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Supporting Information

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Highly Efficient Chemoenzymatic Synthesis of Naturally Occurring and Unnatural α2,6-linked Sialosides: A *P. damsela* α2,6-Sialyltransferase with Extremely Flexible Donor Substrate Specificity

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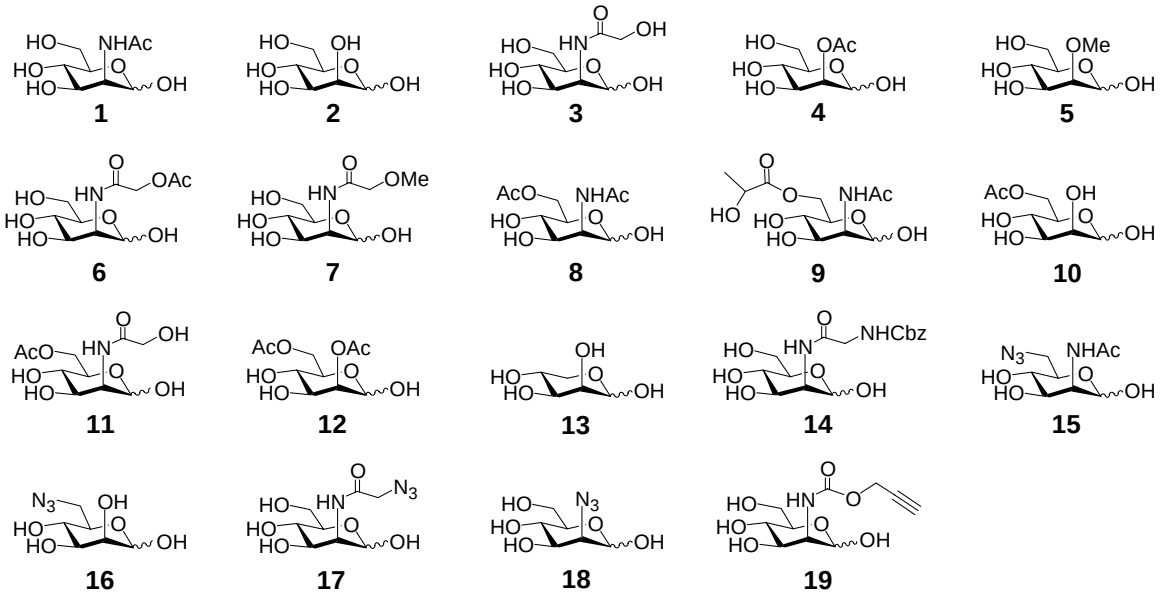
Table of Contents

General Methods

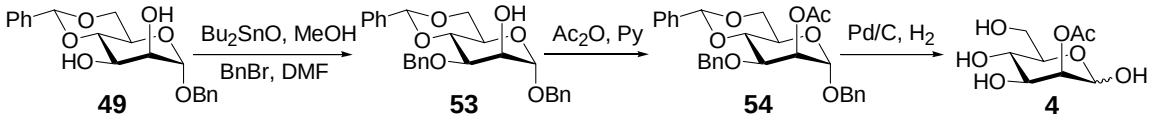
^{1}H NMR (300, 400 or 600 MHz) and ^{13}C NMR (75 or 100 MHz) spectra were recorded on a Varian Mercury-300, a Varian Inova-400, or a Varian Inova-600 spectrometer. Low and high resolution electrospray ionization (ESI) mass spectra were obtained at the Mass Spectrometry Facility in the University of Minnesota. Silica gel 60 Å (40-63 μm, Sorbent technologies) was used for flash column chromatography. Analytical thin-layer chromatography was performed on silica gel plates 60 GF_{254} (Sorbent technologies) using anisaldehyde 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).

Synthesis of ManNAc, Mannose, and ManNGc Derivatives as Precursors for Sialic Acid Derivatives:



ManNAc **1**, mannose **2**, and lyxose **13** were bought from Sigma. ManNGc **3**, ManNAc and mannose derivatives **7** and **14-19** were synthesized as described previously.[1,2] Compounds **4-6** and **8-12** were synthesized as described in the following:

2-*O*-Acetyl-D-mannopyranose (4).

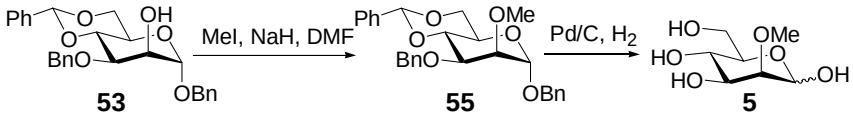


Compound **49** (0.56g, 1.56 mmol) and Bu_2SnO (0.43 g, 1.72 mmol) in CH_3OH (10 mL) were refluxed for 1.5 h to provide a clear solution. After cooled to room temperature, the solvent was removed under reduced pressure. The resulted benzyl 4,6-*O*-benzylidene-2,3-*O*-(dibutylstannylene)-α-D-mannopyranoside was dried under vacuum for 6 h and taken up in DMF (8 mL). The solution was treated with $PhCH_2Br$ (0.37 mL, 3.12 mmol) and heated at 100 °C with stirring for 30 minutes. The cooled reaction mixture was diluted with EtOAc (15 mL) and saturated aqueous $NaHCO_3$ (10 mL) with vigorous stirring. The organic layer was washed with brine and dried with Na_2SO_4. The residue was purified by flash column chromatography (hexanes: ethyl acetate, 4:1) to afford **53** (0.60 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52-7.33 (m, 15H), 5.65 (s, 1H, PhCH), 4.98 (d, 1H, *J* = 1.2 Hz, H-1), 4.91 (d, 1H, *J* = 12.0 Hz), 4.75 (d, 1H, *J* = 3.0 Hz), 4.72 (d, 1H, *J* = 3.0 Hz), 4.54 (d, 1H, *J* = 12.0 Hz), 4.35-3.89 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 138.23, 137.64, 137.16, 129.20, 128.80, 128.73, 128.51, 128.41, 128.32, 128.18, 128.11, 126.32, 101.83, 99.52, 79.18, 76.07, 73.42, 70.28, 69.62, 69.14, 63.81.

A solution of **53** (0.18 g, 0.40 mmol) in pyridine (3 mL) was treated with acetic anhydride (3 mL) at 0 °C and the mixture was stirred for overnight at r.t. The reaction mixture was concentrated. Purification of the residue by flash column chromatography (hexanes:ethyl acetate = 4:1, v/v) afforded **54** (0.18 g, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53-7.25 (m, 15 H), 5.65 (s, 1H), 5.47 (dd, 1H, *J* = 1.6 and 2.8 Hz, H-2), 4.89 (d, 1H, *J* = 1.6 Hz, H-1), 4.73-4.51 (m, 4H), 4.25 (dd, 1H, *J* = 4.0 and 9.6 Hz), 4.27-3.83(m, 4H), 2.17 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$), δ 170.43, 138.24, 137.67,136.79, 129.20, 128.82, 128.59, 128.45, 128.40, 128.33, 127.94, 127.89, 126.34, 101.81, 98.34, 78.58, 74.29, 72.48, 70.00, 69.82, 68.96, 64.33, 21.30.

To a solution of **54** (0.18 g, 0.37 mmol) in MeOH (3 mL) was added 10% Pd-C (60 mg), and the resulting mixture was hydrogenated for 18 h with stirring vigorously under hydrogen atmosphere. The resulted suspension was filtered and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2: MeOH, 4:1) to afford **4** (60mg, 75%). ^{1}H NMR (400 MHz, D_2O) δ 5.03 (d, 1H, *J* = 1.2 Hz), 4.91 (m, 1H), 3.77-3.51(m, 5H), 2.00 (m, 3H); ^{13}C NMR (100 MHz, D_2O) δ 173.51, 91.52, 73.15, 72.50, 68.82, 67.13, 60.85, 20.52.

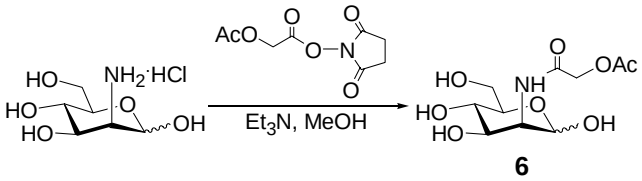
2-*O*-methyl-D-mannopyranose (5).



A solution of **53** (0.43 g, 0.96 mmol) in anhydrous DMF (5 mL) was stirred for 1 h with NaH (0.13 g, 5.50 mmol) at room temperature and then cooled to 0 °C. Methyl iodide (0.32 mL, 5.14 mmol) was added in three minutes, and the reaction was stirred at r.t. for 3 h. Dichloromethane (50 mL) was then added. The mixture was washed with water, dried, and evaporated to dryness. Purification of the residue by flash column chromatography (hexane: ethyl acetate = 1:1, v/v) afforded **55** (0.44 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56-7.34 (m, 15H), 5.67 (s, 1H), 5.00 (d, 1H, *J* = 1.2 Hz), 4.95 (d, 1H, *J* = 12.4 Hz), 4.77-4.52 (m, 2H), 4.74 (d, 1H, *J* = 12.0 Hz), 4.31-3.58 (m, 6H), 2.18 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$), δ 138.13, 137.53, 137.04, 129.11, 128.80, 128.59, 128.48, 128.29, 128.24, 127,85, 127.80, 126.32, 101.73, 98.45, 79.93, 79.47, 76.63, 73.59, 69.55, 69.10, 64.64, 60.48.

To a solution of **55** (0.44 g, 0.95 mmol) in MeOH (5 mL) was added 10% Pd-C (50 mg), and the resulted mixture was hydrogenated for 18 h with stirring vigorously under hydrogen atmosphere. The resulting suspension was filtered and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2: MeOH, 4:1) to afford **5** (0.15 g, 80%). ^{1}H NMR (400 MHz, D_2O) δ 5.15 (d, 1H, *J* = 1.2 Hz), 3.70-3.38 (m, 6H), 3.29 (s, 3H); ^{13}C NMR (100 MHz, D_2O), δ 90.85, 80.73, 72.40, 70.07, 67.34, 61.08, 58.85.

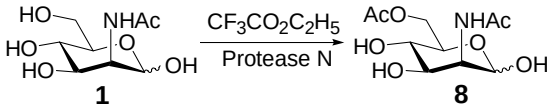
***N*-Acetoxyacetate-D-mannosamine (6).**



6

Mannosamine hydrochloride salt (0.25 g, 1.14 mmol) was dissolved in 10 mL of dry MeOH under argon. To this solution was added 0.19 mL of triethylamine. The mixture was stirred for 10 min until the solution turned clear. *N*-hydroxysuccinimidyl acetyloxyacetate[4] (0.28 g) was then added and the resulted solution was stirred at room temperature for overnight. The reaction mixture was concentrated and the residue was purified by flash column chromatography (CH_2Cl_2: MeOH, 10:1) to afford **6** as a white solid (0.29 g, 92%). NMR data of the product was consistent with that reported.[6]

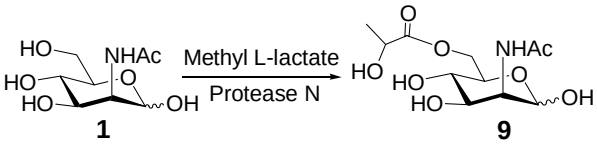
6-*O*-Acetyl *N*-Acetylmannosamine (8)



1 **8**

ManNAc (200 mg) was dissolved in a mixture of CH_3CN and DMSO (3:1, v/v, 6 mL). Trifluoroethyl acetate (1 mL) and Protease N (100 mg) were added and the suspension was stirred vigorously at 45 °C for 2 days. The enzyme was filtrated off and the filtrate was concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH = 5:1, v/v) to afford product **8** (121 mg, 51%). NMR data of the product was consistent with that reported.[5]

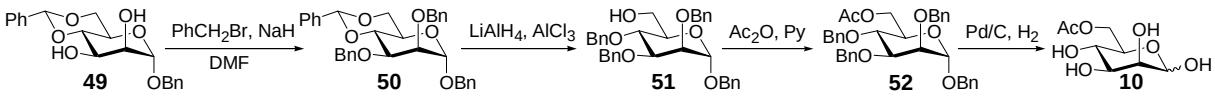
6-*O*-Lactyl *N*-Acetylmannosamine (9)



1 **9**

ManNAc (200 mg) was dissolved in DMF (6 mL). Methyl L-lactate (1 mL) and Protease N (100 mg) were added and the suspension was stirred vigorously at 45 °C for 2 days. The enzyme was filtrated off and the filtrate was concentrated. The residue was purified by flash column chromatography (EtOAc:MeOH = 5:1, v/v) to afford product **9** (84 mg, 32%). ^{13}C NMR (100 MHz, D_2O) δ 174.97, 165.08, 93.13, 69.89, 68.70, 67.18, 66.86, 64.22, 53.43, 20.02, 19.36.

6-*O*-Acetyl-D-mannopyranose (10).



49 OBn **50** OBn **51** OBn **52** OBn **10**

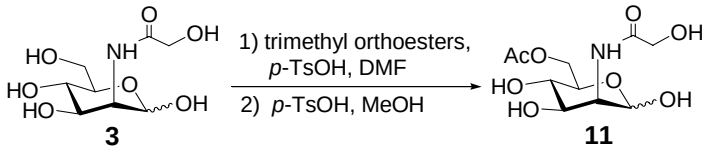
To a solution of benzyl 4,6-*O*-benzylidene-α-D-mannopyranoside[3] (3.0 g, 8.37 mmol) in DMF (15 mL) was added NaH (1.0 g, 41.85 mmol), followed by the addition of benzyl bromide (3.0 mL, 25.1 mmol). After 15 h, the remaining NaH was quenched by addition of MeOH. The mixture was diluted with ethyl acetate, washed with H_2O, dried with $MgSO_4$, and concentrated. The sample was purified by flash column chromatography (hexane:ethyl acetate = 4:1, v/v) to afford **50** (3.36g, 74%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71-7.41 (m, 20H), 5.80 (s, 1H), 5.08 (d, 1H, *J* = 1.6 Hz, H-1), 5.00-4.77 (m, 5H), 4.60 (d, 1H, *J* = 12.0 Hz), 4.50-4.05 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.11, 138.46, 138.01, 137.38, 129.22, 128.90, 128.70, 128.58, 128.48, 128.34, 128.24, 128.17, 127.92, 127.88, 126.48, 101.83, 99.03, 79.62, 76.92, 76.79, 73.96,73.60, 69.54, 69.23, 64.84.

Compound **50** (0.58 g, 1.08 mmol) was dissolved in dry CH_2Cl_2 (5 mL)and dry Et_2O (5 mL). $LiAlH_4$ (0.172 g, 4.53 mmol) was added in three portions with stirring. The mixture was then slowly heated to boiling point. To the hot solution, $AlCl_3$ (0.51 g, 3.82 mmol) in ether (5 mL) was added during a 30 minute-period. Reflux was continued for 1.5-2 hours. When TLC indicated the absence of starting material, the mixture was cooled down to room temperate. Excess $LiAlH_4$ was decomposed with ethyl acetate (8 mL). $Al(OH)_3$ was precipitated by the addition of water (15 mL). The mixture was diluted with ether and the organic layer was separated and concentrated. Purification of the residue by flash column chromatography afforded **51** (0.26g, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-7.25 (m, 20H), 4.95 (d, 1H, *J* = 11.2 Hz), 4.91 (d, 1H, *J* = 1.6 Hz, H-1), 4.78-4.21 (m, 7H), 4.01-3.72 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.57, 128.69, 128.62, 128.37, 128.11, 128.06, 128.02, 127.97, 127.88, 127.83, 97.76, 80.42, 75.55, 75.12, 75.01, 73.15, 72.64, 72.54, 69.36, 62.60.

A solution of **51** (0.25 g, 0.46 mmol) in pyridine (3 mL) was treated with acetic anhydride (3 mL) at 0 °C. The reaction mixture was stirred overnight at r.t. The reaction mixture was concentrated and purified by flash column chromatography (hexanes: ethyl acetate, 4:1) to afford **52** (0.27g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42-7.33 (m, 20H), 5.03 (d, 1H, *J* = 1.6 Hz, H-1), 4.77-4.48 (m, 9H), 4.06-3.86 (m, 4H), 2.12 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.22, 138.62, 138.43, 137.38, 128.78, 128.75, 128.70, 128.64, 128.50, 128.21, 128.14, 127.99, 97.45, 80.46, 75.56, 74.77, 74.72, 72.85, 72.42, 70.58, 69.50, 63.85. 21.25.

To a solution of **52** (0.27 g, 0.46 mmol) in MeOH (3 mL) was added 10% Pd-C (30 mg). The resulted mixture was hydrogenated for 18 h with vigorous stirring under hydrogen atmosphere. The resulted suspension was filtered and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2: MeOH = 4:1, v/v) to afford **10** (90 mg, 88%). ^{1}H (400 MHz, D_2O) δ 5.04 (d, 1H, *J* = 0.8 Hz), 4.37 (d, 1H, *J* = 12.0 Hz, H-6a), 4.22 (m, 1H), 3.93 (m, 1H), 3.79-3.73 (m, 2H), 3.63 (t, 1H, *J* = 9.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 171.90, 94.70, 71.57, 70.99, 70.42, 67.62, 64.10, 19.64.

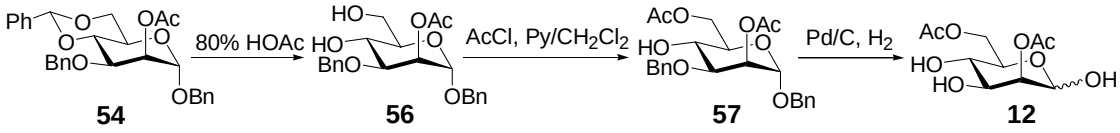
6-*O*-Acetyl *N*-Glycolylmannosamine (11)



3 **11**

To a solution of ManNGc (250 mg) in DMF (4 mL) was added trimethyl orthoacetate (1.5 eq.) and a catalytic amount of *p*-TsOH. After being stirred for 30 min, the reaction was neutralized by adding Et_3N and the solvent was removed. The residue was dissolved in MeOH (4 mL). *p*-TsOH (10 mg) was added and the mixture was stirred for 20 min. After Et_3N (50 μL) was added, the reaction mixture was concentrated and purified by flash column chromatography (EtOAc:MeOH, 3:1) to afford product **11** (84 mg, 32%). ^{13}C NMR (100 MHz, D_2O) δ 175.20, 174.17, 93.04, 69.95, 68.64, 67.05, 63.52, 60.98, 52.92, 20.33.

2,6-Di-*O*-acetyl-D-mannopyranose (12).

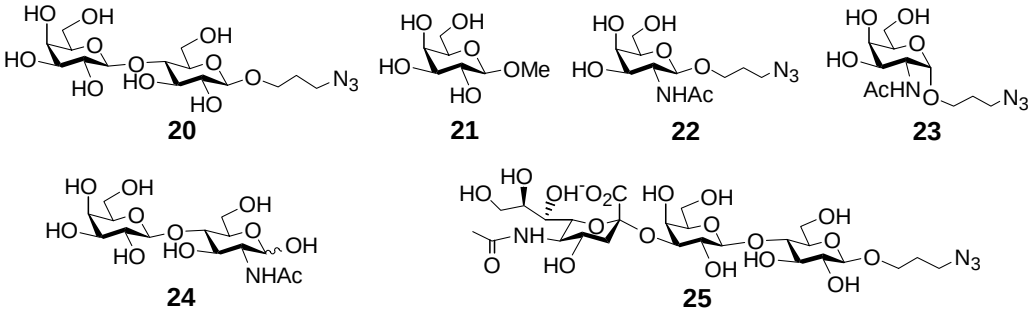


54 **56** **57** **12**

Compound **54** (500 mg, 1.02 mmol) was dissolved in 80% HOAc (5 mL) and heated at 60 °C for 1 hour. After removing the solvent, the residue was purified by silica gel column (EtOAc:Hexanes = 1:2, v/v) to afford **56** (377 mg, 92%). AcCl (49 mg, 0.62 mmol, in 2 mL of dry CH_2Cl_2) was added to a solution of **56 (**226 mg, 0.56 mmol) in dry pyridine (5 mL) at 0 °C. The mixture was stirred at 0 °C for 10 hours and the reaction was quenched by adding MeOH. After removed the solvent, the residue was purified by flash column chromatography (EtOAc:Hexanes = 1:1, v/v) to afford **57** (212 mg, 85%). ^1H NMR (400 MHz, CD_3Cl) δ 5.40 (t, 1H, *J* = 1.6 Hz), 4.91 (d, 1H, *J* = 1.2 Hz), 4.72 (s, 1H), 4.69 (s, 1H), 4.53-4.39 (m, 4H), 4.29 (dd, 1H, *J* = 1.6 Hz and 12 Hz), 3.85-3.80 (m, 2H), 2.10 (s, 3H), 2.09 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.48, 170.48, 137.75, 136.85, 128.81-128.30, 97.54, 72.00, 70.82, 69.74, 68.22, 66.66, 63.77, 21.20, 21.14.

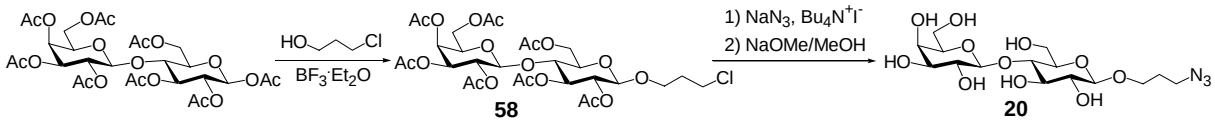
To a solution of **57** (212 mg, 0.48 mmol) in MeOH (5 mL) was added 10% Pd-C (100 mg). The resulted mixture was hydrogenated on a hydrogenation apparatus for 18 h under hydrogen atmosphere. The resulted suspension was filtered and concentrated. The residue was purified by flash column chromatography (CH_2Cl_2: MeOH, 6:1) to afford **12** (110 mg, 88%). ^1H NMR (400 MHz, D_2O) δ 5.02 (s, 1H), 4.93 (t, 1H, *J* = 1.2 Hz), 4.19 (d, 1H, *J* = 2.4 Hz), 3.90-3.87(m, 2H), 3.62 (t, 1H, *J* = 6.4 Hz), 3.54 (s, 1H); ^{13}C NMR (100 MHz, D_2O) δ 174.25, 173.40, 91.68, 73.04, 70.05, 68.69, 66.94, 63.55, 20.48, 20.35.

Synthesis of Derivatives of Gal, GalNAc, and lactose as Potential Acceptors for Pd2,6ST



GalβOMe **21** and LacNAc **24** were purchased from Sigma. Compound **25** were synthesized as described previously.[2] LacβProN$_3$ **20**, GalNAcβProN$_3$ **22**, and GalNAcαProN$_3$ **23** were synthesized as described in the following:

3-Azidopropyl β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (LacβProN$_3$, 20)

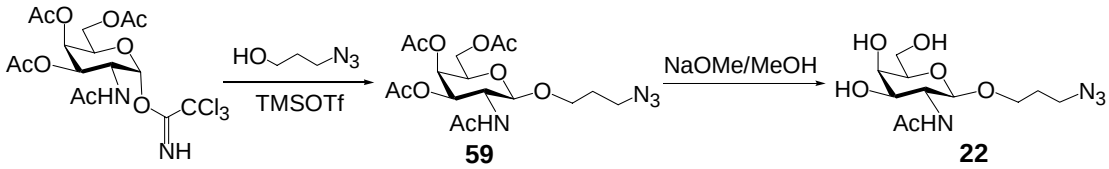


Peracetylated lactose (3.12 g, 4.60 mmol) and 3-chloro-1-propanol (0.63 mL) were dissolved in anhydrous CH_2Cl_2 (30 mL) with 4 Å powdered molecular sieves (2 g). After the mixture was stirred for 30 min, $BF_3{\cdot}Et_2O$ (2.33 mL, 15.44 mmol) was added at -10 °C. The reaction mixture was allowed to warm up to 0 °C and was stirred for overnight. Et_3N (1 mL) was added and the mixture was filtered over Celite and concentrated. The residue was purified by a silica gel column (Hexane:EtOAc = 1:1, v/v) to give peracetylated LacβProCl as a white solid (1.81 g, 52%). A mixture containing the above product (0.90 g), sodium azide (0.45 g, 6.92 mmol), and a catalytic amount of tetra-*n*-butylammonium iodide (50 mg) in dry DMF (20 mL) was stirred at 60 °C for 2 h. The organic layer was diluted with EtOAc, washed with water and brine, dried with Na_2SO_4, filtered and concentrated. Column chromatography of the residue on silica gel with 1:1 EtOAc-Hexane gave the product **58** as white foam (0.85 g, 100%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.21 (d, 1H, *J* = 2.4 Hz, H-4'), 5.06 (t, 1H, *J* = 9.6 Hz, H-3), 4.96 (dd, 1H, *J* = 10.4 and 8.0 Hz, H-2'), 4.83 (dd, *J* = 10.4 and 3.6 Hz, H-3'), 4.75 (dd, *J* = 10.0 and 7.6 Hz, H-2), 4.40-4.35 (m, 3H, H-1, H-1' and H-6'a), 4.03-3.93 (m, 3H, H-5', H-6'b and H-6a), 3.80-3.75 (m, 2H, OCH_{2a}, H-6b), 3.68 (t, *J* = 9.6 Hz, H-4), 3.52-3.44 (m, 2H, OCH_{2b} and H-5), 3.28-3.19 (m, 2H, CH_2Br), 2.02, 1.99, 1.93, 1.92, 1.83 (s, 21H, 7 × $COCH_3$), 1.76-1.64 (m, 2H, -CH_2-); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.56, 170.54, 170.34, 170.25, 169.95, 169.83, 169.27, 101.25, 100.74, 76.42, 72.94, 72.84, 71.81, 71.15, 70.85, 69.27, 66.80, 66.65, 62.12, 61.00, 48.12, 29.14, 21.04, 20.98, 20.89, 20.82, 20.70.

Compound **58** (0.85 g, 1.18 mmol) was dissolved in dry methanol (20 mL) containing a catalytic amount of sodium methoxide. The resulted mixture was stirred at room temperature for 12 h and neutralized with DOWEX HCR-W2 (H^+) resin. Filtration and concentration afforded

deacetylated product **20** as a white solid (0.48 g, 96%). ^{1}H NMR (400 MHz, D_2O) δ 4.29 (d, 1H, *J* = 8.0 Hz, H-1), 4.25 (d, 1H, *J* = 7.6 Hz, H-1'), 3.81-3.77 (m, 2H), 3.73 (d, 1H, *J* = 3.2 Hz), 3.63-3.33 (m, 12H), 3.29 (t, 2H, *J* = 6.8 Hz), 3.12 (m, 1H), 1.72 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ 103.45, 102.25, 78.49, 75.47, 74.89, 74.49, 72.92, 72.64, 71.07, 68.67, 67.47, 61.16, 60.20, 47.99, 28.37.

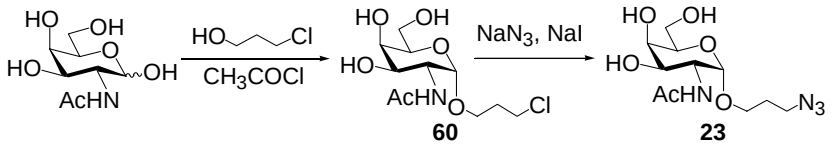
3-Azidopropyl 2-acetamido-2-deoxy-β-D-galactopyranoside (GalNAcβProN$_3$, 22)



2-Acetamido-3,4,5-tri-*O*-acetyl-2-deoxy-D-galactosyl trichloroacetimidate[7] (400 mg, 0.81 mmol) was dissolved in CH_2Cl_2 (10 mL) with 4 Å powdered molecular sieves (0.5 g). 3-azidopropanol (411 mg, 4.05 mmol) was added and the reaction mixture was cooled down to -30 °C. After the mixture was stirred for 30 min, TMSOTf (60 μL) was added in a 5 minute-period. The reaction mixture was allowed to warm up to 0 °C and stirred for 4 h. Et_3N (0.3 mL) was added to quench the reaction. The mixture was filtered over Celite and concentrated. The crude material was purified by flash silica gel column (Hexane:EtOAc = 2:1, v/v) to yield the peracetylated GalNAc derivative **59** as a white solid (276 mg, 79%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.01 (s, 1H, NH), 5.29 (d, 1H, *J* = 3.2 Hz, H-4), 5.17 (dd, 1H, *J* = 11.2 and 3.2 Hz, H-3), 4.58 (d, 1H, *J* = 8.4 Hz, H-1), 4.12-3.86 (m, 5H), 3.56 (m, 1H), 3.32 (m, 2H), 2.08, 1.98, 1.93, 1.90 (s, 12H, 4 × $COCH_3$), 1.86-1.70 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$)) δ 170.75, 170.70 (2C), 170.54, 101.47, 70.80, 70.22, 66.98, 66.42, 61.81, 51.32, 48.20, 29.11, 23.56, 20.89 (3C).

Compound **59** (0.25 g, 0.58 mmol) was dissolved in dry methanol (8 mL) containing a catalytic amount of sodium methoxide. The resulted mixture was stirred at room temperature for overnight. The reaction mixture was then neutralized with DOWEX HCR-W2 (H^+) resin, filtered and concentrated to give desired product **22** as a white solid (0.17 g, 97%). ^{1}H NMR (400 MHz, D_2O) δ 4.25 (d, 1H, *J* = 7.6 Hz, H-1), 3.78-3.62 (m, 3H), 3.59-3.52 (m, 5H), 3.19 (t, 2H, *J* = 6.4 Hz), 1.86 (s, 3H, $COCH_3$), 1.65 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 174.78, 101.81, 75.20, 71.08, 67.91, 67.10, 61.10, 52.56, 47.91, 28.27, 22.37.

