Advanced Synthesis & Catalysis

Supporting Information

Semi-Synthetic Sialic Acid Probes for Challenging the Substrate Promiscuity of Enzymes in the Sialoconjugation Pathway

Alexander Mertsch, Silvan Poschenrieder, and Wolf-Dieter Fessner*

Semi-Synthetic Sialic Acid Probes for Challenging the Substrate Promiscuity of Enzymes in the Sialoconjugation Pathway

Alexander Mertsch, Silvan Poschenrieder and Wolf-Dieter Fessner*

Institute of Organic Chemistry and Biochemistry
Technische Universität Darmstadt
Alarich-Weiss-Str. 4, 64287 Darmstadt, Germany
Fax: +49 6151 1623645
E-mail: fessner@tu-darmstadt.de

Supporting information

Index

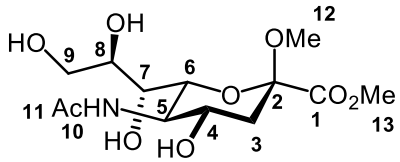
Materials and methods

The CSS from *N. meningitidis* was produced by recombinant expression and purified as described earlier.[1] The engineered α2,3-SiaT$_{pph}$_A151D from *P. phosphoreum* was produced and purified as described recently.[2] Cresol red and CTP were purchased from Carl Roth, Germany. Neu5Ac was from R&S Pharmchem, China. Column chromatography was performed on *Roth* silica gel (0.040–0.063 mesh); analytical thin layer chromatography was performed on Merck silica gel plates 60 F_{254} using anisaldehyde staining or *N*-(1-Naphthyl)-ethylenediamine for detection. Commercial reagents/solvents were used as received without further purification. Organic solvents were dried freshly by standard methods. NMR spectra were recorded on Bruker DRX500 spectrometer; chemical shifts are referenced to HOD, δ = 4.79 ppm. For quantitative NMR measurements an accurate manual phase correction and a baseline correction with Whittaker Smoother method were performed using MestReNova 11.0.3. High-resolution mass data were determined on a Bruker Impact II ESI-Q-TOF spectrometer. Assays were measured using a SpectraMax 190 plate reader from Molecular Devices and *SoftMax Pro 6.5.1* software. TLC: all R_f values are reported for the solvent mixture n-BuOH:acetone:H_2O:AcOH = 35:35:23:7. Organic solvents were removed under vacuum using a rotary evaporator, and residues were dissolved in H_2O and lyophilized.

Compounds **4**, **5** and **6** were prepared by adaption of reported literature procedures,[3] however, no spectral data had been reported. More recently ^{1}H NMR data for **4** and **5** were recorded in MeOD solution.[4] NMR-data for **6** are in accordance with recent literature data.[5]

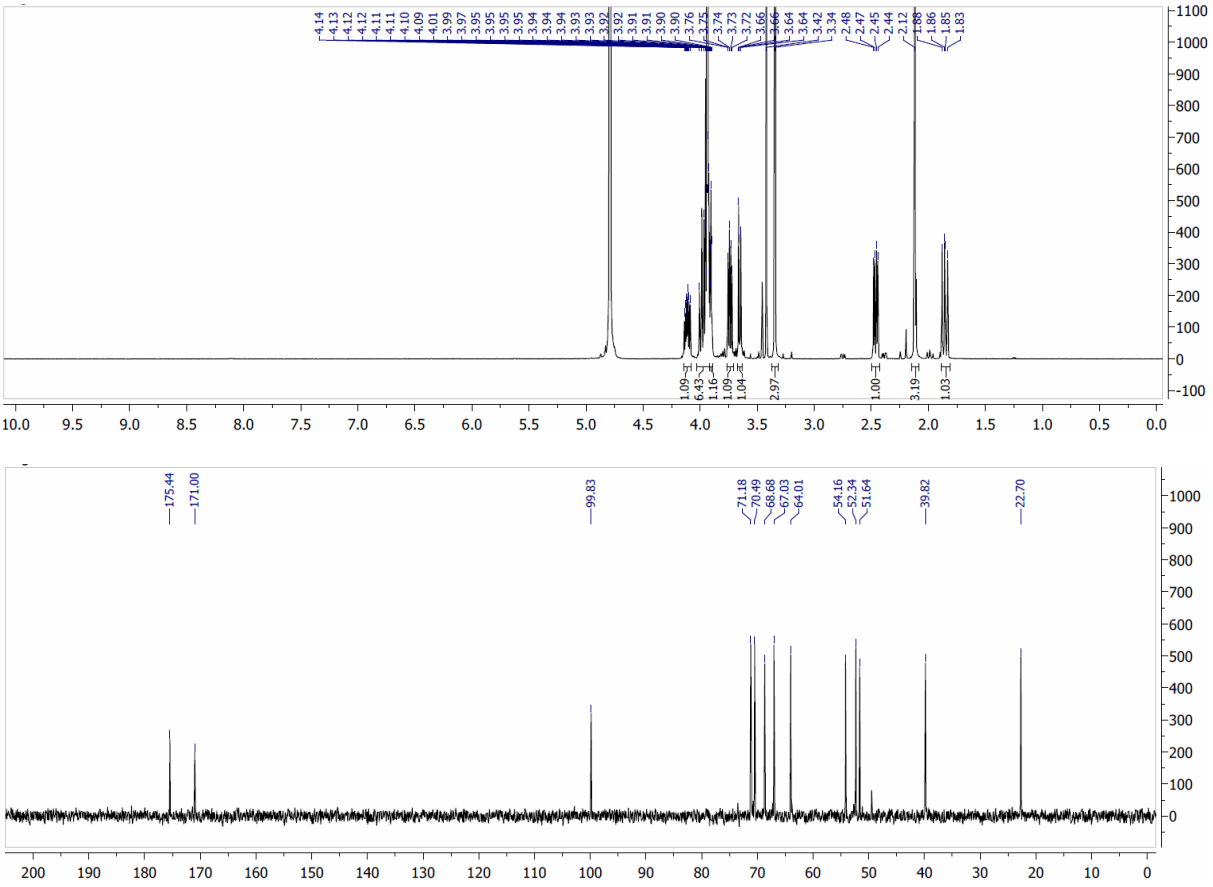
N-Acetyl-2-*O*-methyl-β-D-neuraminic acid methyl ester (**4**)

A mixture of *N*-acetyl-neuraminic acid (**1**) (4.00 g, 12.9 mmol, 1.0 eq), freshly regenerated dry Dowex-50 resin (H^+ form; 7.80 g) and molecular sieves (3 Å) in dry methanol (660 mL) was heated to reflux for 24 h. The warm reaction mixture was filtered through celite and the solvent was removed in vacuo to obtain a yellow oil. The residue was dissolved in methanol (17 mL), diluted with diethyl ether (45 mL), and the mixture was refrigerated at –25 °C for 24 h. The crystalline solid was filtered off and washed with ether. This procedure was repeated for the filtrate (MeOH:ether = 1:3). The product was obtained as a colorless crystalline solid (3.31 g, 76%). R_f = 0.63.



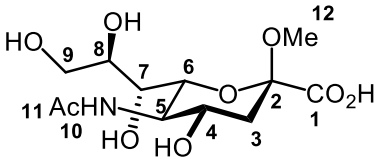
^{1}H NMR (500 MHz, D_2O) δ = 4.11 (ddd, J = 11.4, 9.4, 5.0 Hz, 1H, 4-H), 4.01–3.92 (m, 6H, 5-, 6-, 8-, 13-H), 3.92-3.89 (m, 1H, 9a-H), 3.74 (dd, J = 6.2, 5.8 Hz, 1H, 9b-H), 3.65 (dd, J = 9.3, 1H, 7-H), 3.34 (s, 3H, 12-H), 2.46 (dd, J = 13.3, 4.9 Hz, 1H, 3_{eq}-H), 2.12 (s, 3H, 11-H), 1.86 (dd, J = 13.3, 11.4 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.44 (C-10), 171.00 (C-1), 99.83 (C-2), 71.18 (C-6), 70.49 (C-8), 68.68 (C-7), 67.03 (C-4), 64.01 (C-9), 54.16 (C-13), 52.34 (C-5), 51.64 (C-12), 39.82 (C-3), 22.70 (C-11).



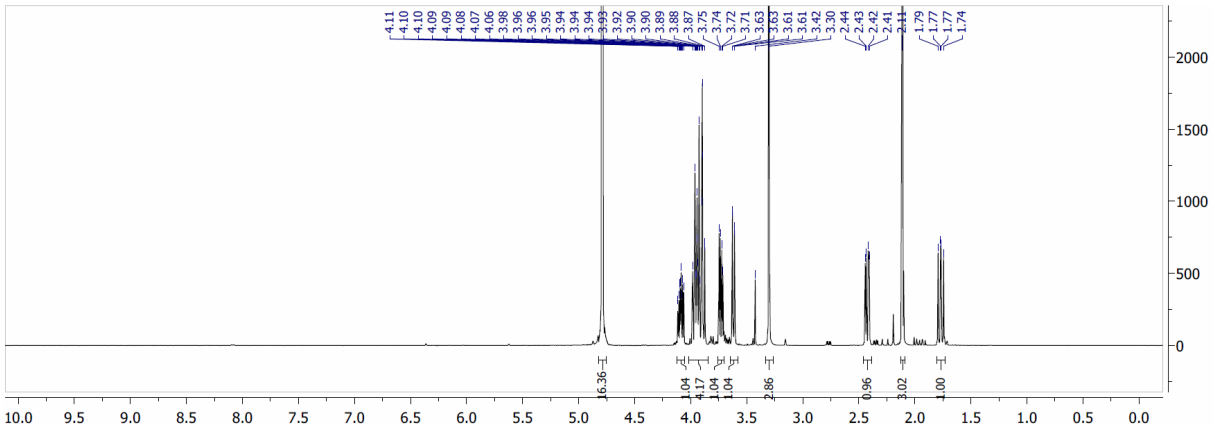
N-Acetyl-2-*O*-methyl-β-D-neuraminic acid (**5**)

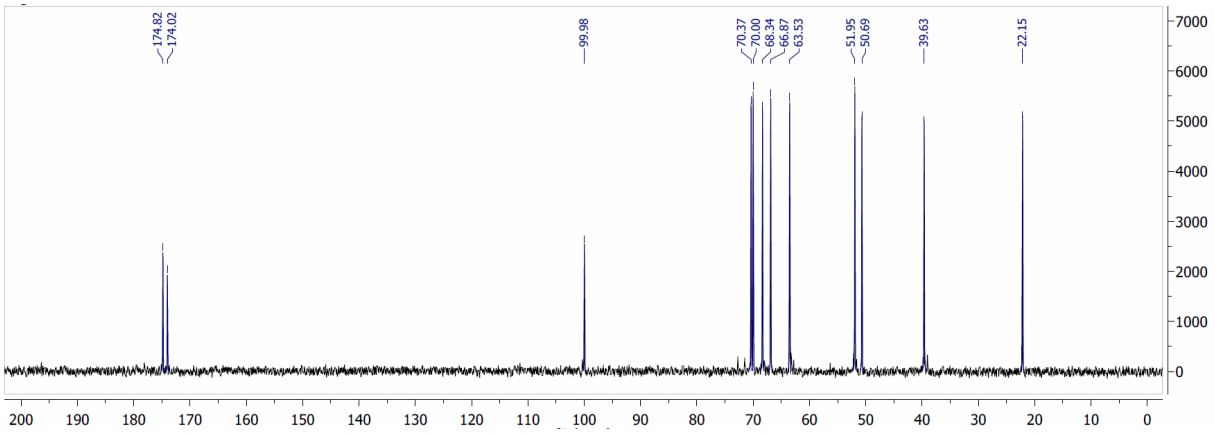
A solution of **4** (2.20 g, 6.52 mmol, 1.0 eq) in 0.06 N NaOH (165 mL, 9.90 mmol, 1.5 eq) was stirred at room temperature for 2.5 h. After completion the mixture was neutralized to pH 7.0 by swirling with Dowex-50 resin (H^+ form; 2.5 g). The suspension was filtered and the volume of the filtrate was reduced to 10 mL in vacuo. This solution was lyophilized to obtain a colorless crystalline solid (2.10 g, quant.). R_f = 0.21.



^{1}H NMR (500 MHz, D_2O) δ = 4.09 (ddd, *J* = 11.4, 9.8, 5.0 Hz, 1H, 4-H), 3.98-3.87 (m, 4H, 5-, 6-, 8-, 9a-H), 3.73 (dd, *J* = 11.7, 5.5 Hz, 1H, 9b-H), 3.62 (dd, *J* = 9.2, 1.1 Hz, 1H, 7-H), 3.30 (s, 3H, 12-H), 2.42 (dd, *J* = 13.2, 5.0 Hz, 1H, 3_{eq}-H), 2.11 (s, 3H, 11-H), 1.77 (dd, *J* = 13.2, 11.4 Hz, 1H, 3_{ax}-H).

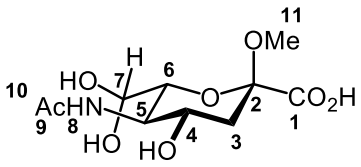
^{13}C NMR (126 MHz, D_2O) δ = 174.82 (C-10), 174.02 (C-1), 99.98 (C-2), 70.37 (C-8), 70.00 (C-6), 68.34 (C-7), 66.87 (C-4), 63.53 (C-9), 51.95(C-5), 50.69 (C-12), 39.63 (C-3), 22.15 (C-11).





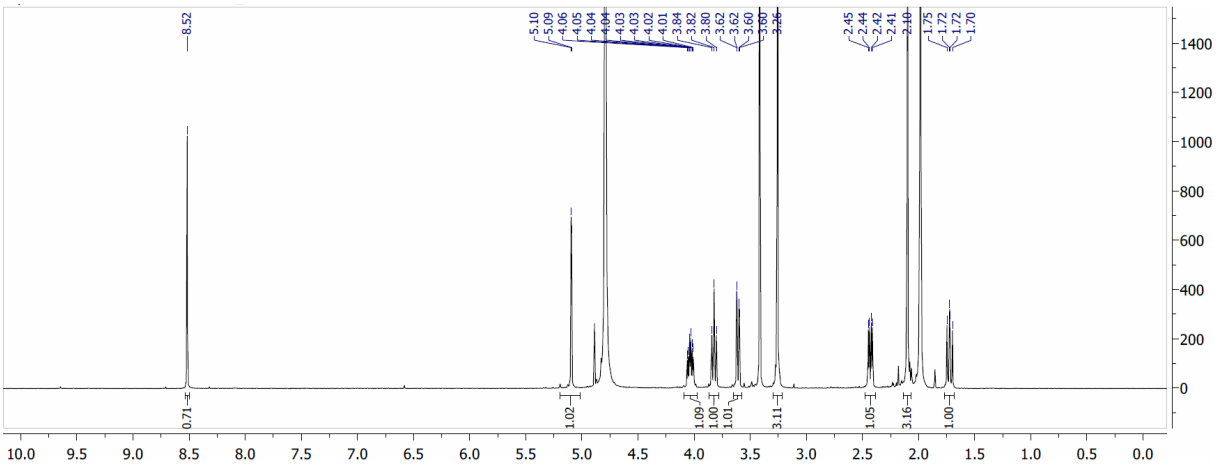
Methyl 3-(acetylamino)-3,5-dideoxy-L-*lyxo*-(6*S*)-heptulo-2,6-pyranosiduronic acid – trunctated C_7-aldehyde (**6**)

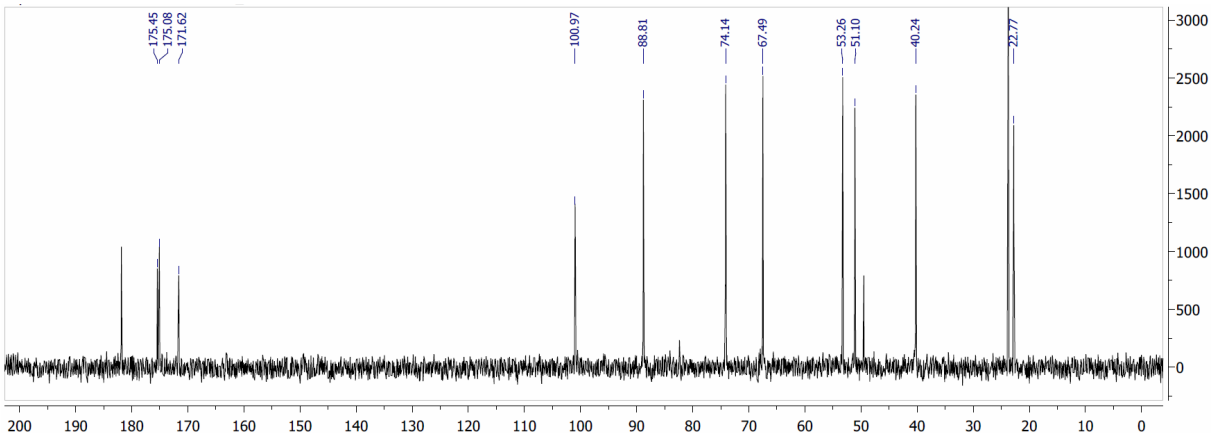
To a solution of **5** (1.00 g, 3.09 mmol, 1 eq. in 35 mL H_2O) was added 0.2 M $NaIO_4$ (80.5 mL). After standing for 2 h in the dark at RT TLC showed completion. A solution of $Ba(OAc)_2$ (117 mL, 0.1 M) was added to remove IO_4^- and I^- -salts. After complete precipitation, salts were filtered off and CO_2 was bubbled through the solution to precipitate excess of barium. After filtration the solvent was removed under vacuum. To remove residual salts, column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 0.79 g colorless solid (98%). R_f = 0.34.



^{1}H NMR (500 MHz, D_2O) δ = 8.52 (s, 1NH, 8-H), 5.09 (d, *J* = 1.7 Hz, 1H, 7-H), 4.03 (ddd, *J* = 11.4, 10.0, 5.0 Hz, 1H, 4-H), 3.82 (dd, *J* = 10.0, 10.5 Hz, 1H, 5-H), 3.61 (dd, *J* = 10.5, 1.8 Hz, 1H, 6-H), 3.26 (s, 3H, 11-H), 2.43 (dd, *J* = 13.2, 5.0 Hz, 1H, 3_{eq}-H), 2.10 (s, 3H, 10-H), 1.72 (dd, *J* = 13.2, 11.4 Hz, 1H, 3_{ax}-H).

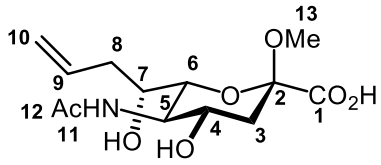
^{13}C NMR (126 MHz, D_2O) δ = 175.45 (C-1), 175.08 (C-9), 100.97 (C-2), 88.81 (C-7), 74.14 (C-6), 67.49 (C-4), 53.26 (C-5), 51.10 (C-11), 40.24 (C-3), 22.77 (C-10).





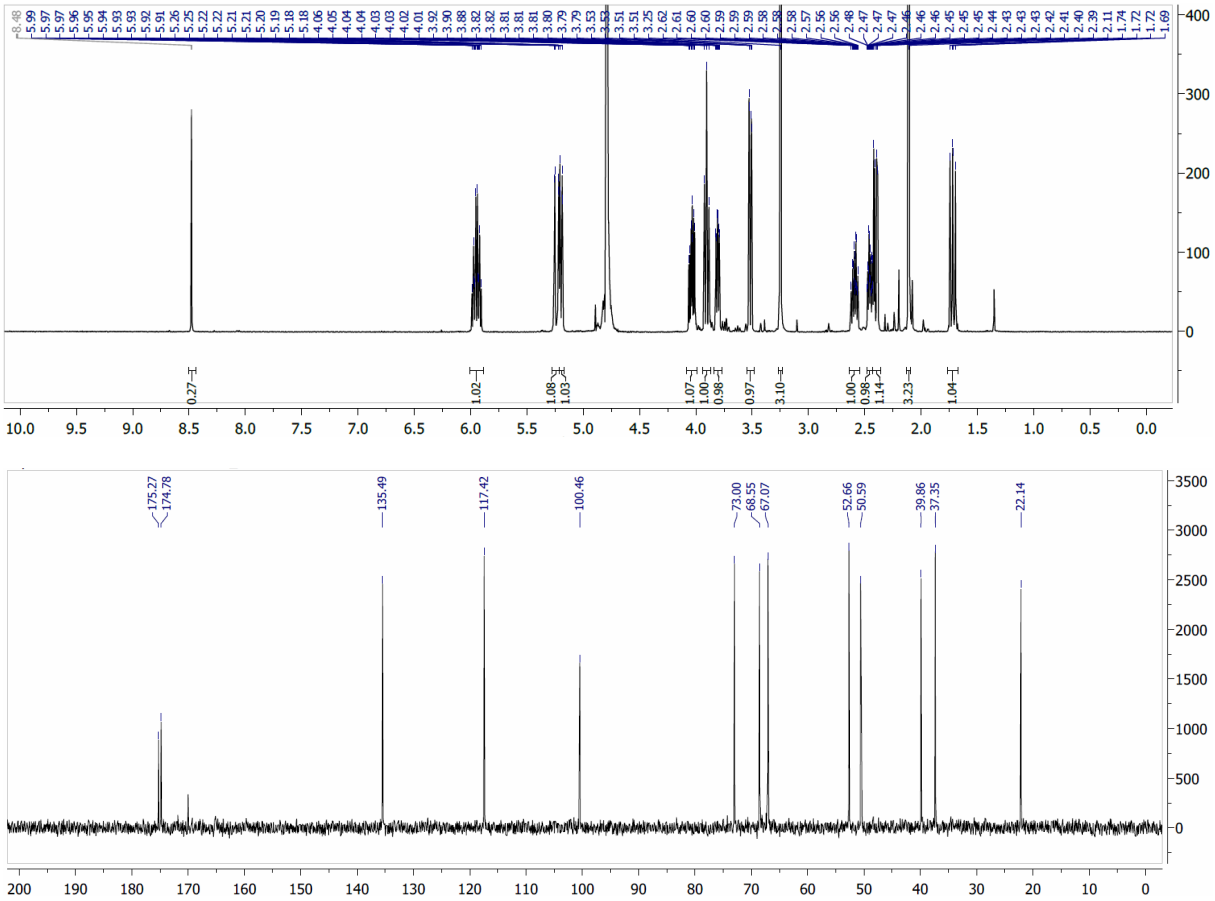
8,9-Dideoxy-2-*O*-methyl-9-methylidene-β-Neu5Ac (**7**)

For allylation see general procedure in main paper.
Aldehyde **6** (622 mg, 2.38 mmol), indium powder (546 mg, 4.76 mmol), allyl bromide (864 mg, 7.14 mmol). Column chromatography: MeOH:acetone:H_2O:DCM = 4:2:1:8 (R_f = 0.18). Yield: 700.5 mg colorless solid (97%). R_f = 0.64.



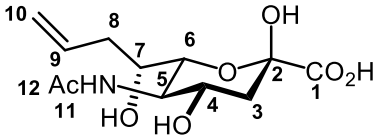
^{1}H NMR (500 MHz, D_2O) δ = 5.95 (ddt, *J* = 17.2, 10.2, 6.9 Hz, 1H, 9-H), 5.23 (dm, *J* = 17.2 Hz, 1H, 10a-H), 5.20 (dm, *J* = 10.2 Hz, 1H, 10b-H), 4.04 (ddd, *J* = 11.4, 10.1, 5.0 Hz, 1H, 4-H), 3.90 (dd, *J* = 10.1, 10.3 Hz, 1H, 5-H), 3.81 (ddd, *J* = 8.5, 5.7, 1.3 Hz, 1H, 7-H), 3.52 (dd, *J* = 10.3, 1.2 Hz, 1H, 6-H), 3.25 (s, 3H, 13-H), 2.64 – 2.55 (m, 1H, 8a-H), 2.50 – 2.43 (m, 1H, 8b-H), 2.40 (dd, *J* = 13.1, 5.0 Hz, 1H, 3_{eq}-H), 2.11 (s, 3H, 12-H), 1.72 (dd, *J* = 13.2, 11.4 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.27(C-1), 174.78 (C-11), 135.49 (C-9), 117.42 (C-10), 100.46 (C-2), 73.00 (C-6), 68.55 (C-7), 67.07 (C-4), 52.66 (C-5), 50.59 (C-13), 39.86 (C-3), 37.35 (C-8), 22.14 (C-12).



8,9-Dideoxy-9-methylidene-Neu5Ac (**8**)

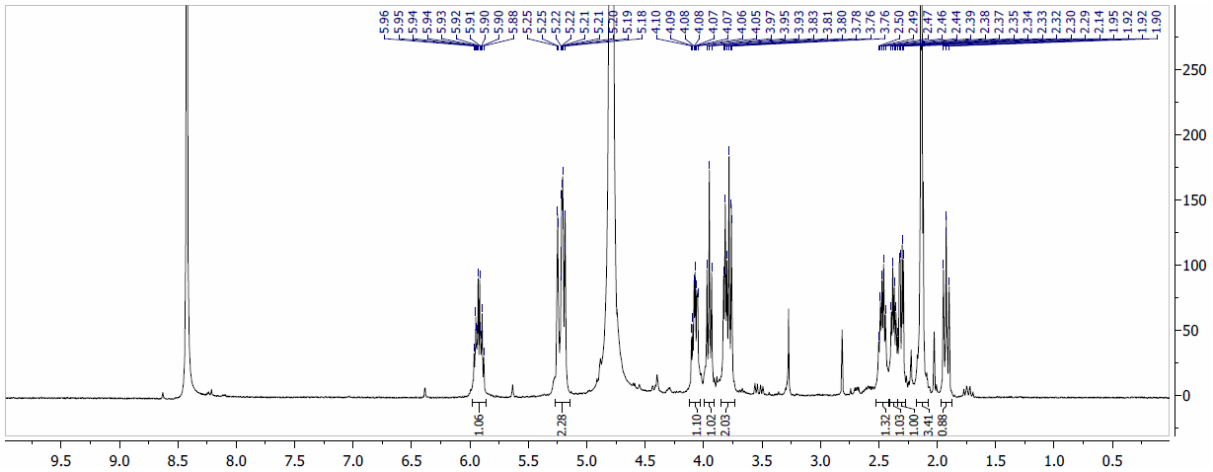
For glycoside deprotection see general procedure in main paper.
Compound **7** (102.3 mg, 0.34 mmol, 1 eq.), H_2O (10 mL), formic acid (500 µL, 610 mg, 13.3 mmol, 39 eq.). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8 (R_f = 0.18). Yield: 89.1 mg colorless solid (91%). R_f = 0.65.

