Multivalent Homo and Hetero Glycodendrimers Allow Sequence-Defined Carbohydrate-Protein Interactions

Thesis submitted towards the partial fulfillment of

BS-MS dual degree programme

By

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Under the guidance of

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CERTIFICATE

This is to certify that this dissertation entitled "Multivalent Homo and Hetero Glycodendrimers Allow Sequence-Defined Carbohydrate-Protein Interactions" towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents the research carried out by "**Catherine Alex** at IISER Pune" under the supervision of "**Dr. Raghavendra Kikkeri**, Assistant Professor, Department of Chemistry" during the academic year 2015-2016.

Date: 28/03/2016

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DECLARATION

I hereby declare that the matter embodied in the report entitled "Multivalent Homo and Hetero Glycodendrimers Allow Sequence-Defined Carbohydrate-Protein Interactions" are the results of the investigations carried out by me at the Department of Chemistry, IISER Pune, under the supervision of Dr. Raghavendra Kikkeri and the same has not been submitted elsewhere for any other degree.

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ABBREVIATION

µl	micro-litre
$BF_3.Et_2O$	Boron trifluoride diethyl etherate
Boc_2O	Di-tert-butyl-dicarbonate
Con A	Concanavalin A
CPI	Carbohydrate-Protein Interactions
DCM	Dichloromethane
DIC	N, N'-Diisopropylcarbodiimide
EtOAc	Ethyl Acetate
EtOH	Ethanol
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
J	Coupling Constant
MeOH	Methanol
MHz	Megahertz
mmol	millimole
NaOMe	Sodium Methoxide
NIS	*N*-Iodosuccinimide
NMR	Nuclear Magnetic Resonance
PFP	2, 3, 4, 5, 6 - Pentafluorophenol
PPh_3	Triphenylphosphine
TEA	Triethylamine
TFA	Trifluoroacetic acid
TfOH	Trifluoromethanesulfonic acid
TLC	Thin Layer Chromatography
HOBt	1-Hydroxybenzotriazole hydrate

ABSTRACT

We present the synthesis of multivalent glycodendrimers in homo and hetero patterns utilizing different mannose and galactose carbohydrate residues. Highly efficient mannose thiophenol donor in presence of promoter and linker is the key step to generate a library of mono, di and tri α(1-2) mannose glycans in one-pot method. Introduction of these carbohydrate ligands on a tripodal backbone in stoichiometric ratio allows for the straightforward synthesis of multivalent hetero and homo glycodendrimers. Surprisingly, all hetero glycodendrimers show high affinities toward Concanavalin A lectin receptors in comparison to their homo-analogs. Detailed studies of *E.coli* ORN 178 binding further demonstrated the significance of heterogeneity in the glycodendrimers, which promote steric shielding of the glycans to modulate the binding avidity. Overall, these results shed light to the cell surface carbohydrate-protein interactions, which is heterogeneous in nature.

INTRODUCTION

Multivalency plays a crucial role in bolstering the interaction between different molecules or interfaces[1]. The characteristic feature for selective and strong association of components which include biomolecules, cells and tissues is the presentation of multiple binding partners with multiple binding sites[2], which is essential to increase avidity as well as selectivity[3]. The surface of mammalian cells are heavily glycosylated and these glycans are attached to proteins (glycoproteins) or lipids (glycolipids). The interaction with the extracellular matrix is carried out by the communication between these glycans and lectins (carbohydrate binding proteins) present on other cells which can be a mammalian cell or a pathogen[4]. Targeting these lectin interactions is an excellent way to shed light into pathogens as well as cell based therapies[5]. Recently, several tools have been developed for mimicking the carbohydrate-protein interactions (CPIs) which include dendrimers, peptides, polymers, supramolecular complexes and nanoparticles [6-8] (**Fig. 1**).

The natural carbohydrate-protein interactions are far more selective, sensitive and spontaneous and hence these probes are still far way in mimicking these naturally occurring interactions. Hence, design of multivalent probes taking into consideration additional parameters such as symmetry, size and shape is of utmost importance in order to optimize the carbohydrate-lectin binding events. For example, C_5-symmetric GM1 ganglioside resulted in potential inhibition of cholera toxin *via* specific multivalent binding[9], while the C_3-symmetric GM3 ganglioside inhibited influenza virus hemagglutination[10]. The rod-shaped gold nanoparticles coated with mannose monosaccharide exhibited sensitive and selective inhibition of bacterial infection[11]. On the other hand, monodisperse glycopolymers[12] and glycodendrimers with different sugar topologies provided useful information about the spatial display of glycans and the optimal carbohydrate density essential for carbohydrate-lectin interactions[13-15]. These parameters provided valuable insight into the multivalency and dendrimer structures.

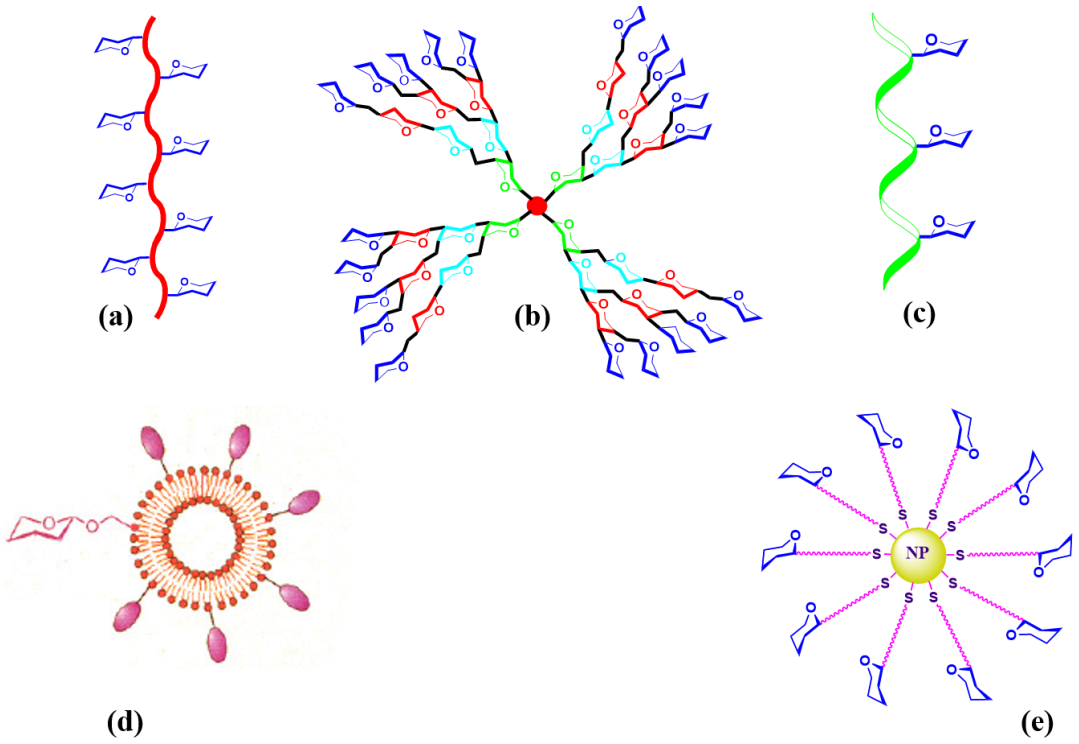


Figure. 1. Carbohydrate based multivalent probes: **(a)** glyco-polymers; **(b)** glyco-dendrimers; **(c)** glyco-peptides; **(d)** glyco-liposomes; **(e)** glyco gold-nanoparticles.

Carbohydrate recognition events are of utmost importance in progression of diseases like AIDS and Cancer. Mimicking this genuine composition has become one of the mainstream goals in glycoscience[16]. Because of the presence of a number of free reducing hydroxyl groups and also due to different configurations, sugars can branch in a variety of ways and hence the study of glycans gets more complicated. Many studies have been done for understanding the composition and representation of glycans on cell surface but none has been reached to the mark for being developed as a therapeutic agent. CPI can be enhanced by multivalency in certain areas; but it can also be hindered due to steric factors[17-18]. The heterogeneity on cell surface glycans hence has to be probed well to understand more about CPI. Recently, Hartmann group designed artificial homo/hetero-glycoclusters to study Con A mediated interactions[12]. Their study revealed better binding of ligands on heteromultivalent system as compared to homomultivalent system; it was observed that in homo system, multiple ligands were

attached to the receptors resulting in low binding due to steric factor whereas in hetero system, this steric factor was compromised as only one ligand will attach to the particular receptor resulting in better binding affinity. Similarly, Percec group synthesized hybrid Janus dendrimers to decipher to role of mannose-Con A and galactose - galectin mediated interactions[19]. In all these studies, hybrid dendrimers were prepared by using two carbohydrate units with distinct binding affinities. The intrinsic heterogeneity of the glycodendrimers leading to the establishment of carbohydrate-mediated interactions has yet to be deciphered.

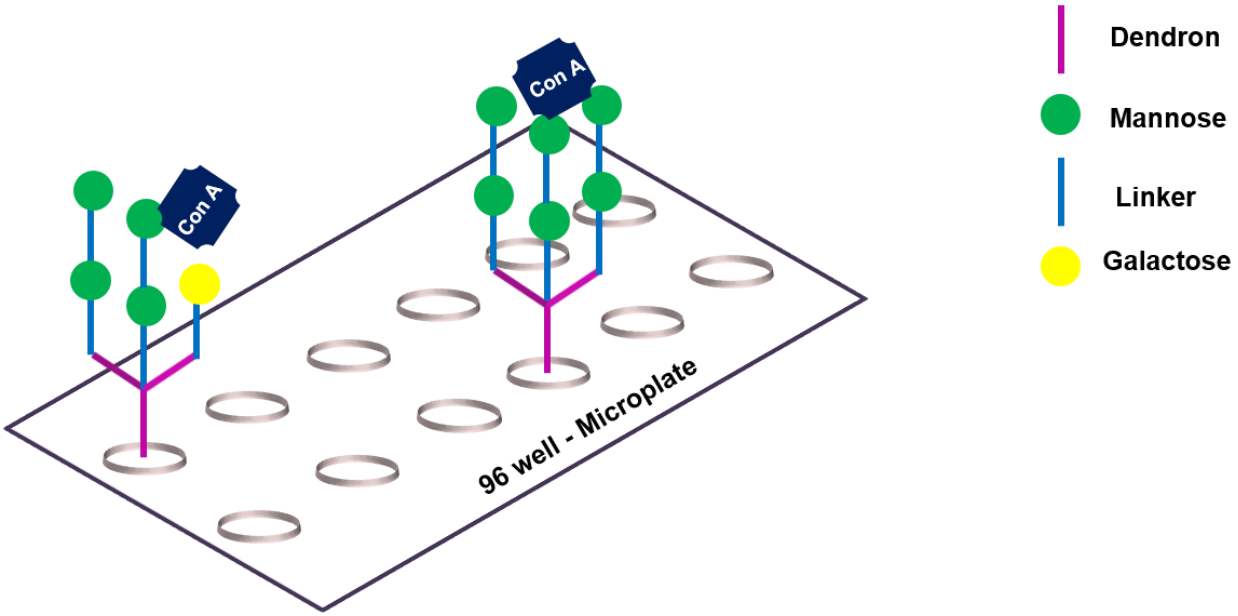


Figure 2. Representation of binding of homo and hetero multivalent glycoclusters with lectins (Con A).

Understanding how the hybrid form of the glycodendrimers translate its information to the final carbohydrate-protein interactions will generate a new set of rules to synthesize smart glycodendrimers to target and also to image cell surface lectins. To address this issue, we proposed to synthesize hybrid dendrimers with enantiomeric form of the sugars to optimize the binding affinities. With the aim of designing homo and hetero-glycoclusters, we synthesized mannosylated hybrid dendrimers via divergent strategy. The immobilization of these glycoclusters were then carried out on amine binding,

maleic anhydride activated 96 well microplates and were used for binding assay with lectins (**Fig. 2**).

We also used one-pot strategy to synthesize mannose glycans. The purity of the complexes was characterized by ^{1}H-NMR and MALDI-TOF MS. The functionalization of these dendrimers on ELISA plate interprets the binding affinity with different lectins and bacteria and thereby deciphers the role of hybrid dendrimers in carbohydrate-protein interactions.

OBJECTIVES

The main objective of this work was to develop a series of hybrid glycodendrimers and to optimize the binding affinity with different plant and human lectins. These analogs were modified with surface-adhesive functionalities, attached to ELISA and microarray slides and used as tools to target specific bacteria.

The particular aims are:

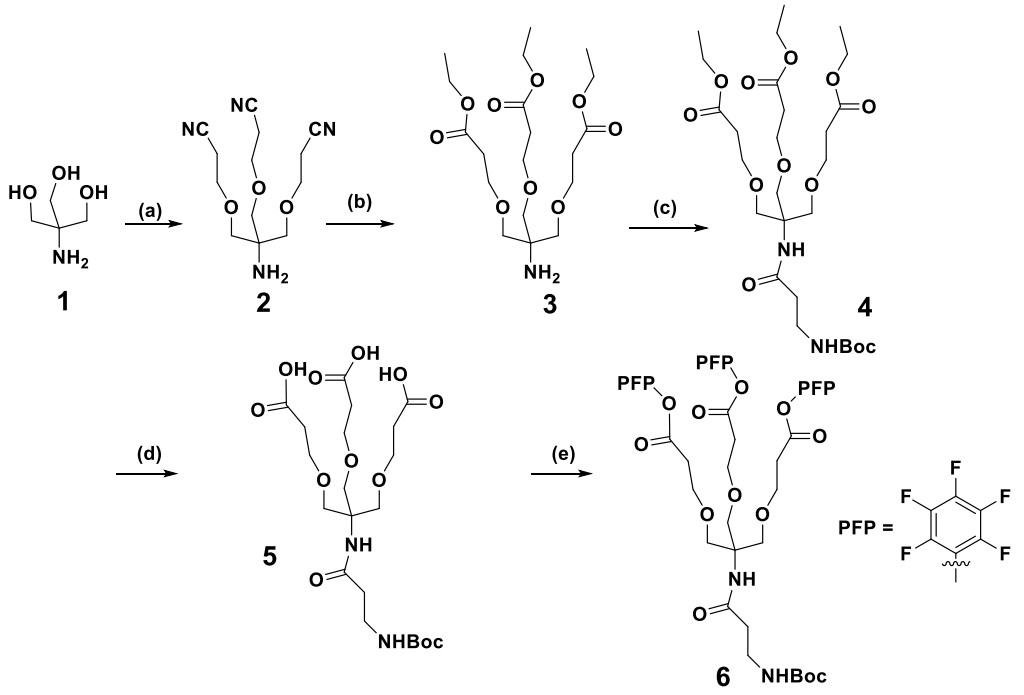
1. Design, synthesis and biological evaluation of a library of hybrid glycodendrimers.
2. Bio-screening of synthesized analogs to elucidate lectins and bacterial affinity and identify compounds with pronounced specificity.
3. Decipher the role of heterogeneity of carbohydrates on cell surfaces.

RESULTS AND DISCUSSION

Homo and hetero-glycodendrimers were readily accessible in high yield using active ester coupling between carbohydrate containing an amine linker and tripodal-active ester. Subsequent removal of protecting groups on carbohydrate moieties will provide access to the desired tripodal dendrimers. The formation of desired compounds was confirmed by MALDI-TOF MS and then purified by silica column chromatography and were then characterized by ^{1}H and ^{13}C NMR. NMR of final compounds have been given in the Supplementary Data section.

Synthesis of Tripod

Tripod-active ester **6** was readily synthesized from tris base (**Scheme 1**). Michael addition of acrylonitrile and tris base followed by hydrolysis of nitrile and esterification in the presence of conc. HCl and ethanol yielded compound **3**. Coupling between boc-β-alanine and tripodal-ester **3** yielded compound **4**, which was hydrolyzed in the presence of 1N NaOH, followed by coupling with Pentafluorophenol to yield **6** active ester[20]. The formation of desired compounds was confirmed by MALDI-TOF MS and then purified by silica column chromatography and were then characterized by ^{1}H and ^{13}C NMR.



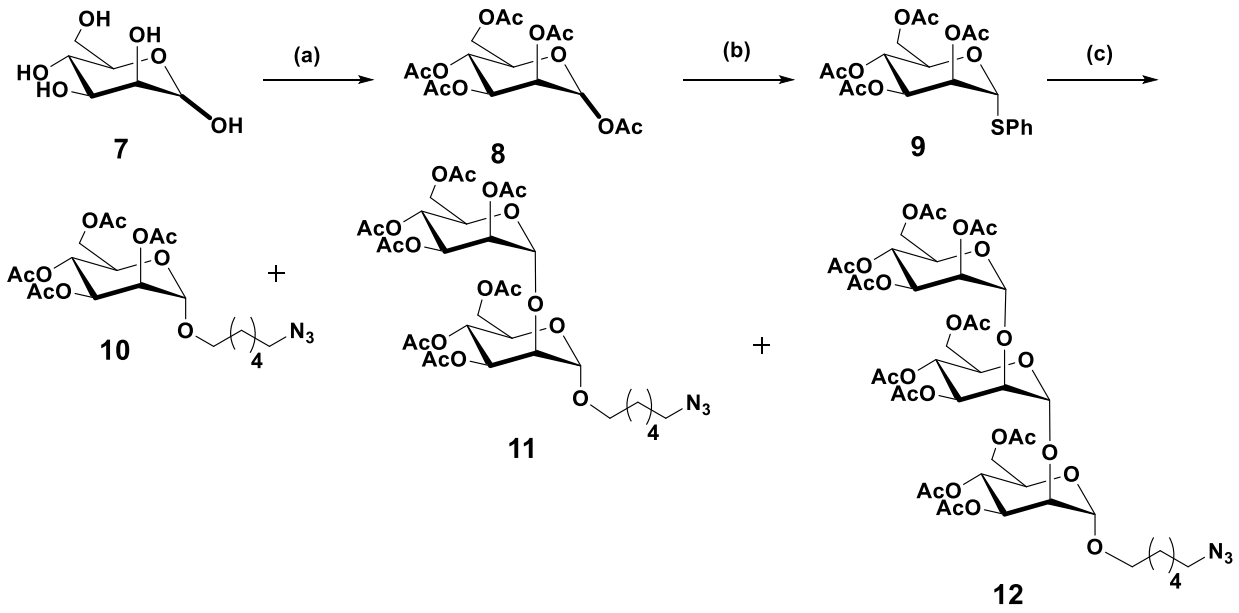
Scheme 1. *Synthesis of Tripod.* **Reagents** : (a) Acrylonitrile/KOH (40%); (b) Conc. HCl, EtOH; (c) Boc-β-Alanine, DIC, HOBt, TEA, DCM; (d) NaOH (2N), MeOH; (e) Pentafluorophenol (PFP), DIC, HOBt, Et_3N, DCM

^{1}H NMR and ^{13}C NMR Peak Characterization:

Ester formation was confirmed by the peak at δ. 1.25 ppm with an integration of 9 confirming the presence of terminal methyl groups in **3**. Formation of Boc-β-Alanine coupled compound **4** was confirmed by the peak at δ: 1.43 ppm with an integration of 9 resulting from the 3 methyl groups in Boc. Acid formed in the next step showed peak at δ. 176 ppm corresponding to the carboxylic group. The active ester **6** showed peaks around 140 ppm corresponding to PFP carbons.

One Pot Glycosylation for synthesis of Mono, Di and Tri Mannose derivatives with Azide Linker

Acetylated thioglycoside **8** was chosen as a model mannose donor and in the presence of 0.8 equivalence of linker with NIS/TfOH activator conditions, we got not only the expected monosaccharide, but also di and trisaccharides[21] (**Scheme 2**).



Scheme 2. *One pot polyglycosylation*. **Reagents**: (a) Acetic anhydride, Pyridine; (b) Thiophenol, $BF_3.Et_2O$; (c) NIS, TfOH, 6- azidohexan-1-ol, Acetic anhydride.

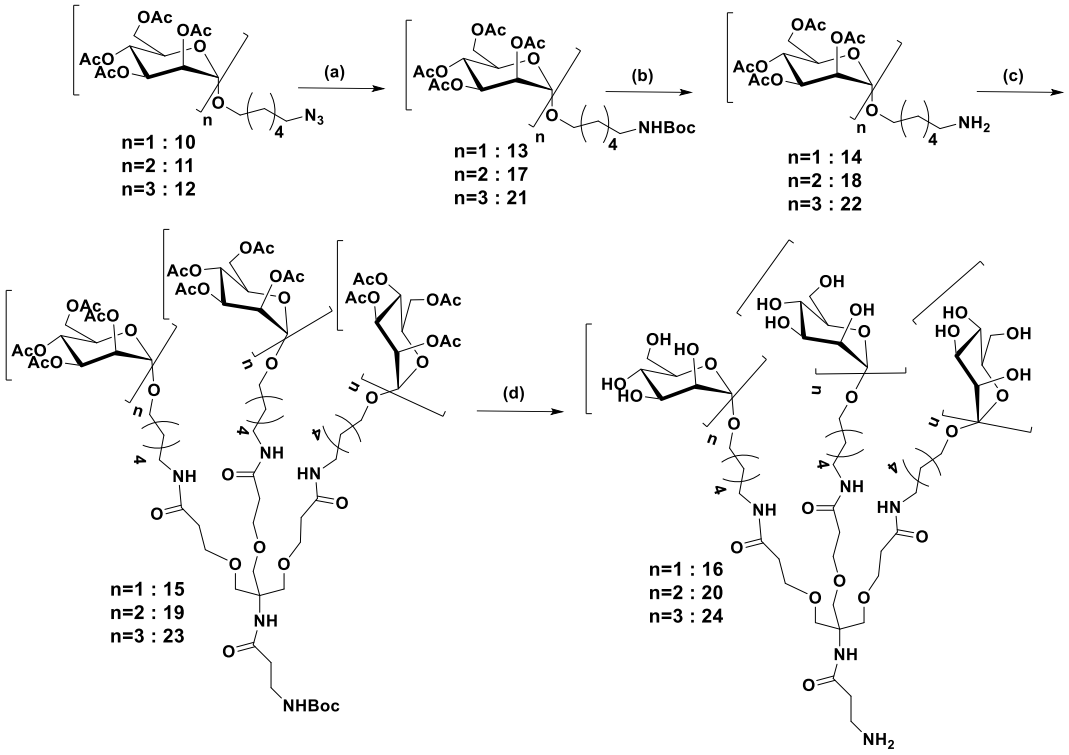
^{1}H NMR and ^{13}C NMR Peak Characterization:

Monomannose glycosylated with azide linker was confirmed to be of exclusively α- form. Four acetate groups in monomannose showed distinct singlet peaks at around δ. 2.0 ppm each with an integration of 3. The anomeric proton showed *J* = 1.6 Hz. Linker glycosylation was confirmed with the peaks of CH_2 groups at around 3 ppm. Dimannose with α(1 - 2) linkage was confirmed by NMR with its anomeric protons integrating for 2 Hydrogens at δ. 4.93 ppm with *J* = 1.8 Hz thereby confirming the linkage. Similar way, trimannose containing 3 anomeric protons showed peaks at 4.94 (*J* = 1.8 Hz), 4.93 (*J* = 2.1 Hz) and 4.92 (*J* = 1.7 Hz) ppm confirming the formation of desired product.