3-Azidopropyl 2-acetamido-2-deoxy-α-D-galactopyranoside (GalNAcαProN$_3$, 23)



To a solution of *N*-acetyl-D-galactosamine (1.0 g, 4.5 mmol) in 3-chloropropanol (15 mL) was added dropwisely acetyl chloride (0.43 g, 5.4 mmol) at 0 °C. The reaction mixture was heated at

70 °C for 8 h. The solution was concentrated and the residue was purified by silica gel chromatography to yield 3-chloropropyl GalNAc (1.17 g, 87%) as a syrup residue. 3-chloropropyl GalNAc (0.10 g, 0.353 mmol) was dissolved in CH_3CN (3 mL) by heating the solution. NaN_3 (0.23 g, 3.53 mmol) and NaI (0.053 g, 0.353 mmol) were then added. The reaction mixture was heated at 60 °C for 7 h. The reaction mixture was concentrated and the residue was purified using a short silica gel column with $CHCl_3$: MeOH = 95:5 as mobile phase to yield **23** (0.097 g, 95%) as an solid. ^{1}H NMR (400 MHz, D_2O) δ 4.72 (d, 1H, *J* = 4.0 Hz, H-1), 3.97 (dd, 1H, *J* = 10.8 and 3.6 Hz), 3.81-3.73 (m, 3H), 3.65-3.58 (m, 3H), 3.38-3.24 (m, 3H), 1.86 (s, 3H), 1.72 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 174.65, 97.17, 71.06, 68.65, 67.76, 65.04, 61.39, 50.12, 48.29, 28.09, 22.10.

Enzymatic Synthesis of Sialosides

General procedure for one-pot three enzyme preparative synthesis of α2,6-linked sialosides using Pd2,6ST. A prospective acceptor for Pd2,6ST (e.g. galactose, lactose, GalNAc, LacNAc or their derivatives, 50-100 mg), a sialic acid precursor or its analog (e.g. mannose, ManNAc, or their derivatives, 1.5 equiv.), sodium pyruvate (7.5 equiv.), and CTP (1.5 equiv.) were dissolved in H_2O. Stock solutions of Tris-HCl buffer (1 M, pH 8.8, 1 mL) and $MgCl_2$(0.5 M, 0.4 mL) were added. After the addition of appropriate amount of an *E. coli* sialic acid aldolase, an *N. meningitidis* CMP-sialic acid synthetase, and Pd2,6ST, H_2O 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 2 h at 37 °C (or for 15 h at room temperature) with agitation at 140 rpm. The product formation was monitored by TLC developed with EtOAc:MeOH:H_2O:HOAc = 4:2:1:0.5 (by volume) and stained with *p*-anisaldehyde sugar stain. The reaction was quenched by adding the same volume of ice-cold EtOH and incubation at 4 °C for 30 min. The mixture was then centrifuged to remove insoluble precipitates. The supernatant was concentrated and passed through a BioGel P-2 gel filtration column with water to obtain desired product.

3-Azidopropyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5Acα2,6LacβProN$_3$, 26). Yield, 98%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.34 (d, 1H, *J* = 8.0 Hz), 4.28 (d, 1H, *J* = 8.0 Hz), 3.88-3.78 (m, 4H), 3.75-3.58 (m, 7H), 3.73-3.36 (m, 9H), 3.33 (t, 2H, *J* = 6.4 Hz), 3.19 (t, 1H, *J* = 8.8 Hz), 2.56 (dd, 1H, *J* = 12.4 and 4.8 Hz, H-3$_{eq}$''), 1.88 (s, 3H), 1.77 (m, 2H), 1.58 (t, 1H, *J* = 12.4 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 175.08, 173.64, 103.36, 102.16, 100.45, 79.76, 74.79, 74.70, 73.84, 72.89, 72.68, 72.52, 71.95, 70.95, 68.67, 68.51, 68.53, 67.49, 63.71, 62.80, 60.41, 51.95, 48.04, 40.26, 28.40, 22.24. HRMS (ESI) *m/z* calcd for $C_{26}H_{43}N_4O_{19}Na_2$ (M+Na) 761.2317, found 761.2339.

3-Azidopropyl *O*-(3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (KDNα2,6LacβProN$_3$, 27). Yield, 97%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.31 (d, 1H, *J* = 8.0 Hz), 4.24 (d, 1H, *J* = 8.0 Hz), 3.85-3.71 (m, 6H), 3.65-3.32 (m, 14H), 3.28 (t, 2H, *J* = 6.8 Hz), 3.15 (t, 1H, *J* = 8.4 Hz), 2.47 (dd, 1H, *J* = 4.8 and 12.8 Hz, H-3$_{eq}$''), 1.74 (m, 2H), 1.52 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 173.82, 103.34, 102.13, 100.36, 79.79, 74.73, 74.69, 73.85, 73.65, 72.85, 72.46, 72.18, 70.88, 70.31, 69.96, 68.63, 68.16, 67.45, 63.72, 62.78, 60.35, 47.98, 39.85, 28.35. HRMS (ESI) *m/z* calcd for $C_{24}H_{40}O_{19}N_3Na_2$ (M+Na) 720.2051, found 720.2066.

3-Azidopropyl *O*-(5-glycolylamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5Gcα2,6LacβProN$_3$, 28). Yield, 95%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.32 (d, 1H, *J* = 8.0 Hz), 4.26 (d, 1H, *J* = 8.0 Hz), 3.94 (s, 2H), 3.86-3.56 (m, 12H), 3.53-3.34 (m, 8H), 3.29 (t, 2H, *J* = 6.8 Hz), 3.17 (t, 1H, *J* = 8.4 Hz), 2.55 (dd, 1H, *J* = 4.4 and 12.4 Hz, H-3$_{eq}$''), 1.74 (m, 2H), 1.58 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 175.82, 173.65, 103.33, 102.12, 100.41, 79.73, 74.76, 74.75, 73.83, 72.85, 72.47, 72.37, 71.96, 71.01, 68.64, 68.42, 68.21, 67.45, 63.74, 62.70, 61.11, 60.36, 51.61, 47.99, 40.27, 28.36. HRMS (ESI) *m/z* calcd for $C_{26}H_{43}N_4O_{20}Na_2$ (M+Na) 777.2266, found 777.2277.

3-Azidopropyl *O*-(5-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (KDN5Acα2,6LacβProN$_3$, 29). Yield, 75%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.76 (t, 1H, *J* = 10.0 Hz, H-5''), 4.31 (d, 1H, *J* = 8.0 Hz), 4.24 (d, 1H, *J* = 8.0 Hz), 3.85-3.68 (m, 9H), 3.63-3.33 (m, 10H), 3.29 (t, 2H, *J* = 6.8 Hz), 3.16 (t, 1H, *J* = 8.0 Hz), 2.53 (dd, 1H, *J* = 4.0 and 12.4 Hz, H-3$_{eq}$''), 1.95 (s, 3H), 1.73 (m, 2H), 1.63 (t, 1H, *J* = 11.6 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 173.51(2C), 103.35, 102.13, 100.42, 79.83, 74.73, 73.83, 72.83, 72.44, 71.83, 71.69, 70.88, 68.62, 68.50, 67.96, 67.45, 63.84, 62.72, 60.35, 47.98, 39.68, 28.36, 20.54. HRMS (ESI) *m/z* calcd for $C_{26}H_{42}N_3O_{20}Na_2$ (M+Na) 762.2157, found 762.2174.

3-Azidopropyl *O*-(5-*O*-methyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (KDN5Meα2,6LacβProN$_3$, 30). Yield, 76%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.31 (d, 1H, *J* = 7.8 Hz), 4.24 (d, 1H, *J* = 7.8 Hz), 3.83-3.72 (m, 6H), 3.63-3.33 (m, 13H), 3.39 (s, 3H, OCH_3), 3.29 (t, 2H, *J* = 6.6 Hz), 3.17 (m, 2H), 2.46 (dd, 1H, *J* = 4.8 and 13.2 Hz, H-3$_{eq}$''), 1.74 (m, 2H), 1.63 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 173.71, 103.32, 102.10, 100.17, 79.86, 74.66, 73.82, 72.77, 72.40, 72.20, 70.83, 69.97, 69.68, 68.54, 68.36, 67.42, 63.72, 62.74, 60.31, 60.19, 47.94, 39.90, 28.31. HRMS (ESI) *m/z* calcd for $C_{25}H_{42}N_3O_{19}Na_2$ (M+Na) 734.2208, found 734.2224.

3-Azidopropyl *O*-(5-acetoxyacetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5GcAcα2,6LacProN$_3$, 31). Yield, 87%; white foam. ^{1}H NMR (300 MHz, D_2O) δ 4.53 (s, 2H, $\underline{CH_2}$OAc), 4.32 (d, 1H, *J* = 8.0 Hz), 4.25 (d, 1H, *J* = 8.0 Hz), 3.84-3.33 (m, 20H), 3.29 (t, 2H, *J* = 6.4 Hz), 3.16 (t, 1H, *J* = 8.0 Hz), 2.53 (dd, 1H, *J* = 4.4 and 12.0 Hz, H-3$_{eq}$''), 2.00 (s, 3H), 1.74 (m, 2H), 1.57 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (75 MHz, D_2O) δ 173.64, 173.43, 170.92, 103.35, 102.13, 100.42, 79.76, 74.76, 73.84, 72.86, 72.55, 72.35, 71.99, 70.91, 68.64, 68.47, 68.21, 67.46, 63.73, 63.02, 62.76, 60.37, 51.78, 48.00, 40.28, 28.37, 20.16. HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_4O_{21}Na_2$ (M+Na) 819.2372, found 819.2410.

3-Azidopropyl *O*-(5-methoxyacetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5GcMeα2,6LacProN$_3$, 32). Yield, 99%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.32 (d, 1H, *J* = 7.6 Hz), 4.25 (d, 1H, *J* = 8.0 Hz), 3.86 (d, 2H, *J* = 1.2 Hz), 3.84-3.33 (m, 20H), 3.29 (t, 2H, *J* = 6.8 Hz), 3.24 (s, 3H, OMe), 3.17 (t, 1H, *J* = 8.4 Hz), 2.55 (dd, 1H, *J* = 4.8 and 12.4 Hz, H-3$_{eq}$''), 1.74 (m, 2H), 1.58 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 173.66, 173.50, 103.34, 102.13, 100.42, 79.74, 74.76, 73.83, 72.86, 72.47, 72.35, 71.99, 71.01, 70.90, 70.31, 68.64, 68.51, 68.22, 67.45, 63.75, 62.72, 60.36, 59.11, 51.58, 48.00, 40.34, 28.36. HRMS (ESI) *m/z* calcd for $C_{27}H_{45}N_4O_{20}Na_2$ (M+Na) 791.2422, found 791.2458.

3-Azidopropyl *O*-(9-*O*-acetyl-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5,9Ac$_2$α2,6LacβProN$_3$, 33). Yield, 84%; white foam. ^{1}H NMR (300 MHz, D_2O) δ 4.32 (d, 1H, *J* = 7.8 Hz), 4.26-4.22 (m, 2H, H-1 and H-6a''), 4.05-3.75 (m, 6H), 3.73-3.32 (m, 13H), 3.29 (t, 2H, *J* = 6.6 Hz), 3.16 (t, 1H, *J* = 8.4 Hz), 2.53 (dd, 1H, *J* = 4.5 and 12.6 Hz, H-3$_{eq}$''), 1.96, 1.86 (2s, 6H, 2CH_3), 1.74 (m, 2H), 1.56 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (75 MHz, D_2O) δ

174.98, 174.52, 173.61, 103.34, 102.13, 100.44, 79.77, 74.76, 73.86, 72.87, 72.46, 72.43, 70.89, 69.37, 68.67, 68.52, 67.45, 65.81, 63.83, 60.37, 51.88, 48.00, 40.25, 28.37, 22.21, 20.41. HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_4O_{20}Na_2$ (M+Na) 803.2422, found 803.2430.

3-Azidopropyl *O*-(9-*O*-lactyl-5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5Ac9Ltα2,6LacβProN$_3$, 34). Yield, 72%; white foam. (600 MHz, D_2O) δ 4.32 (d, 1H, *J* = 7.8Hz), 4.30-4.19 (m, 3H), 3.95 (m, 1H), 3.84-3.75 (m, 4H), 3.68 (t, 1H, *J* = 10.2 Hz), 3.64-3.41 (m, 12H), 3.36 (dd, 1H, *J* = 7.8 and 9.6 Hz), 3.29 (t, 2H, *J* = 6.6 Hz), 3.16 (t, 1H, *J* = 8.4 Hz), 2.54 (dd, 1H, *J* = 4.2 and 13.2 Hz, H-3$_{eq}$''), 1.85 (s, 3H), 1.74(m, 2H), 1.56(t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''), 1.25 (d, 3H, *J* = 6.6 Hz); ^{13}C NMR (75 MHz, D_2O) 174.96, 174.93, 173.61, 103.33, 102.12, 100.46, 79.76, 74.74, 73.82, 72.85, 72.46, 72.39, 70.88, 69.28, 68.63, 68.44, 68.25, 67.44, 66.75, 66.05, 63.77, 60.36, 51.89, 47.99, 40.24, 28.36, 22.15, 19.36. HRMS (ESI) *m/z* calcd for $C_{29}H_{47}N_4O_{21}Na_2$ (M+Na) 833.2528, found 833.2552.

3-Azidopropyl *O*-(9-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (KDN9Acα2,6LacβProN$_3$, 35). Yield, 75%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.31 (d, 1H, *J* = 8.4 Hz), 4.25 (dd, 1H, H-9a), 4.24 (d, 1H, *J* = 7.6 Hz), 4.08 (dd, 1H, *J* = 5.6 and 11.6 Hz), 3.93 (m, 1H), 3.84-3.70 (m, 4H), 3.64-3.33 (m, 13H), 3.28 (t, 2H, *J* = 6.8 Hz), 3.15 (t, 1H, *J* = 8.0 Hz), 2.48 (dd, 1H, *J* = 4.0 and 12.4 Hz, H-3$_{eq}$''), 1.96 (s, 3H), 1.74 (m, 2H), 1.53 (t, 1H, *J* = 11.6 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 174.57, 173.81, 103.34, 102.13, 100.38, 79.82, 74.73, 73.90, 73.46, 72.86, 72.44, 70.86, 70.31, 69.94, 69.58, 68.66, 68.03, 67.45, 65.79, 63.83, 60.35, 47.99, 39.88, 28.35, 20.42. HRMS (ESI) *m/z* calcd for $C_{26}H_{42}N_3O_{20}Na_2$ (M+Na) 762.2157, found 762.2154.

3-Azidopropyl *O*-(9-*O*-acetyl-5-glycolylamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu9Ac5Gcα2,6LacβProN$_3$, 36). Yield, 78%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.32 (d, 1H, *J* = 8.4 Hz), 4.26-4.23 (m, 2H), 4.02 (dd, 1H, *J* = 6.0 and 12.0 Hz), 3.95 (s, 2H), 3.93 (m, 1H), 3.85-3.76 (m, 5H), 3.70 (d, 1H, *J* = 10.2 Hz), 3.65-3.42 (m, 10H), 3.36 (t, 1H, *J* = 9.6 Hz), 3.29 (t, 2H, *J* = 6.6 Hz), 3.17 (t, 1H, *J* = 8.4 Hz), 2.56 (dd, 1H, *J* = 4.8 and 12.6 Hz, H-3$_{eq}$''), 1.96(s, 3H), 1.74(m, 2H), 1.59 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (75 MHz, D_2O) δ 175.76, 174.53, 173.63, 103.33, 102.11, 100.41, 79.77, 74.73, 73.87, 72.84, 72.43, 72.14, 70.86, 69.40, 68.66, 68.40, 68.23, 67.44, 65.74, 63.88, 60.10, 60.34, 51.55, 47.94, 40.27, 28.35, 20.40. HRMS (ESI) *m/z* calcd for $C_{28}H_{45}N_4Na_2O_{21}$ (M+Na) 819.2372, found 819.2382.

3-Azidopropyl *O*-(3-deoxy-5,9-di-*O*-acetyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (KDN5,9Ac$_2$α2,6LacβProN$_3$, 37). Yield, 70%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.82 (t, 1H, *J* = 10.0 Hz, H-5''), 4.36 (d, 1H, *J* = 8.4 Hz), 4.29 (d, 1H, *J* = 7.6 Hz), 4.25 (dd, 1H, H-9a), 4.07-3.77 (m, 7H), 3.67-3.40 (m, 11H), 3.33 (t, 2H, *J* = 6.8 Hz), 3.21 (t, 1H, *J* = 8.0 Hz), 2.58 (dd, 1H, *J* = 4.0 and 12.4 Hz, H-3$_{eq}$''), 2.02 (s, 3H), 2.00 (s, 3H), 1.79 (m, 2H), 1.67 (t, 1H, *J* = 12.4 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 174.51, 173.49 (2C), 103.34, 102.13, 100.43, 79.87, 74.72, 73.87, 72.82, 72.42, 71.78, 71.50, 70.84, 69.21, 68.64, 68.51, 67.87, 67.44, 65.71, 63.97, 60.34, 47.97, 39.70, 28.35, 20.54, 20.38. HRMS (ESI) *m/z* calcd for $C_{28}H_{44}N_3O_{21}Na_2$ (M+Na) 804.2263, found 804.2289.

3-Azidopropyl *O*-(3-deoxy-D-*glycero*-α-D-*galacto*-2-octulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (4,6-bis-*epi*-KDNα2,6LacProN_3, 38). Yield, 92%; white foam. ^{1}H NMR (300 MHz, D_2O) δ 4.32 (d, 1H, *J* = 8.1 Hz), 4.25 (d, 1H, *J* = 8.1 Hz), 3.81-3.73 (m, 5H), 3.64-3.32 (m, 14H), 3.29 (t, 2H, *J* = 6.6 Hz), 3.15 (t, 1H, *J* = 8.4 Hz), 2.42 (dd, 1H, *J* = 4.5 and 12.6 Hz, H-3_{eq}''), 1.74 (m, 2H), 1.49 (t, 1H, *J* = 12.0 Hz, H-3_{ax}''); ^{13}C NMR (75 MHz, D_2O) δ 173.86, 103.33, 102.15, 100.60, 79.71, 74.76, 74.70, 74.39, 73.87, 72.86, 72.45, 70.91, 70.20, 69.98, 69.01, 68.62, 67.46, 63.62, 63.37, 60.34, 47.99, 39.85, 28.36. HRMS (ESI) *m/z* calcd for $C_{23}H_{38}N_3O_{18}Na_2$ (M+Na) 690.1946, found 690.1957.

3-Azidopropyl *O*-[5-(N-benzyloxycarboxyamido)glycylamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid]-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5GlyCbzα2,6LacProN_3, 39). Yield, 99%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 7.28-7.21 (m, 5H), 4.95 (s, 2H, Ph$\underline{CH_2}$), 4.30 (d, 1H, *J* = 8.0 Hz), 4.24 (d, 1H, *J* = 7.6 Hz), 3.84-3.33 (m, 22 H), 3.27 (t, 2H, *J* = 6.8 Hz), 3.16 (t, 1H, *J* = 8.6 Hz), 2.53 (dd, 1H, *J* = 4.8 and 12.4 Hz, H-3_{eq}''), 1.73 (m, 2H), 1.56 (t, 1H, *J* = 12.0 Hz, H-3_{ax}''); ^{13}C NMR (100 MHz, D_2O) δ 173.61, 173.09, 158.64, 136.33, 128.94, 128.60, 127.92, 103.33, 102.13, 100.39, 79.73, 74.75, 73.80, 72.84, 72.47, 72.00, 70.90, 68.63, 68.42, 68.25, 67.45, 63.68, 62.79, 60.36, 51.99, 47.99, 43.84, 40.31, 28.36, 26.55. HRMS (ESI) calcd. for $C_{34}H_{50}N_5Na_2O_{21}$ (M+Na), 910.2794, found 910.2810.

Methyl *O*-(5-acetamido-9-azido-3,5,9-trideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5Ac9N_3α2,6GalβOMe, 40). Yield, 93%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.12 (d, 1H, *J* = 8.0 Hz), 3.83-3.70 (m, 3H), 3.67-3.28 (m, 10H), 3.38 (s, 3H, OMe), 2.53 (dd, 1H, *J* = 4.0 and 12.8 Hz, H-3_{eq}'), 1.85 (s, 3H, Ac), 1.50 (t, 1H, *J* = 12.0 Hz, H-3_{ax}'); ^{13}C NMR (100 MHz, D_2O) δ 175.12, 173.58, 104.12, 100.62, 73.56, 72.70, 72.56, 70.74, 70.44, 68.94, 68.76, 68.31, 63.55, 57.53, 53.22, 51.96, 40.32, 22.16. HRMS (ESI) *m/z* calcd for $C_{18}H_{29}N_4O_{13}Na_2$ (M+Na) 555.1526, found 555.1541.

Methyl *O*-(9-azido-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (KDN9N_3α2,6GalβOMe, 41). Yield, 90%; white foam. ^{1}H NMR (300 MHz, D_2O) δ 4.12 (d, 1H, *J* = 8.1 Hz), 3.87-3.67 (m, 4H), 3.63-3.27 (m, 9H), 3.39 (s, 3H, OMe), 2.49 (dd, 1H, *J* = 4.5 and 12.6 Hz, H-3_{eq}'), 1.47 (t, 1H, *J* = 12.0 Hz, H-3_{ax}'); ^{13}C NMR (75 MHz, D_2O) δ 173.78, 104.01, 100.60, 73.57(2C), 72.69, 70.74(2C), 70.23, 70.01, 68.78, 68.62, 63.59, 57.53, 53.31, 39.90. HRMS (ESI) *m/z* calcd for $C_{16}H_{26}N_3O_{13}Na_2$ (M+Na) 514.1261, found 514.1245.

Methyl *O*-(5-azidoacetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (Neu5AcN_3α2,6GalβOMe, 42). Yield, 91%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.12 (d, 1H, *J* = 8.0 Hz), 3.88 (s, 2H), 3.78-3.51 (m, 8H), 3.47-3.40 (m, 4H), 3.38 (s, 3H, OMe), 3.30 (dd, 1H, *J* = 3.6 and 10.0 Hz), 2.55 (dd, 1H, *J* = 4.8 and 12.4 Hz, H-3_{eq}'), 1.58 (t, 1H, *J* = 12.0 Hz, H-3_{ax}'); ^{13}C NMR (100 MHz, D_2O) δ 173.56, 171.33, 104.00, 100.56, 73.54, 72.70, 72.42, 71.93, 70.75, 68.76, 68.25, 68.14, 63.54, 62.72, 57.52, 52.04, 52.01, 40.31. HRMS (ESI) *m/z* calcd for $C_{18}H_{29}N_4O_{14}Na_2$ (M+Na) 571.1476, found 571.1478.

Methyl *O*-(5-azido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside (KDN5N$_3$α2,6GalβOMe, 43). Yield, 86%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.12 (d, 1H, *J* = 7.6 Hz), 3.86 (d, 2H, *J* = 1.2 Hz), 3.84-3.33 (m, 9H), 3.29 (t, 2H, *J* = 6.8 Hz), 3.24 (s, 3H, OMe), 3.17 (t, 1H, *J* = 8.4 Hz), 2.55 (dd, 1H, *J* = 4.8 and 12.4 Hz, H-3$_{eq}$''), 1.74 (m, 2H), 1.58 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 173.47, 104.00, 100.55, 73.54, 72.70, 72.04, 70.75, 69.54, 68.77, 68.60, 63.61, 62.83, 62.69, 57.51, 40.09, 26.54. HRMS (ESI) *m/z* calcd for $C_{16}H_{26}N_3O_{13}Na_2$ (M+Na) 514.1261, found 514.1264.

Methyl *O*-(5-[(2-propynyloxy)carbonyl]amido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranoside. (NeuAlkyneα2,6GalβOMe, 44). Yield, 87%; white foam. ^{1}H NMR (300 MHz, D_2O) δ 4.52 (d, 1H, *J* = 1.5 Hz), 4.13 (d, 1H, *J* = 8.1 Hz), 3.80-3.69 (m, 4H), 3.62-3.41 (m, 9H), 3.40 (s, 3H, OMe), 3.31 (dd, 1H, *J* = 8.1 and 9.9 Hz), 2.74 (t, 1H, *J* = 2.4 Hz); 2.55 (dd, 1H, *J* = 4.2 and 12.3 Hz, H-3$_{eq}$'), 1.52 (t, 1H, *J* = 12.3 Hz, H-3$_{ax}$'); ^{13}C NMR (75 MHz, D_2O) δ 173.61, 157.72, 104.01, 100.55, 78.61, 75.91, 73.55, 72.70, 72.07, 70.76, 68.77, 68.51, 68.26, 63.53, 62.79, 57.54, 53.45, 53.01, 40.27. HRMS (ESI) *m/z* calcd for $C_{20}H_{30}NO_{15}Na_2$ (M+Na) 570.1411, found 570.1429.

3-Azidopropyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-2-acetamido-2-deoxy-β-D-galactopyranoside (Neu5Acα2,6GalNAcβProN$_3$, 45). Yield, 87%; white foam. ^{1}H NMR (300 MHz, D_2O) δ 4.25 (d, 1H, *J* = 8.4 Hz), 3.83-3.65 (m, 6H), 3.62-3.38 (m, 9H), 3.20 (t, 2H, *J* = 6.6 Hz), 2.55 (dd, 1H, *J* = 3.9 and 12.0 Hz, H-3$_{eq}$''), 1.87, 1.86 (2s, 2CH$_3$), 1.66 (m, 2H), 1.51 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 175.17, 174.82, 173.56, 101.87, 100.55, 73.54, 72.76, 71.87, 70.90, 68.32, 67.87, 67.36, 63.58, 62.75, 52.48, 51.97, 47.89, 40.31, 28.29, 22.35, 22.16. HRMS (ESI) *m/z* calcd for $C_{22}H_{37}N_5O_{14}Na$ (M+H) 618.2235, found 618.2213.

3-Azidopropyl *O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-2-acetamido-2-deoxy-α-D-galactopyranoside (Neu5Acα2,6GalNAcαProN$_3$, 46). Yield, 61%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 4.70 (d, 1H, *J* = 3.6 Hz), 3.97 (dd, 1H, *J* = 3.6 and 10.8 Hz), 3.87 (dd, 1H, *J* = 4.2 and 7.8 Hz), 3.83 (d, 1H, *J* = 3.0 Hz), 3.76-3.61 (m, 5H), 3.55-3.26 (m, 9H), 2.56 (dd, 1H, *J* = 4.8 and 12.0 Hz, H-3$_{eq}$''), 1.87, 1.86 (2s, 2CH$_3$), 1.74 (m, 2H), 1.51 (t, 1H, *J* = 12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (100 MHz, D_2O) δ 175.16, 174.68, 173.57, 100.49, 97.20, 72.69, 71.91, 69.74, 69.62, 68.66, 68.38, 67.61, 65.28, 63.97, 62.75, 51.99, 50.07, 48.29, 40.40, 28.06, 22.18, 22.08. HRMS (ESI) *m/z* calcd for $C_{22}H_{36}N_5O_{14}Na_2$ (M+Na) 640.2054, found 640.2070.

***O*-(5-Acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)-*O*-β-D-galactopyranosyl-(1→4)-2-acetamido-2-deoxy-D-glucopyranose (Neu5Acα2,6LacNAc, 47).** Yield, 92%; white foam. ^{1}H NMR (600 MHz, D_2O) δ 5.02 (d, 0.3H, *J* = 2.4 Hz, H-1α), 4.57 (d, 0.7H, *J* = 7.8 Hz, H-1β), 4.27 (d, 1H, *J* = 7.8 Hz, H-1'), 3.82-3.63 (m, 8H), 3.55-3.35 (m, 11H), 2.49 (dd, 1H, *J* = 4.8 and 12.6 Hz, H-3$_{eq}$''), 1.89 (s, 3H), 1.85 (s, 3H), 1.54 (t, 1H, *J* =12.0 Hz, H-3$_{ax}$''); ^{13}C NMR (75 MHz, D_2O) δ 175.06, 174.61, 173.72, 103.64, 100.29, 94.84, 90.72, 81.07, 80.82, 74.67, 73.83, 72.68, 72.54, 71.84, 70.87, 70.12, 69.75,

69.50, 68.53, 68.36, 63.53, 62.77, 60.48, 60.31, 56.08, 53.55, 52.01, 48.99, 40.22, 22.42, 22.12; HRMS (ESI) m/z calcd for $C_{25}H_{41}N_2O_{19}Na_2$ (M+Na) 719.2099, found 719.2125.

3-Azidopropyl *O*-(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→3)-*O*-[(5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosylonic acid)-(2→6)]-*O*-β-D-galactopyranosyl-(1→4)-β-D-glucopyranoside (Neu5Acα2,3(Neu5Acα2,6)LacβProN$_3$, 48). Yield, 91%; white foam. ^{1}H NMR (400 MHz, D_2O) δ 4.32 (d, 1H, *J* = 7.6 Hz), 4.30 (d, 1H, *J* = 7.6 Hz), 3.93 (dd, 1H), 3.84-3.55 (m, 10H), 3.53-3.58 (m, 6H), 3.73-3.32 (m, 10H), 3.28 (t, 2H, *J* = 6.4 Hz), 3.15 (t, 1H, *J* = 8.4 Hz), 2.53 (m, 2H, H-3_{eq}'', H-3_{eq}'''), 1.84 (s, 6H), 1.73 (m, 2H), 1.61 (t, 1H, *J* = 12.4 Hz, H-3_{ax}''), 1.54 (t, 1H, *J* = 12.8 Hz, H-3_{ax}'''); ^{13}C NMR (100 MHz, D_2O) δ 175.09, 175.00, 173.99, 173.61, 103.06, 102.13, 100.39, 100.06, 79.72, 75.36, 74.71, 73.60, 72.97, 72.84, 72.62, 71.84, 69.35, 68.51, 68.20, 67.71, 67.45, 63.65, 62.75, 62.68, 60.37, 51.90, 51.80, 48.00, 40.18, 39.60, 28.36, 22.21. HRMS (ESI) *m/z* calcd for $C_{37}H_{59}N_5NaO_{27}$ (M-Na) 1028.3295, found 1028.3284.

