

Synthesis and Microarray-Assisted Binding Studies of Core Xylose and Fucose Containing N-Glycans

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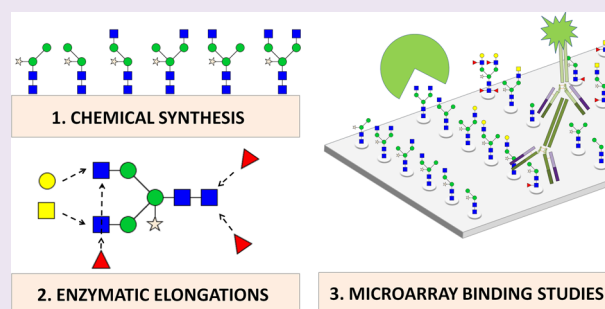
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S Supporting Information

ABSTRACT: The synthesis of a collection of 33 xylosylated and core-fucosylated N-glycans found only in nonmammalian organisms such as plants and parasitic helminths has been achieved by employing a highly convergent chemo-enzymatic approach. The influence of these core modifications on the interaction with plant lectins, with the human lectin DC-SIGN (Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Nonintegrin), and with serum antibodies from schistosome-infected individuals was studied. Core xylosylation markedly reduced or completely abolished binding to several mannose-binding plant lectins and to DC-SIGN, a C-type lectin receptor present on antigen presenting cells. Employing the synthetic collection of core-fucosylated and core-xylosylated N-glycans in the context of a larger glycan array including structures lacking these core modifications, we were able to dissect core xylose and core fucose specific antiglycan antibody responses in *S. mansoni* infection sera, and we observed clear and immunologically relevant differences between children and adult groups infected with this parasite. The work presented here suggests that, quite similar to bisecting N-acetylglucosamine, core xylose distorts the conformation of the unsubstituted glycan, with important implications for the immunogenicity and protein binding properties of complex N-glycans.



N-glycosylation is a major and complex post-translational modification of eukaryotic proteins. Glycosylation not only affects considerably the solubility, conformation, and charge of a protein, but it also provides ligands for specific recognition by lectins involved in cell–cell interaction, signal transduction, and the immune response.^{1,2} The biosynthesis of a common N-glycan precursor in the Endoplasmic Reticulum (ER) is conserved for all species, but further processing by the concerted action of glycosidases and glycosyltransferases in the Golgi leads to high-mannose, hybrid and complex N-glycans with a high degree of structural heterogeneity due to variability in the number of antennae, terminal sugars, and core modifications.

Core modification with simple monosaccharides like bisecting N-acetylglucosamine, α -1,3/ α -1,6-fucose, and β -1,2-xylose can have a profound effect on subsequent glycan processing. Fucose is itself an important ligand which is recognized by a large number of lectin receptors, whereas bisecting GlcNAc is a structural modifier that distorts the native conformation of glycans with important consequences for their recognition.^{3,4}

Importantly, core-xylose and core α -1,3-fucose are immunogenic glycan motifs often present in plants and invertebrates,

but never in mammals. IgM, IgG, and IgE antibodies against these structural elements present on food allergens are widely observed in allergic patients, but the immunopathological significance of these cross-reactive anticarbohydrate antibodies is still largely unknown.^{5,6} Core xylose and fucose modifications are also present on glycans of various parasitic nematodes and trematodes, including *Schistosoma* spp. which are responsible for over 200 million infections worldwide. Schistosomes abundantly express core-xylosylated N-glycan structures in the infectious cercariae and in the eggs. Since such core-modified glycans appear to form targets of antibody responses in the infected host, their availability would support further studies on glycan-based intervention tools for schistosomiasis and related parasitic infections.^{7–9}

To facilitate analysis of the antigenic properties of xylose and α -1,3 fucose modifications as well as various core extensions, we chose to synthesize a range of core N-glycan structural variants. These were subsequently diversified with recombinant

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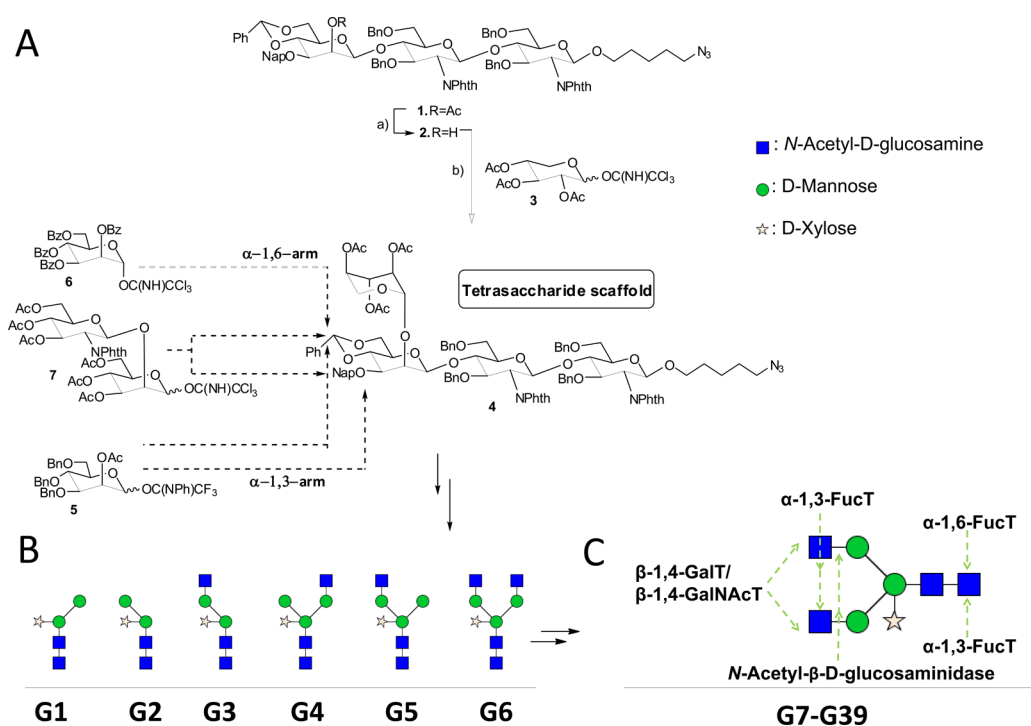


Figure 1. A general strategy for the chemoenzymatic synthesis of xylosylated N-glycans. (A) Convergent synthesis of glycan core structures from tetrasaccharide scaffold 4 and glycosyl donors 5–7. Reaction conditions a) $\text{Mg}(\text{OMe})_2$, DCM, MeOH, 80°C, MW, 86% b) TMSOTf, DCM, 0°C, 68%. (B) Target structures with a common xylosylated tetrasaccharide core (G1–G6). (C) Exemplified enzymatic diversifications (G7–G39) of core structures shown for the example of biantennary glycan G6.

glycosyltransferases to create a large set of defined extended glycans that would be difficult to obtain by full chemical synthesis or isolation from natural sources. This approach limits the necessity of chemical synthesis to the preparation of a few conserved core structures (Figure 1A,B) which are further enzymatically modified to install core fucosylation and terminal structural elements (Figure 1B,C). Here we present the chemoenzymatic synthesis of 33 core-xylosylated glycans and study their interaction with selected lectins and schistosomiasis serum antibodies on a glycan microarray comprising over 120 synthetic N-glycans and fragments of glycolipids and O-glycans.

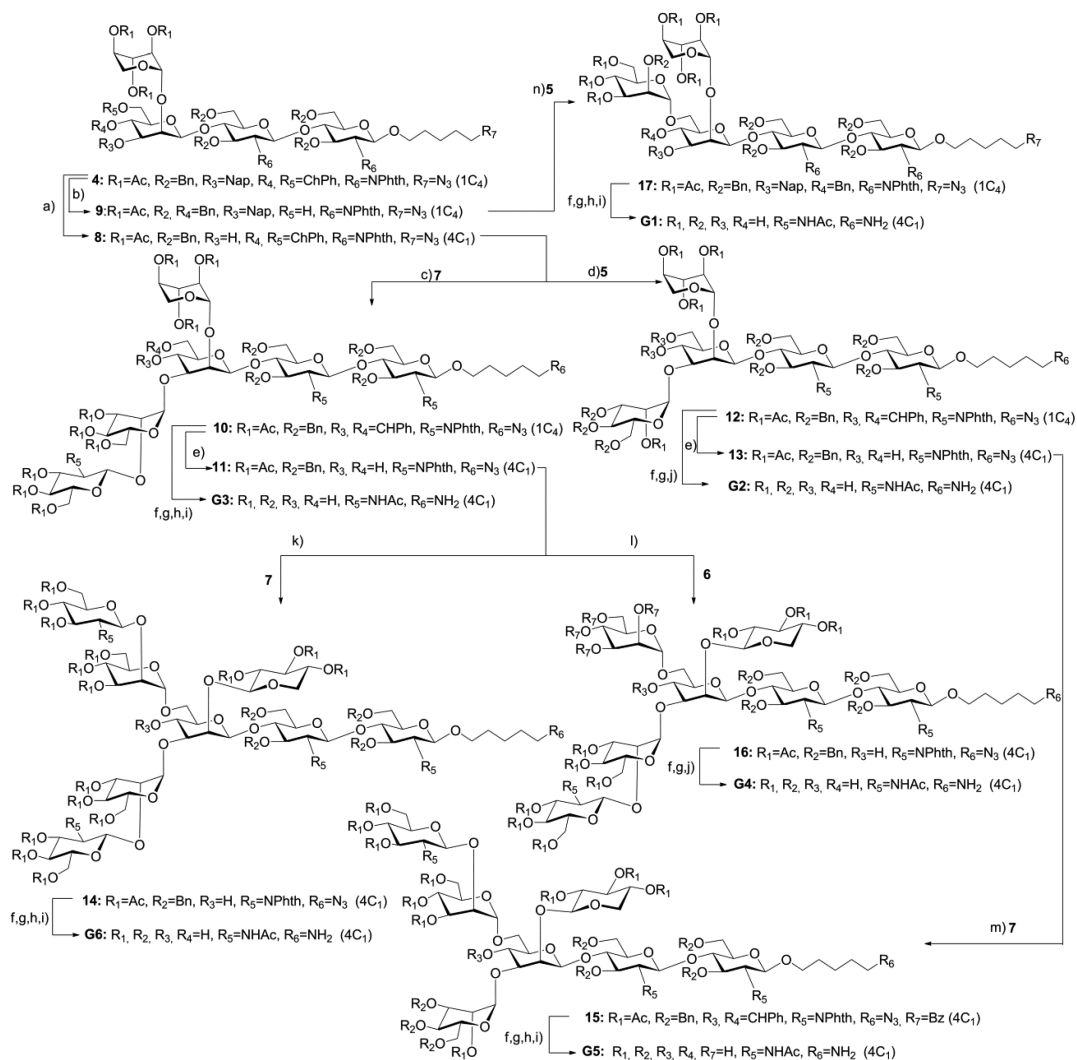
RESULTS AND DISCUSSION

In spite of their wide occurrence in plants and invertebrates, their involvement in food allergies, and their implication in infection with parasitic helminths, the synthesis of core-xylosylated glycans has received only little attention.^{10–14} With the exception of a publication by Nakano et al.,¹⁵ this work is the only report on the synthesis of these N-glycans, and the first one focused on studies of their interaction with lectins and serum antibodies using glycan array technology.

Synthesis of Xylosylated N-Glycans. All N-glycans included in our array are accessible via the previously described protected trisaccharide precursor 1¹⁶ (Figure 1), comprising a terminal mannose unit orthogonally protected with an acetate in C-2, a 2-naphthylether in C-3, and a benzylidene-acetal in C-4/C-6. Deacetylation of O-2 with magnesium methoxide provided acceptor 2 in 87% yield. This allowed the introduction of a β -xylose residue early in the synthesis by subsequent coupling with trichloroacetimidate 3 to furnish tetrasaccharide 4 in 73% yield. In line with previous reports, this glycosylation required an excess of donor, high concentration, and a strong promoter to achieve satisfactory conversion presumably due to

hindrance from the neighboring 2-naphthyl ether.¹⁵ Depending on the protecting groups regime employed, we could observe the known^{5–18} conformational flipping of the core xylose from the $^1\text{C}_4$ (4) to the $^4\text{C}_1$ conformation which is reflected by an increase in the $^3J_{1,2}$ coupling constant from the diequatorial (3.6 Hz) to the diaxial conformation (7 Hz) for the xylose skeleton protons H1 and H2.

To access the xylosylated glycans G1–G6 required as core structures for the enzymatic diversification with recombinant glycosyltransferases, the 2-naphthylmethyl ether group in 4 was first removed (Scheme 1) under standard conditions employing DDQ to give 8 in variable yields ranging from 50 to 60% due to the partial cleavage of primary benzyl groups even under buffered conditions.¹⁷ The best results were obtained when the reaction was worked up before the complete conversion and the unreacted starting material recovered. Glycosylation of 8 with either mannose donor 5 or disaccharide donor 7¹⁴ under TMSOTf activation produced pentasaccharide 12 and hexasaccharide 10 in excellent yields of 93% and 82%, respectively. Removal of the benzylidene acetal in 12 and 10 by BF_3OEt_2 catalyzed transacetalation with thioethane afforded the corresponding diols 13 (86%) and 11 (79%). Acceptors 13 and 11 were reacted with disaccharide donor 7¹⁸ under TMSOTf activation to produce heptasaccharide 16 (73%) and octasaccharide 14 (70%) with complete stereo- and regioselectivity. For the synthesis of glycan G5, the glycosyl acceptor 11 was coupled with the bulky benzoylated imide 6 in an attempt to avoid both bis-glycosylation of O-4 and O-6 and the formation of the corresponding orthoester which had been observed for the glycosylation with the acetylated donor. However, the formation of the O-4-regioisomer could not be suppressed completely, and the heptasaccharide was obtained in a medium yield of 53% after chromatographic purification.

Scheme 1. Chemical Synthesis of Xylosylated Glycans G1–G4^a

^aReagents and conditions: (a) DDQ, DCM/MeOH (4:1) 60%; (b) BH₃·THF, *n*-Bu₂BOTf, 0 °C; 65%; (c) 7, TMSOTf, DCM, 4 Å MS, −40 °C, 82%; (d) 5, TMSOTf, DCM, 4 Å MS, −20 °C, 94%; (f) NH₂CH₂CH₂NH₂/*n*BnOH, MW (120 °C, 3 × 30'); (g) Ac₂O, pyr.; (h) NaOMe, MeOH; (i) Hcube, 10% Pd/C, MeOH, 0.1%TFA; (j) Birch reduction, Na, NH₃(aq.), THF, −78 °C; (e) BF₃·Et₂O, EtSH, DCM; (k) 7, TMSOTf, DCM, 4 Å MS, −45 °C, 73%; (l) 6, TMSOTf, DCM, 4 Å MS, −45 °C, 47%; (m) 7, TMSOTf, DCM, 4 Å MS, −45 °C, 73%; (n) 5, TMSOTf, DCM, 4 Å MS, −20 °C, 52%.

For the synthesis of the O-6-substituted derivative **G1**, the benzylidene-acetal in **4** was regioselectively opened under reducing conditions with BH₃·THF and *n*-Bu₂BOTf catalysis with excellent selectivity furnishing the primary alcohol **9** in 70% yield. Coupling of O-6 acceptor **9** with glycosyl donor **5** provided the pentasaccharide **17** in 52% yield. This route was preferred to the regioselective glycosylation in O-6 of the corresponding diol as initial attempts had led also to the formation of the undesired O-4 regioisomer, presumably via an orthoester intermediate.^{19,20} The N-glycan precursors **11**, **13**, and **14**–**17** were deprotected toward the target glycans **G1**–**G6** either in a three or four step sequence, starting in both cases with the aminolysis of phthalimide groups followed by reacylation of the intermediate amines. Birch reduction readily removed acetates and benzyl groups and reduced the linker azide to an amine in a single step for the glycans **G2** (69%) and **G4** (56%). For the other glycans, the deprotection under the dissolving metal conditions of the Birch reduction was accompanied by the formation of a side product with a

mass defect of 18 Da, presumably formed by the elimination of an acetate group. This side reaction could be avoided by methanolysis of the acyl groups with sodium methoxide followed by the removal of remaining benzyl ether protection by hydrogenation. Following this procedure, the fully deprotected target glycans **G1** (49%), **G3** (65%), **G5** (58%), and **G6** (69%) were obtained in good yields. With **G1**–**G6** in hand, we focused on the preparation of a library of core-xylosylated compounds by enzymatic diversification with recombinant glycosyltransferases.

On-Chip Evaluation of Xylosylated Structures As Substrates for Enzymatic Extensions. As β-1,2-xylosylation is known biosynthetically to occur prior to other core modifications,²¹ we could rapidly evaluate our synthetic compounds as potential substrates by microarray technology. The six glycans **G1**–**G6** were immobilized in different subarrays on NHS activated glass slides and individual wells incubated with bovine milk β-1,4 galactosyltransferase 2.4.1.22 (GalT), *C. elegans* β-1,4 N-acetylgalactosaminyltransferase

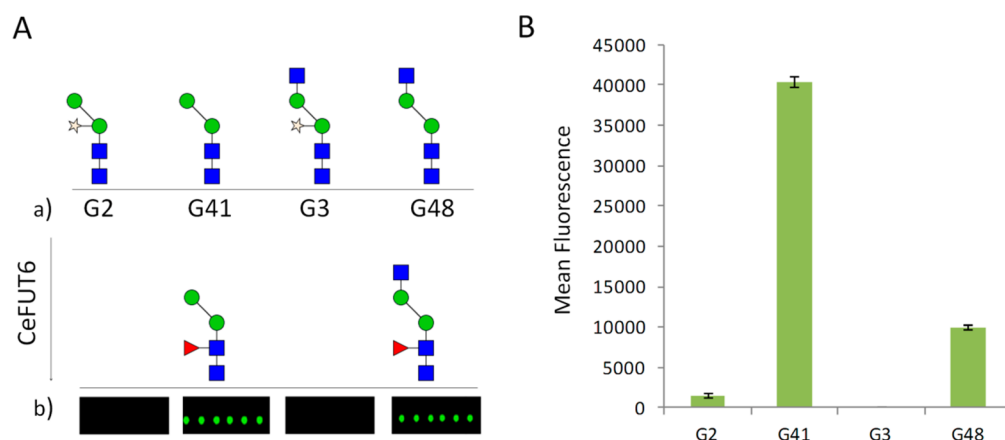


Figure 2. Screening for CeFUT6 substrates on the glycan array. (A) Enzymatic on-chip elongation with CeFUT6 (a); the N-glycan microarray image after incubation with fucose-recognizing AAL-555 lectin (b). (B) Mean fluorescence intensities of the CeFUT6-incubated (fucosylated) array after incubation with AAL-555 lectin.

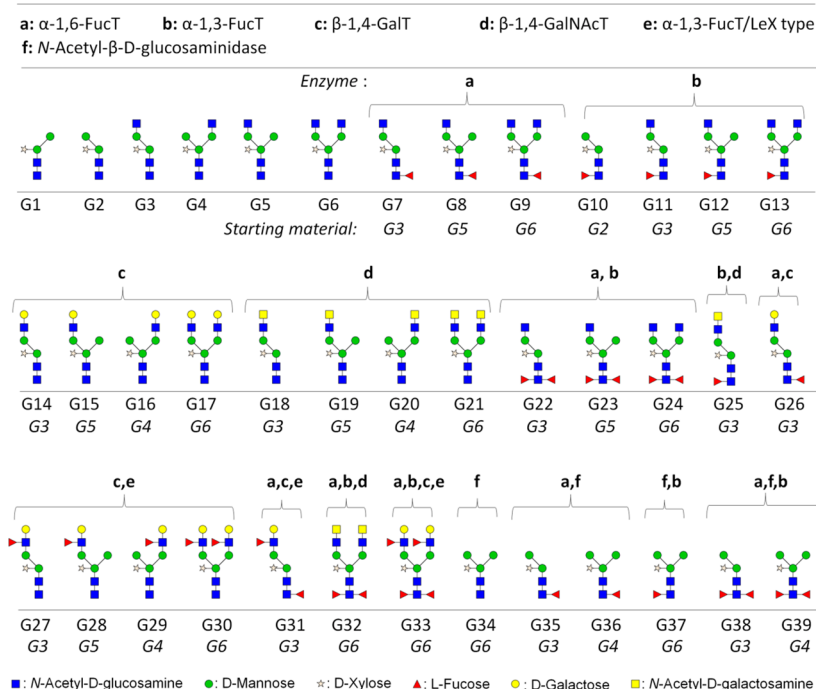


Figure 3. Enzymatic derivatizations performed on the six synthetic N-glycans (G1–G6). Compounds were subsequently diversified with (a) α -1,6-fucosyltransferase (AgFUT6) (G7–G9); (b) α -1,3-fucosyltransferase (AtFucTA/CeFUT1) (G10–G13); (c) β -1,4-galactosyltransferase (GalT) (G14–G17); (d) β -1,4-N-acetyl-galactosyltransferase (GalNAcT) (G18–G21); (e) LeX-type α -1,6-fucosyltransferase (CeFUT6); (f) β -N-acetylglucosaminidase.

(GalNAcT), *Arabidopsis thaliana* core type α -1,3 fucosyltransferase (AtFucTA), *C. elegans* core type α -1,3 fucosyltransferase (CeFUT1), *C. elegans* α -1,3 fucosyltransferase (CeFUT6), and *C. elegans* core type α -1,6 fucosyltransferase (CeFUT8).^{22–26} The presence of enzymatically extended structures was verified by incubating wells with the fluorescently tagged lectins, *Ricinus communis* (RCA-I), *Wisteria floribunda* (WFL), and *Aleuria aurantia* (AAL-555), that specifically recognize β -galactose, N-acetylgalactosamine, and α -fucose, respectively.²⁷ With the exception of CeFUT6, enzymatic elongations of xylosylated glycans were possible as long as other previously reported basic structural requirements were fulfilled. Besides the known Lewis-type fucosyltransferase activity of CeFUT6, we recently demonstrated as an additional activity of the enzyme the fucosylation of the inner GlcNAc of pauci-mannosidic N-

glycans (G41) and structures with nonreducing GlcNAc on the α 1,3-arm (G48).²³

In both cases, only glycans without further substituents in C6 of the central β -mannose are tolerated. As an additional constraint, we found that the presence of a core xylose (Figure 2, compounds G2, G3) impedes the fucosylation with CeFUT6 on glycans which are otherwise good substrates for the enzyme (G41, G48). On-chip fucosylation was assessed by incubation with the fucose-specific lectin AAL (Figure 2B), while enzyme reactions in solution were followed by MALDI-TOF MS.

Enzymatic Solution Phase Synthesis of the Glycan Library. On the basis of the results of the glycosyltransferase screen and previous knowledge of substrate specificity, we selected a number of enzymatic transformations to prepare a library of 33 core-xylosylated structures from the only six

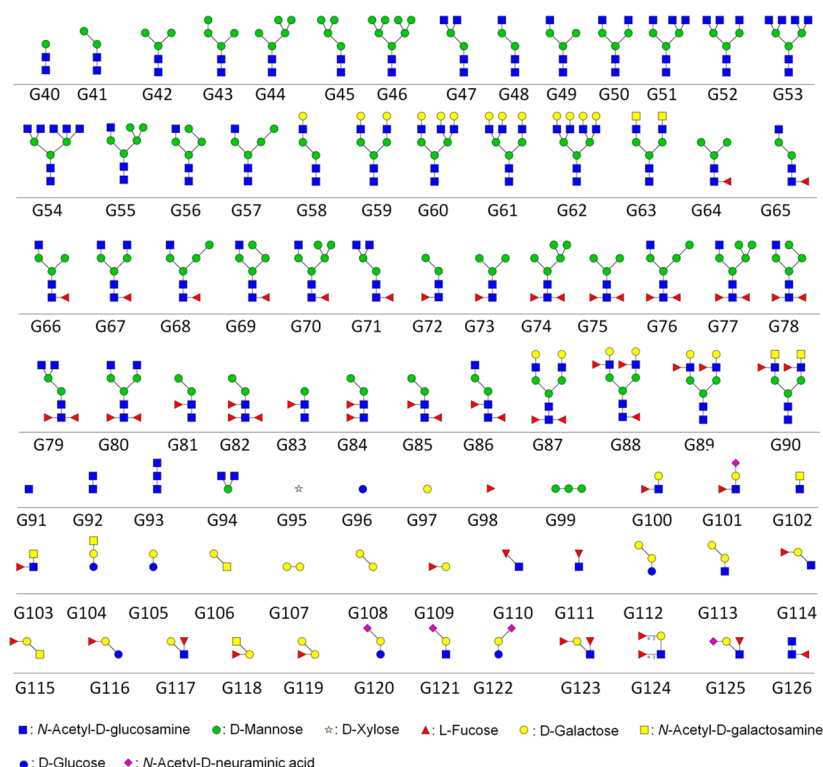


Figure 4. Collection of synthetic nonxylosylated glycans included on the microarray.

starting compounds (**G1–G6**) on a preparative scale in solution, which allowed their concise characterization by NMR and mass spectrometry. The synthesis of target compounds involved up to four subsequent incubations with the glycosyltransferases, starting with core fucosylation/s (α -1,6 prior α -1,3; CeFUT8, AtFucTA, CeFUT1), followed by terminal β -1,4 GalT or β -1,4 GalNAcT modifications and final completion of Lewis X (Gal(β 1,4)[Fuc(α 1,3)]GlcNAc, e.g. **G27–G31**, Figure 4) or LDNF (GalNAc(β 1,4)[Fuc(α 1,3)]GlcNAc, e.g. **G90**, Figure 3) structural elements (employing CeFUT6).

In addition, the pauci-mannosidic compounds **G35–G39** were accessible from **G3**, **G4**, and **G6** by incubation with a β -N-acetyl-glucosaminidase and further derivatization with recombinant glycosyltransferases (see Supporting Information for experimental details).

This collection of core-xylosylated compounds was complemented by a library of nonxylosylated N-glycans obtained from the core structures **G40–G57**^{16,19,22,27} by analogous enzymatic derivatizations in solution. Details for the enzymatic transformations and the chemical synthesis of high-mannose type N-glycan **G43** and the penta-antennary **G54** are provided in the Supporting Information. Together with a number of smaller structural elements (**G91–126**, see Supporting Information) either reported earlier or commercially available the newly synthesized compounds composed an array of 126 well-defined synthetic structures with a unique focus on core-xylosylated and core-fucosylated N-glycans (Figures 3 and 4).

We then studied the influence of N-glycan core modifications (β -1,2-xylose, α -1,3 and α -1,6-fucose) on binding to selected plant lectins (Figure 5), to a C-type lectin receptor (CLR) involved in pathogen recognition by dendritic cells (DC-SIGN; Figure 6) and to antibodies in human *Schistosoma mansoni* infection sera (Figure 7).

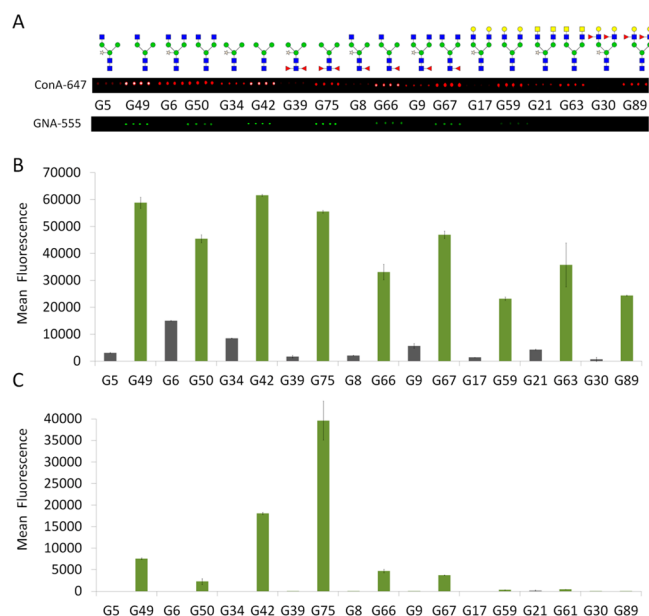


Figure 5. Validation of the glycan array by incubation with fluorescently labeled lectins. (A) Selected ligands in the interaction with plant lectins ConA and GNA. (B) Decrease of binding for xylosylated (gray bars) and nonxylosylated structures (green bars) in the interaction with ConA. (C) Lack of recognition of xylosylated structures compared to the corresponding nonxylosylated N-glycans (green bars) after incubation with fluorescently labeled GNA.

Binding Studies with Plant Lectins and DC-SIGN. Plant lectins have been defined as proteins that possess at least one noncatalytic domain that binds reversibly to a mono- or oligosaccharide.²⁸ They are widely distributed among plant tissues but often found highly concentrated in plant seeds and

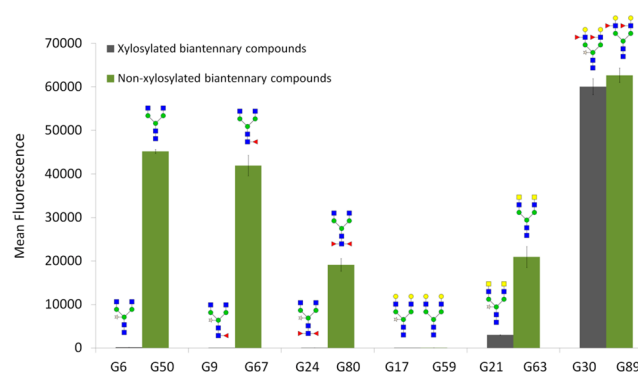


Figure 6. Interactions of DC-SIGN-Fc with xylosylated and nonxylosylated glycan series of biantennary compounds.

storage organs where they exert a dual function as storage proteins and defense toxins against herbivores and pathogens.²⁸ This defense strategy has been exploited for crop protection against insect predators by engineering the gene encoding a plant lectin like *Galantus nivalis* lectin from snowdrop into the crop plant genome.²⁹

The glycan array was incubated with lectins from *Galanthus nivalis* (GNA) reported to bind to high mannose structures, *Wisteria floribunda* (WFL) recognizing *N*-acetyl-D-galactosamine, *Aleuria aurantia* (AAL) which preferentially binds fucose, *Bandeiraea simplicifolia* (BSL II) specific for terminal, nonreducing α - or β -linked *N*-acetyl-D-glucosamine, *Canavalia ensiformis* (Con A) binding to α -mannose and glucose, and *Ricinus communis* (RCA-I) which exhibits a specificity for β -galactose residues. The presence of core xylose strongly reduced the binding of ConA when compared to the nonxylosylated counterparts (Figure 5A,C), and xylosylation completely abolished binding to GNA (Figure 5B,C). Binding to other plant lectins was not altered significantly by core xylosylation. Core fucosylation also reduced binding to ConA, but to a lesser extent (see ligand pairs G66/G49 and G42/G75) and not in all cases (G50/G67). The core trimannoside fragment 3,6-di-*O*-(α -D-mannopyranosyl)- α -D-mannopyranoside core, common to all N-glycans present on the array, binds to ConA with high specificity as it completely fills the ConA binding site.³⁰

This core trimannoside fragment is even recognized in the context of a complex biantennary N-glycan with two bulky terminal LacDiNAc (GalNAc(β 1,4)GlcNAc) or Lewis X epitopes (Figure 5A) but core xylosylation of the central mannose with xylose leads to a very strong reduction in binding. GNA on the other hand binds to terminal α -1,3 and α -1,6 linked mannose residues and unlike ConA tolerates further extension of the β -mannose with GlcNAc but not with LacNAc or LDN residues.

As these lectins are involved in plant defense against predators presumably by attaching to insect or vertebrate type glycans lacking the core xylose motif, the strongly reduced binding affinity of ConA and GNA toward core-xylosylated plant glycans might be interpreted as a tolerance mechanism to avoid interference with self-proteins.

Next, we studied the binding of DC-SIGN to core-modified structures (Figure 6). This C-type lectin signaling receptor expressed by human dendritic cells binds and internalizes mannose, glucose, and fucose containing antigens.

DC-SIGN targeting is an efficient strategy of pathogens like HIV, hepatitis virus, Ebola virus, or *Mycobacteria tuberculosis* to

evade the immune system and has practical consequences for the treatment of allergy and autoimmune diseases. DC-SIGN is also one of the receptors for the recognition and uptake of *S. mansoni* antigens via LeX, LDN-F, and other mannosylated and fucosylated glycan motifs.³¹ Incubation on our array confirmed the known specificity of DC-SIGN for high-mannose, LDNF, blood type antigens and perhaps less known, complex glycans with terminal GlcNAc on the 3-arm.^{32–34} The best binders in terms of fluorescence signal intensity were Lewis X presenting N-glycans (G27–G31, G33, G88, G89; see Figure S-9).

Interestingly, the presence of a core xylose abolished binding to the GlcNAc terminal (3-arm) oligosaccharides including biantennary and hybrid N-glycans (Figure 6). Again, the small xylose residues must distort the glycan conformation in such a way that the lectin can no longer make favorable contacts with the binding element. No significant effect of xylose on binding to the LeX presenting N-glycans structures was seen (G30, G33); the conformation and accessibility of the LeX branches is clearly not compromised by the additional xylose core modification. Xylosylated glycans which do not bind DC-SIGN but conserve binding to other C-type lectins could be welcome lead structures for the more specific glycan mediated targeting of antigens to C-type lectin receptors on dendritic cells in immune therapy.³⁵

Binding Studies with Serum Antibodies from *S. mansoni* Infected Patients. Antibodies against glycans dominate the antibody response of individuals infected with parasitic schistosome worms, but many of the underlying immunogenic glycan structures are still unknown.³⁶ Shotgun microarrays presenting the entire *S. mansoni* glycome at different stages of their life cycle are a key tool to match antibody responses to parasite glycan structures and understand glycan mediated immune responses.³⁶ Fully synthetic glycan arrays still lack the complexity and glycan coverage of a shotgun array, but ligand concentration and purity can be more precisely controlled when using synthetic glycans. Moreover, tagging procedures of natural sugars required for purification and immobilization often lead to an open ring formation of the reducing end *N*-acetyl glucosamine. Such a non-natural presentation may in particular cause a disturbed conformation and recognition of motifs associated with the glycan core. At the present time, shotgun and synthetic arrays are therefore highly complementary tools in glycomics research, in particular in studies addressing antibodies to immunogenic N-glycan core modifications often found in parasitic helminths and plant allergens.

In the last part of our study, we employed our carbohydrate microarray to determine the glycan binding profile of antibodies in sera from groups of *S. mansoni* infected children and adults. Acquired immunity against *S. mansoni* infection is the result of a slow process that includes repeated infections and maturation of the immune system, which is reflected in an age-dependent antibody response against parasite antigens.^{36,37} As a recent study employing a large shotgun array of schistosome glycans revealed an age-dependent immune response toward a range of glycan elements, we were intrigued to evaluate whether an age-related differentiation of sera can be defined on the basis of antibody reactivity to our array of core-modified glycans.³⁶

We incubated our synthetic glycan array with selected sera from a schistosomiasis study comprising pooled samples from children (age 5–11) and adults (age 26–46),³⁷ respectively, and measured binding of total IgG (Figure 7) and IgM. The

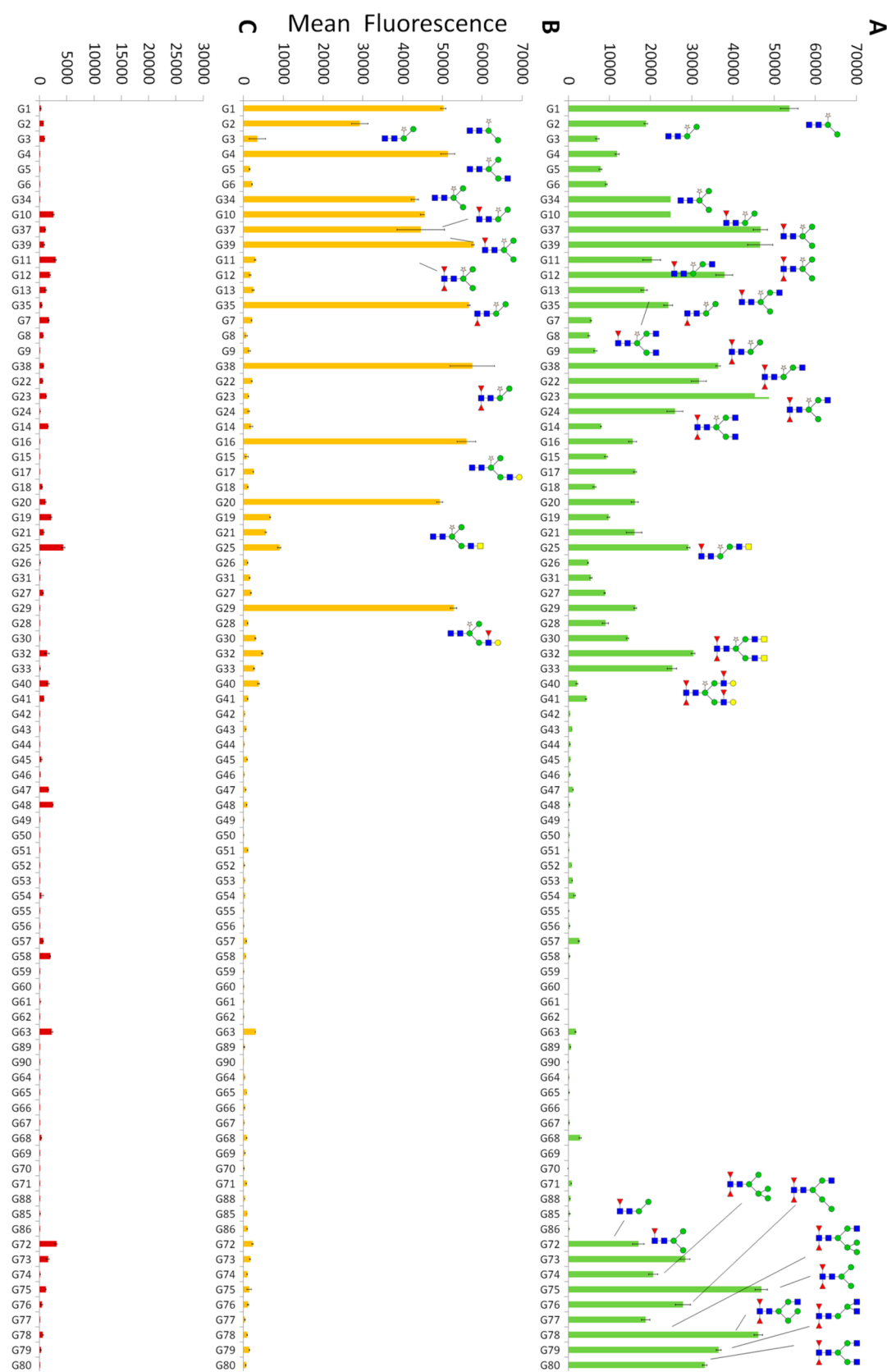


Figure 7. Comparison of IgG antibody response on pooled serum samples from *S. mansoni* infected children and adults. (A) IgG from pooled sera of infected children bind strongly to xylosylated and fucosylated structures. (B) IgG from pooled sera of infected adults bind only the fraction of xylosylated structures lacking bulky substituents on the 3-arm. (C) No significant binding to the same xylosylated or fucosylated structures was found for IgG in sera of healthy European individuals.

IgM responses to the glycans on our array (see Supporting Information Figure S-13) are of weak to moderate intensity and of quite broad specificity in both age groups. For IgG binding, however, we found a more pronounced specific binding pattern, and a strong differentiation between the two age groups based on core β -1,2 xylosylated and core α -1,3 fucosylated glycan structures. This observation highlights the antigenic nature of both these motifs as well as the value of our synthetic core-xylosylated structures to detect specific antibodies in different serum cohorts. While the children-derived serum pool contains IgG antibodies binding to both core-xylosylated and α -1,3 core-fucosylated structures, in the adult pool, IgG antibodies only bound to certain core-xylosylated compounds, but not to core α -1,3 fucosylated structures that lack xylose. Importantly the glycans recognized by either the adults or children were bound at only very low levels by serum antibodies from individuals uninfected with schistosomes (see Figure 7). Structures which were truncated on the 3-arm and presenting substituents no larger than a single α -1,3 mannose residue were bound best both in children and adults. Core-xylosylated structures presenting more sterically disturbing substituents on the 3-arm were still bound by the children serum pool albeit with lower intensity. In adults, no antibodies against these glycans were detected.

A further interpretation of the serum antibody binding profiles, e.g. with respect to identifying structures as potential vaccine candidates or specific diagnostic purposes, has to be made with caution, as core-xylosylated and core α -1,3 fucosylated glycans are not unique to the *S. mansoni* glycome but also present on several other parasites as well as on dietary proteins, e.g., from maize, rice, or peas.⁵ Consequently, low antibody titers for IgG and IgM against these structural motifs have been observed in a considerable part of the healthy population.⁵ Nevertheless, the fact that we see strong IgG binding to core-xylosylated glycans in schistosomiasis sera and not in controls indicates that this response is indeed against the parasite. The possible protective nature of the antibody response cannot be determined by glycan array screening alone but would require additional immunological studies and an immunization trial with putative glycan conjugate vaccine candidates in a relevant animal model.

Conclusions. In conclusion, we have developed convergent chemo-enzymatic protocols for the synthesis of invertebrate and plant N-glycans, with a focus on structures with core fucose and xylose, and highlighted their role in selected, biomedically relevant molecular recognition studies. Core-xylosylated compounds were not substrates for the fucosyltransferase CeFUT 6 that introduces α -1,3-fucose residues on the inner GlcNAc of the chitobiose moiety. Likewise, binding to mannose binding plant lectins and the C-type lectin receptor DC-SIGN was reduced significantly or even abolished completely by core xylosylation, opening interesting possibilities for the design of more selective compounds for CLR targeting. Finally, within our focused glycan array, xylosylated compounds next to α -1,3 core-fucosylated compounds could differentiate clearly the antiglycan antibody response between a cohort of children and adults infected with schistosomes, highlighting their potential use in studies into glycan-based immunity and diagnosis. Ongoing studies on immunoglobulin isotype and subclass specific binding will help clarify the functional role of the detected antibodies. Focused glycan arrays like the one presented here will be valuable tools in screenings of larger cohorts of noninfected, infected, and

resistant cohorts where comprehensive metadata are available, with the aim of defining prospective parasite antigens for the design of glycoconjugate vaccines.⁹

METHODS

5-Azidopentyl 4,6-O-Benzylidene-3-O-(2-naphthylmethyl)- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (2). To a solution of trisaccharide **1**¹⁶ (200 mg, 132.9 μ mol) in CH_2Cl_2 (3 mL) was added 12 mL of $\text{Mg}(\text{OMe})_2$. The reaction mixture was heated to 80 °C employing microwave irradiation (four cycles of 30 min). The suspension was filtered through a pad of Celite, and the filtrate was neutralized with 10% acetic acid. The organic layer was washed with saturated NaHCO_3 , water, and saturated NaCl ; dried over MgSO_4 ; and concentrated. The obtained crude product was purified by flash column chromatography (SiO_2 , hexane/EtOAc, 3:1 to 2:1), concentrated, and dried under high vacuum conditions to afford the title compound as a white foam (167 mg, 86%); $[\alpha]_{589}^{20} = +14.2$ (c, 0.5, CHCl_3). ¹H NMR (500 MHz, CDCl_3): δ 7.87–7.66 (m, 12H, Ar), 7.48–7.46 (m, 5H, Ar), 7.39–7.29 (m, 8H, Ar), 7.24–7.14 (m, 5H, Ar), 7.02–7.00 (m, 2H, Ar), 6.97–6.88 (m, 5H, Ar), 6.78–6.74 (m, 3H, Ar), 5.51 (s, 1H, OCHPh), 5.27 (d, $J = 8.7$ Hz, 1H, H-1A β), 4.93–4.90 (m, 2H, H-1B β , CH_2Ph), 4.87–4.82 (m, 3H, CH_2Ph), 4.65 (s, 1H, H-1C β), 4.54–4.35 (m, 7H, H-3A, CH_2Ph), 4.24–4.20 (m, 2H, H-2A, H-4B), 4.14–4.09 (m, 4H, H-2B, H-6aC, H-3B, H-4A), 4.05–4.00 (m, 2H, H-2C, H-4C), 3.69–3.62 (m, 2H, H-6aA, OCHH(CH_2)₄N₃), 3.58–3.48 (m, 4H, H-6bA, H-6aB, H-6bC, H-3C), 3.41 (dd, $J_{6a-6b} = 11.1$ Hz, $J_{6b-5} = 3.7$ Hz, 1H, H-6bB), 3.34–3.22 (m, 3H, H-5A, H-5B, OCHH(CH_2)₄N₃), 3.14–3.09 (m, 1H, H-5C), 2.91–2.81 (m, 2H, $-\text{CH}_2\text{N}_3$), 1.40–1.28 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.10–1.02 (m, 2H, O-(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl_3): δ 138.7–123.3, 101.7, 100.8, 98.2, 97.1, 79.1, 78.3, 75.8, 74.8, 74.5, 74.5, 73.4, 72.7, 72.6, 69.9, 69.0, 68.6, 68.3, 67.9, 66.8, 56.7, 55.8, 51.2, 29.8, 28.4, 23.1. HRMS (MALDI-ToF) m/z $[\text{M} + \text{Na}]^+$ calculated for $\text{C}_{85}\text{H}_{83}\text{N}_5\text{O}_{18}$: 1484.5625. Found: 1484.5649.

5-Azidopentyl (2,3,4-Tri-O-acetyl- β -D-xylopyranosyl)-(1 $\rightarrow$ 2)-(4,6-O-benzylidene-3-O-(2-naphthylmethyl)- β -D-mannopyranosyl)-(1 $\rightarrow$ 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (4). A mixture of **2** (172 mg, 117.6 μ mol) and **3** (125 mg, 297.1 μ mol) was dried under high vacuum overnight conditions. The mixture was dissolved in anhydrous CH_2Cl_2 (1.1 mL) with freshly activated molecular sieves (3 Å) added. After stirring for 40 min at room temperature, the mixture was cooled to 0 °C, and a 5% solution of trifluoromethanesulfonic acid (50 μ L, 0.28 equiv) was slowly added. The mixture was allowed to warm up to room temperature again, stirred for 2 h, and then quenched with Et_3N . The reaction mixture was filtered through a pad of Celite and concentrated. The crude product was purified by automated flash chromatography (toluene/acetone 10:1), concentrated, and dried under high vacuum conditions to afford the title compound (128 mg, 68%) as a white foam. $[\alpha]_{589}^{20} = -4$ (c, 0.5, CHCl_3). ¹H NMR (500 MHz, CDCl_3): δ 7.87–7.78 (m, 4H, Ar), 7.74–7.63 (m, 7H, Ar), 7.51–7.43 (m, 5H, Ar), 7.40–7.28 (m, 9H, Ar), 7.23–7.16 (m, 2H, Ar), 7.15–7.09 (m, 3H, Ar), 7.06–7.02 (m, 2H, Ar), 6.99–6.93 (m, 4H, Ar), 6.91–6.86 (m, 1H, Ar), 6.80–6.73 (m, 3H, Ar), 5.46 (s, 1H, OCHPh), 5.30 (d, $J = 8.4$ Hz, 1H, H-1A β), 5.10–5.07 (m, 2H, H-1X β , H-3X), 5.04 (dd, $J = 3.47$ Hz, $J = 5.4$ Hz, 1H, H-2X), 4.94–4.92 (m, 1H, H-1B β), 4.90–4.82 (m, 5H, H-4X, OCH₂Ph), 4.60 (dd, $J_{5\text{Xa}-5\text{Xb}} = 13$ Hz, $J_{5\text{Xa}-4} = 3.4$ Hz, 1H, H-5Xa), 4.58–4.48 (m, 4H, OCH₂Ph, H-1C β), 4.43 (d, $J = 12.1$ Hz, 1H, OCH₂Ph), 4.38–4.32 (m, 2H, H-3A, OCH₂Ph), 4.26–4.19 (m, 3H, OCH₂Ph, H-4B, H-2A), 4.14–4.06 (m, 4H, H-2C, H-3B, H-2B, H-6_aC), 4.03–3.99 (m, 1H, H-4A), 3.89 (t, $J = 9.5$ Hz, 1H, H-4C), 3.70–3.62 (m, 2H, OCHH(CH_2)₄N₃, H-6aB), 3.55 (dd, $J = 10.6$ Hz, 1H, H-6bB), 3.52–3.35 (m, 5H, H-5Xb, H-6_bC, H-3C, H-6aA, H-6bA), 3.34–3.28 (m, 2H, H-5A, H-5B), 3.27–3.21 (m, 1H, OCHH(CH_2)₄N₃), 3.12–3.05 (m, 1H, H-5C), 2.93–2.80 (m, 2H, $-\text{CH}_2\text{N}_3$), 2.10 (s, 3H, CH_3CO), 2.02, 1.99 (2s, 6H, CH_3CO), 1.41–

1.28 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.12–1.01 (m, 2H, O(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.1, 169.5, 169.1, 167.7, 138.8–131.8, 129.9–123.3, 102.0, 101.7, 99.4, 98.2, 97.2, 80.6, 78.4, 75.9, 76.0, 75.8, 75.2, 74.8, 74.6, 74.5, 73.5, 73.4, 72.8, 72.1, 69.1, 69.0, 68.7, 68.5, 68.4, 68.3, 68.3, 67.4, 60.3, 56.7, 55.9, 51.2, 28.8, 28.4, 23.1, 21.1–20.8. HRMS (ESI) *m/z* [M+NH₄]⁺ calculated for C₉₆H₉₇N₅O₂₅NH₄: 1737.6811. Found: 1737.6862.

5-Azidopentyl [2,3,4-Tri-O-acetyl-β-D-xylopyranosyl-(1→2)-(4,6-O-benzylidene-β-D-mannopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (8). A mixture of tetrasaccharide 4 (110 mg, 63.9 μmol) was dissolved in a mixture of CH₂Cl₂/MeOH (0.6 mL, 4:1) and treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ; 35 mg, 154 μmol). After 90 min of stirring at room temperature, the reaction was quenched with saturated NaHCO₃ and extracted with EtOAc (3×). The combined organic layers were washed with saturated NaHCO₃, water, and saturated NaCl; dried over MgSO₄; and concentrated. The crude product was purified by flash column chromatography (SiO₂, hexane/EtOAc 4:2 to 2:2) to afford the tetrasaccharide (60 mg, 60%) as a white solid. [α]_D²⁰ = −8.6° (c, 0.5, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.85–7.28 (m, 23H, Ar), 7.04–6.78 (m, 10H, Ar), 5.41 (s, 1H, OCHPh), 5.28 (d, *J* = 8.1 Hz, 1H, H-1Aβ), 5.24 (t, 1H, *J* = 8.4 Hz, H-3X), 5.02–4.95 (m, 2H, H-2X, H-4X), 4.94–4.92 (m, 1H, H-1Bβ), 4.86 (d, 1H, *J* = 12.8 Hz, OCHHPh), 4.81–4.78 (m, 2H, *J*_{1–2} = 6.7 Hz, *J* = 11.9 Hz, OCHHPh, H-1Xβ), 4.66 (s, 1H, H-1Cβ), 4.61–4.44 (m, 5H, 3× OCH₂Ph), 4.32 (d, 1H, *J* = 12.2 Hz, OCHHPh), 4.29–4.20 (m, 4H, H-4A, H-3B, H-5X, H-2A), 4.15–4.11 (m, 2H, H-2B, H-4B), 4.05–4.00 (m, 2H, H-6C, H-3A), 3.91 (d, *J* = 2.7 Hz, 1H, H-2C), 3.70–3.65 (m, 2H, H-6aA, OCHH(CH₂)₄N₃), 3.59–3.47 (m, 4H, H-6aB, H-6bA, H-4C, H-3C), 3.38–3.35 (m, 3H, H-5X, H-6C, H-6bB), 3.34–3.28 (m, 2H, H-5A, H-5B), 3.27–3.21 (m, 1H, OCHH(CH₂)₄N₃), 3.12–3.05 (m, 1H, H-5C), 2.93–2.80 (m, 2H, −CH₂N₃), 2.10, 2.02, 1.99 (3s, 9H, CH₃CO), 1.41–1.28 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.12–1.01 (m, 2H, O(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.0, 169.9, 169.4, 138.8, 138.6, 137.7, 137.3, 134.0, 131.5, 129.2–126.5, 123.3, 102.2, 102.0, 101.0, 98.3, 97.2, 80.9, 79.0, 78.9, 76.1, 74.7, 74.6, 74.5, 73.7, 72.7, 71.1, 70.8, 70.4, 69.0, 68.6, 68.5, 68.3, 68.1, 67.5, 62.1, 56.6, 55.9, 51.2, 29.8, 29.7, 28.8, 28.4, 23.1, 21.1, 20.9, 20.9. HRMS (ESI) *m/z* [M+NH₄]⁺ calculated for C₉₆H₉₇N₅O₂₅: 1737.6811. Found: 1737.6862.

5-Azidopentyl 2,3,4-Tri-O-acetyl-β-D-xylopyranosyl-(1→2)-4-O-benzyl-3-O-(2-naphthylmethyl)-β-D-mannopyranosyl-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (9). A solution of BH₃·THF (0.310 mL, 310 μmol, 1.0 M in THF) was added to the tetrasaccharide 8 (50 mg, 29.05 μmol) and the reaction mixture cooled to 0 °C. A total of 92 μL of *n*-Bu₄BOTf (1.0 M solution in Et₂O, 92 μmol) was then added and the reaction mixture stirred at 0 °C for 2 h, quenched with Et₃N and MeOH, and concentrated *in vacuo*. The crude mixture was coevaporated with MeOH (two times) and purified by flash column chromatography (SiO₂, toluene/EtOAc, 3:1). The pure compound was obtained as a white solid (32 mg, 64%). [α]_D²⁰ = +9.6° (c, 0.52, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.85–7.77 (m, 5H, Ar), 7.73–7.61 (m, 7H, Ar), 7.49–7.45 (m, 3H, Ar), 7.36–7.27 (m, 11H, Ar), 7.14–7.10 (m, 3H, Ar), 7.10–7.07 (m, 2H, Ar), 7.02–6.91 (m, 6H, Ar), 6.77–6.75 (m, 3H, Ar), 5.29 (d, *J*_{1–2} = 8.3 Hz, 1H, H-1Aβ), 5.09 (d, *J*_{1–2} = 3.35 Hz, 1H, H-1Xβ), 5.07 (t, *J*_{3–2/1} = 5.06 Hz, 1H, H-3X), 5.01 (q, 1H, *J*_{2–1} = 3.40 Hz, *J*_{2–3} = 4.82 Hz, H-2X), 4.98 (d, *J* = 12.5 Hz, 1H, OCHHPh), 4.94–4.92 (m, 1H, H-1Bβ), 4.90 (d, *J* = 11.0 Hz, 1H, OCHHPh), 4.85–4.82 (m, 3H, OCH₂Ph, H-4X), 4.68 (d, *J* = 11.9 Hz, 1H, OCHHPh), 4.58–4.49 (m, 6H, H-5Xa, H-1Cβ, 2xOCH₂Ph), 4.42 (d, *J* = 12.5 Hz, 2H, OCH₂Ph), 4.38–4.36 (m, 1H, H-3A), 4.28 (d, *J* = 12.1 Hz, 1H, OCHHPh), 4.24–4.17 (m, 2H, H-2A, H-4B), 4.11–4.07 (m, 3H, H-2B, H-3B, H-2C), 3.96 (t, 1H, *J* = 9.30 Hz, H-4A), 3.75–3.54 (m, 5H, H-4C, H-6Ca, H-6Aa, H-6Ba, OCHH(CH₂)₄N₃), 3.49–3.38 (m, 5H, H-5Xb, H-6Ab, H-6Bb, H-6Cb, H-3C), 3.33–3.22 (m, 3H, H-5A, H-5B, OCHH(CH₂)₄N₃), 3.12–3.08 (m, 1H, H-5C), 2.92–2.82 (m, 2H, −CH₂N₃), 2.11, 2.04, 1.99 (3s, 9H, CH₃CO),

1.43–1.31 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.12–1.01 (m, 2H, O(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.1, 169.6, 169.2, 138.9, 138.8, 138.6, 138.3, 137.8, 135.5, 133.7, 133.4, 133.1, 128.6, 128.5, 128.4, 128.3, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.6, 127.6, 127.1, 127.0, 126.7, 126.6, 126.4, 126.2, 125.9, 123.3, 101.2, 98.9, 98.2, 97.2, 81.1, 80.3, 76.0, 75.7, 75.2, 74.6, 74.6, 74.4, 74.0, 73.8, 73.4, 72.8, 71.7, 70.7, 69.0, 68.8, 68.4, 68.3, 68.1, 62.1, 59.9, 56.7, 55.8, 51.2, 29.8, 28.8, 28.4, 23.1, 21.1, 21.1, 20.9. HRMS (ESI) *m/z* [M+Na]⁺ calculated for C₉₆H₉₉N₅O₂₅Na: 1744.6521. Found: 1744.6563.

5-Azidopentyl [2,3,4-Tri-O-acetyl-β-D-xylopyranosyl-(1→2)-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→3)]-(4,6-O-benzylidene-β-D-mannopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (12). A mixture of 8 (43 mg, 27.2 μmol) and 5 (25 mg, 37.6 μmol) was dried under a high vacuum overnight. The mixture was dissolved in anhydrous CH₂Cl₂ (0.6 mL) and with freshly activated molecular sieves (3 Å) added. After stirring for 40 min at room temperature, the mixture was cooled to −20 °C, and 0.1 equiv of trimethylsilyl trifluoromethanesulfonate TMSOTf (10% dilution, 4.5 μL, 2.75 μmol) was slowly added. After stirring for 45 min, the suspension was quenched with Et₃N, filtered through a pad of Celite, and concentrated. The crude product was purified by flash column chromatography (SiO₂, hexane/EtOAc, 4:2 to 3:2) to afford the pentasaccharide as a white solid (52 mg, 94%). [α]_D²⁰ = −5° (c, 0.5, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.91–7.66 (m, 12H, Phth), 7.44–7.16 (m, 30H, Ar), 7.18–6.76 (m, 10H, Ar), 5.60–5.55 (m, 1H, H-2M), 5.42 (s, 1H, OCHPh), 5.28 (d, 1H, *J*_{1–2} = 8.35 Hz, H-1Aβ), 5.24–5.20 (m, 1H, H-1Mα), 5.09 (t, *J*_{3/2/4} = 6.6 Hz, 1H, H-3X), 5.03–4.97 (m, *J*_{2–3} = 6.6 Hz, *J*_{2–1} = 4.4 Hz, 1H, H-2X), 4.95 (d, 1H, *J*_{1–2} = 4.4 Hz, H-1Xβ), 4.93–4.88 (m, 2H, H-1B, CH₂O), 4.84 (dd, *J* = 12.5 Hz, *J* = 5.3 Hz, 2H, OCH₂Ph), 4.80–4.74 (q, 1H, *J*_{4–3} = 6.6 Hz, *J*_{4–5b} = 5.7 Hz, H-4X), 4.72 (d, *J* = 11.3 Hz, 1H, OCHHPh), 4.63 (d, 2H, OCHHPh, H-1Cβ), 4.59–4.44 (m, 8H, 4xOCH₂Ph), 4.41–4.30 (m, 3H, OCH₂Ph, H-3A), 4.26 (dd, *J*_{5a–5b} = 12.6 Hz, *J*_{5a–4} = 3.9 Hz, 1H, H-5Xa), 4.23–4.19 (m, 2H, H-2A, H-4B), 4.11–4.09 (m, 2H, H-2B, H-3B), 4.05–3.96 (m, 5H, H-6C, H-2C, H-4A, H-3C, H-3M), 3.87–3.72 (m, 4H, H-4M, H-4C, H-5M, H-6M), 3.69–3.63 (m, 3H, H-6M, H-6A, OCHH(CH₂)₄N₃), 3.54 (d, *J* = 10.9 Hz, 1H, H-6A), 3.47–3.39 (m, 2H, H-6abB), 3.38–3.27 (m, 4H, H-6C, H-5bX, H-5A, H-5B), 3.27–3.21 (m, 1H, OCHH(CH₂)₄N₃), 3.05–2.99 (td, *J*_{5–6} = 9.5 Hz, *J*_{5–4} = 4.7 Hz, 1H, H-5C), 2.92–2.82 (m, 2H, −CH₂N₃), 2.11, 2.03, 2.01, 1.93 (4xs, 12H, CH₃CO), 1.37–1.27 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.10–1.03 (m, 2H, OCH₂CH₂CH₂CH₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.1, 169.7, 169.6, 169.2, 168.6, 167.7, 138.8, 138.7, 138.5, 138.3, 138.1, 137.8, 137.2, 133.7, 131.8, 131.5, 128.9–127.0, 126.0, 123.8, 123.3, 102.1, 101.2, 99.7, 99.1, 98.2, 97.1, 80.6, 78.2, 77.8, 76.7, 76.0, 75.2, 74.7, 74.6, 74.6, 74.5, 74.5, 74.3, 73.6, 73.5, 73.4, 72.7, 72.1, 71.7, 69.6, 69.1, 69.0, 68.5, 68.4, 68.3, 68.3, 68.1, 67.2, 60.7, 56.6, 55.8, 51.2, 28.8, 28.4, 23.1, 21.2–21.0. HRMS (MALDI-ToF) *m/z* [M + Na]⁺ calculated for C₁₁₄H₁₁₉N₅O₃₁: 2076.7781. Found: 2076.7880.

5-Azidopentyl [3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→3)-[2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)]-(β-D-mannopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (11). Hexasaccharide 10 (69 mg, 30.18 μmol) was dissolved in dry CH₂Cl₂ (0.32 mL), and subsequently EtSH (13.5 μL) and a 10% solution of BF₃·Et₂O (7.4 μL, 0.2 equiv) were added. After 2 h of stirring at room temperature, the reaction mixture was quenched with Et₃N and concentrated. The crude product was purified by flash column chromatography (SiO₂, hexane/EtOAc, 2:1 to 1:2) to afford compound 11 as a white solid (52 mg, 79%). [α]_D²⁰ = −3.5° (c, 0.5, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.86–7.55 (m, 12H, Ar), 7.38–7.23 (m, 10H, Ar), 7.06–6.78 (m, 10H, Ar), 5.78 (dd, *J*_{3–2} = 10.75 Hz, *J*_{3–4} = 9.21 Hz, 1H, H-3G), 5.42 (d, *J*_{1–2} = 8.4 Hz, 1H, H-1Gβ), 5.27 (d, *J*_{1–2} = 8.5 Hz, 1H, H-1Aβ), 5.19–5.09 (m, 3H, H-4G, H-3X, H-4M), 5.05–5.02 (m, 2H, H-2X, H-3M), 4.95 (d, 1H, *J*_{1–2} = 1.4 Hz, H-1Mα), 4.93–4.84 (m, 5H,

H-1B β , H-4X, H-1X β , OCH₂Ph), 4.59–4.48 (m, 5H, 2xOCH₂Ph, H-1C β), 4.43–4.37 (m, 4H, OCH₂Ph, H-2G, H-3A), 4.32 (dd, 1H, J_{6a-6b} = 12.4 Hz, J_{6a-5} = 4.8 Hz, H-6_aG), 4.28 (t, 1H, J_{2-4} = 3.6 Hz, J_{2-3} = 2.4 Hz, H-2M), 4.24–4.17 (m, 3H, H-2A, H-5_aX, H-4B), 4.14–4.08 (m, 3H, H-2B, H-3B, H-6_aG), 3.99–3.97 (m, 1H, H-4A), 3.95–3.90 (m, 2H, H-5M, H-5G), 3.84 (d, 1H, J = 2.9 Hz, H-2C), 3.78 (dd, 1H, J_{6a-6b} = 12.1 Hz, J_{6a-5} = 2.6 Hz, H-6_aM), 3.73–3.64 (m, 4H, H-6_bM, H-4C, H-6_aA, OCHH(CH₂)₄N₃), 3.61 (dd, 1H, J_{6a-6b} = 12.0 Hz, J_{6a-5} = 2.7 Hz, H-6_aC), 3.54–3.46 (m, 3H, H-6_bA, H-6_bB, H-6_bC), 3.43–3.39 (m, 2H, H-6_bB, H-5_bX), 3.33–3.29 (m, 2H, H-5A, H-5B), 3.26–3.19 (m, 2H, OCHH(CH₂)₄N₃, H-3C), 2.96–2.93 (m, 1H, H-5C), 2.92–2.81 (m, 2H, -CH₂N₃), 2.11, 2.07, 2.04, 2.02, 1.94, 1.91, 1.86 (9xs, 27H, CH₃CO), 1.35–1.29 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.09–1.04 (m, 2H, O(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.9, 170.6, 170.5, 170.3, 169.8, 169.6, 169.5, 138.8, 138.5, 137.7, 134.4, 134.1, 133.8, 131.8, 131.5, 128.8–127.0, 123.7, 123.3, 101.2, 99.8, 98.2, 97.8, 97.1, 96.9, 80.1, 79.9, 76.5, 76.1, 75.9, 74.7, 74.6, 74.6, 74.3, 74.2, 73.5, 72.7, 71.9, 70.7, 70.3, 69.9, 69.9, 69.1, 69.0, 68.7, 68.6, 68.3, 67.7, 66.3, 66.1, 63.1, 62.2, 61.1, 56.6, 55.8, 54.6, 51.2, 29.8, 28.8, 28.4, 23.1, 21.1–20.6. HRMS (MALDI-ToF) m/z [M + Na]⁺ calculated for C₁₁₀H₁₂₀N₆O₄₂: 2219.7331. Found: 2219.7351.

5-Azidopentyl [2,3,4-tri-O-acetyl- β -D-xylopyranosyl-(1 $\rightarrow$ 2)]-[2-O-acetyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-(β -D-mannopyranosyl)-(1 $\rightarrow$ 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (13). Pentasaccharide 12 (51 mg, 24.81 μ mol) was dissolved in dry CH₂Cl₂ (0.24 mL) and subsequently EtSH (10 μ L, 5.7 equiv), and a 10% solution of BF₃·Et₂O (8.5 μ L, 0.27 equiv) was added. After 3 h of stirring at room temperature, the reaction mixture was quenched with Et₃N and concentrated. The crude product was purified by flash column chromatography (SiO₂, hexane: EtOAc, 2:1 to 1:1) to afford the pure compound as a white solid (42 mg, 86%). [α]_D²⁰ = +0.8, (c, 0.35, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.84–7.64 (m, 6H, Ar), 7.39–7.24 (m, 25H, Ar), 7.19–7.17 (m, 2H, Ar), 7.04–7.02 (m, 2H, Ar), 6.99–6.97 (m, 2H, Ar), 6.92–6.86 (m, 3H, Ar), 6.78–6.767 (m, 2H, Ar), 5.57 (dd, 1H, J_{2-3} = 3.2 Hz, J_{2-1} = 1.8 Hz, H-2M), 5.43 (d, 1H, J_{1-2} = 1.8 Hz, H-1M α), 5.26 (d, J_{1-2} = 8.3 Hz, 1H, H-1A β), 5.21 (t, 1H, $J_{3-4/2}$ = 8.4 Hz, H-3X), 5.04 (dt, 1H, J_{4-3} = 8.4 Hz, J_{4-5a} = 5.1 Hz, H-4X), 4.95 (d, 1H, J_{1-2} = 6.6 Hz, H-1X β), 4.94–4.91 (m, 1H, H-1B β), 4.90–4.77 (m, 5H, H-2X, 2x OCH₂Ph), 4.69 (d, 1H, J_{1-2} = 8.4 Hz, H-1B β), 4.56–4.41 (m, 10H, 5x OCH₂Ph), 4.28 (dd, J = 10.6 Hz, J = 8.6 Hz, 1H, H-3A), 4.22–4.10 (m, 7H, H-5X_a, H-5M, H-2A, H-2C, H-4B, H-3B, H-2B), 4.0–3.94 (m, 2H, H-3M, H-4A), 3.85 (t, 1H, $J_{4-3/5}$ = 9.7 Hz, H-4C), 3.74 (dd, 1H, J_{6a-6b} = 10.3 Hz, J_{6a-5} = 1.7 Hz, H-6_aM), 3.68–3.57 (m, 5H, H-6C_a, H-4M, OCHH(CH₂)₄N₃, H-6M_b, H-6Ba), 3.53–3.46 (m, 3H, H-6A_b, H-6C_b), 3.44 (dd, 1H, J_{3-4} = 9.7 Hz, J_{3-2} = 3.05 Hz, H-3C), 3.40 (dd, 1H, J_{6a-6b} = 10.90 Hz, J_{6a-5} = 4.10 Hz, H-6Bb), 3.31–3.22 (m, 4H, H-5A, H-5B, H-5X_b, OCHH(CH₂)₄N₃), 3.08–3.04 (m, 1H, H-5C), 2.91–2.81 (m, 2H, -CH₂N₃), 2.17, 2.07, 2.01, 1.79 (4xs, 12H, CH₃CO), 1.37–1.24 (m, 4H, OCH₂CH₂CH₂CH₂N₃), 1.19–1.03 (m, 2H, OCH₂CH₂CH₂CH₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.4, 170.0, 169.8, 169.6, 138.7, 138.6, 138.5, 138.3, 138.1, 137.7, 134.1, 133.9, 133.8, 131.8, 131.5, 128.7–127.0, 123.7, 123.3, 101.9, 99.5, 98.2, 97.1, 96.9, 80.5, 79.7, 77.9, 76.1, 76.1, 75.3, 75.1, 74.8, 74.7, 74.7, 74.6, 74.6, 73.6, 73.5, 72.7, 71.8, 71.4, 71.0, 70.8, 69.7, 69.1, 69.0, 68.6, 68.3, 67.8, 62.9, 62.1, 56.6, 55.8, 51.2, 29.8, 28.8, 28.4, 23.1, 21.2, 21.0, 20.8, 20.7. HRMS (MALDI-ToF) m/z [M + Na]⁺ calculated for C₁₀₇H₁₁₅N₅O₃₁: 1988.7468. Found: 1988.7417.

5-Azidopentyl (3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4,6-tri-O-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4-tri-O-acetyl- β -D-xylopyranosyl-(1 $\rightarrow$ 2)]-(4,6-O-benzylidene- β -D-mannopyranosyl)-(1 $\rightarrow$ 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (10). A mixture of 8 (55 mg, 34.79 μ mol) and 7 (50 mg, 57.6 μ mol) was dried under high vacuum conditions overnight, then dissolved in anhydrous CH₂Cl₂ (1.1 mL), and freshly activated, ground molecular sieves (3 Å) added. After 1 h of stirring at room temperature, the mixture was cooled to –40 °C, and the 0.1 equiv of trimethylsilyl

trifluoromethanesulfonate TMSOTf (1 μ L, 5.52 μ mol) was added. After 1 h, the reaction mixture was quenched with Et₃N, filtered through a pad of Celite, and concentrated. The crude product was purified by flash column chromatography (hexane/EtOAc, 3:2 to 2:3) to afford the hexasaccharide 10 as a white foam (63 mg, 82%). [α]_D²⁰ = –21 (c, 0.5, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.86–7.53 (m, 12H, Ar), 7.77–7.29 (m, 15H, Ar), 7.02–6.79 (m, 10H, Ar), 5.49 (dd, J_{3-2} = 10.9 Hz, J_{3-4} = 9.3 Hz, 1H, H-3G), 5.36 (s, 1H, OCHPh), 5.31 (d, J_{1-2} = 8.4 Hz, 1H, H-1A β), 5.09–5.06 (m, 2H, H-3M, H-3X), 5.01 (t, J = 10.01, J = 11.1 Hz, 1H, H-4M), 4.98–4.95 (m, 2H, H-4G, H-2X), 4.95–4.87 (m, 5H, H-1B β , H-1M α , H-1X β , OCHHPh, H-1G β), 4.85–4.80 (m, 2H, H-4X, OCHHPh), 4.66 (d, J = 11.8 Hz, 1H, OCH₂Ph), 4.57–4.48 (m, 3H, OCH₂Ph), 4.43 (s, 1H, H-1C β), 4.41–4.36 (m, 2H, H-5_aX, H-3A), 4.31–4.24 (m, 3H, OCH₂Ph, H-2G), 4.24–4.18 (m, 2H, H-2A, H-4B), 4.14–4.09 (m, 2H, H-2B, H-3B), 4.07–4.02 (m, 3H, H-6_aC, H-2M, H-4A), 3.95 (dd, J_{6a-6b} = 12.4 Hz, J_{6a-5} = 3.7 Hz, 1H, H-6_aG), 3.90–3.87 (m, 1H, H-5M), 3.79 (d, J = 3.4 Hz, 1H, H-2C), 3.72–3.63 (m, 6H, H-6_bG, OCHH(CH₂)₄N₃, H-6_aA, H-4C, H-6_abM), 3.59 (dd, J_{5a-5b} = 12.4 Hz, J_{5b-4} = 4.2 Hz, 1H, H-5_bX), 3.55–3.50 (m, 2H, H-6_aA, H-6_aB), 3.45–3.41 (m, 2H, H-3C, H-6_bB), 3.36–3.29 (m, 3H, H-5A, H-5B, H-6_bC), 3.27–3.22 (m, 1H, OCHH(CH₂)₄N₃), 2.94–2.90 (m, 1H, H-5C), 2.89–2.81 (m, 2H, -CH₂N₃), 2.11–2.10 (m, 1H, H-5G), 2.07, 2.05, 2.03, 1.98, 1.97, 1.94, 1.87, 1.86 (9xs, 27H, CH₃CO), 1.38–1.29 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.10–1.03 (m, 2H, O(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.7, 170.5, 170.4, 170.3, 169.8, 169.5, 169.3, 169.0, 138.9, 138.8, 138.6, 137.9, 137.6, 134.35, 133.8, 131.5, 130.3, 129.1, 128.9, 128.7, 128.7, 128.6, 128.3, 128.2, 128.0, 127.8, 127.5, 127.5, 127.1, 127.0, 123.7, 123.3, 102.5, 101.3, 99.6, 98.4, 98.2, 97.2, 95.8, 79.8, 78.4, 76.6, 76.1, 74.6, 74.6, 73.6, 72.8, 72.7, 71.2, 70.5, 69.2, 69.0, 68.8, 68.7, 68.5, 68.3, 67.9, 66.7, 66.2, 65.5, 63.2, 61.1, 60.3, 56.6, 55.8, 54.2, 51.2, 29.8, 28.8, 28.4, 23.1, 21.1, 21.0, 20.9, 20.8, 20.7, 20.6. HRMS (MALDI-ToF) m/z [M + Na]⁺ calculated for C₁₁₇H₁₂₄N₆O₄₂: 2307.7644. Found: 2307.7554.

5-azidopentyl (3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4,6-tri-O-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 3)-[2,3,4-tri-O-acetyl- β -D-xylopyranosyl-(1 $\rightarrow$ 2)]-(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 2)-(3,4,6-tri-O-acetyl- α -D-mannopyranosyl)-(1 $\rightarrow$ 6)]-(β -D-mannopyranosyl)-(1 $\rightarrow$ 4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl)-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (14). A mixture of 11 (28 mg, 12.73 μ mol) and 7 (14.3 mg, 16.5 μ mol) was dried under high vacuum conditions over the night. The mixture was dissolved in anhydrous CH₂Cl₂ (3 mL, 250 mL/mmol) with freshly activated, ground 3 Å MS. After 1 h at room temperature, the mixture was cooled to –45 °C and 0.15 equiv of trimethylsilyl trifluoromethanesulfonate TMSOTf (3.4 μ L, 1.9 μ mol) was slowly added. After 2 h, the reaction mixture was quenched with Et₃N, filtered through a pad of Celite, and concentrated. The crude product was purified by flash chromatography (SiO₂, hexane/acetone, 3:2) to afford the octasaccharide as a white solid (25 mg, 70%); [α]_D²⁰ = +8.2, (c, 0.5, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 5.76–5.69 (m, 2H, H-3G, H-3G'), 5.45 (d, 1H, J_{1-2} = 8.5 Hz, H-1G β), 5.29 (d, 1H, J_{1-2} = 8.8 Hz, H-1G' β), 5.25 (d, 1H, J_{1-2} = 8.4 Hz, H-1A β), 5.20–5.11 (m, 5H, H-4M, H-4G, H-4M', H-4G', H-3X), 5.07 (dd, 1H, J_{3-4} = 10.1 Hz, J_{3-2} = 3.3 Hz, H-3M), 4.96–4.86 (m, 6H, H-3M', H-2X, H-1B β , H-1M α , H-1X β , H-4X), 4.84 (d, 1H, J = 12.8 Hz, OCHHPh), 4.72 (d, 1H, J = 12.5 Hz, OCHHPh), 4.58 (d, 1H, J = 12.0 Hz, OCH₂Ph), 4.52–4.43 (m, 4H, OCH₂Ph, H-1C β), 4.41–4.30 (m, 7H, H-1M' α , H-2G, H-2G', OCH₂Ph, H-2C, H-6Ga), 4.27–4.21 (m, 3H, H-6G'a, H-5Xa, H-3A), 4.17–4.08 (m, 6H, H-4B, H-2M', H-3B, H-2A, H-2B, H-6Gb), 3.97–3.93 (m, 4H, H-5G, H-5Xb, H-4A, H-5M), 3.83–3.71 (m, 5H, H-6Mab, H-6M'ab, H-2C), 3.68–3.55 (m, 6H, H-5G', H-5M', H-6Cab, H-4C, OCHH(CH₂)₄N₃), 3.51–3.36 (m, 4H, H-6Aab, H-6Ba, H-5Xb), 3.29–3.21 (m, 4H, H-5A, H-5B, H-6Bb, OCHH(CH₂)₄N₃), 3.15 (dd, 1H, J_{3-4} = 9.6 Hz, J_{3-2} = 3.0 Hz, H-3C), 3.04–3.00 (m, 1H, H-5C), 2.91–2.81 (m, 2H, -CH₂N₃), 2.08, 2.06 (2s, 6H, CH₃CO), 2.04–2.03 (3s, 9H, CH₃CO), 2.00–1.96 (m, 21H, CH₃CO), 1.84 (s, 6H, CH₃CO), 1.81 (s, 3H,

CH₃CO), 1.37–1.27 (m, 4H, OCH₂CH₂CH₂CH₂N₃), 1.09–1.02 (m, 2H, O(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 171.0–168.4, 138.8, 138.6, 137.8, 134.4, 133.8, 131.5, 128.8–127.0, 123.7, 123.3, 101.2, 99.6, 99.1, 98.2, 98.0, 97.4, 97.2, 79.9, 79.6, 76.0, 75.4, 74.7–74.4, 73.6, 73.5, 72.7, 71.8, 71.7, 70.8, 70.2, 70.1, 69.9, 69.9, 69.7, 69.4, 69.0, 68.9, 68.7, 68.2, 67.7, 66.1, 65.6, 62.9, 62.4, 61.9, 61.7, 61.1, 56.7, 55.8, 54.7, 54.6, 51.2, 29.8, 28.8, 28.4, 23.1, 20.8–20.7. HRMS (MALDI-Tof) *m/z* [M + Na]⁺ calculated for C₁₄₂H₁₅₅N₇O₅₉: 2924.9236. Found: 2924.9144.