Synthesis of Tripodal Dendrimers of corresponding Mannose derivatives

Azide derivatives were converted to amine bearing components for ease of coupling with the PFP active ester **6** to form the desired glycodendrimers under basic condition (**Scheme 3**). For one equivalence of the active ester, 3.3 equivalence of sugar derivative was necessary. The deprotection of the compounds were then carried out in

NaOMe/MeOH condition where deprotection of one Acetate group required 1.2 equivalence of NaOMe.



Scheme 3. *Synthesis of tripodal structures of mannose*. **Reagents**: (a) PPh_3, water, Boc_2O; (b) TFA (20%) in DCM; (c) **6**, TEA, DCM; (d) NaOMe, MeOH.

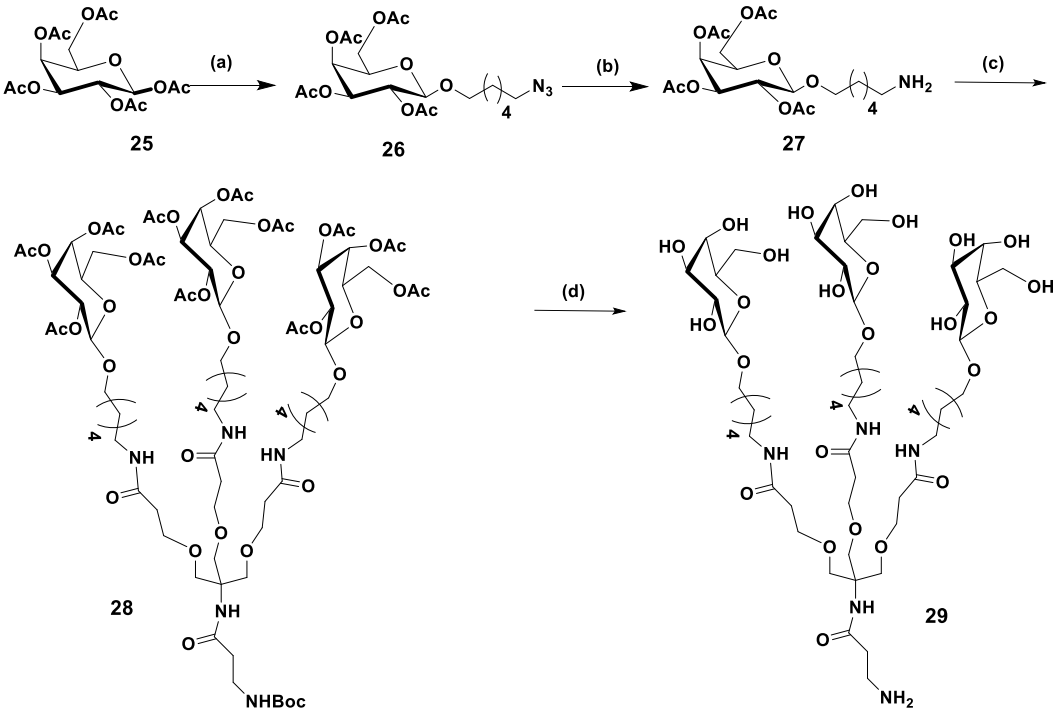
<u>^{1}H NMR and ^{13}C NMR Peak Characterization:</u>

Boc protected form of sugar derivatives were confirmed by the characteristic singlet peak of three methyl groups at δ. 1.4 ppm with an integration of 9. The next step which included the boc deprotection was confirmed by staining the TLC in Ninhydrin solution which helps in specific staining of free amine group and thereby confirming the consumption of starting material. Dendrimer formation was confirmed with MALDI-TOF MS. In both mono and di mannose dendrimer case, anomeric protons were observed as singlet peaks with integrations 3 and 6 respectively. If higher magnetic field is used, we may be able to resolve it better. In tri mannose case, anomeric protons with peaks at 5.12 (J = 2.0 Hz, 3H), 4.96 (J = 1.8 Hz, 3H) and 4.94 (J = 2.0 Hz, 3 H) ppm were observed, thus confirming 9 anomeric protons of the desired product. Also the

increment in the no. and intensity of peaks in ^{13}C NMR also confirms the formation of homo dendrimers of mannose derivatives.

Synthesis of Galactose Dendrimer

Homo dendrimer of Galactose was also prepared by coupling amine derivative of galactose with PFP active ester (**Scheme 4**).



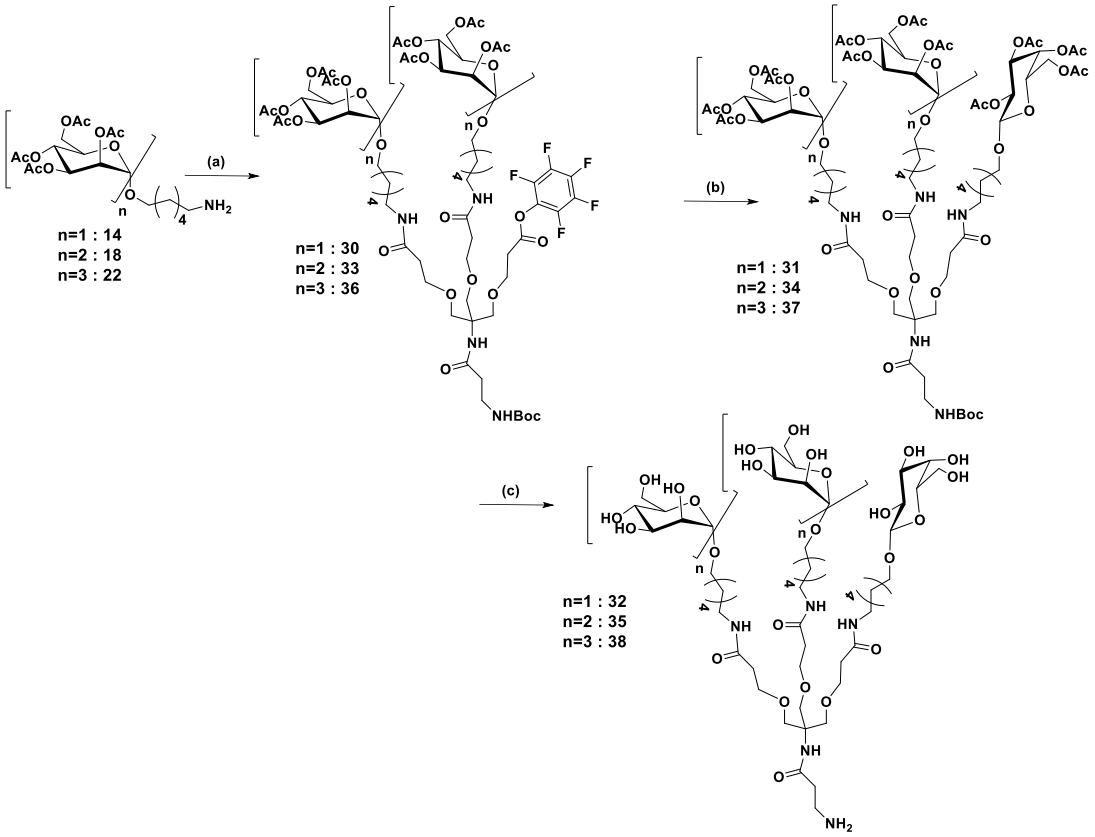
Scheme 4. *Synthesis of tripod of galactose.* **Reagents**: (a) 6- azidohexan-1-ol, $BF_3.Et_2O$; (b) H_2/Pd/MeOH; (c) **6**, TEA, DCM; (d) NaOMe, MeOH.

^1H NMR and ^{13}C NMR Peak Characterization:

Galactose showed anomeric proton peak with J = 7.9 Hz at δ. 4.44 ppm and singlet acetate peaks at δ. around 2.02 ppm thereby confirming the formation of beta product exclusively in glycosylation. The formation of glycodendrimer was confirmed from the number of anomeric protons in the integration which showed 3 protons at δ. 4.48 ppm. Also the characteristic peak of methyl groups in boc was observed at 1.45 ppm which showed integration as 9.

Synthesis of Hetero Glycodendrimers of Mannose and Galactose

Hetero glycodendrimers of mannose derivatives with galactose (**Scheme 5**) were synthesized by altering the equivalence.



Scheme 5. *Synthesis of hybrid dendrimers of mannose and galactose.* **Reagents**: (a) **6**, TEA, DCM; (b) **27**, TEA, DCM; (c) MeOH, NaOMe.

^{1}H NMR and ^{13}C NMR Peak Characterization:

The formation of di substituted compound was confirmed by MALDI as well as by NMR. The hetero dendrimer of each derivative showed two distinct anomeric protons, one with low coupling constant indicating mannose and other with high coupling constant indicating galactose. In deprotection of trimannose heterodendrimer, galactose anomeric proton showed peak at δ. 4.21 ppm and J = 8.0 Hz and mannose anomeric protons showed peaks at around δ. 4.88 ppm with weak coupling constants.

Binding Assay Experiment

The compounds synthesized were then subjected to binding affinity study with FITC conjugated Concanavalin A (Con A) lectin. Con A is a tetrameric leguminous plant lectin with specific affinity towards α-D-mannoside[5]. Different concentrations (100 µM, 50 µM, 20 µM, 10 µM, 5 µM, 2 µM and 1µM) of the 11 compounds were immobilized on amine binding maleic anhydride activated 96 well microplate by incubating 100µl of each compound in each well overnight for better binding. For immobilization, PBS buffer was used and for amine activation, pH was adjusted to 7.8. The unbound molecules were removed by washing with wash buffer containing 0.05% tween in PBS. The unreacted maleic anhydride residues were blocked by incubating each well for 1 hour with 100µl block buffer containing 5µl ethanol amine in PBS. This was followed by washing of each well with 200µl wash buffer 2-3 times to remove excess of block buffer. Then 100µl of FITC conjugated Con A was added to each well and was incubated for 1 hour. Then binding study was done using plate reader after one wash with PBS buffer. The control used in the experiment was Galactose which does not have affinity towards Con A. Out of the 7 different concentrations, 5 µM and 2 µM showed significant difference in binding. Of this, data obtained for 5 µM concentration was used to plot the graphs and previous experiments done has showed that 5 µM is best concentration as it gives an excess of sugar density as compared to surface capacity of microtiter plate[18].

The major pattern observed was the better binding of hetero glycodendrimer than homo glycodendrimer of each mannose derivative and this is in agreement with previous work[14, 18]. In the case of sugars as such, tri mannose showed better binding affinity with Con A; the reason behind it being the difference in no. of mannose sugar as compared to di or mono mannose derivative (**Fig. 3. (a)**). As the no. of sugar increases, the no. of lectins binding also increases and hence the fluorescence value obtained will be higher. In the case of glycodendrimers, as expected, trimannose substituted tripod showed better binding and fluorescence as compared to mono and di mannose substituted homo glycodendrimer (**Fig. 3. (b)**). In case of hetero glycodendrimer, tri mannose substituted hetero glycodendrimer showed better binding but not much of significant difference was observed in comparison with other two heterodendrimers (**Fig. 3. (c)**).

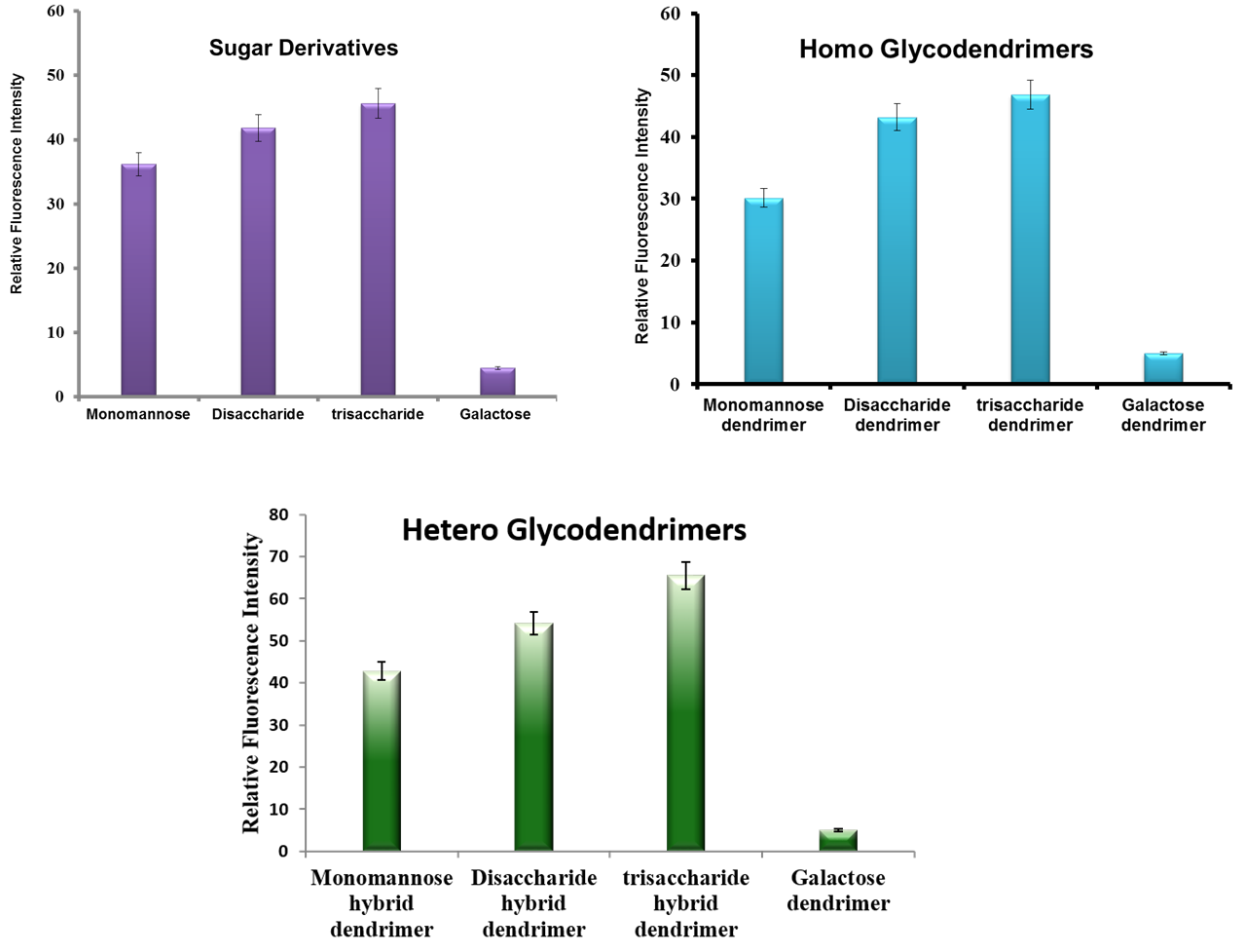


Figure 3. Comparitive binding assay study between **(a)** sugar derivatives, **(b)** homo dendrimers and **(c)** hetero dendrimers with FITC-conjugated Con A

For each mannose derivative component, corresponding hetero dendrimer gave the best response. This can be considered as an outcome of minor steric hindrance as compared to homodendrimer. We are not sure where exactly the sugars bind to tripod in hetero case; it can get di substituted either at first and second branch or first and third branch. If latter is the case, then galactose can sit in between the two sugar derivatives and since Con A does not bind to galactose there won't be large no. of Con A molecules trying to bind mannose residues. Hence, whatever binding happens, it will be a strong one; one washing with buffer will not remove most of these strongly bound lectin. This will give better fluorescence for hetero when we take the reading. In homo case, since we have same kind of mannose residue attached, Con A population trying to bind with

all of these will be high. Larger the population, lesser the efficiency in binding. Even though Con A shows specificity to mannose, it is not able to bind efficiently because of steric crowding. Hence in one wash, most of Con A can get removed. Just the same happens for mannose sugar derivatives as such. Con A is binding but not that strongly and hence gets washed away.

Each well hoisting the amine bound sugar component is a mimic of cell surface and the solution containing the lectin mimics the extra cellular fluid matrix containing lectins and other substances for binding the cell surface. Understanding the binding affinity of each components to the glycan surface will be helpful in developing. For this to happen, the study must be extended to varieties of lectins as well as bacteria and pathogens.

CONCLUSION

We synthesized mono, di and tri mannose derivatives in one pot method and these glycans were successfully conjugated to tripod to yield homo-glycodendrimers. The hetero dendrimer form of the above sugar derivatives with galactose moiety were also synthesized by mixing stoichiometric amount of mannose and galactose carbohydrate ligands. All the final compounds were characterized using traditional NMR and mass spectrometry. From the preliminary lectin and bacterial binding study, it became clear that heterogeneity on dendrimers promoted better binding with Con A lectin and *E.coli* ORN 178 bacteria as compared to homo glycodendrimers. With this simple model, we learnt the significance of heterogeneity on cell surfaces. Currently, we have extended this work with C-type human lectins, which are involved in heterogeneous carbohydrate-protein interactions on human cell surfaces.

METHODS

Experimental Section

General Information

All chemicals were reagent grade and were used as supplied except where noted. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254

plates (0.25 mmol). Compounds were visualized by UV irradiation or dipping the plate in CAM/$KMnO_4$/Ninhydrin solution followed by heating. Column chromatography was carried out using force flow of the indicated solvent on Fluka Kieselgel 60 (100–200 mesh). ^1H and ^{13}C NMR spectra were recorded on Jeol 400 MHz, 100 MHz respectively with cryo probe using residual solvents signals as an internal reference ($CDCl_3$ δ_H. 7.26 ppm, δ_C 77.3 ppm and D_2O δ_H 4.79 ppm). The chemical shifts (δ) are reported in ppm and coupling constants (*J*) in Hz. Binding assay study of 11 compounds with FITC conjugated Con A was done using 96 well microtiter plate reader at excitation wavelength of 494 nm and fluorescence was noted at emission wavelength of 518 nm.

3-amino-3-(cyanomethyl) pentanedinitrile (**2**)

To the mixture of Tris Base **1** (5g, 41.322 mmol, 1.0 eq.) and acrylonitrile (16.223 ml, 247.934 mmol, 6.0 eq.), 40% KOH (2 ml) was added dropwise at 0 ºC. Then, 1, 4 - Dioxane (5 ml) was added and the mixture was allowed to stir at RT for 16 hr to yield 3-amino-3-(cyanomethyl) pentanedinitrile **2**. Pale yellow colored oil is formed. On completion of reaction, evaporate off dioxane and extract the reaction mixture with EtOAc (100 ml) and minimum amount of water (10 ml) thrice. Give the organic layer washing with brine. Dry over Na_2SO_4 and concentrate under reduced pressure. Continue for next reaction without purification.

Diethyl 3, 3'-((2-amino-2-((3-ethoxy-3-oxopropoxy) methyl) propane-1, 3-diyl) bis (oxy)) dipropionate (**3**)

Compound **2** (8.4g, 56.757 mmol) was dissolved in Conc. HCl (30 ml) and refluxed for 24 hr. To this, ethanol (30 ml) was added and was refluxed for 24 hr. After completion, Ethanol was evaporated and 10 N NaOH was added to the white precipitate till it reached a pH of 8.6. The mixture was then extracted with EtOAc (100 ml) and water (5 ml) twice. The organic layer was given brine wash once and then dried over Na_2SO_4 and concentrated *in vacuo* and was purified by silica gel column chromatography using EtOAc/Pet-ether (80:20) to yield **3** (10.99g, 46%). ^1H NMR (400 MHz, $CDCl_3$): δ. 4.39 (bs, 2H), 4.14 (q, *J* = 7.13, 7.13Hz, 6H), 3.73 - 3.68 (m, 8H), 3.35 (s, 4H), 2.53 (t, *J* = 6.28Hz, 6H), 1.25 (t, *J* = 7.12Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 177.65, 71.90,

66.96, 66.17, 60.35, 56.47, 35.03, 14.01. HRMS (m/z) calc'd for $[M+H]^+$ $C_{19}H_{36}NO_9$: 422.2390; observed: 422.2395.

