Supplementary Material (ESI) for Chemical Communications

Supporting Information

for

Highly Efficient Chemoenzymatic Synthesis of β1–3-Linked Galactosides

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Cloning and expression of D-galactosyl-β1–3-*N*-acetyl-D-hexosamine phosphorylase from *Bifidobacterium longum* subsp. *infantis* ATCC 15697 (BiGalHexNAcP) encoded by *Blon_2174* gene (GenBank Accession number NC_011593)

Bacterial strains, plasmids, and materials

Electrocompetent *E. coli* DH5α cells were purchased from Invitrogen (Carlsbad, CA). Chemical competent *E. coli* Origami™ B(DE3) cells and vector plasmid pET15b was purchased from Novagen (EMD Biosciences, Inc. Madison, WI). Ni^{2+}–NTA agarose (nickel–nitrilotriacetic acid–agarose), QIAprep spin miniprep kit, and QIAEX II gel extraction kit were from Qiagen (Valencia, CA). Herculase enhanced DNA polymerase was from Stratagene (La Jolla, CA). T4 DNA ligase, 1 kb DNA ladder, and *Xho*I restriction enzyme were obtained from Promega (Madison, WI). *Nde*I restriction enzyme was from New England Biolabs, Inc. (Beverly, MA). Bicinchoninic acid (BCA) protein assay kit was from Pierce Biotechnology, Inc. (Rockford, IL). Genomic DNA of *Bifidobacterium longum* subsp. *infantis* ATCC 15697 was a kind gift from David Mills in the University of California-Davis.

Cloning

BiGalHexNAcP encoded by gene *Blon_2174* (GenBank accession number NC_011593) was cloned from *Bifidobacterium longum* subsp. *infantis* (ATCC 15697) genomic DNA in pET15b vector and expressed in *Escherichia coli* as an N-terminal His_6-tagged fusion protein. Primers used were: forward primer 5'GATCCATATGACCAACACCGGCCGCTTCACGCTGCC3' (*Nde*I restriction site is underlined) and reverse primer 5'CCGCTCGAGTTAGGCTTCACGCCAGGCGATACCGC3' (*Xho*I restriction site is underlined). PCR amplification of the target gene was performed in a 50 μL reaction containing genomic DNA (1 μg), forward and reverse primers (1 μM each), 10 × Herculase buffer (5 μL), dNTP mixture (1 mM), and 5 units (1 μL) of Herculase enhanced DNA polymerase. The reaction mixture was subjected to 30 cycles of amplification with an annealing temperature of 50°C. The resulting PCR product was digested with restriction enzymes, purified, and ligated with pET15b vector predigested with *Nde*I and *Xho*I restriction enzymes. Ligation product was transformed into electrocompetent *E. coli* DH5α cells. Selected clones were grown for minipreps and positive

clones were verified by restriction mapping and DNA sequencing performed by Davis Sequencing Facility.

Expression

Positive plasmid was selected and subsequently transformed into *E. coli* Origami^{TM} B(DE3) chemical competent cells. *E. coli* cells harboring the pET15b-Blon2174 plasmid were cultured in LB medium (10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl) with ampicillin (100 μg/mL) and kanamycin (15 μg/mL) until the $OD_{600\ nm}$ of the culture reached 0.8–1.0. Overexpression of the recombinant protein was achieved by adding 0.1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) followed by incubation at 25°C for 18–20 h with rigorous shaking at 250 rpm in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ).

Protein purification

His_6-tagged target proteins were purified from cell lysate using Ni^{2+}–NTA affinity column. To obtain cell lysate, cells were harvested by centrifugation at 4,000 rpm (Sorvall) at 4°C for 3 h. The cell pellet was resuspended in lysis buffer (pH 8.0, 100 mM Tris–HCl containing 0.1% Triton X-100). Lysozyme (100 μg/mL) and DNaseI (5 μg/mL) were then added to the cell suspension. The mixture was incubated at 37°C for 1 h with vigorous shaking (200 rpm). Cell lysate was obtained by centrifugation at 11,000 rpm (Sorvall) at 4°C for 45 min as the supernatant. Purification is performed by loading the supernatant onto a Ni^{2+}-NTA column pre-equilibrated with 10 column volumes of binding buffer (10 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.5). The column was wash with 10 column volumes of binding buffer and 10 column volumes of washing buffer (50 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.5). Protein of interest was eluted with Tris-HCl (pH 7.5, 50 mM) containing imidazole (200 mM) and NaCl (0.5 M). The fractions containing the purified enzymes were collected and dialyzed against Tris-HCl (pH 8.0, 20 mM) containing 10% glycerol. Dialyzed proteins were stored at 4°C. Typically, 55 mg of BiGalHexNAcP was purified from one liter cell culture.

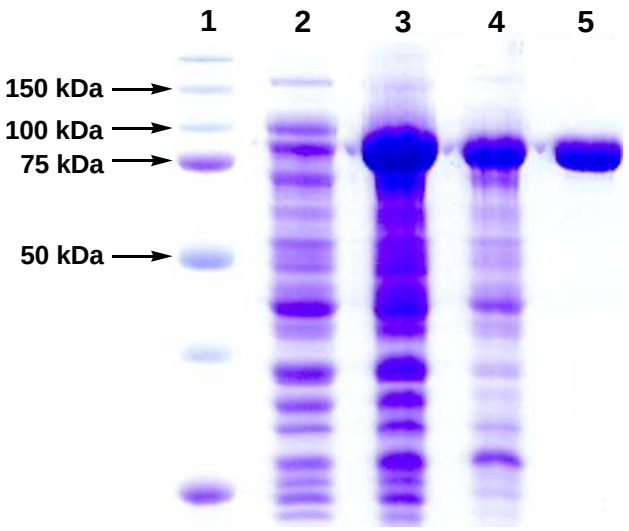


Figure S1. SDS–PAGE analysis of BiGalHexNAcP. Lanes: 1, protein standards; 2, whole cell extraction before induction; 3, whole cell extraction after induction; 4, cell lysate after induction; 5, Ni^{2+}-column purified protein. The calculated molecular weight of BiGalHexNAcP is 86.5 kDa.

Quantification of purified protein

The concentration of purified enzyme was obtained in a 96-well plate using a Bicinchoninic acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, IL) with bovine serum albumin as a protein standard. The absorbance of samples was measured at 562 nm by a BioTek Synergy^{TM} HT Multi-Mode Microplate Reader.

BiGalHexNAcP pH profile by HPLC assays

Typical enzymatic assays were performed in a total volume of 20 μL in a buffer (250 mM) with pH varying from 4.0–9.0 containing 10 mM $MgCl_2$, 1 mM GlcNAcαProNH2AA, 1 mM Gal-1-P, and 0.9 μg enzyme in an Eppendorf's tube. Reactions were allowed to proceed for 15 min at 37°C and quenched by the addition of ice-cold 12% acetonitrile (580 μL) to make 30-fold dilution. The samples were then kept on ice until analyzed by a Shimadzu LC-2010A HPLC system equipped with a membrane on-line degasser, a temperature control unit and a fluorescence detector. A reverse phase Premier C18 column (250 × 4.6 mm I.D., 5 μm particle size, Shimadzu) protected with a C18 guard column cartridge was used. The mobile phase was

12% acetonitrile. The fluorescent compound GlcNAcαProNH2AA and the product Galβ1–3GlcNAcαProNH2AA were detected by excitation at 315 nm and emission at 400 nm.[1] All assays were carried out in duplicate.

As shown in Figure 3S, the pH profile of BiGalHexNAcP using GlcNAcαProNH2AA as an acceptor indicates an optimal pH range of 5.0 to 6.5. Optimum activity was observed in MES buffer with pH varying from pH 5.5 to 6.0. Very low activity was observed in the pH range of 7.0–9.0.

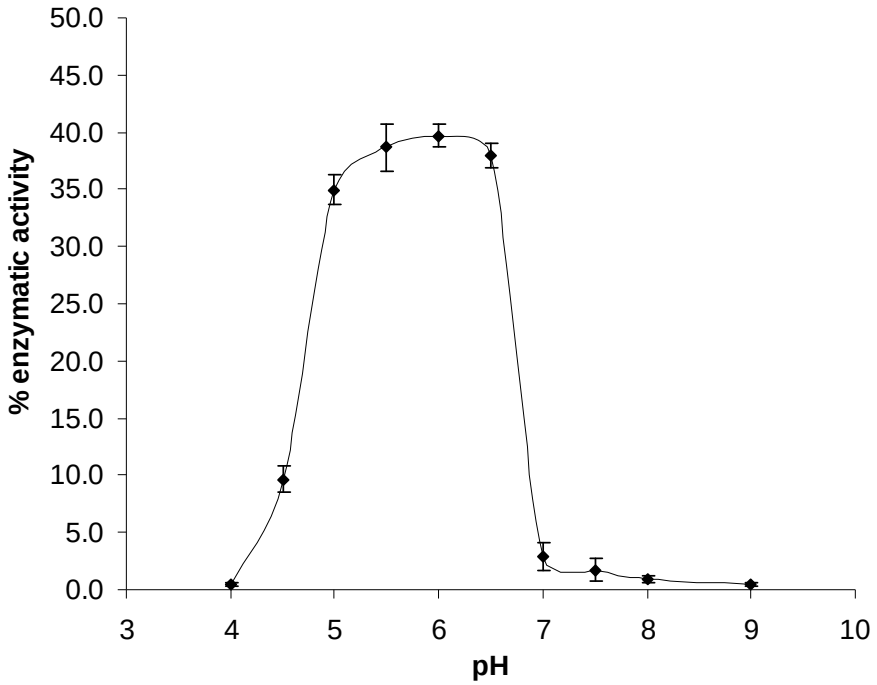


Figure S2. The pH profile of BiGalHexNAcP by HPLC analysis. Buffers (250 mM) used were: acetate-NaOH, pH 4.0–5.5; MES-KOH, pH 5.5–6.5; Tris-HCl, pH 7.0–9.0.

General Methods

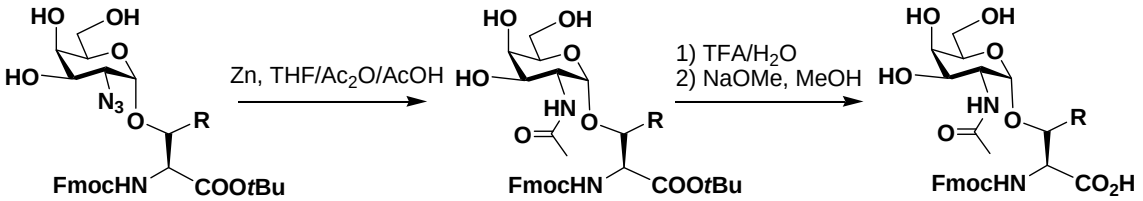
^{1}H NMR (300, 400 or 600 MHz) and ^{13}C NMR (75 or 100 MHz) spectra were recorded on a Varian Mercury-300, a Varian Inova-400, or a Varian Inova-600 spectrometer. . High resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility in the University of California at Davis. Silica gel 60 Å (40–63 μm, Sorbent technologies) was used for flash chromatography. Analytical thin-layer chromatography was performed on silica gel plates 60 GF_{254} (Sorbent technologies) using *p*-anisaldehyde sugar stain for detection. Gel filtration chromatography was performed using a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad, Hercules, CA). Chemicals were purchased and used without further purification.

Chemical synthesis of GlcNAc and GalNAc derivatives

Synthesis of GalNAc/GlcNAc derivatives as potential acceptors for BiGalHexNAcP

GlcNAc **1** and GalNAc **11** were purchased from Sigma. Compounds **2**–**10** and **12**–**20** were synthesized as described previously.[2-5] GalNAcα1-*O*-Ser **41**, GalNAcα1-*O*-Thr **42**, and GlcNAcαProNH2AA were synthesized as described below.

Synthesis of GalNAcα1-*O*-Ser 41 and GalNAcα1-*O*-Thr 42



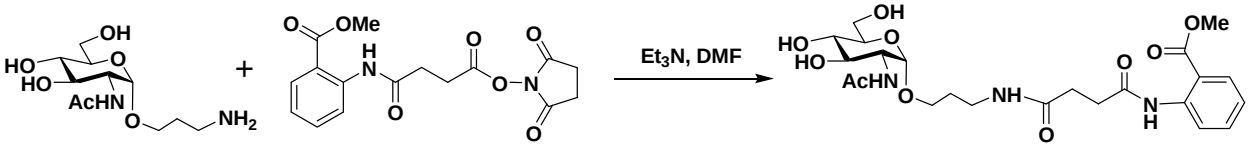
To the solution of compound **S1**[6] (2.5 g, 4.86 mmol) or **S2**[7] (2.1 g, 4.08 mmol) in THF/HOAc/Ac_2O (60 mL, 3:2:1) was added a solution of Zn (4 g) in 50 mL of $CuSO_4$ (2%) water solution. The mixture was stirred for 1 h. After filtration, the solvent mixture was removed *in vacuo* and the residue was purified by a flash chromatography using a silica gel column. The obtained the product was dissolved in trifluoroacetic acid (9.5 mL) and H_2O (0.5 mL). After stirring at room temperature for 1 h, the solvent mixture was removed *in vacuo*. Toluene was added to the residue and removed *in vacuo*. The process was repeated two times. The crude

product was dissolved in dry MeOH (30 mL), and a solution of 2% NaOMe in methanol was added to ajust the pH to 8.5. After stirred for 4 h, the pH was adjusted to 7.0 by adding resin (H^+). After filtration and evaporation of the solution, the residue was purified by silica gel chromatography to produce the desired product **41** or **42**.

GalNAcα1-*O*-Ser (**41**, 84% in three steps): δ 7.23–6.82 (m, 8 H), 4.82 (d, 1 H, *J* = 3.6 Hz), 4.13–3.31 (m, 12 H), 1.91 (s, 3H). ^{13}C (150 MHz, D_2O), 174.53, 170.04, 156.86, 143.76, 143.52, 140.80, 127.59, 127.11, 124.91, 119.81, 97.94, 71.05, 68.63, 68.53, 67.78, 66.71, 61.26, 55.69, 49.89, 46.62, 22.26.

GalNAcα1-*O*-Thr (**42**, 81% in three steps): δ 7.24–6.78 (m, 8 H), 4.83 (d, 1 H, *J* = 3.6 Hz), 4.25–3.31 (m, 11 H), 1.97 (s, 3H), 1.02 (d, 1 H, *J* = 6.0 Hz). ^{13}C (150 MHz, D_2O), 174.87, 174.51, 157.80, 143.79, 143.47, 140.82, 127.56, 127.02, 124.97, 119.77, 99.01, 76.45, 71.15, 68.70, 67.81, 66.62, 61.34, 59.52, 50.03, 46.75, 22.50, 18.36.