References

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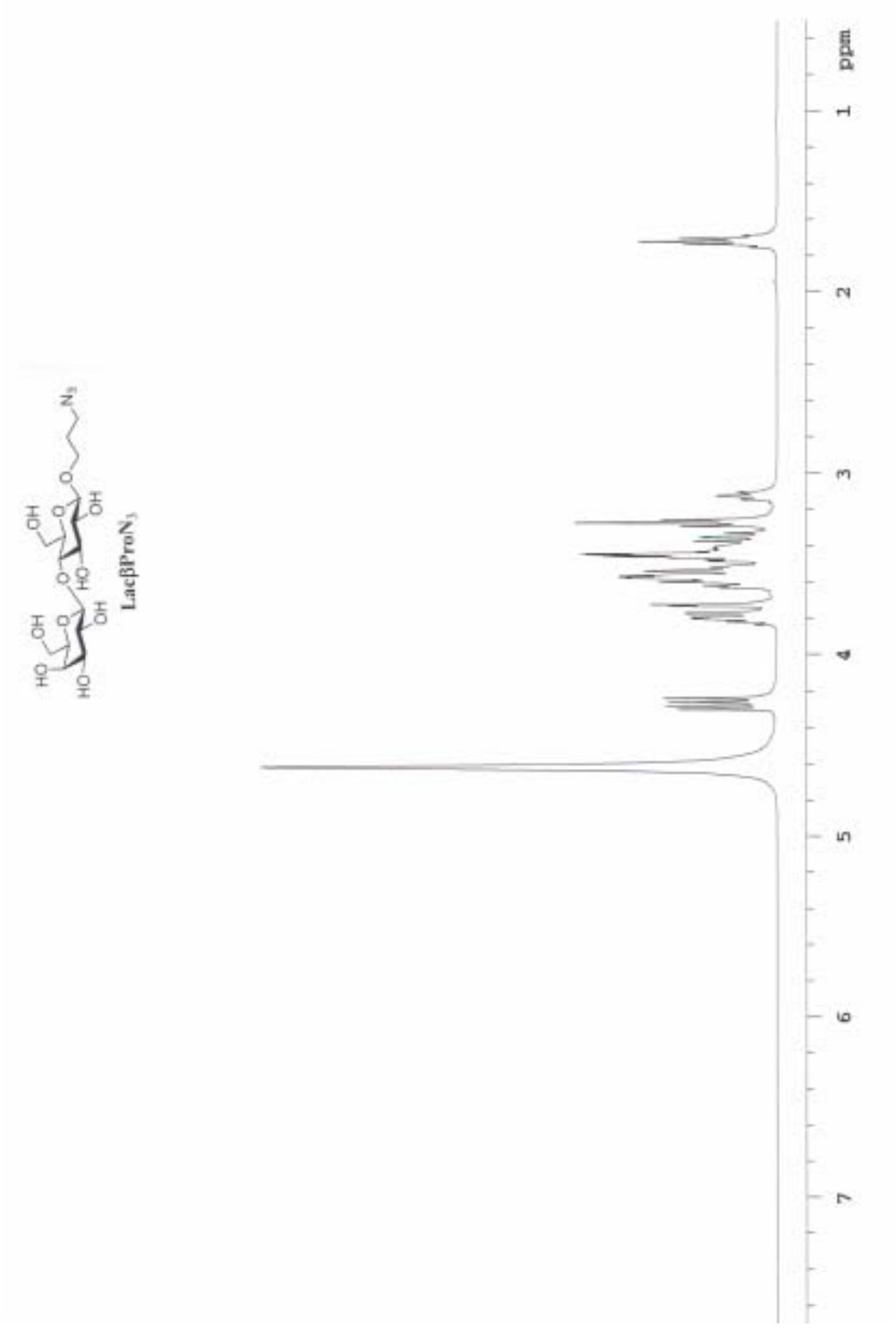
Compound **20**



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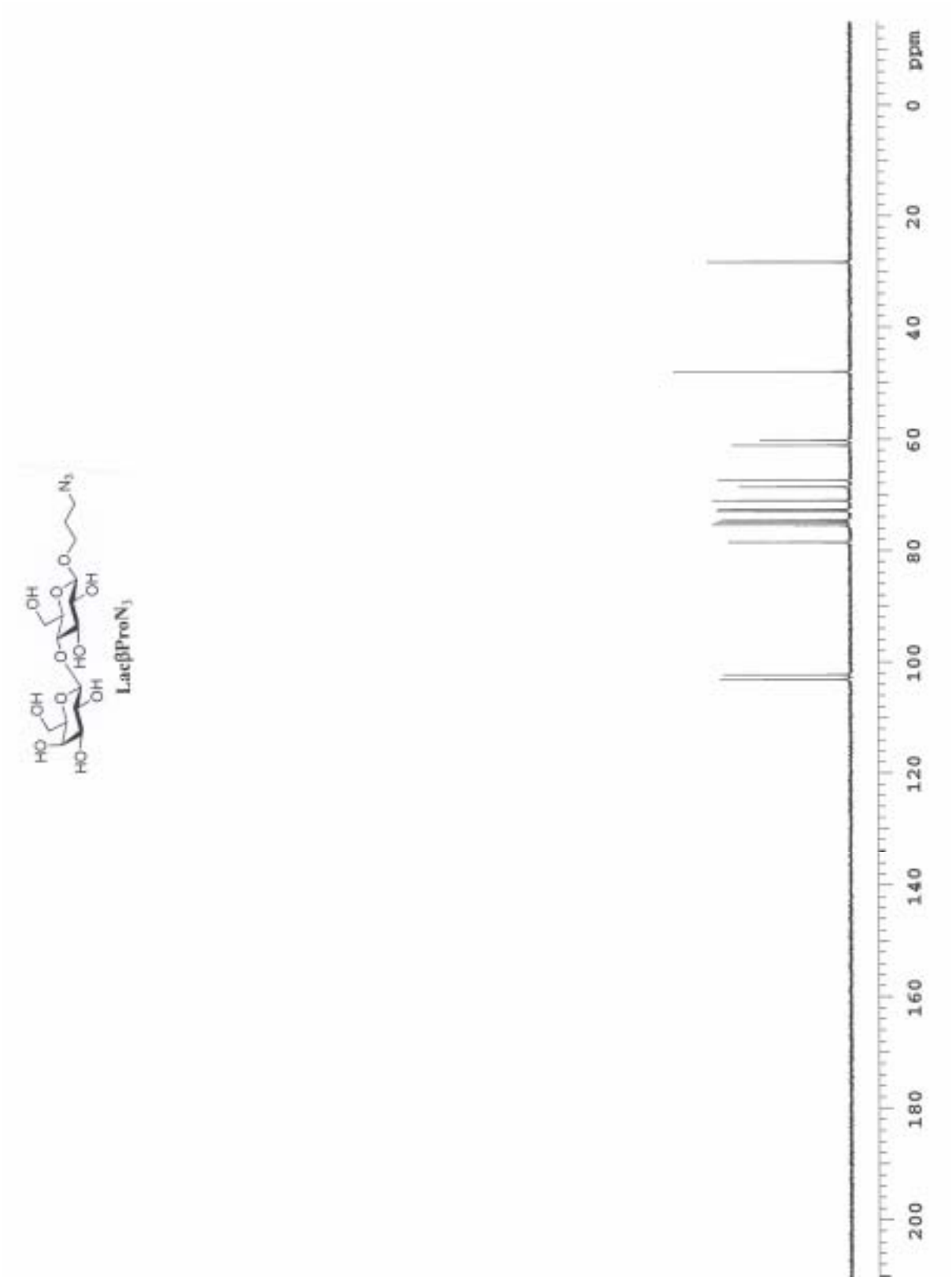

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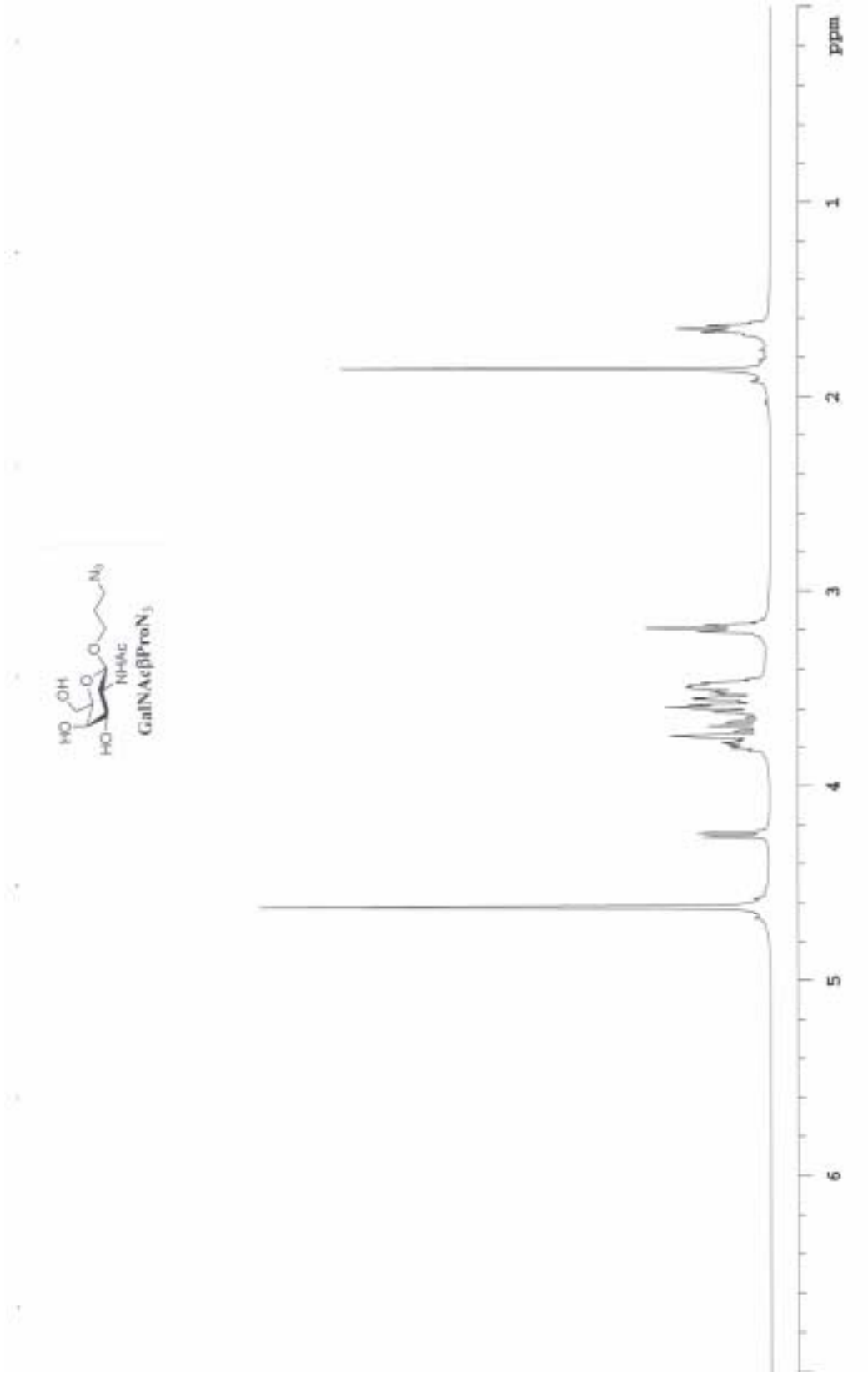

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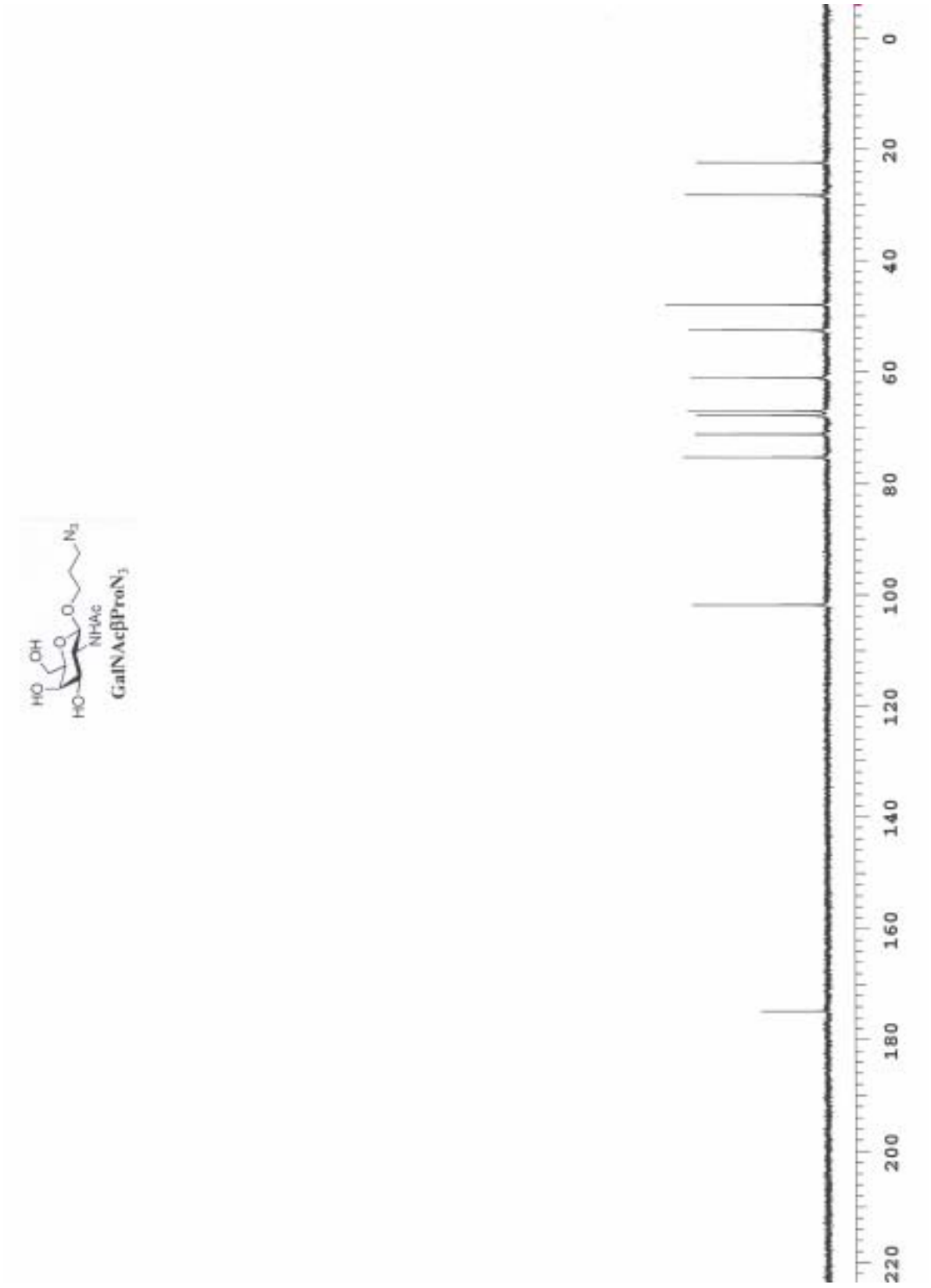

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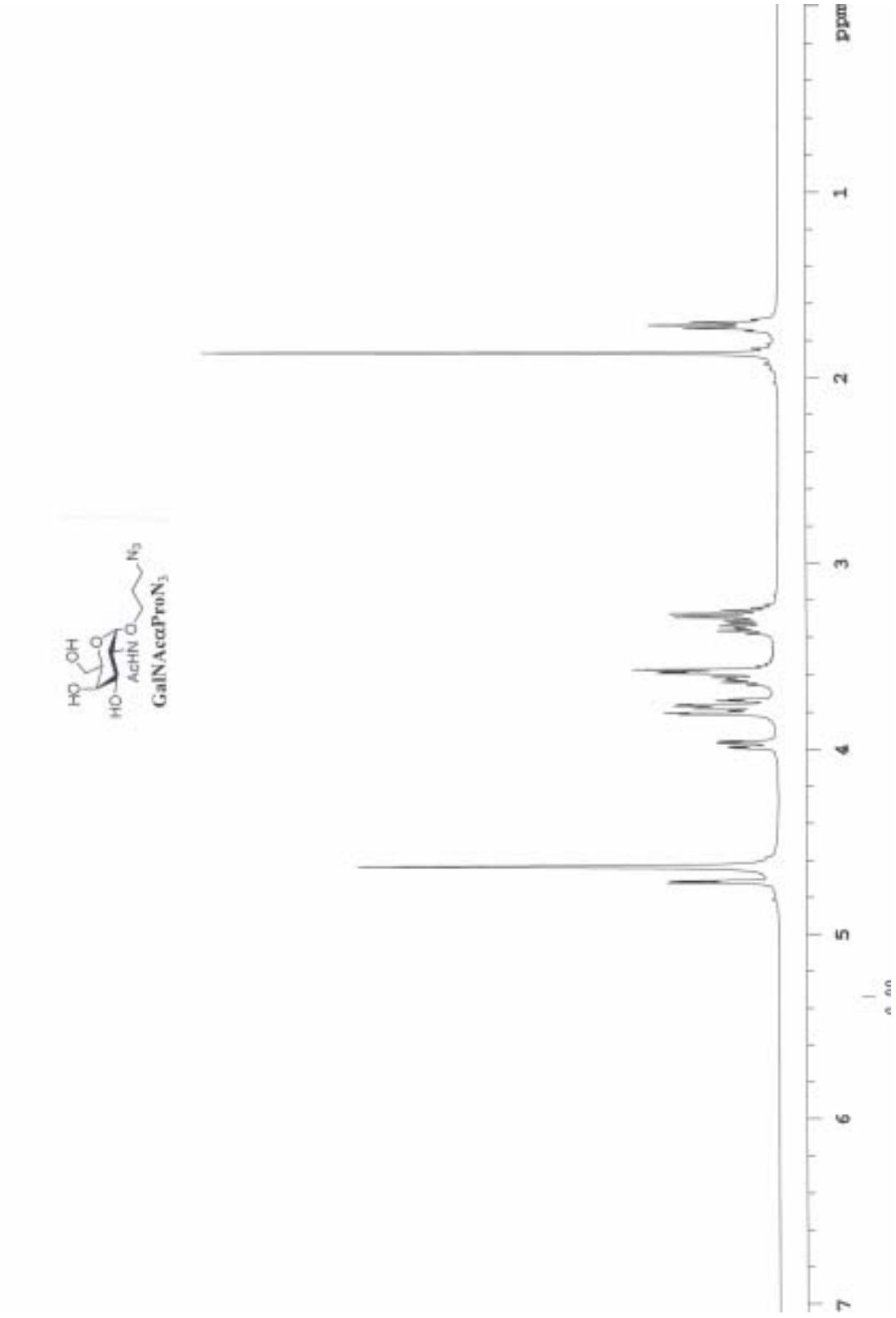

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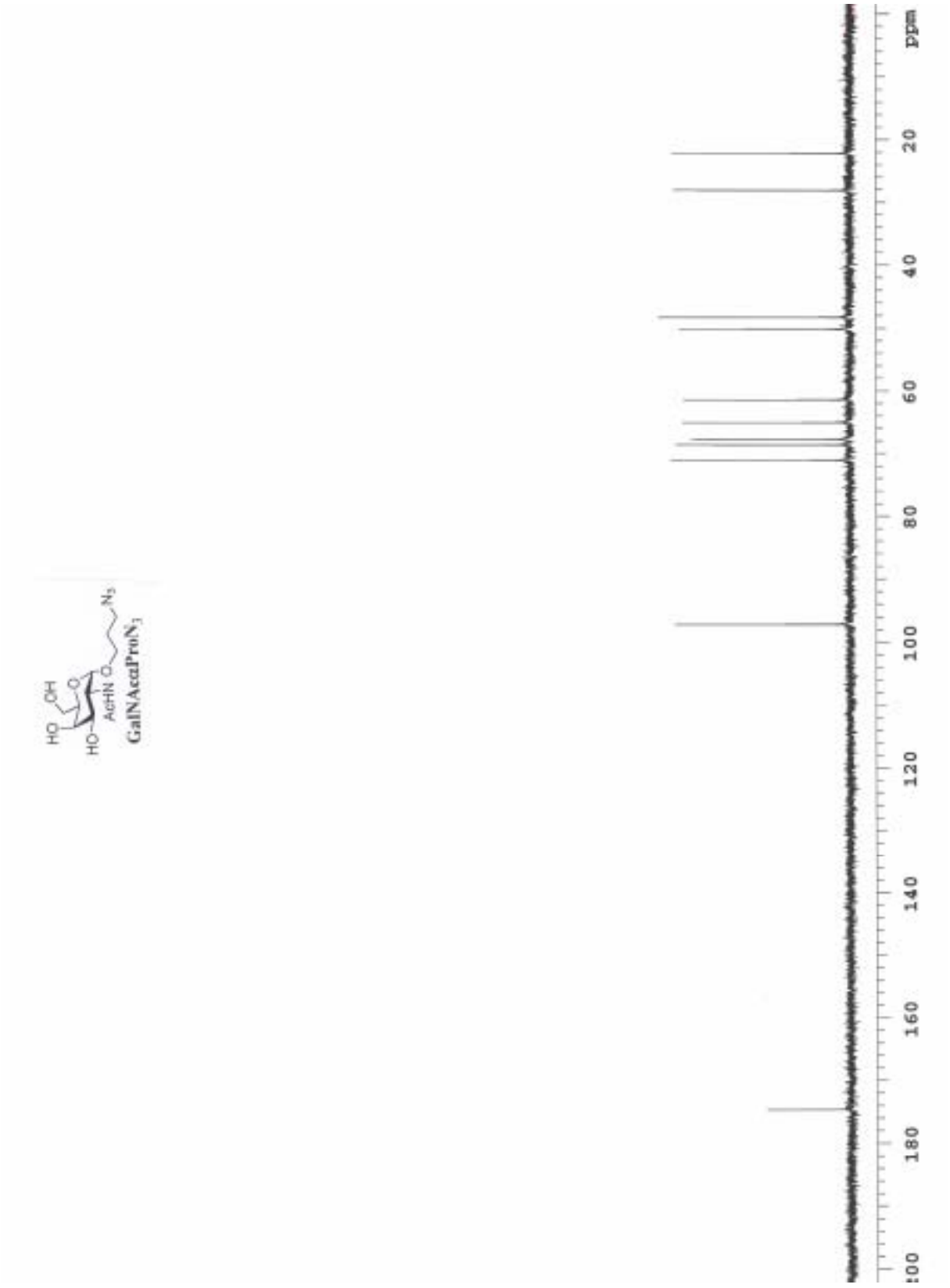
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80
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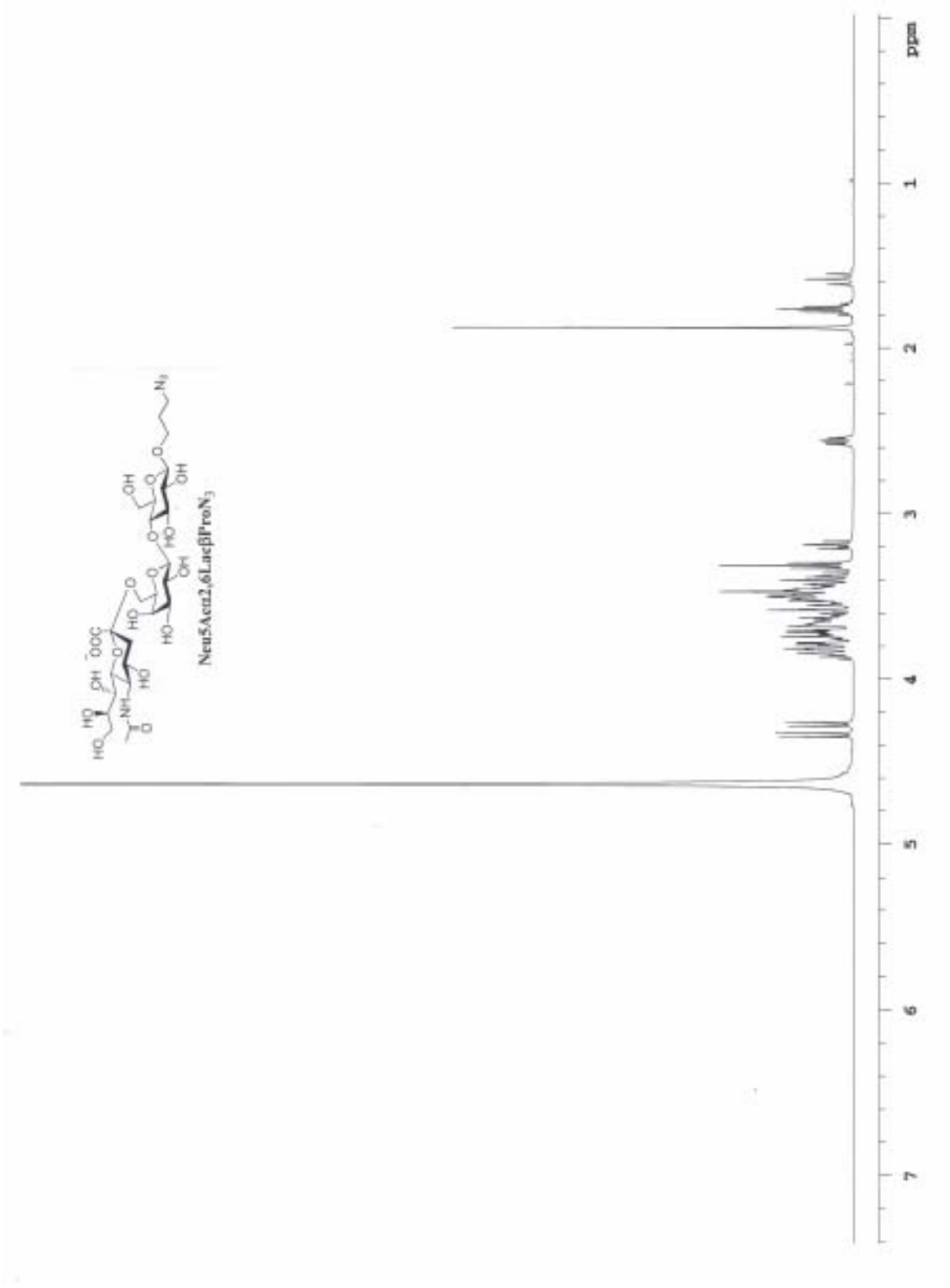
Compound **26**



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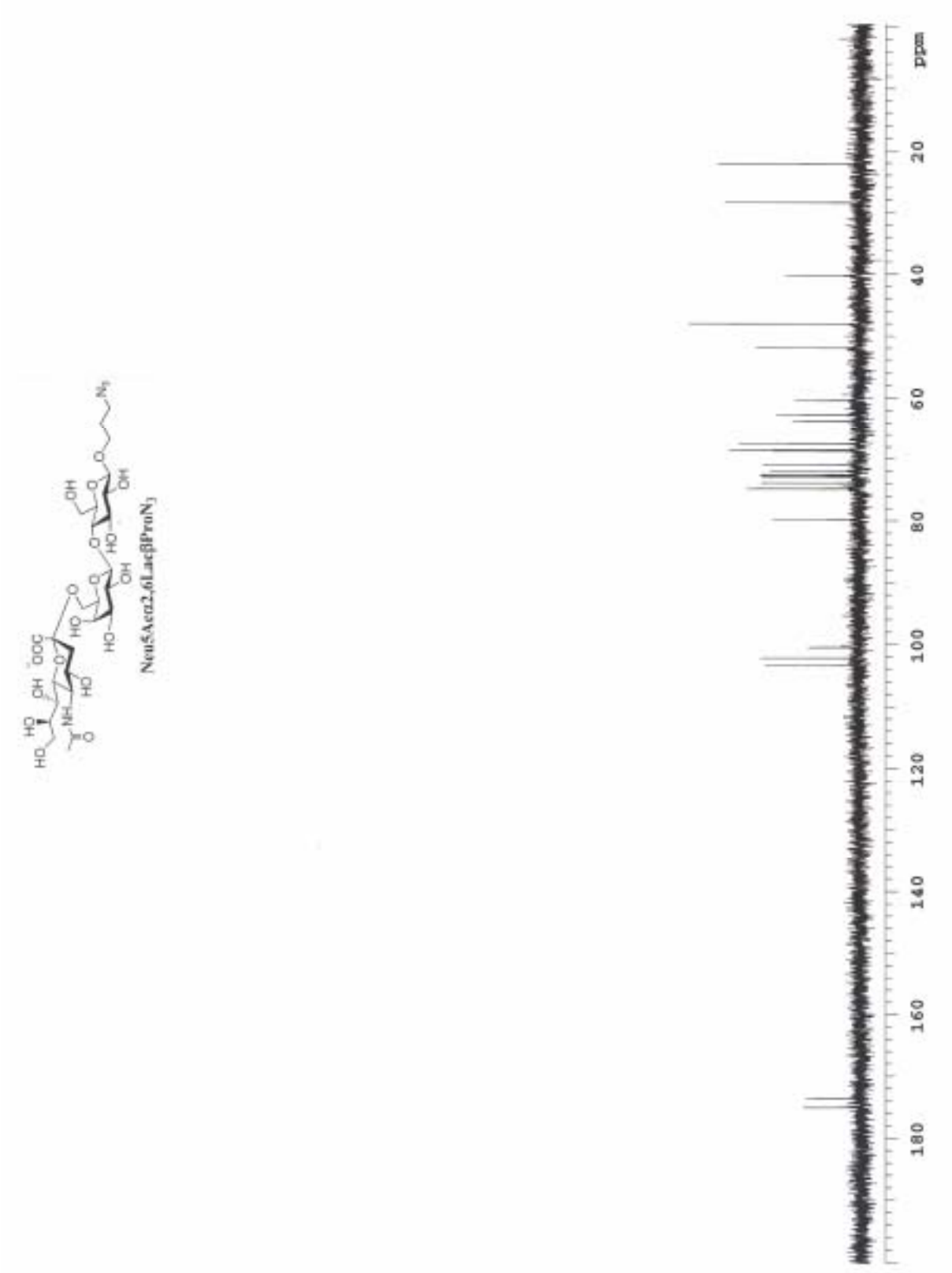

