

## Decoding glycan protein interactions by a new class of asymmetric *N*-glycans

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## I. Materials and enzymes

EndoM N175Q from *Mucor hiemalis* (EndoM N175Q)<sup>1</sup> was expressed and purified as previously described. Two recombinant hemoagglutinins (HAs) of A/New York/18/2009 (H1N1) and HA of A/Czech Republic/32/2011 (H1N1) were obtained from the BEI Resources Repository (NR19941 and NR42486) (<https://www.beiresources.org>). Other reagents were purchased from Sigma.

## II. General methods for EndoM N175Q treatment

The EndoM N175Q reaction mixtures contain 50 mM PBS (pH 6.5), 10 nM glycan as acceptor, 50 nM glycan oxazoline as donor, and 50  $\mu$ g EndoM N175Q. Reactions were incubated at 30 °C for 1 h, and were analyzed by HPLC-HILIC-ELSD (amide column, 4.6 mm  $\times$  250 mm under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.2; solvent B: acetonitrile; flow rate: 1 mL/min; B%: 65-50% within 25 min)). Then, the target glycans were purified by HPLC-HILIC-UV<sub>210nm</sub> using a semi-preparative amide column (130 Å, 5  $\mu$ m, 10 mm  $\times$  250 mm) according to the retention time shown on HPLC-HILIC-ELSD chromatograph. The gradient running condition is the same as that for HPLC-HILIC-ELSD analysis except for the flow rate is 4 mL/min for purification.

## III. General methods for HPLC analysis and purification of *N*-glycans

### A) General methods for HILIC-ELSD analysis of *N*-glycans

Column: Waters XBridge BEH amide column, 130 Å, 5  $\mu$ m, 4.6 mm  $\times$  250 mm

Solvent A: 100 mM ammonium formate, pH 3.2

Solvent B: Acetonitrile

Temperature: 40 °C

Gradient elution:	Time	B%	Flow rate (mL/min)
	0	65	1
	25	50	1
	26	0	0.5
	27	0	0.5
	28	65	1
	30	65	1

Monitor: Evaporative light scattering detector, 60 °C (Shimadzu ELSD-LTII)

### B) General methods for HILIC-UV<sub>210nm</sub> purification of *N*-glycans (1a-9a, 2b, 6b, 7b)

Column: Waters XBridge BEH amide column, 130 Å, 5  $\mu$ m, 4.6 mm  $\times$  250 mm

Solvent A: 100 mM ammonium formate, pH 3.2

Solvent B: Acetonitrile

Temperature: 40 °C

Gradient elution:	Time	B%	Flow rate (mL/min)
	0	65	1
	25	50	1
	26	0	0.5
	27	0	0.5
	28	65	1
	30	65	1

Monitor: A<sub>210nm</sub>

### C) Methods for HILIC-UV<sub>210nm</sub> purification of *N*-glycans (10b)

Column: Waters XBridge BEH amide column, 130 Å, 5 µm, 10 mm × 250 mm

Solvent A: 100 mM ammonium formate, pH 3.2

Solvent B: Acetonitrile

Temperature: 40 °C

Gradient elution:	Time	B%	Flow rate (mL/min)
	0	65	4
	25	50	4
	26	0	2
	27	0	2
	28	65	4
	30	65	4

Monitor: A<sub>210nm</sub>

## IV. General methods for mass spectrometry analyses

### A) Method of ESI-MS analyses

ESI-MS experiments were performed on an LTQ-Orbitrap Elite mass spectrometer (Thermo Fisher) equipped with EASY-spray source and nano-LC UltiMate 3000 high performance liquid chromatography system (Thermo Fisher). Samples were transmitted into MS with a silica column. LTQ-Orbitrap Elite mass spectrometer was operated in the data dependent mode. A full-scan survey MS experiment (*m/z* range from 375 to 1600; automatic gain control target, 1,000,000 ions; resolution at 400 *m/z*, 240,000; maximum ion accumulation time, 200 ms) was acquired by the Orbitrap mass spectrometer.

### B) Method of MALDI-TOF MS analyses

MALDI-TOF MS analyses were performed on UltrafleXtreme MALDI TOF/TOF Mass Spectrometer (Bruker). Scan range of MS<sup>1</sup> was set according to the molecular weight of *N*-glycans, and reflector mode was used for *N*-glycan analysis. Mass spectra were obtained in both positive and negative extraction mode with the following voltage settings: ion source 1 (19.0 kV), ion source 2 (15.9 kV), and lens (9.3 kV). The reflector voltage was set to 20 kV. The laser was pulsed at 7 Hz and the pulsed ion extraction time was set at 400 ns. The laser power was kept in the range of 25–40%.

## V. Synthesis of acceptor *N*-glycans, *N*-glycan oxazolines (as donor) and deacetylation of asymmetric *N*-glycan products

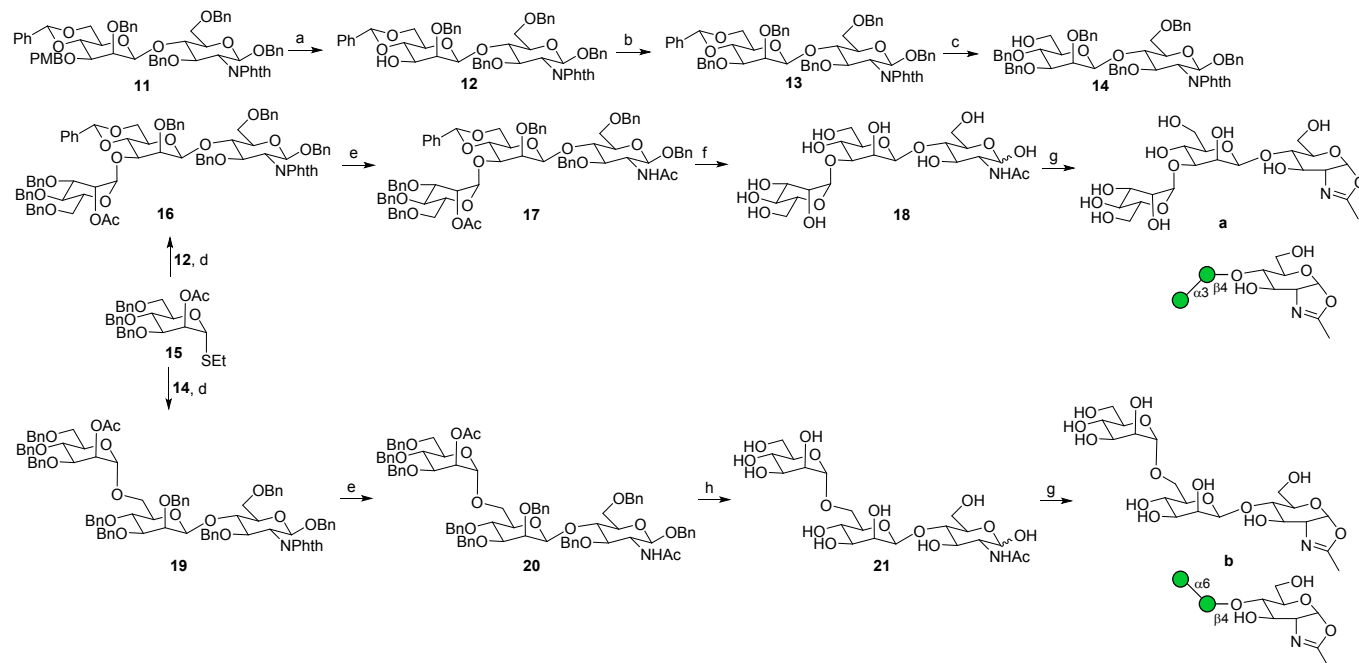
### A) Synthesis of acceptor *N*-glycans

The acceptor *N*-glycans were prepared as previously described.<sup>2</sup>

### B) Synthesis of *N*-glycan oxazolines

**Materials:** All chemicals were purchased as reagent grade and used without further purification. Anhydrous dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), acetonitrile (CH<sub>3</sub>CN), tetrahydrofuran (THF), *N,N*-dimethyl formamide (DMF), toluene, and methanol (MeOH) were purchased from a commercial source without further distillation. Pulverized Molecular Sieves MS-4 Å (Aldrich) for glycosylation was activated by heating at 350 °C for 3 h. Reactions were monitored by

analytical thin-layer chromatography (TLC) in EM silica gel 60 F254 plates and visualized under UV (254 nm) and/or by staining with acidic ceric ammonium molybdate or *p*-anisadehyde. Flash chromatography was performed on silica gel (Merck) of 40-63 $\mu$ m particle size and P2 gel (Biorad).  $^1\text{H}$  NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz), and Bruker AVANCE 500 (500 MHz) spectrometer at 25  $^\circ\text{C}$ . All  $^1\text{H}$  Chemical shifts (in ppm) were assigned according to  $\text{CDCl}_3$  ( $\delta = 7.24$  ppm) and  $\text{D}_2\text{O}$  ( $\delta = 4.79$  ppm).  $^{13}\text{C}$  NMR spectra were obtained with Bruker AVANCE 600 spectrometer and calibrated with  $\text{CDCl}_3$  ( $\delta = 77.00$  ppm). Coupling constants ( $J$ ) are reported in hertz (Hz). Splitting patterns are described using the following abbreviations: s, singlet; brs, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet.  $^1\text{H}$  NMR spectra are reported in the following order: chemical shift, multiplicity, coupling constant(s), and number(s) of protons. All NMR signals were assigned on the basis of  $^1\text{H}$  NMR, COSY, HSQC, HMQC, and  $^{13}\text{C}$  NMR experiments. High resolution MALDI mass spectra were recorded on a Bruker Ultraflex extreme spectrometer.



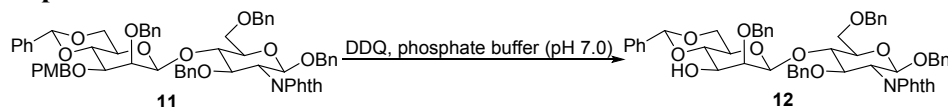
**Scheme S1.** Synthesis of *N*-glycan oxazolines.

Reagents and conditions: a: DDQ, phosphate buffer (pH 7.0), DCM, 89%; b: BnBr, NaH, DMF, 90%; c:  $\text{PhBCl}_2$ ,  $\text{Et}_3\text{SiH}$ , DCM,  $-78$   $^\circ\text{C}$ , 92%; d: NIS, AgOTf, 4  $\text{\AA}$  MS, DCM, 0  $^\circ\text{C}$ , **16**: 95%; **19**: 88%; e: (i) ethylenediamine, *n*-butanol, 90  $^\circ\text{C}$ , 10 h; (ii) Py,  $\text{Ac}_2\text{O}$ , over two steps, **17**: 65%; **20**: 63%; f:  $\text{Pd}(\text{OH})_2$ ,  $\text{H}_2$ , MeOH, 90%; g: 2-chloro-1,3-dimethylimidazolium chloride (DMC),  $\text{Et}_3\text{N}$ ,  $\text{H}_2\text{O}$ , quantitative; h: (i) MeONa, MeOH; (ii)  $\text{Pd}(\text{OH})_2$ ,  $\text{H}_2$ , MeOH, over two steps, 85%.

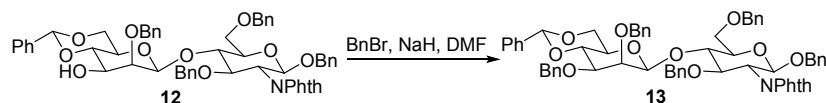
For the synthesis of *N*-glycan oxazolines (**a** and **b**), we adopted the same synthetic strategy to install the mannose residue at the 3- and 6-positions of the  $\beta$ -mannose moiety (**Scheme S1**). We envisaged that disaccharide **11** containing a crucial  $\beta$ -mannoside would be a versatile precursor for the synthesis of *N*-glycan oxazolines, which can transform to disaccharide acceptors **12** and **14** by sequential introducing and removal of the protecting groups. The versatile precursor disaccharide **11**<sup>3</sup> and donor fragment **15** were prepared as previously reported. To synthesize oxazoline **a**, thioether donor **15** was stereoselectively installed onto C3-hydroxyl of disaccharide **12** in the presence of *N*-iodosuccinimide (NIS)/AgOTf with a yield of 95%. The two phthalimides of **16** were then converted into acetamides in two steps by treatment with hydrazine hydrate followed by acetylation with  $\text{Ac}_2\text{O}$ /pyridine to afford **17**, which was subject to the removal of acetyl group by MeONa/MeOH and the global deprotection of Bn and benzylidene acetal by catalytic hydrogenolysis with  $\text{Pd}(\text{OH})_2/\text{H}_2$  in MeOH/ $\text{H}_2\text{O}$  (10:1) to give the trisaccharide **36**. Following Wang's method,<sup>4</sup> treatment of **18** with an excess of 2-chloro-1,3-dimethylimidazolium chloride (DMC) and  $\text{Et}_3\text{N}$  in water afforded the desired sugar oxazoline **a** in quantitative yield, which was readily purified by gel

filtration on a Sephadex G-15 column. Similarly, sugar oxazoline **b** was synthesized by installing **15** onto C6-hydroxyl of  $\beta$ -man of **14**, and then following the previous procedure to provide the desired sugar oxazoline **b**.

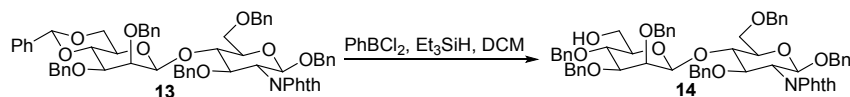
## Experimental Procedures



**Benzyl 2-O-benzyl-4,6-O-benzylidene- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-phthalimido- $\beta$ -D-glucopyranoside (12):** The compound **11** (1.67 g, 1.61 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  (30 mL) and phosphate buffer (6 mL, pH 7), and DDQ (729 mg, 3.21 mmol) was added at 0 °C. The reaction mixture was allowed to warm to room temperature for 3 h before it was quenched with saturated aqueous  $\text{NaHCO}_3$ , washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , and concentrated. The residue was purified by chromatography on a silica gel column to afford the desired product **12** (1.36 g, 89%).  $[\alpha]_{\text{D}}^{20}$  5.68 (c 1,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.68-7.71 (m, 2 H), 7.52-7.54 (m, 2 H), 7.35-7.46 (m, 14 H), 7.22-7.24 (m, 1 H), 7.09-7.15 (m, 5 H), 6.98-7.01 (m, 2 H), 6.89-6.93 (m, 3 H), 5.50 (s, 1 H), 5.20-5.22 (m, 1 H), 5.06 (d,  $J$  = 11.6 Hz, 1 H), 4.93 (d,  $J$  = 12.4 Hz, 1 H), 4.85 (d,  $J$  = 12.4 Hz, 1 H), 4.84 (d,  $J$  = 12.1 Hz, 1 H), 4.75 (d,  $J$  = 11.6 Hz, 1 H), 4.70 (s, 1 H), 4.58 (d,  $J$  = 12.4 Hz, 1 H), 4.56 (d,  $J$  = 12.0 Hz, 1 H), 4.49 (d,  $J$  = 12.3 Hz, 1 H), 4.31-4.33 (m, 2 H), 4.27 (dd,  $J$  = 10.6, 5.0 Hz, 1 H), 4.14-4.20 (m, 1 H), 3.78-3.80 (m, 2 H), 3.74-3.75 (m, 2 H), 3.56-3.61 (m, 3 H), 3.18-3.23 (m, 1 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  167.8, 138.8, 138.3, 137.8, 137.4, 137.2, 133.7, 129.2, 129.1, 128.7, 128.6, 128.3, 128.2, 128.1, 128.0 (2 C), 127.9, 127.8, 127.7 (2 C), 127.0, 126.4, 125.4, 123.3, 102.1, 102.0, 97.5, 79.6, 79.2, 78.9, 77.5, 77.2, 77.1, 76.9, 75.7, 74.8, 74.6, 73.8, 71.0, 70.8, 68.6, 68.5, 67.0, 55.8. MALDI-MS:  $[\text{M}+\text{Na}]^+$   $\text{C}_{55}\text{H}_{53}\text{NO}_{12}\text{Na}$  calcd for 942.3465, found 942.4147.

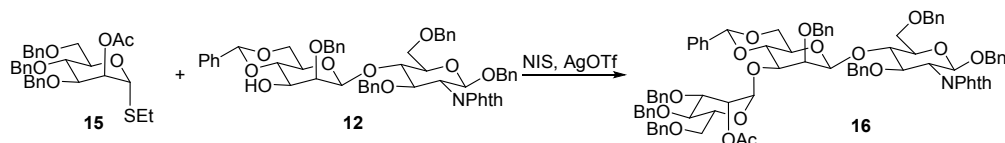


**Benzyl 2,3-O-di-benzyl-4,6-O-benzylidene- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-phthalimido- $\beta$ -D-glucopyranoside (13):** Compound **12** (1 g, 1.09 mmol) was dissolved in dry DMF (15 mL).  $\text{NaH}$  (78 mg, 1.96 mmol),  $\text{Bu}_4\text{NI}$  (36 mg, 0.1 mmol), followed by  $\text{BnBr}$  (194  $\mu\text{L}$ , 1.64 mmol) were added at 0 °C, the reaction mixture was stirred under argon for 4 h. The solvent was evaporated, and the residue was diluted with ethyl acetate and washed with water and a brine solution. After dried over  $\text{Na}_2\text{SO}_4$ , the organic layer was evaporated. The residue was purified on a silica gel column (Hexanes: EtOAc = 5:1) to afford the desired product **13** (989 mg, 90%) as colorless oil.  $[\alpha]_{\text{D}}^{20}$  10.7 (c 1,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.70 (brs, 2 H), 7.52-7.59 (m, 4 H), 7.35-7.43 (m, 18 H), 7.11-7.16 (m, 5 H), 6.98-6.99 (m, 2 H), 6.87-6.91 (m, 3 H), 7.98 (s, 1 H), 5.20 (d,  $J$  = 7.8 Hz, 1 H), 4.91-4.95 (m, 3 H), 4.87 (d,  $J$  = 12.3 Hz, 1 H), 4.81 (d,  $J$  = 12.4 Hz, 1 H), 4.75 (d,  $J$  = 12.0 Hz, 1 H), 4.66 (d,  $J$  = 12.4 Hz, 1 H), 4.63 (d,  $J$  = 8.8 Hz, 1 H), 4.57 (d,  $J$  = 12.4 Hz, 1 H), 4.50 (d,  $J$  = 12.4 Hz, 1 H), 4.48 (d,  $J$  = 12.1 Hz, 1 H), 4.29-4.32 (m, 2 H), 4.26 (dd,  $J$  = 10.4, 4.6 Hz, 1 H), 4.17 (dd,  $J$  = 7.1, 4.3 Hz, 1 H), 4.11 (dd,  $J$  = 9.2, 2.8 Hz, 1 H), 3.82 (d,  $J$  = 2.8 Hz, 1 H), 3.75 (dd,  $J$  = 10.6, 0.8 Hz, 1 H), 3.62-3.66 (m, 2 H), 3.53-3.56 (m, 1 H), 3.49 (dd,  $J$  = 9.8, 2.9 Hz, 1 H), 3.18-3.24 (m, 1 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  167.8, 138.8, 138.7 (2 C), 137.9, 137.7, 137.3, 133.7, 131.7, 129.1, 128.9, 128.6, 128.4 (2 C), 128.3 (2 C), 128.2 (3 C), 128.0, 127.9, 127.8, 127.7, 127.6 (2 C), 127.5, 127.0, 126.2, 123.3, 102.0, 101.4, 97.5, 79.5, 78.8, 78.4, 77.1, 77.0, 75.1, 74.8, 74.7, 73.7, 72.6, 70.8, 68.6 (2 C), 67.4, 55.8. MALDI-MS:  $[\text{M}+\text{Na}]^+$   $\text{C}_{62}\text{H}_{58}\text{NO}_{12}\text{Na}$  calcd for 1032.3935, found 1032.3148.

