Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Automated Glycan Assembly of Peptidoglycan Backbone Fragments

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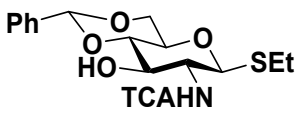
Contents

1. General Information

Solvents and reagents were used as supplied without any further purification. The automated syntheses were performed on a home-built synthesizer, developed at the Max Planck Institute of Colloids and Interfaces. Anhydrous solvents were taken from a dry solvent system (JC-Meyer Solvent Systems). Analytical thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in staining solution (*p*-anisaldehyde stain: 135 mL of EtOH, 5 mL of H_2SO_4, 1.5 mL of AcOH and 3.7 mL of *p*-anisaldehyde; $KMnO_4$ stain: 1.5 g of $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL water). Column chromatography was carried out using Sigma Aldrich silica gel 60 (230–400 mesh). Products were lyophilized using a Christ Alpha 2–4 LD plus freeze dryer. NMR spectra were recorded on a Bruker Ascend (400 MHz), Varian 400-MR (400 MHz), Varian 600-MR (600 MHz), or Bruker Biospin AVANCE700 (700 MHz) spectrometer. Spectra were recorded in $CDCl_3$ (7.26 ppm ^1H, 77.0 ppm ^{13}C) or DMSO-d^6 (2.50 ppm ^1H, 39.5 ppm ^{13}C) or CD_3OD (3.31 ppm, 49.3 ppm ^{13}C) or D_2O (4.79 ppm ^1H) by using the solvent residual peak chemical shift as internal standard. Optical rotations were measured using a UniPol 7000 polarimeter (Schmidt&Haensch) with concentrations expressed as g/100 mL. IR spectra were recorded on an FT-IR spectrometer from Perkin-Elmer. High-resolution mass spectra were obtained using a UPLC Acquity H-class coupled with ESI-QTOF G2-Xevo-XS from Waters and a MALDI-TOF-autoflex™ (Bruker). Low-resolution mass spectra were obtained using an HPLC-System Serie 1100 coupled with ESI-single quadrupole from Agilent. Analytical normal phase HPLC was performed on an HPLC-System Serie 1100 from Agilent using YMC-Diol-300 column (150 x 4.6 mm). Preparative normal phase HPLC was performed on an Agilent 1200 using a preparative YMC-Diol-300 column (150 x 20 mm). Analytical reverse phase HPLC was performed on an HPLC-System Serie 1200 from Agilent using Hypercarb column (150 x 4.6 mm). Preparative reverse phase HPLC was performed on an Agilent 1200 using a preparative Hypercarb column (150 x 10 mm). Photocleavage using E-Series Photochemical reactor (UV and LED) from Vapourtec.

2. Building Blocks



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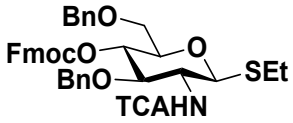
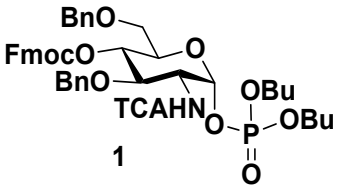


S1



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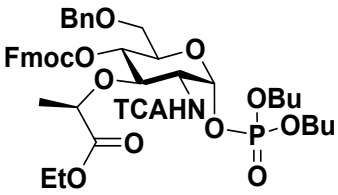

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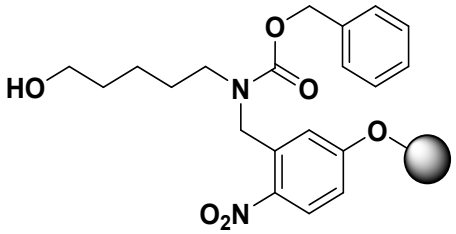
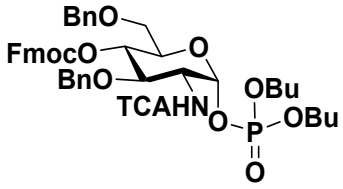


7

Intermediates **3** and **S1** were synthesized as reported in literature.[1, 2] Linker-functionalized resin **7** was prepared according to literature.[3] The Resin loading was determined as described previously.[4]

Dibutylphosphoryloxy 3,6-di-*O*-benzyl-2-deoxy-4-*O*-fluorenylmethoxycarbonyl-2-trichloroacetamido-α-D-glucopyranoside (1)

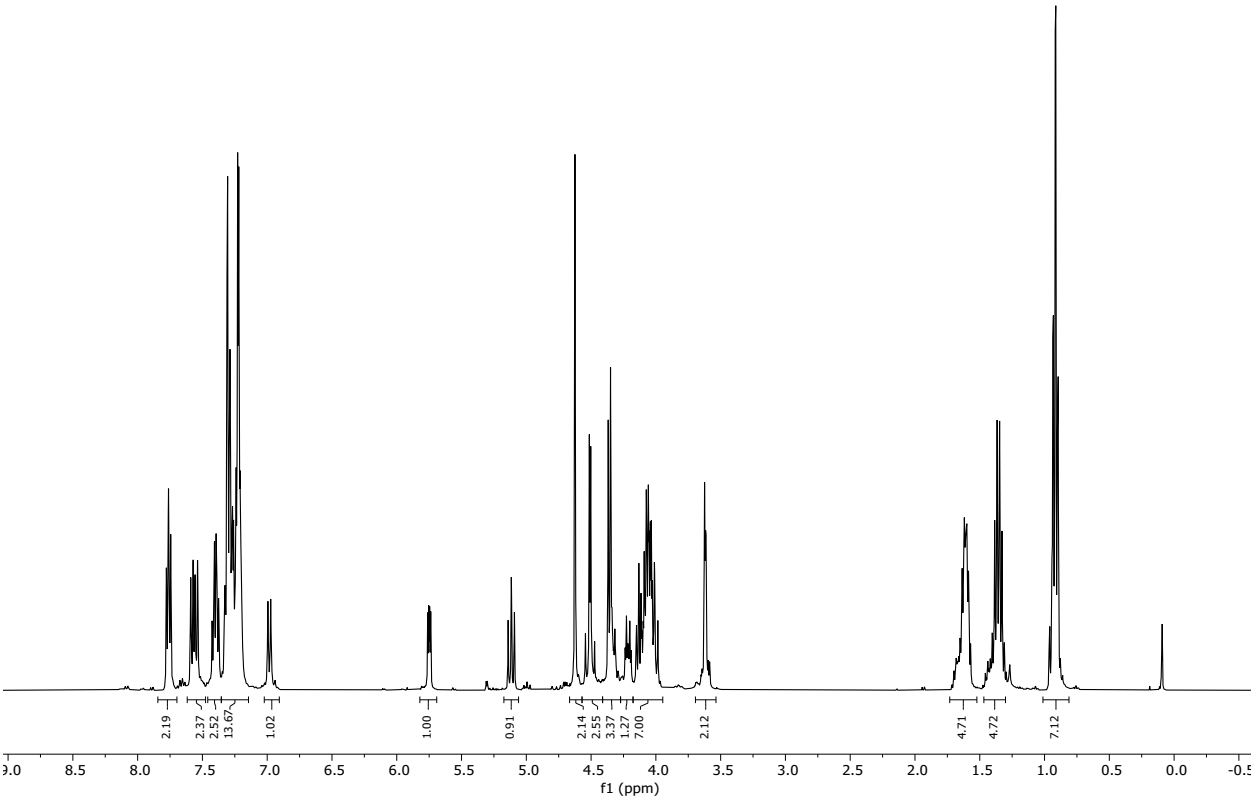


A solution of dibutylphosphate (5.00 mL, 25.2 mmol) in DCM (15 mL) was dried over molecular sieves. After 1 h the supernatant (4.10 mL, 5.10 mmol, 1.7 eq) was added to a solution of **S1** (2.33 g, 3.00 mmol, 1 eq) in DCM (20 mL) and cooled to 0°C. Than NIS (878 mg, 3.90 mmol, 1.3 eq) was added and the reaction was stirred for 2 h. Then the mixture was quenched with a sat. aq. solution of $Na_2S_2O_3$/$NaHCO_3$ (1:1, 100 mL) and extracted with DCM (100 mL). The organic layer was dried over Na_2SO_4 and purified with a column chromatography (Hexane/EtOAc 4:1) obtaining **1** (2.24 g, 81%).

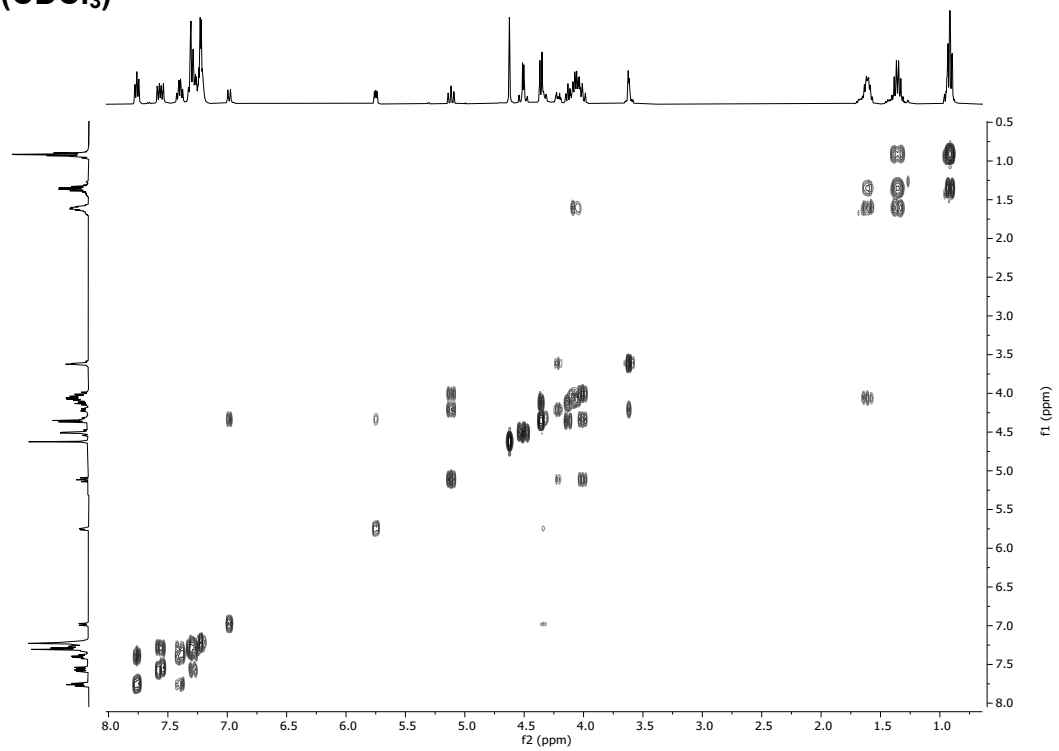
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79–7.74 (m, 2H), 7.63–7.49 (m, 2H), 7.46–7.36 (m, 2H), 7.34–7.13 (m, 12H), 6.98 (d, *J* = 8.7 Hz, 1H, N*H*), 5.75 (dd, *J* = 6.1, 3.3 Hz, 1H, H-1), 5.15–

5.07 (m, 1H, H-4), 4.62 (s, 2H, CH_2-Ph), 4.57–4.43 (m, 2H, CH_2-Ph), 4.39–4.28 (m, 3H, CH_2-Fmoc, H-2), 4.24–4.18 (m, 1H, H5), 4.18–3.95 (m, 6H, H-3, *CH*-Fmoc, Bu), 3.78–3.50 (m, 2H, H-6a, H6-b), 1.88–1.52 (m, 4H, Bu), 1.47–1.21 (m, 4H, Bu), 1.00–0.78 (m, 6H, Bu). ^{13}C NMR (101 MHz, $CDCl_3$): 13C NMR (101 MHz, CDCl3) δ 161.87, 153.96, 143.07, 142.93, 141.23, 141.19, 137.40, 137.02, 128.41, 128.28, 128.26, 127.88, 127.82, 127.75, 127.73, 127.70, 127.67, 127.12, 127.10, 124.96, 124.89, 120.04, 95.41 & 95.35 (m, C-1), 92.00 (*C*Cl_3), 75.94, 74.58, 73.99, 73.57, 70.85, 70.03, 68.42, 68.29, 68.23, 54.21, 54.13, 46.55, 32.15, 32.10, 32.08, 32.03, 32.02, 18.60, 18.50, 13.54, 13.51, 13.49. ^{31}P NMR (162 MHz, DMSO-d^6) δ −2.74. δ $[\alpha]_D^{25}$ = 42.75 (*c* 1.0, $CHCl_3$). ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{45}H_{51}Cl_3NNaO_{11}P$: 942.2158; found 942.2183. IR (neat): ν_{max} = 3234, 2963, 2876, 1755, 1715, 1522, 1453, 1386, 1258, 1029, 962 cm^{-1}.

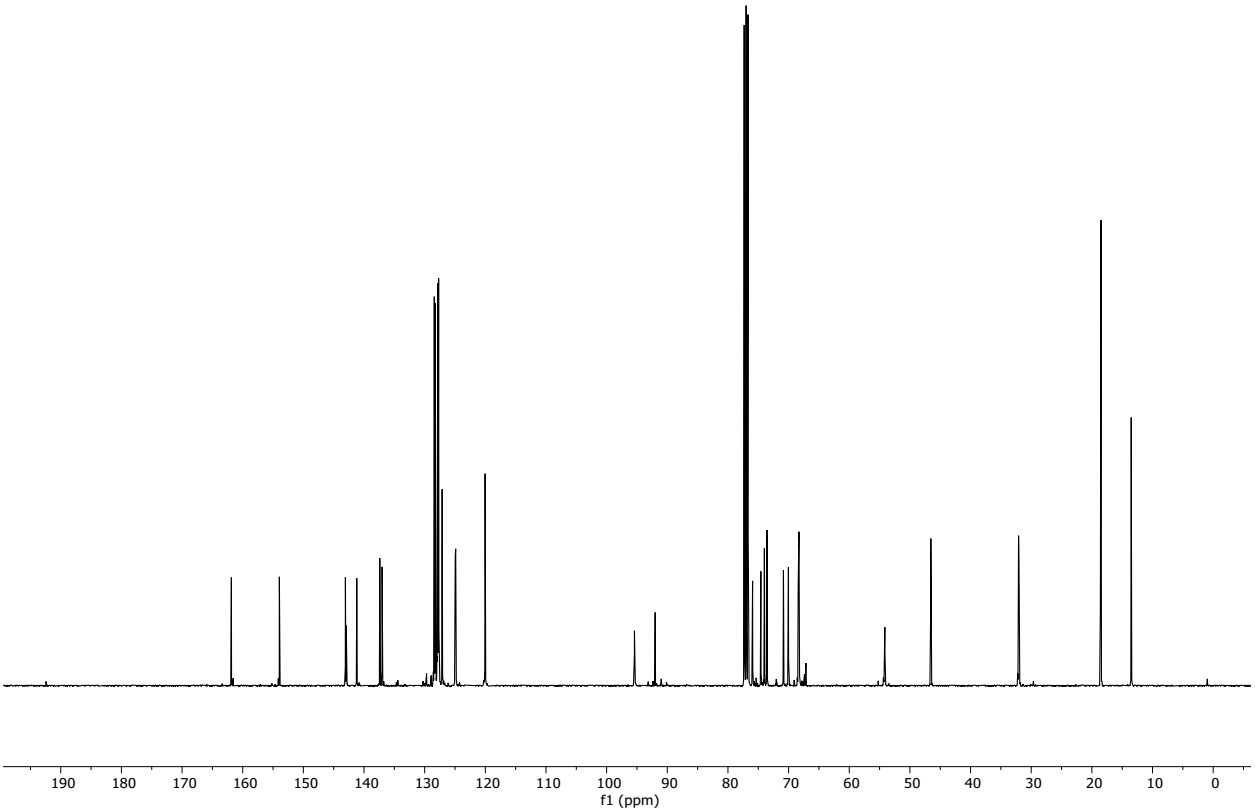
^{1}H NMR ($CDCl_3$)



COSY ($CDCl_3$)



^{13}C NMR ($CDCl_3$)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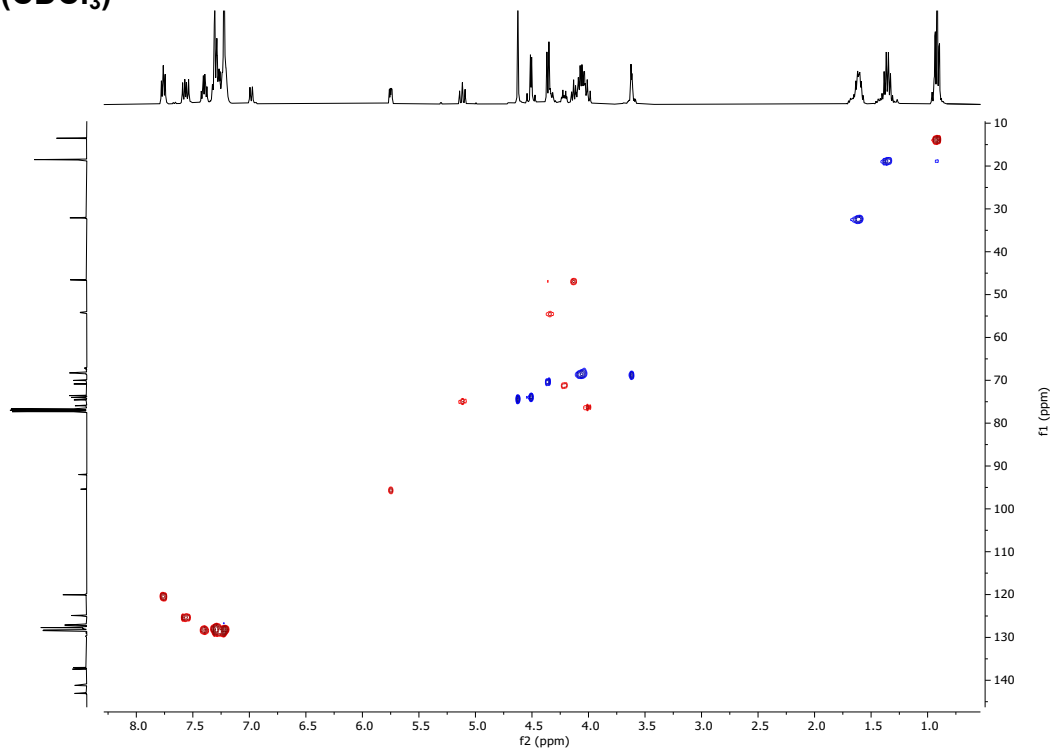
f1 (ppm)

HSQC ($CDCl_3$)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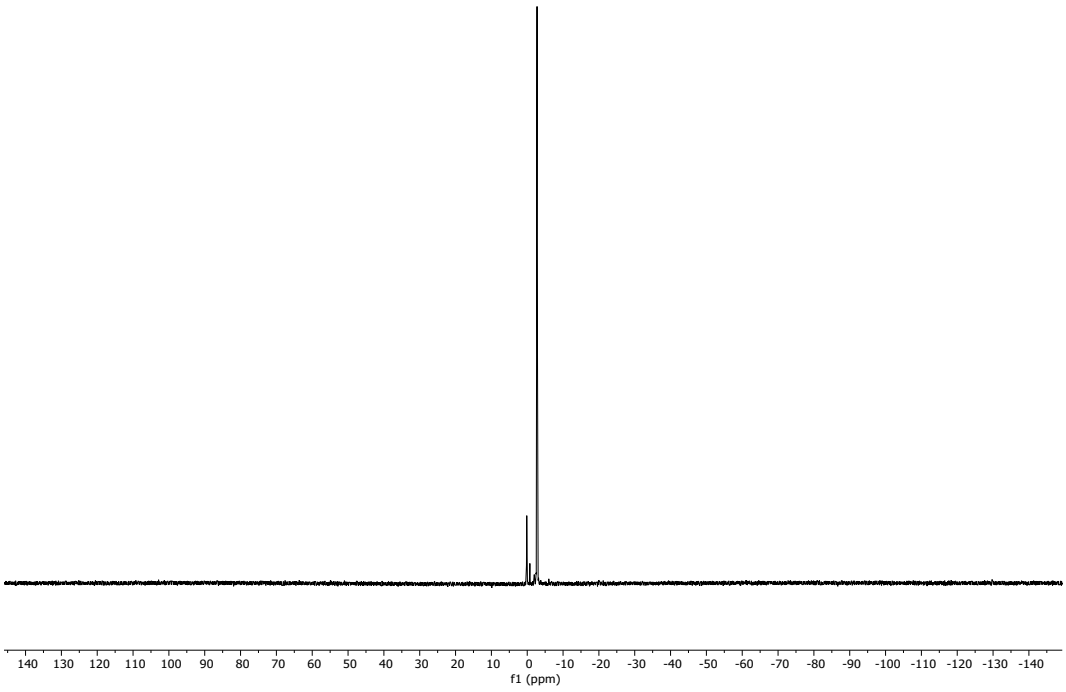
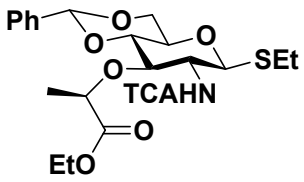
f2 (ppm)
10 20 30 40 50 60 70 80 90 100 110 120 130 140
f1 (ppm)

^{31}P NMR (162 MHz, DMSO-d^6)



Ethyl 2-deoxy-3-*O*-((*R*)-1'-ethoxycarbonylethyl)-4,6-*O*-benzylidene-1-thio-2-trichloroacetamido-β-D-glucopyranoside (4)

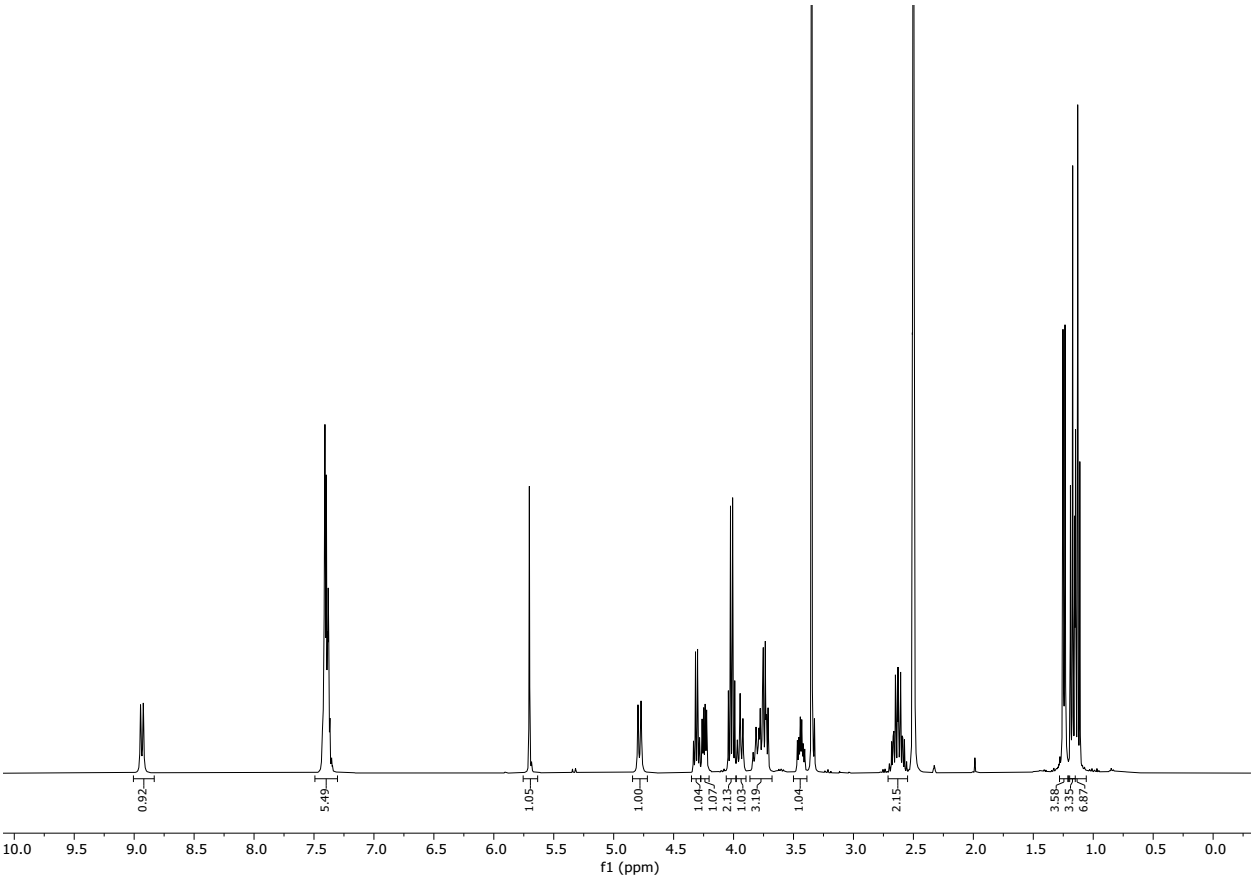


To a solution of (*S*)-LacOEt (0.91 mL, 7.92 mmol, 1 eq) in dry DCM (2.3 mL) was added 2,6-lutidine (0.93 mL, 7.92 mmol, 1 eq) under Ar atmosphere. The reaction was cooled to −78°C and Tf_2O (1.36 mL, 7.92 mmol, 1 eq) was added dropwise. The reaction mixture was stirred for 40 min and then warmed up to rt and stirred for 1 h. The organic solution was diluted with DCM/hexane (1:1) and directly purified through a short silica gel column (DCM/hexane 1:1) to afford the lactic triflate. To a mixture of **3** (600 mg, 1.32 mmol, 1 eq) in dry DCM (2.5 mL) under Ar atmosphere some drops of dry DMF were added in order to completely dissolve the starting material. Afterwards, NaH (260 mg, 6.60 mmol, 60% oil dispersion, 5 eq) was added to the solution and the reaction mixture was stirred for 1 h

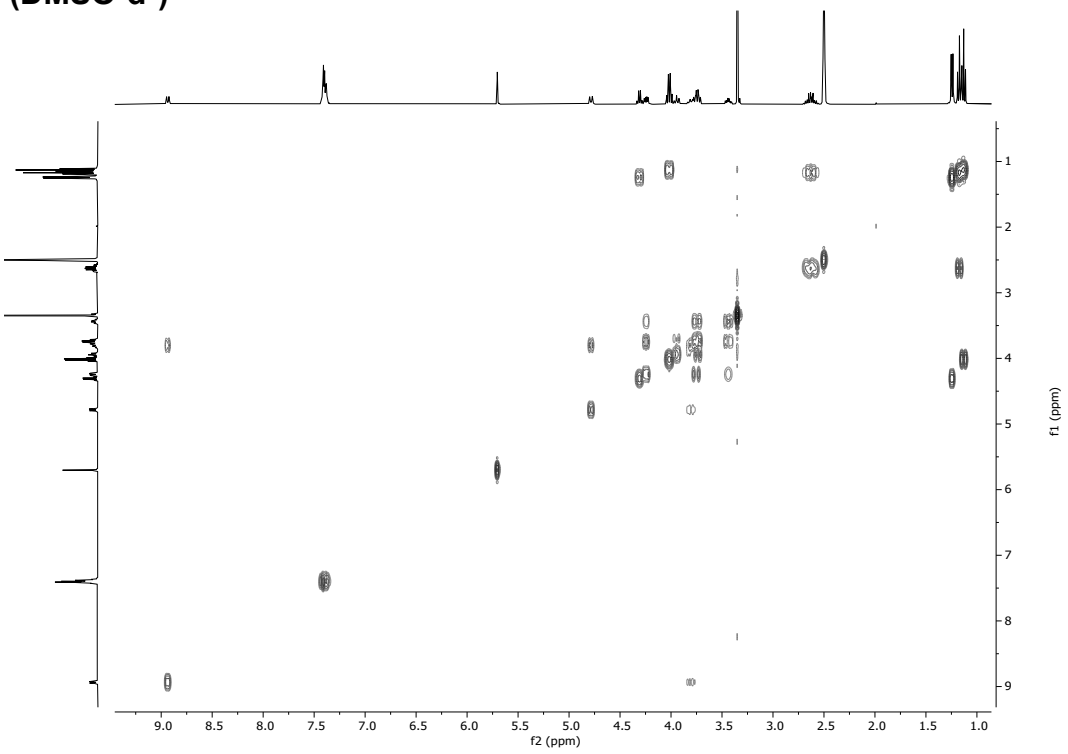
at rt. The lactic triflate was added dropwise to the reaction mixture and stirred for 2 h. The reaction mixture was diluted with EtOAc (25 mL), washed with sat. aq. solution of $NaHCO_3$ (25 mL) and brine (25 mL), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by column chromatography (hexane/EtOAc 5:1) to give **4** (386 mg, 53%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d^6): δ 8.94 (d, *J* = 9.2 Hz, 1H, N*H*), 7.40 (m, 5H, Ar), 5.70 (s, 1H, C*H*-Ph), 4.78 (d, *J* = 10.3 Hz, 1H, H-1), 4.31 (q, *J* = 6.7 Hz, 1H, C*H*-CH_3), 4.24 (dd, *J* = 10.1, 5.0 Hz, 1H, H-6a), 4.02 (q, *J* = 7.1 Hz, 2H, OEt), 3.95 (t, *J* = 9.3 Hz, 1H, H-3), 3.85–3.70 (m, 3H, H-2, H-4, H-6b), 3.48–3.39 (m, 1H, H-5), 2.70–2.55 (m, 2H, SEt), 1.24 (d, *J* = 6.7 Hz, 3H, CH-*CH$_3$*), 1.17 (t, *J* = 7.4 Hz, 3H, SEt), 1.15 (t, *J* = 7.1 Hz, 3H, OEt). ^{13}C NMR (101 MHz, DMSO-d^6): δ 172.11, 161.55, 137.95, 129.29, 128.64, 126.27, 100.46, 93.38, 84.24, 80.82, 78.40, 75.29, 70.33, 68.04, 60.71, 55.86, 23.93, 19.17, 15.52, 14.44. $[\alpha]_D^{25}$ = −28.30 (*c* 1.0, $CHCl_3$). ESI-HRMS: m/z [M+Na]$^+$ calcd. for $C_{22}H_{28}Cl_3NNaO_7S$: 580.0552; found 580.0525. IR (neat): ν_{max} = 3308, 2983, 2929, 2880, 1757, 1687, 1537, 1082, 1028, 1007 cm^{-1}.

