

## Site-selective chemoenzymatic construction of synthetic glycoproteins using endoglycosidases

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## 1. General considerations

Melting points (m.p.) were recorded on a Leica Galen III hot stage microscope equipped with a Testo 720 thermocouple probe and are uncorrected. Proton nuclear magnetic resonance ( $^1\text{H}$  NMR) spectra were recorded on a Bruker AV400 (400 MHz) or a Bruker AVII500 (500 MHz) spectrometer, as indicated. Carbon nuclear magnetic resonance ( $^{13}\text{C}$  NMR) spectra were recorded on a Bruker AV400 (100 MHz) spectrometer or on a Bruker AVII500 (125 MHz) spectrometer, as indicated. NMR Spectra were fully assigned using COSY, HMQC, HMBC and NOESY. All chemical shifts are quoted on the  $\delta$  scale in ppm using residual solvent as the internal standard ( $^1\text{H}$  NMR:  $\text{CDCl}_3 = 7.26$ ,  $\text{CD}_3\text{OD} = 4.87$ ;  $\text{DMSO-}d_6 = 2.50$  and  $^{13}\text{C}$  NMR:  $\text{CDCl}_3 = 77.0$ ;  $\text{CD}_3\text{OD} = 49.0$ ;  $\text{DMSO-}d_6 = 39.5$ ). Coupling constants ( $J$ ) are reported in Hz with the following splitting abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, and a = apparent.

Infrared (IR) spectra were recorded on a Bruker Tensor 27 Fourier Transform spectrophotometer using thin films on NaCl plates for liquids and oils and KBr discs for solids and crystals. Absorption maxima ( $\nu_{\text{max}}$ ) are reported in wavenumbers ( $\text{cm}^{-1}$ ).

Low resolution mass spectra (LRMS) were recorded on a Waters Micromass LCT Premier TOF spectrometer using electrospray ionization (ESI) and high resolution mass spectra (HRMS) were recorded on a Bruker MicroTOF ESI mass spectrometer. Nominal and exact  $m/z$  values are reported in Daltons.

Optical rotations were measured on a Perkin–Elmer 241 polarimeter with a path length of 1.0 dm and are reported with implied units of  $10^{-1} \text{ deg cm}^2 \text{ g}^{-1}$ . Concentrations (c) are given in g/100 ml.

Thin layer chromatography (TLC) was carried out using Merck aluminium backed sheets coated with 60F254 silica gel. Visualization of the silica plates was achieved using a UV lamp ( $\lambda_{\text{max}} = 254 \text{ nm}$ ), and/or ammonium molybdate (5% in 2 M  $\text{H}_2\text{SO}_4$ ), and/or potassium permanganate (5%  $\text{KMnO}_4$  in 1 M NaOH with 5% potassium carbonate). Flash column chromatography was carried out using BDH 40–63  $\mu\text{m}$

silica gel (VWR). Mobile phases are reported in relative composition (*e.g.* 1:2:4 H<sub>2</sub>O/*i*PrOH/EtOAc).

Anhydrous solvents were purchased from Fluka or Acros. Triethylamine was stored over NaOH pellets. All other solvents were used as supplied (Analytical or HPLC grade), without prior purification. Distilled water was used for chemical reactions and Milli-QR purified water for protein manipulations. Reagents were purchased from Aldrich and used as supplied, unless otherwise indicated. 'Petrol' refers to the fraction of light petroleum ether boiling in the range 40–60 °C. All reactions using anhydrous conditions were performed using flame-dried apparatus under an atmosphere of argon or nitrogen. Brine refers to a saturated solution of sodium chloride. Anhydrous magnesium sulfate (MgSO<sub>4</sub>) was used as drying agents after reaction workup, as indicated.

DOWEX 50WX8 (H<sup>+</sup> form) was conditioned as follows: 100 g of the commercial resin was placed in a 500 mL sintered filter funnel and allowed to swell with 200 mL of acetone for 5 minutes. The solvent was removed by suction and the resin was washed successively with 800 mL of acetone, 500 mL methanol, 500 mL 5 M HCl, and then 1 L of water or until the pH of filtrate was ~ 7, as indicated by pH paper. The resin was partially dried on the filter and then stored and used as needed.

In addition to those specified above, the following abbreviations, designations, and formulas are used throughout the Supporting Information:

MeOH = methanol	H <sub>2</sub> O = water	Et <sub>2</sub> O = diethyl ether
EtOAc = ethyl acetate	CH <sub>2</sub> Cl <sub>2</sub> = dichloromethane	DMF = dimethylformamide
<i>i</i> PrOH = isopropanol	PBu <sub>3</sub> = tributylphosphine	Et <sub>3</sub> N = triethylamine
Boc = <i>tert</i> -butoxycarbonyl	K <sub>2</sub> CO <sub>3</sub> = potassium carbonate	Cs <sub>2</sub> CO <sub>3</sub> = cesium carbonate
NaHCO <sub>3</sub> = sodium bicarbonate	NaOH = sodium hydroxide	NH <sub>4</sub> Cl = ammonium chloride
NH <sub>4</sub> OH = ammonium hydroxide	aq. = aqueous	sat. = saturated

### *Solid Phase Peptide Synthesis*

Peptides were synthesized by means of Fmoc-chemistry on Rink amide MBHA-polystyrene resin [1% divinyl benzene, GL Biochem] using a microwave assisted Liberty CEM peptide synthesizer. Side-chain protection for the amino acids was *t*Bu for Asp, Ser, Thr; Trt for Asn, Cys, Gln, His and Boc for Lys.

Standard steps included:

*Fmoc deprotection*: resin treated with 20% piperidine in DMF (10 mL) and irradiated at 60 W for 35 s followed by a second portion of 20% piperidine/DMF (10 mL) and irradiate at 65 W for 180 s (max. temp. 77 °C).

*Coupling*: resin treated with protected amino acid (5 mL of a 0.2 M solution in DMF), HBTU (2 mL of a 0.45 M solution in DMF), DIEA (1 mL of a 2 M solution in NMP) and irradiated at 25 W for 300 s (max. temp. 75 °C). “His/Cys cycle” uses a modified method in which irradiation was carried out at 25 W for 11 min (max. temp. 53 °C).

*Capping*: resin treated with 20% acetic anhydride in DMF (10 mL) and irradiated at 50 W for 60 s. (max. Temp. 65 °C).

*Cleavage and side-chain deprotection*: resin treated with TFA/TIS/H<sub>2</sub>O/EDT (94:1:2.5:2.5, 10 mL) and irradiated at 15 W for 18 min (max. temp. 38 °C).

*Work up*: the cleavage peptide solution was precipitated with iced Et<sub>2</sub>O (20 mL), centrifuged (3000 rpm, 5 min) and decanted. New washes with iced Et<sub>2</sub>O (20 mL) were repeated twice. The pellet was then dissolved in water and lyophilized.

### *HPLC analysis*

HPLC was performed on a Dionex UltiMate 3000 instrument coupled to a UltiMate 3000 variable wavelength detector, using Chromeleon software (version 6.80). Analytical analyses were carried out using a Phenomenex Onyx Monolithic C18 (100 × 4.6mm) column and Phenomenex Synergi 4u Fusion-RP 80A (100 × 4.6mm) column. Preparative purifications were carried out using a Phenomenex Onyx Monolithic C18 (100 × 10mm) column and Phenomenex Synergi 4u Fusion-RP 80A (100 × 21.20mm) column. Water (solvent A) and acetonitrile (solvent B), each containing 0.1% TFA, were used as the mobile phase in all the cases.

### *Protein and peptide Mass Spectrometry*

Liquid chromatography-mass spectrometry (LC-MS) was performed on a Micromass LCT (ESITOF-MS) coupled to a Waters Alliance 2790 HPLC using a Phenomenex Jupiter C4 column (250 × 4.6 mm × 5 μm) for proteins, and Phenomenex Synergi 4u Hydro-RP 80A (150 × 2mm) and Phenomenex Onyx Monolithic C18 (100 × 4.6mm) for peptides. Water (solvent A) and acetonitrile (solvent B), each containing 0.1% formic acid, were used as the mobile phase at a flow rate of 1.0 mL min<sup>-1</sup> (0.2 mL min<sup>-1</sup> for Hydro-RP column). The gradient was programmed as follows: 95% A (5 min isocratic) to 100% B after 15 min then isocratic for 5 min. The electrospray source was operated with a capillary voltage of 3.2 kV and a cone voltage of 25 V for subtilisin *Bacillus lentus* (SBL) and Np276, 35 V for β-galactosidase (SsβG) and 10 V for peptides. Nitrogen was used as the nebulizer and desolvation gas at a total flow of 600 L hr<sup>-1</sup>. Spectra were calibrated using a calibration curve constructed from a minimum of 17 matched peaks from the multiply charged ion series of equine myoglobin obtained at a cone voltage of 25 V. Total mass spectra were reconstructed from the ion series using the MaxEnt algorithm preinstalled on MassLynx software (v. 4.0 from Waters) according to the manufacturer's instructions.

### *Materials*

GST-Endo-A fusion protein was overproduced in *Escherichia coli* strain BL21(DE3) and purified by affinity column (Glutathione Sepharose 4B) as previously described.<sup>1</sup> *N*-α-Fmoc-L-Asn[GlcNAc]-OH was prepared as previously reported.<sup>2,3</sup> (*S*)-4-Azido-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid, (*S*)-2-(9H-fluoren-9-ylmethoxycarbonylamino)hex-5-ynoic acid, and propargyl 2-*N*-acetylamino-2-deoxy-β-D-glucopyranoside were prepared as previously described.<sup>4</sup> Precursors 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-β-D-glucopyranosyl azides along with 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-1-thio-β-D-manno, and glucopyranose were prepared following an adapted methodology described by Albert and Dax.<sup>5</sup> 1-*O*-(2'-Propargyl)-α-D-mannopyranoside was prepared as previously described.<sup>6</sup> 2-Acetamido-2-deoxy-1-thio-β-D-glucopyranose and 1-thio-β-D-glucopyranose were prepared as previously reported.<sup>7</sup> Disaccharide oxazoline **1** was prepared as previously reported.<sup>8</sup> SsβG Aha43 C344S 9Ile and SsβG Hpg1 Hpg43 C344S 9Ile were cloned and expressed as previously described.<sup>4</sup> Tristriazole ligand triethyl

2,2',2''-(4,4',4''-nitrilotris(methylene)tris(1H-1,2,3-triazole-4,1-diyl))triacetate was prepared as previously described.<sup>9</sup>

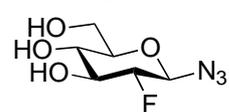
## 2. Experimental section

### 2.1. Preparation of glycoamino acids 3a–11a

#### General procedure for the synthesis of 2-deoxy-2-fluoro-glycosyl azides

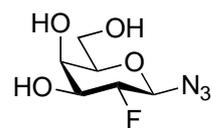
The corresponding glycopyranosyl azide (1 mmol) was treated with 0.1M NaOMe in MeOH (7 mL/mmol) at room temperature. The reaction mixture was stirred at the same temperature for 15 minutes and neutralized with Dowex (H<sup>+</sup> 50WX8-200). The ion exchanger was filtered off and washed with MeOH. The resulting solution was concentrated under reduced pressure and the residue purified by chromatographic techniques.

#### 2-Deoxy-2-fluoro- $\beta$ -D-glucopyranosyl azide<sup>[11]</sup>



The title compound was prepared following the general procedure above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl azide<sup>5</sup> (25 mg, 0.075 mmol) and 0.1M NaOMe in MeOH (525  $\mu$ L). After concentration under reduced pressure crude product (15.5 mg, 100%) was obtained as a white foam.  $R_f$  (7:1 EtOAc/MeOH): 0.43;  $[\alpha]_D^{20}$ : -9.5 (0.9, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta$  in ppm: 4.83 (dd, 1H,  $J_{1,2}$  = 8.8 Hz,  $J_{1,F}$  = 2.3 Hz, H-1), 3.93 (ddd, 1H,  $J_{2,F}$  = 51.6 Hz,  $J_{1,2}$  =  $J_{2,3}$  = 8.8 Hz, H-2), 3.88 (dd,  $J_{6a,b}$  = 12 Hz,  $J_{5,6a}$  = 2.2 Hz, H-6a), 3.71–3.60 (m, 2H, H-3,6b), 3.45–3.33 (m 2H, H-4,5); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125.8 MHz)  $\delta$  in ppm: 93.3(d,  $J_{2,F}$  = 186.9 Hz, C-2), 89.2 (d,  $J_{1,F}$  = 22.9 Hz, C-1), 80.4 (C-5), 76.4 (d,  $J_{3,F}$  = 16.2 Hz, C-3), 70.9 (d,  $J_{4,F}$  = 8.6 Hz, C-4), 62.4 (C-6); <sup>19</sup>F NMR (CD<sub>3</sub>OD, 470.4 MHz)  $\delta$  in ppm: -199.4 (ddd,  $J_{2,F}$  = 51.6 Hz,  $J_{3,F}$  = 14.9 Hz,  $J_{1,F}$  = 2.3 Hz, F-2); FT-IR (KBr)  $\nu$  in cm<sup>-1</sup>: 3358, 3075, 2733, 2687, 2105; HRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>6</sub>H<sub>9</sub>FN<sub>3</sub>O<sub>4</sub> ( $m/z$ ): calc. 206.0583; found 206.0583; spectroscopic data was identical to that previously reported.<sup>10</sup>

#### 2-Deoxy-2-fluoro- $\beta$ -D-galactopyranosyl azide



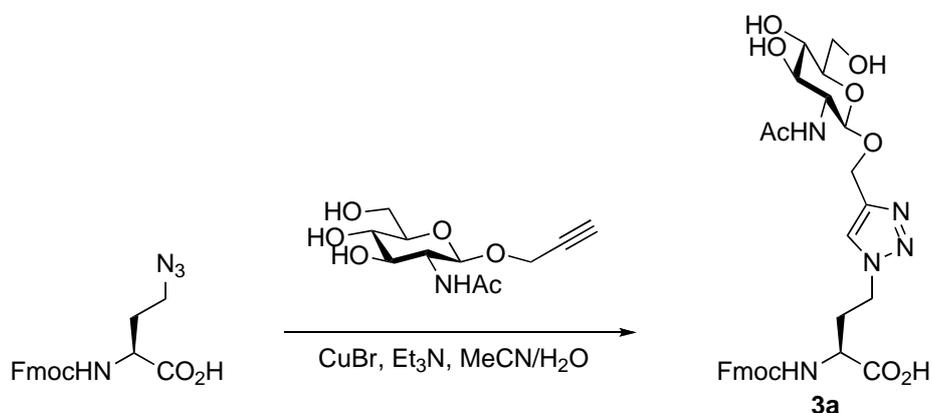
The title compound was prepared following the general procedure above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro- $\beta$ -D-galactopyranosyl azide<sup>11</sup> (27.5 mg, 0.083 mmol) and 0.1M NaOMe in MeOH (578  $\mu$ L). After concentration under reduced pressure crude product (17.1 mg, 100%) was obtained as a white solid.  $R_f$  (7:1 EtOAc/MeOH): 0.43; mp: 92–94 °C;  $[\alpha]_D^{20}$ : +2.9 (0.53, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz)  $\delta$  in ppm: 4.74 (dd, 1H,

$J_{1,2} = 8.4$  Hz,  $J_{1,F} = 3.7$  Hz, H-1), 4.29 (ddd, 1H,  $J_{2,F} = 52.0$  Hz,  $J_{1,2} = J_{2,3} = 8.4$  Hz, H-2), 3.92 (m, H-4), 3.80–3.66 (m, 4H, H-3, 5, 6a,b);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125.8 MHz)  $\delta$  in ppm: 92.5 (d,  $J_{2,F} = 183.1$  Hz, C-2), 89.6 (d,  $J_{1,F} = 23.8$  Hz, C-1), 79.2 (C-5), 73.3 (d,  $J_{3,F} = 17.2$  Hz, C-3), 71.0 (d,  $J_{4,F} = 8.6$  Hz, C-4), 62.4 (C-6);  $^{19}\text{F}$  NMR ( $\text{CD}_3\text{OD}$ , 470.4 MHz)  $\delta$  in ppm:  $-207.9$  (dddd,  $J_{2,F} = 52.0$  Hz,  $J_{3,F} = 13.9$  Hz,  $J_{1,F} = 3.7$  Hz,  $J_{4,F} = 3.5$  Hz, F-2); FT-IR (KBr)  $\nu$  in  $\text{cm}^{-1}$ : 3356, 2925, 2122, 1373, 1251, 1071; HRMS (TOF ES $^-$ ) for (M-H)  $\text{C}_6\text{H}_9\text{FN}_3\text{O}_4$  ( $m/z$ ): calc. 206.0583; found 206.0582.

## 2-Deoxy-2-fluoro- $\beta$ -D-mannopyranosyl azide

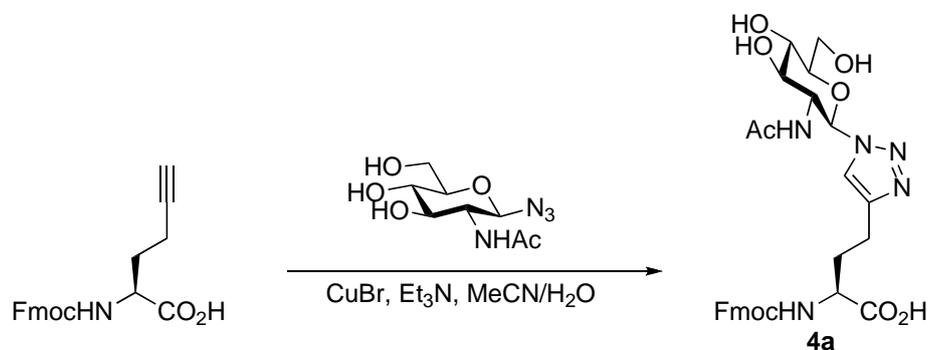
 The title compound was prepared following the general procedure above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro- $\beta$ -D-mannopyranosyl azide (89 mg, 0.267 mmol) and 0.1M NaOMe in MeOH (1.9 mL). After concentration under reduced pressure the residue was purified by column chromatography (7:1 EtOAc/MeOH) to afford the title compound (19.9 mg, 36%) as a white hygroscopic solid.  $R_f$  (7:1 EtOAc/MeOH): 0.33;  $[\alpha]_D^{20}$ : +3.2 (0.60, MeOH);  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  in ppm: 4.71 (d, 1H,  $J_{2,F} = 51.9$  Hz), 4.63 (d, 1H,  $J_{1,F} = 22.2$  Hz, H-1), 3.93 (d,  $J_{6a,b} = 12.1$  Hz, H-6a), 3.73 (dd,  $J_{6a,b} = 12.1$  Hz,  $J_{5,6b} = 6.3$  Hz, H-6b), 3.62–3.50 (m, 2H, H-3,4), 3.38 (m, 1H, H-5);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ , 125.8 MHz)  $\delta$  in ppm: 93.2 (d,  $J_{2,F} = 185.1$  Hz, C-2), 86.6 (d,  $J_{1,F} = 15.9$  Hz, C-1), 80.8 (C-5), 74.0 (d,  $J_{3,F} = 17.5$  Hz, C-3), 68.2 (d,  $J_{4,F} = 1.9$  Hz, C-4), 62.7 (C-6);  $^{19}\text{F}$  NMR ( $\text{CD}_3\text{OD}$ , 470.4 MHz)  $\delta$  in ppm:  $-220.2$  (m, F-2); FT-IR (KBr)  $\nu$  in  $\text{cm}^{-1}$ : 3357, 2920, 2122, 1071; HRMS (TOF ES $^-$ ) for (M-H)  $\text{C}_6\text{H}_9\text{FN}_3\text{O}_4$  ( $m/z$ ): calc. 206.0583; found 206.0584.

## (*S*)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{4-[(2-*N*-acetylamino-2-deoxy- $\beta$ -D-glucopyranosyloxy)methyl]-[1,2,3]-triazol-1-yl}butanoic acid (**3a**)



To a deoxygenated (argon was bubbled for 20 min) solution of propargyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (57 mg, 0.219 mmol) and (*S*)-4-Azido-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid (73 mg, 0.199 mmol) in MeCN (7 mL), cuprous bromide (0.5 mL of 13 mg/mL deoxygenated MeCN, 0.045 mmol) was added, followed by Et<sub>3</sub>N (28  $\mu$ L, 0.201 mmol) and water (0.5 mL). After 22 h, TLC (3:2 EtOAc/MeOH) indicated the formation of a product ( $R_f$  0.09) and almost complete consumption of the amino acid starting material ( $R_f$  0.62). The mixture was concentrated *in vacuo* and purified by flash column chromatography (7:3 to 3:2 EtOAc/MeOH) to afford the triazole **3a** (44 mg, 35%) as a white solid;  $[\alpha]_D^{20}$ : -10.2 (0.12, MeOH); <sup>1</sup>H NMR (500 MHz, MeOD):  $\delta$  7.96 (s, 1H, ArH<sub>triazole</sub>), 7.74-7.62 (m, 4H, ArH), 7.35-7.24 (m, 4H, ArH), 4.81 (d,  $J$  = 12.7 Hz, 1H, OCH<sub>2</sub>), 4.73 (d,  $J$  = 12.6 Hz, 1H, OCH<sub>2</sub>), 4.44-4.38 (m, 3H, H-1, H $\gamma$ , H $\gamma'$ ), 4.36-4.29 (m, 2H, CH<sub>2</sub><sub>Fmoc</sub>), 4.17 (t,  $J$  = 6.6 Hz, 1H, CH<sub>Fmoc</sub>), 3.96 (dd,  $J$  = 7.7 Hz,  $J$  = 4.8 Hz, 1H, H $\alpha$ ), 3.87 (ad,  $J$  = 11.7 Hz, 1H, H-6), 3.69-3.65 (m, 2H, H-2, H-6'), 3.43-3.39 (m, 1H, H-3), 3.29-3.27 (m, 2H, H-4, H-5), 2.42-2.35 (m, 1H, H $\beta$ ), 2.20-2.13 (m, 1H, H $\beta'$ ), 1.88 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  176.9 (C=O), 172.9 (CO<sub>2</sub>H), 157.3 (OC(O)N), 144.4, 144.2, 144.1, 141.62, 141.58 (4 $\times$ Ar-C, C=C<sub>triazole</sub>), 127.8, 127.2, 125.3, 119.9 (8 $\times$ Ar-H), 124.9 (CH<sub>triazole</sub>), 100.1 (C-1), 77.1 (C-5), 75.1 (C-3), 71.1 (C-4), 66.8 (CH<sub>2</sub><sub>Fmoc</sub>), 61.7 (C-6), 61.3 (OCH<sub>2</sub>), 56.1 (C-2), 54.1 (C $\alpha$ ), 47.56 (C $\gamma$ ), 47.49 (CH<sub>Fmoc</sub>), 33.9 (C $\beta$ ), 22.1 (CH<sub>3</sub>); HRMS (ES<sup>+</sup>) for (M+Na) C<sub>30</sub>H<sub>35</sub>N<sub>5</sub>NaO<sub>10</sub> ( $m/z$ ): calc. 648.2282; found 648.2262.

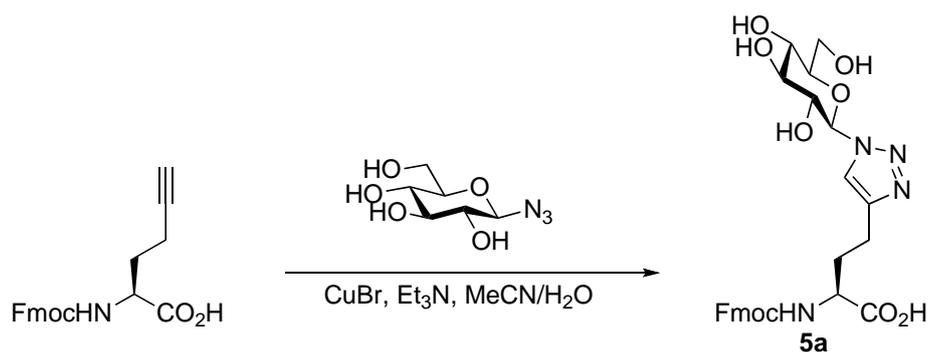
**(*S*)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{1-(2-*N*-acetylamino-2-deoxy- $\beta$ -D-glucopyranosyl)[1,2,3]-triazol-4-yl}butanoic acid (**4a**)**



To a deoxygenated (argon was bubbled for 20 min) solution of 2-*N*-acetylamino-2-deoxy- $\beta$ -D-glucopyranosyl azide (40 mg, 0.162 mmol) and (*S*)-2-(9H-fluoren-9-

ylmethoxycarbonylamino)hex-5-ynoic acid (51 mg, 0.146 mmol) in MeCN (5 mL), cuprous bromide (0.4 mL of 13 mg/mL deoxygenated MeCN, 0.036 mmol) was added, followed by Et<sub>3</sub>N (22 μL, 0.158 mmol) and water (0.4 mL). After 21 h, TLC (3:2 EtOAc/MeOH) indicated the formation of a product (R<sub>f</sub> 0.04) and almost complete consumption of the amino acid starting material (R<sub>f</sub> 0.68). The mixture was concentrated *in vacuo* and purified by flash column chromatography (4:1 to 3:2 EtOAc/MeOH) to afford the triazole **4a** (54.2 mg, 62%) as a white solid. [α]<sub>D</sub><sup>20</sup>: -7.0 (0.13, MeOH); <sup>1</sup>H NMR (400 MHz, MeOD): δ 7.89 (s, 1H, ArH<sub>triazole</sub>), 7.70-7.57 (m, 4H, ArH), 7.31-7.20 (m, 4H, ArH), 5.71 (d, *J* = 9.8 Hz, 1H, H-1), 4.30-4.21 (m, 2H, CH<sub>2</sub> Fmoc), 4.18-4.11 (m, 2H, H-2, CH<sub>Fmoc</sub>), 4.00 (dd, *J* = 7.7 Hz, *J* = 4.9 Hz, 1H, Hα), 3.78 (dd, *J* = 11.9 Hz, *J* = 6.2 Hz, 1H, H-6), 3.69-3.65 (m, 2H, H-3, H-6'), 3.51-3.49 (m, 2H, H-4, H-5), 2.69 (t, *J* = 7.7 Hz, 2H, H<sub>γ</sub>, H<sub>γ'</sub>), 2.15-2.05 (m, 1H, H<sub>β</sub>), 1.97-1.89 (m, 1H, H<sub>β'</sub>), 1.71 (s, 3H, CH<sub>3</sub>); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 179.1 (C=O), 173.6 (CO<sub>2</sub>H), 158.3 (OC(O)N), 148.7, 148.5, 145.5, 145.3, 142.6 (4×Ar-C, C=C<sub>triazole</sub>), 128.8, 128.2, 126.3, 120.9 (8×Ar-H), 122.1 (CH<sub>triazole</sub>), 87.9 (C-1), 81.3 (C-4), 75.7 (C-3), 71.4 (C-5), 67.9 (CH<sub>2</sub> Fmoc), 62.4 (C-6), 57.0 (Cα), 56.9 (C-2), 48.4 (CH<sub>Fmoc</sub>), 33.8 (C<sub>β</sub>), 22.9, (C<sub>γ</sub>), 22.7 (CH<sub>3</sub>); HRMS (ES<sup>+</sup>) for (M+Na) C<sub>29</sub>H<sub>33</sub>N<sub>5</sub>NaO<sub>9</sub> (*m/z*): calc. 618.2176; found 618.2169.

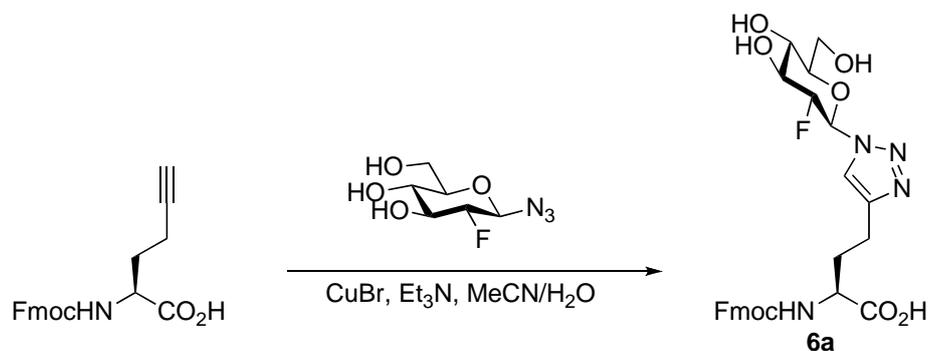
**(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{1-(β-D-glucopyranosyl)[1,2,3]-triazol-4-yl}butanoic acid (5a)**



To a deoxygenated (argon was bubbled for 20 min) solution of β-D-glucopyranosyl azide (35 mg, 0.172 mmol) and (S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)hex-5-ynoic acid (51 mg, 0.147 mmol) in MeCN (5 mL), cuprous bromide (0.5 mL of 10 mg/mL deoxygenated MeCN, 0.036 mmol) was added, followed by Et<sub>3</sub>N (36 μL, 0.258 mmol) and water (0.42 mL). After 24h, fresh cuprous bromide (0.4 mL of

10mg/mL solution) was added. After 3 days, TLC (3:2 EtOAc/MeOH) indicated the formation of a product ( $R_f$  0.19) and almost complete consumption of the amino acid starting material ( $R_f$  0.68). The mixture was concentrated *in vacuo* and purified by flash column chromatography (4:1 to 3:2 EtOAc/MeOH), and the isolated compound was dissolved in water and lyophilized to afford the triazole **5a** (16.5 mg, 20%) as a white solid.  $[\alpha]_D^{20}$ :  $-10.4$  (0.21, MeOH);  $^1\text{H}$  NMR (500 MHz, MeOD): 7.97 (s, 1H, ArH<sub>triazole</sub>), 7.79-7.66 (m, 4H, ArH), 7.39-7.29 (m, 4H, ArH), 5.54 (dd,  $J = 9.8$  Hz,  $J = 2.7$  Hz, 1H, H-1), 4.38-4.31 (m, 2H, CH<sub>2</sub> Fmoc), 4.22 (t,  $J = 6.9$  Hz, 1H, CH<sub>Fmoc</sub>), 4.07 (m, 1H, H $\alpha$ ), 4.38-4.31 (m, 2H, H-2, H-6), 3.69 (dd,  $J = 12.3$  Hz,  $J = 5.2$  Hz, 1H, H-6'), 3.57-3.47 (m, 3H, H-3, H-4, H-5), 2.78 (t,  $J = 7.7$  Hz, 2H, H $\gamma$ , H $\gamma'$ ), 2.22-2.15 (m, 1H, H $\beta$ ), 2.04-1.97 (m, 1H, H $\beta'$ );  $^{13}\text{C}$  NMR (125 MHz, MeOD):  $\delta$  179.1 (CO<sub>2</sub>H), 158.3 (C(O)N), 148.7, 145.5, 145.3, 142.6 (4 $\times$ Ar-C, C=C<sub>triazole</sub>), 128.8, 128.2, 126.3, 120.9 (8 $\times$ Ar-H), 122.5 (CH<sub>triazole</sub>), 89.6 (C-1), 81.1 (C-5), 78.4 (C-3), 74.1 (C-2), 70.9 (C-4), 67.8 (CH<sub>2</sub> Fmoc), 62.5 (C-6), 57.0 (C $\alpha$ ), 48.5 (CH<sub>Fmoc</sub>), 33.9 (C $\beta$ ), 22.8 (C $\gamma$ ); HRMS (ESI<sup>+</sup>) calcd for C<sub>27</sub>H<sub>30</sub>N<sub>4</sub>NaO<sub>9</sub> (M+Na)<sup>+</sup>, 577.1910, found 577.1906.

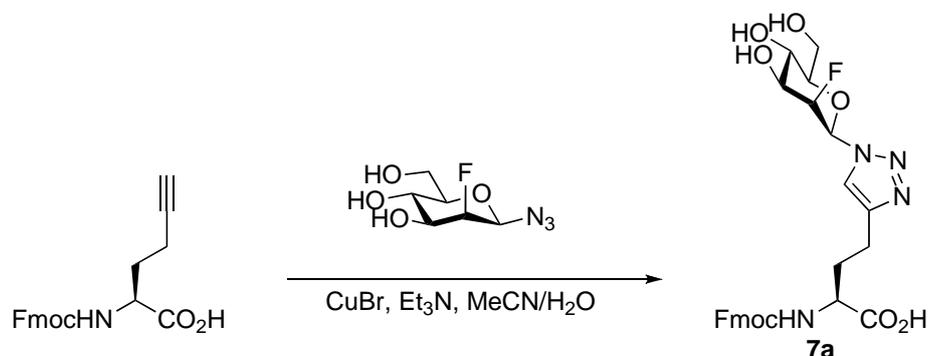
**(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{1-(2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl)[1,2,3]-triazol-4-yl}butanoic acid (**6a**)**



To a deoxygenated (argon was bubbled for 20 min) solution of 2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl azide (13 mg, 0.063 mmol) and (S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)hex-5-ynoic acid (20 mg, 0.057 mmol) in MeCN (2 mL), cuprous bromide (0.2 mL of 10 mg/mL deoxygenated MeCN, 0.014 mmol) was added, followed by Et<sub>3</sub>N (14  $\mu$ L, 0.100 mmol) and water (0.16 mL). After 18 h, TLC (65:35 EtOAc/MeOH) indicated the formation of a product ( $R_f$  0.19) and complete consumption of the amino acid starting material ( $R_f$  0.44). The mixture was

concentrated *in vacuo* and purified by flash column chromatography (7:3 to 3:2 EtOAc/MeOH), and the isolated compound was dissolved in water and lyophilized to afford the triazole **6a** (14 mg, 44%) as a white solid.  $[\alpha]_D^{20}$ : +5.8 (0.03, MeOH);  $^1\text{H}$  NMR (500 MHz, MeOD):  $\delta$  8.05 (s, 1H, ArH<sub>triazole</sub>), 7.79-7.66 (m, 4H, ArH), 7.39-7.28 (m, 4H, ArH), 5.87 (m, 1H, H-1), 4.84-4.73 (m, 1H, H-2), 4.39-4.32 (m, 2H, CH<sub>2</sub> Fmoc), 4.22 (t,  $J = 6.7$  Hz, 1H, CH<sub>Fmoc</sub>), 4.08 (m, 1H, H $\alpha$ ), 3.88-3.80 (m, 2H, H-3, H-6'), 3.69 (dd,  $J_{H6-H6'} = 12.3$  Hz,  $J_{H6-H5} = 5.3$  Hz, 1H, H-6), 3.63-3.59 (m, 1H, H-5), 3.54 (t,  $J = 9.5$  Hz, 1H, H-4), 2.78 (t,  $J = 7.6$  Hz, 2H, H $\gamma$ , H $\gamma'$ ), 2.19 (m, 1H, H $\beta$ ), 2.01 (m, 1H, H $\beta'$ );  $^{13}\text{C}$  NMR (125 MHz, MeOD):  $\delta$  158.3 (OC(O)N), 149.1, 145.5, 145.3, 145.65, 142.63 (4 $\times$ Ar-C, C=C<sub>triazole</sub>), 128.8, 128.2, 126.3, 120.9 (8 $\times$ Ar-H), 122.7 (CH<sub>triazole</sub>), 92.2 (d,  $J_{C2-F} = 188.1$  Hz, C-2), 86.5 (d,  $J_{C1-F} = 24.2$  Hz, C-1), 81.2 (C-5), 76.5 (d,  $J_{C3-F} = 16.7$  Hz, C-3), 70.7 (d,  $J_{C4-F} = 7.9$  Hz, C-4), 67.8 (CH<sub>2</sub> Fmoc), 62.2 (C-6), 57.4 (C $\alpha$ ), 48.3 (CH<sub>Fmoc</sub>), 33.8 (C $\beta$ ), 22.9 (C $\gamma$ );  $^{19}\text{F}\{^1\text{H}\}$  NMR (MeOD, 470.4 MHz):  $\delta$  -200.5 (s, F-2).; HRMS (ES<sup>+</sup>) for (M+Na) C<sub>27</sub>H<sub>29</sub>N<sub>4</sub>NaO<sub>8</sub> ( $m/z$ ): calc. 579.1862; found 579.1860.

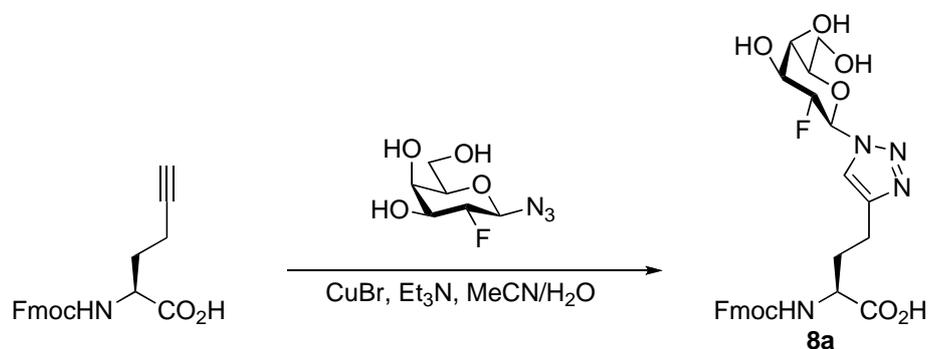
**(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{1-(2-deoxy-2-fluoro- $\beta$ -D-mannopyranosyl)[1,2,3]-triazol-4-yl}butanoic acid (**7a**)**



To a deoxygenated (argon was bubbled for 20 min) solution of 2-deoxy-2-fluoro- $\beta$ -D-mannopyranosyl azide (11 mg, 0.053 mmol) and (S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)hex-5-ynoic acid (17 mg, 0.048 mmol) in MeCN (2 mL), cuprous bromide (0.35 mL of 5 mg/mL deoxygenated MeCN, 0.012 mmol) was added, followed by Et<sub>3</sub>N (12  $\mu$ L, 0.086 mmol) and water (0.13 mL). After 16.5 h, fresh cuprous bromide (0.15 mL, 0.005 mmol) and Et<sub>3</sub>N (4  $\mu$ L, 0.028 mmol) were added. After 44 h, TLC (3:2 EtOAc/MeOH) indicated the formation of a product ( $R_f$  0.18) and almost complete consumption of the amino acid starting material ( $R_f$  0.44).

The mixture was concentrated *in vacuo* and purified by flash column chromatography (3:1 to 3:2 EtOAc/MeOH), and the isolated compound was dissolved in water and lyophilized to afford the triazole **7a** (5.9 mg, 21%) as a white solid.  $[\alpha]_{\text{D}}^{20}$ : -1.4 (0.10, MeOH);  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  in ppm: 7.97 (s, 1H,  $\text{ArH}_{\text{triazole}}$ ), 7.77–7.64 (m, 4H, ArH), 7.37–7.27 (m, 4H, ArH), 6.06 (d,  $J_{1,\text{F}} = 22.1$  Hz, 1H, H-1), 4.92 (d,  $J_{2,\text{F}} = 50.8$  Hz, 1H, H-2), 4.32 (m, 2H,  $\text{CH}_2_{\text{Fmoc}}$ ), 4.20 (m, 1H,  $\text{CH}_{\text{Fmoc}}$ ), 4.07 (m, 1H, H $\alpha$ ), 3.91–3.71 (m, 4H, H-3, H-4, H-6a,b), 3.54 (m, 1H, H-5), 2.74 (m, 2H, H $\gamma$ , H $\gamma'$ ), 2.16 (m, 1H, H $\beta$ ), 1.99 (m, 1H, H $\beta'$ );  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 125 MHz)  $\delta$  in ppm: 158.2 (OC(O)N), 148.8, 145.7, 145.4, 142.8 (4 $\times$ Ar-C, C=C $_{\text{triazole}}$ ), 128.8, 128.3, 126.5, 126.4, 122.9, 121.0 (8 $\times$ Ar-H, CH $_{\text{triazole}}$ ), 91.8 (d,  $J_{2,\text{F}} = 186.5$  Hz, C-2), 86.4 (d,  $J_{1,\text{F}} = 15.8$  Hz, C-1), 81.46, 81.44 (C-5), 73.7 (d,  $J_{3,\text{F}} = 17.7$  Hz, C-3), 67.9, 67.8 (C-4, CH $_{2\text{Fmoc}}$ ), 62.51 (C-6), 57.4 (C $\alpha$ ), 48.3 (CH $_{\text{Fmoc}}$ ), 34.2 (C $\beta$ ), 23.0 (C $\gamma$ );  $^{19}\text{F NMR}$  ( $\text{CD}_3\text{OD}$ , 470.4 MHz)  $\delta$  in ppm: -218.3 (m, F-2); HRMS (ES $^+$ ) for (M+Na) C $_{27}$ H $_{29}$ N $_4$ NaO $_8$  ( $m/z$ ): calc. 579.1862; found 579.1857.

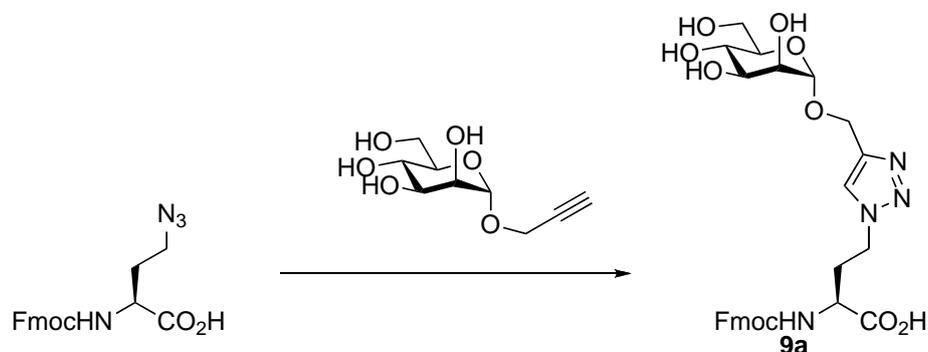
**(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{1-(2-deoxy-2-fluoro- $\beta$ -D-galactopyranosyl)[1,2,3]-triazol-4-yl}butanoic acid (**8a**)**



To a deoxygenated (argon was bubbled for 20 min) solution of 2-deoxy-2-fluoro- $\beta$ -D-galactopyranosyl azide (11 mg, 0.053 mmol) and (S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)hex-5-ynoic acid (16 mg, 0.046 mmol) in MeCN (1 mL), cuprous bromide (0.2 mL of 6 mg/mL deoxygenated MeCN, 0.008 mmol) was added, followed by Et $_3$ N (12  $\mu$ L, 0.086 mmol) and water (0.11 mL). After 2h, fresh cuprous bromide (0.2 mL, 0.008 mmol) was added. After 18 h, TLC (3:2 EtOAc/MeOH) indicated the formation of a product ( $R_f$  0.18) and almost complete consumption of the amino acid starting material ( $R_f$  0.44). The mixture was concentrated *in vacuo* and purified by flash column chromatography (3:1 to 3:2 EtOAc/MeOH), and the isolated compound was dissolved in water and lyophilized to afford the triazole **8a** (8.4 mg,

33%) as a white solid.  $[\alpha]_D^{20}$ : +2.0 (0.3, MeOH);  $^1\text{H NMR}$  (500 MHz, MeOD):  $\delta$  8.55 (br s, 1H, NH), 8.06 (s, 1H, ArH<sub>triazole</sub>), 7.79-7.67 (m, 4H, ArH), 7.39-7.29 (m, 4H, ArH), 5.82 (dd,  $J_{H1-H2} = 8.9$  Hz,  $J_{H1-F} = 3.2$  Hz, 1H, H-1), 5.06 (dt,  $J_{H2-F} = 51.6$  Hz,  $J_{H2-H1} = 9.1$  Hz,  $J_{H2-H3} = 9.1$  Hz, 1H, H-2), 4.40-4.32 (m, 2H, CH<sub>2</sub><sub>Fmoc</sub>), 4.23 (t,  $J = 6.7$  Hz, 1H, CH<sub>Fmoc</sub>), 4.09 (m, 1H, H $\alpha$ ), 4.03 (m, 1H, H-4), 3.99-3.96 (m, 1H, H-3), 3.89 (m, 1H, H-5), 3.78 (dd,  $J_{H6-H6'} = 11.5$  Hz,  $J_{H6-H5} = 6.9$  Hz, 1H, H-6), 3.72 (dd,  $J_{H6'-H6} = 11.6$  Hz,  $J_{H6'-H5} = 4.8$  Hz, 1H, H-6'), 2.78 (t,  $J = 7.8$  Hz, 2H, H $\gamma$ , H $\gamma'$ ), 2.19 (m, 1H, H $\beta$ ), 2.01 (m, 1H, H $\beta'$ );  $^{13}\text{C NMR}$  (125 MHz, MeOD):  $\delta$  170.3 (C=O), 158.3 (OC(O)N), 149.3, 145.6, 145.3, 145.65, 142.63 (4 $\times$ Ar-C, C=C<sub>triazole</sub>), 128.8, 128.2, 126.3, 120.9 (8 $\times$ Ar-H), 122.3 (CH<sub>triazole</sub>), 91.6 (d,  $J_{C2-F} = 184.2$  Hz, C-2), 87.1 (d,  $J_{C1-F} = 25.0$  Hz, C-1), 80.1 (C-5), 73.5 (d,  $J_{C3-F} = 16.8$  Hz, C-3), 71.0 (d,  $J_{C4-F} = 8.5$  Hz, C-4), 67.8 (CH<sub>2</sub><sub>Fmoc</sub>), 62.2 (C-6), 57.5 (C $\alpha$ ), 48.4 (CH<sub>Fmoc</sub>), 33.9 (C $\beta$ ), 22.9 (C $\gamma$ );  $^{19}\text{F NMR}$  (MeOD, 470.4 MHz):  $\delta$  -208.9 (dddd,  $J_{2,F} = 51.4$  Hz,  $J_{3,F} = 13.9$  Hz,  $J_{1,F} = 2.9$  Hz,  $J_{4,F} = 2.9$  Hz, F-2); HRMS (ES<sup>+</sup>) for (M+Na) C<sub>27</sub>H<sub>29</sub>N<sub>4</sub>NaO<sub>8</sub> ( $m/z$ ): calc. 579.1862; found 579.1860.

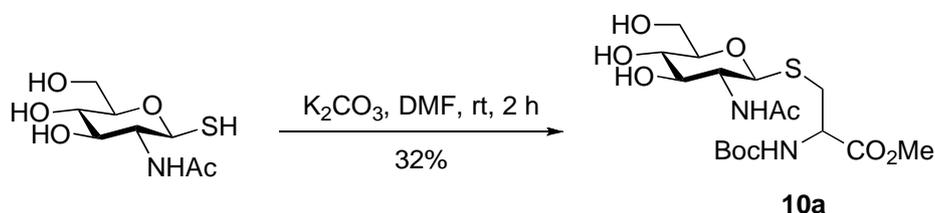
**(S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-4-{4-[( $\alpha$ -D-mannopyranosyloxy)methyl]-[1,2,3]-triazol-1-yl}butanoic acid (9a)**



To a solution of 1-*O*-(2'-Propargyl)- $\alpha$ -D-mannopyranoside (41 mg, 0.188 mmol) and (*S*)-4-Azido-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid (68.8 mg, 0.188 mmol) in H<sub>2</sub>O/tBuOH (2:1, 1.5 mL) under argon conditions, phenylenediamine (75  $\mu$ g of a 375 mM solution in H<sub>2</sub>O, 28.1  $\mu$ mol), sodium ascorbate (75  $\mu$ g of a 250 mM solution in H<sub>2</sub>O, 18.7  $\mu$ mol) and copper sulphate pentahydrate (75  $\mu$ g of a 125 mM solution in H<sub>2</sub>O, 9.37  $\mu$ mol) were added and stirred at rt. After 3h, charcoal (75 mg) was added to the reaction mixture and stirred overnight. TLC (3:2 EtOAc/MeOH) indicated the formation of a product ( $R_f$  0.13) and almost complete consumption of

the amino acid starting material ( $R_f$  0.62). The reaction mixture was then filtered through a Celite plug, eluted with  $H_2O$ , and evaporated. The crude was purified by flash column chromatography (3:1 to 3:2 EtOAc/MeOH), and the isolated compound was dissolved in water and lyophilized to afford the triazole **9a** (10 mg, 9%) as a white solid.  $[\alpha]_D^{20}$ : +42.5 (0.12, MeOH);  $^1H$  NMR (500 MHz, MeOD):  $\delta$  8.05 (s, 1H,  $ArH_{\text{triazole}}$ ), 7.82-7.70 (m, 4H, ArH), 7.42-7.31 (m, 4H, ArH), 4.83 (H1), 4.80 (d,  $J = 12.4$  Hz, 1H,  $OCH_2$ ), 4.65 (d,  $J = 12.4$  Hz, 1H,  $OCH_2$ ), 4.47-4.37 (m, 4H,  $H_\gamma$ ,  $H_{\gamma'}$ ,  $CH_2_{\text{Fmoc}}$ ), 4.25 (t,  $J = 6.5$  Hz, 1H,  $CH_{\text{Fmoc}}$ ), 4.05 (dd,  $J = 7.3$  Hz,  $J = 4.6$  Hz, 1H,  $H_\alpha$ ), 3.87 (ad,  $J = 11.7$  Hz, 1H, H-6), 3.80 (m, 1H, H-2), 3.75-3.70 (m, 2H, H-3, H-6'), 3.64-3.60 (m, 2H, H-4, H-5), 2.49-2.42 (m, 1H,  $H_\beta$ ), 2.25-2.18 (m, 1H,  $H_{\beta'}$ );  $^{13}C$  NMR (125 MHz,  $CDCl_3$ ):  $\delta$  170.4 ( $CO_2H$ ), 158.3 ( $OC(O)N$ ), 145.5, 145.3, 145.2, 142.67, 142.64 ( $4 \times Ar-C$ ,  $C=C_{\text{triazole}}$ ), 128.8, 128.2, 126.3, 120.9 ( $8 \times Ar-H$ ), 125.6 ( $CH_{\text{triazole}}$ ), 100.7 (C-1), 75.0 (C-5), 72.5 (C-3), 72.1 (C-2), 68.7 (C-4), 67.8 ( $CH_2_{\text{Fmoc}}$ ), 63.0 (C-6), 60.7 ( $OCH_2$ ), 55.3 ( $C_\alpha$ ),  $\sim 48.5$  ( $C_\gamma$ ),  $\sim 48.5$  ( $CH_{\text{Fmoc}}$ ), 35.1 ( $C_\beta$ ); HRMS ( $ES^+$ ) for  $(M+Na)$   $C_{28}H_{32}N_4NaO_{10}$  ( $m/z$ ): calc. 607.2016; found 607.2009.

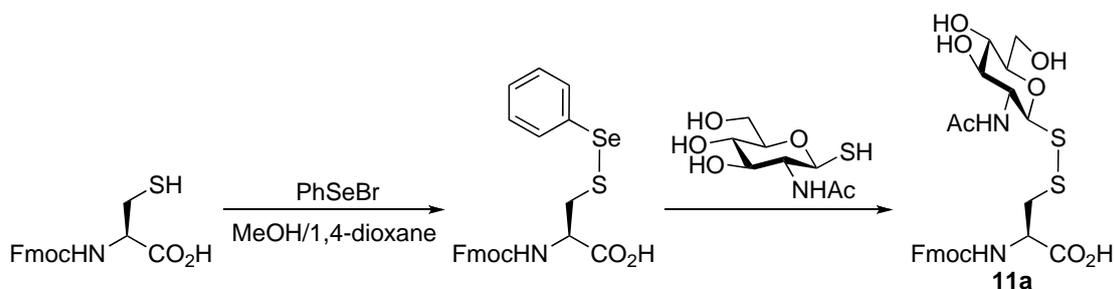
**(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-*N*-(*tert*-butoxycarbonyl)-D/L-cysteine methyl ester (**10a**)**



2-Acetamido-2-deoxy-1-thio- $\beta$ -D-glucopyranose (418.1 mg, 1.76 mmol) and  $K_2CO_3$  (492 mg, 3.52 mmol) were added as solids to a solution of BocDhaOMe<sup>12</sup> (390 mg, 1.94 mmol) in dry DMF (8.8 mL) at room temperature under argon atmosphere. The reaction mixture was stirred at the same temperature for 2 h and then concentrated under reduced pressure. The residue was purified by column chromatography (7:2:1 EtOAc/MeOH/ $H_2O$ ) to afford **10a** (250 mg, 32%) as a white fluffy solid being a diastereomeric mixture (D:L, 1:1.2).  $R_f$  (7:2:1 EtOAc/MeOH/ $H_2O$ ): 0.32.  $^1H$  NMR ( $CD_3OD$ , 500 MHz)  $\delta$  in ppm: 4.53, 4.47 (d,  $J_{1,2} = 10.4$  Hz, 2H, H-1), 4.46–4.41 (m, 2H,  $H_\alpha$ ), 3.93 (d,  $J_{6a,b} = 12.0$  Hz, 1H, H-6a), 3.89 (d,  $J_{6a,b} = 12.9$  Hz, 1H, H-6a), 3.82 (dd,  $J_{1,2} = 10.4$  Hz,  $J_{2,3} = 10.1$  Hz, 1H H-2), 3.74–3.69 (m, 3H, H-2,6b), 3.73, 3.72 (s, 6H,  $OCH_3$ ), 3.44 (dd,  $J_{2,3} = J_{3,4} = 9.4$  Hz, 2H, H-3), 3.40–3.28 (m, 5H, H-4,5,  $SCH_2$ ),

3.10 (dd,  $J = 14.2, 7.6$  Hz, 1H, SCH<sub>2</sub>), 3.00 (dd,  $J = 14.2, 7.6$  Hz, 1H, SCH<sub>2</sub>), 2.81 (dd,  $J = 14.2, 8.5$  Hz, 1H, SCH<sub>2</sub>), 1.97 (s, 6H, CH<sub>3</sub>, NHAc), 1.46 (s, 18H, 6CH<sub>3</sub>, *t*-Bu). <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125.8 MHz)  $\delta$  in ppm: 173.74, 173.73, 173.5, 173.4 (CO<sub>2</sub>Me, NHAc), 158.1, 158.0 (C=O, NHBoc), 87.3, 85.4 (C-1), 82.8, 82.3 (C-5), 81.2, 81.1 (C, *t*-Bu), 77.4, 77.3 (C-3), 72.0, 71.7 (C-4), 63.1, 62.8 (C-6), 56.4 (C-2), 55.8 (C <sub>$\alpha$</sub> ), 55.7 (C-2), 55.5 (C <sub>$\alpha$</sub> ), 53.0 (OCH<sub>3</sub>), 34.3, 32.4 (SCH<sub>2</sub>), 28.8, 28.9 (6CH<sub>3</sub>, *t*-Bu) 23.1 (CH<sub>3</sub>, NHAc). FT-IR (KBr)  $\nu$  in cm<sup>-1</sup>: 3355, 3346, 3335, 2978, 2955, 2933, 1692, 1653, 1420, 1165, 1057. HRMS (TOF ES+) for (M+Na) C<sub>17</sub>H<sub>30</sub>N<sub>2</sub>NaO<sub>9</sub>S ( $m/z$ ): calc. 461.1564; found 461.1557.

***N*-(9H-fluoren-9-ylmethoxycarbonyl)-*S*-(2-*N*-acetylamino-2-deoxy-1-dithio- $\beta$ -D-glucopyranosyl disulfide)-*L*-cysteine (**11a**)**



*N*-(9H-fluoren-9-ylmethoxycarbonyl)-*L*-cysteine (38.8 mg, 0.113 mmol) and phenyl selenyl bromide (60 mg, 0.254 mmol) were dissolved in a mixture of anhydrous dioxane (1 mL) and methanol (0.7 mL). Et<sub>3</sub>N (49  $\mu$ L) was added to the resulting mixture and stirred at rt overnight. TLC (4:1 EtOAc/MeOH) indicated the formation of a major product ( $R_f$  0.29); the solution was concentrated *in vacuo*. The residue was filtrated in a short pad of silica (9:1 EtOAc/MeOH) and used in the next step without further purification (56 mg, quant.); LRMS (ES<sup>-</sup>) for (M-H) C<sub>24</sub>H<sub>20</sub>NO<sub>4</sub>S<sub>2</sub>Se ( $m/z$ ): calc. 498.03; found 498.03.

Finally, *N*-(9H-fluoren-9-ylmethoxycarbonyl)-*S*-(phenylselenenyl)-*L*-cysteine (56 mg, 0.113 mmol) and Et<sub>3</sub>N (31  $\mu$ L, 0.222 mmol) were dissolved in anhydrous methanol (4 mL) under argon conditions at rt. A solution of 2-*N*-acetylamino-2-deoxy-1-thio- $\beta$ -D-glucopyranose (26 mg, 0.109 mmol) in methanol (5 mL) was added dropwise over 5 min and the mixture was stirred at rt. After 2.5 h, TLC (7:2:1 EtOAc/MeOH/H<sub>2</sub>O) indicated the formation of a product ( $R_f$  0.36). The mixture was quenched with TFA (20  $\mu$ L), evaporated *in vacuo* and purified by flash column chromatography (7.5:2:0.5 EtOAc/MeOH/H<sub>2</sub>O) to afford the disulfide **11a** (47.5 mg, 75%) as a white solid;

$[\alpha]_D^{20}$ :  $-56.2$  ( $c$  0.3, MeOH);  $^1\text{H}$  NMR (500 MHz, MeOD):  $\delta$  7.79-7.64 (m, 4H, ArH), 7.39-7.26 (m, 4H, ArH), 4.59 (d,  $J_{1,2} = 10.3$  Hz, 1H, H-1), 4.49 (dd,  $J = 8.3$  Hz,  $J = 3.4$  Hz, 1H, H $\alpha$ ), 4.36 (dd,  $J = 9.2$  Hz,  $J = 7.0$  Hz, 1H, CH<sub>2</sub><sub>Fmoc</sub>), 4.29-4.18 (m, 2H, H-2, CH<sub>2</sub><sub>Fmoc</sub>), 3.96 (t,  $J = 10.0$  Hz, 1H, CH<sub>Fmoc</sub>), 3.90 (ad,  $J_{6,6'} = 12.2$  Hz, 1H, H-6'), 3.73-3.69 (m, 1H, H-6), 3.54-3.50 (m, 1H, H-3), 3.44 (dd,  $J = 13.5$  Hz,  $J = 3.8$  Hz, 1H, SCH<sub>2</sub>), 3.40-3.37 (m, 2H, H-4, H-5), 3.04 (dd,  $J = 13.4$  Hz,  $J = 9.2$  Hz, 1H, SCH<sub>2</sub>), 1.98 (s, 3H, CH<sub>3</sub>);  $^{13}\text{C}$  NMR (125 MHz, MeOD):  $\delta$  177.4 (C=O), 173.6 (CO<sub>2</sub>H), 158.4 (OC(O)N), 145.37, 145.31, 142.5 (4 $\times$ Ar-C), 128.8, 128.23, 128.21, 126.4, 126.3, 120.9 (8 $\times$ Ar-H), 91.4 (C-1), 82.5 (C-5), 77.3 (C-3), 71.6 (C-4), 68.0 (CH<sub>2</sub><sub>Fmoc</sub>), 62.8 (C-6), 56.6 (C $\alpha$ ), 55.4 (CH<sub>Fmoc</sub>), 48.5 (C-2), 43.9 (SCH<sub>2</sub>), 23.0 (CH<sub>3</sub>); HRMS (ES<sup>-</sup>) for (M-H) C<sub>26</sub>H<sub>29</sub>N<sub>2</sub>O<sub>9</sub>S<sub>2</sub> ( $m/z$ ): calc. 577.1314; found 577.1316.

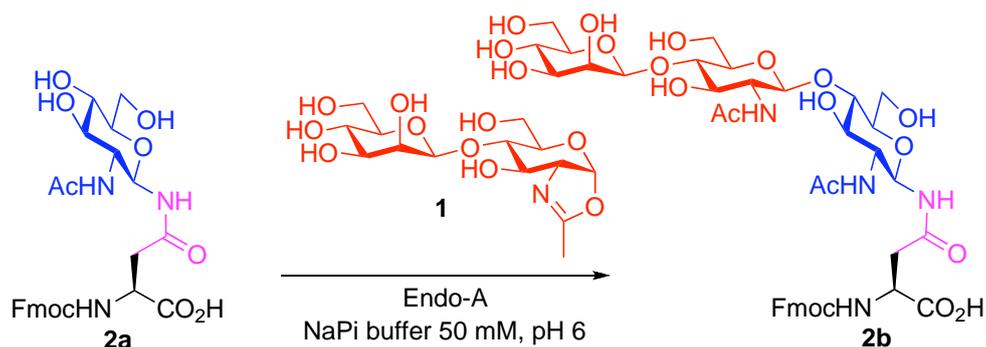
## 2.2. Enzymatic extension of glycoamino acids

### General method for the enzymatic extension of glycoamino acids 2a–11a

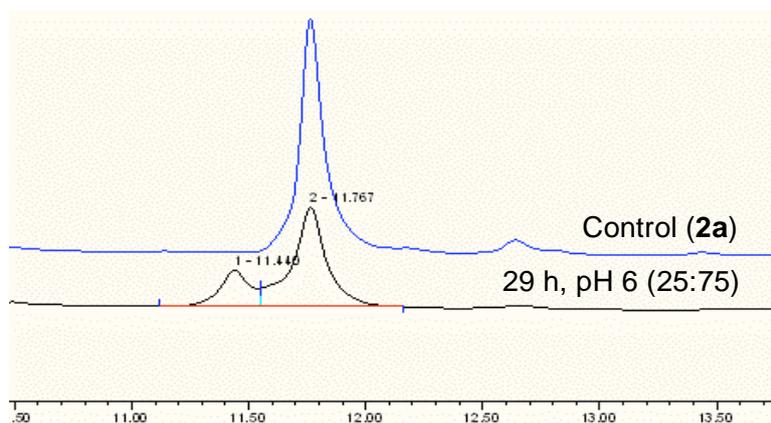
To a solution of glycoamino acid **2a–11a** (0.2  $\mu\text{mol}$ ) in 50 mM sodium phosphate buffer (100 or 1000  $\mu\text{L}$ , pH 6 or 7), disaccharide oxazoline **1** (0.6  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of a 0.01 U/ $\mu\text{L}$ ) were added and the mixture was rotated on a lab rotisserie at 22 or 27  $^{\circ}\text{C}$ . Aliquots were taken through one day, diluted in water and spinned down in a vivaspin-500 concentrator 10KDa MWCO (13.000 g, 8 min). The eluent was analyzed by RP-HPLC and MS (ES<sup>-</sup>). Reaction conditions were reproduced in a similar sample without Endo-A as control reaction.

*Method A*: pH 6.0,  $c$  0.2 mM, 27  $^{\circ}\text{C}$ ; *Method B*: pH 7.0,  $c$  0.2 mM, 27  $^{\circ}\text{C}$ ; *Method C*: pH 7.0,  $c$  2 mM, 22  $^{\circ}\text{C}$ ; *Method D*: pH 7.0,  $c$  0.2 mM, 22  $^{\circ}\text{C}$ ; *Method E*: pH 7.0,  $c$  6 mM, 22  $^{\circ}\text{C}$ .

