Electronic Supporting Information (*ESI*)

Synthesis of a MUC1-Glycopeptide-BSA Conjugate Vaccine Bearing the 3'-Deoxy-3'-Fluoro-Thomson-Friedenreich Antigen

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Contents

General Remarks:

Solvents for moisture-sensitive reactions (toluene, MeCN, CH_2Cl_2, $MeNO_2$) 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 Ac_2O 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 F_{254} 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 H_2SO_4 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, ^{13}C, ^{19}F, 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), br s (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 the saccharide portions were denoted as follows: *N*-acetyl-D-galactosamine (no prime), and D-galactose (’). 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 Phenomenex Luna C18(2) (250×4.6 mm, 10 µm), and Phenomenex Jupiter C18(2) (250×4.6 mm, 10 µm) columns at a flow rate of 1 $mLmin^{-1}$. Preparative HPLC separations were carried out on a JASCO-HPLC System with 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 H_2O–MeCN were used as solvents; if required 0.1% TFA were added.

Gradient **A**:

time (min)	0	10	25	60
MeCN (%)	50	50	77	100
H_2O (%)	50	50	23	0

Gradient **B**:

time (min)	0	5	30	60
MeCN + 0.1% TFA (%)	30	30	90	100
H_2O + 0.1% TFA (%)	70	70	10	0

Gradient **C**:

time (min)	0	1	40	60
MeCN + 0.1% TFA (%)	5	5	70	100
H_2O + 0.1% TFA(%)	95	95	30	0

Gradient **D**:

time (min)	0	5	40	60
MeCN + 0.1% TFA (%)	5	5	25	70
H_2O + 0.1% TFA (%)	95	95	75	30

1,2;5,6-Di-*O*-isopropylidene-α-ribo-hexofuranose-3-ulose (2)
To a stirred suspension of D-glucose (**1**) (20.0 g, 0.11 mmol) in acetone (400 mL) was slowly added conc. H_2SO_4 (16 mL, 0.299 mmol) at 0 °C with a temperature maximum of 10 °C. The mixture was stirred at ambient temperature for 10 h and neutralized with solid NaOH (11.97 g, 0.299 mmol). Thereby, the temperature again was not allowed to exceed 10 °C. After addition of water (30 mL), solid $NaHCO_3$ was added until a pH of 8.0 was reached, and the mixture was allowed to stand over night. The precipitate was filtered through celite, the solvent was removed in vacuo, and the crude product was purified by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 1:1) to give 1,2;5,6-Di-*O*-isopropylidene-D-glucofuranose[S1] as a colorless amorphous solid (18.85 g, 72.42 mmol, 65%). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 5.90 (d, 1H, $J_{H1,H2}$ = 3.65 Hz, 1-H), 4.49 (d 1H, $J_{H2,H1}$ = 3.64 Hz, 2-H), 4.34–4.25 (m, 2H, 6a-H, 6b-H), 4.13 (dd, 1H, $J_{H4,H5}$ = 8.62 Hz, $J_{H4,H3}$ = 7.07 Hz, 4-H), 4.02 (dd, 1H, $J_{H3,H4}$ = 7.04

Hz, $J_{H3,H2}$ = 2.76 Hz, 3-H), 3.96 (dd, 1H, $J_{H5,H4}$ = 8.64 Hz, $J_{H5,H6a/b}$ = 5.26 Hz, 5-H), 2.78 (s, 1H, OH), 1.46, 1.41, 1.33, 1.28 (4s, 12H, 4×CH_3).
To a stirred suspension of pyridinium dichromate PDC (26.23 g, 69.74 mmol) and Ac_2O (19.64 mL, 209.2 mmol) in dry CH_2Cl_2 (80 mL) was slowly added a solution of 1,2;5,6-Di-*O*-isopropylidene-D-glucofuranose (18.15 g, 69.74 mmol) in dry CH_2Cl_2 (40 mL). The mixture was refluxed for 3 h, cooled to room temperature, and the solvent was removed in vacuo. The residue was filtered through a pad of silica (eluent: EtOAc), and the crude 1,2;5,6-di-*O*-isopropylidene-α-ribo-hexofuranose-3-ulose (**2**)[S2] was further used without purification. Yield: 12.74 g, 49.33 mmol, 71%, colorless amorphous solid. R_f= 0.60 (^{c}Hex/EtOAc, 1:1).

