

## Supporting Information

for

### Highly efficient one-pot multienzyme (OPME) synthesis of glycans with fluororous-tag assisted purification

Joel Hwang,<sup>a</sup> Hai Yu,<sup>a</sup> Hamed Malekan,<sup>a</sup> Go Sugiarto,<sup>a</sup> Yanhong Li,<sup>a</sup> Jingyao Qu,<sup>ab</sup> Van Nguyen,<sup>a</sup> Dongyuan Wu,<sup>a</sup> and Xi Chen<sup>\*a</sup>

<sup>a</sup>Department of Chemistry, University of California, One Shields Avenue, Davis, California 95616, USA. E-mail: chen@chem.ucdavis.edu; Fax: +1 530-752-8995; Tel: +1 530-754-6037

<sup>b</sup>Current address: Department of Chemistry, Georgia State University, Atlanta, Georgia 30302, USA.

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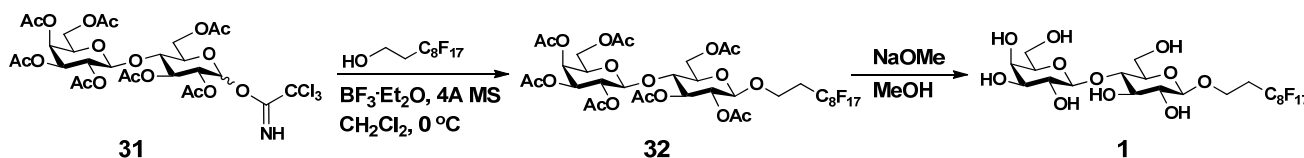
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## General methods

All chemicals were purchased from commercial suppliers and used without further purification. Anhydrous solvents were used to carry out organic reactions under inert argon or nitrogen environment.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Varian Inova-600 spectrometer or a Bruker Avance-800 spectrometer.  $^{19}\text{F}$  NMR spectra were recorded on a Varian Mercury-300 spectrometer. MALDI-TOF analysis of samples was carried out using an Applied Biosystems 4700 MALDI TOF/TOF with each reaction mixture (0.5  $\mu\text{L}$ ) diluted 100 fold using a solvent mixture ( $\text{H}_2\text{O}:\text{MeOH}:\text{TFA} = 50:50:0.1$ , by volume). The diluted reaction mixture (0.5  $\mu\text{L}$ ) was mixed with the same volume of 2,5-dihydroxybenzoic acid solution (10 mg/mL in 50% of acetonitrile in water) on a well spot of a stainless steel plate (ABI 01-192-6-AB). The glycans were analyzed in the positive ion reflector mode with a 355 nm (200 Hz) Nd:YAG laser. The instrument was calibrated with ABI peptide standards (4700 Mass standards kit, 4333604). Spectra were analyzed using the GPS Explorer software (v. 3.0) (Applied Biosystems). High resolution electrospray ionization (HR-ESI) mass spectra were obtained using Thermo Electron LTQ-Orbitrap Hybrid MS at the Mass Spectrometry Facility in the University of California, Davis. Silica gel 60  $\text{\AA}$  (200–425 mesh, Fisher Chemical) was used for flash column chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF254 (Sorbent technologies) using *p*-anisaldehyde sugar stain or 5% sulfuric acid in ethanol stain for detection.

## Synthesis of fluorous-tagged lactosides 1–8

### Synthesis of Lac $\beta$ C $_8$ F $_{17}$ (1)

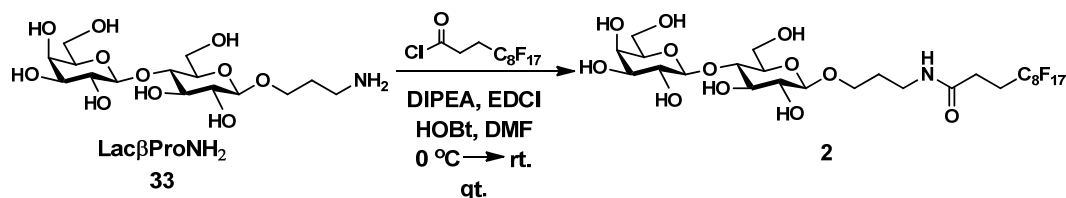


$\text{BF}_3\cdot\text{Et}_2\text{O}$  (25  $\mu\text{L}$ , 0.20 mmol) in  $\text{CH}_2\text{Cl}_2$  (2 mL) was added drop-wise to a solution of lactosyl trichloroacetimidate **31** (123 mg, 0.158 mmol), 1*H*,1*H*,2*H*,2*H*-perfluoro-1-decanol (46 mg, 0.1 mmol), activated 4  $\text{\AA}$  molecular sieves (400 mg), and  $\text{CH}_2\text{Cl}_2$  (5 mL) at 0  $^\circ\text{C}$ . The mixture was allowed to slowly warm up to room temperature and stirred for 20 h. The mixture was filtered over Celite, concentrated, and then purified via silica gel column chromatography ( $\text{EtOAc}:\text{Hexane} = 1:5$  to 1:1 by volume) to afford peracetylated lactoside (**32**) as a white solid (90 mg, 82%).  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  5.34 (dd, 1H,  $J = 1.2$  Hz and 3.6 Hz), 5.19 (t, 1H,  $J = 9.0$  Hz), 5.10 (dd, 1H,  $J = 7.8$  Hz and 10.2 Hz), 4.95 (dd, 1H,  $J = 3.0$  Hz and 10.2 Hz), 4.88 (dd, 1H,  $J = 8.4$  Hz and 9.6 Hz), 4.50–4.46 (m, 3H), 4.14–4.05 (m, 4H), 3.88–3.77 (m, 3H), 3.61 (m, 1H), 2.50–2.33 (m, 2H), 2.14 (s, 3H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (s, 6H), 2.01 (s, 3H), 1.96 (s, 3H).  $^{13}\text{C}$  (151 MHz,  $\text{CDCl}_3$ )  $\delta$  170.30, 170.27, 170.09, 170.02, 169.67, 169.56, 160.02, 105.00, 101.06, 100.60, 76.16, 72.75, 72.57, 71.31, 70.94, 70.69, 69.08, 66.56, 61.82, 60.76, 31.43, 20.75, 20.71, 20.61, 20.59 (2C), 20.48, 20.45.  $^{19}\text{F}$  NMR (282 MHz,  $\text{CDCl}_3$ )  $\delta = -81.18$  (s, 3F,  $\text{CF}_3$ ),  $-113.86$  (s, 2F,  $\text{CF}_2$ ),  $-122.36$  (m, 6F,  $3\text{CF}_2$ ),  $-123.17$  (s, 2F,  $\text{CF}_2$ ),  $-124.01$  (s, 2F,  $\text{CF}_2$ ),  $-126.56$  (s, 2F,  $\text{CF}_2$ ).

Sodium methoxide was added to a mixture of compound **32** (294 mg, 0.27 mmol) and MeOH (30 mL) until pH  $\sim 10$  under room temperature. After 2 h, the mixture was neutralized with DOWEX HCR-W2 ( $\text{H}^+$ ) resin. After filtration, the residue was concentrated and purified via FSPE to afford Lac $\beta$ C $_8$ F $_{17}$  (**1**) as a white solid (156 mg, 73%).  $^1\text{H}$  NMR (800 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  4.36 (d, 1H,  $J = 7.7$  Hz), 4.35 (d, 1H,  $J = 7.8$  Hz), 4.18 (dt, 1H,  $J = 7.0$  Hz and 10.3 Hz), 3.93–3.83 (m, 3H), 3.83–3.75 (m, 2H), 3.70 (dd, 1H,

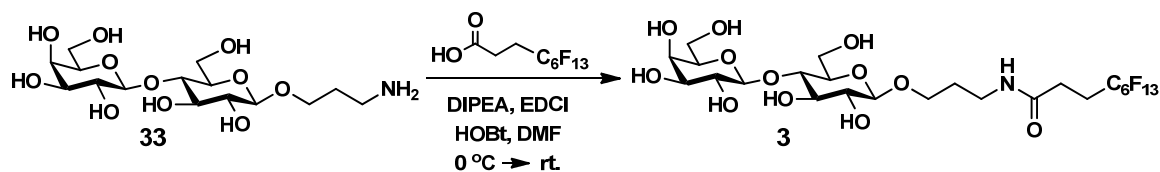
$J = 4.6$  Hz and  $11.5$  Hz),  $3.61$ – $3.51$  (m, 4H),  $3.48$  (dd, 1H,  $J = 3.3$  Hz and  $9.7$  Hz),  $3.44$  (m, 1H),  $3.26$  (dd, 1H,  $J = 7.9$  Hz and  $9.1$  Hz),  $2.63$ – $2.54$  (m, 2H).  $^{13}\text{C}$  NMR (201 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  105.10, 104.45, 80.46, 77.10, 76.56, 76.37, 74.82, 74.60, 72.56, 70.32, 70.30, 62.51, 61.82, 32.51 (t,  $J = 21.2$  Hz).  $^{19}\text{F}$  NMR (282 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  -82.79 (s, 3F,  $\text{CF}_3$ ), -114.90 (s, 2F,  $\text{CF}_2$ ), -123.33 (s, 2F,  $\text{CF}_2$ ), -124.17 (m, 6F,  $3\text{CF}_2$ ), -125.07 (s, 2F,  $\text{CF}_2$ ), -127.72 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$  calcd for  $[\text{C}_{22}\text{H}_{25}\text{F}_{17}\text{O}_{11}+\text{H}]^+$  789.1213, found 789.1198.

### Synthesis of Lac $\beta$ ProNH-C $_8$ F $_{17}$ (2)



To a solution of Lac $\beta$ ProNH $_2$  (**33**)<sup>1</sup> (71 mg, 0.18 mmol) and 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11-heptadecafluoroundecanoylchloride (117 mg, 0.23 mmol) in 7 mL anhydrous DMF, dry diisopropylethylamine (40  $\mu\text{L}$ ) was added under argon atmosphere at 0 °C. The mixture was allowed to slowly warm up to room temperature and stirred for 48 h. After monitoring the reaction with TLC, *N*-ethyl-*N*'-dimethylaminopropylcarbodiimide hydrochloride (34 mg, 0.18 mmol) and 1-hydroxybenzotriazole (24 mg, 0.18) was added to the reaction mixture under 0 °C and the mixture was stirred overnight under room temperature. The mixture was purified directly with an FSPE cartridge to afford Lac $\beta$ ProNH-C $_8$ F $_{17}$  (**2**) as a white solid (162 mg, qt.).  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  4.36 (d, 1H,  $J = 7.7$  Hz), 4.30 (d, 1H,  $J = 7.8$  Hz), 3.92 (m, 2H), 3.87–3.74 (m, 3H), 3.70 (dd, 1H,  $J = 11.4$  Hz, 4.5 Hz), 3.66–3.46 (m, 7H), 3.41 (m, 1H), 3.38–3.20 (m, 2H), 2.52 (m, 4H), 1.85–1.75 (m, 2H).  $^{13}\text{C}$  (151 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  105.11, 104.17, 80.63, 77.09, 76.48, 76.43, 74.83, 74.76, 72.55, 70.30, 68.31, 62.50, 61.89, 37.80, 30.28, 27.77, 27.51.  $^{19}\text{F}$  (282 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  -82.79 (s, 3F,  $\text{CF}_3$ ), -116.18 (s, 2F,  $\text{CF}_2$ ), -123.16 (s, 2F,  $\text{CF}_2$ ), -123.31 (s, 2F,  $\text{CF}_2$ ), -123.34 (s, 2F,  $\text{CF}_2$ ), -124.17 (s, 2F,  $\text{CF}_2$ ), -124.97 (s, 2F,  $\text{CF}_2$ ), -127.72 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$  calcd for  $[\text{C}_{26}\text{H}_{32}\text{F}_{17}\text{NO}_{12}+\text{H}]^+$  874.1726, found 874.1757.

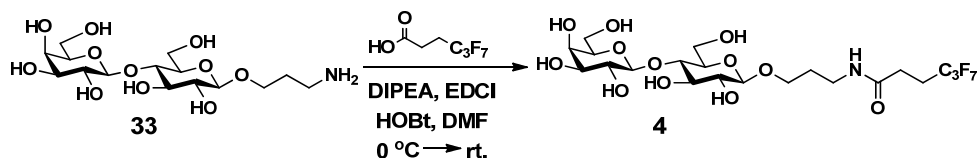
### Synthesis of Lac $\beta$ ProNH-C $_6$ F $_{13}$ (3)



To a solution of Lac $\beta$ ProNH $_2$  (**33**)<sup>1</sup> (28 mg, 0.07 mmol), 2*H*, 2*H*, 3*H*, 3*H*-perfluorounonanoic acid (30 mg, 0.08 mmol), *N*-ethyl-*N*'-dimethylaminopropylcarbodiimide hydrochloride (17 mg, 0.09 mmol), and 1-hydroxybenzotriazole (10 mg, 0.08 mmol) in 5 mL anhydrous DMF, dry diisopropylethylamine (16  $\mu\text{L}$ , 0.09 mmol) was added under argon atmosphere at 0 °C. The mixture was allowed to slowly warm up to room temperature and stirred overnight. The mixture was purified directly with an FSPE cartridge and then with flash column chromatography (EtOAc:MeOH:H $_2$ O = 9:2:1 by volume) to afford Lac $\beta$ ProNH-C $_6$ F $_{13}$  (**3**) as a white solid (21 mg, 59%).  $^1\text{H}$  NMR (600 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  4.36 (d, 1H,  $J = 7.7$  Hz), 4.30 (d, 1H,  $J = 7.8$  Hz), 3.92 (m, 2H), 3.88–3.74 (m, 3H), 3.70 (dd, 1H,  $J = 4.6$  Hz and 11.5 Hz), 3.66–3.45 (m, 7H), 3.41 (m, 1H), 3.38–3.22 (m, 2H), 2.61–2.43 (m, 4H), 1.86–1.76 (m, 2H).  $^{13}\text{C}$  (151 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  172.75, 105.11, 104.16, 80.64, 77.08, 76.47, 76.42, 74.82, 74.76, 72.55,

70.29, 68.31, 62.50, 61.89, 37.80, 30.28, 27.75, 27.52.  $^{19}\text{F}$  (282 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  -82.79 (s, 3F,  $\text{CF}_3$ ), -116.18 (s, 2F,  $\text{CF}_2$ ), -123.33 (s, 2F,  $\text{CF}_2$ ), -124.17 (s, 2F,  $\text{CF}_2$ ), -124.97 (s, 2F,  $\text{CF}_2$ ), -127.72 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$  calcd for  $[\text{C}_{24}\text{H}_{32}\text{F}_{13}\text{NO}_{12}+\text{H}]^+$  774.1790, found 774.1801.

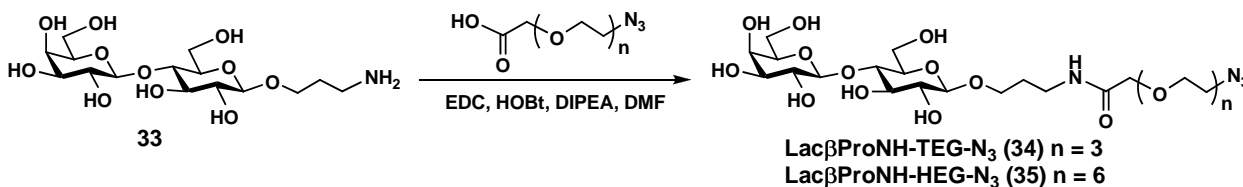
### Synthesis of Lac $\beta$ ProNH- $\text{C}_3\text{F}_7$ (**4**)



To a solution of Lac $\beta$ ProNH $_2$  (**33**)<sup>1</sup> (29 mg, 0.07 mmol), 2*H*, 2*H*, 3*H*, 3*H*-perfluorohexanoic acid (19 mg, 0.08 mmol), *N*-ethyl-*N'*-dimethylaminopropylcarbodiimide hydrochloride (18 mg, 0.09 mmol), and 1-hydroxybenzotriazole (11 mg, 0.08 mmol) in 5 ml anhydrous DMF, dry diisopropylethylamine (16  $\mu\text{L}$ , 0.09 mmol) was added under argon atmosphere at 0 °C. The mixture was allowed to slowly warm up to room temperature and stirred overnight. The mixture was purified directly with an FSPE cartridge and then with flash column chromatography (EtOAc:MeOH:H $_2$ O = 9:2:1 by volume) to produce Lac $\beta$ ProNH- $\text{C}_3\text{F}_7$  (**4**) as a white solid (21 mg, 48%).  $^1\text{H}$  NMR (800 MHz,  $\text{D}_2\text{O}$ )  $\delta$  4.49 (d, 1H,  $J$  = 8.0 Hz), 4.47 (d, 1H,  $J$  = 7.8 Hz), 4.03 – 3.93 (m, 3H), 3.85 – 3.71 (m, 5H), 3.71 – 3.64 (m, 3H), 3.61 (s, 1H), 3.59 – 3.54 (m, 2H), 3.38 – 3.29 (m, 3H), 1.86 (p, 2H,  $J$  = 6.5 Hz).  $^{13}\text{C}$  (201 MHz,  $\text{D}_2\text{O}$ )  $\delta$  173.72, 102.86, 102.00, 78.35, 75.28, 74.69, 74.30, 72.76, 72.45, 70.87, 68.47, 67.71, 60.93, 60.01, 36.28, 26.79.  $^{19}\text{F}$  (282 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  -82.59 (s, 3F,  $\text{CF}_3$ ), -117.17 (s, 2F,  $\text{CF}_2$ ), -129.53 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$  calcd for  $[\text{C}_{21}\text{H}_{32}\text{F}_7\text{NO}_{12}+\text{H}]^+$  624.1885, found 624.1894.

