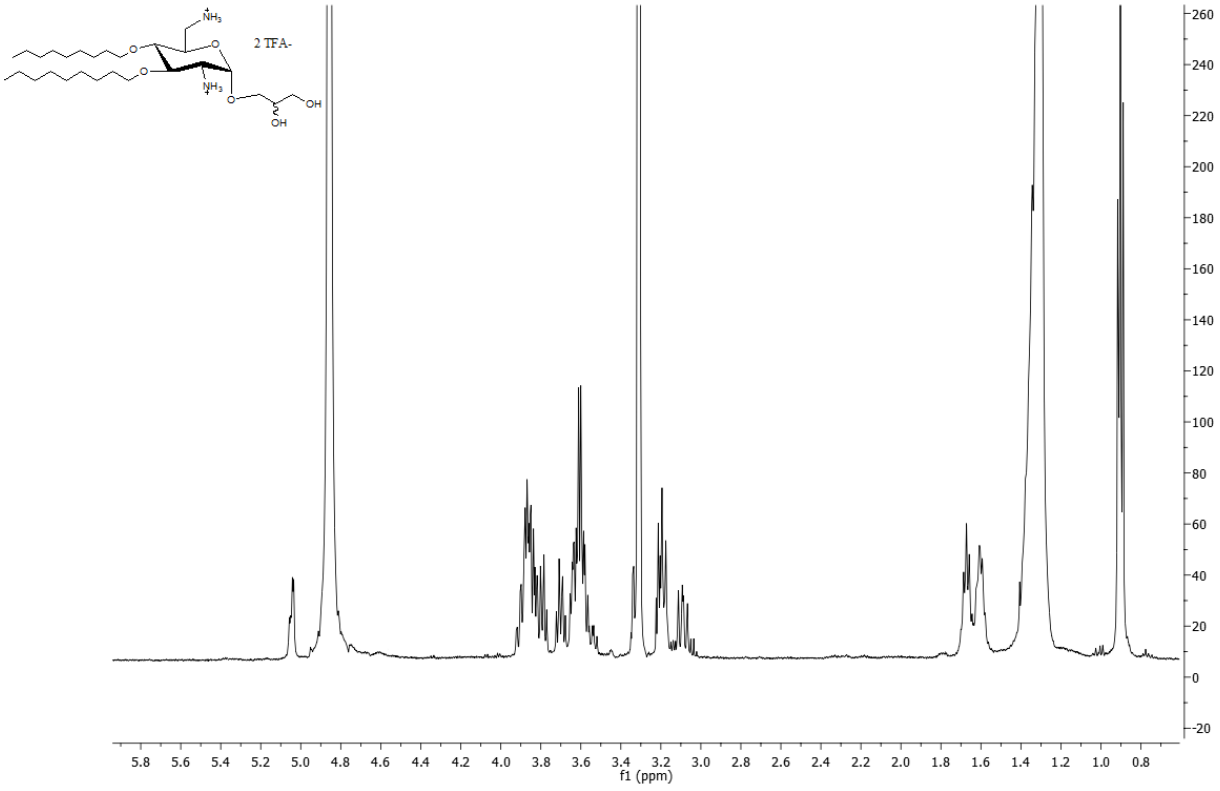



$^{13}$C NMR $CD_3OD$

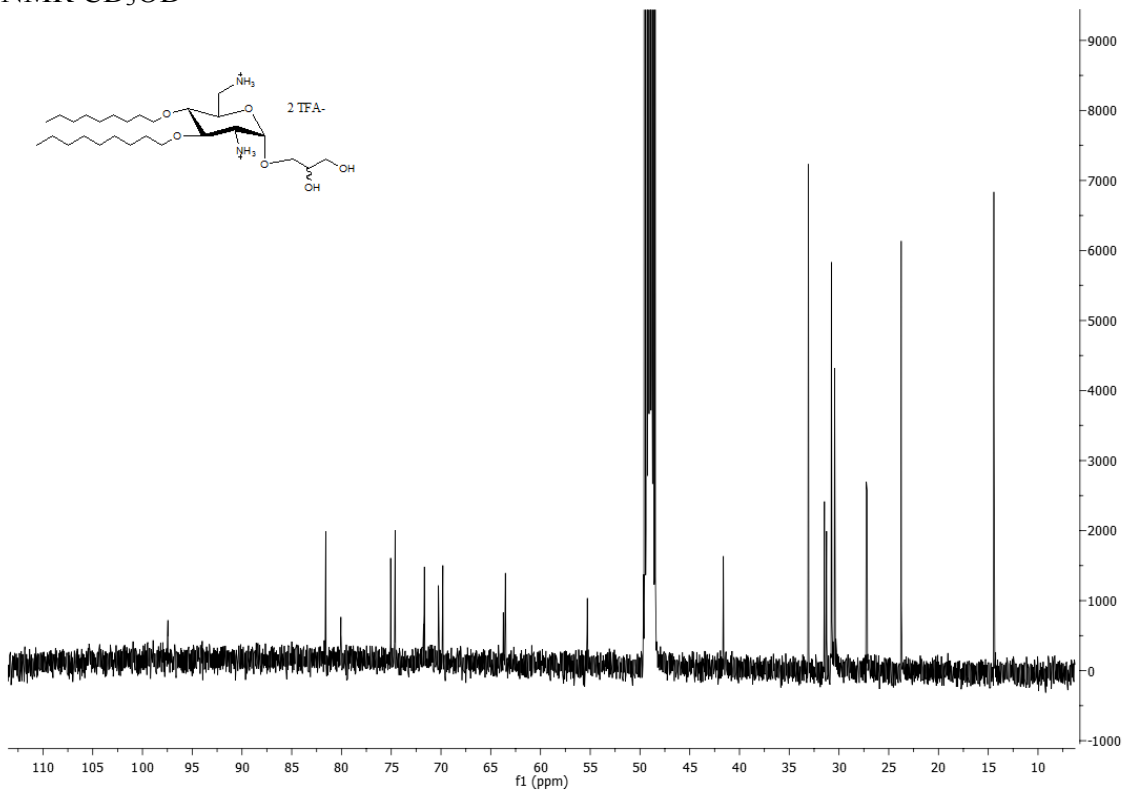


Compound **39a,b**

$^{1}$H NMR $CD_3OD$