5-Azidopentyl (3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→6)-[2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)]-[2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→3)]-(β-D-mannopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (16). A mixture of 13 (40 mg, 20.33 μmol) and 7 (21 mg, 24.19 μmol) was dried under high vacuum conditions overnight, then dissolved in anhydrous CH₂Cl₂ (5 mL, 250 mL/mmol) and freshly activated, with ground molecular sieves added (3 Å). After stirring for 1 h, the reaction mixture was cooled to –45 °C, and 0.2 equiv of trimethylsilyl trifluoromethanesulfonate TMSOTf (7 μL, 4.06 μmol) was slowly added with the help of a syringe. After 2 h of stirring, the suspension was quenched with Et₃N, filtered through a pad of Celite, and concentrated. The crude product was purified by flash chromatography (SiO₂, hexane/acetone, 3:2) to afford the octasaccharide as a white solid (40 mg, 73%); [α]_D²⁰ = –0.6 (c, 0.35, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.79–7.55 (m, 12H, Ar), 7.38–7.27 (m, 24H, Ar), 7.16–7.15 (m, 2H, Ar), 7.04–7.03 (m, 2H, Ar), 6.94–6.93 (m, 2H, Ar), 6.81–6.74 (m, 5H, Ar), 5.66 (t, 1H, J = 9.9 Hz, H-3G), 5.59 (s, 1H, H-2M), 5.36 (s, 1H, H-1Ma), 5.24 (t, 2H, J = 7.9 Hz, H-1Aβ, H-1Gβ), 5.19 (t, J = 8.4 Hz, H-3X), 5.14 (t, 1H, J = 9.9 Hz, H-4M), 5.08 (t, 1H, J = 9.7 Hz, H-4G), 5.02–4.99 (m, 1H, H-4X), 4.98–4.93 (m, 2H, H-3M', H-1Xβ), 4.91–4.83 (m, 4H, H-2X, H-1Bβ, OCH₂Ph), 4.78 (d, 1H, J = 11.3 Hz, OCHHPh), 4.67 (d, 1H, J = 12.6 Hz, OCHHPh), 4.59–4.54 (m, 5H, H-C1β, 2xOCH₂Ph), 4.51–4.33 (m, 8H, H-1M'a, H-2G, 3xOCH₂Ph), 4.23–4.06 (m, 10H, H-5Xa, H-6aG, H-3A, H-5M, H-2A, H-2B, H-2M', H-2C, H-3B, H-4B), 4.00 (dd, 1H, J₃₋₄ = 9.9 Hz, J₃₋₂ = 3.4 Hz, H-3M), 3.95 (t, 1H, J = 9.0 Hz, H-4A), 3.87 (d, 1H, J = 12.0 Hz, H-6Gb), 3.80 (dd, 1H, J_{6a-6b} = 12.2 Hz, J_{6a-5} = 4.3 Hz, H-6M'a), 3.72–3.61 (m, 8H, H-4C, H-4M, H-5M', H-6M'b, OCHH(CH₂)₄N₃, H-6Mab, H-6Aa), 3.56–3.35 (m, 7H, H-5G, H-3C, H-6Ab, H-6Bab, H-6Cab), 3.30–3.22 (m, 4H, H-5A, H-5B, H-5Xb, OCHH(CH₂)₄N₃), 3.13–3.10 (m, 1H, H-5C), 2.91–2.80 (m, 2H, –CH₂N₃), 2.16, 2.05, 2.02, 2.00, 1.99, 1.98, 1.93, 1.92, 1.82, 1.71 (10xs, 30H, CH₃CO), 1.41–1.29 (m, 4H, OCH₂CH₂CH₂CH₂N₃), 1.107–1.03 (m, 2H, OCH₂CH₂CH₂CH₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.9–167.4, 138.8, 138.5, 138.1, 138.0, 134.4, 134.0, 133.7, 131.9, 131.7, 131.5, 128.7–127.0, 123.6, 123.3, 101.8, 99.6, 98.2, 97.8, 97.7, 97.2, 80.7, 79.5, 77.8, 76.1, 75.1, 75.1, 74.8, 74.7, 74.6, 74.4, 73.5, 73.4, 72.7, 71.8, 71.7, 71.6, 71.0, 70.7, 70.0, 69.4, 69.1, 69.0, 68.9, 68.7, 68.6, 68.4, 68.3, 68.0, 66.8, 65.6, 62.5, 62.0, 61.7, 56.5, 55.8, 54.5, 51.2, 29.8, 28.8, 28.4, 23.1, 21.2–20.6. HRMS (ESI) *m/z* [M + Na]⁺ calculated for C₁₃₉H₁₅₀N₆O₄₈: 2693.9373. Found: 2693.9343.

5-Azidopentyl (3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→2)-(3,4,6-tri-O-acetyl-α-D-mannopyranosyl)-(1→3)-[2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)]-[2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl)-(1→6)]-(β-D-mannopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (15). A mixture of 11 (21 mg, 9.55 μmol) and 6 (8.5 mg, 11.47 μmol) was dried under high vacuum conditions overnight, dissolved in anhydrous CH₂Cl₂ (2.2 mL, 250 mL/mmol), and freshly activated, with ground molecular sieves (3 Å) added. After 1 h at room temperature, the mixture was cooled to –45 °C, and 0.4 equiv of trimethylsilyl trifluoromethanesulfonate TMSOTf (6.5 μL, 3.82 μmol) was added slowly with the help of a syringe. After 5 h of stirring at the same temperature, the reaction mixture was quenched with Et₃N, filtered through a pad of Celite, and concentrated. The crude product was purified by HPLC (acetonitrile/H₂O; initial condition 85:15; 2 min 90:10; 35 min 99:1; 36 min

85:15) to afford the final product as a white solid (12.4 mg, 47%). [α]_D²⁰ = –8.8 (c, 0.52, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 8.12–8.11 (m, 2H, Ar), 7.99–7.97 (m, 2H, Ar), 7.91–7.89 (m, 2H, Ar), 7.83–7.71 (m, 5H, Ar), 7.66–7.61 (m, 5H, Ar), 7.57–7.47 (m, 6H, Ar), 7.43–7.39 (m, 3H, Ar), 7.35–7.28 (m, 12H, Ar), 7.24–7.21 (m, 4H, Ar), 7.05 (d, J = 7.92 Hz, 2H, Ar), 6.98–6.96 (m, 2H, Ar), 6.83 (t, J = 7.92 Hz, 2H, Ar), 6.78–6.76 (m, 3H, Ar), 6.74–6.71 (m, 1H, Ar), 6.09 (t, J = 10.0 Hz, H-4M'), 5.83–5.79 (m, 2H, H-3M', H-3G), 5.66–5.64 (m, 1H, H-2M'), 5.49 (d, J_{2-1/3} = 8.5 Hz, 1H, H-1Gβ), 5.27 (d, J₁₋₂ = 8.43 Hz, 1H, H-1Aβ), 5.21–5.08 (m, 4H, H-3X, H-4M, H-4G, H-3M), 5.04–5.02 (m, 3H, H-2X, H-1M, H-1M'), 4.95–4.90 (m, 4H, H-1Xβ, H-1Bβ, H-4X, OCHHPh), 4.87 (d, J = 12.9 Hz, 1H, OCHHPh), 4.80–4.77 (m, 1H, H-6M'a), 4.61–4.33 (m, 12H, 6xOCH₂Ph, H-1Cβ, H-5M', H-2G, H-5M, H-3A, H-6M'b), 4.25–4.20 (m, 3H, H-2A, H-6Ga, H-5Xa), 4.18–4.09 (m, 3H, H-4B, H-3B, H-2B), 4.04 (t, J = 9.18 Hz, 1H, H-4A), 3.99–3.96 (m, 2H, H-5M, H-6Gb), 3.91–3.84 (m, 3H, H-6Ca, H-2C, H-5G), 3.80–3.64 (m, 6H, H-6Cb, H-4C, H-6Mab, H-6Aa, OCHH(CH₂)₄N₃), 3.56–3.47 (m, 2H, H6Ab, H6Ba), 3.42–3.33 (m, 2H, H6Bb, H5Xb), 3.35–3.22 (m, 4H, OCHH(CH₂)₄N₃, H-3C, H-5A, H-5B), 3.18–3.15 (m, 1H, H-5C), 2.90–2.81 (m, 2H, –CH₂N₃), 2.06, 2.04, 2.03, 2.015, 2.01, 1.93, 1.91, 1.85 (9s, 27H, CH₃CO), 1.41–1.29 (m, 4H, OCH₂CH₂CH₂CH₂N₃), 1.06–1.05 (m, 2H, OCH₂CH₂CH₂CH₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.9, 170.6, 170.5, 170.3, 169.9, 169.8, 169.6, 169.5, 169.2, 166.5, 165.6, 165.5, 165.2, 138.8, 138.7, 138.5, 137.8, 134.3, 133.7, 133.4, 133.2, 131.6, 130.0, 129.9, 129.9, 129.4, 129.3, 129.2, 128.9, 128.8, 128.6, 128.6, 128.5, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0, 127.8, 127.5, 127.4, 127.1, 127.0, 101.9, 100.1, 98.8, 98.3, 98.2, 97.2, 97.0, 80.5, 79.3, 76.1, 75.9, 74.8, 74.7, 74.6, 74.5, 74.2, 73.4, 72.7, 71.8, 70.9, 70.7, 70.5, 70.4, 70.3, 70.2, 69.9, 69.4, 69.0, 68.7, 68.3, 67.9, 67.8, 66.7, 66.2, 63.1, 62.4, 62.0, 61.4, 56.7, 55.8, 54.7, 51.2, 32.0, 28.8, 28.4, 23.1, 20.9–20.6. HRMS (ESI) *m/z* [M + Na]⁺ calculated for C₁₄₄H₁₄₆N₆O₅₁Na: 1410.4399. Found: 1410.4358.

5-Azidopentyl 2-O-Acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl-(1→6)-[2,3,4-tri-O-acetyl-β-D-xylopyranosyl-(1→2)]-4-O-benzyl-3-O-(2-naphthylmethyl)-β-D-mannopyranosyl)-(1→4)-(3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranoside (17). A mixture of 9 (24 mg, 13.93 μmol) and 5 (13.4 mg, 20.19 μmol) was dried under high vacuum conditions overnight, then dissolved in anhydrous CH₂Cl₂ (0.4 mL) and freshly activated, with ground molecular sieves (3 Å) added. After 30 min of stirring at room temperature, the mixture was cooled to –20 °C, and 0.15 equiv of trimethylsilyl trifluoromethanesulfonate TMSOTf (3.6 μL, 2.08 μmol) was slowly added with the help of a syringe. After 1 h of stirring at the same temperature, the suspension was quenched with Et₃N, filtered through a pad of Celite, and concentrated. The crude product was purified by preparative thin layer chromatography (TLC) on silica gel 60 F₂₅₄ Merck KGaA, Germany (hexane/EtOAc 3:1 to 3:2) to afford the product in 52% yield (16 mg). [α]_D²⁰ = +2.1 (c, 0.45, CHCl₃). ¹H NMR (500 MHz, CDCl₃): δ 7.83–7.76 (m, 4H, Ar), 7.71–7.62 (m, 7H, Ar), 7.48–7.44 (m, 3H, Ar), 7.33–7.29 (m, 7H, Ar), 7.24–7.15 (m, 20H, Ar), 7.17–7.11 (m, 2H, Ar), 7.07–7.04 (m, 2H, Ar), 7.04–6.98 (m, 2H, Ar), 6.93–6.92 (m, 2H, Ar), 6.77–6.68 (m, 6H, Ar), 5.38–5.37 (dd, J₂₋₃ = 3.35 Hz, J₂₋₁ = 1.83 Hz, 1H, H-2M), 5.26–5.22 (m, 2H, H-3X, H-1Aβ), 5.10–5.06 (m, 2H, H-1X, H-2X), 4.98–4.89 (m, 4H, H-4X, H-1Bβ, OCH₂Ph), 4.82–4.75 (m, 4H, H1M-α, OCH₂Ph), 4.62–4.26 (m, 16H, H-1Cβ, OCH₂Ph, H-5Xa, H-3A), 4.21–4.15 (m, 3H, H-2A, H-2C, H-4B), 4.09–4.06 (m, 2H, H-2B, H-3B), 4.01 (t, J_{4-3/5} = 9.1 Hz, 1H, H-4A), 3.91 (dd, J₃₋₄ = 9.6 Hz, J₃₋₂ = 3.35 Hz, 1H, H-3M), 3.77 (t, J_{4-3/5} = 9.6 Hz, 1H, H-4M), 3.73–3.63 (m, 5H, H-4C, H-6Ca, H-6Aa, H-6Ba, OCHH(CH₂)₄N₃), 3.60–3.54 (m, 2H, H-5M, H-6Cb), 3.53–3.49 (m, 2H, H-6Ab, H-6Bb), 3.45–3.37 (m, 4H, H-5Xb, H-3C, H-6Mab), 3.33–3.21 (m, 4H, H-5A, H-5B, H-5C, OCHH(CH₂)₄N₃), 2.91–2.81 (m, 2H, –CH₂N₃), 2.10, 1.98, 1.975, 1.97, (4s, 12H, CH₃CO), 1.38–1.29 (m, 4H, OCH₂CH₂CH₂CH₂CH₂N₃), 1.09–1.03 (m, 2H, O-(CH₂)₂CH₂(CH₂)₂N₃). ¹³C NMR (126 MHz, CDCl₃): δ 170.4–169.1, 138.7–133.1, 128.4–123.2, 102.7, 99.8, 98.0, 97.4, 96.9, 81.3,

78.1, 76.5, 76.0, 75.0–74.1, 73.3, 72.9, 72.7, 71.6, 71.3, 70.4, 69.0–68.3, 66.7, 56.7, 55.8, 51.2, 28.8, 28.4, 23.1, 21.0, 20.9. HRMS (ESI) m/z [M + H + Na]²⁺ calculated for C₁₂₅H₁₂₉N₅O₃₁: 1120.9227. Found: 1120.9224.

■ ASSOCIATED CONTENT

■ Supporting Information

Description of the chemical synthesis of the nonxylosylated oligosaccharides and their enzymatic diversification, the deprotection to final compounds, commercial sources for glycan fragments, spectroscopic data for all newly synthesized compounds, and detailed information for the microarray experiments. This material is available free of charge via the Internet at <http://pubs.acs.org>

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Notes

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Synthesis and Microarray-Assisted Binding Studies of Core Xylose and Fucose Containing N-Glycans

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1. General Methods

Materials. Chemicals were purchased from Sigma-Aldrich or Acros Organics and were used without further purification. All reaction solvents were dried over activated 4Å or 3Å molecular sieves. Thin layer chromatography was carried out using Merck aluminium sheets silica gel 60 F₂₅₄ and visualized by UV irradiation (254 nm) or by staining with vanillin solution

The recombinant enzymes α -1,3-fucosyltransferases (AtFucTA) from *Arabidopsis Thaliana*, α -1,6-fucosyltransferase, (AgFUT6) from *Anopheles gambiae*, core α -1,3-fucosyltransferase CeFUT6 and β -1,4-N-acetylgalactosaminyltransferase from *C. elegans*, bovine milk β -1,4-galactosyltransferase were expressed as described previously.^{1,2,3,4,5} β -N-acetylglucosaminidase from *Canavalia ensiformis* (Jack bean) was purchased from Sigma Aldrich. Uridine 5'-diphosphogalactose disodium salt (UDP-Gal), uridine 5'-diphospho-N-acetylgalactosamine disodium salt (UDP-GalNAc) and guanosine 5'-diphospho- β -L-fucose sodium salt (GDP-Fuc) were purchased from Carbosynth.

All aqueous solutions were prepared from nanopure water produced with a Diamond UV water purification system (Branstead International).

Canavalia ensiformis lectin (ConA), Wheat germ agglutinin (WGA), *Ricinus communis* agglutinin -120 (RCA-120), *Aleuria aurantia* lectin (AAL), *Pisum sativum* agglutinin (PSA), *Galantus nivalis* agglutinin (GNA), *Griffonia (Bandeiraea) simplicifolia* lectin II (BSL-II) and *Wisteria floribunda* lectin (WFL) were purchased from VectorLabs. Lectins were labeled with Hilyte PlusTM 647 and Hilyte PlusTM 555 protein labeling kits from AnaSpec. Recombinant human DC-SIGN/Fc Chimera was purchased from Sino Biological Inc.. Anti-human IgG (Fc specific)-Cy3, developed in goat, was purchased from Sigma-Aldrich and Anti-human IgM-Alexa fluor 647 (μ specific) was purchased from Invitrogen. Anti-human antibodies were used in dilution 1:1000. Schistosomiasis infected sera samples were obtained from 41 individuals living in Piida community, Butiaba, Uganda. Glycan arrays (36x18) were prepared as indicated in the section 6 and compartmentalized with 8 Well ProPlate Module 7 x 16 mm Well from Electron Microscopy Sciences. NHS activated glass slides, Nexterion[®] H were purchased from Schott AG.

Instrumentation. Microwave irradiation was performed on Biotage Initiator monomode oven, (Biotage AB). Hydrogenation reactions were performed in continuous-flow hydrogenation reactor H-Cube[®] from ThalesNano Nanotechnology Inc. Purifications of compounds were performed on: size-exclusion chromatography using LP2 gel from Biorad, SampliQ high performance graphitized carbon cartridges (1 mL) from Agilent Technologies, C18 Sep-Pak Cartridges (1 mL) from Waters (Milford), flash chromatography using Merck 62Å 230-400 mesh silica gel or on a Biotage SP4 automated flash chromatography system, (Biotage AB) employing

¹ Serna, S.; Yan, S.; Martin-Lomas, M.; Wilson, I. B. H.; Reichardt, N. C. *J. Am. Chem. Soc.* **2011**, *133*, 16495–16502.

² Yan, S.; Serna, S.; Reichardt, N. C.; Paschinger, K.; Wilson, I. B. H. *J. Biol. Chem.* **2013**, *288*, 21015–21028

³ Serna, S.; Hokke, C. H.; Weissenborn, M.; Flitsch, S.; Martin-Lomas, M.; Reichardt, N. C. *ChemBioChem* **2013**, *14*, 862 – 869.

⁴ Boeggeman, E. E.; Ramakrishnan, B.; Qasba, P. K. *Protein Expres. Purif.* , **2003**, *30*, 219–229.

⁵ Kwar, Z. S.; Van Die, I.; Cummings, R. D. *J. Biol. Chem.* **2002**, *277*, 34924–34932.

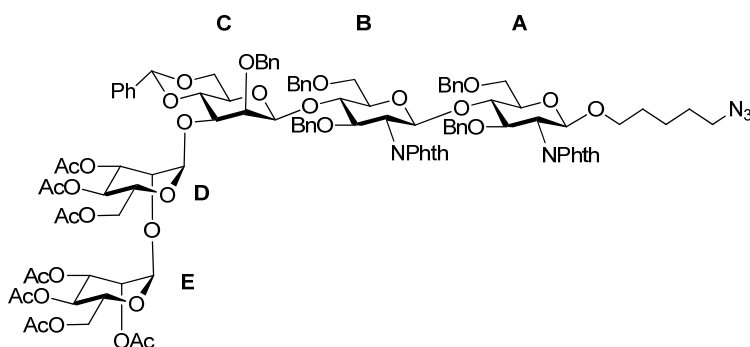
prepacked silica cartridges. All aqueous solutions were prepared from nanopure water produced with a Diamond UV water purification system (Branstead International).

Pooled glycan containing fractions were lyophilized on an ALPHA-2-4 LSC freeze-dryer from Christ. All organic solvents were concentrated using rotary evaporation.

^1H and ^{13}C spectra were acquired on Bruker 500 MHz spectrometer or on Bruker BioSpin GmbH 800 MHz. ^{13}C -HSQC spectra recorded with Bruker BioSpin GmbH 800 MHz use a folded window in the F1(^{13}C) dimension (with a sweep width value of 40 ppm). Chemical shifts were reported in ppm (δ) and referenced to the residual signal of the solvent used (MeOD 4.87 ppm; CDCl_3 7.26 ppm; D_2O 4.79 ppm). Splitting patterns are designated as s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants (J) are reported in Hz. High resolution mass spectra were acquired on a Waters LCT Premier XE instrument, (Waters) equipped with a standard ESI source by direct injection. The instrument was operated with a capillary voltage of 1.0 kV and a cone voltage of 200 V. Cone and desolvation gas flow were set to 50 and 600 L/h, respectively; source and desolvation temperatures were 100 °C. MALDI-TOF mass analyses were performed on an Ultraflextreme III time-of-flight mass spectrometer equipped with a pulsed N_2 laser (337 nm) and controlled by FlexControl 3.3 software (Bruker Daltonics). The acquisitions were carried out in positive ion reflectron mode at a laser frequency of 500 Hz. Microarrays were printed employing a robotic non contact piezoelectric SciFLEXARRAYER spotter S11 (Scienion). Fluorescence measurements were performed in an Agilent G2565BA microarray scanner system (Agilent Technologies) with 10 μm resolution, using 2 lasers (532 nm, 633 nm). Quantification of fluorescence was achieved by ProScanArray[®] Express software from Perkin Elmer.

2. Chemical synthesis of compounds G43 and G54

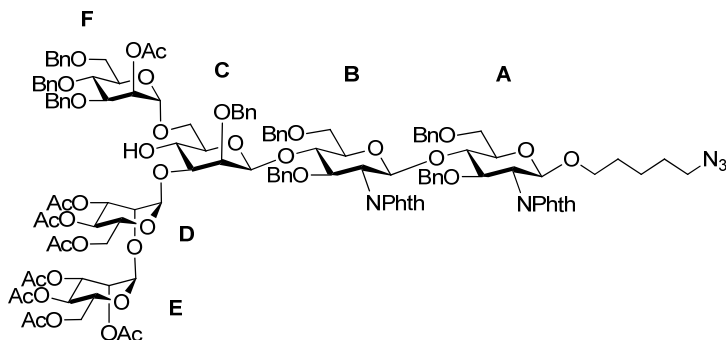
S1



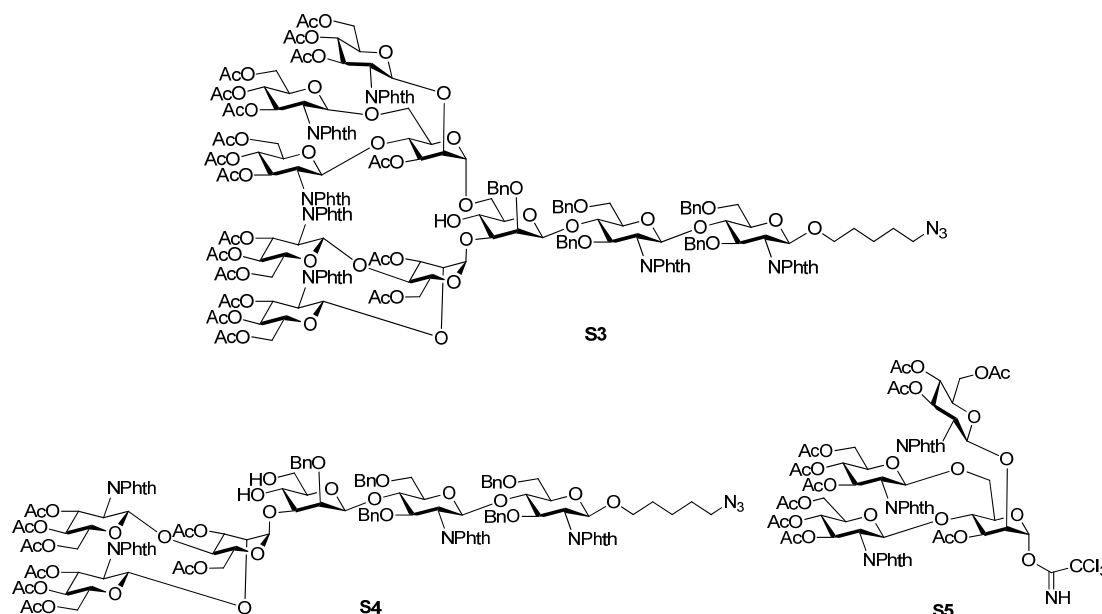
5-azidopentyl 2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3,4,6-tri-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl-4,6-O-benzylidene- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (S1): The acceptor⁶ (58 mg, 0.41 mmol) and the disaccharide donor⁷ (40 mg, 0.50 mmol) were stirred in dry CH₂Cl₂ (1 mL) in the presence of 3Å molecular sieves for 1 h at room temperature, then TMSOTf added (1 μ L, 0.05 mmol, 0.13 eq) and resulting reaction mixture stirred for 2 h, quenched with triethylamine, filtrated through a plug of Celite® and concentrated. The residue was purified by flash column chromatography (hexanes: EtOAc, 1:1) to yield the title compound as a white solid (66 mg, 80%); ¹H NMR (500 MHz, CDCl₃) δ 7.97-6.71 (m, 40H, Ar), 5.43 (s, 1H, H-1D), 5.40-5.29 (m, 5H, CHPh, H-3D, H-1B, H-3E, H-4D), 5.24 (t, J = 9.9 Hz, 1H, H-4E), 5.20 (dd, J = 3.4, 1.9 Hz, 1H, H-2E), 4.96-4.81 (m, 5H, CH₂Ph, H-1A), 4.71 (d, J = 1.8 Hz, 2H, H-1C, H-1E), 4.60 (t, J = 13.3 Hz, 1H, CH₂Ph), 4.57-4.49 (m, 4H, CH₂Ph), 4.41 (d, J = 12.2 Hz, 1H, CH₂Ph), 4.32 (dd, J = 10.7, 8.4 Hz, 1H, H-3B), 4.27-4.17 (m, 3H, H-4A, H-2B, H-6aD), 4.17-4.08 (m, 5H, H-4A, H-3A, H-2A, H-2D, H-6aC), 4.07-3.95 (m, 4H, H-6bD, H-5E, H-4C, H-6aE), 3.85-3.74 (m, 3H, H-2C, H-3C, H-5D), 3.74-3.64 (m, 3H, H-6bE, H-6aB, OCH₂), 3.59-3.51 (m, 2H, H-6bB, H-6aA), 3.48-3.40 (m, 2H, H-6bA, H-6bC), 3.36-3.22 (m, 3H H-5A, H-5B, OCH₂), 3.11 (td, J = 9.6, 4.8 Hz, 1H, H-5C), 2.93-2.81 (m, 2H, CH₂N₃), 2.11 (s, 3H, CH₃CO), 2.10 (s, 3H, CH₃CO), 2.07 (s, 3H, CH₃CO), 2.06 (s, 3H, CH₃CO), 2.01 (s, 3H, CH₃CO), 1.98 (s, 3H, CH₃CO), 1.94 (s, 3H, CH₃CO), 1.43-1.26 (m, 4H, CH₂, linker), 1.07 (m, 2H, CH₂ linker); ¹³C NMR from HSQC experiment (126 MHz, CDCl₃) δ : 134.1, 133.7, 128.4, 127.9, 127.8, 127.6, 127.3, 126.1, 123.6, 123.2, 123.1, 101.4, 99.3, 98.8, 98.1, 97.1, 78.5, 78.4, 78.2, 77.0, 76.9, 75.9, 75.4, 74.9, 74.7, 74.6, 74.4, 74.3, 73.4, 72.8, 69.8, 69.6, 69.1, 69.0, 68.9, 68.8, 68.6, 68.4, 68.3, 68.2, 67.6, 67.6, 67.0, 66.4, 66.0, 62.4, 62.0, 61.9, 56.6, 55.7, 51.1, 28.7, 28.2, 23.1, 20.8, 20.8, 20.7.

⁶ Serna, S.; Etzebarria, J.; Ruiz, N.; Martin-Lomas, M.; Reichardt, N.-C. *Chem. Eur. J.*, **2010**, *16*, 13163–13175.

⁷ Upreti, M.; Ruhel, D.; Vishwakarma, R. A.; *Tetrahedron*, **2000**, *56*, 6577-6584.



5-azidopentyl 2-O-acetyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl-(1 $\rightarrow$ 6)-[2,3,4,6-tetra-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 2)-3,4,6-tri-O-acetyl- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]-2-benzyl- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido- β -D-glucopyranoside (S2): Following the general procedure for benzylidene acetal deprotection (see experimental part), compound **S1** (66 mg, 32.5 μ mol) was treated with $\text{BF}_3 \cdot \text{OEt}_2$. After purification, the diol acceptor was obtained (45 mg, 70%). Intermediate diol (65 mg, 33.4 μ mol) and donor **5** (23 mg, 36.1 μ mol) in dry CH_2Cl_2 (1 mL) were stirred for 1h with 3 $\text{\AA}$ molecular sieves, the mixture was cooled to -78°C and TMSOTf (2 μ L, 11.1 μ mol, 0.33 eq) added. After 1h, triethylamine was added to quench the acid and the crude was filtrated through a plug of Celite[®] and concentrated. The residue was purified by flash column chromatography (toluene:EtOAc, 3:1) to yield the title compound as a white solid (26 mg, 32%); ¹H NMR (500 MHz, CDCl_3) δ 7.84-6.67 (m, 48H, Ar), 5.38 (dd, J = 10.0, 3.4 Hz, 1H, H-3E), 5.32-5.26 (m, 4H, H-4D, H-3D, H-1D, H-1B), 5.26-5.23 (m, 2H, H-4E, H-2F), 5.22 (dd, J = 3.3, 1.9 Hz, 1H, H-2E), 5.00 (d, J = 12.1 Hz, 1H, OCH_2Ph), 4.96-4.88 (m, 2H, OCH_2Ph , H-1A), 4.82 (dd, J = 12.5, 10.5 Hz, 2H, OCH_2Ph), 4.76 (s, 2H, H-1E, H-1F), 4.72 (d, J = 10.9 Hz, 1H, OCH_2Ph), 4.67 (s, 1H, H-1C), 4.60 (dd, J = 12.4, 9.4 Hz, 2H, OCH_2Ph), 4.56-4.44 (m, 6H, OCH_2Ph), 4.36 (d, J = 10.8 Hz, 1H, OCH_2Ph), 4.28-4.16 (m, 5H, OCH_2Ph , H-3B, H-4A, H-2B, H-6aD), 4.16-4.05 (m, 8H, H-6bD, H-6aE, H-2A, H-4C, H-5E, H-4B, H-3A, H-2D), 4.02 (m, 2H, H-6bE, OCH_2Ph), 3.92 (dd, J = 10.7, 3.3 Hz, 1H, H-6aC), 3.84-3.74 (m, 4H, H-5D, H-5F, H-2C, H-3F), 3.74-3.58 (m, 6H, H-4F, H-6aA, H-6bA, H-6aF, H-6bF, CH_2O), 3.57-3.44 (m, 3H, H-6bA, H-6aB, H-3C), 3.40 (dd, J = 10.9, 3.9 Hz, 1H, H-6bB), 3.33-3.27 (m, 2H, H-5A, H-5B), 3.27-3.20 (m, 1H CH_2O), 3.13 (t, J = 9.5, 3.2 Hz, 1H, H-2C), 2.92-2.80 (m, 2H, $-\text{CH}_2\text{N}_3$), 2.10 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.06 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 1.37-1.28 (m, 4H, 2x CH_2), 1.11-1.00 (m, 2H, CH_2); ¹³C NMR from HSQC experiment (126 MHz, CDCl_3) δ : 133.8, 128.4, 128.1, 128.0, 127.8, 127.7, 127.1, 123.4, 101.4, 100.2, 98.5, 98.4, 98.2, 97.2, 81.2, 78.8, 78.4, 77.9, 77.1, 76.9, 76.5, 76.0, 75.2, 75.0, 74.7, 74.5, 74.5, 74.2, 73.4, 72.8, 72.0, 71.6, 70.2, 69.8, 69.1, 69.0, 68.9, 68.6, 68.5, 68.3, 68.0, 67.8, 67.4, 66.6, 62.5, 56.7, 55.8, 51.2, 28.5, 23.2, 20.9, 20.8, 19.7.



5-azidopentyl **{[3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-O-[(3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→4)]-O-3,6-di-O-acetyl-α-D-mannopyranosyl-(1→3)]-O-[3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→2)-O-[3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)]-O-[3,4,6-tri-O-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→6)]-O-(3-O-acetyl-α-D-mannopyranosyl)-(1→6)]-O-2-O-benzyl-β-D-mannopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-3,6-di-O-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranose (S3):** Acceptor **S4**¹ (61 mg, 25.3 μmol), donor **S5**⁸ (65 mg, 40.2 μmol) and 3 Å molecular sieves were stirred in dry CH₂Cl₂ (2 mL) for 45 min. The reaction was cooled to 0°C and 10% of TMSOTf in CH₂Cl₂ (5 μL, 2.7 μmol) was added. After 2h the reaction was quenched with triethylamine, concentrated and purified by flash column chromatography (hexane:acetone, 3:2) to obtain the title compound as a white foam (70 mg, 72%); ¹H NMR (500 MHz, CDCl₃) δ 7.98-7.46 (m, 28H, Ar), 7.39-7.18 (m, 15H, Ar), 7.17-7.11 (m, 1H, Ar), 7.02-6.64 (m, 9H, Ar), 5.85-5.73 (m, 2H,), 5.60-5.44 (m, 4H), 5.40 (d, *J* = 8.4 Hz, 1H), 5.21 (dd, *J* = 5.5, 2.8 Hz, 1H), 5.19-5.11 (m, 4H), 5.08 (t, *J* = 9.6 Hz, 1H), 5.03-4.86 (m, 6H), 4.83 (d, *J* = 12.8 Hz, 1H), 4.78 (s, 1H), 4.73 (d, *J* = 12.0 Hz, 1H), 4.62-4.42 (m, 7H), 4.42-4.22 (m, 9H), 4.21-3.85 (m, 20H), 3.76 (d, *J* = 9.1 Hz, 1H), 3.71-3.61 (m, 2H), 3.56 (d, *J* = 10.3 Hz, 3H), 3.53-3.44 (m, 4H), 3.44-3.30 (m, 6H), 3.31-3.21 (m, 2H), 3.16 (d, *J* = 9.9 Hz, 1H), 3.05-2.80 (m, 5H), 2.66-2.54 (m, 2H), 2.11 (s, 3H, CH₃CO), 2.10-2.06 (m, 9H, 3x CH₃CO), 2.05 (s, 6H, 2x CH₃CO), 2.04-2.01 (m, 9H, 3x CH₃CO), 2.00 (d, *J* = 6.4 Hz, 6H, 2x CH₃CO), 1.95 (s, 3H, CH₃CO), 1.91 (s, 3H, CH₃CO), 1.87-1.83 (m, 9H, 3x CH₃CO), 1.80 (2xs, 6H, 2x CH₃CO), 1.42-1.29 (m, 4H, 2xCH₂), 1.12-1.01 (m, 2H, CH₂); ¹³C NMR (126 MHz, CDCl₃)δ: 171.0, 171.0, 170.7, 170.6, 170.1, 170.0, 169.9, 169.9, 169.8, 169.5, 169.4, 169.4, 168.2, 167.5, 167.2, 138.9, 138.7, 138.6, 138.4, 137.8, 134.4, 133.9, 133.6, 131.8, 131.7, 131.6, 131.5, 131.3, 128.6, 128.5, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 127.3, 127.3, 127.1, 126.9, 123.8, 123.8, 123.6, 123.5, 123.1, 123.1, 101.0, 99.0, 98.9, 98.1, 97.8, 97.6, 97.3, 97.0, 96.3, 78.9, 77.7, 77.2, 77.1, 76.9, 76.9, 75.8, 75.3, 74.8, 74.5, 74.5, 74.4, 74.3, 73.2, 72.8, 72.6, 71.6, 71.5, 71.5, 71.4, 71.0, 70.7, 70.5, 70.4, 70.3, 70.2, 69.4, 69.3, 69.0, 68.9, 68.8,

⁸ Unverzagt, C.; Gundel, G.; Eller, S.; Schuberth, R.; Seifert, J.; Weiss, H.; Niemietz, M.; Pischl, M.; Raps, C. *Chem. Eur. J.*, **2009**, *15*, 12292-12302.

68.6, 68.5, 68.3, 68.2, 67.7, 67.4, 62.3, 62.1, 61.7, 61.3, 56.6, 55.7, 55.0, 54.7, 54.6, 54.4, 54.3, 51.1, 29.7, 28.7, 28.3, 23.0, 20.9, 20.8, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4, 20.4, 20.2.

3. General procedures

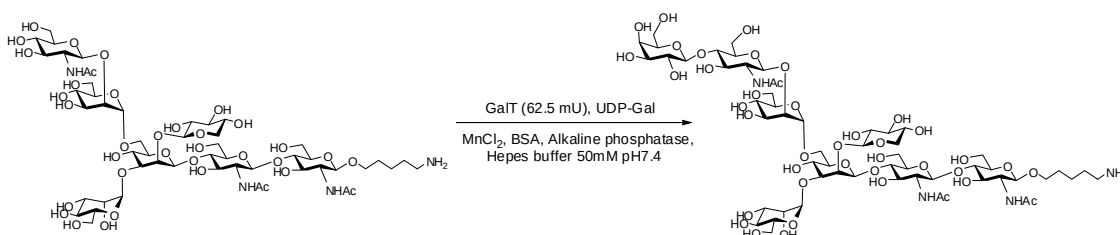
3.1 General procedure A: Deprotection of compounds G1, G3, G5, G6

A mixture of the glycan (1 eq.) and ethylenediamine in *n*BuOH (1:4; 42 ml/mmol) was heated to 120 °C under microwave irradiation for 3 cycles of 30 minutes each. The reaction mixture was concentrated and coevaporated with toluene (3 times) and EtOH (3 times). The crude product was dissolved in anhydrous pyridine (0.06 mL/μmol), cooled to 0 °C and Ac₂O (0.028 mL/μmol) was added dropwise. The reaction was warmed to room temperature and stirred overnight. The mixture was concentrated and co-evaporated with toluene (3 times) to remove traces of pyridine. The crude product was purified by size exclusion chromatography (CH₂Cl₂:MeOH, 1:2). The compound was dissolved in MeOH and 0.5M NaOMe in MeOH was added (0.2 eq. per each acetate group). After 2 h of stirring at room temperature, the reaction mixture was quenched with Amberlite® IR120 (H), filtered and concentrated. The crude product was dissolved in MeOH containing 0.1% of trifluoroacetic acid (TFA) and hydrogenated with H-Cube® reactor on 10% Pd/C cartridge (full H₂ mode; 0.5 ml/min; 50 °C). The solution of the final compound was quenched with NaHCO₃ and purified on graphitized carbon cartridge.

3.2 General procedure B: Deprotection of compounds G2, G4, G43 and G54

A mixture of the **glycans** (1 eq.) and ethylenediamine in *n*BuOH (1:4; 42 ml/mmol) was heated to 120 °C under microwave irradiation for 3 cycles of 30 minutes each. The reaction mixture was concentrated and coevaporated with toluene (3 times) and EtOH (3 times). The crude product was dissolved in anhydrous pyridine (0.06 mL/μmol), cooled to 0 °C and Ac₂O (0.028 mL/μmol) was added drop wise. The reaction was warmed to room temperature and stirred overnight. The mixture was concentrated and coevaporated with toluene (3 times). The crude product was purified by flash column chromatography (SiO₂, EtOAc:MeOH, 99:1). Sodium (25 mg) was added to liquid ammonia (15 mL) at -78 °C and the resulting blue mixture was stirred for 5 minutes. A solution of the protected product in anhydrous THF (0.11 mL/μmol) was added drop wise and the resulting mixture was stirred at -78 °C until the blue color disappeared (1 h). The reaction was quenched by adding solid NH₄Cl until neutral pH and evaporated to dryness. The crude product was purified by graphitized carbon cartridge with the elution conditions (H₂O:MeOH, 1:1).

3.3 General procedure C: Reaction with bovine milk β-1,4-galactosyltransferase

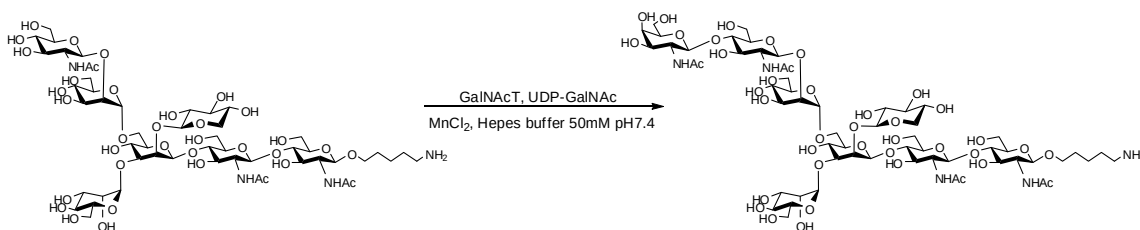


A solution (500 $\mu\text{L}/\text{mg}$) of glycan (1eq.), UDP-Gal (Uridine 5'-diphosphogalactose disodium salt) (1.2 eq.), bovine serum albumin BSA (0.5 mg), bovine milk β -1,4-galactosyltransferase, alkaline phosphatase and MnCl_2 (10 mM) in HEPES buffer (50 mM, $\text{pH}=7.4$) was incubated at 37 $^\circ\text{C}$ overnight. The resulting mixture was heated at 95 $^\circ\text{C}$ for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge (ACN/water + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried to obtain the title compound

3.4 General procedure D: Reaction with fucosyltransferases

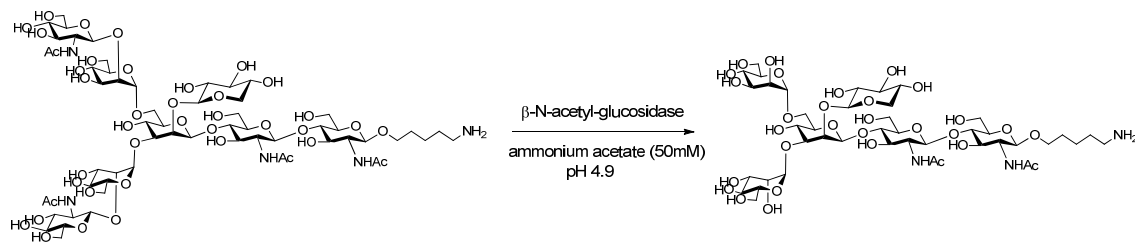
A solution (500 $\mu\text{L}/\text{mg}$) of glycan (1 eq.), GDP-Fuc (guanosine 5'-diphospho- β -L-fucose sodium salt) (1.2eq./fucose residue), CeFUT6, AtFucTA, CeFUT1 or AgFUT6 and 20 mM MnCl_2 in MES buffer (40 mM, $\text{pH}=6.5$) was incubated at room temperature overnight. The resulting mixture was heated at 95 $^\circ\text{C}$ for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge with the elution conditions (ACN/water, 1/1 + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried.

3.5 General procedure E: Reaction with β -1,4-N-acetylgalactosaminyltransferase



A solution (500 $\mu\text{L}/\text{mg}$) of glycan (1 eq.), UDP-GalNAc (Uridine 5'-diphospho-N-acetylgalactosamine disodium salt) (1.2 eq.), concentrated *P. pastoris* extract containing GalNAcT and MnCl_2 (10 mM) in HEPES buffer (40 mM, $\text{pH}=7.4$) was incubated at room temperature for 72h. The resulting mixture was heated at 95 $^\circ\text{C}$ for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge with the elution conditions (ACN/water, 1/1 + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried.

3.6 General procedure F: Reaction with Jack bean β -N-acetylglucosaminidase



A solution (500 μ L/mg) of compound glycan (1 eq.), Jack bean β -N-acetylglucosaminidase in ammonium acetate buffer (50mM, pH 4.9) was incubated at room temperature for 96h. The resulting mixture was heated at 95°C for 5 minutes to precipitate the enzyme. After centrifugation the supernatant was purified on the C18 cartridge with the elution conditions (ACN/water, 1/1 + 0.1% TFA). Fractions were analyzed by MALDI-TOF and the compound containing fractions were pooled and freeze dried.

4. List of final compounds

5-Aminopentyl **[α -D-mannopyranosyl]-(1 $\rightarrow$ 6),[β -D-xylopyranosyl]-(1 $\rightarrow$ 2)- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside (G1):** Following the general procedure A, compound **17** (24.5 mg, 11.17 μ mol) was deprotected to give **G1** as a white solid (5.32 mg, 49 % over 4 steps); $^1\text{H NMR}$ (500 MHz, D_2O) δ 4.93 (s, 1H), 4.87 (s, 1H), 4.62 (d, J = 7.5 Hz, 1H), 4.50 (d, J = 7.5 Hz, 1H), 4.46 (d, J = 7.5 Hz, 1H), 4.28 (m, 1H), 3.99–3.59 (m, 33H), 3.54–3.37 (m, 4H), 3.27 (t, J = 11.1Hz, 1H), 2.94 (t, J = 7.54 Hz, 2H, CH_2NH_2), 2.09, 2.04 (2xs, 6H, -NHAc), 1.66–1.58 (m, 4H, CH_2 linker), 1.43–1.37 (m, 2H, CH_2 linker); $^{13}\text{C NMR}$ (126 MHz, D_2O) δ 174.7, 174.4, 104.2, 101.3, 101.0, 100.7, 99.7, 79.7, 79.3, 78.0, 75.3, 74.5, 74.4, 73.2, 72.7, 72.4, 72.1, 70.4, 70.1, 69.8, 69.2, 67.0, 66.7, 65.8, 65.0, 60.9, 60.1, 55.0, 39.5, 28.1, 27.0, 22.2, 22.1; **HRMS (ESI) m/z [M+H] $^+$** calculated for $\text{C}_{38}\text{H}_{67}\text{N}_3\text{O}_{25}\text{H}$ 966.4142, found 966.4161.

5-Aminopentyl **[α -D-mannopyranosyl]-(1 $\rightarrow$ 3),[β -D-xylopyranosyl]-(1 $\rightarrow$ 2)- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside (G2):** Following the general procedure B, compound **12** (31 mg, 15.08 μ mol) was deprotected to give **G2** as a white solid (7.43 mg, 50 % over 3 steps); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.14 (s, 1H), 4.86 (s, 1H), 4.60 (d, J = 7.6 Hz, 1H), 4.50 (d, J = 7.8 Hz, 1H), 4.47(d, J = 7.6 Hz, 1H), 4.26 (d, J = 3.0 Hz, 1H), 4.06–4.04 (m, 1H), 4.03–3.99 (m, 2H), 3.93–3.57 (m, 27H), 3.52–3.43 (m, 3H), 3.38 (t, J = 8.6 Hz, 1H), 3.25 (t, J = 11.3 Hz, 1H), 2.93 (t, J = 7.2 Hz, 2H), 2.08, 2.04 (2xs, 6H, -NHAc), 1.67–1.56 (m, 4H, CH_2 linker), 1.42–1.36 (m, 2H CH_2 linker); $^{13}\text{C NMR}$ (126 MHz, D_2O , peaks assigned from HSQC) δ 104.8, 102.2, 101.3, 101.0, 100.1, 79.3, 79.0, 78.7, 77.3, 76.2, 74.5, 74.5, 73.5, 73.2, 72.4, 72.0, 70.3, 70.3, 70.1, 70.0, 69.2, 66.8, 66.3, 64.9, 64.9, 60.3, 60.1, 60.1, 55.1, 55.0, 39.5, 28.2, 27.2, 22.3, 22.2, 22.2; **HRMS (ESI) m/z [M+Na] $^+$** calculated for $\text{C}_{38}\text{H}_{67}\text{N}_3\text{O}_{25}\text{Na}$ 988.3956, found 988.3972.

5-Aminopentyl [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),[β-D-xylopyranosyl]-(1→2)- β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G3): Following the general procedure A, compound **10** (36 mg, 15.74 μmol) was deprotected to give **G3** as a white solid (12.5 mg, 65% over 4 steps); ¹H NMR (500 MHz, D₂O) δ 5.15 (s, 1H, H1M), 4.86 (s, 1H, H1M'), 4.60 (d, *J* = 7.7 Hz, 1H), 4.53-4.49 (m, 2H), 4.45 (d, *J* = 7.6 Hz, 1H, H1X), 4.25 (d, *J* = 2.8 Hz, 1H, H2M), 4.16 (m, 1H, H2M'), 4.04-4.00 (m, 1H), 3.93-3.85 (m, 10H), 3.80– 3.42(m, 28H), 3.42-3.39 (m, 1H), 3.27-3.23 (m, 1H), 2.92 (t, *J* = 7.6 Hz, 2H, -CH₂NH₂), 2.07, 2.06, 2.04 (3xs, 9H, -NHAc), 1.66-1.57 (m, 4H, CH₂ linker), 1.42-1.37 (m, 2H, CH₂ linker); ¹³C NMR (126 MHz, D₂O) δ 174.7, 174.6, 174.4, 104.9, 101.3, 101.0, 100.1, 99.7, 99.2, 79.3, 79.1, 78.7, 77.1, 76.6, 76.3, 75.8, 75.3, 74.5, 73.6, 73.3, 73.2, 72.4, 71.9, 70.1, 69.9, 69.4, 69.3, 67.2, 66.5, 64.9, 61.7, 60.6, 60.2, 60.1, 55.3, 55.1, 55.0, 39.5, 28.1, 27.3, 22.3, 22.1, 22.1, 22.1; HRMS (ESI) *m/z* [M+H]⁺ calculated for C₄₆H₈₀N₄O₃₀H 1169.493, found 1169.4907.

5-Aminopentyl [α-D-mannopyranosyl]-(1→3), [2-acetamido-2-deoxy-β-D-glucopyranosyl]-(1→2)-α-D-mannopyranosyl]-(1→6), [β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G4): Following the general procedure B compound **16** (40 mg, 14.96 μmol) was deprotected to give **G4** as a white solid (11.85 mg, 56 %); ¹H NMR (500 MHz, D₂O) δ 5.13 (s, 1H), 4.91 (s, 1H), 4.88 (s, 1H), 4.60 (d, *J* = 7.8 Hz, 1H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.50 (m, *J*=7.8 Hz, 1H), 4.45 (d, *J* = 7.5 Hz, 1H), 4.27 (d, *J* = 2.8 Hz, 1H), 4.12-4.11 (m, 1H), 4.06-4.04 (m, 1H), 4.02-3.98 (m, 2H), 3.94-3.36 (m, 43H), 3.25 (t, 11.2 Hz, 1H), 2.92 (t, *J*=7.5 Hz, 2H, CH₂NH₂), 2.09, 2.06, 2.04 (3xs, 9H, -NHAc), 1.65-1.58 (m, 4H, CH₂ linker), 1.40-1.37 (m, 2H, CH₂ linker); ¹³C NMR (126 MHz, D₂O) δ 174.8, 174.6, 174.4, 105.1, 102.2, 101.3, 101.0, 100.6, 99.6, 96.9, 79.5, 79.3, 77.3, 76.2, 75.8, 75.3, 74.5, 74.4, 74.3, 73.5, 73.3, 72.8, 72.4, 72.0, 70.3, 70.1, 69.9, 69.9, 69.7, 69.5, 69.3, 67.3, 66.7, 66.4, 65.4, 64.9, 61.6, 61.2, 60.6, 60.1, 60.0, 55.3, 55.0, 39.5, 28.2, 28.1, 27.4, 27.2, 22.3, 22.2, 22.1; HRMS (ESI) *m/z* [M+H]⁺ calculated for C₅₂H₉₀N₄O₃₅H 1331.5458, found 1331.5494.

5-Aminopentyl [2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-mannopyranosyl]-(1→3),[α-D-mannopyranosyl]-(1→6),[β-D-xylopyranosyl]-(1→2)-β-D-mannopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (G5): Following the general procedure A, compound **15** (24.8 mg, 8.93 μmol) was deprotected to give **G5** as a white solid (58% over 4 steps); ¹H NMR (500 MHz, D₂O) δ 5.15 (s, 1H), 4.93 (d, 1H, *J*=1.7 Hz), 4.88 (s, 1H), 4.61 (d, *J* = 8.0 Hz, 1H), 4.53 (d, 1H, *J*=8.3 Hz), 4.50 (d, *J* = 8.2Hz, 1H), 4.45 (d, *J* = 7.6 Hz, 1H), 4.27 (d, *J*=3.2 Hz, 1H), 4.20-4.15 (dd, *J*=3.2, 1.4 Hz, 1H), 4.05-3.37 (m, 45H), 3.26 (t, *J* = 11.1 Hz, 1H), 2.99 (t, *J*=7.8 Hz, 1H, linker), 2.09, 2.06, 2.04 (3xs, 9H, -NHAc), 1.71-1.65 (m, 2H, linker), 1.64-1.58 (m, 2H, linker), 1.43-1.39 (m, 2H, linker); ¹³C NMR (126 MHz, D₂O) δ 105.1, 101.3, 101.0, 100.4, 99.7, 99.5, 99.1, 79.5, 79.3, 79.2, 77.0, 76.5, 75.6, 74.5, 74.2, 73.7, 73.3, 73.2, 72.7, 72.4, 71.8, 70.3, 70.2, 70.1, 69.8, 69.7, 69.7, 69.4, 69.2, 67.2, 66.6, 66.5, 65.2, 65.2, 64.9, 64.8, 61.7, 60.6, 60.6, 60.1, 54.9, 55.1, 39.4, 28.1, 26.5, 22.3, 22.1; HRMS (ESI) *m/z* [M+H]⁺ calculated for C₅₂H₉₀N₄O₃₅H 1331.5458, found 1331.5502.

5-Aminopentyl di[2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranosyl]-(1 $\rightarrow$ 3),(1 $\rightarrow$ 6)-[β -D-xylopyranosyl]-(1 $\rightarrow$ 2)- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside (G6): Following the general procedure A, compound **14** (95 mg, 54.2 μ mol) was deprotected to give **G6** as a white solid (50.2 mg, 60 % over 4 steps); ^1H NMR (500 MHz, D_2O) δ 5.13 (s, 1H), 4.90 (s, 1H), 4.86 (s, 1H), 4.59 (d, J = 7.7 Hz, 1H), 4.57 (d, J = 8.4 Hz, 1H), 4.52 (d, J = 8.3 Hz, 1H), 4.51 (d, J = 7.6 Hz, 1H), 4.45 (d, J = 7.7 Hz, 1H), 4.26 (d, J = 2.6 Hz, 1H), 4.15-4.12 (m, 1H), 4.12-4.07 (m, 1H), 4.05-3.35 (m, 51H), 3.24 (t, J = 11.2 Hz, 1H), 2.91 (t, J = 7.8 Hz, 2H), 2.07, 2.05, 2.04, 2.02 (4xs, 12H, -NHAc), 1.65-1.55 (m, 4H, linker), 1.40-1.34 (m, 2H, linker); ^{13}C NMR (126 MHz, D_2O) δ 174.8, 174.7, 174.5, 105.1, 101.4, 101.0, 100.5, 99.7, 99.6, 99.2, 96.9, 79.6, 79.4, 76.6, 76.3, 75.8, 75.4, 74.5, 74.4, 73.7, 73.4, 73.3, 72.9, 72.5, 72.1, 70.2, 69.9, 69.6, 69.5, 69.3, 67.3, 67.2, 66.5, 65.0, 61.8, 61.6, 60.7, 60.1, 55.4, 55.1, 39.6, 28.1, 27.3, 22.4, 22.2, 22.2; HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{60}\text{H}_{103}\text{N}_5\text{O}_{40}$ 1534.6252, found 1534.6322.

5-aminopentyl α -D-mannopyranosyl-(1 $\rightarrow$ 6)-[α -D-mannopyranosyl-(1 $\rightarrow$ 2)- α -D-mannopyranosyl-(1 $\rightarrow$ 3)]- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-2-acetamido-2-deoxy- β -D-glucopyranoside (G43): Following the general procedure B, compound **S1** (40 mg, 16.54 μ mol) was deprotected to give **G43** (8.76 mg, 46% over 3 steps); ^1H NMR (500 MHz, D_2O) δ 5.35 (d, J = 1.9 Hz, 1H, H-1 $_{\alpha\text{Man}}$), 5.05 (d, J = 1.9 Hz, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (d, J = 1.8 Hz, 1H, H-1 $_{\alpha\text{Man}}$), 4.77 (s, 1H), 4.60 (d, J = 7.7 Hz, 1H), 4.49 (d, J = 8.0 Hz, 1H), 4.24 (d, J = 1.7 Hz, 1H), 4.10 (dd, J = 3.3, 1.7 Hz, 1H), 4.07 (dd, J = 3.3, 1.8 Hz, 1H), 4.01-3.96 (m, 2H), 3.95-3.54 (m, 32H), 3.53-3.45 (m, 1H), 2.94-2.87 (m, 2H, linker), 2.08 (s, 3H, -NHAc), 2.03 (s, 3H, -NHAc), 1.66-1.51 (m, 4H, 2x CH_2 linker), 1.42-1.33 (m, 2H, CH_2 linker); ^{13}C NMR (126 MHz, D_2O , peaks assigned from HSQC) δ : 174.7, 174.4, 102.2, 101.4, 101.0, 100.8, 100.3, 99.6, 80.6, 79.6, 79.3, 78.4, 74.5, 74.3, 74.2, 73.4, 73.2, 72.7, 72.4, 71.9, 70.4, 70.3, 70.1, 70.1, 70.0, 69.9, 69.8, 67.0, 66.8, 66.7, 65.8, 61.7, 61.1, 60.9, 60.1, 60.0, 55.0, 54.9, 39.5, 28.1, 27.3, 22.1; HRMS (ESI) m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{45}\text{H}_{79}\text{N}_3\text{O}_{31}\text{Na}$ 1180.4595, found 1180.4685.

5-aminopentyl {2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-O-[2-acetamido-2-deoxy- β -D-glucopyranosyl]-(1 $\rightarrow$ 4)]-O- α -D-mannopyranosyl-(1 $\rightarrow$ 3)}-O-{2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 2)-O-[2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)]-O-[2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 6)]-O- α -D-mannopyranosyl-(1 $\rightarrow$ 6)}-O- β -D-mannopyranosyl-(1 $\rightarrow$ 4)-O-2-acetamido-2-deoxy- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-O-2-acetamido-2-deoxy- β -D-glucopyranose (G54): Following the general procedure B, compound **S2** (30 mg, 7.76 μ mol) was deprotected to give **G54** (3.26 mg, 21% over 3 steps); ^1H NMR (500 MHz, D_2O) δ 5.13 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.84 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.61 (d, J = 7.5 Hz, 1H), 4.55-4.47 (m, 6H), 4.24 (d, J = 2.8 Hz, 1H), 4.20 (d, J = 3.1 Hz, 1H), 4.16-4.02 (m, 3H), 4.01-3.36 (m, 57H), 2.93 (dd, J = 8.5, 6.7 Hz, 2H, linker), 2.15-1.99 (m, 21H, 7x -NHAc), 1.69-1.53 (m, 4H, 2x CH_2 , linker), 1.39 (m, J = 6.3 Hz, 2H, CH_2); ^{13}C NMR (126 MHz, D_2O , peaks assigned from HSQC) δ 101.6, 101.6, 101.4, 101.4, 101.0, 100.4, 99.9, 99.8, 99.1, 97.0, 80.1, 79.3, 79.3, 79.2, 78.3, 78.1, 76.2, 76.0, 75.9, 75.8, 74.52, 74.51, 74.4, 73.5, 73.4, 73.3, 72.4, 71.9, 70.17, 70.15, 70.12, 70.11, 69.8, 69.48, 69.46, 68.3, 68.1, 65.5, 65.2, 61.3, 60.7, 60.6, 60.09, 60.06, 55.7, 55.6, 55.2, 54.8, 39.4, 28.1, 26.8, 22.5, 22.4, 22.4, 22.2, 22.14, 22.12; HRMS (ESI) m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{79}\text{H}_{134}\text{N}_8\text{O}_{51}\text{Na}$ 2033.8036, found 2033.8073.

5. List of compounds enzymatically diversified in solution

Heptasaccharide G7: Following the general procedure D, compound **G7** (1.35 mg, 98.5%) was prepared from **G3** (1.22 mg, 1.04 μ mol) using core α -1,6-fucosyltransferase AgFUT6 (30 μ L, 1.05 mg/mL) and GDP-Fuc (792 μ g, 1.25 μ mol). ; $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.15 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (d, J = 4.0 Hz, 1H, H-1 $_{\text{Fuc}}$), 4.86 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.68 (d, J = 7.6 Hz, 1H), 4.51 (t, J = 8.4 Hz, 2H), 4.46 (d, J = 7.6 Hz, 1H), 4.26-4.25 (m, 1H, H-2 $_{\text{Man}}$), 4.17-4.16 (m, 1H, H-2 $_{\text{Man}}$), 4.16-4.12 (m, 1H, H-5 $_{\text{Fuc}}$), 4.02 (dd, J = 11.5, 5.4 Hz, 1H), 3.96-3.44 (m, 38H), 3.40-3.35 (m, 1H), 3.26 (t, J = 11.1 Hz, 1H), 2.99 (t, J = 7.6 Hz, 2H, linker), 2.08, 2.06, 2.04 (3xs, 9H, NHAc), 1.69-1.65 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.44-1.38 (m, 2H, linker), 1.24 (d, J = 6.7 Hz, 3H, CH_3_{Fuc}); $^{13}\text{C NMR}$ (126 MHz, D_2O) δ 105.0, 101.2, 101.0, 100.1, 99.7, 99.4, 99.2, 79.2, 78.7, 78.6, 77.17, 76.6, 76.3, 75.7, 73.6, 73.4, 73.3, 73.1, 72.3, 72.0, 71.8, 70.0, 69.8, 69.6, 69.5, 69.5, 69.4, 69.3, 68.1, 68.0, 67.2, 66.8, 66.5, 66.5, 64.9, 64.9, 61.7, 60.3, 60.3, 55.2, 55.1, 39.3, 28.1, 26.4, 22.3(x3), 22.1, 15.4; **HRMS (ESI) m/z** $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{52}\text{H}_{90}\text{N}_4\text{O}_{34}\text{H}$ 1315.5509, found 1315.5499.

Octasaccharide G8: Following the general procedure D, compound **G8** (1.24 mg, 99%) was prepared from **G5** (1.03 mg, 0.755 μ mol) using core α -1,6-fucosyltransferase AgFUT6 (30 μ L, 1.05 mg/mL), and GDP-Fuc (589 μ g, 0.93 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.15 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.93 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.92 (d, J = 4.0 Hz, 1H, H-1 $_{\text{Fuc}}$), 4.88 (s, 1H), 4.68 (d, J = 8.1 Hz, 1H), 4.53 (d, J = 8.1 Hz, 1H), 4.51 (d, J = 8.1 Hz, 1H), 4.45 (d, J = 7.6 Hz, 1H), 4.27-4.26 (m, 1H, H-2 $_{\text{Man}}$), 4.18-4.16 (m, 1H, H-2 $_{\text{Man}}$), 4.15-4.12 (m, 1H, H-5 $_{\text{Fuc}}$), 4.04-3.51 (m, 45H), 3.40-3.36 (m, 1H), 3.26 (t, J = 11.3 Hz, 1H), 2.99 (t, J = 7.5 Hz, 2H, linker), 2.10, 2.06, 2.04 (3xs, 9H, NHAc), 1.71-1.65 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.43-1.38 (m, 2H, linker), 1.24 (d, J = 6.7 Hz, 3H, CH_3_{Fuc}); $^{13}\text{C NMR}$ (126 MHz, D_2O) δ 105.2, 101.1, 101.0, 100.6, 99.7, 99.5, 99.1, 79.6, 79.6, 79.4, 78.6, 76.9, 76.5, 75.6, 74.4, 74.3, 73.6, 73.3, 73.3, 72.8, 72.6, 72.1, 71.8, 70.2, 70.0, 69.9, 69.8, 69.6, 69.5, 69.3, 68.1, 68.0, 67.2, 66.8, 66.7, 66.6, 66.5, 65.4, 65.3, 64.9, 64.9, 61.8, 60.7, 60.6, 55.20, 54.3, 54.8, 39.3, 28.1, 26.4, 22.3, 22.1, 22.1 (x2), 15.3; **HRMS (ESI) m/z** $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{58}\text{H}_{100}\text{N}_4\text{O}_{39}\text{H}$ 1477.6037, found 1477.6073.

Nonasaccharide G9: Following the general procedure D, compound **G9** (0.82 mg, 75%) was prepared from **G6** (1 mg, 0.651 μ mol) using core α -1,6-fucosyltransferase AgFUT6 (30 μ L, 1.05 mg/mL) and GDP-Fuc (589 μ g, 0.93 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.16 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.92 (s, 2H, H-1 $_{\alpha\text{Man}}$, H-1 $_{\alpha\text{Fuc}}$), 4.89 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.68 (d, J = 7.9 Hz, 1H), 4.57 (d, J = 8.3 Hz, 1H), 4.53 (d, J = 8.3 Hz, 1H), 4.51 (d, J = 8.0 Hz, 1H), 4.45 (d, J = 7.6 Hz, 1H), 4.27-4.25 (m, 1H, H-2 $_{\text{Man}}$), 4.18-4.16 (m, 1H, H-2 $_{\text{Man}}$), 4.14-4.12 (m, 2H), 4.04-4.00 (m, 2H), 3.94-3.37 (m, 49H), 3.26 (t, J = 11.1 Hz, 1H), 3.00 (t, J = 7.7 Hz, 2H, linker), 2.10, 2.07, 2.06, 2.04 (4xs, 12H, NHAc), 1.69-1.66 (m, 2H, linker), 1.62-1.59 (m, 2H, linker), 1.42-1.38 (m, 2H, linker), 1.24 (d, J = 6.6 Hz, 3H, CH_3_{Fuc}); $^{13}\text{C NMR}$ (126 MHz, D_2O) δ 105.2, 101.2, 101.1, 100.6, 99.7, 99.3, 99.2, 99.2, 99.1, 81.8, 79.6, 79.6, 79.4, 79.2, 77.1, 76.6, 76.3, 76.2, 75.7, 74.3, 73.9, 73.3, 73.3, 73.1, 73.0, 72.2, 71.8, 71.70, 71.1, 70.2, 69.8, 69.5, 69.4, 68.2, 68.0, 67.1, 66.8, 66.5, 66.3, 66.3, 65.5, 65.1, 64.9, 64.2,

61.7, 61.7, 60.1, 60.6, 60.3, 55.2, 55.1, 54.9, 53.5, 39.3, 28.1, 26.5, 22.2, 22.2, 22.1, 15.4; **HRMS (ESI) m/z $[M+Na]^+$** calculated for $C_{66}H_{113}N_5O_{44}Na$ 1702.6651, found 1702.6577.

Hexasaccharide G10: Following the general procedure D, compound **G10** (0.72 mg, 99%) was prepared from **G2** (548 μ g, 0.57 μ mol) using core α -1,3-fucosyltransferase CeFUT1 (26 μ L, 0.9 mg/ml), and GDP-Fuc (430 μ g, 0.68 μ mol); **^1H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-1_{Fuc}), 5.14 (s, 1H, H-1 _{α Man}), 4.84 (s, 1H, H-1 _{β Man}), 4.74-4.70 (m, 1H, H-5_{Fuc}), 4.55 (d, J = 7.9 Hz, 1H), 4.49 (t, J = 7.6 Hz, 2H), 4.26 (d, J = 3.2 Hz, 1H, H-2_{Man}), 4.06-3.39 (m, 29H), 3.26 (t, J = 11.2 Hz, 1H), 2.95 (t, J = 7.7 Hz, 2H, linker), 2.06, 2.03 (2xs, 9H, -NHAc), 1.68-1.57 (m, 4H, linker), 1.41-1.37 (m, 2H, linker), 1.28 (d, J = 6.7 Hz, 3H, CH₃_{Fuc}); **^{13}C NMR (126 MHz, D_2O) δ** 104.9, 102.2, 100.9, 100.4, 100.1, 98.4, 79.9, 78.9, 77.4, 76.2, 75.3, 75.3, 74.7, 73.9, 73.5, 73.3, 72.2, 72.0, 70.3, 70.3, 70.2, 69.9, 69.4, 69.1, 67.7, 66.8, 66.7, 66.3, 64.9, 64.7, 61.2, 61.0, 60.1, 55.7, 55.2, 39.4, 28.1, 26.9(2x), 22.3, 22.2, 15.5; **HRMS (ESI) m/z $[M+H]^+$** calculated for $C_{44}H_{77}N_3O_{29}$ 1112.4716 found 1112.4758.

Heptasaccharide G11: Following the general procedure D, compound **G11** (??? mg, 85 %) was prepared from **G3** (1.27 mg, 1.08 μ mol) using AtFucTA (50 μ L, 0.55 mg/ml) and GDP-Fuc (guanosine 5'-diphospho- β -L-fucose sodium salt) (0.8 mg, 1.27 μ mol); **^1H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-1_{Fuc}), 5.14 (d, J = 4.2 Hz, 1H, H-1 _{α Man}), 4.72 (m, 1H, H-5_{Fuc}), 4.85 (s, 1H, H-1 _{β Man}), 4.55 (d, J = 7.91 Hz, 1H), 4.53 (d, J = 8.4 Hz, 1H), 4.50 (d, J = 7.91 Hz, 1H), 4.48 (d, J = 7.7 Hz, 1H), 4.25 (d, J = 2.27 Hz, 1H, H-2_{Man}), 4.19-4.17 (m, 1H, H-2_{Man}), 4.03 (dd, J = 11.7, 5.5 Hz, 1H), 3.99 (dd, J = 10.5, 3.26 Hz, 1H), 3.94-3.43 (m, 34H), 3.38 (dd, J = 9.8, 7.9 Hz, 1H), 3.27 (t, J = 11.1 Hz, 1H), 2.91 (t, J = 7.6 Hz, 2H, linker), 2.06, 2.05, 2.03 (3xs, 9H, -NHAc), 1.66-1.57 (m, 4H, linker), 1.42-1.37 (m, 2H, linker), 1.28 (d, J = 6.9 Hz, 3H, CH₃_{Fuc}); **^{13}C NMR (126 MHz, D_2O) δ** 104.9, 101.0, 100.4, 100.1, 99.7, 99.1, 98.3, 79.9, 79.7, 79.1, 77.0, 76.6, 76.1, 75.9, 75.4, 74.7, 73.3, 73.7, 73.2, 72.2, 71.9, 70.2, 70.2, 69.8, 69.4, 69.3, 69.2, 67.8, 67.2, 66.8, 66.1, 64.9, 64.9, 61.8, 60.9, 60.5, 60.3, 60.2, 55.6, 55.3, 39.7, 28.1, 27.3, 22.2 (3x), 22.1, 15.5; **HRMS (MALDI-tof) m/z $[M+Na]^+$** calculated for $C_{52}H_{90}N_4O_{34}Na$ 1337.5329, found 1337.5347.

Octasaccharide G12: Following the general procedure D, compound **G12** (0.47 mg, 84%) was prepared from **G5** (0.56 mg, 0.38 μ mol) using core α -1,3-fucosyltransferase AtFucTA (25 μ L, 0.25 mg/mL), and GDP-Fuc (291 μ g, 0.46 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.15 (s, 1H, H-1 _{α Man}), 5.13 (d, J = 3.9 Hz, 1H, H-1_{Fuc}), 4.91 (d, J = 1.3 Hz, 1H, H1- H _{α Man}), 4.85 (s, 1H, H1 _{β Man}), 4.71-4.69 (m, 1H, H-5_{Fuc}), 4.54 (d, J = 8.3 Hz, 1H, H1- H_{GlcNAc}), 4.52 (d, J = 8.7 Hz, 1H, H1- H_{GlcNAc}), 4.49 (d, J = 8.3 Hz, 1H, H1- H_{GlcNAc}), 4.45 (d, J = 7.4 Hz, 1H, H1- H_{xyI}), 4.25 (d, J = 3.4 Hz, 1H, H2- H_{Mann}), 4.16-4.15 (m, 1H, H2- H_{Man}), 4.02 (dd, J = 11.6, 5.3 Hz, 1H), 3.98-3.95 (m, 2H), 3.93-3.83 (m, 12H), 3.81-3.80 (m, 1H), 3.80-3.72 (m, 9H), 3.68-3.43 (m, 17H), 3.37 (dd, J = 9.4, 7.8 Hz, 1H), 3.26 (t, J = 11 Hz, 1H), 2.98 (t, J = 7.8 Hz, 2H, linker), 2.05, 2.05, 2.02 (3xs, 9H, NHAc), 1.68-1.64 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.41-1.37 (m, 2H, linker), 1.27 (d, J = 6.6 Hz, 3H, CH₃ Fuc); **^{13}C NMR (201 MHz, D_2O , peaks from HSQC experiment) δ** 107.8, 103.6, 103.2, 103.0, 102.3, 102.3, 101.7, 100.9, 83.3, 81.8, 79.6, 79.2, 78.4, 78.0, 77.1, 77.1, 76.9, 76.5, 76.4, 75.9, 75.8, 75.4, 74.9, 74.7, 74.4, 73.1, 72.5, 72.4, 72.0, 71.9, 71.8, 69.8, 69.4, 69.3, 69.1, 68.0, 67.8, 67.8, 64.4, 64.4, 63.5, 63.5, 63.0, 62.6, 62.4, 58.2, 58.1, 57.7, 41.9, 30.7, 29.0, 24.8, 24.8, 24.8, 18.1; **HRMS (ESI) m/z $[M+Na+H]^2+$** calculated for $C_{58}H_{101}N_4O_{39}Na$ 851.8362, found 851.8347.

Nonasaccharide G13: Following the general procedure D, compound **G13** (0.62 mg, 99%) was prepared from **G6** (0.57 mg, 0.37 μ mol) using core α -1,3-fucosyltransferase AtFucTA (25 μ L, 0.25 mg/mL), and GDP-Fuc (267 μ g, 0.42 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.16 (s, 1H, H-1 $_{\alpha\text{Man}}$), 5.15 (d, J =3.7 Hz, 1H, H-1 $_{\text{Fuc}}$), 4.91 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.87 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.75 (m, 1H, H-5 $_{\text{Fuc}}$), 4.55 (m, 3H), 4.50 (d, J = 8.0 Hz, 1H), 4.46 (d, J = 7.6 Hz, 1H), 4.27-4.26 (m, 1H, H-2 $_{\text{Man}\beta}$), 4.17-4.16 (m, 1H, H-2 $_{\text{Man}\alpha}$), 4.12-4.11 (m, 1H, H-2 $_{\text{Man}\alpha}$), 4.05-3.43 (m, 47H), 3.40 (t, J =8.8 Hz 1H), 3.27 (t, J =11.5 Hz, 1H), 3.00 (t, J =7.8 Hz, 2H, linker), 2.07, 2.06, 2.03 (3xs, NHAc, 12H), 1.71-1.58 (m, 4H, linker), 1.43-1.37 (m, 2H, linker), 1.28 (d, J = 6.5 Hz, 3H, CH_3_{Fuc}); $^{13}\text{C NMR}$ (201 MHz, D_2O) δ : 104.7, 100.5, 100.1, 99.9, 99.3, 99.1, 98.6, 97.8, 96.5, 80.2, 78.8, 76.6, 76.0, 75.7, 75.3, 74.9, 74.8, 74.1, 74.1, 73.8, 73.3, 72.8, 72.8, 72.4, 71.8, 71.5, 71.2, 69.7, 69.7, 69.4, 69.0, 68.9, 68.8, 68.7, 68.0, 67.3, 66.7, 66.2, 65.9, 64.9, 64.5, 64.4, 61.2, 61.2, 61.1, 60.5, 60.5, 60.1, 60.1, 60.1, 59.5, 59.4, 55.1, 54.8, 54.5, 38.9, 27.6, 26.0, 21.8, 21.7, 21.6, 15.0; **HRMS (MALDI-tof) m/z** $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{66}\text{H}_{113}\text{N}_5\text{O}_{44}\text{Na}$ 1702.6651, found 1702.6577.

Heptasaccharide G14: Following the general procedure C, compound **G14** (0.63 mg, 99%) was prepared from **G3** (0.5 mg, 0.427 μ mol) using 5mU of bovine milk β -1,4-galactosyltransferase and, UDP-Gal (0.39 mg, 0.64 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.16 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.86 (s, 1H, H-1 $_{\beta\text{-Man}}$), 4.61 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 7.6 Hz, 1H, H-1 $_{\beta\text{Gal}}$), 4.51 (d, J = 8.0 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.46 (d, J = 7.7 Hz, 1H), 4.26 (d, J = 2.8 Hz, 1H, H-2 $_{\text{Man}}$), 4.18-4.17 (m, 1H, H-2 $_{\text{Man}}$), 4.04-3.98 (m, 2H), 3.94-3.43 (m, 37H), 3.40-3.36 (m, 1H) 3.26 (t, J = 11.4 Hz, 1H), 2.95 (t, J = 7.4 Hz, 2H, linker), 2.08, 2.06, 2.04 (3xs, 9H, -NHAc), 1.70-1.56 (m, 4H, linker) 1.44-1.37 (m, 2H, linker); $^{13}\text{C NMR}$ (126 MHz, D_2O) δ 105.0, 102.9, 101.3, 101.0, 100.2, 99.6, 99.2, 79.1, 78.5, 77.2, 76.5, 76.4, 75.3, 75.3, 74.7, 74.6, 74.5, 73.7, 73.3, 72.4, 71.9, 71.0, 70.2, 70.2, 70.1, 69.4, 68.5, 67.2, 66.5, 65.0, 64.9, 61.8, 61.8, 60.9, 60.3, 60.3, 60.2, 59.9, 59.9, 58.3, 55.1, 54.9, 39.4, 28.0, 26.8, 22.2, 22.1; **HRMS (ESI) m/z** $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{52}\text{H}_{90}\text{N}_4\text{O}_{35}\text{Na}$ 1353.5278, found 1353.5264.

Octasaccharide G15: Following the general procedure C, compound **G15** (0.64 mg, 94.5%) was prepared from **G5** (0.6 mg, 0.450 μ mol) applying using 94 mU of bovine milk β -1,4-galactosyltransferase and, UDP-Gal (0.59 mg, 0.98 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.16 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.93 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.88 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.61 (d, J = 7.7 Hz, 1H), 4.55 (d, J = 7.7 Hz, 1H), 4.50 (d, J = 8.0 Hz, 1H), 4.48 (d, J = 8.0 Hz, 1H), 4.45 (d, J = 7.7 Hz, 1H), 4.27 (d, J = 2.8 Hz, 1H, H-2 $_{\text{Man}}$), 4.18-4.17 (m, 1H, H-2 $_{\text{Man}}$), 4.04-3.43 (m, 46H), 3.26 (t, J =11.3 Hz, 1H), 2.96 (t, 2H, J =7.5 Hz, linker), 2.09, 2.06, 2.04 (3xs, 9H, -NHAc), 1.67-1.59 (m, 4H, linker), 1.43-1.39 (m, 2H, linker); $^{13}\text{C NMR}$ (201 MHz, D_2O , peaks from HSQC experiment) δ : 107.8, 105.5, 104.0, 103.7, 103.2, 102.3, 102.2, 101.8, 82.1, 82.0, 81.9, 81.1, 79.7, 79.1, 78.0, 77.9, 77.1, 77.1, 77.0, 76.3, 75.3, 75.0, 74.5, 73.8, 73.1, 72.8, 72.4, 71.9, 71.2, 69.8, 69.4, 69.3, 68.0, 67.6, 67.6, 64.4, 64.4, 63.6, 63.5, 62.6, 62.6, 62.6, 60.8, 57.6, 57.6, 57.5, 42.1, 30.7, 29.5, 24.9, 24.8, 24.7, 24.7; **HRMS (ESI) m/z** $[\text{M}+\text{Na}+\text{H}]^{2+}$ calculated for $\text{C}_{58}\text{H}_{101}\text{N}_4\text{O}_{40}\text{Na}$ 758.2939 found 758.2911.

Octasaccharide G16: Following the general procedure C, compound G16 (0.65 mg, 99 %) was prepared from **G4** (0.582 mg, 0.437 μmol) using 62.5 mU of bovine milk β -1,4-galactosyltransferase and UDP-Gal (Uridine 5'-diphosphogalactose disodium salt) (0.306 mg, 0.502 μmol); **^1H NMR (500 MHz, D_2O) δ** 5.14 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.92 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.89 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.61 (d, J = 8.4 Hz, 1H), 4.59 (d, J = 7.90 Hz, 1H), 4.51 (d J = 7.9 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.46 (d, J = 7.6 Hz, 1H), 4.29-4.27 (m, 1H, H-2 $_{\text{Man}}$), 4.13-4.11 (m, 1H, H-2 $_{\text{Man}}$), 4.06-4.05 (m, 1H, H-2 $_{\text{Man}}$), 4.02-3.50 (m, 45H), 3.45 (t, J = 9.5 Hz, 1H), 3.41-3.36 (m, 1H), 3.26 (t, J = 11.1 Hz, 1H), 3.00 (t, J = 7.6 Hz, 2H, linker), 2.09, 2.06, 2.04 (3xs, 9H, NHAc), 1.71– 1.65 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.44-1.38 (m, 2H, linker); **^{13}C NMR (126 MHz, D_2O) δ** 105.1, 102.9, 102.2, 101.4, 101.0, 100.6, 99.4, 96.9, 79.7, 79.4, 79.2, 78.5, 78.3, 77.3, 76.2, 75.3, 74.5, 74.4, 73.6, 73.3, 73.1, 72.9, 72.6, 72.3, 72.0, 70.9, 70.2, 70.09, 69.8, 69.9, 69.7, 69.6, 69.5, 69.2, 68.5, 66.6, 66.3, 65.5, 65.1, 65.0, 61.5, 61.4, 61.1, 60.1, 60.0, 60.0, 55.0, 54.9, 39.3, 28.0, 26.3, 22.2, 22.1, 22.1; **HRMS (ESI) m/z $[\text{M}+\text{H}]^+$** calculated for $\text{C}_{58}\text{H}_{101}\text{N}_4\text{O}_{40}$ 1493.5987, found 1493.6013.

