Identification of the binding roles of terminal and internal glycan epitopes using enzymatically synthesized *N*-glycans containing tandem epitopes

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Supporting Information

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I. Materials and enzymes

N-Glycolylneuraminic acid (Neu5Gc) and cytidine 5'-triphosphate (CTP) were purchased from Carbosynth Limited. Cyanine 5 (Cy5)-streptavidin was from Invitrogen. FastAP was from Promega. Monoclonal anti-CD15s (sialyl Lewis x, sLe^x) antibody, and goat anti-mouse IgM-Alexa Fluor® 488 conjugate were purchased from ThermoFisher Scientific. Pronase E, monoclonal anti-CD15 (Lewis x, Le^x) antibody and other chemicals were purchased from Sigma. They were used without further purification. Biotinylated plant lectins were purchased from Vector Laboratories. Other enzymes including sialidase BiNanH2 from *Bifidobacterium longum* subsp. infantis ATCC15697,[1] CMP-sialic acid synthetase from *Neisseria meningitidis* (NmCSS),[2] double mutant E271F/R313Y of *Pasteurella multocida* α2–3-sialyltransferase 1 (PmST1 E271F/R313Y),[3] *Photobacterium damselae* α2–6-sialyltransferase (Pd2,6ST),[4] *Campylobacter jejuni* sialyltransferase (CjCstII, serves as an α2–8-sialyltransferase in this work),[5] β1–3-*N*-acetylglucosaminyltransferase from *N. meningitides* (NmLgtA),[6] β1–4-galactosyltransferase from *N. meningitides* (NmLgtB),[7] bovine α1–3-galactosyltransferase (Bα1,3GalT),[8] and C-terminal 66 amino acids truncated α1–3-fucosyltransferase from *Helicobacter pylori* (Hpα1,3FT)[9,10] were expressed and purified as previously described. Enzymes were then desalted against 50 mM Tris-HCl, and 10% glycerol, and stored at -20 °C for long term use. Sugar nucleotides uridine 5'-diphospho-galactose (UDP-Gal),[11] UDP-*N*-acetylglucosamine (UDP-GlcNAc)[12] and guanosine 5'-diphospho-L-fucose (GDP-Fuc)[12] were prepared as described previously.

II. General methods for glycan preparation

A) Preparation of sialylglycopeptide (SGP)

SGP was isolated from fresh chicken egg yolk by the method reported in Ref. 13.

B) Preparation of the core *N*-glycan

The core *N*-glycan was prepared by sequential digestion of SGP with pronase E and sialidase.

i. Digestion of SGP by pronase E

SGP (5 mg) in Tris-HCl (50 mM, pH 8.8) was digested by pronase E (5 mg) at 37 °C for 2 days until no residual SGP was detected by HPLC-HILIC-ELSD. The reaction was terminated by boiling for 5 min. Semi-praparative Waters XBridge BEH amide column (130 Å, 5 μm, 10 mm × 250 mm) was applied for purification. A gradient condition is solvent A: ammonium formate (100 mM, pH 3.4); solvent B: acetonitrile; flow rate: 4 mL/min; B%: 65–50% within 25 min.

The peak corresponding to **BA-01** was pooled and lyophilized twice to remove ammonium formate. **BA-01** (3.5 mg) was obtained as a white powder.

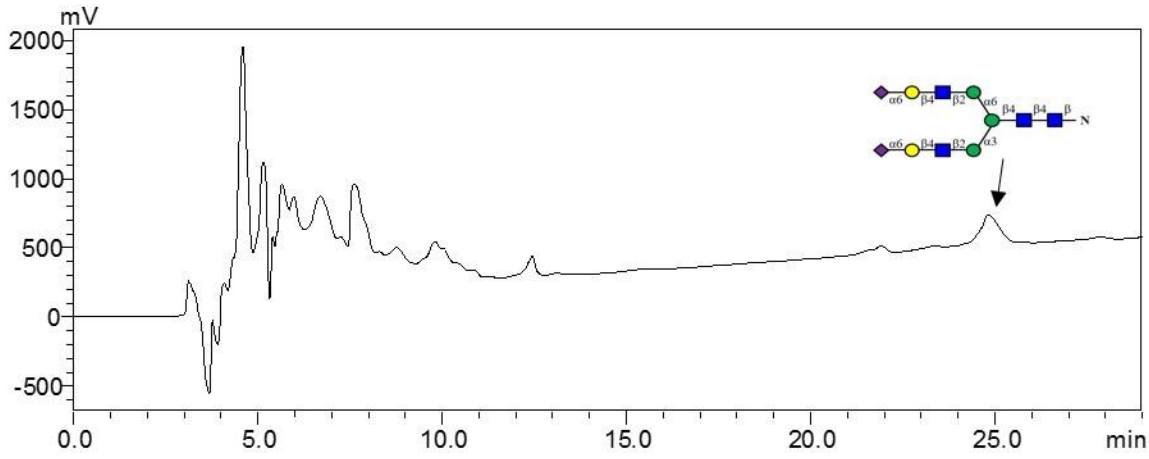


Fig. S1 HILIC-UV purification of **BA-01**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: ammonium formate (100 mM, pH 3.4); solvent B: acetonitrile; flow rate: 4 mL/min; B%: 65–50% within 25 min).

ii. Sequential digestion of SGP by pronase E and sialidase

SGP (100 mg) in Tris-HCl (50 mM, pH 8.8) was digested by 100 mg pronase E at 37 °C for 2 days until no SGP could be detected by HPLC-HILIC-ELSD. The pH was adjusted to 8.0 before sialidase BiNanH2 (5 mg) was added, and reaction was incubated at 37 °C overnight. Semi-praparative Waters XBridge BEH amide column (130 Å, 5 μm, 10 mm × 250 mm) was applied for purification. A gradient condition is solvent A: ammonium formate (100 mM, pH 3.4); solvent B: acetonitrile; flow rate: 4 mL/min; B%: 65–50% within 25 min. The peak corresponding to **BA-02** was pooled and lyophilized twice to remove ammonium formate. **BA-02** (54 mg) was obtained as a white powder.

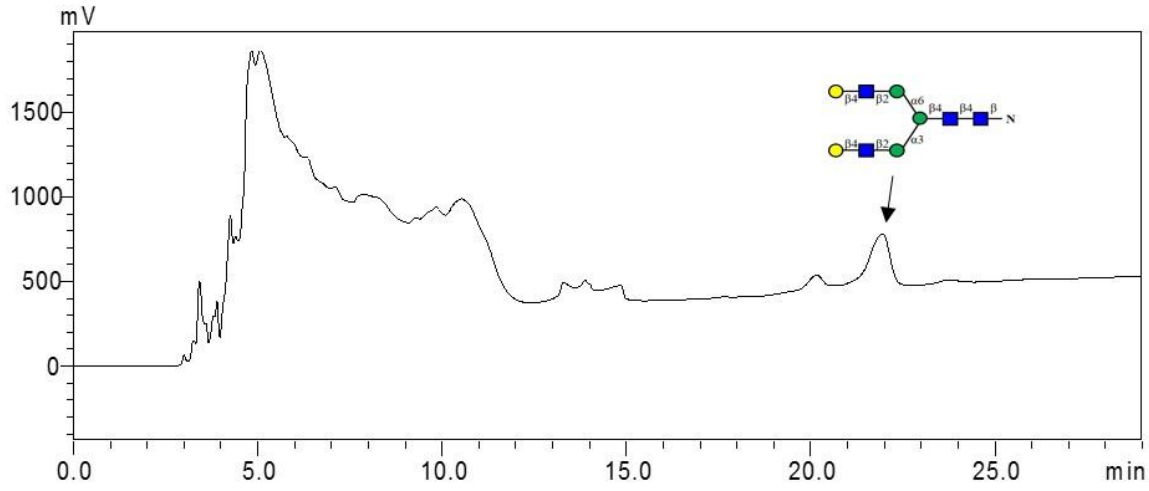


Fig. S2 HILIC-UV purification of **BA-02**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: ammonium formate (100 mM, pH 3.4); solvent B: acetonitrile; flow rate: 4 mL/min; B%: 65–50% within 25 min).

C) β1–3-GlcNAcylation catalyzed by NmLgtA

Reaction mixtures contained Tris-HCl (50 mM, pH 8.0), an acceptor glycan (4 mM), UDP-GlcNAc (8 mM), $MnCl_2$ (5 mM), and varying amounts of NmLgtA. FastAP (1 U/200 μL) was also added to digest the reaction byproduct UDP to drive reaction forward. Reactions were incubated at 37 °C for 2 days, and were monitored by MALDI-TOF. After all acceptor was converted, the reaction was quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-$A_{210\ nm}$ was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

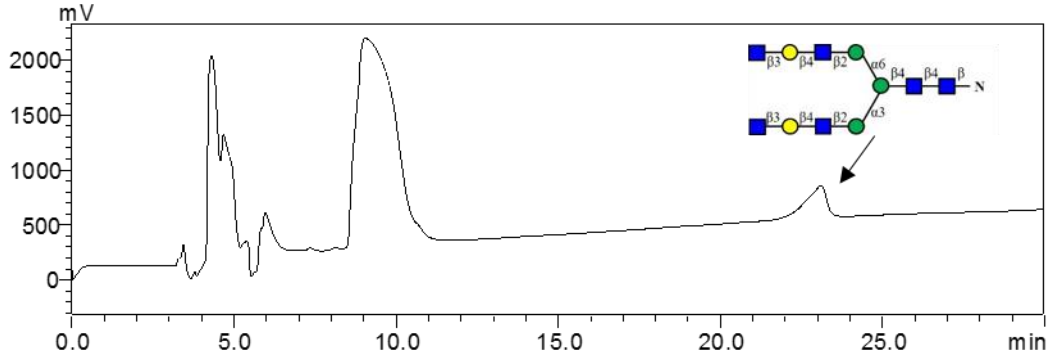


Fig. S3 HILIC-UV purification of **BA-02' (**product of LgtA reaction with **BA-02** as acceptor). Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: ammonium formate (100 mM, pH 3.4); solvent B: acetonitrile; flow rate: 4 mL/min; B%: 65–50% within 25 min).

D) β1–4-Galactosylation catalyzed by NmLgtB

Reaction mixtures contained Tris-HCl (50 mM, pH 8.0), an acceptor glycan (4 mM), UDP-Gal (8 mM), $MnCl_2$ (5 mM), and varying amounts of NmLgtB. FastAP (1 U/200 μL) was also added to digest the reaction byproduct UDP to drive reaction forward. Reactions were incubated at 37 °C overnight, and were monitored by MALDI-TOF. After all acceptor was converted, the reaction was quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-$A_{210\ nm}$ was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

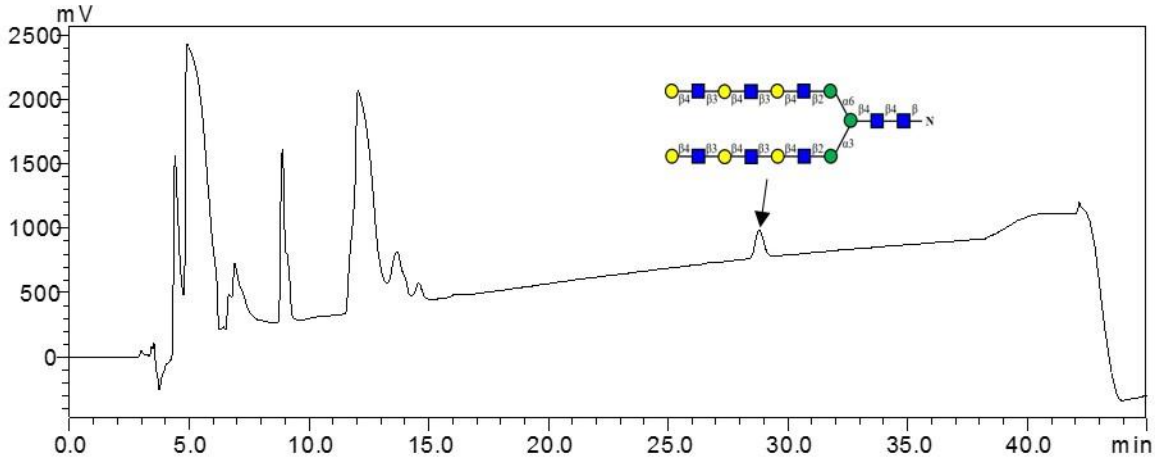


Fig. S4 HILIC-UV purification of **BA-25**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.4; solvent B: acetonitrile; flow rate: 4 mL/min; B%: 70-40% within 35 min).

E) α1–3-Galactosylation catalyzed by Bα1,3GalT

Reaction mixtures contained Tris-HCl (50 mM pH 8.0), 4 mM acceptor glycans, UDP-Gal (8 mM), $MnCl_2$ (5 mM), and varying amounts of Bα1,3GalT. FastAP (1 U/200 μL) was also added to digest the reaction byproduct UDP to drive reaction forward. Reactions were incubated at 37 °C overnight, and were monitored by MALDI-TOF. After all acceptor was converted, the reaction was quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-A_{210nm} was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

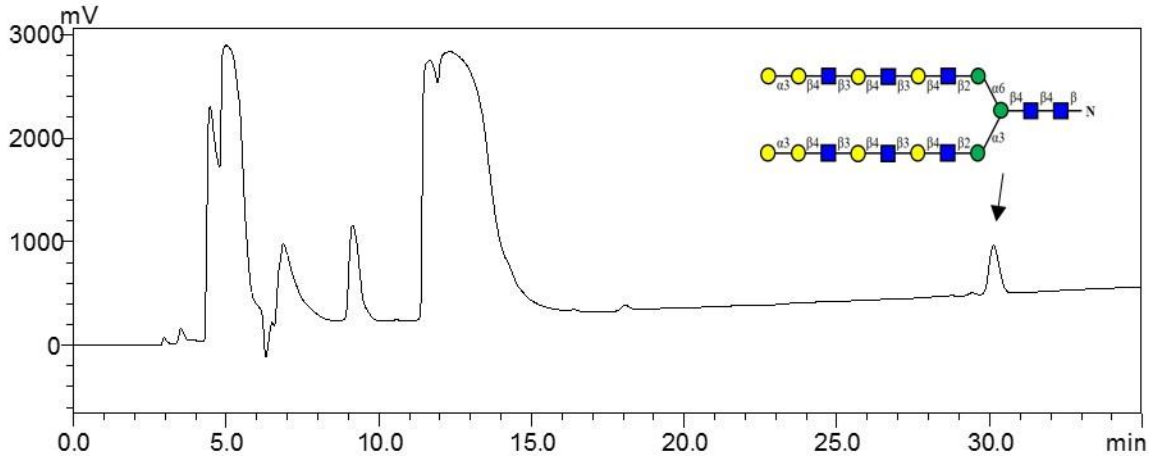


Fig. S5 HILIC-UV purification of **BA-27**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.4; solvent B: acetonitrile; flow rate: 4 mL/min; B%: 70-40% within 35 min).

F) α1–3-Fucosylation catalyzed by Hpα1,3FT

Reaction mixtures contained Tris-HCl (50 mM, pH 8.0), acceptor glycans (4 mM), GDP-Fuc (8 mM), $MnCl_2$ (5 mM), and varying amounts of Hpα1,3FT. FastAP (1 U/200 μL) was also added to digest the reaction byproduct GDP to drive reaction forward. Reactions incubated at 37 °C overnight, and monitored by MALDI-TOF. After all acceptor was converted, the reaction was quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-A_{210nm} was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

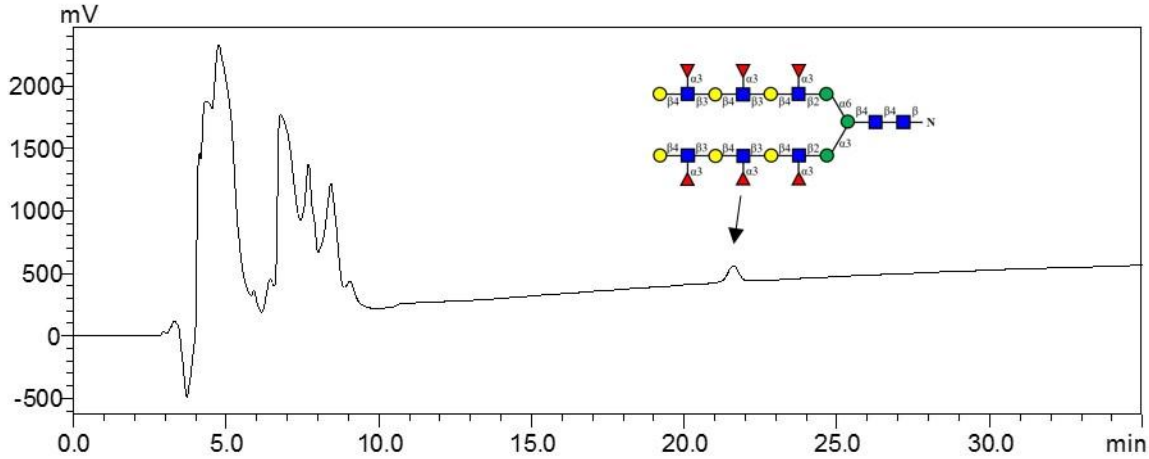
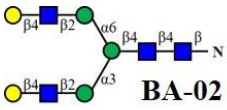
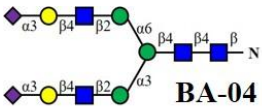
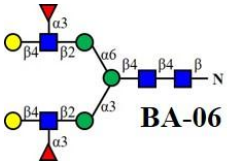
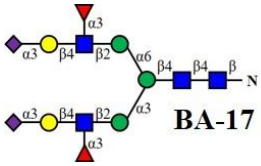
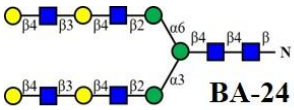
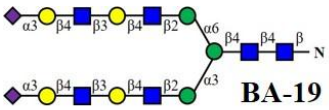
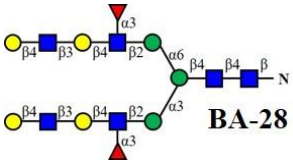
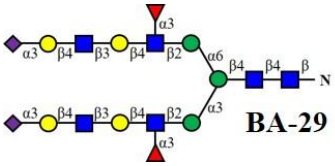


Fig. S6 HILIC-UV purification of **BA-34**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.4; solvent B: acetonitrile; flow rate: 4 mL/min; B%: 60-30% within 35 min).

G) α2–3-Sialylation catalyzed by PmST1 E271F/R313Y

Reaction mixtures contained Tris-HCl (100 mM, pH 8.0), acceptor glycans (4 mM), CTP (8 mM), Neu5Ac/Neu5Gc (8 mM), $MgCl_2$ (5 mM), NmCSS (5 μg/mL), and PmST1 E271F/R313Y (2 μg/mL). Reactions were incubated at 37 °C for 45 min and quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-A_{210nm} was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

Table S1 Acceptor substrate specificity of PmST1.

Acceptor	Product	Percentage Conversion (%)
 BA-02	 BA-04	71[a]
 BA-06	 BA-17	ND[b]
 BA-24	 BA-19	96[a]
 BA-28	 BA-29	99[a]

a. The percentage conversion is determined from HPLC-HILIC-ELSD data.

b. Not detected.

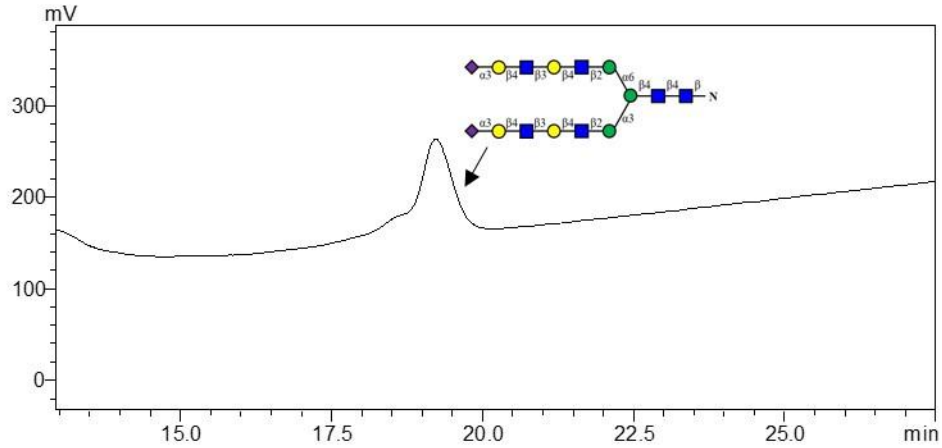


Fig. S7 HILIC-UV purification of **BA-19**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.4; solvent B: acetonitrile; flow rate: 4 mL/min; B%: 57-47% within 25 min).

H) α2–6-Sialylation catalyzed by Pd2,6ST

Reaction mixtures contained Tris-HCl (100 mM, pH 8.0), acceptor glycans (4 mM), CTP (8 mM), Neu5Ac/Neu5Gc (8 mM), $MgCl_2$ (5 mM), NmCSS (5 μg/mL), and Pd2,6ST (5 μg/mL). Reactions were incubated at 37 °C overnight and quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-A_{210nm} was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

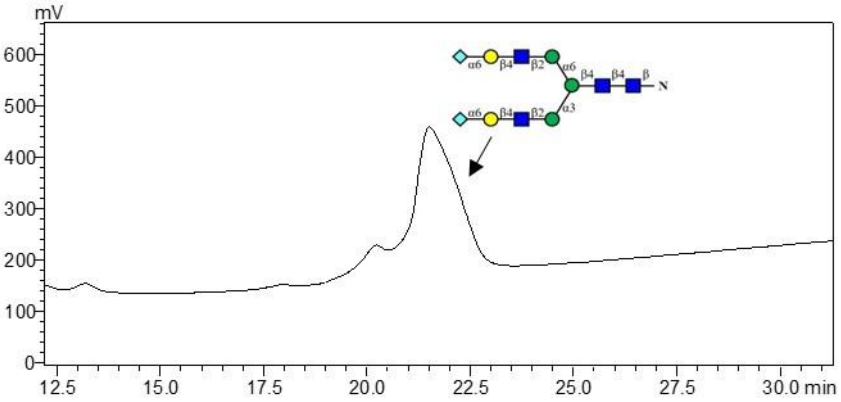
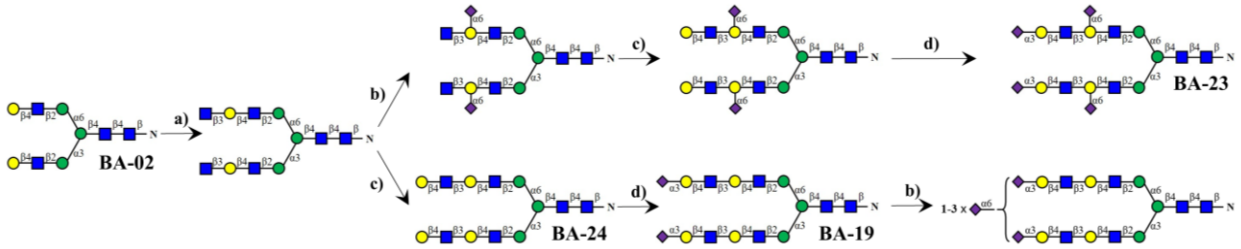


Fig. S8 HILIC-UV purification of **BA-03**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.4; solvent B: acetonitrile; flow rate: 4 mL/min; B%: 65-50% within 25 min).

Scheme S1 Synthetic scheme for **BA-23**.



I) α2–8-Sialylation catalyzed by CjCstII

Reaction mixtures contained Tris-HCl (100 mM, pH 8.0), acceptor glycans (4 mM), CTP (8 mM), Neu5Ac/Neu5Gc (8 mM), $MgCl_2$ (5 mM), NmCSS (5 μg/mL), and CjCstII (2 μg/mL). Reactions were incubated at 37 °C for 45 min and quenched by boiling for 5 min, followed by concentration using a vacufuge concentrator. HPLC-A_{210nm} was then used to purify target glycans using a semi-preparative amide column (130 Å, 5 μm, 10 mm × 250 mm).

Table S2 Conversion percentage of reactions catalyzed by NmCSS and CjCstII.

Acceptor	Donor	Product (2SA)	Yield (%)[a] (0SA)	Yield (%)[a] (1SA)	Yield (%)[a] (2SA)	Yield (%)[a] (3SA)
BA-01	CMP-Neu5Ac	**BA-09**	0	4	82	14
	CMP-Neu5Gc	**BA-10**	2	22	76	0
BA-03	CMP-Neu5Ac	**BA-11**	0	30	70	0
	CMP-Neu5Gc	**BA-12**	19	63	18	0
BA-04	CMP-Neu5Ac	**BA-13**	0	12	82	6
	CMP-Neu5Gc	**BA-14**	1	19	80	0
BA-05	CMP-Neu5Ac	**BA-15**	3	52	45	0
	CMP-Neu5Gc	**BA-16**	11	63	25	0

a. The percentage yield is calculated from HPLC-HILIC-ELSD data.

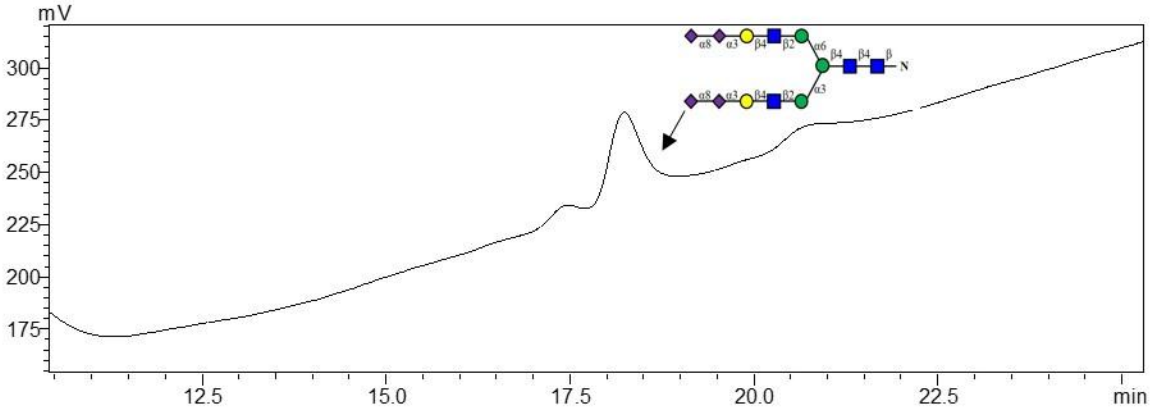


Fig. S9 HILIC-UV purification of **BA-13**. Waters XBridge BEH amide column was used (130 Å, 5 μm, 10 mm × 250 mm) under a gradient running condition (solvent A: 100 mM ammonium formate, pH 3.4; solvent B: acetonitrile; flow rate: 4 mL/min; B%: 60-45% within 35 min).

J) Summary of one-pot enzymatic extension of glycans

The sequences of one-pot enzymatic extension for glycan synthesis are summarized in **Table S3**.

Table S3 Sequential one-pot enzymatic extension of glycans.

Acceptor	Donor and Enzyme	Product	Yield
BA-02	c	**BA-06**	>99%
	d	**BA-07**	>99%
	d+c	**BA-08**	>99%
	a+b	**BA-24**	>99%
	a+b+a+b	**BA-25**	>99%
	a+b+d	**BA-26**	>99%
	a+b+a+b+d	**BA-27**	>99%
	a+c+b	**BA-28**	>99%
	a+b+c	**BA-30**	>99%
	a+c+b+d	**BA-31**	>99%
	a+b+c+d	**BA-32**	>99%
	a+b+a+c+b	**BA-33**	>99%
	a+b+a+b+c	**BA-34**	>99%
	a+b+a+c+b+d	**BA-35**	>99%
	a+b+a+b+c+d	**BA-36**	>99%

(a) UDP-GlcNAc, Mn^{2+} and NmLgtA; (b) UDP-Gal, Mn^{2+} and NmLgtB; (c) GDP-Fuc, Mn^{2+} and Hpα1,3FT; (d) UDP-Gal, Mn^{2+} and Bα1,3GalT. The plus sign denotes the subsequent reactions were performed without purification of the product from the previous reaction.

III. General methods for HPLC-HILIC-UV purification of *N*-glycans

A) General methods for HILIC-UV210 purification of *N*-glycans

1) **BA-01**, **BA-02**, **BA-03**, **BA-04**, **BA-05**, **BA-06**, **BA-07**.
Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 10 mm × 250 mm
Solvent A: 100 mM ammonium formate, pH 3.4
Solvent B: Acetonitrile
Temperature: 40 °C

Gradient elution:	Time (min)	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_{210nm}

2) **BA-08**, **BA-17**, **BA-18**, **BA-24**, **BA-25**, **BA-26**, **BA-27**, **BA-28**, **BA-29**, **BA-30**, **BA-31**, **BA-32**, **BA-33**, **BA-34.**
Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 10 mm × 250 mm
Solvent A: 100 mM ammonium formate, pH 3.4
Solvent B: Acetonitrile
Temperature: 40 °C

Gradient elution:	Time (min)	B%	Flow rate (mL/min)
	0	70	4
	35	40	4
	36	0	2
	37	0	2
	38	70	4
	45	70	4

Monitor: A_{210nm}

3) **BA-09**, **BA-10**, **BA-11**, **BA-12**, **BA-13**, **BA-14**, **BA-15**, **BA-16.**
Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 10 mm × 250 mm
Solvent A: 100 mM ammonium formate, pH 3.4
Solvent B: Acetonitrile
Temperature: 40 °C

Gradient elution:	Time (min)	B%	Flow rate (mL/min)
	0	60	4
	35	45	4
	36	0	2
	37	0	2
	38	60	4
	45	60	4

Monitor: A_{210nm}

4) **BA-19**, **BA-20**, **BA-21**, **BA-22**, **BA-23**.

Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 10 mm × 250 mm
Solvent A: 100 mM ammonium formate, pH 3.4
Solvent B: Acetonitrile
Temperature: 40 °C

Gradient elution:	Time (min)	B%	Flow rate (mL/min)
	0	57	4
	35	47	4
	36	0	2
	37	0	2
	38	57	4
	45	57	4

Monitor: A_{210nm}

5) **BA-35**, **BA-36**.

Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 10 mm × 250 mm
Solvent A: 100 mM ammonium formate, pH 3.4
Solvent B: Acetonitrile
Temperature: 40 °C

Gradient elution:	Time (min)	B%	Flow rate (mL/min)
	0	60	4
	35	30	4
	36	0	2
	37	0	2
	38	70	4
	45	70	4

Monitor: A_{210nm}

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

Column: Waters XBridge BEH amide column, 130 Å, 5 μm, 4.6 mm × 250 mm
Solvent A: 100 mM ammonium formate, pH 3.4
Solvent B: Acetonitrile
Temperature: 40 °C

Gradient elution:	Time (min)	B%	Flow rate (mL/min)
	0	60	1
	35	30	1
	36	0	0.5
	37	0	0.5
	38	60	1
	45	60	1

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

IV. General methods for mass spectrometry analysis

A) ESI-MS analysis of *N*-glycans

In this study, HPLC-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

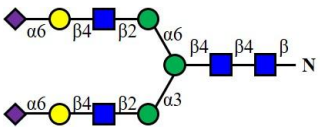
MALDI-TOF MS analyses were performed on UltrafleXtreme MALDI TOF/TOF Mass Spectrometer (Bruker). Scan range of MS^1 was set according to the molecular weight of *N*-glycans, and reflector mode was used for *N*-glycan analysis. Mass spectra were obtained in both positive and negative extraction mode with 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. General methods for glycan microarray assay

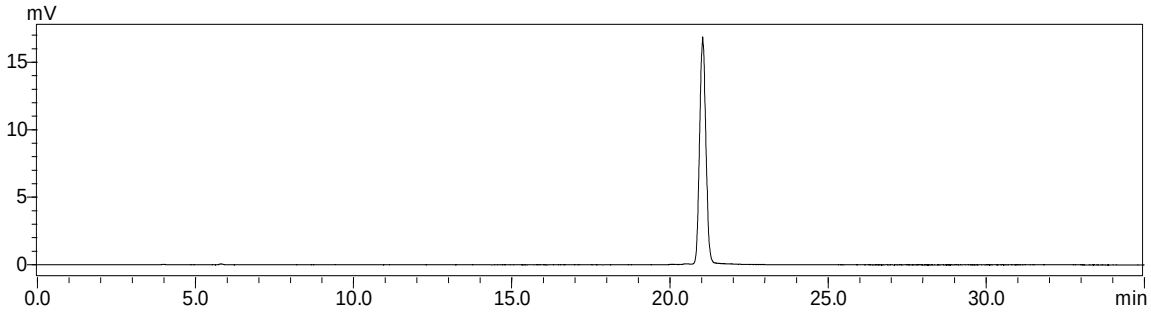
The glycans were printed on *N*-hydroxysuccinimide (NHS)-derivatized slides, each in replicates of 6 in a subarray, and 8 subarrays were printed on each slide. Contact printing was performed using 2470 Arrayer (Aushon Biosystems). All glycans were prepared at a concentration of 100 μM in phosphate buffer (100 mM sodium phosphate buffer, pH 8.5). The slides were fitted with 8-chamber adapter to separate the subarray into individual well for assay. Before assay, slides were rehydrated for 5 min in TSMW buffer (20 mM Tris-HCl, 150 mM NaCl, 0.2 mM $CaCl_2$, and 0.2 mM $MgCl_2$, 0.05% Tween). And then the analysis was performed for plant lectins, antibodies, and viruses as previously described.[14,15] Eight subarrays were assayed with *Maackia amurensis* lectin I (MAL-I, binds NeuAcα2–3-Galβ1–4GlcNAc, 10 μg/mL),[16] *Sambucus nigra* lectin (SNA, binds NeuAcα2–6Galβ1–4GlcNAc, 10 μg/mL),[17] *Erythrina cristagalli* lectin (ECL, binds Galβ1–4GlcNAc, 10 μg/mL),[18] B subunit of *Griffonia simplicifolia* lectin I (GSL-I-B_4, binds α-Gal, 10 μg/mL),[19] and wheat germ agglutinin (WGA, binds GlcNAc and sialic acids, 0.5 μg/mL, 2 μg/mL, 5 μg/mL, and 10 μg/mL).[20,21] The biotinylated lectins were detected by Cy5-streptavidin (1 μg/mL). Two subarrays were assayed with anti-CD15 antibody (binds Le^x epitope, 5 μg/mL) and anti-CD15s antibody (binds sLe^x epitope, 20 μg/mL), respectively. The primary antibodies were then bound by goat anti-mouse IgM-Alexa Fluor® 488 conjugate (5 μg/mL). Three subarrays were assayed with three influenza A viruses, including swine, avian and human vaccine strains.[15] All viruses were labeled with Alexa Fluor 488 as previously described,[15,22] and were incubated on the slide at 4 °C for 1 hour. The slides were scanned with a microarray scanner (InnoScan 1100 AL).

VI. HPLC profiles, MS and NMR data of purified *N*-glycans

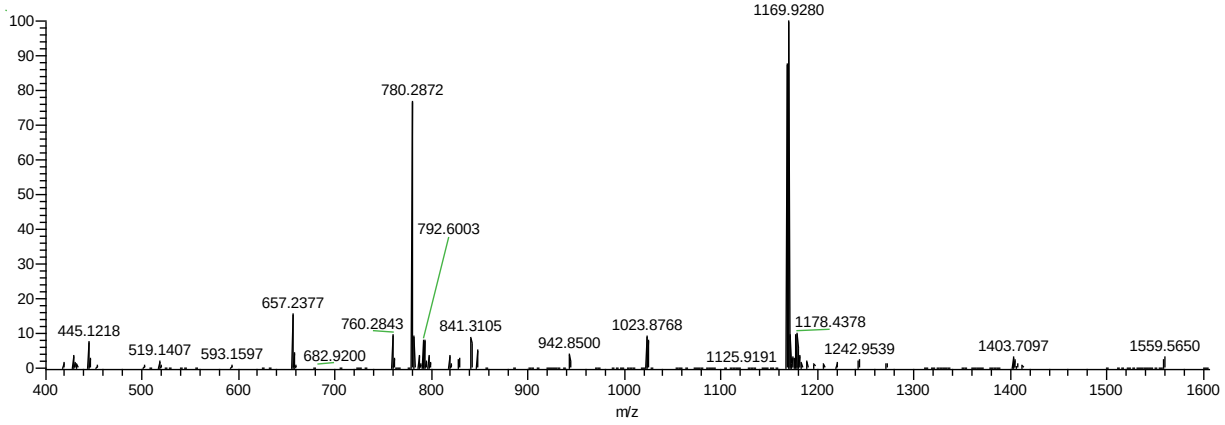
BA-01



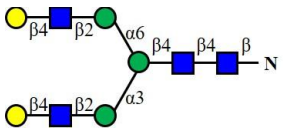
HILIC-ELSD, T_R = 21.034 min.



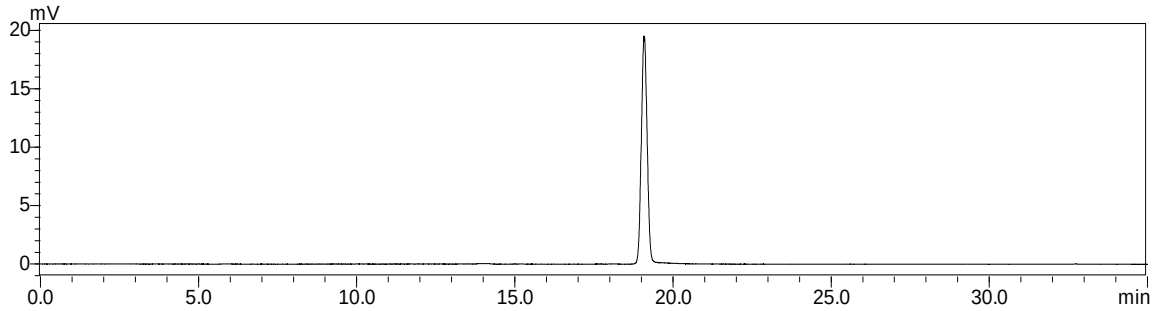
ESI-MS, calculated 2337.9003; found $[M+3H]^{3+}$ 780.2872, $[M+2H]^{2+}$ 1169.9280.



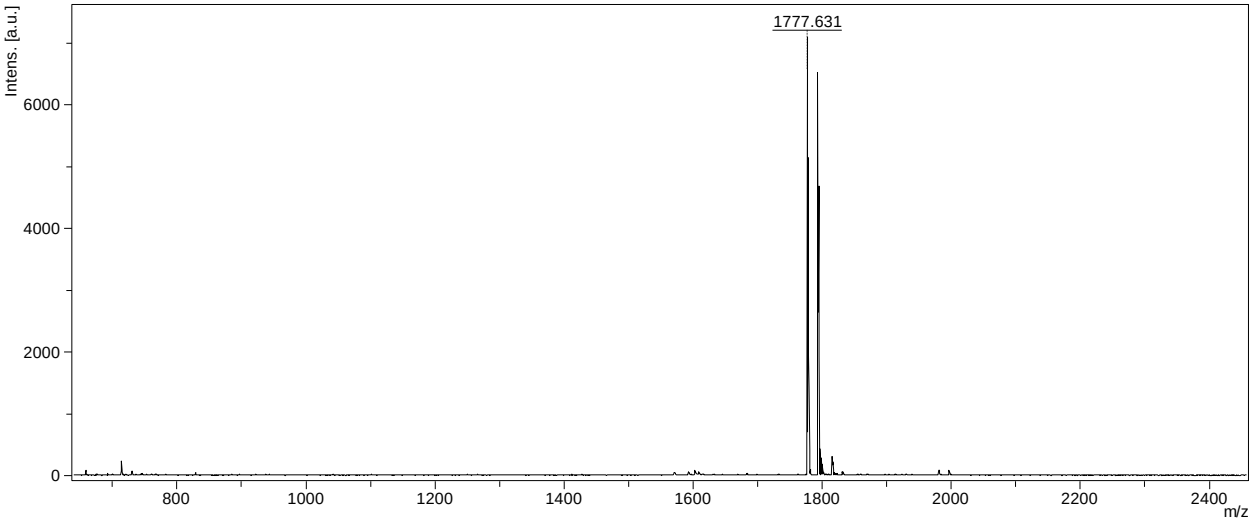
^{1}H NMR (D_2O, 500 MHz): δ 5.14 (s, 1 H, Man4 H-1), 5.08 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.78 (s, 1 H, Man3 H-1), 4.60-4.63 (m, 3 H, GlcNAc 2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.45 (d, *J* = 7.3 Hz, 2 H, Gal6 H-1, Gal6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.21 (brs, 1 H, Man4 H-2), 4.12 (brs, 1 H, Man4' H-2), 3.50-4.02 (m, 66 H), 2.94 (dd, *J* = 17.3, 4.2 Hz, 1 H, Asn-βCH_2), 2.87 (d, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.65-2.69 (m, 2 H, Neu5Ac H-3eq), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, 2 Ac), 2.04 (s, 6 H, 2 Ac), 2.02 (s, 3 H, Ac), 1.73 (t, *J* = 12.2 Hz, 2 H, Neu5Ac H-3ax).