3-*O*-Acetyl-1,2;5,6-di-*O*-isopropylidene-α-D-*erythro*-3-hexenofuranose (3)
A solution of ketone **2** (12.74 g, 49.33 mmol,) in a mixture of diethyl ether (200 mL) and water (30 mL) was refluxed for 7 h. The solvent was removed in vacuo and the residue was co-evaporated with toluene (2×50 mL). The crude product was dried (vacuo), dissolved in a mixture of pyridine (100 mL) and Ac_2O (40 mL), and heated to 75 °C over night. The solvent was removed in vacuo and the residue was co-evaporated with toluene (2×50 mL). Purification by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 1:1) provided **3**[S3,S4] (8.18 g, 27.25 mmol, 59%) as a yellowish amorphous solid. R_f = 0.21 (^{c}Hex/EtOAc, 5:1). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 5.99 (d, 1H, $J_{H1,H2}$ = 5.52 Hz, 1-H), 5.35 (d, 1H, $J_{H2,H1}$ = 5.54 Hz, 2-H), 4.66 (t, 1H, $J_{H5,H6a/b}$ = 6.45, 5-H), 4.02 (dd, 2H, $J_{H6a/b,H6a/b}$ = 6.37 Hz, $J_{H6a/b,H5}$ = 2.92 Hz, 6a/b-H), 2.16 (s, 3H, CH_3-OAc), 1.49, 1.42, 1.40, 1.33 (4s, 12H, 4×CH_3).

1,2;5,6-Di-*O*-isopropylidene-α-D-gulofuranose (4)
Palladium-on-activated-charcoal (300 mg) was evacuated in a Schlenk-flask and flushed with H_2. Then, compound **3** (6.57 g, 21.88 mmol), dissolved in MeOH (50 mL), was added and the mixture was stirred at room temperature for 5 d. After filtration through celite, the solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 4:1) to give 3-*O*-acetyl-1,2;5,6-di-*O*-isopropylidene-α-D-gulofuranose[S4] (5.02 g, 16.62 mmol, 76%) as a colorless amorphous solid. R_f= 0.07 (^{c}Hex/EtOAc, 4:1). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 5.80 (d, 1H, $J_{H1,H2}$ = 4.15 Hz, 1-H), 5.05 (dd, 1H, $J_{H3,H2}$ = 5.62 Hz, $J_{H3,H4}$ = 6.78 Hz, 3-H), 4.79 (dd, 1H, $J_{H2,H1}$ = 4.17 Hz, $J_{H2,H3}$ = 5.66 Hz , 2-H), 4.67–4.54 (m, 1H, 5-H), 4.14–4.02 (m, 2H, 4-H, 6a/b-H), 3.58–3.43 (m, 1H, 6a/b-H), 2.12 (s, 3H, CH_3-OAc), 1.56, 1.43, 1.37, 1.33 (4s, 12H, 4×CH_3).

To a stirred solution of 3-*O*-acetyl-1,2;5,6-di-*O*-isopropylidene-α-D-gulofuranose (4.83 g, 15.96 mmol) in MeOH (10 mL) was added a methanolic solution of NaOMe (54.02 mg Na in 75 mL MeOH), so that a pH value of 9.5 was reached. The solution was stirred for 3 h at ambient temperature and neutralized with dry ice. The solvent was removed in vacuo and the product was purified by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 1:1) furnishing **4**[S1,S4] (3.90 g, 14.98 mmol, 94%) as a colorless amorphous solid. R_f= 0.31 (^{c}Hex/EtOAc, 1:1). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 5.73 (d, 1H, $J_{H1,H2}$ = 4.12 Hz, 1-H), 4.61 (dd, 1H, $J_{H2,H1}$ = 4.14 Hz, $J_{H2,H3}$ = 6.27 Hz, 2-H), 4.44 (dt, 1H, $J_{H5,H4}$ = 8.63 Hz, $J_{H5,H6a/b}$ = 7.02 Hz , 5-H), 4.22–4.14 (m, 2H, 3-H, 6a/b-H), 3.85 (dd, 1H, $J_{H4,H5}$ = 8.72 Hz, $J_{H4,H3}$ = 5.86 Hz, 4-H), 3.66 (dd, 1H, $J_{H6a/b,H5}$ = 8.62 Hz, $J_{H6a/b,H6a/b}$ = 7.36 Hz, 6a/b-H), 2.68 (d, 1H, $J_{OH,H3}$ = 6.42 Hz, OH), 1.58, 1.40, 1.37, 1.33 (4s, 12H, 4×CH_3).