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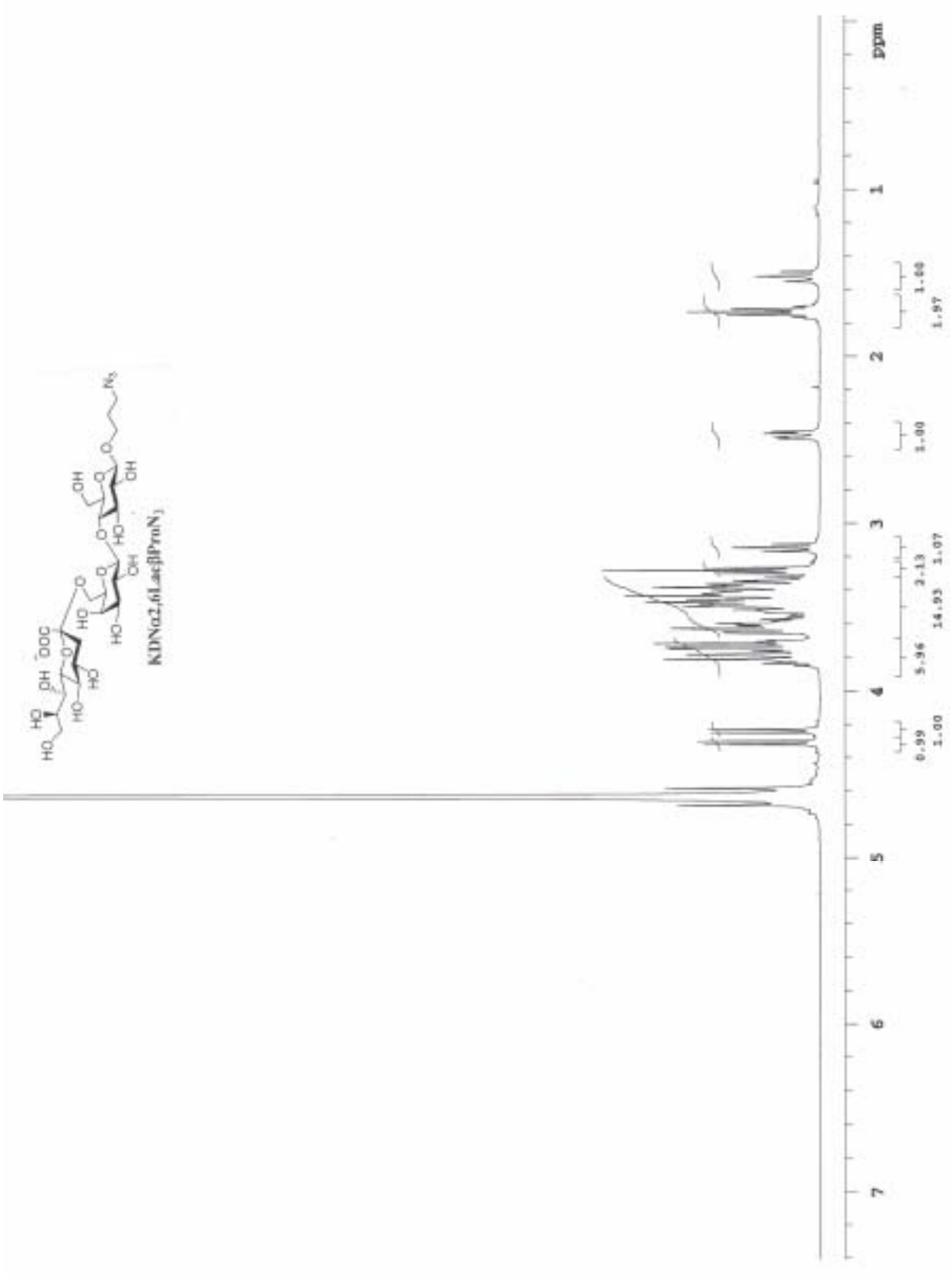
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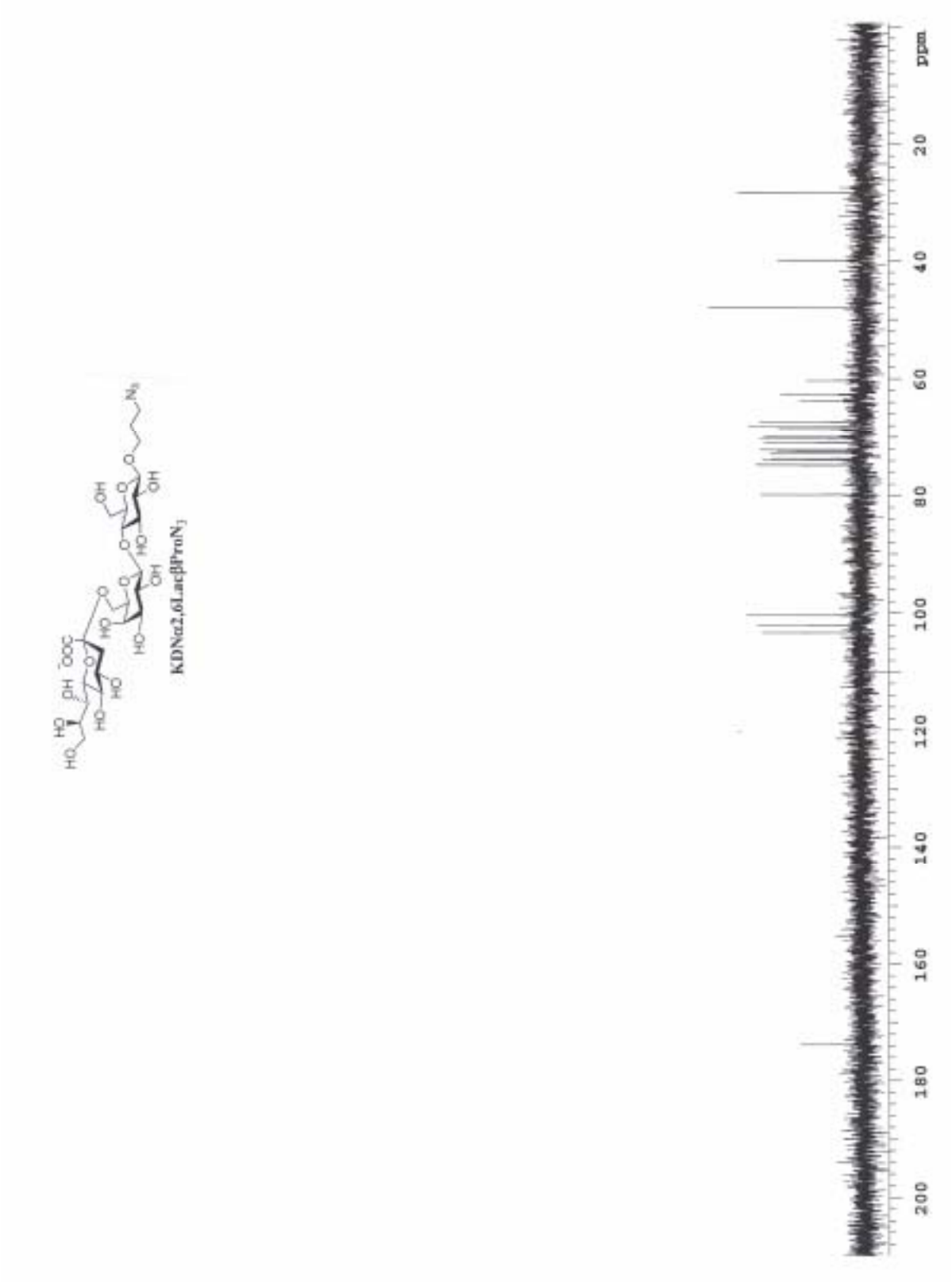
0.99
1.00

Compound **27**



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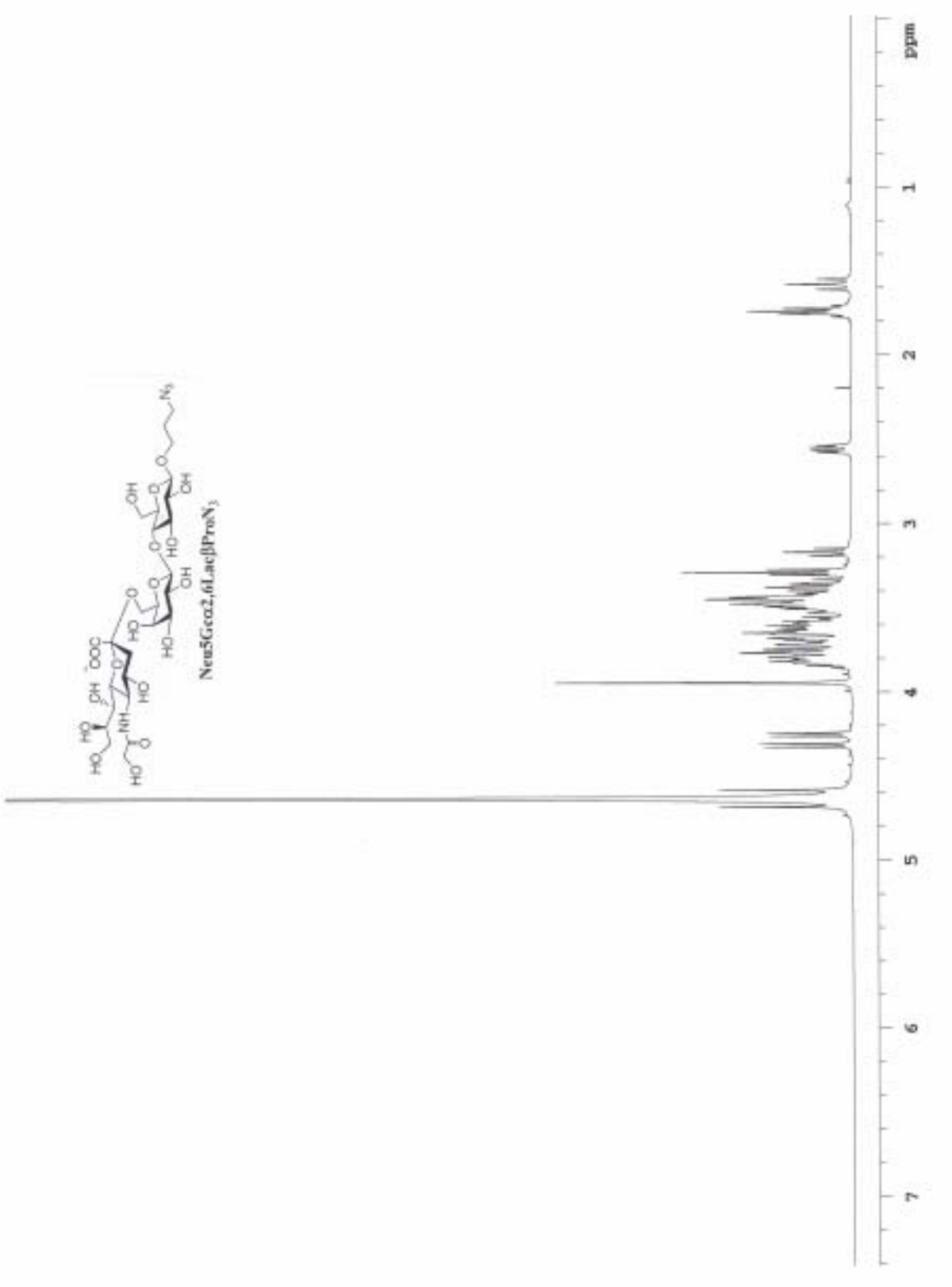

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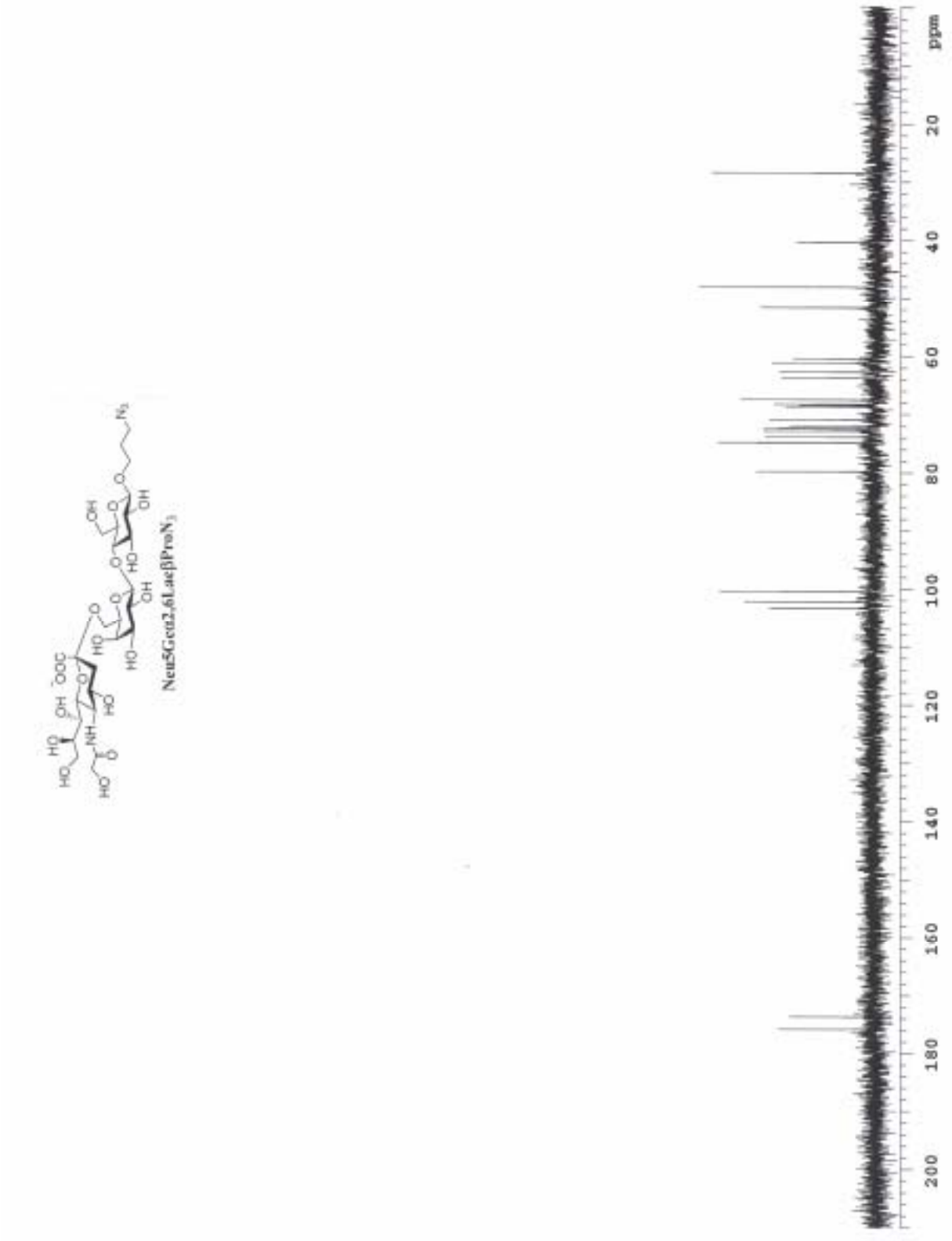

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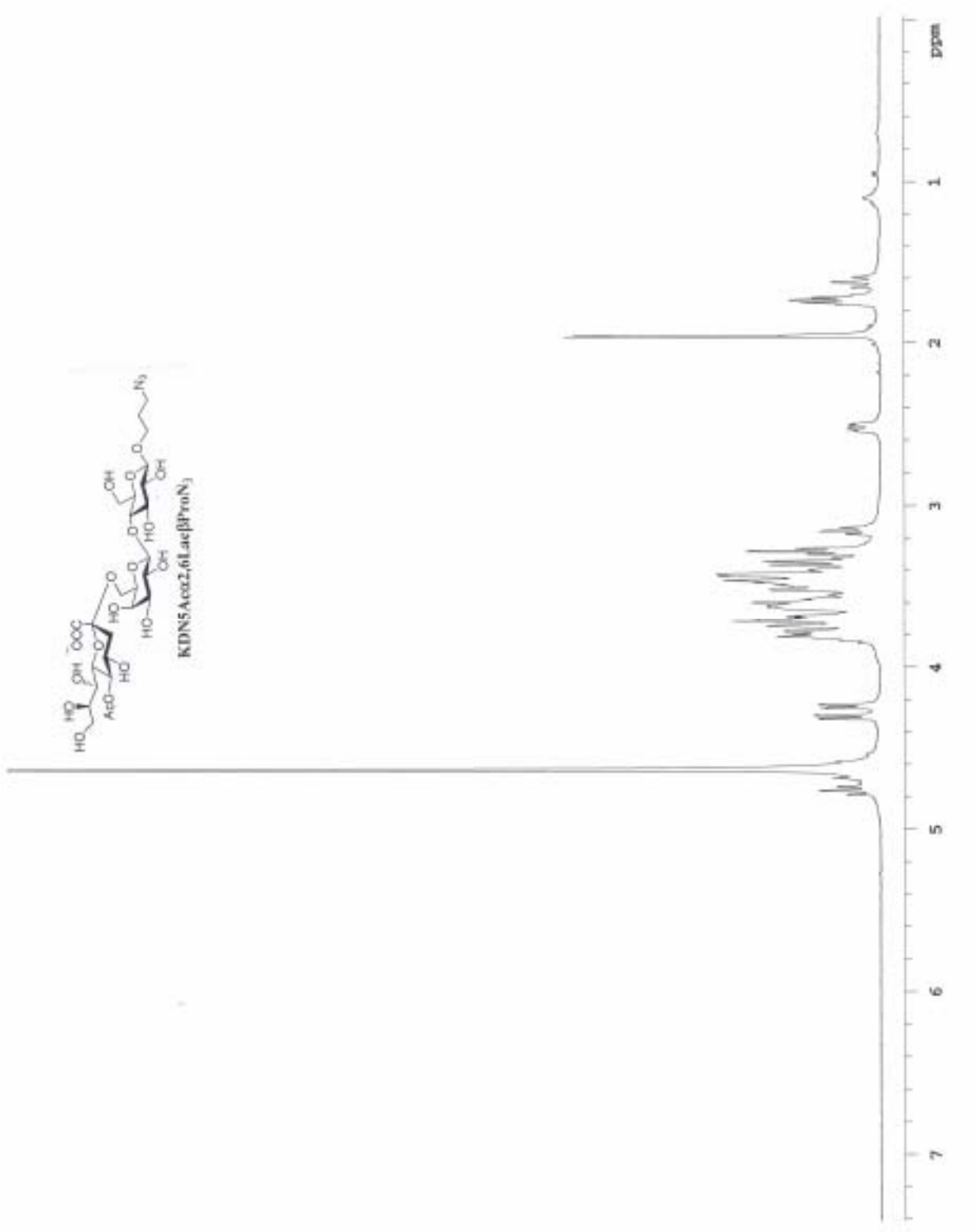

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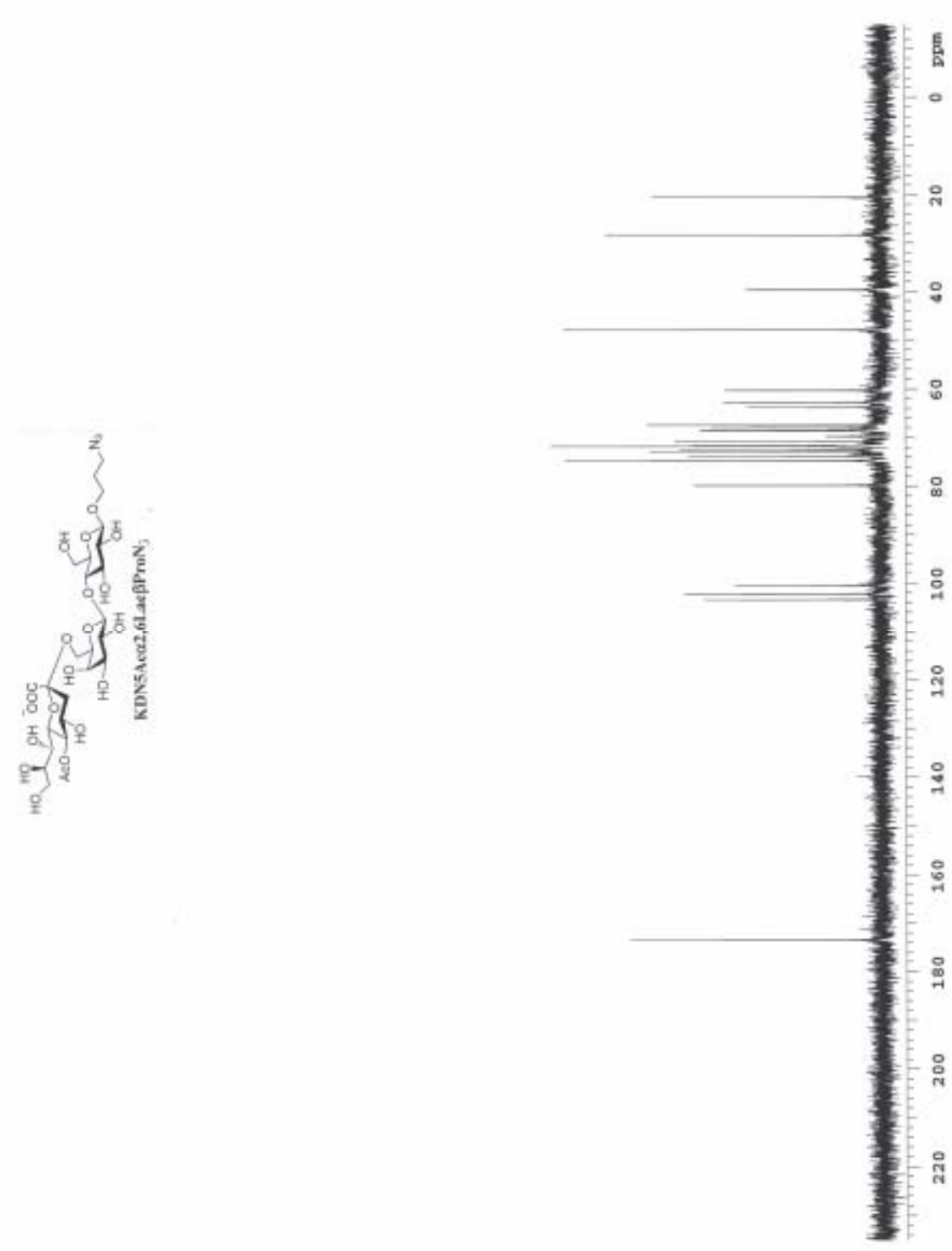
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200
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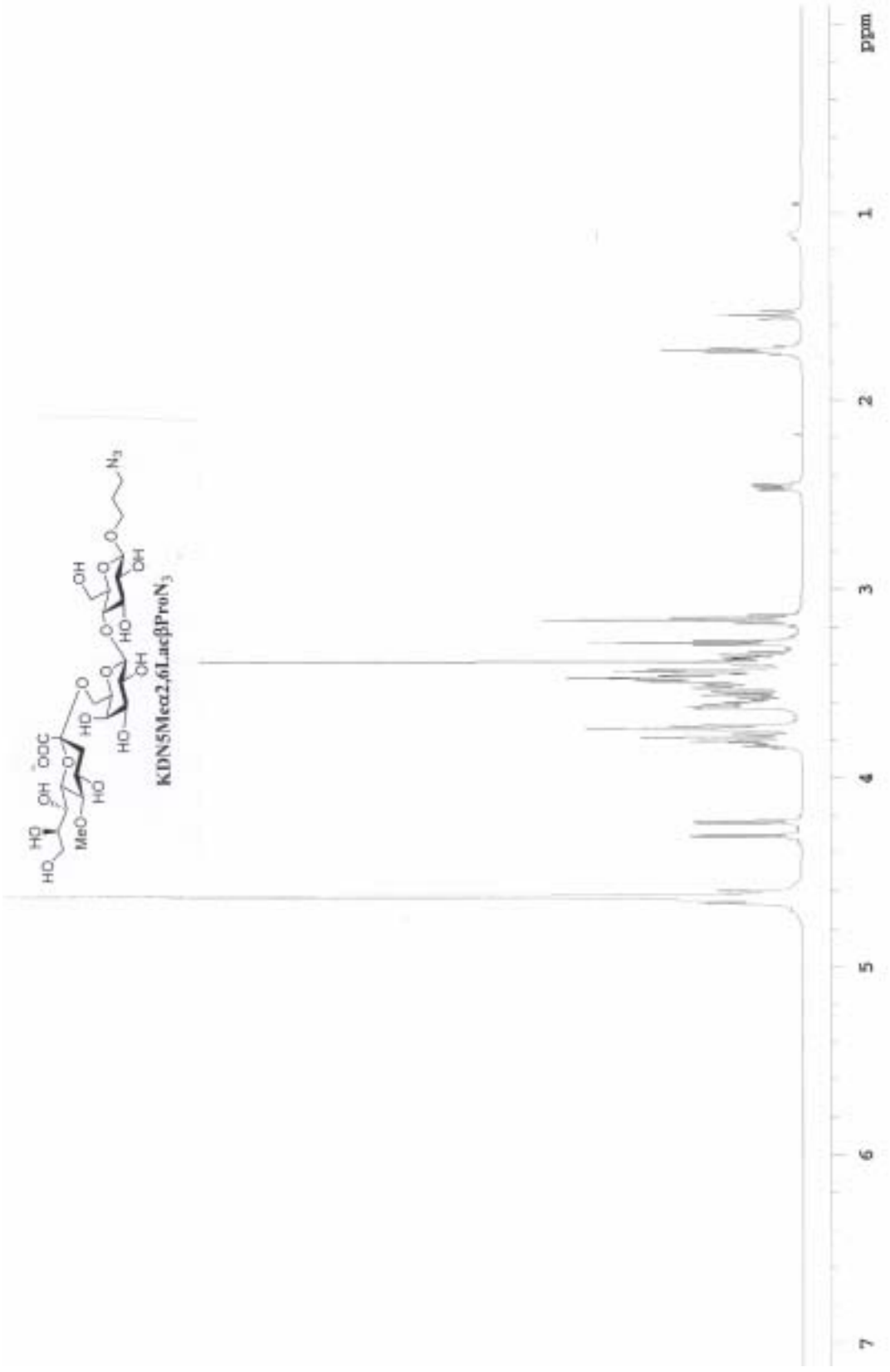
Compound **30**



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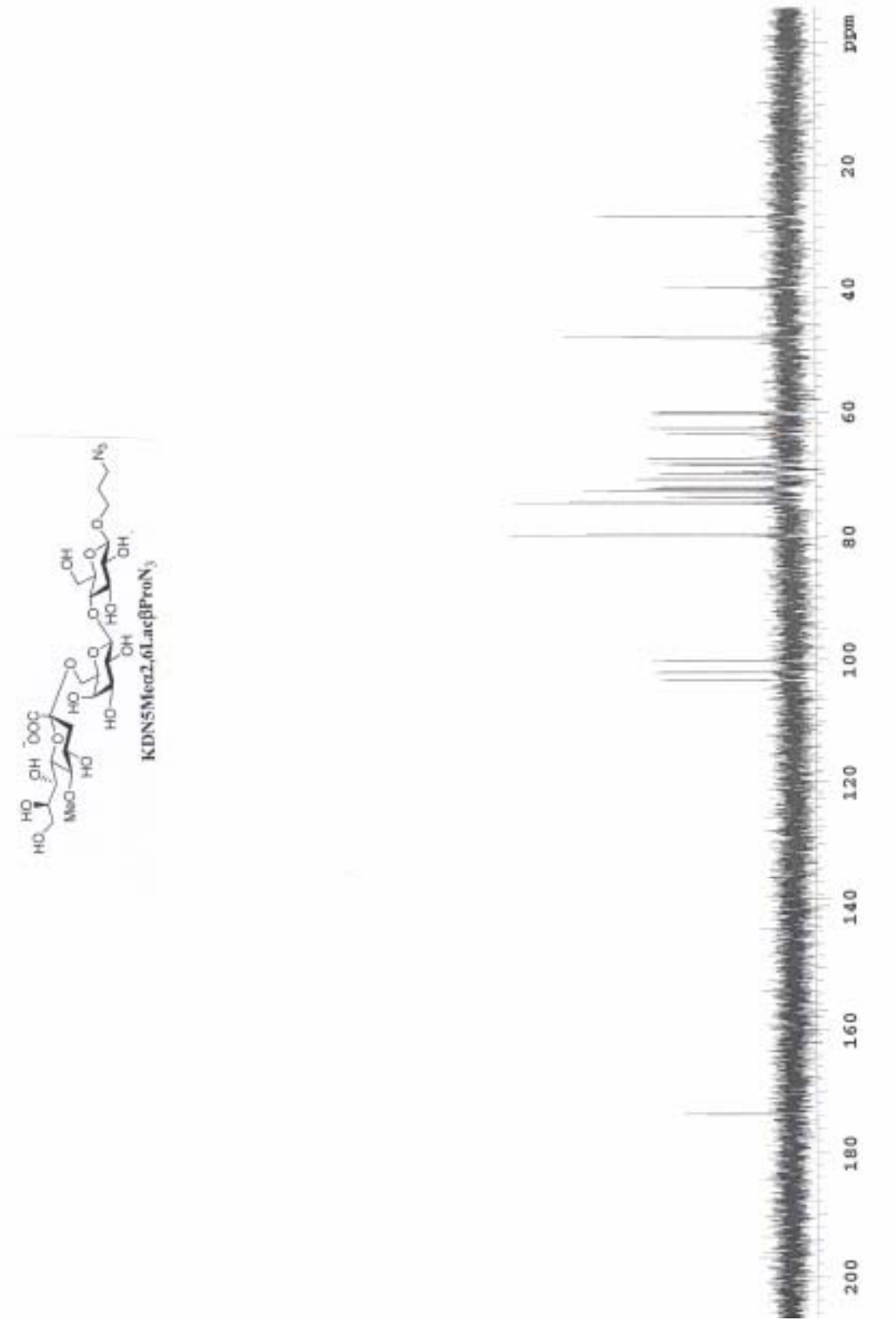