BA-02 

HILIC-ELSD, T_R = 19.076 min.

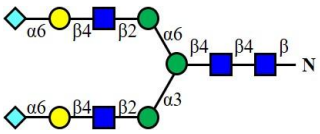


MALDI-MS, calculated 1755.7094; found $[M+Na]^+$ 1777.631, $[M+K]^+$ 1793.619.

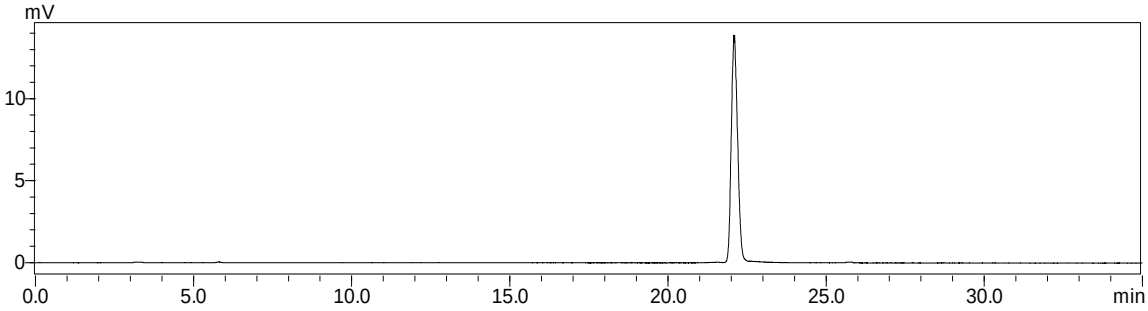


^{1}H NMR (D_2O, 500 MHz): δ5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.66 (d, *J* = 7.8 Hz, 1 H, GlcNAc 2 H-1), 4.62 (d, *J* = 7.8 Hz, 2 H, GlcNAc5 H-1, GlcNAc5' H-1), 4.51 (d, *J* = 7.8 Hz, 1 H, Gal6 H-1), 4.50 (d, *J* = 7.8 Hz, 1 Gal6' H-1), 4.29 (d, *J* = 1.8 Hz, 1 H, Man3 H-2), 4.23 (d, *J* = 1.7 Hz, 1 H, Man4 H-2), 4.15 (d, *J* = 1.8 Hz, 1 H, Man4' H-2), 3.51-4.04 (m, 52 H), 2.98 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (d, *J* = 17.2, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.05 (s, 3 H, Ac).

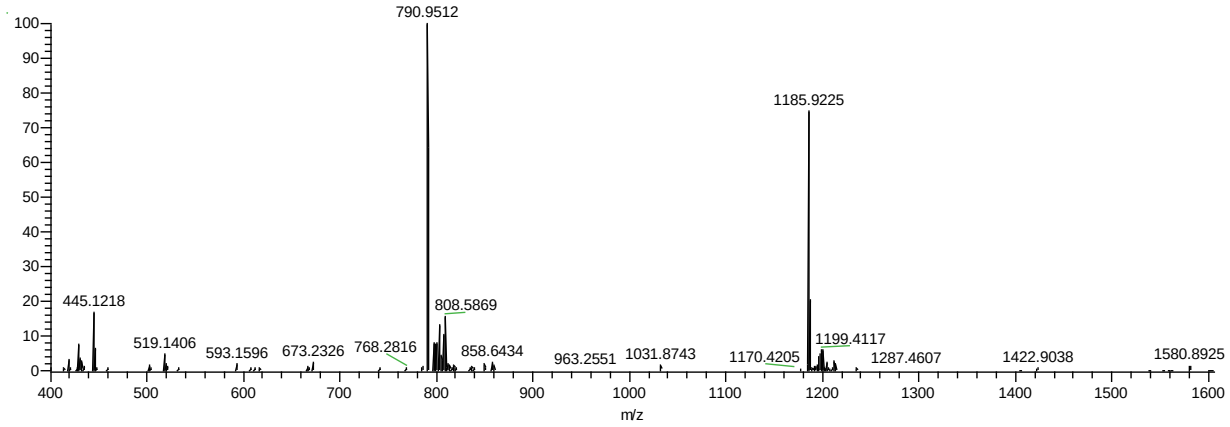
BA-03



HILIC-ELSD, T_R = 22.080 min.

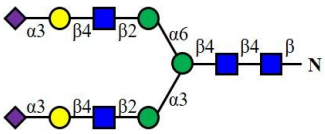


ESI-MS, calculated 2369.8901; found $[M+3H]^{3+}$ 790.9512, $[M+2H]^{2+}$ 1185.9225.

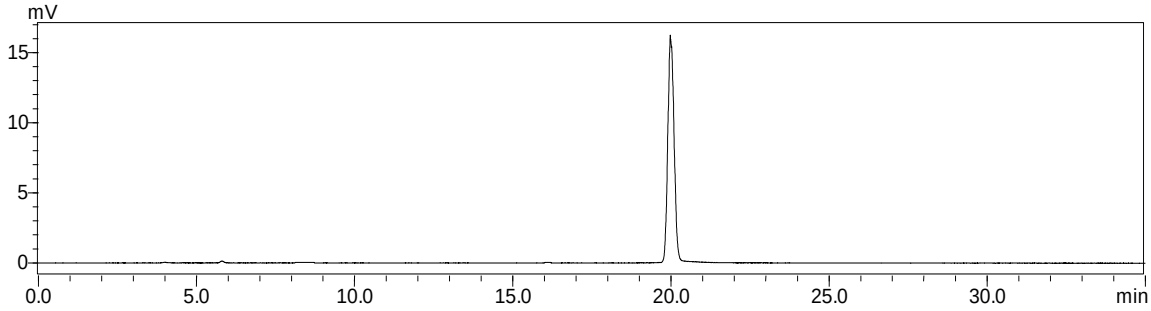


^{1}H NMR (D_2O, 500 MHz): δ 5.18 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.99 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.46-4.66 (m, 3 H, GlcNAc 2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.50 (d, *J* = 7.9 Hz, 1 H, Gal6 H-1), 4.49 (d, *J* = 7.8 Hz, 1 H, Gal6' H-1), 4.30 (s, 1 H, Man3 H-2), 4.24 (d, *J* = 2.2 Hz, 1 H, Man4 H-2), 4.16 (brs, 4 H, Man4' H-2), 3.52-4.06 (m, 70 H), 2.98 (dd, *J* = 17.3, 4.2 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.71-2.75 (m, 2 H, Neu5Gc H-3eq), 2.16 (s, 3 H, Ac), 2.12 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 1.78 (t, *J* =12.3 Hz, 2 H, Neu5Gc H-3ax).

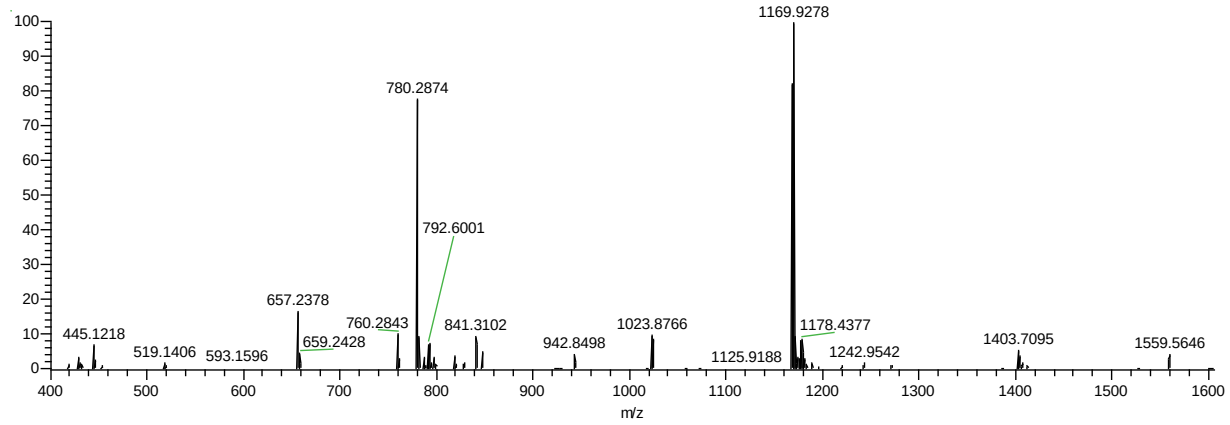
BA-04



HILIC-ELSD, T_R = 19.947 min.

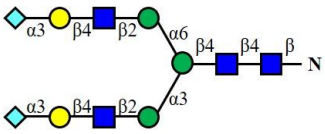


ESI-MS, calculated 2337.9003; found $[M+3H]^{3+}$ 780.2874, $[M+2H]^{2+}$ 1169.9278.

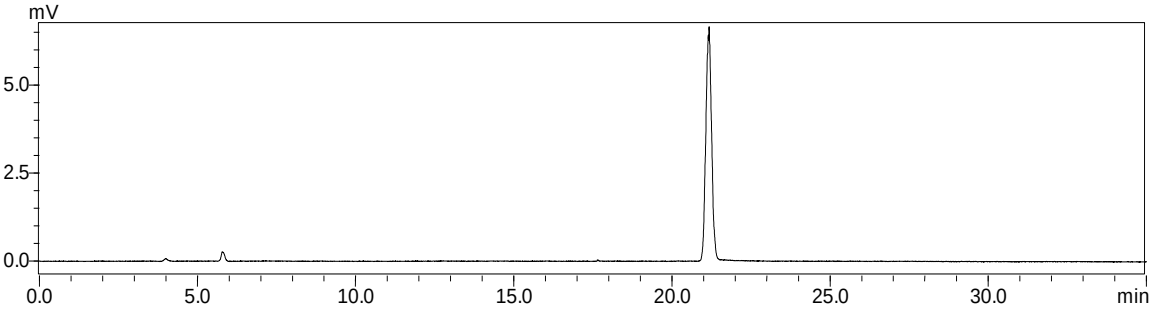


^{1}H NMR (D_2O, 500 MHz): δ5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.66 (d, *J* = 7.9 Hz, 1 H, GlcNAc1 H-1), 4.58-4.62 (m, 4 H, GlcNAc5 H-1, GlcNAc5' H-1, Gal6 H-1, Gal6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.23 (d, *J* = 1.9 Hz, 1 H, Man4 H-2), 4.15-4.16 (m, 3 H), 3.51-4.04 (m, 64 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.80 (dd, *J* = 12.4, 4.3 Hz, 2 H, Neu5Ac H-3eq), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 3 H, 2 Ac), 2.06 (s, 3 H, Ac), 1.84 (t, *J* =12.4 Hz, 2 H, Neu5Ac H-3ax).

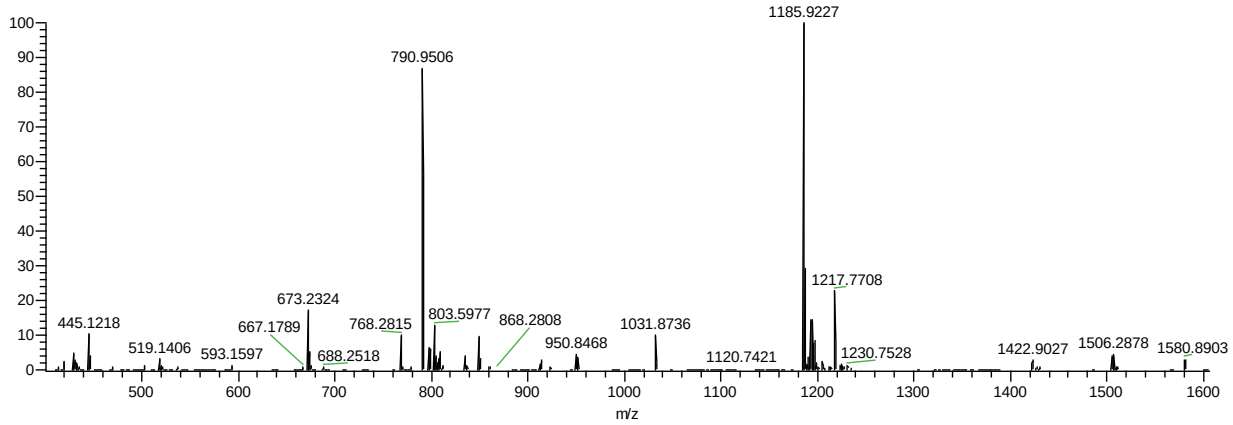
BA-05



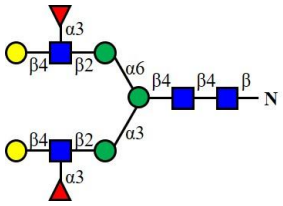
HILIC-ELSD, T_R = 21.148 min.



ESI-MS, calculated 2369.8901; found $[M+3H]^{3+}$ 790.9506, $[M+2H]^{2+}$ 1185.9227.

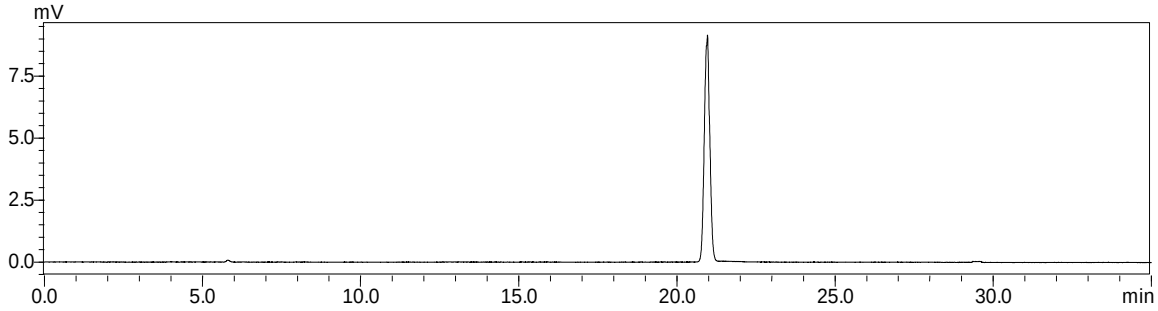


^{1}H NMR (D_2O, 500 MHz): δ 5.14 (s, 1 H, Man4 H-1), 5.07 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.78 (s, 1 H, Man3 H-1), 4.59-4.62 (m, 3 H, GlcNAc1 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.45 (d, *J* = 7.4 Hz, 2 H, Gal6 H-1, Gal6' H-1), 4.26 (brs, 1 H, Man3 H-2), 4.20 (brs, 1 H, Man4 H-2), 4.12 (brs, 4 H), 3.50-4.03 (m, 67 H), 2.94 (dd, *J* = 17.3, 4.1 Hz, 1 H, Asn-βCH_2), 2.87 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.66-2.72 (m, 2 H, Neu5Gc H-3eq), 2.09 (s, 3 H, Ac), 2.08 (s, 6 H, 2 Ac), 2.02 (s, 3 H, Ac), 1.75 (t, *J* =12.2 Hz, 2 H, Neu5Gc H-3ax).

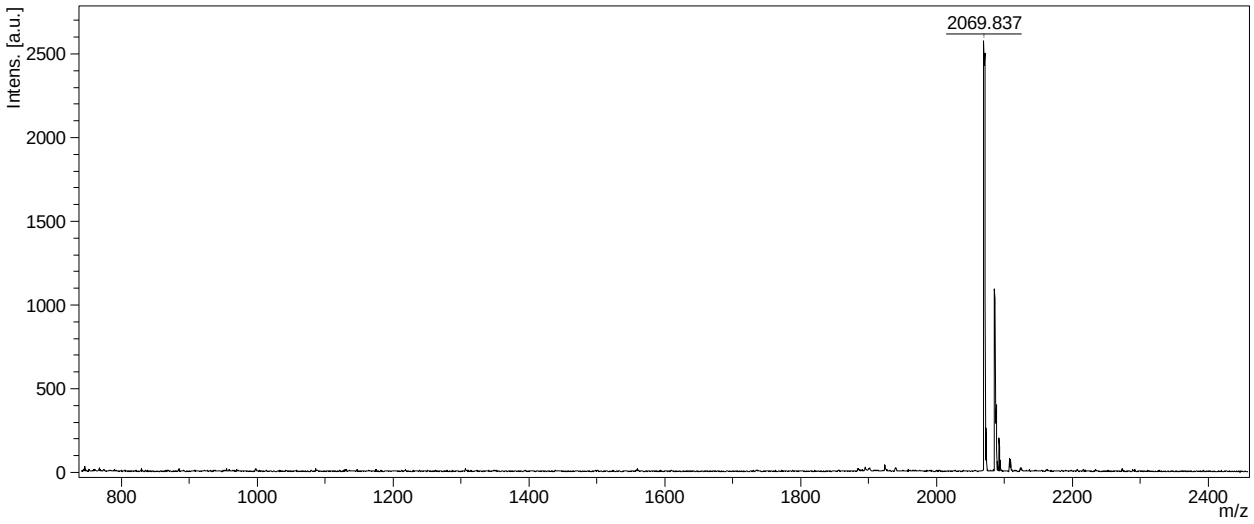


BA-06

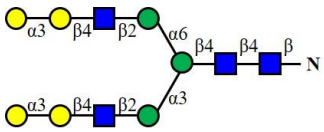
HILIC-ELSD, T_R = 20.965 min.



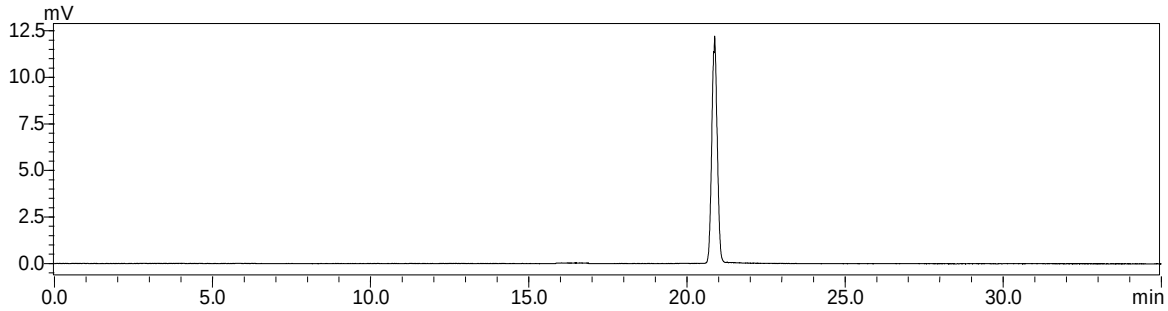
MALDI-MS, calculated 2047.8253; found $[M+Na]^+$ 2069.837, $[M+K]^+$ 2085.819.



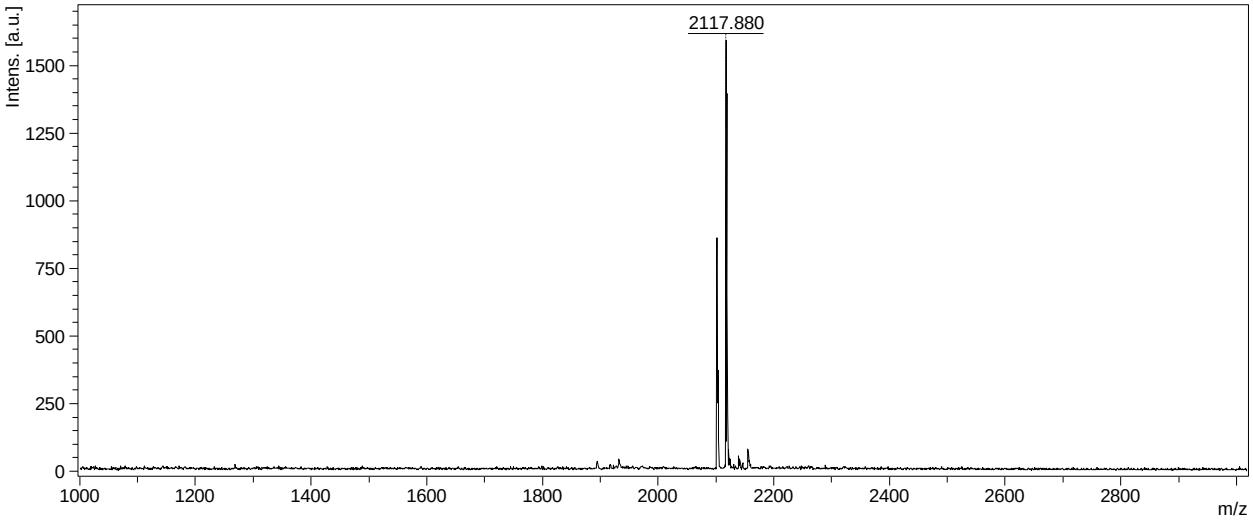
^{1}H NMR (D_2O, 500 MHz): δ 5.17 (d, *J* = 3.4 Hz, 1 H, Fuc H-1), 5.16 (d, *J* = 3.5 Hz, 1 H, Fuc H-1), 5.14 (s, 1 H, Man4 H-1), 5.10 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.85-4.88 (m, 2 H, Man3 H-1, GlcNAc1 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc5 H-1), 4.62 (d, *J* = 8.1 Hz, 1 H, GlcNAc5' H-1), 4.49 (d, *J* = 7.7 Hz, 1 H, Gal6 H-1), 4.48 (d, *J* = 7.6 Hz, 1 H, Gal6' H-1), 4.29 (brs, 1 H), 4.22 (d, *J* = 2.0 Hz, 1 H), 4.14 (d, *J* = 2.2 Hz, 1 H), 3.50-4.04 (m, 60 H), 2.96 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.89 (dd, *J* = 17.2, 6.9 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 6 H, 2 Ac), 2.06 (s, 3 H, Ac), 1.22 (d, *J* = 6.4 Hz, 3 H, Fuc CH_3), 1.21 (d, *J* = 6.4 Hz, 3 H, Fuc CH_3).

BA-07 

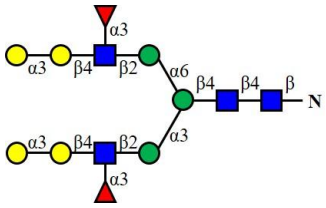
HILIC-ELSD, T_R = 20.877 min.



MALDI-MS, calculated 2079.8151; found $[M+Na]^+$ 2101.899, $[M+K]^+$ 2117.880.

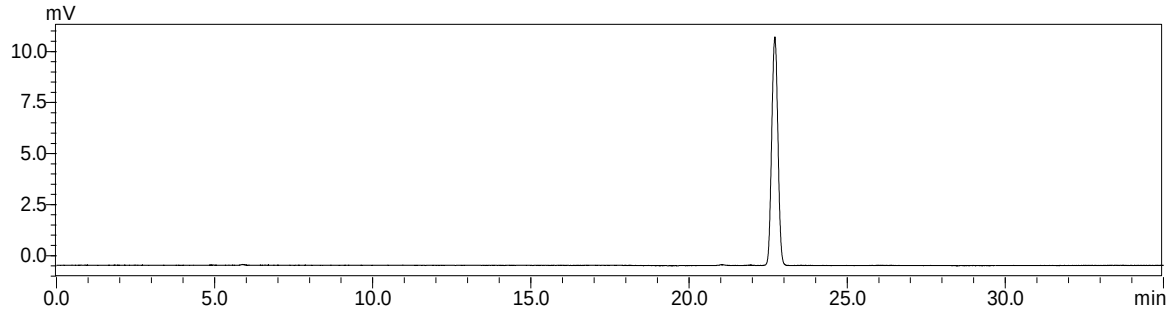


^{1}H NMR (D_2O, 500 MHz): δ 5.12 (s, 1 H, Man4 H-1), 5.08 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.93 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.69 (d, *J* = 8.4 Hz, 2 H, GlcNAc1 H-1, GlcNAc5 H-1), 4.62 (d, *J* = 7.9 Hz, 1 H,GlcNAc5' H-1), 4.58 (d, *J* = 7.6 Hz, 2 H, Gal6 H-1, Gal6' H-1), 4.47 (d, *J* = 7.5 Hz, 1 H, Gal7 H-1), 4.46 (d, *J* = 7.6 Hz, 1 H, Gal7' H-1), 4.26 (brs, 1 H, Man3 H-2), 4.19 (brs, 1 H, Man4 H-2), 4.16 (brs, 2 H), 4.11 (brs, 1 H), 3.46-4.00 (m, 61 H), 2.94 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.87 (dd, *J* = 17.2, 6.8 Hz, 1 H, Asn-βCH_2), 2.08 (s, 3 H, Ac), 2.05 (s, 6 H, 2 Ac), 2.02 (s, 3 H, Ac).

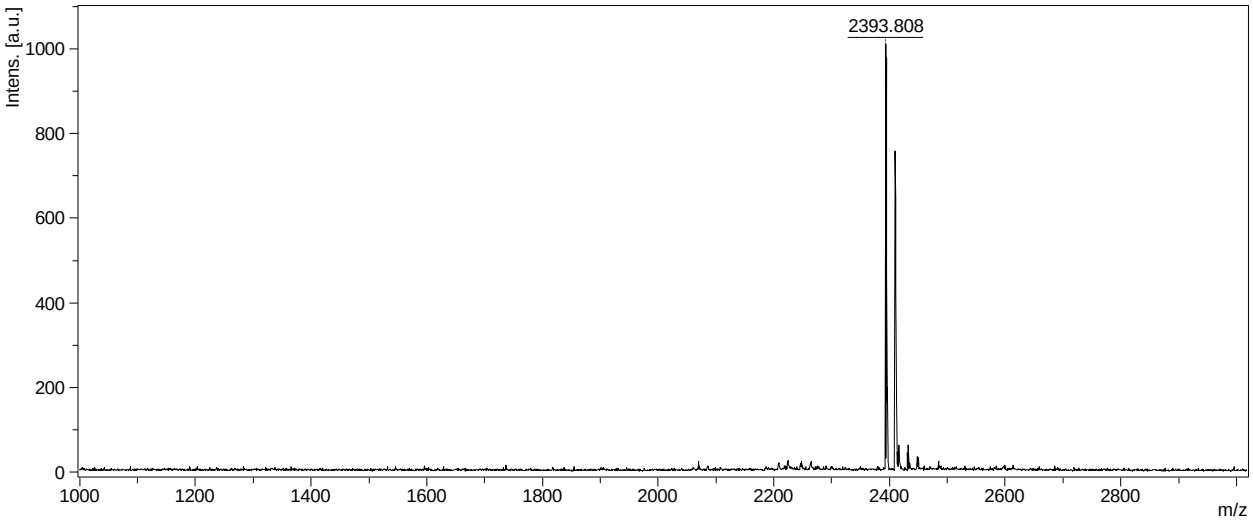


BA-08

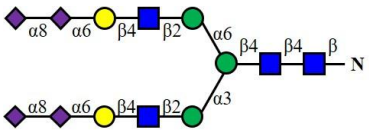
HILIC-ELSD, T_R = 22.716 min.



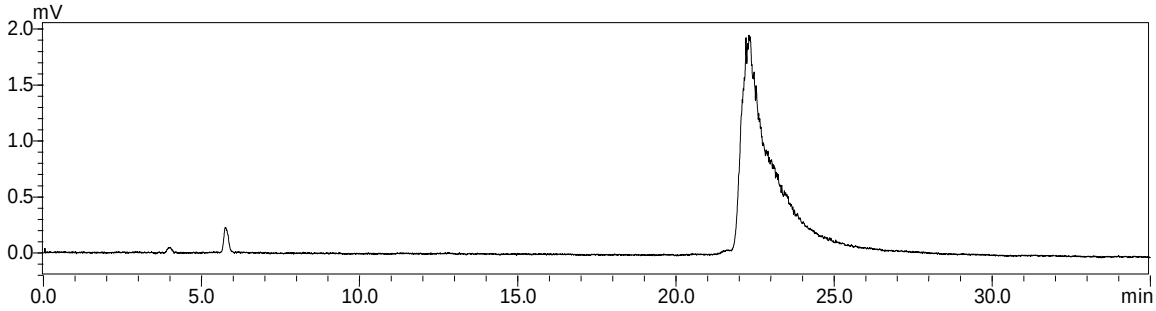
MALDI-MS, calculated 2371.9309; found $[M+Na]^+$ 2393.808, $[M+K]^+$ 2409.785.



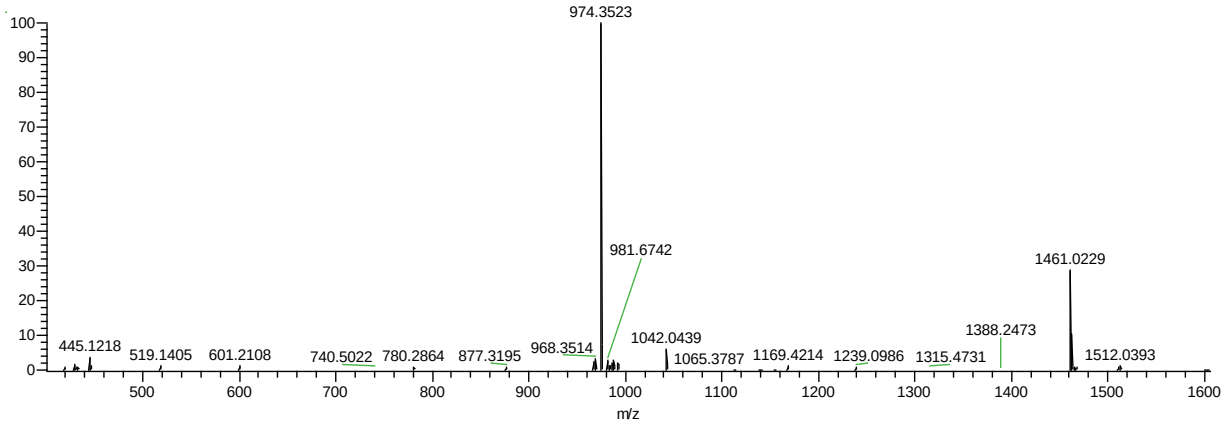
^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, *J* = 3.5 Hz, 2 H, Fuc H-1), 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.85-4.87 (m, 2 H, GlcNAc2 H-1, GlcNAc5 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.62-4.66 (m, 3 H, GlcNAc5' H-1, Gal6 H-1, Gal6' H-1), 4.56 (d, *J* = 7.7 Hz, 1 H, Gal7 H-1), 4.55 (d, *J* = 7.7 Hz, 1 H, Gal7 H-1), 4.29 (brs, 1 H, Man3 H-2), 4.20-4.24 (m, 5 H), 4.14 (d, *J* = 2.3 Hz, 1 H), 3.50-4.06 (m, 68 H), 2.98 (dd, *J* = 17.2, 4.4 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.2, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.09 (s, 6 H, 2 Ac), 2.08 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.23 (d, *J* = 6.3 Hz, 3 H, Fuc CH_3), 1.22 (d, *J* = 6.3 Hz, 3 H, Fuc CH_3).

BA-09 

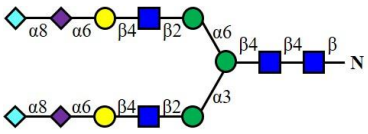
HILIC-ELSD, T_R = 22.309 min.



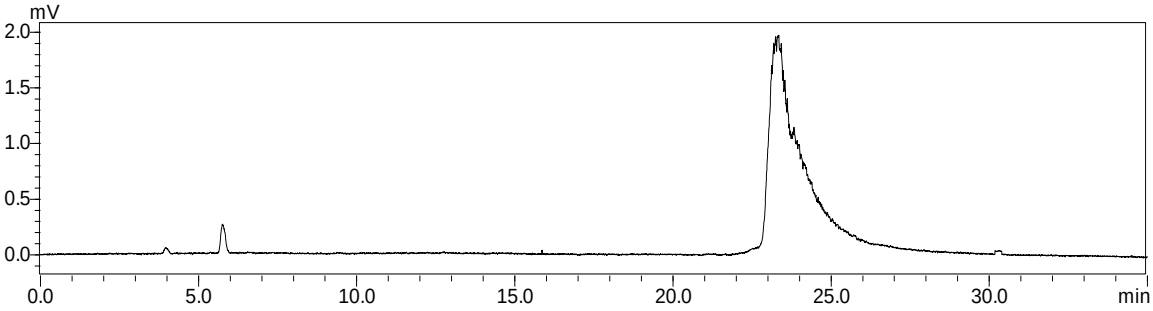
ESI-MS, calculated 2920.0911; found $[M+3H]^{3+}$ 974.3523, $[M+2H]^{2+}$ 1461.0229.



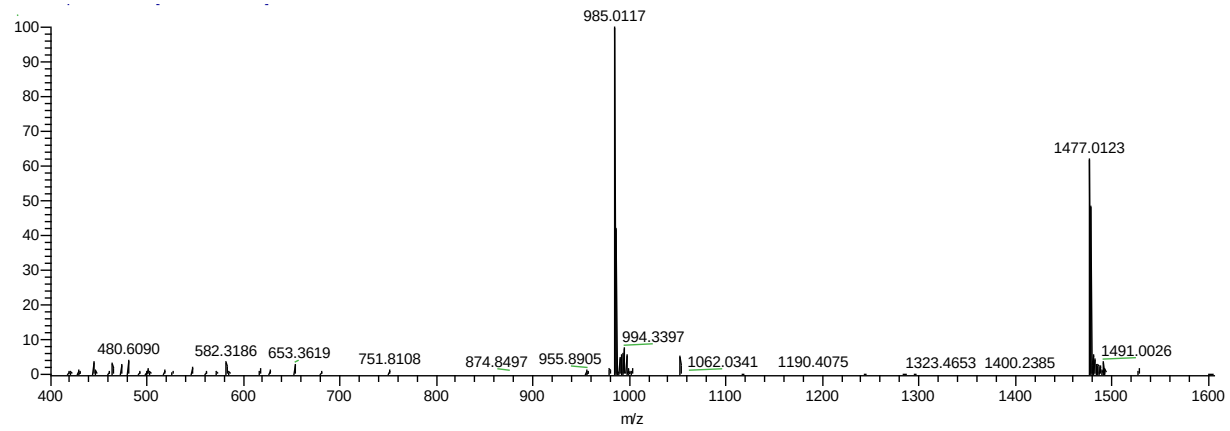
^{1}H NMR (D_2O, 500 MHz): δ 5.17 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 5.00 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.64-4.67 (m, 3 H, GlcNAc2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.48 (d, *J* = 7.6 Hz, 2 H, Gal6 H-1, Gla6' H-1), 4.30 (brs, 1 H, Man3 H-2), 4.20-4.24 (m, 3 H), 4.14-4.16 (m, 3 H), 3.54-4.03 (m, 76 H), 2.98 (dd, *J* = 17.3, 4.2 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 7.0 Hz, 1 H, Asn-βCH_2), 2.82 (dd, *J* = 12.3, 4.4 Hz, 2 H, Neu5Ac H-3eq), 2.60-2.63 (m, 2 H, Neu5Ac H-3eq), 2.12 (s, 6 H, 2 Ac), 2.11 (s, 3 H, Ac), 2.10 (s, 6 H, 2 Ac), 2.07 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 1.79 (t, *J* = 12.3 Hz, 2 H, Neu5Ac H-3ax), 1.69 (t, *J* = 12.1 Hz, 2 H, Neu5Ac H-3ax).

BA-10 

HILIC-ELSD, T_R = 23.342 min.

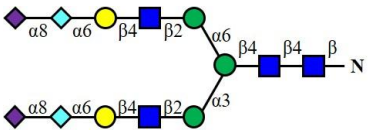


ESI-MS, calculated 2952.0809; found $[M+3H]^{3+}$ 985.0117, $[M+2H]^{2+}$ 1477.0123.

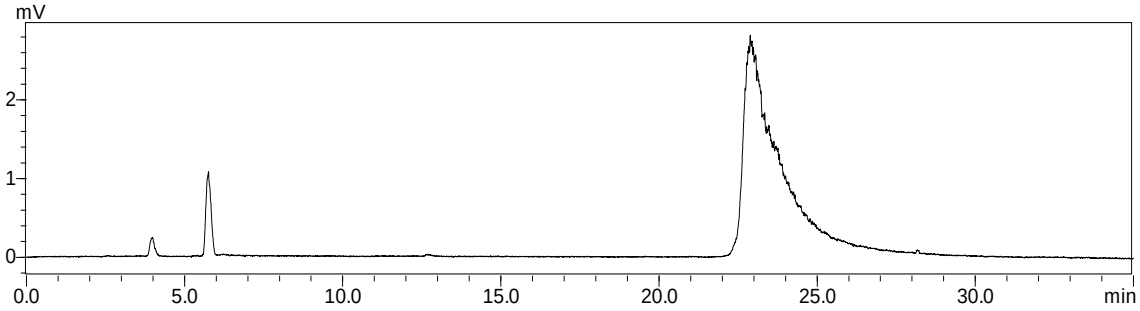


^{1}H NMR (D_2O, 500 MHz): δ 5.17 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 5.00 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.64-4.67 (m, 3 H, GlcNAc2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.48 (d, *J* = 6.8 Hz, 2 H, Gal6 H-1, Gla6' H-1), 4.30 (brs, 1 H, Man3 H-2), 4.22-4.24 (m, 3 H), 4.14-4.16 (m, 3 H), 3.54-4.04 (m, 80 H), 2.98 (dd, *J* = 17.2, 4.1 Hz, 1 H, Asn-βCH_2), 2.93 (dd, *J* = 17.2, 7.0 Hz, 1 H, Asn-βCH_2), 2.84 (dd, *J* = 12.4, 4.5 Hz, 2 H, Neu5Ac H-3eq), 2.60-2.63 (m, 2 H, Neu5Gc H-3eq), 2.12 (s, 6 H, 2 Ac), 2.11 (s, 3 H, Ac), 2.10 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 1.80 (t, *J* = 12.4 Hz, 2 H, Neu5Ac H-3ax), 1.69 (t, *J* = 12.1 Hz, 2 H, Neu5Gc H-3ax).

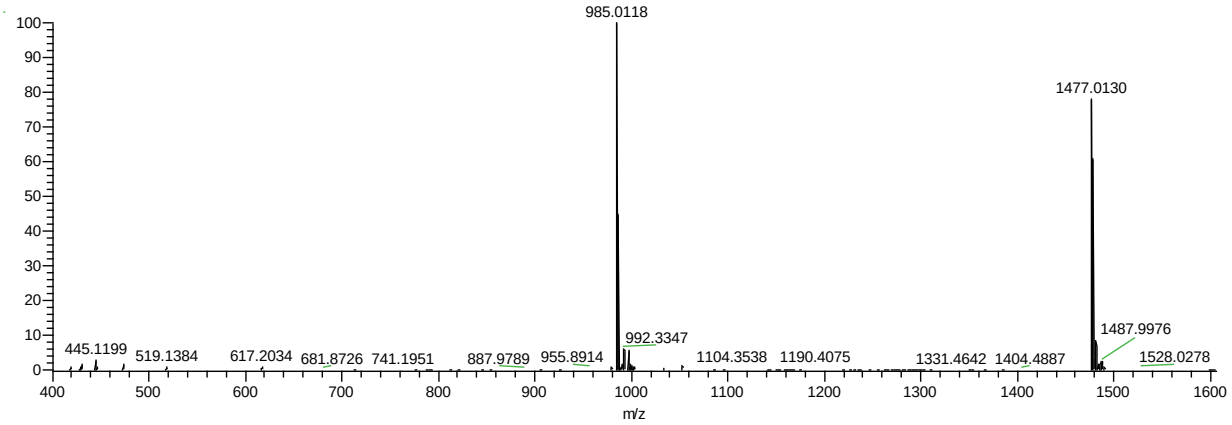
BA-11



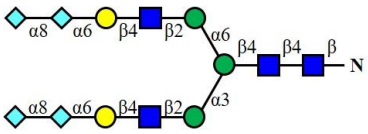
HILIC-ELSD, T_R = 22.883 min.



