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A multifunctional anomeric linker for the chemoenzymatic synthesis of complex oligosaccharides

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Materials and methods

General methods and reagents

^{1}H spectra were recorded on a 300 MHz Varian Mercury, 500 MHz Varian Inova, 600 MHz Agilent DD2, or 800 MHz Agilent DD2 spectrometer. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as the internal standard. Glycans **6**, **7**, and **8** were referenced by H1 and C1 of Glc for trisaccharide **5** at δ 3.98 and δ 92.92, respectively. Compounds **11**, **12**, **13**, and **14** were referenced against H1 and C1 of Glc for the biotinylated tetrasaccharide **14** at δ 4.02 and δ 92.85, respectively. NMR data is represented as follows: Chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublets, m = multiplet and / or multiple resonances, br. = broad signal), *J* coupling, integration, and peak identity. NMR signals were assigned on the basis of ^{1}H NMR, gCOSY, gHSQC, zTOCSY, and NOESY experiments. Enzymatic reactions were monitored by mass spectrometry recorded on an Applied Biosystems SCIEX MALDI TOF / TOF 5800 using 2,5-dihydroxybenzoic acid (DHB) as a matrix. Normal phase TLC was performed on glass plates coated with HPTLC Silica get 60 F_{254} and developed with (EtOAc:EtOH:H_2O, 5:2:1 *v:v:v)* or EtOAc:EtOH:H_2O, 5:2:1.5 *v:v:v*). Size exclusion chromatography was performed using Bio-Gel® P-2 Gel 45-90 mm (wet) (Bio-Rad Laboratories, Inc.) using 5% *n*-butanol in water as the eluent or Sephadex®G-25 superfine size exclusion chromatography eluting with 0.1 M NH_4HCO_3. Normal phase column chromatography was performed on silica gel G60 (Silicycle, 60-2000 mm, 60Å). Thin layer chromatography (TLC) analysis was conducted on Silica gel 60 F_{254} (EMD Chemicals, Inc.) with detection by UV-absorption (254 nm) where applicable. Visualization of TLC plates was accomplished by spraying with 10% sulfuric acid in ethanol or Hanessian's Stain, followed by charring at ~150 ^{o}C. Reverse phase (RP) TLC was performed on glass plates coated with HPTLC Silica gel 69 RP-18 WF_{254S} (EMD Chemicals, Inc.) and developed with 20-40% acetonitrile in H_2O. RP column chromatography was accomplished using Bondapak® C18 125Å 37-55 mm bulk packing material (Waters Corp). CH_2Cl_2 was freshly distilled from calcium hydride under nitrogen prior to use. Reagents were purchased from Sigma-Aldrich and used without further purification. HPLC purification of compounds **17** and **19** was performed on an Agilent Technologies 1200 Series HPLC equipped with a Zorbax Eclipse XD8-C8 4.6 x 150 mm, 5 mm column. HPLC grade acetonitrile and water were purchased from Fischer. Samples were eluted in a 50 mM solution of ammonium bicarbonate with a linear gradient of 0-100% acetonitrile over 1 h. Spectra were monitored at a wavelength of 262 nm using an Agilent 1200 series diode array with a multiple wavelength detector. Glycan symbols: *N*-acetyl-D-glucosamine (GlcNAc, ■); *N*-acetyl neuraminic acid (Neu5Ac, ◆); L-fucose (Fuc, ▲); D-galactose (Gal, ●); D-glucose (Glu, ●); and D-mannose (Man, ●).

General biochemical reagents

The recombinant enzymes, *Helicobacter pylori* β1-3-*N*-acethylglucosaminyltransferase (β3GlcNAcT) and *H. pylori* α1-3-fucosyltransferase (HPα1-3FucT) used were produced and purified as previously described.[1] ST3Gal-IV (α2-3-sialyltransferase) and ST6Gal-I (α2-6-sialyl transferase) were provided by Dr. K.W. Moremen (Complex Carbohydrate Research Center, Athens, GA). GalT-I (β1-4-Galactosyltransferase from bovine milk) was purchased from Sigma-Aldrich. Alkaline phosphate from calf intestine (CIAP) was purchased from Calbiochem EMD Millipore. Uridine 5'-diphospho-N-acetylglucosamine (UDP-GlcNAc), uridine 5'-diphosphogalactose (UDP-Gal), cytidine 5'-monophospho-*N*-acetylneuraminic (CMP-Neu5Ac) acid, and guanosine 5'-diphospho-L-fucose (GDP-Fuc) were purchased from Carbosynth Limited.

General method for Fmoc deprotection

A saccharide (0.32 μmol) was dissolved in a solution of 20% piperidine in water (500 μL) and agitated at room temperature (RT) for 2 h at which time, MS indicated removal of the Fmoc group. The compound was lyophilized and used without further purification for biotinylation.

Galectin-3 binding

Biotin-conjugated oligosaccharides **11-15** (10 μM) were allowed to bind for 2 h in NeutrAvidin-coated plates (40 μL/well). After washing, recombinant human galectin-3 (5 μg/mL) was added to the biotin-compound-coated plate (40 μL/well) and incubated for 1 h. Next, rabbit anti-human galectin-3 antibody (5 μg/mL) was added to the wells (40 μL/well) for 1 h. After incubation of 1 h with Alexa Fluor 488-labelled anti-rabbit IgG (20 μg/mL, 40 μL/well) and a final wash, fluorescence was read at 485 nm exitation/520 nm emission. Blank well measurements were substracted from all values. Data shown are mean ± SD (n=3).

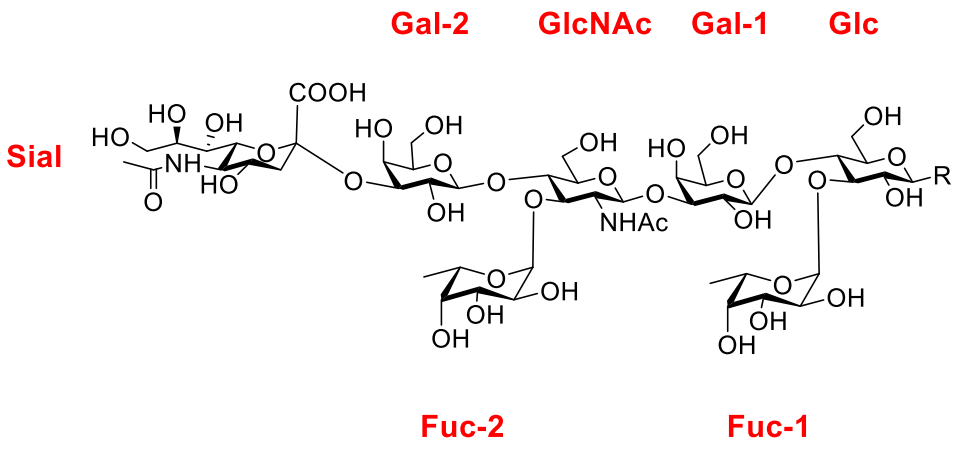


Figure S1. Glycan numbering for NMR assignment.

Synthetic procedures

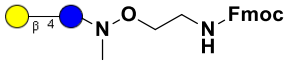
***N*-(2-Fluorenylmethyloxycarbamate)-aminoethanol (2).** Fluorenylmethyloxycarbonyl chloride (4.1 g, 15.9 mmol) was dissolved in DCM (50 mL). To this solution was added an aqueous saturated K_2CO_3 solution (25 mL). In an addition funnel, ethanolamine (1.0 g, 16.4 mmol) was dissolved in DCM (100 mL) and added dropwise to the fluorenylmethyloxycarbonyl chloride solution. The mixture was stirred for 18 h at RT. The two layers were separated and the aqueous phase was washed twice with DCM (50 mL portions). The combined organic layers were dried ($MgSO_4$), filtered and the filtrate was concentrated *in vacuo* to yield compound **2** (3.31g, 74%) as a white cotton-like material. ^{1}H (300 MHz, $CDCl_3$) δ (ppm) 7.76 (d, *J* = 7.4 Hz, 2H, 2 x CH Ar), 7.59 (d, *J* = 7.4 Hz, 2H, 2 x CH Ar), 7.40 (t, *J* = 7.3 Hz, 2H, 2 x CH Ar), 7.31 (t, *J* = 7.4 Hz, 2H, 2 x CH Ar), 4.43 (d, *J* = 6.5 Hz, 2H, CH_2 Fmoc), 4.21 (t, *J* = 6.5 Hz, 1H, CH Fmoc), 3.70 (br. d, *J* = 4.5 Hz, 2H, CH_2O), 3.34 (br. d, *J* = 4.9 Hz, 2H, CH_2N); ^{13}C from HSQC (75 MHz, $CDCl_3$) δ 127.52 (Ar), 127.53 (Ar), 124.18 (Ar), 119.32 (Ar), 74.22 (CH_2O), 67.08 (CH_2 Fmoc), 46.86 (CH Fmoc), 43.62(CH_2N); MALDI TOF-MS *m/z* calcd for $C_{17}H_{17}NO_3Na$ $(M + Na)^+$ 306.11, found 306.09.

***N*-*t*-Butoxycarbamate-*N*-methyl-*O*-(2-(2-fluorenylmethyloxycarbamate)ethyl)-hydroxylamine (3).** A cooled (0 °C) solution of compound **2** (1.0 g, 2.9 mmol), triphenylphosphine (916 mg, 3.5 mmol), and *N*-*t*-butyloxycarbamate-*N*-methylhydroxylamine (426 mg, 2.9 mmol) in DCM (50 mL) was placed under an atmosphere of Ar. Diisopropyl azodicarboxylate (0.75 mL, 3.38 mmol) was added dropwise to produce a clear solution. The reaction mixture was allowed to warm to RT and after stirring for 9 h, it was quenched by the addition of MeOH. The solution was concentrated under reduced pressure and the resulting oil was purified by flash silica column chromatography using 10% acetone in toluene as the eluent to yield compound **3** (725 mg, 65%) as a thick oil. ^{1}H (300 MHz, $CDCl_3$) δ 7.75 (d, *J* = 7.4, 2H, 2 x CH Ar), 7.62 (d, *J* = 7.4, 2H, 2 x CH Ar), 7.39 (t, *J* = 7.3 Hz, 2H, 2 x CH Ar), 7.31 (t, *J* = 7.4 Hz, 2H, 2 x CH Ar), 4.39 (d, *J* = 6.9 Hz, 2H, CH_2 Fmoc), 4.23 (t, *J* = 7.22, 1H, CH Fmoc), 3.94 - 3.84 (m, 2H, CH_2O), 3.46 - 3.39 (m, 2H, CH_2N), 3.08 (s, 3H, CH_3N), 1.50 (s, 9H, 3 x CH_3 Boc); ^{13}C from HSQC (75 MHz, $CDCl_3$) δ 128.00 (Ar), 127.53 (Ar), 125.74 (Ar), 119.89 (Ar), 73.40 (CH_2O), 67.08 (CH_2 Fmoc), 47.50 (CH Fmoc), 39.77 (CH_2N), 37.28 (CH_3N), 28.70 (CH_3 Boc). MALDI TOF-MS *m/z* calcd for $C_{23}H_{28}N_2O_5Na$ $(M + Na)^+$ 435.19, found 435.12.

***N*-Methyl-*O*-(2-(2-fluorenylmethyloxycarbamate)ethyl)-hydroxylammonium chloride (1).** Compound **3** (700 mg, 1.7 mmol) was dissolved in 4 M HCl in dioxane (8 mL). After 30 min, diethyl ether (10 mL) was

added resulting in the formation of a white precipitate, which was filtered off and washed with ice-cold diethyl ether (20 mL) to yield, after drying *in vacuo,* compound **1** (406 mg, 70%) as the ammonium chloride salt. ^{1}H (500 MHz, MeOD) δ 7.36 (d, *J* = 5.9 Hz, 2H, 2 x CH Ar), 7.29 (br. d, 2H, 2 x CH Ar), 7.11 - 7.02 (m, 4H, 4 x CH Ar), 4.08 (br. s, 2H,cc CH_2 Fmoc), 3.89 (br. t, 2H, CH_2O), 3.80 (t, *J* = 5.7 Hz, 1H, CH Fmoc), 3.14 (br. s, 2H, CH_2N), 2.74 (s, 3H, CH_3N); ^{13}C from HSQC (125 MHz, $CDCl_3$) δ 127.40 (Ar), 126.94 (Ar), 124.55 (Ar), 119.50 (Ar), 66.36 (CH_2 Fmoc), 61.49 (CH_2O), 46.26 (CH Fmoc), 43.27 (CH_2N), 23.86 (CH_3N). MALDI TOF-MS *m/z* calcd for $C_{18}H_{21}N_2O_3$ $(M + H)^+$ 313.15, found 313.14.

_N_-(_N_-methyl-_O_-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (4).



Compound **1** (100 mg, 0.29 mmol) and lactose (20 mg, 0.058 mmol) were dissolved in a sodium acetate buffer (0.1 M, pH 4.2). The reaction mixture was stirred for 48 h at 40 °C after which it was concentrated under reduced pressure. The solid residue was purified by reverse phase C18 column chromatography using a gradient of acetonitrile in water (0 - 40%) to yield compound **4** (12 mg, 33%) as a white solid.

^{1}H (500 MHz, MeOD): δ (ppm)

	H1	H2	H3	H4	H5	H6
Glc	4.05 (d, *J* = 8.9 Hz, 1H)	3.50	3.56	3.38	N/A[a]	3.75, 3.70
Gal-1	4.35	3.55	3.48	3.82	3.56	3.90, 3.87

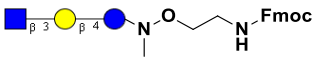
[a] N/A indicates not assigned

Peak	1H Signal
Fmoc CH Aromatic	7.82 (d, *J* = 7.5 Hz, 2H)
Fmoc CH Aromatic	7.68 (d, *J* = 5.8 Hz, 2H)
Fmoc CH Aromatic	7.41 (t, *J* = 7.5 Hz, 2H)
Fmoc CH Aromatic	7.34 (t, *J* = 7.4 Hz, 2H)
Fmoc CH_2	4.36
Fmoc CH	4.23 (t, *J* = 6.7 Hz, 1H)
CH_2-N	3.35
CH_2-O-N	3.82
N-CH_3	2.73 (s, 3H)

^{13}C from HSQC (125 MHz, MeOD): δ(ppm) 39.71, 46.55, 47.91, 60.65, 61.09, 61.12, 66.39, 68.95, 70.15, 71.04, 71.23, 73.46, 76, 76.81, 79.13, 93.94, 103.8, 119.62, 124.89, 126.93, 127.49.

MALDI TOF-MS *m/z* calcd for $C_{30}H_{40}N_2O_{13}Na$ $(M + Na)^+$ 659.24, found 659.21.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino)-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (5).**



Compound **4** (0.63 μmol) and UDP-GlcNAc (0.94 μmol) were dissolved in HEPES buffer (113 μL, 50 mM, pH 7.3) with KCl (25 mM), $MgCl_2$ (2 mM), and DTT (1 mM). CIAP (10 mU) and β3GlcNAcT (27.8 mU/μmol substrate) were added to achieve a final saccharide concentration of 5 mM. The resulting reaction mixture was incubated at 37 °C until no starting material could be detected by MALDI TOF. The reaction mixture was centrifuged and the supernatant was purified by reverse phase C18 column chromatography using a gradient of acetonitrile in water (0-40%). Fractions containing the product were combined and lyophilized to yield compound **5** (0.5 mg, 96%) as a white fluffy solid.

^{1}H (500 MHz,D_2O) δ (ppm)

	H1	H2	H3	H4	H5	H6	NH-Acetyl
Glc	3.98	3.39	3.49	3.35	N/A[a]	N/A	-[b]
Gal-1	4.12	3.44	3.51	4.00	N/A	N/A	-
GlcNAc	4.58 (d, *J* = 8.1 Hz, 1H)	3.66	3.45	3.36	N/A	N/A	1.93

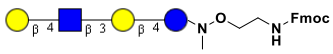
[a] N/A indicates not assigned
[b] - indicates position not available

Peak	1H Signal
Fmoc CH Aromatic	7.81 (d, *J* = 7.3 Hz, 2H)
Fmoc CH Aromatic	7.61 (d, *J* = 7.3 Hz, 2H)
Fmoc CH Aromatic	7.40 (t, *J* = 7.4 Hz, 2H)
Fmoc CH Aromatic	7.33 (t, *J* = 7.4 Hz, 2H)
Fmoc CH_2	4.55 - 4.38 (dm)
Fmoc CH	4.23 (t, *J* = 6.7 Hz, 1H)
CH_2-N	3.14 (br. s)
CH_2-O-N	3.66
N-CH_3	2.57 (s, 3H)

^{13}C from HSQC (125 MHz, D_2O): δ(ppm) 45.12, 47.1, 47.51, 48.07, 55.66, 60.41, 60.44, 60.7, 68.24, 69.69, 69.87, 71.23, 73.54, 74.72, 75.54, 76.19, 78.6, 81.88, 92.96, 102.16, 102.84, 103.09, 118.57, 119.69.

MALDI TOF-MS *m/z* calcd for $C_{38}H_{53}N_3O_{18}Na$ $(M + Na)^+$ 862.32, found 862.26.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino)-β-D-galactopyranosyl(1→4)-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (6).**



Compound **5** (0.83 μmol) and UDP-Gal (1.67 μmol) were dissolved in TRIS buffer (128 μL, 50 mM, pH 7.5) containing BSA (1.0% V_t) and CIAP (10 mU, 1.0% V_t). To this solution was added GalT-I (12.0 mU/μmol substrate) to yield a final saccharide concentration of 5 mM. The reaction mixture was incubated at 37 °C until no further starting material was detected by MALDI TOF. The reaction mixture was centrifuged and the supernatant was purified by reverse phase C18 column chromatography using a gradient of acetonitrile in water (0 - 40%). Fractions containing the product were combined and lyophilized to yield compound **6** (0.7 mg, 84%) as a white fluffy solid.

