The Importance of Hydration for Inhibiting Ice Recrystallization with *C*-Linked Antifreeze Glycoproteins.

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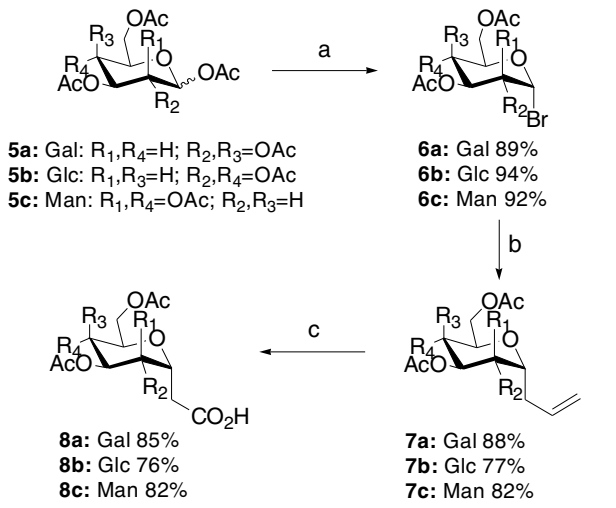
I. Synthesis of *C*-Linked Antifreeze Glycoprotein Analogues (**1**-**4**)

(i) General Experimental

All anhydrous reactions were performed in flame-dried or oven-dried glassware under a positive pressure of dry argon or nitrogen. Air or moisture-sensitive reagents and anhydrous solvents were transferred with oven-dried syringes or cannulae. All flash chromatography was performed with E. Merck silica gel 60 (230-400 mesh). All solution phase reactions were monitored using analytical thin layer chromatography (TLC) with 0.2mm pre-coated silica gel aluminum plates 60 F254 (E. Merck). Components were visualized by illumination with a short-wavelength (254 nm) ultra-violet light and/or staining (ceric ammonium molybdate, potassium permanganate, or phosphomolybdate stain solution). All solvents used for anhydrous reactions were distilled. Tetrahydrofuran (THF) and diethyl ether were distilled from sodium/benzophenone under nitrogen. Dichloromethane, acetonitrile, triethylamine, benzene and diisopropylethylamine (DIPEA) were distilled from calcium hydride. Methanol was distilled from calcium sulfate. *N,N*-dimethylformamide (DMF) was stored over activated molecular sieves 4Å under argon.
^{1}H (300, 360, 400, or 500 MHz) and ^{13}C NMR (75, 90, 100 or 125 MHz) spectra were recorded at ambient temperature on a Bruker Avance 300, Bruker AM 360, Bruker Avance 400 or Bruker Avance 500 spectrometer. Deuterated chloroform ($CDCl_3$), methanol (CD_3OD), or water (D_2O) were used as NMR solvents, unless otherwise stated. Chemical shifts are reported in ppm downfield from TMS and corrected using the solvent residual peak or TMS as an internal standard. Splitting patterns are designated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet and br, broad. Low resolution mass spectrometry (LRMS) was performed on a Micromass Quatro-LC Electrospray spectrometer with a pump rate of 20μL/min using electrospray ionization (ESI) or a Voyager DE-Pro matrix-assisted desorption ionization-time of flight (MALDI-TOF), (Applied Biosystem, Foster City, CA) mass spectrometer operated in the reflectron/positive-ion mode with DHB in 20% EtOH/H_2O as the MALDI matrix. High resolution mass spectrometry (HRMS) data was acquired on Applied Biosystems/Sciex QStar (Concord, ON). Samples in CH_2Cl_2/MeOH 1:1 were mixed with Agilent ES tuning mix for internal calibration, and infused into the mass spectrometer at 5 μL/min. Infrared absorption spectra (IR) were recorded on a Shimadzu FTIR-8400S spectrometer as a neat film from 4000 cm^{-1} to 650 cm^{-1}. Analytical and preparatory scale RP-HPLC were carried out with C-18 columns on a Varian Dynomax HPLC system equipped with a variable wavelength detector (ProStar 330 PDA) or a Waters Delta 600E HPLC system equipped with a variable wavelength detector. Automated solid phase peptide synthesis (SPPS) was performed on APEX 396 (Advanced ChemTech) equipped with a 40 well reaction vessel.

(ii) Synthesis of Carbohydrate Component

Scheme 1



5a: Gal: R_1,R_4=H; R_2,R_3=OAc
5b: Glc: R_1,R_3=H; R_2,R_4=OAc
5c: Man: R_1,R_4=OAc; R_2,R_3=H

6a: Gal 89%
6b: Glc 94%
6c: Man 92%

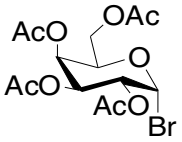
8a: Gal 85%
8b: Glc 76%
8c: Man 82%

7a: Gal 88%
7b: Glc 77%
7c: Man 82%

Conditions: (a) HBr/HOAc; (b) $(^nBu)_6Sn_2$, allylphenylsulfone, benzene, hν; (c) $RuCl_3{\cdot}H_2O$, $NaIO_4$, $CCl_4/CH_3CN/H_2O$

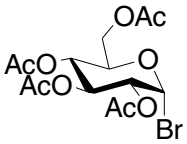
General protocol for Bromination of D-galactose pentaacetate, D-glucose pentaacetate and D-mannose pentaacetate

To commercially available D-pyranose pentaacetate (5 g, 12.81 mmol), 30 mL of HBr in AcOH (33% solution) was added at room temperature. The reaction was stirred for 40 minutes and then diluted with CH_2Cl_2. The solution was transferred to a separatory funnel containing ice and the organic layer was washed with ice water until neutral pH. The organic extract was dried over $MgSO_4$, filtered and concentrated to afford product in ~90% yield. The crude product was crystallized from diethyl ether to form a white powder.



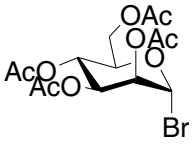
2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide (6a)

^{1}H NMR (360 MHz, $CDCl_3$) δ 6.56 (1H, d, *J*=3.9 Hz), 5.39 (1H, m), 5.22 (1H, dd, *J*=4.2, 8.2 Hz), 4.85 (1H, d, *J*=4.3 Hz), 4.82 (1H, d, *J*=4.2 Hz), 4.25 (1H, m), 3.97 (2H, m), 1.98 (3H, s), 1.98 (3H, s), 1.87 (3H, s), 1.82 (3H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.98, 169.70, 169.64, 169.42, 88.19, 70.94, 67.73, 67.51, 66.78, 60.67, 20.47, 20.37, 20.31; LRMS (ESI): Calcd for $C_{14}H_{23}BrNO_9$ $[M+NH_4]^+$ 429.2, found 429.9.



2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (6b)

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.16 (1H, d, *J*=1.3 Hz), 5.53 (1H, dd, *J*=3.6, 7.8 Hz), 5.27 (2H, m), 4.15 (1H, dd, *J*=9.6, 18 Hz), 4.07 (1H, m), 3.98 (1H, dd, *J*=2.1, 12 Hz), 2.01, (3H, s), 1.98 (3H, s), 1.91 (3H, s), 1.84 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.28, 169.51, 169.39, 169.35, 83.20, 72.71, 71.90, 67.78, 65.05, 61.27, 20.60, 20.49, 20.41; LRMS (ESI): Calcd for $C_{14}H_{23}BrNO_9$ $[M+NH_4]^+$ 429.2, found 429.9.



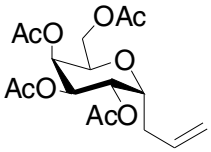
2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide (6c)

^{1}H NMR (360 MHz, $CDCl_3$) δ 6.45 (1H, d, *J*=3.9 Hz), 5.39 (1H, dd, *J*=9.6, 10 Hz), 5.01 (1H, dd, *J*=9.6, 9.9Hz), 4.67, (1H, ddd, *J*=1.5, 3.9, 5.4Hz), 4.16 (2H, m), 3.95 (1H, d, *J*=12.0 Hz), 1.92 (3H, s), 1.91 (3H, s), 1.91 (3H, s), 1.90 (3H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ 169.98, 169.70, 169.64, 169.42, 88.19, 70.94, 76.73, 67.57, 66.78, 60.67, 20.47, 20.37, 20.31; LRMS (ESI): Calcd for $C_{14}H_{23}BrNO_9$ $[M+NH_4]^+$ 429.2, found 429.9.

General protocol for photochemical allylation of 2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl bromide, 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide, 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl bromide[1]

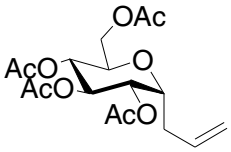
To a solution of 2,3,4,6-tetra-O-acetyl-α-D-pyranosyl bromide (1.24 g, 3.01 mmol) in benzene (15 mL), allyl phenyl sulfone (1.418 mL, 7.52 mmol), and bis-tributyl tin (2.19 mL, 4.21 mmol) were added. The solution was degassed and sonicated for 30 minutes under argon atmosphere; the sealed flask was irradiated for 9 hours under a 450W mercury arc lamp. The reaction was monitored using TLC at time intervals of 2, 6 and 8 hours. The reaction mixture was loaded directly onto a silica gel column packed with hexanes. The organostannanes were flushed with 3 void volumes of hexanes, after which the solvent system was changed to 5:1 hexanes/EtOAc to remove the unreacted allyl phenyl sulfone. The product was eluted with 3:1 hexanes/EtOAc and concentrated to afford the allylated derivative as colorless oil (~95% yield).

[1] Pontén, F.; Magnusson, G. *J. Org. Chem.* **1996**, 61, 7463.



Allyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (7a)

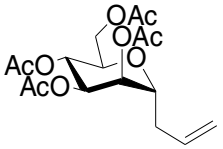
^{1}H NMR (360 MHz, $CDCl_3$) δ 5.76 (1H, dddd, J=6.5, 7.1, 10.0, 16.6 Hz), 5.42 (1H, dd, J=2.4, 3.1 Hz), 5.28 (1H, dd, J=4.8, 9.3 Hz), 5.22 (1H, dd, J=3.1, 9.3 Hz), 5.13 (1H, ddd, J=1.6, 3.0, 16.6 Hz), 5.12 (1H, ddd, J=1.4, 3.0, 10.0 Hz), 4.30 (1H, ddd, J=4.7, 5.2, 10.3 Hz), 4.21 (1H, dd, J=8.9, 12.5 Hz), 4.09 (2H, m), 2.46 (1H, m), 2.29 (1H, m), 2.12 (1H, s), 2.07 (1H, s), 2.04 (1H, s), 2.03 (1H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ 170.5, 170.1, 169.9, 169.8, 133.3, 117.6, 71.4, 68.2, 67.9, 61.4, 30.9, 20.8, 20.7, 20.6; LRMS (ESI): Calcd for $C_{17}H_{25}O_9$ $[M+H]^+$ 373.4, found 373.1.



Allyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (7b)

The product was co-eluted with starting material with 3:1 hexanes to EtOAc and concentrated. The mixture was re-dissolved in a 1:1 mixture of acetone and water, to which silver carbonate was added. The reaction was allowed to stir for 30 minutes, after which it was filtered. The product was extracted with CH_2Cl_2, washed with water, brine, dried over $MgSO_4$ and concentrated. The colorless oil was purified with flash column chromatography in 3:1 hexanes/EtOAc, and afforded **7b** as a colorless oil in ~70% yield over two steps.

^{1}H NMR (360 MHz, $CDCl_3$) δ 5.76 (1H, dddd, *J*=6.6, 8.0, 16.0Hz), 5.41 (1H, dd, *J*=6.8, 13.2Hz), 5.18 (2H, m), 5.01 (1H, dd, *J*=6.5, 12.1Hz), 4.35 (1H, m), 4.23 (1H, dd, *J*=5.9, 12.8Hz), 4.10 (1H, dd, *J*=3.0, 13.2Hz), 3.89 (1H, m), 2.59 (1H, m), 2.38 (1H, m), 2.19 (3H, s), 2.01 (3H, s), 1.95 (3H, s), 1.91 (3H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ 177.30, 174.48, 170.58, 169.55, 169.43, 168.66, 163.66, 140.11, 115.29, 76.97, 76.61, 69.31, 68.76, 67.71, 67.47, 32.83, 26.55, 20.51, 20.48, 20.36, 20.22, 20.09, 20.06, 19.86; HRMS (ESI): Calcd for $C_{17}H_{25}O_9$ $[M+H]^+$ 373.1498, found 373.1461.

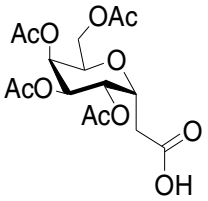


Allyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (7c)

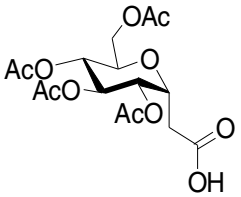
^{1}H NMR (360 MHz, $CDCl_3$) δ 5.77 (1H, dddd, J=6.8, 10.0, 10.4Hz), 5.23 (1H, dd, J=3.24, 6.8Hz), 5.15 (1H, m), 5.07 (1H, m), 4.29 (1H, dd, J=6.4, 12.2Hz), 4.06 (1H, dd, J=2.9, 8.2 Hz), 4.03 (1H, m), 3.86 (1H, ddd, J=2.9, 6.8, 8.6Hz), 2.52 (1H, m), 2.29 (1H, m), 2.07 (3H, s), 2.02 (3H, s), 1.99 (3H, s), 1.94 (3H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ 174.30, 171.58, 170.38, 169.98, 169.13, 168.16, 163.11, 140.21, 114.21, 73.91, 68.81, 68.36, 67.69, 61.49, 35.81, 26.95, 22.53, 22.49, 22.31, 20.20, 20.01, 19.98, 19.45; LRMS (ESI): Calcd for $C_{17}H_{25}O_9$ $[M+H]^+$ 373.4, found 373.1.

General protocol for oxidation of allyl 2,3,4,6-tetra-O-acetyl-*α*-D-pyranoside to 2,3,4,6-tetra-O-acetyl-*α*-D-pyranosyl acid

Allylated pyranose tetraacetate (0.83 g, 2.23 mmol) was dissolved in 14 mL of a solution of (2:2:3) acetonitrile: carbon tetrachloride: water, followed by the addition of sodium periodate (1.90 g. 8.92 mmol). A catalytic amount of ruthenium trichloride trihydrate was then added, and the reaction was allowed to stir at room temperature for 2-3 hours. The solution was filtered through celite and transferred to a separatory funnel with dichlroromethane. The organic layer was washed successively with saturated ammonium chloride solution, saturated brine solution, dried over $MgSO_4$, filtered and concentrated to afford the carboxylic acid derivative in >75 % yield.

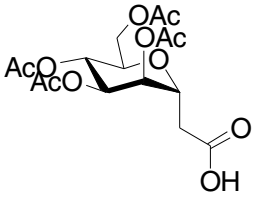


2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl acid (8a)
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.43 (1H, t, *J*=2.8 Hz), 5.33 (1H, dd, *J*=8.9, 5.0 Hz,), 5.17 (1H, dd, *J* =8.9, 3.3 Hz), 4.70 (1H, ddd, *J*=9.3, 8.3, 5.3 Hz), 4.30-4.08 (3H, m), 2.73 (1H, dd, *J*=15.6, 8.7 Hz), 2.63 (1H, dd, *J*=15.6, 5.7 Hz), 2.13 (3H, s), 2.07 (3H, s), 2.04 (6H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ: 175.5, 170.8, 170.1, 170.0, 169.7, 69.4, 68.9, 67.8, 67.6, 67.1, 61.2, 33.15, 20.7; IR (thin film): 3706-2355, 1748 cm^{-1}.



2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl acid (8b)

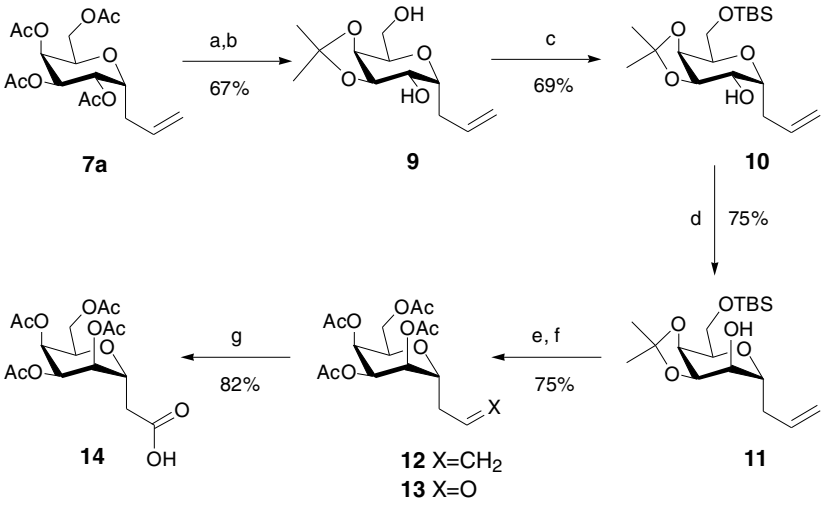
^{1}H NMR (360 MHz, $CDCl_3$) δ 5.28 (1H, ddd, *J*=4.6, 9.7Hz), 5.17 (1H, ddd, *J*=6.7, 9.9Hz), 5.03 (1H, q, *J*=8.9Hz), 4.84 (1H, m), 4.68 (1H, m), 4.26 (1H, ddd, *J*=6.2, 9.9Hz), 4.06 (1H, m), 3.94 (1H, m), 3.86 (1H, m), 2.81 (1H, m), 2.04 (3H, s), 2.02 (3H, s), 2.00 (3H, s), 1.99 (3H, s); ^{13}C NMR (90 MHz, $CDCl_3$) δ 177.28, 174.50, 170.62, 169.84, 101.95, 69.31, 68.76, 67.71, 67.50, 67.47, 67.01, 66.73, 66.43, 61.00, 32.92, 20.89, 20.77, 20.72, 20.67, 20.65, 20.60; HRMS (ESI): Calcd for $C_{16}H_{23}O_{11}$ $[M+H]^+$ 391.1240, found 391.1216.



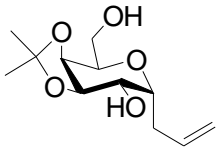
2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl acid (8c)

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (1H, dd, *J*=5.4, 7.8 Hz), 5.14 (1H, m), 4.49 (1H, m), 4.21 (1H, dd *J*=5.7, 13.5 Hz), 4.01 (1H, m), 2.63 (1H, ddd, *J*=6.4, 7.9, 9.3 1H), 2.10 (3H, s), 1.99 (3H, s), 1.89 (3H, s), 1.75 (3H, s), 1.44 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.53, 171.15, 170.30, 170.10, 169.99, 133.96, 116.38, 73.19, 68.81, 68.34, 67.63, 63.13, 61.79, 35.76, 35.13, 26.93, 24.79, 21.17, 21.09, 21.06, 20.56, 19.23, 14.22; IR (thin film): 3300-2350, 1750 cm^{-1}; HRMS (ESI): Calcd for $C_{16}H_{23}O_{11}$ $[M+H]^+$ 391.1240, found 391.1228.

Scheme 2



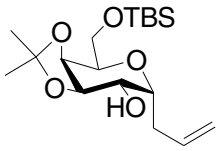
Conditions: (a) NaOMe, MeOH; (b) $(CH_3)_2C(OCH_3)_2$, *p*-TsOH, CH_3CN; (c) TBDMSCl, DMAP, py; (d) $(CO)_2Cl_2$, DMSO, Et_3N, then $NaBH_4$, CH_3OH; (e) 80% HOAc-H_2O, then Ac_2O, py-CH_2Cl_2; (f) O_3, PPh_3, CH_2Cl_2, -78 ºC; (g) $NaClO_2$, KH_2PO_4, 2-methyl-2-butene, ^tBuOH-^iPrOH-H_2O.



3,4-O-isopropylidene-1-allyl-α-D-galactopyranoside (9)

To a solution of allylglycoside (0.81 g, 3.97 mmol) and dimethoxypropane (2.44 mL, 19.85 mmol) in dry acetonitrile (15 mL), p-TsOH (40 mg) was added and the reaction mixture was stirred for 2h. Water (3 mL) was then added and after 30 min the reaction was neutralized with triethylamine and evaporated. Flash column chromatography (10% CH_2Cl_2/EtOAc) afforded **9** (0.69 g, 70%) as white crystalline solid.

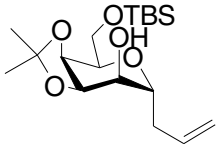
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.86 (1H, dddd, *J*=7.1, 10.2, 17.2 Hz), 5.15 (1H, dd, *J*=1.8, 17.2 Hz), 5.09 (1H, dddd, *J*=1.5, 17.2 Hz), 4.33-4.25 (2H, m), 4.08-4.02 (2H, m), 3.87-3.77 (2H, m), 2.45-2.30 (2H, m), 2.20 (1H, dd, *J*=3.2, 9.3 Hz), 2.00 (1H, d, *J*=4.5 Hz), 1.47 (3H, s), 1.32 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.3, 117.7, 109.7, 74.8, 73.1, 70.6, 69.6, 69.1, 63.6, 34.9, 26.8, 24.6; HRMS (ESI): Calcd for $C_{12}H_{21}O_5$ $[M+H]^+$ 245.1388, found 245.1377.



3,4-O-isopropylidene-6-O-tert-butyldimethylsilyl-1-allyl-α-D-galactopyranoside (10)

To a solution of diol **9** (0.30 g, 1.21 mmol) in dry pyridine:CH_2Cl_2 (5:5 mL), tert-butyldimethylsilyl chloride (0.22 g, 1.45 mmol) and DMAP (7 mg) were added sequentially. The reaction mixture was stirred overnight, diluted with CH_2Cl_2 and washed successively with 0.5 N HCl, water and brine. The organic extract was dried over $MgSO_4$ and concentrated *in vacuo*. Flash column chromatography (25% EtOAc/hexanes) gave the product in quantitative yield (0.43 g, >99%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (1H, dddd, *J*=7.0, 7.0, 10.2, 17.1 Hz), 5.16-5.10 (1H, m), 5.08-5.04 (1H, m), 4.34 (1H, dd, *J*=1.8, 7.1 Hz), 4.17 (1H, dd, *J*=3.4, 7.1 Hz), 3.99-3.93 (2H, m), 3.76-3.64 (3H, m), 2.45 (1H, br s), 2.35-2.28 (2H, m), 1.43 (3H, s), 1.28 (3H, s), 0.84 (9H, s), 0.02 (3H, s), 0.02 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.4, 117.5, 109.0, 74.6, 71.7, 70.5, 70.0, 69.7, 62.6, 35.0, 27.0, 25.9, 24.5, -5.3 ,-5.4; HRMS (ESI): Calcd for $C_{18}H_{35}O_5Si$ $[M+H]^+$ 359.2253, found 359.2257.

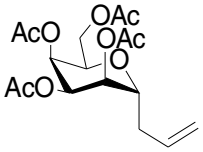


3,4-O-isopropylidene-6-O-tert-butyldimethylsilyl-1-allyl-α-D-talopyranoside (11)

To a solution of oxalyl chloride (0.13 mL, 1.51 mmol) in CH_2Cl_2 (10 mL) at -78 °C, DMSO (0.23 mL, 3.31 mmol) was added dropwise and the reaction mixture was stirred for 30 min. Then, **10** (0.27 g, 0.75 mmol) in CH_2Cl_2 (10 mL) was added over 10 min. After an additional 30 min of stirring, triethylamine (0.73 mL, 5.27 mmol) was added at -60°C and the reaction mixture was brought to room temperature. After 1h, the reaction mixture was diluted with CH_2Cl_2 and water was added. The layers were separated and the organic extract was washed with water, brine, dried over $MgSO_4$, and concentrated. The crude product was immediately dissolved in MeOH (20 mL) and $NaBH_4$ (0.06 g, 1.51 mmol) was added. After stirring for 30 min, the reaction mixture was quenched with acetic acid and the solvent was evaporated. The residue was taken up in CH_2Cl_2, washed with water, brine and then dried over $MgSO_4$. The organic phase was concentrated *in vacuo* and after flash column chromatography (25% EtOAc/hexanes), compound **11** was obtained as an oil (0.41 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.93 (1H, dddd, *J*=6.7, 7.4, 10.2, 17.1 Hz), 5.19-5.07 (2H, m), 4.50 (1H, dd, *J*=3.6, 8.0 Hz), 4.42 (1H, dd, *J*=1.5, 8.0 Hz), 3.77 (1H, ddd, *J*=4.24, 6.50, 9.65 Hz), 3.74-3.57 (4H, m), 2.52-2.27 (2H, m), 1.99 (1H, br s), 1.51 (3H, s), 1.36 (3H, s), 0.89 (9H, s), 0.06 (3H, s), 0.06 (3H,s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 134.4,

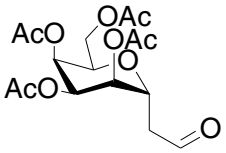
117.6, 109.8, 73.4, 73.1, 72.5, 70.5, 68.2, 62.3, 36.9, 26.2, 25.9, 24.7, -5.3, -5.5; HRMS (ESI): Calcd for $C_{18}H_{38}NO_5Si$ $[M+NH_4]^+$ 376.2519, found 376.2508.



Allyl 2,3,4,6-tetra-O-acetyl-α-D-talopyranoside (12)

A solution of acetal **11** (0.22 g, 0.61 mmol) in 80% acetic acid (20 mL) was heated for 1h at 80 °C. The reaction mixture was cooled and the solvent was evaporated. The residue was taken up in pyridine (20 mL), followed by addition of acetic anhydride (10 mL) and DMAP (10 mg). The reaction mixture was stirred overnight, then diluted with CH_2Cl_2 and washed successively with saturated $CuSO_4$ solution, water and brine. The organic extract was dried over $MgSO_4$ and concentrated. Flash column chromatography afforded compound **12** as a white crystalline solid (0.17 g, 75%).

^{1}H NMR (500 MHz, $CDCl_3$) δ 5.77 (1H, dddd, *J*=6.9, 6.9, 10.7, 16.6 Hz), 5.44 (1H, t, *J*=3.2 Hz), 5.18 (1H, dd, *J*=3.2, 5.1 Hz), 5.11-5.08 (1H, m), 5.08-5.06 (1H, m), 4.83 (1H, dd, *J*=3.2, 7.3 Hz), 4.62 (1H, dd, *J*=8.9, 12.1 Hz), 4.18 (1H, ddd, *J*=3.8, 4.8, 8.8 Hz), 4.09 (1H, dd, *J*=3.6, 12.2 Hz), 4.01 (1H, ddd, *J*=4.8, 7.5, 7.5 Hz), 2.39-2.23 (2H, m), 2.08 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 2.02 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 169.6, 169.4, 169.3, 132.8, 117.7, 70.5, 69.2, 68.7, 67.1, 66.4, 60.3, 34.6, 20.7, 20.6, 20.5; HRMS (ESI): Calcd for $C_{17}H_{28}NO_9$ $[M+NH_4]^+$ 390.1764, found 390.1777.

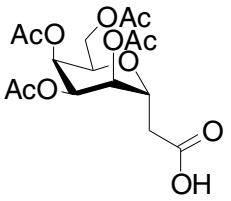


2,3,4,6-tetra-O-acetyl-α-D-talopyranosyl aldehyde (13)

Ozone gas was bubbled through a solution of **12a** (0.86 g, 2.33 mmol) in 15 mL of dry CH_2Cl_2 at -78 ^{o}C until the solution turned blue in color. Nitrogen was then bubbled through the solution until it turned colorless. Triphenylphosphine (1.5 g, 5.7 mmol) was then added, the solution was allowed to warm up to room temperature and stirred overnight. Dichloromethane was removed under reduced pressure and the residue was purified by flash column chromatography (toluene/acetone 4:1) to afford **13** in 85% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 9.70 (1H, t, *J*=2.1 Hz), 5.56 (1H, t, *J*=3.0 Hz), 5.18 (1H, dd, *J*=3.0, 6.3 Hz), 4.90 (1H, dd, *J*=9.8, 12.6 Hz), 4.77 (1H, dd, *J*=3.0, 9.3 Hz), 4.53 (1H, ddd, *J*=5.2, 7.4, 9.2 Hz), 4.23 (1H, ddd, *J*=2.7, 6.3, 9.6 Hz), 4.07 (1H, dd, *J*=2.8, 12.7 Hz), 2.57-2.54 (2H, m), 2.15 (3H, s), 2.07 (3H, s), 2.05 (3H, s), 2.00 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.1, 171.0, 169.6, 169.3, 169.1, 71.8, 69.1, 67.4, 66.5, 63.3, 59.5,

44.9, 20.8, 20.8, 20.6, 20.5; HRMS (ESI): Calcd for $C_{16}H_{26}NO_{10}$ $[M+NH_4]^+$ 392.1556, found 392.1568.