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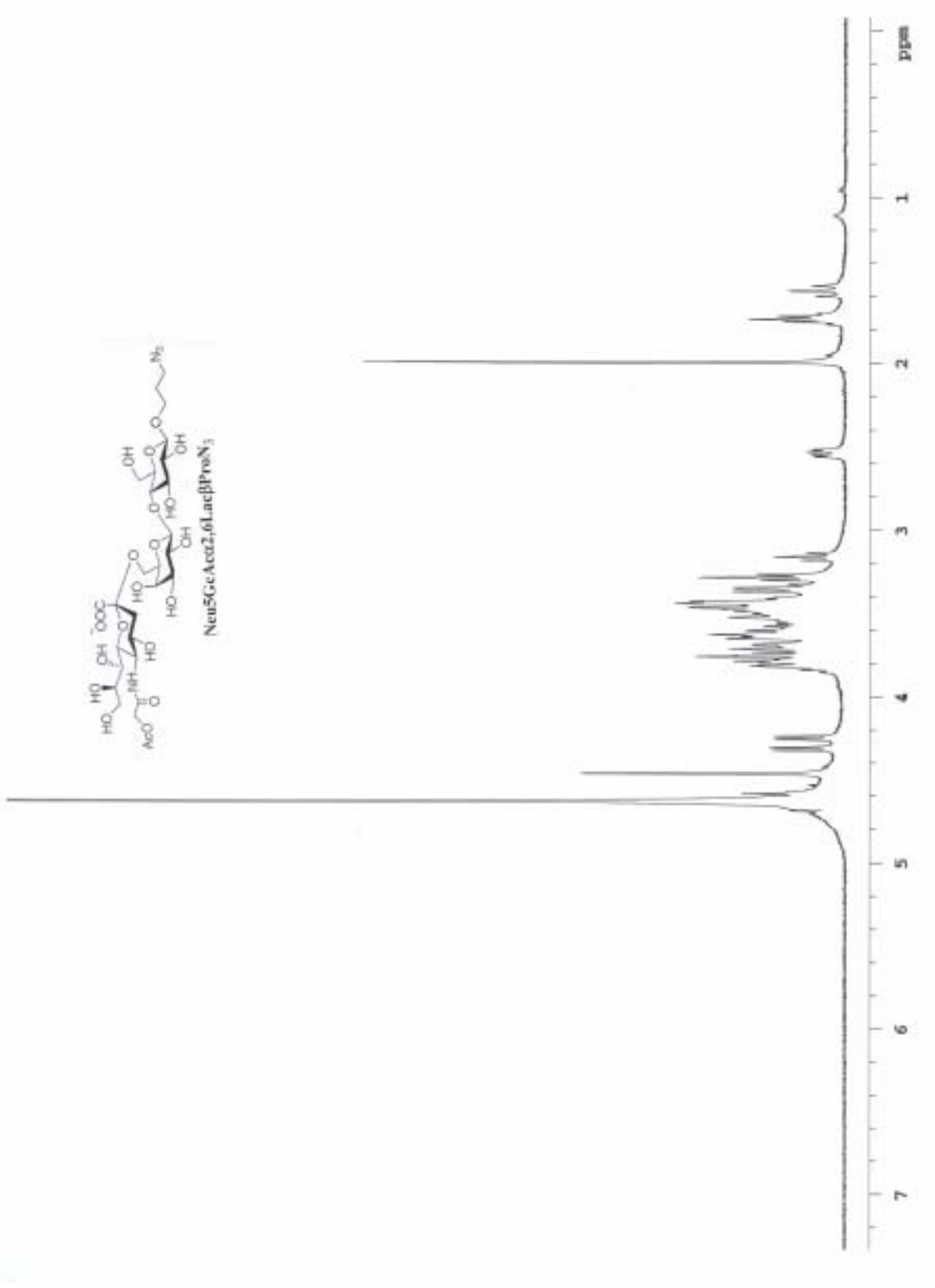
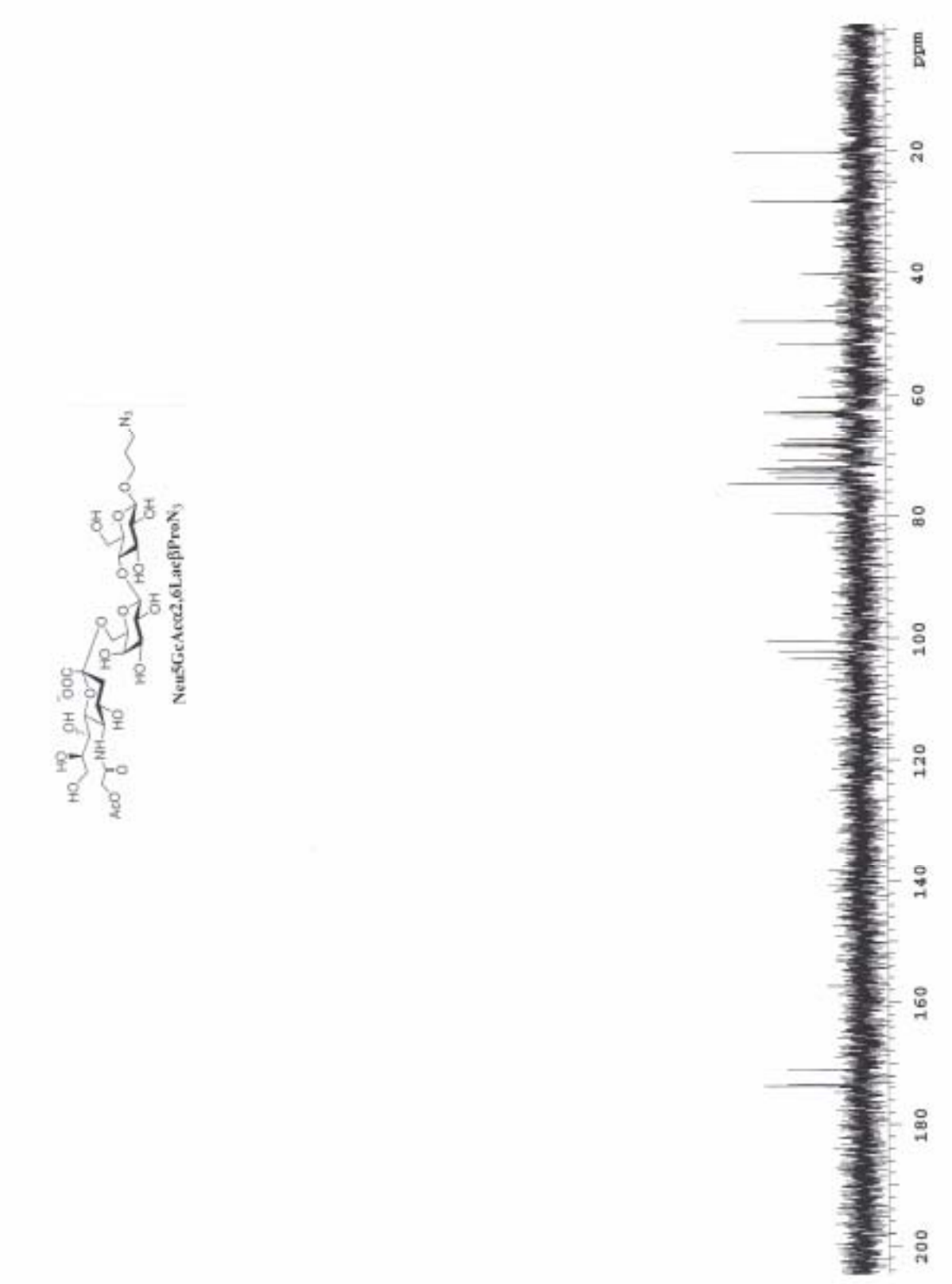
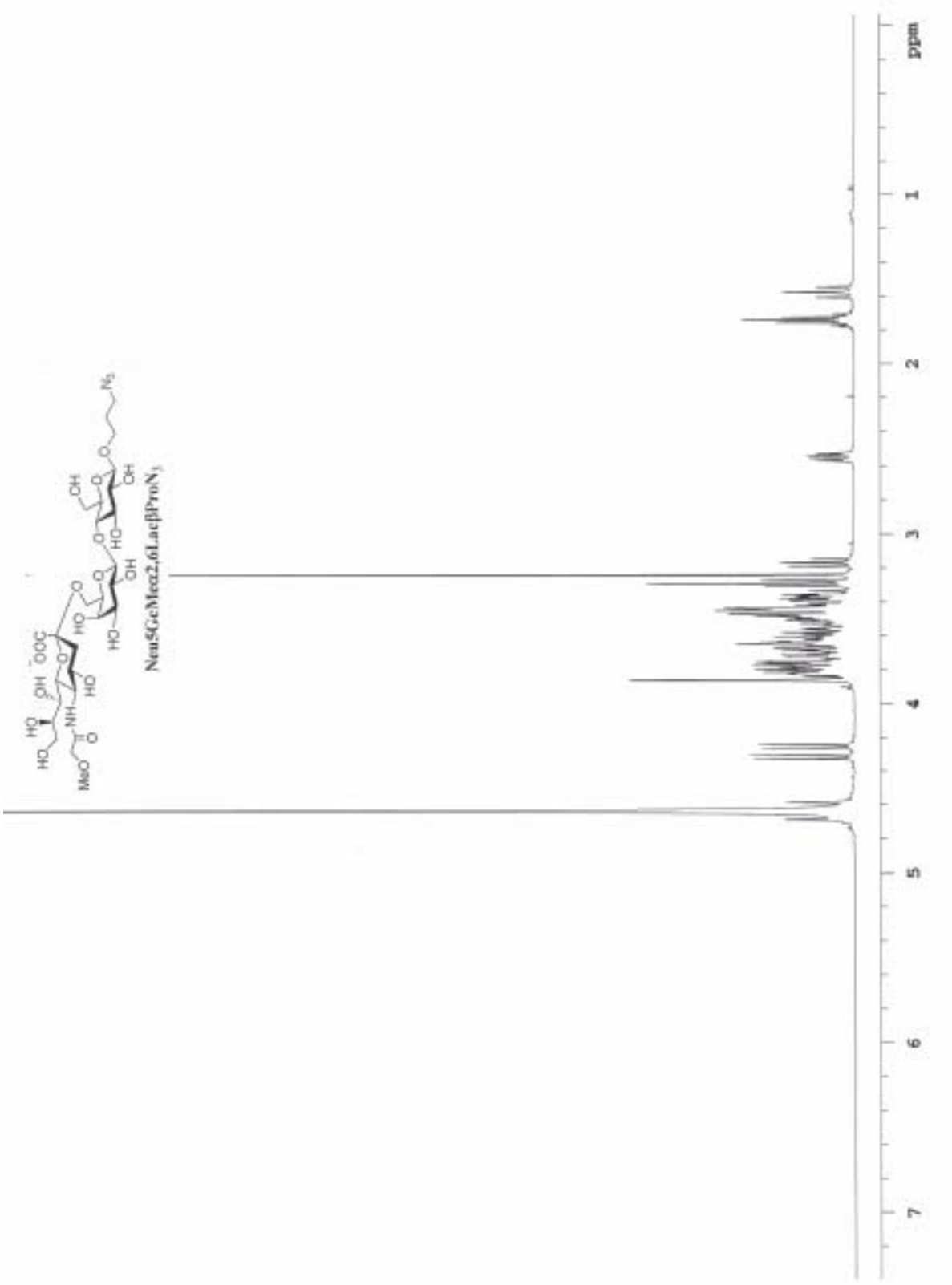