Decasaccharide G17: Following the general procedure C, compound **G17** (0.7 mg, 99%) was prepared from **G4** (0.470 mg, 0.306 μmol) using 88mU of bovine milk β -1,4-galactosyltransferase and, UDP-Gal (0.55 mg, 0.91 μmol); **^1H NMR (800 MHz, D_2O) δ** 5.14 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.87 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.59 (dd, J = 8.6, 7.9 Hz, 2H), 4.54 (d, J = 7.9 Hz, 1H), 4.49 (d, J = 7.9 Hz, 1H), 4.47 (dd, J = 7.8, 4.7 Hz, 2H), 4.44 (d, J = 7.7 Hz, 1H), 4.26-4.25 (d, J = 3.2 Hz, 1H, H-2 $_{\text{Man}}$), 4.16-4.15 (m, 1H, H-2 $_{\text{Man}}$), 4.11-4.10 (m, 1H, H-2 $_{\text{Man}}$), 4.02-3.97 (m, 4H), 3.93-3.48 (m, 51H), 3.45-3.42 (td, J = 9.2, 2.3 Hz, 1H), 3.38-3.36 (m, 1H), 3.25 (t, J = 11.3 Hz, 1H), 2.98 (t, 2H, J = 7.7 Hz, linker), 2.08, 2.05, 2.04, 2.03 (4xs, 12H, NHAc), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.40-1.38 (m, 2H, linker); **^{13}C NMR (201 MHz, D_2O) δ** : 107.7, 105.6, 105.6, 104.0, 103.7, 103.2, 102.2, 102.1, 101.7, 99.7, 82.1, 82.05, 81.9, 81.1, 79.7, 79.1, 78.9, 78.0, 77.9, 77.9, 77.4, 77.4, 77.1, 77.0, 76.3, 76.2, 75.9, 75.5, 75.1, 74.7, 74.6, 74.4, 73.6, 73.5, 72.8, 72.7, 72.2, 72.1, 71.9, 71.9, 71.4, 71.2, 69.8, 69.1, 68.0, 67.9, 67.6, 67.6, 64.3, 64.2, 63.6, 62.9, 62.7, 62.6, 62.6, 62.6, 62.6, 62.5, 57.6, 57.6, 57.5, 41.9, 30.7, 29.0, 24.9, 24.9, 24.8, 24.7; **HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$** calculated for $\text{C}_{72}\text{H}_{123}\text{N}_5\text{O}_{50}\text{Na}$ 1880.7128, found 1880.7042.

Heptasaccharide G18: Following the general procedure E, compound **G18** (0.68 mg, 99%) was prepared from **G3** (0.59 mg, 0.50 μmol) using concentrated *P. pastoris* extract containing GalNAcT (40 μL) and UDP-GalNAc (0.39 mg, 0.60 μmol); **^1H NMR (500 MHz, D_2O) δ** 5.15 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.86 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.61 (d, J = 7.5 Hz, 1H, H-1 $_{\beta\text{GlcNAc}}$), 4.54-4.50 (m, 3H), 4.46 (d, J = 7.5 Hz, 1H, H-1 $_{\beta\text{Xyl}}$), 4.26-4.24 (m, 1H, H-2 $_{\text{Man}}$), 4.18-4.16 (m, 1H, H-2 $_{\text{Man}}$), 4.05-3.99 (dd, J = 11.4, 5.5 Hz, 1H), 3.96-3.43 (m, 38H), 3.40-3.36 (m, 1H), 3.26 (t, J = 11.1 Hz, 1H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.06, 2.04 (3xs, 9H, NHAc), 1.71-1.65 (m, 2H, linker), 1.62-1.58 (m, 2H, linker), 1.44-1.39 (m, 2H, linker); **^{13}C NMR (201 MHz, D_2O , peaks from HSQC experiment) δ** : 107.6, 104.4, 104.0, 103.7, 102.7, 102.2, 101.8, 81.9, 81.8, 81.7, 81.3, 79.8, 79.1, 78.9, 78.0, 77.9, 77.2, 77.1, 76.3, 75.9, 75.0, 74.6, 74.4, 73.3, 72.8, 72.8, 72.1, 72.0, 70.2, 69.8, 69.1, 67.6, 64.4, 64.4, 63.6, 63.0, 62.7, 62.7, 62.6, 57.8, 57.6, 57.2, 55.1, 41.9, 30.7, 29.0, 24.8, 24.7, 24.7, 24.7; **HRMS (ESI) m/z $[\text{M}+\text{H}]^+$** calculated for $\text{C}_{54}\text{H}_{94}\text{N}_5\text{O}_{35}$ 1372.5724, found 1372.5740.

Octasaccharide G19: Following the general procedure E, compound **G19** (0.5 mg, 99%) was prepared from **G4** (0.450 mg, 0.338 μ mol), using concentrated *P. pastoris* extract containing GalNAcT (75 μ L) and UDP-GalNAc (0.27 mg, 0.41 μ mol); **^1H NMR (800 MHz, D₂O) δ** 5.14 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.92 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.87 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.59 (d, J = 8.3 Hz, 1H), 4.52-4.50 (m, 2H), 4.49 (d, J = 7.9 Hz, 1H), 4.44 (d, J = 7.6 Hz, 1H), 4.25 (d, J = 3.3 Hz, 1H, H-2 $_{\text{Man}}$), 4.14 (m, 1H, H-2 $_{\text{Man}}$), 4.01 (dd, J = 11.7, 5.6 Hz, 1H), 3.99-3.98 (m, 1H), 3.97-3.54 (m, 39H), 3.52-3.49 (m, 3H), 3.43 (t, J = 9.2 Hz, 1H), 3.37 (dd, J = 9.3, 7.6 Hz, 1H), 3.25 (t, J = 11.1 Hz, 1H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.07, 2.04, 2.03 (4xs, 12H, NHAc), 1.69-1.64 (m, 2H, linker), 1.62-1.57 (m, 2H, linker), 1.40-1.37 (m, 2H); **^{13}C NMR(201 MHz, D₂O, peaks from HSQC experiment)** 105.0, 101.6, 101.2, 101.0, 100.4, 99.5, 99.4, 98.8, 82.2, 81.9, 81.9, 81.8, 79.7, 79.1, 77.9, 77.9, 77.1, 77.0, 77.0, 76.3, 75.9, 75.3, 75.1, 74.6, 74.4, 73.3, 73.1, 72.7, 72.7, 72.4, 72.2, 72.0, 71.9, 70.2, 69.8, 69.3, 68.0, 64.4, 64.4, 63.5, 63.5, 62.9, 62.7, 62.7, 57.6, 57.6, 57.2, 55.1, 41.9, 30.7, 29.0, 24.9, 24.8, 24.8, 24.8, 24.7; **HRMS (ESI) m/z [M+H]⁺** calculated for C₆₀H₁₀₄N₅O₄₀ 1534.6252, found 1534.6299.

Octasaccharide G20: Following the general procedure E, compound **G20** (0.7 mg, 99 %) was prepared from **G4** (0.61 mg, 0.458 μ mol) using concentrated *P. pastoris* extract containing GalNAcT (120 μ L) and UDP-GalNAc (Uridine 5'-diphospho-N-acetylgalactosamine disodium salt) (0.752 mg, 1.15 μ mol); **^1H NMR (500 MHz, D₂O) δ** 5.14 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.89 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.61 (d, J = 7.9 Hz, 1H), 4.58-4.55 (d, J = 7.8 Hz, 1H), 4.52 (d, J = 8.3 Hz, 1H), 4.51 (d, J = 7.8 Hz, 1H), 4.46 (d, J = 7.6 Hz, 1H), 4.28 (d, J = 3.0 Hz, 1H, H-2 $_{\text{Man}}$), 4.12-4.10 (m, 1H, H-2 $_{\text{Man}}$), 4.05 (dd, J = 3.2, 1.4 Hz, 1H, H-2 $_{\text{Man}}$), 4.03-3.58 (m, 40H), 3.53-3.49 (m, 3H), 3.45 (t, J = 9.26 Hz, 1H), 3.40-3.37 (m, 1H), 3.26 (t, J = 11.1 Hz, 1H), 3.00 (t, J = 7.7 Hz, 2H, linker), 2.10, 2.09, 2.05, 2.04 (4xs, 12H, NHAc), 1.71-1.65 (m, 2H, linker), 1.62-1.58 (m, 2H, linker), 1.44-1.38 (m, 2H, linker); **^{13}C NMR(201 MHz, D₂O, peaks from HSQC experiment) δ** : 107.6, 104.8, 104.4, 104.0, 103.7, 103.3, 102.1, 99.5, 82.3, 82.2, 81.9, 79.9, 78.8, 78.0, 78.0, 77.1, 77.0, 76.2, 75.9, 75.5, 75.0, 74.7, 74.3, 73.3, 72.9, 72.8, 72.7, 72.6, 72.2, 72.2, 71.9, 70.2, 69.9, 69.4, 69.0, 68.0, 67.6, 67.5, 64.2, 63.8, 63.7, 62.8, 62.7, 62.7, 57.6, 57.6, 57.3, 55.1, 41.9, 30.7, 29.0, 24.9, 24.9, 24.8, 24.8, 24.7; **HRMS (ESI) m/z [M+H]⁺** calculated for C₆₀H₁₀₄N₅O₄₀ 1534.6252, found 1534.6201.

Decasaccharide G21: Following the general procedure E, compound **G21** (0.70 mg, 99%) was prepared from **G6** (0.57 mg, 0.37 μ mol), using concentrated *P. pastoris* extract containing GalNAcT (150 μ L) and UDP-GalNAc (0.96 mg, 1.48 μ mol); **^1H NMR (800 MHz, D₂O) δ** 5.15 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.88 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.61 (d, J = 8.0 Hz, 1H), 4.58-4.50 (m, 5H), 4.45 (d, J = 8.1 Hz, 1H), 4.28-4.26 (m, 1H, H-2 $_{\text{Man}}$), 4.16-4.14 (m, 1H, H-2 $_{\text{Man}}$), 4.01-3.42 (m, 56H), 3.38 (s, 2H), 3.28-3.23 (m, 1H), 2.97 (t, J = 7.4 Hz, 2H), 2.10-2.07 (m, 9H, NHAc), 2.06-2.04 (m, 9H, NHAc), 1.69-1.63 (m, 2H, linker), 1.62-1.57 (m, 2H, linker), 1.42-1.38 (m, 2H, linker); **HRMS (MALDI-tof) m/z [M+Na]⁺** calculated for C₇₆H₁₂₉N₇O₅₀Na 1962.7659, found 1962.7672.

Octasaccharide G22: Following the general procedure D, compound **G22** (0.65 mg, 99%) was prepared from **G7** (0.47 mg, 0.36 μ mol) using core α -1,3-fucosyltransferase AtFucTA (25 μ L, 0.25 mg/mL), and GDP-Fuc (267 μ g, 0.42 μ mol); **^1H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-1 $_{\alpha\text{Man}}$), 5.14 (d, J = 4.1 Hz, 1H, H-1 $_{\text{Fuc } \alpha 1,3}$), 4.94 (d, J = 4.1 Hz, 1H, H-1 $_{\text{Fuc } \alpha 1,6}$), 4.84 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.74-4.72 (m, 1H, H-5 $_{\text{Fuc } \alpha 1,3}$), 4.69 (d, J = 7.9 Hz, 1H), 4.52 (d, J = 8.5 Hz, 1H), 4.49 (t, J = 8.9 Hz, 2H), 4.25 (d, J = 3.17 Hz, 1H, H-2 $_{\text{Man}}$), 4.18-4.16 (m, 2H, H-2 $_{\text{Man}}$, H-5 $_{\text{Fuc } \alpha 1,6}$), 4.03-3.46 (m, 39H), 3.40-3.35 (m, 1H), 3.27 (t, J = 11.3 Hz, 1H), 2.99 (t, J = 7.7 Hz, 2H, linker), 2.06, 2.05, 2.03 (3xs, 9H, NHAc), 1.71-1.65 (m, 2H, linker), 1.62-1.57 (m, 2H, linker), 1.41-1.36 (m, 2H, linker), 1.28 (d, J = 6.8 Hz, 3H, CH $_{3\text{Fuc } \alpha 1,3}$), 1.24 (d, J = 6.7 Hz, 3H, CH $_{3\text{Fuc } \alpha 1,6}$); **^{13}C NMR (201 MHz, D_2O) δ** 107.6, 103.6, 102.7, 102.69, 102.4, 101.8, 101.6, 100.9, 82.7, 81.8, 79.7, 79.2, 78.8, 78.5, 77.9, 77.7, 77.3, 76.6, 76.3, 76.0, 75.9, 74.8, 74.4, 72.7, 72.5, 72.1, 72.0, 71.9, 71.8, 70.7, 70.4, 69.8, 69.4, 69.1, 67.6, 67.5, 65.3, 64.4, 64.3, 63.2, 63.0, 58.3, 57.9, 57.9, 42.0, 30.7, 29.2, 24.8, 24.8, 24.8, 24.8, 18.1, 18.0; **HRMS (ESI) m/z [M+Na] $^+$** calculated for C $_{58}$ H $_{100}$ N $_4$ O $_{38}$ Na 1483.5908 found 1483.5907.

Nonasaccharide G23: Following the general procedure D, compound **G23** (0.34 mg, 91.5%) was prepared from **G8** (0.34 mg, 0.23 μ mol) using core α -1,3-fucosyltransferase AtFucTA (25 μ L, 0.25 mg/mL), and GDP-Fuc (228 μ g, 0.36 μ mol); **^1H NMR (500 MHz, D_2O) δ** 5.16 (s, 1H, H-1 $_{\alpha\text{Man}}$), 5.14 (d, J = 3.9 Hz, 1H, H-1 $_{\text{Fuc } \alpha 1,3}$), 4.94 (d, J = 3.9 Hz, 1H, H-1 $_{\text{Fuc } \alpha 1,6}$), 4.9 (d, J = 1.6 Hz, 1H, H-1 $_{\alpha\text{Man}}$), 4.87 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.74-4.72 (m, 1H, H-5 $_{\text{Fuc } \alpha 1,3}$), 4.70 (d, J = 7.9 Hz, 1H), 4.53 (d, J = 8.6 Hz, 1H), 4.50 (d, J = 8.2 Hz, 1H), 4.48 (d, J = 7.8 Hz, 1H), 4.27 (d, J = 3.1 Hz, 1H, H-2 $_{\text{Man}}$), 4.18-4.15 (m, 2H, H-2 $_{\text{Man}}$, H-5 $_{\text{Fuc } \alpha 1,6}$), 4.05-3.45 (m, 42H), 3.41-3.36 (m, 1H), 3.28 (t, J = 11.3 Hz, 1H), 3.00 (t, J = 7.5 Hz, 2H, linker), 2.07, 2.06, 2.03 (3xs, 12H, NHAc), 1.71-1.64 (m, 2H, linker), 1.63-1.58 (m, 2H, linker), 1.43-1.38 (m, 2H, linker), 1.29 (d, J = 6.5 Hz, 3H, CH $_{3\text{Fuc } \alpha 1,3}$), 1.24 (d, J = 6.7 Hz, 3H, CH $_{3\text{Fuc } \alpha 1,6}$); **^{13}C NMR (201 MHz, D_2O) δ** 107.8, 103.6, 103.2, 102.6, 102.4, 102.3, 101.8, 101.6, 100.9, 83.6, 82.05, 79.6, 79.2, 78.4, 77.9, 77.3, 77.1, 77.0, 76.6, 76.3, 76.0, 75.9, 75.8, 75.3, 75.00, 74.5, 73.1, 72.7, 72.4, 72.3, 72.1, 72.0, 71.9, 71.8, 70.7, 70.4, 70.0, 69.4, 69.4, 69.3, 69.2, 68.0, 68.1, 67.6, 67.5, 64.4, 64.3, 63.5, 63.2, 58.3, 57.9, 57.7, 42.0, 30.7, 29.3, 24.9, 24.8, 24.8, 24.8, 18.1, 18.0; **HRMS (ESI) m/z [M+Na] $^+$** calculated for C $_{64}$ H $_{110}$ N $_4$ O $_{43}$ Na 1645.6436 found 1645.6415.

Decasaccharide G24: Following the general procedure D, compound **G24** (1.64 mg, 93 %) was prepared from **G9** (1.62 mg, 0.96 μ mol) using core α -1,3-fucosyltransferase AtFucTA (30 μ L, 0.25 mg/mL), and GDP-Fuc (728 μ g, 1.15 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.15 (s, 1H, H-1 $_{\alpha\text{Man}}$), 5.13 (d, J = 3.6 Hz, 1H, H-1 $_{\text{Fuc } \alpha 1,3}$), 4.94 (d, J = 3.6 Hz, 1H, H-1 $_{\text{Fuc } \alpha 1,6}$), 4.90 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.86 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.68 (d, J = 8.3 Hz, 1H), 4.56 (d, J = 8.6 Hz, 1H), 4.51 (d, J = 8.6 Hz, 1H), 4.49-4.48 (m, 1H), 4.46 (d, J = 7.6 Hz, 1H), 4.26 (d, J = 3.2 Hz, 1H, H-2 $_{\text{Man}}$), 4.16-4.15 (m, 2H, H-2 $_{\text{Man}}$, H-5 $_{\text{Fuc } \alpha 1,6}$), 4.11-4.10 (m, 1H), 4.03-3.42 (m, 51H), 3.38 (dt, J = 9.8, 1.8 Hz, 1H), 3.27 (t, J = 11.3 Hz, 1H), 2.97-2.94 (m, 2H, linker), 2.06 (s, 3H, NHAc), 2.05 (m, 6H, NHAc), 2.02 (s, 3H, NHAc), 1.67-1.63 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.40-1.37 (m, 2H, linker), 1.27 (d, J = 6.6 Hz, 3H, CH $_{3\text{Fuc } \alpha 1,3}$), 1.23 (d, J = 6.5 Hz, 3H, CH $_{3\text{Fuc } \alpha 1,6}$); **^{13}C NMR (201 MHz, D_2O) δ** : 106.6, 102.4, 102.0, 101.4, 101.3, 100.9, 100.5, 100.4, 99.7, 98.5, 82.3, 80.8, 78.4, 77.9, 77.5, 77.2, 76.6, 76.0, 75.9, 75.8, 75.3, 75.0, 74.8, 74.7, 74.7, 74.2, 73.8, 73.3, 73.1, 71.4, 71.3, 70.9, 70.8, 70.6, 70.5, 69.9, 69.5, 69.1, 68.6, 68.2, 68.1, 67.8, 67.4, 67.4, 66.9, 66.4, 66.3, 63.1, 63.1, 62.9,

62.5, 62.4, 62.0, 62.0, 61.7, 57.0, 56.7, 56.4, 40.8, 29.5, 28.1, 23.7, 23.6, 23.6, 23.6, 23.5, 16.9, 16.8; **HRMS (ESI) m/z $[M+Na+H]^{2+}$** calculated for $C_{72}H_{123}N_5O_{48}$ 924.8651, found 924.8627.

Octasaccharide G25: Following the general procedure E, compound **G25** (0.37 mg, 99%) was prepared from **G11** (0.32 mg, 0.24 μ mol) using concentrated *P. pastoris* extract containing GalNAcT (50 μ L) and UDP-GalNAc (189 μ g, 0.291 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.14-5.13 (m, 2H, H-1 $_{\alpha Man}$, H-1 $_{Fuc}$), 4.83 (s, 1H, H-1 $_{\beta Man}$), 4.71-4.69 (m, 1H, H-5 $_{Fuc}$), 4.54 (d, 1H, J = 8.5 Hz), 4.52-4.50 (m, 2H), 4.48 (d, J = 8.2 Hz, 1H), 4.45 (d, J = 7.8 Hz, 1H), 4.25-4.23 (m, 1H, H-2 $_{Man}$), 4.15-4.14 (m, 1H, H-2 $_{Man}$), 4.00 (dd, J = 11.4, 5.6 Hz, 1H), 3.97 (dd, J = 10.5, 3.4 Hz, 1H), 3.94-3.43 (m, 40H), 3.37 (dd, J = 10.0, 7.8 Hz, 1H), 3.25 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.9 Hz, 2H, linker), 2.07, 2.04, 2.04, 2.02 (4xs, 12H), 1.69-1.65 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.26 (d, J = 6.6 Hz, 3H, CH $_{3Fuc}$); **^{13}C NMR(201 MHz, D_2O , peaks from HSQC experiment) δ :** 107.6, 104.4, 103.7, 103.1, 102.7, 102.2, 101.7, 100.9, 82.5, 81.8, 81.7, 79.7, 79.0, 78.9, 78.0, 78.0, 77.9, 77.1, 76.4, 76.4, 75.9, 74.8, 74.7, 74.4, 73.3, 72.8, 72.7, 72.1, 72.0, 71.9, 70.4, 70.2, 69.8, 69.4, 69.1, 67.6, 67.5, 64.4, 64.4, 63.6, 63.6, 63.5, 62.9, 62.7, 62.7, 62.7, 58.2, 57.9, 57.2, 55.2, 30.7, 29.0, 24.9, 24.8, 24.8, 24.7, 18.1; **HRMS (MALDI-tof) m/z $[M+Na]^+$** calculated for 1540.6122 $C_{60}H_{103}N_5O_{39}$, found 1540.6124.

Octasaccharide G26: Following the general procedure C, compound **G26** (0.83mg, 89%) was prepared from **G7** (0.830 mg, 0.631 μ mol) using 87 mU of bovine milk β -1,4-galactosyltransferase and UDP-Gal (464 μ g, 0.76 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.14 (s, 1H, H-1 $_{\alpha Man}$), 4.91 (s, 1H), 4.85 (s, 1H), 4.66 (d, J = 8.3 Hz, 1H), 4.54 (d, J = 7.6 Hz, 1H), 4.50 (d, J = 8.6 Hz, 1H), 4.45 (dd, J = 7.9, 7.7 Hz, 2H), 4.24 (s, 1H, H-2 $_{Man}$), 4.16 (s, 1H, H-2 $_{Man}$), 4.14-4.12 (m, 1H, H-5 $_{Fuc}$), 4.02-3.97 (m, 2H), 3.92-3.44 (m, 40H), 3.37 (t, J = 8.5 Hz, 1H), 3.24 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.9 Hz, 2H), 2.08, 2.05, 2.03 (3xs, 9H, NHAc), 1.68-1.65 (m, 2H, linker), 1.62-1.58 (m, 2H, linker), 1.41-1.37 (m, 2H, linker), 1.23 (d, J = 6.6 Hz, 3H, CH $_{3Fuc}$); **^{13}C NMR(201 MHz, D_2O , peaks from HSQC experiment) δ :** 106.4, 104.3, 102.5, 102.5, 101.5, 101.0, 100.8, 100.5, 80.5, 79.8, 78.5, 77.9, 77.7, 76.7, 76.6, 76.1, 75.9, 75.0, 74.9, 74.7, 73.3, 73.1, 72.9, 72.3, 71.4, 70.9, 70.8, 70.7, 69.9, 69.5, 68.6, 68.2, 67.9, 67.8, 66.3, 66.3, 63.2, 63.1, 62.4, 61.6, 61.4, 61.3, 61.3, 61.3, 56.6, 56.5, 56.4, 40.7, 29.5, 27.81, 23.7, 23.6, 23.5, 23.5, 16.8; **HRMS (MALDI-tof) m/z $[M+Na]^+$** calculated for $C_{58}H_{100}N_4O_{39}Na$ 1499.5857, found 1499.5786.

Octasaccharide G27: Following the general procedure D, compound **G27** (0.38 mg, 99%) was prepared from **G14** (0.35 mg, 0.26 μ mol) using core α -1,3-fucosyltransferase CeFUT6 (20 μ L, 1.07 mg/mL) and GDP-Fuc (202 μ g, 0.32 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.13 (s, 1H, H-1 $_{\alpha Man}$), 5.13 (d, J = 4.1 Hz, 1H, H-1 $_{Fuc}$), 4.85(s, 1H, H-1 $_{\beta Man}$), 4.84-4.82 (m, 1H, H-5 $_{Fuc}$), 4.59 (d, J = 8.1 Hz, 1H), 4.55-4.53 (m, 1H), 4.49 (d, J = 7.7 Hz, 1H), 4.44 (d, J = 8.2 Hz, 2H), 4.24 (d, J = 2.8 Hz, 1H, H-2 $_{Man}$), 4.16-4.15 (m, 1H, H-2 $_{Man}$), 4.03-3.99 (m, 2H), 3.96-3.84 (m, 14H), 3.80-3.56 (m, 21H), 3.52-3.46 (m, 4H), 3.43 (t, J = 9.3 Hz, 1H), 3.37 (dd, J = 9.5, 7.7 Hz, 1H), 3.24 (t, J = 11.1 Hz, 1H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.06, 2.04, 2.03 (3xs, 9H, NHAc), 1.69-1.64 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.42-1.37 (m, 2H, linker), 1.17 (d, J = 6.6 Hz, 3H, CH $_{3Fuc}$); **^{13}C NMR(201 MHz, D_2O , peaks from HSQC experiment) δ :** 106.4, 103.2, 102.8, 102.4, 101.3, 100.8, 100.5, 99.93, 80.7, 80.5, 80.0, 78.5, 77.8, 77.7, 76.3, 75.9, 75.8, 75.1, 74.7, 74.6, 73.8, 73.3, 73.0, 72.5, 71.5, 70.8, 70.7, 70.6, 69.8, 69.1, 68.6, 68.1, 67.8, 66.4, 66.3, 63.2, 63.1, 62.9, 61.7, 61.6, 61.5, 61.4,

61.3, 61.1, 57.1, 56.6, 56.4, 40.7, 29.4, 27.8, 23.8, 23.6, 23.5, 23.4, 16.7; **HRMS (ESI) m/z [M+H]** calculated for $C_{58}H_{101}N_4O_{39}$ 1477.6037, found 1477.6034.

Nonasaccharide G28: Following the general procedure D, compound **G28** (0.38 mg, 99%) was prepared from **G15** (0.325 mg, 0.217 μ mol) using α -1,3-fucosyltransferase CeFUT6 (15 μ L, 1.07 mg/mL) and GDP-Fuc (164 μ g, 0.26 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.13 (s, 1H, H-1 $_{\alpha Man}$), 5.12(d, J = 3.9 Hz, 1H, H-1 $_{Fuc}$), 4.92 (s, 1H, H-1 $_{\alpha Man}$), 4.87 (s, 1H, H-1 $_{\beta Man}$), 4.60 (d, J = 8.0 Hz, 1H), 4.56-4.54 (m, 1H), 4.5 (d, J = 7.8 Hz, 1H), 4.45-4.43 (m, 2H), 4.26 (d, J = 2.7 Hz, 1H, H-2 $_{Man}$), 4.16-4.15 (m, 1H, H-2 $_{Man}$), 4.13-4.09 (m, 1H), 4.02-3.48 (m, 48H), 3.44 (t, J = 9.3 Hz, 1H), 3.37 (dd, J = 9.6, 8.0 Hz, 1H), 3.25 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.8 Hz, 2H, linker), 2.08, 2.04, 2.03 (3xs, 9H, NHAc), 1.68-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.18 (d, J = 6.5 Hz, 3H, CH_3Fuc); **^{13}C NMR (201 MHz, D_2O , peaks from HSQC experiment) δ** 107.8, 104.4, 104.0, 103.7, 103.2, 102.3, 101.9, 101.7, 101.2, 82.1, 82.0, 81.9, 79.7, 79.0, 77.9, 77.9, 77.6, 77.2, 77.1, 77.0, 76.3, 75.9, 75.9, 75.3, 75.0, 75.0, 74.6, 74.6, 74.4, 73.7, 73.4, 72.7, 72.7, 72.4, 72.0, 71.9, 71.7, 70.9, 70.3, 69.8, 69.5, 69.3, 69.2, 68.2, 67.6, 67.5, 64.8, 64.1, 63.5, 62.9, 63.0, 62.3, 57.6, 57.6, 30.7, 29.1, 25.0, 24.8, 24.8, 24.7, 17.9; **HRMS (ESI) m/z [M+Na+H] $^{2+}$** calculated for $C_{64}H_{110}N_4O_{44}$ 831.3229, found 831.3253.

Nonasaccharide G29: Following the general procedure D, compound **G29** (0.26 mg, 74%) was prepared from **G16** (0.32 mg, 0.22 μ mol) using core α -1,3-fucosyltransferase CeFUT6 (15 μ L, 1.07 mg/mL) and GDP-Fuc (164 μ g, 0.26 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.14-5.12 (m, 2H, H-1 $_{\alpha Man}$, H-1 $_{Fuc\alpha-1,3}$), 4.90 (s, 1H, H-1 $_{\alpha Man}$), 4.88 (s, 1H, H-1 $_{\beta Man}$), 4.59-4.58 (m, 2H), 4.49 (d, J = 7.62 Hz, 1H), 4.44 (dd, J = 7.91, 2.8 Hz, 2H), 4.27 (d, J = 2.8 Hz, 1H, H-2 $_{Man}$), 4.11-4.10 (m, 1H), 4.04-3.54 (m, 45H), 3.51-3.47 (m, 3H), 3.44 (t, J = 9.4 Hz, 1H), 3.39-3.36 (m, 1H), 3.25 (t, J = 11.1 Hz, 1H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.04, 2.03 (3xs, 9H, NHAc), 1.68-1.63 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.40-1.38 (m, 2H, linker), 1.17 (d, J = 6.6 Hz, 3H, $CH_3Fuc\alpha-1,3$); **^{13}C NMR(201 MHz, D_2O , peaks from HSQC experiment) δ** :107.7, 104.8, 104.4, 104.0, 103.7, 103.3, 101.8, 101.2, 99.4, 82.2, 81.9, 81.9, 79.9, 78.7, 77.9, 77.9, 77.6, 77.3, 77.1, 77.0, 76.2, 75.9, 75.9, 75.5, 75.1, 75.1, 74.7, 74.6, 74.3, 73.7, 72.9, 72.7, 72.6, 72.2, 71.8, 71.1, 71.0, 70.3, 69.9, 69.4, 69.3, 69.1, 67.5, 64.1, 63.8, 63.0, 62.7, 62.7, 62.3, 62.1, 58.3, 57.6, 57.6, 41.9, 30.7, 28.9, 25.0, 24.8, 24.8, 22.6, 17.9; **HRMS (ESI) m/z [M+Na+H] $^{2+}$** calculated for $C_{64}H_{111}N_4O_{44}Na$ 831.3229, found 831.3199.

Dodecasaccharide G30: Following the general procedure D, compound **G30** (0.4 mg, 99%) was prepared from **G17** (0.36 mg, 0.19 μ mol) using α -1,3-fucosyltransferase CeFUT6 (15 μ L, 1.07 mg/mL) and GDP-Fuc (291 μ g, 0.46 μ mol); **^1H NMR (800 MHz, D_2O) δ** 5.13-5.11 (m, 3H, H-1 $_{\alpha Man}$, H-1 $_{Fuc\alpha-1,3}$, H-1 $_{Fuc\alpha-1,3}$), 4.89 (s, 1H, H-1 $_{\alpha Man}$), 4.87(s, 1H, H-1 $_{\beta Man}$), 4.84 (m, 1H H-5 $_{Fuc\alpha-1,3}$), 4.60-4.58 (m, 2H), 4.55-4.53 (m, 1H), 4.48 (d, J = 8.1 Hz, 1H), 4.45-4.43 (m, 3H), 4.25 (d, J = 3.1 Hz, 1H, H-2 $_{Man}$), 4.16-4.15 (m, 1H, H-2 $_{Man}$), 4.11-4.10 (m, 1H, H-2 $_{Man}$), 4.02-3.55 (m, 57H), 3.50-3.47 (m, 5H), 3.44-3.42 (m, 1H), 3.39-3.36 (m, 1H), 3.24 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.04, 2.04, 2.03 (4xs, 12H, NHAc), 1.68-1.64 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.17 (m, 6H, $CH_3Fuc\alpha-1,3$, $CH_3Fuc\alpha-1,3$); **^{13}C NMR(201 MHz, D_2O , peaks from HSQC experiment) δ** :108.0, 104.5, 104.4, 103.9, 103.7, 103.2, 101.9, 101.8, 101.7, 101.2, 101.2, 99.5, 82.2, 82.0, 81.9, 79.9, 79.0, 78.7, 77.9, 77.9, 77.6, 77.2, 77.1, 76.9, 76.3, 75.9, 75.9, 75.5, 75.1, 75.0, 75.0, 74.7, 74.5, 74.4, 73.7, 73.7, 72.7, 72.2, 72.2, 72.2, 72.1, 71.9, 71.8, 71.7,

71.0, 70.3, 70.3, 69.8, 69.3, 69.3, 69.1, 68.3, 68.1, 67.6, 67.6, 64.4, 64.3, 64.2, 64.1, 63.0, 62.8, 62.7, 62.7, 62.6, 62.4, 62.3, 58.3, 58.3, 57.6, 57.6, 41.9, 30.7, 29.0, 25.0, 25.0, 24.8, 24.8, 24.7, 17.9, 17.9; **HRMS (ESI) m/z [M+K]⁺** calculated for C₈₄H₁₄₃N₅O₅₇K 2172.8286, found 2172.8364.

Nonasaccharide G31: Following the general procedure D, compound **G31** (0.42 mg, 79%) was prepared from **G26** (0.480 mg, 0.324 μ mol) using α -1,3-fucosyltransferase CeFUT6 (30 μ L, 1.07 mg/mL) and GDP-Fuc (297 μ g, 0.47 μ mol); **¹H NMR (800 MHz, D₂O) δ** 5.13 (s, 1H, H-1 _{α Man}), 5.12 (d, J = 4.1 Hz, 1H, H-1_{Fuc α -1,3}), 4.90 (d, J = 3.9 Hz, 1H, H-1_{Fuc α -1,6}), 4.85 (s, 1H, H-1 _{β Man}), 4.83-4.81 (m, 1H, H-5_{Fuc α -1,3}), 4.65 (d, J = 8.0 Hz, 1H), 4.55-4.53 (m, 1H), 4.50 (d, J = 8.5, 1H), 4.44 (dd, J = 7.4, 3.9 Hz, 2H), 4.24 (d, J = 2.94 Hz, 1H, H-2_{Man β}), 4.16-4.15 (m, 1H, H-2_{Man α}), 4.12 (q, J = 6.7 Hz, 1H, H-5_{Fuc α -1,6}), 4.02-3.99 (m, 2H), 3.96-3.56 (m, 39H), 3.52-3.48 (m, 3H), 3.45 (t, J = 9.2 Hz, 1H), 3.38-3.36 (m, 1H), 3.25 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.04, 2.03 (3xs, 9H, NHAc), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.37 (m, 2H, linker), 1.22 (d, J = 6.6 Hz, 3H, CH₃_{Fuc α -1,3}), 1.17 (d, J = 6.5 Hz, 3H, CH₃_{Fuc α -1,6}); **¹³C NMR(201 MHz, D₂O) δ** : 106.4, 103.2, 102.5, 102.4, 101.5, 100.8, 100.7, 100.4, 99.9, 80.6, 80.1, 78.5, 77.8, 77.7, 76.7, 76.6, 76.0, 75.9, 75.1, 74.9, 74.7, 74.6, 73.8, 73.4, 73.3, 73.1, 72.5, 71.4, 71.4, 70.8, 70.8, 70.6, 69.7, 69.5, 69.1, 68.6, 68.2, 68.1, 67.9, 67.8, 66.3, 66.3, 63.1, 62.9, 61.6, 61.6, 61.5, 61.1, 61.0, 57.1, 56.5, 56.4, 40.6, 29.4, 27.8, 23.7, 23.6, 23.5, 23.5, 16.7, 16.7; **HRMS (ESI) m/z [M+Na]⁺** calculated for C₆₄H₁₁₀N₄O₄₃Na 1645.6436, found 1645.6499.

Dodecasaccharide G32: Following the general procedure E, compound **G32** (0.62 mg, 63%) was prepared from **G24** (0.900 mg, 0.493 μ mol) using concentrated *P. pastoris* extract containing GalNAcT (210 μ L) and UDP-GalNAc (0.32 mg, 1.97 μ mol); **¹H NMR (800 MHz, D₂O) δ** 5.15-5.13 (m, 2H, H-1 _{α Man}, H-1_{Fuc α -1,3}), 4.95, (m, 1H, H-1_{Fuc α -1,6}), 4.90 (m, 1H, H-1 _{α Man}), 4.86 (s, 1H, H-1 _{β Man}), 4.69 (m, 1H), 4.57-4.45 (m, 6H), 4.26-4.25 (m, 1H, H-2_{Man}), 4.16-4.15 (m, 2H, H-2_{Man}, H-5_{Fuc α -1,6}), 4.10-4.09 (m, 2H), 4.03-3.34 (m, 69H), 2.98 (m, 2H, linker), 2.08, 2.07, 2.05, 2.02 (4xs, 12H, NHAc), 1.69-1.64 (m, 2H, linker), 1.62-1.55 (m, 2H, linker), 1.42-1.36 (m, 2H, linker), 1.28 (d, J = 6.7 Hz, 3H, CH₃_{Fuc α -1,3}), 1.24 (d, J = 6.6 Hz, 3H, CH₃_{Fuc α -1,6}); **HRMS (ESI) m/z [M+Na]⁺** calculated for C₈₈H₁₄₉N₇O₅₈ 2254.8817, found 2254.885.

Tetradecasaccharide G33: Following the general procedure B, compound **G24** (1.18mg, 0.689mmol) was first galactosylated employing bovine GalT (35 μ L) and UDP-Gal. The galactosylated intermediate (0.714 mg, 0.331 μ mol) was purified and fucosylated using α -1,3-fucosyltransferase CeFUT6 (75 μ L, 1.07 mg/mL) and GDP-Fuc (633 μ g, 1.0 μ mol) to arrive at compound **G33** (0.8 mg, 99%); **¹H NMR (500 MHz, D₂O) δ** 5.16-5.13 (m, 4H, H-1 _{α Man}, H-1_{Fuc α -1,3}, H-1_{Fuc α -1,3}, H-1_{Fuc α -1,3}), 4.95 (d, J = 3.61 Hz, 1H), 4.91 (s, 1H, H-1 _{α Man}), 4.87-4.83 (m, 3H, H-1 _{β Man}, 2xH-5_{Fuc α -1,3/LewisX}), 4.73-4.69 (m, 1H, H-5_{Fuc α -1,3}), 4.69 (d, J = 8.0 Hz, 1H), 4.60 (d, J = 8.4 Hz, 1H), 4.56 (d, J = 7.6 Hz, 1H), 4.50 (d, J = 8.4 Hz, 1H), 4.48-4.45 (m, 3H), 4.27-4.26 (m, 1H, H-2_{Man}), 4.17-4.16 (m, 1H, H-2_{Man}), 4.11-4.10 (m, 1H, H-2_{Man}), 4.04 –3.47 (m, 70H), 3.42-3.37 (m, 1H), 3.28 (t, J = 10.8 Hz, 1H), 3.01 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.05, 2.03 (3xs, 12H, NHAc), 1.70-1.57 (m, 4H, linker), 1.43-1.38 (m, 2H, linker), 1.29 (d, J = 6.54 Hz, 3H, CH₃_{Fuc α -1,3}), 1.24 (d, J = 6.54 Hz, 3H, CH₃_{Fuc α -1,6}), 1.17 (m, 6H, 2xCH₃_{Fuc/LewisX}); **HRMS (ESI) m/z [M+H]⁺** calculated for C₉₆H₁₆₄N₅O₆₆ 1232.9759, found 1232.9803.

Hexasaccharide G34: Following the general procedure F, compound G34 (0.67 mg, 99 %) was prepared from **G6** (0.924 mg, 0.602 μ mol) using 1.4 U of Jack bean β -N-acetylglucosaminidase; ^1H NMR (800 MHz, D_2O) δ 5.12 (d, J = 1.6 Hz, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (d, J = 1.8 Hz, 1H, H-1 $_{\alpha\text{Man}}$), 4.87 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.60 (d, J = 8.2 Hz, 1H, H-1 $_{\text{GlcNAc}}$), 4.50 (d, J = 8.0 Hz, 1H, H-1 $_{\text{GlcNAc}}$), 4.45 (d, J = 7.7, 1H, H-1 $_{\text{Xyl}}$), 4.27 (d, J = 3.1 Hz, 1H, H-2 $_{\text{Man}\beta}$), 4.04 (dd, J = 3.38 Hz, J = 1.6 Hz, 1H, H-2 $_{\text{Man}\alpha}$), 4.01 (dd, J = 11.62 Hz, J = 5.4 Hz, 1H), 4.00-3.94 (m, 3H), 3.92-3.58 (m, 27H), 3.51-3.49 (m, 1H), 3.44 (td, J = 9.2 Hz, J = 2.3 Hz, 1H), 3.39-3.37 (m, 1H), 3.25 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.9 Hz, 2H, linker), 2.08, 2.03 (2xs, 6H, NHAc), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker); ^{13}C NMR (201 MHz, D_2O) δ 107.7, 104.8, 103.9, 103.7, 103.2, 102.2, 82.2, 81.9, 81.9, 79.9, 77.9, 77.2, 77.1, 76.1, 75.9, 75.2, 75.0, 74.7, 73.1, 72.6, 72.4, 72.2, 71.9, 69.1, 68.0, 67.6, 67.5, 63.9, 63.8, 63.5, 63.5, 62.8, 62.7, 62.7, 57.6, 57.6, 41.9, 30.7, 28.9, 24.8, 24.7, 24.7; HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{44}\text{H}_{77}\text{N}_3\text{O}_{30}\text{Na}$ 1150.4484, found 1150.4433.

Hexasaccharide G35: Following the general procedure F, compound G35 (1 mg, 90%) was prepared from **G7** (1.3 mg, 0.99 μ mol) using 0.5 U of jack bean β -N-acetylglucosaminidase; ^1H NMR (500 MHz, D_2O) δ 5.14 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.92 (d, J = 3.9 Hz, 1H, H-1 $_{\alpha\text{Fuc}}$), 4.87 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.67 (d, J = 7.7 Hz, 1H), 4.51 (d, J = 7.7 Hz, 1H), 4.47 (d, J = 7.9 Hz, 1H), 4.25 (d, J = 3.1 Hz, 1H, H-2 $_{\text{Man}}$), 4.14 (dd, J = 13.4 Hz, J = 6.22 Hz, 1H, H-5 $_{\text{Fuc}}$), 4.06 (dd, J = 3.3, 1.5 Hz, 1H, H-2 $_{\text{Man}}$), 4.04-3.99 (m, 2H), 3.94-3.58 (m, 20H), 3.51-3.45 (m, 2H), 3.41-3.36 (m, 1H), 3.26 (t, J = 11.4 Hz, 1H), 3.00 (t, J = 7.6 Hz, 2H, linker), 2.09, 2.05 (2xs, 6H, NHAc), 1.71-1.67 (m, 2H, linker), 1.64-1.58 (m, 2H, linker), 1.44-1.39 (m, 2H, linker), 1.24 (d, J = 6.7 Hz, 3H, CH_3_{Fuc}); ^{13}C NMR (126 MHz, D_2O) δ 104.9, 102.2, 101.2, 101.1, 100.1, 99.4, 79.1, 78.7, 77.3, 75.4, 74.5, 73.5, 73.3, 72.4, 72.3, 72.0, 71.8, 71.7, 70.3, 70.0, 69.9, 69.6, 69.1, 68.1, 66.8, 66.7, 66.5, 66.43, 64.90, 64.87, 61.7, 60.2, 60.2, 55.1, 55.1, 39.3, 28.1, 26.4, 22.2, 22.1, 22.1, 15.3; HRMS (ESI) m/z $[\text{M}+\text{H}]^+$ calculated for $\text{C}_{45}\text{H}_{77}\text{N}_3\text{O}_{29}$ 1112.4716, found 1112.4758.

Heptasaccharide G37: Following the general procedure D, compound **G37** (0.43 mg, 99%) was prepared from **G34** (0.34 mg, 0.30 μ mol) using core α -1,3-fucosyltransferase CeFUT1 (20 μ L, 0.9 mg/mL), and GDP-Fuc (272 μ g, 0.43 μ mol); ^1H NMR (800 MHz, D_2O) δ 5.13 (d, J = 3.7 Hz, 1H, H-1 $_{\text{Fuc}}$), 5.12 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.91 (s, 1H, H-1 $_{\alpha\text{Man}}$), 4.85 (s, 1H, H-1 $_{\beta\text{Man}}$), 4.72-4.70 (m, 1H, H-5 $_{\text{Fuc}}$), 4.54 (d, J = 8.3 Hz, 1H), 4.49-4.46 (m, 2H), 4.26 (d, J = 3.1 Hz, 1H, H-2 $_{\text{Man}\beta}$), 4.04-4.03 (m, 1H, H-2 $_{\text{Man}\alpha}$), 4.03-3.44 (m, 36H), 3.39-3.37 (m, 1H), 3.26 (t, J = 11.0 Hz, 1H), 2.98 (t, J = 7.8 Hz, 2H, linker), 2.05, 2.02 (2xs, 6H, NHAc), 1.69-1.65 (m, 2H, linker), 1.59-1.57 (m, 2H, linker), 1.41-1.38 (m, 2H, linker), 1.27 (d, J = 6.7 Hz, 3H, CH_3_{Fuc}); ^{13}C NMR (201 MHz, D_2O , peaks from HSQC experiment) δ : 106.4, 103.7, 102.4, 101.9, 101.8, 101.1, 99.7, 82.1, 80.6, 78.7, 76.7, 76.7, 75.9, 75.9, 75.7, 75.2, 74.9, 74.7, 74.0, 73.7, 73.4, 73.1, 71.7, 71.7, 71.3, 71.2, 70.7, 70.5, 69.1, 68.2, 68.1, 67.9, 66.6, 66.4, 62.6, 62.4, 62.3, 62.3, 61.7, 57.0, 56.5, 40.7, 29.5, 27.8, 23.6, 23.5, 23.6, 16.8; HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{50}\text{H}_{87}\text{N}_3\text{O}_{34}$ 1296.5063, found 1296.512.

Heptasaccharide G38: Following the general procedure D, compound **G38** (0.57 mg, 99%) was prepared from **G35** (0.46 mg, 0.41 μ mol) using core α -1,3-fucosyltransferase CeFUT1 (20 μ L, 0.9 mg/mL) and GDP-Fuc (310 μ g, 0.49 μ mol); $^1\text{H NMR}$ (800 MHz, D_2O) δ 5.13 (d, J = 3.8 Hz, 1H, H-1_{Fuc α 1,3}), 5.12 (s, 1H, H-1 _{α Man}), 4.93 (d, J = 3.8 Hz, 1H, H-1_{Fuc α 1,6}), 4.91 (s, 1H, H-1 _{α Man}), 4.85 (s, 1H, H-1 _{β Man}), 4.73-4.71 (m, 1H, H-5_{Fuc α 1,3}), 4.69 (d, J = 8.6 Hz, 1H), 4.48 (d, J = 8.9 Hz, 1H), 4.47 (d, J = 7.6 Hz, 1H), 4.26 (d, J = 3.1 Hz, 1H, H-2_{Man β}), 4.15 (q, J = 6.8 Hz, 1H, H-5_{Fuc α 1,6}), 4.04 (dd, J = 3.4, 1.4 Hz, 1H, H-2_{Man α}), 3.99-3.55 (m, 37H), 3.49 (t, J = 9.2 Hz, 1H), 3.46 (t, J = 8.9 Hz, 1H), 3.38 (dd, J = 9.4, 7.5 Hz, 1H), 3.27 (t, J = 11.3 Hz, 1H), 2.98 (t, J = 7.9 Hz, 2H, linker), 2.06, 2.02 (2xs, 6H, NHAc), 1.68-1.64 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.40-1.36 (m, 2H, linker), 1.28 (d, J = 6.6 Hz, 3H, CH₃Fuc α 1,3), 1.23 (d, J = 6.6 Hz, 3H, CH₃Fuc α 1,6); $^{13}\text{C NMR}$ (201 MHz, D_2O , peaks from HSQC experiment) δ : 104.5, 101.9, 100.6, 99.6, 99.6, 98.0, 97.9, 79.6, 78.7, 76.8, 75.8, 74.8, 74.3, 74.2, 73.6, 73.0, 72.9, 71.5, 71.3, 69.8, 69.6, 69.5, 69.5, 69.1, 68.7, 67.6, 66.5, 66.4, 65.7, 65.3, 64.9, 64.4, 64.4, 60.7, 59.9, 59.9, 58.7, 56.7, 55.3, 55.0, 38.9, 27.7, 26.1, 21.8, 21.7, 21.7, 15.1, 14.9; **HRMS (ESI) m/z** [$\text{M}+\text{H}$] $^+$ calculated for $\text{C}_{50}\text{H}_{88}\text{N}_3\text{O}_{33}$ 1258.5295, found 1258.5330.

Octasaccharide G39: Following the general procedure D, compound **G39** (0.36 mg, 99%) was prepared from **G36** (0.32 mg, 0.26 μ mol) using core α -1,3-fucosyltransferase CeFUT1 (20 μ L, 0.9 mg/mL) and GDP-Fuc (190 μ g, 0.31 μ mol); $^1\text{H NMR}$ (800 MHz, D_2O) δ 5.13 (d, J = 3.8 Hz, 1H, H-1_{Fuc α 1,3}), 5.12 (s, 1H, H-1 _{α Man}), 4.93 (d, J = 3.8 Hz, 1H, H-1_{Fuc α 1,6}), 4.91 (s, 1H, H-1 _{α Man}), 4.85 (s, 1H, H-1 _{β Man}), 4.73-4.71 (m, 1H, H-5_{Fuc α 1,3}), 4.69 (d, J = 8.6 Hz, 1H), 4.48 (d, J = 8.9 Hz, 1H), 4.47 (d, J = 7.6 Hz, 1H), 4.26 (d, J = 3.1 Hz, 1H, H-2_{Man β}), 4.15 (q, J = 6.8 Hz, 1H, H-5_{Fuc α 1,6}), 4.04 (dd, J = 3.4, 1.4 Hz, 1H, H-2_{Man α}), 3.99-3.55 (m, 37H), 3.49 (t, J = 9.2 Hz, 1H), 3.46 (t, J = 8.9 Hz, 1H), 3.38 (dd, J = 9.4, 7.5 Hz, 1H), 3.27 (t, J = 11.3 Hz, 1H), 2.98 (t, J = 7.9 Hz, 2H, linker), 2.06, 2.02 (2xs, 6H, NHAc), 1.68-1.64 (m, 2H, linker), 1.60-1.57 (m, 2H, linker), 1.40-1.36 (m, 2H, linker), 1.28 (d, J = 6.6 Hz, 3H, CH₃Fuc α 1,3), 1.23 (d, J = 6.6 Hz, 3H, CH₃Fuc α 1,6); $^{13}\text{C NMR}$ (201 MHz, D_2O) δ : 106.5, 103.6, 102.4, 102.0, 101.4, 101.1, 100.34, 99.71, 82.3, 80.8, 78.6, 76.6, 76.0, 75.9, 75.8, 75.4, 74.9, 74.8, 74.7, 74.1, 73.7, 73.3, 71.8, 71.3, 71.3, 71.2, 70.9, 70.9, 70.6, 70.5, 69.4, 68.2, 68.2, 68.1, 67.8, 67.5, 66.8, 66.8, 66.3, 62.6, 62.6, 62.4, 62.3, 62.3, 56.9, 56.4, 40.7, 29.5, 27.8, 23.6, 23.6, 23.5, 16.9, 16.7; **HRMS (ESI) m/z** [$\text{M}+\text{H}$] $^+$ calculated for $\text{C}_{56}\text{H}_{98}\text{N}_3\text{O}_{38}$ 1420.5823, found 1420.5786.

Nonasaccharide G63: Following the general procedure E, compound **G63** (1.30 mg, 99%) was prepared from **G50** (1.07 mg, 0.76 μ mol) using concentrated extract of *P. pastoris* containing GalNacT (260 μ L) and UDP-GalNAc (1.88 mg, 2.89 μ mol); $^1\text{H NMR}$ (800 MHz, D_2O) δ 5.11 (s, 1H, H-1 _{α Man}), 4.92 (s, 1H, H-1 _{α Man}), 4.76 (s, 1H, H-1 _{β Man}), 4.60 (d, J = 8.2 Hz, 1H), 4.57-4.55 (m, 2H), 4.52 (dd, J = 8.9, 6.7 Hz, 2H), 4.50 (d, J = 7.9 Hz, 1H), 4.24 (d, J = 2.9 Hz, 1H, H-2_{Man}), 4.18 (dd, J = 3.5, 1.6 Hz, 1H, H-2_{Man}), 4.09 (dd, J = 3.5, 1.6 Hz, 1H), 3.95-3.57 (m, 47H), 3.51-3.46 (m, 6H), 2.98 (t, J = 7.7 Hz, 2H, linker), 2.08, 2.07, 2.07, 2.05, 2.04, 2.03 (6xs, 18H, NHAc), 1.69-1.65 (m, 2H, linker), 1.61-1.57 (m, 2H, linker), 1.40-1.38 (m, 2H, linker); $^{13}\text{C NMR}$ (201 MHz, D_2O) δ : 104.4, 104.4, 104.0, 103.7, 103.1, 102.2, 102.1, 102.0, 99.7, 83.1, 82.1, 82.0, 81.9, 81.9, 79.0, 79.0, 78.0, 77.9, 77.5, 77.1, 77.0, 76.2, 75.4, 75.2, 75.0, 74.7, 73.3, 73.2, 73.0, 72.8, 72.8, 72.7, 72.7, 72.1, 72.1, 70.2, 69.6, 69.5, 68.4, 68.3, 64.3, 64.3, 63.7, 63.6, 63.6, 62.7, 62.7, 62.6, 62.6, 57.6, 57.6, 57.2, 57.2, 55.2, 55.1, 41.9, 30.7, 29.0, 24.9, 24.9, 24.8, 24.8, 24.8, 24.8, 24.7; **HRMS (ESI) m/z** [$\text{M}+\text{H}$] $^+$ calculated for $\text{C}_{71}\text{H}_{122}\text{N}_7\text{O}_{46}$ 1808.7417, found 1808.7428.

Hexasaccharide G64: Following the general procedure F, compound **G64** (300 µg, 93%) was prepared from **G66** (377 µg, 0.28 µmol) using 50 mU of jack bean β-N-acetylglucosaminidase; ¹H NMR (500 MHz, D₂O) δ 5.12 (d, *J* = 1.7 Hz, 1H), 4.93 (d, *J* = 1.6 Hz, 1H), 4.91 (d, *J* = 4.0 Hz, 1H), 4.68 (d, *J* = 7.9 Hz, 1H), 4.51 (d, *J* = 8.1 Hz, 1H), 4.27 (d, *J* = 1.9 Hz, 1H), 4.14 (q, *J* = 6.8 Hz, 1H), 4.08 (dd, *J* = 3.4, 1.5 Hz, 1H), 3.98 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.97-3.56 (m, 30H), 3.05-2.94 (m, 2H, linker), 2.11 (s, 3H, NHAc), 2.04 (s, 3H, NHAc), 1.73-1.55 (m, 4H, linker), 1.47-1.35 (m, 2H, linker), 1.24 (d, *J* = 6.6 Hz, 3H); HRMS (MALDI-tof) *m/z* [M+Na]⁺ calculated for C₄₅H₇₉N₃NaO₃₀ 1164.4646, found 1164.4664.

Heptasaccharide G66: Following the general procedure D, compound **G66** (580 µg, 86%) was prepared from **G49** (700 µg, 0.58 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (380 µg, 0.60 µmol); ¹H NMR (500 MHz, D₂O) δ 5.12 (s, 1H), 4.92 (d, *J* = 1.5 Hz, 1H), 4.90 (d, *J* = 3.7 Hz, 1H), 4.67 (d, *J* = 8.0 Hz, 1H), 4.56 (d, *J* = 8.5 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.26 (d, *J* = 1.9 Hz, 1H), 4.19 (dd, *J* = 3.3, 1.4 Hz, 1H), 4.13 (q, *J* = 6.6 Hz, 1H), 3.98 (dd, *J* = 3.4, 1.7 Hz, 1H), 3.96-3.40 (m), 3.02-2.93 (m, 2H, linker), 2.10 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 1.74-1.53 (m, 4H), 1.47-1.34 (m, 2H), 1.23 (d, *J* = 6.6 Hz, 3H); HRMS (MALDI-tof) *m/z* [M+Na]⁺ calculated for C₅₃H₉₂N₄NaO₃₅ 1367.5440, found 1367.5493.

Octasaccharide G68: Following the general procedure D, compound **G68** (750 µg, 79%) was prepared from **G57** (860 µg, 0.63 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (437 µg, 0.69 µmol); ¹H NMR (500 MHz, D₂O) δ 5.12 (s, 1H), 4.95-4.88 (m, 2H), 4.67 (d, *J* = 8.1 Hz, 1H), 4.56 (d, *J* = 8.4 Hz, 1H), 4.49 (d, *J* = 8.0 Hz, 1H), 4.26 (s, 1H), 4.20 (d, *J* = 3.1 Hz, 1H), 4.12 (q, *J* = 6.8 Hz, 1H), 4.06-3.41 (m, 16H), 3.05-2.91 (m, 2H, linker), 2.09 (s, 3H, NHAc), 2.06 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.74-1.51 (m, 4H, linker), 1.47-1.31 (m, 2H, linker), 1.23 (d, *J* = 6.6 Hz, 3H, CH₃Fuc1α,6); HRMS (MALDI-tof) *m/z* [M+Na]⁺ calculated for C₅₉H₁₀₂N₄NaO₄₀ 1529.5968, found 1529.5904.

Octasaccharide G69: Following the general procedure D, compound **G69** (800 µg, 91%) was prepared from **G56** (788 µg, 0.58 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (405 µg, 0.64 µmol); ¹H NMR (500 MHz, D₂O) δ 5.13 (d, *J* = 0.6 Hz, 1H), 5.11 (d, *J* = 1.2 Hz, 1H), 4.95-4.87 (m, 2H), 4.67 (d, *J* = 8.1 Hz, 1H), 4.57 (d, *J* = 8.4 Hz, 1H), 4.50 (d, *J* = 7.9 Hz, 1H), 4.26 (d, *J* = 0.6 Hz, 1H), 4.20 (s, 1H), 4.15 (dd, *J* = 3.1, 1.9 Hz, 1H), 4.08 (dd, *J* = 3.4, 1.4 Hz, 1H), 4.04-3.39 (m, 21H), 3.02-2.96 (m, 2H, linker), 2.09 (s, 3H, NHAc), 2.07 (s, 3H, NHAc), 2.04 (s, 3H, NHAc), 1.75-1.52 (m, 4H, linker), 1.47-1.34 (m, 2H, linker), 1.24 (d, *J* = 6.6 Hz, 3H, CH₃Fucα1,6); HRMS (MALDI-tof) *m/z* [M+Na]⁺ calculated for C₅₉H₁₀₂N₄NaO₄₀ 1529.5968, found 1529.5942.

Heptasaccharide G71: Following the general procedure D, compound **G71** (650 µg, 99%) was prepared from **G47** (600 µg, 0.48 µmol) using core α-1,6-fucosyltransferase AgFUT6 (16.5 µg, 1.05 mg/mL) and GDP-Fuc (336 µg, 0.53 µmol); ¹H NMR (500 MHz, D₂O) δ 5.13 (s, 1H), 4.92 (d, *J* = 3.5 Hz, 1H), 4.67 (d, *J* = 7.5 Hz, 3H), 4.57-4.48 (m, 3H), 4.25-4.19 (m, 2H), 4.13 (q, *J* = 6.4 Hz, 1H), 4.05 (dd, *J* = 9.2, 3.2 Hz, 1H), 3.98-3.39 (m, 45H), 3.04-2.94 (m, 2H, linker), 2.09 (s, 3H), 2.09 (s, 3H, NHAc), 2.06 (s, 3H, NHAc), 2.04 (s, 3H, NHAc), 1.71-1.56 (m, 4H, linker), 1.47-1.36 (m, 2H, linker), 1.24 (d, *J* = 6.5 Hz, 3H, CH₃Fucα1,6); HRMS (MALDI-tof) *m/z* [M+Na]⁺ calculated for C₅₅H₉₅N₅NaO₃₅ 1408.5705, found 1408.5769.

Pentasaccharide G72: Following the general procedure D, compound **G72** (1.44 mg, 1.47 μ mol, 98%) was prepared from **G41** (1.27 mg, 1.50 μ mol) using core α -1,3-fucosyltransferase CeFUT1 (27 μ g), and GDP-Fuc (1.0 mg, 1.7 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.11 (d, J = 3.5 Hz, 1H), 5.08 (s, 1H), 4.77 (s, 1H), 4.72 (dd, J = 10.9, 5.2 Hz, 1H), 4.58 (d, J = 7.1 Hz, 1H), 4.53 (d, J = 7.0 Hz, 1H), 4.30 (d, J = 2.6 Hz, 1H), 4.16 (dd, J = 2.9, 1.4 Hz, 1H), 4.10-3.58 (m), 3.23-3.15 (m, 2H), 2.36 (s, 3H, NHAc), 2.34 (s, 3H, NHAc), 2.09-1.87 (m, 4H, linker), 1.84-1.72 (m, 2H, linker), 1.67 (d, J = 5.9 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1-3}$); **HRMS (MALDI-tof)** m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{39}\text{H}_{69}\text{N}_3\text{NaO}_{25}$ 1002.4118, found 1002.4063.

Pentasaccharide G73: Following the general procedure D, compound **G73** (365 μ g, 0.32 μ mol, 99%) was prepared from **G42** (320 μ g, 0.32 μ mol) using core α -1,3-fucosyltransferase (CeFUT1, 15 μ g) and GDP-Fuc (222 μ g, 0.35 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.14 (d, J = 4.0 Hz, 1H), 5.10 (d, J = 1.0 Hz, 1H), 4.54 (d, J = 7.6 Hz, 1H), 4.49 (d, J = 7.9 Hz, 2H), 4.26 (d, J = 1.4 Hz, 1H), 4.07 (s, 1H), 4.02-3.41 (m), 3.05-2.92 (m, 2H, linker), 2.06 (s, 3H, NHAc), 2.02 (s, 3H, NHAc), 1.74-1.51 (m, 4H, linker), 1.48-1.35 (m, 2H, linker), 1.28 (d, J = 6.3 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1-3}$); **HRMS (MALDI-tof)** m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{45}\text{H}_{79}\text{N}_3\text{NaO}_{30}$ 1164.4646, found 1164.4701.

Nonasaccharide G74: Following the general procedure F, compound **G74** (340 μ g, 84%) was prepared from **G77** (453 μ g, 0.25 μ mol) using 50 mU of jack bean β -N-acetylglucosaminidase; $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.15 (d, J = 3.7 Hz, 1H), 5.11 (s, 1H), 5.08 (s, 1H), 4.94 (d, J = 3.8 Hz, 1H), 4.92 (s, 1H), 4.88 (s, 2H), 4.67 (d, J = 8.0 Hz, 1H), 4.50 (d, J = 8.0 Hz, 1H), 4.27 (d, J = 2.7 Hz, 1H), 4.20-4.13 (m, 1H), 4.12-3.53 (m, 50H), 3.04-2.95 (m, 2H, linker), 2.07 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.73-1.55 (m, 4H, linker), 1.45-1.36 (m, 2H, linker), 1.30 (d, J = 6.7 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1,3}$), 1.24 (d, J = 6.5 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1,6}$); **HRMS (MALDI-tof)** m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{63}\text{H}_{109}\text{N}_3\text{NaO}_{44}$ 1634.6282, found 1634.6301.

Heptasaccharide G75: Following the general procedure D, compound **G75** (190 μ g, 99%) was prepared from **G64** (171 μ g, 0.15 μ mol) using core α -1,3-fucosyltransferase (CeFUT1, 18 μ g) and GDP-Fuc (101 μ g, 0.16 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.14 (d, J = 3.8 Hz, 1H), 5.11 (s, 1H), 4.97-4.91 (m, 2H), 4.55 (d, J = 7.3 Hz, 1H), 4.49 (d, J = 8.1 Hz, 1H), 4.26 (d, J = 0.8 Hz, 1H), 4.15 (dd, J = 13.0, 6.6 Hz, 1H), 4.09-4.06 (m, 1H), 4.05-3.53 (m, 40H), 3.48-3.42 (m, 1H), 2.07 (s, 3H, NHAc), 2.02 (s, 3H, NHAc), 1.75-1.52 (m, 4H, linker), 1.48-1.35 (m, 2H, linker), 1.29 (d, J = 6.6 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1,3}$), 1.23 (d, J = 6.7 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1,6}$); **HRMS (MALDI-tof)** m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{51}\text{H}_{89}\text{N}_3\text{NaO}_{34}$ 1310.5225, found 1310.5254.

Nonasaccharide G76: Following the general procedure D, compound **G76** (410 μ g, 98%) was prepared from **G68** (376 μ g, 0.25 μ mol) using core α -1,3-fucosyltransferase AtFucTA (11 μ g) and GDP-Fuc (171 μ g, 0.27 μ mol); $^1\text{H NMR}$ (500 MHz, D_2O) δ 5.15-5.13 (m, 2H), 4.94 (d, J = 3.5 Hz, 1H), 4.92 (s, 1H), 4.91 (s, 1H), 4.69 (d, J = 7.8 Hz, 1H), 4.57 (d, J = 8.2 Hz, 1H), 4.50 (d, J = 7.5 Hz, 1H), 4.27 (s, 1H), 4.24-4.19 (q, J = 6.6 Hz, 1H), 4.19-4.12 (m, 1H), 4.07-3.41 (m, 26H), 3.07-2.93 (m, 2H, linker), 2.08 (s, 3H, NHAc), 2.07 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.73-1.54 (m, 4H, linker), 1.46-1.36 (m, 4H, linker), 1.29 (d, J = 6.6 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1,3}$), 1.24 (d, J = 6.6 Hz, 3H, $\text{CH}_3\text{Fuc}\alpha_{1,6}$); **HRMS (MALDI-tof)** m/z $[\text{M}+\text{Na}]^+$ calculated for $\text{C}_{65}\text{H}_{112}\text{N}_4\text{NaO}_{44}$ 1675.6547, found 1675.6625.

Decasaccharide G77: Following the general procedure D, compound **G77** (640 μ g, 77%) was prepared from **G55** (700 μ g, 0.46 μ mol) using core α -1,6-fucosyltransferase AgFUT6 (16.5 μ g, 1.05 mg/mL), and GDP-Fuc (316 μ g, 0.50 μ mol). After complete conversion of starting material GDP-Fuc (316 μ g, 0.50 μ mol) and AtFucTA (16 μ g, 0.37 mg/mL) were added and the resulting mixture was incubated at room temperature for 48 hours; **$^1\text{H NMR}$ (500 MHz, D_2O) δ** 5.14 (d, J = 4.1 Hz, 1H), 5.12 (s, 1H), 5.07 (d, J = 1.2 Hz, 1H), 4.93 (d, J = 3.8 Hz, 1H), 4.91 (s, 1H), 4.87 (s, 1H), 4.66 (d, J = 7.0 Hz, 1H), 4.57-4.55 (m, 2H), 4.49 (d, J = 7.9 Hz, 1H), 4.25-4.24 (m, 1H), 4.21-4.20 (m, 2H), 4.15-4.14 (m, 2H), 4.30-3.33 (m, 78H), 3.00-2.96 (m, 2H), 2.06 (s, 3H, NHAc), 2.06 (s, 3H, NHAc), 2.02 (s, 3H, NHAc), 1.75-1.53 (m, 4H, linker), 1.44-1.36 (m, 2H, linker), 1.29 (d, J = 6.5 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1-3}$), 1.23 (d, J = 6.5 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1-6}$); **HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$** calculated for $\text{C}_{71}\text{H}_{122}\text{N}_4\text{NaO}_{49}$ 1837.7075, found 1837.7139.

Nonasaccharide G78: Following the general procedure D, compound **G78** (400 μ g, 92%) was prepared from **G69** (376 μ g, 0.25 μ mol) using core α -1,3-fucosyltransferase AtFucTA (11 μ g) and GDP-Fuc (171 μ g, 0.27 μ mol); **$^1\text{H NMR}$ (500 MHz, D_2O) δ** 5.15-5.12 (m, 2H), 5.09 (s, 1H), 4.94 (d, J = 3.7 Hz, 1H), 4.90 (s, 1H), 4.67 (d, J = 8.0 Hz, 1H), 4.57 (d, J = 8.2 Hz, 1H), 4.50 (d, J = 7.8 Hz, 1H), 4.26 (d, J = 2.6 Hz, 1H), 4.21 (d, J = 2.4 Hz, 1H), 4.17-4.13 (m, 2H), 4.11-3.42 (m, 74H), 3.05-2.95 (m, 2H, linker), 2.07 (s, 6H, NHAc), 2.03 (s, 3H, NHAc), 1.77-1.51 (m, 4H, linker), 1.49-1.34 (m, 2H, linker), 1.30 (d, J = 6.6 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1,3}$), 1.24 (d, J = 6.6 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1,6}$); **HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$** calculated for $\text{C}_{65}\text{H}_{112}\text{N}_4\text{NaO}_{44}$ 1675.6547, found 1675.6541.

Octasaccharide G79: Following the general procedure D, compound **G79** (355 μ g, 92%) was prepared from **G71** (346 μ g, 0.25 μ mol) using core α -1,3-fucosyltransferase (AtFucTA, 11 μ g) and GDP-Fuc (171 μ g, 0.27 μ mol); **$^1\text{H NMR}$ (500 MHz, D_2O) δ** 5.16-5.10 (m, 2H), 4.95 (d, J = 3.8 Hz, 1H), 4.68 (d, J = 8.3 Hz, 1H), 4.57-4.52 (m, 2H), 4.50 (d, J = 7.8 Hz, 1H), 4.24-4.19 (m, 2H), 4.19-4.13 (m, 1H), 4.09-3.42 (m, 19H), 3.05-2.96 (m, 2H), 2.10 (s, 3H, NHAc), 2.07 (s, 3H, NHAc), 2.06 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.73-1.54 (m, 4H, linker), 1.46-1.33 (m, 2H, linker), 1.28 (d, J = 6.6 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1,3}$), 1.24 (d, J = 6.5 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1,6}$); **HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$** calculated for $\text{C}_{61}\text{H}_{105}\text{N}_5\text{NaO}_{39}$ 1554.6284, found 1554.6315.

Pentasaccharide G81: Following the general procedure D, compound **G81** (950 μ g, 91%) was prepared from **G41** (894 μ g, 1.07 μ mol) using core α -1,3-fucosyltransferase (CeFUT6, 24 μ g) and GDP-Fuc (760 μ g, 1.20 μ mol); **$^1\text{H NMR}$ (500 MHz, D_2O) δ** 5.22 (d, J = 3.9 Hz, 1H), 5.09 (d, J = 1.2 Hz, 1H), 4.71 (s, 1H), 4.66 (q, J = 6.6 Hz, 1H), 4.62 (d, J = 7.9 Hz, 1H), 4.50 (d, J = 8.0 Hz, 1H), 4.21 (d, J = 3.3 Hz, 1H), 4.08 (dd, J = 3.4, 1.6 Hz, 1H), 4.06-3.56 (m, 10H), 3.54-3.48 (m, 1H), 3.44-3.37 (m, 1H), 3.04-2.95 (m, 2H, linker), 2.07 (s, 3H, NHAc), 2.04 (s, 3H, NHAc), 1.75-1.53 (m, 4H, linker), 1.45-1.35 (m, 2H, linker), 1.17 (d, J = 6.5 Hz, 3H, $\text{CH}_{3\text{Fuc}\alpha 1,3}$); **HRMS (MALDI-tof) m/z $[\text{M}+\text{Na}]^+$** calculated for $\text{C}_{39}\text{H}_{69}\text{N}_3\text{NaO}_{25}$ 1002.4118, found 1002.4061.

Hexasaccharide G84: Following the general procedure D, compound **G84** (510 μ g, 96%) was prepared from **G72** (461 μ g, 0.47 μ mol) using α -1,3-fucosyltransferase (CeFUT6, 16 μ g) and GDP-Fuc (317 μ g, 0.50 μ mol); **$^1\text{H NMR}$ (500 MHz, D_2O) δ** 5.23 (d, J = 3.8 Hz, 1H), 5.16 (d, J = 3.9 Hz, 1H), 5.09 (s, 1H), 4.69 (s, 3H), 4.66 (dd, J = 13.8, 6.5 Hz, 3H), 4.53 (d, J = 8.1 Hz, 1H), 4.50 (d, J = 7.7 Hz, 1H), 4.21 (d, J = 3.1 Hz, 1H), 4.13-3.48 (m, 28H), 3.45-3.36 (m, 1H), 3.04-2.93 (m, 2H, linker), 2.05 (s, 3H, NHAc), 2.03 (s, 3H, NHAc), 1.76-1.50 (m, 4H, linker), 1.47-1.33 (m, 2H,

linker), 1.28 (d, $J = 6.6$ Hz, 3H, CH₃Fuc α 1,3), 1.17 (d, $J = 6.6$ Hz, 3H, CH₃Fuc α 1,6); **HRMS (MALDI-tof)** m/z [M+Na]⁺ calculated for C₄₅H₇₉N₃NaO₂₉ 1148.4697, found 1148.4689.

Dodecasaccharide G87: Following the general procedure C, compound **G87** (1.25 mg, 89%) was prepared from **G80** (1.18 mg, 0.67 μ mol) using 0.72U of bovine milk β -1,4-galactosyltransferase and UDP-Gal (1.63 mg, 2.67 μ mol); **¹H NMR (500 MHz, D₂O)** δ 5.15 (s, 1H, H-1 α Man), 5.13 (d, $J = 3.3$ Hz, 1H, H-1_{Fuc α 1-3}), 4.94 (d, $J = 4.0$ Hz, 1H, H-1_{Fuc α 1-6}), 4.92 (s, 1H, H-1 α Man), 4.76-4.73 (m, 2H, H-1 β Man, H-5_{Fuc α 1-3}), 4.70-4.66 (d, $J = 8.2$ Hz, 1H), 4.60-4.58 (m, 2H), 4.51-4.46 (m, 3H), 4.26-4.25 (d, $J = 2.8$ Hz, 1H), 4.21-4.20 (m, 1H), 4.15-4.14 (m, 1H, H-5_{Fuc1 α -6}), 4.13-4.11 (m, 1H), 4.04-3.46 (m, 55H), 2.99 (t, $J = 7.7$ Hz, 2H, linker), 2.09-2.07 (s, 4H), 2.08, 2.06, 2.06, 2.03 (4xs, 12H, NHAc), 1.71-1.65 (m, 2H, linker), 1.61-1.56 (m, 2H, linker), 1.43-1.38 (m, linker), 1.29 (d, $J = 6.7$ Hz, 3H, CH₃Fuc α 1-3), 1.24 (d, $J = 6.7$ Hz, 3H, CH₃Fuc α 1-6); **¹³C NMR (126 MHz, D₂O)** δ 15.6, 15.7, 22.3, 28.1, 26.5, 22.6, 22.3, 22.4, 22.3, 39.2, 80.8, 67.4, 74.4, 70.9, 74.6, 70.0, 74.8, 72.9, 72.9, 61.5, 72.4, 60.7, 69.5, 67.6, 78.6, 75.3, 78.4, 72.0, 54.9, 80.4, 54.9, 73.5, 66.3, 61.1, 67.9, 71.9, 59.9, 74.4, 55.7, 61.6, 68.5, 61.5, 69.2, 61.6, 61.3, 69.2, 68.8, 66.0, 60.0, 73.3, 76.2, 66.8, 76.3, 70.2, 102.8, 102.8, 101.2, 99.3, 99.3, 100.1, 66.7, 100.4, 96.9, 98.8, 99.5, 98.4; **HRMS (MALDI-tof)** m/z [M+Na]⁺ calculated for C₇₉H₁₃₅N₅O₅₄ 2240.7864, found 2240.7828.

Dodecasaccharide G88: Following the general procedure C, compound **G88** (0.79 mg, 81%) was prepared from the G67 (0.54 mg, 0.348 μ mol) using 0.15U of bovine milk β -1,4-galactosyltransferase and UDP-Gal (0.506 mg, 0.83 μ mol). After complete conversion of starting material, GDP-Fuc (696 μ g, 1.1 μ mol) and α -1,3-fucosyltransferase CeFUT6 (70 μ L, 1.07 mg/mL) were added. The resulting mixture was incubated at room temperature for 3 days; **¹H NMR (500 MHz, D₂O)** δ 5.14 (m, 2H), 5.12 (s, 1H), 4.92-4.91 (m, 2H), 4.68-4.67 (m, 1H), 4.61-4.59 (m, 2H), 4.50 (d, $J = 7.8$ Hz, 1H), 4.46 (dd, $J = 7.4$ Hz, $J = 2.3$ Hz, 2H), 4.27 (broad s, 1H), 4.20-4.19 (m, 1H), 4.14-4.11 (m, 2H), 4.02-3.47 (m), 2.99 (t, $J = 7.7$ Hz, 2H, linker), 2.11, 2.06, 2.04 (3xs, 12H, NHAc), 1.71-1.65 (m, 4H, linker), 1.62-1.58 (m, 2H, linker), 1.43-1.38 (m, 2H, linker), 1.24 (d, $J = 6.6$ Hz, 3H, CH₃Fuc), 1.18 (2xd, $J = 6.88$ Hz, $J = 2.42$ Hz, 6H, 2xCH₃Fuc); **¹³C NMR (126 MHz, D₂O)** δ 101.8, 101.8, 101.0, 101.0, 100.6, 99.5, 99.5, 99.2, 99.1, 98.6, 98.6, 97.4, 80.3, 79.8, 76.3, 76.2, 75.1, 74.8, 74.5, 74.4, 73.7, 73.4, 73.2, 72.6, 72.1, 71.8, 71.1, 70.2, 70.1, 70.0, 69.7, 69.2, 69.1, 68.0, 67.7, 67.4, 67.1, 66.7, 66.7, 66.2, 66.1, 62.6, 61.6, 61.6, 61.5, 59.8, 59.8, 55.8, 55.7, 55.1, 54.9, 39.3, 27.8, 26.4, 22.4, 22.3, 22.2, 22.1, 15.4, 15.3, 15.3; **HRMS (MALDI-tof)** m/z [M+Na]⁺ calculated for C₈₅H₁₄₅N₅O₅₈Na 2186.8443, found 2186.8404.

Undecasaccharide G89: Following the general procedure D, compound **G89** (670 μ g, 97%) was prepared from **G59** (590 μ g, 0.34 μ mol) using α -1,3-fucosyltransferase (CeFUT6, 48 μ g) and GDP-Fuc (667 μ g, 1.05 μ mol); **¹H NMR (500 MHz, D₂O)** δ 5.10-5.00 (m, 3H), 4.84 (s, 1H), 4.56-4.49 (m, 2H), 4.41 (d, $J = 7.9$ Hz, 1H), 4.40-4.34 (m, 2H), 4.15 (s, 1H), 4.08 (s, 1H), 4.00 (s, 1H), 3.97-3.36 (m), 2.95-2.86 (m, 2H, linker), 2.01 (s, 3H, NHAc), 1.97 (s, 6H, NHAc), 1.95 (s, 3H, NHAc), 1.66-1.44 (m, 4H, linker), 1.39-1.25 (m, 2H, linker), 1.15-1.04 (m, 6H, 2xCH₃Fuc α 1,3); **HRMS (MALDI-tof)** m/z [M+Na]⁺ calculated for C₇₉H₁₃₅N₅NaO₅₄ 2040.7869, found 2040.7877.

Undecasaccharide G90: Following the general procedure D, compound **G90** (200 µg, 25%) was prepared from **G63** (670 µg, 0.37 µmol) using core α-1,3-fucosyltransferase CeFUT6 (170 µL, 1.07 mg/mL) and GDP-Fuc (128.9 µL, 2.57 µmol); **HRMS (MALDI-tof)** m/z $[M+Na]^+$ calculated for $C_{83}H_{141}N_7O_{54}$ 2122.8395, found 2122.8315. Amount of final product was not enough for a NMR analysis.

6. Microarray Fabrication and analysis

6.1 Immobilization of the ligands and test for biofunctionality of the printed ligands

Ligand solutions were prepared from stock solutions (1 mM in water) by dilution with sodium phosphate buffer (300 mM, pH 8.4, 0.005% Tween 20) to a final concentration of 50 µM. Total amount of 40 µl of each glycans solutions were placed into a 384 well source plate (Scienion, Berlin, Germany) which was stored at -20°C and reused if necessary. These solutions (1.25 nL, 5 drops of 250 pL) were spatially arrayed onto NHS functionalized glass slides. After printing, the slides were placed in a 75 % humidity chamber (saturated NaCl solution) at 25 °C for 18 hours. The unreacted NHS groups were quenched by placing the slides in a 50 mM solution of ethanolamine in sodium borate buffer 50 mM, pH 9.0, for 1h. The standard washing of the slides was performed with PBST (PBS solution containing 0.5% Tween 20), PBS and water. The slides were dried in a slide spinner.

18x18 array:

27 N-glycans (Figure S-1) were spotted with a distance of 350 µm. Ligands were printed in 6 replicates (3 different N-glycans/row), establishing the complete 18x18 array which was printed in 14 copies into each slide.

The array was used to predict possible enzymatic elongations of xylosylated compounds (G1-G6) and to compare enzyme specificities with corresponding, non-xylosylated structures.