### General procedure for the synthesis of oligo(ethylene glycol)-tagged lactosides

Lac $\beta$ ProNH-TEG- $\text{N}_3$  (**34**) and Lac $\beta$ ProNH-HEG- $\text{N}_3$  (**35**):



To a solution of Lac $\beta$ ProNH $_2$  (**33**)<sup>1</sup> (1 eq.) in anhydrous DMF, an ethylene glycol linker (11-azido-3,6,9-trioxaundecanoic acid<sup>2</sup> or 20-azido-3,6,9,12,15,18-hexaoxaicosanoic acid<sup>3</sup>) (1.2 eq.) and *N*-hydroxybenzotriazole (HOBt, 2.0 eq.) were added. After being stirred for 30 mins, *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide hydrochloride (EDC, 2.0 eq.) and DIEPA (2.0 eq.) were added at 0 °C. The mixture was stirred at 0 °C for 30 min. and then at room temperature for 24 h. After removed the solvent *in vacuo*, the residue was purified by flash column chromatography on silica gel (EtOAc:MeOH:H $_2$ O = 2:1:0.05 by volume) to produce the corresponding ethylene glycol-linked lactosides, Lac $\beta$ ProNH-TEG- $\text{N}_3$  (**34**) and Lac $\beta$ ProNH-HEG- $\text{N}_3$  (**35**) as white solids.

Lac $\beta$ ProNH-TEG- $\text{N}_3$  (**34**): 1.17 g, 76%.  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  4.47 (d, 1H,  $J$  = 8.0 Hz), 4.44 (d, 1H,  $J$  = 7.8 Hz), 4.08 (s, 2H), 4.02–3.89 (m, 3H), 3.86–3.53 (m, 20H), 3.50 (t, 2H,  $J$  = 4.8 Hz), 3.37 (t, 2H,  $J$  = 6.8 Hz), 3.34–3.29 (m, 1H), 1.87 (p, 2H,  $J$  = 6.5 Hz).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 101 MHz):  $\delta$  172.56,

103.09, 102.23, 78.57, 75.50, 74.92, 74.51, 72.99, 72.67, 71.10, 70.46, 69.77, 69.69, 69.65 (2C), 69.39, 68.69, 67.93, 61.17, 60.25, 50.29, 36.10, 28.66. HRMS (ESI)  $m/z$   $[M+H]^+$  calcd for  $C_{23}H_{43}N_4O_{15}$  615.2725, found 615.2727;  $[M+Na]^+$   $C_{23}H_{42}N_4NaO_{15}$  637.2544, found 637.2539.

Lac $\beta$ ProNH-HEG-N<sub>3</sub> (**35**): 0.82 g, 73%. <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O):  $\delta$  4.49 (d, 1H,  $J = 7.8$  Hz), 4.46 (d, 1H,  $J = 8.4$  Hz), 4.09 (s, 2H), 3.98–3.93 (m, 3H), 3.83–3.51 (m, 34H), 3.38 (t, 2H,  $J = 6.6$  Hz), 3.33 (t, 1H,  $J = 8.4$  Hz), 1.90–1.86 (m, 2H). <sup>13</sup>C NMR (D<sub>2</sub>O, 151 MHz):  $\delta$  172.35, 102.88, 102.02, 78.38, 75.30, 74.72, 74.31, 72.79, 72.47, 70.89, 70.24, 69.55, 69.53, 69.50, 69.48, 69.46, 69.16, 68.48, 68.05, 67.73, 60.95, 60.05, 50.09, 35.90, 28.47. HRMS (ESI)  $m/z$   $[M+H]^+$  calcd for  $C_{29}H_{55}N_4O_{18}$  747.3511, found 747.3518;  $[M+Na]^+$   $C_{29}H_{54}N_4NaO_{18}$  769.3331, found 769.3329.

### General procedure for the synthesis of fluoros-tagged lactosides 5–8:

Lac $\beta$ ProNH-TEG-N<sub>3</sub> (**34**) or Lac $\beta$ ProNH-HEG-N<sub>3</sub> (**35**) (50–100 mg) was dissolved in 10 mL of H<sub>2</sub>O/MeOH (1:1) and 50 mg Pd/C was added. The mixture was shaken under H<sub>2</sub> (4 Bar) for 2 h and filtered. The filtrate was evaporated to dryness to afford the corresponding amine product and used directly for the next coupling reaction. To a solution of corresponding amino-containing lactosides (TEG or HEG linker) (1.2 eq.) and HOBt (2.0 eq.) in 10 mL of dry DMF, EDC (2.0 eq.) and DIPEA (2.0 eq.) were added at 0 °C. The mixture was stirred at 0 °C for 30 min and then at room temperature for overnight. The solvent was then removed *in vacuo* and the crude product was purified by flash column chromatography on silica gel (EtOAc:MeOH = 2:1 by volume) to produce the corresponding fluoros-tagged oligosaccharides **5–8**.

Lac $\beta$ ProNH-TEG-C<sub>8</sub>F<sub>17</sub> (**5**): 0.22 g, 68%. <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O):  $\delta$  4.44 (d, 1H,  $J = 7.2$  Hz), 4.43 (d, 1H,  $J = 6.7$  Hz), 4.01 (s, 2H), 3.98–3.86 (m, 3H), 3.86–3.46 (m, 20H), 3.45–3.24 (m, 5H), 2.63–2.28 (m, 4H), 1.90–1.76 (m, 2H). <sup>13</sup>C NMR (D<sub>2</sub>O, 201 MHz):  $\delta$  172.26, 171.83, 102.85, 102.09, 78.22, 75.21, 74.66, 74.26, 72.77, 72.46, 70.83, 70.06, 69.55, 69.47, 69.41, 69.38, 68.86, 68.47, 67.49, 60.89, 59.95, 38.93, 38.85, 35.80, 28.62, 26.18. <sup>19</sup>F NMR (D<sub>2</sub>O, 282 MHz):  $\delta$  -83.81 (s, 3F, CF<sub>3</sub>), -116.28 (s, 2F, CF<sub>2</sub>), -123.33 to -124.89 (m, 10F, 5CF<sub>2</sub>), -128.48 (s, 2F, CF<sub>2</sub>). HRMS (ESI)  $m/z$   $[M+H]^+$  calcd for  $C_{34}H_{48}F_{17}N_2O_{16}$  1063.2732, found 1063.2744;  $[M+Na]^+$   $C_{34}H_{47}F_{17}N_2NaO_{16}$  1085.2552, found 1085.2543.

Lac $\beta$ ProNH-HEG-C<sub>8</sub>F<sub>17</sub> (**6**): 0.091 g, 67%. <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O):  $\delta$  4.48 (d, 1H,  $J = 8.6$  Hz), 4.47 (d, 1H,  $J = 8.5$  Hz), 4.08 (s, 2H), 4.02–3.93 (m, 3H), 3.86–3.55 (m, 34H), 3.45–3.32 (m, 3H), 2.59–2.38 (m, 4H), 1.89 (p, 2H,  $J = 6.4$  Hz). <sup>13</sup>C NMR (D<sub>2</sub>O, 201 MHz):  $\delta$  172.03, 171.82, 102.92, 102.09, 78.45, 75.30, 74.72, 74.34, 72.81, 72.51, 70.89, 70.21, 69.65, 69.61, 69.57, 69.55, 69.51, 68.89, 68.49, 67.61, 60.96, 60.09, 38.98, 35.89, 28.64, 26.12. <sup>19</sup>F NMR (D<sub>2</sub>O, 282 MHz):  $\delta$  -83.77 (s, 3F, CF<sub>3</sub>), -116.29 (s, 2F, CF<sub>2</sub>), -123.24 to -124.80 (m, 10F, 5CF<sub>2</sub>), -128.44 (s, 2F, CF<sub>2</sub>). HRMS (ESI)  $m/z$   $[M+Na]^+$  calcd for  $C_{40}H_{59}F_{17}N_2NaO_{19}$  1217.3338, found 1217.3301.

Lac $\beta$ ProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**7**): 0.22 g, 70%. <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O):  $\delta$  4.43 (d, 2H,  $J = 7.2$  Hz), 4.01 (s, 2H), 3.98–3.88 (m, 3H), 3.83–3.51 (m, 20H), 3.42–3.27 (m, 5H), 2.58–2.35 (m, 4H), 1.89–1.78 (m, 2H). <sup>13</sup>C NMR (D<sub>2</sub>O, 201 MHz):  $\delta$  172.27, 171.89, 102.85, 102.07, 78.24, 75.22, 74.66, 74.27, 72.76, 72.45, 70.83, 70.07, 69.54, 69.47, 69.43, 69.36, 68.84, 68.45, 67.51, 60.89, 59.95, 38.91, 38.87, 35.79,



28.57, 26.17.  $^{19}\text{F}$  NMR ( $\text{D}_2\text{O}$ , 282 MHz):  $\delta$  -83.50 (s, 3F,  $\text{CF}_3$ ), -116.25 (s, 2F,  $\text{CF}_2$ ), -123.51 (s, 2F,  $\text{CF}_2$ ), -124.61 (s, 2F,  $\text{CF}_2$ ), -125.00 (s, 2F,  $\text{CF}_2$ ), -128.28 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$   $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{32}\text{H}_{48}\text{F}_{13}\text{N}_2\text{O}_{16}$  963.2796, found 963.2809;  $[\text{M}+\text{Na}]^+$   $\text{C}_{32}\text{H}_{47}\text{F}_{13}\text{N}_2\text{NaO}_{16}$  985.2616, found 985.2602.

Lac $\beta$ ProNH-HEG- $\text{C}_6\text{F}_{13}$  (**8**): 0.100 g, 66%.  $^1\text{H}$  NMR (600 MHz,  $\text{D}_2\text{O}$ ):  $\delta$  4.47 (d, 1H,  $J = 7.5$  Hz), 4.46 (d, 1H,  $J = 7.5$  Hz), 4.06 (s, 2H), 4.02–3.91 (m, 3H), 3.88–3.48 (m, 34H), 3.45–3.28 (m, 3H), 2.59 – 2.34 (m, 4H), 1.87 (p, 2H,  $J = 6.6$  Hz).  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ , 151 MHz):  $\delta$  172.24, 172.14, 103.09, 102.26, 78.61, 75.48, 74.90, 74.51, 72.97, 72.67, 71.07, 70.38, 69.80, 69.77, 69.74, 69.71, 69.65, 69.37, 69.33, 69.03, 68.66, 67.88, 67.79, 61.14, 60.25, 39.17, 36.06, 28.79, 26.31.  $^{19}\text{F}$  NMR ( $\text{D}_2\text{O}$ , 282 MHz):  $\delta$  -83.42 (s, 3F,  $\text{CF}_3$ ), -116.13 (s, 2F,  $\text{CF}_2$ ), -123.45 (s, 2F,  $\text{CF}_2$ ), -124.54 (s, 2F,  $\text{CF}_2$ ), -124.86 (s, 2F,  $\text{CF}_2$ ), -128.19 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$   $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{38}\text{H}_{60}\text{F}_{13}\text{N}_2\text{O}_{19}$  1095.3583, found 1095.3550;  $[\text{M}+\text{Na}]^+$   $\text{C}_{38}\text{H}_{59}\text{F}_{13}\text{N}_2\text{NaO}_{19}$  1117.3402, found 1117.3374.

### Small scale one-pot multienzyme (OPME) glycosylation reactions

#### $\alpha 2$ -3-Sialylation

PmST1 E271F/R313Y mutant (0.07  $\mu\text{g}$ ) was added to a 0.5 mL centrifuge tube containing 0.01  $\mu\text{mol}$  of a lactoside acceptor (chosen from **1**–**8**), Neu5Ac (1.2 eq.), CTP (2 eq.), Tris-HCl buffer (100 mM, pH 8.5),  $\text{MgCl}_2$  (20 mM), NmCSS (0.08  $\mu\text{g}$ ), and water (total volume = 10  $\mu\text{L}$ ). The reactions were monitored by TLC (EtOAc:MeOH:H<sub>2</sub>O:AcOH = 5:2:1:0.1).

#### $\alpha 2$ -6-Sialylation

Pd2,6ST (0.72  $\mu\text{g}$ ) was added to a 0.5 mL centrifuge tube containing 0.01  $\mu\text{mol}$  (1 eq.) of a lactoside acceptor (chosen from **1**–**8**), Neu5Ac (1.2 eq.), CTP (2 eq.), Tris-HCl buffer (100 mM, pH 8.5),  $\text{MgCl}_2$  (20 mM), NmCSS (0.08  $\mu\text{g}$ ), and water (total volume = 10  $\mu\text{L}$ ). The reactions were monitored by TLC (EtOAc:MeOH:H<sub>2</sub>O:AcOH = 5:2:1:0.1).

#### $\alpha 1$ -3-Galactosylation

$\alpha 1$ -3GalT (0.4  $\mu\text{g}$ ) was added to a 0.5 mL centrifuge tube containing 0.01  $\mu\text{mol}$  (1 eq.) of a lactoside acceptor (chosen from **1**–**8**), galactose (1 eq.), ATP (2 eq.), UTP (2 eq.),  $\text{MgCl}_2$  (20 mM),  $\text{MnCl}_2$  (20 mM), Tris-HCl buffer (100 mM, pH = 7.0), *E. coli* GalK (1.75  $\mu\text{g}$ ), BLUSP (4.3  $\mu\text{g}$ ), PmPpA (5.0  $\mu\text{g}$ ), and water (total volume = 10  $\mu\text{L}$ ). The reactions were monitored by TLC (EtOAc:MeOH:H<sub>2</sub>O = 5:2:1).

### TLC-ImageQuant verification of reaction conversion rates

Reaction conversion rates were determined by staining the TLC plates with *p*-anisaldehyde sugar stain and then using ImageQuant 5.2 software to compare the relative intensities between the glycosylation product spot and the lactoside acceptor spot of each reaction (Figure S1). For external standard comparison, each lactoside acceptor spot was also compared with its corresponding standard 10 mM stock solution spot to verify the conversion rates of each reaction.

## Preparative scale OPME synthesis of fluorinated glycosylated glycans **11**, **14**, **23**, **25**, **27**, and **29**

### Neu5Ac $\alpha$ 2-3Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub> (**11**)

Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub> (**3**) (10 mg, 1 eq.), Neu5Ac (5 mg, 1.2 eq.), and CTP (15 mg, 2 eq.) were dissolved in water in a 1 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and MgCl<sub>2</sub> (20 mM). After the addition of PmST1\_E271F/R313Y (0.033 mg) and NmCSS (0.2 mg), millipore water was added to bring the total volume of the reaction mixture to 1 mL. The reaction was gently shaken in an isotherm incubator for 2 h at 37 °C. After monitoring the reaction with TLC (EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume), 0.5 eq. of additional CTP was added to the mixture. After another hour, 0.5 eq. of CTP was added again to drive the reaction towards completion. One hour later, the reaction underwent centrifugation to remove the precipitants. The supernatant was purified directly via an FSPE cartridge and concentrated to give **10** as a white solid (10 mg, 72%). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  4.53 (d, 1H,  $J$  = 7.8 Hz), 4.45 (d, 1H,  $J$  = 7.9 Hz), 4.11 (d, 1H,  $J$  = 8.0 Hz), 3.96 (m, 3H), 3.92–3.80 (m, 4H), 3.78–3.51 (m, 12H), 3.31 (d, 3H,  $J$  = 7.8 Hz), 2.76 (dd, 1H,  $J$  = 12.1 Hz, 4.1 Hz), 2.61–2.41 (m, 4H), 2.03 (s, 3H), 1.89–1.74 (m, 3H). <sup>13</sup>C (201 MHz, CD<sub>3</sub>OD)  $\delta$  174.05, 173.54, 171.31, 103.55, 102.66, 99.62, 79.34, 76.09, 75.55, 74.96, 74.78, 73.43, 73.26, 71.50, 69.33, 68.54, 67.83, 67.47, 66.78, 62.98, 61.24, 61.07, 60.39, 52.45, 40.59, 36.25, 28.80, 26.19 (dd,  $J$  = 20.9 Hz and 42.7 Hz), 21.15. <sup>19</sup>F (282 MHz, D<sub>2</sub>O)  $\delta$  -82.39 (s, 3F, CF<sub>3</sub>), -115.64 (s, 2F, CF<sub>2</sub>), -123.02 (s, 2F, CF<sub>2</sub>), -124.04 (s, 2F, CF<sub>2</sub>), -124.70 (s, 2F, CF<sub>2</sub>), -127.42 (s, 2F, CF<sub>2</sub>). HRMS (ESI)  $m/z$  calcd for [C<sub>35</sub>H<sub>48</sub>F<sub>13</sub>N<sub>2</sub>O<sub>20</sub>] 1063.2598, found 1063.2588.