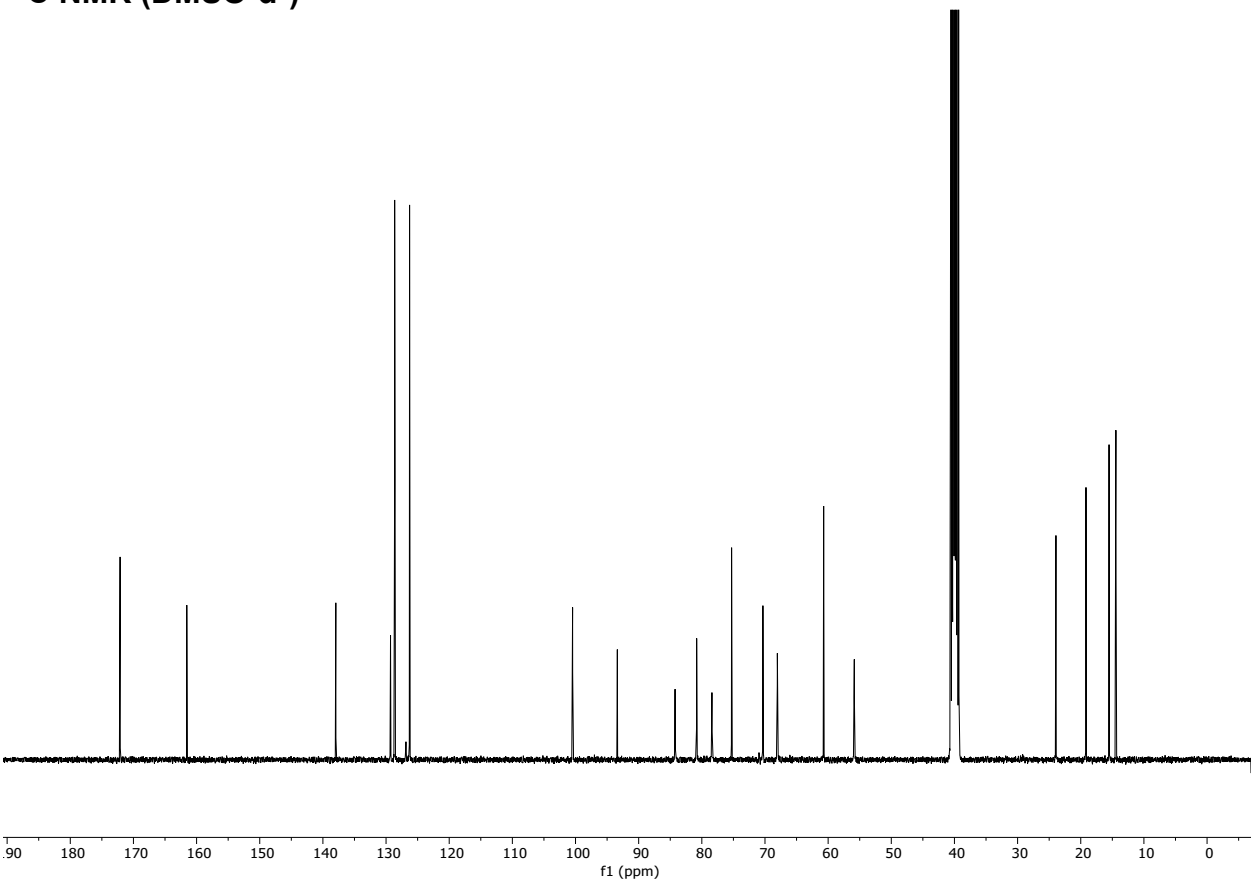
^{1}H NMR (DMSO-d^6)


0.92
5.49
1.05
1.00
1.04
1.07
2.13
1.03
3.19
1.04
2.15
3.58
3.35
6.87
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

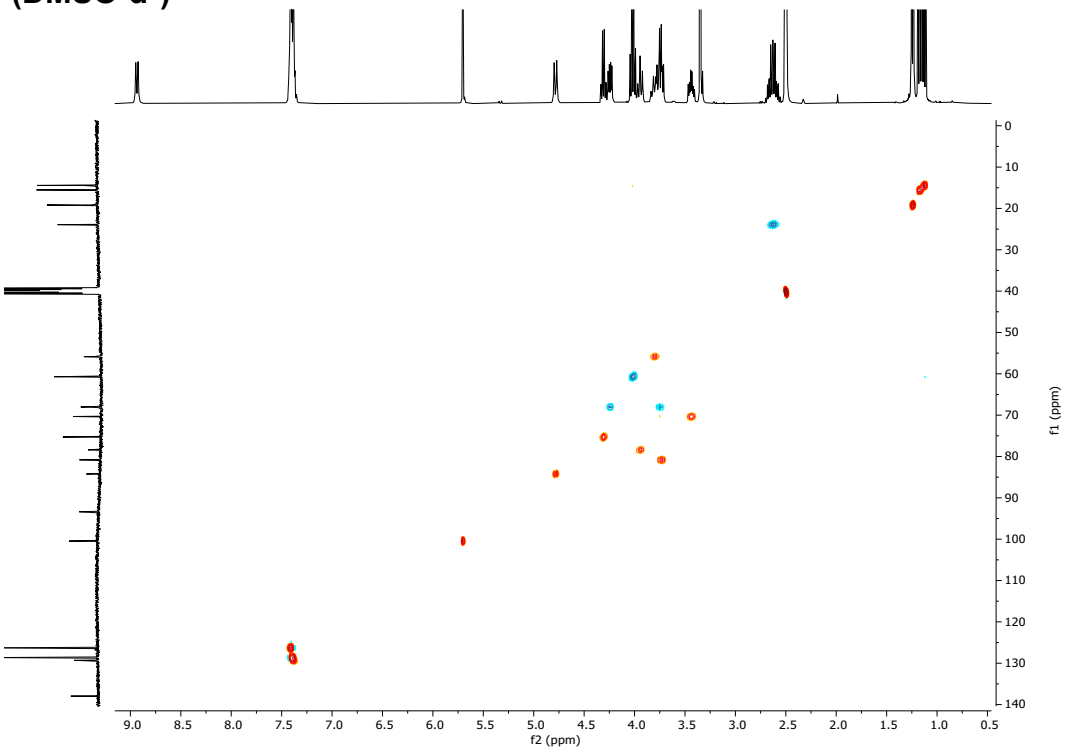
COSY (DMSO-d^6)


9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0
f2 (ppm)
1 2 3 4 5 6 7 8 9
f1 (ppm)

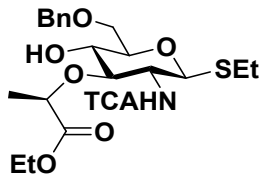
^{13}C NMR (DMSO-d^6)



HSQC (DMSO-d^6)



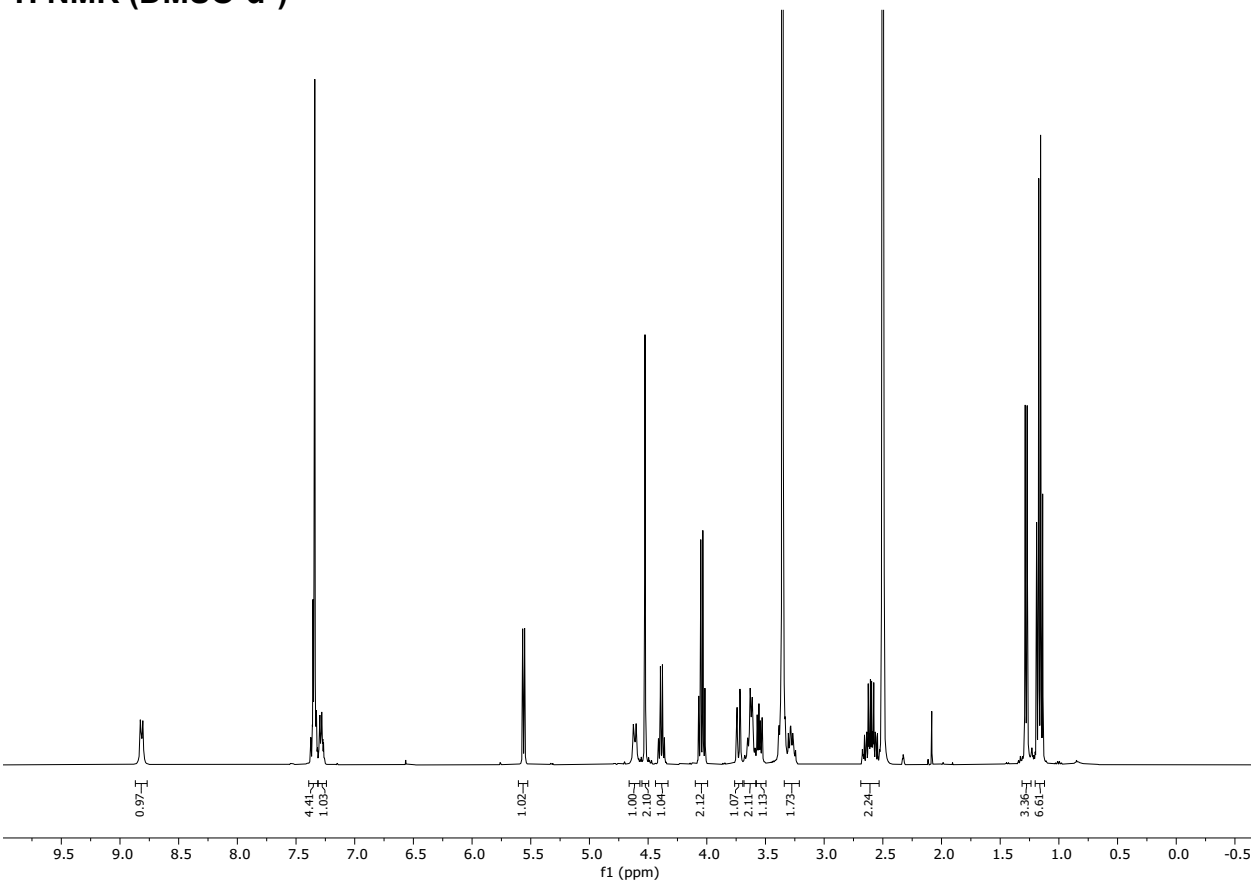
Ethyl 6-*O*-benzyl-2-deoxy-3-*O*-((*R*)-1'-ethoxycarbonylethyl)-1-thio-2-trichloroacetamido-β-D-glucopyranoside (5)



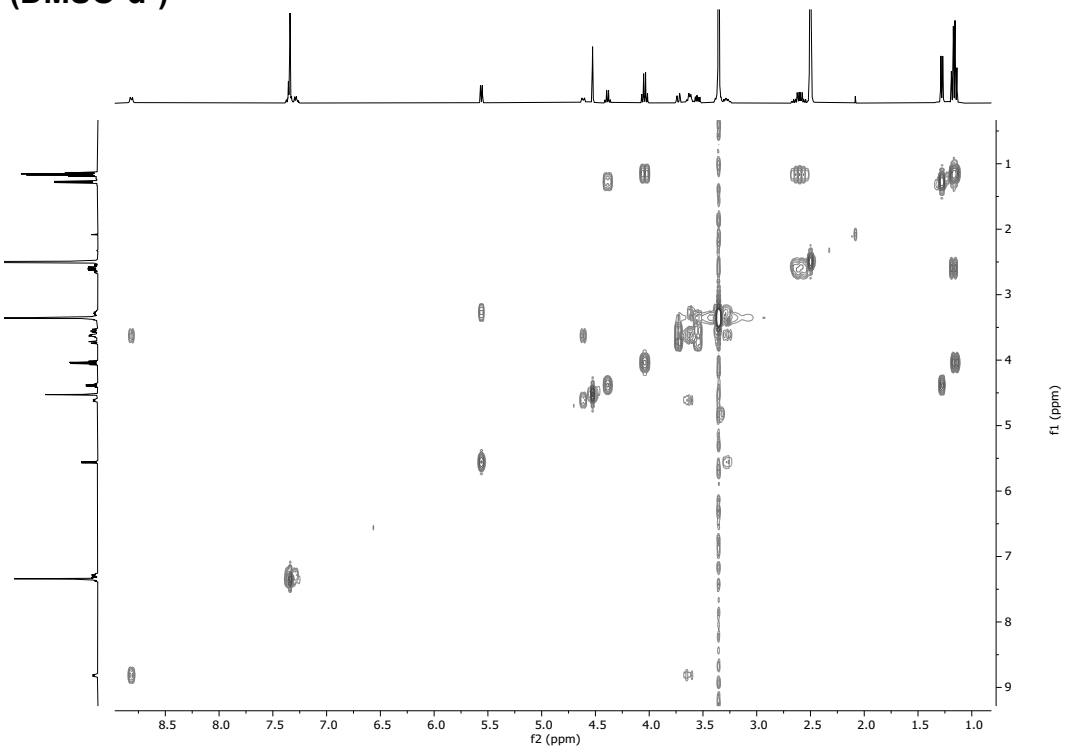
4 (347 mg, 0.62 mmol, 1 eq) was dissolved in dry CH_3CN (40 mL) under Ar and treated with $Me_3N{\bullet}BH_3$ (137 mg, 1.86 mmol, 3 eq) and $BF_3{\bullet}OEt_2$ (0.23 mL, 1.86 mmol, 3 eq) at 0 °C. After 1 h the mixture was allowed to reach rt and was diluted with ethyl acetate and quenched with saturated cold aq. $NaHCO_3$, 5% citric acid (2 x 50 mL), sat. aq. $NaHCO_3$ solution (50 mL), brine (50 mL) and the organic layer was dried over Na_2SO_4, concentrated *in vacuo* and purified with column chromatography (Hexane/EtOAc 7:3) obtaining **5** (227 mg, 65%).

^{1}H NMR (400 MHz, DMSO-d^6): δ 8.82 (d, *J* = 8.2 Hz, 1H, N*H*), 7.36–7.24 (m, 5H, Ar), 5.56 (d, *J* = 6.6 Hz, 1H, O*H*), 4.61 (d, *J* = 9.1 Hz, 1H, H-1), 4.53 (s, 2H, *CH*$_2$-Ph), 4.39 (q, *J* = 6.7 Hz, 1H, *CH*-CH$_3$), 4.04 (q, *J* = 7.1 Hz, 2H, OEt), 3.73 (dd, *J* = 11.1, 1.7 Hz, 1H, H-6a), 3.62 (m, 2H, H-2, H-3), 3.55 (dd, *J* = 11.1, 5.9 Hz, 1H, H-6b), 3.35 (m, 1H, H-5), 3.28 (m, 1H, H-4), 2.68–2.53 (m, 2H, SEt*),* 1.28 (d, *J* = 6.7 Hz, 3H, CH-C*H*$_3$), 1.20–1.13 (m, 6H, SEt, OEt). ^{13}C NMR (101 MHz, DMSO-d^6): δ 172.70, 161.47, 139.03, 128.69, 128.48, 128.01, 127.83, 93.47, 83.54, 82.08, 80.04, 75.49, 72.75, 70.36, 69.94, 60.64, 55.87, 23.91, 19.25, 15.51, 14.47. $[\alpha]_D^{25}$ = −30.26 (*c* 1.0, $CHCl_3$). ESI-HRMS: m/z [M+Na]$^+$ calcd. for $C_{22}H_{30}Cl_3NNaO_7S$: 582.0701; found 582.0707. IR (neat): ν_{max} = 3481, 3324, 2983, 2930, 2873, 1723, 1695, 1531, 1136, 1075 cm^{-1}.

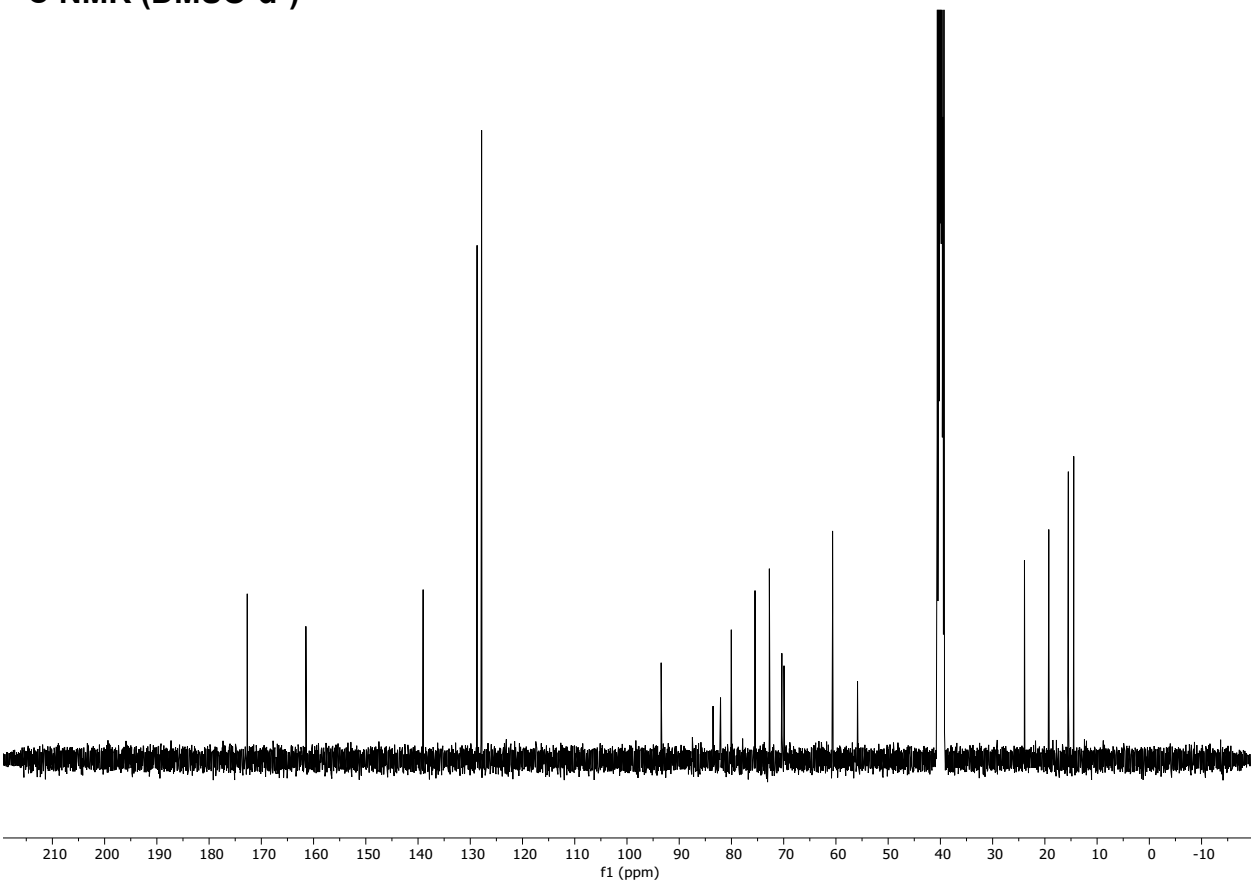
^{1}H NMR (DMSO-d^6)



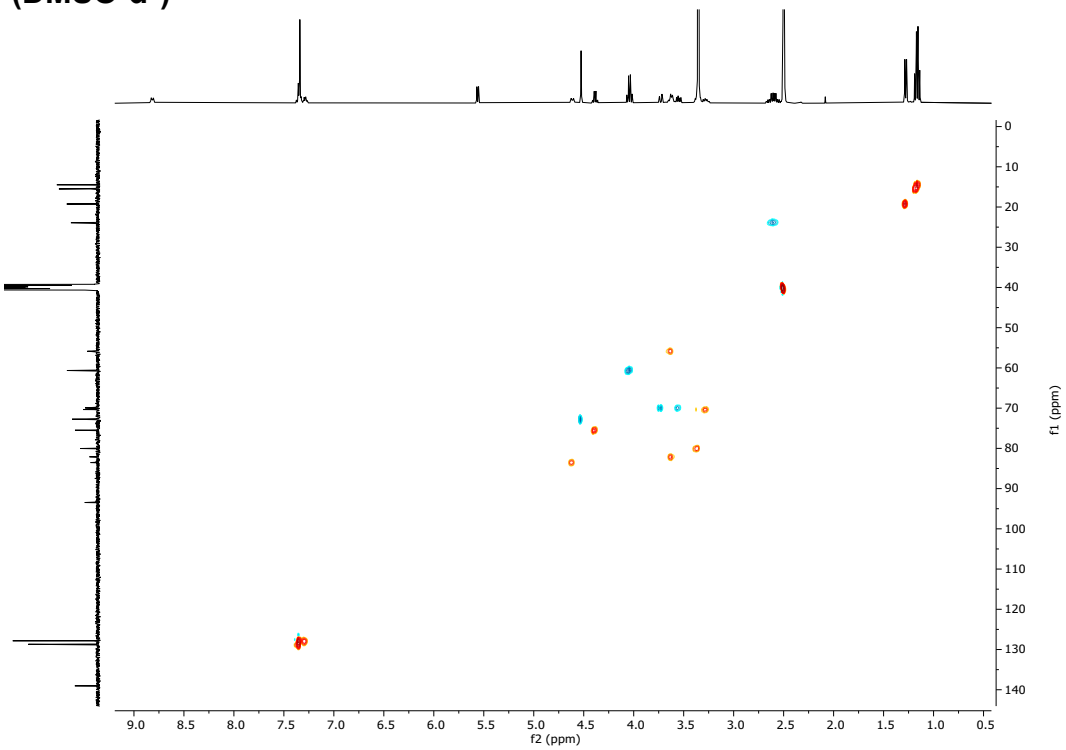
COSY (DMSO-d^6)



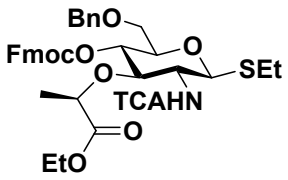
^{13}C NMR (DMSO-d^6)



HSQC (DMSO-d^6)



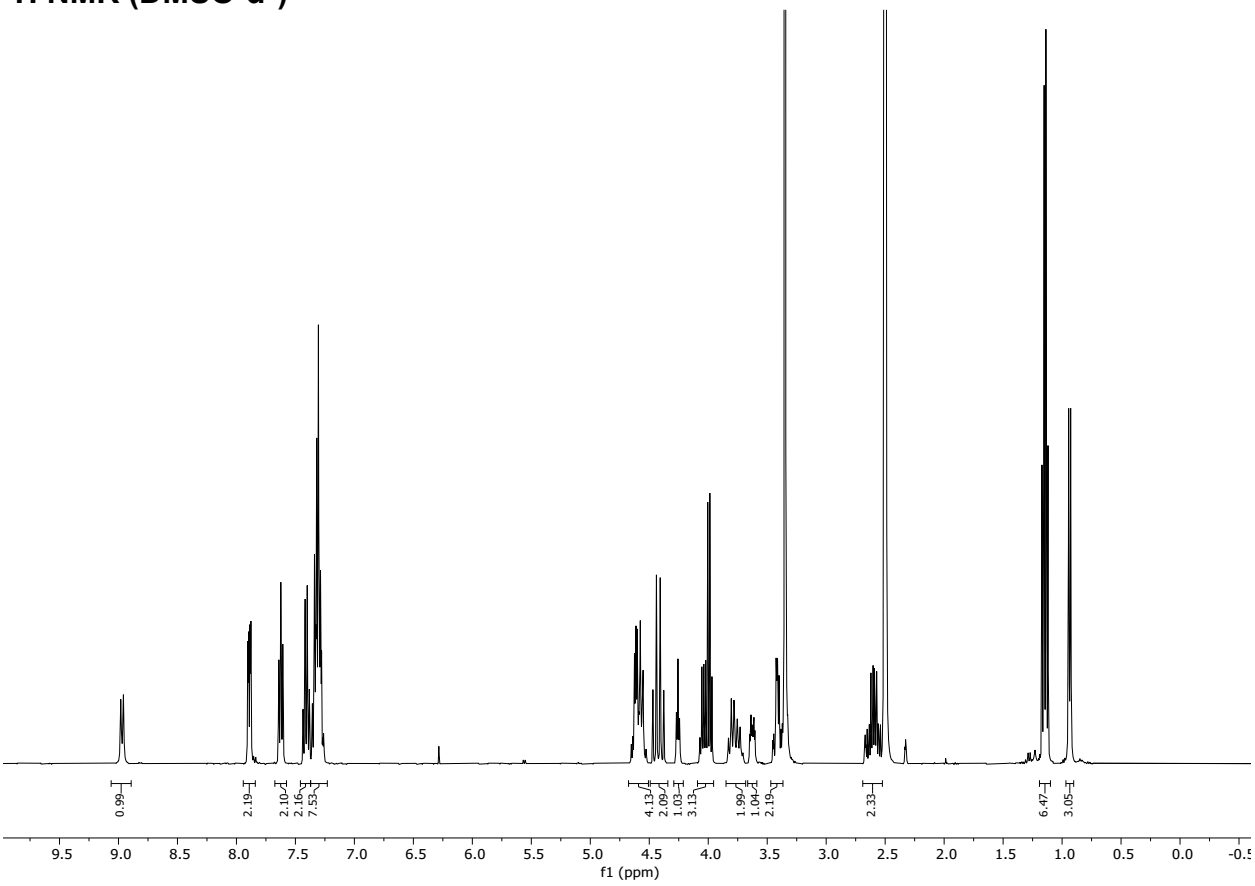
Ethyl 6-*O*-benzyl-2-deoxy-3-*O*-((*R*)-1'-ethoxycarbonylethyl)-4-*O*-fluorenylmethoxycarbonyl-1-thio-2-trichloroacetamido-β-D-glucopyranoside (6)



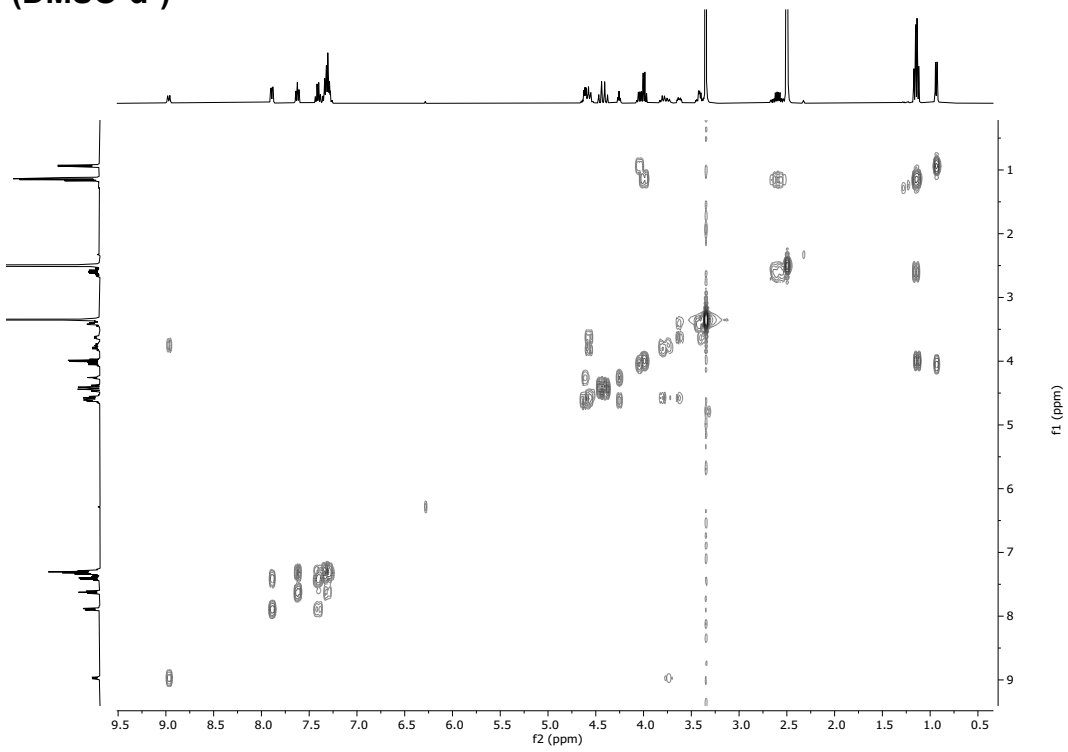
To a solution of **5** (838 mg, 1.50 mmol, 1 eq) in DCM (10 mL) and pyridine (1.85 mL, 1.95 mmol, 1.3 eq), FmocCl (780 mg, 3.00 mmol, 2 eq) was added. The reaction mixture was stirred for 2 h at rt and then diluted with DCM (100 mL) and washed with aq. 0.1 M HCl solution (100 mL) and brine (100 mL). The organic layer was dried over Na_2SO_4 and purified with a column cromatography (Hexane/EtOAc 8:2) to give compound **6** (1.09 g, 93%) as a white solid.