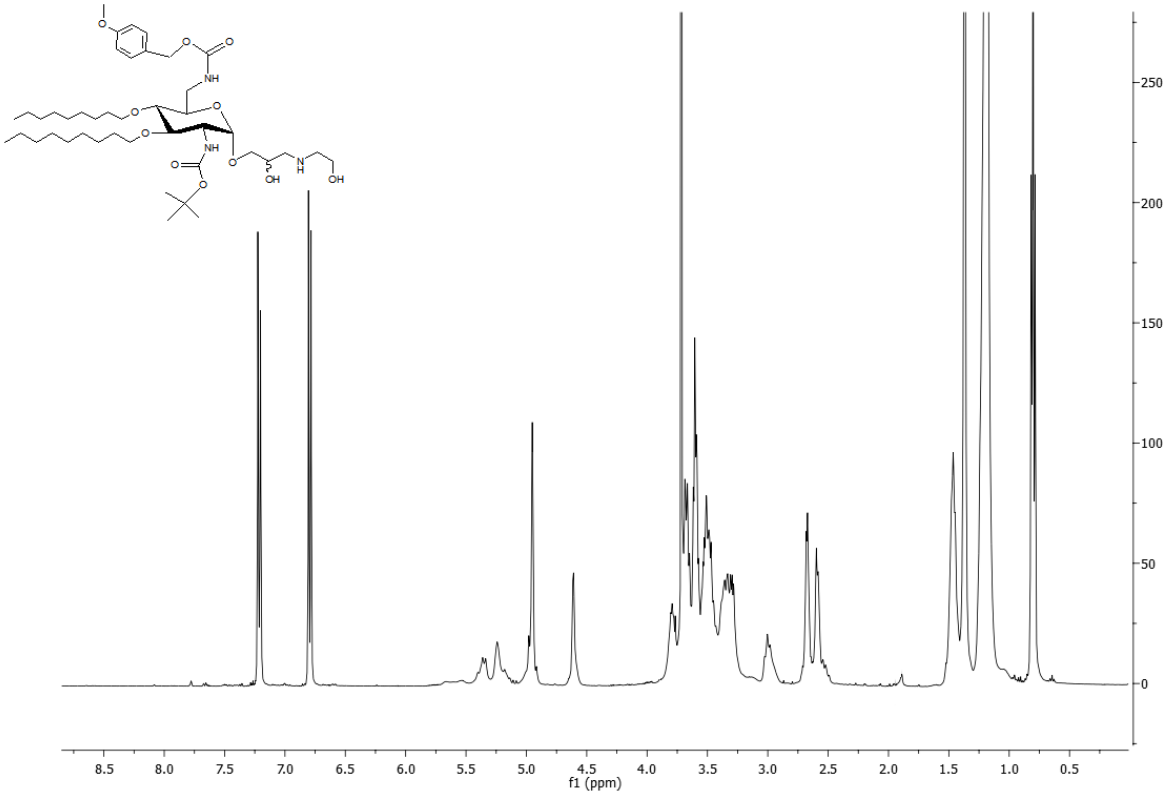



$^{13}$C NMR $CD_3OD$

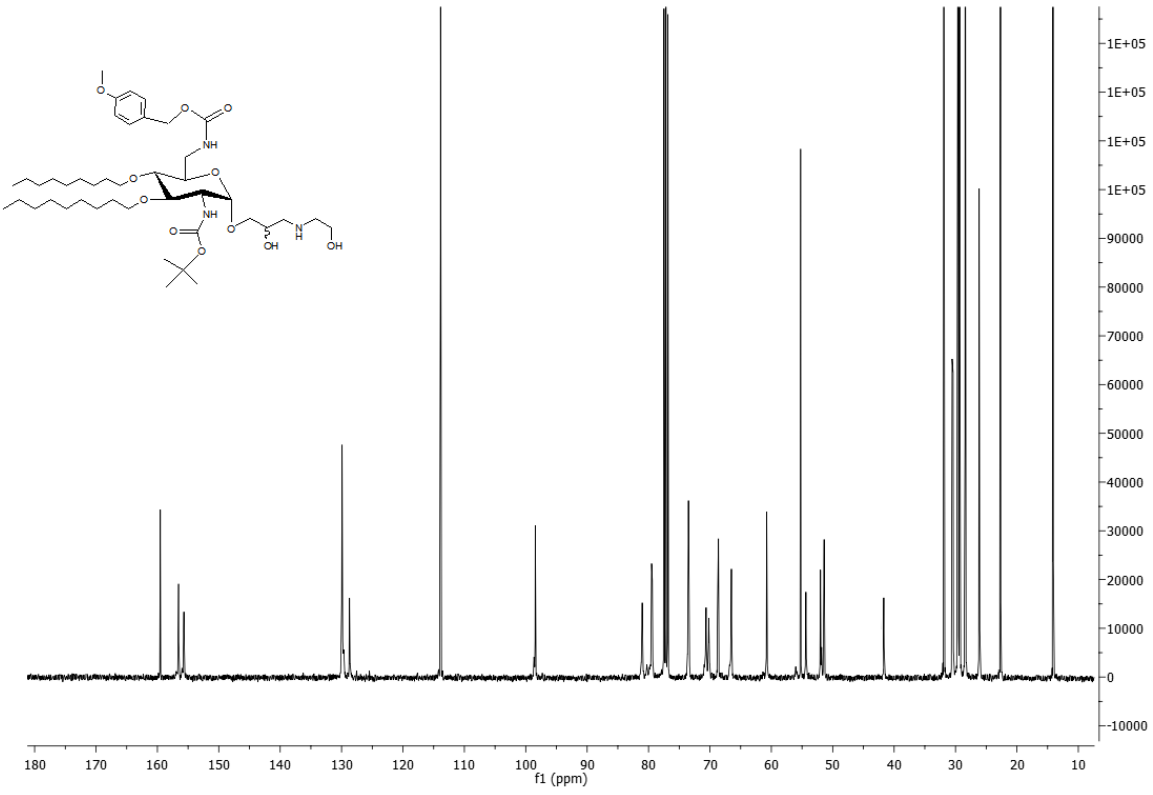


Compound **40a,b**

$^{1}$H NMR $CDCl_3$

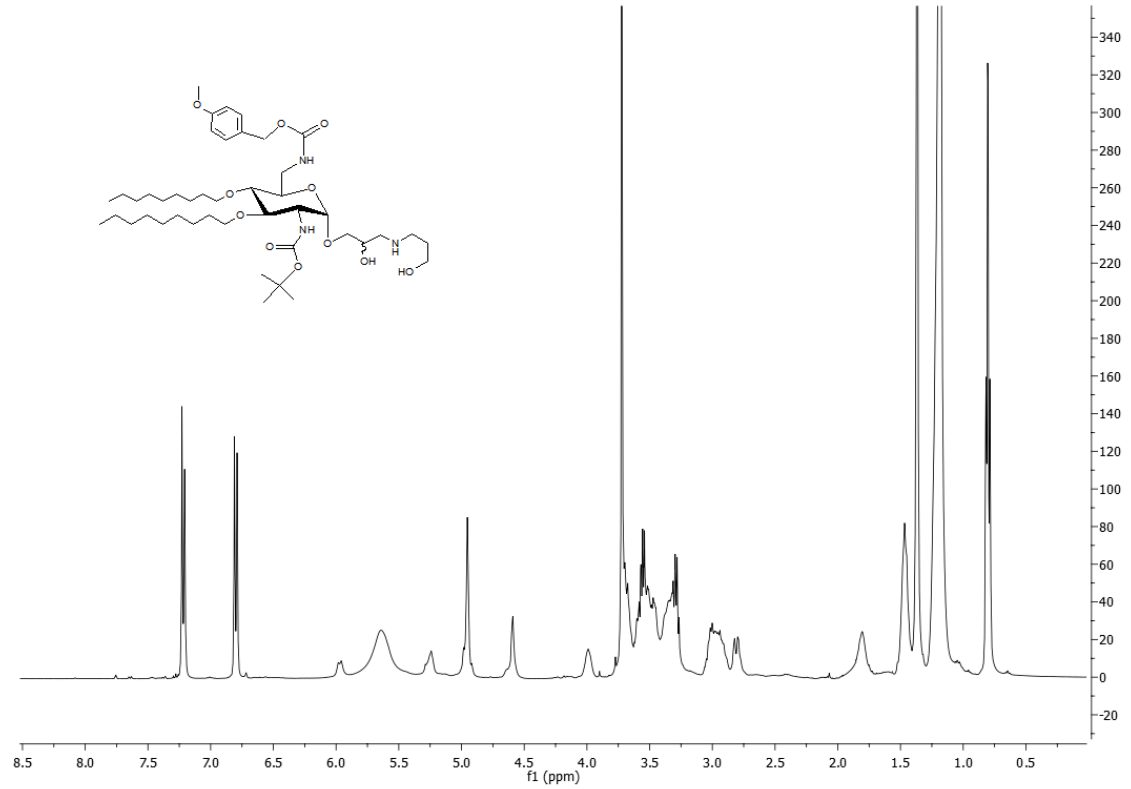