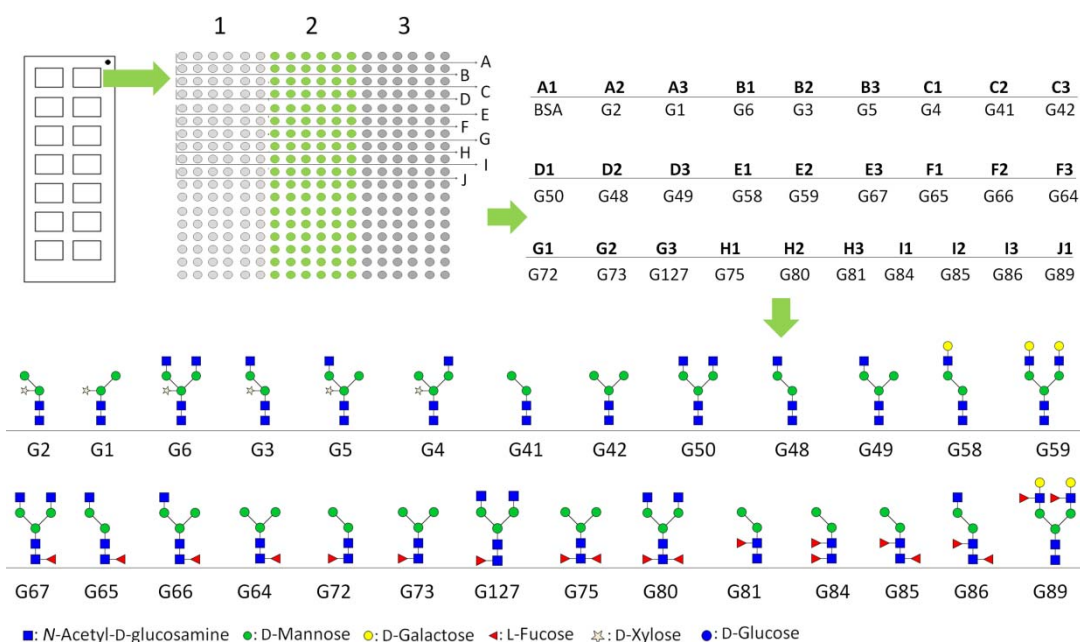


Figure S-1. 27 ligands printed on the NHS activated glycan array (18x18) used for the evaluation of enzyme specificity.

The immobilized glycans were probed with solutions of fluorescently labelled lectins. Solutions of *Aleuria aurantia* lectin, fucose recognition (AAL-555, 50 µg/mL) and *Ricinus communis* agglutinin, galactose recognition (RCA-120, 10 µg/mL) were prepared in PBS, 2 mM CaCl₂, 2 mM MgCl₂ containing 0.01% Tween-20 (Figure S-2). For incubations, 100 µL of each lectin solution was applied to each microarray using using 16 well Fast Frame[®] incubation chambers for 1h in the dark at room temperature. The slides were washed under standard conditions, dried with argon and the emitted fluorescence was analyzed with a microarray scanner.

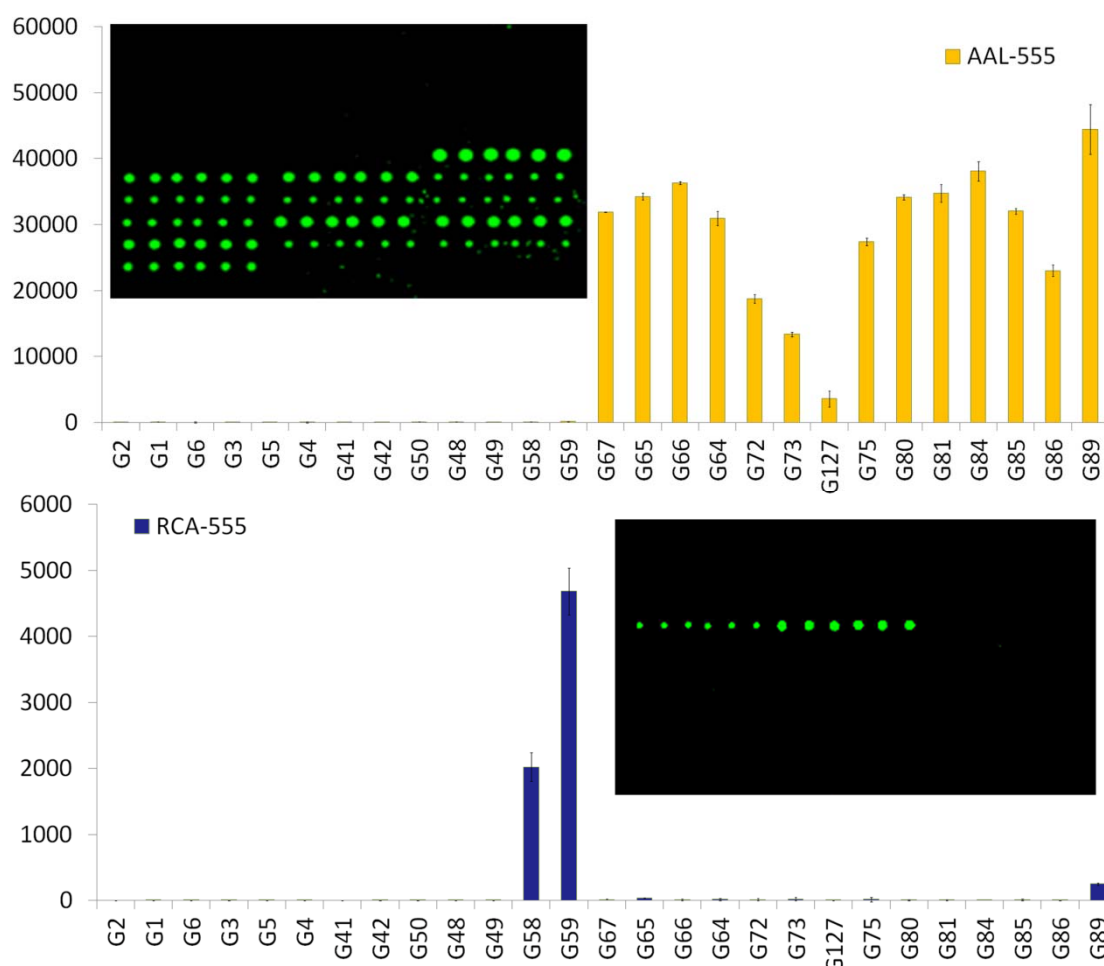


Figure S-2. Binding profiles for **AAL-555** (orange) and **RCA-555** (blue) on the 27 ligand array.

6.2 Data analysis

Quantification of spots was performed using an adaptive circle with minimum diameter of 50 µm and maximum diameter of 200 µm. Histograms represents average of mean fluorescence of spots with local background subtraction. Protein incubations and enzymatic screening reactions were performed using 16 well Fast Frame[®] incubation chambers from Whatman, Kent, UK and 8 Well ProPlate Module 7 x 16 mm Well from Electron Microscopy Sciences, Hatfield, UK.

6.3 Enzymatic Extension in well format

The 18x18 glycan microarrays were compartmentalized using 16 well Fast Frame[®] incubation chambers from Whatman[®] and treated with the corresponding enzyme containing solutions .

Fucosylation: A solution (100 μ L) containing a fucosyltransferase (either AtFucTA, CeFUT6, CeFUT8 or CeFUT1, 10 μ L), GDP-Fuc (2 mM), $MnCl_2$ (20 mM) in MES buffer (80 mM, pH 6.5) was added o each well and the slide was incubated at room temperature for 24 hours. The slide was then washed with PBS and water. To test efficient fucosylation one well was incubated with *Aleuria aurantia* lectin (AAL-555, 20 μ g/mL) in PBS, 5mM $CaCl_2$, 5 mM $MgCl_2$ containing 0.1% Tween-20, for 1h at room temperature. The slide was washed with PBST, PBS and water, dried under a stream of Ar and analyzed (Figure S-3).

Galactosylation: A solution (100 μ L) containing of bovine β -1,4-GalT (10 mU), UDP-Gal (2 mM), $MnCl_2$ (10 mM), and alkaline phosphatase (50 mU) in HEPES buffer (50 mM, pH 7.4) was added into each well and the slide incuabted at 37°C for 24 hours and the slide subsequently washed with PBS and water. Galactosylation was qualitatively evaluated by incubating a well with fluoresecnetly tagged *Ricinus communis* agglutinin (RCA120-555, 1 μ g/mL) lectin in PBS, 5mM $CaCl_2$, 5 mM $MgCl_2$ containing 0.1% Tween-20, for 1h at room temperature. The slide was washed with PBST, PBS and water, dried under a stream of Ar and analyzed (Figure S-4)

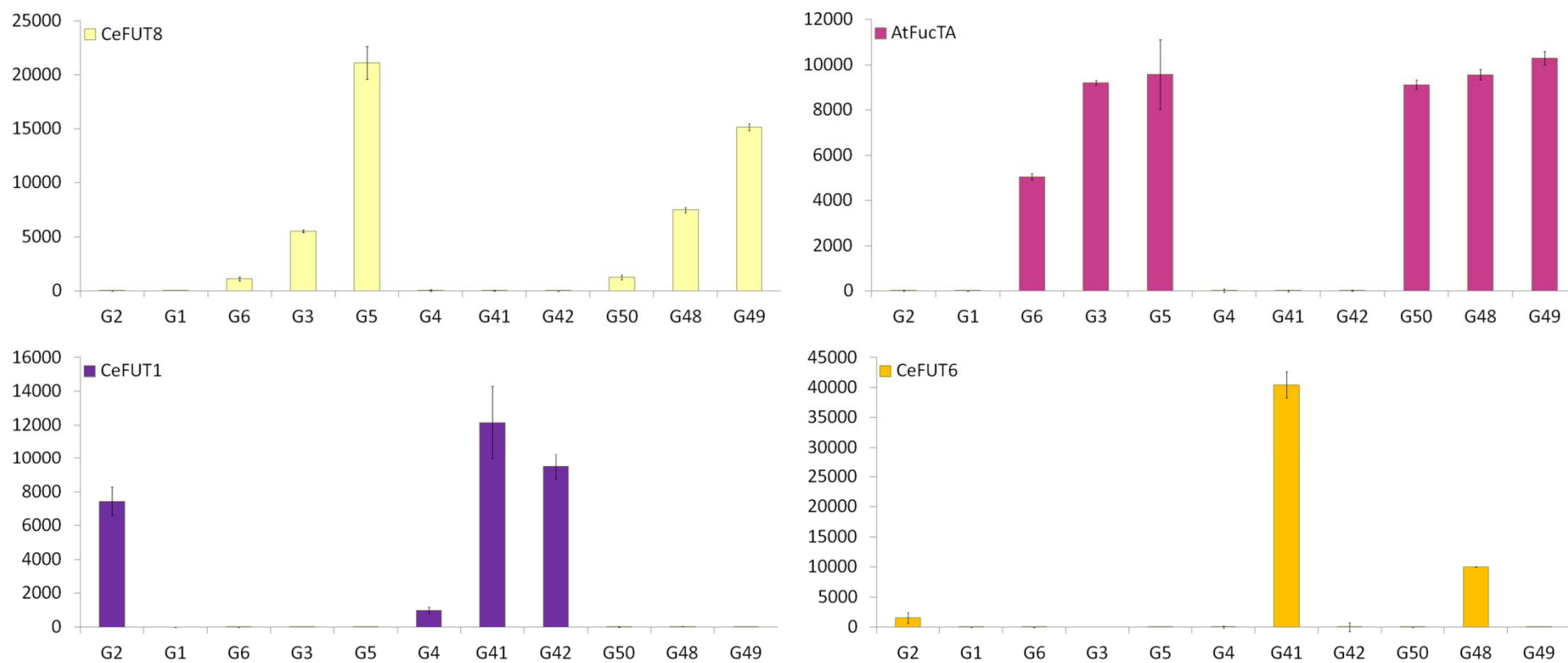


Figure S-3. **AAL-555** binding profile after on chip enzymatic extension (24h of incubation) with four different fucosyltransferases: α -1,6-FucT (yellow bars-CeFUT8) and α -1,3-FucT (pink-AtFucTA, violet-CeFUT1, orange-CeFUT6); each histogram shows the average fluorescence from 6 spots including standard deviation.

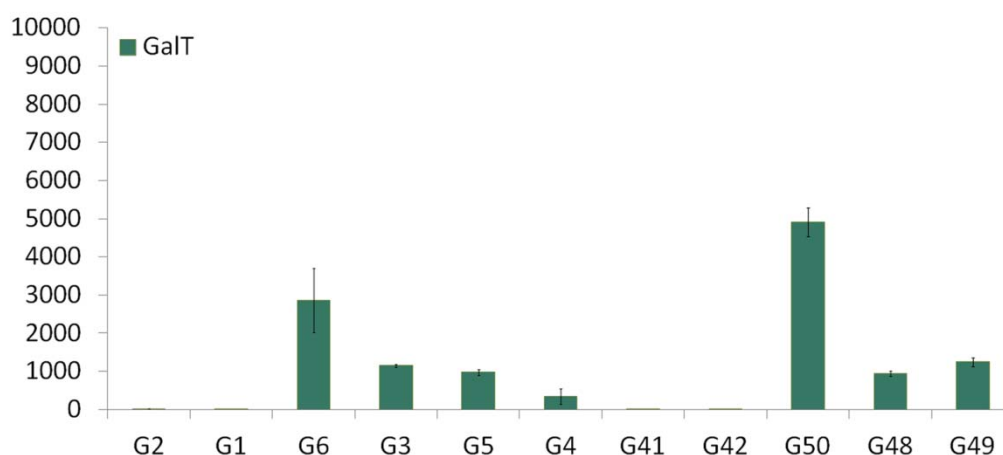


Figure S-4. **RCA120-555** binding profile after on chip enzymatic extension (24 h incubation) with β -1,4-galactosyltransferase; each histogram shows the average fluorescence from 6 spots including standard deviation

Reaction with β -1,4-N-acetylgalactosaminyltransferase: A solution (100 μ L) containing of β -1,4-GalNAcT (20 μ L), UDP-GalNAc (2 mM), $MnCl_2$ (20 mM) in HEPES buffer (50 mM, pH 7.4) was added to each well and the slide left at room temperature for 24 hours. The slide was then washed with PBS and water. Incubation of a single well with *Wisteria floribunda* lectin (WFL-555, 10 μ g/mL) in in PBS, 5mM $CaCl_2$, 5 mM $MgCl_2$ containing 0.1% Tween-20 for 1h at room temperature, washing and subsequent scanning of the slide showed the successful introduction of GalNAc residues on selected ligands (Figure S-5).

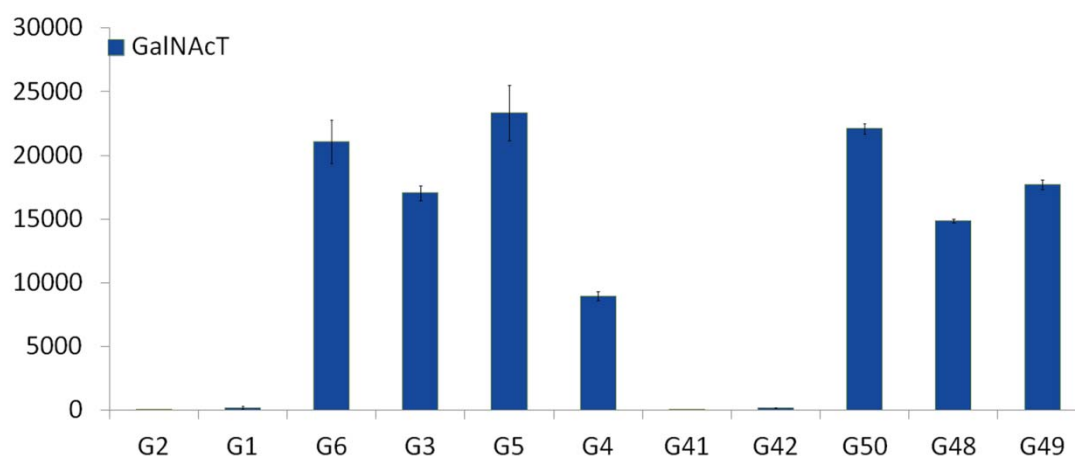


Figure S-5. **WFL-555** binding profile after on chip enzymatic extensions (24h incubation) with β -1,4-N-acetylgalactosaminyltransferase; each histogram shows the average of fluorescence from 6 spots including standard deviation.

7 Glycan array Screening Experiments

7.1 Interaction with plant lectins

36x18 array:

Volumes (1.25 nL, 5 drops of 250 pL) of the buffered solutions were spotted with a distance of 400 μm . 127 N-glycans were spotted in 4 replicates (9 different N-glycans/row), establishing the complete 36x18 array which was printed in 7 copies into each slide (Figure S-6).

The immobilized glycans were probed with solutions of fluorescently labelled lectins (Figure S-4). Solutions of *Galanthus nivalis* agglutinin (GNA-555, 22 $\mu\text{g/mL}$), *Concanavalin A* (ConA-647, 22 $\mu\text{g/mL}$) (Figure S-7), *Aleuria aurantia* lectin (AAL-555, 20 $\mu\text{g/mL}$), *Bandeiraea simplicifolia* lectin (BSL-II-647, 24 $\mu\text{g/mL}$) (Figure S-8), *Wisteria floribunda* lectin (WFL-555, 10 $\mu\text{g/mL}$) and *Ricinus communis* agglutinin (RCA-120-555, 1 $\mu\text{g/mL}$) (Figure S-9), were prepared in PBS, 2 mM CaCl_2 , 2 mM MgCl_2 containing 0.01% Tween-20. For incubations, 200 μL of each lectin solution was applied to each microarray using 8 Well ProPlate Module incubation chambers for 1h in the dark at room temperature. The slides were washed under standard conditions, dried with Ar and their fluorescence was analyzed with a microarray scanner.

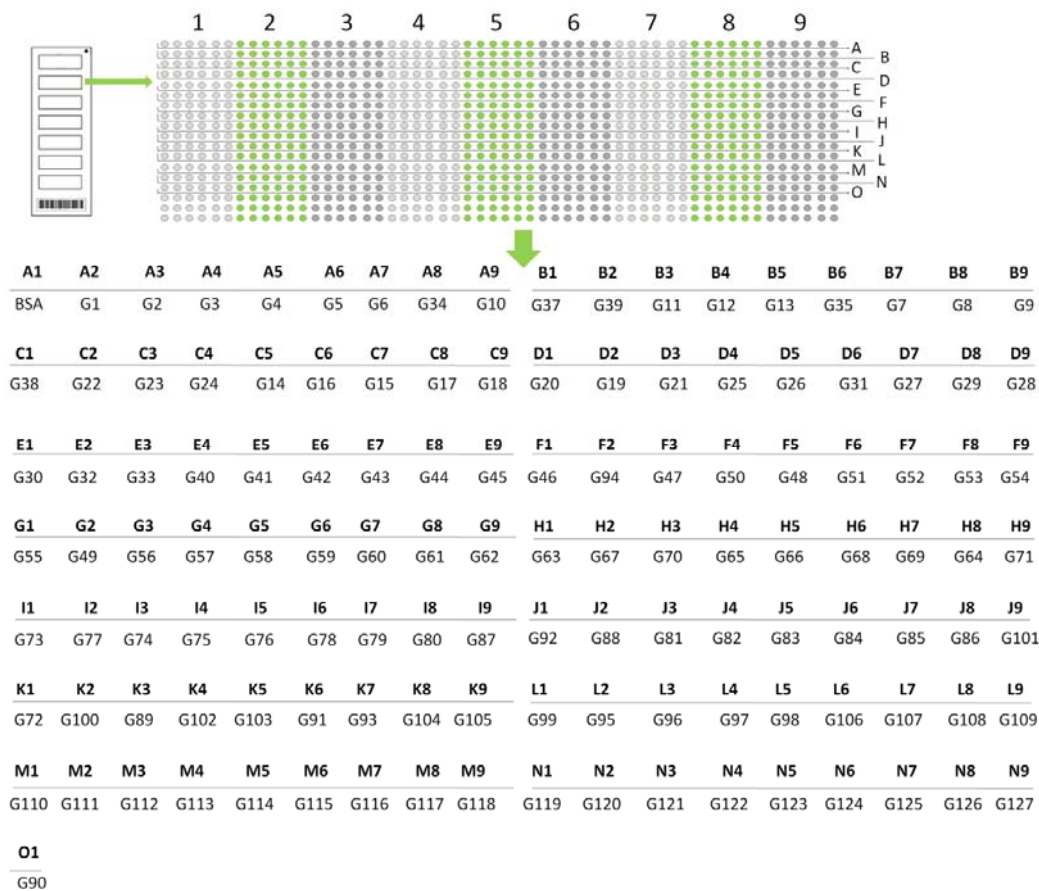


Figure S-6. 127 ligands printed on the NHS activated glycan array (36x18) used for screening of GBP's.

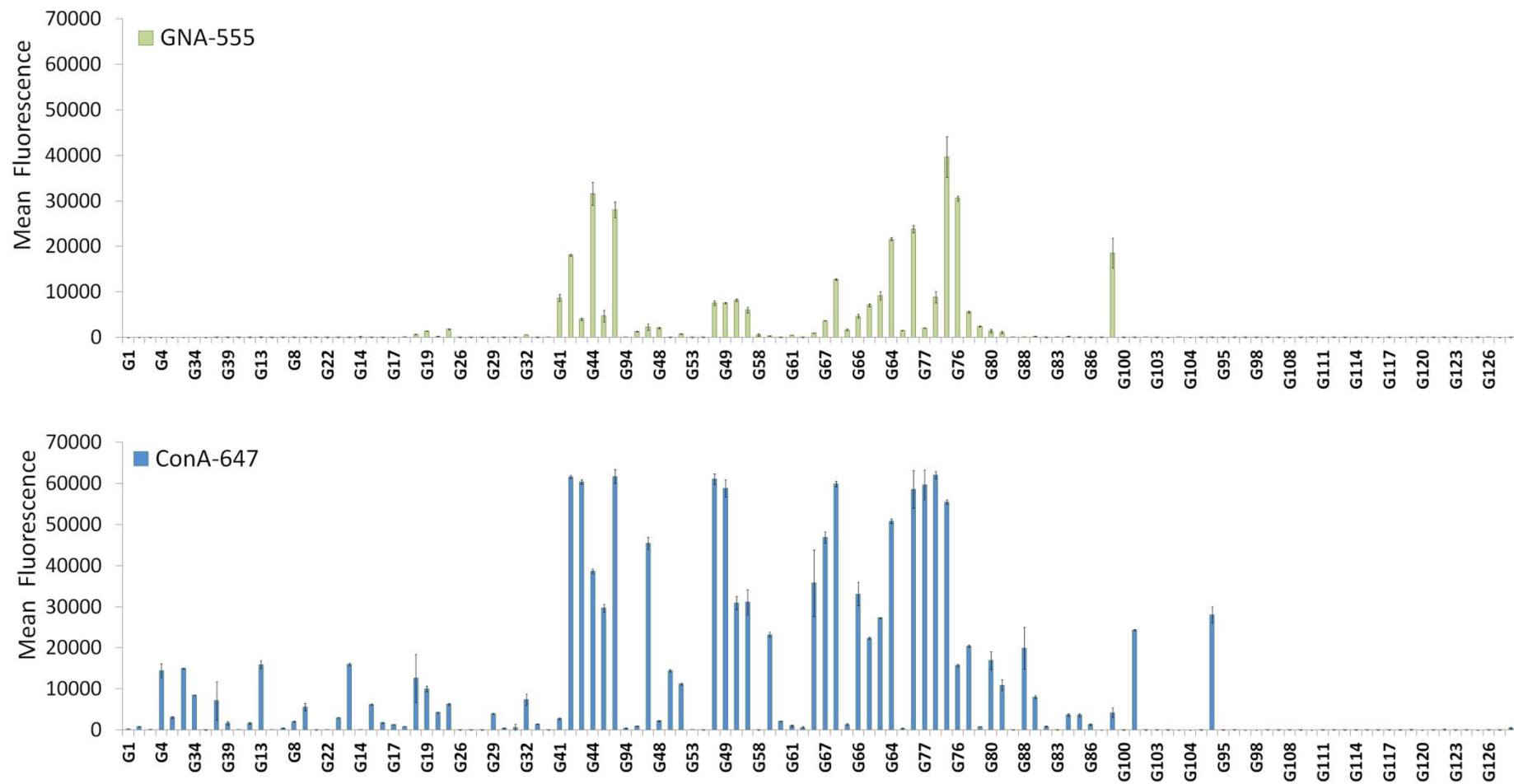


Figure S-7. Fluorescence intensities for binding of mannose recognizing plant lectins: **GNA-555** (green) and **ConA-647** (blue) on large glycan array.

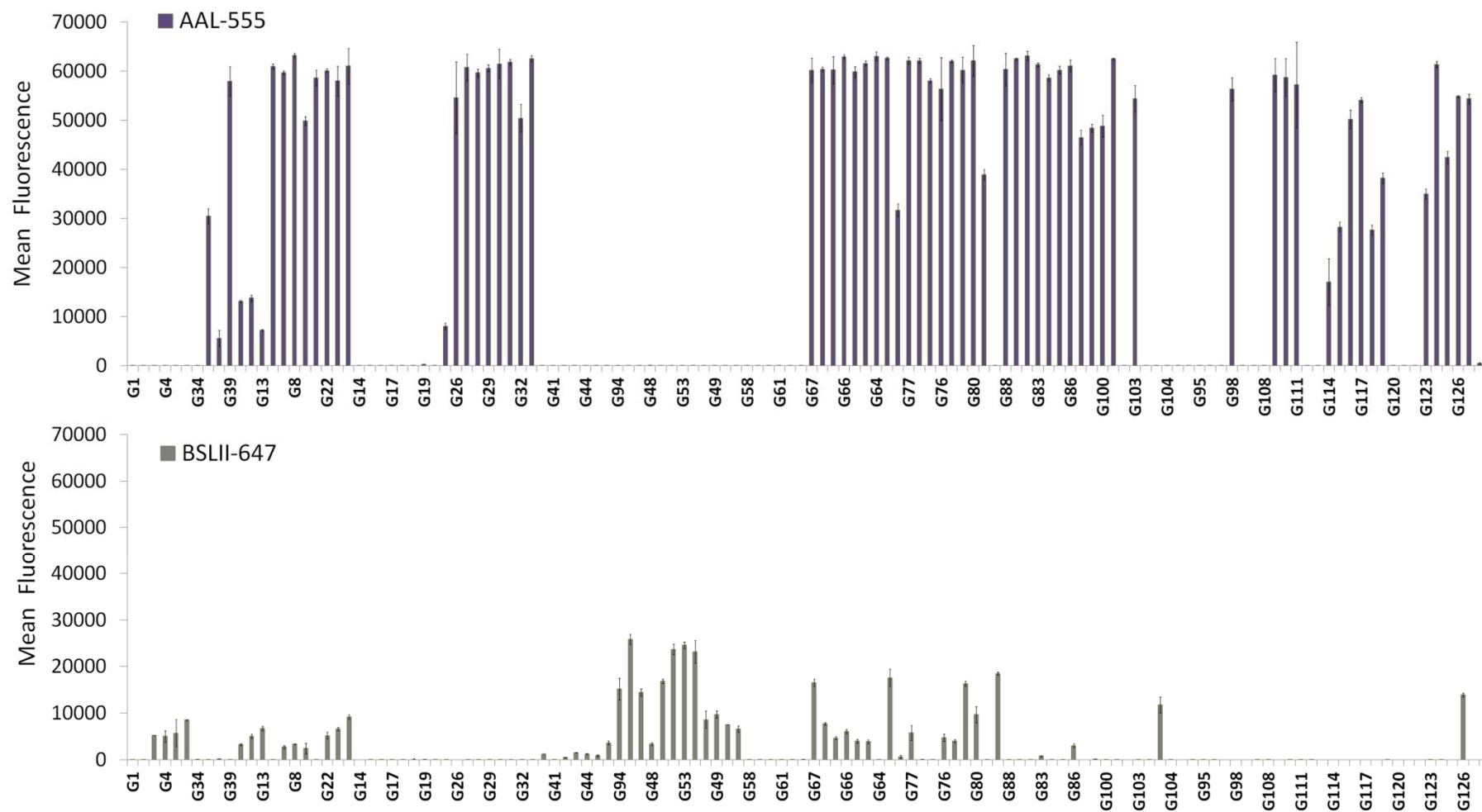


Figure S-8. Large (18x36) glycan array probed with fucose specific lectin **AAL-555** (violet) and **BSLII-647** (gray) recognizing terminal, non-reducing α - or β -linked GlcNAc.

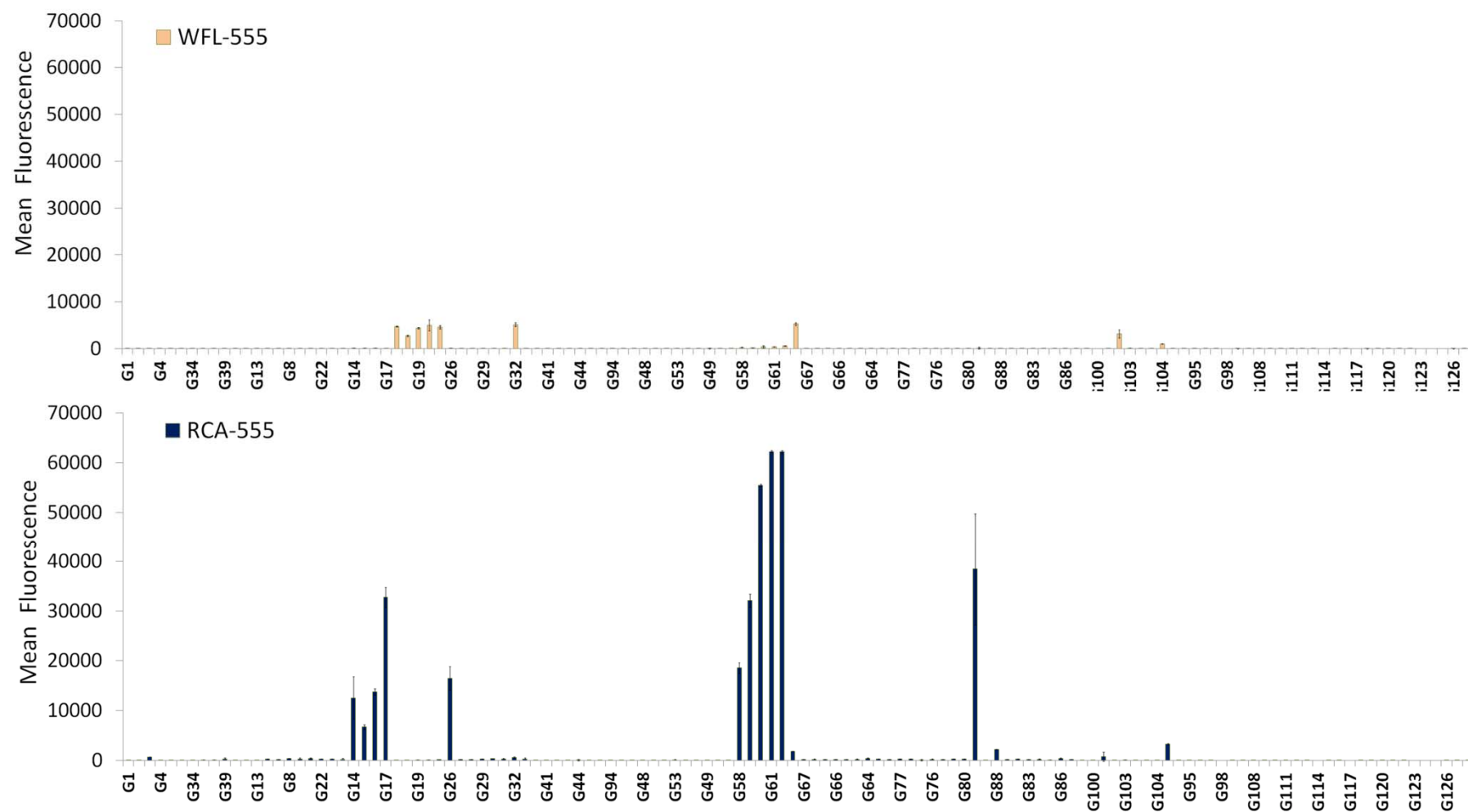


Figure S-9. Large (18x36) glycan array probed with WFL-555 (orange) specific for GalNAc residues and RCA-555 (blue) recognizing terminal β -linked galactose.

7.2 Glycan binding analysis of a DC-SIGN-Fc fusion protein

DC-SIGN/Fc solutions were prepared from stock solution (0.25 mg/mL in PBS) by dilution with TSM_T binding buffer pH=7.5 (20 mM Tris·HCl, 150 mM NaCl, 2 mM CaCl₂, 2 mM MgCl₂, 1% BSA and 0.005% Tween-20) to a final concentration of 10 µg/mL (Figure 9a). These solutions (200 µL) were placed into subarrays and incubated overnight at 0°C with gently shaking. Solutions containing protein were removed and each subarray was washed with binding buffer, followed by 1h incubation with anti-human IgG (Fc specific)-Cy3 in TSM binding buffer (1:1000). The washing of slides was performed with TSM_T (TSM containing 0.005% Tween-20), TSM and water. Slides were dried in a slide spinner. The fluorescence was analysed with a microarray scanner (Figure S-10).

7.3 Glycan binding analysis of anti-carbohydrate polyclonal antibodies in sera from *S. mansoni* infected patients

Previously collected selected sera of schistosomiasis patients were pooled into two age-groups: children (21 individuals, age between 5 to 11 years) and adults (20 individuals from 20 to 46 years)⁹. In addition, sera from 3 healthy Europeans were analyzed and used as negative controls for the study (Figure S-11, pooled binding data). In both groups the ratio female/male ratio was 3:2. Sera were diluted 1:100 with PBS containing 1% BSA and 0.01% Tween-20, and aliquots (180 µL per array) used to incubate individual wells on a glycan array slide at room temperature for 1h. The slides were washed with PBST (PBS containing 0.05% Tween-20) followed by PBS. Slides were then incubated with anti-human IgG(Fc specific)-Cy3 (Figure S-12) and anti human-IgM(µ specific)-Alexa fluor 647 (Figure S-13) in PBS containing 1% BSA and 0.01% Tween-20 for 30 minutes in the dark. Slides were washed subsequently with PBST, PBS and H₂O and dried in a slide spinner prior to scanning the fluorescence emission in a microarray scanner.

⁹ van Diepen, A., Smit, C.H, van Egmond, L., Kabatereine, N.B, de Moira, A. P, Dunne, D.W, Hokke, C.H. *PLOS NTD*, **2012**,6, 1922.

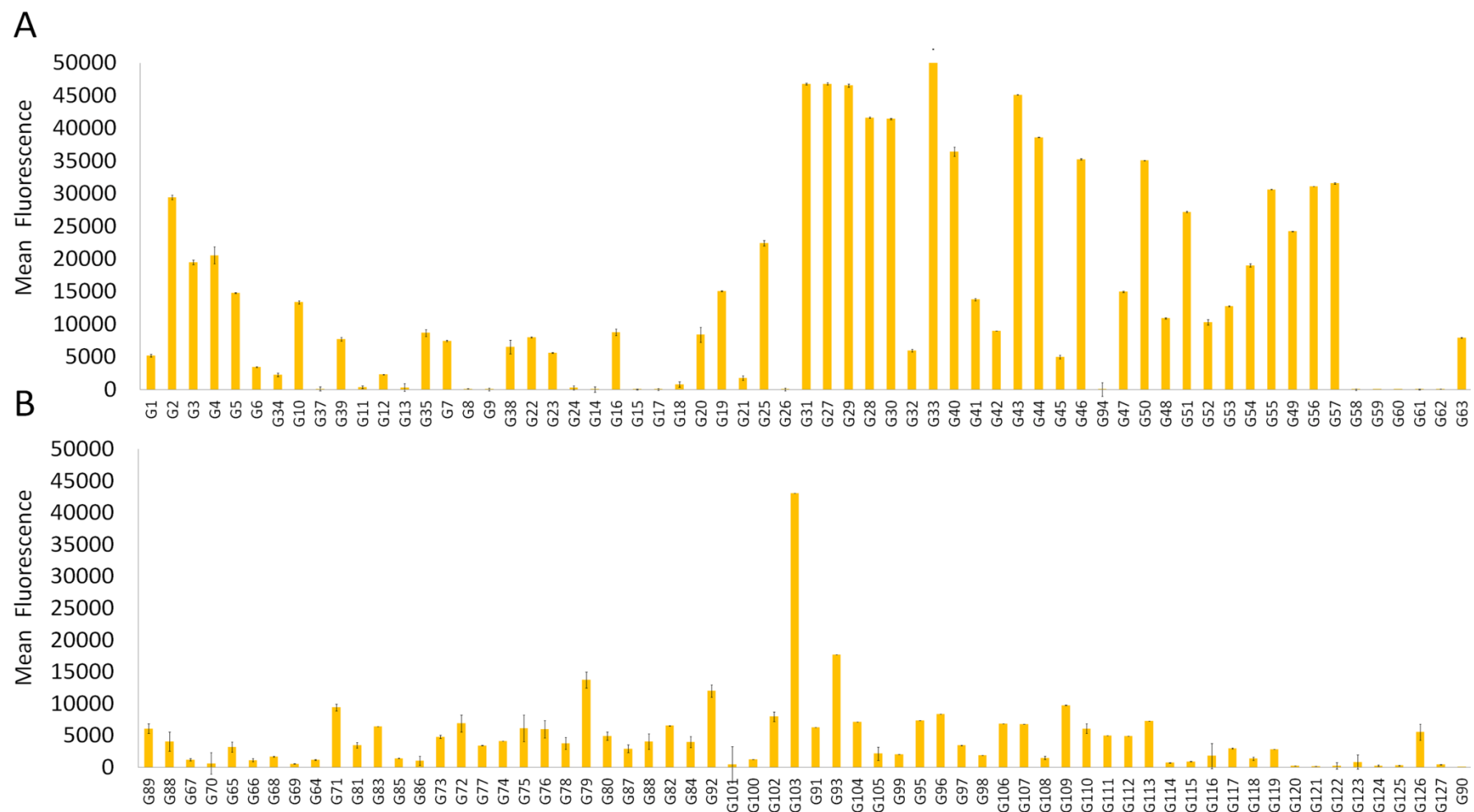


Figure S-10. DC-SIGN-Fc binding profile on a large (18x36) glycan array. Mean fluorescence after incubation with DC-SIGN-Fc (10 $\mu\text{g/mL}$) followed incubation with secondary cnetly labeled anti-human antibody. Each bar in the histogram shows the average of fluorescence from 4 spots and the standard deviation as error bar.

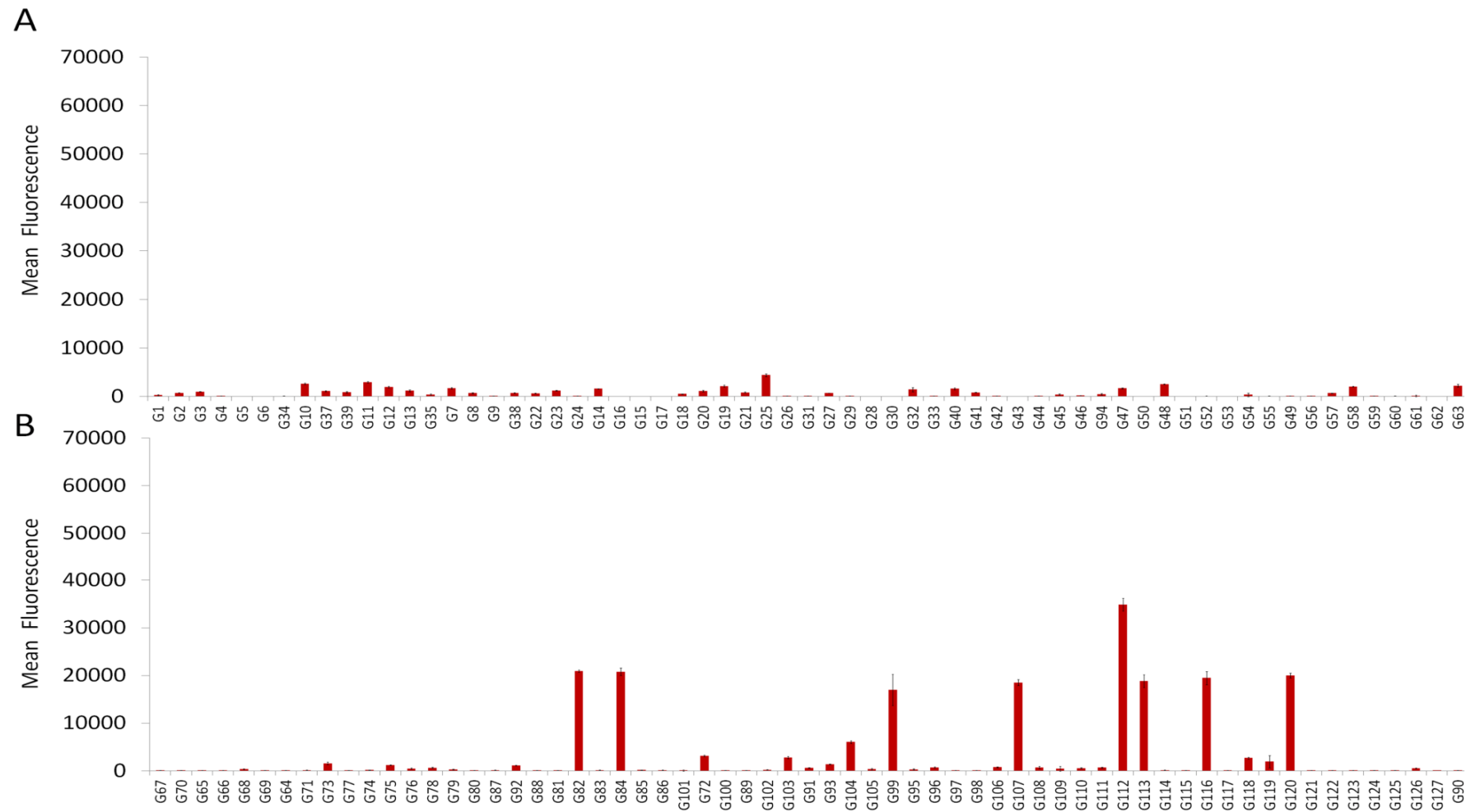


Figure S-11. Glycan array screening sera of healthy Europeans followed by incubation with secondary anti human-IgG-(Fc specific)-Cy3; **A.** Mean fluorescence for IgG binding to glycans **G1-G63**; **B.** Mean fluorescence for IgG binding to glycans **G89-G90**; Histogram shows pooled binding data of 3 individuals. Fluorescence values are averaged from 4 replicate spots.

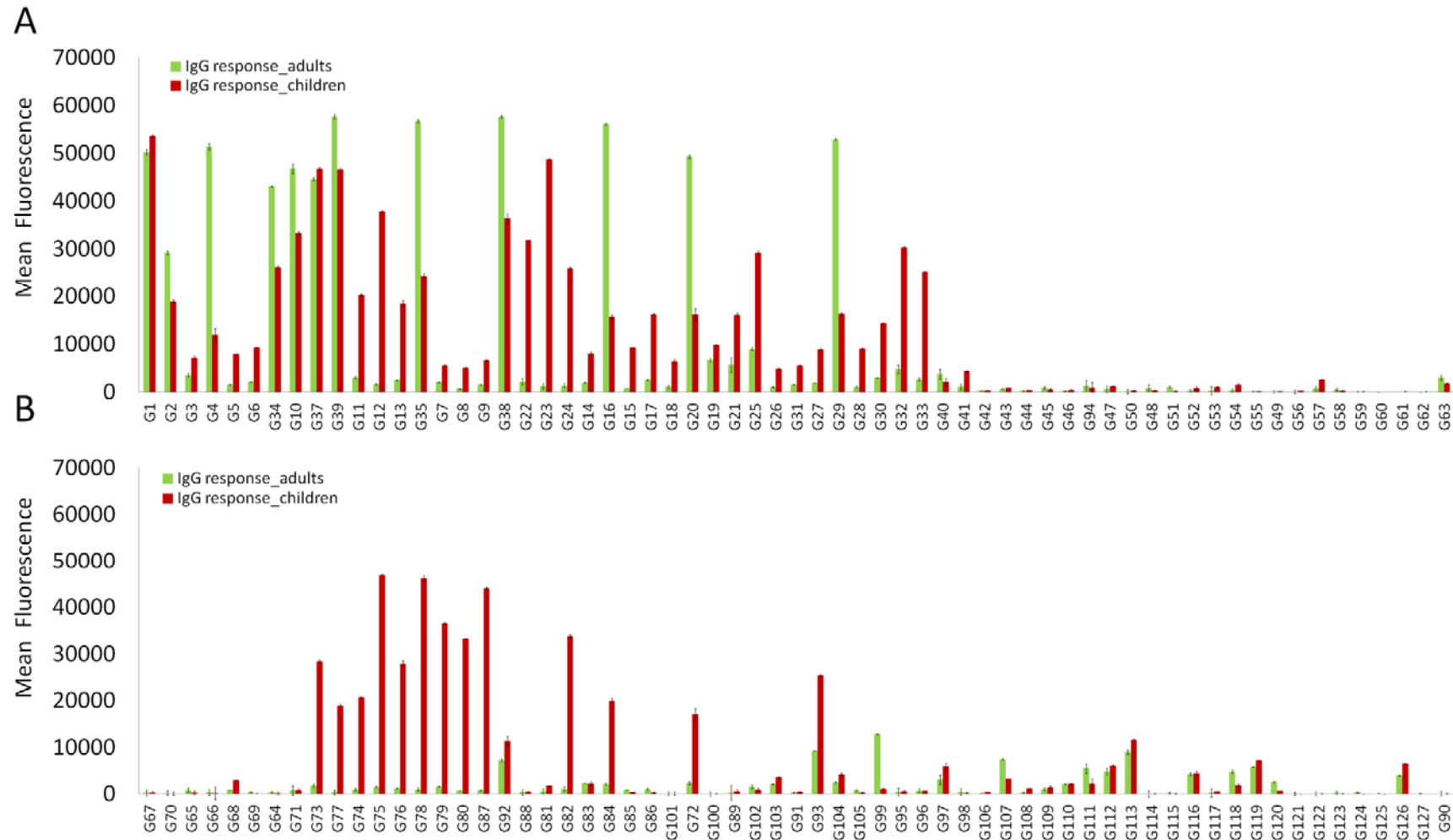


Figure S-12. Glycan array screening of schistosomiasis infected sera followed by incubation with anti human-IgG-(Fc specific)-Cy3; **A**. IgG response observed in pooled sera from children and adults against glycans **G1-G63**; **B**. IgG response observed in pooled sera from children and adults against glycans **G89-G90**; Error bars show the standard deviation of mean fluorescence for 4 replicate spots quantified for each ligand

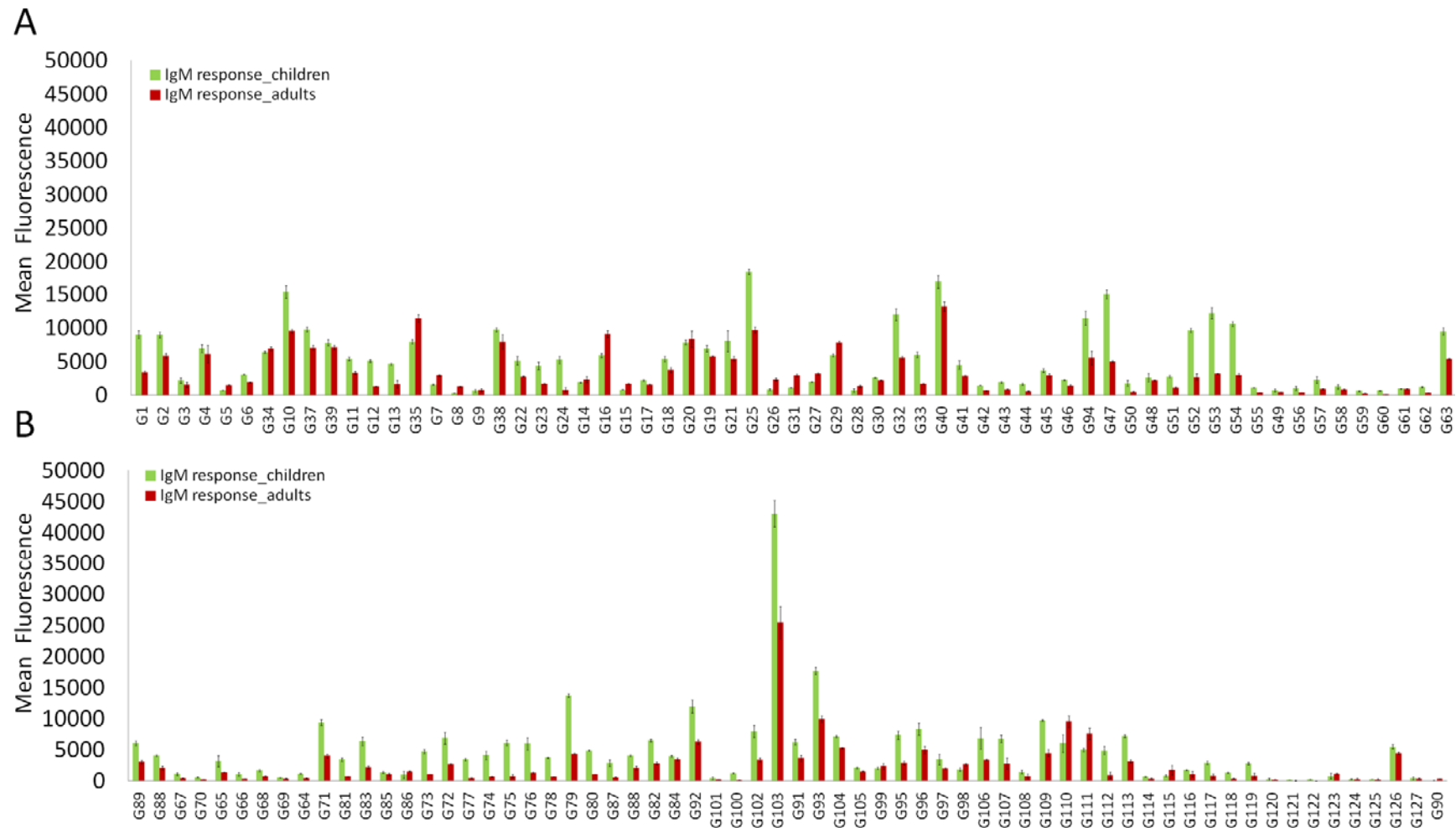
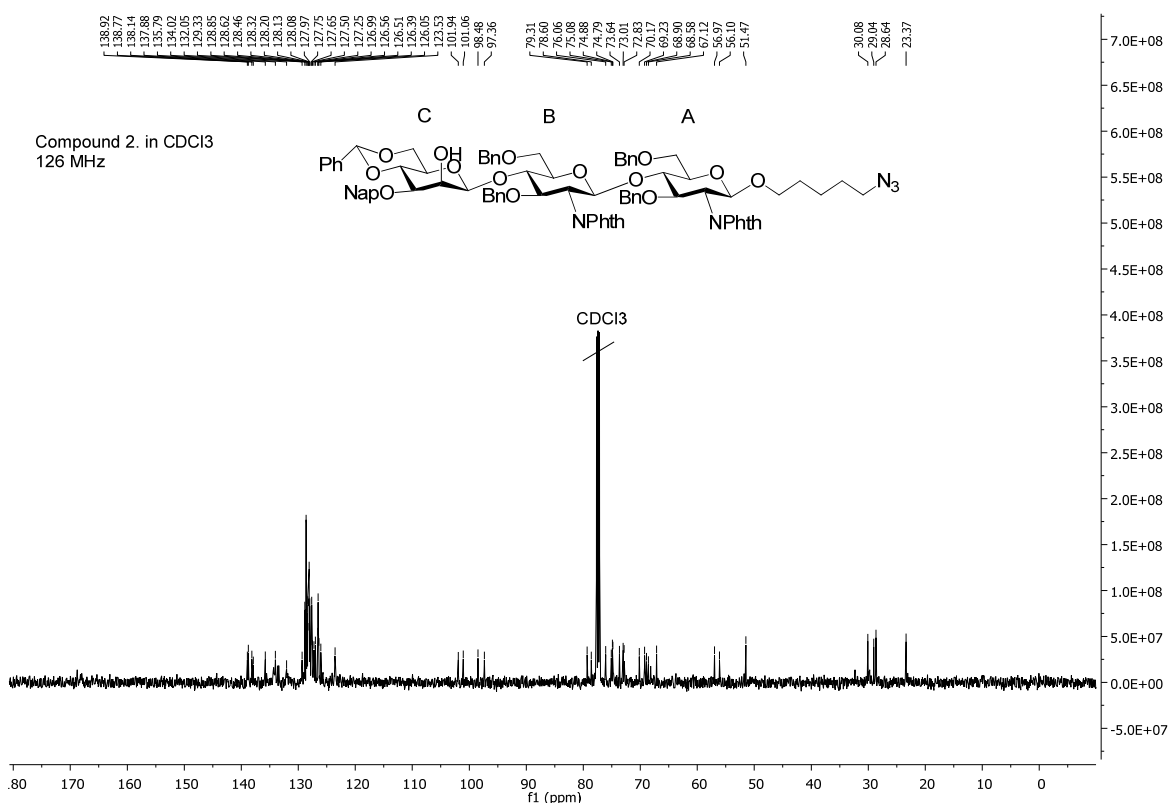
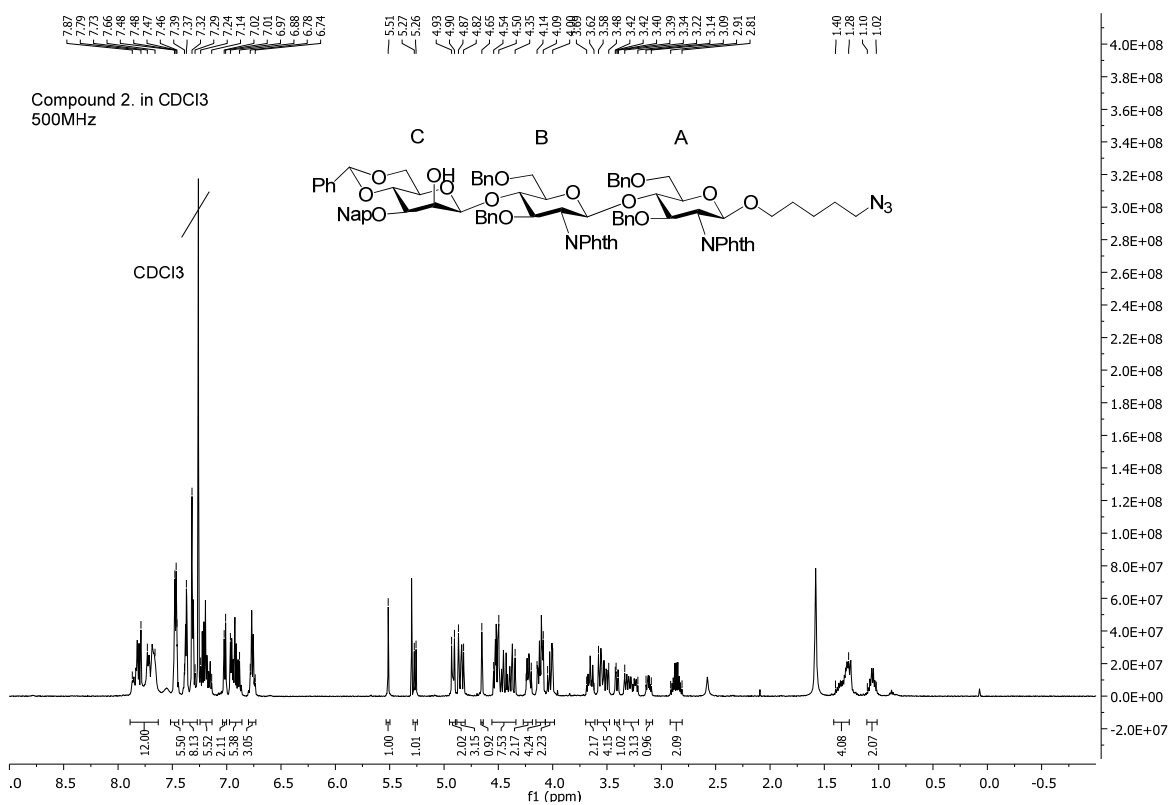


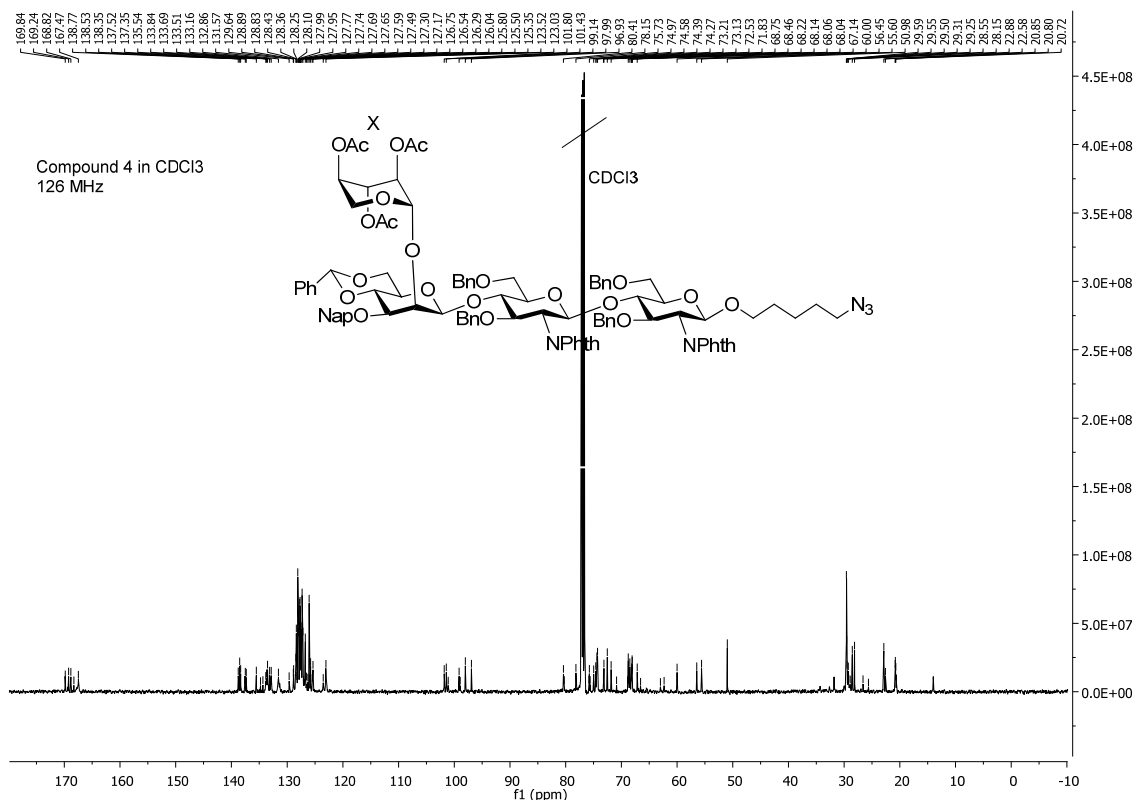
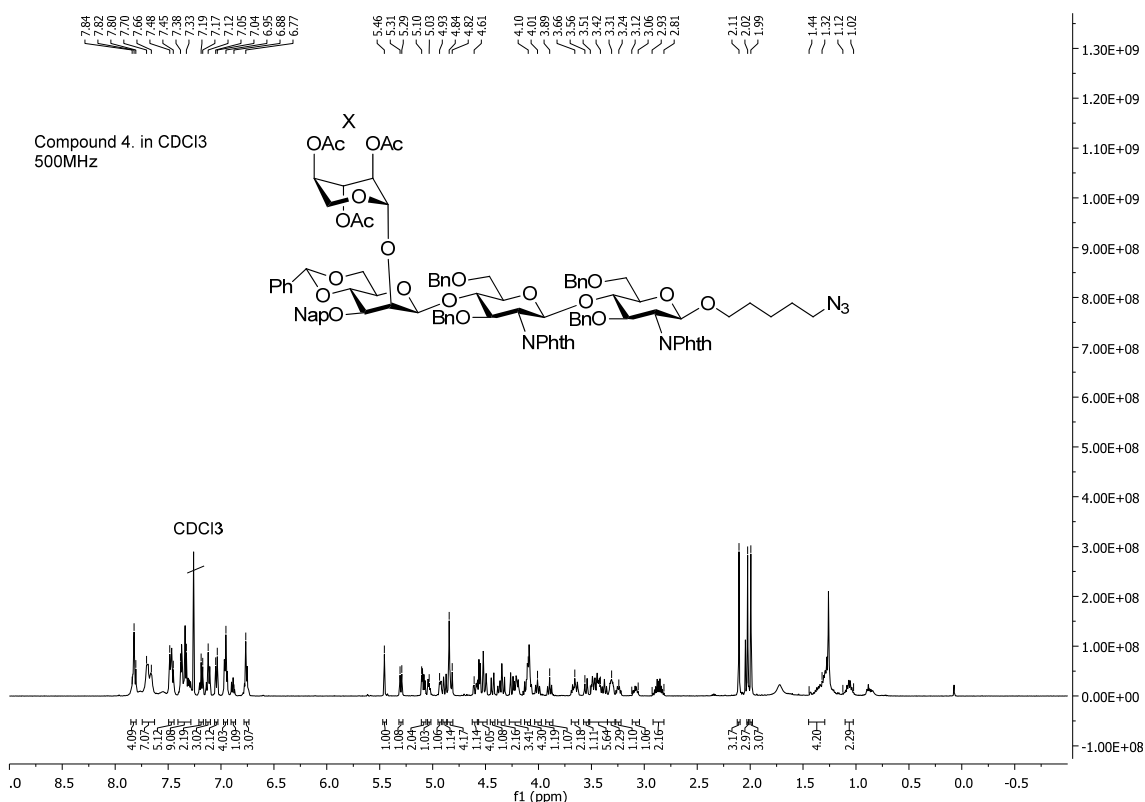
Figure S-13. Glycan array screening of schistosomiasis infected sera followed by incubation with anti human-IgM-647; **A.** IgM from pooled sera of children (green) and adults (red) binding to glycans **G1-G63**; **B.** IgM from pooled sera of children (green) and adults (red) binding to glycans **G89-G90**; Error bars show the standard deviation of mean fluorescence for 4 replicate spots quantified for each ligand

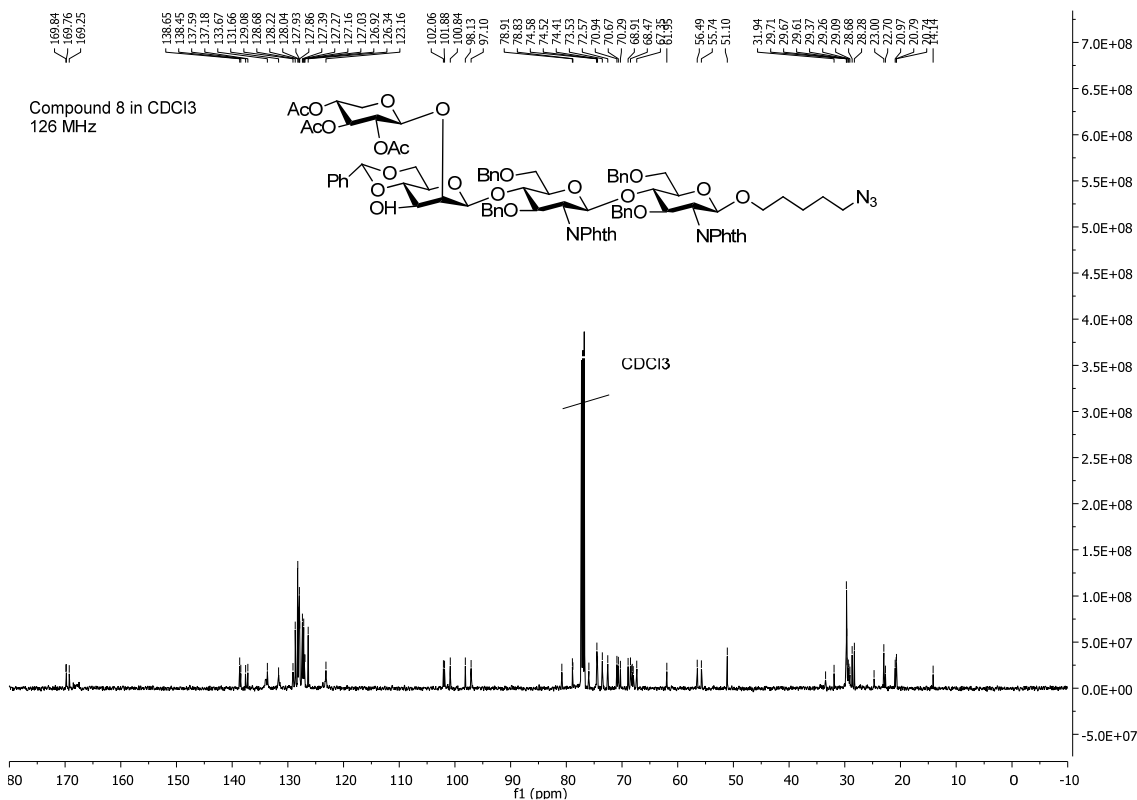
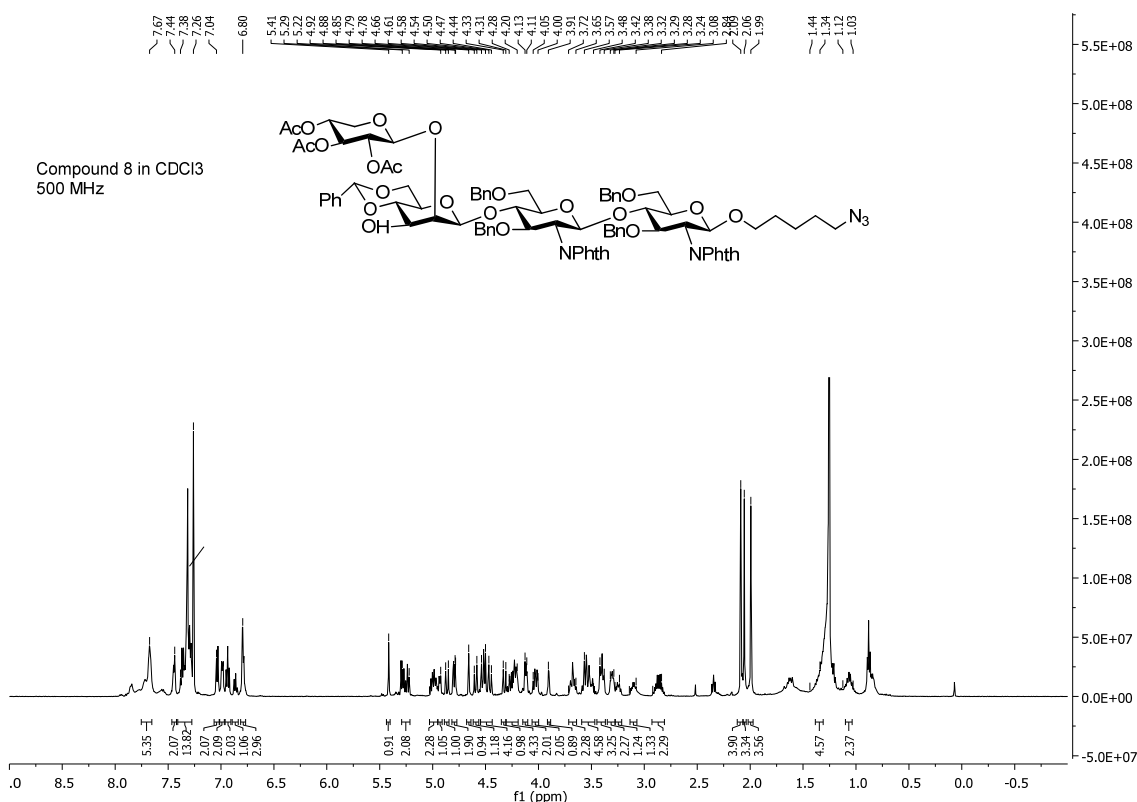
8. NMR spectra

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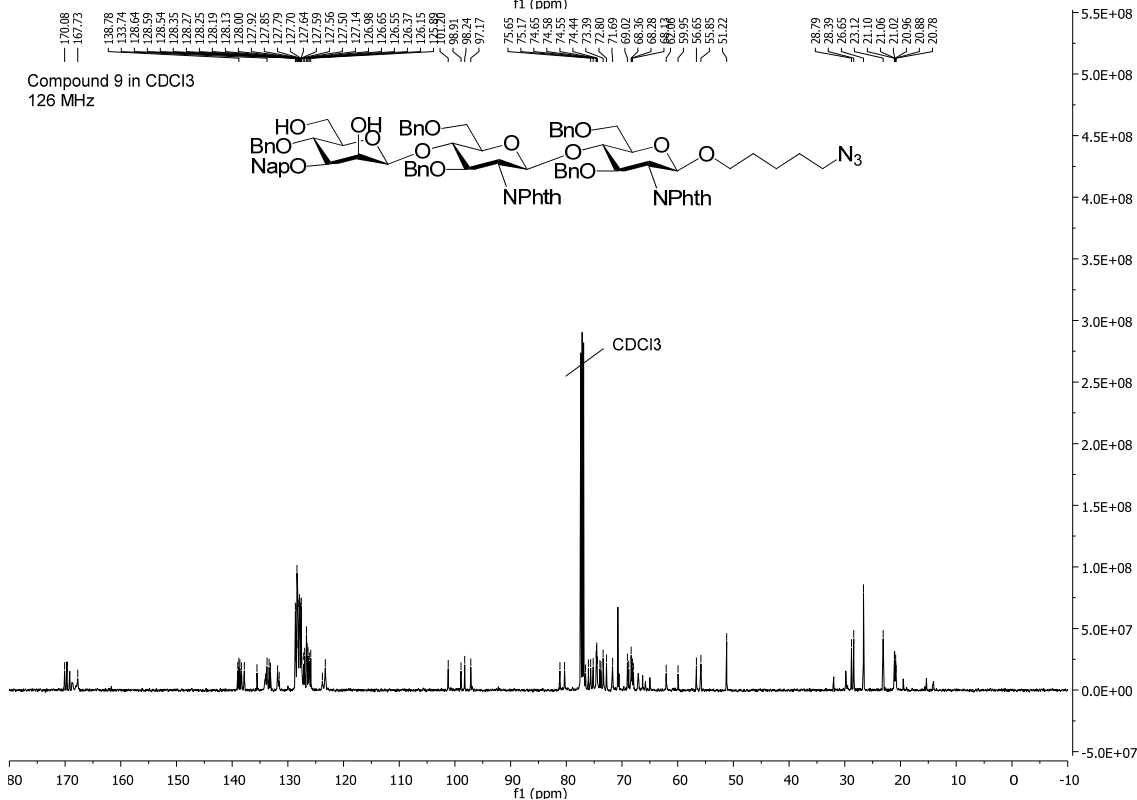
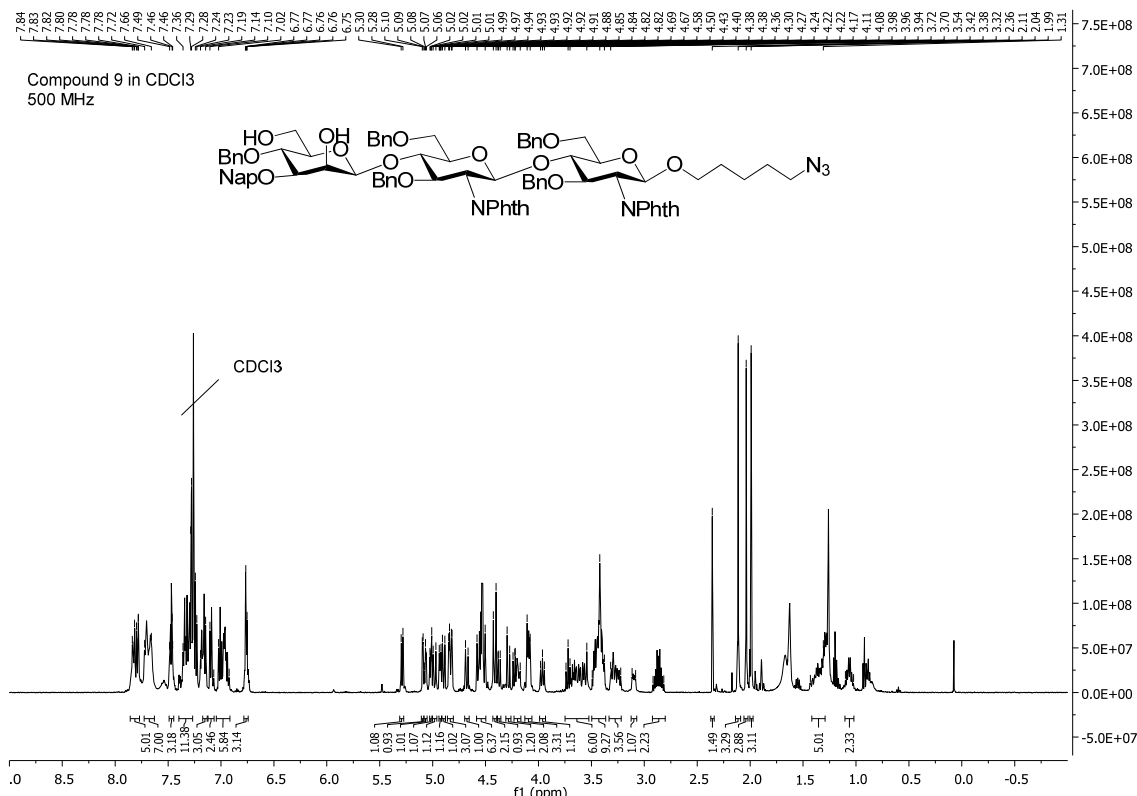


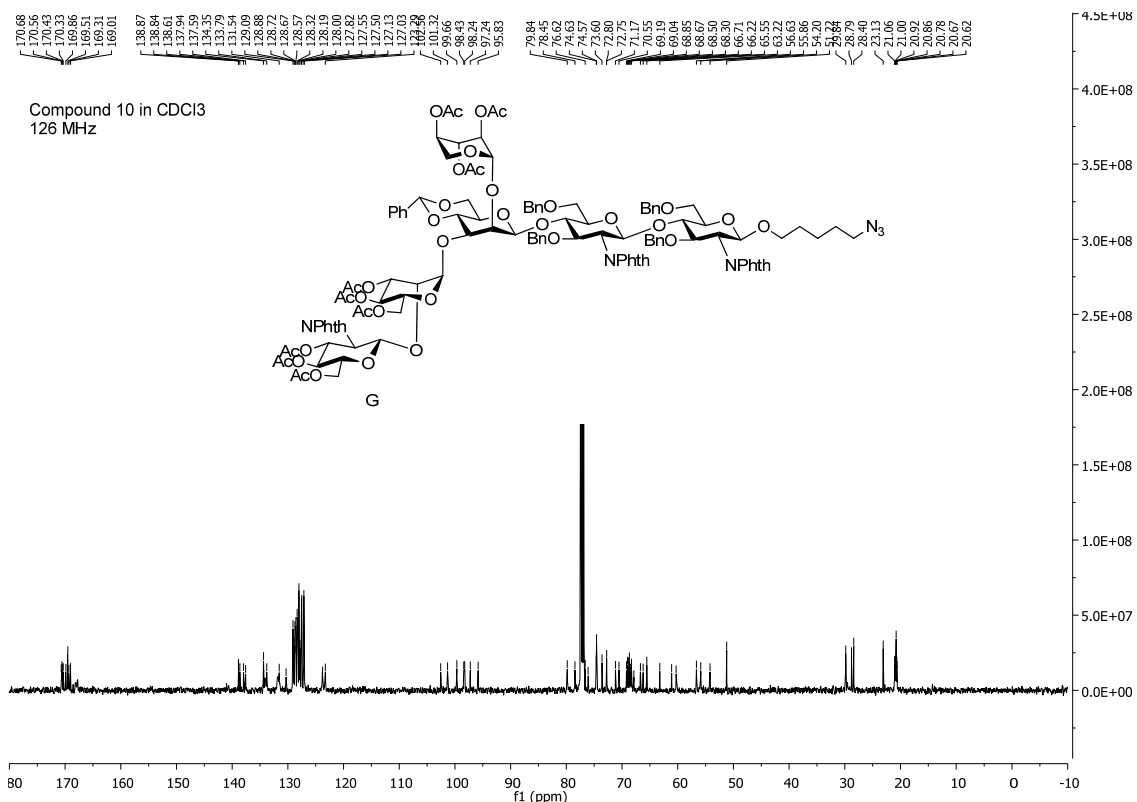
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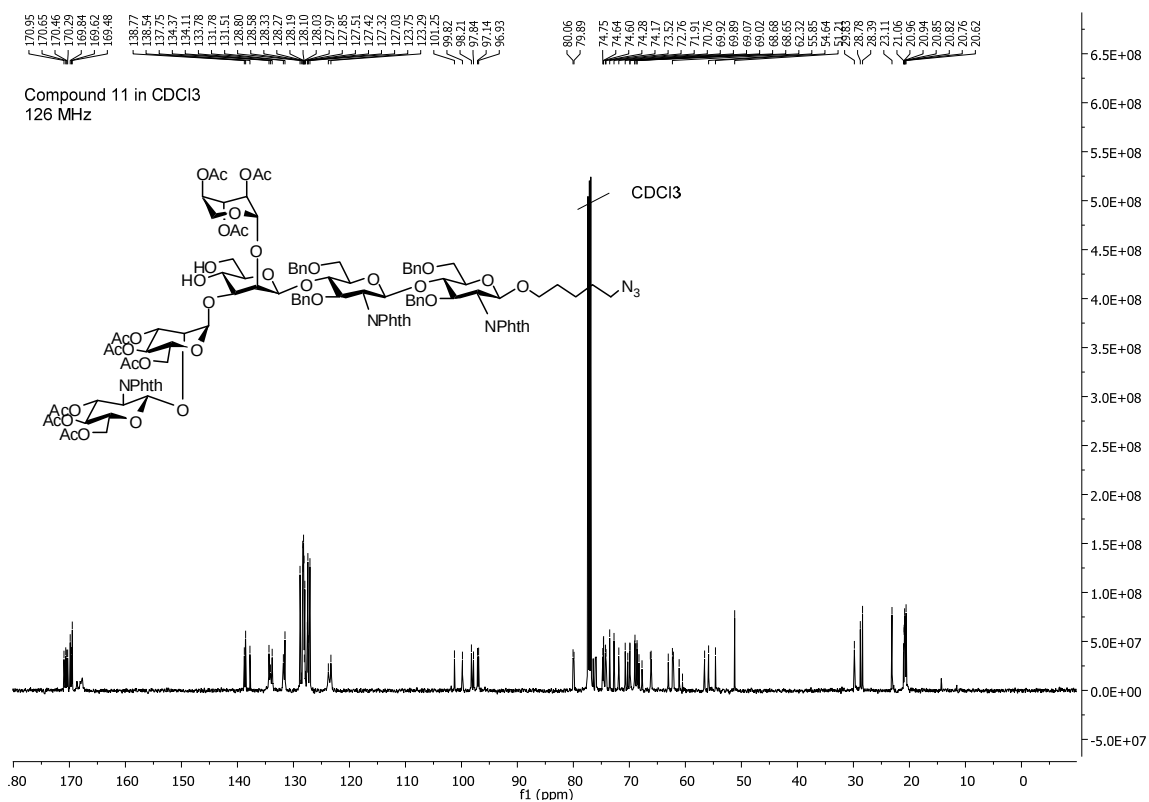


