

Electronic Supporting Information (ESI)

Antibody recognition of fluorinated MUC1 glycopeptide antigens

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Contents	1
General Remarks	2
Compound 2	3
Compound 4	3
Compound 8	3
Compound 9	5
Compound 10	6
Compound 11	7
Compound 12	8
Compound 13	10
General Protocol for SPPS	10
Compound 14	11
Compound 15	13
Compound 16	14
Compound 17	16
Serum neutralisation test	18
References	19
NMR spectra of selected compounds	20

General Remarks:

Solvents for moisture-sensitive reactions (toluene, MeCN, CH₂Cl₂, MeNO₂) were distilled and dried according to standard procedures. Glycosylations were performed in flame-dried glassware under inert argon atmosphere. DMF (amine-free, for peptide synthesis) and NMP were purchased from Roth, and Ac₂O in p.a. quality from Acros. Reagents were purchased in the highest available commercial quality and used as supplied except where noted. Fmoc-protected amino acids were purchased from Orpegen Pharma. For solid-phase synthesis, pre-loaded TentaGel S resin (Rapp Polymere) was employed. Reactions were monitored by TLC with pre-coated silica gel 60 F₂₅₄ aluminium plates (Merck KGaA, Darmstadt) using UV light as the visualizing agent and by dipping the plate into a 1:1 mixture of 1 M H₂SO₄ in EtOH and 3% 3-methoxyphenol solution in EtOH followed by heating. Flash column chromatography was performed with silica gel (230-400 mesh) from Merck.

¹H, ¹³C, ¹⁹F and 2D NMR spectra were recorded on a Bruker AC-300 or a Bruker AM-400 spectrometer. The chemical shifts are reported in ppm relative to the signal of the deuterated solvent. Multiplicities are given as: s (singlet), bs (broad singlet), d (doublet), t (triplet), and m (multiplet). Assignment of proton and carbon signals was achieved by additional COSY, HMQC, and HMBC experiments when noted. The signals of molecule-fragments were denoted as follows: amino acids (greek indices), *N*-acetyl-D-galactosamine (no prime), D-galactose (') and glycosylated Thr (*). ESI- and HR-ESI-mass spectra were recorded on a Micromass Q TOF Ultima 3 spectrometer, while MALDI-TOF mass spectra were acquired on a Micromass Tofspec E spectrometer. Optical rotations were measured at 546 nm and 578 nm with a Perkin-Elmer polarimeter 241.

RP-HPLC analyses were performed on a JASCO-HPLC system with PerfectSil C18(2) (250×4.6 mm, 5 μm), Phenomenex Luna C18(2) (250×4.6 mm, 5 μm) and Phenomenex Jupiter C18(2) (250×4.6 mm, 5 μm) columns at a flow rate of 1 mLmin⁻¹. Preparative HPLC separations were carried out on a JASCO-HPLC System with PerfectSil C18(2) (250×20 mm, 5 μm), Phenomenex Luna C18(2) (250×30 mm, 10 μm) and Phenomenex Jupiter C18(2) (250×30 mm, 10μm) columns at a flow rate of 20 mLmin⁻¹ or 10 mLmin⁻¹. Mixtures of H₂O–MeCN were used as solvents; if required 0.1% TFA were added.

***N*-(9H-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-2,6-dideoxy-6-fluoro-3-*O*-[2,3,4-tri-*O*-benzyl-2-deoxy-2-fluoro-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine (2)**

To Fmoc-Thr-(β-2F-Bn₃Gal-(1-3)-α-6F-GalNAc)-*O**t*Bu^[S1] **1**, 220 mg, 0.21 mmol), dissolved in water (0.7 mL) was added TFA (7.0 mL) and the solution was stirred for 2.5 h at ambient temperature. The mixture was co-evaporated with toluene (4×25 mL) and CH₂Cl₂ (25 mL) and the residue was purified by flash chromatography (SiO₂, EtOAc → EtOAc/MeOH/AcOH, 10:1:0.1) to afford **2** (183 mg, 0.19 mmol, 90%) as a colorless amorphous solid. *R_f* = 0.63 (EtOAc/MeOH/AcOH, 4:1:0.05). *Analytical RP-HPLC* (Luna C18(2), λ = 214 nm, H₂O-MeCN, 70:30, 5 min → 10:90, 25 min → 0:100, 30 min, *t_R* = 36.3 min). *ESI-MS (positive)*, (*m/z*): 1003.41 ([M+Na]⁺, calc.: 1003.38), 1019.45 ([M+K]⁺, calc.: 1019.35). *HR-ESI-MS (positive, m/z)* calc. for C₅₄H₅₈F₂N₂NaO₁₃: 1003.3805 ([M+Na]⁺), found : 1003.3808.

***N*-(9H-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-2,6-dideoxy-6-fluoro-3-*O*-[2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine (4)**

To Fmoc-Thr-(β-Ac₄Gal-(1-3)-α-6F-GalNAc)-*O**t*Bu (**3**)^[S2] (306 mg, 0.33 mmol), dissolved in water (0.5 mL) was added TFA (5.0 mL) and the solution was stirred for 6 h at ambient temperature. The mixture was co-evaporated with toluene (4×20 mL) and CH₂Cl₂ (20 mL) and the residue was purified by flash chromatography (SiO₂, EtOAc/MeOH/AcOH, 4:1:0.05) to afford **4** (219 mg, 0.25 mmol, 76%) as a colorless amorphous solid. *R_f* = 0.29 (EtOAc/MeOH/AcOH, 4:1:0.05). *Analytical RP-HPLC* (Luna C18(2), λ = 214 nm, H₂O-MeCN (+0.1% TFA), 70:30, 5 min → 10:90, 25 min → 0:100, 30 min, *t_R* = 20.2 min). *ESI-MS (positive)*, (*m/z*): 899.37 ([M+Na]⁺, calc.: 899.28), 915.34 ([M+K]⁺, calc.: 915.39). *HR-ESI-MS (positive, m/z)* calc. for C₄₁H₄₉FN₂NaO₁₈: 899.2861 ([M+Na]⁺), found: 899.2864.

3,4-Di-*O*-benzyl-2,6-dideoxy-2,6-fluoro-α-D-galactopyranosyl trichloroacetimidate (8)

To a stirred solution of 3,4-Di-*O*-acetyl-6-deoxy-6-fluoro-D-galactal (**7**)^[S2] (2.54 g, 10.94 mmol) in MeOH (50 mL) was added a methanolic solution of NaOMe (54 mg Na in 75 mL MeOH) until a pH value of 10.5 was reached. The solution was stirred for 3 h at ambient temperature. The solvent was removed in vacuo and the residue was co-evaporated with toluene (30 mL), before it was dissolved in anhyd. DMF (40 mL). The solution was

cooled to 0 °C and sodium hydride dispersion in mineral oil (60%, 1.31 g, 32.82 mmol) was carefully added. The mixture was stirred for further 20 min at 0 °C and benzyl bromide (2.86 mL, 24.07 mmol) was slowly added. After stirring for 18 h at ambient temperature, the solution was diluted with toluene (200 mL) and water (100 mL). The organic phase was separated and the aqueous phase was washed with toluene (2×50 mL). The combined organic phases were dried (MgSO₄), filtered and the solvent was removed in vacuo. Purification by flash chromatography (SiO₂, toluene) provided 3,4-Di-*O*-benzyl-6-deoxy-6-fluoro-D-galactal (3.26 g, 9.74 mmol, 89%) as a pale yellow oil. $R_f = 0.25$ (toluene). $[\alpha]_D^{23} = -76.8$ ($c = 1$, CHCl₃). *ESI-MS* (positive), (m/z): 351.17 ([M+Na]⁺, calc.: 351.14). *HR-ESI-MS* (positive, m/z) calc. for C₂₀H₂₁FNao₃: 351.1373 ([M+Na]⁺), found: 351.1367. ¹H-NMR (400 MHz, CDCl₃), δ (ppm): 7.42-7.29 (m, 10H, H_{ar}), 6.38 (dd, 1H, $J_{H1,H2} = 6.2$ Hz, $J_{H1,H3} = 0.9$ Hz, 1-H), 4.96-4.90 (m, 2H, 2-H {4.95}), 6a-H {4.84 (ddd, 1H, $J_{H6a,F} = 89.1$ Hz, $J_{H6a,H6b} = 10.5$ Hz, $J_{H6a,H5} = 8.1$ Hz)}, 4.83 (d, 1H, $J_{CH2,CH2} = 11.8$ Hz, CH₂-Bn), 4.73 (ddd, 1H, $J_{H6b,F} = 89.1$ Hz, $J_{H6b,H6a} = 10.4$ Hz, $J_{H6b,H5} = 3.0$ Hz, 6b-H), 4.71 (d, 1H, $J_{CH2,CH2} = 12.1$ Hz, CH₂-Bn), 4.68 (d, 1H, $J_{CH2,CH2} = 11.7$ Hz, CH₂-Bn), 4.65 (d, 1H, $J_{CH2,CH2} = 11.9$ Hz, CH₂-Bn), 4.47-4.38 (m, 1H, 5-H), 4.13-4.09 (m, 1H, 3-H), 3.97 (dt, 1H, $J_{H4,H5} = 3.9$ Hz, $J_{H4,H3} = 2.0$ Hz, 4-H). ¹³C-NMR (100.6 MHz, CDCl₃, HMQC), δ (ppm): 143.9 (C1), 138.3, 137.8 (C_q-Bn), 128.4, 128.4, 127.9, 127.9, 127.7, 127.6, 127.5 (C_{ar}), 99.5 (C2), 82.3 (CH₂-Bn), 81.5 (d, $J_{C6,F} = 165.5$ Hz, C6), 80.7 (CH₂-Bn), 74.9 (d, $J_{C5,F} = 20.8$ Hz, C5), 74.4 (d, $J_{C4,F} = 7.6$ Hz, C4), 68.1 (C3). ¹⁹F NMR (376.5 MHz, CDCl₃), δ (ppm): -224.8 - -225.8 (m).

To a solution of 3,4-Di-*O*-benzyl-6-deoxy-6-fluoro-D-galactal (1.04 g, 3.17 mmol) in nitromethane and water (4:1, 75 mL) was added Selectfluor™ (1.85 g, 5.22 mmol) and the mixture was stirred 18 h at ambient temperature before it was additionally refluxed for 3 h. After cooling to room temperature, ice-water (75 mL) was added, and the mixture was extracted with EtOAc (3×250 mL). The organic phase was washed with sat. aq. NaHCO₃ (100 mL), dried (MgSO₄), and concentrated in vacuo. Purification by flash chromatography (SiO₂, °Hex/EtOAc, 3:1) provided 3,4-Di-*O*-benzyl-2,6-dideoxy-2,6-fluoro- α/β -D-galactopyranose (1.08 g, 2.96 mmol, 97%) as a colorless oil. $R_f = 0.31$ (°Hex/EtOAc, 3:1). *ESI-MS* (positive), (m/z): 387.16 ([M+Na]⁺, calc.: 387.14). *HR-ESI-MS* (positive, m/z) calc. for C₂₀H₂₂F₂NaO₄: 387.1384 ([M+Na]⁺), found: 387.1395. ¹⁹F NMR (376.5 MHz, CDCl₃), δ (ppm): α -anomer: -204.7 (dd, $J_{F2,H2} = 53.7$ Hz, $J_{F2,H3} = 12.1$ Hz, 2-F), -229.2 - -229.7 (m, 6-F); β -anomer: -206.8 (dd, $J_{F2,H2} = 50.1$ Hz, $J_{F2,H3} = 10.3$ Hz, 2-F), -229.2 - -229.7 (m, 6-F).

To a solution of 3,4-Di-*O*-benzyl-2,6-dideoxy-2,6-fluoro- α/β -D-galactopyranose (340 mg, 0.93 mmol) in anhyd. CH₂Cl₂ (20 mL) at 0 °C were added trichloroacetonitrile (0.4 mL,

3.99 mmol) and 5 drops of DBU. The mixture was stirred for 16 h at ambient temperature and the solvent was removed under reduced pressure. Purification of the residue by flash chromatography (SiO₂, ^cHex/EtOAc, 3:1) afforded **8** (420 mg, 0.83 mmol, 89%) as a colorless oil. R_f = 0.72 (^cHex/EtOAc, 3:1). *ESI-MS (positive)*, (*m/z*): 530.07 ([M+Na]⁺, calc.: 530.05). *HR-ESI-MS (positive)*, (*m/z*) calc. for C₂₂H₂₂Cl₃F₂NNaO₄: 530.0480 ([M+Na]⁺), found: 530.0488. ¹H-NMR (400 MHz, CDCl₃), δ (ppm): 8.68 (s, 1H, NH), 7.42-7.29 (m, 10H, H_{ar}), 6.59 (d, 1H, J_{H1,H2} = 3.7 Hz, 1-H), 5.18 (ddd, 1H, J_{H2,F} = 49.2 Hz, J_{H2,H3} = 9.9 Hz, J_{H2,H1} = 3.7 Hz, 2-H), 5.00 (d, 1H, J_{CH2,CH2} = 11.3 Hz, CH₂-Bn), 4.87 (d, 1H, J_{CH2,CH2} = 11.9 Hz, CH₂-Bn), 4.75 (d, 1H, J_{CH2,CH2} = 11.9 Hz, CH₂-Bn), 4.64 (d, 1H, J_{CH2,CH2} = 11.3 Hz, CH₂-Bn), 4.49 (ddd, 1H, J_{H6a,F} = 45.4 Hz, J_{H6a,H6b} = 10.3 Hz, J_{H6a,H5} = 6.4 Hz, 6a-H), 4.38 (ddd, 1H, J_{H6b,F} = 46.0 Hz, J_{H6b,H6a} = 8.4 Hz, J_{H6b,H5} = 6.3 Hz, 6b-H), 4.24-4.17 (m, 1H, 3-H), 4.13 (dt, 1H, J_{H5,H6a/b} = 9.9 Hz, J_{H5,H4} = 2.9 Hz, 5-H), 4.03 (pt, 1H, J_{H4,H5} = 3.5 Hz, 4-H). ¹³C-NMR (100.6 MHz, CDCl₃, HMQC), δ (ppm): 160.7 (C=NH(CCl₃)), 137.7, 137.6 (C_q-Bn), 129.0, 128.5, 128.4, 128.3, 128.2, 128.0, 127.9, 127.9 (C_{ar}), 93.8 (d, J_{C1,F2} = 23.4 Hz, C1), 91.0 (CCl₃), 88.3 (d, J_{C2,F2} = 189.7 Hz, C2), 81.1 (d, J_{C6,F6} = 168.0 Hz, C6), 75.9 (d, J_{C5,F6} = 16.1 Hz, C5), 75.0 (CH₂-Bn), 74.4 (dd, J_{C4,F2} = 8.7 Hz, J_{C4,F6} = 4.4 Hz, C4), 73.2 (CH₂-Bn), 71.6 (d, J_{C3,F2} = 24.8 Hz, C4) ¹⁹F-NMR (376.5 MHz, CDCl₃), δ (ppm): -208.9 (ddd, J_{F2,H2} = 49.2 Hz, J_{F2,H3} = 9.7 Hz, J_{F2,H1} = 3.2 Hz, 2-F), -230.4 (dt, J_{F6,H6a/b} = 46.5 Hz, J_{F6,H5} = 10.8 Hz, 6-F).

***N*-(9H-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-2,6-dideoxy-6-fluoro-3-*O*-[3,4-di-*O*-benzyl-2,6-dideoxy-2,6-fluoro-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine *tert*-butyl ester (**9**)**

Glycosyl acceptor **6**^[S1] (390 mg, 0.65 mmol) and donor **8** (420 mg, 0.83 mmol) were dissolved in anhyd. CH₂Cl₂ (25 mL) and stirred with activated, powdered molecular sieves (4 Å, 150 mg) for 30 min at ambient temperature under an argon atmosphere. The suspension was cooled to -10 °C, and TMSOTf (20 μL, 0.11 mmol) was added. The reaction mixture was slowly warmed to room temperature over 20 h, before it was neutralized with solid NaHCO₃ and filtered through Hyflo Super Cel[®]. The organic phase was concentrated in vacuo and purified by flash chromatography on silica gel (^cHex/EtOAc, 2:1) to afford **9** as a colorless amorphous solid (354 mg, 0.37 mmol, 57%, α/β = 1:2).