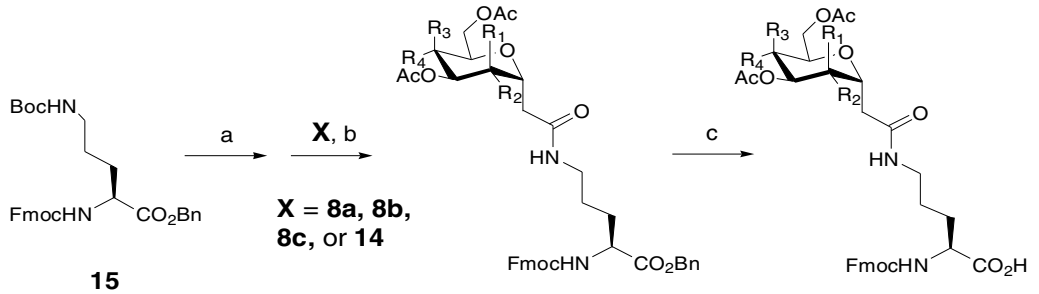
2,3,4,6-tetra-O-acetyl-α-D-talopyranosyl acid (14)

To a solution of **13** (2.0 g, 5.4 mmol) in 1:1 (v:v) mixture of *tert*-butyl alcohol and 2-methyl-2-butene (20 mL), an aqueous solution of potassium dihydrogen phosphate (3.7 g) and sodium chlorite (2.4 g) in 10 mL distilled water was added. The mixture was stirred vigorously at 0 °C for 6 hours. The product was extracted with EtOAc and washed with 100 mL water and 50 mL brine solution. The organic layer was then dried over $MgSO_4$ and concentrated to afford **14** in 96% yield.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.57 (1H, t, *J*=3.0 Hz), 5.20 (1H, dd, *J*=3.1, 6.2 Hz), 4.83 (1H, dd, *J*=3.0, 9.2 Hz), 4.76 (1H, dd, *J*=9.5,12.5 Hz), 4.38 (1H, ddd, *J*=3.7, 8.9, 8.9 Hz), 4.29 (1H, ddd, *J*=3.0, 6.2, 9.3 Hz), 4.17 (1H, dd, 3.0, 12.6 Hz), 2.62 (1H, dd, *J*=3.7, 15.8 Hz), 2.53 (1H, dd, *J*=8.8, 15.8 Hz), 2.16 (3H, s), 2.08 (3H, s), 2.07 (3H, s), 2.03 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 171.0, 169.7, 169.3, 169.2, 71.6, 68.9, 67.4, 66.5, 65.0, 59.8, 36.1, 20.9, 20.7, 20.6, 20.6; LRMS (ESI): Calcd for $C_{16}H_{23}O_{11}$ $[M+H]^+$ 391.3, found 391.4; HRMS (ESI): Calcd for $C_{16}H_{26}NO_{11}$ $[M+NH_4]^+$ 408.1505, found 408.1512.

(iii) Synthesis of C-linked glycoconjugate

Scheme 3



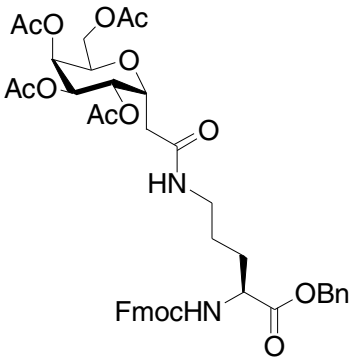
16: Gal: 83%
17: Glc: 85%
18: Man: 93%
19: Tal: 87%

20: Gal: 84%
21: Glc: 87%
22: Man: 82%
23: Tal: 85%

Conditions: (a) TFA/CH_2Cl_2; (b) HBTU, DIPEA, CH_2Cl_2; (c) 10% Pd/C, H_2, EtOH-EtOAc.

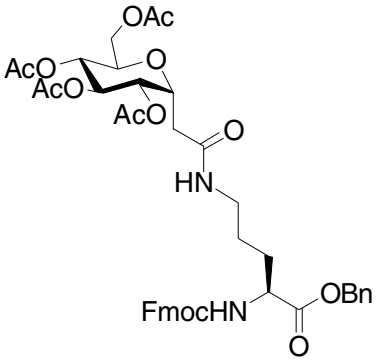
General Protocol for amide coupling of C-linked glycoconjugate

To a solution of fully protected amino acid derivative **15** (0.21 g, 0.38 mmol) in 10 mL of CH_2Cl_2, 2 mL of TFA was added. The reaction was stirred for 40 minutes and then concentrated under reduced pressure. The syrup was re-dissolved in a 1:1 mixture of toluene and CH_2Cl_2, concentrated, then re-dissolved in diethyl ether and concentrated to afford a white solid. To a solution of carboxylic acid carbohydrate derivative (0.15 g, 0.38 mmol), HBTU (0.17 g, 0.46 mmol) in 15 mL of CH_2Cl_2, and 0.20 mL (0.15 g, 1.14 mmol) of DIPEA were added. The reaction was allowed to stir for 20 minutes and then the deprotected amino acid derivative was added. After stirring overnight, the reaction mixture was washed successively with saturated ammonium chloride, water, brine, dried over $MgSO_4$ and concentrated. The product was purified by flash column chromatography in 50:1 CH_2Cl_2 and MeOH to afford a C-linked glycosyl amide building block in ~80% yield.



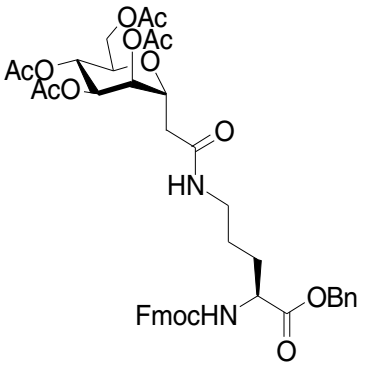
Benzyl Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside) (16)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (2H, d, *J*=7.5 Hz), 7.59 (2H, d, *J*=7.21 Hz), 7.42-7.28 (9H, m), 6.12 (1H, br s), 5.55 (1H, d, *J*=8.4 Hz), 5.4 (1H, t, *J*=3.3 Hz), 5.29-5.23 (1H, m), 5.17-5.12 (3H, m), 4.67-4.61 (1H, m), 4.46-4.34 (3H, m), 4.25-4.19 (2H, m), 4.15-4.09 (2H, m), 3.29-3.23 (2H, m), 2.57-2.47 (1H, m), 2.41-2.34 (1H, m), 2.10 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 2.00 (3H, s), 1.93-1.82 (1H, m), 1.73-1.47 (3H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 170.5, 169.9, 169.7, 169.5, 169.2, 156.0, 143.6, 141.2, 135.0, 128.6, 128.5, 127.7, 127.0, 125.0, 119.9, 69.4, 68.7, 67.8, 67.7, 67.2, 67.0, 66.8, 61.0, 53.5, 47.0, 38.6, 34.4, 30.0, 25.3, 20.7, 20.6; LRMS (ESI): Calcd for $C_{43}H_{49}N_2O_{14}$ $[M+H]^+$ 817.8, found 817.4.



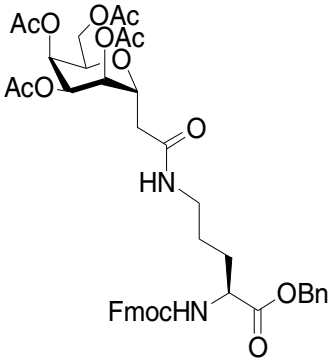
Benzyl Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside) (17)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (2H, d, *J*=7.4 Hz), 7.60 (2H, d, *J*=7.1 Hz), 7.45-7.25 (9H, m), 6.17 (1H, br s), 5.64 (1H, d, *J*=7.9 Hz), 5.23 (1H, t, *J*=8.4 Hz), 5.20-5.04 (3H, m), 4.97 (1H, t, *J*=8.3 Hz), 4.25-4.12 (3H, m), 3.97-3.90 (1H, m), 3.35-3.17 (2H, m), 2.60 (1H, dd, *J*=9.6, 12.4 Hz), 2.45 (1H, dd, *J*=4.4, 15.4 Hz), 2.07-1.98 (12H, m), 1.95-1.77 (1H, m), 1.76-1.60 (1H, m), 1.60-1.46 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 170.6, 169.9, 169.4, 169.0, 156.0, 143.8, 143.6, 141.2, 135.1, 128.6, 128.5, 128.5, 128.3, 127.7, 127.0, 125.0, 119.9, 69.9, 69.7, 69.4, 69.3, 68.2, 67.3, 67.0, 62.0, 53.5, 47.1, 38.9, 38.6, 34.2, 30.0, 25.3, 20.7, 20.6, 20.6; HRMS (ESI): Calcd for $C_{43}H_{49}N_2O_{14}$ $[M+H]^+$ 817.3183, found 817.3192.



Benzyl Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside) (18)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (2H, d, *J*=7.4 Hz), 7.60 (2H, d, *J*=7.1 Hz), 7.44-7.25 (9H, m), 6.18 (1H, br s), 5.71 (1H, d, *J*=8.1 Hz), 5.25 (1H, dd, *J*=3.2, 7.2 Hz), 5.20-5.07 (3H, m), 4.47-4.30 (5H, m), 4.25-4.15 (2H, m), 4.02-3.94 (1H, m), 3.40-3.20 (2H, m), 2.52 (2H, d, *J*=6.87 Hz), 2.10-2.06 (12H, m), 2.00-1.82 (1H, m), 1.80-1.65 (1H, m), 1.64-1.50 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 170.6, 169.9, 169.8, 169.5, 168.8, 156.1, 143.8, 143.6, 141.2, 135.1, 128.6, 128.6, 128.5, 128.5, 128.3, 127.7, 127.6, 127.0, 126.9. 125.0, 119.9, 72.0, 69.9, 69.1, 68.0, 67.3, 67.2, 67.0, 65.2, 61.7, 53.6, 47.1, 38.9, 38.6, 37.3, 29.9, 25.5, 20.7, 20.7, 20.6; HRMS (ESI): Calcd for $C_{43}H_{49}N_2O_{14}$ $[M+H]^+$ 817.3183, found 817.3198.

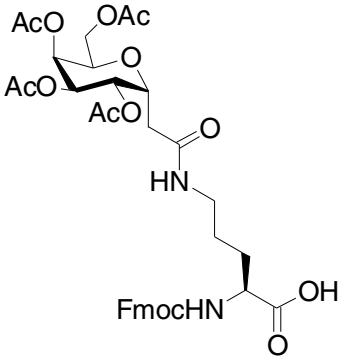


Benzyl Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-talopyranoside) (19)

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.69 (2H, d, *J*=7.3 Hz), 7.52 (2H, d, *J*=7.8 Hz), 7.35-7.17 (9H, m), 6.18-6.11 (1H, m), 5.54 (1H, d, *J*=7.6 Hz), 5.46 (1H, t, *J*=3.3 Hz), 5.14-5.05 (3H, m), 4.70 (1H, dd, *J*=2.6, 9.0 Hz), 4.55 (1H, dd, *J*=9.8, 12.6 Hz), 4.40-4.00 (7H, m), 3.23-3.10 (2H, m), 2.37 (1H, dd, *J*=2.7, 15.1 Hz), 2.23 (1H, dd, *J*=9.0, 14.8 Hz), 2.06 (3H, s), 1.99 (3H, s), 1.96 (3H, s), 1.93 (3H, s), 1.87-1.77 (1H, m), 1.67-1.57 (1H, m), 1.53-1.42 (2H, m); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.0, 170.7, 169.6, 169.4, 169.2, 169.2, 156.0, 143.8, 143.6, 141.2, 135.1, 128.6, 128.5, 128.2, 127.6, 127.0, 125.0, 125.0, 119.9, 71.7, 69.0, 67.4, 67.2, 67.0, 66.4, 65.6, 60.1, 53.6, 47.0, 38.9, 38.5, 38.2, 29.9, 25.4, 20.8, 20.8, 20.6; HRMS (ESI): Calcd for $C_{43}H_{49}N_2O_{14}$ $[M+H]^+$ 817.3183, found 817.3149.

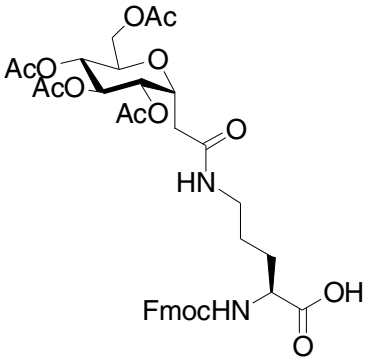
General protocol for debenzylation of *C*-linked glycoconjugate

To a solution of benzyl ester in 1:1 ethanol/ethyl acetate, a catalytic amount (8% w/w) of palladium on charcoal (10% w/w) was added. The reaction mixture was stirred under H_2 atmosphere and monitored via TLC. After the reaction was completed, the catalyst was gravity filtered through Whatman No.2 filter paper. The combined filtrates were concentrated and the compound was purified by column chromatography (5% MeOH/CH_2Cl_2) to afford a crystalline solid in >80% yield.



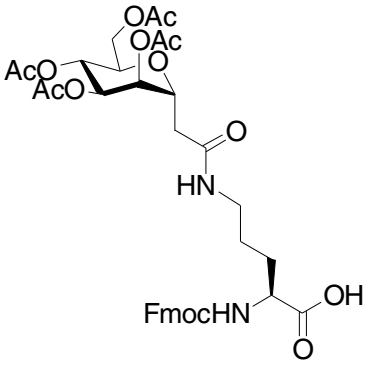
Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside) (20)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (2H, d, *J*=7.2 Hz), 7.60 (2H, d, *J*=6 Hz), 7.42-7.37 (2H, m), 7.33-7.28 (2H, m) 6.25 (1H, br s), 5.6 (1H, d, *J*=7.5 Hz), 5.39 (1H, t, *J*=3.3 Hz), 5.27-5.23 (1H, m), 5.16-5.12 (1H, m), 4.68-4.63 (1H, m), 4.39 (2H, d, *J*=6.6), 4.33-4.05 (5H, m), 3.37-3.25 (2H, m), 2.60 (1H, dd, *J*=15.5, 9.8 Hz), 2.44 (1H, dd, *J*=15.5, 4.2 Hz), 2.11 (3H, s), 2.05 (3H, s), 2.04 (3H, s), 2.02 (3H, s), 1.98-1.86 (1H, m), 1.82-1.69 (1H, m), 1.70-1.54 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.4, 172.4, 170.9, 170.4, 170.0, 169.8, 156.1, 143.6, 141.2, 127.7, 127.0, 125.0, 119.9, 69.1, 68.9, 67.8, 67.7, 66.9, 61.2, 53.4, 50.6, 47.0, 39.1, 34.1, 31.7, 28.6, 22.1, 20.7; IR (thin film): 3367, 1748 cm^{-1}; LRMS (ESI): Calcd for $C_{36}H_{43}N_2O_{14}$ $[M+H]^+$ 726.3, found 727.0.



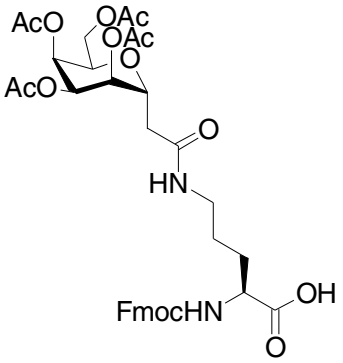
Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside) (21)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (2H, d, *J*=7.6 Hz), 7.63-7.55 (2H, m), 7.38 (2H, t, *J*=7.4 Hz), 7.33-7.25 (2H, m), 6.64-6.53 (1H, m), 5.83 (1H, d, *J*=7.4 Hz), 5.24 (1H, t, *J*=8.3 Hz), 5.07 (1H, dd, *J*=5.2, 8.6 Hz), 4.98 (1H, t, *J*=8.3 Hz), 4.70-4.60 (1H, m), 4.45-4.30 (3H, m), 4.25-4.12 (2H, m), 4.05-3.90 (3H, m), 3.40-3.20 (2H, m), 2.66 (2H, dd, *J*=9.9, 15.2 Hz), 2.49 (2H, dd, *J*=4.3, 15.3 Hz), 2.05 (3H, s), 2.03 (3H, s), 2.02 (3H, s), 2.01 (3H, s), 1.97-1.82 (1H, m), 1.81-1.67 (1H, m), 1.67-1.51 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 170.0, 169.6, 169.5, 156.3, 143.8, 143.7, 141.3, 127.7, 127.1, 125.1, 120.0, 77.2, 69.9, 69.7, 69.5, 69.4, 68.2, 67.1, 62.1, 53.5, 47.1, 39.1, 34.2, 29.8, 25.2, 20.7, 20.7, 20.6, 20.6; HRMS (ESI): Calcd for $C_{36}H_{43}N_2O_{14}$ $[M+H]^+$ 727.2714, found 727.2739.



Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside) (22)

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (2H, d, *J*=7.4 Hz), 7.58 (2H, dd, *J*=4.8, 7.1 Hz), 7.37 (2H, dd, 7.2, 7.9 Hz), 7.27 (2H, dt, *J*=1.1, 7.7, 7.8 Hz), 6.72 (1H, br m), 5.94 (1H, d, *J*=7.5 Hz), 5.24 (1H, dd, *J*=3.1, 7.4 Hz), 5.15-5.08 (2H, m), 4.45-4.30 (4H, m), 4.22-4.12 (2H, m), 4.02-3.92 (1H, m), 3.38-3.20 (2H, m), 2.65-2.45 (2H, m), 2.05 (3H, s), 2.04 (3H, s), 2.03 (6H, s), 1.97-1.82 (1H, m), 1.80-1.65 (1H, m), 1.65-1.52 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.5, 170.9, 170.2, 169.9, 169.8, 169.5, 156.3, 143.7, 143.6, 141.2, 127.7, 127.0, 125.0, 119.9, 77.2, 71.7, 70.1, 69.3, 68.0, 67.2, 67.0, 61.8, 53.4, 47.0, 39.1, 36.9, 29.6, 25.2, 20.7, 20.7, 20.7, 20.6; HRMS (ESI): Calcd for $C_{36}H_{43}N_2O_{14}$ $[M+H]^+$ 727.2714, found 727.2715.



Fluorenylmethoxycarbonyl-L-ornithine-1-(2,3,4,6-tetra-O-acetyl-α-D-talopyranoside) (23)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.3-8.2 (1H, br s), 7.74 (2H, d, *J*=7.4 Hz), 7.59 (2H, d, *J*=6.9 Hz), 7.33 (4H, dt, *J*=7.1, 28.6 Hz), 6.73 (1H, br s), 5.99 (1H, br d, *J*=5.6 Hz), 5.52 (1H, br s), 5.16 (1H, br dd, *J*=3.0, 5.2), 4.80 (1H, br dd, *J*=2.2, 8.3 Hz), 4.62 (1H, br dd, *J*=9.8, 12.6 Hz), 4.50-4.11 (6H, m), 3.4-3.15 (2H, br m), 2.58-2.30 (2H, br m), 2.12 (3H, s), 2.06 (3H, s), 2.03 (3H, s), 2.00 (3H, s), 1.96-1.83 (1H, m), 1.80-1.66 (1H, m), 1.66-1.52 (2H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.0, 170.2, 169.7, 169.5, 156.3, 143.8, 143.6, 141.2, 127.7, 127.0, 125.1, 120.0, 71.5, 68.9, 67.3, 66.9, 66.4, 66.0, 60.3, 47.0, 39.0, 37.9, 29.7, 25.3, 20.8, 20.8, 20.6; HRMS (ESI): Calcd for $C_{36}H_{43}N_2O_{14}$ $[M+H]^+$ 727.2714, found 727.2701.

(iv) Solid Phase Synthesis of C-Linked Glycopeptides

General protocol for manual and automated solid phase synthesis of C-linked analogues

All polypeptides were prepared by linear solid-phase synthesis using standard Fmoc chemistry.[2] A typical procedure started from Fmoc-glycine Wang resin with loading capacities of ~0.066 mmol/g. The resin was swollen in DMF for 30 minutes. The solvent was then drained and 20% piperidine solution in DMF was added. The solution was allowed to stir for 1 hour, then it was drained, and the resin was washed with three aliquots of DMF. Kaiser and TNBS tests for free amine were then performed. Building block (1.5 equivalents) was premixed with 1.5 equivalents of HBTU in DMF, followed by the addition of 1.5 equivalents of DIPEA in DMF. The reaction was stirred for 30 minutes, transferred to the SPS flask and stirred for 24 hours. The flask was drained and the resin was rinsed three times with DMF. Kaiser and TNBS tests were performed to verify the coupling had reached completion (negative test outcome). The resin was treated with 20% piperdine in DMF solution for 1 hour, the flask was drained and the resin was washed thoroughly with DMF. Kaiser and TNBS tests were performed to ensure the presence of free amine. Then the next successive building block (5 equivalents) to be coupled (commercially available amino acid) was premixed with 5 equivalents of HBTU and 5 equivalents of DIPEA in DMF for 20 minutes. The reaction solution was then transferred to the resin, and the reaction mixture was allowed to stir for 4 hours. The Fmoc deprotection and coupling steps were repeated until twelve amino acid residues were coupled to the resin. To cleave the glycopeptide from the resin, the resin was successively rinsed with DMF, MeOH, and CH_2Cl_2, and stirred for 2 hours in 1:1 (v:v) trifluoroacetic acid and CH_2Cl_2. The solution was filtered and concentrated, and the product crystallized from diethyl ether to produce a white powder. The glycopolymer was dissolved in a sodium methoxide (pH=10) solution (4:1 sodium methoxide/ water) and stirred for 5 hours. The solution was neutralized with IR-120 ion exchange resin, filtered, concentrated to remove MeOH and lyophilized to give the product as a white powder. Purification via HPLC using reverse phase chromatography (C-18 column, liquid phase: 0.1% TFA in acetonitrile/water) was then performed to obtain the desired glyopeptide.

[L-Ornithine(galactose)-glycine-glycine]$_4$-glycine (1)

^{1}H NMR (300 MHz, D_2O) δ 4.42-4.32 (4H, m), 4.28-4.18 (4H, m), 4.00-3.80 (29H, m), 3.75-3.67 (4H, m), 3.67-3.47 (14H, m), 3.20-3.05 (8H, m), 2.62-2.42 (8H, m), 1.85-1.70 (5H, m), 1.70-1.57 (4H, m), 1.57-1.37 (9H, m); ^{13}C NMR (75 MHz, D_2O) δ 175.0, 174.9, 174.1, 173.9, 173.6, 172.3, 172.3, 172.2, 172.0, 171.9, 171.8, 170.7, 163.6, 163.1, 73.3, 72.8, 70.0, 69.1, 67.9, 61.2, 54.1, 53.2, 42.9, 42.7, 41.5, 41.4, 39.1, 32.7, 28.4, 25.2; LRMS (MALDI-TOF): Calcd for $C_{70}H_{117}N_{17}O_{38}$ $[M+H]^+$ 1805.8, found 1805.3.

[2] Fields, G. B.; Fields, C. G. *J. Am. Chem. Soc.* **1991**, 113, 4202.

[L-Ornithine(glucose)-glycine-glycine]$_4$ -glycine (2)

^{1}H NMR (360 MHz, D_2O) δ 4.47 (32H, s), 4.08 (2H, br s), 3.73 (6H, m), 3.32 (10H, m), 3.01 (6H, m), 2.45 (2H, m), 2.19 (18H, s), 1.98 (8H, s), 1.68 (8H, m), 1.30 (4H, m), 0.87 (2H, m); ^{13}C NMR (90 MHz, D_2O) δ 187.76, 186.77, 185.19, 184.83, 184.24, 180.00, 159.30, 143.77, 142.41, 142.34, 139.011, 138.55, 97.19, 86.21, 85.68, 82.81, 81.66, 80.76, 80.63, 74.08, 71.31, 66.13, 60.96, 55.79, 55.60, 51.98, 45.61, 45.43, 38.05, 36.468, 19.55; LRMS (ESI): Calcd for $C_{70}H_{117}N_{17}O_{38}$ $[M+H]^+$ 1805.8, found 1805.2

[L-Ornithine(mannose)-glycine-glycine]$_4$ -glycine (3)

^{1}H NMR (500 MHz, D_2O) δ 4.83 (32H, m), 4.45 (4H, m), 4.07 (6H, m), 3.93 (8H, m), 3.65 (4H, m), 3.46 (4H, m), 3.21 (3H, m), 2.98 (1H, m), 2.80 (4H, m), 2.68 (1H, m), 2.58 (1H, m), 2.25 (4H, m), 2.07 (16H, m), 1.79 (4H, m), 1.60 (8H, m), 1.33 (8H, m), 1.06 (4H, m); ^{13}C NMR (125 MHz, D_2O) δ 181.01, 170.59, 117.47, 117.05, 74.60, 74.33, 74.07, 70.34, 70.06, 66.70, 62.04, 60.51, 42.73, 42.07, 41.95, 38.29, 35.01, 34.79, 29.77, 27.69, 24.29, 22.81; LRMS (ESI): Calcd for $C_{70}H_{117}N_{17}O_{38}$ $[M+H]^+$ 1805.8, found 1804.1.

[L-Ornithine(2,3,4,6-peracetylated talose)-glycine-glycine]$_4$ -glycine

^{1}H NMR (300 MHz, MeOD) δ 5.51 (1H, br s), 5.21 (1H, br s), 4.65-4.53 (1H, m), 4.46-3.82 (10H, m), 3.24 (3H, m), 2.60-2.45 (2H, m), 2.11 (3H, s), 2.08 (3H, s), 2.06 (6H, s), 1.97-1.83 (1H, m), 1.83-1.72 (1H, m), 1.72-1.52 (2H, m); ^{13}C NMR (75 MHz, MeOD) δ 174.3, 174.0, 171.9, 171.5, 171.4, 171.1, 170.8, 170.5, 170.5, 170.4, 71.1, 69.4, 68.1, 67.5, 67.0, 60.9, 53.9, 53.9, 53.2, 42.7, 40.9, 39.2, 38.9, 37.6, 29.8, 28.7, 25.9, 25.0, 24.9, 22.8, 20.0, 19.8, 19.7, 19.7; LRMS (MALDI-TOF): Calcd for $C_{102}H_{149}N_{17}O_{54}$ $[M+H]^+$ 2478.4, found 2476.8.

[L-Ornithine(talose)-glycine-glycine]$_4$ -glycine (4)

^{1}H NMR (500 MHz, D_2O) δ 4.27-4.17 (5H, m), 3.92-3.72 (20H, m), 3.67-3.55 (7H, m), 3.45-3.40 (1H, m), 3.14-3.07 (6H, m), 2.54-2.40 (6H, m), 1.80-1.70 (2H, m), 1.70-1.55 (3H, m), 1.55-1.37 (6H, m); ^{13}C NMR (125 MHz, D_2O) δ 174.4, 172.6, 171.8, 171.5, 170.9, 99.9, 73.9, 72.9, 72.8, 70.6, 68.8, 66.8, 60.0, 53.8, 53.5, 43.0, 42.4, 42.3, 42.2, 38.8, 38.6, 36.0, 28.0, 24.7, 24.6, 24.4; LRMS (MALDI-TOF): Calcd for $C_{70}H_{117}N_{17}O_{38}$ $[M+H]^+$ 1805.8, found 1804.8.

II. Assessing Antifreeze Activity:

Antifreeze activity of analogues **1-4** was evaluated using two standard methods. Thermal hysteresis (TH) was measured using a Clifton Nanoliter Osmometer[3] and recrystallization-inhibition (RI) activity was assessed using the splat cooling technique previously described by Knight and co-workers.[4] Briefly, the latter technique involves generating a frozen wafer from the analyte which is dissolved in phosphate buffered saline (PBS) solution; a 10 uL drop of this solution is then dropped from a pipette through a two metre high plastic tube (10 cm in diameter) onto a block of polished aluminum pre-cooled to approximately -80 ^{o}C. The droplet freezes instantly on the polished aluminum block and is approximately 1 cm in diameter and 20 um thick. This wafer is then carefully removed from the surface of the block and transferred to a cryostage (refrigerated microscope stage) held at -6.4 ^{o}C. The wafer is left to anneal at this temperature for a period of 30 mins and then photographed between crossed polarizing filters using a digital camera (Nikon CoolPix 5000) fitted to the microscrope. A total of six different images are taken from each wafer; three from the middle and three from the edge. The images are then analyzed using the mean elliptical method. In this method, the ten largest ice crystals were chosen from the field of view (FOV) in each image.[5,6] Selection of these crystals was arbitrary in that they were chosen after a visual inspection of the image. The two dimensional surface area of each of these ten crystals was then calculated via approximation of the crystal as an elliptical area. The major and minor elliptical axes were defined by the two largest orthogonal dimensions across the ice grain surface. The surface area of each ice grain was then calculated based on the formula: $A = \pi ab$, in which A represented area; a and b represented the length of the major and minor elliptical axes. Totalling all individual measurements for each FOV produces a value for the average grain surface area referred to as the mean largest grain size (MLGS). Error was calculated using standard error of the mean (SEM). T-tests were performed to a 95% confidence level.