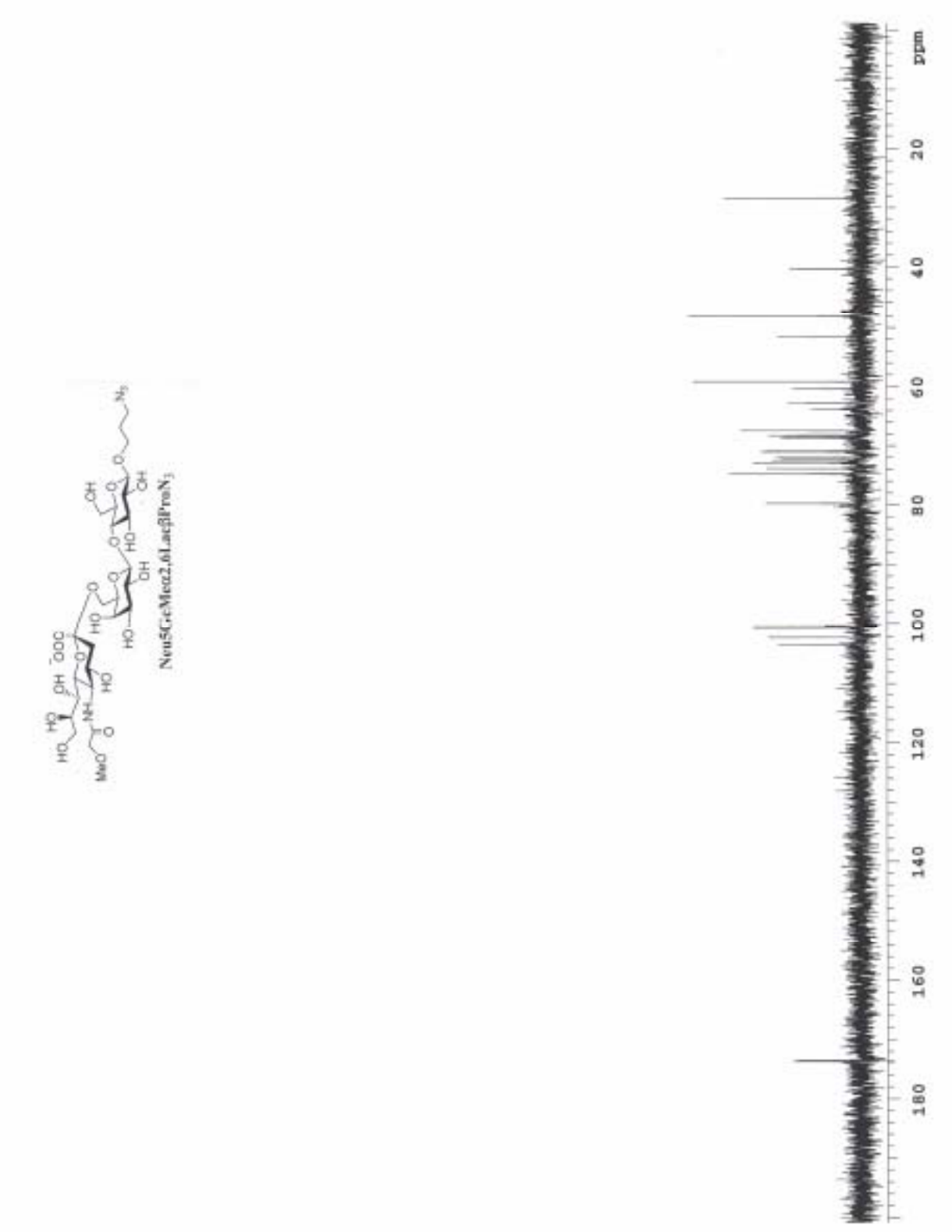
Compound **31**



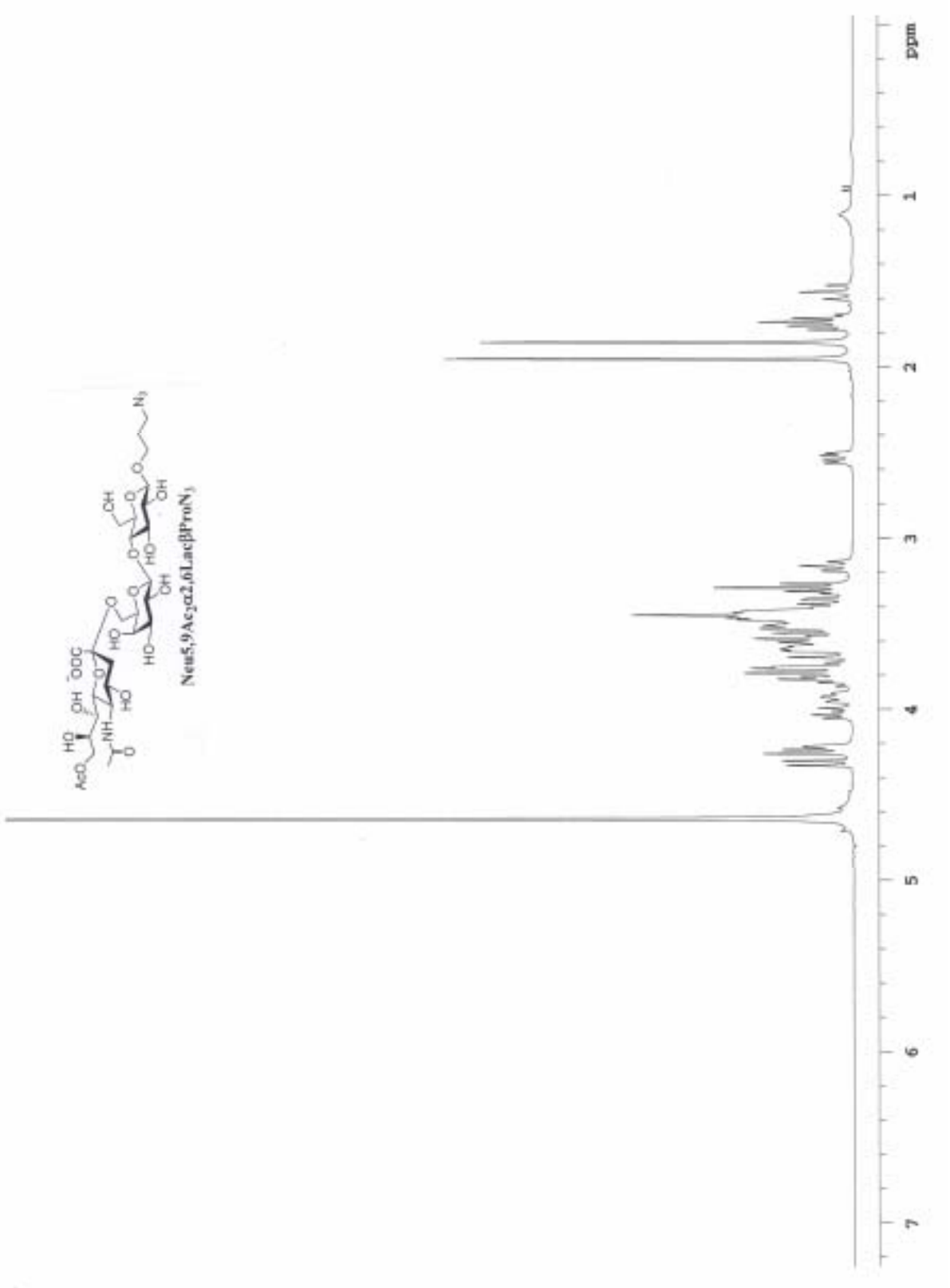
Compound **32**



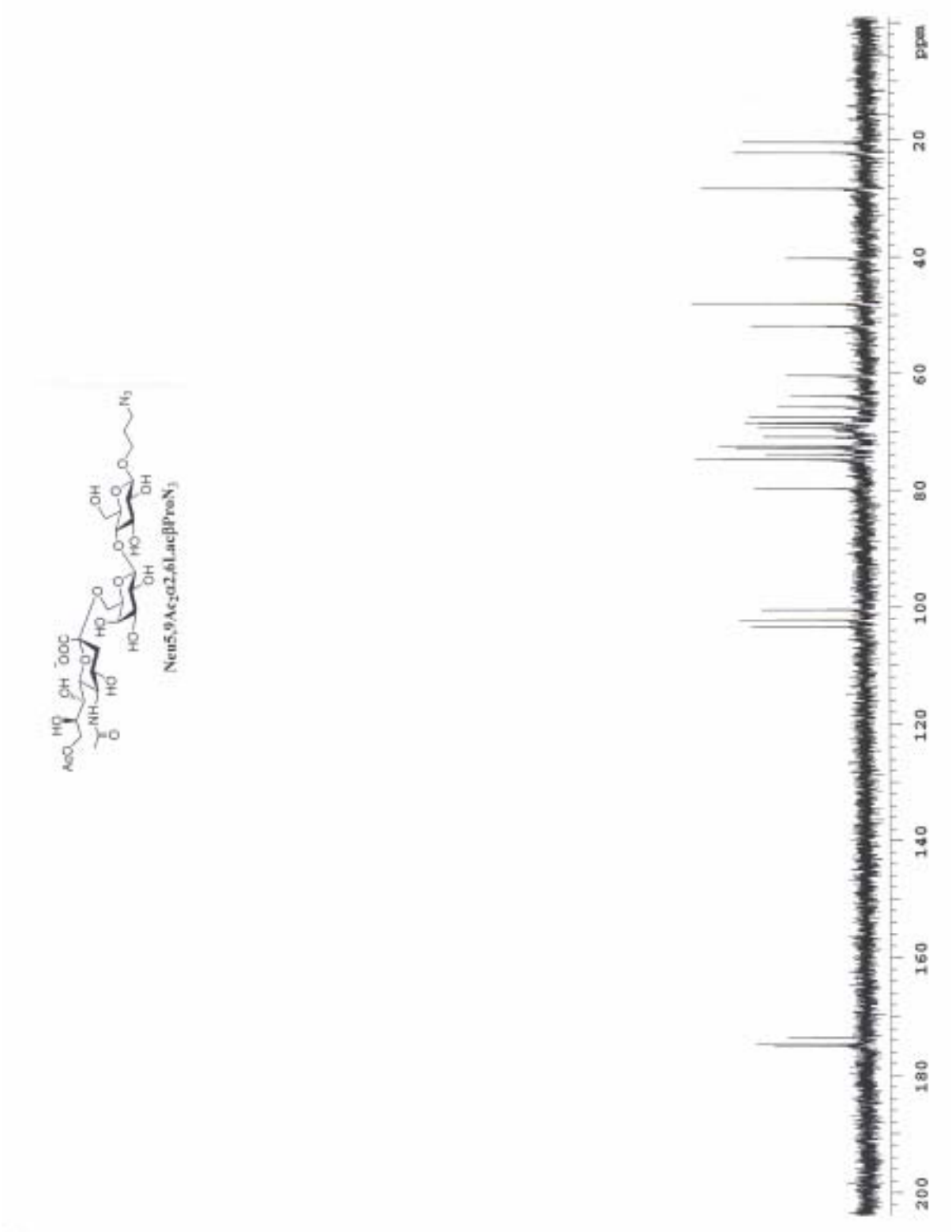
Compound **32**


Neu5Gcα2,6LacβPreN$_3$
ppm
20
40
60
80
100
120
140
160
180

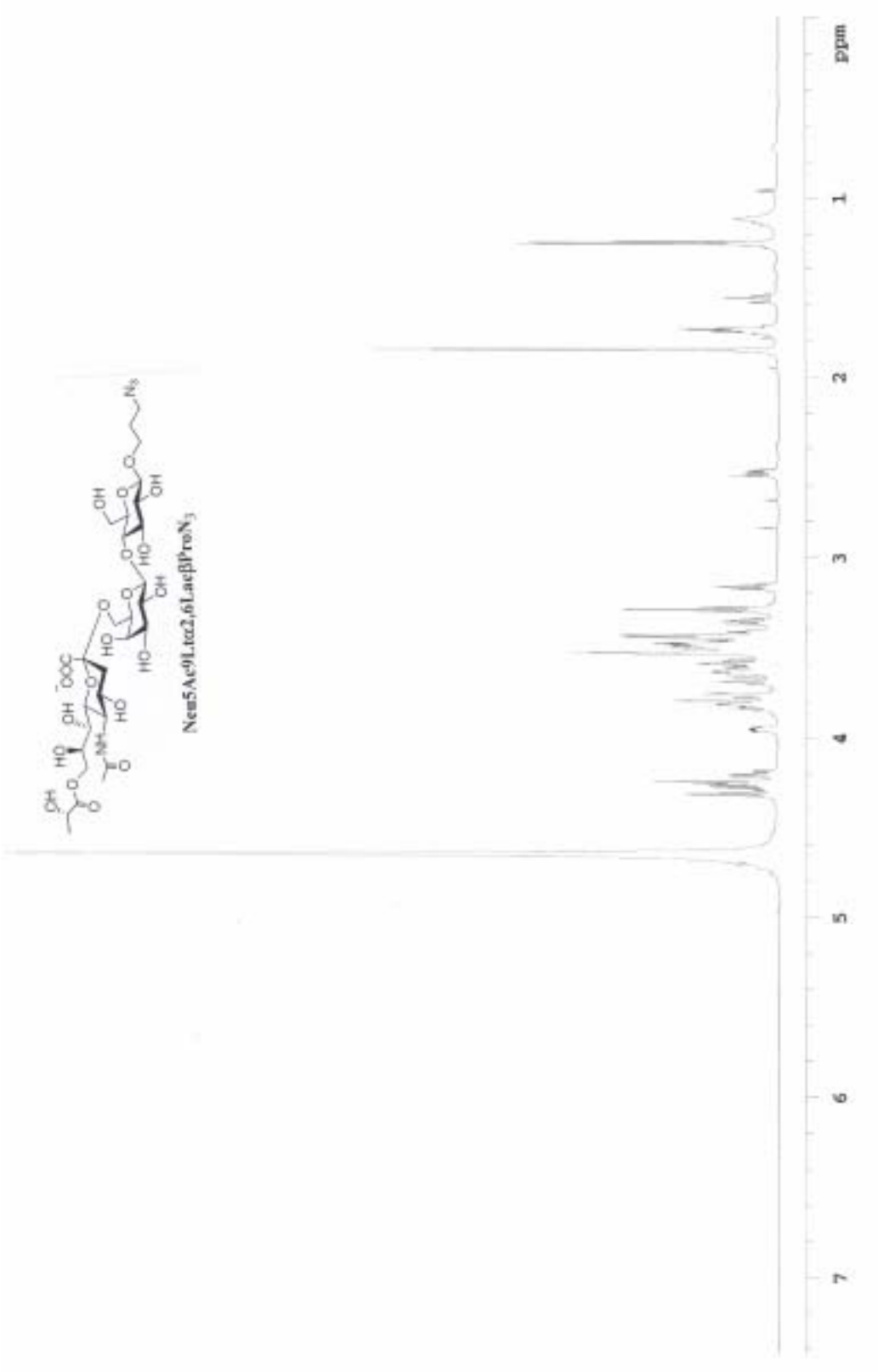
Compound **33**



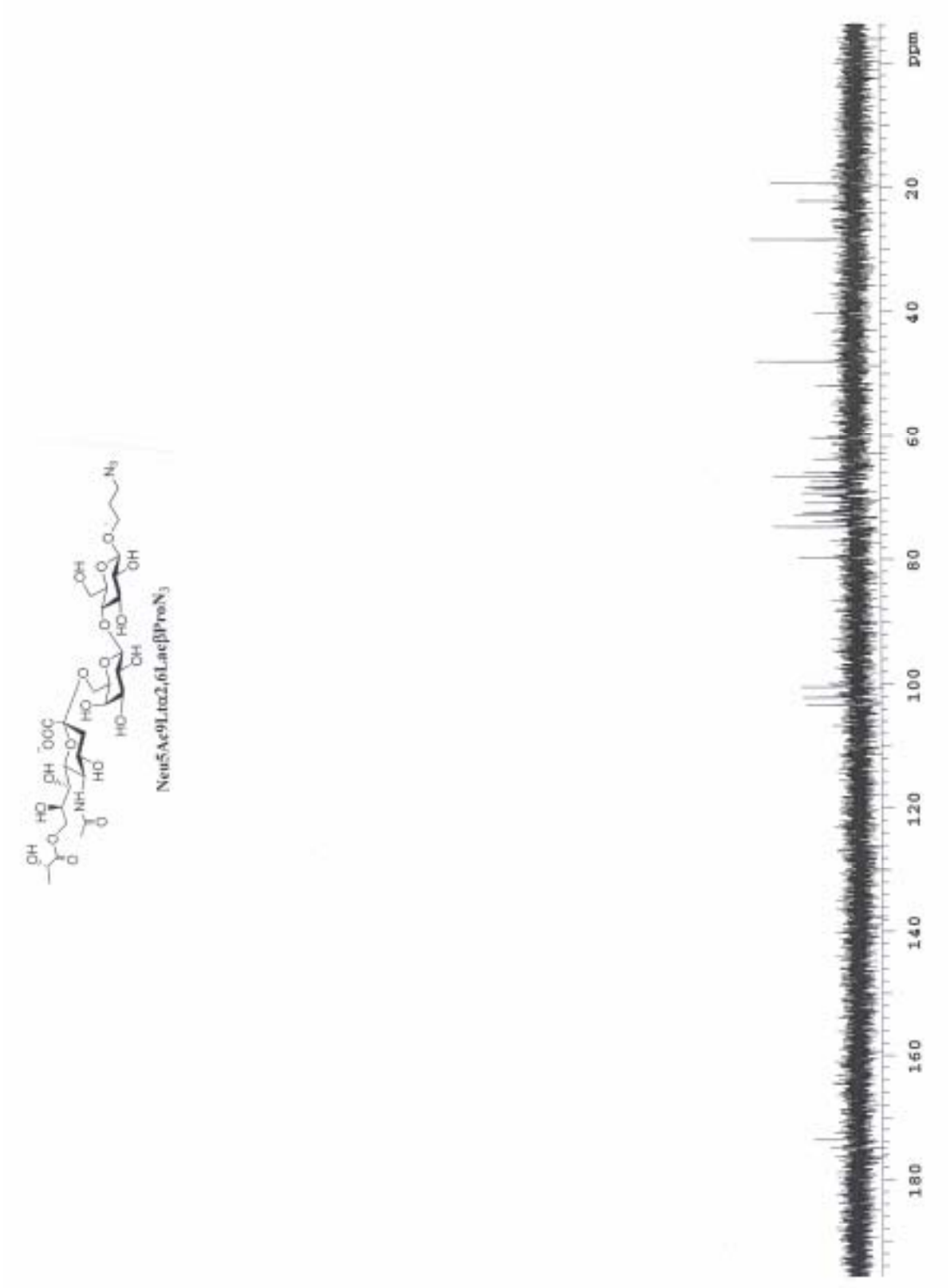
Compound **33**



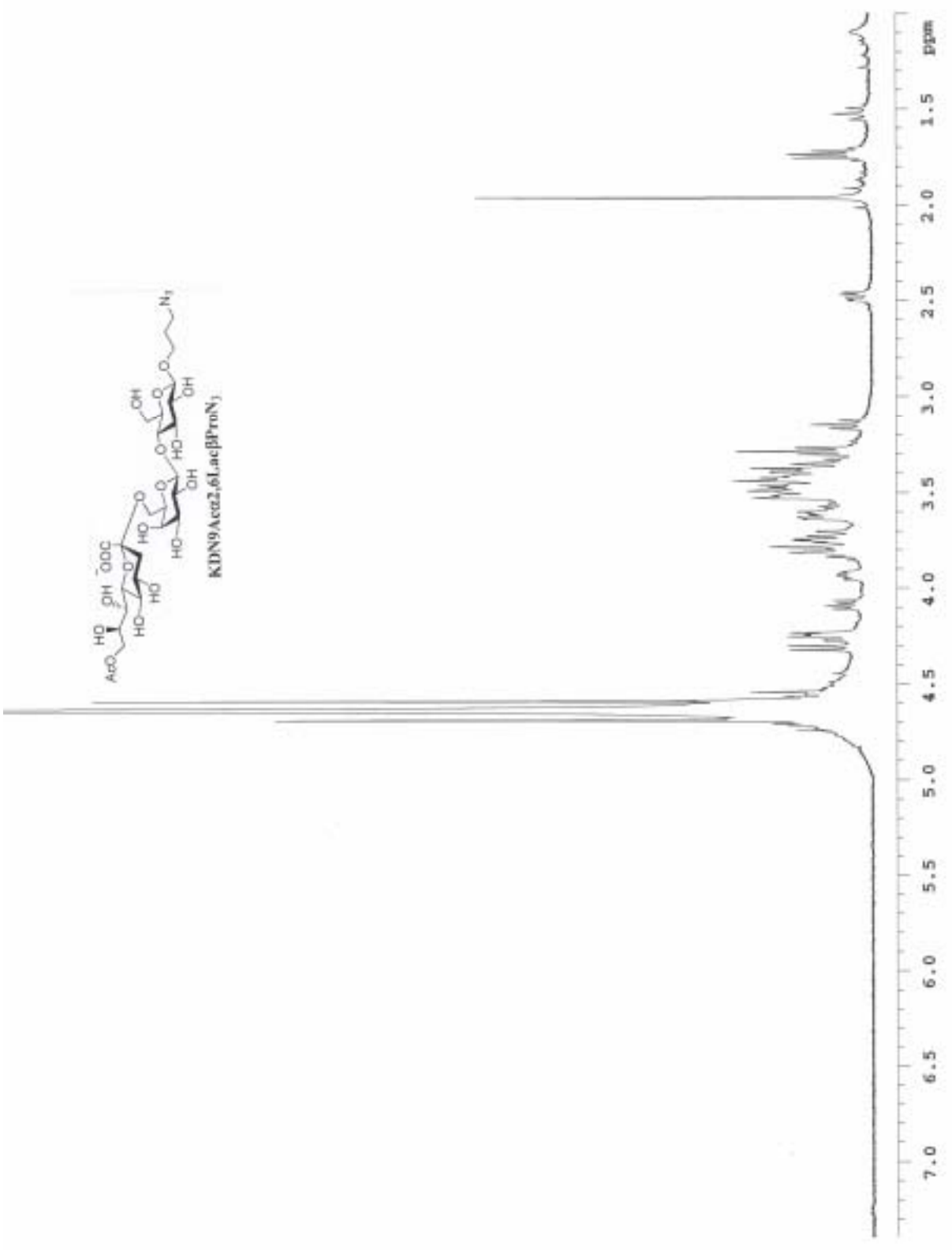
Compound **34**



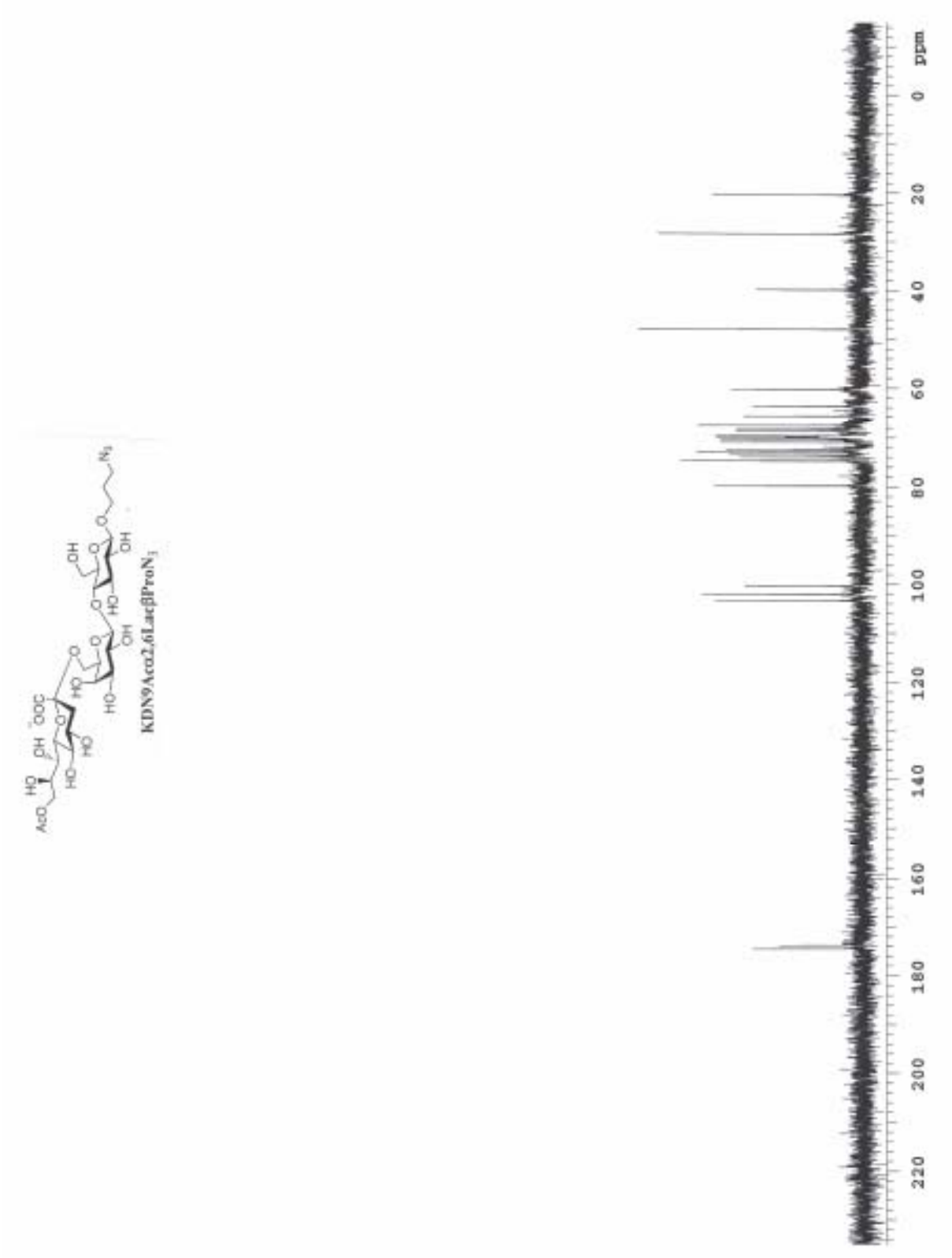
Compound **34**



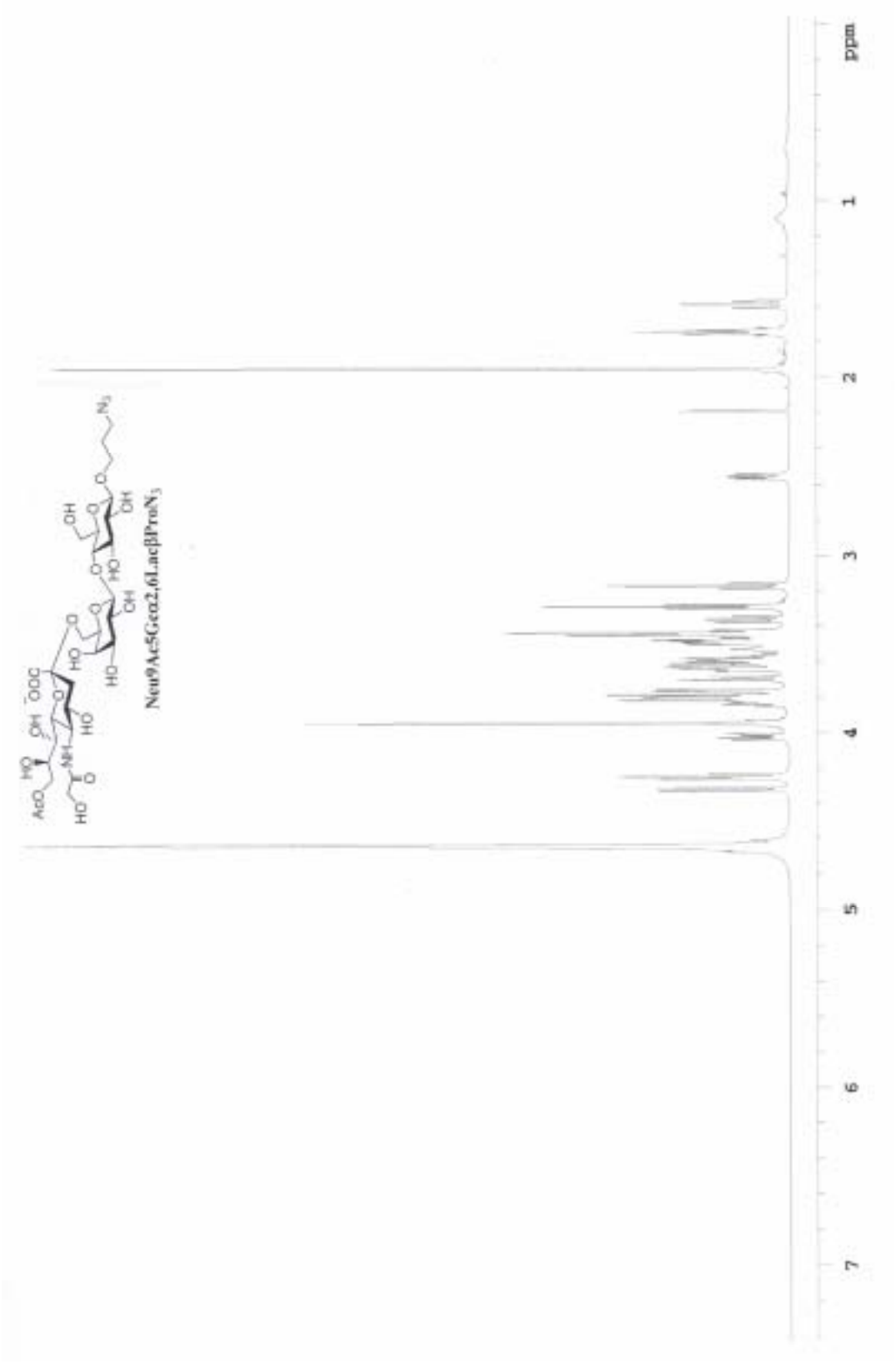
Compound **35**



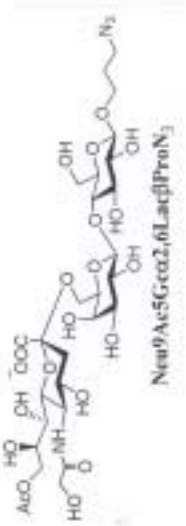
Compound **35**

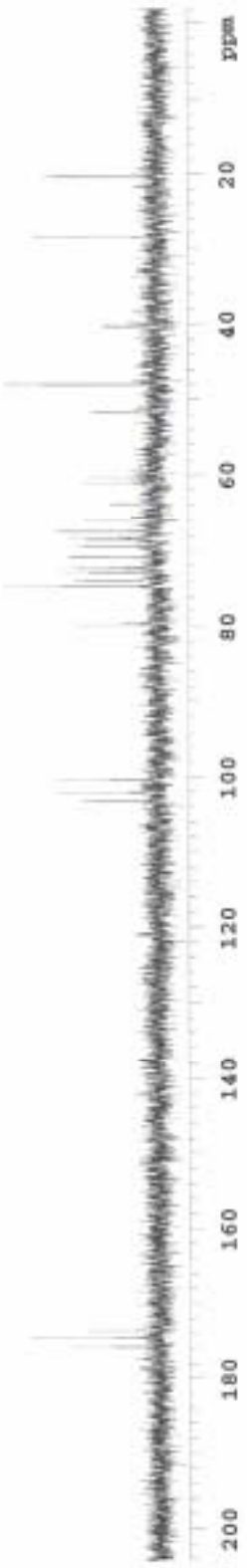

KDN9Acα2,6Lacβ1ProN3
ppm
0
20
40
60
80
100
120
140
160
180
200
220

Compound **36**

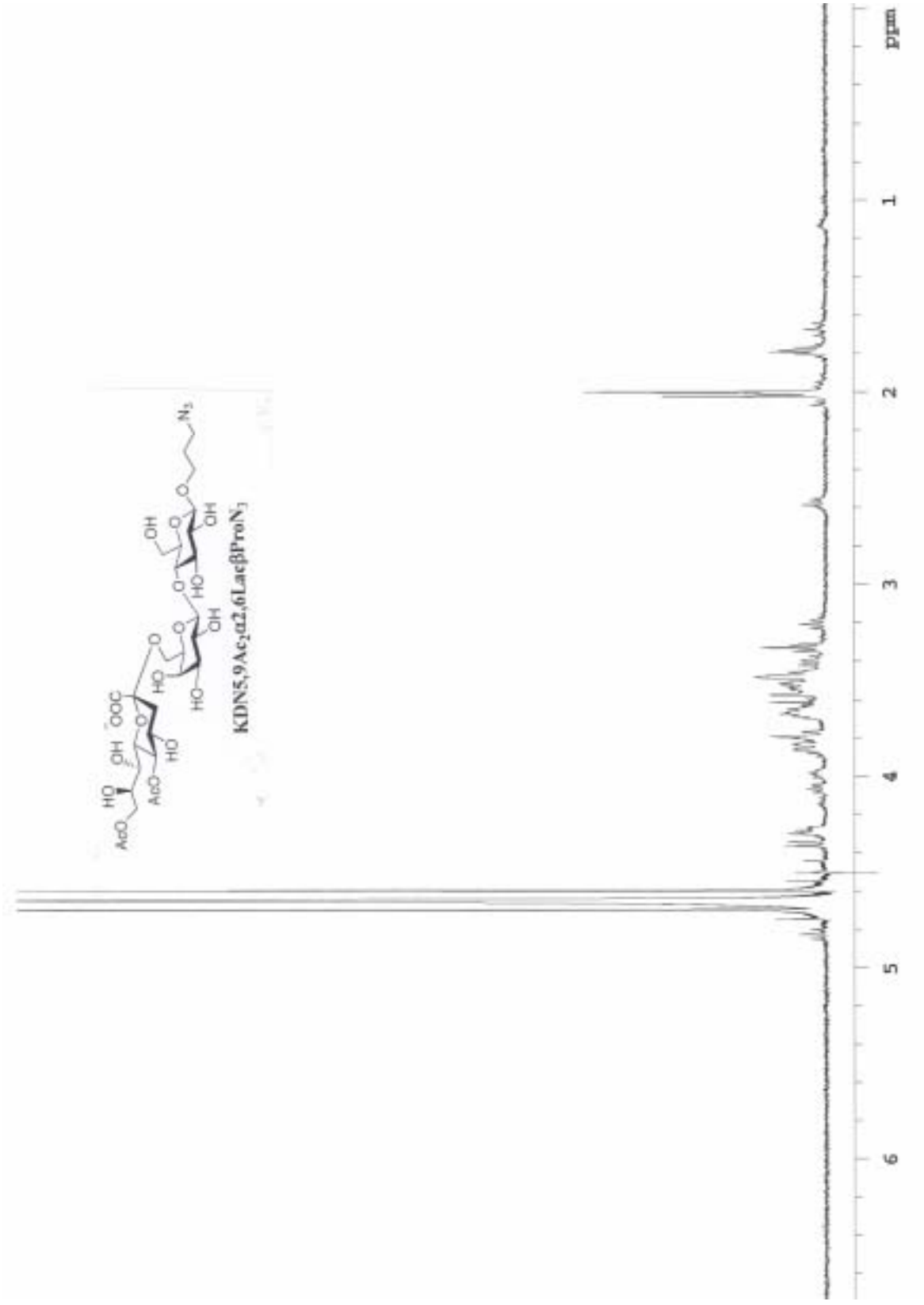


Compound **36**

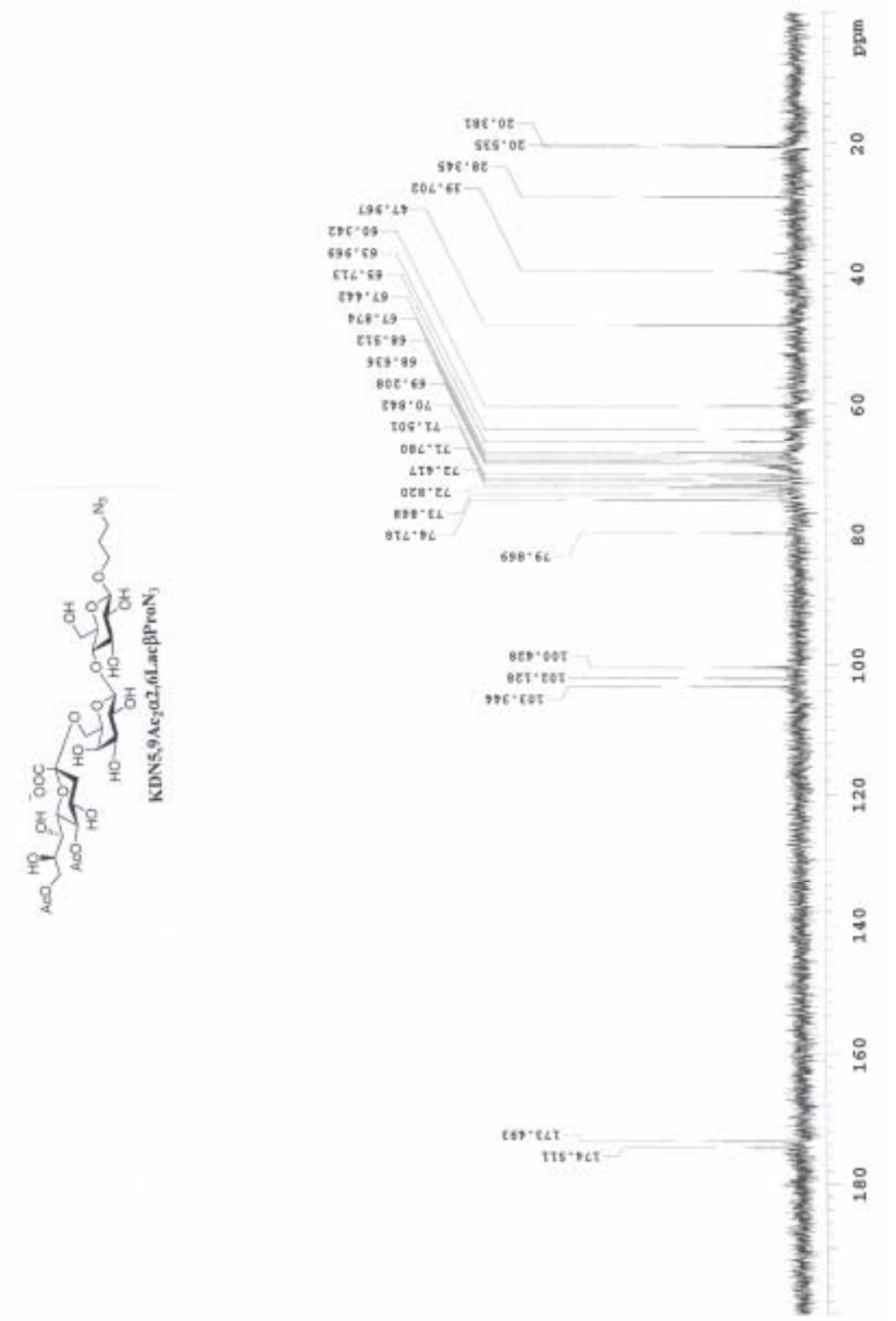

Neu9Ac5Gcα2,6Lactβ1ProN3


ppm
20
40
60
80
100
120
140
160
180
200

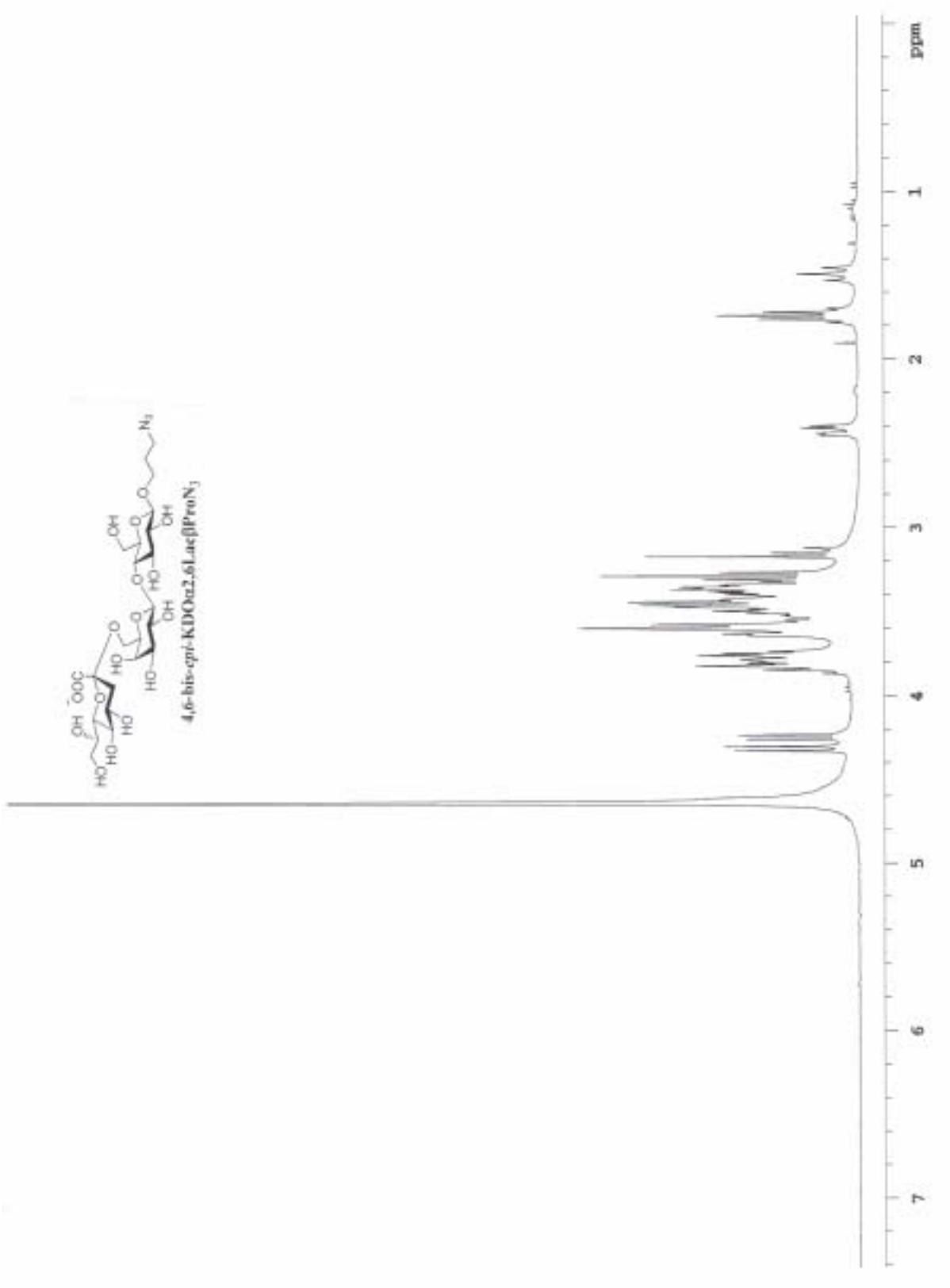
Compound **37**



Compound **37**

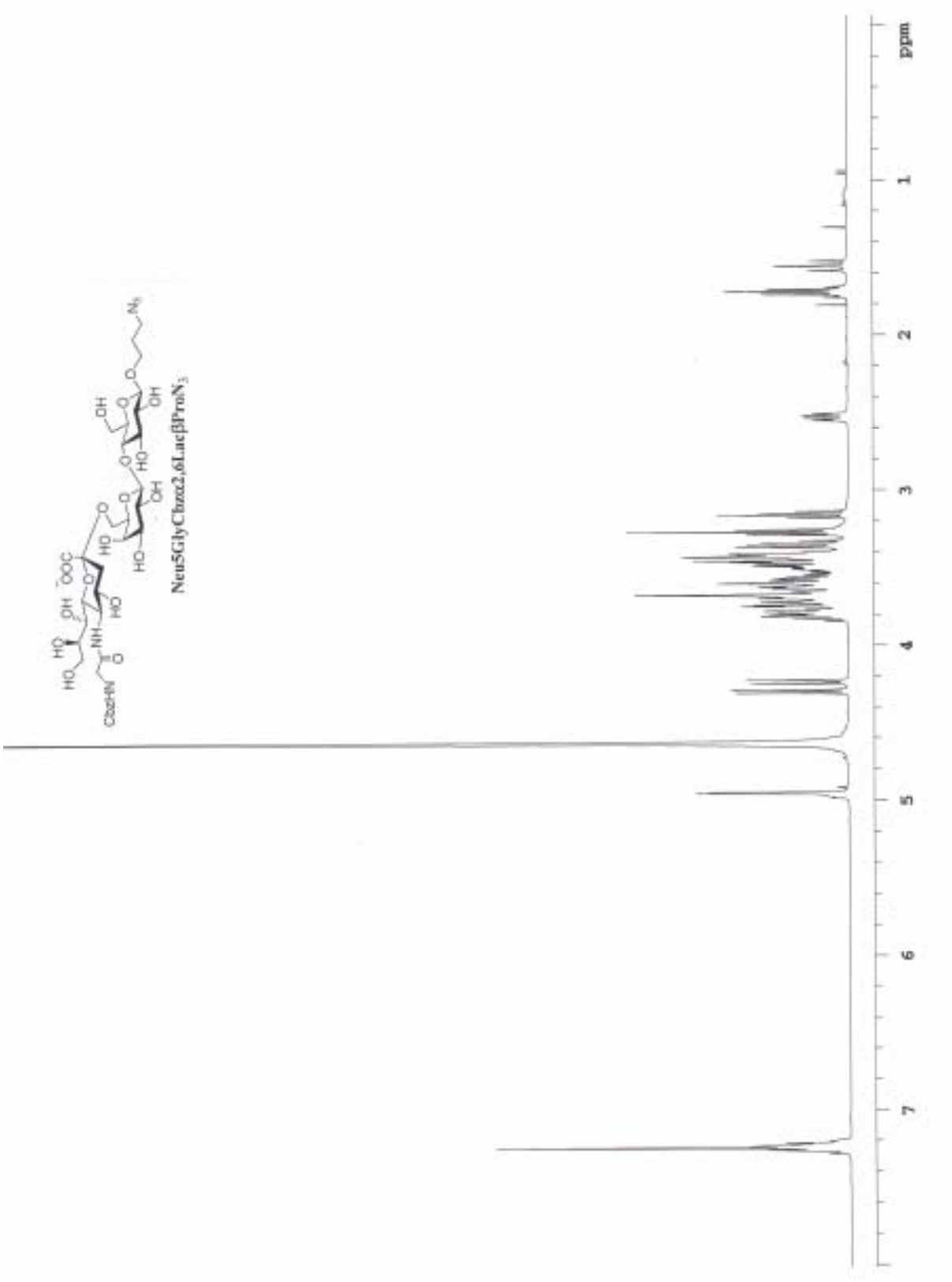

KDN5,9Ac2α2,6LacβProN3
20.381
20.535
38.345
39.703
47.967
60.342
63.969
69.713
67.443
67.874
68.513
68.636
69.308
70.843
71.501
71.780
73.417
73.820
73.868
74.718
79.869
100.438
103.128
103.344
173.493
174.511
ppm
20
40
60
80
100
120
140
160
180