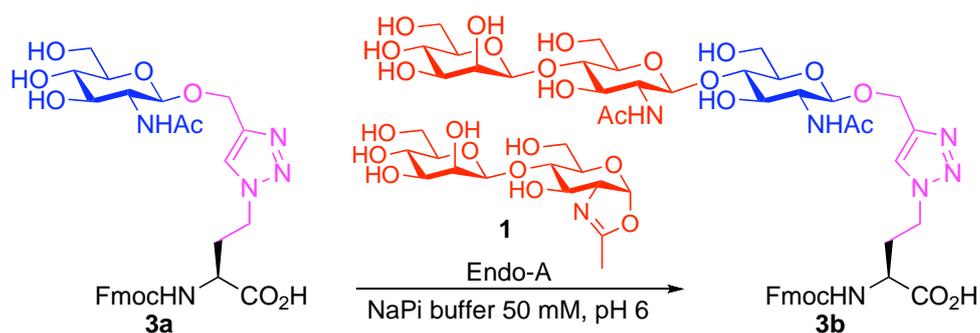
Conditions for RP-HPLC: For compounds **2b–9b** [Phenomenex Onyx Monolithic C18 (100  $\times$  4.6mm) column; flow rate was 1 mL/min; gradient run, 0–1 min 5%B, 1–15 min linear gradient 5 to 50%B, 18–20min linear gradient 50 to 90%B, 20–22 min 90%B, 22-24 min linear gradient 90-5%B, 24-26 min 5%B; UV monitoring 214 nm]; For compounds **10b–11b** [Phenomenex Synergi 4u Fusion-RP 80A (100  $\times$  4.6mm) column; flow rate was 1 mL/min; gradient run, 0-3 min 5%B, 3-15 min linear gradient 5 to 55%B, 15-18min linear gradient 55 to 90%B, 18–20 min 90%B, 20-22 min linear gradient 90-5%B, 22-24 min 5%B; UV monitoring 214 nm].

**Endo A-catalyzed glycosylation of GlcNAc-Asn 2a**

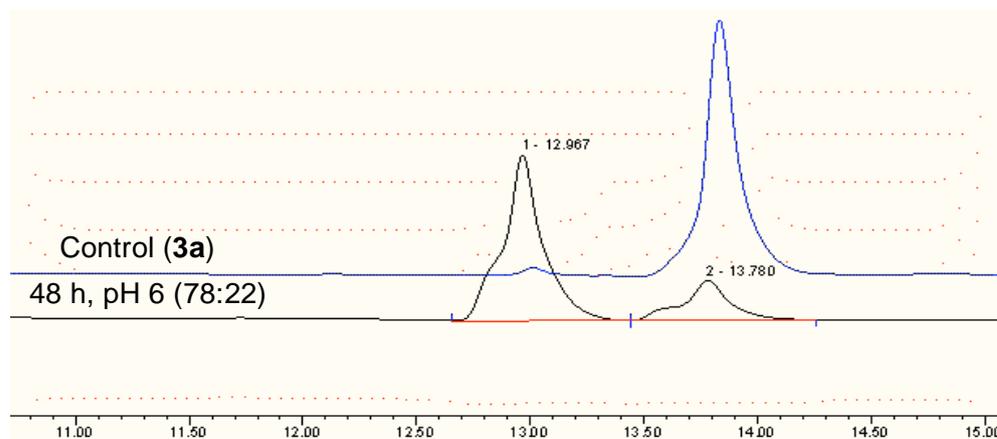
Man-GlcNAc-GlcNAc-Asn **2b** was prepared following the method A above, starting from GlcNAc-Asn **2a** (37  $\mu$ L of 1.5 mg/0.5 mL buffer, 0.199  $\mu$ mol), oxazoline **1** (58  $\mu$ L of 1.9 mg/0.5 mL buffer, 0.603  $\mu$ mol) and Endo A (2  $\mu$ L of 0.01 U/ $\mu$ L stock) in 50 mM sodium phosphate buffer (903  $\mu$ L, pH 6) at 27  $^{\circ}$ C. Additional amount of oxazoline **1** (58  $\mu$ L at 2.75, 5.25 and 23 h) and Endo-A (5  $\mu$ L at 2.75 and 23 h) were added to the reaction. 40  $\mu$ L samples were taken through 1 day (0.5 h, 1 h, 2 h, 4 h, 6 h, 22 h, 24 h, 29 h), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 25% at 29h; LRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>41</sub>H<sub>54</sub>N<sub>4</sub>O<sub>20</sub> (*m/z*): calc. 921.3; found 921.3.



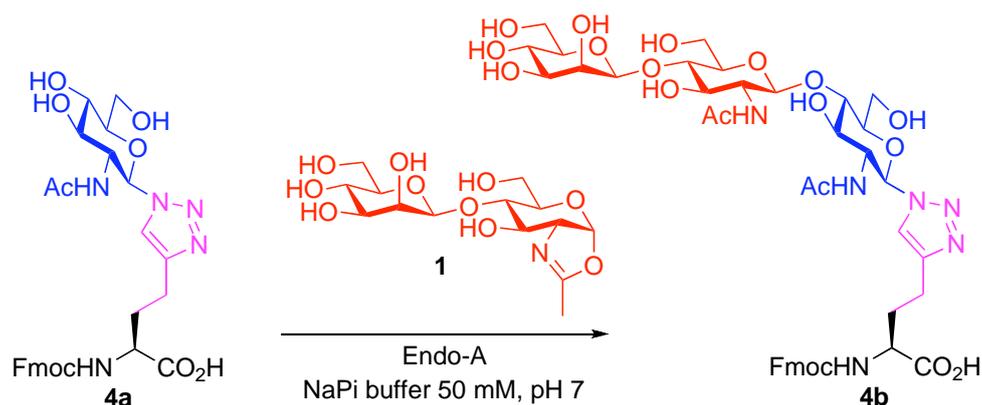
**Figure S1.** HPLC analysis after 29 h incubation of **2a** (0.2 mM) with Endo A.

**Endo A-catalyzed glycosylation of GlcNAc-OCH<sub>2</sub>-triazole-amino acid **3a****

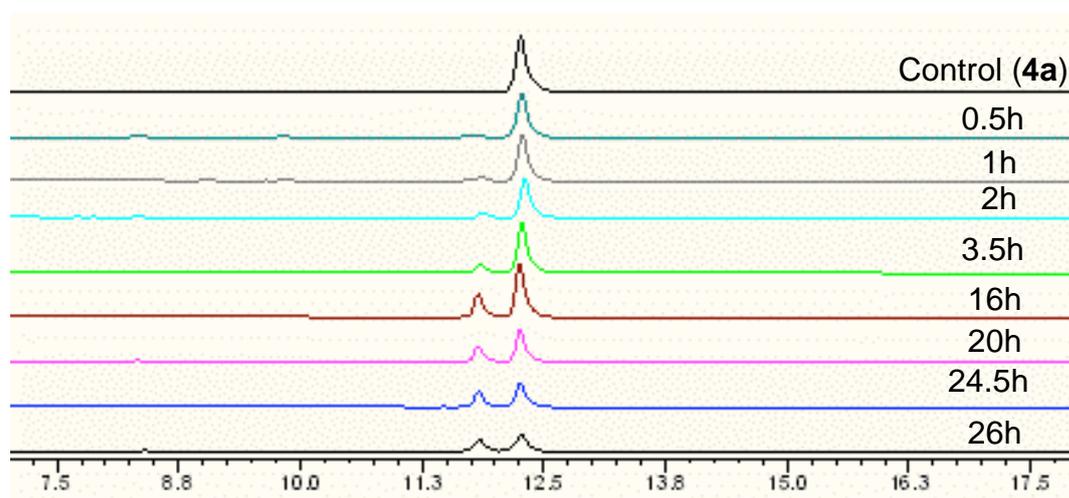
Trisaccharide amino acid **3b** was prepared following the method A above, starting from glycoamino acid **3a** (37  $\mu$ L of 1.7 mg/0.5 mL buffer, 0.201  $\mu$ mol), oxazoline **1** (55  $\mu$ L of 2 mg/0.5 mL buffer, 0.602  $\mu$ mol) and Endo A (2  $\mu$ L of 0.01 U/ $\mu$ L stock) in 50 mM sodium phosphate buffer (906  $\mu$ L, pH 6) at 27  $^{\circ}$ C. Additional amount of oxazoline **1** (55  $\mu$ L at 1.5, 18.75 and 23.5 h) and Endo-A (5  $\mu$ L at 1.5 h) were added to the reaction. 40  $\mu$ L samples were taken through 2 days (0.5 h, 1 h, 2.5 h, 3.5 h, 18 h, 22.5 h, 24.5 h, 48 h), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 78% at 48h; LRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>44</sub>H<sub>57</sub>N<sub>6</sub>O<sub>20</sub> (*m/z*): calc. 989.3; found 989.3.



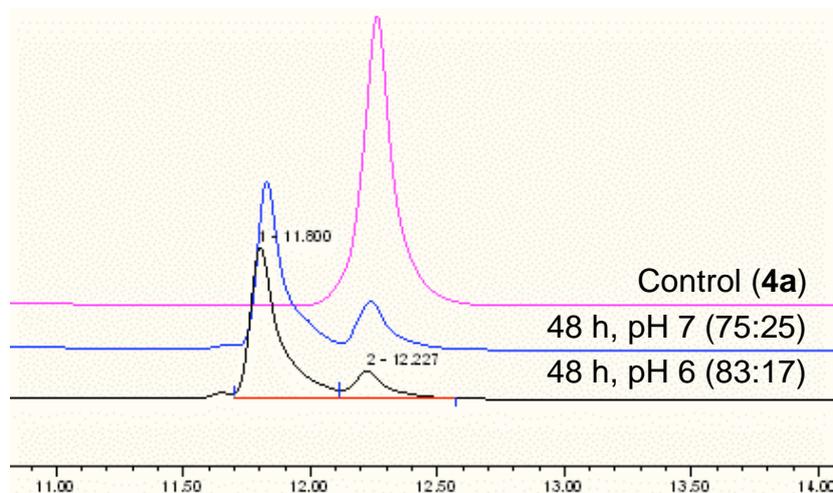
**Figure S2.** HPLC analysis after incubation of **3a** with Endo A following method A.

**Endo A-catalyzed glycosylation of GlcNAc-triazole-amino acid **4a****

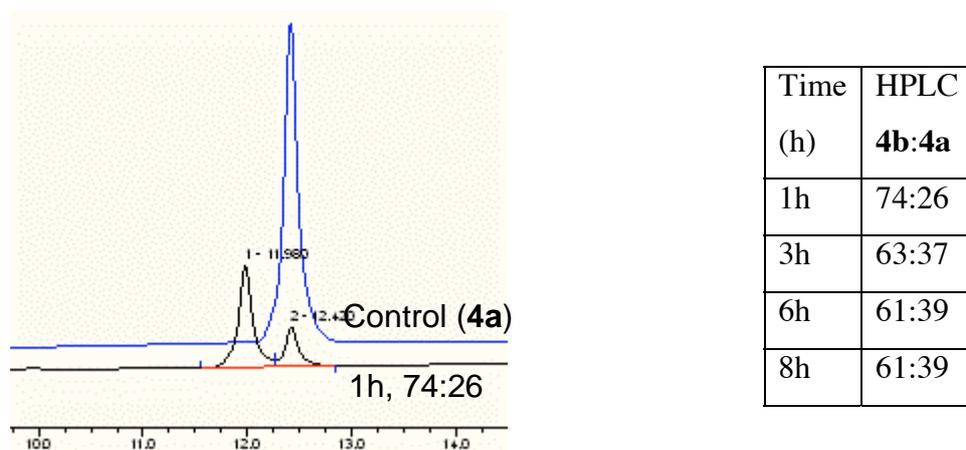
Trisaccharide amino acid **4b** was prepared following the method A above, starting from glycoamino acid **4a** (50  $\mu\text{L}$  of 1.2 mg/0.5 mL buffer, 0.201  $\mu\text{mol}$ ), oxazoline **1** (73  $\mu\text{L}$  of 1.5 mg/0.5 mL buffer, 0.599  $\mu\text{mol}$ ) and Endo A (2  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (875  $\mu\text{L}$ , pH 6) at 27  $^{\circ}\text{C}$ . Additional amount of oxazoline **1** (73  $\mu\text{L}$  at 4, 9, 25.5 and 32 h) and Endo-A (2  $\mu\text{L}$  at 7 and 25.5 h and 5  $\mu\text{L}$  at 32 h) were added to the reaction. 40  $\mu\text{L}$  samples were taken through 2 days (0.5 h, 1 h, 2 h, 4 h, 5 h, 6 h, 8 h, 24 h, 27 h, 30 h, 31 h, 33 h, 48 h), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 83% at 48h; HRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>43</sub>H<sub>54</sub>N<sub>6</sub>O<sub>19</sub> ( $m/z$ ): calc. 959.3522; found 959.3530.



**Figure S3.** HPLC analysis after incubation of **4a** with Endo A following method A.

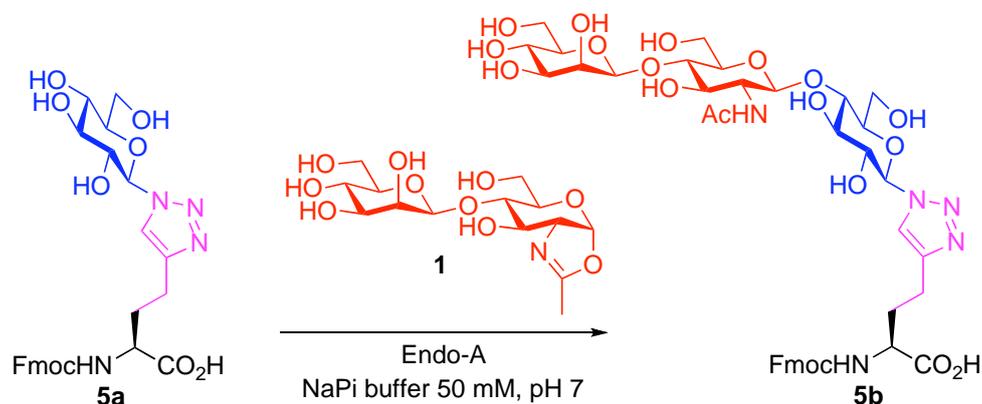


**Figure S4.** HPLC comparison of glycosylation of **4a** following methods A and B.



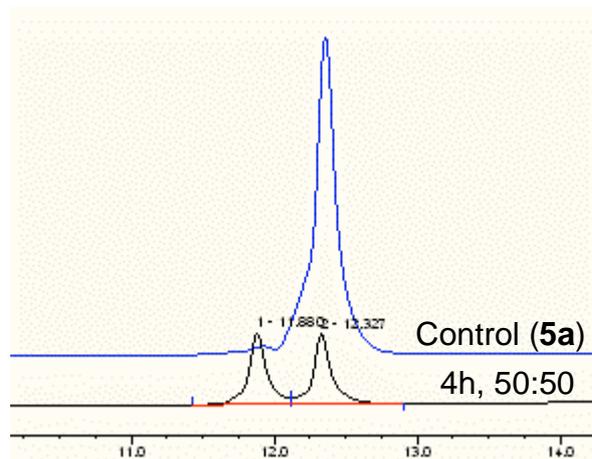
**Figure S5.** HPLC analysis after incubation of **4a** with Endo A following method C.

### Endo A-catalyzed glycosylation of Glc-triazole-amino acid **5a**



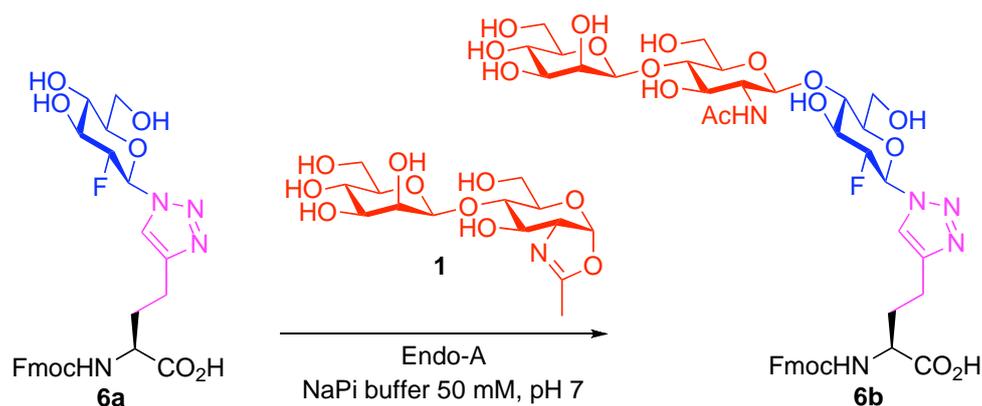
Trisaccharide amino acid **5b** was prepared following the method C above, starting from glycoamino acid **5a** (112  $\mu\text{g}$ , 0.202  $\mu\text{mol}$ ), oxazoline **1** (220  $\mu\text{g}$ , 0.602  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (100  $\mu\text{L}$ ,

pH 7) at 22 °C. 8  $\mu\text{L}$  samples were taken through 1 day, diluted with water (30  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 50% at 4h; HRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>41</sub>H<sub>52</sub>N<sub>5</sub>O<sub>19</sub> ( $m/z$ ): calc. 918.3256; found 918.3291.

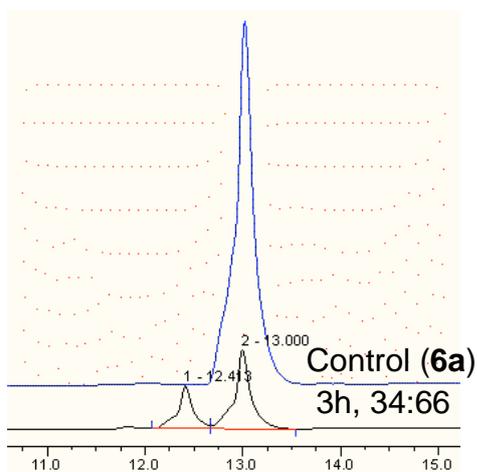


Time (h)	HPLC <b>5b:5a</b>
2h	46:54
4h	50:50
6h	37:63
8h	37:63
21h	37:63

**Figure S6.** HPLC analysis after incubation of **5a** with Endo A following method C.

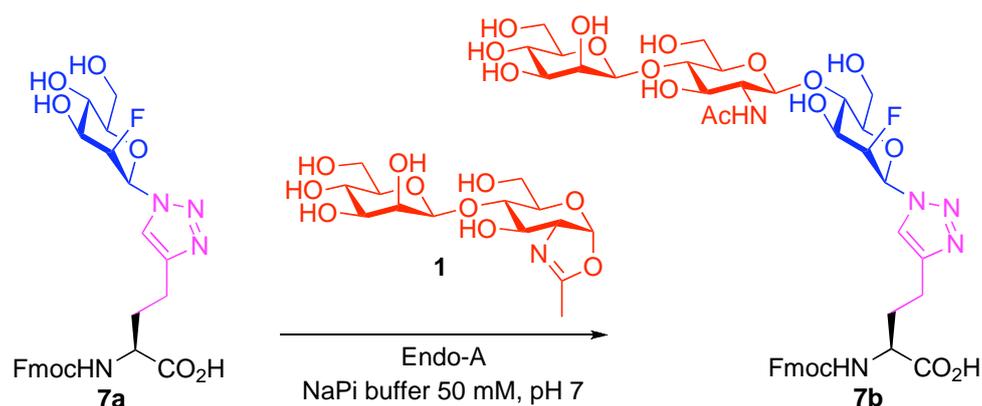
Endo A-catalyzed glycosylation of Glc2F-triazole-amino acid **6a**

Trisaccharide amino acid **6b** was prepared following the method C above, starting from glycoamino acid **6a** (110  $\mu\text{g}$ , 0.198  $\mu\text{mol}$ ), oxazoline **1** (221  $\mu\text{g}$ , 0.604  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (100  $\mu\text{L}$ , pH 7) at 22  $^\circ\text{C}$ . 8  $\mu\text{L}$  samples were taken through 1 day, diluted with water (30  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 34% at 3h; HRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>41</sub>H<sub>51</sub>FN<sub>5</sub>O<sub>18</sub> ( $m/z$ ): calc. 920.3213; found 920.3227.

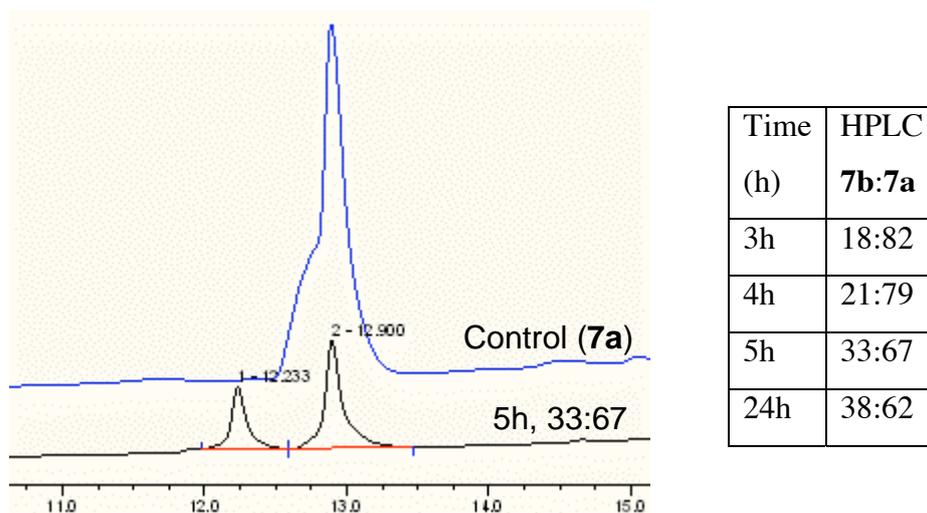


Time (h)	HPLC <b>6b:6a</b>
0.5h	16:84
1h	24:76
3h	34:66
4h	34:66
21h	34:66

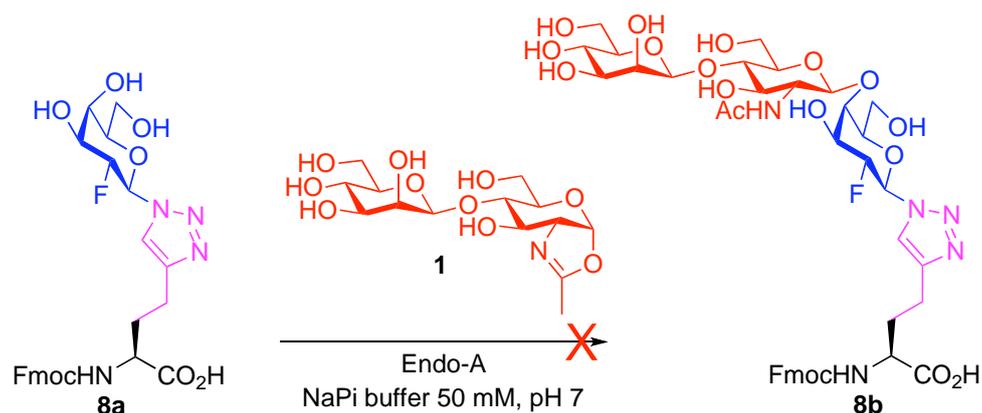
**Figure S7.** HPLC analysis after incubation of **6a** with Endo A following method C.

Endo A-catalyzed glycosylation of Man2F-triazole-amino acid **7a**

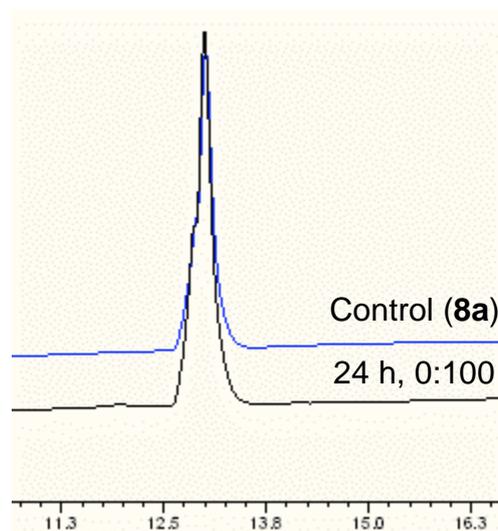
Trisaccharide amino acid **7b** was prepared following the method C above, starting from glycoamino acid **7a** (110  $\mu\text{g}$ , 0.198  $\mu\text{mol}$ ), oxazoline **1** (221  $\mu\text{g}$ , 0.604  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (100  $\mu\text{L}$ , pH 7) at 22  $^\circ\text{C}$ . 8  $\mu\text{L}$  samples were taken through 1 day, diluted with water (30  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 38% at 24h; LRMS (TOF ES<sup>-</sup>) for (M-H) C<sub>41</sub>H<sub>51</sub>FN<sub>5</sub>O<sub>18</sub> ( $m/z$ ): calc. 920.3; found 920.3.



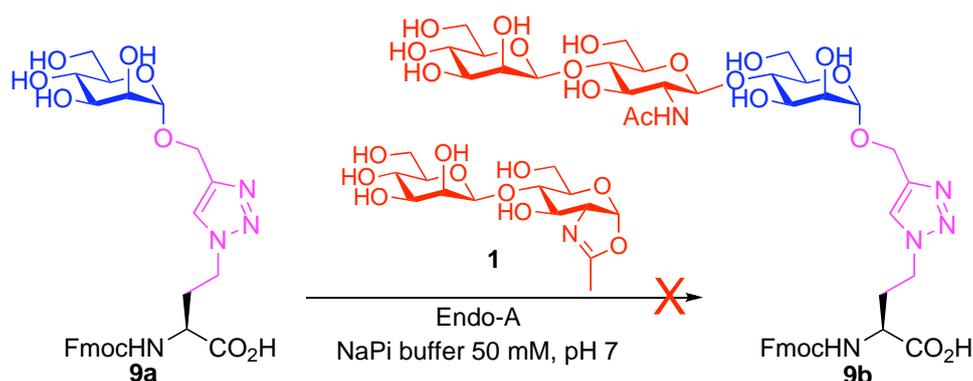
**Figure S8.** HPLC analysis after incubation of **7a** with Endo A following method C.

**Endo A-catalyzed glycosylation of Gal2F-triazole-amino acid 8a**

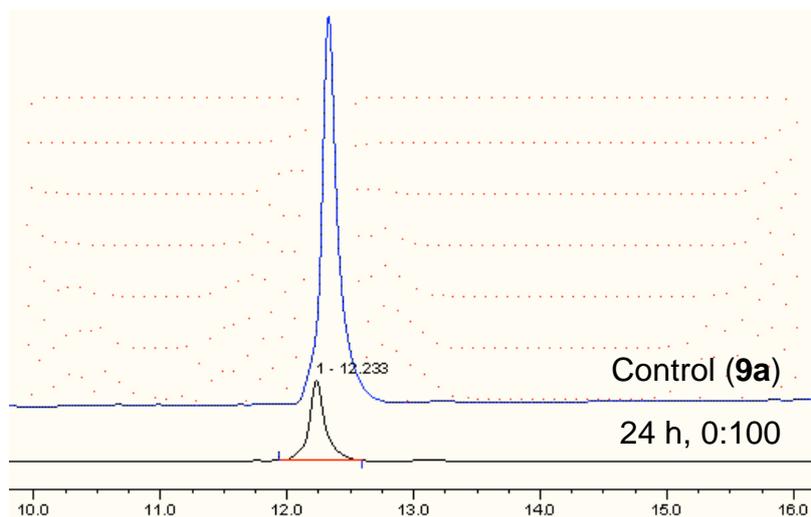
Trisaccharide amino acid **8b** was prepared following the method C above, starting from glycoamino acid **8a** (110  $\mu\text{g}$ , 0.198  $\mu\text{mol}$ ), oxazoline **1** (221  $\mu\text{g}$ , 0.604  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (100  $\mu\text{L}$ , pH 7) at 22  $^{\circ}\text{C}$ . 8  $\mu\text{L}$  samples were taken through 1 day, diluted with water (30  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 0% at 24h.



**Figure S9.** HPLC analysis after incubation of **8a** with Endo A following method C.

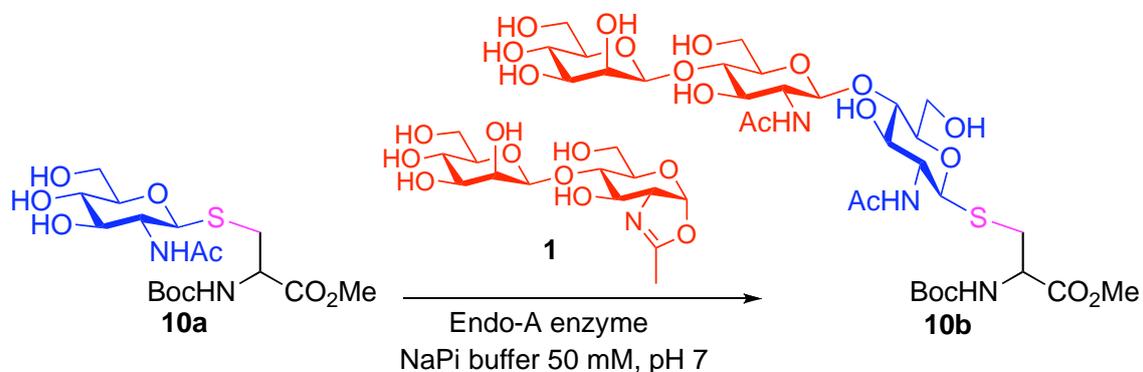
**Endo A-catalyzed glycosylation of Man-triazole-amino acid 9a**

Trisaccharide amino acid **9b** was prepared following the method C above, starting from glycoamino acid **9a** (117  $\mu\text{g}$ , 0.201  $\mu\text{mol}$ ), oxazoline **1** (219  $\mu\text{g}$ , 0.600  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (100  $\mu\text{L}$ , pH 7) at 22  $^{\circ}\text{C}$ . 8  $\mu\text{L}$  samples were taken through 1 day, diluted with water (30  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 0% at 24h.

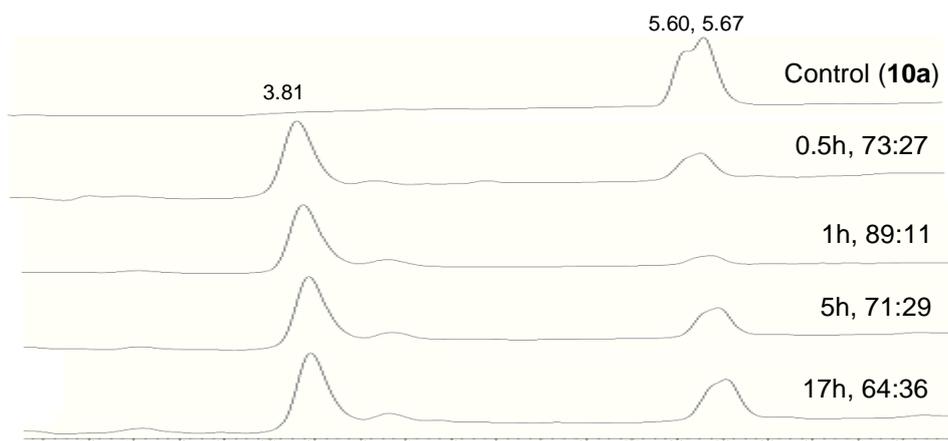


**Figure S10.** HPLC analysis after incubation of **9a** with Endo A following method C.

#### Endo A-catalyzed glycosylation of GlcNAc-S-amino acid **10b**

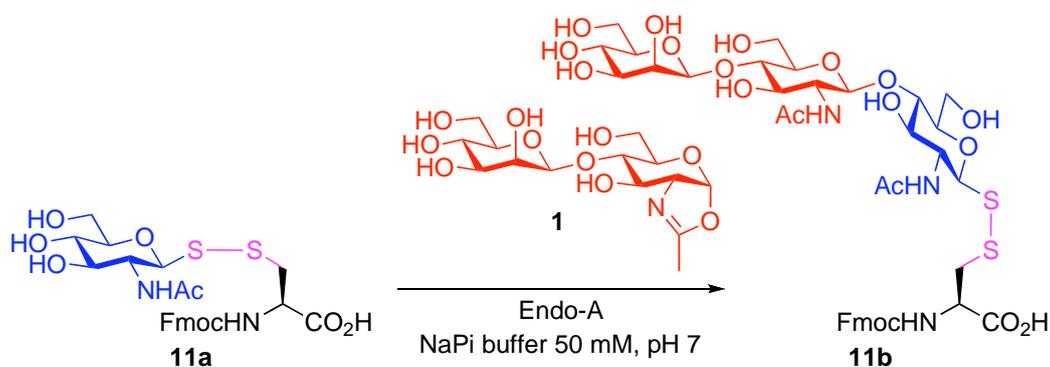


Trisaccharide amino acid **10b** was prepared following the method C above, starting from a 1:1.2 mixture of diastereomers of glycoamino acid **10a** (87.7  $\mu\text{g}$ , 0.2  $\mu\text{mol}$ ), oxazoline **1** (731  $\mu\text{g}$ , 2  $\mu\text{mol}$ ) and Endo A (9  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (100  $\mu\text{L}$ , pH 7) at 22  $^{\circ}\text{C}$ . 18  $\mu\text{L}$  samples were taken through 17 h, diluted with water (25  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 89% at 1h. HRMS (TOF ES<sup>+</sup>) for (M+Na) C<sub>31</sub>H<sub>53</sub>N<sub>3</sub>NaO<sub>19</sub>S (*m/z*): calc. 826.2886; found 826.2891.

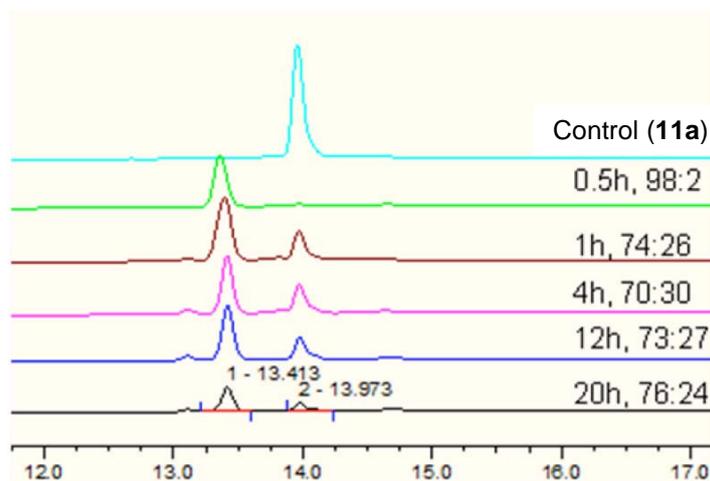


**Figure S11.** HPLC analysis after 0–17h incubation of **10a** with Endo A following method C with excess of oxazoline **1** and Endo A.

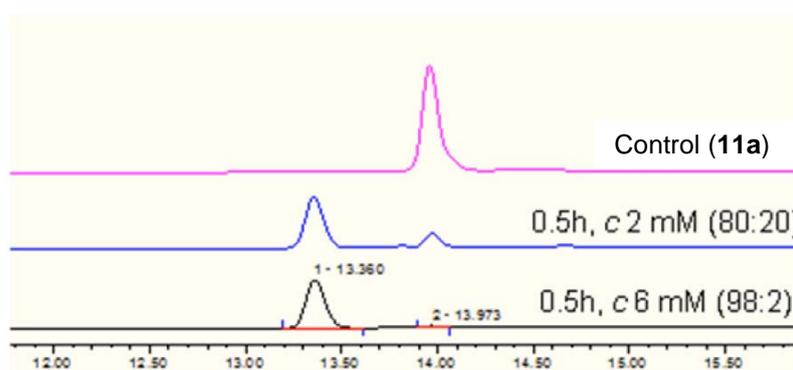
### Endo A-catalyzed glycosylation of GlcNAc-SS-amino acid **11a**



Trisaccharide amino acid **11b** was prepared following the method E above, starting from glycoamino acid **11a** (109  $\mu\text{g}$ , 0.199  $\mu\text{mol}$ ), oxazoline **1** (218  $\mu\text{g}$ , 0.600  $\mu\text{mol}$ ) and Endo A (3  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) in 50 mM sodium phosphate buffer (33  $\mu\text{L}$ , pH 7) at 22  $^{\circ}\text{C}$ . 5  $\mu\text{L}$  samples were taken through 1 day, diluted with water (20  $\mu\text{L}$ ), centrifuged in vivaspin-500 and analyzed by HPLC and MS; Conversion by HPLC: 98% at 0.5h. HRMS (TOF  $\text{ES}^-$ ) for (M–H)  $\text{C}_{40}\text{H}_{52}\text{N}_3\text{O}_{19}\text{S}_2$  ( $m/z$ ): calc. 942.2636; found 942.2671.



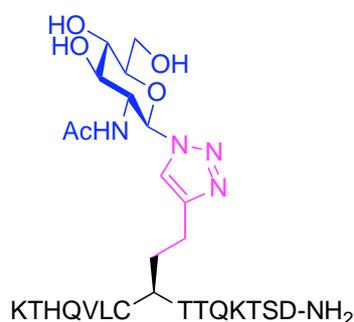
**Figure S12.** HPLC after incubation of **11a** with Endo A following method E.



**Figure S13.** HPLC comparison of glycosylation of **11a** following methods C and E.

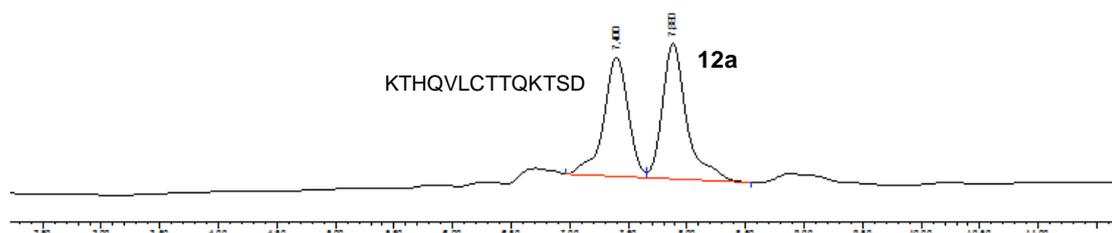
### 2.3. Preparation of glycopeptides **12a–14a**

#### GlcNAc-triazole linked peptide (**12a**)

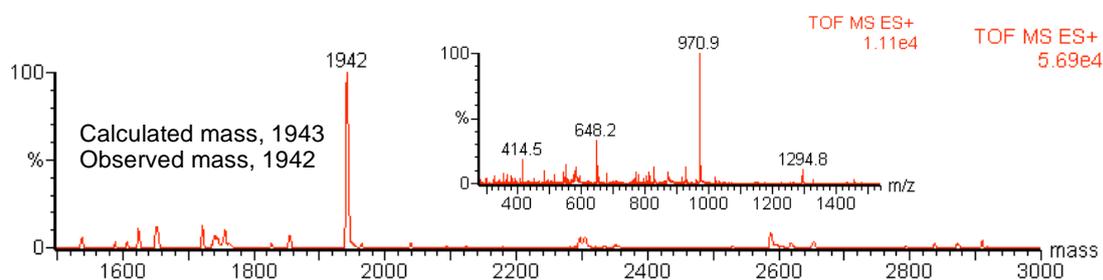


Glycopeptide **12a** was synthesized by means of Fmoc-chemistry on Rink amide MBHA-polystyrene resin [1% divinyl benzene, GL Biochem]. Every Fmoc-amino acid, included glycoamino acid **4a**, was incorporated in the sequence using a microwave assisted Liberty CEM peptide synthesizer. Analytical HPLC analysis shown the formation of side peptide KTHVQVLCTTQKTSD in around 50% yield, thus, the crude was purified onto semi-preparative RP-HPLC [Phenomenex Onyx-monolithic C18 (100x10mm) column; the flow rate was 10 mL/min; gradient run, 0-1 min 5%B, 1-15 min linear gradient 5 to 10%B, 15-18 min

10%B, 18–20 min linear gradient 10 to 90%B, 20–22 min 90%B, 22–24 min linear gradient 90–5%B, 24–26 min 5%B; UV monitoring 214 nm]. The pure fraction was lyophilized to afford glycopeptide **12a** as an amorphous solid. LC–MS analysis (calculated mass 1943, observed mass 1942).

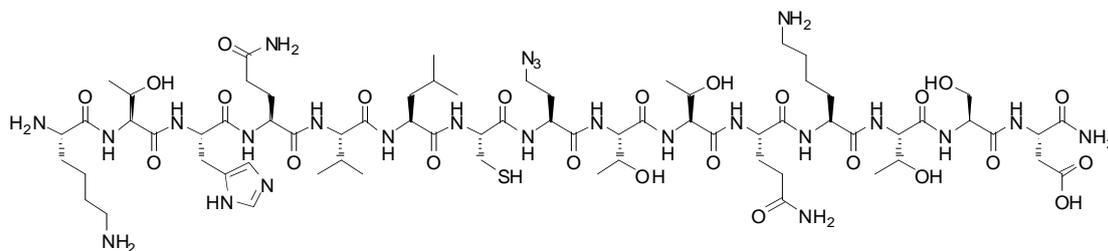


**Figure S14.** Analytical-HPLC analysis of **12a** solid phase synthesis.

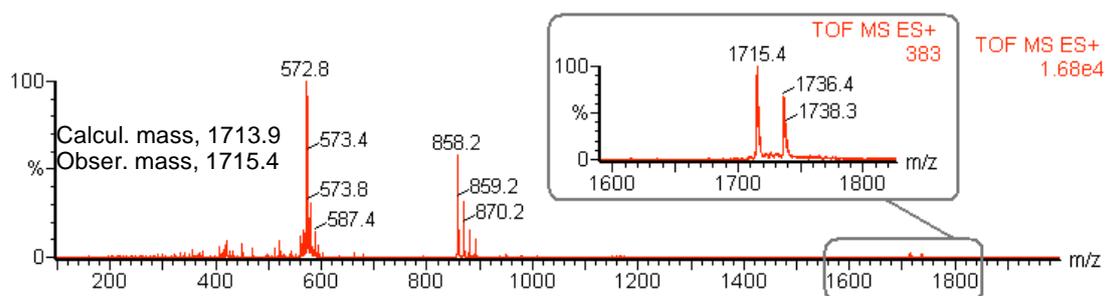


**Figure S15.** ESI–MS spectrum of purified glycopeptide **12a**.

**H<sub>2</sub>N-Lys-Thr-His-Gln-Val-Leu-Cys-Aha-Thr-Thr-Gln-Lys-Thr-Ser-Asp-C(O)NH<sub>2</sub>**

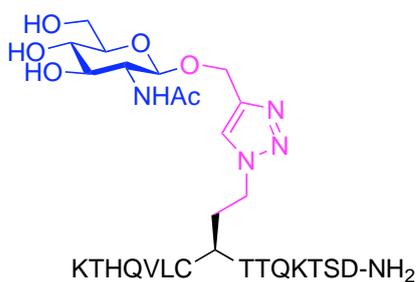


Gp90 peptide fragment<sup>13</sup> (361–375 Lys-Thr-His-Gln-Val-Leu-Cys-Aha-Thr-Thr-Gln-Lys-Thr-Ser-Asp) was synthesized by means of Fmoc-chemistry on Rink amide MBHA-polystyrene resin [1% divinyl benzene, GL Biochem]. Every Fmoc-amino acid, included (*S*)-4-Azido-2-(9H-fluoren-9-ylmethoxycarbonylamino)butanoic acid, was incorporated in the sequence using a microwave assisted Liberty CEM peptide synthesizer. Analysis by LC–MS (calculated mass 1713.9, observed mass 1715.4).

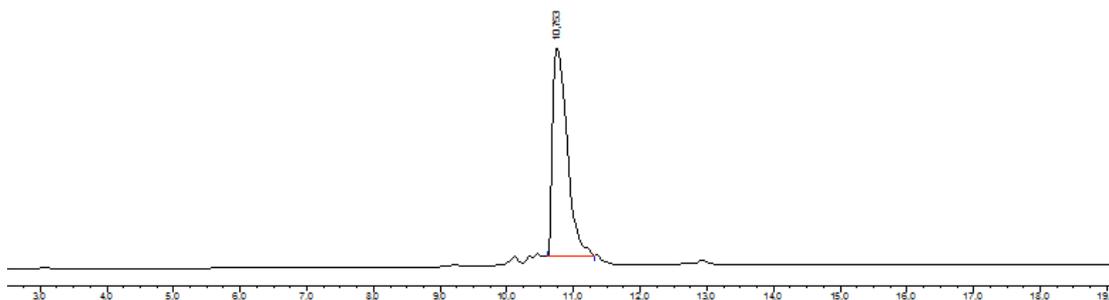


**Figure S16.** ESI-MS spectrum of peptide KTHQVLCaHaTTQKTSD.

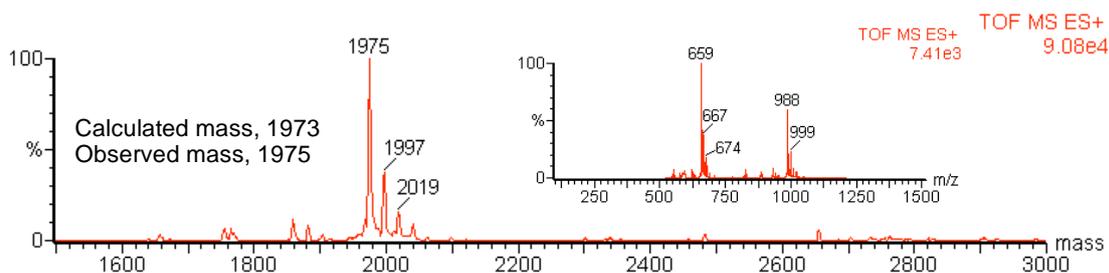
### GlcNAc-OCH<sub>2</sub>-triazole linked peptide (**13a**)



A freshly prepared solution of copper(I) bromide (99.999%) in acetonitrile (660  $\mu$ L of 10 mg/mL) was mixed with a solution of tris-triazolyl amine ligand 2,2',2''-(4,4',4''-nitrilotris(methylene)tris(1H-1,2,3-triazole-4,1-diyl))triacetate in acetonitrile (250  $\mu$ L of 120 mg/mL). The preformed Cu-complex solution (760  $\mu$ L) was added to a solution of peptide KTHQVLCaHaTTQKTSD (14 mg, 8.163  $\mu$ mol) and propargyl 2-*N*-acetylamino-2-deoxy- $\beta$ -D-glucopyranoside (22 mg, 84.8  $\mu$ mol) in 50 mM sodium phosphate buffer (3.3 mL, pH 8.2), and mixed on a lab rotisserie for 2 h at rt. The mixture was then centrifuged to remove any precipitate of Cu(II) salts (3000 rpm, 10 min, 4  $^{\circ}$ C), and the supernatant was concentrated and lyophilized. The resulting foam was then purified by HPLC [Phenomenex Onyx Monolithic C18 (100  $\times$  10mm) column; flow rate was 15 mL/min; gradient run, 0–3 min 5%B, 3–15 min linear gradient 5 to 20%B, 15–18 min 20%B, 18–20 min linear gradient 20 to 90%B, 20–22 min 90%B, 22–24 min linear gradient 90–5%B, 24–26 min 5%B; UV monitoring 214 nm] to afford peptide **13a** (7.1 mg, 44%) as a foam. LC-MS analysis (calculated mass 1973, observed mass 1975).

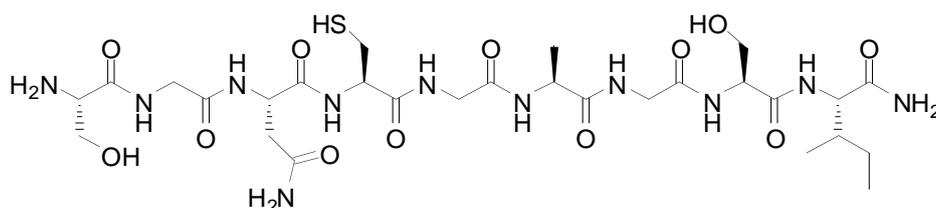


**Figure S17.** Analytical-HPLC analysis of **13a** after purification.



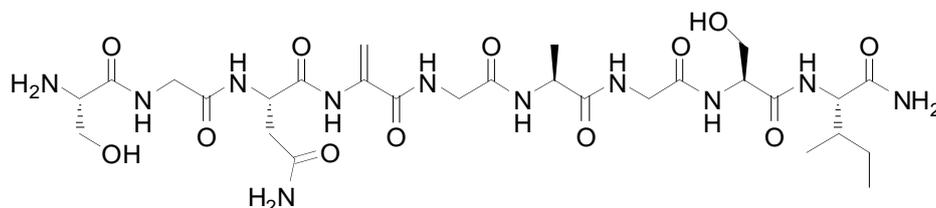
**Figure S18.** ESI-MS spectrum of glycopeptide **13a**.

**H<sub>2</sub>N-Ser-Gly-Asn-Cys-Gly-Ala-Gly-Ser-Ile-C(O)NH<sub>2</sub>**



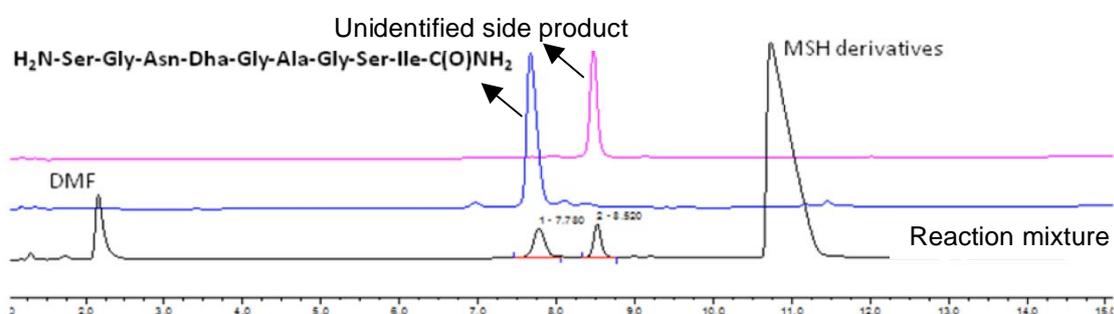
SBL peptide fragment (153-161; Ser-Gly-Asn-Cys-Gly-Ala-Gly-Ser-Ile) was synthesized by means of Fmoc-chemistry on Rink amide MBHA-polystyrene resin [1% divinyl benzene, GL Biochem] using a microwave assisted Liberty CEM peptide synthesizer. HRMS (ES<sup>+</sup>) for (M+H) C<sub>28</sub>H<sub>50</sub>F<sub>2</sub>N<sub>11</sub>O<sub>12</sub>S<sub>1</sub> (*m/z*): calc. 764.3361; found 764.3354.

**H<sub>2</sub>N-Ser-Gly-Asn-Dha-Gly-Ala-Gly-Ser-Ile-C(O)NH<sub>2</sub>**



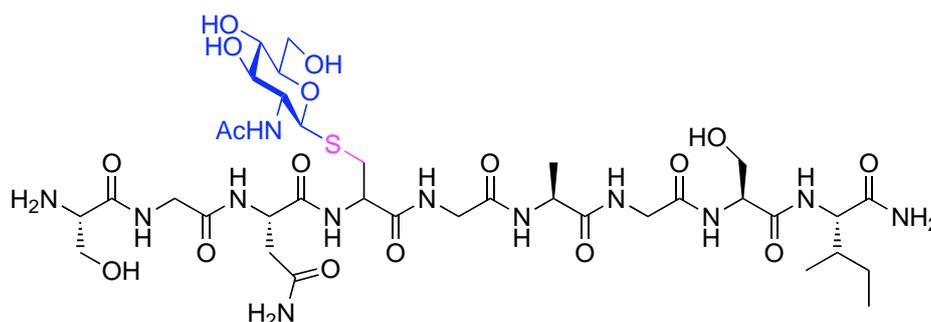
In a round bottom flask, *o*-mesitylenesulfonylhydroxylamine - MSH<sup>12</sup> (13.9 mg, 0.064 mmol) was dissolved in DMF (0.65 mL). In a separate vial, peptide SGNCGAGSI (8 mg, 0.010 mmol) was dissolved in deoxygenated H<sub>2</sub>O (2.5 mL) and sodium carbonate was added (11 mg, 0.104 mmol). The resulting solution was added by pipette over a period of 3 min to the stirred MSH solution at room temperature. The vial was washed with 0.5 mL of H<sub>2</sub>O. The reaction was stirred for additional 9 min, and quenched with TFA (pH 4). The reaction mixture was purified by preparative HPLC [Phenomenex Synergi 4u Fusion-RP 80A (100 × 21.20mm) column; flow rate was 12 mL/min; gradient run, 0-3 min 5%B, 3-13 min linear gradient 5 to 15%B, 13-15min linear gradient 15 to 85%B, 15-17 min 85%B, 17-19 min linear gradient 85-5%B, 19-21 min

5%B; UV monitoring 214 nm] and the collected fractions were lyophilized to provide the desired peptide SGNDhaGAGSI (4.2 mg, 55%) along with an as of yet unidentified side product (3.1 mg, 41%,  $m/z$  737.3200) as white solids; HRMS ( $ES^+$ ) for SGNDhaGAGSI ( $M+H$ )  $C_{28}H_{48}N_{11}O_{12}$  ( $m/z$ ): calc. 730.3484; found 730.3473.

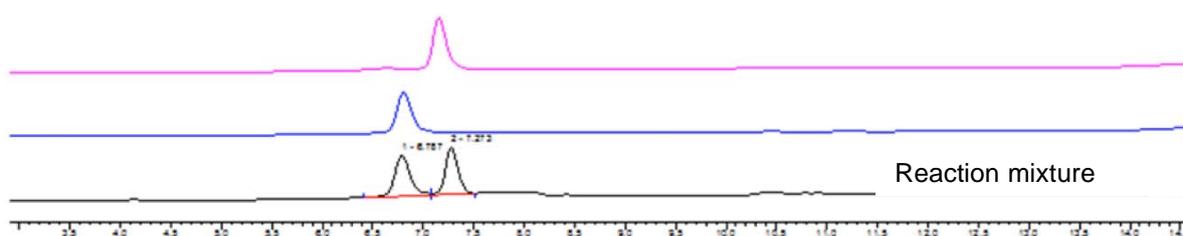


**Figure S19.** Analytical HPLC analysis of purification of SGNDhaGAGSI.

#### GlcNAc-S-linked peptide (14a)



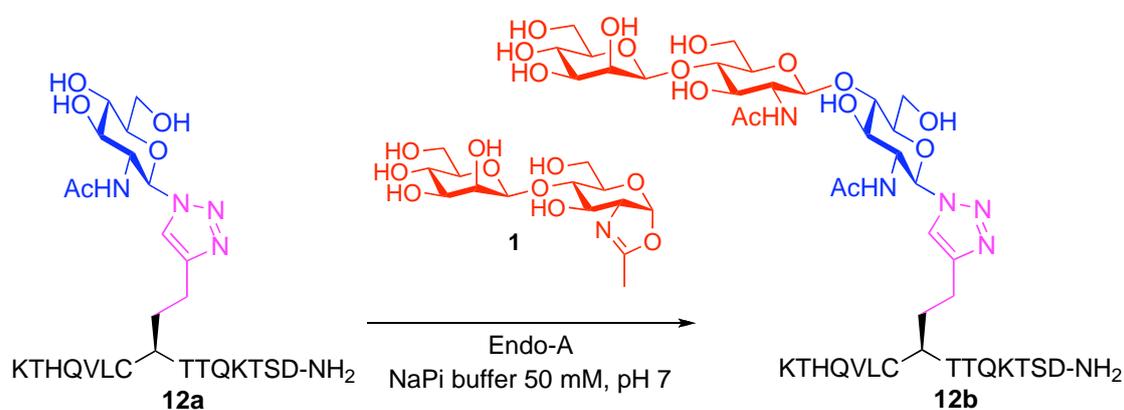
Peptide SGNDhaGAGSI( $NH_2$ ) (4 mg, 5.48  $\mu$ mol) was dissolved in 250 mM sodium phosphate buffer (1 mL, pH 8.1, previously deoxygenated with argon). 2-acetamido-2-deoxy-1-thio- $\beta$ -D-glucopyranose (10 mg, 0.042 mmol) was added as a solid and the reaction was stirred at room temperature under argon conditions. After 2.5 h, the reaction mixture was purified by preparative HPLC [Phenomenex Synergi 4u Fusion-RP 80A (100  $\times$  21.20mm) column; flow rate was 12 mL/min; gradient run, 0–3 min 5%B, 3–13 min linear gradient 5 to 15%B, 13–15min linear gradient 15 to 85%B, 15–17 min 85%B, 17–19 min linear gradient 85–5%B, 19–21 min 5%B; UV monitoring 214 nm] to provide **14a** as a mixture of diastereoisomers of ( $R_t$  8.5: 1.7 mg, and  $R_t$  8.9: 1.5 mg, 60%) as white solids; HRMS ( $ES^+$ ) for ( $M+H$ )  $C_{36}H_{63}N_{12}O_{17}S$  ( $m/z$ ): calc. 967.4155; found 967.4152.



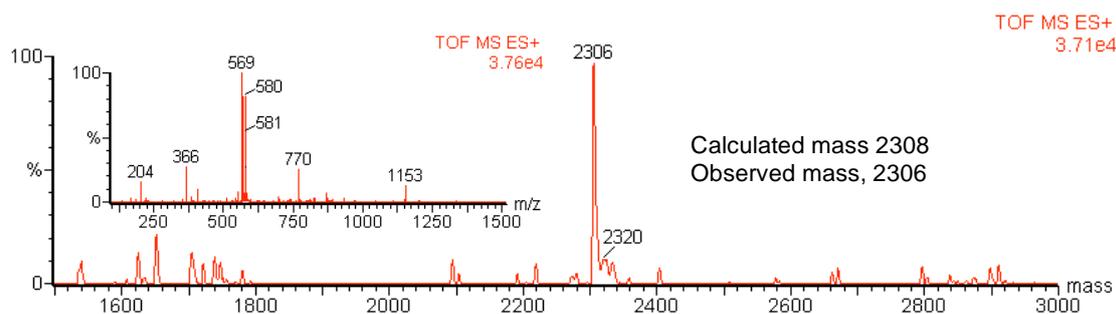
**Figure S20.** Analytical-HPLC analysis of the purified two diastereomers **14a** ( $R_t$  6.78 and  $R_t$  7.27).

## 2.4. Enzymatic extension of glycopeptides

### Endo A-catalyzed glycosylation of GlcNAc-triazole-peptide **12a**

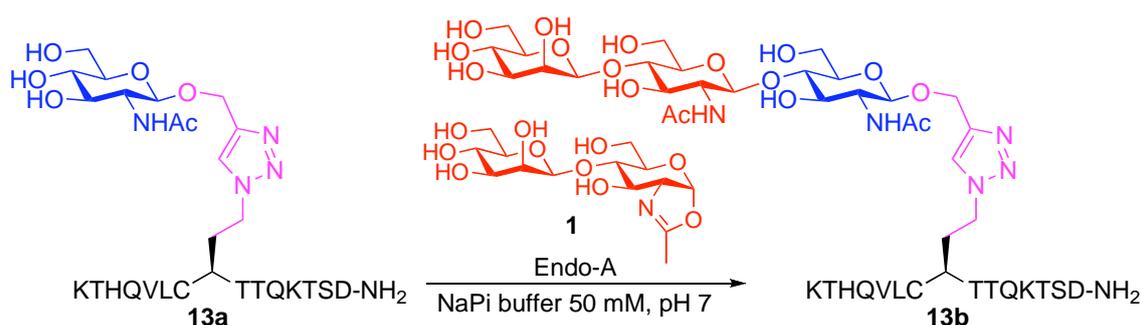


To a solution of oxazoline **1** (1.22 mg, 3.335  $\mu\text{mol}$ ) and glycopeptide **12a** (572  $\mu\text{g}$ , 0.294  $\mu\text{mol}$ ) in 50 mM sodium phosphate buffer (450  $\mu\text{L}$ , pH 7), Endo-A (4  $\mu\text{L}$  of 0.01 U/ $\mu\text{L}$  stock) was added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}\text{C}$ . Aliquots were taken through one day, centrifuged in a vivaspin-500 concentrator 10KDa MWCO (12.000 g, 8 min) and analyzed by LC-MS (Onyx-monolithic C18 100x4.6mm column; ESI<sup>+</sup>). After 2h, >95% conversion was observed (calculated mass 2308, observed mass 2306).

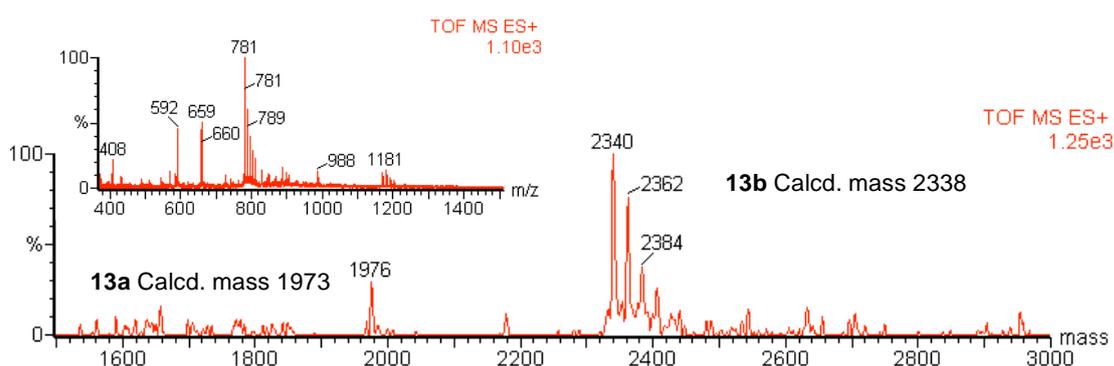


**Figure S21.** ESI-MS spectra after 2 h incubation of **12a** with Endo A.

### Endo A-catalyzed glycosylation of GlcNAc-OCH<sub>2</sub>-triazole-peptide **13a**

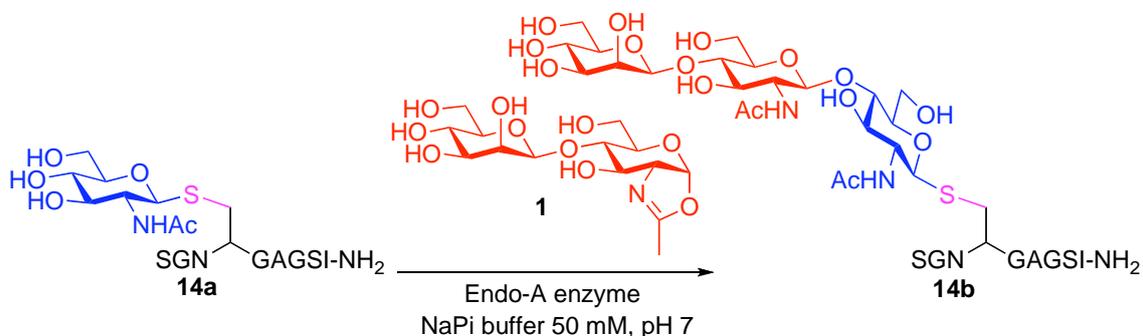


To a solution of oxazoline **1** (1.77 mg, 4.844  $\mu$ mol) and glycopeptide **13a** (1.06 mg, 0.537  $\mu$ mol) in 50 mM sodium phosphate buffer (800  $\mu$ L, pH 7), Endo-A (6  $\mu$ L of 0.01 U/ $\mu$ L stock) was added and the mixture was rotated on a lab rotisserie at 22 °C. Aliquots were taken through one day, centrifuged in a vivaspin-500 concentrator 10KDa MWCO (12.000 g, 8 min) and analyzed by LC-MS (Onyx-monolithic C18 100x4.6mm column; ESI<sup>+</sup>). Conversion of 77% was observed after 8 h (calculated mass 2338, observed mass 2340).