### Neu5Ac $\alpha$ 2-6Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub> (**14**)

Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub> (**3**) (10 mg, 1 eq.), Neu5Ac (5 mg, 1.2 eq.), and CTP (15 mg, 2 eq.) were dissolved in water in a 1 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and MgCl<sub>2</sub> (20 mM). After the addition of Pd2,6ST (0.09 mg) and NmCSS (0.2 mg), millipore water was added to bring the total volume of the reaction mixture to 1 mL. The reaction was gently shaken in an isotherm incubator for 2 h at 37 °C. After monitoring the reaction with TLC (EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume), 0.5 eq. of additional CTP was added to the mixture. After another hour, 0.5 eq. of CTP was added again to drive the reaction towards completion. One hour later, the reaction underwent centrifugation to remove the precipitants. The supernatant was purified directly via an FSPE cartridge and concentrated to produce **14** as a white solid (10 mg, 79%). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O)  $\delta$  4.47 (d, 1H,  $J$  = 7.7 Hz), 4.44 (d, 1H,  $J$  = 7.5 Hz), 4.06–3.93 (m, 4H), 3.93–3.78 (m, 6H), 3.77–3.54 (m, 10H), 3.39–3.29 (m, 3H), 2.72 (dd, 1H,  $J$  = 12.1, 4.0 Hz), 2.62–2.48 (m, 4H), 2.04 (s, 3H), 1.85 (p, 2H,  $J$  = 6.0 Hz), 1.75 (t, 1 H,  $J$  = 12.1 Hz). <sup>13</sup>C (151 MHz, D<sub>2</sub>O)  $\delta$  174.84, 173.41, 103.18, 101.95, 100.24, 79.54, 74.57, 73.61, 72.68, 72.47, 72.33, 71.72, 70.74, 68.45, 68.32, 67.61, 63.45, 62.58, 61.74, 60.17, 57.97, 51.74, 40.04, 36.28, 28.32, 26.74, 26.43, 22.00. <sup>19</sup>F (282 MHz, D<sub>2</sub>O)  $\delta$  -81.92 (s, 3F, CF<sub>3</sub>), -115.34 (s, 2F, CF<sub>2</sub>), -122.81 (s, 2F, CF<sub>2</sub>), -123.78 (s, 2F, CF<sub>2</sub>), -124.54 (s, 2F, CF<sub>2</sub>), -127.07 (s, 2F, CF<sub>2</sub>). HRMS (ESI)  $m/z$  calcd for [C<sub>35</sub>H<sub>48</sub>F<sub>13</sub>N<sub>2</sub>O<sub>20</sub>] 1063.2598, found 1063.2588.

### Gal $\alpha$ 1-3Lac $\beta$ ProNH-TEG-C<sub>8</sub>F<sub>17</sub> (**23**)

Lac $\beta$ ProNH-TEG-C<sub>8</sub>F<sub>17</sub> (**5**) (11 mg, 1 eq.) and galactose (1.5 eq.) were dissolved in water in a 1 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 7.0), ATP (2 eq.), UTP (2 eq.), MgCl<sub>2</sub> (10 mM), and MnCl<sub>2</sub> (10 mM). After the addition of *E. coli* GalK (1 mg), BLUSP (0.2 mg),  $\alpha$ 1-3GalT (0.08 mg), and PmPpA (0.25 mg), millipore water was added to bring the total volume of the reaction mixture to 1 mL. The reaction was gently shaken in an isotherm incubator for 16 h at 37 °C. After monitoring the reaction with TLC (EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume), an additional amount of ATP (0.5 eq.), UTP (0.5 eq.), EcGalK (0.5 mg), BLUSP (0.05 mg),  $\alpha$ 1-3GalT (0.02 mg), and PmPpA

(0.05 mg) was added to the mixture. After another 17 h, the reaction was still incomplete and the mixture was purified directly via an FSPE cartridge and concentrated to go through another round of the enzymatic reaction as described above to drive the reaction towards completion. The reaction underwent centrifugation to remove the precipitates and was purified using FSPE to produce **23** as a white solid (10 mg, 82%). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O) δ 5.18 (d, 1H, *J* = 2.9 Hz), 4.54 (d, 1H, *J* = 7.5 Hz), 4.46 (d, 1H, *J* = 7.7 Hz), 4.27–4.13 (m, 2H), 4.10–3.91 (m, 6H), 3.91–3.45 (m, 23H), 3.45–3.26 (m, 5H), 3.59–2.30 (m, 4H), 1.90–1.76 (m, 2H). <sup>13</sup>C (151 MHz, D<sub>2</sub>O) δ 172.01, 171.77, 102.78, 102.06, 95.24, 78.62, 77.01, 74.91, 74.60, 74.32, 72.69, 70.67, 70.06, 69.49, 69.40, 69.15, 68.98, 68.85, 68.09, 67.48, 64.64, 62.34, 60.89, 60.79, 60.05, 56.84, 38.90, 35.82, 28.63, 26.14. <sup>19</sup>F (282 MHz, D<sub>2</sub>O) δ -83.76 (s, 3F, CF<sub>3</sub>), -116.31 (s, 2F, CF<sub>2</sub>), -123.32 (s, 2F, CF<sub>2</sub>), -123.70 (m, 4F, 2CF<sub>2</sub>), -124.68 (s, 2F, CF<sub>2</sub>), -124.94 (s, 2F, CF<sub>2</sub>), -128.46 (s, 2F, CF<sub>2</sub>). HRMS (ESI) *m/z* calcd for [C<sub>40</sub>H<sub>57</sub>F<sub>17</sub>N<sub>2</sub>O<sub>21</sub>+H]<sup>+</sup> 1225.3481, found 1225.3481.

### Neu5Acα2–3LacβProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**25**)

LacβProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**7**) (10 mg, 1 eq.), Neu5Ac (1.2 eq.), and CTP (2 eq.) were dissolved in water in a 1 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and MgCl<sub>2</sub> (20 mM). After the addition of PmST1\_E271F/R313Y mutant (0.033 mg) and NmCSS (0.2 mg), millipore water was added to bring the total volume of the reaction mixture to 1 mL. The reaction was gently shaken in an isotherm incubator for 2 h at 37 °C. After monitoring the reaction with TLC (EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume), 0.5 eq. of additional CTP was added to the mixture. After another hour, 0.5 eq. of CTP was added again to drive the reaction towards completion. After 1 h, the reaction mixture was purified directly via an FSPE cartridge and concentrated by rotavap to produce compound **25** as a white solid (14 mg, 86%). <sup>1</sup>H NMR (800 MHz, D<sub>2</sub>O) δ 4.55 (d, 1H, *J* = 7.8 Hz), 4.48 (d, 1H, *J* = 7.9 Hz), 4.13 (dd, 1H, *J* = 2.3 Hz and 9.8 Hz), 4.07 (s, 2H), 4.03–3.94 (m, 3H), 3.94–3.81 (m, 4H), 3.81–3.55 (m, 22H), 3.43 (t, 2H, *J* = 5.1 Hz), 3.37 (t, 2H, *J* = 6.9 Hz), 3.34 (t, 1H, *J* = 8.3 Hz), 2.78 (dd, 1H, *J* = 4.4 Hz and 12.4 Hz), 2.59 (t, 2H, *J* = 7.0 Hz), 2.54 (dd, 2H, *J* = 6.8 Hz and 18.8 Hz), 2.06 (s, 3H), 1.88 (p, 2H, *J* = 6.7 Hz), 1.83 (t, 1H, *J* = 12.1). <sup>13</sup>C (201 MHz, D<sub>2</sub>O) δ 174.92, 173.83, 173.21, 172.14, 102.61, 102.07, 99.75, 78.26, 75.41, 75.07, 74.70, 74.29, 72.81, 72.76, 71.67, 70.16, 69.57, 69.47, 69.32, 69.30, 68.75, 68.29, 68.03, 67.64, 67.41, 62.50, 61.27, 60.94, 60.01, 58.59, 51.63, 39.55, 38.97, 35.85, 28.49, 26.47 (d, *J* = 49.1 Hz), 21.97. <sup>19</sup>F (282 MHz, D<sub>2</sub>O) δ -82.78 (s, 3F, CF<sub>3</sub>), -115.82 (s, 2F, CF<sub>2</sub>), -123.17 (s, 2F, CF<sub>2</sub>), -124.23 (s, 2F, CF<sub>2</sub>), -124.78 (s, 2F, CF<sub>2</sub>), -127.73 (s, 2F, CF<sub>2</sub>). HRMS (ESI) *m/z* calcd for [C<sub>43</sub>H<sub>63</sub>F<sub>13</sub>N<sub>3</sub>O<sub>24</sub>]<sup>-</sup> 1252.3599, found 1252.3586.

### Neu5Acα2–6LacβProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**27**)

LacβProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**7**) (10 mg, 1 eq.), Neu5Ac (5 mg, 1.2 eq.), and CTP (15 mg, 2 eq.) were dissolved in water in a 1 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 8.5) and MgCl<sub>2</sub> (20 mM). After the addition of Pd2,6ST (0.09 mg) and NmCSS (0.2 mg), millipore water was added to bring the total volume of the reaction mixture to 1 mL. The reaction was gently shaken in an isotherm incubator for 2 h at 37 °C. After monitoring the reaction with TLC (EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume), 0.5 eq. of additional CTP was added to the mixture. After another hour, 0.5 eq. of CTP was added again to drive the reaction towards completion. After 1 h, the reaction was purified directly by passing through an FSPE cartridge and concentrated by rotavap to produce **27** as a white solid (13 mg, qt.). <sup>1</sup>H NMR (600 MHz, D<sub>2</sub>O) δ 4.46 (d, 1H, *J* = 7.8 Hz), 4.42 (d, 1H, *J* = 7.7 Hz), 4.05 (s, 2H), 4.00–3.77 (m, 8H), 3.74–3.49 (m, 22H), 3.43–3.29 (m, 5H), 2.71 (dd, 1H, *J* = 4.4 Hz and 12.1 Hz), 2.62–2.43 (m, 4H), 2.02 (s, 3H), 1.90–1.81 (m, 2H), 1.73 (t, 1H, *J* = 12.2 Hz). <sup>13</sup>C (151 MHz, D<sub>2</sub>O) δ 174.80, 173.40, 173.20, 172.14, 103.18, 101.93, 100.22, 79.57, 74.57, 73.59, 72.67, 72.46, 72.30, 71.71, 70.72, 70.16, 69.57, 69.49, 69.46, 69.32, 68.75, 68.43, 68.29, 68.27, 67.63, 63.43, 62.56, 62.22, 60.18, 57.27,



51.73, 40.05, 38.96, 35.86, 28.51, 26.59, 21.99.  $^{19}\text{F}$  (282 MHz,  $\text{D}_2\text{O}$ )  $\delta$  -82.48 (s, 3F,  $\text{CF}_3$ ), -115.63 (s, 2F,  $\text{CF}_2$ ), -123.04 (s, 2F,  $\text{CF}_2$ ), -124.07 (s, 2F,  $\text{CF}_2$ ), -124.72 (s, 2F,  $\text{CF}_2$ ), -127.50 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$  calcd for  $[\text{C}_{43}\text{H}_{63}\text{F}_{13}\text{N}_3\text{O}_{24}]^-$  1252.3599, found 1252.3599.

### Gal $\alpha$ 1-3Lac $\beta$ ProNH-TEG-C $_6$ F $_{13}$ (29)

Lac $\beta$ ProNH-TEG-C $_6$ F $_{13}$  (7) (12 mg, 1 eq.) and galactose (1.5 eq.) were dissolved in water in a 1 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 7.0), ATP (2 eq.), UTP (2 eq.),  $\text{MgCl}_2$  (10 mM), and  $\text{MnCl}_2$  (10 mM). After the addition of *E. coli* GalK (1 mg), BLUSP (0.2 mg),  $\alpha$ 1-3GalT (0.16 mg), and PmPpA (0.25 mg), millipore water was added to bring the total volume of the reaction mixture to 1 mL. The reaction was gently shaken in an isotherm incubator for 16 h at 37 °C. After monitoring the reaction with TLC (EtOAc:MeOH:H $_2$ O = 5:2:1 by volume), an additional amount of ATP (0.5 eq.), UTP (0.5 eq.), EcGalK (0.5 mg), BLUSP (0.1 mg),  $\alpha$ 1-3GalT (0.04 mg), and PmPpA (0.25 mg) was added to the mixture. After another 19 h, more ATP (0.1 eq.), UTP (0.1 eq.), EcGalK (0.2 mg), BLUSP (0.01 mg),  $\alpha$ 1-3GalT (0.004 mg), and PmPpA (0.025 mg) was added to the mixture. After 36 h, the reaction was purified directly via an FSPE cartridge and concentrated to produce 29 as a white solid (13 mg, 89%).  $^1\text{H}$  NMR (800 MHz,  $\text{D}_2\text{O}$ )  $\delta$  5.17 (d, 1H,  $J$  = 3.3 Hz), 4.54 (d, 1H,  $J$  = 7.7 Hz), 4.48 (d, 1H,  $J$  = 7.6 Hz), 4.27–4.16 (m, 2H), 4.11–3.93 (m, 6H), 3.89 (dd, 1H,  $J$  = 3.3 Hz and 10.3 Hz), 3.86–3.48 (m, 22H), 3.46–3.30 (m, 5H), 2.60–2.35 (m, 4H), 1.88 (p, 2H,  $J$  = 6.2 Hz).  $^{13}\text{C}$  (201 MHz,  $\text{D}_2\text{O}$ )  $\delta$  172.22, 171.90, 102.86, 102.13, 95.36, 78.75, 77.17, 74.98, 74.69, 74.41, 72.77, 70.76, 70.16, 69.61, 69.56, 69.50, 69.43, 69.25, 69.08, 68.89, 68.16, 67.59, 64.75, 62.61, 60.94, 60.88, 60.16, 56.76, 38.97, 35.91, 28.68, 26.20.  $^{19}\text{F}$  (282 MHz,  $\text{D}_2\text{O}$ )  $\delta$  -83.32 (s, 3F,  $\text{CF}_3$ ), -116.14 (s, 2F,  $\text{CF}_2$ ), -123.44 (s, 2F,  $\text{CF}_2$ ), -124.51 (s, 2F,  $\text{CF}_2$ ), -124.93 (s, 2F,  $\text{CF}_2$ ), -128.13 (s, 2F,  $\text{CF}_2$ ). HRMS (ESI)  $m/z$  calcd for  $[\text{C}_{38}\text{H}_{57}\text{F}_{13}\text{N}_2\text{O}_{21}+\text{H}]^+$  1125.3319, found 1125.3351.

### Fluorous solid-phase extraction (FSPE) cartridge purification

For fluorous-solid phase extractions, the chemical reaction mixtures were directly loaded to FluoroFlash® SPE cartridges (2 g fluorous silica gel in 10 mL cartridge, conditioned with deionized water) (Fluorous Tech. Inc.) and washed with deionized water (3 mL  $\times$  4) to remove non-fluorous reaction components. The fluorous-tagged products were eluted with methanol (3 mL  $\times$  4). For enzymatic reactions, the reaction mixtures were centrifuged at 13,226  $\times$   $g$  for 10 min. to remove precipitates. Next, the supernatants were loaded to the conditioned FSPE cartridges and the cartridges were then washed with deionized water (3 mL  $\times$  4) to remove non-fluorous components. Lastly, the fluorous-tagged products were eluted by the following washes of methanol (3 mL  $\times$  4).

### References

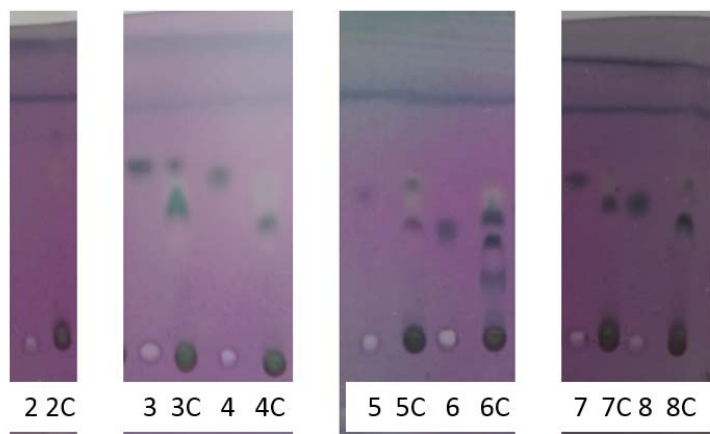
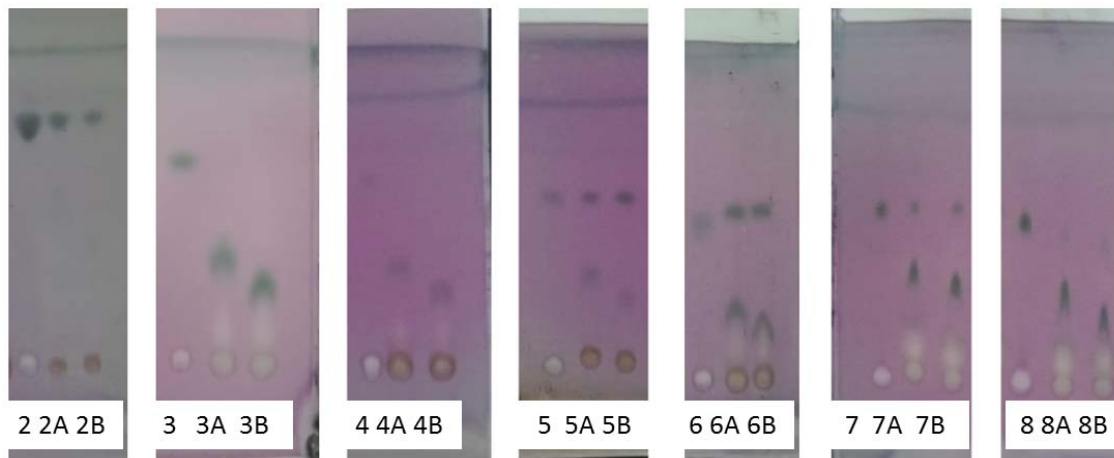
1. H. A. Chokhawala, S. Huang, K. Lau, H. Yu, J. Cheng, V. Thon, N. Hurtado-Ziola, J. A. Guerrero, A. Varki and X. Chen, *ACS Chem. Biol.* 2008, **3**, 567–576.
2. H. Shi, K. Liu, A. Xu and S. Q. Yao, *Chem. Commun.* 2009, 5030–5032.
3. Y. Ma, H. Zhang and X. L. Sun, *Bioconjug. Chem.* 2010, **21**, 1994–1999.