Compound **38**

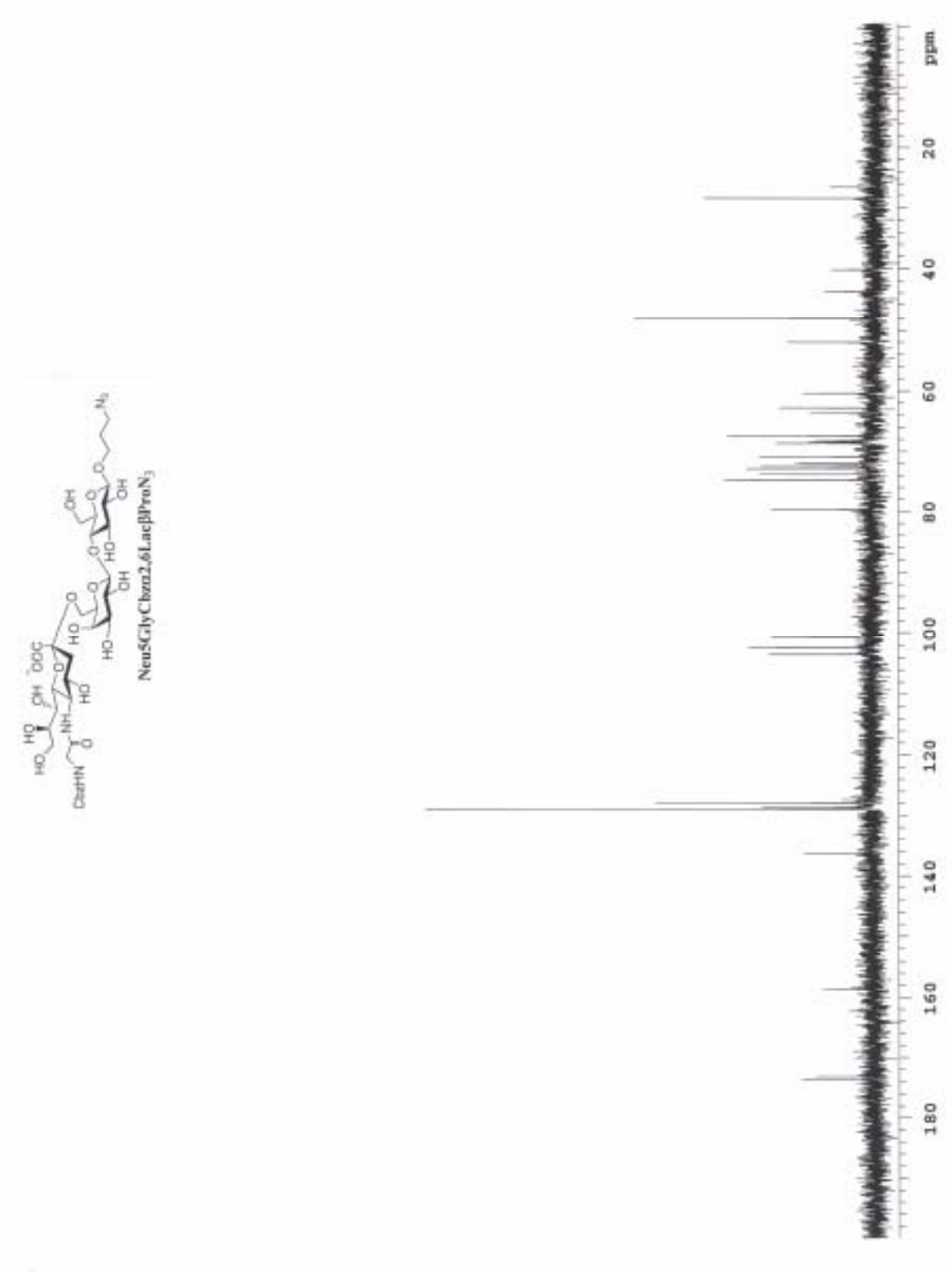


Compound **38**

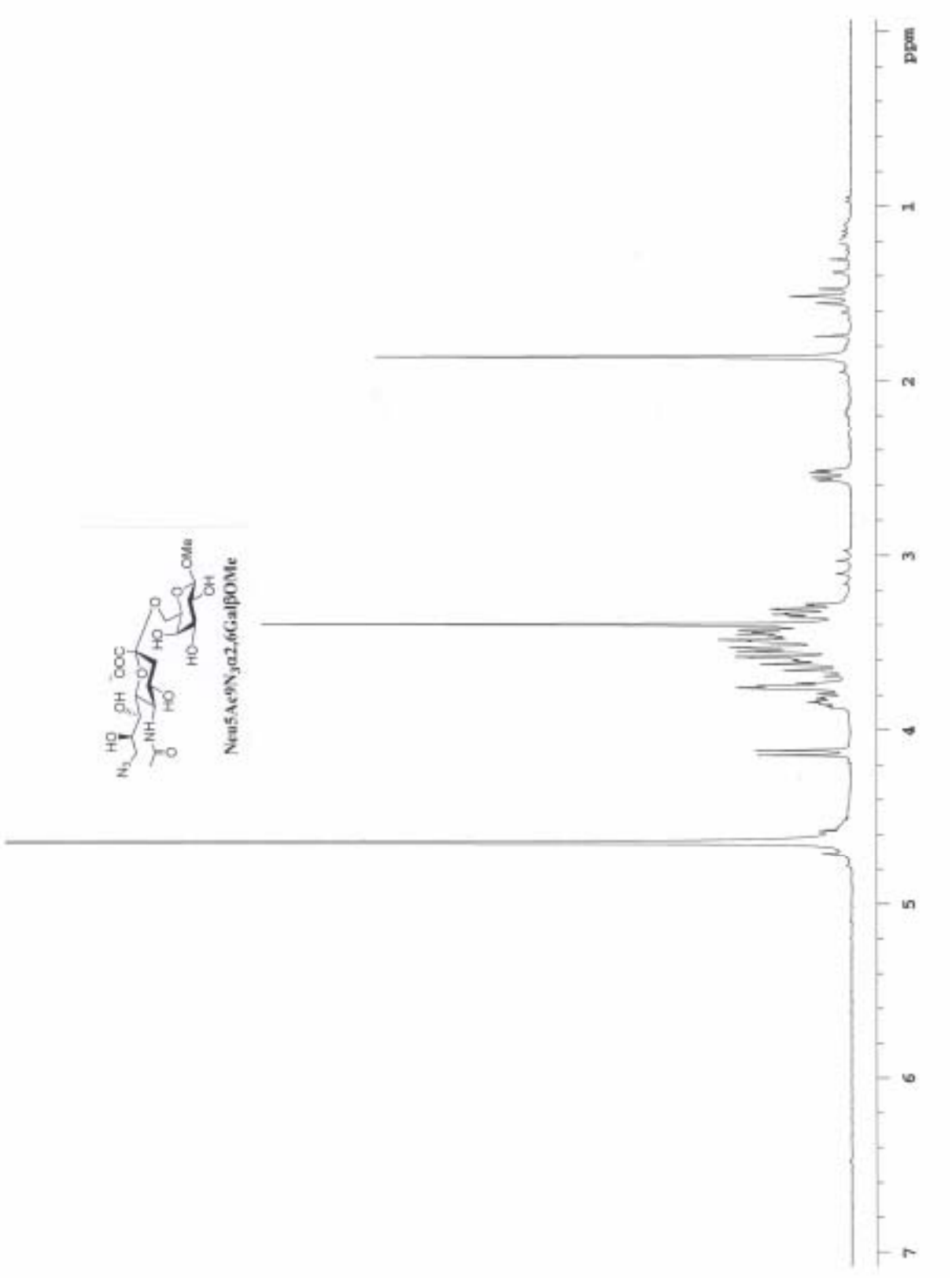
Compound **39**



Compound **39**

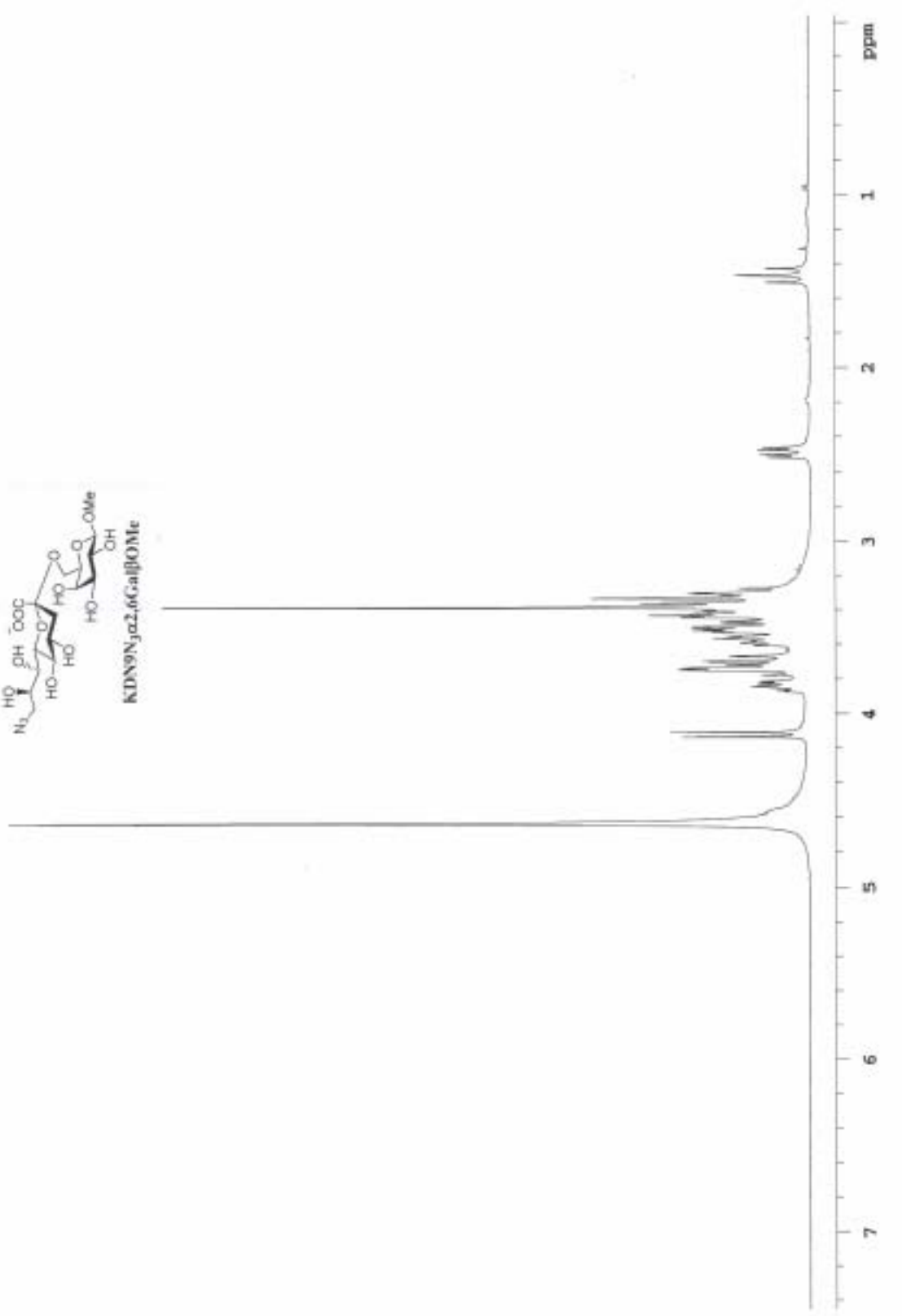


Compound **40**

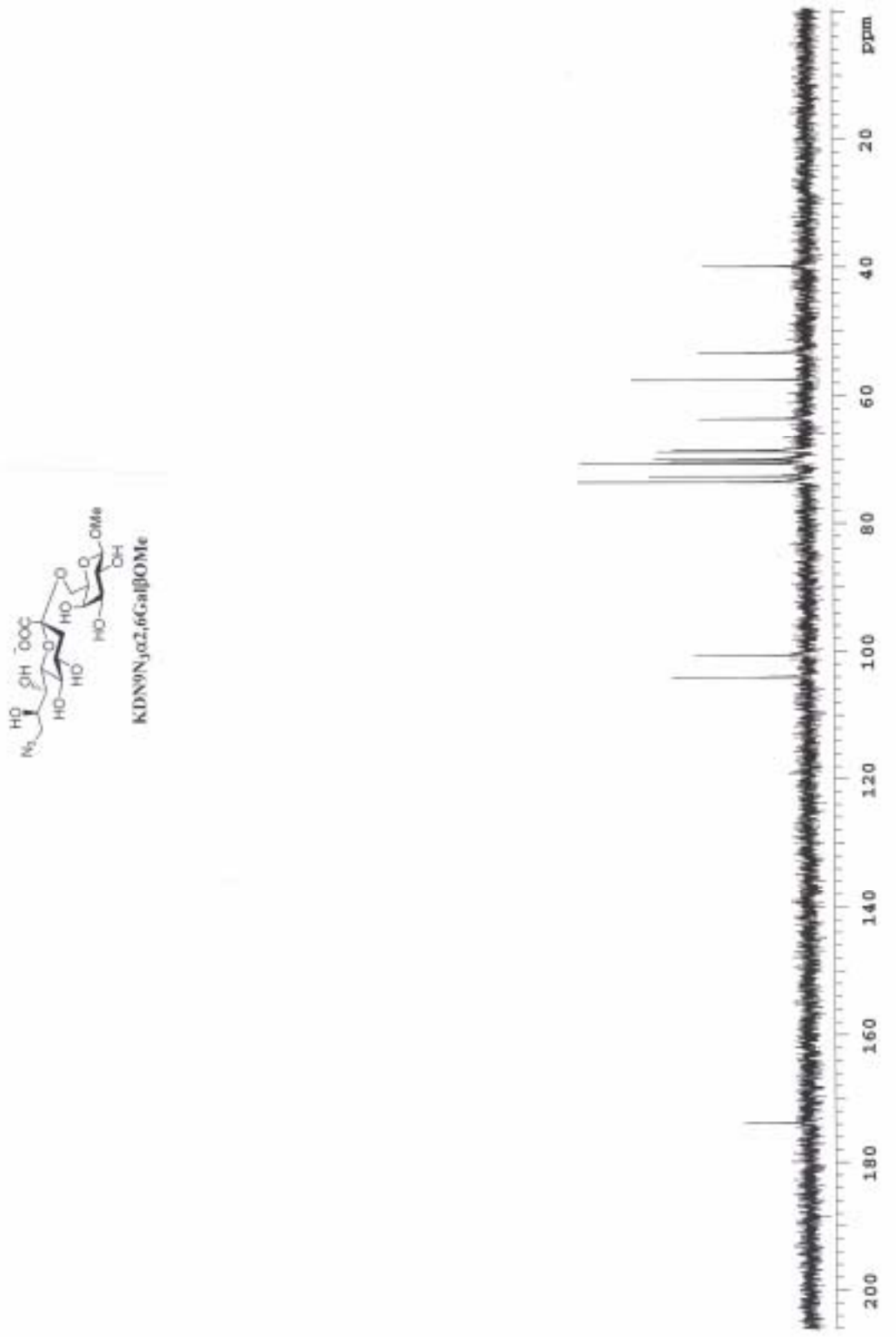


Compound **40**

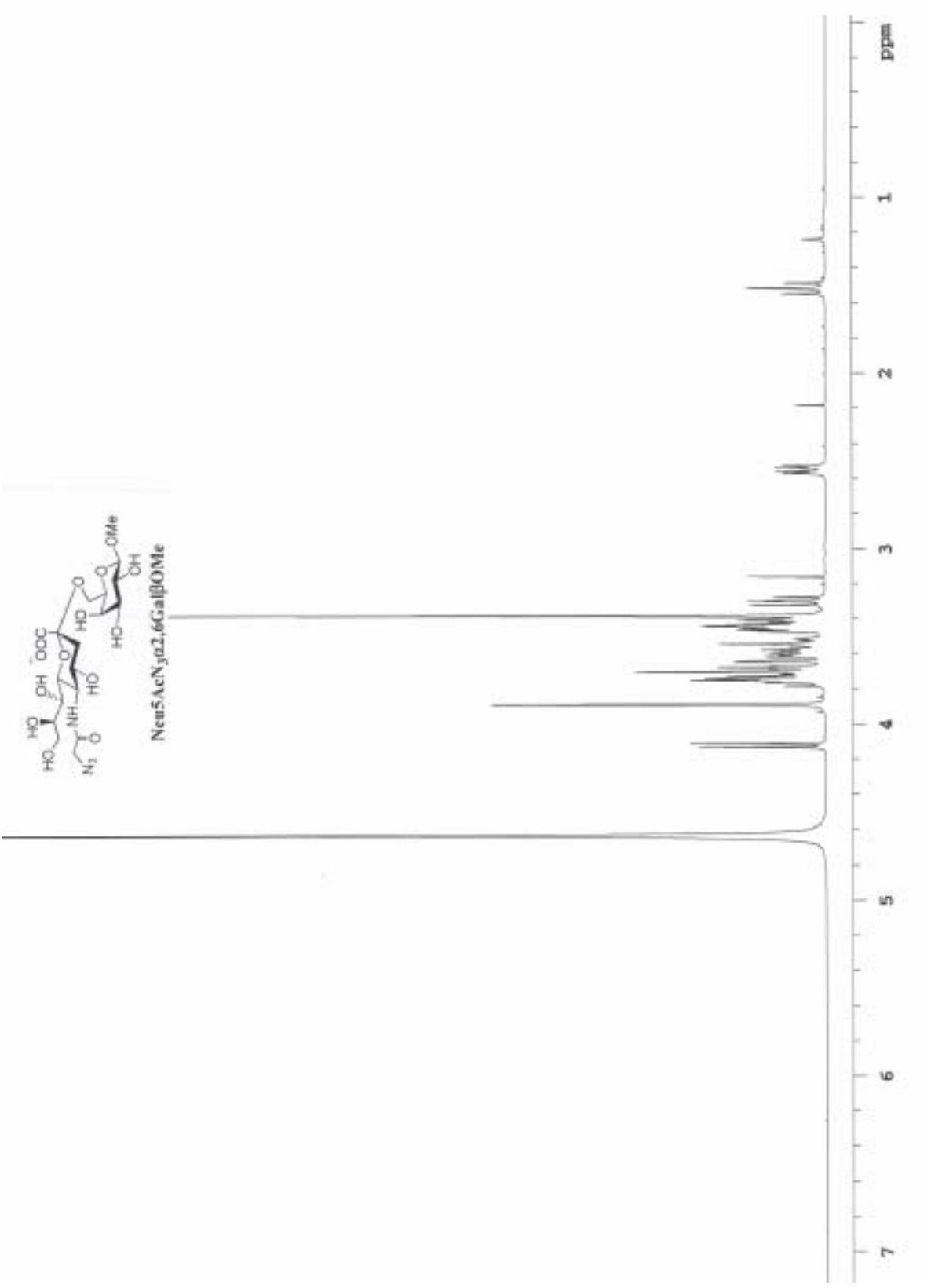
Compound **41**



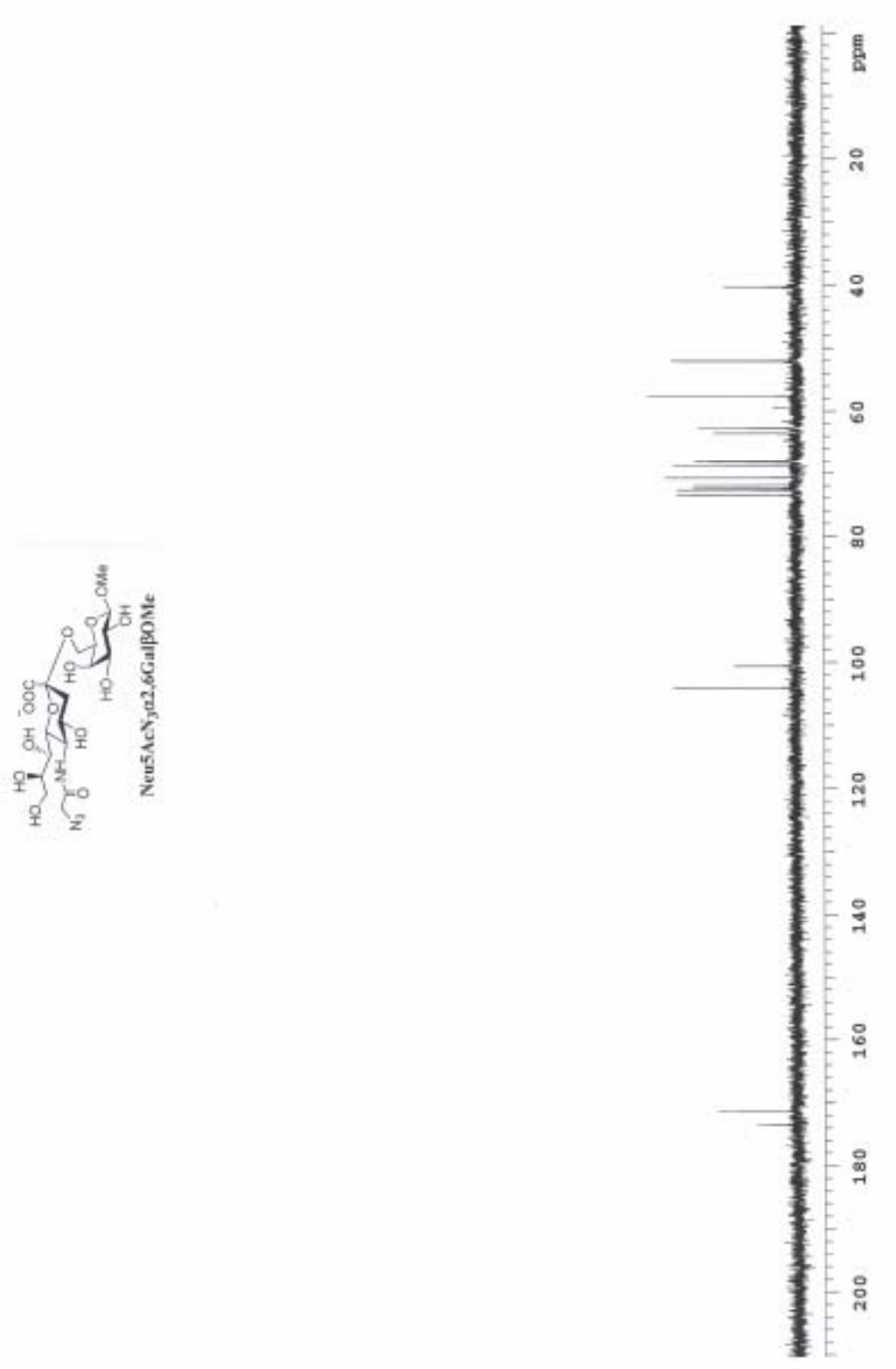
Compound **41**



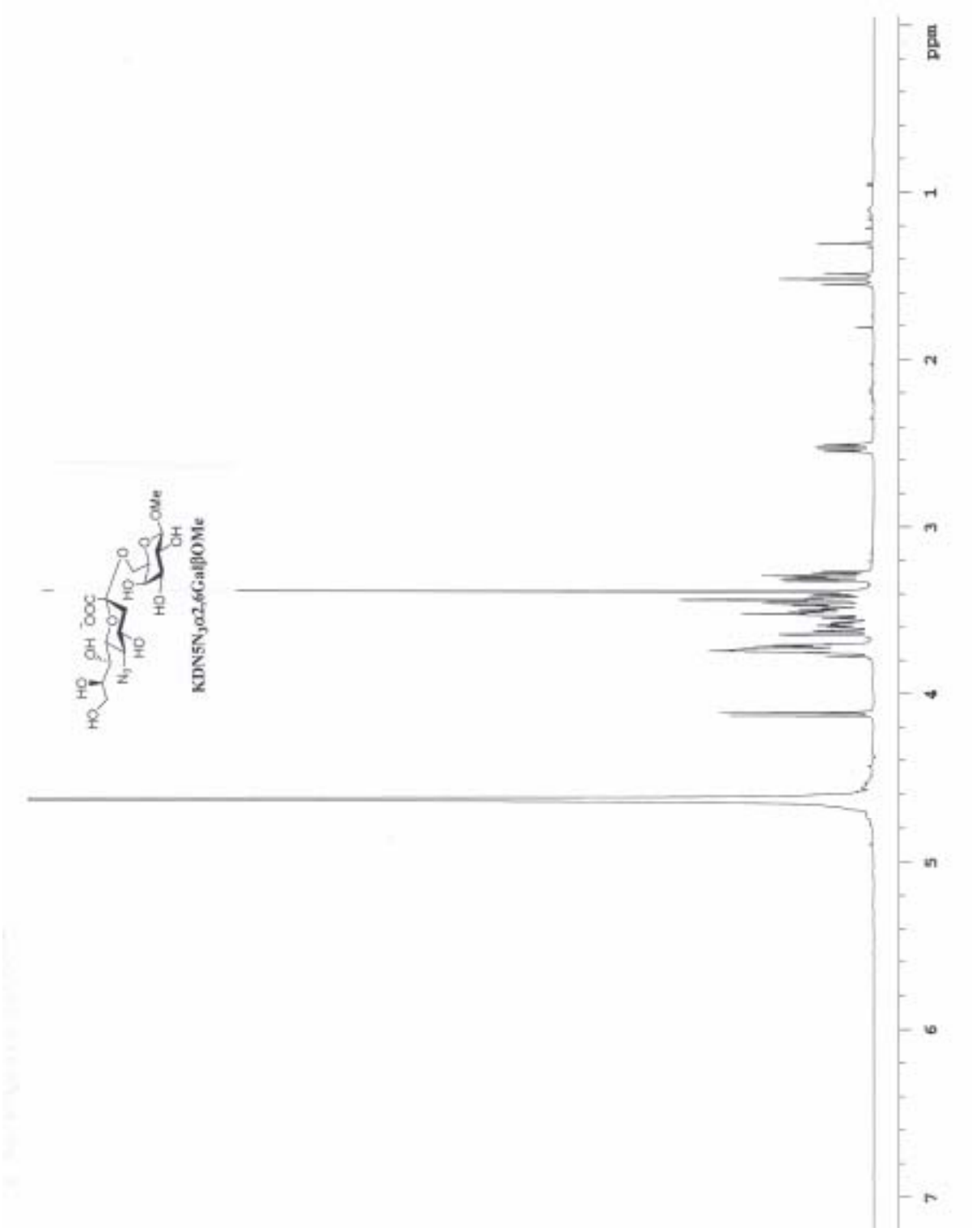
Compound **42**



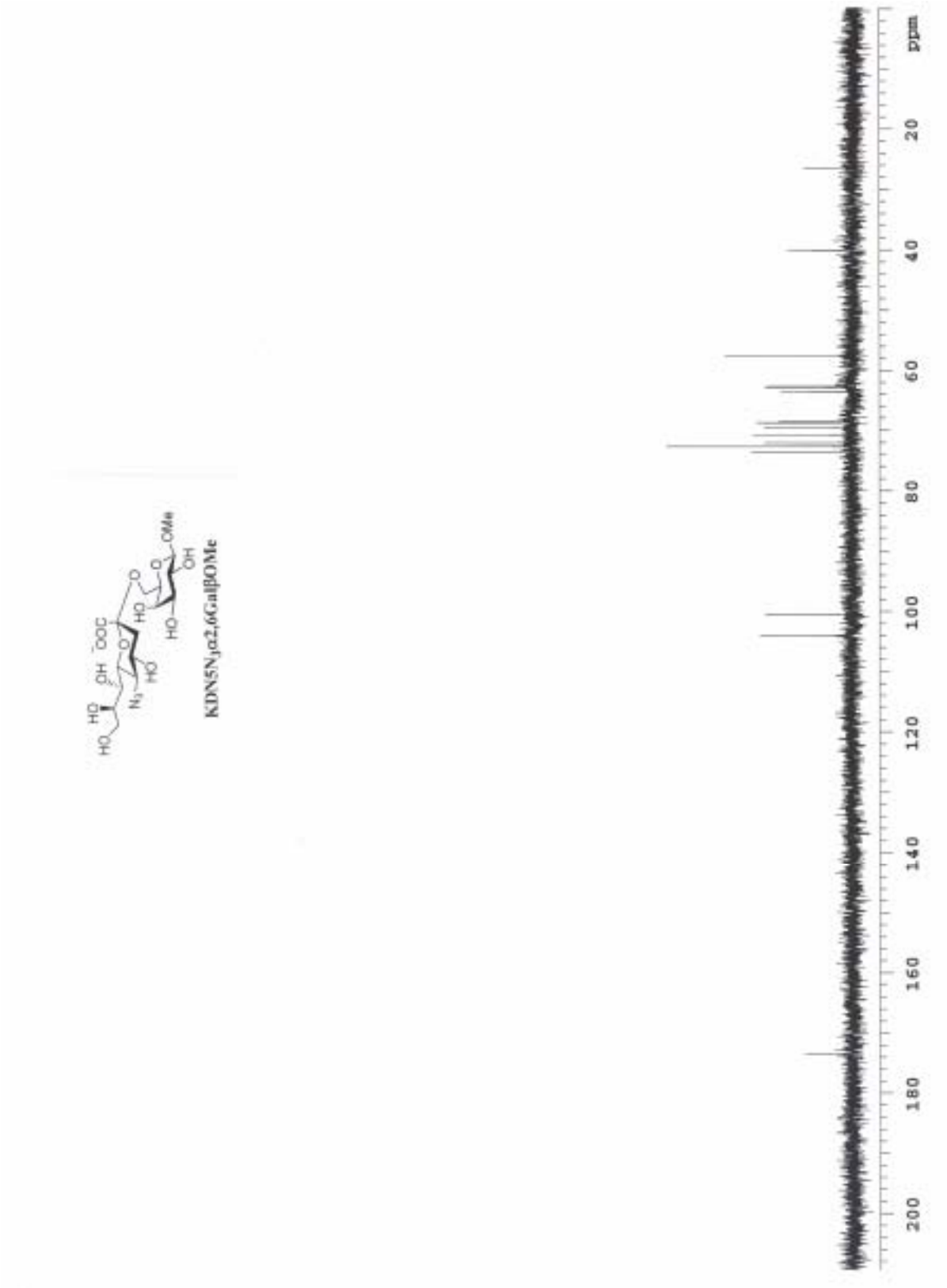
Compound **42**



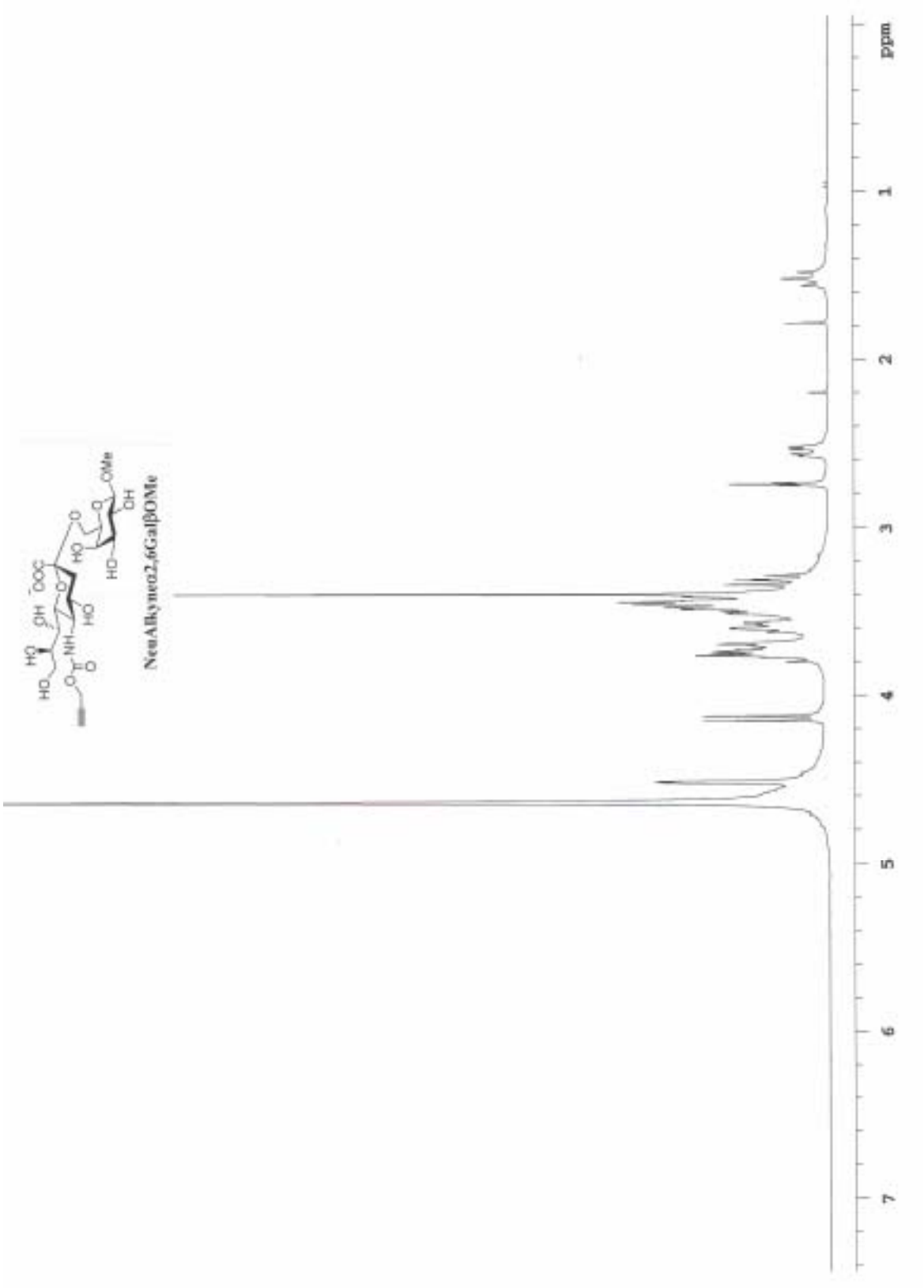
Compound **43**



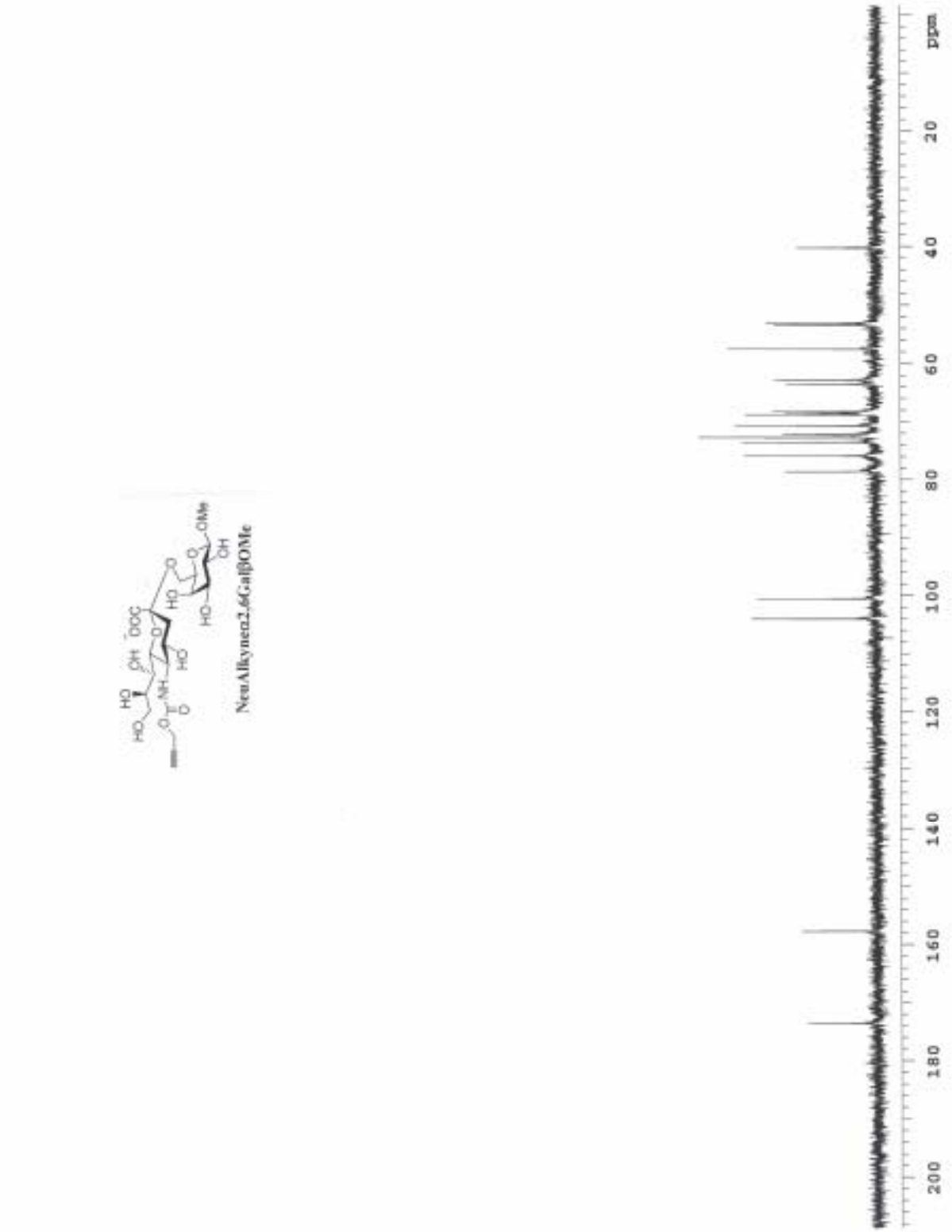
Compound **43**