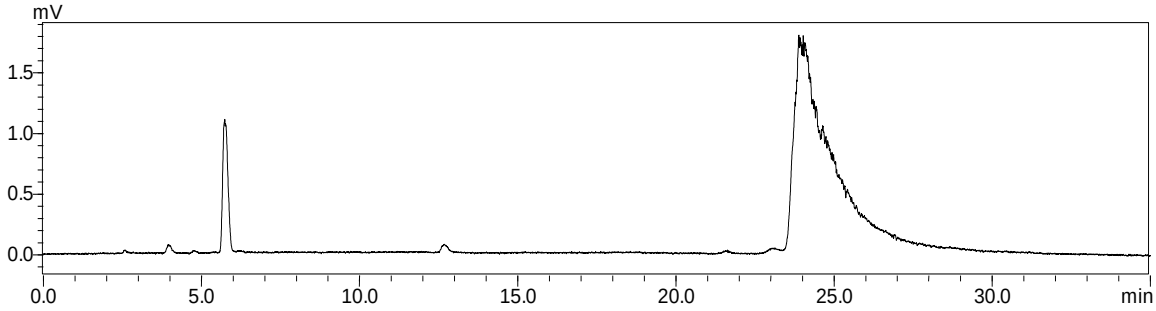
ESI-MS, calculated 2952.0809; found $[M+3H]^{3+}$ 985.0118, $[M+2H]^{2+}$ 1477.0130.



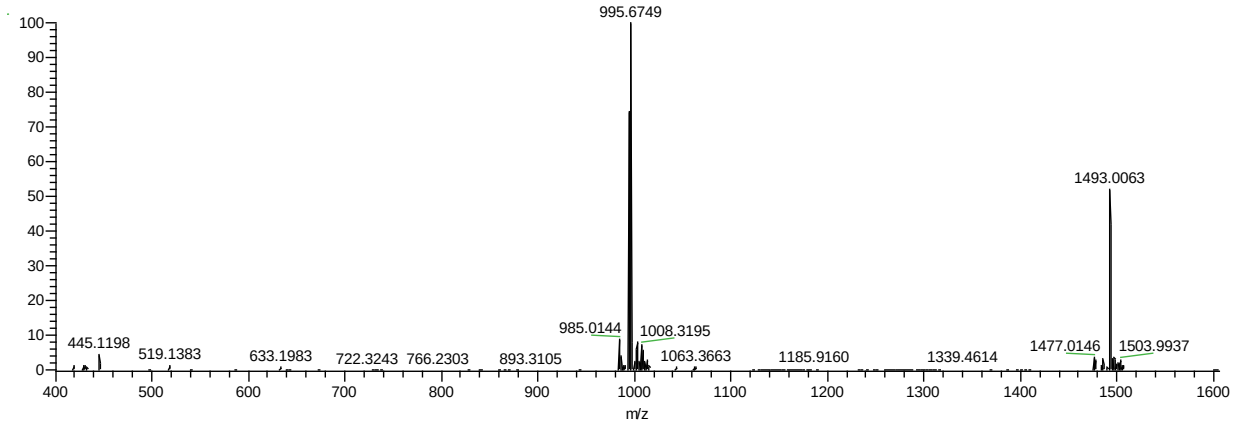
^{1}H NMR (D_2O, 500 MHz): δ 5.17 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 5.00 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.63-4.66 (m, 3 H, GlcNAc2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.48 (d, *J* = 7.6 Hz, 2 H, Gal6 H-1, Gla6' H-1), 4.31 (brs, 1 H, Man3 H-2), 4.22-4.25 (m, 4 H), 4.13-4.16 (m, 4 H), 3.54-4.04 (m, 78 H), 2.98 (dd, *J* = 17.3, 4.2 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 7.1 Hz, 1 H, Asn-βCH_2), 2.81 (dd, *J* = 12.5, 4.4 Hz, 2 H, Neu5Ac H-3eq), 2.61-2.64 (m, 2 H, Neu5Gc H-3eq), 2.13 (s, 6 H, 2 Ac), 2.12 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.06 (s, 3 H, Ac), 1.78 (t, *J* = 12.1 Hz, 2 H, Neu5Ac H-3ax), 1.71 (t, *J* = 12.3 Hz, 2 H, Neu5Gc H-3ax).

BA-12 

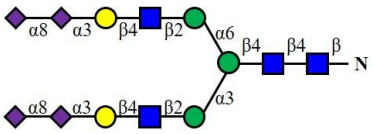
HILIC-ELSD, T_R = 23.883 min.



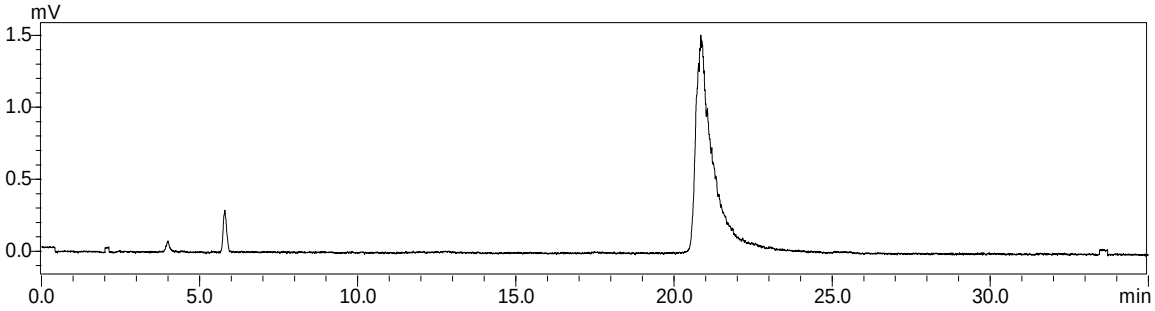
ESI-MS, calculated 2984.0708; found $[M+3H]^{3+}$ 995.6749, $[M+2H]^{2+}$ 1493.0063.



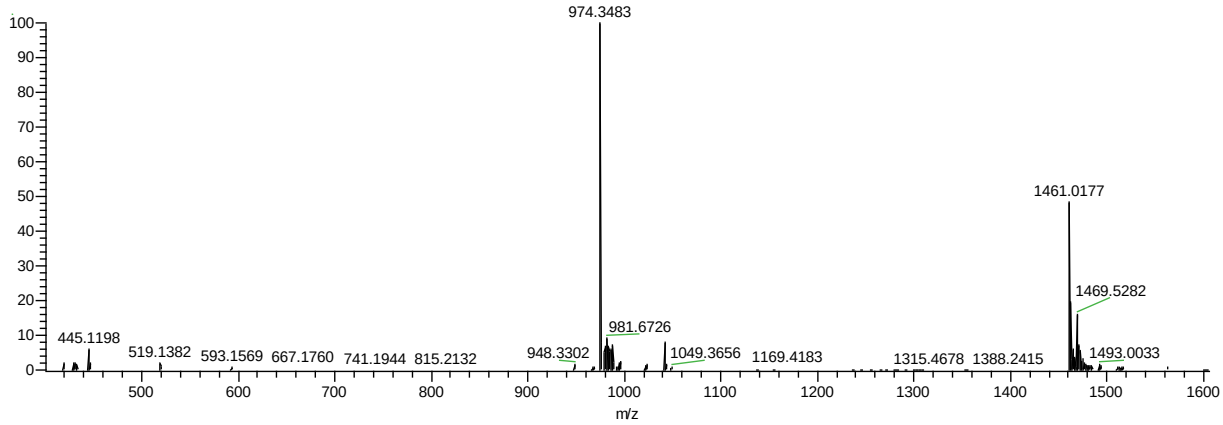
^{1}H NMR (D_2O, 500 MHz): δ 5.17 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 5.00 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.64-4.67 (m, 3 H, GlcNAc2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.48 (d, *J* = 7.6 Hz, 2 H, Gal6 H-1, Gla6' H-1), 4.30 (brs, 1 H, Man3 H-2), 4.22-4.26 (m, 5 H), 4.12-4.16 (m, 5 H), 3.54-4.04 (m, 80 H), 2.98 (dd, *J* = 17.3, 4.0 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.4, 7.1 Hz, 1 H, Asn-βCH_2), 2.83 (dd, *J* = 12.4, 4.5 Hz, 2 H, Neu5Gc H-3eq), 2.61-2.64 (m, 2 H, Neu5Gc H-3eq), 2.13 (s, 6 H, 2 Ac), 2.12 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.80 (t, *J* = 12.4 Hz, 2 H, Neu5Gc H-3ax), 1.71 (t, *J* = 12.1 Hz, 2 H, Neu5Gc H-3ax).

BA-13 

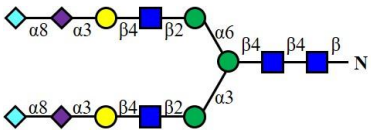
HILIC-ELSD, T_R = 20.851 min.



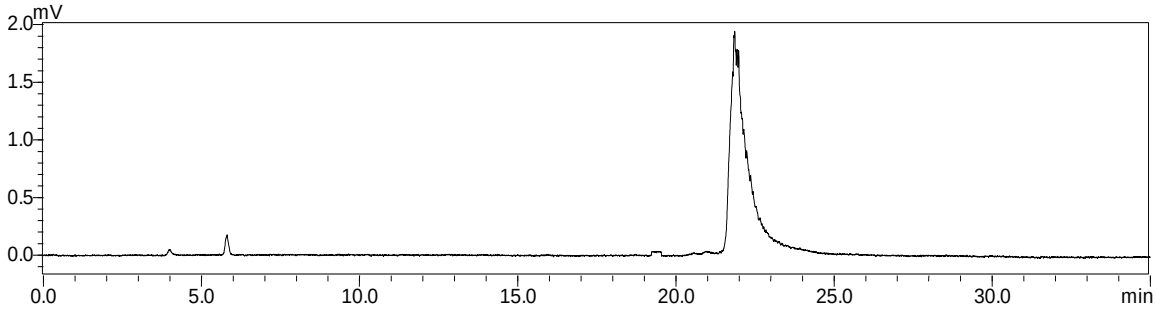
ESI-MS, calculated 2920.0911; found $[M+3H]^{3+}$ 974.3483, $[M+2H]^{2+}$ 1461.0177.



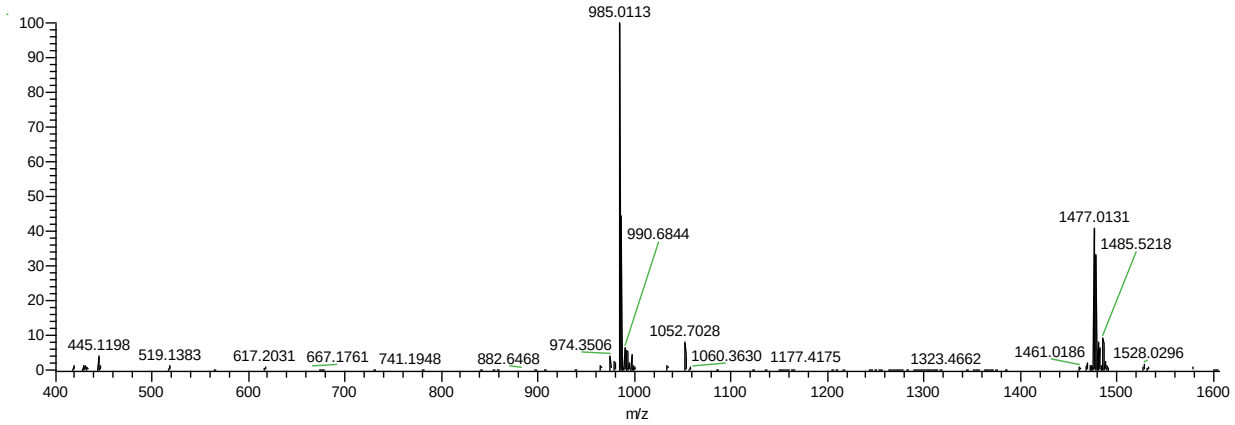
^{1}H NMR (D_2O, 500 MHz): δ 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.6 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc2 H-1), 4.48 (m, 4 H, , GlcNAc5 H-1, GlcNAc5' H-1, Gal6 H-1, Gla6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.13-4.23 (m, 8 H), 3.54-4.04 (m, 74 H), 2.98 (dd, *J* = 17.4, 3.7 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.4, 6.8 Hz, 1 H, Asn-βCH_2), 2.81-2.83 (m, 2 H, Neu5Ac H-3eq), 2.71-2.73 (m, 2 H, Neu5Ac H-3eq), 2.12 (s, 3 H, Ac), 2.11 (s, 6 H, 2 Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 1.78 (t, *J* = 11.5 Hz, 4 H, Neu5Ac H-3ax).

BA-14 

HILIC-ELSD, T_R = 21.858 min.

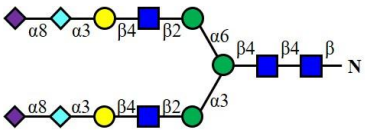


ESI-MS, calculated 2952.0809; found $[M+3H]^{3+}$ 985.0113, $[M+2H]^{2+}$ 1477.0131.

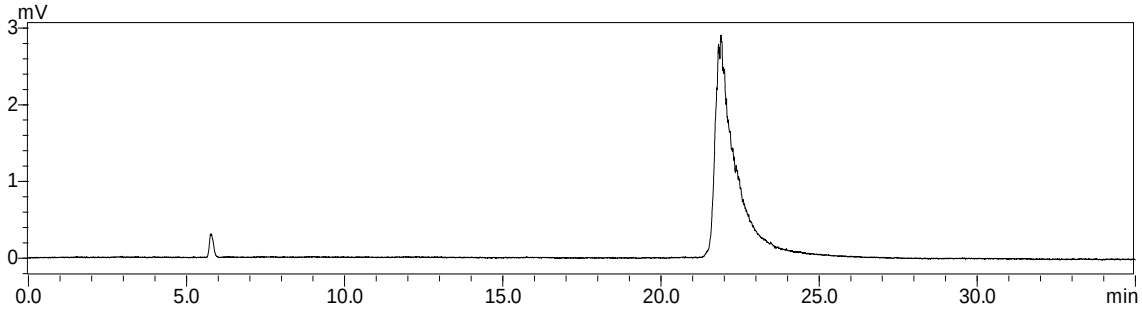


^{1}H NMR (D_2O, 500 MHz): δ 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc2 H-1), 4.57-4.62 (m, 4 H, GlcNAc5 H-1, GlcNAc5' H-1, Gal6 H-1, Gla6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.12-4.23 (m, 10 H), 3.54-4.04 (m, 76 H), 2.98 (dd, *J* = 17.3, 4.0 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.83 (dd, *J* = 12.0, 3.9 Hz, 2 H, Neu5Ac H-3eq), 2.71-2.73 (m, 2 H, Neu5Gc H-3eq), 2.12 (s, 3 H, Ac), 2.11 (s, 6 H, 2 Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.75-1.82 (m, 4 H, Neu5Ac H-3ax, Neu5Gc H-3ax).

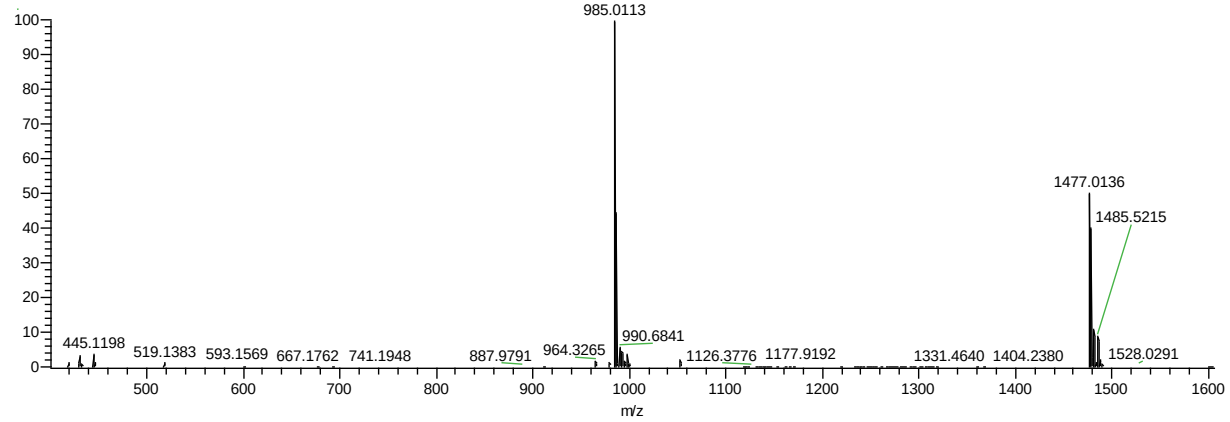
BA-15



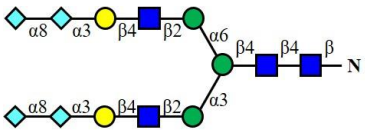
HILIC-ELSD, T_R = 21.895 min.



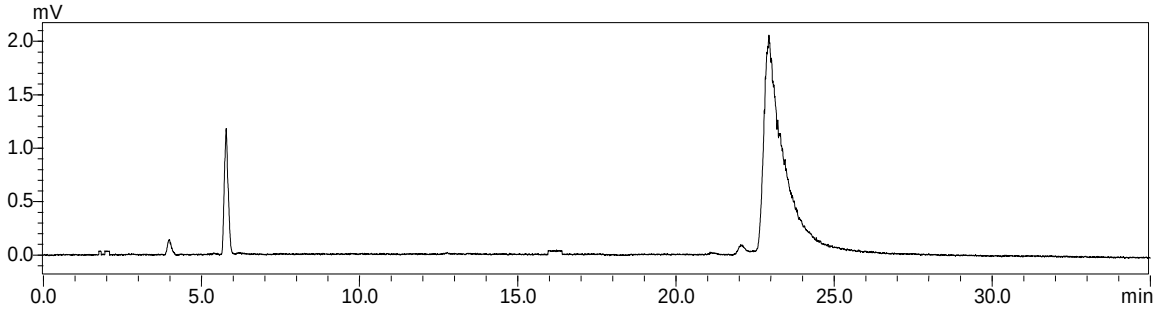
ESI-MS, calculated 2952.0809; found $[M+3H]^{3+}$ 985.0113, $[M+2H]^{2+}$ 1477.0136.



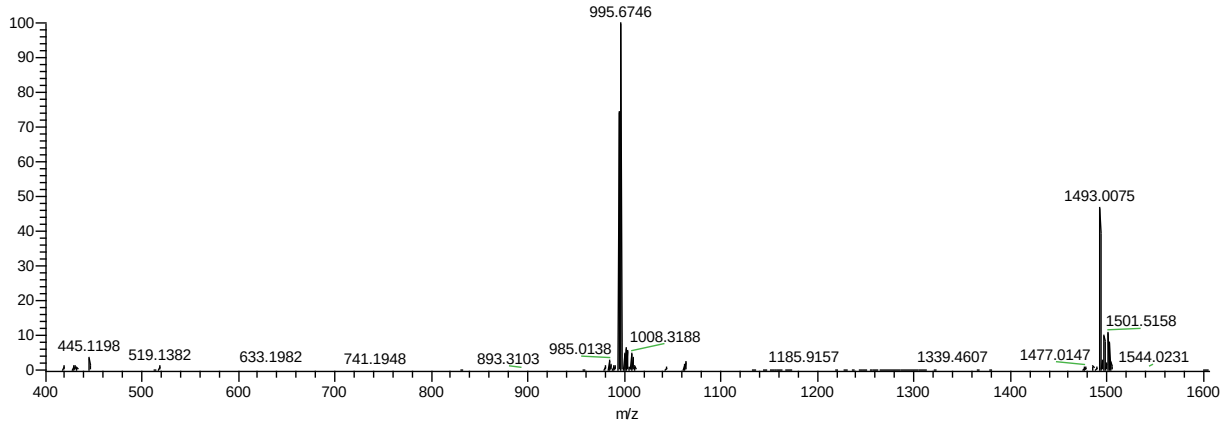
^{1}H NMR (D_2O, 500 MHz): δ 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.65 (d, *J* = 7.9 Hz, 1 H, GlcNAc2 H-1), 4.57-4.62 (m, 4 H, GlcNAc5 H-1, GlcNAc5' H-1, Gal6 H-1, Gla6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.26 (brs, 1 H), 4.13-4.23 (m, 9 H), 3.54-4.06 (m, 76 H), 2.98 (dd, *J* = 17.3, 3.9 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.79-2.83 (m, 2 H, Neu5Ac H-3eq), 2.72-2.76 (m, 2 H, Neu5Gc H-3eq), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 1.75-1.80 (m, 4 H, Neu5Ac H-3ax, Neu5Gc H-3ax).

BA-16 

HILIC-ELSD, T_R = 22.941 min.

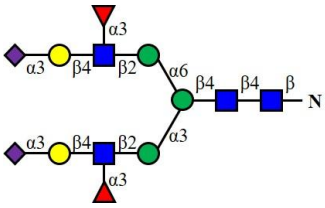


ESI-MS, calculated 2984.0708; found $[M+3H]^{3+}$ 995.6746, $[M+2H]^{2+}$ 1493.0075.

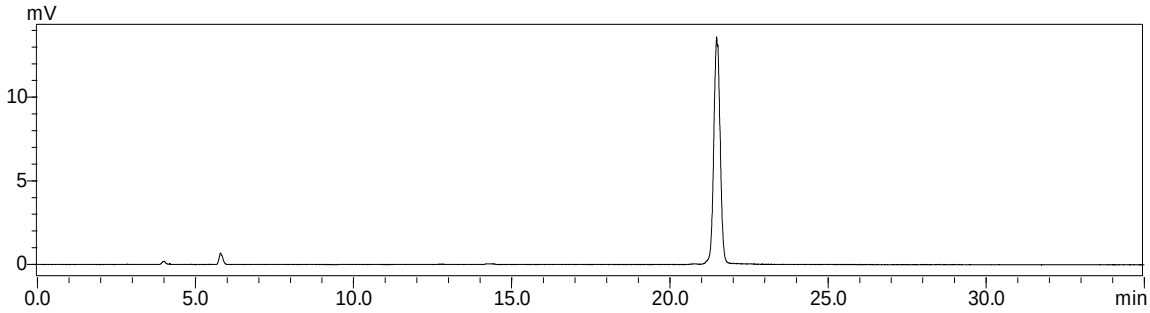


^{1}H NMR (D_2O, 500 MHz): δ 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.65 (d, *J* = 7.9 Hz, 1 H, GlcNAc2 H-1), 4.57-4.62 (m, 4 H, GlcNAc5 H-1, GlcNAc5' H-1, Gal6 H-1, Gla6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.26 (brs, 1 H), 4.13-4.23 (m, 11 H), 3.54-4.06 (m, 78 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 7.1 Hz, 1 H, Asn-βCH_2), 2.83 (dd, *J* = 12.3, 4.4 Hz, 2 H, Neu5Gc H-3eq), 2.74 (dd, *J* = 12.8, 4.7 Hz, 2 H, Neu5Gc H-3eq), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.79 (t, *J* = 12.3 Hz, 4 H, Neu5Gc H-3ax).

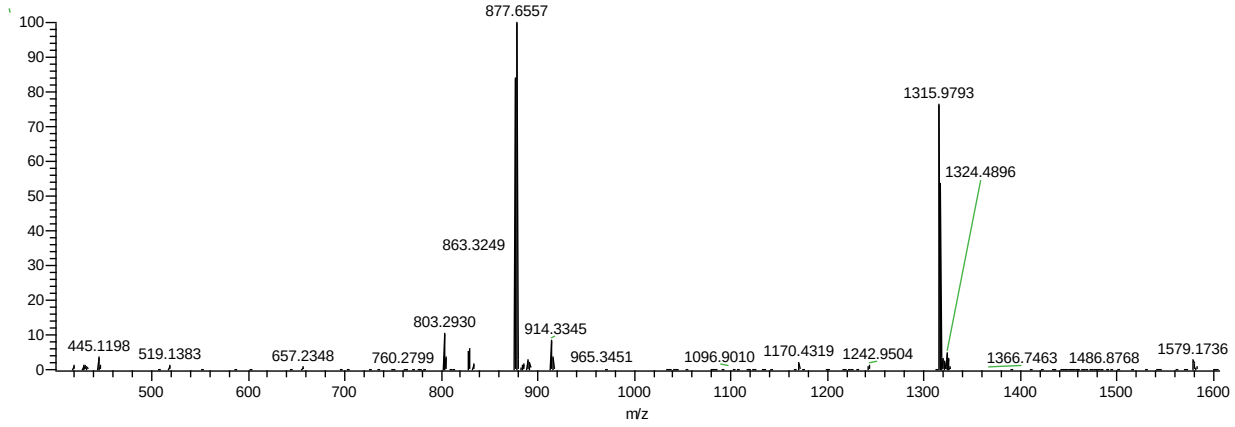
BA-17



HILIC-ELSD, T_R = 21.476 min.

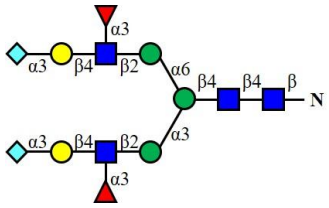


ESI-MS, calculated 2630.0161; found $[M+3H]^{3+}$ 877.6557, $[M+2H]^{2+}$ 1315.9793.

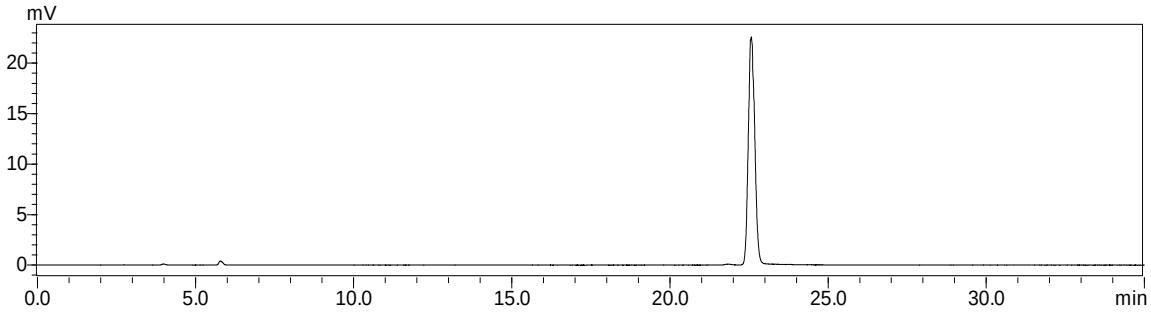


^{1}H NMR (D_2O, 500 MHz): δ 5.17 (d, *J* = 3.7 Hz, 1 H, Fuc H-1), 5.16 (d, *J* = 3.9 Hz, 1 H, Fuc H-1), 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc2 H-1), 4.62 (d, *J* = 8.2 Hz, 2 H, GlcNAc5 H-1, GlcNAc5' H-1), 4.56 (d, *J* = 7.5 Hz, 1 H, Gal6 H-1), 4.55 (d, *J* = 7.5 Hz, 1 H, Gal6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (brs, 1 H), 4.11-4.14 (m, 4 H), 3.50-4.04 (m, 71 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.80 (dd, *J* = 12.3, 4.2 Hz, 2 H, Neu5Ac H-3eq), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 1.84 (t, *J* = 12.0 Hz, 2 H, Neu5Ac H-3ax), 1.21 (d, *J* = 6.1 Hz, 3 H, Fuc CH_3), 1.20 (d, *J* = 6.2 Hz, 3 H, Fuc CH_3).

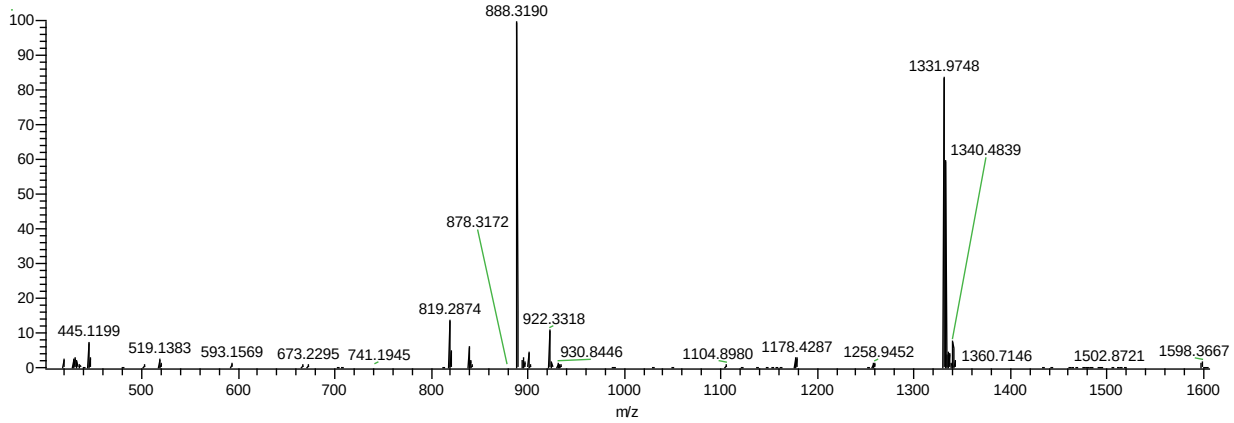
BA-18



HILIC-ELSD, T_R = 22.572 min.

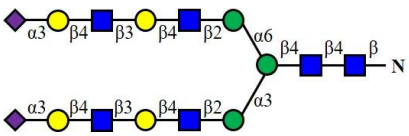


ESI-MS, calculated 2662.0059; found $[M+3H]^{3+}$ 888.3190, $[M+2H]^{2+}$ 1331.9748.

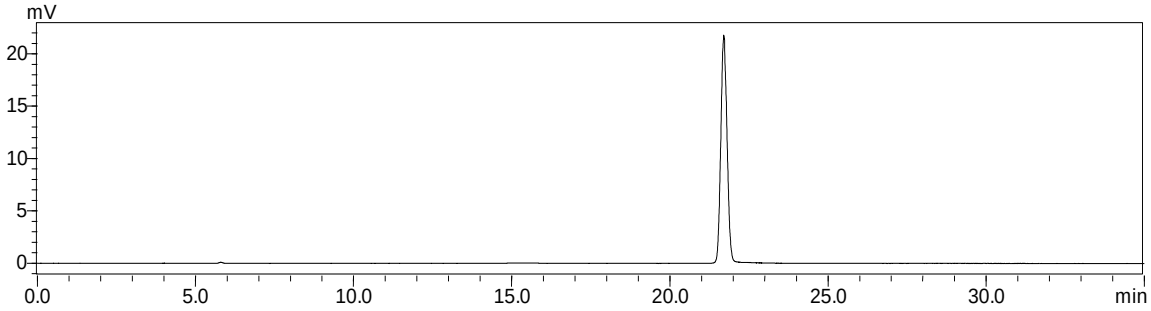


^{1}H NMR (D_2O, 500 MHz): δ 5.16 (d, *J* = 3.6 Hz, 1 H, Fuc H-1), 5.15 (d, *J* = 3.8 Hz, 1 H, Fuc H-1), 5.14 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.86 (t, *J* = 6.0 Hz, 1 H), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.65 (d, *J* = 7.6 Hz, 1 H, GlcNAc2 H-1), 4.62 (d, *J* = 7.8 Hz, 2 H, GlcNAc5 H-1, GlcNAc5' H-1), 4.56 (d, *J* = 7.7 Hz, 1 H, Gal6 H-1), 4.55 (d, *J* = 7.6 Hz, 1 H, Gal6' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (d, *J* = 2.1 Hz, 1 H), 4.12-4.16 (m, 5 H), 3.49-4.06 (m, 73 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.82 (dd, *J* = 12.3, 4.3 Hz, 2 H, Neu5Gc H-3eq), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.05 (s, 6 H, 2 Ac), 1.85 (t, *J* = 12.1 Hz, 2 H, Neu5Gc H-3ax), 1.21 (d, *J* = 6.2 Hz, 3 H, Fuc CH_3), 1.20 (d, *J* = 6.2 Hz, 3 H, Fuc CH_3).

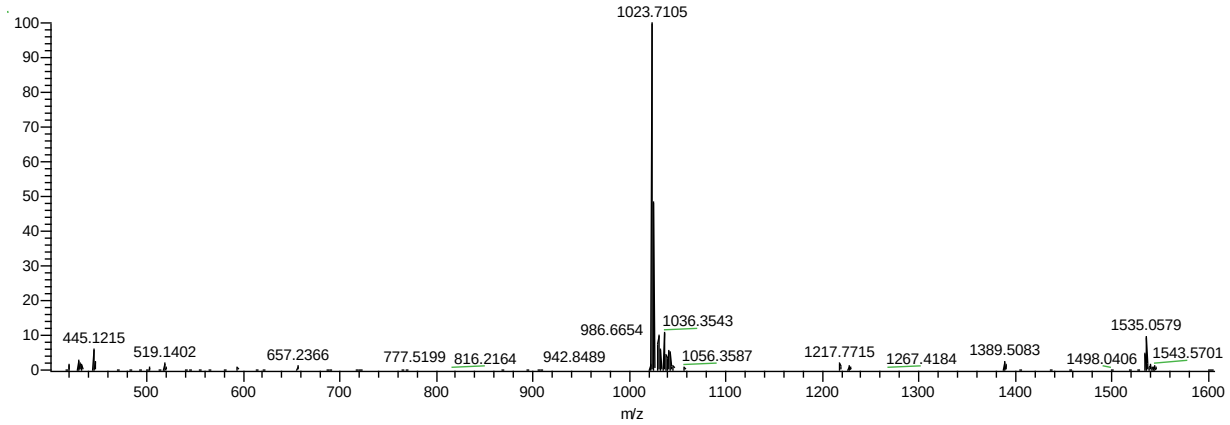
BA-19



HILIC-ELSD, T_R = 21.703 min.

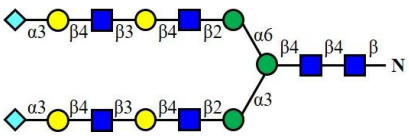


ESI-MS, calculated 3068.1647; found $[M+3H]^{3+}$ 1023.7105, $[M+2H]^{2+}$ 1535.0579.

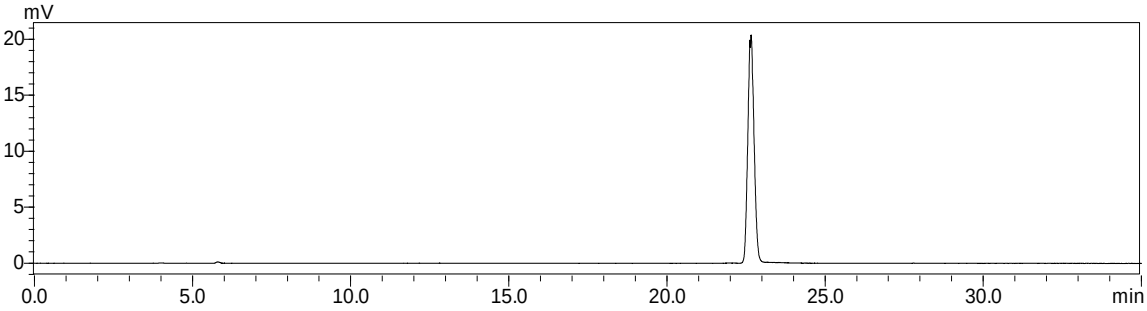


^{1}H NMR (D_2O, 500 MHz): δ 5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.2 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.66 (d, *J* = 7.9 Hz, 1 H, GlcNAc5' H-1), 4.59-4.62 (m, 4 H, GlcNAc7 H-1, GlcNAc7' H-1, Gal6 H-1, Gal6' H-1), 4.50 (d, *J* = 7.6 Hz, 1 H, Gal8 H-1), 4.49 (d, *J* = 7.6 Hz, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.23 (brs, 1 H), 4.20 (brs, 2 H), 4.16 (d, *J* =2.7 Hz, 1 H), 4.14 (d, *J* = 2.5 Hz, 2 H), 3.51-4.03 (m, 86 H), 2.98 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.3, 6.9 Hz, 1 H, Asn-βCH_2), 2.80 (dd, *J* = 12.4, 4.5 Hz, 2 H, Neu5Ac H-3eq), 2.11 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 12 H, 4 Ac), 2.05 (s, 3 H, Ac), 1.84 (t, *J* = 12.4 Hz, 2 H, Neu5Ac H-3ax).

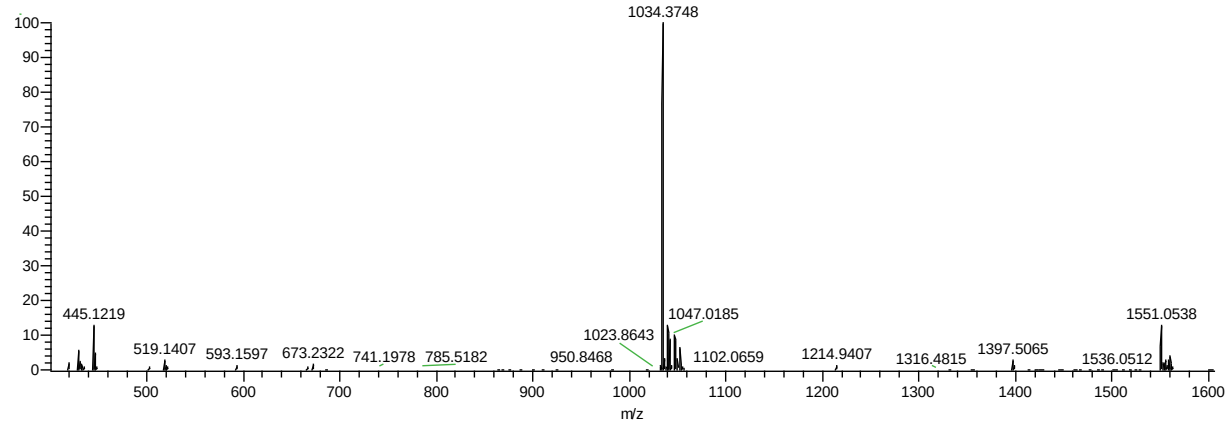
BA-20



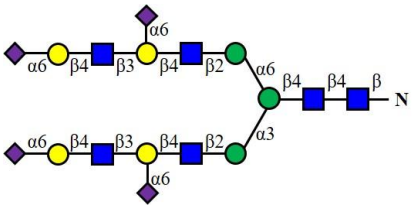
HILIC-ELSD, T_R = 22.661 min.



ESI-MS, calculated 3100.1545; found $[M+3H]^{3+}$ 1034.3748, $[M+2H]^{2+}$ 1551.0538.

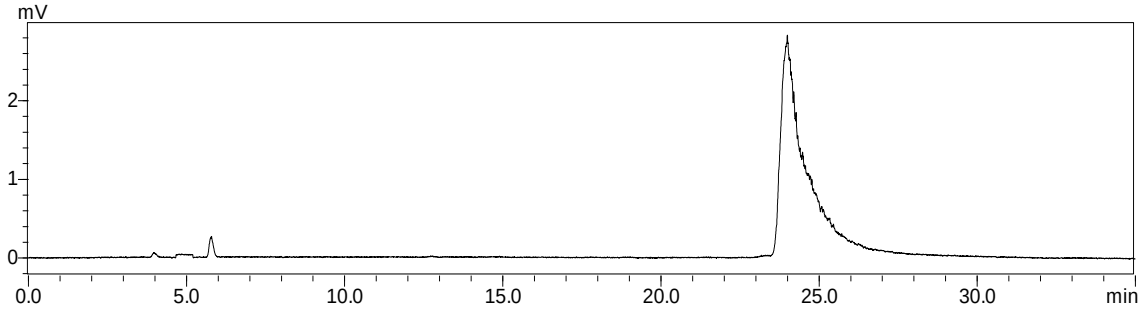


^{1}H NMR (D_2O, 500 MHz): δ 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.1 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc5' H-1), 4.59-4.62 (m, 4 H, GlcNAc7 H-1, GlcNAc7' H-1, Gal6 H-1, Gal6' H-1), 4.50 (d, *J* = 7.6 Hz, 1 H, Gal8 H-1), 4.49 (d, *J* = 7.6 Hz, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.23 (brs, 1 H), 4.20 (brs, 2 H), 4.13-4.18 (m, 5 H), 3.51-4.03 (m, 88 H), 2.98 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.81 (dd, *J* = 12.4, 4.5 Hz, 2 H, Neu5Ac H-3eq), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 12 H, 4 Ac), 2.05 (s, 3 H, Ac), 1.85 (t, *J* = 12.4 Hz, 2 H, Neu5Ac H-3ax).