Ethyl 10, 10- bis ((3-ethoxy-3-oxopropoxy) methyl)-2, 2-dimethyl-4, 8-dioxo-3, 12-dioxa-5, 9-diazapentadecan-15-oate (**4**)

To a mixture of **3** (3g, 7.126 mmol, 1.0 eq.) and Boc-β-Alanine (2.02g, 10.689 mmol, 1.5 eq.) in DCM at 0 ºC, Diisopropyl carbodiimide (DIC) (1.322 ml, 8.551 mmol, 1.2 eq.) and 1 - Hydroxybenzotriazole (HOBt) (0.096g, 0.712 mmol, 0.1 eq.) were added. Finally to adjust the pH of the reaction mixture to slightly basic condition (pH-8), Triethylamine was also added and the reaction mixture was allowed to stir at RT for 12 hr. After completion of reaction, concentrate the reaction mixture under reduced pressure and purify by silica column chromatography using EtOAc/Pet-ether (60:40) to yield **4** (2.74g, 66%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.15 (s, 1H), 5.35 (s, 1H), 4.15 (q, *J* = 8.00, 8.00Hz, 6H), 3.70 - 3.67 (m, 12H), 3.37 - 3.36 (m, 2H), 2.54 (t, *J* = 6.19Hz, 6H), 2.35 (t, *J* = 5.81Hz, 2H), 1.43 (s, 9H), 1.26 (t, *J* = 8.00Hz, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.64, 155.95, 68.81, 66.79, 60.18, 59.75, 34.89, 28.76, 23.33, 14.21. HRMS (m/z) calc'd for $[M+H]^+$ $C_{27}H_{49}N_2O_{12}$: 593.3286; observed: 593.3276.

Perfluorophenyl-2, 2-dimethyl-4, 8-dioxo-10, 10-bis ((3-oxo-3-(perfluorophenoxy) propoxy) methyl)-3, 12-dioxa-5, 9-diazapentadecan-15-oate (**5**)

Compound **4** (2.5g, 4.223 mmol) was mixed with 2 N NaOH (2 ml) in MeOH (8 ml) and was allowed to stir for 12 hr at RT. After completion, solvent was evaporated under reduced pressure and the crude residue was neutralized with 1 N HCl. This was followed by extraction of the mixture with EtOAc (100 ml). The organic layer was then dried over Na_2SO_4, filtered and concentrated *in vacuo* to yield **5** (1.3g, 61%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.40 (s, 1H), 5.49 (s, 1H), 3.79 - 3.71 (m, 12H), 3.42 - 3.36 (m, 2H), 2.59 (t, *J* = 5.48Hz, 6H), 2.40 - 2.33 (m, 2H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 176.14, 175.99, 171.42, 156.49, 80.05, 69.11, 66.92, 42.79, 37.07, 35.04, 28.28, 22.82, 20.94, 20.61, 14.26. HRMS (m/z) calc'd for $[M+H]^+$ $C_{21}H_{37}N_2O_{12}$: 509.2346; Observed: 509.2341.

Perfluorophenyl-2, 2-dimethyl-4, 8-dioxo-10, 10-bis ((3-oxo-3-(perfluorophenoxy) propoxy) methyl)-3, 12-dioxa-5, 9-diazapentadecan-15-oate (**6**)

The acid formed **5** (1.8g, 3.543 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 and 2, 3, 4, 5, 6 -Pentafluorophenol (2.282g, 12.402 mmol, 3.5 eq.) was added to it. Diisopropyl carbodiimide (DIC) (0.665 ml, 4.252 mmol, 3.6 eq.) and Hydroxybenzotriazole (HOBt) (0.048g, 0.354 mmol, 0.1 eq.) were added to the reaction mixture at 0 ºC. Later, Triethylamine was added to adjust the pH of the system to slightly basic condition (pH-8). The reaction mixture was stirred at RT for 12 hr. On completion, the solvent was evaporated under reduced pressure and the crude residue was purified by silica column chromatography using EtOAc/Pet-ether (50:50) to yield u. v. active product **6** (2.5g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.84 (s, 1H), 5.20 (s, 1H), 3.83 (t, *J* = 5.85Hz, 6H), 3.79 (s, 6H), 3.37 - 3.35 (m, 2H), 2.92 (t, *J* = 5.85Hz, 6H), 2.33 (t, *J* = 5.85Hz, 2H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.95 167.41, 155.76, 142.33, 139.86, 139.58, 139.03, 138.38, 137.00, 136.61, 79.37, 69.78, 66.88, 61.70, 36.73, 34.44, 28.33. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{25}H_{41}NO_{12}Na$: 570.2526; observed: 570.2525.

Synthesis of 6-azidohexanol sugar derivatives of thiophenol mannoside

Compound **9** (2.083g, 4.734 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 and 6-azidohexan-1-ol (0.542g, 3.787 mmol, 0.8 eq.) was added to it. 4 Å Molecular sieves were added and the reaction mixture was stirred at RT for 1 hr. After 1 hr, the reaction mixture was brought to -4 ºC using ice and common salt and was allowed to stir for 20 min at the same temperature. To the reaction mixture, N- Iodosuccinimide (1.272g, 5.681 mmol, 1.2 eq.) was added followed by Trifluoromethanesulfonic acid (0.106 ml, 0.947 mmol, 0.2 eq.) and was stirred for 1 hr at -4 ºC. Check for the consumption of starting material using TLC. On completion, take out RB and allow it to attain RT. Ac_2O (1.418 ml, 14.202 mmol, 3.0 eq.) was added to it and was stirred for 30 minutes. Then contents in RB were filtered through celite bed using sintered funnel. The filtrate was then extracted twice with CH_2Cl_2 and aqueous $Na_2S_2O_4$. The organic layer was given one wash with brine and dried over Na_2SO_4. The solvent was evaporated and the crude residue was purified by silica column chromatography using EtOAc/Pet-ether (60:40) to yield mono **10** (0.423g, 19%), di **11** (0.862g, 25%) and tri **12** (0.341g, 7%) mannose derivatives.

6-azidohexanol sugar derivative of monomannose (**10**)

^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.37, 5.35 (dd, *J* = 3.4, 10.0Hz, 1H), 5.32 - 5.29 (m, 1H), 5.27 - 5.24 (m, 1H), 4.82 (d, *J* = 1.6Hz, 1H), 4.31, 4.29 (dd, *J* = 12.2, 5.3Hz, 1H), 4.14, 4.11 (dd, *J* = 12.2, 2.5Hz, 1H), 4.01 - 3.97 (m, 1H), 3.97 - 3.65 (m, 1H), 3.50 - 3.44 (m, 1H), 3.30 (t, *J* = 6.9Hz, 2H), 2.17 (s, 3H), 2.12 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.67 - 1.61 (m, 4H), 1.43 - 1.40 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.64, 170.11, 169.92, 169.75, 97.57, 69.70, 69.12, 68.44, 68.28, 66.27, 62.54, 51.35 29.12, 28.74, 26.47, 25.72, 20.91, 20.74, 20.70. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{20}H_{31}N_3O_{10}Na$: 496.1907; observed: 496.1907.

6-azidohexanol sugar derivative of dimannose (**11**)

^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.42, 5.40 (dd, *J* = 10.0, 3.4Hz, 1H), 5.31 - 5.21 (m, 4H), 4.93 (d, *J* = 1.8Hz, 2H), 4.26, 4.25 (dd, *J* = 11.7, 4.9Hz, 2H), 4.19 - 4.09 (m, 3H), 4.03 - 4.01 (m, 1H), 3.93 - 3.89 (m, 1H), 3.74 - 3.67 (m, 1H), 3.47 - 3.41 (m, 1H), 3.28 (t, *J* = 6.8Hz, 2H), 2.15 (s, 3H), 2.14 (s, 3H), 2.08 (s, 6H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.64 - 1.58 (m, 4H), 1.41 - 1.39 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.86, 170.44, 170.42, 169.85, 169.73, 169.45, 169.41, 99.19, 98.28, 70.36, 69.79, 69.15, 68.56, 68.42, 68.28, 66.41, 66.28, 62.55, 62.25, 51.33, 29.57, 29.21, 28.73, 26.48, 25.76, 20.84, 20.72, 20.65, 20.65. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{32}H_{47}NO_{18}Na$: 784.2752; observed: 784.2750.

6-azidohexanol sugar derivative of trimannose (**12**)

^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.38 - 5.35 (m, 2H), 5.33 - 5.30 (m, 4H), 4.94 (d, *J* = 1.8Hz, 1H), 4.93 (d, *J* = 2.1Hz, 1H), 4.92 (d, *J* = 1.7Hz, 1H), 4.44 - 4.41 (m, 1H), 4.34, 4.32 (dd, *J* = 12.2, 4.4Hz, 1H), 4.25 - 4.19 (m, 3H), 4.16 - 4.11 (m, 4H), 4.07 - 4.04 (m, 1H), 3.92 - 3.88 (m, 1H), 3.81 - 3.78 (m, 1H), 3.73 - 3.67 (m, 1H), 3.45 - 3.43 (m, 1H), 3.28 (t, *J* = 6.8Hz, 2H), 2.15 (s, 3H), 2.13 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.04 (s, 6H), 2.00 (s, 3H), 1.68 - 1.90 (m, 4H), 1.42 - 1.39 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.87, 170.66, 170.49, 170.42, 169.99, 169.75, 169.46, 169.32, 169.26, 168.24, 99.83, 99.28, 99.01, 98.30, 98.28, 90.97, 74.65, 73.03, 72.43, 70.51, 70.38, 69.79, 69.73, 69.46, 68.82, 68.57, 68.49, 68.41, 66.39, 66.31, 66.11, 65.75, 62.55, 62.24, 62.22, 62.14, 51.33, 42.41, 29.68, 29.24, 28.74, 26.49,

25.76, 20.89, 20.82, 20.80, 20.76, 20.72, 20.70, 20.66, 20.63. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{44}H_{63}N_3O_{26}Na$: 1072.3597; Observed: 1072.3623.

Synthesis of Boc protected sugar derivative of monomannose (**13**)

Compound **10** (0.4g, 0.846 mmol, 1.0 eq.) was dissolved in THF (9 ml) and Triphenylphosphine (0.266g, 1.015 mmol, 1.2 eq.) was added and the reaction mixture was allowed to stir at RT for 4 hr. On consumption of starting, water (10 ml) and di-*tert*-butyl dicarbonate (Boc_2O) (0.233 ml, 1.015 mmol, 1.2 eq.) was added to the RB and was stirred for 12 hr at RT. On completion, THF was evaporated and reaction mixture was extracted with EtOAc (100 ml). Aqueous layer was washed with EtOAc thrice. Then, organic layer was washed once with brine and dried over Na_2SO_4. The compound was concentrated and the crude residue was purified by silica column chromatography using EtOAc/Pet-ether (60:40) to yield **13** (0.286g, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.35, 5.33 (dd, *J* = 3.23, 9.98Hz, 1H), 5.29 - 5.26 (m, 1H), 5.24 - 5.22 (m, 1H), 4.79 (d, *J* = 1.5Hz, 1H), 4.55 (s, 1H), 4.26, 4.26 (dd, *J* = 12.2, 5.3Hz, 1H), 4.11 - 4.08 (m, 1H), 3.98 - 3.97 (m, 1H), 3.70 - 3.64 (m, 1H), 3.46 - 3.42 (m, 1H), 3.11 - 3.05 (m, 2H), 2.15 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H), 1.63 - 1.57 (m, 4H), 1.52 - 1.47 (s, 2H), 1.43 (s, 9H), 1.35 - 1.34 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.60, 170.21, 169.89, 169.67, 156.00, 97.51, 69.72, 69.14, 68.32, 66.17, 62.46, 53.13, 40.43, 29.89, 29.17, 28.74, 26.65, 25.85, 20.02, 20.75, 20.72, 20.70. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{25}H_{41}NO_{12}Na$: 570.2526; observed: 570.2525.

Synthesis of Boc deprotected sugar derivative of monomannose (**14**)

Compound **13** (0.224g) was dissolved in TFA (2 ml) and dry DCM (8 ml) and was stirred for 2 hr at RT. On consumption of starting, the reaction mixture was concentrated *in vacuo* and co-evaporated with Toluene and DCM to yield **14** (0.162g, 89%) and was used as such without purification for next reaction.

Synthesis of monomannose substituted tripod (**15**)

Compound **14** (0.254g, 0.567 mmol, 3.2 eq.) was dissolved in DCM and the PFP ester **6** (0.173g, 0.172 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using Triethylamine. The mixture was then allowed to stir at RT for 12 hr.

On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography using MeOH/DCM (4:96) was done to get **15** (0.22g, 49%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.79 (s, 1H), 6.54 (s, 1H), 6.43 (bs, 3H), 5.34 - 5.30 (m, 8H), 5.28 - 5.25 (m, 3H), 4.82 (bs, 3H), 4.32, 4.30 (dd, *J* = 12.1, 5.2Hz, 4H), 4.15 (d, *J* = 12.3Hz, 4H), 4.02, 3.98 (m, 3H), 3.74 - 3.68 (m, 12H), 3.49 - 3.38 (m, 6H), 3.27, 3.26 (dd, *J* = 12.3, 5.7Hz, 6H), 2.24 (t, *J* = 5.6Hz, 6H), 2.18 (s, 9H), 2.13 (s, 9H), 2.07 (s, 9H), 2.02 (s, 9H), 1.57 - 1.52 (m, 12H), 1.46 (s, 9H), 1.40 - 1.39 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.57, 170.43, 170.12, 169.91, 169.63, 155.97, 97.50, 69.48, 69.19, 68.52, 67.48, 66.18, 62.49, 59.71, 39.49, 36.58, 29.55, 29.00, 26.48, 25.62, 20.90, 20.74, 20.70. HRMS (m/z) calc'd for $[M+H]^+$ $C_{81}H_{130}N_2O_{39}$: 1796.8343; observed: 1796.8033.

Synthesis of deprotected monomannose substituted tripod (**16**)

Compound **15** (0.060g, 0.033 mmol) was then kept for Boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). After de-protection, compound obtained was co-evaporated with toluene and DCM several times. Acetate de-protection of the compound (0.044g, 0.026 mmol, 1.0 eq.) was done in NaOMe (0.017g, 0.311 mmol, 14.4 eq.) and MeOH (10 ml) for 2 hr at RT. The crude residue on evaporation of MeOH was purified by sephadex column to yield **16** (0.023g, 58% over two step).^{1}H NMR (400 MHz, D_2O): δ. 4.75 (m, 6H), 3.82 (bs, 3H), 3.79 (bs, 2H), 3.76 (bs, 2H), 3.69 - 3.66 (m, 6H), 3.64 - 3.62 (m, 12H), 3.57 - 3.55 (m, 7H), 3.50 - 3.49 (m, 5H), 3.46 - 3.41 (m, 4H), 2.57 (t, *J* = 6.7Hz, 2H), 2.38 (t, *J* = 5.7Hz, 6H), 1.51 - 1.50 (m, 6H), 1.44 - 1.41 (m, 6H), 1.26 (m, 12H) ; ^{13}C NMR (100 MHz, D_2O): δ. 173.69, 171.76, 162.63, 162.27, 99.61, 72.65, 70.63, 70.08, 68.42, 67.69, 67.56, 66.68, 60.84, 60.33, 39.38, 36.13, 35.50, 28.43, 28.30, 25.89, 25.07. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{52}H_{97}N_5O_{25}Na$: 1214.6370; observed: 1214.6830.

Synthesis of Boc protected sugar derivative of dimannose (**17**)

Compound **11** (0.842g, 1.106 mmol, 1.0 eq.) was dissolved in THF (12 ml) and Triphenylphosphine (0.348g, 1.328 mmol, 1.2 eq.) was added and the reaction mixture was allowed to stir at RT for 4 hr. On consumption of starting, water (10 ml) and di-*tert*-

butyl dicarbonate (Boc_2O) (0.305 ml, 1.328 mmol, 1.2 eq.) was added to the RB and was allowed to stir for 12 hr at RT. On completion, THF was evaporated and the reaction mixture was extracted with EtOAc (100 ml). The aqueous layer was extracted with EtOAc thrice. Then the organic layer was washed once with brine and dried over Na_2SO_4. The organic layer was then concentrated and the crude residue was purified by silica column chromatography using EtOAc/Pet-ether (60:40) yield **17** (0.59g, 64%).^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.40, 5.38 (dd, *J* = 3.36, 10.02Hz, 1H), 5.33 - 5.24 (m, 4H), 4.90 (bs, 2H), 4.58 (bs, 1H), 4.24 - 4.18 (m, 2H), 4.16 - 4.08 (m, 3H), 4.00 - 3.99 (m, 1H), 3.90 - 3.87 (m, 1H), 3.69 - 3.63 (m, 1H), 3.44 - 3.35 (m, 1H), 3.09 - 3.08 (m, 2H), 2.13 (s, 3H), 2.12 (s, 3H), 2.06 (s, 6H), 2.02 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.60 - 1.55 (m, 2H), 1.50 - 1.45 (m, 2H), 1.41 (s, 9H), 1.34 - 1.32 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.79, 170.37, 170.34, 170.03, 169.77, 169.67, 169.39, 169.37, 155.97, 99.10, 98.19, 70.32, 69.73, 69.11, 68.89, 68.48, 68.36, 68.10, 66.36, 66.24, 65.61, 62.51, 62.18, 40.44, 29.93, 29.60, 29.56, 29.22, 28.37, 26.50, 25.83, 20.85, 20.77, 20.66, 20.59, 20.56. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{37}H_{57}NO_{20}Na$: 858.3372; observed: 858.3372.

Synthesis of Boc deprotected sugar derivative of dimannose (**18**)

Compound **17** (0.3g, 0359 mmol) was dissolved in TFA (20%) and dry DCM (8 ml) and was stirred for 2 hr at RT. On consumption of starting, the reaction mixture was concentrated *in vacuo* and co-evaporated with toluene and DCM to yield **18** (0.187g, 71%) and was used as such without purification for next reaction.

Synthesis of dimannose substituted tripod (**19**)

Compound **18** (0.465g, 0.633 mmol, 3.2 eq.) was dissolved in DCM and the PFP ester (0.199g, 0.198 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using Triethylamine. The mixture was then allowed to stir at RT for 12 hr. On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography using MeOH/DCM (4:96) was done to get **19** (0.565g, 46%).^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.87 (s, 1H), 6.53 (s, 1H), 6.49 (s, 2H), 6.35 (s,

1H), 5.40, 5.38 (dd, *J* = 3.34, 10.0Hz, 3H), 5.90 (s, 6H), 5.28 - 5.54 (m, 8H), 4.91 (bs, 6H), 4.23 - 4.17 (m, 7H), 4.14 - 4.10 (m, 8H), 4.00 (t, *J* = 2.0Hz, 3H), 3.91 - 3.88 (m, 3H), 3.70 - 3.65 (m, 12H), 3.44 - 3.35 (m, 6H), 3.24 - 3.19 (m, 6H), 2.40 (t, *J* = 5.3Hz, 8H), 2.14 (s, 9H), 2.13 (s, 9H), 2.07 (s, 18H), 2.03 (s, 9H), 2.02 (s, 9H), 2.00 (s, 9H), 1.60 - 1.57 (m, 8H), 1.53 - 1.50 (m, 8H), 1.41 (s, 9H), 1.38 - 1.34 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.26, 171.26, 170.87, 170.47, 170.45, 169.84, 169.73, 169.70, 169.44, 169.39, 156.01, 99.15, 98.23, 70.37, 69.76, 69.14, 68.49, 68.41, 67.49, 66.37, 6.26, 62.52, 62.21, 53.42, 45.59, 39.49, 36.54, 29.53, 29.30, 28.41, 26.79, 25.93, 20.83, 20.72, 20.65, 20.64, 20.618.14. HRMS (m/z) calc'd for $[M+Na]^+$ $C_{117}H_{177}N_5O_{63}Na$: 2683.0698; observed: 2683.3967.

Synthesis of deprotected dimannose substituted tripod (**20**)

Compound **19** (0.2g, 0.075 mmol) was then kept for Boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). Boc de-protected compound (0.127g, 62%) was obtained after co-evaporation with toluene and DCM several times. Acetate de-protection of compound (0.0.127g, 0.049 mmol, 1.0 eq.) was done in NaOMe (0.66g, 1.24 mmol, 23.2 eq.) and MeOH (10 ml) for 2 hr at RT. The crude residue on evaporation of MeOH was purified by sephadex column to yield **20** (0.028g, 62%). ^{1}H NMR (400 MHz, D_2O): δ. 4.99 (s, 3H), 4.92 (s, 3H), 3.97 (bs, 3H), 3.84 (bs, 3H), 3.80 - 3.79 (m, 6H), 3.77 - 3.75 (m, 8H), 3.74 - 3.73 (m, 2H), 3.68 - 3.67 (m, 4H), 3.64 - 3.61 (m, 16H), 3.59 - 3.57 (m, 9H), 3.54 - 3.49 (m, 7H), 3.46 - 3.40 (m, 4H), 2.56 (t, *J* = 6.6Hz, 2H), 2.38 (t, *J* = 5.7Hz, 6H), 1.51 - 1.50 (m, 6H), 1.44 - 1.41 (m, 6H), 1.29 (m, 12H); ^{13}C NMR (100 MHz, D_2O): δ. 173.63, 171.63, 102.48, 97.95, 78.86, 73.15, 72.58, 70.06, 69.82, 68.40, 67.85, 67.43, 66.81, 66.64, 60.94, 60.75, 60.05, 39.08, 36.16, 35.22, 28.34, 28.19, 25.65, 25.08. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{70}H_{127}N_5O_{40}Na$: 1700.7955; observed: 1700.7780.