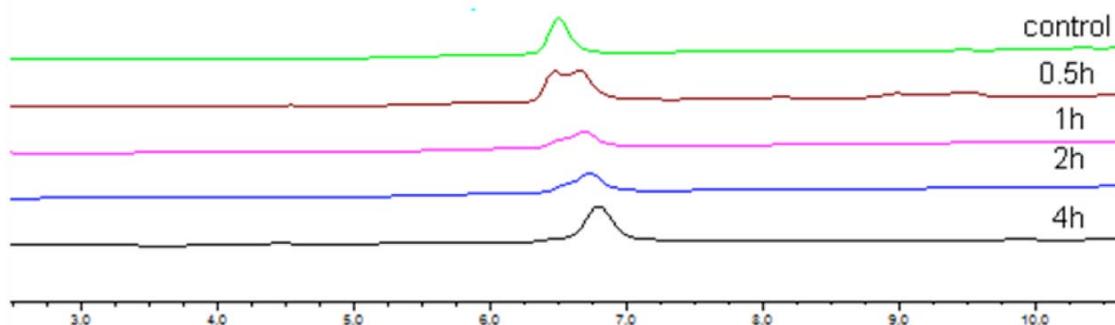
**Figure S22.** ESI-MS spectra after 8 h incubation of **13a** with Endo A.

### Endo A-catalyzed glycosylation of GlcNAc-S-peptide **14a** (R<sub>t</sub> 6.78 and R<sub>t</sub> 7.27)

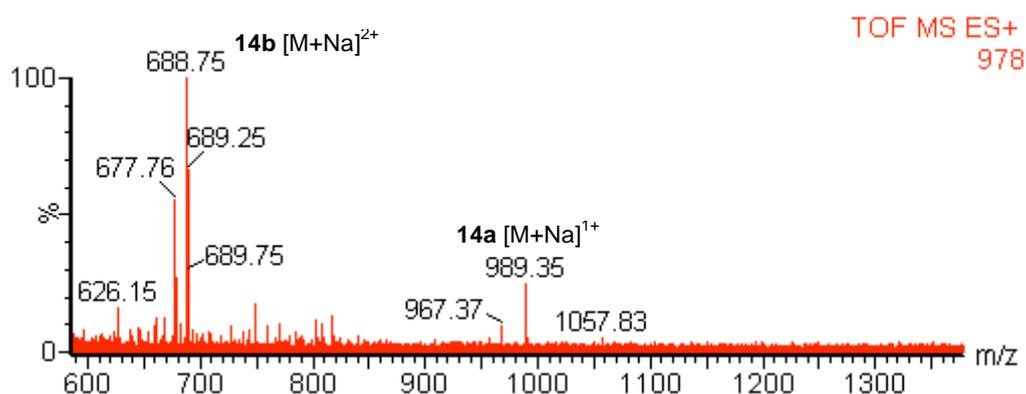


To a solution of oxazoline **1** (1.23 mg, 3.36  $\mu$ mol) and one diastereomer **14a** (441  $\mu$ g, 0.45  $\mu$ mol) in 50 mM sodium phosphate buffer (225  $\mu$ L, pH 7), Endo-A (3  $\mu$ L of 0.01 U/ $\mu$ L stock) was added and the mixture was rotated on a lab rotisserie at 22 °C. Aliquots

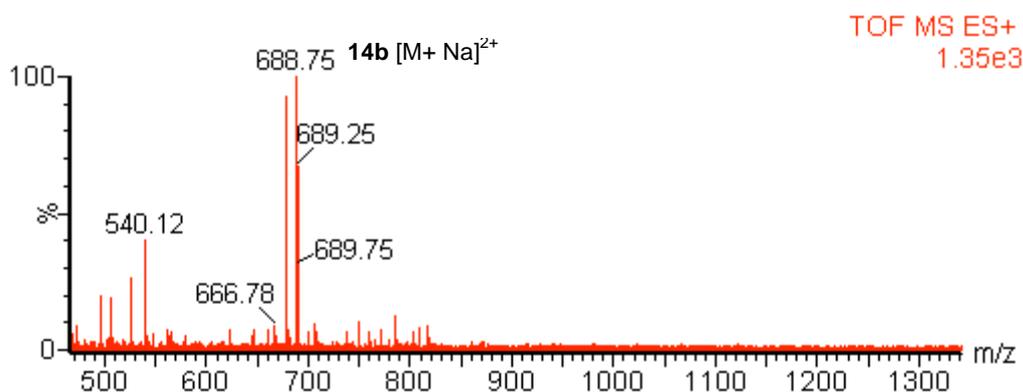
were taken, centrifuged in a vivaspin-500 concentrator 10KDa MWCO (12.000 g, 8 min) and analyzed by HPLC [Phenomenex Synergi 4u Fusion-RP 80A (100 × 4.6mm) column; flow rate was 1 mL/min; gradient run, 0-3 min 5%B, 3-12 min linear gradient 5 to 25%B, 12-18min linear gradient 25 to 85%B, 18-20 min 85%B, 20-22 min linear gradient 85-5%B, 22-24 min 5%B; UV monitoring 214 nm] and MS (ESI<sup>+</sup>). After 4h, >80% and >95% conversion were observed for diastereoisomer *Rt* 6.78 and *Rt* 7.27, respectively.



**Figure S23.** HPLC analysis after incubation of **14a** (isomer *Rt* 6.78) with Endo A.



**Figure S24.** ESI-MS spectra after 4 h incubation of **14a** (isomer *Rt* 6.78) with Endo A.



**Figure S25.** ESI-MS spectra after 4 h incubation of **14a** (isomer *Rt* 7.27) with Endo A.

## 2.5. Preparation of *S*-, *SeS*- and *SS*-linked glycoproteins 15a–24a

### 2.5.1 Synthesis of 2-deoxy-2-fluoro-1-thiosugars

#### 2-Deoxy-2-fluoro-1-thio- $\beta$ -D-mannopyranose



3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-1-thio- $\beta$ -D-mannopyranose (32 mg, 0.087 mmol) was treated with 0.1M NaOMe in MeOH (610  $\mu$ L) at room temperature. The reaction mixture was stirred at the same temperature for 15 minutes and neutralized with Dowex ( $H^+$  50WX8–200). The ion exchanger was filtered off and washed with MeOH. After concentration under reduced pressure, crude product 2-Deoxy-2-fluoro-1-thio- $\beta$ -D-mannopyranose (17.2 mg, 100%) was used in the next step without further purification. LRMS (TOF ES<sup>-</sup>) for (M–H) C<sub>6</sub>H<sub>10</sub>FO<sub>4</sub>S (*m/z*): calc. 197.0; found 197.1.

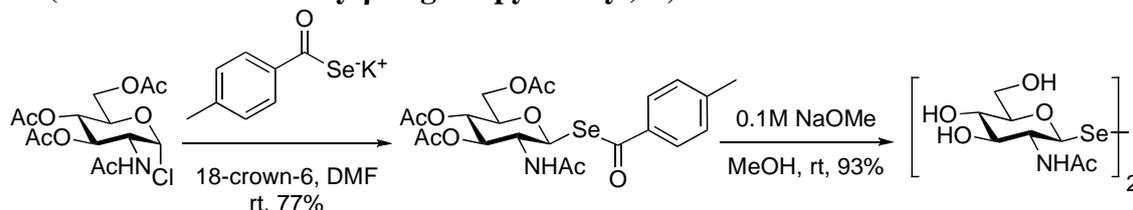
#### 2-Deoxy-2-fluoro-1-thio- $\beta$ -D-glucopyranose<sup>14</sup>



3,4,6-Tri-*O*-acetyl-2-deoxy-2-fluoro-1-thio- $\beta$ -D-glucopyranose (42 mg, 0.115 mmol) was treated with 0.1M NaOMe in MeOH (805  $\mu$ L) at room temperature. The reaction mixture was stirred at the same temperature for 15 minutes and neutralized with Dowex ( $H^+$  50WX8–200). The ion exchanger was filtered off and washed with MeOH. The resulting solution was concentrated under reduced pressure to afford 2-deoxy-2-fluoro-1-thio- $\beta$ -D-glucopyranose (22.8 mg, 100%) as a white solid. Used in the next step without further purification. LRMS (TOF ES<sup>-</sup>) for (M–H) C<sub>6</sub>H<sub>10</sub>FO<sub>4</sub>S (*m/z*): calc. 197.0; found 197.1.

### 2.5.2 Synthesis of sugar diselenides

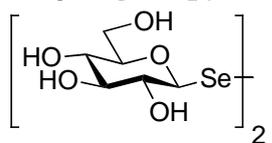
#### Bis(2-Acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-1,1'-diselenide



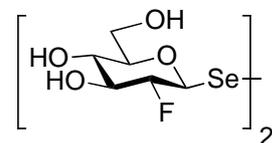
Potassium 4-methylselenobenzoate<sup>15</sup> (215.7 mg, 0.910 mmol) was added to a mixture of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy- $\alpha$ -D-glucopyranosyl chloride<sup>16</sup> (250 mg, 0.455 mmol) and 18-crown-6 (240.4 mg, 0.910 mmol) in dry and degassed DMF (1.7 mL) at room temperature. The reaction mixture was stirred for 2 h. The crude was then diluted

with EtOAc and washed with water. The combined organic layers were dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (from 1:1 to 2:1 EtOAc/petrol) to afford 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*Se*-(4-methylbenzoyl)-1-seleno-β-D-glucopyranose (185 mg, 77%) as a white solid. R<sub>f</sub> (2:1 EtOAc/petrol): 0.26; mp: 176–178 °C; [α]<sub>D</sub><sup>20</sup>: –30.0 (0.17, CH<sub>2</sub>Cl<sub>2</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 500 MHz) δ in ppm: 7.75 (d, *J* = 8.2 Hz, 2H, Ar), 7.27 (d, *J* = 8.2 Hz, 2H, Ar), 5.70 (d, *J*<sub>2,NH</sub> = 9.6 Hz, 1H, NH), 5.40 (d, *J*<sub>1,2</sub> = 10.7 Hz, 1H, H-1), 5.21 (t, *J*<sub>3,4</sub> = *J*<sub>4,5</sub> = 9.6 Hz, 1H, H-4), 5.14 (t, *J*<sub>2,3</sub> = *J*<sub>3,4</sub> = 9.6 Hz, 1H, H-3), 4.56 (dd, *J*<sub>1,2</sub> = 10.7 Hz, *J*<sub>2,3</sub> = *J*<sub>2,NH</sub> = 9.6 Hz, 1H, H-2), 4.28 (dd, *J*<sub>6a,b</sub> = 12.6 Hz, *J*<sub>5,6a</sub> = 4.7 Hz, 1H, H-6a), 4.13 (dd, *J*<sub>6a,b</sub> = 12.6 Hz, *J*<sub>5,6b</sub> = 1.9 Hz, 1H, H-6b), 3.84 (ddd, *J*<sub>4,5</sub> = 9.6, *J*<sub>5,6a</sub> = 4.7 Hz, *J*<sub>5,6b</sub> = 1.9 Hz, 1H, H-5), 2.41 (s, 3H, CH<sub>3</sub>, Ar), 2.09, 2.06 (s, 9H, 3CH<sub>3</sub>, OAc), 1.88 (s, 3H, CH<sub>3</sub>, NAc); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 125.8 MHz) δ in ppm: 193.0 (C=O, SeBz), 171.3, 170.7, 170.0, 169.2 (C=O, 3OAc, NAc), 145.6, 135.5 (C, Ar), 129.7, 135.5 (CH, Ar), 81.4 (C-1), 77.6 (C-5), 74.2 (C-3), 67.8 (C-4), 61.9 (C-6), 53.5 (C-2), 23.2 (CH<sub>3</sub>, NAc), 21.8 (CH<sub>3</sub>, Ar), 20.8, 20.7, 20.6 (3CH<sub>3</sub>, OAc); FT-IR (KBr) ν in cm<sup>-1</sup>: 3283, 3062, 2957, 1747, 1685, 1664, 1232; HRMS (TOF ES+) for (M+Na) C<sub>22</sub>H<sub>27</sub>NNaO<sub>9</sub>Se (*m/z*): calc. 552.0749; found 552.0748.

Finally, the title compound was prepared following the general procedure for the synthesis of 2-deoxy-2-fluoro-glycosyl azides described above, starting from 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-*Se*-(4-methylbenzoyl)-1-seleno-β-D-glucopyranose (100 mg, 0.189 mmol) and 0.1M NaOMe in MeOH (1.3 mL). After 30 min stirring at room temperature the reaction mixture was neutralized and concentrated under reduced pressure. The crude product was recrystallized from 1:2 MeOH/EtOAc to afford bis(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-1,1'-diselenide (50.1 mg, 93%) as a white solid. R<sub>f</sub> (2:5 MeOH/EtOAc): 0.09; mp: 165 °C dec. (1:2 MeOH/EtOAc); [α]<sub>D</sub><sup>20</sup>: –241.4 (0.03, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ in ppm: 5.06 (d, *J*<sub>1,2</sub> = 10.1 Hz, 1H, H-1), 3.89–3.77 (m, 3H, H-2,6a,b), 3.52 (dd, *J*<sub>2,3</sub> = 8.8 Hz, *J*<sub>3,4</sub> = 9.1 Hz, 1H, H-3), 3.44 (dd, *J*<sub>4,5</sub> = 8.8 Hz, *J*<sub>5,6a</sub> = 9.1 Hz, 1H, H-5), 3.32 (m, 1H, H-4), 1.99 (s, 3H, CH<sub>3</sub>, NAc); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125.8 MHz) δ in ppm: 173.7 (C=O, NAc), 84.6 (C-1), 83.4 (C-4), 77.1 (C-3), 71.6 (C-5), 62.6 (C-6), 57.9 (C-2), 23.3 (CH<sub>3</sub>, NAc); FT-IR (KBr) ν in cm<sup>-1</sup>: 3267, 3096, 2925, 2908, 1640, 1562, 1056; HRMS (TOF ES+) for (M+Na) C<sub>16</sub>H<sub>28</sub>N<sub>2</sub>NaO<sub>10</sub>Se<sub>2</sub> (*m/z*): calc. 590.9967; found 590.9959.

**Bis( $\beta$ -D-glucopyranosyl)-1,1'-diselenide**

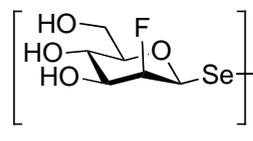
The title compound was prepared following the general procedure for the synthesis of 2-deoxy-2-fluoro-glycosyl azides described above, starting from bis(2,3,4,6-tetra-*O*-acetyl- $\beta$ -D-glucopyranosyl)-1,1'-diselenide<sup>15, 17</sup> (64.1 mg, 0.078 mmol) and 0.1M NaOMe in MeOH (546  $\mu$ L). After 30 min stirring at room temperature the reaction mixture was neutralized and concentrated under reduced pressure. The crude product was recrystallized from 1:2 MeOH/EtOAc to afford bis( $\beta$ -D-glucopyranosyl)-1,1'-diselenide (27.2 mg, 72%) as a white hygroscopic solid together with ca. 26% of the corresponding 1-selenopyranose.  $R_f$  (2:5 MeOH/EtOAc): 0.1;  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  in ppm: 4.76 (d, 1H,  $J_{1,2} = 9.8$  Hz, H-1), 3.88 (d,  $J_{6a,b} = 12$  Hz, H-6a), 3.71–3.64 (m, 2H, H-4,6b), (appt,  $J_{1,2} = J_{2,3} = 9.8$  Hz, H-2), 3.41–3.27 (m 2H, H-3,5);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 125.8 MHz)  $\delta$  in ppm: 84.9 (C-1), 83.6 (C-5), 79.5 (C-3), 75.1 (C-2), 71.4 (C-4), 63.0 (C-6); LRMS (TOF ES+) isotope ratios for (M+Na)  $\text{C}_{12}\text{H}_{22}\text{NaO}_{10}\text{Se}_2$  ( $m/z$ ) (%): calc. 508.9 (100), 506.9 (86.6); found 509.0 (100), 507.0 (87). Spectroscopic data are in agreement with those reported in the literature.<sup>17</sup>

**Bis(2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl)-1,1'-diselenide**

The title compound was prepared following the general procedure for the synthesis of 2-deoxy-2-fluoro-glycosyl azides described above, starting from bis(3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl)-1,1'-diselenide (35 mg, 0.047 mmol) and 0.1M NaOMe in MeOH (330  $\mu$ L). After 15 min stirring at room temperature the reaction mixture was neutralized and concentrated under reduced pressure. The residue was purified by column chromatography (7:1 EtOAc/MeOH) to afford bis(2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl)-1,1'-diselenide (16.1 mg, 100%) as a pale yellow solid.  $R_f$  (7:1 MeOH/EtOAc): 0.26; mp: 240  $^{\circ}\text{C}$  dec;  $[\alpha]_D^{20}$ : +10.2 (0.16, MeOH);  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 500 MHz)  $\delta$  in ppm: 4.96 (dd, 1H,  $J_{1,2} = 9.5$  Hz,  $J_{1,F} = 1.6$  Hz, H-1), 4.41 (ddd, 1H,  $J_{2,F} = 49.6$  Hz,  $J_{1,2} = J_{2,3} = 9.5$  Hz, H-2), 3.89 (d,  $J_{6a,b} = 12$  Hz, H-6a), 3.70–3.63 (m, 2H, H-3,6b), 3.36 (m 2H, H-4,5);  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 125.8 MHz)  $\delta$  in ppm: 93.1 (d,  $J_{2,F} = 185.0$  Hz, C-2), 83.7 (C-5), 80.9 (d,  $J_{1,F} = 26.7$  Hz, C-1), 77.6 (d,  $J_{3,F} = 18.1$  Hz, C-3), 71.2 (d,  $J_{4,F} = 7.6$  Hz, C-4), 62.7 (C-6);  $^{19}\text{F NMR}$  ( $\text{CD}_3\text{OD}$ , 470.4 MHz)  $\delta$  in ppm: –190.0 (ddd,  $J_{2,F} = 49.6$  Hz,  $J_{3,F} = 14.8$  Hz,  $J_{1,F} = 1.6$  Hz, F-2); FT-IR (KBr)  $\nu$  in  $\text{cm}^{-1}$ :

3385, 2924, 2854, 1378, 1065, 1043; HRMS (TOF ES+) for (M+Na) C<sub>12</sub>H<sub>20</sub>F<sub>2</sub>NaO<sub>8</sub>Se<sub>2</sub> (*m/z*): calc. 512.9352; found 512.9350.

**Bis(2-deoxy-2-fluoro-β-D-mannopyranosyl)-1,1'-diselenide**



The title compound was prepared following the general procedure for the synthesis of 2-deoxy-2-fluoro-glycosyl azides described above, starting from 3,4,6-tri-*O*-acetyl-2-deoxy-2-fluoro-1-*Se*-(4-methylbenzoyl)-1-seleno-β-D-mannopyranose (35 mg, 0.066 mmol) and 0.1M NaOMe in MeOH (462 μL). After 30 min stirring at room temperature the reaction mixture was neutralized and concentrated under reduced pressure. The residue was purified by column chromatography (7:1 EtOAc/MeOH) to afford bis(2-deoxy-2-fluoro-β-D-mannopyranosyl)-1,1'-diselenide (16.1 mg, 100%) as a pale yellow solid. *R<sub>f</sub>* (7:1 MeOH/EtOAc): 0.13; mp: 200 °C dec; [α]<sub>D</sub><sup>20</sup>: +26.8 (0.08, MeOH); <sup>1</sup>H NMR (CD<sub>3</sub>OD, 500 MHz) δ in ppm: 5.42 (d, 1H, *J*<sub>1,F</sub> = 30.3 Hz, H-1), 4.97 (dd, 1H, *J*<sub>2,F</sub> = 50.4 Hz, *J*<sub>2,3</sub> = 2.5 Hz, H-2), 3.91 (dd, *J*<sub>6a,b</sub> = 12 Hz, *J*<sub>5,6a</sub> = 1.9 Hz, H-6a), 3.70–3.59 (m, 3H, H-3,4,6b), 3.35 (m 1H, H-5); <sup>13</sup>C NMR (CD<sub>3</sub>OD, 125.8 MHz) δ in ppm: 93.4 (d, *J*<sub>2,F</sub> = 179.3 Hz, C-2), 84.0 (C-5), 83.7 (d, *J*<sub>1,F</sub> = 19.1 Hz, C-1), 74.7 (d, *J*<sub>3,F</sub> = 19.1 Hz, C-3), 68.5 (C-4), 63.0 (C-6); <sup>19</sup>F NMR (CDCl<sub>3</sub>, 470.4 MHz) δ in ppm: –210.1 (ddd, *J*<sub>2,F</sub> = 50.4 Hz, *J*<sub>1,F</sub> = 30.3 Hz, *J*<sub>3,F</sub> = 29.3 Hz, F-2); FT-IR (KBr) ν in cm<sup>-1</sup>: 3356, 2924, 2854, 1375, 1064, 1043; HRMS (TOF ES+) for (M+Na) C<sub>12</sub>H<sub>20</sub>F<sub>2</sub>NaO<sub>8</sub>Se<sub>2</sub> (*m/z*): calc. 512.9352; found 512.9362.

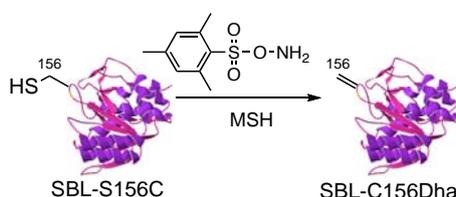
### 2.5.3 Preparation of glycoproteins 15a–24a

#### Sequence of SBL-S156C (BPN' numbering, PDB code for wild type SBL = 1GCI)

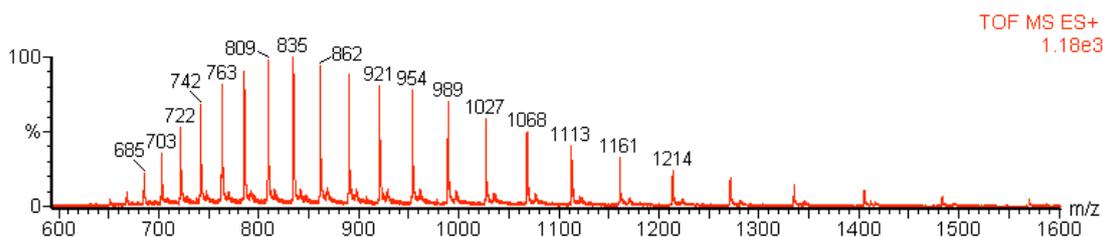
AQSVPWGISRVQAPAAHNRGLTGSGVKVAVLDTGISTHPDLNIRGGASFVPGEP  
 STQDGNGHGHVAGTIAALNNSIGVLGVAPSAELYAVKVLGASGSGSVSSIAQ  
 GLEWAGNNGMHVANLSLGSPPSATLEQAVNSATSRGVLVVAASGNCGAGSIS  
 YPARYANAMAVGATDQNNNRASFSQYGAGLDIVAPGVNVQSTYPGSTYASLN  
 GTSMATPHVAGAAALVKQKNPSWSNVQIRNHLKNTATSLGSTNLYGSGLVNA  
 EAATR

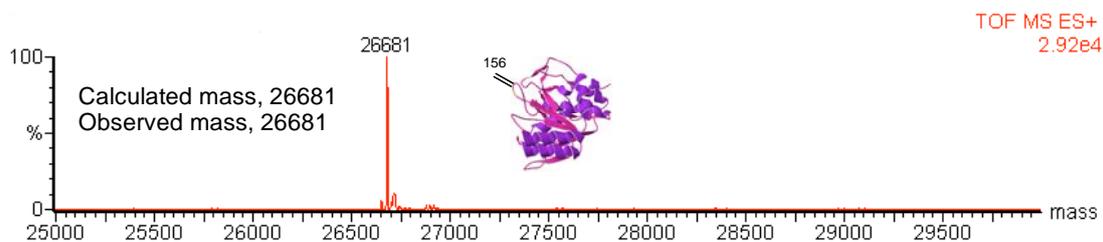
Calculated average isotopic mass = 26714.5

#### Conversion of SBL-S156C to SBL-C156Dha<sup>12</sup>



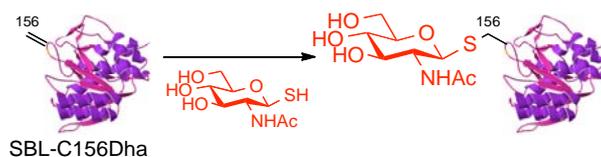
All manipulations were carried out in a cold room maintained at 4 °C. A 1 mg sample of lyophilized SBL-S156C (0.037 μmol) was dissolved in 1.0 mL in 50 mM sodium phosphate buffer (pH 8.0). A solution of *O*-mesitylsulfonylhydroxylamine<sup>12</sup> (MSH) was prepared by dissolving 1.8 mg (8.36 μmol) in 100 μL DMF. A 50 μL portion of the MSH solution was added to the protein by micropipette. The reaction was vortexed periodically over 1 minute and then rotated on a lab rotisserie for an additional 19 minutes at 4 °C. A 50 μL aliquot was analyzed by LC–MS to confirm the conversion of Cys156 to Dha156 (26681 calculated, 26681 found). Small molecules were removed from the reaction mixture by loading the sample onto a PD10 desalting column (GE Healthcare) previously equilibrated with 10 column volumes of pH 8.0 sodium phosphate buffer (50 mM) and eluting with 3.50 mL of the same buffer. The collected sample (now diluted to 0.29 mg/mL) was split into 300 μL aliquots, flash frozen with liquid nitrogen, and stored at -80 °C.



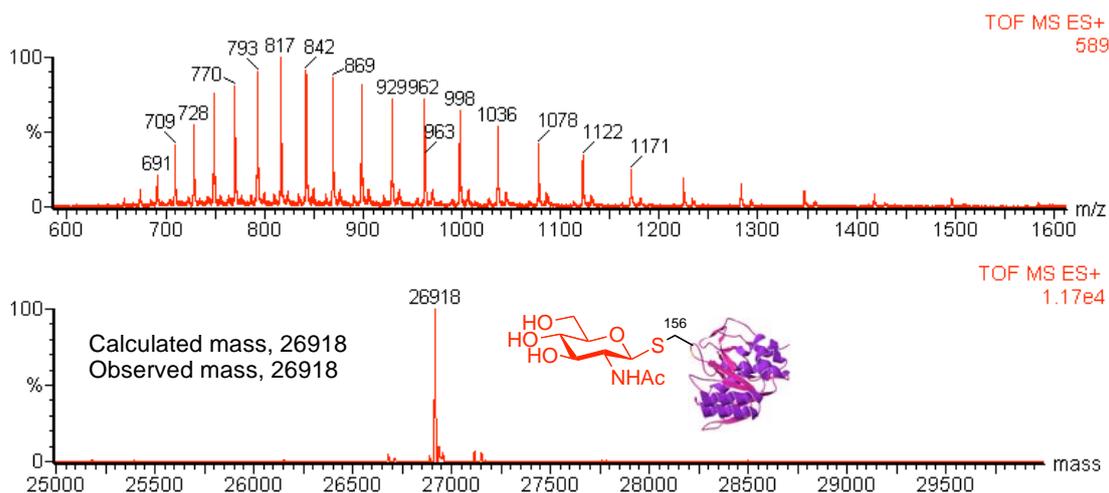


**Figure S26.** ESI-MS spectrum of SBL-C156Dha.

### Preparation of SBL-C156SGlcNAc (**15a**)

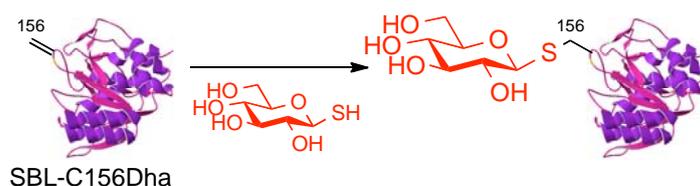


All manipulations were carried out in a cold room maintained at 4 °C. A 250  $\mu$ L aliquot of SBL-C156Dha (0.29 mg/mL, 2.72 nmol) previously prepared was thawed. To the protein solution was added 2-acetamido-2-deoxy-1-thio- $\beta$ -D-glucopyranose as a solid (8.8 mg, 1000 equiv.) to give a 39 mM solution in thiol. After 90 minutes of shaking at 4 °C, the reaction was analyzed directly by LC-MS. Complete conversion to SBL-C156SGlcNAc **15a** was observed (calculated mass, 26918; observed mass, 26918). The reaction mixture was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at -20 °C.

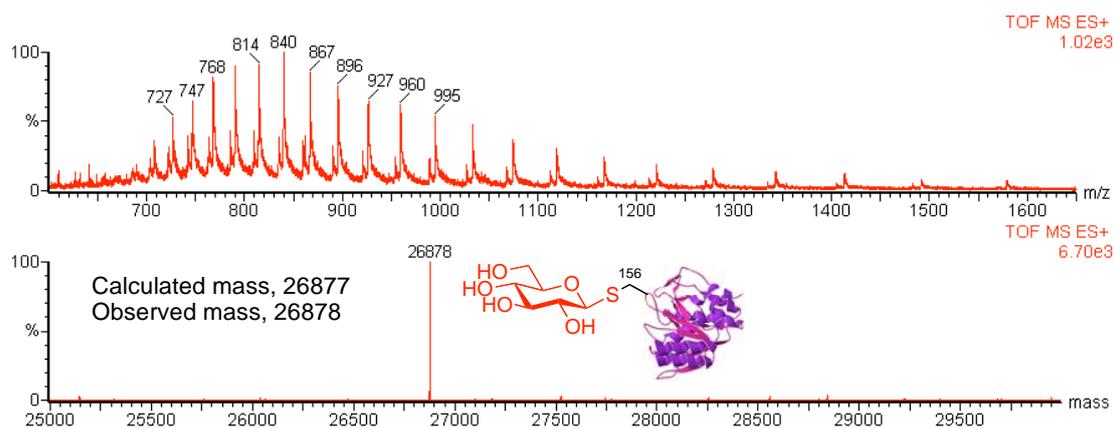


**Figure S27.** ESI-MS spectrum of SBL-C156SGlcNAc **15a**.

### Preparation of SBL-C156SGlc (16a)

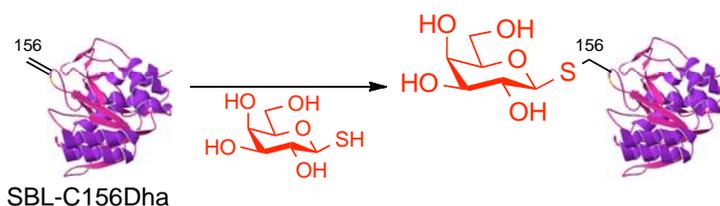


All manipulations were carried out in a cold room maintained at 4 °C. A 250  $\mu$ L aliquot of SBL-C156Dha (0.29 mg/mL, 2.72 nmol) previously prepared was thawed. To the protein solution was added 1-thio- $\beta$ -D-glucopyranose as a solid (5.3 mg, 0.027 mmol) and the resulting mixture vortexed for 1 minute. After 50 minutes of shaking, the reaction was analyzed directly by LC-MS and complete conversion to SBL-C156SGlc **16a** was observed (calculated mass, 26877; observed mass, 26878). The reaction mixture was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at -20 °C.



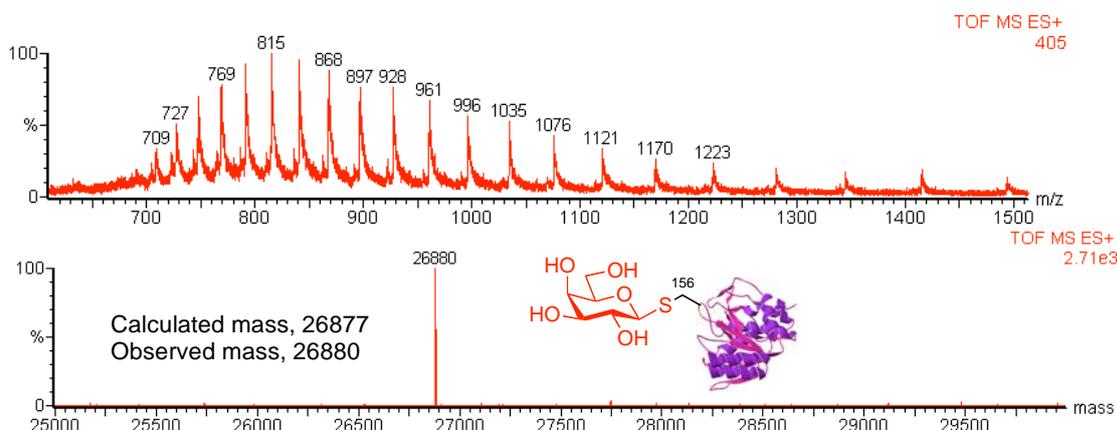
**Figure S28.** ESI-MS spectrum of SBL-C156SGlc **16a**.

### Preparation of SBL-C156SGal (17a)



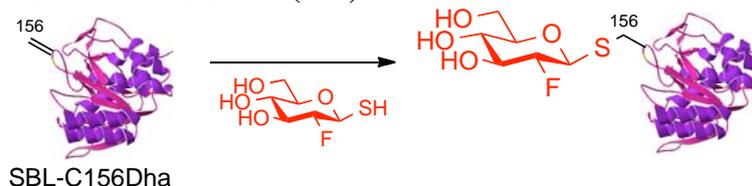
All manipulations were carried out in a cold room maintained at 4 °C. A 235  $\mu$ L aliquot of SBL-C156Dha (0.29 mg/mL, 2.55 nmol) previously prepared was thawed. To the protein solution was added 1-thio- $\beta$ -D-galactopyranose sodium salt as a solid (6 mg, 0.027 mmol) and the resulting mixture vortexed for 30 seconds. After 100 minutes of shaking, the reaction was analyzed directly by LC-MS and complete conversion to SBL-C156SGal **17a** was observed (calculated mass, 26877; observed mass, 26880).

After 2 h, the protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at  $-20\text{ }^{\circ}\text{C}$ .

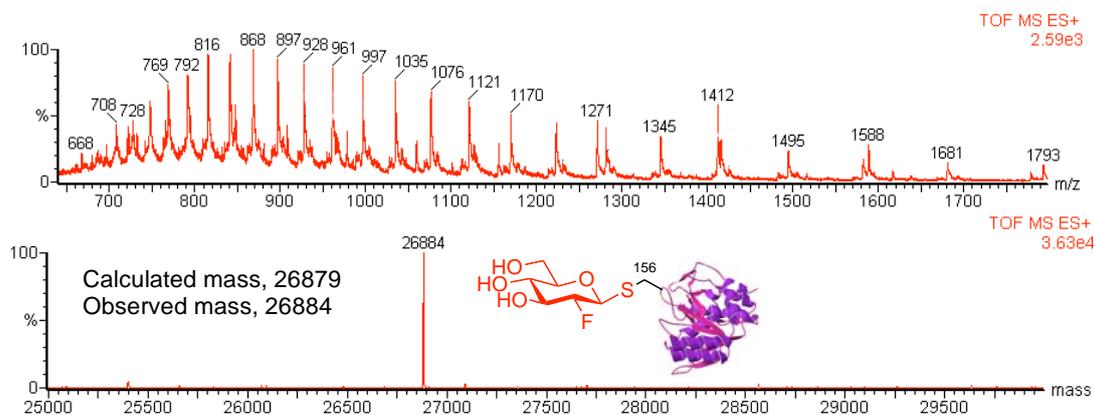


**Figure S29.** ESI-MS spectrum of SBL-C156-SGal **17a**.

### Preparation of SBL-C156-SGlc2F (**18a**)

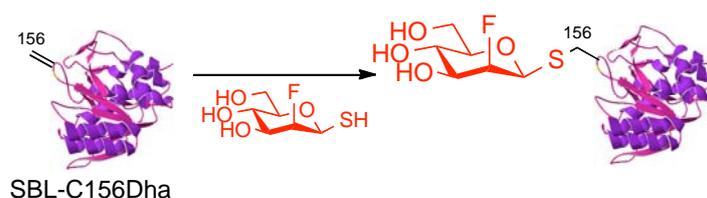


All manipulations were carried out in a cold room maintained at  $4\text{ }^{\circ}\text{C}$ . A  $300\text{ }\mu\text{L}$  aliquot of SBL-C156Dha ( $0.4\text{ mg/mL}$ ,  $4.49\text{ nmol}$ ) previously prepared was thawed. To the protein solution was added 2-deoxy-2-fluoro-1-thio- $\beta$ -D-glucopyranose as a solid ( $3.2\text{ mg}$ ,  $0.016\text{ mmol}$ ) and the resulting mixture vortexed for 30 seconds. After 3h of shaking, the reaction was analyzed directly by LC-MS and complete conversion to SBL-C156SGlc2F **18a** was observed (calculated mass,  $26879$ ; observed mass,  $26884$ ). The protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at  $-20\text{ }^{\circ}\text{C}$ .

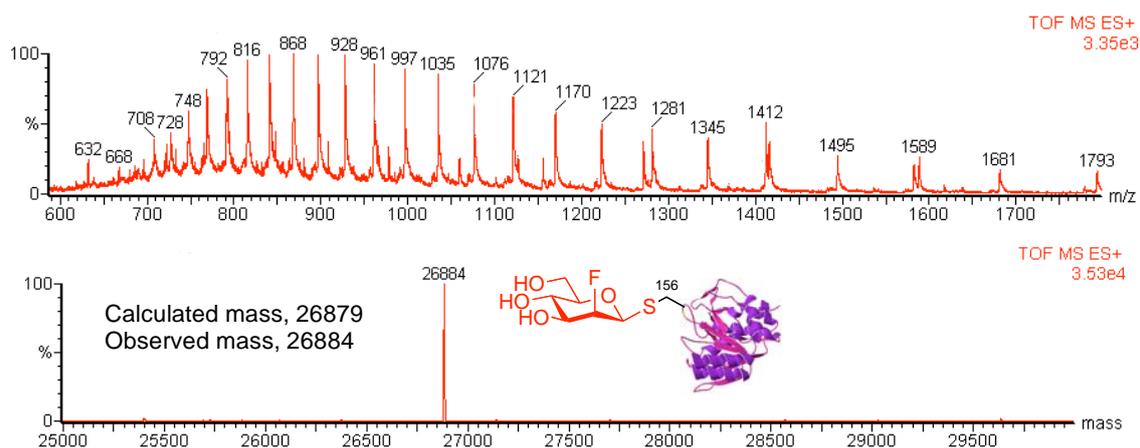


**Figure S30.** ESI-MS spectrum of SBL-C156-SGlc2F **18a**.

### Preparation of SBL-C156-SMan2F (19a)

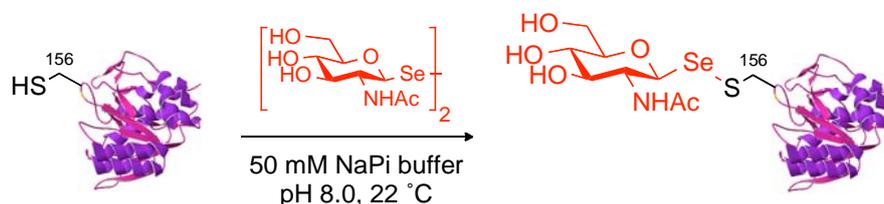


All manipulations were carried out in a cold room maintained at 4 °C. A 300  $\mu$ L aliquot of SBL-C156Dha (0.4 mg/mL, 4.49 nmol) previously prepared was thawed. To the protein solution was added 2-deoxy-2-fluoro-1-thio- $\beta$ -D-mannopyranose as a solid (3.7 mg, 0.018 mmol) and the resulting mixture vortexed for 30 seconds. After 2h of shaking, the reaction was analyzed directly by LC-MS and complete conversion to SBL-C156SGlc2F **19a** was observed (calculated mass, 26879; observed mass, 26884). The protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at -20 °C.



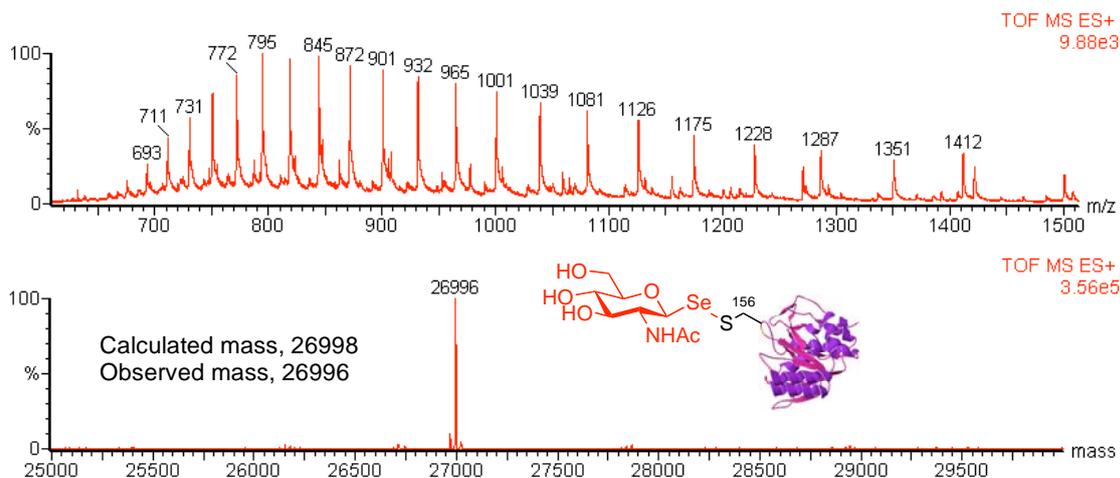
**Figure S31.** ESI-MS spectrum of SBL-C156-SMan2F **19a**.

### Preparation of SBL-C156-SSeGlcNAc (20a)



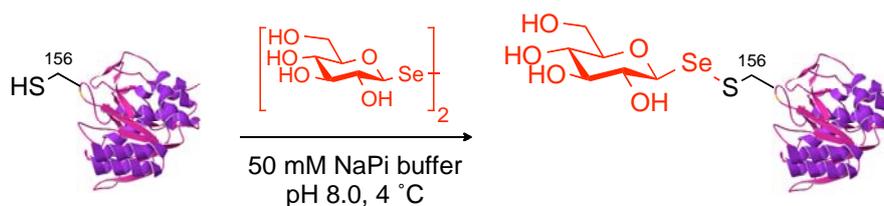
To a solution of SBL-S156C (1 mL of 1 mg/mL, 0.037  $\mu$ mol) in 50 mM sodium phosphate buffer (pH 8.0), bis(2-acetamido-2-deoxy- $\beta$ -D-glucopyranosyl)-1,1'-diselenide (2.1 mg, 3.708  $\mu$ mol) was added as a solid and the resulting mixture vortexed for 30 seconds. After 135 minutes of additional shaking at 22 °C, the reaction was analyzed directly by LC-MS and complete conversion to SBL-C156SSeGlcNAc **20a**

was observed (calculated mass, 26998; observed mass, 26996). The protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at  $-20\text{ }^{\circ}\text{C}$ .

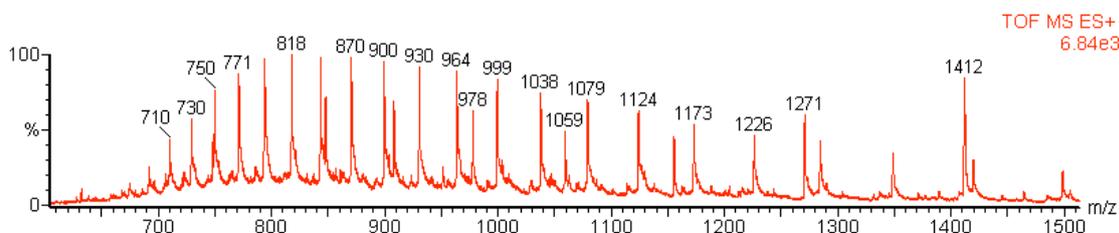


**Figure S32.** ESI-MS spectrum of SBL-C156-SSeGlcNAc **20a**.

### Preparation of SBL-C156-SSeGlc (**21a**)



All manipulations were carried out in a cold room maintained at  $4\text{ }^{\circ}\text{C}$ . To a solution of SBL-S156C (1 mL of 1 mg/mL,  $0.037\text{ }\mu\text{mol}$ ) in 50 mM sodium phosphate buffer (pH 8.0), bis( $\beta$ -D-glucopyranosyl)-1,1'-diselenide (1.9 mg,  $3.923\text{ }\mu\text{mol}$ ) was added as a solid and the resulting mixture vortexed for 30 seconds. After 1 h of additional shaking, the reaction was analyzed directly by LC-MS and complete conversion to SBL-C156-SSeGlc **21a** was observed (calculated mass, 26956; observed mass, 26955). The protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at  $-20\text{ }^{\circ}\text{C}$ .



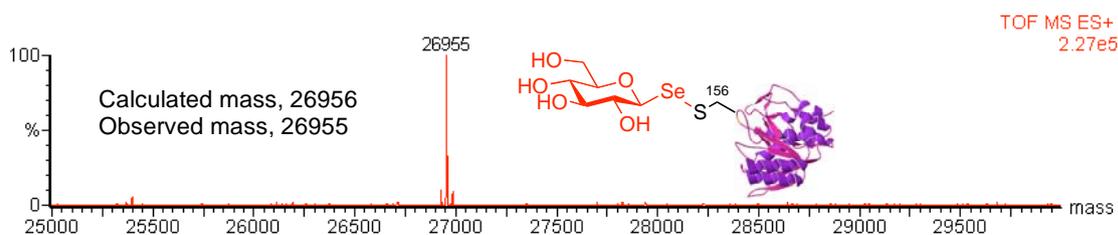
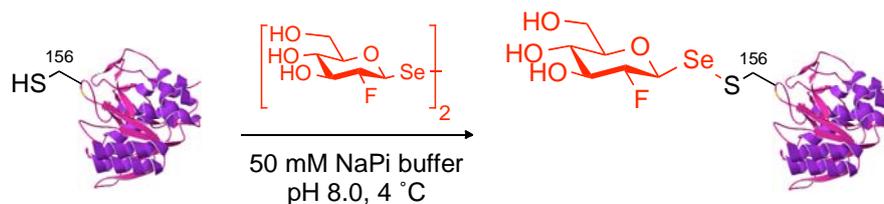


Figure S33. ESI-MS spectrum of SBL-C156-SSeGlc **21a**.

### Preparation of SBL-C156-SSeGlc2F (**22a**)



All manipulations were carried out in a cold room maintained at 4 °C. To a solution of SBL-S156C (250  $\mu$ L of 1 mg/mL, 9.35 nmol) in 50 mM sodium phosphate buffer (pH 8.0), bis(2-deoxy-2-fluoro- $\beta$ -D-glucopyranosyl)-1,1'-diselenide (0.45 mg, 0.922  $\mu$ mol) was added as a solid and the resulting mixture vortexed for 1 min. After 2 h of additional shaking, a 40  $\mu$ L aliquot was analyzed directly by LC-MS and complete conversion to SBL-C156-SSeGlc2F **22a** was observed (calculated mass, 26958; observed mass, 26960). The protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at -20 °C.

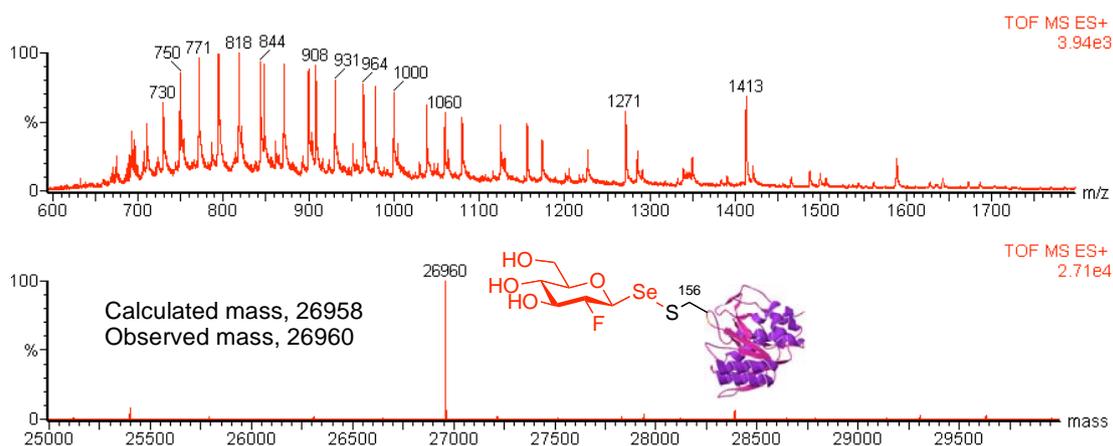
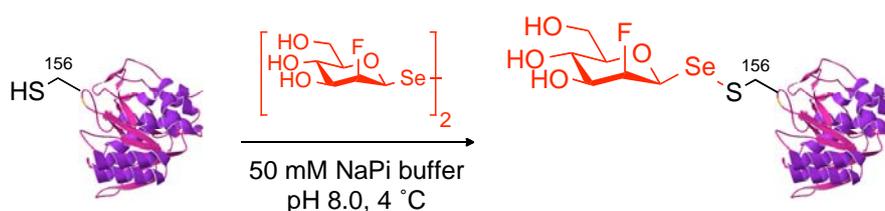
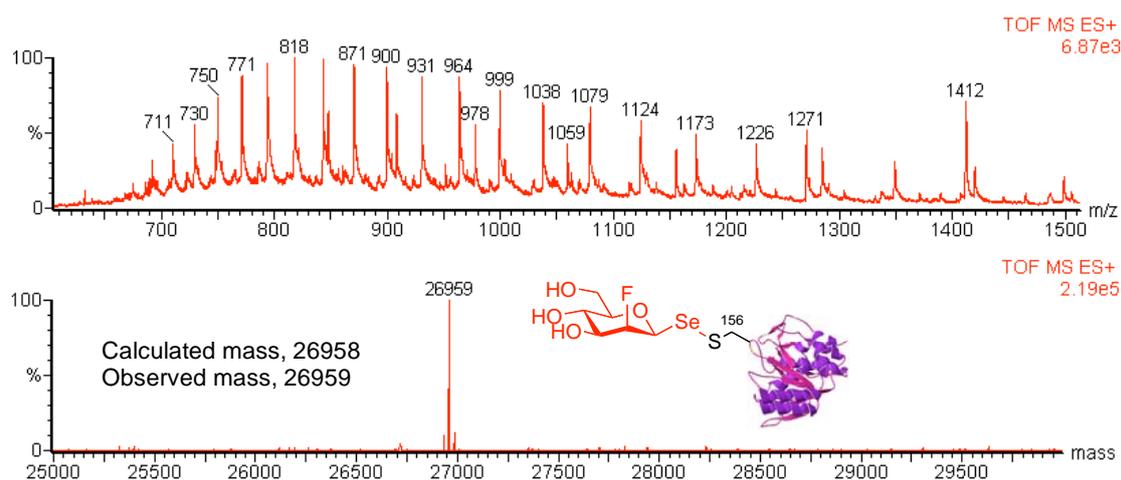


Figure S34. ESI-MS spectrum of SBL-C156-SSe-Glc2F **22a**.

### Preparation of SBL-C156-SSeMan2F (23a)

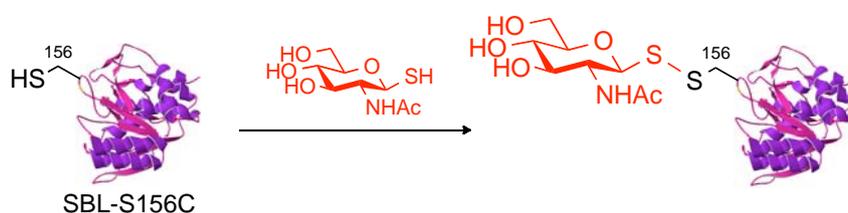


All manipulations were carried out in a cold room maintained at 4 °C. To a solution of SBL-S156C (500  $\mu$ L of 1 mg/mL, 0.018  $\mu$ mol) in 50 mM sodium phosphate buffer (pH 8.0), bis(2-deoxy-2-fluoro- $\beta$ -D-mannopyranosyl)-1,1'-diselenide (0.9 mg, 1.858  $\mu$ mol) was added as a solid and the resulting mixture vortexed for 30 seconds. After 2 h of additional shaking, a 25  $\mu$ L aliquot was analyzed directly by LC-MS and complete conversion to SBL-C156-SSeMan2F **23a** was observed (calculated mass, 26958; observed mass, 26959). The protein solution was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) and stored at -20 °C.



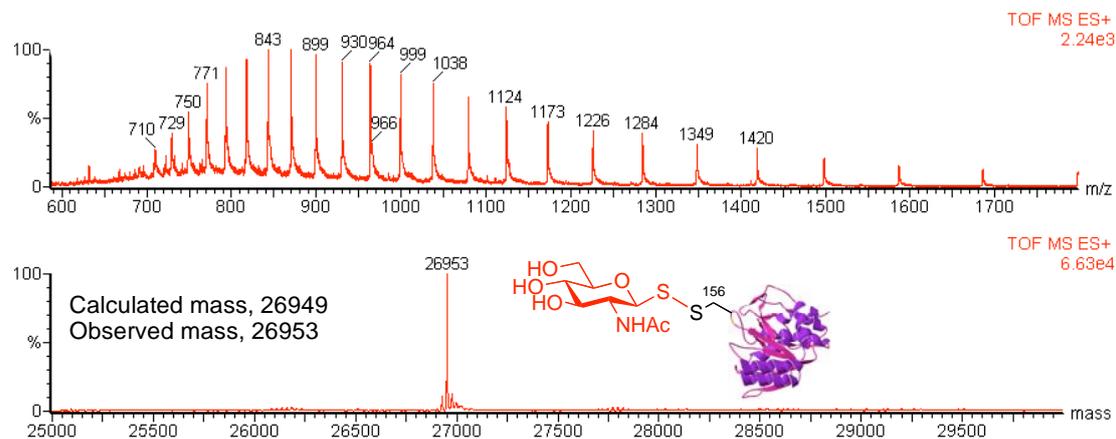
**Figure S35.** ESI-MS spectrum of SBL-C156-SSeMan2F **23a**.

### Preparation of SBL-C156-SSGlcNAc (24a)



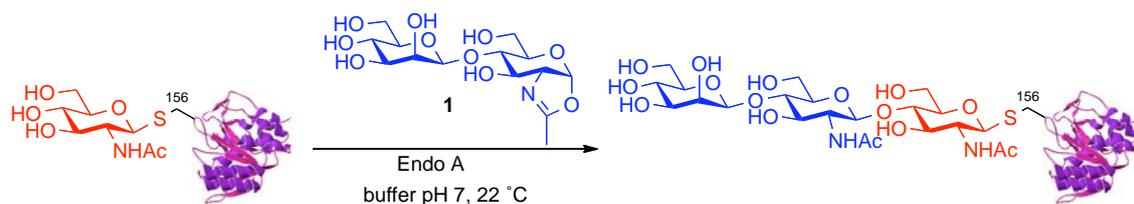
SBL-S156C (3.0 mg, 0.11  $\mu$ mol) was added to a 1.5 mL plastic tube as a lyophilized solid and dissolved in 1.0 mL buffer comprised of 70 mM CHES, 5.0 mM MES, and 2.0 mM  $\text{CaCl}_2$  (pH 9.5). 1-Thio-2-acetamido-2-deoxy- $\beta$ -D-glucopyranose (2.7 mg, 11  $\mu$ mol) was added as a solid to the protein solution along with 200  $\mu$ L acetonitrile. The reaction

was vortexed to homogenize and rotated on a lab rotisserie for 1 h. LC–MS analysis of a 50  $\mu$ L aliquot of the reaction mixture revealed complete conversion to the corresponding disulfide (SBL-SS-GlcNAc) **24a** (calculated mass, 26949; observed mass 26953). The reaction mixture was dialyzed against 50 mM sodium phosphate buffer (pH 7.0) for 4 h and stored at  $-20$   $^{\circ}$ C.



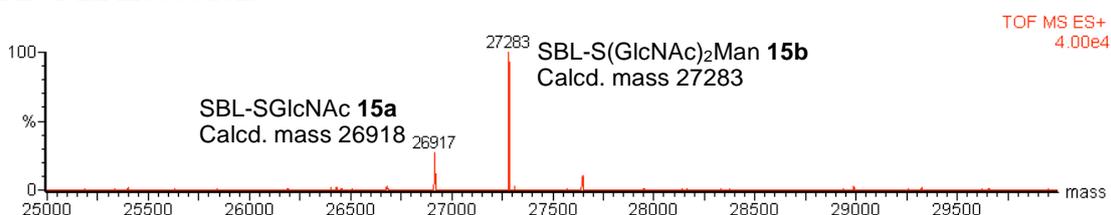
**Figure S36.** ESI–MS spectrum of SBL-C156-SS-GlcNAc **24a**.

## 2.6. Enzymatic extension of glycoproteins 15-24a

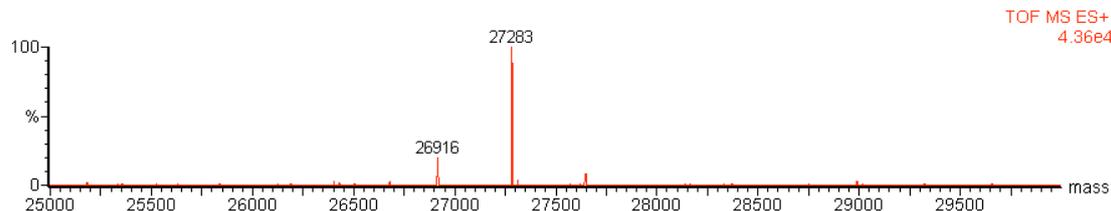
Endo A-catalyzed glycosylation of SBL-C156-SGlcNAc (**15a**)

To a solution of SBL-C156SGlcNAc **15a** (140  $\mu$ L of 1 mg/mL, 5.20 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (420  $\mu$ g, 1.149  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22 °C. The reaction was analyzed directly by LC-MS. Reaction conditions were reproduced in a similar sample without Endo-A as control reaction.

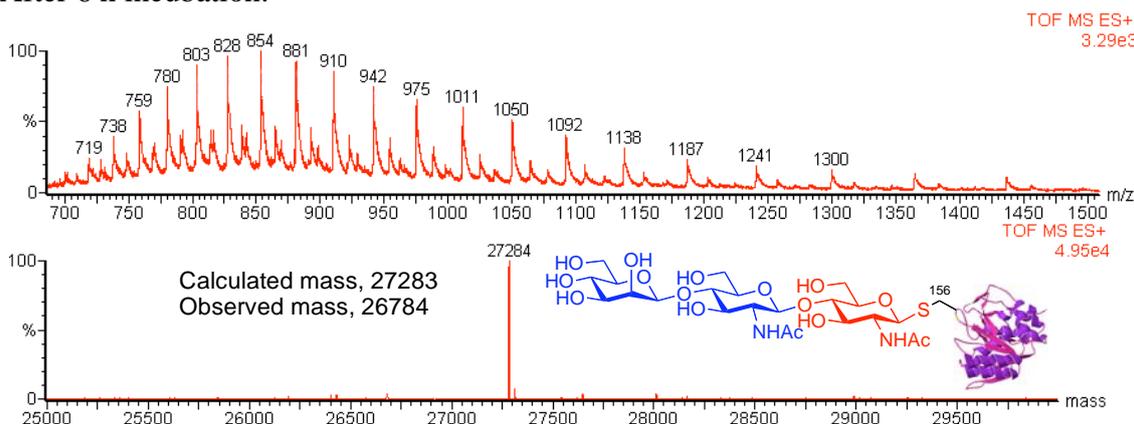
After 2h incubation:



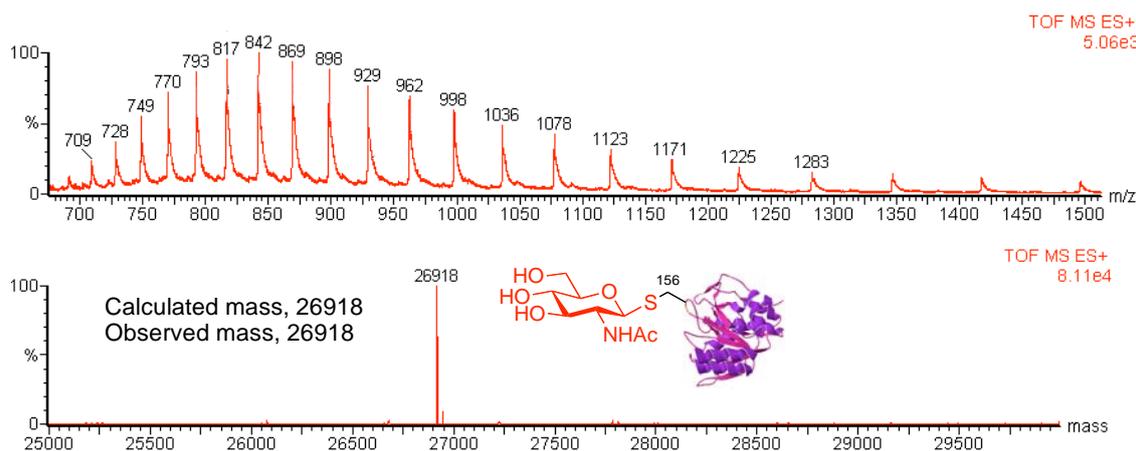
After 4 h incubation:



After 6 h incubation:

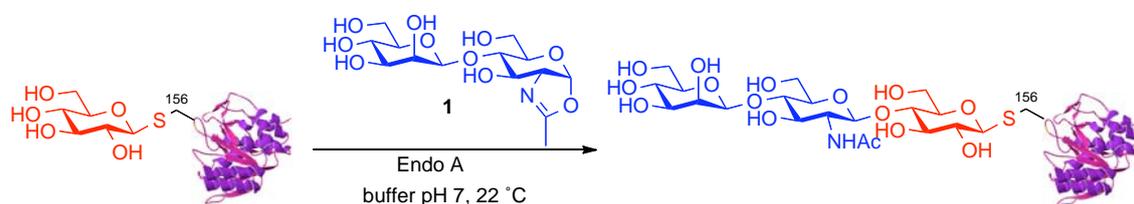


**Figure S37.** ESI-MS spectra after 2, 4 and 6 h incubation of **15a** with Endo A.



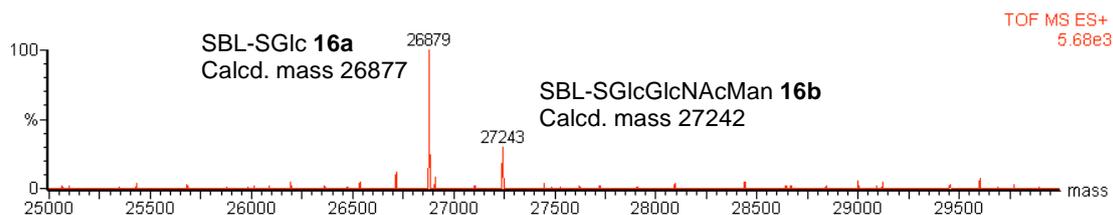
**Figure S38.** ESI–MS spectrum of control reaction without Endo A after 6 h incubation.