^{1}H (500 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6	NH-Acetyl
Glc	3.98	3.39	3.50	3.64	N/A[a]	3.68	-[b]
Gal-1	4.13	3.44	3.51	4.00	N/A	3.83	-
GlcNAc	4.60 (d, *J* = 7.7 Hz, 1H)	3.70	3.63	3.64	N/A	3.55	1.93
Gal-2	4.40 (d, *J* = 8.0 Hz, 1H)	3.44	3.56	3.68	N/A	3.79 3.89	-

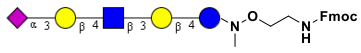
[a] N/A indicates not assigned
[b] - indicates position not available

Peak	1H Signal
Fmoc CH Aromatic	7.82 (d, *J* = 6.3 Hz, 2H)
Fmoc CH Aromatic	7.62 (d, *J* = 7.2 Hz, 2H)
Fmoc CH Aromatic	7.41 (t, *J* = 6.9 Hz, 2H)
Fmoc CH Aromatic	7.34 (t, *J* = 6.9 Hz, 2H)
Fmoc CH_2	4.49 - 4.41 (dm)
Fmoc CH	4.26 (br. s, 1H)
CH_2-N	3.13 (br. s, 2H)
CH_2-O-N	3.66
N-CH_3	2.57 (s, 3H)

^{13}C from HSQC (125 MHz, D_2O): δ(ppm) 39.94, 47.05, 55.12, 59.83, 60.95, 68.14, 69.78, 70.91, 71.21, 72.1, 74.57, 74.61, 75.3, 75.48, 76.1, 78.12, 78.64, 81.95, 102.84, 102.97, 120.13, 125.08, 127.49, 148.56, 149.31.

MALDI TOF-MS *m/z* calcd for $C_{44}H_{63}N_3O_{23}Na$ $(M + Na)^+$ 1024.38, found 1024.20.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino)-α-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid-(2,3)-β-D-galactopyranosyl-(1→4)-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (7).**



Compound **6** (0.70 μmol) and CMP-Neu5Ac (1.4 μmol) were dissolved in sodium cacodylate buffer (136 μL, 50 mM, pH 7.6) containing BSA (1.0% V_t) and CIAP (10 mU, 1.0% V_t). To this solution was added ST3Gal-IV (1.92 mU/μmol substrate) to yield a final saccharide concentration of 5 mM. The reaction mixture was incubated at 37 °C until no further starting material could be detected by TLC. Upon completion, the reaction mixture was centrifuged and the supernatant was purified by reverse phase C18 column chromatography using a gradient of acetonitrile in water (0-20%). Fractions containing the product were combined and lyophilized to yield compound **7** (0.7 mg, 78%) a white fluffy solid.

^{1}H (600 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6	H7	H8	H9	NH Acetyl
Glc	3.98	3.40	3.49	3.37	N/A[a]	N/A	-[b]	-	-	-
Gal-1	4.12 (d, *J* = 7.8 Hz, 1H)	3.44	3.51	3.99	3.59	3.87, 3.78	-	-	-	-
GlcNAc	4.58 (d, *J* = 8.3 Hz, 1H)	3.70	3.49	3.63	N/A	3.59	-	-	-	1.93
Gal-2	4.46 (d, *J* = 7.8 Hz, 1H)	3.48	3.86	4.02	3.49	3.82, 3.66	-	-	-	-
Neu5Ac	-	-	2.53 -eq (dd, *J* = 12.4, 4.6 Hz, 1H) 1.57 - axial (t, *J* = 12.2 Hz, 1H)	3.46	3.74	N/A	N/A	3.51	3.77 3.54	1.93

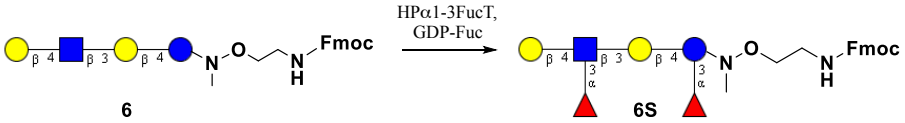
[a] N/A indicates not assigned
[b] - indicates position not available

Peak	1H Signal
Fmoc CH Aromatic	7.79 (d, *J* = 7.6 Hz, 2H)
Fmoc CH Aromatic	7.59 (d, *J* = 7.3 Hz, 2H)
Fmoc CH Aromatic	7.38 (t, *J* = 7.1 Hz, 2H)
Fmoc CH Aromatic	7.31 (t, *J* = 7.4Hz, 2H)
Fmoc CH_2	4.51 - 4.41 (m, 2H)
Fmoc CH	4.21 (br. s, 1H)
CH_2-N	3.15 - 3.05 (m, 2H)
CH_2-O-N	3.63
N-CH_3	2.51 (s, 3H)

^{13}C from HSQC (150 MHz, D_2O): δ(ppm) 40.10, 45.04, 47.06, 47.48, 47.99, 51.63, 55.09, 60.71, 60.97, 62.49, 67.43, 68.01, 68.25, 68.31, 68.47, 69.35, 69.36, 71.14, 71.70, 72.47, 72.86, 74.5, 74.63, 75.11, 75.46, 75.49, 78.13, 80.88, 82.05, 92.92, 92.92, 102.11, 102.51, 102.69, 102.87, 103.08, 118.6, 119.4.

MALDI TOF-MS *m/z* calcd for $C_{55}H_{79}N_4O_{31}Na_2$ $(M + 2Na)^+$ 1337.45, found 1337.38.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-D-glucopyranoside (6S).**



Compound **6** (0.6 μmol) and GDP-Fuc (2.39 μmol) were dissolved in TRIS buffer (113 μL, 50 mM, pH 7.3) containing $MnCl_2$ (10 mM) and CIAP (10 mU, 1.0% V_t). To this solution was added HP α1-3FucT (2.8 mU/μmol of substrate) to yield a final saccharide concentration of 5 mM. The reaction mixture was incubated at 37 °C until no starting material could be detected by MALDI TOF. In the event starting material persisted after 6 h, additional GDP-Fuc and HPα1-3FucT were added. Upon completion, the reaction mixture was centrifuged and the supernatant was purified by reverse phase C18 column chromatography using a gradient of acetonitrile in water (0-40%). Fractions containing the product were combined and lyophilized to yield compound **6S** (0.6 mg, 78%) as a white fluffy solid.

^{1}H (800 MHz,D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6	NH Acetyl	Fuc-CH_3
Glc	3.98 (d, *J* = 8.3 Hz, 1H)	3.56	3.38	3.65	N/A[a]	N/A	-[b]	-
Gal-1	4.26 (d, *J* = 7.3 Hz, 1H)	3.36	3.57	3.96	N/A	N/A	-	-
GlcNAc	4.58 (d, *J* = 8.4 Hz, 1H)	3.84	3.46	3.76	N/A	N/A	1.90	-
Gal-2	4.34 (d, *J* = 7.8 Hz, 1H)	3.38	3.54	3.78	N/A	N/A	-	-
Fuc-1	5.26 (d, *J* = 3.8 Hz, 1H)	3.69	3.81	3.64	4.67	-	-	1.03 (d, *J* = 6.6 Hz, 3H)
Fuc-2	5.01 (d, *J* = 3.8 Hz, 1H)	3.57	3.80	3.69	4.72	-	-	1.05 (d, *J* = 6.6 Hz, 3H)

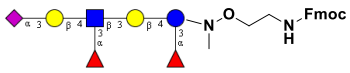
[a] N/A indicates not assigned
[b] - indicates position not available

Peak	1H Signal
Fmoc CH Aromatic	7.79 (d, *J* = 7.6 Hz, 2H)
Fmoc CH Aromatic	7.59 (d, *J* = 7.3 Hz, 2H)
Fmoc CH Aromatic	7.38 (t, *J* = 7.1 Hz, 2H)
Fmoc CH Aromatic	7.31 (t, *J* = 7.4Hz, 2H)
Fmoc CH_2	4.51 - 4.41 (m, 2H)
Fmoc CH	4.21 (br. s, 1H)
CH_2-N	3.15 - 3.05 (m, 2H)
CH_2-O-N	3.63
N-CH_3	2.51 (s, 3H)

^{13}C from HSQC (200 MHz, D_2O): δ(ppm) 22.07, 24.83, 24.9, 25.27, 38.18, 39.12, 46.92, 55.77, 61.25, 65.95, 66.33, 66.51, 67.52, 67.86, 68.01, 68.18, 68.61, 69.06, 70.43, 70.85, 70.89, 71.32, 71.73, 71.74, 72.3, 72.84, 74.2, 74.75, 74.89, 76.68, 81.43, 92.92, 98.38, 98.43, 101.54, 101.56, 102.32, 119.54, 120.01, 124.34, 124.83, 125.32, 127.36, 127.83, 127.89, 135.01, 135.23, 135.46, 145.11, 145.19.

MALDI TOF-MS *m/z* calcd for $C_{56}H_{83}N_3O_{31}Na$ $(M + Na)^+$ 1316.49, found 1316.47.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino-α-5-(acetamindo)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-D-glucopyranoside (8).**



Compound **7** (0.31 μmol) and GDP-Fuc (1.24 μmol) were dissolved in TRIS buffer (59 μL, 50 mM, pH 7.3) containing $MnCl_2$ (10 mM) and CIAP (10 mU, 1.0% V_t). To this solution was added HP α1-3FucT (2.8 mU/μmol of substrate) to yield a final saccharide concentration of 5 mM. The reaction mixture was incubated at 37 °C until no starting material could be detected by TLC. In the event starting material persisted after 6 h, additional GDP-Fuc and HPα1-3FucT were added. Upon completion, the reaction mixture was centrifuged and the supernatant was purified by reverse phase C18 column chromatography using a gradient of acetonitrile in water (0-20%). Fractions containing the product were combined and lyophilized to yield compound **8** (0.4 mg, 82%) as a white fluffy solid.

^{1}H (800 MHz, D_2O) δ (ppm)

	H1	H2	H3	H4	H5	H6	H7	H8	H9	NH Acetyl	Fuc CH_3
Glc	3.98 (d, *J* = 8.9 Hz, 1H)	3.55	3.33	3.64	N/A[a]	N/A	-[b]	-	-	-	-
Gal-1	4.27 (d, *J* = 7.5 Hz, 1H)	3.35	3.56	3.96	N/A	N/A	-	-	-	-	-
GlcNAc	4.57	3.84	3.45	3.74	N/A	N/A	-	-	-	1.89	-
Gal-2	4.41 (d, *J* = 7.8 Hz, 1H)	3.40	3.80	3.96	N/A	N/A	-	-	-	-	-
Fuc-1	5.28 (d, *J* = 3.9 Hz, 1H)	3.67	3.80	3.63	4.69	-	-	-	-	-	1.03 (d, *J* = 6.6 Hz, 3H)
Fuc-2	5.00 (d, *J* = 3.8 Hz, 1H)	3.55	3.77	3.66	4.69	-	-	-	-	-	1.05 (d, *J* = 6.6 Hz, 3H)
Neu5Ac	-	-	2.65 - eq. (dd, *J* = 12.4, 4.5 Hz, 1H) 1.67 - axial (t, *J* = 12.2 Hz, 1H)	3.57	3.72	N/A	N/A	N/A	3.84 3.73	1.91	-

[a] N/A indicates not assigned

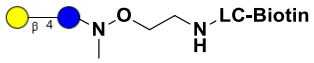
[b] - indicates position not available

Peak	1H Signal
Fmoc CH Aromatic	7.81 (d, *J* = 7.5 Hz, 2H)
Fmoc CH Aromatic	7.60 (d, *J* = 7.1 Hz, 2H)
Fmoc CH Aromatic	7.39 (t, *J* = 7.1 Hz, 2H)
Fmoc CH Aromatic	7.32 (t, *J* = 7.4 Hz, 2H)
Fmoc CH_2	4.51 - 4.43 (m, 2H)
Fmoc CH	4.22 (br. s, 1H)
CH_2-N	3.15 - 3.05 (m, 2H)
CH_2-O-N	3.63
N-CH_3	2.51 (s, 3H)

^{13}C from HSQC (200 MHz, D_2O): δ(ppm) 39.98, 44.81, 46.95, 47.33, 47.87, 59.38, 61.3, 62.43, 62.44, 67.09, 67.60, 67.95, 68.03, 69.03, 69.14, 69.99, 71.14, 71.15, 71.79, 71.82, 72.77, 74.27, 74.83, 75.56, 76.71, 78.38, 81.51, 92.92, 95.14, 98.42, 98.52, 101.50, 101.61, 101.93, 102.14, 118.26, 119.18, 119.67, 119.84.

MALDI TOF-MS *m/z* calcd for $C_{67}H_{99}N_4O_{39}Na_2$ $(M+2Na)^+$ 1629.57, found 1629.44.

***N*-(*N*-methyl-*O*-[2-(acetamido-LC-biotin)-ethyl]hydroxylamino)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (15).**

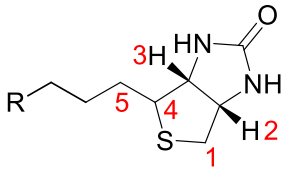


Deprotected saccharide **4** (1.7 μmol) and EZ-Link® Sulfo-NHS-LC-biotin (5.1 μmol, Pierce Biotechnology) were dissolved in PBS buffer (340 μL, 0.1 M, pH 8.01) to give a glycan concentration of 5 mM. The solution was agitated at RT for 3 h at which time MS showed complete disappearance of the free amine starting material. The reaction mixture was purified by P-2 size exclusion eluting with 5% *n*-butanol/water solution. Product fractions were pooled and lyophilized to yield compound **15** (1.0 mg, 80%) as a white fluffy powder.

^{1}H (500 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6
Glc	4.11 (d, *J* = 9.2 Hz, 1H)	3.45	3.53	3.48	3.83	3.87, 3.72
Gal-1	4.33 (d, *J* = 7.8 Hz, 1H)	3.46	3.57	3.65	3.85	3.72, 3.66

Peak	1H Signal
1	2.90 (ddd, *J* = 13.1, 5.0, 1.1 Hz, 1H) 2.68 (d, *J* = 13.0 Hz, 1H)
2	4.54 (dd, *J* = 7.9, 5.0 Hz, 1H)
3	4.34 (dd, *J* = 7.9, 4.5 Hz, 1H)
4	3.24
5	1.64, 1.52
CH_2-N	3.29 (dt, *J* = 9.8, 4.6 Hz, 2H)
CH_2-O-N	3.77
N-CH_3	2.65 (s, 3H)

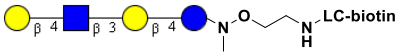


Biotin Numbering for NMR Assignment

^{13}C from HSQC (125 MHz, D_2O): δ (ppm) 24.92, 27.49, 27.65, 27.88, 35.39, 38.28, 38.98, 39.51, 55.18, 60.04, 60.85, 61.91, 68.38, 69.43, 70.76, 70.80, 72.84, 75.13, 75.89, 76.13, 78.10, 92.89, 102.74.

MALDI TOF-MS *m/z* calcd for $C_{31}H_{55}N_5O_{14}SNa$ $(M+Na)^+$ 776.34, found 776.36.

***N*-(*N*-methyl-*O*-[2-(acetamido-LC-biotin)-ethyl]hydroxylamino)-β-D-galactopyranosyl-(1→4)-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (14)**.



Deprotected saccharide **6** (0.6 μmol) and EZ-Link® Sulfo-NHS-LC-biotin (1.8 μmol, Pierce Biotechnology) were dissolved in PBS buffer (297 μL, 0.1 M, pH 8.01) to give a glycan concentration of 2.5 mM. The solution was agitated at RT for 3 h at which time MS showed complete disappearance of the free amine starting material. The reaction mixture was purified by P-2 size exclusion eluting with 5% *n*-butanol/water solution. Product fractions were pooled and lyophilized to yield compound **14** (0.5 mg, 75%) as a white fluffy powder.

^{1}H (800 MHz, D_2O): δ (ppm)

	H1	H2	H3	H4	H5	H6	NH Acetyl
Glc	4.02 (d, *J* = 9.2 Hz, 1H)	3.39	3.50	3.46	N/A[a]	N/A	-[b]
Gal-1	4.29 (d, *J* = 8.5 Hz, 1H)	3.44	3.57	4.01 (d, *J* = 2.8 Hz, 1H)	N/A	N/A	-
GlcNAc	4.56 (d, *J* = 8.3 Hz, 1H)	3.66	3.44	3.49	N/A	N/A	1.87
Gal-2	4.33 (d, *J* = 7.8 Hz, 1H)	3.40	3.52	3.78	N/A	N/A	-

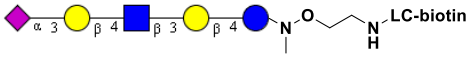
[a] N/A indicates not assigned
[b] - indicates position not available

Peak	1H Signal
1	2.85 (dd, *J* = 13.1, 5.0 Hz, 1H) 2.64 (d, *J* = 13.0 Hz, 1H)
2	4.46 (t, *J* = 6.7 Hz, 1H)
3	4.27 (dd, *J* = 7.9, 4.5 Hz, 1H)
4	3.19 (dt, *J* = 9.7, 5.0 Hz, 1H)
5	1.57, 1.43
CH_2-N	3.24 (dt, *J* = 9.8, 4.6 Hz, 2H)
CH_2-O-N	3.71
N-CH_3	2.60 (s, 3H)

^{13}C from HSQC (200 MHz, D_2O): 22.00, 24.90, 25.38, 27.48, 27.65, 27.83, 35.33, 37.03, 38.11, 38.29, 38.95, 39.47, 39.48, 39.57, 55.03, 55.21, 59.8, 60.07, 60.82, 61.91, 68.18, 68.39, 69.37, 69.80, 70.75, 70.79, 72.02, 72.35, 74.39, 74.76, 75.13, 75.42, 76.16, 77.98, 78.01, 81.89, 92.87, 102.62, 102.71, 102.79.

MALDI TOF-MS *m/z* calcd for $C_{45}H_{78}N_6O_{24}SNa$ $(M+Na)^+$ 1141.47, found 1141.45.