Synthesis of Boc protected sugar derivative of trisaccharide mannose (**21**)

Compound **12** (0.341g, 0.325 mmol, 1.0 eq.) was dissolved in THF (12 ml) and Triphenylphosphine (0.102g, 0.390 mmol, 1.2 eq.) was added and the reaction mixture was allowed to stir at RT for 4 hr. On consumption of starting, water (10 ml) and di-*tert*-

butyl dicarbonate (Boc_2O) (0.089 ml, 0.390 mmol, 1.2 eq.) was added to the RB and was allowed to stir for 12 hr at RT. On completion, THF was evaporated and the reaction mixture was extracted with EtOAc (100 ml). The aqueous layer was extracted with EtOAc thrice. Then the organic layer was washed with brine and dried over Na_2SO_4. The organic layer was concentrated and the crude residue was purified by silica column chromatography using EtOAc/Pet-ether (60:40) to yield **21** (0.295g, 81%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.43, 5.40 (dd, *J* = 10.1, 3.3Hz, 1H), 5.36 - 5.29 (m, 6H), 5.12 (d, *J* = 2.0Hz, 1H), 4.97 (d, *J* = 1.6Hz, 1H), 4.95 (d, *J* = 1.9Hz, 1H), 4.60 (s, 1H), 4.27, 4.25 (dd, *J* = 12.3, 4.6Hz, 1H), 4.18 - 4.11 (m, 7H), 4.03 (t, *J* = 2.3Hz, 1H), 3.94 - 3.92 (m, 1H), 3.77 - 3.68 (m, 2H), 3.49 - 3.43 (m, 1H), 3.13 - 3.12 (m, 2H), 2.17 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.10 (s, 3H), 2.08 (s, 3H), 2.18 (s, 3H), 2.06 (s, 9H), 2.02 (s, 3H), 1.64 - 1.60 (m, 3H), 1.52 - 1.49 (m, 2H), 1.46 (s, 9H), 1.38 - 1.35 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.86, 170.72, 170.44, 170.44, 170.12, 170.04, 169.77, 169.70, 169.48, 169.37, 155.99, 99.79, 99.37, 98.24, 69.72, 69.60, 69.45, 68.58, 66.40, 66.29, 66.20, 31.91, 30.00, 29.68, 29.31, 28.42, 26.57, 25.88, 22.68, 20.83, 20.71 20.63, 20.63, 14.10. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{49}H_{73}NO_{28}Na$: 1146.4217; observed: 1146.5172.

Synthesis of Boc deprotected sugar derivative of trisaccharide mannose (**22**)

Compound **21** (0.295g, 0.320 mmol) was dissolved in TFA (20%) and dry DCM (8 ml) and was stirred for 2 hr at RT. On consumption of starting, the reaction mixture was concentrated *in vacuo* and co-evaporated with Toluene and DCM to yield **22** (0.186g, 77%) and was used as such without purification for next reaction.

Synthesis of trisaccharide mannose substituted tripod (**23**)

Compound **22** (0.177g, 0.173 mmol, 3.2 eq.) was dissolved in DCM and the PFP ester **6** (0.054g, 0.054 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using triethylamine. The mixture was then allowed to stir at RT for 12 hr. On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography using MeOH/DCM (4:96) was done to get **23** (0.279g,

45%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.57 (s, 2H), 6.50 (s, 2H), 6.34 (s, 1H), 5.41, 5.40 (dd, *J* = 10.0, 3.3Hz, 4H), 5.36 - 5.34 (m, 6H), 5.31 (s, 6H), 5.12 (d, *J* = 1.7Hz, 3H), 4.96 (d, *J* = 1.7Hz, 3H), 4.94 (d, *J* = 1.7Hz, 3H), 4.33 - 4.23 (m, 7H), 4.21 - 4.14 (m, 23 H), 4.02 (s, 3H), 3.96 - 3.91 (m, 3H), 3.74 - 3.70 (m, 16H), 3.49 - 3.41 (m, 6H), 3.27 - 3.22 (m, 6H), 2.59 - 2.53 (m, 2H), 2.44 - 2.38 (m, 6H), 2.17 (s, 9H), 2.14 (s, 9H), 2.14 (s, 9H), 2.10 (s, 9H), 2.08 (s, 9H), 2.07 (s, 9H), 2.05 (s, 27H), 2.02 (s, 9H), 1.63 - 1.59 (m, 6H), 1.55 - 1.50 (m, 6H), 1.44 (s, 9H), 1.37 - 1.33 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.29, 170.01, 169.78, 169.41, 169.38, 99.76, 99.34, 98.29, 70.53, 69.75, 69.53, 69.41, 69.25, 68.50, 68.38, 66.72, 66.31, 66.23, 66.16, 62.46, 62.24, 62.09, 38.97, 34.60, 29.61, 29.37, 29.22, 28.31, 26.82, 26.64, 25.84, 20.80, 20.60. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{153}H_{225}N_5O_{87}Na$: 3547.3233; observed: 3547.3728.

Synthesis of deprotected trisaccharide mannose substituted tripod (**24**)

Compound **23** (0.2g, 0.056 mmol) was then kept for Boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). Boc deprotected compound (0.11g, 59%) was obtained after co-evaporation with toluene and DCM several times. Further acetate de-protection (0.11g, 0.033 mmol, 1.0 eq.) was done in NaOMe (0.064g, 1.20 mmol, 36.0 eq.) and MeOH (10 ml) for 2 hr at RT. The crude residue on evaporation of MeOH was purified by sephadex column to yield **24** (0.021g, 55%). ^{1}H NMR (400 MHz, D_2O): δ. 5.20 (bs, 3H), 4.99 (bs, 3H), 4.95 (s, 3H), 4.02 (bs, 3H), 3.98 (bs, 3H), 3.88 - 3.74 (m, 20H), 3.73 - 3.51 (m, 42H), 3.15 - 3.08 (m, 8H), 2.57 (t, *J* = 6.2Hz, 6H), 2.39 (t, *J* = 5.5Hz, 6H), 1.55 - 1.48 (m, 6H), 1.45 - 1.41 (m, 6H), 1.31 - 1.23 (m, 12H); ^{13}C NMR (100 MHz, D_2O): δ. 171.92, 163.45, 163.10, 162.74, 102.20, 100.66, 98.02, 78.91, 78.54, 73.21, 72.73, 70.30, 69.93, 67.88, 67.05, 66.93, 66.81, 61.07, 60.86, 60.19, 39.39, 36.22, 35.52, 34.29, 32.26, 28.43, 28.31, 25.89, 25.06. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{88}H_{157}N_5O_{55}Na$: 2186.9540; observed: 2186.9497.

Synthesis of 6-azidohexanol sugar derivative of monogalactose (**26**)

Compound **25** (0.612g, 1.348 mmol, 1.0 eq.) was dissolved in DCM and 6- Azidohexan-1-ol (0.193g, 1.348 mmol, 1.0 eq.) was added. Then slow addition of $BF_3.Et_2O$ (0.556g, 3.92 mmol) was carried out over 30 min. and the reaction was stirred overnight. TLC

was checked for ensuring the consumption of starting material. Later, contents in RB were extracted with $NaHCO_3$ and DCM twice. One wash with brine was given for the organic layer and dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by silica column chromatography using EtOAc/Pet-ether (50:50) to yield **26** (0.55g, 75%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 5.39 - 5.38 (m, 1H), 5.21 - 5.18 (m, 1H), 5.03, 5.01 (dd, *J* = 10.5, 3.4Hz, 1H), 4.46 (d, *J* = 7.9Hz, 1H), 4.21 - 4.10 (m, 2H), 3.92 - 3.86 (m, 2H), 3.51 - 3.85 (m, 1H), 3.27 (t, *J* = 6.9Hz, 2H), 2.15 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.69 - 1.56 (m, 4H), 1.42 - 1.37 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.40, 170.28, 170.18, 169.34 101.34, 70.94, 70.59, 69.96, 68.92, 67.27, 51.24, 29.27, 28.76, 28.58, 26.40, 25.41, 20.75, 20.68, 20.60. HRMS (m/z) calc'd for $C_{20}H_{31}N_3O_{10}Na$: 496.1907; observed: 496.1913.

Synthesis of Amine derivative of monogalactose (**27**)

Hydrogenation of compound **26** (0.20g, 0.422 mmol) was done using Pd/Charcoal in methanol with hydrogen balloon overnight at RT. After completion of reaction, contents were filtered and then concentrated under reduced pressure to yield **27** (0.15g, 81%). The resulting compound was used as such without purification for next reaction.

Synthesis of monogalactose substituted tripod (**28**)

Compound **27** (0.175g, 0.393 mmol, 3.2 eq.) was dissolved in DCM and the PFP ester **6** (0.120g, 0.119 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using Triethylamine. The mixture was then allowed to stir at RT for 12 hr. On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography using MeOH/DCM (4:96) was done to get **28** (0.11g, 52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.79 (bs, 1H), 6.48 (bs, 1H), 6.37 (bs, 1H), 6.29 (bs, 1H), 5.41 - 5.40 (m, 3H), 5.22 - 5.18 (m, 2H), 5.04, 5.03 (dd, *J* = 10.5, 3.4Hz, 3H), 4.48 (d, *J* = 7.9Hz, 3H), 4.23 - 4.12 (m, 8H), 3.94 - 3.87 (m, 9H), 3.73 - 3.68 (m, 8H), 3.52 - 3.46 (m, 4H), 3.41 - 3.35 (m, 2H), 3.27 - 3.22 (m, 3H), 2.92 - 2.87 (m, 4H), 2.58 (t, *J* = 6.0Hz, 2H), 2.42 (t, *J* = 5.6Hz, 4H), 2.17 (s, 12H), 2.07 (s, 12H), 2.00 (s, 12H), 1.78 - 1.72 (m, 6H), 1.63 - 1.59 (m, 12H), 1.53 - 1.50 (m, 6H), 1.45 (s, 9H); ^{13}C NMR (100

MHz, $CDCl_3$): δ. 170.41, 170.25, 170.14, 169.48, 100.40, 70.82, 70.59, 70.14, 69.90, 69.04, 67.03, 61.22, 56.38, 40.06, 39.36, 36.21, 29.67, 29.36, 29.12, 29.08, 28.41, 26.82, 26.52, 25.60, 25.40, 24.18, 20.84, 20.81, 20.70, 20.69, 20.60. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{81}H_{129}N_5O_{39}Na$: 1818.8162; observed: 1818.8561.

Synthesis of deprotected galactose substituted tripod (**29**)

Compound **28** (0.15g, 0.083 mmol) was then kept for Boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). Boc deprotected compound (0.090g, 63%) was obtained after co-evaporation with toluene and DCM several times. Further acetate de-protection (0.09g, 0.053 mmol, 1.0 eq.) was done in NaOMe (0.041g, 0.764 mmol, 14.4 eq.) and MeOH (10 ml) for 2 hr at RT. The crude residue on evaporation of MeOH was purified by sephadex column to yield **29** (0.059g, 60%). ^1H NMR (400 MHz, D_2O): δ. 4.31 (d, *J* = 7.9Hz, 3H), 3.85 - 3.83 (m, 6H), 3.69 - 3.64 (m, 12H), 3.58 - 3.53 (m, 17H), 3.43 - 3.38 (m, 4H), 3.15 - 3.08 (m, 6H), 3.02 - 2.95 (m, 1H), 2.63 - 2.56 (m, 3H), 2.39 (t, *J* = 5.7Hz, 3H), 1.68 - 1.62 (m, 3H), 1.57 - 1.53 (m, 6H), 1.47 - 1.40 (m, 3H), 1.32 - 1.28 (m, 12H); ^{13}C NMR (100 MHz, D_2O): δ. 162.74, 102.73, 75.08, 72.98, 70.66, 70.42, 70.23, 68.75, 60.92, 55.79, 39.59, 39.38, 36.14, 28.75, 28.30, 25.80, 25.26, 24.80, 24.54, 23.40. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{52}H_{97}N_5O_{25}Na$: 1214.6370; observed: 1214.6843.

Synthesis of disubstituted monomannose tripod derivative (**30**)

Compound **14** (0.254g, 0.567 mmol, 2.0 eq.) was dissolved in DCM and the PFP ester **6** (0.173g, 0.172 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using Triethylamine. The mixture was then allowed to stir at RT for 12 hr. On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography was done to get **30** (0.092g, 35%) using Acetone/EtOAc (30:70). ^1H NMR (400 MHz, $CDCl_3$): δ. 6.44 (bs, 1H), 6.35 (bs, 1H), 5.37 - 5.31 (m, 2H), 5.29 - 5.26 (m, 1H), 5.23 - 5.22 (m, 2H), 4.81 (d, *J* = 1.2Hz, 2H), 4.35 (t, *J* = 6.6Hz, 1H), 4.31 - 4.27 (m, 2H), 4.13, 4.10 (dd, *J* = 12.2, 2.3Hz, 2H), 4.00 - 3.96 (m, 1H), 3.84 - 3.80 (m, 6H), 3.77 - 3.68 (m, 8H), 3.47 - 3.43 (m, 1H), 3.40 - 3.36 (m, 2H), 3.27 - 3.22 (m,

6H), 2.92 (t, *J* = 5.9Hz, 2H), 2.43 (t, *J* = 4.8Hz, 6H), 2.17 (s, 6H), 2.11 (s, 6H), 2.06 (s, 6H), 2.00 (s, 6H), 1.63 - 1.59 (m, 4H), 1.55 - 1.52 (m, 4H), 1.44 (s, 9H), 1.38 - 1.37 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.01, 169.78, 169.65, 169.49, 162.67, 162.45, 162.12, 141.03, 140.53, 139.82, 99.76, 99.34, 98.22, 70.53, 69.66, 69.53, 69.41, 69.14, 68.50, 68.38, 66.31, 66.23, 66.11, 62.52, 62.24, 29.65, 29.31, 28.31, 26.74, 25.86, 20.80, 20.69, 20.60. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{67}H_{97}F_5N_4O_{30}Na$: 1555.6005; observed: 1555.6175.

Synthesis of monomannose and monogalactose tripod derivative (**31**)

Compound **30** (0.09g, 0.058 mmol, 1.0 eq.) was taken in an RB and **27** (0.031g, 0.07 mmol, 1.2 eq.) dissolved in DCM was added to it. The pH of the reaction mixture was adjusted to 8 using Triethylamine. It was allowed to stir for 12 hr at RT. On completion, the solvent was evaporated under reduced pressure and the crude residue was purified using silica column chromatography using MeOH/DCM (4:96) to yield **31** (0.066g, 63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.88 (s, 1H), 6.52 (s, 2H), 6.42 (s, 2H), 5.37 (m, 1H), 5.34 - 5.28 (m, 2H), 5.21 - 5.14 (m, 3H), 5.01, 4.99 (dd, *J* = 10.5, 3.4Hz, 2H), 4.78 (d, *J* = 1.6Hz, 2H), 4.44 (d, *J* = 7.9Hz, 1H), 4.27, 4.26 (dd, *J* = 12.2, 5.2Hz, 2H), 4.20 - 4.07 (m, 6H), 3.97 - 3.93 (m, 1H), 3.90 - 3.83 (m, 4H), 3.74 - 3.64 (m, 13H), 3.47 - 3.40 (m, 4H), 3.26 - 3.18 (m, 6H), 2.39 (t, *J* = 5.2Hz, 8H), 2.14 (s, 3H), 2.13 (s, 6H), 2.09 (s, 3H), 2.03 (s, 18H), 1.98 (s, 3H), 1.97 (s, 3H), 1.58 - 1.48 (m, 12H), 1.41 (s, 9H), 1.35 - 1.31 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 170.67, 170.42, 170.26, 170.16, 169.72, 155.94, 101.35, 97.64, 70.87, 70.59, 70.21, 69.74, 69.23, 68.98, 68.43, 67.50, 67.06, 66.24, 62.74, 61.67, 39.45, 39.21, 36.84, 32.35, 29.62, 29.49, 29.35, 29.13, 28.58, 26.79, 26.71, 26.46, 26.01, 25.59, 25.40, 20.83, 20.80, 20.76, 20.71, 20.69, 20.67, 20.60. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{81}H_{129}N_5O_{39}Na$: 1818.8162; observed: 1818.8322.

Synthesis of Deprotected monomannose and monogalactose tripod derivative (**32**)

Compound **31** (0.04g, 0.022 mmol) was kept for Boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). After de-protection, the

compound was obtained after co-evaporation with toluene and DCM several times. Acetate de-protection of the compound (0.035g, 0.020 mmol, 1.0 eq.) was done in NaOMe (0.016g, 0.297 mmol, 14.4 eq.) and MeOH (5 ml) for 2 hr at RT. The crude residue on evaporation of MeOH was purified by sephadex column to yield **32** (0.02g, 76%). ^{1}H NMR (400 MHz, D_2O): δ. 4.76 (bs, 2H), 4.30 (d, *J* = 7.9Hz, 1H), 3.83 - 3.77 (m, 6H), 3.67 - 3.61 (m, 15H), 3.61 - 3.50 (m, 15H), 3.46 - 3.38 (m, 4H), 3.15 - 3.09 (m, 4H), 2.57 (t, *J* = 10.7Hz, 4H), 2.39 (t, *J* = 5.4Hz, 4H), 1.54 (bs, 6H), 1.45 - 1.42 (m, 6H), 1.28 (bs, 12H); ^{13}C NMR (100 MHz, D_2O): δ. 102.72, 99.62, 75.06, 72.83, 72.72, 70.71, 70.64, 70.38, 70.16, 68.55, 67.72, 67.61, 66.71, 39.31, 36.20, 35.51, 28.70, 28.40, 28.32, 25.82. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{52}H_{97}N_5O_{25}Na$: 1214.6370; observed: 1214.7262.

Synthesis of disubstituted dimannose tripod derivative (**33**)

Compound **18** (0.233g, 0.318 mmol, 2.0 eq.) was dissolved in DCM and the PFP ester **6** (0.16g, 0.159 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using Triethylamine. The mixture was then allowed to stir at RT for 12 hr. On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography using Acetone/EtOAc (30:70) was done to get **33** (0.124g, 37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.73 (s, 1H), 6.36 (s, 1H), 6.26 (s, 2H), 5.40, 5.38 (dd, *J* = 10.0, 3.3Hz, 2H), 5.31 - 5.29 (m, 2H), 5.27 - 5.26 (m, 2H), 5.25 - 5.24 (m, 3H), 4.90 (m, 4H), 4.22 - 4.16 (m, 3H), 4.15 - 4.08 (m, 6H), 3.99 - 3.98 (m, 2H), 3.90 - 3.86 (m, 2H), 3.80 - 3.76 (m, 4H), 3.68 - 3.61 (m, 12H), 3.42 - 3.32 (m, 4H), 3.23 - 3.18 (m, 4H), 2.89 (t, *J* = 5.9Hz, 2H), 2.39 (t, *J* = 5.7Hz, 6H), 2.16 (s, 3H), 2.13 (s, 3H), 2.12 (s, 6H), 2.06 (s, 12H), 2.02 (s, 6H), 2.01 (s, 6H), 1.99 (s, 6H), 1.58 - 1.56 (m, 4H), 1.49 - 1.48 (m, 4H), 1.40 (s, 9H), 1.34 - 1.32 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.31, 170.84, 170.38, 169.88, 169.75, 169.47, 169.23, 156.03, 142.24, 140.04, 139.18, 99.10, 98.14, 70.36, 69.79, 69.54, 69.45, 69.37, 69.01, 68.50, 68.36, 67.48, 67.45, 66.45, 66.25, 66.15, 62.52, 62.20, 53.78, 39.50, 37.09, 36.61, 34.26, 33.73, 31.96, 29.69, 29.66, 29.60, 29.36, 29.31, 29.26, 28.41, 26.81, 25.96, 22.66, 20.87, 20.76, 20.71,

20.67, 20.65. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{91}H_{129}F_5N_4O_{46}Na$: 2131.7696; observed: 2131.9399.

Synthesis of dimannose and monogalactose tripod derivative (**34**)

Compound **33** (0.1g, 0.047 mmol, 1.0 eq.) was taken in an RB and **27** (0.056g, 0.025 mmol, 1.2 eq.) dissolved in DCM was added to it. The pH of the reaction mixture was adjusted to 8 using triethylamine. It was allowed to stir for 12 hr at RT. On completion, the solvent was evaporated under reduced pressure and the crude residue was purified using silica column chromatography using DCM/MeOH (4:96) to yield **34** (0.066g, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.83 (s, 1H), 6.71 (s, 1H), 6.49 (s, 1H), 6.41 (s, 2H), 5.43 - 5.40 (m, 4H), 5.36 - 5.34 (m, 2H), 5.31 - 5.30 (m, 2H), 5.29 - 5.27 (m, 6H), 5.21 - 5.15 (m, 1H), 5.03, 5.01 (dd, *J* = 10.5, 3.4Hz, 2H), 4.93 (bs, 3H), 4.45 (d, *J* = 7.9Hz, 1H), 4.25 - 4.10 (m, 12H), 4.02 (bs, 2H), 3.93 - 3.86 (m, 4H), 3.72 - 3.67 (m, 12H), 3.50 - 3.37 (m, 6H), 3.26 - 3.23 (m, 6H), 2.41 (t, *J* = 5.5Hz, 6H), 2.16 (s, 9H), 2.15 (s, 6H), 2.09 (s, 12H), 2.06 - 2.04 (m, 18 H), 2.02 (s, 6H), 1.99 (s, 3H), 1.61 - 1.58 (m, 6H), 1.53 - 1.52 (m, 6H), 1.44 (s, 9H), 1.40 - 1.31 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.21,170.77, 170.41, 170.26, 169.90, 169.65, 169.45, 169.40, 155.99, 101.24, 99.12, 98.32, 70.84, 70.65, 70.54, 70.52, 70.36, 70.17, 69.85, 69.77, 69.22, 68.84, 68.41, 67.58, 67.10, 66.42, 66.21, 62.52, 62.19, 39.49, 39.45, 36.60, 29.56, 29.31, 28.49, 28.37, 26.80, 26.67, 25.94, 25.54, 20.85, 20.77, 20.74, 20.69, 20.66, 20.63, 20.57. MALDI-TOF (m/z): $[M+K]^+$ calc'd. for $C_{105}H_{161}N_5O_{55}K$: 2410.9592; observed: 2410.8785.