β-9: R_f = 0.39 (^cHex/EtOAc, 1:1); *Analytical RP-HPLC* (PerfectSil C18(2), λ = 214 nm, H₂O-MeCN, 50:50 → 25:75, 30 min → 0:100, 5 min, t_R = 26.1 min); [α]_D²³ = +63.2 (*c* = 1, CHCl₃); *ESI-MS (positive)*, (*m/z*): 971.42 ([M+Na]⁺, calc.: 971.39); 1919.79 ([2M+Na]⁺,

calc.: 1919.79). *HR-ESI-MS* (positive, m/z) calc. for $C_{51}H_{59}F_3N_2NaO_{12}$: 971.3918 ($[M+Na]^+$), found: 971.3959. 1H -NMR (400 MHz, $CDCl_3$), δ (ppm): 7.78 (d, 2H, $J_{H_4,H_3} = J_{H_5,H_6} = 7.3$ Hz, 4-H-, 5-H-Fmoc), 7.63 (m, 2H, 1-H-, 8-H-Fmoc), 7.44-7.39 (m, 2H, 3-H-, 6-H-Fmoc), 7.38-7.24 (m, 12H, 2-H-, 7-H-Fmoc, H_{ar} -Bn), 5.81 (d, 1H, $J_{NH,H_2} = 9.6$ Hz, NHAc), 5.42 (d, 1H, $J_{NH,T\alpha} = 9.4$ Hz, NH-urethane), 4.93 (d, 2H, $J_{CH_2,CH_2} = 11.4$ Hz, CH_2 -Bn), 4.86 (d, 1H, $J_{H_1,H_2} = 3.3$ Hz, 1-H), 4.84-4.63 (m, 5H, CH_2 -Bn {4.82, d, $J_{CH_2,CH_2} = 12.0$ Hz}, 2'-H {4.76}, CH_2 -Bn {4.68, d, $J_{CH_2,CH_2} = 12.0$ Hz}, 6a,b-H {4.64}, 2-H {4.63}), 4.63-4.37 (m, 5H, CH_2 -Bn {4.59, d, $J_{CH_2,CH_2} = 11.4$ Hz}, CH_2 -Fmoc {4.50, d, $J_{CH_2,CH} = 6.6$ Hz}, 1'-H {4.55}, 6a'-H {4.49, ddd, $J_{H_{6a'},H_5} = 6.9$ Hz, $J_{H_{6a'},H_{6b'}} = 9.5$ Hz, $J_{H_{6a'},F_6'} = 47.4$ Hz}), 4.37-4.13 (m, 4H, 6b'-H {4.31, ddd, $J_{H_{6b'},H_5} = 5.1$ Hz, $J_{H_{6b'},H_{6a'}} = 9.5$ Hz, $J_{H_{6b'},F_6'} = 47.4$ Hz}, CH-Fmoc {4.29}, T^α {4.22, d, $J_{T\alpha,T\beta} = 9.4$ Hz}, T^β {4.18}), 4.14-4.00 (m, 2H, 4-H, 5-H), 3.85-3.79 (m, 1H, 4'-H), 3.72-3.62 (m, 2H, 3-H {3.71}, 5'-H {3.68}), 3.62-3.53 (m, 3'-H), 2.60 (bs, OH), 2.01 (s, 3H, CH_3 -NHAc), 1.47 (s, 9H, CH_3 -*t*Bu), 1.30 (d, 3H, $J_{T\gamma,T\beta} = 6.2$ Hz, T^γ). ^{13}C -NMR (100.6 MHz, $CDCl_3$, HMQC), δ (ppm): 170.4 (C=O-NHAc), 170.2 (C=O-ester), 156.4 (C=O-urethane), 143.8, 143.6 (C_{1a} -, C_{8a} -Fmoc), 141.3, 141.3 (C_{4a} -, C_{5a} -Fmoc), 137.6, 137.5 (C_q -Bn), 128.5, 128.4, 128.4, 127.9, 127.8, 127.6, 127.1 (C_{ar} -Bn, C2-, C3-, C6-, C7-Fmoc), 125.0, 125.0 (C_{1-} , C8-Fmoc), 120.0 (C_{4-} , C_{5-} -Fmoc), 102.5 (d, $J_{C_1',F_2'} = 24.5$ Hz, C_1'), 100.4 (C_1), 91.2 (d, $J_{C_2',F_2'} = 183.5$ Hz, C_2'), 83.2 (d, $J_{C_6,F_6} = 167.8$ Hz, C6), 83.1 (C_q -*t*Bu), 81.8 (d, $J_{C_6',F_6'} = 169.3$ Hz, C_6'), 79.9 (C3), 79.6 (d, $J_{C_3',F_2'} = 16.2$ Hz, C_3'), 77.2 (T^β), 74.7 (CH_2 -Bn), 73.6-73.2 (m, C_4' , C_5'), 73.0 (CH_2 -Bn), 69.2 (d, $J_{C_5,F_6} = 20.7$ Hz, C5), 68.2 (d, $J_{C_4,F_6} = 6.5$ Hz, C4), 67.0 (CH_2 -Fmoc), 59.2 (T^α), 47.4 (C2), 47.2 (CH-Fmoc), 28.1 (CH_3 -*t*Bu), 23.3 (CH_3 -NHAc), 18.8 (T^γ). ^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm): -204.7 (dd, $J_{F_2',H_3} = 12.1$ Hz, $J_{F_2',H_2} = 50.9$ Hz, 2'-F), -228.9 (td, $J_{F_6',H_5'} = 15.1$ Hz, $J_{F_6',H_6} = 47.4$ Hz, 6'-F), -228.9 (td, $J_{F_6',H_5'} = 11.7$ Hz, $J_{F_6',H_6} = 46.6$ Hz, 6-F).

***N*-(9H-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-2,6-dideoxy-6-fluoro-3-*O*-[3,4-di-*O*-benzyl-2,6-dideoxy-2,6-fluoro- β -D-galactopyranosyl]- α -D-galactopyranosyl)-L-threonine (10)**

To ester **9** (186 mg, 0.20 mmol), dissolved in water (0.7 mL), was added TFA (7.0 mL), and the solution was stirred for 3 h at ambient temperature. The mixture was co-evaporated with toluene (4×30 mL) and CH_2Cl_2 (30 mL), and the residue was purified by flash chromatography (SiO_2 , EtOAc \rightarrow EtOAc/MeOH/AcOH 10:1:0.01) to afford **10** (144 mg, 0.16 mmol, 80%) as a colorless amorphous solid. $R_f = 0.28$ (EtOAc/MeOH/AcOH, 10:1:0.01). *ESI-MS* (positive), (m/z): 915.3 ($[M+Na]^+$, calc.: 915.3).

***N*-(9*H*-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-2-deoxy-4,6-*O*-benzyliden-3-*O*-[3,4-tri-*O*-benzyl-2,6-dideoxy-2,6-difluoro- β -D-galactopyranosyl]- α -D-galactopyranosyl)-*L*-threonine *tert*-butyl ester (**11**)**

Glycosyl acceptor **5**^[S3] (378 mg, 0.55 mmol) and donor **8** (355 mg, 0.70 mmol) were dissolved in anhyd. CH₂Cl₂ (20 mL) and stirred with activated, powdered molecular sieves (4 Å, 200 mg) for 1 h at ambient temperature under an argon atmosphere. The suspension was cooled to 0 °C, and TMSOTf (15 μ L, 0.08 mmol) was added. The reaction mixture was slowly warmed to room temperature over 20 h, before it was neutralized with solid NaHCO₃ and filtered through Hyflo Super Cel[®]. The organic phase was concentrated in vacuo and purified by flash chromatography on silica gel (^cHex/EtOAc, 1:1) to afford **11** as a colorless amorphous solid (412 mg, 0.40 mmol, 72%, α/β = 1:3).

β -11: R_f = 0.43 (^cHex/EtOAc, 1:1); *Analytical RP-HPLC* (Jupiter C18(2), λ = 214 nm, H₂O-MeOH, 30:70 \rightarrow 0:100, 30 min, t_R = 17.9 min); $[\alpha]_D^{23}$ = 67.5 (c = 1, CHCl₃); *ESI-MS* (*positive*), (m/z): 1057.41 ([M+Na]⁺, calc.: 1057.43). *HR-ESI-MS* (*positive*, m/z) calc. for C₅₈H₆₄F₂N₂NaO₁₃: 1057.4274 ([M+Na]⁺), found: 1057.4304. ¹H-NMR (400 MHz, CDCl₃), δ (*ppm*): 7.78 (d, 2H, J_{H_4,H_3} = J_{H_5,H_6} = 6.9 Hz, 4-H-, 5-H-Fmoc), 7.64 (d, 2H, J_{H_1,H_2} = J_{H_8,H_7} = 7.5 Hz, 1-H-, 8-H-Fmoc), 7.54 (d, 2H, J_{H_3,H_4} = J_{H_6,H_5} = 6.6 Hz, 3-H-, 6-H-Fmoc), 7.44-7.22 (m, 17H, 2-H-, 7-H-Fmoc, H_{ar}), 5.77 (d, 1H, J_{NH,H_2} = 9.3 Hz, NHAc), 5.60-5.51 (m, 2H, NH-Fmoc, CH-Bzn), 5.02 (d, 1H, J_{H_1,H_2} = 3.3 Hz, 1-H), 4.96-4.56 (m, 6H, CH₂-Bn {4.92, d, J_{CH_2,CH_2} = 11.5 Hz}, CH₂-Bn {4.82, d, J_{CH_2,CH_2} = 12.2 Hz}, CH₂-Bn {4.67, d, J_{CH_2,CH_2} = 12.3 Hz}, CH₂-Bn {4.59, d, J_{CH_2,CH_2} = 11.5 Hz}, 2'-H {4.85, 4.73}, 1'-H {4.63}), 4.64-4.52 (m, 1H, 6'a-H), 4.52-4.25 (m, 1H, 6'b-H), 4.56-4.44 (m, 2H, CH₂-Fmoc), 4.40-4.18 (m, 5H, 4-H {4.36}, CH-Fmoc {4.27}, T ^{β} {4.24}, T ^{α} {4.22}, 6a-H {4.21}), 4.08-3.98 (m, 1H, 6b-H), 3.90 (dd, 1H, J_{H_3,H_2} = 11.3 Hz, J_{H_3,H_4} = 2.5 Hz, 3-H), 3.82 (s, 1H, 4'-H), 3.74-3.67 (m, 2H, 5-H {3.70}, 5'-H {3.69}), 3.61 (t, 1H, $J_{H_3',H_2'}$ = 10.7 Hz, 3'-H), 2.01 (s, 3H, CH₃-NHAc), 1.46 (s, 9H, CH₃-*t*Bu), 1.28 (d, 3H, $J_{T\gamma,T\beta}$ = 6.3 Hz, T ^{γ}). ¹³C-NMR (100.6 MHz, CDCl₃, HMQC), δ (*ppm*): 170.3, 170.1 (C=O-ester, -NHAc), 156.5 (C=O-urethane), 143.8, 143.7 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 137.8, 137.6 (C_q-Bn), 128.7, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.6, 127.1, 126.4 (C_{ar}-Bn, C2-, C3-, C6-, C7-Fmoc), 125.2, 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C5-Fmoc), 102.3 (d, J_{C_1',F_2} = 24.2 Hz, C1'), 100.8 (CH-Bzn), 100.0 (C1), 90.9 (d, J_{C_2',F_2} = 183.7 Hz, C2'), 83.1 (C_q-*t*Bu), 82.2 (d, J_{C_6',F_6} = 167.9 Hz, C6'), 79.5 (d, J_{C_3',F_2} = 16.0 Hz, C3'), 76.5 (C3, T ^{β}), 75.8 (C4), 74.4 (CH₂-Bn), 73.4 (C4'), 73.3 (d, J_{C_5',F_6} = 22.1 Hz, C5'), 72.9 (CH₂-Bn), 69.1 (C6), 67.0 (CH₂-Fmoc), 63.8 (C5), 59.2 (T ^{α}), 48.0 (C2), 47.2 (CH-Fmoc), 28.1 (CH₃-*t*Bu), 23.4 (CH₃-NHAc), 19.1 (T ^{γ}). ¹⁹F NMR (376.5 MHz,

$CDCl_3$), δ (ppm): -203.6 (d, $J_{F_2,H_2} = 49.4$ Hz, 2-F), -228.7 (dt, $J_{F_6,H_{6a/b}} = 46.4$ Hz, $J_{F_6,H_5} = 10.8$ Hz, 6-F).

***N*-(9H-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[3,4-tri-*O*-benzyl-2,6-dideoxy-2,6-difluoro- β -D-galactopyranosyl]- α -D-galactopyranosyl)-L-threonine *tert*-butyl ester (12)**

To a solution of disaccharide **11** (414 mg, 0.40 mmol) in a mixture of CH_2Cl_2 and MeOH (4:1, 50 mL) were added $NaHSO_4-SiO_2$ ^[S4] (450 mg), and the suspension was stirred for 18 h at ambient temperature. The suspension was diluted with CH_2Cl_2 (150 mL) and the catalyst was filtered off. The filtrate was washed with sat. aq. $NaHCO_3$ (2×50 mL), and brine (2×50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo. Flash chromatography (SiO_2 , $^{\circ}Hex/EtOAc$, 1:10) afforded Fmoc-Thr-(β -2,6F-Bn₂Gal-(1-3)- α -GalNAc)-*O**t*Bu (288 mg, 0.30 mmol, 76%) as a colorless amorphous solid. $R_f = 0.24$ ($^{\circ}Hex/EtOAc$, 1:10). *Analytical RP-HPLC* (Jupiter C18(2), $\lambda = 214$ nm, H_2O -MeOH, 30:70 \rightarrow 0:100, 30 min, $t_R = 14.3$ min). $[\alpha]_D^{23} = +42.8$ ($c = 1$, $CHCl_3$). *ESI-MS* (positive), (m/z): 947.45 ($[M+H]^+$, calc.: 947.41); 969.44 ($[M+Na]^+$, calc.: 969.40). *HR-ESI-MS* (positive, m/z) calc. for $C_{51}H_{61}F_2N_2O_{13}$: 947.4142 ($[M+H]^+$), found: 947.4120; calc. for $C_{51}H_{60}F_2N_2NaO_{13}$: 969.3961 ($[M+Na]^+$), found: 969.3967. *¹H-NMR* (400 MHz, $CDCl_3$, COSY, HSQC), δ (ppm): 7.78 (d, 2H, $J_{H_4,H_3} = J_{H_5,H_6} = 7.4$ Hz, 4-H-, 5-H-Fmoc), 7.63 (d, 2H, $J_{H_{11},H_2} = J_{H_8,H_7} = 7.0$ Hz, 1-H-, 8-H-Fmoc), 7.41 (t, 2H, $J_{H_2,H_1} = J_{H_7,H_8} = 6.0$ Hz, 2-H-, 7-H-Fmoc), 7.38-7.24 (m, 12H, H_{ar}), 5.80 (d, 1H, $J_{NH,H_2} = 9.7$ Hz, NHAc), 5.45 (d, 1H, $J_{NH,T\alpha} = 9.6$ Hz, NH-Fmoc), 4.93 (d, 1H, $J_{CH_2,CH_2} = 11.6$ Hz, CH_2 -Bn), 4.90 (d, 1H, $J_{H_1,H_2} = 3.4$ Hz, 1-H), 4.83-4.70 (m, 1H, 2'-H), 4.81 (d, 1H, $J_{CH_2,CH_2} = 12.1$ Hz, CH_2 -Bn), 4.68 (d, 1H, $J_{CH_2,CH_2} = 12.0$ Hz, CH_2 -Bn), 4.64 (dd, 1H, $J_{H_2,H_3} = 10.6$ Hz, $J_{H_2,H_1} = 3.5$ Hz, 2-H), 4.58 (d, 1H, $J_{CH_2,CH_2} = 11.4$ Hz, CH_2 -Bn), 4.56-4.52 (m, 1H, 1'-H), 4.49 (d, 2H, $J_{CH_2,CH} = 6.6$ Hz, CH_2 -Fmoc), 4.44 (ddd, 1H, $J_{H_6'a,F_6} = 74.4$ Hz, $J_{H_6'a,H_6'b} = 9.5$ Hz, $J_{H_6'a,H_5'} = 5.1$ Hz, 6'a-H), 4.32 (ddd, 1H, $J_{H_6'b,F_6} = 69.8$ Hz, $J_{H_6'b,H_6'a} = 9.6$ Hz, $J_{H_6'b,H_5'} = 6.9$ Hz, 6'b-H), 4.31-4.08 (m, 3H, CH-Fmoc {4.27}, T^{α} {4.23}, T^{β} {4.19}), 3.97-3.90 (m, 1H, 6a-H), 3.88-3.75 (m, 2H, 4'-H {3.85}, 5'-H {3.82}), 3.73-3.62 (m, 2H, 3-H {3.71}, 5-H {3.66}), 3.58 (pt, 1H, 3'-H), 2.81 (bs, 1H, OH), 2.50 (bs, 1H, OH), 2.01 (s, 3H, CH_3 -NHAc), 1.46 (s, 9H, CH_3 -*t*Bu), 1.29 (d, 3H, $J_{T\gamma,T\beta} = 6.3$ Hz, T^{γ}). *¹³C-NMR* (100.6 MHz, $CDCl_3$, HMQC), δ (ppm): 170.4, 170.1 (C=O-NHAc, -ester), 156.4 (C=O-urethane), 143.8, 143.6 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 137.6, 137.5 (C_q -Bn), 128.5, 128.4, 128.0, 127.9, 127.9, 127.6, 127.1 (C_{ar} -Bn, C2-, C3-, C6-, C7-Fmoc), 125.0, 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C5-Fmoc), 102.5 (d, $J_{C1',F_2} = 24.3$ Hz, C1'), 100.3 (C1), 91.2 (d, $J_{C2',F_2} = 184.1$

Hz, C2'), 83.1 (C_q-*t*Bu), 81.7 (d, $J_{C6',F6} = 168.9$ Hz, C6'), 80.0 (C3), 79.6 (d, $J_{C3',F2} = 15.5$ Hz, C3'), 76.6 (T^β), 74.7 (CH₂-Bn), 73.4 (C5), 73.3 (d, $J_{C5',F6} = 22.0$ Hz, C5'), 73.0 (CH₂-Bn), 69.8 (C4, C4'), 67.0 (CH₂-Fmoc), 63.0 (C6), 59.1 (T^α), 47.4 (C2), 47.2 (CH-Fmoc), 28.1 (CH₃-*t*Bu), 23.3 (CH₃-NHAc), 18.9 (T^γ). ¹⁹F NMR (376.5 MHz, CDCl₃), δ (ppm): -204.7 (dd, $J_{F2,H2} = 51.2$ Hz, $J_{F2,H3} = 10.6$ Hz, 2-F), -229.4 (dt, $J_{F6,H6a} = 46.8$ Hz, $J_{F6,H6b} = 46.5$ Hz, $J_{F6,H5} = 11.0$ Hz, 6-F).