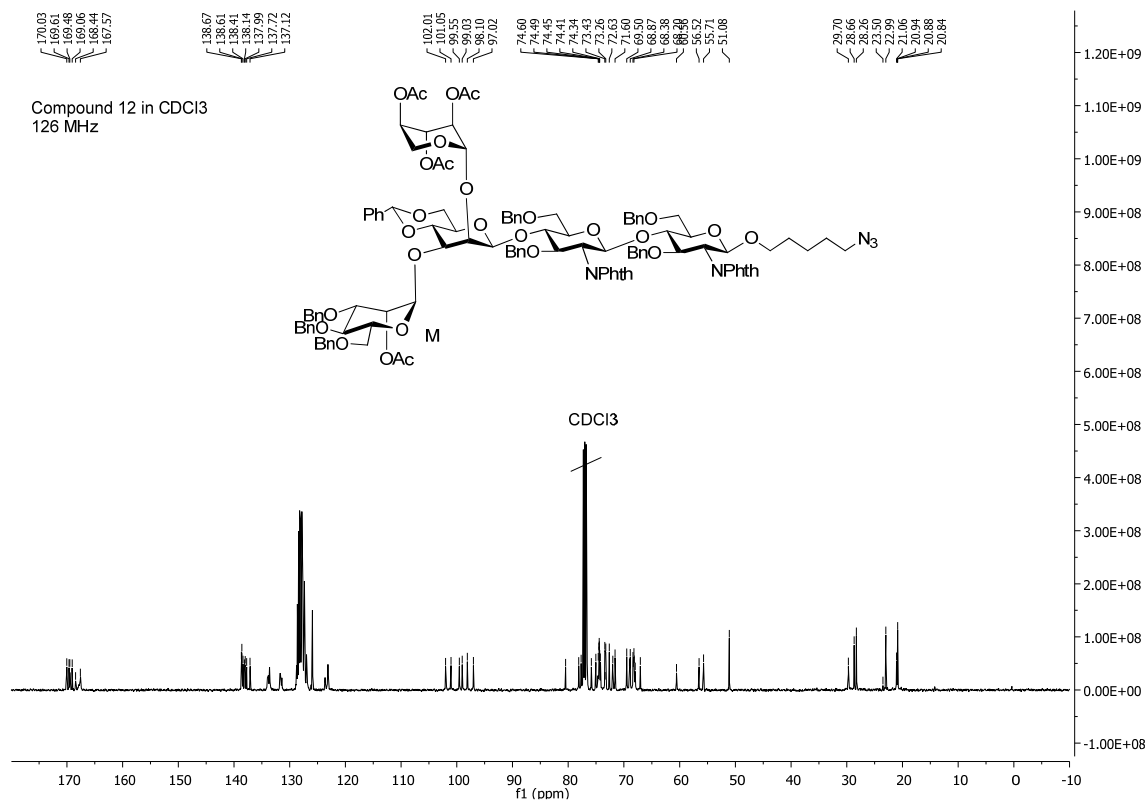
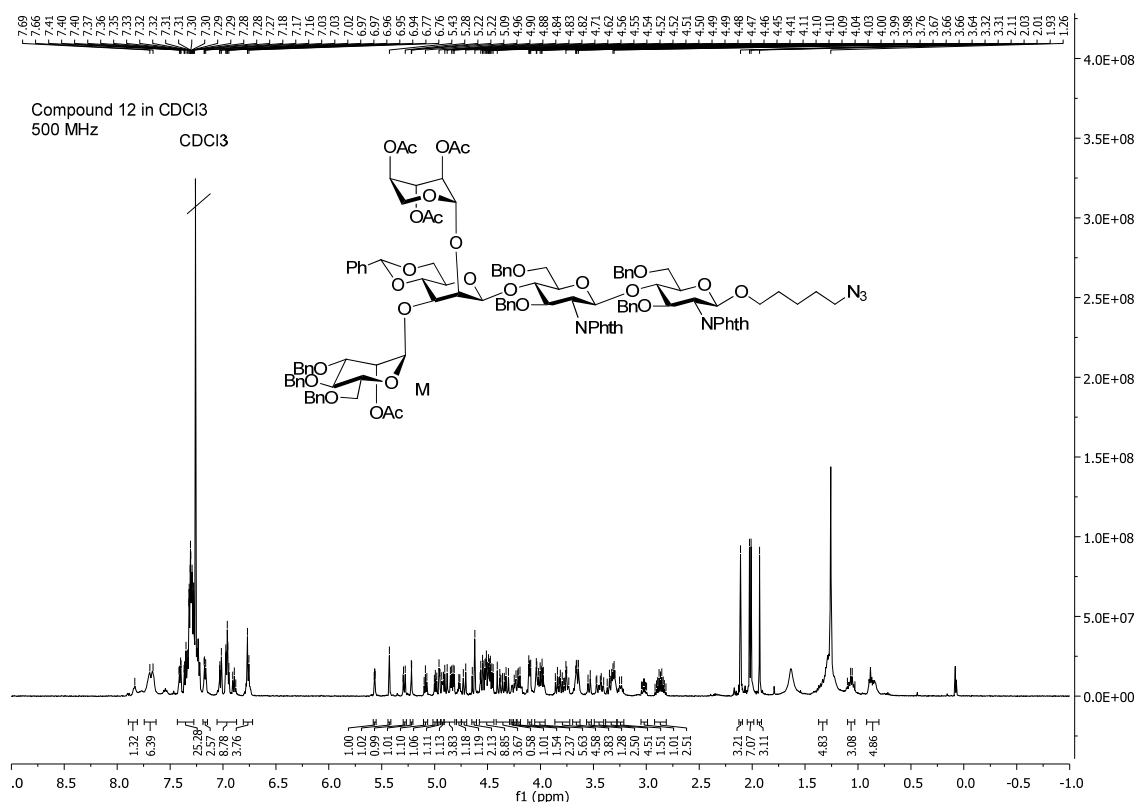


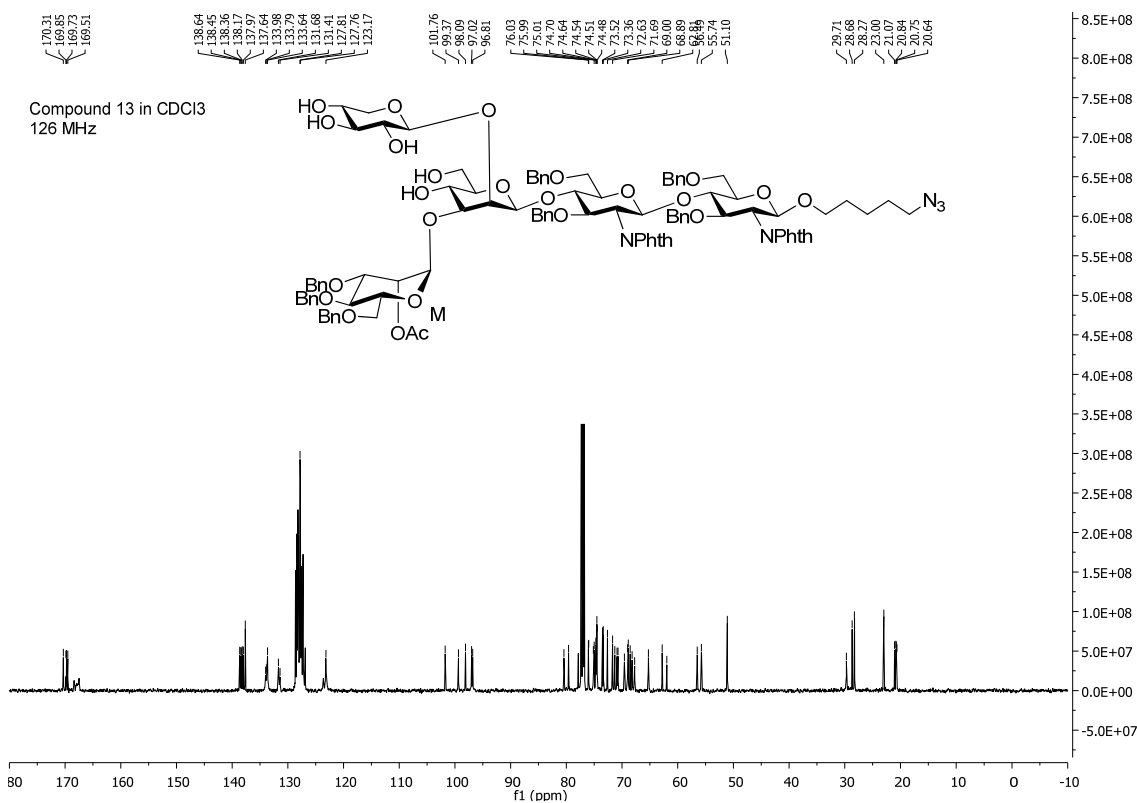
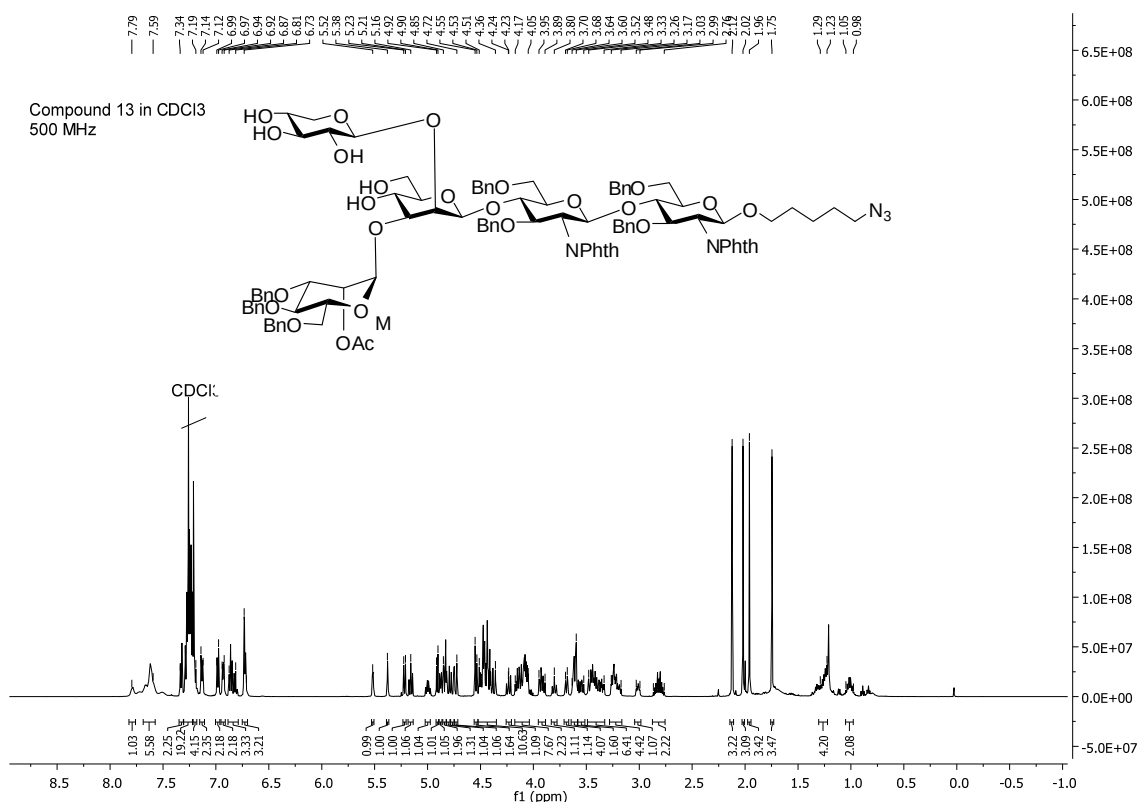
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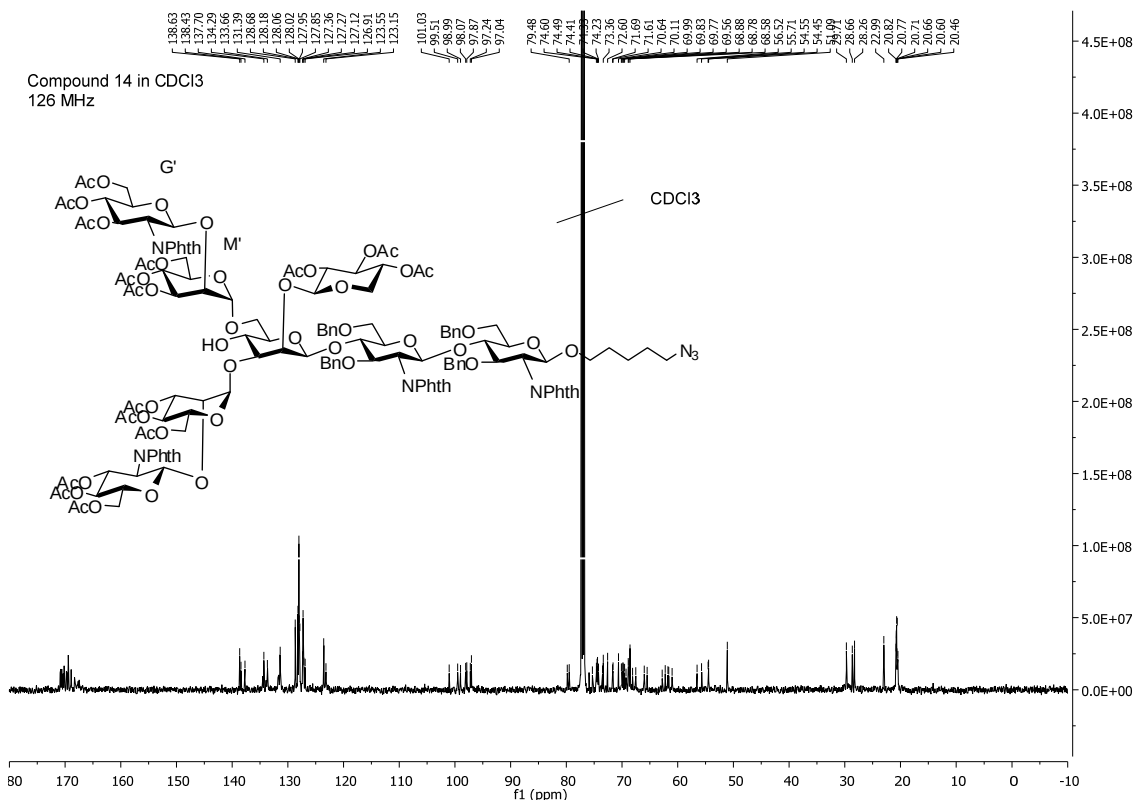
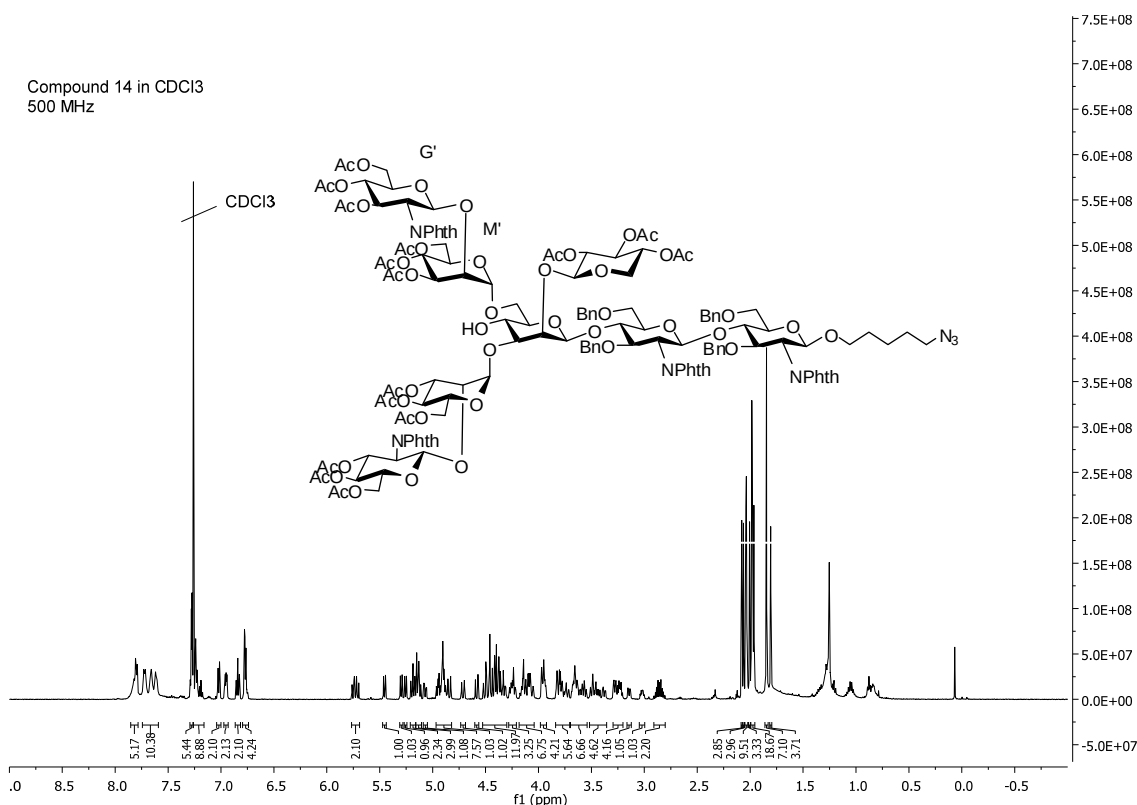


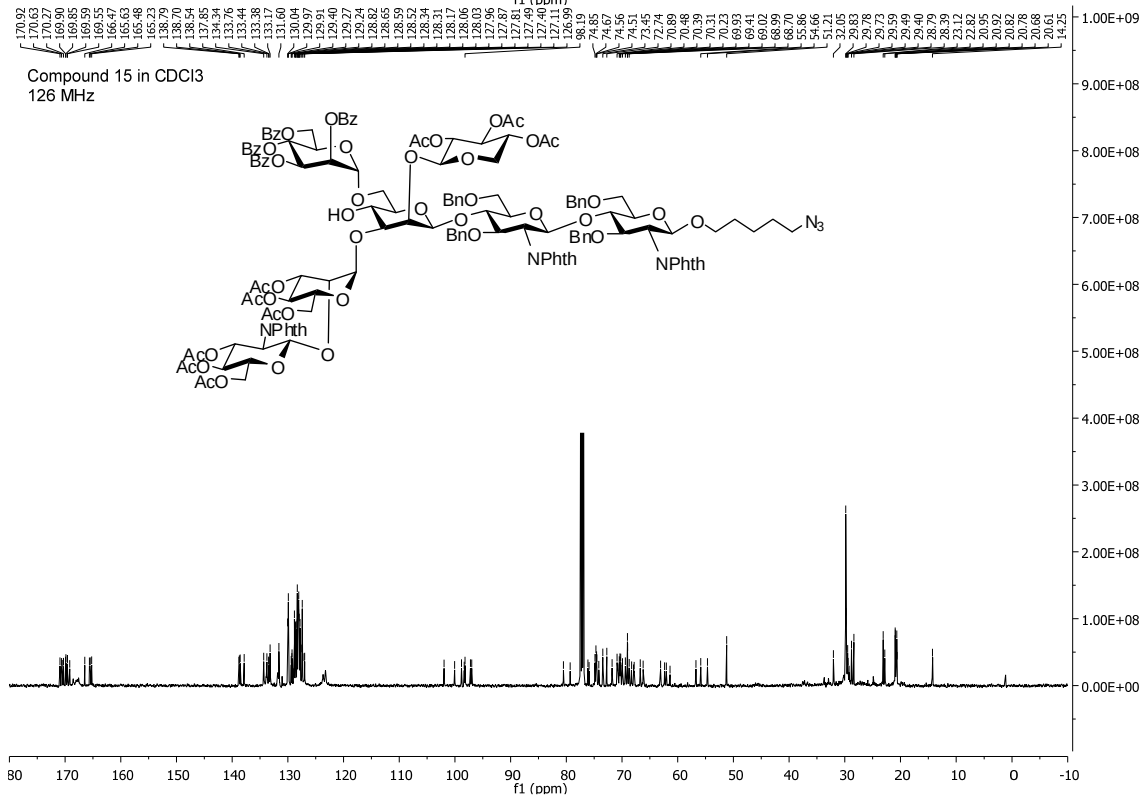
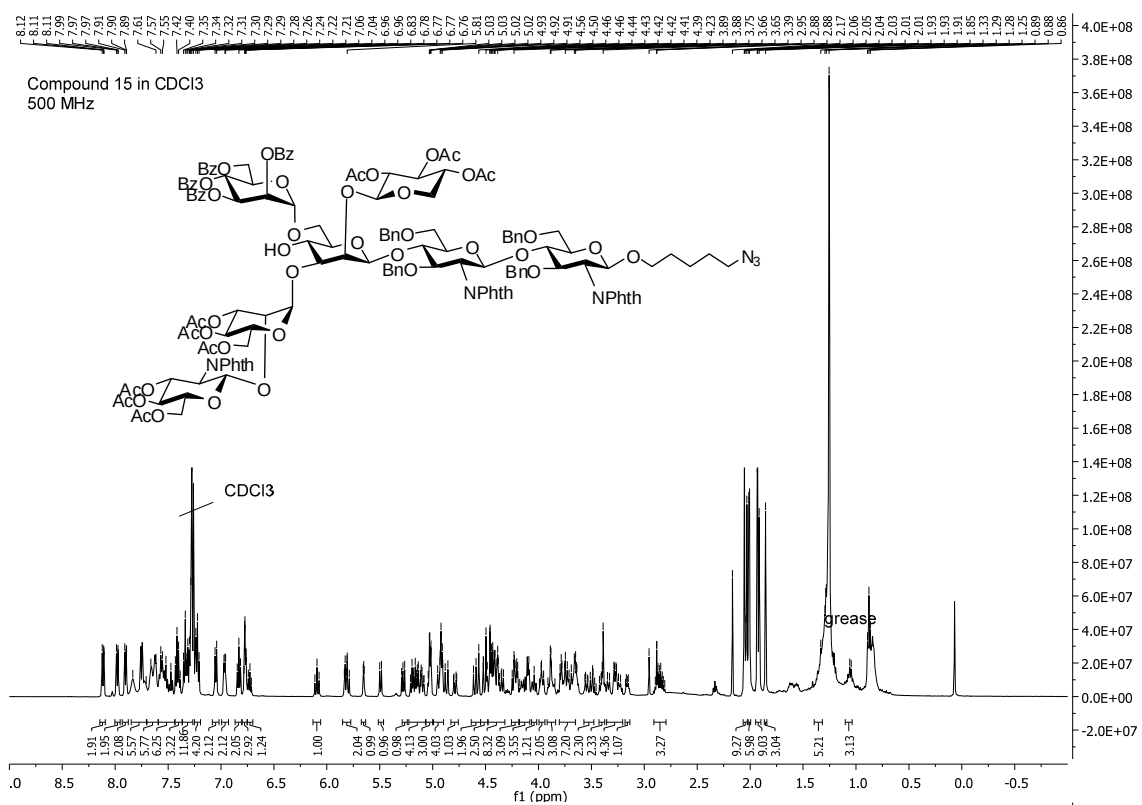


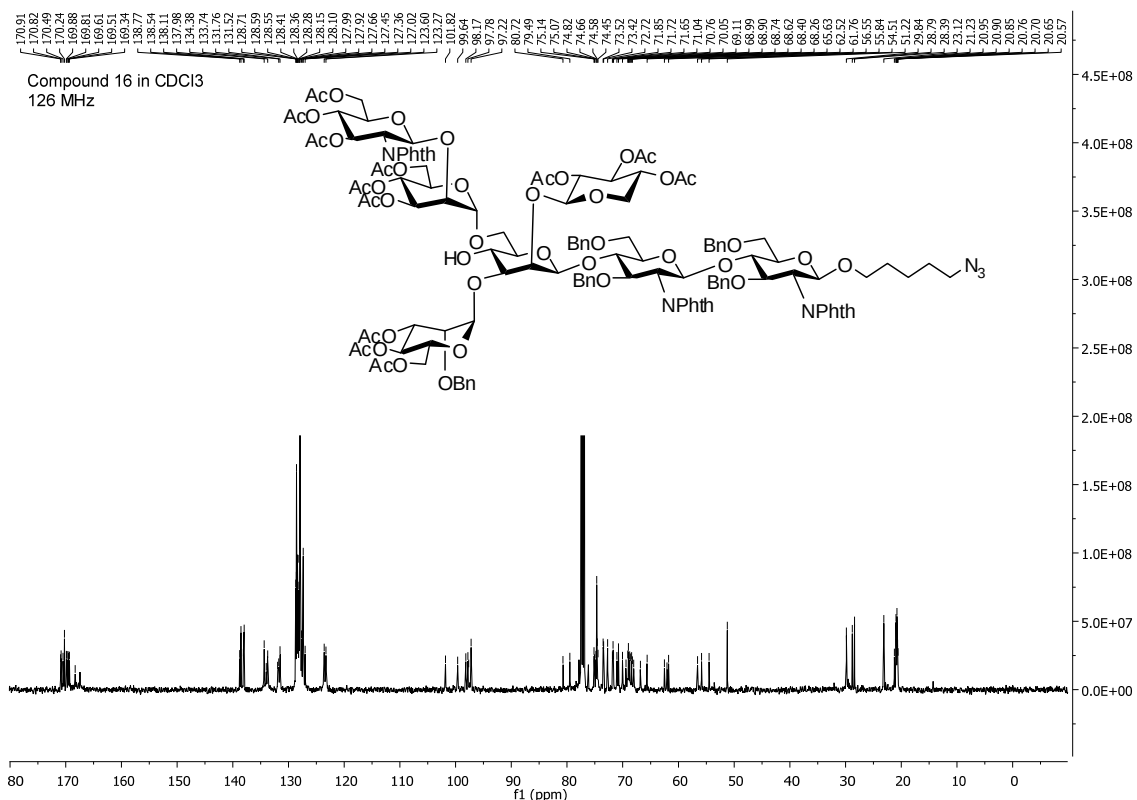




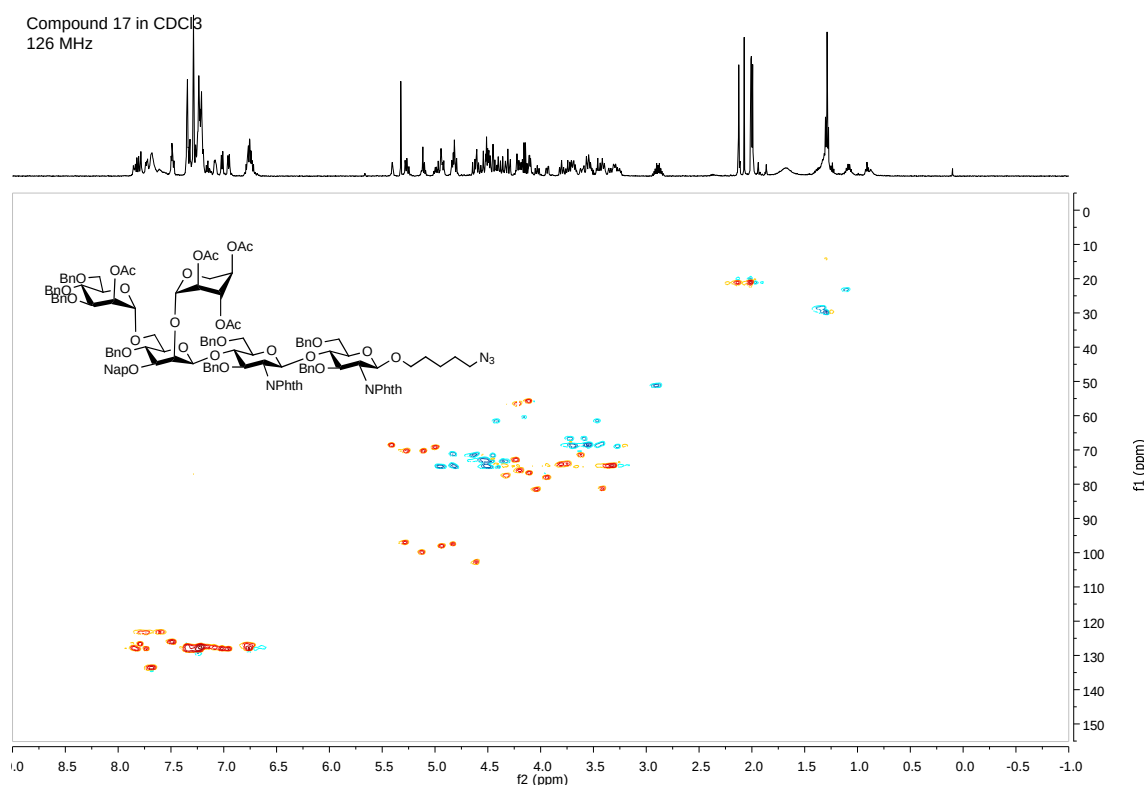
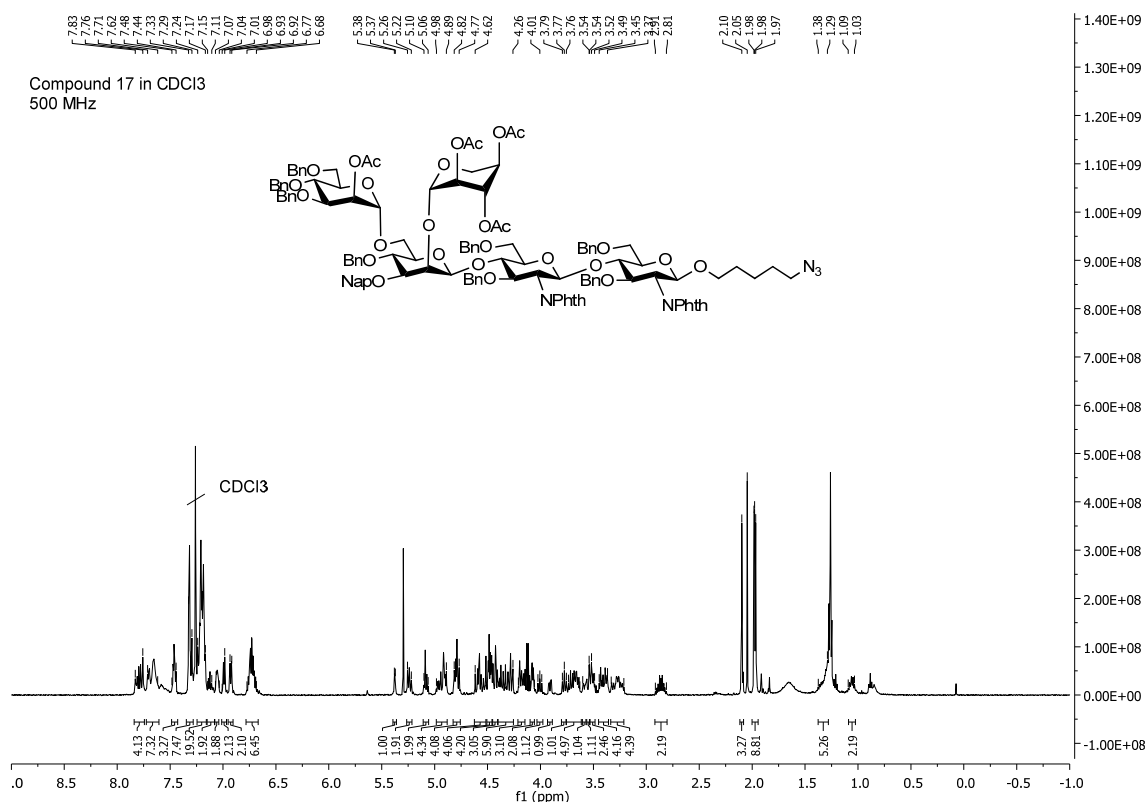




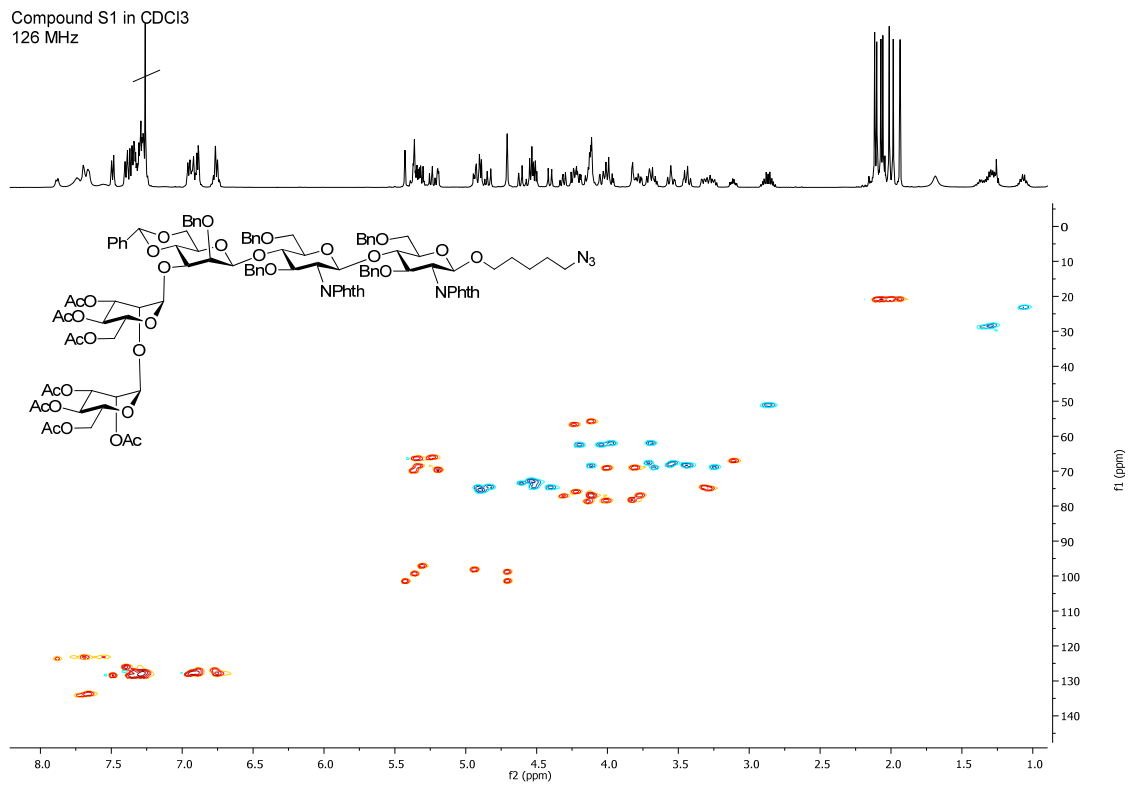
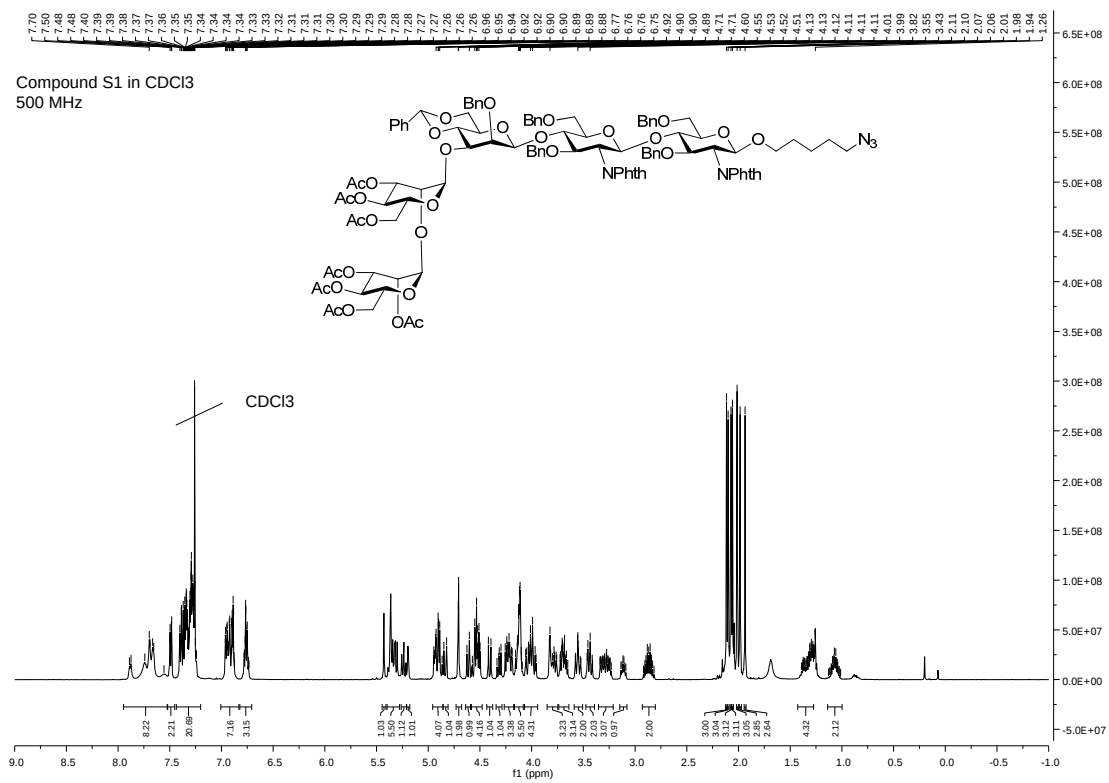


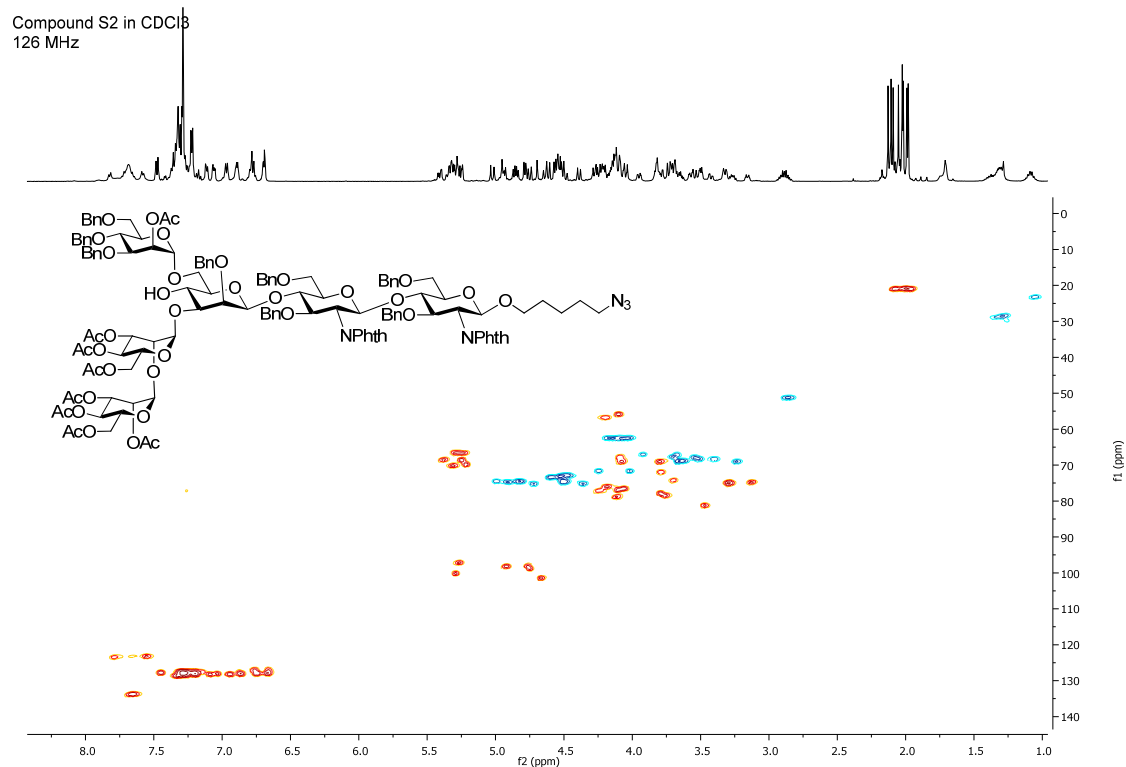


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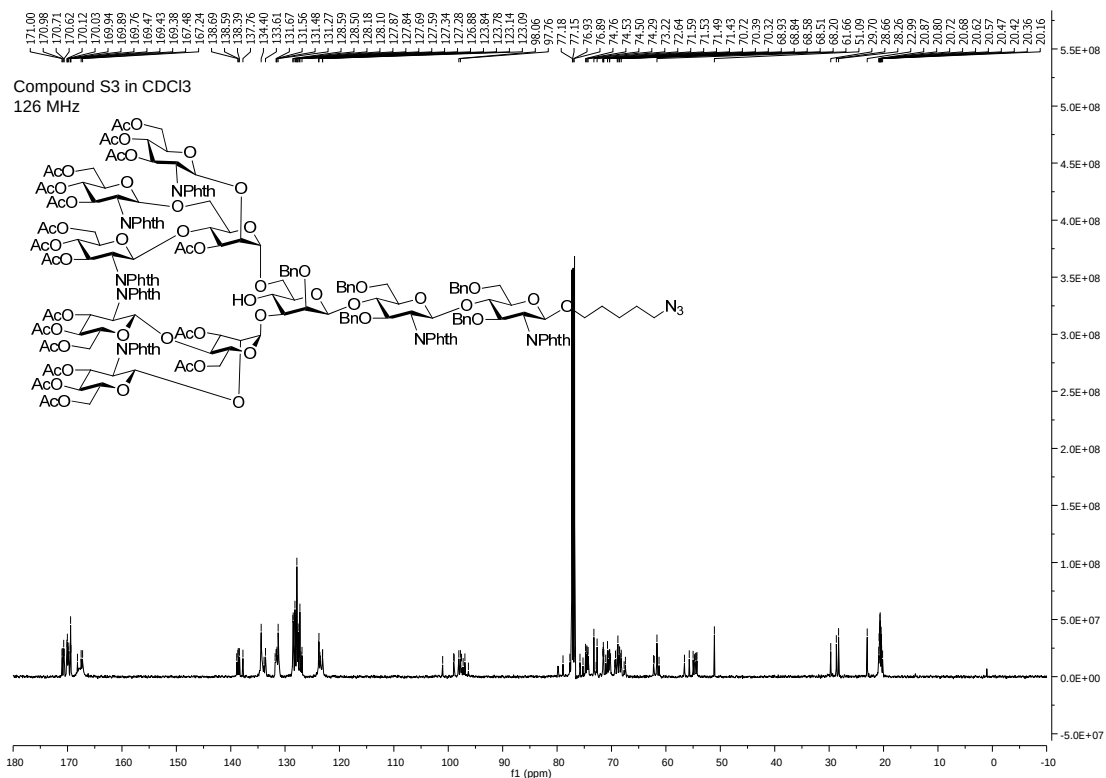
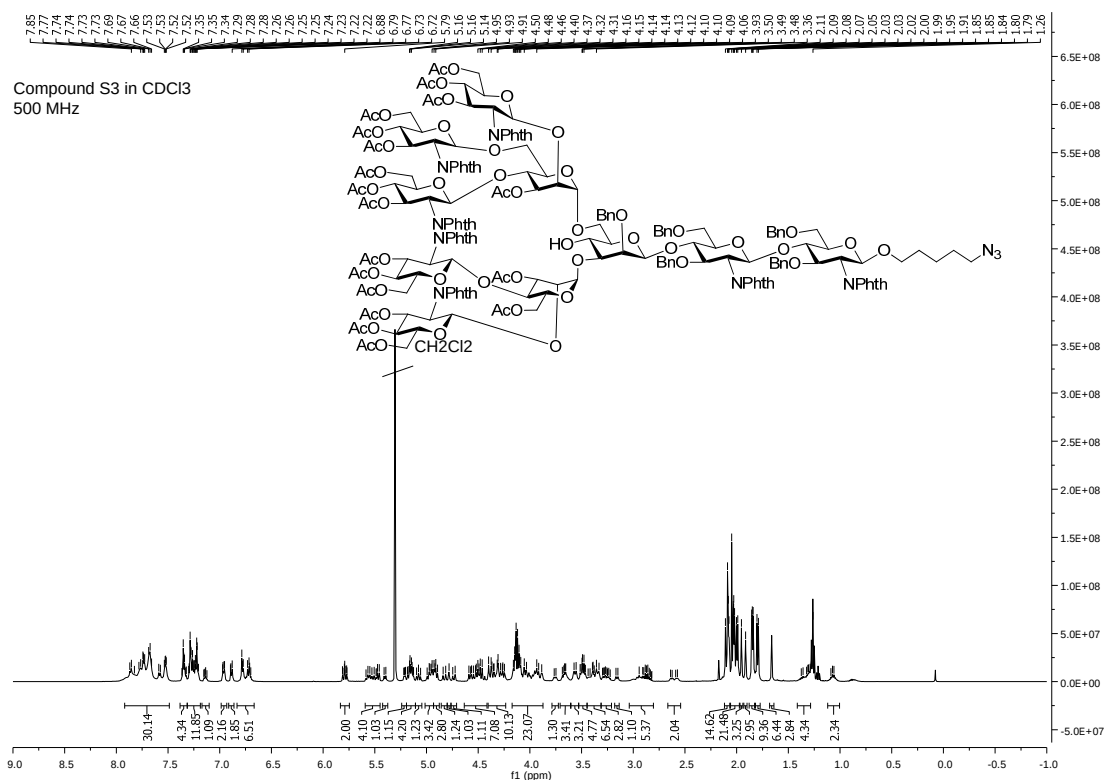


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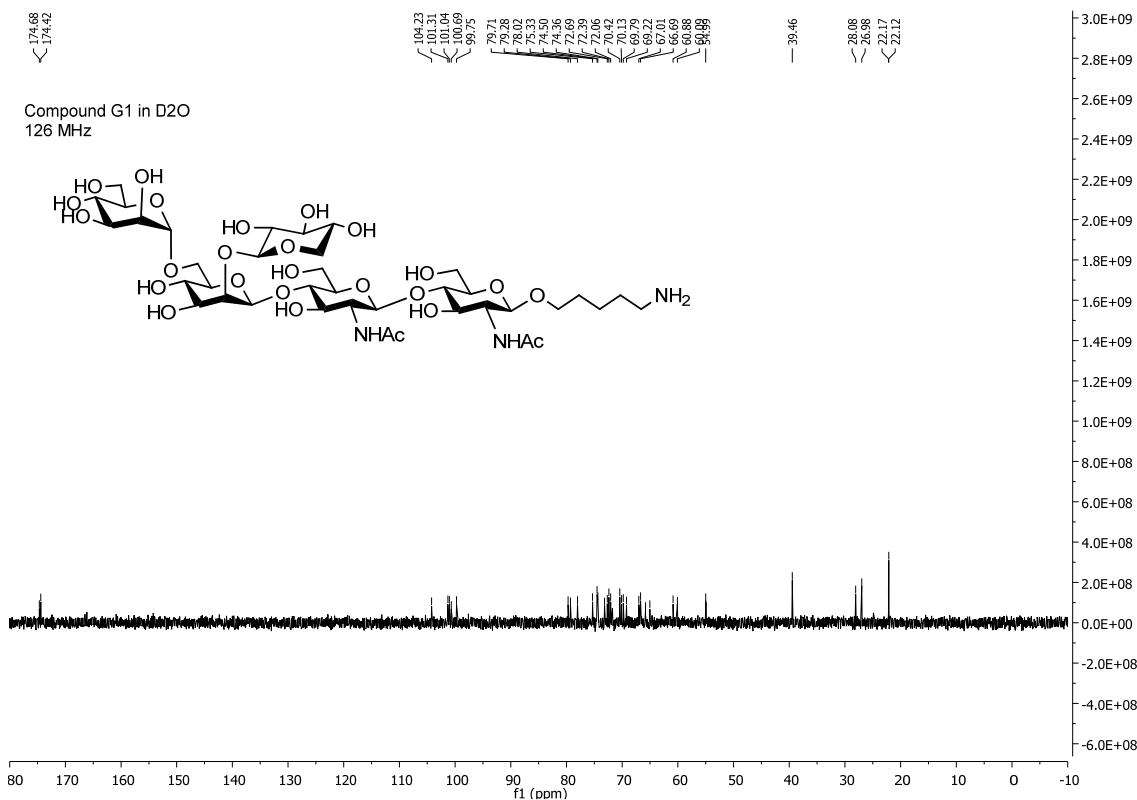
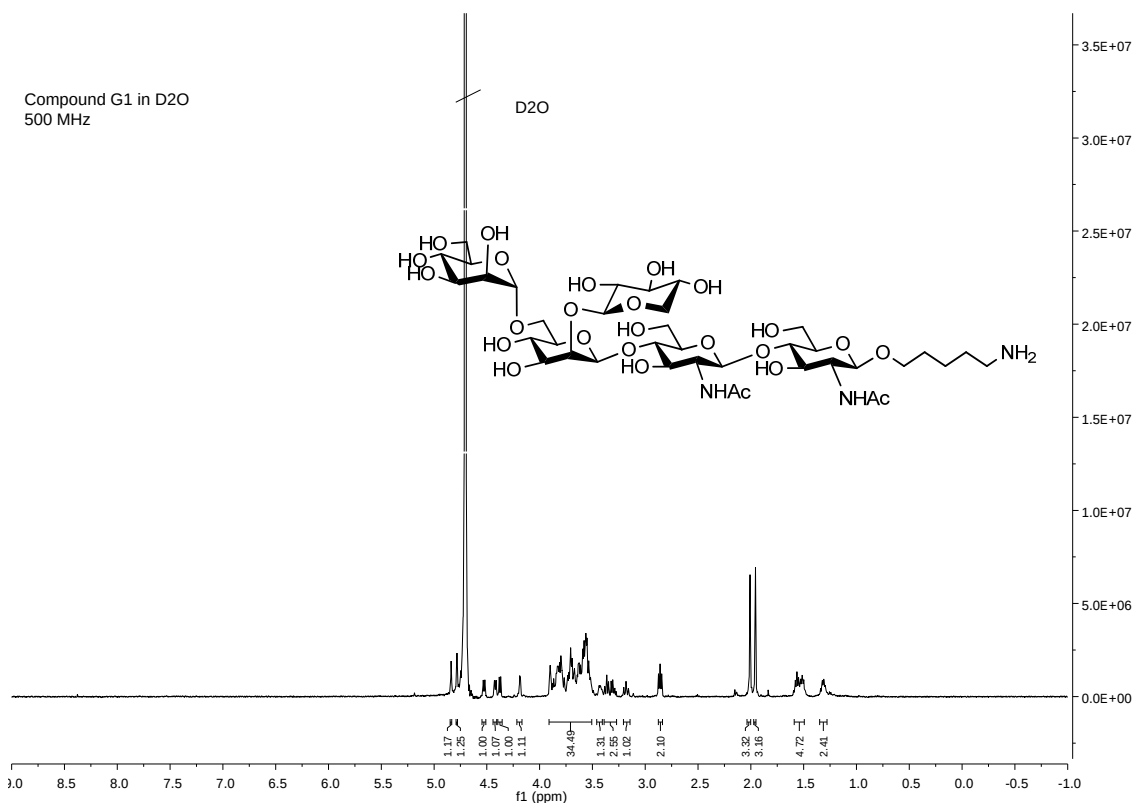




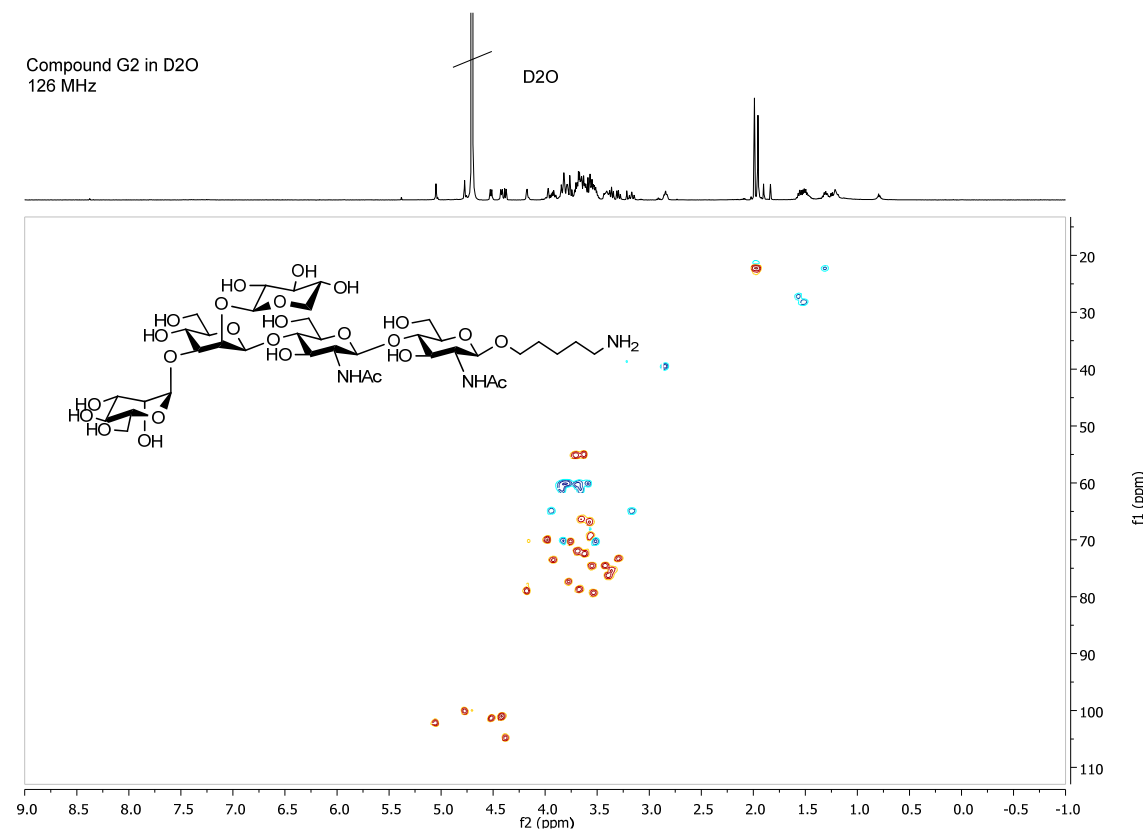
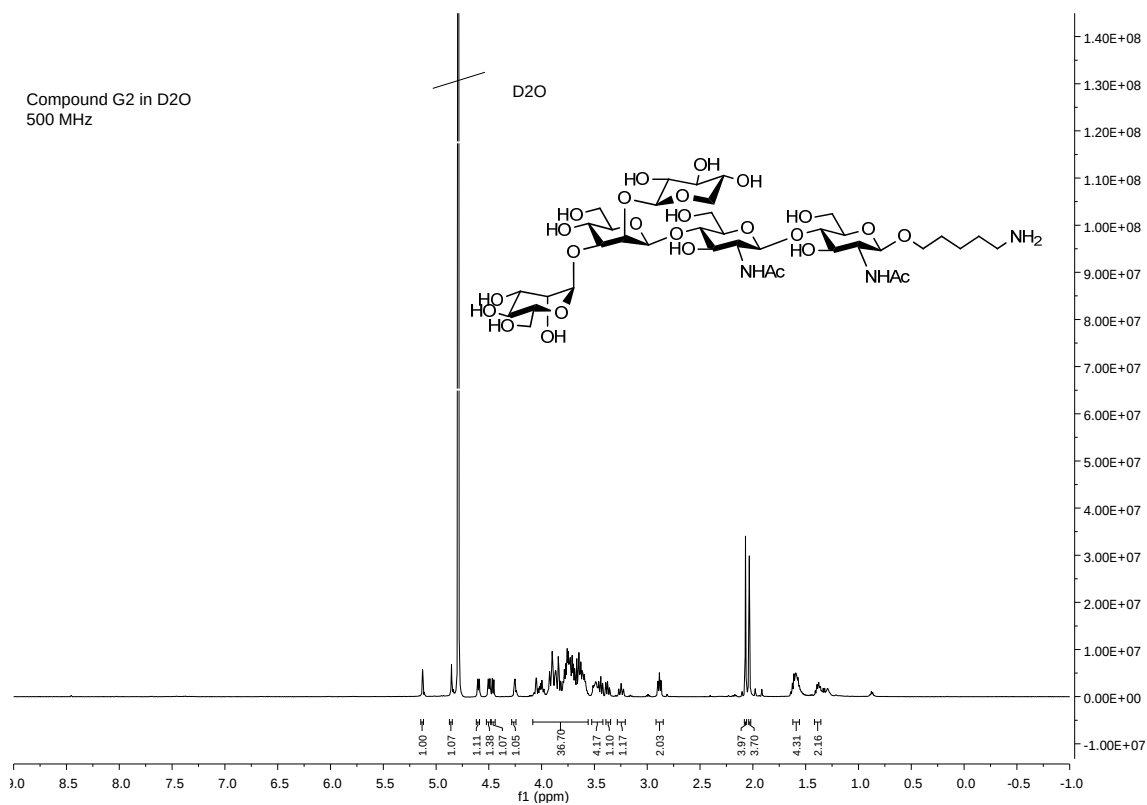
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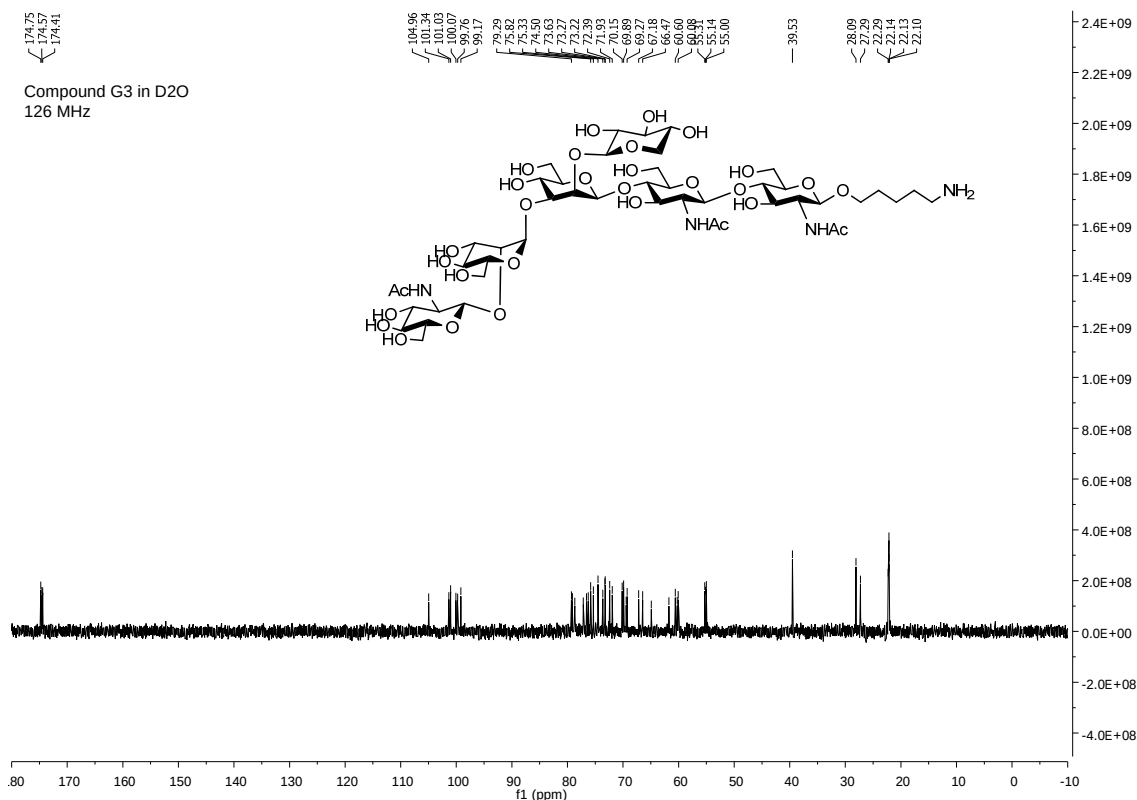
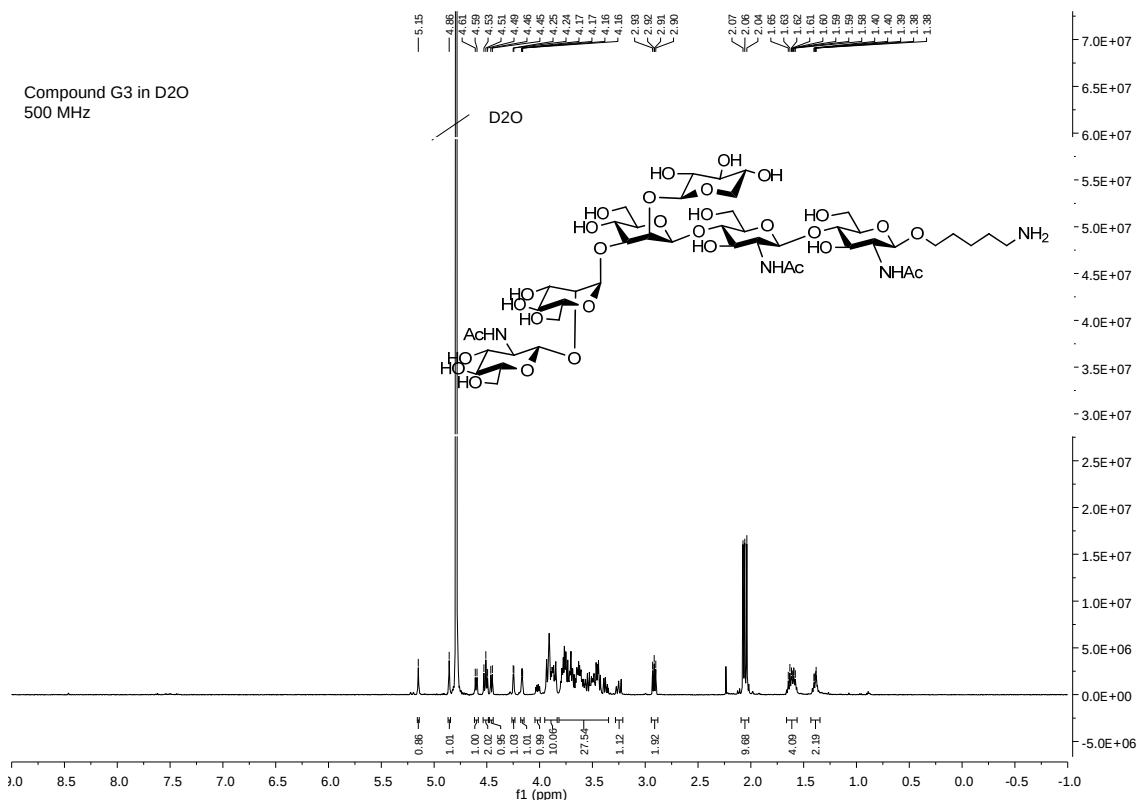
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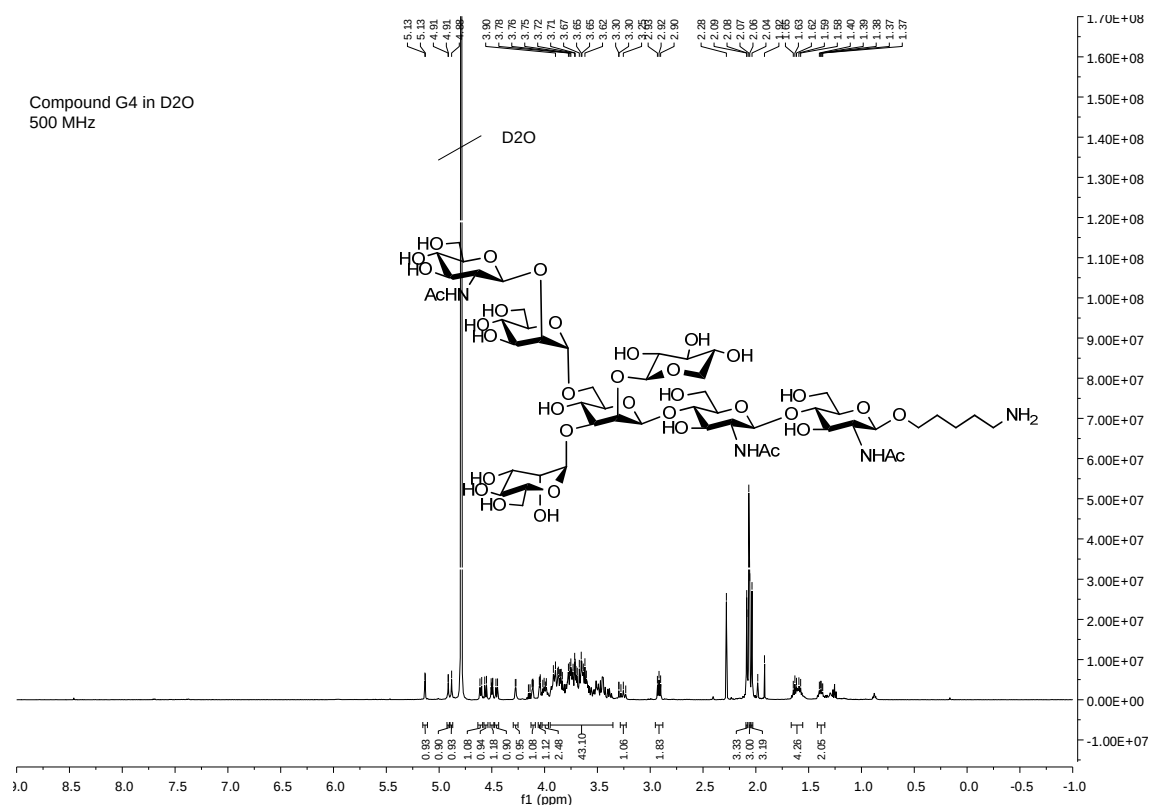


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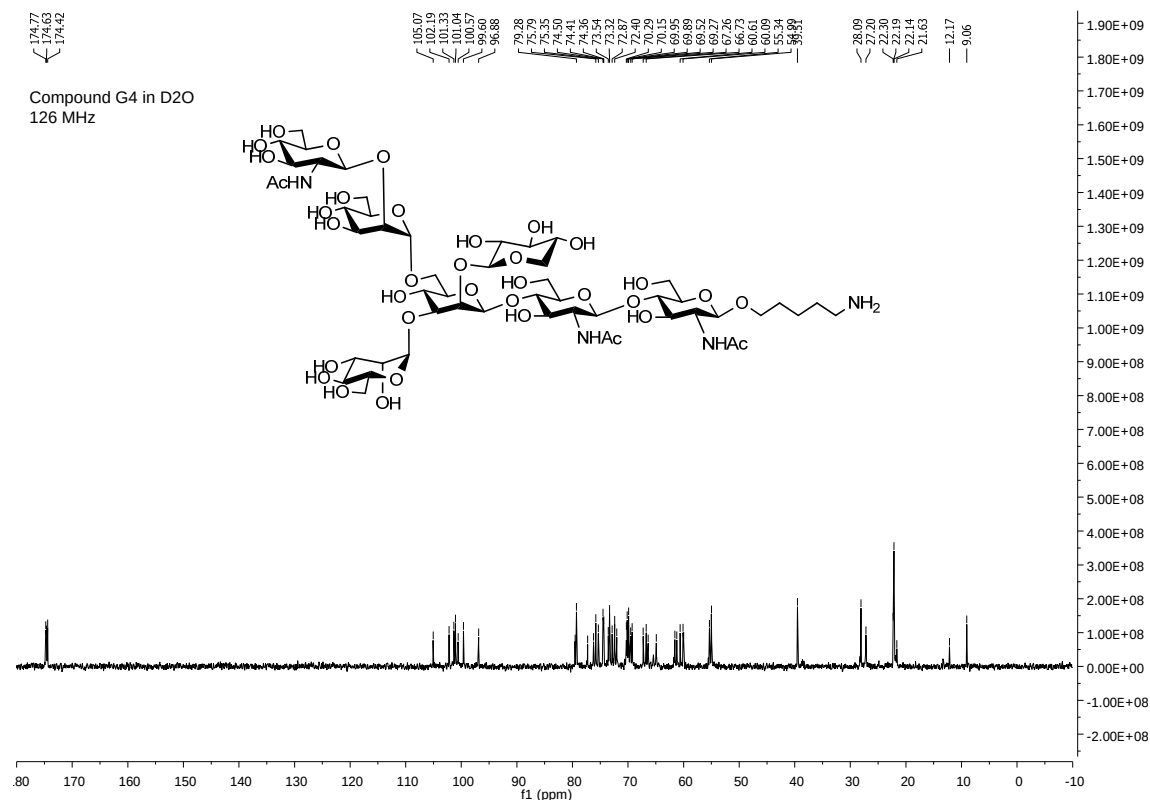


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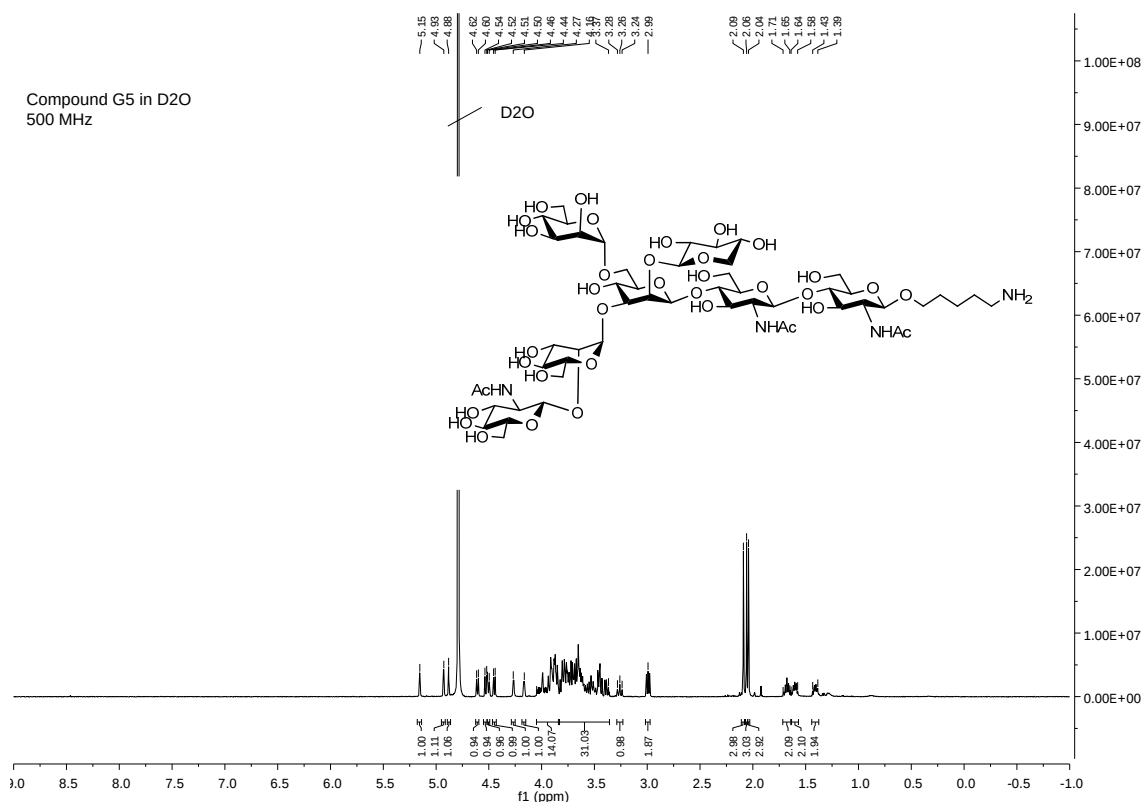


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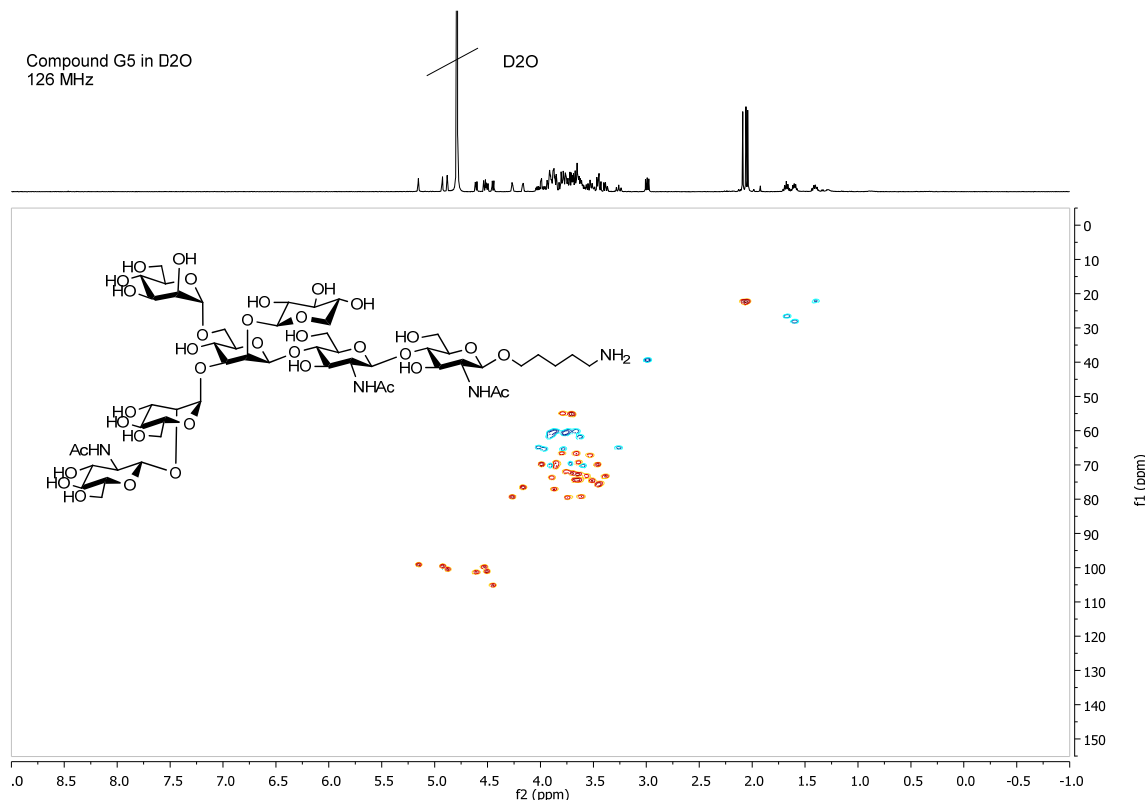


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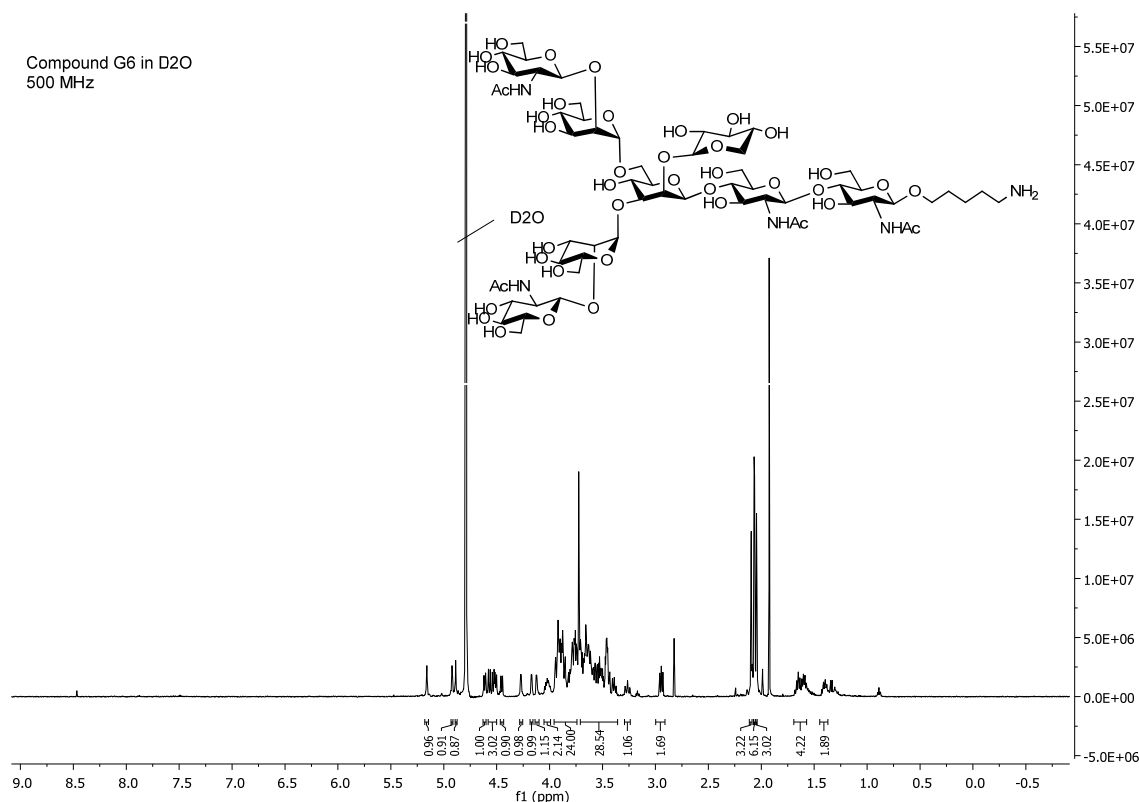


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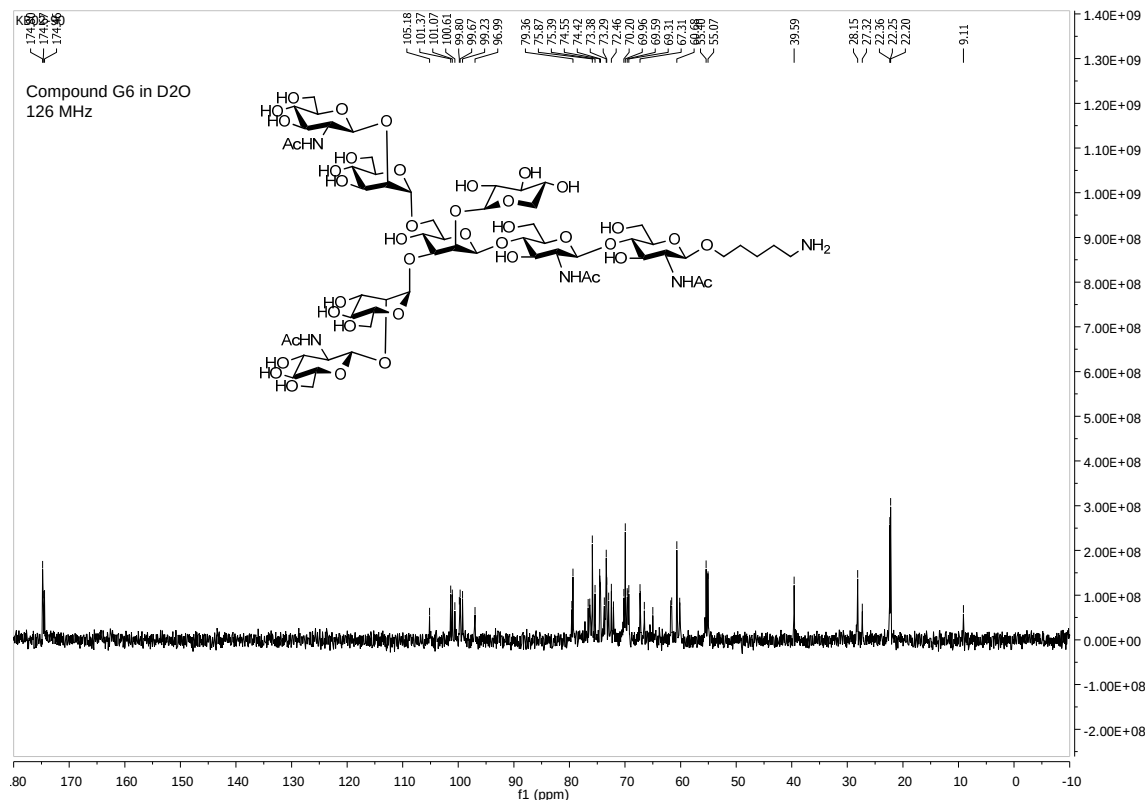