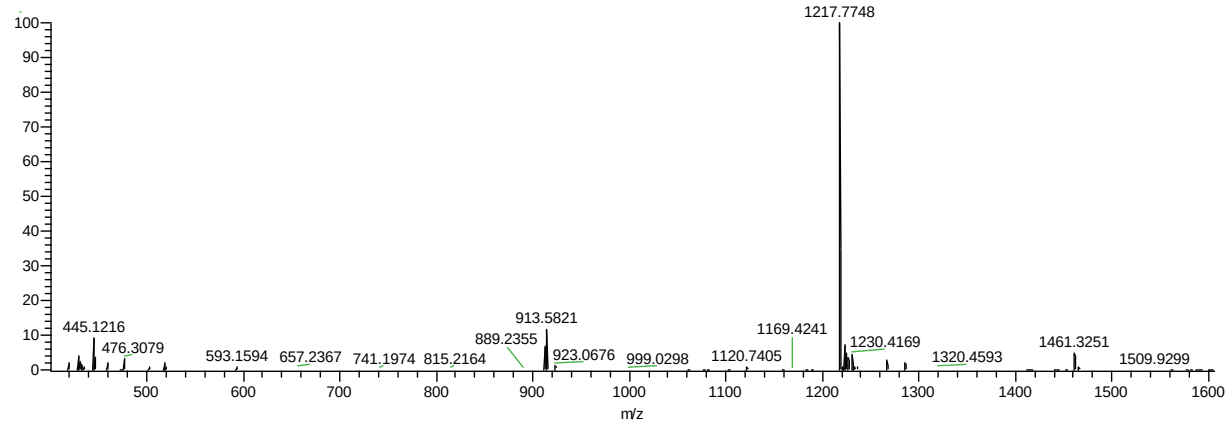


BA-21

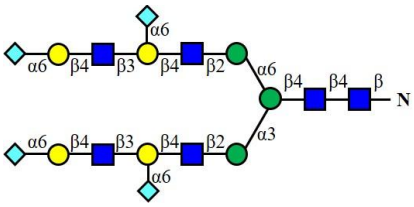
HILIC-ELSD, T_R = 23.996 min.



ESI-MS, calculated 3650.3555; found $[M+4H]^{4+}$ 913.5821, $[M+3H]^{3+}$ 1217.7748.

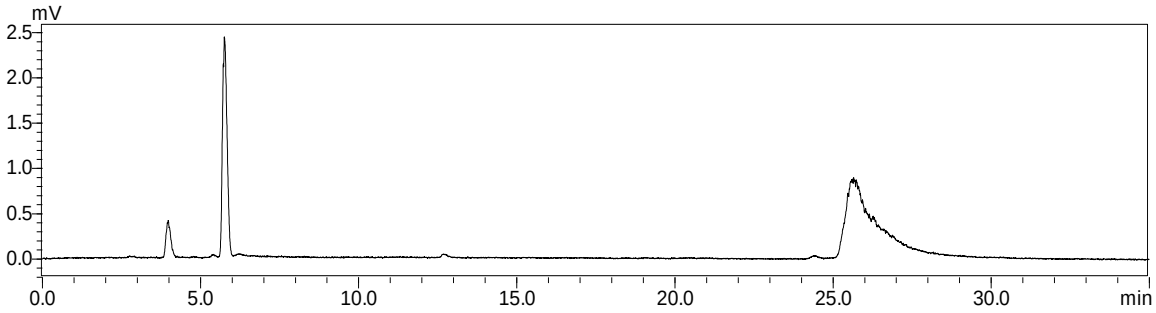


^{1}H NMR (D_2O, 500 MHz): δ 5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.6 Hz, 1 H, GlcNAc1 H-1), 4.98 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 7.3 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.63-4.66 (m, 3 H, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1), 4.45-4.50 (m, 4 H, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (brs, 1 H), 4.20 (brs, 2 H), 4.15 (m, 2 H), 3.51-4.05 (m, 101 H), 2.98 (dd, *J* = 17.2, 3.6 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.5 Hz, 1 H, Asn-βCH_2), 2.68-2.71 (dd, 4 H, Neu5Ac H-3eq), 2.11 (s, 3 H, Ac), 2.09 (s, 6 H, 2 Ac), 2.08 (s, 3 H, Ac), 2.06 (s, 12 H, 4 Ac), 2.04 (s, 3 H, Ac), 1.71-1.77 (m, 4 H, Neu5Ac H-3ax).

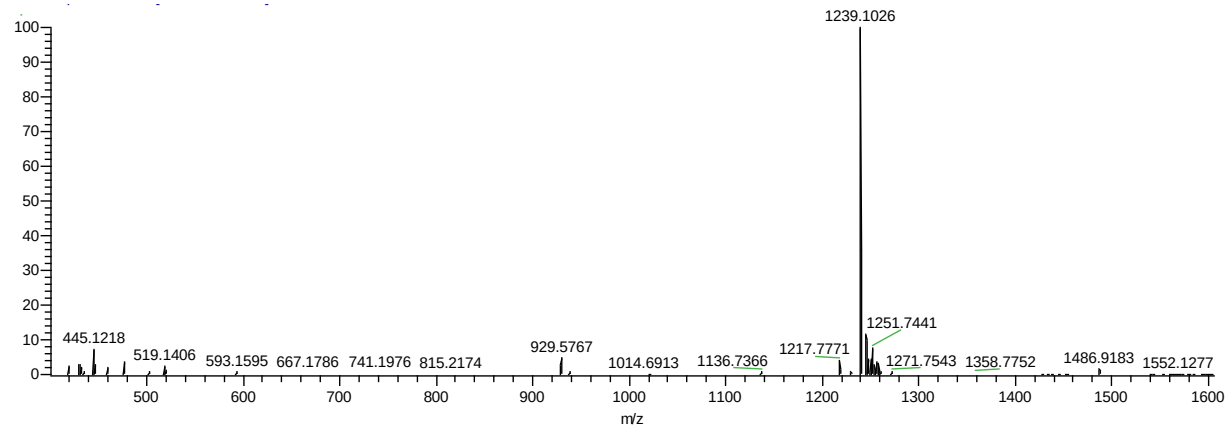


BA-22

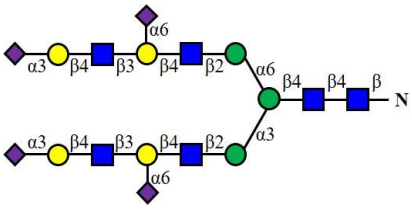
HILIC-ELSD, T_R = 25.652 min.



ESI-MS, calculated 3714.3352; found $[M+4H]^{4+}$ 929.5767, $[M+3H]^{3+}$ 1239.1026.

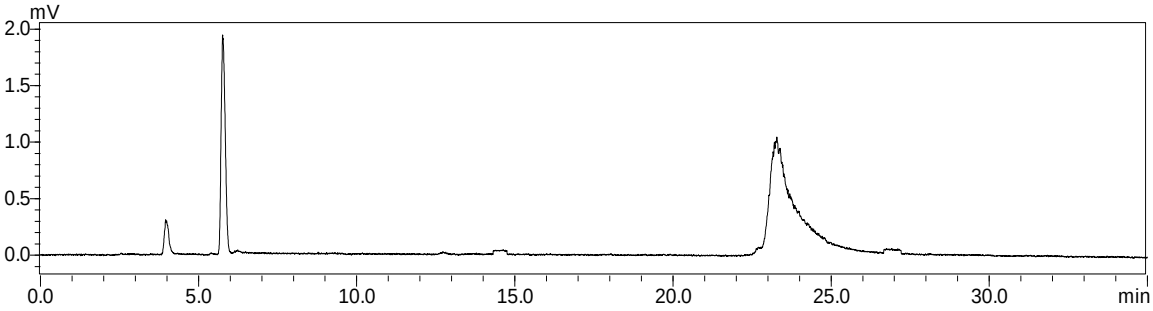


^{1}H NMR (D_2O, 500 MHz): δ 5.17 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.98 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.75 (d, *J* = 7.6 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.63-4.66 (m, 3 H, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1), 4.47-4.51 (m, 4 H, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1), 4.30 (brs, 1 H, Man3 H-2), 4.21-4.23 (m, 4 H), 4.15-4.17 (m, 4 H), 3.54-4.03 (m, 106 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 7.0 Hz, 1 H, Asn-βCH_2), 2.73 (dd, *J* =12.3, 4.3 Hz, 4 H, Neu5Gc H-3eq), 2.12 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 1.74-1.80 (m, 4 H, Neu5Gc H-3ax).

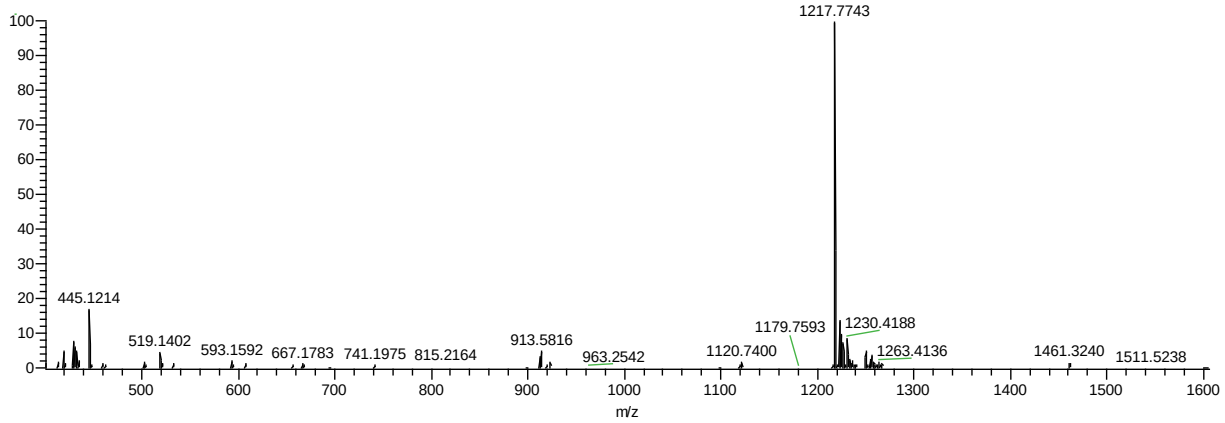


BA-23

HILIC-ELSD, T_R = 23.283 min.

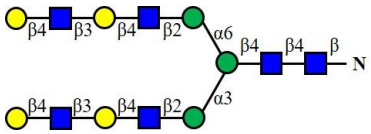


ESI-MS, calculated 3650.3555; found $[M+4H]^{4+}$ 913.5816, $[M+3H]^{3+}$ 1217.7743.

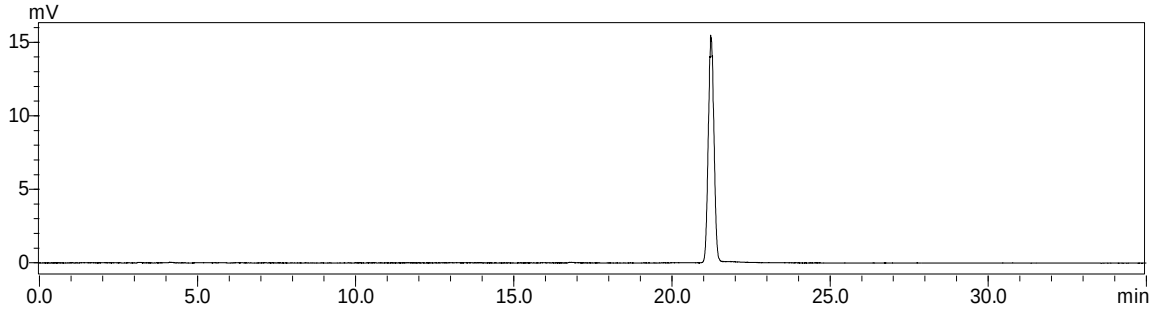


^{1}H NMR (D_2O, 500 MHz): δ 5.17 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.99 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.72 (d, *J* = 8.2 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.63-4.66 (m, 3 H, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1), 4.59 (d, *J* = 7.8 Hz, 2 H, Gal6 H-1, Gal6' H-1), 4.46-4.48 (m, 2 H, Gal8 H-1, Gal8' H-1), 4.30 (brs, 1 H, Man3 H-2), 4.23 (d, *J* = 1.9 Hz, 1 H), 4.21 (brs, 2 H), 4.14-4.17 (m, 3 H), 3.51-4.04 (m, 100 H), 2.98 (dd, *J* = 17.2, 4.2 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 7.0 Hz, 1 H, Asn-βCH_2), 2.80 (dd, *J* =12.5, 4.5 Hz, 2 H, Neu5Ac H-3eq), 2.68-2.72 (m, 2 H, Neu5Ac H-3eq), 2.12 (s, 3 H, Ac), 2.11 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.07 (s, 18 H, 6 Ac), 2.05 (s, 3 H, Ac), 1.84 (t, *J* = 12.5 Hz, 2 H, Neu5Ac H-3ax), 1.75 (t, *J* = 12.1 Hz, 2 H, Neu5Ac H-3ax).

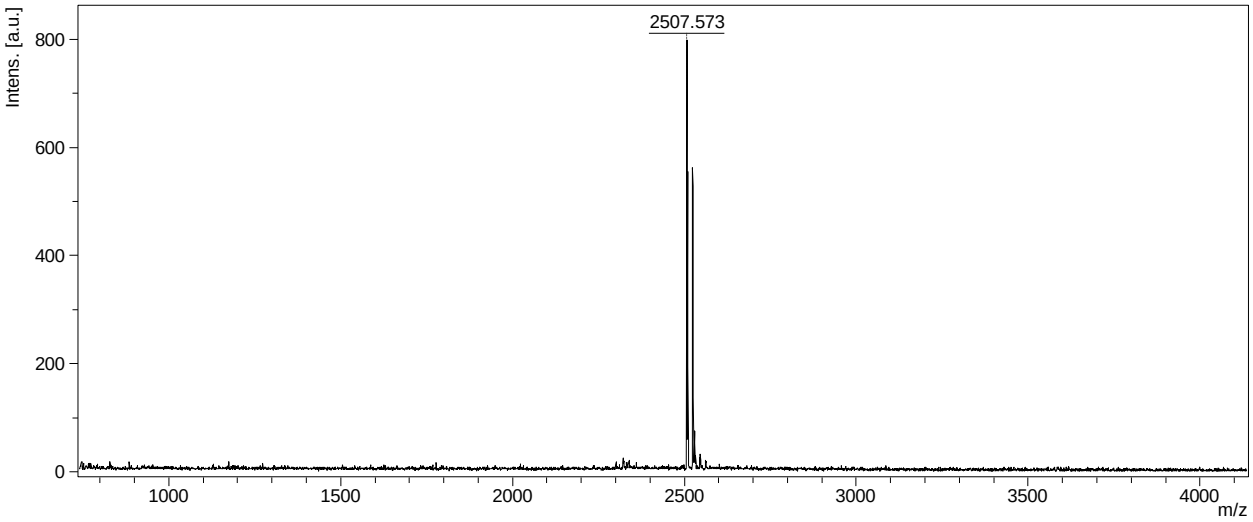
BA-24



HILIC-ELSD, T_R = 21.225 min.

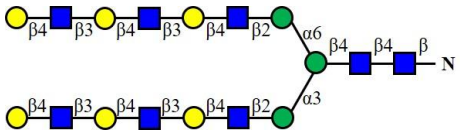


MALDI-MS, calculated 2485.9738; found $[M+Na]^+$ 2507.573, $[M+K]^+$ 2523.541.

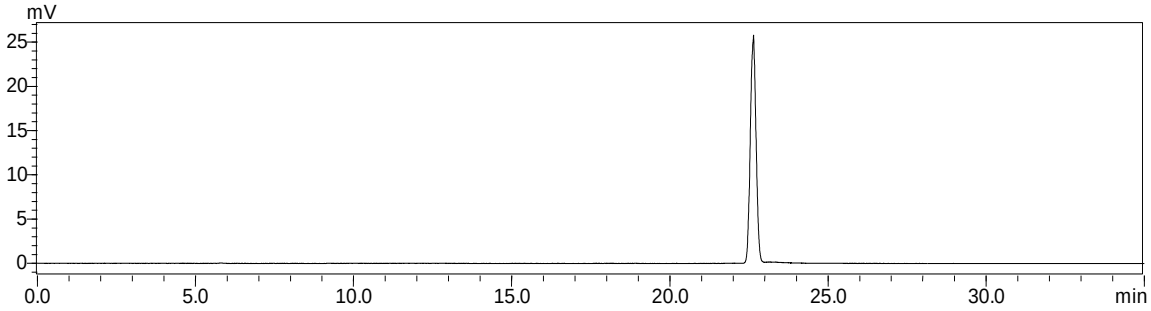


^{1}H NMR (D_2O, 500 MHz): δ 5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.3 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc5' H-1), 4.61 (d, *J* = 7.7 Hz, 2 H, GlcNAc7 H-1, GlcNAc7' H-1), 4.49-4.53 (m, 4 H, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (d, *J* = 2.1 Hz, 1 H), 4.20 (brs, 2 H), 4.14 (d, *J* = 2.3 Hz, 1 H), 3.51-4.03 (m, 74 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.80 (dd, *J* =12.5, 4.5 Hz, 2 H, Neu5Ac H-3eq), 2.68-2.72 (m, 2 H, Neu5Ac H-3eq), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), 2.05 (s, 3 H, Ac).

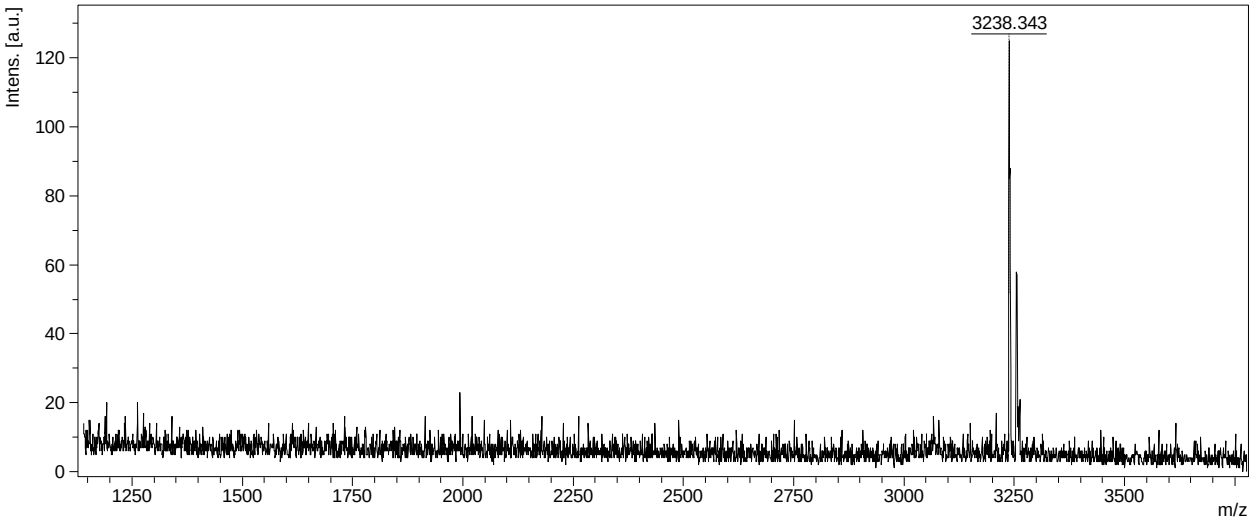
BA-25



HILIC-ELSD, T_R = 22.644 min.

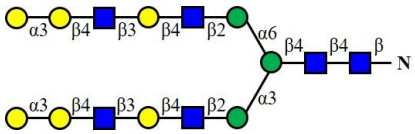


MALDI-MS, calculated 3216.2382; found $[M+Na]^+$ 3238.343, $[M+K]^+$ 3254.335.

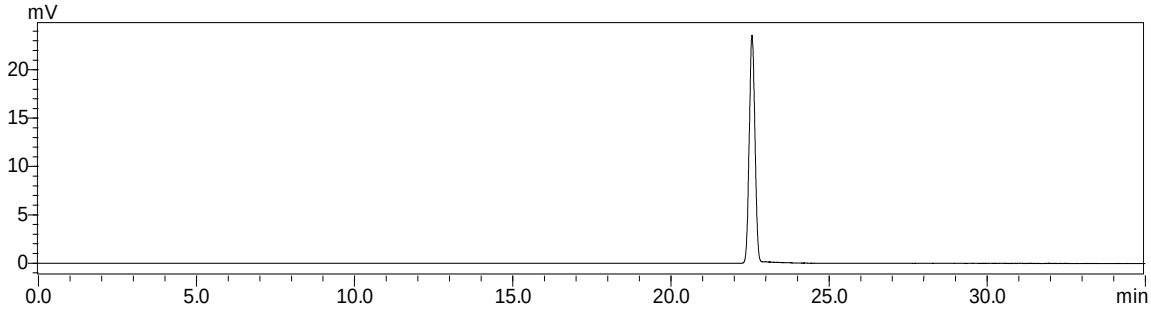


^{1}H NMR (D_2O, 500 MHz): δ 5.15 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.96 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.2 Hz, 3 H, GlcNAc 2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc7 H-1), 4.62 (d, *J* = 7.6 Hz, 2 H, GlcNAc7' H-1, GlcNAc8 H-1), 4.47-4.53 (m, 7 H, GlcNAc8' H-1, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1, Gal10 H-1, Gal10' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (brs, 1 H), 4.19 (brs, 4 H), 4.14 (d, *J* = 1.8 Hz, 1 H), 3.51-4.04 (m, 96 H), 2.97 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.11 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 12 H, 4 Ac), 2.05 (s, 3 H, Ac).

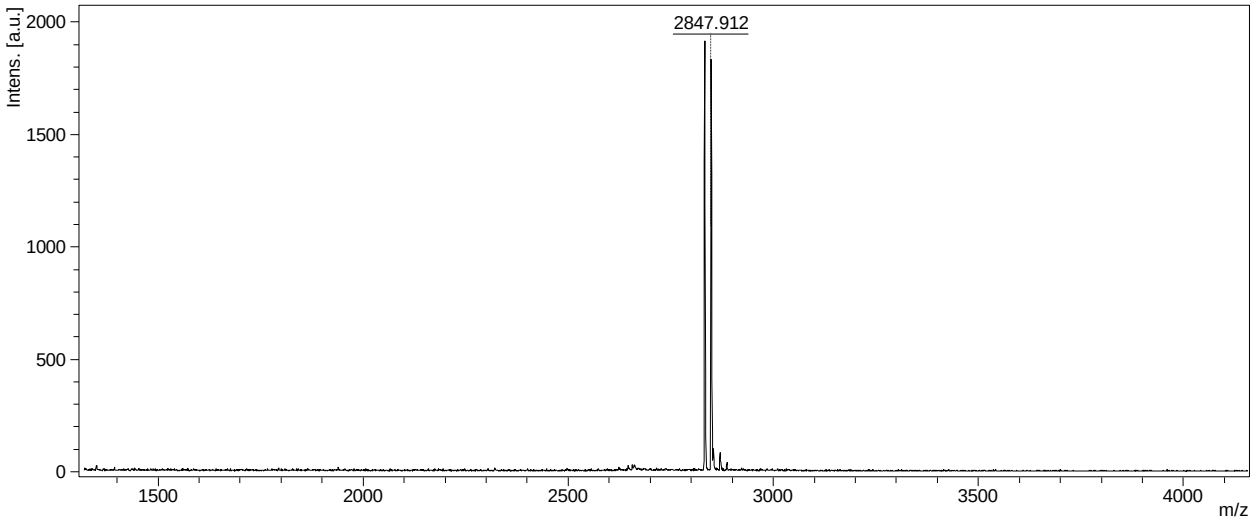
BA-26



HILIC-ELSD, T_R = 22.564 min.

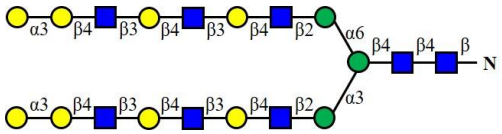


MALDI-MS, calculated 2810.0795; found $[M+Na]^+$ 2831.924, $[M+K]^+$ 2847.912.

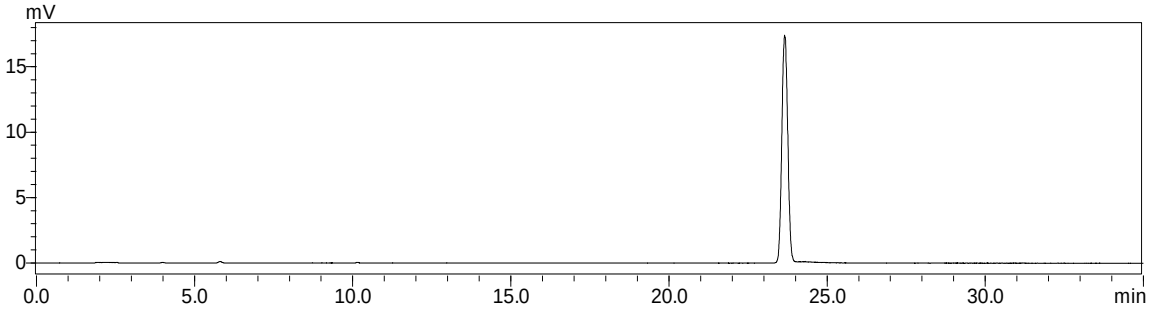


^{1}H NMR (D_2O, 500 MHz): δ 5.19 (d, *J* = 3.7 Hz, Gal-9 H-1, Gal9' H-1), 5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.2 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc5' H-1), 4.58-4.63 (m, 4 H, GlcNAc7 H-1, GlcNAc7' H-1, Gal6 H-1, Gal6' H-1), 4.50 (d, *J* = 7.6 Hz, 1 H, Gal8 H-1), 4.49 (d, *J* = 7.5 Hz, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.20-4.24(m, 7 H), 4.14 (brs, 1 H), 4.05-4.06 (m, 2 H), 3.97-4.04 (m, 8 H), 3.69-3.91 (m, 60 H), 3.51-3.56 (m, 2 H), 2.98 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac).

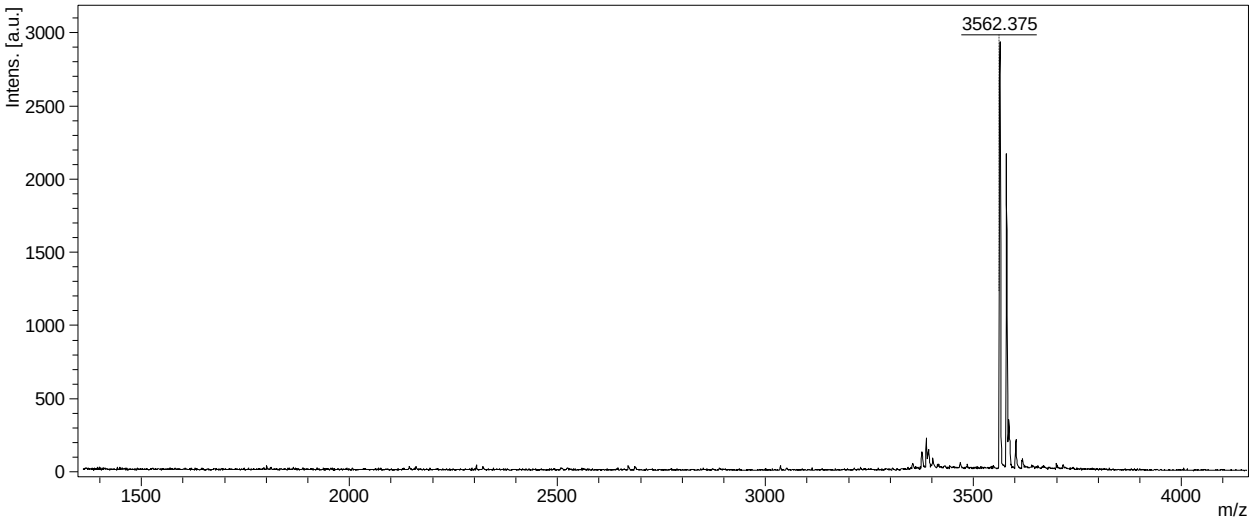
BA-27



HILIC-ELSD, T_R = 23.654 min.

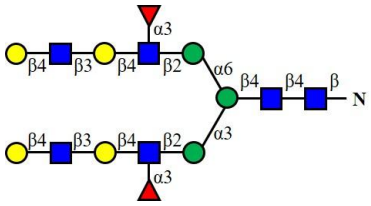


MALDI-MS, calculated 3540.3439; found $[M+Na]^+$ 3562.375, $[M+K]^+$ 3578.362.

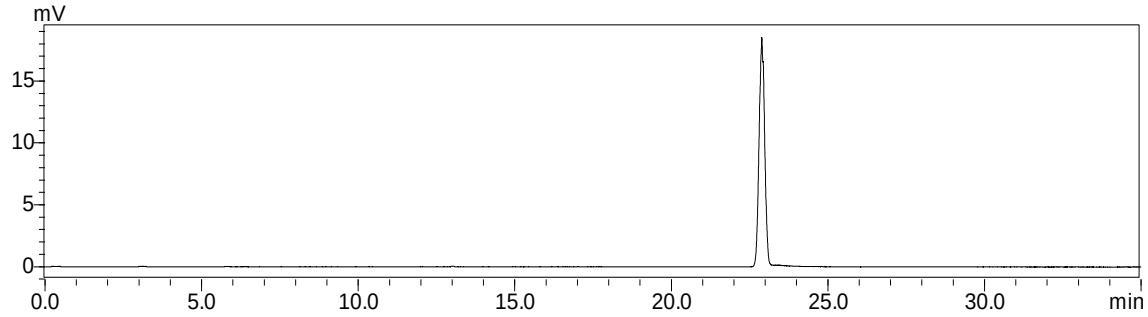


^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, *J* = 3.7 Hz, Gal-11 H-1, Gal11' H-1), 5.16 (s, 1 H, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.97 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74-4.76 (m, 3 H, GlcNAc 2 H-1, GlcNAc5 H-1, GlcNAc5' H-1), 4.65 (d, *J* = 7.8 Hz, 1 H, GlcNAc7 H-1), 4.58-4.62 (m, 5 H, , GlcNAc7' H-1, GlcNAc9 H-1, GlcNAc9' H-1, Gal6 H-1, Gal6' H-1), 4.48-4.52 (m, 4 H, Gal8 H-1, Gal8' H-1, Gal10 H-1, Gal10' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.20-4.24(m, 8 H), 4.14 (d, *J* = 2.2 Hz, 1 H), 4.05-4.06 (m, 2 H), 3.50-4.03 (m, 103 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 12 H, 4 Ac), 2.05 (s, 3 H, Ac).

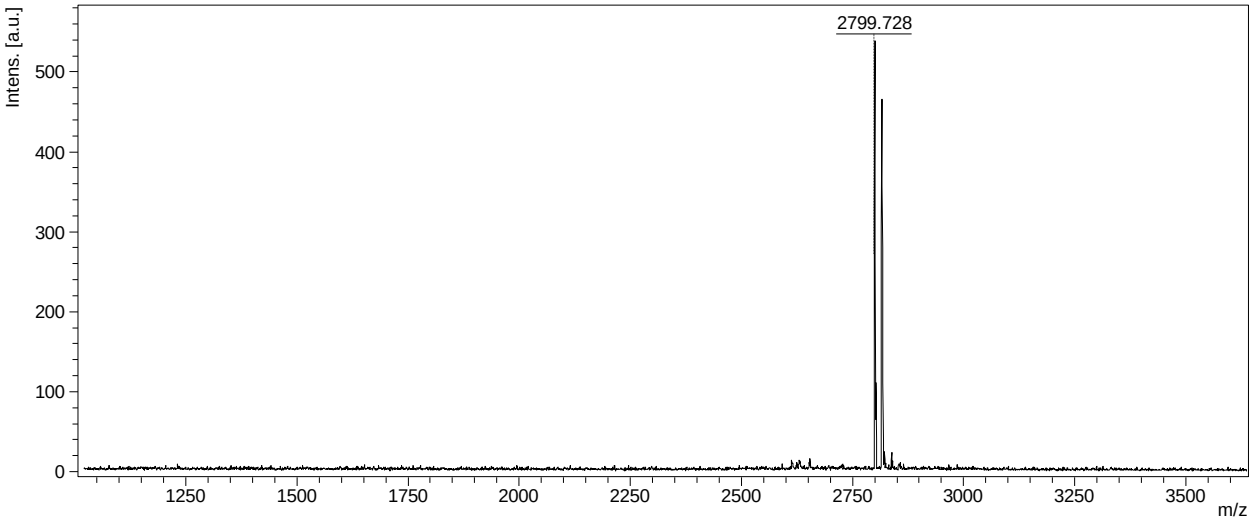
BA-28



HILIC-ELSD, T_R = 22.891 min.

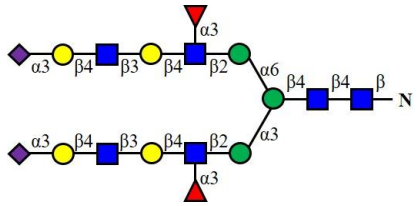


MALDI-MS, calculated 2778.0897; found $[M+Na]^+$ 2799.728, $[M+K]^+$ 2815.709.

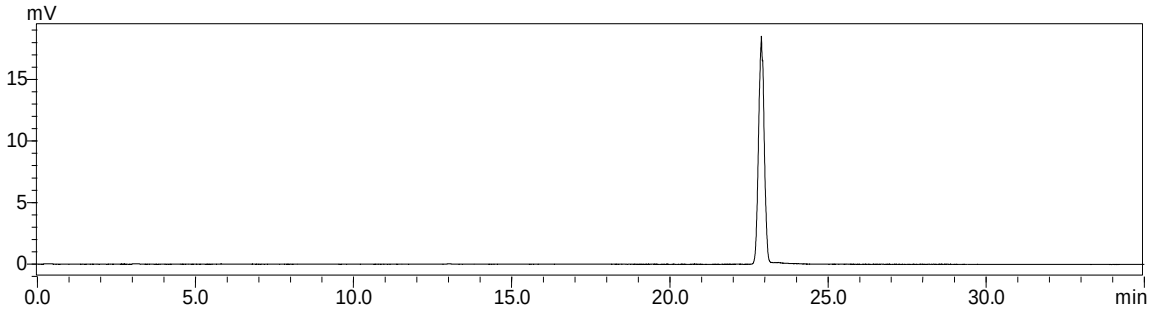


^{1}H NMR (D_2O, 500 MHz): δ 5.14-5.16 (m, 3 H, Fuc H-1, Man4 H-1), 5.10 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.0 Hz, GlcNAc 2 H-1), 4.62-4.66 (m, 3 H, GlcNAc5 H-1, GlcNAc5' H-1, GlcNAc7 H-1), 4.51-4.53 (m, 3 H, GlcNAc7' H-1, Gal6 H-1, Gal6' H-1), 4.47 (d, *J* = 7.6 Hz, 1 H, Gal8 H-1), 4.46 (d, *J* = 7.6 Hz, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (d, *J* = 2.3 Hz, 1 H), 4.14 (brs, 3 H), 3.50-4.04 (m, 81 H), 2.98 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.2, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.06 (s, 9 H, 3 Ac), 2.05 (s, 3 H, Ac), 1.19 (d, *J* = 6.2 Hz, 3 H, Fuc CH_3), 1.18 (d, *J* = 6.2 Hz, 3 H, Fuc CH_3).

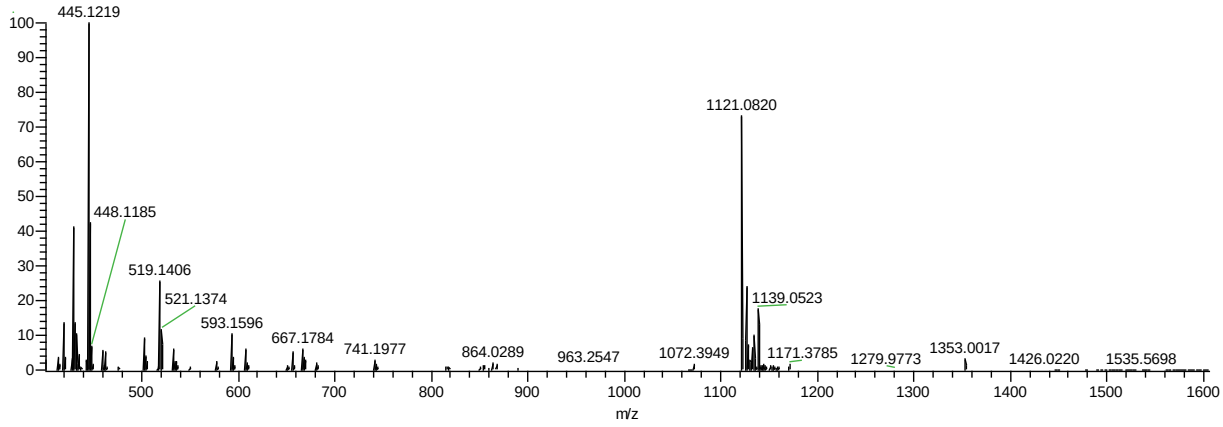
BA-29



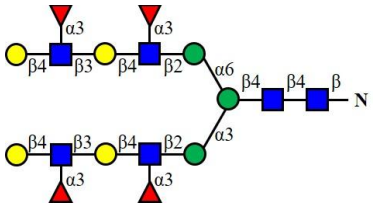
HILIC-ELSD, T_R = 23.063 min.



ESI-MS, calculated 3360.2805; found $[M+3H]^{3+}$ 1121.0820.

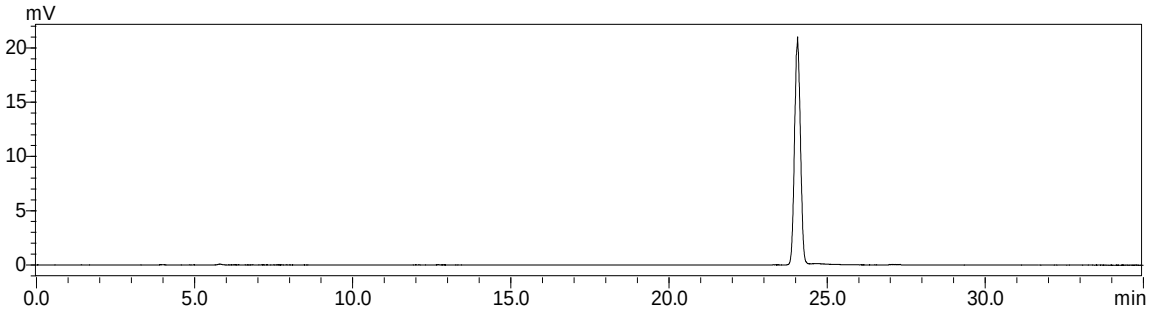


^{1}H NMR (D_2O, 500 MHz): δ 5.14-5.16 (m, 3 H, Fuc5 H-1, Fuc5' H-1, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 7.7 Hz, 1 H, GlcNAc 2 H-1), 4.59-4.66 (m, 5 H, , GlcNAc5 H-1, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1, Gal6 H-1), 4.51 (d, *J* = 7.8 Hz, 1 H, Gal6' H-1), 4.47 (d, *J* = 7.3 Hz, 1 H, , Gal8 H-1), 4.46 (d, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (brs, 1 H), 4.16 (d, J = 2.6 Hz, 1 H), 4.14 (brs, 4 H), 3.50-4.06 (m, 94 H), 2.97 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.79 (dd, *J* = 12.4, 4.5 Hz, 2 H, Neu5Ac H-3eq), 2.11 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.06 (s, 12 H, 4 Ac), 2.05 (s, 3 H, Ac), 1.83 (t, *J* = 12.4 Hz, 1 H, Neu5Ac H-3ax), 1.19 (d, *J* = 5.9 Hz, 3 H, Fuc CH_3), 1.18 (d, *J* = 5.9 Hz, 3 H, Fuc CH_3).

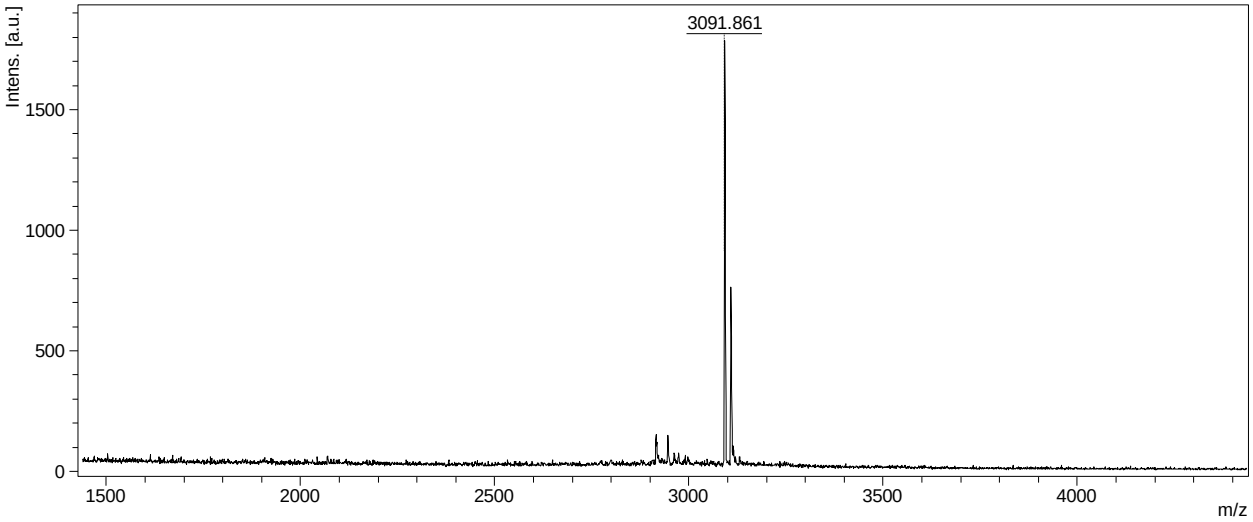


BA-30

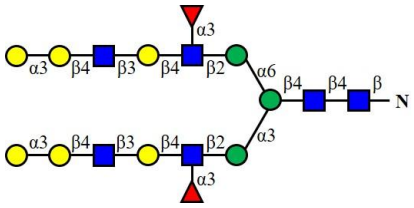
HILIC-ELSD, T_R = 24.067 min.