Synthesis of GlcNAcαProNH2AA



To a solution of GlcNAcαProNH$_2$[3] (130 mg, 0.46 mmol) in 10 mL anhydrous DMF, dry triethylamine (120 μL) was added under argon. Then 2-(methoxycarbonyl)succinanilic acid NHS ester[1] (2AA-OSu, 325 mg, 0.93mmol) was added at 0°C. The reaction mixture was stirred at room temperature for overnight. The reaction mixture was concentrated and the residue was purified by silica gel chromatography (EtOAc:MeOH:H_2O = 8:2:1) to afford pure GlcNAcαProNH2AA (208 mg, 87%). ^{1}H NMR (300 MHz, D_2O) δ 8.48 (d, 1 H, *J* = 8.4 Hz), 8.02 (dd, 1 H, *J* = 7.2 and 8.4 Hz), 7.56 (t, 1 H, *J* = 7.8 Hz), 7.14 (t, 1 H, *J* = 7.8 Hz), 4.72 (d, 1 H, *J* = 3.0 Hz), 3.86–3.58 (m, 7 H), 3.82 (s, 3 H), 3.56 (m, 1 H), 3.23 (t, 2 H, *J* = 6.6 Hz), 2.66 (t, 2 H, *J* = 6.9 Hz), 2.54 (t, 2 H, *J* = 6.9 Hz), 1.97 (s, 3 H), 1.72 (m, 2 H). ^{13}C NMR (75 MHz, D_2O) δ 171.62, 171.58, 170.09, 166.96, 139.10, 132.55, 129.21, 121.36, 119.05, 114.69, 95.76, 71.11, 70.34, 69.63, 63.08, 60.01, 52.63, 50.22, 34.58, 31.20, 28.89, 27.49, 19.89.

Enzymatic synthesis of β1–3 linked galactosides

General one-pot two-enzyme preparative synthesis of β1–3-linked galactosides. A prospective hexosamine acceptor for BiGalHexNAcP (GalNAc, GlcNAc, or one of their derivatives, 50–100 mg), galactose (1.2 equiv. was used for **3–5** and **13–15**; 1.5 equiv. was used for **1–2**, **6–7**, **10–12**, **16**, **20**, and **41–42**), and ATP (1.2 equiv. was used for **3–5** and **13–15**; 1.5 equiv. was used for **1–2**, **6–7**, **10–12**, **16**, **20**, and **41–42**) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 6.5) and $MgCl_2$ (20 mM). After the addition of appropriate amount of GalK (2.0–4.5 mg) and BiGalHexNAcP (1.5–3.0 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 24 h at 37°C with agitation at 140 rpm. The product formation was monitored by TLC developed with CH_3CN:H_2O = 4:1 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by adding the same volume (10 mL) of ice-cold EtOH and incubating at 4°C for 30 min. The mixture was then centrifuged to remove precipitates. The supernatant was concentrated and purified by silica gel chromatography (EtOAc:MeOH:H_2O = 4:1:0.1) (a ratio of 2:1:0.1 was used for purifying **21**, **31**, **43**, and **44**). A BioGel P-2 gel filtration column was then used for additional purification.

β-D-Galactopyranosyl-(1–3)-2-acetamido-2-deoxy-D-glucopyranose (Galβ1–3GlcNAc, 21). Yield, 95%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.00 (d, 0.6 H, *J* = 3.6 Hz, H'-1α), 4.58 (d, 0.4 H, *J* = 8.4 Hz, H'-1β), 4.29 (d, 0.6 H, *J* = 7.8 Hz), 4.25 (d, 0.4 H, *J* = 7.8 Hz), 3.90–3.33 (m, 12 H), 1.86 (s, 3 H). ^{13}C NMR (150 MHz, D_2O) δ 174.90, 174.64, 103.67, 103.54, 94.82, 91.13, 82.67, 80.21, 75.55, 75.38, 75.33, 72.63, 72.60, 71.31, 70.81, 70.76, 68.79, 68.75, 68.64, 61.11, 60.81, 60.64, 55.70, 52.99, 22.34, 22.08. HRMS (ESI) *m/z* calcd for $C_{14}H_{25}NO_{11}$ HRMS (M+H) 384.1506, found 384.1504.

3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-β-D-glucopyranoside (Galβ1–3GlcNAcβProN$_3$, 22). Yield, 96%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.51 (d, 1 H, *J* = 8.4 Hz), 4.39 (d, 1 H, *J* = 7.8 Hz), 3.96–3.92 (m, 1 H), 3.90–3.87 (m, 2 H), 3.80–3.59 (m, 8 H), 3.51–3.44 (m, 3 H), 3.35–3.32 (m, 2 H), 2.00 (s, 3 H), 1.80 (m, 2 H). ^{13}C NMR (75 MHz, D_2O) δ 174.73, 103.64, 101.04, 82.48, 75.44, 75.38, 72.56, 70.77, 68.81, 68.61, 67.26, 61.13,

60.80, 54.66, 47.88, 28.21, 22.32. HRMS (ESI) *m/z* calcd for $C_{17}H_{31}N_4O_{11}$ (M+H) 467.1989, found 467.1989.

3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-α-D-glucopyranoside (Galβ1–3GlcNAcαProN$_3$, 23). Yield, 94%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.82 (d, 1 H, *J* = 2.4 Hz),, 4.40 (d, 1 H, *J* = 7.8 Hz), 4.07 (dd, 1 H, *J* = 1.8 and 10.8 Hz), 3.91–3.67 (m, 8 H), 3.61 (dd, 1 H, *J* = 1.8 and 10.2 Hz), 3.57–3.42 (m, 6 H), 2.00 (s, 3 H), 1.88 (m, 2 H). ^{13}C NMR (150 MHz, D_2O) δ 174.56, 103.54, 97.19, 80.32, 75.34, 72.62, 71.68, 70.80, 68.73, 68.66, 65.04, 61.12, 60.62, 52.63, 48.28, 28.09, 22.07. HRMS (ESI) *m/z* calcd for $C_{17}H_{31}N_4O_{11}$ (M+H) 467.1989, found 467.1986.

β-D-Galactopyranosyl-(1–3)-2-deoxy-2-trifluoroacetamido-D-glucopyranoside (Galβ1–3GlcNTFA, 24). Yield, 93%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.19 (d, 0.5 H, *J* = 3.6 Hz, H'-1α), 4.76 (d, 0.5 H, *J* = 8.4 Hz, H'-1β), 4.40 (d, 0.5 H, *J* = 7.8 Hz), 4.35 (d, 0.5 H, *J* = 7.8 Hz), 4.13 (dd, 0.5 H, *J* = 3.6 and 10.8 Hz), 4.00 (t, 0.5 H, *J* = 7.2 Hz), 3.77–3.32 (m, 11 H). ^{13}C NMR (150 MHz, D_2O) δ 159.68, 159.44, 159.36, 159.11, 116.86, 114.94, 103.75, 103.52, 94.22, 90.63, 82.30, 79.82, 75.63, 75.45, 75.38, 72.64, 71.24, 70.72, 70.67, 68.77, 68.70, 68.63, 68.61, 61.10, 61.08, 60.74, 60.60, 56.17, 53.67. HRMS (ESI) *m/z* calcd for $C_{14}H_{23}F_3NO_{11}$ (M+H) 438.1223, found 438.1219.

β-D-Galactopyranosyl-(1–3)-2-azidoacetamido-2-deoxy-D-glucopyranoside (Galβ1–3GlcNAcN$_3$, 25). Yield, 91%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.14 (d, 0.5 H, *J* = 3.0 Hz, H'-1α), 4.74 (d, 0.5 H, *J* = 7.8 Hz, H'-1β), 4.41 (d, 0.5 H, *J* = 7.8 Hz), 4.37 (d, 0.5 H, *J* = 8.4 Hz), 4.10–3.45 (m, 14 H). ^{13}C NMR (150 MHz, D_2O) δ 171.30, 170.92, 103.70, 103.54, 94.57, 91.05, 82.19, 80.01, 75.62, 75.40, 75.35, 72.68, 72.64, 71.34, 70.79, 70.73, 68.78, 68.73, 68.66, 62.56, 61.16, 61.13, 60.81, 60.64, 55.82, 53.08, 52.14, 51.88. HRMS (ESI) *m/z* calcd for $C_{14}H_{25}N_4O_{11}$ (M+H) 425.1520, found 425.1512.

β-D-Galactopyranosyl-(1–3)-2-deoxy-2-propionamido-D-glucopyranoside (Galβ1–3GlcNPr, 26). Yield, 86%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, 0.6 H, *J* = 3.6 Hz, H'-1α),

4.72 (d, 0.4 H, *J* = 7.2 Hz, H'-1β), 4.44 (d, 0.6 H, *J* = 7.8 Hz), 4.39 (d, 0.4 H, *J* = 7.8 Hz), 4.05–3.40 (m, 12 H), 2.28 (m, 2 H), 1.09 (m, 3 H). ^{13}C NMR (150 MHz, D_2O) δ 178.75, 178.54, 103.58, 103.45, 94.91, 91.20, 82.38, 80.01, 75.59, 75.43, 75.38, 72.67, 72.63, 71.34, 70.93, 70.87, 68.87, 68.83, 68.70, 68.69, 61.17, 61.15, 60.90, 60.74, 55.77, 52.96, 29.51, 29.23, 9.48, 9.40. HRMS (ESI) *m/z* calcd for $C_{15}H_{28}NO_{11}$ (M+H) 398.1662, found 398.1664.

β-D-Galactopyranosyl-(1–3)-2-butyramido-2-deoxy-D-glucopyranoside (Galβ1–3GlcNBu, 27). Yield, 78%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.13 (d, 0.6 H, *J* = 3.6 Hz, H'-1α), 4.71 (d, 0.4 H, *J* = 7.2 Hz, H'-1β), 4.44 (d, 0.6 H, *J* = 7.8 Hz), 4.40 (d, 0.4 H, *J* = 7.8 Hz), 4.05–3.45 (m, 12 H), 2.23 (m, 2 H), 1.58 (m, 2 H), 0.89 (m, 3 H). ^{13}C NMR (150 MHz, D_2O) δ 177.95, 177.72, 103.48, 103.36, 94.92, 91.18, 82.16, 79.76, 75.57, 75.42, 75.38, 72.66 72.61, 71.30, 70.93, 70.87, 68.86, 68.81, 68.70, 68.68, 61.17, 61.15, 60.88, 60.72, 55.81, 53.03, 49.00, 38.23, 37.85, 19.05, 19.00, 12.98. HRMS (ESI) *m/z* calcd for $C_{16}H_{30}NO_{11}$ (M+H) 412.1819, found 412.1812.

β-D-Galactopyranosyl-(1–3)-2-acetamido-2,6-dideoxy-D-glucopyranoside Galβ1–3GlcNAc6Deoxy, 30). Yield, 84%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.06 (d, 0.5 H, *J* = 3.6 Hz, H'-1α), 4.67 (d, 0.5 H, *J* = 8.4 Hz, H'-1β), 4.40 (d, 0.5 H, *J* = 7.8 Hz), 4.36 (d, 0.5 H, *J* = 7.8 Hz), 4.03 (dd, 0.5 H, *J* = 3.0 and 7.8 Hz), 3.90 (m, 0.5 H), 3.85–3.24 (m, 9 H), 1.97 (s, 3 H), 1.26 (d, 1.5 H, *J* = 6.6 Hz), 1.23 (d, 1.5 H, *J* = 6.6 Hz). ^{13}C NMR (150 MHz, D_2O) δ 174.88, 174.63, 103.62, 103.47, 94.62, 90.95, 82.31, 79.81, 75.36, 75.30, 74.34, 73.99, 72.63, 72.60, 71.60, 70.78, 70.75, 68.84, 68.62, 67.36, 61.09, 61.08, 55.90, 53.20, 22.33, 22.08, 17.09, 17.07. HRMS (ESI) *m/z* calcd for $C_{14}H_{26}NO_{10}$ (M+H) 368.1557, found 368.1547.

β-D-Galactopyranosyl-(1–3)-2-acetamido-2-deoxy-D-galactopyranose (Galβ1–3GalNAc, 31). Yield, 93%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.19 (d, 0.6 H, *J* = 3.6 Hz, H'-1α), 4.67 (d, 0.4 H, *J* = 8.4 Hz, H'-1β), 4.47 (d, 0.6 H, *J* = 7.8 Hz), 4.41 (d, 0.4 H, *J* = 7.8 Hz), 4.27 (dd, 0.4 H, *J* = 3.6 and 11.4 Hz), 4.22 (d, 0.6 H, *J* = 3.0 Hz), 4.15 (d, 0.4 H, *J* = 3.0 Hz), 4.12 (t, 0.6 H, *J* = 6.0 Hz), 4.00 (dd, 0.6 H, *J* = 3.0 and 11.4 Hz), 3.96 (t, 0.4 H, *J* = 11.4 Hz), 3.90–3.58 (m, 8 H), 3.50 (t, 1 H, *J* = 9.0 Hz), 2.00 (s, 3 H). ^{13}C NMR (150 MHz, D_2O) δ 175.07,

174.78, 104.99, 104.81, 95.30, 91.31, 80.18, 77.17, 75.08, 74.90, 72.66, 72.61, 70.75, 70.69, 70.31, 68.86, 68.69, 68.19, 61.29, 61.08, 61.07, 52.56, 49.01, 22.37, 22.14. HRMS (ESI) *m/z* calcd for $C_{14}H_{26}NO_{11}$ (M+H) 384.1506, found 384.1508.

3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-β-D-galactopyranoside (Galβ1–3GalNAcβProN$_3$, 32). Yield, 95%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.48 (d, 1 H, *J* = 8.4 Hz), 4.42 (d, 1 H, *J* = 7.8 Hz), 4.16 (d, 1 H, *J* = 3.0 Hz), 3.99–3.94 (m, 2 H), 3.88 (d, 1 H, *J* = 3.0 Hz), 3.84 (dd, 1 H, *J* = 3.0 Hz and 10.8 Hz), 3.80–3.58 (m, 10 H), 3.50 (dd, 1 H, *J* = 8.4 Hz), 3.37–3.32 (m, 2 H), 2.01 (s, 3 H), 1.82 (m, 2 H). ^{13}C NMR (150 MHz, D_2O) δ 174.87, 105.00, 101.54, 80.05, 75.13, 74.89, 72.61, 70.73, 68.72, 68.15, 67.16, 61.15, 61.08, 51.43, 47.94, 28.26, 22.38. HRMS (ESI) *m/z* calcd for $C_{17}H_{31}N_4O_{11}$ (M+H) 467.1989, found 467.1991.