III. Conformational Analysis via Circular Dichroism

Circular Dichroism was performed on JASCO Model J-810 automatic spectrophotometer interfaced with Acer computer. Spectral data were obtained with the following parameters: 1nm bandwidth; four scans at a scan speed of 50nm/min; 190-350nm wavelength range. Samples were measured at 22 ^{o}C in a quartz cell (Hellma QS) with a 1cm path length. A molar concentration of 43 μM was used for each sample, diluted in doubly distilled water. Data obtained from CD spectroscopy was converted into molar ellipticity (deg cm^2 dmol^{-1}). Glycopeptide secondary structures were estimated using the deconvolution software CD Pro (SELCON 3 program and IBASIS 4 protein dataset).[7]

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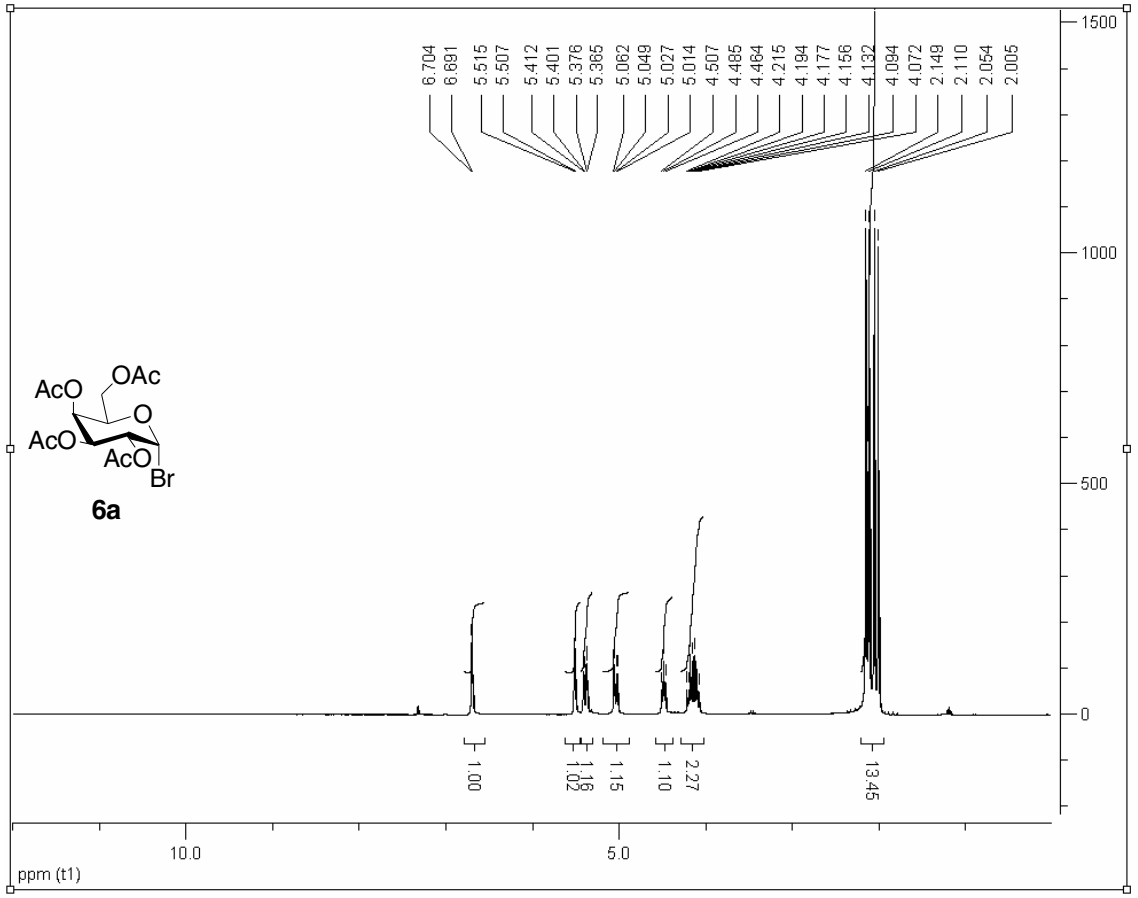
[3] Chakrabartty, A.; Hew, C.L.; *Eur. J. Biochem.* **1991,** 202, 1057.
[4] Knight, C. A.; Hallett, J.; DeVries, A.L. *Cryobiology* **1988,** 25, 55.
[5] Eniade, A.; Purushotham, M.; Ben, R.N.; Wang, J.B.; Horwath, K. *Cell Biochem. Biophys.* **2003**, 38, 115.
[6] Liu, S.; Ben, R.N. *Org. Lett.* **2005**, 7, 2385.
[7] (a) Sreerama, N.; Venyaminov, S.Y.; Woody, R.W. *Anal. Biochem.* **2000,** 287, 243. (b) Sreerama, N.; Woody, R.W. *Anal. Biochem.* **2000,** 287, 252-260. (c) Greenfield, N. J. *Anal. Biochem.* **1996,** 35, 1-10.

IBASIS 4	Conformation							
	Regular α-Helix	Distorted α-Helix	Regular β-Strand	Distorted β-Strand	Turn	Random coil	Sum	NRMSD
OGG Gal (**1**)	-0.011	0.160	0.031	0.092	0.256	0.504	1.032	0.543
OGG Glc (**2**)	-0.012	0.172	-0.004	0.073	0.232	0.568	1.029	0.425
OGG Man (**3**)	0.010	0.164	0.008	0.007	0.225	0.540	0.954	0.440
OGG Tal (**4**)	0.020	0.049	0.084	0.101	0.248	0.529	1.031	0.407
AFGP-8	0.049	0.114	0.164	0.094	0.237	0.378	1.036	0.833

Table 1. Deconvolution data for AFGP-8, *C*-linked analogues **1**-**4**, using SELCON 3 and IBASIS 4.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4.507
4.485
4.464
4.215
4.194
4.177
4.156
4.132
4.094
4.072
2.149
2.110
2.054
2.005
1.00
1.02
1.18
1.15
1.10
2.27
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5.0
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OAc
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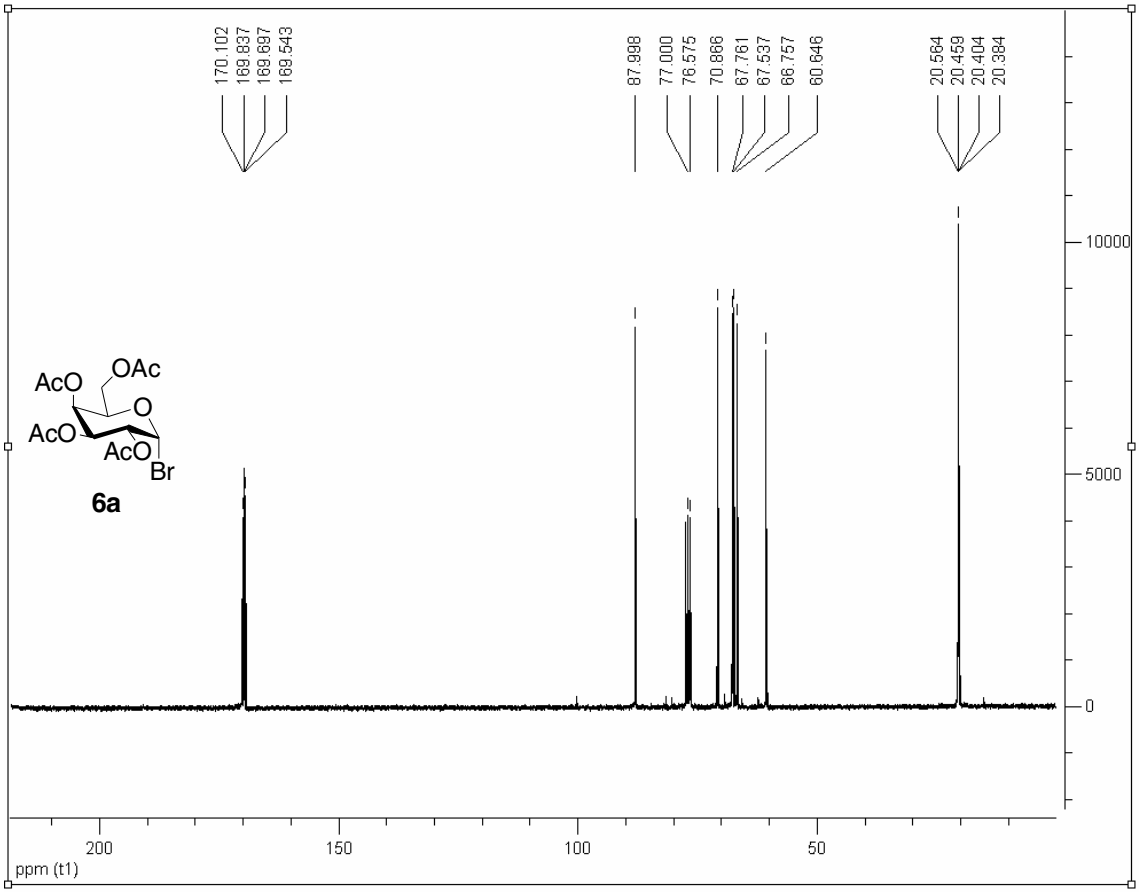
AcO
AcO
Br
6a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20.459
20.404
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200
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100
50
ppm (t1)
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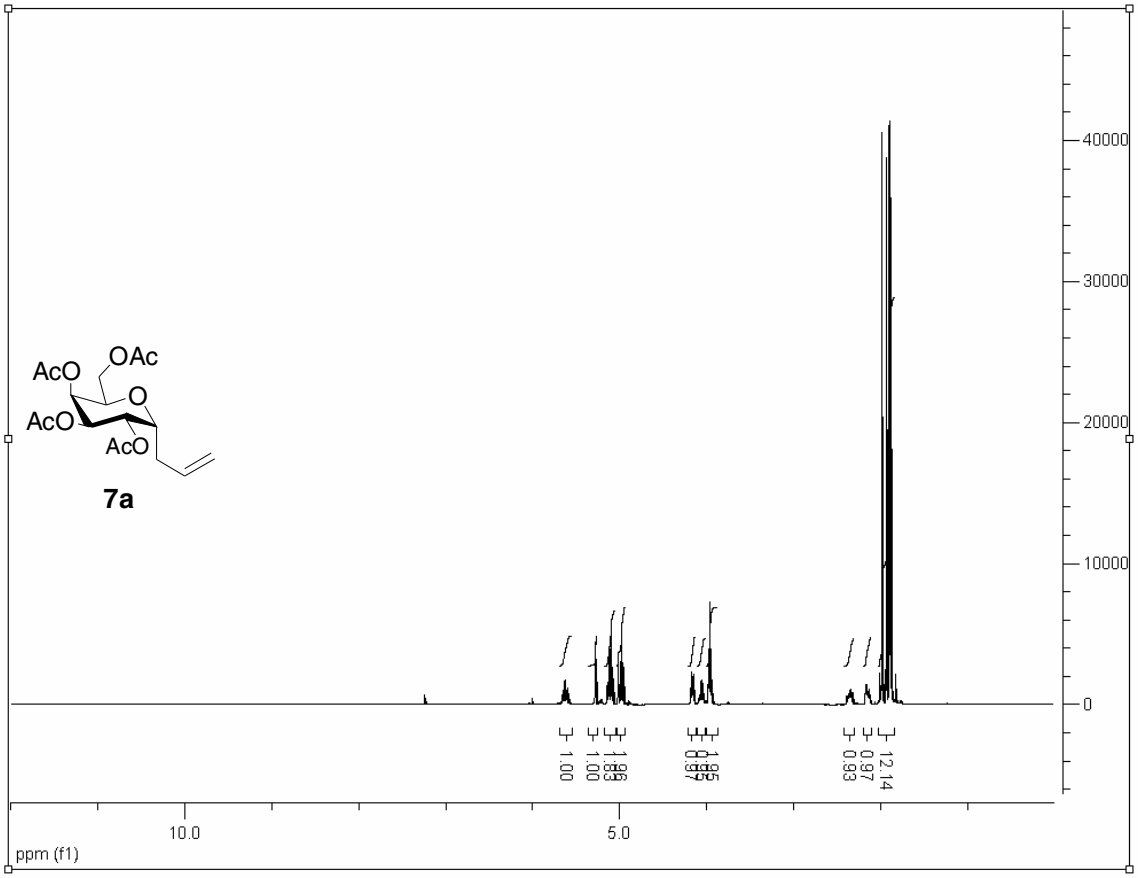
OAc
O
AcO
AcO
Br
6a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AcO
AcO
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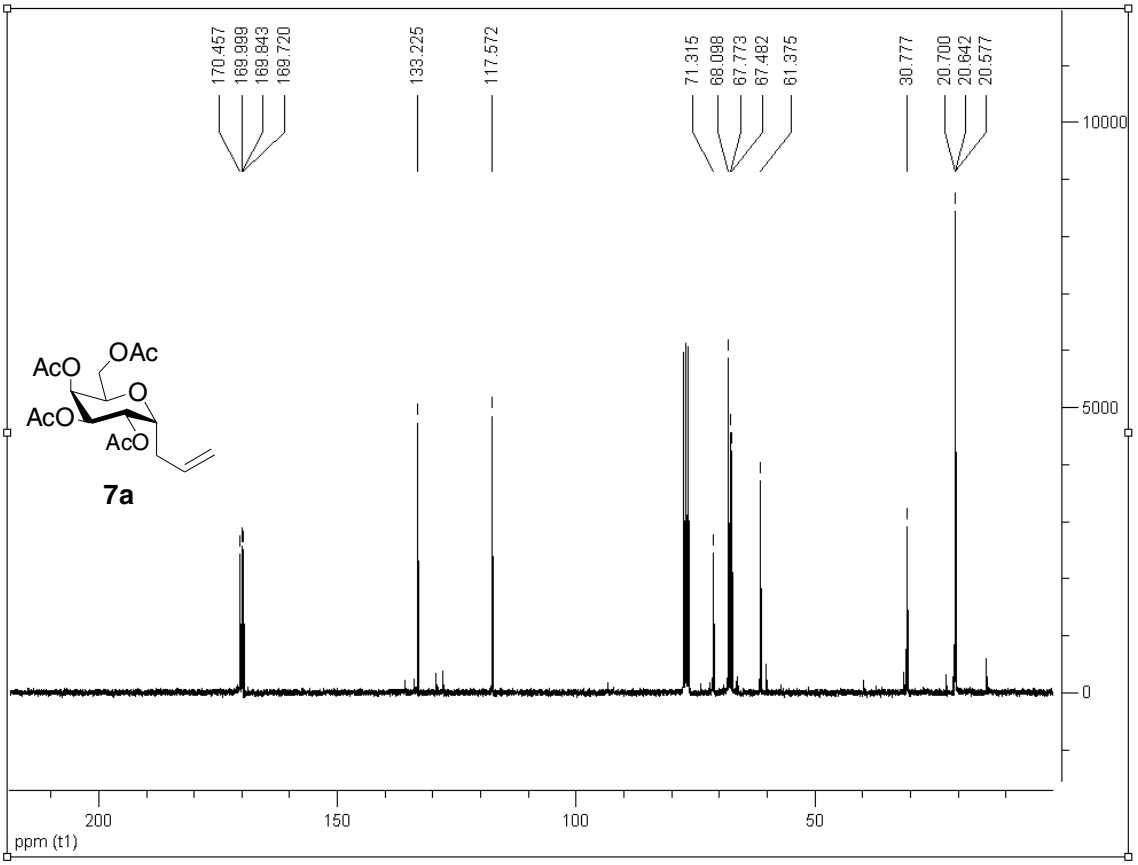
10.0
5.0
ppm (f1)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67.482
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20.700
20.642
20.577
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OAc
O
AcO
AcO
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0
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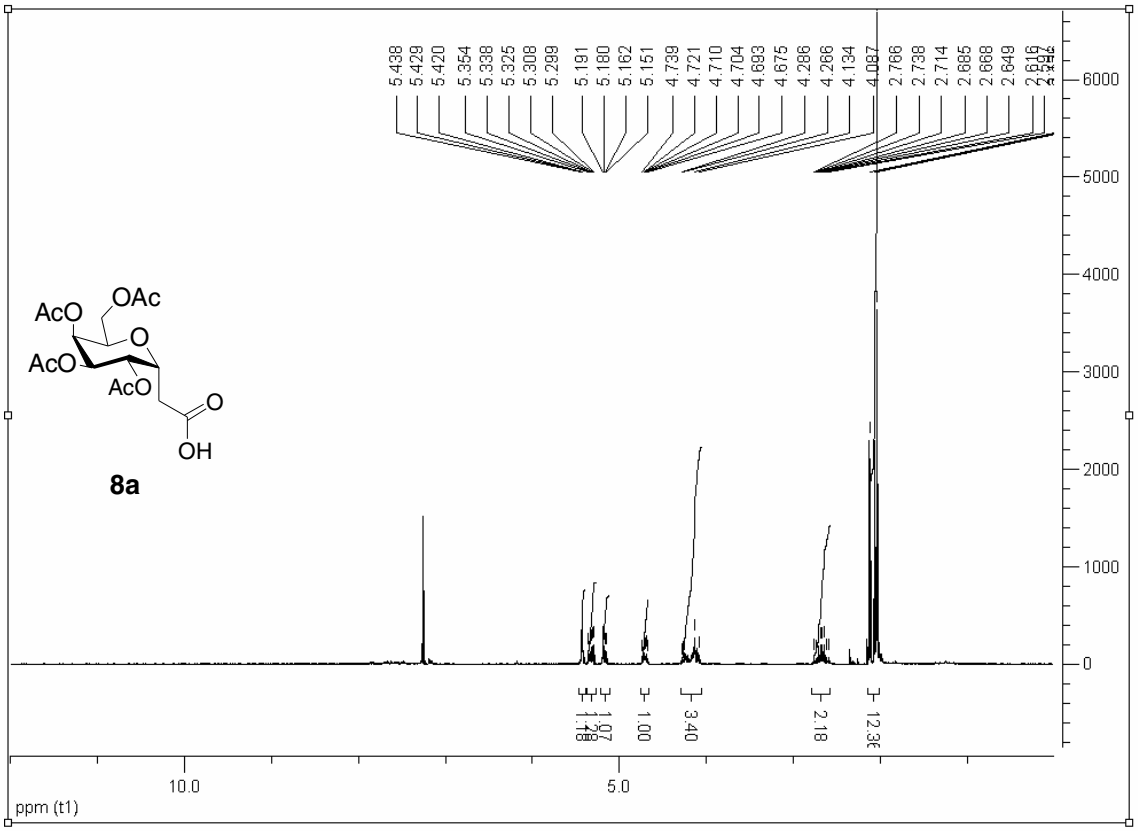
150
100
50
ppm (t1)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4.721
4.710
4.704
4.693
4.675
4.286
4.266
4.134
4.087
2.766
2.738
2.714
2.685
2.668
2.649
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5000
4000
3000
2000
1000
0
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ppm (t1)
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OAc
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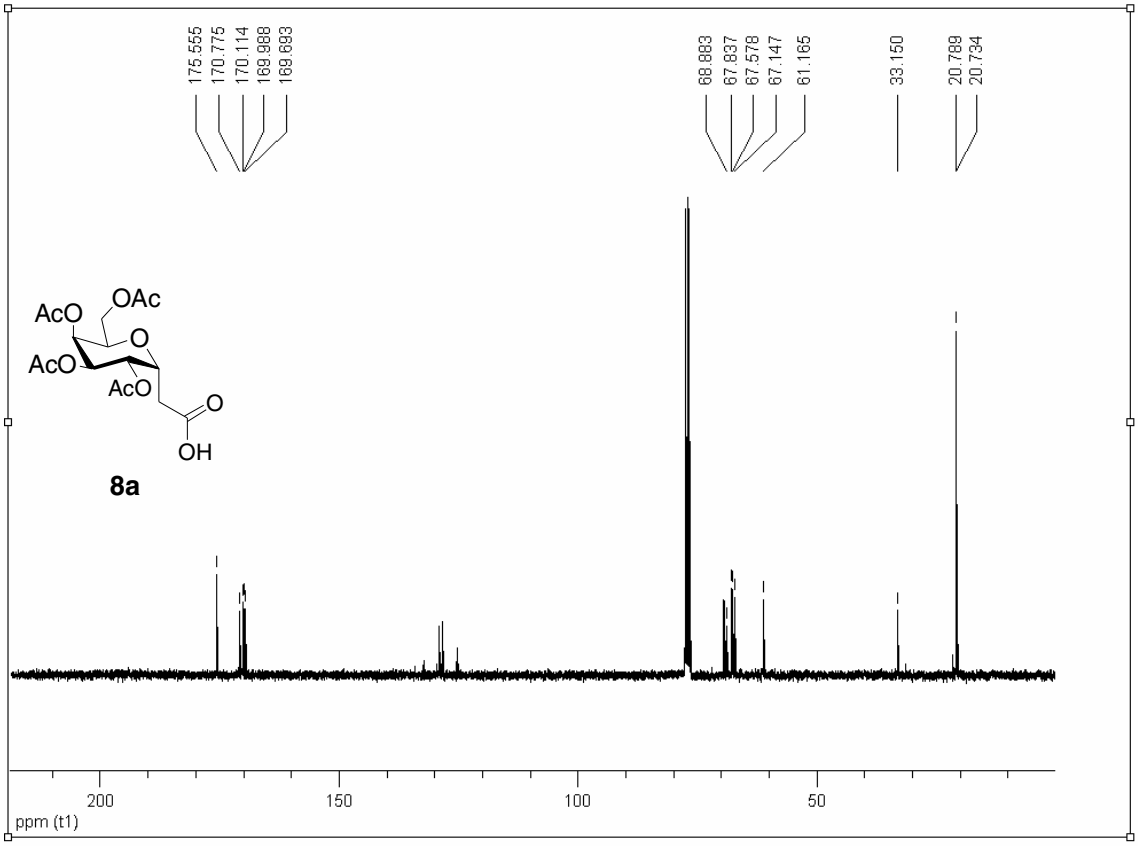
AcO
O
OH
8a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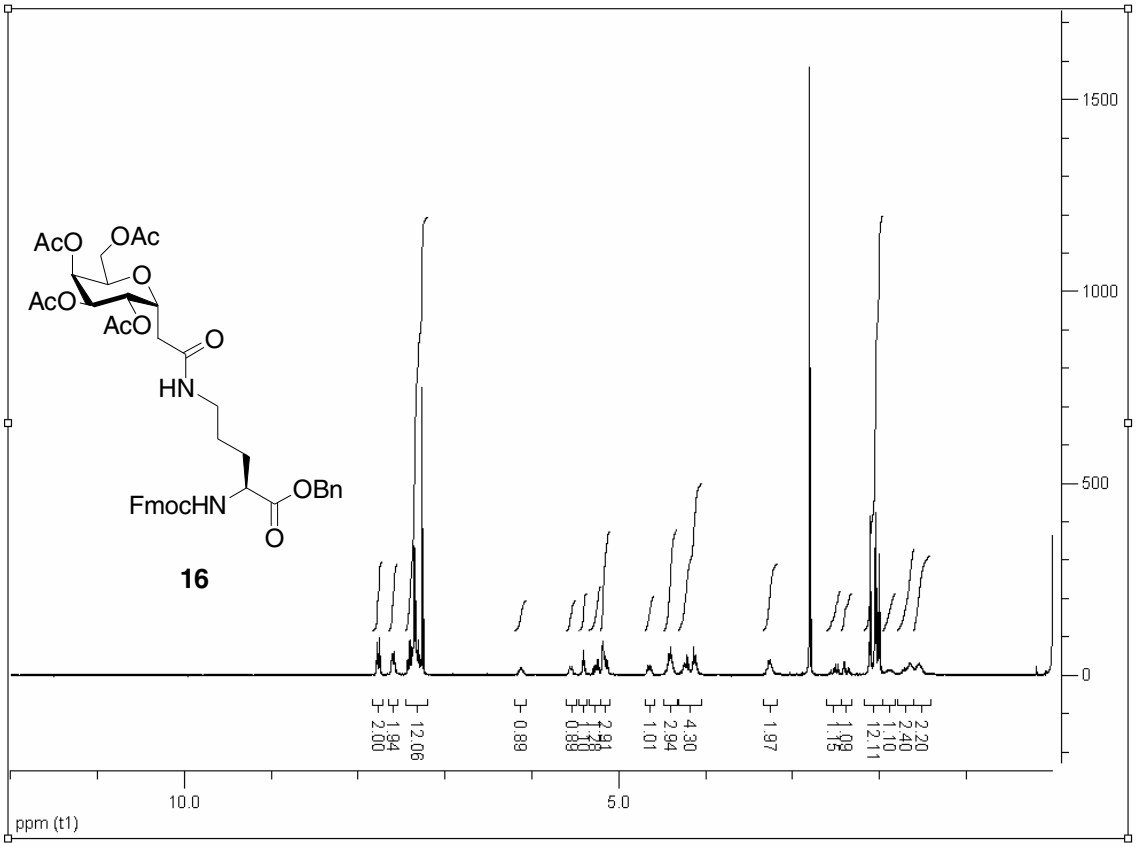
OH
8a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OBn
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2.84
4.30
1.01
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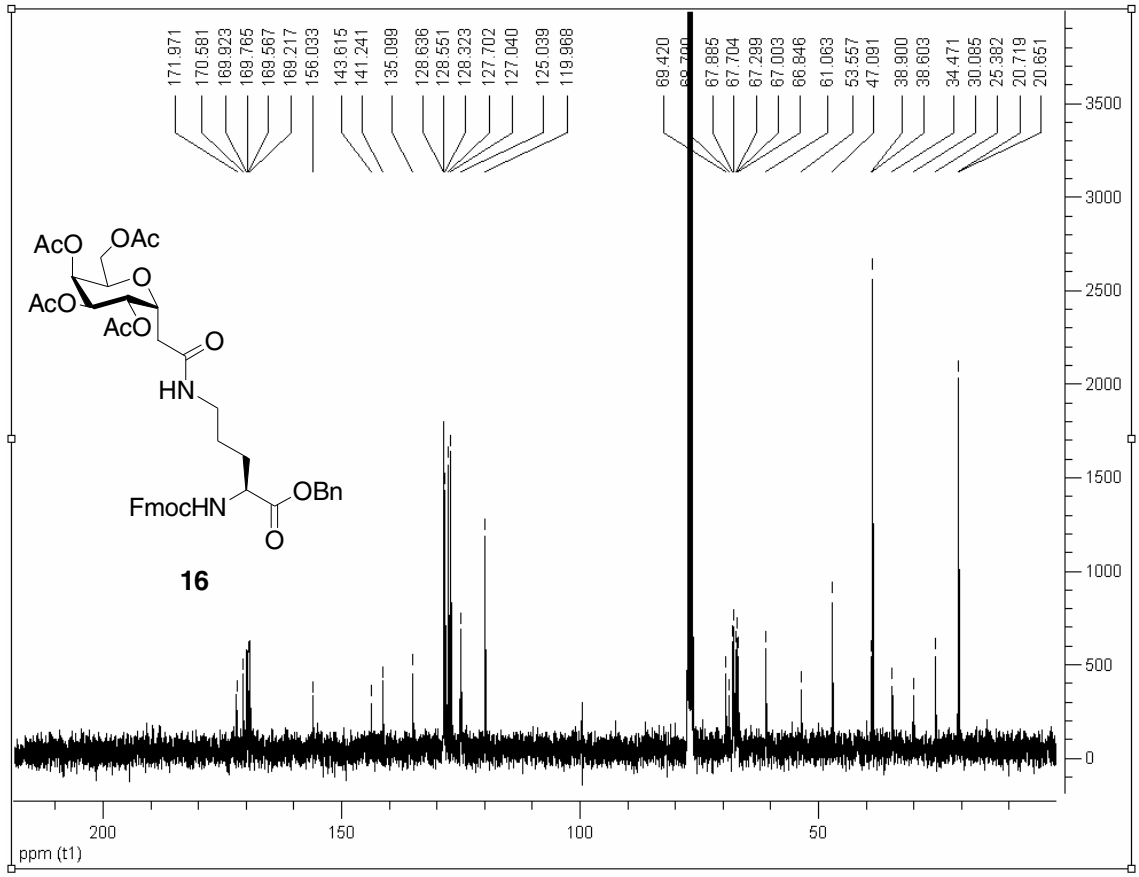
ppm (t1)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67.704
67.299
67.003
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61.063
53.557
47.091
38.900
38.603
34.471
30.085
25.382
20.719
20.651
AcO
OAc
AcO
AcO
O
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FmocHN
OBn
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500
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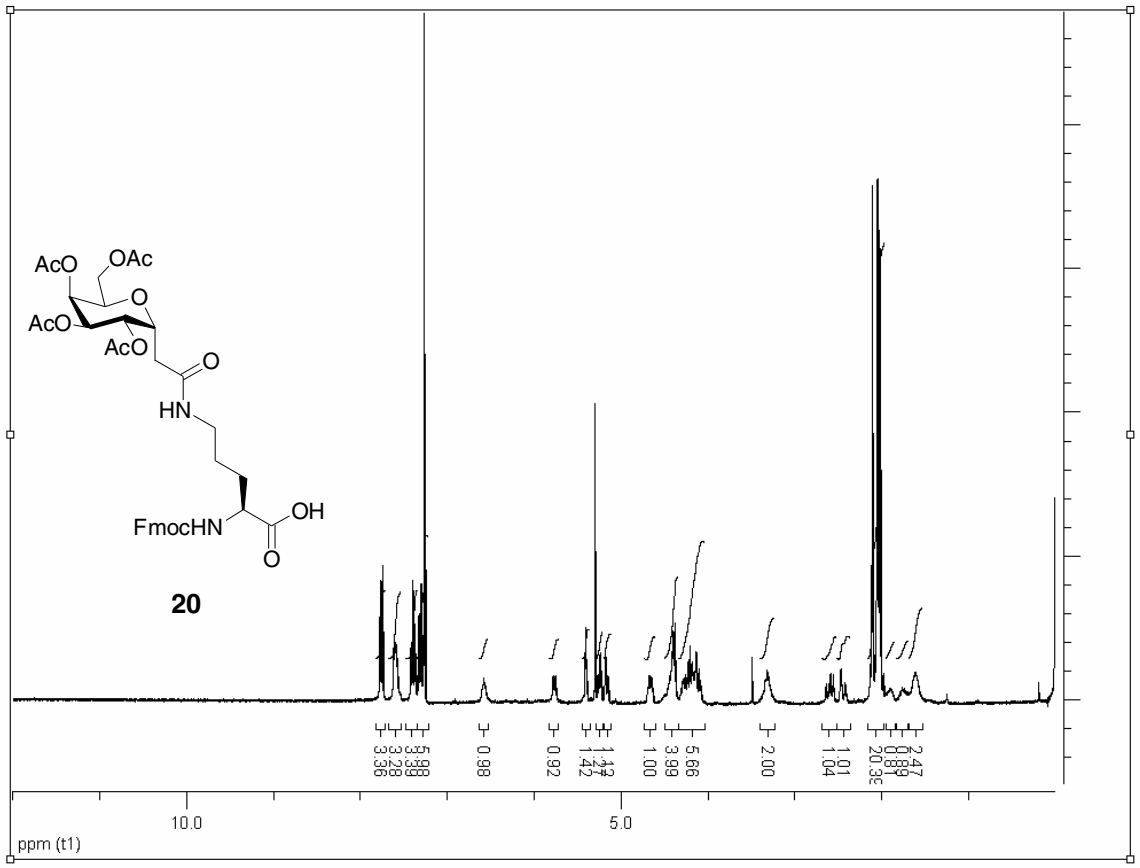
100
50
ppm (t1)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2.92
1.42
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2.00
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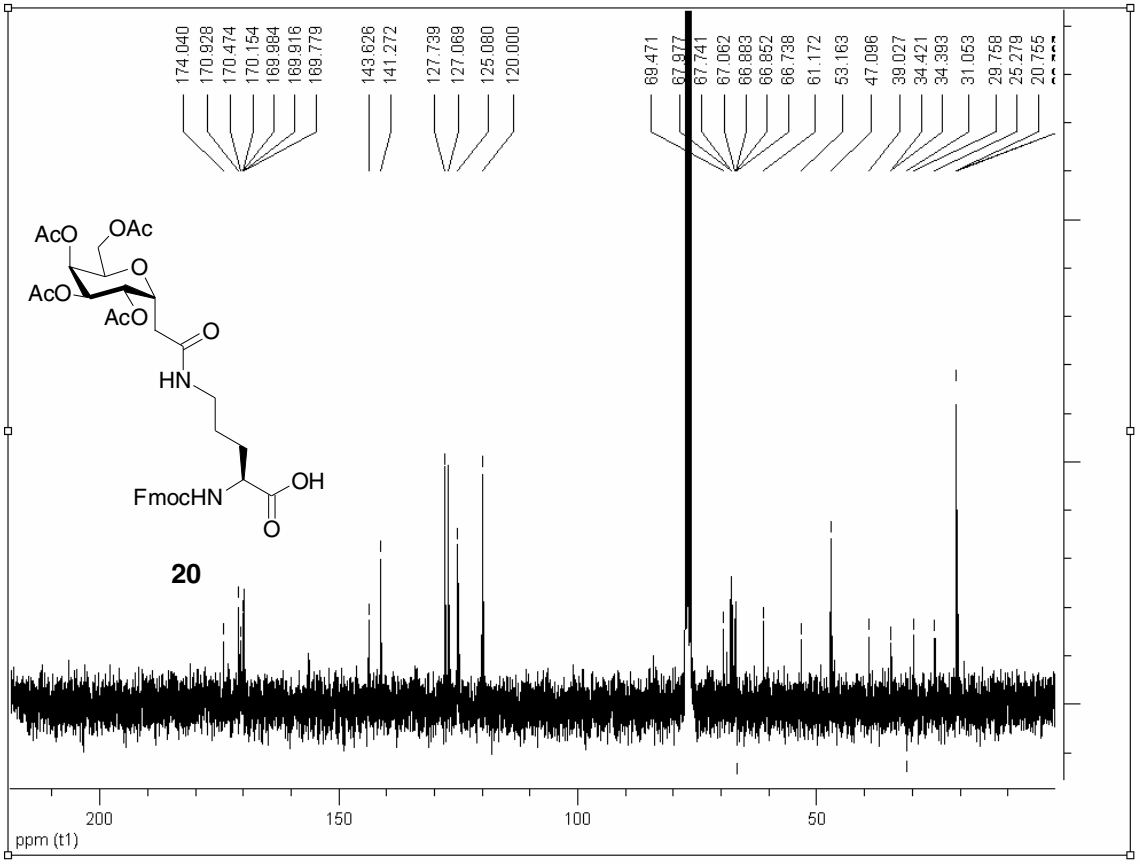
5.0
ppm (t1)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141.272
127.739
127.069
125.080
120.000
69.471
67.977
67.741
67.062
66.883
66.852
66.738
61.172
53.163
47.096
39.027
34.421
34.393
31.053
29.758
25.279
20.755
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OH
O
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100
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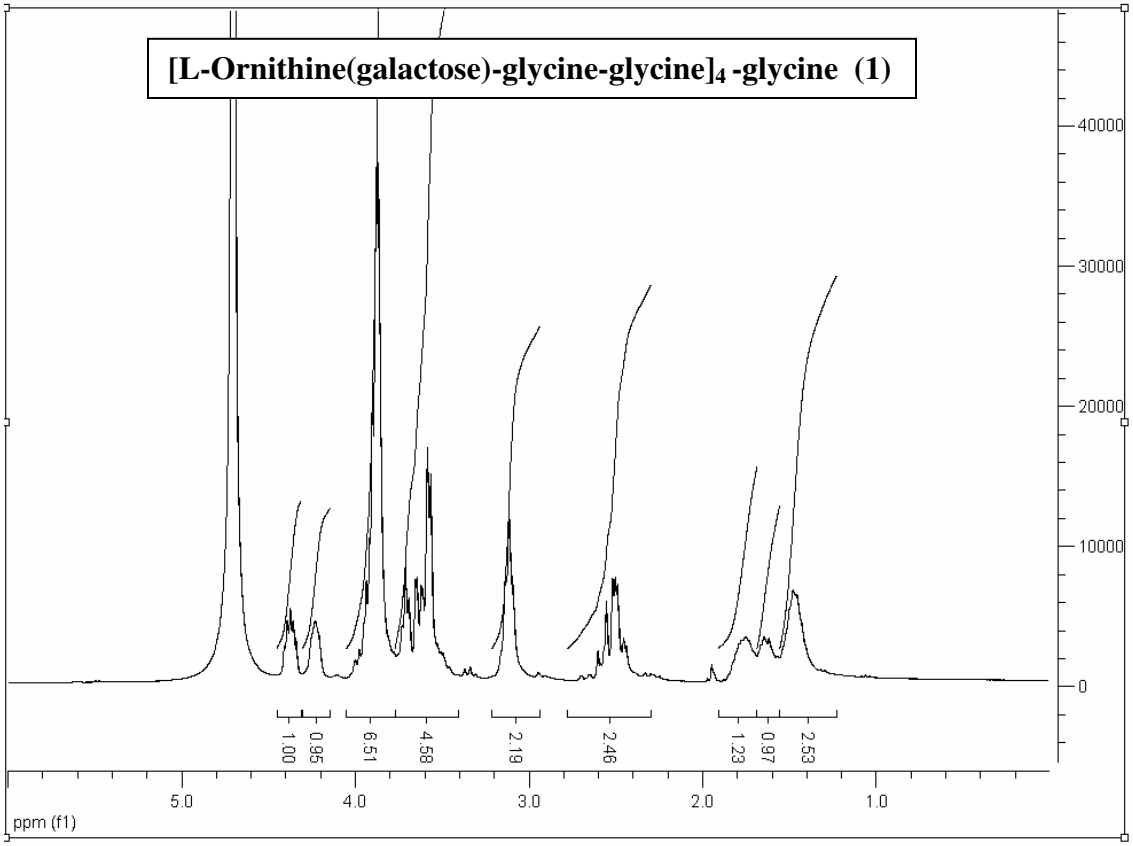
ppm (t1)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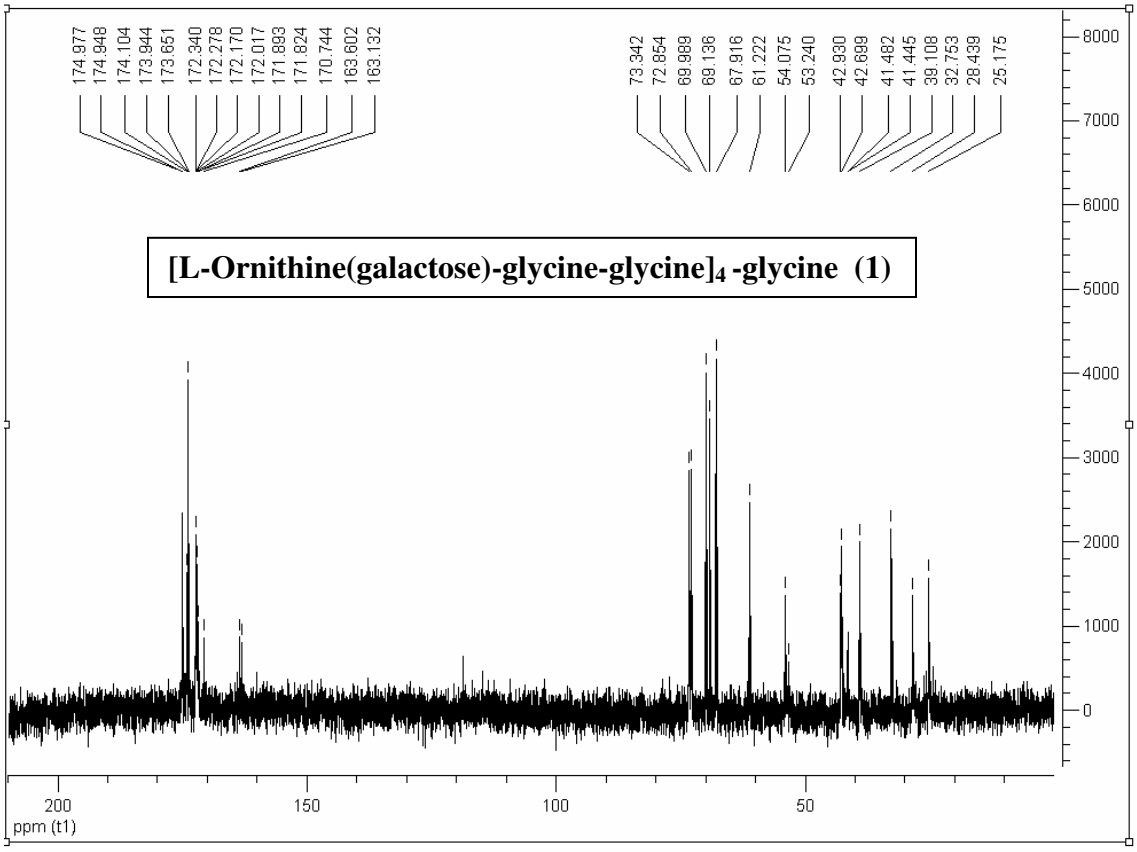
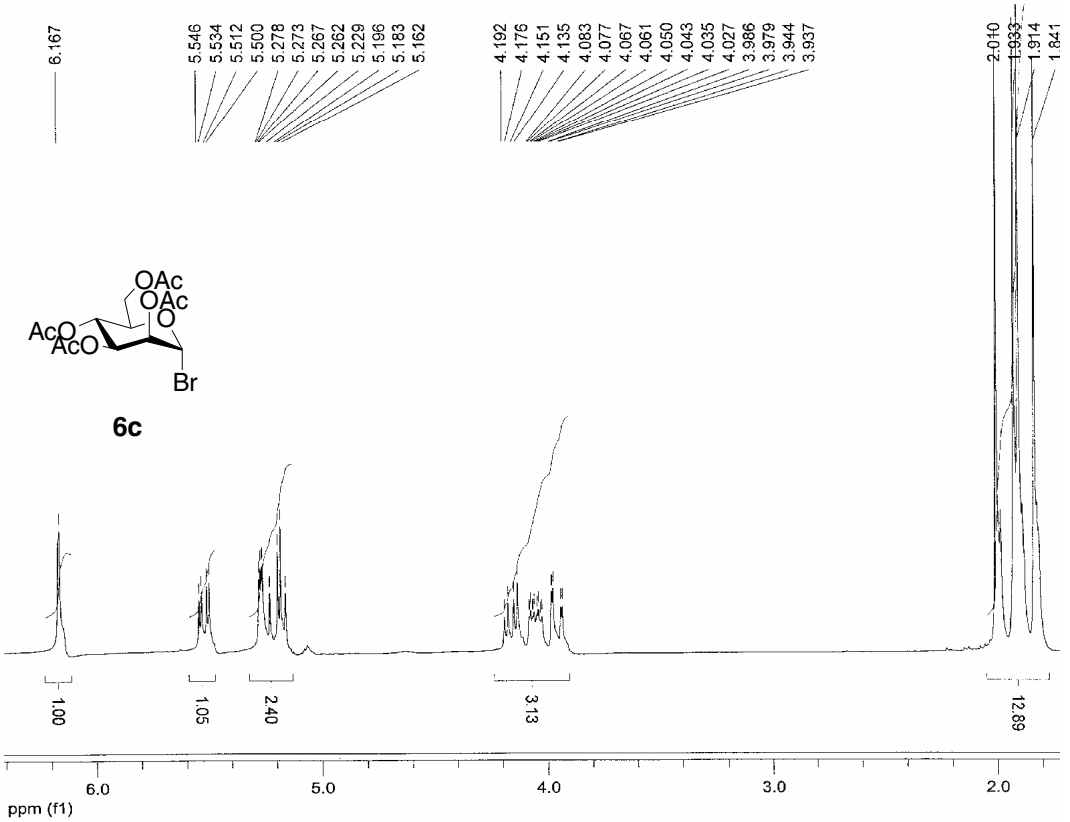
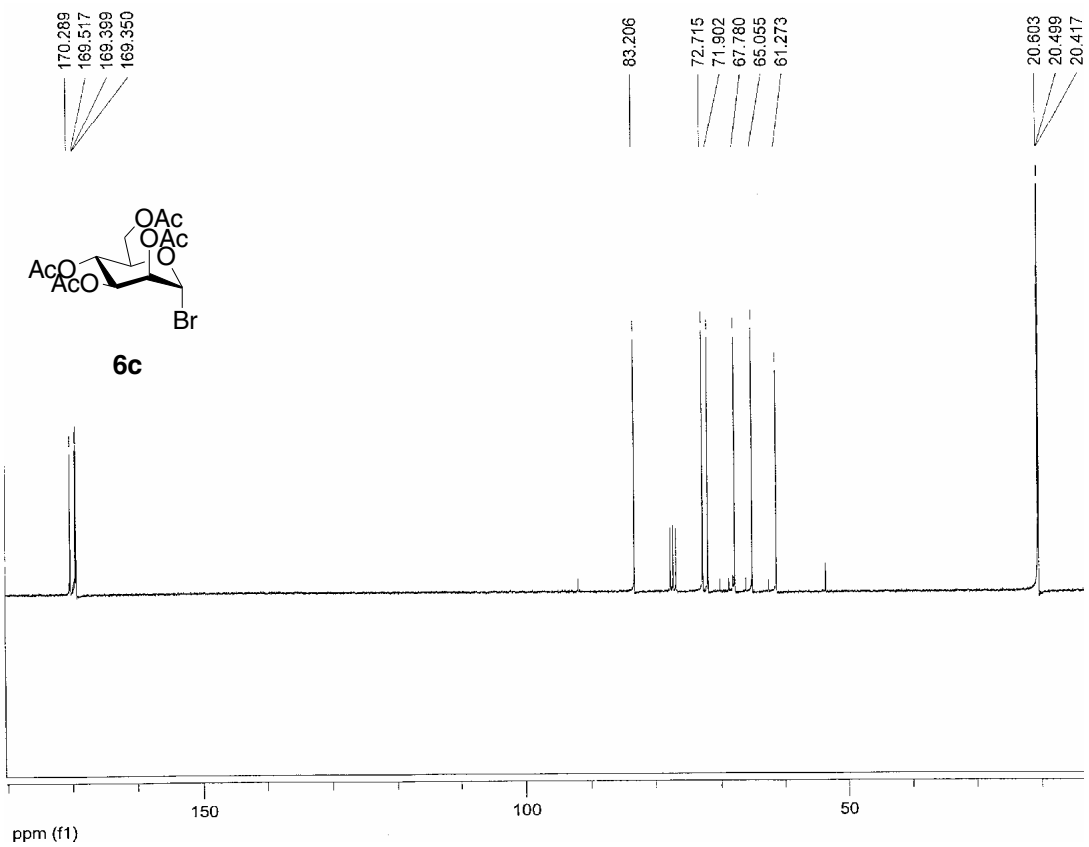
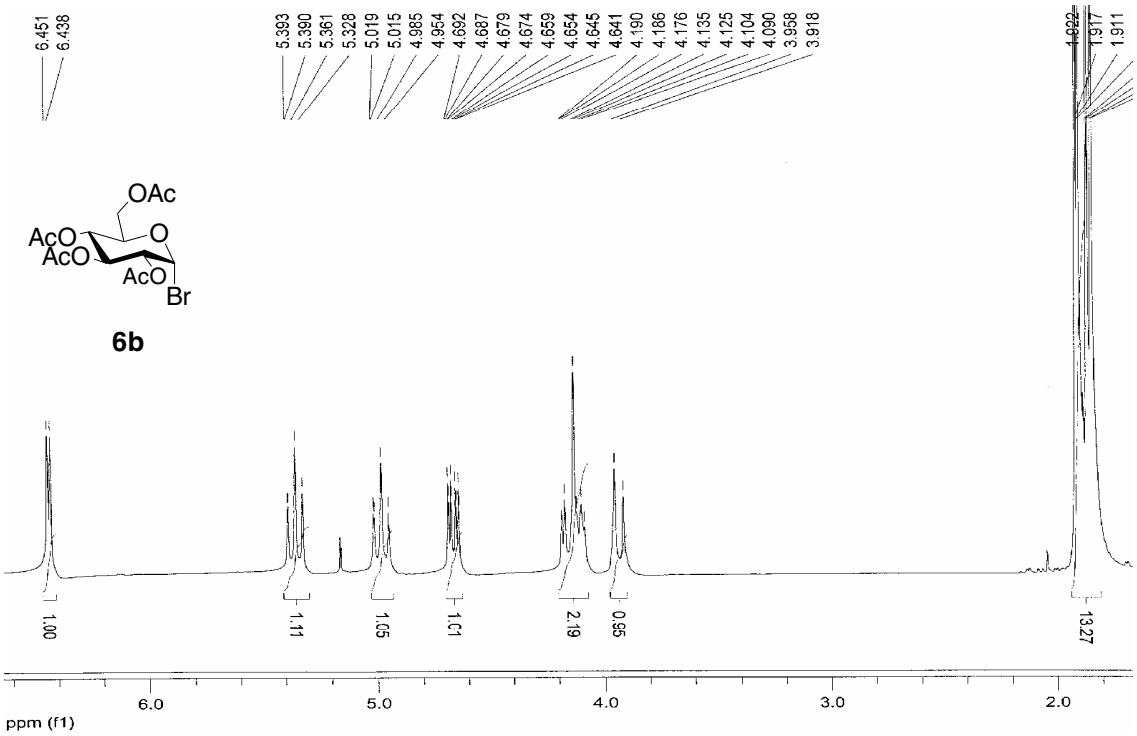
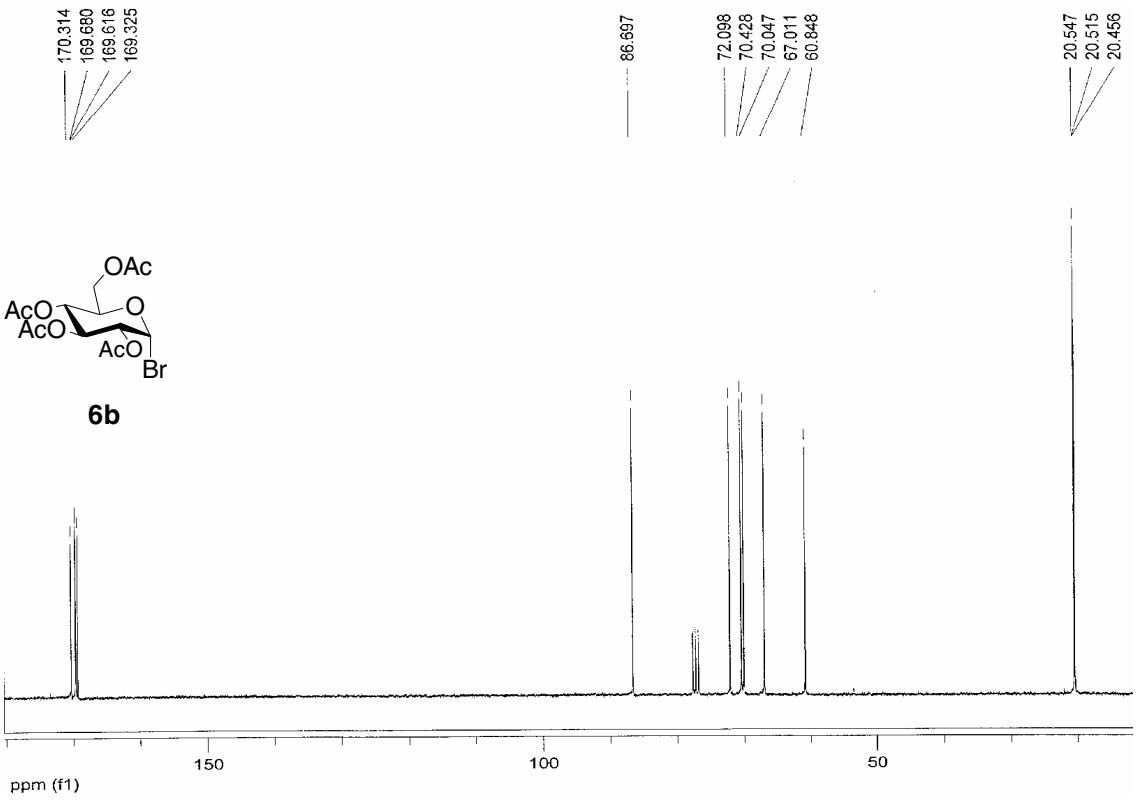
2.19
2.46
1.23
0.87
2.53
5.0
4.0
3.0
2.0
1.0
ppm (f1)