**Figure S1.** Reaction conversion rates of Table 1 and Table 2 were determined by first staining the TLC plates with *p*-anisaldehyde sugar stain and then using ImageQuant 5.2 to compare the relative intensities (under greyscale) between the glycosylation product spot and the lactoside acceptor spot of each reaction. For external standard comparison, each lactoside acceptor spot was also compared with its corresponding standard 10 mM stock solution spot (of compounds **2–8**) to verify the conversion rates of each reaction.

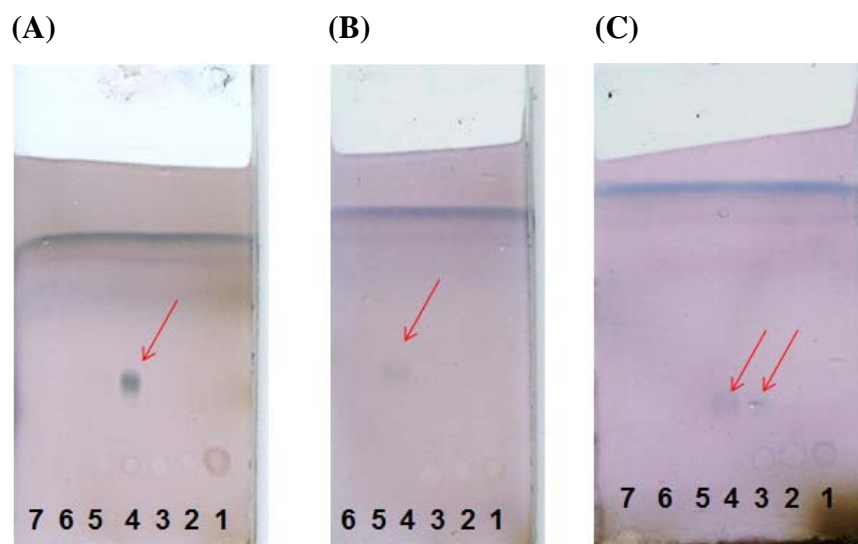
A: One-pot two-enzyme sialylation reaction with PmST1 E271F/R313Y

B: One-pot two-enzyme sialylation reaction with Pd2,6ST

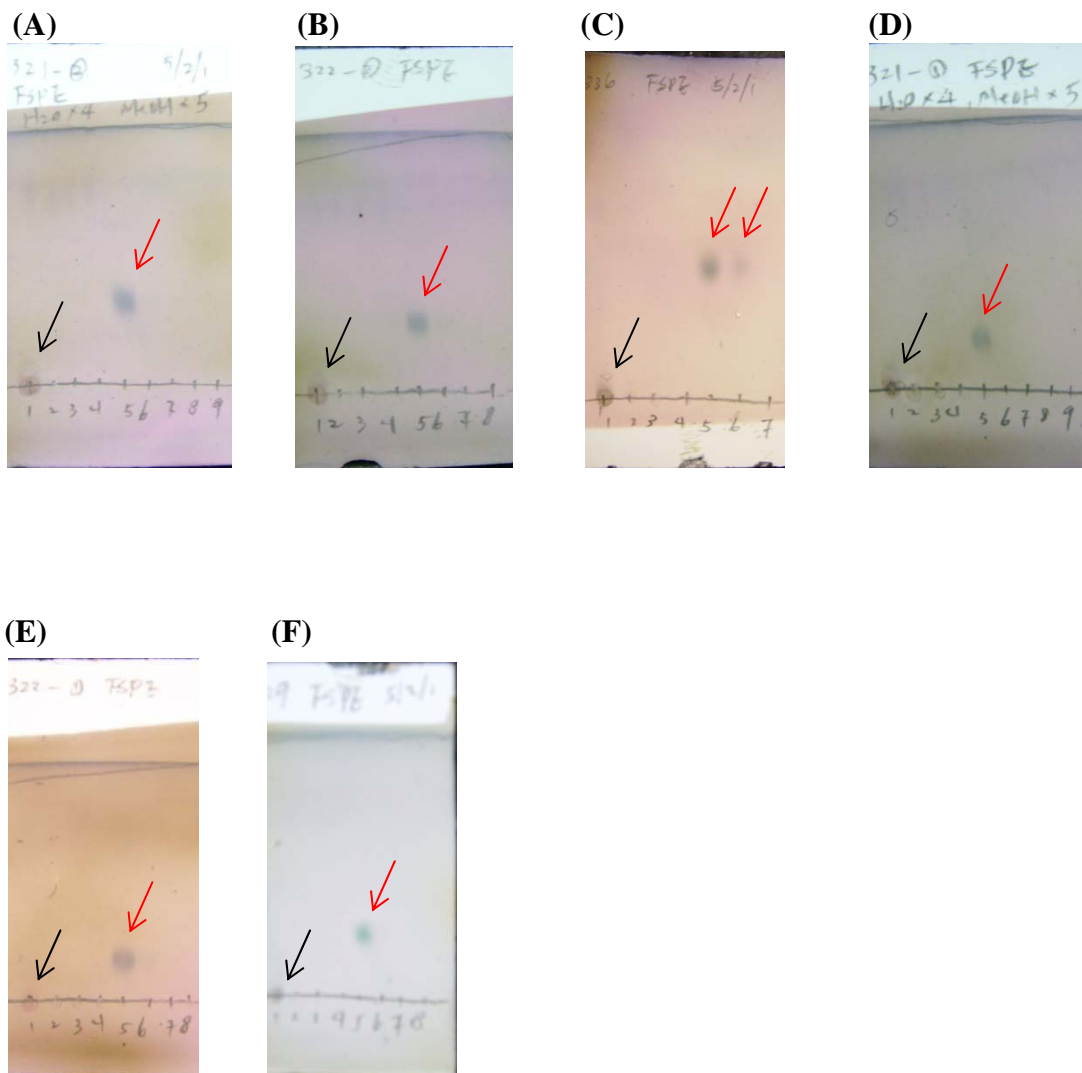
C: One-pot four-enzyme galactosylation reaction with  $\alpha$ 1–3GalT



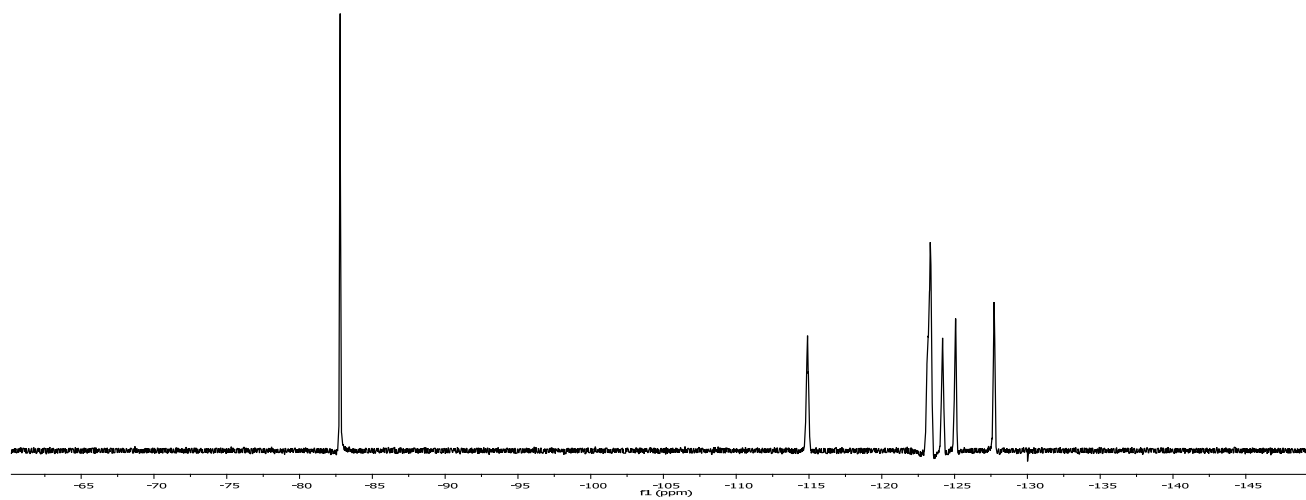
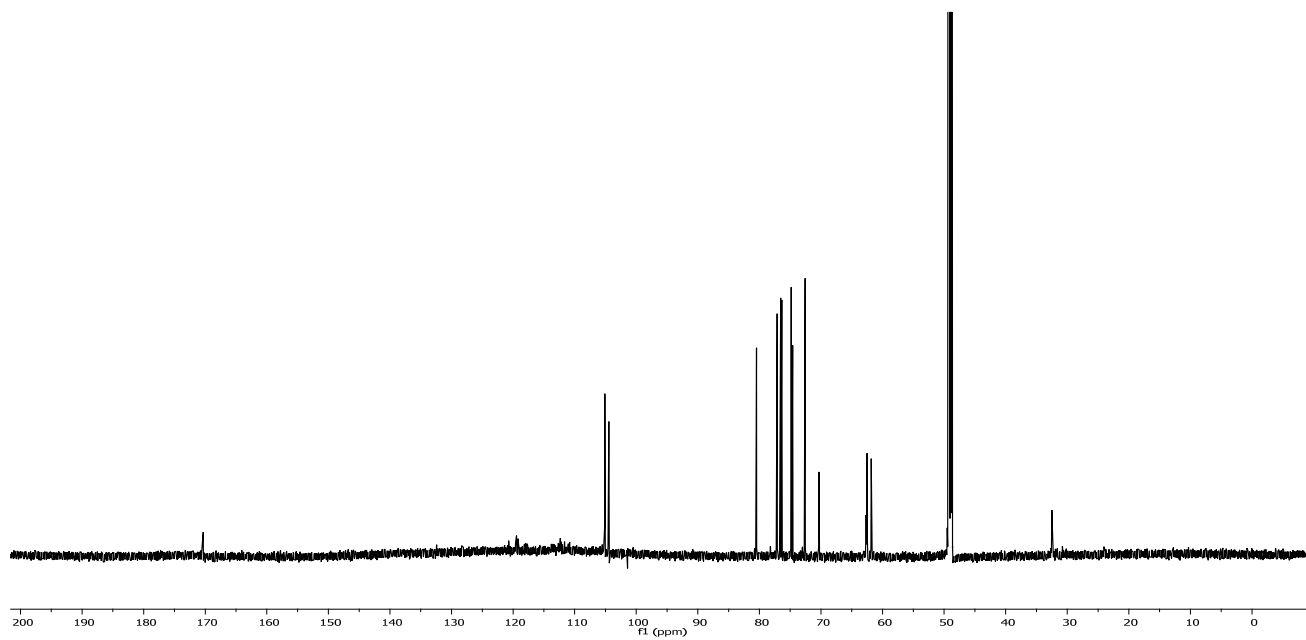
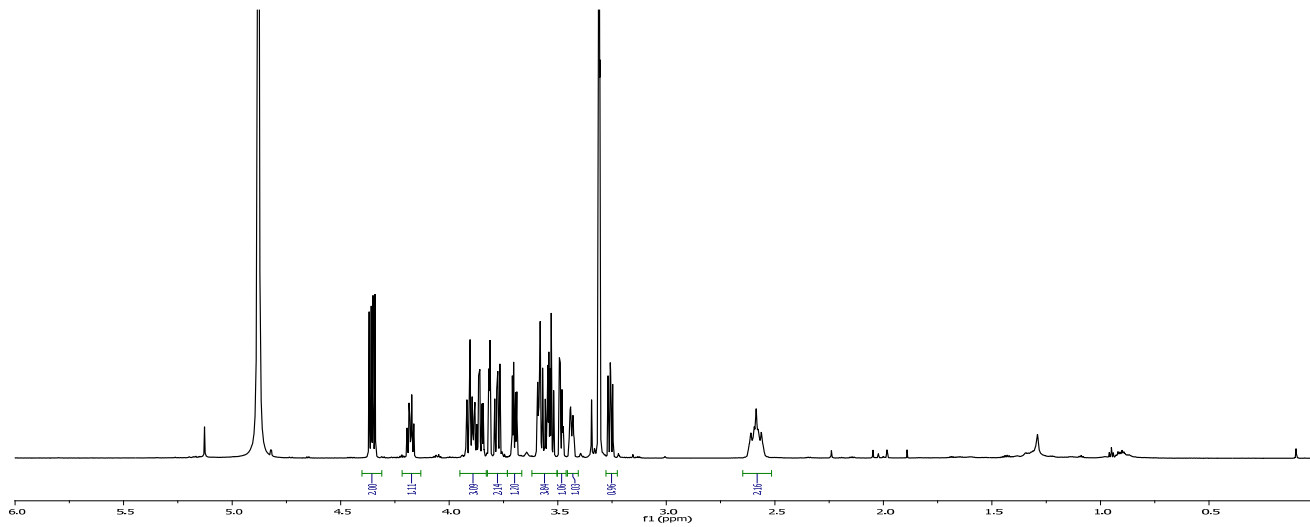
**Figure S2.** FSPE purification of (A) Neu5Ac $\alpha$ 2–3Lac $\beta$ ProNH-C<sub>8</sub>F<sub>17</sub>, (B) Neu5Ac $\alpha$ 2–3Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub>, and (C) Neu5Ac $\alpha$ 2–3Lac $\beta$ ProNH-C<sub>3</sub>F<sub>7</sub>. After loading the reaction mixture to FSPE cartridge, 3  $\times$  3.5 mL of H<sub>2</sub>O (numbers 1–3) was used to wash out non-fluorous components of the mixture. 3–4  $\times$  3.5 mL of MeOH (numbers 4–7) was then used to elute the fluorous-tagged glycans. As shown on thin-layer chromatography (TLC) plates, the C<sub>3</sub>F<sub>7</sub>-tagged sialoside was not retained in the cartridge during the third water wash and eluted prematurely prior to the methanol wash. Developing solvent used for TLC was EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume.



**Figure S3.** FSPE purification of (A) Neu5Ac $\alpha$ 2-3Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub> (**11**), (B) Neu5Ac $\alpha$ 2-6Lac $\beta$ ProNH-C<sub>6</sub>F<sub>13</sub> (**14**), (C) Gal $\alpha$ 1-3Lac $\beta$ ProNH-TEG-C<sub>8</sub>F<sub>17</sub> (**23**), (D) Neu5Ac $\alpha$ 2-3Lac $\beta$ ProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**25**), (E) Neu5Ac $\alpha$ 2-6Lac $\beta$ ProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**27**), (F) Gal $\alpha$ 1-3Lac $\beta$ ProNH-TEG-C<sub>6</sub>F<sub>13</sub> (**29**) monitored by thin-layer chromatography (TLC). After loading reaction mixtures to FSPE cartridges, 4  $\times$  3.5 mL of H<sub>2</sub>O was used to wash out non-fluorous components of the mixture (numbers 1-4). 3-5  $\times$  3.5 mL of MeOH was then used to elute the fluorous-tagged glycans (numbers 5-9). Developing solvent used for TLC was EtOAc:MeOH:H<sub>2</sub>O = 5:2:1 by volume. (Red arrow: fluorous-tagged product. Black arrows: reaction components without fluorous tag.)