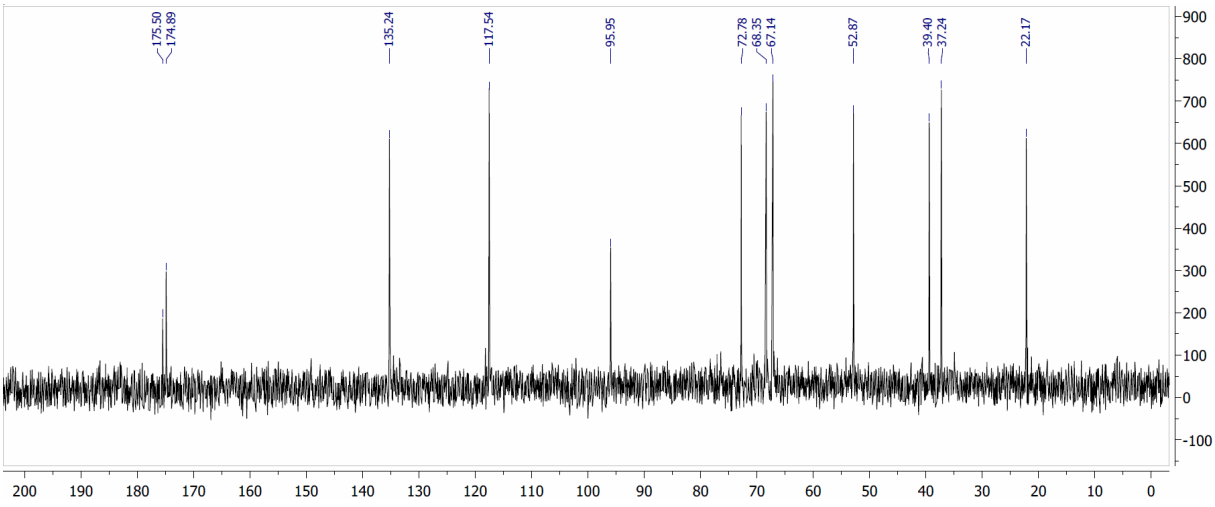


^{1}H NMR (500 MHz, D_2O) δ = 8.42 (1H, NH), 5.92 (ddt, *J* = 17.3, 10.3, 7.0 Hz, 1H, 9-H), 5.23 (dm, *J* = 17.3 Hz, 1H, 10a-H), 5.19 (dm, *J* = 10.3 Hz, 1H, 10b-H), 4.08 (ddd, *J* = 11.5, 9.9, 4.9 Hz, 1H, 4-H), 3.95 (dd, *J* = 9.9, 10.0Hz, 1H, 5-H), 3.81 (m, 1H, 7-H), 3.77 (d, *J* = 10.0 Hz, 1H, 6-H), 2.47 (ddd, *J* = 14.9, 7.5, 7.0 Hz, 1H, 8a-H), 2.40 – 2.33 (m, 1H, 8b-H), 2.47 (dd, *J* = 13.0, 4.9 Hz, 1H, 3_{eq}-H), 2.14 (s, 3H, 12-H), 1.92 (dd, *J* = 13.0, 11.5 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.50(C-1), 174.89 (C-11), 135.24 (C-9), 117.54 (C-10), 96.95 (C-2), 72.78 (C-6), 68.35 (C-7), 67.14 (C-4), 52.87 (C-5), 39.40 (C-3), 37.24 (C-8), 22.17 (C-12).

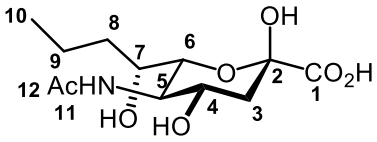
HRMS (ESI)$^+$: *m/z* calcd for $[C_{12}H_{19}N_1O_7]+Na^+$ = 312.1054; found 312.1054





8,9-Dideoxy-9-methyl-Neu5Ac (**9**)

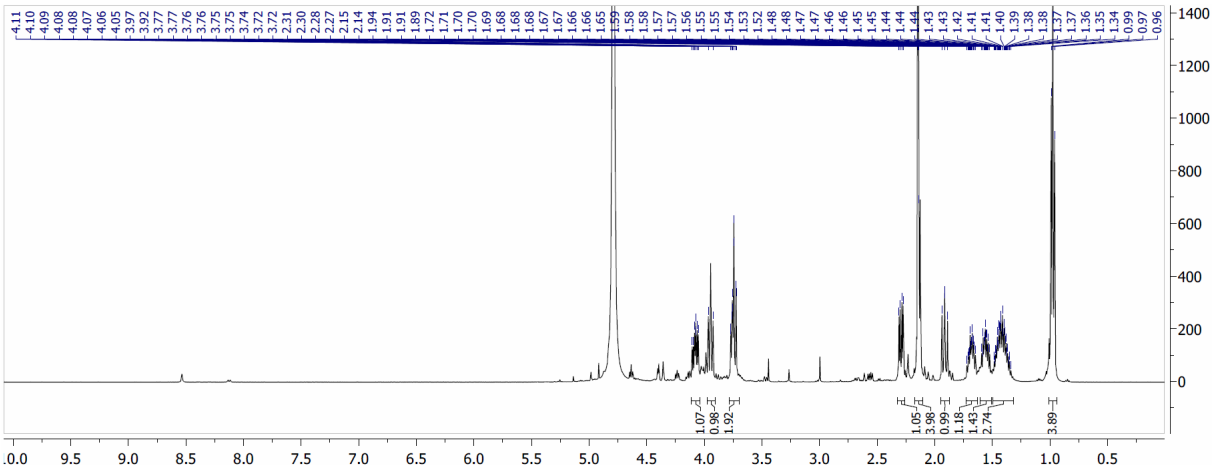
Compound **8** (62.7 mg, 0.207 mmol) was dissolved in dry methanol (11 mL), palladium on carbon (50 mg) was added and an orsat rubber expansion bag with ~2 bar of H_2 was placed on the flask for 24 h at RT with vigorous stirring. After filtration through celite the solvent was removed under vacuum. Yield: 63.1 mg colorless solid (98%). R_f = 0.59.

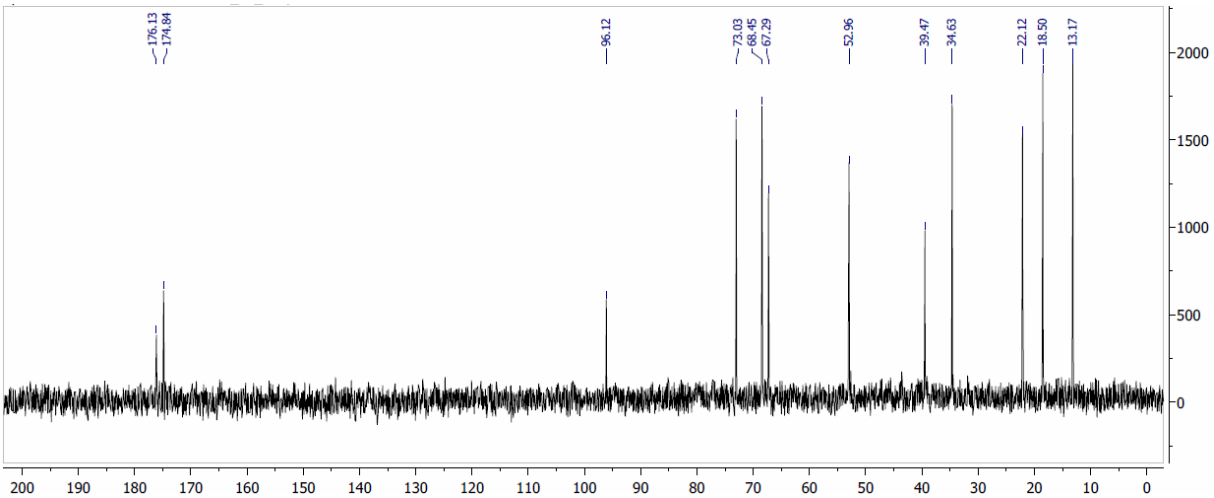


^{1}H NMR (500 MHz, D_2O) δ = 4.08 (ddd, *J* = 11.4, 10.0, 4.9 Hz, 1H, 4-H), 3.94 (dd, *J* = 10.2, 10.1 Hz, 1H, 5-H), 3.79-3.71 (m, 2H, 6-, 7-H), 2.29 (dd, *J* = 12.9, 5.0 Hz, 1H, 3_{eq}-H), 2.14 (s, 3H, 12-H), 1.91 (dd, *J* = 13.0, 11.5 Hz, 1H, 3_{ax}-H), 1.67 (m, 1H, 8a-H), 1.55 (m, 1H, 8b-H), 1.41 (m, 2H, 9-H), 0.97 (t, 3H, 10-H).

^{13}C NMR (126 MHz, D_2O) δ = 176.13(C-1), 174.84 (C-11), 96.12 (C-2), 73.03 (C-6), 68.45 (C-7), 67.29 (C-4), 52.96 (C-5), 39.47 (C-3), 34.63 (C-8), 22.12 (C-12), 18.50 (C-9), 13.17 (C-10).

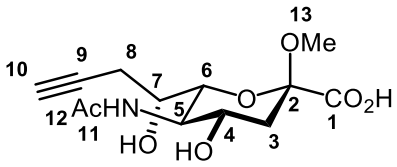
MS (ESI)$^-$: *m/z* calcd for $[C_{12}H_{21}N_1O_7]$-H^+ = 290.12; found 290.12





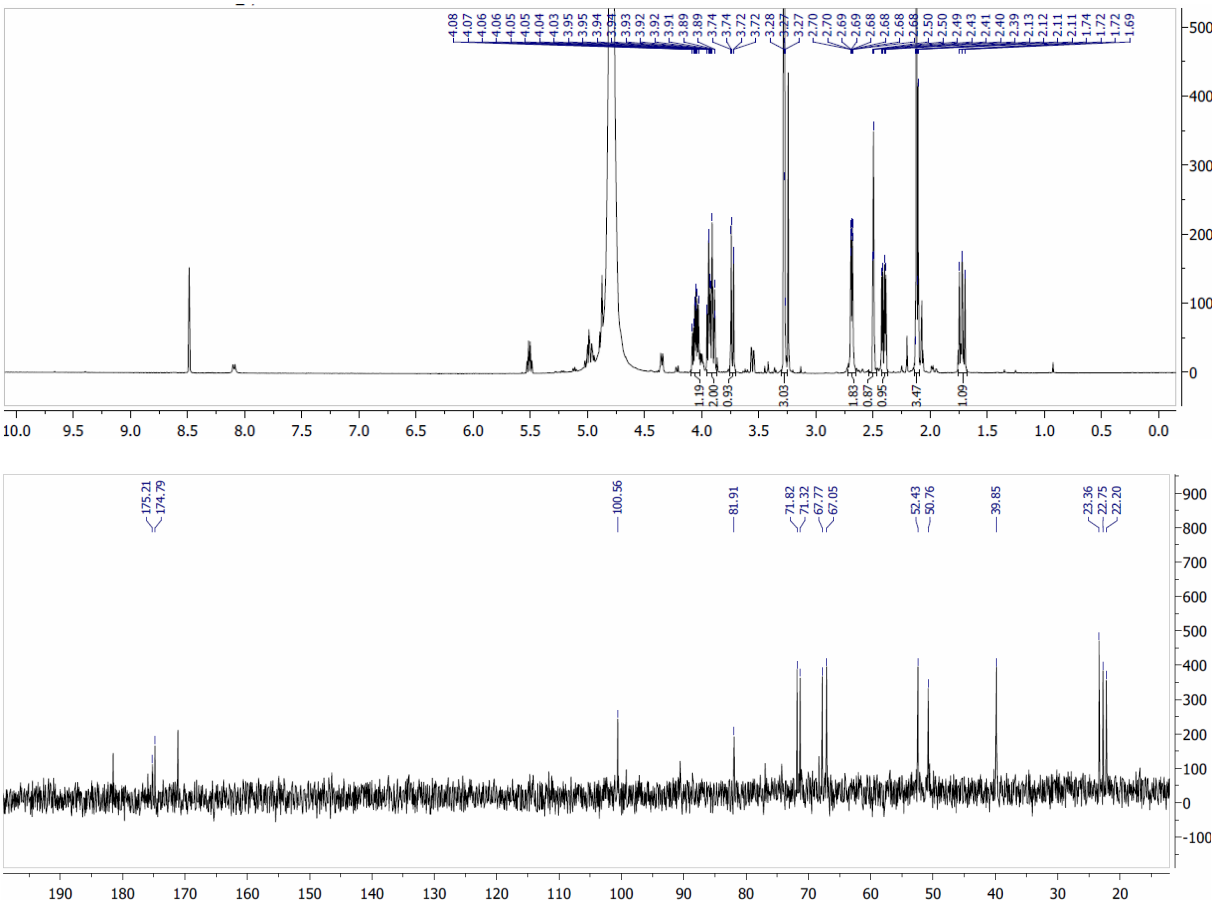
8,9-Dideoxy-9-methylidyne-2-*O*-methyl-β-Neu5Ac (**10**)

For allylation see general procedure in main paper.
Aldehyde **6** (300 mg, 1.15 mmol), indium powder (266.1 mg, 2.30 mmol), propargyl bromide (416.8 mg, 3.45 mmol). Column chromatography: MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 264.1 mg colorless solid (76%). R_f = 0.55.



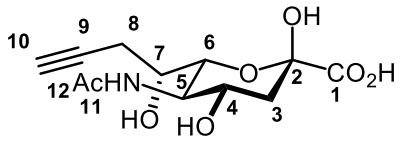
^{1}H NMR (500 MHz, D_2O) δ = 4.05 (ddd, *J* = 11.4, 10.0, 5.0 Hz, 1H, 4-H), 3.96-3.92 (m, 1H, 5-H), 3.90 (d, *J* = 10.4, 1H, 7-H), 3.73 (dd, *J* = 10.4, 1.3 Hz, 1H, 6-H), 3.28 (s, 3H, 13-H), 2.69 (ddd, *J* = 7.3, 2.7, 1.1 Hz, 2H, 8-H), 2.50 (t, *J* = 2.7 Hz, 1H, 10-H), 2.41 (dd, *J* = 13.3, 5.0 Hz, 1H, 3_{eq}-H) 2.11 (s, 3H, 12-H), 1.72 (dd, *J* = 13.3, 11.5 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.17(C-1), 174.76 (C-11), 100.50 (C-2), 81.92 (C-9), 71.78 (C-6), 71.29 (C-10), 67.79 (C-7), 67.05 (C-4), 52.44 (C-5), 50.74 (C-13), 39.86 (C-3), 22.71 (C-8), 22.18 (C-12).



8,9-Dideoxy-9-methylidyne-Neu5Ac (**11**)

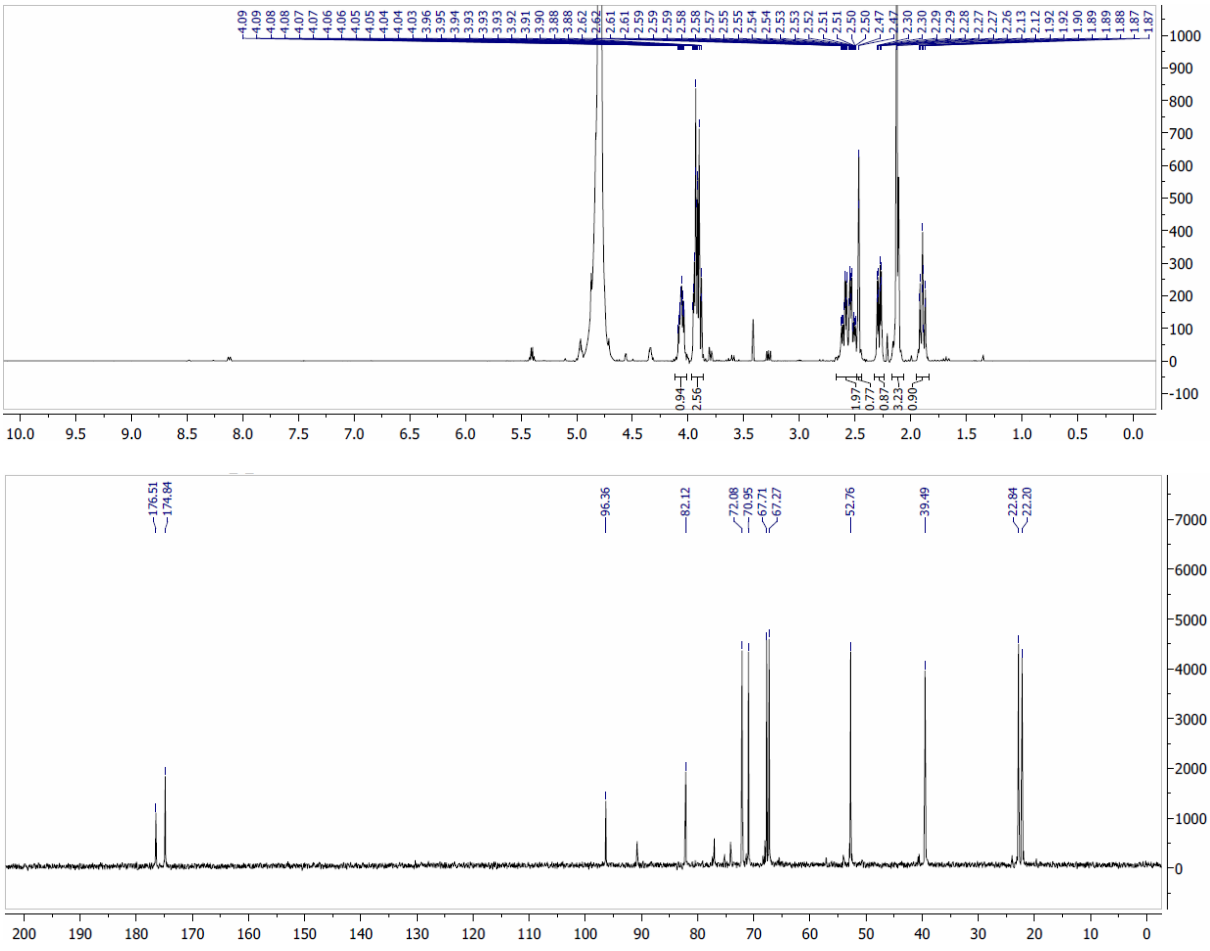
For glycoside deprotection see general procedure in main paper. Compound **10** (437.1 mg, 1.451 mmol, 1 eq.), H_2O (10 mL), formic acid (90 µL, 109.8 mg, 2.38 mmol, 1.6 eq.). 70°C for 24h, repeated four times (NMR control). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 73.2 mg colorless solid (18%). R_f = 0.54.



^{1}H NMR (500 MHz, D_2O) δ = 4.06 (ddm, *J* = 9.4, 4.9 Hz, 1H, 4-H), 4.00-3.86 (m, 3H, 5-, 7-, 6-H), 2.60 (ddd, *J* = 16.8, 7.4, 2.3 Hz, 1H, 8a-H), 2.52 (ddd, *J* = 16.8, 7.0, 2.3 Hz, 1H, 8b-H), 2.47 (dm, *J* = 2.3 Hz, 1H, 10-H), 2.28 (ddd, *J* = 13.0, 5.0 Hz, 1H, 3_{eq}-H) 2.13 (s, 3H, 12-H), 1.89 (dd, *J* = 13.0, 11.4 Hz, 1H, 3_{ax}-H).

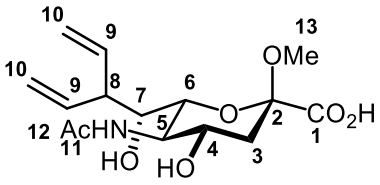
^{13}C NMR (126 MHz, D_2O) δ = 176.51(C-1), 174.84 (C-11), 96.36 (C-2), 82.12 (C-9), 72.08 (C-6), 70.95 (C-10), 67.71 (C-7), 67.27 (C-4), 52.76 (C-5), 39.49 (C-3), 22.84 (C-8), 22.20 (C-12).

MS (ESI)$^-$: *m/z* calcd for $[C_{12}H_{17}N_1O_7]$-H^+ = 286.09; found 286.09



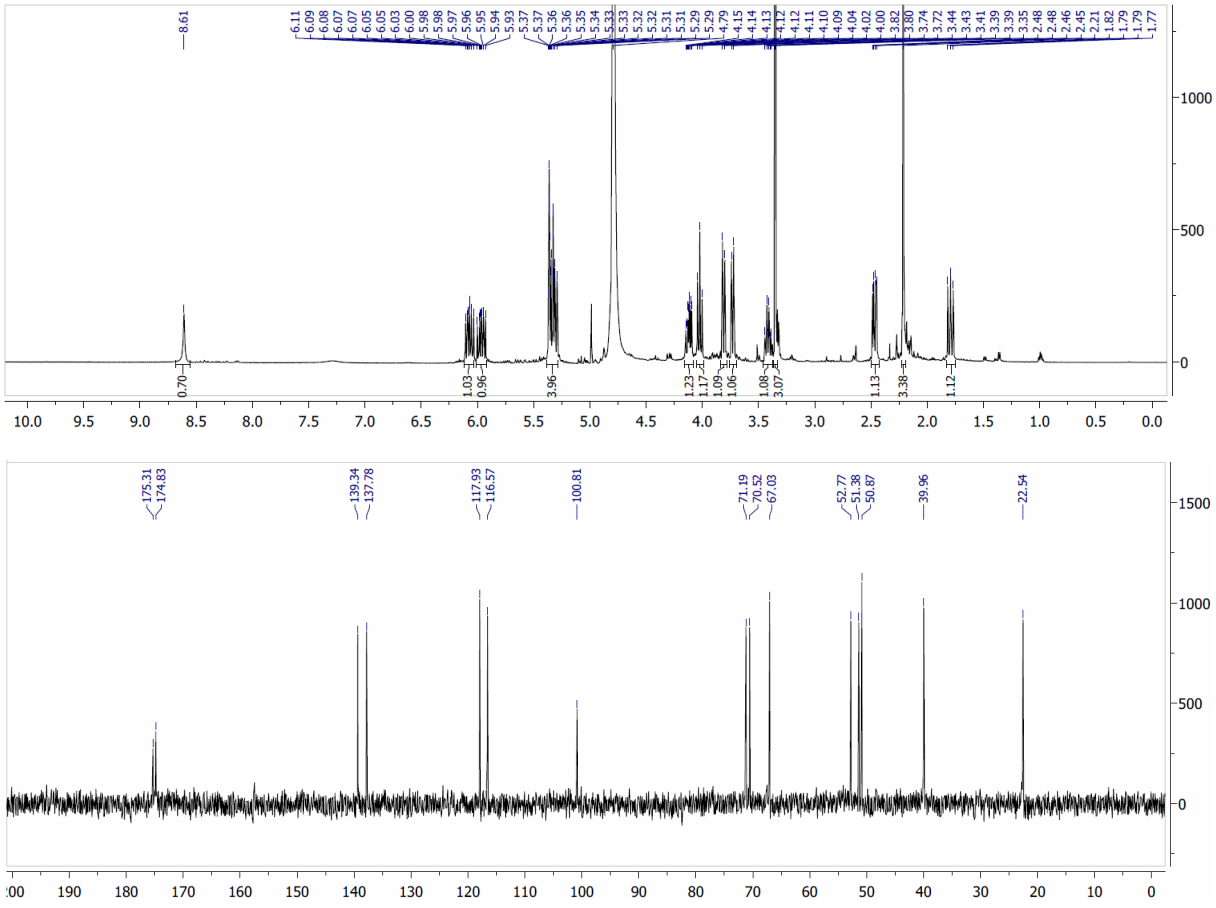
8,9-Dideoxy-8-ethenyl-2-*O*-methyl-9-methylidene-β-Neu5Ac (**12**)

For allylation see general procedure in main paper.
Aldehyde **6** (1.00 g, 3.83 mmol), indium powder (879.1 mg, 7.66 mmol), pentadienyl bromide (1.688 g, 11.48 mmol). Column chromatography: MeOH:acetone:H_2O:DCM = 4:2:1:10 Yield: 556.0 mg colorless solid (44%). R_f = 0.68.



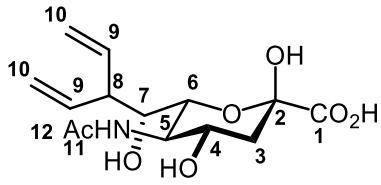
^{1}H NMR (500 MHz, D_2O) δ = 8.61 (s, 1H, NH), 6.07 (ddd, *J* = 17.1, 10.2, 8.1 Hz, 1H, 9a-H), 5.96 (ddd, *J* = 17.0, 10.7, 8.6 Hz, 1H, 9b-H), 5.38 – 5.28 (m, 4H, 10a,b-H), 4.12 (ddd, *J* = 11.4, 10.2, 4.9 Hz, 1H, 4-H), 4.02 (dd, *J* = 10.2 Hz, 1H, 5-H), 3.81 (d, *J* = 10.2 Hz, 1H, 6-H), 3.73 (d, *J* = 9.9 Hz, 1H, 7-H), 3.42 (m, 1H, 8-H), 3.35 (s, 3H, 13-H), 2.47 (dd, *J* = 13.1, 4.9 Hz, 1H, 3_{eq}-H), 2.21 (s, 3H, 12-H), 1.79 (dd, *J* = 13.1, 11.4 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.31 (C-1), 174.83 (C-11), 139.34 (C-9a), 137.78 (C-9b), 117.93 (C-10a), 116.57 (C-10b), 100.81 (C-2), 71.19 (C-6), 70.52 (C-7), 67.03 (C-4), 52.77 (C-5), 51.38 (C-8), 50.87 (C-13), 39.96 (C-3), 22.54 (C-12).



8,9-Dideoxy-8-ethenyl-9-methylidene-Neu5Ac (**13**)

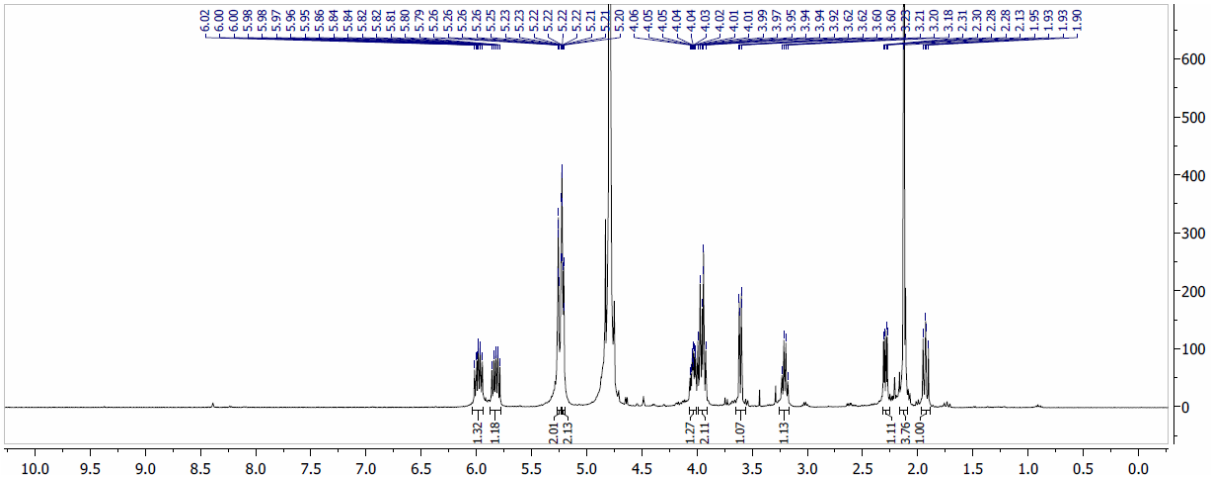
For glycoside deprotection see general procedure in main paper. Compound **12** (503.3 mg, 1.451 mmol, 1 eq.), H_2O (10 mL), formic acid (500 µL, 610 mg, 13.3 mmol, 9 eq.). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:10. Yield: 294.0 mg colorless solid (61%). R_f = 0.67.