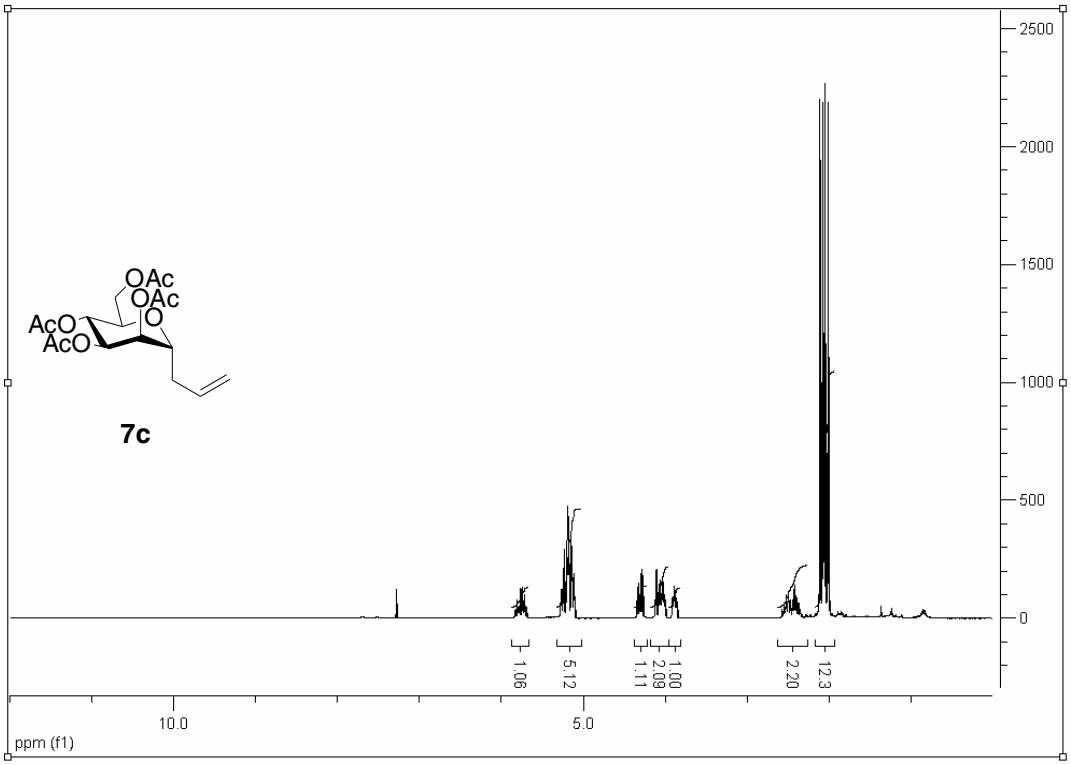

174.977
174.948
174.104
173.944
173.651
172.340
172.278
172.170
172.017
171.893
171.824
170.744
163.602
163.132
73.342
72.854
69.989
69.136
67.916
61.222
54.075
53.240
42.930
42.689
41.482
41.445
39.108
32.753
28.439
25.175
[L-Ornithine(galactose)-glycine-glycine]4 -glycine (1)
8000
7000
6000
5000
4000
3000
2000
1000
0
200
150
100
50
ppm (t1)

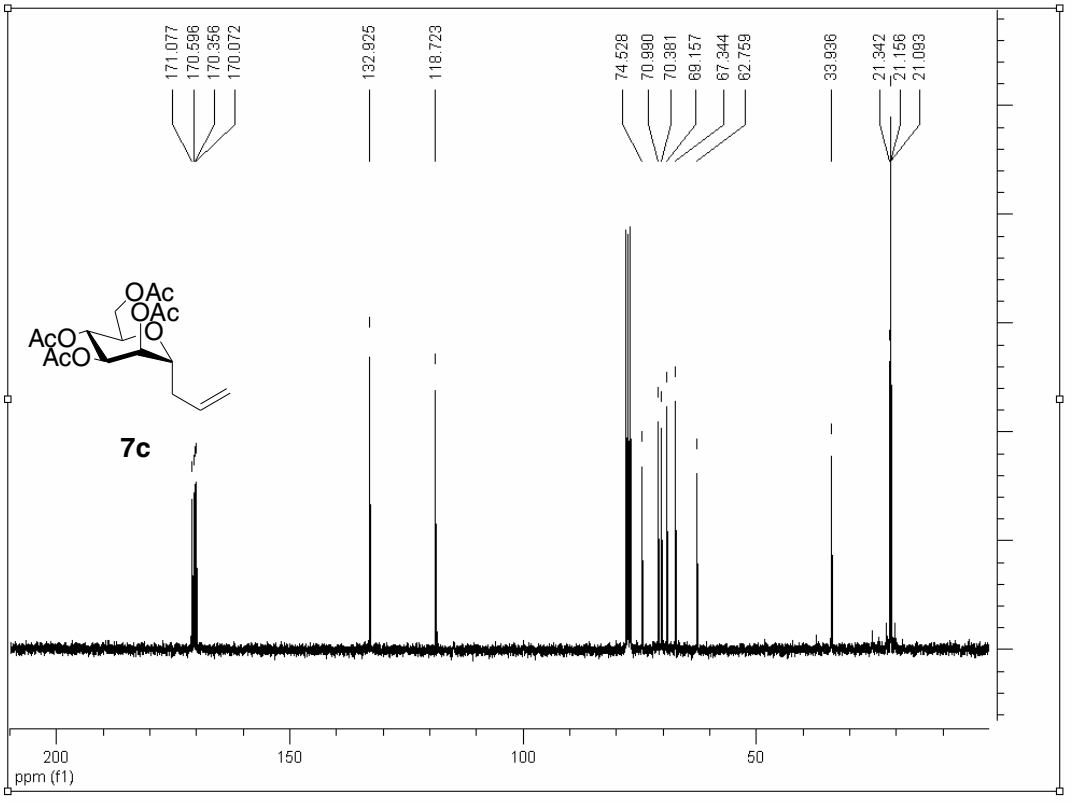

6.167
5.546
5.534
5.512
5.500
5.278
5.273
5.267
5.262
5.229
5.196
5.183
5.162
4.192
4.176
4.151
4.135
4.083
4.077
4.067
4.061
4.050
4.043
4.035
4.027
3.986
3.979
3.944
3.937
2.010
1.933
1.914
1.841
OAc
OAc
AcO
AcO
O
Br
6c
1.00
1.05
2.40
3.13
12.89
6.0
5.0
4.0
3.0
2.0
ppm (f1)