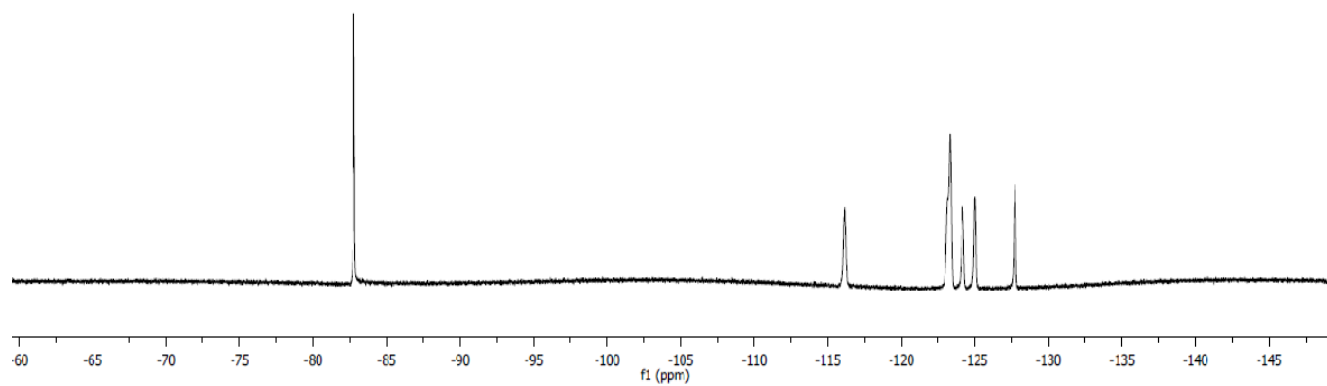
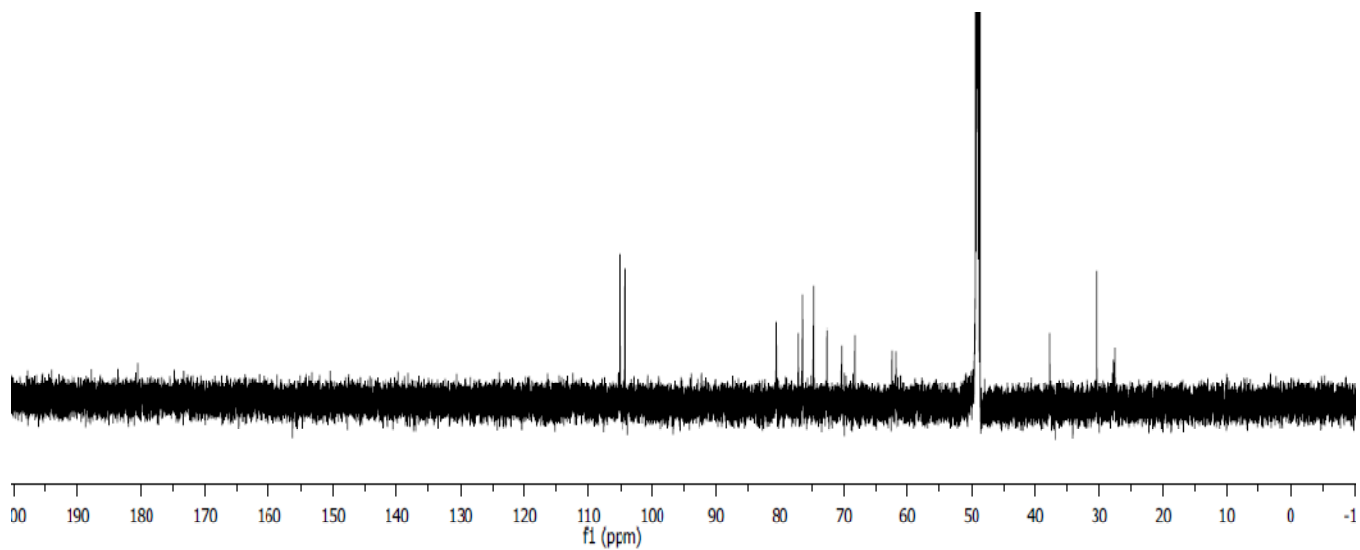
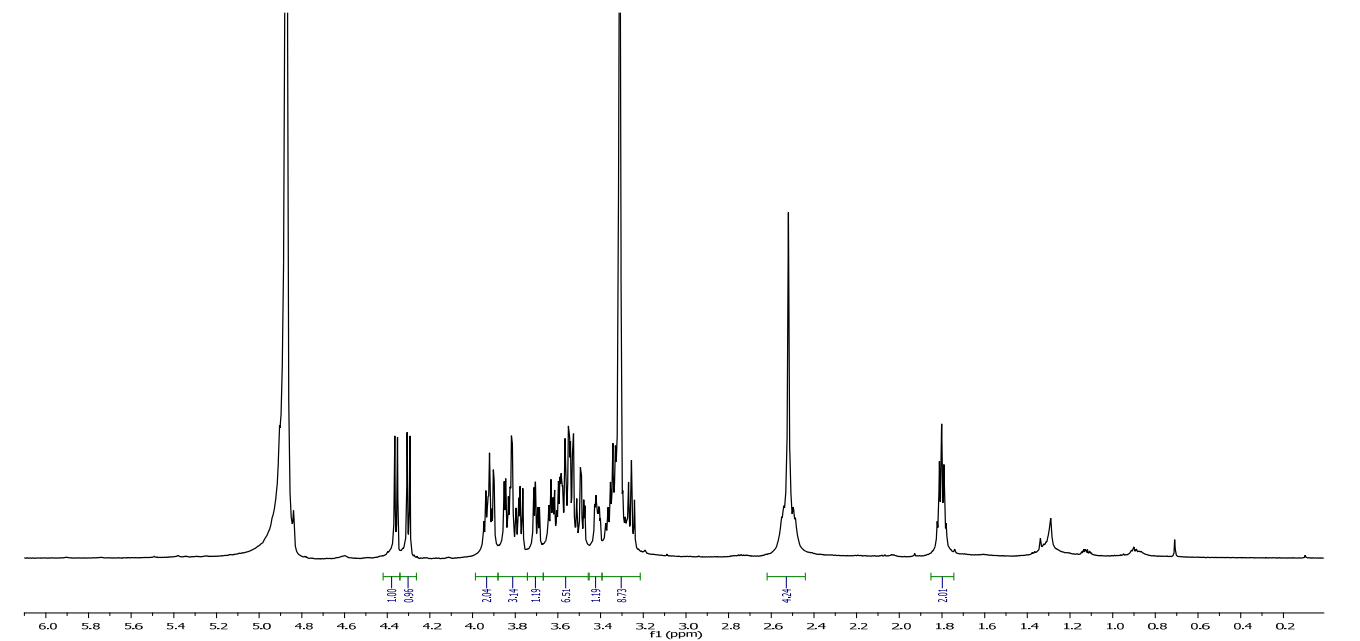


$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Lac $\beta\text{C}_8\text{F}_{17}$  (1)

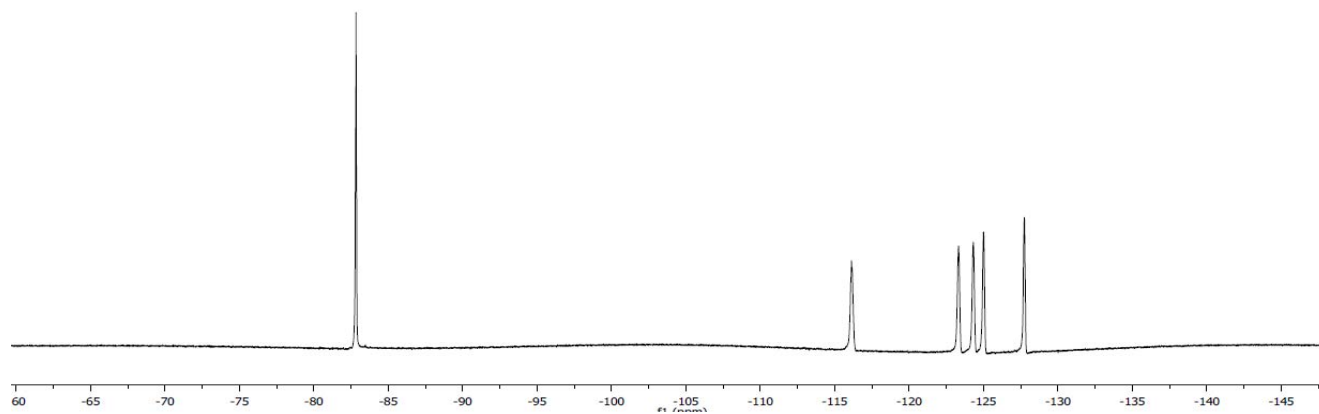
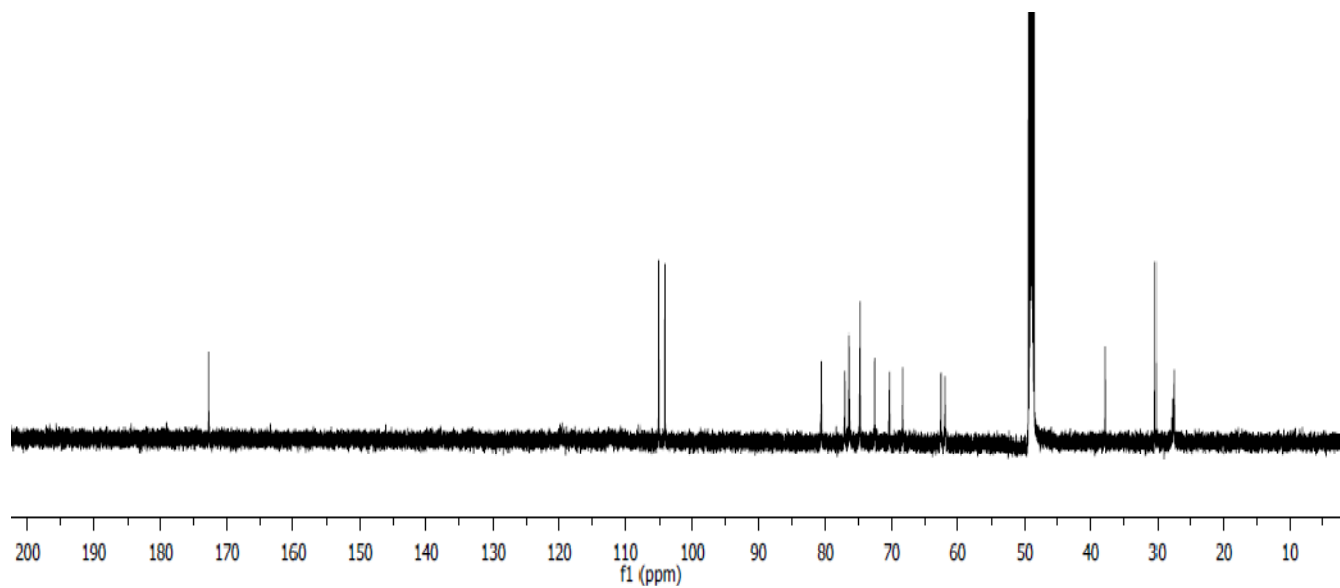
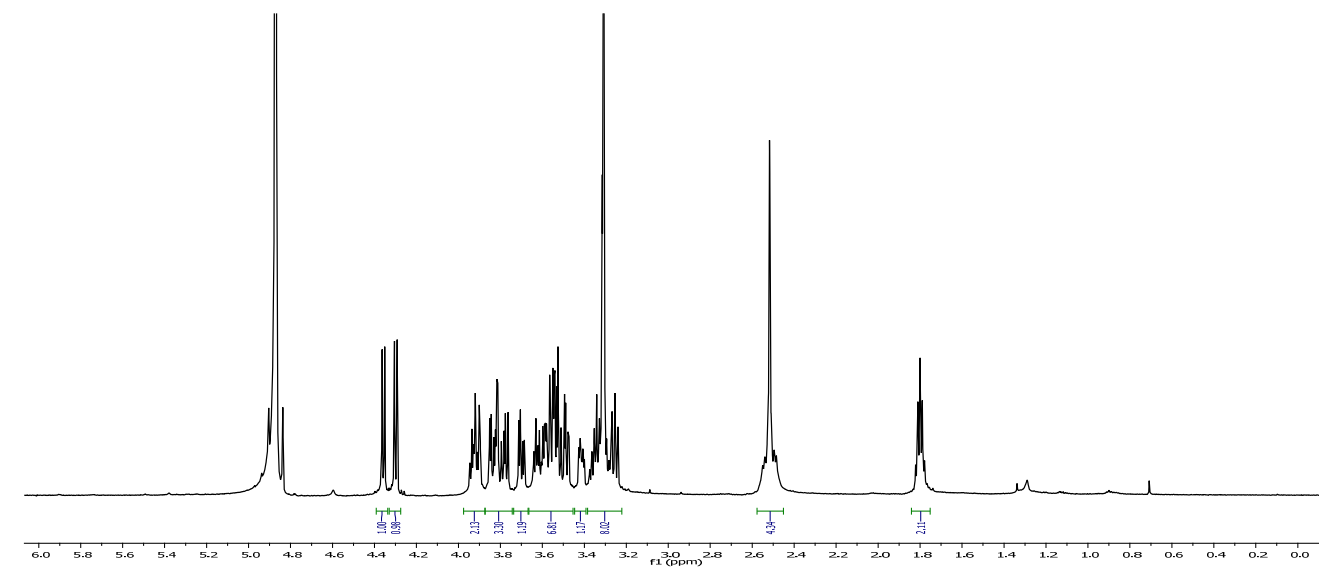




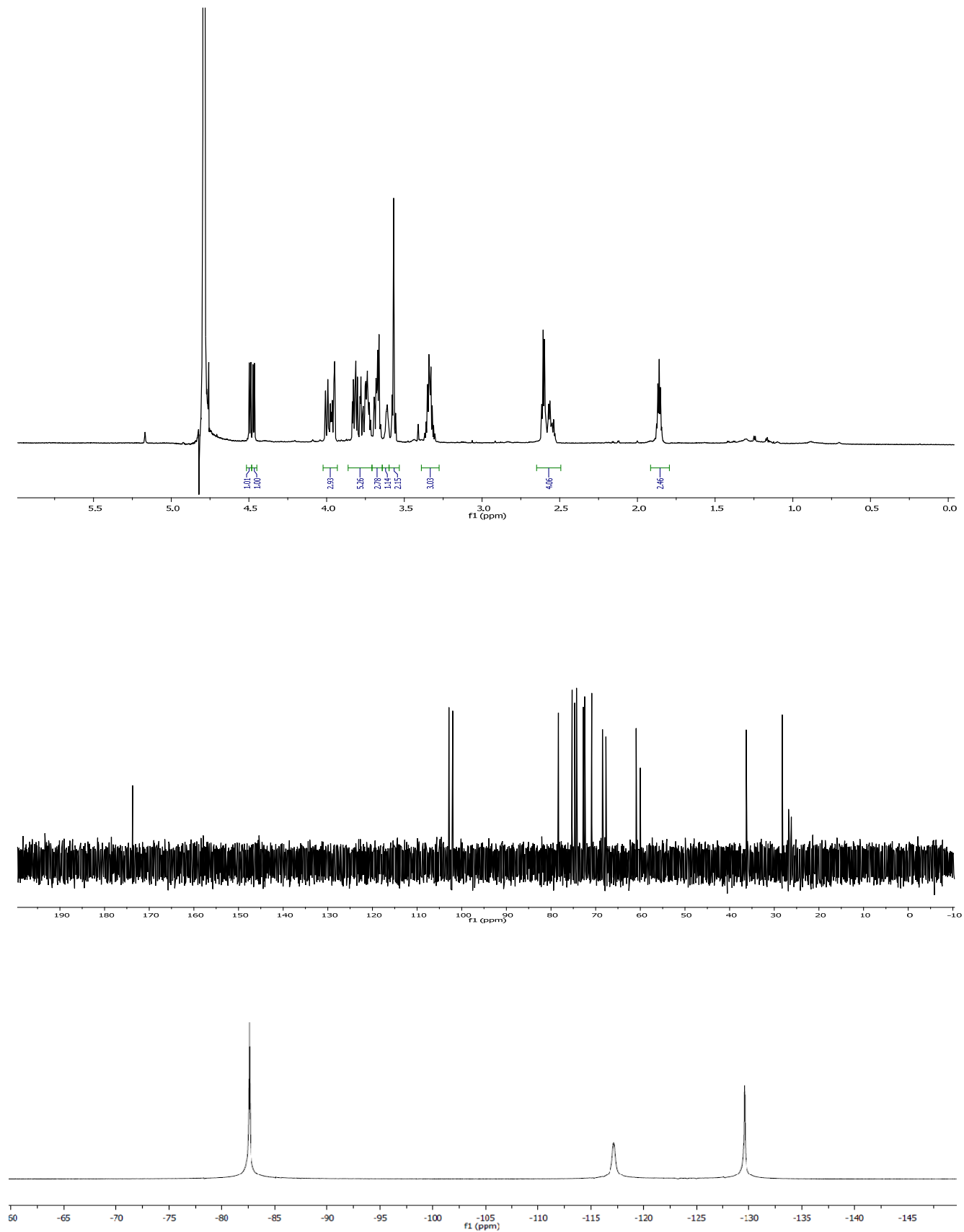
$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Lac $\beta$ ProNH-C $_8$ F $_{17}$  (**2**)