MALDI-MS, calculated 3070.2055; found $[M+Na]^+$ 3091.861, $[M+K]^+$ 3107.847.

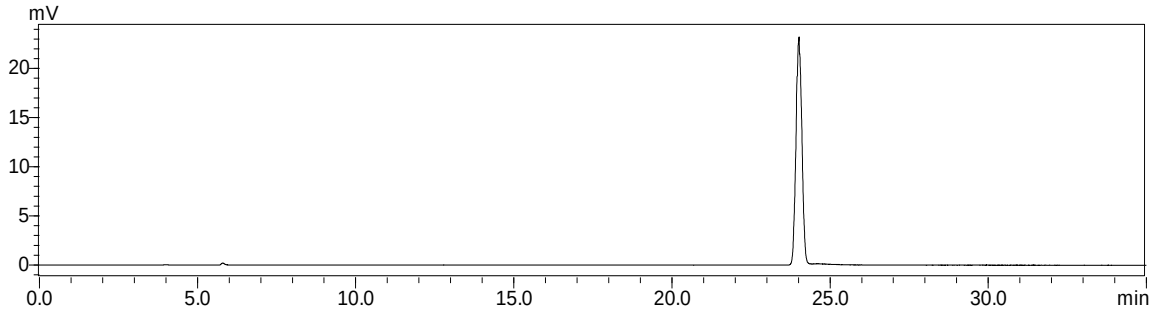


^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, *J* = 3.7 Hz, 2 H, Fuc H-1), 5.16 (s, 1 H, Man4 H-1), 5.15 (d, *J* = 3.9 Hz, 2 H, Fuc H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.87 (d, *J* = 6.7 Hz, 1 H, GlcNAc 2 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.0 Hz, GlcNAc5 H-1), 4.62-4.66 (m, 3 H, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1), 4.50 (d, *J* = 7.8 Hz, 2 H, Gal6 H-1, Gal6' H-1), 4.47 (d, *J* = 7.5 Hz, 1 H, Gal8 H-1), 4.46 (d, *J* = 7.1 Hz, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (d, *J* = 1.9 Hz, 1 H), 4.14 (brs, 4 H), 3.50-4.04 (m, 80 H), 2.98 (dd, *J* = 17.3, 4.4 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.2, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.06 (s, 6 H, 2 Ac), 2.05 (s, 6 H, 2 Ac), 1.21 (d, *J* = 6.6 Hz, 6 H, 2 Fuc CH_3), 1.19 (d, *J* = 6.3 Hz, 3 H, Fuc CH3), 1.18 (d, *J* = 6.1 Hz, 3 H, Fuc CH_3).

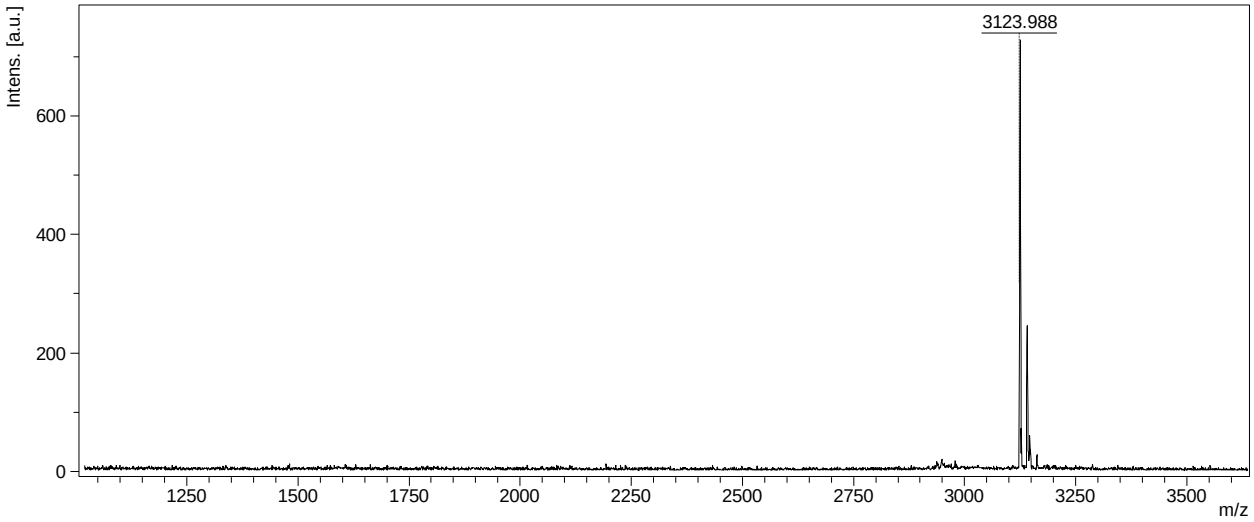


BA-31

HILIC-ELSD, T_R = 24.016 min.

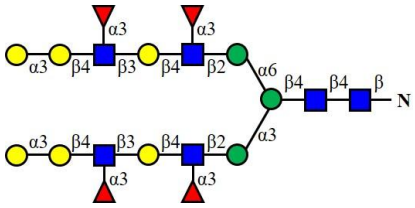


MALDI-MS, calculated 3102.1953; found $[M+Na]^+$ 3123.988, $[M+K]^+$ 3139.978.

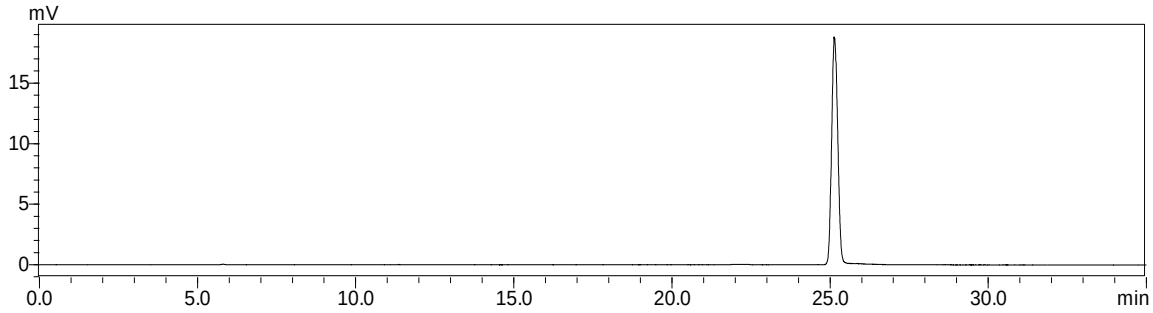


^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, *J* = 3.7 Hz, 2 H, Gal9 H-1, Gal9' H-1), 5.15 (s, 1 H, Man4 H-1), 5.14 (d, *J* = 3.7 Hz, 2 H, Fuc H-1), 5.10 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.84 (d, *J* = 6.8 Hz, 1 H, GlcNAc 2 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 7.6 Hz, 1 H, GlcNAc5 H-1), 4.58-4.66 (m, 5 H, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1, Gal6 H-1, Gal6' H-1), 4.47 (d, *J* = 7.2 Hz, 1 H, Gal8 H-1), 4.46 (d, *J* = 7.0 Hz, 1 H, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.21-4.24 (m, 5 H), 4.13 (m, 3 H), 3.51-4.06 (m, 80 H), 2.98 (dd, *J* = 17.4, 4.1 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.4, 7.0 Hz, 1 H, Asn-βCH_2), 2.11 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 6 H, 2 Ac), 2.06(s, 6 H, 2 Ac), 2.05 (s, 3 H, Ac), 1.19 (d, *J* = 5.9 Hz, 3 H, Fuc CH_3), 1.18 (d, *J* = 6.0 Hz, 3 H, Fuc CH_3).

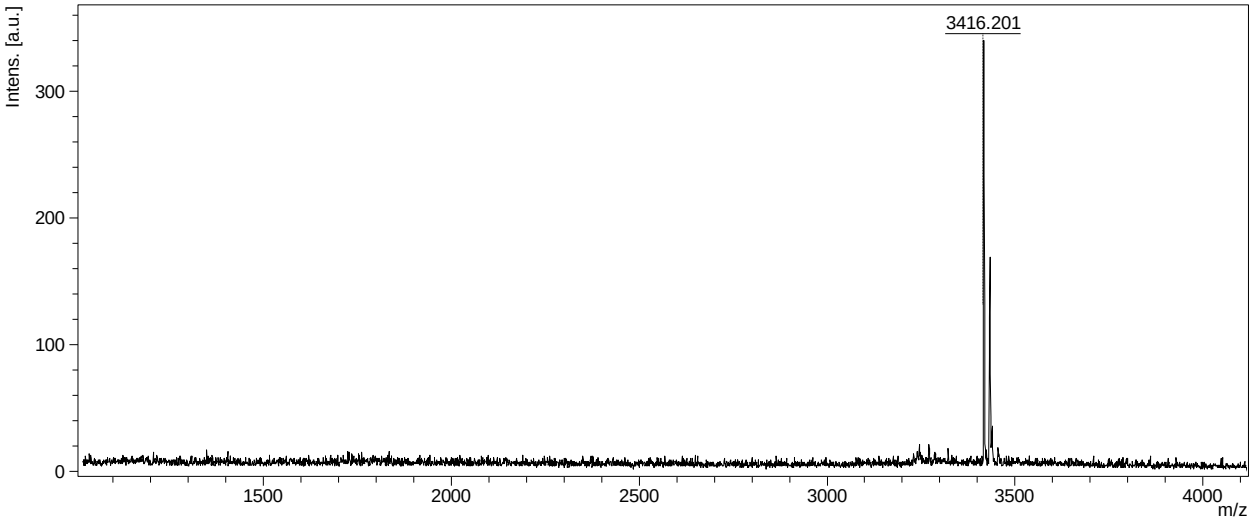
BA-32



HILIC-ELSD, T_R = 25.127 min.

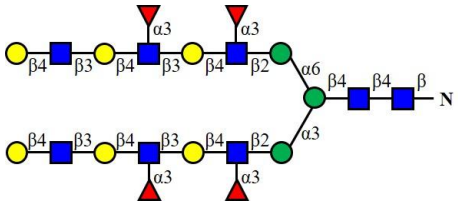


MALDI-MS, calculated 3394.3111; found $[M+Na]^+$ 3416.201, $[M+K]^+$ 3432.191.

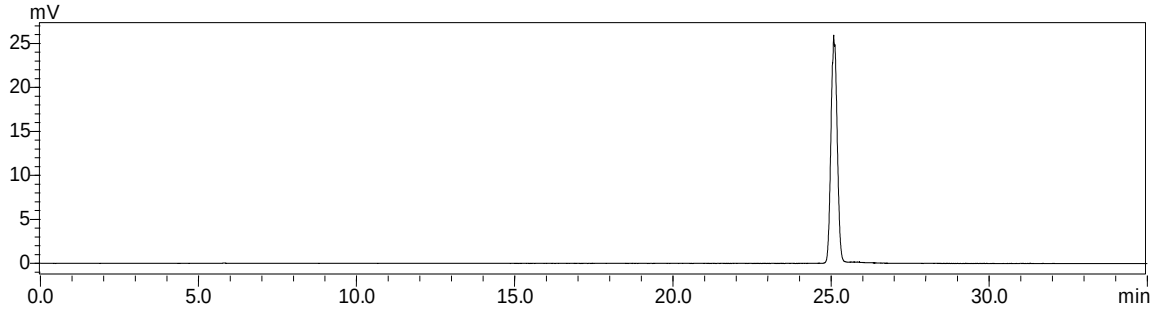


^{1}H NMR (D_2O, 500 MHz): δ 5.19 (d, *J* = 3.6 Hz, 4 H, Gal9 H-1, Gal9' H-1, Fuc7 H-1, Fuc7' H-1), 5.16 (s, 1 H, Man4 H-1), 5.15 (d, *J* = 3.7 Hz, 2 H, Fuc5 H-1, Fuc5' H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.87 (d, *J* = 6.8 Hz, 1 H, GlcNAc 2 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.75 (d, *J* = 8.2 Hz, 1 H, GlcNAc5 H-1), 4.62-4.66 (m, 3 H, GlcNAc5' H-1, GlcNAc7 H-1, GlcNAc7' H-1), 4.58 (d, *J* = 7.7 Hz, 2 H, , Gal6 H-1, Gal6' H-1), 4.45-4.48 (m, 2 H, Gal8 H-1, Gal8' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.20-4.25 (m, 5 H), 4.13 (brs, 3 H), 3.50-4.06 (m, 88 H), 2.98 (dd, *J* = 17.4, 4.4 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.4, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.06 (s, 12 H, 4 Ac), 1.22 (d, *J* = 6.5 Hz, 6 H, 6 H, Fuc CH_3), 1.20 (d, *J* = 5.9 Hz, 3 H, Fuc CH_3), 1.19 (d, *J* = 5.8 Hz, 3 H, Fuc CH_3).

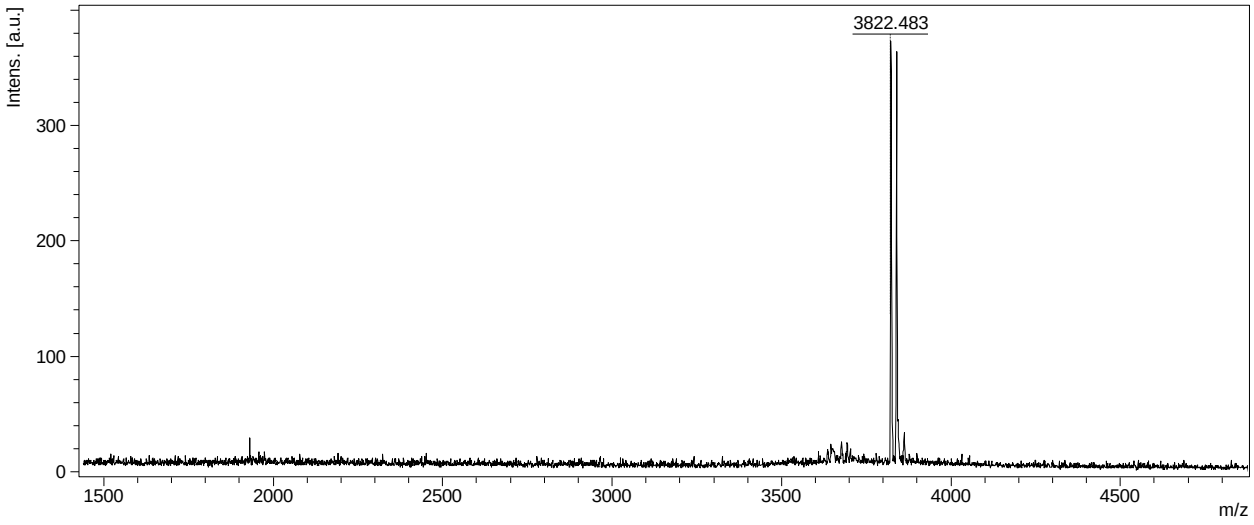
BA-33



HILIC-ELSD, T_R = 25.084 min.

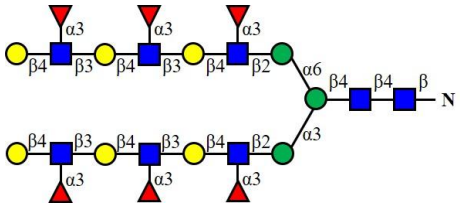


MALDI-MS, calculated 3800.4699; found $[M+Na]^+$ 3822.483, $[M+K]^+$ 3838.475.

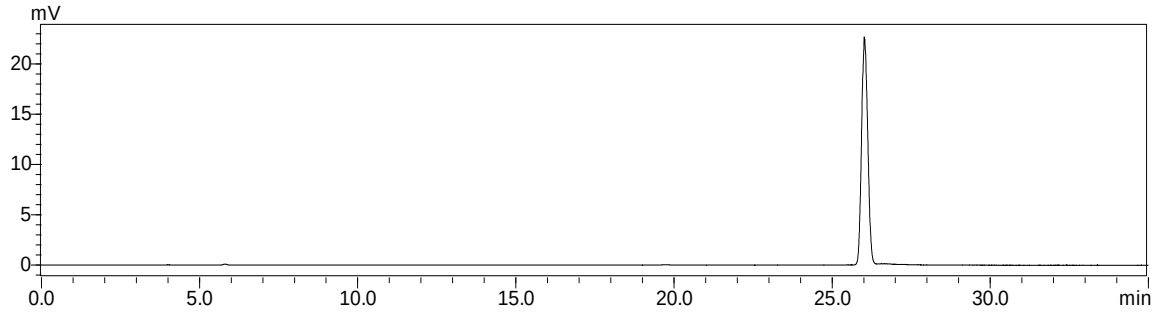


^{1}H NMR (D_2O, 500 MHz): δ 5.16 (brs, 5 H, Fuc7 H-1, Fuc7' H-1, Fuc5 H-1, Fuc5' H-1, Man4 H-1), 5.11 (d, *J* = 9.8 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.85 (d, *J* = 6.8 Hz, 1 H, GlcNAc 2 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.0 Hz, 2 H, GlcNAc5 H-1, GlcNAc5' H-1), 4.62-4.66 (m, 3 H, GlcNAc7 H-1, GlcNAc7' H-1, GlcNAc9 H-1), 4.46-4.53 (m, 7 H, GlcNAc9' H-1, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1, Gal10 H-1, Gal10' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (brs, 1 H), 4.13 (m, 8 H), 3.50-4.04 (m, 109 H), 2.97 (dd, *J* = 17.2, 3.3 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.2, 6.6 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 9 H, 3 Ac), 2.05 (s, 9 H, Ac), 1.19 (d, *J* = 5.3 Hz, 12 H, 4 Fuc CH_3).

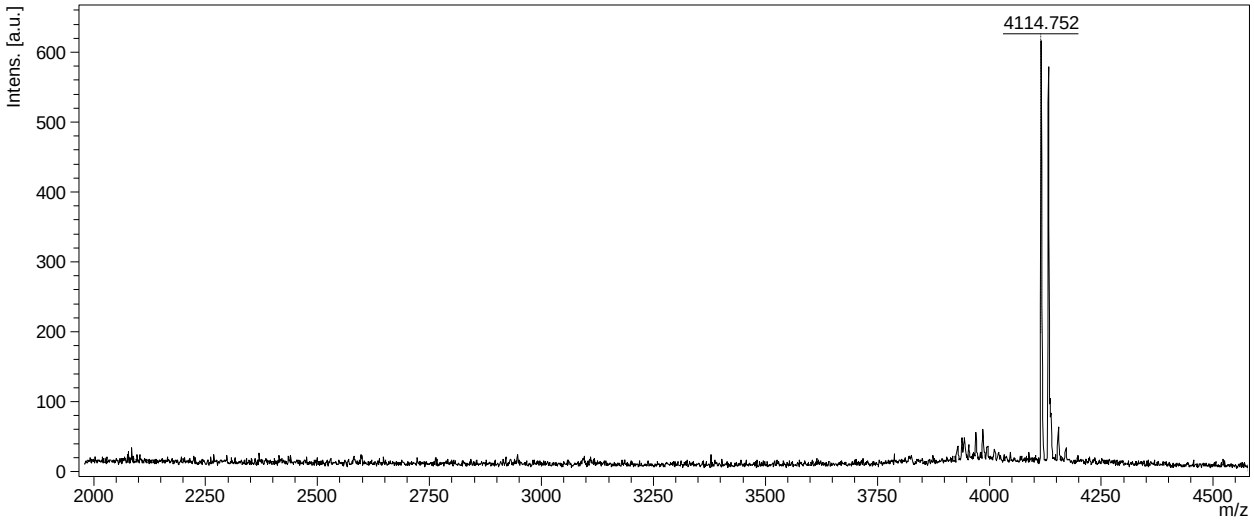
BA-34



HILIC-ELSD, T_R = 26.016 min.

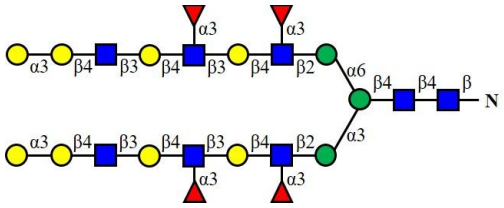


MALDI-MS, calculated 4092.5857; found $[M+Na]^+$ 4114.752, $[M+K]^+$ 4130.760.

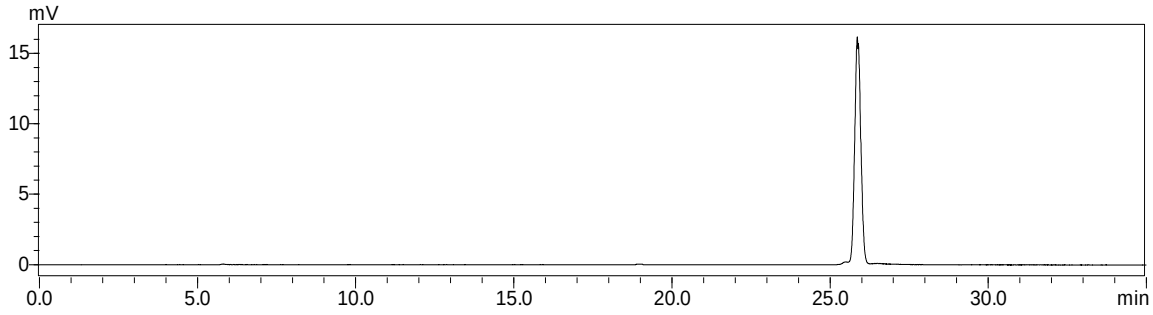


^{1}H NMR (D_2O, 500 MHz): δ 5.15-5.18 (m, 7 H, Fuc5 H-1, Fuc5' H-1, Fuc7 H-1, Fuc7' H-1, Fuc9 H-1, Fuc9' H-1, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.84-4.88 (m, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.2 Hz, 2 H, GlcNAc5' H-1, GlcNAc7 H-1), 4.62-4.66 (m, 3 H, GlcNAc7' H-1, GlcNAc9 H-1, GlcNAc9' H-1), 4.45-4.51 (m, 6 H, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1, Gal10 H-1, Gal10' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22 (brs, 1 H), 4.14 (m, 7 H), 3.50-4.04 (m, 118 H), 2.98 (dd, *J* = 17.2, 4.3 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.2, 6.7 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 6 H, 2 Ac), 2.06 (s, 15 H, 5 Ac), 1.21 (d, *J* = 6.6 Hz, 6 H, 2 Fuc CH_3), 1.19 (d, *J* = 6.1 Hz, 12 H, 4 Fuc CH_3).

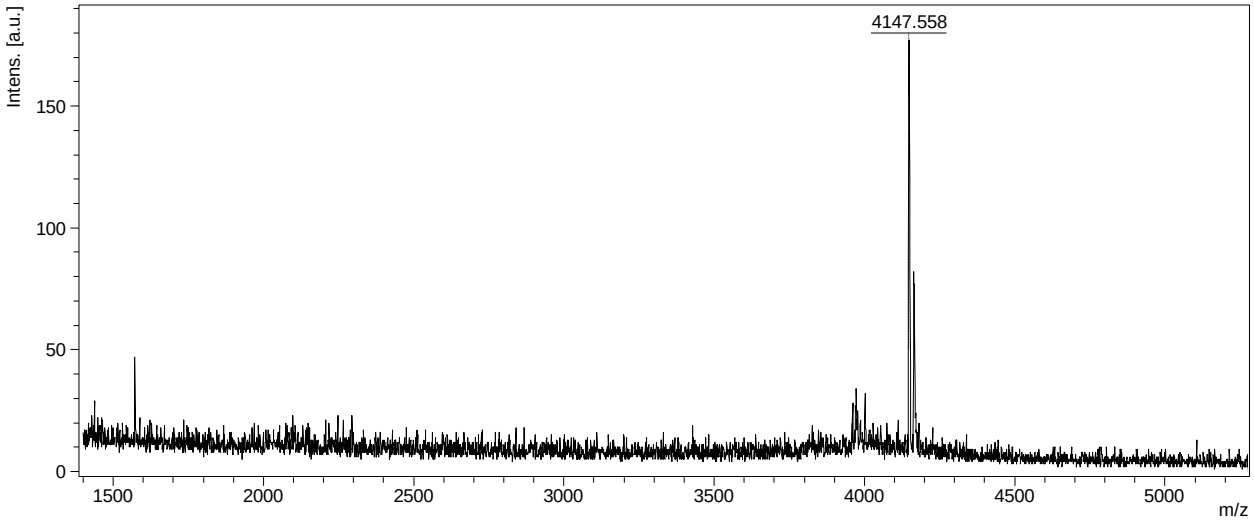
BA-35



HILIC-ELSD, T_R = 25.860 min.

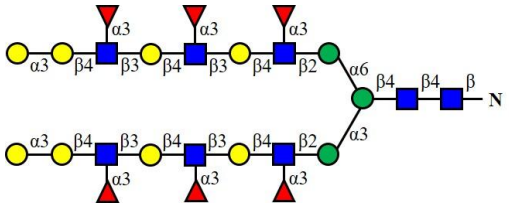


MALDI-MS, calculated 4124.5755; found $[M+Na]^+$ 4147.558, $[M+K]^+$ 4163.586.

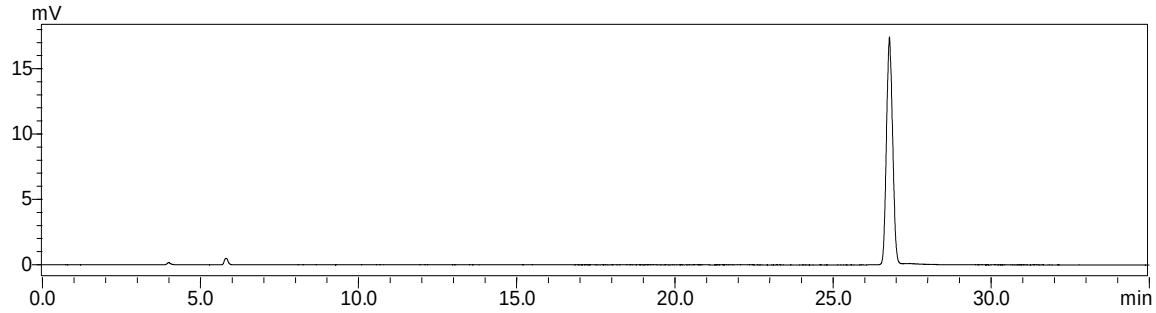


^{1}H NMR (D_2O, 500 MHz): δ 5.18 (d, *J* = 3.8 Hz, 2 H, Gal11 H-1, Gal11' H-1), 5.12-5.16 (m, 5 H, Fuc5 H-1, Fuc5' H-1, Fuc7 H-1, Fuc7' H-1, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.85 (d, *J* = 6.2 Hz, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.74 (d, *J* = 8.1 Hz, 2 H, GlcNAc5' H-1, GlcNAc7 H-1), 4.65 (d, *J* = 7.9 Hz, 1 H, GlcNAc7' H-1), 4.63 (d, *J* = 8.1 Hz, 1 H, GlcNAc9 H-1), 4.59 (d, *J* = 7.8 Hz, 1 H, GlcNAc9' H-1), 4.45-4.50 (m, 6 H, Gal6 H-1, Gal6' H-1, Gal8 H-1, Gal8' H-1, Gal10 H-1, Gal10' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22-4.24 (m, 5 H), 4.14 (brs, 5 H), 3.50-4.06 (m, 120 H), 2.98 (dd, *J* = 17.3, 4.3 Hz, 1 H, Asn-βCH_2), 2.90 (dd, *J* = 17.3, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 3 H, Ac), 2.07 (s, 9 H, 3 Ac), 2.05 (s, 9 H, 3 Ac), 1.19 (d, *J* = 6.1 Hz, 12 H, 4 Fuc CH_3).

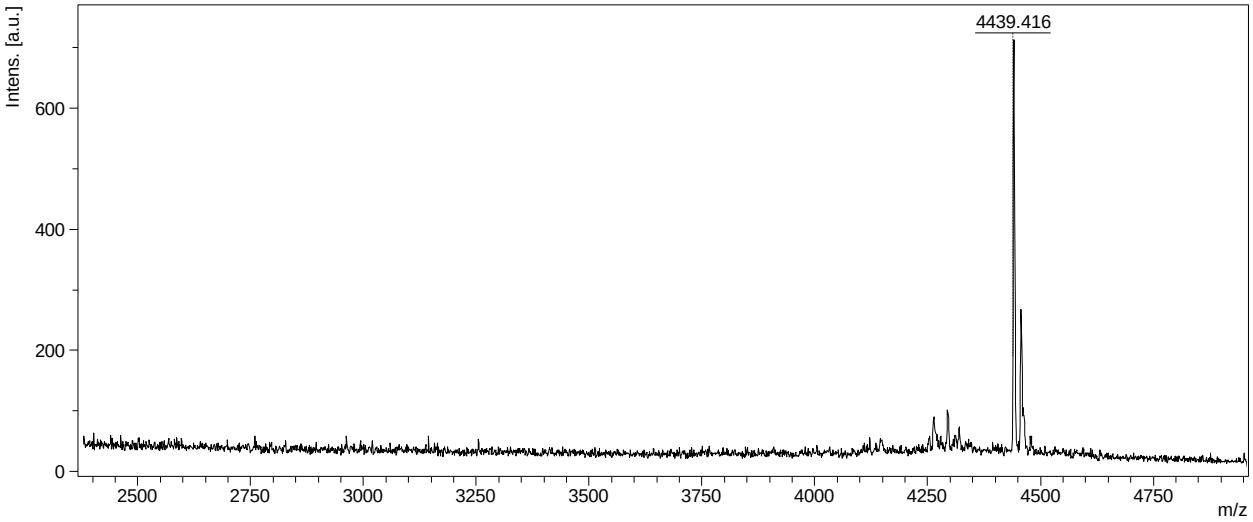
BA-36



HILIC-ELSD, T_R = 26.784 min.



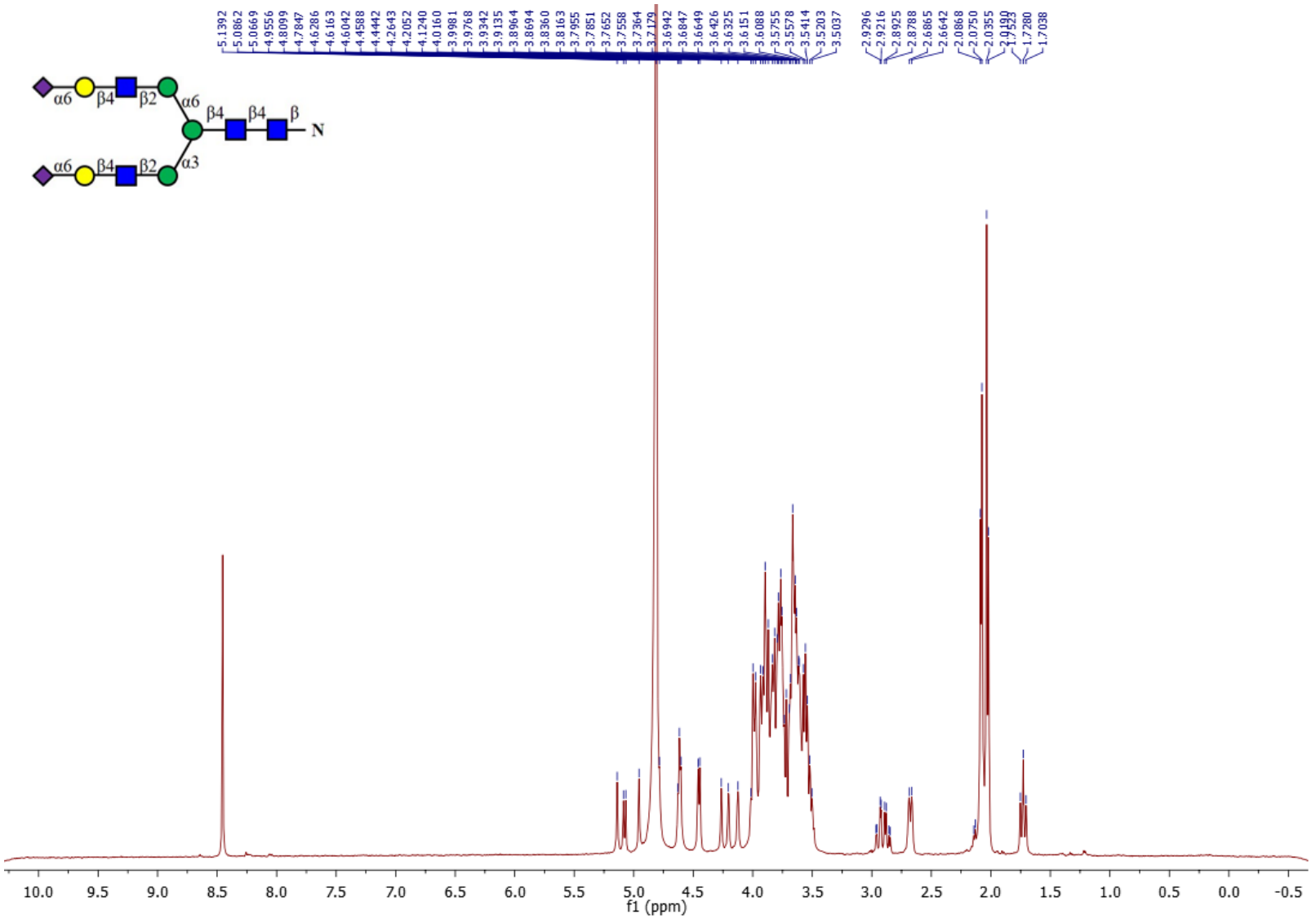
MALDI-MS, calculated 4416.6913; found $[M+Na]^+$ 4439.416, $[M+K]^+$ 4455.475.



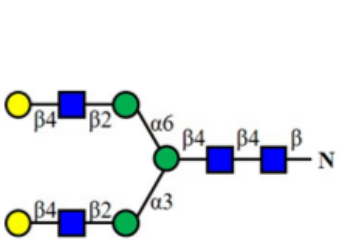
^{1}H NMR (D_2O, 500 MHz): δ 5.15-5.19 (m, 9 H, Gal11 H-1, Gal11' H-1, Fuc5 H-1, Fuc5' H-1, Fuc7 H-1, Fuc7' H-1, Fuc9 H-1, Fuc9' H-1, Man4 H-1), 5.11 (d, *J* = 9.7 Hz, 1 H, GlcNAc1 H-1), 4.95 (s, 1 H, Man4' H-1), 4.84-4.87 (m, 2 H, GlcNAc 2 H-1, GlcNAc5 H-1), 4.81 (s, 1 H, overlap with D_2O, Man3 H-1), 4.75 (d, *J* = 8.0 Hz, 2 H, GlcNAc5' H-1, GlcNAc7 H-1), 4.62-4.66 (m, 2 H, GlcNAc7' H-1, GlcNAc9 H-1), 4.57 (d, *J* = 7.7 Hz, 2 H, GlcNAc9' H-1, Gal6 H-1), 4.46-4.49 (m, 5 H, Gal6' H-1, Gal8 H-1, Gal8' H-1, Gal10 H-1, Gal10' H-1), 4.29 (brs, 1 H, Man3 H-2), 4.22-4.25 (m, 5 H), 4.14 (brs, 5 H), 3.50-4.06 (m, 128 H), 2.98 (dd, *J* = 17.4, 4.2 Hz, 1 H, Asn-βCH_2), 2.91 (dd, *J* = 17.4, 6.8 Hz, 1 H, Asn-βCH_2), 2.12 (s, 3 H, Ac), 2.08 (s, 6 H, 2 Ac), 2.06 (s, 15 H, 5 Ac), 1.19 (d, *J* = 6.0 Hz, 18 H, 6 Fuc CH_3).