### Endo A-catalyzed glycosylation of SBL-C156SGlc 16a

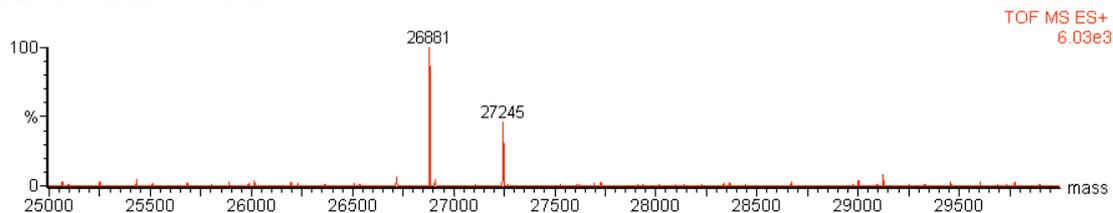


An aliquot of SBL-C156SGlc **16a** (140  $\mu$ L of 0.29 mg/mL, 1.51 nmol) in 50 mM sodium phosphate buffer (pH 7.0) previously prepared was thawed. To the protein solution, disaccharide oxazoline **1** (350  $\mu$ g, 0.958  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC–MS.

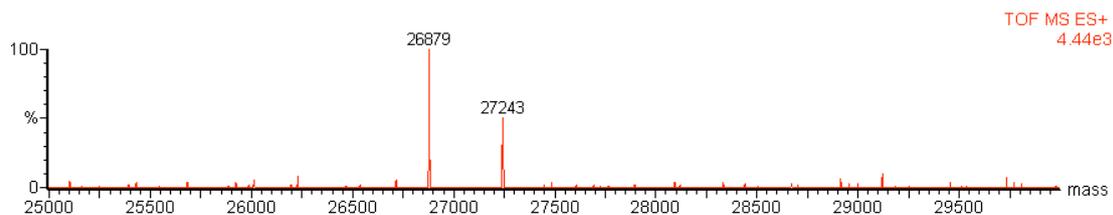
After 2h incubation:



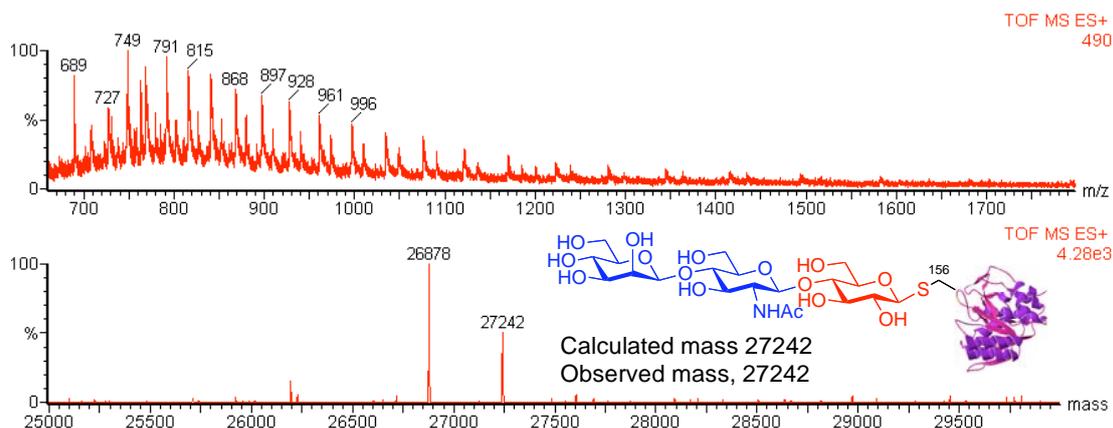
After 4 h incubation:



After 6 h incubation:

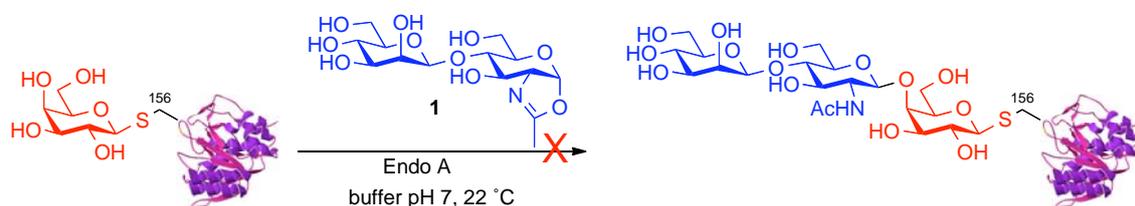


After 12 h incubation:



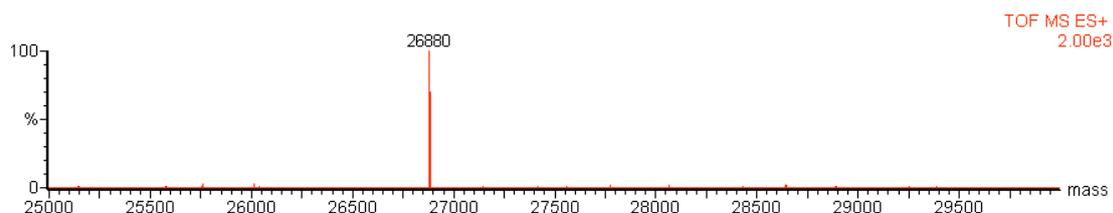
**Figure S39.** ESI–MS spectra after 2, 4, 6 and 12 h incubation of **16a** (0.29 mg/mL) with Endo A.

### Endo A-catalyzed glycosylation of SBL-C156SGal **17a**

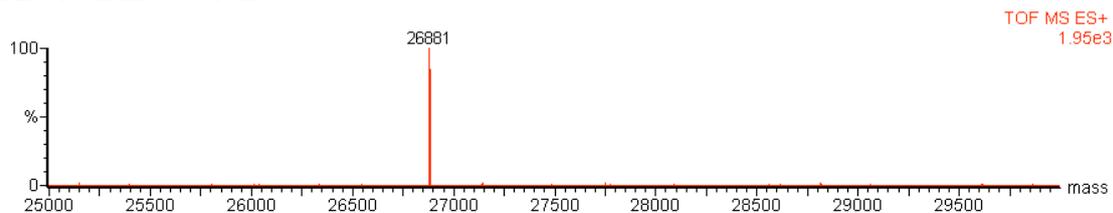


To a solution of SBL-C156Gal **17a** (150  $\mu$ L of 0.29 mg/mL, 1.618 nmol) in sodium phosphate buffer (50 mM solution, pH 7.0), disaccharide oxazoline **1** (280  $\mu$ g, 0.766  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC–MS.

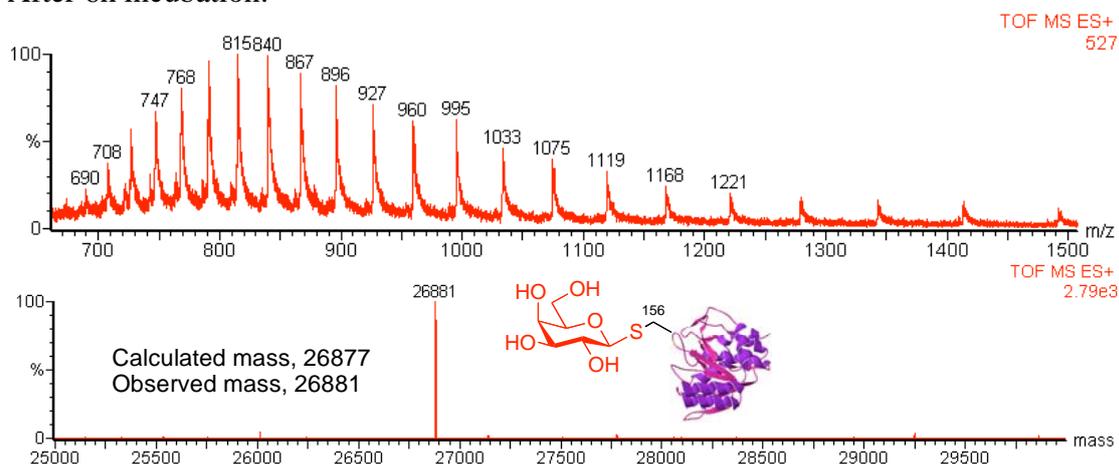
After 2h incubation:



After 4h incubation:

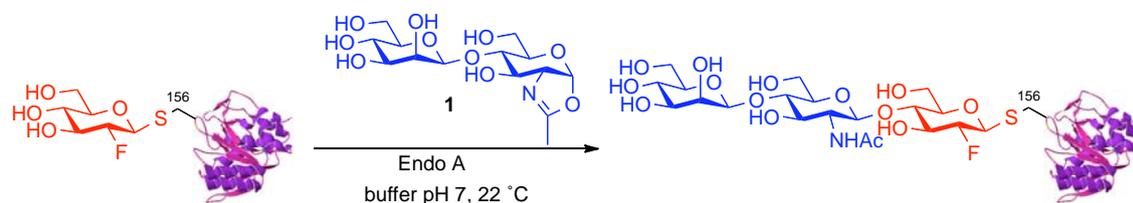


After 6h incubation:



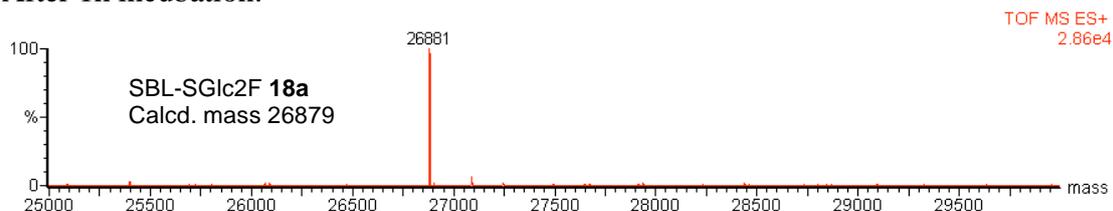
**Figure S40.** ESI–MS spectra after 2, 4 and 6 h incubation of **17a** (0.29 mg/mL) with Endo A.

### Endo A-catalyzed glycosylation of SBL-C156-SGlc2F **18a** (0.29 mg/mL)

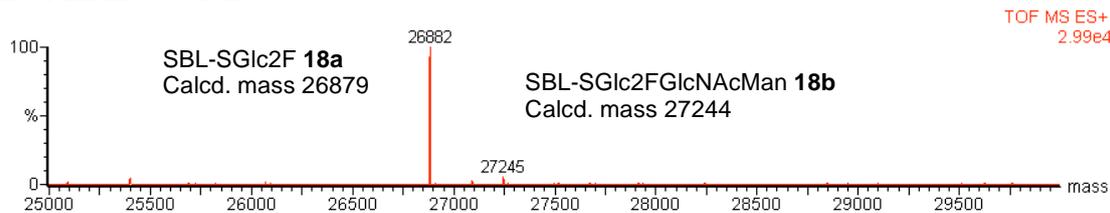


To a solution of SBL-C156-SGlc2F **18a** (145  $\mu$ L of 0.4 mg/mL, 2.16 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (55  $\mu$ L of 4.69 mg/mL, 0.707  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22 °C. The reaction was analyzed directly by LC–MS.

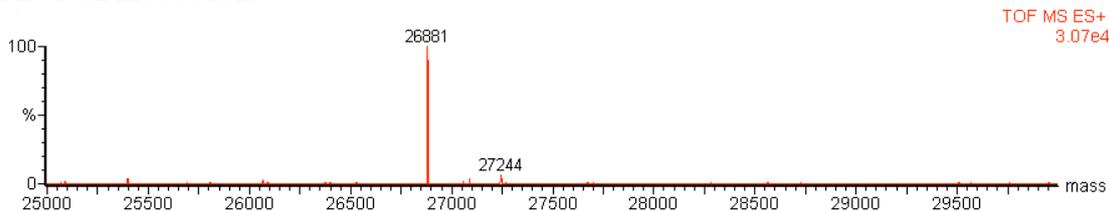
After 1h incubation:



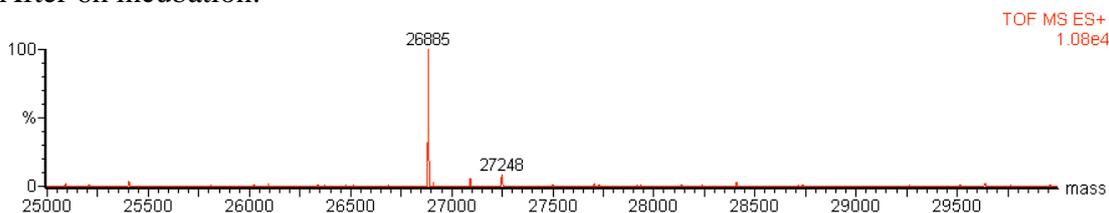
After 2h incubation:



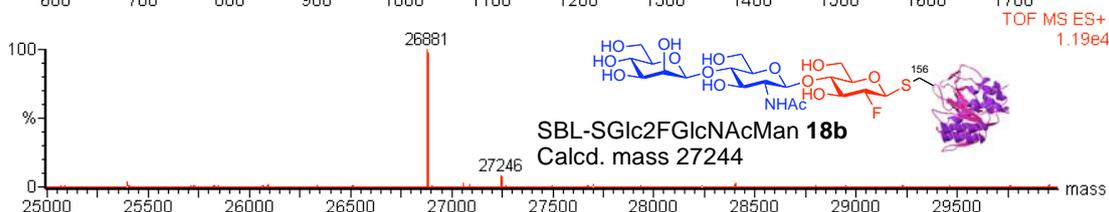
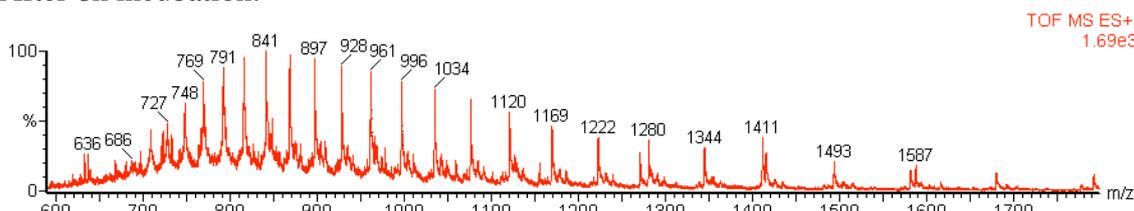
After 4h incubation:



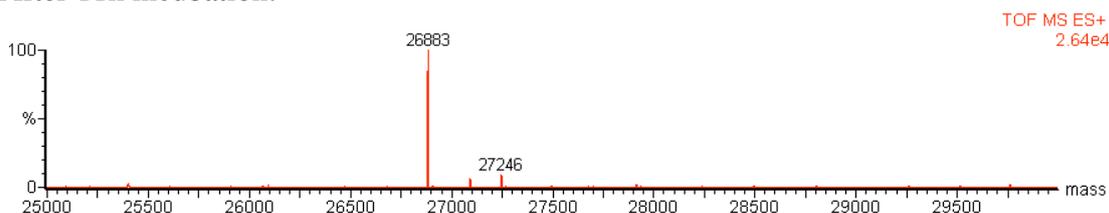
After 6h incubation:



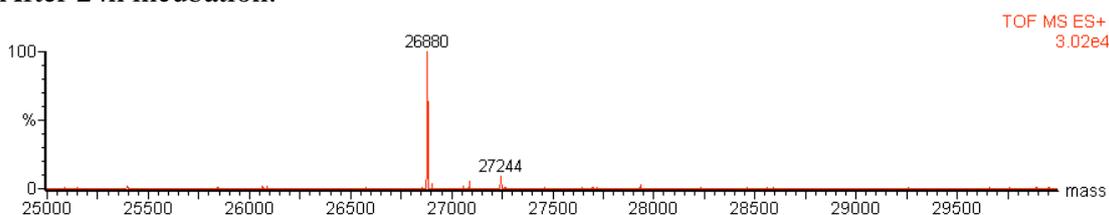
After 8h incubation:



After 11h incubation:

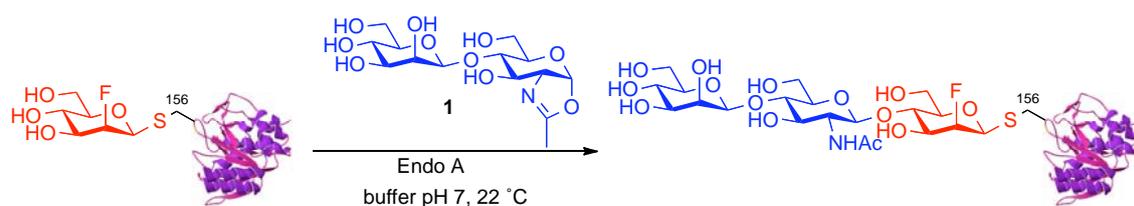


After 24h incubation:



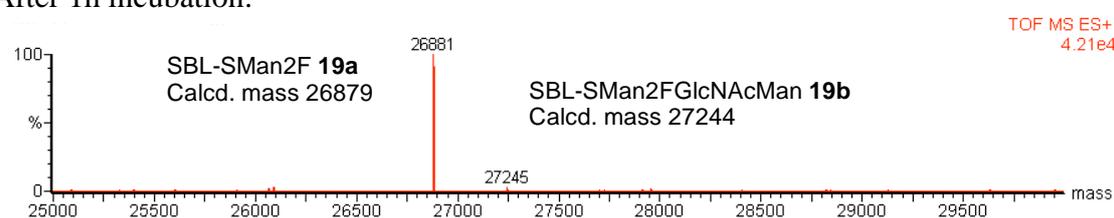
**Figure S41.** ESI-MS spectra after 1, 2, 4, 6, 8, 11 and 24 h incubation of **18a** (0.29 mg/mL) with Endo A.

### Endo A-catalyzed glycosylation of SBL-C156-SMan2F **19a** (0.29 mg/mL)

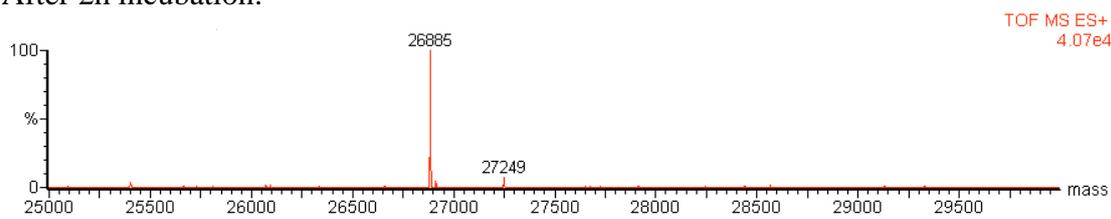


To a solution of SBL-C156-SGlc2F **19a** (145  $\mu$ L of 0.4 mg/mL, 2.16 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (55  $\mu$ L of 4.69 mg/mL, 0.707  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22 °C. The reaction was analyzed directly by LC-MS.

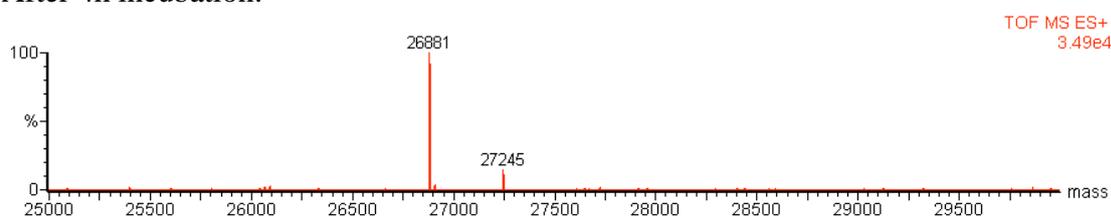
After 1h incubation:



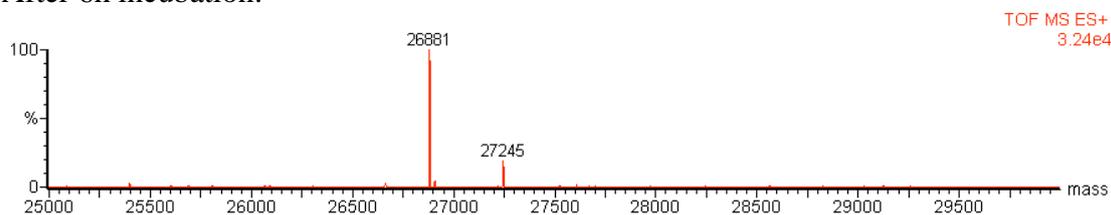
After 2h incubation:



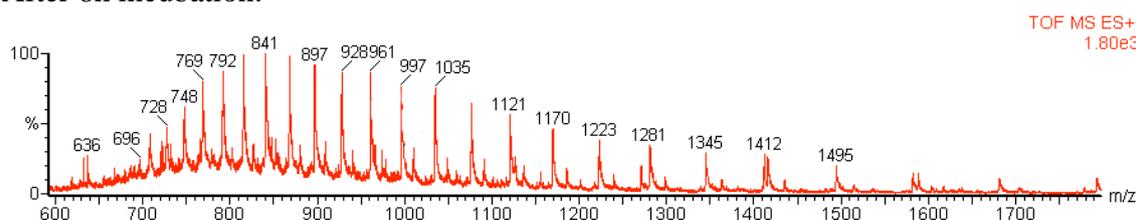
After 4h incubation:

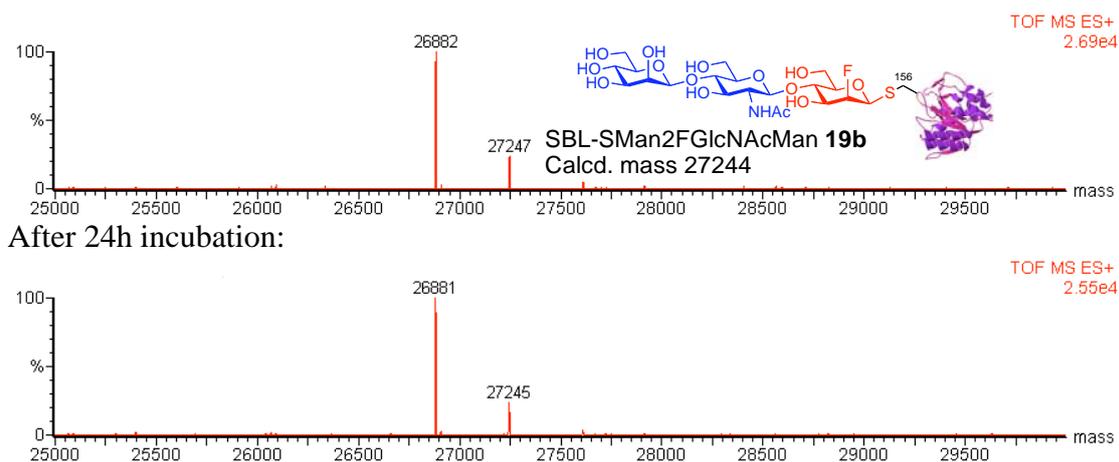


After 6h incubation:



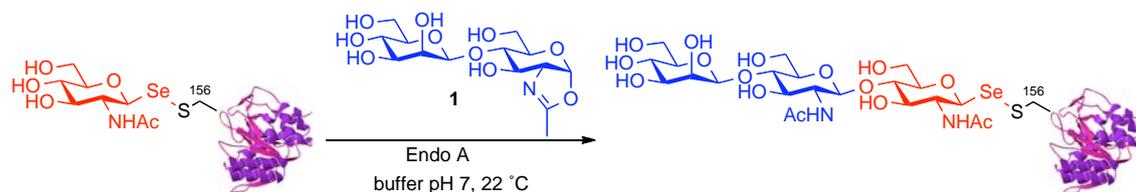
After 8h incubation:





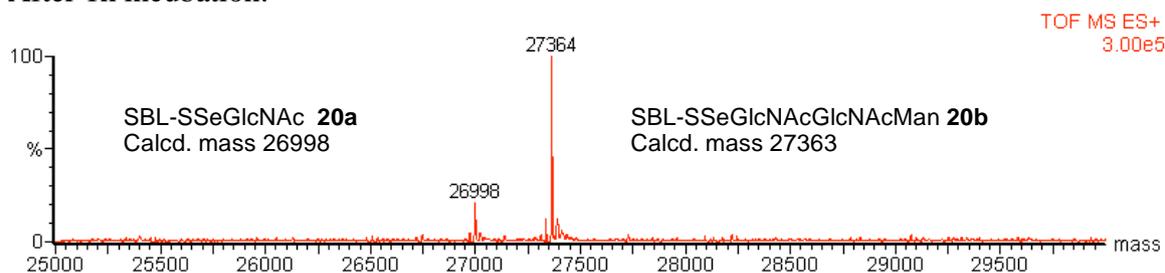
**Figure S42.** ESI-MS spectra after 1, 2, 4, 6, 8 and 24 h incubation of **19a** (0.29 mg/mL) with Endo A.

### Endo A-catalyzed glycosylation of SBL-C156-SSeGlcNAc **20a** (1 mg/mL)

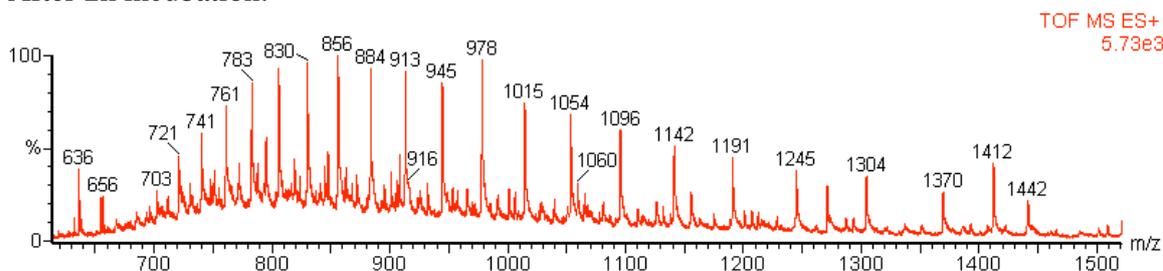


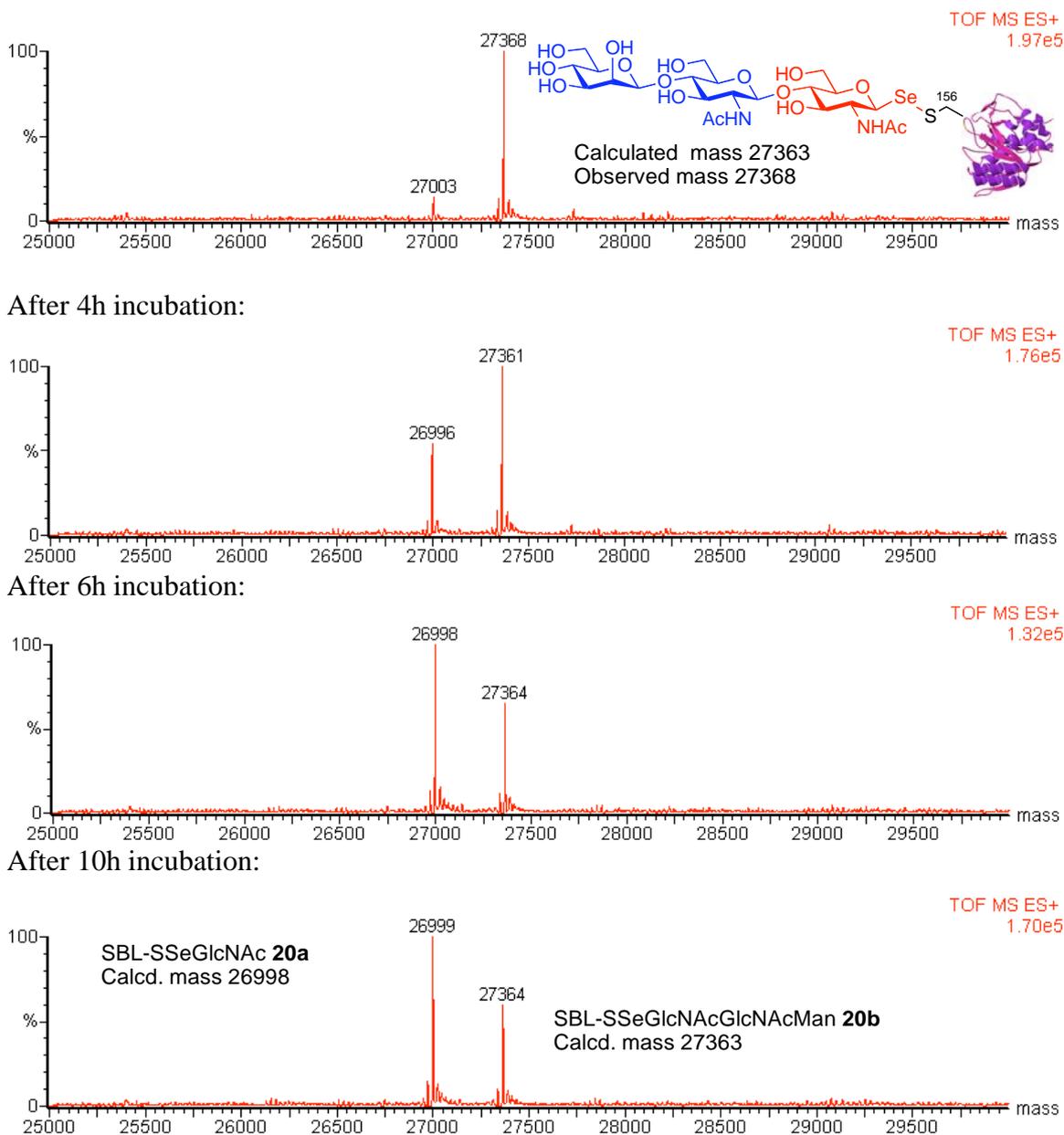
To a solution of SBL-C156-SSeGlcNAc **20a** (150  $\mu$ L of 1 mg/mL, 5.55 nmol) in sodium phosphate buffer (50 mM solution, pH 7.0), disaccharide oxazoline **1** (550  $\mu$ g, 1.505  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22 °C. The reaction was analyzed directly by LC-MS.

After 1h incubation:



After 2h incubation:





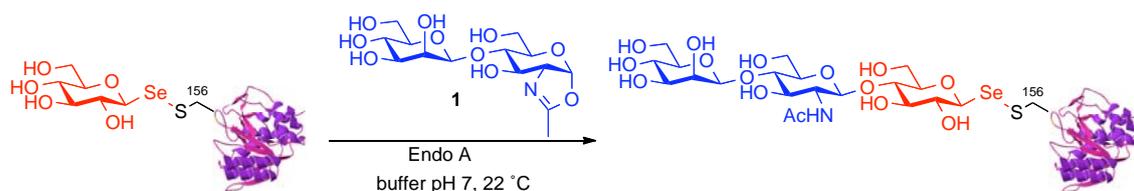
**Figure S43.** ESI-MS spectra after 1, 2, 4, 6 and 10 h incubation of **20a** (1 mg/mL) with Endo A.

#### Endo A-catalyzed glycosylation of SBL-C156-SSeGlcNAc **20a** (0.29 mg/mL)

To a solution of SBL-C156-SSeGlcNAc **20a** (200 mL of 0.29 mg/mL, 2.14 nmol) in sodium phosphate buffer (50 mM solution, pH 7.0), disaccharide oxazoline **1** (252 mg, 0.691 mmol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22 °C. The reaction was analyzed directly by LC-MS.

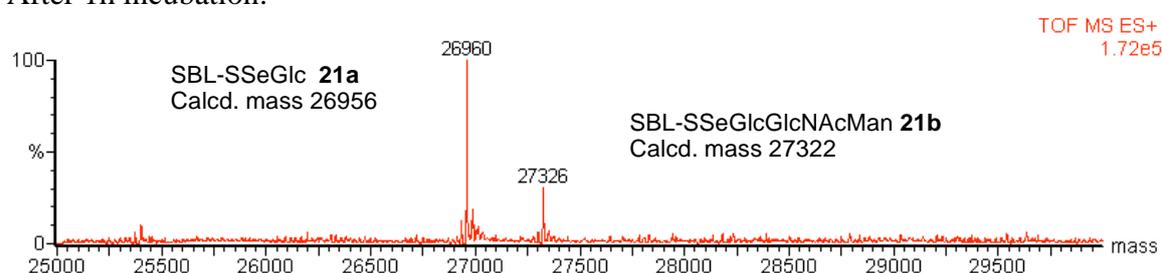


## Endo A-catalyzed glycosylation of SBL-C156-SSeGlc 21a (1 mg/mL)

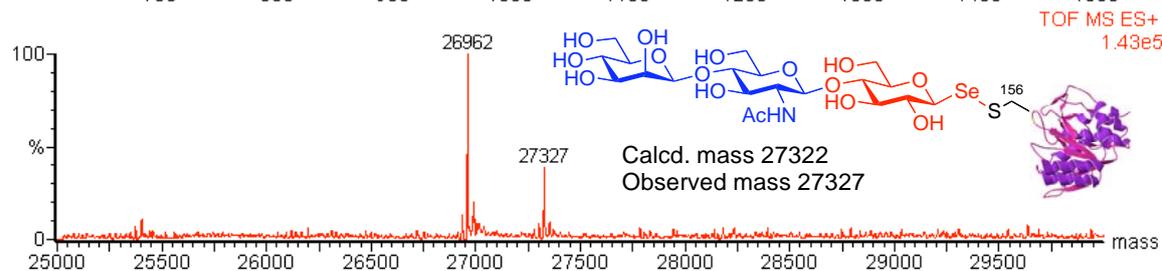
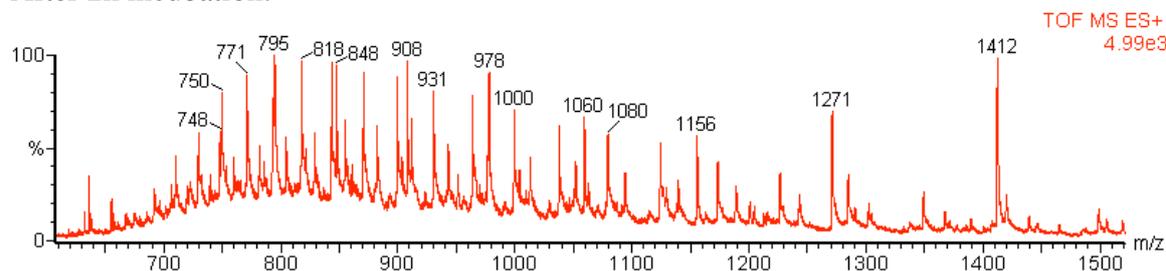


To a solution of SBL-C156-SSeGlc **21a** (150 mL of 1 mg/mL, 5.56 nmol) in sodium phosphate buffer (50 mM solution, pH 7.0), disaccharide oxazoline **1** (490 mg, 1.341 mmol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22 °C. The reaction was analyzed directly by LC-MS.

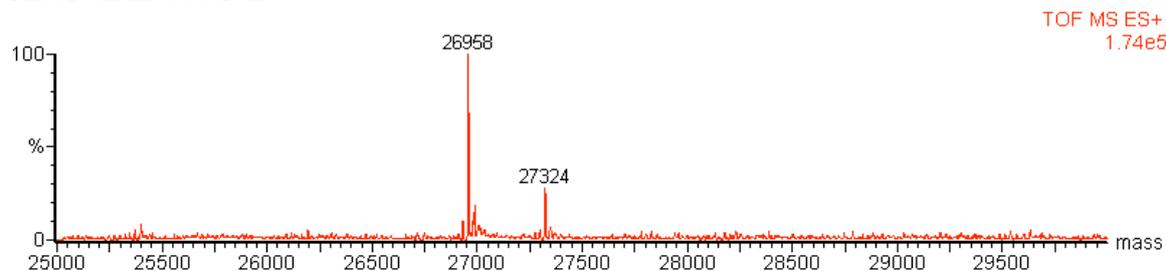
After 1h incubation:



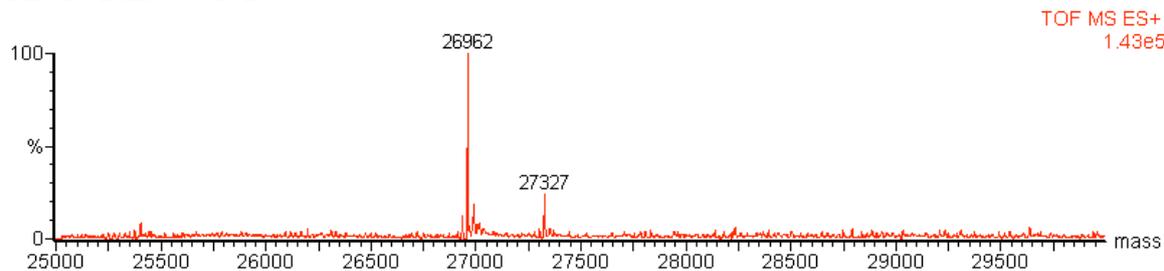
After 2h incubation:



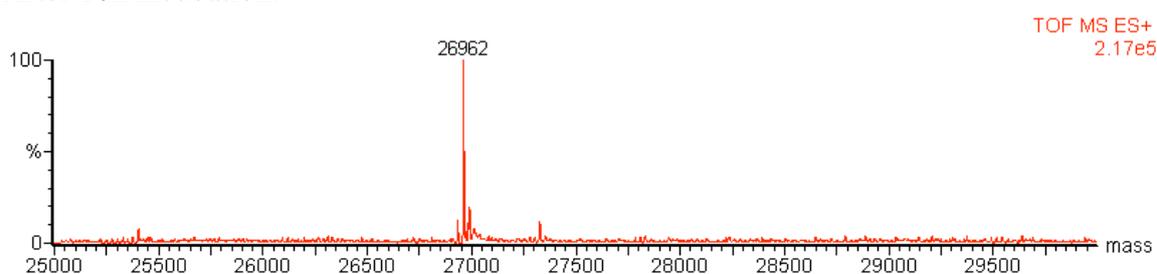
After 4h incubation:



After 6h incubation:



After 10h incubation:

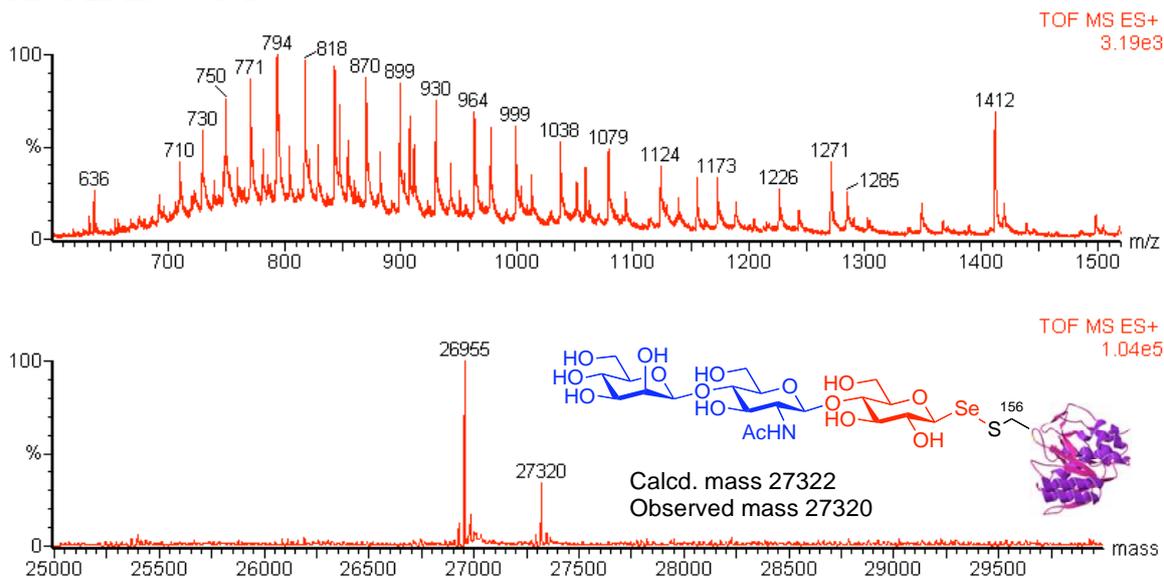


**Figure S45.** ESI–MS spectra after 1, 2, 4, 6 and 10 h incubation of **21a** (1 mg/mL) with Endo A.

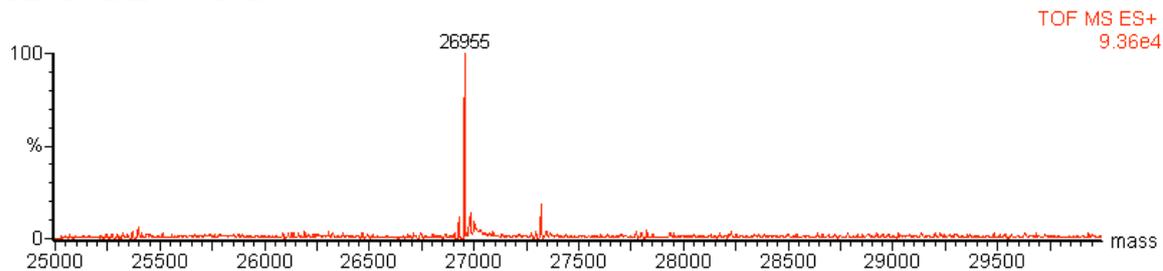
### Endo A-catalyzed glycosylation of SBL-C156-SSeGlc **21a** (0.29 mg/mL)

To a solution of SBL-C156-SSeGlc **21a** (200  $\mu$ L of 0.29 mg/mL, 2.15 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (252  $\mu$ g, 0.691  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC–MS.

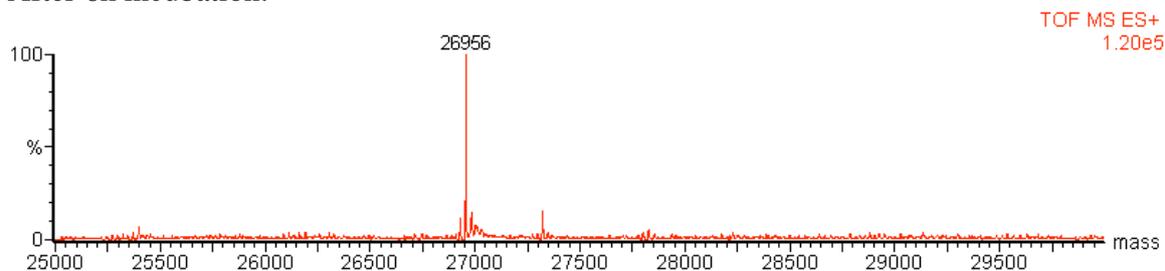
After 2h incubation:



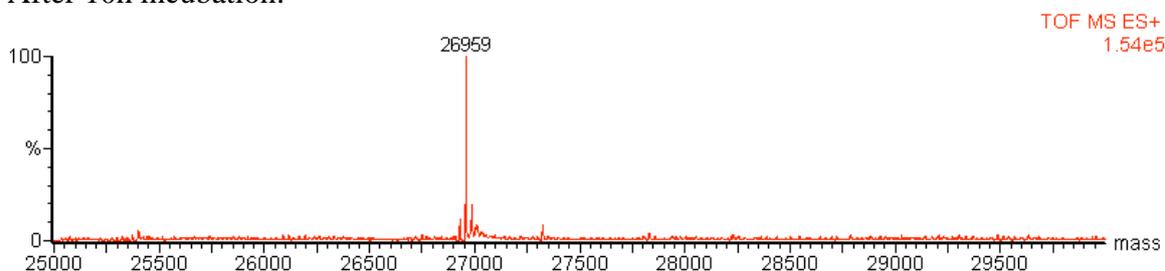
After 4h incubation:



After 6h incubation:

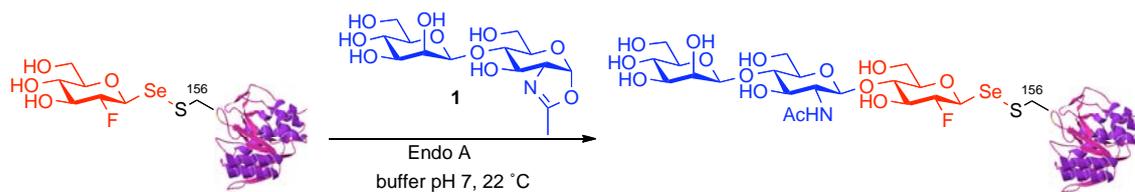


After 10h incubation:



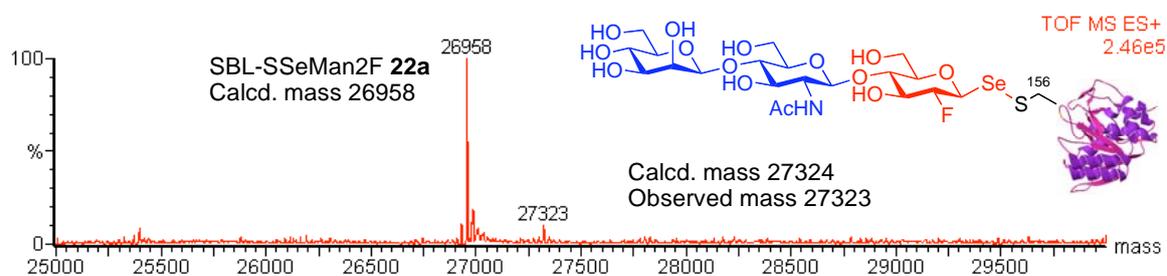
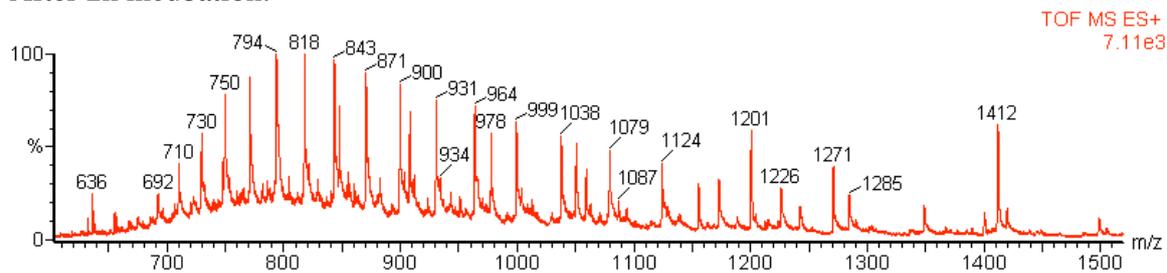
**Figure S46.** ESI-MS spectrum after 2, 4, 6 and 10 h incubation of **21a** (0.29 mg/mL) with Endo A.

#### Endo A-catalyzed glycosylation of SBL-C156-SSeGlc2F **22a** (0.5 mg/mL)

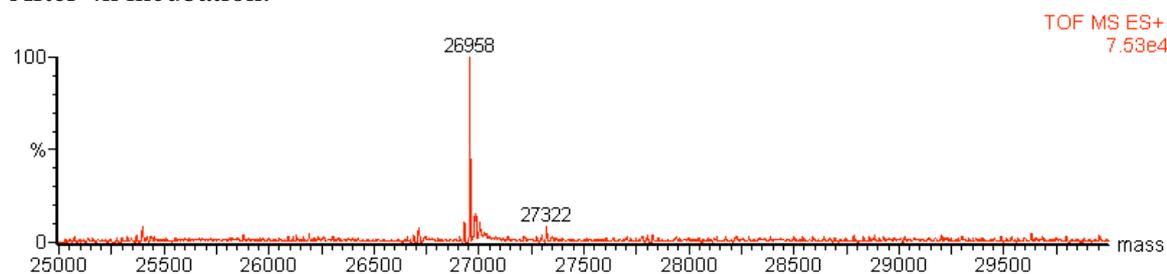


To a solution of SBL-C156-SSeGlc2F **22a** (200  $\mu$ L of 0.5 mg/mL, 3.709 nmol) in sodium phosphate buffer (50 mM solution, pH 7.0), disaccharide oxazoline **1** (370  $\mu$ g, 1.012  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC-MS.

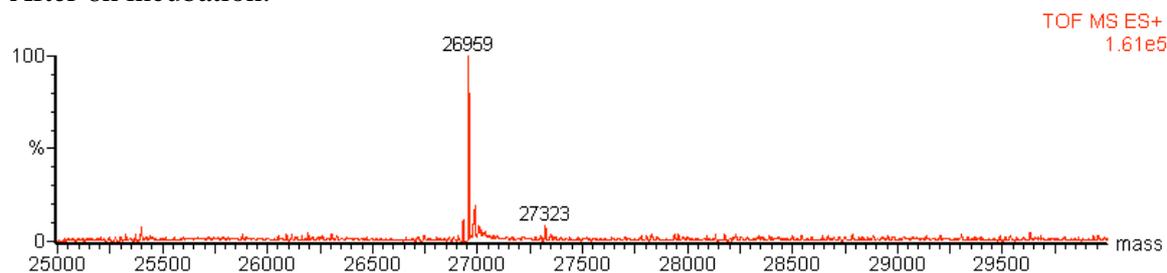
After 2h incubation:



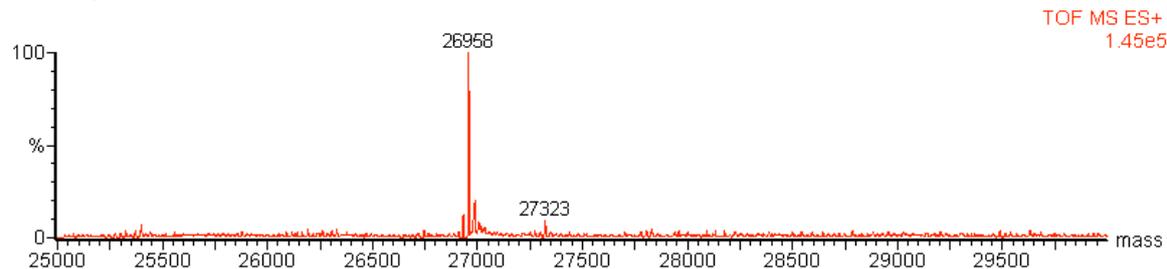
After 4h incubation:



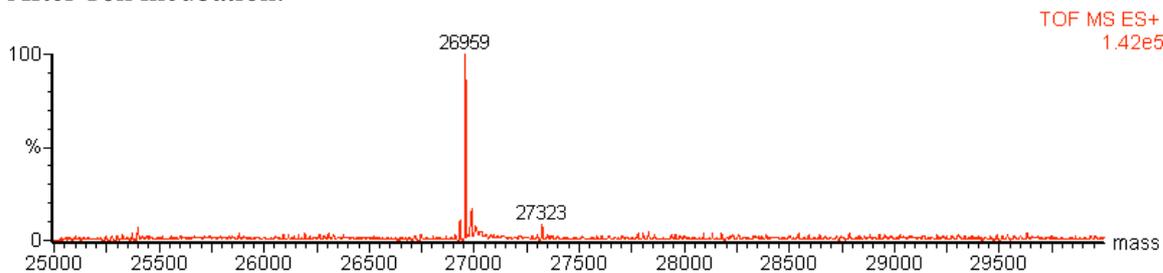
After 6h incubation:



After 8h incubation:

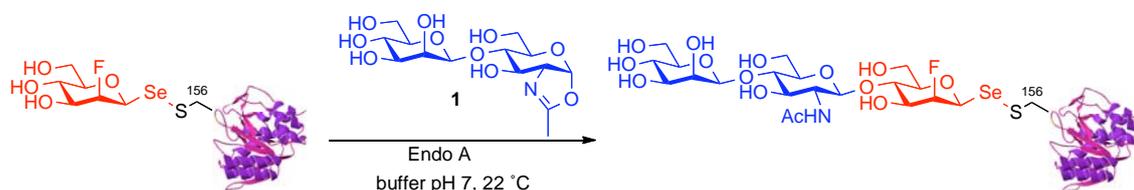


After 10h incubation:



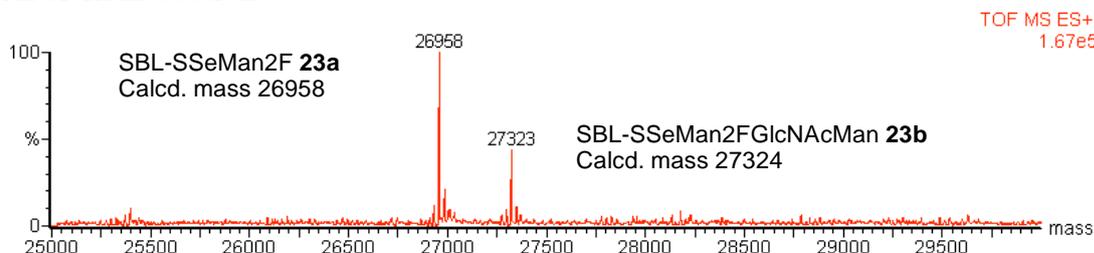
**Figure S47.** ESI–MS spectra after 2, 4, 6, 8 and 10 h incubation of **22a** (0.5 mg/mL) with Endo A.

**Endo A-catalyzed glycosylation of SBL-C156-SSeMan2F **23a** (1 mg/mL)**

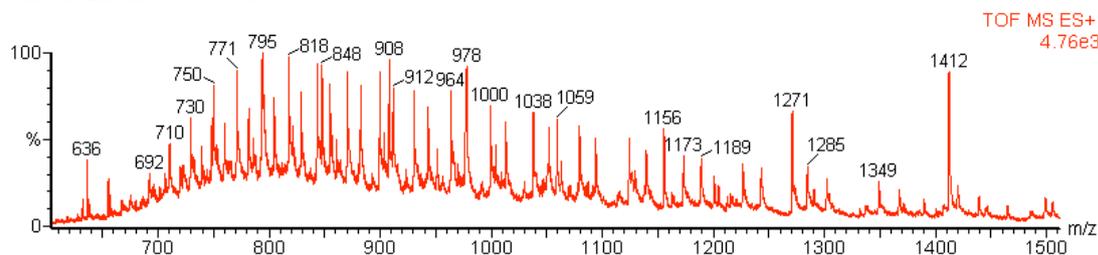


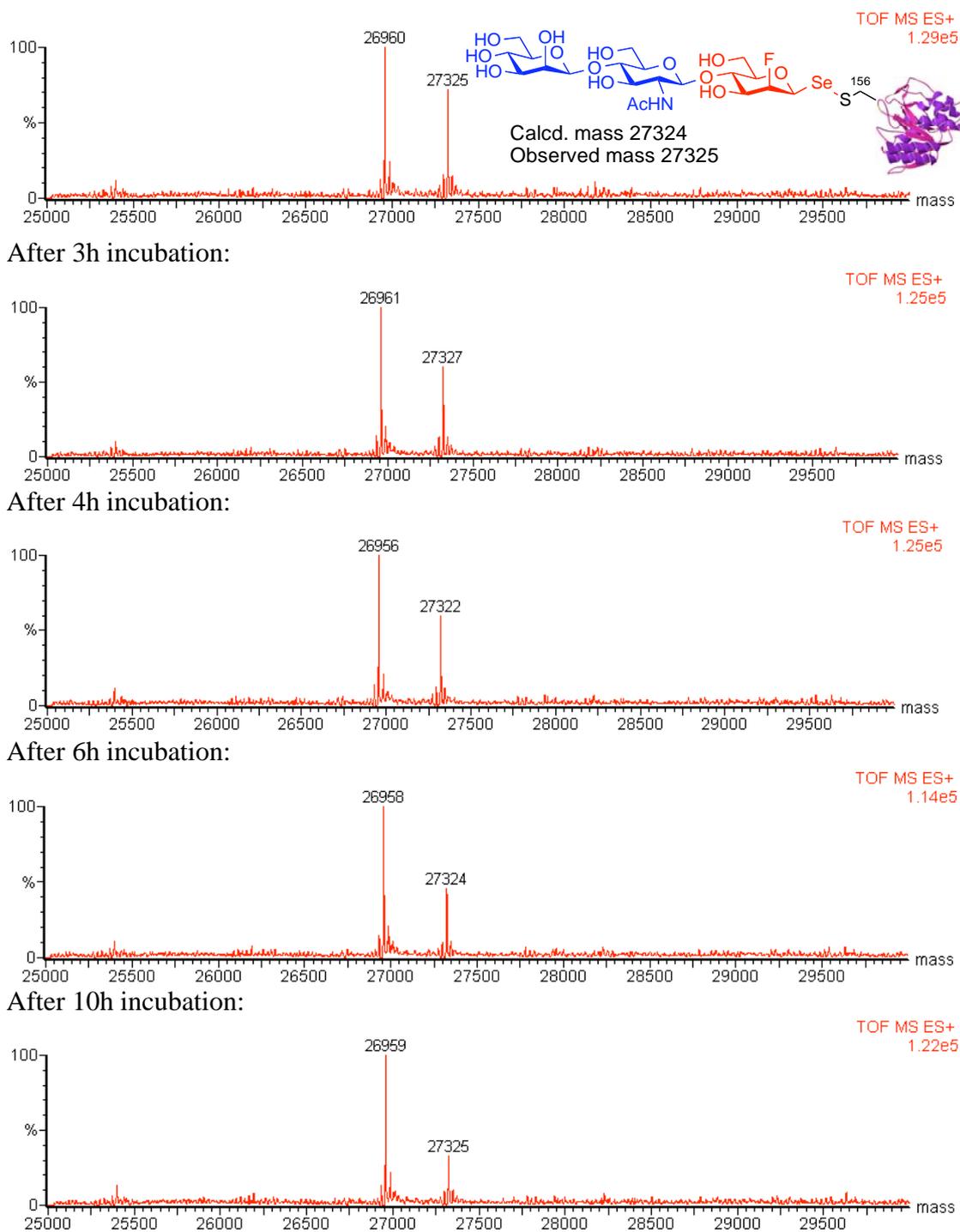
To a solution of SBL-C156-SSeMan2F **23a** (150  $\mu$ L of 1 mg/mL, 5.56 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (510  $\mu$ g, 1.395  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC–MS.

After 1h incubation:



After 2h incubation:



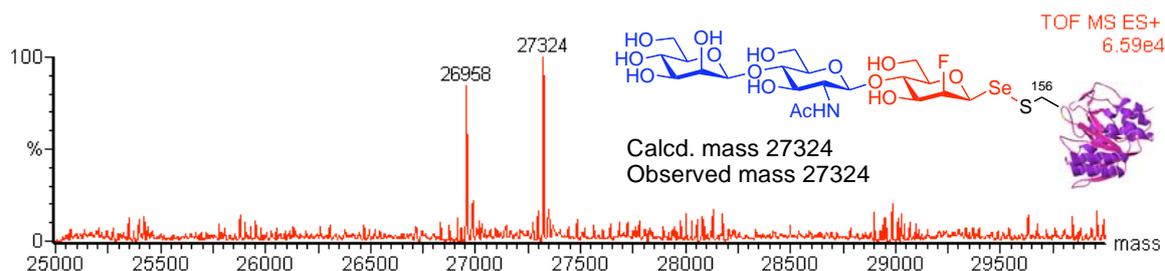
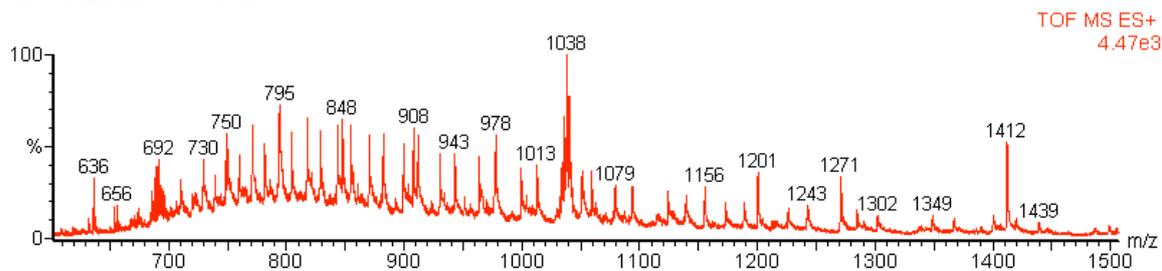


**Figure S48.** ESI-MS spectrum after 1, 2, 3, 4, 6 and 10 h incubation of **23a** (1 mg/mL) with Endo A.

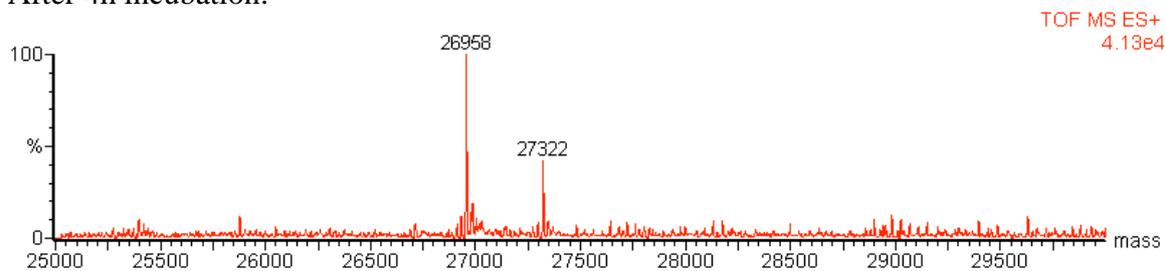
#### Endo A-catalyzed glycosylation of SBL-C156-SSeMan2F **23a** (0.43 mg/mL)

To a solution of SBL-C156-SSeMan2F **23a** (200  $\mu$ L of 0.43 mg/mL, 3.22 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (390  $\mu$ g, 1.067  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC-MS.