***N*-(*N*-methyl-*O*-[2-(acetamido-LC-biotin)-ethyl]hydroxylamino)-α-5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid-(2,3)-β-D-galactopyranosyl-(1→4)-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-β-D-glucopyranoside (13).**



Deprotected saccharide **7** (0.39 μmol) and EZ-Link® Sulfo-NHS-LC-biotin (0.77 μmol, Pierce Biotechnology) were dissolved in PBS buffer (76 μL, 0.1 M, pH 8.01) to give a glycan concentration of 5 mM. The solution was agitated at RT for 3 h at which time MS showed complete disappearance of the free amine starting material. The reaction mixture was purified by P-2 size exclusion eluting with 5% *n*-butanol/water solution. Product fractions were pooled and lyophilized to yield compound **13** (0.4 mg, 74%) as a white fluffy powder.

^{1}H (800 MHz, D_2O) δ (ppm)

	H1	H2	H3	H4	H5	H6	H7	H8	H9	NH Acetyl
Glc	4.02 (d, *J* = 9.2 Hz, 1H)	3.39	3.50	3.45	N/A[a]	N/A	-[b]	-	-	-
Gal-1	4.28 (d, *J* = 8.2 Hz, 1H)	3.43	3.57	4.00 (d, *J* = 3.3 Hz, 1H)	N/A	N/A	-	-	-	-
GlcNAc	4.54 (d, *J* = 8.4 Hz, 1H)	3.65	3.42	3.58	N/A	N/A	-	-	-	1.88
Gal-2	4.40 (d, *J* = 7.9 Hz, 1H)	3.41	3.80	3.97 (dd, *J* = 9.9, 3.1 Hz, 1H)	N/A	N/A	-	-	-	-
Neu5Ac	-	-	2.60 (dd, *J* = 12.3, 4.8 Hz, 1H), 1.64 (t, *J* = 12.1 Hz, 1H)	3.54	3.69	3.48	N/A	N/A	3.80 3.71	1.88

[a] N/A indicates not assigned

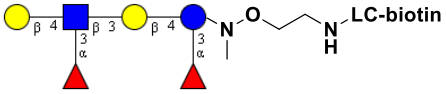
[b] - indicates position not available

Peak	1H Signal
1	2.84 (dd, *J* = 13.1, 5.0 Hz, 1H) 2.62 (d, *J* = 13.0 Hz, 1H)
2	4.46 (dd, *J* = 8.0, 4.6 Hz, 1H)
3	4.26 (dd, *J* = 7.9, 4.5 Hz, 1H)
4	3.18 (dt, *J* = 9.7, 5.0 Hz, 1H)
5	1.57, 1.42
CH_2-N	3.23 (dt, *J* = 9.8, 4.6 Hz, 2H)
CH_2-O-N	3.70
N-CH_3	2.58 (s, 3H)

^{13}C from HSQC (200 MHz, D_2O) δ (ppm) 21.86, 24.71, 27.30, 27.31, 27.44, 27.66, 35.16, 36.02, 38.14, 38.76, 39.29, 55.02, 55.60, 59.28, 59.37, 59.54, 59.90, 61.14, 61.73, 66.24, 66.32, 67.33, 67.65, 67.91, 68.01, 68.86, 68.93, 70.49, 70.53, 70.58, 70.84, 71.57, 71.97, 72.11, 72.66, 74.04, 74.43, 74.58, 74.77, 76.59, 78.09, 81.24, 92.85, 98.24, 98.26, 101.43, 101.44, 102.19, 155.05.

MALDI TOF-MS *m/z* calcd for $C_{56}H_{94}N_7O_{32}S$ $(M - H)^-$ 1408.57, found 1408.69 (negative mode).

***N*-(*N*-methyl-*O*-[2-(acetamido-LC-biotin)-ethyl]hydroxylamino)-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-D-glucopyranoside (12).**



Deprotected saccharide **6S** (0.23 μmol) and EZ-Link® Sulfo-NHS-LC-biotin (0.7 μmol, Pierce Biotechnology) were dissolved in PBS buffer (124 μL, 0.1 M, pH 8.01) to give a glycan concentration of 5 mM. The solution was agitated at RT for 3 h at which time MS showed complete disappearance of the free amine starting material. The reaction mixture was purified by P-2 size exclusion eluting with 5% *n*-butanol/water solution. Product fractions were pooled and lyophilized to yield compound **12** (0.3 mg, 94%) as a white fluffy powder.

^{1}H (800 MHz, D_2O) δ (ppm)

	H1	H2	H3	H4	H5	H6	NH Acetyl	Fuc-CH_3
Glc	4.02 (d, *J* = 9.2 Hz, 1H)	3.59	3.35	3.66	3.63	N/A[a]	-[b]	-
Gal-1	4.26 (d, *J* = 8.0, 1H)	3.34	3.54	3.94 (d, *J* = 3.0 Hz, 1H)	3.33	N/A	-	-
GlcNAc	4.54 (d, *J* = 8.5 Hz, 1H)	3.81	3.41	3.71	N/A	N/A	1.86	-
Gal-2	4.30 (d, *J* = 7.7 Hz, 1H)	3.33	3.49	3.74	N/A	N/A	-	-
Fuc-1	5.28 (d, *J* = 3.9 Hz, 1H)	3.59	3.79	3.63	5.66	-	-	1.04 (d, *J* = 6.6 Hz, 3H)
Fuc-2	4.97 (d, *J* = 3.8 Hz, 1H)	3.53	3.74	3.63	4.60	-	-	1.04 (d, *J* = 6.5 Hz, 3H)

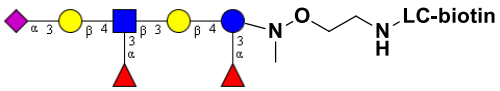
[a] N/A indicates not assigned
[b] - indicates position not available

Peak	1H Signal
1	2.84 (dd, *J* = 13.0, 5.0 Hz, 1H) 2.61 (d, *J* = 13.0 Hz, 1H)
2	4.46 (t, *J* = 6.4 Hz, 1H)
3	4.28 - 4.24 (m, 1H)
4	3.18 (dt, *J* = 9.7, 5.0 Hz, 1H)
5	1.56, 1.43
CH_2-N	3.22 (dt, *J* = 9.8, 4.6 Hz, 2H)
CH_2-O-N	3.69
N-CH_3	2.58 (s, 3H)

^{13}C from HSQC (200 MHz, D_2O) δ (ppm) 21.86, 24.71, 27.3, 27.31, 27.44, 27.66, 35.16, 36.02, 37.91, 38.14, 38.76, 39.29, 55.02, 55.61, 59.37, 59.90, 61.14, 61.73, 66.24, 66.32, 67.33, 67.65, 67.90, 68.01, 68.86, 68.9, 70.49, 70.53, 70.58, 70.84, 71.57, 71.97, 72.11, 72.66, 74.04, 74.42, 74.58, 74.77, 76.61, 77.58, 81.25, 92.85, 98.24, 98.29, 101.43, 101.44, 102.19, 155.05.

MALDI TOF-MS *m/z* calcd for $C_{57}H_{98}O_{32}N_6SNa$ $(M + Na)^+$ 1433.58, found 1433.70.

***N*-(*N*-methyl-*O*-[2-(acetamido-LC-biotin)-ethyl]hydroxylamino)-α-5-(acetamindo)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid-β-D-galactopyranosyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)–[α-L-fucopyranosyl (1→3)]-β-D-glucopyranoside (11).**



Deprotected saccharide **8** (0.32 μmol) and EZ-Link® Sulfo-NHS-LC-biotin (0.95 μmol, Pierce Biotechnology) were dissolved in PBS buffer (63 μL, 0.1 M, pH 8.01) to give a glycan concentration of 5 mM. The solution was agitated at RT for 3 h at which time MS showed complete disappearance of the free amine starting material. The reaction mixture was purified by P-2 size exclusion eluting with 5% *n*-butanol/water solution. Product fractions were pooled and lyophilized to yield compound **11** (0.4 mg, 75%) as a white fluffy powder.

^{1}H (800 MHz, D_2O) δ (ppm)

	H1	H2	H3	H4	H5	H6	H7	H8	H9	NH Acetyl	Fuc CH_3
Glc	4.02 (d, *J* = 9.1 Hz, 1H)	3.58	3.36	3.66	3.63	N/A[a]	-[b]	-	-	-	-
Gal-1	4.29 - 4.25 (m, 2H)	3.33	3.54	3.95 (d, *J* = 3.4 Hz, 1H)	N/A	N/A	-	-	-	-	-
GlcNAc	4.54 (d, *J* = 8.0 Hz, 1H)	3.81	3.42	3.71	N/A	N/A	-	-	-	1.86	-
Gal-2	4.38 (d, *J* = 7.9 Hz, 1H)	3.37	3.78	3.92 (dd, *J* = 10.0, 3.0 Hz, 1H)	N/A	N/A	-	-	-	-	-
Neu5Ac	-	-	2.61 eq. 1.64 axial (t, *J* = 12.1 Hz, 1H)	3.54	3.70	N/A	N/A	N/A	3.86 3.69	1.88	-
Fuc-1	5.25 (d, *J* = 4.2 Hz, 1H)	3.52	3.80	3.66	4.66	-	-	-	-	-	1.00 (d, *J* = 6.2 Hz, 3H)
Fuc-2	4.97 (d, *J* = 3.7 Hz, 1H)	3.65	3.77	3.63	4.66	-	-	-	-	-	1.00 (d, *J* = 6.2 Hz, 3H)

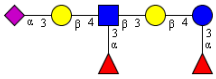
[a] N/A indicates not assigned

[b] - indicates position not available

Peak	1H Signal
1	2.85 (dd, *J* = 13.0, 5.0 Hz, 1H) 2.62 (d, *J* = 13.0 Hz, 1H)
2	4.46 (dd, *J* = 7.9, 5.1 Hz, 1H)
3	4.28 - 4.25 (m, 2H)
4	3.18 (dt, *J* = 10.0, 5.1 Hz, 1H)
5	1.56, 1.43
CH_2-N	3.23 (dt, *J* = 9.8, 4.6 Hz, 2H)
CH_2-O-N	3.69
N-CH_3	2.58 (s, 3H)

MALDI TOF-MS *m/z* calcd for $C_{68}H_{114}O_{40}N_7S$ $(M - H)^-$ 1700.68, found 1700.87 (negative mode).

α/β-(α-5-(acetamindo)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid-(2→3)-β-D-galactopyranosyl-(1→4) -[α-L-fucopyranosyl (1→3)]-β-2-acetamido-2-deoxy-D-glucopyranosyl-(1→3)-β-D-galactopyransoyl-(1→4)-[α-L-fucopyranosyl (1→3)]-β-D-glucopyranose (9).



Glycan **8** (0.13 μmol) was dissolved in an aqueous 0.25% (*v:v)* (63 μL) solution of trifluoroacetic acid (TFA) to give a final glycan concentration of 2 mM. Solution was agitated at RT for 2 h, at which time, MS showed complete hydrolysis of *N*-methyl-hydroxylamine linker. Reaction was quenched with saturated sodium bicarbonate and purified via size exclusion P-2 chromatography eluting with a 5% *n*-butanol/H_2O solution. Product fractions were pooled and lyophilized to yield compound **9** (0.2 mg, 100%) as a white powder.

^{1}H (800 MHz,D_2O) δ (ppm)

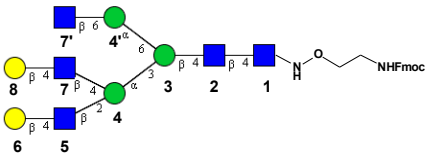
	H1	H2	H3	H4	H5	H6	H7	H8	H9	NH Acetyl	Fuc CH_3
Glc (β)	4.48 (d, *J* = 7.8 Hz, 1H)	3.32	3.62	3.72	3.44	N/A[a]	-[b]	-	-	-	-
Glc (α)	5.02 (d, *J* = 3.5 Hz, 1H)	3.62	3.78	3.72	N/A	N/A	-	-	-	-	-
Gal-1	4.27 (d, *J* = 7.7 Hz, 1H)	3.35	3.56	3.96	N/A	N/A	-	-	-	-	-
GlcNAc	4.53 (d, *J* = 8.3 Hz, 1H)	3.33	3.63	3.72	3.44	N/A	-	-	-	1.86	-
Gal-2	4.37 (d, *J* = 7.9 Hz, 1H)	3.39	3.79	3.94	N/A	N/A	-	-	-	-	-
Neu5Ac	-	-	2.61 eq. (dd, *J* = 11.8, 4.9 Hz, 1H) 1.65 axial (t, *J* = 11.8 Hz, 1H)	3.54	3.71	N/A	N/A	N/A	3.80 3.73	1.88	-
Fuc-1 (β)	5.27 (d, *J* = 4.1 Hz, 1H)	3.64	3.80	3.42	4.65	-	-	-	-	-	1.04 - 0.99 (m, 6H)
Fuc-1 (α)	5.21 (d, *J* = 3.6 Hz, 1H)	N/A	N/A	N/A	N/A	-	-	-	-	-	1.04 - 0.99 (m, 6H)
Fuc-2	4.97 (d, *J* = 4.0 Hz, 1H)	3.54	3.76	3.64	4.65	-	-	-	-	-	1.04 - 0.99 (m, 6H)

[a] N/A indicates not assigned
[b] - indicates position not available

^{13}C from HSQC (200 MHz, D_2O) δ (ppm) 51.51, 55.78, 59.45, 59.45, 61.35, 62.39, 62.43, 66.43, 67.2, 68.01, 68.03, 68.16, 68.52, 69.11, 69.11, 70.53, 71.77, 71.82, 72.18, 72.91, 74.49, 74.77, 75.42, 75.53, 76.89, 81.50, 90.13, 91.99, 94.91, 95.65, 96.93, 97.11, 98.13, 98.34, 98.42, 98.45, 101.42, 101.62, 101.84, 102.41, 103.55, 112.52, 114.68, 114.69.

MALDI TOF-MS *m/z* calcd for $C_{49}H_{81}O_{37}N_2Na_2$ $(M + 2Na)^+$ 1335.43, found 1335.31.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino-[[β-D-Acetyllactosamine-(1-2)]-[β-D-Acetyllactosamine-(1-4)]-α-D-mannopyranosyl-(1-3)]-[2-deoxy-2-acetamido-β-D-glucopyranosyl-(1-6)-α-D-mannopyranosyl-(1-6)]-[-β-D-mannopyranosyl]-(1-4)-[2-deosy-2-acetamido-β-D-glycopyranosyl]-(1-4)-deoxy-2-acetamido-β-D-glycopyranoside (18).**



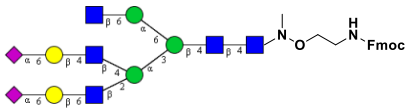
Decasaccharide **16** (0.4 mg, 0.21 μmol) and 2-((methylamino)oxy)ethanamine dihydrochloride (3.1 mg, 19 μmol) was dissolved in a sodium acetate buffer (0.1 M, pH 6.0) at 35 °C for 48 h. The reaction mixture was lyophilyzed and purified using Sephadex®G-25 eluting with 0.1 M ammonium bicarbonate to yield compound **17** as white powder. MALDI-MS *m/z* calcd for $C_{73}H_{125}N_7O_{51}Na$ $(M+Na)^+$ 1938.73, found 1938.30. The resulting powder was dissolved in 102 μL of $NaHCO_3$ (0.1M). To this solution was added Fmoc-Cl (0.77 μmol) dissolved in ACN. The resulting mixture was agitated at RT for 4 h. The reaction mixture was reduced *in vacuo* and purified by C8 HPLC as previously described to yield compound **18** (0.4 mg, 86 %) as a white fluffy powder. Full NMR assignment for intermediate **17** is given in Ref. 2.

^1H (800 MHz,D_2O) δ (ppm)

	H1	Peak	1H
GlcNAc-1	4.00	**Fmoc CH Aromatic**	7.77 (d, *J* = 6.4 Hz, 2H)
GlcNAc-2	4.33 - 4.28 (m, 3H)	**Fmoc CH Aromatic**	7.58 (dd, *J* = 11.7, 7.6 Hz, 2H)
Man-3	4.55 (s, 1H)	**Fmoc CH Aromatic**	7.36 (dd, *J* = 12.0, 7.0 Hz, 2H)
Man-4	4.96 (s, 1H)	**Fmoc CH Aromatic**	7.29 (dd, *J* = 11.2, 7.4 Hz, 2H)
Man-4'	4.72 (s, 1H)	**Fmoc CH_2**	4.44 - 4.34 (m, 5H)
GlcNAc-5	4.44 - 4.34 (m, 5H)	**Fmoc CH**	4.20 - 41.6 (m, 1H)
Gal-6	4.33 - 4.28 (m, 3H)	**CH_2-N**	3.16 - 3.08 (m, 2H)
GlcNAc-7	4.44 - 4.34 (m, 5H	**CH_2-O-N**	3.50
GlcNAc-7'	4.44 - 4.34 (m, 5H	**N-CH_3**	2.50 (s, 3H)
Gal-8	4.33 - 4.28 (m, 3H)		

MALDI TOF-MS *m/z* calcd for $C_{88}H_{135}N_7O_{53}Na$ $(M+Na)^+$ 2160.80, found 2160.31.

***N*-(*N*-methyl-*O*-[2-(2-fluorenylmethyloxycarbamate)ethyl]hydroxylamino-[[α-5-(acetamindo)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid (2→6)-β-D-Acetyllactosamine-(1-2)]-[α-5-(acetamindo)-3,5-dideoxy-D-glycero-α-D-galacto-non-2-ulopyranosonyl acid (2→6)-β-D-Acetyllactosamine-(1-4)]-α-D-mannopyranosyl-(1-3)]-[2-deoxy-2-acetamido-β-D-glucopyranosyl-(1-6)-α-D-mannopyranosyl-(1-6)]-[-β-D-mannopyranosyl]-(1-4)-[2-deoxy-2-acetamido-β-D-glycopyranosyl]-(1-4)-deoxy-2-acetamido-β-D-glycopyranoside (19).**



Decasaccharide **18** (0.14 μmol) and CMP-Neu5Ac (0.56 μmol) were dissolved in sodium cacodylate buffer (66.7 μL, 50 mM, pH 7.6) containing BSA (1% V_t) and CIAP (10 mU, 1.0% V_t). To this solution was added ST6Gal-I (1.28 mU/μmol of substrate) to yield a final decasaccharide concentration of 2 mM. The reaction mixture was incubated at 37 °C until no further starting material was detected. The reaction was centrifuged and the supernatant was purified by C8 reverse phase HPLC to yield dodecasaccharide **19** as a white fluffy powder (0.3 mg, 79% yield). MALDI TOF-MS *m/z* calcd for $C_{110}H_{167}N_9O_{69}Na$ $(M-2H+Na)^-$ 2740.97, found 2740.66 (negative mode).