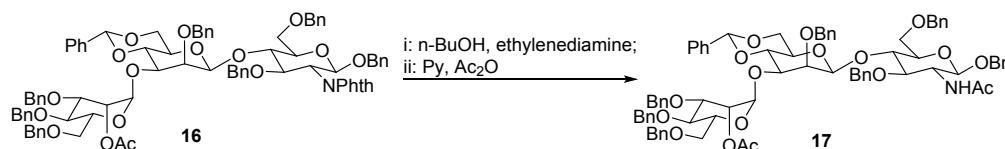


**Benzyl 2,3,4-O-tri-benzyl- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-phthalimido- $\beta$ -D-glucopyranoside (14):** A mixture of compound **13** (300 mg, 0.297 mmol) and 4 Å molecular sieves in dry  $\text{CH}_2\text{Cl}_2$  (20 mL) was stirred at room temperature under argon for 2 h.  $\text{Et}_3\text{SiH}$  (142  $\mu\text{L}$ , 0.892 mmol) and  $\text{PhBCl}_2$  (116  $\mu\text{L}$ , 0.892 mmol) were added at -78 °C. The reaction mixture was stirred at -78 °C for 1 h and quenched upon addition of solid  $\text{NaHCO}_3$  and MeOH. The resulting mixture was filtered. The filtrate was diluted with  $\text{CH}_2\text{Cl}_2$  and washed with

aqueous NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified on a silica gel column (EA: Hex = 1:3) to afford the product **14** (276 mg, 92%). [ $\alpha$ ]<sub>D</sub><sup>20</sup> -4.68 (c 1, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.70 (brs, 2 H), 7.50-7.52 (m, 2 H), 7.28-7.41 (m, 20 H), 7.09-7.11 (m, 5 H), 6.92-6.98 (m, 5 H), 5.23 (d, *J* = 7.9 Hz, 1 H, GlcNAc-H-1), 4.94-4.99 (m, 4 H), 4.88 (d, *J* = 12.4 Hz, 1 H), 4.76 (d, *J* = 12.1 Hz, 1 H), 4.48-4.66 (m, 7 H), 4.30-4.37 (m, 2 H), 4.10 (t, *J* = 9.4 Hz, 1 H), 3.80-3.89 (m, 4 H), 3.74 (dd, *J* = 11.0, 3.3 Hz, 1 H), 3.63-3.67 (m, 1 H), 3.54 (dd, *J* = 11.7, 5.7 Hz, 1 H), 3.44 (dd, *J* = 9.4, 2.7 Hz, 1 H), 3.27-3.31 (m, 1 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  167.9, 138.8, 138.6, 138.4, 137.9, 137.3, 133.8, 131.7, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.7 (3 C), 127.6 (2 C), 127.5, 127.1, 123.3, 101.0, 97.5, 82.6, 78.9, 77.0, 75.8, 75.2, 75.1, 74.9, 74.8, 74.5 (2 C), 73.7, 71.8, 70.8, 68.7, 62.4, 55.8; MALDI-MS: [M+Na]<sup>+</sup> C<sub>62</sub>H<sub>61</sub>NO<sub>12</sub>Na calcd for 1034.4091, found 1034.3687.

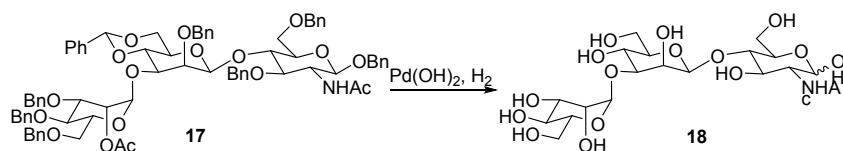


**Benzyl 2-O-acetyl-3,4,6-O-tri-benzyl- $\alpha$ -D-Mannopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl-4,6-O-benzylidene- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-phthalimido- $\beta$ -D-glucopyranoside (**16**):** A mixture of donor **15** (90 mg, 0.167 mmol), acceptor **12** (130 mg, 0.129 mmol) and 4 Å molecular sieves in dry CH<sub>2</sub>Cl<sub>2</sub> was stirred at room temperature under argon for 1 h. NIS (49 mg, 0.219 mmol) and AgOTf (5 mg, 0.019 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h before it was quenched with a few drops of triethylamine. The resulting mixture was filtered. The filtrate was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with 5% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, aqueous NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified on a silica gel column to get the desired title compound **16** (181 mg, 95%) as white foam. [ $\alpha$ ]<sub>D</sub><sup>20</sup> 6.38 (c 1, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.70-7.73 (m, 2 H), 7.55-7.60 (m, 4 H), 7.32-7.50 (m, 28 H), 7.14-7.23 (m, 5 H), 7.05-7.07 (m, 2 H), 6.96-6.99 (m, 3 H), 5.77 (brs, 1 H), 5.64 (s, 1 H), 5.48 (d, *J* = 0.8 Hz, 1 H), 5.28 (d, *J* = 7.8 Hz, 1 H), 4.92-5.05 (m, 5 H), 4.83 (d, *J* = 11.2 Hz, 1 H), 4.79 (d, *J* = 11.9 Hz, 1 H), 4.76 (d, *J* = 11.8 Hz, 1 H), 4.74 (d, *J* = 8.9 Hz, 1 H), 4.51-4.66 (m, 6 H), 4.40 (t, *J* = 4.4 Hz, 2 H), 4.32 (dd, *J* = 10.4, 4.7 Hz, 1 H), 4.19-4.23 (m, 2 H), 4.12-4.15 (m, 1 H), 3.99-4.00 (m, 2 H), 3.94-3.97 (m, 2 H), 3.88-3.91 (m, 1 H), 3.80 (t, *J* = 10.5 Hz, 2 H), 3.65-3.69 (m, 2 H), 3.58-3.61 (m, 2 H), 3.19-3.25 (m, 1 H), 2.22 (s, 3 H, Ac); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  170.1, 138.9, 138.7, 138.5, 138.3, 138.0, 137.9, 137.5, 137.3, 133.8, 131.8, 128.9, 128.8, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0 (2 C), 127.9, 127.8 (3 C), 127.1, 126.2, 123.3, 101.7, 101.2, 98.9, 97.6, 79.1, 78.8, 78.4, 78.1, 77.6, 75.7, 75.5, 75.2, 75.0, 74.6, 74.4, 73.7, 73.6, 72.5, 71.7, 70.9 (2 C), 69.3, 68.5, 68.3, 67.1, 55.9, 21.1; MALDI-MS: [M+Na]<sup>+</sup> C<sub>84</sub>H<sub>83</sub>NO<sub>18</sub>Na calcd for 1416.5508, found 1416.5089.

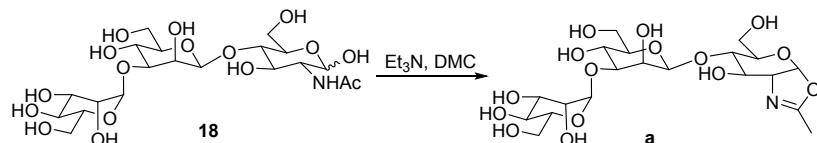


**Benzyl 2-O-acetyl-3,4,6-O-tri-benzyl- $\alpha$ -D-Mannopyranosyl-(1 $\rightarrow$ 3)-2-O-benzyl-4,6-O-benzylidene- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-acetamido- $\beta$ -D-glucopyranoside (**17**):** The compound **16** (150 mg, 0.101 mmol) was dissolved in *n*BuOH at room temperature, followed by addition of ethylenediamine (*n*BuOH: ethylenediamine = 2:1). After being stirred at 90 °C for 12 h, the mixture was evaporated *in vacuo* to give a residue for the next step without further purification. To a solution of the residue in pyridine was added Ac<sub>2</sub>O. After being stirred at room temperature for 12 h, the solution was diluted with EtOAc and washed with aqueous HCl (1 M), saturated aqueous NaHCO<sub>3</sub>, and brine solution. The organic layer was dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography to give compound **17** (85 mg, 65% over two steps). [ $\alpha$ ]<sub>D</sub><sup>20</sup> 1.60 (c 1, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  7.53-7.54 (m, 2 H), 7.26-7.46 (m, 40 H), 5.99 (d, *J* = 8.0 Hz, 1 H, NH), 5.70 (brs, 1 H), 5.61 (s, 1 H), 5.42 (s, 1 H), 5.03 (d, *J* = 6.7 Hz, 1 H), 4.99 (brs, 1 H), 4.96 (t, *J* = 5.0 Hz, 2 H), 4.87 (d, *J* = 12.2 Hz, 1 H), 4.84 (d, *J* = 12.5 Hz, 1 H), 4.77 (d, *J* = 11.2 Hz, 1 H), 4.72 (d, *J* = 12.5 Hz, 1 H), 4.66-4.69 (m, 3 H), 4.64 (d, *J* = 7.6 Hz, 1 H), 4.55-4.58 (m, 2 H), 4.51 (d, *J* = 11.0 Hz, 1 H), 4.49 (d, *J* = 11.9 Hz, 1 H), 4.12-4.20 (m, 3 H), 4.00-4.06 (m, 2 H), 3.88-3.97 (m, 4 H), 3.78-3.86 (m, 3 H), 3.62-3.75 (m, 4 H), 3.15-3.19 (m, 1 H), 2.18 (s, 3 H, Ac), 1.79 (s, 3 H, Ac); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  170.4, 170.1, 138.9, 138.6, 138.3, 138.1, 137.9, 137.7, 137.3, 128.9, 128.6, 128.5 (2 C), 128.4, 128.3, 128.2 (2 C), 128.1,

128.0, 127.9 (2 C), 127.8 (2 C), 127.7, 127.6, 126.1, 101.5, 101.2, 99.3, 98.8, 78.8, 78.5, 78.0, 77.8, 77.5, 75.7, 75.4, 75.3, 75.2, 74.3, 73.7, 73.6, 72.4, 71.7, 70.8, 69.2, 69.1, 68.5, 68.2, 67.6, 55.0, 23.2, 21.1; MALDI-MS:  $[M+Na]^+$   $C_{78}H_{83}NO_{17}Na$  calcd for 1328.5559, found 1328.6794.

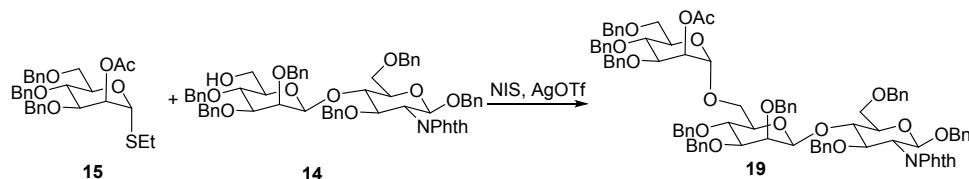


**$\alpha$ -D-Mannopyranosyl-(1 $\rightarrow$ 3)-O- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-2-deoxy-acetamido- $\beta$ -D-glucopyranoside (18):** Pd(OH)<sub>2</sub> on carbon was added to a solution of compound **17** (70 mg, 0.054 mmol) in MeOH/H<sub>2</sub>O (10/1). The mixture was stirred under 1 atmosphere of hydrogen. After being stirred for 24 h, the mixture was filtered through a PTFE syringe filter and concentrated *in vacuo*. The residue was purified by Bio-Gel P-2 (BIO-RAD) column chromatography using water as eluent. The product was then lyophilized to get compound **18** (28 mg, 90%) as white powder.  $[\alpha]_D^{20}$  6.78 (c 0.5, H<sub>2</sub>O); <sup>1</sup>H NMR (D<sub>2</sub>O, 400 MHz):  $\delta$  5.15-5.17 (m, 1 H), 5.12 (s, 1 H), 5.06 (s, 0.5 H), 4.74 (s, 1 H), 4.18-4.21 (m, 1 H), 4.03-3.06 (m, 1 H), 3.78-3.90 (m, 6 H), 3.56-3.75 (m, 9 H), 3.40-3.43 (m, 1 H), 2.12 (s, 1.5 H, Ac), 2.00 (s, 3 H, Ac); <sup>13</sup>C NMR (D<sub>2</sub>O, 100 MHz):  $\delta$  174.4, 173.2, 102.4, 99.9, 99.4, 94.9, 90.5, 80.5 (2 C), 79.3, 78.9, 76.1, 74.6, 73.4, 72.3, 72.2, 70.3, 70.2, 70.1, 70.0 (4 C), 69.1, 68.7, 66.9 (2 C), 65.8 (2 C), 61.1, 60.8, 60.2, 60.1, 56.1, 53.6, 21.8, 20.4; ESI-MS:  $[M+Na]^+$   $C_{20}H_{35}NO_{16}Na$  calcd for 568.1854, found 568.1810.



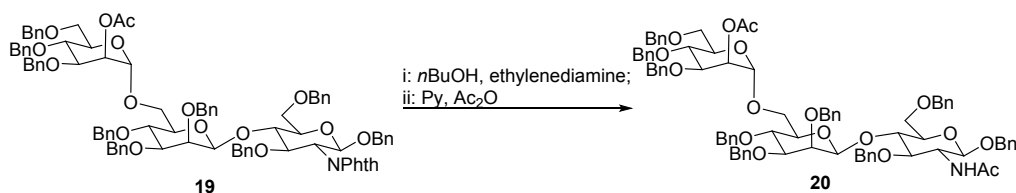
#### Synthesis of the $\alpha$ 2, 6 oxazoline (a)

Compound **18** (10 mg, 0.018 mmol) and 2-chloro-1,3-dimethylimidazolium chloride (DMC, 45 mg, 0.264 mmol) were dissolved in 0.2 mL of water at 4 °C. Then Et<sub>3</sub>N (108  $\mu$ L, 0.774 mmol) was added and the mixture was shaken at 4 °C for 30 min. The residue was subject to gel filtration on a Sephadex G-15 column. The column was eluted by water containing 0.05% Et<sub>3</sub>N. The fractions containing the glycan-oxazoline were combined. The fractions were mixed with an aqueous solution of NaOH (0.1 M, 10  $\mu$ L) and were then lyophilized to give the glycan-oxazoline (**a**) as a white powder (9.5 mg, quantitative yield). The inclusion of a catalytic amount of NaOH ensures an alkaline situation for the oxazoline for its stability after freeze-dry. <sup>1</sup>H NMR (D<sub>2</sub>O, 400 MHz):  $\delta$  6.07 (d,  $J$  = 7.4 Hz, 1 H, H-1 of oxazoline), 5.08 (s, 1 H, H-1 of  $\alpha$ -Man), 4.72 (s, 1 H, H-1 of  $\beta$ -Man), 4.37 (s, 1 H), 4.17 (d,  $J$  = 6.7 Hz, 1 H), 4.12 (s, 1 H), 4.04 (s, 1 H), 3.86-3.95 (m, 3 H), 3.59-3.75 (m, 9 H), 3.36-3.43 (m, 2 H), 2.05 (s, 3 H, CH<sub>3</sub> of oxazoline); <sup>13</sup>C NMR (D<sub>2</sub>O, 100 MHz):  $\delta$  168.6, 102.4, 100.9, 99.8, 80.3, 77.3, 76.2, 73.3, 70.9, 70.2, 70.1, 70.0, 69.1, 66.8, 66.0, 65.0, 61.7, 61.0, 60.9, 12.9; ESI-MS:  $[M+Na]^+$   $C_{20}H_{33}NO_{15}Na$  calcd for 550.1748, found 550.1790.

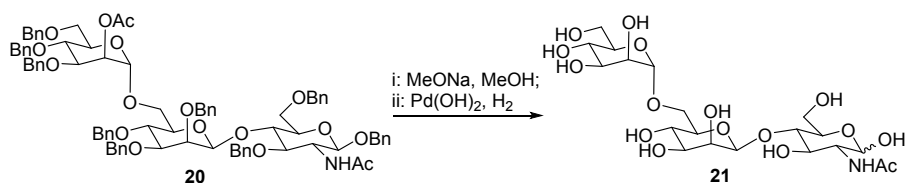


**Benzyl 2-O-acetyl-3,4,6-O-tri-benzyl- $\alpha$ -Mannopyranosyl-(1 $\rightarrow$ 6)-2,3,4-O-tri-benzyl- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-phthalimido- $\beta$ -D-glucopyranoside (19):** A mixture of donor **15** (103 mg, 0.192 mmol), acceptor **14** (150 mg, 0.148 mmol) and 4 Å molecular sieves in dry CH<sub>2</sub>Cl<sub>2</sub> was stirred at room temperature under argon for 1 h. NIS (56 mg, 0.252 mmol) and AgOTf (8 mg, 0.030 mmol) were added at 0 °C. The reaction mixture was stirred at 0 °C for 0.5 h before it was quenched with a few drops of triethylamine. The resulting mixture was filtered. The filtrate was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with 5% Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, aqueous NaHCO<sub>3</sub>, brine, dried over Na<sub>2</sub>SO<sub>4</sub>, and concentrated. The residue was purified on a silica gel column to get the desired title compound **19** (193 mg, 88%) as white foam.  $[\alpha]_D^{20}$  -5.92 (c 1, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz):  $\delta$  7.68 (brs, 2 H), 7.55-7.57 (m, 2

H), 7.19-7.42 (m, 36 H), 7.08-7.12 (m, 4 H), 6.97-7.00 (m, 2 H), 6.71-6.75 (m, 3 H), 5.46 (brs, 1 H), 5.20 (d,  $J = 7.8$  Hz, 1 H), 4.99-5.03 (m, 3 H), 4.96 (t,  $J = 4.0$  Hz, 1 H), 4.89 (d,  $J = 11.3$  Hz, 1 H), 4.85 (d,  $J = 12.6$  Hz, 1 H), 4.75 (d,  $J = 12.1$  Hz, 1 H), 4.64-4.70 (m, 3 H), 4.50-4.59 (m, 7 H), 4.46 (d,  $J = 12.1$  Hz, 1 H), 4.29-4.33 (m, 3 H), 4.19 (dd,  $J = 14.3, 7.2$  Hz, 1 H), 4.14 (t,  $J = 8.0$  Hz, 1 H), 3.87-4.03 (m, 5 H), 3.73-3.84 (m, 5 H), 3.60-3.63 (m, 2 H), 3.40-3.47 (m, 2 H), 1.98 (s, 3 H, Ac);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  171.2, 170.0, 139.0, 138.9, 138.7, 138.6, 138.5, 138.3, 138.1 (2 C), 137.3, 133.5, 131.8, 128.6, 128.5 (2 C), 128.4 (2 C), 128.3 (2 C), 128.2 (2 C), 127.9 (2 C), 127.8 (4 C), 127.7 (3 C), 127.6, 127.5 (3 C), 127.4, 127.0, 123.1, 102.0, 98.4, 97.5, 82.8, 79.9, 77.9, 76.7, 75.0, 74.9, 74.8, 74.4, 74.2, 74.0, 73.6, 73.4, 71.8, 71.7, 71.3, 70.8, 68.9, 68.4, 67.1, 60.4, 55.8, 20.9; MALDI-MS:  $[\text{M}+\text{Na}]^+ \text{C}_{91}\text{H}_{91}\text{NO}_{18}\text{Na}$  calcd for 1508.6134, found 1508.5891.