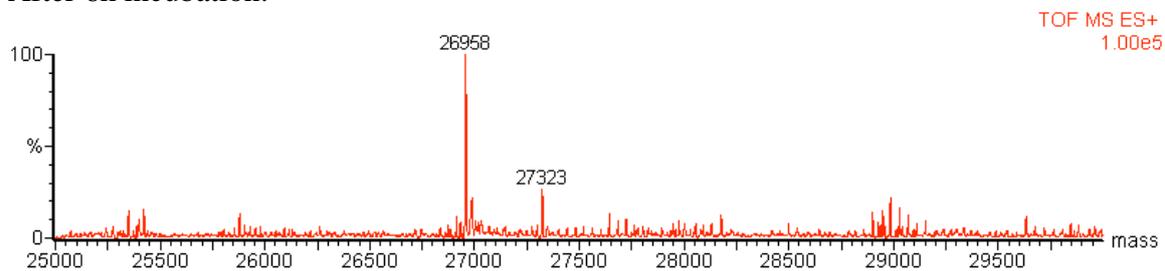
After 2h incubation:



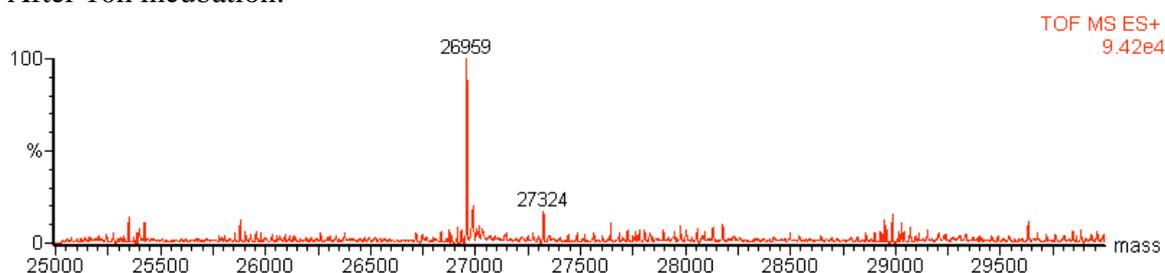
After 4h incubation:



After 6h incubation:



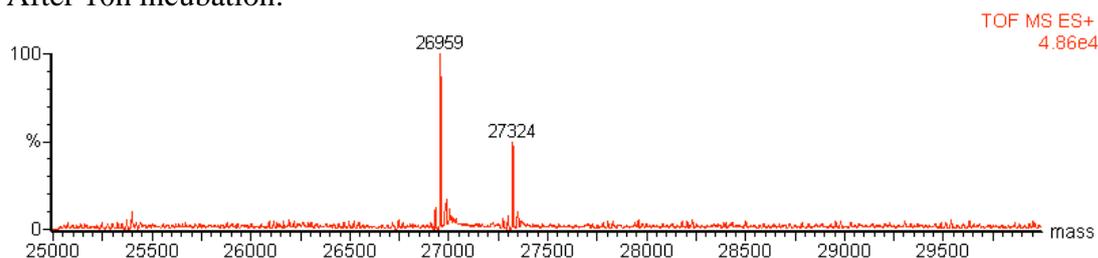
After 10h incubation:



**Figure S49.** ESI-MS spectrum after 2, 4, 6 and 10 h incubation of **23a** (0.43 mg/mL) with Endo A.

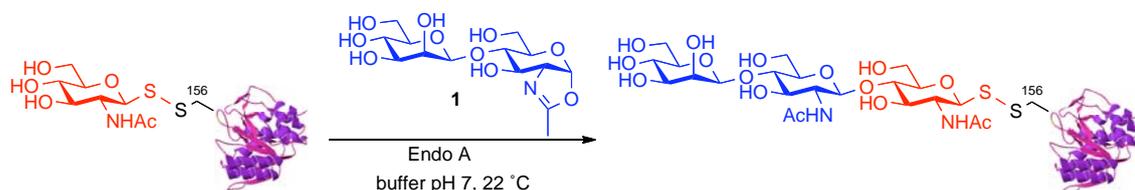


After 10h incubation:



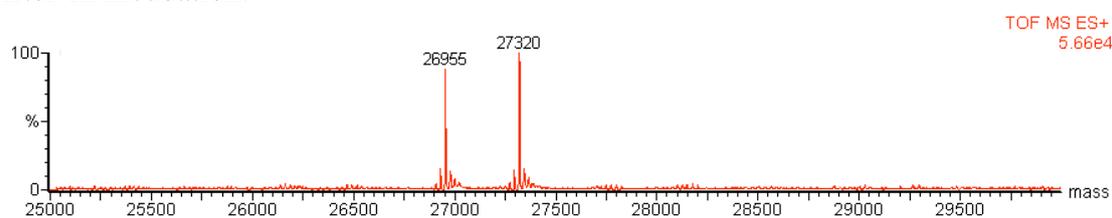
**Figure S50.** ESI–MS spectrum after 1, 2, 4, 6 and 10 h incubation of **23a** (0.29 mg/mL) with Endo A.

### Endo A-catalyzed glycosylation of SBL-C156-SSGlcNAc **24a** (1 mg/mL)

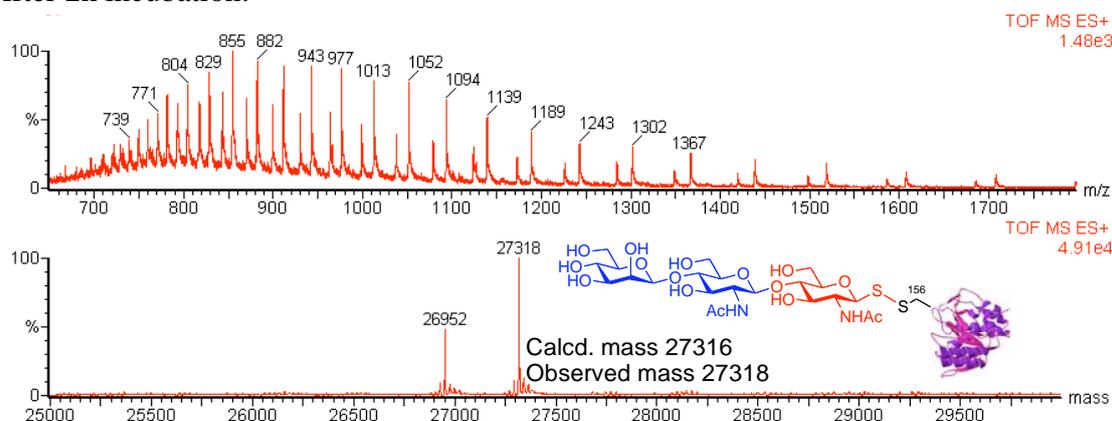


To a solution of SBL-C156-SSGlcNAc **24a** (200  $\mu$ L of 1 mg/mL, 7.42 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (530  $\mu$ g, 1.45  $\mu$ mol) and Endo A (11 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC–MS.

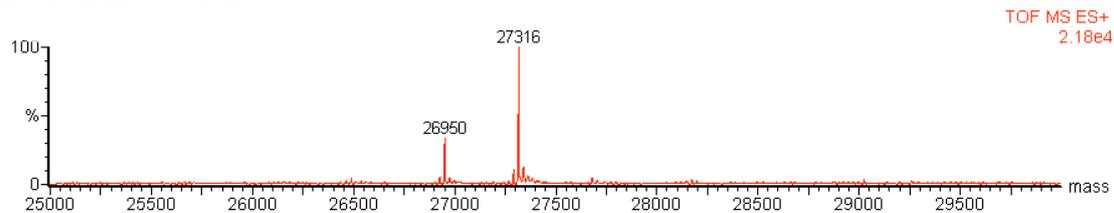
After 1h incubation:



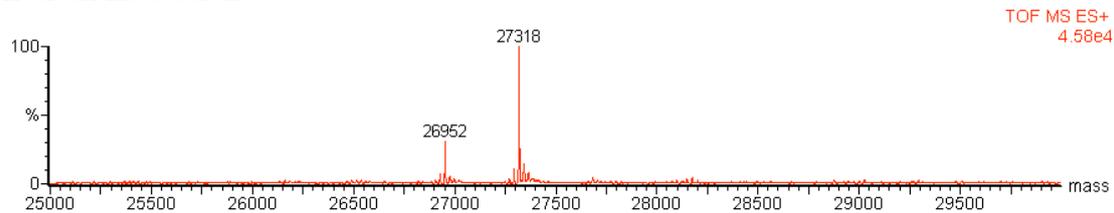
After 2h incubation:



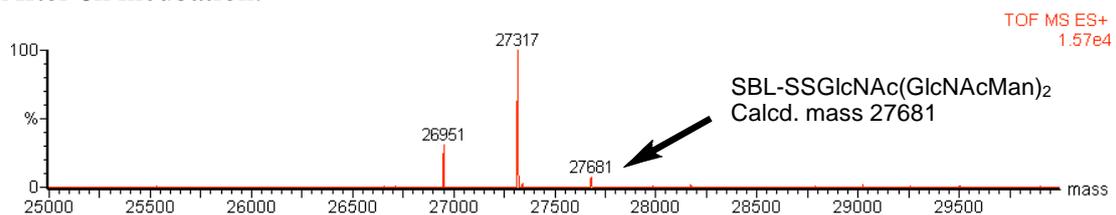
After 4h incubation:



After 6h incubation:



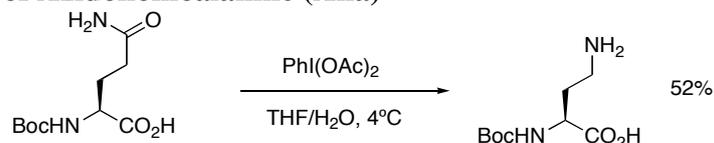
After 8h incubation:



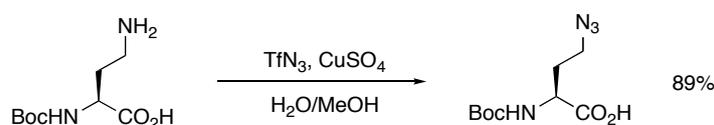
**Figure S51.** ESI-MS spectra after 1, 2, 4, 6 and 8 h incubation of **24a** (1 mg/mL) with Endo A.

## 2.7 Data for comparison of triazole vs. thioether on Np276

### 2.7.1 Synthesis of Azidohomoalanine (Aha)



*N*-Boc-glutamine (11.49 g, 46.66 mmol) was added to a 500 mL round bottom flask and dissolved in a mixture of THF (100 mL) and H<sub>2</sub>O (25 mL). The solution was stirred at 4 °C (cold room) and PhI(OAc)<sub>2</sub> (18.03 g, 55.99 mmol) was added in several portions to give a cloudy suspension. The reaction was stirred vigorously at 4 °C for 6 hours after which the reaction was judged to be complete by TLC (4:1:1 <sup>i</sup>PrOH:H<sub>2</sub>O:AcOH). The solvent was removed to give a white residue that was then dissolved in H<sub>2</sub>O (60 mL) and washed with EtOAc (3 × 60 mL). The organic fractions were discarded. The aqueous layer was concentrated under reduced pressure to give a white/yellow solid. The solid was triturated with cold Et<sub>2</sub>O (100 mL), isolated by filtration, and dried under vacuum to give 5.26 g (52%) of the Hoffman-rearrangement product as a white solid. m.p.= 200-201 °C. (Lit<sup>18</sup>: 190-191 °C). [α]<sub>D</sub><sup>20</sup> = -8.1 (c = 1.0, H<sub>2</sub>O) (Lit<sup>18</sup>: [α]<sub>D</sub><sup>20</sup> = -9.6, c = 1.0, H<sub>2</sub>O). IR (ν<sub>max</sub>, KBr): 3490, 2973, 2217, 1697, 1392, 1319, 1256, 1182, 1054, 1019. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 1.28 (9H, s, Boc), 1.76-1.85 (1H, m, CHHCH<sub>2</sub>NH<sub>2</sub>), 1.94-2.03 (1H, m, CHHCH<sub>2</sub>NH<sub>2</sub>), 2.91 (2H, t, *J* = 7.8, CH<sub>2</sub>NH<sub>2</sub>), 3.82 (1H, dd, *J* = 8.0, 5.2, H<sub>α</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): δ = 28.0 (Boc), 30.1 (CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>), 37.1 (CH<sub>2</sub>NH<sub>2</sub>), 53.9 (C<sub>α</sub>), 81.6 (Boc), 157.9, 178.3 (2 × C=O). LRMS m/z (ESI<sup>+</sup>): 219.2 [M+H]<sup>+</sup>.

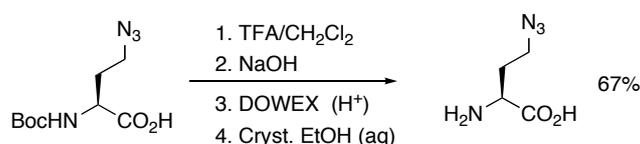


#### Preparation of TfN<sub>3</sub><sup>19</sup>

Sodium azide (14.89 g, 229 mmol) was dissolved in H<sub>2</sub>O (25mL) and CH<sub>2</sub>Cl<sub>2</sub> (50 mL) was added to the reaction flask. Triflic anhydride (10.0 g, 35.44 mmol) was added dropwise to the stirred solution at 0 °C and the biphasic mixture stirred vigorously from 0 °C to room temperature over two hours. After this time, the organic layer was separated and the aqueous layer extracted with CH<sub>2</sub>Cl<sub>2</sub> (2 × 60 mL). The combined organics were washed with sat. NaHCO<sub>3</sub> (100 mL). The solution of TfN<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> was used immediately in the next step.

*Diazotransfer:*

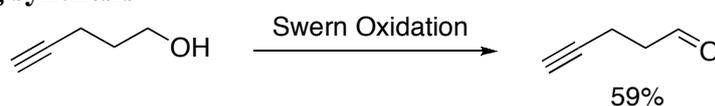
The primary amine derived from the Hoffman rearrangement of BocGlnOH (3.87 g, 17.73 mmol) and CuSO<sub>4</sub> (44 mg, 0.17 mmol) were dissolved in H<sub>2</sub>O (60 mL). To this solution was added MeOH (90 mL) and K<sub>2</sub>CO<sub>3</sub> (3.67 g, 26.60 mmol). The resulting blue/green solution was stirred vigorously at room temperature while the TfN<sub>3</sub> solution prepared above was poured into the reaction mixture. The cloudy solution was stirred at room temperature overnight. TLC (4:1:1 *i*PrOH:H<sub>2</sub>O:AcOH, ninhydrin) indicated complete consumption of the starting material. The organic solvent was removed under reduced pressure and the remaining aqueous solution was washed with CH<sub>2</sub>Cl<sub>2</sub> (2 × 50 mL). The organic layer was discarded and the aqueous layer was acidified carefully to pH 2 (pH paper) with 10M HCl. The aqueous solution was extracted with EtOAc (4 × 80 mL) and the combined organics were washed with brine (200 mL). The solution was dried (MgSO<sub>4</sub>), filtered, and the solvent removed under reduced pressure to give Boc-azidohomoalanine (BocAhaOH) as a clear oil (3.85 g, 89% yield). This material was used in the next step without purification.  $[\alpha]_D^{20} = +19.0$  (c = 1.0, CHCl<sub>3</sub>). IR ( $\nu_{\max}$ , film): 2982, 2106, 1695, 1517, 1396, 1157, 1060. <sup>1</sup>H NMR (400 MHz, MeOD):  $\delta = 1.44$  (9H, s, Boc), 1.82-1.91 (1H, m, CHHCH<sub>2</sub>N<sub>3</sub>), 2.04-2.10 (1H, m, CHHCH<sub>2</sub>N<sub>3</sub>), 3.35-3.48 (2H, m, CH<sub>2</sub>N<sub>3</sub>), 4.22 (1H, dd, *J* = 9.4, 4.6, H <sub>$\alpha$</sub> ). <sup>13</sup>C NMR (50 MHz, MeOD):  $\delta = 28.8$  (Boc), 32.0 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 49.2 (CH<sub>2</sub>N<sub>3</sub>), 52.4 (C <sub>$\alpha$</sub> ), 80.8 (Boc), 158.1, 175.6 (2 × C=O). HRMS *m/z* (ESI<sup>+</sup>): Found 267.1066 (M+Na)<sup>+</sup>; C<sub>9</sub>H<sub>16</sub>N<sub>4</sub>O<sub>4</sub>Na requires 267.1064.



To a solution of BocAhaOH (5.72 g, 23.42 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (15 mL) at 0 °C was added TFA (4.0 mL). The solution was stirred from 0 °C to room temperature over an hour. An additional 5.0 mL of TFA was then added and the solution stirred for 4 hours at room temperature. TLC analysis indicated complete consumption of the starting material. The solvent was removed under reduced pressure to give a thick yellow oil. This oil was dissolved in 5.0 mL of H<sub>2</sub>O, cooled to 0 °C, and the resulting solution adjusted to pH ~10 with 2.5M NaOH (pH paper). Dowex-50WX8 was prewashed with MeOH, acetone, 2M HCl, and then H<sub>2</sub>O until the filtrate was neutral. This conditioned

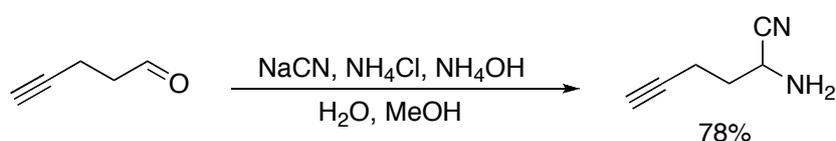
resin was then added to the solution until the pH of the reaction mixture was approximately neutral (pH paper). The neutralized solution was poured onto a column of DOWEX-50WX8 (100 g conditioned resin). The resin was washed with H<sub>2</sub>O (500 mL) to remove TFA salts and then the product eluted with 5% NH<sub>4</sub>OH (aq). The fractions containing azidohomoalanine (ammonium carboxylate) were combined and evaporated under reduced pressure at 50 °C to give the azidoamoalanine as the free amino acid. This white solid was recrystallized by suspending in ~20 mL of boiling EtOH and then adding distilled H<sub>2</sub>O dropwise until the solution was clear. Once the solution was clear, an additional 10 mL of EtOH was added. Upon slow cooling (over 6 hours to rt), azidohomoalanine crystallized as small white cubes. The crystals were isolated by filtration and dried under vacuum (2.12 g, 67% yield). m.p.= 231-232 °C.  $[\alpha]_D^{20} = +6.60$  (c = 0.50, H<sub>2</sub>O). IR (KBr): 2954, 2112, 1587, 1507, 1446, 1415, 1359, 1322, 1196, 1160, 1091. <sup>1</sup>H NMR (400 MHz, D<sub>2</sub>O): δ = 1.99 (2H, m, CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.44 (2H, app dt, J = 6.6, 2.3, CH<sub>2</sub>N<sub>3</sub>), 3.69 (1H, dd, J = 5.6, 7.2, H<sub>α</sub>). <sup>13</sup>C NMR (100 MHz, D<sub>2</sub>O): δ = 29.9 (CH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 47.9 (CH<sub>2</sub>N<sub>3</sub>), 53.2 (C<sub>α</sub>), 174.2 (C=O). HRMS m/z (ESI+): Found 167.0539 (M+Na)<sup>+</sup>; C<sub>4</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>Na requires 167.0545. Anal. calc. for C<sub>4</sub>H<sub>8</sub>N<sub>4</sub>O<sub>2</sub>: C, 33.33; H, 5.59; N, 38.87; found C, 33.37; H, 5.59; N, 38.85.

### 2.7.1 (rac)-Hpg synthesis



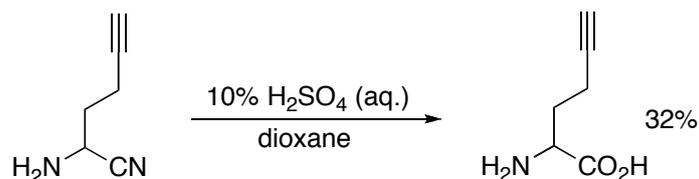
This procedure was adapted from the literature.<sup>20</sup> CH<sub>2</sub>Cl<sub>2</sub> (200 mL, anhydrous) was added to a flame-dried 500 mL round bottom flask under a stream of nitrogen. Oxalyl chloride (10.3 mL, 118 mmol) was added and the stirred solution was cooled to -78 °C. A solution of DMSO (16.8 mL) in CH<sub>2</sub>Cl<sub>2</sub> (30 mL) was added dropwise to the reaction at -78 °C under a stream of nitrogen over a period of 15 minutes. After 30 minutes, 4-pentyn-1-ol (10.0 mL, 108 mmol) was added over a period of 5 minutes. The reaction was stirred for 1 hour at -78 °C before Et<sub>3</sub>N (75.0 mL, 538 mmol) was added over a period of 15 minutes. The reaction was stirred vigorously to accommodate the formation of triethylamine hydrochloride and was warmed to 0 °C over a period of 1 hour. After this time, the reaction was stirred at 0 °C for two additional hours. The reaction was then quenched at 0 °C by the addition of 1M HCl (100 mL). The mixture was then transferred to a separatory funnel and diluted with an additional 200 mL of 1M HCl. The organic layer was separated and the aqueous layer was extracted with CH<sub>2</sub>Cl<sub>2</sub>

(2 × 100 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. The resulting residue was purified by column chromatography (50% CH<sub>2</sub>Cl<sub>2</sub> in 30-40 petrol) to afford the aldehyde as a thin, yellow oil (5.23 g, 59%). Note that the aldehyde is volatile and care must be taken when concentrating under reduced pressure. Spectroscopic data was consistent with that previously reported.<sup>20</sup> IR ( $\nu_{\max}$ , film): 3291, 2922, 2835, 2735, 2360 2341, 2120, 1724, 1059. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 1.95 (1H, t,  $J$  = 2.7, C≡CH), 2.45 (2H, td,  $J$  = 7.1, 2.7, HC≡CCH<sub>2</sub>), 2.64 (2H, t,  $J$  = 7.1, CH<sub>2</sub>CHO), 9.74 (1H, app. s., CHO). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz):  $\delta$  = 11.4 (HC≡CCH<sub>2</sub>), 42.1 (CH<sub>2</sub>CHO), 69.1 (HC≡C), 82.2 (HC≡C), 200.0 (C=O).



*Caution: NaCN is extremely toxic. All manipulations must be carried out in a fume hood and aqueous waste should be kept basic and disposed of safely.*

This synthesis was adapted from the literature.<sup>21</sup> NH<sub>4</sub>Cl (1.41 g, 26.4 mmol) and NaCN (1.28 g, 26.1 mmol) were added as solids to a 100 mL round bottom flask and dissolved in 16 mL of 12.5% NH<sub>4</sub>OH (aq.). The solution was stirred at room temperature for 1 hour before 4-pentynal (1.25 g, 15.2 mmol) was added as a solution in 6 mL MeOH. The reaction was heated to 40 °C and stirred for 4 hours. After this time, the reaction was cooled to room temperature and then extracted with CH<sub>2</sub>Cl<sub>2</sub> (3 × 50 mL). The combined organic layers were dried (MgSO<sub>4</sub>), filtered, and concentrated under reduced pressure. The product amino nitrile (1.28 g, 78%, yellow oil) was sufficiently pure to carry on to the next step and was not purified further. IR ( $\nu_{\max}$ , film): 2937, 2867, 2360, 2341, 2230, 2117, 1606, 1446, 1433, 1313, 1116, 1042. <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz):  $\delta$  = 1.64 (2H, br. s., NH<sub>2</sub>), 1.91-2.02 (2H, m, H $\beta$ ), 2.04 (1H, t,  $J$  = 2.6, C≡CH), 2.38-2.55 (2H, m, H $\gamma$ ), 3.90 (1H, quin.,  $J$  = 7.6, H $\alpha$ ). <sup>13</sup>C NMR (CDCl<sub>3</sub>, 50 MHz):  $\delta$  = 14.6, 33.5 (CH<sub>2</sub>CH<sub>2</sub>C≡CH), 41.8 (C $\alpha$ ), 69.9 (C≡CH), 81.7 (C≡CH), 121.6 (C≡N). LRMS  $m/z$  (ESI<sup>+</sup>): 109.1 [M+H]<sup>+</sup>.



This synthesis was adapted from the literature.<sup>21</sup> The crude amino nitrile prepared above (1.19 g, 11.0 mmol) was dissolved in 1,4-dioxane (4 mL) in a 50 mL round bottom flask. An ice cold solution of 10% H<sub>2</sub>SO<sub>4</sub> (aq.) was added to the stirred solution. The reaction was then heated to reflux (bath temp = 120 °C) and stirred for 24 hours. The resulting solution (now very dark in color) was cooled to room temperature and then poured onto a column of DOWEX-50WX8 (100 g, H<sup>+</sup> form). The column was washed with water (500 mL) and the flow through was discarded. The product was then eluted with 500 mL of 5% NH<sub>4</sub>OH (aq.). The product was collected and the solvent was removed under reduced pressure (bath temp = 65 °C). The resulting off-white solid was recrystallized (EtOH / H<sub>2</sub>O) to give racemic Hpg as small off-white needles (451 mg, 32%). m.p. = 230 °C (decomposes to red solid). IR (ν<sub>max</sub>, KBr): 3425, 3295, 3037, 2590, 2117, 1608, 1453, 1411, 1360, 1344, 1320, 1280. <sup>1</sup>H NMR (D<sub>2</sub>O, 500 MHz): δ = 1.91-2.10 (2H, m, Hβ), 2.26-2.37 (3H, m, CH<sub>2</sub>C≡CH), 3.77 (1H, dd, J = 7.6, 5.4, Hα). <sup>13</sup>C NMR (D<sub>2</sub>O, 125 MHz): δ = 14.4 (CH<sub>2</sub>C≡CH), 29.2 (Cβ), 53.9 (Cα), 70.8 (C≡CH), 83.0 (C≡CH), 174.2 (C=O). LRMS m/z (ESI<sup>+</sup>): 128.1 [M+H]<sup>+</sup>.

### 2.7.3 Expression of Np276 Aha61, Np276 Hpg61, Np276 Cys61, Np276 Aha101

#### **Expression of Np276 Aha61**

A glycerol stock of *E. coli* B834(DE3) cells containing the pET28d plasmid carrying the gene for Np276 (M21I) was used to inoculate a small starter culture of LB media (25 ml) containing kanamycin (50 µg/ml). This starter culture was grown overnight at 37 °C and then used to inoculate SelenoMet media (1L) containing kanamycin (50 µg/ml) and filter-sterilised L-Methionine (40 µg/ml). The cells were incubated at 37 °C and grown to an optical density value between 0.6 and 0.8 before being harvested by centrifugation (10 mins, 8000 rpm, 4 °C). The cells were washed thrice by re-suspending in SelenoMet media containing kanamycin (50 µg/ml) but absent of either L-Methionine or azidohomoalanine. The cells were then transferred to pre-warmed SelenoMet media (1L) containing kanamycin (50 µg/ml) and supplemented with filter-sterilised azidohomoalanine (100 µg/ml). The culture was incubated for 30 mins at 37 °C followed by 30 mins at 30 °C. Expression was induced with IPTG (0.24 g, 1 mmol) and incubation continued overnight at 20 °C (220 rpm).

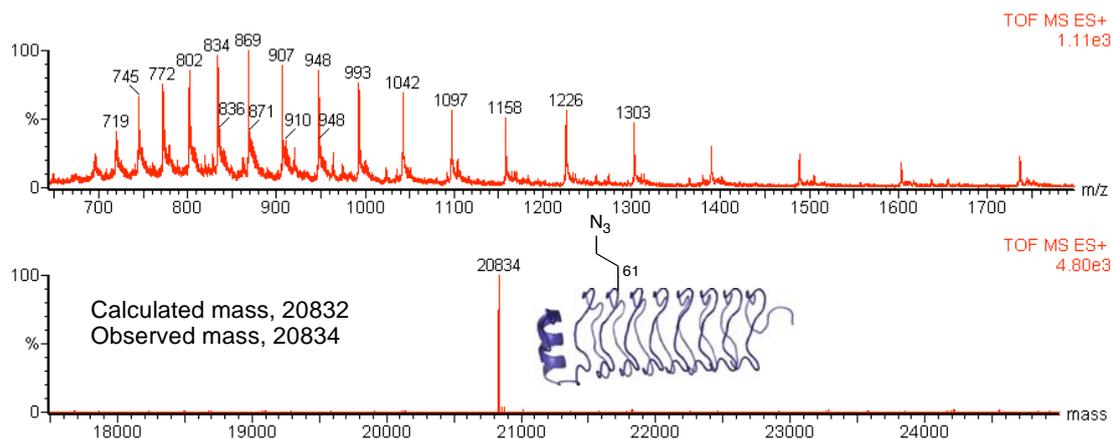
The cells were then harvested by centrifugation (10 mins, 8000 rpm, 4°C) and the pellets collected and incubated on ice for 1 hour with stirring following the addition of DNase (10 mg) and lysozyme (10 mg). The cells were then sonicated (20% power, 5 bursts of 30 seconds with 1 minute waiting times between bursts) and the resulting cell debris pelleted by centrifugation (30 minutes, 20,000 rpm, 4 °C). Purification of the protein was achieved by incubating the cell lysate overnight with HisBind resin (10 ml) at 4 °C. The resin was then washed with 150 ml of binding buffer (20 mmol Tris-HCl, 15 mmol imidazole, 0.3 M NaCl, pH 7.8) and finally eluted with 60 ml of elution buffer (20 mmol Tris-HCl, 500 mmol imidazole, 0.3 M NaCl, pH 7.8). All fractions were analysed by SDS-PAGE (4-12% Bis-Tris gel, MES buffer).

The fractions containing protein were combined and dialysed twice into 4 L of phosphate buffer (50 mmol Na<sub>2</sub>HPO<sub>4</sub>, pH 8) to remove the imidazole. The resulting protein, now in phosphate buffer was analysed by LC-MS and stored at -20 °C.

**Sequence of NP276 M61Aha**

GSSHHHHHHSSGLVPRGSHIDVVGKLRQLYAAGERDFSIV DLRGAVLENI  
 NLSGAILHGA**Aha**LDEANLQQANLSRADLSGATLNGADLRGANLSKADLSDA  
 ILDNAILEGAILDEAVLNQANLKAANLEQAILSHANIREADLSEANLEAADLSG  
 ADLAIADLHQANLHQAAALERANLTGANLEDANLEGTILEGGNNLAT

Calculated average isotopic mass = 20832 (N-terminal Met cleaved)



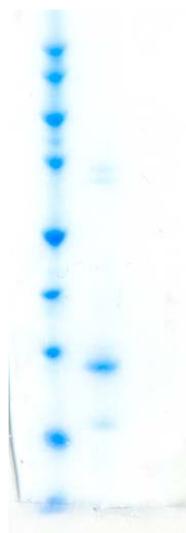
**Figure S52.** ESI-MS spectrum of Np276 Aha61.

**Expression of Np276 Hpg61**

A glycerol stock of *E. coli* B834(DE3) cells containing the pET28d plasmid carrying the gene for Np276 (M21I) was used to inoculate a small starter culture of LB media (25 ml) containing kanamycin (50 µg/ml). This starter culture was grown overnight at 37 °C and then used to inoculate SelenoMet media (1L) containing kanamycin (50 µg/ml) and filter-sterilised L-Methionine (20 µg/ml). The cells were incubated at 37 °C and grown to an optical density value between 0.6 and 0.8 before being harvested by centrifugation (10 mins, 8000 rpm, 4 °C). The cells were washed five times by re-suspending in SelenoMet media containing kanamycin (50 µg/ml) but absent of either L-Methionine or azidohomoalanine. The cells were then transferred to pre-warmed SelenoMet media (1L) containing kanamycin (50 µg/ml) and supplemented with filter-sterilised homopropargylglycine (250 µg/ml). The culture was incubated for two hours at 37 °C followed by 30 mins at 30 °C. Expression was induced with IPTG (0.24 g, 1 mmol) and incubation continued overnight at 20 °C (220 rpm).

The cells were then harvested by centrifugation (10 mins, 8000 rpm, 4 °C) and the pellets collected and incubated on ice for 1 hour with stirring following the addition of DNase (10 mg) and lysozyme (10 mg). The cells were then sonicated (20% power, 5 bursts of 30 seconds with 1 minute waiting times between bursts) and the resulting cell

debris pelleted by centrifugation (30 minutes, 20,000 rpm, 4 °C). Purification of the protein was achieved by incubating the cell lysate overnight with HisBind resin (10 ml) at 4 °C. The resin was washed with 150 ml of binding buffer (20 mmol Tris-HCl, 15 mmol imidazole, 0.3 M NaCl, pH 7.8) and finally eluted with 60 ml of elution buffer (20 mmol Tris-HCl, 500 mmol imidazole, 0.3 M NaCl, pH 7.8). All fractions were analysed by SDS-PAGE (4-12% Bis-Tris gel, MES buffer).



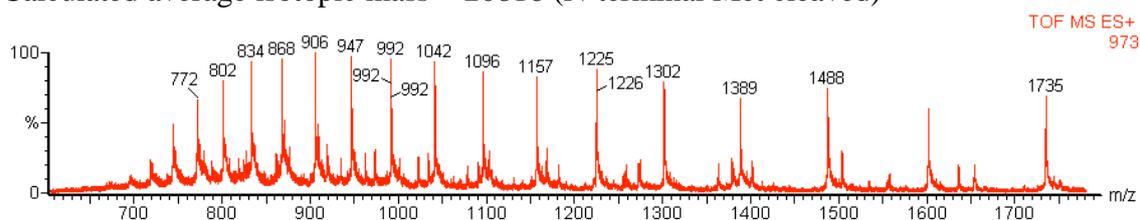
### Np276-Hpg61 eluted fractions

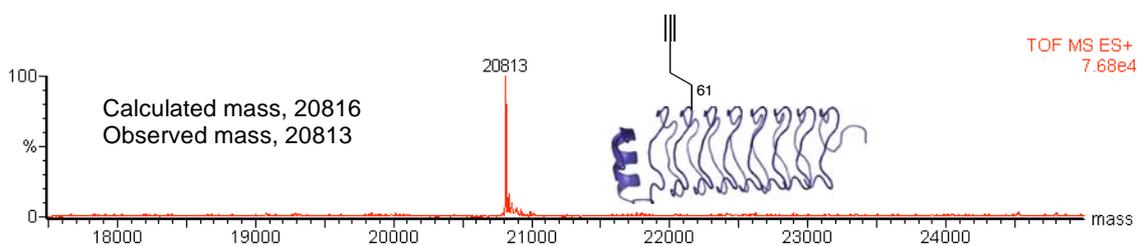
The fractions containing protein were combined and dialysed twice into 4 L of phosphate buffer (50 mmol Na<sub>2</sub>HPO<sub>4</sub>, pH 8) to remove the imidazole. The resulting protein, now in phosphate buffer was analysed by LCT-MS and stored at -20 °C.

### Sequence of Np276 M61Hpg

GSSHHHHHSSGLVPRGSHIDVGKLRQLYAAGERDFSIV DLRGAVLENI  
NLSGAILHGA**Hpg**LDEANLQQANLSRADLSGATLNGADLRGANLSKADLSDA  
ILDNAILEGAILDEAVLNQANLKAANLEQAILSHANIREADLSEANLEAADLSG  
ADLAIADLHQANLHQAALERANLTGANLEDANLEGTTILEGGNNLAT

Calculated average isotopic mass = 20816 (N-terminal Met cleaved)





**Figure S53.** ESI-MS spectrum of Np276 Hpg61.

### **Expression of Np276 Cys61**

A pET28d plasmid carrying the gene for Np276 (M21I) was modified by site-directed mutagenesis in order to introduce the mutation *M61C* into the proteins primary sequence using the following primers:

- M61C (F) – [5'-CTACATGGGGCGTGCCTAGATGAAGC-3']
- M61C (R) – [5'-GCTTCATCTAGGCACGCCCATGTAG-3'].

DNA sequencing confirmed the presence of both mutations (GeneService, Oxford):

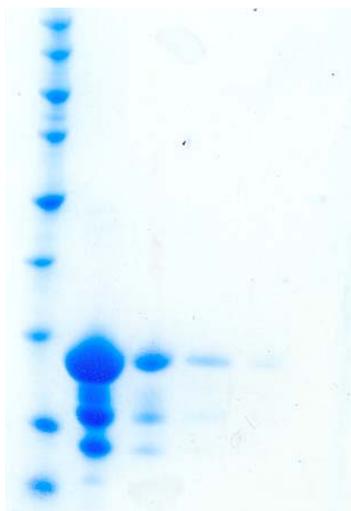
#### **Forward DNA sequence - T7F**

ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCG CGC GGC AGC CAT  
**ATT** GAC GTA GGA AAA CTC AGG CAA CTA TAT GCC GCA GGA GAG CGA GAC TTT AGT ATC GTT  
 GAC TTG AGG GGT GCA GTC TTG GAA AAC ATC AAT CTC AGT GGT GCA ATT CTA CAT GGG GCG  
**ATT** CTA GAT GAA GCA AAT TTG CAA CAG GCA AAT CTC AGT CGG GCT GAC TTA AGT GGG GCT  
 ACG CTC AAT GGT GCA GAT TTA AGA GGG GCT AAT TTA AGC AAA GCC GAT TTG AGT GAT GCA  
**ATG** CTT GAC AAT GCA ATA TTA GAA GGT GCA ATT CTT GAT GAA GCC GTT TTA AAT CAG GCT  
 AAT CTC AAA GCT GCT AAC TTG GAG CAG GCG ATT CTT AGT CAC GCT AAC ATC CGT GAA GCT  
 GAT TTG AGT GAA GCT AAT TTG GAA GCA GCA GAT TTG AGC GGG GCA GAT TTA GCG ATC GCG  
 GAT TTG CAT CAG GCA AAT CTG CAC CAA GCT GCA TTA GAA AGA GCC AAT CTT ACA GGA GCT  
 AAT CTA GAA GAT GCC AAT TTA GAG GGG ACT ATT TTA GAG GGC GGC AAC AAC AAT CTT GCA  
 ACC TAA

1  $\mu$ L of the above mutant was transformed into *E. coli* B834(DE3) methionine-auxotrophic cells. A glycerol stock of the same cells was used to inoculate a small starter culture of LB media (25 ml) containing kanamycin (50  $\mu$ g/ml). This starter culture was grown overnight at 37 °C and then used to inoculate LB media (1L) containing kanamycin (50  $\mu$ g/ml). The cells were incubated at 37 °C and grown to an optical density value between 0.6 and 0.8. Expression was induced with IPTG (0.24 g, 1 mmol) and incubation continued overnight at 20 °C (220 rpm).

The cells were then harvested by centrifugation (10 mins, 8000 rpm, 4 °C) and the pellets collected and incubated on ice for 1 hour with stirring following the addition of DNase (10 mg) and lysozyme (10 mg). The cells were then sonicated (20% power, 5 bursts of 30 seconds with 1 minute waiting times between bursts) and the resulting cell

debris pelleted by centrifugation (30 minutes, 20,000 rpm, 4 °C). Purification of the protein was achieved by incubating the cell lysate overnight with HisBind resin (10 ml) at 4 °C. The resin was washed with 150 ml of binding buffer (20 mmol Tris-HCl, 15 mmol imidazole, 0.3 M NaCl, pH 7.8) and finally eluted with 60 ml of elution buffer (20 mmol Tris-HCl, 500 mmol imidazole, 0.3 M NaCl, pH 7.8). All fractions were analysed by SDS-PAGE (4-12% Bis-Tris gel, MES buffer).



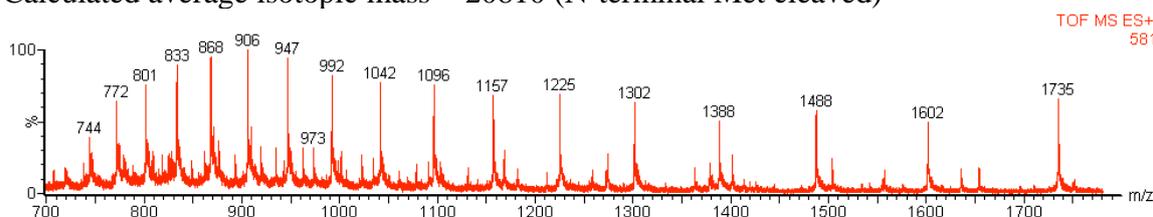
**Np276-Cys61 eluted fractions**

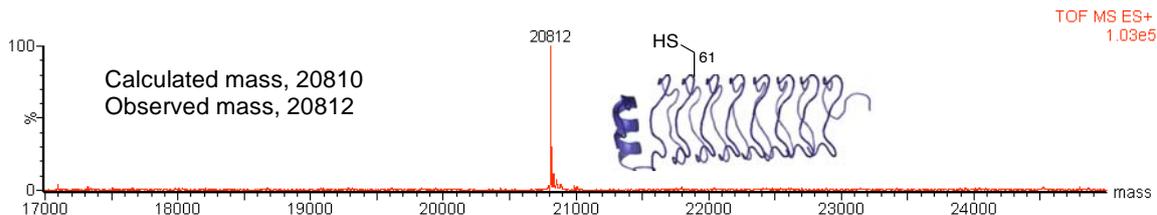
The fractions containing protein were combined and dialysed twice into 4 L of phosphate buffer (50 mmol Na<sub>2</sub>HPO<sub>4</sub>, pH 8) to remove the imidazole. The resulting protein, now in phosphate buffer was analysed by LCT-MS and stored at -20 °C .

### Sequence of Np276 Cys61

GSSHHHHHSSGLVPRGSHIDVGKLRQLYAAGERDFSIV DLRGAVLENI  
NLSGAILHGACLD<sup>●</sup>EANLQQANLSRADLSGATLNGADLRGANLSKADLSDA  
ILDNAILEGAILDEAVLNQANLKAANLEQAILSHANIREADLSEANLEAADLSG  
ADLAIADLHQANLHQAALERANLTGANLEDANLEGTILEGGNNLAT

Calculated average isotopic mass = 20810 (N-terminal Met cleaved)





**Figure S54.** ESI-MS spectrum of Np276 Cys61.

### **Expression of Np276 Aha101**

A pET28d plasmid carrying the gene for Np276 (M21I) was modified by multi site-directed mutagenesis in order to introduce the mutations *M61I* and *I101M* into the proteins primary sequence using the following primers:

- M61I (F) – [5'-CATGGGGCGATTCTAGATGAAGC-3']
- M61I (R) – [5'-GCTTCATCTAGAATCGCCCCATG-3']
- I101M (F) – [5'-GAGTGATGCAATGCTTGACAATGC-3']
- I101M (R) – [5'-GCATTGTCAAGCATTGCATCACTC-3'].

DNA sequencing confirmed the presence of both mutations (GeneService, Oxford):

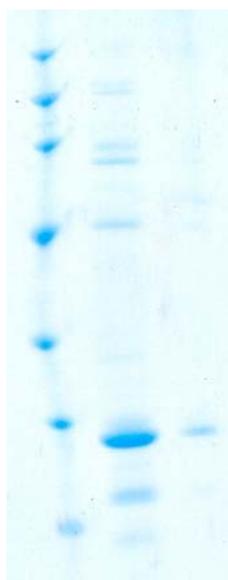
#### **Forward DNA sequence - T7F**

ATG GGC AGC AGC CAT CAT CAT CAT CAC AGC AGC GGC CTG GTG CCG CGC GGC AGC CAT  
**ATT** GAC GTA GGA AAA CTC AGG CAA CTA TAT GCC GCA GGA GAG CGA GAC TTT AGT ATC GTT  
 GAC TTG AGG GGT GCA GTC TTG GAA AAC ATC AAT CTC AGT GGT GCA ATT CTA CAT GGG GCG  
**ATT** CTA GAT GAA GCA AAT TTG CAA CAG GCA AAT CTC AGT CGG GCT GAC TTA AGT GGG GCT  
 ACG CTC AAT GGT GCA GAT TTA AGA GGG GCT AAT TTA AGC AAA GCC GAT TTG AGT GAT GCA  
**ATG** CTT GAC AAT GCA ATA TTA GAA GGT GCA ATT CTT GAT GAA GCC GTT TTA AAT CAG GCT  
 AAT CTC AAA GCT GCT AAC TTG GAG CAG GCG ATT CTT AGT CAC GCT AAC ATC CGT GAA GCT  
 GAT TTG AGT GAA GCT AAT TTG GAA GCA GCA GAT TTG AGC GGG GCA GAT TTA GCG ATC GCG  
 GAT TTG CAT CAG GCA AAT CTG CAC CAA GCT GCA TTA GAA AGA GCC AAT CTT ACA GGA GCT  
 AAT CTA GAA GAT GCC AAT TTA GAG GGG ACT ATT TTA GAG GGC GGC AAC AAC AAT CTT GCA  
 ACC TAA

1  $\mu$ L of the above mutant was transformed into *E. coli* B834(DE3) methionine-auxotrophic cells. A glycerol stock of the same cells was used to inoculate a small starter culture of LB media (25 ml) containing kanamycin (50  $\mu$ g/ml). This starter culture was grown overnight at 37 °C and then used to inoculate SelenoMet media (1L) containing kanamycin (50  $\mu$ g/ml) and filter-sterilised L-Methionine (40  $\mu$ g/ml). The cells were incubated at 37 °C and grown to an optical density value between 0.6 and 0.8 before being harvested by centrifugation (10 mins, 8000 rpm, 4°C). The cells were washed thrice by re-suspending in SelenoMet media containing kanamycin (50  $\mu$ g/ml) but absent of either L-Methionine or azidohomoalanine. The cells were then transferred

to pre-warmed SelenoMet media (1L) containing kanamycin (50  $\mu\text{g/ml}$ ) and supplemented with filter-sterilised azidohomoalanine (100  $\mu\text{g/ml}$ ). The culture was incubated for 30 mins at 37  $^{\circ}\text{C}$  followed by 30 mins at 30  $^{\circ}\text{C}$ . Expression was induced with IPTG (0.24 g, 1 mmol) and incubation continued overnight at 20  $^{\circ}\text{C}$  (220 rpm).

The cells were then harvested by centrifugation (10 mins, 8000 rpm, 4 $^{\circ}\text{C}$ ) and the pellets collected and incubated on ice for 1 hour with stirring following the addition of DNase (10 mg) and lysozyme (10 mg). The cells were then sonicated (20% power, 5 bursts of 30 seconds with 1 minute waiting times between bursts) and the resulting cell debris pelleted by centrifugation (30 minutes, 20,000 rpm, 4  $^{\circ}\text{C}$ ). Purification of the protein was achieved by incubating the cell lysate overnight with HisBind resin (10 ml) at 4  $^{\circ}\text{C}$ . The resin was washed with 150 ml of binding buffer (20 mmol Tris-HCl, 15 mmol imidazole, 0.3 M NaCl, pH 7.8) and finally eluted with 60 ml of elution buffer (20 mmol Tris-HCl, 500 mmol imidazole, 0.3 M NaCl, pH 7.8). All fractions were analysed by SDS-PAGE (4-12% Bis-Tris gel, MES buffer).



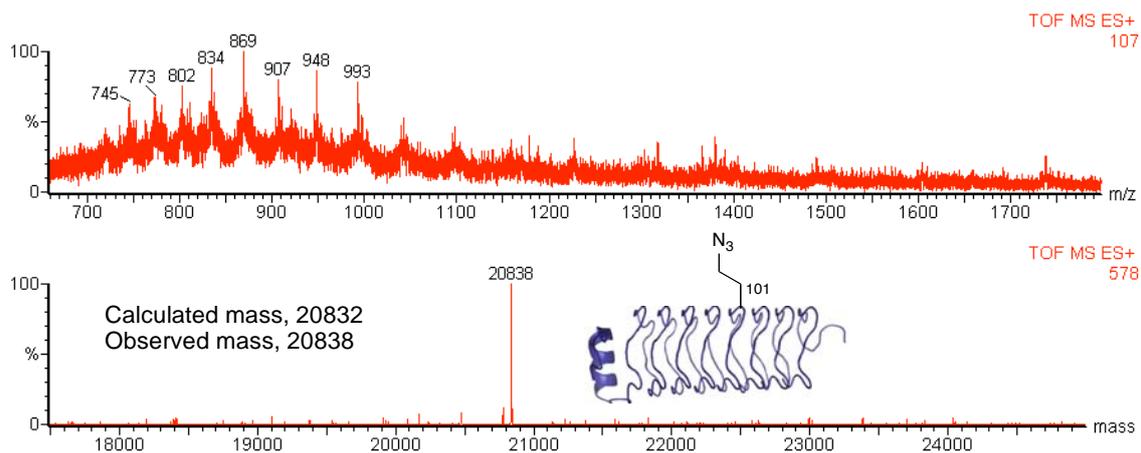
#### **Np276-Aha101 eluted fractions**

The fractions containing protein were combined and dialysed twice into 4 L of phosphate buffer (50 mmol  $\text{Na}_2\text{HPO}_4$ , pH 8) to remove the imidazole. The resulting protein, now in phosphate buffer was analysed by LCT-MS and stored at -20  $^{\circ}\text{C}$ .

**Sequence of NP276 Aha101**

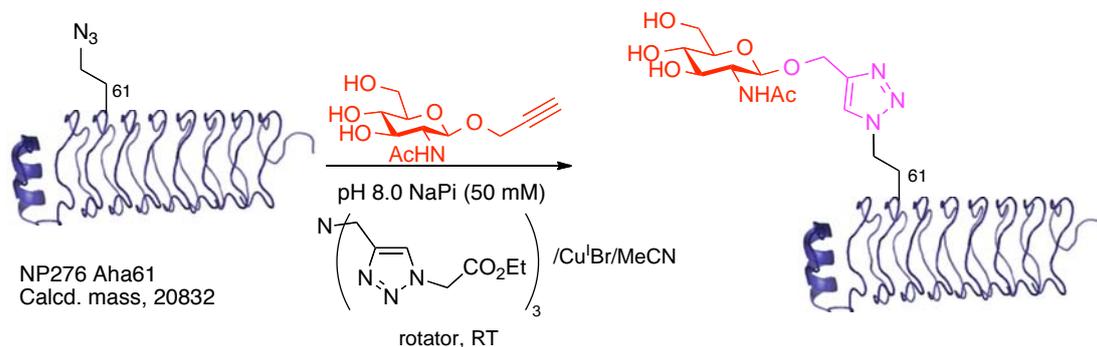
GSSHHHHHHSSGLVPRGSHIDVGKLRQLYAAGERDFSIV DLRGAVLENI  
NLSGAILHGAILDEANLQQANLSRADLSGATLNGADLRGANLSKADLSDA  
**Aha**LDNAILEGAILDEAVLNQANLKAANLEQAILSHANIREADLSEANLEAADLS  
GADLAIADLHQANLHQAAALERANLTGANLEDANLEGTILEGGNNLAT

Calculated average isotopic mass = 20832 (N-terminal Met cleaved)

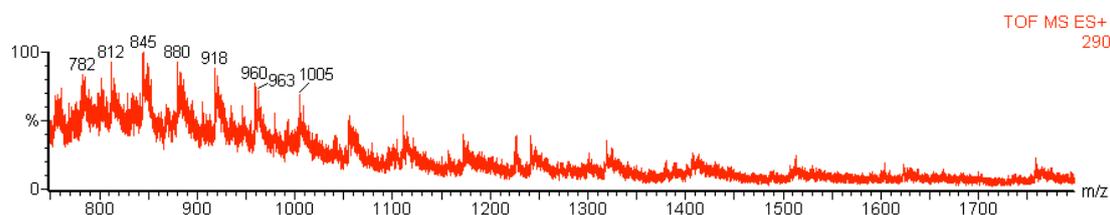


## 2.7.4 Preparation of substrates for Endo-A catalysed glycosylation of Np276

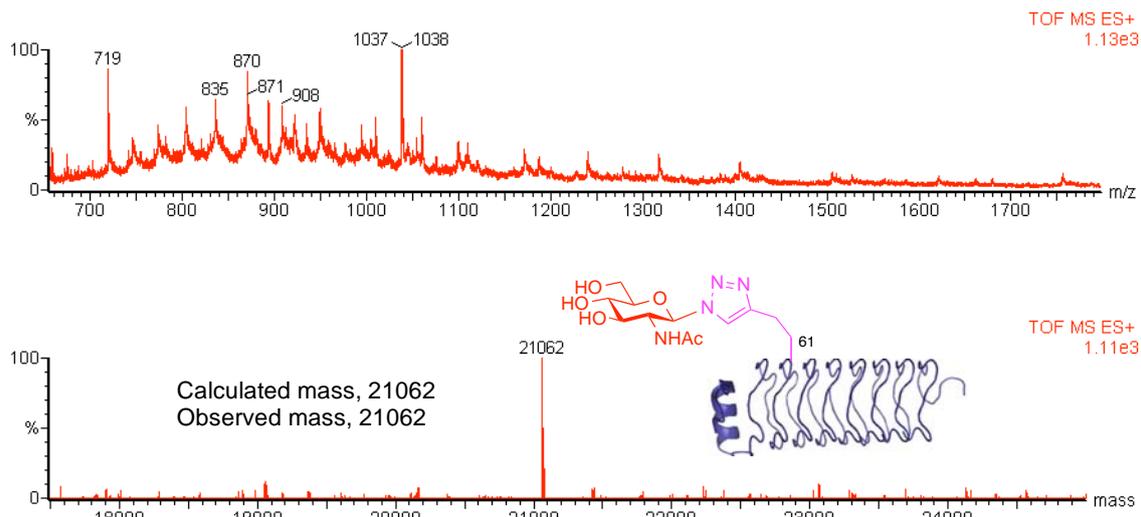
### Preparation of GlcNAc-OCH<sub>2</sub>-triazole-61Np276 (25a)



A freshly prepared solution of copper(I) bromide (99.999%) in acetonitrile (89  $\mu\text{L}$ , 10 mg/mL) was premixed with an acetonitrile solution of tris-triazolyl amine ligand (36  $\mu\text{L}$ , 127 mg/mL). The preformed Cu-complex solution (125  $\mu\text{L}$ ) was added to a NP276 Aha61 protein solution (500  $\mu\text{L}$ , 0.66 mg/mL) and mixed thoroughly. Propargyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (12.3 mg, 47.5  $\mu\text{mol}$ ) in sodium phosphate buffer (400  $\mu\text{L}$ , 50 mM, pH 8.2) was added to the above mixture and the reaction was agitated on a rotator for 6 h at room temperature. Small molecules were removed from a 500  $\mu\text{L}$  reaction mixture aliquot by loading the sample onto a PD10 desalting column (GE Healthcare) previously equilibrated with 10 column volumes of pH 7.0 sodium phosphate buffer (50 mM) and eluting with 1 mL of the same buffer. The solution was concentrated on a vivaspin membrane concentrator (10 kDa molecular weight cut off) and washed with sodium phosphate buffer (3 x 100  $\mu\text{L}$ , 50 mM, pH 7.0). Finally, the solution was concentrated to 250  $\mu\text{L}$  and the product was characterized by LC-MS (calc. 21091; found 21090). The collected sample (0.34 mg/mL by BCA assay) was flash frozen with liquid nitrogen and stored at  $-20\text{ }^\circ\text{C}$ .

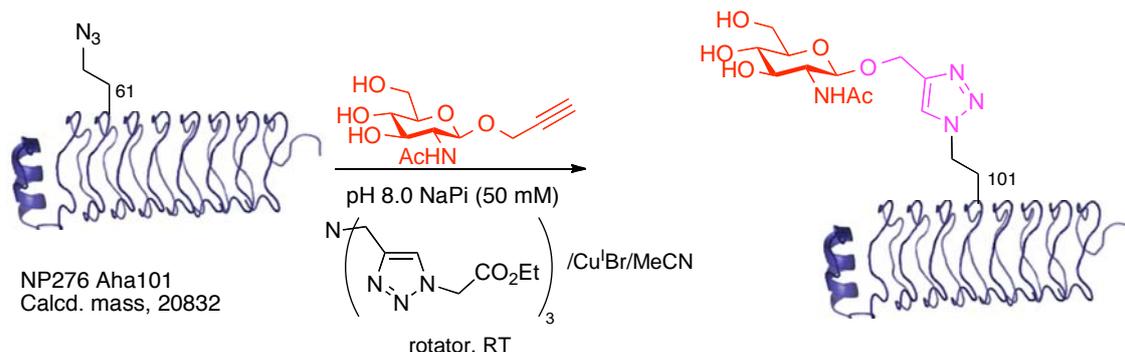






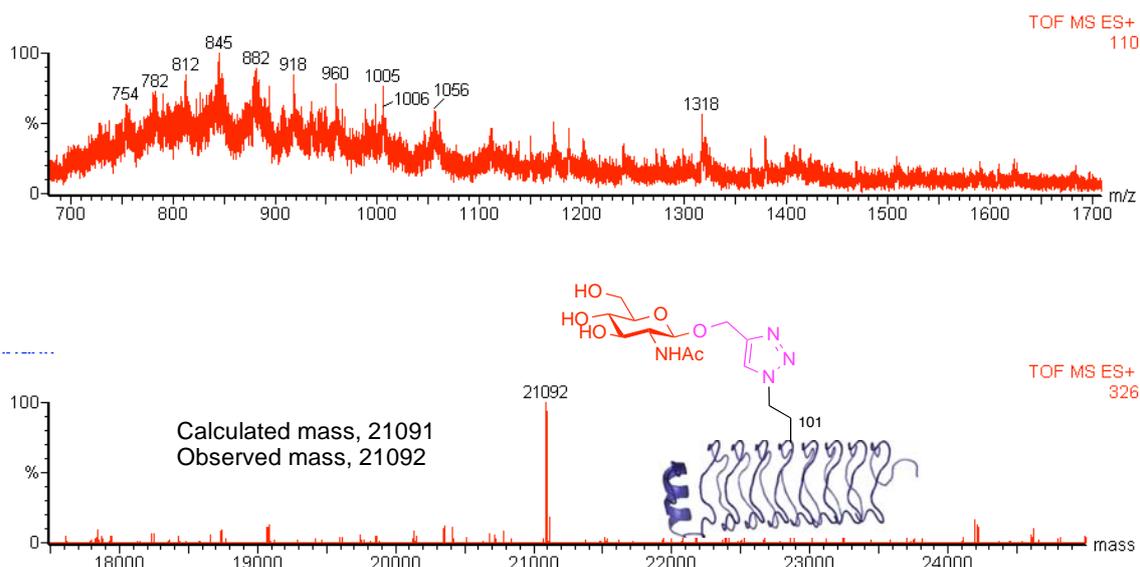
**Figure S57.** ESI-MS spectrum of GlcNAc-triazole-61Np276 (0.37 mg/mL by BCA assay) after 6 h of reaction (**26a**).

### Preparation of GlcNAc-OCH<sub>2</sub>-triazole-101Np276 (**28a**)



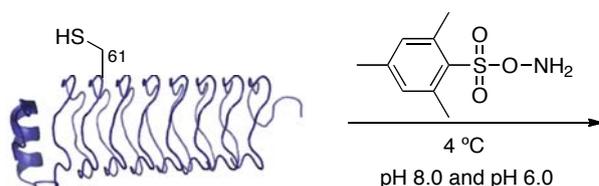
A freshly prepared solution of copper(I) bromide (99.999%) in acetonitrile (147  $\mu$ L, 10 mg/mL) was premixed with an acetonitrile solution of tris-triazolyl amine ligand (73  $\mu$ L, 127 mg/mL). The preformed Cu-complex solution (220  $\mu$ L) was added to a NP276-101Aha protein solution (2 mL, 0.29 mg/mL) and mixed thoroughly. Propargyl 2-acetamido-2-deoxy- $\beta$ -D-glucopyranoside (21.7 mg, 83.5  $\mu$ mol) in sodium phosphate buffer (50  $\mu$ L, 50 mM, pH 8.2) was added to the above mixture and the reaction was agitated on a rotator for 3 h at room temperature. Small molecules were removed from the reaction mixture by loading the sample onto a PD10 desalting column (GE Healthcare) previously equilibrated with 10 column volumes of pH 7.0 sodium phosphate buffer (50 mM) and eluting with 3.5 mL of the same buffer. The solution was concentrated on a vivaspin membrane concentrator (10 kDa molecular weight cut off) and washed with sodium phosphate buffer (3 x 100  $\mu$ L, 50 mM, pH 7.0). Finally, the solution was concentrated to 500  $\mu$ L and the product was characterized by LC-MS

(calc. 21091; found 21092). The collected sample (0.22 mg/mL by BCA assay) was flash frozen with liquid nitrogen and stored at  $-20\text{ }^{\circ}\text{C}$ .

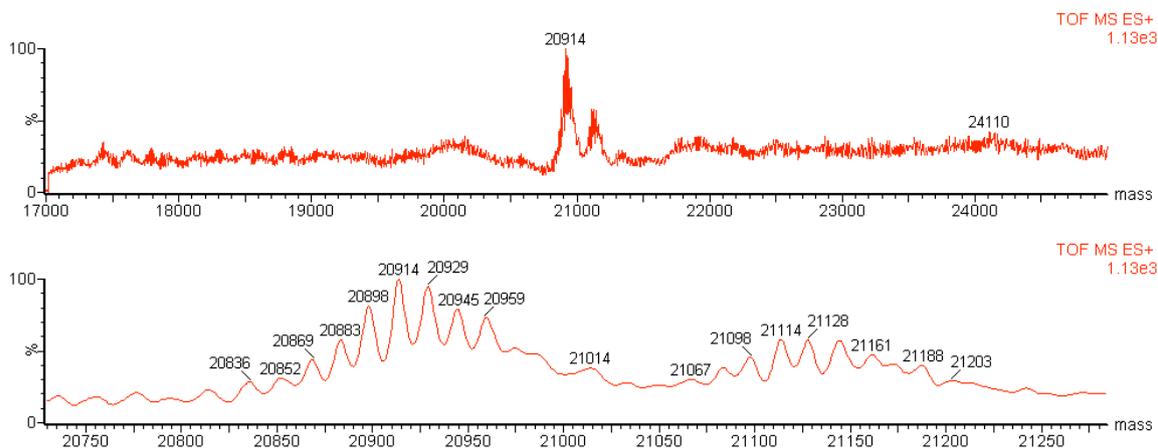
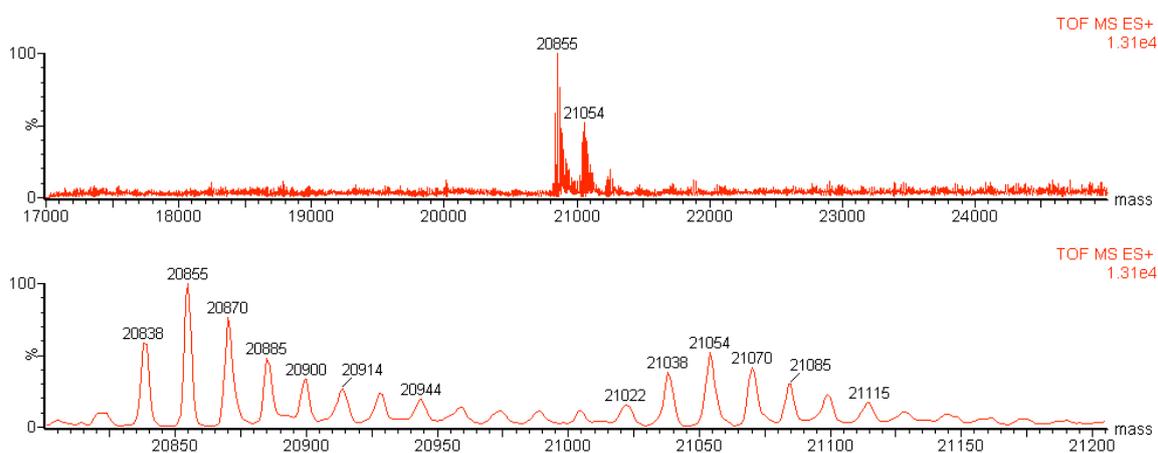


**Figure S58.** ESI-MS spectrum of GlcNAc-OCH<sub>2</sub>-triazole-101Np276 (0.22 mg/mL by BCA assay) after 3 h of reaction (**28a**).

### Preparation of GlcNAc-S-61NP276 (**27a**)

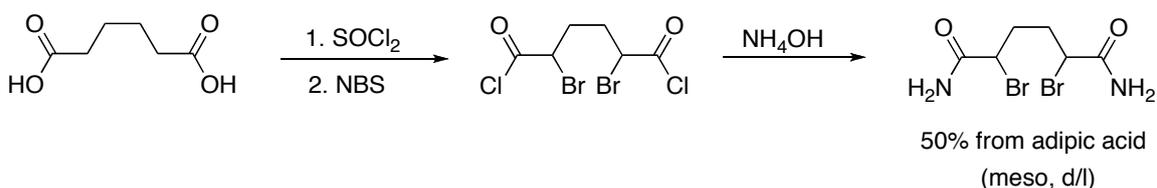


Np276 Cys61 (1.00 mL,  $c = 0.79\text{ mg/mL}$ , pH 8.0 sodium phosphate) was reduced with DTT (5 mg) (15 min. RT). 500  $\mu\text{L}$  of the reduced protein was passed through a PDmini column into pH 8.0 sodium phosphate buffer and the remaining 500  $\mu\text{L}$  was passed through a PDmini column into pH 6.0 sodium phosphate buffer. The proteins solutions were kept on ice. A stock solution of MSH was prepared by dissolving 3.4 mg, 567  $\mu\text{L}$  of DMF. Two reactions were set up: one at pH 8.0 and one at pH 6.0. Accordingly, 100  $\mu\text{L}$  of the protein solution was mixed with 5  $\mu\text{L}$  of the MSH solution at 4  $^{\circ}\text{C}$ . The reaction was shaken at 4  $^{\circ}\text{C}$  and then analyzed by LCMS. A mixture NH<sub>2</sub> adducts were observed at both pH values. Fewer NH<sub>2</sub> groups were observed at pH 6.0. ESI-MS are shown below.