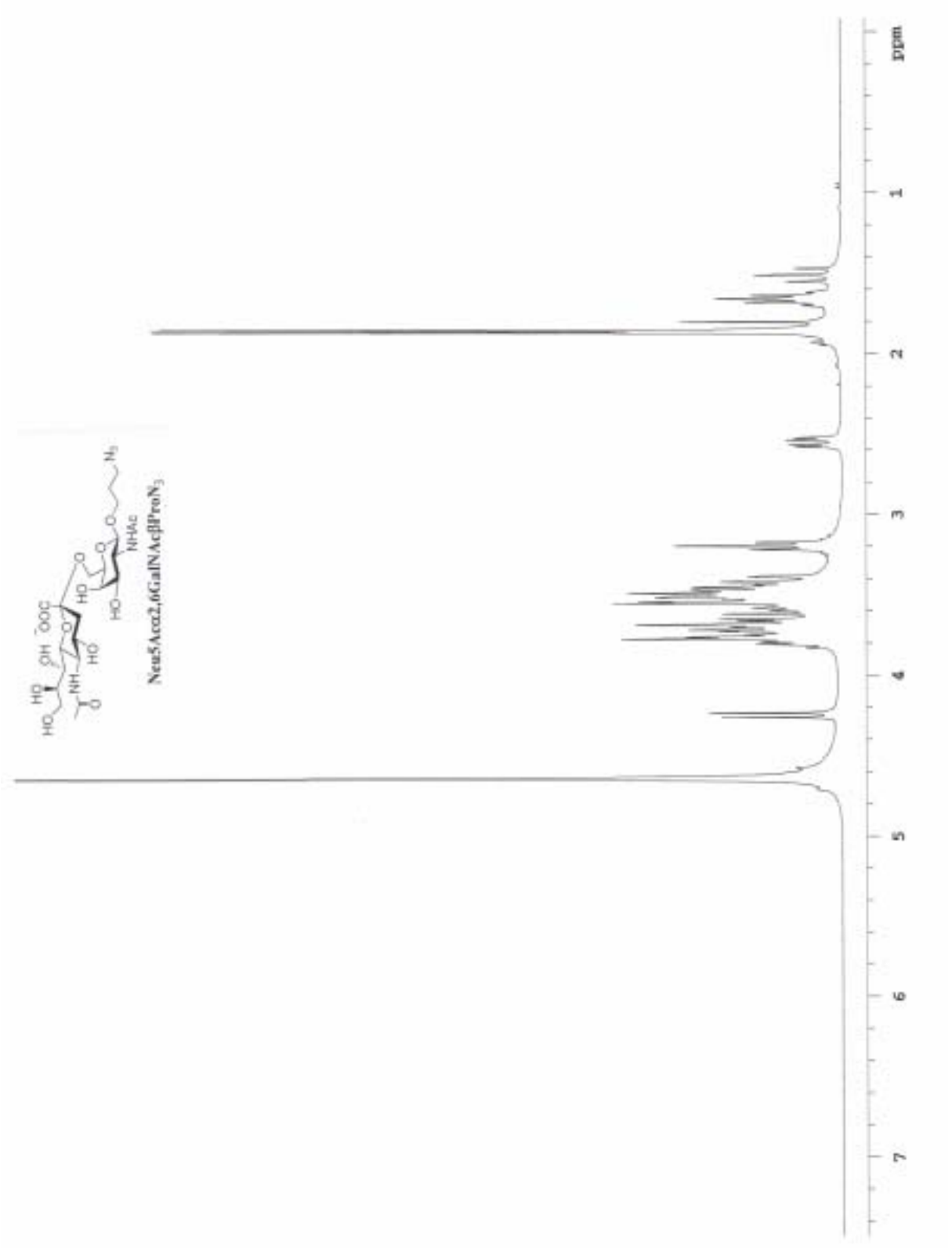
Compound **44**



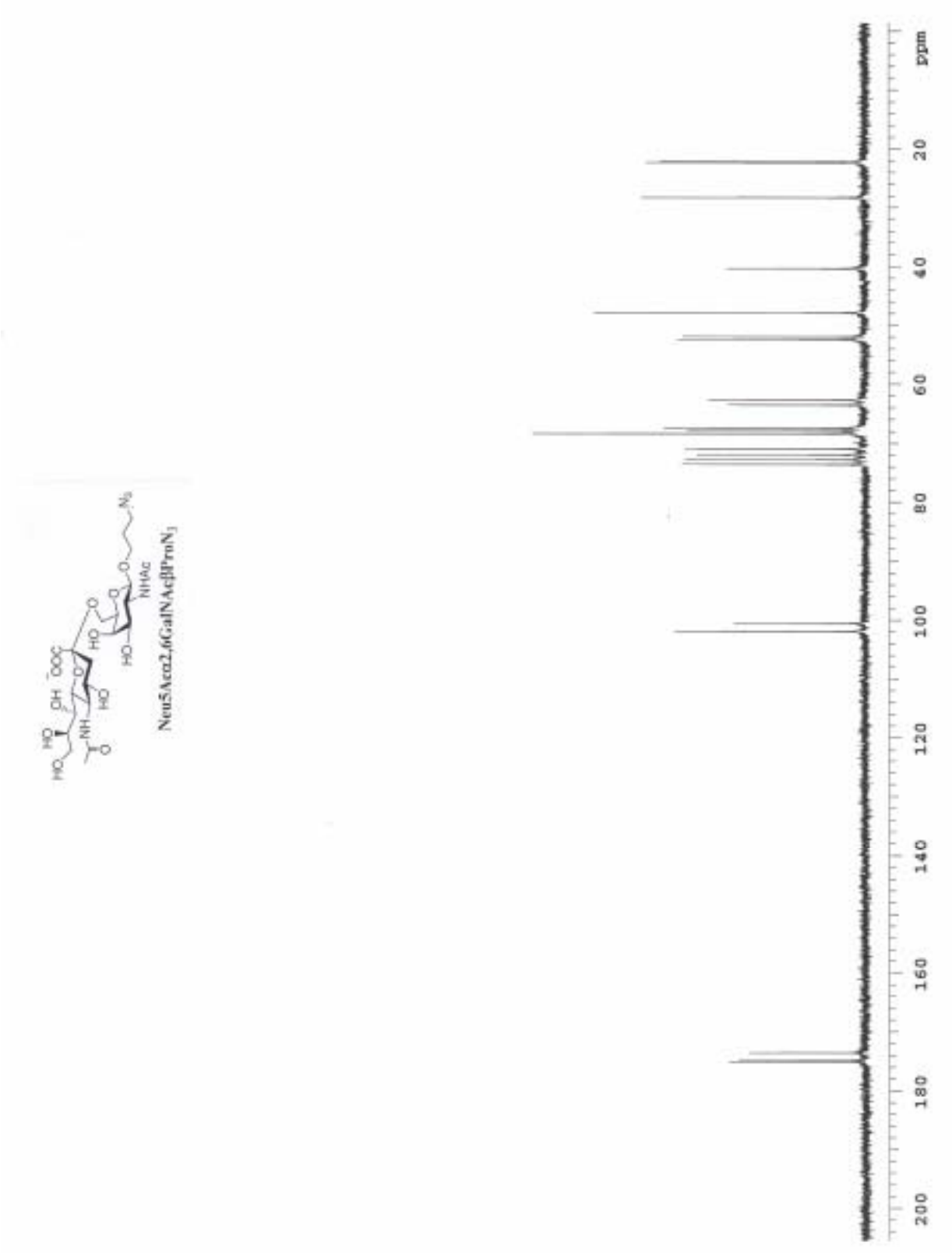
Compound **44**



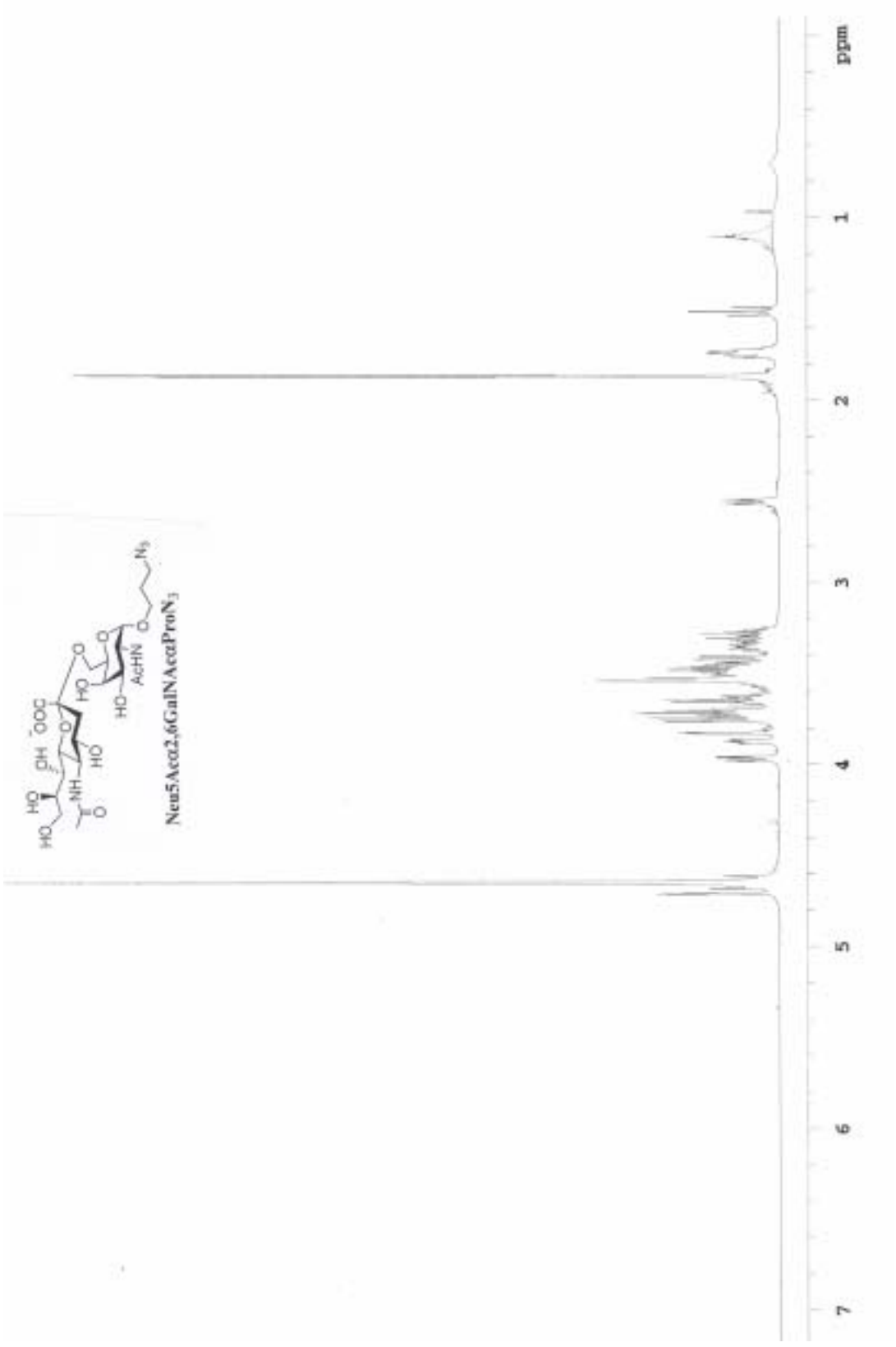
Compound **45**



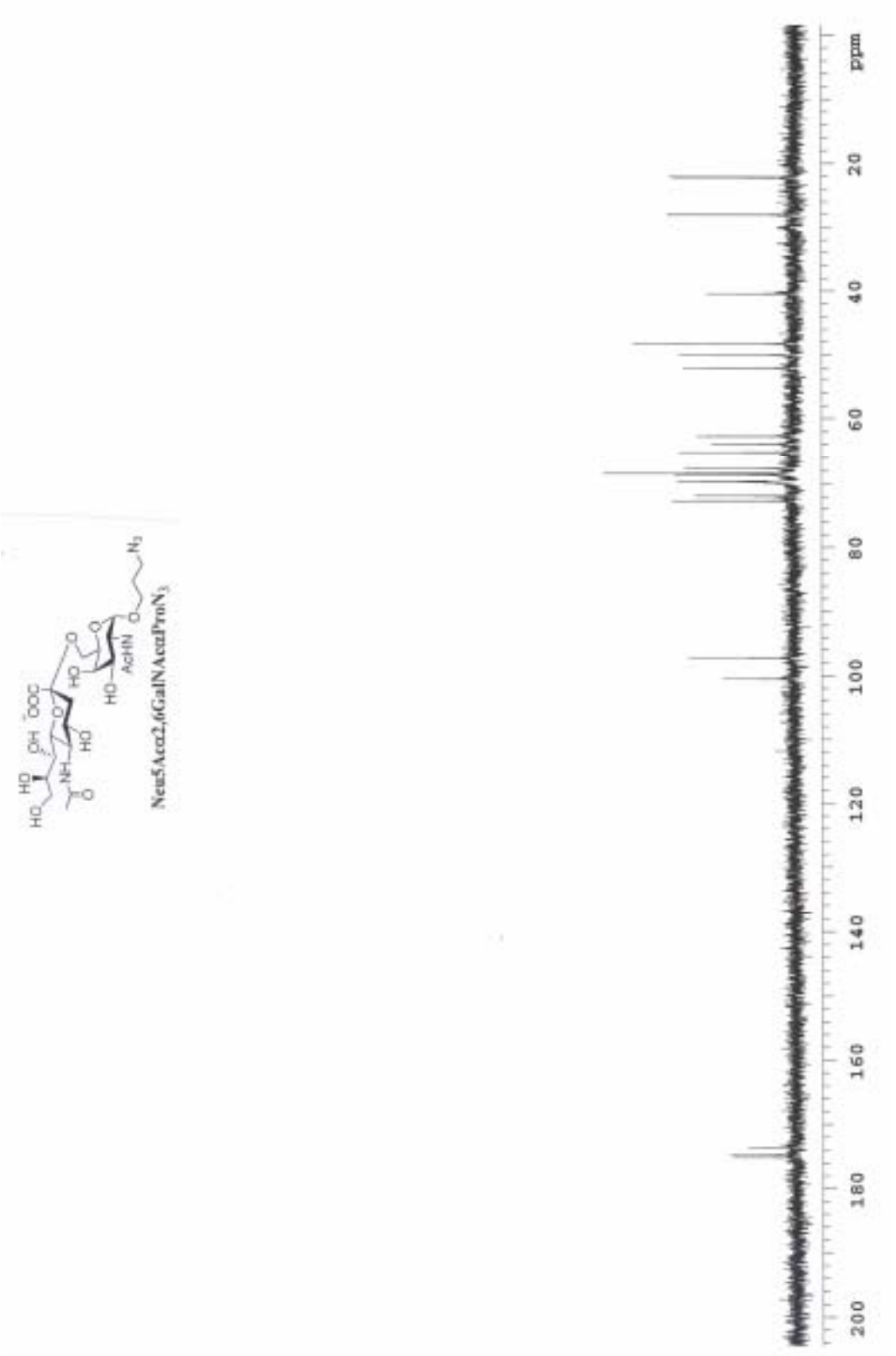
Compound **45**


Neu5Acα2,6GalNAcβProN3
ppm
20
40
60
80
100
120
140
160
180
200

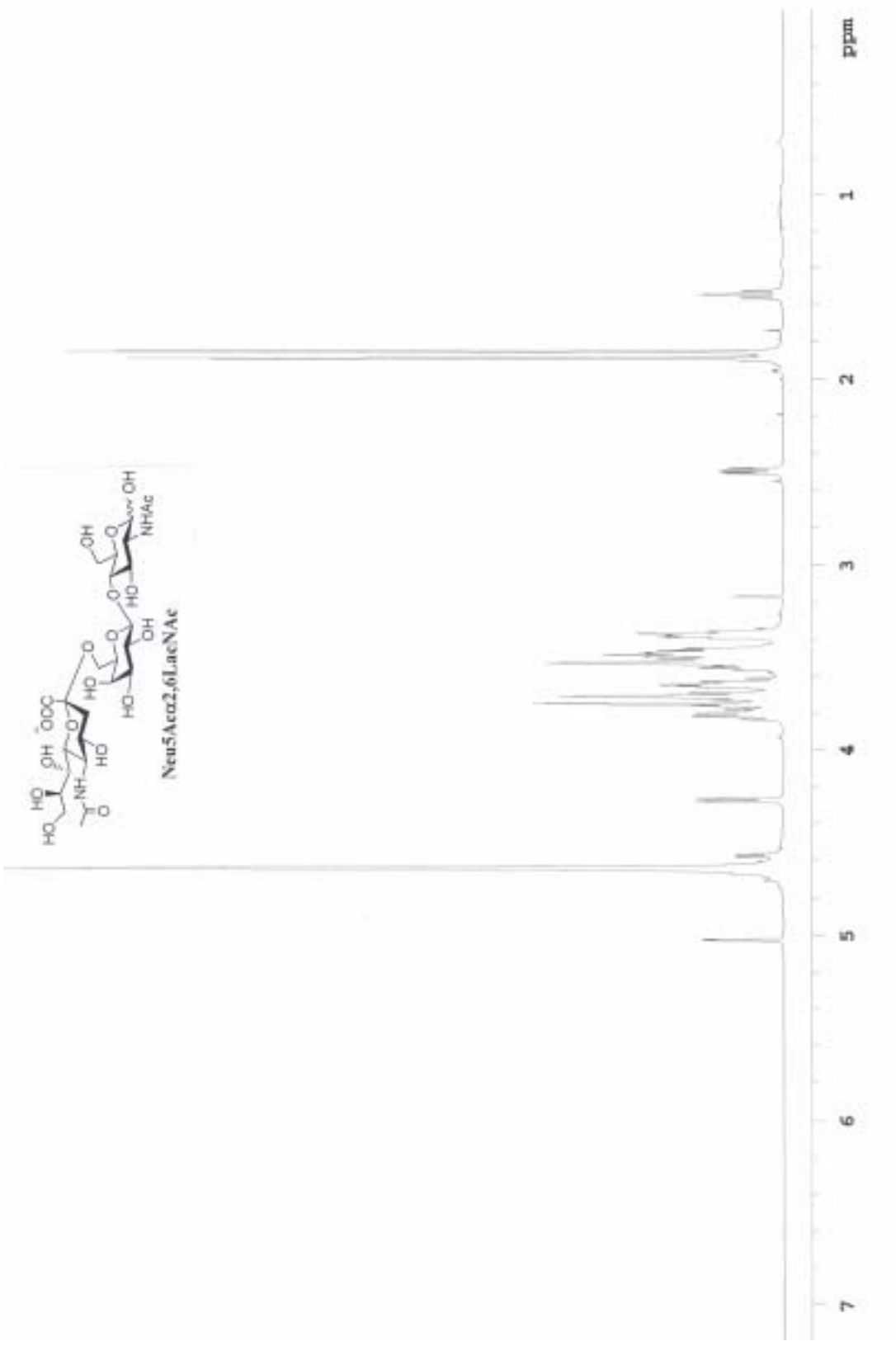
Compound **46**



Compound **46**

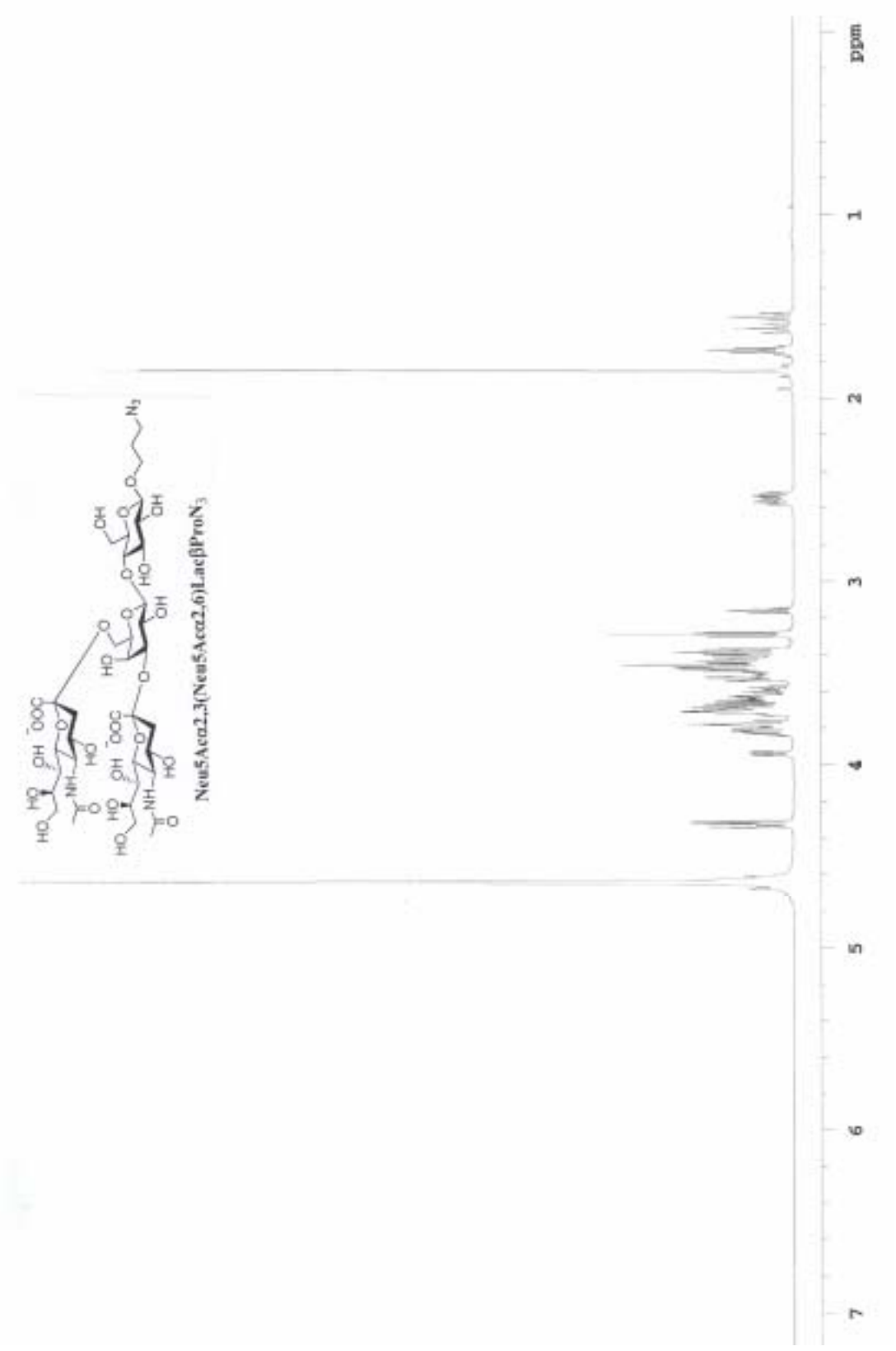

Neu5Acα2,6GalNAcαProN3
ppm
20
40
60
80
100
120
140
160
180
200

Compound **47**

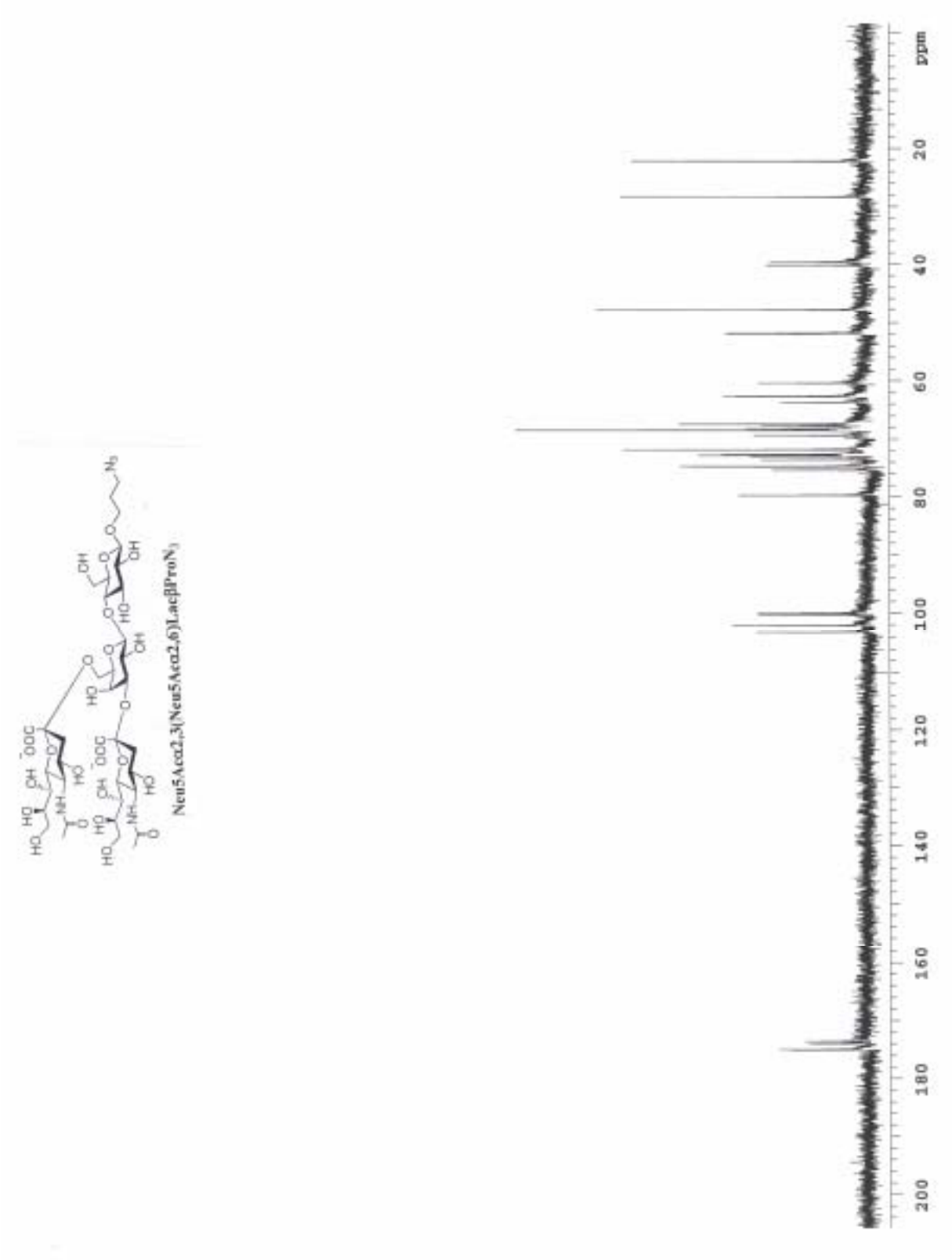


Compound **47**

Compound **48**



Compound **48**


Neu5Acα2,3(Neu5Acα2,6)LacβProN3
ppm
20
40
60
80
100
120
140
160
180
200