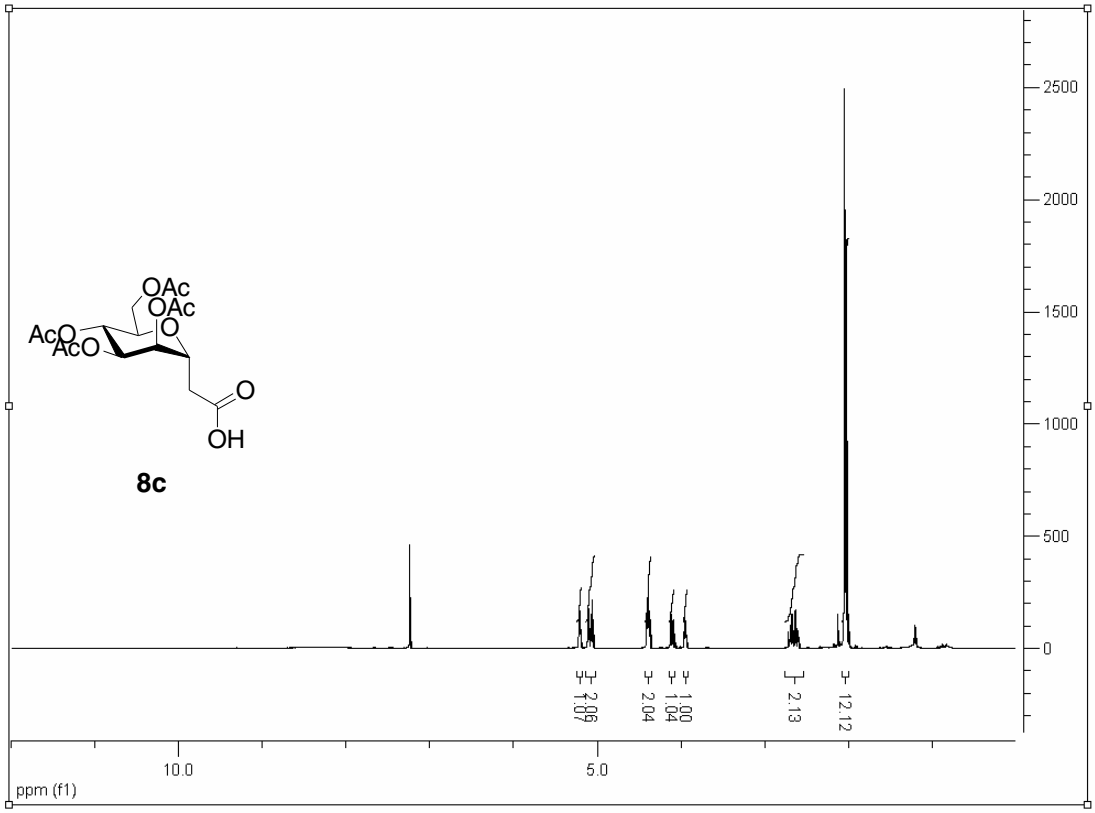

170.289
169.517
169.399
169.350
83.206
72.715
71.902
67.780
65.055
61.273
20.603
20.499
20.417
OAc
OAc
AcO
AcO
O
Br
6c
150
100
50
ppm (f1)

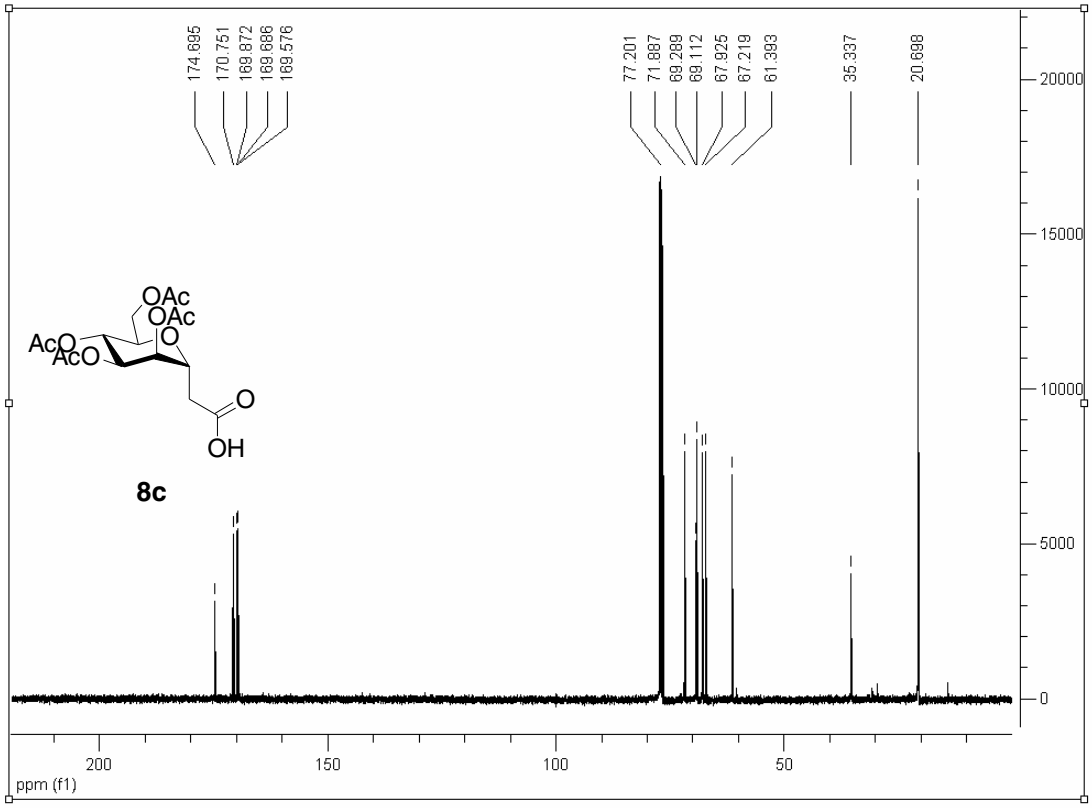

6.451
6.438
5.393
5.390
5.361
5.328
5.019
5.015
4.985
4.954
4.692
4.687
4.679
4.674
4.659
4.654
4.645
4.641
4.190
4.186
4.176
4.135
4.125
4.104
4.090
3.958
3.918
1.922
1.917
1.911
OAc
AcO
AcO
O
AcO
Br
6b
1.00
1.11
1.05
1.01
2.19
0.95
13.27
6.0
5.0
4.0
3.0
2.0
ppm (f1)

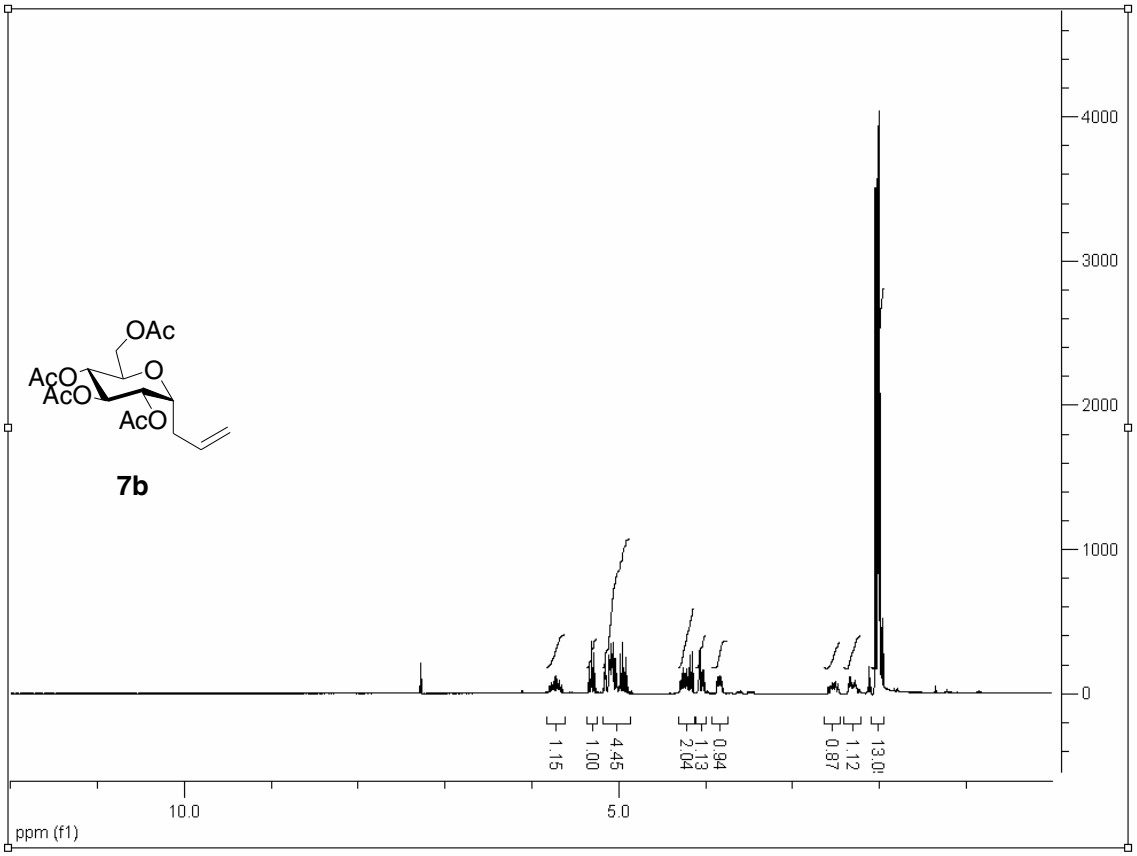

170.314
169.680
169.616
169.325
86.697
72.098
70.428
70.047
67.011
60.848
20.547
20.515
20.456
OAc
AcO
AcO
O
AcO
Br
6b
150
100
50
ppm (f1)

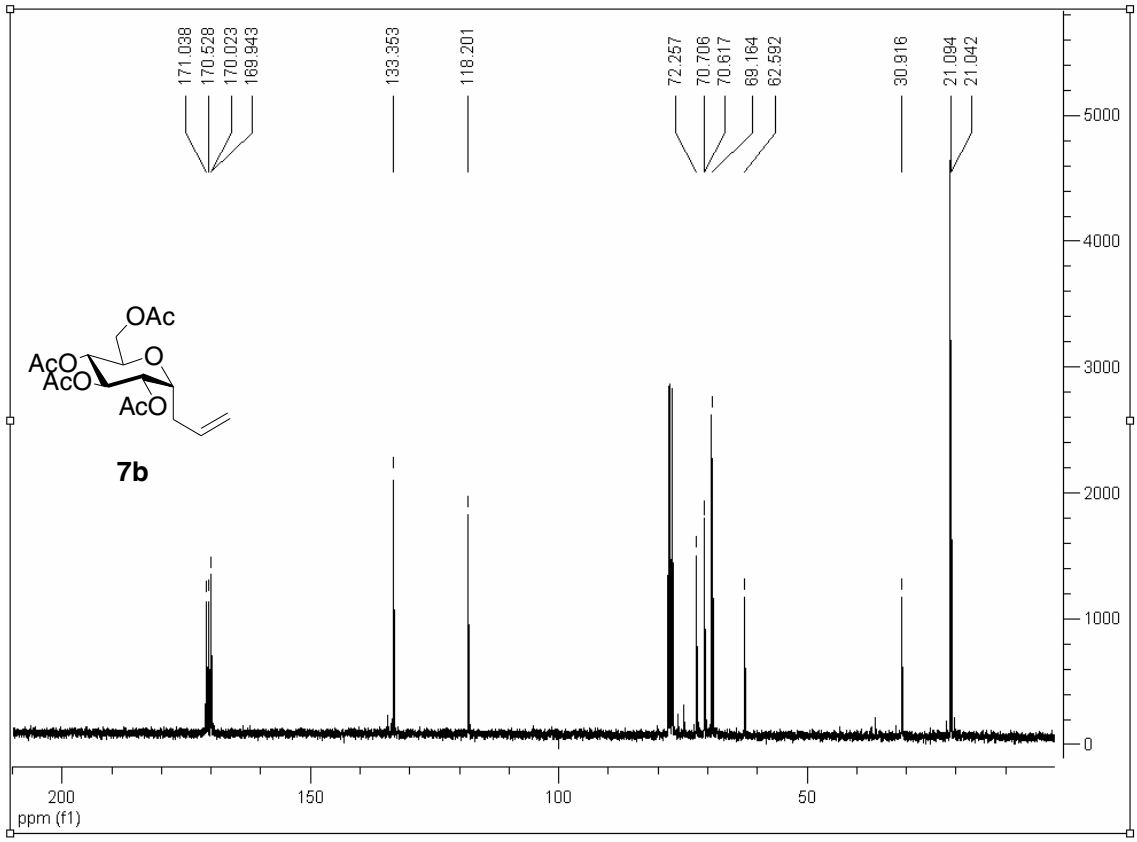

OAc
OAc
AcO
AcO
O
7c
2500
2000
1500
1000
500
0
1.06
5.12
1.11
2.09
1.00
2.20
12.3
10.0
5.0
ppm (f1)

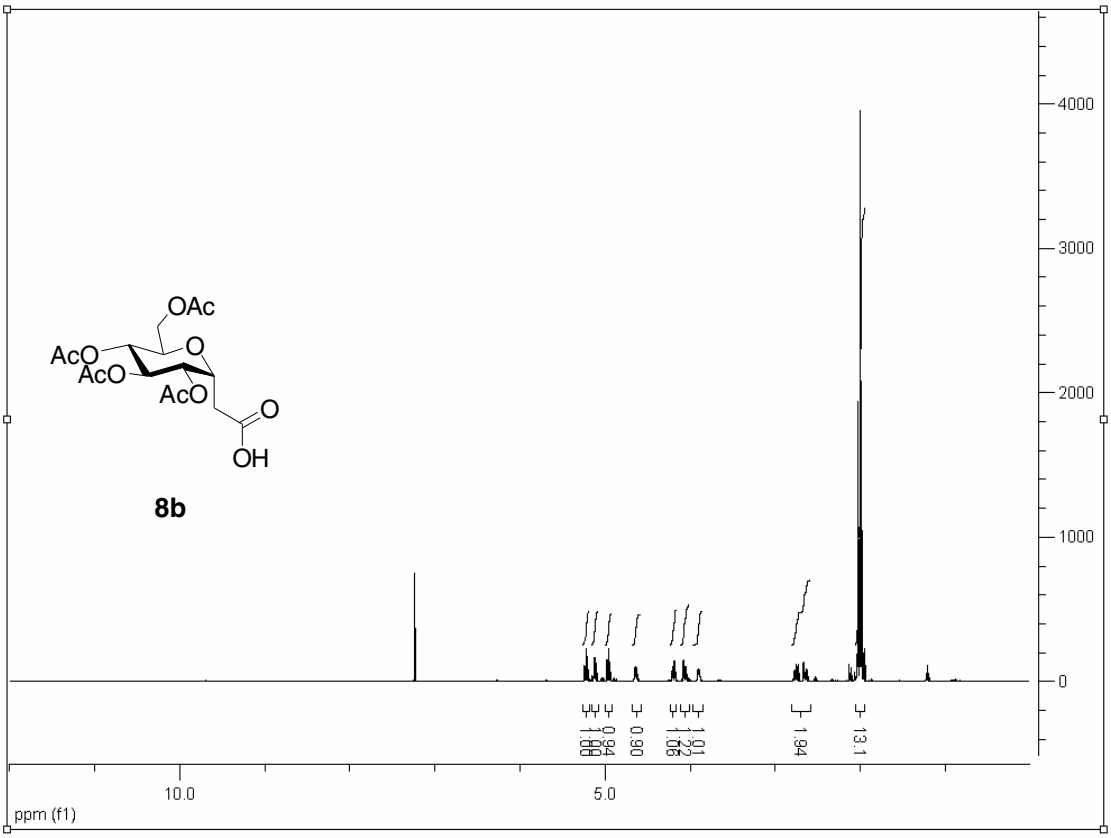

171.077
170.596
170.356
170.072
132.925
118.723
74.528
70.990
70.381
69.157
67.344
62.759
33.936
21.342
21.156
21.093
OAc
OAc
AcO
AcO
O
7c
200
150
100
50
ppm (f1)

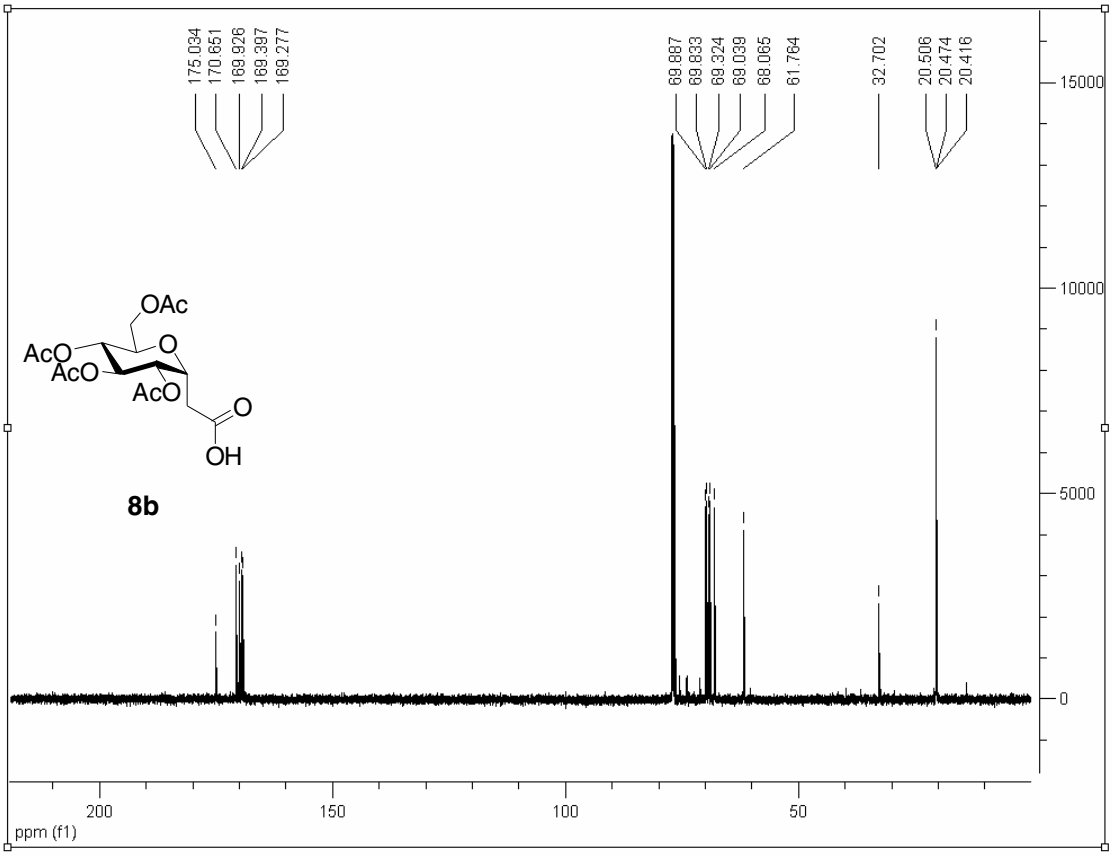

OAc
OAc
AcO
AcO
O
O
OH
8c
2500
2000
1500
1000
500
0
3.09
2.06
2.04
1.00
1.04
2.13
12.12
10.0
5.0
ppm (f1)

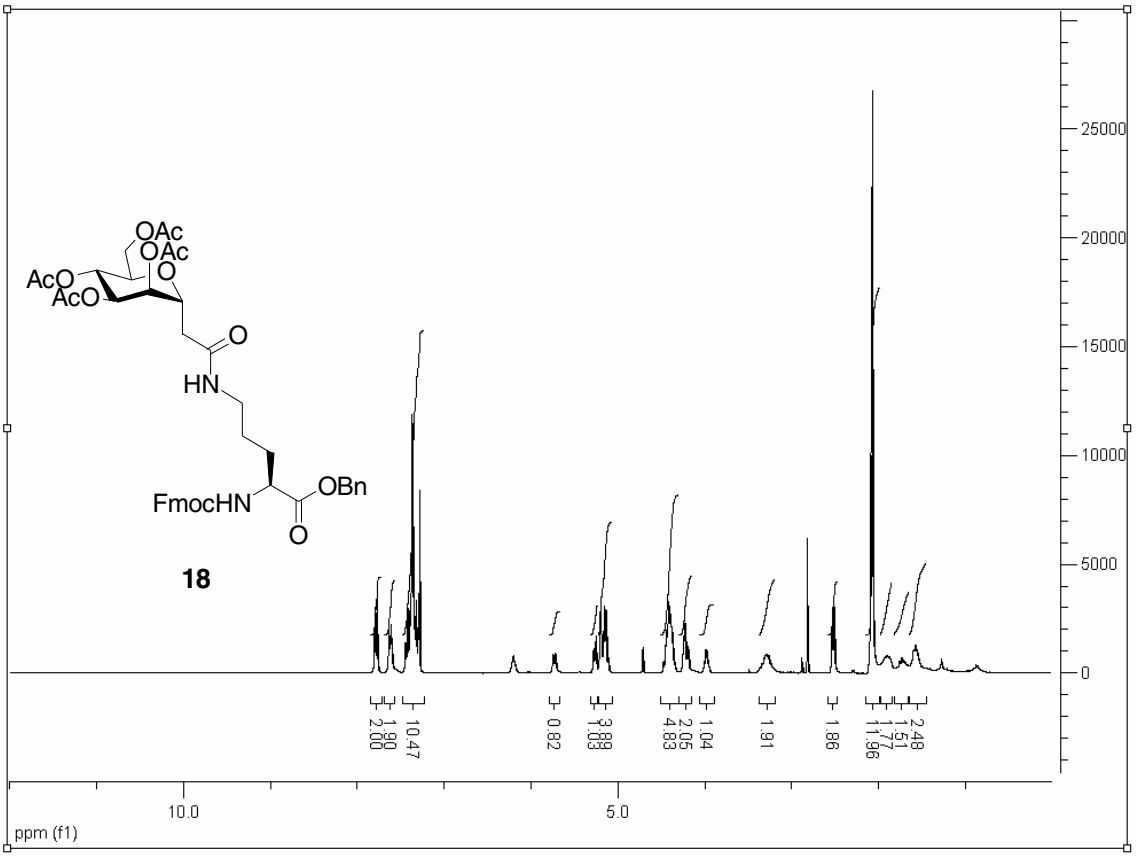

174.685
170.751
169.872
169.686
169.576
77.201
71.887
69.289
69.112
67.925
67.219
61.393
35.337
20.898
OAc
OAc
AcO
AcO
O
O
OH
8c
20000
15000
10000
5000
0
200
150
100
50
ppm (f1)

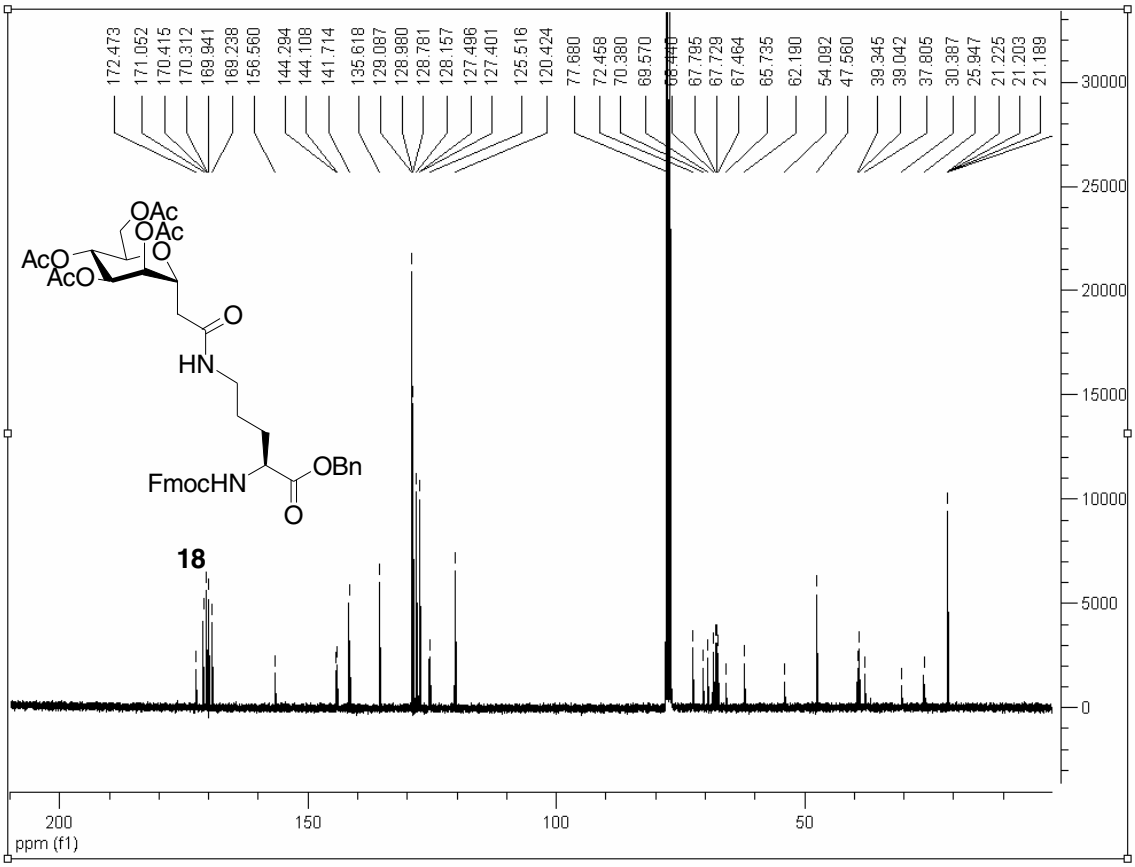

OAc
AcO
AcO
O
AcO
7b
1.15
1.00
4.45
2.04
1.13
0.94
0.87
1.12
13.0
4000
3000
2000
1000
0
10.0
5.0
ppm (f1)

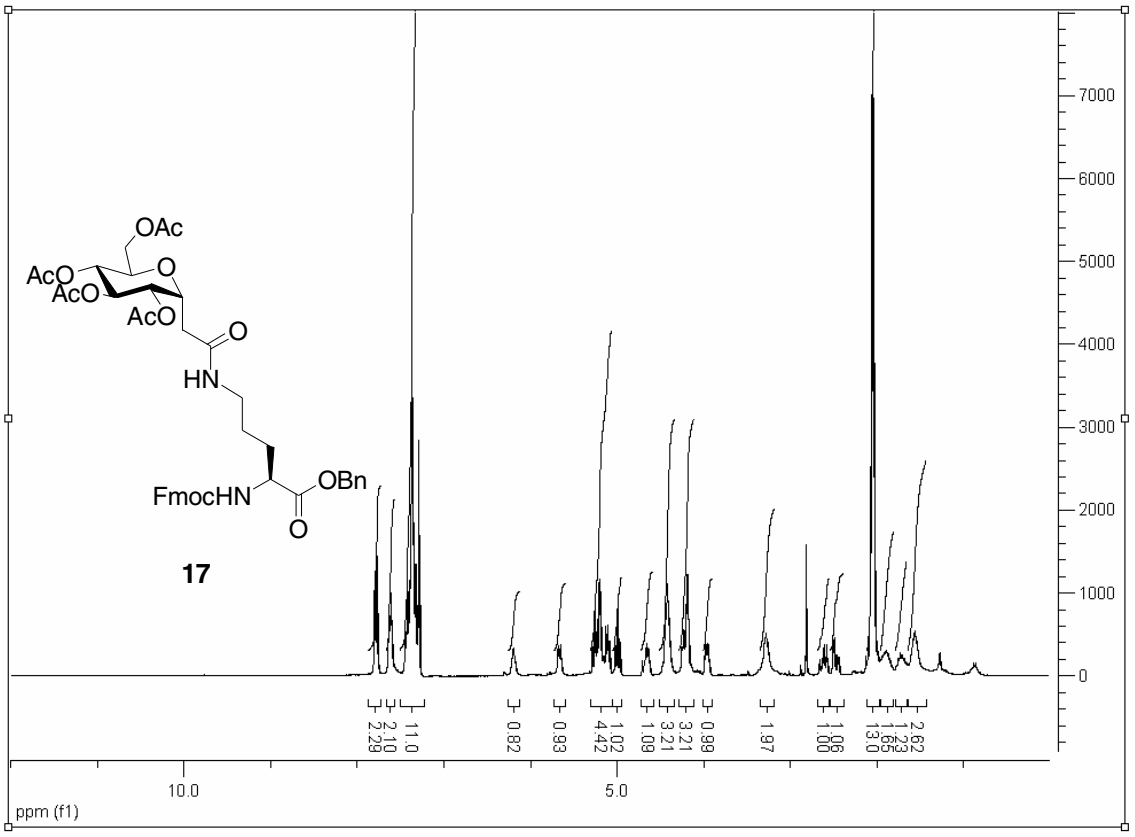

171.038
170.528
170.023
169.943
133.353
118.201
72.257
70.706
70.617
69.164
62.592
30.916
21.094
21.042
OAc
AcO
AcO
O
AcO
7b
5000
4000
3000
2000
1000
0
200
150
100
50
ppm (f1)