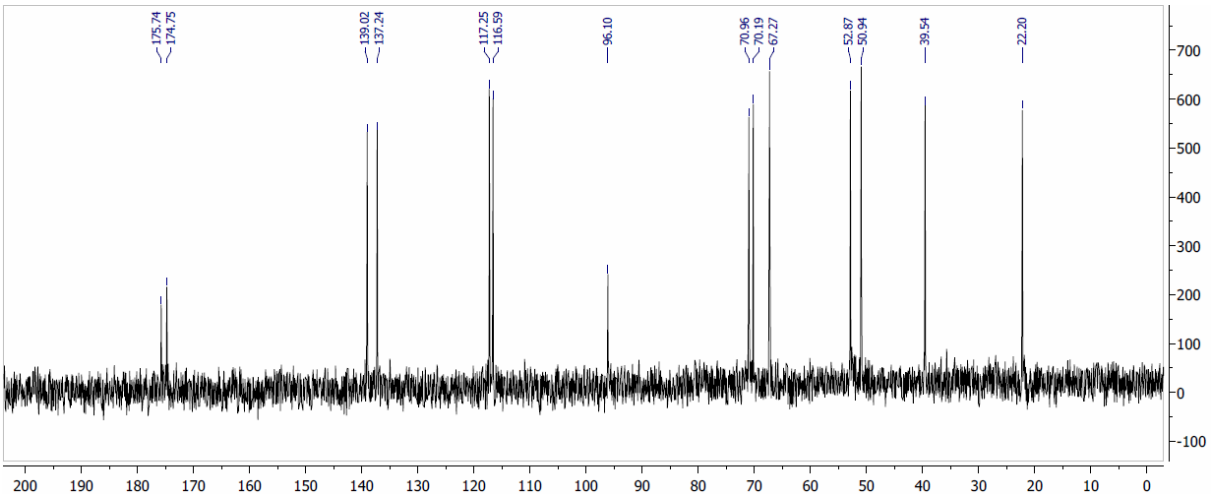


^{1}H NMR (500 MHz, D_2O) δ = 5.98 (ddd, *J* = 17.1, 10.3, 8.3 Hz, 1H, 9a-H), 5.82 (ddd, *J* = 17.1, 10.3, 8.7 Hz, 1H, 9b-H), 5.27 – 5.19 (m, 4H, 10a,b-H), 4.10-4.00 (m, 1H, 4-H), 4.02 (dd, *J* = 10.0, 9.8 Hz, 1H, 5-H), 3.93 (d, *J* = 10.0 Hz, 1H, 6-H), 3.61 (dd, *J* = 10.0, 1.0 Hz, 1H, 7-H), 3.20 (ddd, *J* = 10.0, 8.7, 8.3 Hz, 1H, 8-H), 2.29 (dd, *J* = 12.9, 4.7 Hz, 1H, 3_{eq}-H), 2.13 (s, 3H, 12-H), 1.93 (dd, *J* = 12.9, 11.3 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.74 (C-1), 174.75 (C-11), 139.02 (C-9a), 137.24 (C-9b), 117.25 (C-10a), 116.59 (C-10b), 96.10 (C-2), 70.96 (C-6), 70.19 (C-7), 67.27 (C-4), 52.87 (C-5), 50.94 (C-8), 39.54 (C-3), 22.20 (C-12).

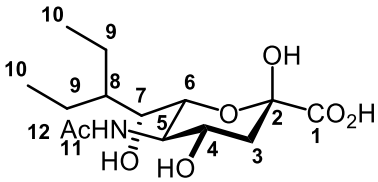
HRMS (ESI)$^+$: *m/z* calcd for $[C_{14}H_{21}N_1O_7]+Na^+$ = 338.1210; found 338.1210





8,9-Dideoxy-8-ethyl-9-methyl-Neu5Ac (**14**)

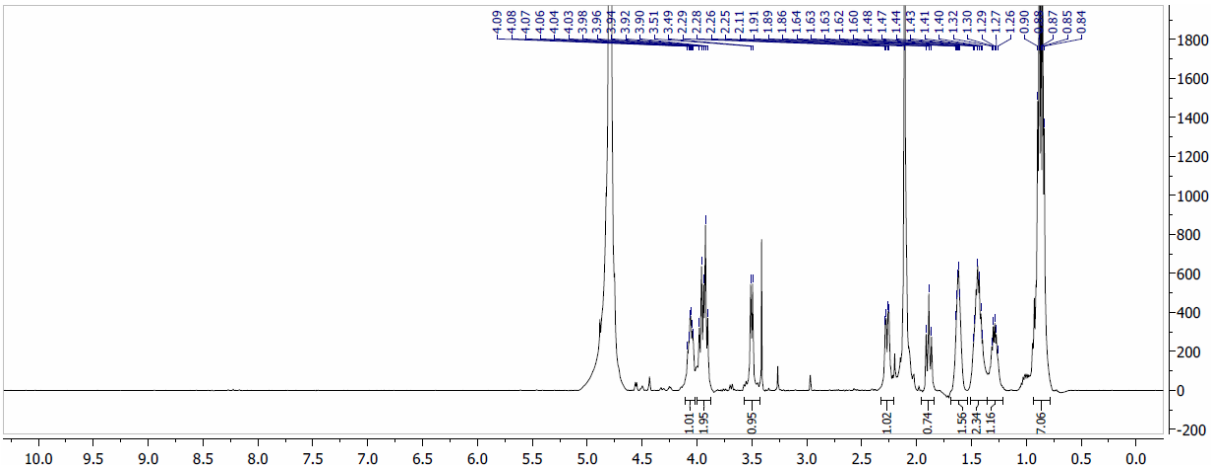
Compound **13** (50 mg, 0.16 mmol, 1 eq.) was dissolved in dry methanol (5 mL), palladium on coal (30 mg) was added and an orsat rubber expansion bag with ~2 bar of H_2 was placed on the flask for 24 h at RT with vigorous stirring. After filtration through celite the solvent was removed under vacuum. Yield: 48.8 mg colorless solid (96%).

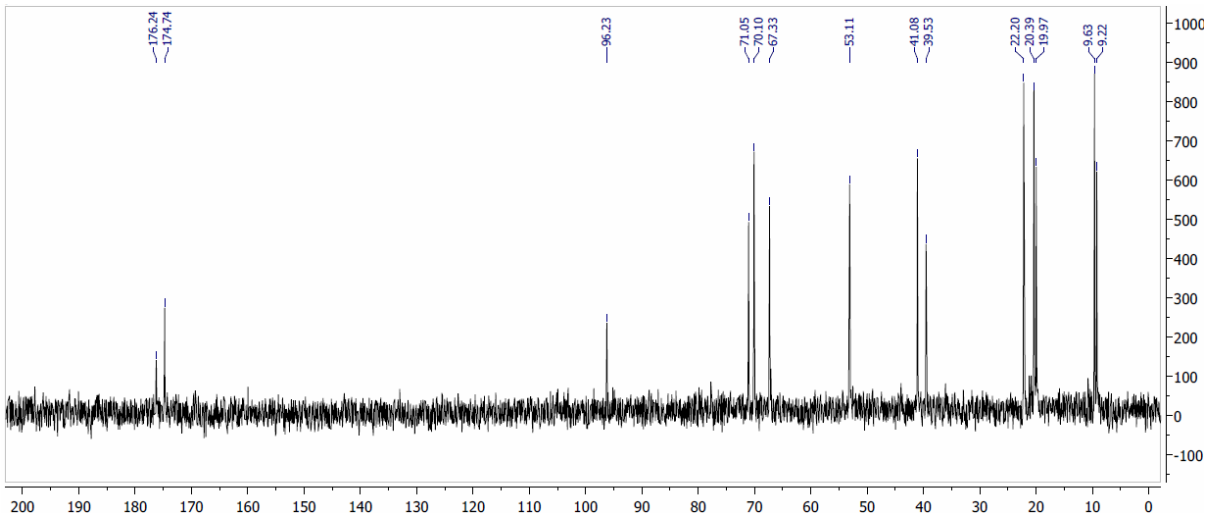


^{1}H NMR (500 MHz, D_2O) δ = 4.06 (dm, *J* = 15.4 Hz, 1H, 4-H), 4.00-3.87 (m, 2H, 5-, 6-H), 3.50 (d, *J* = 8.9 Hz, 1H, 7-H), 2.27 (dd, *J* = 12.9, 5.0 Hz, 1H, 3_{eq}-H), 2.11 (s, 3H, 12-H), 1.89 (dm, *J* = 12.2 Hz, 1H, 3_{ax}-H), 1.69-1.54 (m, 2H, 8-, 9-H), 1.51-1.36 (m, 2H, 9-H) 1.33-1.24 (m, 2H, 9-H), 0.88 (t, *J* = 7.2 Hz, 3H, 10-H), 0.85 (t, *J* = 7.2 Hz, 3H, 10-H).

^{13}C NMR (126 MHz, D_2O) δ = 176.24 (C-1), 174.74 (C-11), 96.23 (C-2), 71.05 (C-6), 70.10 (C-7), 67.33 (C-4), 53.11 (C-5), 41.08 (C-8), 39.53 (C-3), 22.20 (C-12), 20.39 (C-9), 19.97 (C-9), 9.63 (C-10), 9.22 (C-10).

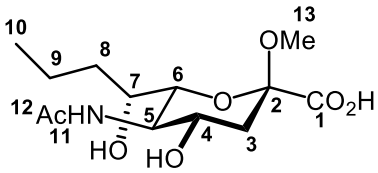
MS (ESI)$^-$: *m/z* calcd for $[C_{14}H_{25}N_1O_7]$-H^+ = 318.16; found 318.16





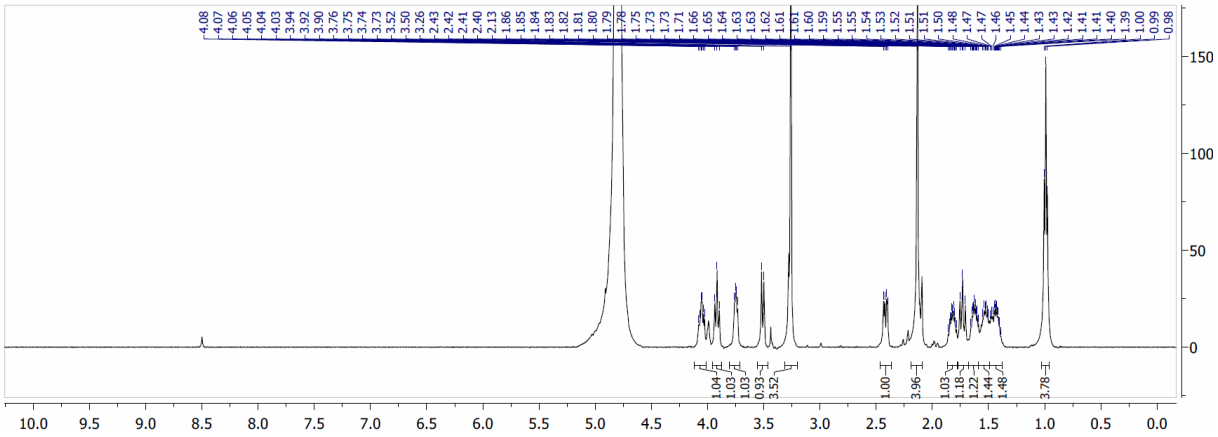
8,9-Dideoxy-9-methyl-2-*O*-methyl-β-Neu5Ac (**15**)

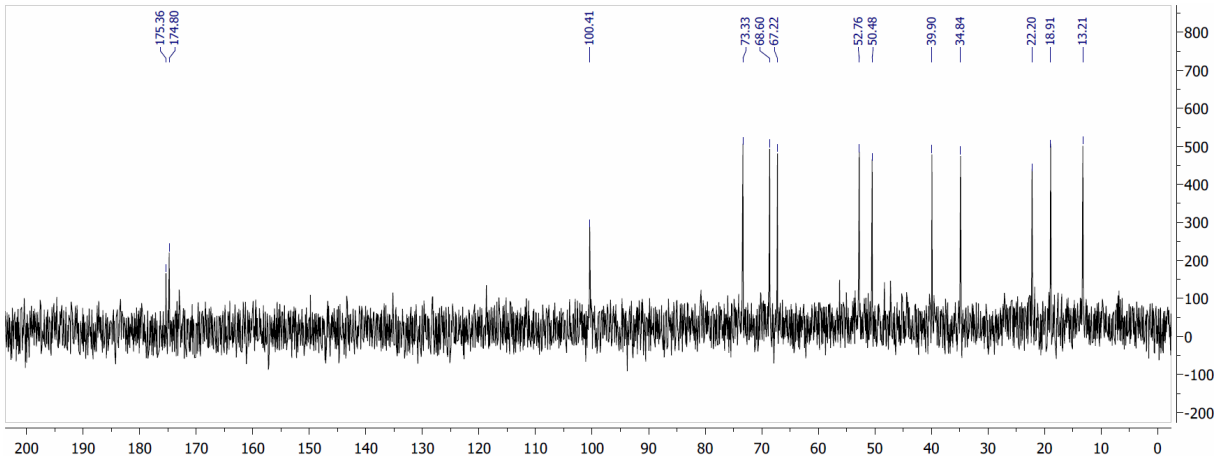
Prepared by the same procedure as for **9.** Yield: colorless solid (98%). R_f = 0.59.



^{1}H NMR (500 MHz, D_2O) δ = 4.05 (ddd, *J* = 10.7, 10.7, 5.0 Hz, 1H, 4-H), 3.92 (dd, *J* = 10.1, 10.1 Hz, 1H, 5-H), 3.75 (dd, *J* = 8.6, 5.1 Hz, 1H, 7-H), 3.51 (d, *J* = 10.3 Hz, 1H, 6-H) 3.26 (s, 3H, 13-H), 2.41 (dd, *J* = 13.2, 5.0 Hz, 1H, 3_{eq}-H), 2.13 (s, 3H, 12-H), 1.82 (dtd, *J* = 13.6, 8.9, 5.2 Hz, 1H, 8a-H), 1.73 (dd, *J* = 13.2, 11.4 Hz, 1H, 3_{ax}-H), 1.62 (dtd, *J* = 13.6, 8.9, 5.7 Hz, 1H, 8b-H), 1.55-1.49 (m, 1H, 9a-H), 1.48-1.37 (m, 1H, 9b-H), 0.99 (t, *J* = 7.1, 3H, 10-H).

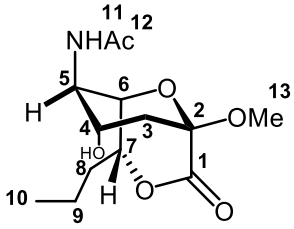
^{13}C NMR (126 MHz, D_2O) δ = 175.36(C-1), 174.80 (C-11), 100.41 (C-2), 73.33 (C-6), 68.65 (C-7), 67.22 (C-4), 52.76 (C-5), 50.48 (C-13), 39.90 (C-3), 34.84 (C-8), 22.20 (C-12), 18.91 (C-9), 13.21 (C-10).





1,7-Lactone (**16**)

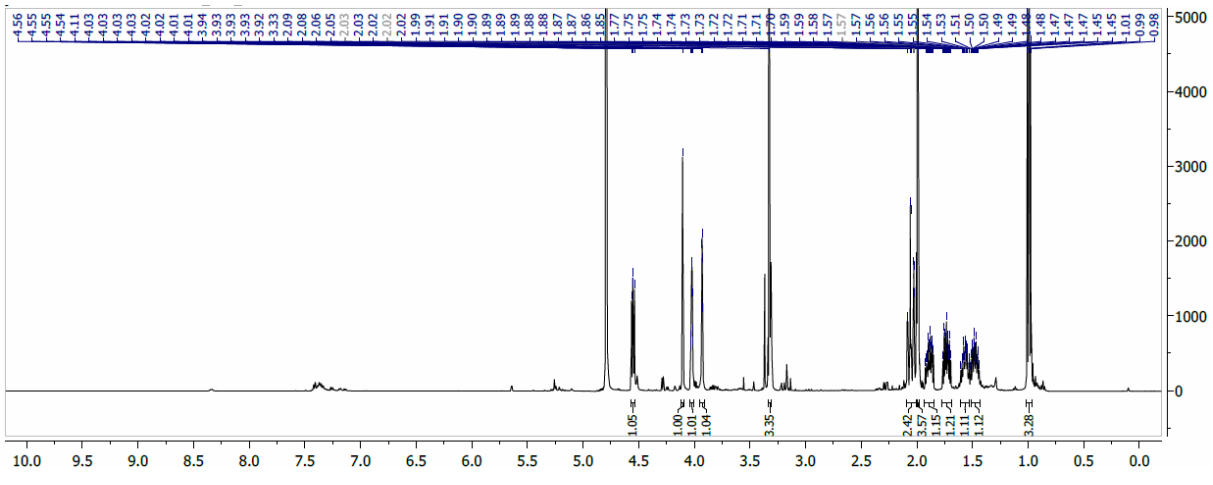
The synthesis of **16** was conducted by a procedure adapted from the literature.[6] For details, see main paper. Column chromatography was performed with EtOAc:MeOH = 9:1. Yield: 28.8 mg colorless solid (16%). R_f = 0.85.

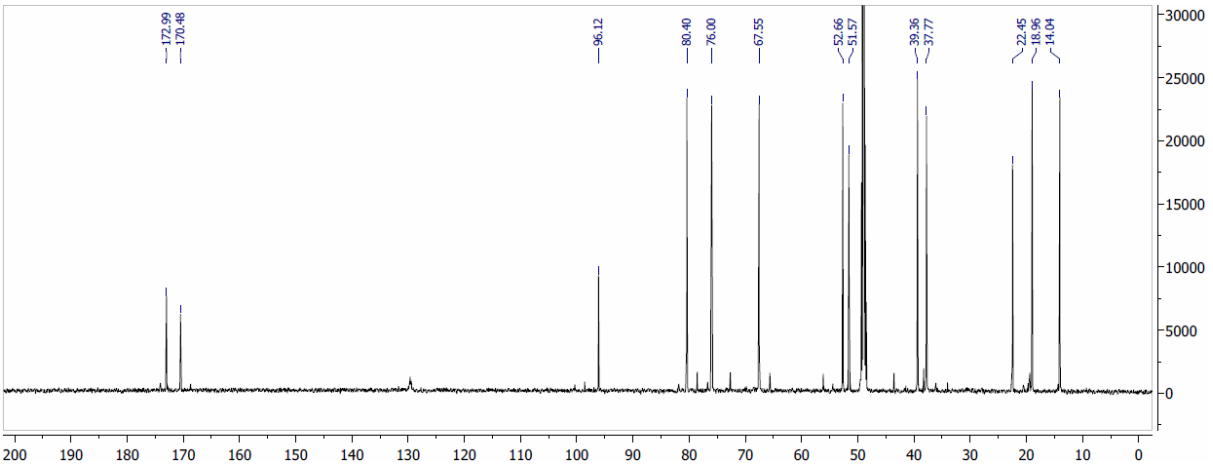


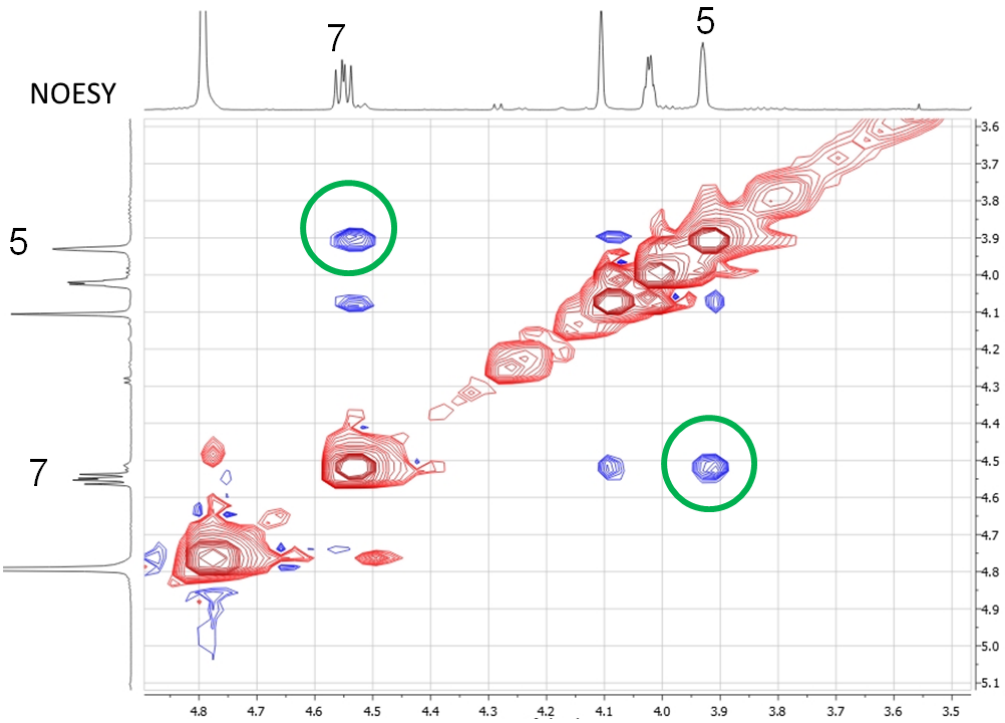
^{1}H NMR (500 MHz, methanol-d_4) δ = 4.55 (dd, J = 7.7, 5.4 Hz, 1H, 7-H), 4.11 (s, 1H, 6-H), 4.05 – 3.99 (m, 1H, 4-H), 3.93 (m, 1H, 5-H), 3.33 (s, 3H, 13-H), 2.09-2.00 (dm, J = 14.1Hz, 2H, $3_{ax/eq}$-H), 1.99 (s, 3H, 12-H), 1.94 – 1.83 (m, 1H, 8a-H), 1.77-1.70 (m, 1H, 8b-H), 1.62 – 1.53 (m, 1H, 9a-H), 1.51-1.44 (m, 1H, 9b-H) 0.99 (t, J = 7.4 Hz, 3H, 10-H).

^{13}C NMR (126 MHz, MeOD) δ = 172.99 (C-12), 170.48 (C-1), 96.12 (C-2), 80.40 (C-7), 76.00 (C-6), 67.55 (C-4), 52.66 (C-5), 51.57 (C-13), 39.36 (C-8), 37.77 (C-3), 22.45 (C-12), 18.96 (C-9), 14.04 (C-10).

HRMS (ESI)$^+$: m/z calcd for $[C_{13}H_{21}N_1O_6]+H^+$ = 288.1442; found 288.1443

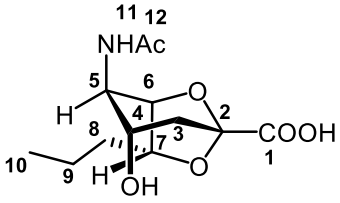





NOESY
7
5
5
7
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
5.0
5.1
4.8
4.7
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5

2,7-Anhydro-9-methyl-Neu5Ac (**17**) / 8,9-dideoxy-9-methyl-Neu5Ac (**9**)

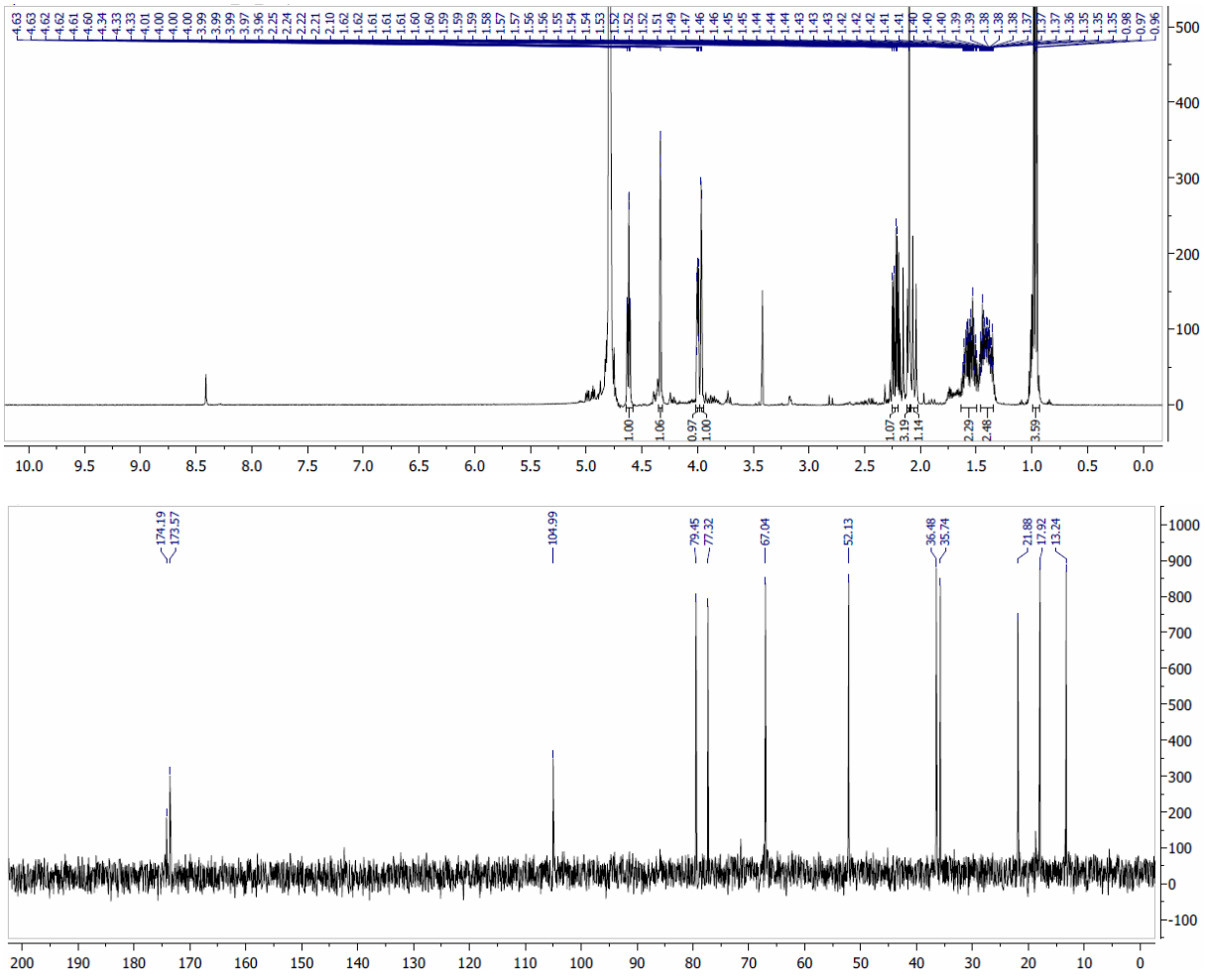
Compound **15** (221.7 mg, 0.73 mmol, 1 eq.) was dissolved in H_2O (10 mL) and formic acid (30.16 µL, 1.1 eq.) was added. The solution was heated to 85°C for 24 h. The solvents were removed under vacuum and the solid was purified by column chromatography with MeOH:acetone:H_2O:DCM = 4:2:1:10. The deprotected product (**9**) was furnished as a colorless solid: 71.2 mg (34%), and the 2,7-lactone (**17**) was isolated with 40.6 mg (20%).