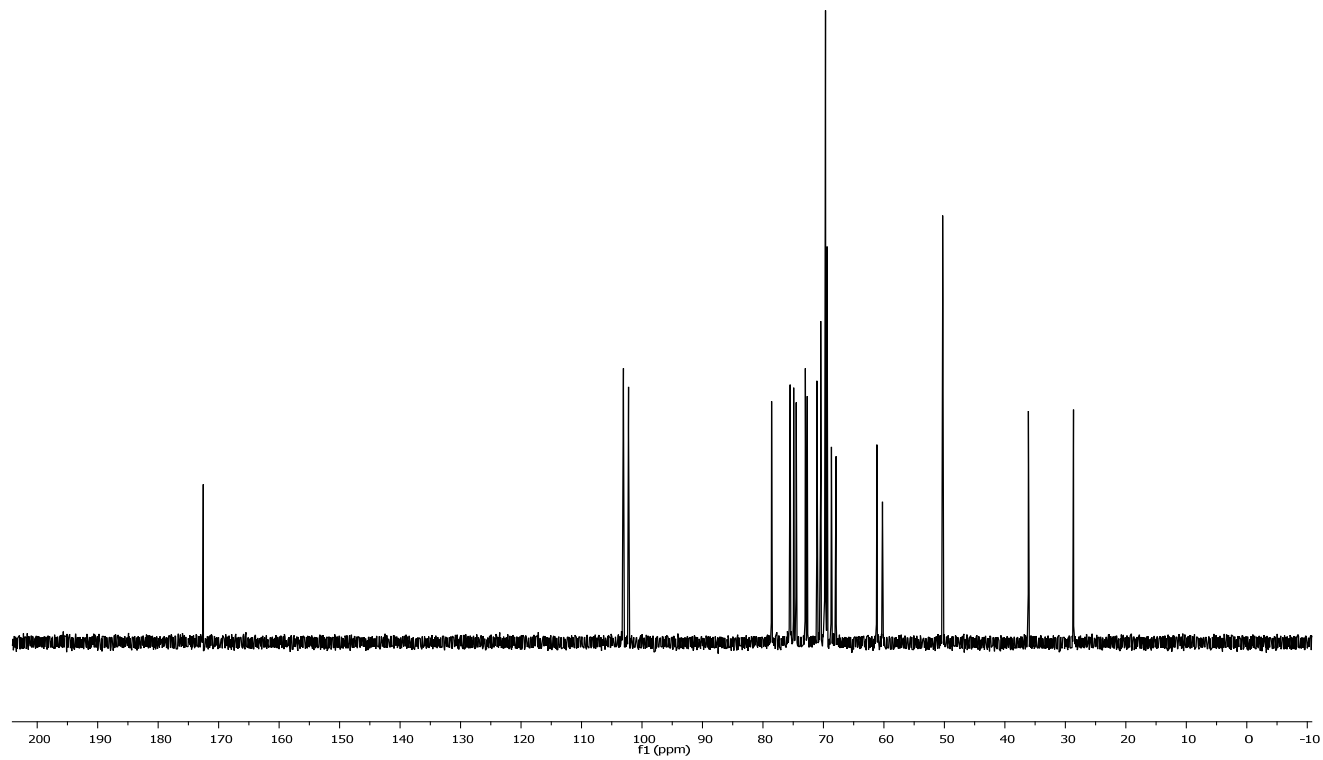
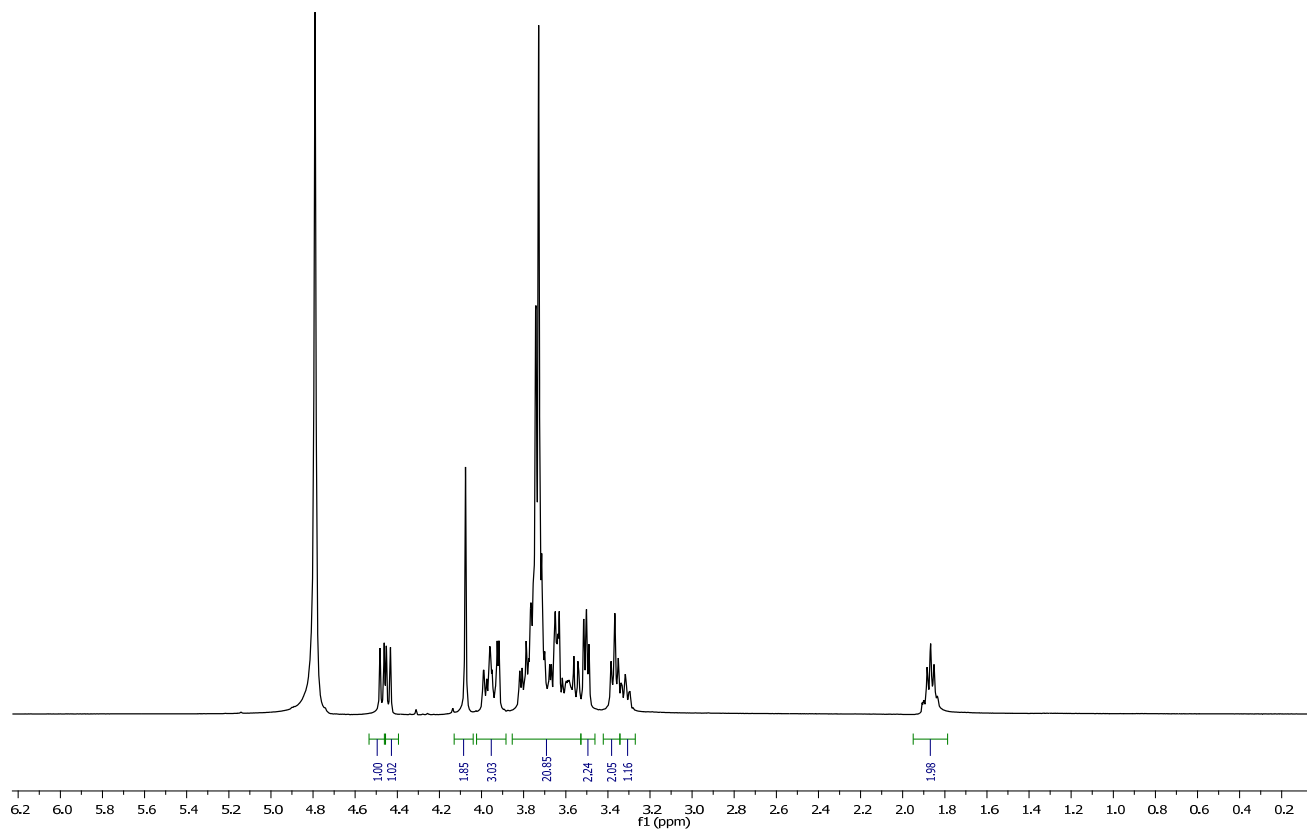
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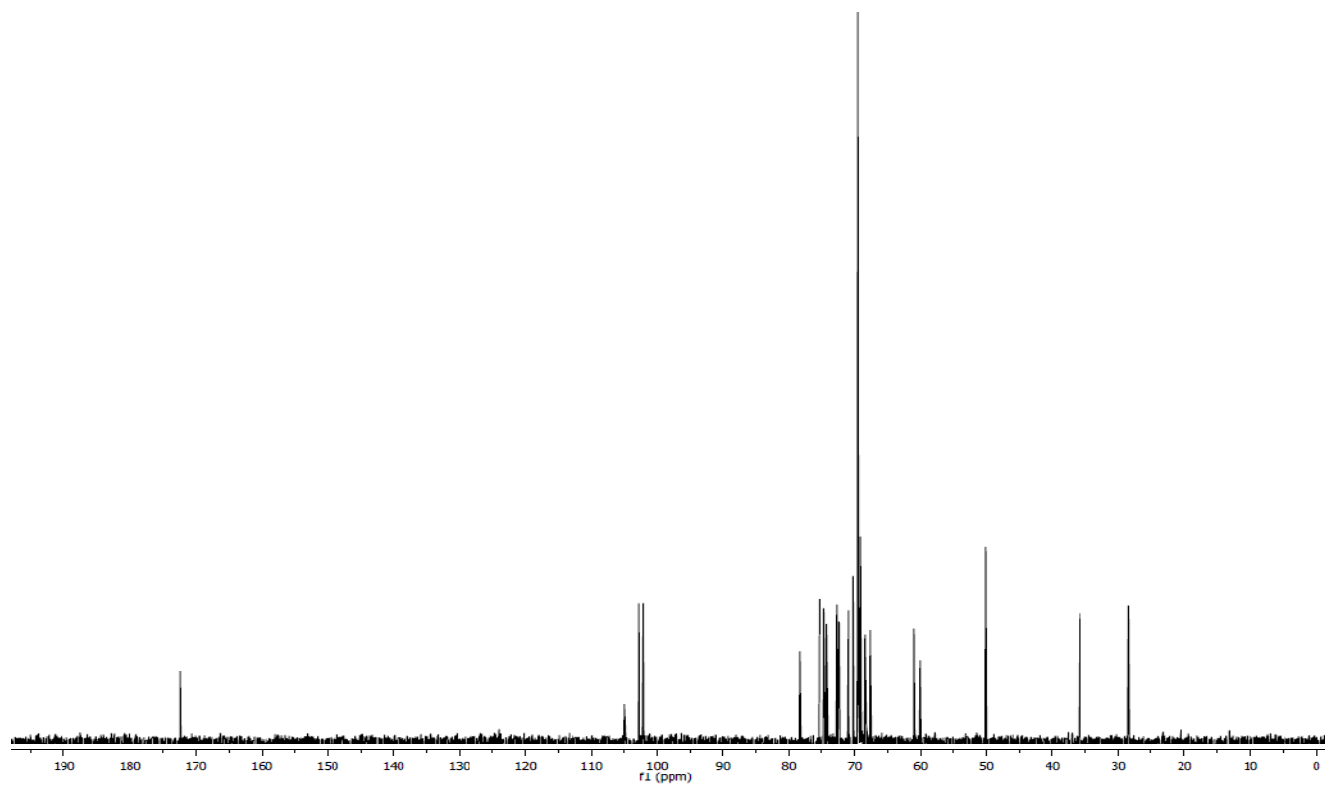
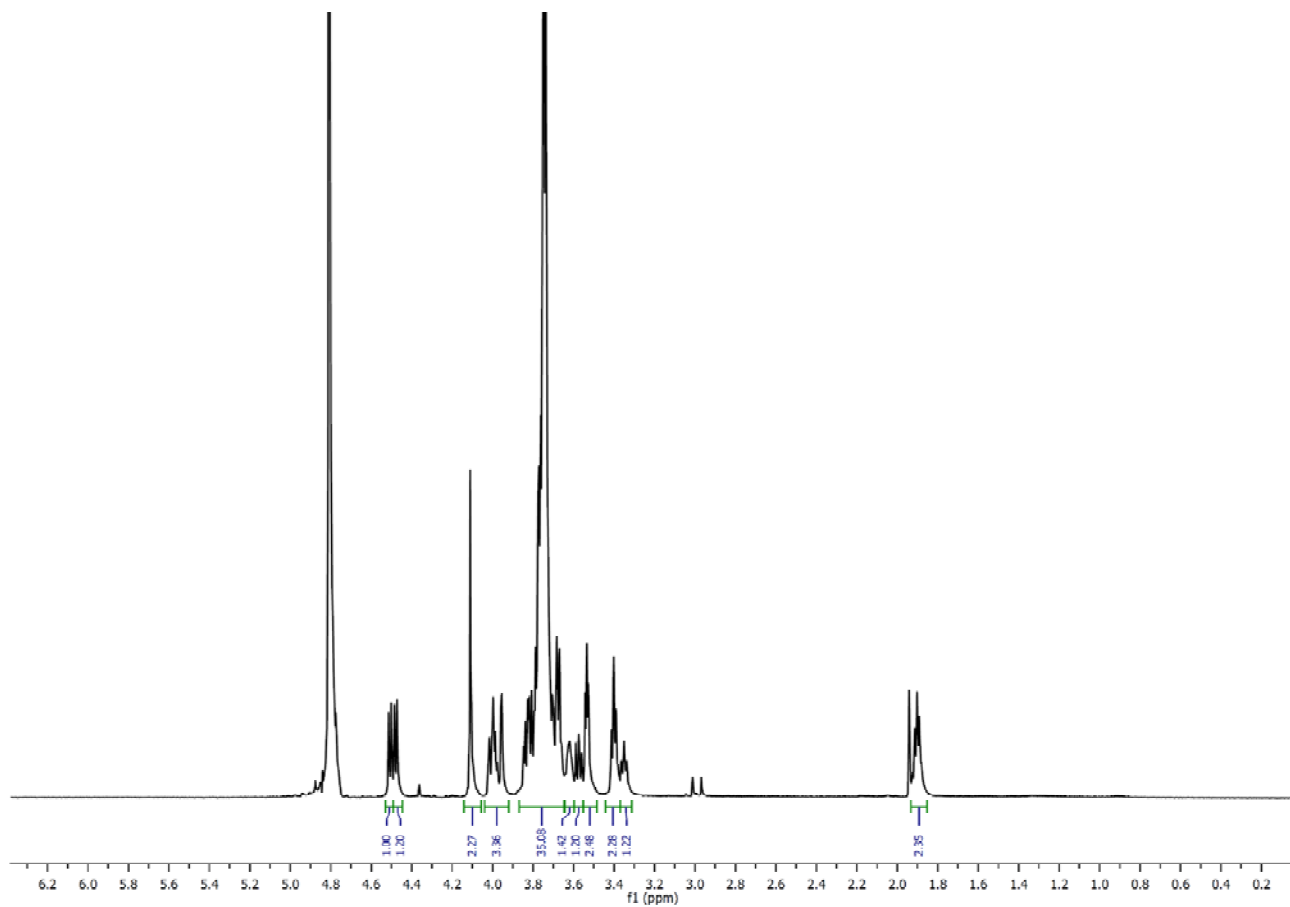
$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Lac $\beta$ ProNH-C $_3$ F $_7$  (**4**)



$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of Lac $\beta$ ProNH-TEG- $\text{N}_3$  (**34**)