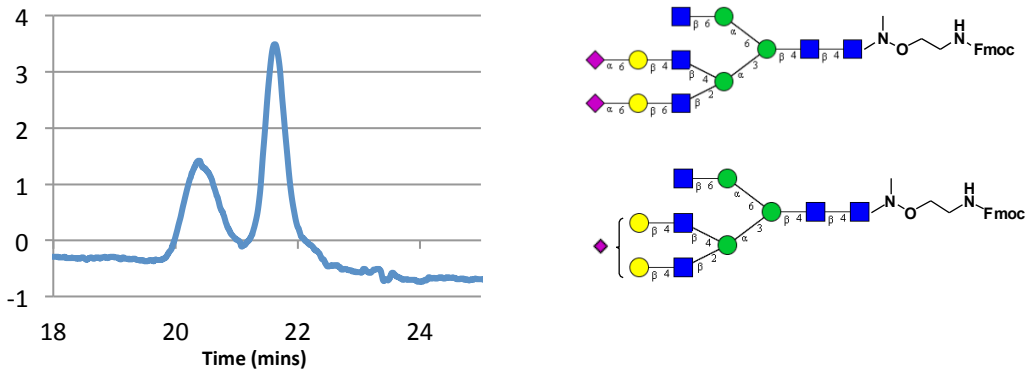


Figure S2. C8 HPLC trace of incomplete bis-sialylation reaction.

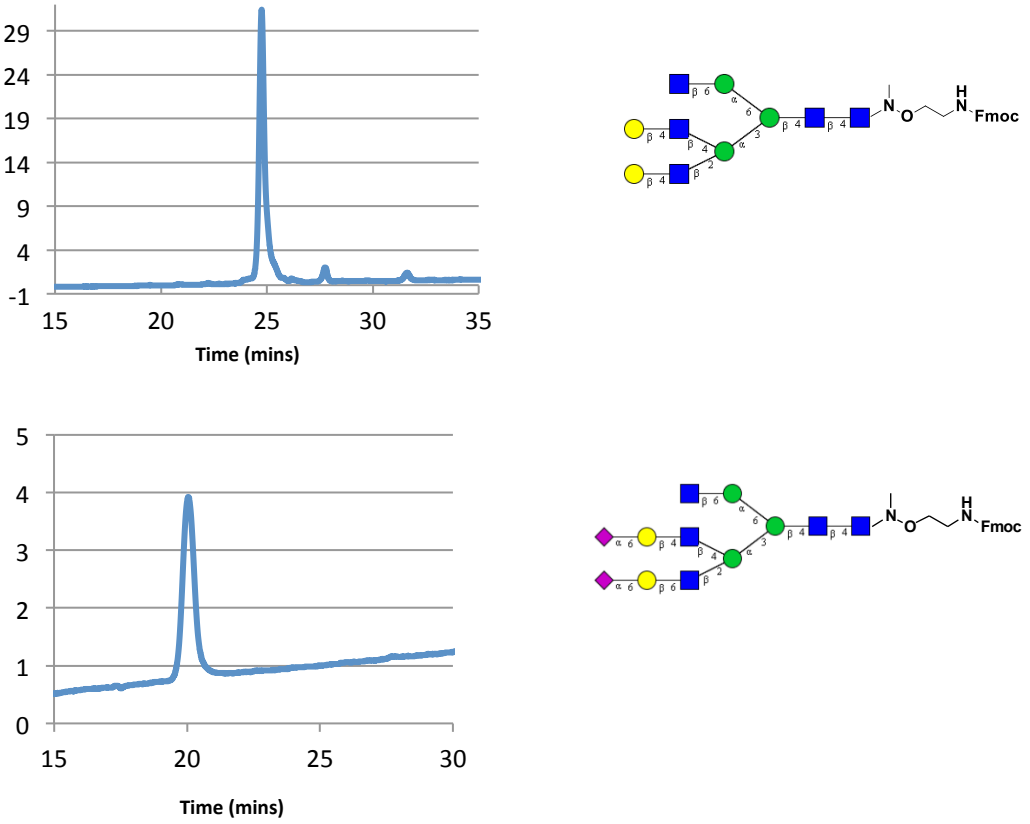
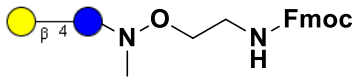


Figure S3. C8 HPLC trace of pure deca (**18**)- and dodecasaccharide (**19**).

NMR spectra



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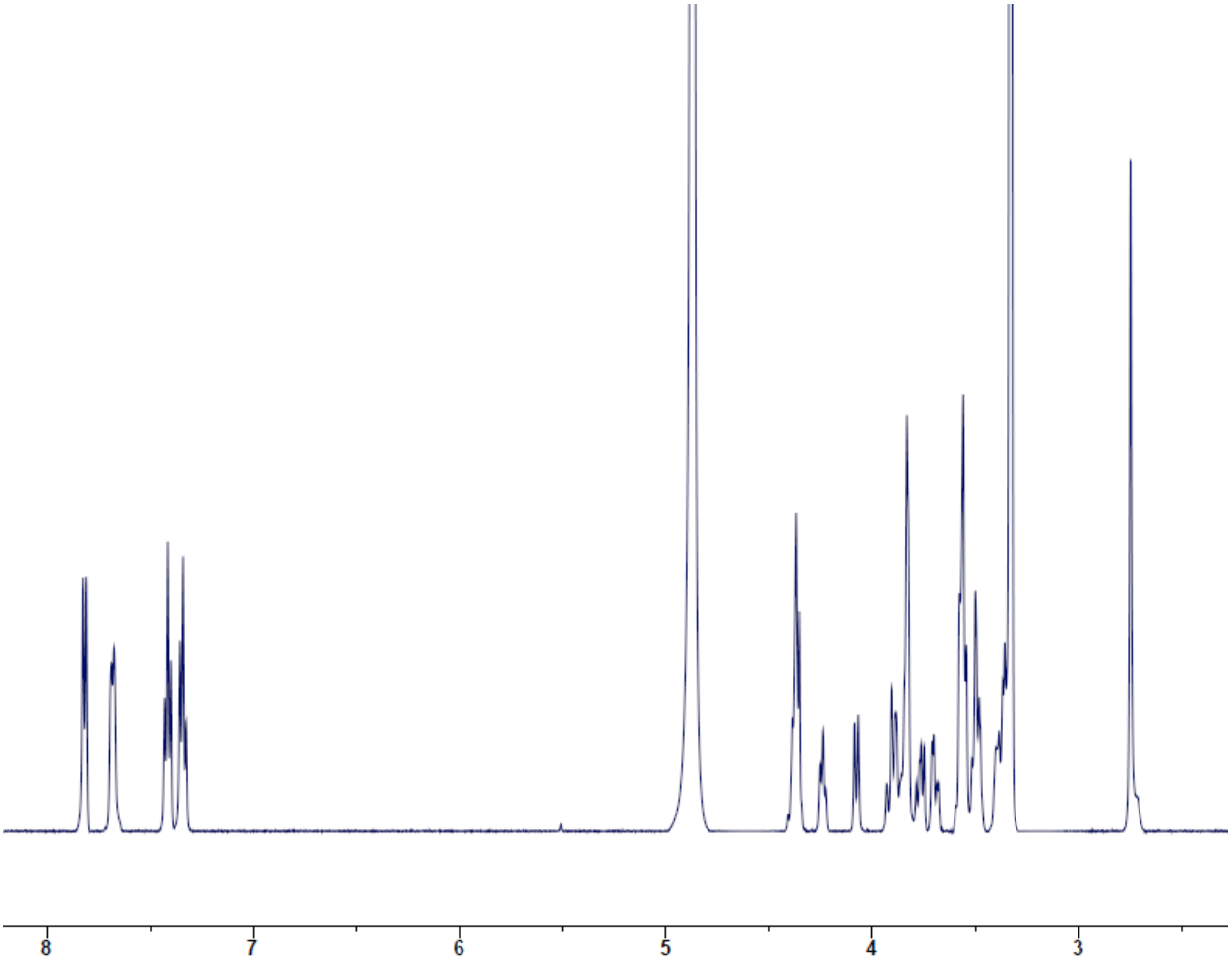

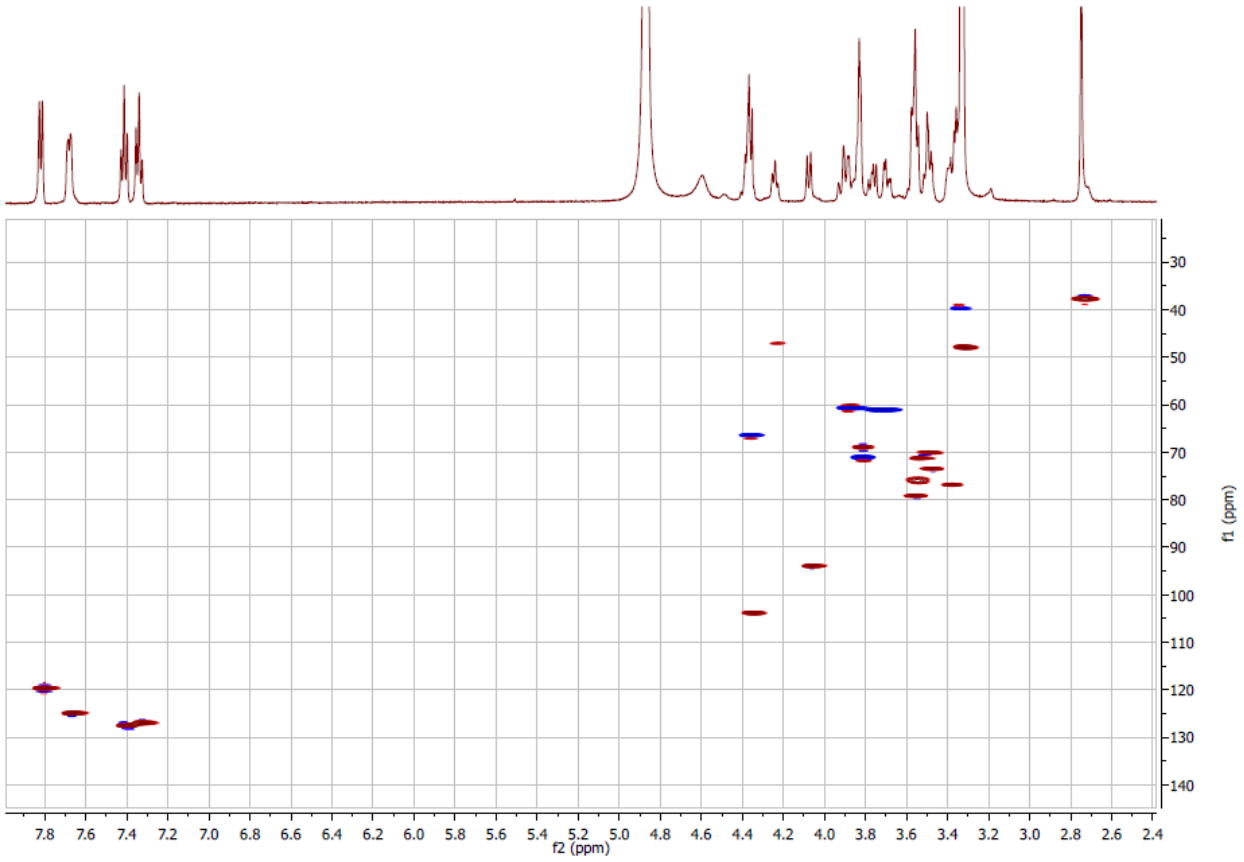
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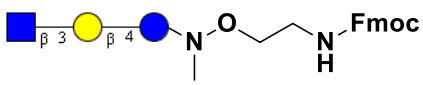
f1 (ppm)



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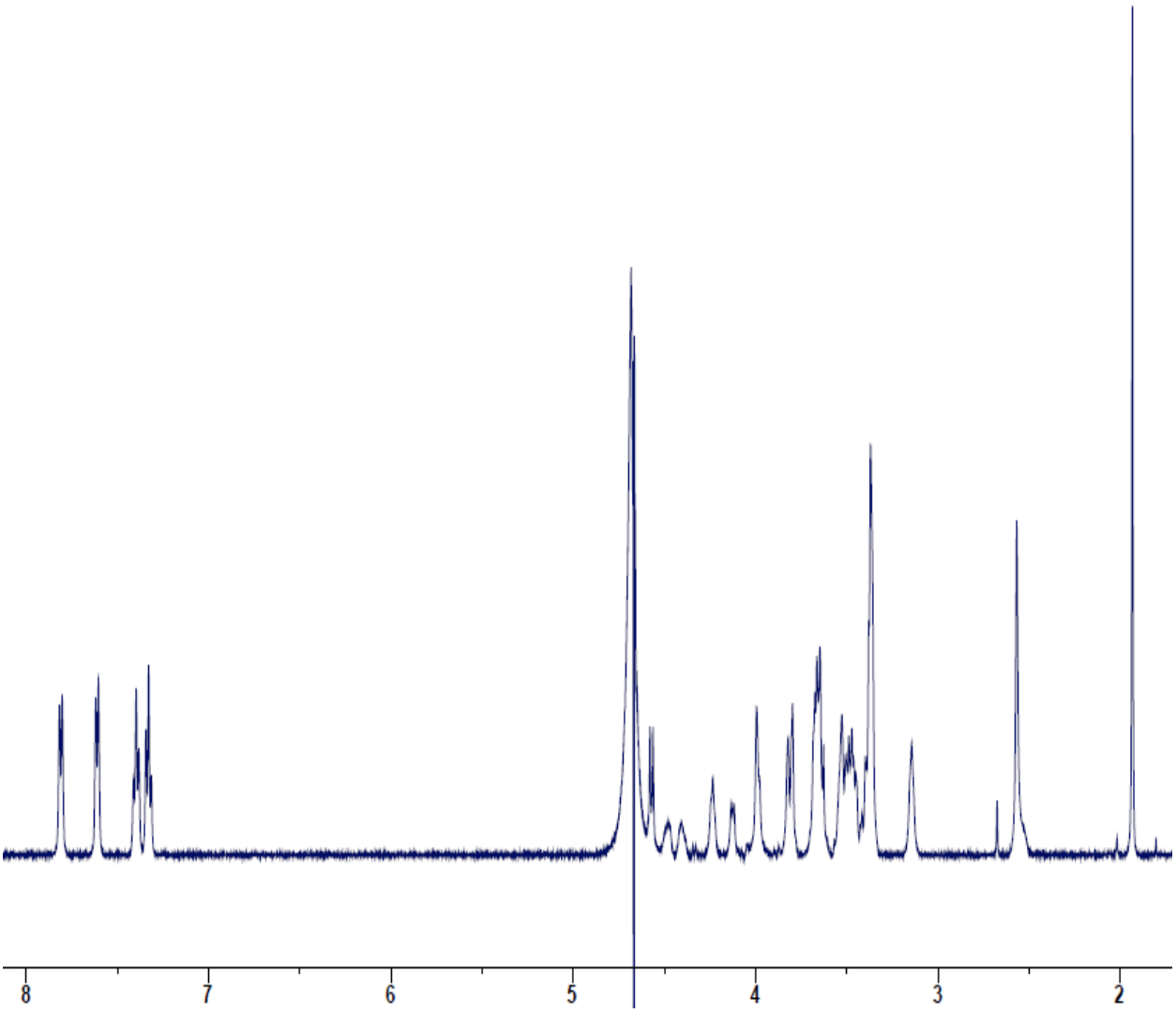

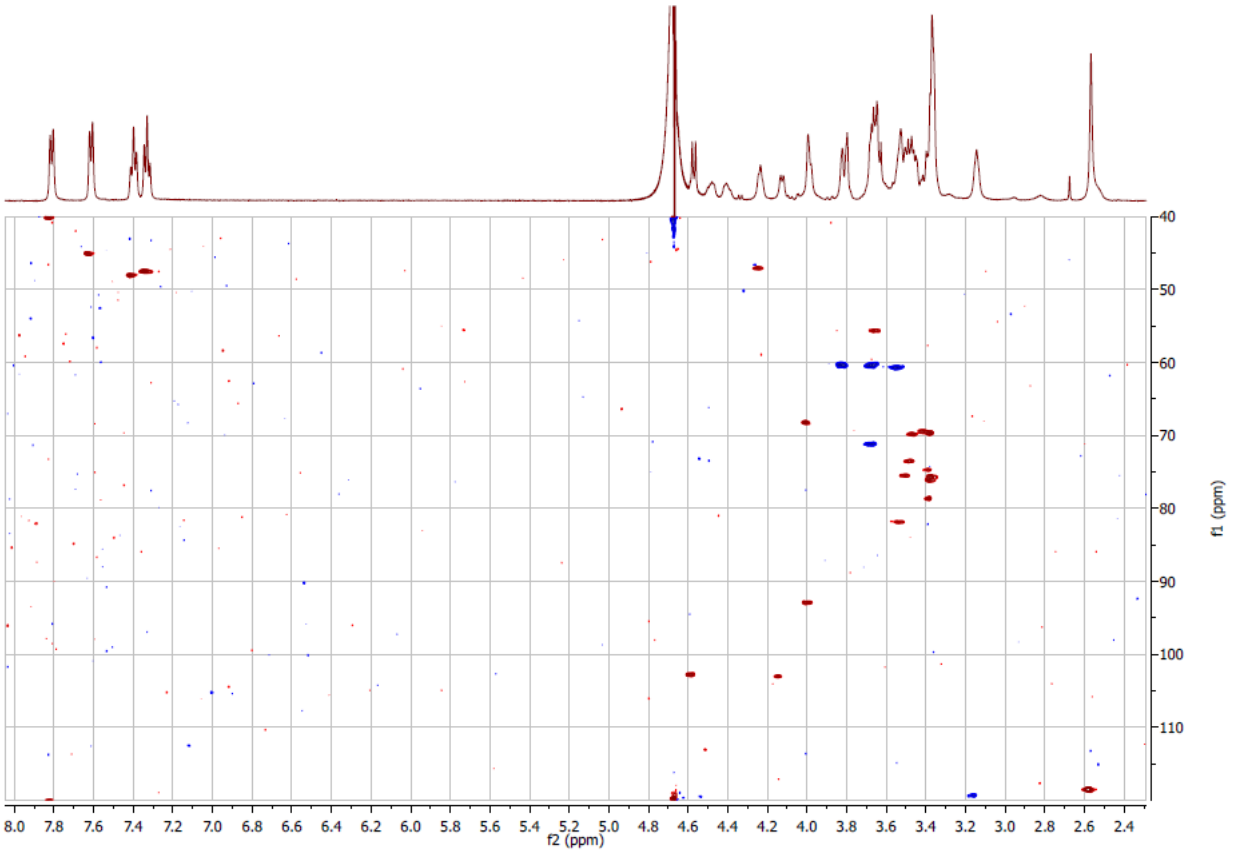
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f2 (ppm)
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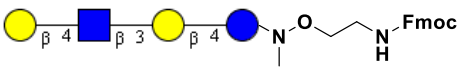
f1 (ppm)