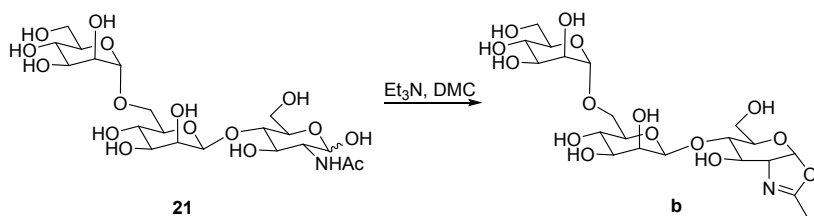
**Benzyl 2-O-acetyl-3,4,6-O-tri-benzyl- $\alpha$ -Mannopyranosyl-(1 $\rightarrow$ 6)-2,3,4-O-tri-benzyl- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-3,6-O-di-benzyl-2-deoxy-acetamido- $\beta$ -D-glucopyranoside (20):** The compound **19** (160 mg, 0.108 mmol) was dissolved in *n*BuOH at room temperature, followed by addition of ethylenediamine (*n*BuOH: ethylenediamine = 2:1). After being stirred at 90 °C for 12 h, the mixture was evaporated *in vacuo* to give a residue for the next step without further purification. To a solution of the residue in pyridine was added  $\text{Ac}_2\text{O}$ . After being stirred at room temperature for 12 h, the solution was diluted with EtOAc and washed with aqueous HCl (1 M), saturated aqueous  $\text{NaHCO}_3$ , and brine solution. The organic layer was dried over  $\text{Na}_2\text{SO}_4$ , filtered, and evaporated *in vacuo* to give a residue, which was purified by silica gel column chromatography to give compound **20** (95 mg, 63% over two steps).  $[\alpha]_{\text{D}}^{20} -3.60$  (c 1,  $\text{CH}_2\text{Cl}_2$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ , 400 MHz):  $\delta$  7.47-7.48 (m, 2 H), 7.32-7.40 (m, 41 H), 7.18-7.19 (m, 2 H), 5.46 (s, 1 H), 5.45 (d,  $J = 9.4$  Hz, 1 H), 5.06 (d,  $J = 7.0$  Hz, 1 H), 4.92-5.01 (m, 5 H), 4.86 (d,  $J = 10.9$  Hz, 1 H), 4.59-4.69 (m, 6 H), 4.53-4.56 (m, 3 H), 4.52 (d,  $J = 12.1$  Hz, 1 H), 4.47 (d,  $J = 10.9$  Hz, 1 H), 4.40 (d,  $J = 12.1$  Hz, 1 H), 3.37 (d,  $J = 12.7$  Hz, 1 H), 4.17-4.21 (m, 1 H), 4.11 (t,  $J = 7.8$  Hz, 1 H), 3.97-3.99 (m, 2 H), 3.90-3.92 (m, 1 H), 3.80-3.88 (m, 5 H), 3.73-3.76 (m, 2 H), 3.62 (dd,  $J = 10.9, 2.3$  Hz, 1 H), 3.45-3.54 (m, 3 H), 3.28 (d,  $J = 10.8$  Hz, 1 H), 2.13 (s, 3 H, Ac), 1.60 (s, 3 H, Ac);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ , 100 MHz):  $\delta$  170.5, 170.3, 139.3, 138.9, 138.7, 138.5, 138.4, 138.2 (2 C), 138.1, 138.0, 128.6, 128.5, 128.4 (2 C), 128.3 (3 C), 128.2, 128.1 (2 C), 127.9, 127.8 (2 C), 127.7 (2 C), 127.6 (2 C), 127.5 (2 C), 127.3, 100.7, 99.4, 97.8, 82.9, 78.5, 77.0, 75.3, 75.0, 74.9, 74.7, 74.5, 73.6, 73.3, 71.8, 71.7, 71.5, 70.9, 69.5, 69.0, 68.7, 67.1, 60.5, 55.7, 23.3, 21.2; MALDI-MS:  $[\text{M}+\text{Na}]^+ \text{C}_{85}\text{H}_{91}\text{NO}_{17}\text{Na}$  calcd for 1420.6185, found 1420.5988.



**$\alpha$ -D-Mannopyranosyl-(1 $\rightarrow$ 6)-O- $\beta$ -D-Mannopyranosyl-(1 $\rightarrow$ 4)-2-deoxy-acetamido- $\beta$ -D-glucopyranoside (21):** Compound **20** (80 mg, 0.057 mmol) was dissolved in MeOH, and NaOMe in MeOH was added until pH was 10. After stirring at room temperature for 2 h, the solution was neutralized with ion-exchange resin ( $\text{H}^+$ ), and then filtered. The residue was dissolved in MeOH/ $\text{H}_2\text{O}$  (10/1), and  $\text{Pd}(\text{OH})_2$  on carbon was added to the solution. The mixture was stirred under 1 atmosphere of hydrogen. After being stirred for 24 h, the mixture was filtered through a PTFE syringe filter and concentrated *in vacuo*. The residue was purified by Bio-Gel P-2 (BIO-RAD) column chromatography using water as eluent. The product was then lyophilized to get compound **21** (31 mg, 85% over two steps) as white powder.  $[\alpha]_{\text{D}}^{20} -2.78$  (c 0.5,  $\text{H}_2\text{O}$ );  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 400 MHz):  $\delta$  5.16 (s, 0.6 H), 4.86 (s, 1 H), 4.72 (s, 1 H), 4.67 (d, 1 H,  $J = 5.0$  Hz, 1 H), 4.03 (s, 1 H), 3.80-3.90 (m, 6 H), 3.75-3.77 (m, 2 H), 3.54-3.59 (m, 9 H), 2.00 (s, 3 H, Ac);  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 100 MHz):  $\delta$  174.6, 100.5, 100.4, 99.6, 94.8, 90.4, 80.1, 79.8, 74.4, 74.3, 72.7, 72.3, 70.4 (2 C), 70.3, 70.2,



69.8 (2 C), 69.1, 66.8, 66.2, 66.1, 60.9, 60.2, 60.1, 56.0, 53.6, 22.2, 21.9; ESI-MS:  $[M+Na]^+$   $C_{20}H_{35}NO_{16}Na$  calcd for 568.1854, found 568.1790.



### Synthesis of the $\alpha$ 2,6 oxazoline (**b**)

Compound **21** (10 mg, 0.018 mmol) and 2-chloro-1,3-dimethylimidazolium chloride (DMC, 45 mg, 0.264 mmol) were dissolved in 0.2 mL of water at 4 °C. Then  $Et_3N$  (108  $\mu$ L, 0.774 mmol) was added and the mixture was shaken at 4 °C for 30 min. The residue was subject to gel filtration on a Sephadex G-15 column. The column was eluted by water containing 0.05%  $Et_3N$ . The fractions containing the glycan-oxazoline were combined. The fractions were mixed with an aqueous solution of NaOH (0.1 M, 10  $\mu$ L) and were then lyophilized to give the glycan-oxazoline (**b**) as a white powder (9.5 mg, quantitative yield). The inclusion of a catalytic amount of NaOH ensures an alkaline situation for the oxazoline for its stability after freeze-dry.  $^1H$  NMR ( $D_2O$ , 400 MHz):  $\delta$  6.10 (d,  $J = 7.3$  Hz, 1 H, H-1 of oxazoline), 4.95 (s, 1 H, H-1 of  $\alpha$ -Man), 4.73 (s, 1 H, H-1 of  $\beta$ -Man), 4.38 (dd,  $J = 2.9, 1.9$  Hz, 1 H), 4.18-4.21 (m, 1 H), 4.01 (dd,  $J = 3.4, 1.7$  Hz, 1 H), 3.99 (d,  $J = 1.2$  Hz, 1 H), 3.93-3.95 (m, 1 H), 3.90-3.92 (m, 1 H), 3.85-3.89 (m, 2 H), 3.76-3.79 (m, 2 H), 3.73-3.74 (m, 1 H), 3.68-3.70 (m, 2 H), 3.62-3.66 (m, 3 H), 3.53-3.57 (m, 1 H), 3.41-3.45 (m, 1 H), 2.07 (s, 3 H,  $CH_3$  of oxazoline);  $^{13}C$  NMR ( $D_2O$ , 100 MHz):  $\delta$  168.6, 101.5, 99.9, 99.6, 77.8, 74.5, 72.8, 72.7, 70.9, 70.4, 69.9, 69.2, 66.8, 66.7, 66.0, 65.1, 61.7, 60.9, 13.0. ); ESI-MS:  $[M+Na]^+$   $C_{20}H_{33}NO_{15}Na$  calcd for 550.1748, found 550.1790.

### C) Deacetylation of asymmetric N-glycan products

Glycan was dissolved in a mixture of  $H_2O$  and 28%-30%  $NH_4OH$  (10% in volume) to achieve a 341  $\mu$ M final concentration of glycan. The reaction mixture was shaken at room temperature for 2 h. Upon completion, as indicated by MALDI-TOF MS, the reaction mixture was lyophilized and the residue was reconstituted in water and subjected to gel filtration over Sephadex G-25 (eluent 0.1 M  $NH_4HCO_3$ ). Fractions containing product were combined and lyophilized to give the respective products as an amorphous white solid.

## VI. Labeling of N-glycans with AEAB

AEAB (*N*-(aminoethyl)-2-amino benzamide) was used to label compounds **1-10**, **1a-9a**, **2b**, **6b**, **7b**, and **10b** to assist their printing onto slides. The AEAB labeling reactions were performed<sup>5</sup> using the following conditions: Dissolve 8 mg of AEAB in 100  $\mu$ L solution of DMSO/AcOH (7:3 v/v) (labelling solution A), and dissolve 6 mg of  $NaCNBH_3$  in another 100  $\mu$ L solution of DMSO/AcOH (7:3 v/v) (labelling solution B). To every 100  $\mu$ g glycan sample, 25  $\mu$ L of labelling solution A and 25  $\mu$ L of labelling solution B were added, mixed well and incubated in a water bath for 2 h at 65 °C. Labeled glycans were precipitated with acetonitrile (10 volumes) and incubated at -20 °C for 1 h. After centrifugation (13,000 g for 10 min), the supernatant was removed. The pellet was dried in a vacufuge for 15 min.<sup>5</sup> Labeled glycan was dissolved in 100  $\mu$ L of water and purified using a Waters XBridge BEH amide column (130 Å, 5  $\mu$ m, 4.6 mm  $\times$  250 mm) as described. Sample concentration was determined using the peak area and comparing to 10 nmol of known standard (AEAB labelled lactose).

## VII. General methods for glycan microarray

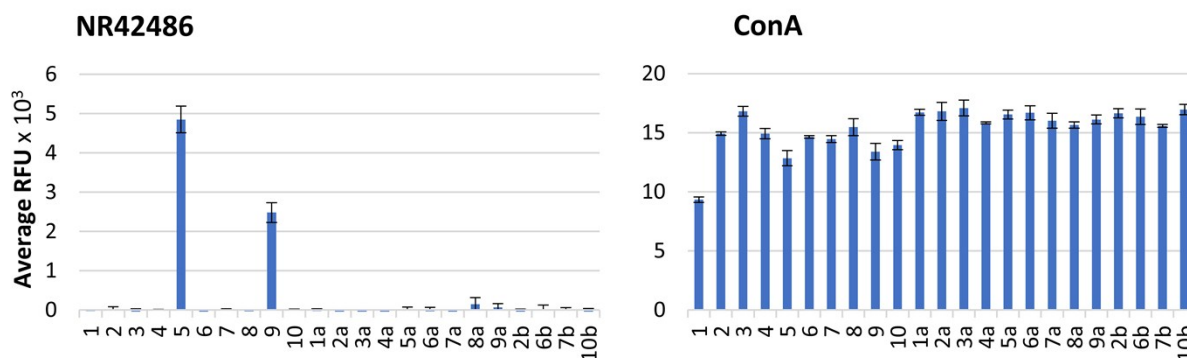
### A) Customized glycan array preparation

Customized glycan arrays were printed in an 8-subarray format by Z Biotech (Aurora, CO, USA). The AEAB-tagged glycans were printed onto microarray substrates coated with NHS surface chemistry. Within each subarray, the glycans were printed in 6 replicates at 100  $\mu$ M concentration in print buffer (300 mM sodium phosphate, pH 8.5, 0.005% Tween-20), 0.6 nL for each replicate, and print buffer or AEAB alone was printed as a negative binding control. A biotinylated PEG amine was printed within each subarray to serve as a positive control. Covalent coupling was processed at 80% humidity for 30 min. Then, the unreacted NHS groups were blocked with blocking buffer (50 mM ethanolamine in 50 mM sodium borate, pH 9.2) for 1 h and the slide was rinsed with water, dried and stored at  $-20^{\circ}$ C until use.

## B) Methods of glycan microarray analysis

Binding studies using plant lectins were performed as previously described.<sup>6</sup>

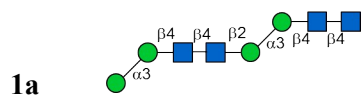
Glycan microarray involving viral lectins was analyzed as follows: Recombinant HA proteins (1 mg/mL, 15  $\mu$ L) were pre-mixed with Penta-His Alexa Fluor 647 Conjugate (0.2 mg/mL, 35  $\mu$ L) and Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody Alexa Fluor 647 (2 mg/mL, 2.3  $\mu$ L) in a molar ratio of 4:2:1 and were incubated on ice for 15 min, followed by dilution to 200  $\mu$ L with TSMTB buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 2 mM CaCl<sub>2</sub>, 2 mM MgCl<sub>2</sub>, 0.05 Tween-20, and 1% BSA). The array slide was mounted with a ProPlate 8 Well Module, and 200  $\mu$ L of TSMTB buffer was added to each well and the slide was shaken for 30 min. Then the buffer was removed and 200  $\mu$ L of HA-antibody pre-mixed complexes was added to each well, and the slide was sealed and incubated at room temperature for 2 h. The slide was then washed by TSMT buffer, TSM buffer and deionized water. After dried by brief centrifuge, the slide was scanned with a GenePix 4000B microarray scanner by recording the fluorescent signal of Cy5. The average relative fluorescence units (RFUs) and standard error measurement (SEM) were calculated and shown in bar graphs (SEM value as error bars).



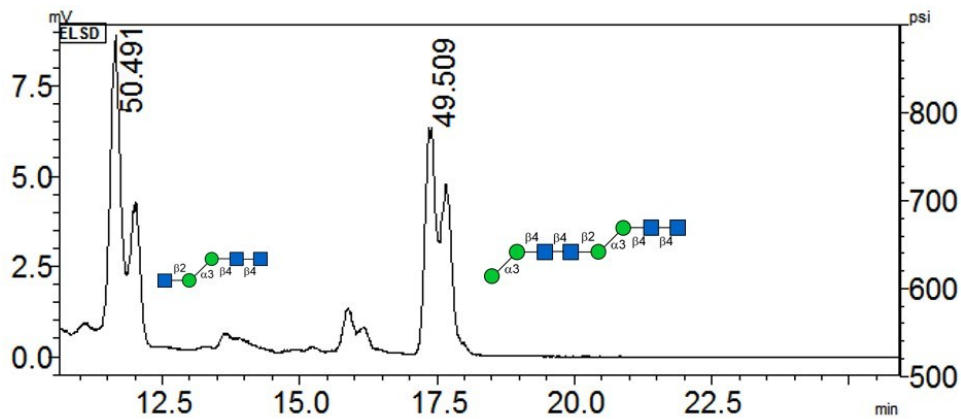
**Figure S1.** Glycan microarray analyses. Binding profiles with viral lectin hemoagglutinin (HA) NR42486 from human influenza viruses and plant lectin Concanavalin A from *Canavalia ensiformis* (ConA) were examined.

### VIII. HPLC analyses of *N*-glycan synthesizing mixtures and MS data of purified *N*-glycans

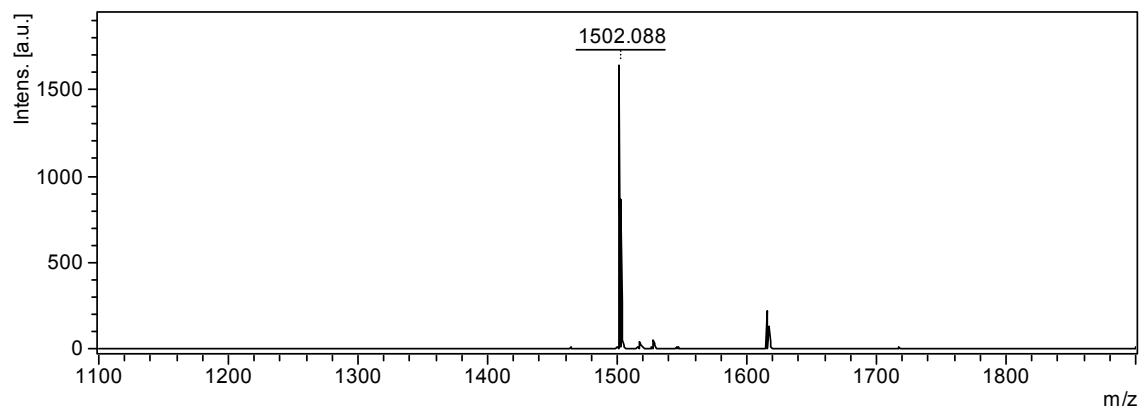
The peak area percentages of acceptor and product *N*-glycans are shown in HPLC chromatographs.

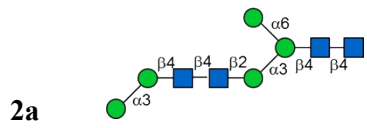


HILIC-ELSD,  $T_R = 17.35$  min.

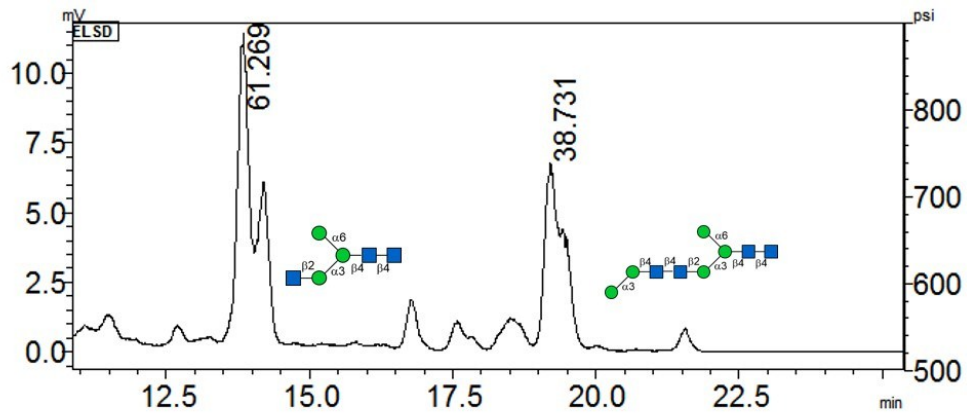


MALDI-MS, calculated 1478.5393; found  $[M+Na]^+$  1502.088.

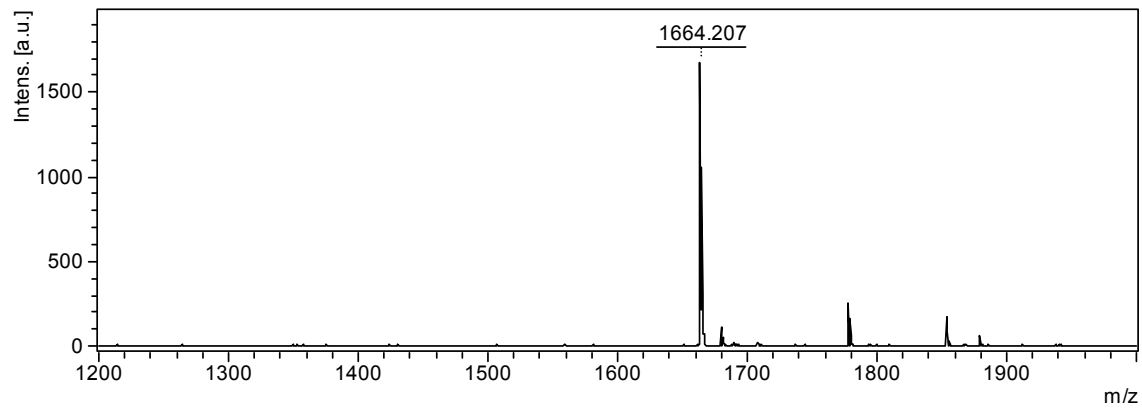


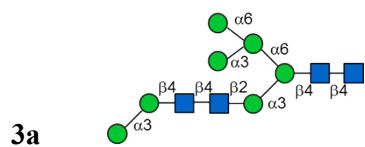


HILIC-ELSD,  $T_R = 19.20$  min.

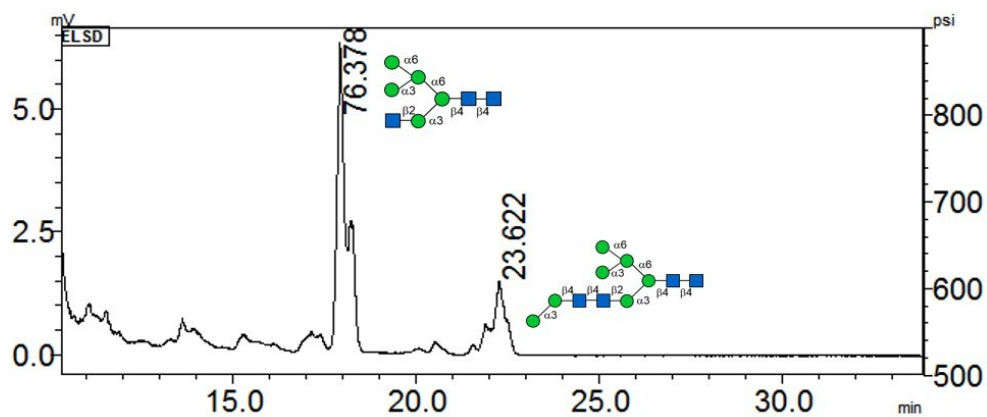


MALDI-MS, calculated 1640.5922; found  $[M+Na]^+$  1664.207.