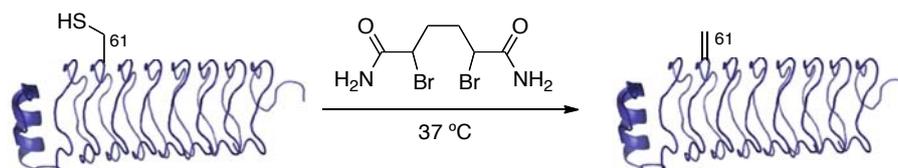
**pH 8.0:****pH 6.0:**

**Figure S59.** ESI-MS spectra of the reaction of Np276 Cys61 with MSH at pH 8.0 and pH 6.0, respectively.

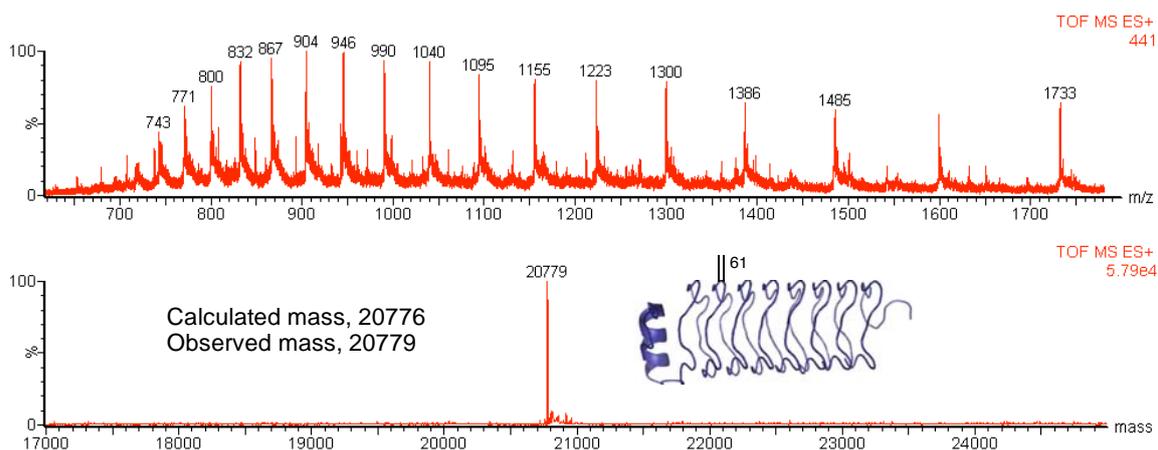
MSH was not suitable for selectively converting cysteine to dehydroalanine on NP276 at position 61. An alternative method was used that is adapted from a recent dialkylation, elimination of cysteine to dehydroalanine reported by Kajihara.<sup>22</sup> A full account of dehydroalanine synthesis on protein substrates, including the use of dibromide, is forthcoming.<sup>23</sup>

**Synthesis of dibromide reagent**

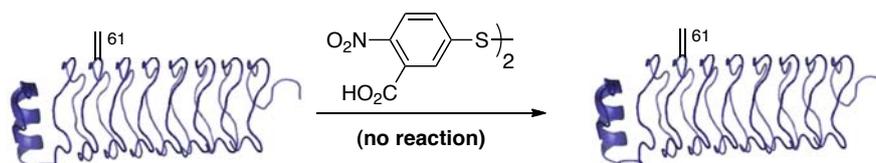
$\alpha,\alpha'$ -Di-bromo-adipyl(bis)amide was prepared using a procedure adapted from the literature.<sup>24, 25</sup> Adipic acid (25.00 g, 171.1 mmol) was added to a 500 mL round bottom flask and suspended in thionylchloride (75.0 mL, 1034 mmol). The flask was equipped with a condenser and the reaction was heated to reflux (open to air, bath temp 80 °C). After 30 minutes at reflux, all adipic acid had dissolved. The reaction was stirred for an additional 60 minutes at reflux and then cooled to room temperature. CCl<sub>4</sub> (100 mL) was added to the reaction followed by NBS (73.1 g, 410.7 mmol). The reaction was stirred vigorously and 10 drops of HBr (48% aq) was added by pipette. The reaction was heated to reflux, again open to air. The reaction gradually turns from red to black over the course of an hour. After 2 hours at reflux, the reaction was cooled to room temperature and then to 0 °C. The mixture was stirred at 0 °C to ensure all succinimide had precipitated. The solid was removed by filtration. Et<sub>2</sub>O (50 mL) was used to rinse and complete the filtration. The filtrate was concentrated under reduced pressure to give a thick, dark red liquid. In a 500 mL round bottom flask, 200 mL of NH<sub>4</sub>OH (25% aq.) was cooled to 0 °C. The crude acid chloride was added dropwise over 20 minutes to the ammonia solution with rapid stirring. After the addition was complete, the reaction was stirred vigorously at 0 °C for 1 hour. The bis-amide product precipitated from the reaction mixture. The dark solid was isolated by filtration and partially dried. The product was purified by triturating in MeOH/H<sub>2</sub>O: The solid was suspended in H<sub>2</sub>O (100 mL) and MeOH (100 mL) and heated to 60 °C. The mixture was stirred rapidly at 60 °C for 30 minutes. After this time, the mixture was cooled to room temperature. The resulting white solid (a mixture of *meso* and *d/l* diastereomers) was isolated by filtration and washed with MeOH (200 mL). The product was dried under high vacuum (25.75 g, 50%). IR ( $\nu_{\max}$ , KBr): 3302, 2946, 2801, 1684, 1418, 1319, 1277, 1250, 1220, 1194, 978, 876. <sup>1</sup>H NMR (400 MHz, DMSO):  $\delta$  = 1.75-2.08 (4H, m, CH<sub>2</sub>CH<sub>2</sub>), 4.28-4.36 (2H, m, 2 × CHBr), 7.30 (2H, s), 7.69 (2H, s) (2 × NH<sub>2</sub>). <sup>13</sup>C NMR (100 MHz, DMSO): (both diastereomers reported),  $\delta$  = 32.5, 32.6 (CH<sub>2</sub>CHBr), 48.2, 48.5 (CH<sub>2</sub>CHBr), 169.87, 169.92 (C=O). LRMS m/z (ESI<sup>+</sup>): 324.9 [M+Na]<sup>+</sup>.



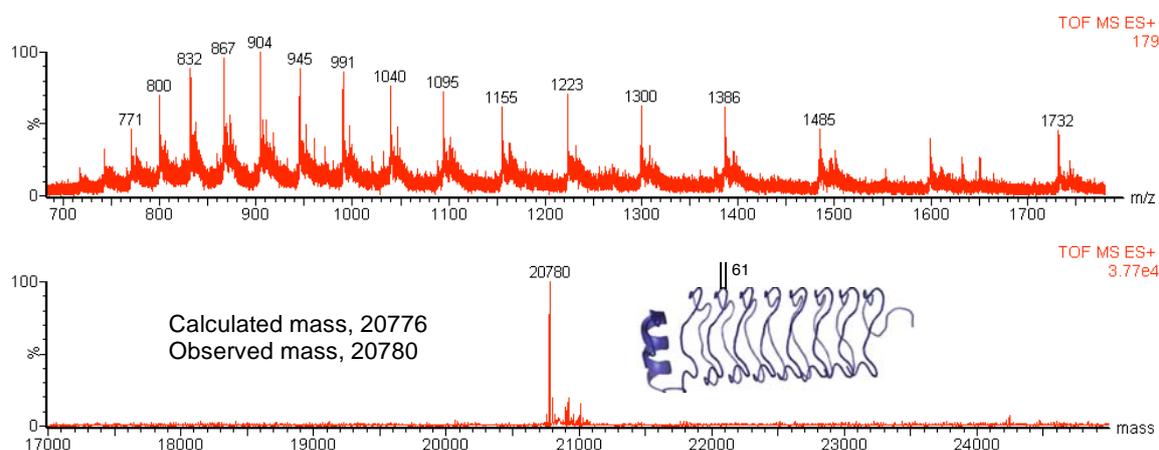
Np276 Cys61 (2.5 mL, 1.1 mg / mL in pH 8.0 sodium phosphate, 50 mM) was reduced by the addition of 10 mg DTT. The solution was shaken at room temperature for 15 minutes to reduce and contaminant disulfide. After this time, the protein solution was passed through a PD10 column (GE Healthcare), eluting with the same buffer. The resulting protein solution was used immediately. Accordingly, 2.5 mL of the Np276 protein ( $c = 0.79$  mg/mL) was added to a 15 mL plastic tube and kept at room temperature. A stock solution of di-bromide was prepared by dissolving 218 mg in 1.91 mL DMF. A 125  $\mu$ L aliquot of the di-bromide solution was added to the protein solution and the reaction was vortexed to homogenize and immediately placed in a 37 °C incubator with shaking. After 15 minutes, an additional 125  $\mu$ L aliquot of the di-bromide solution was added to the reaction. After another 15 minutes, a third and final 125  $\mu$ L aliquot of the di-bromide solution was added. The reaction was shaken at 37 °C for a final 30 minutes and then analyzed by LCMS. A protein mass corresponding to the Dha protein was observed (20776 calculated, 20779 found). Precipitated di-bromide was removed by centrifugation and the protein solution was stored on ice. ESI-MS are shown below.



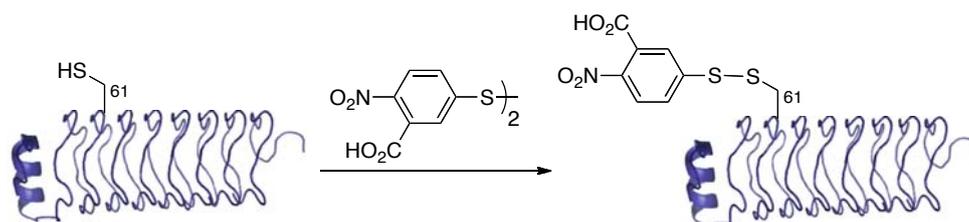
**Figure S60.** ESI-MS spectrum of Np276 Dha61.



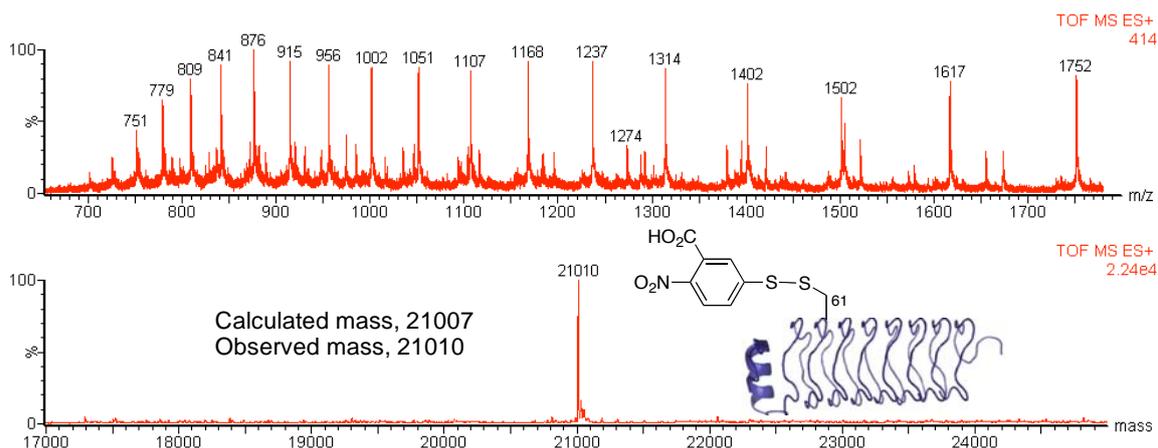
A stock solution of Ellman's Reagent was prepared by dissolving 0.5 mg in 250  $\mu\text{L}$  of sodium phosphate buffer (pH 8.0, 50 mM). A 140  $\mu\text{L}$  aliquot of the dehydroalanine containing protein (0.79 mg/mL in pH 8.0 sodium phosphate buffer) was mixed with 10  $\mu\text{L}$  of the Ellman's solution. The solution was incubated at room temperature for 15 minutes and then analyzed by LCMS. No reaction was observed, indicating complete consumption of cysteine during the elimination reaction (20776 calculated, 20780 observed). ESI-MS are shown below. Under identical conditions, Np276 Cys61 is converted to the Ellman disulfide (see next).



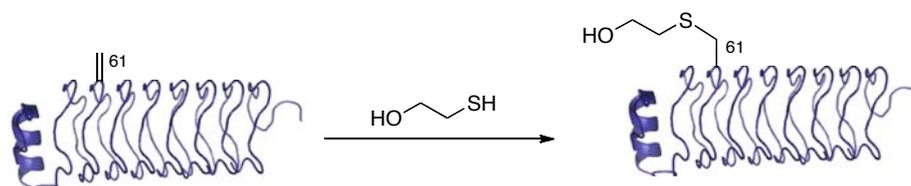
**Figure S61.** ESI-MS spectrum when Np276 Dha61 is incubated with Ellman's reagent – to confirm all Cys was consumed in the previous step.



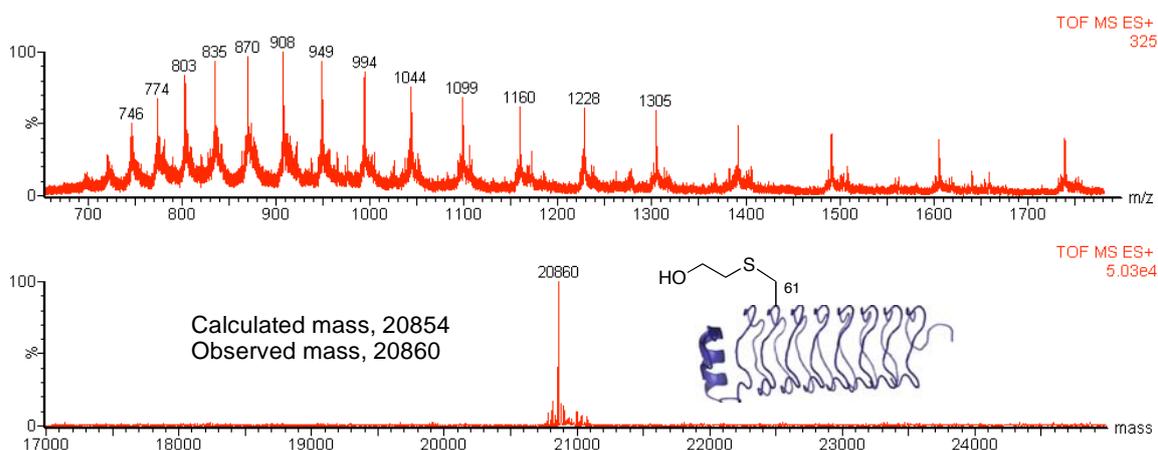
Np276 Cys61 (140  $\mu\text{L}$  of a 0.79 mg/mL solution in pH 8.0 sodium phosphate buffer, 50 mM) was mixed with 10  $\mu\text{L}$  of the Ellman's reagent solution prepared above. The reaction was shaken at room temperature for 15 minutes and then analyzed by LCMS. Full conversion to the Ellman disulfide was observed (21007 calculated, 21010 observed). ESI-MS are shown below.



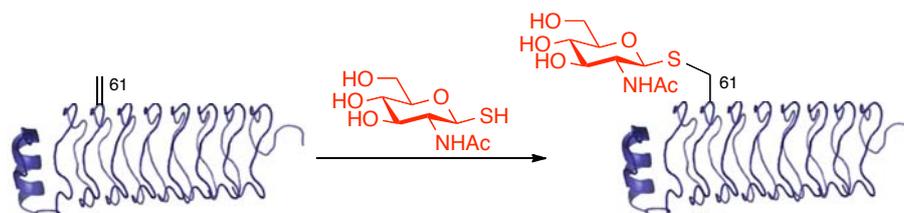
**Figure S62.** ESI-MS spectrum of when Np276 Cys61 is incubated with Ellman's reagent - to confirm presence of Cys.



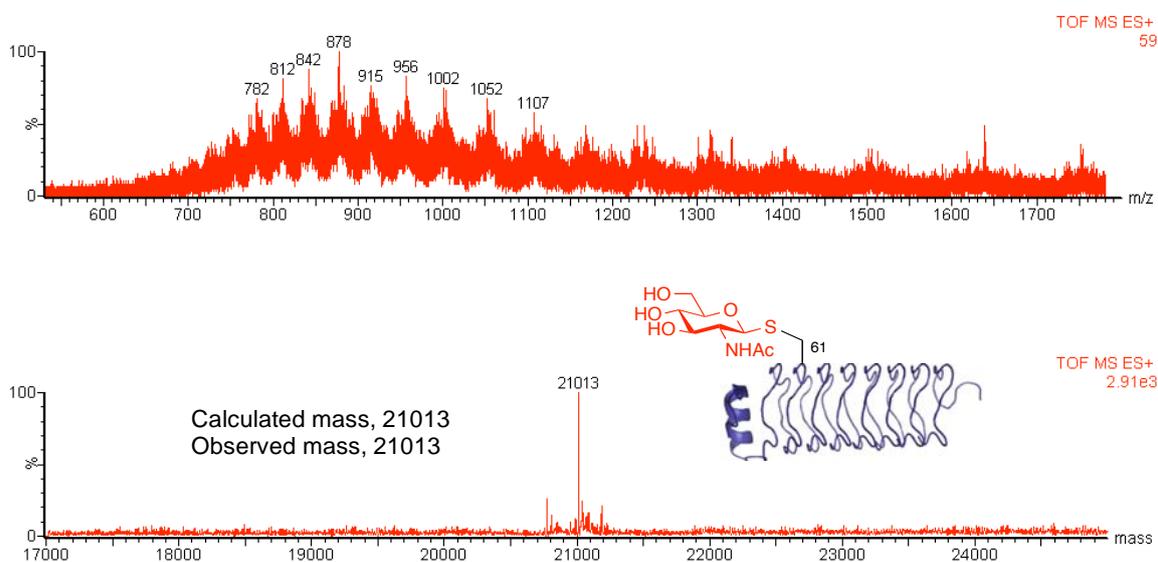
A 100  $\mu$ L aliquot of the Dha containing Np276 was mixed with 5  $\mu$ L of 2-mercaptoethanol. The reaction was shaken at 37  $^{\circ}$ C for 1 hour. After this time, full conversion to the thioether was observed by LCMS (20854 calculated, 20860 observed). ESI-MS are shown below.



**Figure S63.** ESI-MS spectrum of the addition of mercaptoethanol to Np276 Dha61 – to confirm the presence of Dha.



A 1.00 mL aliquot of the Dha containing Np276 ( $c = 0.79$  mg/mL) was mixed with 135 mg of thio-GlcNAc. The reaction was shaken at 37 °C for 2 hours. After this time, small molecules were removed by dialysis ( $2 \times 4$ L pH 7.0 sodium phosphate, 50 mM). Full conversion to the thioether was observed by LCMS (21013 calculated, 21013 observed). ESI-MS are shown below.



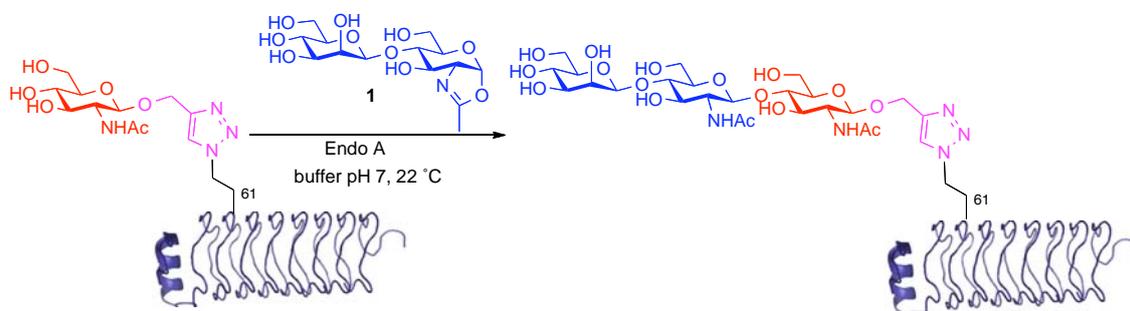
**Figure S64.** ESI-MS spectrum of GlcNAc-S-61Np276 (**27a**).

## 2.7.4 Endo-A enzymatic glycosylation on Np276 substrates

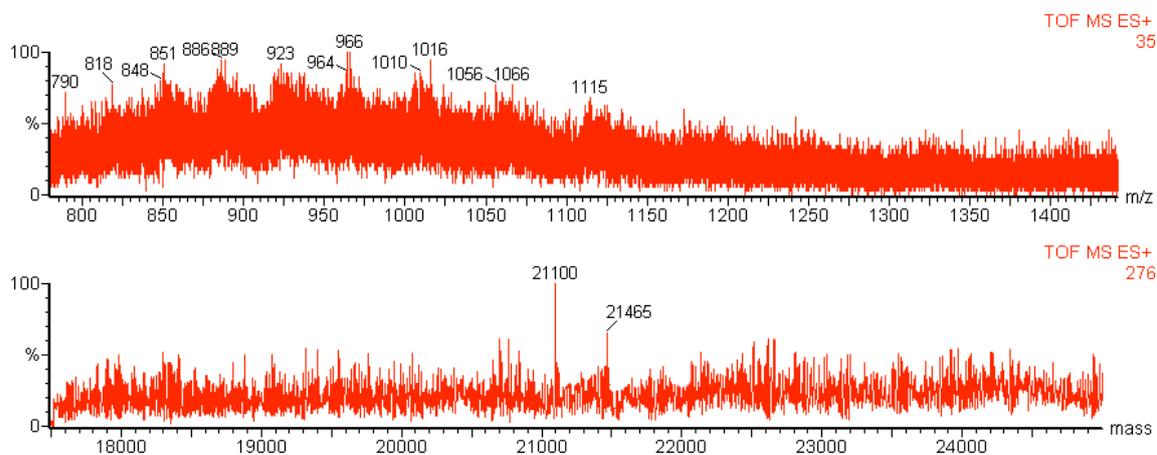
### General procedure for the Endo-A enzymatic glycosylation on Np276 substrates

To a solution of GlcNAc-OCH<sub>2</sub>-*triazole*-61Np276 **25a** (150  $\mu$ L of 0.5 mg/mL, 3.614 nmol) in 50 mM sodium phosphate buffer (pH 7.0), disaccharide oxazoline **1** (370  $\mu$ g, 1.012  $\mu$ mol) and Endo A (10 milliunits) were added and the mixture was rotated on a lab rotisserie at 22  $^{\circ}$ C. The reaction was analyzed directly by LC-MS.

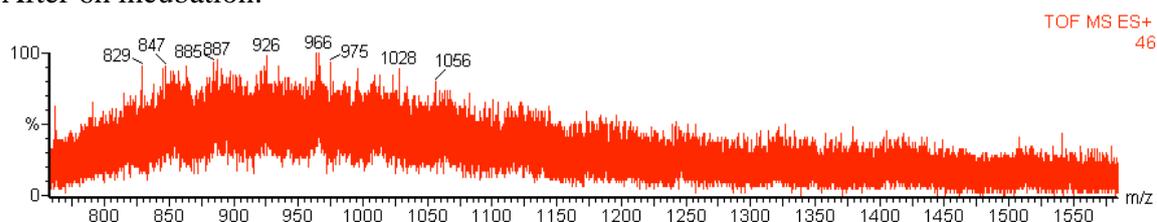
### **Endo A-catalyzed glycosylation of GlcNAc-OCH<sub>2</sub>-*triazole*-61NP276 **25a** (0.34 mg/mL)**

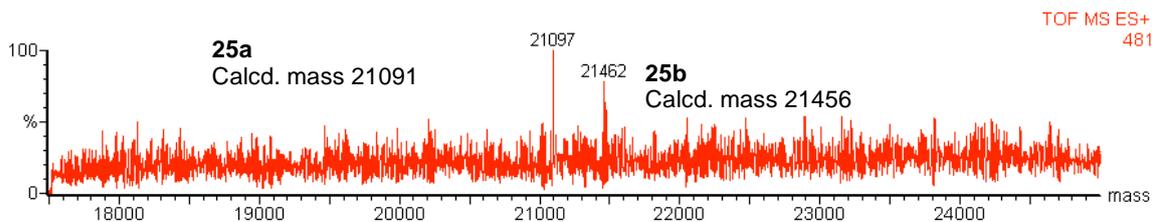


After 2h incubation:



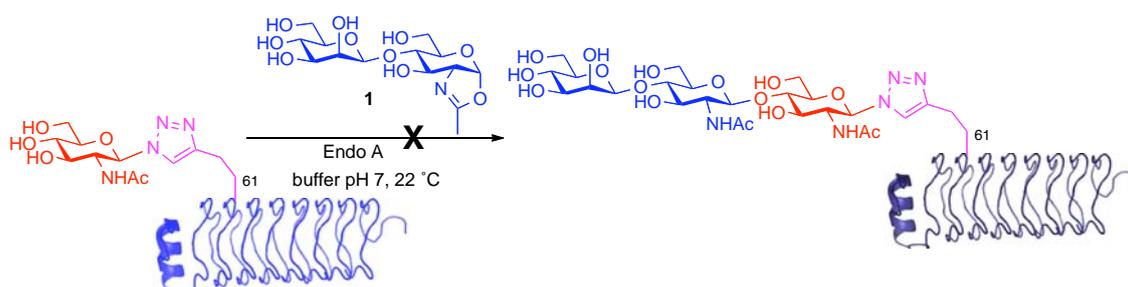
After 6h incubation:



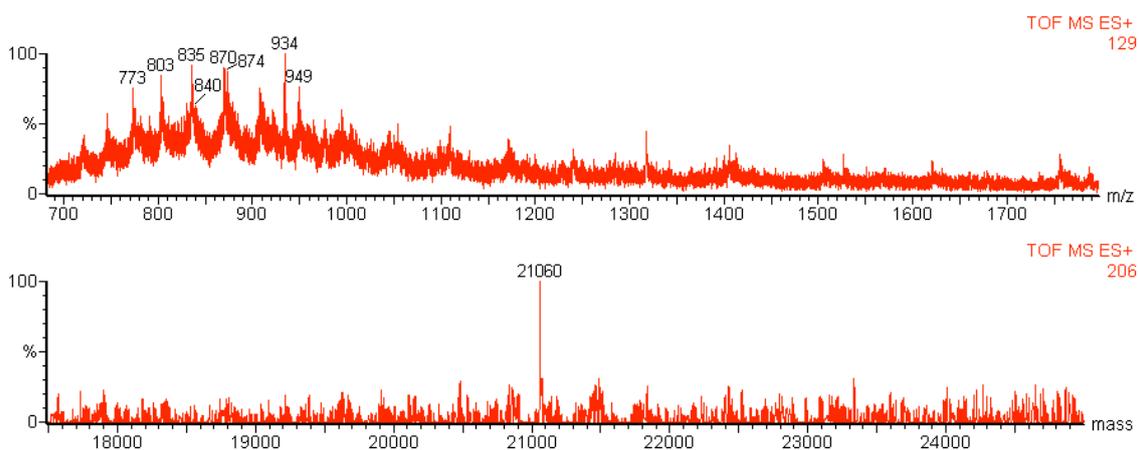


**Figure S65.** ESI-MS spectra after 2 and 6 h incubation of **25a** with Endo A and oxazoline **1**.

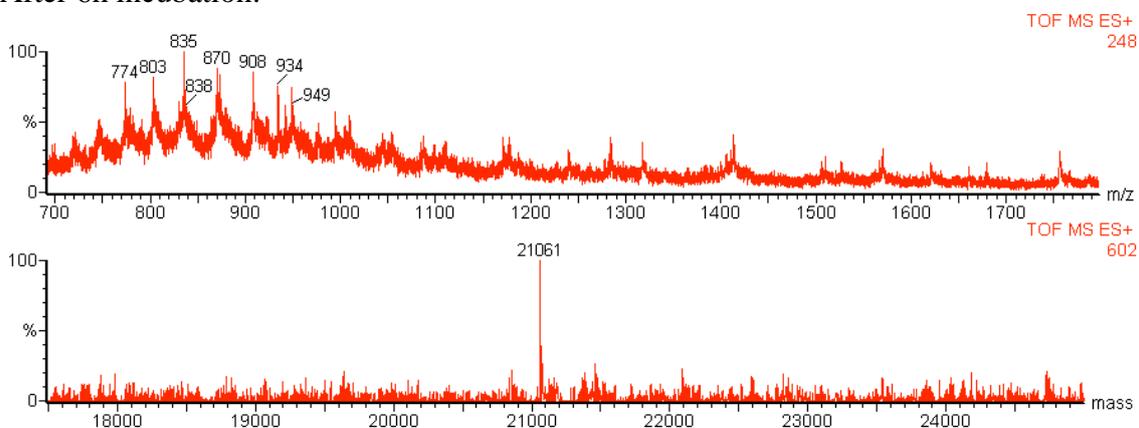
**Endo A-catalyzed glycosylation of GlcNAc-triazole-61NP276 **26a** (0.37 mg/mL)**



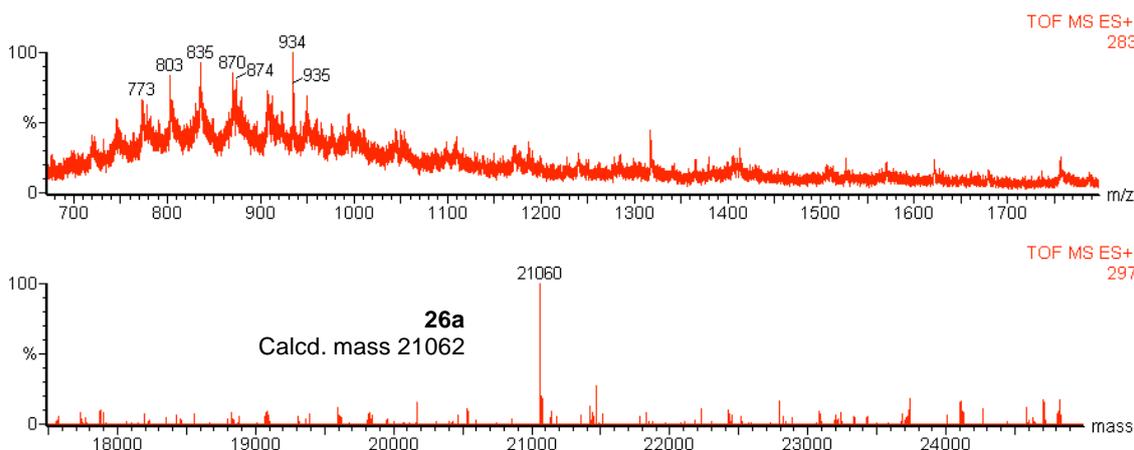
After 2h incubation:



After 6h incubation:

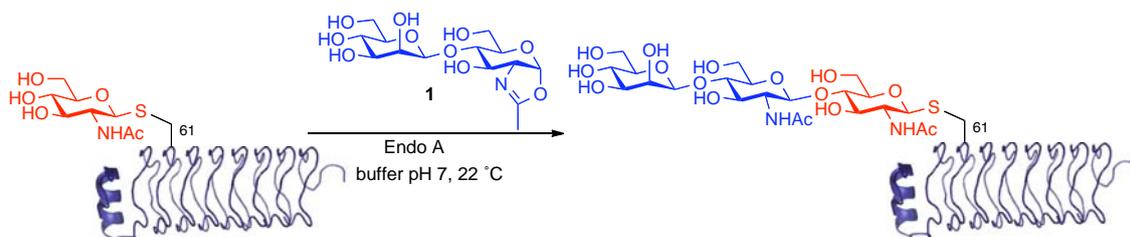


After 8h incubation:

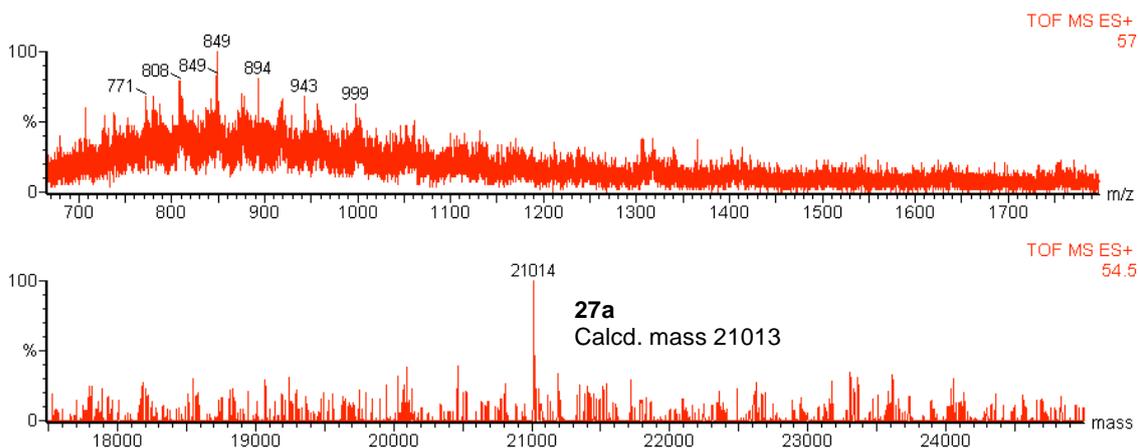


**Figure S66.** ESI-MS spectra after 2, 6 and 8 h incubation of **26a** with Endo A and oxazoline **1**.

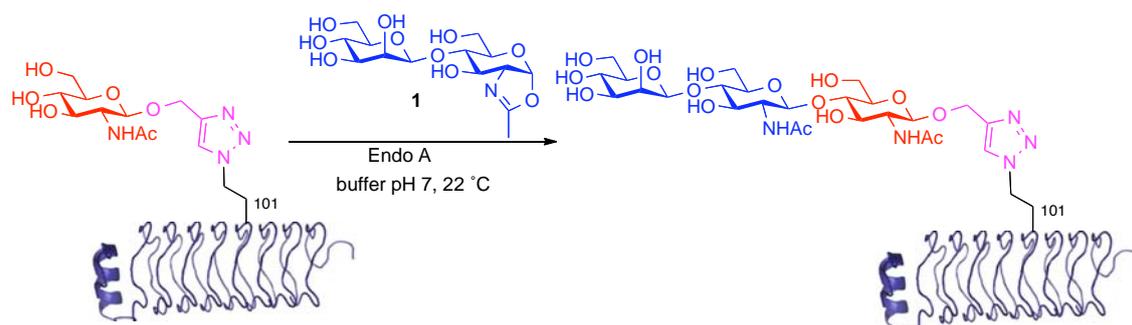
**Endo A-catalyzed glycosylation of GlcNAc-S-61NP276 27a (0.35 mg/mL)**



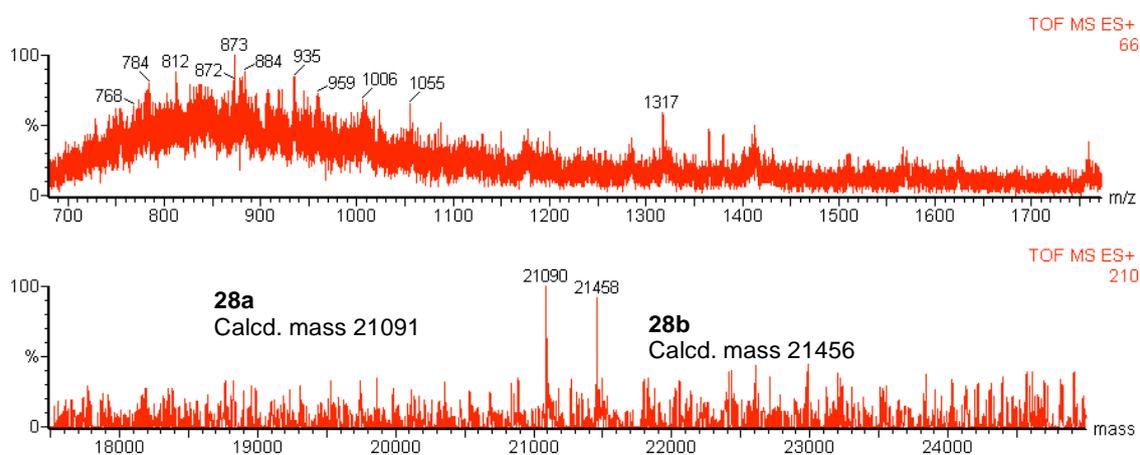
After 6h incubation:



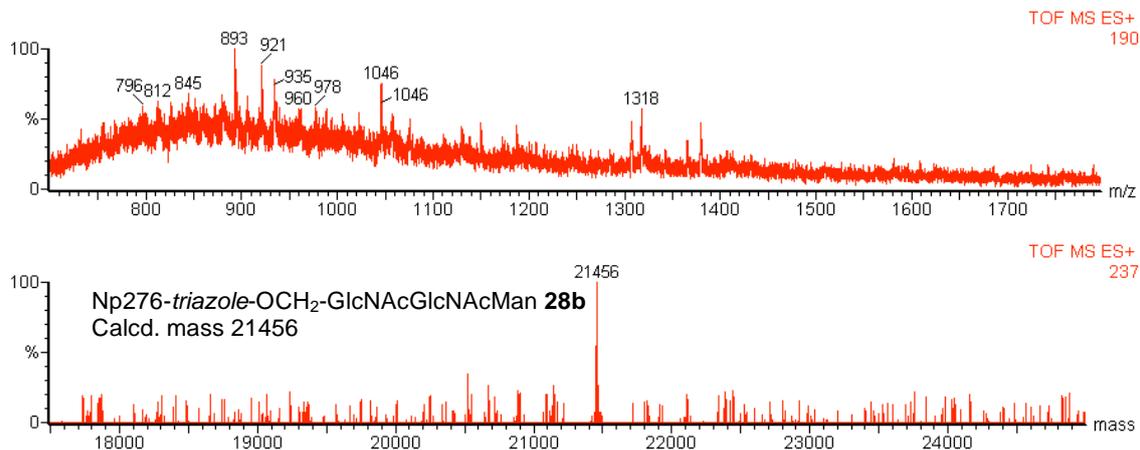
**Figure S67.** ESI-MS spectra after 6 h incubation of **27a** with Endo A and oxazoline **1**.

**Endo A-catalyzed glycosylation of GlcNAc-OCH<sub>2</sub>-triazole-101NP276 28a (0.22 mg/mL)**

After 2h incubation:



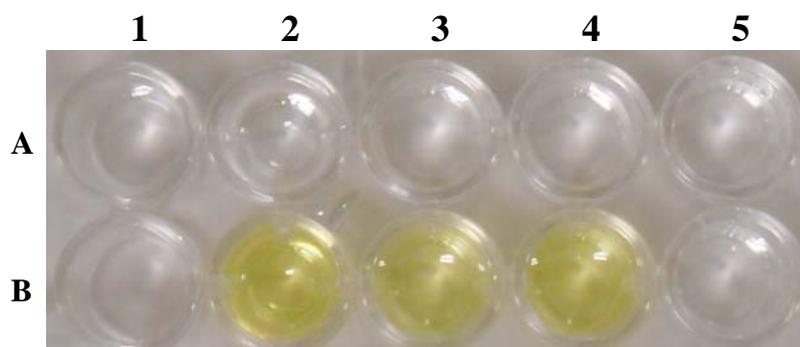
After 4h incubation:

**Figure S68.** ESI-MS spectra after 2 and 4 h incubation of **28a** with Endo A and oxazoline **1**.

## 2.8. Peptidase activity of modified glycoproteins

### S-linked Glycoproteins

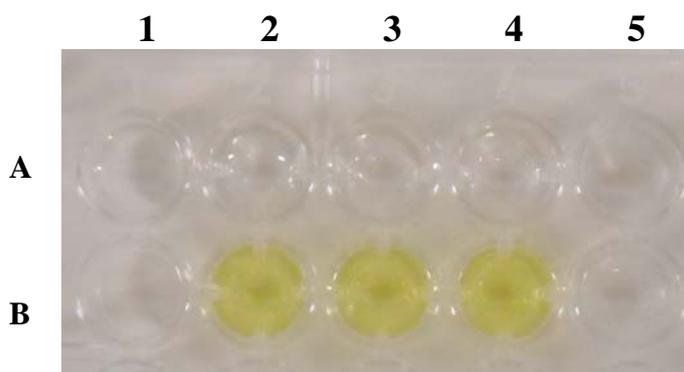
SBL-S156C (unmodified), SBL-156-S-GlcNAc **17a**, and SBL-156-S-GlcNAc-GlcNAc-Man **17b** were prepared at a concentration of 0.01 mg/mL in pH 8.0 sodium phosphate (50 mM). The sample of SBL-156-S-GlcNAc-GlcNAc-Man **17b** contained 0.11 µg/mL of EndoA from the enzymatic extension reaction. EndoA was separately prepared at a concentration of 0.11 µg/mL in the same buffer. 200 µL aliquots of each protein sample were added to a 96-well plate. A 2 µL aliquot of SucAAPFpNA (0.20 M in DMSO, Bachem) was added to each of the protein samples. All SBL samples turned yellow immediately upon addition of the peptide substrate. The yellow solution indicates liberation of *p*-nitroaniline (pNA), confirming peptidase activity of all SBL samples. The solution of EndoA and SucAAPFpNA was colorless. All protein solutions and the peptide solution alone at the same concentration are colorless (See below).



Well A1:	SucAAPFpNA
Well A2:	SBL-S156C
Well A3:	SBL-S-GlcNAc <b>17a</b>
Well A4:	SBL-S-GlcNAc-GlcNAc-Man <b>17b</b>
Well A5:	EndoA
Well B1:	(empty)
Well B2:	SBL-S156C + SucAAPFpNA
Well B3:	SBL-S-GlcNAc <b>17a</b> + SucAAPFpNA
Well B4:	SBL-S-GlcNAc-GlcNAc-Man <b>17b</b> + SucAAPFpNA
Well B5:	EndoA + SucAAPFpNA

**SeS-linked Glycoproteins**

SBL-S156C (unmodified), SBL-C156-SSeGlcNAc **22a**, and SBL-C156-SSe(GlcNAc)<sub>2</sub>Man **22b** were prepared at a concentration of 0.01 mg/mL in pH 8.0 sodium phosphate (50 mM). The sample of SBL-C156-SSe(GlcNAc)<sub>2</sub>Man **22b** contained 0.11 µg/mL of EndoA from the enzymatic extension reaction. EndoA was separately prepared at a concentration of 0.11 µg/mL in the same buffer. 200 µL aliquots of each protein sample were added to a 96-well plate. A 2 µL aliquot of SucAAPFpNA (0.20 M in DMSO, Bachem) was added to each of the protein samples. All SBL samples turned yellow immediately upon addition of the peptide substrate. The yellow solution indicates liberation of *p*-nitroaniline (pNA), confirming peptidase activity of all SBL samples. The solution of EndoA and SucAAPFpNA was colorless. All protein solutions and the peptide solution alone at the same concentration are colorless (See below).

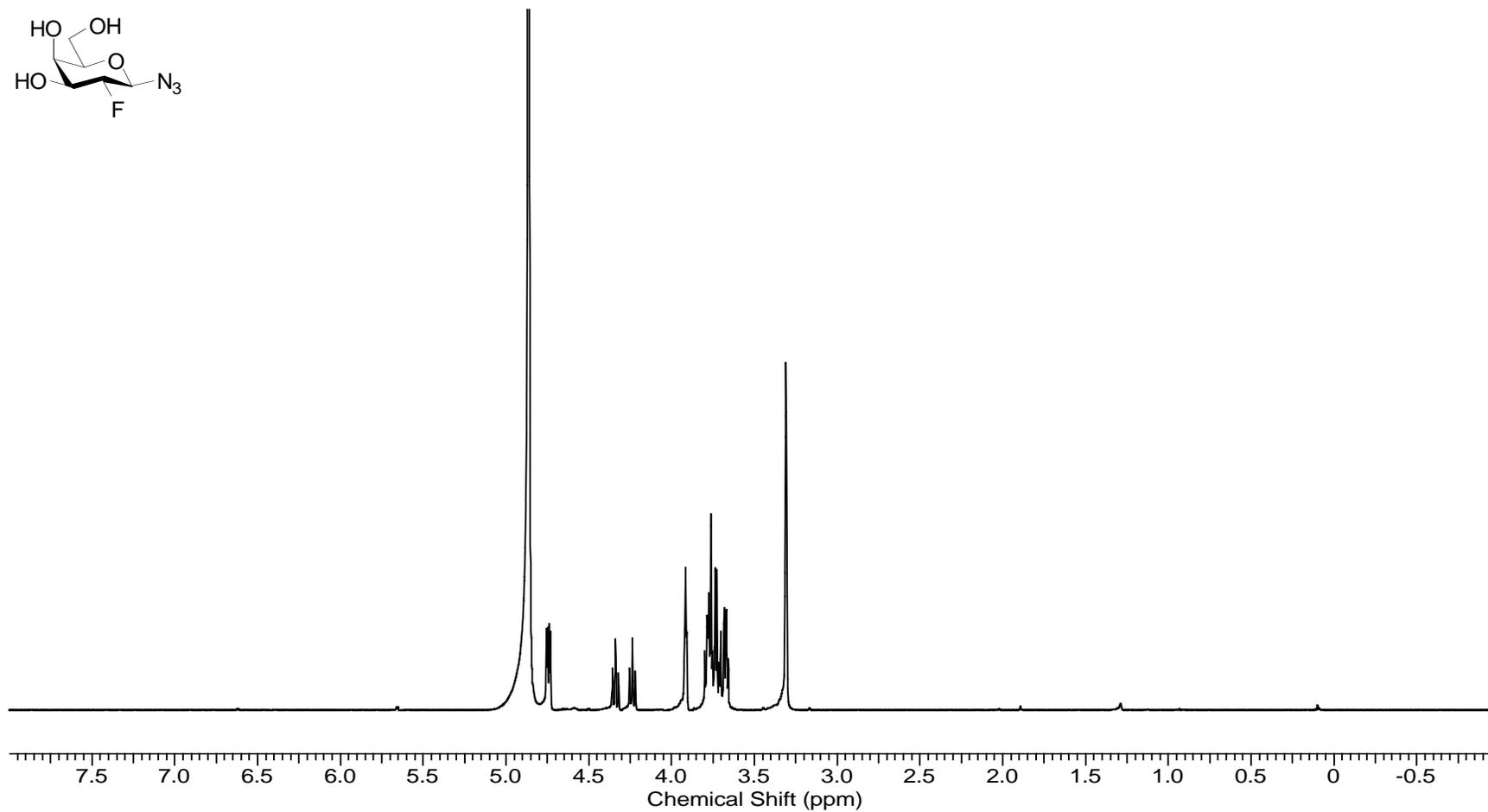


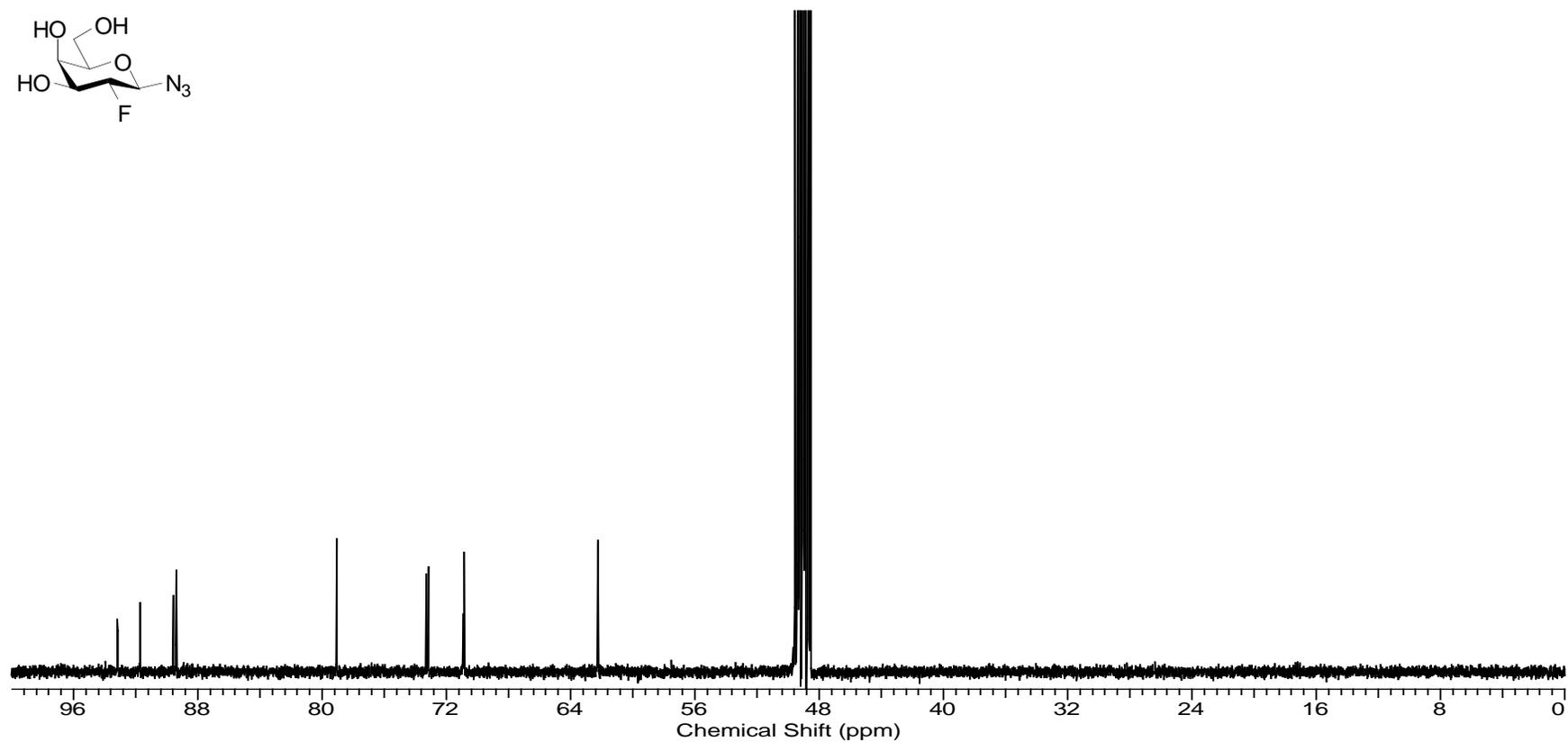
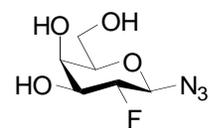
Well A1:	SucAAPFpNA
Well A2:	SBL-S156C
Well A3:	SBL-C156-SSeGlcNAc <b>17a</b>
Well A4:	SBL-C156-SSe(GlcNAc) <sub>2</sub> Man <b>17b</b>
Well A5:	EndoA
Well B1:	(empty)
Well B2:	SBL-S156C + SucAAPFpNA
Well B3:	SBL-C156-SSeGlcNAc <b>17a</b> + SucAAPFpNA
Well B4:	SBL-C156-SSe(GlcNAc) <sub>2</sub> Man <b>17b</b> + SucAAPFpNA
Well B5:	EndoA + SucAAPFpNA

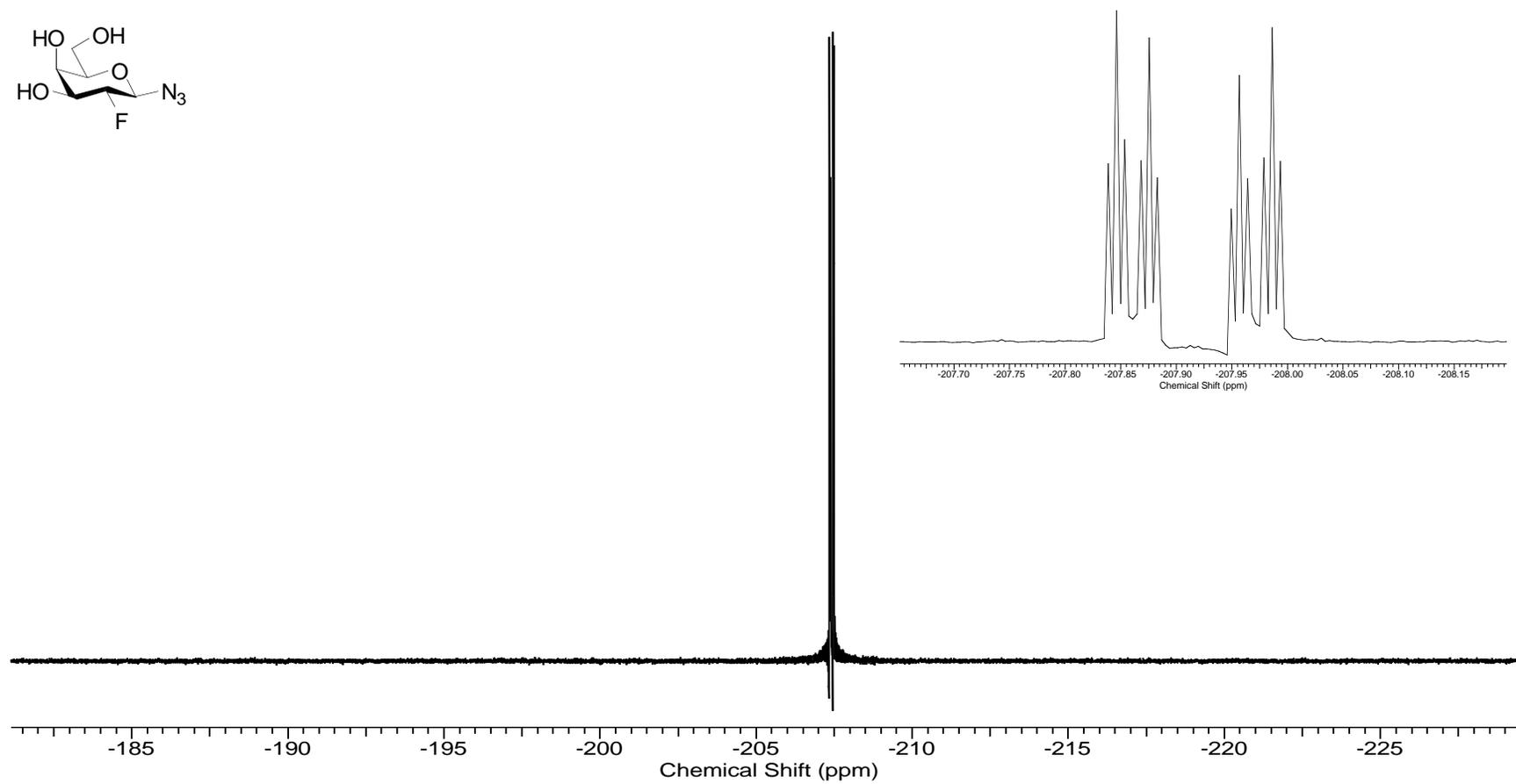
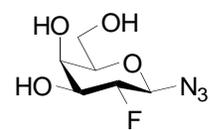
### 3. References

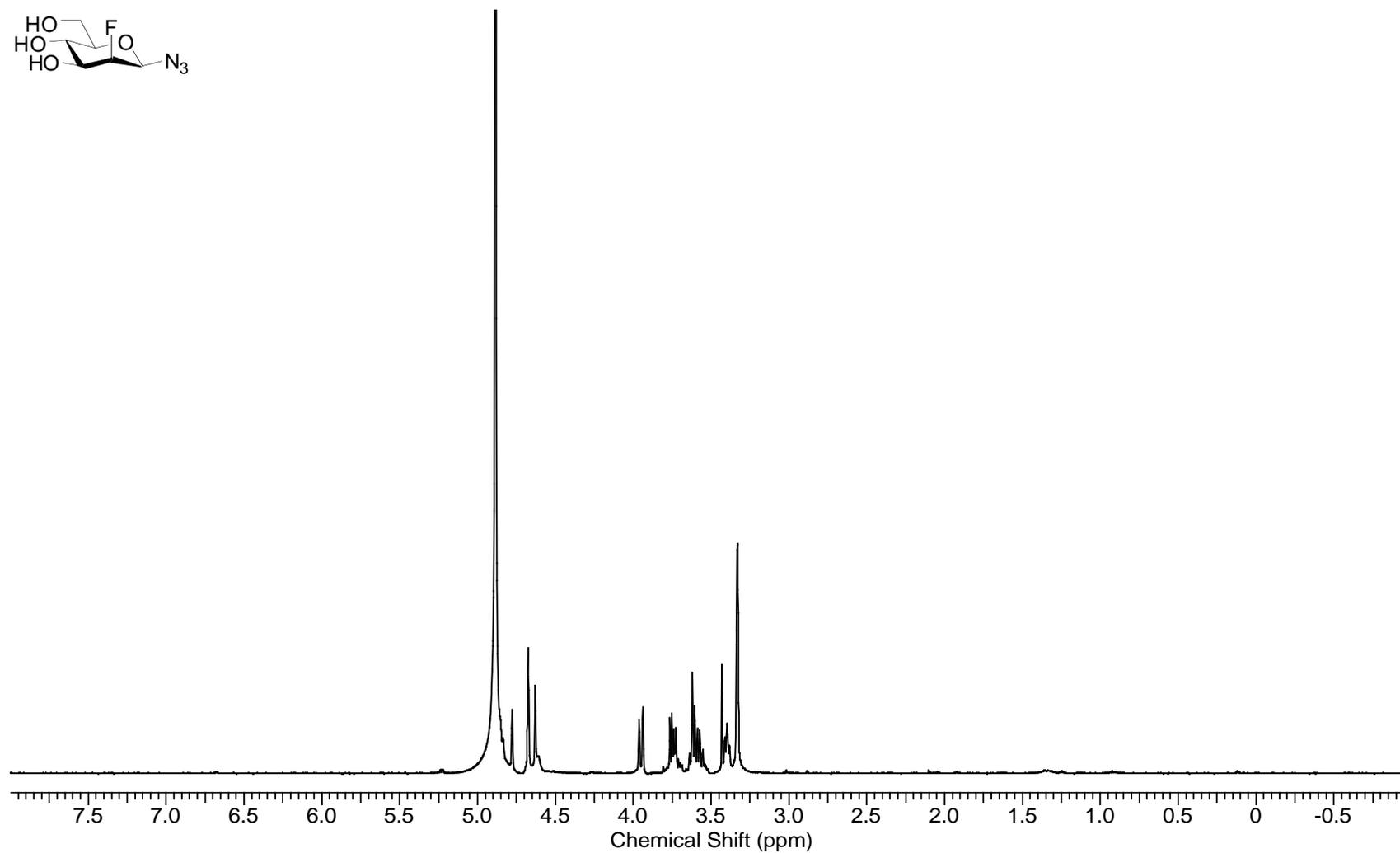
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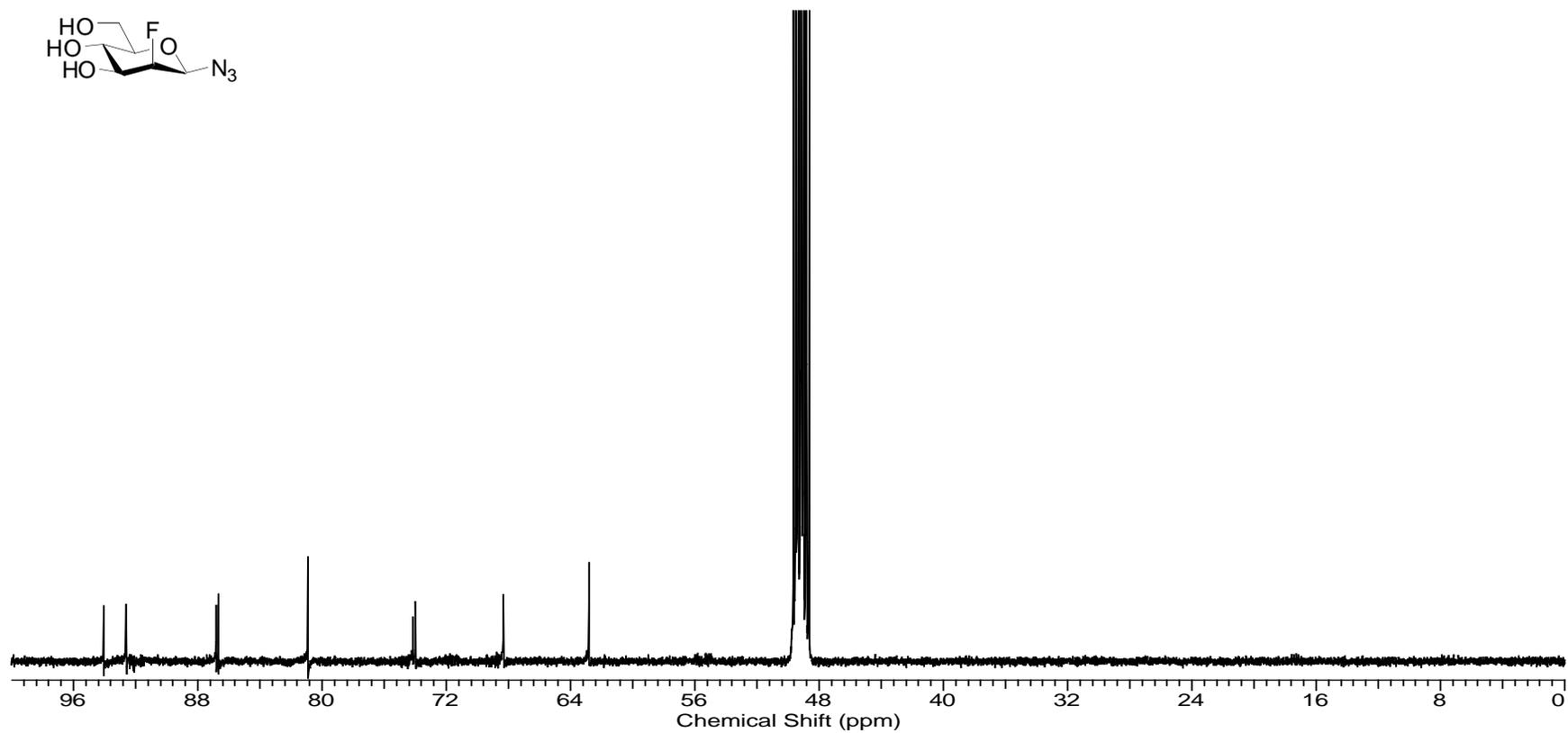
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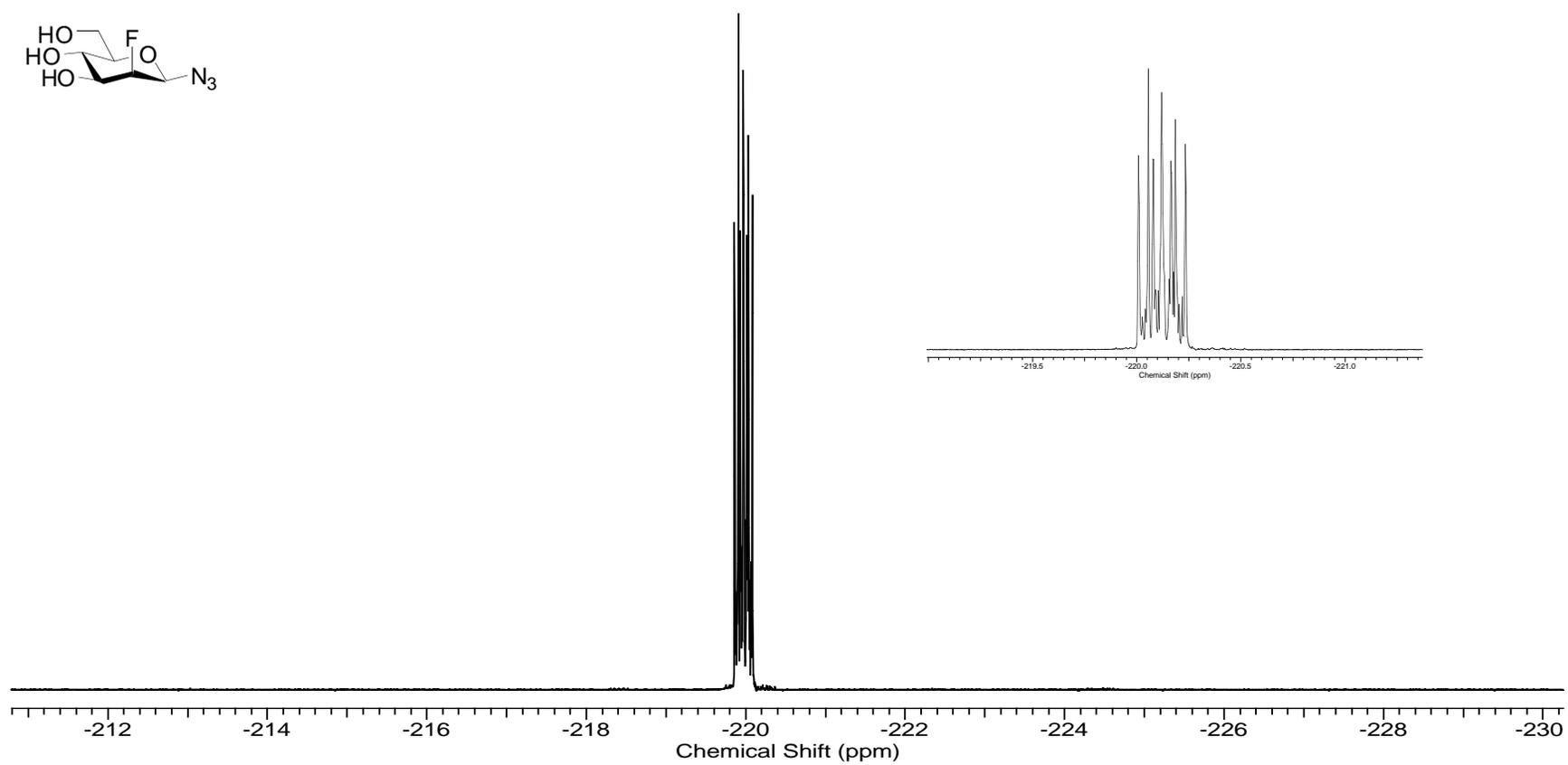