^{1}H NMR (400 MHz, DMSO-d^6): δ 8.97 (d, *J* = 8.9 Hz, 1H, N*H*), 7.89 (dd, *J* = 7.6, 3.1 Hz, 2H, Ar), 7.62 (t, *J* = 6.7 Hz, 2H, Ar), 7.41 (q, *J* = 7.2 Hz, 2H, Ar), 7.36–7.25 (m, 7H, Fmoc, Ar) 4.67–4.50 (m, 4H, H-1, H-4, *CH*$_2$-Fmoc), 4.42 (q, *J* = 12.0 Hz, 2H, *CH*$_2$-Ph), 4.26 (t, *J* = 5.4 Hz, 1H, *CH*-Fmoc), 4.08–3.96 (m, 3H, *CH*-CH$_3$, OEt), 3.85–3.69 (m, 2H, H-2, H-3), 3.66–3.59 (m, 1H, H-5), 3.47–3.37 (m, 2H, H-6a, H-6b), 2.69–2.53 (m, 2H, SEt), 1.20–1.11 (m, 6H, SEt, OEt), 0.94 (d, *J* = 6.8 Hz, 3H, *C*H-C*H*$_3$). ^{13}C NMR (101 MHz, DMSO-d^6): δ 171.57, 161.44, 154.18, 143.79, 143.59, 141.34, 141.31, 138.51, 128.68, 128.20, 128.17, 127.96, 127.94, 127.63, 127.56, 125.28, 125.10, 120.68, 93.36, 83.74, 79.54, 76.44, 75.97, 74.67, 72.91, 69.29, 69.19, 60.83, 56.14, 46.77, 40.63, 40.58, 40.42, 40.37, 40.21, 40.16, 39.95, 39.74, 39.54, 39.33, 23.98, 18.51, 15.49, 14.37. $[\alpha]_D^{25}$ = −13.61 (*c* 1.0, $CHCl_3$). ESI-HRMS: m/z $[M+Na]^+$ calcd. for $C_{37}H_{40}Cl_3NNaO_9S$: 804.1382; found 804.1412. IR (neat): ν_{max} = 3307, 2929, 1752, 1693, 1533, 1451, 1385, 1257, 1124 cm^{-1}.

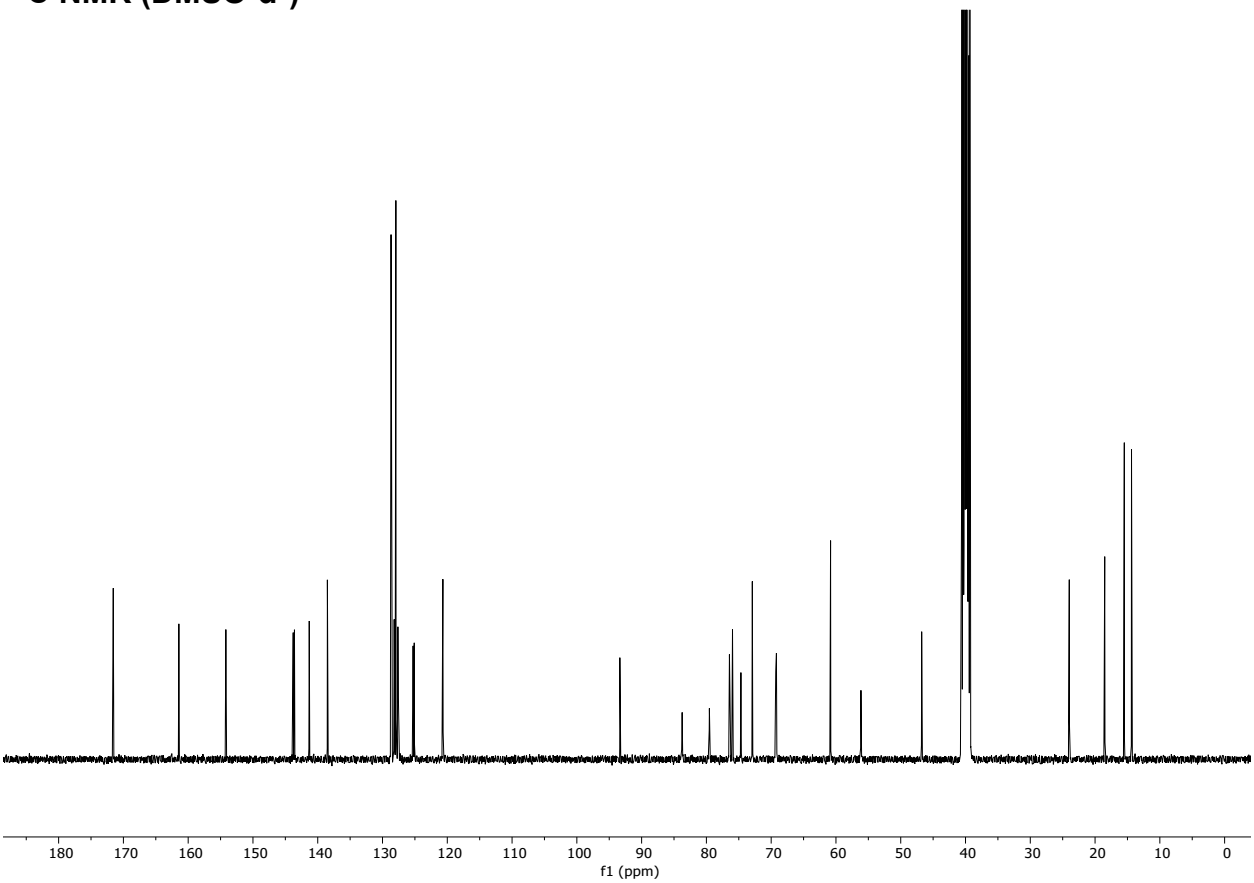
^{1}H NMR (DMSO-d^6)



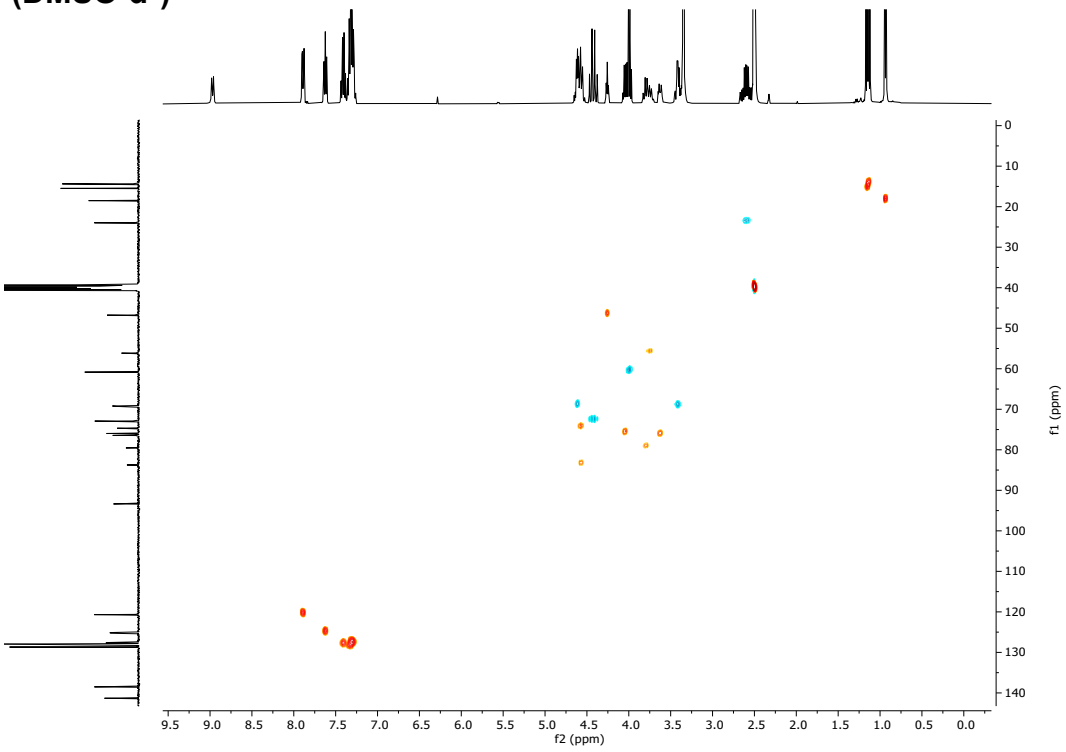
COSY (DMSO-d^6)



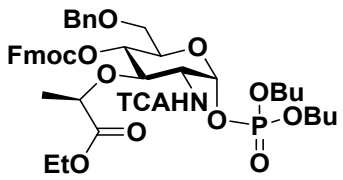
^{13}C NMR (DMSO-d^6)



HSQC (DMSO-d^6)



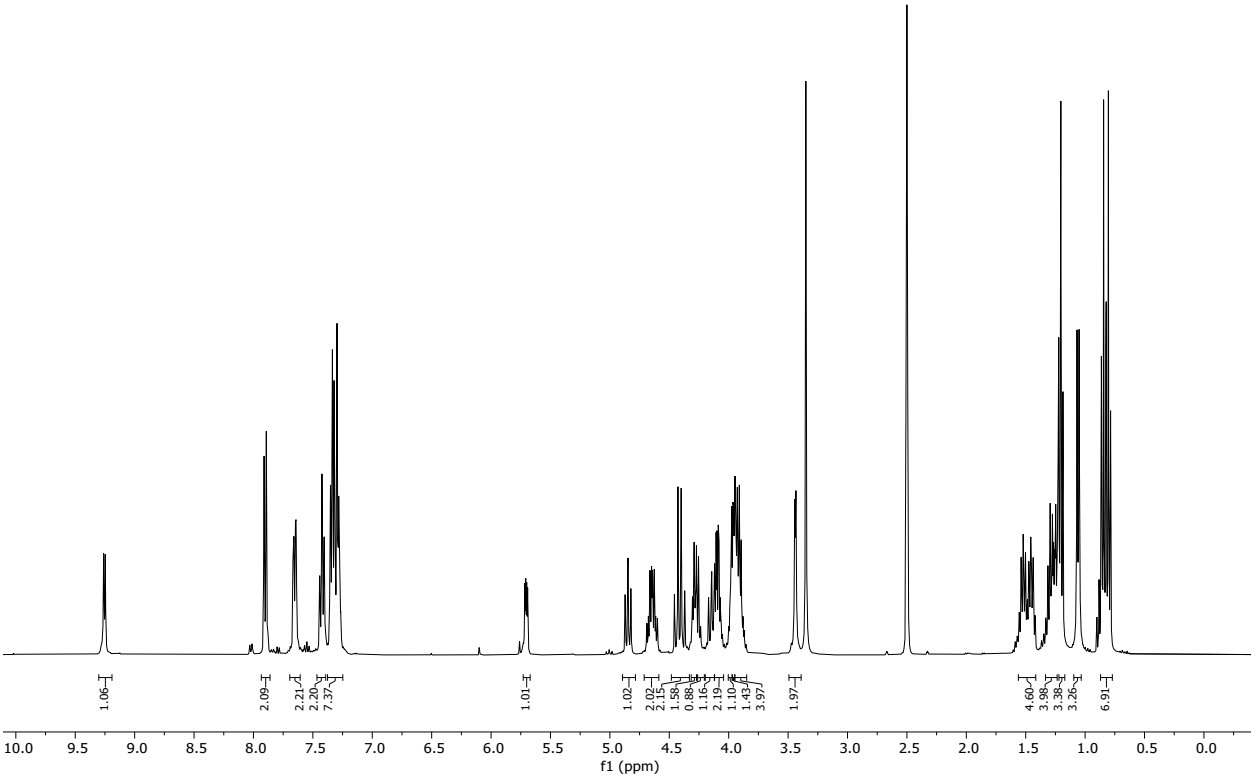
Dibutylphosphoryloxy 6-*O*-benzyl-2-deoxy-3-*O*-((R)-1'-ethoxycarbonylethyl)-4-*O*-fluorenylmethoxycarbonyl-2-trichloroacetamido-β-D-glucopyranoside (2)



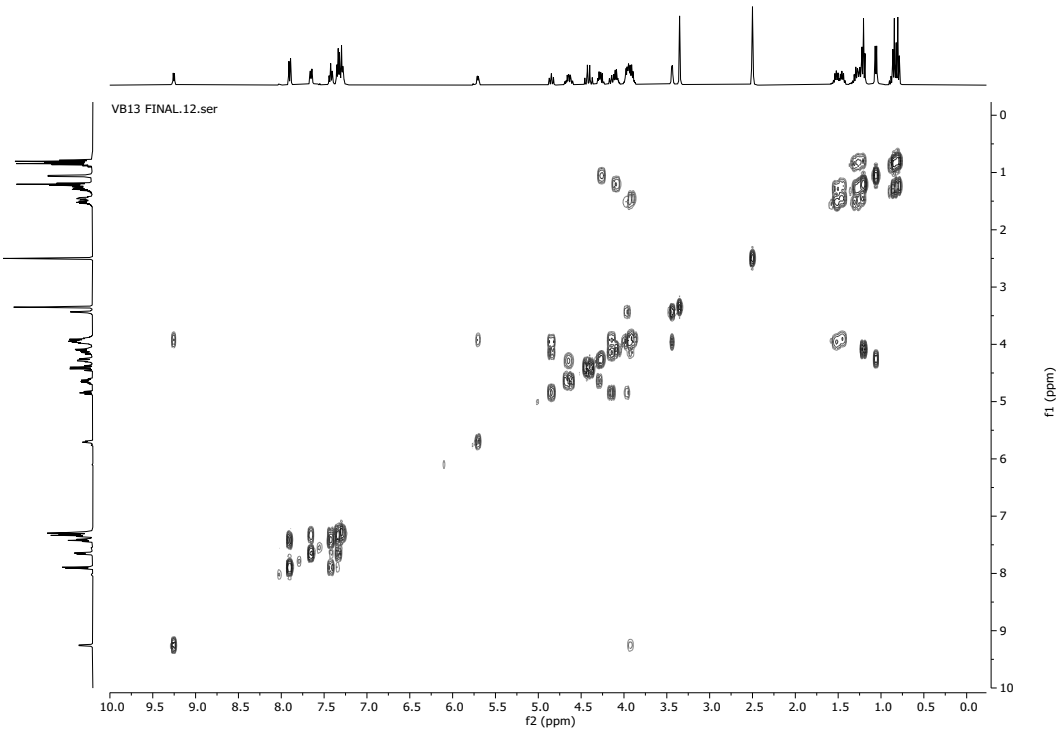
A solution of dibutyl phosphate (2.5 mL, 12.6 mmol) in DCM (10 mL) was dried over molecular sieves. After 1 h the supernatant (3.7 mL, 2.6 eq) was added to a solution of **6** (1.09 g, 1.40 mmol, 1 eq) in DCM (7 mL) and cooled to 0 °C. Then, NIS (926 mg, 4.12 mmol, 1.2 eq) was added. The reaction was stirred for 3 h, quenched with a sat. aq. solution of $Na_2S_2O_3$/$NaHCO_3$ (1:1, 50 mL) and extracted with DCM (50 mL). The organic layer was then washed with water (50 mL) and brine (50 mL), dried over Na_2SO_4 and purified with a column chromatography (Hexane/EtOAc 3:1) obtaining **2** (1.05 g, 83%).

^{1}H NMR (400 MHz, DMSO-d^6) δ 9.25 (d, *J* = 5.5 Hz, 1H, N*H*), 7.90 (d, *J* = 7.5 Hz, 2H, Ar), 7.65 (dd, *J* = 7.5, 2.7 Hz, 2H, Ar), 7.42 (m, 2H, Ar), 7.38–7.22 (m, 7H, Ar), 5.70 (dd, *J* = 6.4, 3.1 Hz, 1H, H-1), 4.84 (t, 9.7 Hz, 1H, H-3), 4.70–4.59 (m, 2H, *CH$_2$*-Fmoc), 4.41 (q, *J* = 11.9 Hz, 2H, *CH$_2$*-Ph), 4.32–4.22 (m, 3H, *CH*-Fmoc, *CH*-CH_3), 4.19–4.04 (m, 3H, Et, H-4), 4.00–3.85 (m, 6H, OBu,, H-2, H-5), 3.48–3.40 (m, 2H, H-6a, H-6b), 1.60–1.40 (m, 4H, OBu), 1.37–1.18 (m, 7H, OBu, Et), 1.06 (d, *J* = 7.0 Hz, 2H, CH-*CH$_3$*), 0.82 (m,6H, Bu). ^{13}C NMR (101 MHz, DMSO-d^6) δ 173.52, 162.09, 153.62, 143.38, 143.04, 140.92, 140.91, 137.88, 128.25, 127.80, 127.57, 127.55, 127.24, 127.17, 124.84, 124.64, 120.30, 120.26, 93.50 & 93.44 (m, C-1), 91.98 (*C*Cl_3), 75.04, 74.42, 73.05, 72.52, 70.37, 69.07, 68.25, 67.38, 67.32, 67.17, 67.11, 61.17, 55.19, 55.10, 46.29, 31.66, 31.62, 31.59, 31.55, 18.40, 18.22, 18.13, 18.11, 13.93, 13.45, 13.42. ^{31}P NMR (162 MHz, DMSO-d^6) δ −2.50. $[\alpha]_D^{25}$ = +52.85 (*c* 1.0, $CHCl_3$). ESI-HRMS: m/z [M+Na]$^+$ calcd. for $C_{43}H_{53}Cl_3NNaO_{13}P$: 950.2213; found 950.2263. IR (neat): ν_{max} = 3289, 2964, 2875, 1758, 1715, 1523, 1453, 1380, 1255, 1120, 1026, 952 cm^{-1}.

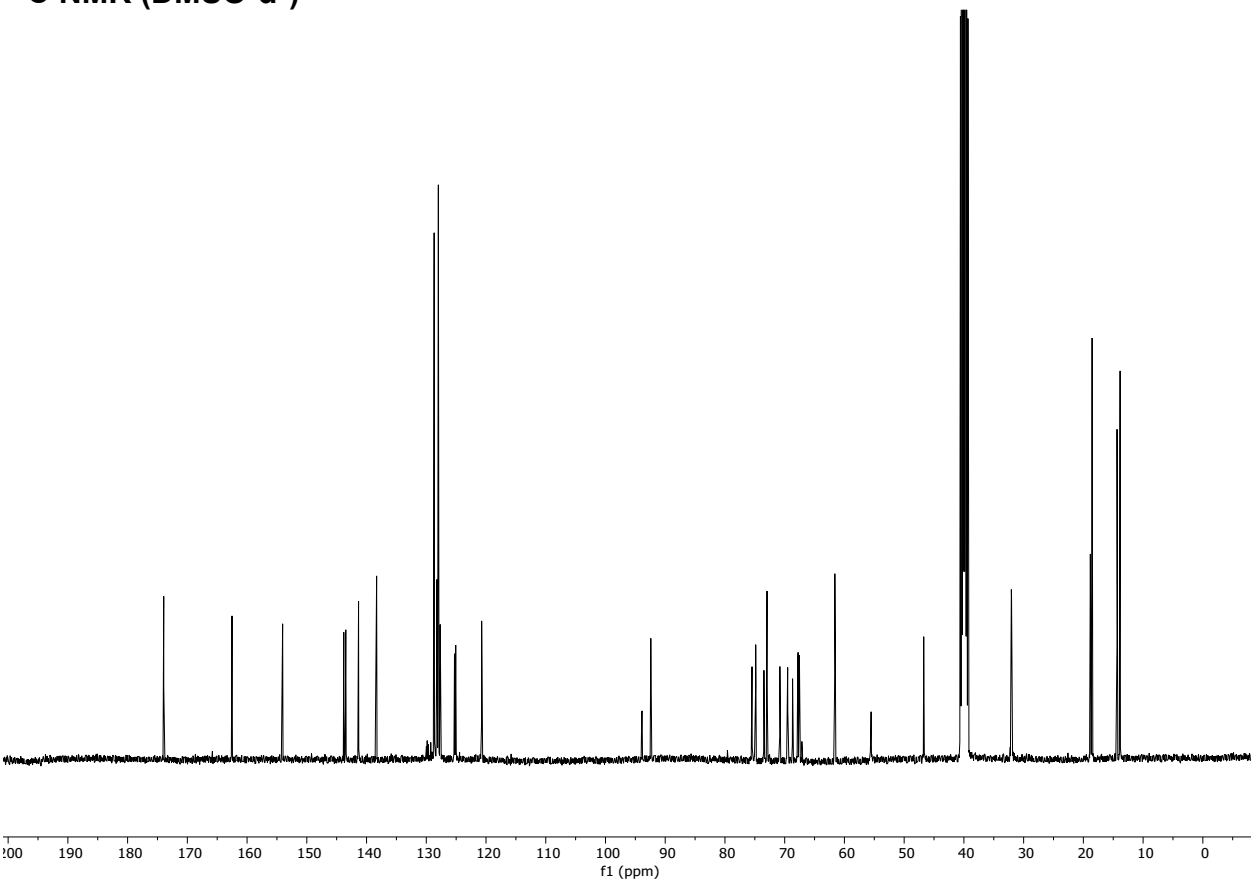
^{1}H NMR (DMSO-d^{6})



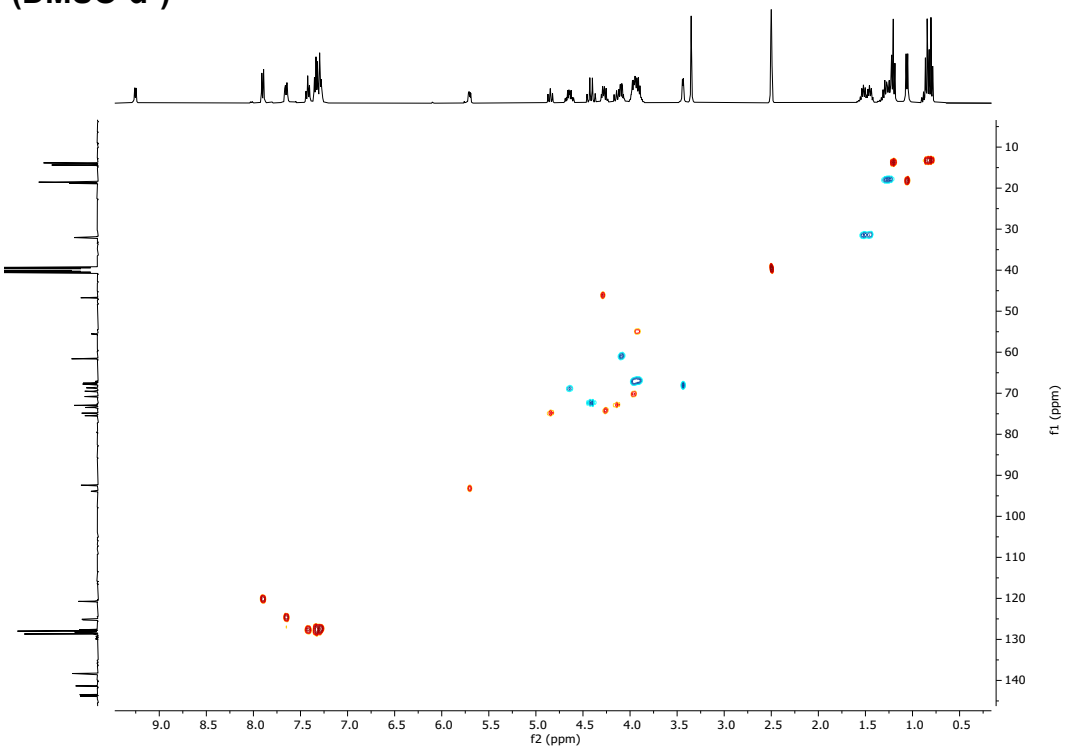
COSY (DMSO-d^{6})



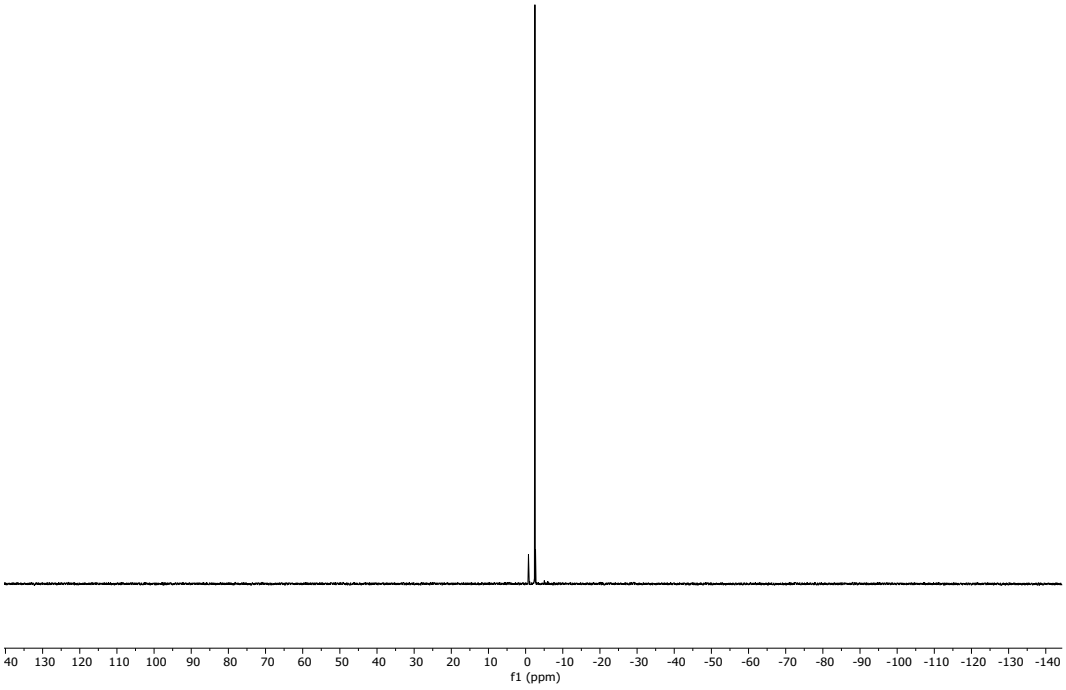
^{13}C NMR (DMSO-d^6)



HSQC (DMSO-d^6)



^{31}P NMR (DMSO-d^{6})



3 Automated Glycan Assembly

General Materials and Methods for AGA

All solvents used were HPLC-grade. The solvents used for the building block, activator, TMSOTf, and capping solutions, were taken from an anhydrous solvent system. The building blocks were co-evaporated three times with DCM and toluene and dried under vacuum before use. Activator, capping, deprotection, acidic wash and building block solutions were freshly prepared and kept under Argon. All yields of products obtained by AGA were calculated on the basis of resin loading. Resin loading was determinated following previously established procedures.[4]

Preparation of stock solutions:

Building Blocks: Every 0.06 mmol (5 equiv.) of glycosyl phosphate was dissolved in 1 mL of DCM.