3-Azidopropyl β-D-galactopyranosyl-(1–3)-2-acetamido-2-deoxy-α-D-galactopyranoside (Galβ1–3GalNAcαProN$_3$, 33). Yield, 92%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.90 (d, 1 H, *J* = 3.6 Hz), 4.48 (d, 1 H, *J* = 7.8 Hz), 4.35 (dd, 1 H, *J* = 10.8 Hz and 3.6 Hz), 4.26 (d, 1 H, *J* = 2.4 Hz), 4.04 (dd, 1 H, *J* = 10.8 Hz and 3.0 Hz), 4.00 (t, 1 H, *J* = 6.0 Hz), 3.92 (d, 1 H, *J* = 3.0 Hz), 3.83–3.74 (m, 5 H), 3.68–3.63 (m, 2 H), 3.58–3.45 (m, 4 H), 2.04 (s, 3 H), 1.92 (m, 2 H). ^{13}C NMR (150 MHz, D_2O) δ 174.70, 104.89, 97.41, 77.40, 75.18, 72.71, 70.81, 68.92, 68.79, 65.13, 61.39, 61.19, 48.87, 48.41, 28.17, 22.22. HRMS (ESI) *m/z* calcd for $C_{17}H_{31}N_4O_{11}$ (M+H) 467.1989, found 467.1985.

β-D-galactopyranosyl-(1–3)-2-deoxy-2-trifluoroacetamido-D-galactopyranoside (Galβ1–3GalNTFA, 34). Yield, 94%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.26 (d, 0.4 H, *J* = 3.6 Hz, H'-1α), 4.75 (d, 0.6 H, *J* = 8.4 Hz, H'-1β), 4.47 (d, 0.4 H, *J* = 8.4 Hz), 4.40 (d, 0.6 H, *J* = 8.4 Hz), 4.36 (dd, 0.4 H, *J* = 3.6 and 10.8 Hz), 4.25 (d, 0.4 H, *J* = 2.4 Hz), 4.20 (d, 0.6 H, *J* = 2.4 Hz), 4.14 (t, 0.6 H, *J* = 6.0 Hz), 4.05 (t, 0.4 H, *J* = 10.8 Hz), 3.93 (dd, 0.6 H, *J* = 3.0 and 10.8 Hz), 3.87–3.55 (m, 8 H), 3.46 (t, 1 H, *J* = 9.0 Hz). ^{13}C NMR (150 MHz, D_2O) δ 159.81, 159.74, 159.57, 159.49, 159.32, 159.24, 158.99, 116.89, 116.86, 114.99, 114.96, 104.99, 104.78, 94.60, 90.80, 79.69, 76.65, 75.09, 75.07, 75.05, 72.65, 72.63, 70.69, 70.62, 70.29, 68.78, 68.67, 68.03,

61.25, 61.09, 61.06, 61.01, 53.22, 49.98. HRMS (ESI) *m/z* calcd for $C_{14}H_{23}F_3NO_{11}$ (M+H) 438.1223, found 438.1222.

β-D-Galactopyranosyl-(1–3)-2-azidoacetamido-2-deoxy-D-galactopyranoside (Galβ1–3GalNAcN_3, 35). Yield, 92%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.07 (d, 0.5 H, *J* = 4.2 Hz, H'-1α), 4.58 (d, 0.5 H, *J* = 8.4 Hz, H'-1β), 4.32 (d, 0.5 H, *J* = 7.2 Hz), 4.27 (d, 0.5 H, *J* = 7.8 Hz), 4.20 (dd, 0.5 H, *J* = 3.6 and 10.8 Hz), 4.09 (d, 0.5 H, *J* = 3.0 Hz), 4.02 (d, 0.5 H, *J* = 3.0 Hz), 3.99 (t, 0.5 H, *J* = 6.6 Hz), 3.93–3.33 (m, 2 H). ^{13}C NMR (150 MHz, D_2O) δ 171.43, 171.03, 104.93, 104.76, 95.00, 91.19, 79.72, 77.00, 75.12, 75.08, 75.01, 72.65, 72.60, 70.72, 70.65, 70.33, 68.82, 68.68, 68.17, 61.27, 61.10, 61.05, 52.71, 52.13, 51.90, 49.22. HRMS (ESI) *m/z* calcd for $C_{14}H_{25}N_4O_{11}$ (M+H) 425.1520, found 425.1519.

β-D-Galactopyranosyl-(1–3)-2-deoxy-2-propionamido-D-galactopyranoside (Galβ1–3GalNPr, 36). Yield, 69%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.16 (d, 0.5 H, *J* = 3.6 Hz, H'-1α), 4.64 (d, 0.5 H, *J* = 8.4 Hz, H'-1β), 4.44 (d, 0.5 H, *J* = 7.2 Hz), 4.38 (d, 0.5 H, *J* = 7.2 Hz), 4.26–3.29 (m, 12 H), 2.25 (m, 2 H), 1.06 (m, 3 H). ^{13}C NMR (150 MHz, D_2O) δ 178.90, 178.63, 104.89, 104.70, 95.32, 91.32, 79.87, 76.93, 75.05, 74.92, 72.62, 72.55, 70.80, 70.74, 70.26, 68.87, 68.68, 68.24, 61.29, 61.07, 59.97, 52.49, 48.97, 29.52, 29.25, 9.54, 9.47. HRMS (ESI) *m/z* calcd for $C_{15}H_{27}NaNO_{11}$ (M+Na) 420.1482, found 420.1472.

β-D-Galactopyranosyl-(1–3)-2-acetamido-6-azido-2,6-dideoxy-D-glucopyranoside (Galβ1–3GlcNAc6N_3, 40). Yield, 87%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.12 (d, 0.6 H, *J* = 3.6 Hz, H'-1α), 4.72 (d, 0.4 H, *J* = 8.4 Hz, H'-1β), 4.41 (d, 0.6 H, *J* = 7.8 Hz), 4.36 (d, 0.4 H, *J* = 7.8 Hz), 4.05–3.45 (m, 12 H), 1.98 (s, 3 H). ^{13}C NMR (150 MHz, D_2O) δ 174.88, 174.63, 103.66, 103.54, 94.84, 91.18, 82.30, 79.88, 75.37, 75.32, 74.17, 72.63, 72.59, 70.79, 70.76, 70.07, 69.63, 69.52, 68.64, 68.62, 61.12, 55.67, 52.94, 51.10, 51.03, 22.33, 22.08. HRMS (ESI) *m/z* calcd for $C_{14}H_{25}N_4O_{10}$ (M+H) 409.1571, found 409.1565.

***N*-(*9H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-3-*O*-[β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-serine (Galβ1–3GalNAcαSer, 43)**. Yield, 92%;

white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.66–7.21 (m, 8 H), 4.79 (d, 1 H, *J* = 3.6 Hz), 4.39–4.26 (m, 4H), 4.15–3.47 (m, 14 H), 1.97 (s, 3H). ^{13}C (150 MHz, D_2O), 175.52, 172.41, 157.85, 144.99, 140.97, 128.04, 127.49, 125.04, 120.17, 104.75, 98.67, 77.47, 74.86, 72.58, 70.75, 70.60, 69.73, 69.10, 68.61, 66.69, 61.17, 60.93, 48.96, 48.52, 46.87, 22.07. HRMS (ESI) *m/z* calcd for $C_{32}H_{41}N_2O_{15}$ (M+H) 693.2507, found 693.2505.

***N*-*(9H*-Fluoren-9-yl)methoxycarbonyl-*O*-(2-acetamido-2-deoxy-3-*O*-[β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine (Galβ1–3GalNAcαThr, 44)**. Yield, 91%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 7.90–7.36 (m, 8 H), 4.85 (d, 1 H, *J* = 3.6 Hz), 4.62 (dd, 1 H, *J* = 3.0 and 12.0 Hz), 4.33 (d, 1 H, *J* = 7.8 Hz), 4.30–4.12 (m, 4H), 3.94–3.55 (m, 10 H), 3.46 (dd, 1 H, *J* = 8.4 and 9.6 Hz), 1.98 (s, 3H), 1.01 (d, 1 H, *J* = 6.0 Hz). ^{13}C (150 MHz, D_2O), 176.26, 174.75, 155.87, 143.79, 143.47, 140.86, 140.85, 127.76, 127.24, 124.93, 119.96, 104.78, 99.16, 77.77, 77.40, 74.86, 72.58, 70.76, 70.64, 68.78, 68.51, 61.21, 60.99, 60.44, 52.08, 48.65, 46.91, 22.53, 18.40. HRMS (ESI) *m/z* calcd for $C_{33}H_{43}N_2O_{15}$ (M+H) 707.2663, found 707.2667.

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(1) Zhang, L.; Lau, K.; Cheng, J.; Yu, H.; Li, Y.; Sugiarto, G.; Huang, S.; Ding, L.; Thon, V.; Wang, P. G.; Chen, X. *Glycobiology,* 2010; doi: doi:10.1093/glycob/cwq068.

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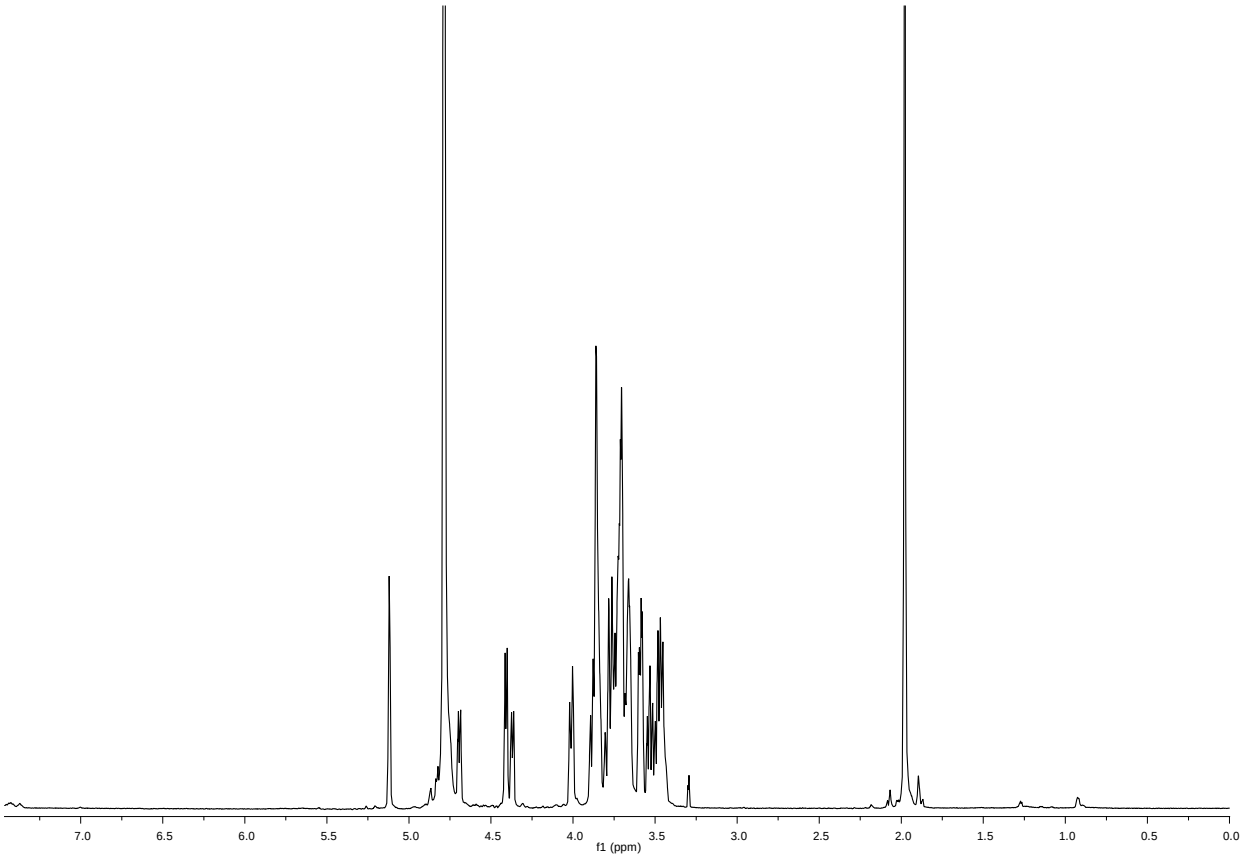
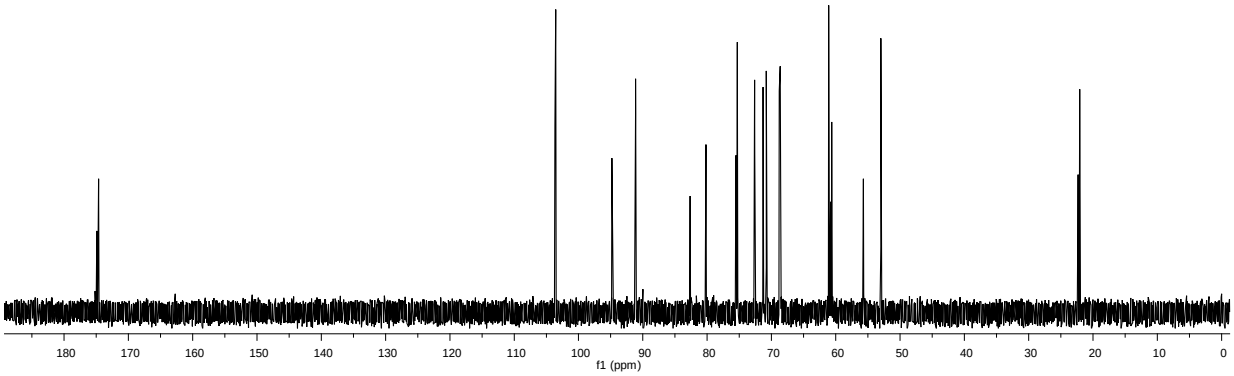
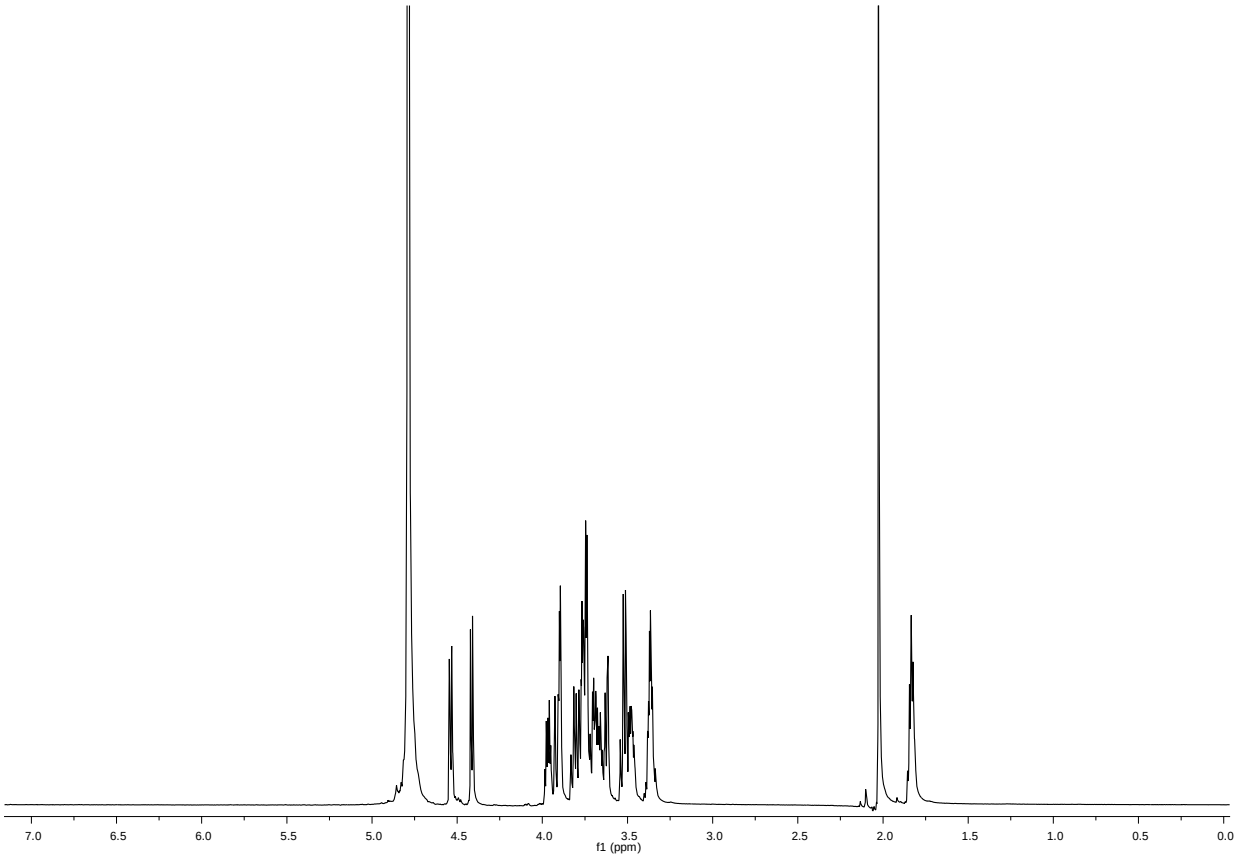
(7) Liebe, B.; Kunz, H. *Angew. Chem. Int. Ed. Engl.*, 1997, *36*, 618–621.