VI. NMR spectra of purified *N*-glycans

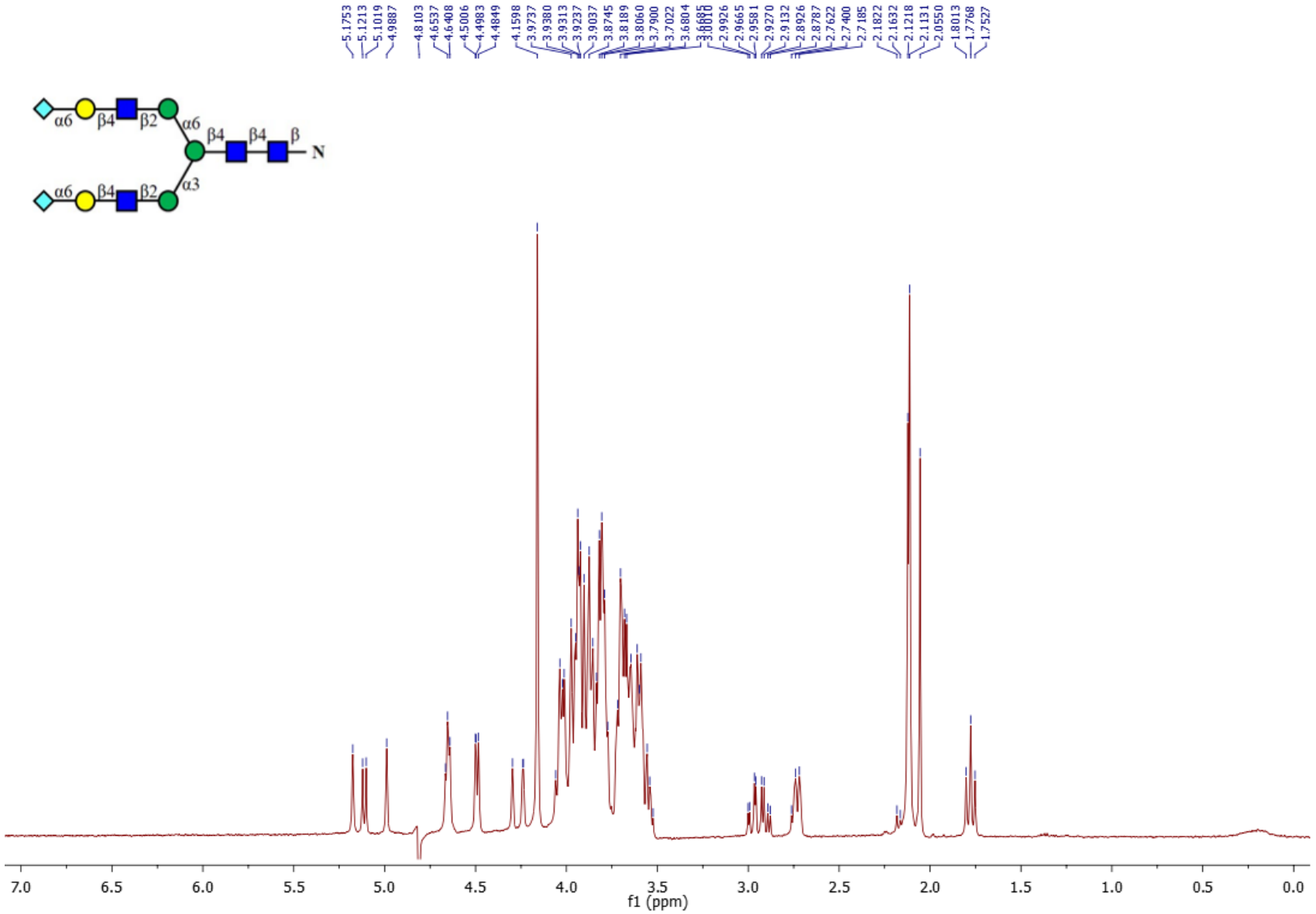
^{1}H NMR of BA-01



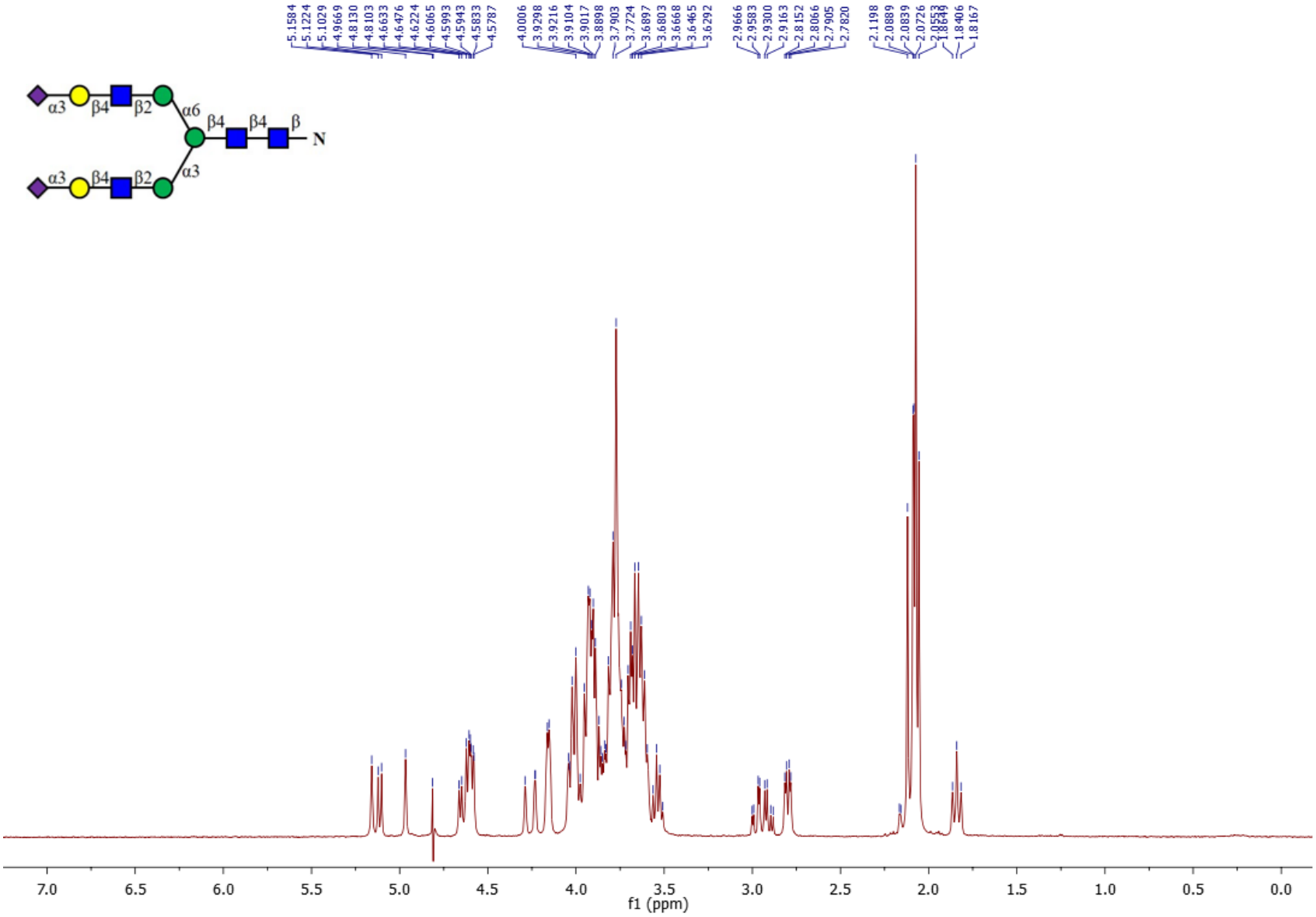
^{1}H NMR of BA-02



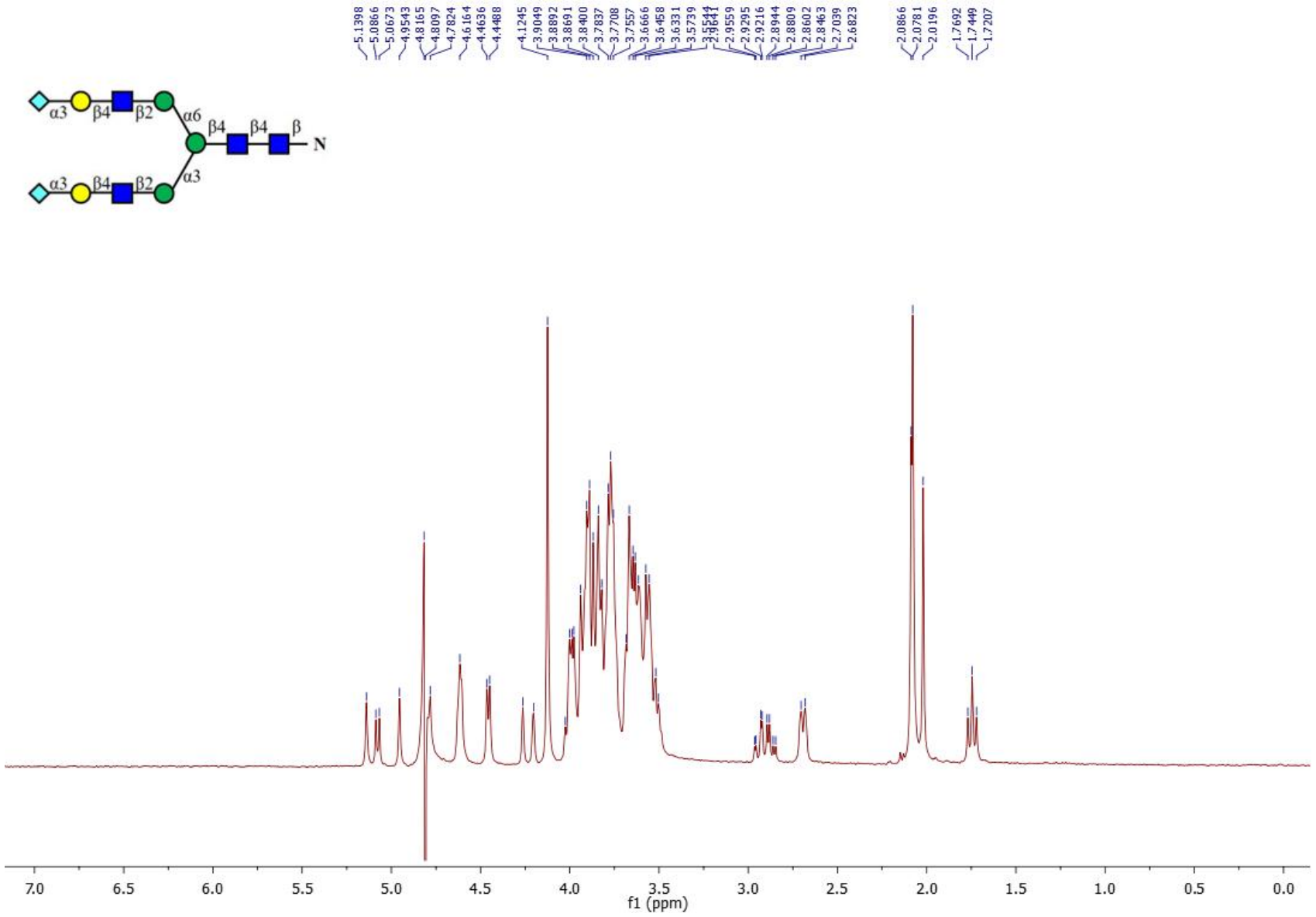
^{1}H NMR of BA-03



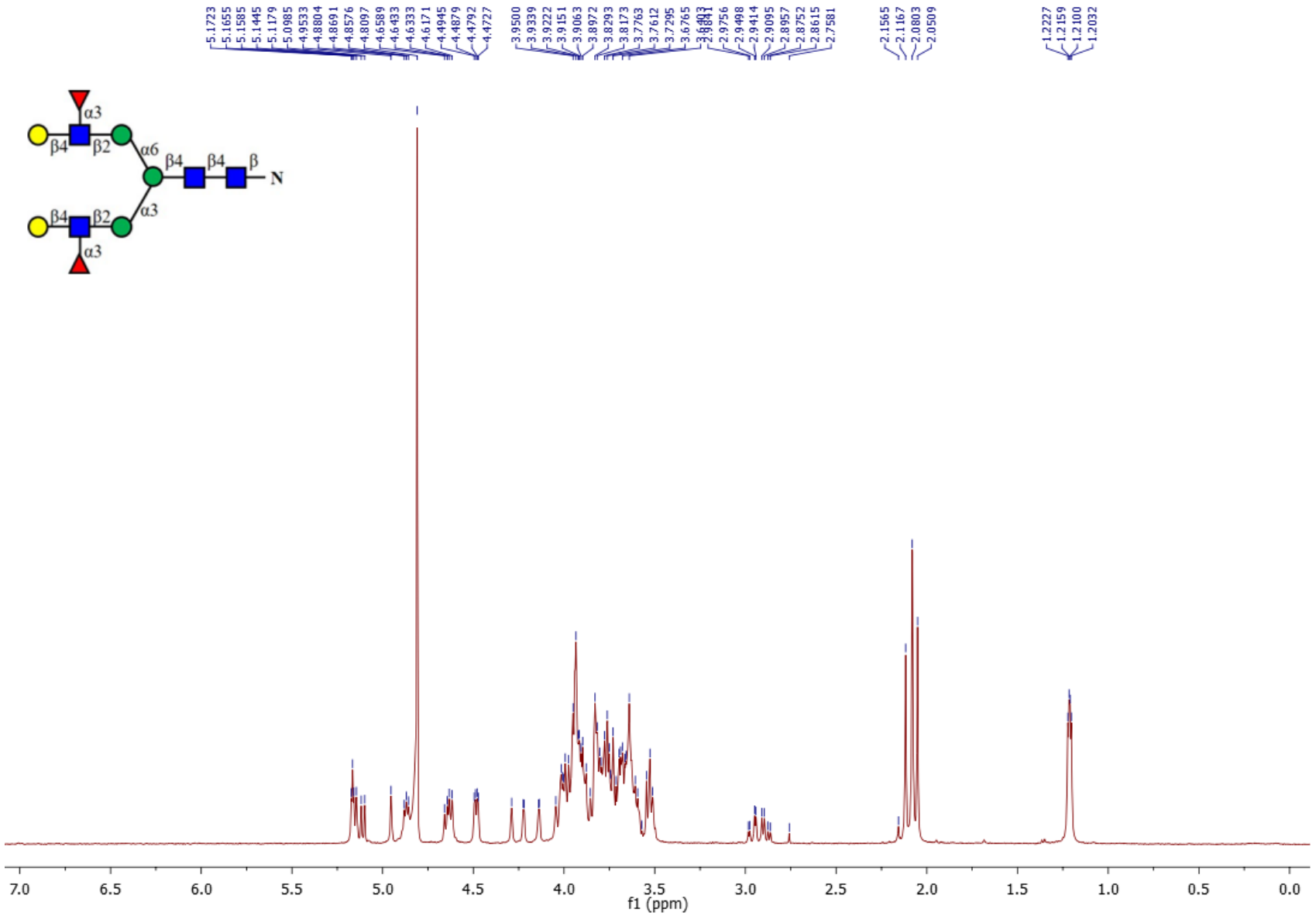
^{1}H NMR of BA-04



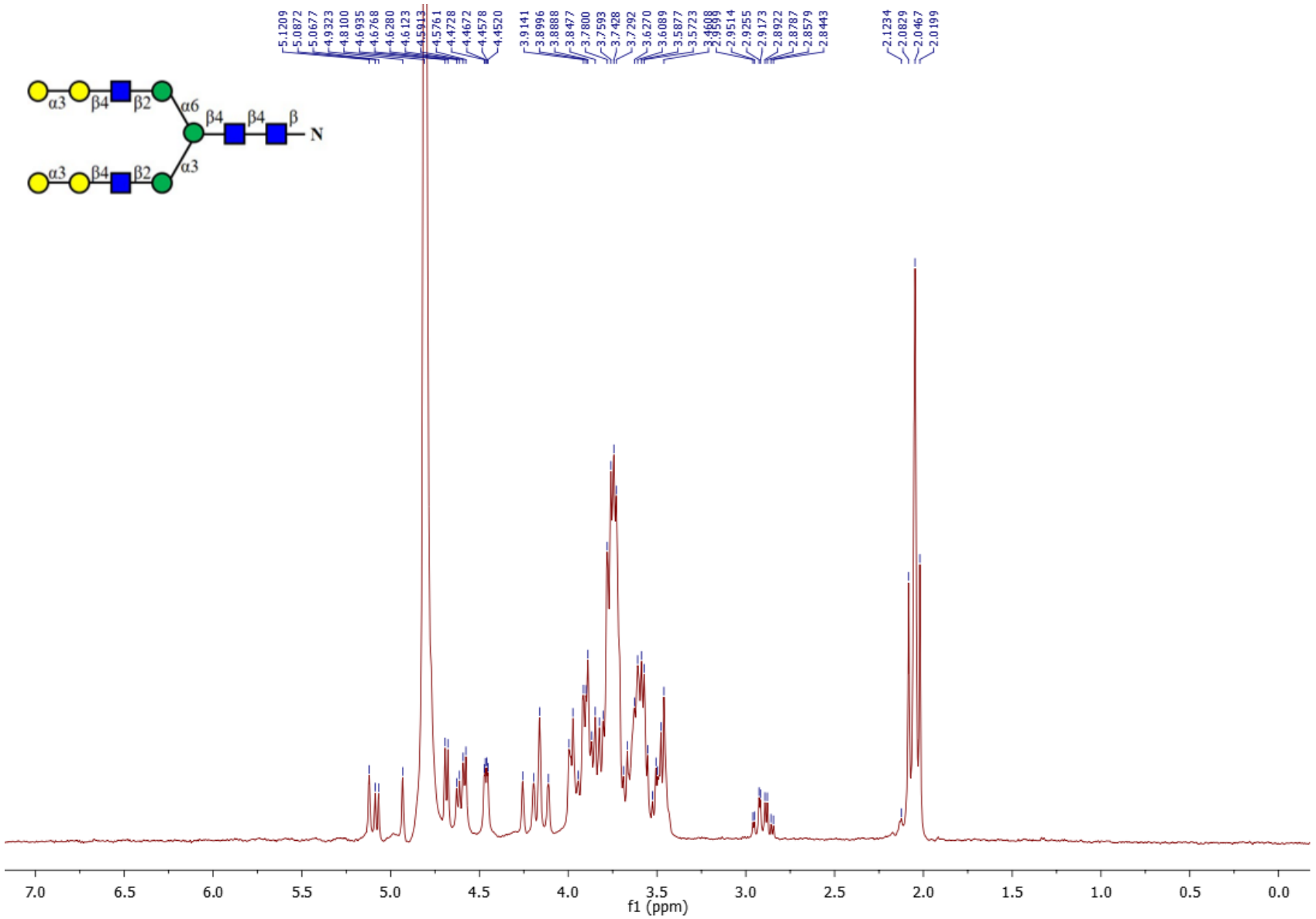
[1]H NMR of BA-05



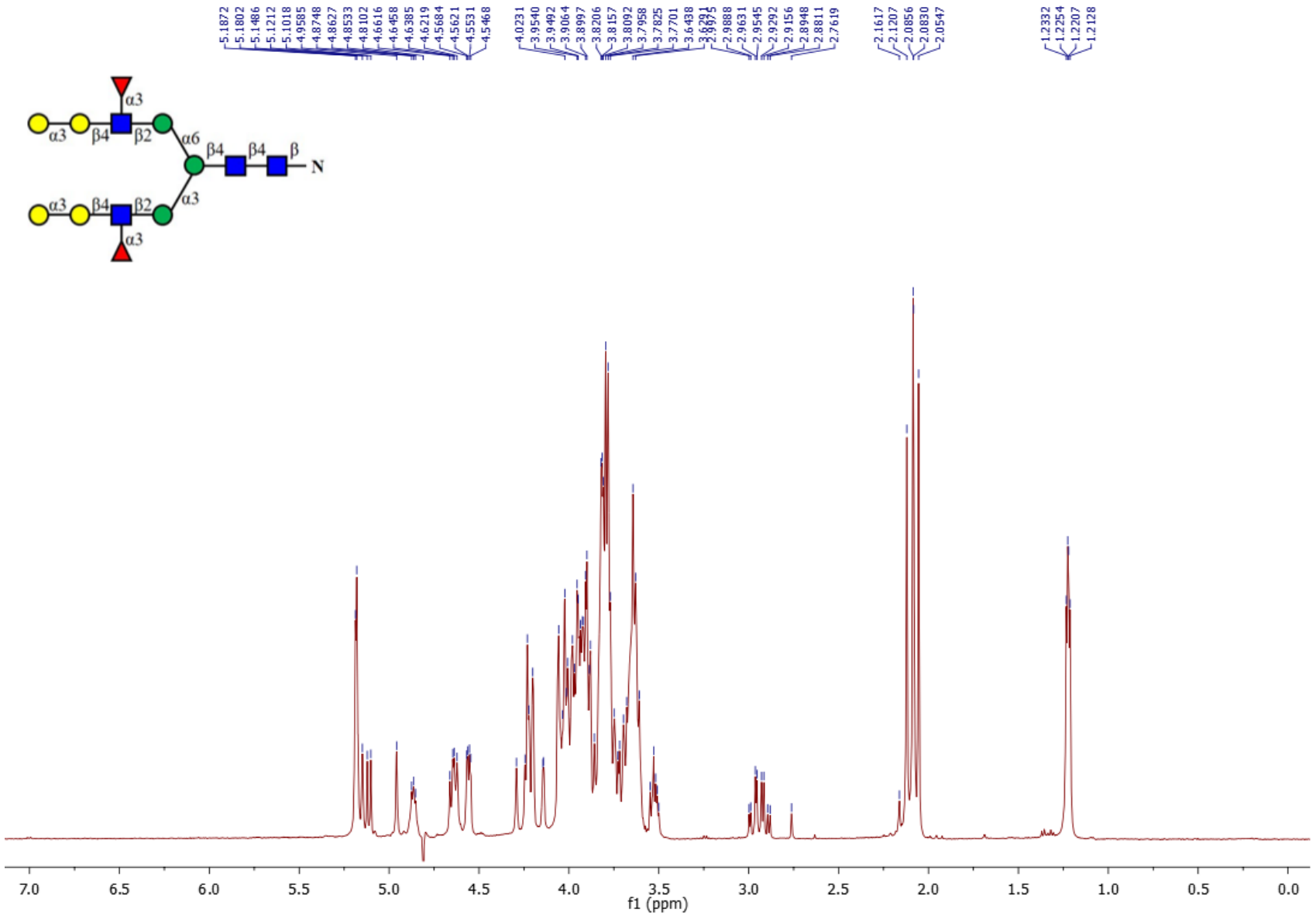
^{1}H NMR of BA-06



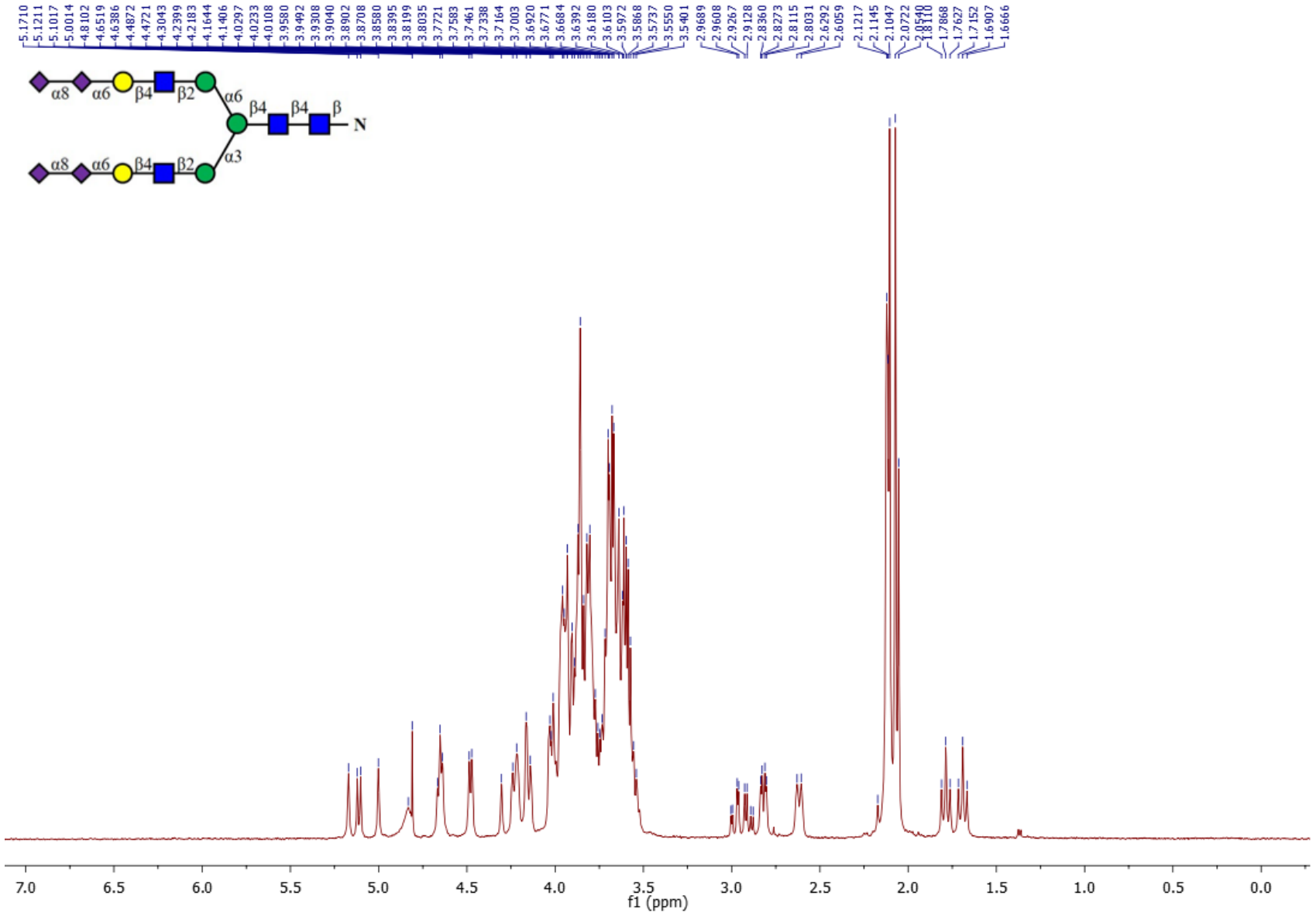
^{1}H NMR of BA-07



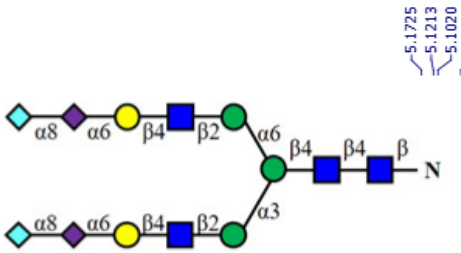
^{1}H NMR of BA-08



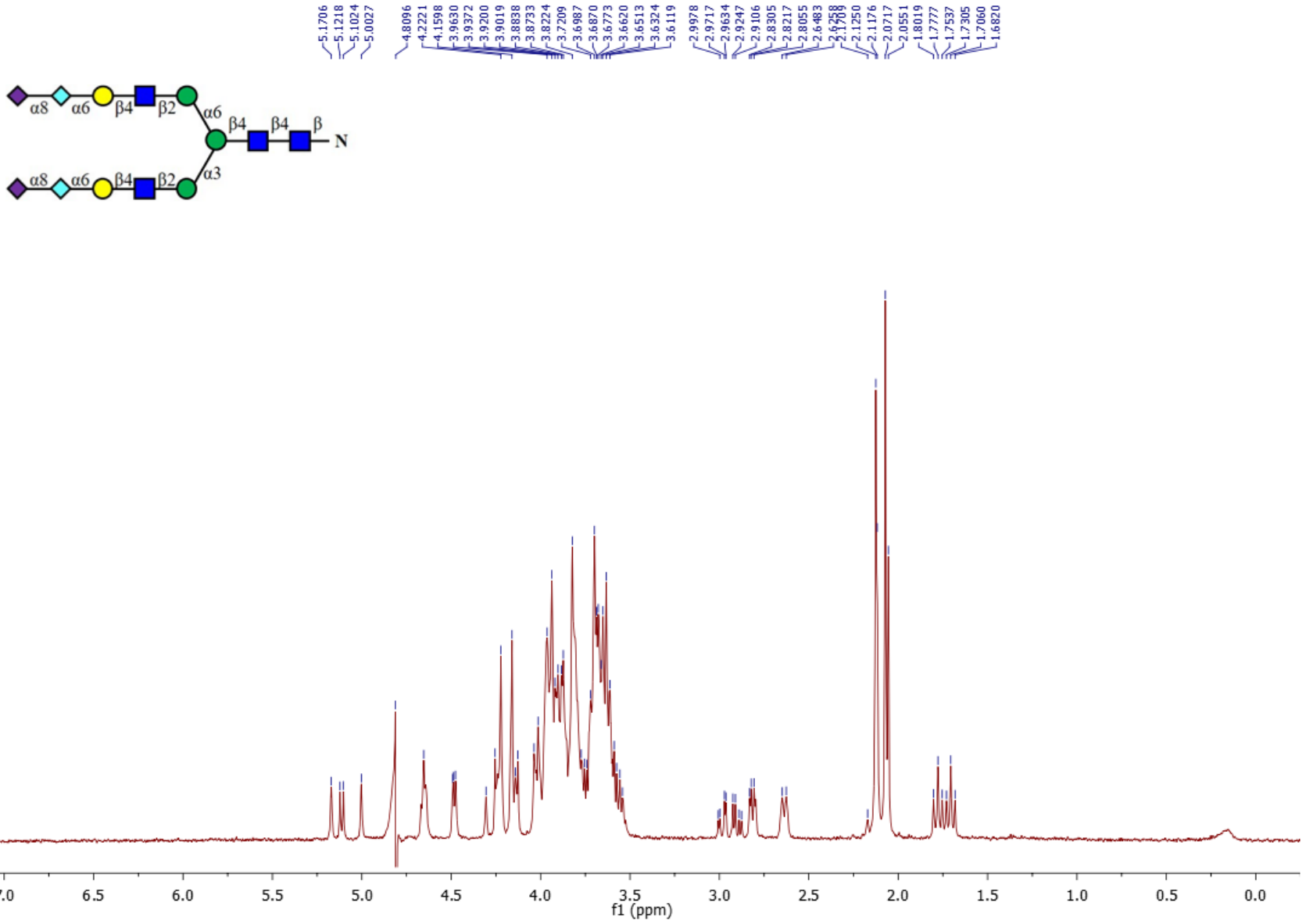
^{1}H NMR of BA-09



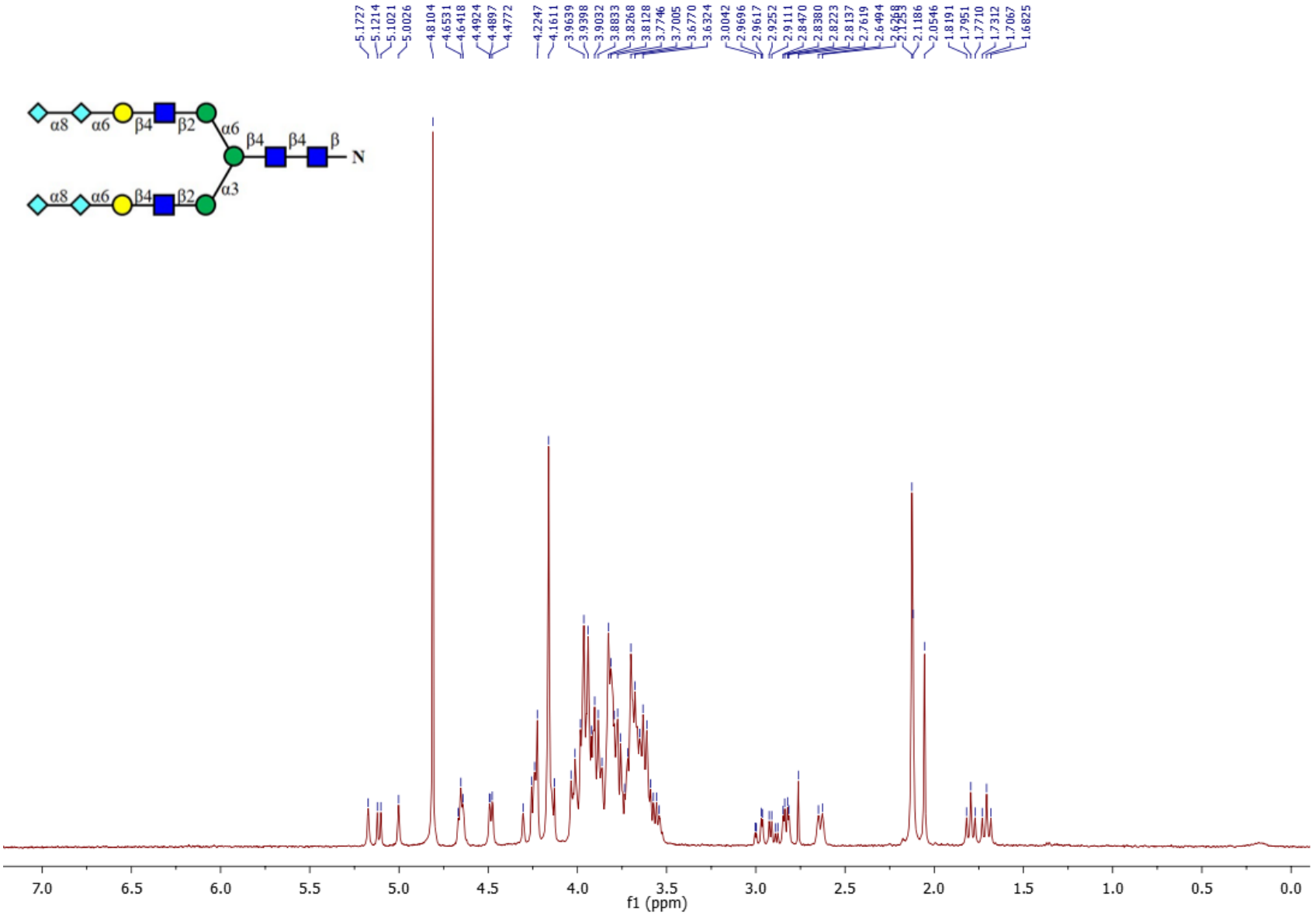
^{1}H NMR of BA-10



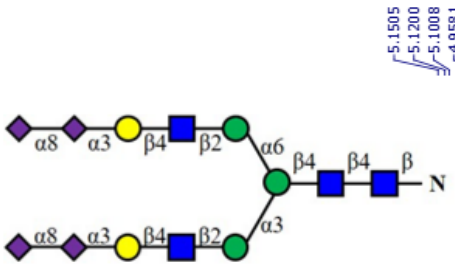
^{1}H NMR of BA-11



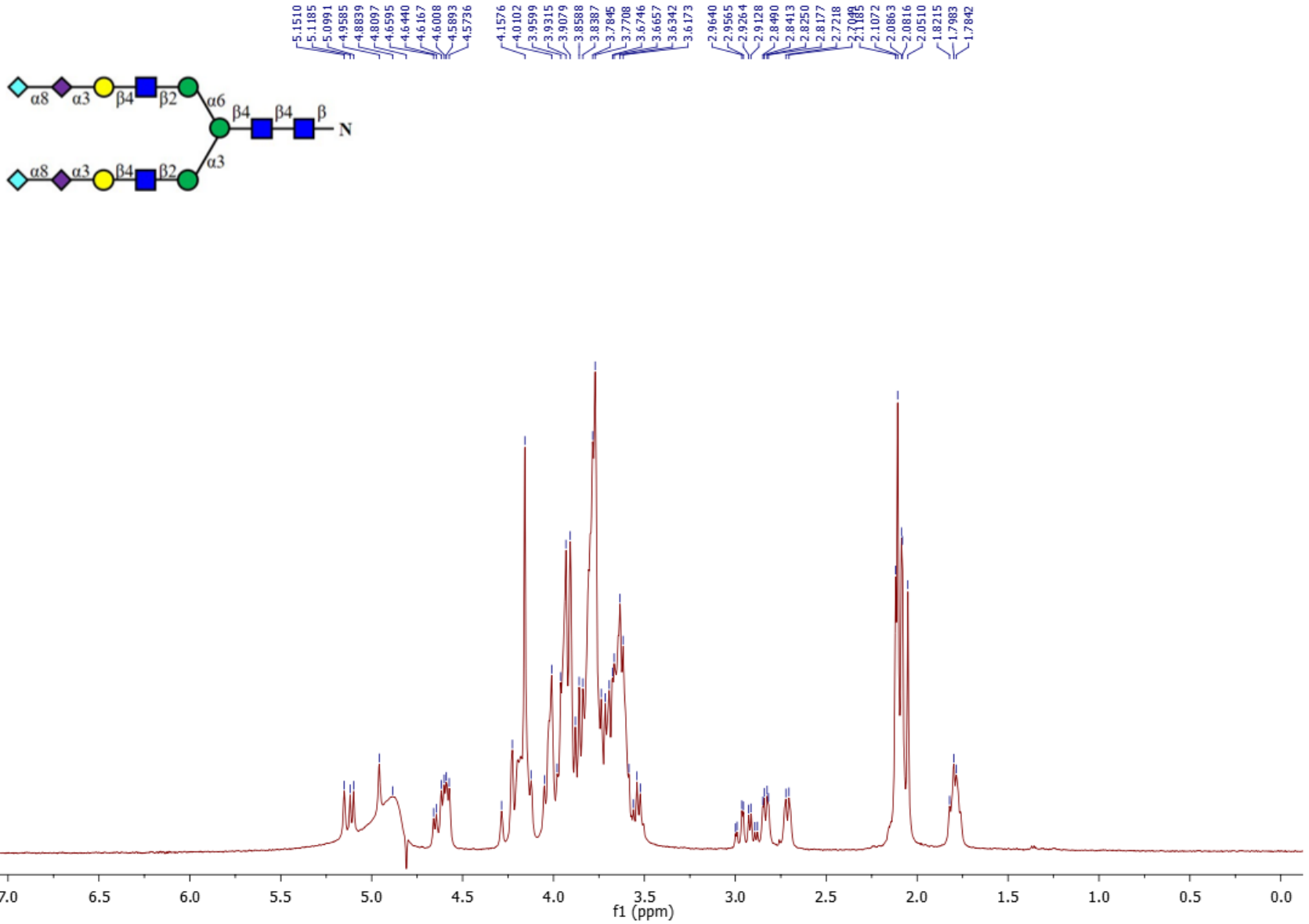
^{1}H NMR of BA-12



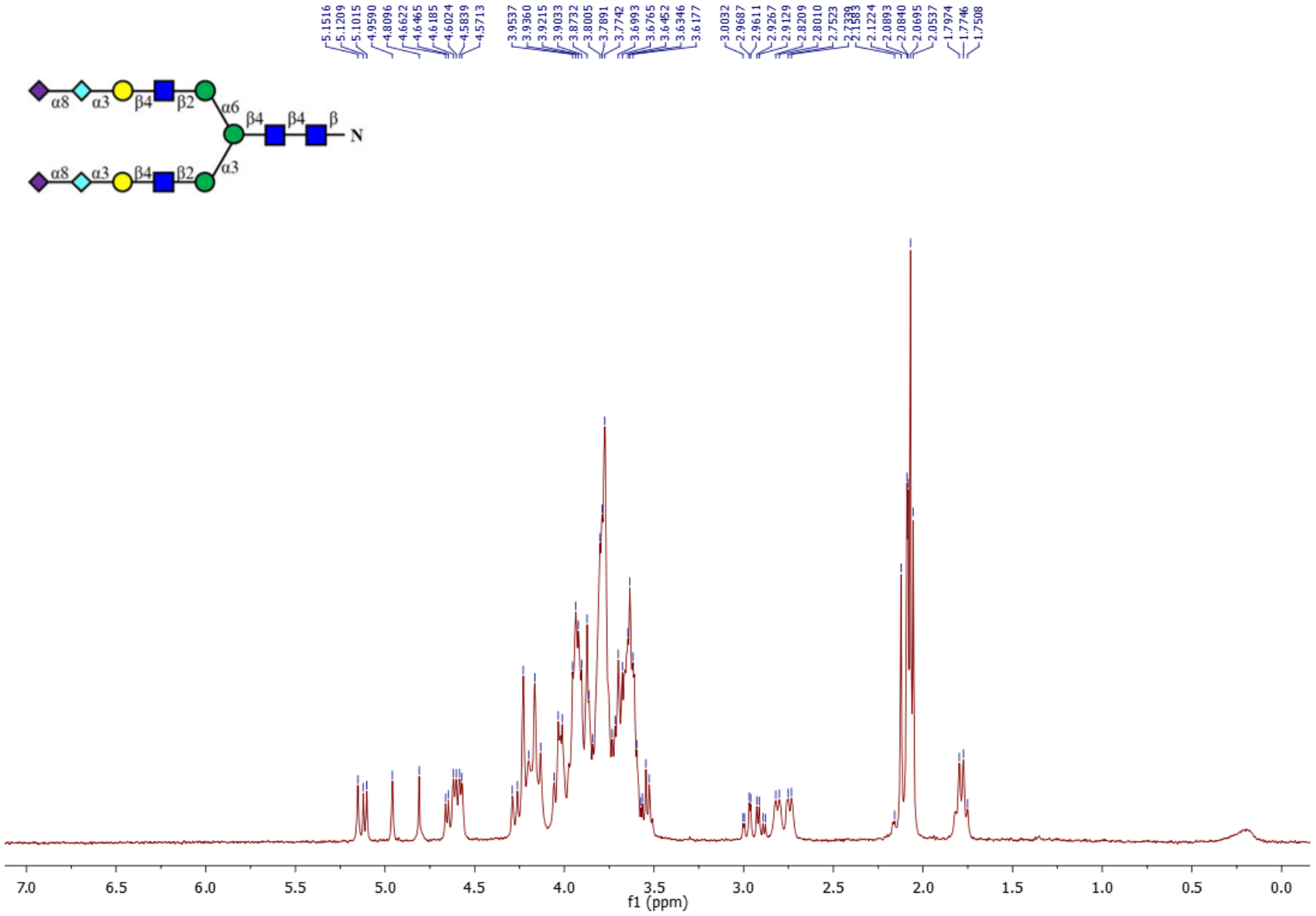
^{1}H NMR of BA-13



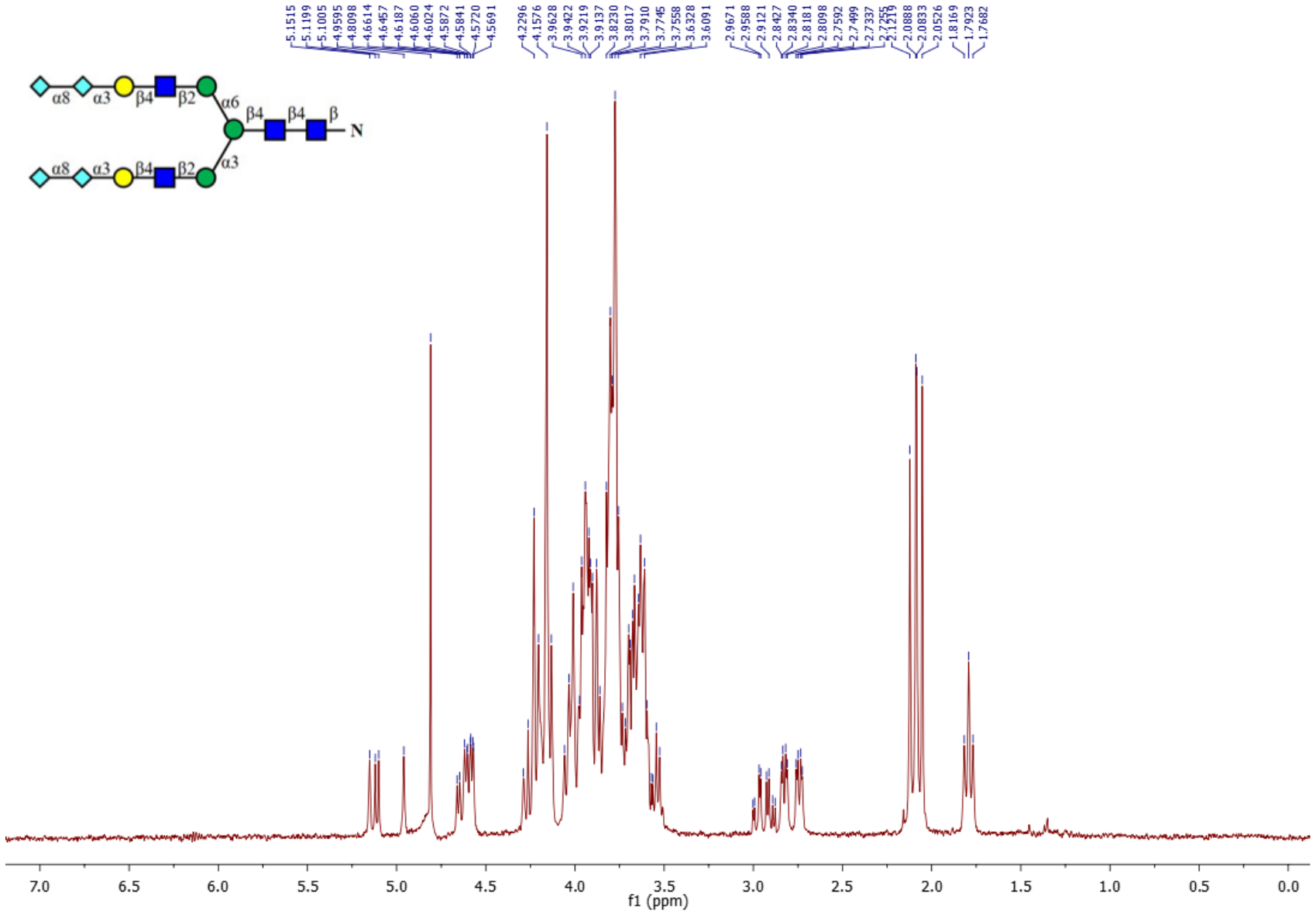
^{1}H NMR of BA-14



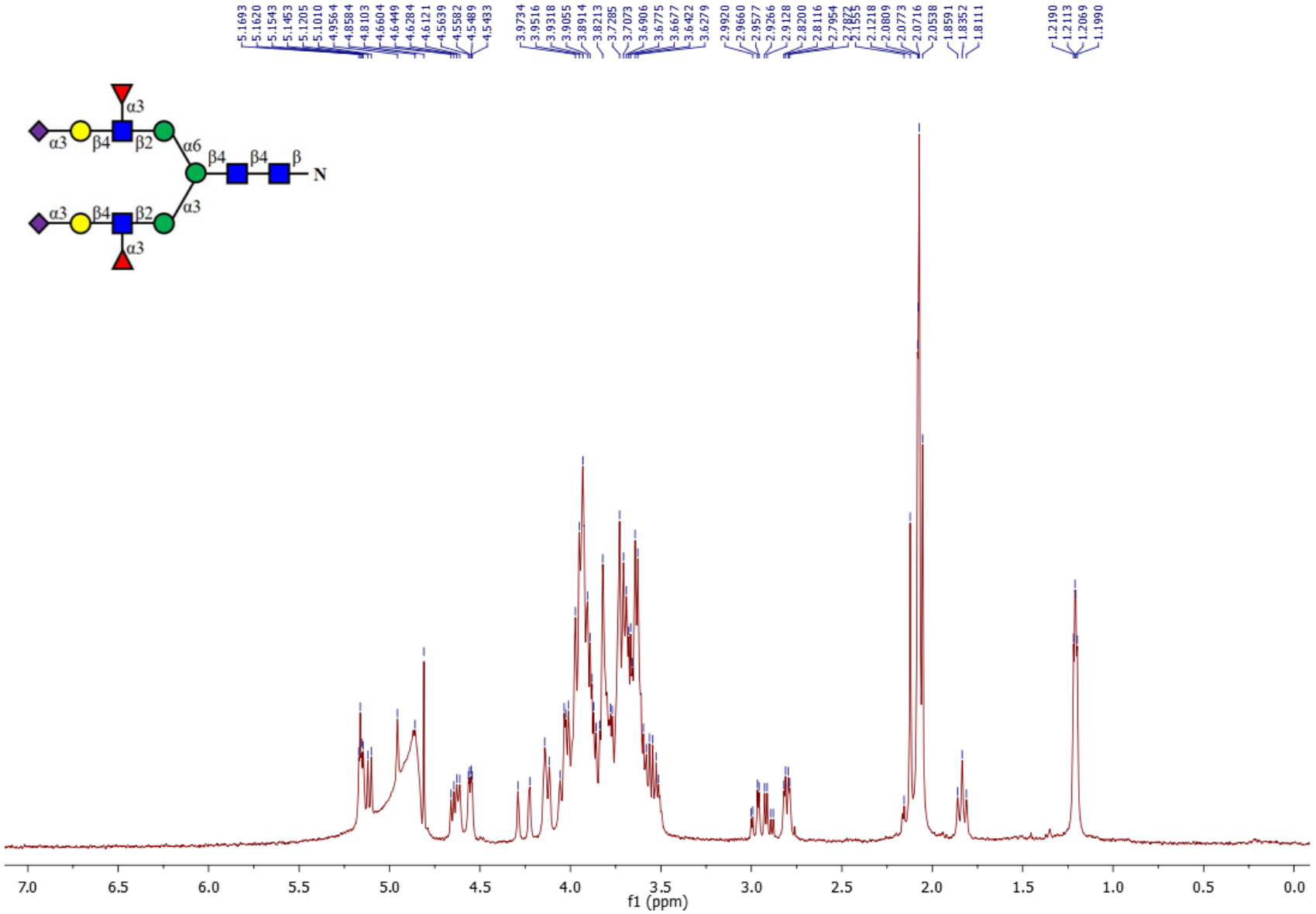
^{1}H NMR of BA-15



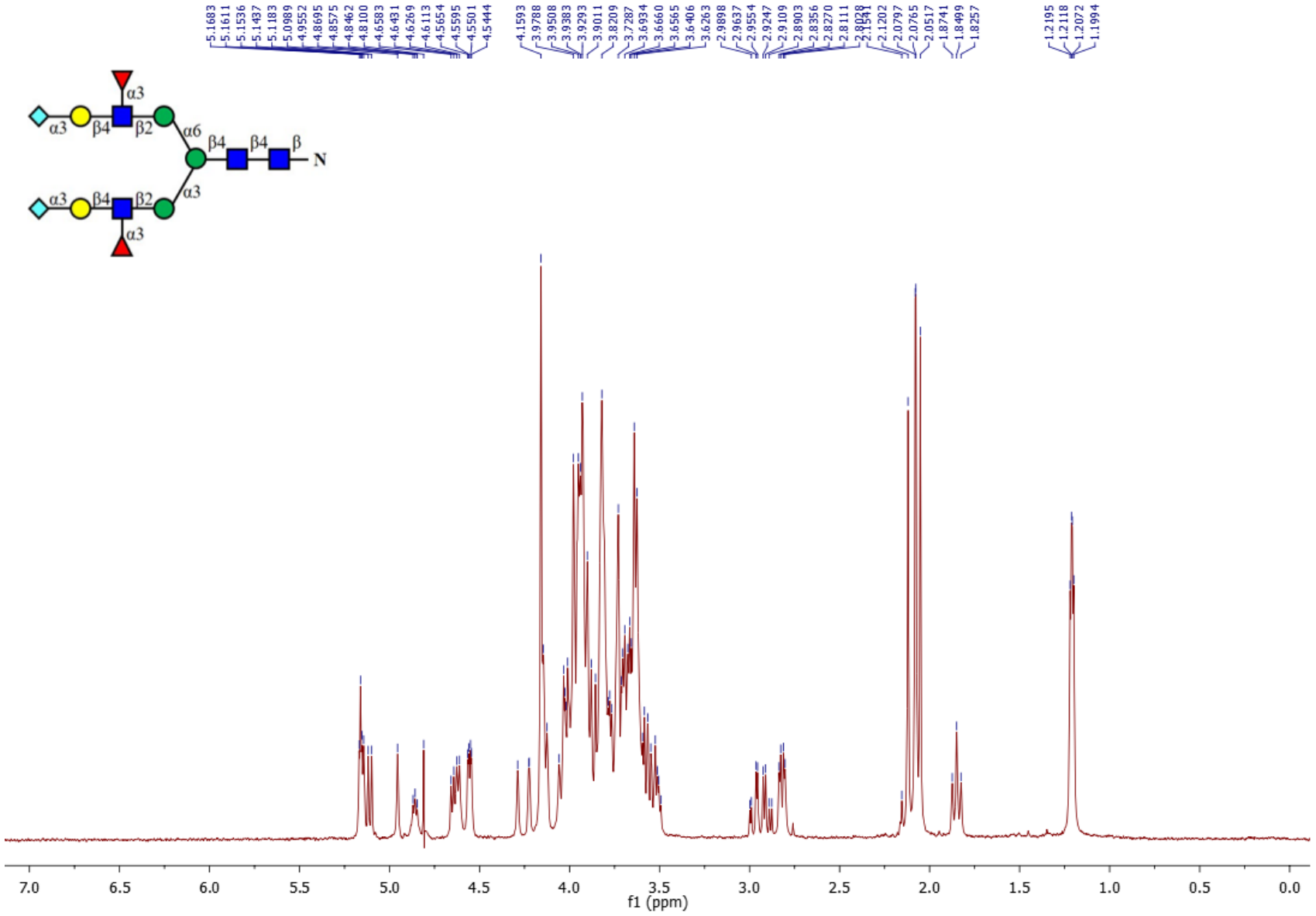
^{1}H NMR of BA-16



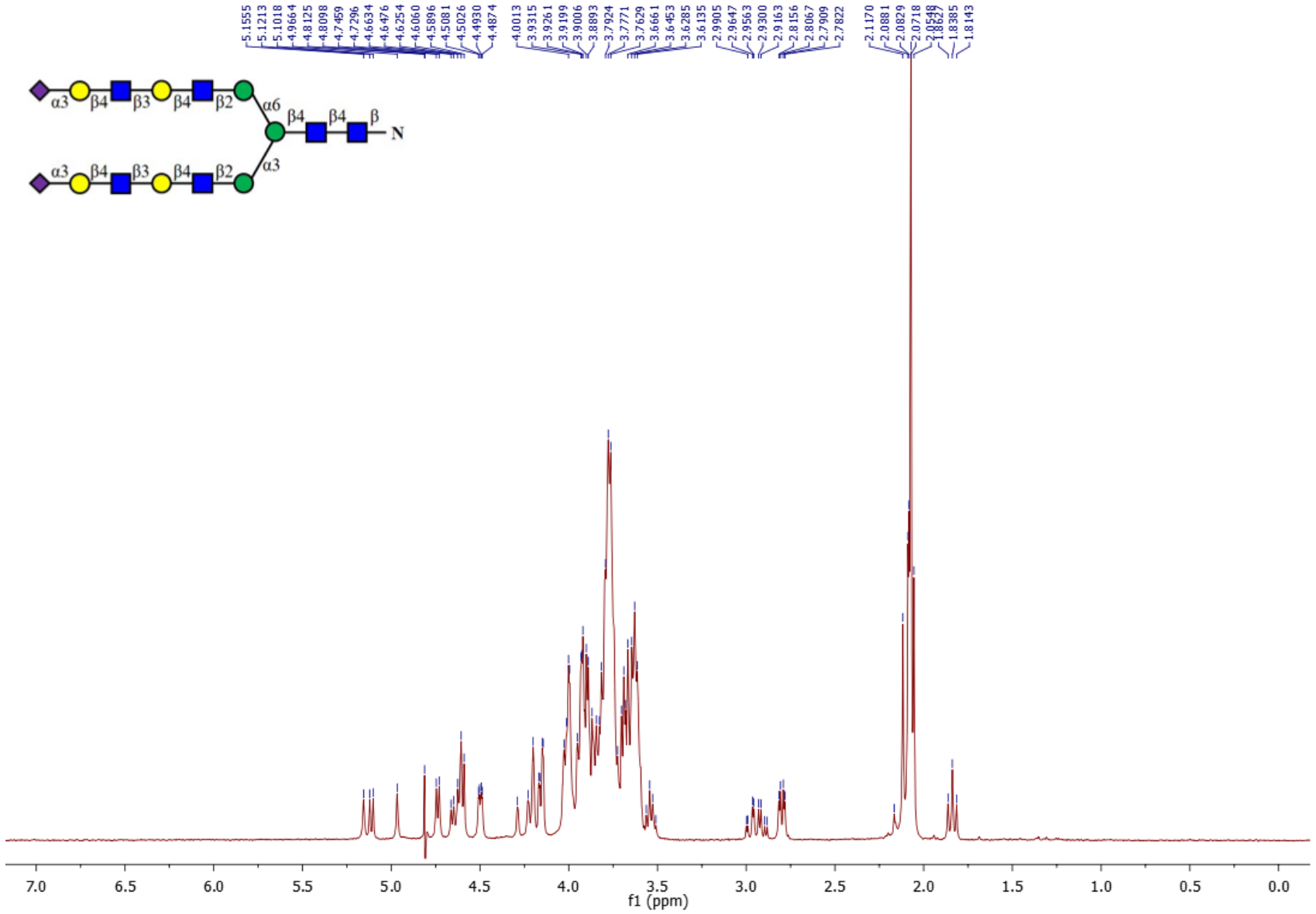
^{1}H NMR of BA-17



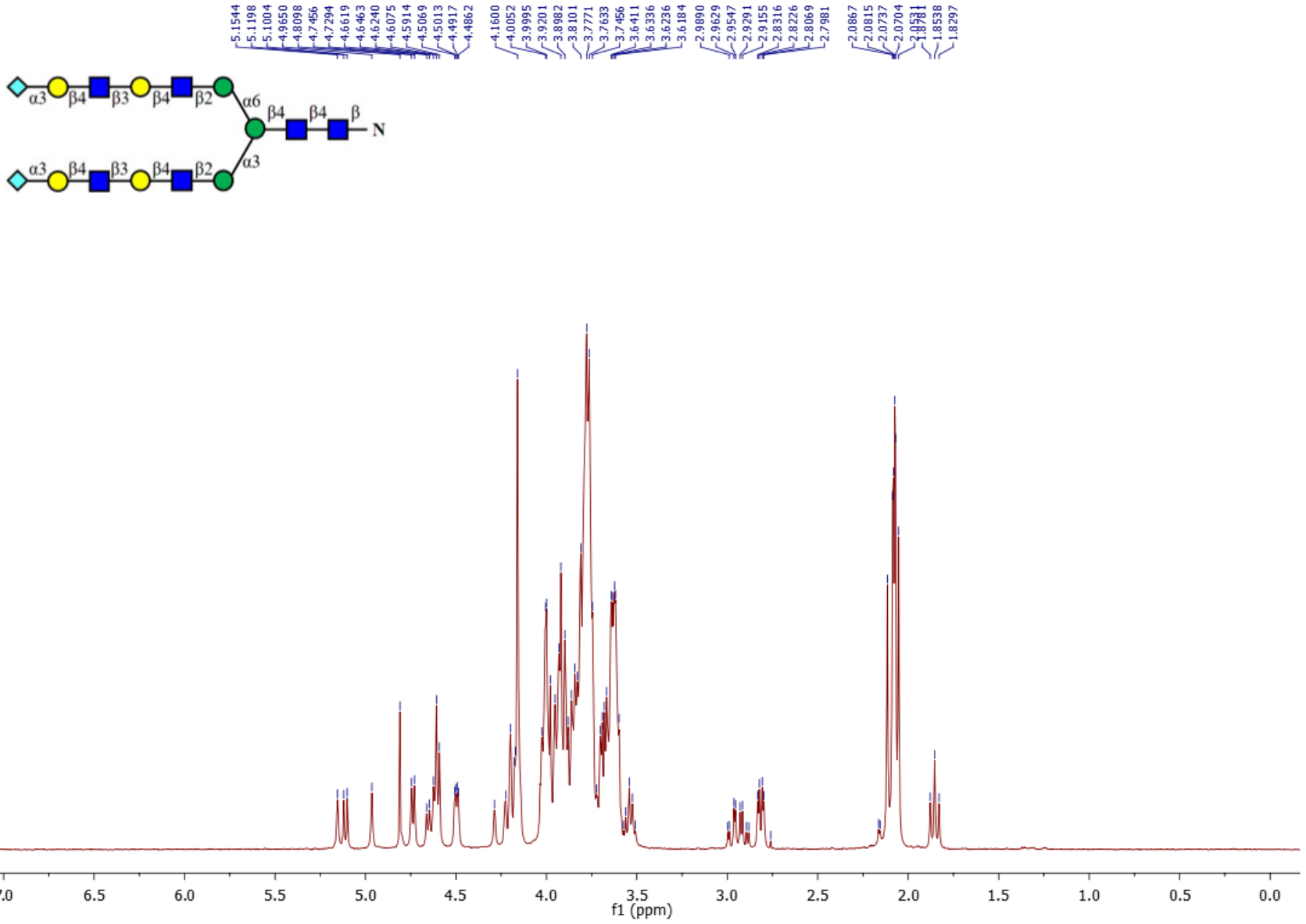
^{1}H NMR of BA-18



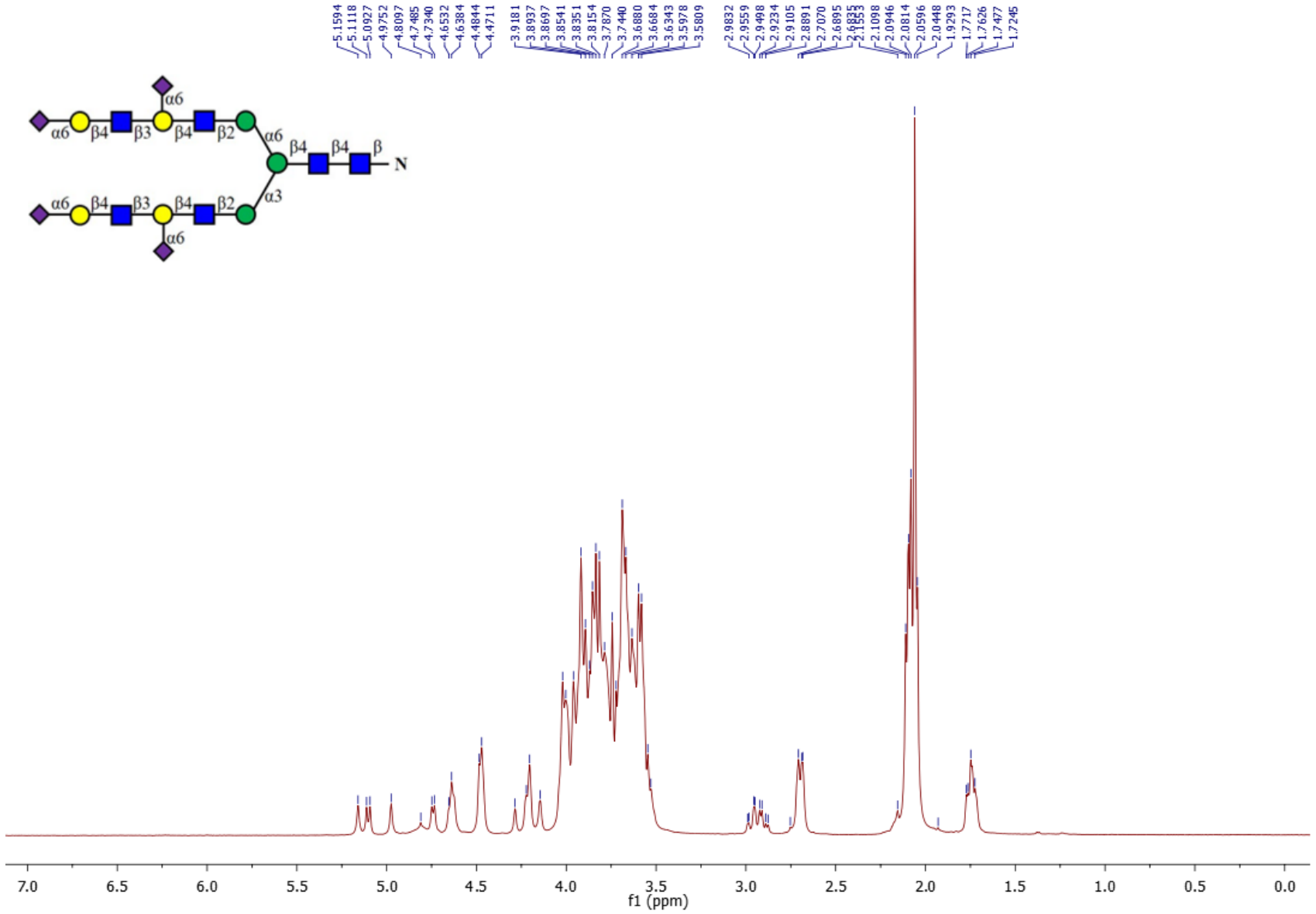
^{1}H NMR of BA-19



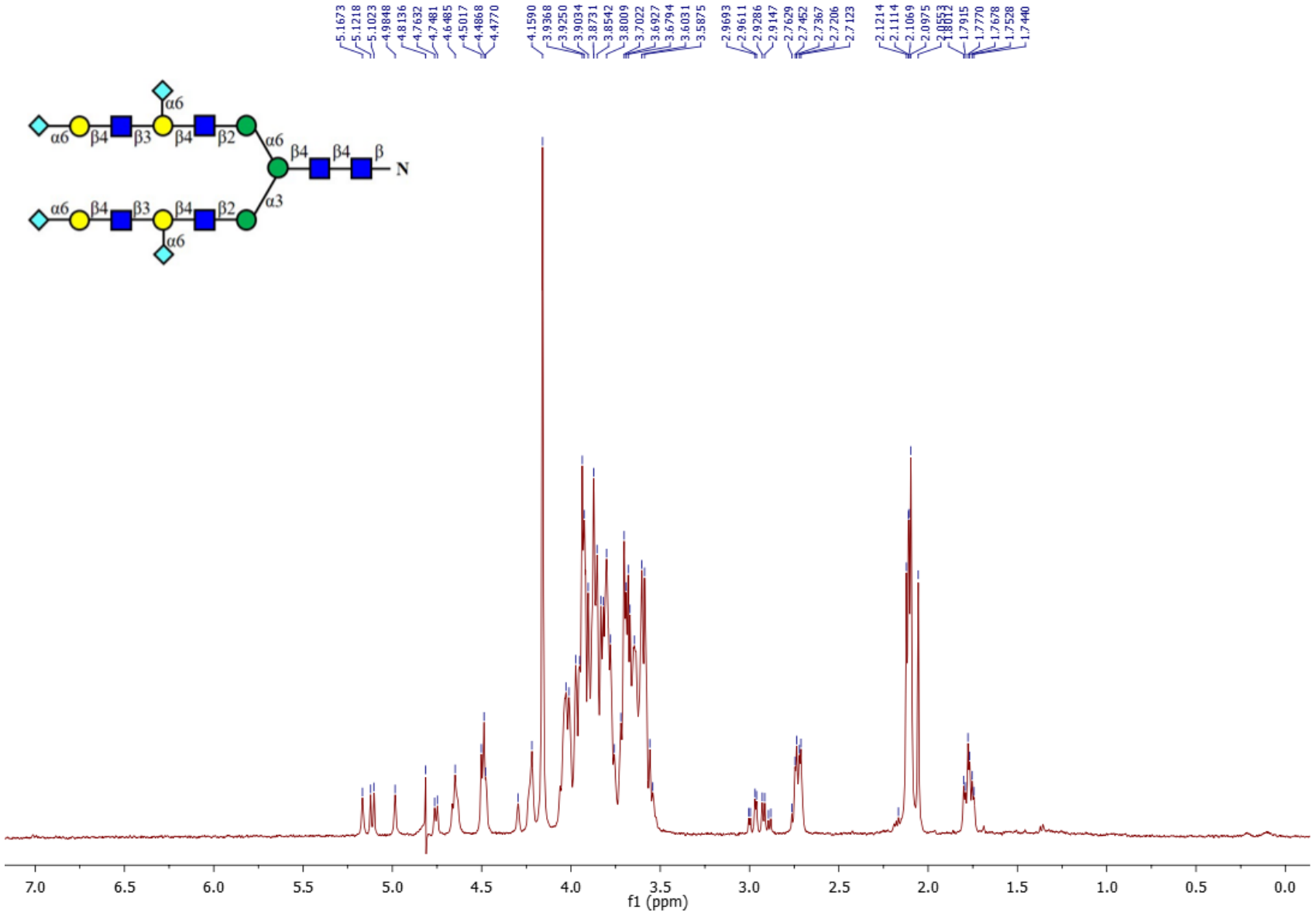
^{1}H NMR of BA-20



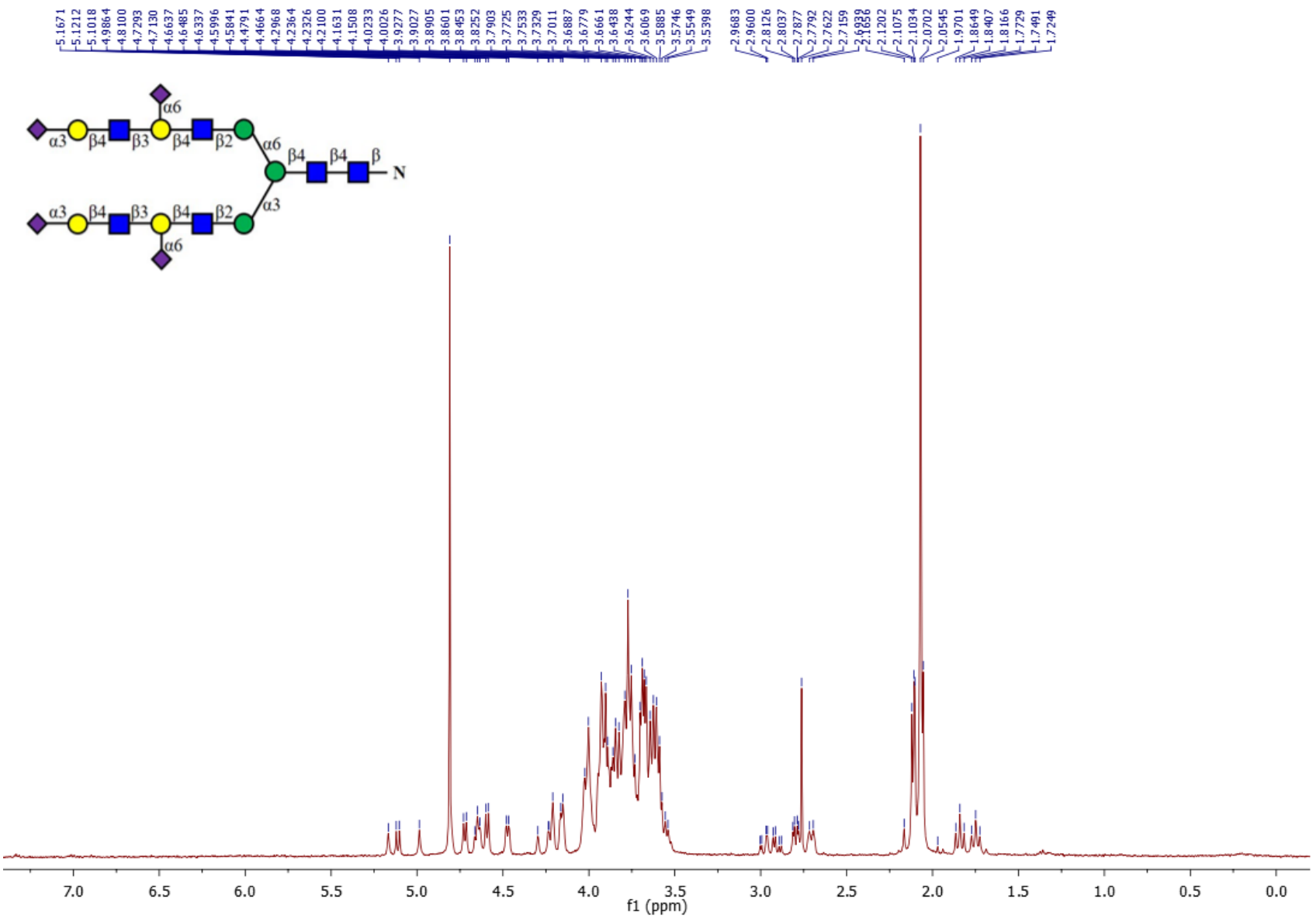
^{1}H NMR of BA-21



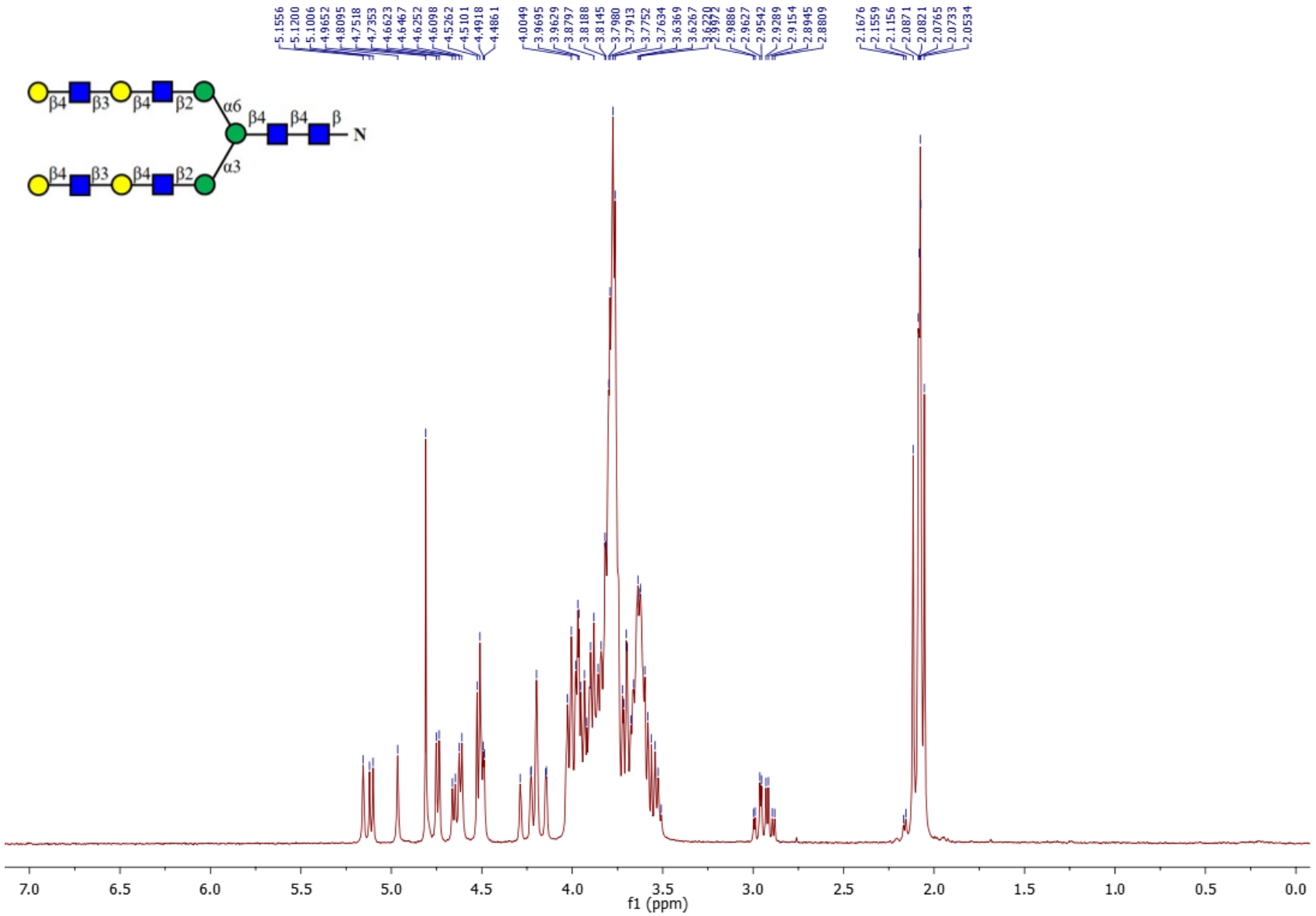
^{1}H NMR of BA-22



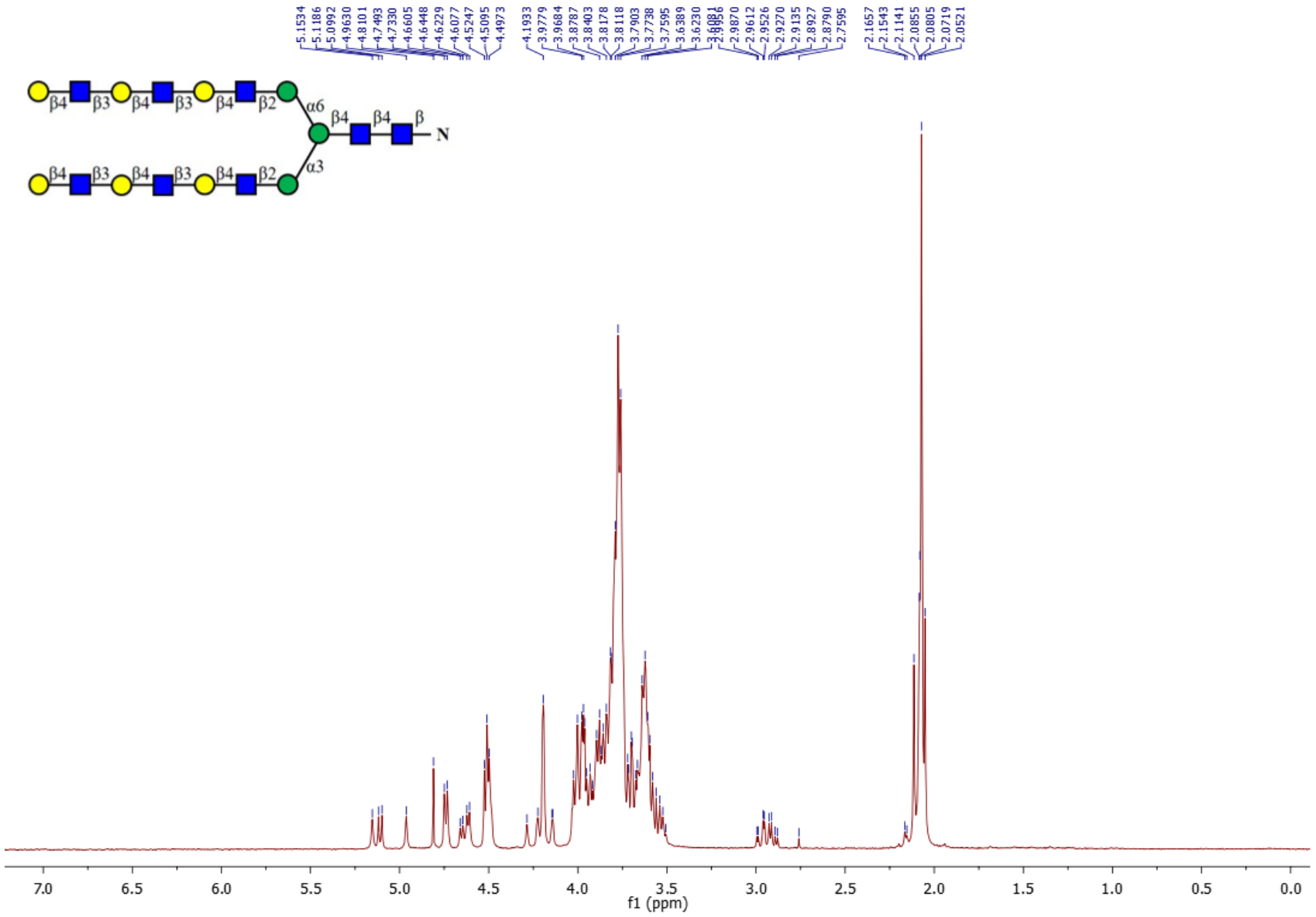
^{1}H NMR of BA-23



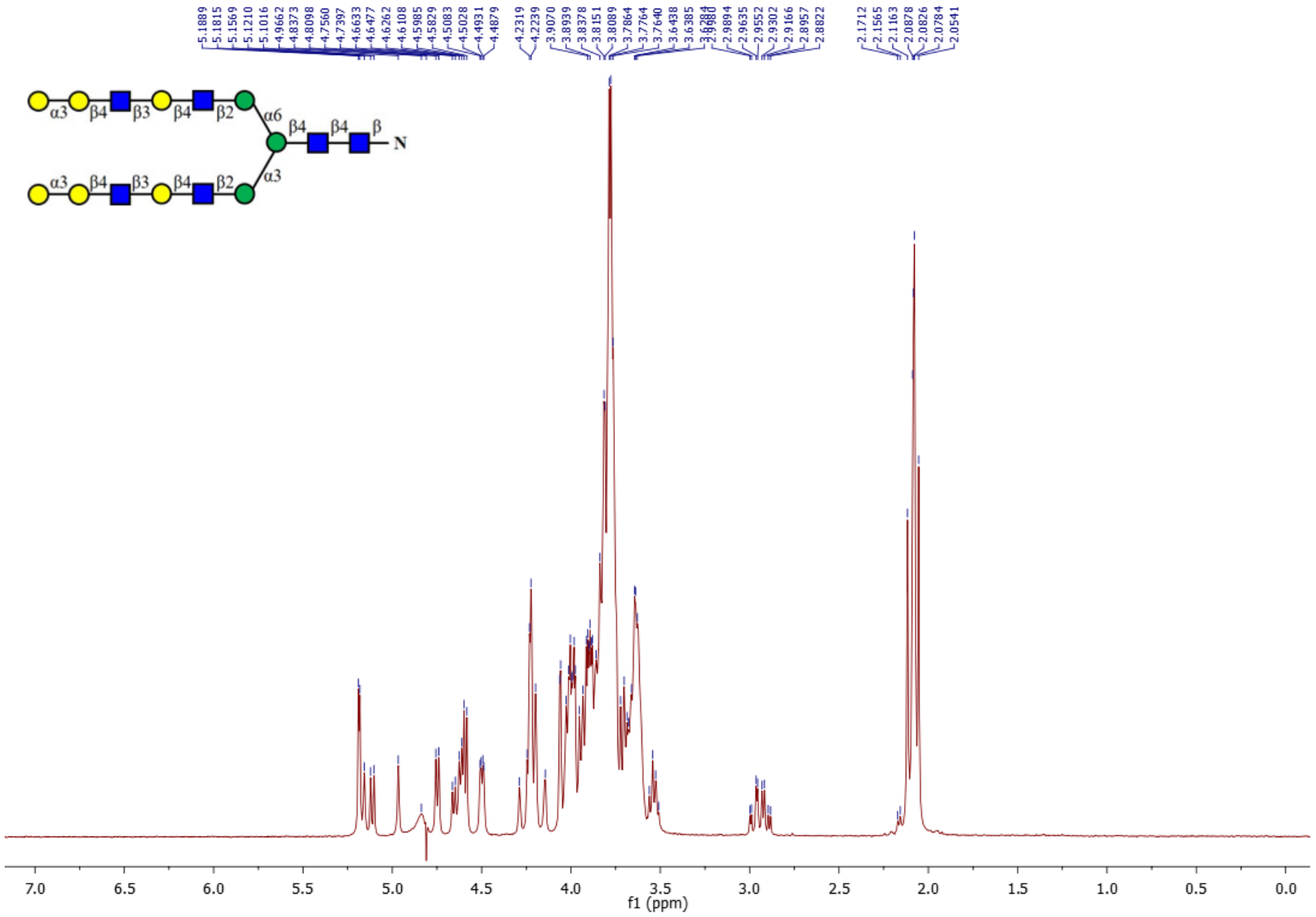
^{1}H NMR of BA-24



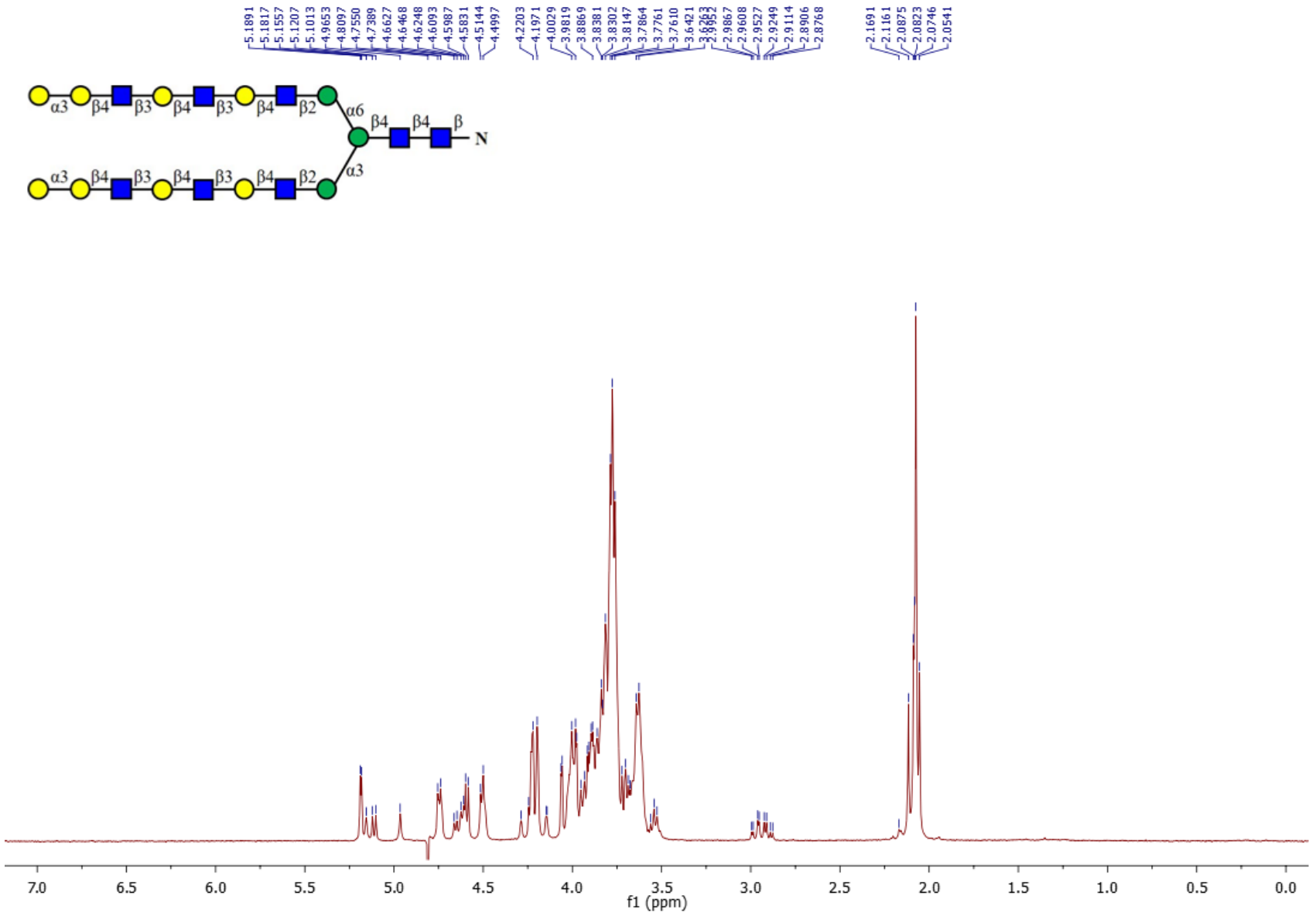
^{1}H NMR of BA-25



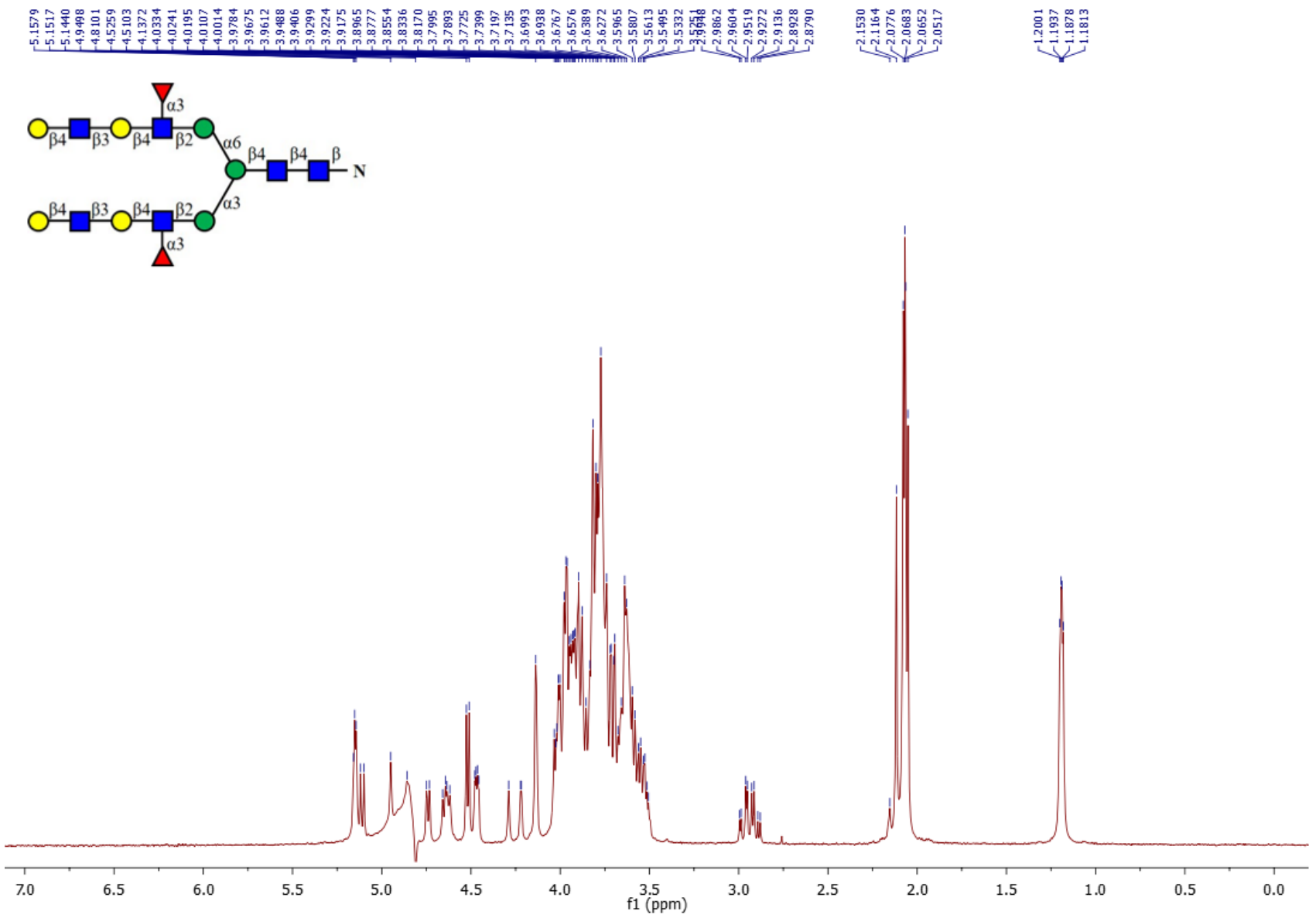