3-Deoxy-3-fluoro-α-D-galactopyranose (5)

A solution of gulofuranose **4** (3.23 g, 12.39 mmol) and 4-(dimethylamino)pyridine DMAP (3.03 g, 24.78 mmol) in dry CH_2Cl_2 (75 mL) was treated with *N*,*N*-(diethylamino)sulfur trifluoride DAST (2.74 mL, 22.31 mmol) at -10 °C. The mixture was allowed to warm to room temperature and stirred for 2 d. After quenching with MeOH (10 mL), the solvent was removed in vacuo. Purification by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 4:1) furnished 1,2;5,6-di-*O*-isopropylidene-3-deoxy-3-fluoro-α-D-galactofuranose[S5,S6] (2.73 g, 10.41 mmol, 84%) as a colorless amorphous solid. R_f = 0.50 (^{c}Hex/EtOAc, 4:1). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 5.89 (d, 1H, $J_{H1,H2}$ = 3.82 Hz, 1-H), 4.79 (dd, 1H, $J_{H3,F}$ = 51.46 Hz, $J_{H3,H4}$ = 3.32 Hz, 3-H), 4.71 (dd 1H, $J_{H2,F}$ = 14.78 Hz, $J_{H2,H1}$ = 3.91 Hz, 2-H), 4.31 (dd, 1H, $J_{H5,H6a}$ = 13.75 Hz, $J_{H5,H6b}$ = 6.62 Hz , 5-H), 4.08 (ddd, 1H, $J_{H4,F}$ = 24.31 Hz, $J_{H4,H3}$ = 7.22 Hz, $J_{H4,H5}$ = 3.35 Hz, 4-H), 4.05 (dd, 1H, $J_{H6a/b,H6a/b}$ = 8.42 Hz, $J_{H6a/b,H5}$ = 6.67 Hz, 6a/b-H), 3.79 (dd, 1H, $J_{H6a/b,H6a/b}$ = 8.42 Hz, $J_{H6a/b,H5}$ = 6.54 Hz, 6a/b-H), 1.52, 1.43, 1.35, 1.33 (4s, 12H, 4×CH_3). *^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm):* -187.2 (ddd, $J_{F,H3}$ = 51.46 Hz, $J_{F,H4}$ = 24.31 Hz, $J_{F,H2}$ = 14.78 Hz,).

To a stirred solution of 1,2;5,6-di-*O*-isopropylidene-3-deoxy-3-fluoro-α-D-galactofuranose (2.14 g, 8.15 mmol) in EtOH (50 mL) and water (100 mL) was added ion exchange resin IR 120H$^+$ (3.00 g), and the mixture was refluxed for 3 d. After filtration, the resin was washed with MeOH (50 mL) and the combined filtrates were concentrated in vacuo. The 3-deoxy-3-fluoro-α-D-galactopyranose (**5**)[S5,S6] was dried (vacuo) and used without further purification. Yield: 1.48 g (8.15 mmol, quant.), colorless amorphous solid. *ESI-MS (positive)* calc. for $C_6H_{11}FO_5$: *(m/z):* 205.05 ([M+Na]$^+$, calc.: 205.05), 387.13 ([2M+Na]$^+$, calc.: 387.12).

2,4,6-Tri-*O*-acetyl-3-deoxy-3-fluoro-α-D-galactosyl bromide (6)