$^{13}$C NMR $CDCl_3$

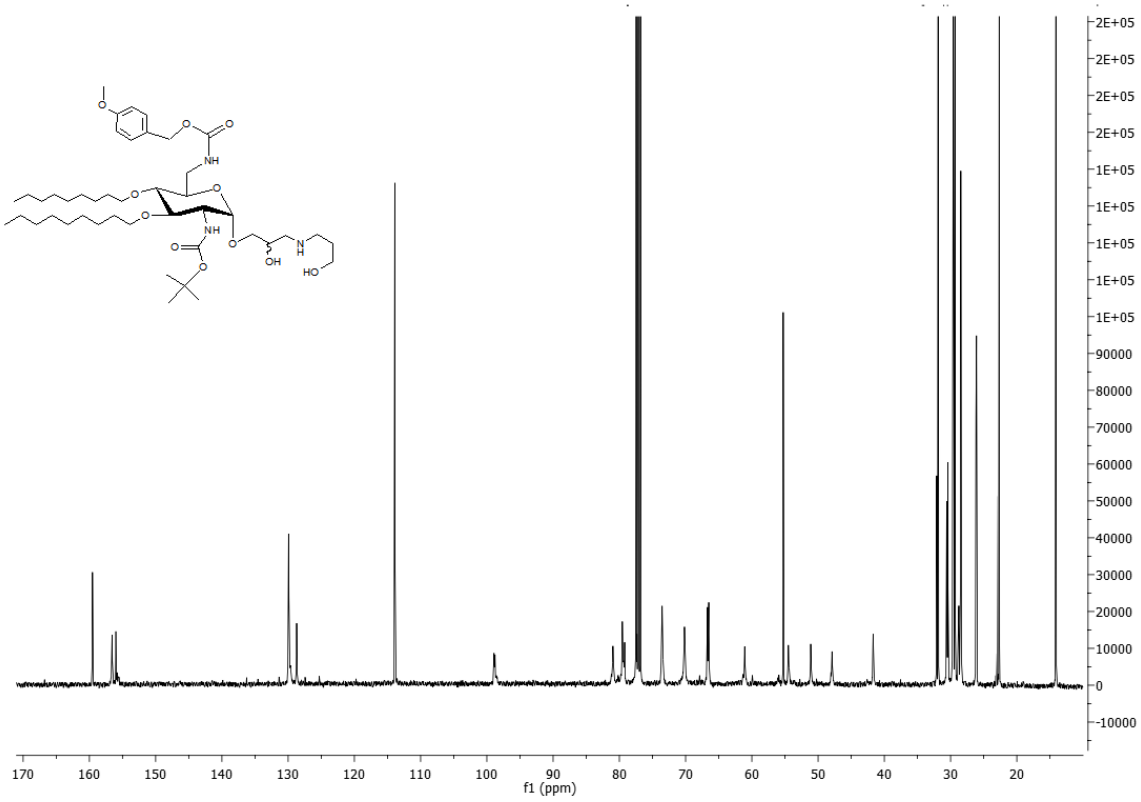


Compound **41a,b**

$^{1}H$ NMR $CDCl_3$

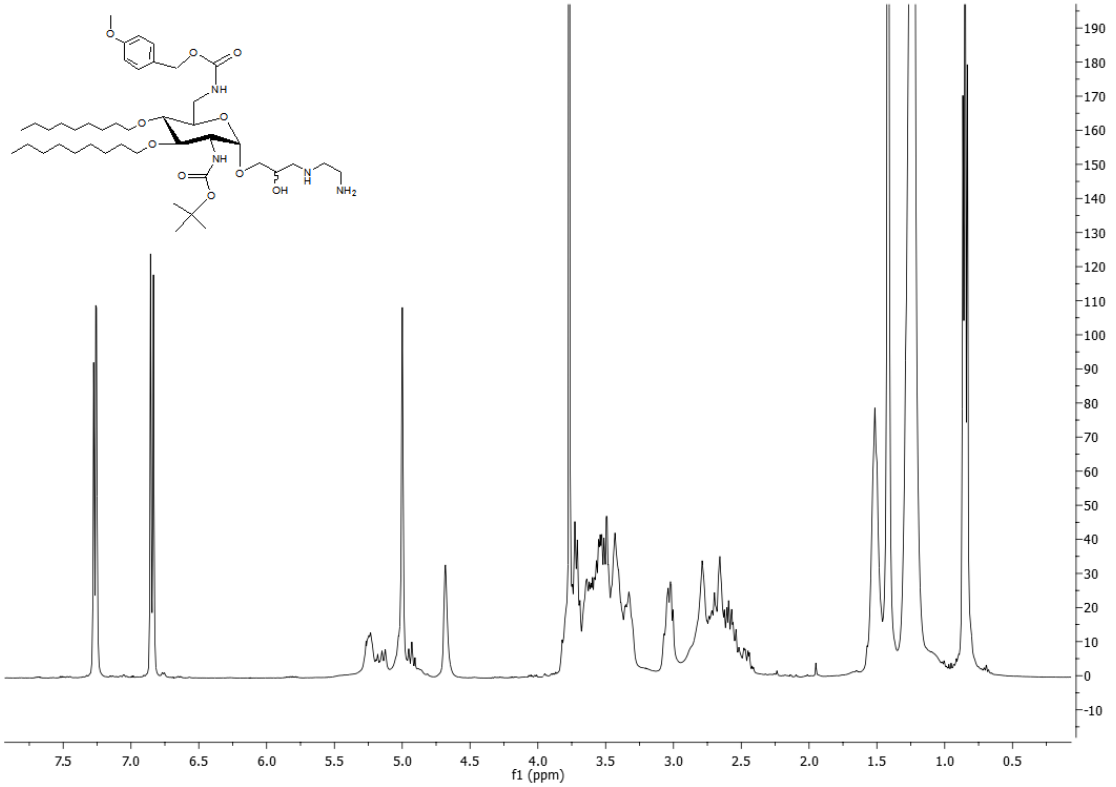