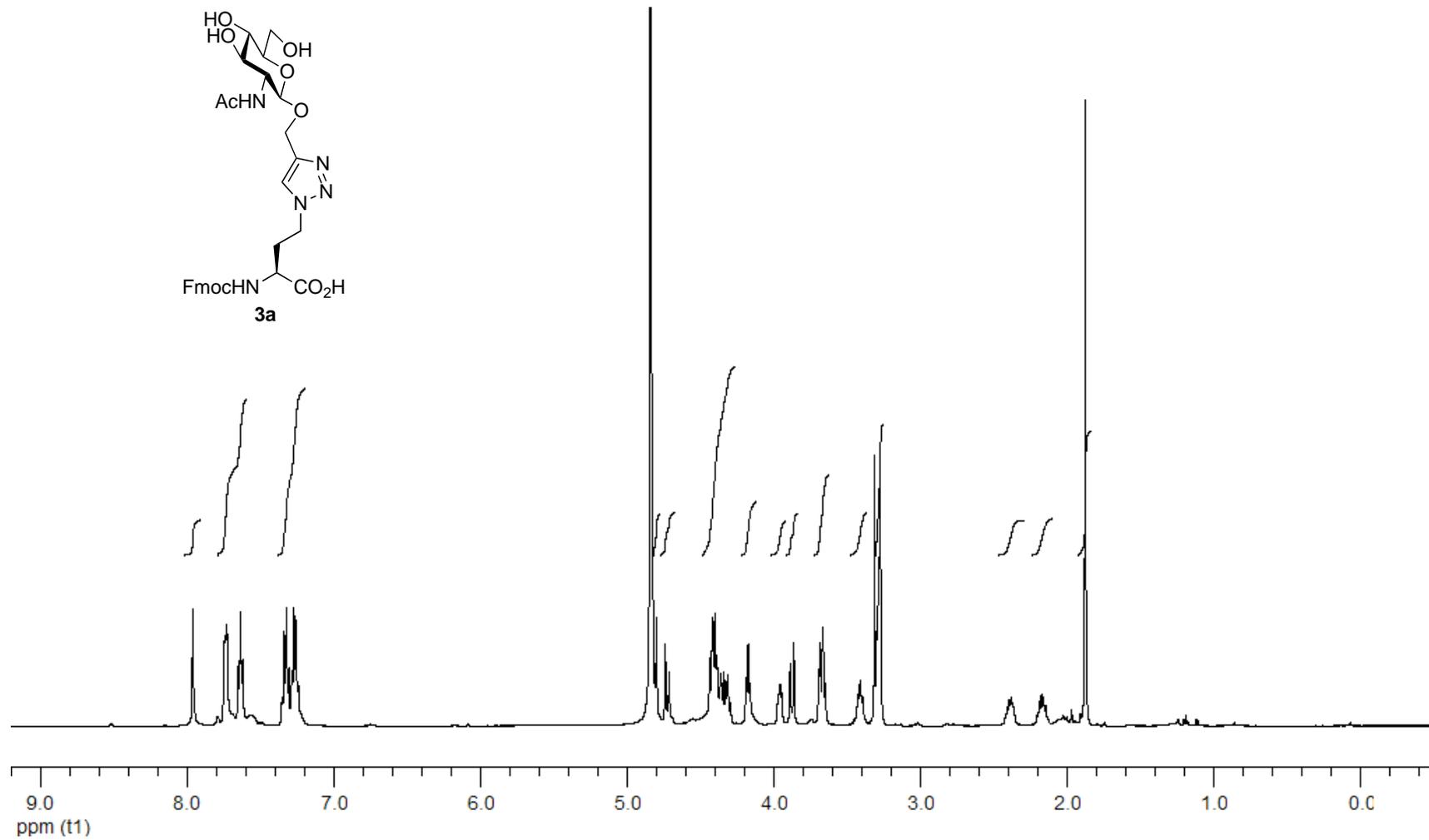
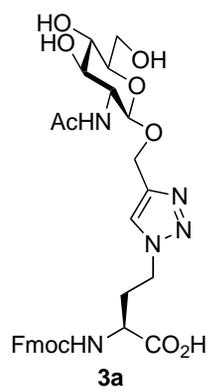


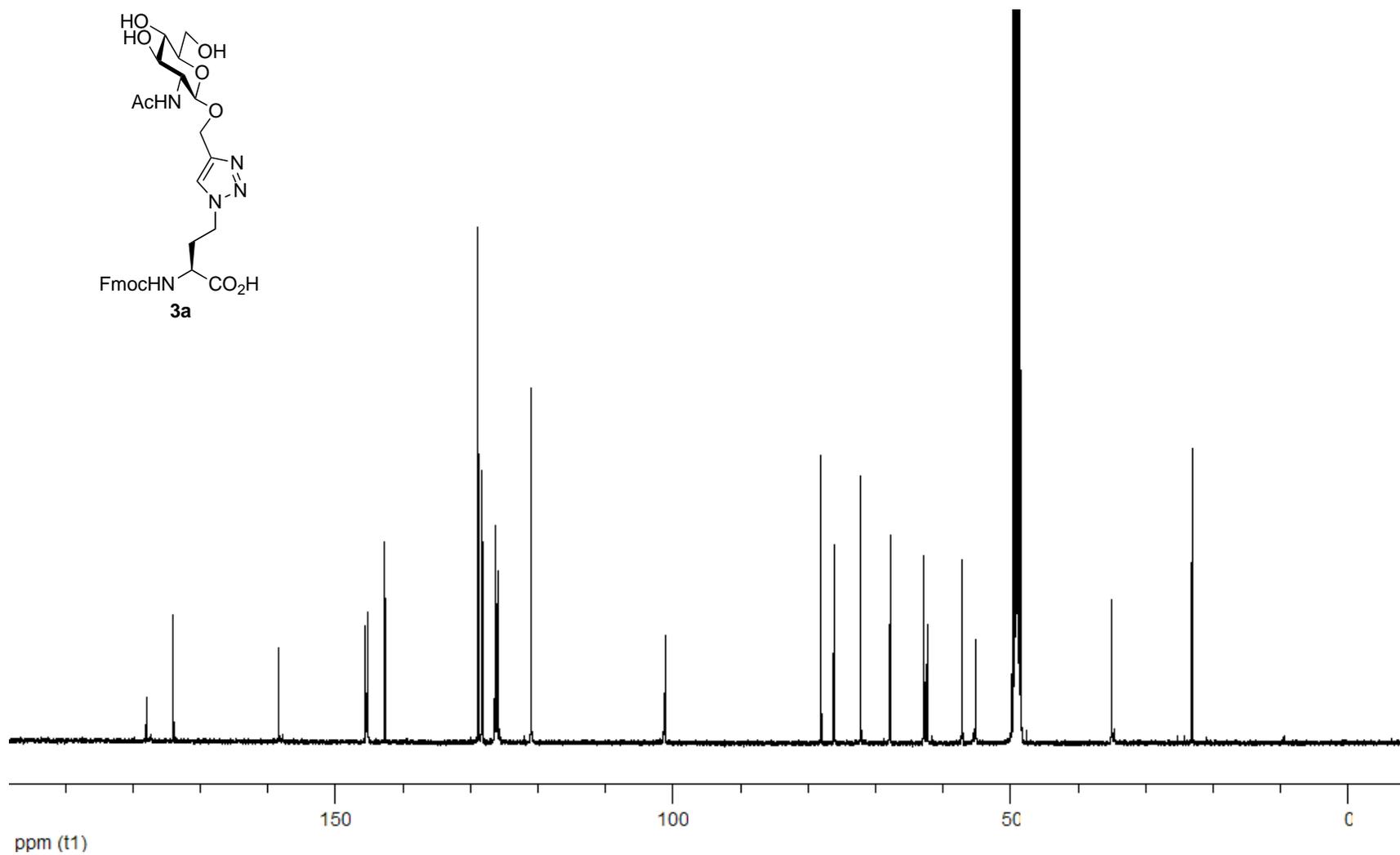
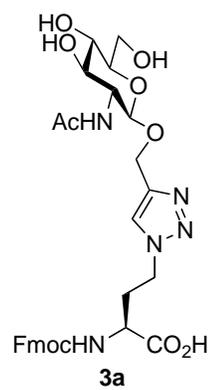


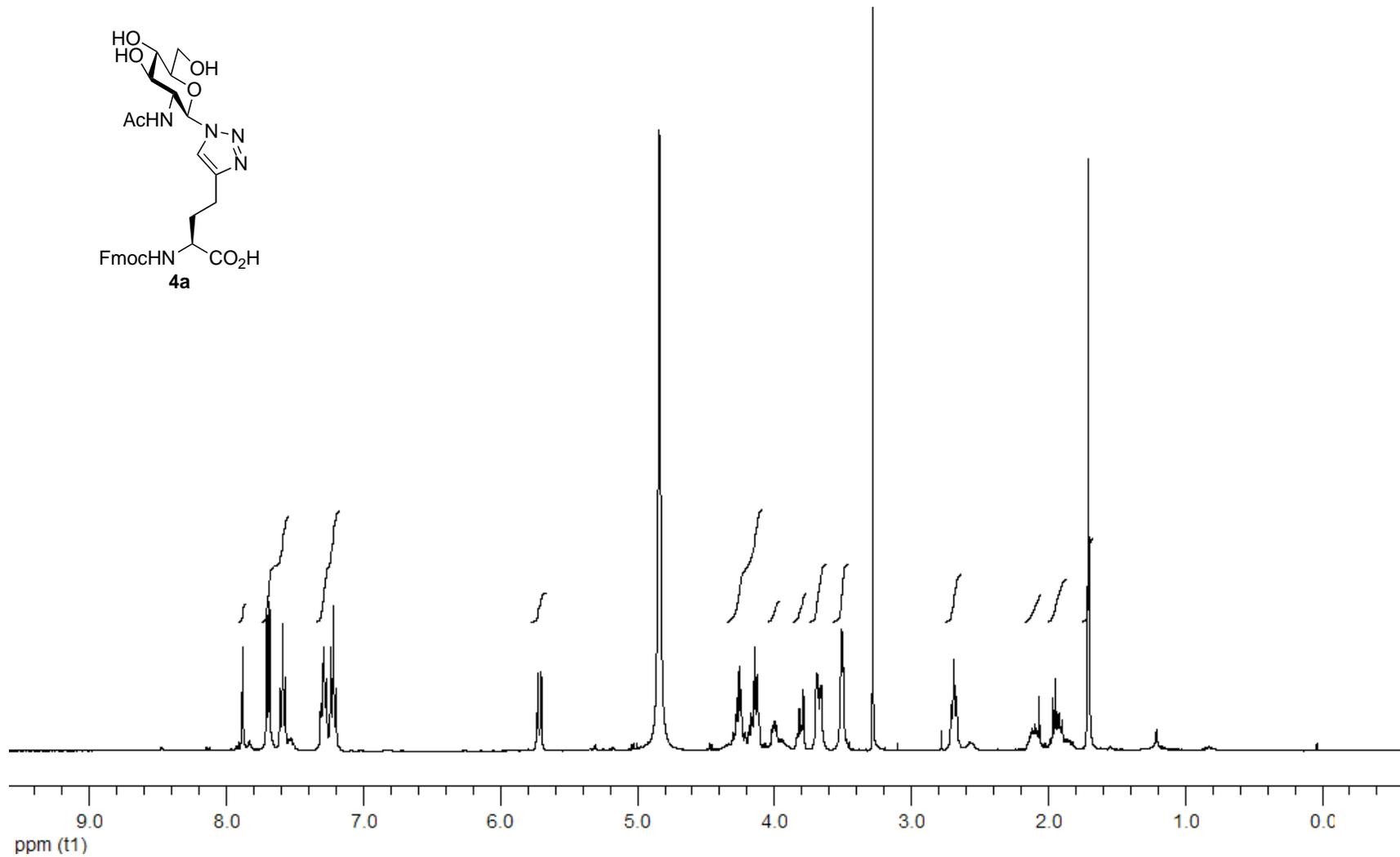
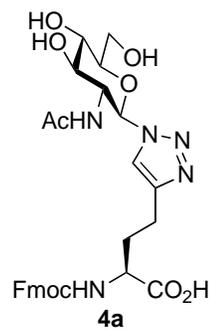


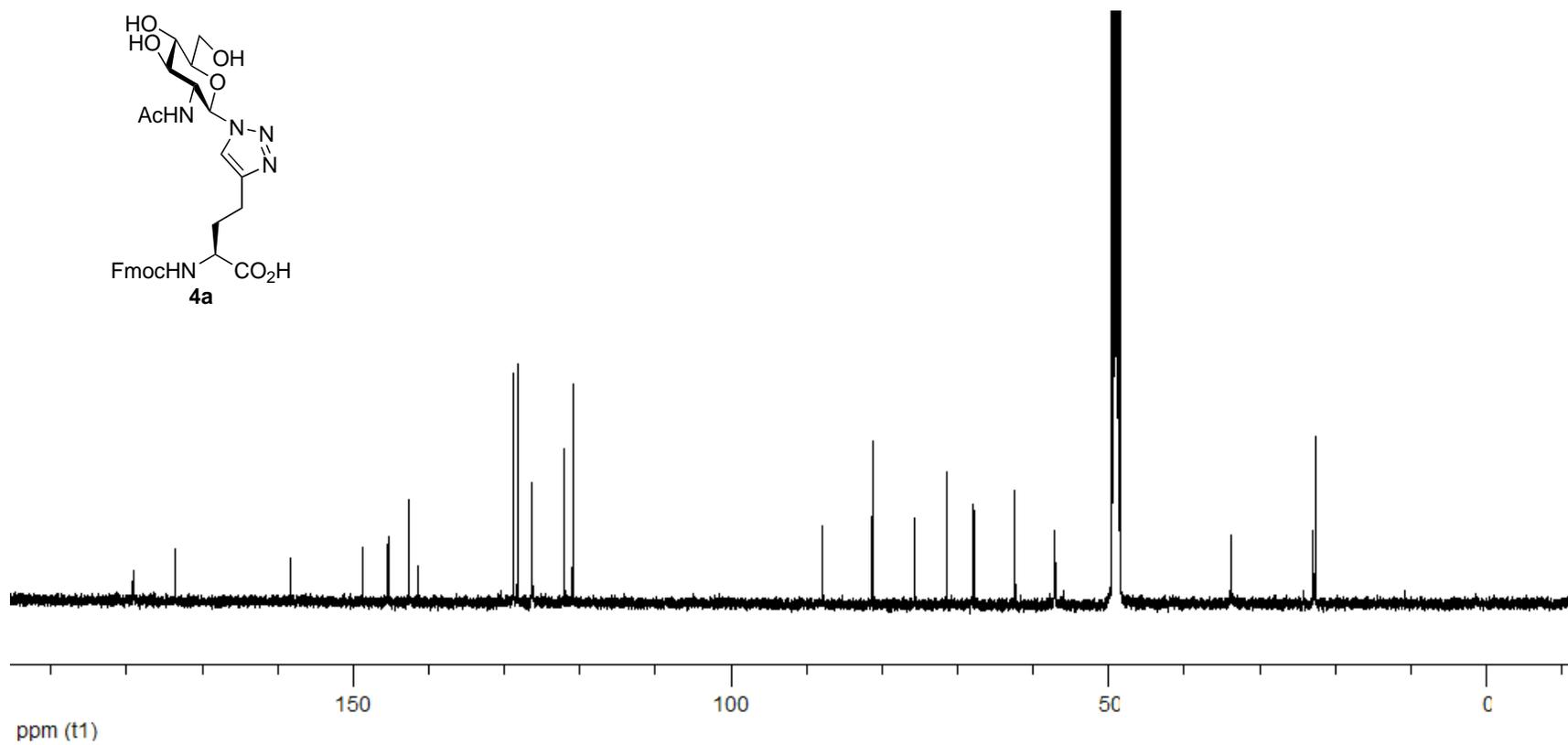
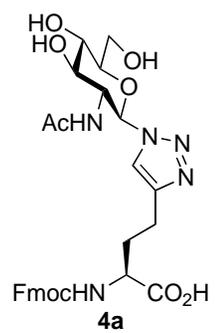


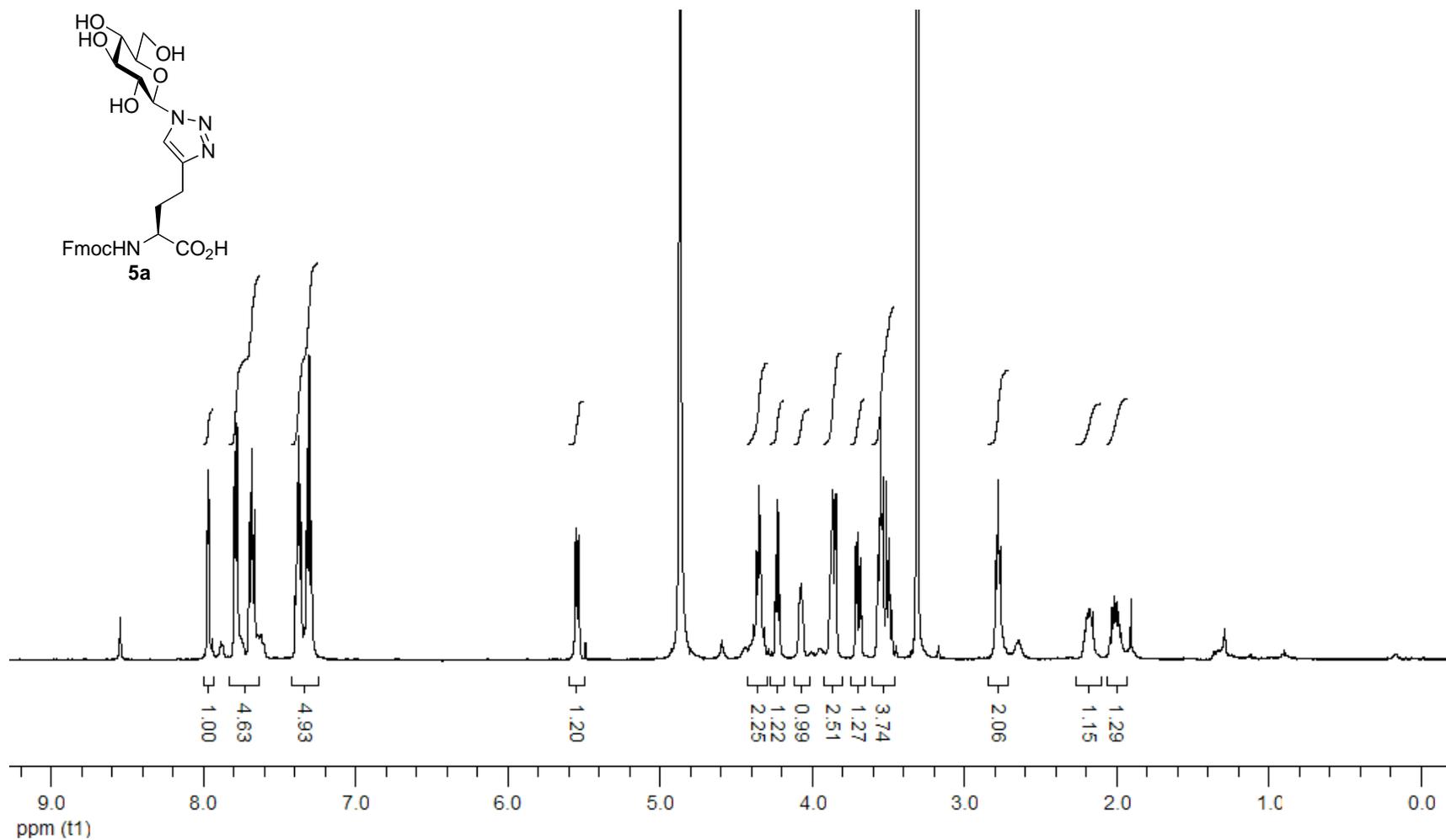


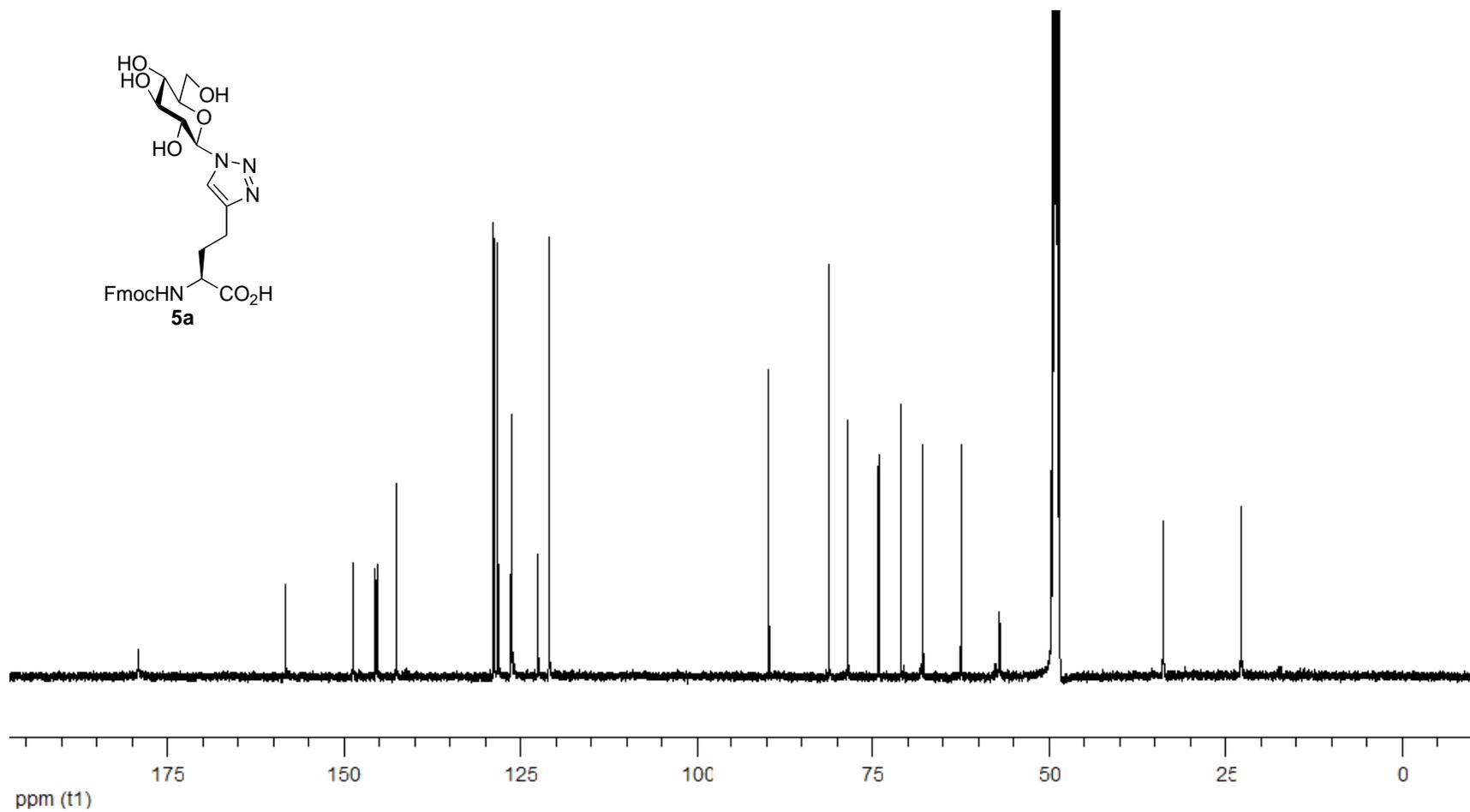


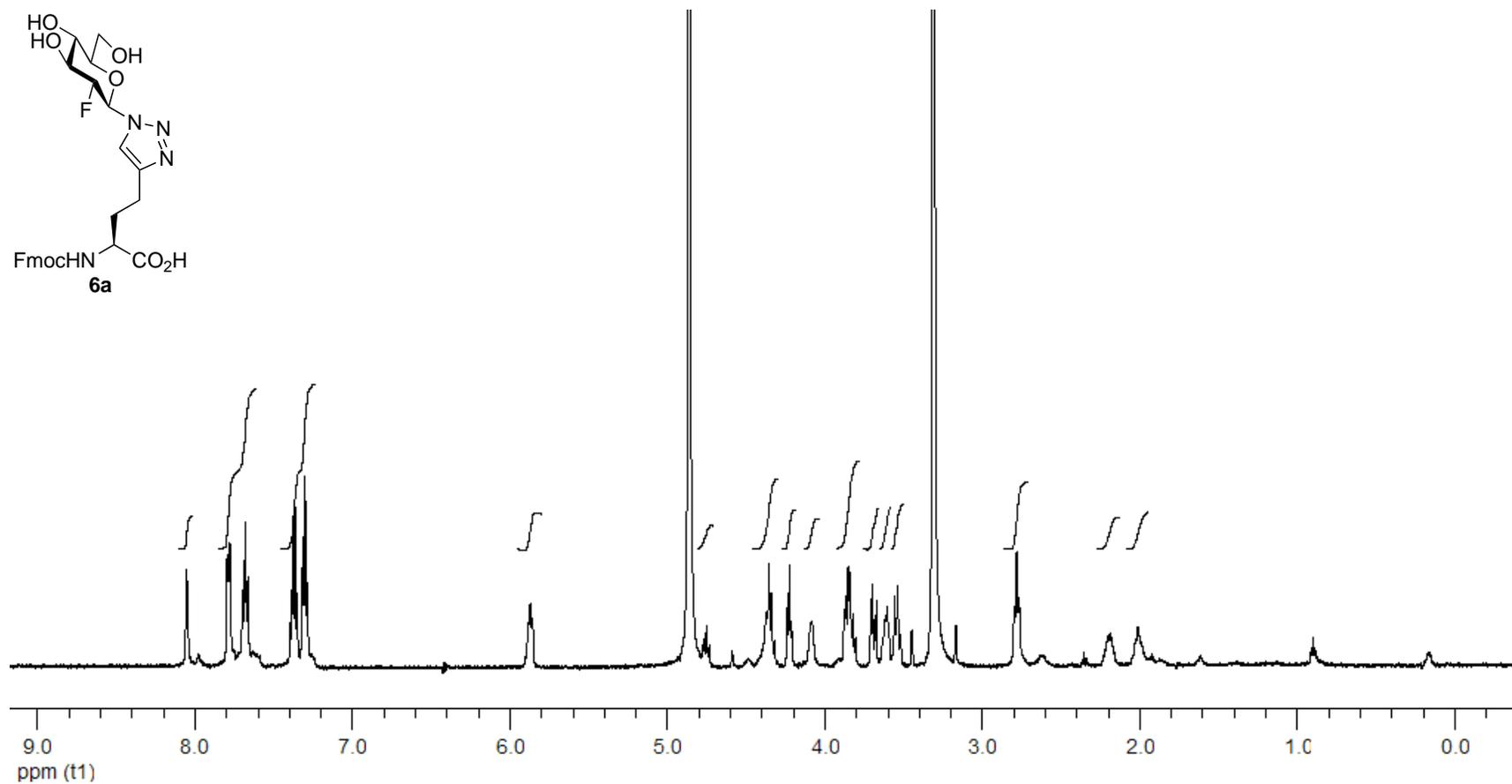
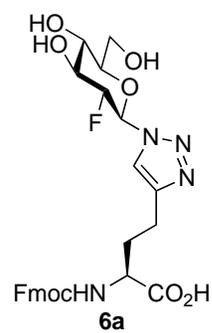


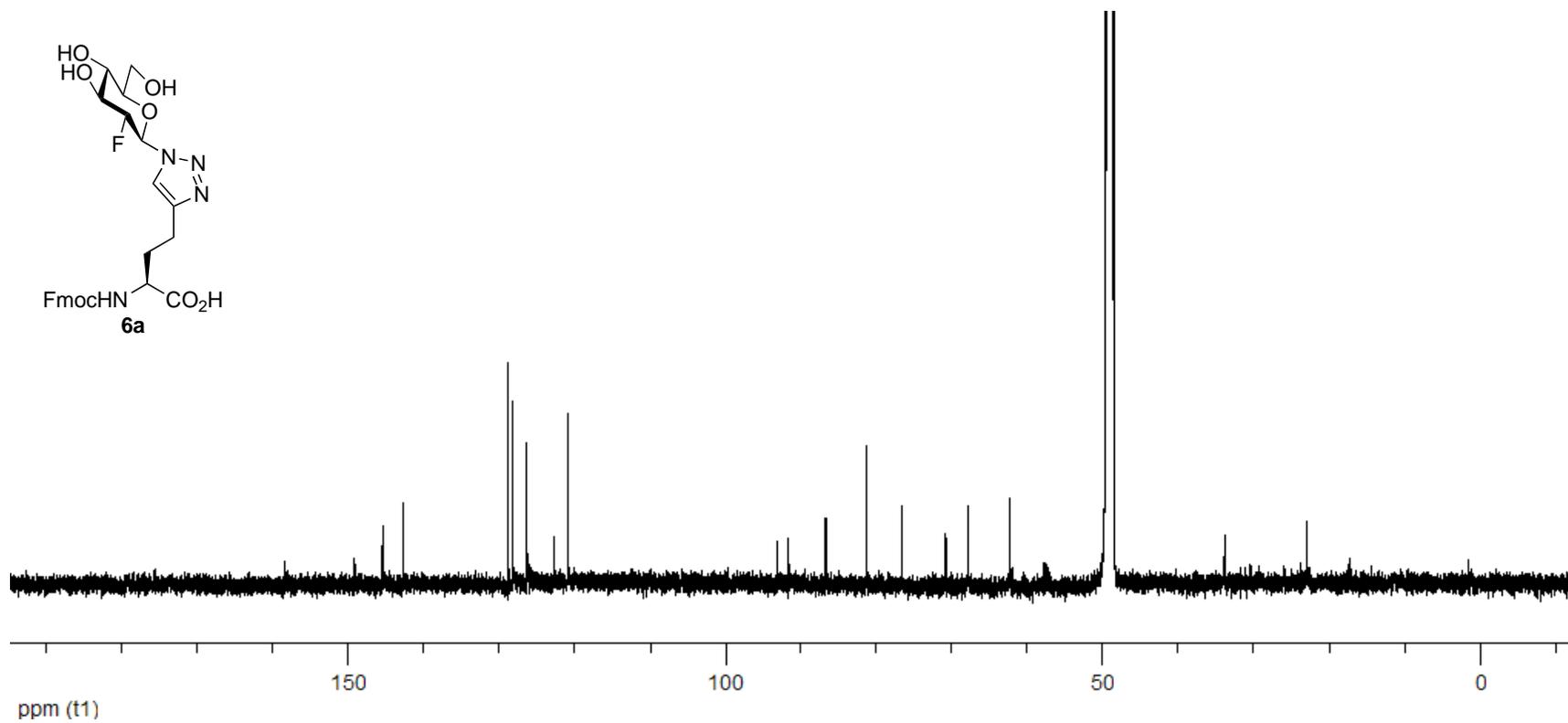


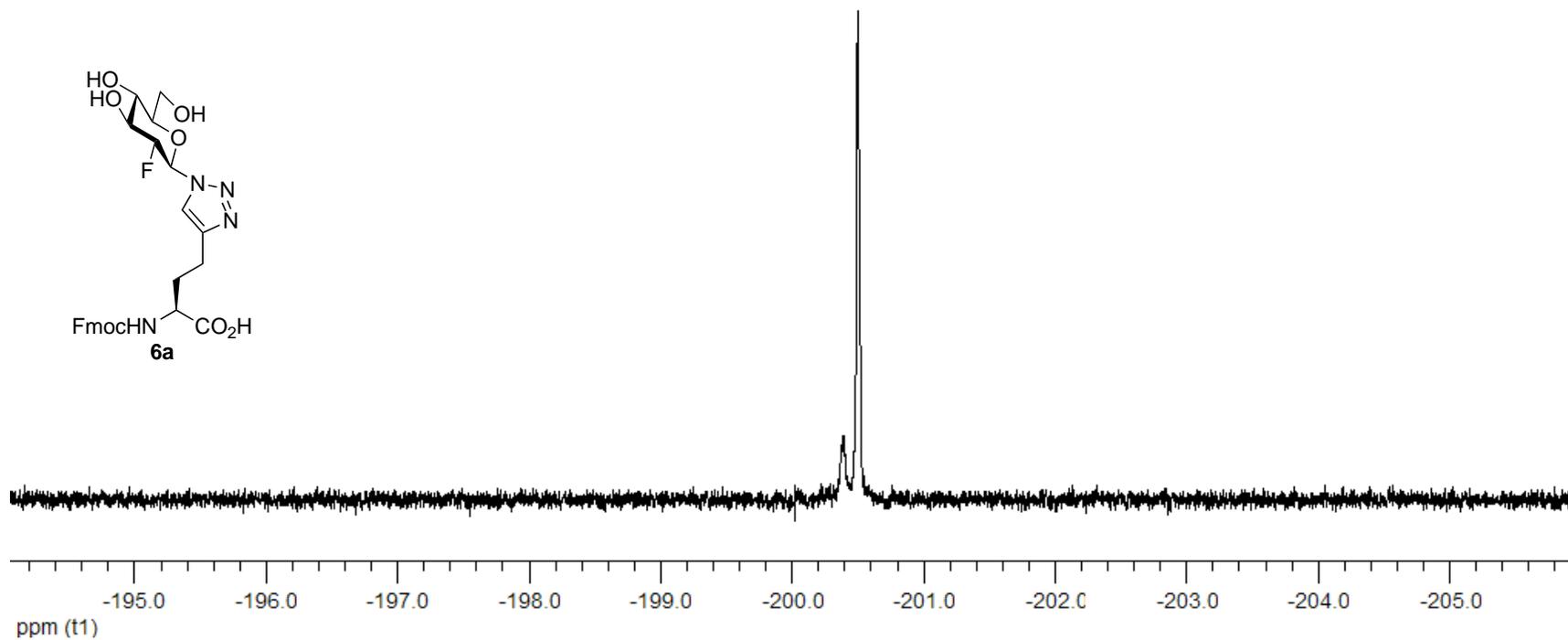
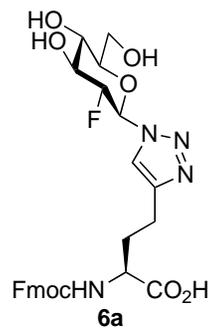


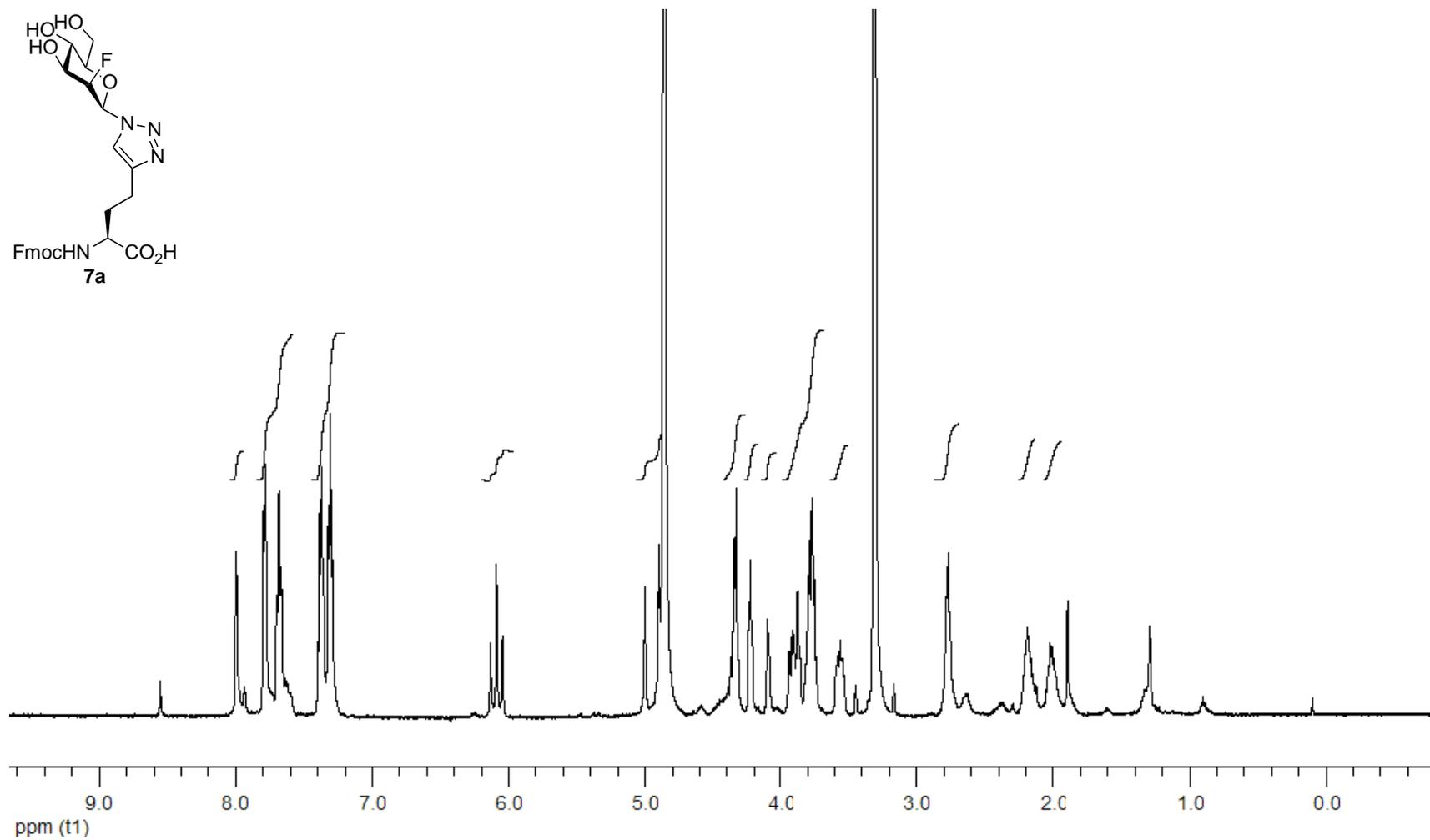
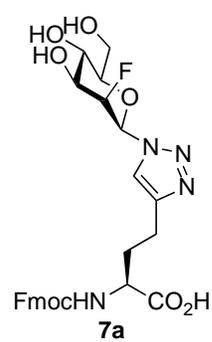


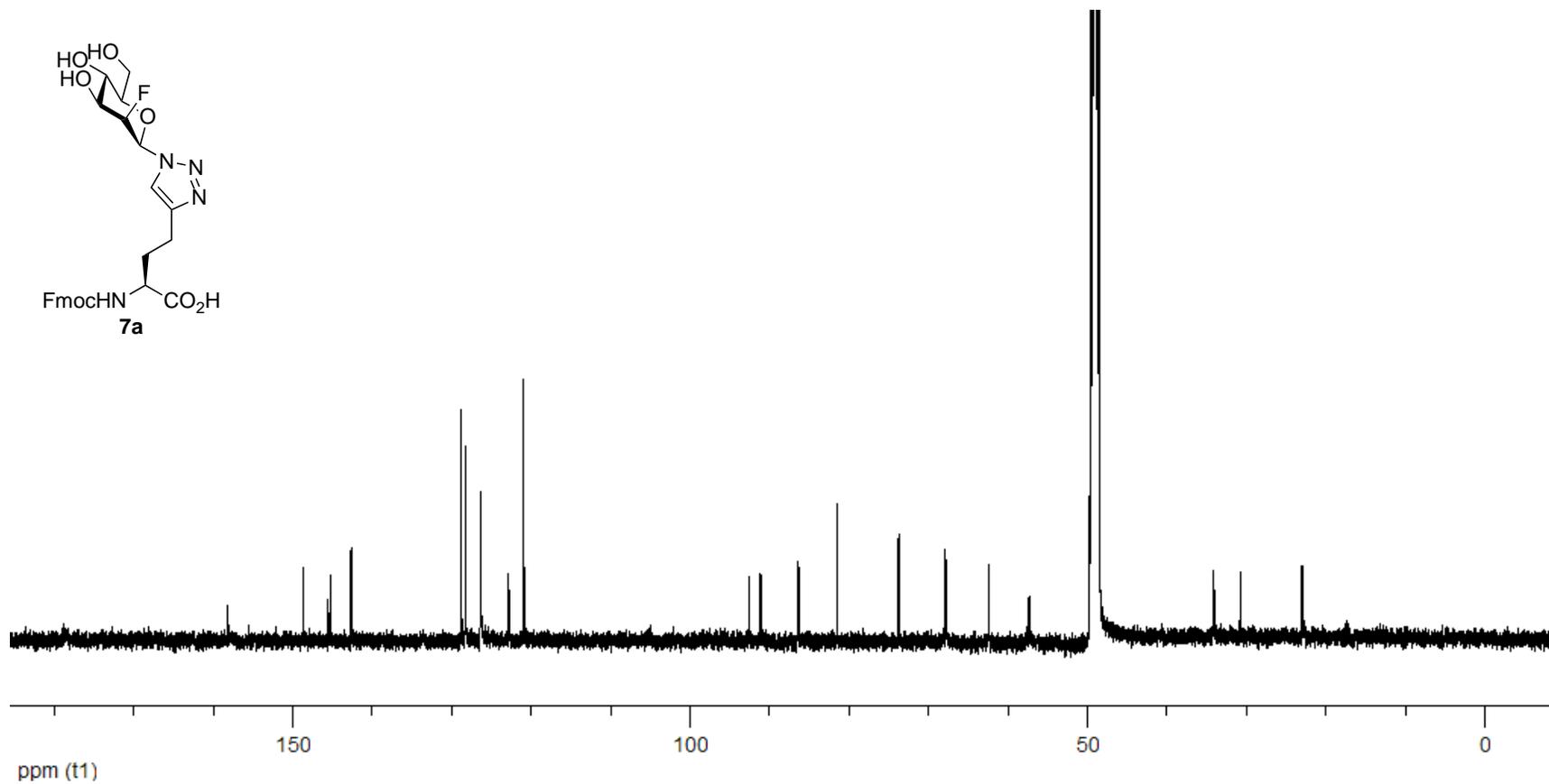
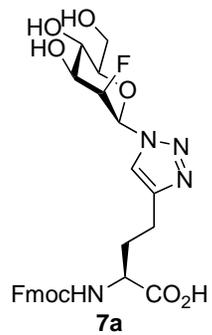


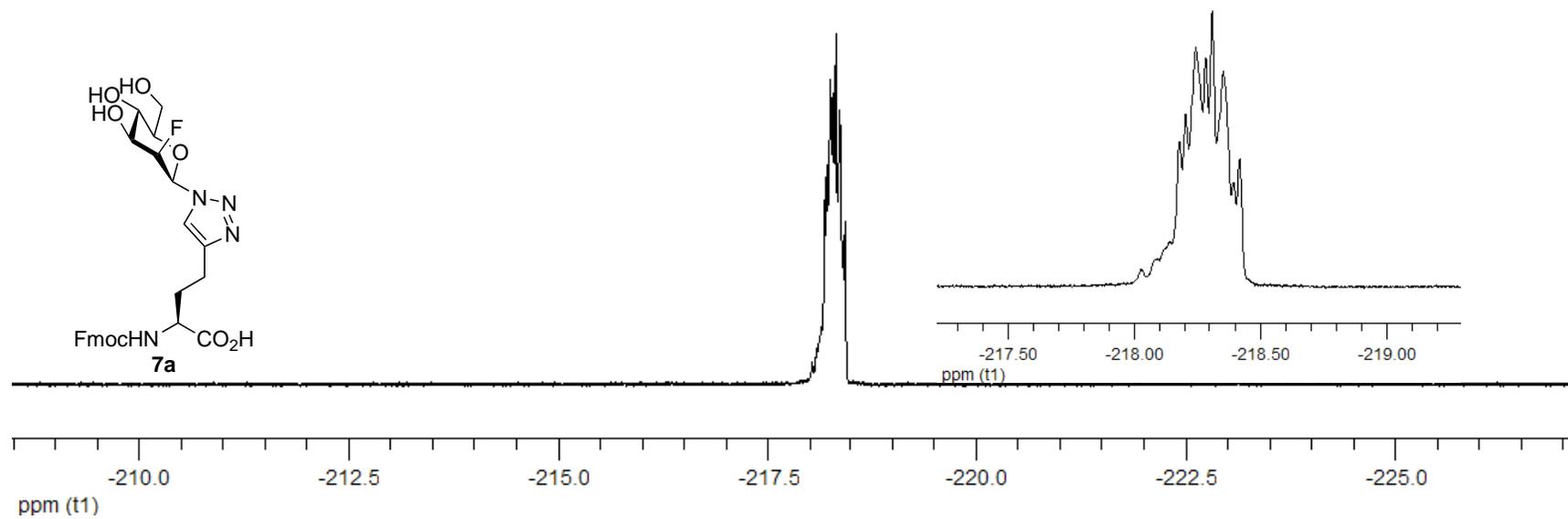


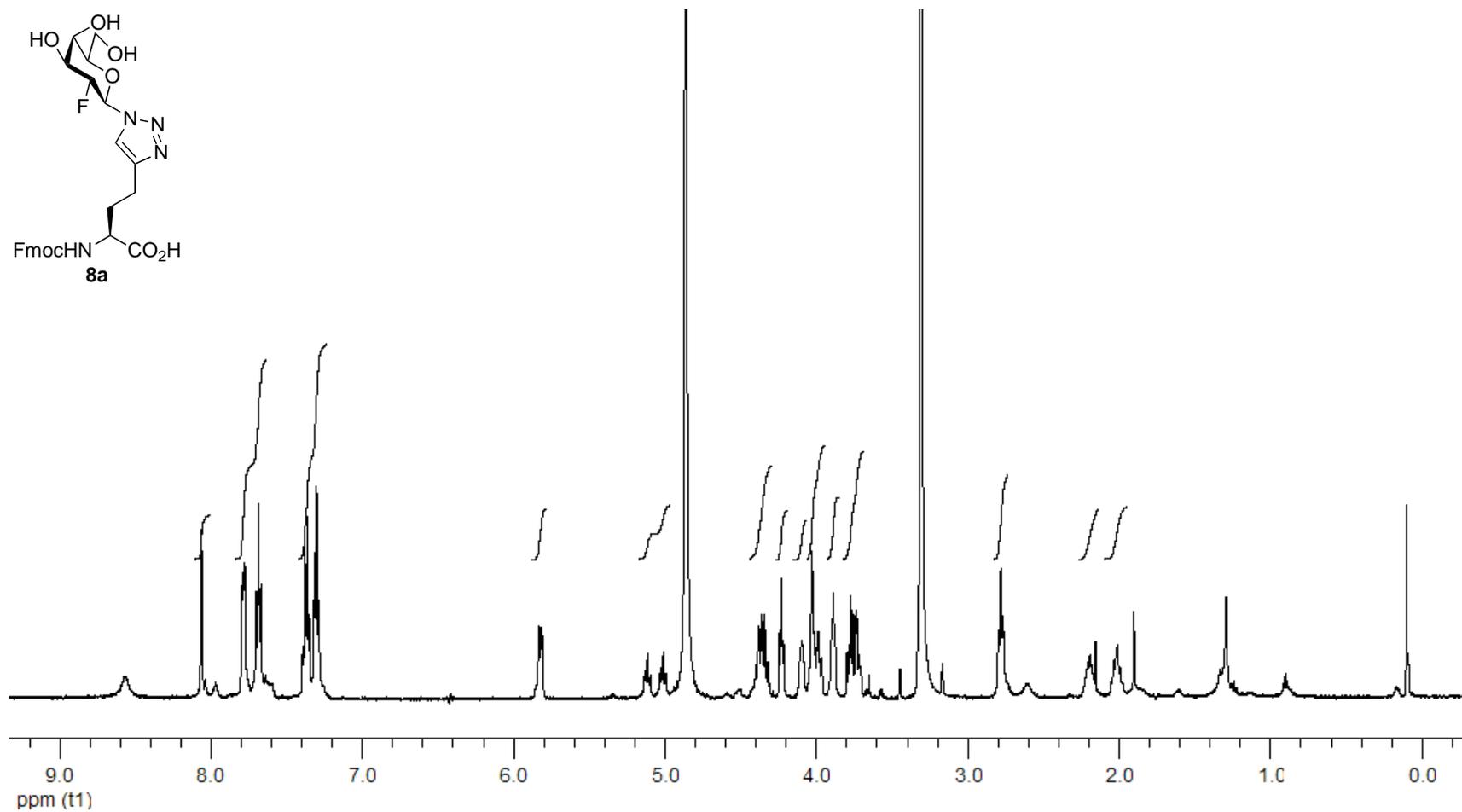
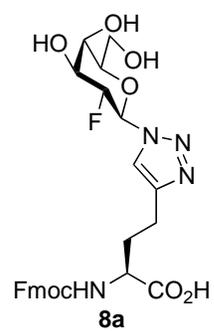


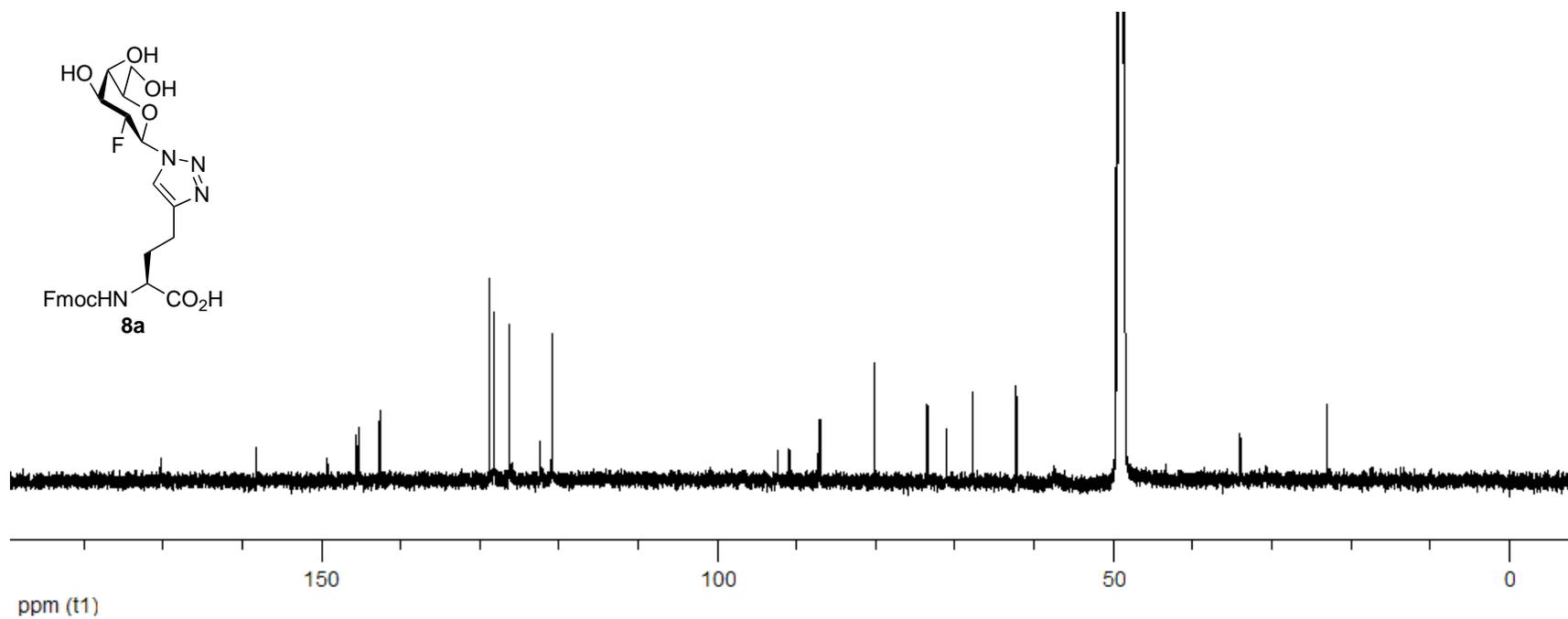


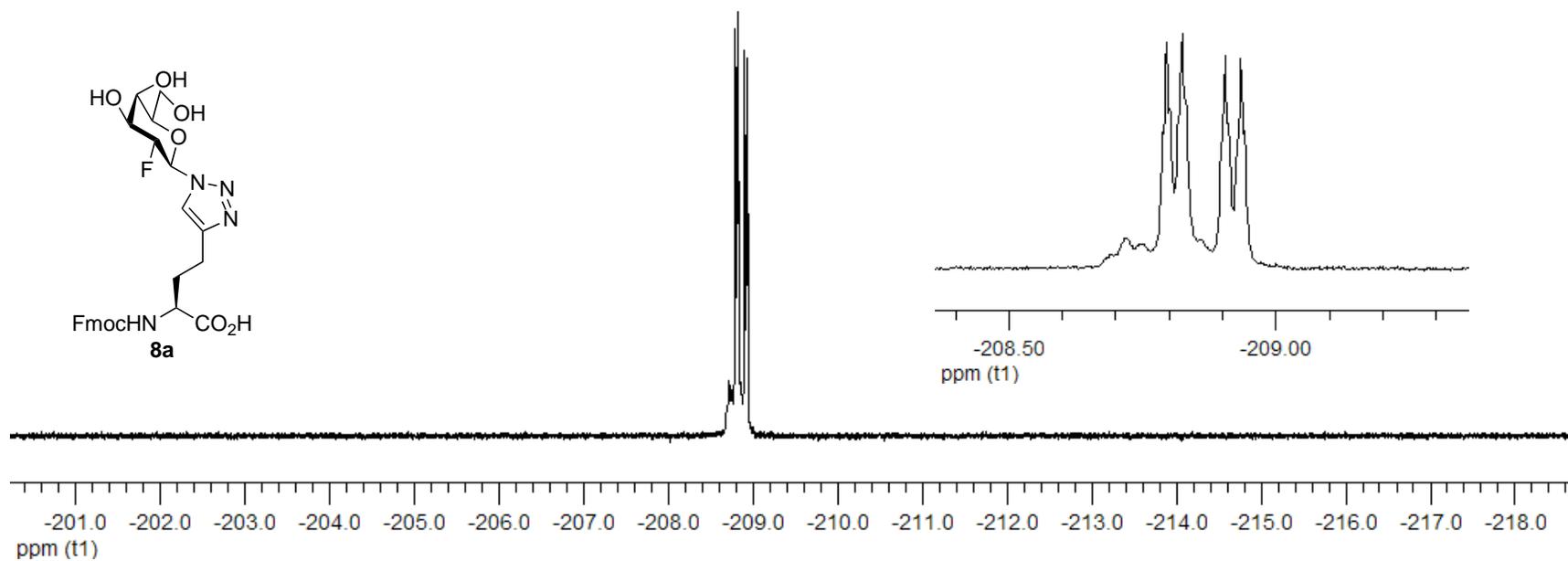


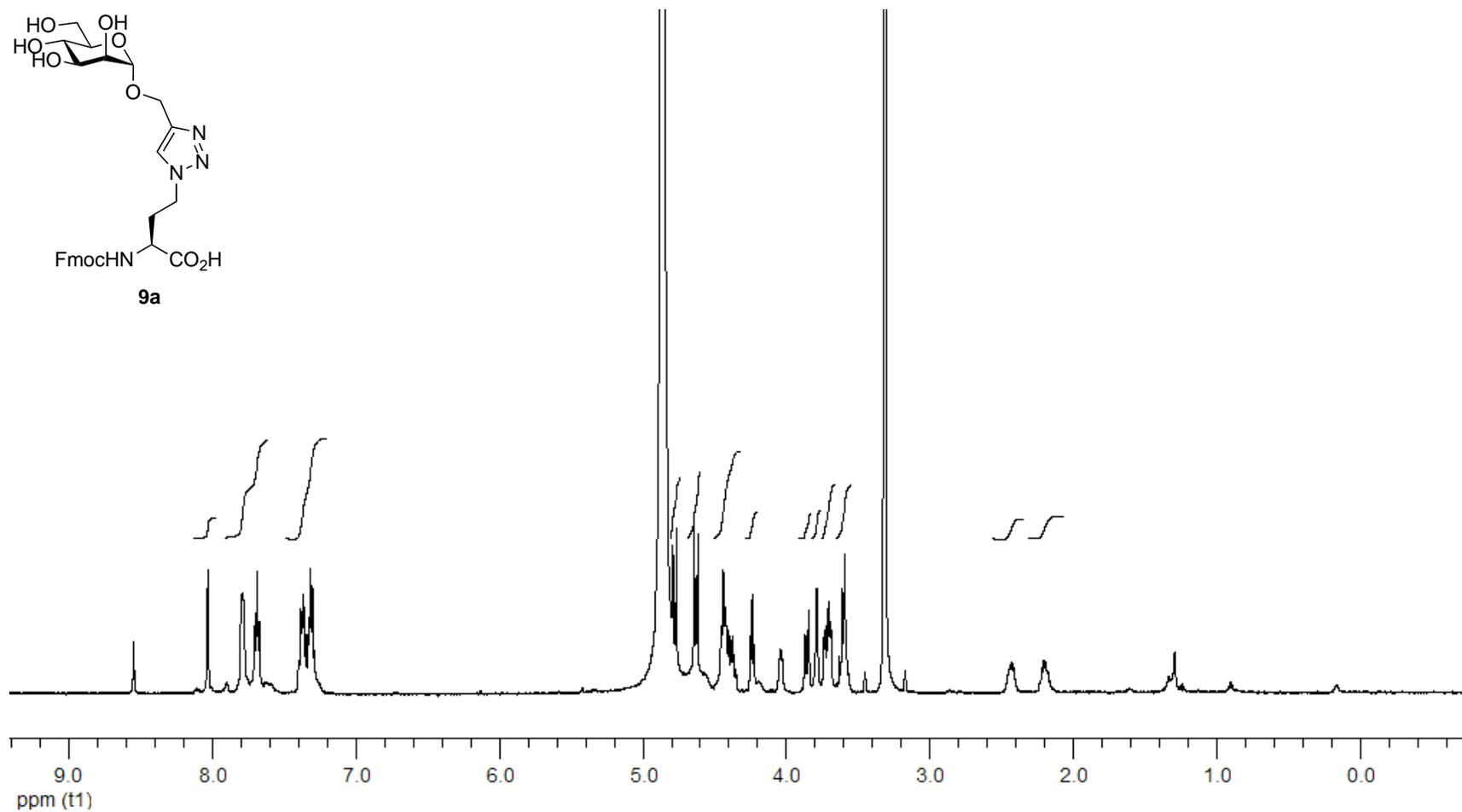
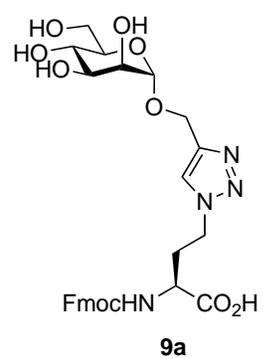