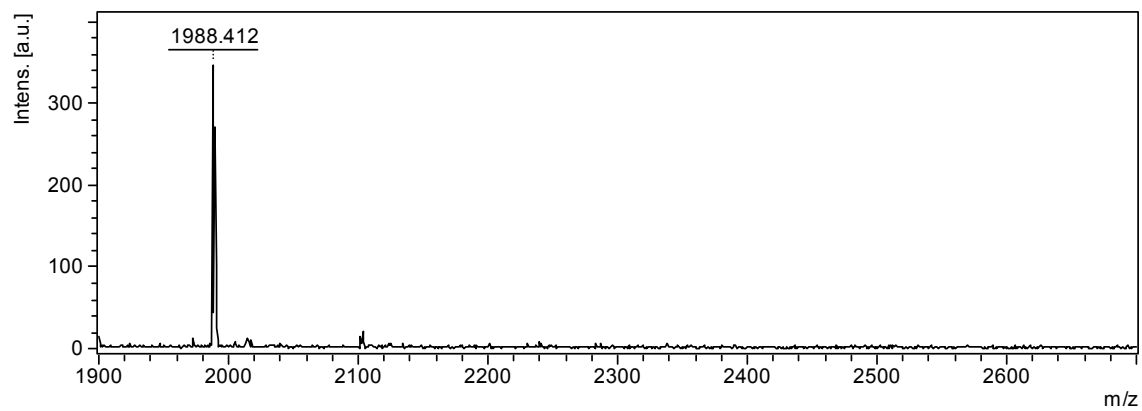


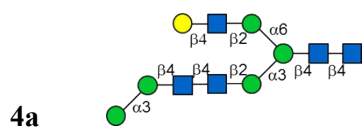


HILIC-ELSD,  $T_R = 22.27$  min.

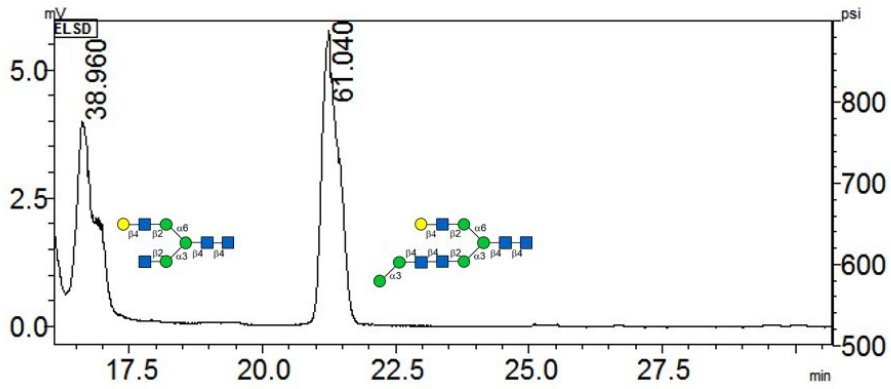


MALDI-MS, calculated 1964.6987; found  $[M+Na]^+$  1988.412.

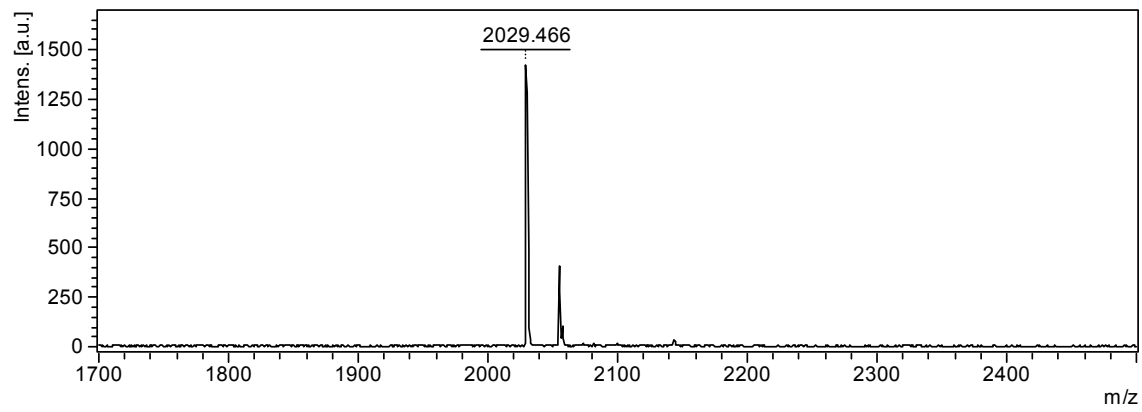


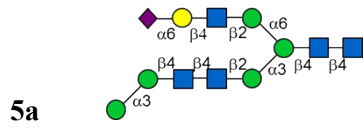


HILIC-ELSD,  $T_R = 21.24$  min.

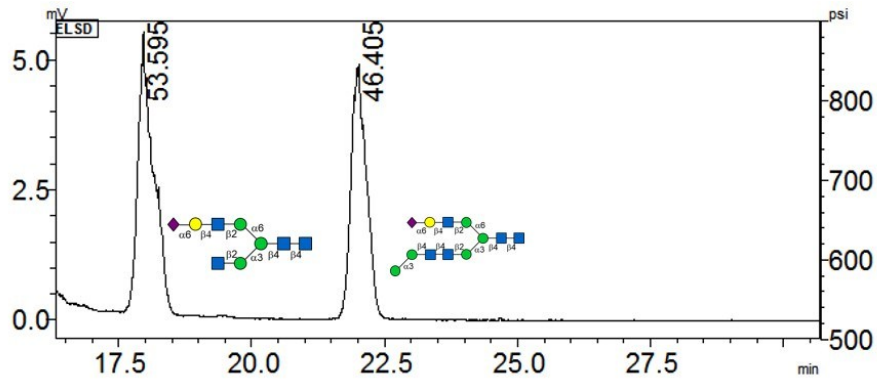


MALDI-MS, calculated 2005.7244; found  $[M+Na]^+$  2029.466.

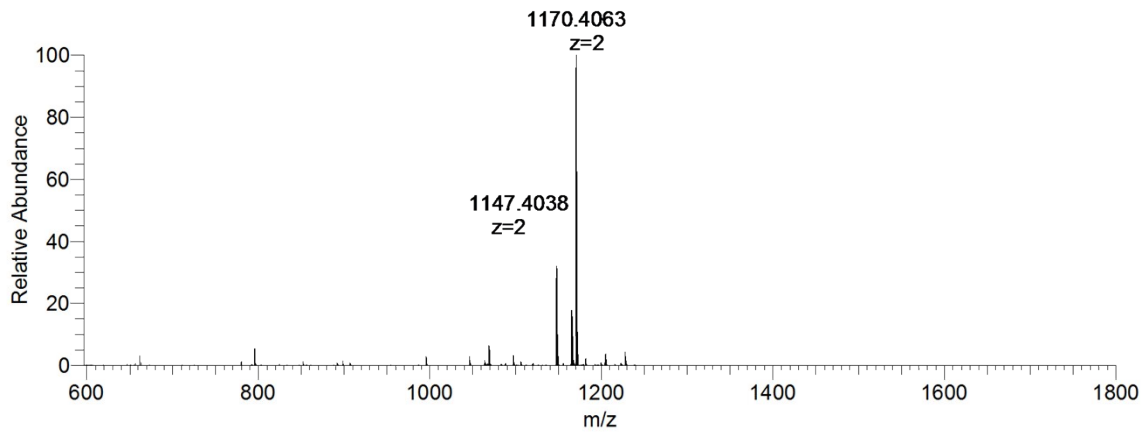


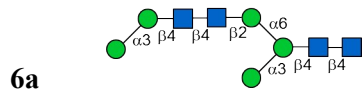


HILIC-ELSD,  $T_R = 22.01$  min.

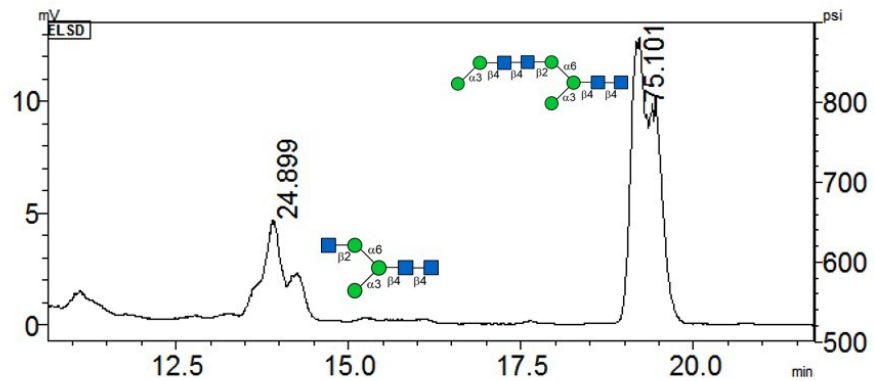


ESI-MS, calculated 2296.8198; found  $[M-2H]^{2-}$  1147.4038,  $[M+HCOOH-2H]^{2-}$  1170.4063.

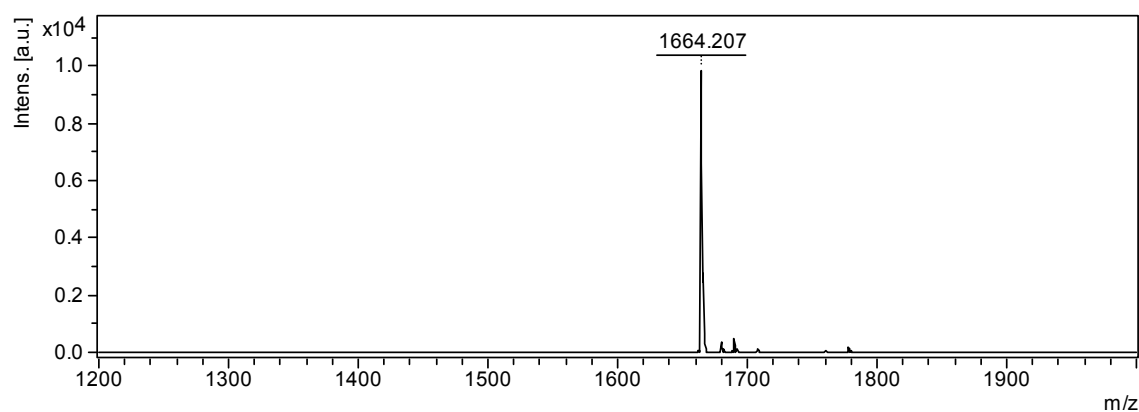




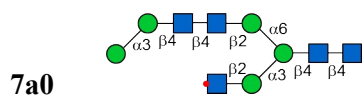
HILIC-ELSD,  $T_R = 22.27$  min.



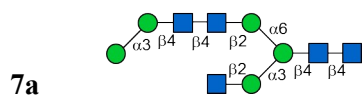
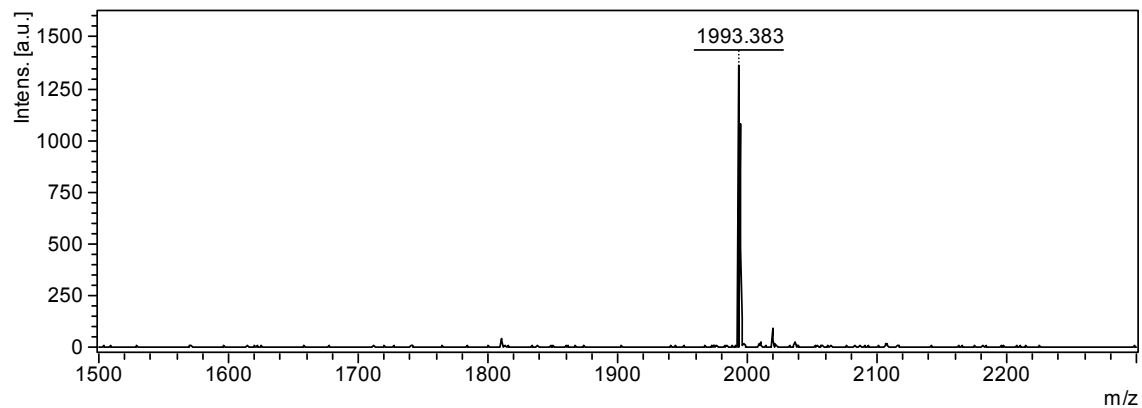
MALDI-MS, calculated 1640.5922; found  $[M+Na]^+$  1664.207.



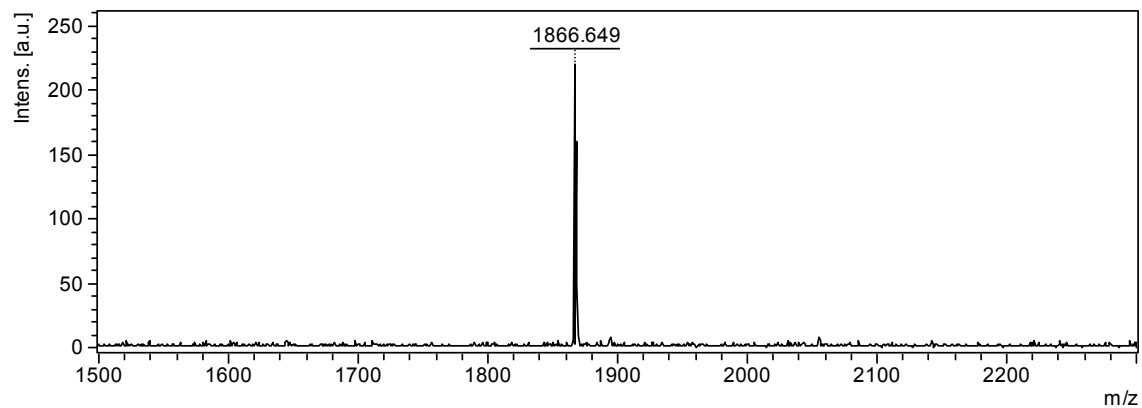


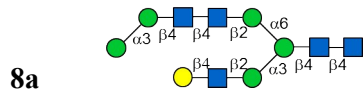


**MALDI-MS**, calculated: 1969.7032; found  $[M+Na]^+$  1993.383.

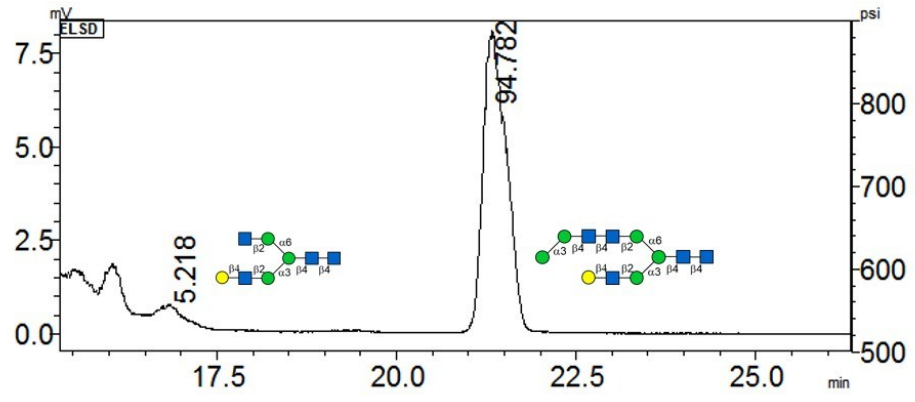


**MALDI-MS**, calculated 1843.6715; found  $[M+Na]^+$  1866.649.

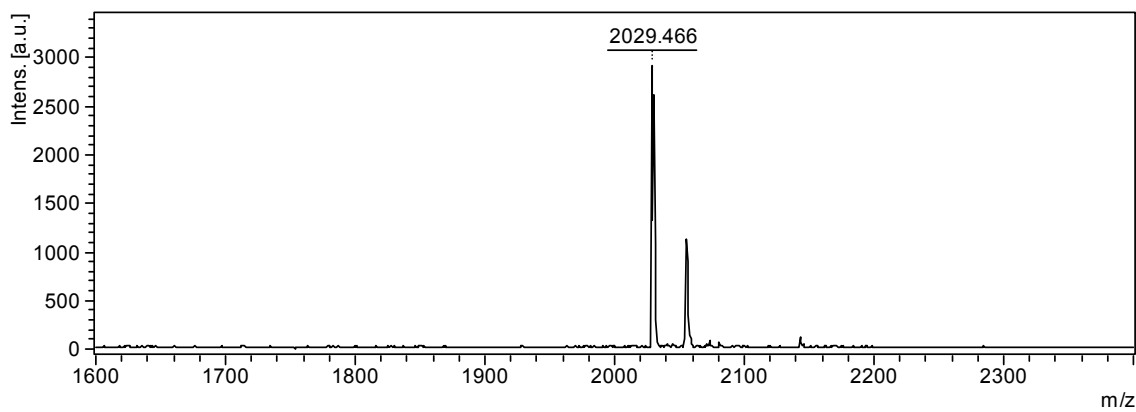


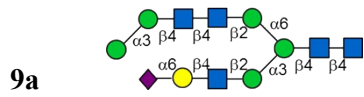


HILIC-ELSD,  $T_R = 21.33$  min.

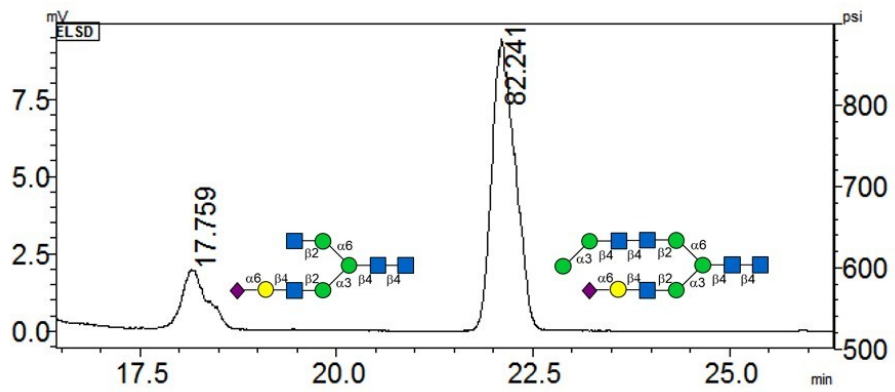


MALDI-MS, calculated 2005.7244; found  $[M+Na]^+$  2029.466.

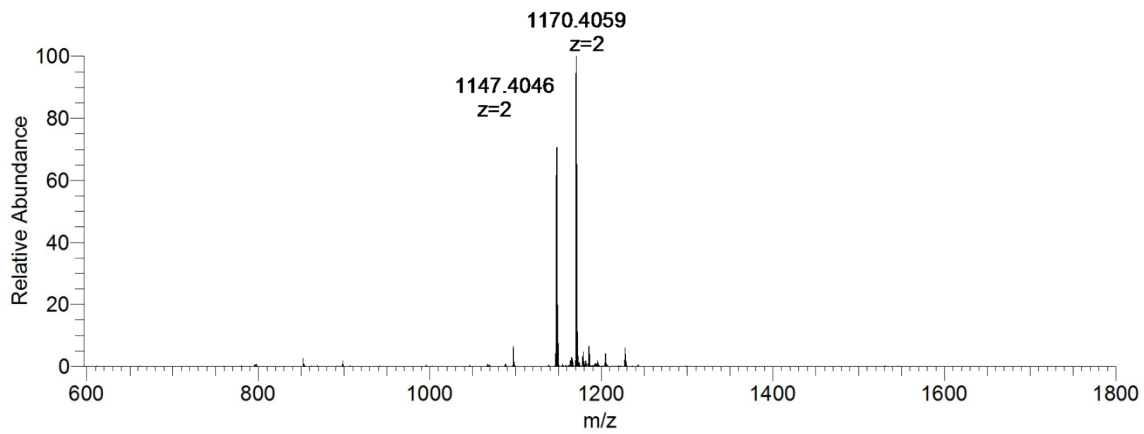


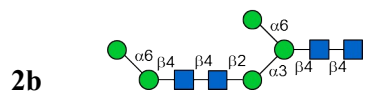


HILIC-ELSD,  $T_R = 22.10$  min.