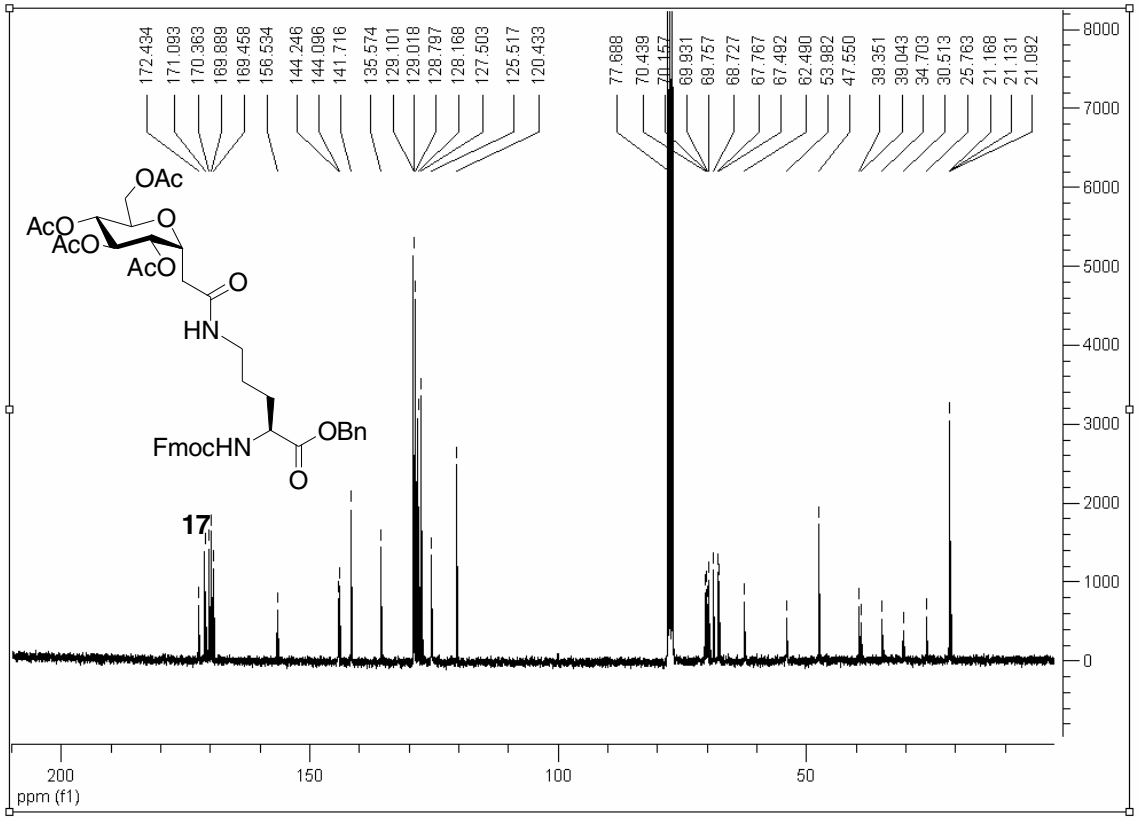

OAc
AcO
AcO
AcO
O
OH
8b
4000
3000
2000
1000
0
3.00
0.84
0.90
1.01
1.22
1.26
1.94
13.1
10.0
5.0
ppm (f1)

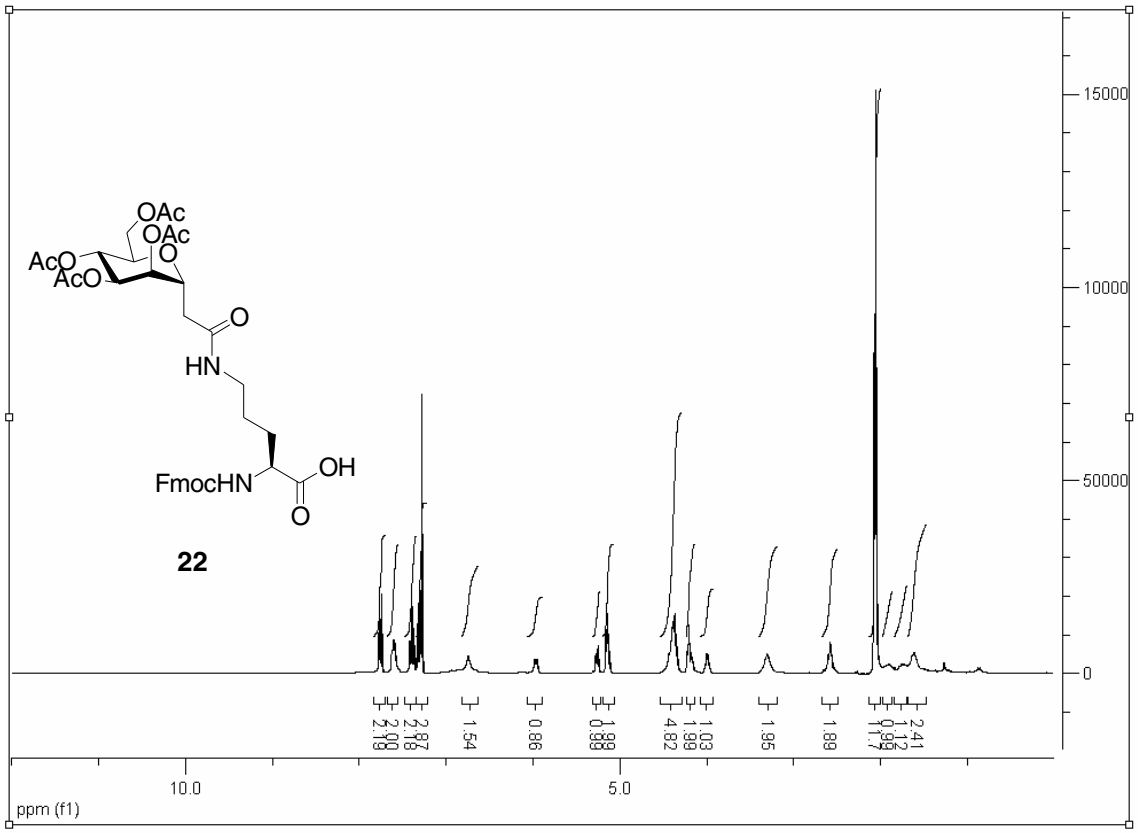

175.034
170.651
169.926
169.397
169.277
69.887
69.833
69.324
69.039
68.065
61.764
32.702
20.506
20.474
20.416
OAc
AcO
AcO
AcO
O
OH
8b
15000
10000
5000
0
200
150
100
50
ppm (f1)

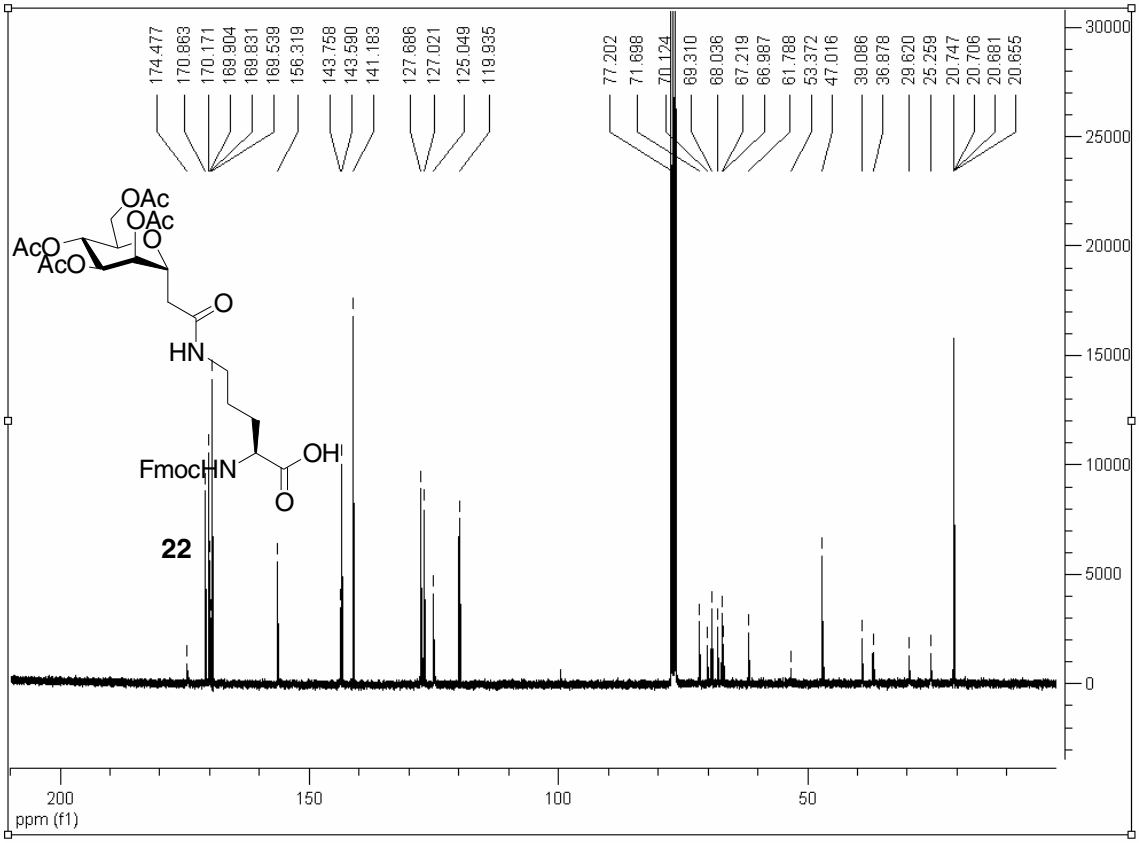

OAc
OAc
AcO
AcO
O
O
HN
FmocHN
OBn
O
18
25000
20000
15000
10000
5000
0
2.00
1.90
10.47
2.82
3.89
4.83
2.05
1.04
1.91
1.86
11.96
1.51
1.77
2.48
10.0
5.0
ppm (f1)

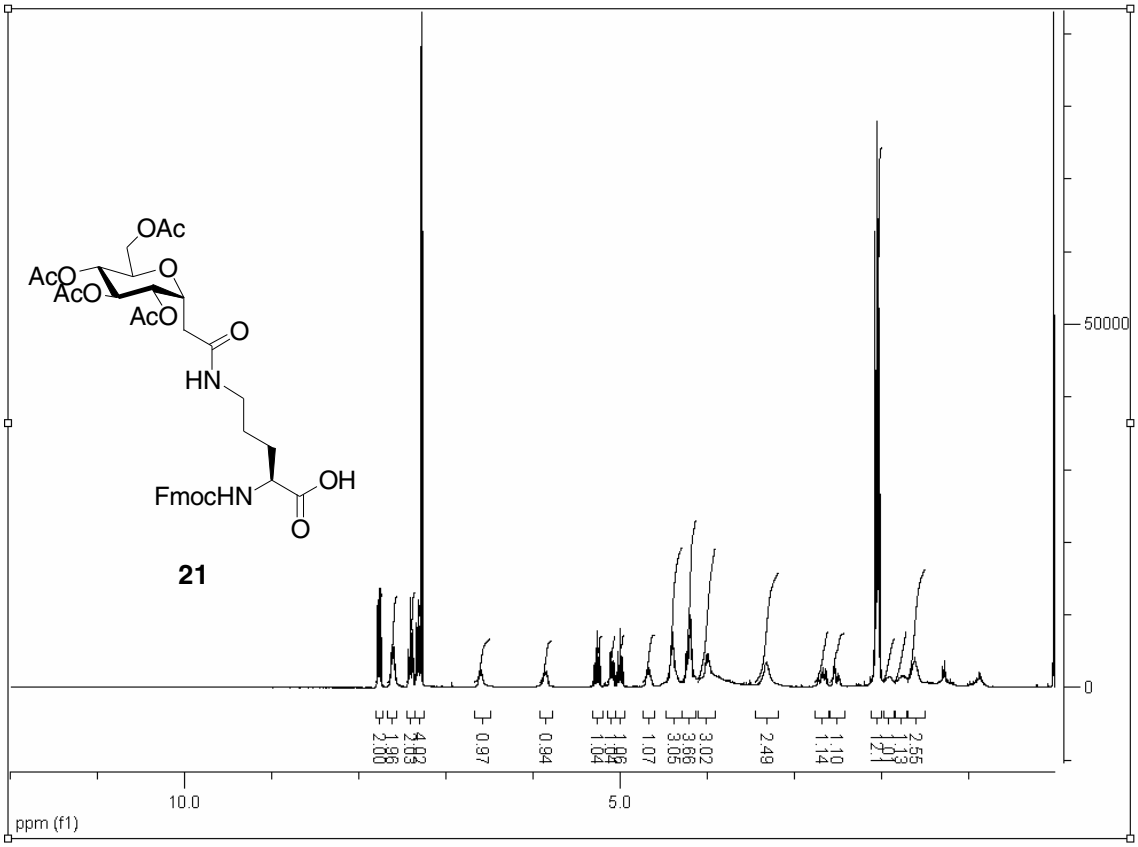

172.473
171.052
170.415
170.312
169.941
169.238
156.560
144.294
144.108
141.714
135.618
129.087
128.980
128.781
128.157
127.496
127.401
125.516
120.424
77.680
72.458
70.380
69.570
68.440
67.795
67.729
67.464
65.735
62.190
54.092
47.560
39.345
39.042
37.805
30.387
25.947
21.225
21.203
21.189
OAc
OAc
AcO
AcO
O
O
HN
FmocHN
OBn
O
18
30000
25000
20000
15000
10000
5000
0
200
150
100
50
ppm (f1)

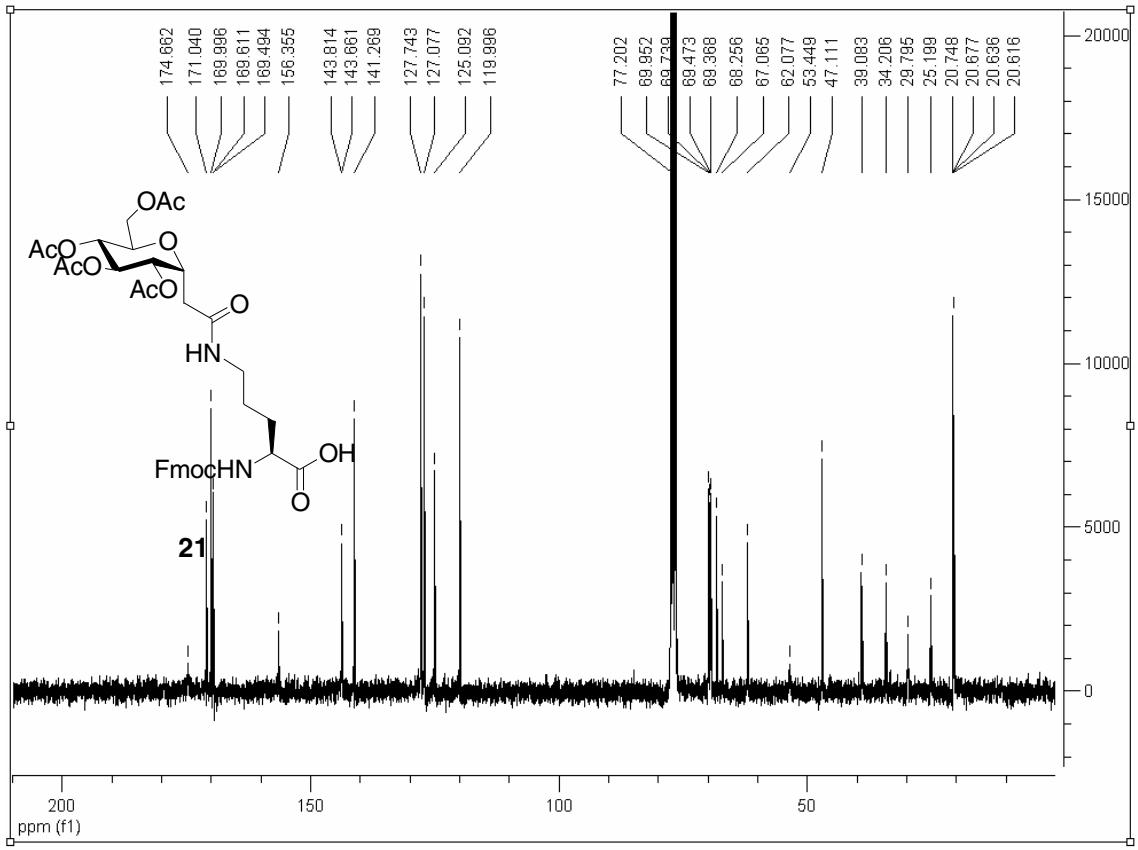

OAc
AcO
AcO
AcO
O
O
HN
FmocHN
OBn
O
17
2.29
2.10
11.0
0.82
0.93
4.42
1.20
1.09
3.21
3.11
0.98
1.97
1.00
1.06
13.0
1.65
1.23
2.92
10.0
5.0
ppm (f1)
7000
6000
5000
4000
3000
2000
1000
0


172.434
171.093
170.363
169.889
169.458
156.534
144.246
144.096
141.716
135.574
129.101
129.018
128.797
128.168
127.503
125.517
120.433
77.688
70.439
70.157
69.931
69.757
68.727
67.767
67.492
62.490
53.982
47.550
39.351
39.043
34.703
30.513
25.763
21.168
21.131
21.092
OAc
AcO
AcO
AcO
O
O
HN
FmocHN
OBn
O
17
200
150
100
50
ppm (f1)
8000
7000
6000
5000
4000
3000
2000
1000
0

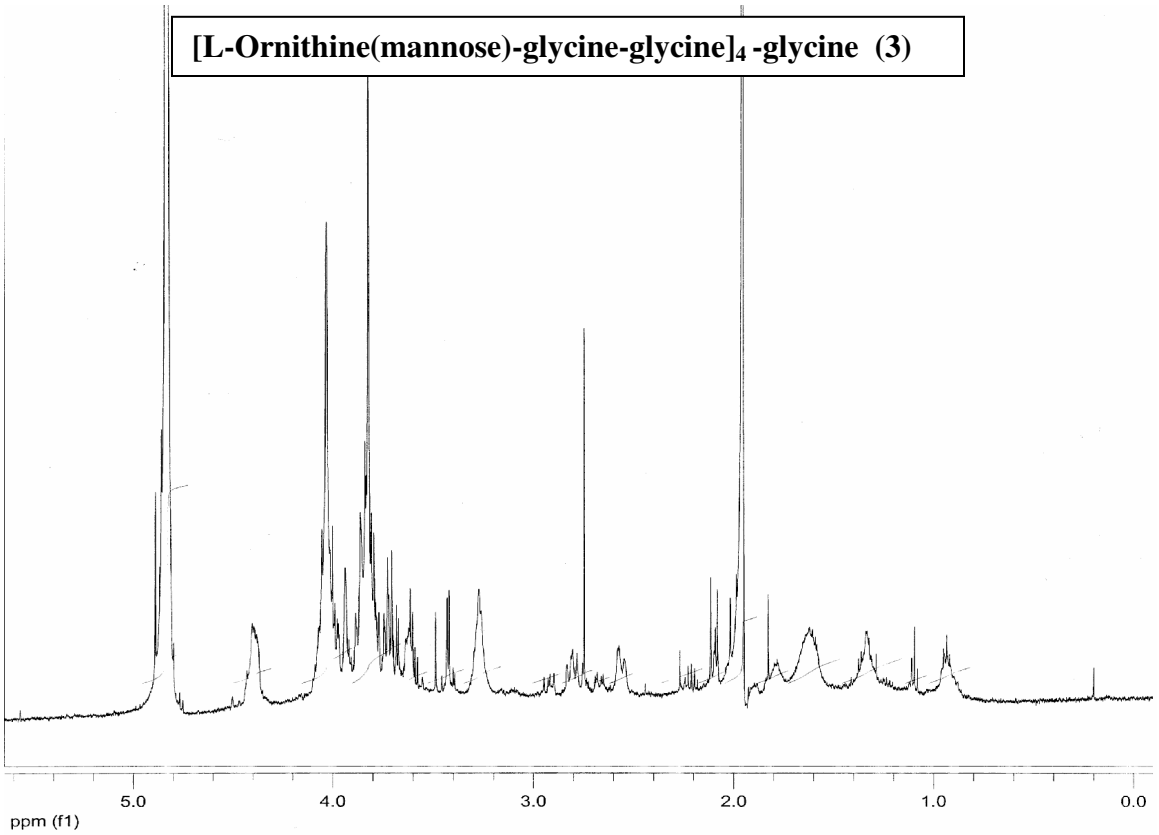

OAc
OAc
AcO
AcO
O
O
HN
FmocHN
OH
O
22
15000
10000
50000
0
2.19
2.00
2.87
2.18
1.54
0.98
0.88
1.86
4.82
1.88
1.03
1.95
1.89
1.17
0.96
1.12
2.41
10.0
5.0
ppm (f1)


174.477
170.863
170.171
169.904
169.831
169.539
156.319
143.758
143.590
141.183
127.686
127.021
125.049
119.935
77.202
71.698
70.124
69.310
68.036
67.219
66.987
61.788
53.372
47.016
39.086
36.878
29.620
25.259
20.747
20.706
20.681
20.655
OAc
OAc
AcO
AcO
O
O
HN
FmocHN
OH
O
22
30000
25000
20000
15000
10000
5000
0
200
150
100
50
ppm (f1)

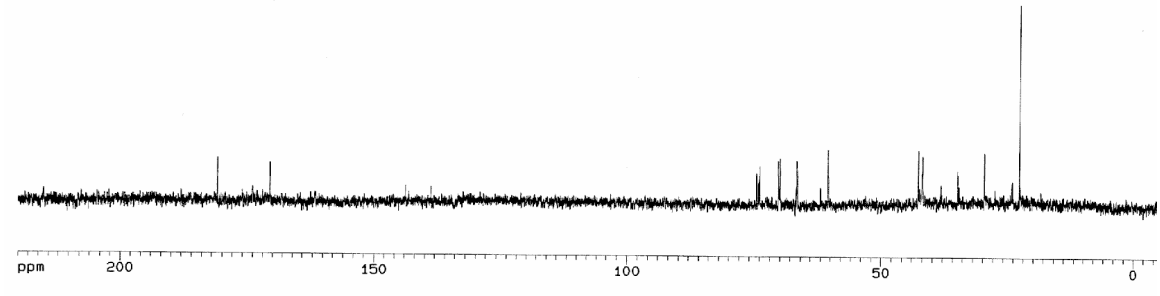

OAc
AcO
AcO
AcO
O
HN
FmocHN
OH
O
21
50000
0
10.0
5.0
ppm (f1)


174.662
171.040
169.996
169.611
169.494
156.355
143.814
143.661
141.269
127.743
127.077
125.092
119.996
77.202
69.952
69.739
69.473
69.368
68.256
67.065
62.077
53.449
47.111
38.083
34.206
29.795
25.199
20.748
20.677
20.636
20.616
OAc
AcO
AcO
AcO
O
HN
FmocHN
OH
O
21
20000
15000
10000
5000
0
200
150
100
50
ppm (f1)

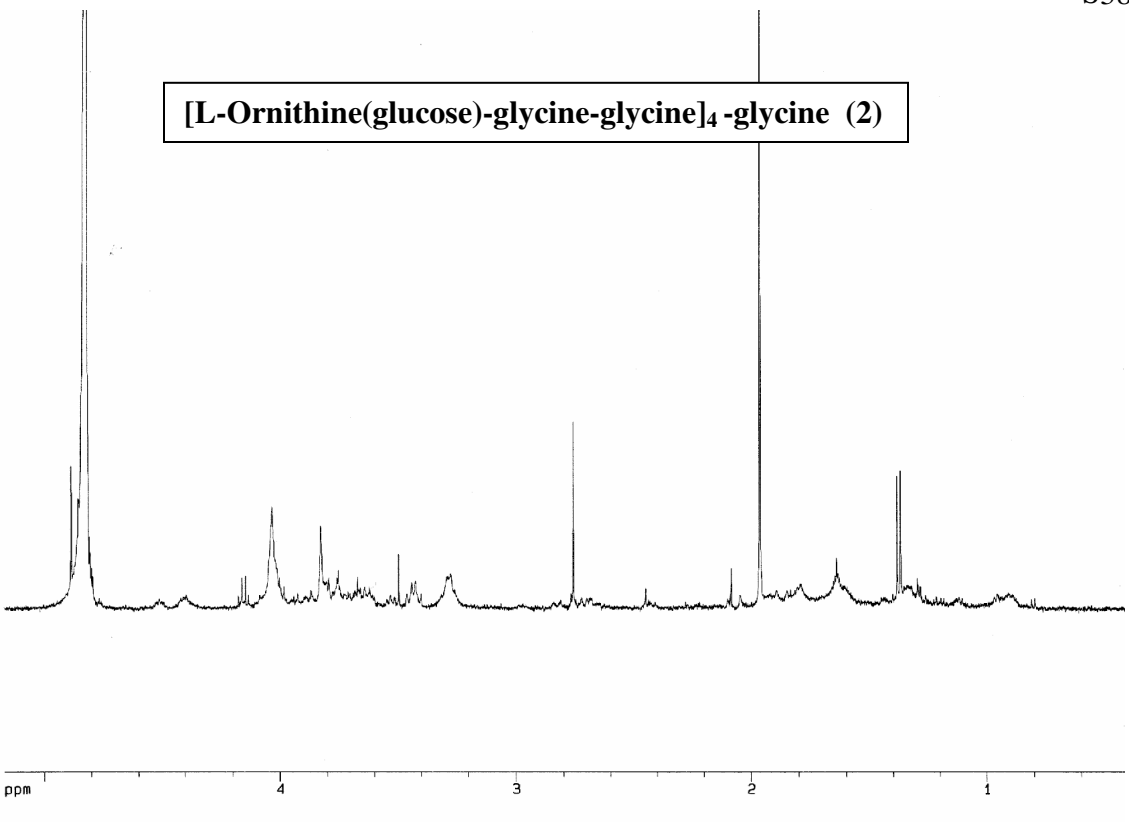
[L-Ornithine(mannose)-glycine-glycine]$_4$ -glycine (3)



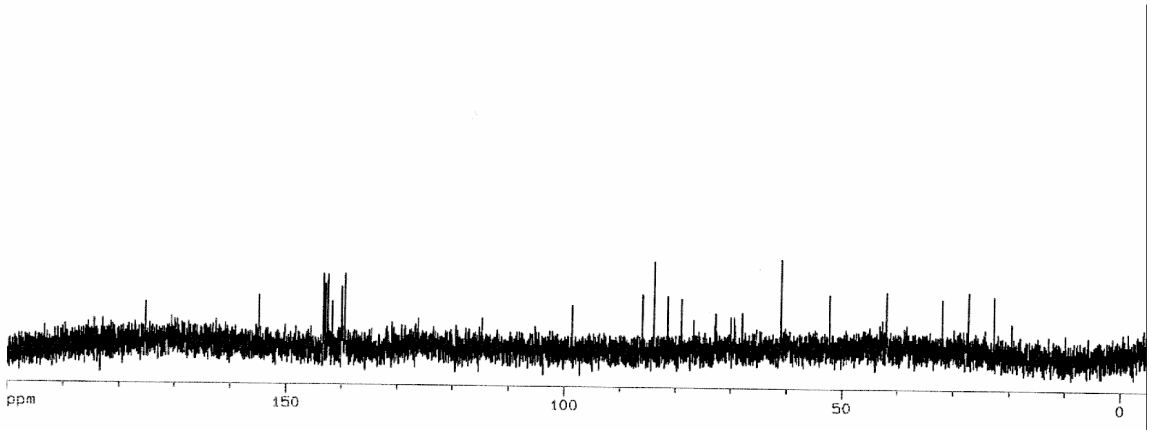
[L-Ornithine(mannose)-glycine-glycine]$_4$ -glycine (3)