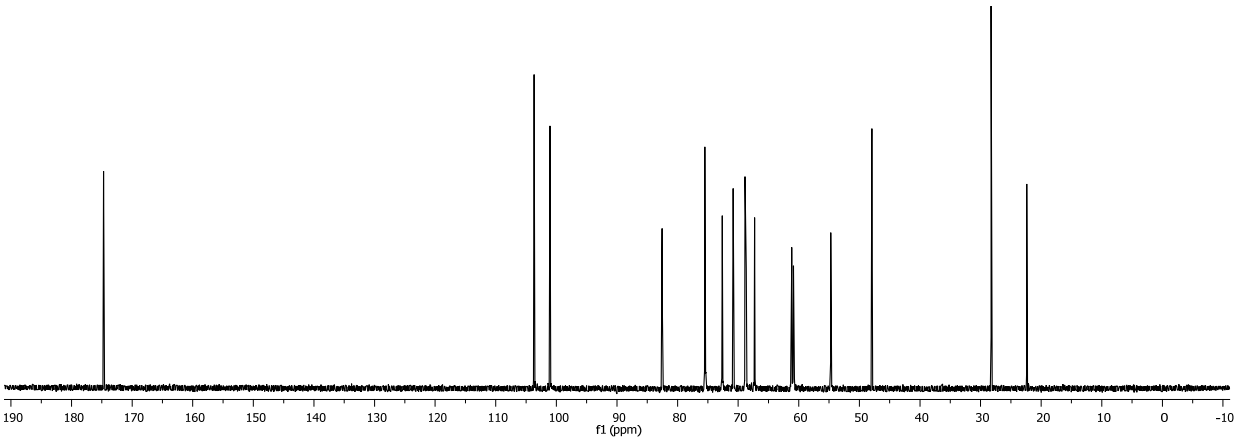
Compound **21**, Galβ1–3GlcNAc



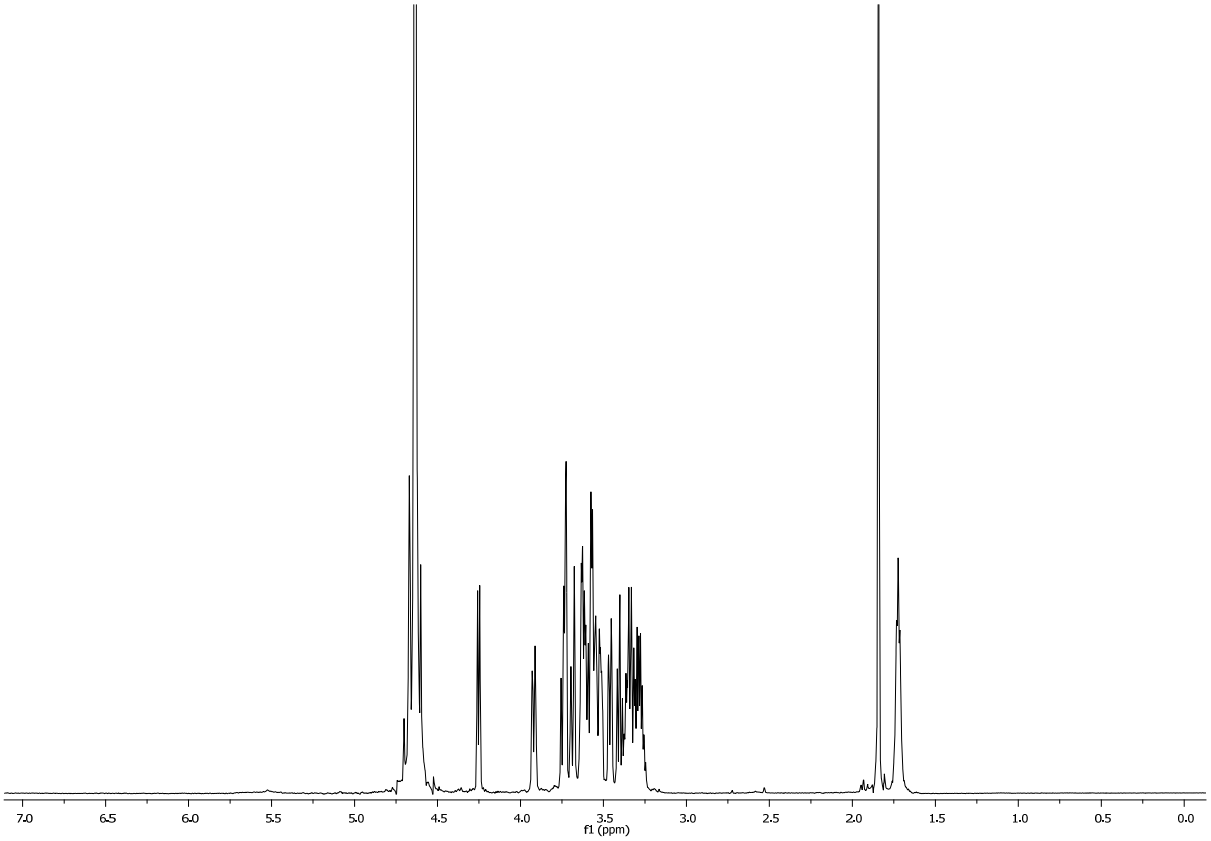


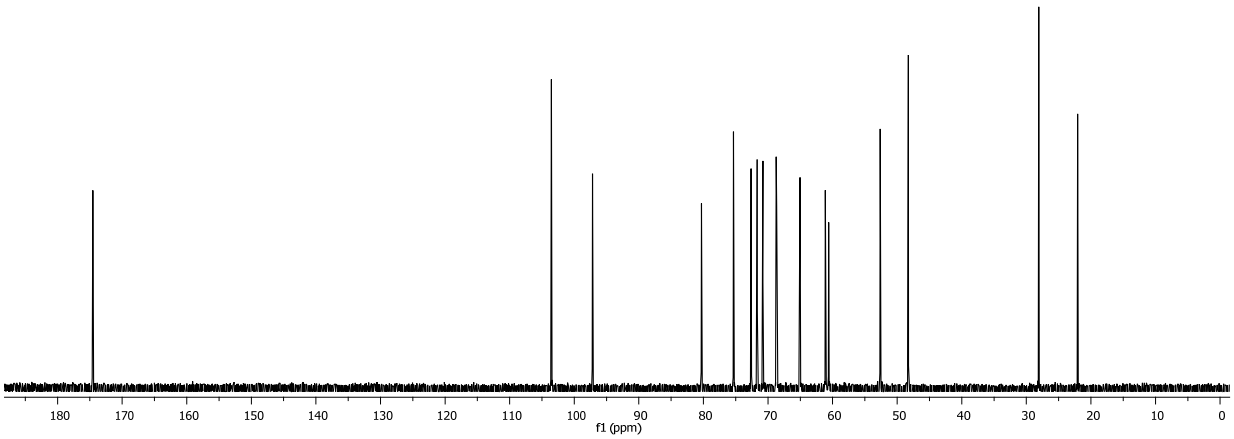
Compound **22**, Galβ1–3GlcNAcβProN_3



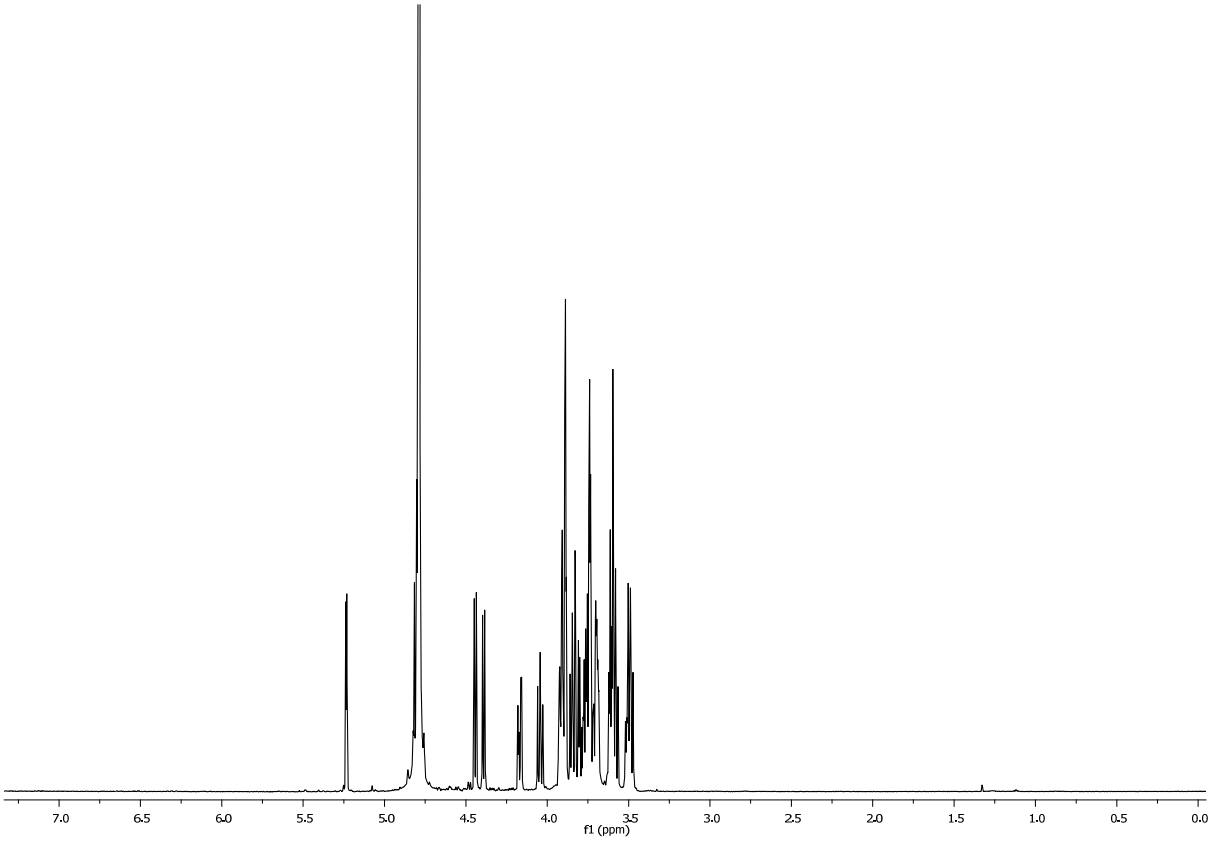


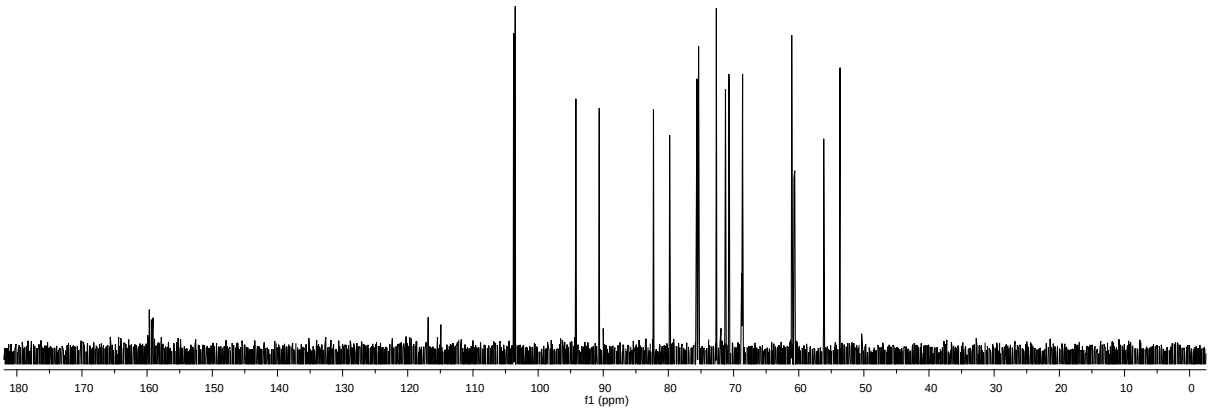
Compound **23**, Galβ1–3GlcNAcαProN_3



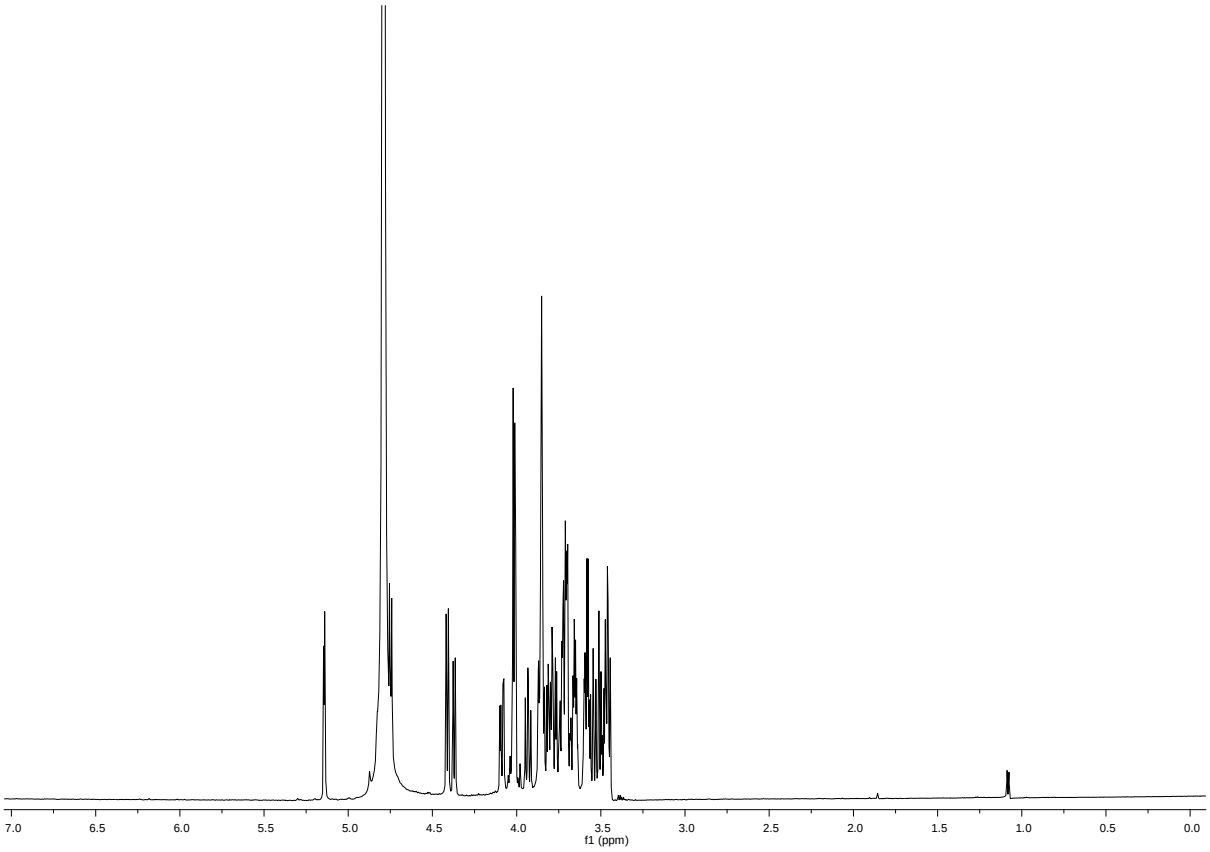