Fmoc deprotection solution: A solution of 20% piperidine in DMF (v/v) was prepared.

TMSOTf solution: TMSOTf (0.45 mL) was added to DCM (40 mL).

Capping solution: A solution of 10% acetic anhydride and 2% methanesulfunic acid in DCM (v/v) was prepared.

Modules for automated synthesis

The linker-functionalized resin was placed in the reaction vessel of the automated oligosaccharide synthesizer and swollen with DCM. Before each synthesis, the resin was washed with DMF, THF, and DCM (Module A). Subsequently, the glycosylation (Module B and C), the capping (Module D) and, in the end, the Fmoc deprotection (Module E) steps were performed. Mixing of the components was accomplished by bubbling Argon through the reaction mixture.

Module A: Resin Preparation for Synthesis (20 min)

All automated syntheses were performed on 12.5 µmol scale. Resin was placed in the reaction vessel and swollen in DCM for 20 min at room temperature prior to synthesis. During this time, all reagent lines needed for the synthesis were washed and primed.

Before the first glycosylation, the resin was washed with DMF, THF, and DCM (three times each with 2 mL for 25 s).

Module B: Acidic Wash with TMSOTf Solution (20 min)

The resin was swollen in 2 mL DCM and the temperature of the reaction vessel was adjusted to −20 °C. Upon reaching the low temperature, TMSOTf solution (1 mL) was added drop wise to the reaction vessel. After bubbling for 3 min, the acidic solution was drained and the resin was washed with 2 mL DCM for 25 s.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	−20	(15 min)*
Deliver	1	DCM	2 mL	−20	-
Deliver	1	TMSOTf solution	1 mL	−20	3 min
Wash	1	DCM	2 mL	−20	25 sec

*Time required to reach the desired temperature.

Module C: Glycosyl Phosphate Glycosylation (70 min)

The building block solution (0.06 mmol of BB in 1 mL of DCM per glycosylation) was delivered to the reaction vessel. After the set temperature was reached (−30 °C), the reaction was started by dropwise addition of the TMSOTf solution (1.0 mL, excess). The temperature increased until −10 °C in 40 min. After completion of the reaction, the solution is drained and the resin washed with 2 mL DCE (2 mL) and with DCM (2 x 2 mL for 20 s). The temperature of the reaction vessel is increased to 25 °C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Cooling	-	-	-	−30	(20 min)*
Deliver	1	BB solution	1 mL	−30	-
Deliver	1	TMSOTf solution	1 mL	−30	-

Reaction time (BB dependet)	1	DCM	2 mL	−30 to −10	5 min 40 min
Wash	1	DCE	2 mL	0	1 min
Wash	1	DCM	2 mL	>0	22 sec
Heating	-	-	-	25	3 min

*Time required to reach the desired temperature.

Module D: Capping (30 min)

The resin was washed with DMF (two times with 2 mL for 25 s) at 25 °C. 2 mL of pyridine solution (10% in DMF) was delivered into the reaction vessel. After 1 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s). 4 mL of capping solution was delivered into the reaction vessel. After 20 min, the reaction solution was drained and the resin washed with DCM (three times with 3 mL for 25 s).

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Heating	-	-	-	25	(5 min)*
Wash	2	DMF	2 mL	25	25 sec
Deliver	1	10% pyridine in DMF	2 mL	25	1 min
Wash	3	DCM	2 mL	25	25 sec
Deliver	1	Capping solution	4 mL	25	20 min
Wash	3	DCM	2 mL	25	25 sec

*Time required to reach the desired temperature.

Module E: Fmoc Deprotection (9 min)

The resin was washed with DMF (three times with 2 mL for 25 s) at 25 °C. 2 mL of Fmoc deprotection solution 1 was delivered to the reaction vessel. After 5 min, the reaction solution was drained and the resin washed with DMF (three times with 3 mL for 25 s) and

DCM (five times each with 2 mL for 25 s). The temperature of the reaction vessel was decreased to −20 °C for the next module.

Action	Cycles	Solution	Amount	T (°C)	Incubation time
Wash	3	DMF	2 mL	25	25 sec
Deliver	1	Fmoc depr. Solution	2 mL	25	5 min
Wash	1	DMF	2 mL	-	
Cooling	-	-	-	−20	-
Wash	3	DMF	2 mL	<25	25 sec
Wash	5	DCM	2 mL	<25	25 sec

Post-synthesizer manipulations

Module F: Cleavage from solid support

The oligosaccharides were cleaved from the solid support using a continuous-flow photoreactor as described previously.[5]

Module G: Ester hydrolysis

To a solution of the compound in THF/dioxane/ H_2O (4:2:1, 500 µL) was added LiOH (1 mg, 0.42 µmol) and stirred at room temperature. The solution was neutralized with Amberlite, filtered, and concentrated.

Module H1: Hydrogenolysis

The crude compound obtained from Module G was dissolved in 2 mL of H_2O/AcOH (1:1). 100% by weight Pd/C (10%) was added and the reaction was stirred in a flask equipped with a H_2 balloon. Upon completion, the reaction was filtered and washed with H_2O/AcOH (1:1) and H_2O. The filtrates were concentrated *in vacuo*.

Module H2: Hydrogenolysis under pressure

The crude compound obtained from Module G was dissolved in 2 mL of H_2O/AcOH (1:1). 100% by weight Pd/C (10%) was added and the reaction was stirred in H_2 bomb with 60 psi pressure. Upon completion, the reaction was filtered and washed with H_2O/AcOH (1:1) and H_2O. The filtrates were concentrated *in vacuo*.

Module I1: Normal Phase Purification

After AGA the crudes were purified using a normal phase HPLC (Agilent 1200 Series, Method A).

- Method A: (YMC-Diol-300 column, 150 x 20 mm, 5 µm) flow rate of 15.0 mL/min with 20% EtOAc in hexane as eluents [isocratic (5 min), linear gradient to 100% EtOAc (35 min)]

Module I2: Reverse Phase Purification

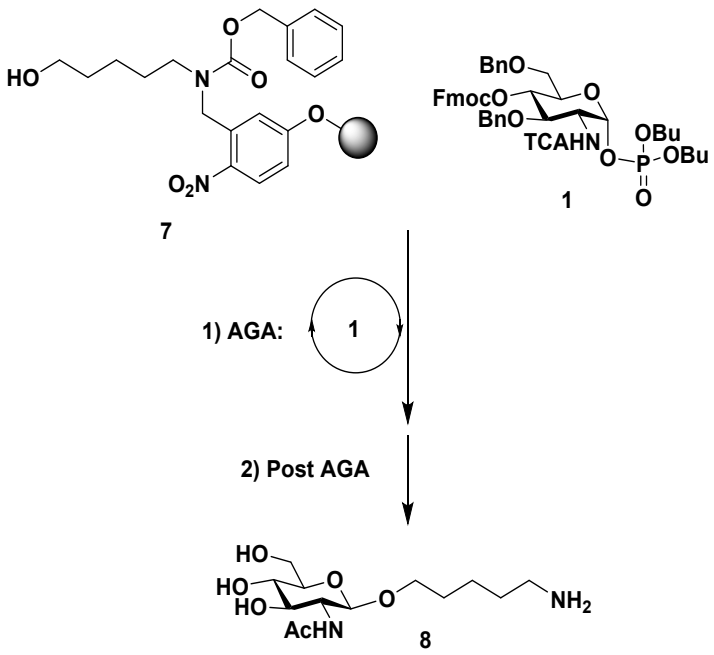
Deprotected compounds were purified using a preparative reverse phase HPLC (Agilent 1200 Series, method B). The pure compounds were analyzed using an analytical HPLC (Agilent 1200 Series, Method C).

- Method B:(Hypercarb column, 150 x 10 mm, 5 µm), flow rate of 3 mL/min with H_2O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 20% ACN (30 min), linear gradient to 100% ACN (5 min)].
- Method C: (Hypercarb column, 150 x 4.6 mm, 3 µm) flow rate of 0.7 mL/min with H_2O (0.1% formic acid) as eluents [isocratic (5 min), linear gradient to 20% ACN (30 min), linear gradient to 100% ACN (5 min)].

Following final purification, all deprotected products were lyophilized on a Christ Alpha 2-4 LD plus freeze dryer prior to characterization.

Oligosaccharides synthesis

Aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranoside (8)

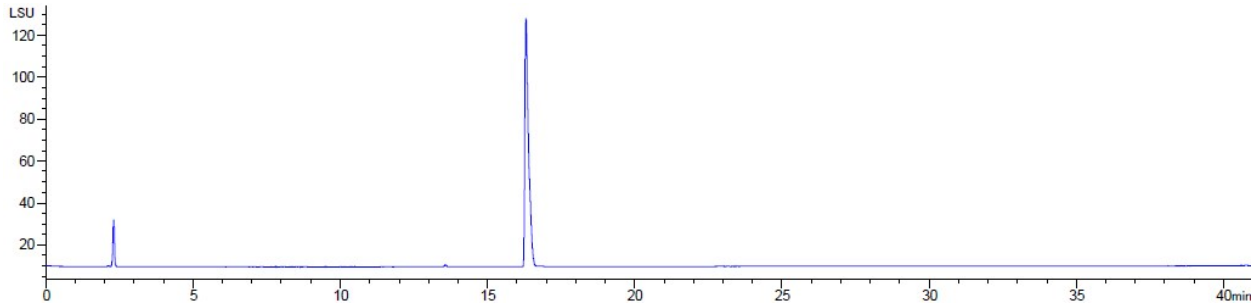


Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	1	**B, C, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**H1, I2**	**H1:** (24 h), **I2:** (Method B)

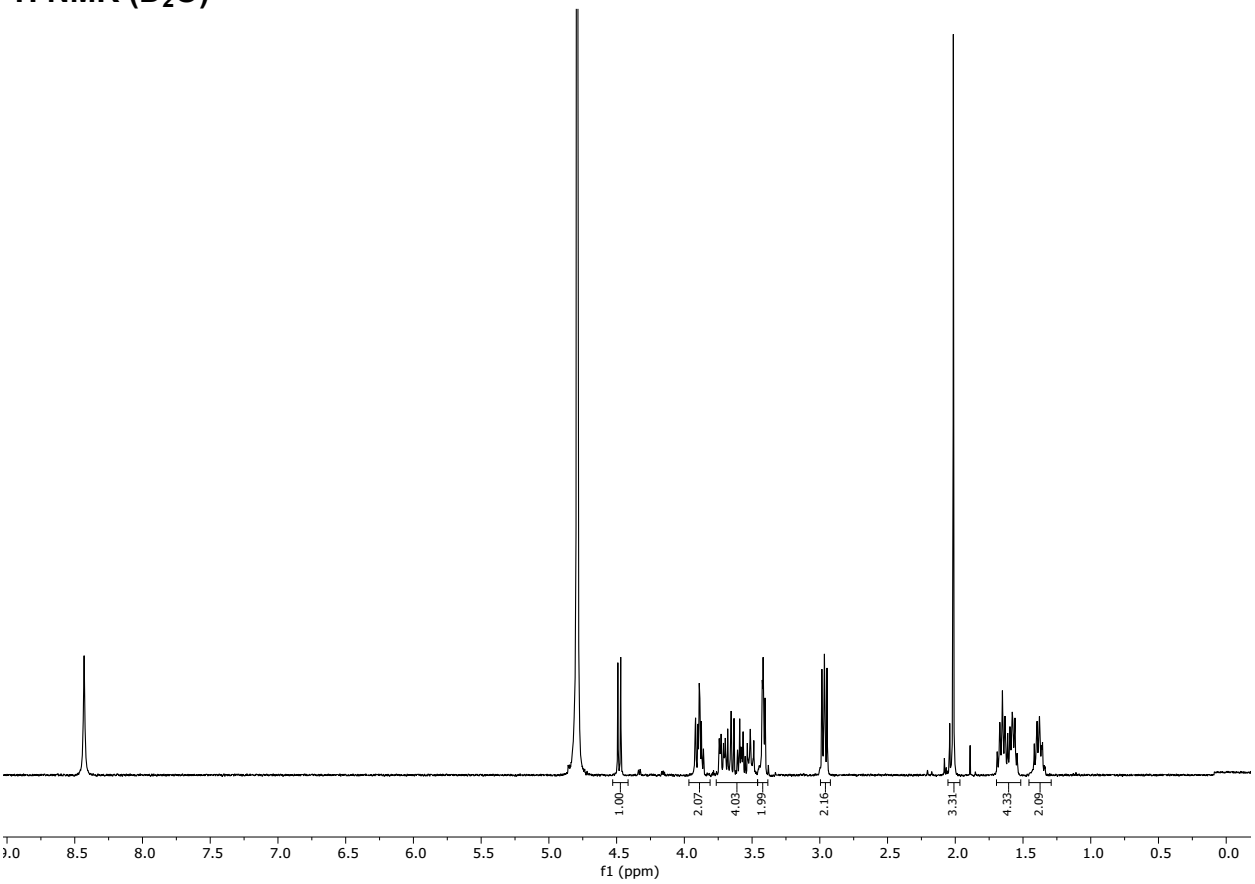
Automated synthesis, global deprotection, and purification afforded **8** as a white solid (1.05 mg, 27% overall yield)

^{1}H NMR (400 MHz, D_2O) δ 8.43 (formic acid), 4.42 (d, *J* = 8.5 Hz, 1H), 3.95–3.75 (m, 2H) 3.73–3.41 (m, 4H), 3.39–3.32 (m, 2H), 3.06–2.79 (m, 2H), 1.95 (s, 3H), 1.67–1.44 (m, 4H), 1.40–1.26 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 174.43, 171.00, 101.14, 75.82, 73.73, 70.00, 69.85, 60.67, 55.53, 39.26, 28.03, 26.32, 22.07. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{13}H_{27}N_2O_6$ 307.1864 found 307.1863.

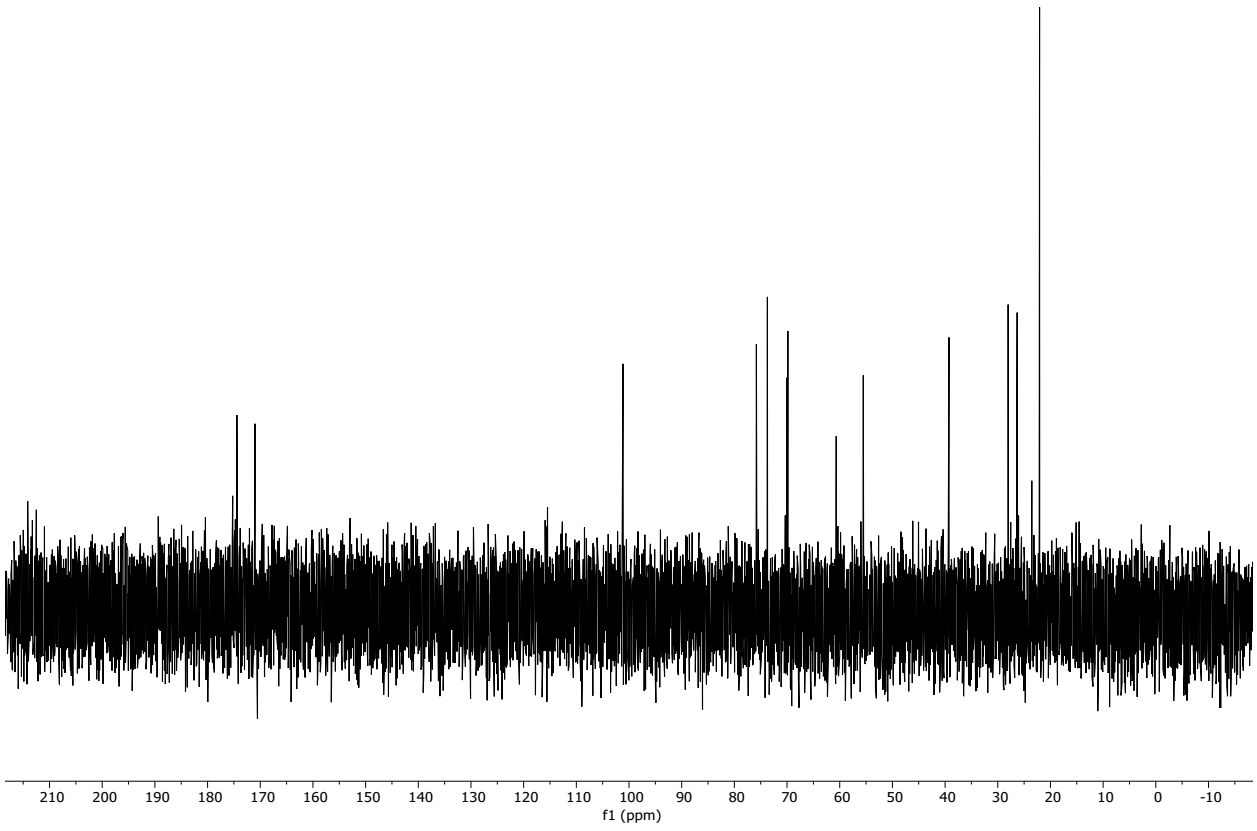
RP-HPLC (ELSD trace, method C, t_R = 16.3 min)



^{1}H NMR (D_2O)



^{13}C NMR (D_2O)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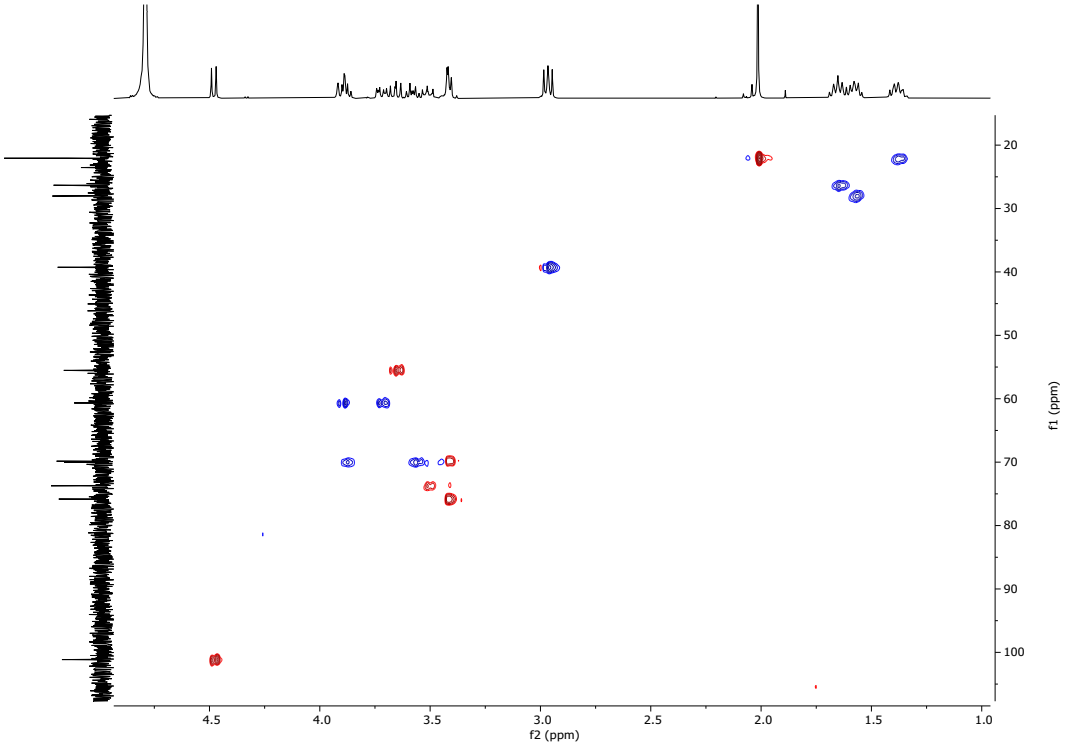
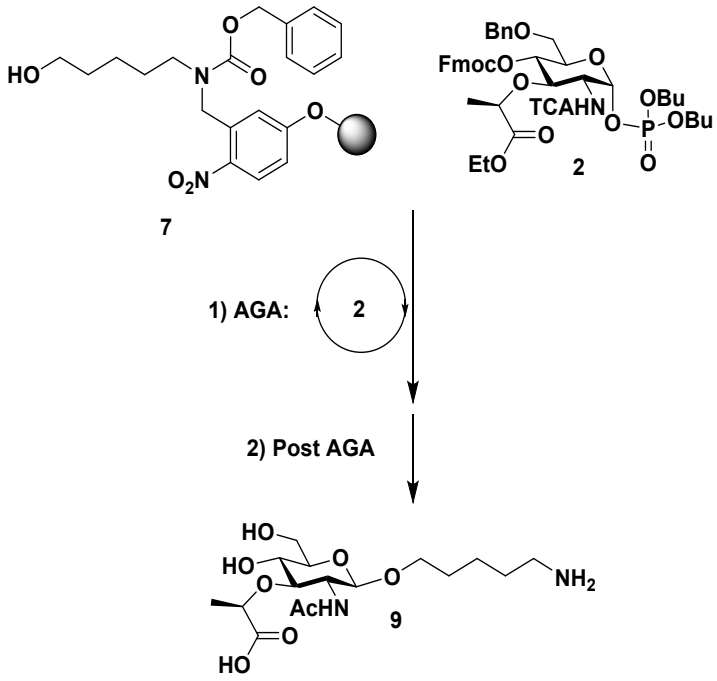
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

HSQC (D_2O)



Aminopentyl 2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranoside (9)

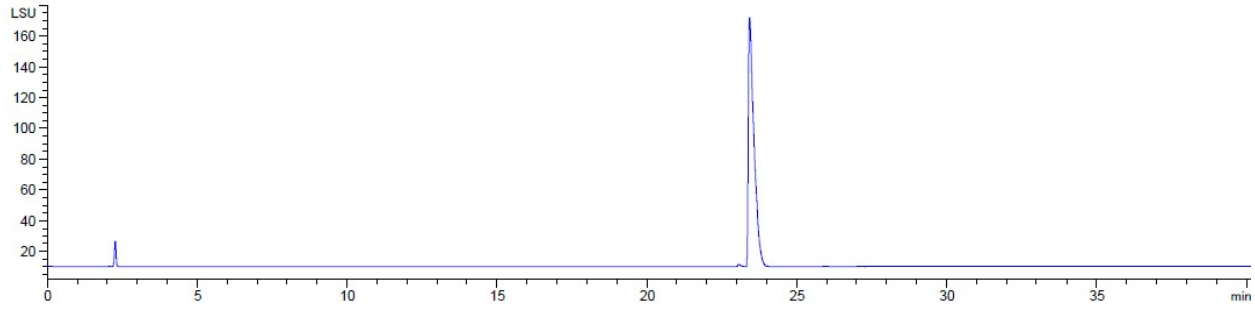


Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	2	**B, C, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**G, H1, I2**	**G:** (2 h), **H1:** (24 h), **I2:** (Method B)

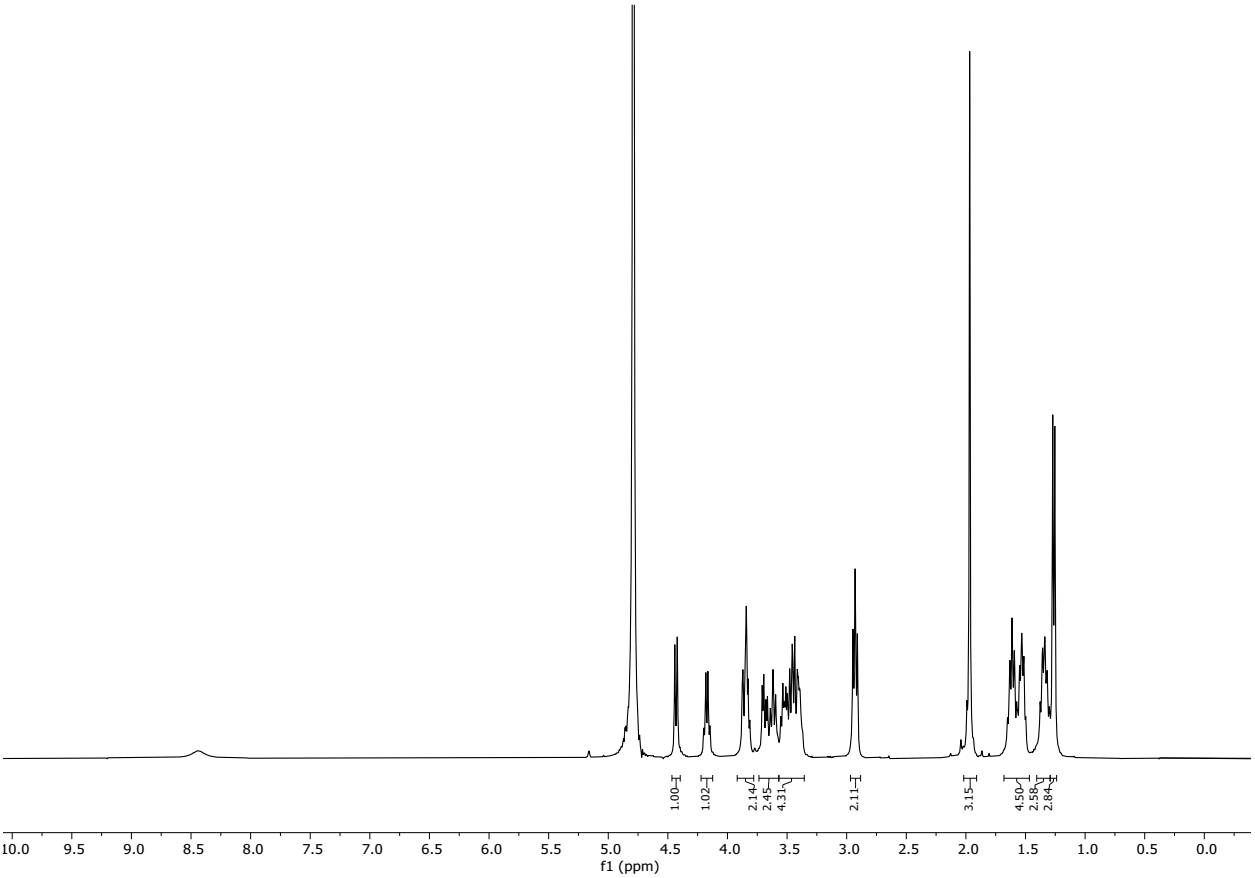
Automated synthesis, global deprotection, and purification afforded **9** as a white solid (1.98 mg, 42% overall yield)

^{1}H NMR (400 MHz, D_2O) δ 4.43 (d, *J* = 8.3 Hz, 1H), 4.17 (q, *J* = 6.9 Hz, 1H), 3.91–3.78 (m, 2H), 3.76–3.57 (m, 2H), 3.57–3.33 (m, 4H), 2.93 (t, *J* = 7.6 Hz, 2H), 1.97 (s, 3H), 1.72–1.44 (m, 4H), 1.44–1.29 (m, 2H), 1.26 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 181.17, 174.21, 101.50, 80.43, 77.84, 75.58, 69.91, 69.23, 60.59, 54.30, 39.23, 27.95, 26.25, 22.22, 22.00, 18.71. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{16}H_{31}N_2O_8$ 379.2075 found 379.2074.