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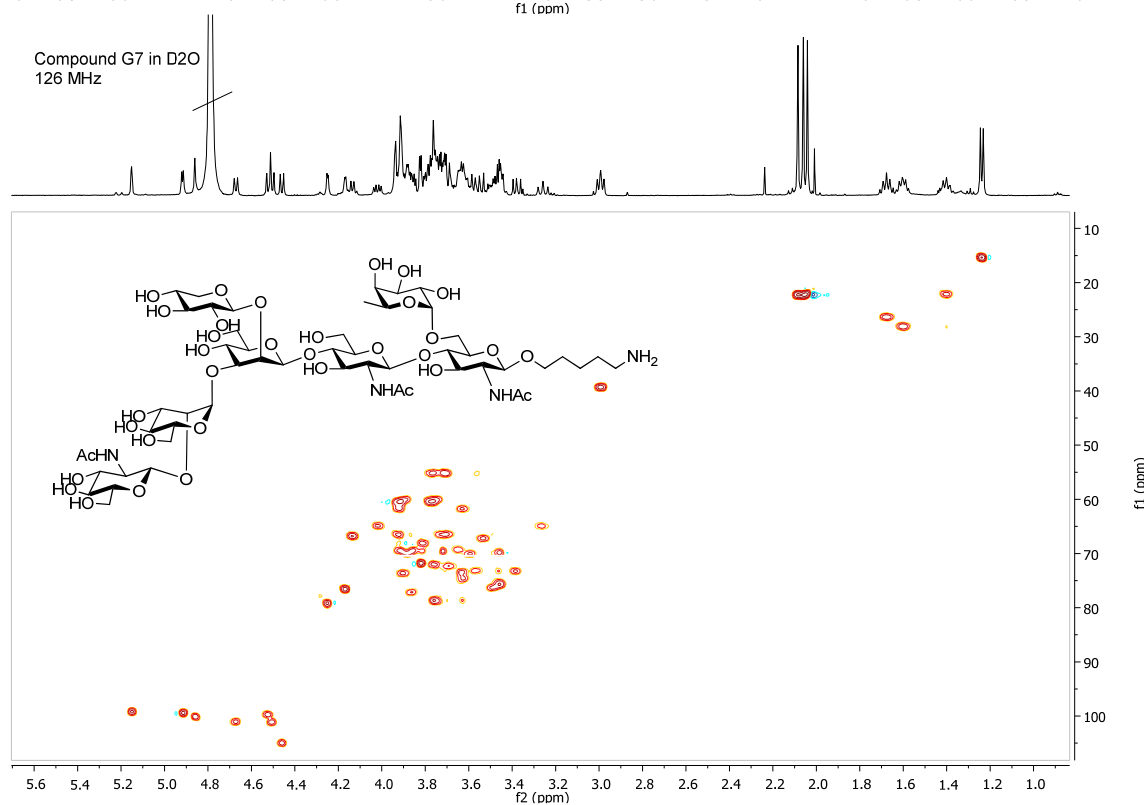
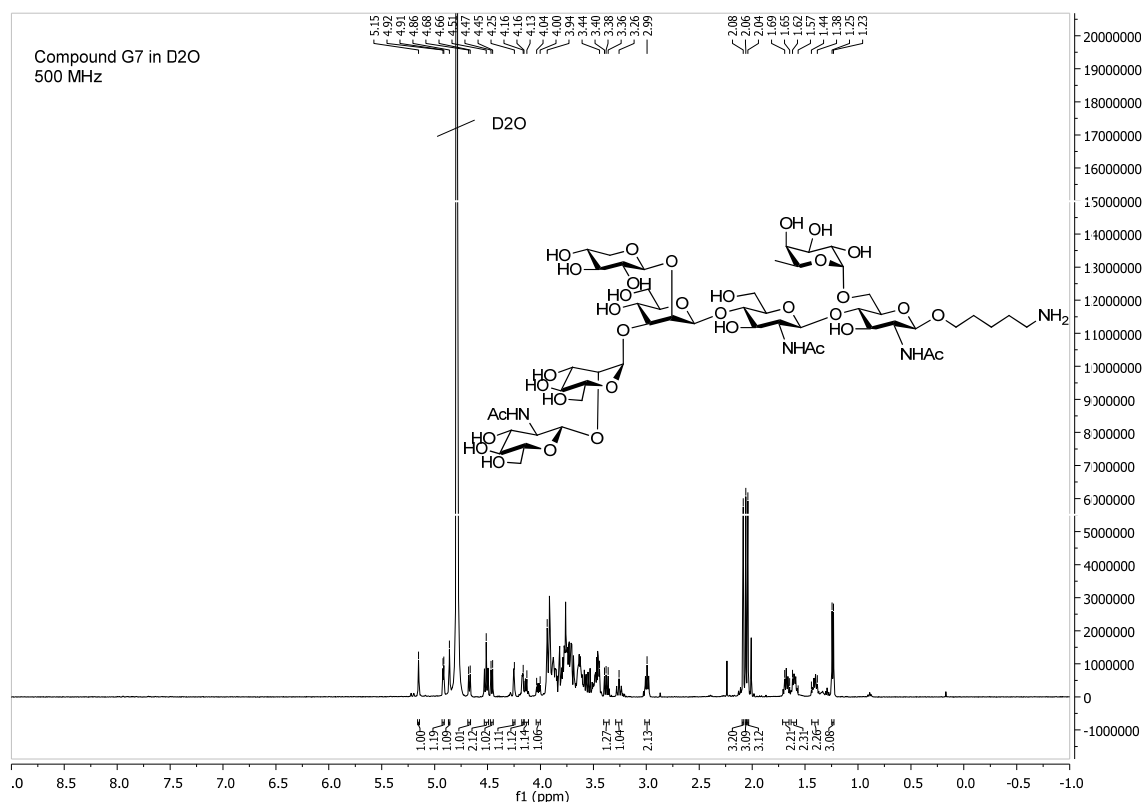
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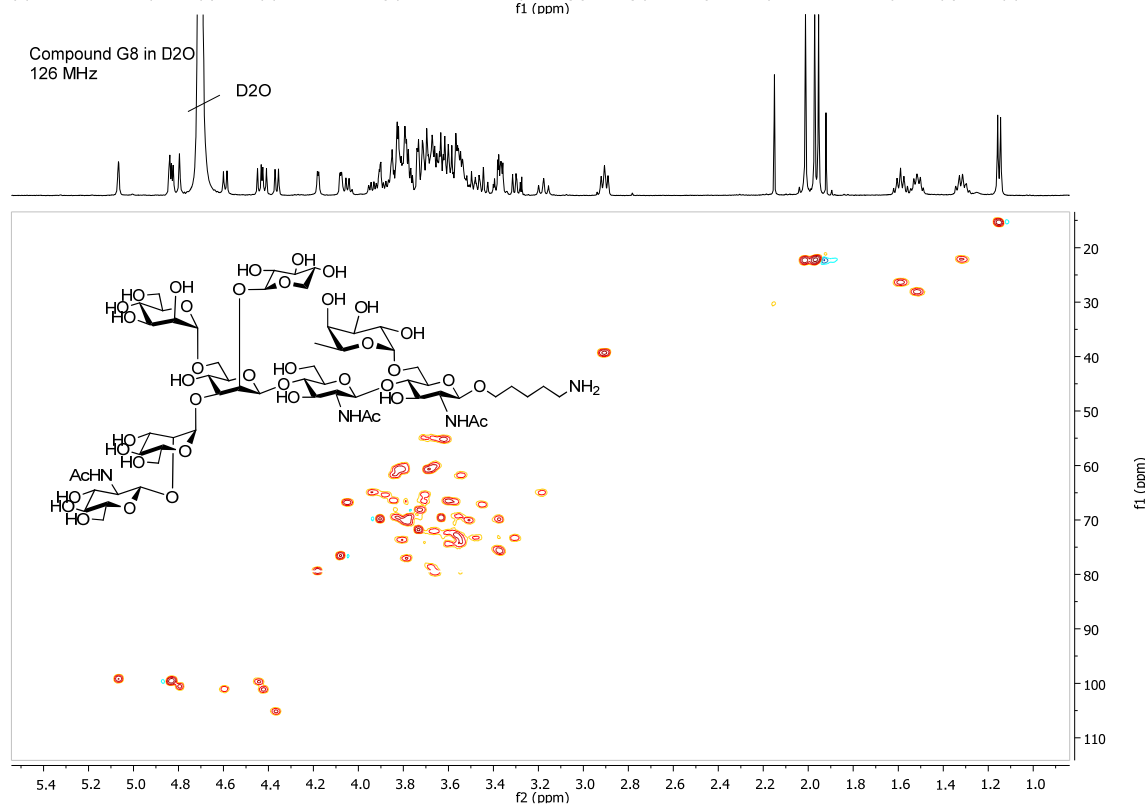
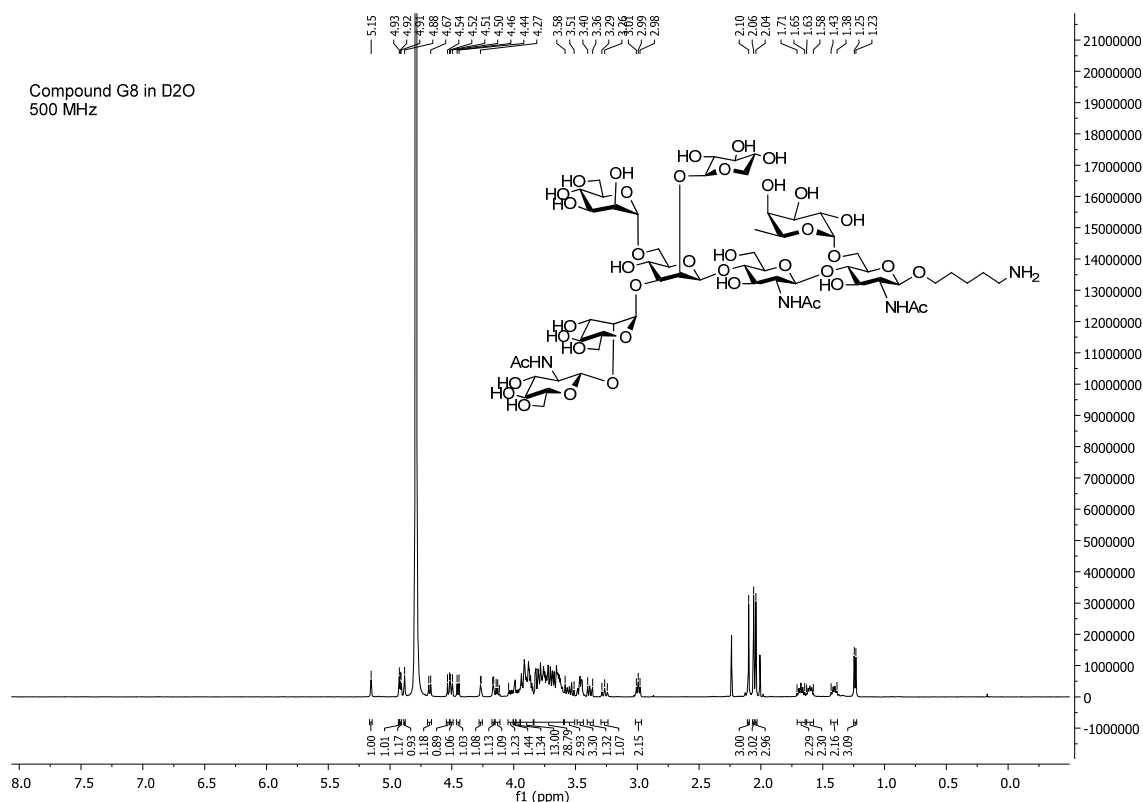
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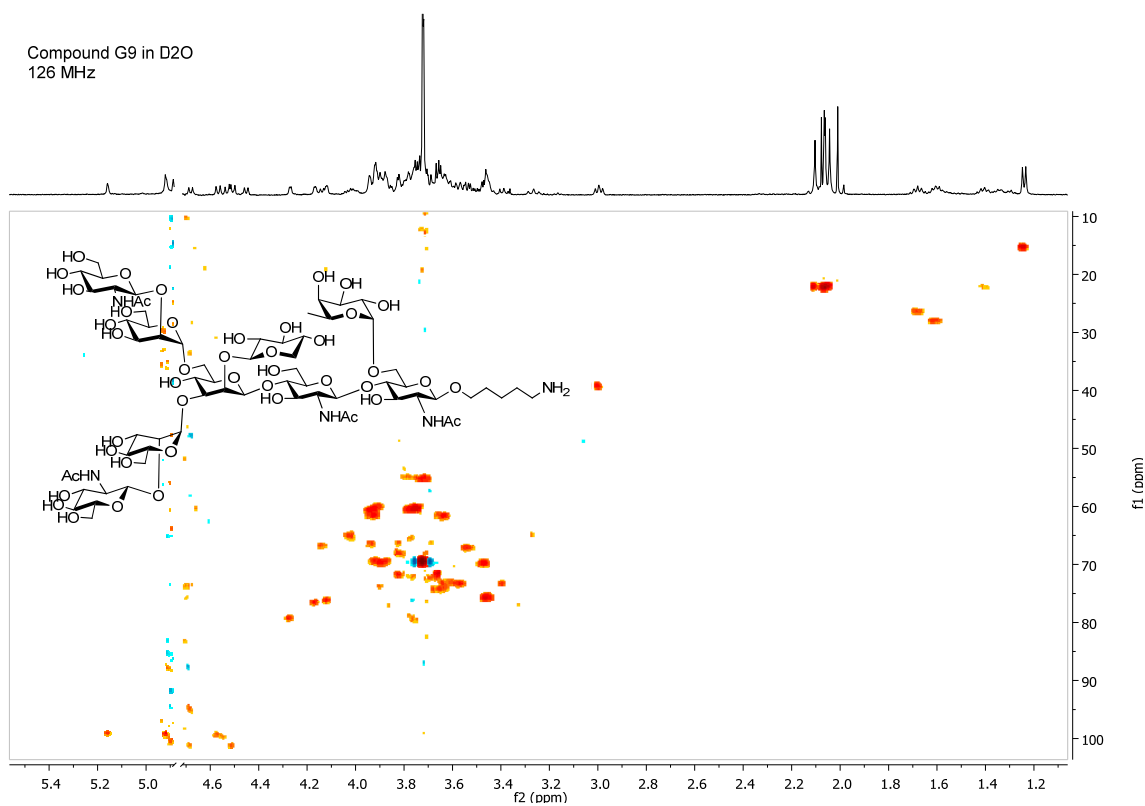
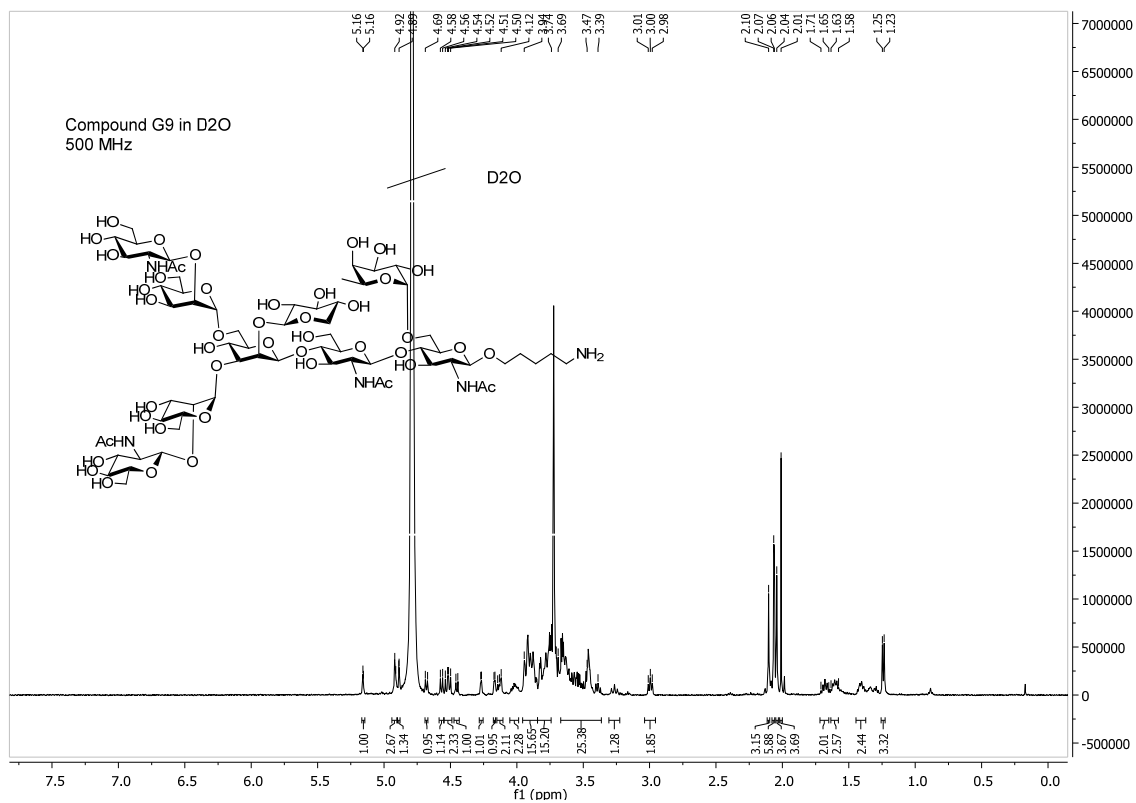
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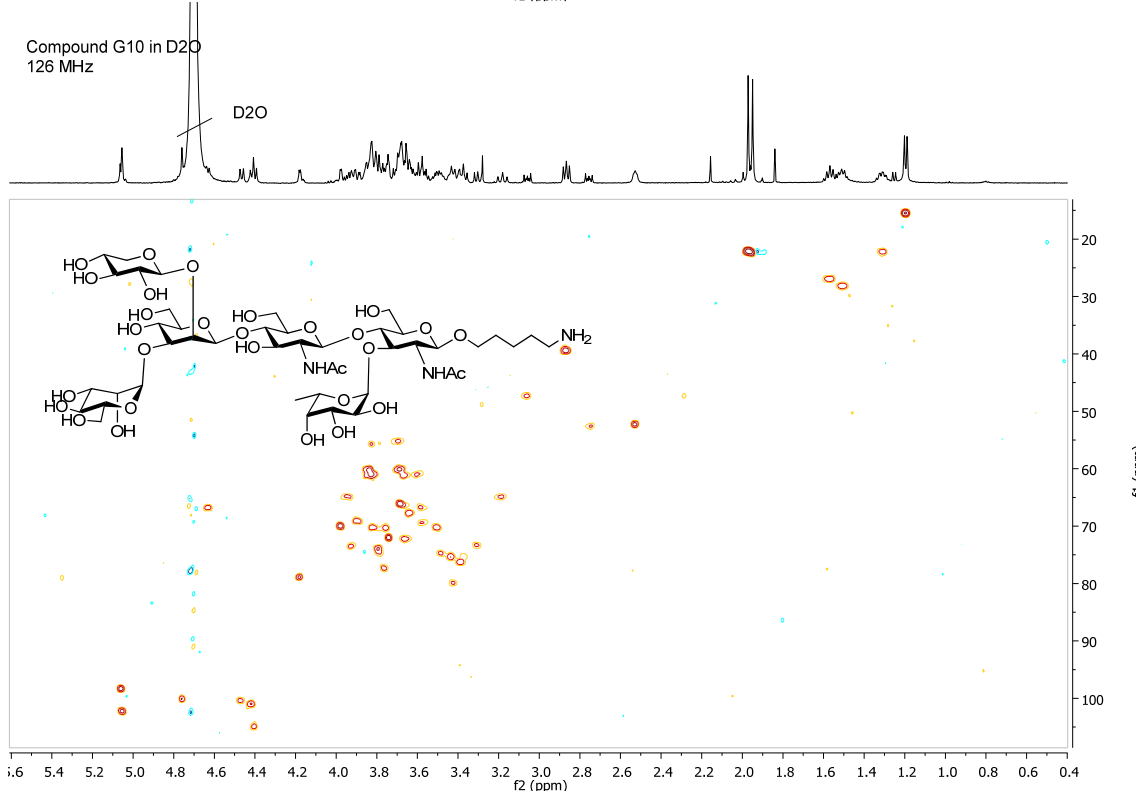
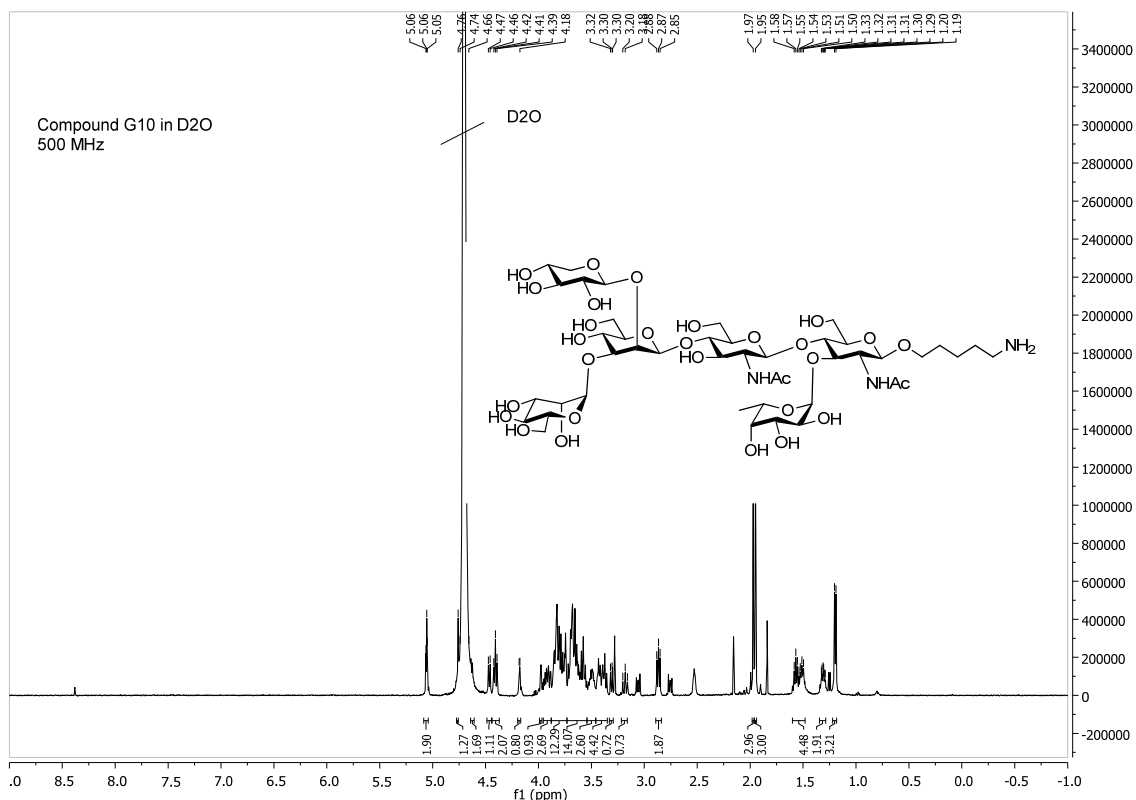
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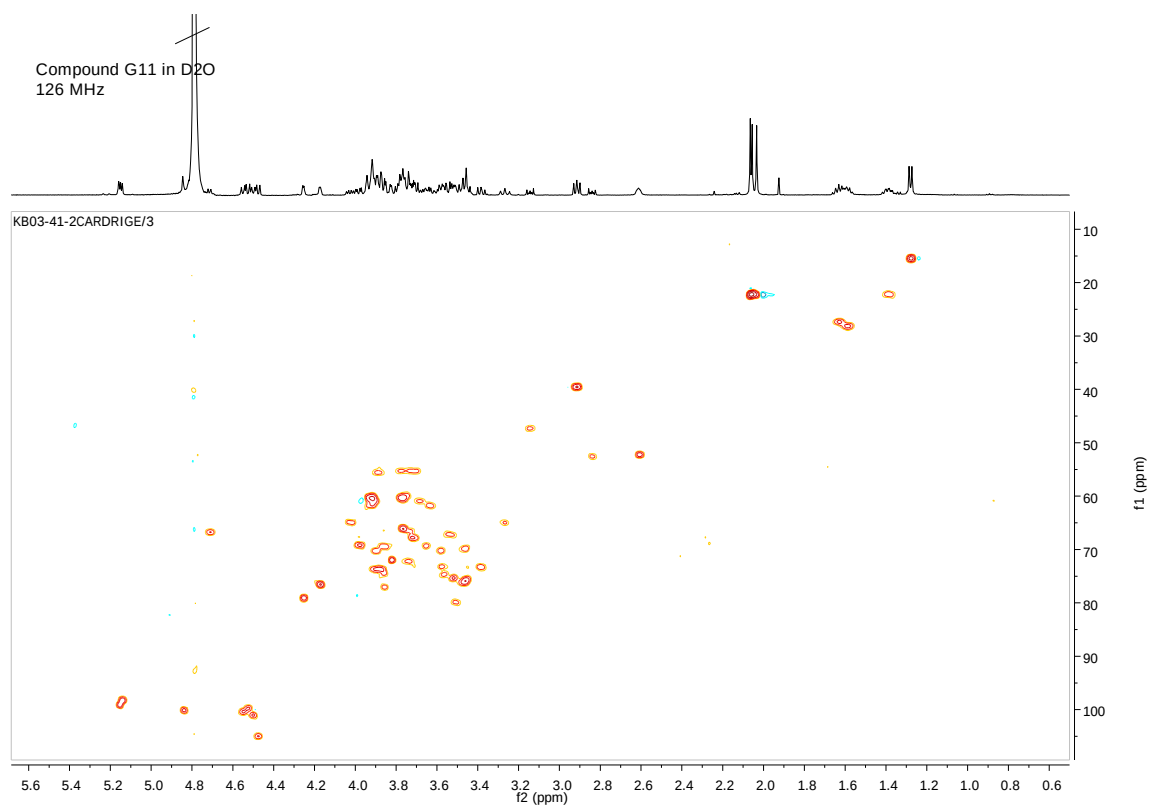
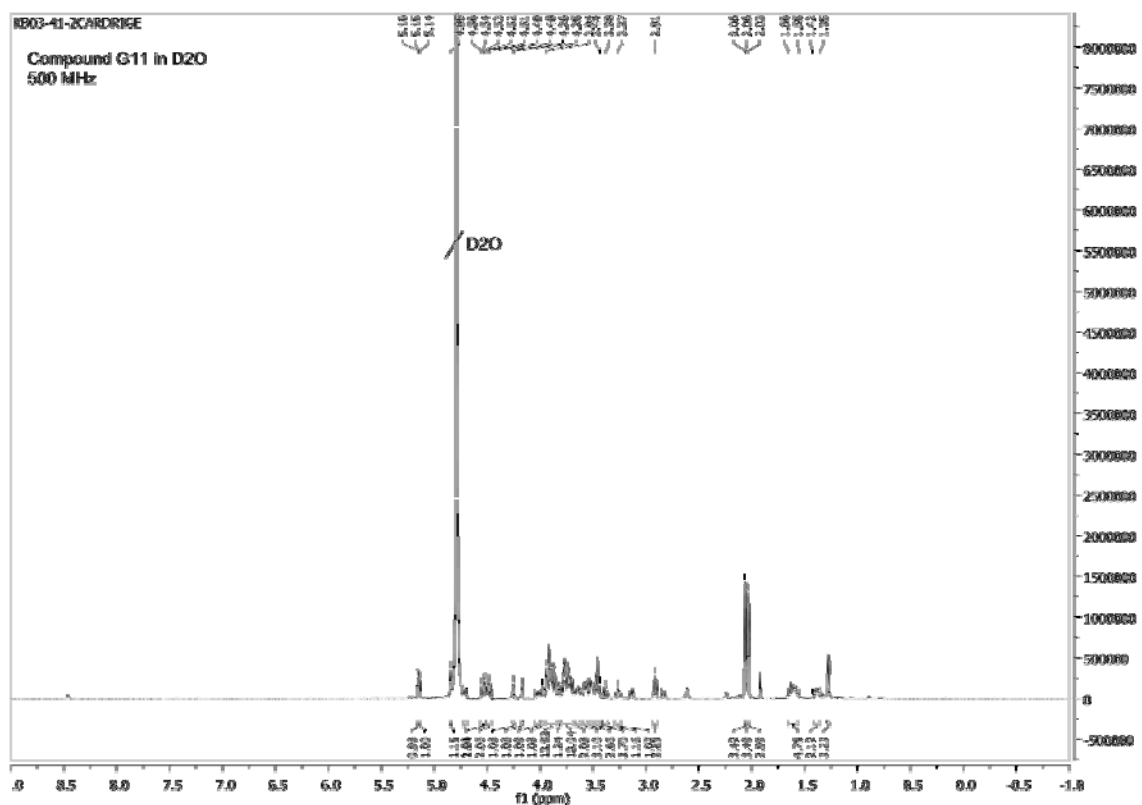
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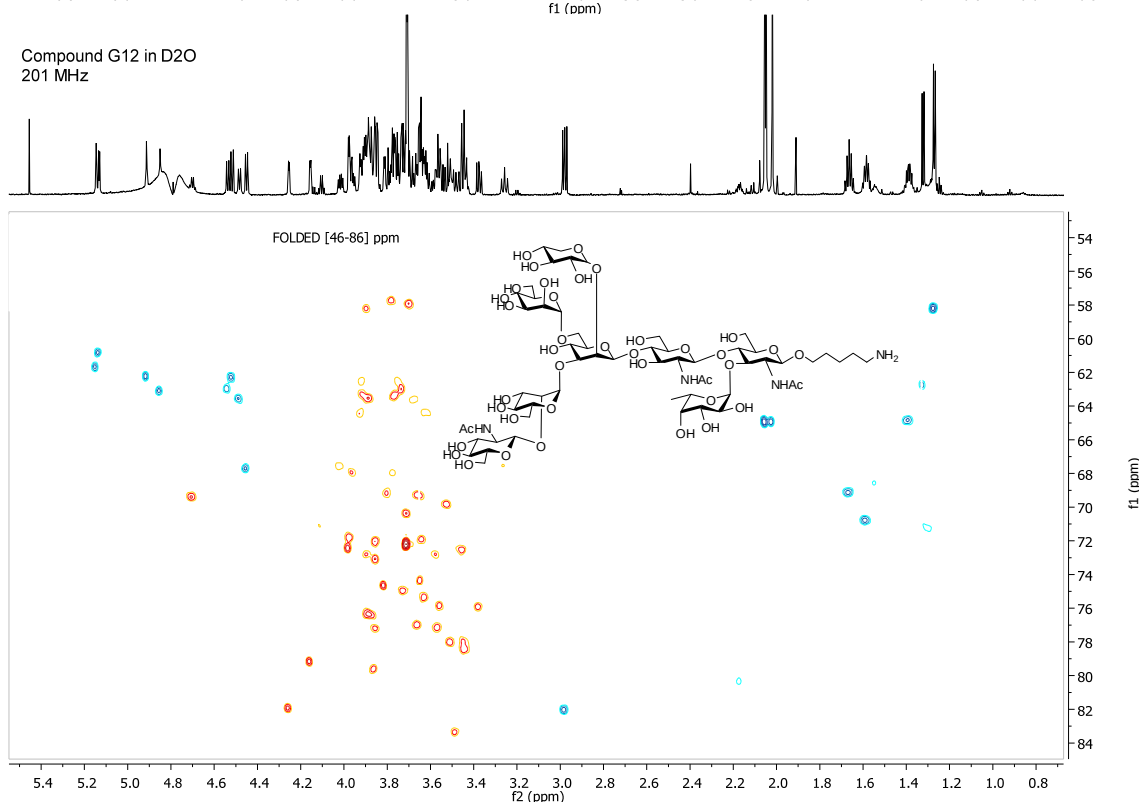
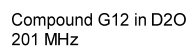
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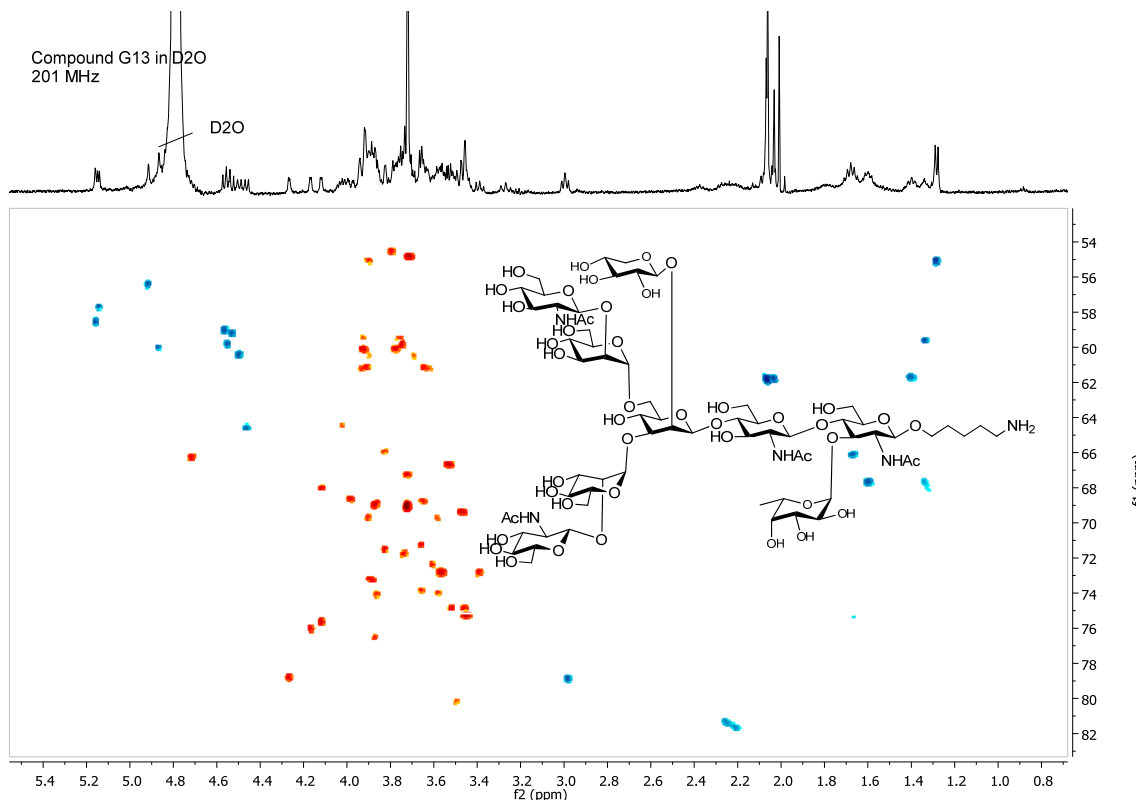
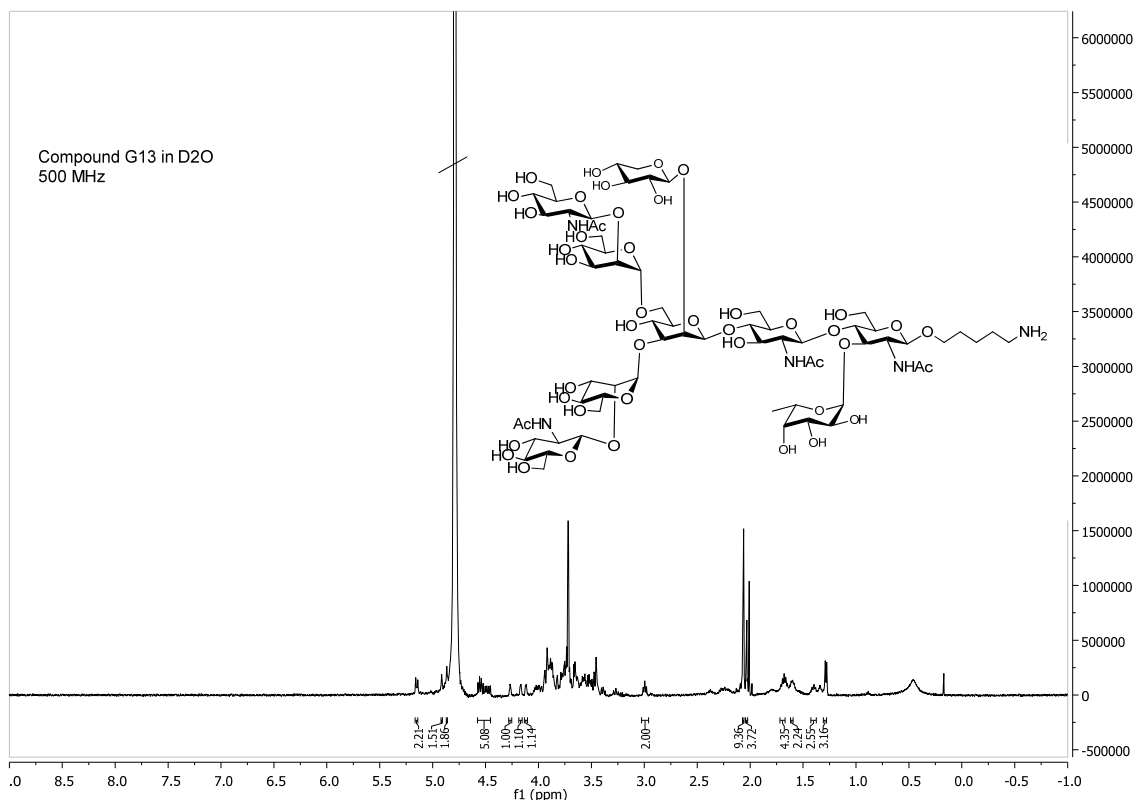
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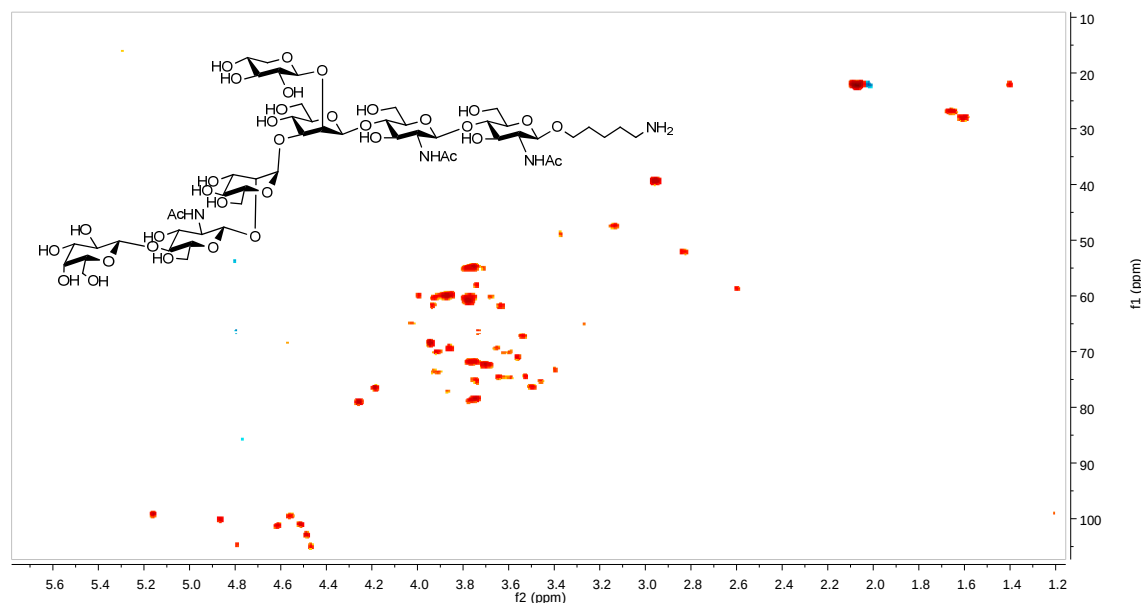
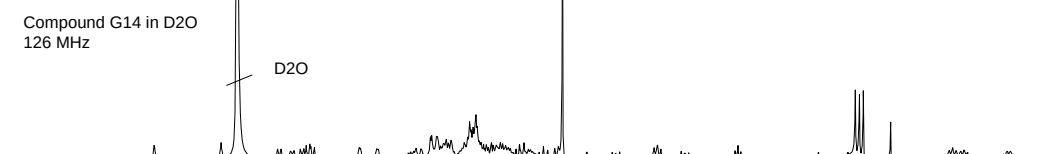
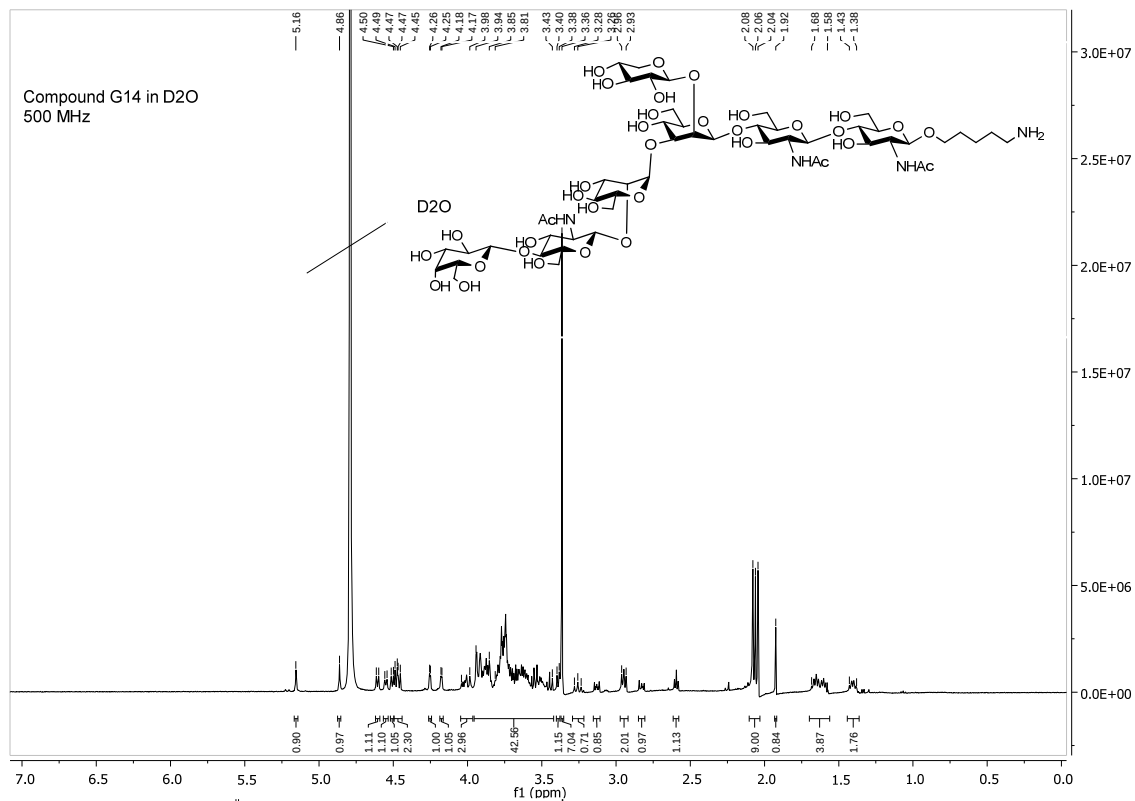
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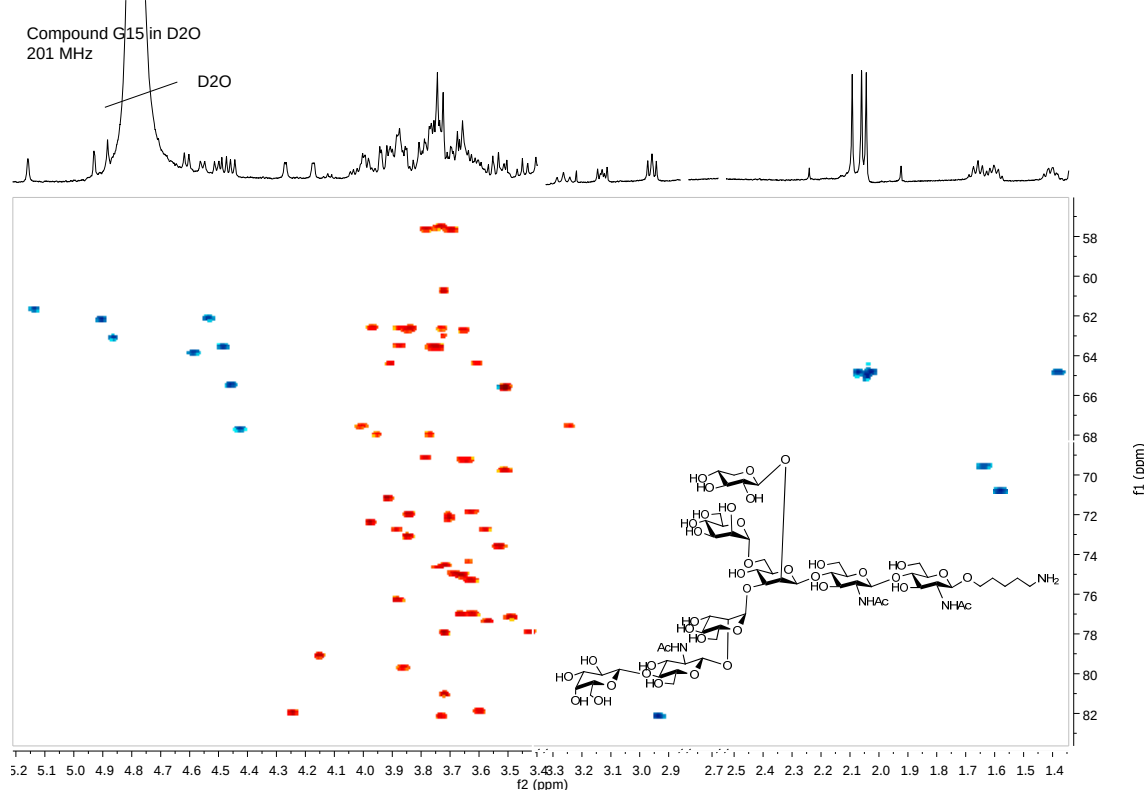
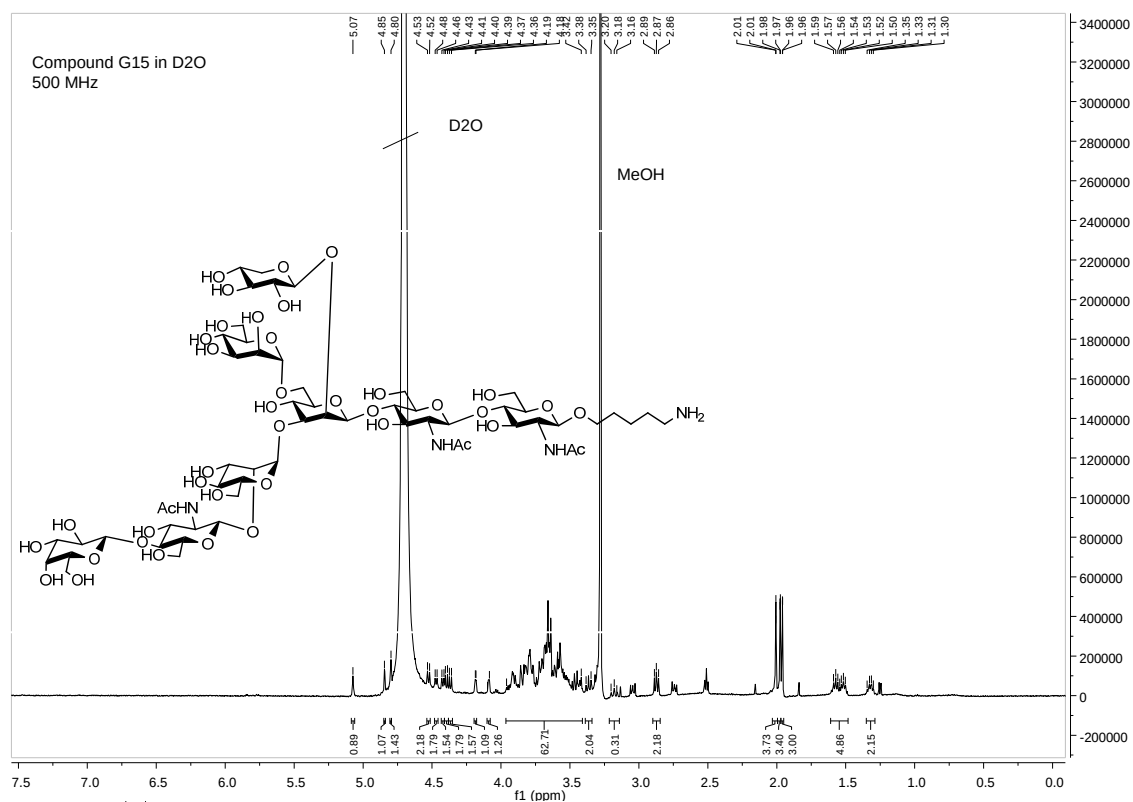
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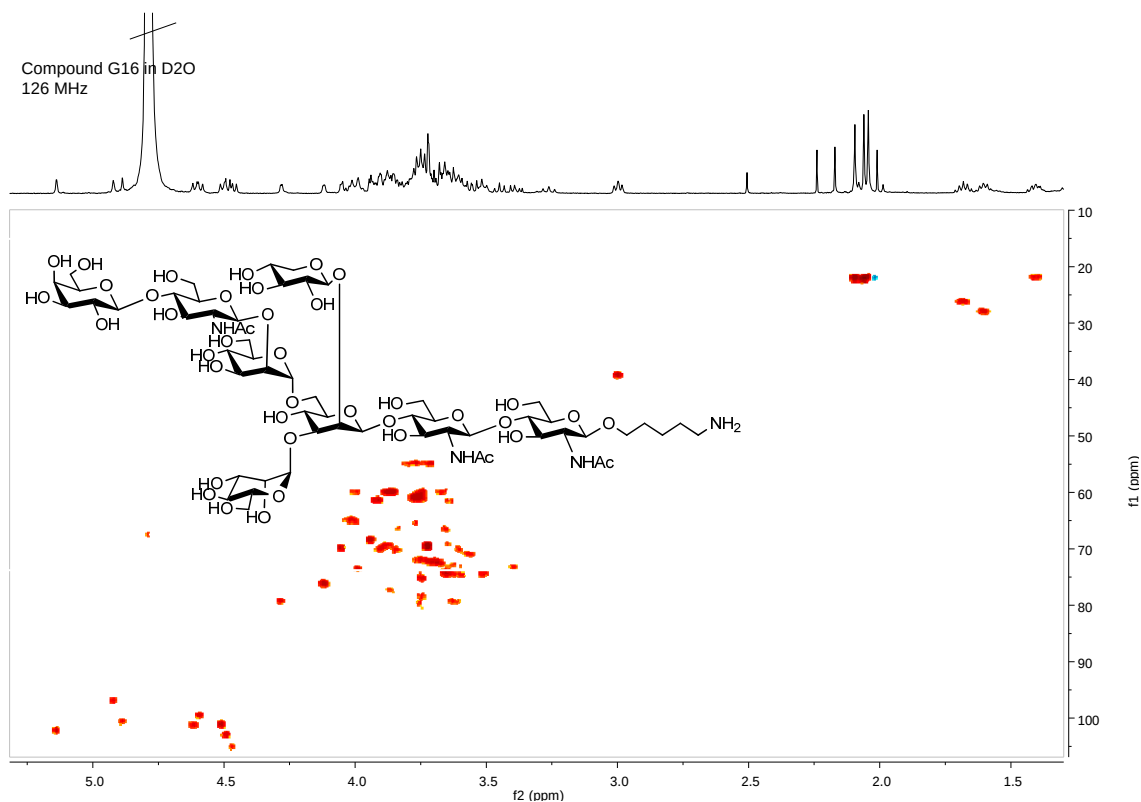
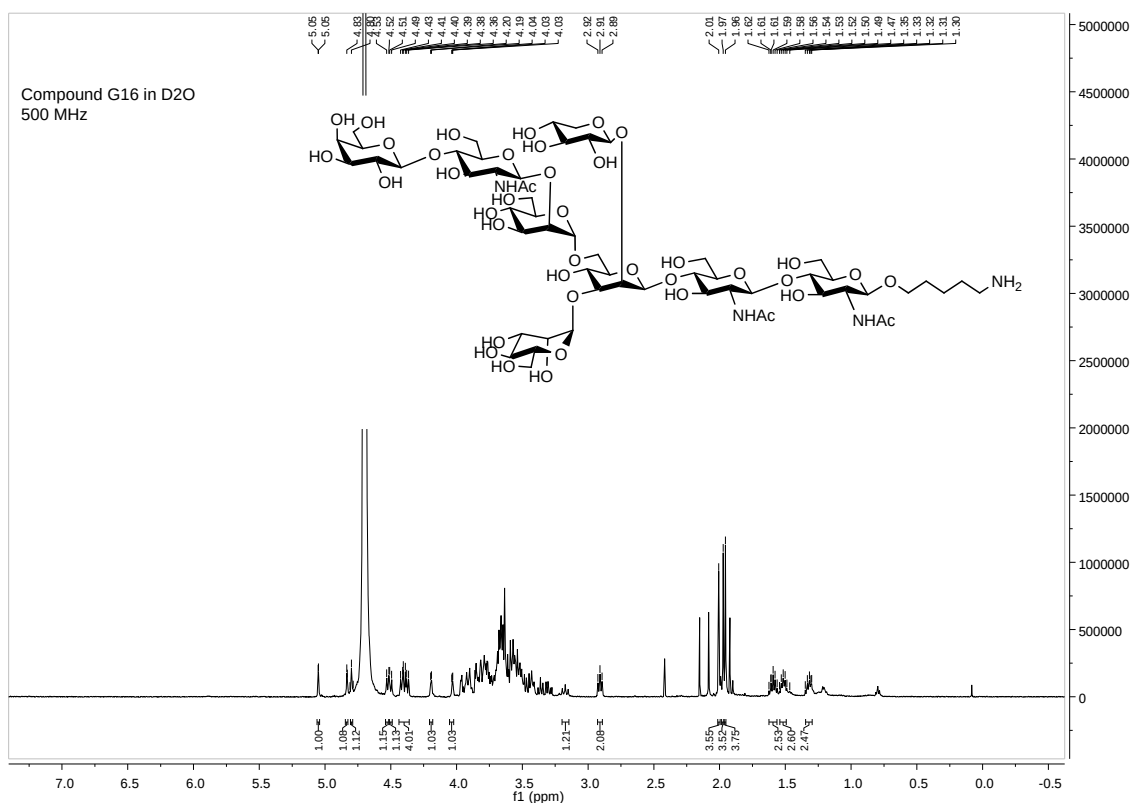
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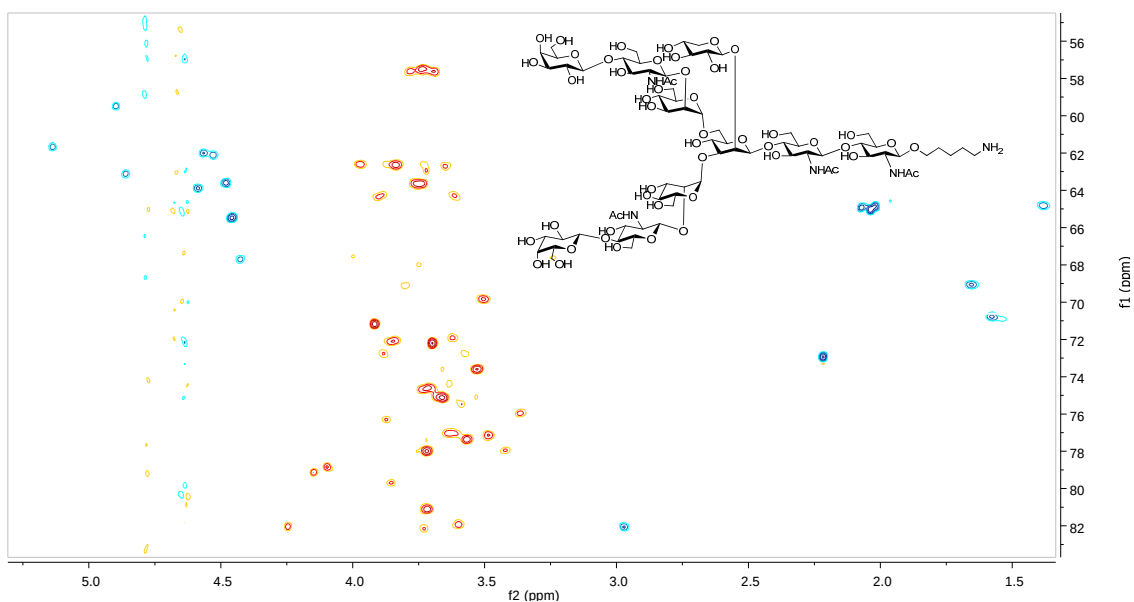
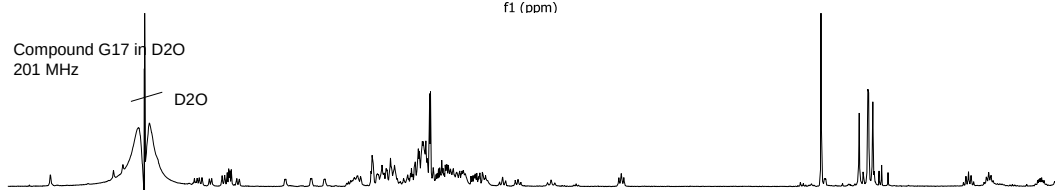
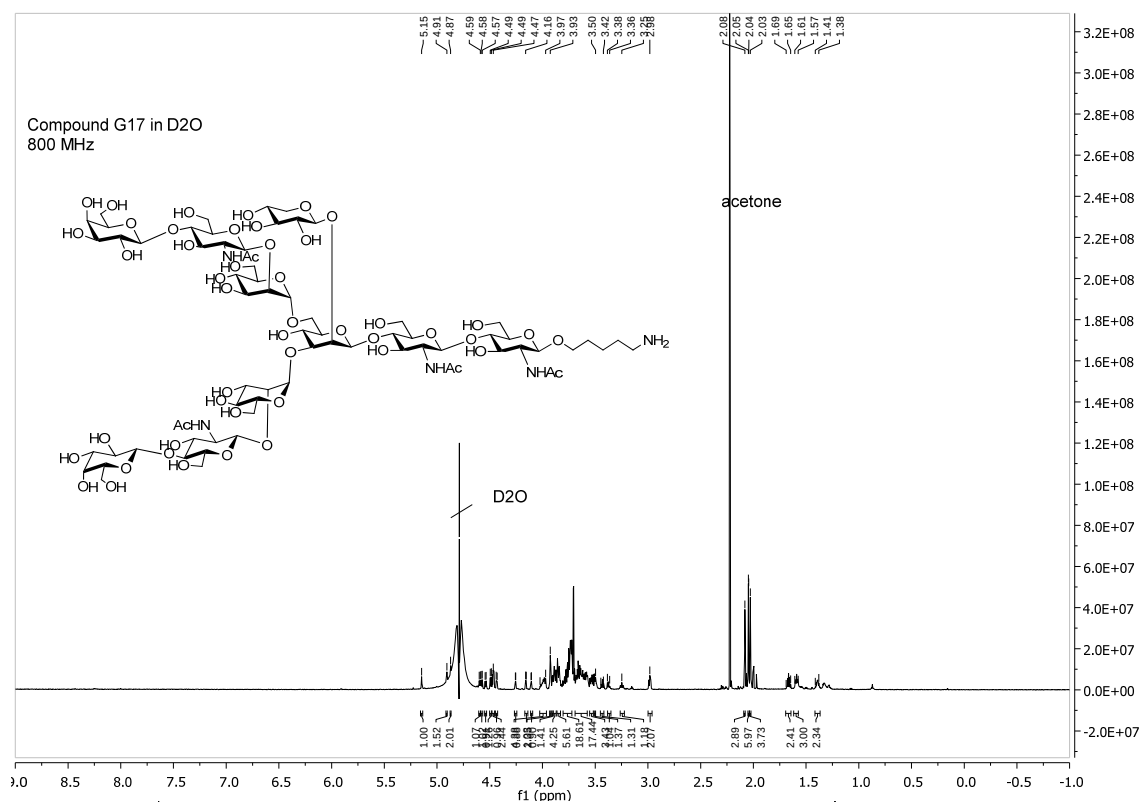
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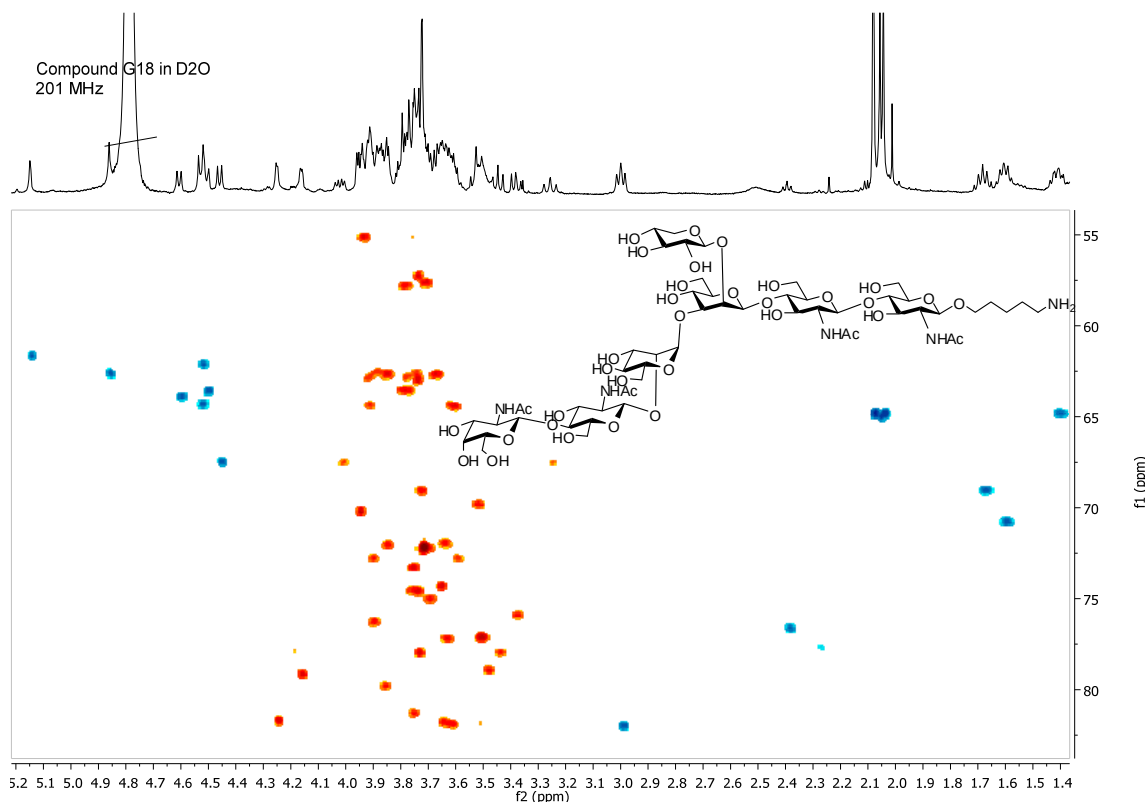
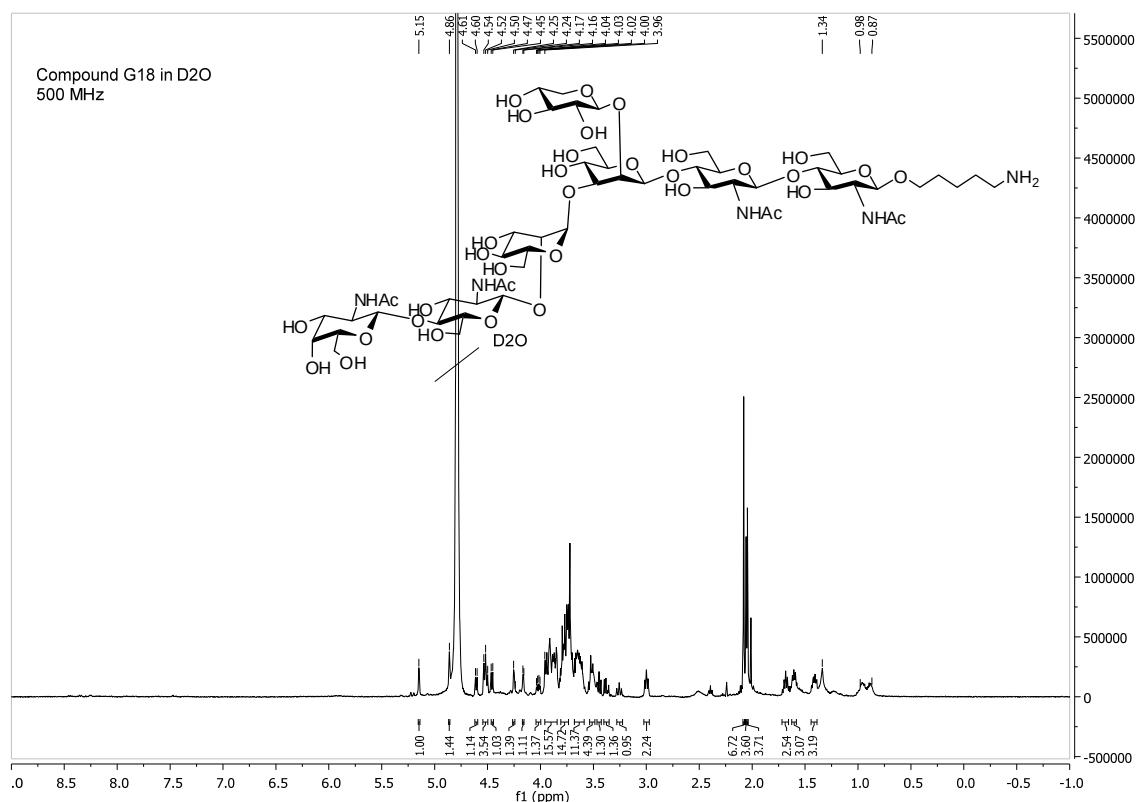
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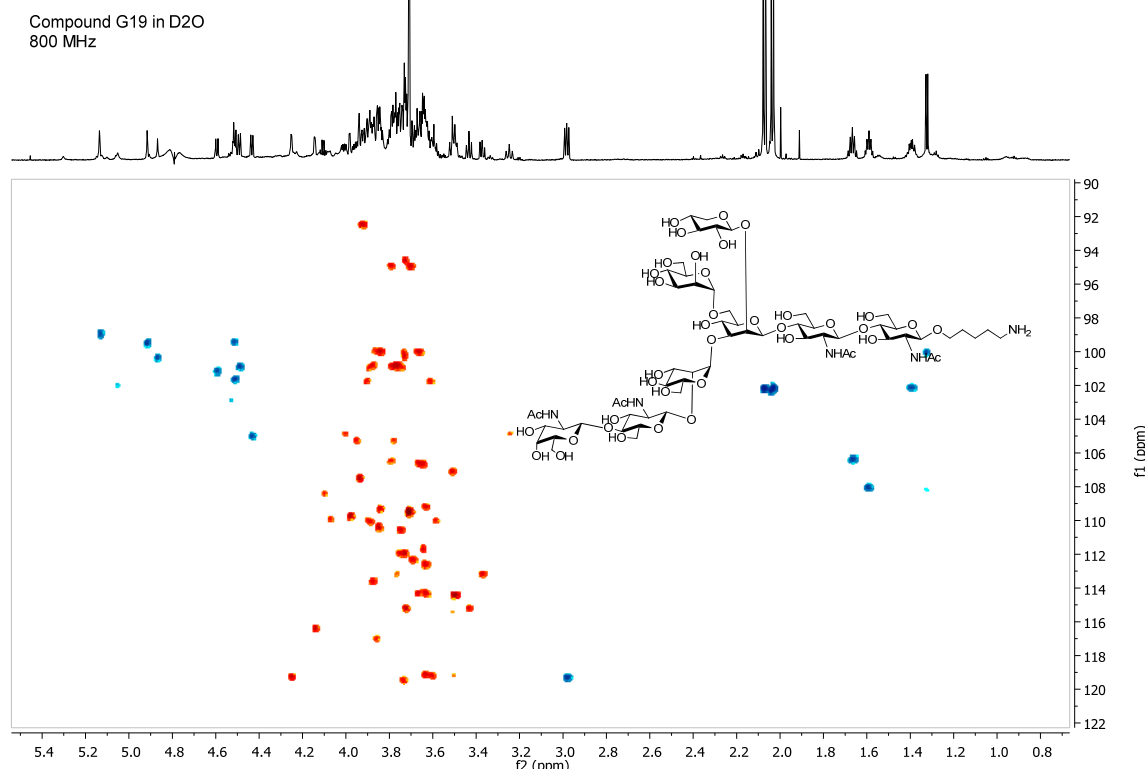
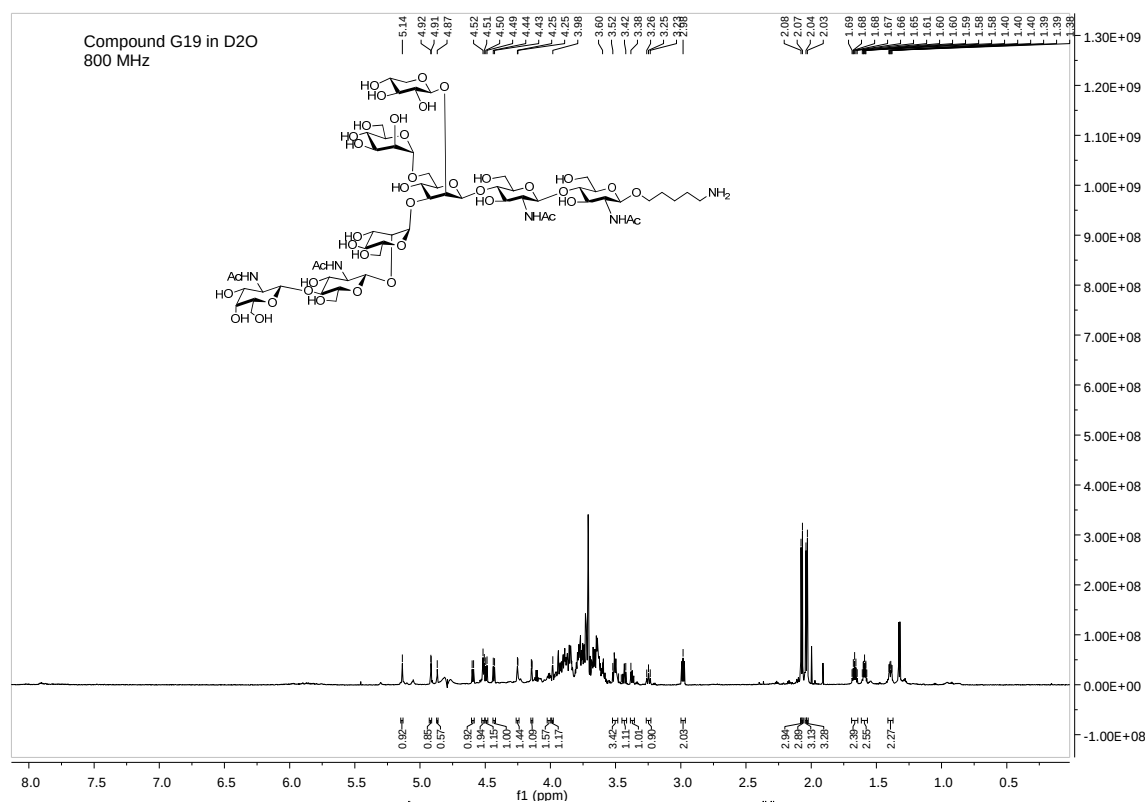
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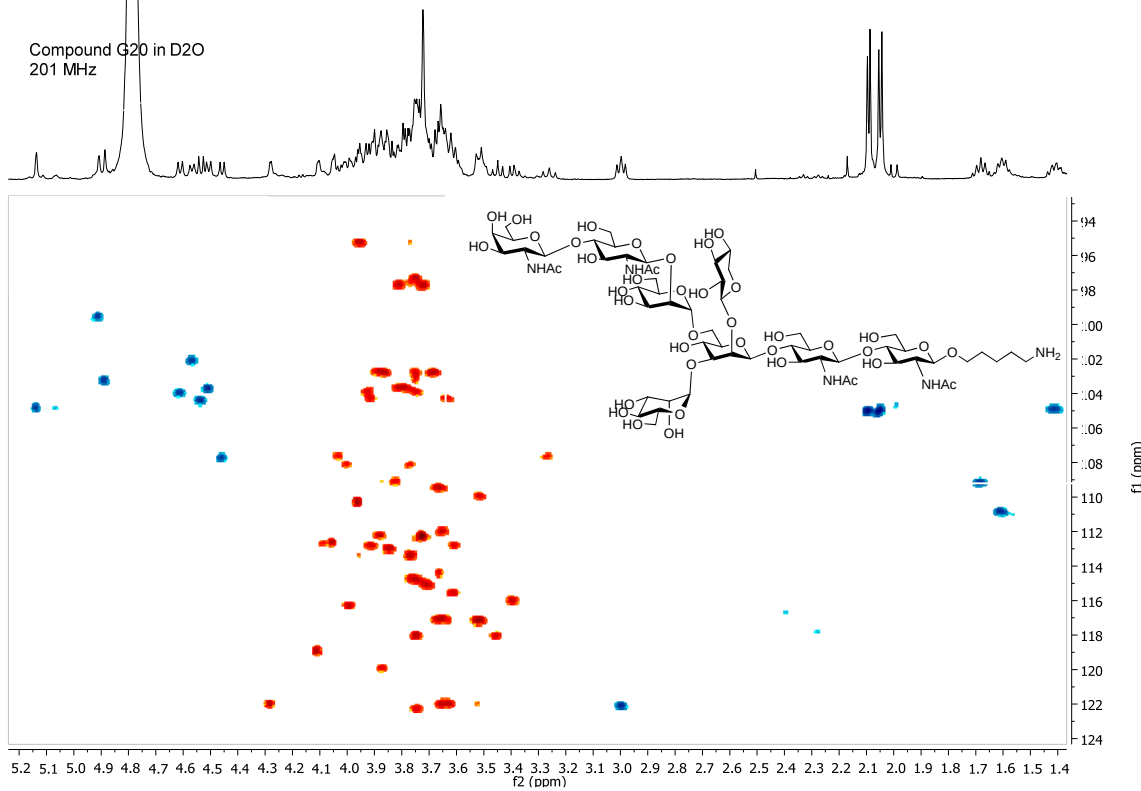
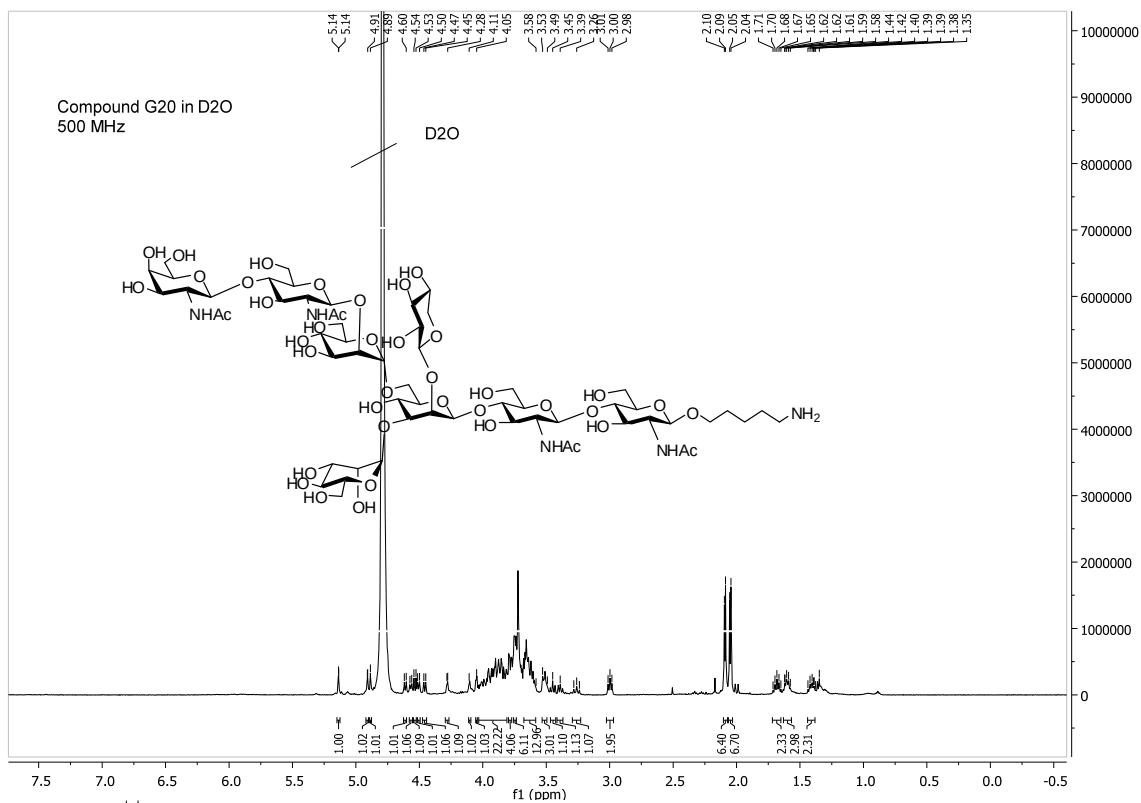
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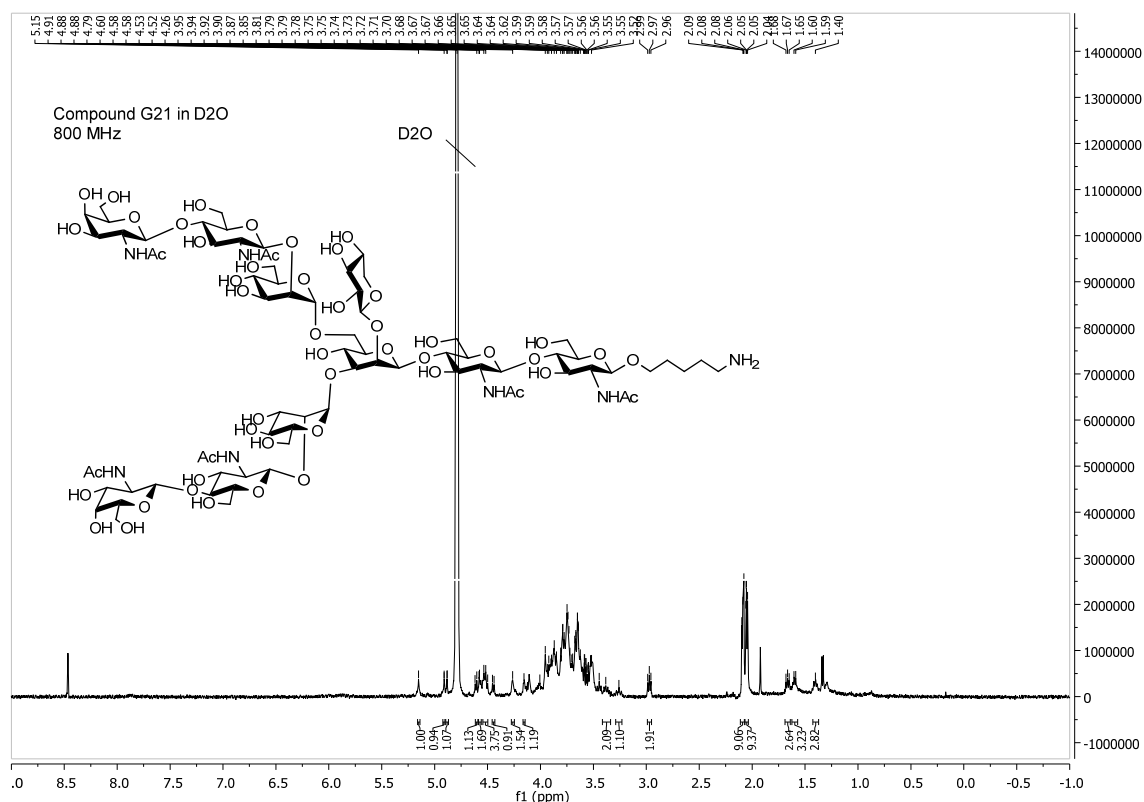
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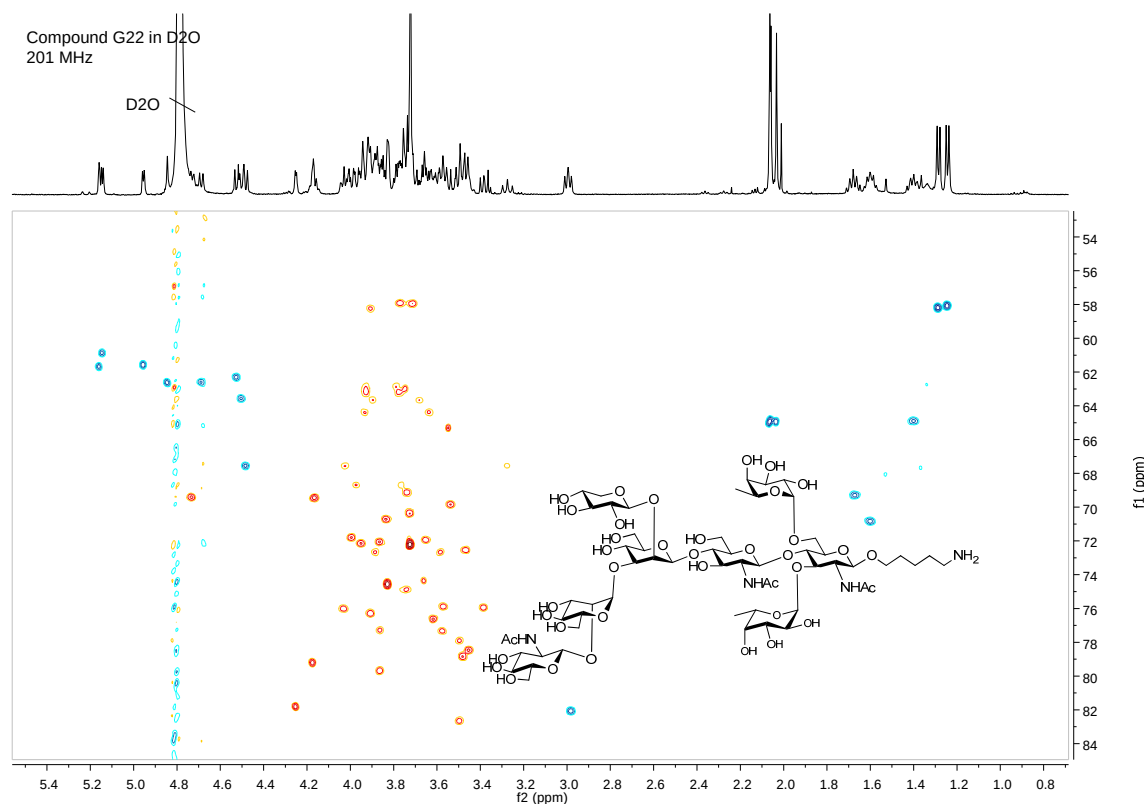
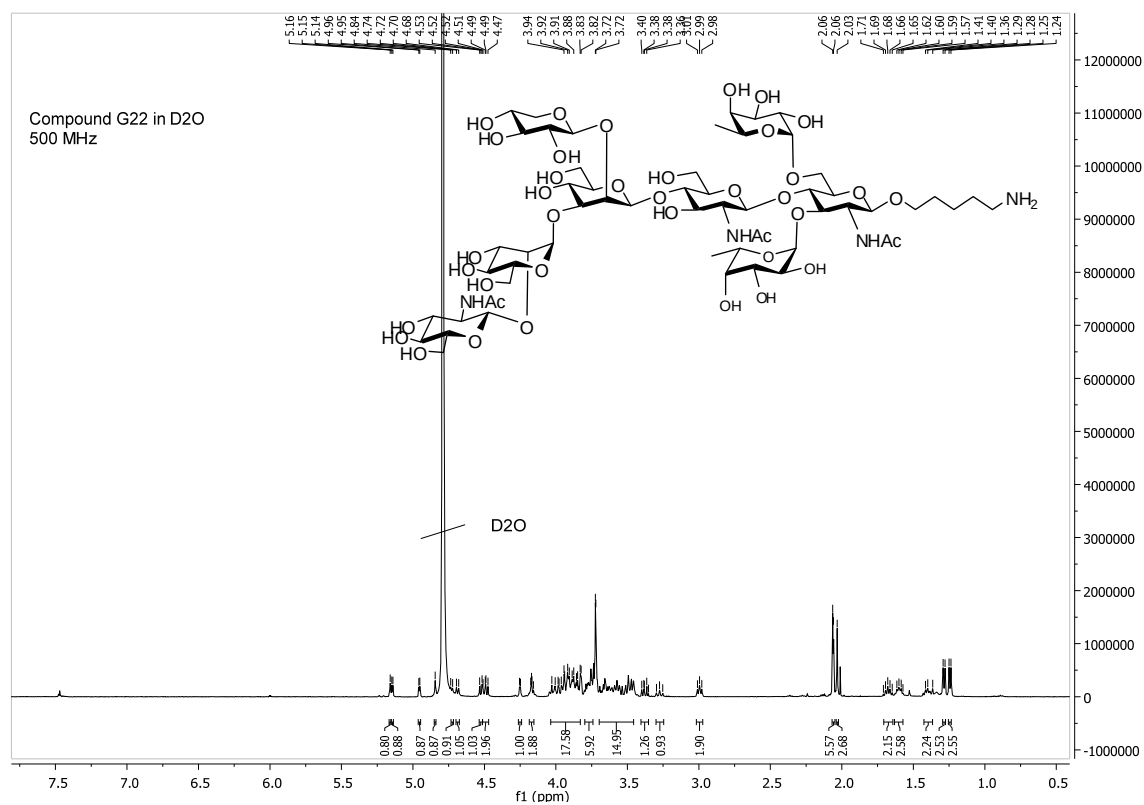
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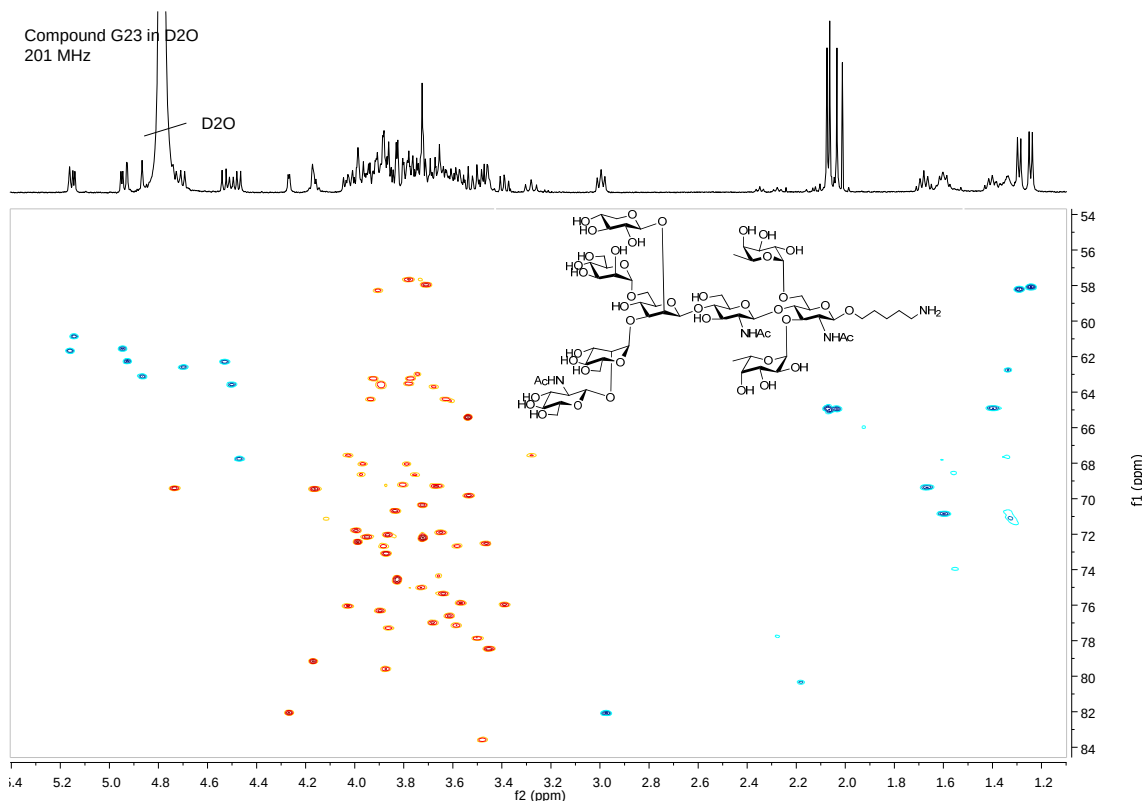
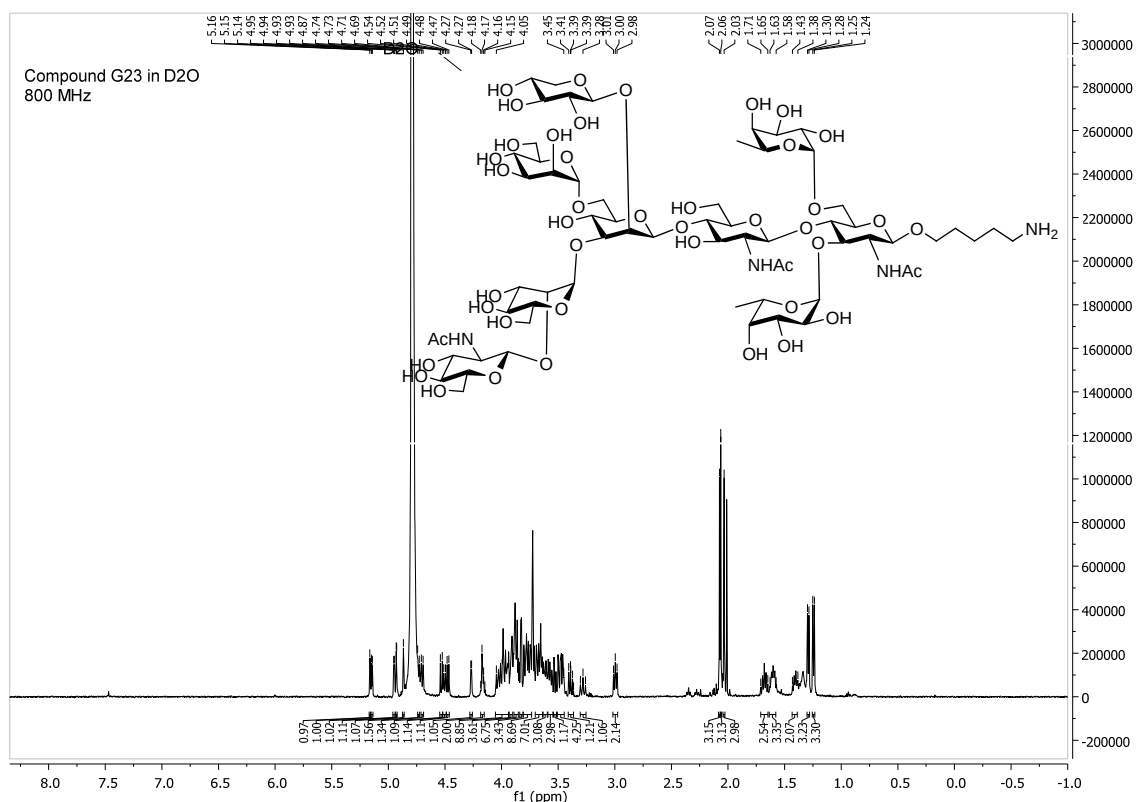
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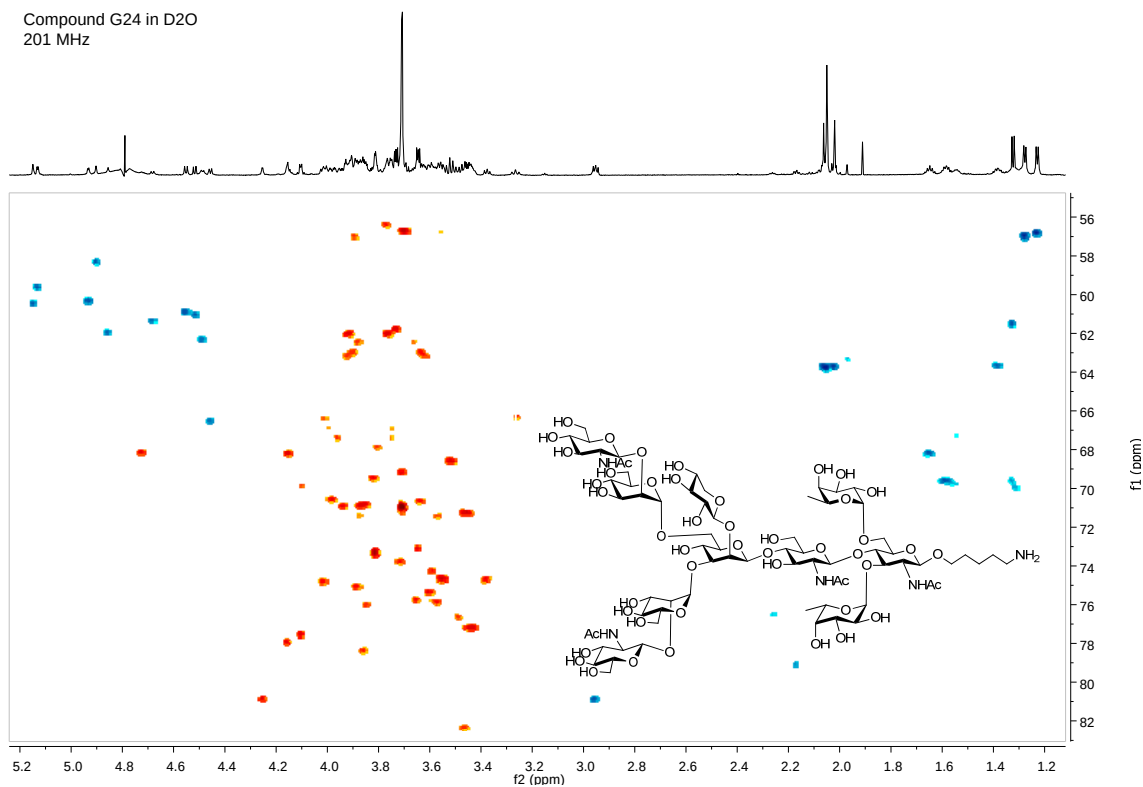
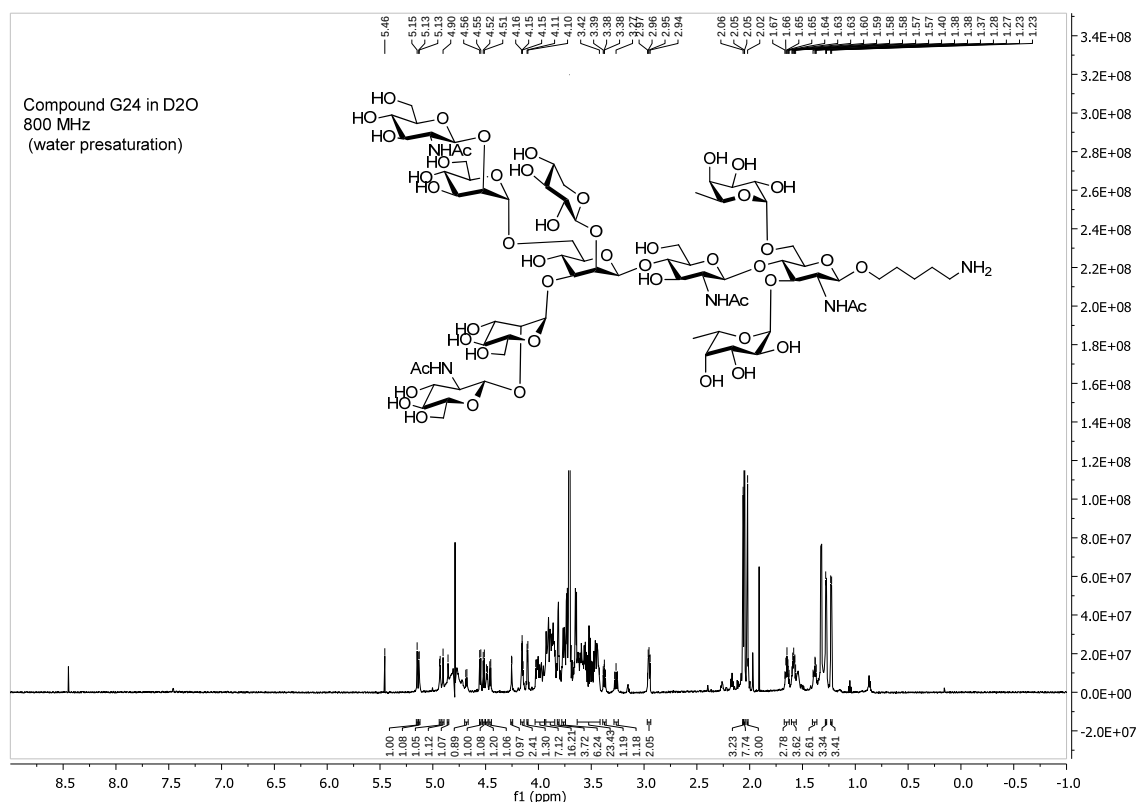
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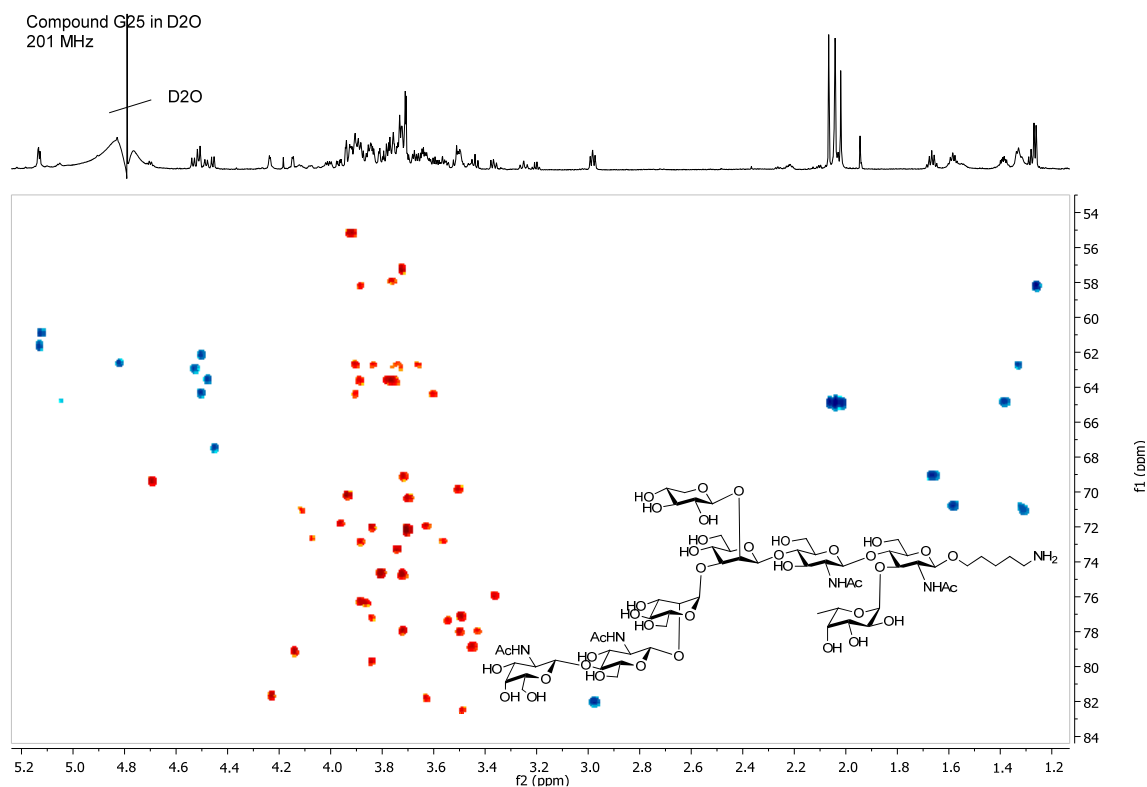
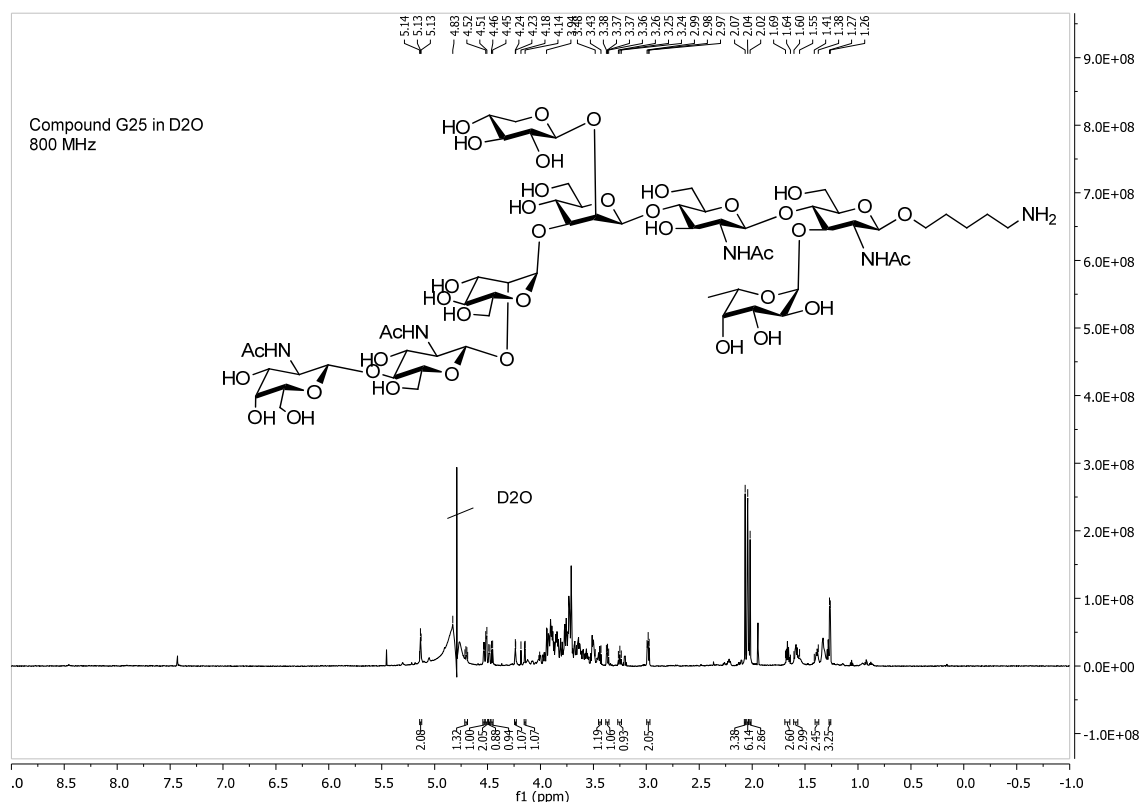
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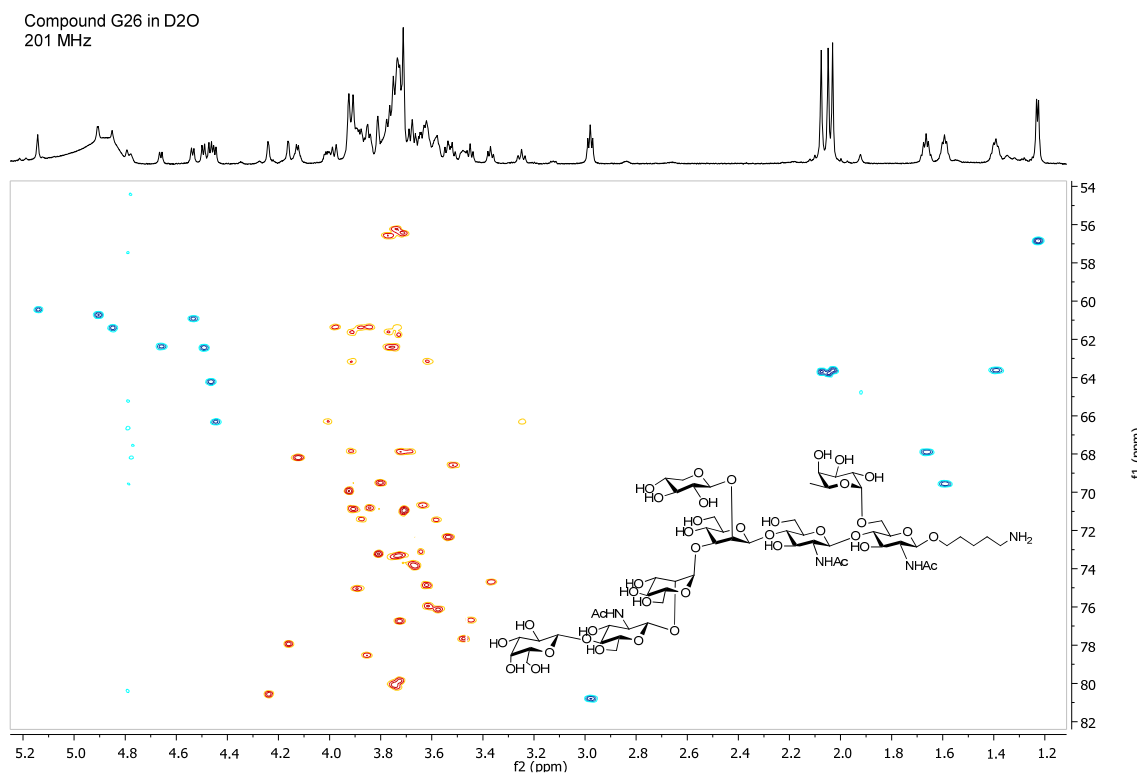
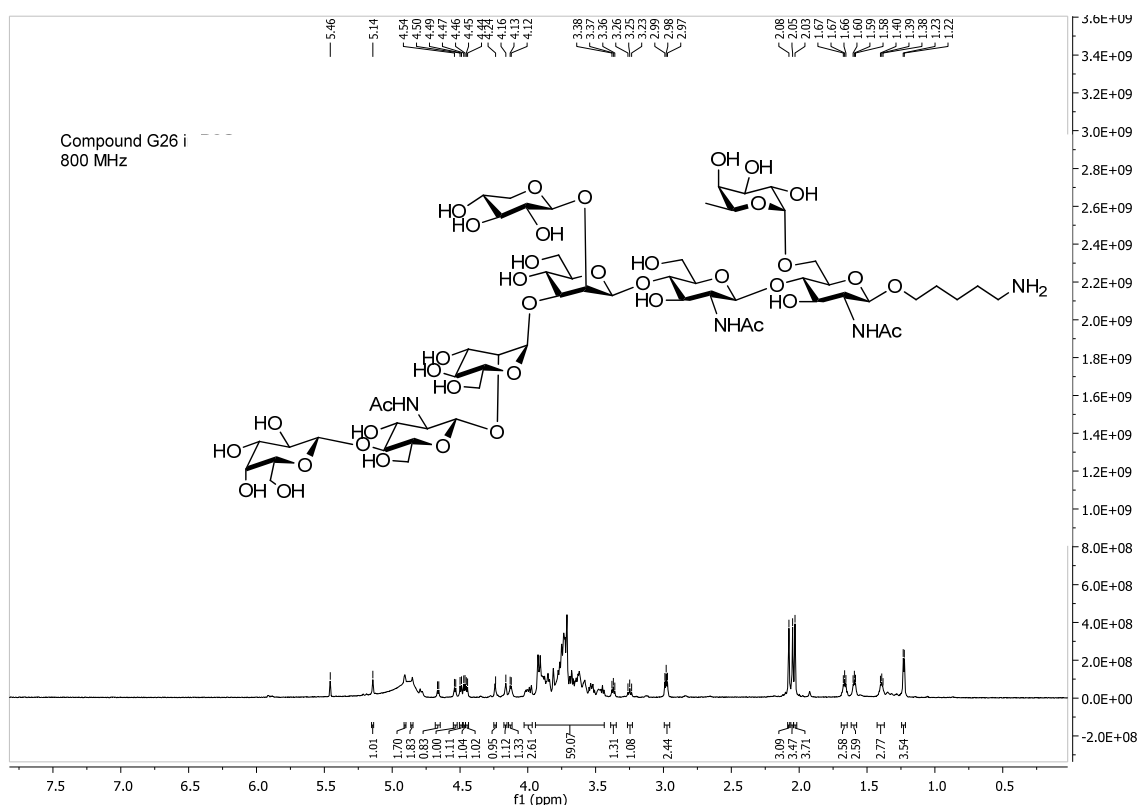
G24