To a solution of Fmoc-Thr-(β-2,6F-Bn₂Gal-(1-3)-α-GalNAc)-O*t*Bu (234 mg, 0.25 mmol) in pyridine (8 mL) was added Ac₂O (4 mL), and the solution was stirred at ambient temperature for 20 h. The reaction mixture was poured onto ice-water, and the solution was extracted with CH₂Cl₂ (5×60 mL). The combined organic phases were washed with sat. aq. NaHCO₃ (75 mL) and brine (75 mL), dried (MgSO₄), filtered and concentrated in vacuo. Purification by flash chromatography (SiO₂, ^cHex/EtOAc, 1:2) afforded **12** (165 mg, 0.16 mmol, 65%) as a colorless amorphous solid. $R_f = 0.61$ (^cHex/EtOAc, 1:2). Analytical RP-HPLC (Jupiter C18(2), λ = 214 nm, H₂O-MeOH, 30:70 → 0:100, 40 min, $t_R = 16.6$ min). $[\alpha]_D^{23} = +67.1$ (c = 1, CHCl₃). ESI-MS (positive), (m/z): calc. for C₅₅H₆₄F₂N₂NaO₁₅: 1053.4173 ([M+Na]⁺), found: 1053.4207. ¹H-NMR (400 MHz, CDCl₃, COSY, HSQC), δ (ppm): 7.78 (d, 2H, $J_{H4,H3} = J_{H5,H6} = 7.4$ Hz, 4-H-, 5-H-Fmoc), 7.64 (d, 2H, $J_{H1,H2} = J_{H8,H7} = 7.5$ Hz, 1-H-, 8-H-Fmoc), 7.41 (t, 2H, $J_{H3,H4} = J_{H6,H5} = 7.3$ Hz, 3-H-, 6-H-Fmoc), 7.37–7.24 (m, 12H, 2-H-, 7-H-Fmoc, H_{ar}), 5.80 (d, 1H, $J_{NH,H2} = 9.6$ Hz, NHAc), 5.46 (d, 1H, $J_{NH,T\alpha} = 9.5$ Hz, NH-Fmoc), 5.43 (s, 1H, 4-H), 4.93–4.88 (m, 2H, CH₂-Bn {4.91, d, $J_{CH2,CH2} = 11.4$ Hz}, 1-H {4.90}), 4.77 (d, 2H, $J_{CH2,CH2} = 11.8$ Hz, CH₂-Bn), 4.78–4.61 (m, 1H, 2'-H), 4.69–4.36 (m, 6H, CH₂-Bn {4.67, d, $J_{CH2,CH2} = 12.2$ Hz}, CH₂-Bn {4.58, d, $J_{CH2,CH2} = 11.5$ Hz}, 6'a-H {4.56, 4.40}, 1'-H {4.51}, CH₂-Fmoc {4.49}), 4.47–4.25 (m, 1H, 6'b-H), 4.30–4.09 (m, 5H, CH-Fmoc {4.27}, T^α {4.23}, 6a-H {4.21}, T^β {4.20}, 5-H {4.13}), 3.95 (dd, 1H, $J_{H6b,H6a} = 11.5$ Hz, $J_{H6b,H5} = 7.8$ Hz, 6b-H), 3.84 (d, 1H, $J_{H4',H3'} = 2.9$ Hz, 4'-H), 3.81 (d, 1H, $J_{H3,H4} = 2.9$ Hz, 3-H), 2.08, 2.04 (2s, 6H, CH₃-Ac), 2.01 (s, 3H, CH₃-NHAc), 1.46 (s, 9H, CH₃-*t*Bu), 1.30 (d, 3H, $J_{T\gamma,T\beta} = 6.3$ Hz, T^γ). ¹³C-NMR (100.6 MHz, CDCl₃, HMQC), δ (ppm): 170.4, 170.1 (C=O-Ac, -ester, -NHAc), 156.4 (C=O-urethane), 143.7, 143.6 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 137.9, 137.7 (C_q-Bn), 128.5, 128.3, 128.0, 127.8, 127.8, 127.7, 127.6, 127.1 (C_{ar}-Bn, C3-, C6-Fmoc, C2-, C7-Fmoc), 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C5-Fmoc), 101.8 (d, $J_{C1',F2} = 24.0$ Hz, C1'), 100.2 (C1), 91.3 (d, $J_{C2',F2} = 183.0$ Hz, C2'), 83.1 (C_q-*t*Bu), 81.1 (d, $J_{C6',F6} = 167.5$ Hz, C6'), 79.5 (d, $J_{C3',F2} = 15.9$ Hz, C3'), 77.0 (T^β), 76.1 (C3), 74.5 (CH₂-Bn), 73.3 (dd, $J_{C4',F6} = 9.1$ Hz, $J_{C4',F6} = 3.5$ Hz, C4'), 72.9 (d, $J_{C5',F6} = 25.0$ Hz, C5'), 72.7 (CH₂-Bn), 69.2 (C4), 68.1 (C5), 67.1 (CH₂-Fmoc), 63.2 (C6), 59.1 (T^α), 48.3 (C2), 47.2 (CH-Fmoc), 28.1 (CH₃-

*t*Bu), 23.3 (CH₃-NHAc), 20.8, 20.7 (CH₃-Ac), 18.6 (T^γ). ¹⁹F NMR (376.5 MHz, CDCl₃), δ (ppm): -204.7 (d, *J*_{F₂,H₂} = 51.5 Hz, *J*_{F₂,H₃} = 10.8 Hz, 2-F), -229.9 (dt, *J*_{F₆,H_{6a/b}} = 46.6 Hz, *J*_{F₆,H₅} = 9.9 Hz, 6-F).

***N*-(9H-Fluoren-9-yl)-methoxycarbonyl-*O*-(2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[3,4-tri-*O*-benzyl-2,6-dideoxy-2,6-difluoro-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonine (13)**

To ester **12** (184 mg, 0.18 mmol), dissolved in anisole (0.7 mL), was added TFA (7.0 mL), and the solution was stirred for 3 h at ambient temperature. The mixture was co-evaporated with toluene (4×30 mL) and CH₂Cl₂ (30 mL), and the residue was purified by flash chromatography (SiO₂, CH₂Cl₂/MeOH 5:0.3) to afford **13** (154 mg, 0.16 mmol, 88%) as a colorless amorphous solid. *R*_f = 0.63 (CH₂Cl₂/MeOH 5:0.3). Analytical RP-HPLC (Luna C18(2), λ = 214 nm, H₂O-MeCN (+0.1% TFA), 50:50 → 10:90, 30 min → 0:100, 10 min, *t*_R = 18.5 min). ESI-MS (positive), (*m/z*): 975.39 ([M+H]⁺, calc.: 975.37), 997.37 ([M+Na]⁺, calc.: 997.35). HR-ESI-MS (positive, *m/z*) calc. for C₅₁H₅₆F₂N₂NaO₁₅: 997.3547 ([M+Na]⁺), found : 997.3524.

General protocol for automated solid-phase glycopeptide synthesis

All syntheses were carried out in an Applied Biosystems ABI 433A peptide synthesizer (standard program Fastmoc 0.1 mmol) using pre-loaded Fmoc-Pro-Trt-Tentagel S resins (455 mg, 0.10 mmol; loading: 0.22 mmol/g). For the coupling reactions, the amino acids Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Asp-OH, Fmoc-Gly-OH, Fmoc-His(Trt)-OH, Fmoc-Pro-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-Thr(*t*Bu)-OH, and Fmoc-Val-OH were employed. In every coupling cycle, the *N*-terminal Fmoc group was removed by treatment of the resin with a solution of piperidine (20%) in NMP for at least 3×2.5 min. The couplings of the amino acids (1 mmol or 10 equiv. based on the loaded resin) were carried out with HBTU (1 mmol), HOBt (1 mmol) and DIPEA (2 mmol) in DMF (20-30 min vortex). After every coupling step, unreacted amino groups were capped by treatment with a mixture of Ac₂O (0.5 M), DIPEA (0.125 M), and HOBt (0.015 M) in NMP (10 min vortex). Couplings of the fluorinated building blocks were performed using HATU, HOAt (both in 1.2 equiv. with respect to the glycosyl amino acid) and NMM (2.4 equiv.) for activation (8 h vortex). Subsequent couplings of the remaining amino acids were again conducted according to the standard procedure. At the end of each synthesis, a triethylene glycol spacer (1 mmol, 10 equiv. based on the loaded resin) was coupled using HBTU (1 mmol), HOBt (1 mmol) and

DIPEA (2 mmol) in DMF (20-30 min vortex). The *N*-terminal Fmoc groups were then removed by piperidine (20 %) in NMP and the glycopeptides were detached from the resins with simultaneous removal of all side chain protecting groups by shaking with TFA (15 mL), TIS (0.8 mL) and H₂O (0.8 mL) in a Merrifield glass reactor for 2 h. The resulting solutions were filtered, the resins were washed with 5 mL of TFA and the combined solutions were concentrated in vacuo to a volume of 1 mL. After co-evaporation with toluene (3×10 mL), the crude products were dissolved in H₂O and subjected to lyophilization.

Amino-4,7,10-trioxadodecanylamido-*N*-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-*O*-(2-acetamido-2,6-dideoxy-6-fluoro-3-*O*-[2-deoxy-2-fluoro-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-L-threonyl-L-alanyl-L-proline (14)

The synthesis was performed according to the general protocol with Fmoc-Thr-(β-2F-Bn₃Gal-(1-3)-α-6F-GalNAc)-OH (**2**) (173 mg, 0.18 mmol) to provide the crude partially protected glycopeptide (252 mg, 0.09 mmol, 90%) as a slightly yellow amorphous solid. *ESI-MS* (*positive*), (*m/z*): 910.83 ([M+3H]³⁺, calc.: 910.44), 918.15 ([M+Na+2H]³⁺, calc.: 917.77), 925.16 ([M+2Na+H]³⁺, calc.: 925.10), 932.48 ([M+3Na]³⁺, calc.: 932.43), 1365.73 ([M+2H]²⁺, calc.: 1365.16), 1376.71 ([M+Na+H]²⁺, calc.: 1376.15), 1387.72 ([M+2Na]²⁺, calc.: 1387.14).

For complete deprotection, the crude peptide (252 mg, 0.09 mmol) was dissolved in MeOH (50 mL, HPLC-grade) and subjected to 6 cycles of vacuum/Ar flush. After addition of Pd(OAc)₂ (15 mg, 0.07 mmol), the argon atmosphere was replaced by hydrogen and the reaction mixture was stirred for 2 d at ambient temperature. The catalyst was removed by filtration through Hyflo Super Cel[®] and the filtrate was concentrated in vacuo. Purification by semi-preparative RP-HPLC provided glycopeptide **14** (46 mg, 0.02 mmol, 22%) as a colorless amorphous solid after lyophilization. *Analytical RP-HPLC* (Luna C18(2), λ = 214 nm, H₂O-MeCN (+0.1% TFA) 95:5 → 90:10, 10 min → 89:11, 10 min → 87.5:12.5, 10 min, *t_R* = 24.7 min). [α]_D²³ = -88.5 (c = 1, H₂O). *ESI-MS* (*positive*), (*m/z*): 820.75 ([M+3H]³⁺, calc.: 820.40), 1230.11 ([M+2H]²⁺, calc.: 1230.09). *HR-ESI-MS* (*positive*, *m/z*) calc. for C₁₀₃H₁₆₇F₂N₂₇O₄₀: 1230.0916 ([M+2H]⁺), found: 1230.0884. *MALDI-TOF-MS* (*DHB*, *positive*), *m/z*: 2460.03 ([M+H]⁺, calc.: 2459.18). ¹*H-NMR* (400 MHz, D₂O, COSY, TOCSY, NOESY), δ (ppm): 8.62 (d, 1H, *J*_{He,Hδ} = 1.3 Hz, H^ε), 7.32 (s, 1H, H^δ), 5.04 (d, 1H, *J*_{H1,H2} = 3.7 Hz, 1-H), 4.75-4.50 (m, 11H, D^α {4.74}, 1'-H {4.72}, H^α {4.70}, 6a,b-H {4.70, 4.58}, T^{α*} {4.68}, R^α {4.65}, A_(3,4)^α {4.61, q, *J*_{A3,4α,A3,4β} = 7.1 Hz}, S₁^α {4.53, t, *J*_{S1α,S1β} = 5.4 Hz}, A₄^α {4.56}), 4.49-4.18