^{1}H NMR of BA-26



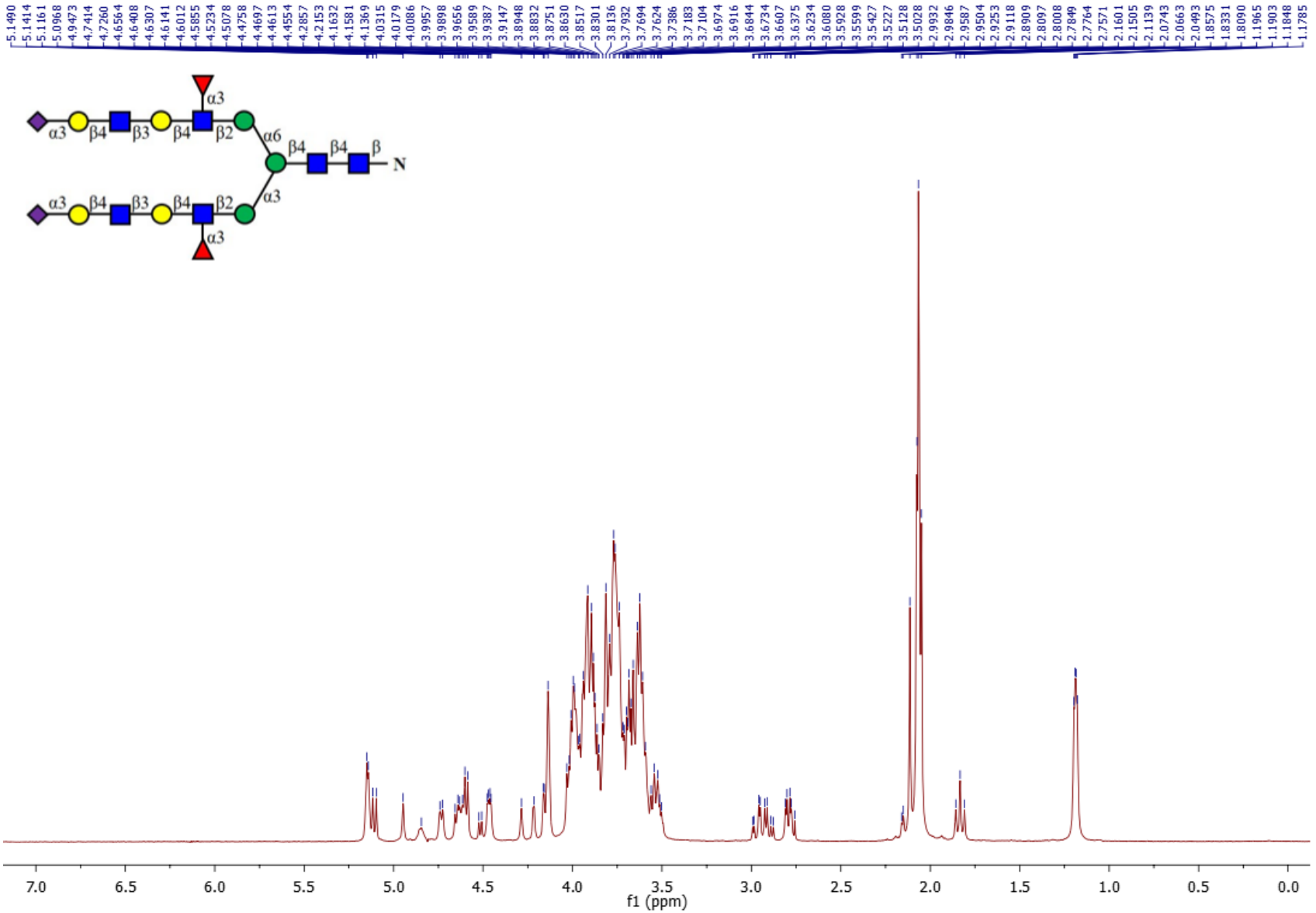
^{1}H NMR of BA-27



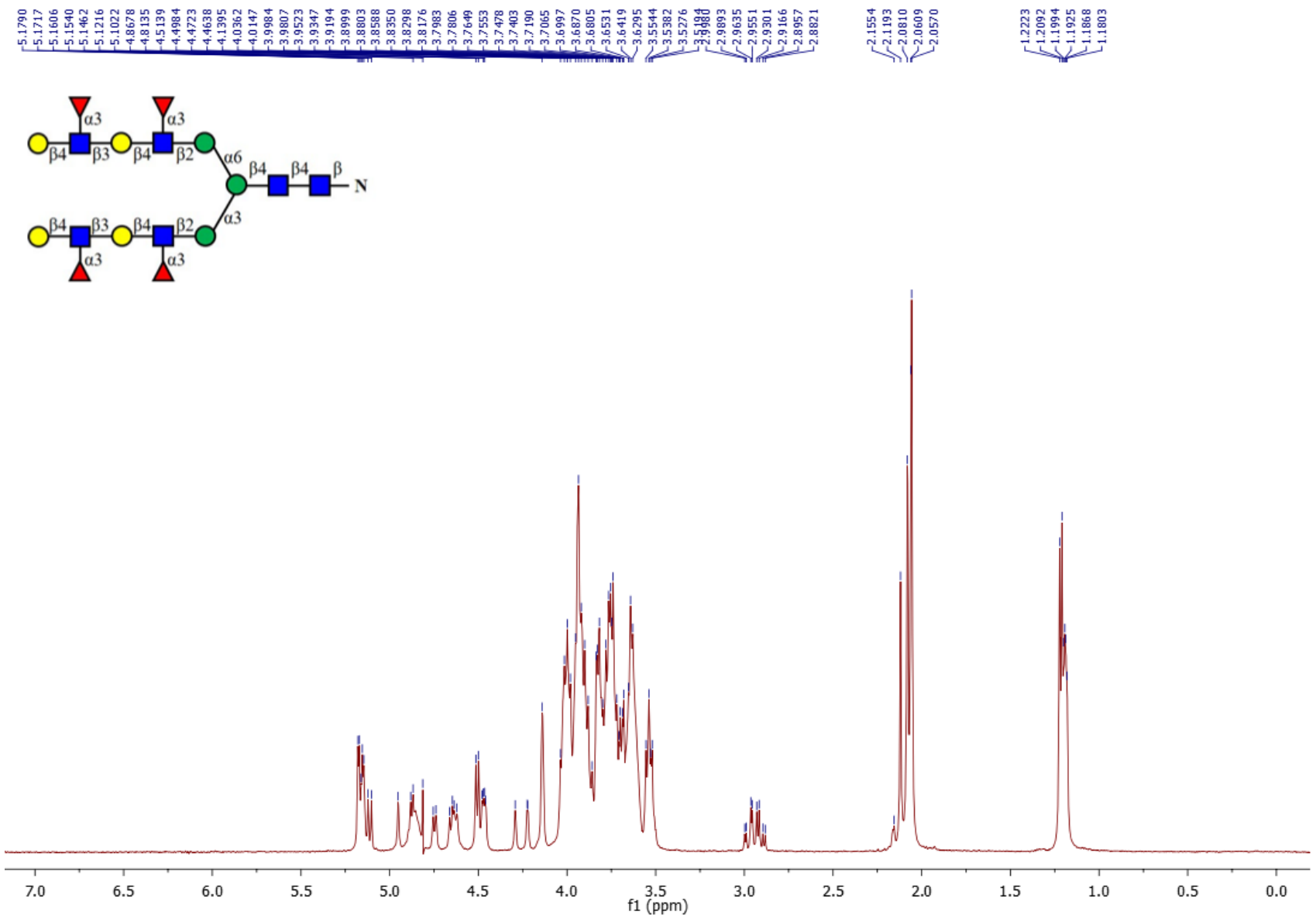
^{1}H NMR of BA-28



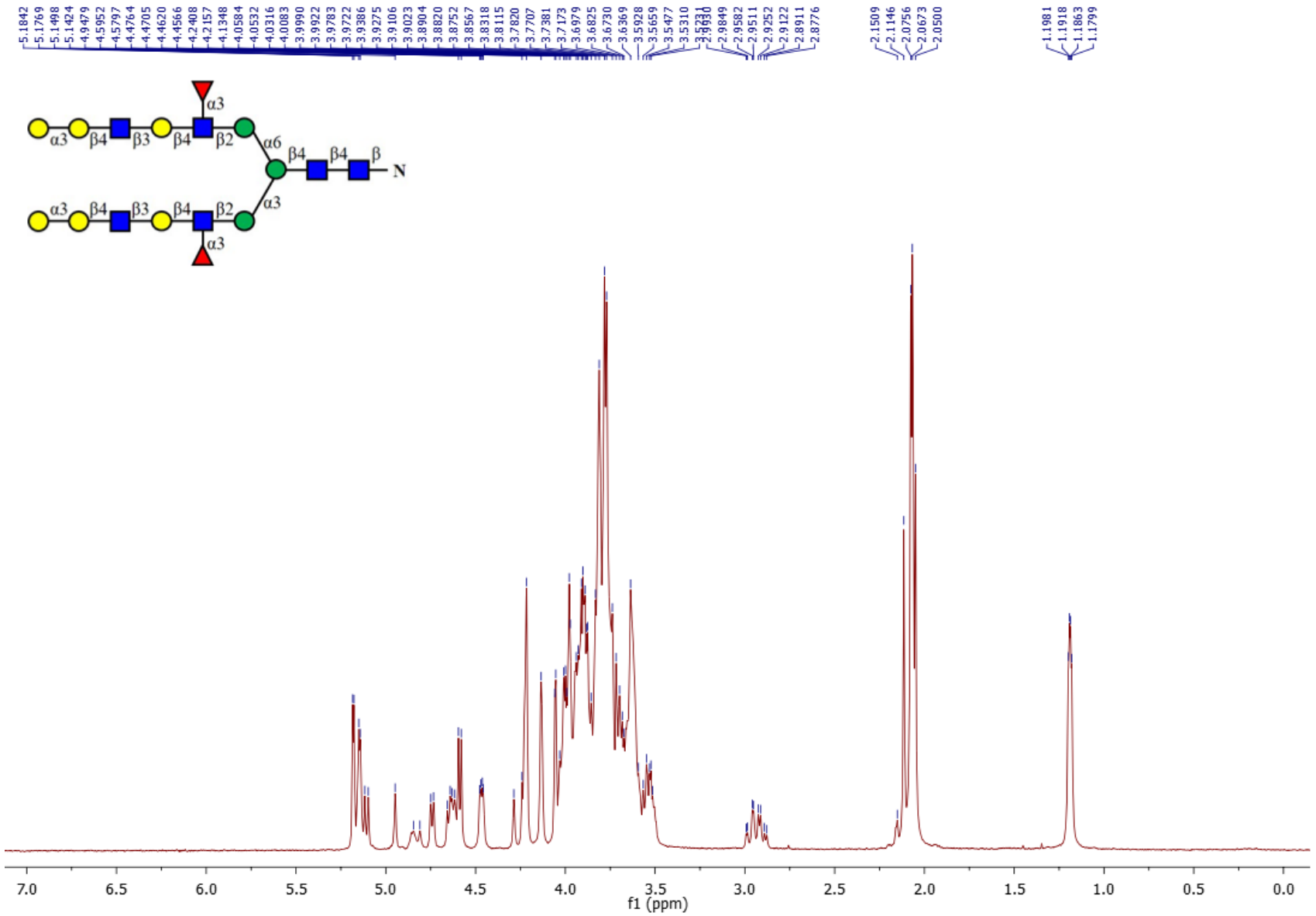
^{1}H NMR of BA-29



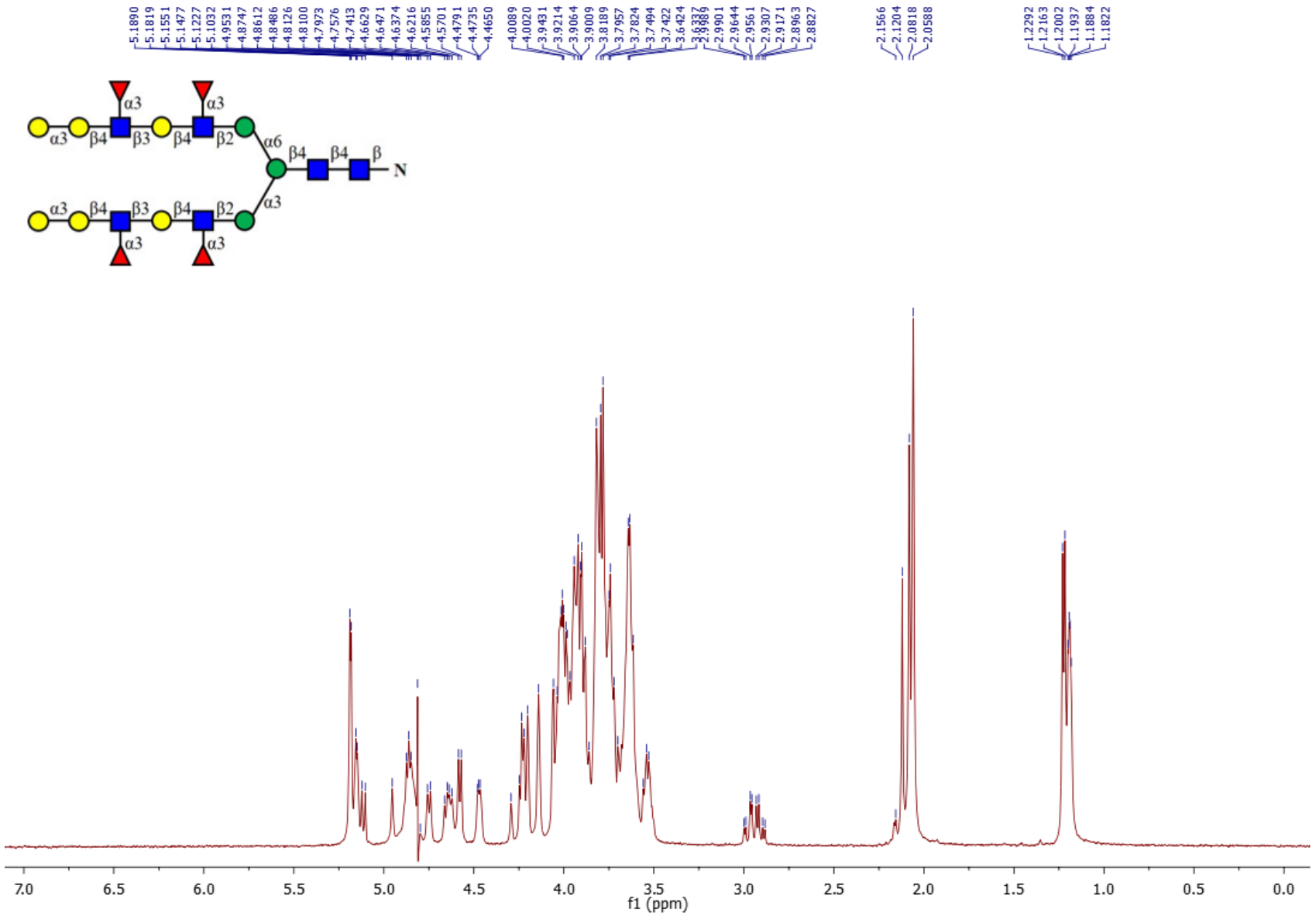
^{1}H NMR of BA-30



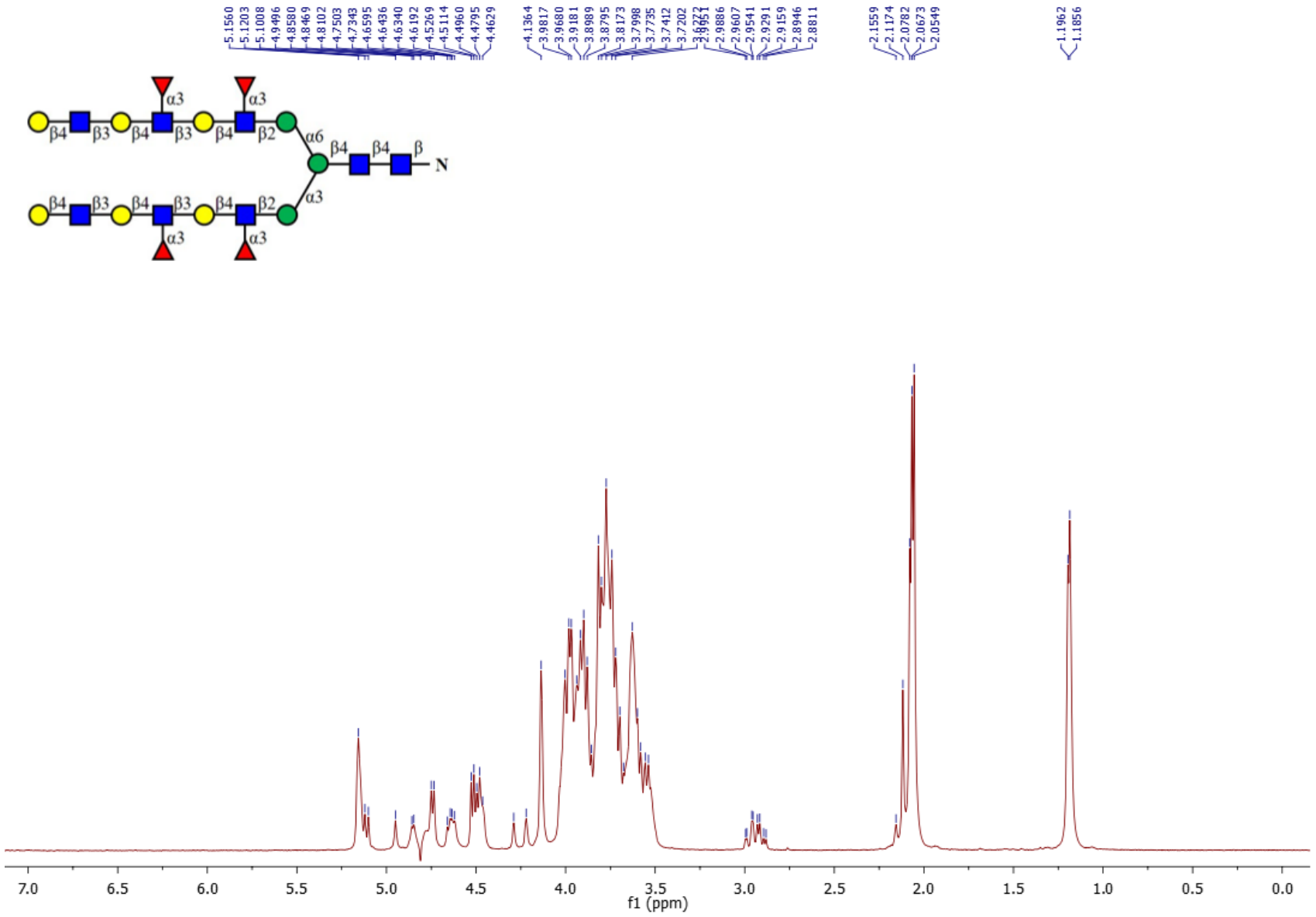
^{1}H NMR of BA-31



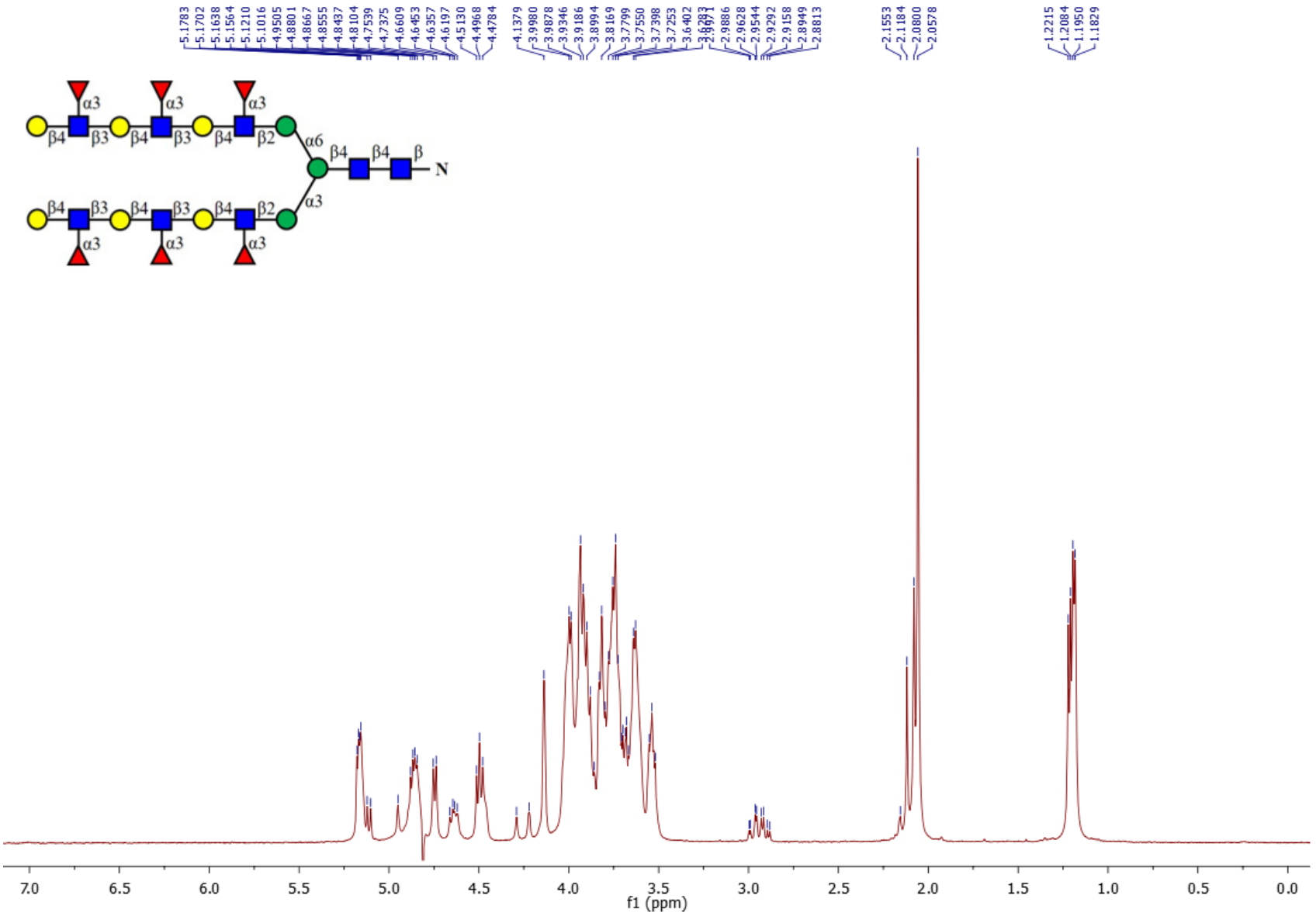
^{1}H NMR of BA-32



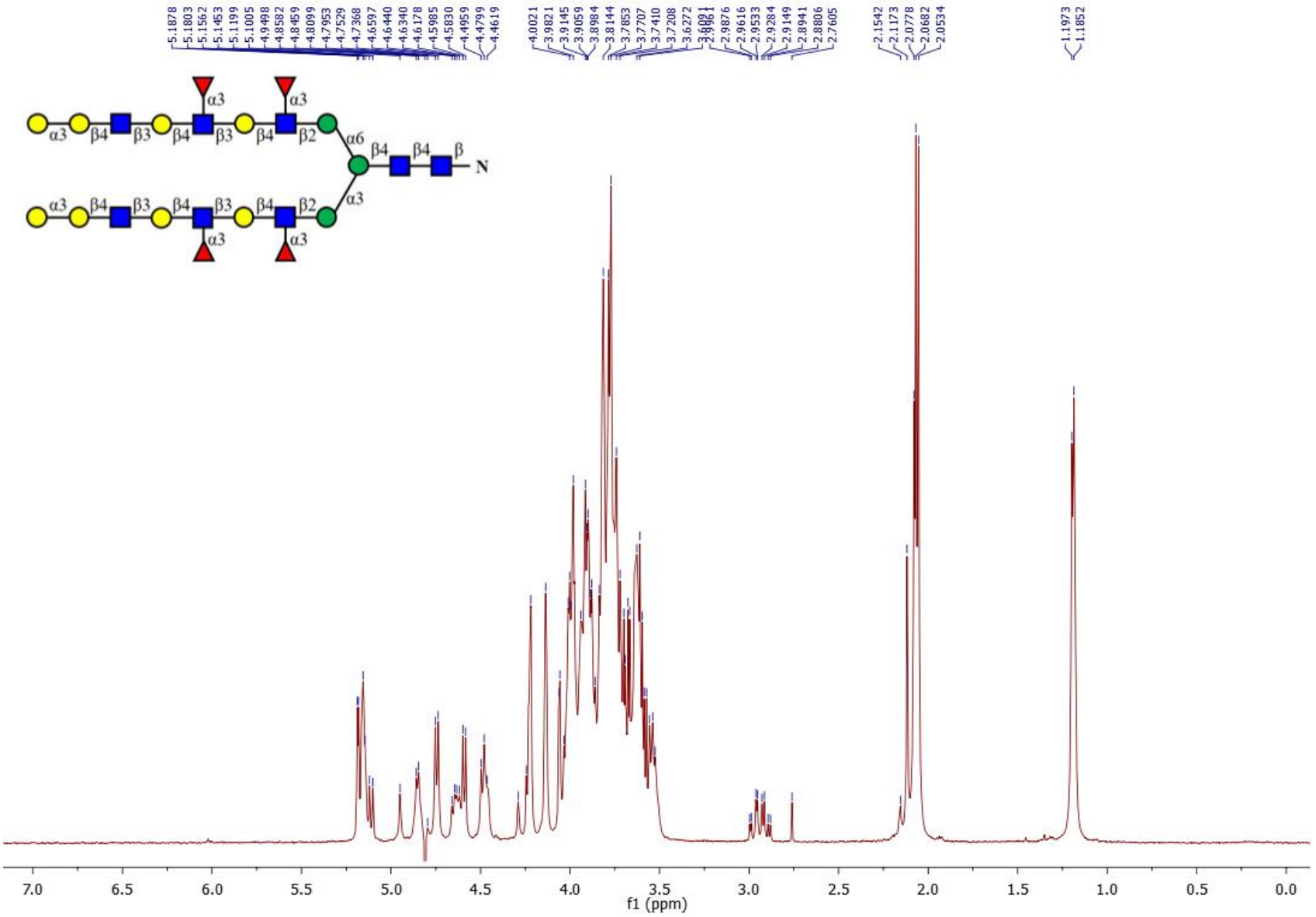
^{1}H NMR of BA-33



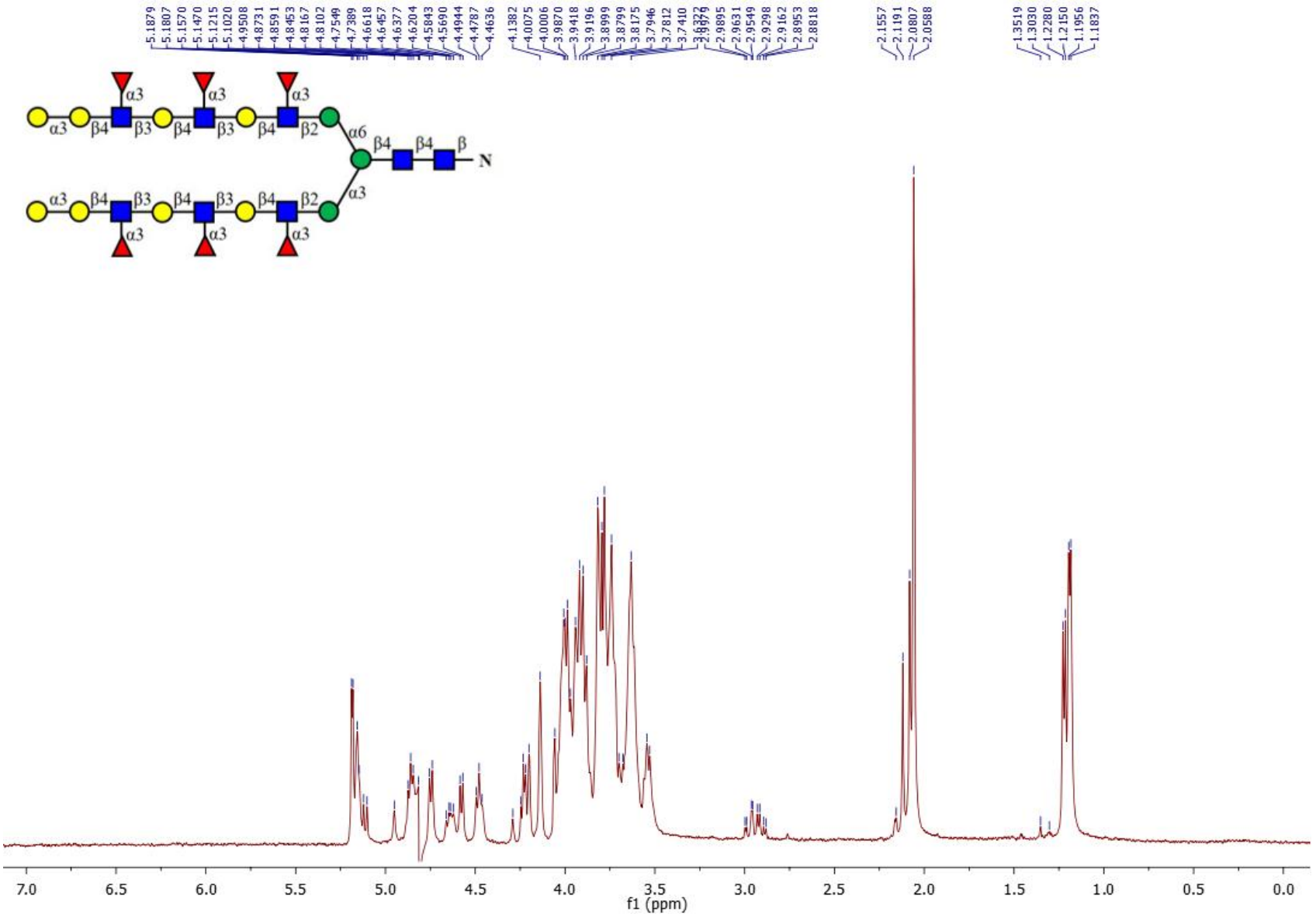
^{1}H NMR of BA-34



^{1}H NMR of BA-35



^{1}H NMR of BA-36



VII. References

1. D. A. Sela, Y. Li, L. Lerno, S. Wu, A. M. Marcobal, J. B. German, X. Chen, C. B. Lebrilla and D. A. Mills, *J. Biol. Chem.*, 2011, **286**, 11909.
2. H. Yu, H. Yu, R. Karpel and X. Chen, *Bioorg. Med. Chem.*, 2004, **12**, 6427.
3. G. Sugiarto, K. Lau, Y. Li, Z. Khedri, H. Yu, D. T. Le and X. Chen, *Mol. Biosyst.*, 2011, **7**, 3021.
4. H. Yu, S. Huang, H. Chokhawala, M. Sun, H. Zheng and X. Chen, *Angew. Chem. Int. Ed. Engl.*, 2006, **45**, 3938.
5. J. Cheng, H. Yu, K. Lau, S. Huang, H. A Chokhawala, Y. Li, V. K. Tiwari and X. Chen, Glycobiology, 2008, **18**, 686.
6. Y. Li, M. Xue, X. Sheng, H. Yu, J. Zeng, V. Thon, Y. Chen, M. M. Muthana, P. G. Wang and X. Chen, *Bioorg. Med. Chem.*, 2016, **24**, 1696.
7. K. Lau, V. Thon, H. Yu, L. Ding, Y. Chen, M. M. Muthana, D. Wong, R. Huang and X. Chen, *Chem. Commun.*, 2010, **46**, 6066.
8. J. Fang, J. Li, X. Chen, Y. Zhang, J. Wang, Z. Guo, W. Zhang, L. Yu, K. Brew and P. G. Wang, *J. Am. Chem. Soc.* 1998, **120**, 6635.
9. S. W. Lin, T. M. Yuan, J. R. Li and C. H. Lin, *Biochemistry*, 2006, **45**, 8108.