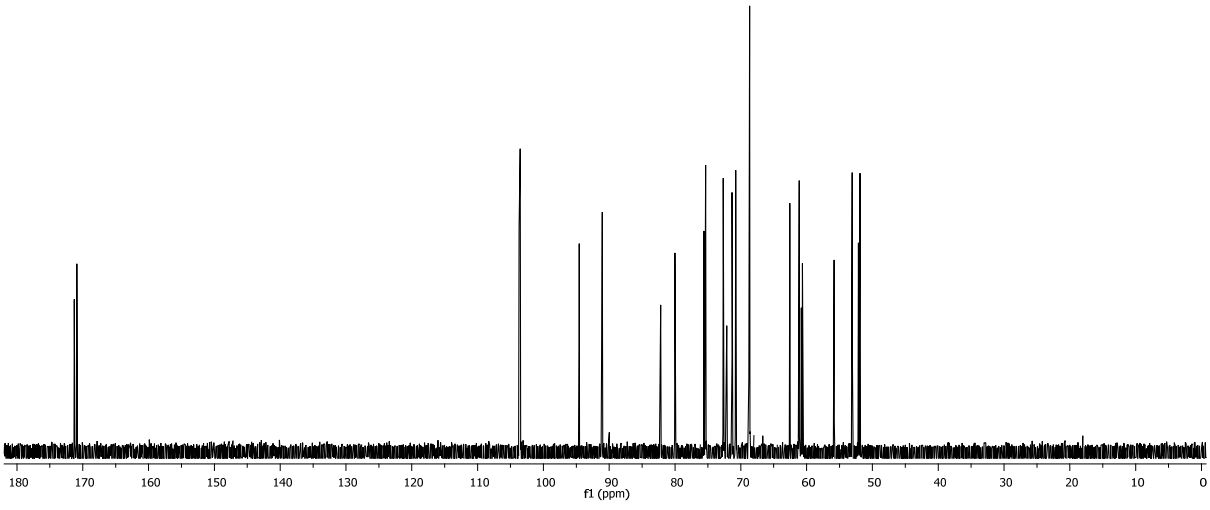
Compound **24**, Galβ1–3GlcNTFA



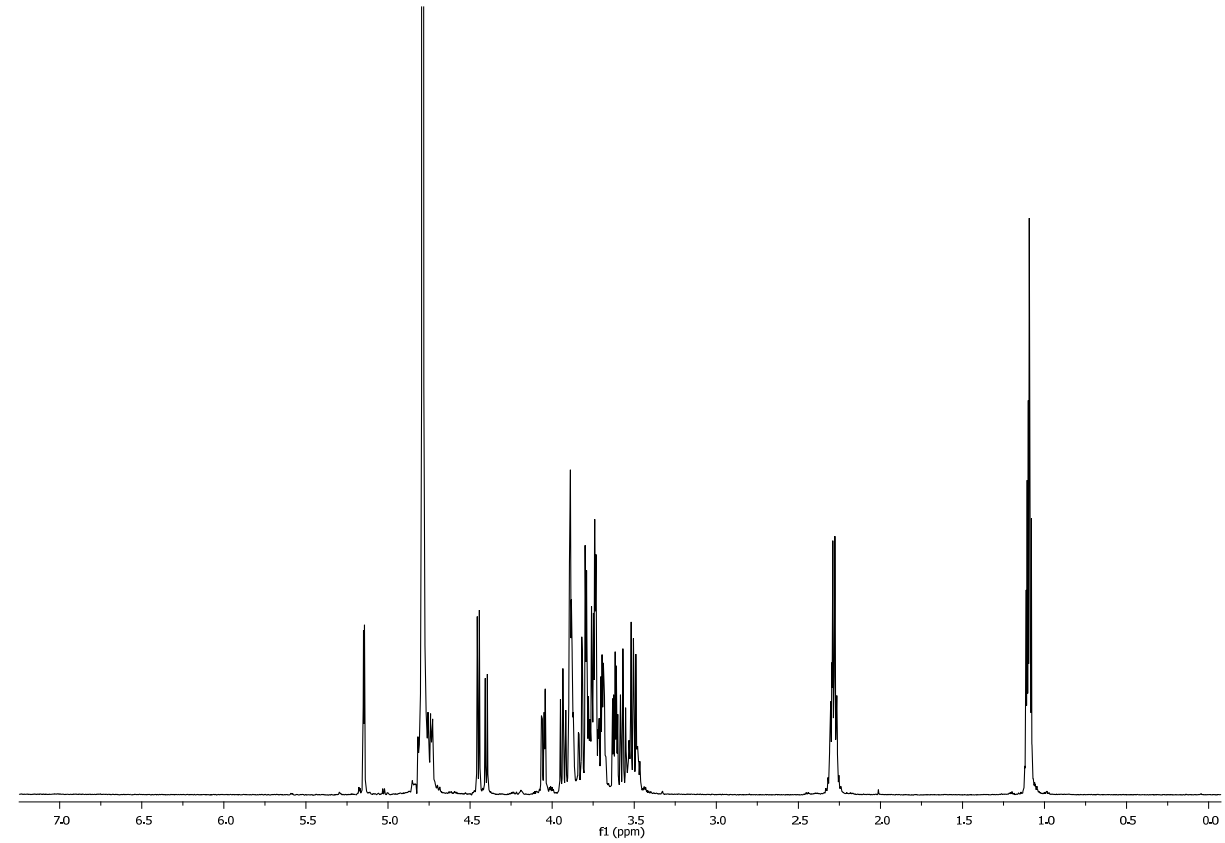


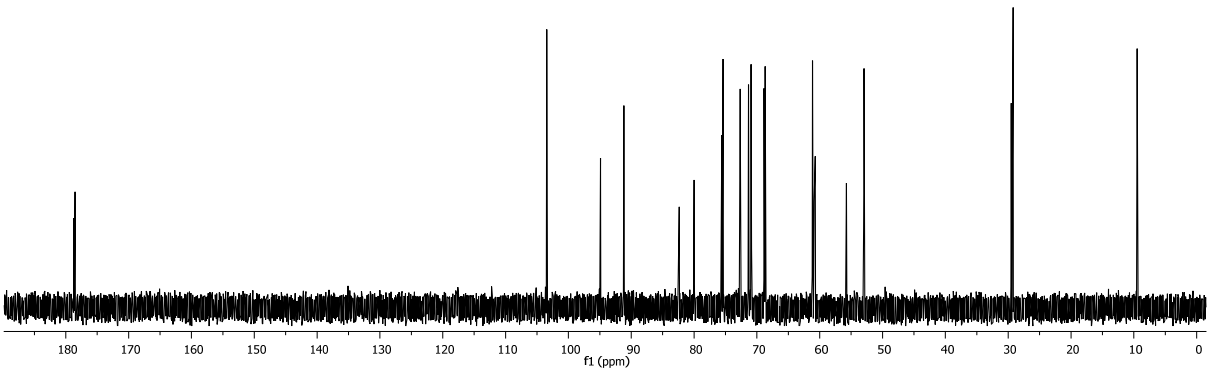
Compound **25**, Galβ1–3GlcNAcN_3



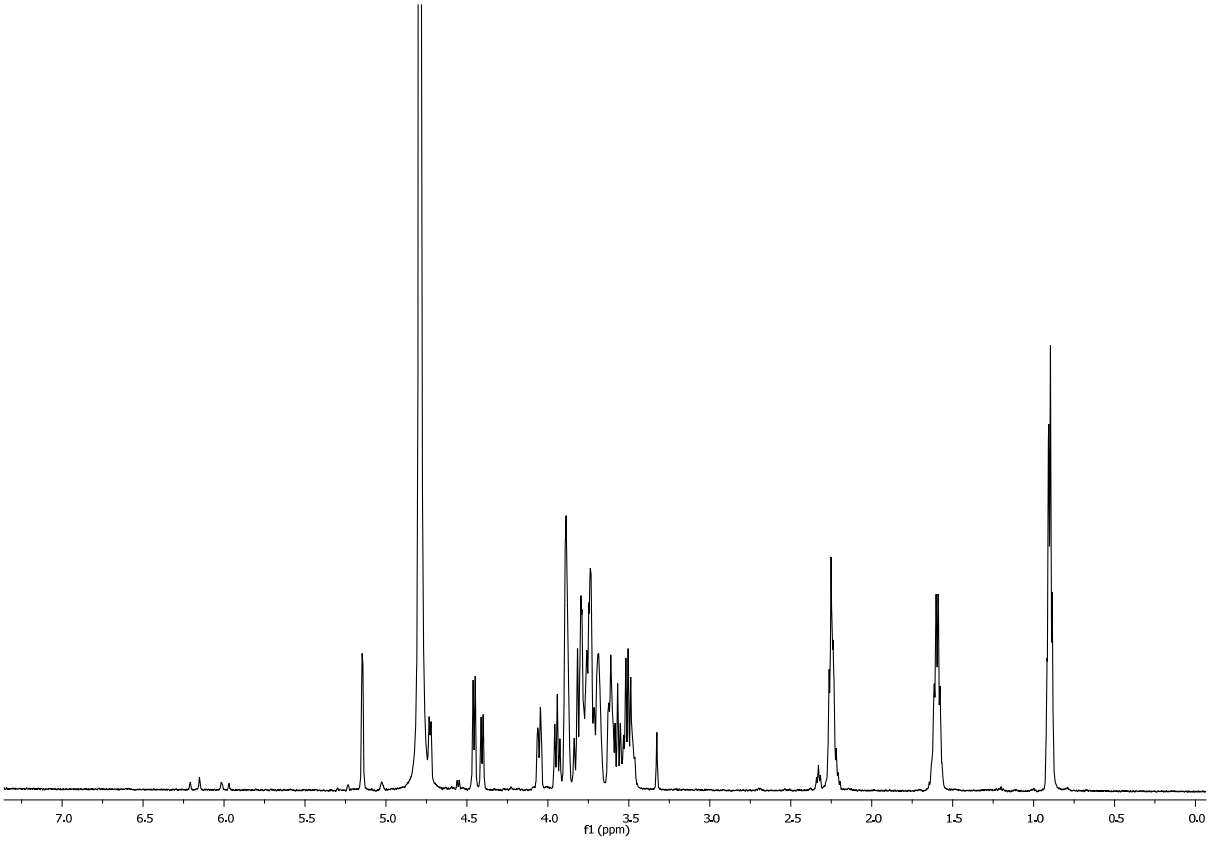


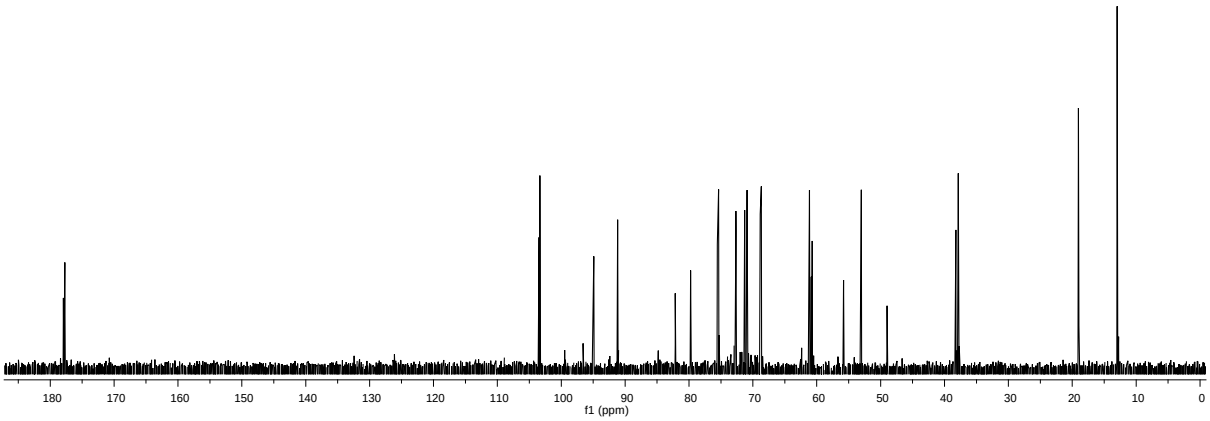
Compound **26**, Galβ1–3GlcNPr



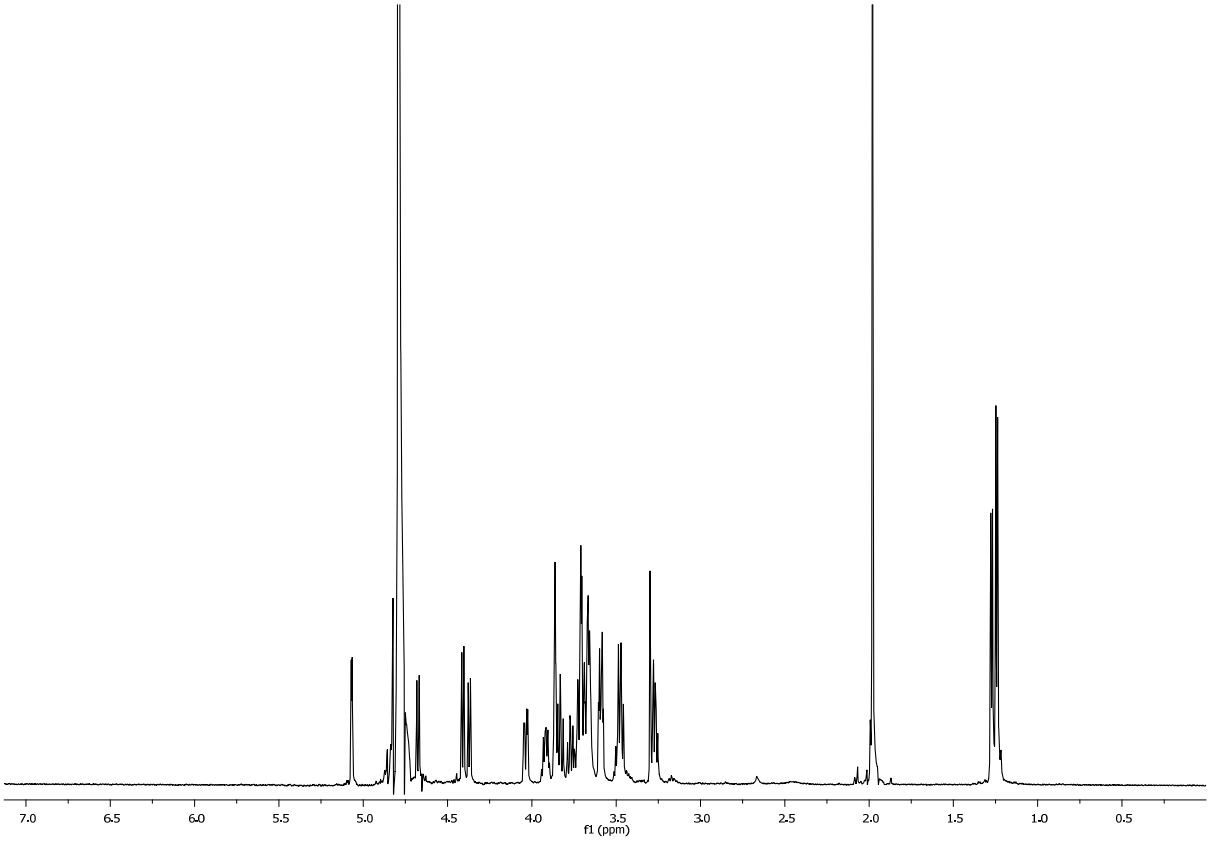