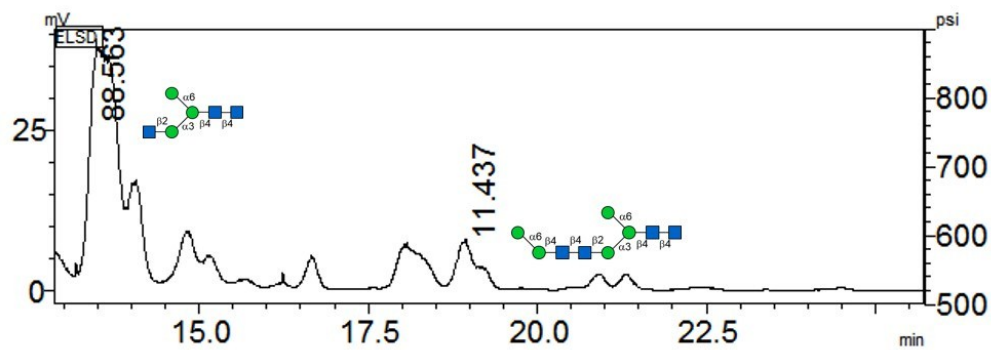


ESI-MS, calculated 2296.8198; found  $[M-2H]^{2-}$  1147.4046,  $[M+HCOOH-2H]^{2-}$  1170.4059.

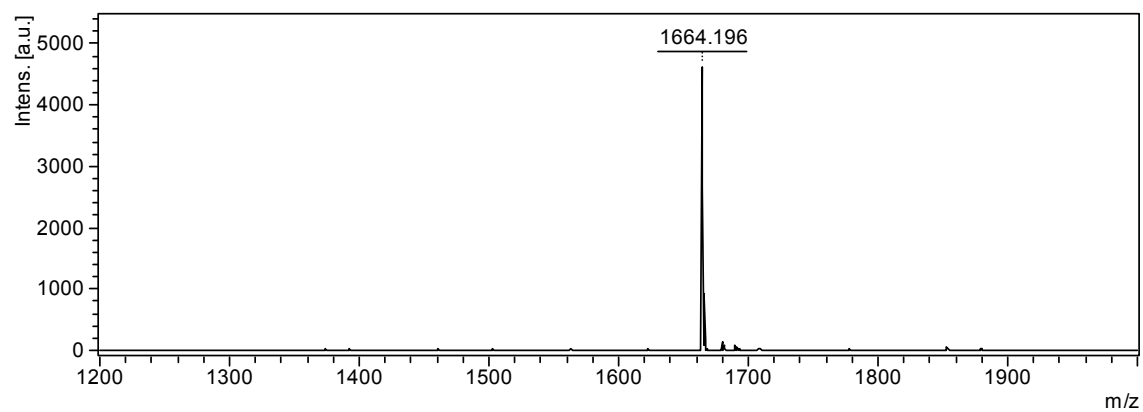


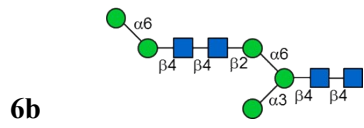


HILIC-ELSD,  $T_R = 18.94$  min.

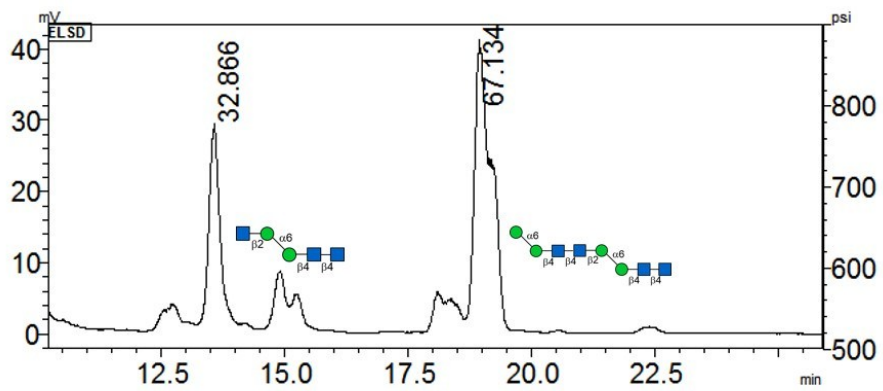


MALDI-MS, calculated 1640.5922; found  $[M+Na]^+$  1664.196.

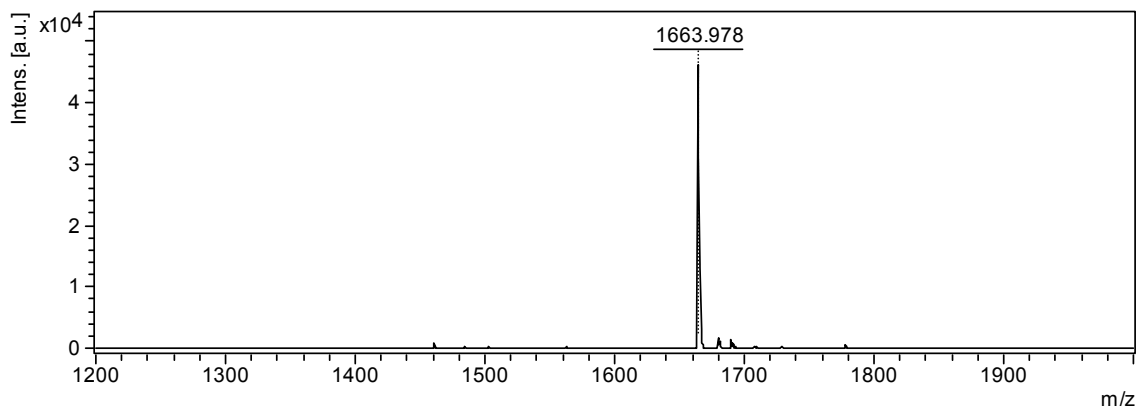




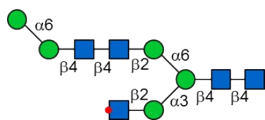
HILIC-ELSD, T<sub>R</sub> = 18.94 min.



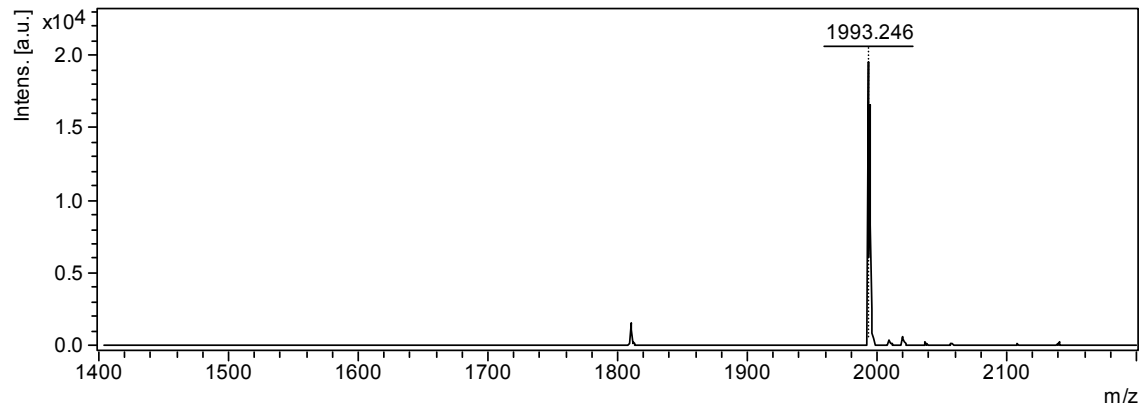
MALDI-MS, calculated 1640.5922; found [M+Na]<sup>+</sup> 1663.978.



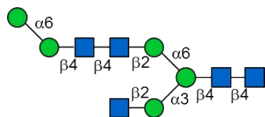
7b0



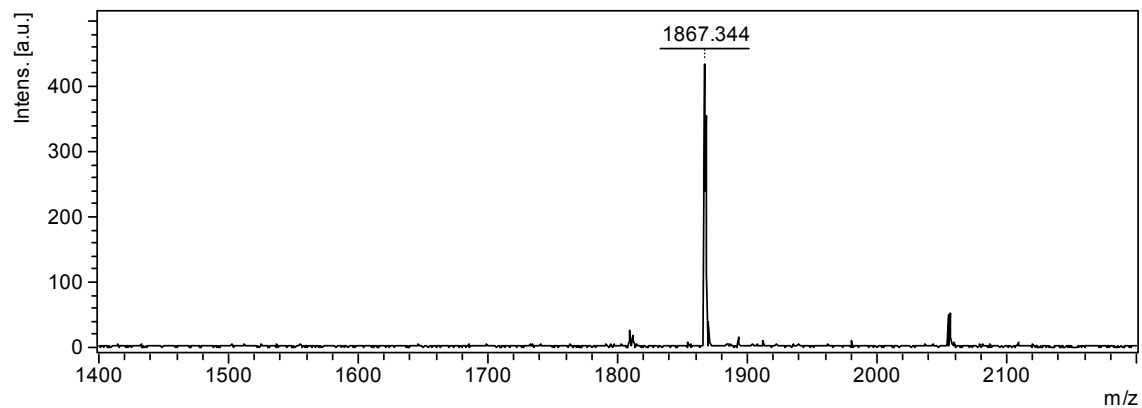
MALDI-MS, calculated 1843.6715; found  $[M+Na]^+$  1993.246.

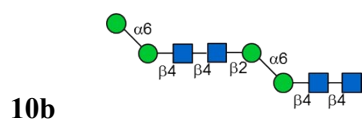


7b

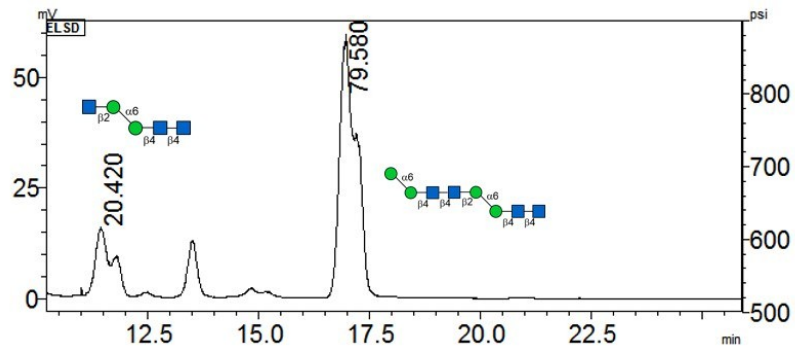


MALDI-MS, calculated 1843.6715; found  $[M+Na]^+$  1867.344.

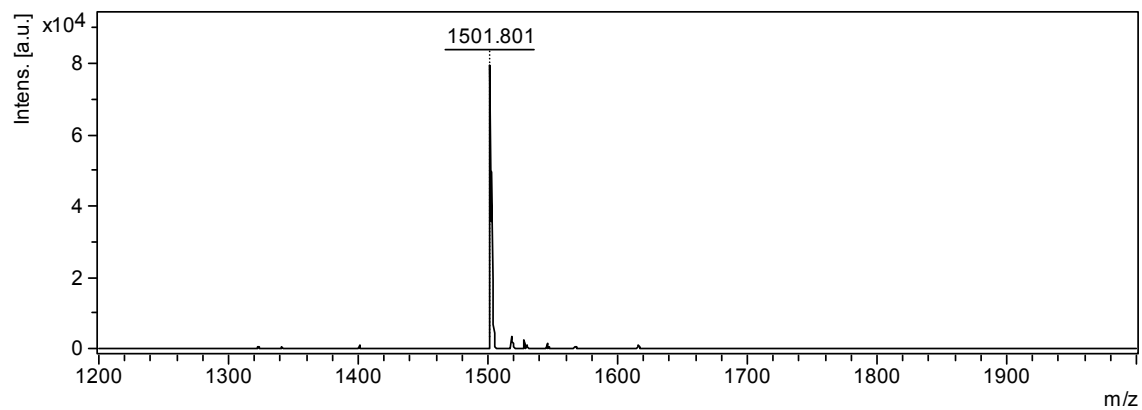




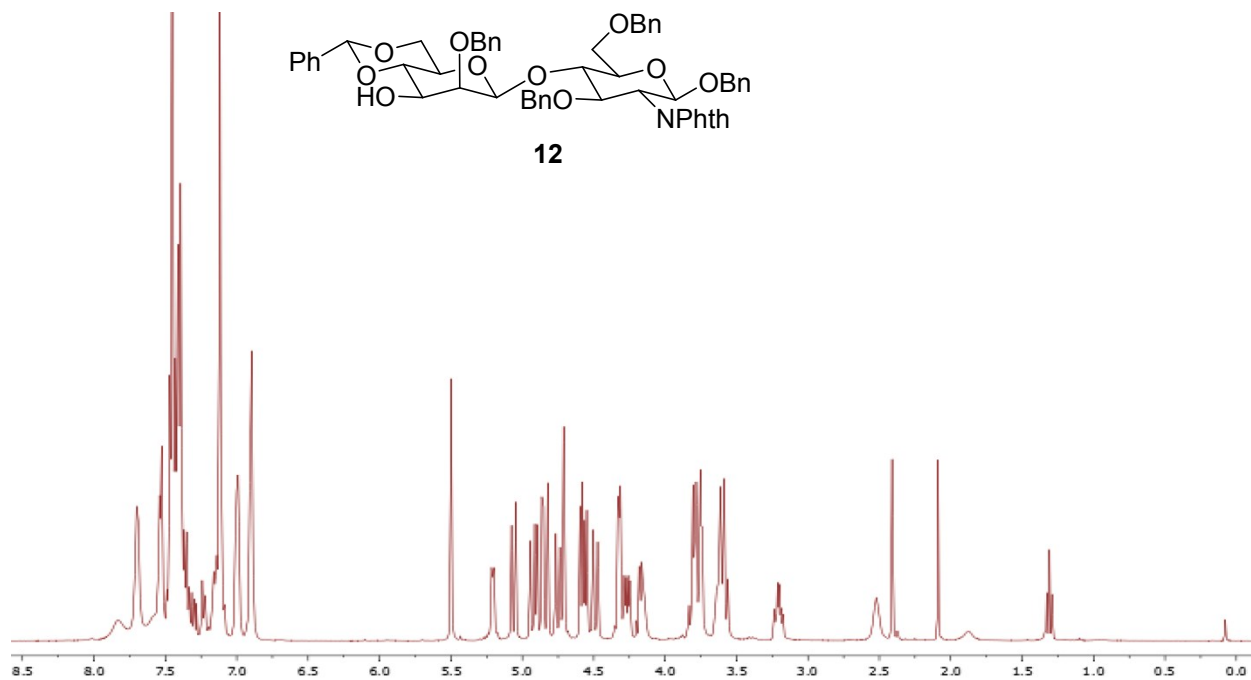
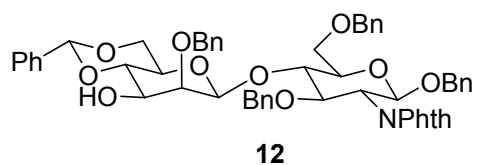
HILIC-ELSD,  $T_R = 16.97$  min.



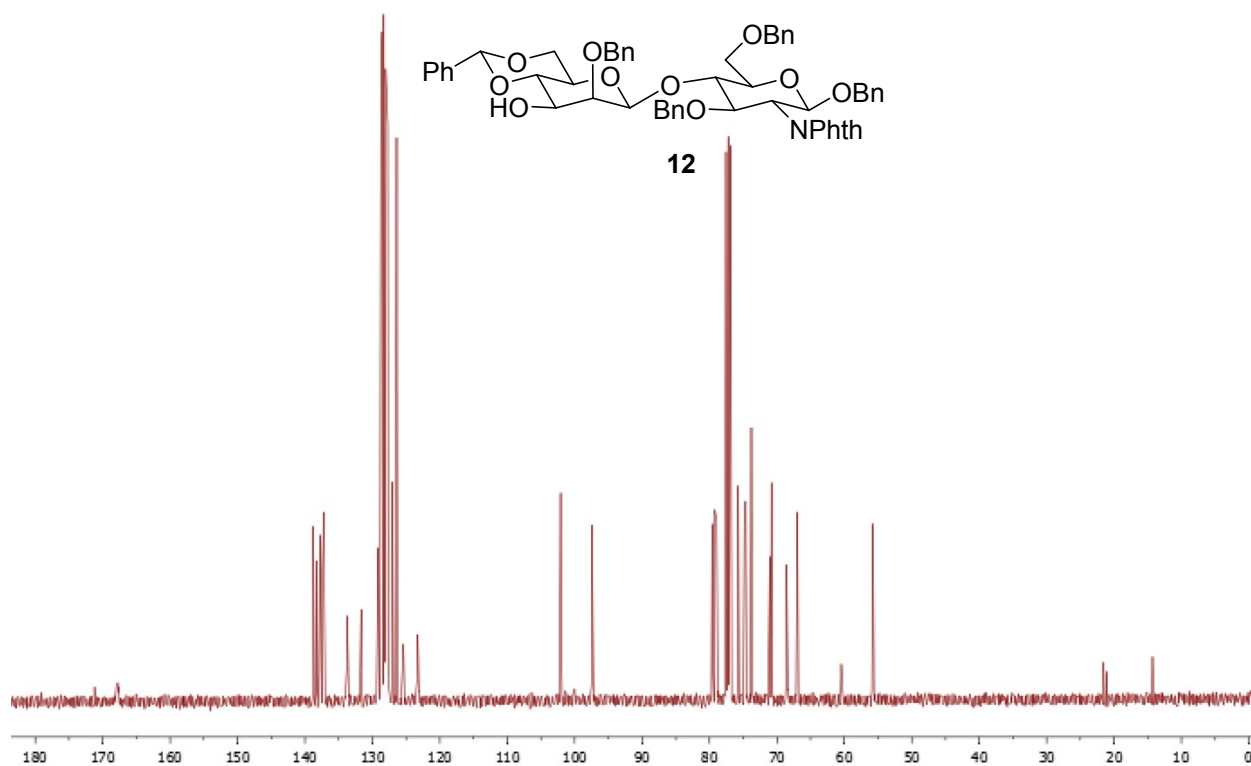
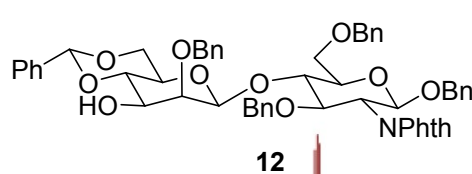
MALDI-MS, calculated 1478.5393, found  $[M+Na]^+$  1501.801.