(m, 15H, S_2^α {4.47}, $P_{(1-5)}^\alpha$ {4.44, 4.40, 4.38, 4.36}, T_1^α {4.36}, $T^{\beta*}$ {4.36}, V^α {4.32}, T_2^α {4.31}, A_1^α {4.26}, 2-H {4.24}, $T_{(1,2)}^\beta$ {4.22, 4.20}, 4'-H {4.21}), 4.38-4.22 (m, 1H, 2'-H), 4.08-3.55 (m, 37H, 3-H {4.06}, G_1^α {3.99}, 4-H {3.97}, G_2^α {3.96}, 3'-H {3.90}, S_1^β {3.88}, 5-H {3.87}, $P_{(1-5)}^\delta$ {3.84, 3.81, 3.68, 3.63, 3.59}, S_2^β {3.78}, 3a,b-H-, 5a,b-H-, 6a,b-H-, 8a,b-H-, 9a,b-H-, 11a,b-H-spacer {3.78, 3.75, 3.72, 3.70, 3.67}, 6'a,b-H {3.75}, 5'-H {3.69}), 3.38-3.31 (m, 1H, $H^{\beta a}$), 3.31-3.14 (m, 5H, R^δ {3.21}, 12a,b-H-spacer {3.20}, $H^{\beta b}$ {3.19}), 3.02-2.85 (m, 2H, $D^{\beta a}$ {2.97}, $D^{\beta b}$ {2.91}), 2.81-2.61 (m, 2H, 2a,b-H-spacer), 2.39-2.20 (m, 4H, $P_{(1,2)}^\beta$ {2.32, 2.28}), 2.12-1.80 (m, 21H, V^β {2.09}, $P_{(1-5)}^\gamma$ {2.08, 2.05, 2.02, 1.98}, $P_{(3-5)}^\beta$ {2.04, 1.94, 1.88}, CH_3-NHAc {1.99}, $R^{\beta a}$ {1.83}), 1.77-1.69 (m, 3H, $R^{\beta b}$ {1.72}, R^γ {1.67}), 1.37 (d, 3H, $J_{A_4\beta, A_4\alpha} = 6.9$ Hz, A_4^β), 1.37 (d, 6H, $J_{A_2,3\beta, A_2,3\alpha} = 7.1$ Hz, $A_{2,3}^\beta$), 1.33 (d, 3H, $J_{A_1\beta, A_1\alpha} = 7.2$ Hz, A_1^β), 1.26 (d, 3H, $J_{T\gamma^*, T\beta^*} = 6.2$ Hz, T^{γ^*}), 1.20 (d, 3H, $J_{T_1\gamma, T_1\beta} = 6.5$ Hz, T_1^γ), 1.18 (d, 3H, $J_{T_2\gamma, T_2\beta} = 6.5$ Hz, T_2^γ), 0.98 (d, 3H, $J_{V\gamma^a, V\beta} = 5.7$ Hz, V^{γ^a}), 0.96 (d, 3H, $J_{V\gamma^b, V\beta} = 6.4$ Hz, V^{γ^b}). $^{13}C-NMR$ (100.6 MHz, D_2O , DEPT, HMQC), δ (ppm): 175.8, 174.9, 174.3, 174.0, 173.7, 173.5, 173.1, 172.6, 172.5, 171.9, 171.8, 171.5, 171.3, 171.3, 171.2, 171.1, 170.9, 170.7 (C=O), 156.7 (C=NH), 133.4 (H^ϵ), 128.4 (H^γ), 117.3 (H^δ), 102.1 (d, $J_{C_1', F_2} = 21.0$ Hz, C_1'), 99.2 (C_1), 90.9 (d, $J_{C_2', F_2} = 187.7$ Hz, C_2'), 83.5 (d, $J_{C_6, F_6} = 163.6$ Hz, C_6), 77.6 (C_3 , $T^{\beta*}$), 75.2 (C_5'), 71.3 (d, $J_{C_5, F_6} = 19.5$ Hz, C_5), 71.3 (d, $J_{C_3', F_2} = 17.7$ Hz, C_3'), 69.6, 69.6, 69.5 (C_5- , C_6- , C_8- , C_{11} -spacer), 69.1 (d, $J_{C_4, F_6} = 9.5$ Hz, C_4), 67.0 ($T_{(1,2)}^\beta$), 66.3, 66.1 (C_3- , C_9 -spacer), 61.4, 61.1 ($S_{(1,2)}^\beta$), 60.9 (C_6), 60.5, 60.1, 60.0 ($P_{(1-5)}^\alpha$), 59.4 (V^α), 58.9 (T_2^α), 58.8 (T_1^α), 57.0 ($T^{\alpha*}$), 55.5 (S_1^α), 55.0 (S_2^α), 52.3 (H^α), 51.1 (R^α), 50.1 (D^α), 49.6 (C_2 , A_1^α), 48.0, 47.9, 47.8 ($P_{(2-5)}^\delta$), 47.7, 47.6 ($A_{(2-4)}^\alpha$), 47.4 (P_1^δ), 42.4 ($G_{(1,2)}^\alpha$), 40.5 (R^δ), 39.0 (C_{12} -spacer), 35.0 (D^β), 34.0 (C_2 -spacer), 30.2 (V^β), 29.6, 29.3, 29.2, 28.7 ($P_{(1-5)}^\beta$), 27.5 (R^β), 26.4 (H^β), 24.7, 24.6, 24.5, 24.3 ($P_{(1-5)}^\gamma$), 24.0 (R^γ), 22.1 (CH_3-NHAc), 18.8, 18.7 ($T_{(1,2)}^\gamma$), 18.4 (V^{γ^b}), 18.2 (T^{γ^*}), 17.9 (V^{γ^a}), 16.3 ($A_{(1)}^\beta$), 15.2, 15.1 ($A_{(2-4)}^\beta$). $^{19}F-NMR$ (376.5 MHz, D_2O), δ (ppm): -207.6 (dd, $J_{F_2, H_3} = 14.9$ Hz, $J_{F_2, H_2} = 52.1$ Hz, 2'-F), -229.9 (dt, $J_{F_6, H_5} = 16.1$ Hz, $J_{F_6, H_6} = 47.2$ Hz, 6-F).

Amino-4,7,10-trioxadodecanylamido-N-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-O-(2-acetamido-2,6-dideoxy-6-fluoro-3-O-[2,6-dideoxy-2,6-difluoro-β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-L-threonyl-L-alanyl-L-proline (15)

The synthesis was performed according to the general protocol with Fmoc-Thr-(β-2,6F-Bn₂Gal-(1-3)-α-6F-GalNAc)-OH (**10**) (134 mg, 0.15 mmol) to provide the crude partially protected glycopeptide (185 mg, 0.08 mmol, 80%) as a slightly yellow amorphous solid. *ESI-MS (positive)*, (*m/z*): 881.42 ([M+3H]³⁺, calc.: 881.09), 1321.62 ([M+2H]²⁺, calc.: 1321.14).

For complete deprotection, the crude peptide (185 mg, 0.08 mmol) was dissolved in MeOH (50 mL, HPLC-grade) and subjected to 6 cycles of vacuum/Ar flush. After addition of Pd(OAc)₂ (15 mg, 0.07 mmol), the argon atmosphere was replaced by hydrogen and the reaction mixture was stirred for 6 d at ambient temperature. The catalyst was removed by filtration through Hyflo Super Cel®, and the filtrate was concentrated in vacuo. Purification by semi-preparative RP-HPLC provided glycopeptide **15** (76 mg, 0.03 mmol, 31%) as a colorless amorphous solid after lyophilization. *Analytical RP-HPLC* (Luna C18(2), λ = 214 nm, H₂O-MeCN (+0.1% TFA) 95:5 → 75:25, 30 min → 100:0, 10 min → 100:0, 30 min, *t_R* = 18.9 min). [α]_D²³ = -91.2 (c = 1, H₂O). *ESI-MS (positive)*, (*m/z*): 821.15 ([M+3H]³⁺, calc.: 821.06), 1231.20 ([M+2H]²⁺, calc.: 1231.09). *HR-ESI-MS (positive, m/z)* calc. for C₁₀₃H₁₆₆F₃N₂₇O₃₉: 1231.0894 ([M+2H]⁺), found: 1231.0852. *¹H-NMR (400 MHz, D₂O, COSY, HMQC)*, δ (*ppm*): 8.57 (d, 1H, *J*_{Hε,Hδ} = 1.3 Hz, H^ε), 7.29-7.27 (m, 1H, H^δ), 4.99 (d, 1H, *J*_{H1,H2} = 3.8 Hz, 1-H), 4.73-4.46 (m, 13H, D^α {4.69}, 1'-H {4.71}, H^α {4.67}, T^{α*} {4.64}, 6a,b-H, 6'a,b-H {4.64, 4.53}, R^α {4.61}, A₍₂₋₄₎^α {4.57, 4.54, 4.51}, S₁^α {4.47, *t*, *J*_{S1α,S1β} = 5.4 Hz}), 4.45-4.14 (m, 17H, S₂^α {4.43}, P₍₁₋₅₎^α {4.37, 4.35, 4.34, 4.33, 4.32}, T₁^α {4.32}, V^α {4.30}, T^{β*} {4.29}, T₂^α {4.27}, 2'-H {4.27}, 5-H {4.25}, 2-H {4.22}, A₁^α {4.20}, T_(1,2)^β {4.19, 4.16}, 4-H {4.15}), 4.02-3.68 (m, 34H, 3-H {3.99}, 4'-H {3.97}, G₁^α {3.94}, 5'-H {3.92}, G₂^α {3.90}, 3'-H {3.87}, S₁^β {3.83}, P₍₁₋₅₎^δ {3.78, 3.76, 3.72, 3.66, 3.63}, S₂^β {3.75}, 3a,b-H, 5a,b-H, 6a,b-H, 8a,b-H, 9a,b-H, 11a,b-H (spacer) {3.68-3.59}), 3.26 (dd, 1H, *J*_{Hβa,Hα} = 5.8 Hz, *J*_{Hβa,Hβb} = 15.9 Hz, H^{βa}), 3.21-3.09 (m, 5H, R^δ {3.17}, 12a,b-H (spacer) {3.16}, H^{βb} {3.15}), 2.93 (dd, 1H, *J*_{Dβa,Dα} = 6.3 Hz, *J*_{Dβa,Dβb} = 17.1 Hz, D^{βa}), 2.85 (dd, 1H, *J*_{Dβb,Dα} = 7.4 Hz, *J*_{Dβb,Dβa} = 16.5 Hz, D^{βb}), 2.76-2.59 (m, 2H, 2a,b-H), 2.35-2.15 (m, 6H, P₍₁₋₃₎^β), 2.08-1.77 (m, 19H, V^β {2.04}, P₍₁₋₅₎^γ {2.01-1.82}, P_(4,5)^β {2.00, 1.89}, CH₃-NHAc {1.94}, R^{βa} {1.80}), 1.77-1.69 (m, 3 H, R^{βb} {1.70}, R^γ {1.64}), 1.33 (d, 3H, *J*_{A4β,A4α} = 6.9 Hz, A₄^β), 1.32 (d, 6H, *J*_{A2,3β,A2,3α} = 7.1 Hz, A_{2,3}^β), 1.28 (d, 3H, *J*_{A1β,A1α} = 7.2 Hz, A₁^β), 1.21 (d, 3H,

$J_{T\gamma^*,T\beta^*} = 6.3$ Hz, T^{γ^*}), 1.16 (d, 3H, $J_{T2\gamma,T2\beta} = 6.5$ Hz, T_2^{γ}), 1.13 (d, 3H, $J_{T1\gamma,T1\beta} = 6.4$ Hz, T_1^{γ}), 0.92 (d, 3H, $J_{V\gamma^a,V\beta} = 6.7$ Hz, V^{γ^a}), 0.92 (d, 3H, $J_{V\gamma^b,V\beta} = 6.6$ Hz, V^{γ^b}). $^{13}\text{C-NMR}$ (100.6 MHz, D_2O , DEPT, HMQC), δ (ppm): 175.8, 175.0, 174.4, 174.0, 174.0, 173.8, 173.7, 173.5, 173.5, 173.1, 173.0, 172.7, 172.5, 172.4, 172.0, 172.0, 171.6, 171.4, 171.3, 171.2, 171.2, 171.0, 170.7 (C=O), 156.7 (C=NH), 133.5 (H^{ϵ}), 128.4 (H^{γ}), 117.3 (H^{δ}), 101.8 (d, $J_{C1',F2'} = 23.8$ Hz, $C1'$), 99.4 ($C1$), 90.9 (d, $J_{C2',F2'} = 180.1$ Hz, $C2'$), 83.6 (d, $J_{C6,F6} = 164.9$ Hz, $C6$), 82.8 (d, $J_{C6',F6'} = 165.8$ Hz, $C6'$), 77.8 (T^{β^*}), 77.7 ($C3$), 73.2 (d, $J_{C5',F6'} = 19.9$ Hz, $C5'$), 71.1 (d, $J_{C3',F2'} = 16.9$ Hz, $C3'$), 69.6 ($C5$), 69.6, 69.5, 69.5 ($C5-$, $C6-$, $C8-$, $C11$ -spacer), 68.7 (d, $J_{C4',F6'} = 9.0$ Hz, $C4'$), 68.2 (d, $J_{C4,F6} = 8.3$ Hz, $C4$), 67.0 ($T_{(1,2)}^{\beta}$), 66.3, 66.1 ($C3-$, $C9$ -spacer), 61.4, 61.2 ($S_{(1,2)}^{\beta}$), 60.8, 60.6, 60.4, 60.1, 60.0 ($P_{(1-5)}^{\alpha}$), 59.4 (V^{α}), 58.9 (T_2^{α}), 58.8 (T_1^{α}), 57.0 (T^{α^*}), 55.6 (S_1^{α}), 55.1 (S_2^{α}), 52.4 (H^{α}), 51.1 (R^{α}), 50.5 (D^{α}), 49.6 ($C2$), 48.1 (A_1^{α}), 47.8, 47.7, 47.7 ($P_{(2-5)}^{\delta}$), 47.9, 47.9, 47.8 ($A_{(2-4)}^{\alpha}$), 47.4 (P_1^{δ}), 42.4, 42.3 ($G_{(1,2)}^{\alpha}$), 40.5 (R^{δ}), 39.1 ($C12$ -spacer), 34.9 (D^{β}), 34.0 ($C2$ -spacer), 30.1 (V^{β}), 29.7, 29.4, 29.3, 29.2, 28.8 ($P_{(1-5)}^{\beta}$), 27.5 (R^{β}), 26.3 (H^{β}), 24.8, 24.7, 24.6, 24.4 ($P_{(1-5)}^{\gamma}$), 24.0 (R^{γ}), 22.1 ($\text{CH}_3\text{-NHAc}$), 18.9, 18.8 ($T_{(1,2)}^{\gamma}$), 18.5 (V^{γ^a}), 18.2 (T^{γ^*}), 17.9 (V^{γ^b}), 16.3, 15.2, 15.1 ($A_{(1-4)}^{\beta}$). $^{19}\text{F-NMR}$ (376.5 MHz, D_2O), δ (ppm): -207.7 (dd, $J_{F2',H3} = 14.7$ Hz, $J_{F2',H2} = 51.4$ Hz, 2'-F), -229.7 - -230.1 (m, 6,6'-F).

Amino-4,7,10-trioxadodecanylamido-*N*-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-*O*-(2-acetamido-2-deoxy-3-*O*-[2,6-dideoxy-2,6-difluoro- β -D-galactopyranosyl]- α -D-galactopyranosyl)-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-L-threonyl-L-alanyl-L-proline (16)

The synthesis was performed according to the general protocol with Fmoc-Thr-(β -2,6F-Bn₂Gal-(1-3)- α -GalNAc)-OH (**13**) (154 mg, 0.16 mmol) to provide the crude partially protected glycopeptide (140 mg, 0.05 mmol, 50%) as a slightly yellow amorphous solid. *ESI-MS* (positive), (m/z): 878.47 ($[\text{M-Bn}+3\text{H}]^{3+}$, calc.: 878.42), 908.49 ($[\text{M}+3\text{H}]^{3+}$, calc.: 908.44), 1317.20 ($[\text{M-Bn}+2\text{H}]^{2+}$, calc.: 1317.13), 1362.23 ($[\text{M}+2\text{H}]^{2+}$, calc.: 1362.15).

For complete deprotection, the crude peptide (136 mg, 0.05 mmol) was dissolved in MeOH (30 mL, HPLC-grade) and subjected to 6 cycles of vacuum/Ar flush. After addition of Pd(OAc)₂ (15 mg, 0.07 mmol), the argon atmosphere was replaced by hydrogen and the reaction mixture was stirred for 2 d at ambient temperature. The catalyst was removed by filtration through Hyflo Super Cel®, and the filtrate was concentrated in vacuo. The residue was again dissolved in MeOH (30 mL, HPLC-grade) and the pH was adjusted to 9.5 with