$^{13}C$ NMR $CDCl_3$

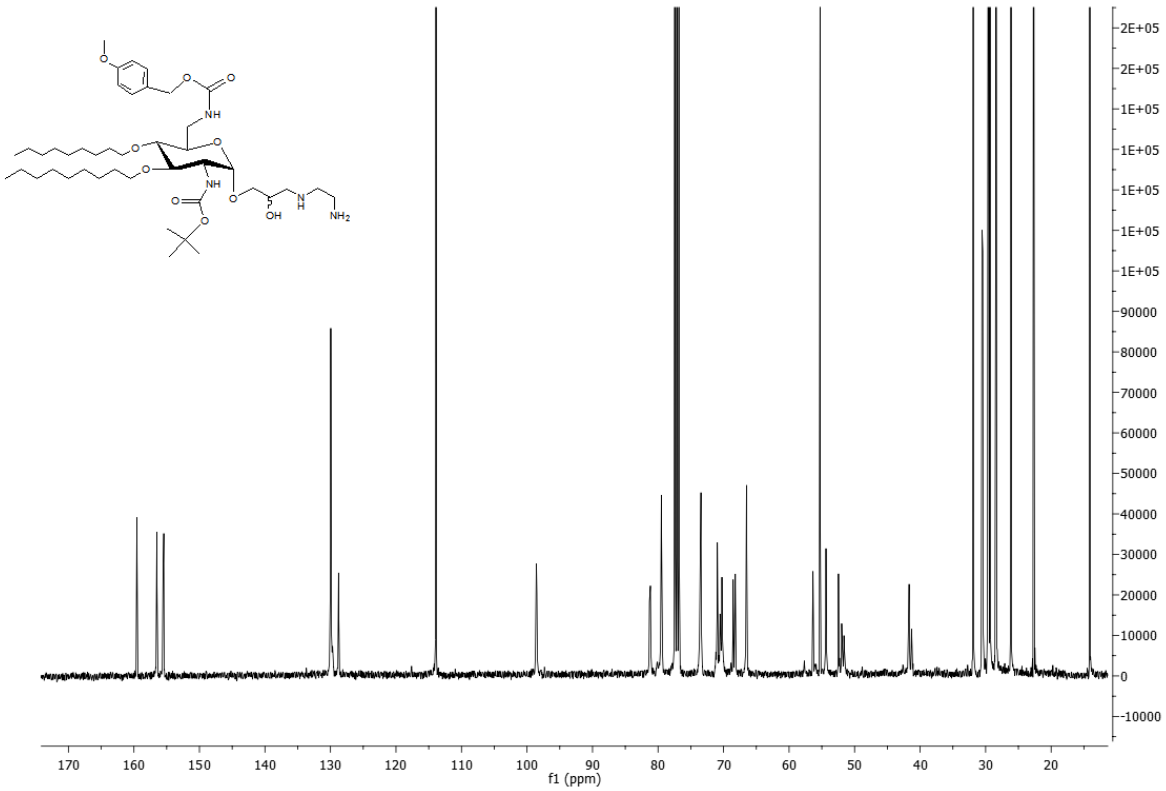


Compound **42a,b**

$^{1}H$ NMR $CDCl_3$

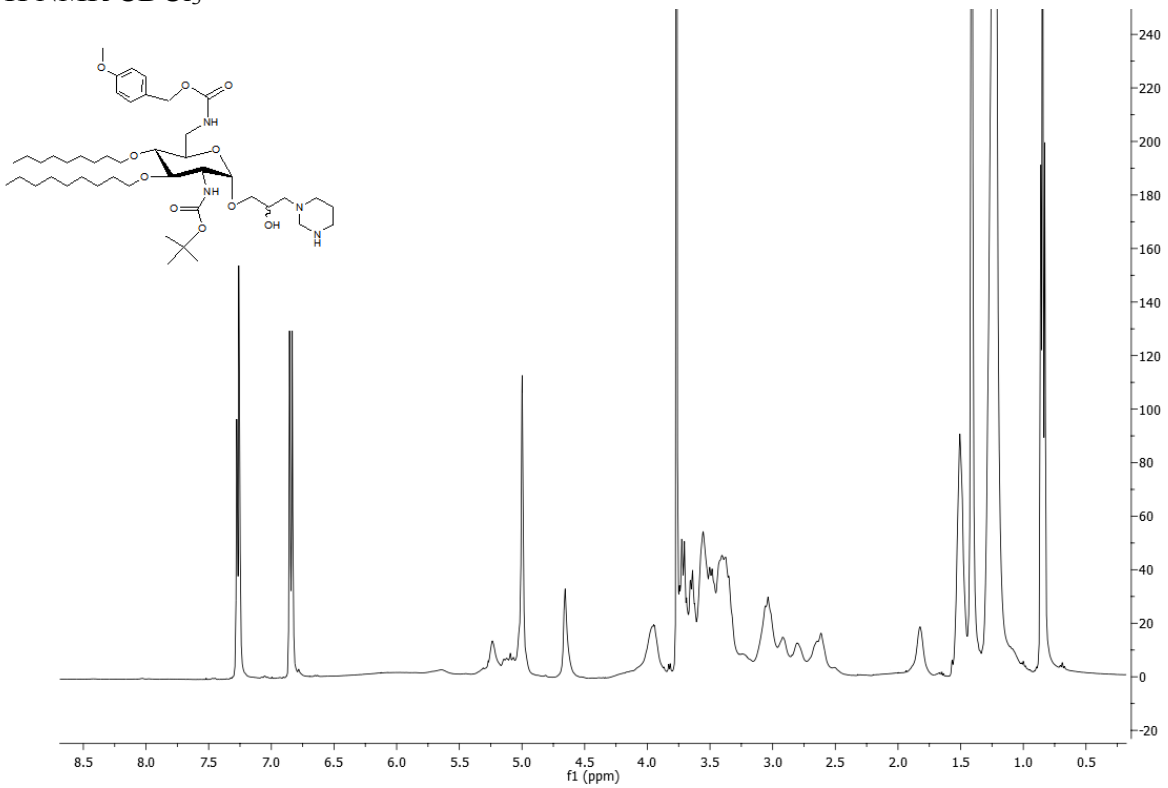



$^{13}C$ NMR $CDCl_3$