A solution of 3-deoxy-3-fluoro-α-D-galactose **5** (1.48 g, 8.15 mmol, 1.0 eq.) in pyridine (50 mL) and Ac_2O (7.65 mL, 81.47 mmol) was stirred over night at ambient temperature. The solvent was removed in vacuo and the residue was co-evaporated with toluene (2×50 mL). Purification by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 2:1) gave 1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-fluoro-α/β-D-galactopyranose[S5,S6] (2.85 g, 8.15 mmol, quant., α:β = 1:1.7) as a colorless amorphous solid. R_f = 0.21 (^{c}Hex/EtOAc, 2:1). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 6.35 (t, 1H, $J_{H1,H2}$ = $J_{H1,F}$ = 4.23 Hz, α-anomer, 1-H), 5.61 (d, 1H, $J_{H1,H2}$ = 8.23 Hz, β-anomer, 1-H). *^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm):* -204.3 (ddt, $J_{F,H3}$ = 48.32 Hz, $J_{F,H4}$ = 10.73 Hz, $J_{F,H2}$ = 5.32 Hz, $J_{F,H1}$ = 5.32 Hz, α-anomer), -200.5 (ddd, $J_{F,H3}$ = 47.34 Hz, $J_{F,H4}$ = 11.54 Hz, $J_{F,H2}$ = 5.61 Hz, β-anomer).

To a solution of 1,2,4,6-tetra-*O*-acetyl-3-deoxy-3-fluoro-α/β-D-galactopyranose (3.90 g, 11.15 mmol) in dry CH_2Cl_2 (50 mL) was slowly added HBr in glacial acetic acid (33%, 7.70 mL 44.59 mmol) at 0 °C. The solution was stirred 3 d at ambient temperature, before HBr/AcOH (33%, 3.00 mL) was again added. After further stirring for 3 d, the mixture was poured onto ice, neutralized with solid $NaHCO_3$, and extracted with CH_2Cl_2 (3×50 mL). The organic phase was washed with sat. aq. $NaHCO_3$ (50 mL), dried ($MgSO_4$), and the solvents were removed in vacuo. Purification by flash chromatography (SiO_2, ^{c}Hex/EtOAc, 2:1) yielded **6**[S7] (2.84 g, 7.65 mmol, 69%) as a colorless amorphous solid. R_f = 0.50 (^{c}Hex/EtOAc, 2:1). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 6.65. (pt, 1H, $J_{H1,H2}$ = 4.23 Hz, $J_{H1,F}$ = 4.23 Hz, 1-H), 5.65 (ddd, 1H, $J_{H4,F}$ = 6.01 Hz, $J_{H4,H3}$ = 3.62 Hz, $J_{H4,H5}$ = 1.17 Hz, 4-H), 5.06 (ptd, 1H, $J_{H2,F}$ = 9.91 Hz, $J_{H2,H3}$ = 9.91 Hz, $J_{H2,H1}$ = 3.87 Hz, 2-H), 5.01 (ddd, 1H, $J_{H3,F}$ = 68.11 Hz, $J_{H3,H2}$ = 9.92 Hz, $J_{H3,H4}$ = 3.81 Hz, 3-H), 4.40 (pt, 1H, $J_{H1,F}$ = 6.41 Hz, $J_{H1,H2}$ = 6.41 Hz, 5-H), 4.20 (dd, 1H, $J_{H6a/b,H6a/b}$ = 11.60 Hz, $J_{H6,H5}$ = 6.08 Hz, $6_{a/b}$-H), 4.07 (ddd, 1H,

$J_{H6a/b,H6a/b}$ = 11.62 Hz, $J_{H6a/b,H5}$ = 6.91 Hz, $J_{H6a/b,H4}$ = 0.88 Hz, $6_{a/b}$-H), 2.14, 2.14, 2.05 (3s, 9H, 3×CH_3). *^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm):* -206.4 – -206.7 (m).

_N_-(9H-Fluoren-9-yl)-methoxycarbonyl-_O_-(2-acetamido-2-deoxy-4,6-_O_-benzyliden-3-_O_-[2,4,6-tri-_O_-acetyl-β-D-3-deoxy-3-fluorogalactopyranosyl]-α-D-galactopyranosyl)-L-threonine _tert_-butyl ester (8)