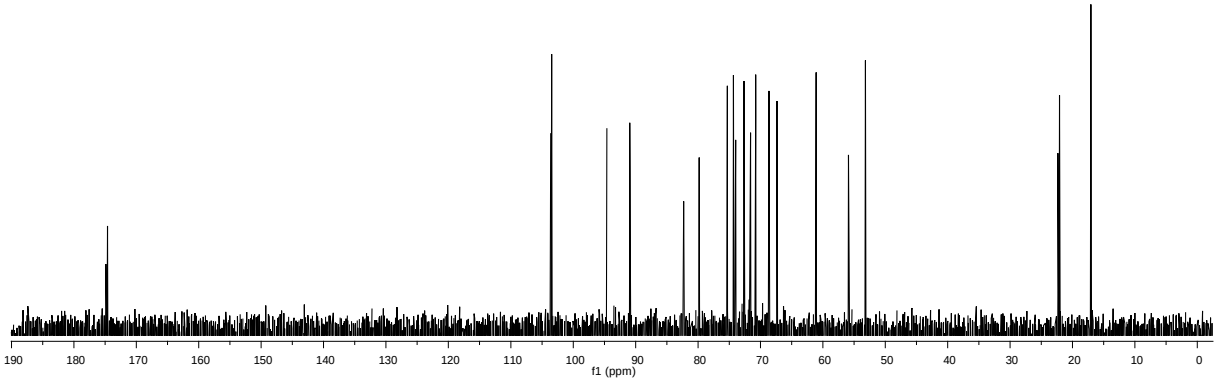
Compound **27**, Galβ1–3GlcNBu



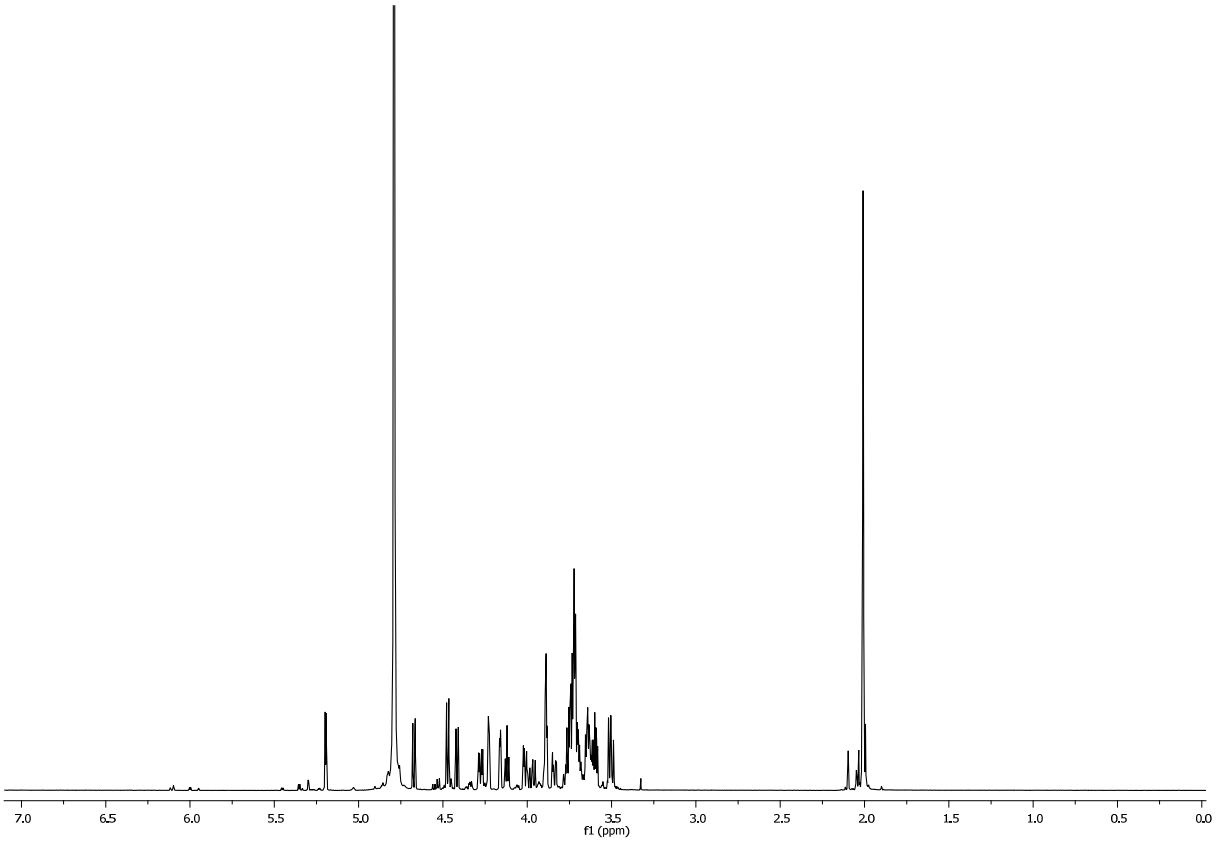


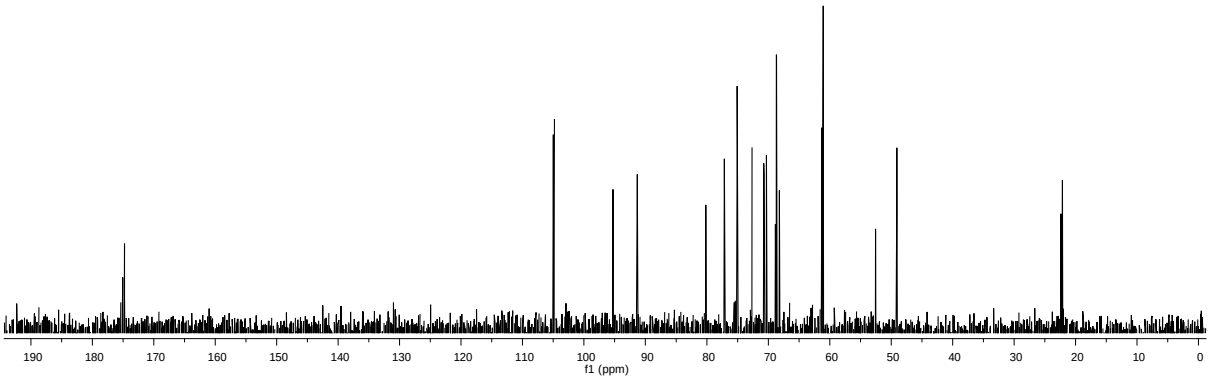
Compound **30**, Galβ1–3GlcNAc6Deoxy



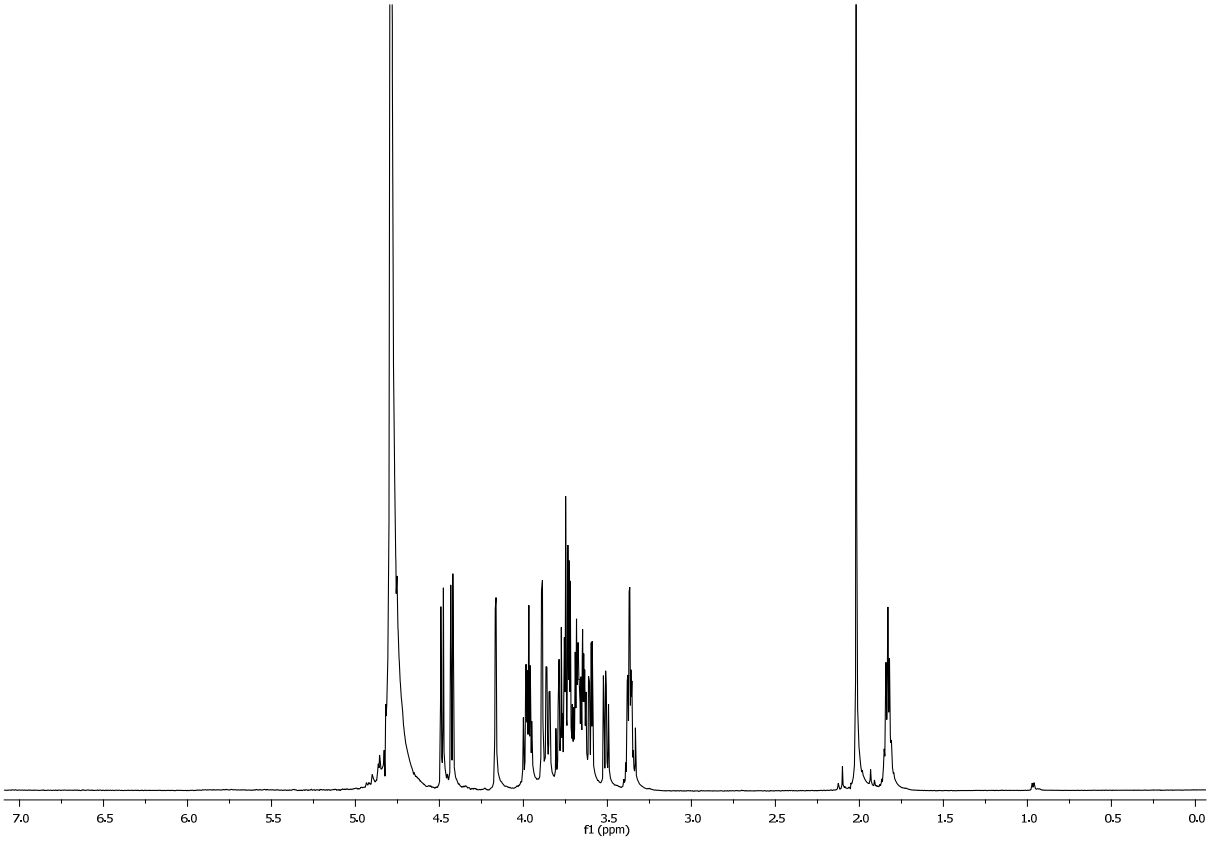


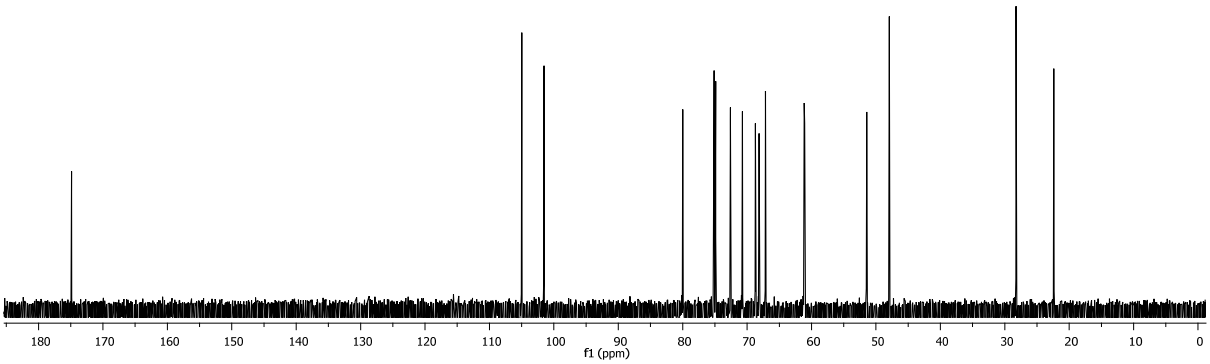
Compound **31**, Galβ1–3GalNAc



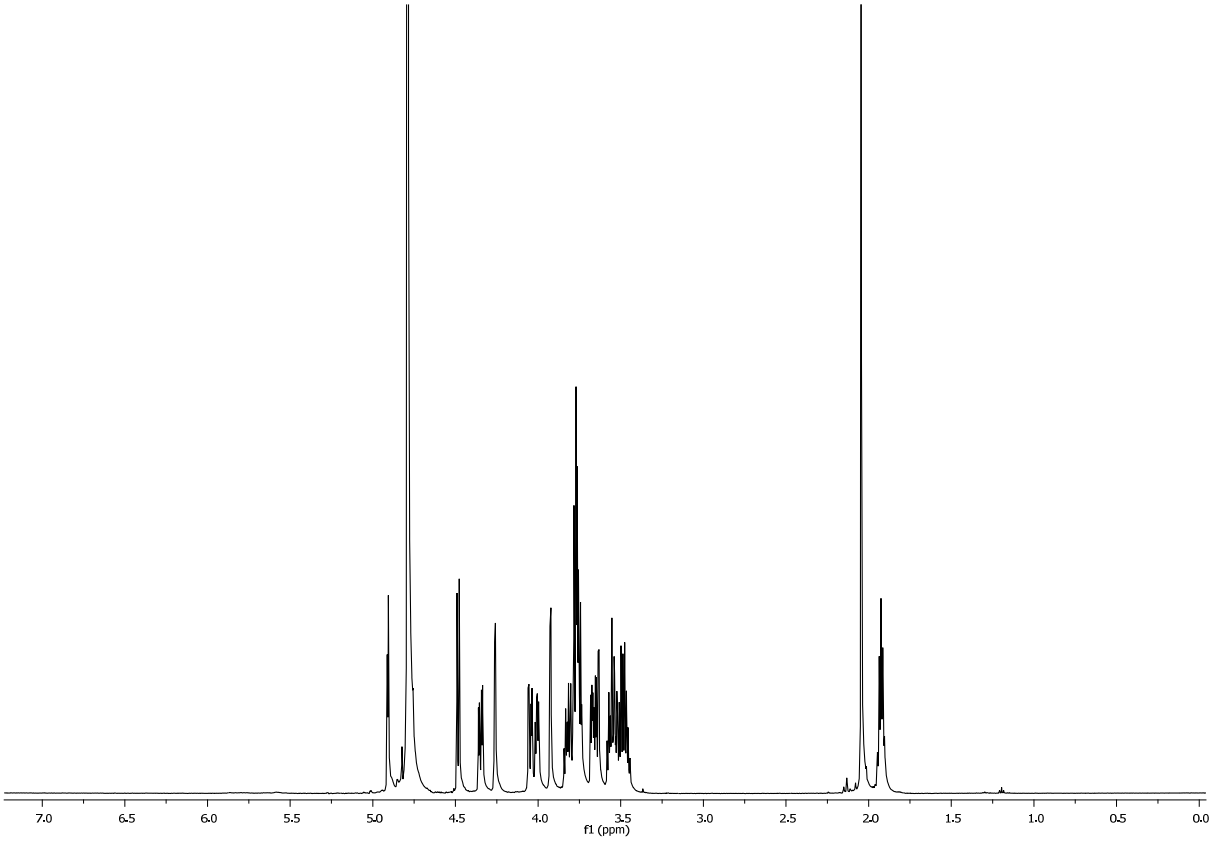


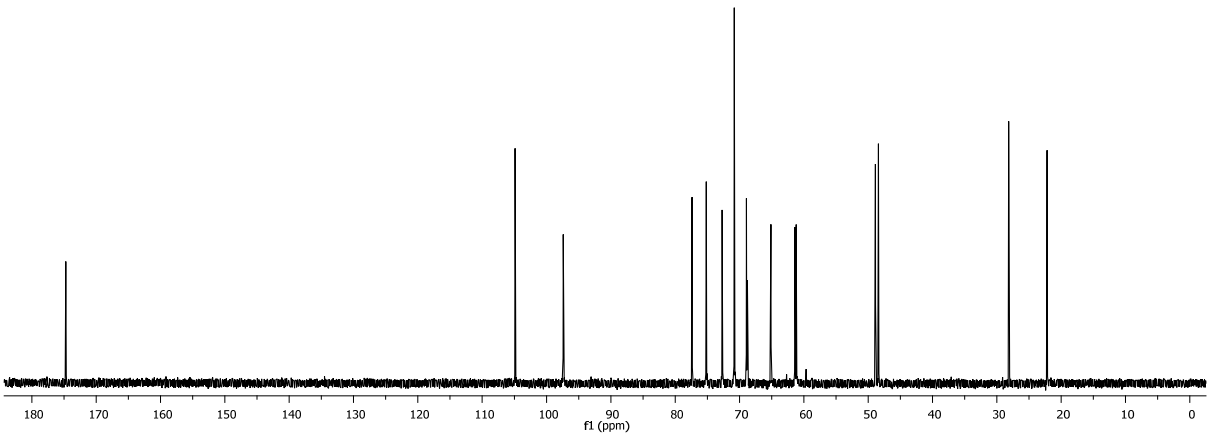