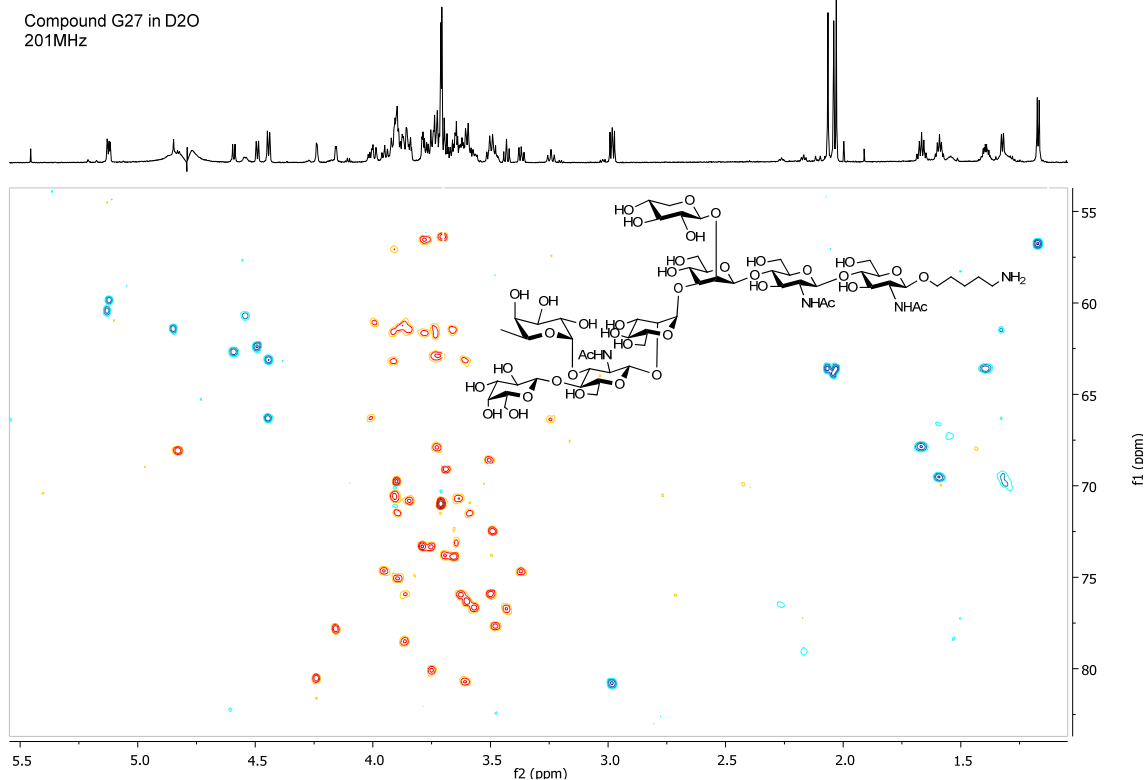
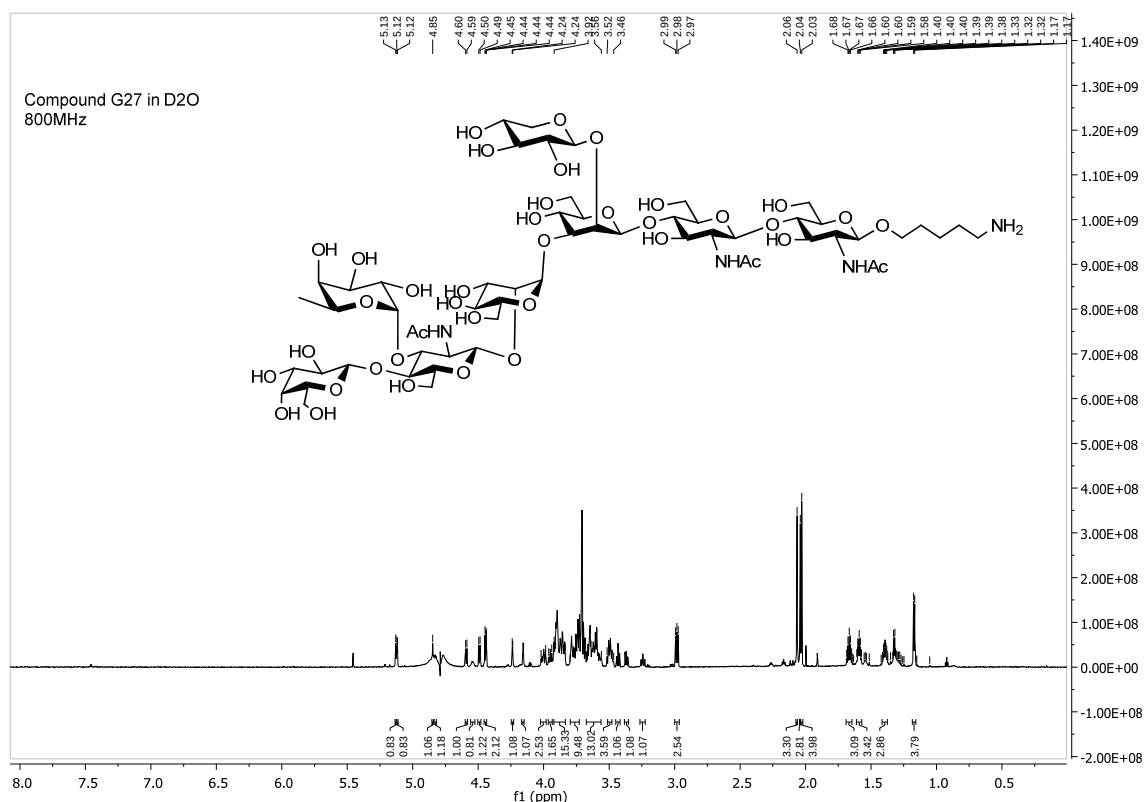
G25



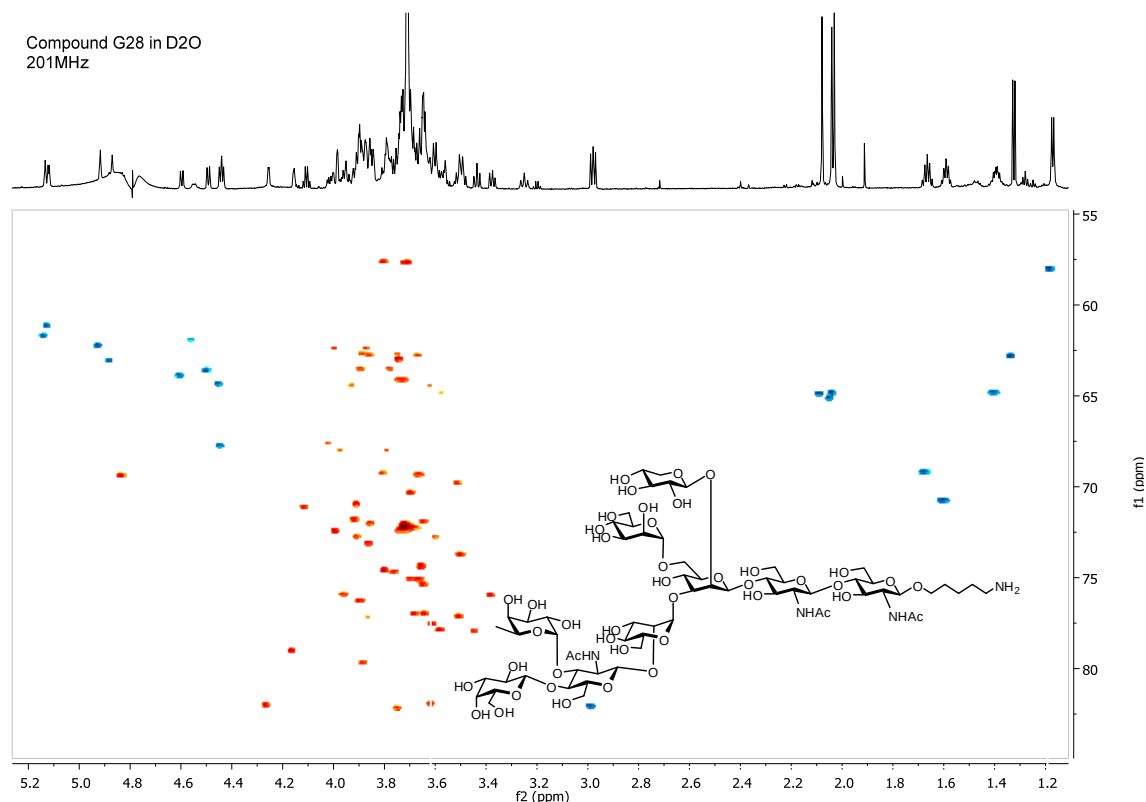
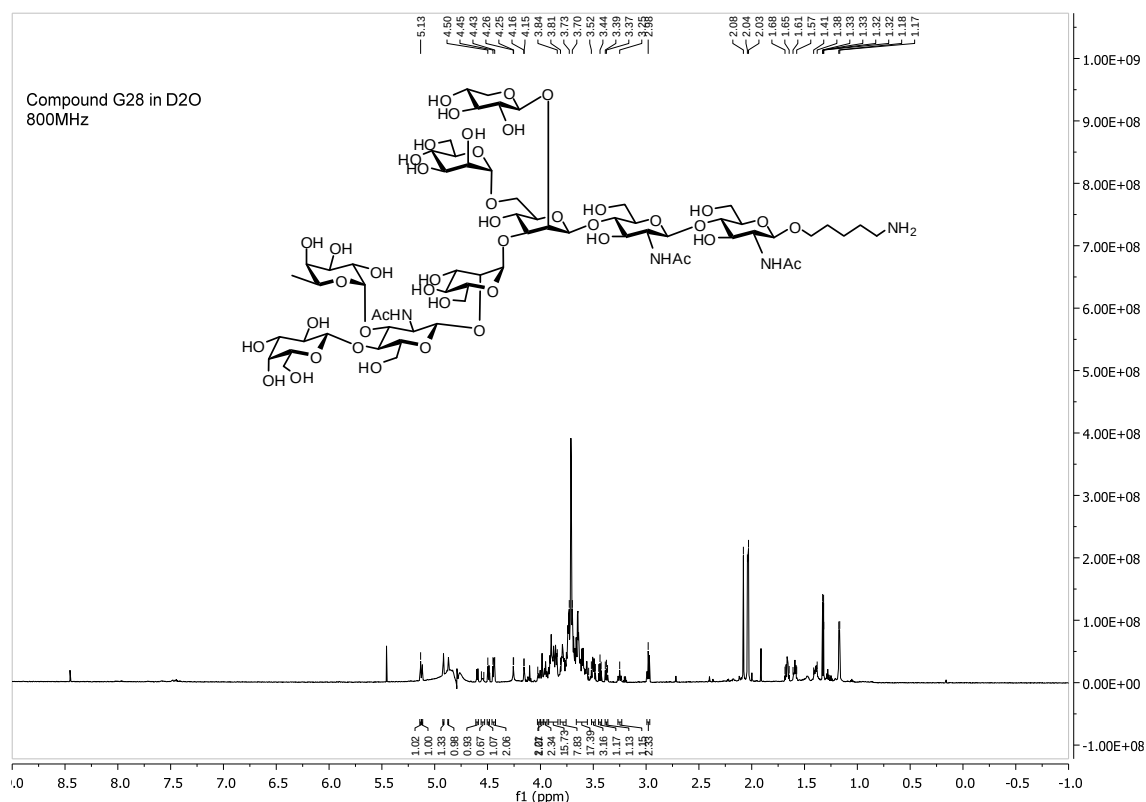
G26



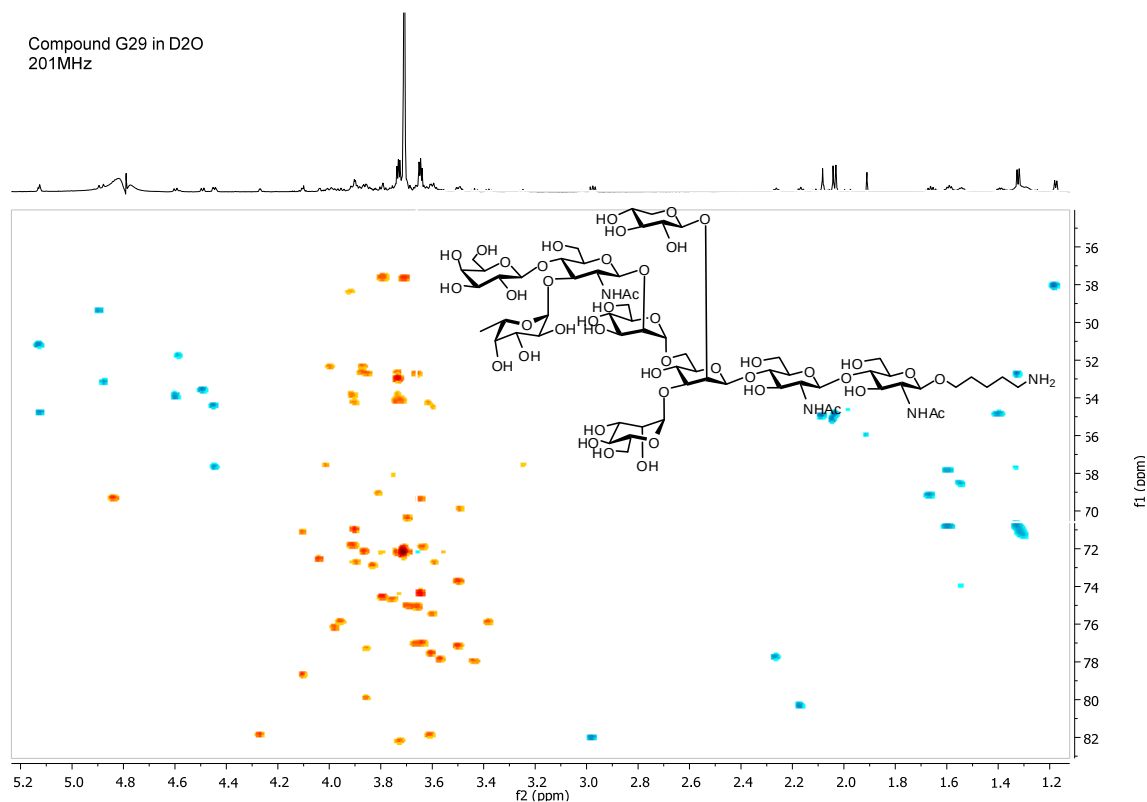
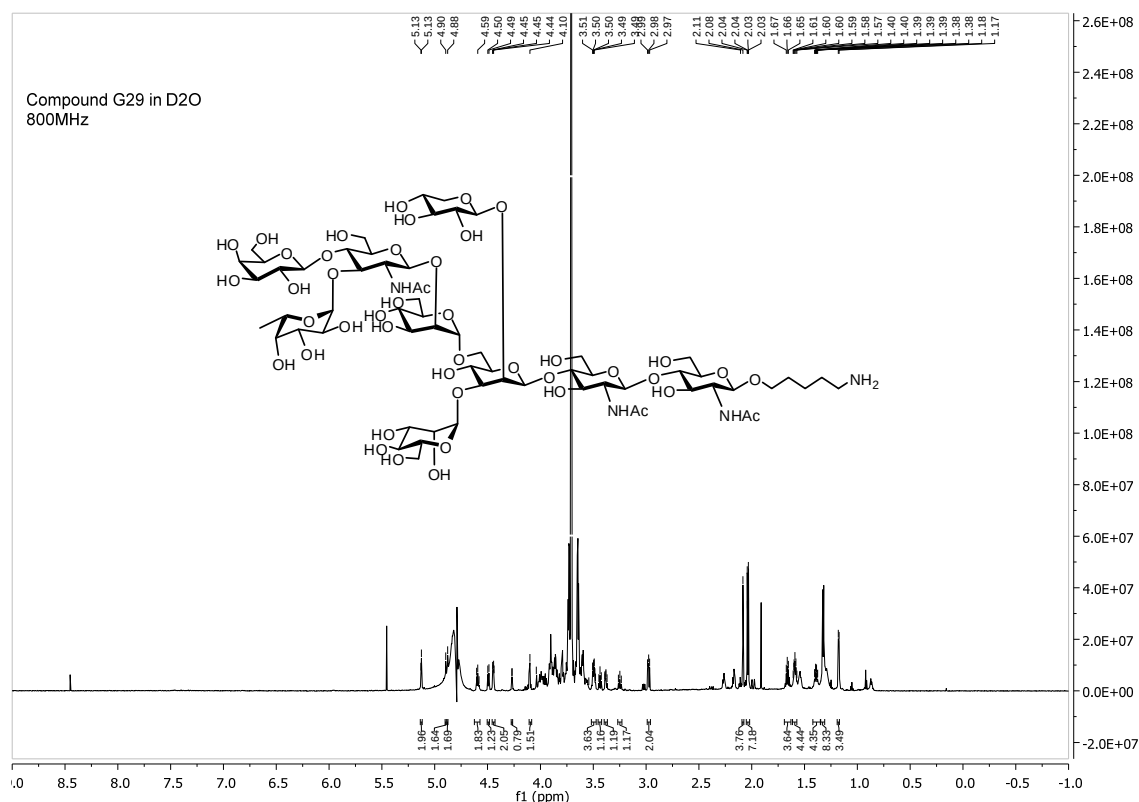
G27



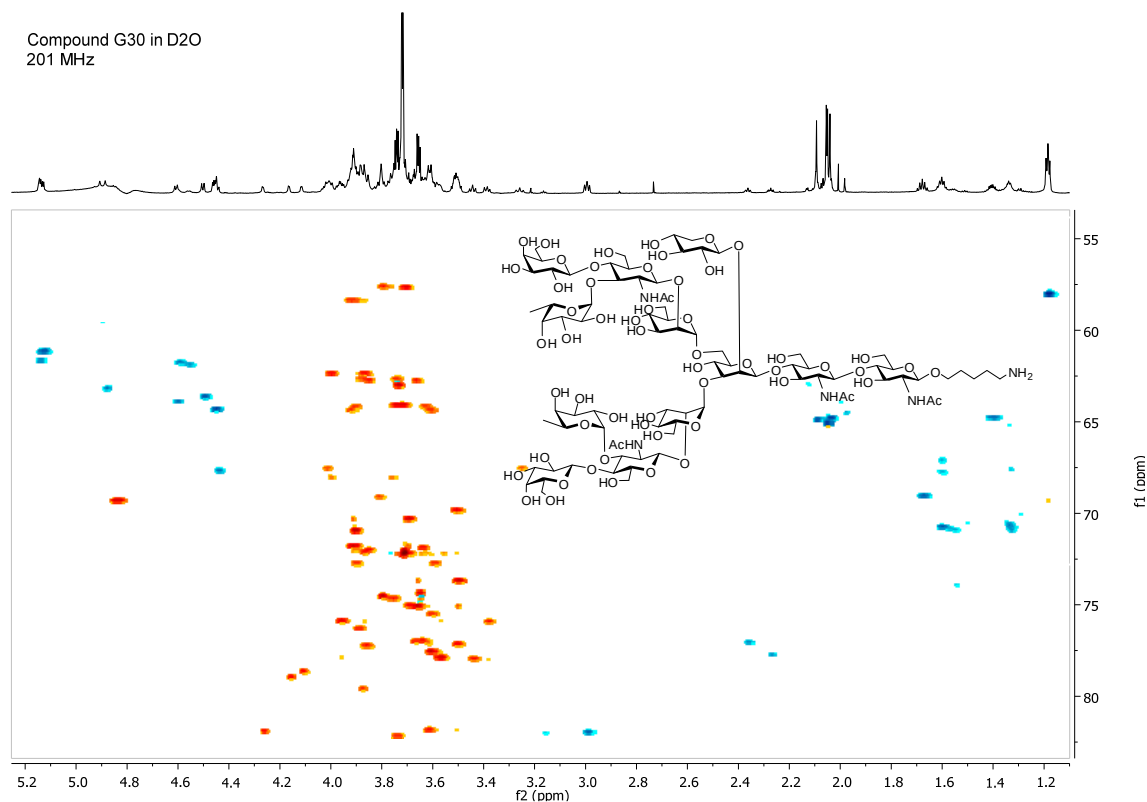
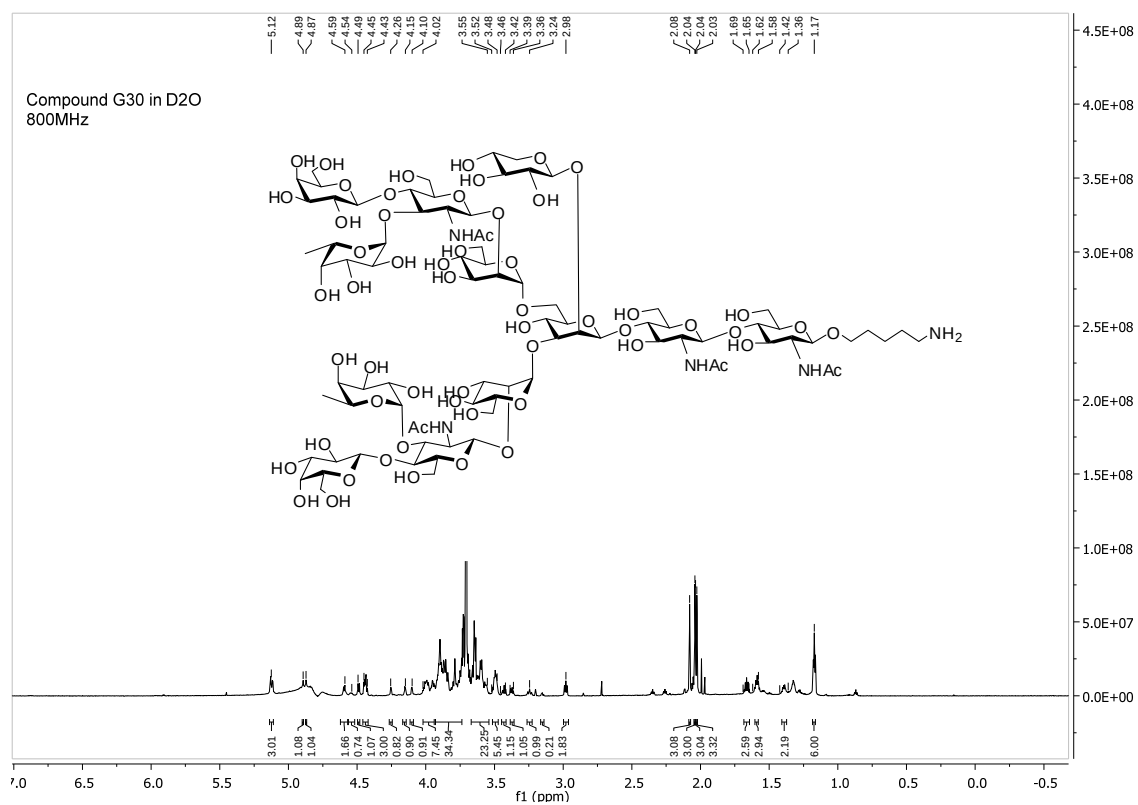
G28



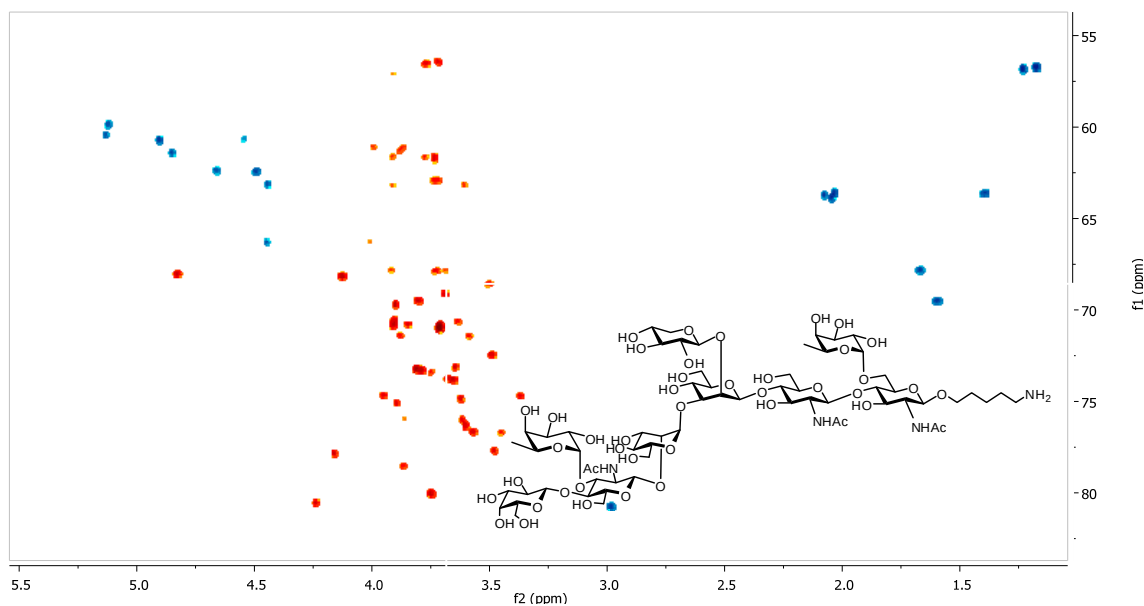
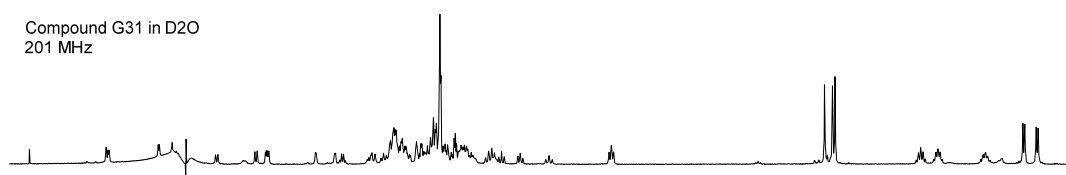
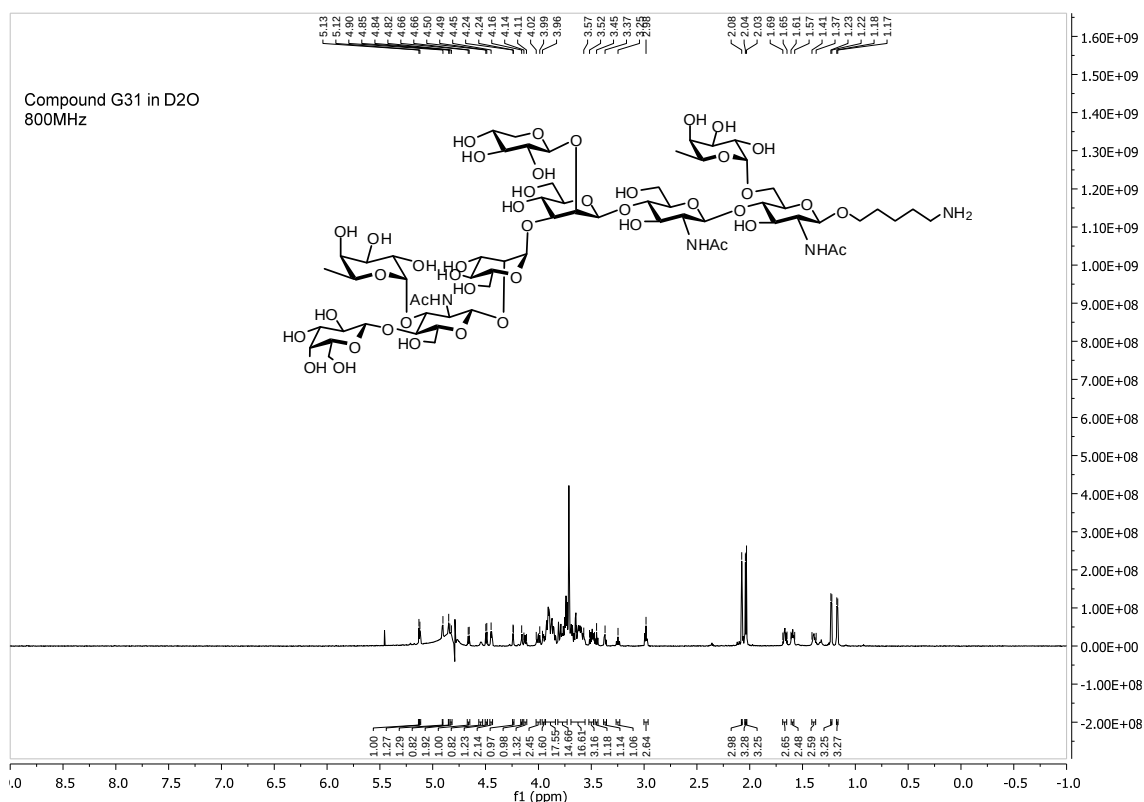
G29



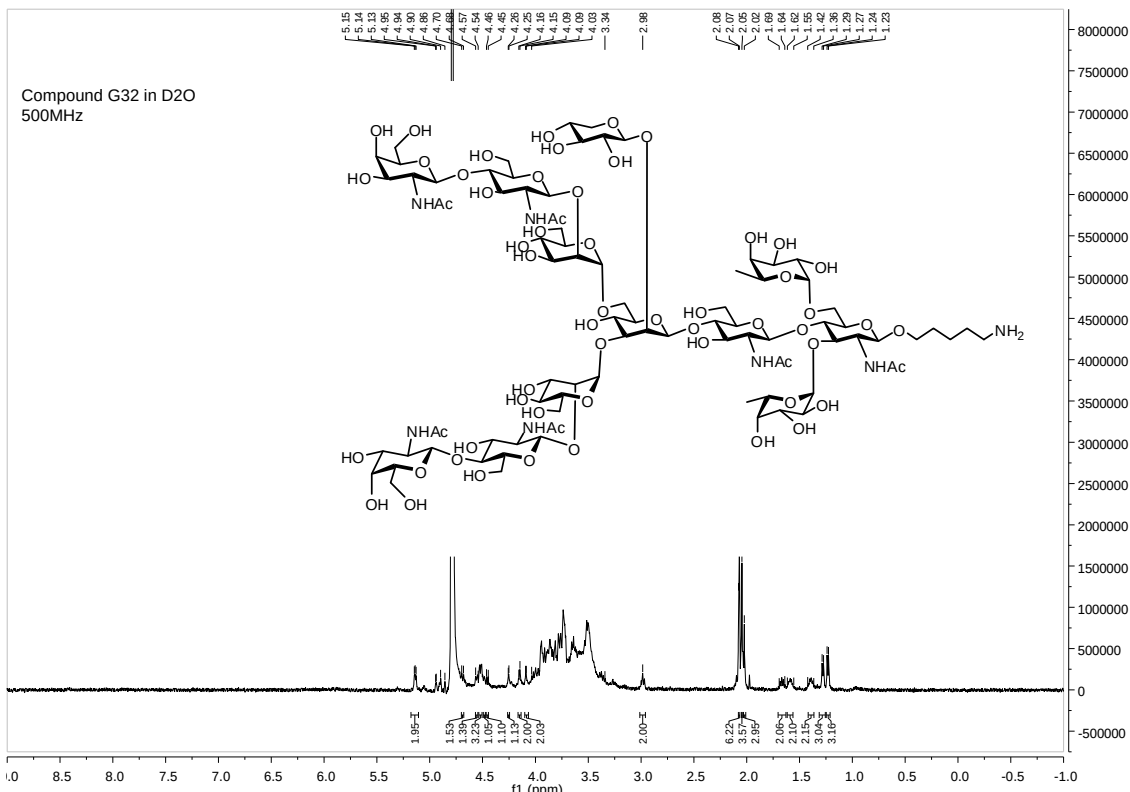
G30



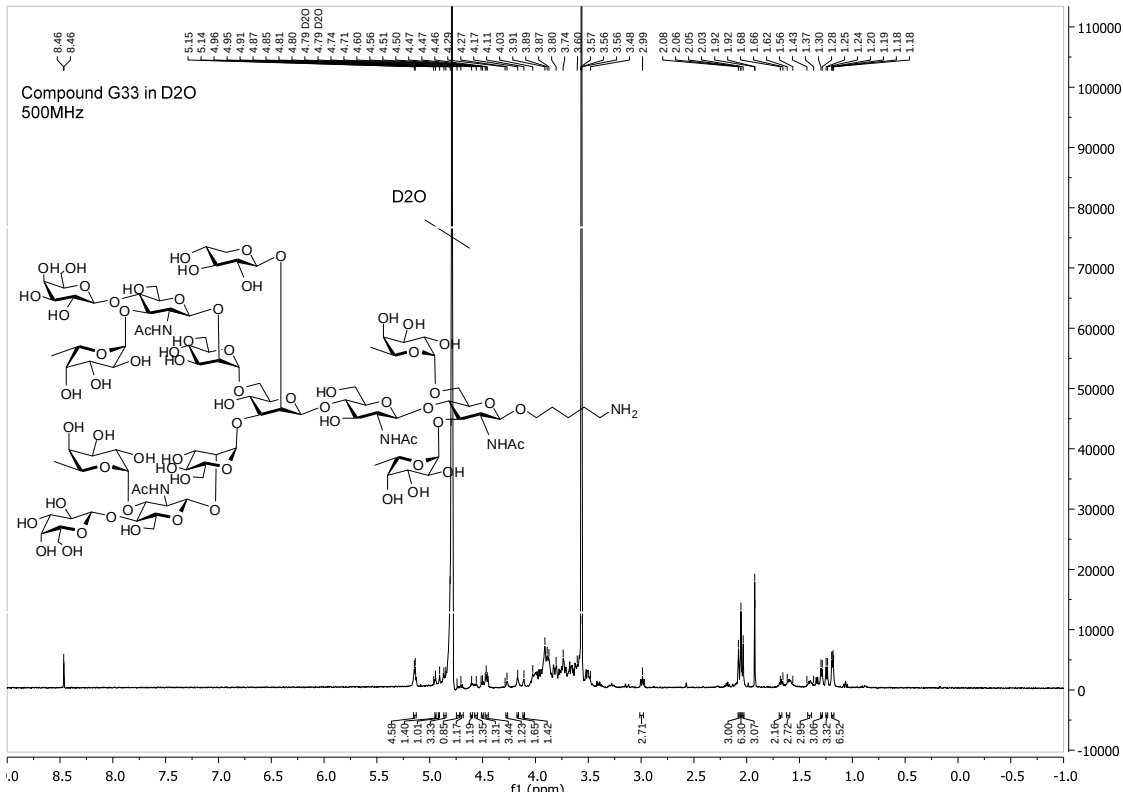
G31



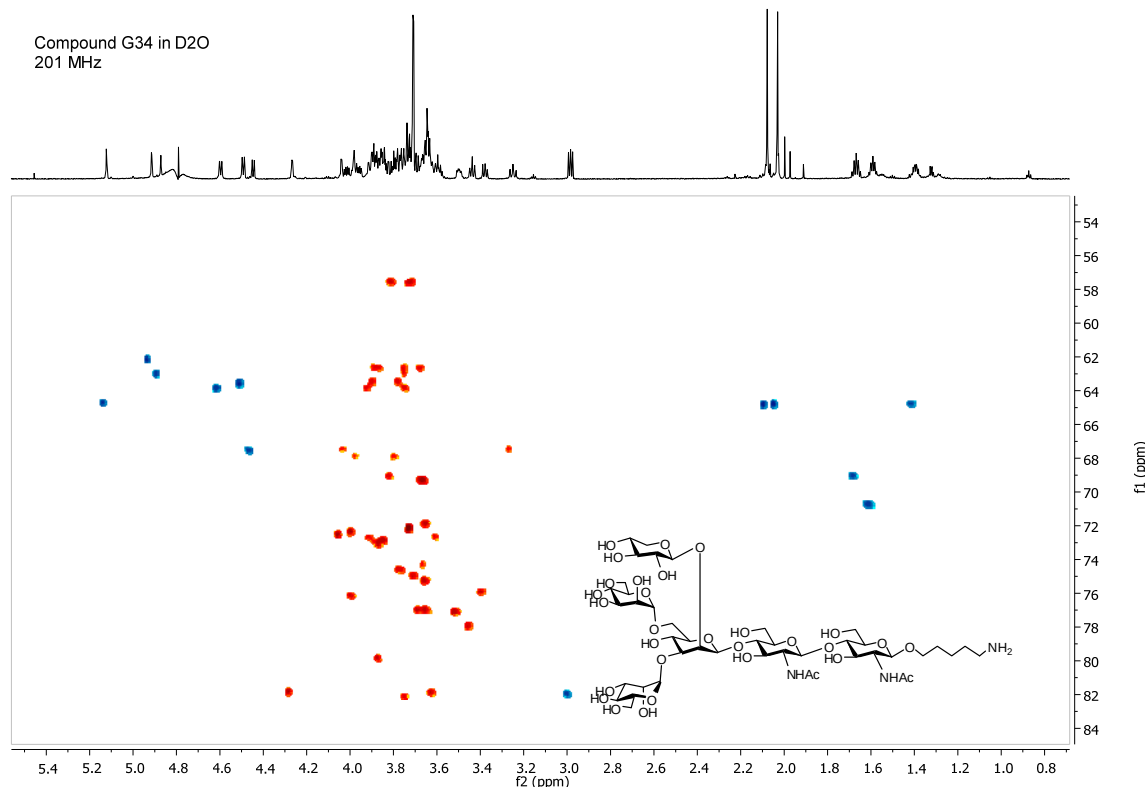
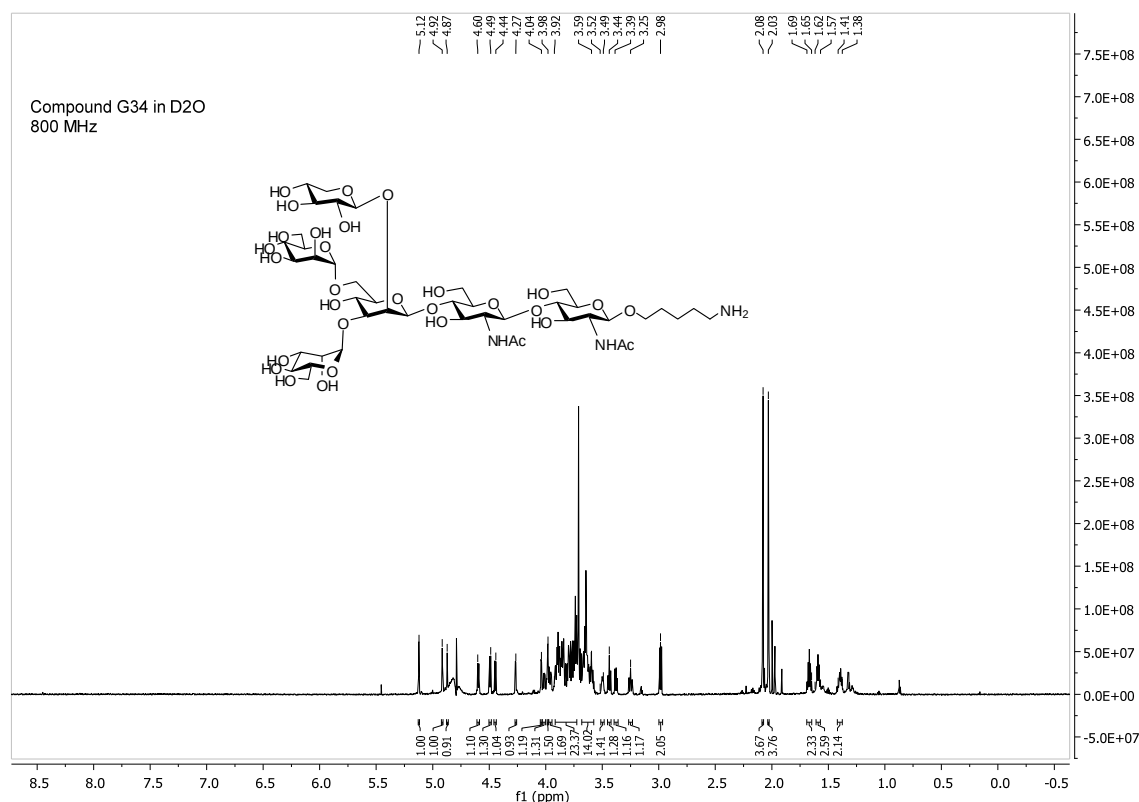
G32



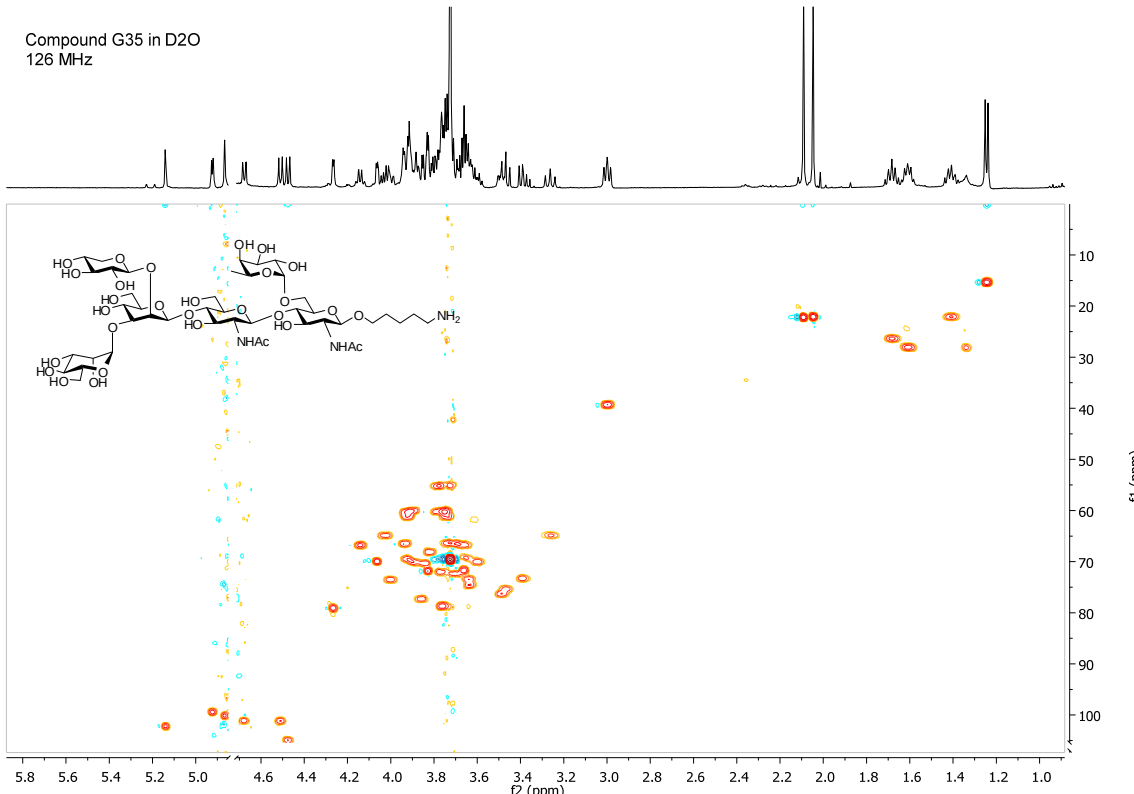
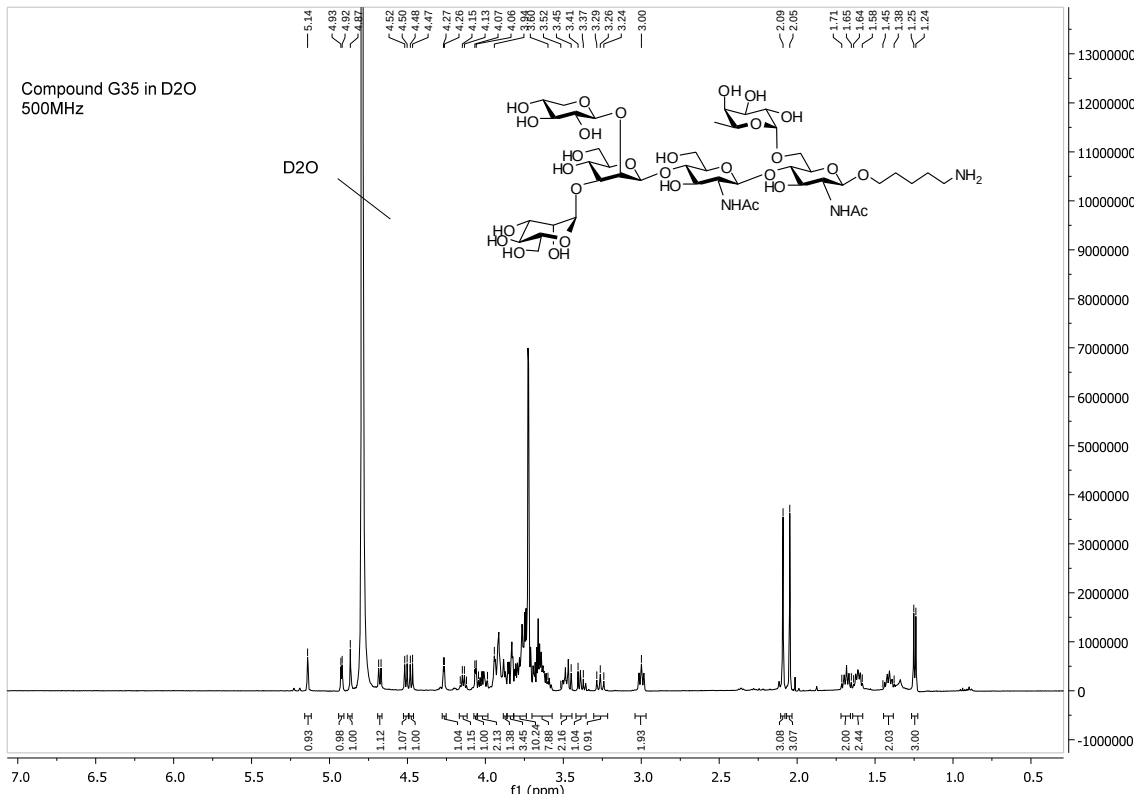
G33



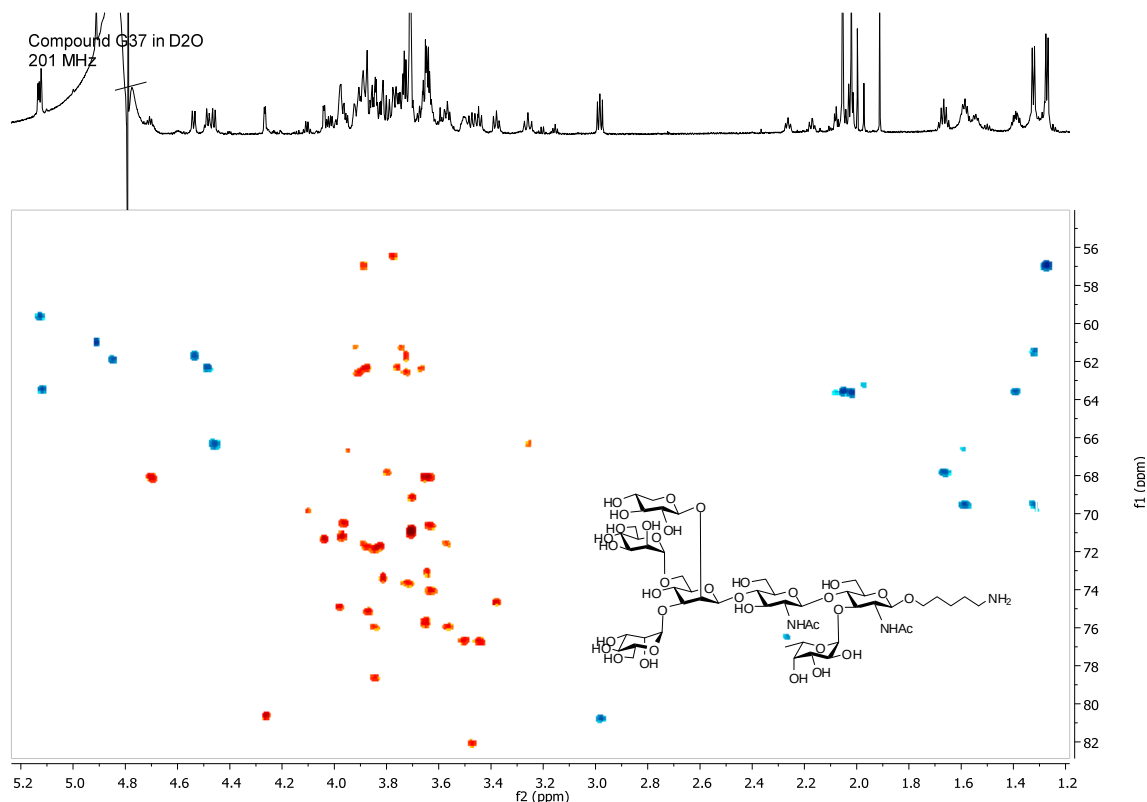
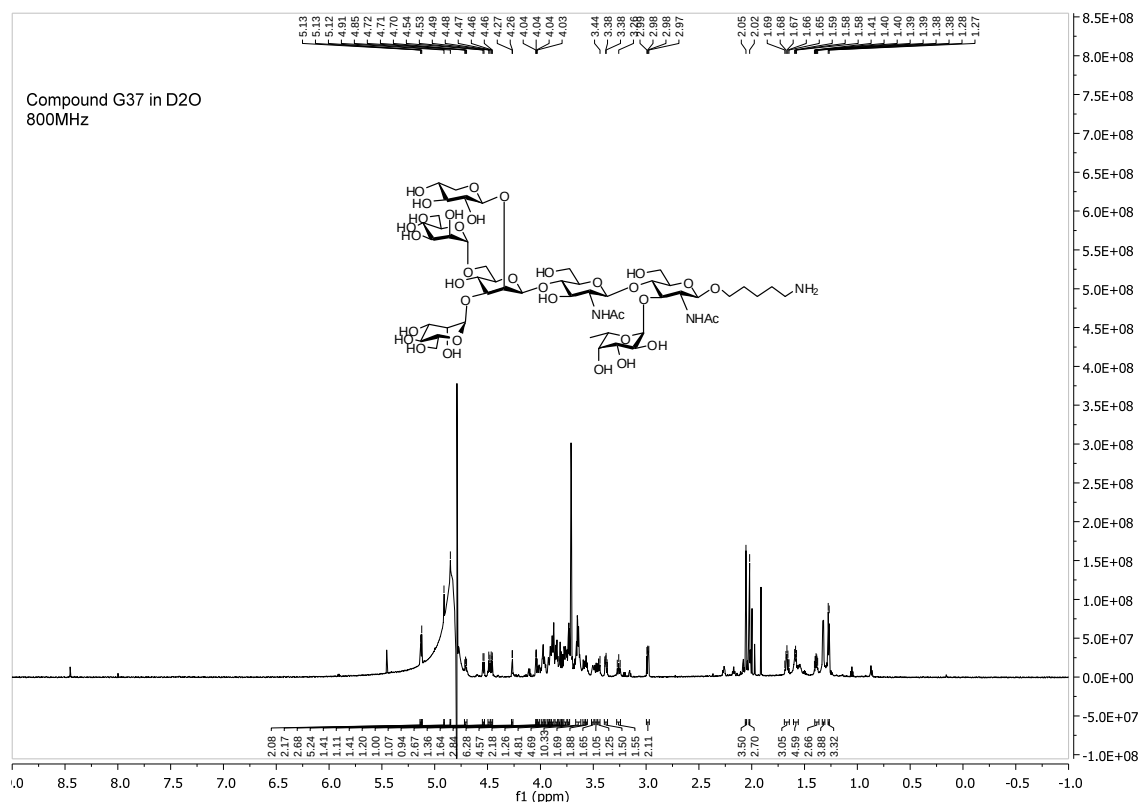
G34