Synthesis of Deprotected dimannose and monogalactose tripod derivative (**35**)

Compound **34** (0.06g, 0.025 mmol) was then kept for boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). After de-protection, the compound was obtained after co-evaporation with toluene and DCM several times. Acetate de-protection of the compound (0.05g, 0.022 mmol, 1.0 eq.) was done in NaOMe (0.025g, 0.47 mmol, 21.6 eq.) and MeOH (10 ml) for 2 hr at RT. The crude

residue on evaporation of MeOH was purified by sephadex column to yield **35** (0.027g, 72%). ^{1}H NMR (400 MHz, D_2O): δ. 4.99 (bs, 2H), 4.92 (bs, 2H), 4.27 (d, *J* = 7.9Hz, 1H), 3.97 (m, 2H), 3.84 - 3.80 (m, 14H), 3.77 - 3.61 (m, 15H), 3.59 - 3.50 (m, 15H), 3.46 - 3.38 (m, 4H), 3.14 - 3.08 (m, 6H), 2.57 (t, *J* = 6.6Hz, 2H), 2.38 (t, *J* = 5.7Hz, 6H), 1.53 - 1.52 (m, 6H), 1.44 - 1.41 (m, 6H), 1.27 (m, 12H); ^{13}C NMR (100 MHz, D_2O): δ. 173.74, 171.78, 102.76, 102.30, 98.04, 78.72, 75.04, 73.23, 72.82, 72.71, 70.74, 70.38, 70.27, 69.90, 68.60, 68.40, 67.82, 67.59, 66.92, 66.84, 61.08, 60.87, 60.36, 39.38, 36.17, 35.41, 28.68, 28.42, 28.31, 25.89, 25.07, 24.73. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{64}H_{117}N_5O_{35}Na$: 1538.7427; observed: 1538.8334.

Synthesis of 2 substituted trimannose tripod derivative (**36**)

Compound **22** (0.291g, 0.298 mmol, 2.0 eq.) was dissolved in DCM and the PFP ester **6** (0.15g, 0.149 mmol, 1.0 eq.) was added to it. The pH of the reaction mixture was then adjusted to 8 using Triethylamine. The mixture was then allowed to stir at RT for 12 hr. On completion, the mixture was concentrated *in vacuo* to obtain the crude residue and silica column chromatography was done using Acetone/EtOAc (30:70) to get **36** (0.14 g, 36%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.39 (s, 1H), 6.27 (s, 3H), 5.42, 5.40 (dd, *J* = 10.0, 3.4Hz, 2H), 5.37 - 5.35 (m, 3H), 5.32 - 5.30 (m, 3H), 5.12 (d, *J* = 1.8Hz, 2H), 4.97 (d, *J* = 1.7Hz, 2H), 4.95 (d, *J* = 1.9Hz, 2H), 4.27, 4.25 (dd, *J* = 12.3, 4.5Hz, 3H),4.18 - 4.15 (m, 12H), 4.03 (m, 2H), 3.94 - 3.91 (m, 2H), 3.84 - 3.80 (m, 5 H), 3.73 - 3.65 (m, 12H), 3.46 - 3.44 (m, 2H), 3.41 - 3.38 (m, 2H), 3.28 - 3.23 (m, 6H), 2.93 (t, *J* = 5.9 Hz, 6H), 2.43 (t, *J* = 5.6Hz, 6H), 2.21 (s, 6H), 2.20 (s, 6H), 2.18 (s, 6H), 2.15 (s, 6H), 2.15 (s, 6H), 2.11 (s, 6H), 2.11 (s, 6H), 2.09 (s, 6H), 2.06 (s, 6H), 2.05 (s, 6H), 2.03 (s, 6H), 1.70 - 1.60 (m, 4H), 1.54 - 1.52 (m, 4H), 1.45 (s, 9H), 1.38 - 1.36 (m, 8H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.18, 170.85, 170.64, 170.64, 170.43, 170.16, 170.03, 169.75, 169.64, 169.51, 169.45, 169.32, 169.29, 155.88, 141.22, 140.38, 139.14, 99.79, 99.19, 98.24, 70.51, 69.69, 69.43, 69.32, 69.12, 68.51, 68.41, 66.29, 66.23, 66.13, 62.47, 62.21, 62.12, 39.47, 36.59, 34.21, 31.65, 29.68, 29.60, 29.34, 29.21, 28.35, 28.75, 28.85, 20.82, 20.70, 20.68, 20.64, 20.60. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{115}H_{161}F_5N_4O_{62}Na$: 2707.9386; observed: 2707.9921.

Synthesis of trimannose and monogalactose tripod derivative (**37**)

Compound **36** (0.1g, 0.037 mmol, 1.0 eq.) was taken in an RB and **27** (0.019g, 0.044 mmol, 1.2 eq.) dissolved in DCM was added to it. The pH of the reaction mixture was adjusted to 8 using triethylamine. It was allowed to stir for 12 hr at RT. On completion, the solvent was evaporated under reduced pressure and the crude residue was purified using silica column chromatography using MeOH/DCM (4: 96) to yield **37** (0.062g, 57%). ^{1}H NMR (400 MHz, $CDCl_3$): δ. 6.44 (bs, 3H), 6.36 (bs, 1H), 6.24 (bs, 1H), 5.43 - 5.40 (m, 6H), 5.37 - 5.28 (m, 15H), 5.14 - 5.11 (m, 3H), 4.97 (d, *J* = 1.3Hz, 3H), 4.95 (d, *J* = 1.4Hz, 3H), 4.48 (d, *J* = 6.1Hz, 1H), 4.28 - 4.12 (m, 17H), 3.94 - 3.91 (m, 6H), 3.75 - 3.68 (m, 12H), 3.51 - 3.45 (m, 4H), 3.42 - 3.37 (m, 2H), 3.28 - 3.22 (m, 6H), 2.58 (t, *J* = 5.7Hz, 2H), 2.44 - 2.42 (m, 6H), 2.18 (s, 9H), 2.15 (s, 12H), 2.11 (s, 9H), 2.09 (s, 3H), 2.08 (s, 3H), 2.07 (s, 9H), 2.06 (s, 15H), 2.03 (s, 9H), 2.01 (s, 3H), 1.72 - 1.68 (m, 6H), 1.64 - 1.59 (m, 12H), 1.55 - 1.52 (m, 6H), 1.45 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ. 171.28, 170.29, 170.04, 169.79, 169.33, 169.24, 156.03, 101.21, 99.49, 98.33, 69.61, 69.50, 69.17, 69.70, 68.63, 68.52, 68.46, 68.23, 67.04, 66.35, 62.66, 62.30, 61.34, 32.00, 29.77, 29.333\, 28.47, 26.89, 26.00, 24.47, 23.07, 22.71, 22.64, 20.76, 20.62, 20.34. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{129}H_{193}N_5O_{71}Na$: 2971.1543; observed: 2971.1541.

Synthesis of Deprotected trimannose and monogalactose tripod derivative (**38**)

Compound **37** (0.081g, 0.027 mmol) was then kept for boc de-protection reaction by dissolving it in 20% TFA in DCM (2ml TFA, 8 ml DCM). After de-protection, the compound was obtained after co-evaporation with toluene and DCM several times. Acetate de-protection of the compound (0.069g, 0.024 mmol, 1.0 eq.) was done in NaOMe (0.037g, 0.697 mmol, 28.8 eq.) and MeOH (10 ml) for 2 hr at RT. The crude residue on evaporation of MeOH was purified by sephadex column to yield **38** (0.024g, 69%). ^{1}H NMR (400 MHz, D_2O): δ. 5.13 (d, *J* = 0.5Hz, 2H), 4.92 (d, *J* = 0.7Hz, 2H), 4.88

(d, *J* = 0.5Hz, 2H), 4.23 (d, *J* = 8.0Hz, 1H), 3.95 - 3.94 (m, 2H), 3.90 - 3.89 (m, 2H), 3.80 - 3.75 (m, 8H), 3.72 - 3.67 (m, 9H), 3.61 - 3.55 (m, 27H), 3.52 - 3.48 (m, 12H), 3.03 (t, *J* = 7.1Hz, 6H), 2.50 (t, *J* = 6.4Hz, 4H), 2.31 (t, *J* = 5.7Hz, 6H), 1.48 - 1.43 (m, 6H), 1.37 - 1.34 (m, 6H), 1.27 - 1.20 (m, 12H). ^{13}C NMR (100 MHz, D_2O): δ. 163.00, 102.57, 102.38, 102.18, 73.22, 72.23, 70.27, 70.20, 69.88, 69.80, 68.25, 67.97, 67.69, 66.90, 66.81, 39.35, 28.50, 28.18, 28.09, 27.28, 26.81, 25.55, 24.98, 24.94, 24.66. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{76}H_{137}N_5O_{45}Na$: 1862.8483; observed: 1862.8563.

General Procedure for deprotection of Sugars

Acetate de-protection of compound was done in NaOMe and MeOH (10 ml) for 2 hr at RT. Then, after completion of reaction, neutralize with IR 120 H^+. The crude residue on evaporation of MeOH was purified by sephadex column to yield deprotected form of sugars.

Deprotected monomannose sugar (**39**)

^{1}H NMR (400 MHz, D_2O): δ. 4.77 (s, 1H), 3.84 (bs, 1H), 3.78 (bs, 1H), 3.73 - 3.62 (m, 4H), 3.59 - 3.51 (m, 2H), 3.50 - 3.43 (m, 1H), 2.74 (t, *J* = 7.3Hz, 1H), 1.59 - 1.43 (m, 4H), 1.30 (bs, 4H); ^{13}C NMR (100 MHz, D_2O): δ. 99.61, 72.70, 70.61, 70.04, 67.69, 66.73, 60.90, 39.68, 28.26, 27.93, 25.46, 24.96. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{12}H_{25}NO_6Na$: 302.1580; observed: 302.1458.

Deprotected disaccharide mannose sugar (**40**)

^{1}H NMR (400 MHz, D_2O): δ. 5.01 (d, *J* = 1.4Hz, 1H), 4.93 (d, *J* = 1.6Hz, 1H), 3.99 - 3.97 (m, 1H), 3.86 - 3.85 (m, 1H), 3.81 - 3.80 (m, 1H), 3.79 - 3.78 (m, 2H), 3.74 - 3.73 (m, 1H), 3.70 - 3.67 (m, 1H), 3.66 - 3.62 (m, 3H), 3.60 - 3.57 (m, 1H), 3.54 - 3.50 (m, 2H), 3.48 - 3.42 (m, 1H), 2.90 (t, *J* = 7.6Hz, 2H), 1.58 - 1.54 (m ,4H), 1.33 - 1.30 (m, 4H); ^{13}C NMR (100 MHz, D_2O): δ. 102.25, 97.98, 78.67, 73.18, 72.66, 70.24, 69.87, 67.68, 66.91, 66.83, 61.04, 60.86, 39.37, 29.36, 28.11, 26.53, 25.25, 24.78. MALDI-TOF (m/z): $[M+Na]^+$ calc'd. for $C_{18}H_{35}NO_{11}Na$: 464.2108; observed: 464.1927.

Deprotected trisaccharide mannose sugar (**41**)

^{1}H NMR (400 MHz, D_2O): δ. 5.34 - 5.19 (m, 1H), 5.09 - 4.96 (m, 2H), 4.77 - 4.67 (m, 1H), 4.57 - 4.42 (m, 1H), 4.06 (bs, 1H), 4.00 (d, *J* = 2.0Hz, 2H), 3.94 - 3.79 (m, 6H), 3.76 - 3.67 (m, 6H), 3.65 - 3.55 (m, 3H), 2.64 (t, *J* = 7.5Hz, 2H), 1.70 - 1.58 (m, 4H), 1.45 - 1.41 (m, 4H); ^{13}C NMR (100 MHz, D_2O): δ. 102.83, 100.94, 98.42, 79.52, 79.13, 73.61, 73.25, 70.75, 70.48, 67.83, 67.70, 67.40, 66.97, 61.92, 61.81, 61.65, 39.24, 29.21, 27.34, 25.55, 25.33. MALDI-TOF (m/z): $[M+K]^+$ calc'd. for $C_{24}H_{45}NO_{16}K$: 642.2375; observed: 642.5877.

Deprotected monogalactose sugar (**42**)

^{1}H NMR (400 MHz, D_2O): δ. 4.31 (d, *J* = 7.9Hz, 1H), 3.88 - 3.82 (m, 2H), 3.70 - 3.65 (m, 2H), 3.61 - 3.54 (m, 3H), 3.44 - 3.39 (m, 1H), 3.24 (t, *J* = 5.9Hz, 2H), 1.58 - 1.52 (m, 4H), 1.33 - 1.30 (m, 4H); ^{13}C NMR (100 MHz, D_2O): δ. 102.75, 75.07, 72.83, 70.76, 70.39, 68.63, 60.90, 51.12, 28.59, 27.87, 25.67, 24.60. HRMS (m/z) calc'd for $C_{20}H_{31}O_{10}N_3Na$: 496.1907; observed: 496.1913.

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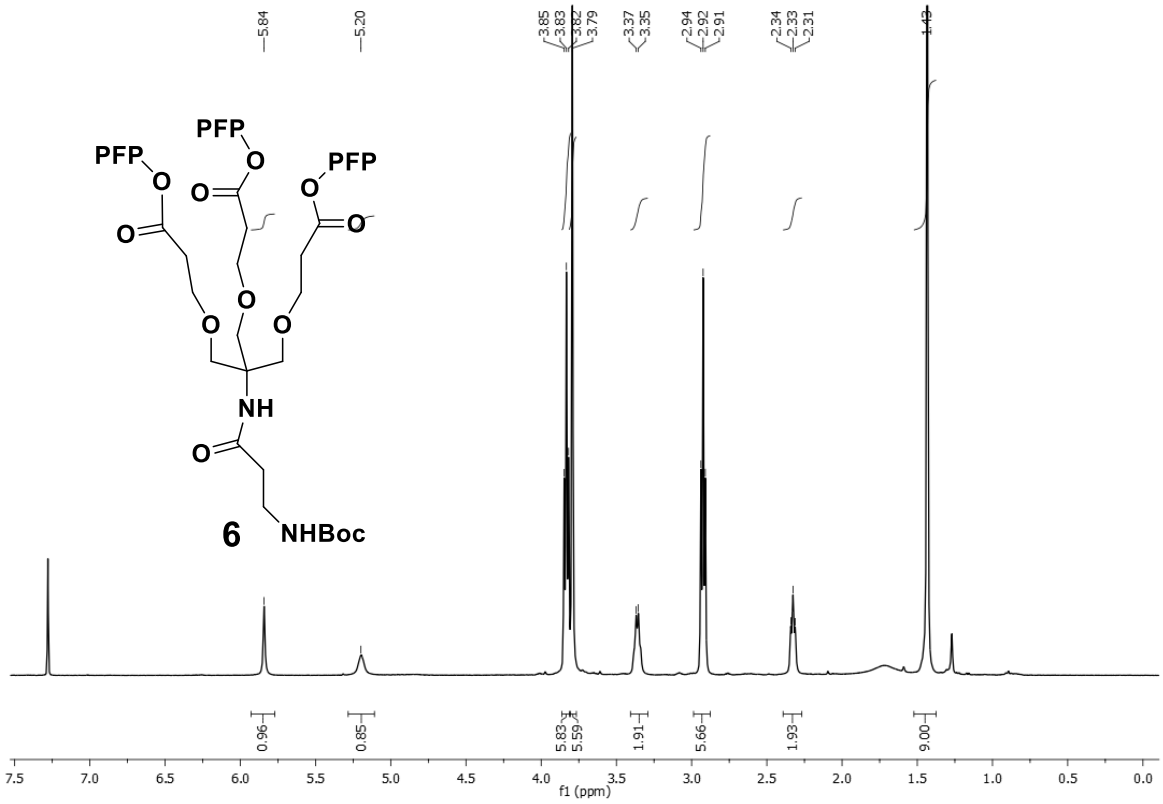
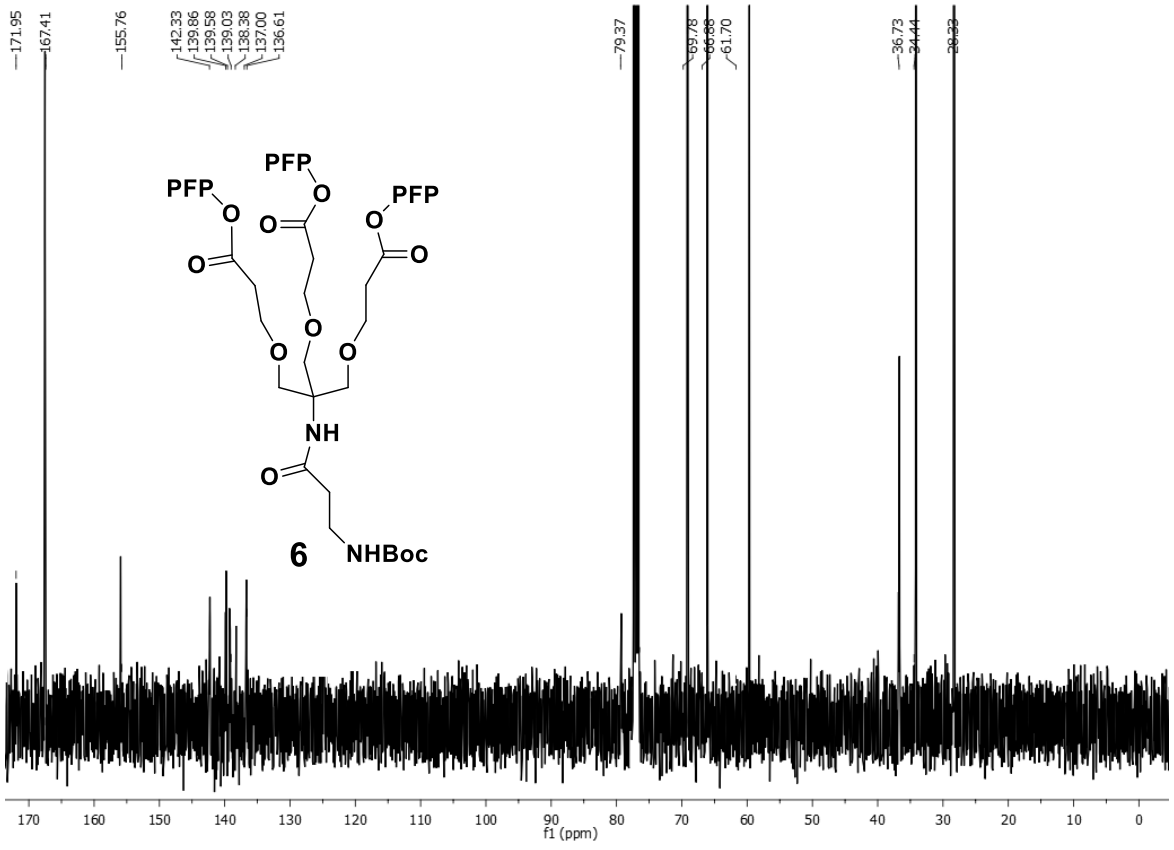
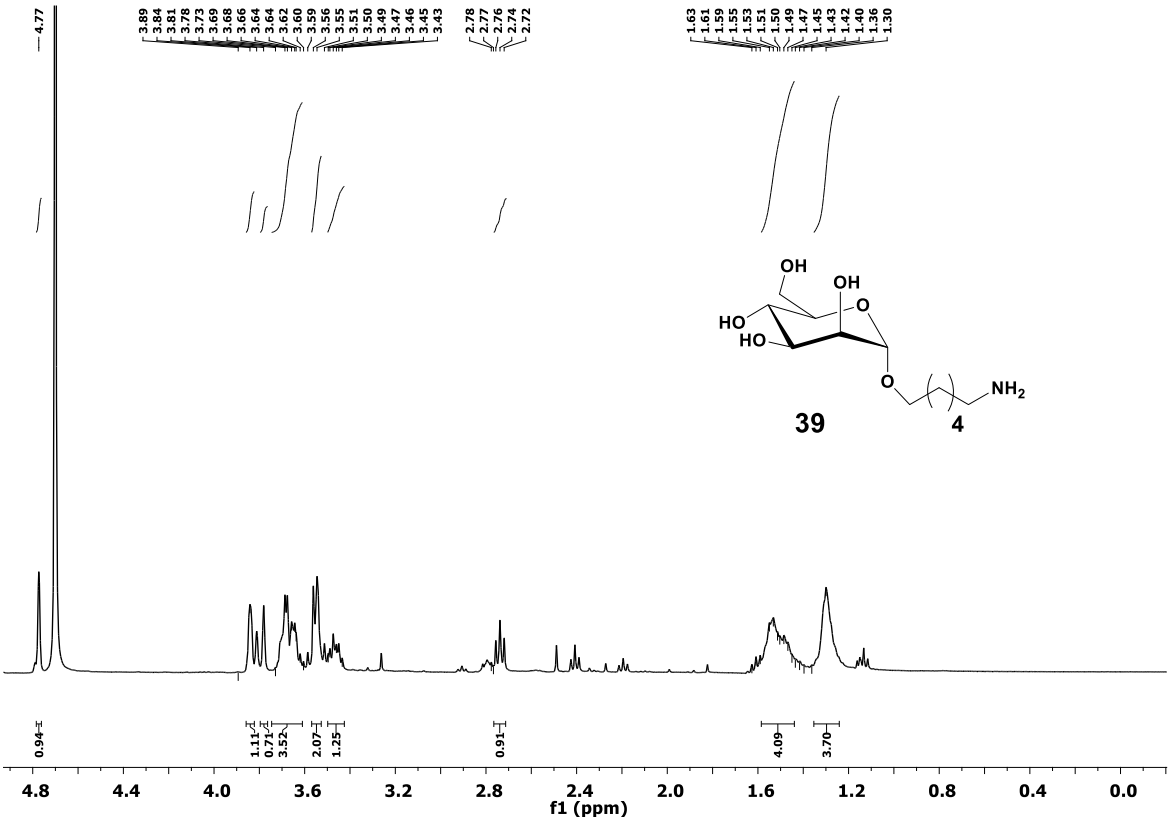
SUPPLEMENTARY DATA