Compound **32**, Galβ1–3GalNAcβProN_3



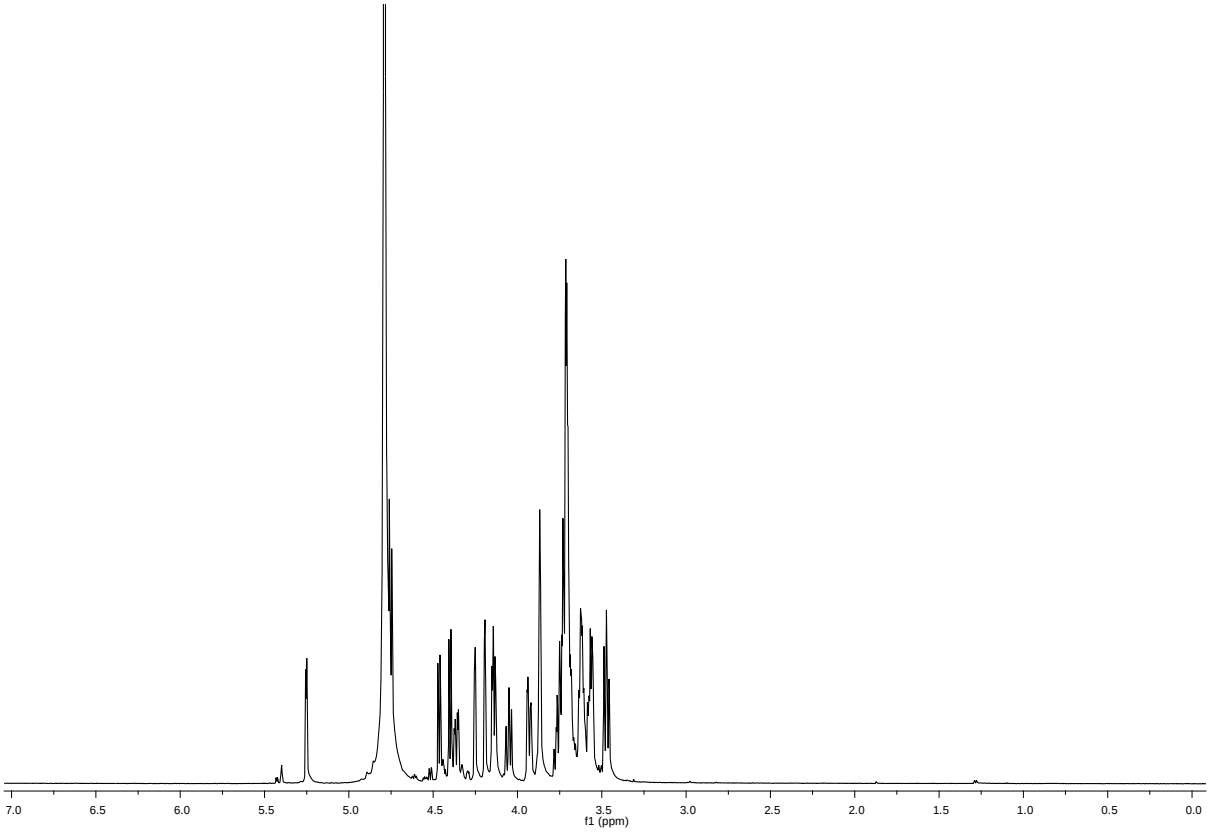


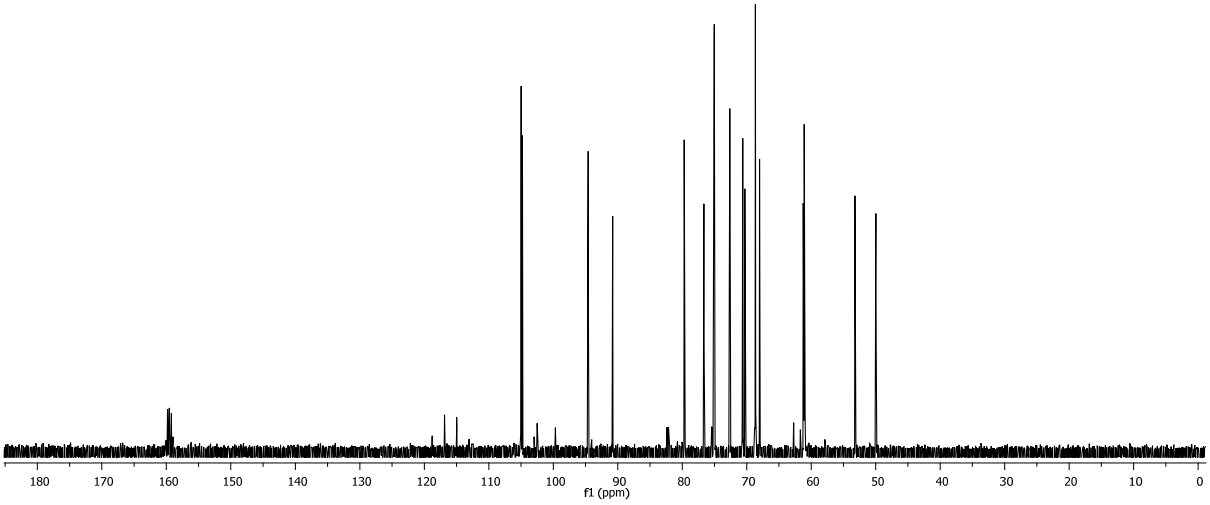
Compound **33**, Galβ1–3GalNAcαProN_3



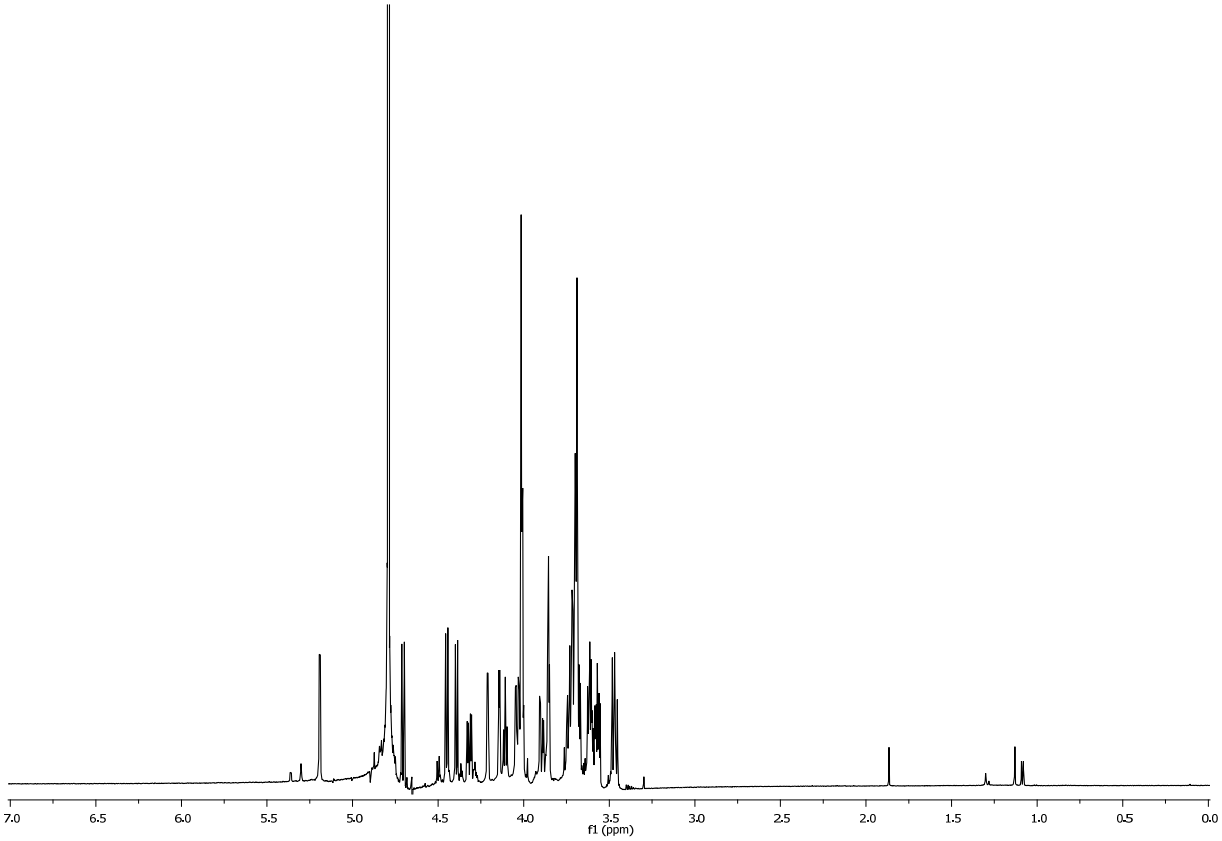


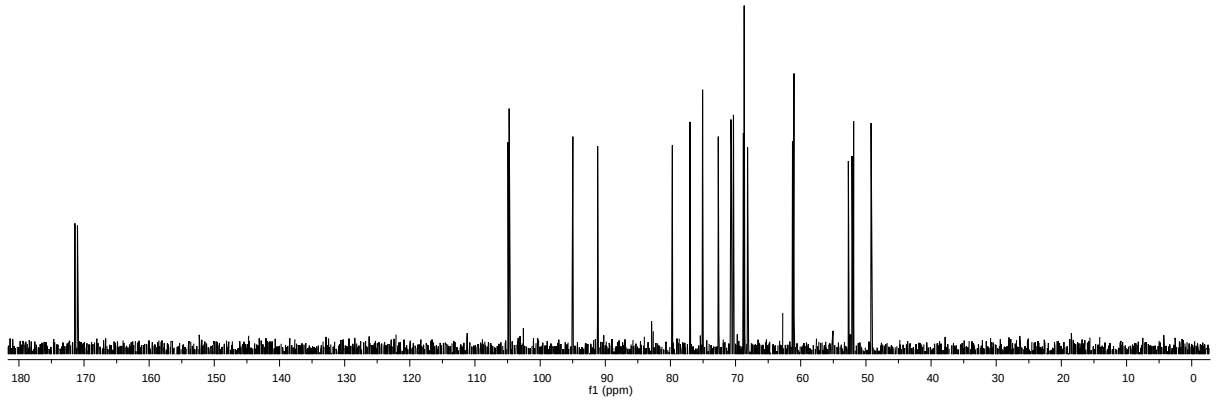
Compound **34**, Galβ1–3GalNTFA



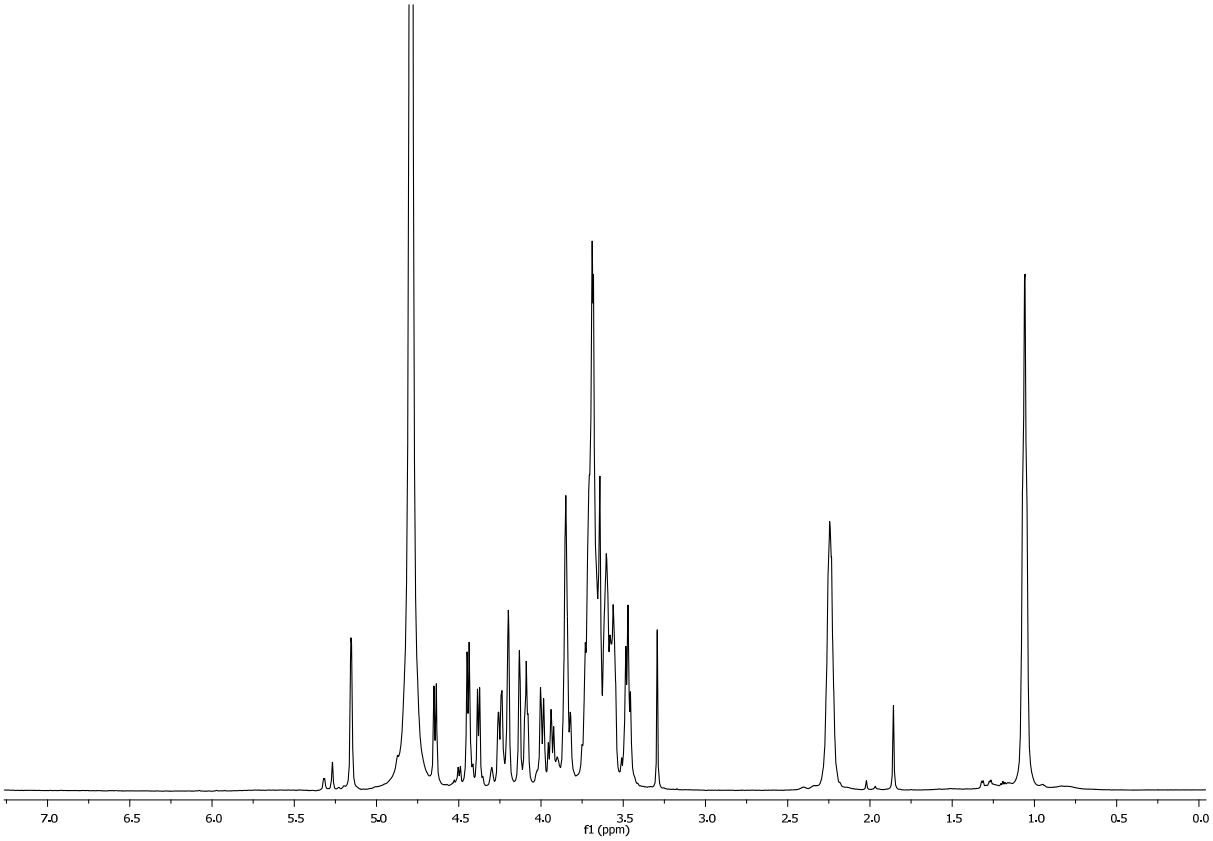


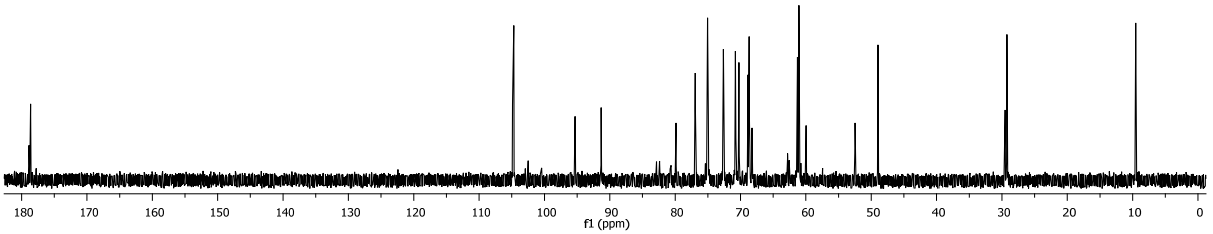