## IX. NMR spectra of compounds for oxazolines a and b synthesis

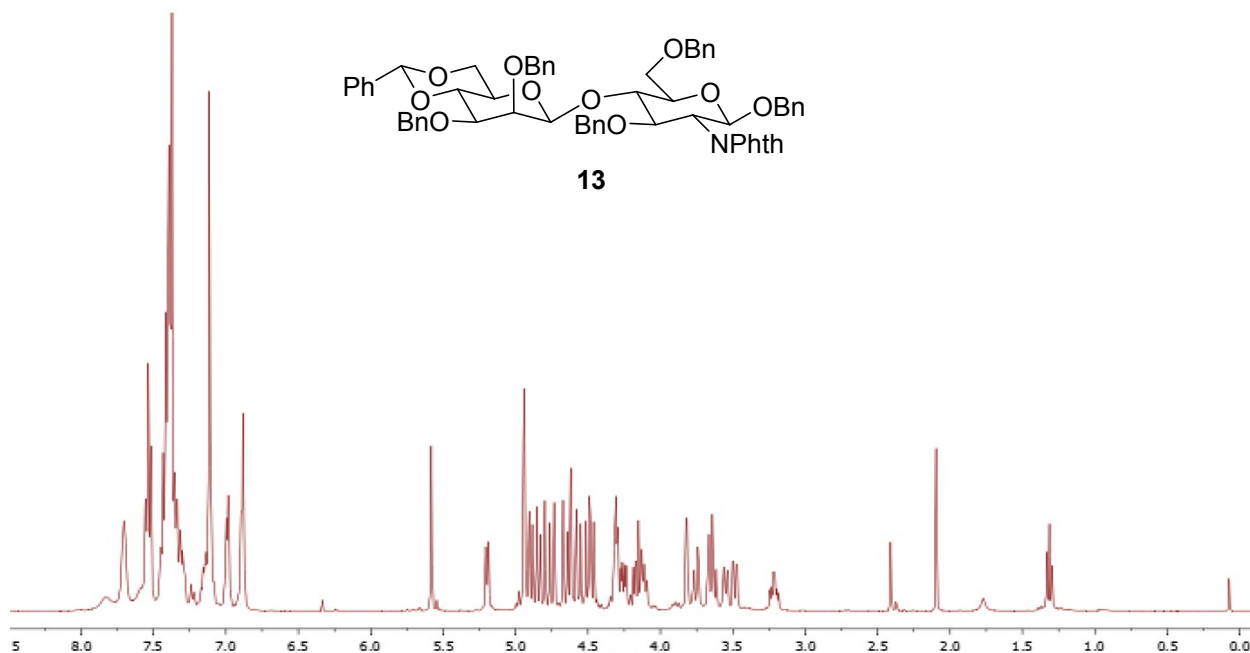
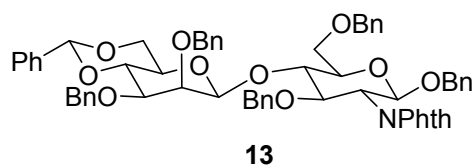


$^1\text{H}$  NMR of Compound 12

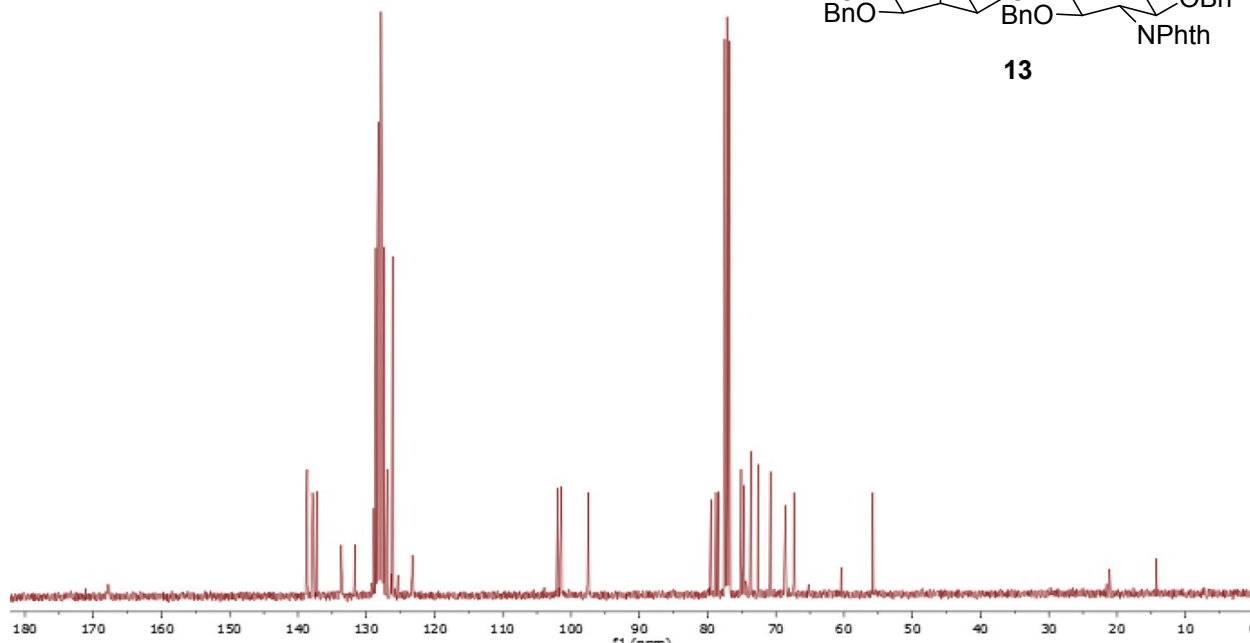
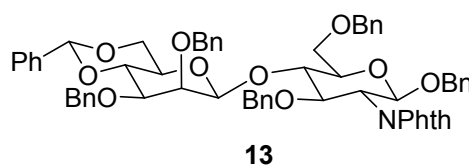


$^{13}\text{C}$  NMR of Compound 12

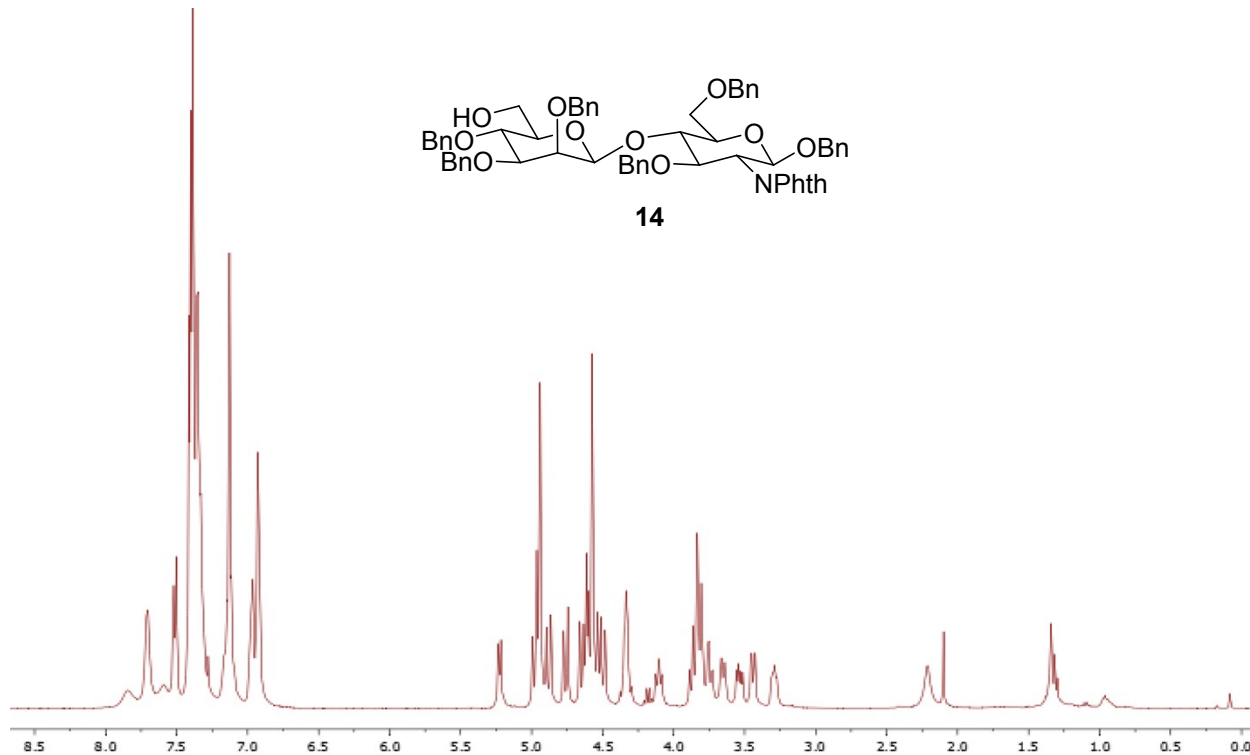
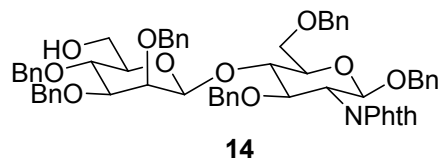




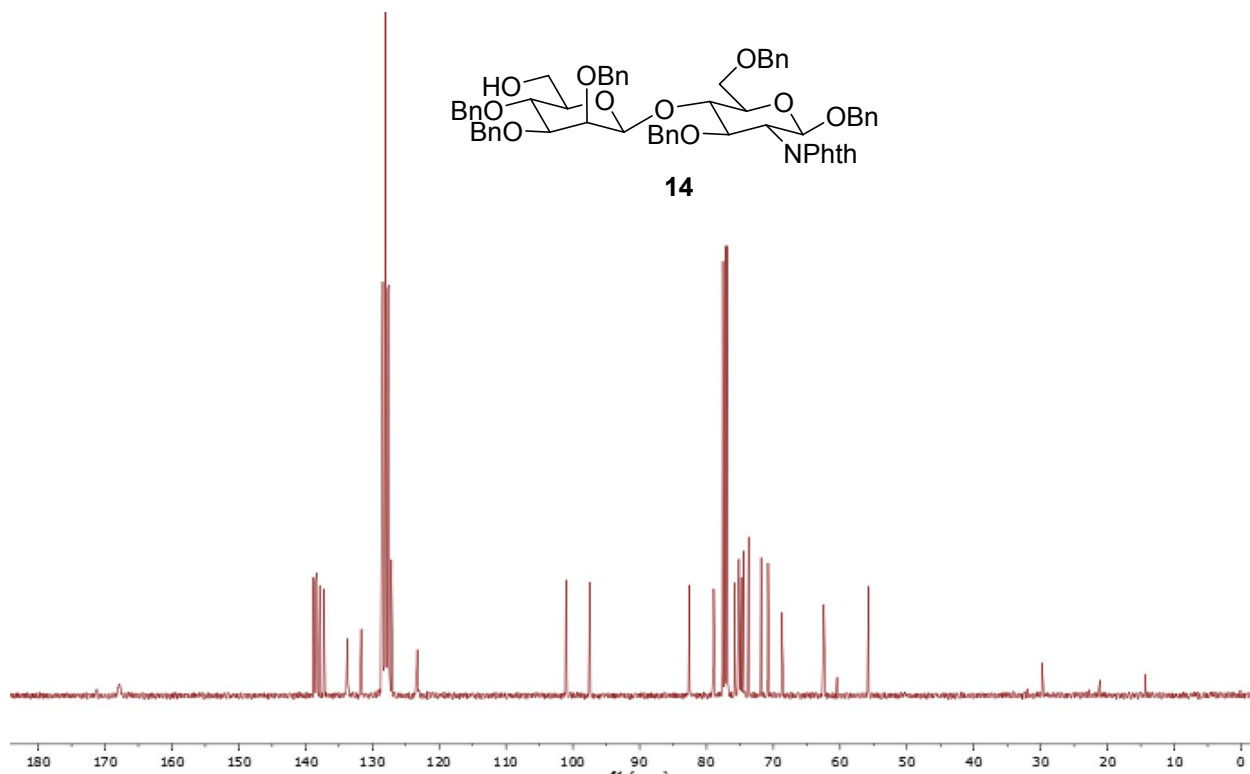
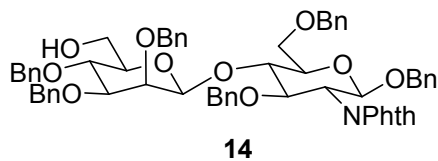
<sup>1</sup>H NMR of Compound 13