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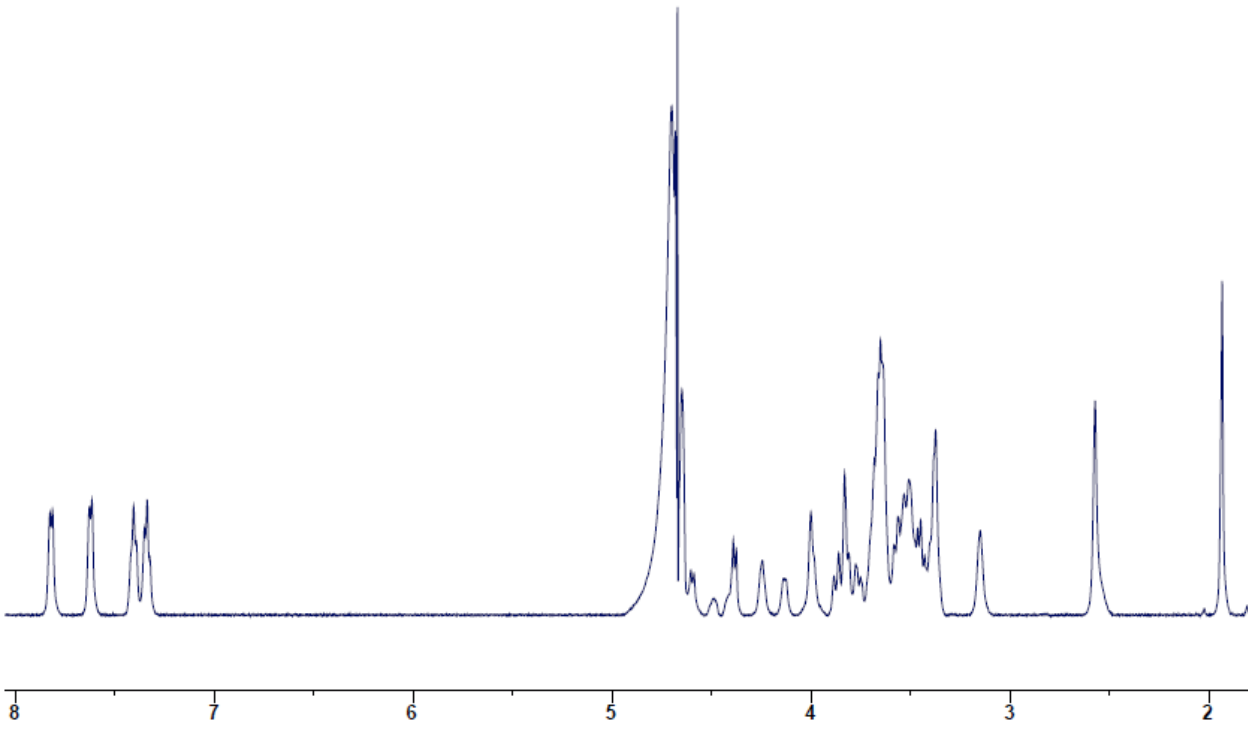

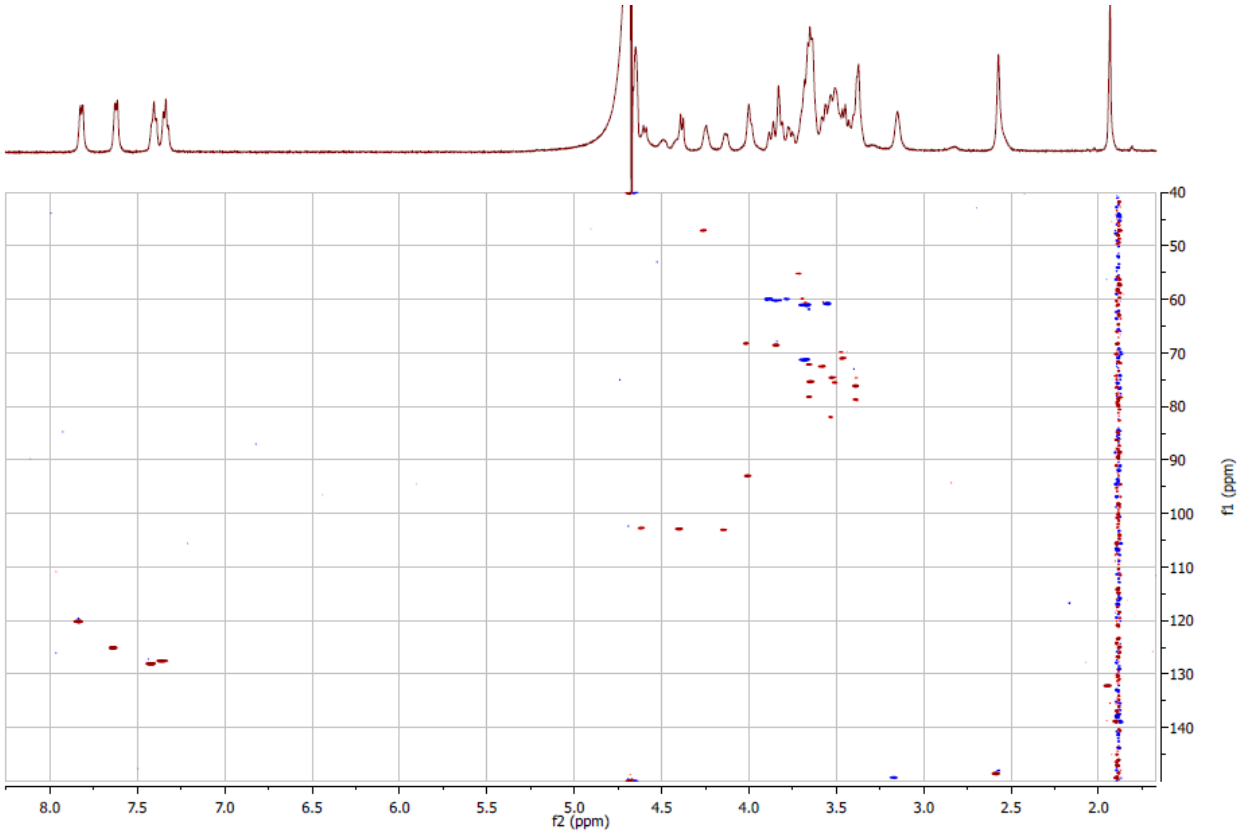
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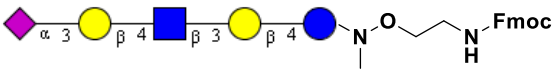
130
140
f1 (ppm)



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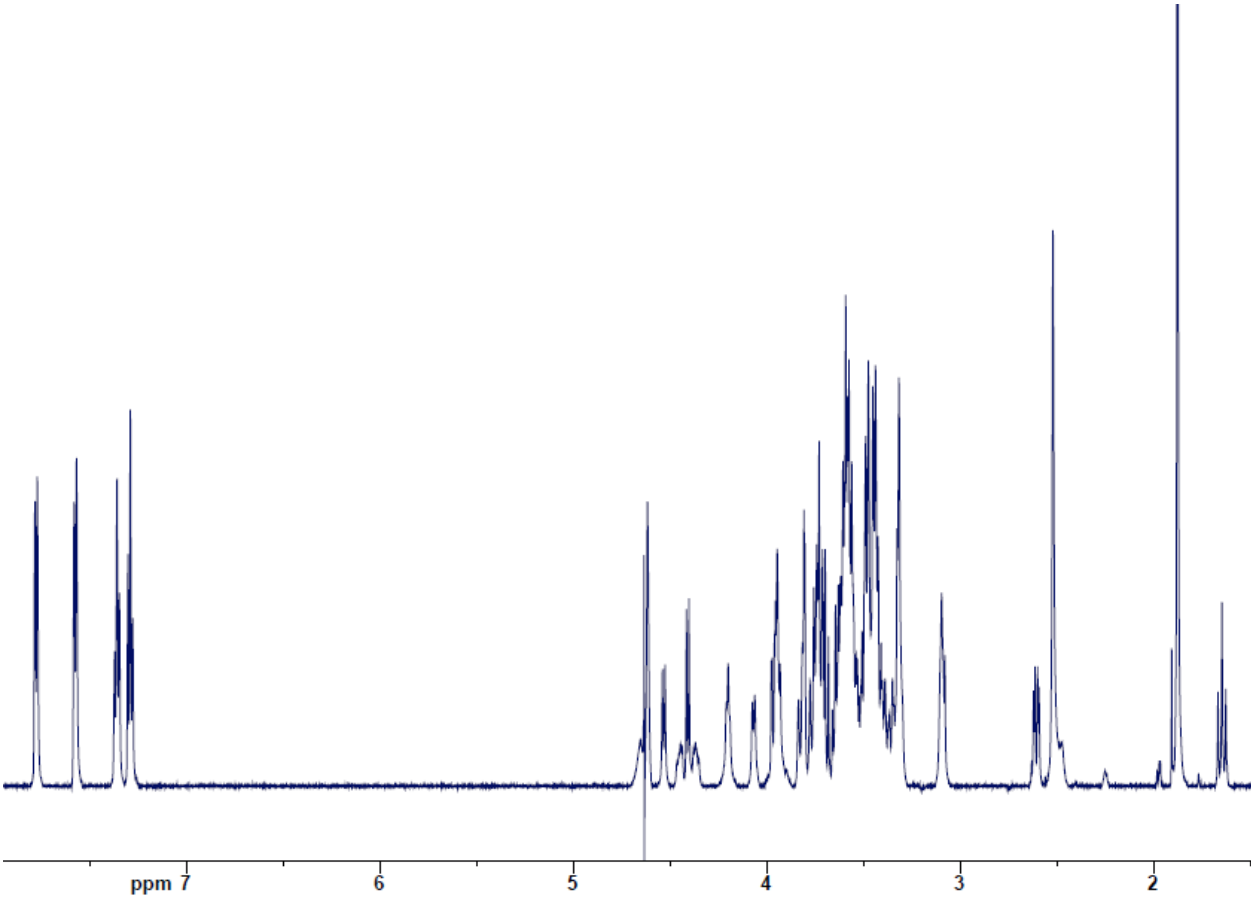

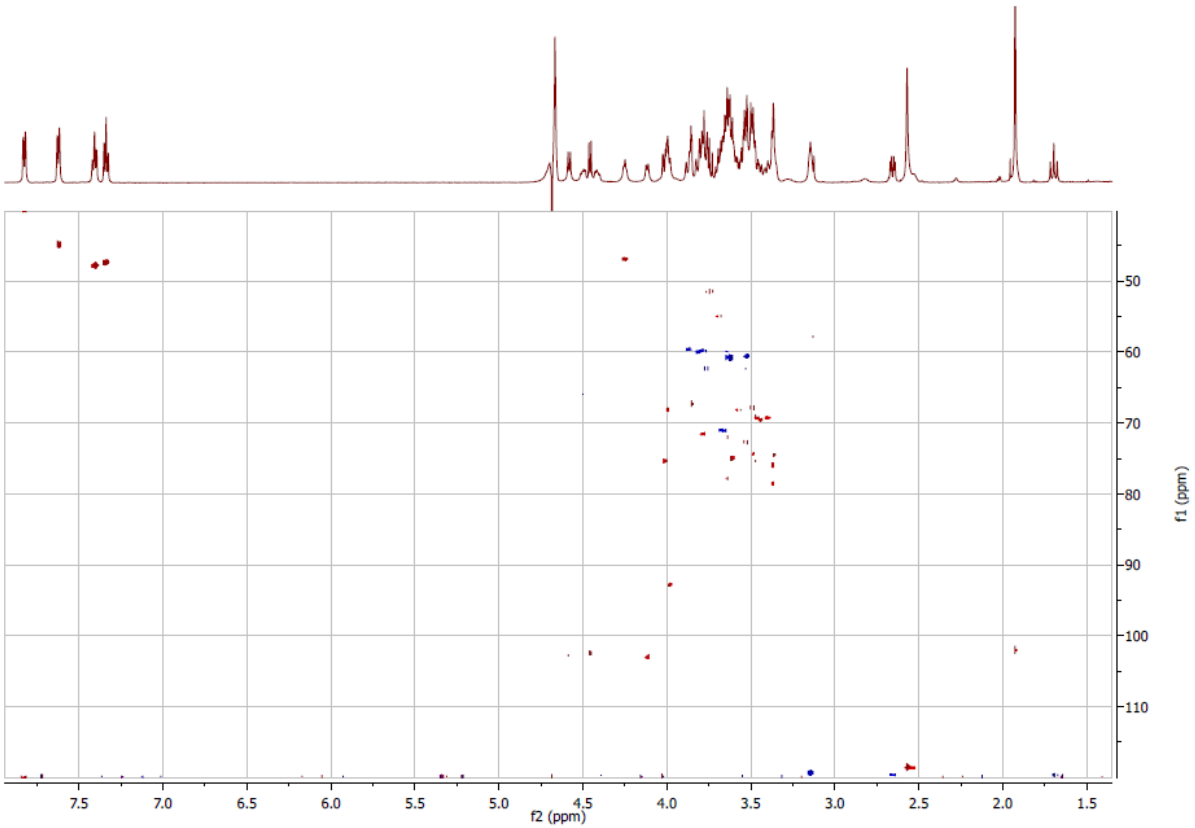
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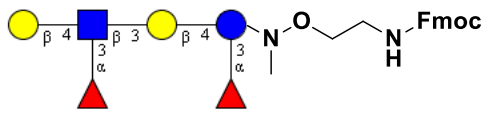
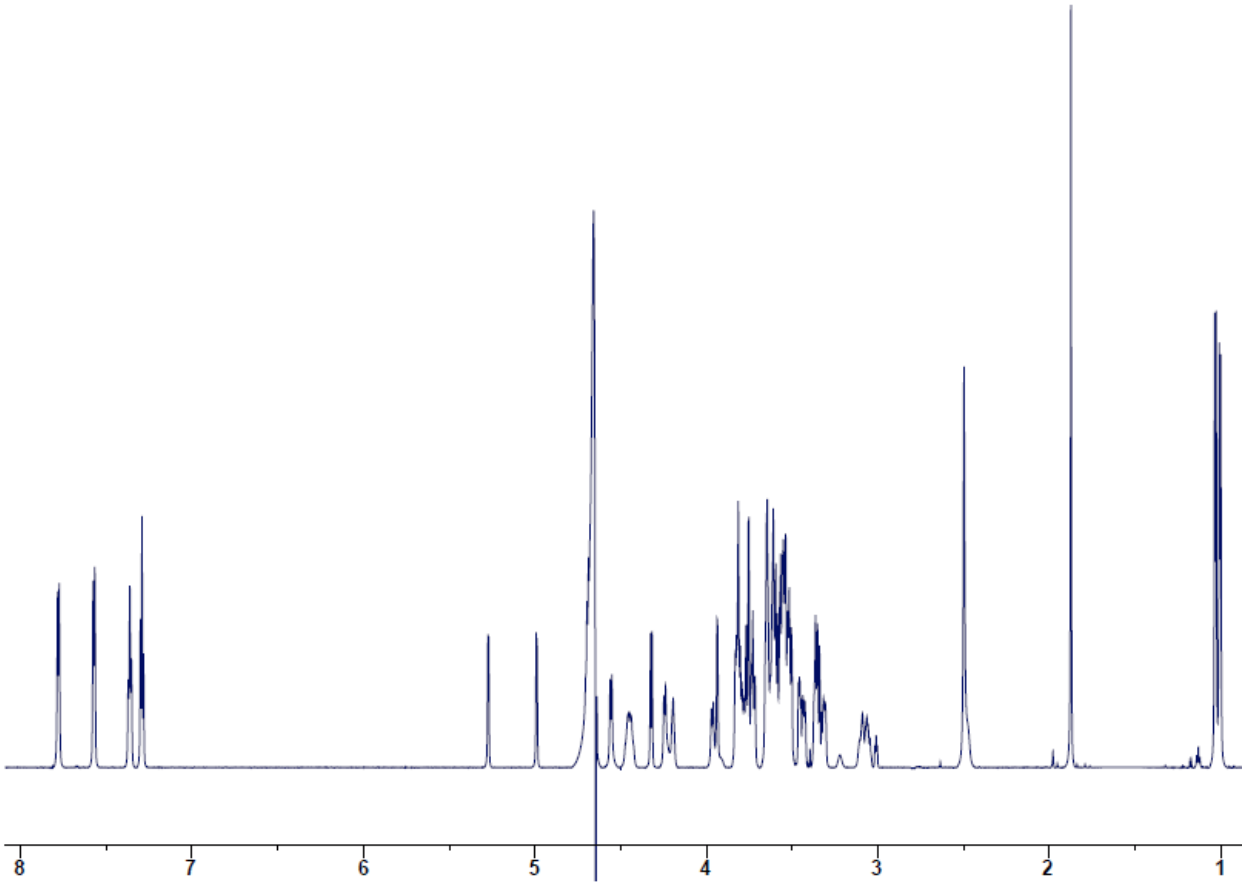
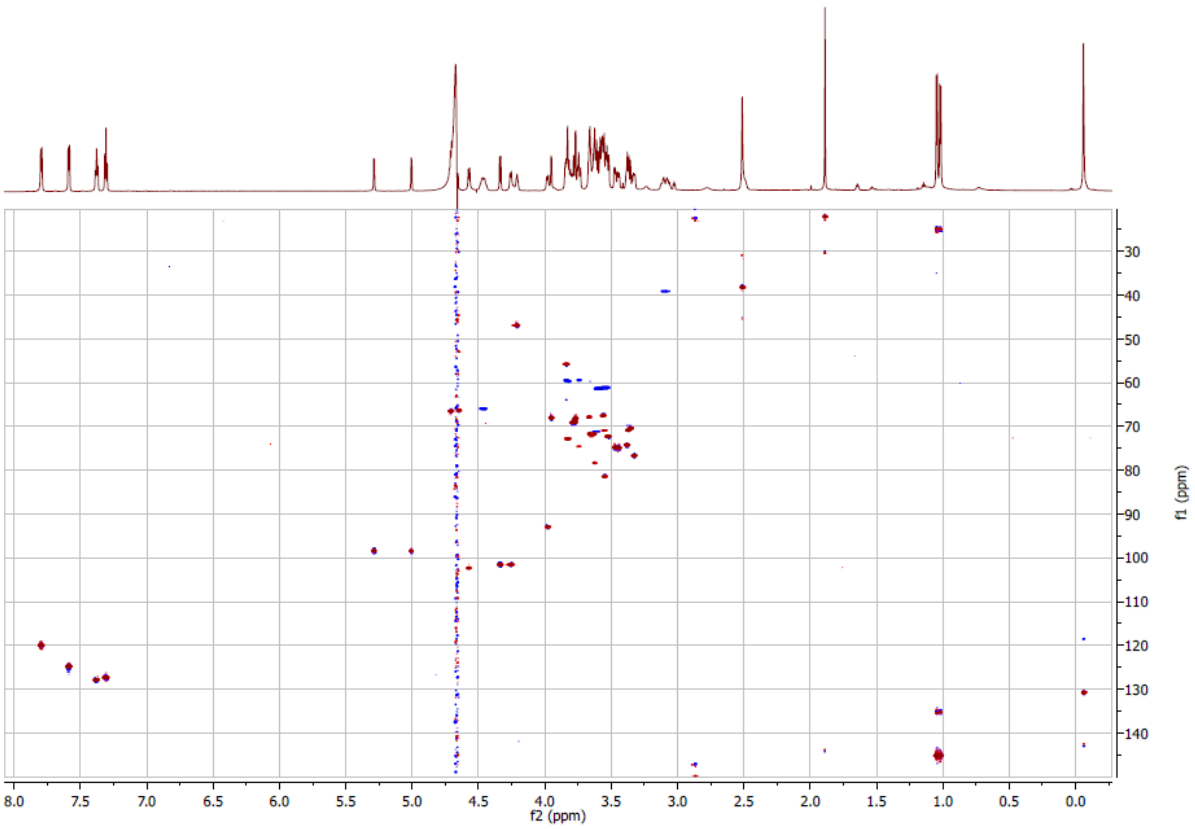
110
f1 (ppm)