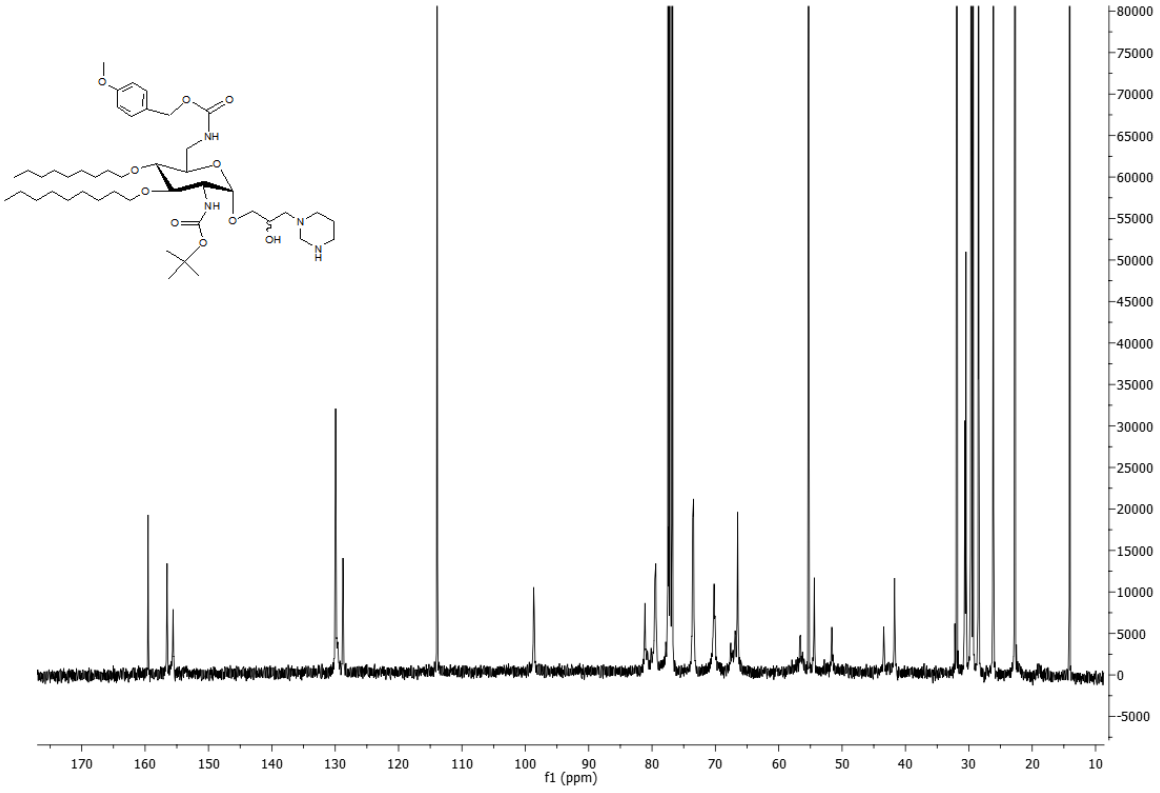


Compound **43a,b**

$^{1}$H NMR $CDCl_3$

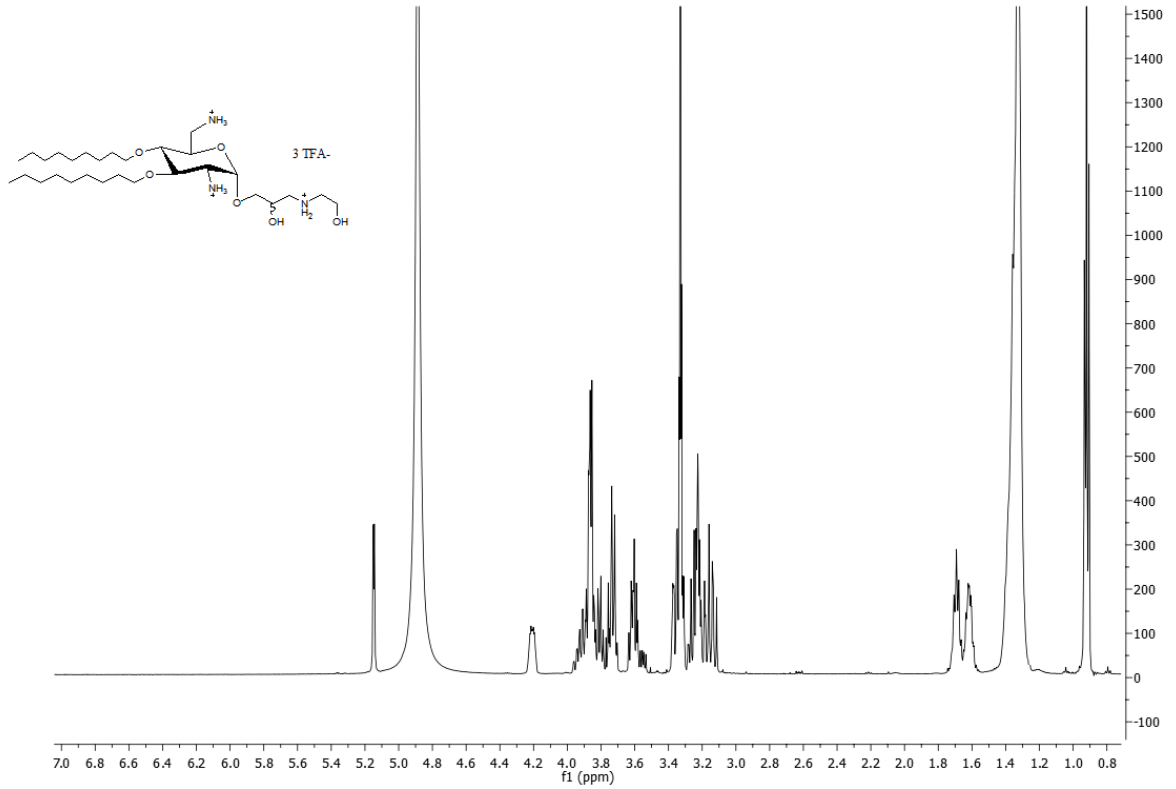



$^{13}$C NMR $CDCl_3$