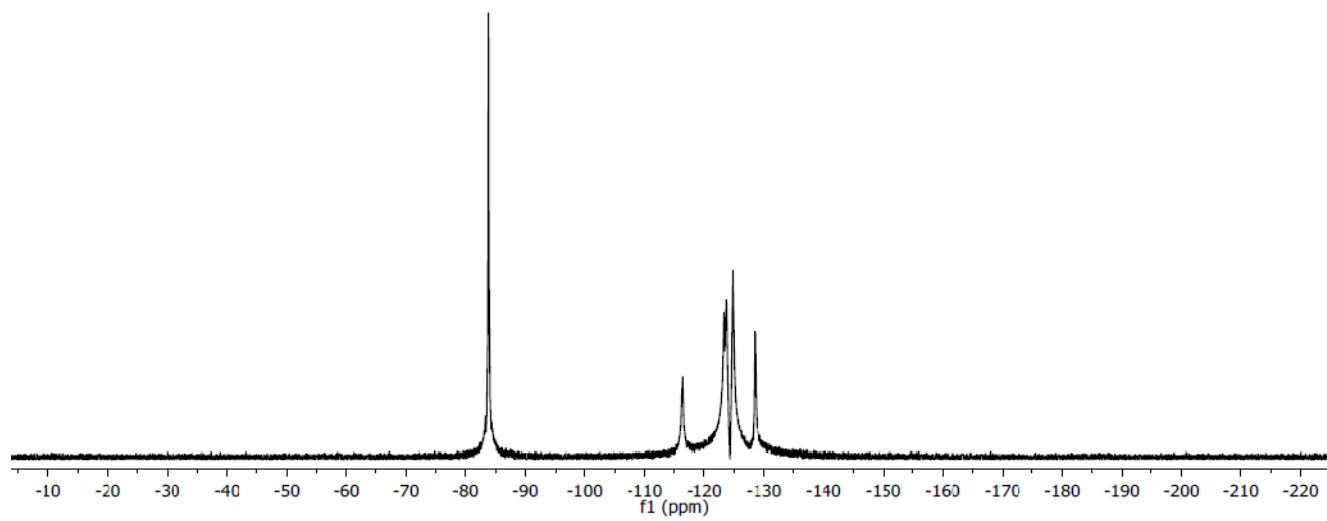
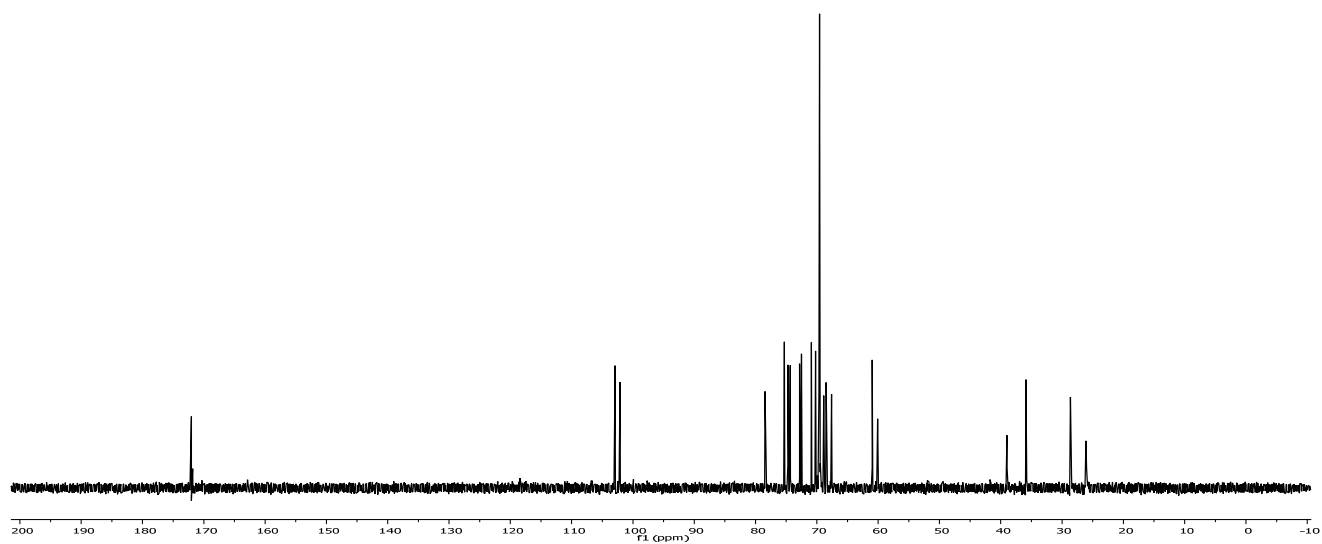
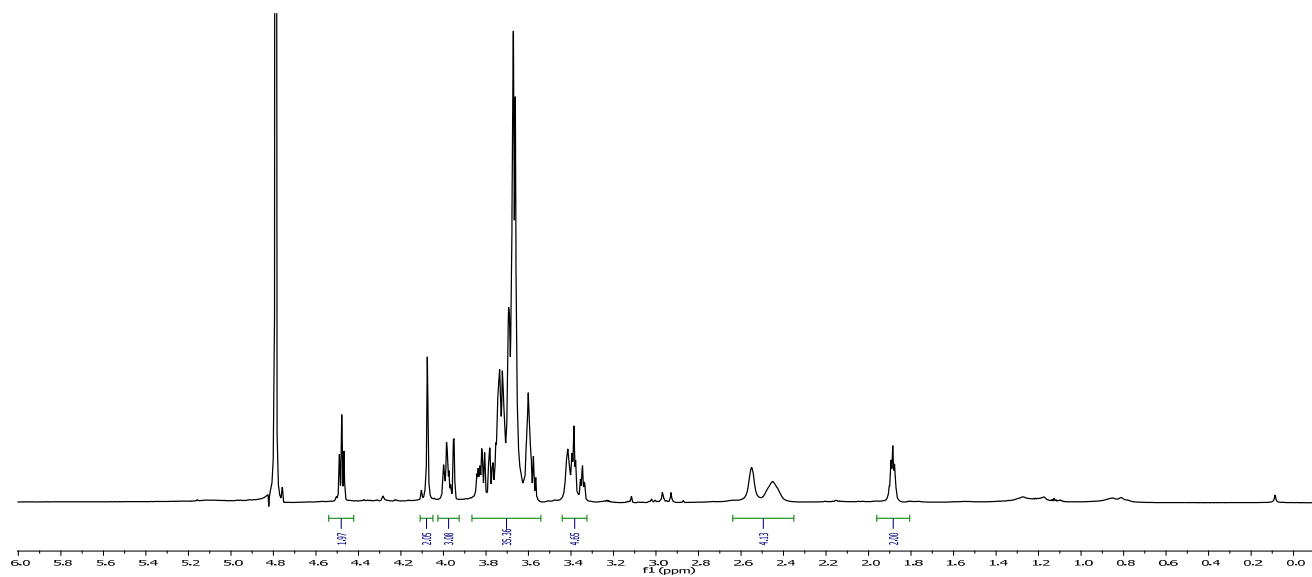
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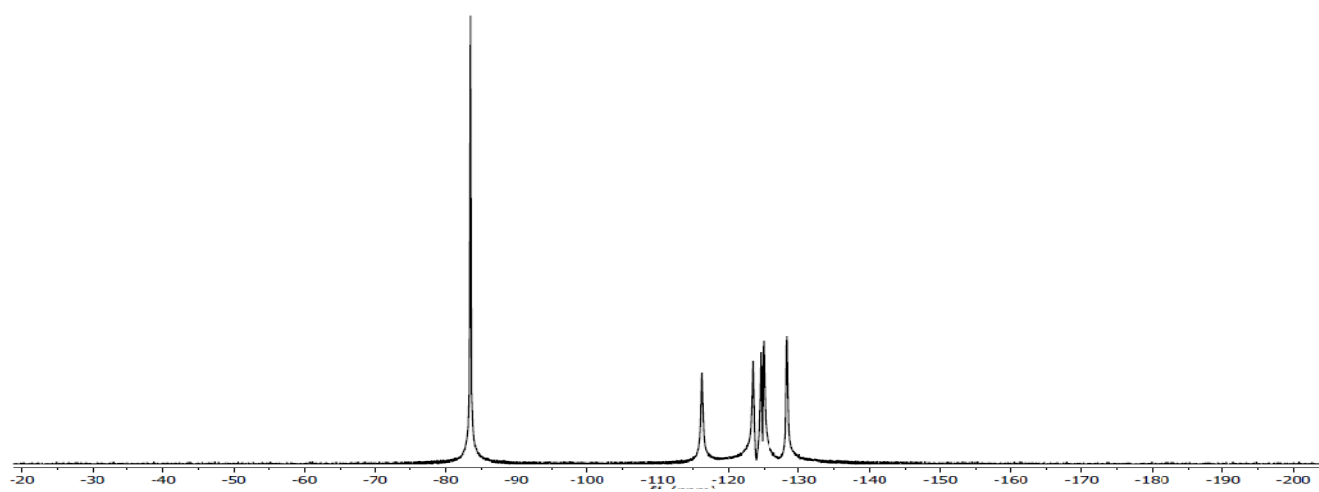
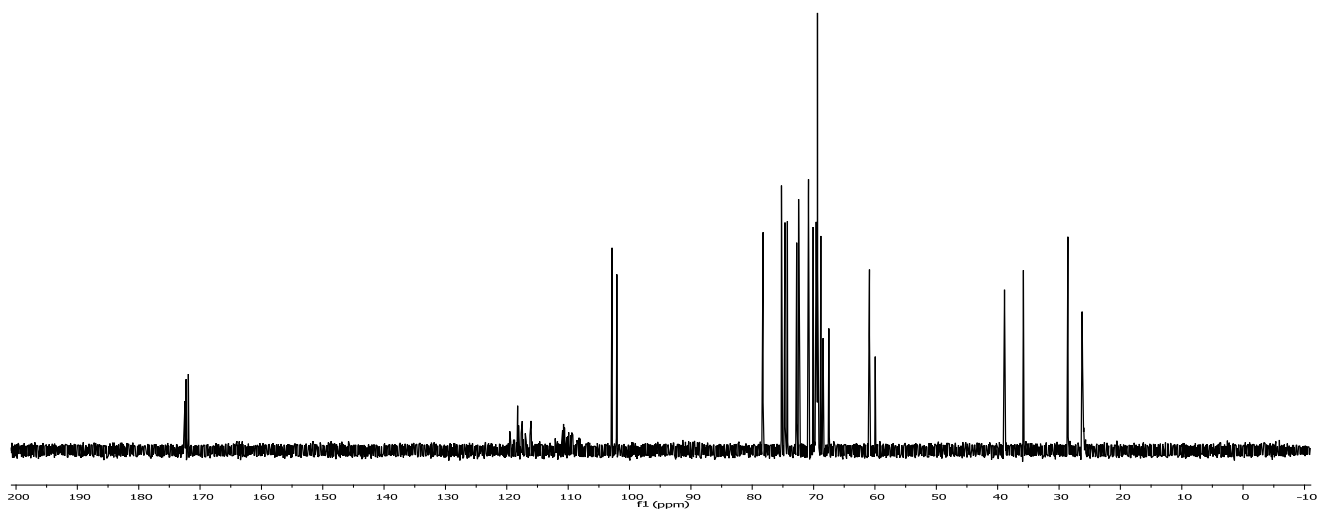
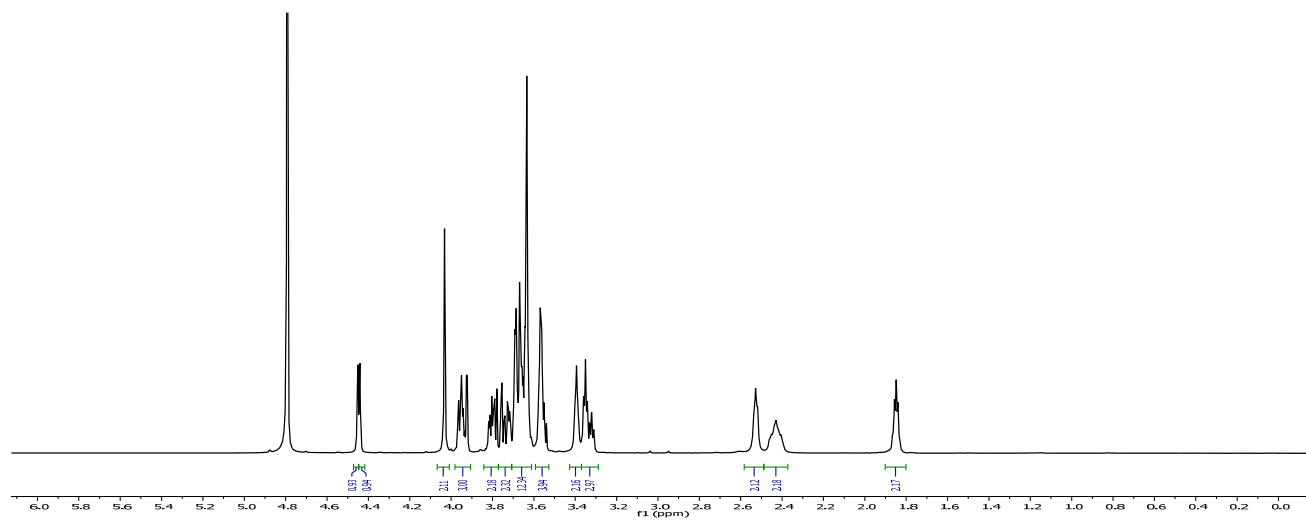




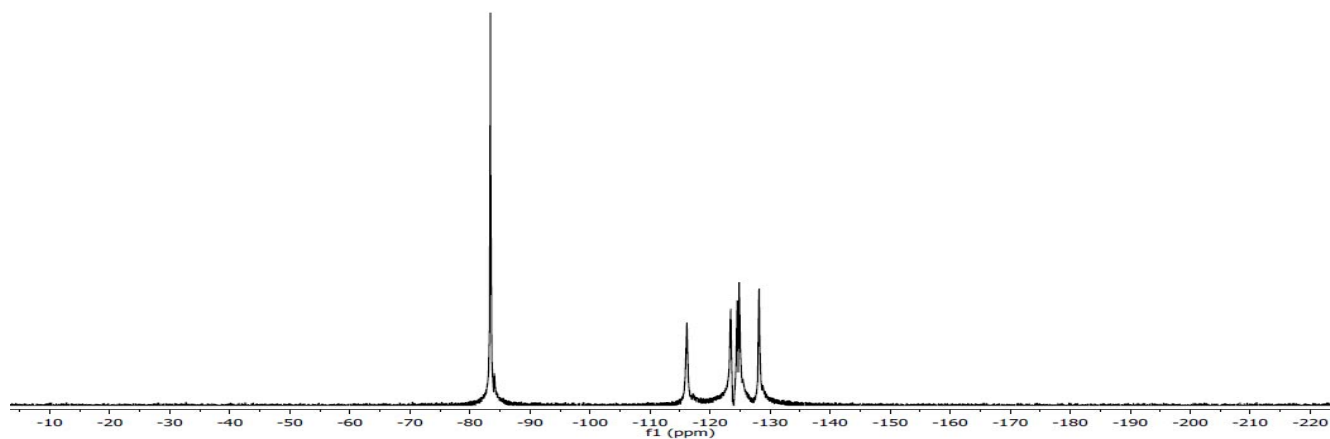
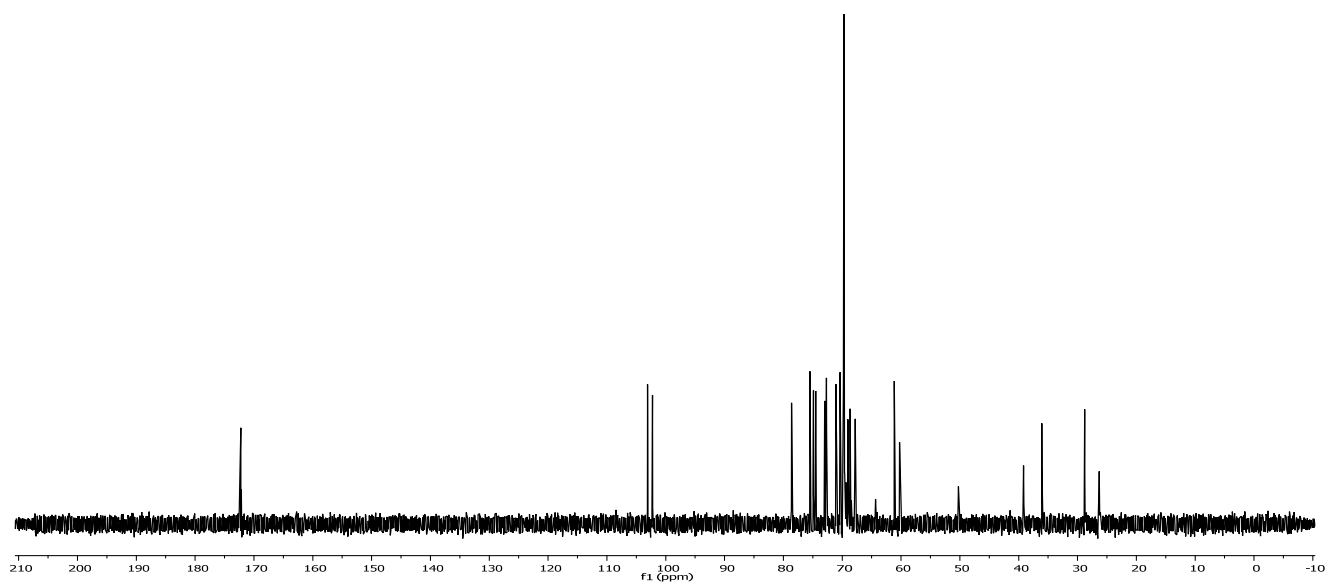
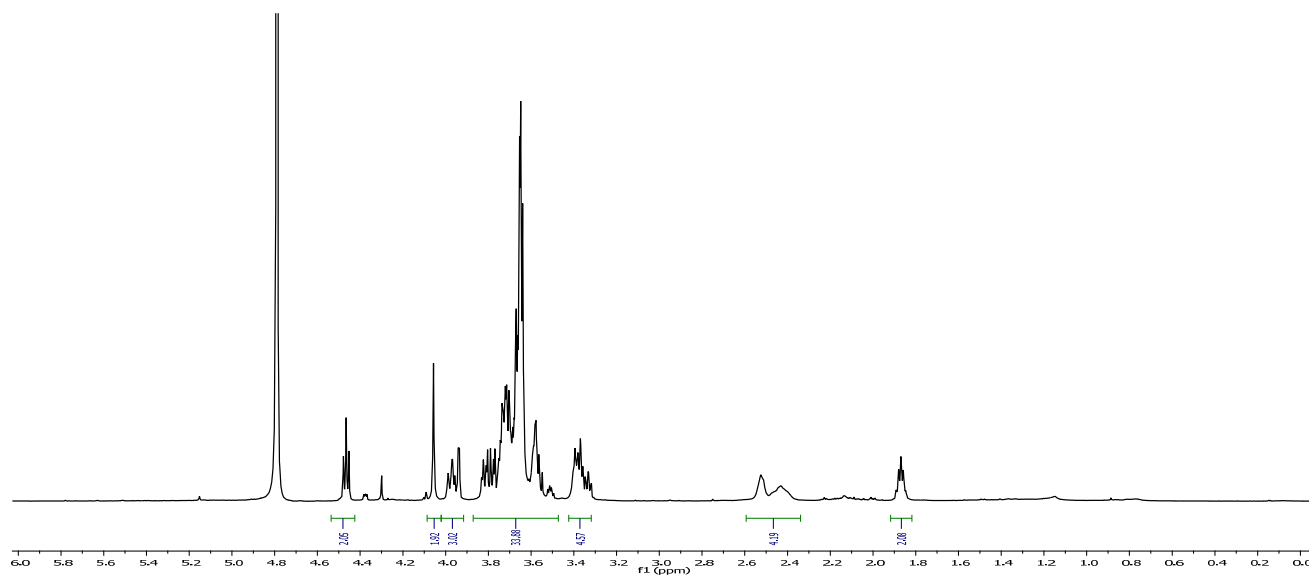
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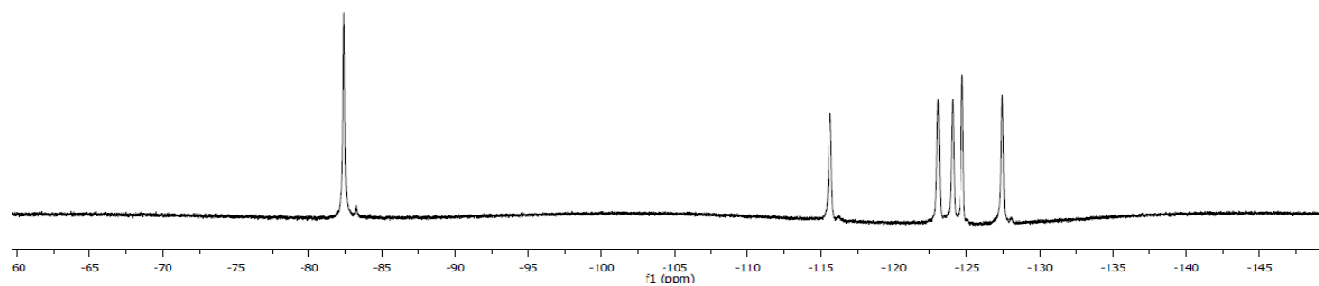
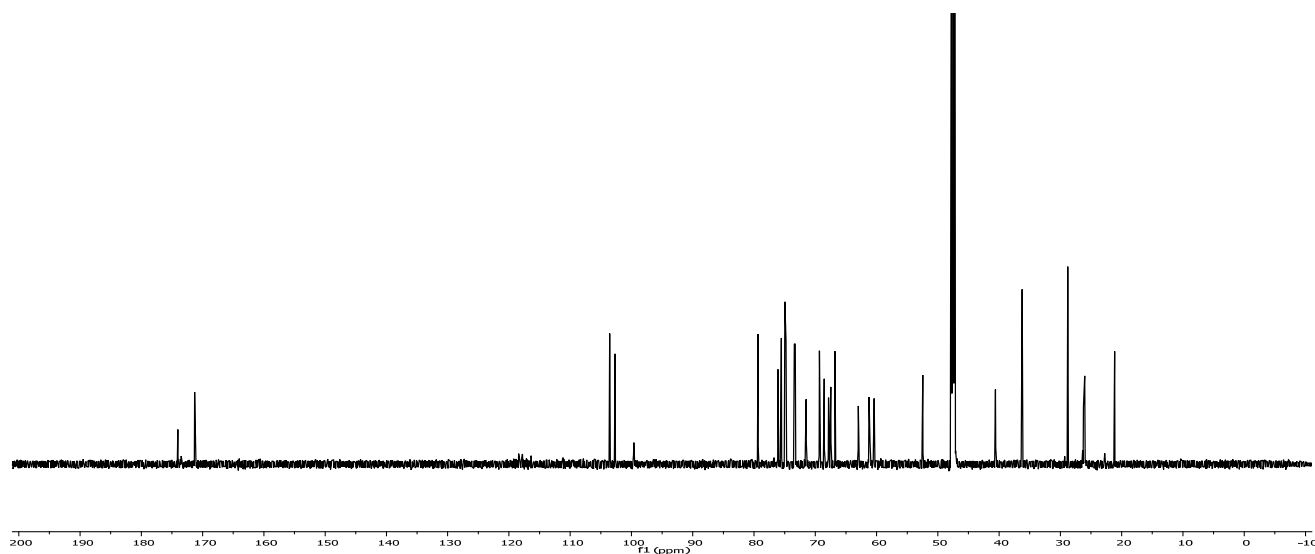
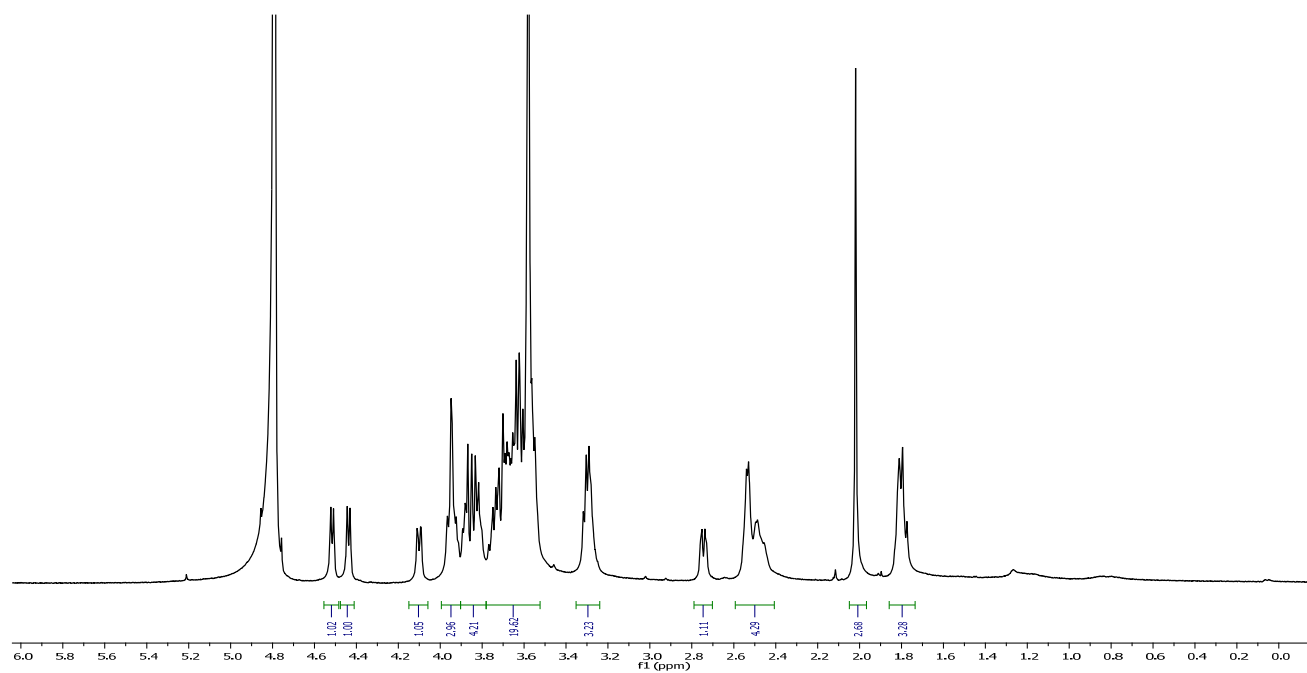
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$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Lac $\beta$ ProNH-HEG- $\text{C}_6\text{F}_{13}$  (**8**)