10. G. Sugiarto, K. Lau, H. Yu, S. Vuong, V. Thon, Y. Li, S. Huang and X. Chen, *Glycobiology*, 2011, **21**, 387.
11. M. M. Muthana, J. Qu, Y. Li, L. Zhang, H. Yu, L. Ding, H. Malekan and X. Chen, *Chem. Commun.*, 2012, **48**, 2728.
12. G. Zhao, W. Guan, L. Cai and P. G. Wang, *Nat. Protoc.*, 2010, **5**, 636.
13. Y. Zou, Z. Wu, L. Chen, X. Liu, G. Gu, M. Xue, P. G. Wang and M. Chen, *J. Carbohydr. Chem.*, 2012, **31**, 436.
14. J. Heimburg-Molinaro, X. Song, D. F. Smith and R. D. Cummings, *Curr. Protoc. Protein Sci.* 2011, ch 12: Unit 12.10.
15. L. Byrd-Leotis, R. Liu, K. C. Bradley, Y. Lasanajak, S. F. Cummings, X. Song, J. Heimburg-Molinaro, S. E. Galloway, M. R. Culhane, D. F. Smith, D. A. Steinhauer and R. D. Cummings, *Proc. Natl. Acad. Sci. U. S. A.*, 2014, **111**, E2241.
16. W. C. Wang and R. D. Cummings,. *J. Biol. Chem.*, 1988, **263**, 4576.
17. N. Shibuya, I. J. Goldstein, W. F. Broekaert, M. Nsimba-Lubaki, B. Peeters and W. J. Peumans, *J. Biol. Chem.*, 1987, **262**, 1596.
18. Y. Itakura, S. Nakamura-Tsuruta, J. Kominami, N. Sharon, K. Kasai and J. Hirabayashi, *J. Biochem.*, 2007, **142**, 459.
19. J. Lescar, R. Loris, E. Mitchell, C. Gautier, V. Chazalet, V. Cox, L. Wyns, S. Perez, C. Breton and A. Imberty, *J. Biol. Chem.*, 2002, **277**, 6608.
20. J. T. Gallagher, A. Morris and T. M. Dexter, *Biochem. J.*, 1985, **231**, 115.
21. P. Parasuraman, V. Murugan, J. F. Selvin, M. M. Gromiha, K. Fukui and K. Veluraja, *J. Mol. Recognit.*, 2014, **27**, 482.
22. J. Heimburg-Molinaro, M. Tappert, X. Song, Y. Lasanajak, G. Air, D. F. Smith and R. D. Cummings, *Methods Mol. Biol.*, 2012, **808**, 251.