A solution of Fmoc-Thr-(α-4,6-Bzn-GalNAc)-O*t*Bu **7**[S8] (680 mg, 0.98 mmol) in dry $MeNO_2$–CH_2Cl_2 (3:2, 12.5 mL) was treated with activated powdered molecular sieves (4 Å, 900 mg) and $Hg(CN)_2$ (500 mg, 1.97 mmol). The mixture was stirred for 30 min at ambient temperature under argon, before glycosyl donor **6** (730 mg, 1.97 mmol), dissolved in dry $MeNO_2$–CH_2Cl_2 (3:2, 12.5 mL), was added. The reaction mixture was irradiated in a CEM *Discover* microwave reactor for 3 h at 100 W (80 °C), filtered through Hyflo Super Cel into sat. aq. $NaHCO_3$ (80 mL), and the aq. phase was extracted with CH_2Cl_2 (2×80 mL). The combined organic phases were washed with sat. aq. $NaHCO_3$ (40 mL) and brine (40 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, ^{c}Hex/EtOAc, 2:3) provided **8** (0.64 g, 0.66 mmol, 67%) as a colorless amorphous solid. R_f = 0.38 (^{c}Hex/EtOAc, 2:3). *Analytical RP-HPLC* (Luna C18, gradient **A**, t_R = 26.7 min, λ = 264 nm). $[\alpha]_D^{23}$ = +67.04 (c = 1, $CHCl_3$). *HR-ESI-MS (positive, m/z)* calc. for $C_{50}H_{59}FN_2O_{17}$: 1001.3715 ([M+Na]$^+$, calc.: 1001.3695). *ESI-MS (positive), (m/z):* 979.41 ([M+H]$^+$, calc.: 979.38), 1002.41 ([M+Na]$^+$, calc.: 1002.37). *^{1}H-NMR (400 MHz, $CDCl_3$, COSY, HSQC), δ (ppm):* 7.76 (d, 2H, $J_{H4,H3}$ = $J_{H5,H6}$ = 7.54 Hz, 4-H-, 5-H-Fmoc), 7.60 (d, 2H, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.79 Hz, 1-H-, 8-H-Fmoc), 7.55–7.47 (m, 2H, H_{ar}-Bzn), 7.43–7.26 (m, 7H, 2-H-, 3-H-, 6-H-, 7-H-Fmoc, H_{ar}-Bzn), 5.84 (d, 1H, $J_{NH,2H}$ = 9.19 Hz, NH-Ac), 5.62 (d, 1H, $J_{NH,T\alpha}$ = 9.22 Hz, NH-carbamate), 5.56–5.50 (m, 2H, CH-Bzn, 4'-H), 5.24 (dd, 1H, $J_{H2',F}$ = 19.15 Hz, $J_{H2',H1'}$ = 9.85 Hz, 2'-H), 4.93 (d, 1H, $J_{H1,H2}$ = 3.26 Hz, 1-H), 4.71–4.62 (m, 2H, 1'-H, 2-H), 4.61–4.36 (m, 3H, CH_2-Fmoc, 3'-H), 4.28–3.98 (m, 8H, T$^\alpha$, CH-Fmoc, 4-H, T$^\beta$, 6a/b-H, 6a/b'-H), 3.88 (dd, 1H, $J_{H3,H2}$ = 10.34 Hz, $J_{H3,H4}$ = 2.73 Hz, 3-H), 3.83–3.74 (m, 1H, 5'-H), 3.71–3.59 (m, 1H, 5-H), 2.13, 2.08, 2.02, 2.00 (4s, 12H, 4×CH_3), 1.43 (s, 9H, *t*Bu), 1.24 (d, 3H, $J_{T\gamma,T\beta}$ = 6.3 Hz, T$^\gamma$). *^{13}C-NMR (100.6 MHz, $CDCl_3$, DEPT, HMQC), δ (ppm):* 170.5 (CO-NHAc), 170.1, 169.8, 169.5 (CO), 156.5 (CO-carbamate), 143.8, 143.7 (C1a-, C8a-Fmoc), 141.4 (C4a-, C5a-Fmoc), 137.5 (Cq-Bzn), 128.9, 128.2, 127.9 (C_{ar}-Bzn), 127.1 (C3-, C6-Fmoc), 126.2 (C2-, C7-Fmoc), 126.0 (C_{ar}-Bzn), 124.9 (C1-, C8-Fmoc), 120.1 (C4-, C5-Fmoc), 100.7 (CH-Bzn), 100.5 (C1), 100.3 (C1'), 88.8 (d, $J_{C3',F}$ = 195.82 Hz, C3'), 83.2 (Cq-*t*Bu), 76.2 (T$^\beta$), 75.6 (C4), 74.0 (C3), 70.3 (d, $J_{C5',F}$ = 6.26 Hz, C5'), 69.8 (d, $J_{C2',F}$ = 19.14 Hz, C-2'), 69.1 (C6), 67.0 (CH_2-Fmoc), 66.9 (d, $J_{C4',F}$ = 14.72 Hz, C4'), 63.7 (C5), 61.4 (d, $J_{C6',F}$ = 2.49 Hz, C6'), 59.1 (T$^\alpha$), 47.8 (C2), 47.3 (CH-Fmoc), 28.1 (CH_3-*t*Bu), 20.8, 20.7, 20.7, 20.7 (4×CH_3-Ac), 19.1 (T$^\gamma$). *^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm):* -200.1 – -200.6 (m).