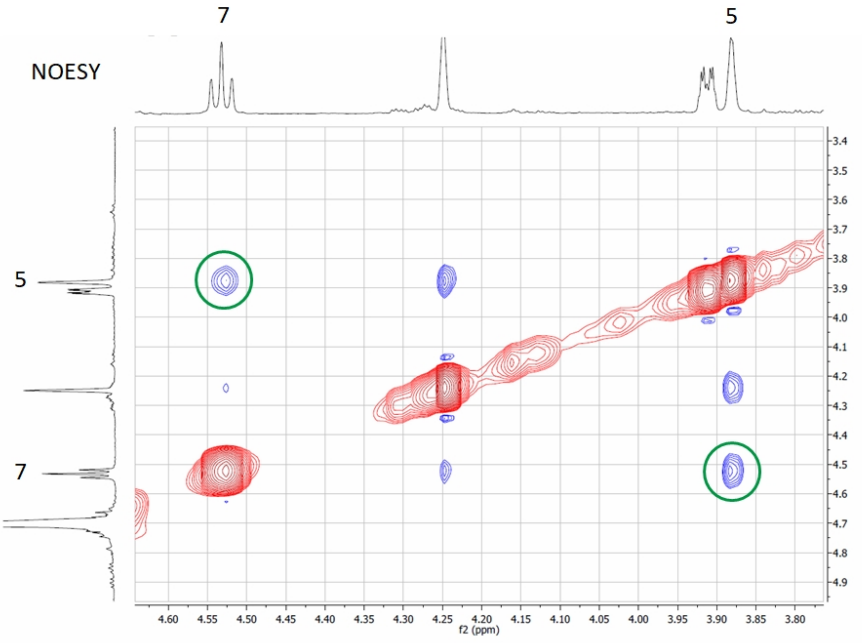


^{1}H NMR (500 MHz, D_2O) δ = 4.62 (ddd, *J* = 6.7, 6.6, 1.0 Hz, 1H, 7-H), 4.33 (dd, *J* = 1.7, 1.0 Hz, 1H, 6-H), 4.00 (ddm, *J* = 5.7, 1.7 Hz, 1H, 4-H), 3.97 (d, *J* = 1.7 Hz, 1H, 5-H), 2.23 (dd, *J* = 15.3, 5.7 Hz, 1H, 3_{ax}-H), 2.10 (s, 3H, 12-H), 2.05 (dd, *J* = 15.3, 1.7 Hz, 1H, 3_{eq}-H), 1.56 (m, 2H, 8-H), 1.41 (m, 2H, 9-H), 0.97 (t, 3H, 10-H).

^{13}C NMR (126 MHz, D_2O) δ = 174.19(C-1), 173.57 (C-11), 104.99 (C-2), 79.45 (C-6), 77.32 (C-7), 67.04 (C-4), 52.13 (C-5), 36.48 (C-8), 35.74 (C-3), 21.88 (C-12), 17.92 (C-9), 13.24 (C-10).

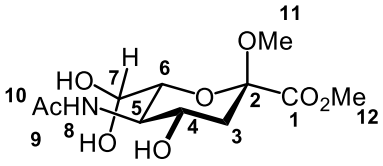
HRMS (ESI)$^+$: *m/z* calcd for $[C_{12}H_{19}N_1O_6]$+Na^+ = 296.1105; found 296.1104




NOESY
7
5
5
7
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.5
4.6
4.7
4.8
4.9
4.60
4.55
4.50
4.45
4.40
4.35
4.30
4.25
4.20
4.15
4.10
4.05
4.00
3.95
3.90
3.85
3.80
f2 (ppm)

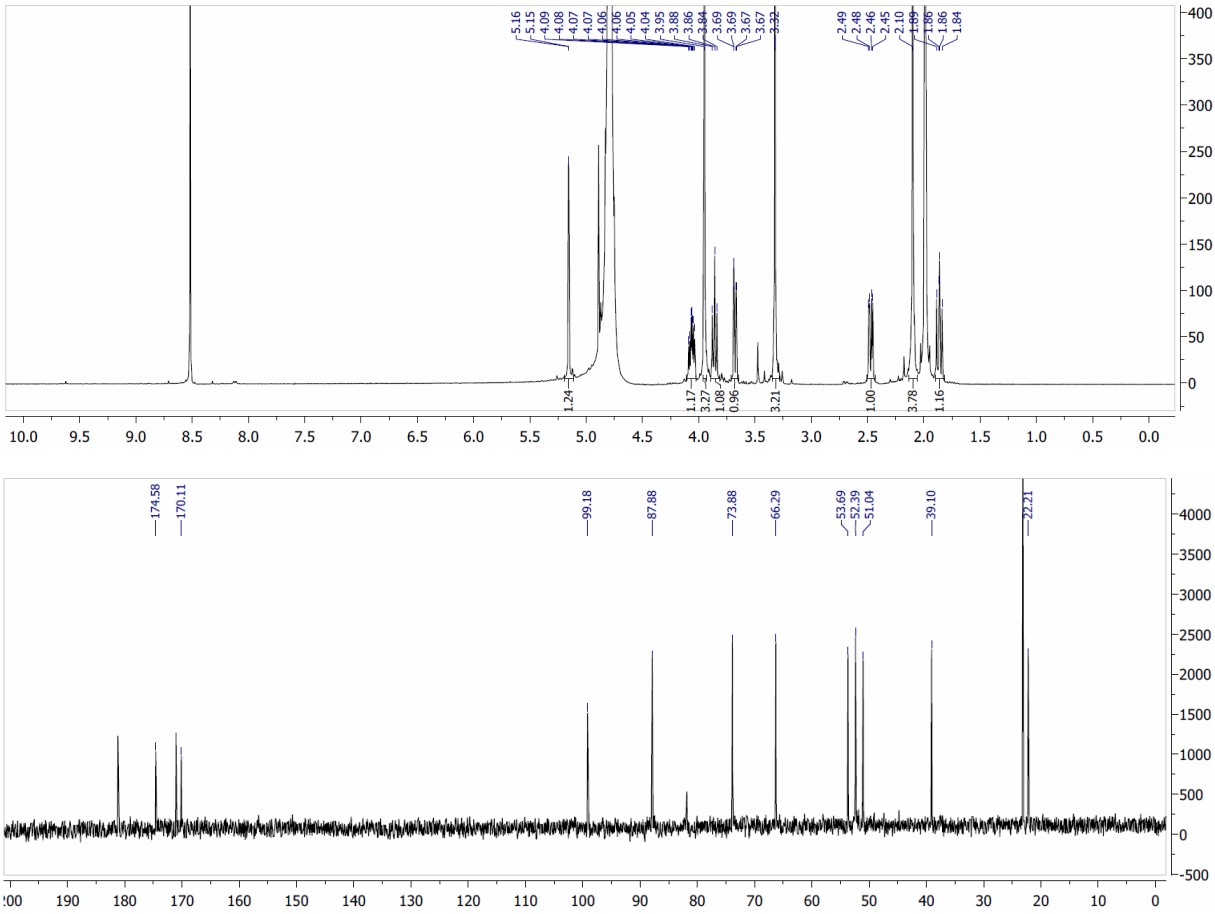
Methyl 3-(acetylamino)-3,5-dideoxy-L-*lyxo*-(6*S*)-heptulo-2,6-pyranosiduronic acid methyl ester (**18**)

To an aqueous solution of **4** (302.7 mg, 0.90 mmol, 1 eq. in 6 mL) was added 0.2 M $NaIO_4$ (23.3 mL). After standing for 2h in the dark at RT TLC showed completion. A solution of $Ba(OAc)_2$ (35 mL, 0.1 M) was added to remove IO_4^- and I -salts. Precipitated salts were filtered off, then CO_2 was bubbled through the solution to precipitate excess of barium ions. After filtration the solvent was removed under vacuum. The residual solid was dissolved in cold MeOH and salts were filtered off. R_f = 0.84.



^{1}H NMR (500 MHz, D_2O) δ = 8.52 (s, 1NH, 8-H), 5.16 (d, *J* = 1.9 Hz, 1-2H, 7-H), 4.06 (ddd, *J* = 11.3, 9.9, 5.0 Hz, 1H, 4-H), 3.95 (s, 3H, 12-H), 3.86 (dd, *J* = 10.3, 10.2 Hz, 1H, 5-H), 3.68 (dd, *J* = 10.6, 1.8 Hz, 1H, 6-H), 3.22 (s, 3H, 11-H), 2.47 (dd, *J* = 13.3, 5.0 Hz, 1H, 3_{eq}-H), 2.10 (s, 3H, 10-H), 1.86 (dd, *J* = 13.3, 11.3 Hz, 1H, 3_{ax}-H).

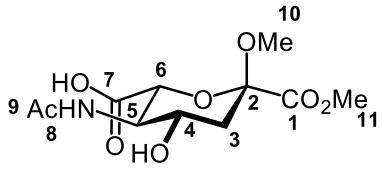
^{13}C NMR (126 MHz, D_2O) δ = 174.58(C-9), 170.11 (C-1), 99.18 (C-2), 87.88 (C-7), 73.88 (C-6), 66.29 (C-4), 53.69 (C-12), 52.39, (C-5), 51.04 (C-11), 39.10 (C-3), 22.21 (C-10).



Methyl 5-(acetylamino)-3,5-dideoxy-α-L-*arabino*-heptulo-2,6-pyranosidaric acid 1-methyl ester (**19**)

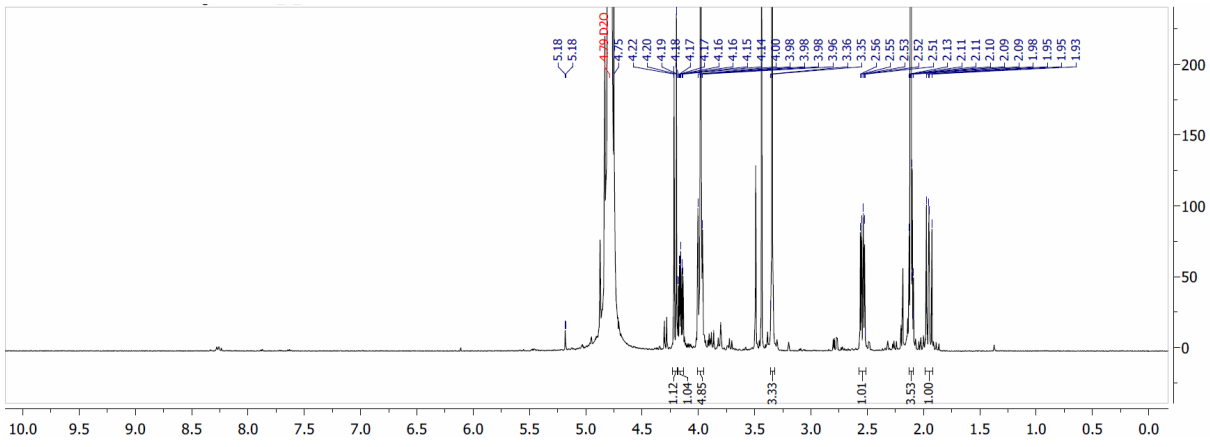
Aldehyde **18** (raw product) was dissolved in H_2O (10 mL) and stirred with $BaCO_3$ (282.6 mg, 1.43 mmol, 1.6 eq.) at 0°C. Then bromine (280 mg, 2.38 mmol, 2.6 eq.) was added. The ice bath was removed and the reaction mixture stirred for additional 12 h at RT in the dark. After TLC showed complete conversion, the reaction mixture was filtered. The solvents were removed under vacuum and the remaining solid residue was purified by column chromatography with MeOH:acetone:H_2O:DCM = 4:2:1:8. Colorless solid R_f = 0.44.

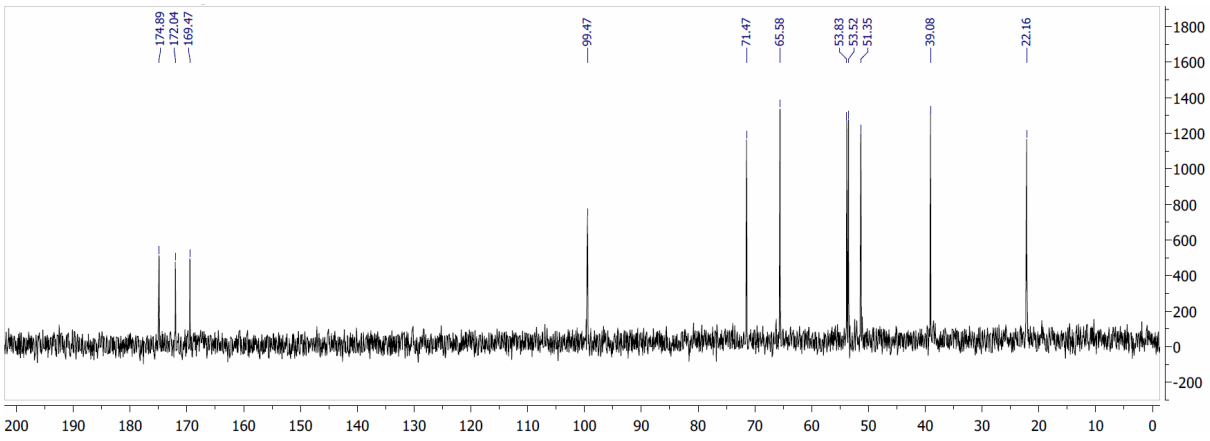
A different synthesis of **19** is described in the literature.[7] No spectral data available.



^{1}H NMR (500 MHz, D_2O) δ = 4.21 (d, J = 10.5 Hz, 1H, 6-H), 4.19-4.13 (m, 1H, 4-H), 4.01-3.95 (m 1H, 5-H), 3.98 (s, 3H, 11-H), 3.35 (s, 3H, 10-H), 2.54 (dd, J = 13.4, 5.0 Hz, 1H, 3_{eq}-H), 2.11 (s, 3H, 9-H), 1.95 (dd, J = 13.3, 11.3 Hz, 1H, 3_{ax}-H).

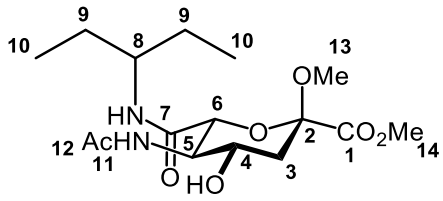
^{13}C NMR (126 MHz, D_2O) δ = 174.89 (C-7), 172.04 (C-8), 169.47 (C-1), 99.47 (C-2), 71.47 (C-6), 65.58 (C-4), 53.83 (C-11), 53.52 (C-5), 51.35 (C-10), 39.08 (C-3), 22.16 (C-9).





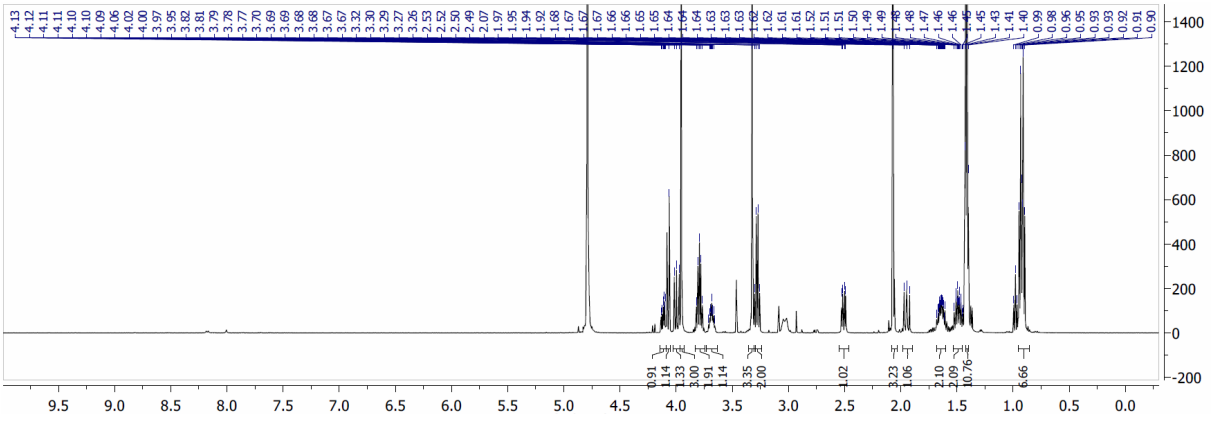
Methyl 5-(acetylamino)-3,5-dideoxy-α-L-*arabino*-heptulo-2,6-pyranosidaric acid 1-methyl ester 7-(3-pentyl)amide (**20**)

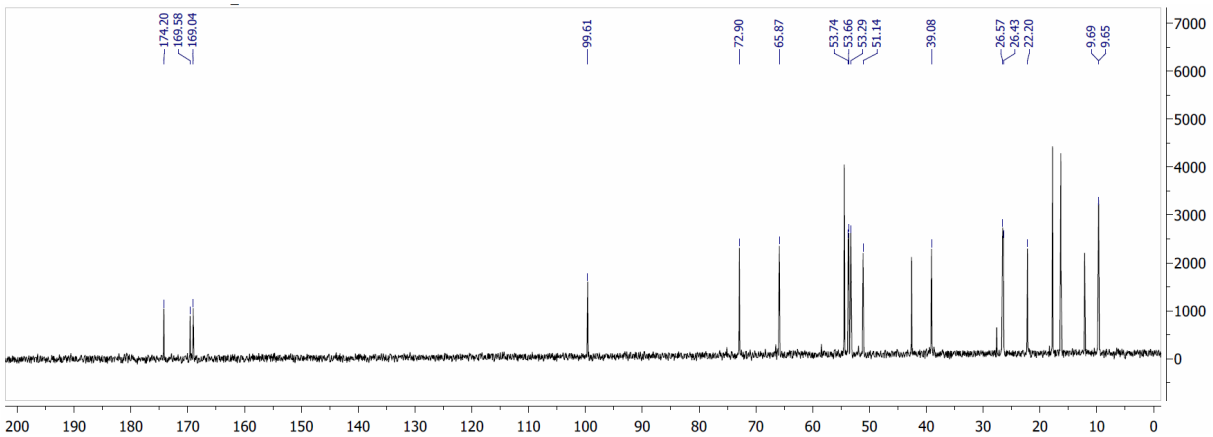
Acid **19** (raw product) was dissolved in DMF (6 mL) and treated with pentan-3-amine (81.9 mg, 0.94 mmol, 1.05 eq.) and HATU (357.2 mg, 0.94 mmol, 1.05 eq.). Then DIPEA (231.3 mg, 1.788 mmol, 2.0 eq.) was added and the reaction mixture stirred for 12 h at RT. The solvents were removed under vacuum and the solid was purified by column chromatography with MeOH:acetone:H_2O:DCM = 4:2:1:11. Yield: 99.99mg (31% from **6**) colorless solid R_f = 0.84.



^{1}H NMR (500 MHz, D_2O) δ = 4.14-4.09 (m, 1H, 4-H), 4.07 (d, *J* = 10.3 Hz, 1H, 6-H), 4.04-3.95 (dd, *J* = 10.3, 10.1 Hz, 1H, 5-H), 3.95 (s, 3H, 14-H), 3.68 (dt, *J* = 8.2, 5.0 Hz, 1H, 8-H), 3.32 (s, 3H, 13-H), 2.51 (dd, *J* = 13.4, 4.9 Hz, 1H, 3_{eq}-H), 2.07 (s, 3H, 12-H), 1.95 (dd, *J* = 13.4, 11.2 Hz, 1H, 3_{ax}-H), 1.69-1.58 (m, 2H, 9-H), 1.53-1.45 (m, 2H, 9'-H), 0.93 (t, 7.7 Hz, 3H, 10-H), 0.91 (t, 7.7 Hz, 3H, 10'-H).

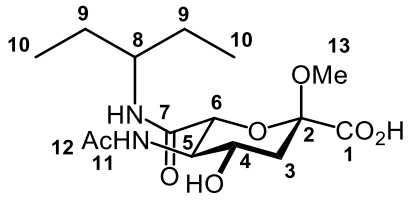
^{13}C NMR (126 MHz, D_2O) δ = 174.20 (C-11), 169.58 (C-1), 169.04 (C-7), 99.61 (C-2), 72.90 (C-6), 65.87 (C-4), 53.74 (C-5), 53.66 (C-14), 53.29 (C-8), 51.14 (C-13), 39.08 (C-3), 26.57 (C-9), 26.43 (C-9'), 22.20 (C-12), 9.69 (C-10), 9.65 (C-10').





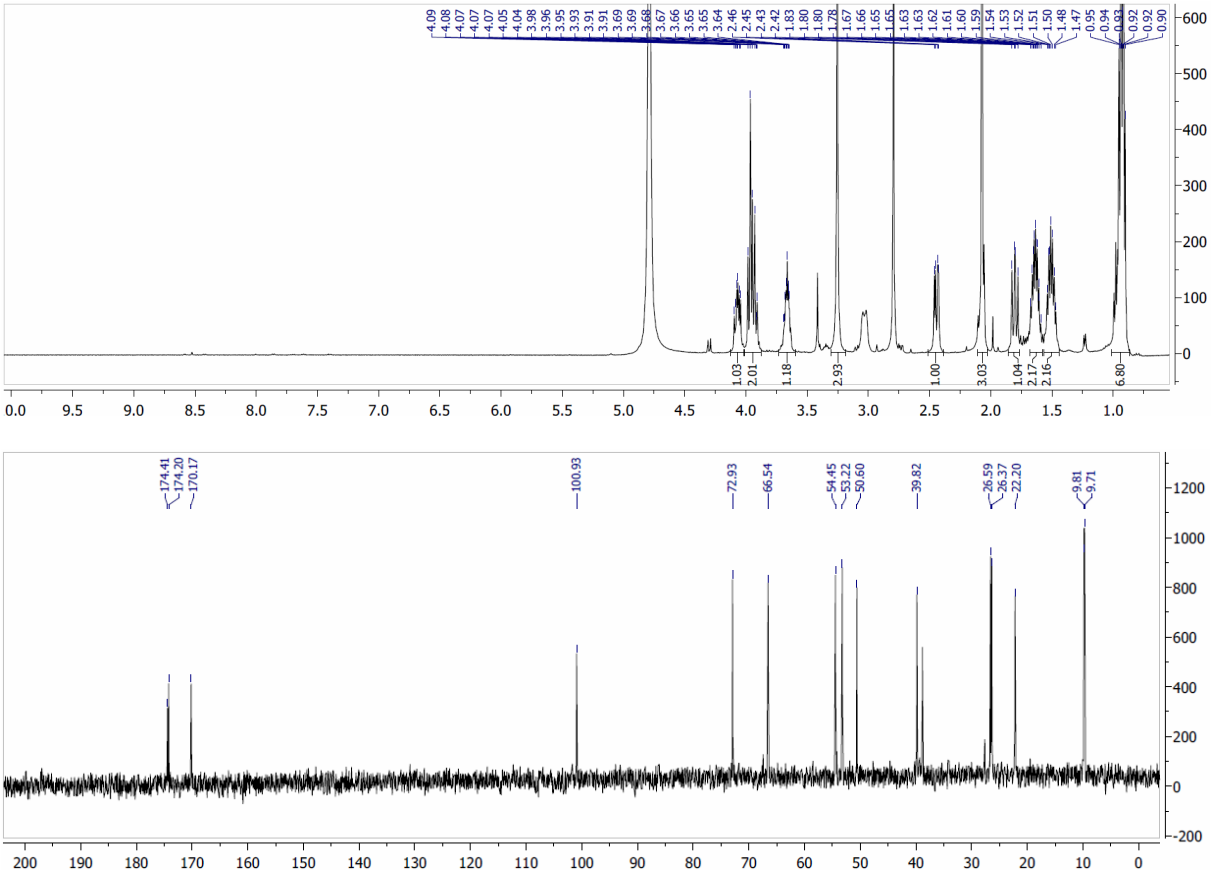
Methyl 5-(acetylamino)-3,5-dideoxy-α-L-*arabino*-heptulo-2,6-pyranosidaric acid 7-(3-pentyl)amide (**21**)

A solution of **20** (375 mg, 1.04 mmol, 1 eq.) in 0.06 N NaOH (30 mL) was stirred at RT for 3 h. After complete conversion the mixture was neutralized to pH 7.0 by addition of Dowex50 H^+ (2.5 g). The suspension was filtered and the volume of the filtrate reduced to approximately 10 mL in vacuo. This solution was lyophilized to obtain a colorless crystalline solid. Yield: 319.9 mg (89%) R_f = 0.59.



^{1}H NMR (500 MHz, D_2O) δ = 4.11-4.02 (m, 1H, 4-H), 4.99-3.94 (m, 1H, 5-H), 3.94-3.90 (d, 1H, 6-H), 3.67 (tt, *J* = 8.6, 4.1 Hz, 1H, 8-H), 3.25 (s, 3H, 13-H), 2.44 (dd, *J* = 13.3, 5.0 Hz, 1H, 3_{eq}-H), 2.07 (s, 3H, 12-H), 1.80 (dd, *J* = 13.3, 11.3 Hz, 1H, 3_{ax}-H), 1.68-1.58 (m, 2H, 9-H), 1.57-1.45 (m, 2H, 9'-H), 0.94 (t, 7.3 Hz, 3H, 10-H), 0.91 (t, 7.3 Hz, 3H, 10'-H).

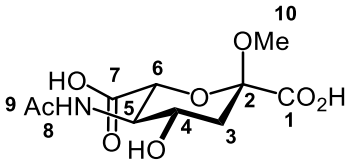
^{13}C NMR (126 MHz, D_2O) δ = 174.41 (C-1), 174.20 (C-11), 170.17 (C-7), 100.93 (C-2), 72.93 (C-6), 66.54 (C-4), 54.45 (C-5), 53.22 (C-8), 50.60 (C-13), 39.82 (C-3), 26.59 (C-9), 26.37 (C-9'), 22.20 (C-12), 9.81 (C-10), 9.71 (C-10').



Methyl 5-(acetylamino)-3,5-dideoxy-α-L-*arabino*-heptulo-2,6-pyranosidaric acid (**23**)

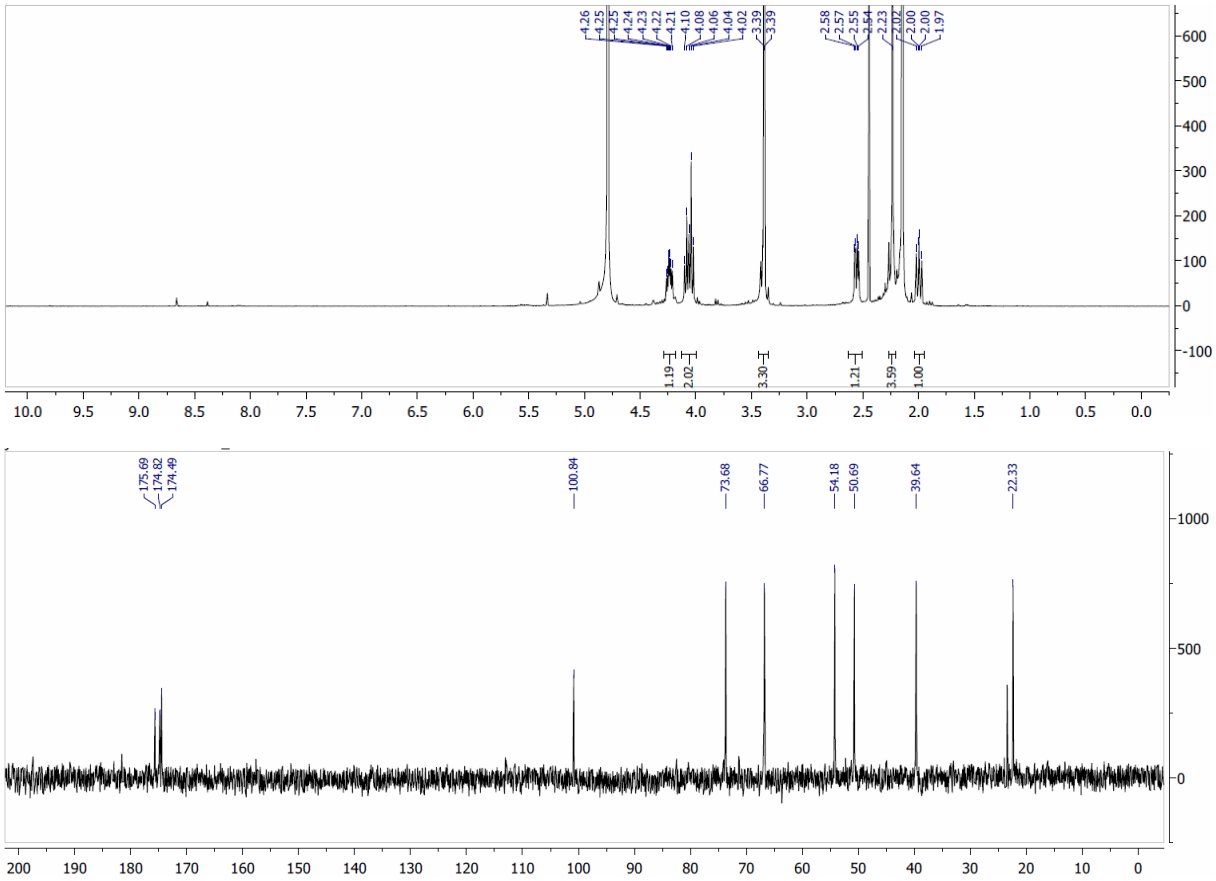
Aldehyde **6** (350 mg, 1.34 mmol, 1 eq.) was dissolved in H_2O (10 mL) and stirred with $BaCO_3$ (362.6 mg, 1.84 mmol, 1.37 eq.) at 0°C, then bromine (244.1 mg, 1.53 mmol, 1.14 eq.) was added. The ice bath was removed and the reaction mixture was stirred for an additional 12 h at RT in the dark. When TLC showed complete conversion, the reaction mixture was filtered. The solvents were removed under vacuum and the remaining solid purified by column chromatography with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 357.6 mg colorless solid (97%). R_f = 0.32.