NaOMe (1% in MeOH). After stirring for 18 h under constant control of the pH at ambient temperature, the solution was neutralized with HOAc (1 M) and concentrated in vacuo. The residue was co-evaporated with toluene (2×20 mL) and purified by semi-preparative RP-HPLC to afford glycopeptide **16** (36 mg, 0.015 mmol, 15%) as a colorless amorphous solid after lyophilization. *Analytical RP-HPLC* (Luna C18(2), $\lambda = 214$ nm, H₂O-MeCN (+0.1% TFA) 95:5 → 60:40, 30 min → 0:100, 10 min, $t_R = 12.0$ min). $[\alpha]_D^{23} = -107.9$ (c = 1, H₂O). *ESI-MS (positive)*, (m/z): 842.42 ([M+3Na]³⁺, calc.: 842.38), 1263.15 ([M+2Na-H]²⁺, calc.: 1263.04). *HR-ESI-MS (positive, m/z)* calc. for C₁₀₃H₁₆₇F₂N₂₇O₄₀: 1230.0917 ([M+2H]⁺), found: 1230.0905. *MALDI-TOF-MS (DHB, positive)*, m/z : 2481.77 ([M+Na]⁺, calc.: 2482.56). *¹H-NMR (400 MHz, D₂O, COSY, TOCSY, NOESY)*, δ (ppm): 8.60 (d, 1H, $J_{H\epsilon, H\delta} = 1.3$ Hz, H ^{ϵ}), 7.30 (d, 1H, $J_{H\delta, H\epsilon} = 1.1$ Hz, H ^{δ}), 4.96 (d, 1H, $J_{H1, H2} = 3.8$ Hz, 1-H), 4.75-4.27 (m, 22 H, 1'-H {4.73}, D ^{α} {4.70}, H ^{α} {4.68}, 6'a,b-H {4.66, 4.54}, T ^{$\alpha*$} {4.63}, R ^{α} {4.62}, A_(3,4) ^{α} {4.56, q, $J_{A3,4\alpha, A3,4\beta} = 7.1$ Hz}, A₂ ^{α} {4.55}, S₁ ^{α} {4.50, t, $J_{S1\alpha, S1\beta} = 5.4$ Hz}, S₂ ^{α} {4.45}, P₍₁₋₅₎ ^{α} {4.39, 4.36, 4.32, 4.28}, T₁ ^{α} {4.34} T ^{$\beta*$} {4.31} T₂ ^{α} {4.29}, V ^{α} {4.28}), 4.36-4.20 (m, 1H, 2'-H), 4.26-4.15 (m, 5H, A₁ ^{α} {4.23}, 2-H {4.21}, T_(1,2) ^{β} {4.20, 4.17}, 4-H {4.12, d, $J_{H4, H3} = 2.0$ Hz}), 4.03-3.54 (m, 35H, 4'-H {4.00}, 5-H {3.99}, 3-H {3.98}, G₂ ^{α} {3.97}, 5'-H {3.95, 3.91}, G₁ ^{α} {3.92}, S₁ ^{β} {3.88}, 3'-H {3.88}, P₍₁₋₅₎ ^{δ} {3.80, 3.76, 3.63, 3.59, 3.55}, S₂ ^{β} {3.77}, 3a,b-H-, 5a,b-H-, 6a,b-H-, 8a,b-H-, 9a,b-H-, 11a,b-H-spacer {3.76, 3.72, 3.67, 3.63}, 6a,b-H {3.72}), 3.29 (dd, 1H, $J_{H\beta a, H\beta b} = 15.5$ Hz, $J_{H\beta a, H\alpha} = 5.5$ Hz, H ^{βa}), 3.22-3.13 (m, 5H, R ^{δ} {3.18}, 12a,b-H-spacer {3.18}, H ^{βb} {3.17}), 2.94 (dd, 1H, $J_{D\beta a, H\beta b} = 17.0$ Hz, $J_{D\beta a, D\alpha} = 6.4$ Hz, D ^{βa}), 2.86 (dd, 1H, $J_{D\beta b, D\beta a} = 17.1$ Hz, $J_{D\beta b, D\alpha} = 6.8$ Hz, D ^{βb}), 2.74 (dt, 1H, $J_{CH2a, CH2b} = 16.1$ Hz, $J_{CH2a, CH2} = 6.5$ Hz, 2a-H-spacer), 2.64 (dt, 1H, $J_{CH2b, CH2a} = 16.2$ Hz, $J_{CH2b, CH2} = 5.9$ Hz, 2b-H-spacer), 2.34-2.17 (m, 4H, P_(1,2) ^{β} {2.29, 2.24}), 2.10-1.78 (m, 21H, V ^{β} {2.06}, P₍₁₋₅₎ ^{γ} {2.05, 2.00, 1.95, 1.93}, P₍₃₋₅₎ ^{β} {1.99, 1.91, 1.85}, CH₃-NHAc {1.96}, R ^{βa} {1.81}), 1.75-1.59 (m, 3H, R ^{βb} {1.71}, R ^{γ} {1.64}), 1.35 (d, 3H, $J_{A4\beta, A4\alpha} = 6.9$ Hz, A₄ ^{β}), 1.34 (d, 6H, $J_{A2,3\beta, A2,3\alpha} = 7.0$ Hz, A_(2,3) ^{β}), 1.31 (d, 3H, $J_{A1\beta, A1\alpha} = 7.2$ Hz, A₁ ^{β}), 1.25 (d, 3H, $J_{T\gamma^*, T\beta^*} = 6.2$ Hz, T ^{$\gamma*$}) 1.18 (d, 3H, $J_{T1\gamma, T1\beta} = 6.5$ Hz, T₁ ^{γ}), 1.16 (d, 3H, $J_{T2\gamma, T2\beta} = 6.5$ Hz, T₂ ^{γ}), 0.95 (d, 3H, $J_{V\gamma a, V\beta} = 6.4$ Hz, V ^{γa}), 0.94 (d, 3H, $J_{V\gamma b, V\beta} = 6.4$ Hz, V ^{γb}). *¹³C-NMR (100.6 MHz, D₂O, DEPT, HMQC)*, δ (ppm): 176.0, 174.9, 174.4, 174.0, 173.9, 173.7, 173.5, 173.1, 172.6, 172.5, 172.4, 172.0, 171.9, 171.5, 171.3, 171.1, 170.9 (C=O), 156.7 (C=NH), 133.4 (H ^{ϵ}), 128.4 (H ^{γ}), 117.3 (H ^{δ}), 102.0 (d, $J_{C1', F2} = 23.6$ Hz, C1'), 99.2 (C1), 90.8 (d, $J_{C2', F2} = 181.1$ Hz, C2'), 82.8 (d, $J_{C6', F6} = 165.8$ Hz, C6'), 78.0 (C3), 77.2 (T ^{$\beta*$}), 73.2 (d, $J_{C5', F6} = 20.4$ Hz, C5'), 71.1 (d, $J_{C3', F2} = 16.3$ Hz, C3'), 71.1 (dd, $J_{C4', F6} = 10.9$ Hz, $J_{C4', F2} = 5.3$ Hz, C4'), 69.6, 69.5, 69.5, 69.4 (C3-, C5-, C6-, C8-, C11-spacer), 68.7 (C4, C5), 67.0 (T_(1,2) ^{β}), 61.4, 61.3, 61.1 (C6, S_(1,2) ^{β}), 60.8,

60.5, 60.1, 60.0, 59.5 (P₍₁₋₅₎^α), 59.4 (V^α), 58.9 (T₂^α), 58.8 (T₁^α), 57.0 (T^{α*}), 55.5 (S₁^α), 55.0 (S₂^α), 52.3 (H^α), 51.1 (R^α), 50.1 (D^α), 49.6 (A₁^α), 48.2 (C₂), 48.0, 47.9, 47.7 (P₍₂₋₅₎^δ), 47.7, 47.6 (A₍₂₋₄₎^α), 47.4 (P₁^δ), 42.4, 42.3 (G_(1,2)^α), 40.5 (R^δ), 39.0 (C₁₂-spacer), 35.0 (D^β), 34.0 (C₂-spacer), 30.1 (V^β), 29.6, 29.3, 29.2, 28.8 (P₍₁₋₅₎^β), 27.4 (R^β), 26.2 (H^β), 24.7, 24.6, 24.5, 24.3 (P₍₁₋₅₎^γ), 24.0 (R^γ), 22.1 (CH₃-NHAc), 18.8, 18.7 (T_(1,2)^γ), 18.4 (V^{γb}), 18.3 (T^{γ*}), 17.8 (V^{γa}), 16.3 (A₁^β), 15.2, 15.1 (A₍₂₋₄₎^β). ¹⁹F NMR (376.5 MHz, D₂O), δ (ppm): -207.2 (dd, J_{F₂,H₂} = 51.8 Hz, J_{F₂,H₃} = 14.7 Hz, 2-F), -228.5 (dt, J_{F₆,H_{6a/b}} = 46.4 Hz, J_{F₆,H₅} = 15.3 Hz, 6-F).

Amino-4,7,10-trioxadodecanylamido-N-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-O-(2-acetamido-2,6-deoxy-6-fluoro-3-O-[β-D-galactopyranosyl]-α-D-galactopyranosyl)-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-L-threonyl-L-alanyl-L-proline (17)

The synthesis was performed according to the general protocol with Fmoc-Thr-(β-Ac₄Gal-(1-3)-α-6F-GalNAc)-OH (**4**) (149 mg, 0.17 mmol) to provide the crude partially protected glycopeptide (166 mg, 0.06 mmol, 60%) as a slightly yellow amorphous solid. *ESI-MS* (positive), (*m/z*): 876.05 ([M+3H]³⁺, calc.: 875.75); 1313.58 ([M+2H]²⁺, calc.: 1313.11).

For complete deprotection, the crude peptide ((166 mg, 0.06 mmol) was dissolved in aq. NaOH (100 mL, pH 10) and stirred for 7 d under constant control of the pH at ambient temperature. Then, the solution was neutralized with HOAc (1 M) and concentrated in vacuo. The residue was co-evaporated with toluene (2×20 mL) and purified by semi preparative RP-HPLC to afford glycopeptide **17** (109 mg, 0.044 mmol, 44%) as a colorless amorphous solid after lyophilization. *Analytical RP-HPLC* (Luna C18(2), λ = 214 nm, H₂O-MeCN (+0.1% TFA) 95:5 → 70:30, 30 min → 0:100, 50 min, *t_R* = 15.4 min). [α]_D²³ = -98.9 (c = 1, H₂O). *ESI-MS* (positive), (*m/z*): 819.72 ([M+3H]³⁺, calc.: 819.73), 1229.61 ([M+2H]²⁺, calc.: 1229.09). *HR-ESI-MS* (positive, *m/z*) calc. for C₁₀₃H₁₆₈FN₂₇O₄₁: 1229.0937 ([M+2H]⁺), found: 1229.0952. *MALDI-TOF-MS* (DHB, positive), *m/z*: 2457.53 ([M+H]⁺, calc.: 2458.58). ¹H-NMR (400 MHz, D₂O, COSY, HMQC), δ (ppm): 8.60 (bs, 1H, H^ε), 7.30 (bs, 1H, H^δ), 4.99 (d, J_{H₁,H₂} = 3.7, 1H, 1-H), 4.75-4.54 (m, 8H, D^α {4.72}, H^α {4.68}, 6a-H {4.66}, T^{α*} {4.66}, R^α {4.63}, A₂^α {4.60}, A₃^α {4.53}, 6b'-H {4.55}), 4.51-4.18 (m, 19H, S₁^α {4.49}, A₄^α {4.46}, S₂^α {4.45}, 1'-H {4.41}, P₁₋₅^α {4.40, 4.38, 4.37, 4.35}, V^α {4.35}, T^{β*} {4.33}, T₁₋₂^α {4.32, 4.30}, 5-H {4.29}, A₁^α {4.23}, 2-H {4.21}, T₁₋₂^β {4.20, 4.19}, 4-H {4.19}), 4.03 (dd, 1H, J_{H₃,H₄} = 2.9 Hz, J_{H₃,H₂} = 10.8 Hz, 3-H), 4.00-3.85 (m, 5H, G^α {3.97, 3.94}, 4'-H {3.87}), 3.90-3.56 (m, 30H, S_{1,2}^β {3.85, 3.75}, P_{1,2}^δ {3.78, 3.76}, 6'a-H {3.78}, 3a,b-H-, 5a,b-H-spacer {3.75,

3.74}, 6'b-H {3.68}, P₃₋₅^δ{3.64, 3.60, 3.57}, 6a,b-H-, 8a,b-H, 9a,b-H, 11a,b-H-spacer {3.66, 3.63, 3.62, 3.61}, 5'-H {3.60}, 3.58 {dd, 1H, $J_{H3',H4'} = 2.9$ Hz, $J_{H3',H2'} = 10.1$ Hz, 3'-H}), 3.47 (dd, 1H, $J_{H2',H1'} = 7.8$ Hz, $J_{H2',H3'} = 9.8$ Hz, 2'-H), 3.29 (dd, 1H, $J_{H\beta a,H\alpha} = 5.6$ Hz, $J_{H\beta a,H\beta b} = 15.6$ Hz, H^{βa}), 3.18-3.14 (m, 5H, R^δ {3.18}, H^β {3.16}), 12a,b-H-spacer {3.14}), 2.96 (dd, 1H, $J_{D\beta a,D\alpha} = 6.2$ Hz, $J_{D\beta a,D\beta b} = 16.9$ Hz, D^{βa}), 2.88 (dd, 1H, $J_{D\beta b,D\alpha} = 6.8$ Hz, $J_{D\beta b,D\beta a} = 17.1$ Hz, D^{βb}), 2.76-2.62 (m, 2H, 2a,b-H-spacer), 2.35-2.20 (m, 6H, P₁₋₃^β {2.35, 2.30, 2.20}), 2.10-1.81 (m, 19H, V^β {2.05}, P₁₋₅^γ {2.04, 2.00, 1.98, 1.95, 1.93}, CH₃-NHAc {1.98}, P_{4,5}^β {1.98, 1.95}, R^{βa} {1.85}), 1.75-1.64 (m, 3H, R^{βb} {1.73}, R^γ {1.65}), 1.36-1.32 (m, 9H, A₁₋₃^β {1.36, 1.34, 1.32}), 1.31 (d, 3H, $J_{A\beta,A\alpha} = 7.2$ Hz, A^β), 1.23 (d, 3H, $J_{T\gamma^*,T\beta^*} = 6.2$ Hz, T^{γ*}), 1.18 (d, 3H, $J_{T\gamma,T\beta} = 6.6$ Hz, T₁^γ), 1.16 (d, 3H, $J_{T\gamma,T\beta} = 6.5$ Hz, T₂^γ), 0.95 (d, 3H, $J_{V\gamma,V\beta} = 6.5$ Hz, V^γ), 0.93 (d, 3H, $J_{V\gamma,V\beta} = 6.6$ Hz, V^γ). ¹³C-NMR (100.6 MHz, D₂O, DEPT, HMQC), δ (ppm): 175.7, 174.9, 174.5, 174.4, 173.9, 173.7, 173.4, 173.1, 173.0, 172.7, 172.6, 172.5, 172.4, 172.3, 172.0, 171.9, 171.5, 171.3, 171.2, 171.1, 171.0, 170.9, 170.7, (C=O), 156.7 (C=NH), 133.4 (H^ε), 128.4 (H^γ), 117.2 (H^δ), 104.5 (C1'), 99.2 (C1), 83.7 (d, $J_{F,C6} = 166.5$ Hz, C-6), 77.4 (T^{β*}), 76.6 (C3), 74.9 (C-5'), 72.4 (C3'), 70.5 (C2'), 69.6, 69.5, 69.4, 69.4 (CH₂O-spacer), 69.5 (C5), 68.4 (C-4'), 68.4 (d, $J_{F,C4} = 6.6$ Hz, C-4), 67.0 (T₁^β), 66.9 (T₂^β), 66.3, 66.1 (CH₂O-spacer), 61.1, 61.0 (S_{1,2}^β), 60.7 (C-6), 60.6, 60.5, 60.1, 60.0 (P₁₋₅^α), 59.3 (V^α), 58.9, 58.7 (T_{1,2}^α), 57.0 (T^{α*}), 55.6, 54.9 (S_{1,2}^α), 52.3 (H^α), 51.1 (R^α), 50.1 (D^α), 49.6, (A^α), 48.2 (C2), 48.1, 47.9, 47.8, (P₁₋₃^δ), 47.8, 47.7 (A^α), 47.5, 47.4 (P_{4,5}^δ), 42.4, 42.3 (G_{1,2}^α), 40.5 (C12-spacer), 39.0 (R^δ), 34.9 (D^β), 34.0 (C2-spacer), 30.2 (V^β), 29.6, 29.3, 29.2, 28.7 (P₁₋₅^β), 27.4 (R^β), 26.2 (H^β), 24.7, 24.6, 24.5, 24.4, 24.3 (P₁₋₅^γ), 24.0 (R^γ), 22.3 (CH₃-NHAc), 18.9, 18.8 (T^γ), 18.5 (V^γ), 18.2 (V^γ), 17.8 (T^{γ*}), 16.2, 15.3, 15.2, 15.1 (A₁₋₄^β). ¹⁹F-NMR (376.4 Hz, D₂O), δ (ppm): -229.4 (dt, $J_{F,H5} = 16.6$ Hz, $J_{F,H6} = 48.5$ Hz, 6'-F).

Neutralisation test:^[S11]

Preparation of the neutralisation solution: 40 μL PBS with 0.1% BSA was titrated in every well of a PS-microtitre plate (Immuno-Plate F96 MaxiSorp, Nunc, Wiesbaden, Germany). The diluted serum is added and increasingly further diluted (1:2 or 1:3). Then 10 μL of the fluorinated glycopeptide **10** ($c = 100\mu\text{g}/\text{mL}$) is added and incubated for 60 minutes.

Coating: The BSA-conjugates with glycopeptide structures similar to those of the TTox-conjugate vaccines were dissolved in a phosphate buffer (0.1 M $\text{Na}_2\text{HPO}_4 \cdot \text{H}_2\text{O}$, pH = 9.3; $c = 5\mu\text{g}/\text{mL}$) and transferred to the wells of a second plate. After incubation for 1 h at 37 °C and three washings with 200 μL phosphate buffer (PBS) pH 7.2 containing 0.01% Tween[®] 20, non-specific binding was blocked by incubation with a solution of BSA (1%) in PBS for 0.5 h at 37 °C. The wells were again washed three times with 200 μL phosphate washing buffer containing 0.01% Tween[®] 20.

Transfer: The neutralisation solution was transferred to the second ELISA plate with the BSA-conjugates and incubated for 60 minutes at 37°C and again washed three times with 200 μL phosphate buffer (PBS) pH 7.2 containing 0.01% Tween[®] 20.