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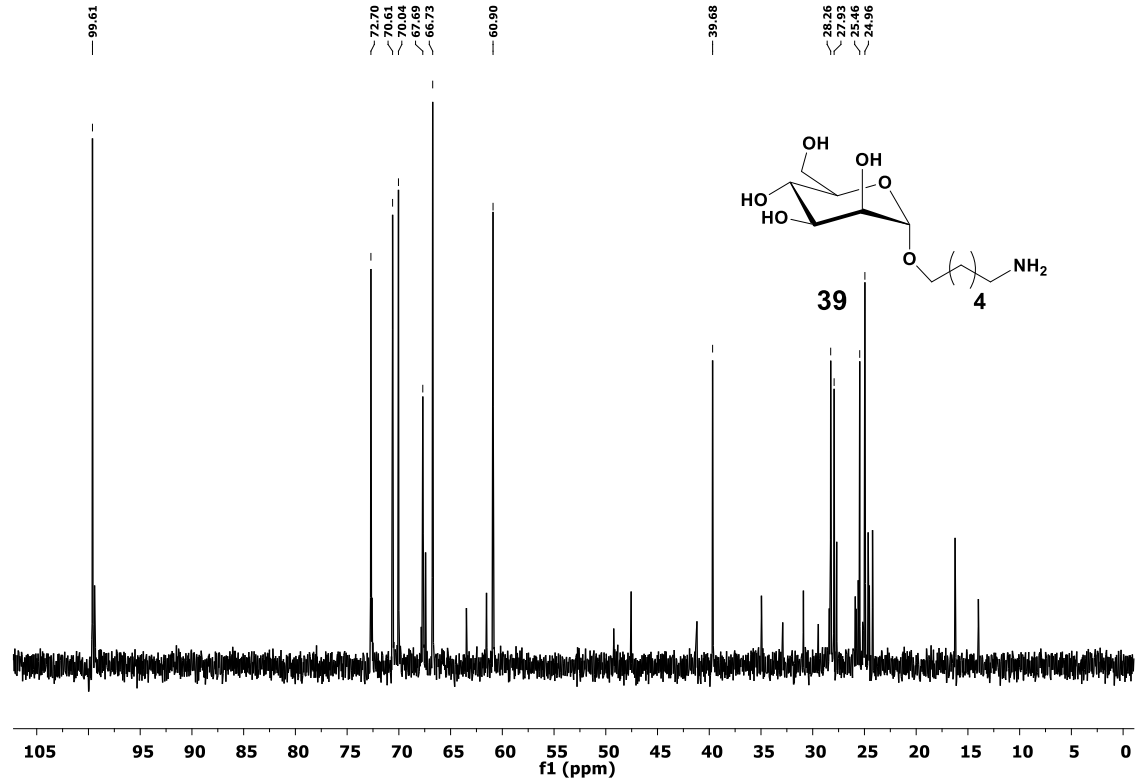
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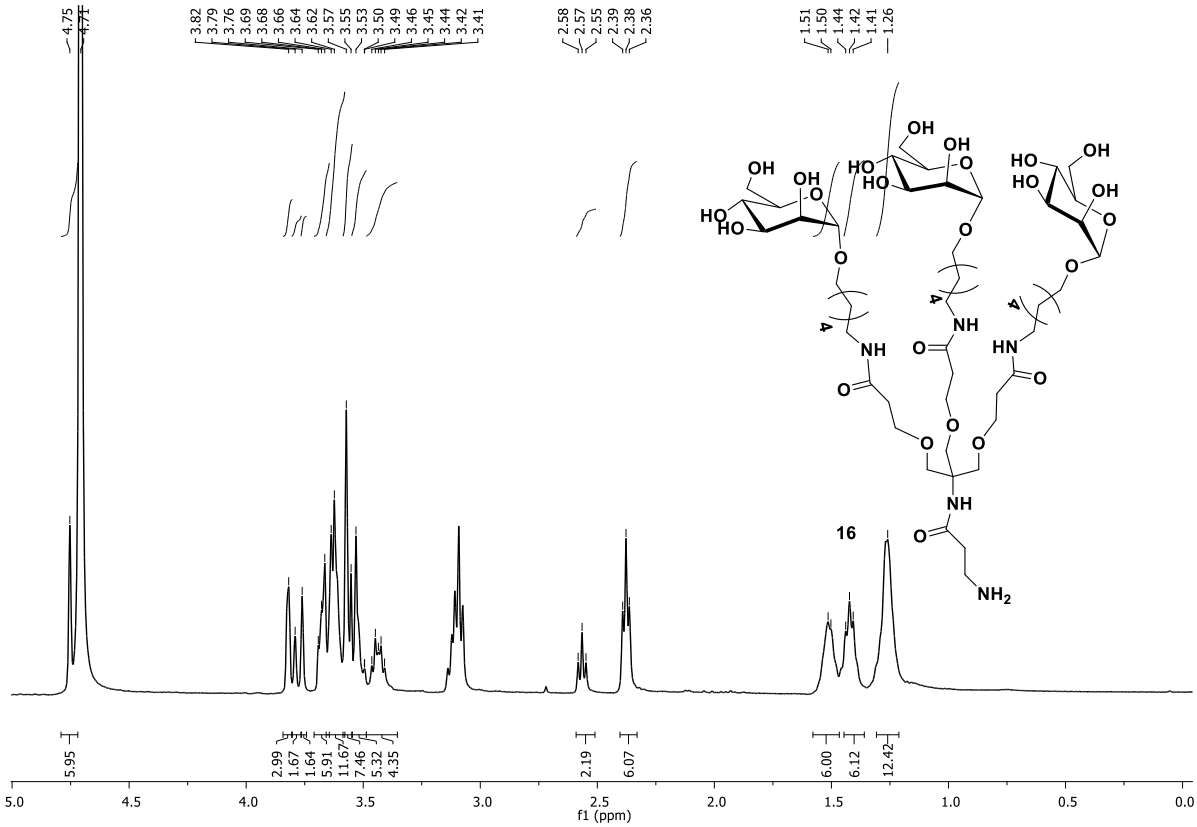
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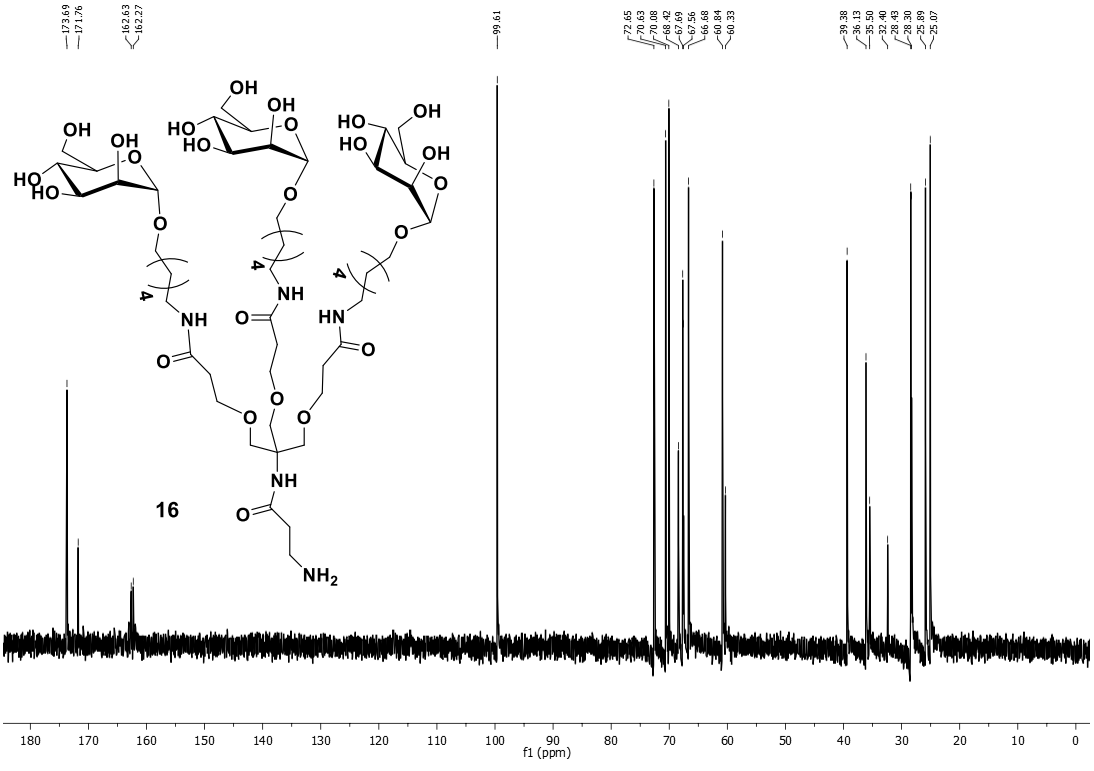
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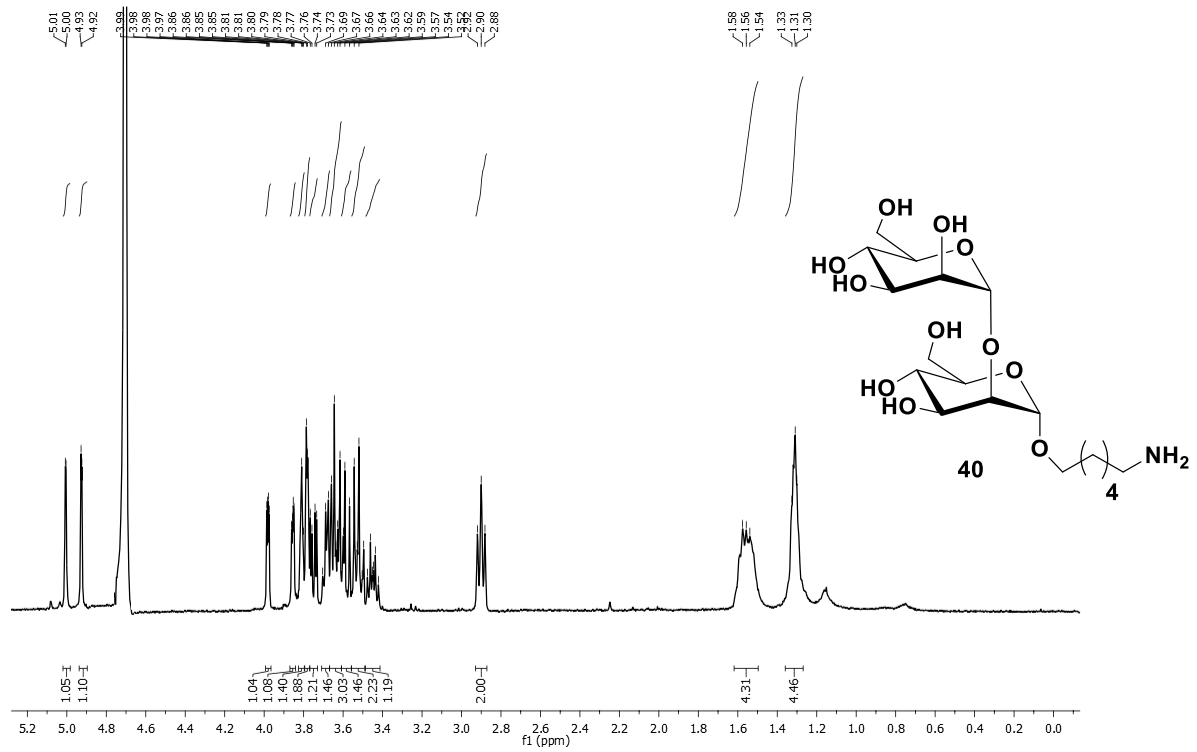
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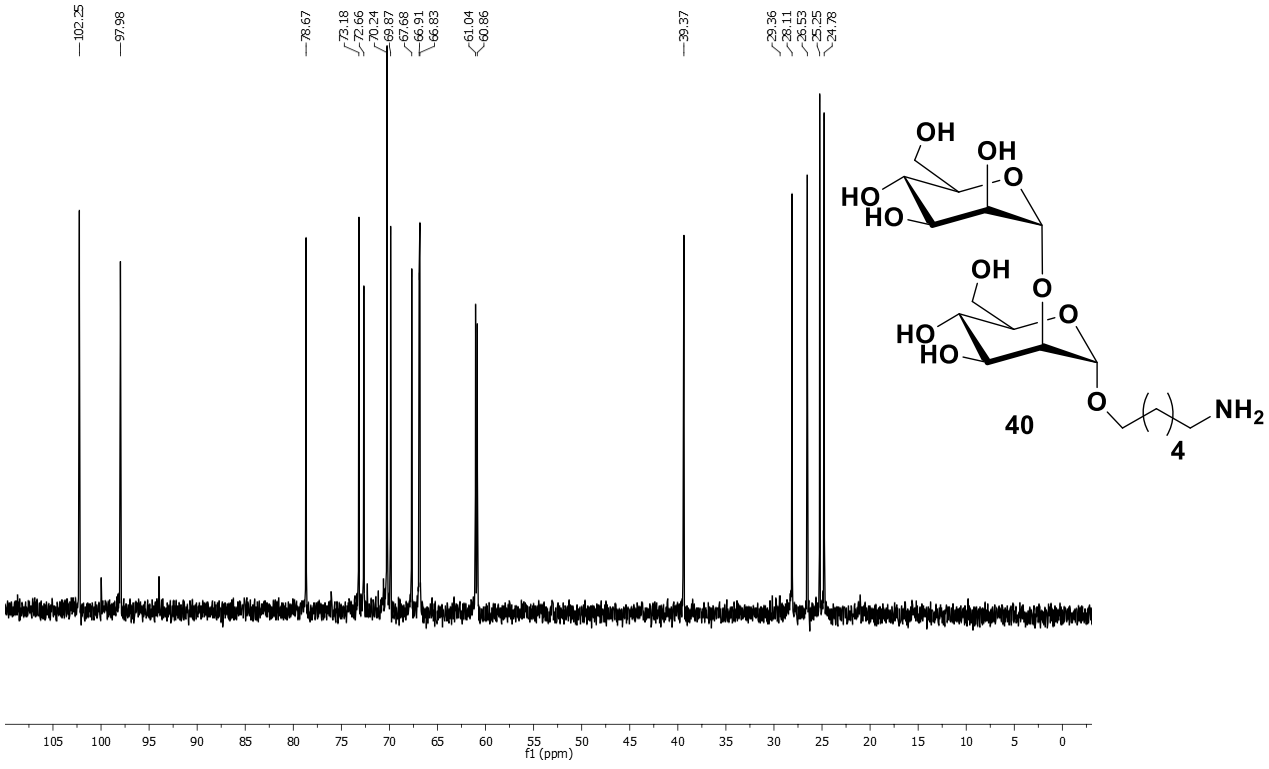
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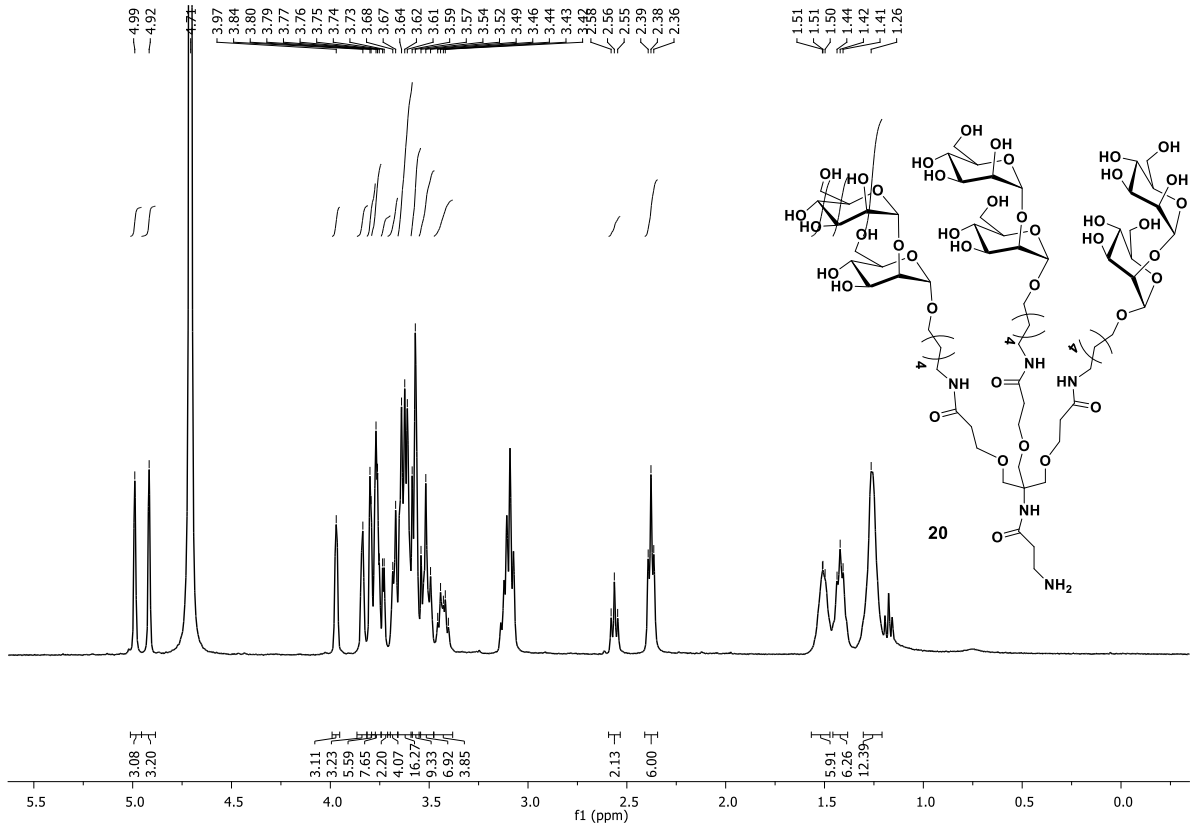
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3.75
3.74
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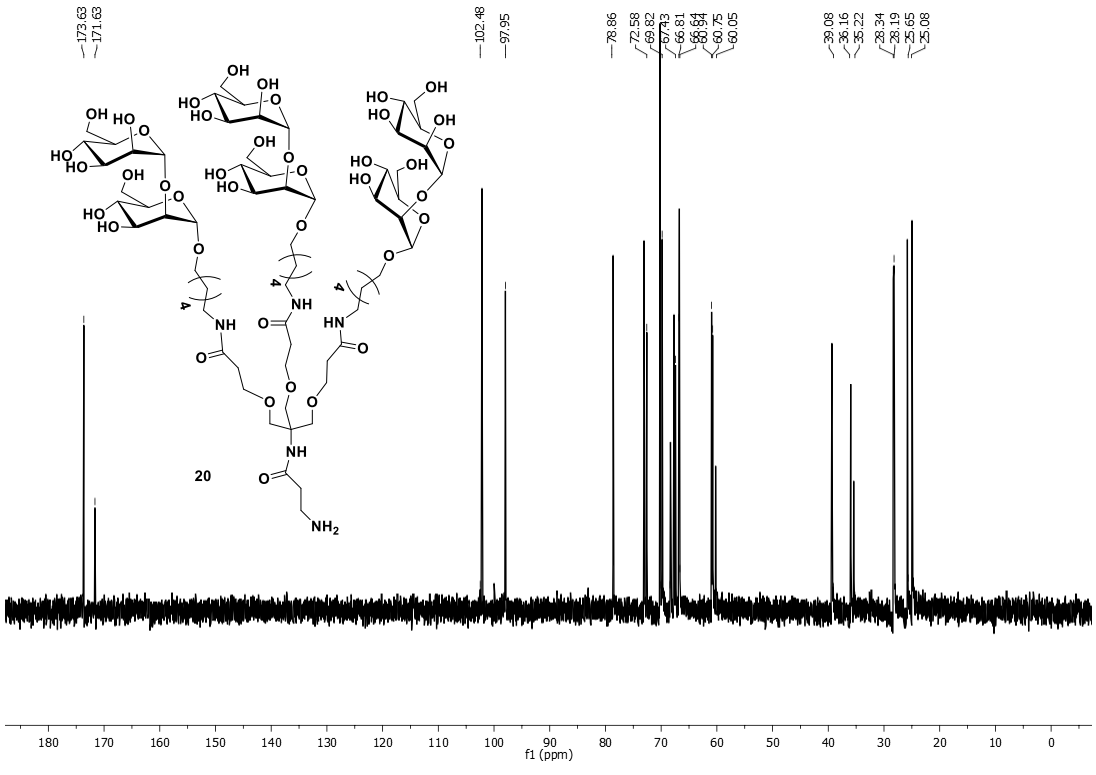
3.68
3.67
3.64
3.62
3.61
3.59
3.57
3.54
3.52
3.49
3.46
3.44
3.43
3.42
2.58
2.56
2.55
2.39
2.38
2.36
1.51
1.51
1.50
1.44
1.42
1.41
1.26
20
3.08
3.20
3.11
3.23
5.59
7.65
2.20
4.07
16.27
9.33
6.92
3.85
2.13
6.00
5.91
6.26
12.39
f1 (ppm)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72.58
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67.73
66.81
66.64
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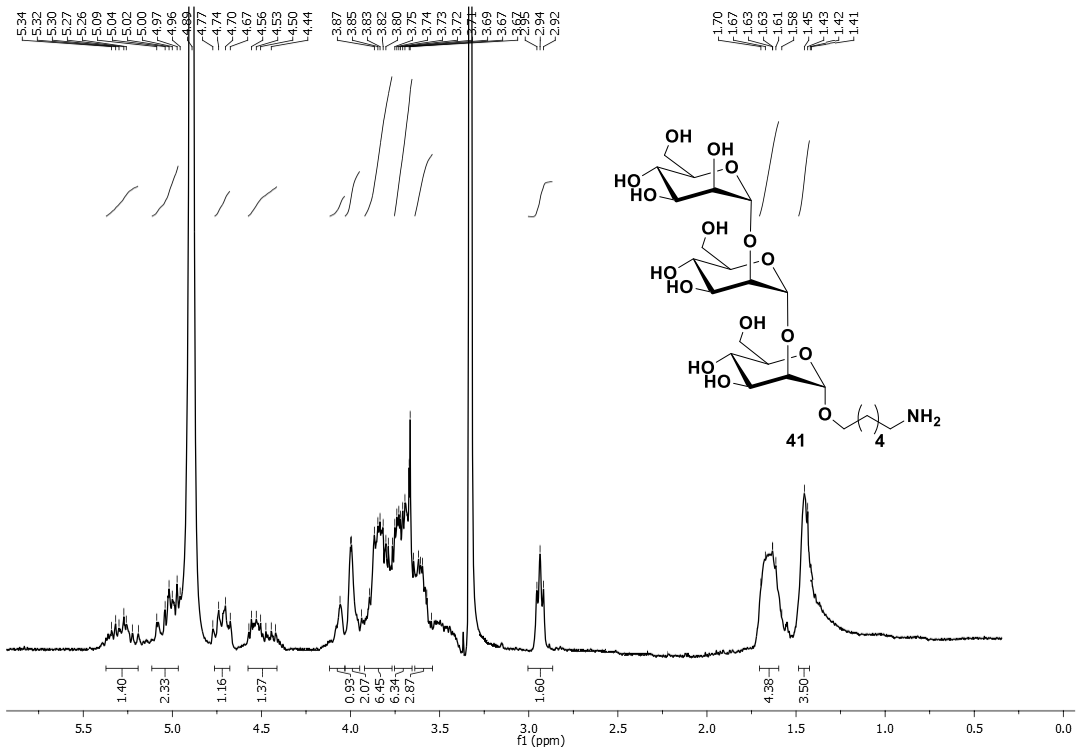
39.08
36.16
35.22
28.34
28.19
25.65
25.08
20
f1 (ppm)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5.02
5.00
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4.96
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4.77
4.74
4.70
4.67
4.56
4.53
4.50
4.44
3.87
3.85
3.83
3.82
3.80
3.75
3.74
3.73
3.72
3.71
3.69
3.67
3.62
2.95
2.94
2.92
1.70
1.67
1.63
1.63
1.61
1.58
1.45
1.43
1.42
1.41
OH
OH
O
HO
HO
OH
O
O
HO
HO
OH
O
O
HO
HO
O
NH2
41
4
1.40
2.33
1.16
1.37
0.93
2.07
6.45
6.34
2.87
1.60
4.38
3.50
5.5
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3.5
3.0
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2.0
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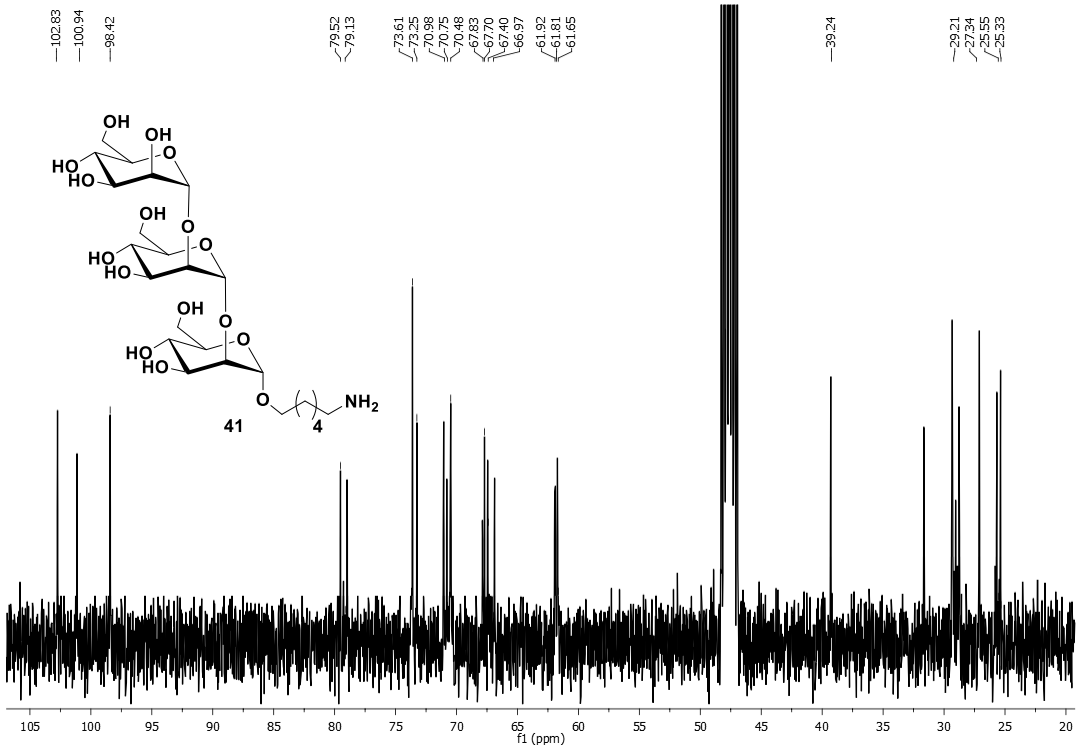
1.0
0.5
0.0
f1 (ppm)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67.83
67.70
67.40
66.97
61.92
61.81
61.65
39.24
29.21
27.34
25.55
25.33
OH
OH
O
HO
HO
OH
O
O
HO
HO
OH
O
O
HO
HO
O
NH2
41
4
105
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95
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85
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75