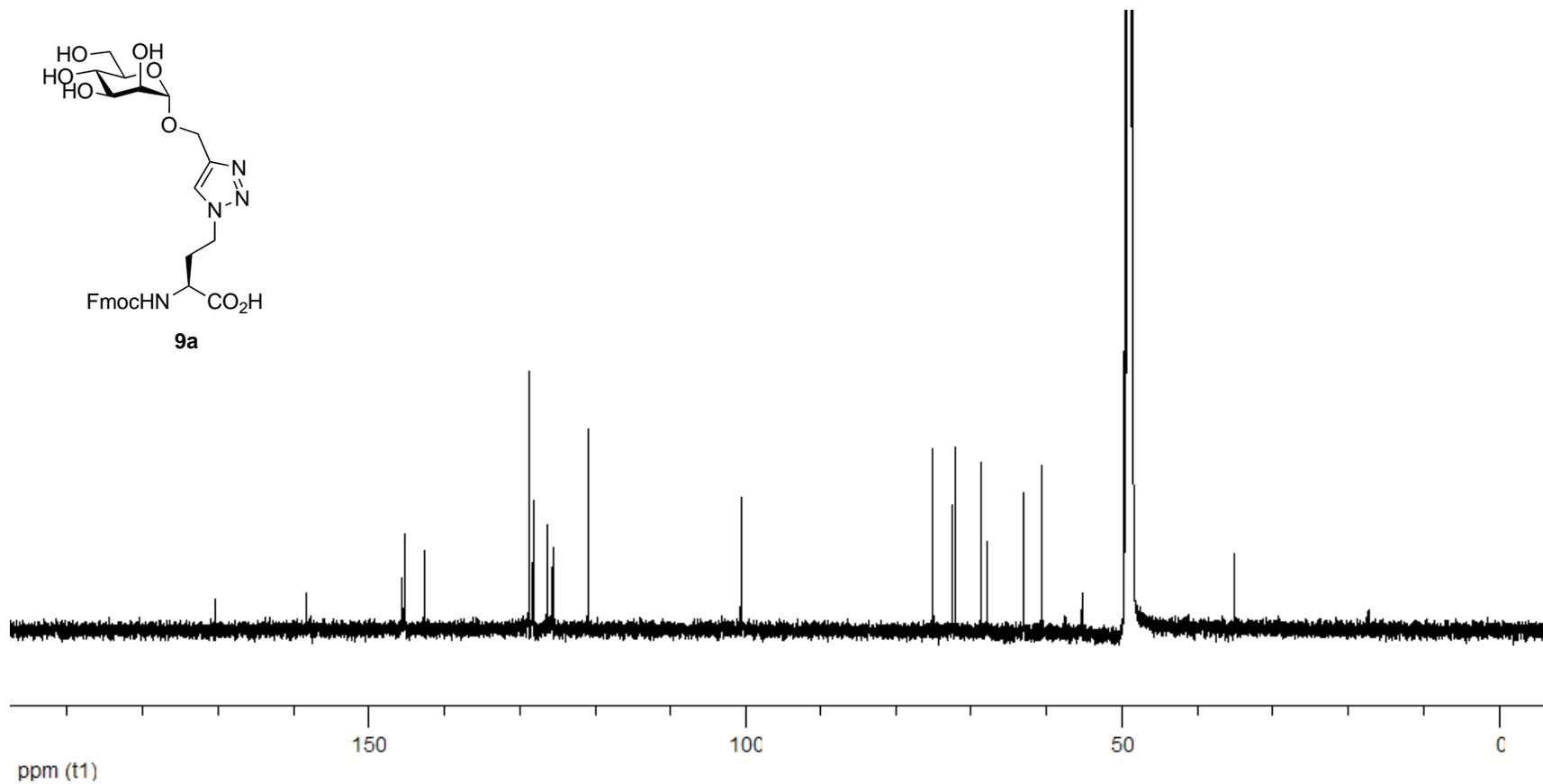
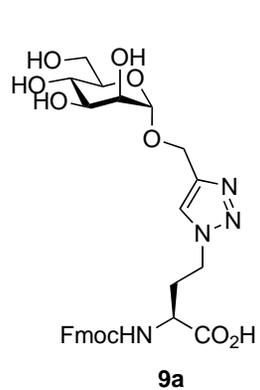


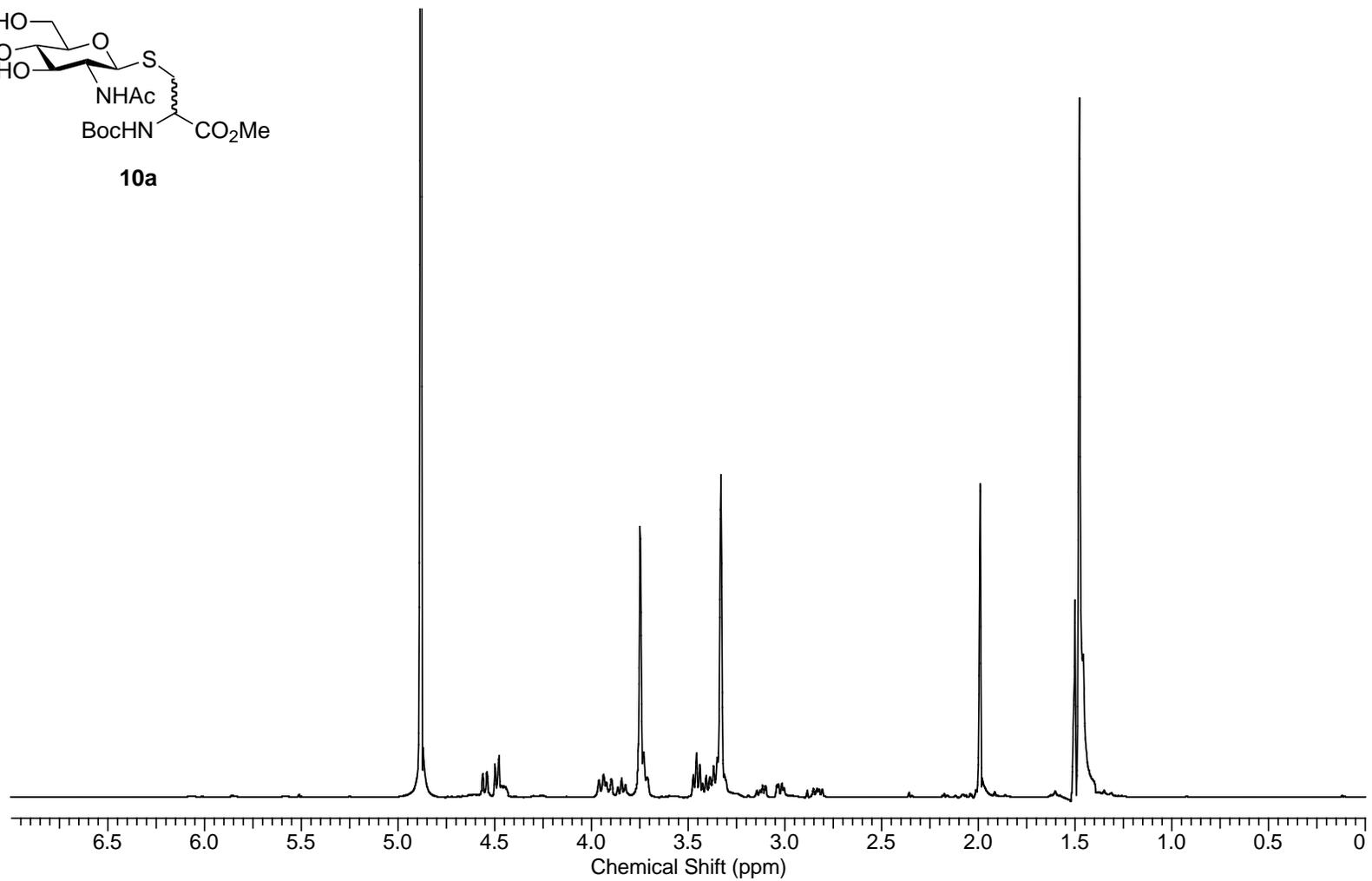
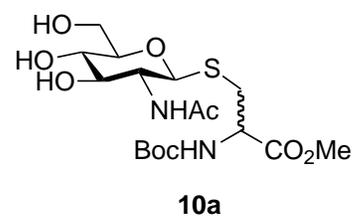


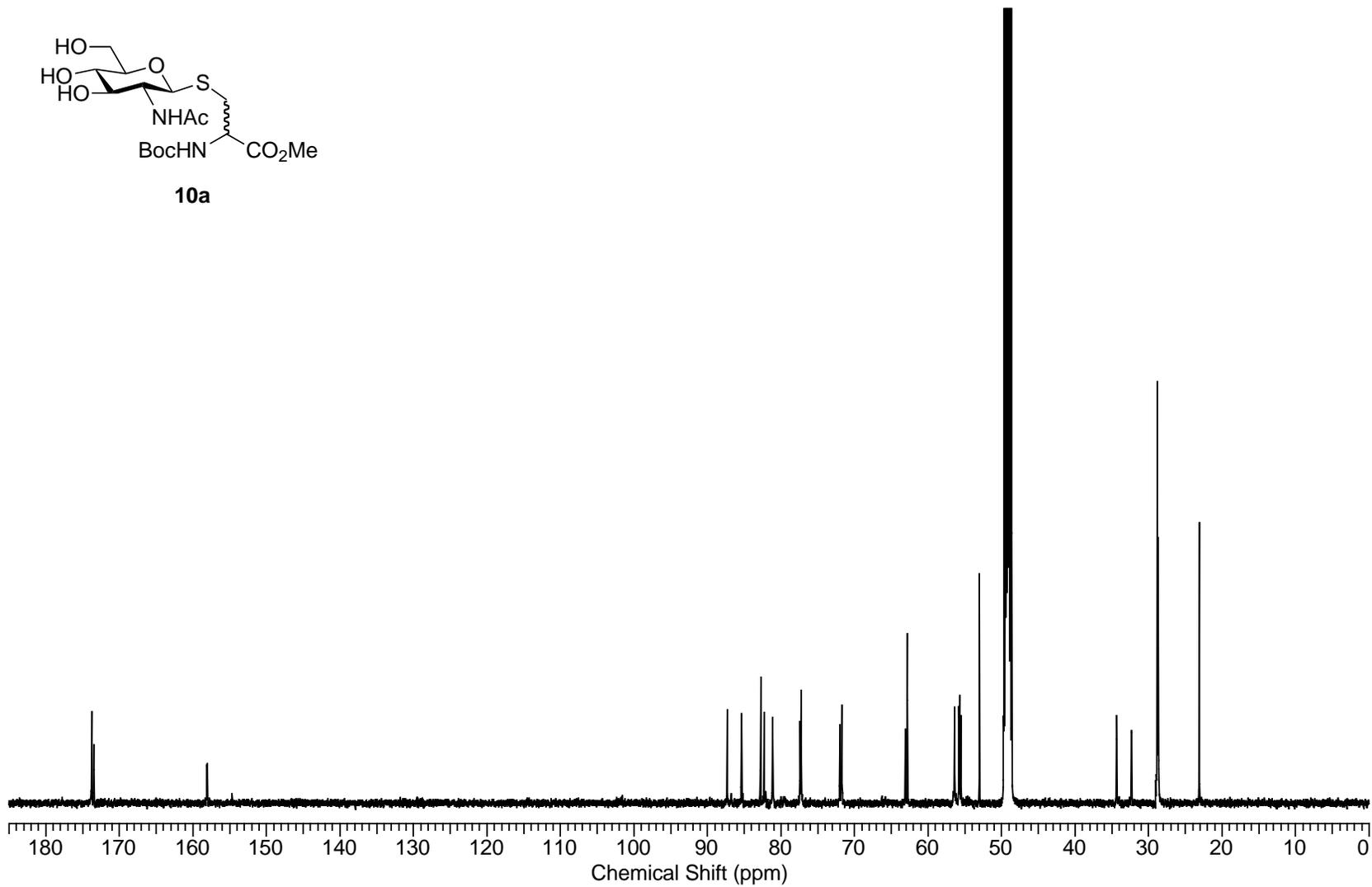
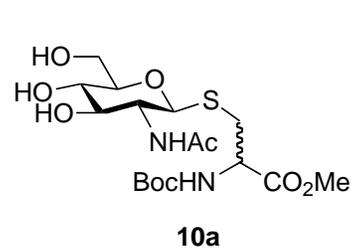


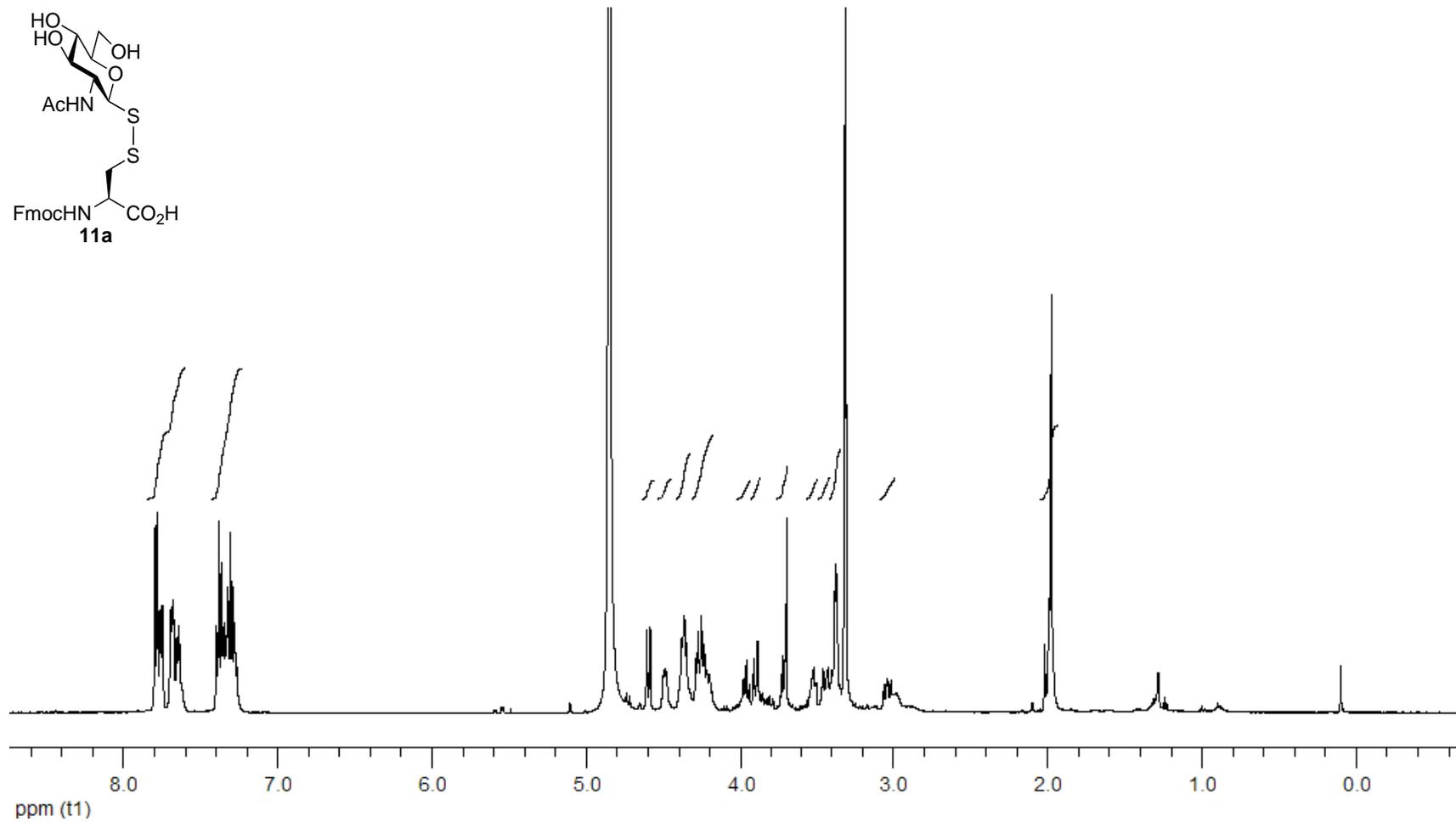
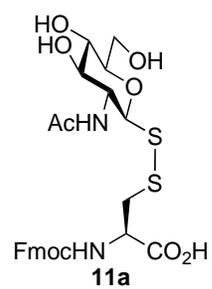


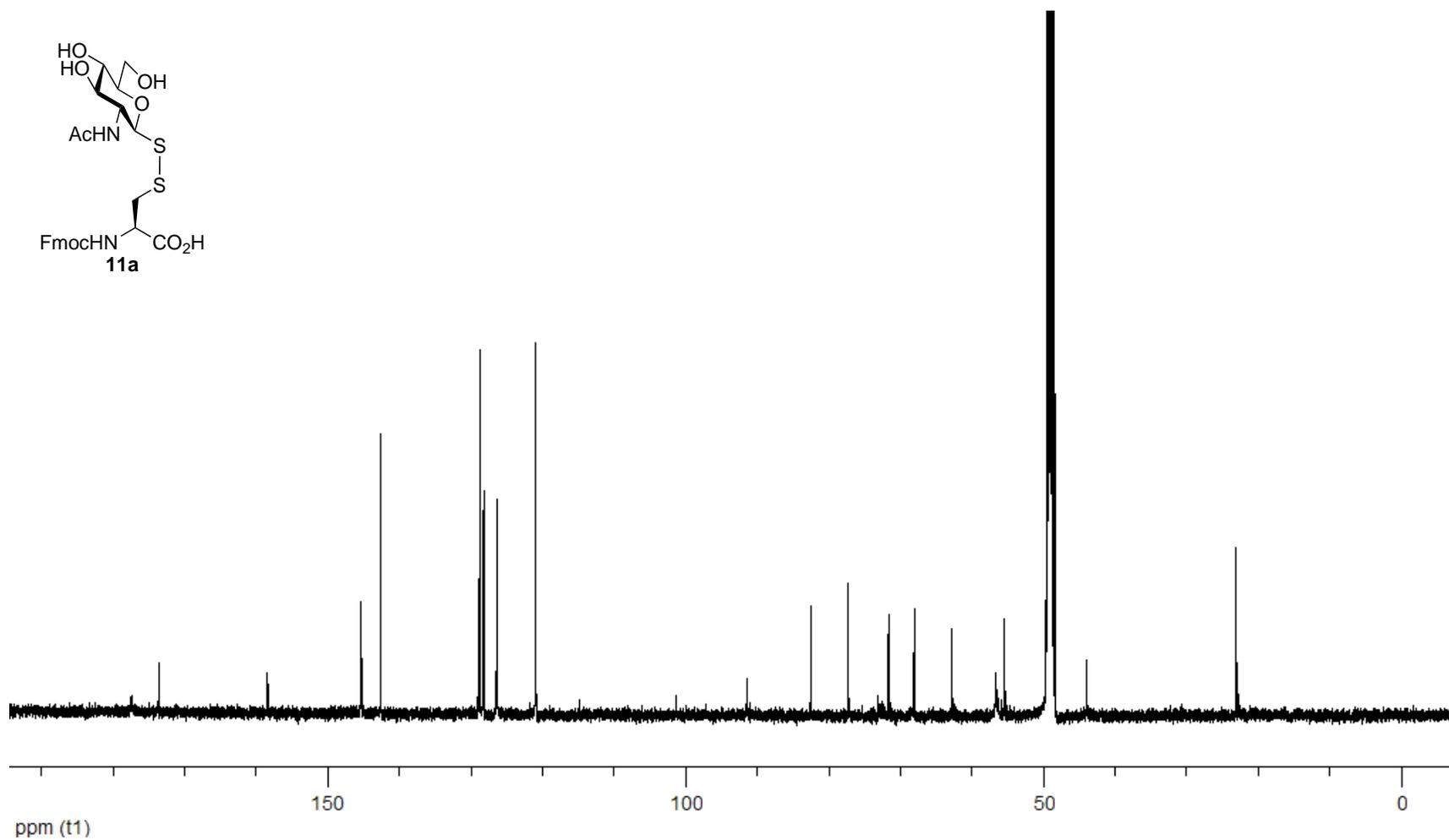
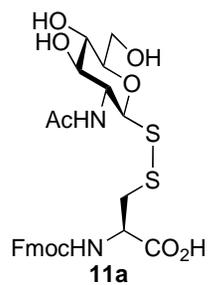


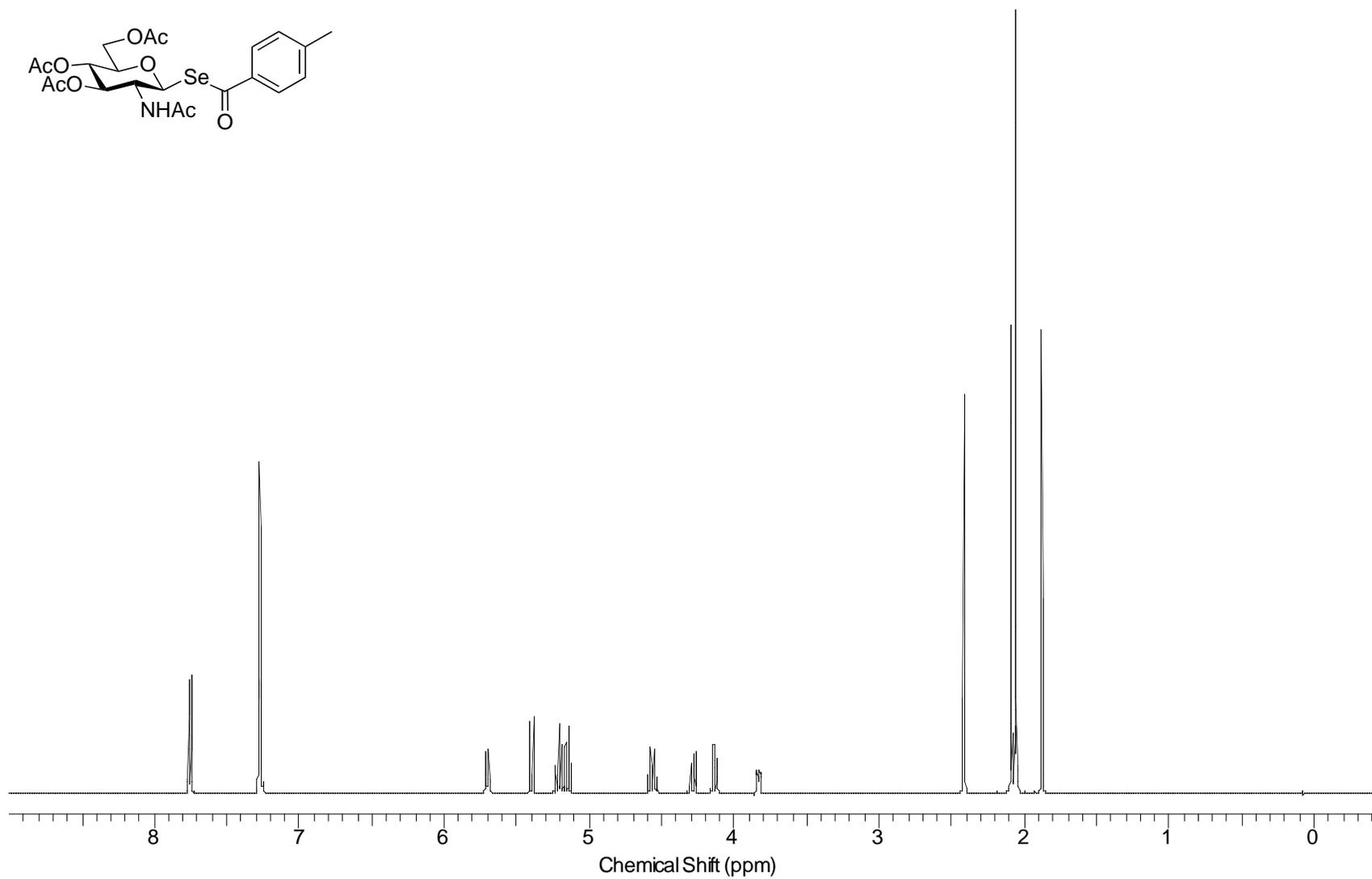


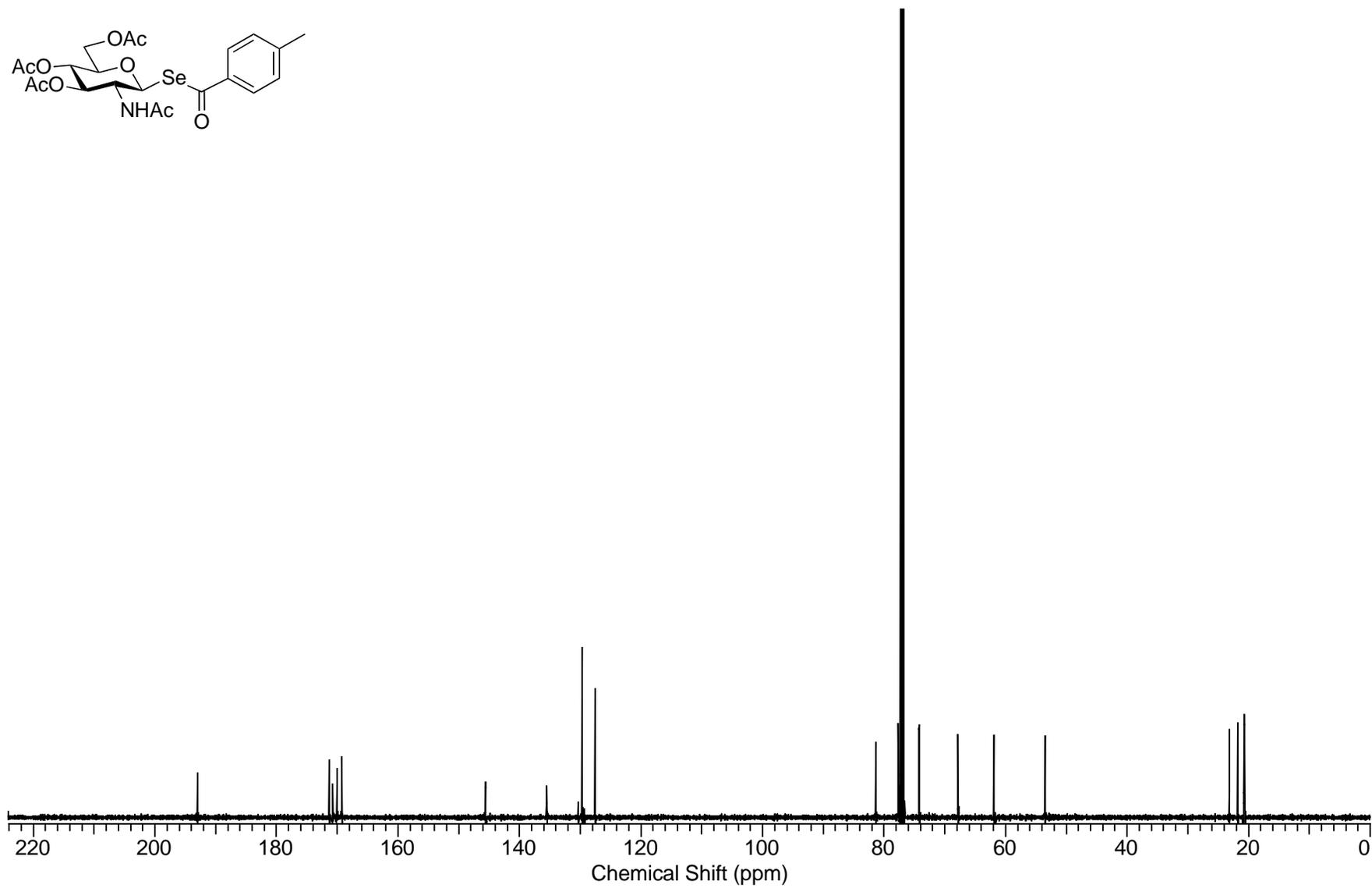


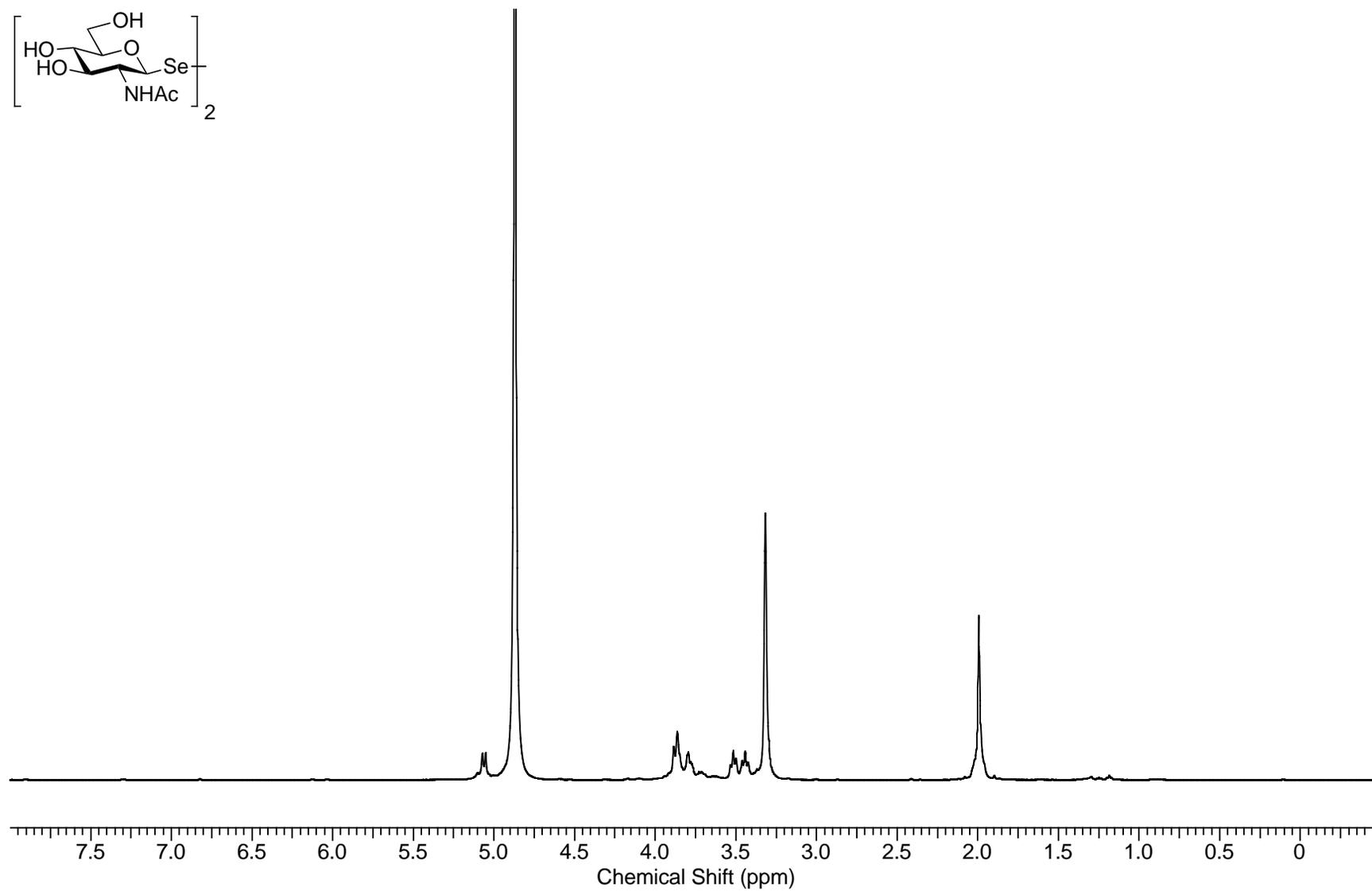
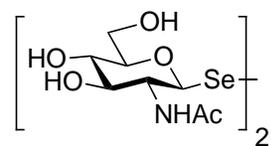


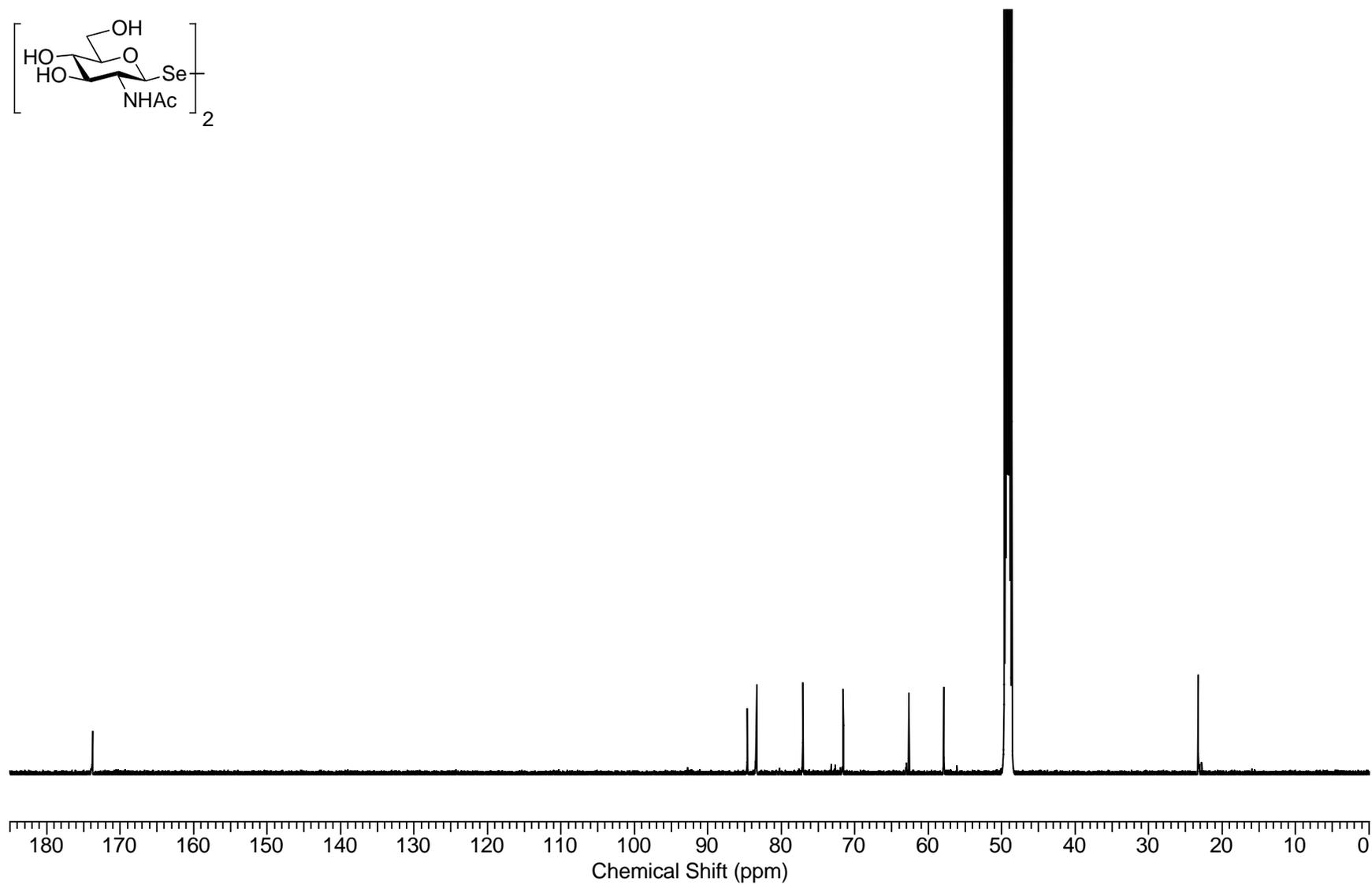
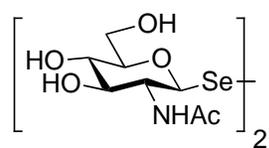


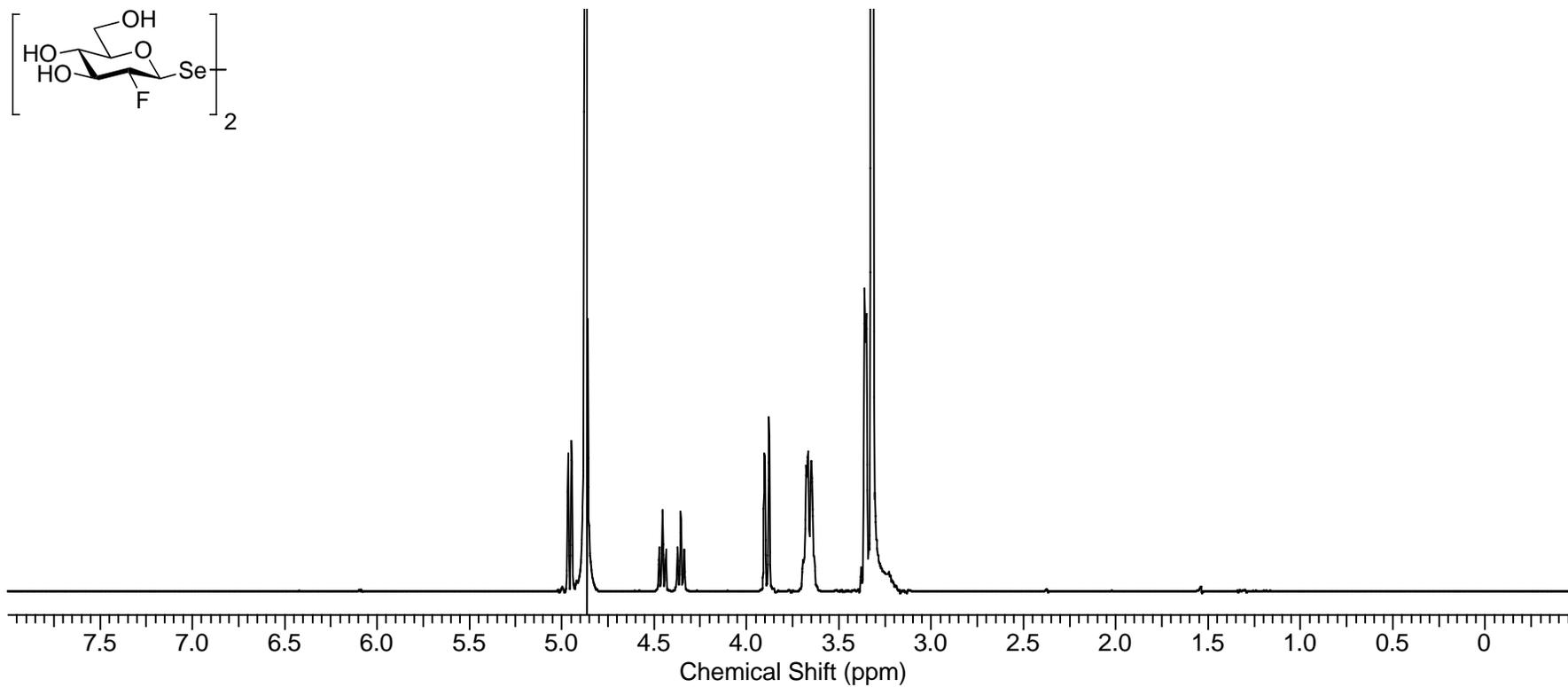


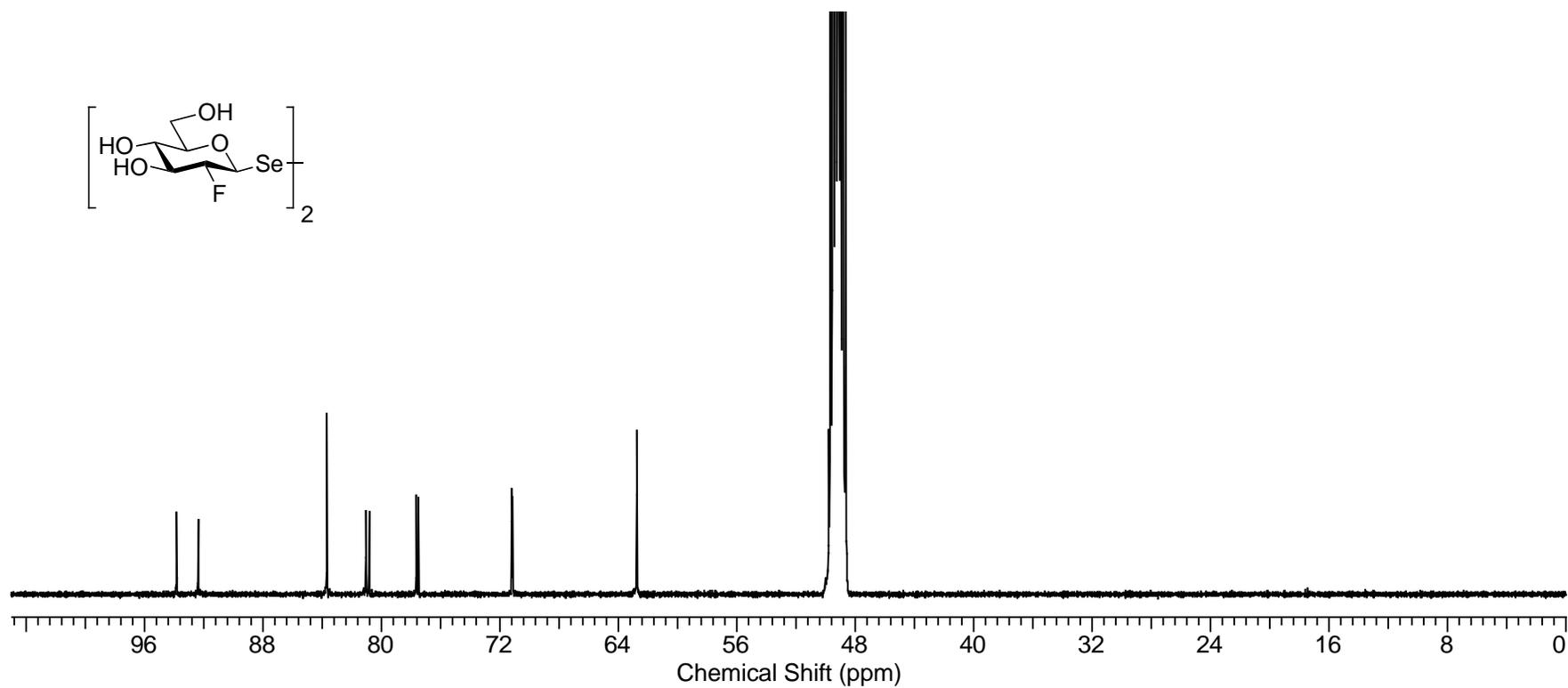


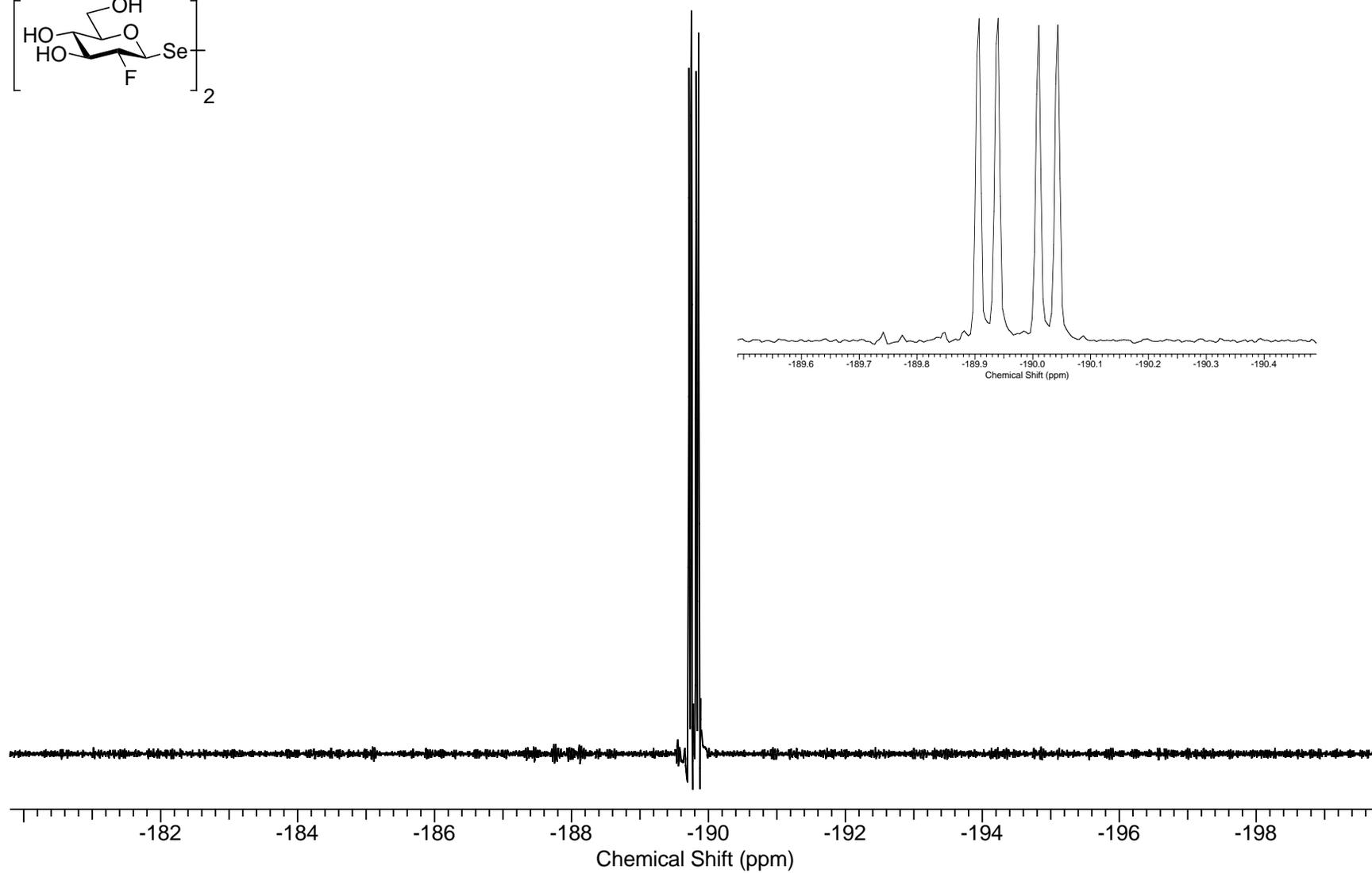
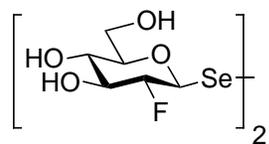


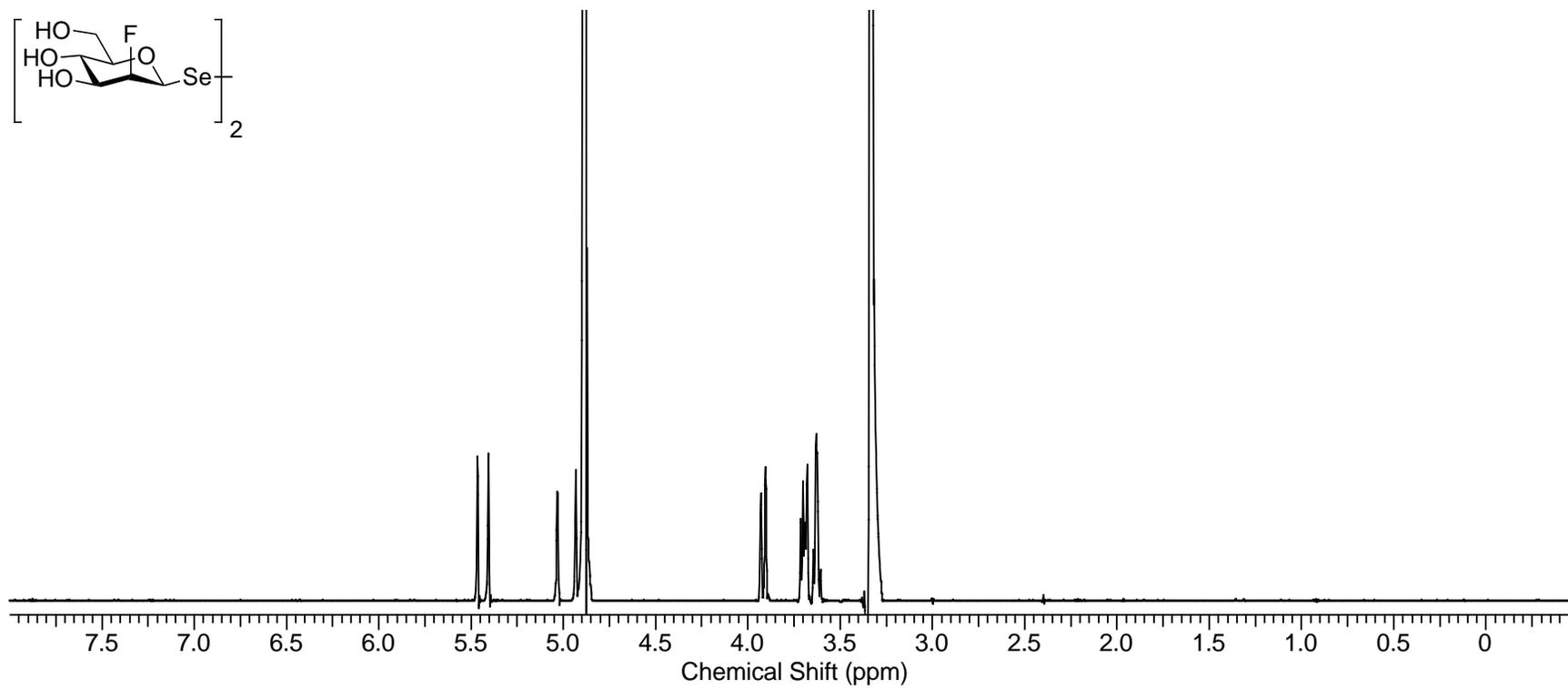


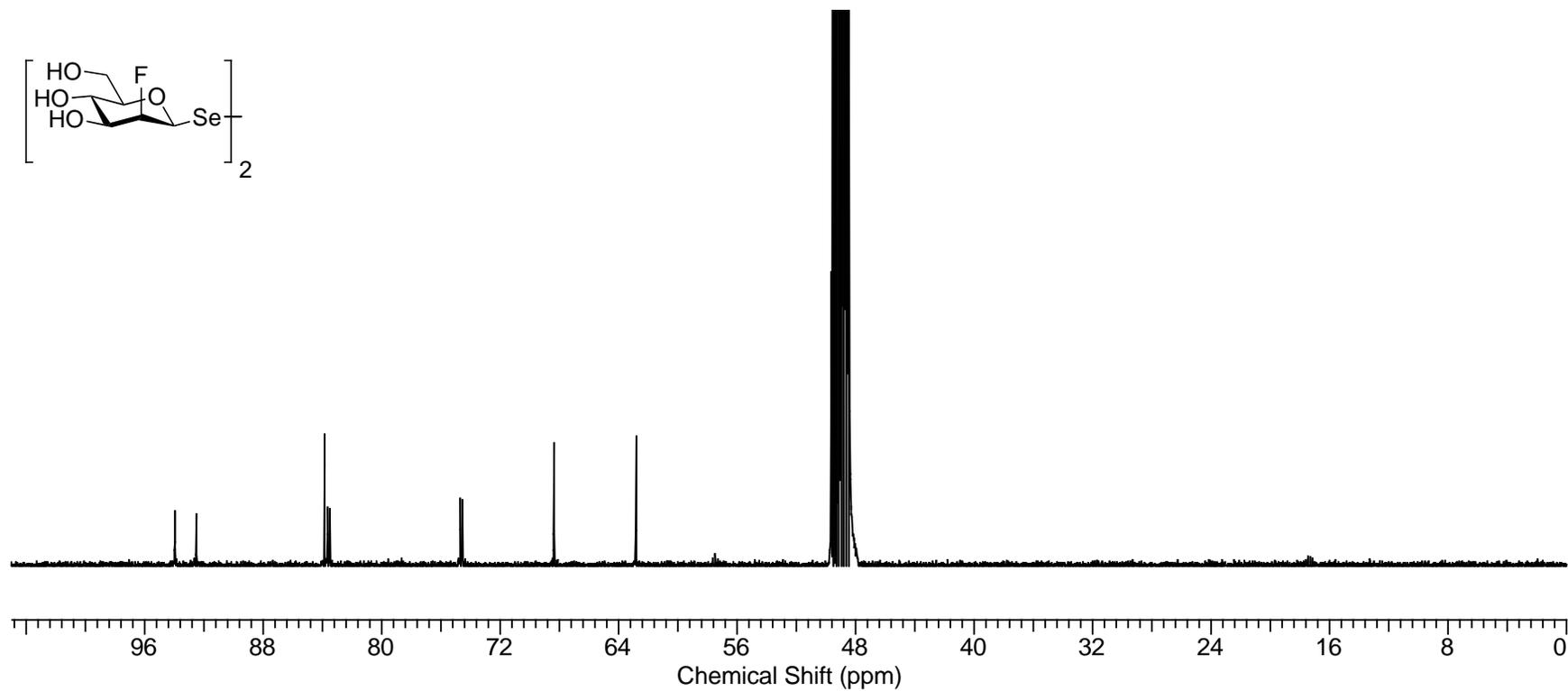


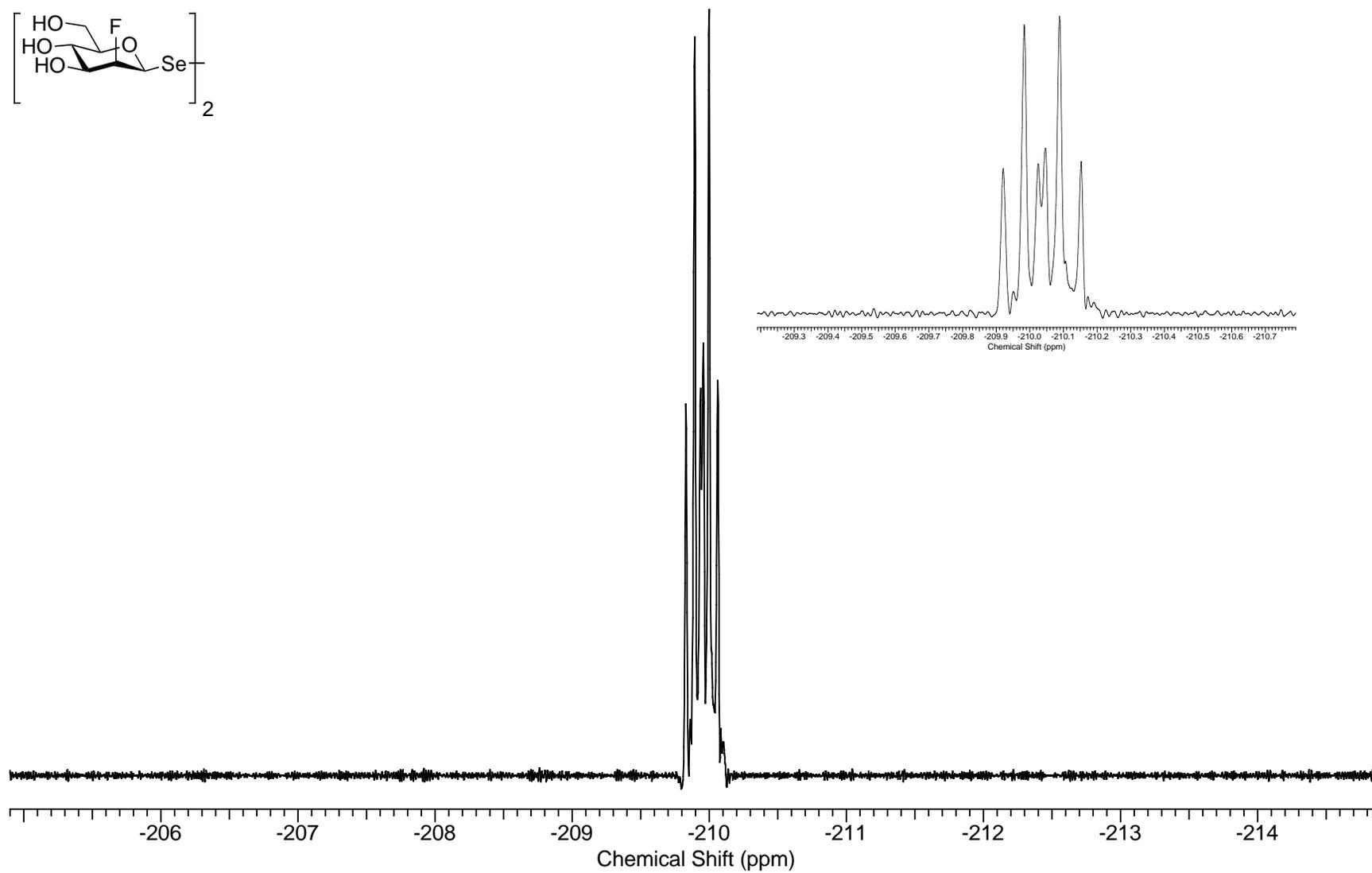
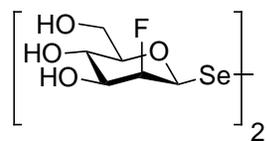


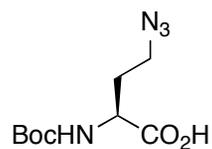




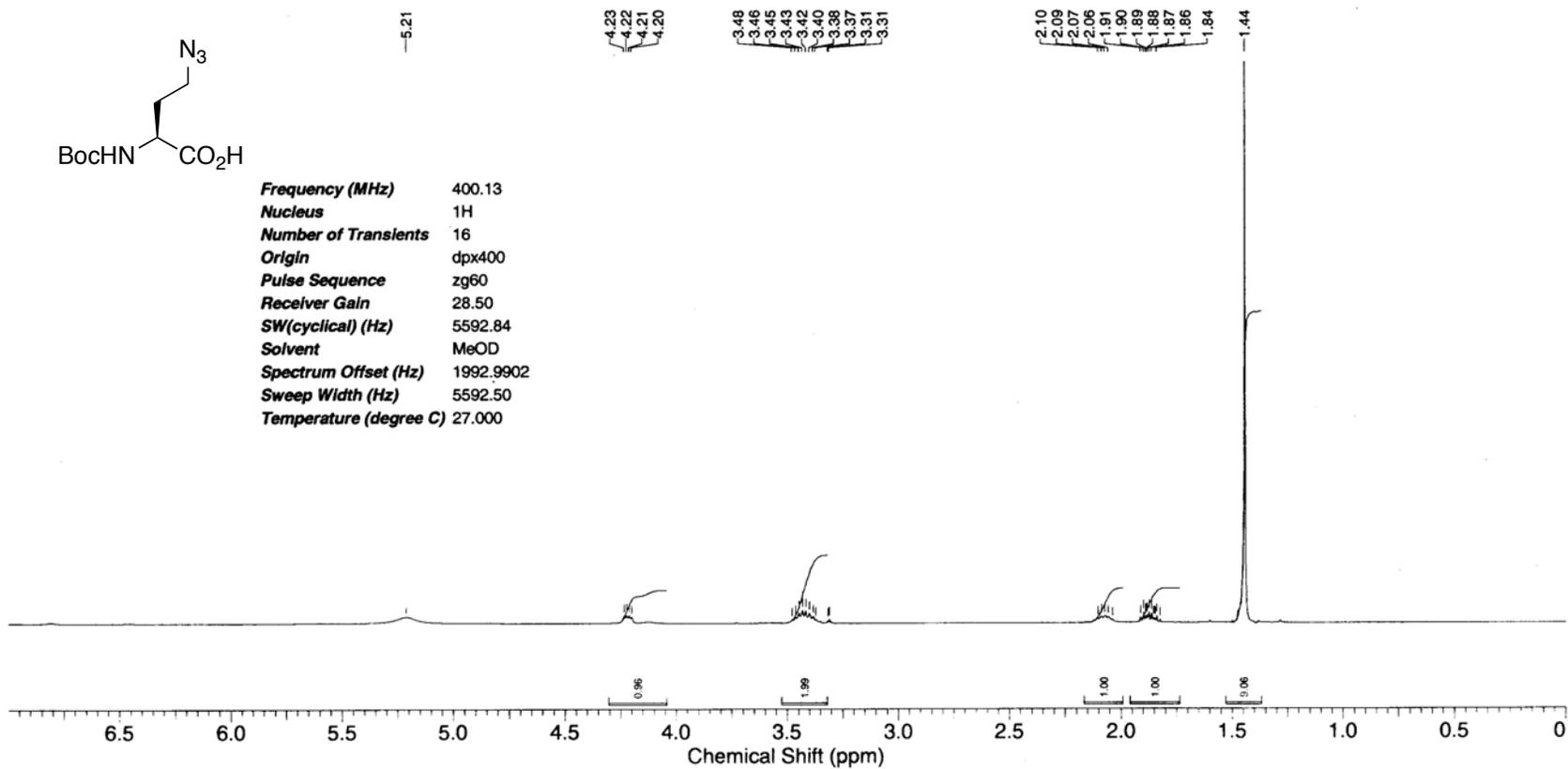


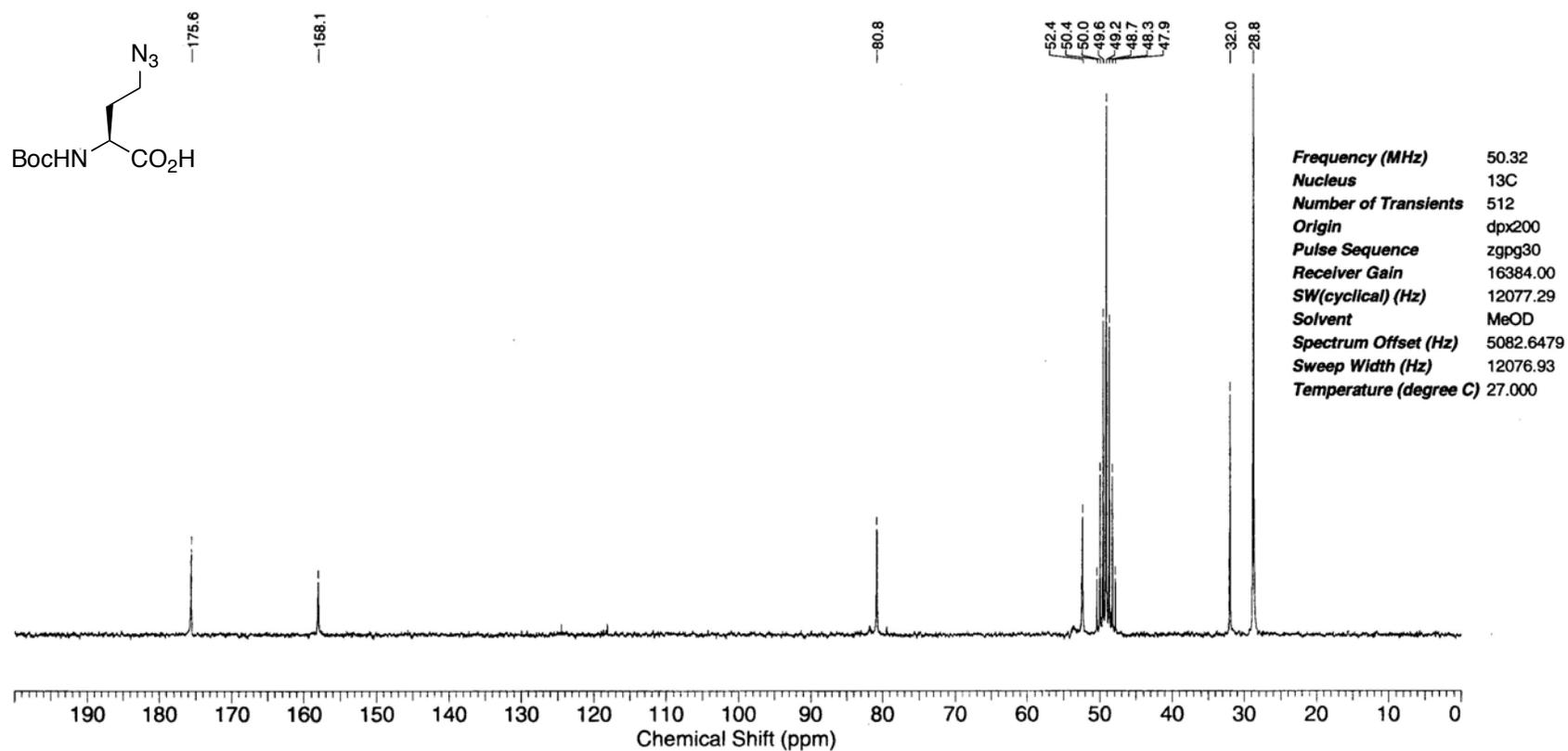


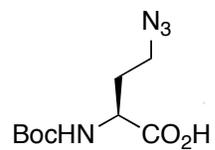




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Receiver Gain 28.50  
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Solvent MeOD  
Spectrum Offset (Hz) 1992.9902  
Sweep Width (Hz) 5592.50  
Temperature (degree C) 27.000







**Frequency (MHz)** 50.32  
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**Pulse Sequence** dept135  
**Receiver Gain** 16384.00  
**SW(cyclical) (Hz)** 12077.29  
**Solvent** MeOD  
**Spectrum Offset (Hz)** 5084.0581  
**Sweep Width (Hz)** 12076.93  
**Temperature (degree C)** 27.000