Detection: A solution of biotinylated sheep anti mouse antibody (1:10000, PBS + 1% gelatine; stock solution with $c = 1.2\mu\text{g}/\text{mL}$) was added to each well. The plate was incubated for 1 h at 37 °C and washed three times with 200 μL phosphate washing buffer containing 0.01% Tween[®] 20. After addition of 50 $\mu\text{L}/\text{well}$ of a solution of streptavidine-horse radish peroxidase (1:10000, PBS + 1% gelatine) the plate was again incubated for 0.5 h at 37 °C and treated with 50 $\mu\text{L}/\text{well}$ ABTS/ H_2O_2 solution ($c(\text{ABTS}) = 1\text{ mg}/\text{mL}$ in citrate buffer pH 4.4-4.5 containing 25 μL H_2O_2 (citrate buffered, 0.3%) per mL ABTS solution). The plate was again incubated for 0.5 h at RT and read with an automated ELISA plate reader (ImmunoReader MJ2000, InterMed) at $\lambda = 410\text{ nm}$. All neutralization experiments were made as triplicates. As a negative control, the ELISA test was performed on coated plates without addition of the antiserum.

PBS = phosphate buffer saline; Tween[®] 20 = poly(oxyethylene)_x-sorbitane-monolaurate; BSA = bovine serum albumine; ABTS = 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)

Data for Fig. 1)

dilution	1/5.000	1/15.000	1/45.000	1/135.000
17	0,5626	0,3131	0,1788	0,1147
16	0,5845	0,3311	0,1836	0,1211
15	0,5775	0,3386	0,1636	0,1097
18	0,5559	0,2707	0,1818	0,1157
14	0,5404	0,2988	0,1775	0,1222
MUC1(20)6Thr-TF	0,6202	0,3236	0,1959	0,1227
positive control	1,5304	1,0270	0,6255	0,3452
negative control	0,0830	0,0845	0,0857	0,0853

Data for Fig. 2)

dilution	1/5.000	1/15.000	1/45.000	1/135.000
17	0,9437	0,5463	0,3552	0,1605
16	0,8959	0,4593	0,2520	0,1460
15	0,9027	0,4512	0,2572	0,1502
18	0,8548	0,4279	0,2611	0,1495
14	0,8108	0,4069	0,2536	0,1484
MUC4	1,2899	0,7659	0,4294	0,2209
positive control	1,3258	0,7661	0,4362	0,2242
negative control	0,0840	0,0792	0,0774	0,0827

Data for Fig. 3)

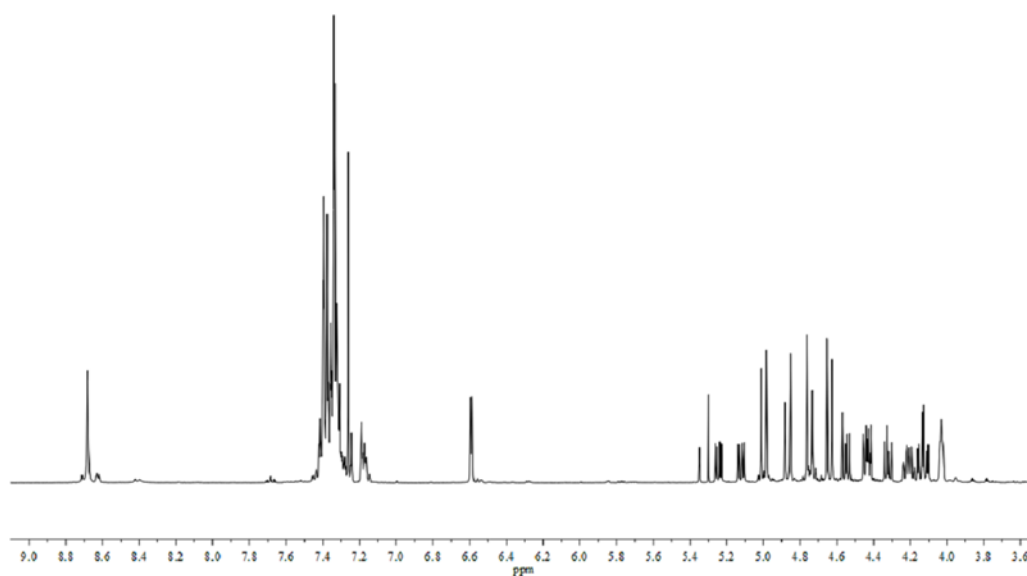
dilution	1/5.000	1/15.000	1/45.000	1/135.000
17	0,8124	0,4204	0,2274	0,1342
16	1,0583	0,5340	0,2794	0,1562
15	0,8178	0,4028	0,2262	0,1389
18	1,1519	0,5573	0,2978	0,1631
14	0,8253	0,3859	0,2105	0,1499
positive control	1,6659	0,9669	0,5070	0,2701
MUC1(20)6Thr- 6F6'F-TF	0,8616	0,4464	0,2358	0,1433
negative control	0,0918	0,0935	0,0973	0,1039

References:

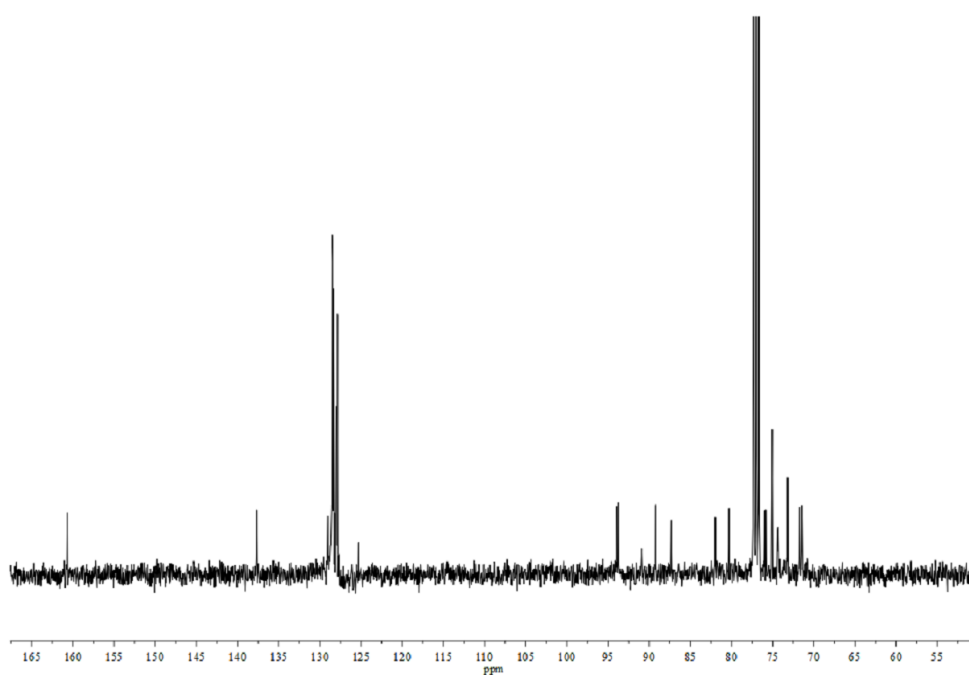
- [S1] M. Johannes, T. Oberbillig, A. Hoffmann-Röder, *Org. Biomol. Chem.*, 2011, **9**, 5541.
- [S2] S. Wagner, C. Mersch, A. Hoffmann-Röder, *Chem.–Eur. J.*, 2010, **16**, 7319.
- [S3] S. Dziadek, C. Brocke, H. Kunz, *Chem–Eur. J.*, 2004, **10**, 4150.
- [S4] Y. Niu, N. Wang, X. Xiao, X.-S. Ye, *Synlett*, 2007, 2116.
- [S5] S. Dziadek, A. Hobel, E. Schmitt, H. Kunz, *Angew. Chem. Int. Ed.*, 2005, **44**, 7630.

NMR spectra of trichloroacetimidate **8**

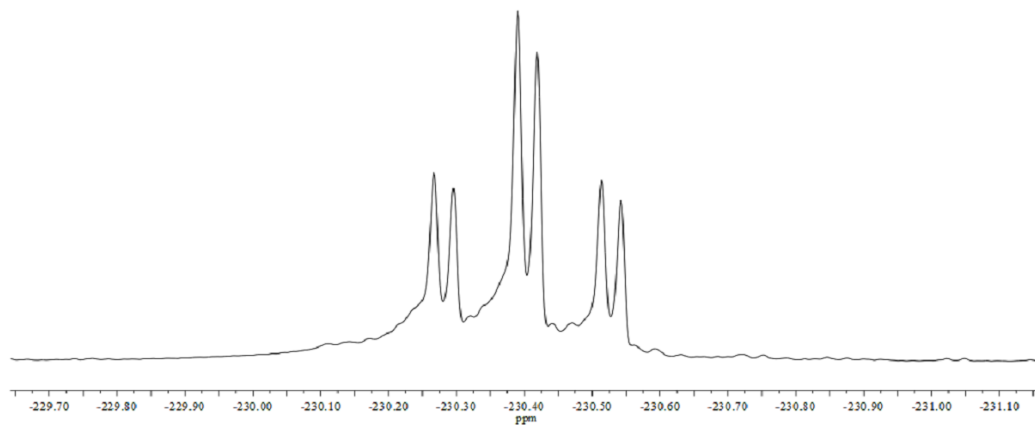
a) ^1H NMR (400 MHz, CDCl_3)



b) ^{13}C NMR (100.6 MHz, CDCl_3)

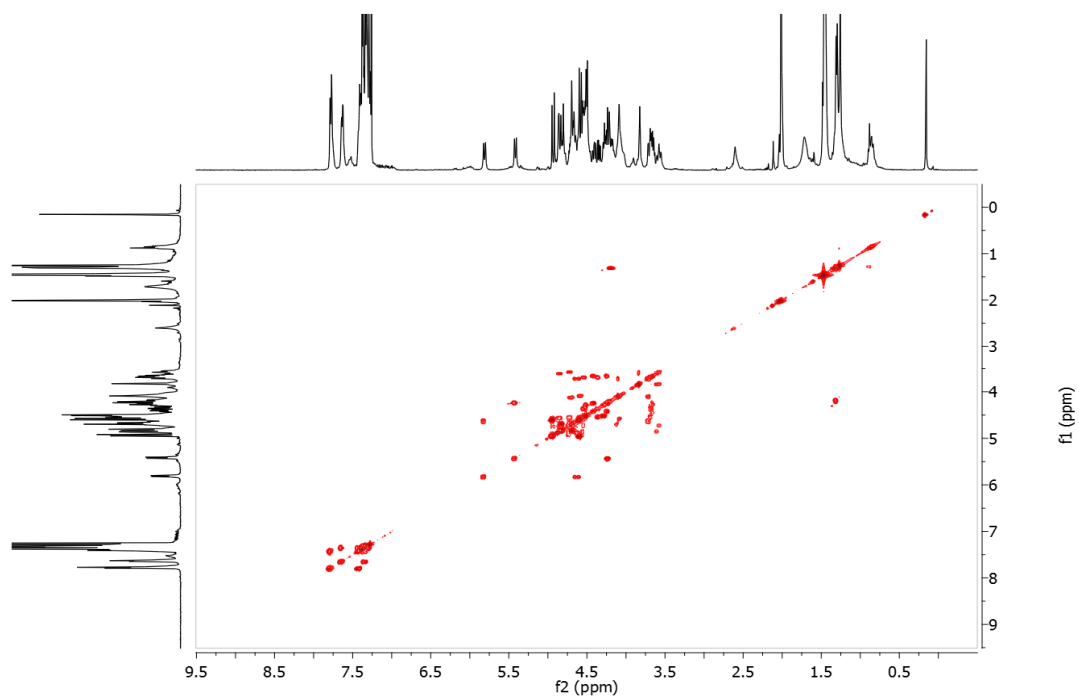


c) ^{19}F NMR (376.5 MHz, CDCl_3)

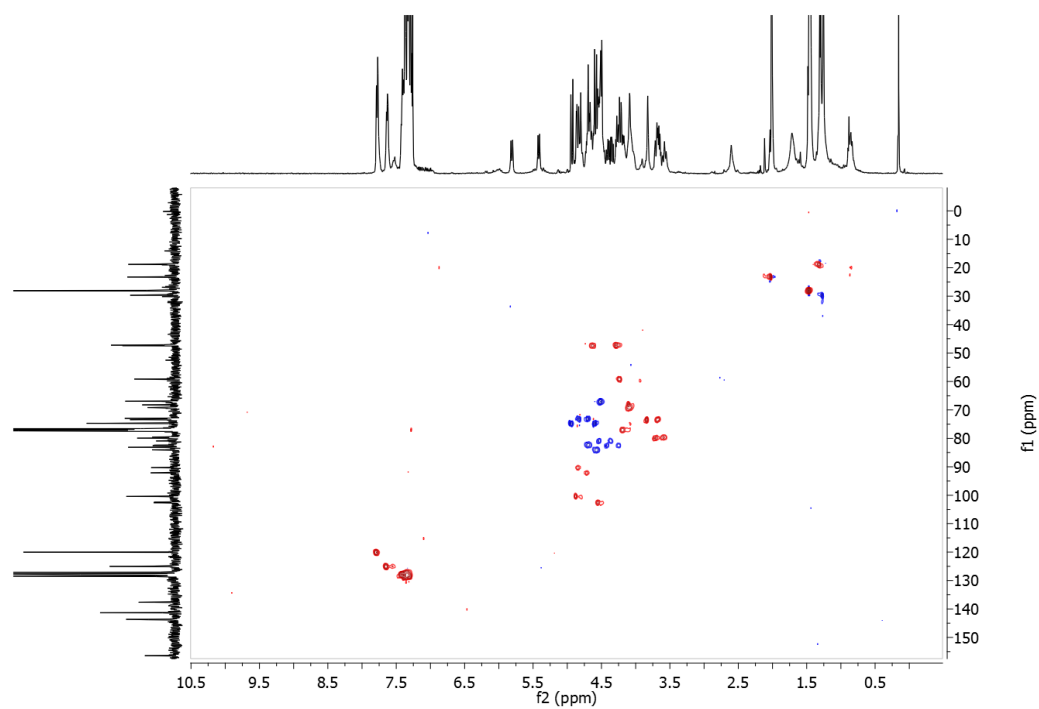


NMR spectra of disaccharide 9

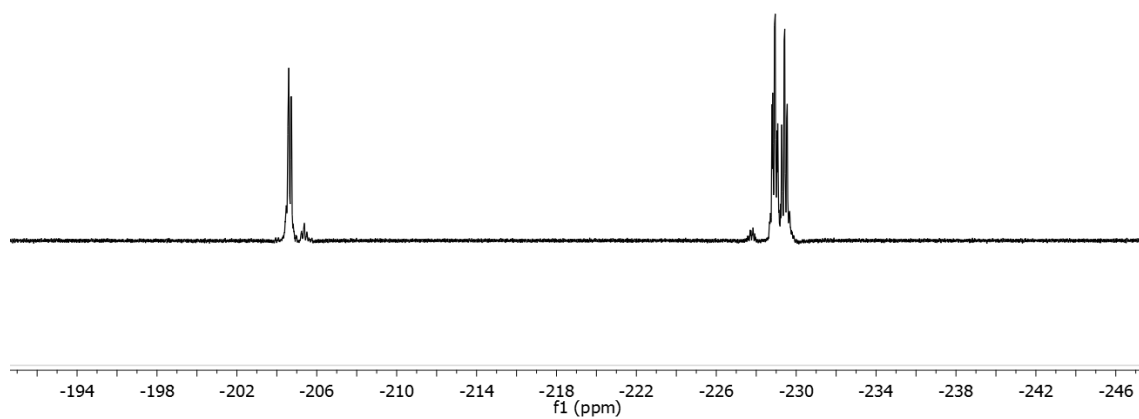
a) ^1H NMR (400 MHz, CDCl_3)



b) HMQC (100.6 MHz, CDCl₃)