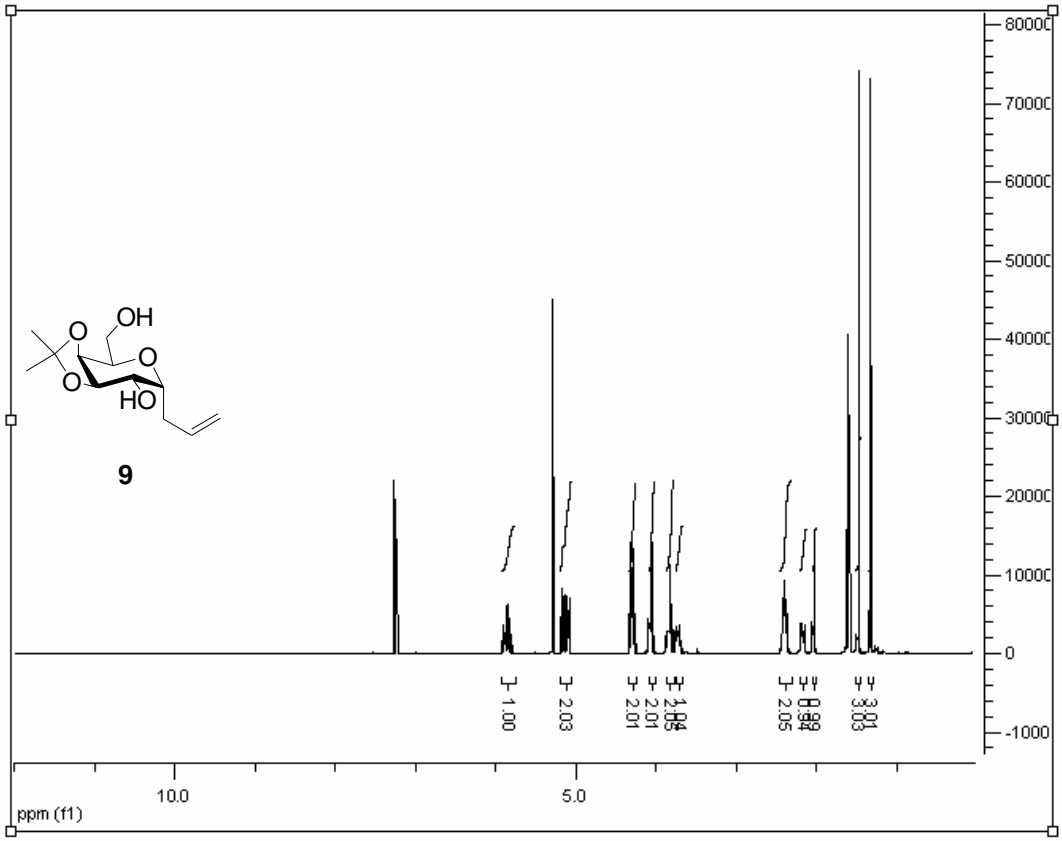


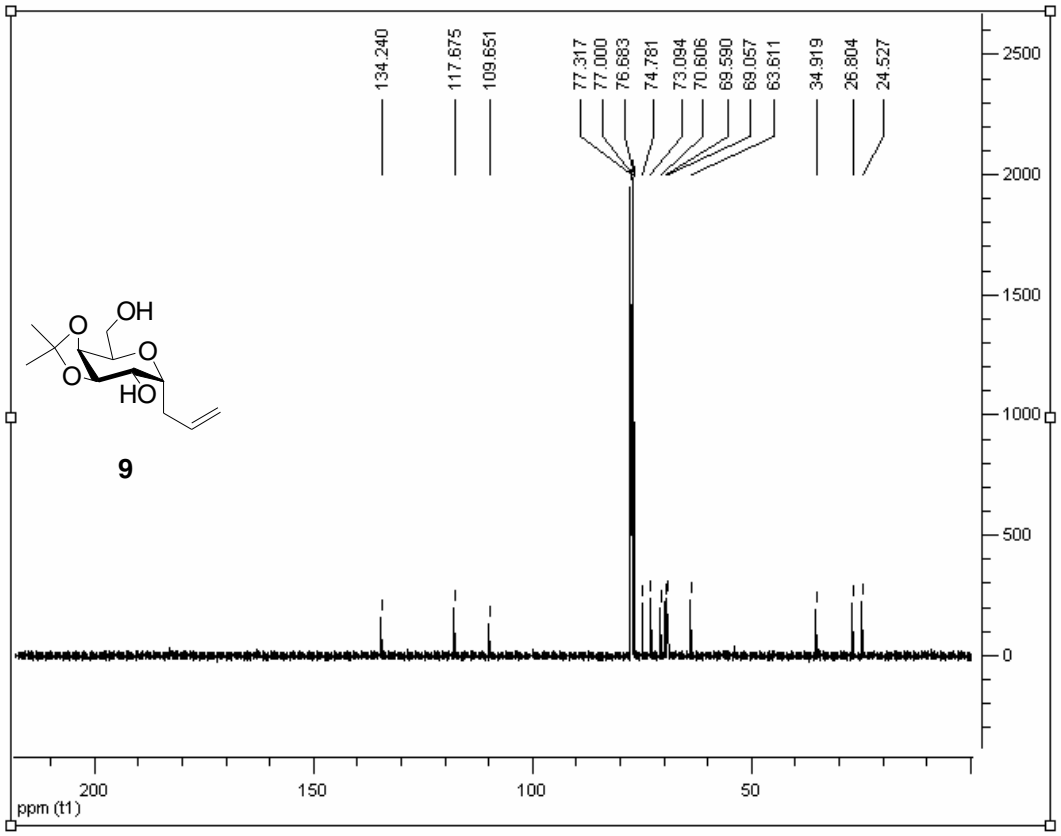
[L-Ornithine(glucose)-glycine-glycine]$_4$ -glycine (2)

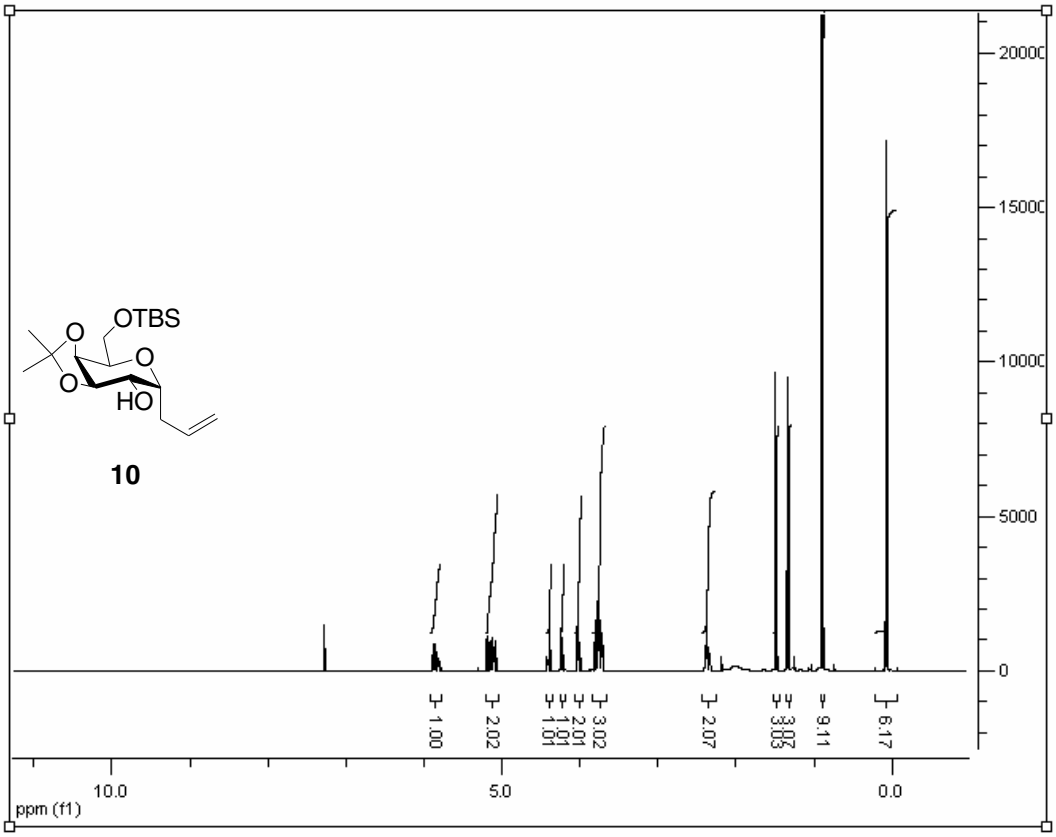


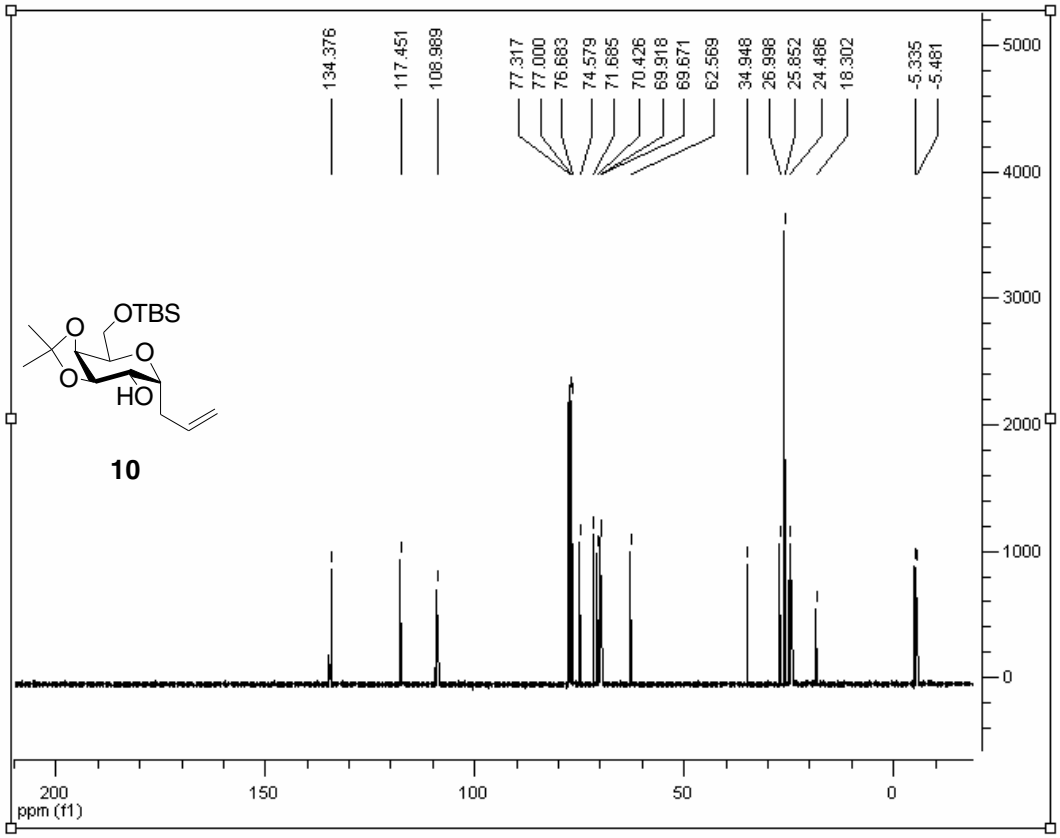
[L-Ornithine(glucose)-glycine-glycine]$_4$ -glycine (2)

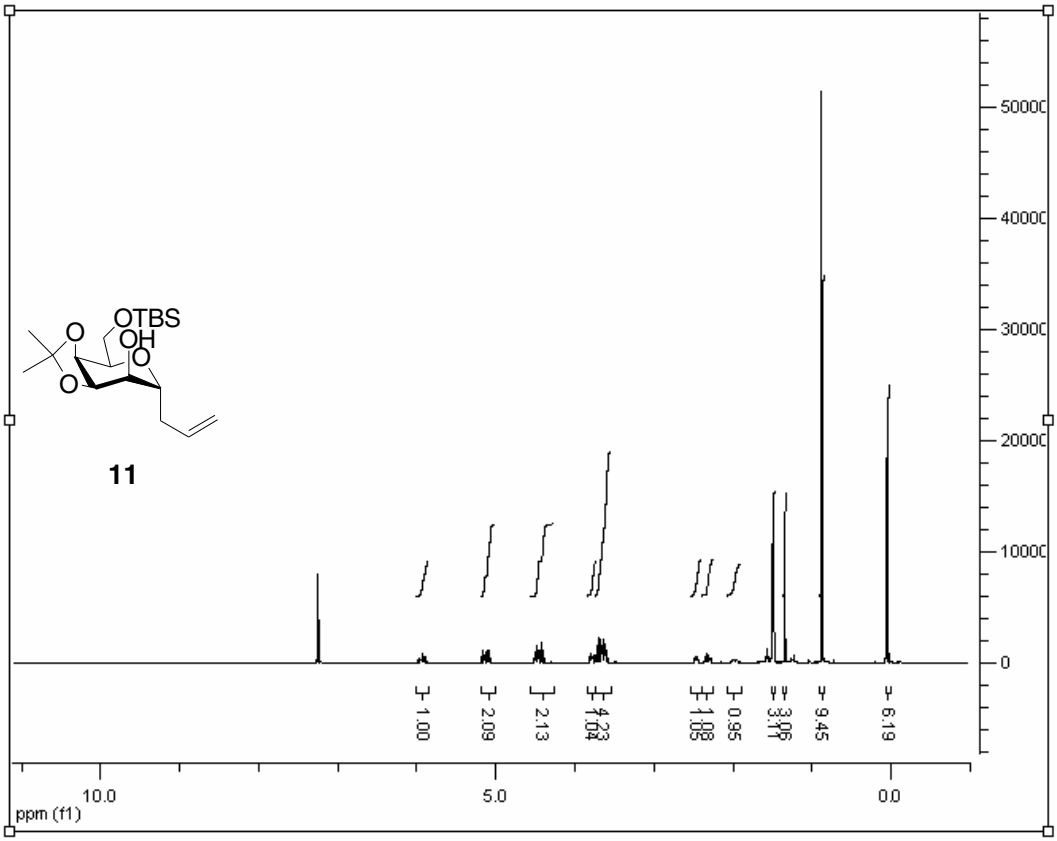


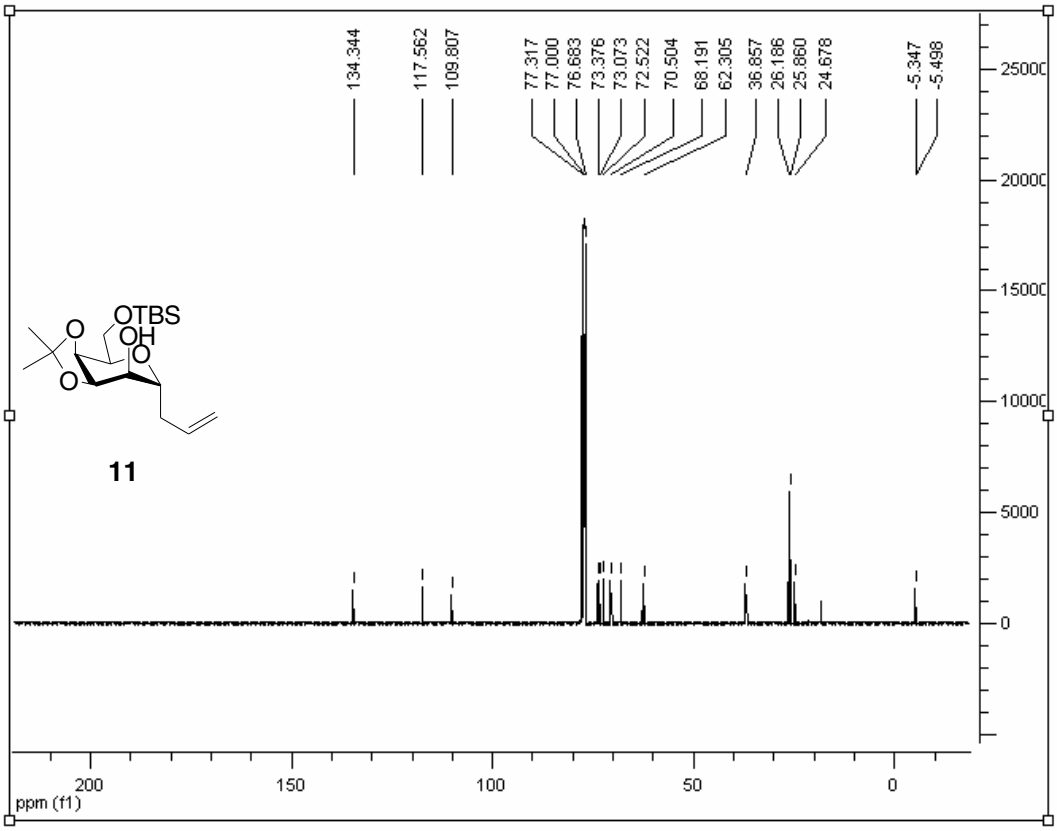

OH
O
O
O
HO
9
80000
70000
60000
50000
40000
30000
20000
10000
0
-1000
1.00
2.03
2.01
2.01
1.98
0.94
0.88
2.05
3.03
10.0
5.0
ppm (f1)

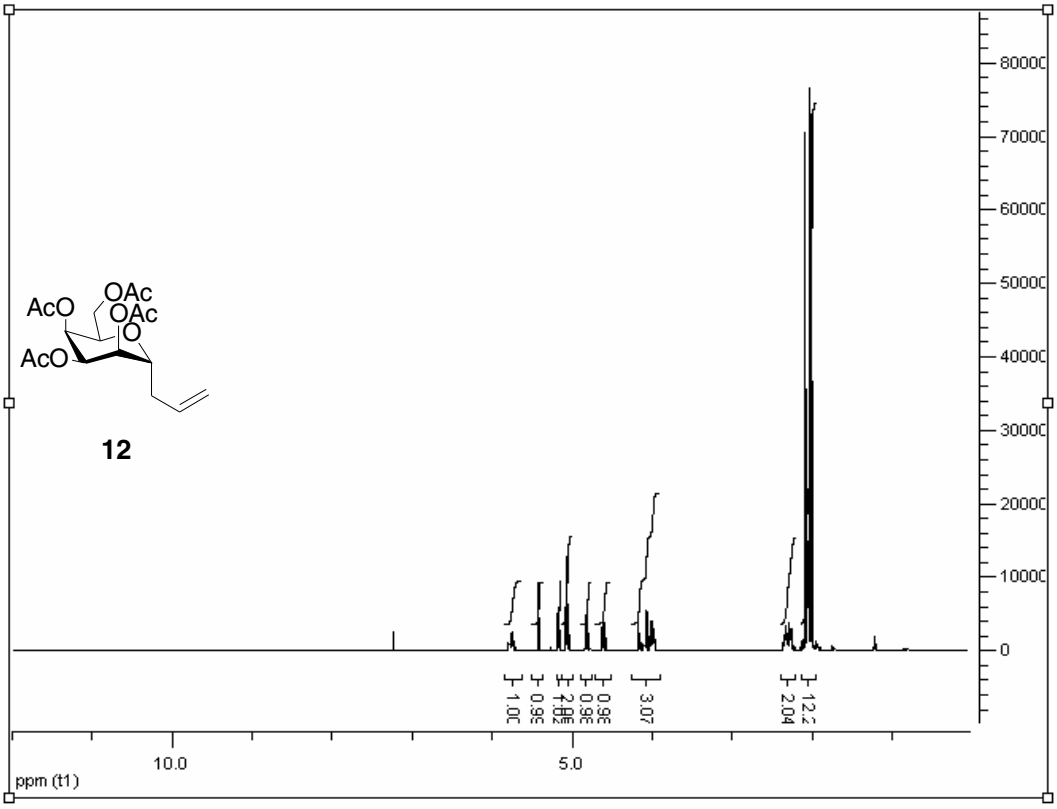

134.240
117.675
109.651
77.317
77.000
76.683
74.781
73.094
70.606
69.590
69.057
63.611
34.919
26.804
24.527
OH
O
O
O
HO
9
2500
2000
1500
1000
500
0
200
150
100
50
ppm (t1)

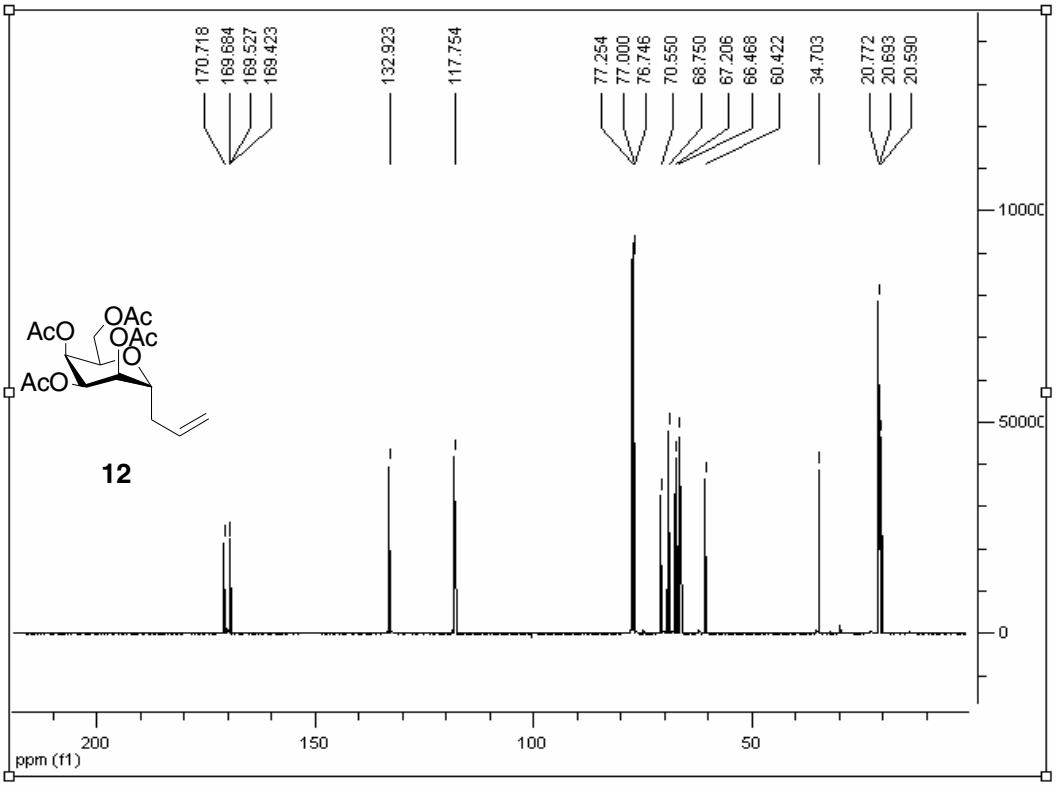

OTBS
O
O
O
HO
10
20000
15000
10000
5000
0
1.00
2.02
1.01
1.01
2.01
3.02
2.07
3.05
3.02
9.11
6.17
10.0
5.0
0.0
ppm (f1)

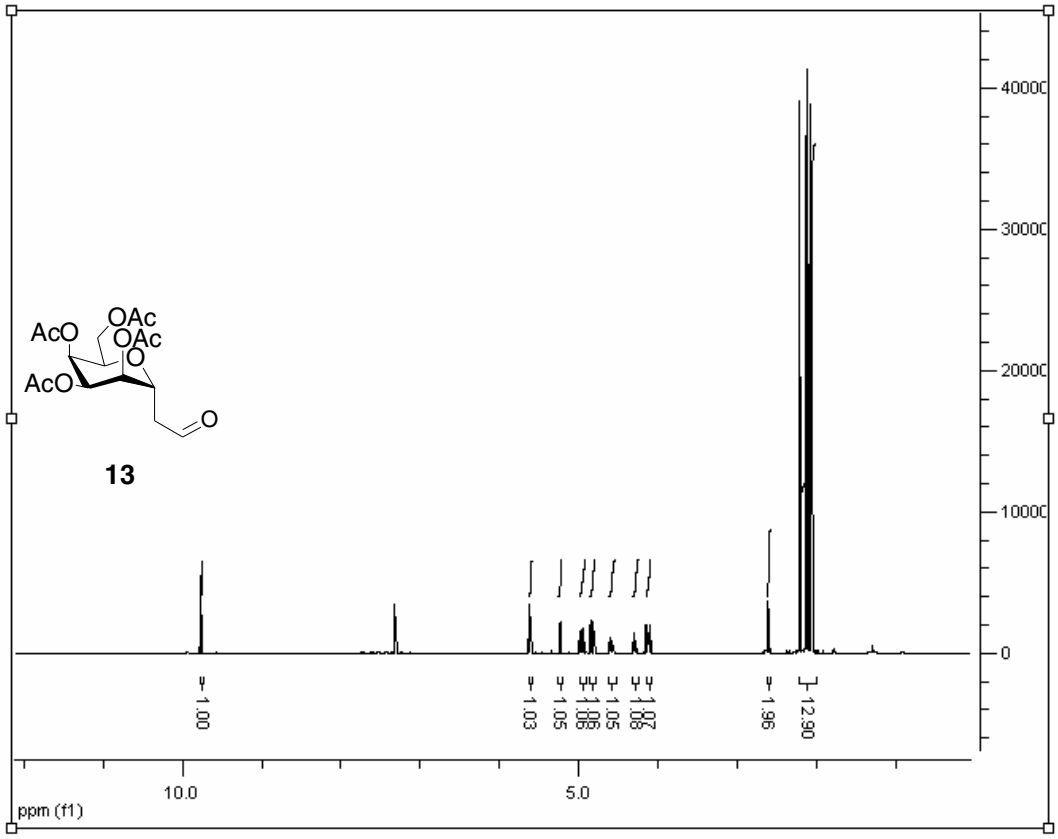

134.376
117.451
108.989
77.317
77.000
76.683
74.579
71.685
70.426
69.918
69.671
62.569
34.948
26.998
25.852
24.486
18.302
-5.335
-5.481
OTBS
O
O
O
HO
10
5000
4000
3000
2000
1000
0
200
150
100
50
0
ppm (f1)

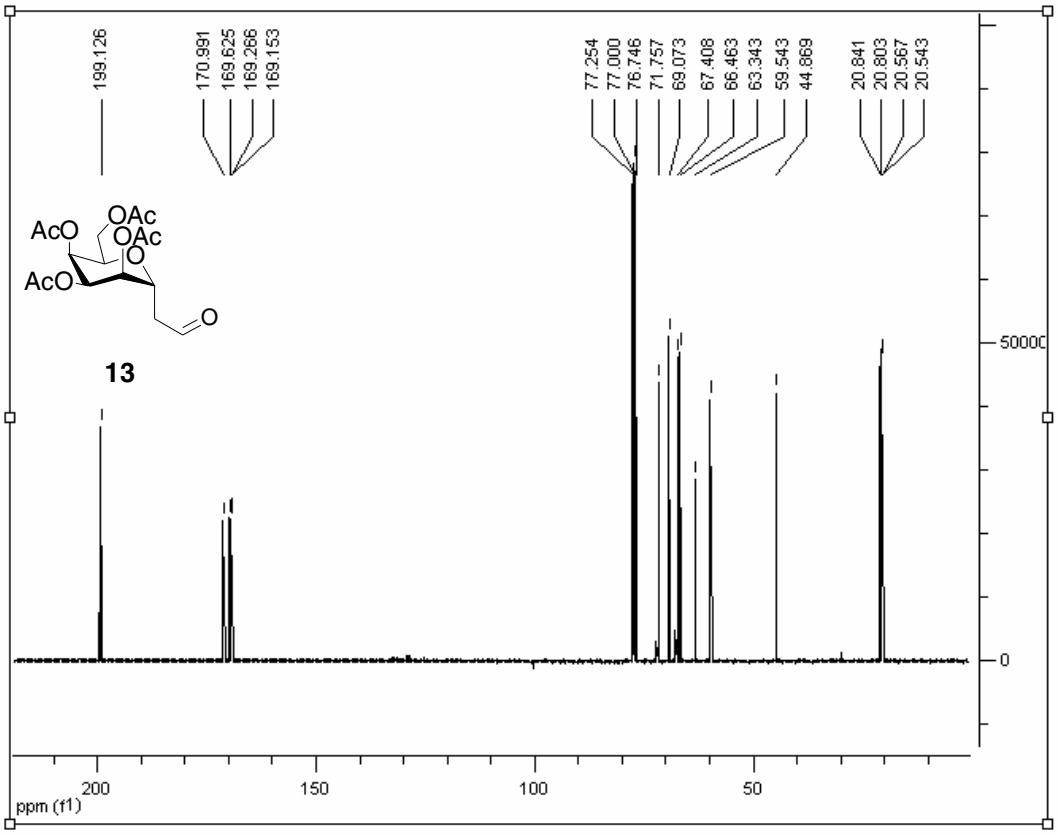

OTBS
OH
O
O
O
11
1.00
2.09
2.13
4.03
1.08
0.95
3.86
9.45
6.19
10.0
5.0
0.0
ppm (f1)

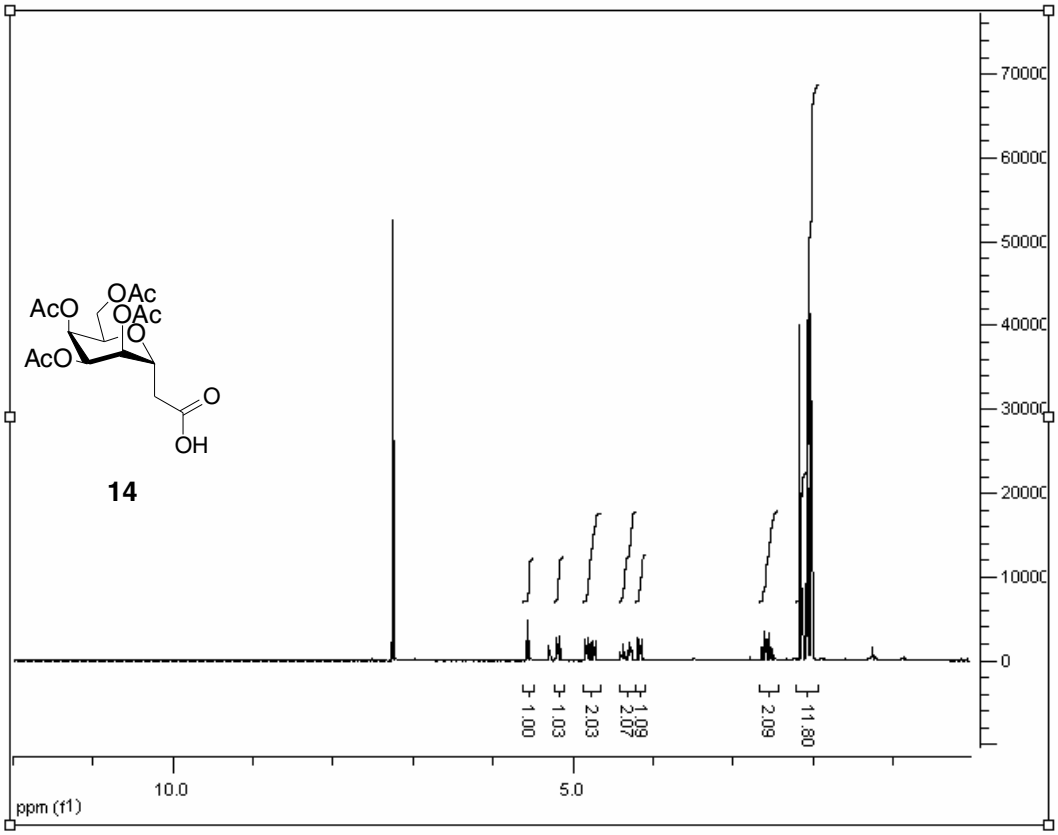

OTBS
OH
O
O
O
11
134.344
117.562
109.807
77.317
77.000
76.683
73.376
73.073
72.522
70.504
68.191
62.305
36.857
26.186
25.860
24.678
-5.347
-5.498
200
150
100
50
0
ppm (f1)