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45
40
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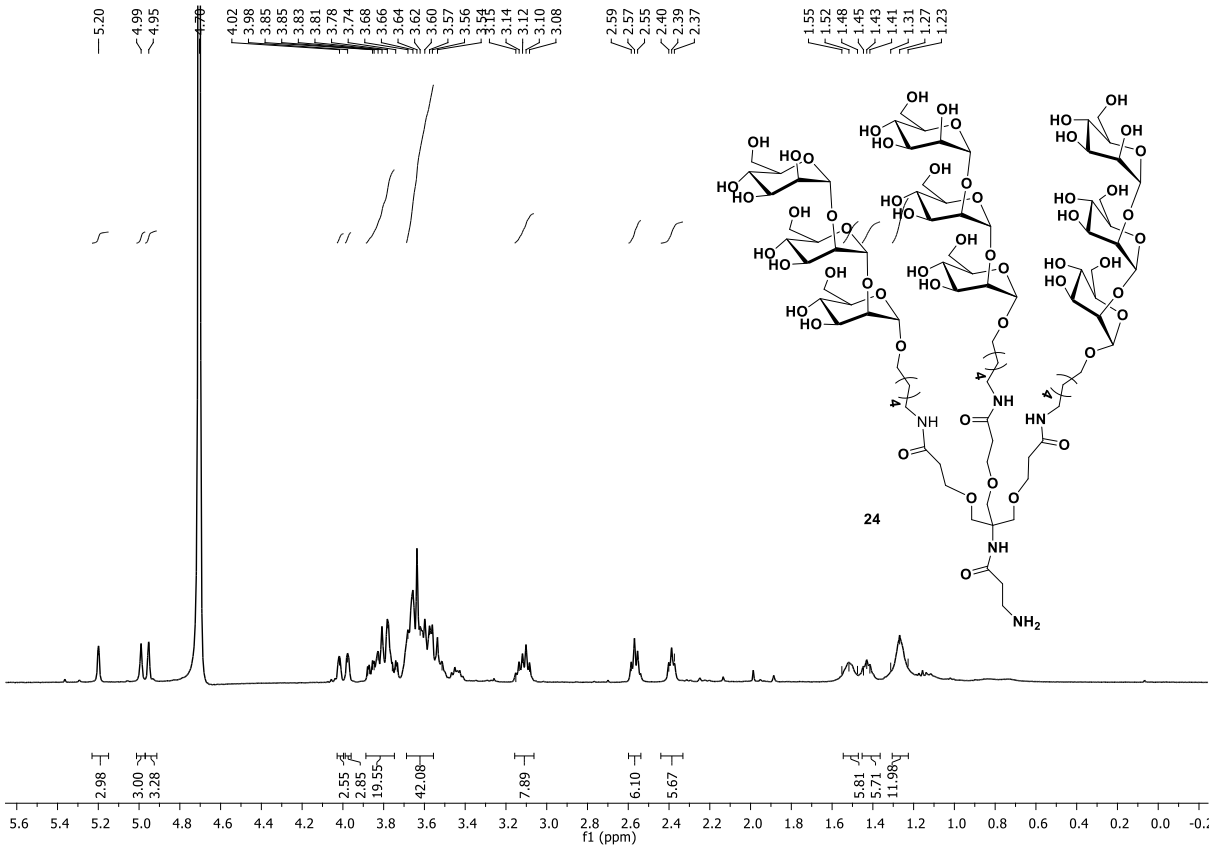
25
20
f1 (ppm)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3.81
3.78
3.74
3.68
3.66
3.64
3.62
3.60
3.57
3.56
3.54
3.15
3.14
3.12
3.10
3.08
2.59
2.57
2.55
2.40
2.39
2.37
1.55
1.52
1.48
1.45
1.43
1.41
1.31
1.27
1.23
24
2.98
3.00
3.28
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19.55
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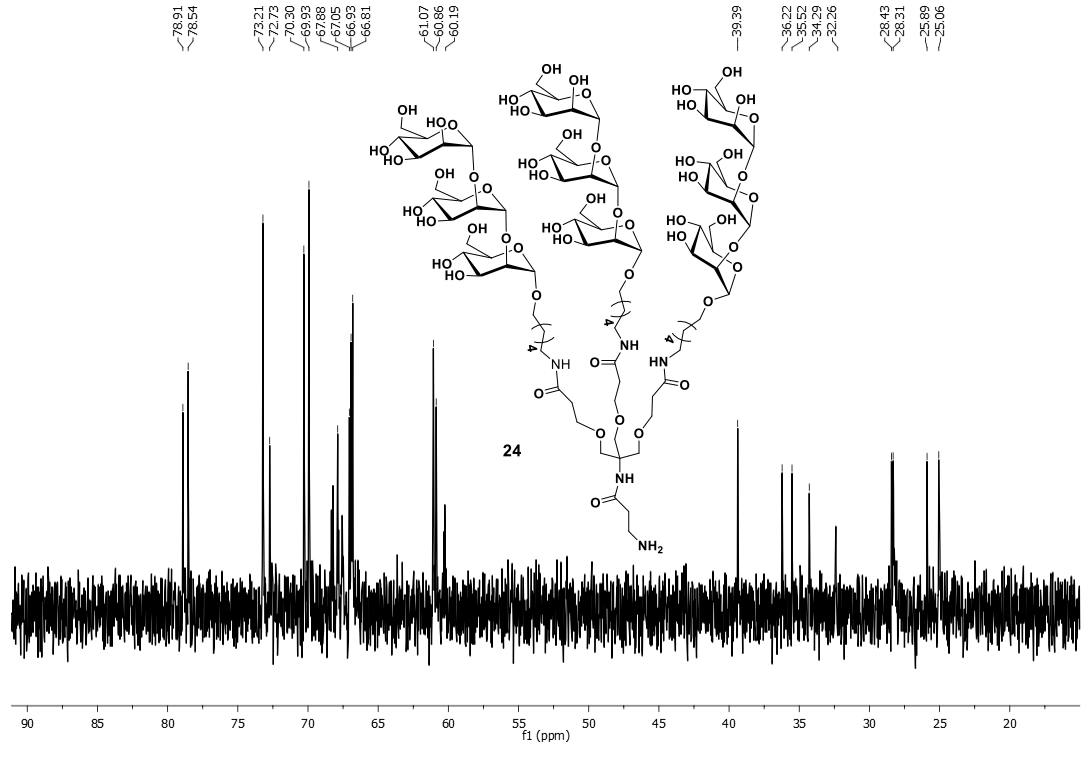
7.89
6.10
5.67
5.81
5.71
11.98
f1 (ppm)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69.93
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67.05
66.93
66.81
61.07
60.86
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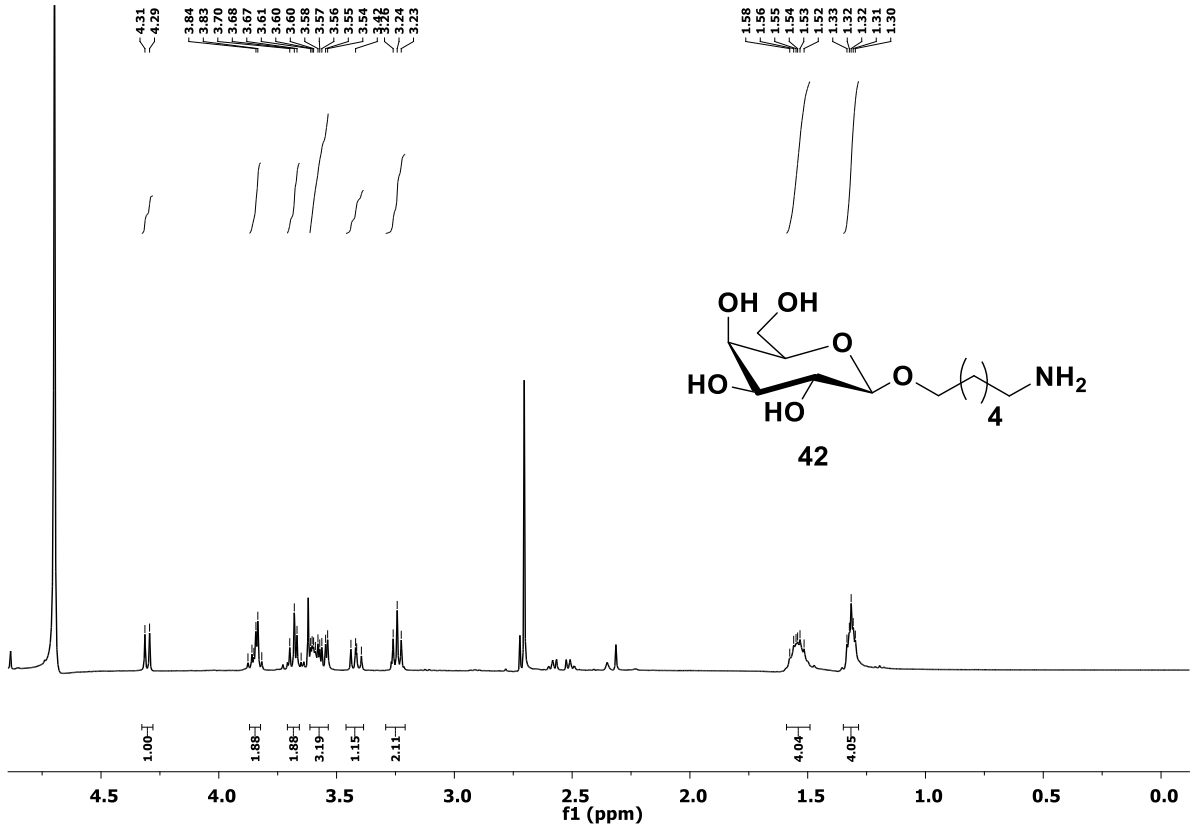
28.43
28.31
25.89
25.06
24
f1 (ppm)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3.68
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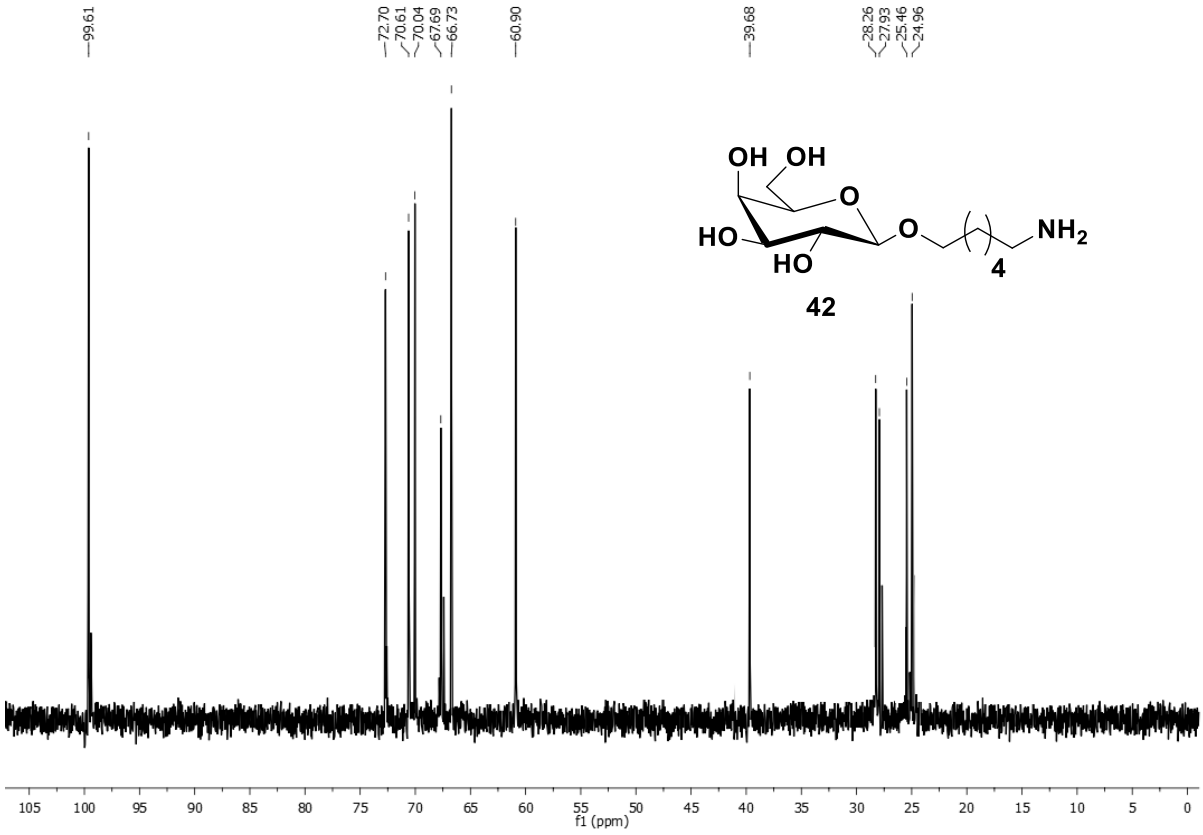
3.61
3.60
3.60
3.58
3.57
3.56
3.55
3.54
3.42
3.24
3.23
1.58
1.56
1.55
1.54
1.53
1.52
1.33
1.32
1.32
1.31
1.30
OH
OH
O
HO
HO
O
NH2
4
42
1.00
1.88
1.88
3.19
1.15
2.11
4.04
4.05
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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66.73
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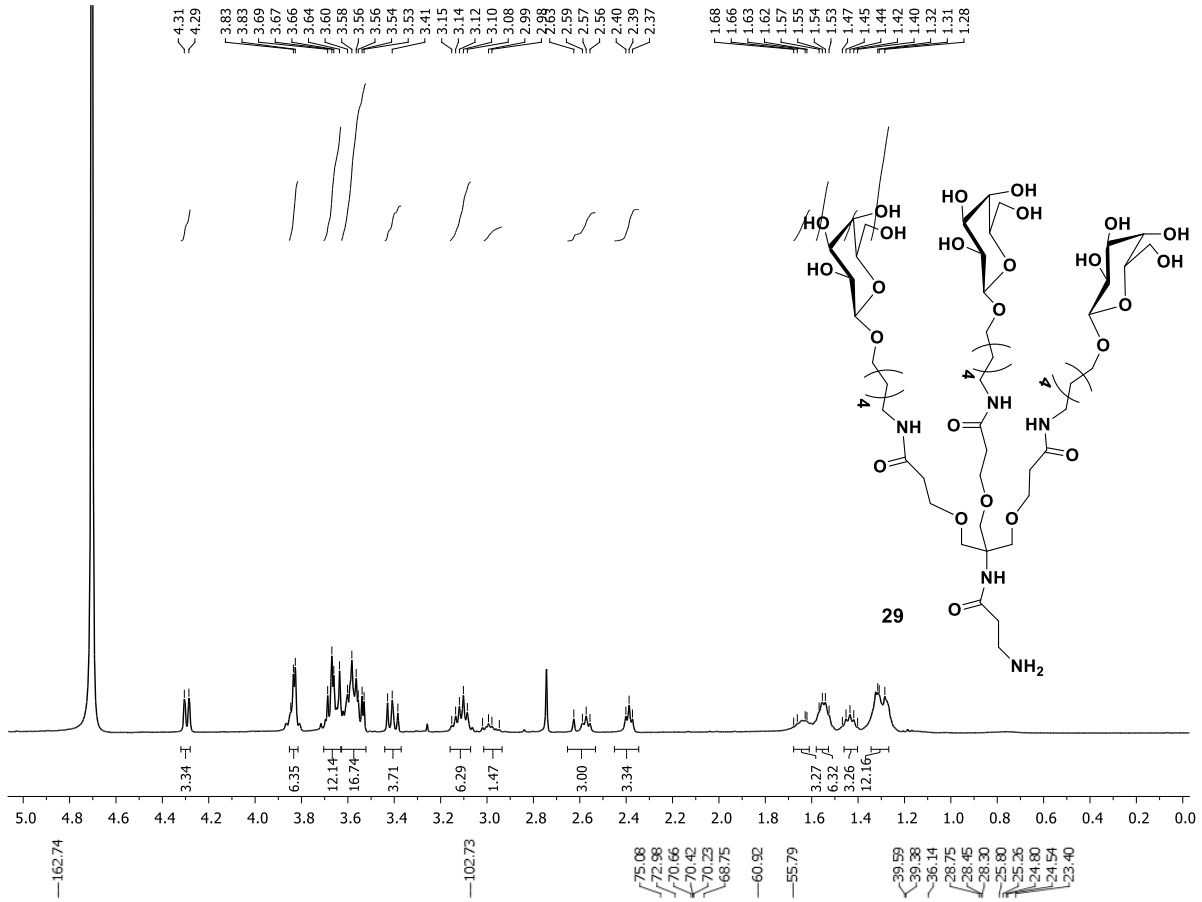
39.68
28.26
27.93
25.46
24.96
OH
OH
O
HO
HO
O
NH2
4
42
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
f1 (ppm)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3.67
3.66
3.64
3.60
3.58
3.56
3.56
3.54
3.53
3.41
3.15
3.14
3.12
3.10
3.08
2.99
2.98
2.63
2.59
2.57
2.56
2.40
2.39
2.37
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1.66
1.63
1.62
1.57
1.55
1.54
1.53
1.47
1.45
1.44
1.42
1.40
1.32
1.31
1.28
29
3.34
6.35
12.14
16.74
3.71
6.29
1.47
3.00
3.34
3.27
6.32
3.26
12.16
5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0 2.8 2.6 2.4 2.2 2.0 1.8 1.6 1.4 1.2 1.0 0.8 0.6 0.4 0.2 0.0
162.74
102.73
75.08
72.98
70.66
70.42
70.23
68.75
60.92
55.79
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39.38
36.14
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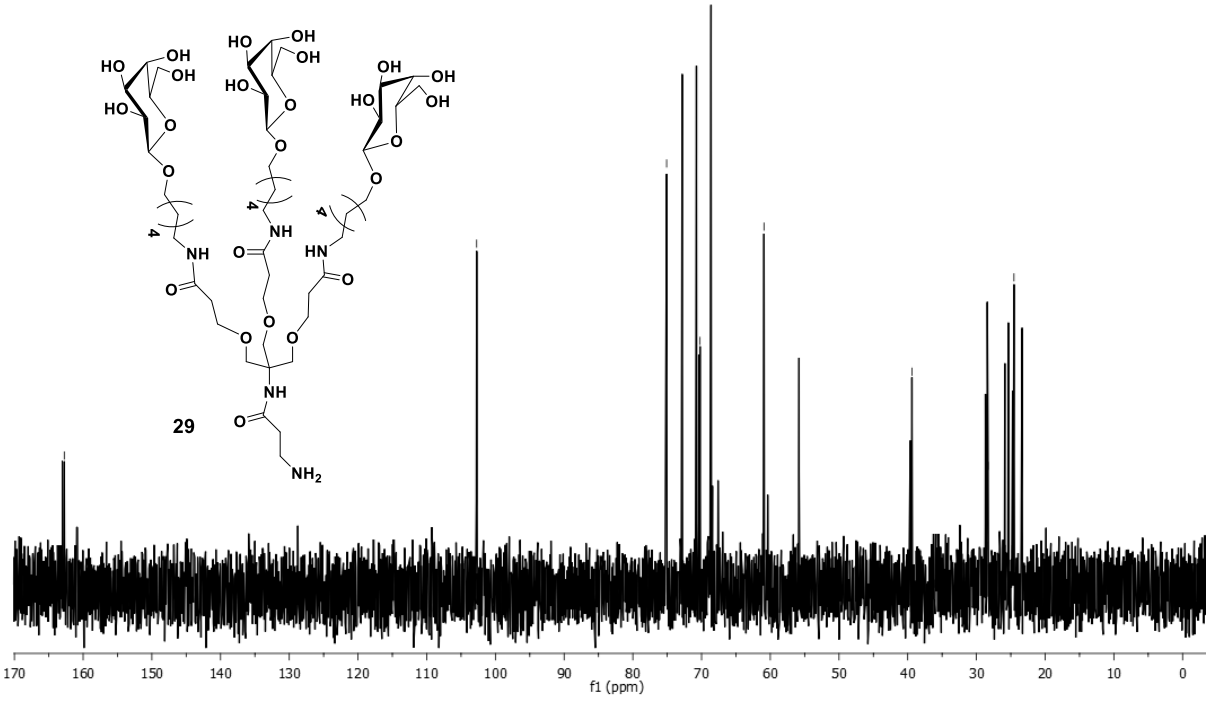
28.30
25.80
25.26
24.80
24.54
23.40