A different synthesis of **23** is described in the literature.[7] No spectral data available.



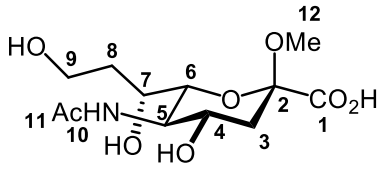
^{1}H NMR (500 MHz, D_2O) δ = 4.24 (ddd, J = 11.4, 10.1, 5.1 Hz, 1H, 4-H), 4.08 (dd, J = 10.5, 10.1 Hz, 1H, 5-H), 4.03 (d, J = 10.5 Hz, 1H, 6-H), 3.39 (m, 3H, 10-H), 2.56 (dd, J = 13.2, 5.1 Hz, 1H, 3_{eq}-H), 2.23 (s, 3H, 9-H), 2.00 (dd, J = 13.2, 11.4 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.69 (C-7), 174.82 (C-1), 174.49 (C-8), 100.84 (C-2), 73.68 (C-6), 66.77 (C-4), 54.18 (C-10), 50.69 (C-5), 39.64 (C-3), 22.23 (C-9).



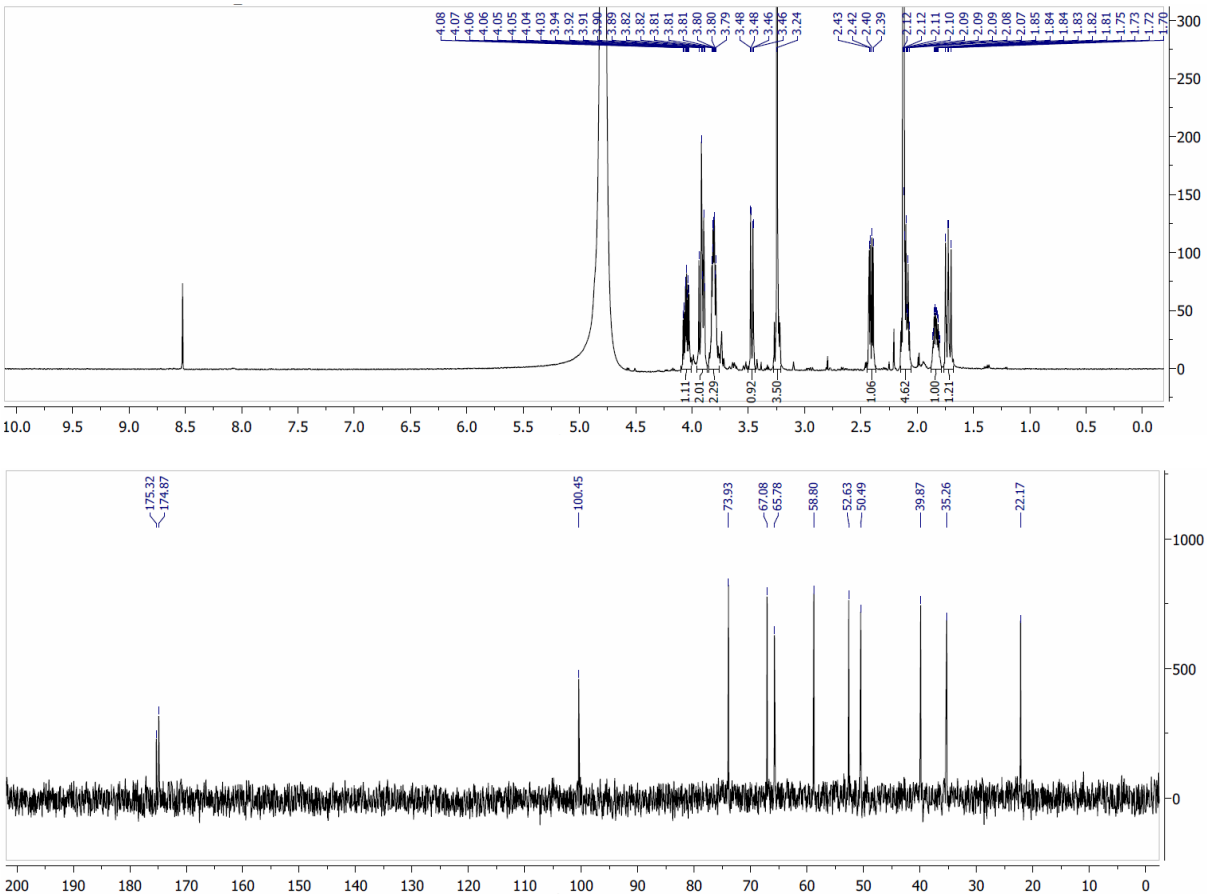
8-Deoxy-2-*O*-methyl-β-Neu5Ac (**25**)

Compound **7** (400.0 mg, 1.32 mmol, 1 eq.) was dissolved in dry MeOH (13 mL) and pyridine (308 mg, 3.90 mmol, 314 µL, 3 eq.) was added. Ozone was bubbled through the solution for 1.28 min with [3g/h] ozone at –78°C. Afterwards, argon was bubbled through the solution for 10 min. $NaBH_4$ (249.4 mg, 6.59 mmol, 5 eq.) and $CeCl_3$ (97.5 mg, 0.40 mmol, 0.3 eq.) was added. The solution was allowed to warm up to RT over 14 h. The solvent was removed and the solid was column purified with MeOH:acetone:H_2O:DCM = 4:2:1:7. Yield: 258.5 mg colorless solid (61%). R_f = 0.44.



^{1}H NMR (500 MHz, D_2O) δ = 4.05 (ddd, *J* = 11.3, 10.0, 5.0 Hz, 1H, 4-H), 3.96-3.87 (m, 2H, 5-, 7-H), 3.81 (ddd, *J* = 10.0, 5.5, 3.1 Hz, 2H, 9-H), 3.47 (dd, *J* = 10.3, 1.2 Hz, 1H, 6-H), 3.24 (s, 3H, 12-H), 2.41 (dd, *J* = 13.1, 5.1 Hz, 1H, 3_{eq}-H), 2.12 (s, 3H, 11-H), 2.12-2.04 (m, 1H, 8a-H), 1.83 (dtd, *J* = 14.5, 7.4, 3.9 Hz, 1H, 8b-H), 1.72 (dd, *J* = 13.2, 11.4 Hz, 1H, 3_{ax}-H).

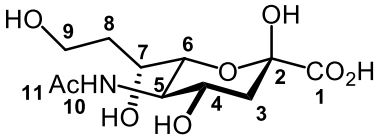
^{13}C NMR (126 MHz, D_2O) δ = 175.32(C-1), 174.87 (C-10), 100.45 (C-2), 73.93 (C-6), 67.08 (C-4), 65.78 (C-7), 58.80 (C-9), 52.63 (C-5), 50.49 (C-12), 39.87 (C-3), 35.26 (C-8), 22.17 (C-11).



8-Deoxy-Neu5Ac (**26**)

For glycoside deprotection see general procedure in main paper. Compound **25** (228.5 mg, 0.74 mmol, 1 eq.), H_2O (10 mL), formic acid (500 µL, 610 mg, 13.3 mmol, 18 eq.). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:7 Yield: 111.74 mg colorless solid (51%). R_f = 0.39.

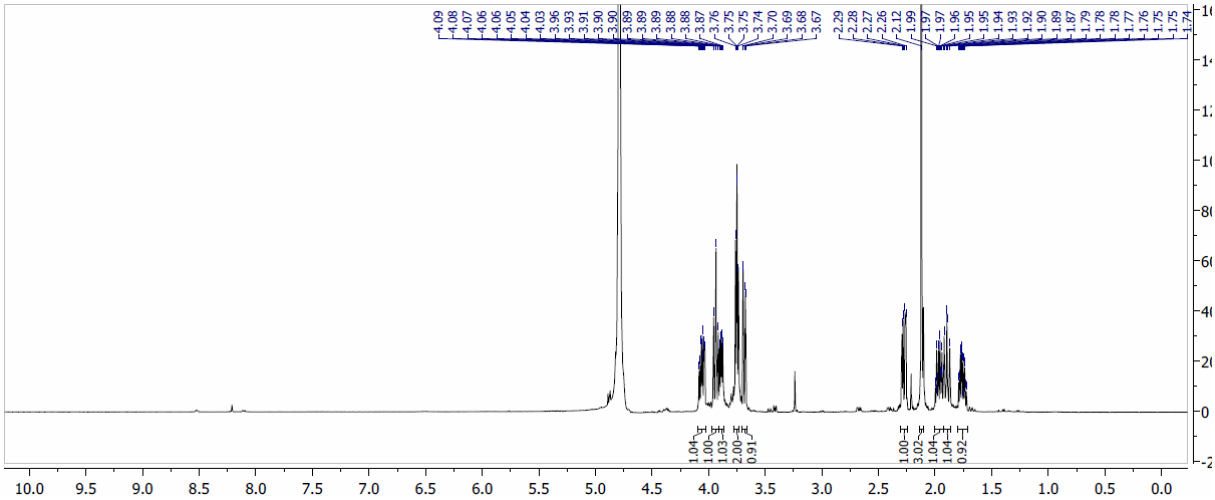
Spectral data are in accordance with the literature.

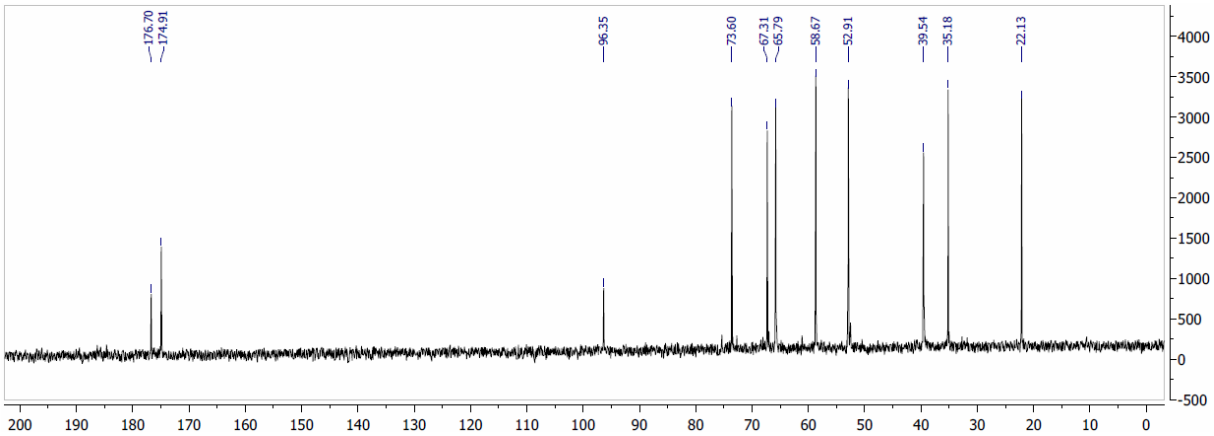


^{1}H NMR (500 MHz, D_2O) δ = 4.06 (ddd, *J* = 11.5, 10.0, 4.9 Hz, 1H, 4-H), 3.94 (dd, *J* = 10.2, 10.1 Hz, 1H, 5-H), 3.89 (ddd, *J* = 9.0, 4.7, 1.3 Hz, 1H, 7-H), 3.75 (dd, *J* = 7.1, 5.8 Hz, 2H, 9-H), 3.68 (dd, *J* = 10.4, 1.4 Hz, 1H, 6-H), 2.27 (dd, *J* = 12.9, 4.9 Hz, 1H, 3_{eq}-H), 2.12 (s, 3H, 11-H), 1.96 (ddt, *J* = 14.5, 9.0, 5.8 Hz, 1H, 8a-H), 1.89 (dd, *J* = 13.0, 11.5 Hz, 1H, 3_{ax}-H), 1.76 (dtd, *J* = 14.5, 7.1, 4.7 Hz, 1H, 8b-H).

^{13}C NMR (126 MHz, D_2O) δ = 176.70(C-1), 174.91 (C-10), 96.35 (C-2), 73.60 (C-6), 67.31 (C-4), 65.79 (C-7), 58.67 (C-9), 52.91 (C-5), 39.54 (C-3), 35.18 (C-8), 22.13 (C-11).

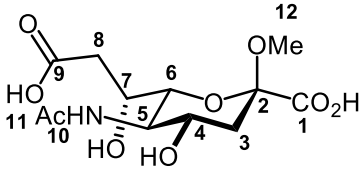
MS (ESI)$^-$: *m/z* calcd for $[C_{11}H_{19}N_1O_8]$-H^+ = 292.10; found 292.10





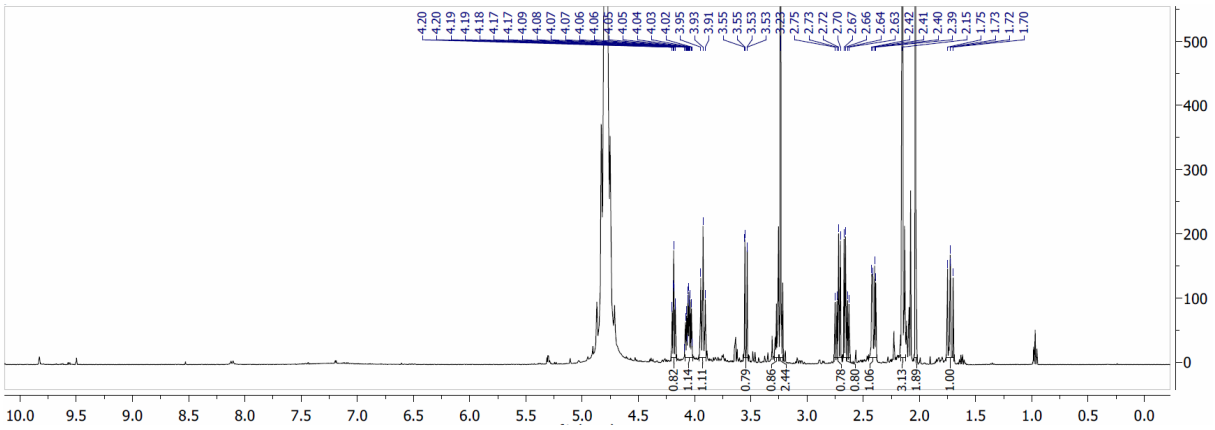
8-Deoxy-2-*O*-methyl-9-oxo-β-Neu5Ac (**27**)

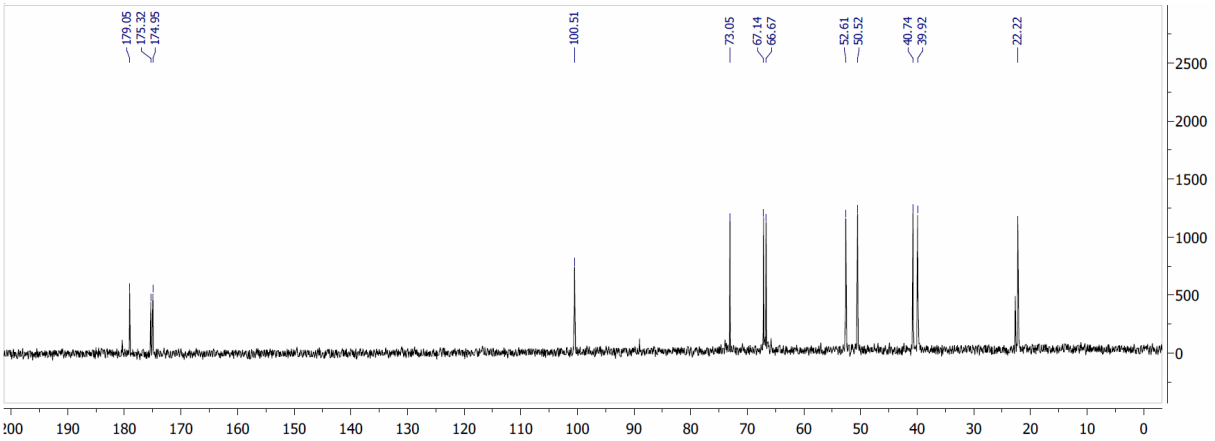
Compound **7** (250.0 mg, 0.82 mmol, 1 eq.) was dissolved in dry MeOH (10 mL) and ozone was bubbled through the solution for 15 min with [3g/h] ozone at –78°C. Afterwards, argon was bubbled through the solution for 10 min. $NaBH_4$ (155.9 mg, 4.12 mmol, 5eq.) and $CeCl_3$ (60.9 mg, 0.25 mmol, 0.3 eq.) was added. The solution was allowed to warm to RT over 14 h. The solvent was removed and the solid was column purified with MeOH:acetone:H_2O:DCM = 4:2:1:7. Yield: 108.9 mg colorless solid (41%). R_f = 0.27.



^{1}H NMR (500 MHz, D_2O) δ = 4.19 (ddd, *J* = 7.8, 6.5, 1.3 Hz, 1H, 7-H), 4.05 (ddd, *J* = 11.4, 10.0, 5.0 Hz, 1H, 4-H), 3.93 (dd, *J* = 10.1, 10.2 Hz, 1H, 5-H), 3.54 (dd, *J* = 10.3, 1.3 Hz, 1H, 6-H), 3.23 (s, 3H, 12-H), 2.73 (dd, *J* = 15.4, 7.8 Hz, 1H, 8a-H), 2.65 (dd, *J* = 15.4, 6.5 Hz, 1H, 8b-H), 2.41 (dd, *J* = 13.1, 5.1 Hz, 1H, 3_{eq}-H), 2.15 (s, 3H, 11-H), 1.73 (dd, *J* = 13.2, 11.4 Hz, 1H, 3_{ax}-H).

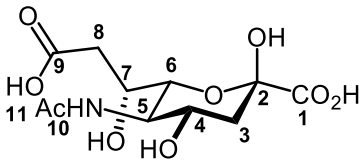
^{13}C NMR (126 MHz, D_2O) δ = 179.05 (C-9), 175.32(C-1), 174.95 (C-10), 100.51 (C-2), 73.05 (C-6), 67.14 (C-4), 66.67 (C-7), 52.61 (C-5), 50.52 (C-12), 40.74 (C-8), 39.92 (C-3), 22.22 (C-11).





8-Deoxy-9-oxo-Neu5Ac (**28**)

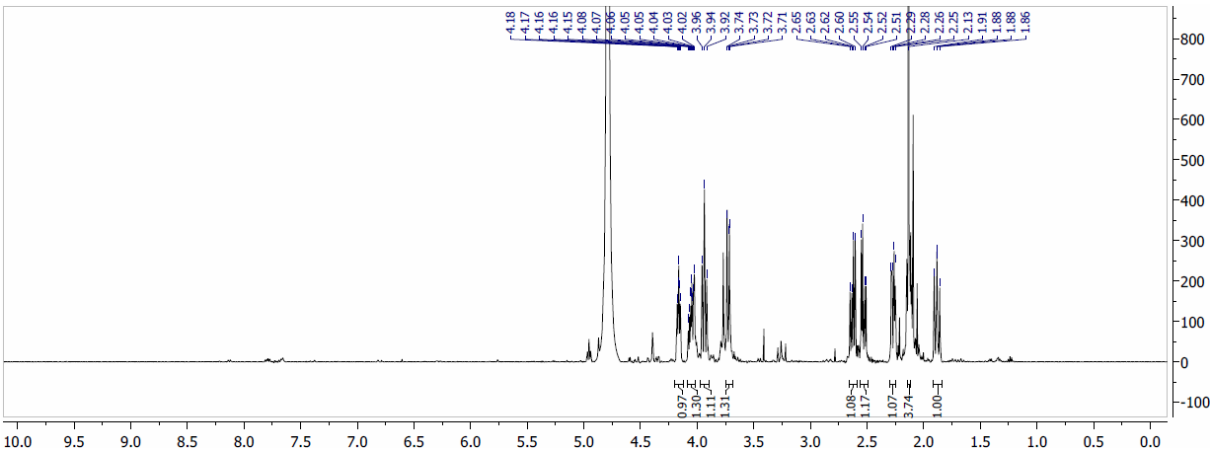
For glycoside deprotection see general procedure in main paper. (3x).
Compound **27** (108.9 mg, 0.34 mmol, 1 eq.), H_2O (10 mL), formic acid (500µL, 610 mg, 13.3 mmol, 39 eq.). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 30.7 mg colorless solid (30%). R_f = 0.25.

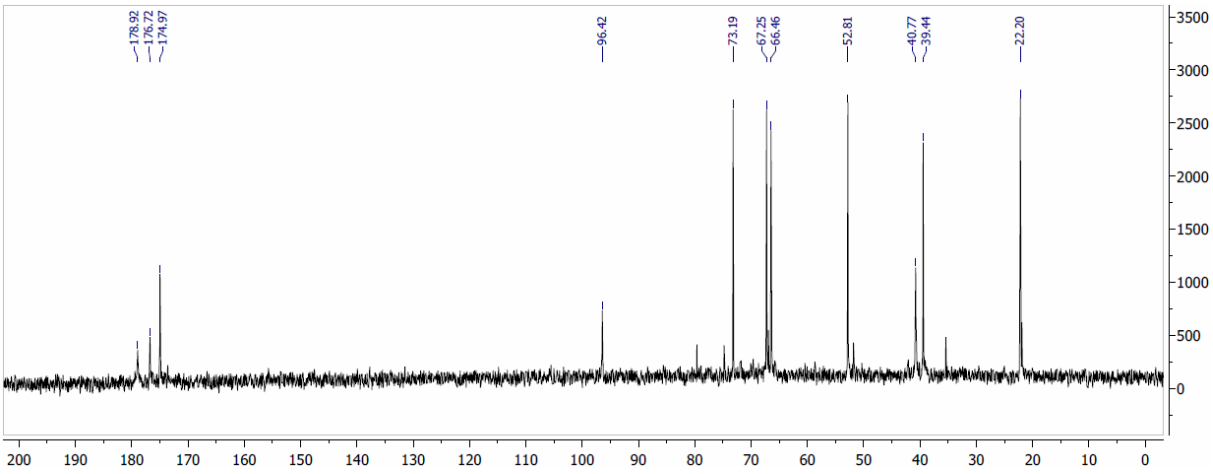


^{1}H NMR (500 MHz, D_2O) δ = 4.19-4.14 (m, 1H, 7-H), 4.05 (ddd, *J* = 11.5, 10.0, 4.9 Hz, 1H, 4-H), 3.94 (dd, *J* = 10.1, 10.2 Hz, 1H, 5-H), 3.73 (dd, *J* = 10.4, 1.2 Hz, 1H, 6-H), 2.63 (dd, *J* = 15.1, 7.9 Hz, 1H, 8a-H), 2.53 (dd, *J* = 15.1, 6.0 Hz, 1H, 8b-H), 2.27 (dd, *J* = 13.0, 4.9 Hz, 1H, 3_{eq}-H), 2.13 (s, 3H, 11-H), 1.88 (dd, *J* = 13.0, 11.5 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 178.92 (C-9), 176.72(C-1), 174.97 (C-10), 96.42 (C-2), 73.19 (C-6), 67.25 (C-4), 66.46 (C-7), 52.81 (C-5), 40.77 (C-8), 39.44 (C-3), 22.20 (C-11).

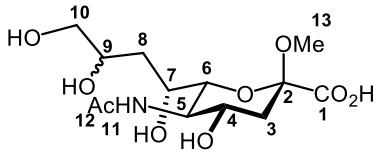
MS (ESI)$^-$: *m/z* calcd for $[C_{11}H_{17}N_1O_9]$-H^+ = 306.08; found 306.08





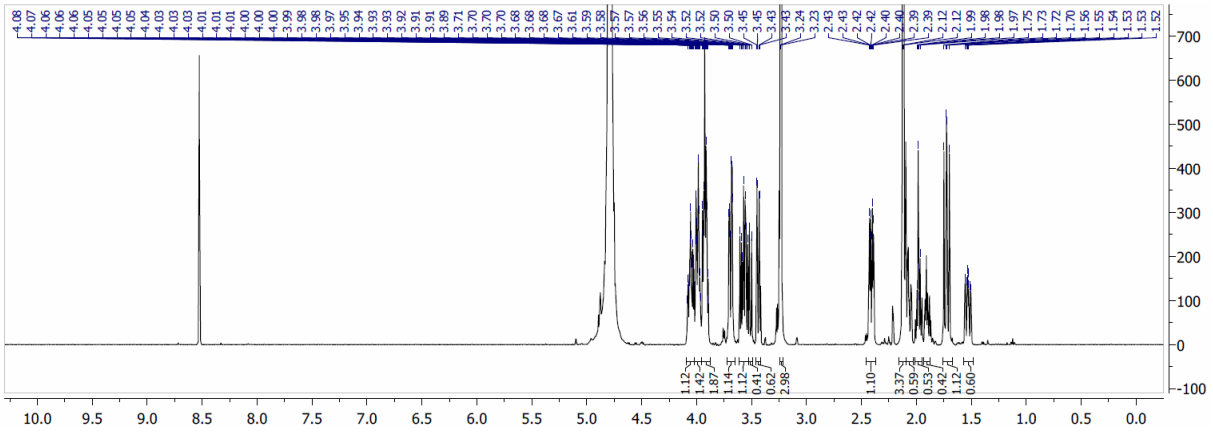
8-Deoxy-9-hydroxymethyl-2-*O*-methyl-β-Neu5Ac (**29**)

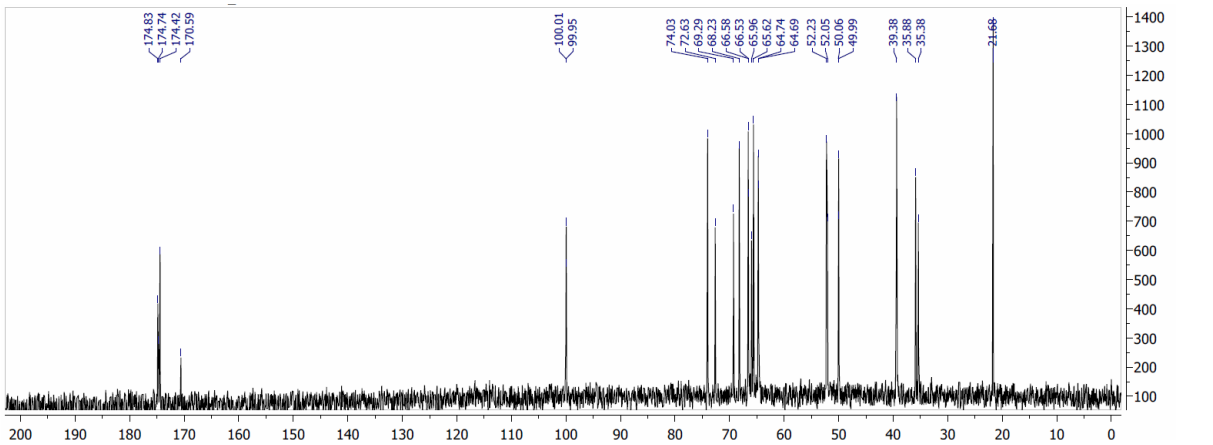
AD-mix β (0.923 g) was dissolved in H_2O (3 mL) and ^tBuOH (3 mL). The solution was cooled to 0°C and compound **7** (140 mg, 0.46 mmol, 1 eq.) was added (dissolved in H_2O (1 mL) and ^tBuOH (1 mL)). The solution was stirred for 24 h and then allowed to warm up to RT. To stop the reaction, Na_2SO_3 solution was added to the slurry. The reaction mixture was filtered over celite and dried directly under vacuum. The resulting slightly orange solid was column purified with MeOH:DCM = 5:1 gradient towards MeOH:acetone:H_2O:DCM = 4:2:1:7. Yield: 64.7 mg colorless solid (42%). R_f = 0.28.