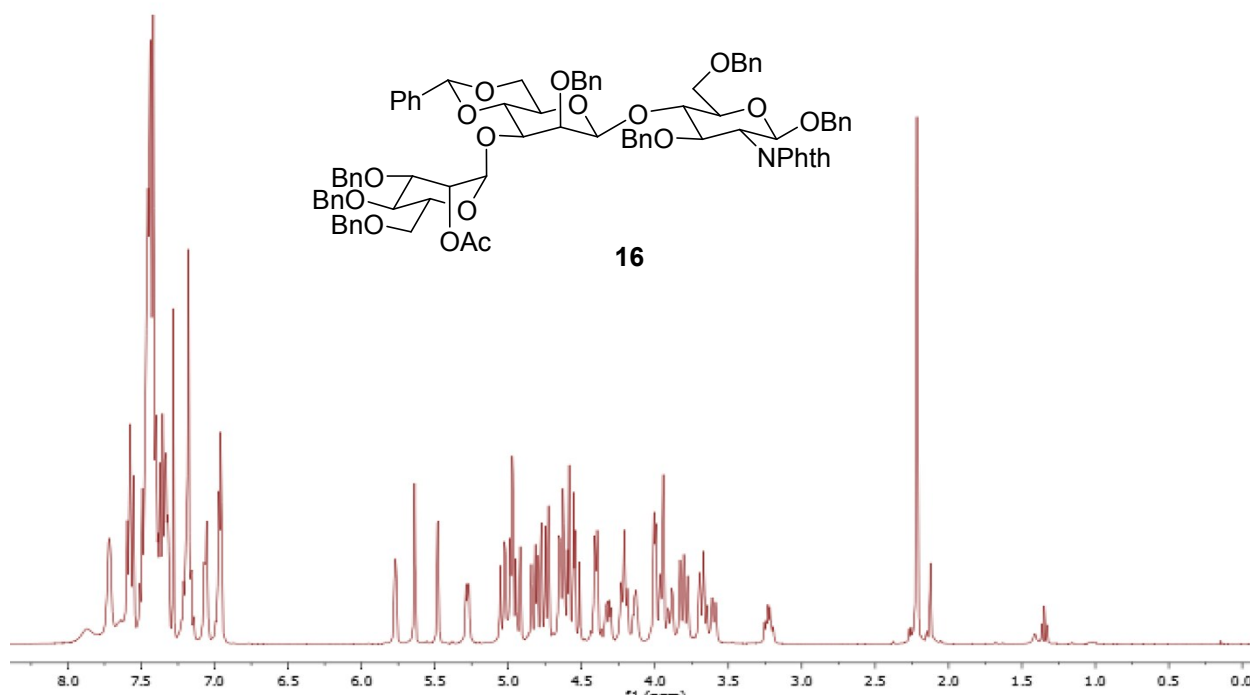
<sup>13</sup>C NMR of Compound 13



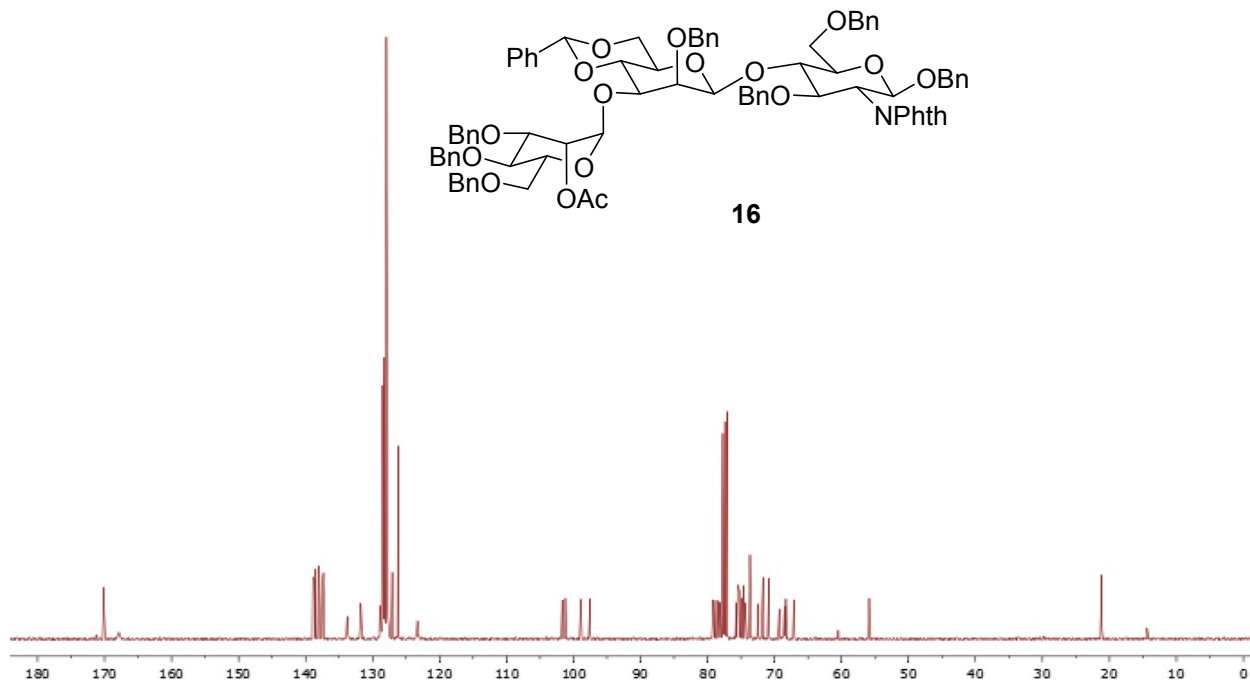
<sup>1</sup>H NMR of Compound 14



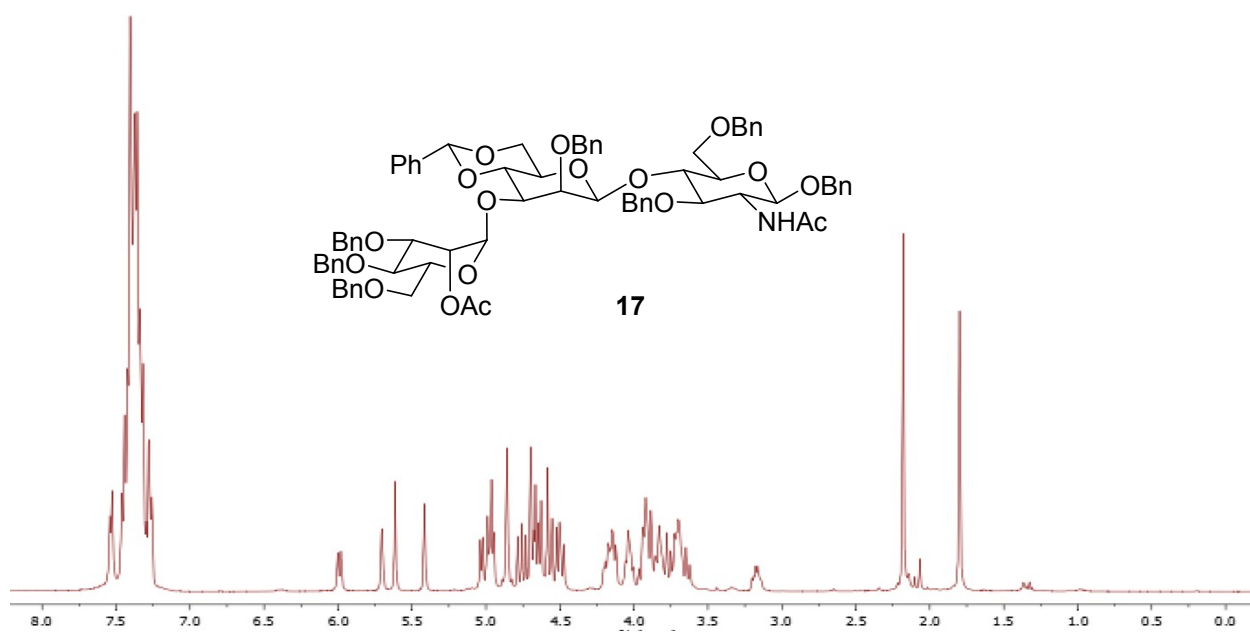
<sup>13</sup>C NMR of Compound 14



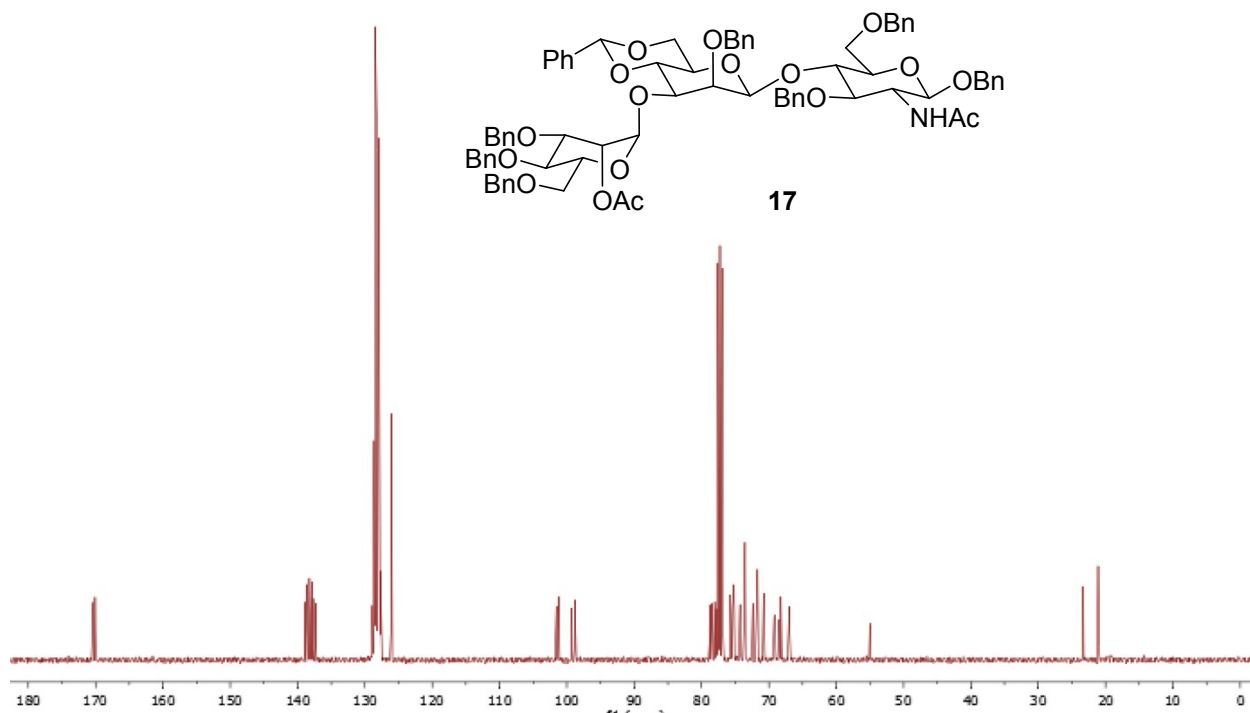
<sup>1</sup>H NMR of Compound **16**



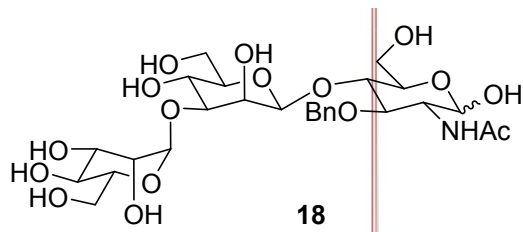
<sup>13</sup>C NMR of Compound **16**



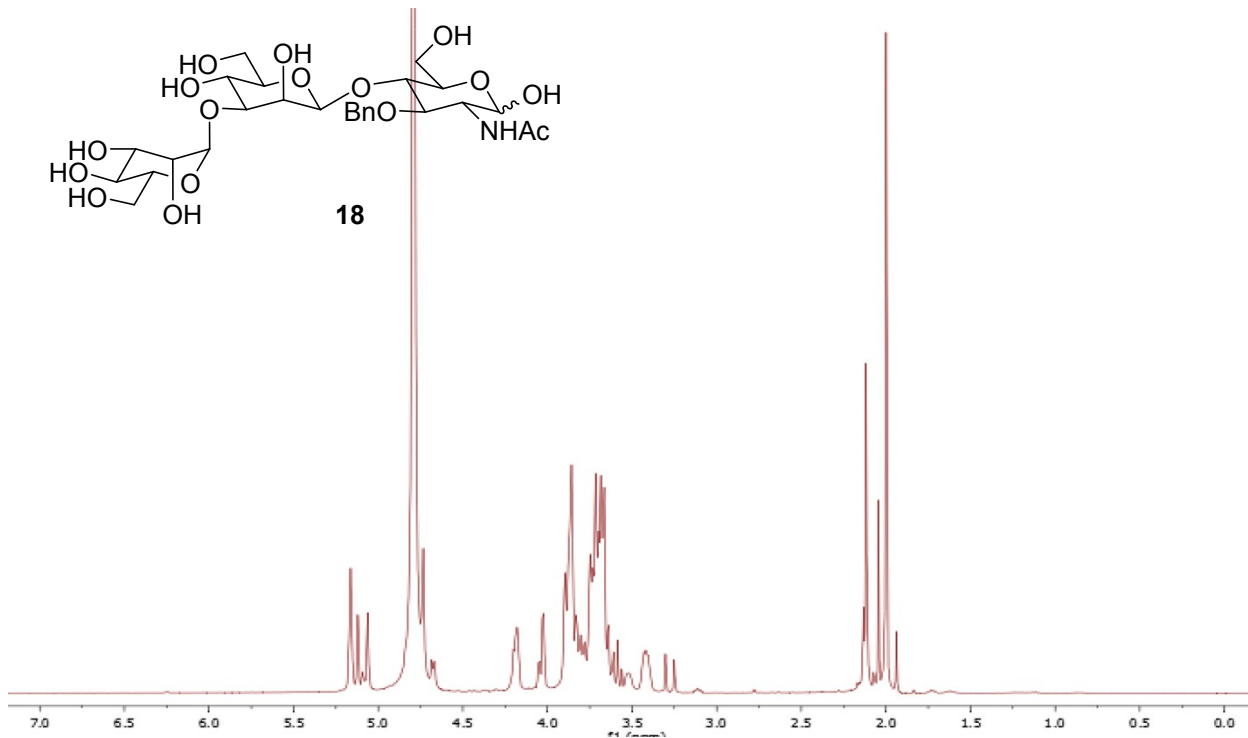
<sup>1</sup>H NMR of Compound 17



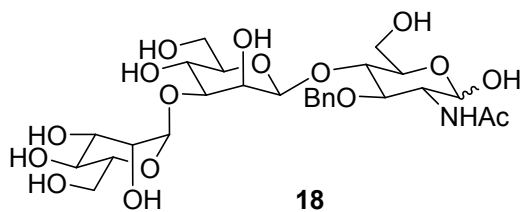
<sup>13</sup>C NMR of Compound 17



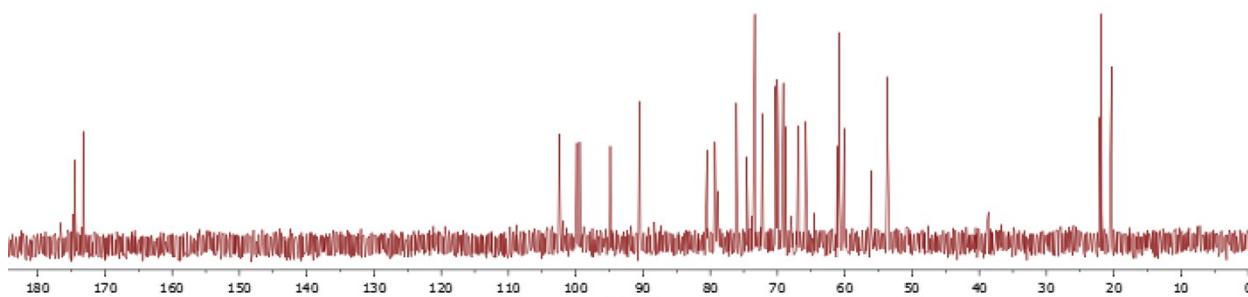
18



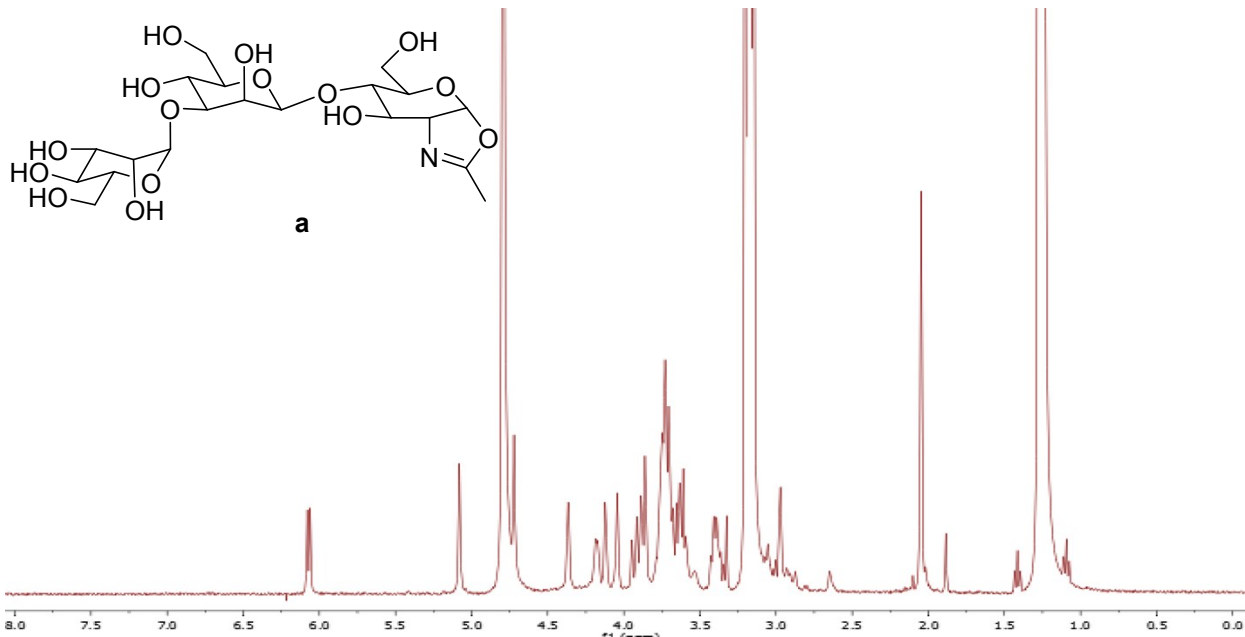
<sup>1</sup>H NMR of Compound 18



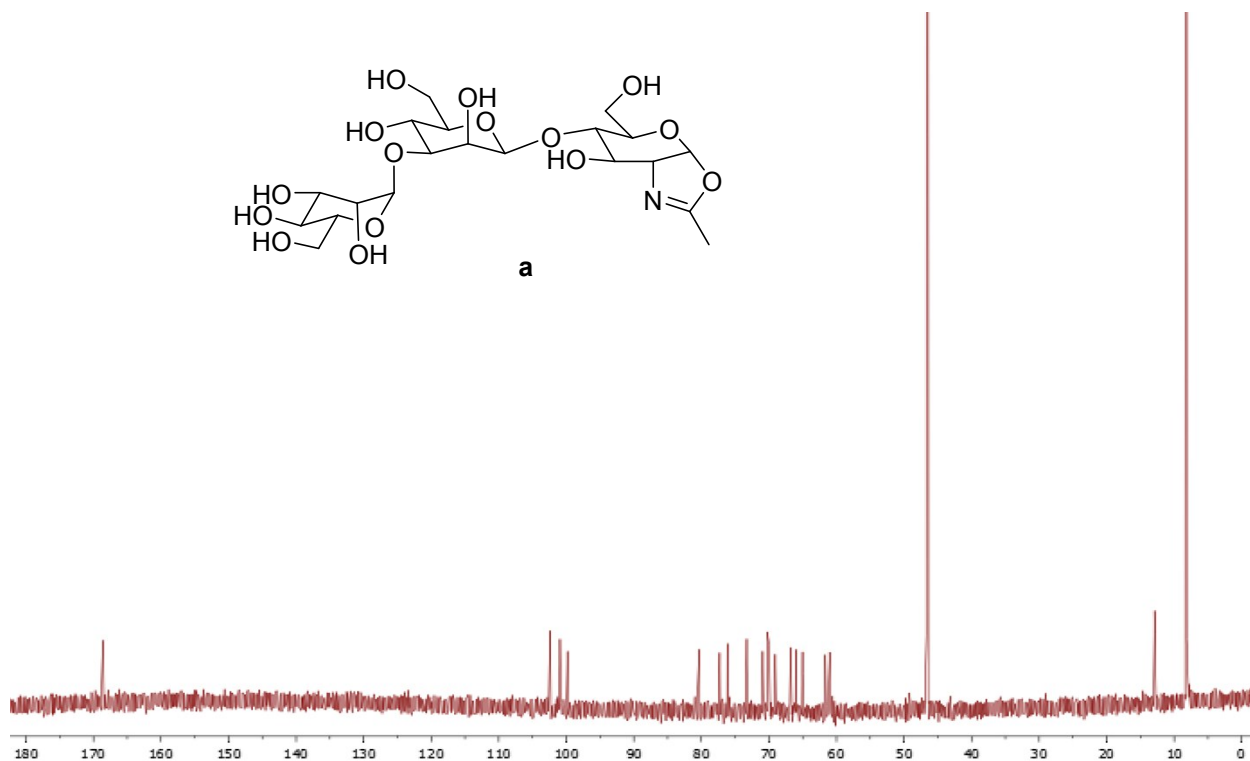
18



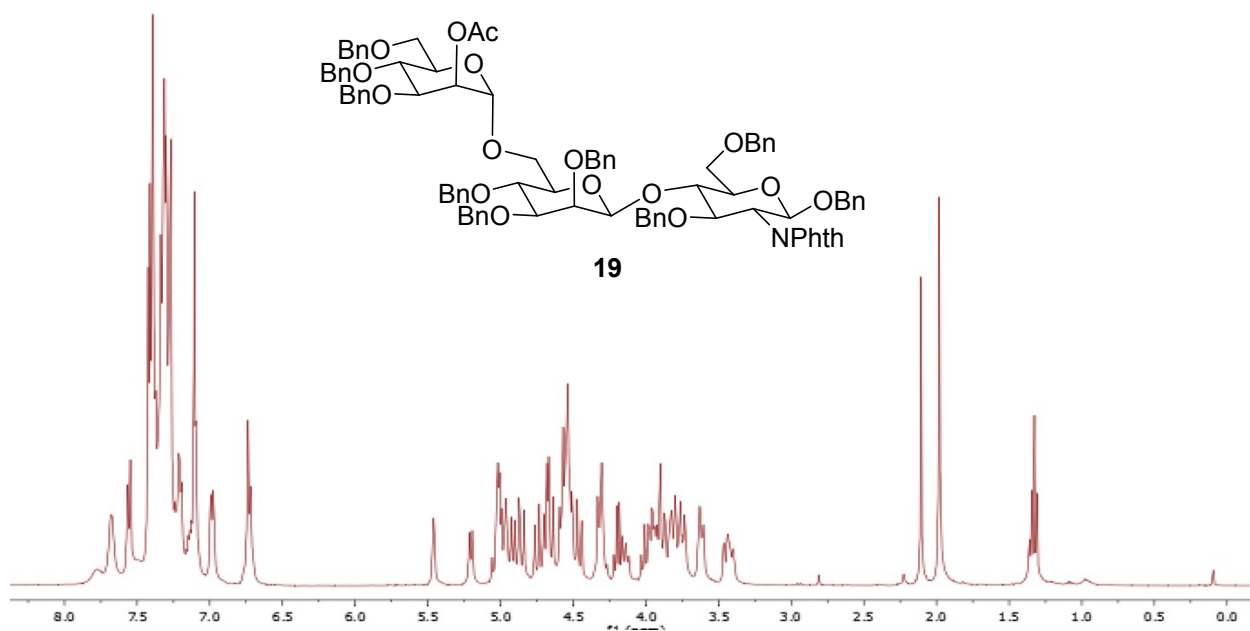
<sup>13</sup>C NMR of Compound 18



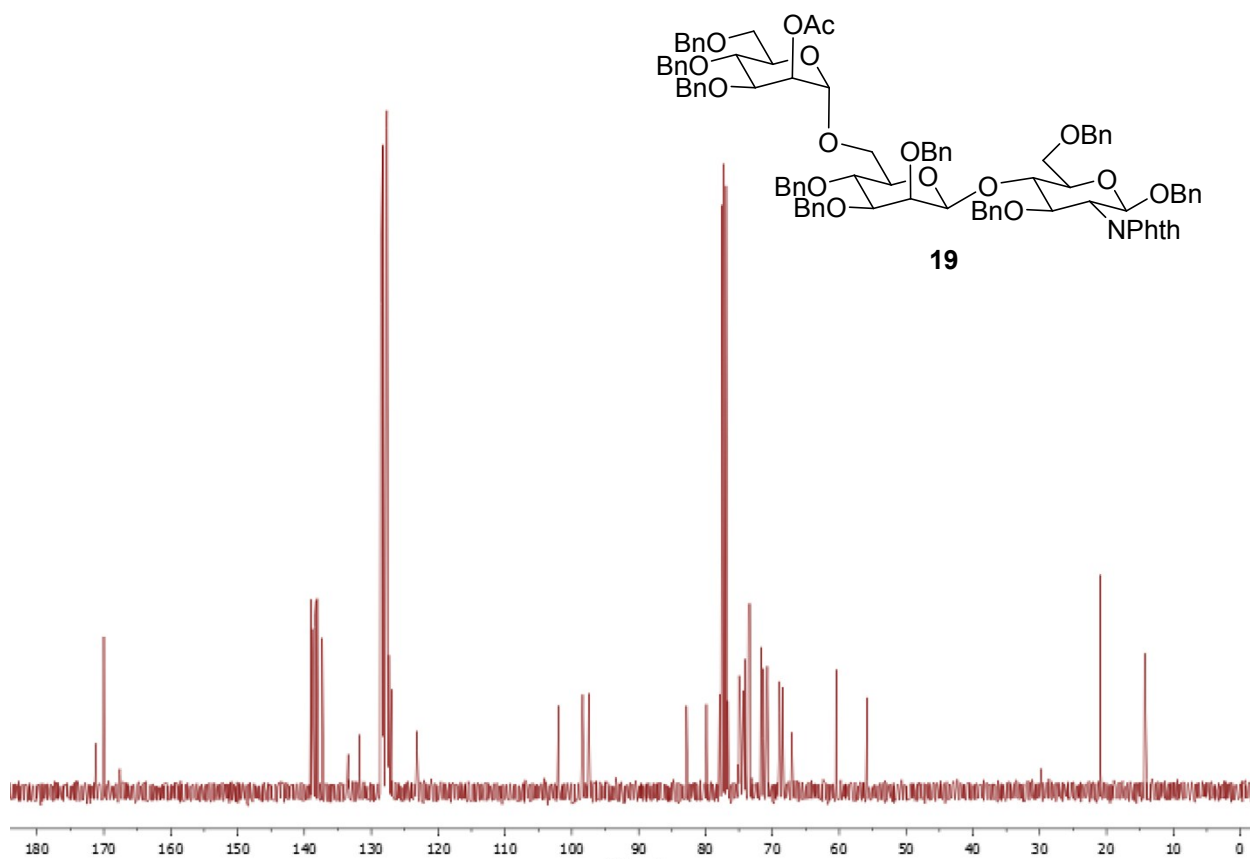
<sup>1</sup>H NMR of Compound **a**



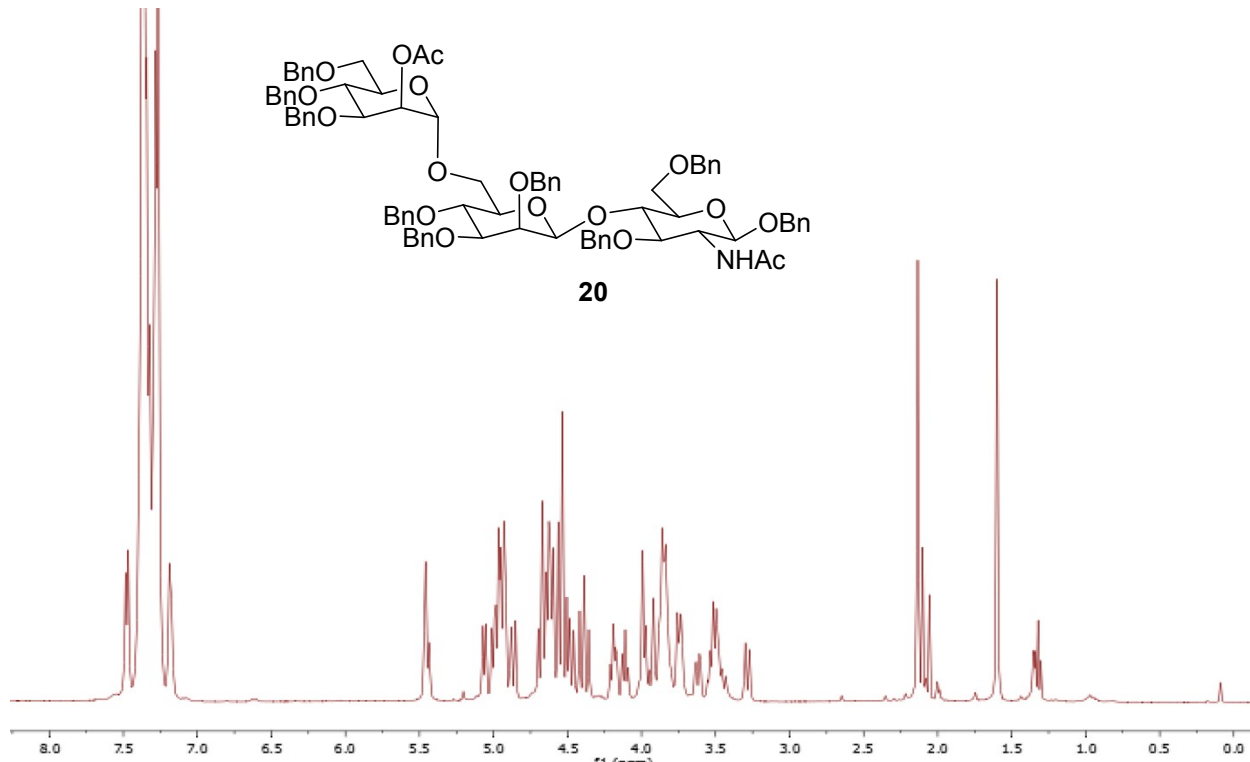
<sup>13</sup>C NMR of Compound **a**



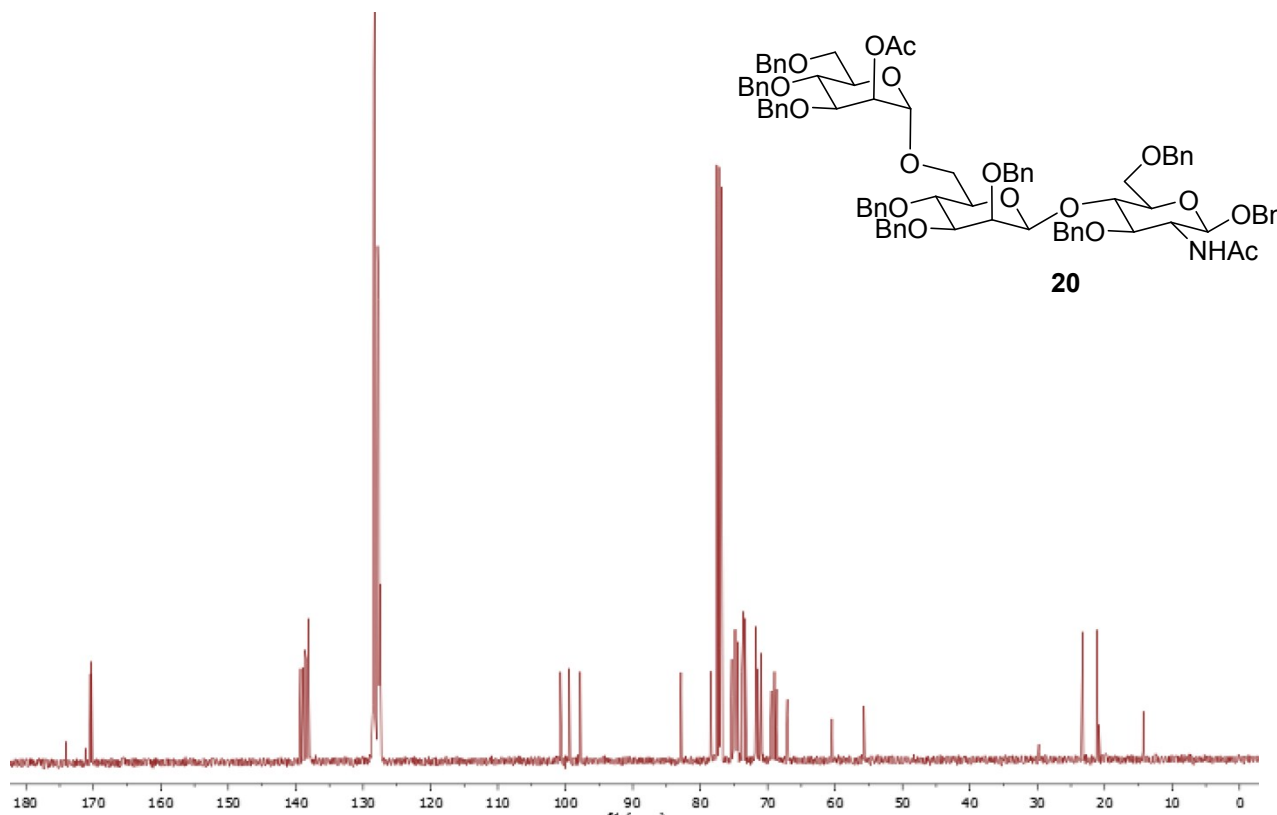
$^1\text{H}$  NMR of Compound **19**



$^{13}\text{C}$  NMR of Compound **19**