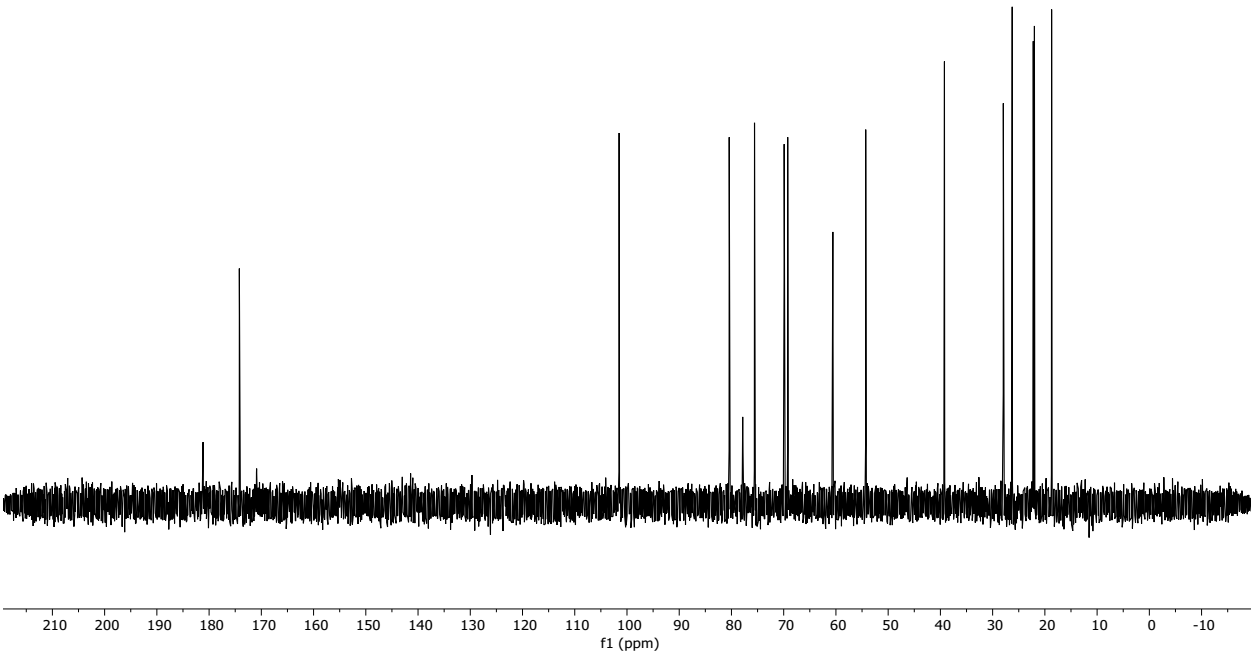
RP-HPLC (ELSD trace, method C, t_R = 23.4 min)



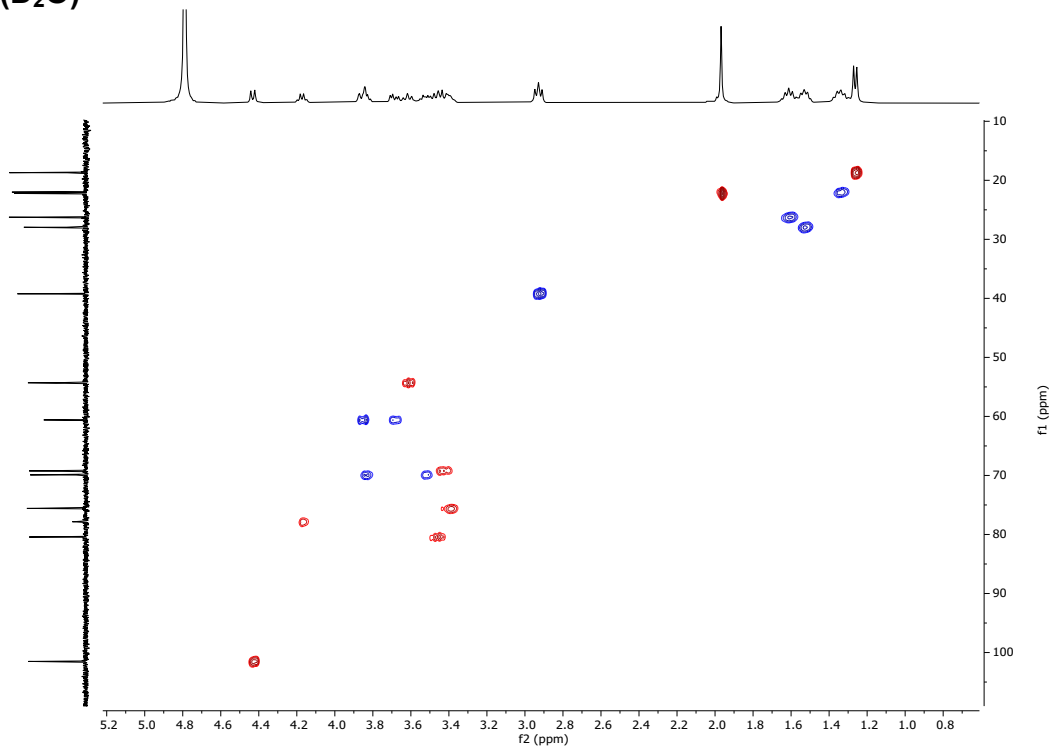
^{1}H NMR (D_2O)



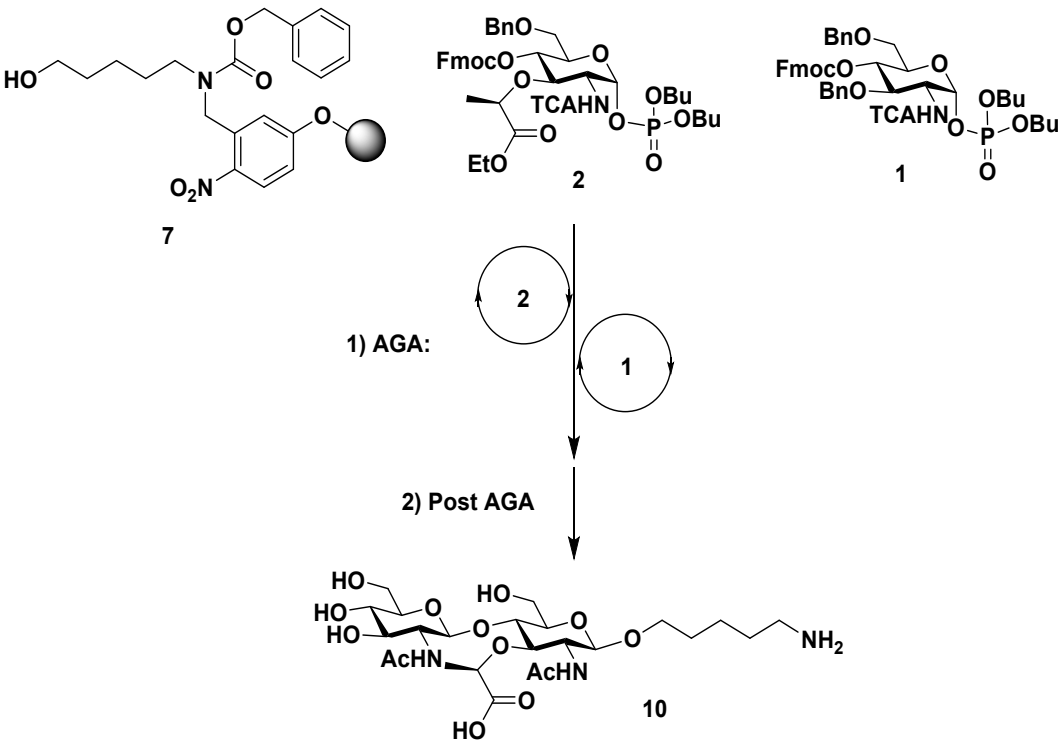
^{13}C NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranoside (10)



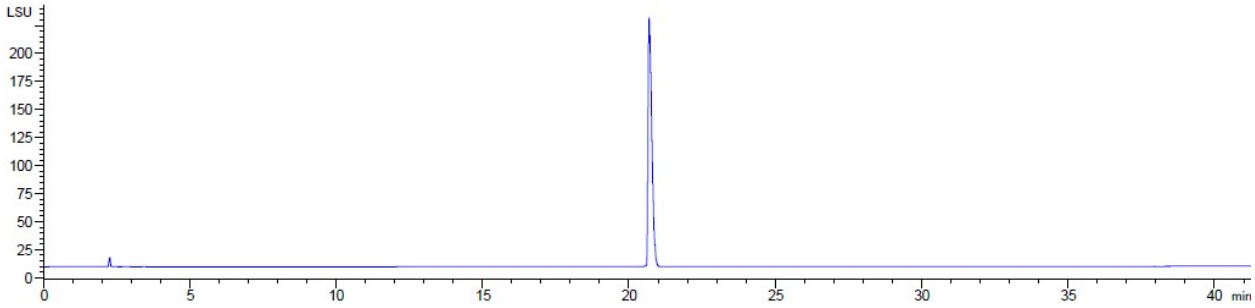
Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**I1, G, H1, I2**	**I1:** (Method A), **G:** (2 h), **H1:** (40 h), **I2:** (Method B)

Automated synthesis, global deprotection, and purification afforded **10** as a white solid (1.44 mg, 20% overall yield)

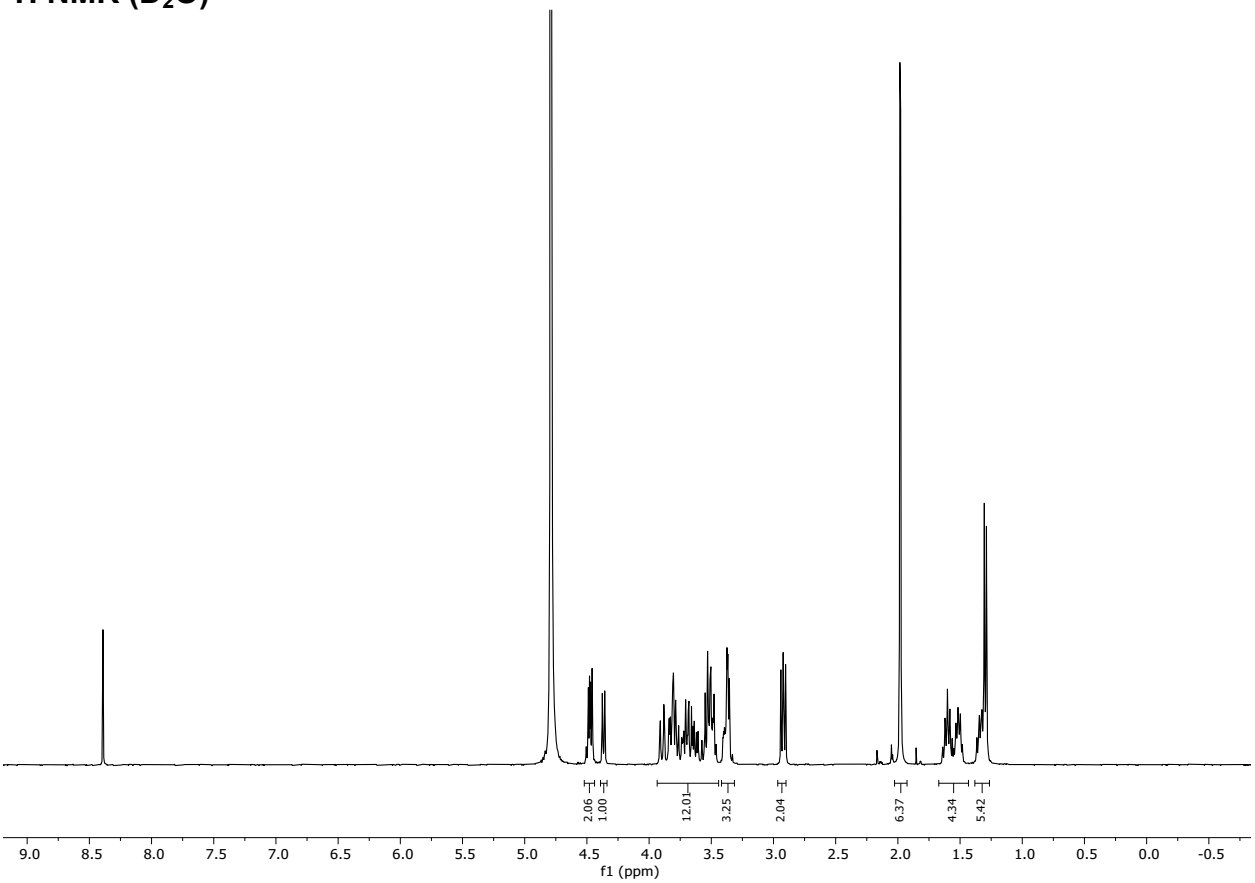
^{1}H NMR (400 MHz, D_2O) δ 8.39 (formic acid), 4.52–4.44 (m, 2H), 4.37 (d, *J* = 7.6 Hz, 1H), 3.97–3.28 (m, 14H), 2.98–2.87 (m, 2H), 1.99 (s, 3H), 1.98 (s, 3H), 1.76–1.44 (m, 4H), 1.37–1.27 (m, 5H). ^{13}C NMR (101 MHz, D_2O) δ 181.45, 174.49, 174.45, 170.96, 101.96,

100.19, 79.06, 77.62, 76.14, 75.53, 75.15, 73.40, 70.22, 69.86, 61.15, 59.76, 56.02, 54.50, 39.23, 27.90, 26.24, 22.34, 21.97, 21.94, 18.26. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{24}H_{44}N_3O_{13}$ 582.2869 found 582.2878.

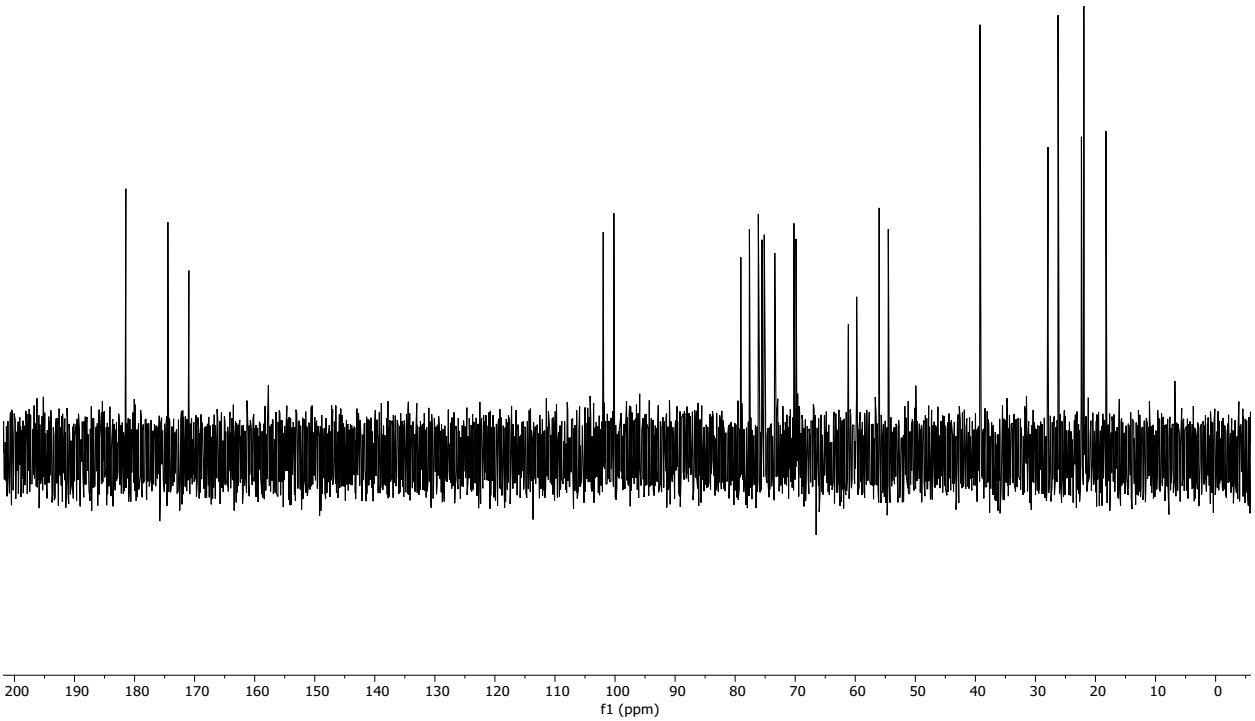
RP-HPLC (ELSD trace, method C, t_R = 20.7)



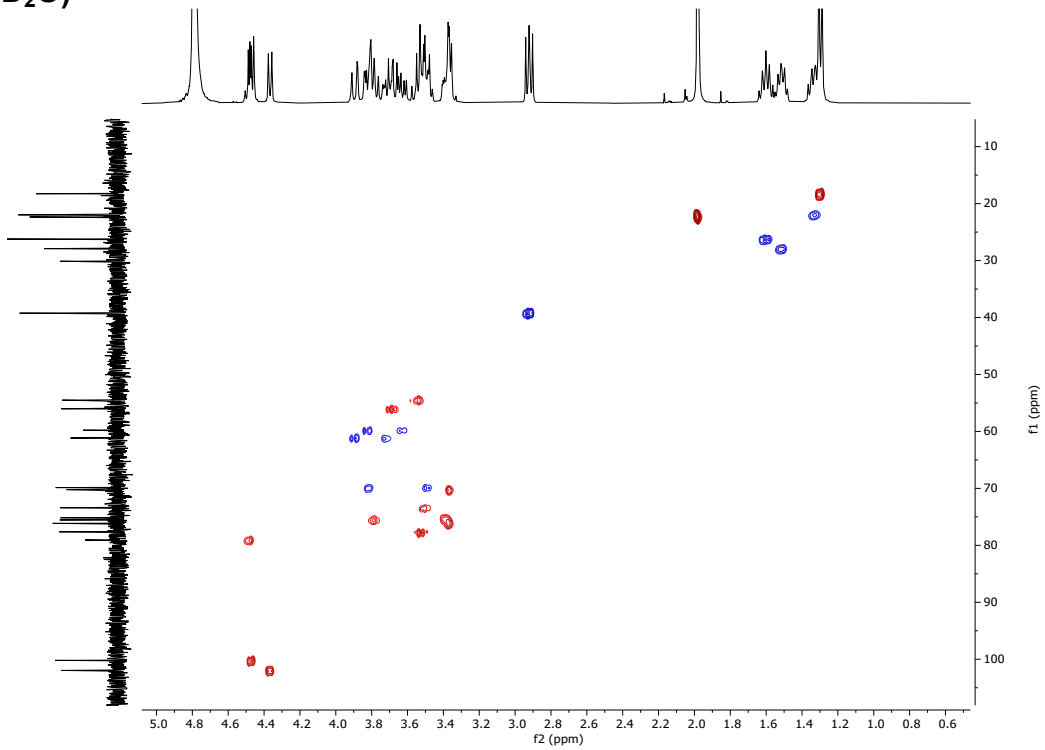
^{1}H NMR (D_2O)



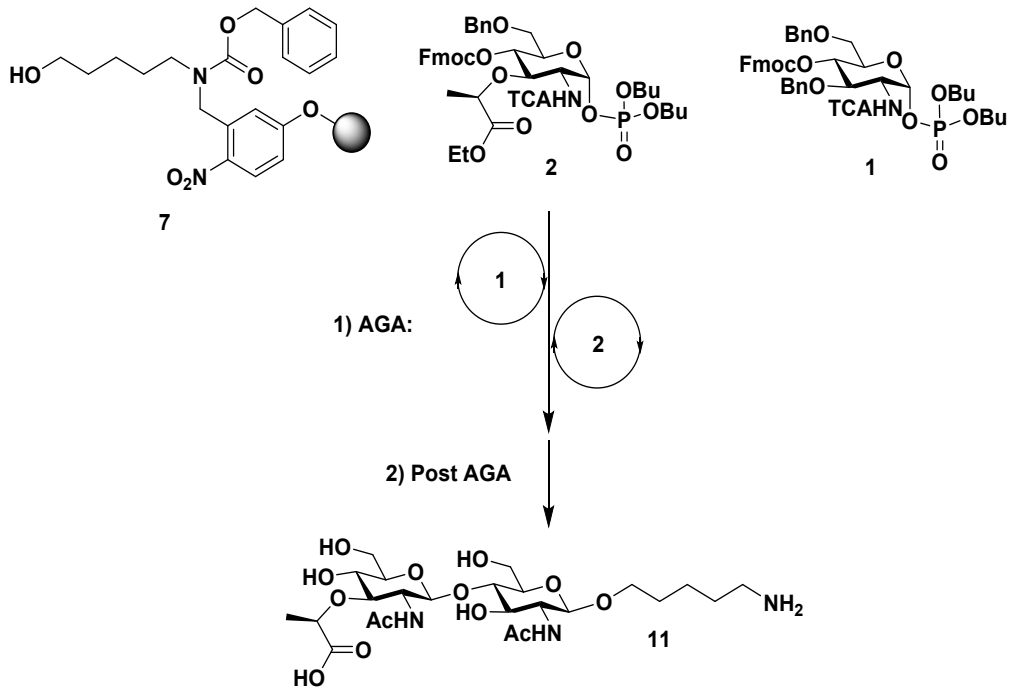
^{13}C NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (11)



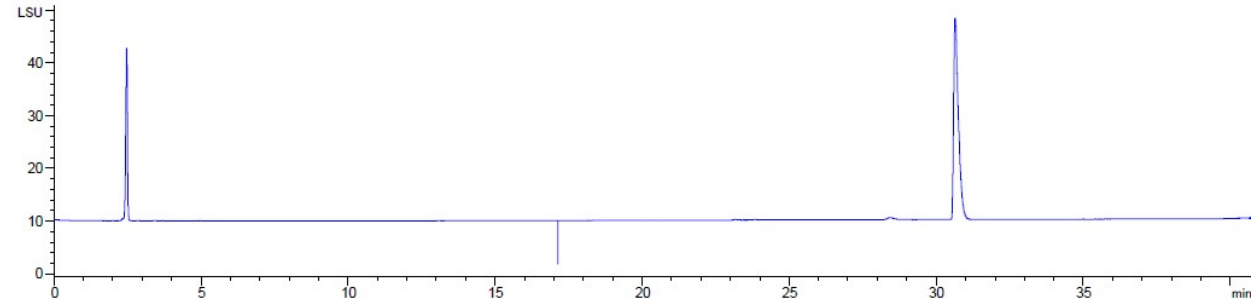
Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**I1, G, H1, I2**	**I1:** (Method A), **G:** (2 h), **H1:** (40 h), **I2:** (Method B)

Automated synthesis, global deprotection, and purification afforded **11** as a white solid (0.84 mg, 10% overall yield)

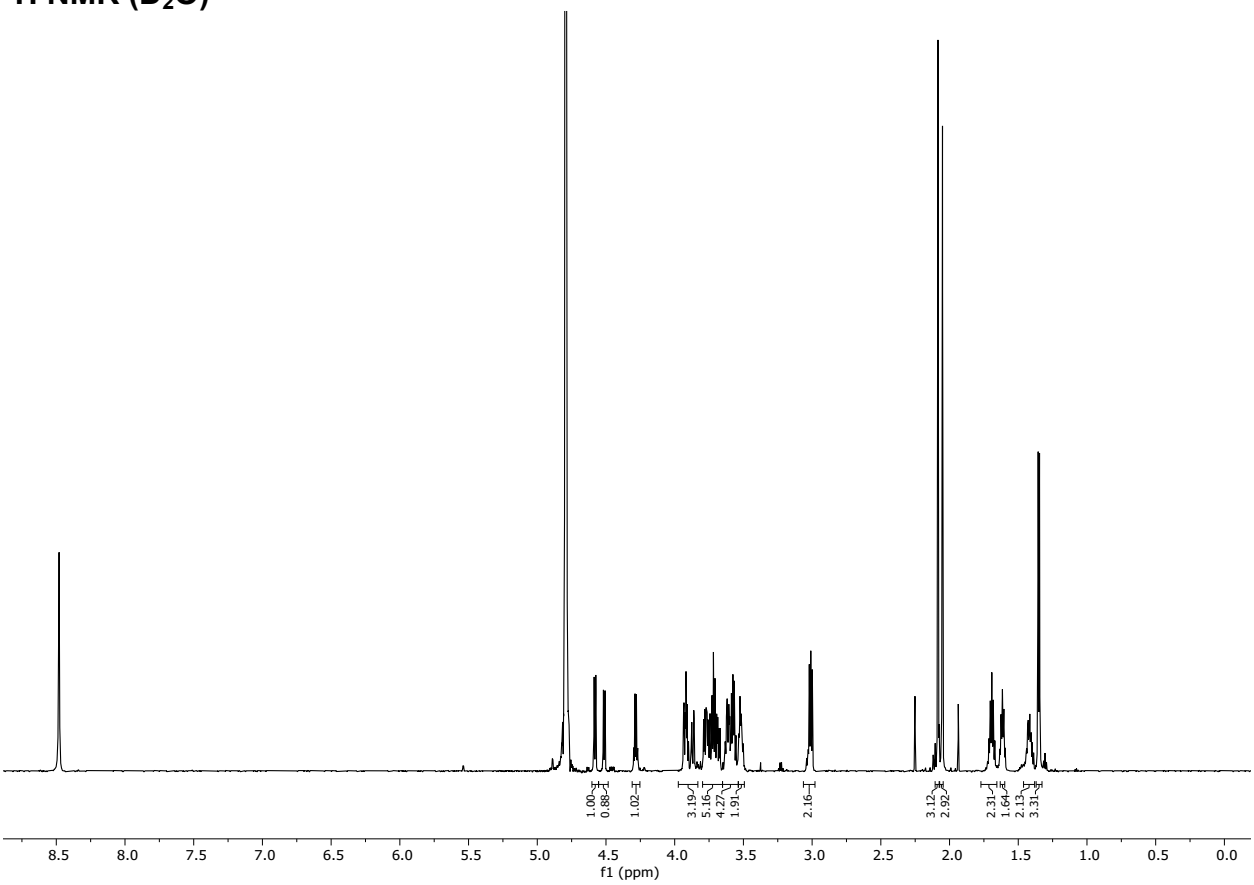
^{1}H NMR (700 MHz, D_2O) δ 8.48 (formic acid), 4.58 (d, *J* = 8.3 Hz, 1H), 4.51 (d, *J* = 7.8 Hz, 1H), 4.28 (q, *J* = 6.8 Hz, 1H), 3.99–3.84 (m, 3H), 3.80–3.65 (m, 5H), 3.65–3.55 (m, 4H), 3.53–3.49 (m, 2H), 3.03–2.98 (m, 2H), 2.08 (s, 3H), 2.05 (s, 3H), 1.74–1.65 (m, 2H), 1.64–1.58 (m, 2H), 1.47–1.38 (m, 2H), 1.35 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (176 MHz, D_2O) δ

181.24, 174.64, 174.45, 171.06, 101.99, 101.10, 80.18, 79.54, 77.93, 75.76, 74.58, 72.44, 70.10, 69.28, 60.50, 60.11, 54.96, 54.36, 39.37, 28.07, 26.38, 22.35, 22.18, 22.11, 18.88. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{24}H_{44}N_3O_{13}$ 582.2869 found 587.2877.