^{1}H NMR (500 MHz, D_2O) δ = 4.09-4.03 (m, 1H, 7(*R*/*S*) -H), 4.02-3.96 (m, 2H, 4(*R*)-, 9(*R*)-, 10(*S*) -H), 3.96-3.89 (m, 1.8H, 5(*R*/*S*)-, 4(*S*)-, 9(*S*)-H), 3.69 (ddd, *J* = 11.8, 3.7, 1.8 Hz, 1.2H, 10a(*R*)-H), 3.57 (ddd, *J* = 17.1, 11.8, 6.8 Hz, 1.2H, 10b(*R*)-H), 3.51 (dd, *J* = 10.3,1.2 Hz, 0.4H, 6(*S*)-H), 3.44 (dd, *J* = 10.3, 1.3 Hz, 0.6H, 6(*R*)-H), 3.24 (2xs, 3H, 13-H), 2.41 (2xdd, *J* = 13.2, 5.1 Hz, 1H, 3_{eq}-H), 2.13, 2.12 (2xs, 3H, 12-H), 1.99 (ddd, *J* = 14.6, 10.9, 2.8 Hz, 0.6H, 8a(*R*)-H), 2.01-1.94 (m, 0.4H, 8a(*S*)-H), 1.94-1.86 (m, 0.4H, 8b(*S*)-H), 1.73 (dd, *J* = 13.2, 11.5 Hz, 1H, 3_{ax}-H), 1.53 (ddd, *J* = 14.6, 10.2, 2.6 Hz, 0.6H, 8b(*R*)-H).

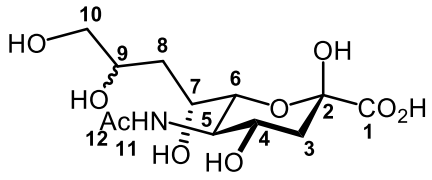
^{13}C NMR (126 MHz, D_2O) δ = 174.83 (C-1), 174.74 (C-1), 174.42 (C-11*R*), 170.59 (C-11*S*), 100.01 (C-2*S*), 99.95 (C-2*R*), 74.03 (C-6*R*), 72.63 (C-6*S*), 69.29 (C-4*S*), 68.23 (C-4*R*), 66.58 (C-7*R*), 66.53 (C-7*S*), 65.96 (C-9*S*), 65.62 (C-10*R*), 64.74 (C-9*R*), 64.69 (C-10*S*), 52.23 (C-5*R*), 52.05 (C-5*S*), 50.06 (C-13*S*), 49.99 (C-13*R*), 39.38 (C-3), 35.88 (C-8*R*), 35.38 (C-8*S*), 21.68 (C-12).





8-Deoxy-9-hydroxymethyl-Neu5Ac (**30**)

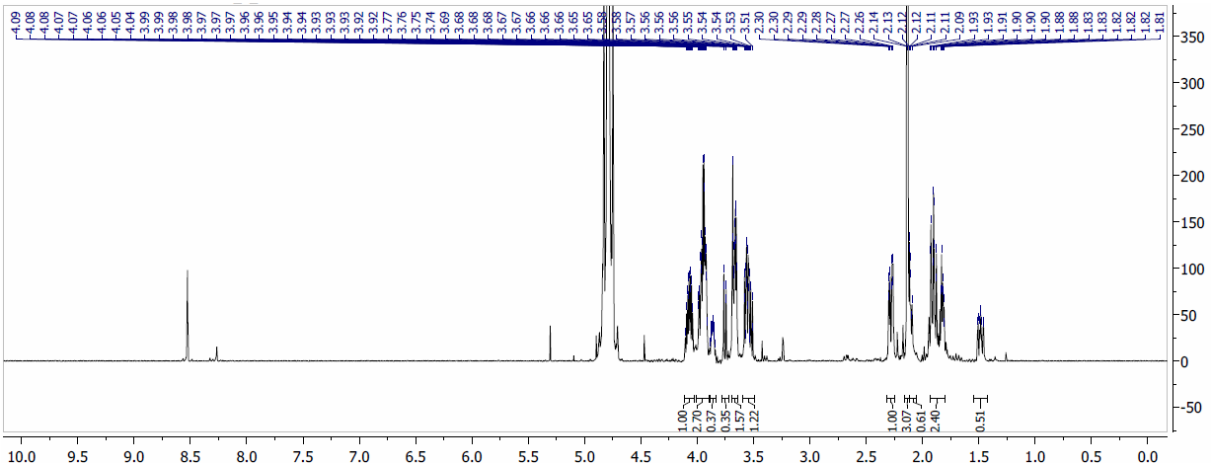
For glycoside deprotection see general procedure in main paper. (2x).
Compound **29** (64.7 mg, 0.192 mmol, 1 eq.), H_2O (10 mL), formic acid (500 µL, 610 mg, 13.3 mmol, 39 eq.). Chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 24.68 mg colorless solid (40%). R_f = 0.27.

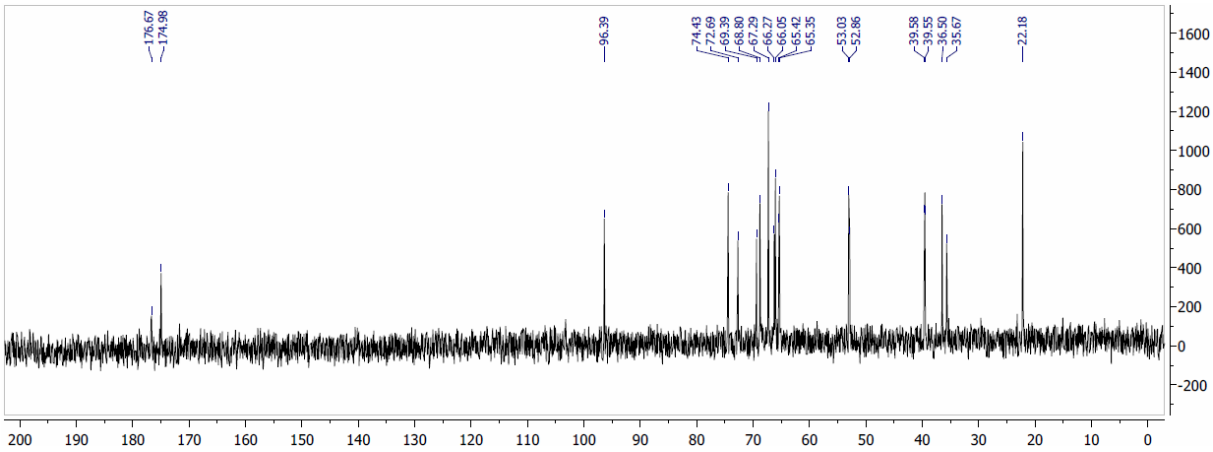


^{1}H NMR (500 MHz, D_2O) δ = 4.11-4.03 (m, 1H, 7(*R*/*S*)-H), 4.00-3.90 (m, 3H, 4(*R*)-, 5(*R*/*S*)-, 9(R/*S*)-, H10(*S*)-H), 3.89-3.82 (m, 0.4H, 4(*S*)-H), 3.76 (dd, *J* = 10.3, 1.3 Hz, 0.4H, 6(*S*)-H), 3.70-3.63 (m, 1.6H, 6(*R*)-, 10a(*R*)-H), 3.60-3.49 (m, 1H, 10b(*R*)-H), 2.32 (2xdd, *J* = 13.1, 5.0 Hz, 1H, 3_{eq}-H), 2.13 (2x s, 3H, 12-H), 2.12-2.09 (m, 0.6H, 8a(*R*)-H), 1.93-1.80 (m, 1.8H, 8a/b(*S*)-, 3_{ax}-H), 1.48 (ddd, *J* = 14.7, 9.9, 2.9 Hz, 0.6H, 8b(*R*)-H).

^{13}C NMR (126 MHz, D_2O) δ = δ 176.67 (C-1), 174.98 (C-11), 96.29 (C-2), 74.43 (C-6*R*), 72.69 (C-6*S*), 69.39 (C-4*S*), 68.80 (C-4*R*), 67.29 (C-7) 66.27 (C-9*S*), 66.05 (C-10), 65.42 (C-10), 65.35 (C-9*R*), 53.03 (C-5*R*), 52.86 (C-5*S*), 39.58 (C-3*S*), 39.55 (C-3*R*), 36.50 (C-8*R*), 35.67 (C-8*S*), 22.18 (C-12).

MS (ESI)$^-$: *m/z* calcd for $[C_{12}H_{21}N_1O_9]$-H^+ = 322.11; found 322.11

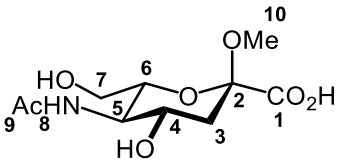




Methyl 5-(acetylamino)-3,5-dideoxy-αL-*arabino*-heptulo-2,6-pyranosidonic acid (**P35**)

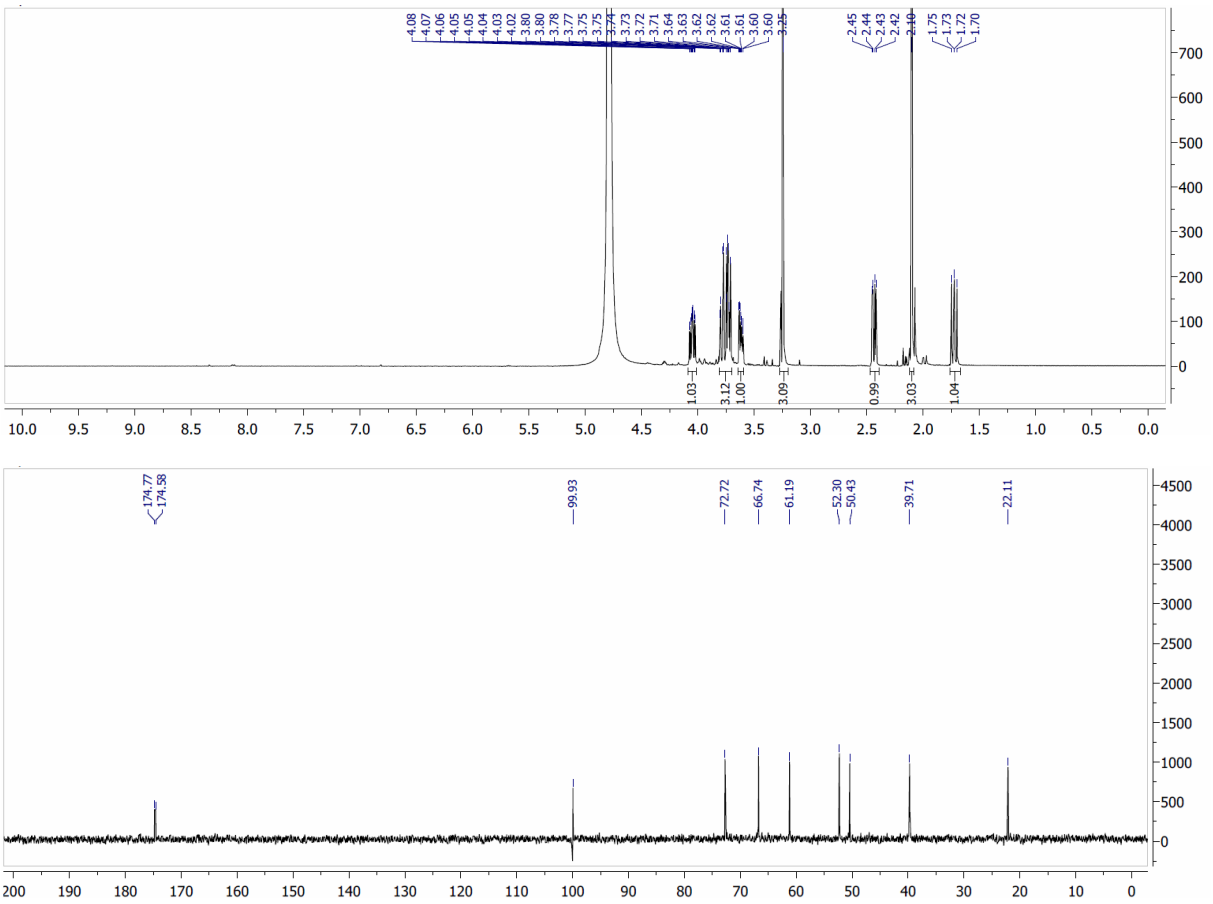
Aldehyde **6** (150 mg, 0.57 mmol, 1 eq.) was dissolved in 10 mM NaOH (40 mL) containing $NaBH_4$ (65.2 mg, 1.72 mmol, 3 eq.) and the mixture stirred for 1 h at RT. When TLC showed complete conversion, the reaction was quenched by addition of Dowex50 (H^+). The solvents were removed under vacuum and the remaining solid was purified by column chromatography using EE:MeOH:AcOH = 80:20:1. Yield: 143.6 mg colorless solid (95%). R_f = 0.35.

Synthesis of **P35** was described in the literature[8] but no spectroscopic data are available.



^{1}H NMR (500 MHz, D_2O) δ = 4.05 (ddd, J = 11.4, 9.9, 5.1 Hz, 1H, 4-H), 3.80-3.71 (m, 3H, 5-, 7-H), 3.62 (ddd, J = 10.5, 5.6, 2.4 Hz, 1H, 6-H), 3.25 (s, 3H, 10-H), 2.44 (dd, J = 13.2, 5.1 Hz, 1H, 3_{eq}-H), 2.10 (s, 3H, 9-H), 1.72 (dd, J = 13.3, 11.4 Hz, 1H, 3_{ax}-H).

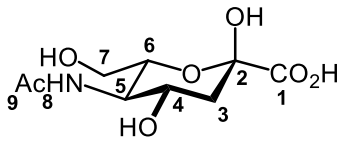
^{13}C NMR (126 MHz, D_2O) δ = 174.77 (C-8), 174.58 (C-1), 99.93 (C-2), 72.72 (C-6), 66.74 (C-4), 61.19 (C-7), 52.30 (C-5), 50.43 (C-10), 39.71 (C-3), 22.11 (C-9).



5-(Acetylamino)-3,5-dideoxy-L-*arabino*-heptulosonic acid (**35**)

For glycoside deprotection see general procedure in main paper. (2x).
Compound **P35** (143.6 mg, 0.55 mmol, 1eq.), H_2O (10 mL), formic acid (500 µL, 610 mg, 13.3 mmol, 24 eq.). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 114.1 mg colorless solid (85%). R_f = 0.34.

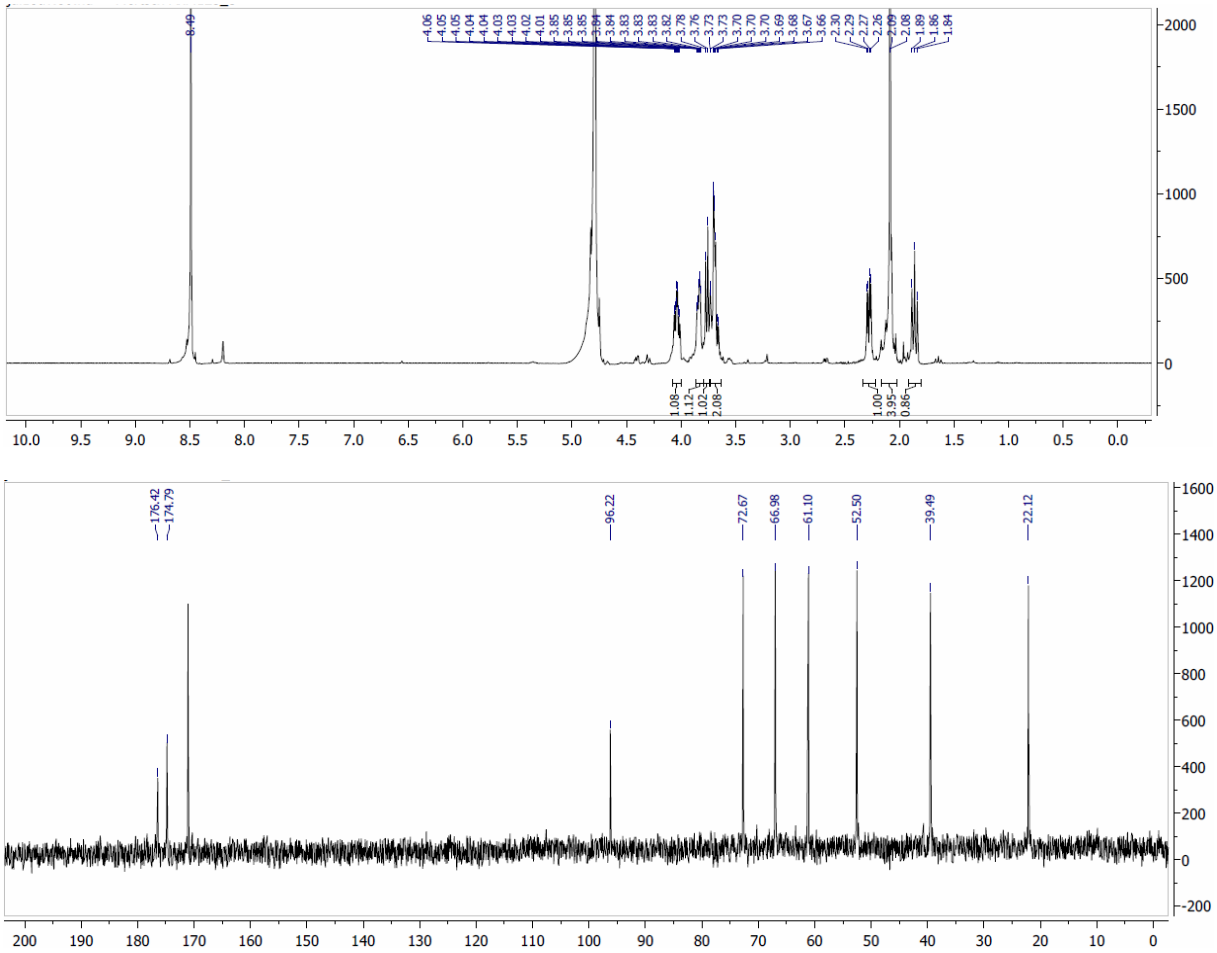
Synthesis of **35** was described in the literature[8] but no spectroscopic data are available.



^{1}H NMR (500 MHz, D_2O) δ = 8.49 (1H, NH), 4.04 (ddd, *J* = 11.8, 9.8, 4.8 Hz, 1H, 4-H), 3.87-3.82 (m, 1H, 6-H), 3.76 (dd, *J* = 10.1, 10.2 Hz, 1H, 5-H), 3.71-3.59 (m, 2H, 7-H), 2.28 (dd, *J* = 13.0, 5.0 Hz, 1H, 3_{eq}-H), 2.09 (s, 3H, 9-H), 1.86 (dd, *J* = 13.0, 11.8 Hz, 1H, 3_{ax}-H).

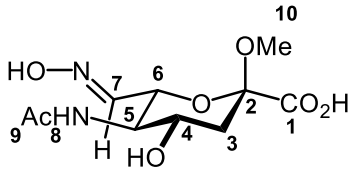
^{13}C NMR (126 MHz, D_2O) δ = 176.42 (C-8), 174.79 (C-1), 96.22 (C-2), 72.67 (C-6), 66.98 (C-4), 61.10 (C-7), 52.50 (C-5), 39.49 (C-3), 22.12 (C-9).

MS (ESI)$^-$: *m/z* calcd for $[C_9H_{15}N_1O_7]$-H^+ = 248.08; found 248.08



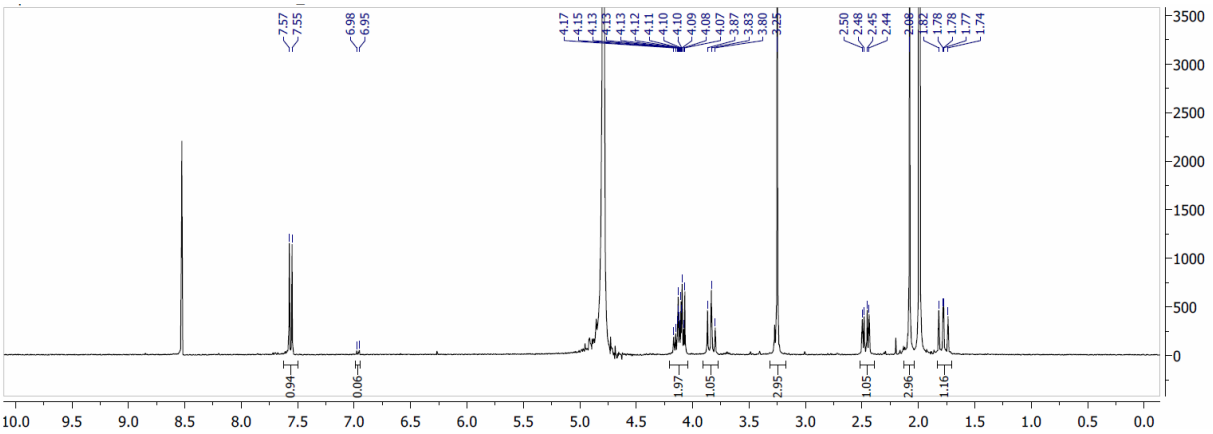
Methyl 3-(acetylamino)-3,5-dideoxy-L-*lyxo*-(6*S*)-heptulo-2,6-pyranosiduronic acid oxime (**31**)

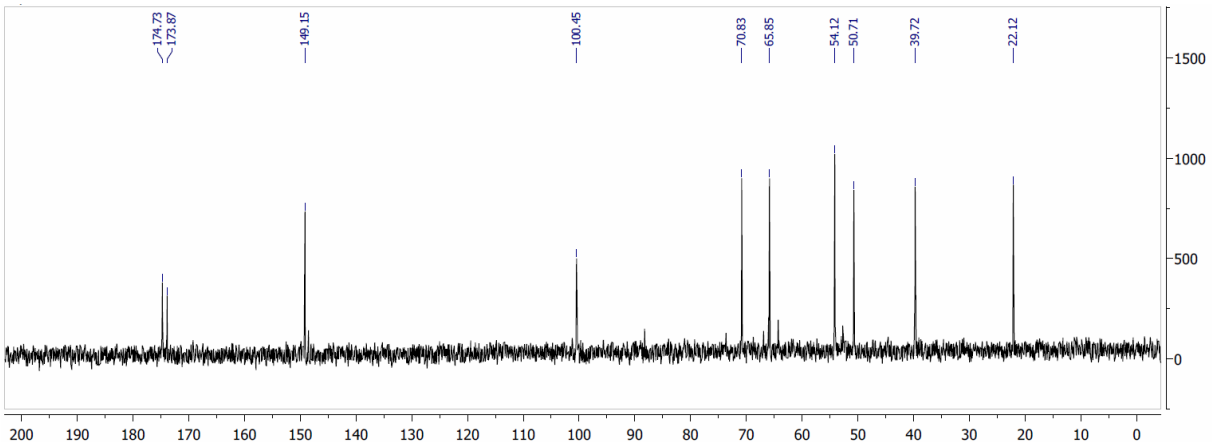
Aldehyde **6** (404 mg, 1.55 mmol, 1 eq.) was dissolved in a mixture of 1 M NaOH (30 mL) and 30 mL THF. Hydroxylammonium chloride (322.4 mg, 4.64 mmol, 3 eq.) was added and the mixture stirred for 6 h at RT. After completion, 2 M HCl was added to pH=7and the solvents were removed under vacuum and the solid was purified by column chromatography with EE:MeOH:DCM = 10:5:1 (R_f = 0.15). Yield: 341.7 mg colorless solid (80%) (E:Z=94:6).



^{1}H NMR (500 MHz, D_2O) δ = 7.56 (d, *J* = 7.3 Hz, 1H, 7E-H), 6.97 (d, *J* = 7.0 Hz, 7Z-H) 4.16 – 4.07 (m, 2H, 4-, 6-H), 3.83 (t, *J* = 10.2 Hz, 1H, 5-H), 3.25 (s, 3H, 10-H), 2.47 (dd, *J* = 13.3, 5.1 Hz, 1H, 3_{eq}-H), 2.08 (s, 3H, 9-H), 1.87 – 1.70 (m, 1H, 3_{ax}-H).

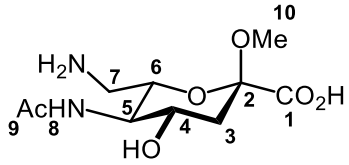
^{13}C NMR (126 MHz, D_2O) δ = 174.73 (C-1), 173.87 (C-8), 149.15 (C-7), 100.45 (C-2), 70.83 (C-6), 65.85 (C-4), 54.12 (C-5), 50.71 (C-10), 39.72 (C-3), 22.12 (C-9).