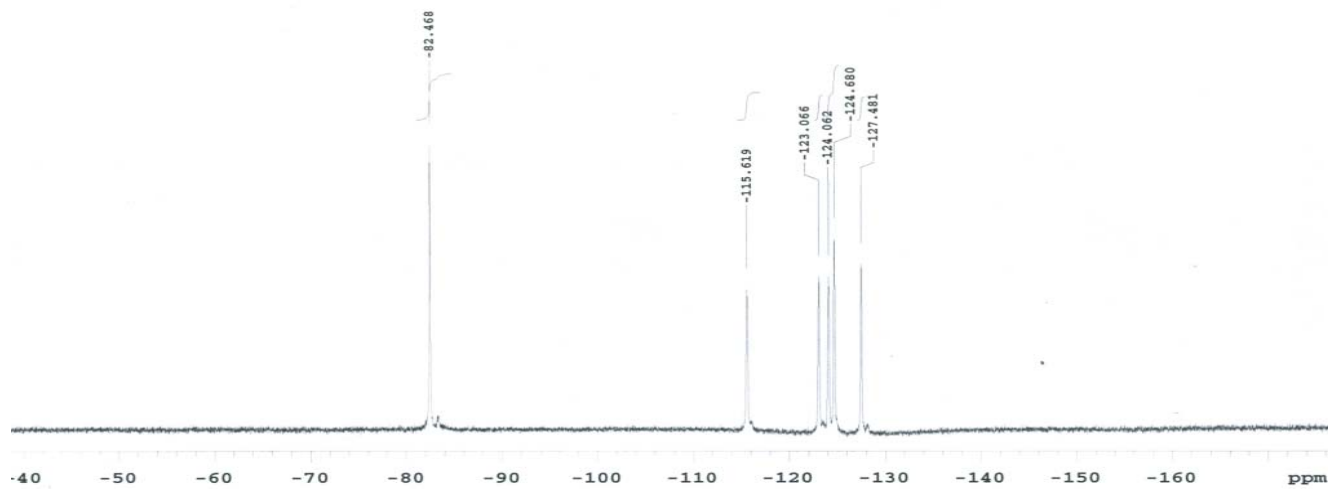
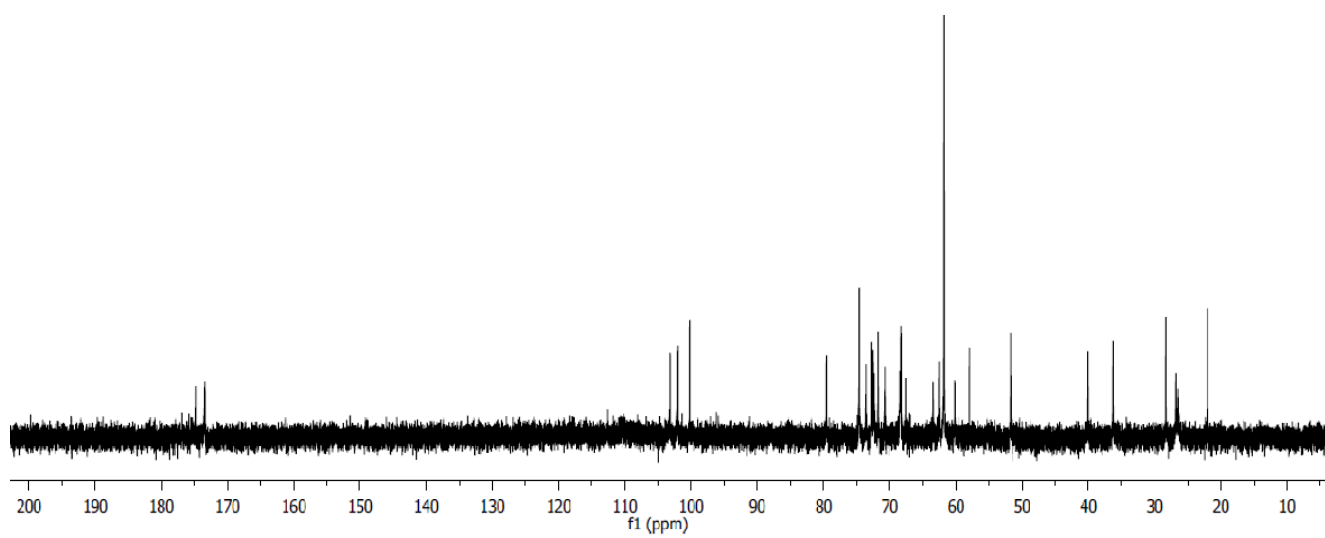
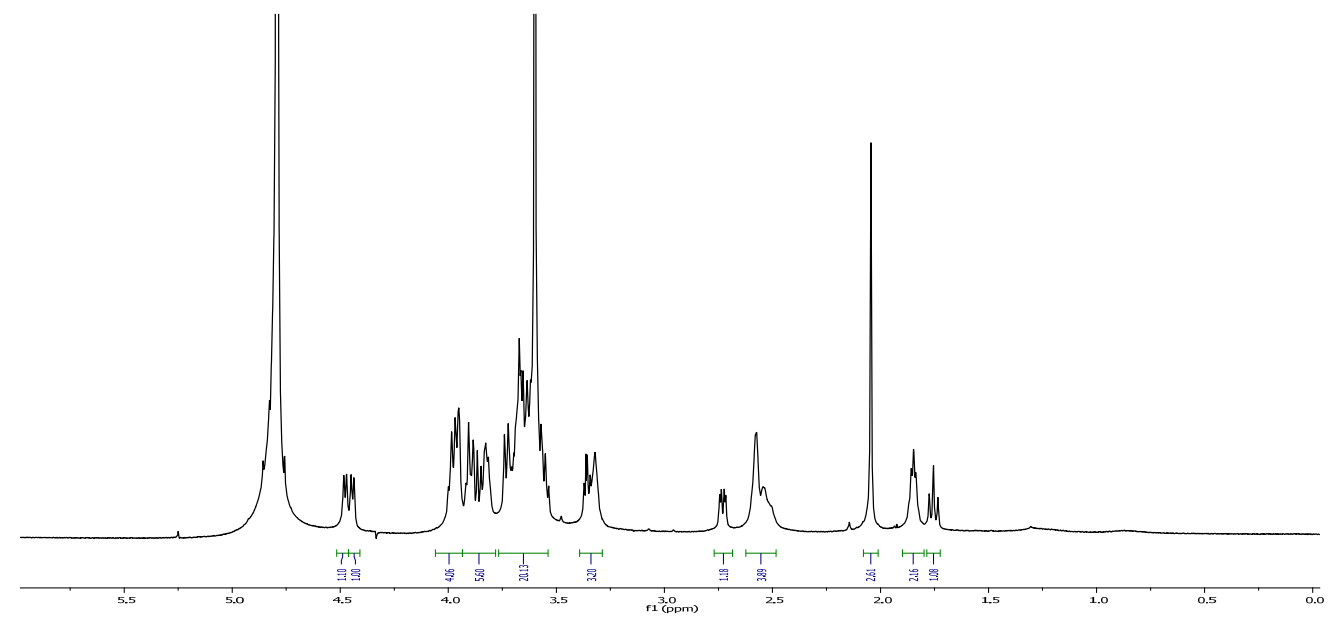


$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Neu5Ac $\alpha$ 2-3Lac $\beta$ ProNH-C $_6\text{F}_{13}$  (**11**)





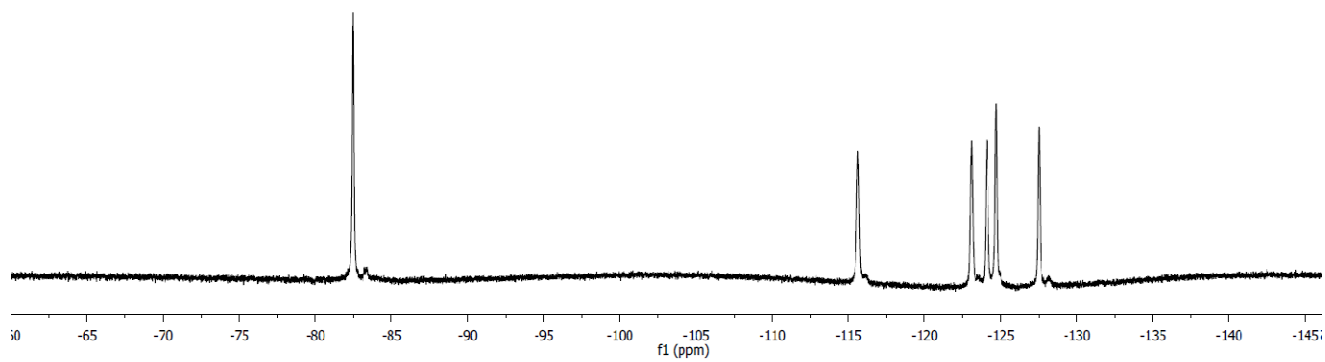
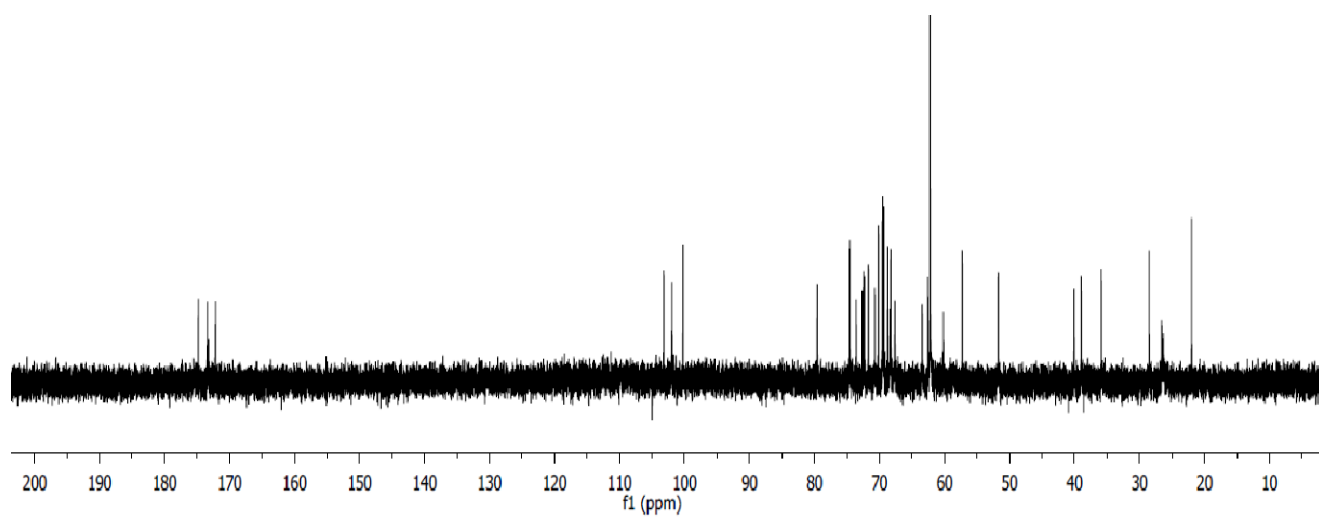
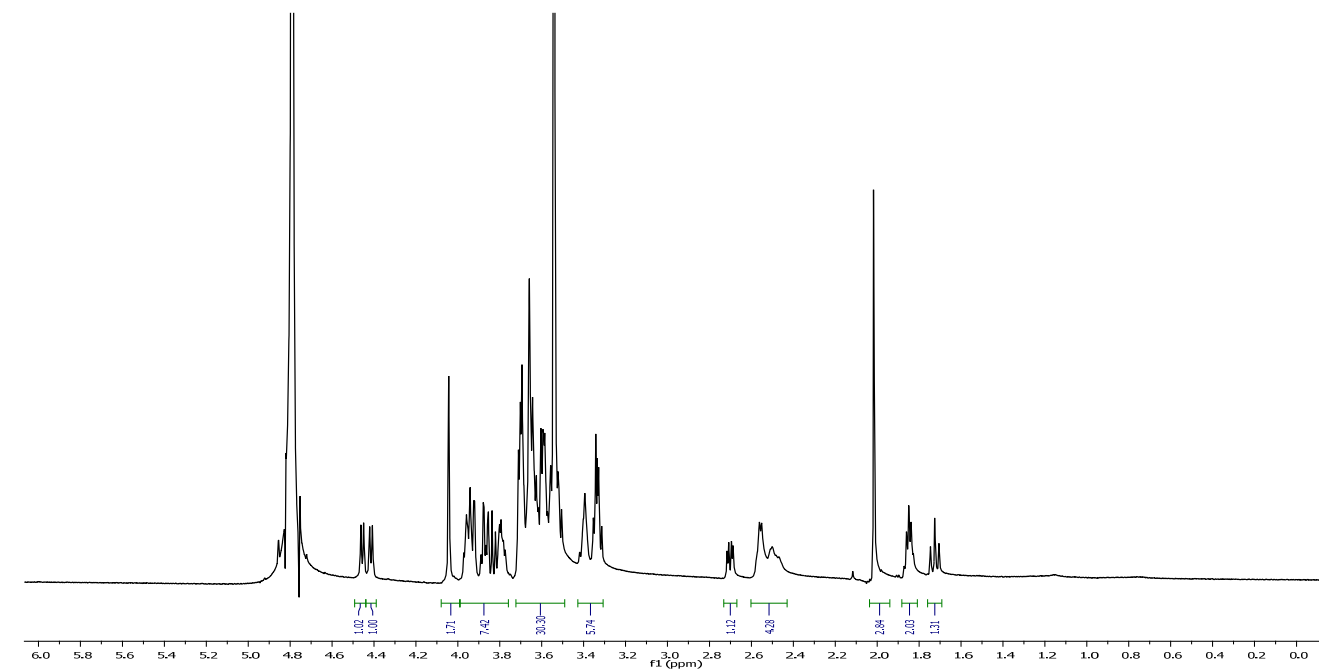
$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Neu5Ac $\alpha$ 2-6Lac $\beta$ ProNH-C $_6\text{F}_{13}$  (**14**)







$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Neu5Ac $\alpha$ 2-6Lac $\beta$ ProNH-TEG-C $_6\text{F}_{13}$  (**27**)



$^1\text{H}$ ,  $^{13}\text{C}$ , and  $^{19}\text{F}$  NMR spectra of Gal $\alpha$ 1-3Lac $\beta$ ProNH-TEG-C $_6$ F $_{13}$  (**29**)