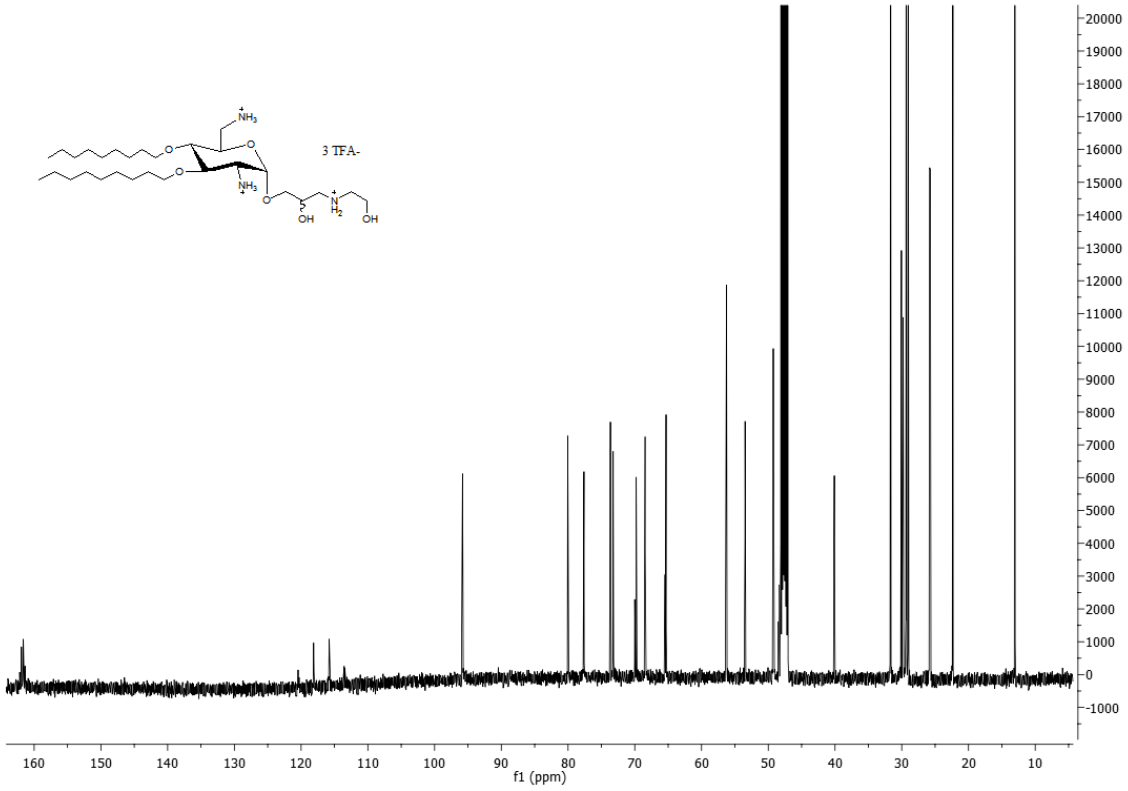


Compound **44a,b**

$^{1}$H NMR $CD_3OD$

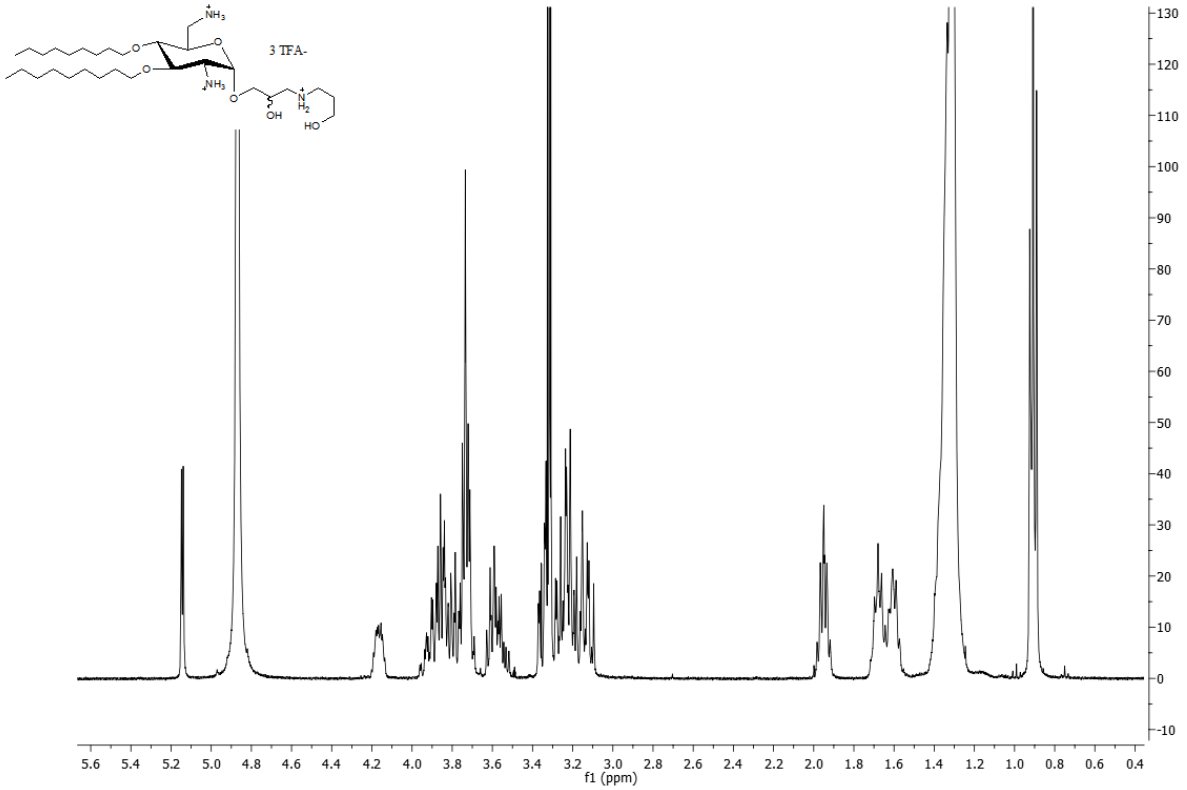



$^{13}$C NMR $CD_3OD$

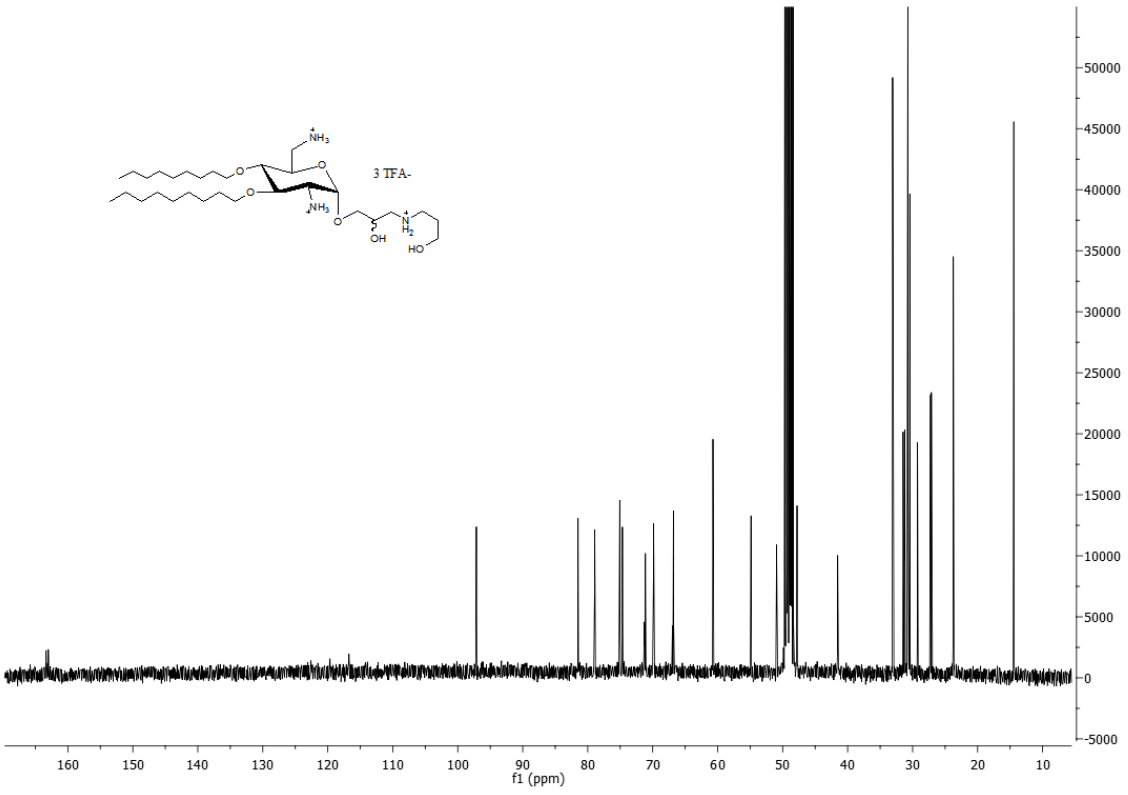