S6

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f2 (ppm)
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90
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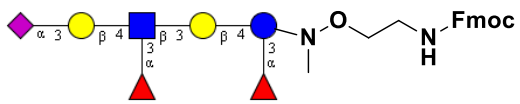
130
140
f1 (ppm)



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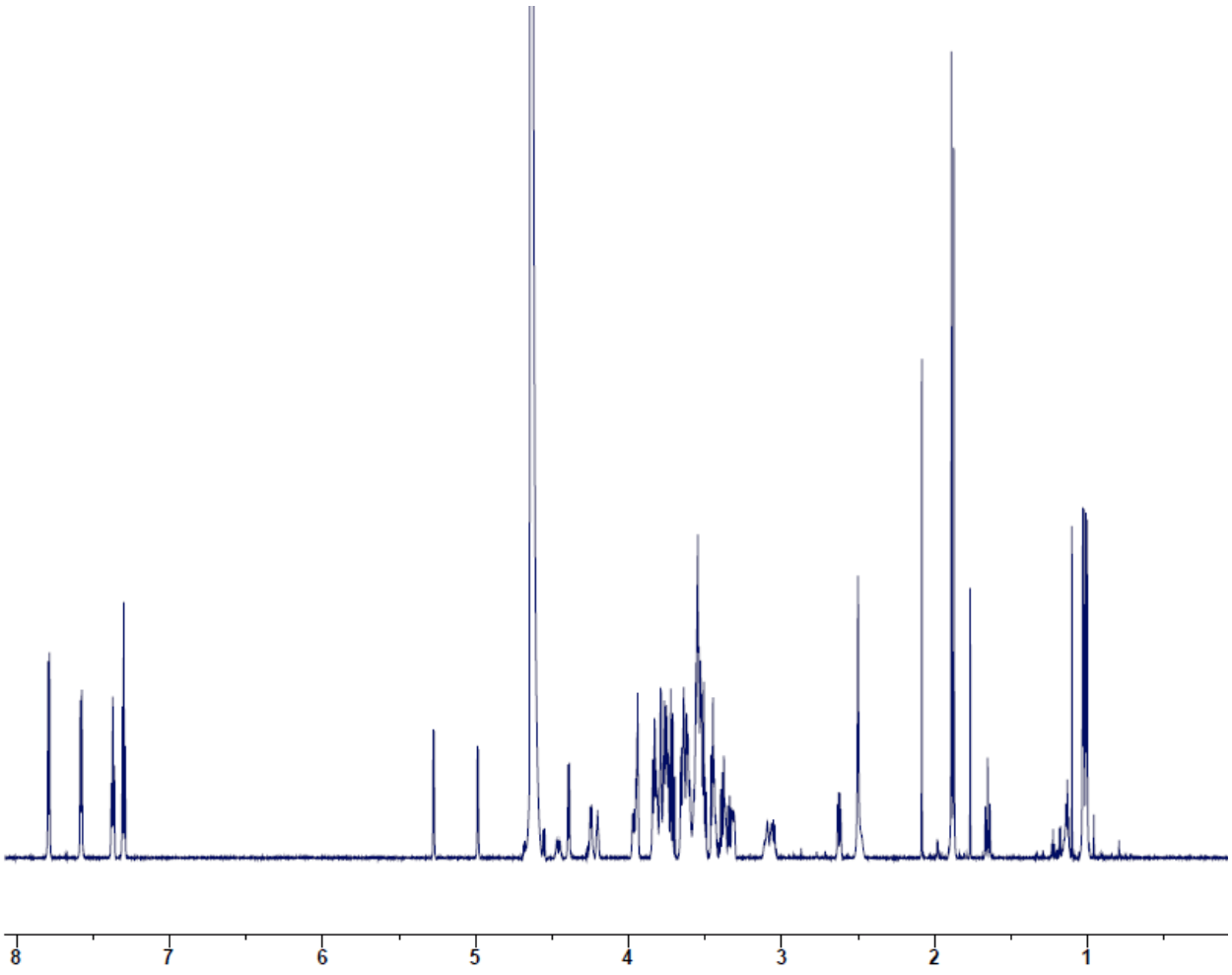

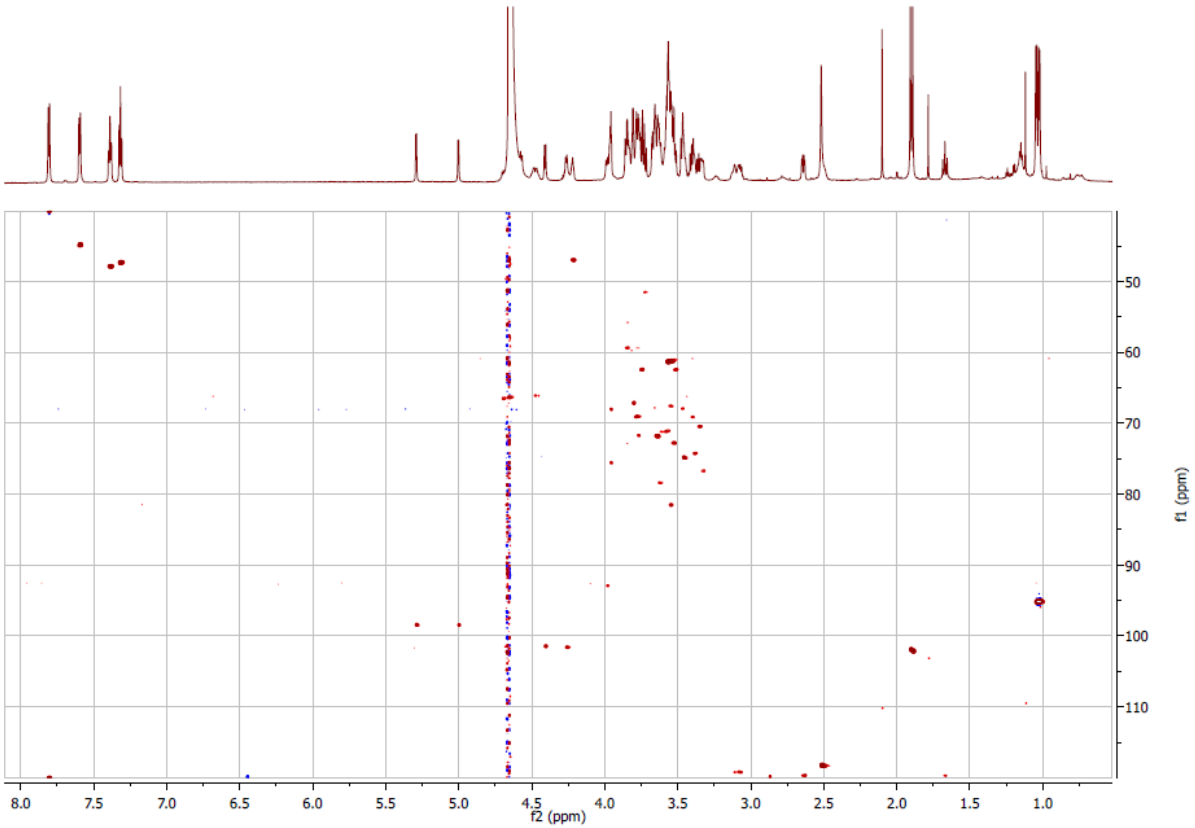
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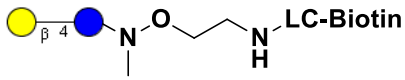
f1 (ppm)



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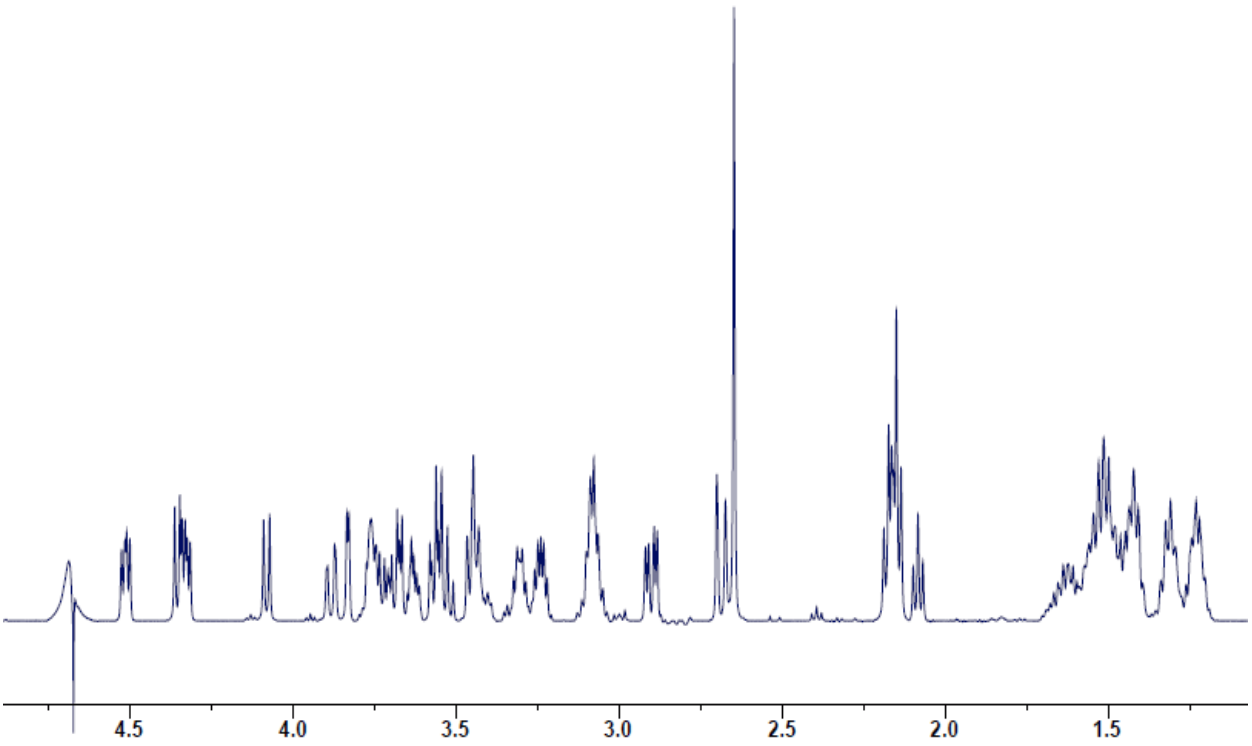

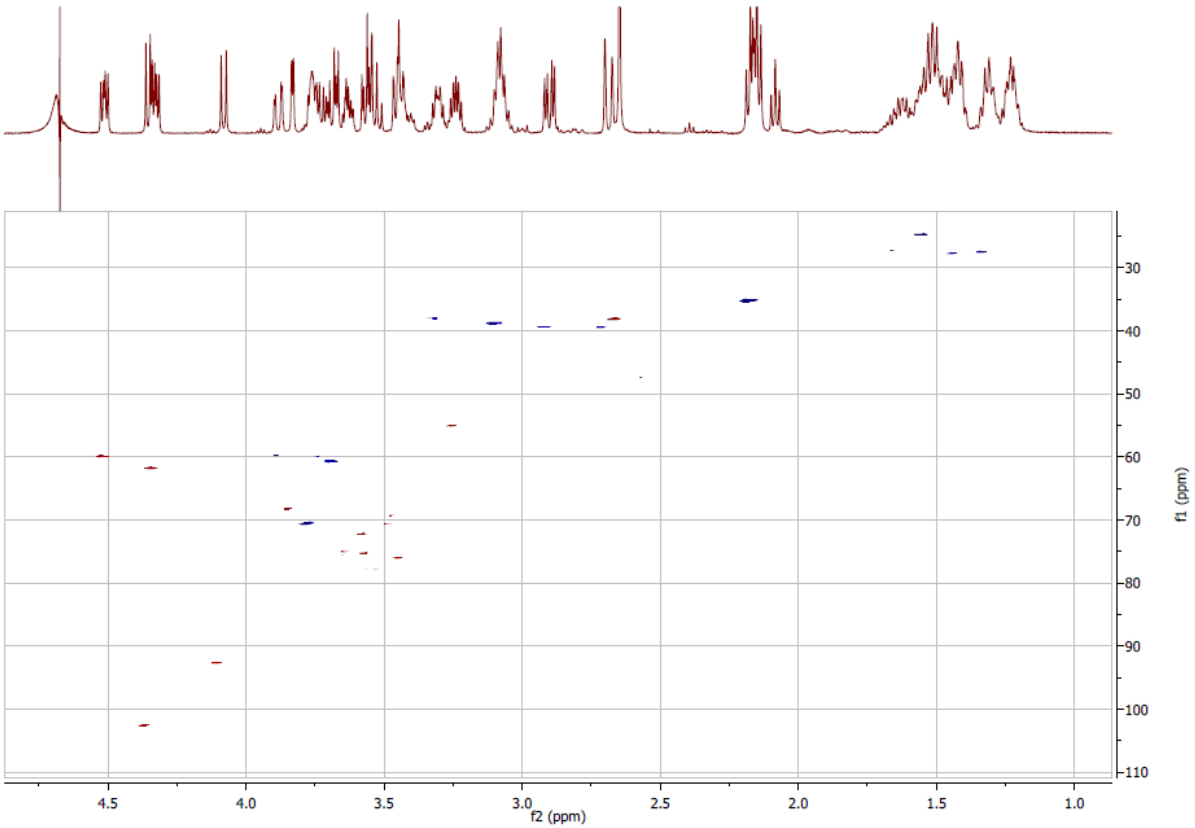
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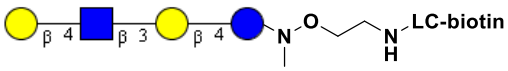
f2 (ppm)