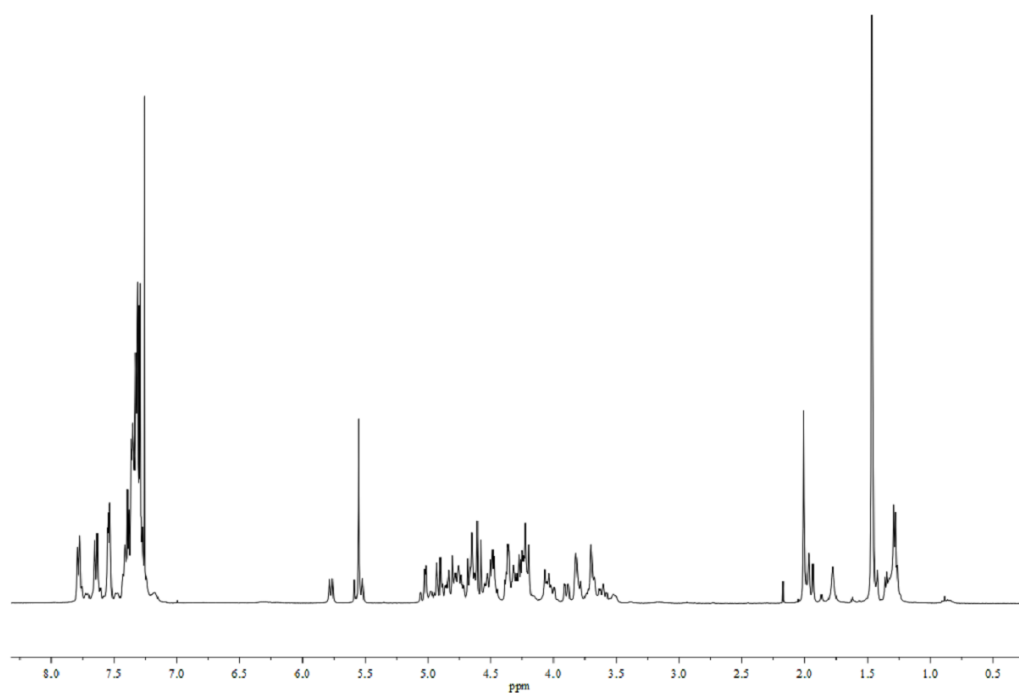


c) ¹⁹F NMR (376.5 MHz, CDCl₃)

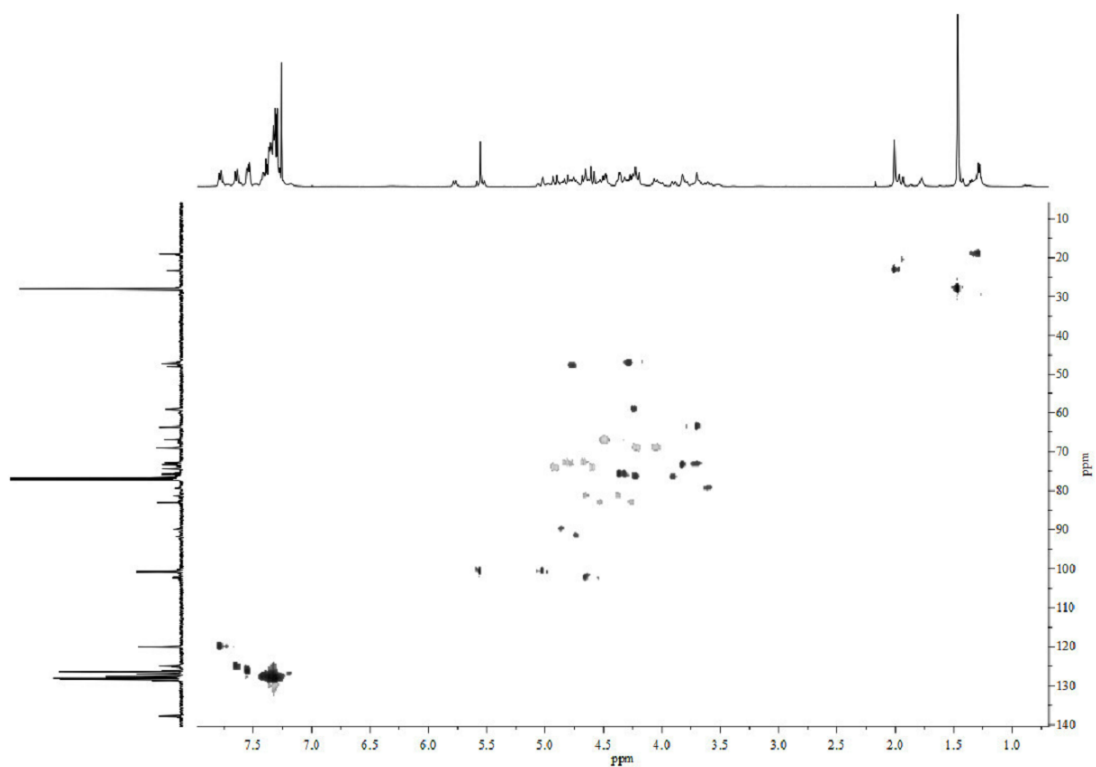


NMR spectra of disaccharide **11**

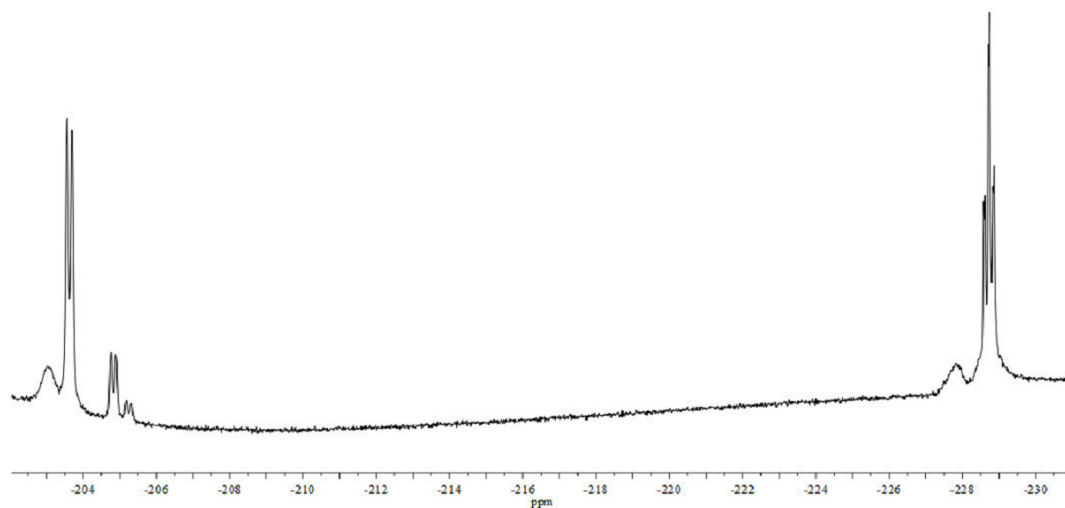
a) ^1H NMR (400 MHz, CDCl_3)



b) HMQC (100.6 MHz, CDCl_3)

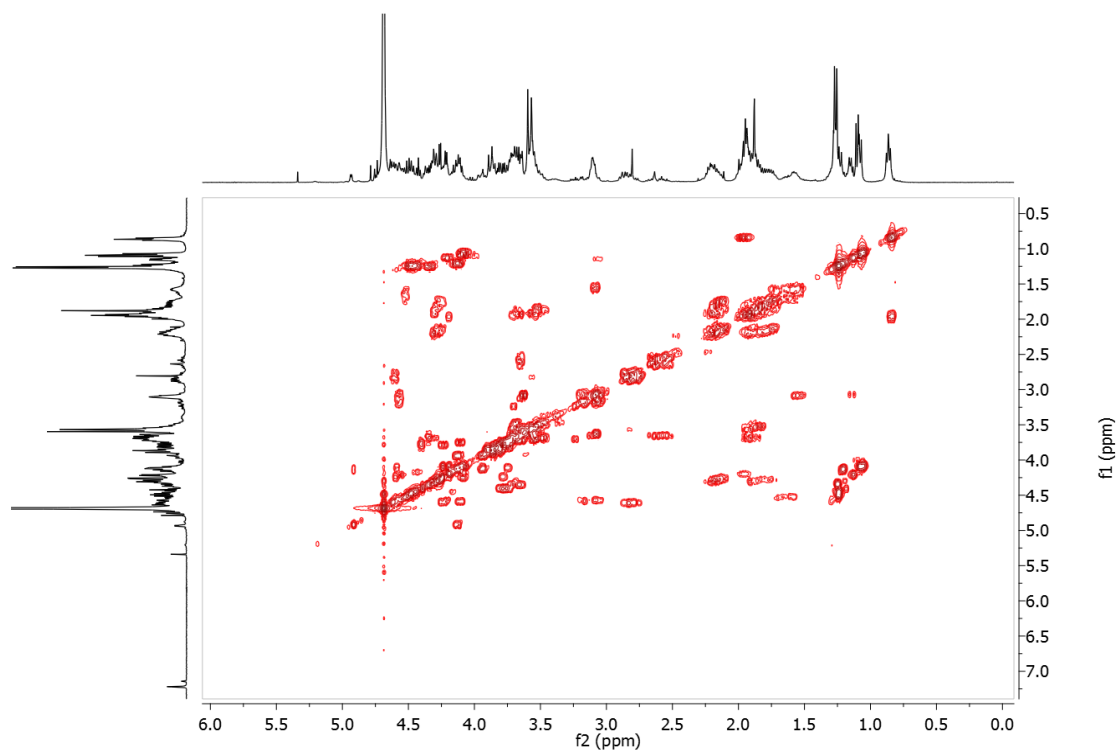


c) ^{19}F NMR (376.5 MHz, CDCl_3)

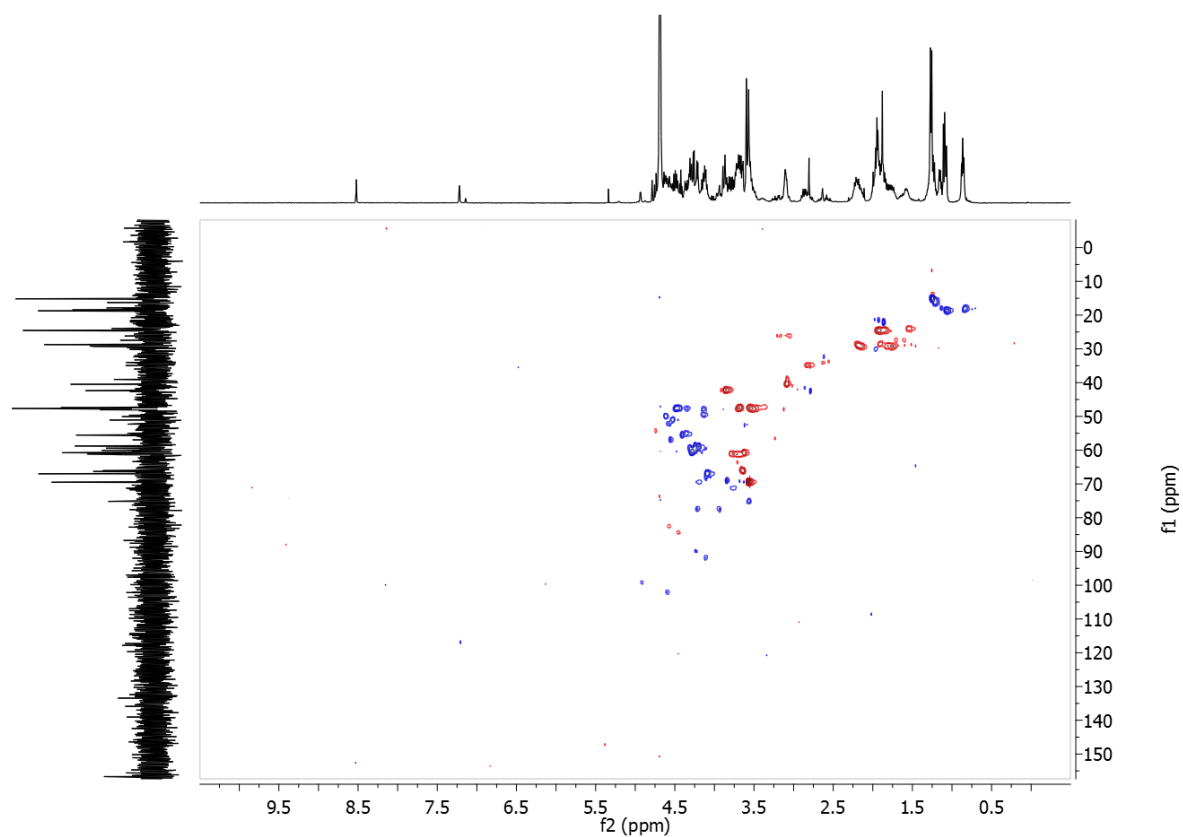


NMR spectra of glycopeptide 14

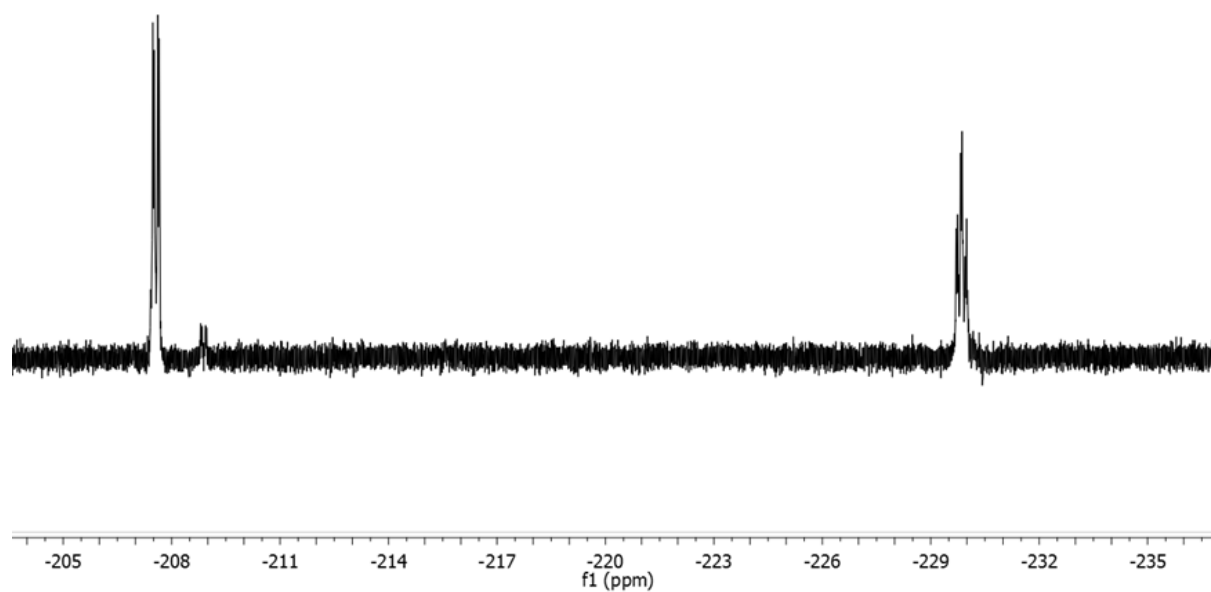
a) H,H-COSY (400 MHz, D_2O)



b) HMQC (100.6 MHz, D₂O)

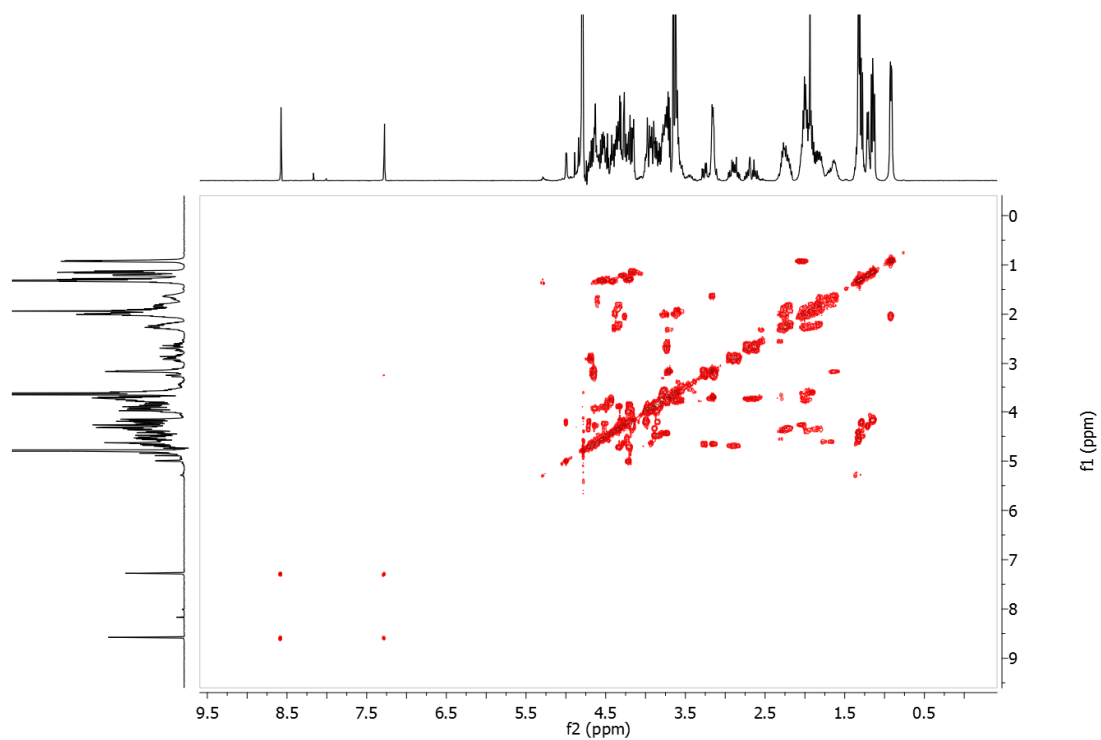


c) ¹⁹F NMR (376.5 MHz, D₂O)