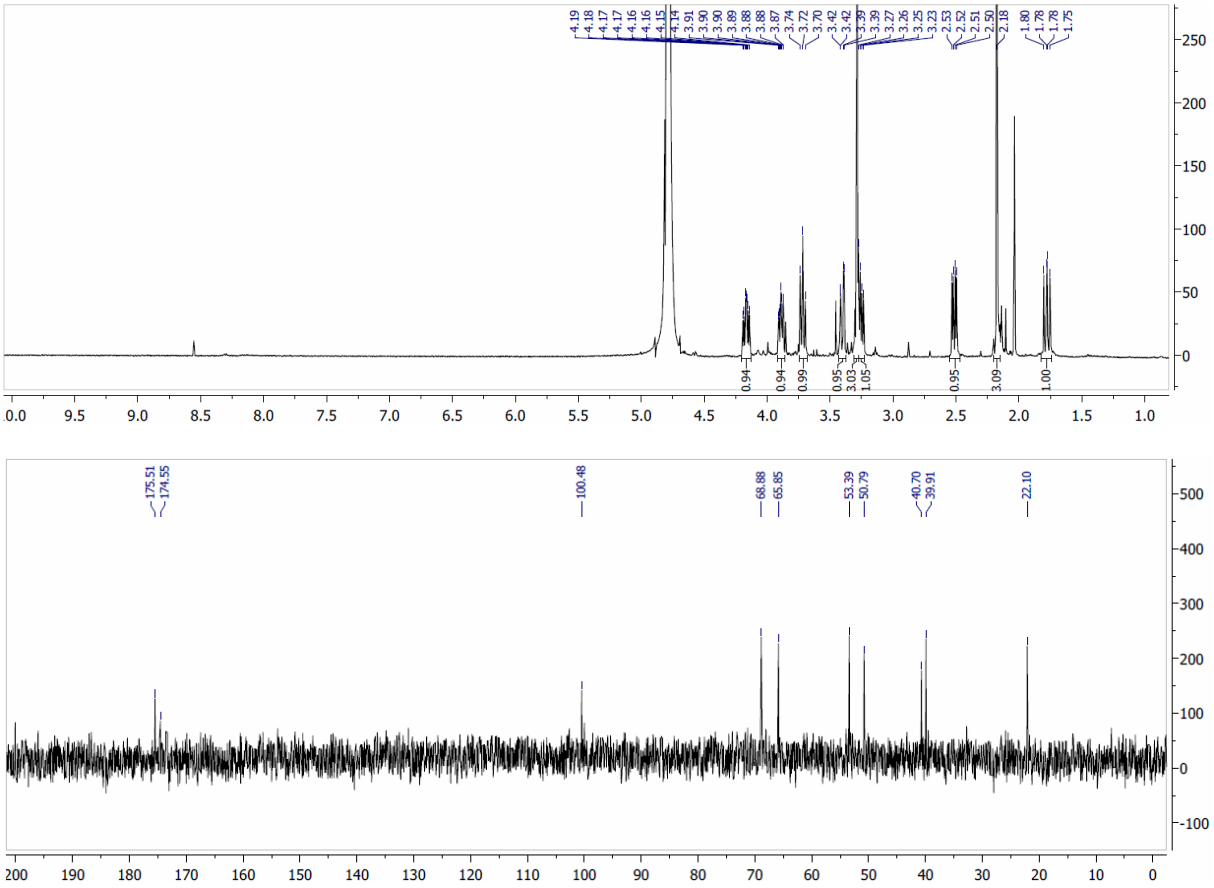
Methyl 5-(acetylamino)-7-amino-3,5,7-trideoxy-α-L-*arabino*-heptulo-2,6-pyranosidonic acid (**32**)

Oxime **31** (211.5 mg, 0.77 mmol, 1 eq.) was dissolved in dry MeOH (5mL). Then premixed $NaBH_3CN$ (192.5 mg, 3.06 mmol, 4 eq.), $MoCl_5$ (209.2 mg, 0.77 mmol, 1 eq.), and $NaHSO_4 \bullet H_2O$ (317.1 mg, 2.30 mmol, 3 eq.) was added at once under argon atmosphere. The slurry was refluxed for 3 hours. The reaction mixture was neutralized with $NaHCO_3$ (5% aq.), the solvents were removed under vacuum, and the solid was purified by column chromatography with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 86.3 mg colorless solid (43%). R_f = 0.32.



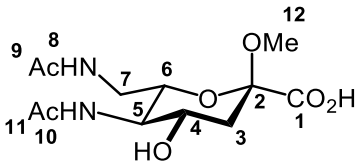
^{1}H NMR (500 MHz, D_2O) δ = 4.17 (ddd, *J* = 11.4, 9.9, 5.0 Hz, 1H, 4-H), 3.89 (ddd, *J* = 9.9, 6.8, 2.7 Hz, 1H, 6-H) 4.72 (dd, *J* = 10.2, 10.3 Hz,1H, 5-H), 3.41 (dd, *J* = 13.6, 2.8 Hz, 1H, 7-H), 3.29 (s, 3H, 10-H), 3.25 (dd, *J* = 13.6, 7.0 Hz, 1H, 7-H), 2.51 (dd, *J* = 13.3, 5.0 Hz, 1H, 3_{eq}-H), 2.18 (s, 3H, 9-H), 1.78 (dd, *J* = 13.3, 11.4 Hz, 1H, 3_{ax}-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.51 (C-1), 174.55 (C-8), 100.48 (C-2), 68.88 (C-6), 65.85 (C-4), 53.39 (C-5), 50.79 (C-10), 40.70 (C-7), 39.91 (C-3), 22.10 (C-9).



Methyl 5,7-di(acetylamino)-3,5,7-trideoxy-α-L-*arabino*-heptulo-2,6-pyranosidonic acid (**33**)

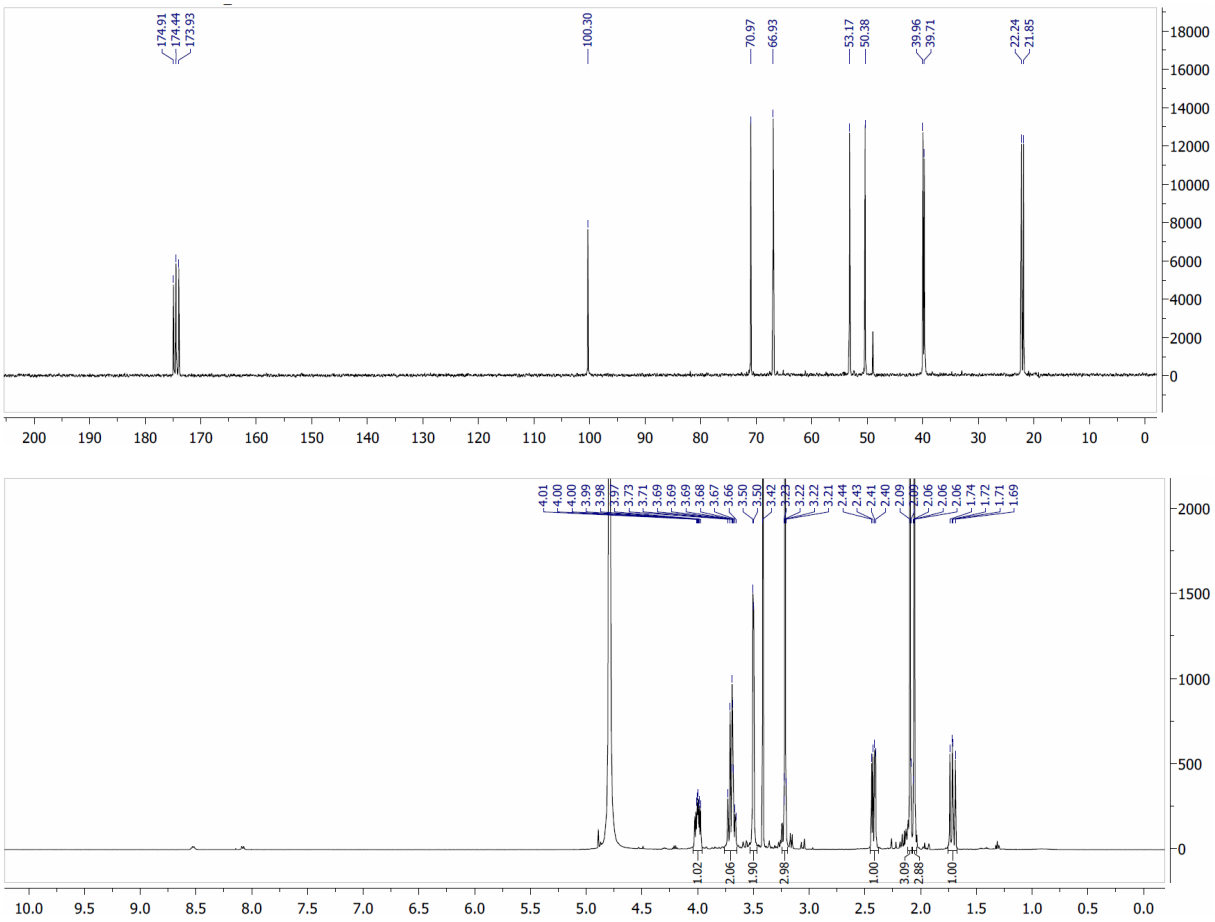
Amine **32** (200.8 mg, 0.77 mmol, 1 eq.) was dissolved in dry MeOH, acetic anhydride (781.7 mg, 7.66 mmol, 10 eq.) was added and the mixture stirred for 3 h at RT. After completion, the solvents were removed and the solid was purified by column chromatography using MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 221.3 mg colorless solid (95%). R_f = 0.44.



^{1}H NMR (500 MHz, D_2O) δ = 4.05-3.92 (m, 1H, 4-H), 3.77-3.61 (m, 2H, 5-, 6-H) 3.50 (d, *J* = 4.0 Hz,1H, 7-H), 3.22 (s, 3H, 12-H), 2.42 (dd, *J* = 13.2, 5.0 Hz, 1H, 3_{eq}-H), 2.09 (s, 3H, 9-H), 2.06 (s, 3H, 11-H), 1.71 (dd, *J* = 13.3, 11.4 Hz, 1H, 3_{ax}-H).

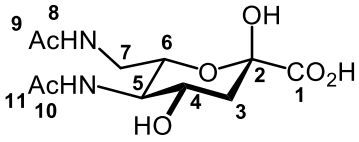
^{13}C NMR (126 MHz, D_2O) δ = 174.91 (C-1), 174.44 (C-8), 173.93 (C-10), 100.30 (C-2), 70.97 (C-6), 66.93 (C-4), 53.17 (C-5), 50.38 (C-12), 39.96 (C-7), 39.71 (C-3), 22.24 (C-9), 21.85 (C-11).

MS (ESI)$^+$: *m/z* calcd for $[C_{12}H_{20}N_2O_7]+H^+$ = 305.13; found 305.13



5,7-Di(acetylamino)-3,5,7-trideoxy-L-*arabino*-heptulosonic acid (**34**)

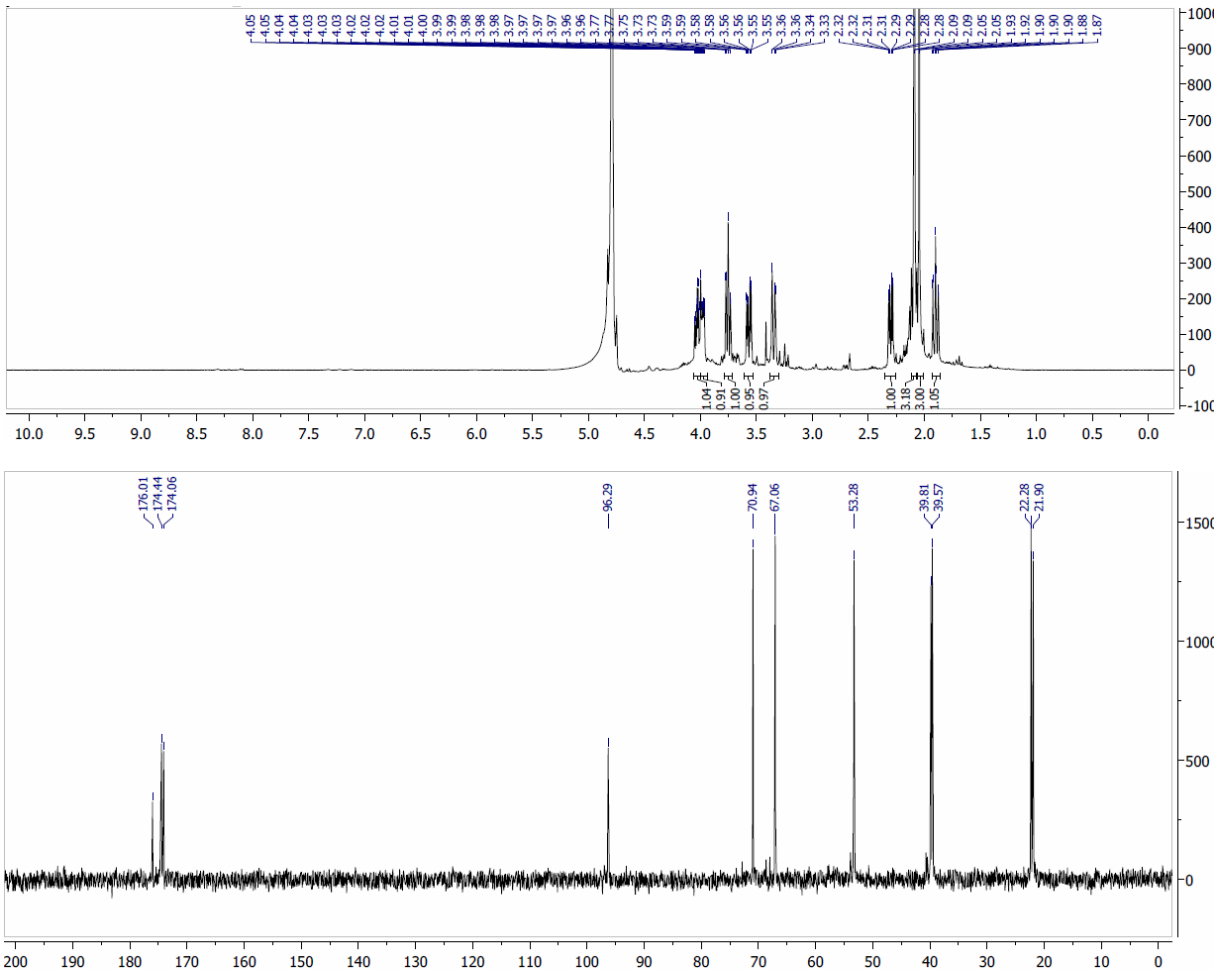
For glycoside deprotection see general procedure in main paper. Compound **33** (137.3 mg, 0.451 mmol, 1 eq.), H_2O (10 mL), formic acid (500 µL, 610 mg, 13.3 mmol, 29 eq.). Column chromatography was performed with MeOH:acetone:H_2O:DCM = 4:2:1:8. Yield: 64.9 mg colorless solid (50%). R_f = 0.43.



^{1}H NMR (500 MHz, D_2O) δ = 4.05 (dd, , *J* = 11.5, 4.9 Hz, 1H, 4-H), 3.98 (m, 1H, 6-H) 3.82-3.71 (m, 1H, 5-H), 3.57 (ddd, *J* = 14.5, 5.3, 1.0 Hz, 1H, 7a-H), 3.35 (dd, , *J* = 14.5, 2.6 Hz, 1H, 7b-H), 2.30 (dd, *J* = 13.1, 4.9 Hz, 1H, 3_{eq}-H), 2.09 (s, 3H, 9-H), 2.05 (s, 3H, 11-H), 1.90 (dd, *J* = 13.1, 11.5, Hz, 1H, 3_{ax}-H).

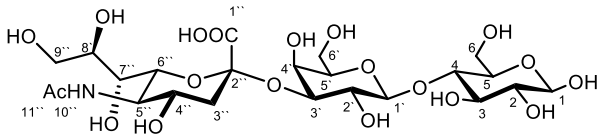
^{13}C NMR (126 MHz, D_2O) δ = 176.01 (C-1), 174.44 (C-8), 174.06 (C-10), 96.29 (C-2), 70.94 (C-6), 67.06 (C-4), 53.28 (C-5), 39.81 (C-7), 39.57 (C-3), 22.28 (C-9), 21.90 (C-11).

MS $(ESI)^-$: *m/z* calcd for $[C_{11}H_{18}N_2O_7]$-H^+ = 289.10; found 289.10



α2,3-Sialyllactose (**36**).

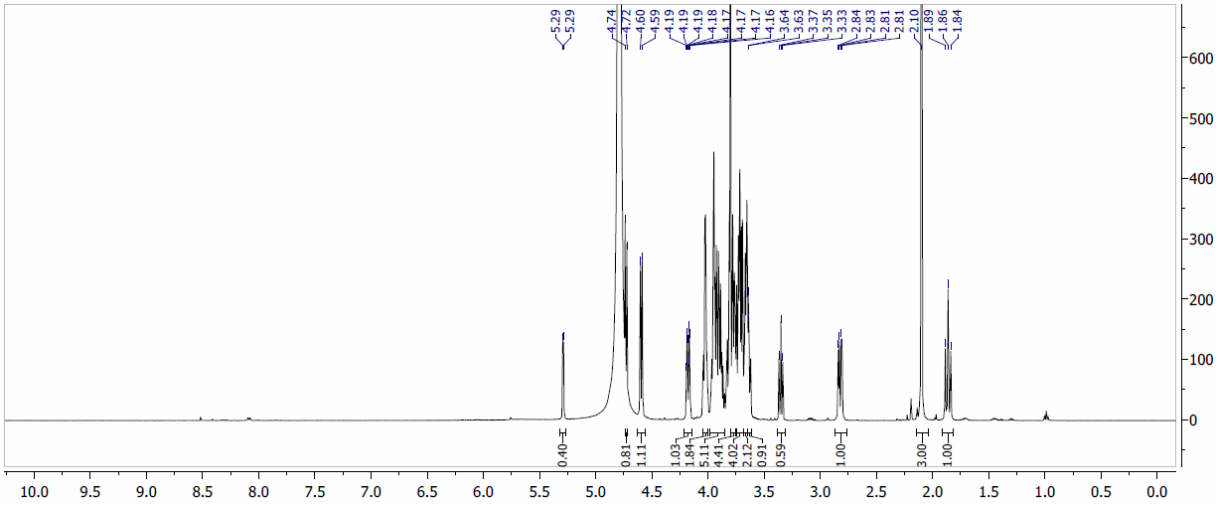
General procedure see main paper.
(50 mg Neu5Ac starting material) Yield: 93.4 mg colorless solid (91%), R_f: 0.24.

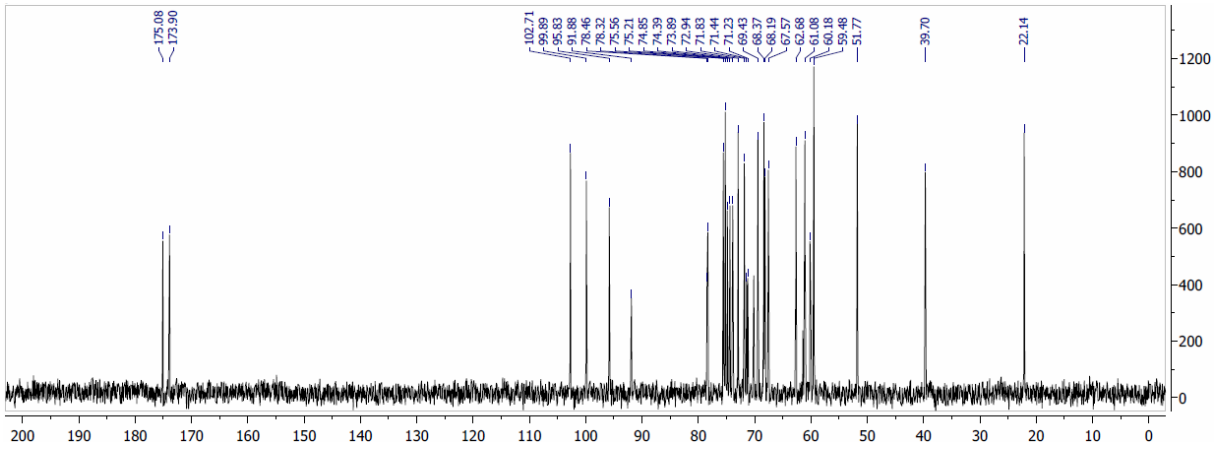


^{1}H NMR (500 MHz, D_2O) δ = 5.29 (d, *J* = 3.7 Hz, 0.4H, 1α-H), 4.73 (d, *J* = 8.1 Hz, 0.6H, 1β-H), 4.59 (d, *J* = 7.9 Hz, 1H, 1´β-H), 4.18 (ddd, *J* = 9.9, 1.9, 2.9 Hz, 1H, 3'-H), 4.06-3.99 (m, 1.8H, 4'-,6α-H),3.98-3.85 (m, 5H, 6''-, 5''-, 5-, 6'-H), 3.82-3.74 (m, 4H, 6β-, 5'-, 4-H) 3.74-3.68 (m, 4H, 4''-, 3-, 9''-H), 3.68-3.65 (m, 2.4H, 7''-,2α-, 8''-H), 3.64-3.61 (m, 1H, 2'-H), 3.35 (dd, *J* = 8.5 Hz, 0.6H, 2β-H), 2.82 (dd, *J* = 12.4, 4.6 Hz, 1H, 3_{eq}''-H),2.10 (s, 3H, 11''-H), 1.86 (dd, *J* = 12.4, 12.1 Hz, 1H, 3_{ax}''-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.08 (C-1''), 173.90 (C-10''), 102.71 (C-1'), 99.89 (C-2''), 95.83 (C-1β), 91.88 (C-1α), 78.46(C-4α), 78.32 (C-4β), 75.56 (C-3'), 75.21 (C-5'), 74.85(C-2α), 74.39 (C-2β), 73.89 (C-4''), 72.94 (C-3), 71.83 (C-6''), 71.44 (C-5α), 71.23 (C-5β), 69.43 (C-2'), 68.37(C-8''), 68.19 (C-7''), 67.57 (C-4'), 62.68 (C-9''), 61.08 (C-6'), 60.18 (C-6α), 59.48 (C-6β), 51.77(C-5''), 39.70 (C-3''), 22.14 (C-11'').

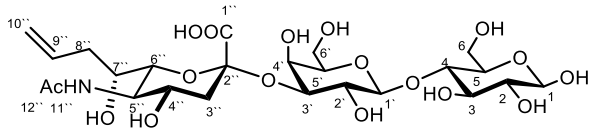
HRMS (ESI)$^+$: *m/z* calcd for $[C_{23}H_{39}N_1O_{19}]+Na^+$ = 656.2009; found 656.2010





Allyl analog of α2,3-sialyllactose (**37**):

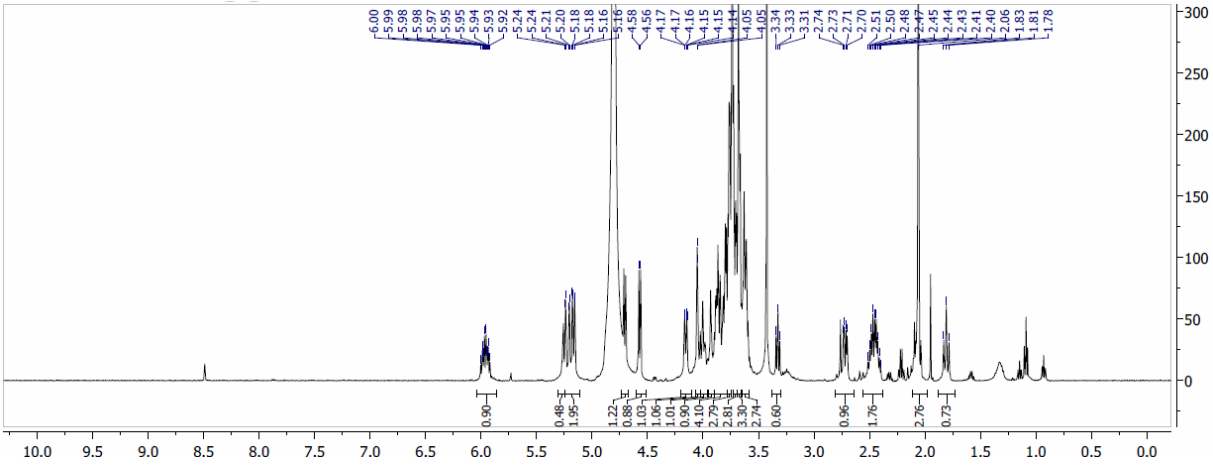
General procedure see main paper.
Yield: 67.8 mg colorless solid (80%), R_f: 0.31.

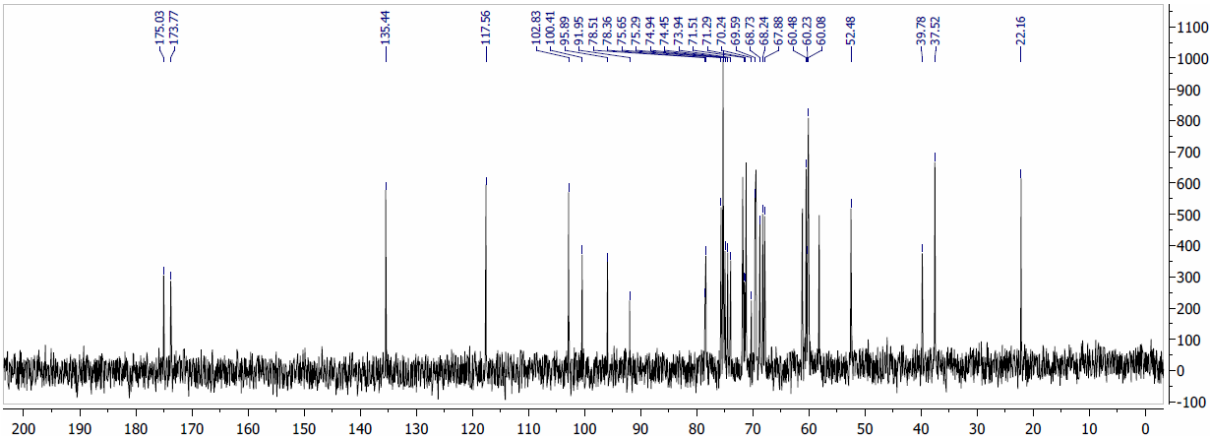


^{1}H NMR (500 MHz, D_2O) δ = 5.96 (ddt, *J* = 17.2, 10.2, 7.0 Hz, 1H, 9''-H), 5.26 (d, *J* = 3.8 Hz, 1H, 1α-H), 5.22 (dd, *J* = 17.2, 1.9 Hz, 1H, 1H, 10''-H), 5.22 (dd, *J* = 10.2, 1.9 Hz, 1H, 10''-H), 4.70 (d, *J* = 7.8 Hz, 1H, 1β-H), 4.57 (d, *J* = 7.9 Hz, 1H, 1'-H), 4.18 – 4.13 (m, 1H, 3'-H), 4.07-4.03 (dm, *J* = 3.1 Hz, 1H, 4'-H) 4.03 – 3.96 (m, 1H, 6β-H), 3.95-3.90 (m, 1H, 6α-H), 3.90-3.87 (m, 4H, 5''-, 6'-, 3β-, 4''-H) 3.87-3.75 (m, 2H, 6''-H), 3.73-3.79 (m, 3H, 5'-, 4α+β-,3α-H), 3.69-3.65 (m, 2H, 7''-, 5α+β-H), 3.65-3.59 (m, 2H, 2'-, 2α-H), 3.33 (dd, *J* = 8.5 Hz, 1H, 2β-H), 2.72 (dd, *J* = 12.2, 4.5 Hz, 1H, 3''-H), 2.56-2.38 (m, 2H, 8''-H), 2.06 (s, 3H, 12''-H), 1.81 (dd, *J* = 12.1 Hz, 1H, 3''-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.03 (C-12''), 173.77 (C-1''), 135.44 (C-9''), 117.56 (C-10''), 102.83 (C-1'), 100.41 (C-2''), 95.89 (C-1β), 91.95 (C-1α), 78.51(C-4α), 78.36 (C-4β), 75.65 (C-3'), 75.29 (C-3β), 75.29 (C-6''), 74.94(C-3α), 74.45 (C-2α), 73.94 (C-2β), 71.51 (C-5β), 71.29 (C-5α), 70.24 (C-7''), 69.59 (C-2'), 68.73 (C-4''), 68.24 (C-4'), 67.88 (C-5'), 60.48 (C-6'), 60.23 (C-6α),60.08 (H-6β), 52.48(C-5''), 39.78 (C-3''), 37.52 (C-8'') 22.16 (C-12'').