Compound **35**, Galβ1–3GalNAcN_3



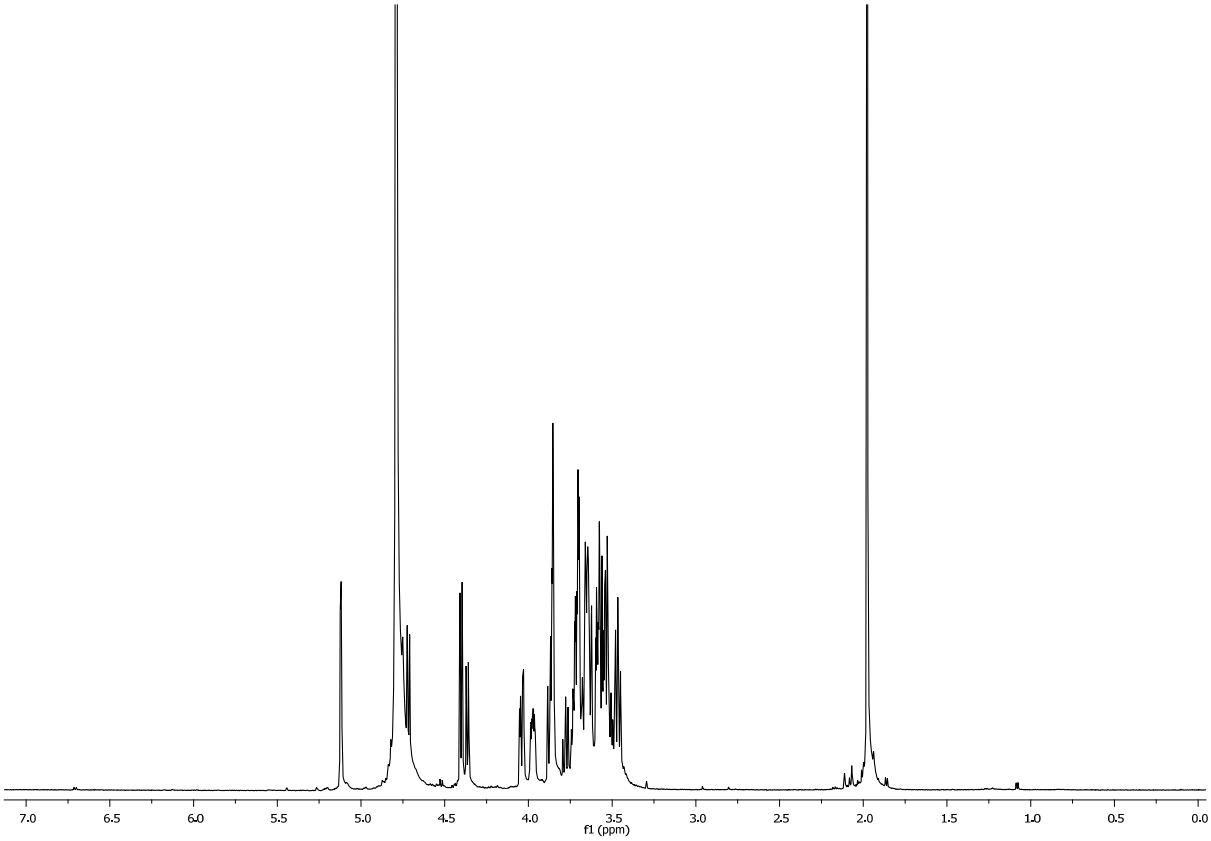


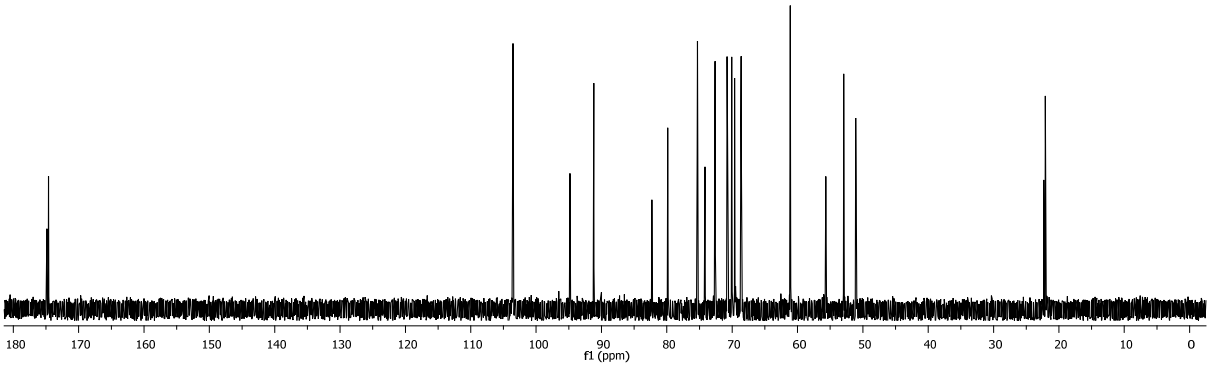
Compound **36**, Galβ1–3GalNPr



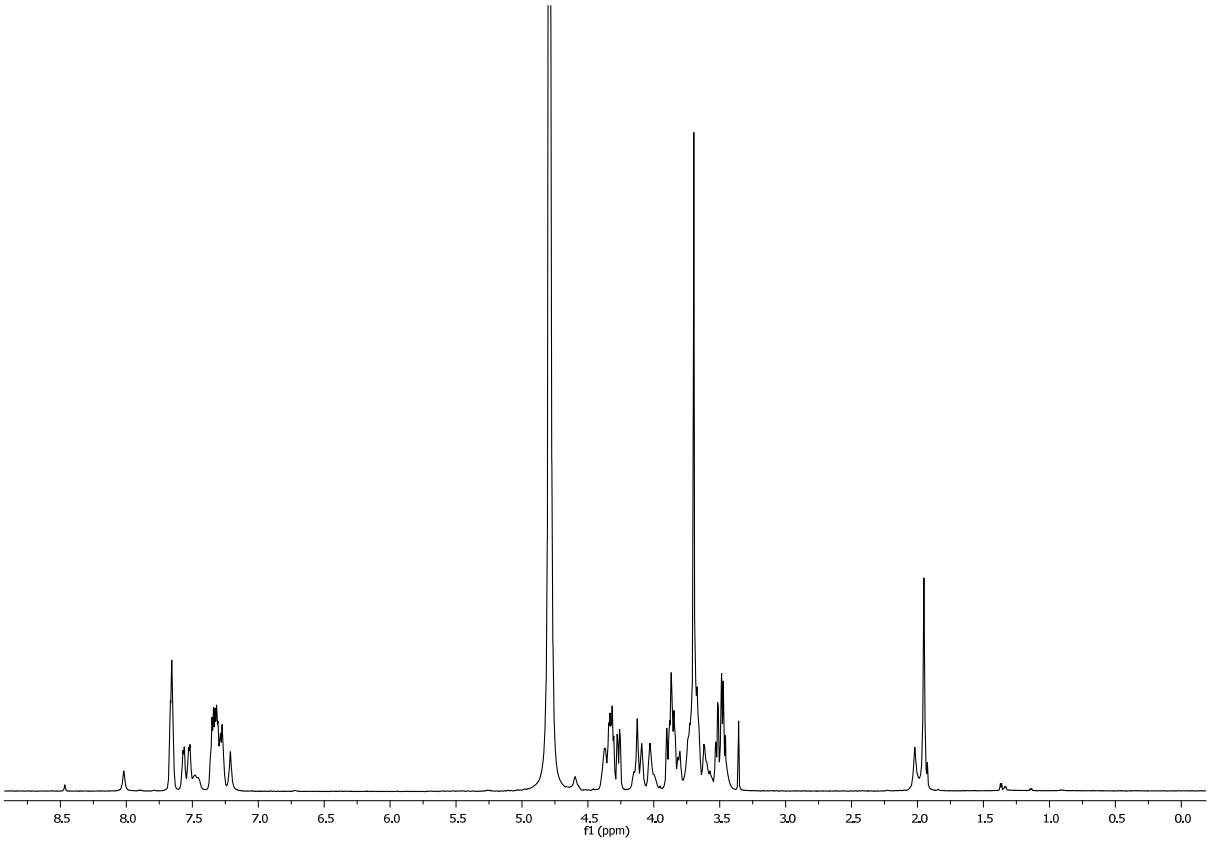


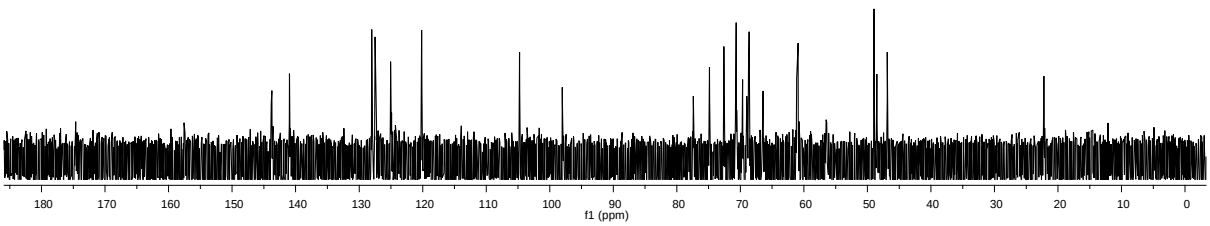
Compound **40**, Galβ1–3GlcNAc6N_3





Compound **43**, Galβ1–3GalNAcαSer





Compound **44**, Galβ1–3GalNAcαThr