Compound **45a,b**

$^{1}$H NMR $CD_3OD$

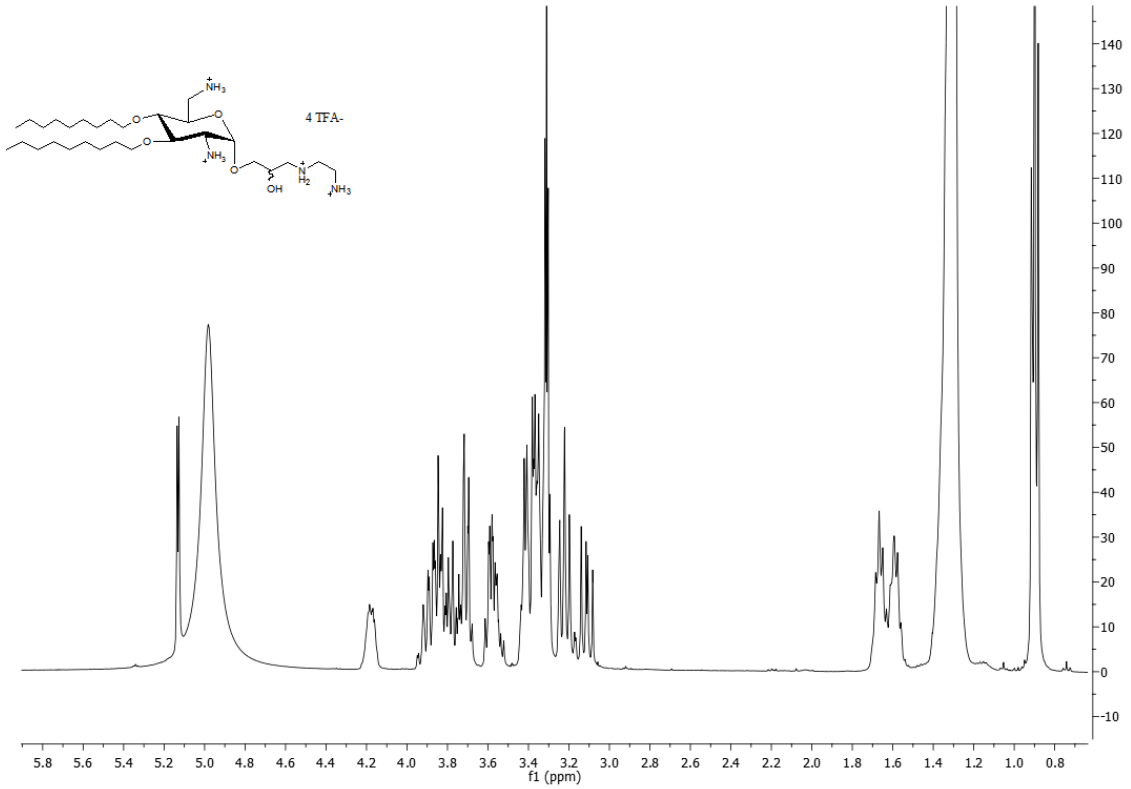



$^{13}$C NMR $CD_3OD$

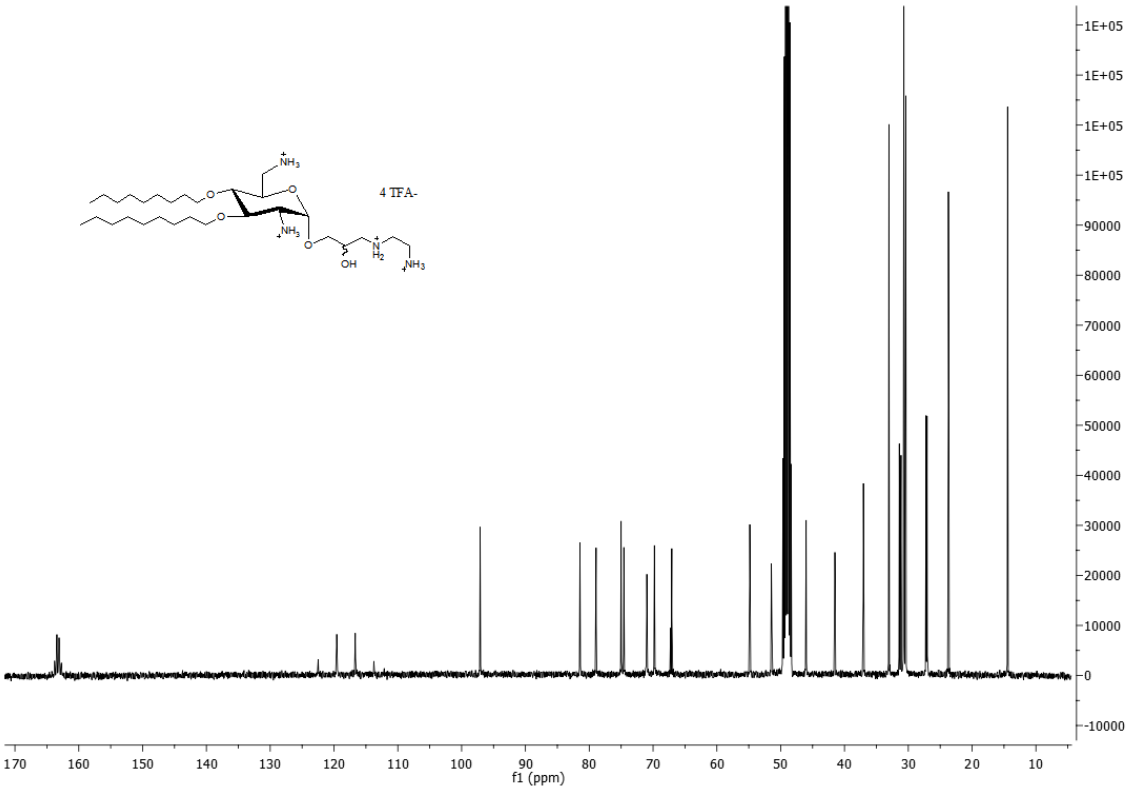