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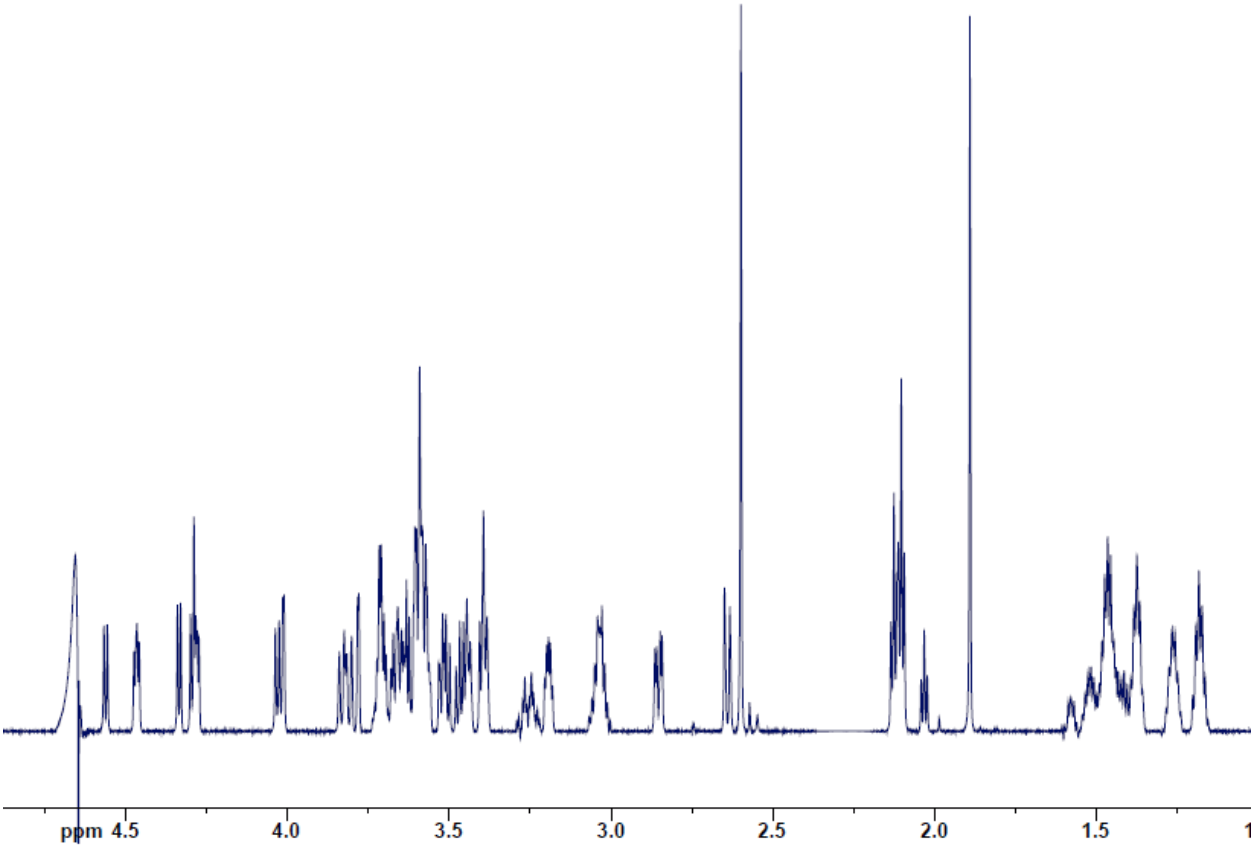

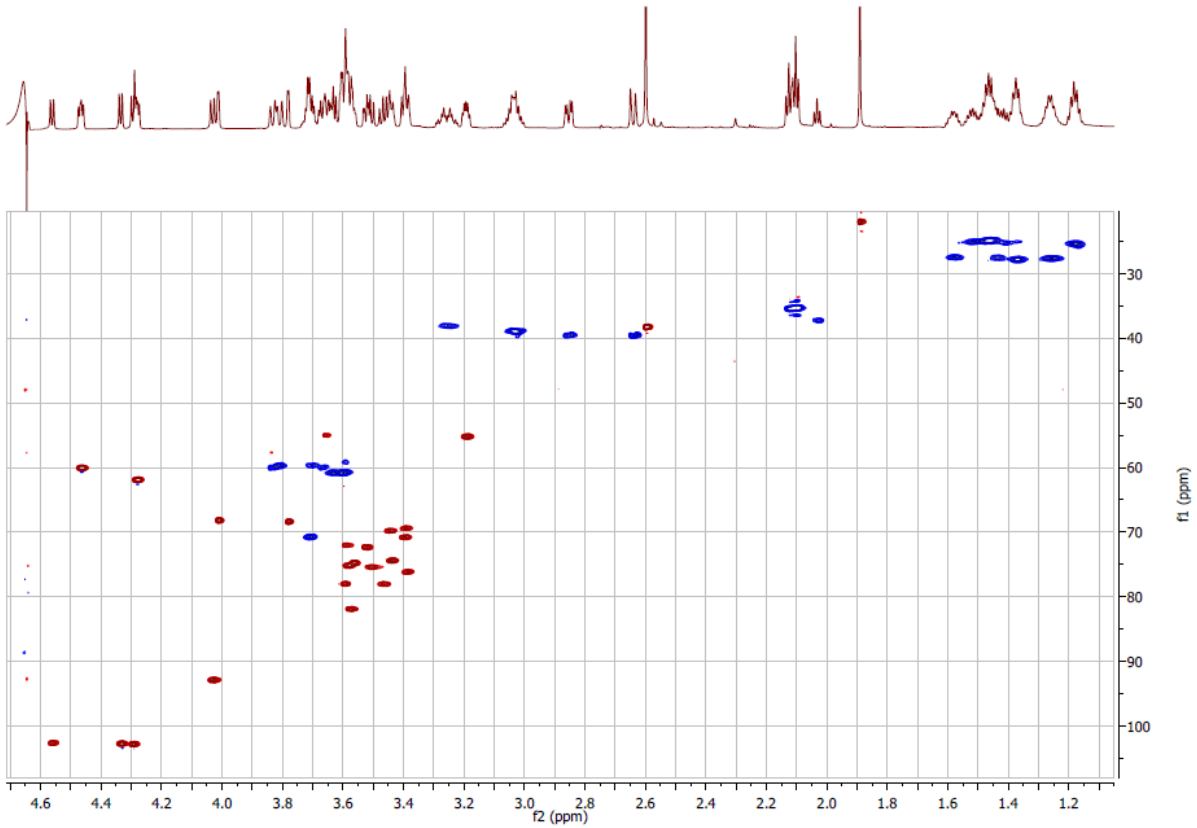
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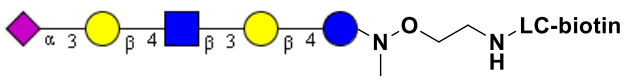
f2 (ppm)



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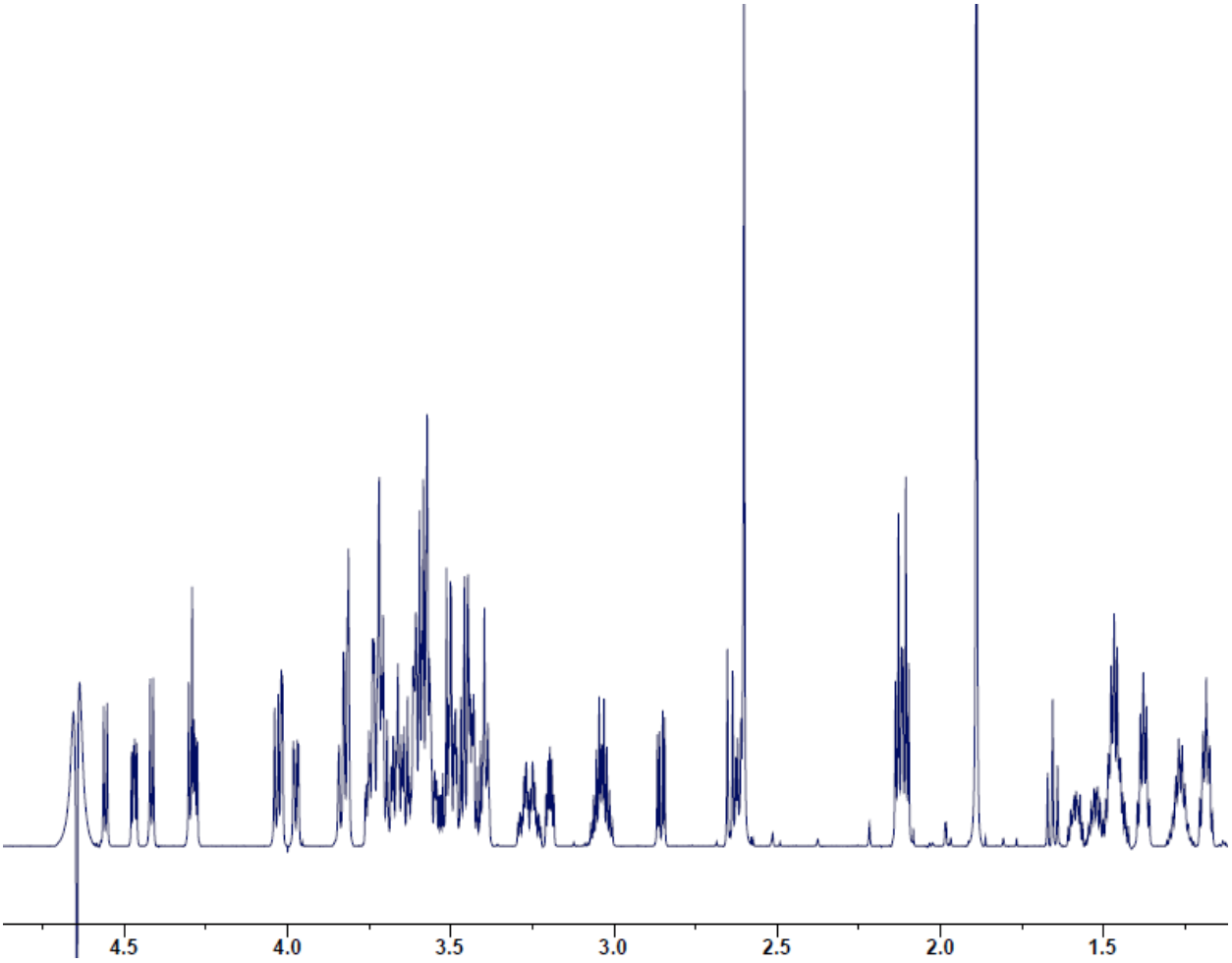

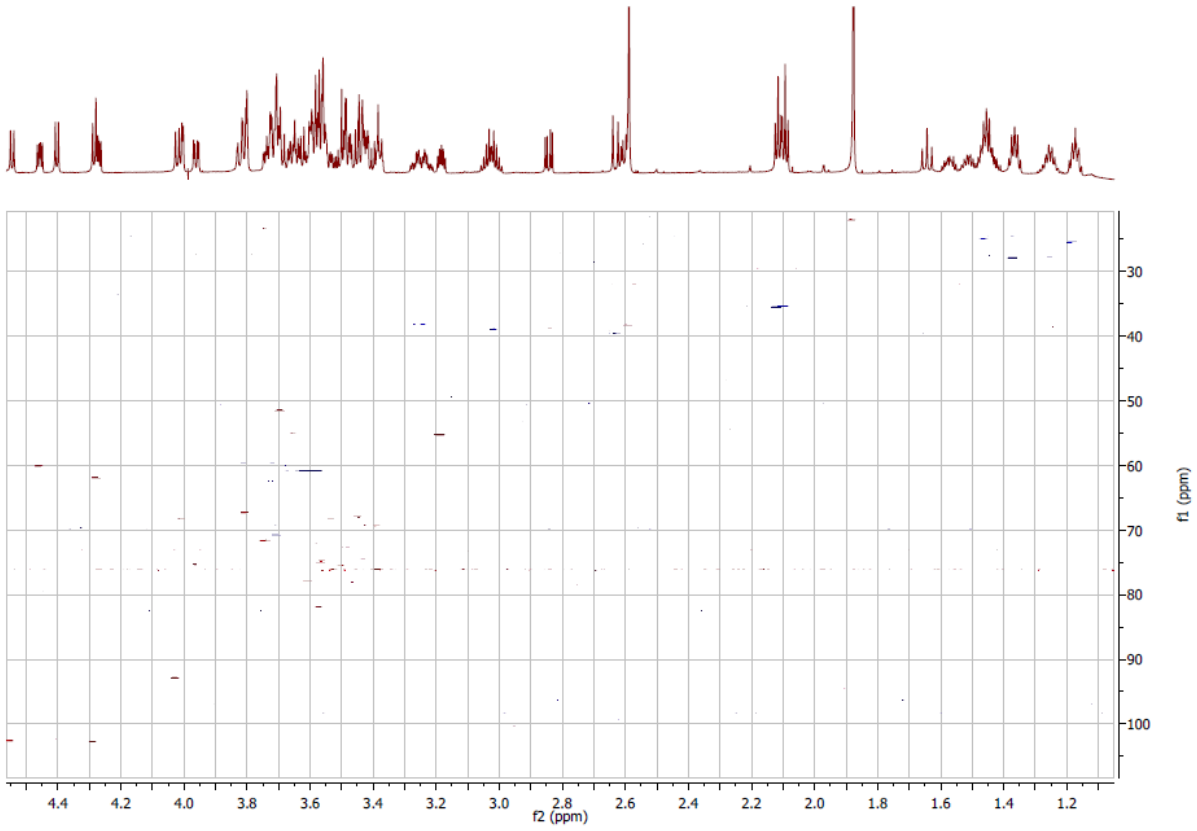
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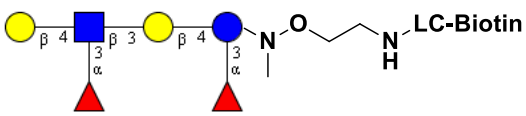
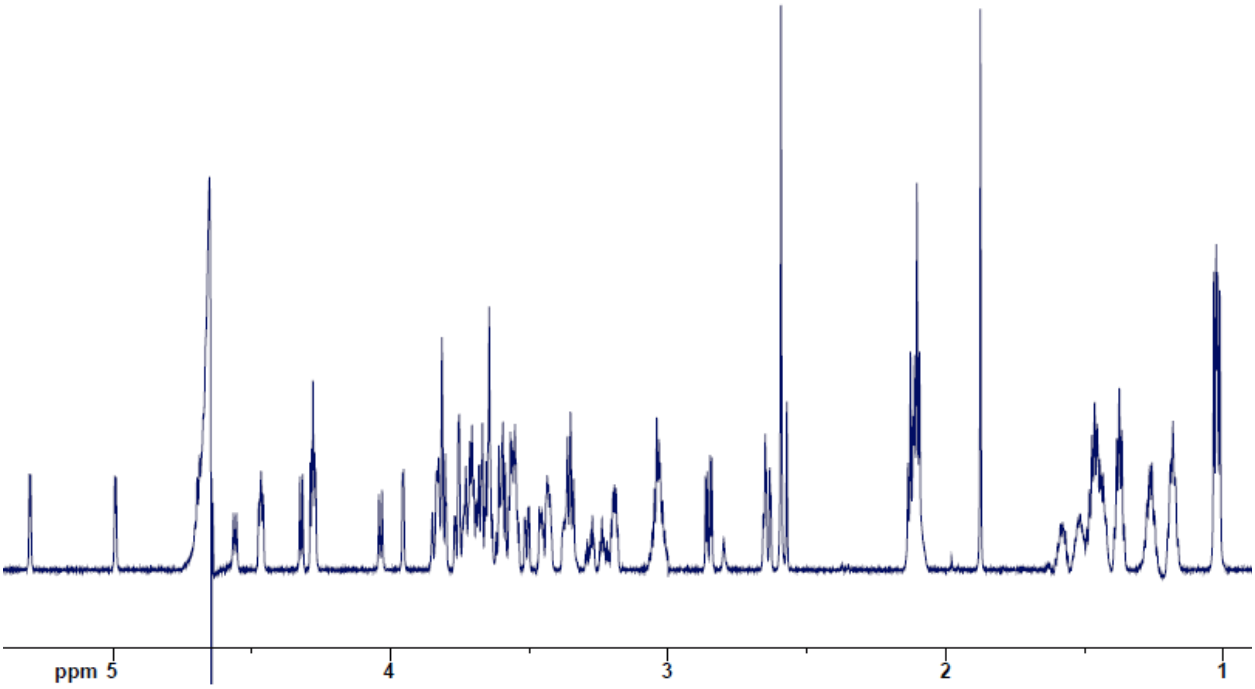
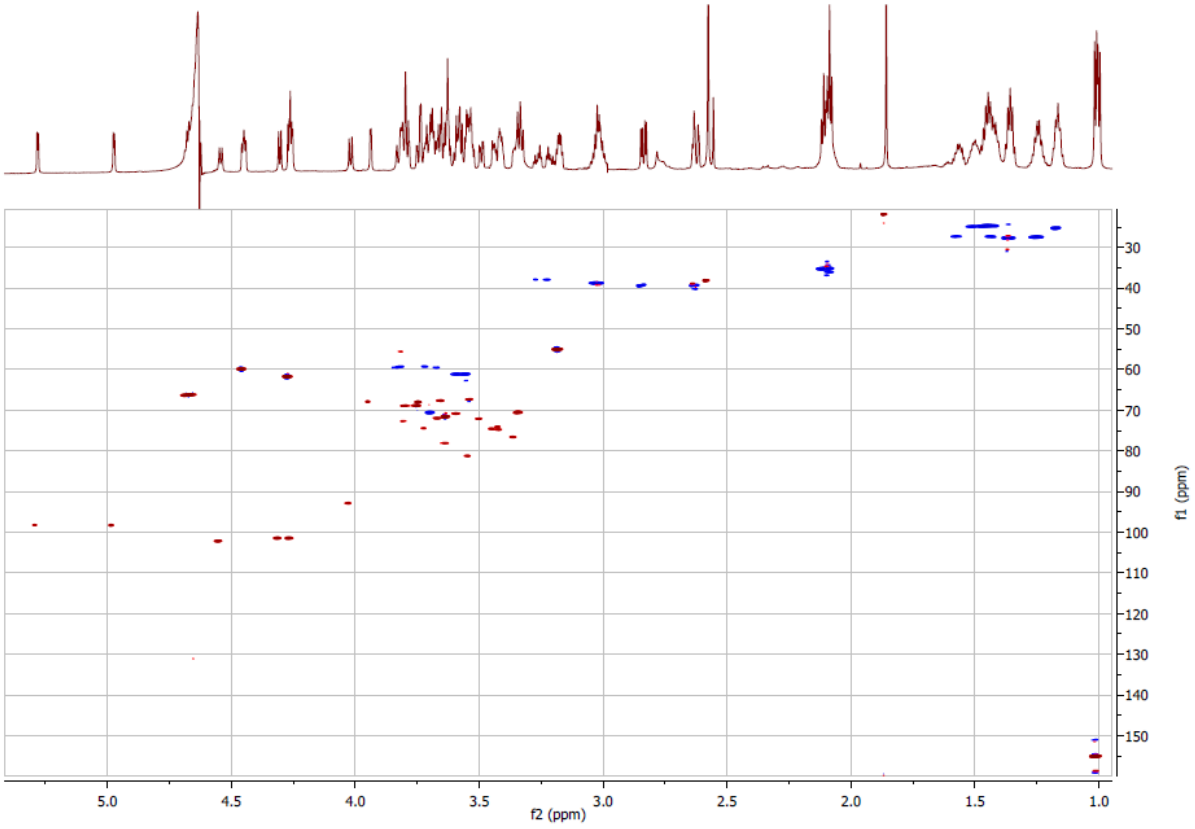
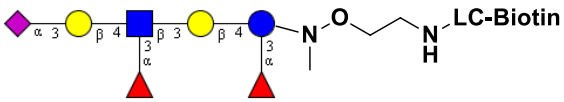
1.4
1.2
f2 (ppm)