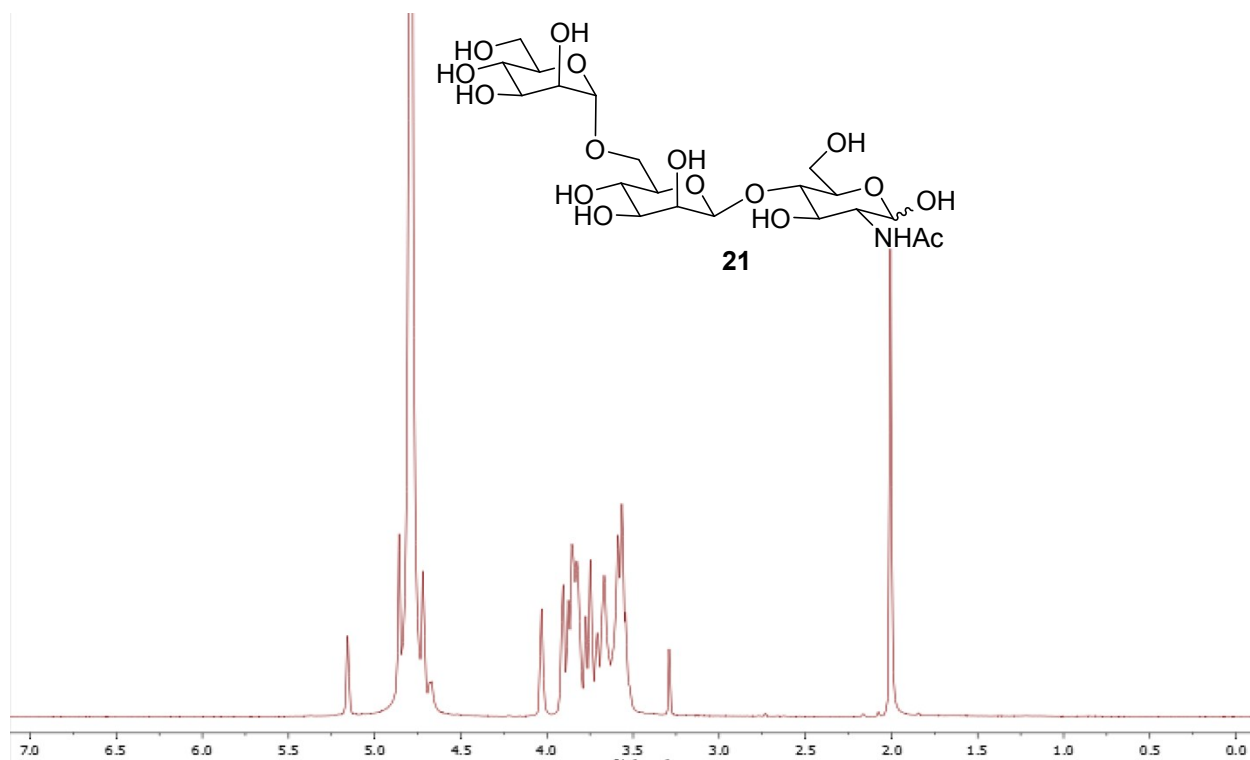


$^1\text{H}$  NMR of Compound **20**

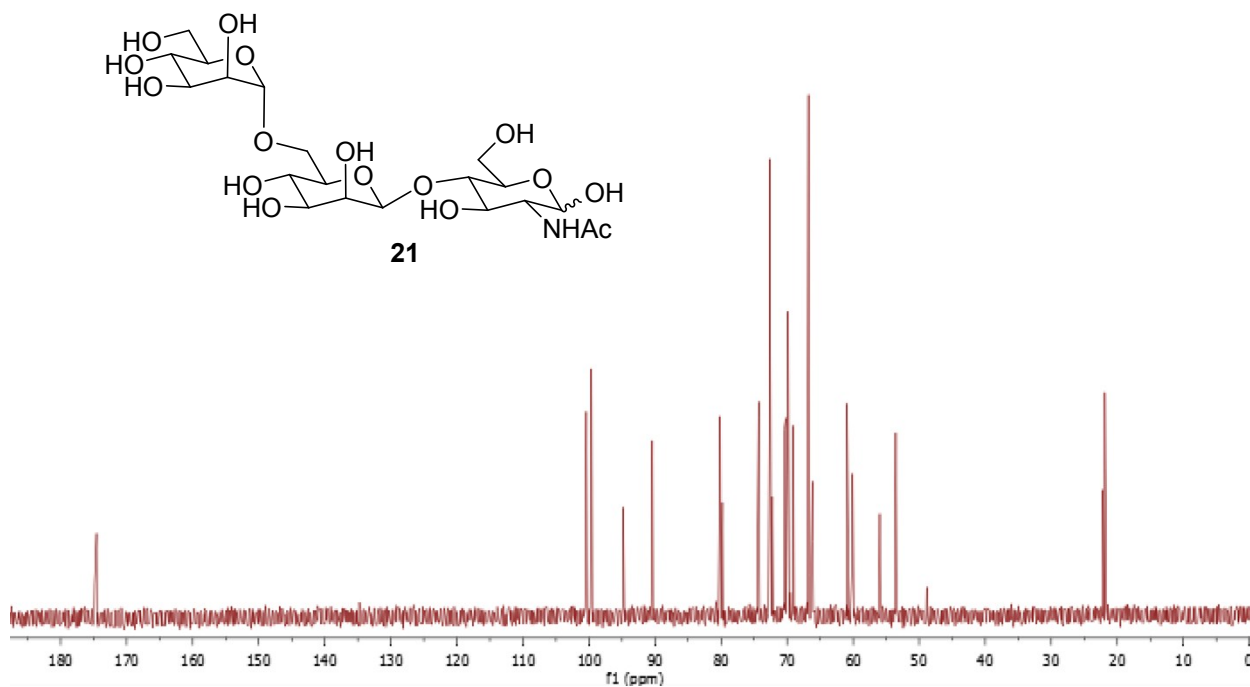


$^{13}\text{C}$  NMR of Compound **20**

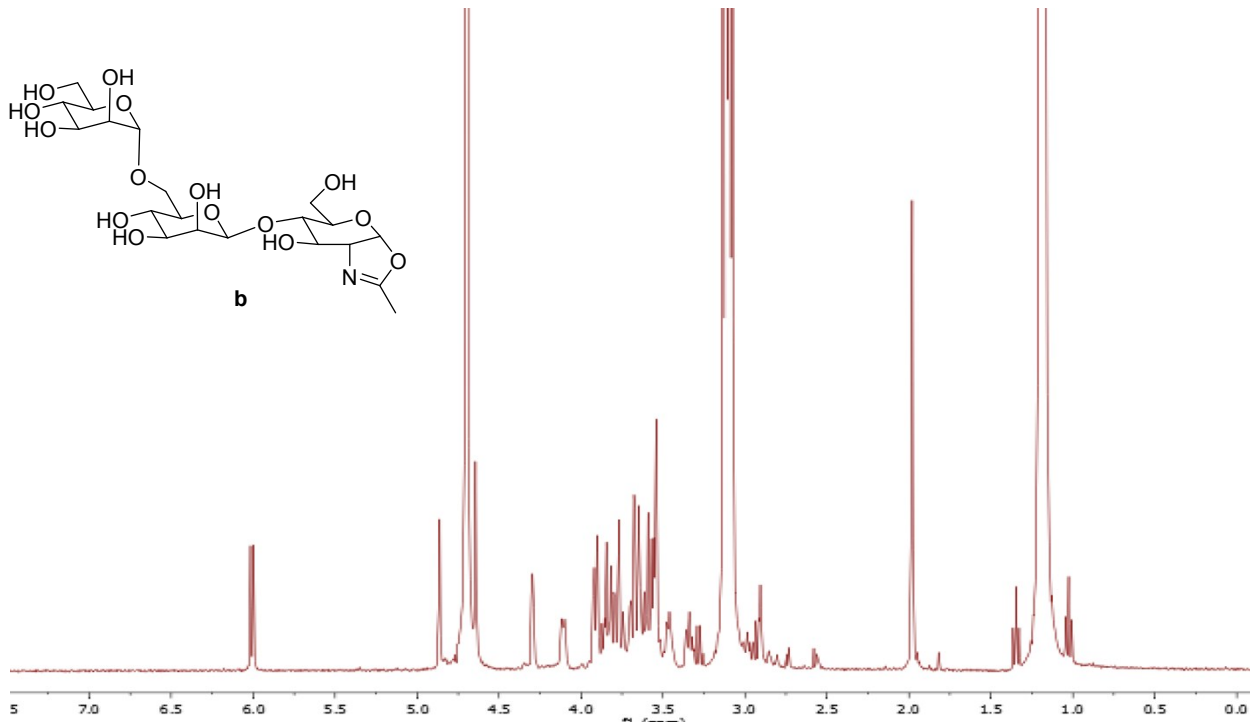




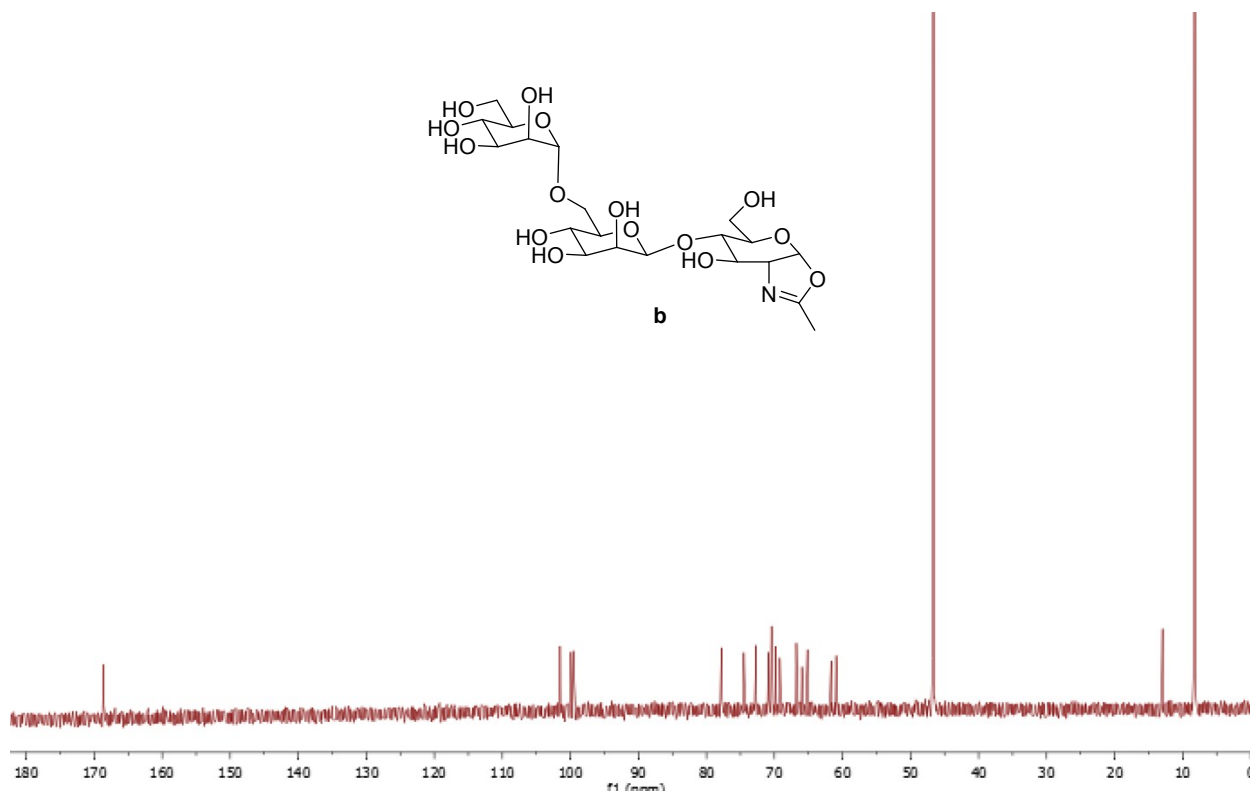
<sup>1</sup>H NMR of Compound 21



<sup>13</sup>C NMR of Compound 21

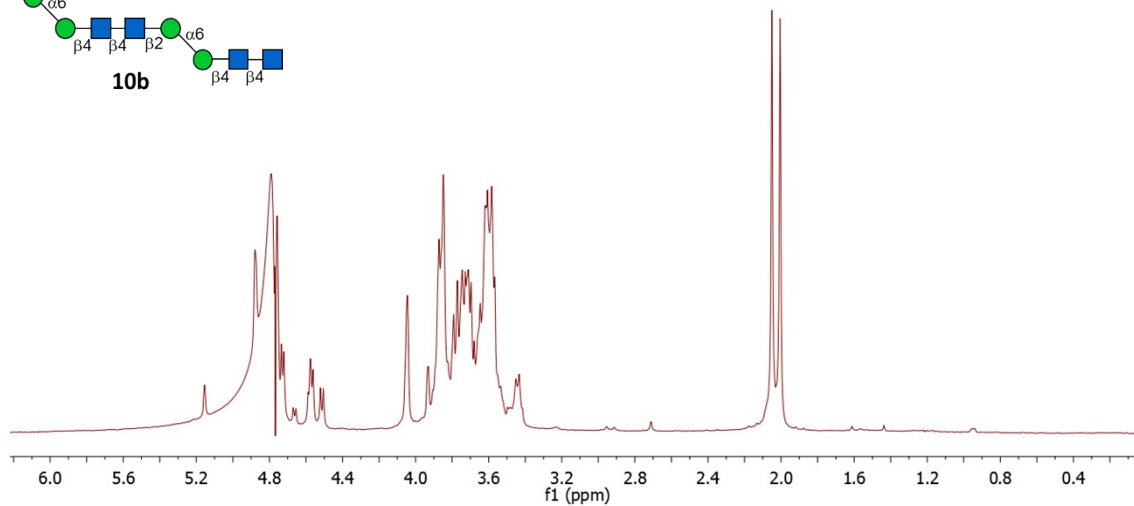
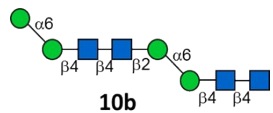


<sup>1</sup>H NMR of Compound **b**



<sup>13</sup>C NMR of Compound **b**

## X. NMR spectrum and data of purified *N*-glycan



$^1\text{H}$  NMR of Compound **10b**

$^1\text{H}$  NMR ( $\text{D}_2\text{O}$ , 500 MHz):  $\delta$  5.15 (s, 1 H), 4.88 (s, 1 H), 4.87 (s, 1 H), 4.73 (d,  $J = 6.6$  Hz, 1.5 H, GlcNAc-1 H-1 of  $\alpha$ -isomer), 4.66 (d,  $J = 7.5$  Hz, 1 H, GlcNAc-1 H-1 of  $\beta$ -isomer), 4.56-4.59 (m, 2 H), 4.51 (d,  $J = 8.0$  Hz, 1 H), 4.40 (brs, 2 H), 3.93 (brs, 1 H), 3.81-3.90 (m, 11 H), 3.67-3.79 (m, 14 H), 3.52-3.65 (m, 17 H), 3.42-3.47 (m, 3 H), 2.05 (s, 6 H, 2 Ac), 2.01 (s, 6 H, Ac).

## XI. References

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