_N_-(9H-Fluoren-9-yl)-methoxycarbonyl-_O_-(4,6-di-_O_-acetyl-2-acetamido-2-deoxy-3-_O_-[2,4,6-tri-_O_-acetyl-β-D-3-deoxy-3-fluorogalacto-pyranosyl]-α-D-galactopyranosyl)-L-threonin (9)

To a solution of disaccharide **8** (324 mg, 0.331 mmol) in a mixture of CH_2Cl_2 and MeOH (4:1, 50 mL) were added $NaHSO_4$–SiO_2[S9] (350 mg), and the suspension was stirred for 18 h at ambient temperature. The catalyst was filtered off and the filtrate was washed with sat. aq. $NaHCO_3$ (3×50 mL) and brine (50 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. Flash chromatography (SiO_2, EtOAc) afforded Fmoc-Thr-(β-3F-Ac_3Gal-(1-3)-α-GalNAc)-O*t*Bu (268 mg, 0.28 mmol, 83%) as a colorless amorphous solid. R_f = 0.33 (EtOAc). *Analytical RP-HPLC* (Luna C18, gradient **A**, t_R = 13.9 min, λ = 264 nm). $[\alpha]_D^{23}$ = +42.00 (c = 1.00, $CHCl_3$). *HR-ESI-MS (positive, m/z)* calc. for $C_{43}H_{55}FN_2O_{17}$: 913.3350 ([M+Na]$^+$, calc.:

913.3382). *ESI-MS (positive), (m/z):* 913.30 ([M+Na]$^+$, calc.: 913.34), 1803.65 ([2M+Na]$^+$, calc.: 1803.69). *^{1}H-NMR (400 MHz, CDCl$_3$, COSY, HSQC), δ (ppm):* 7.74 (d, 2H, $J_{H4,H3}$ = $J_{H5,H6}$ = 7.50 Hz, 4-H-, 5-H-Fmoc), 7.59 (d, 2H, $J_{H1,H2}$ = $J_{H8,H7}$ = 6.77 Hz, 1-H-, 8-H-Fmoc), 7.38 (t, 2H, $J_{H3,H4}$ = $J_{H6,H5}$ = 6.86 Hz, 3-H-, 6-H-Fmoc), 7.28 (t, 2H, $J_{H2,H1}$ = $J_{H7,H8}$ = 7.00 Hz, 2-H-, 7-H-Fmoc), 6.25 (d, 1H, $J_{NH,H2}$ = 9.41 Hz, NH-Ac), 5.89 (d, 1H, $J_{NH,T\alpha}$ = 8.84 Hz, NH-carbamate), 5.53–5.42 (m, 1H, 4'-H), 5.26–5.08 (m, 1H, 2'-H), 4.88–4.74 (m, 1H, 1-H), 4.64–4.19 (m, 6H, 3'-H, 2-H, CH$_2$-Fmoc, 1'-H, CH-Fmoc), 4.19–4.08 (m, 4H, 4-H, T$^\beta$, 6a/b-H), 3.92–3.72 (m, 5H, 6a/b'-H, 5-H, 5'-H, T$^\alpha$), 3.72–3.58 (m, 1H, 3-H), 3.41–3.16 (m, 1H, OH), 2.84 (bs, 1H, OH), 2.11, 2.10, 2.01, 2.00 (4s, 12H, 4×CH$_3$), 1.42 (s, 9H, *t*Bu), 1.27 (d, 3H, $J_{T\gamma,T\beta}$ = 6.22 Hz, T$^\gamma$). *^{13}C-NMR (100.6 MHz, CDCl$_3$, DEPT, HMQC), δ (ppm):* 170.5 (CO-NHAc), 170.3, 170.0, 169.7 (CO), 156.6 (CO-carbamate), 143.7 (C1a-, C8a-Fmoc), 141.3 (C4a-, C5a-Fmoc), 127.8 (C3-, C6-Fmoc), 127.1 (C2-, C7-Fmoc), 125.0 (C1-, C8-Fmoc), 120.0 (C4-, C5-Fmoc), 100.7 (C1'), 100.0 (C1), 88.5 (d, $J_{C3',F}$ = 193.81 Hz, C3'), 83.0 (Cq-*t*Bu), 78.1 (C6), 76.1 (T$^\beta$), 70.0 (C5), 69.9, 68.5, 67.6 (C5', C4, C2'), 66.9 (d, $J_{C4',F}$ = 16.63 Hz, C4'), 66.9 (CH$_2$-Fmoc), 62.4 (C6), 61.4 (C6'), 59.3 (T$^\alpha$), 47.7 (C2), 47.2 (CH-Fmoc), 28.0 (CH$_3$-*t*Bu), 20.8, 20.7, 20.7, 20.7 (4×CH$_3$-Ac), 18.8 (T$^\gamma$). *^{19}F NMR (376.5 MHz, CDCl$_3$), δ (ppm):* -200.0 – -200.5 (m).