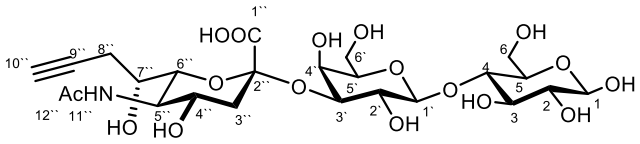
HRMS (ESI)$^+$: *m/z* calcd for $[C_{24}H_{39}N_1O_{17}]+Na^+$ = 636.2110; found 636.2110





Propargyl analog of α2,3-sialyllactose (**38**):

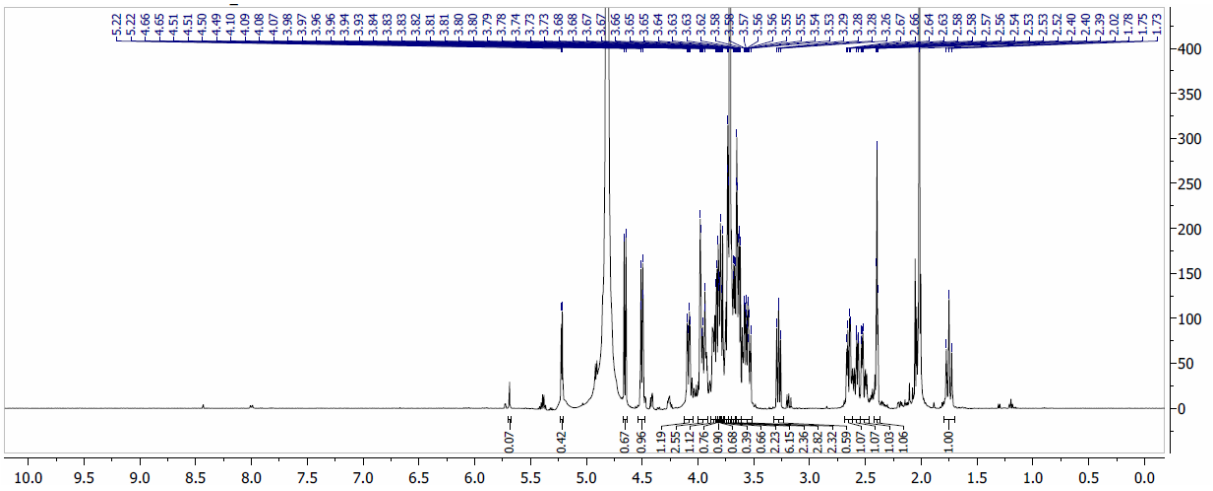
General procedure see main paper.
Yield: 78.2 mg colorless solid (92%), R_f: 0.24.

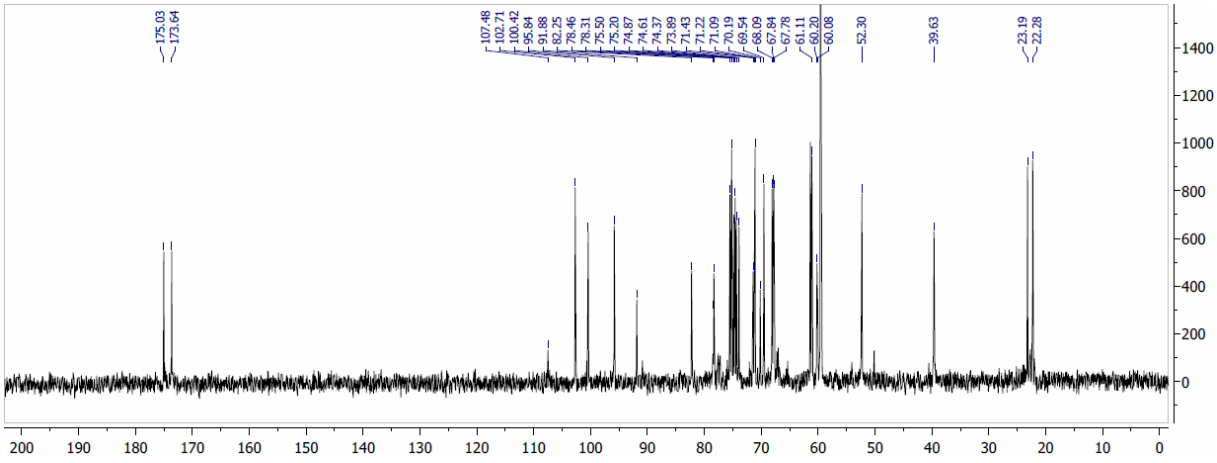


^{1}H NMR (500 MHz, D_2O) δ = 5.22 (d, *J* = 3.8 Hz, 0.4H, 1α-H), 4.65 (d, *J* = 8.0 Hz, 0.6H, 1β-H), 4.50 (d, *J* = 7.9 Hz, 1H, 1'-H), 4.08 (ddm, *J* = 9.9, 2.9 Hz, 1H, 3'-H), 3.98 (dm, *J* = 3.2, 1H, 4'-H) 3.97-3.91 (m, 1.6H, 6''-,6β-H), 3.88-3.82 (m, 2H, 7''-, 6α-H), 3.83-3.77 (m, 2H, 5α/β-, 5''-H) 3.76-3.72 (m, 2H, 6'-H), 3.69-3.61 (m, 4H, 5'-, 4α/β-, 4''-, 3α/β-H), 3.61-3.52 (m, 2H, 2'-, 2α-H), 3.28 (dd, *J* = 9.0, 8.0 Hz, 0.6H, 2β-H), 2.65 (dd, *J* = 12.2, 4.4 Hz, 1H, 3_{eq}''-H), 2.59 (ddd, *J* = 16.9, 7.8, 2.6 Hz, 1H, 8''-H), 2.51 (ddd, *J* = 16.9, 5.9, 2.7 Hz, 1H, 8''-H) 2.40 (t, *J* = 2.6 Hz, 1H, 10''-H), 2.02 (s, 3H, 12''-H), 1.75 (dd, *J* = 12.2, 12.1 Hz, 1H, 3_{ax}''-H).

^{13}C NMR (126 MHz, D_2O) δ = 175.03 (C-1''), 173.64 (C-11''), 107.48 (C-1'), 102.71 (C-2''), 95.84 (C-1β), 91.88 (C-1α), 82.25 (C-9''), 78.46(C-4α), 78.31 (C-4β), 75.50 (C-3'), 75.20 (C-4''), 74.87(C-3α), 74.61 (C-3β), 74.37 (C-2α), 73.89 (C-2β), 71.43 (C-5α), 71.22 (C-5β), 71.09 (C-10''), 70.19 (C-6''), 69.54 (C-2'), 68.09 (C-5'), 67.84 (C-4'), 67.02 (C-7''), 61.11 (C-6'), 60.20 (C-6β), 60.08 (C-6α), 52.30(C-5''), 39.63 (C-3''), 22.68 (C-8''), 22.28 (C-12'').

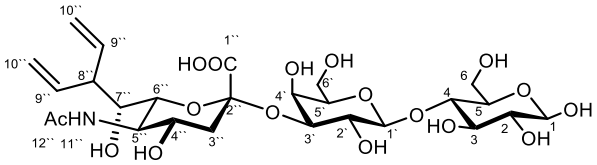
HRMS (ESI)$^+$: *m/z* calcd for $[C_{24}H_{37}N_1O_{17}]+H^+$ = 612.2134; found 612.2135





Penta-1,4-dien-3-yl analog of α2,3-sialyllactose (**39**):

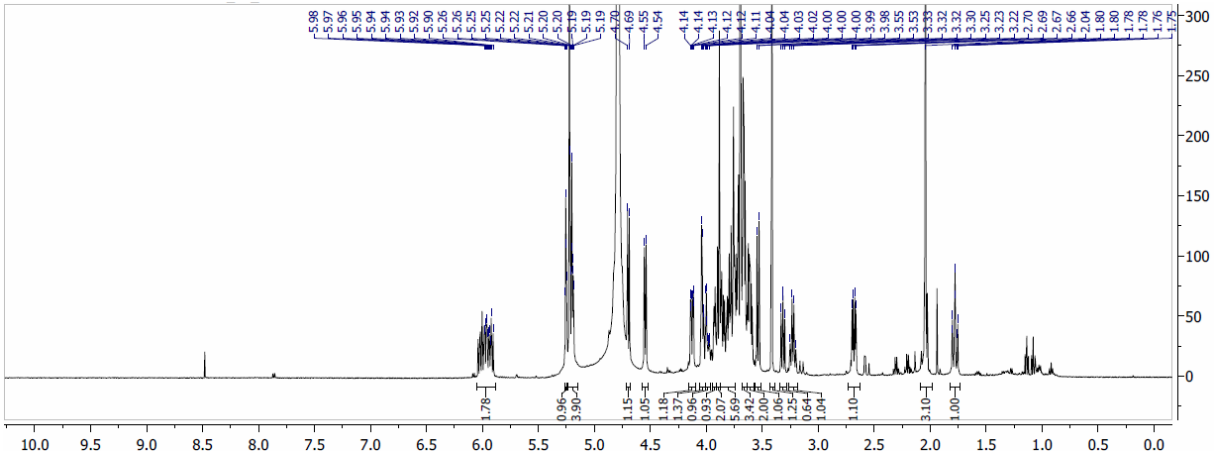
General procedure see main paper.
Yield: 62.9 mg colorless solid (78%), R_f: 0.35.

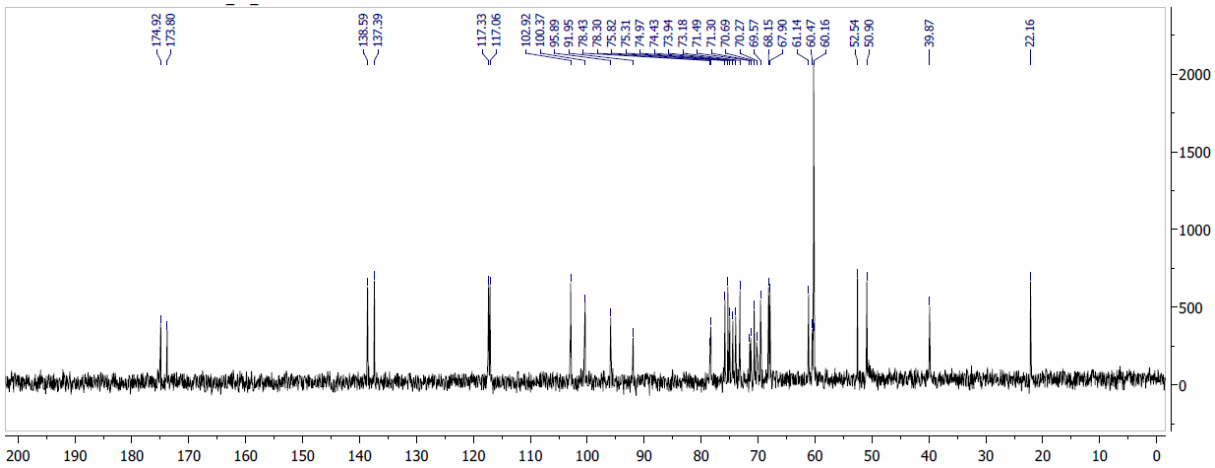


^{1}H NMR (500 MHz, D_2O) δ = 6.00 (ddd, *J* = 17.8, 10.4, 7.9 Hz, 1H, 9''-H), 5.94 (ddd, *J* = 16.5, 11.2, 8.6 Hz, 1H, 9''-H), 5.26 – 5.24 (m, 1H, 1α-H), 5.23 – 5.18 (m, 4H, 10''-H), 4.70 (d, *J* = 8.0 Hz, 1H, 1β-H), 4.55 (d, *J* = 7.9 Hz, 1H, 1'-H), 4.13 (dm, *J* = 9.1 Hz, 1H, 3'-H), 4.04 (dm, *J* = 3.2 Hz, 1H, 4'-H) 4.03 – 3.97 (m, 1H, 6β-H), 3.94-3.91 (m, 1H, 6α-H), 3.91-3.87 (m, 2H, 5''-, 6''-H) 3.87-3.68 (m, 6H, 5α+β-, 3β-, 6'-, 4β-, 3α-H), 3.68-3.64 (m, 2H, 4''-, 5'-H), 3.64-3.58 (m, 3H, 2'-, 4α-, 2α-H), 3.54 (d, *J* = 9.2 Hz, 1H, 7''-H), 3.32 (dd, *J* = 9.1, 8.0 Hz, 1H, 2β-H), 3.23 (dm, *J* = 8.5 Hz, 1H, 8''-H), 2.68 (dd, *J* = 12.2, 4.4 Hz, 1H, 3''-H), 2.04 (s, 3H, 12''-H), 1.78 (dd, *J* = 12.1 Hz, 1H, 3''-H).

^{13}C NMR (126 MHz, D_2O) δ = 174.92 (C-12''), 173.80 (C-1''), 138.59 (C-9''), 137.39 (C-9''), 117.33 (C-10''), 117.06 (C-10''), 102.92 (C-1'), 100.37 (C-2''), 95.90 (C-1β), 91.95 (C-1α), 78.43(C-4α), 78.30 (C-4β), 75.82 (C-3'), 75.31 (C-3β), 74.97(C-3α), 74.44 (C-2α), 73.95 (C-2β), 73.18 (C-6''), 71.49 (C-5β), 71.30 (C-5α), 70.69 (C-2'), 70.27 (C-7''), 69.58 (C-4''), 68.15 (C-4'), 67.91 (C-5'), 61.14 (C-6'), 60.47 (C-6β),60.16 (C-6α), 52.54(C-5''), 50.90 (C-8''), 39.87 (C-3''), 22.16 (C-12'').

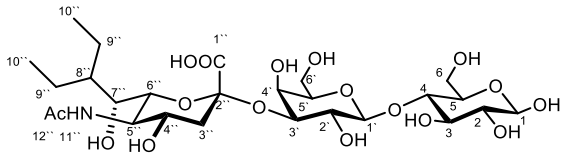
MS (ESI)$^-$: *m/z* calcd for $[C_{26}H_{41}N_1O_{17}]$-H^+ = 638.23; found 638.23





Pentan-3-yl analog of α2,3-sialyllactose (**40**):

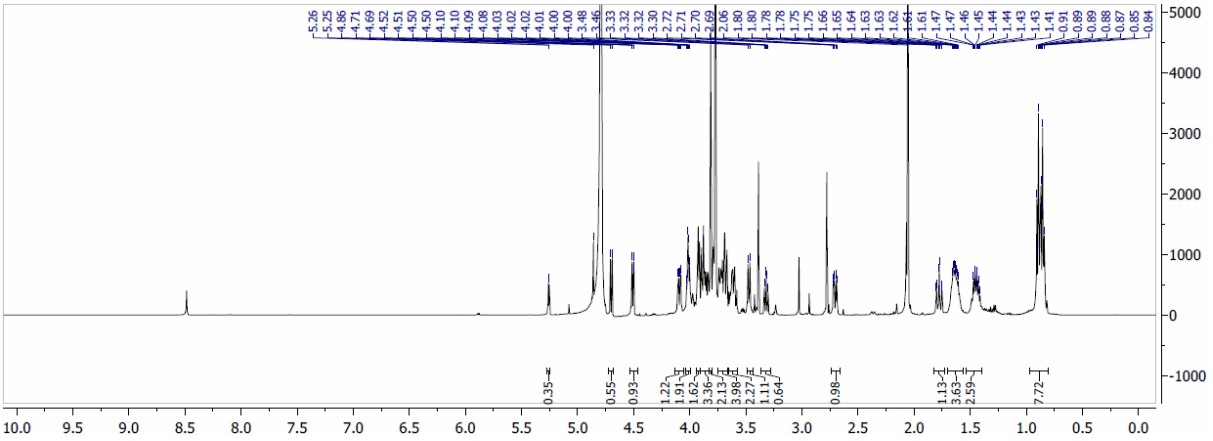
3-Pentenyl-Sia-Lac (24.2 mg) was dissolved in dry methanol (15 mL), then palladium on coal (25 mg) was added and an orsat rubber expansion bag with ~2 bar of H_2 was placed on the flask for 24 h at RT with vigorous stirring. After filtration over celite the solvent was removed under vacuum. Yield: 21.5mg (89%) colorless solid, R_f: 0.31.

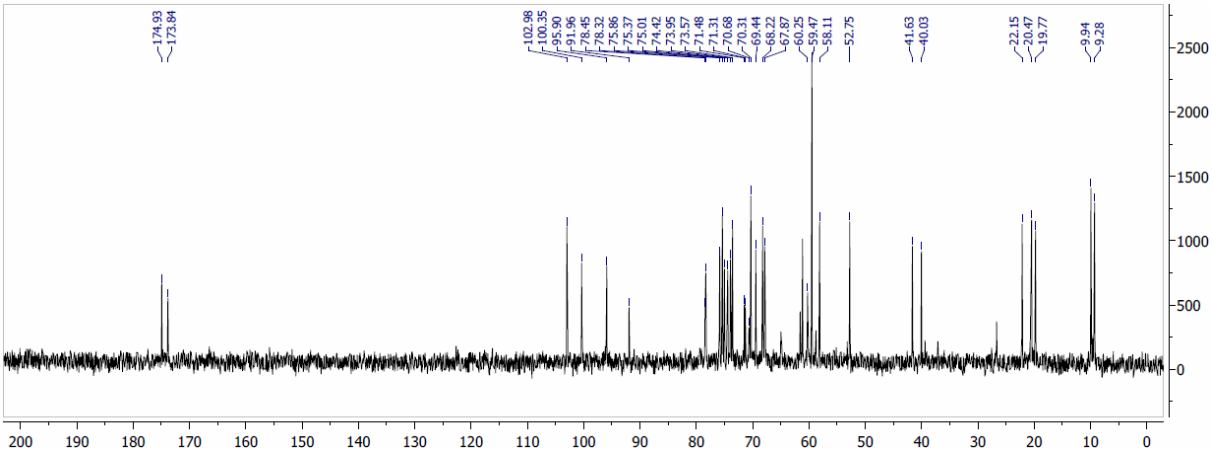


^{1}H NMR (500 MHz, D_2O) δ = 5.25 (d, *J* = 3.8 Hz, 1H, 1α-H), 4.70 (d, *J* = 7.9 Hz, 1H, 1β-H), 4.50 (dd, *J* = 7.9, 1.7 Hz, 1H, 1'-H), 4.09 (dd, *J* = 9.8, 3.1 Hz, 1H, 3'-H), 4.02-3.99 (m, 1H, 4'-H) 3.93 – 3.90 (m, 1H, 6β-H), 3.90-3.82 (m, 4H, 5''-, 6''-, 5β+α-, 6α-H), 3.82-3.79 (m, 2H, 6'-H) 3.74-3.66 (m, 3H, 3β+α-, 4β+α-, 5'-H), 3.65-3.57 (m, 3H, 2α-, 4''-, 2'-H), 3.47 (d, *J* = 8.8, 1H, 7''-H), 3.32 (dd, *J* = 8.9, 7.9 Hz, 1H, 2β-H), 2.70 (dd, *J* = 12.2, 4.4 Hz, 1H, 3''-H), 2.06 (s, 3H, 12''-H), 1.78 (dd, *J* = 12.1, 12.1 Hz, 1H, 3''-H), 1.69-1.56 (m, 3H, 8''-, 9''-H), 1.53-1.39 (m, 2H, 9''-H), 0.89 (t, 3H, *J* = 7.3Hz, 10''-H), 0.85 (t, *J* = 7.3Hz, 3H, 10''-H).

^{13}C NMR (126 MHz, D_2O) δ = 174.93 (C-12''), 173.84 (C-1''), 102.98 (C-1'), 100.35 (C-2''), 95.90 (C-1β), 91.96 (C-1α), 78.45(C-4α), 78.32 (C-4β), 75.86 (C-3'), 75.37 (C-3β), 75.01(C-3α), 74.42 (C-2α), 73.95 (C-2β), 73.57 (C-6''), 71.48 (C-5β), 71.31 (C-5α), 70.68 (C-4''), 70.31 (C-7''), 69.44 (C-2'), 68.22 (C-5'), 67.91 (C-4'), 60.25 (H-6α), 59.47 (C-6β), 58.11 (C-6'), 52.75(C-5''), 41.63 (C-8''), 40.03 (C-3''), 22.15 (C-12''), 20.47 (C-9''), 19.77 (C-9''), 9.94 (C-10''), 9.28 (C-10'').

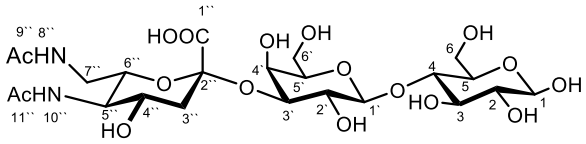
HRMS (ESI)$^+$: *m/z* calcd for $[C_{26}H_{45}N_1O_{17}]+H^+$ = 644.2760; found 644.2761





Truncated acetamido analog of α2,3-sialyllactose (**41**):

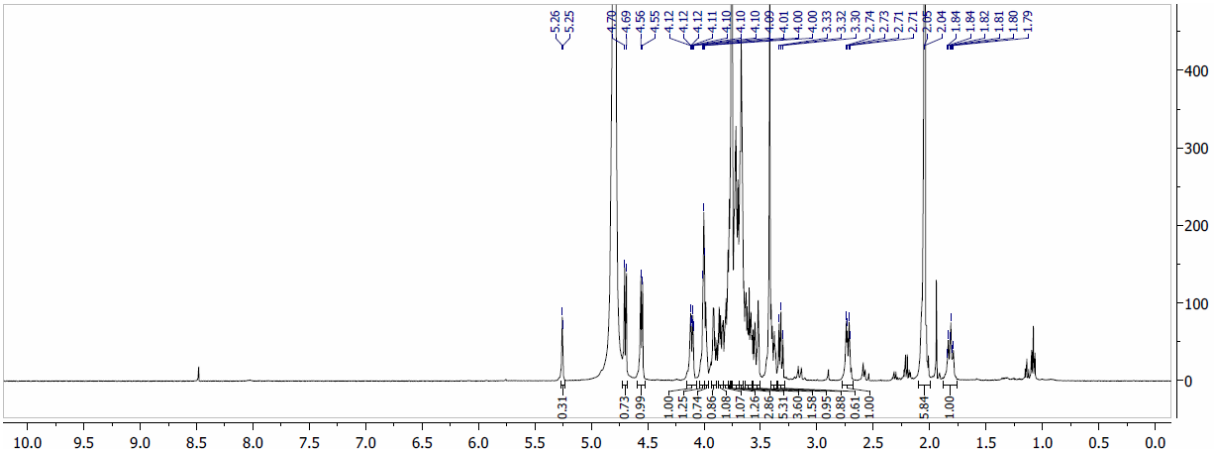
General procedure see main paper.
Yield: 53.7 mg colorless solid (63%), R_f: 0.22.

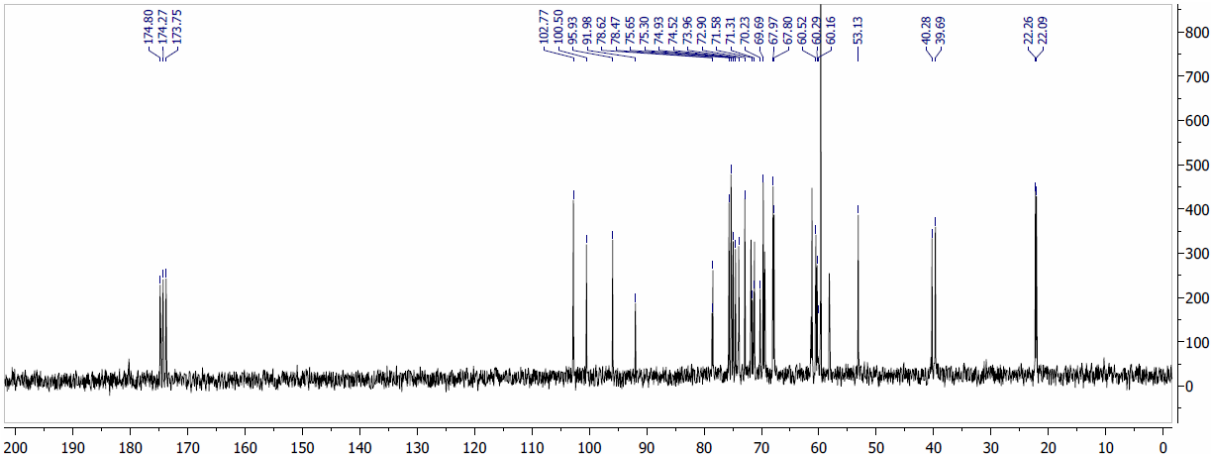


^{1}H NMR (500 MHz, D_2O) δ = 5.25 (d, *J* = 3.7 Hz, 1H, 1α-H), 4.70 (d, *J* = 8.0 Hz, 1H, 1β-H), 4.55 (d, *J* = 7.6 Hz, 1H, 1'-H), 4.11 (ddd, *J* = 9.8, 3.3, 1.9 Hz, 1H, 3'-H), 4.02-3.99 (m, 1H, 4'-H) 4.00-3.99 (m, 1H, 6α-H), 3.94-3.89 (m, 1H, 6β-H), 3.88-3.79 (m, 2H, 6'-H) 3.79-3.77 (m, 1H, 4''-H), 3.75-3.68 (m, 5H, 6''-, 4α/β-, 3β-, 5''-, 5α/β-H), 3.69-3.65 (m, 2H, 5'-, 3α-H), 3.63-3.58 (m, 2H, 2α-, H2'-H), 3.56-3.50 (dm, *J* = 14.2 Hz, 1H, 7''-H), 3.41-3.36 (dm, , *J* = 14.2 Hz, 1H, 7''-H), 3.32 (m, 0.6H, 2β-H), 2.73 (dd, *J* = 12.3, 4.5 Hz, 1H, 3''-H) 2.04 (s, 6H, 9''-, 11''-H), 1.81 (dd, *J* = 12.3, 12.2 Hz, 1H, 3''-H).

^{13}C NMR (126 MHz, D_2O) δ = 174.80 (C-11''), 174.27 (C-8''), 173.75 (C-1'') 102.77 (C-1'), 100.50 (C-2'), 95.93 (C-1β), 91.98 (C-1α), 78.62 (C-4α), 78.47 (C-4β), 75.65 (C-3'), 75.30 (C-3β), 74.93(C-3α), 74.52 (C-2α), 73.96 (C-2β), 72.90 (C-6''), 71.58 (C-5α), 71.31 (C-5β), 70.23 (C-4''), 69.96 (C-2'), 67.97 (C-5'), 67.80 (C-4'), 60.52 (C-6'), 60.29 (C-6β), 60.16 (C-6α), 53.13 (C-5''), 40.28 (C-7''), 39.69 (C-3''), 22.26 (C-9''), 22.09 (C-10'').

HRMS (ESI)$^+$: *m/z* calcd for $[C_{23}H_{38}N_2O_{17}]+H^+$ = 615.2243; found 615.2239





References

[1] M. Knorst, W.-D. Fessner, *Adv. Synth. Catal.* **2001**, *343*, 698-710.

[2] A. Mertsch, N. He, D. Yi, M. Kickstein, W. D. Fessner, *Chem. Eur. J.* **2020**, *26,* 11614-11624.

[3] P. A. Veronesi, P. E. A. Rodriguez, E. Peschechera, S. L. Veronesi, WO 2008/090151 A1, **2008**.

[4] S. Buchini, F. X. Gallat, I. R. Greig, J. H. Kim, S. Wakatsuki, L. M. Chavas, S. G. Withers, *Angew. Chem. Int. Ed.* **2014**, *53*, 3382-3386.

[5] G. J. Ray, N. Ravenscroft, J. Siekmann, Z. Zhang, P. Sanders, U. Shaligram, C. M. Szabo, P. Kosma, *Bioconjug. Chem.* **2014**, *25*, 665-676.

[6] P. Allevi, P. Rota, R. Scaringi, R. Colombo, M. Anastasia, *J. Org. Chem.* **2010**, *75*, 5542-5548.

[7] R. Brossmer, L. Holmquist, *FEBS Lett.* **1974**, *40*, 250-252.

[8] a) R. L. McLean, M. Suttajit, J. Beidler, R. J. Winzler, *J. Biol. Chem.* **1971**, *246*, 803-809; b) N. He, D. Yi, W.-D. Fessner, *Adv. Synth. Catal.* **2011**, *353*, 2384-2398.