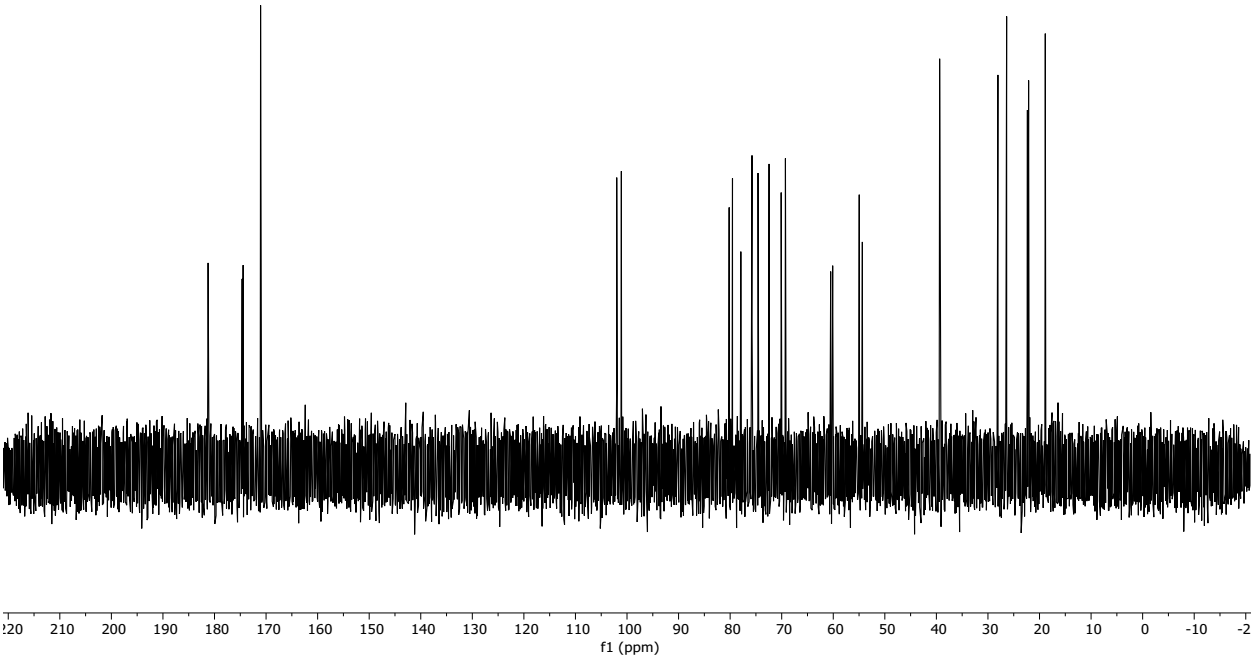
RP-HPLC (ELSD trace, method C, t_R = 30.6 min)



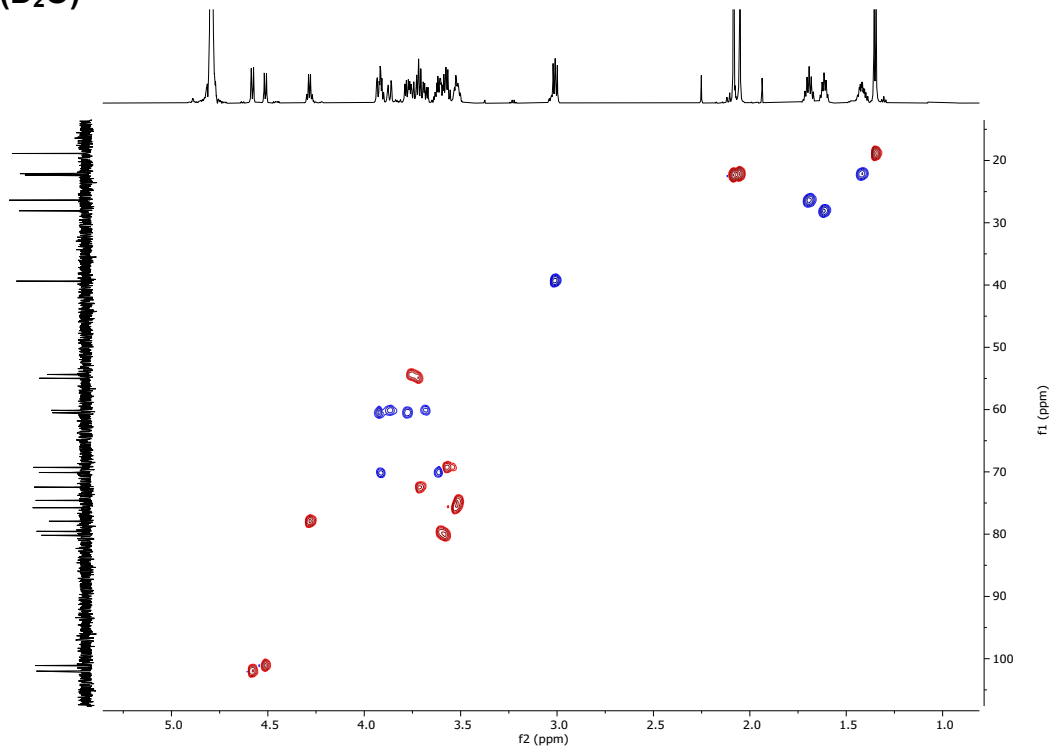
^{1}H NMR (D_2O)



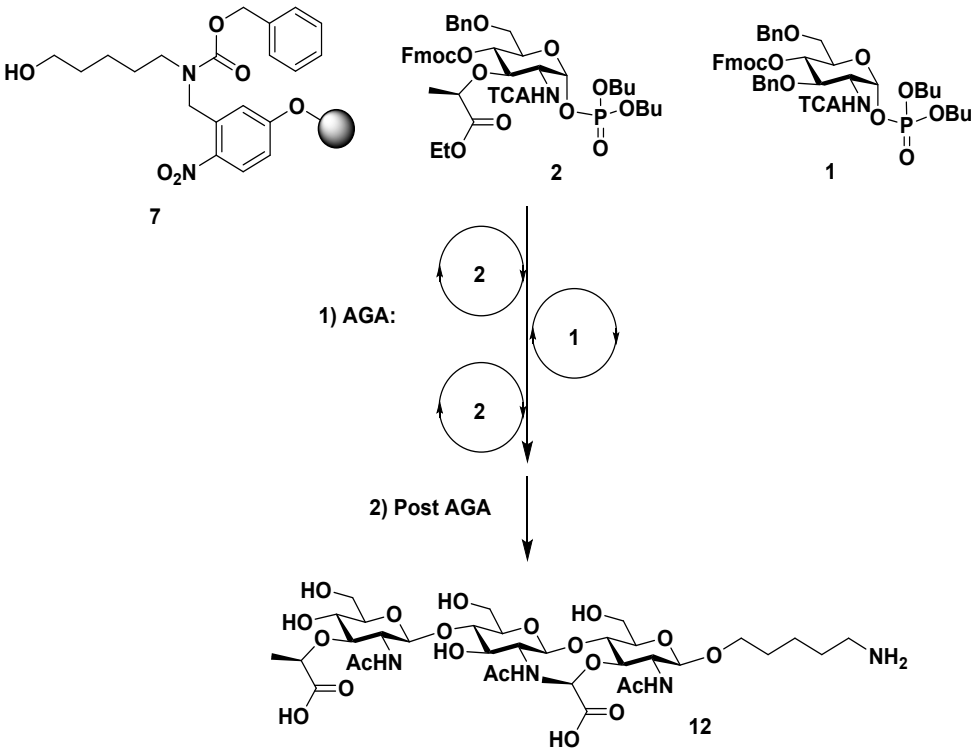
^{13}C NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranoside (12)

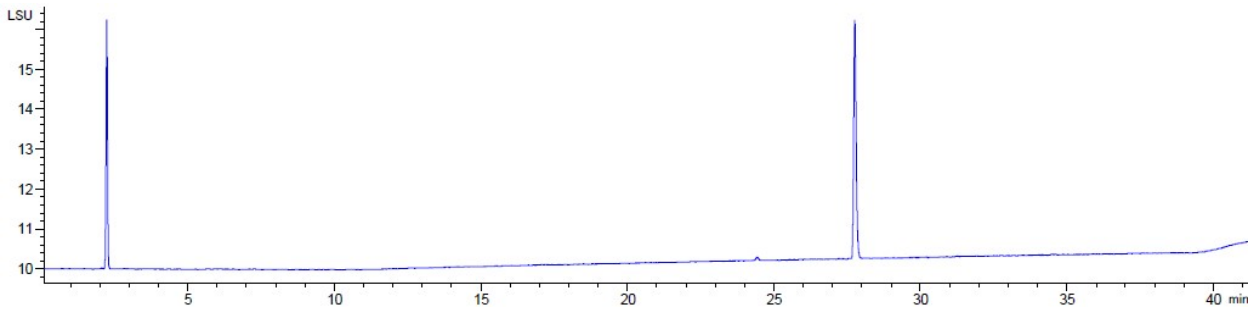


Action	BB	Modules	Notes
AGA		A	**7** swelling
	2	B, C, D, E	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	B, C, D, E	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	B, C, D, E	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		I1, G, H1, I2	**I1:** (Method A), **G:** (20 h), **H1:** (96 h), **I2:** (Method B)

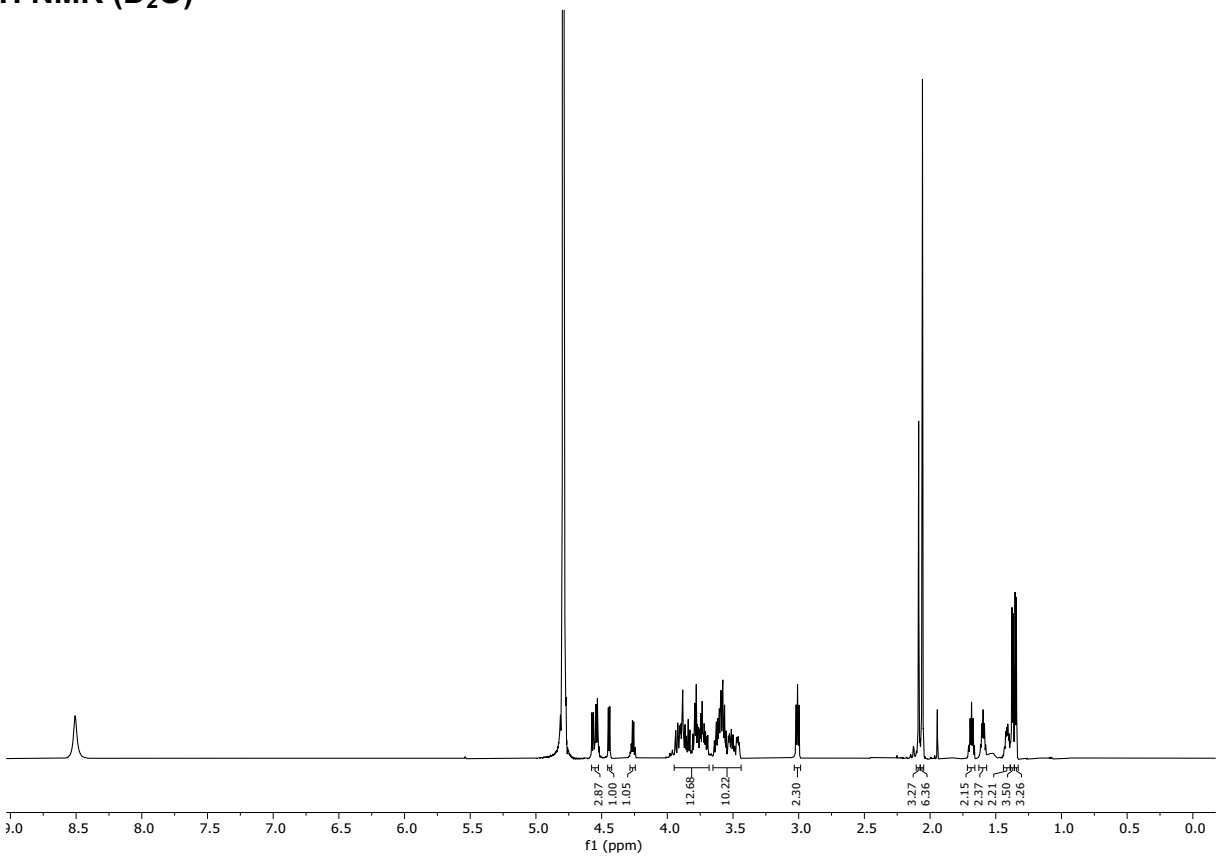
Automated synthesis, global deprotection, and purification afforded **12** as a white solid (1.14 mg, 11% overall yield)

^{1}H NMR (700 MHz, D_2O) δ 8.42 (formic acid), 4.54–4.41 (m, 3H), 4.36 (d, *J* = 7.9 Hz, 1H), 4.18 (q, *J* = 6.7 Hz, 1H), 3.89–3.58 (m, 11H), 3.58–3.34 (m, 9H), 2.98–2.86 (m, 2H), 2.00 (s, 3H), 1.97 (s, 6H), 1.63–1.50 (m, 2H), 1.51 (m, 2H), 1.33 (m, 2H), 1.29 (d, *J* = 6.8 Hz, 3H), 1.26 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (176 MHz, D_2O) δ 174.57, 171.01, 101.98, 100.24, 80.24, 80.03, 77.75, 75.70, 74.92, 72.23, 69.96, 69.11, 60.50, 59.93, 55.50, 54.64, 39.38, 28.01, 26.32, 22.32, 22.05, 18.87, 18.41. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{35}H_{61}N_4O_{20}$ 857.3874 found 857.3909.

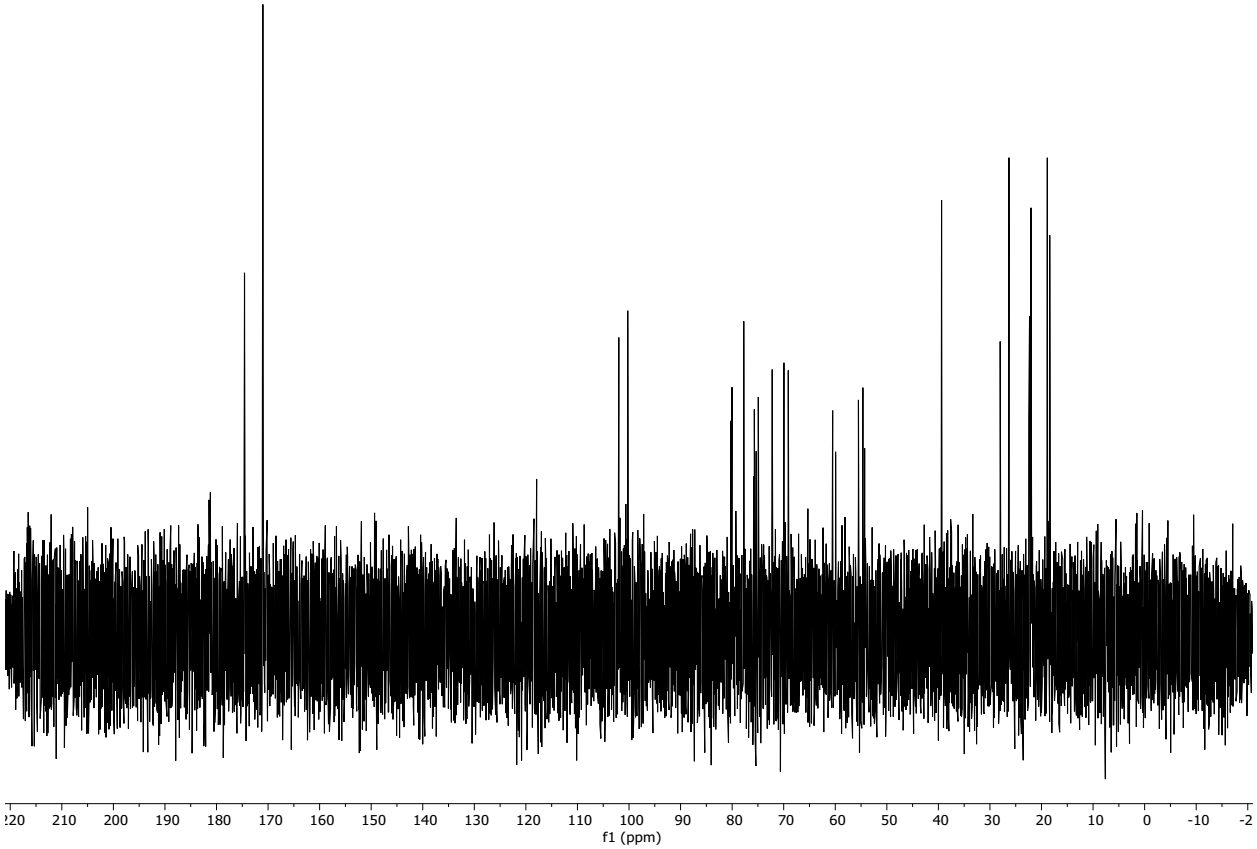
RP-HPLC (ELSD trace, method C, t_R = 27.7 min)



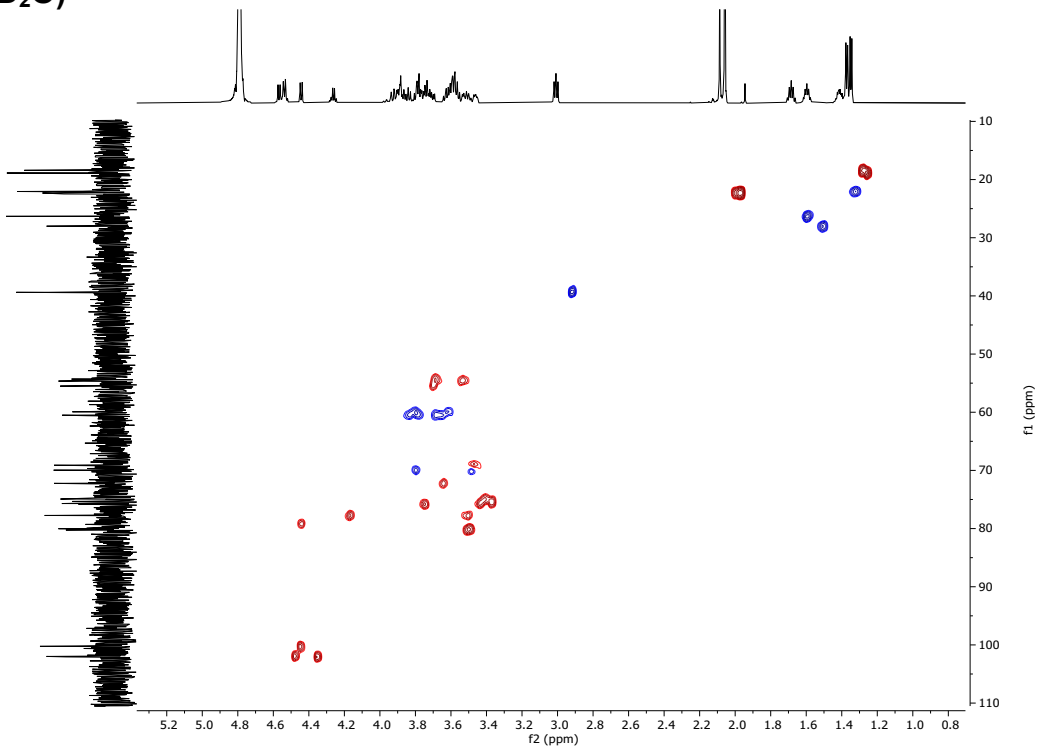
^{1}H NMR (D_2O)


2.87
1.00
1.05
12.68
10.22
2.30
3.27
6.36
2.15
2.37
2.21
3.50
3.26
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

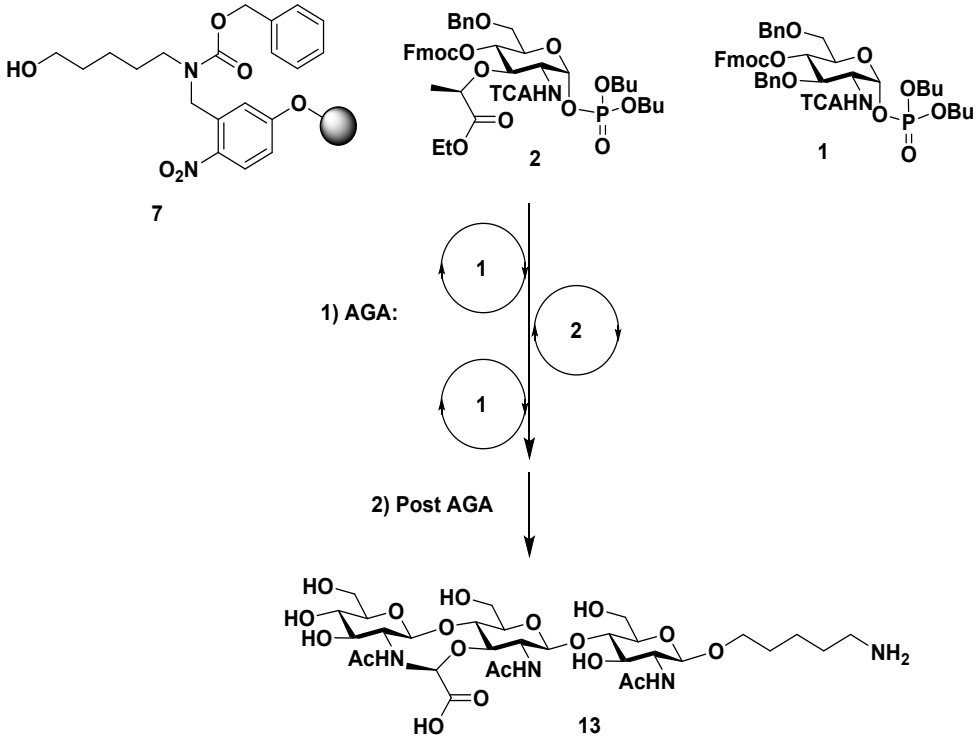
^{13}C NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (13)

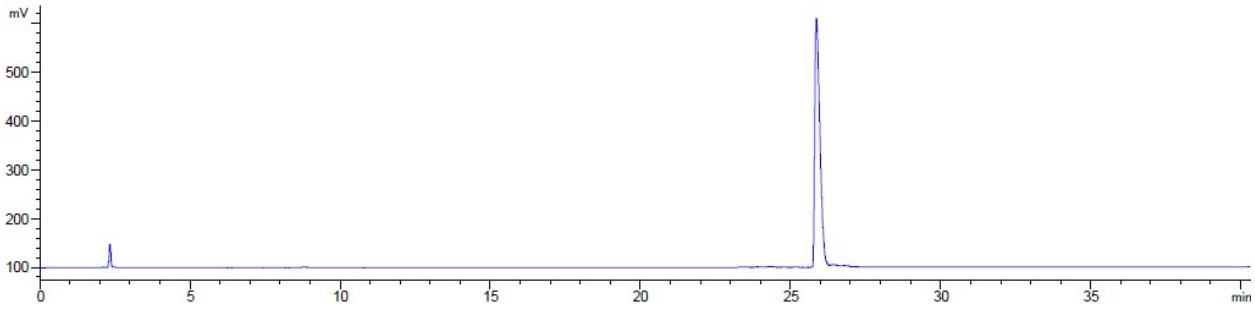


Action	BB	Modules	Notes
AGA		A	**7** swelling
	1	B, C, D, E	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	B, C, D, E	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	B, C, D, E	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		I1, G, H1, I2	**I1:** (Method A), **G:** (20 h), **H1:** (48 h), **I2:** (Method B)

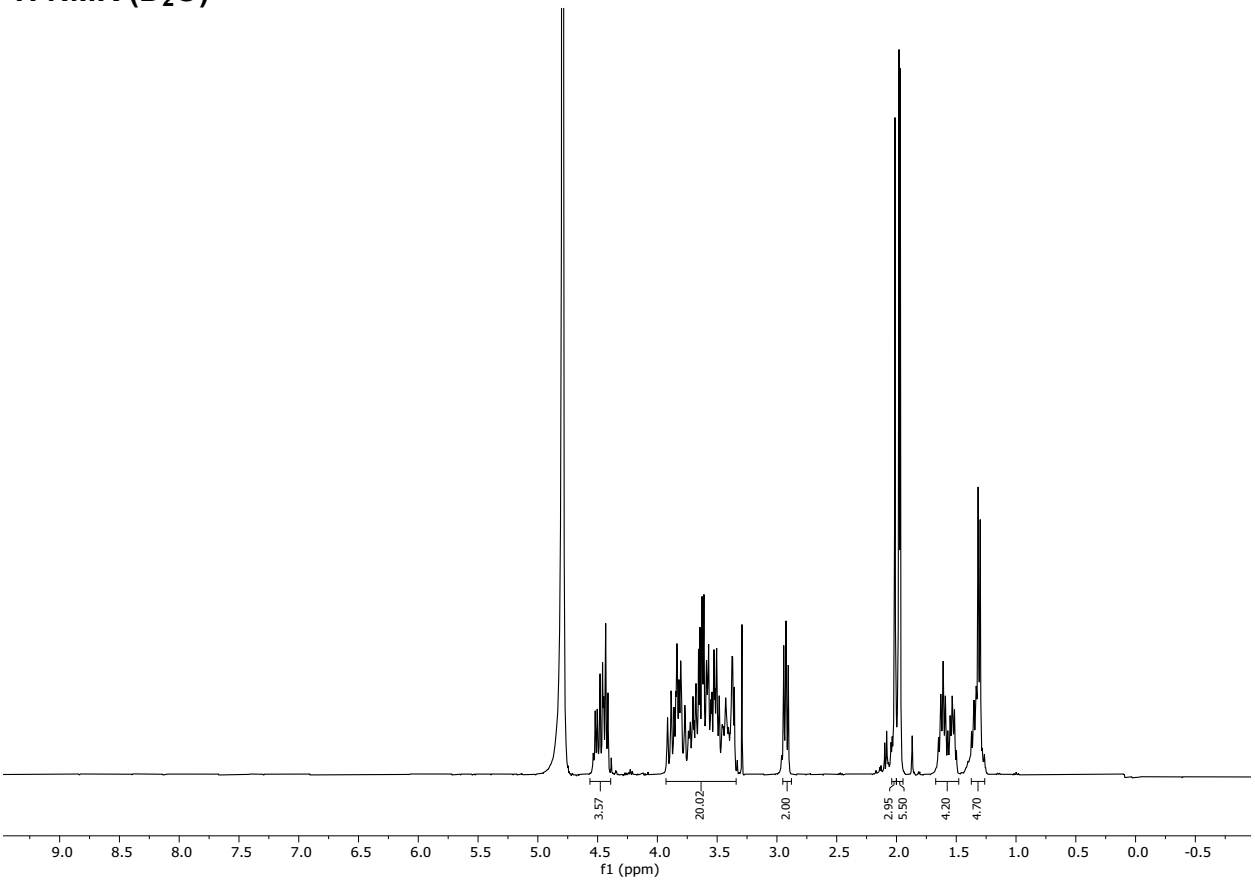
Automated synthesis, global deprotection, and purification afforded **13** as a white solid (1.03 mg, 9% overall yield)

^{1}H NMR (400 MHz, D_2O) δ 4.60–4.37 (m, 4H), 3.91–3.26 (m, 20H), 2.92 (t, *J* = 7.6 Hz, 2H), 2.01 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.68–1.49 (m, 4H), 1.39–1.32 (m, 2H), 1.31 (d, *J* = 6.9 Hz, 3H). ^{13}C NMR (101 MHz, D_2O) δ 174.89, 174.34, 102.26, 100.98, 100.17, 79.24, 77.23, 76.17, 75.15, 74.50, 73.38, 72.22, 70.25, 69.98, 59.82, 56.02, 54.79, 54.55, 39.21, 27.95, 26.26, 22.29, 22.02, 21.98, 18.29. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{32}H_{57}N_4O_{18}$ 735.3663 found 735.3672.