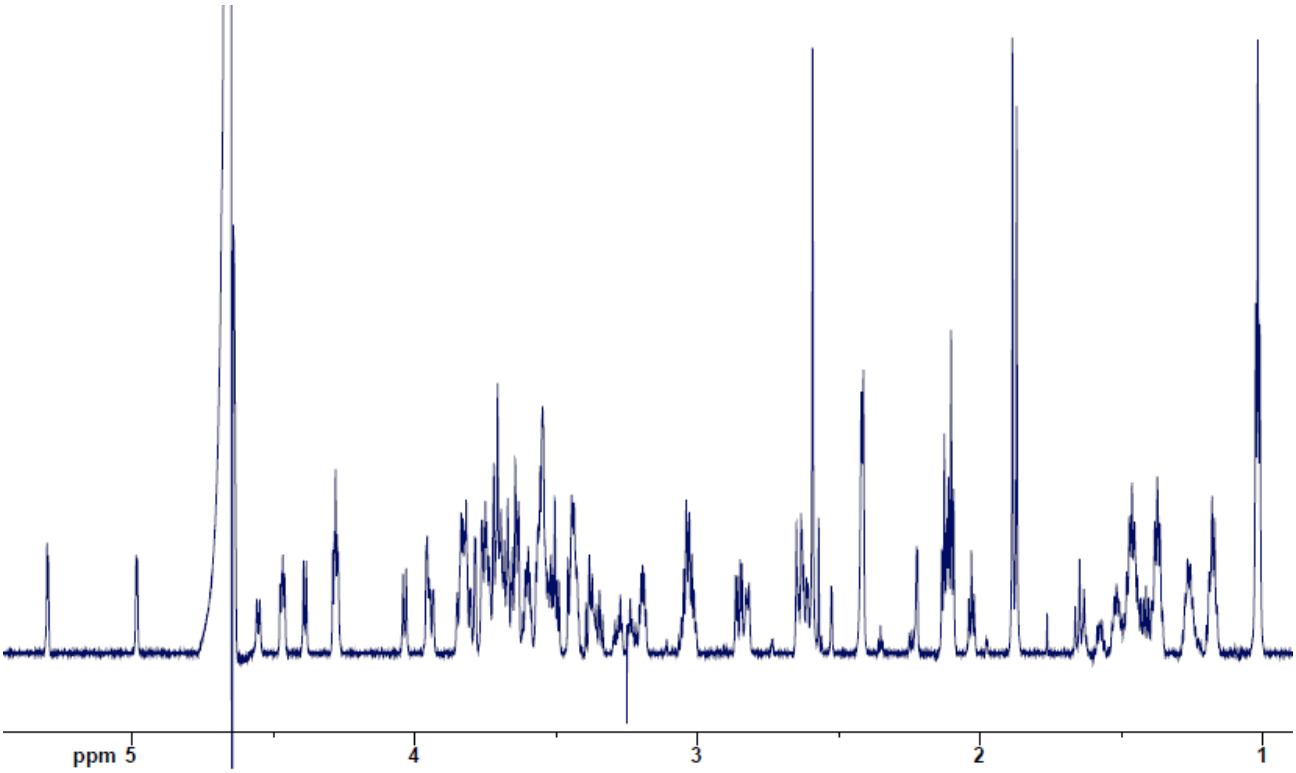
12

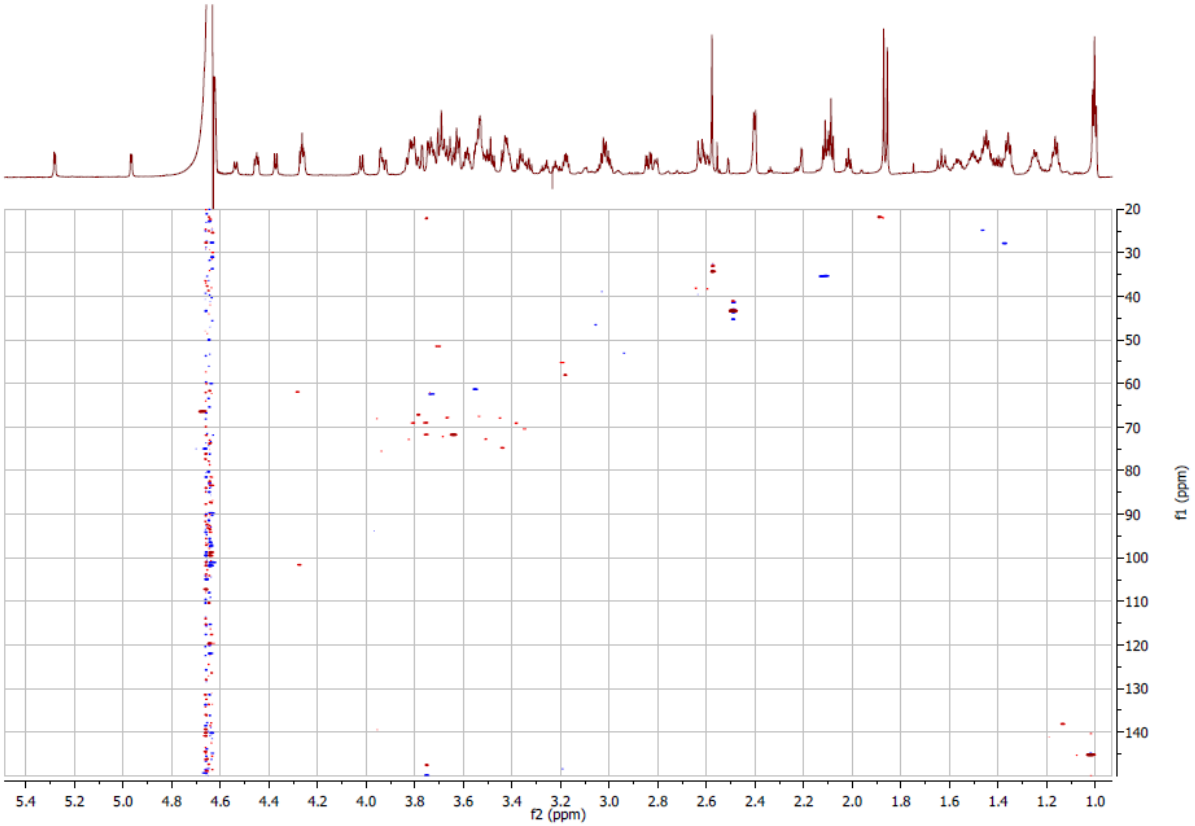


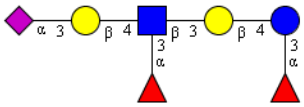

30
40
50
60
70
80
90
100
110
120
130
140
150
f1 (ppm)
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)



11




5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
f2 (ppm)
20
30
40
50
60
70
80
90
100
110
120
130
140
f1 (ppm)



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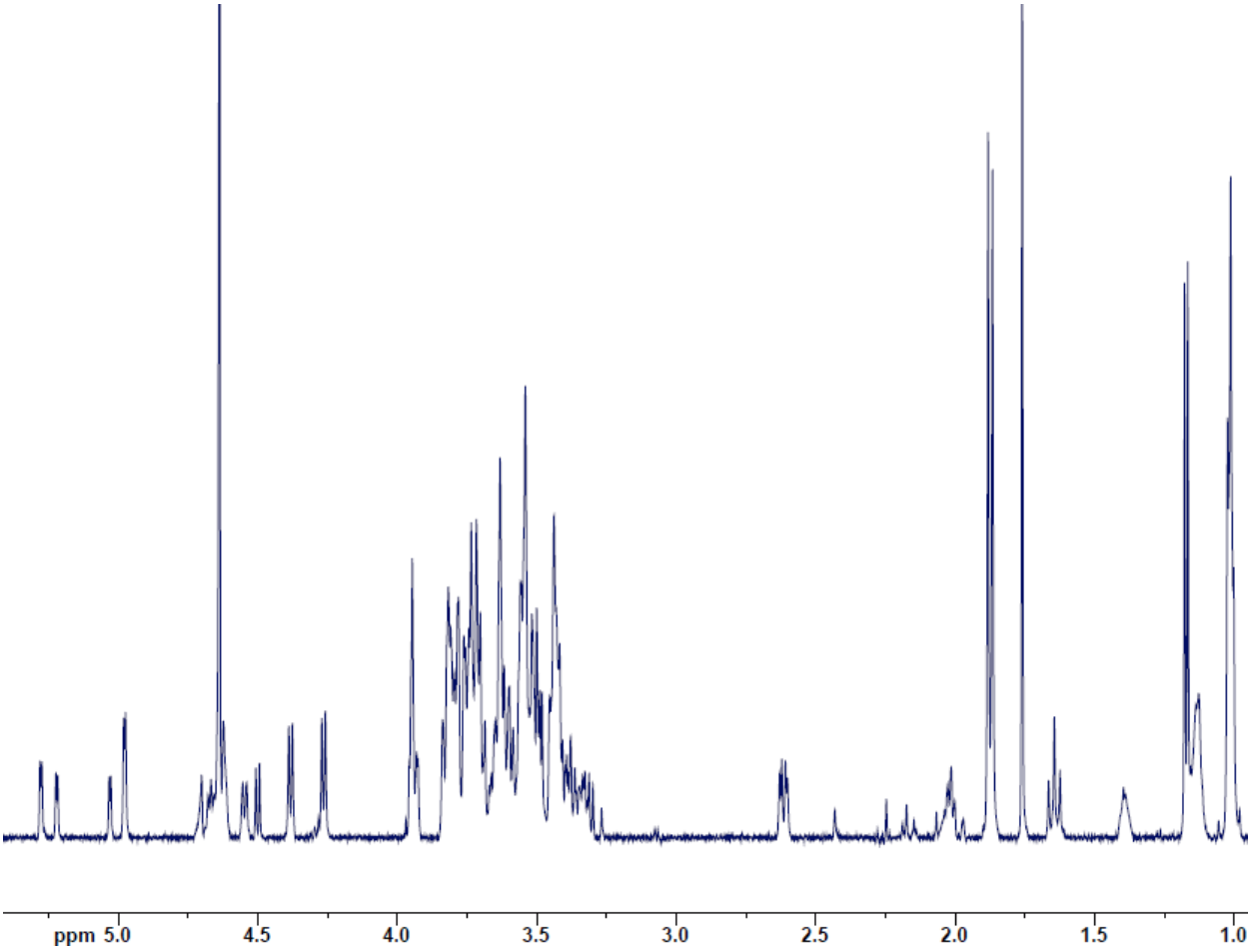
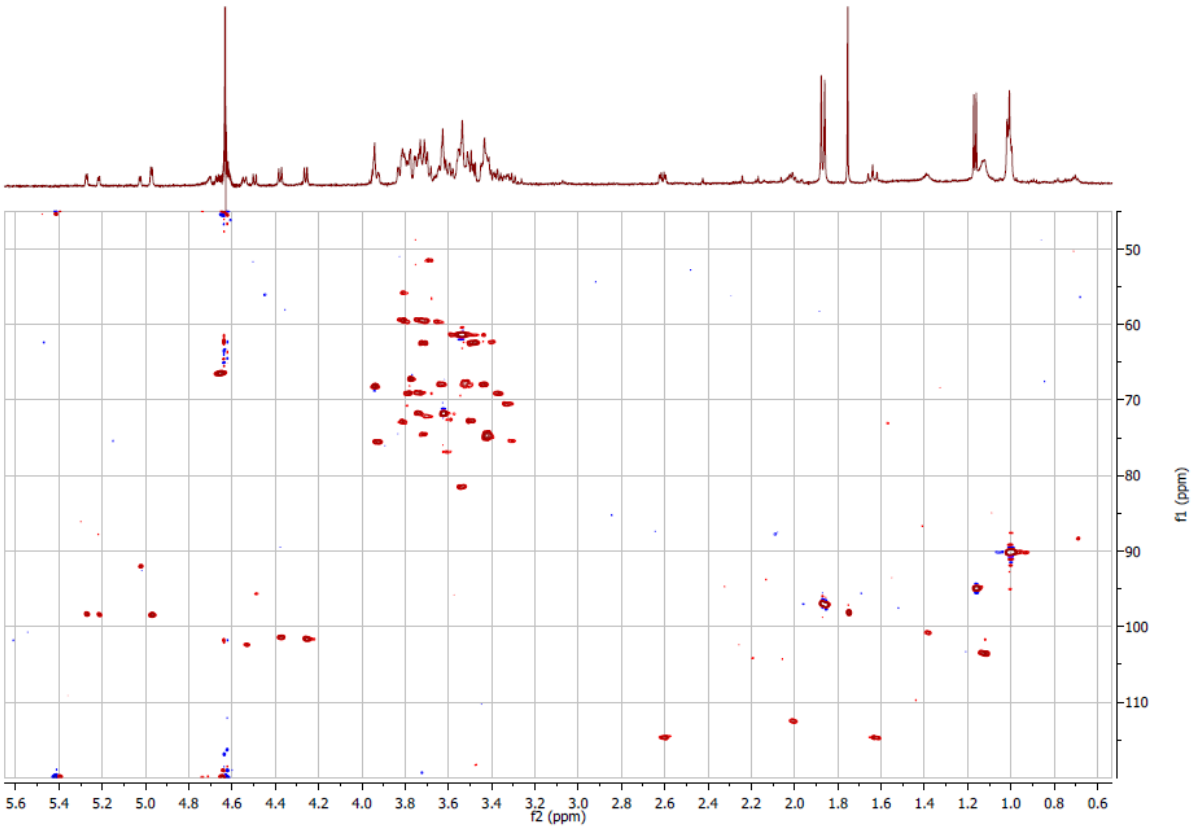
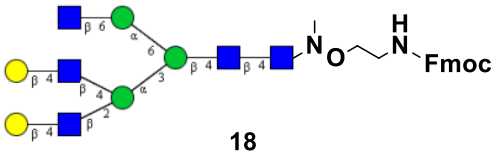



5.6
5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
f2 (ppm)
50
60
70
80
90
100
110
f1 (ppm)



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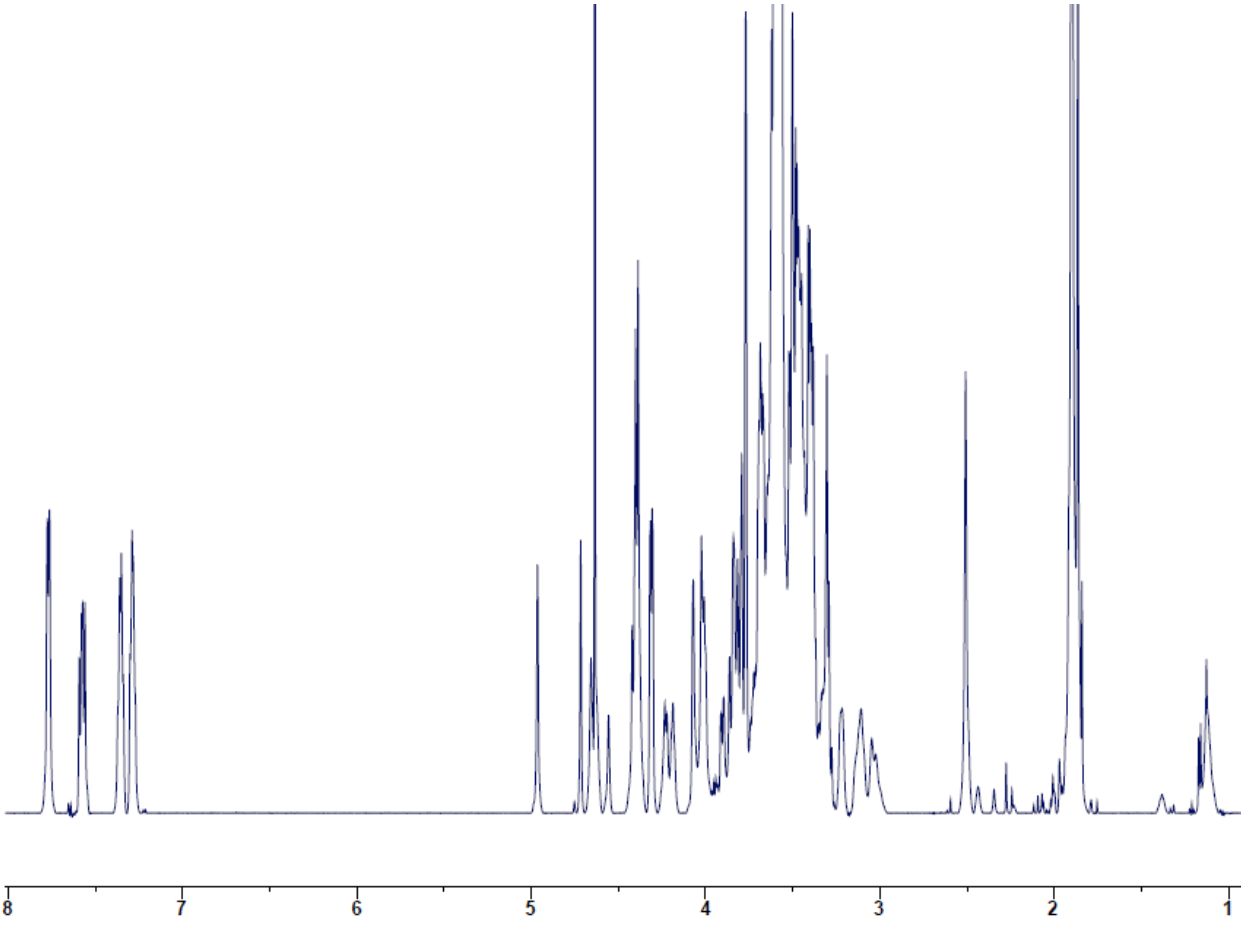

References

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