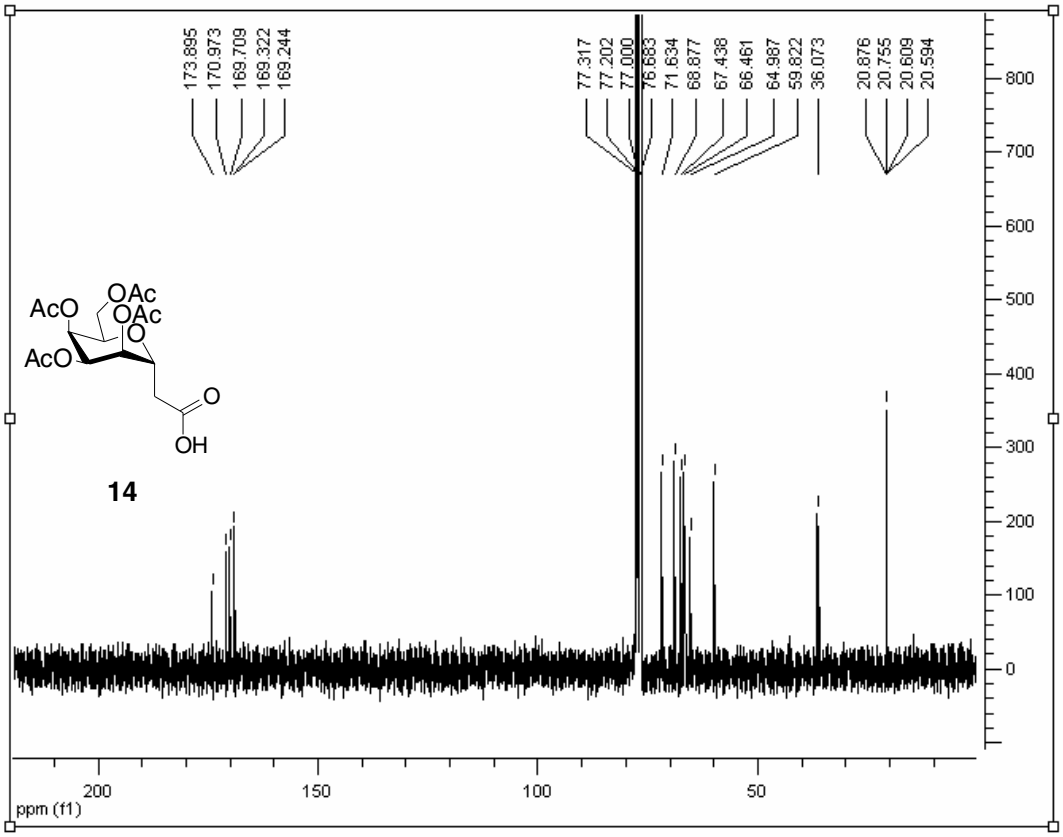

AcO
OAc
OAc
O
AcO
12
1.00
0.95
2.82
0.96
0.96
3.07
2.04
12.2
80000
70000
60000
50000
40000
30000
20000
10000
0
10.0
5.0
ppm (t1)

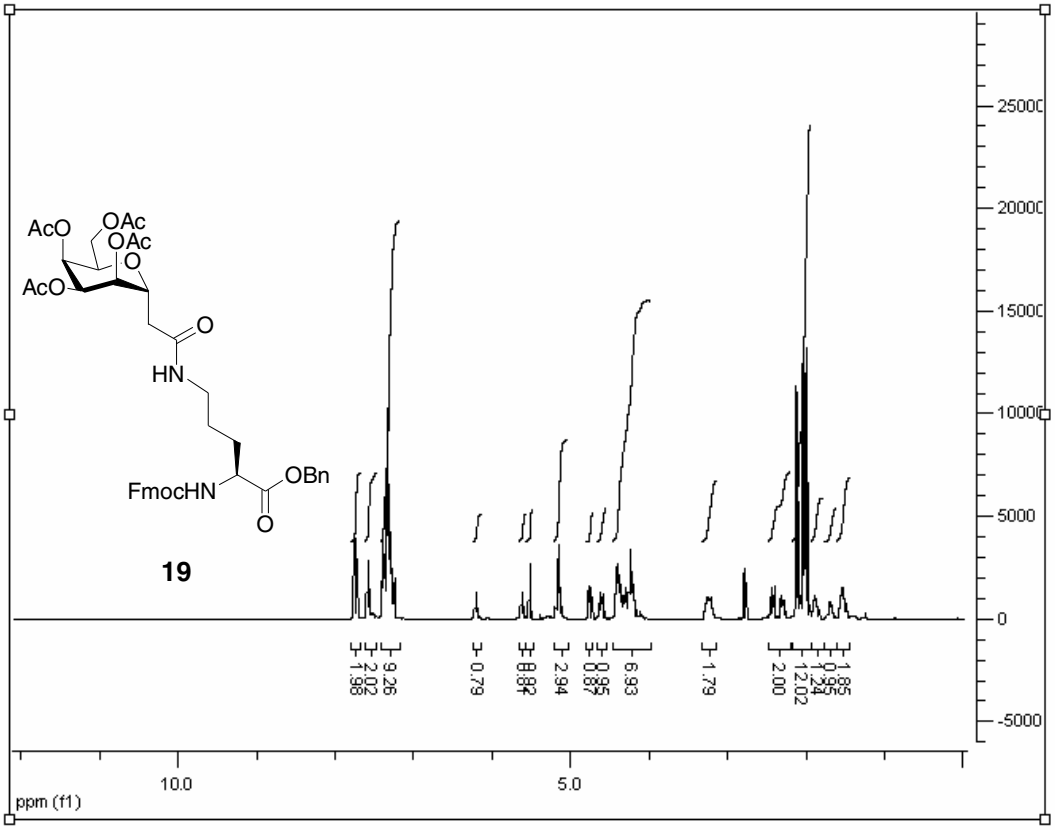

170.718
169.684
169.527
169.423
132.923
117.754
77.254
77.000
76.746
70.550
68.750
67.206
66.468
60.422
34.703
20.772
20.693
20.590
AcO
OAc
OAc
O
AcO
12
10000
50000
0
200
150
100
50
ppm (f1)

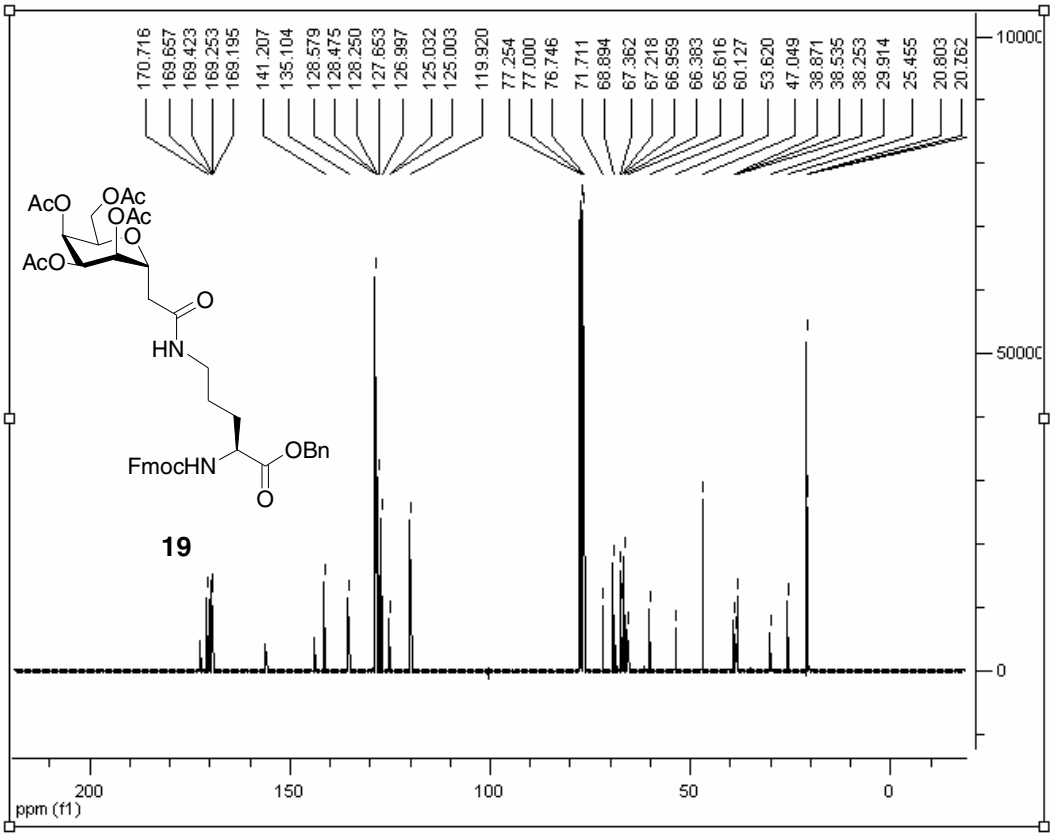

AcO
OAc
OAc
AcO
O
O
13
1.00
1.03
1.05
1.05
1.08
1.08
1.96
12.90
40000
30000
20000
10000
0
10.0
5.0
ppm (f1)

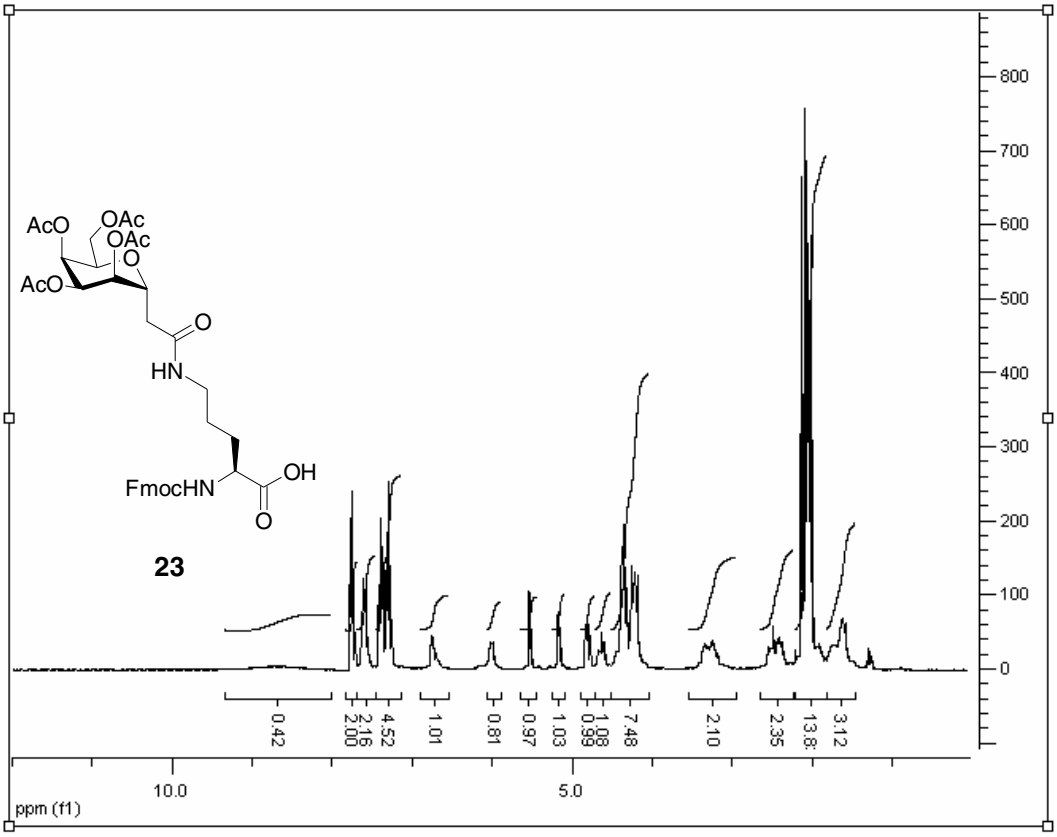

199.126
170.991
169.625
169.266
169.153
77.254
77.000
76.746
71.757
69.073
67.408
66.463
63.343
59.543
44.869
20.841
20.803
20.567
20.543
AcO
OAc
OAc
AcO
O
O
13
50000
0
200
150
100
50
ppm (f1)

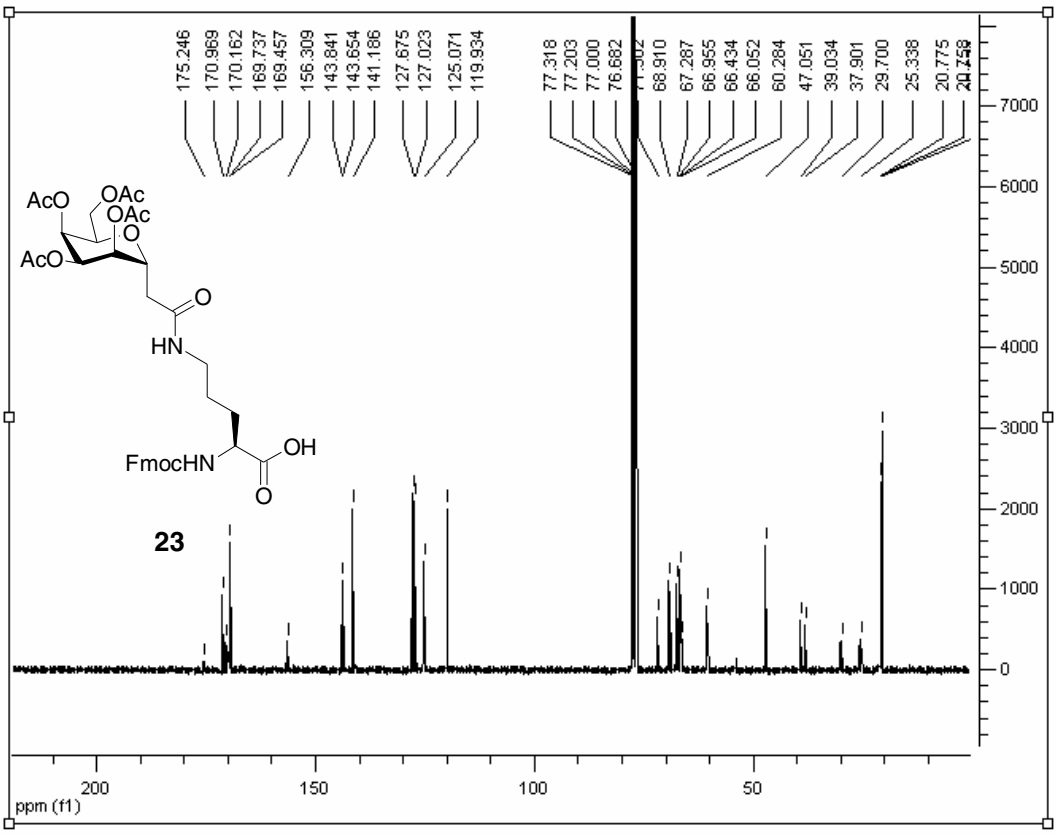

AcO
OAc
OAc
AcO
O
O
OH
14
1.00
1.03
2.03
2.07
1.09
2.09
11.00
10.0
5.0
ppm (f1)

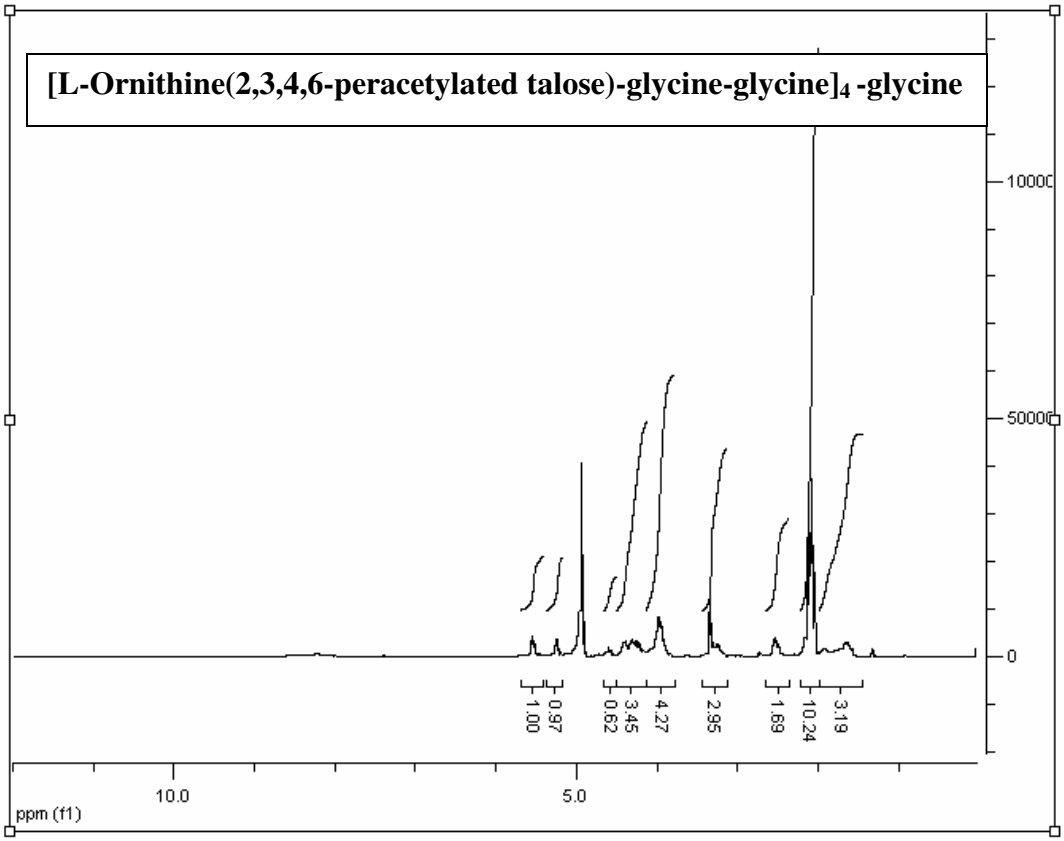

173.895
170.973
169.709
169.322
169.244
77.317
77.202
77.000
76.683
71.634
68.877
67.438
66.461
64.987
59.822
36.073
20.876
20.755
20.609
20.594
AcO
OAc
OAc
AcO
O
O
OH
14
200
150
100
50
ppm (f1)

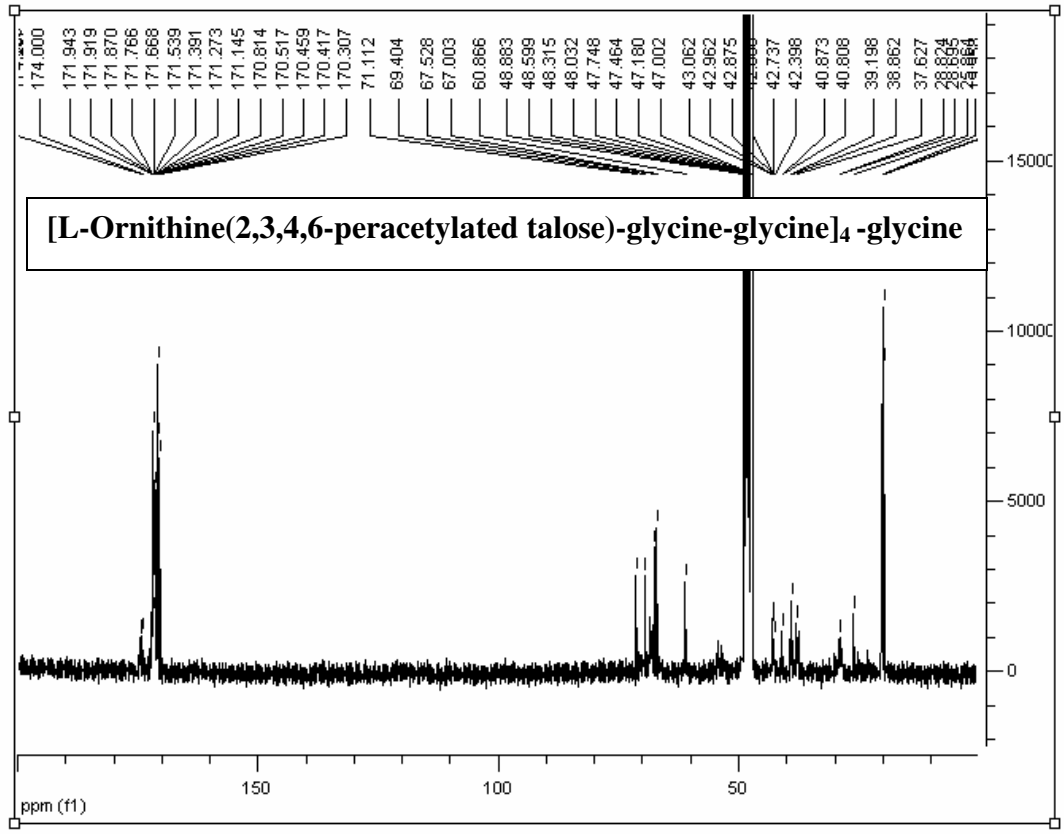

AcO
OAc
OAc
O
AcO
O
HN
FmocHN
OBn
O
19
25000
20000
15000
10000
5000
0
-5000
1.68
2.02
6.26
0.79
8.82
2.64
0.85
0.87
6.93
1.79
2.00
12.02
1.85
0.86
0.94
10.0
5.0
ppm (f1)

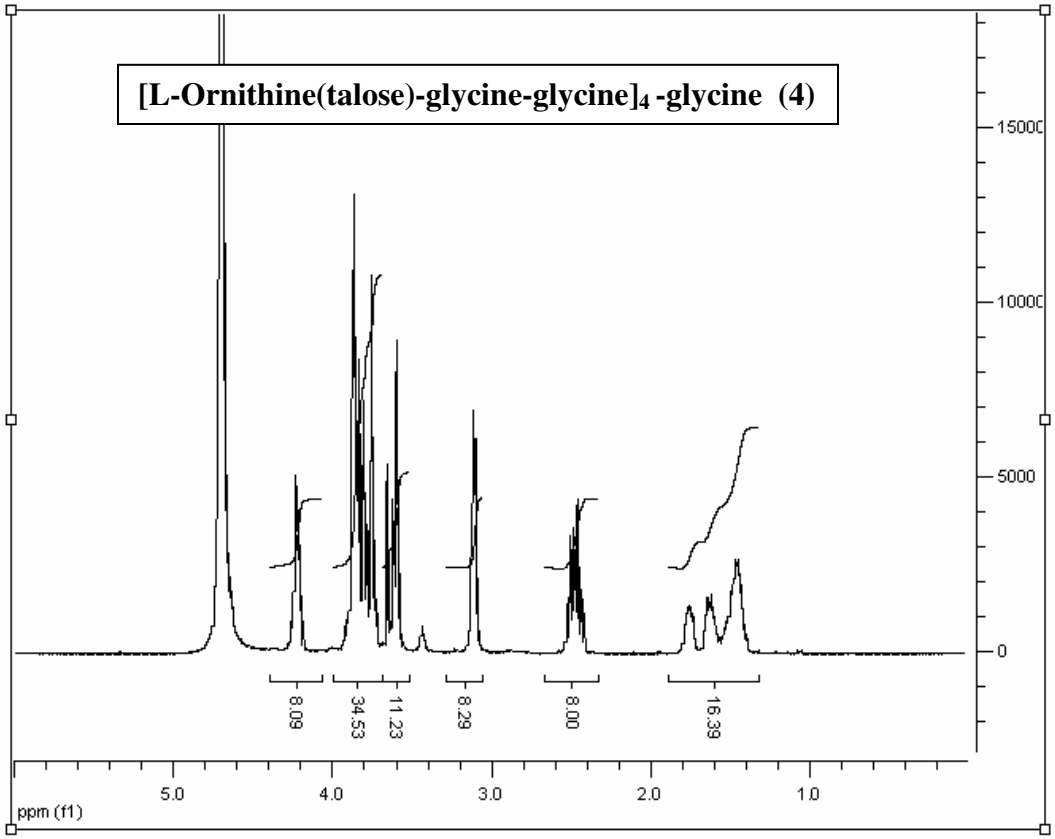

170.716
169.657
169.423
169.253
169.195
141.207
135.104
128.579
128.475
128.250
127.653
126.997
125.032
125.003
119.920
77.254
77.000
76.746
71.711
68.894
67.362
67.218
66.959
66.383
65.616
60.127
53.620
47.049
38.871
38.535
38.253
29.914
25.455
20.803
20.752
AcO
OAc
OAc
O
AcO
O
HN
FmocHN
OBn
O
19
100000
50000
0
200
150
100
50
0
ppm (f1)

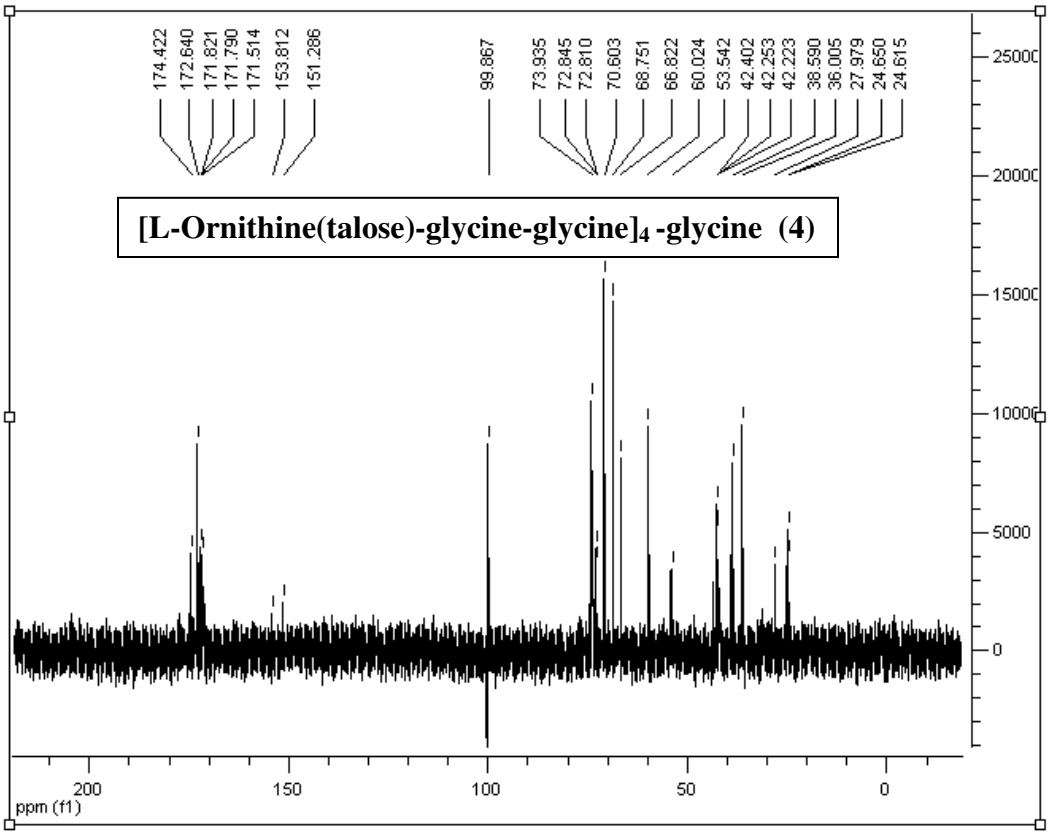

AcO
OAc
OAc
O
AcO
O
HN
FmocHN
OH
O
23
0.42
2.00
2.16
4.52
1.01
0.81
0.97
1.03
0.69
1.08
7.48
2.10
2.35
13.8
3.12
10.0
5.0
ppm (f1)


175.246
170.969
170.162
169.737
169.457
156.309
143.841
143.654
141.186
127.675
127.023
125.071
119.934
77.318
77.203
77.000
76.682
68.910
67.287
66.955
66.434
66.052
60.284
47.051
39.034
37.901
29.700
25.338
20.775
AcO
OAc
OAc
O
AcO
O
HN
FmocHN
OH
O
23
200
150
100
50
ppm (f1)


[L-Ornithine(2,3,4,6-peracetylated talose)-glycine-glycine]4 -glycine
10000
50000
0
1.00
0.97
0.62
3.45
4.27
2.95
1.89
10.24
3.19
10.0
5.0
ppm (f1)


174.000
171.943
171.919
171.870
171.766
171.668
171.539
171.391
171.273
171.145
170.814
170.517
170.459
170.417
170.307
71.112
69.404
67.528
67.003
60.866
48.883
48.599
48.315
48.032
47.748
47.464
47.180
47.002
43.062
42.962
42.875
42.737
42.398
40.873
40.808
39.198
38.862
37.627
[L-Ornithine(2,3,4,6-peracetylated talose)-glycine-glycine]4 -glycine
15000
10000
5000
0
150
100
50
ppm (f1)


[L-Ornithine(talose)-glycine-glycine]4 -glycine (4)
8.09
34.53
11.23
8.29
8.00
19.39
5.0
4.0
3.0
2.0
1.0
ppm (f1)
15000
10000
5000
0


174.422
172.640
171.821
171.790
171.514
153.812
151.286
99.867
73.935
72.845
72.810
70.603
68.751
66.822
60.024
53.542
42.402
42.253
42.223
38.590
36.005
27.979
24.650
24.615
[L-Ornithine(talose)-glycine-glycine]4 -glycine (4)
200
150
100
50
0
ppm (f1)
25000
20000
15000
10000
5000
0