29
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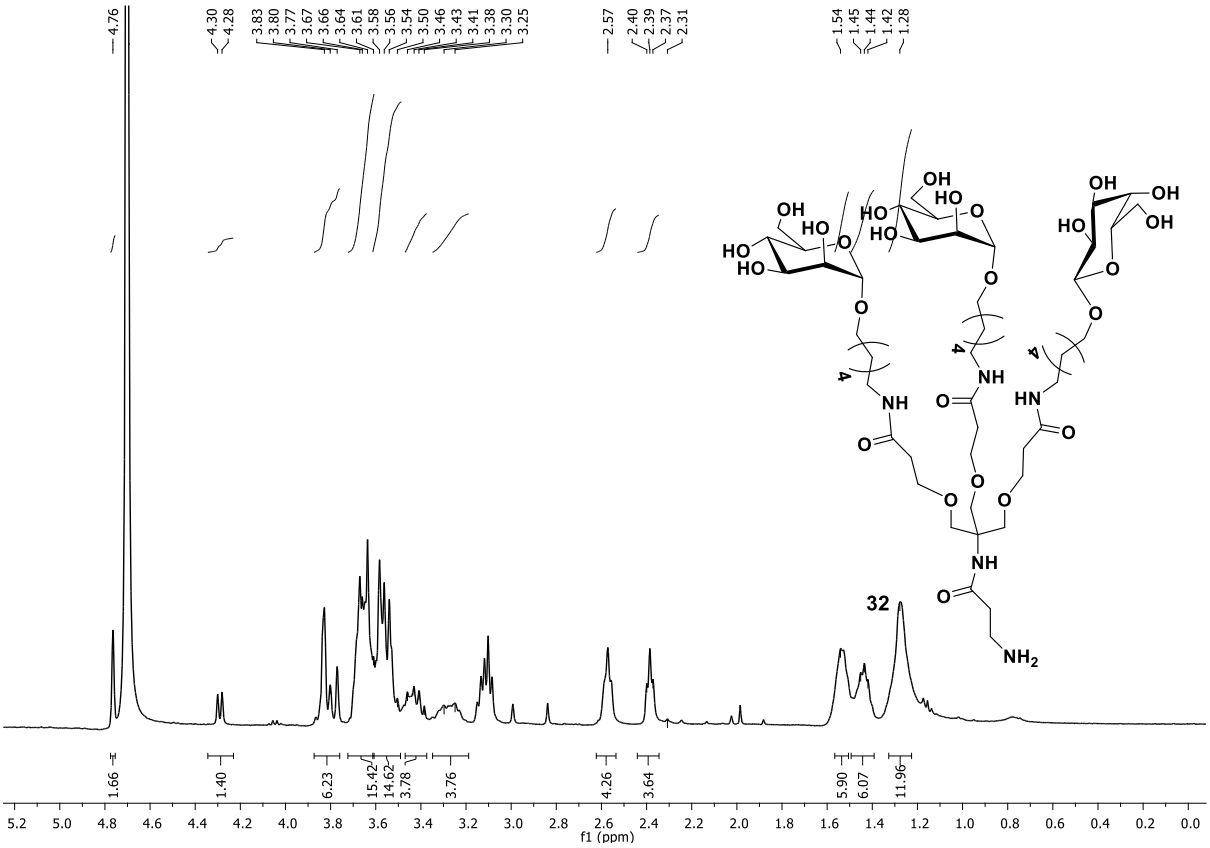
f1 (ppm)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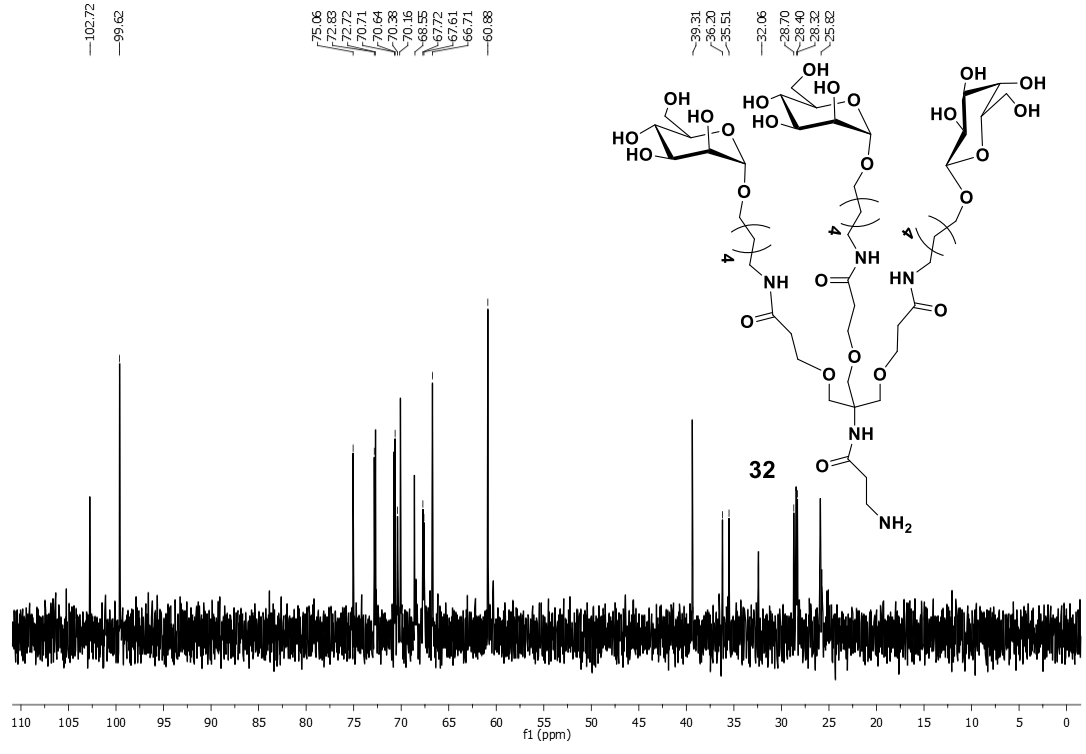
3.67
3.66
3.64
3.61
3.58
3.56
3.54
3.50
3.46
3.43
3.41
3.38
3.30
3.25
2.57
2.40
2.39
2.37
2.31
1.54
1.45
1.44
1.42
1.28
1.66
1.40
6.23
15.42
14.62
3.78
3.76
4.26
3.64
5.90
6.07
11.96
f1 (ppm)
32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67.61
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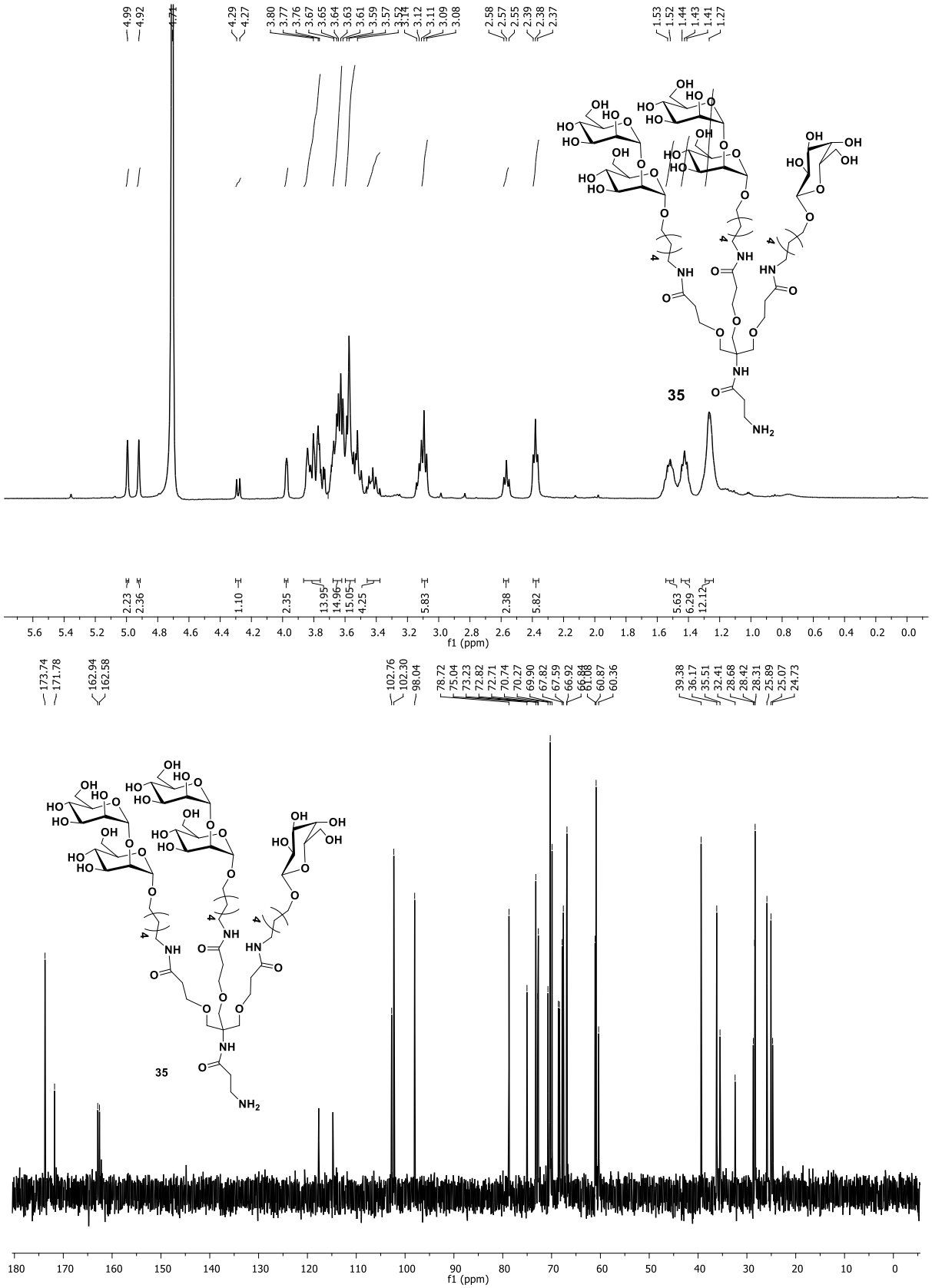
39.31
36.20
35.51
32.06
28.70
28.40
28.32
25.82
f1 (ppm)
32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4.29
4.27
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3.77
3.76
3.67
3.65
3.64
3.63
3.61
3.59
3.57
3.12
3.11
3.09
3.08
2.58
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2.55
2.39
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1.52
1.44
1.43
1.41
1.27
35
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1.10
2.35
13.95
14.96
15.05
4.25
5.83
2.38
5.82
5.63
6.29
12.12
f1 (ppm)
173.74
171.78
162.94
162.58
102.76
102.30
98.04
78.72
75.04
73.23
72.82
72.71
70.74
70.27
69.90
67.82
67.59
66.92
61.08
60.87
60.36
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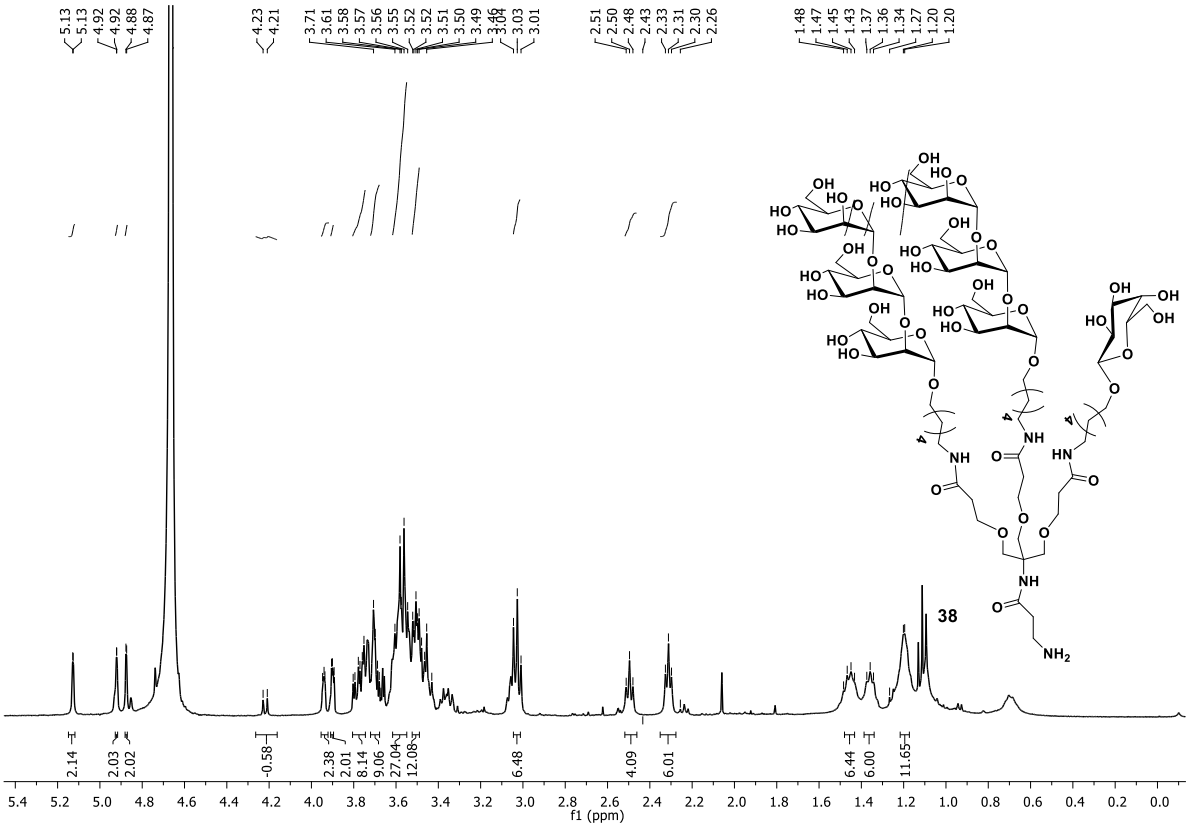
25.89
25.07
24.73
35
f1 (ppm)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3.61
3.58
3.57
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2.33
2.31
2.30
2.26
1.48
1.47
1.45
1.43
1.37
1.36
1.34
1.27
1.20
1.20
38
2.14
2.03
2.02
0.58
2.38
2.01
8.14
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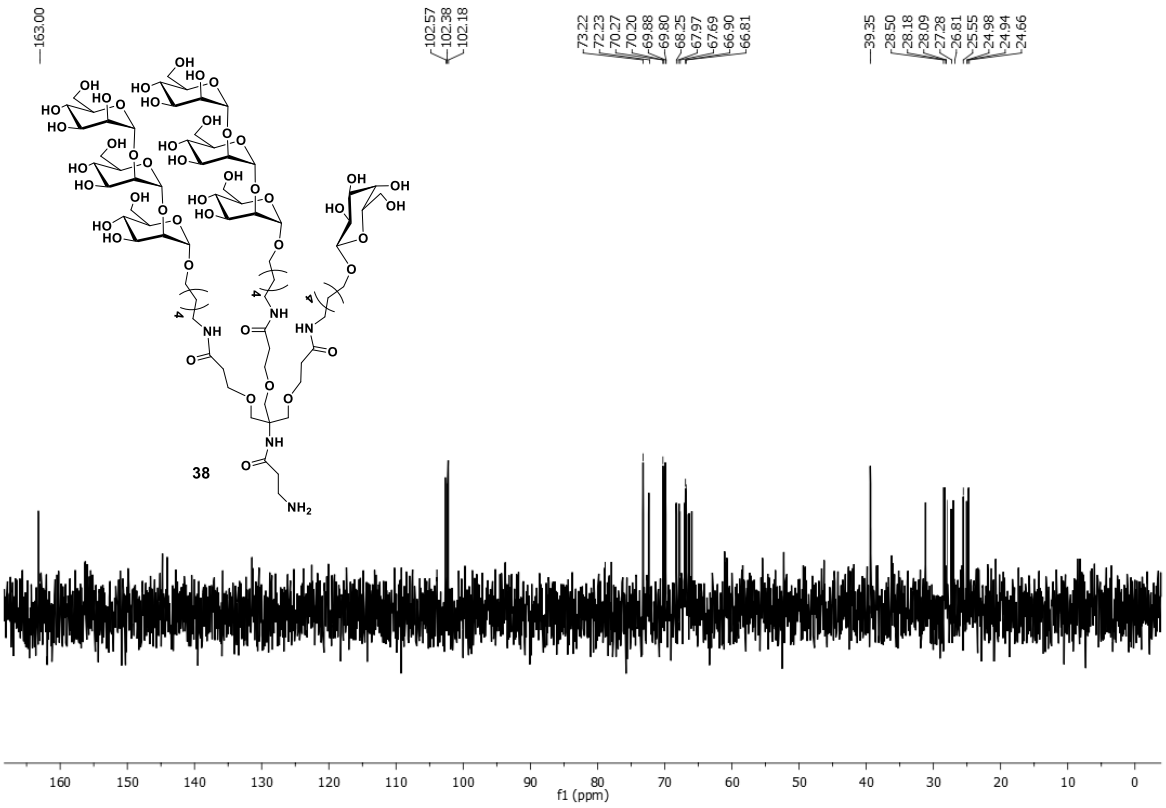
27.04
12.08
6.48
4.09
6.01
6.44
6.00
11.65
f1 (ppm)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69.80
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66.81
39.35
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28.18
28.09
27.28
26.81
25.55
24.98
24.94
24.66
38
f1 (ppm)