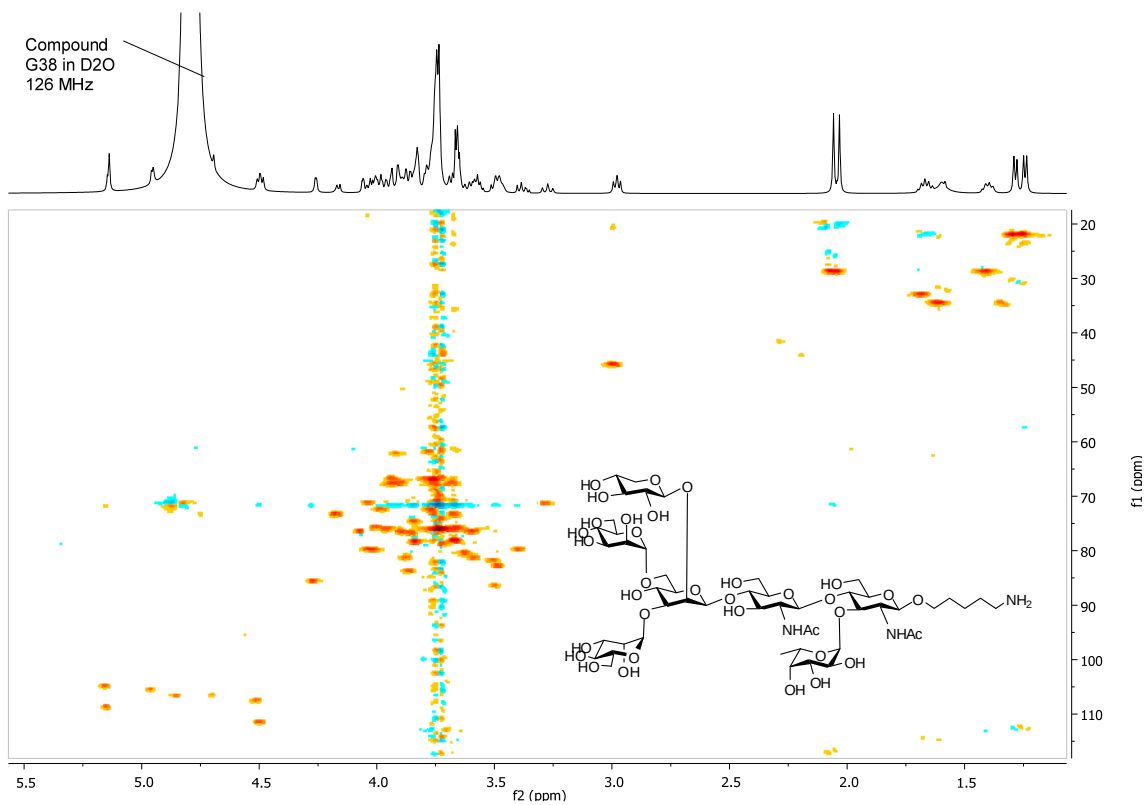
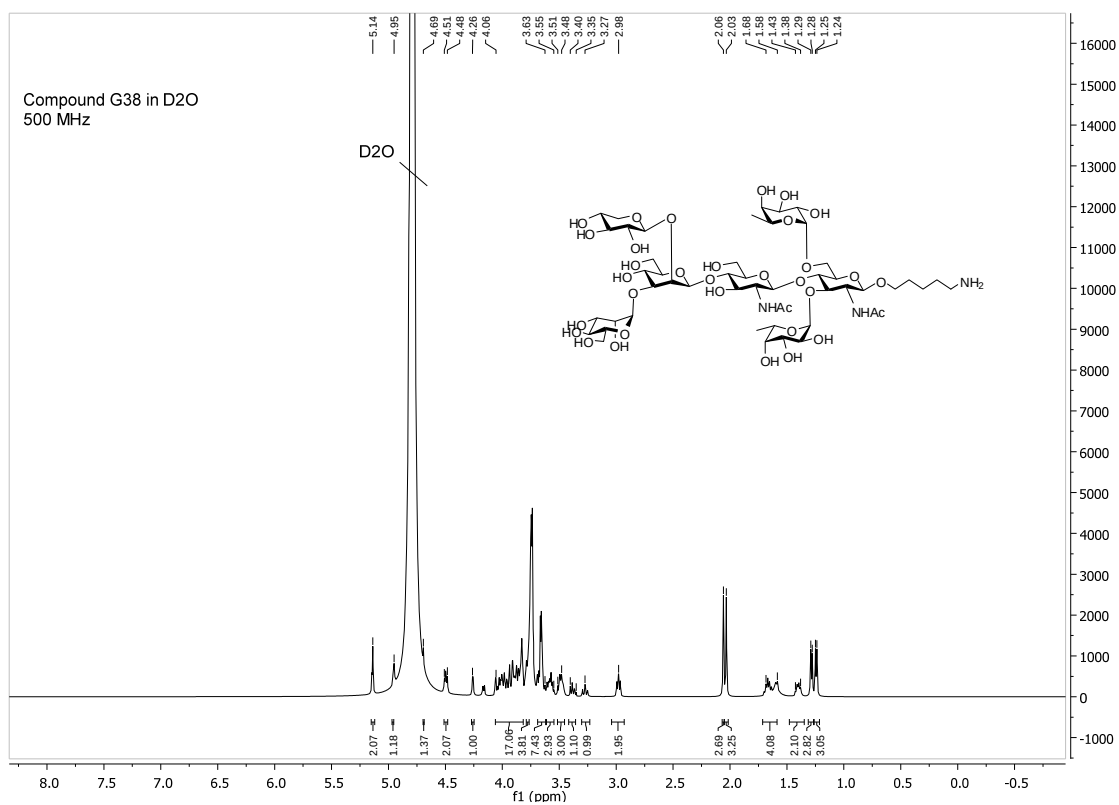
G35



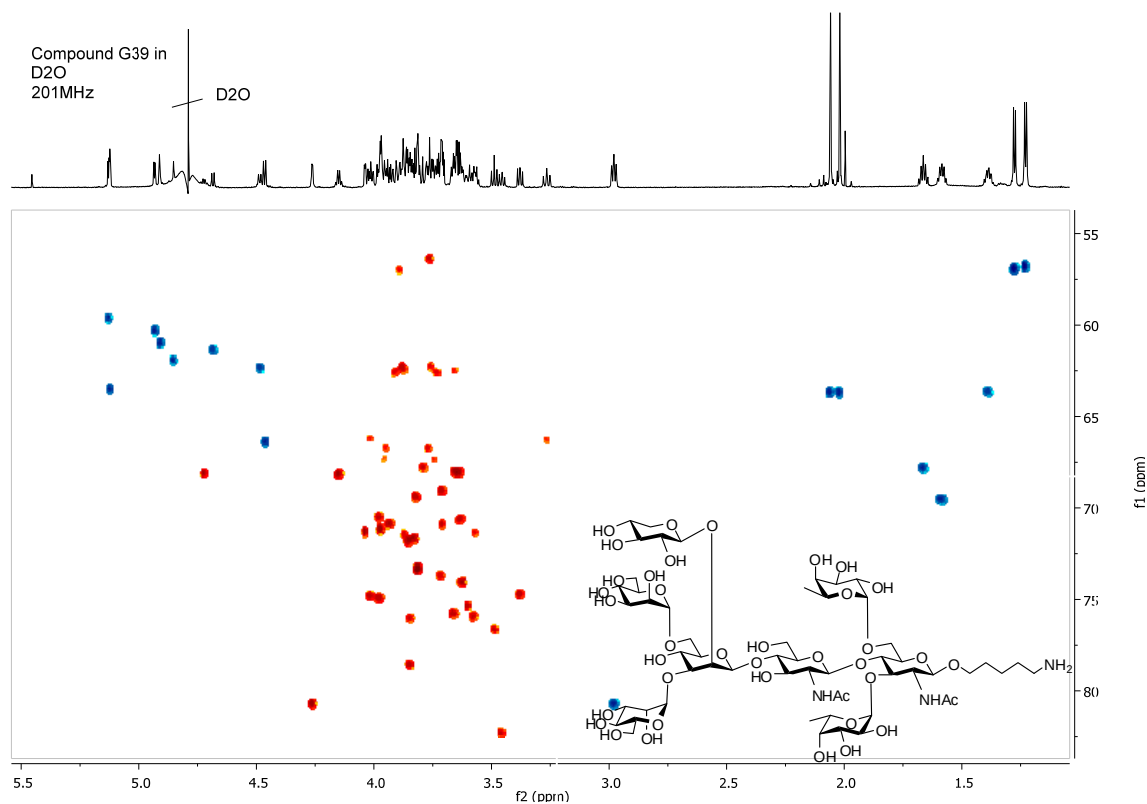
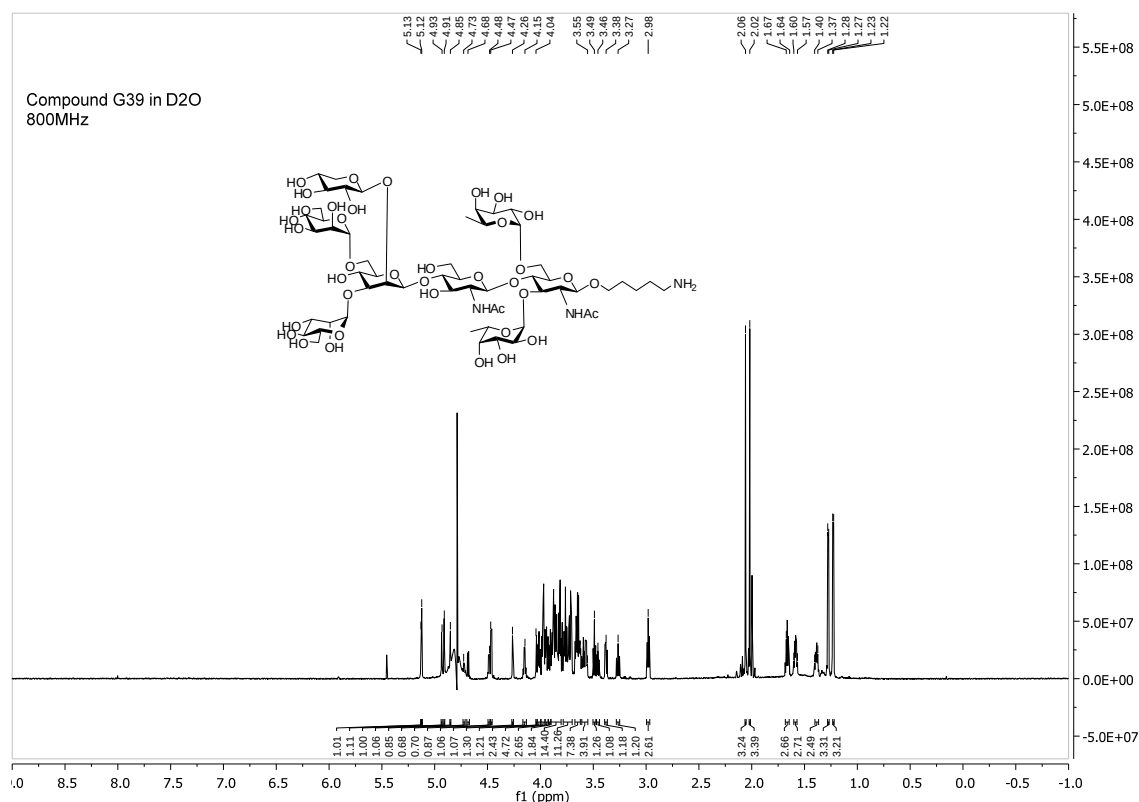
G37



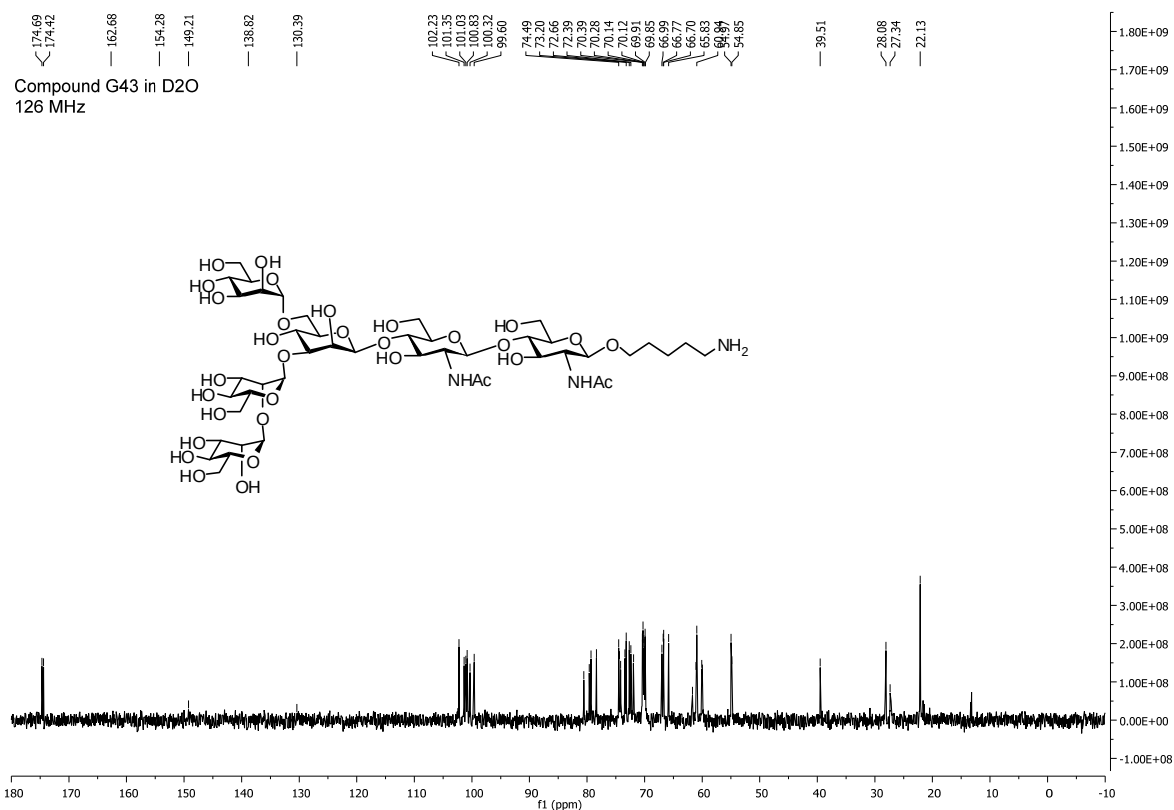
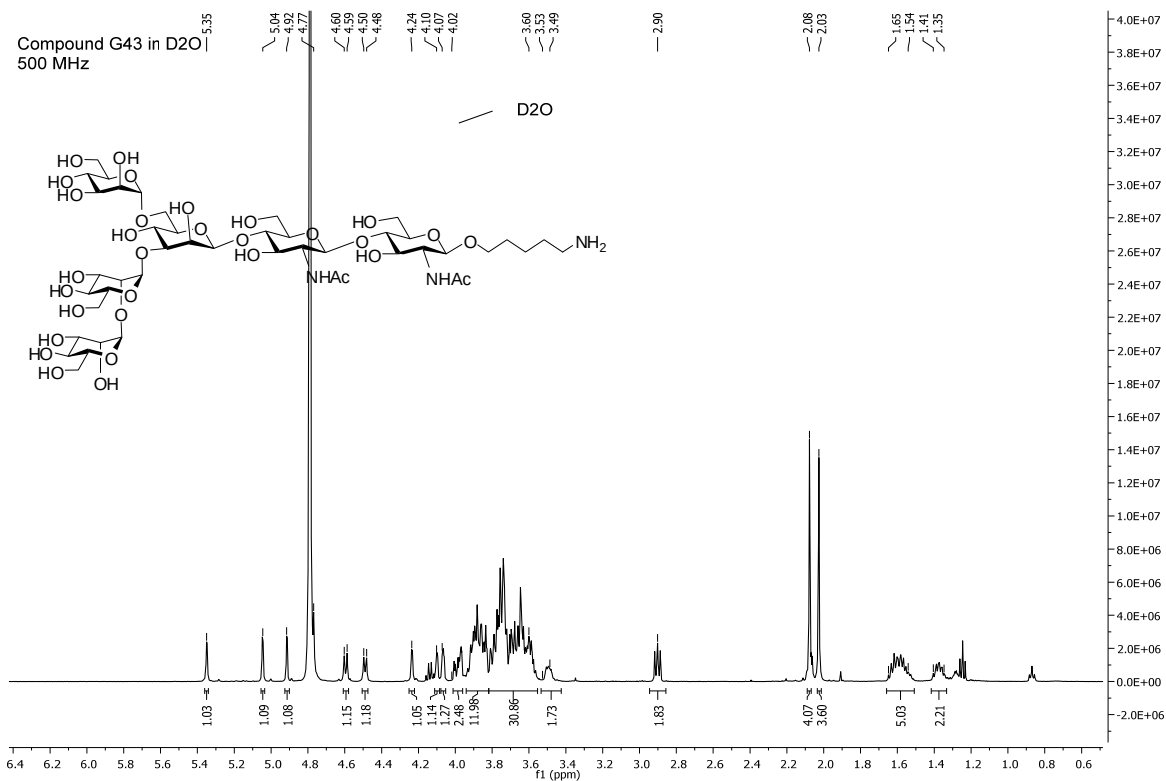
G38



G39

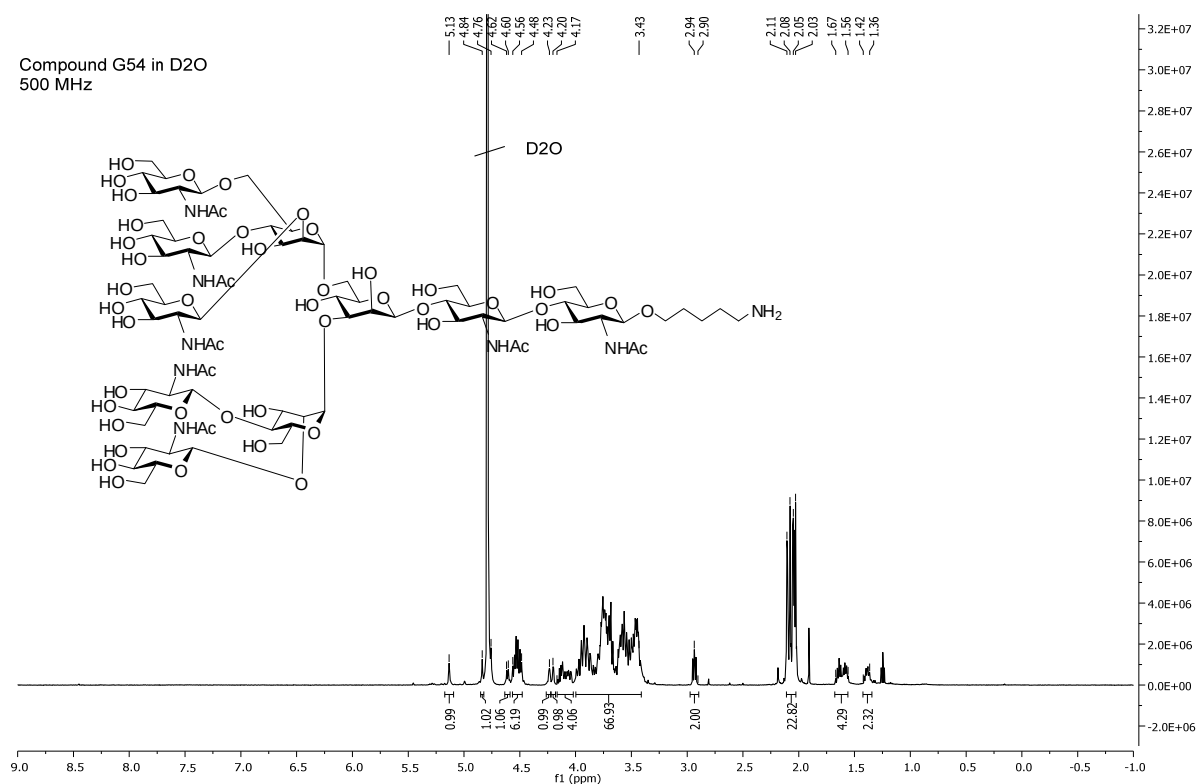


G43

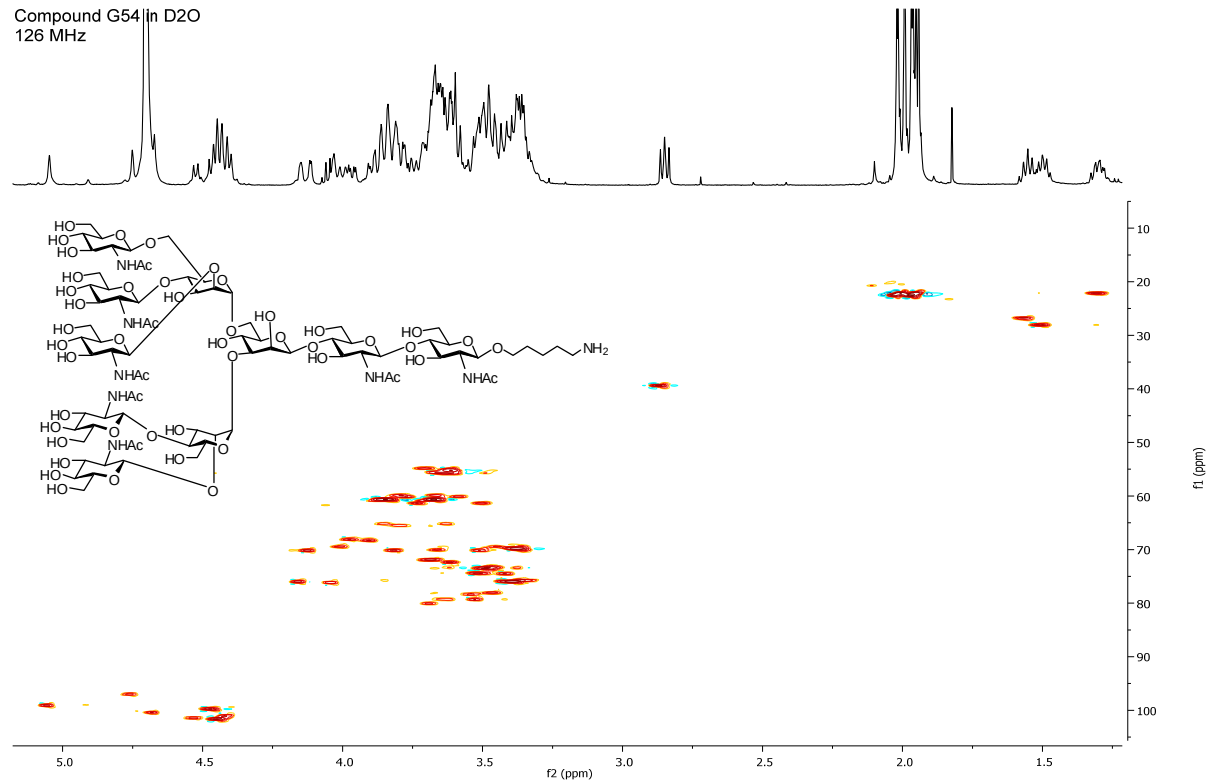


G54

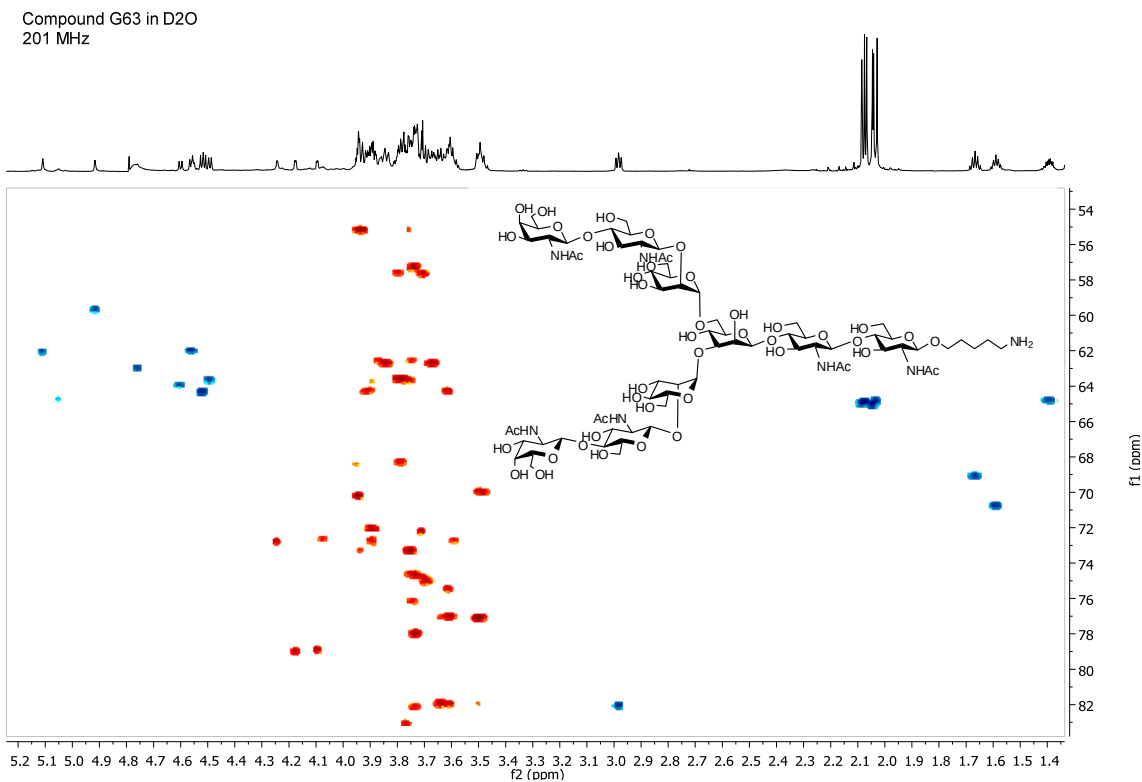
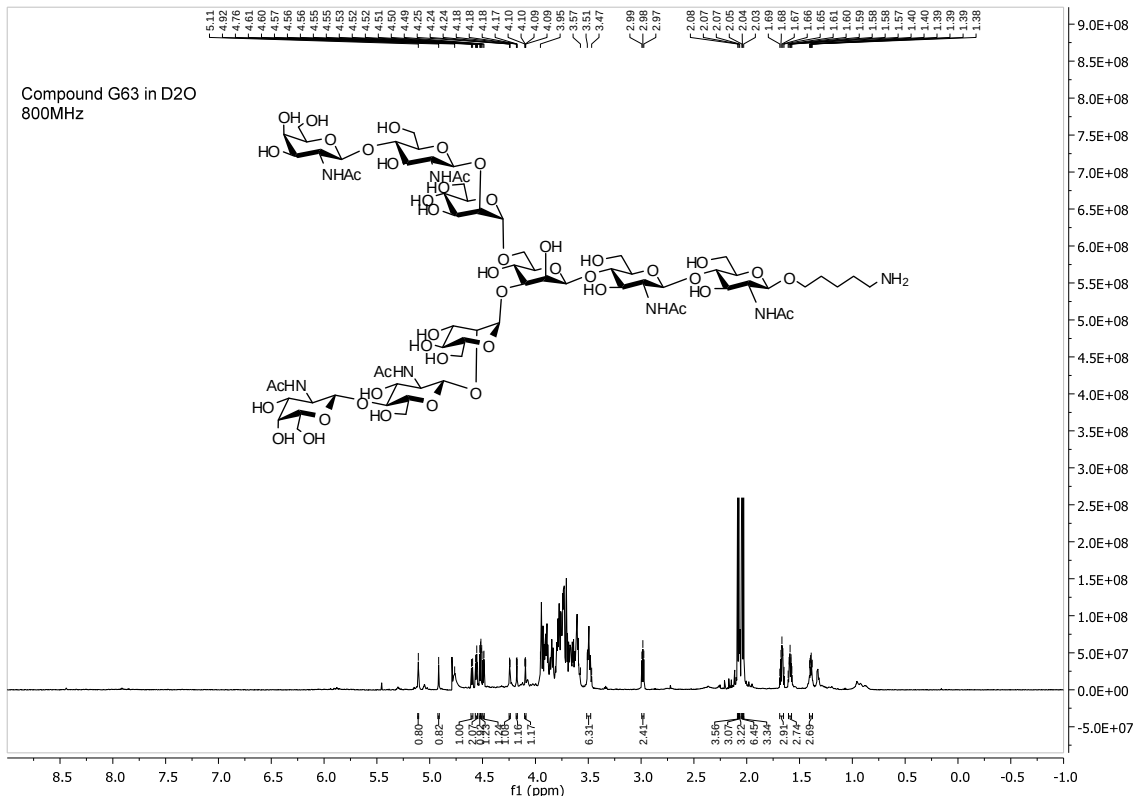
Compound G54 in D2O
500 MHz



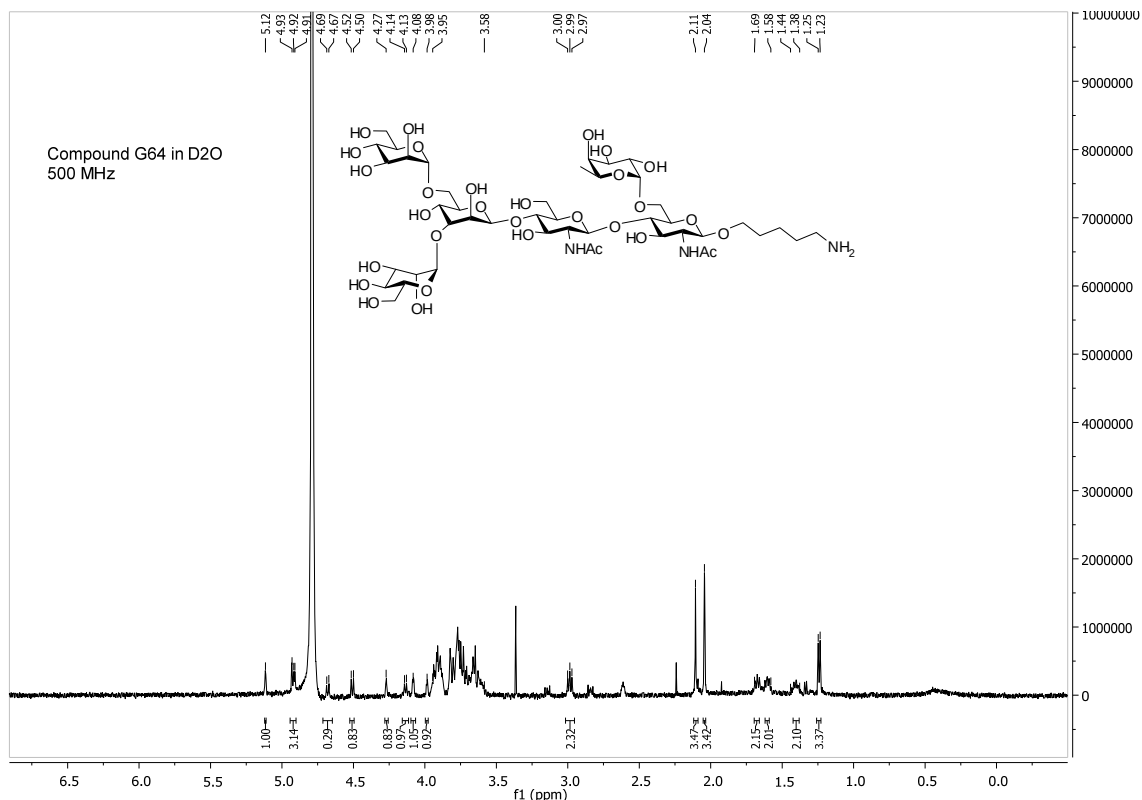
Compound G54 in D2O
126 MHz



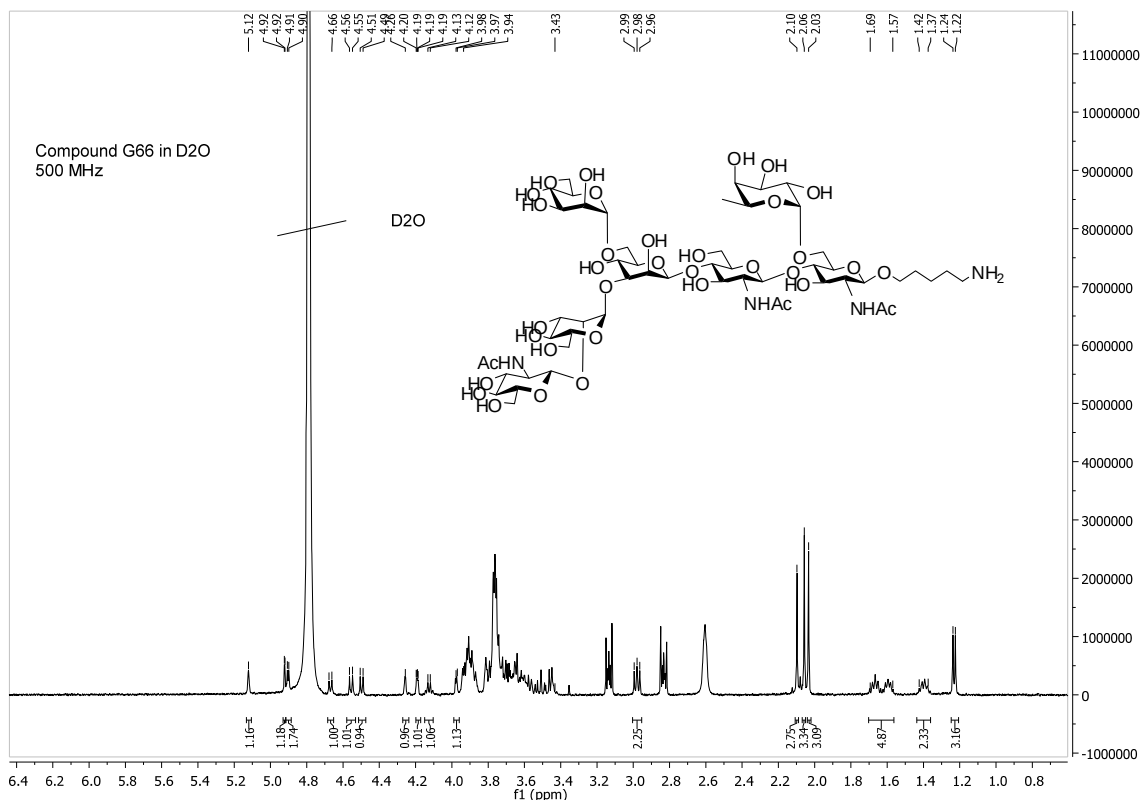
G63



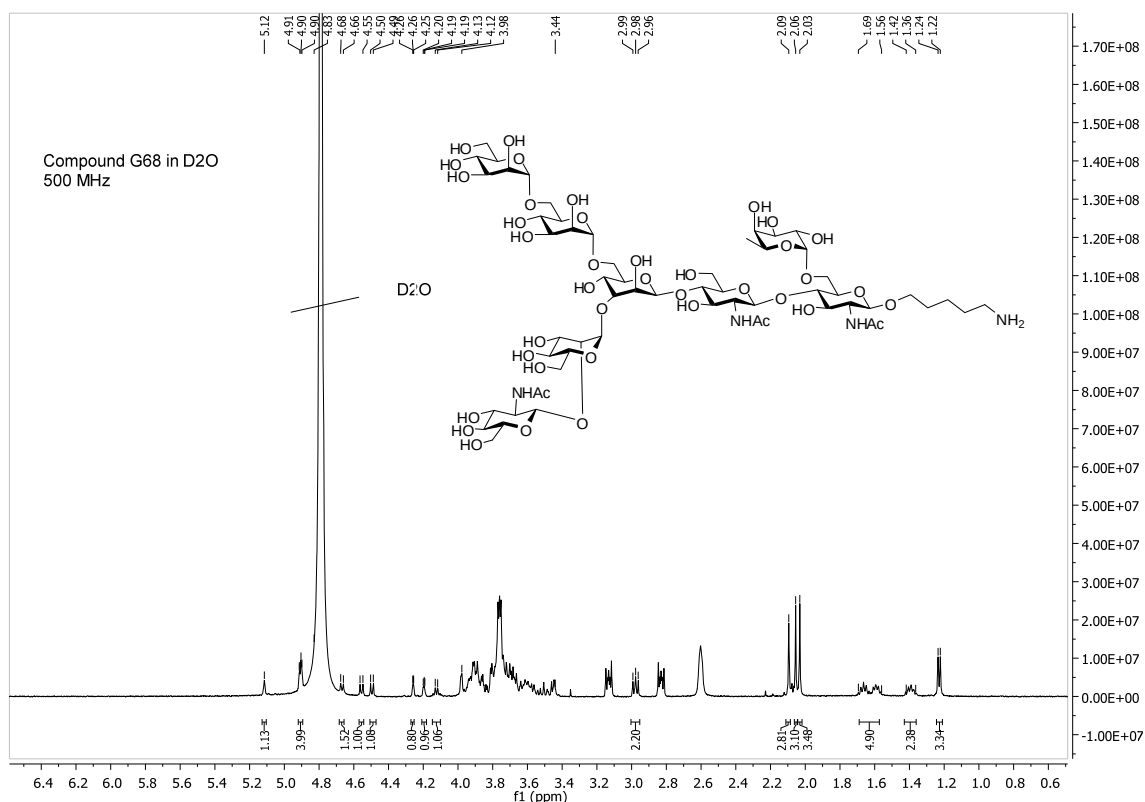
G64



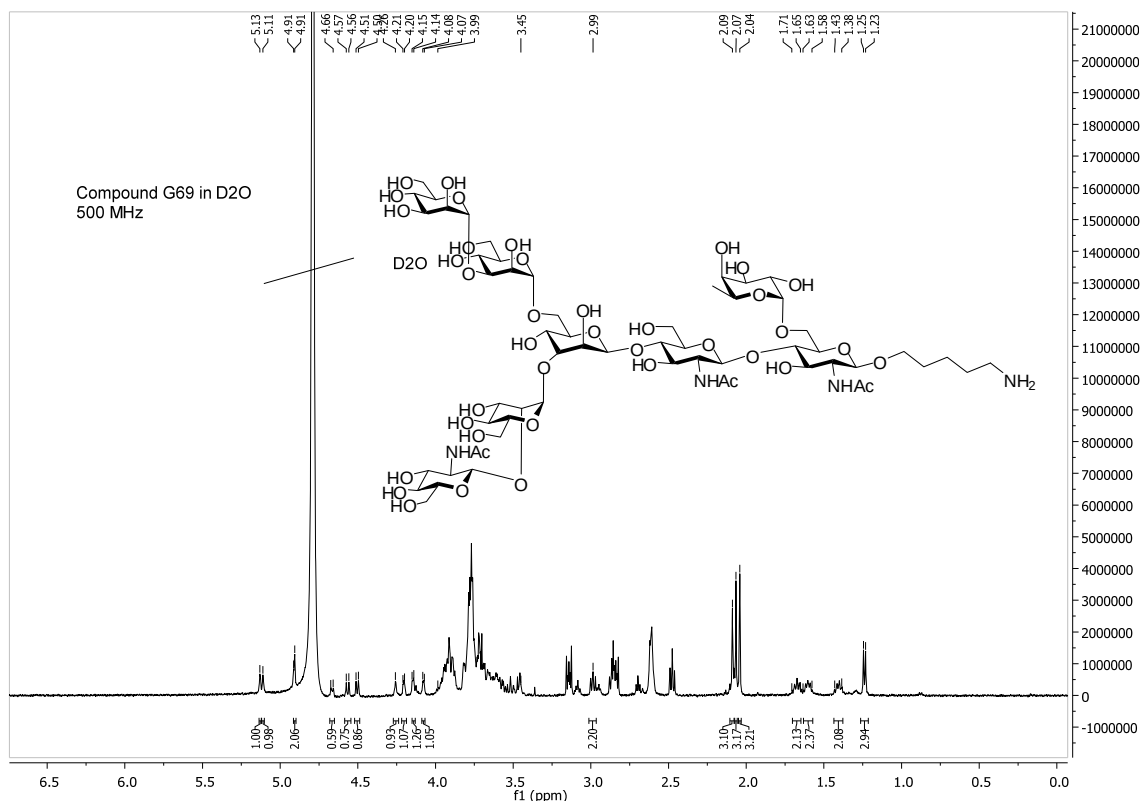
G66



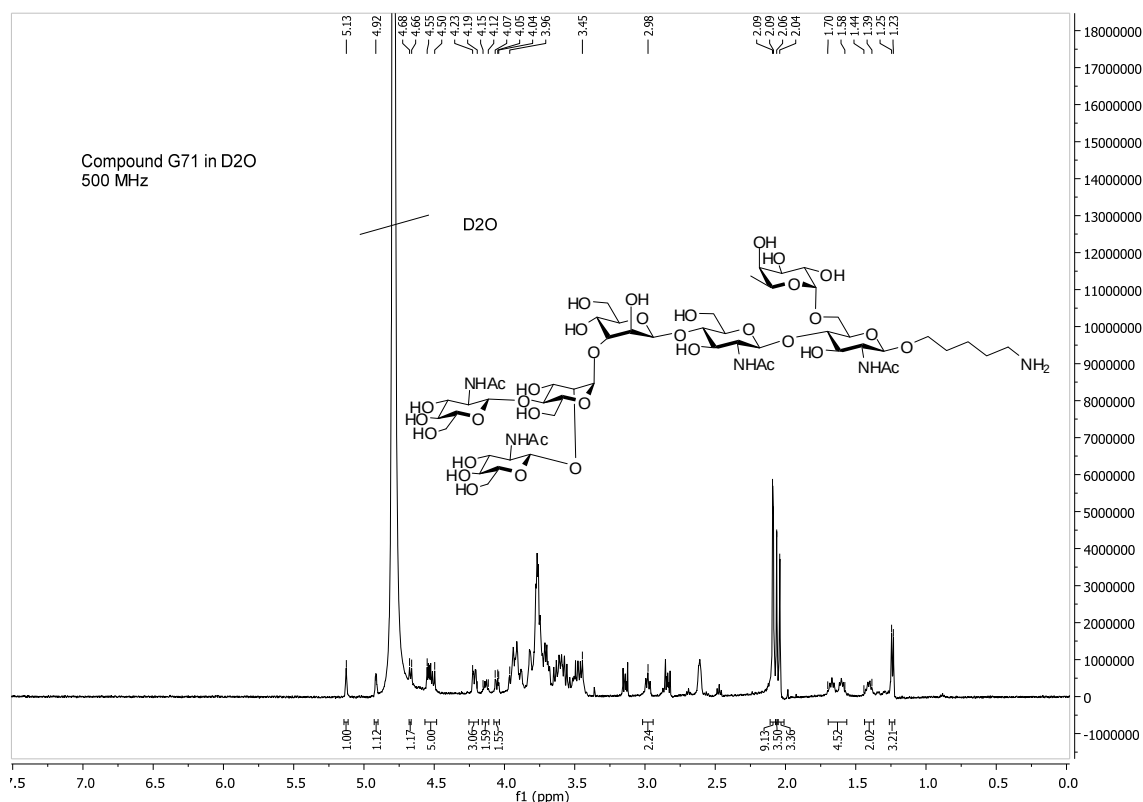
G68



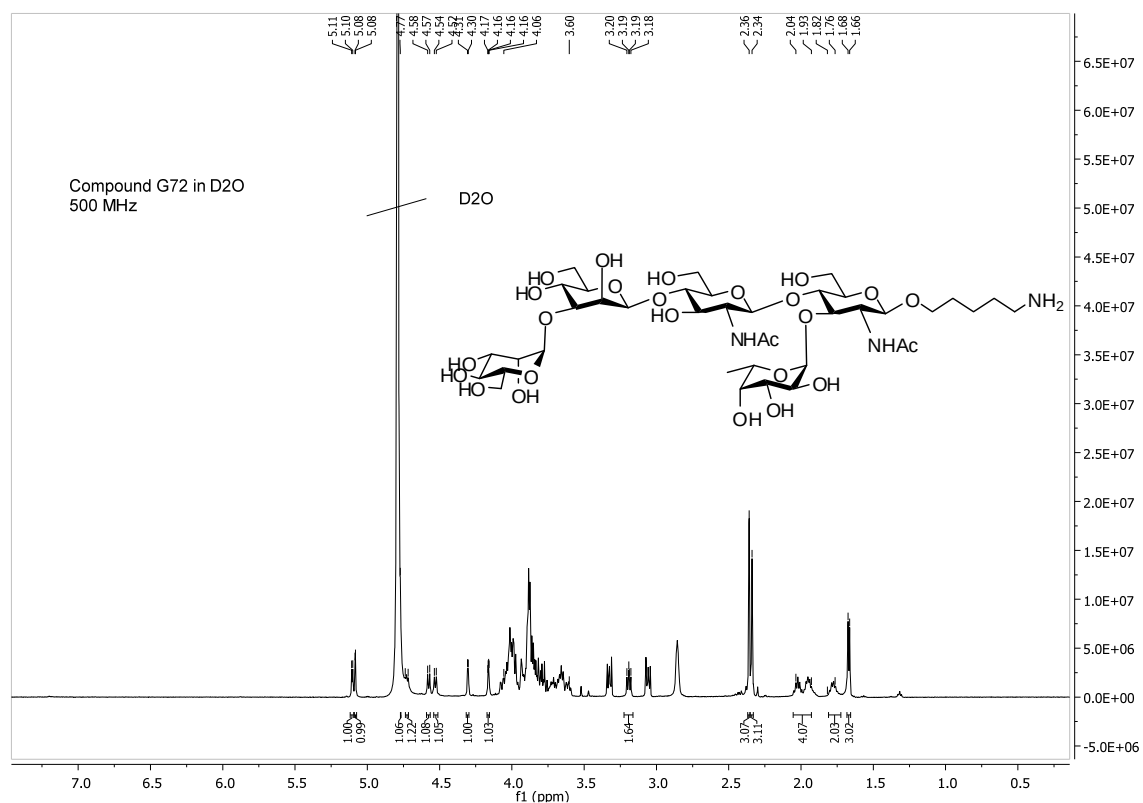
G69



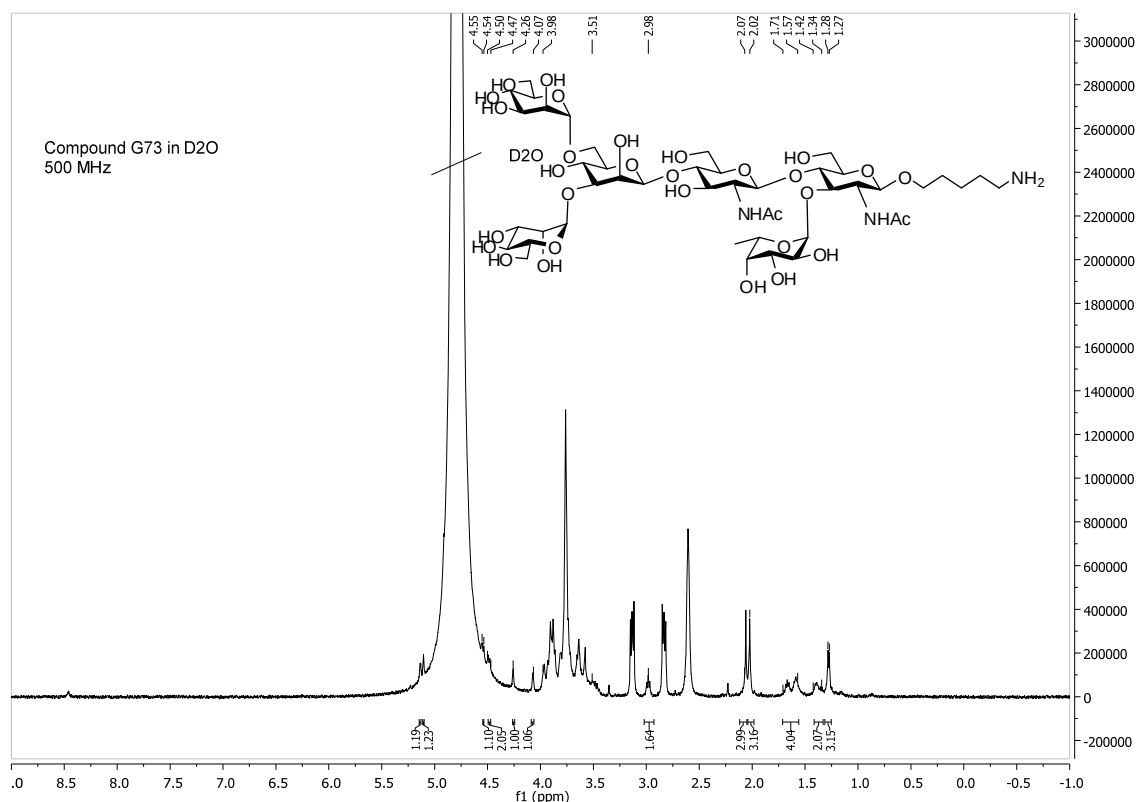
G71



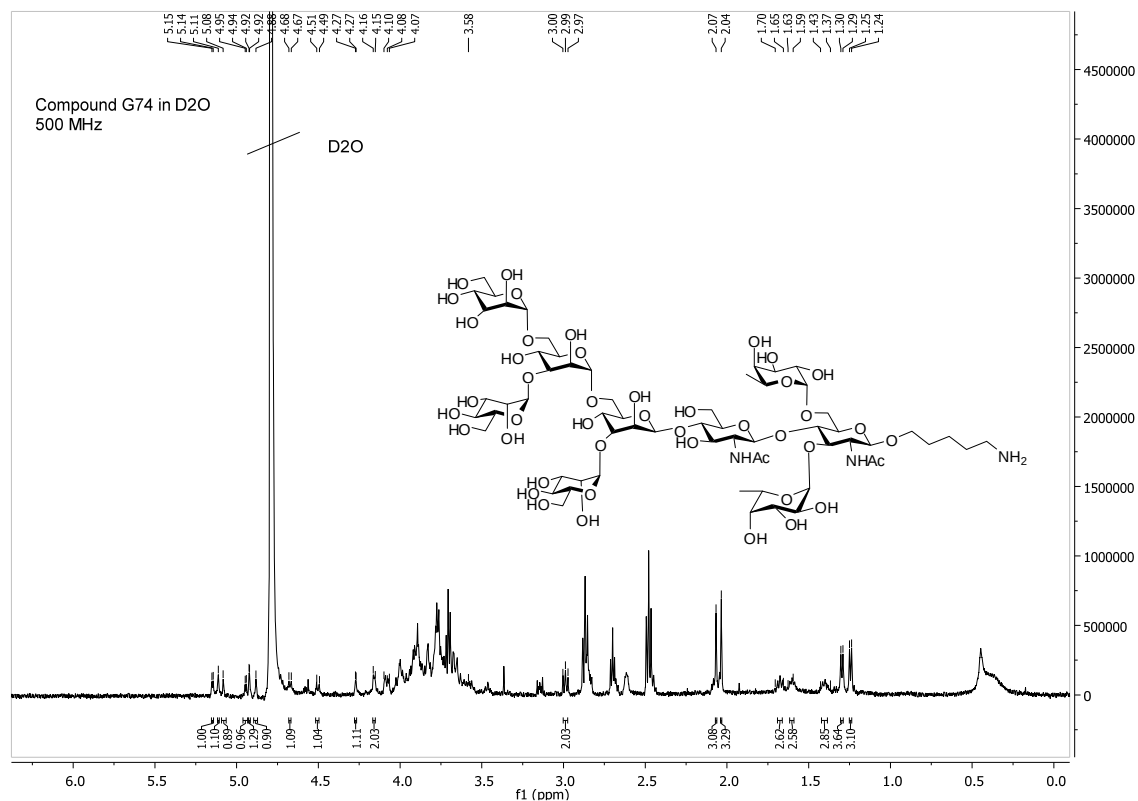
G72



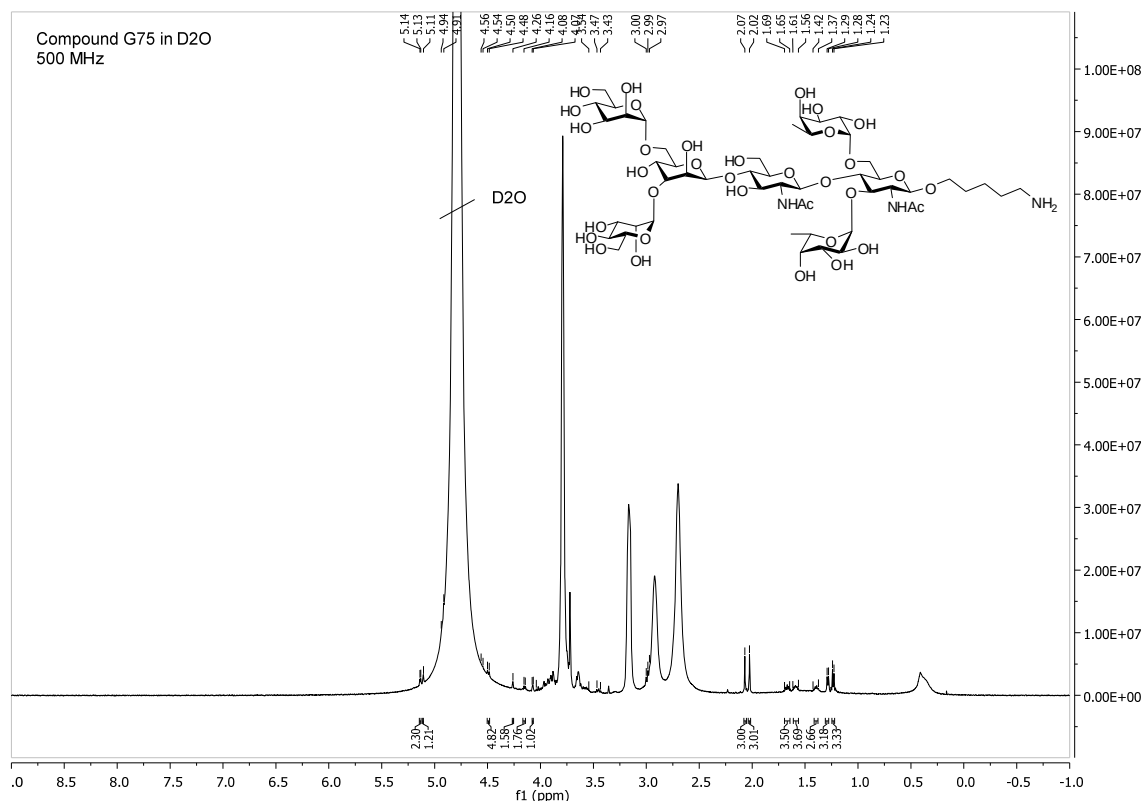
G73



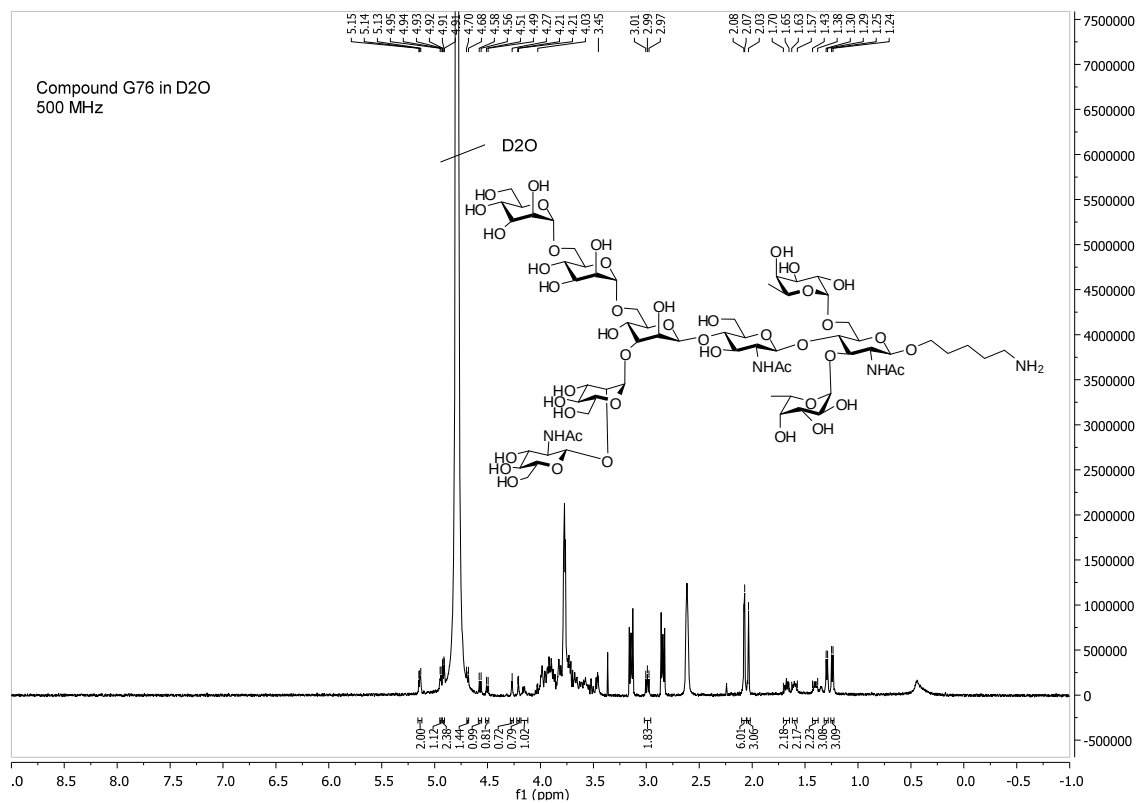
G74



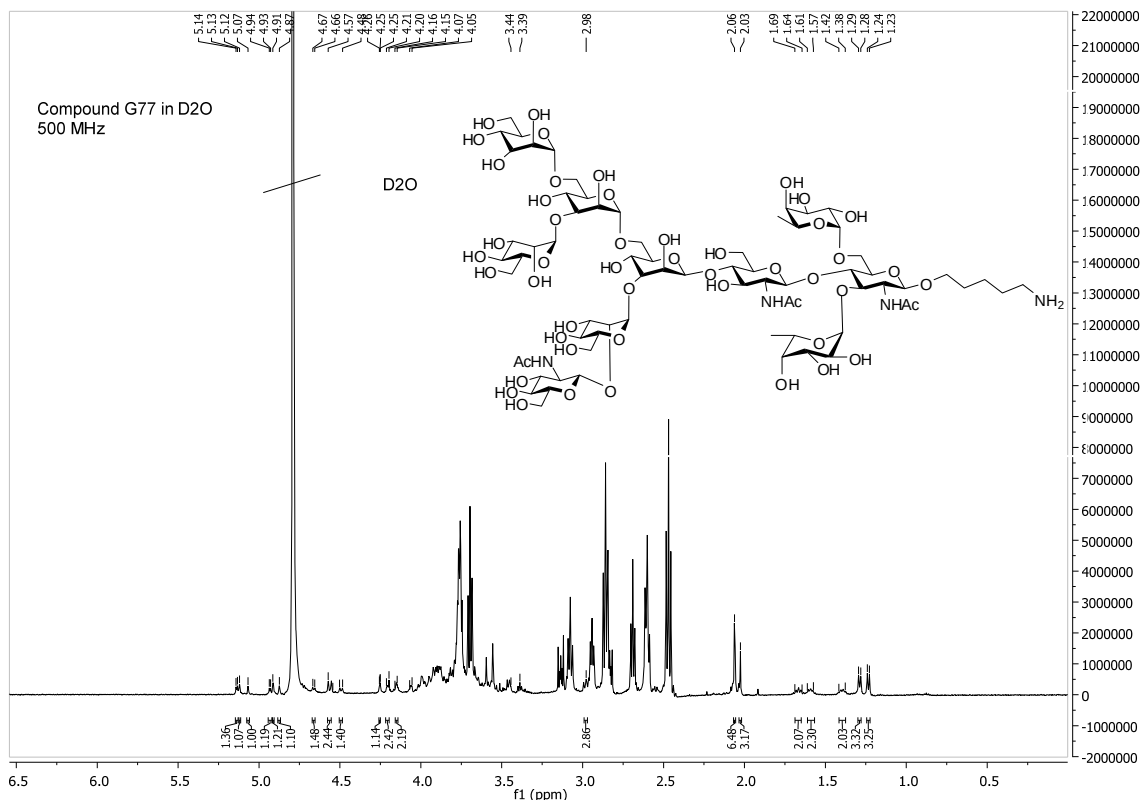
G75



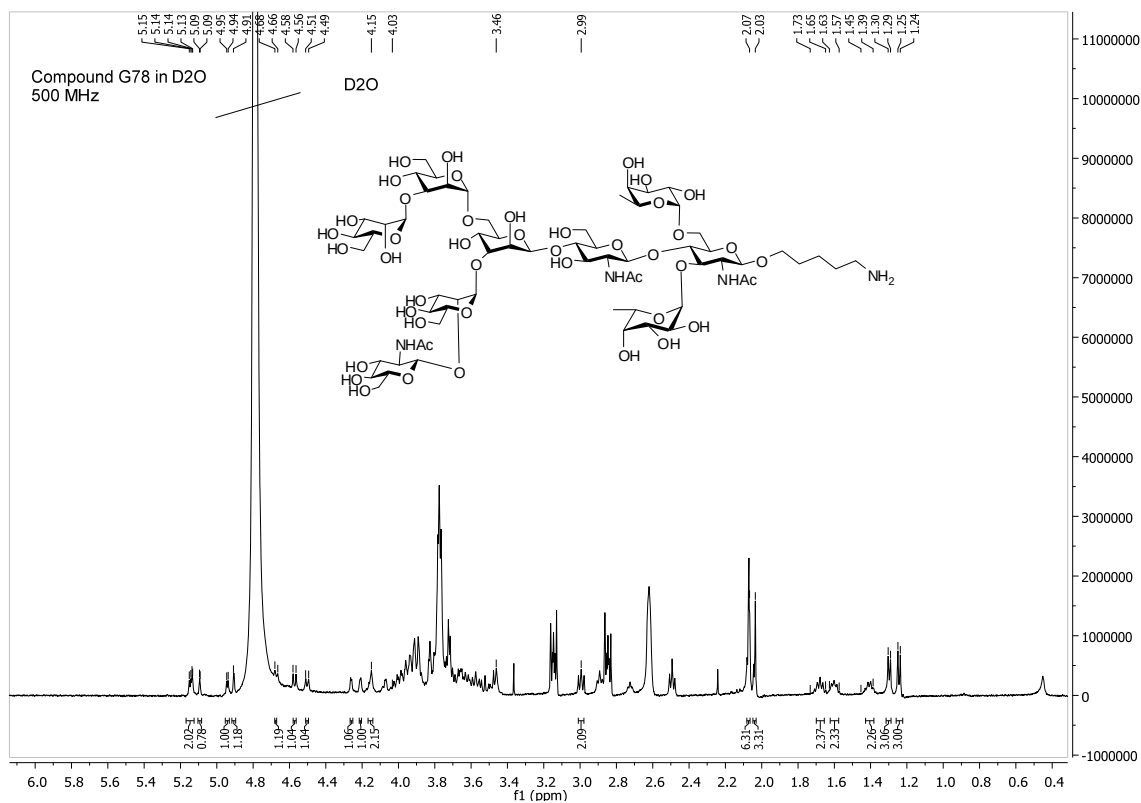
G76



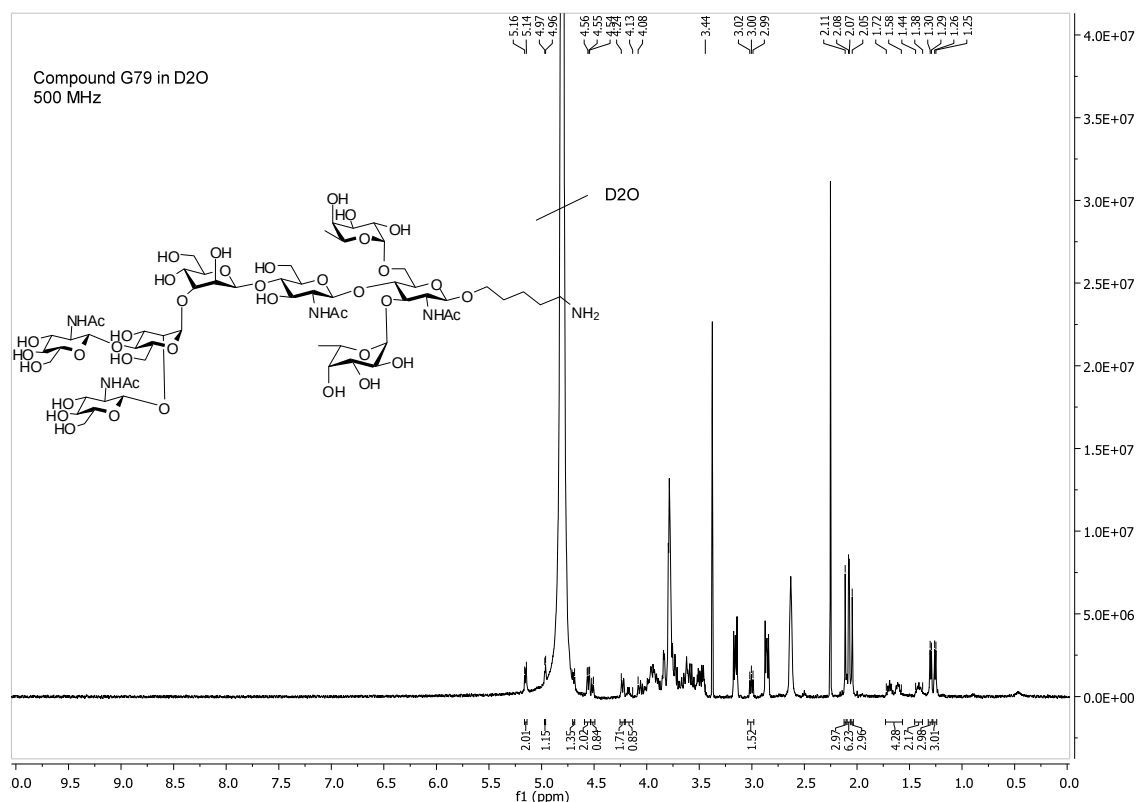
G77



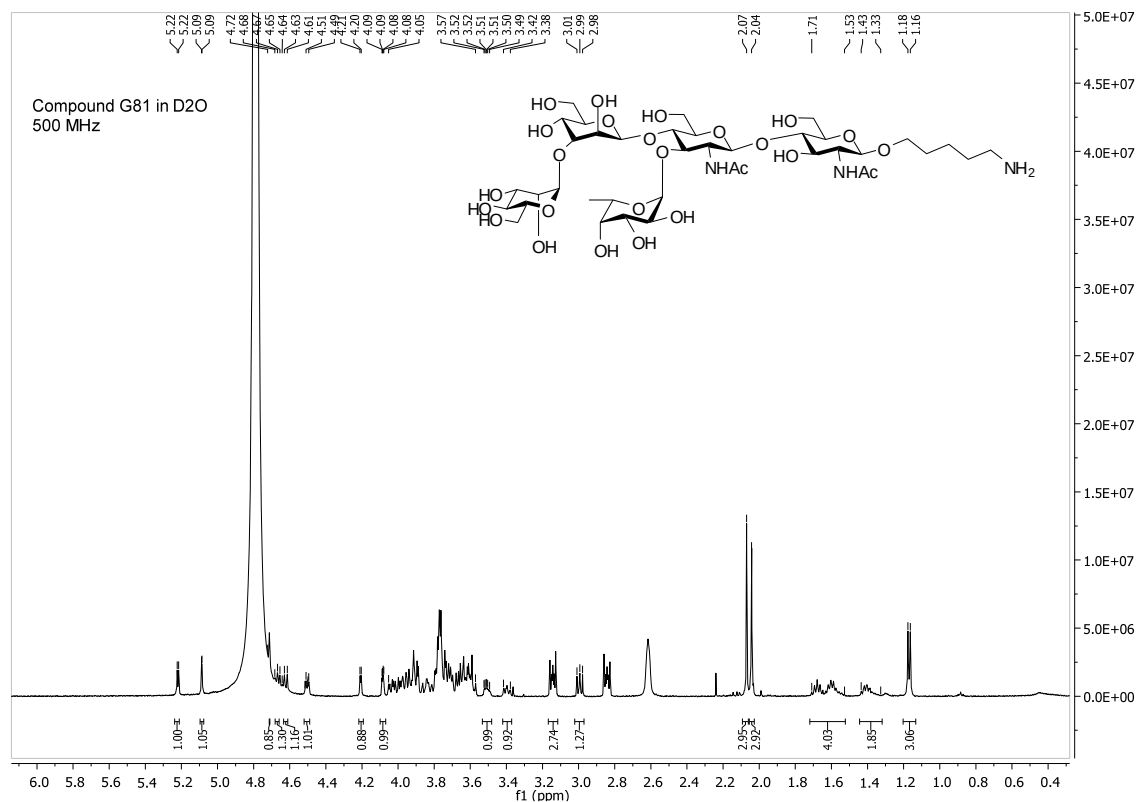
G78



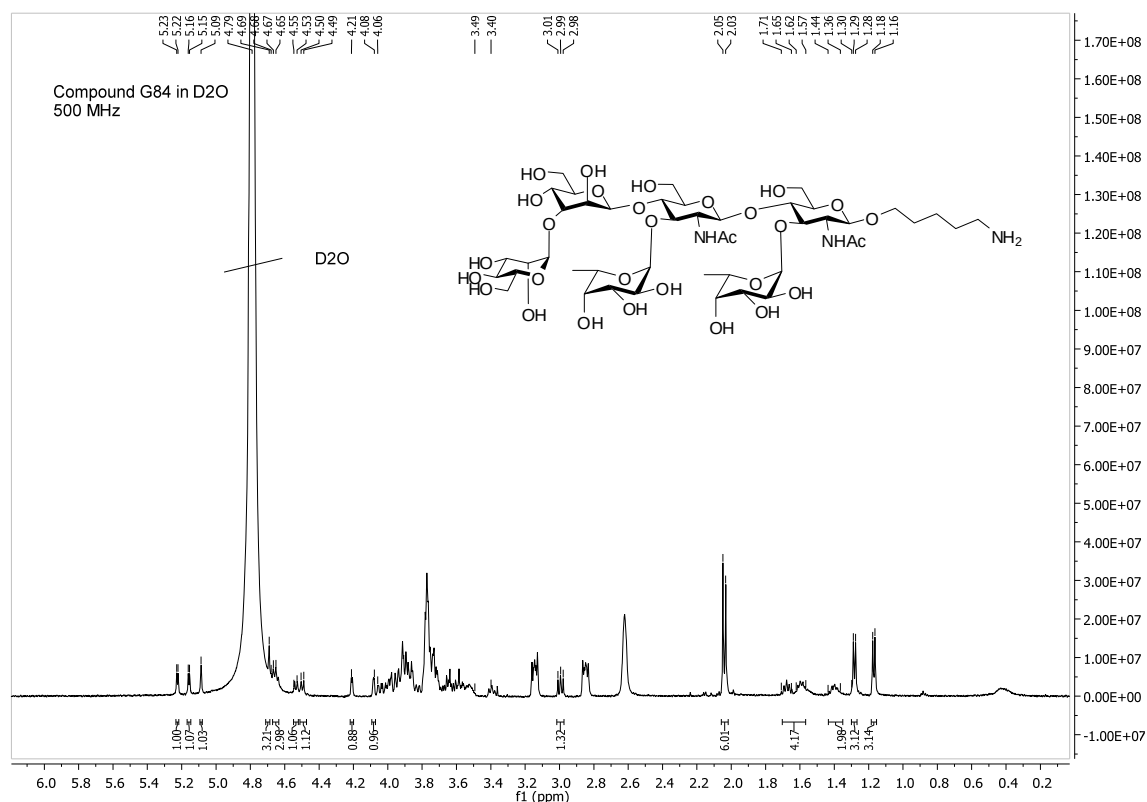
G79



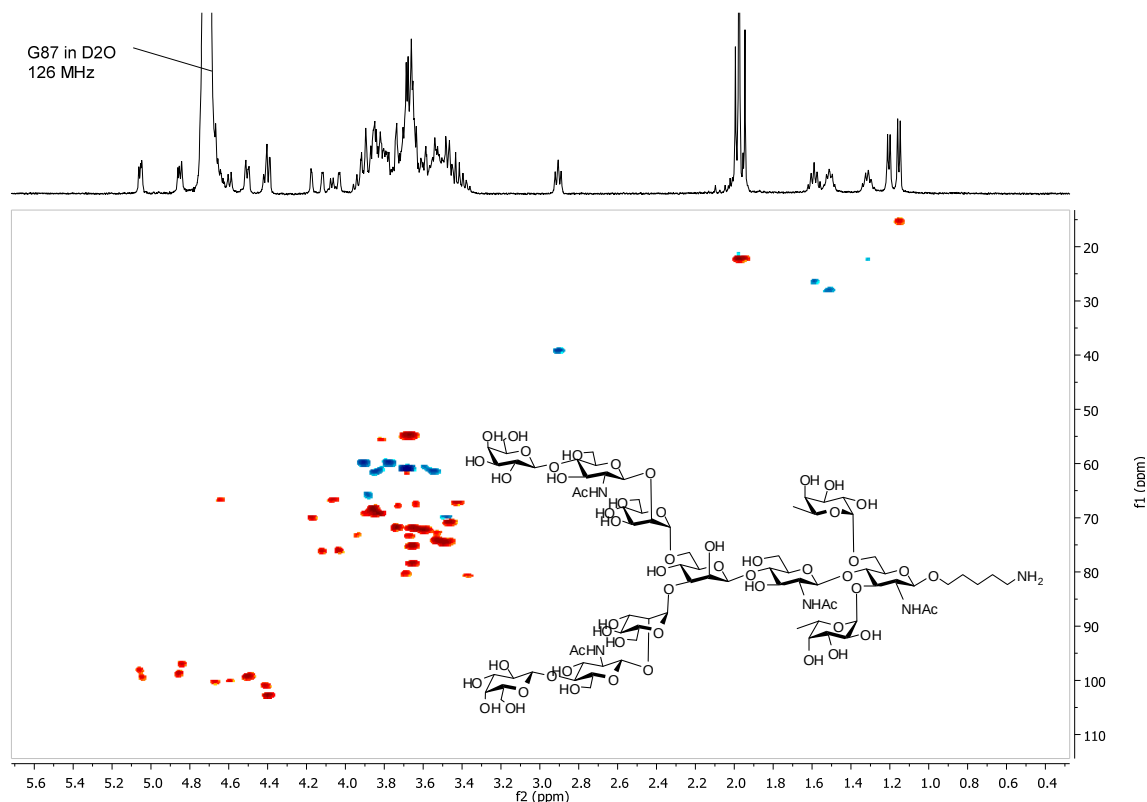
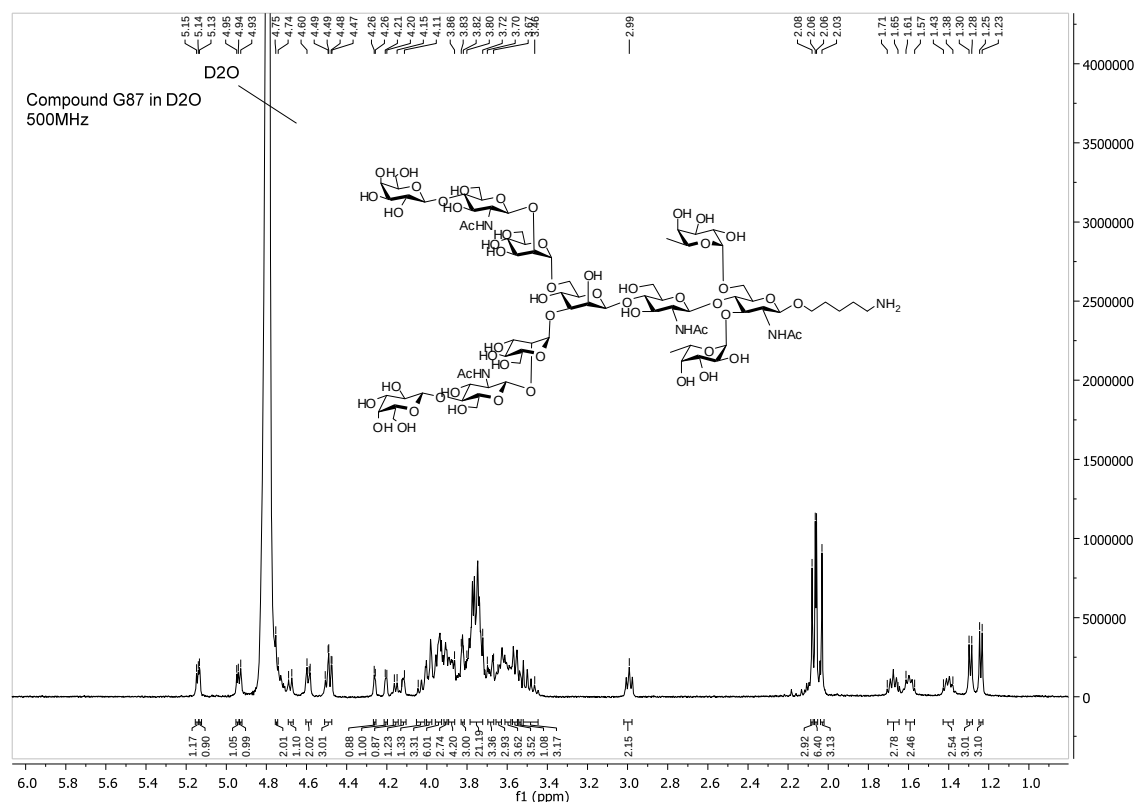
G81



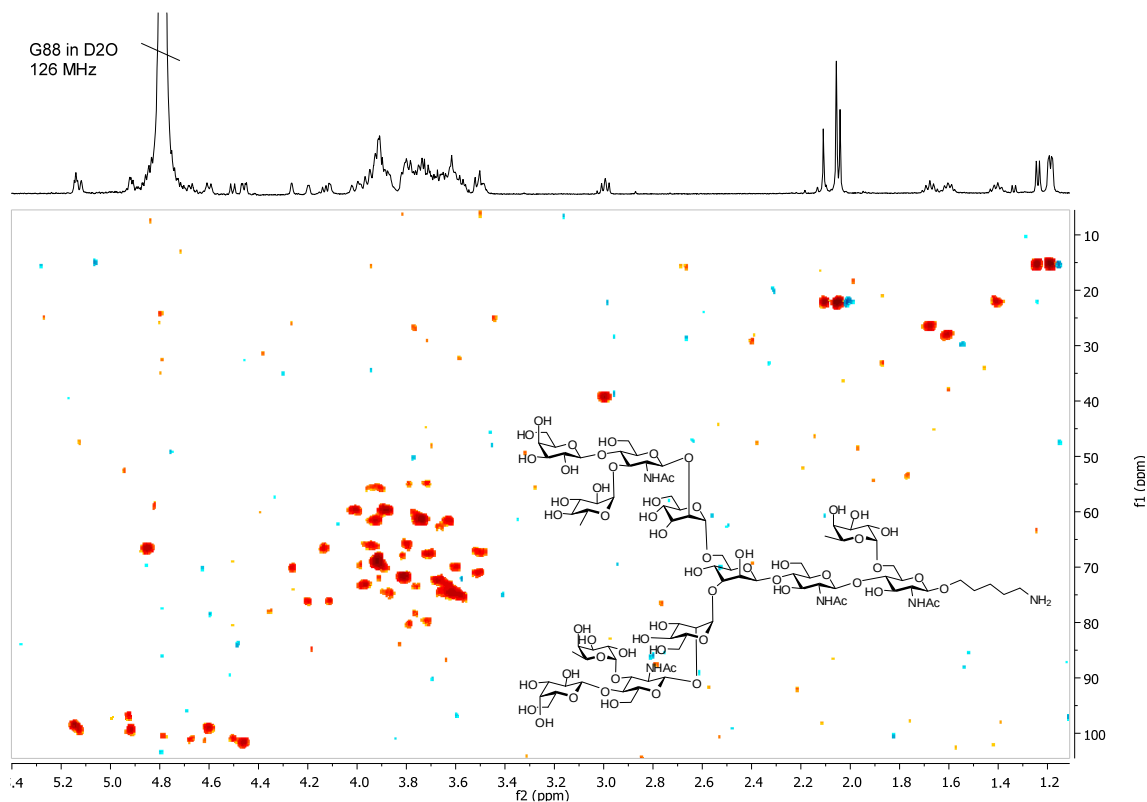
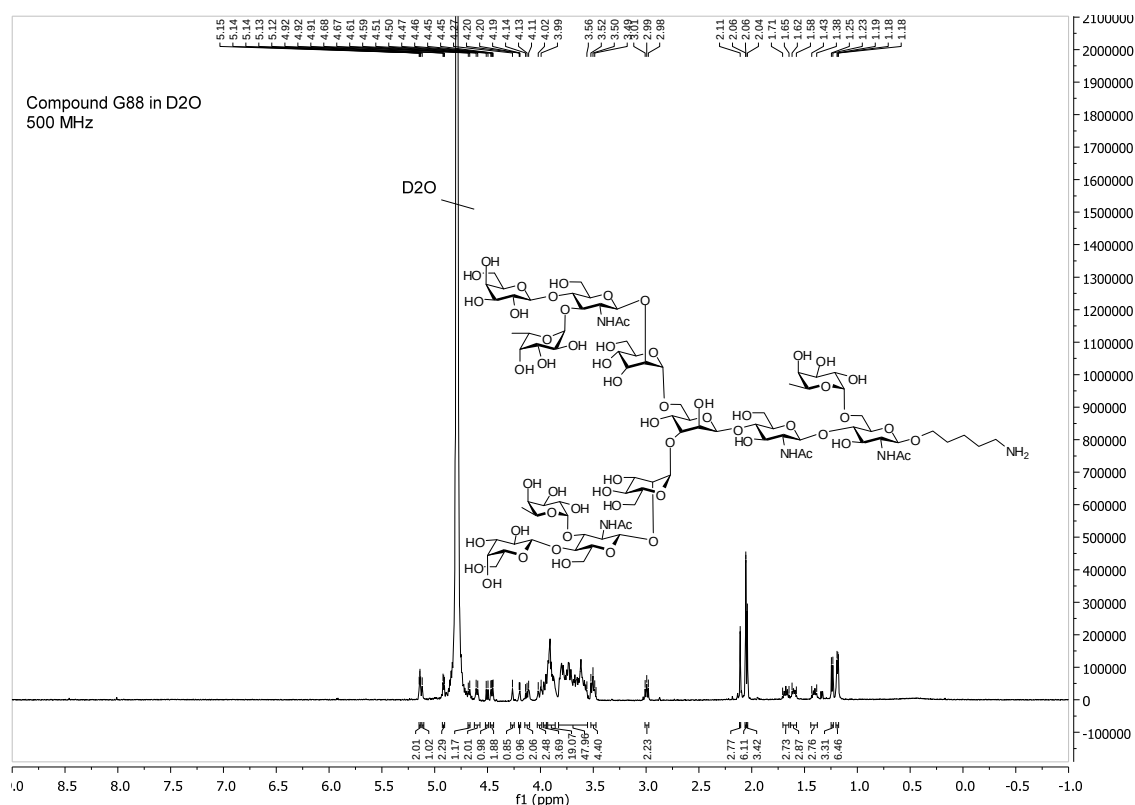
G84



G87



G88



G89