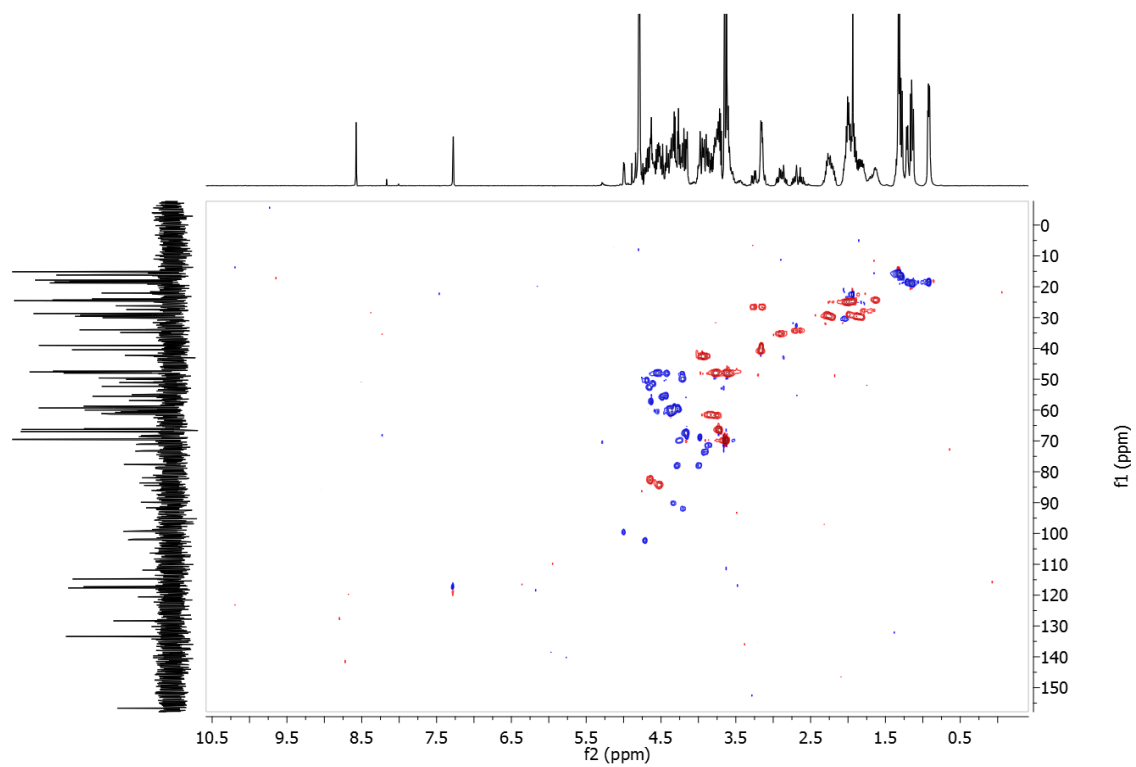


NMR spectra of glycopeptide 15

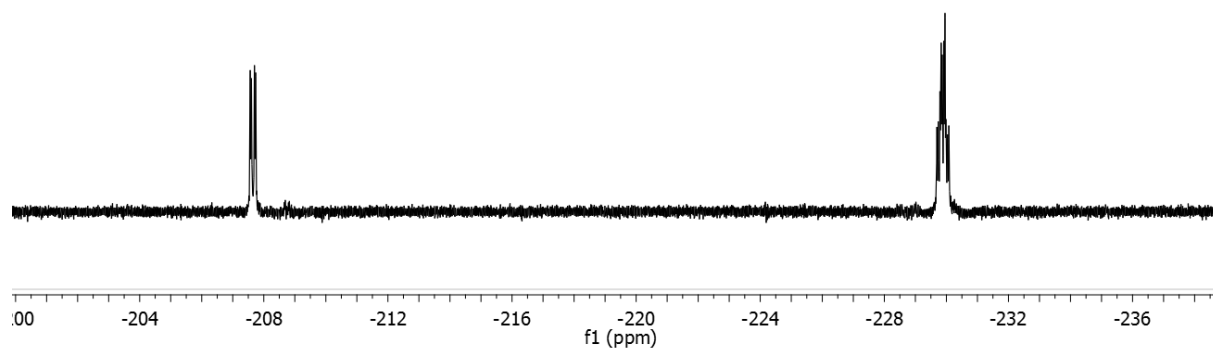
a) H,H-COSY (400 MHz, D₂O)



b) HMQC (100.6 MHz, D₂O)

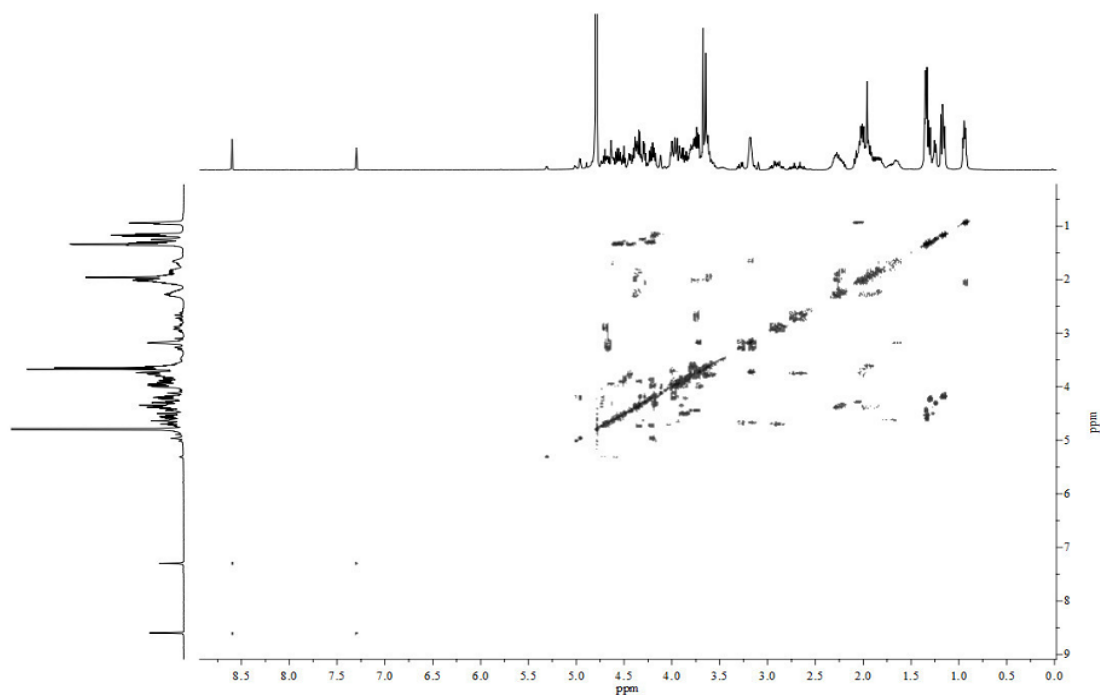


c) ^{19}F NMR (376.5 MHz, D_2O)

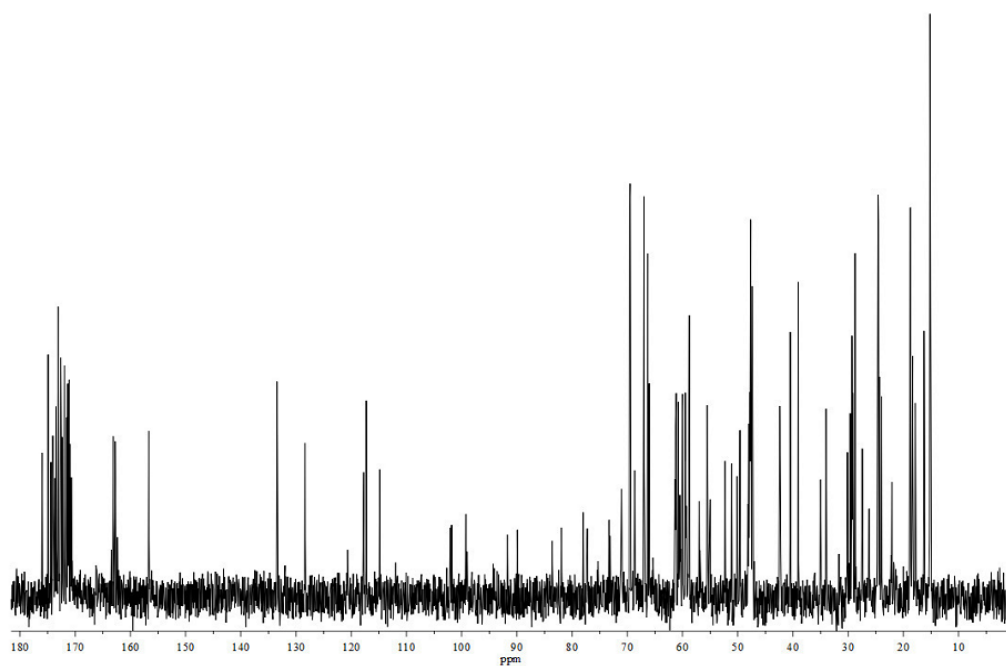


NMR spectra of glycopeptide 16

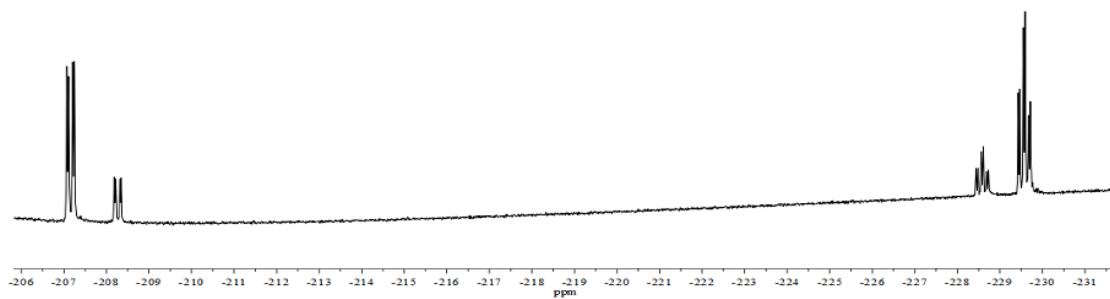
a) H,H-COSY (400 MHz, D_2O)



b) HMQC (100.6 MHz, D₂O)

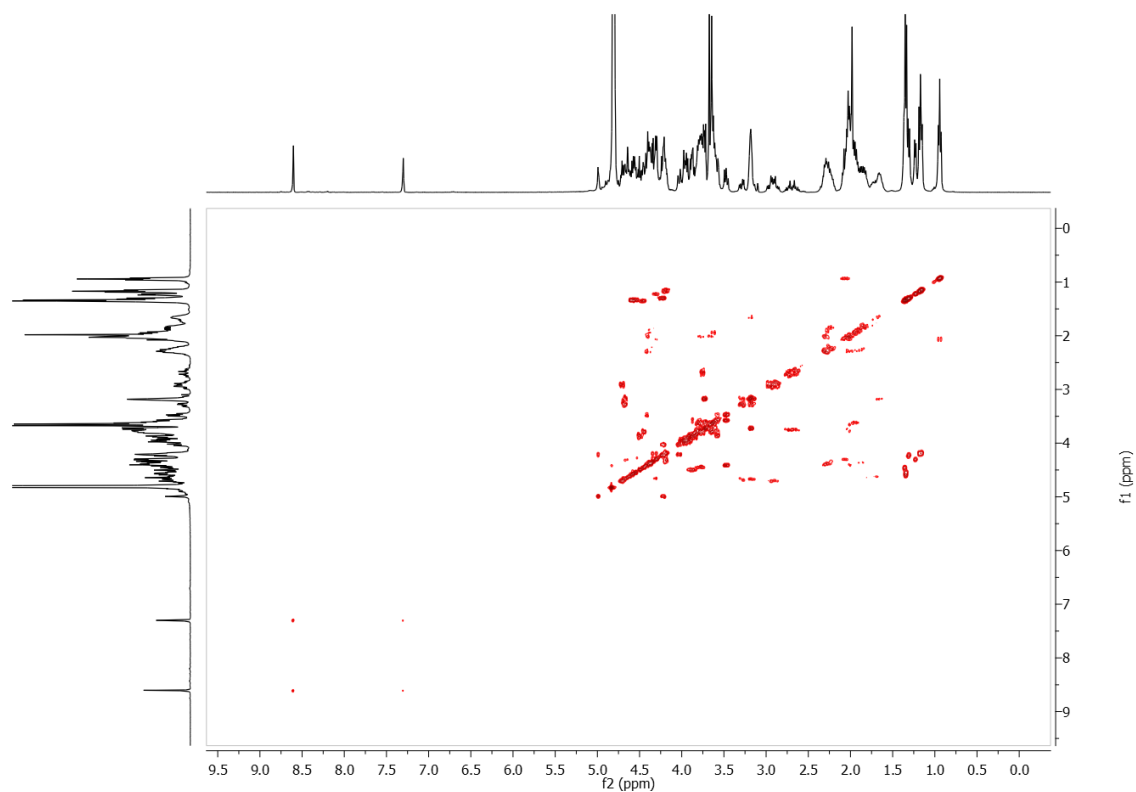


c) ¹⁹F NMR (376.5 MHz, D₂O)

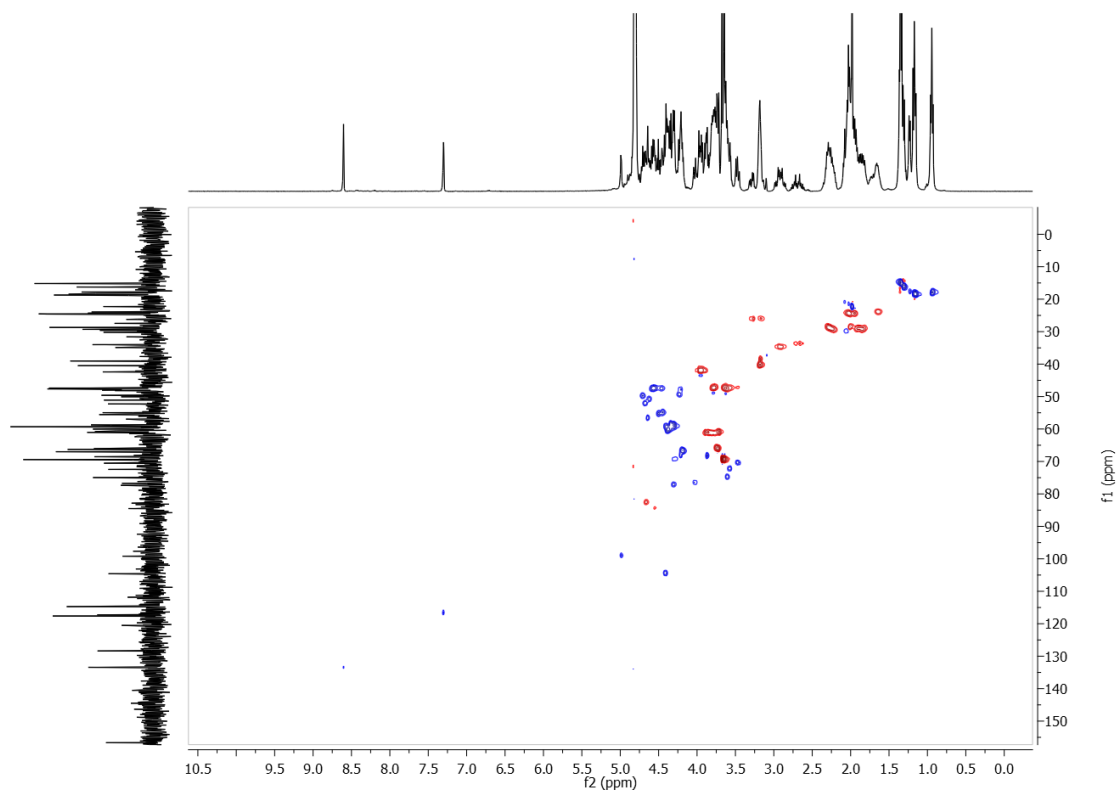


NMR spectra of glycopeptide 17

a) H,H-COSY (400 MHz, D₂O)



b) HMQC (100.6 MHz, D₂O)



c) ^{19}F NMR (376.5 MHz, D_2O)