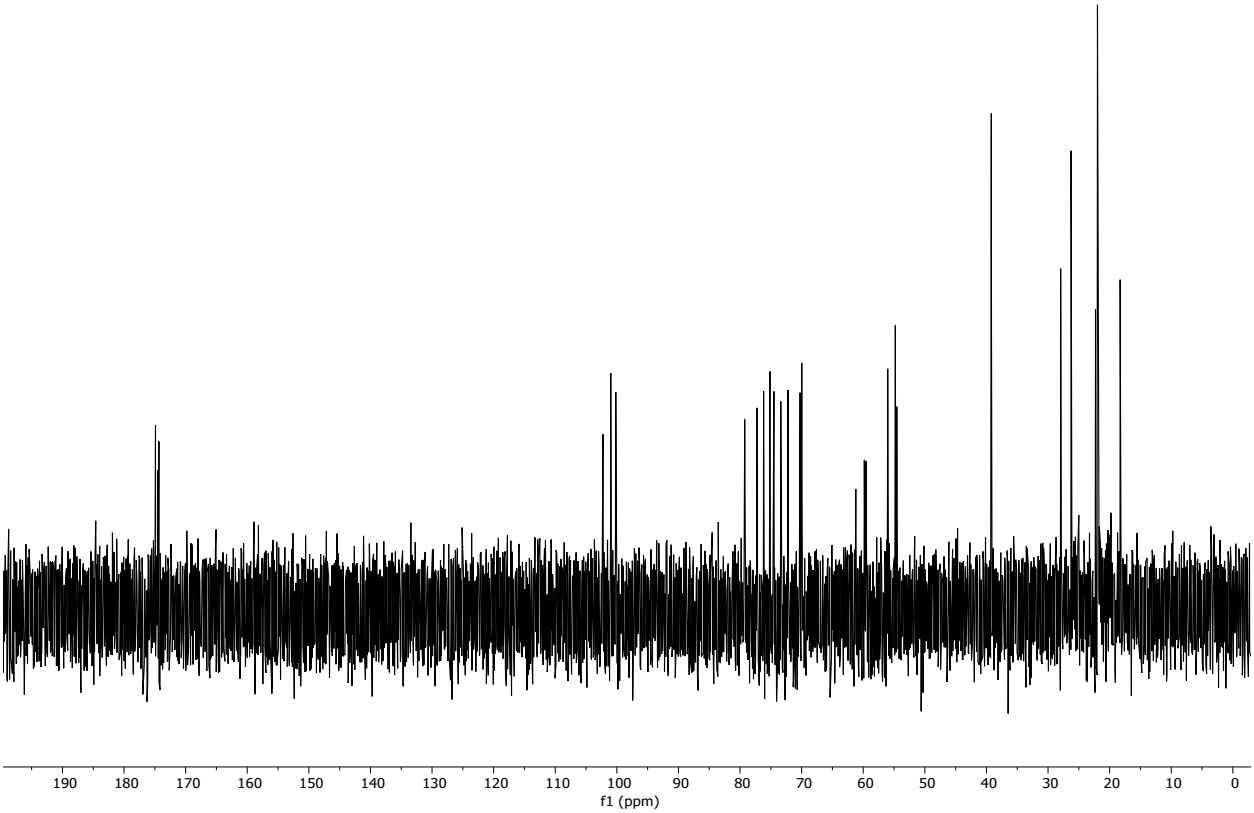
RP-HPLC (ELSD trace, method C, t_R = 25.90)



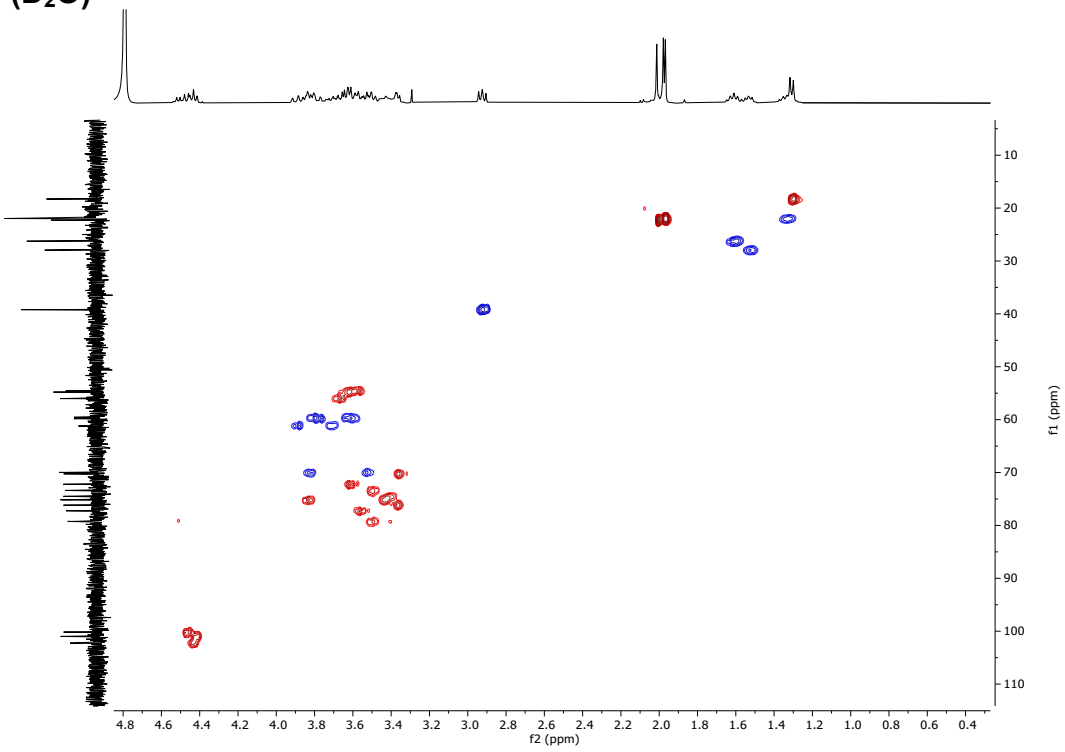
^{1}H NMR (D_2O)



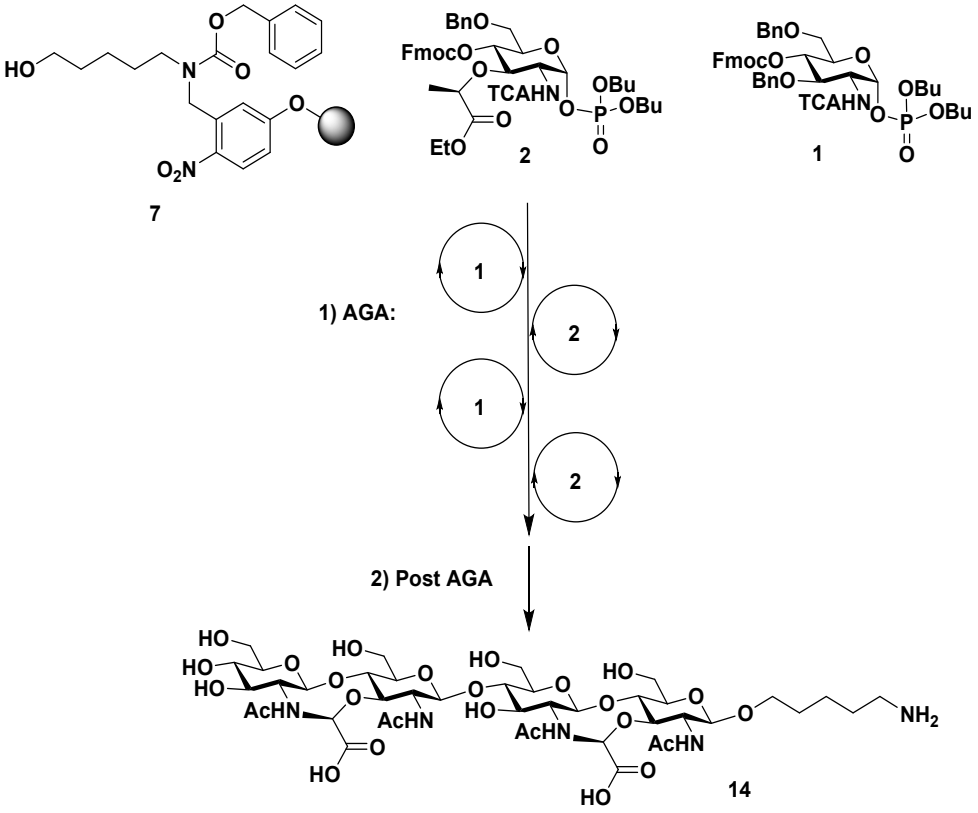
^{13}C NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranoside (14)

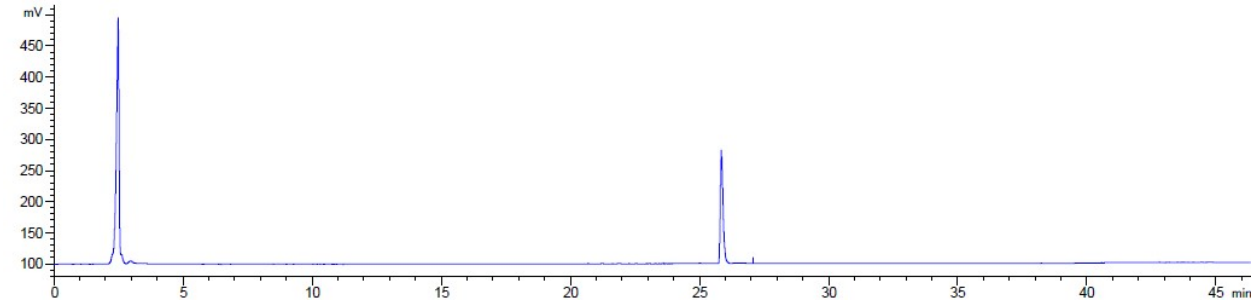


Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**I1, G, H1, I2**	**I1:** (Method B), **G:** (20 h), **H1:** (48 h), **I2:** (Method C)

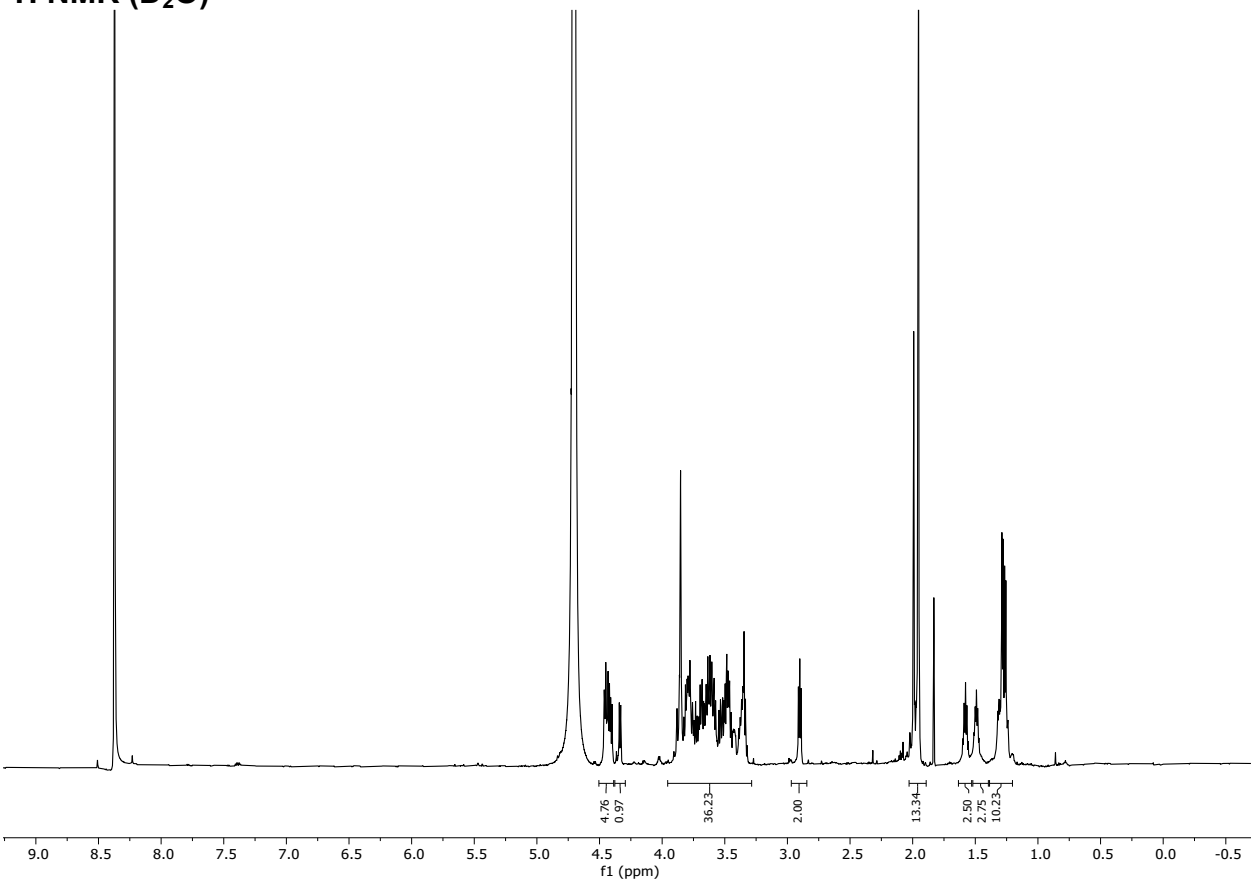
Automated synthesis, global deprotection, and purification afforded **14** as a white solid (0.35 mg, 3% overall yield)

^{1}H NMR (700 MHz, D_2O) δ 8.46 (formic acid), 4.60–4.46 (m, 5H), 4.42 (d, *J* = 7.8 Hz, 1H), 4.08–3.32 (m, 26H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.08 (s, 3H), 2.06–2.02 (m, 9H), 1.71–1.64 (m, 2H), 1.61–1.56 (m, 2H), 1.41–1.31 (m, 8H). ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{43}H_{74}N_5O_{25}$ 1060.4668 found 1060.4714.

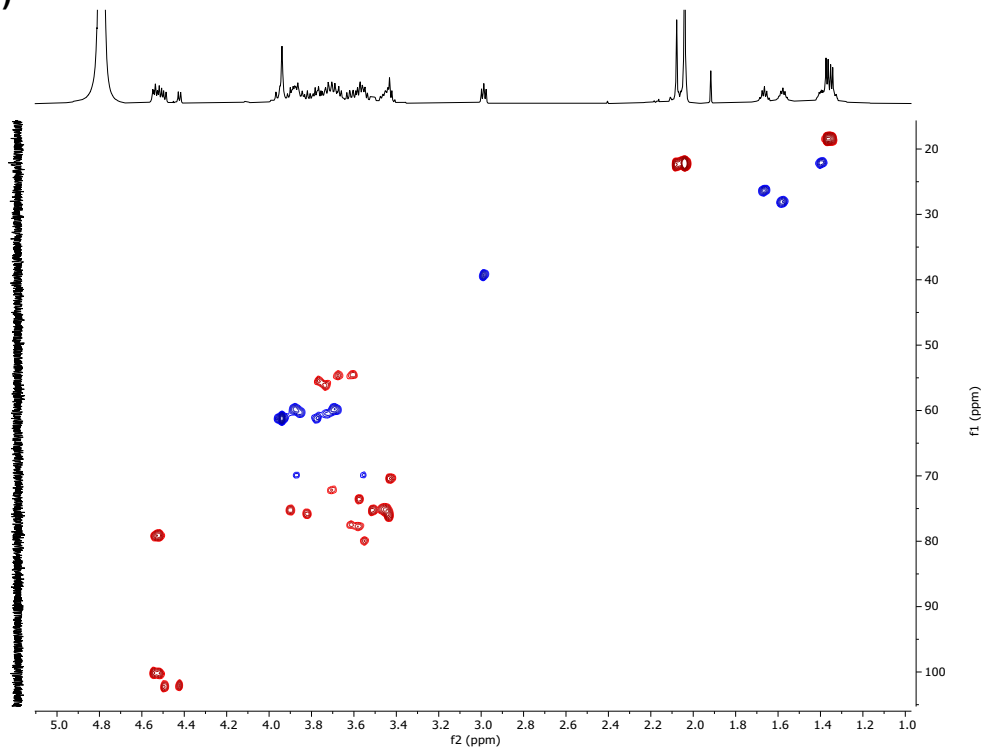
RP-HPLC (ELSD trace, method C, t_R = 25.89 min)



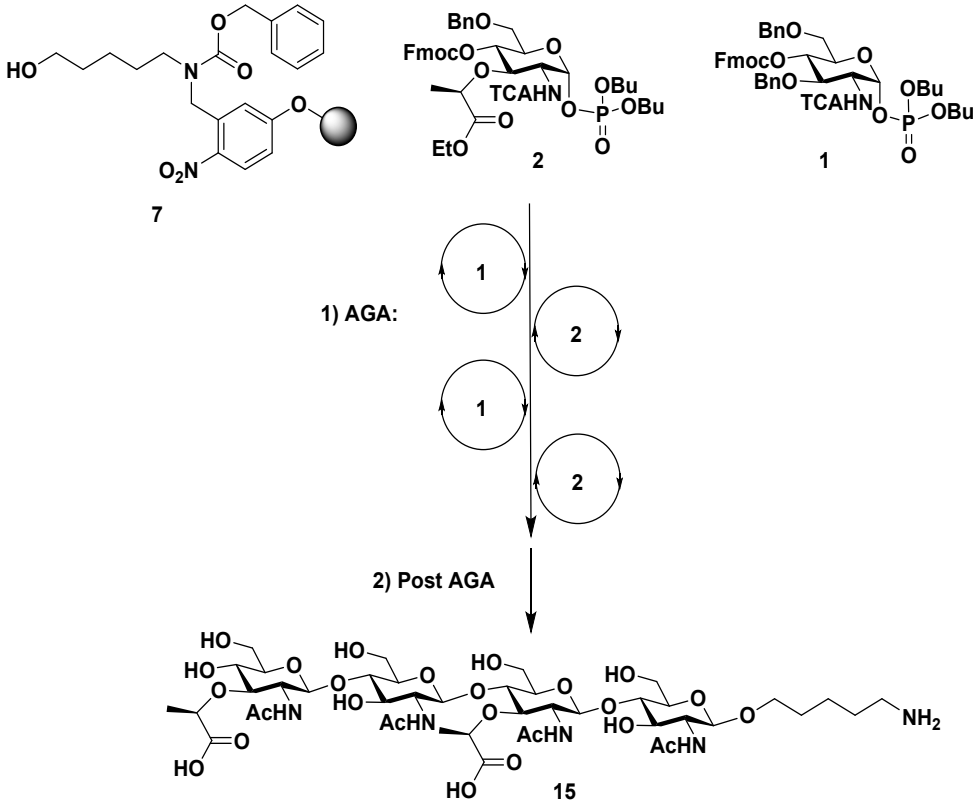
^{1}H NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (15)

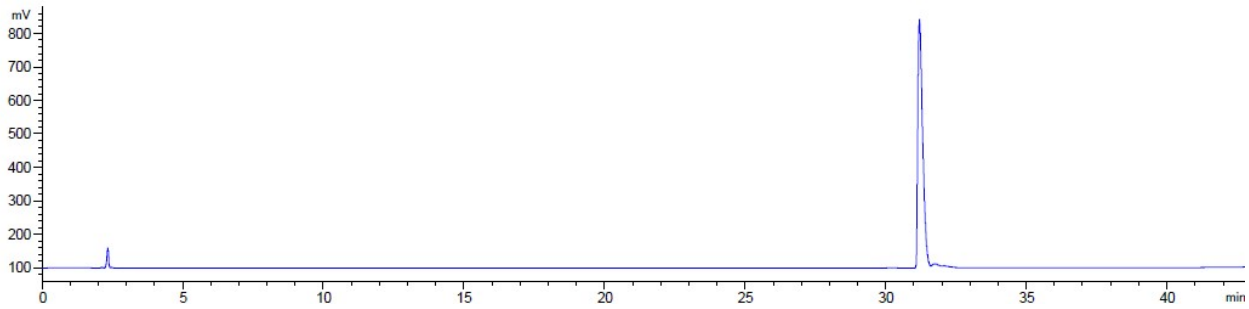


Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**I1, G, H2, I2**	**I1:** (Method A), **G:** (20 h), **H2:** (48 h), **I2:** (Method B)

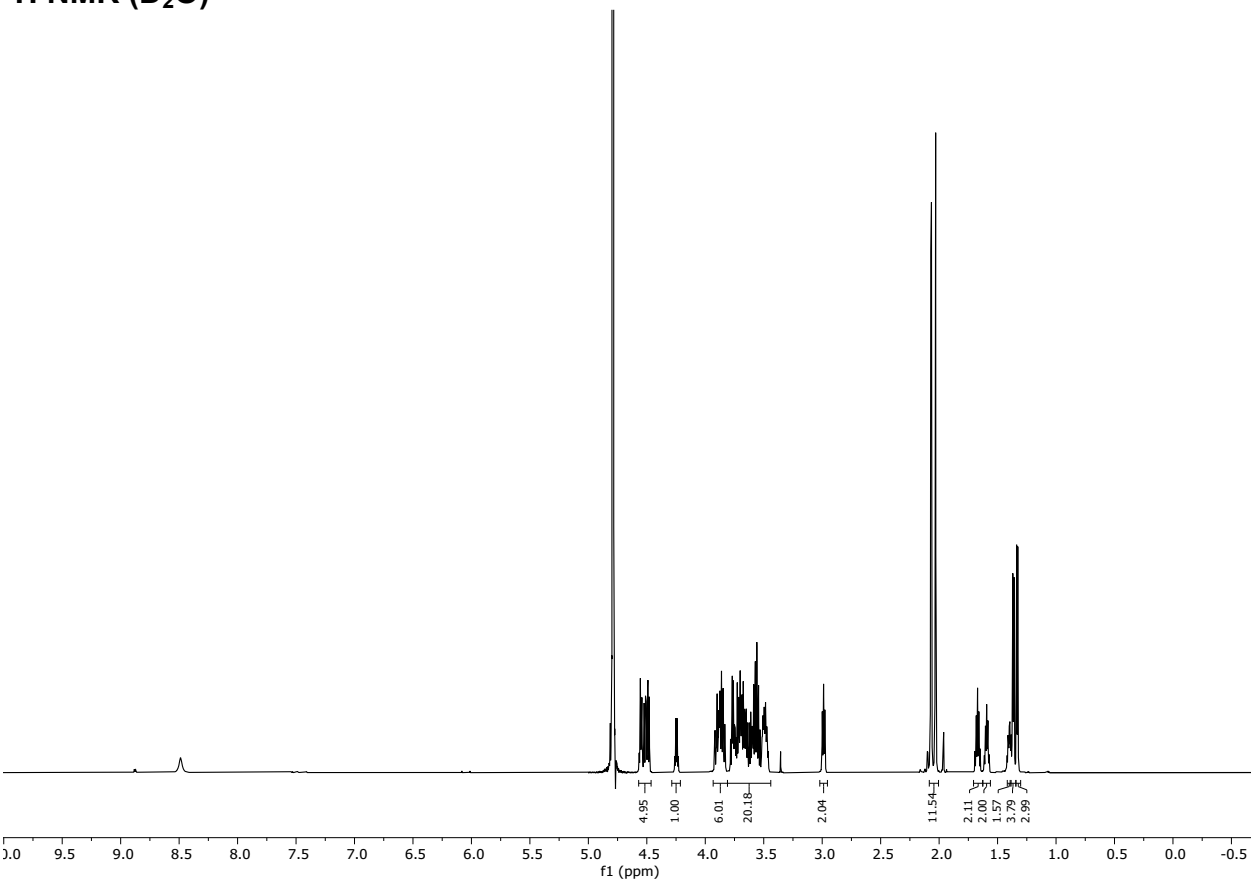
Automated synthesis, global deprotection, and purification afforded **15** as a white solid (1.06 mg, 8% overall yield)

^{1}H NMR (700 MHz, D_2O) δ 4.59–4.42 (m, 5H), 4.24 (q, *J* = 6.9 Hz, 1H), 4.01–3.42 (m, 26H), 3.06–2.87 (t, *J* = 7.8 Hz, 2H), 2.07 (s, 3H), 2.07 (s, 3H), 2.03 (m, 6H), 1.70–1.64 (m, 2H), 1.62–1.56 (m, 2H), 1.43–1.37 (m, 2H), 1.36 (d, *J* = 6.9 Hz, 3H), 1.33 (d, *J* = 6.9 Hz, 3H).^{13}C NMR (176 MHz, D_2O) δ 181.35, 174.95, 174.54, 174.41, 170.85, 102.32, 101.95, 101.07, 100.20, 80.33, 80.02, 79.44, 77.39, 75.68, 75.47, 75.26, 74.93, 74.60, 72.33, 72.19, 70.06, 69.11, 60.60, 60.48, 59.66, 55.46, 54.90, 54.66, 54.32, 39.33, 28.04, 26.34, 22.40, 22.29, 22.14, 22.09, 22.08, 18.85, 18.41. ESI-HRMS: m/z $[M+H]^+$ calcd. for $C_{43}H_{74}N_5O_{25}$ 1060.4668 found 1060.4675.

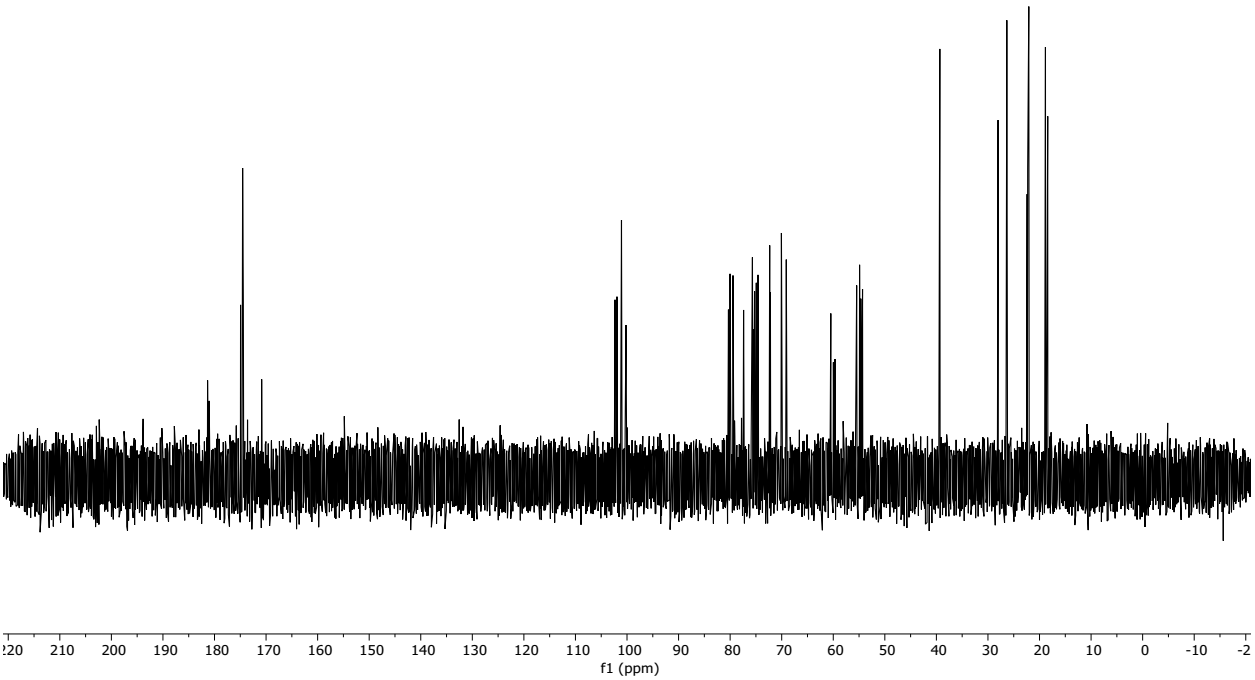
RP-HPLC (ELSD trace, method C, t_R = 31.24 min)



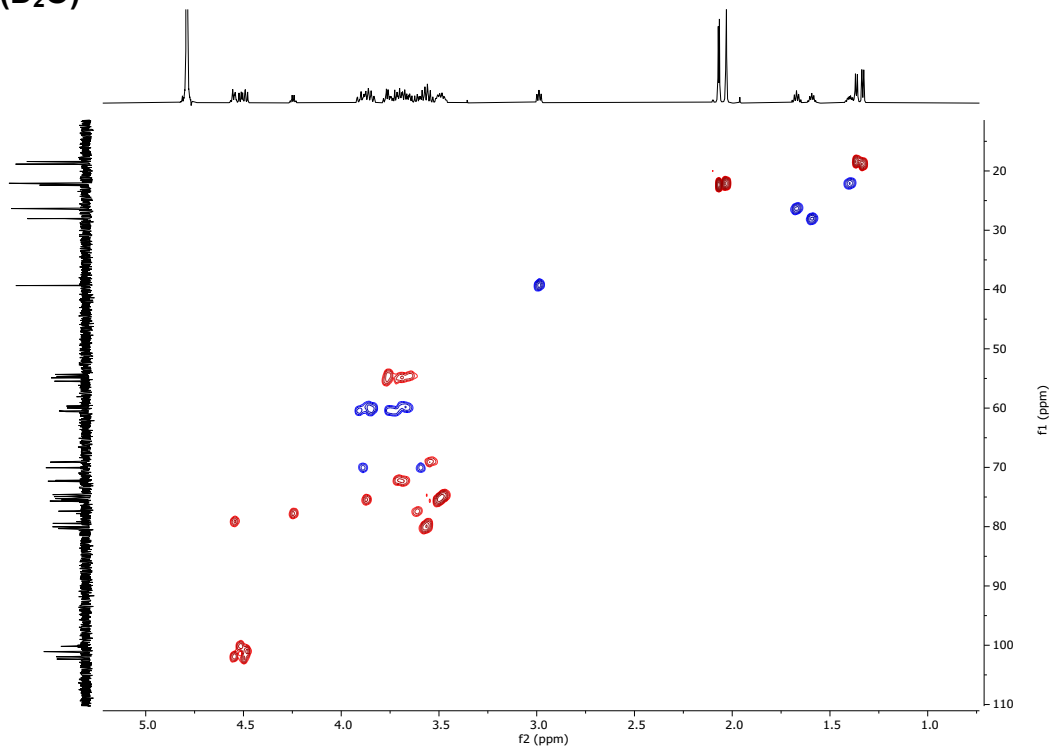
^{1}H NMR (D_2O)