Compound **46a,b**

$^{1}$H NMR $CD_3OD$

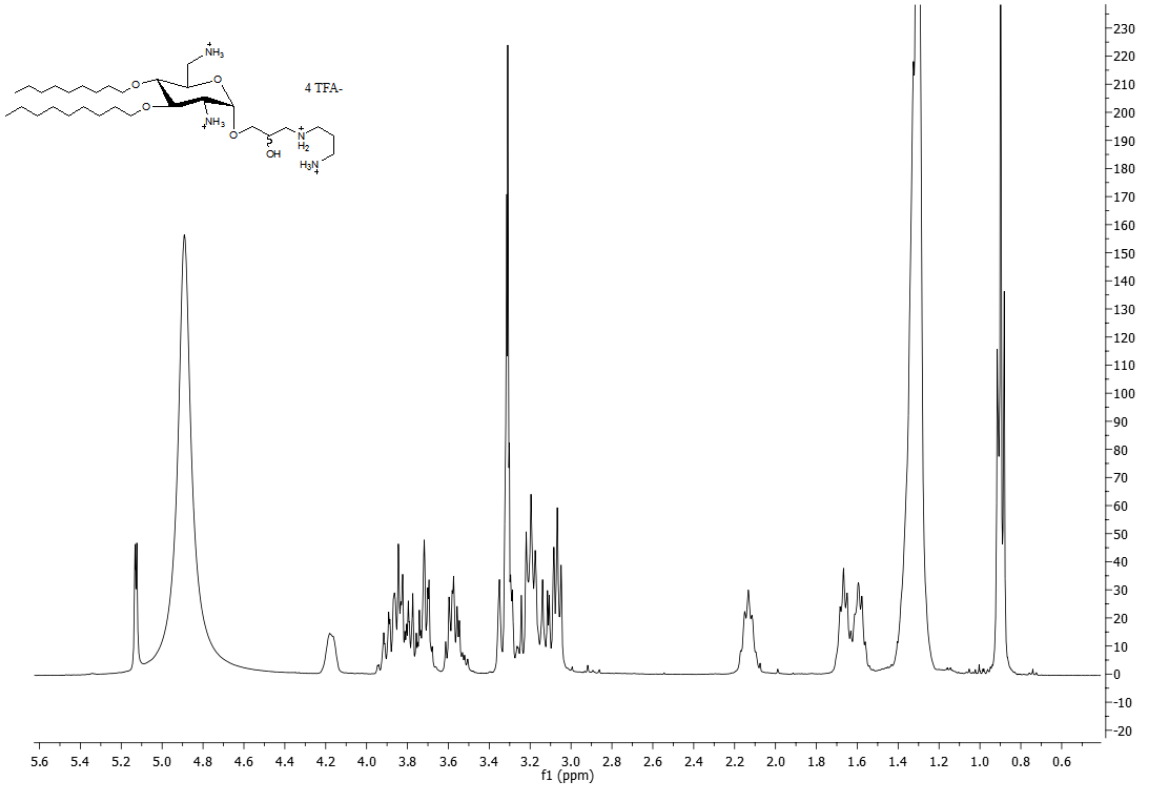



$^{13}$C NMR $CD_3OD$



Compound **47a,b**

$^{1}$H NMR $CD_3OD$




$^{13}$C NMR $CD_3OD$