4.95
1.00
6.01
20.18
2.04
11.54
2.11
2.00
1.57
3.79
2.99
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

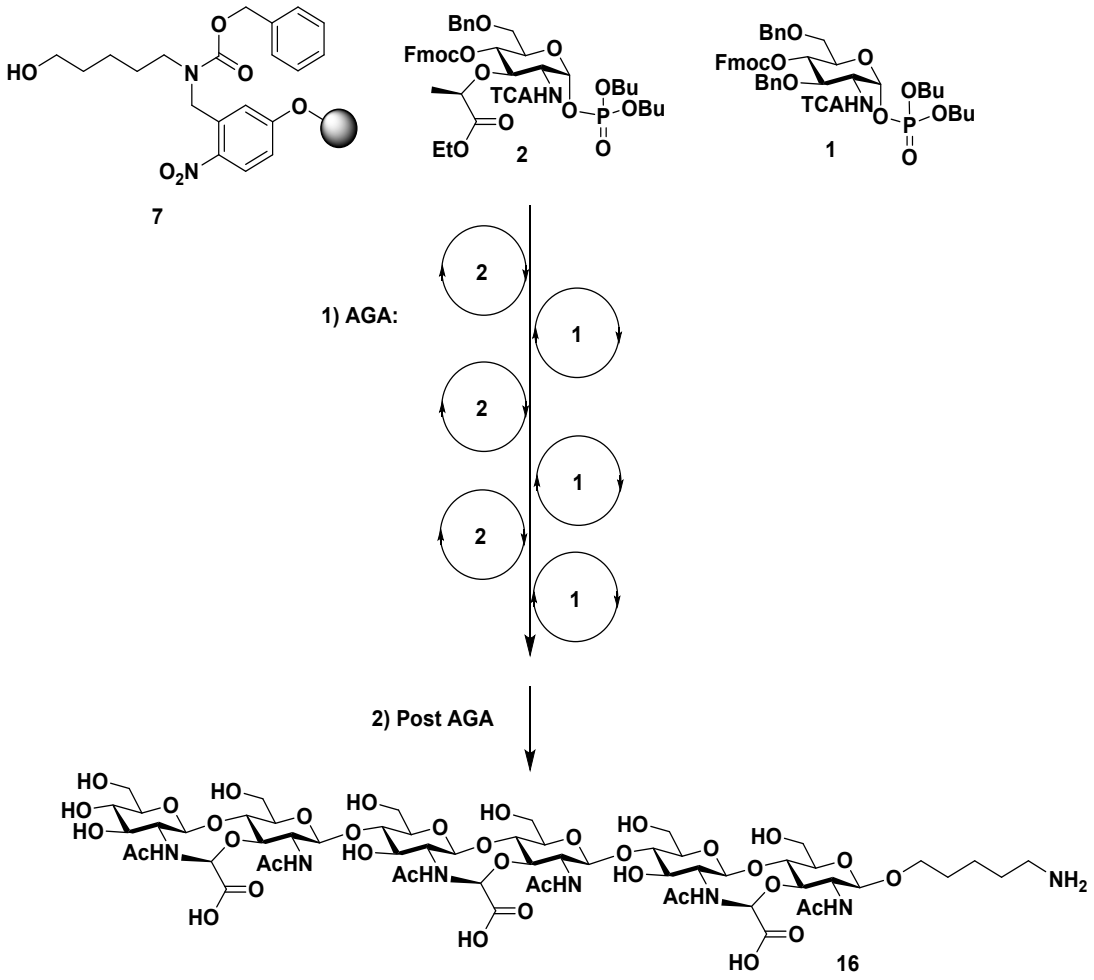
^{13}C NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranoside (16)

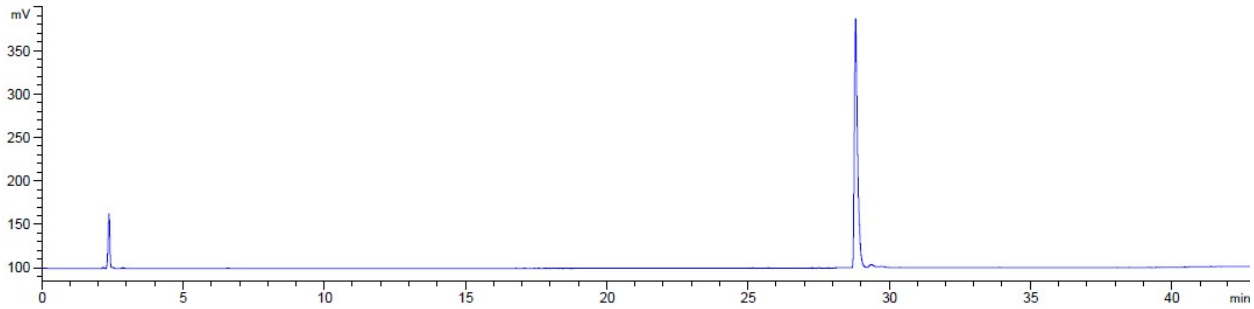


Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**I1, G, H2, I2**	**I1:** (Method A), **G:** (24 h), **H2:** (48 h) **I2:** (Method B)

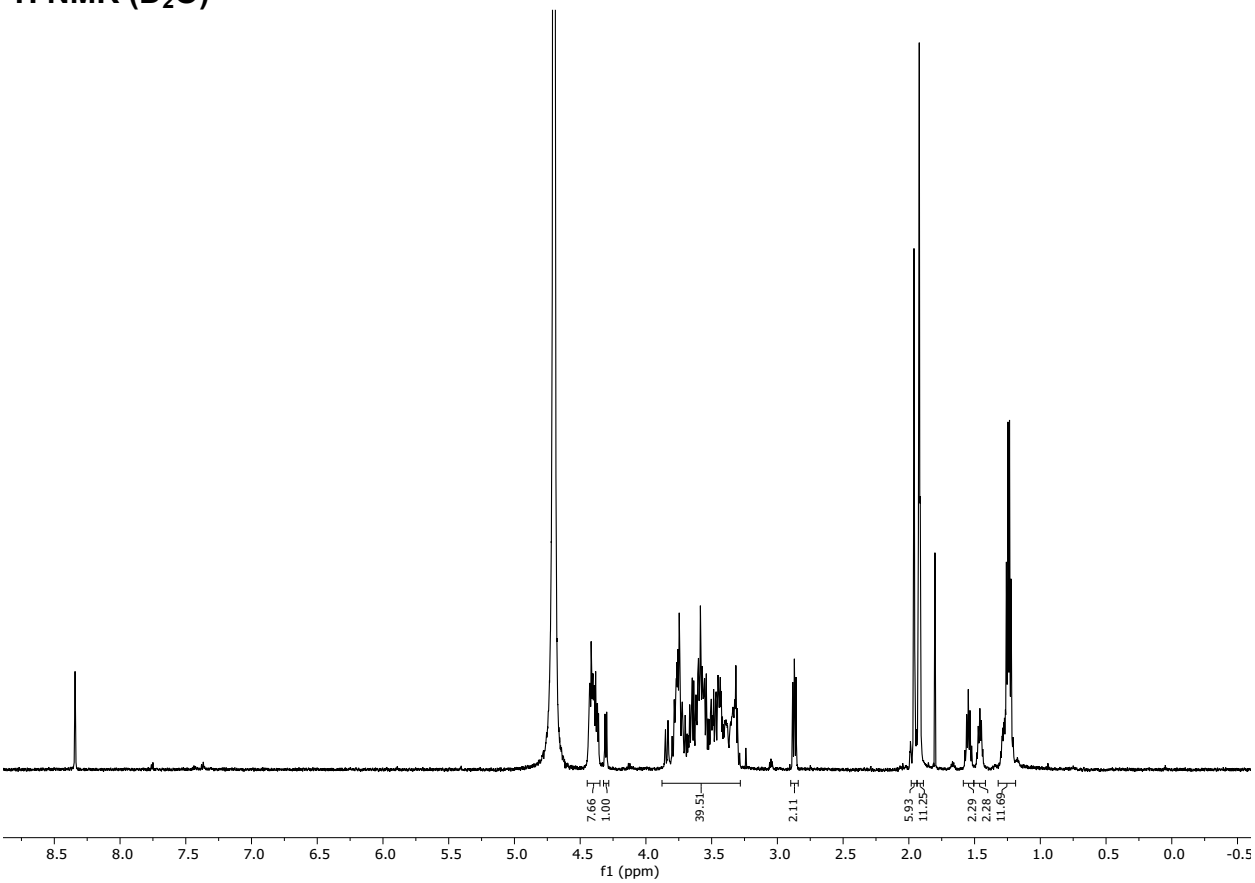
Automated synthesis, global deprotection, and purification afforded **16** as a white solid (0.51 mg, 3% overall yield).\

^{1}H NMR (600 MHz, D_2O) δ 8.34 (formic acid), 4.50–4.34 (m, 8H), 4.30 (d, *J* = 7.7 Hz, 1H), 3.86–3.24 (m, 38H), 2.95–2.82 (m, 2H), 2.00–1.89 (m, 18H), 1.59–1.51 (m, 2H), 1.49–1.41 (m, 2H), 1.31–1.15 (m, 11H). ESI-HRMS: m/z $[M+2H]^{2+}$ calcd. for $C_{62}H_{105}N_7O_{37}$ 769.8270 found 769.8269.

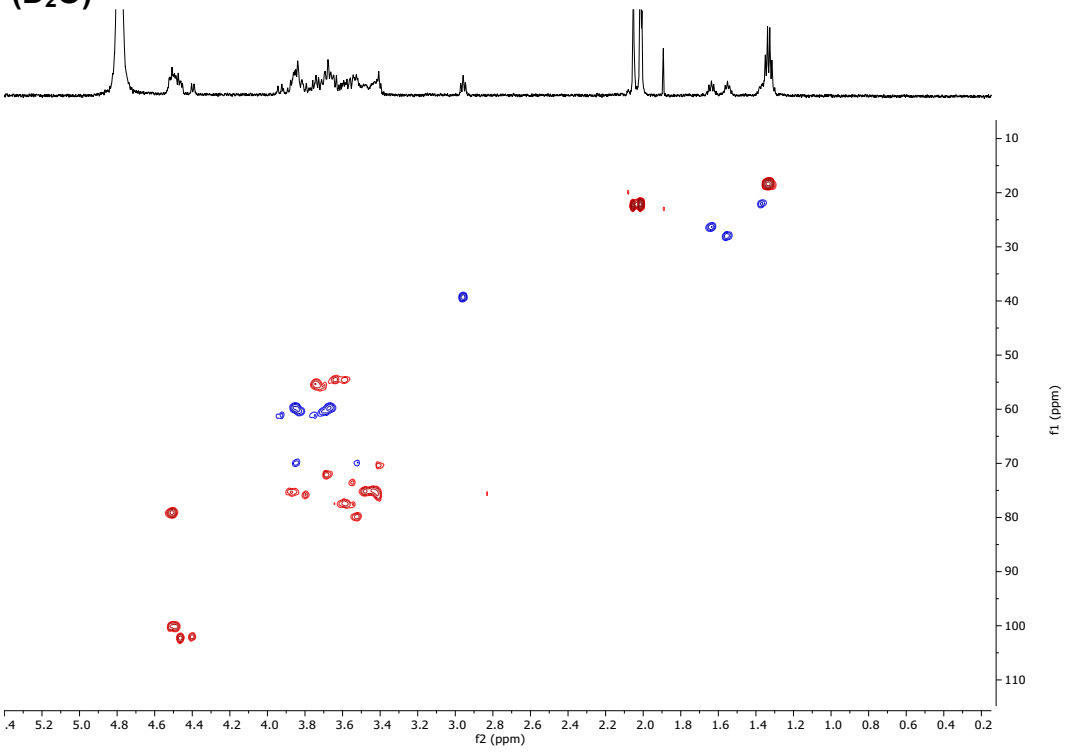
RP-HPLC (ELSD trace, method C, t_R = 28.84 min)



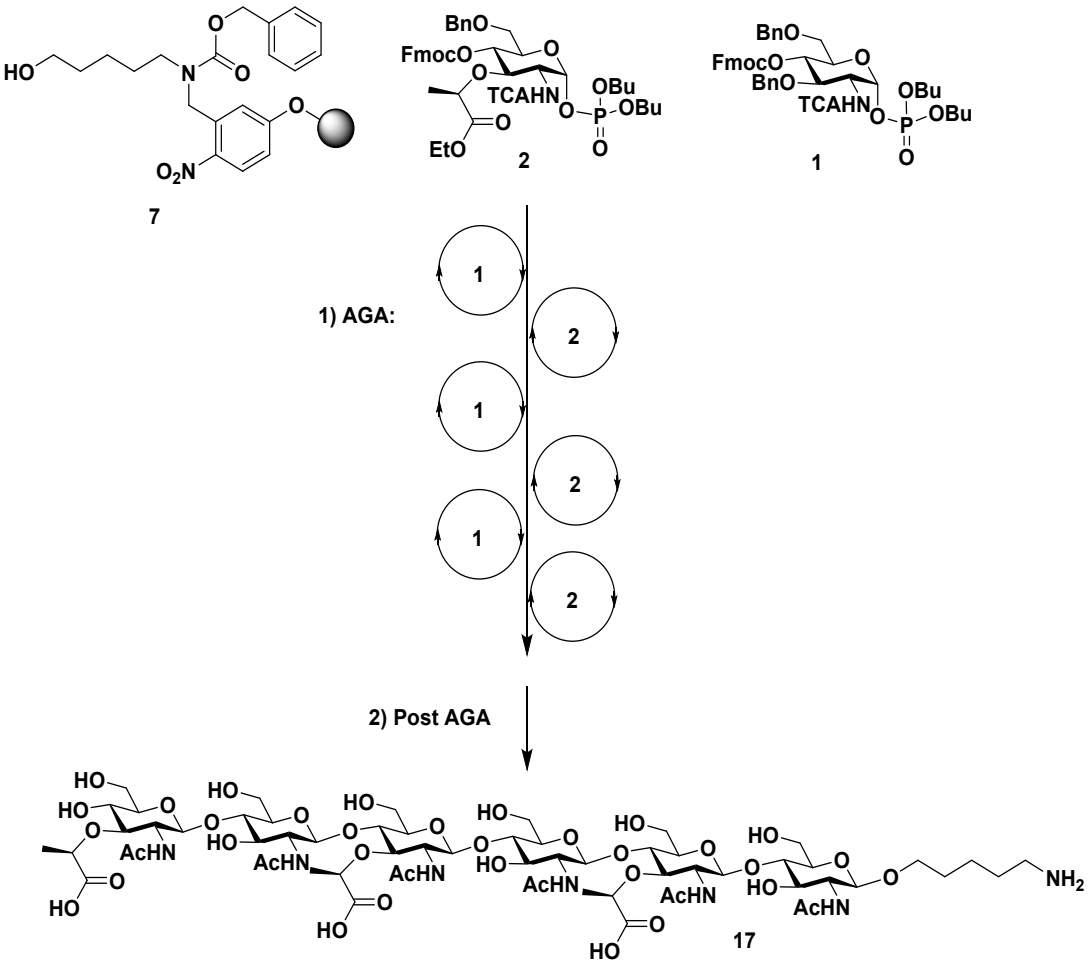
^{1}H NMR (D_2O)



HSQC (D_2O)



Aminopentyl 2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3-*O*-[(R)-1`-carboxyethyl]-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranoside (17)

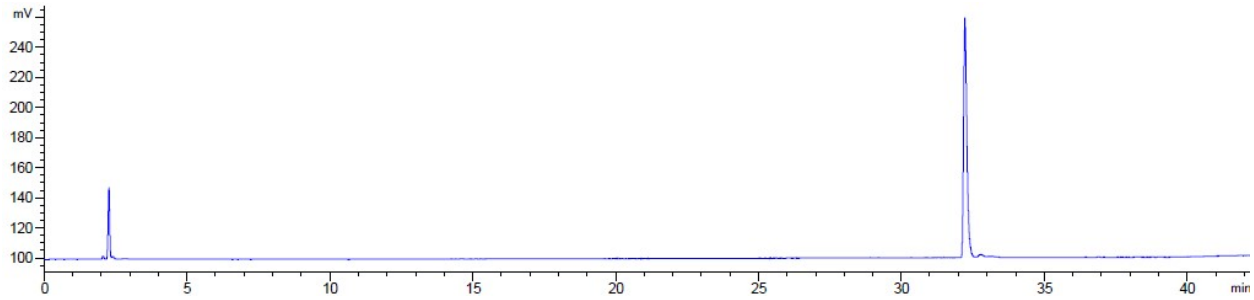


Action	BB	Modules	Notes
AGA		**A**	**7** swelling
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	1	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
	2	**B, C, D, E**	**C:** (−30°C for 5 min, −10°C for 40 min)
Post AGA		**I1, G, H2, I2**	**I1:** (Method A), **G:** (24 h), **H2:** (48 h) **I2:** (Method B)

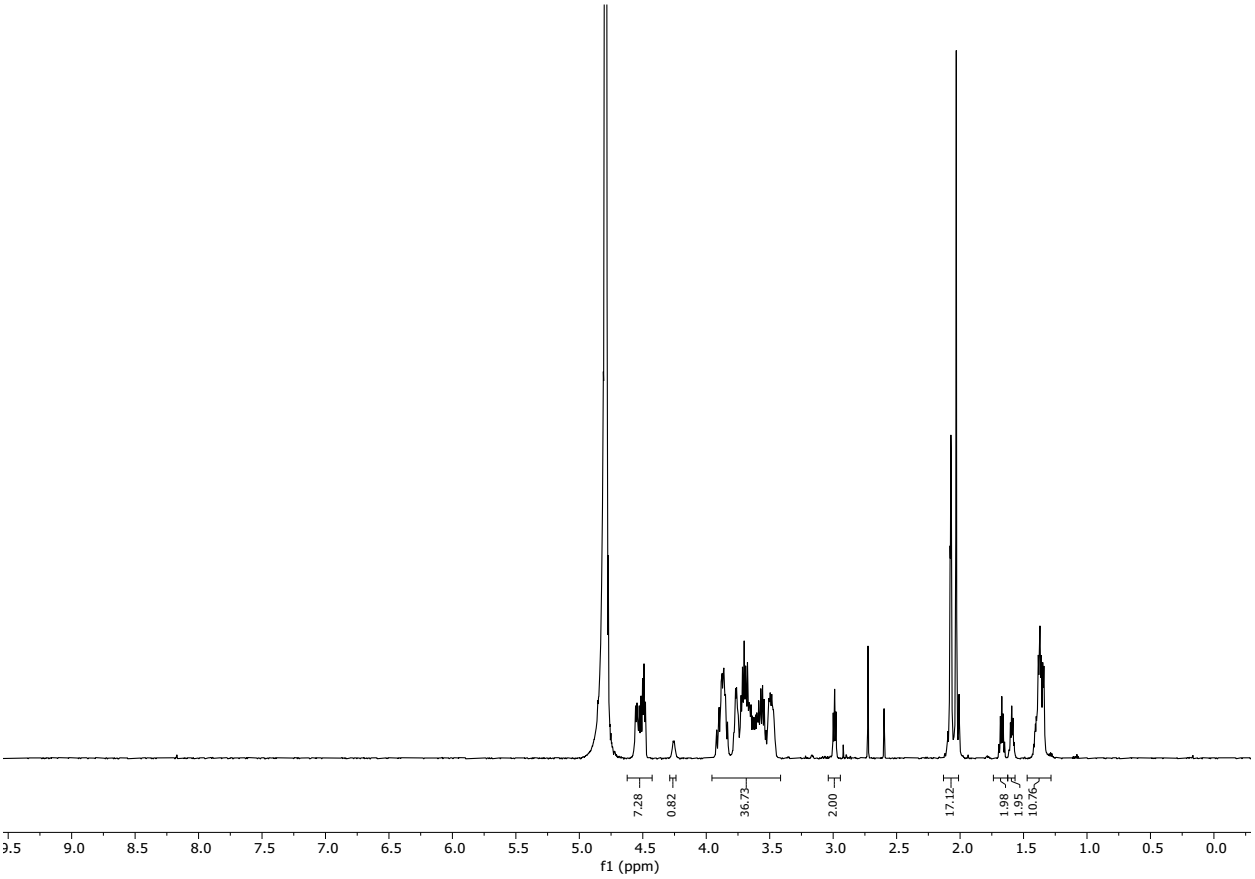
Automated synthesis, global deprotection, and purification afforded **17** as a white solid (0.43 mg, 2% overall yield).

^{1}H NMR (700 MHz, D_2O) δ 4.69–4.44 (m, 8H), 4.26 (d, *J* = 7.4 Hz, 1H), 4.03–3.36 (m, 38H), 2.99 (t, *J* = 7.6 Hz, 2H), 2.20–1.93 (m, 18H), 1.82–1.62 (m, 2H), 1.64–1.54 (m, 2H), 1.45–1.21 (m, 11H) ppm. ^{13}C NMR (176 MHz, D_2O) δ 174.55, 101.07, 80.35, 74.61, 70.07, 69.10, 59.67, 39.34, 28.03, 26.34, 22.82, 22.09, 18.82, 18.38. ESI-HRMS: m/z ESI-HRMS: m/z $[M+2H]^{2+}$ calcd. for $C_{62}H_{105}N_7O_{37}$ 769.8270 found 769.8284.

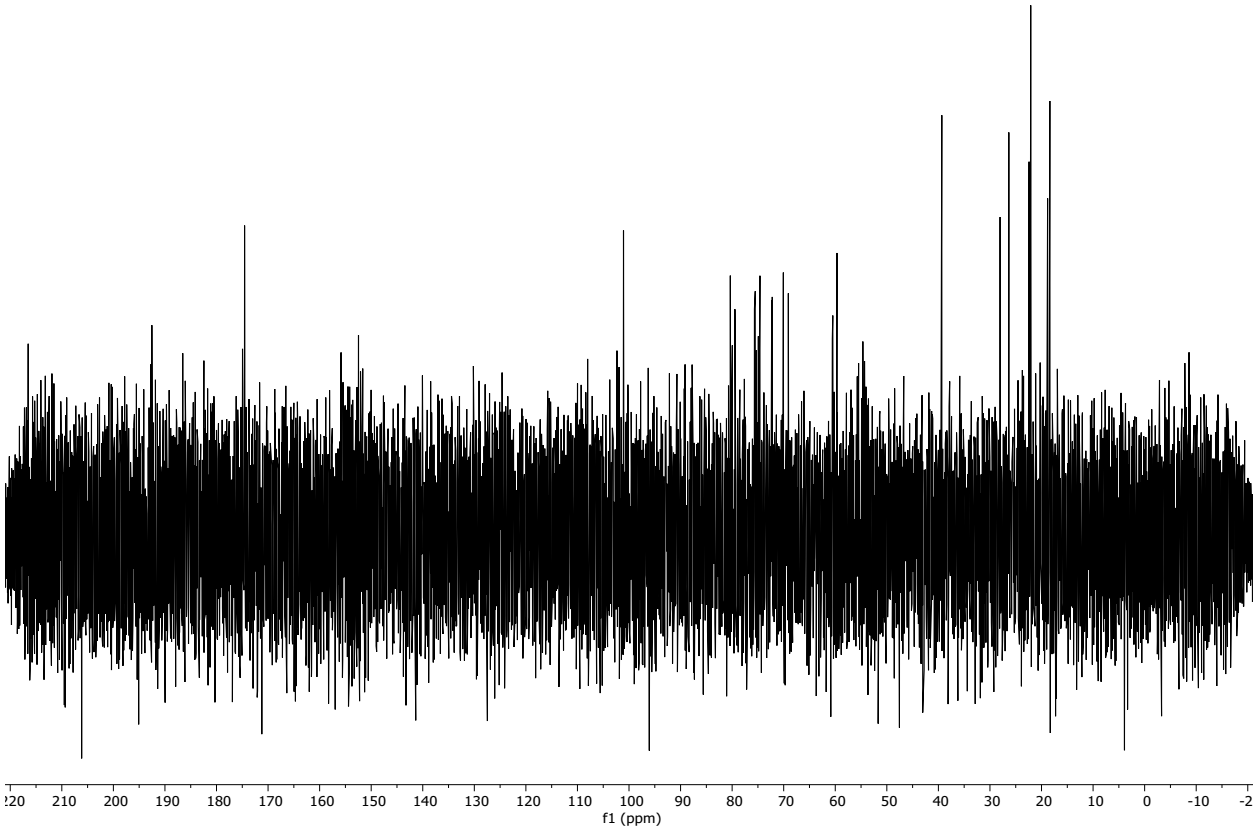
RP-HPLC (ELSD trace, method C, t_R = 32.25 min)



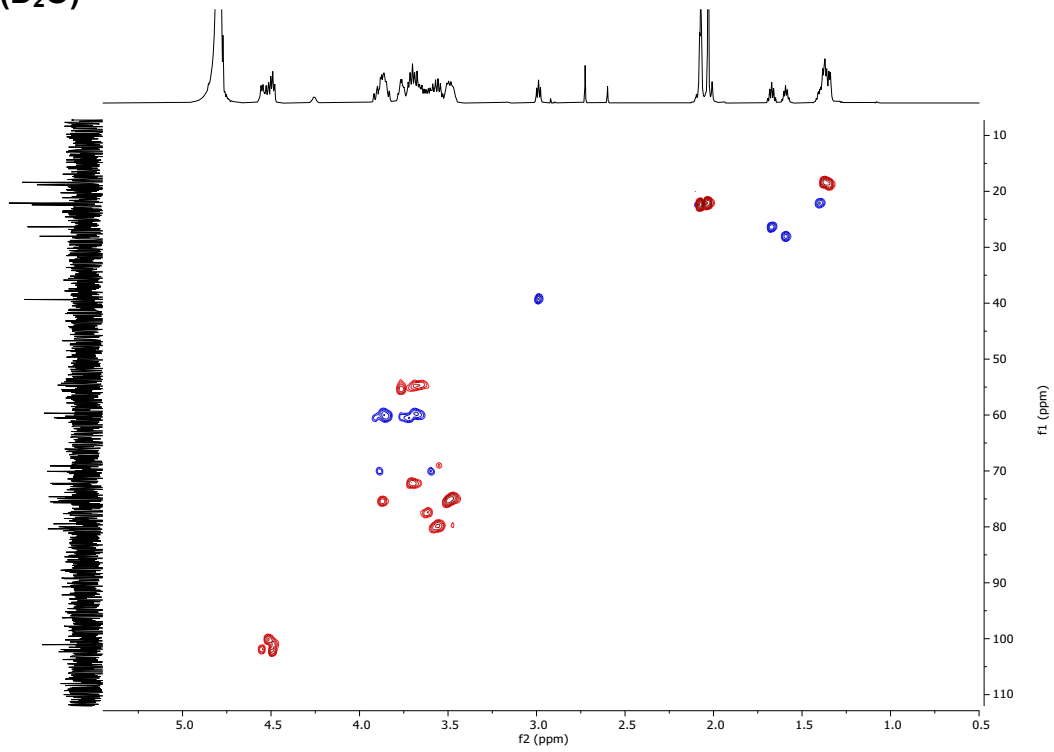
^{1}H NMR (D_2O)


7.28
0.82
36.73
2.00
17.12
1.98
1.95
10.76
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

^{13}C NMR (D_2O)



HSQC (D_2O)



4. Literature

1. J. Kandasamy, F. Schuhmacher, H. S. Hahm, J. C. Klein and P. H. Seeberger, *Chem. Commun.*, 2014, **50**, 1875-1877.
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4. M. Gude, J. Ryf and P. D. White, *Lett. Pept. Sci.*, 2002, **9**, 203-206.
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