To a solution of Fmoc-Thr-(β-3F-Ac$_3$Gal-(1-3)-α-GalNAc)-O*t*Bu (227 mg, 0.255 mmol) in pyridine (10 mL) was added Ac$_2$O (5 mL), and the solution was stirred at ambient temperature over night. The solvent was removed in vacuo, and the residue was co-evaporated with toluene (3×30 mL). Purification by flash chromatography (SiO$_2$, ^{c}Hex/EtOAc, 1:3) afforded Fmoc-Thr-(β-3F-Ac$_3$Gal-(1-3)-α-Ac$_2$GalNAc)-O*t*Bu (248 mg, 0.26 mmol, quant.) as a colorless amorphous solid. R$_f$ = 0.51 (^{c}Hex/EtOAc, 1:3). *Analytical RP-HPLC* (Luna C18, gradient **A**, t_R = 22.1 min, λ = 264 nm). $[\alpha]_D^{23}$ = +48.70 (c = 1, CHCl$_3$). *HR-ESI-MS (positive, m/z)* calc. for $C_{47}H_{59}FN_2O_{19}$: 997.3593 ([M+Na]$^+$, calc.: 997.3594). *ESI-MS (positive), (m/z):* 997.34 ([M+Na]$^+$, calc.: 997.36), 1971.73 ([2M+Na]$^+$, calc.: 1971.73). *^{1}H-NMR (400 MHz, CDCl$_3$, COSY, HSQC), δ (ppm):* 7.76 (d, 2H, $J_{H4,H3}$ = $J_{H5,H6}$ = 7.53 Hz, 4-H-, 5-H-Fmoc), 7.59 (d, 2H, $J_{H1,H2}$ = $J_{H8,H7}$ = 7.17 Hz, 1-H-, 8-H-Fmoc), 7.40 (t, 2H, $J_{H3,H4}$ = $J_{H6,H5}$ = 7.44 Hz, 3-H-, 6-H-Fmoc), 7.31 (t, 2H, $J_{H2,H1}$ = $J_{H7,H8}$ = 7.41 Hz, 2-H-, 7-H-Fmoc), 5.91 (d, 1H, $J_{NH,H2}$ = 9.37 Hz, NH-Ac), 5.51–5.48 (m, 1H, 4'-H), 5.46 (d, 1H, $J_{NH,T\alpha}$ = 9.10 Hz, NH-carbamate), 5.33 (s, 1H, 4-H), 5.19–5.10 (m, 1H, 2'-H), 4.82 (d, 1H, $J_{H1,H2}$ = 2.83 Hz, 1-H), 4.61–4.36 (m, 5H, 3'-H, 2-H, CH$_2$-Fmoc, 1'-H), 4.28–4.01 (m, 7H, 5-H, T$^\alpha$, T$^\beta$, 6a-H, 6'-Ha/b, CH-Fmoc), 3.96 (dd, 1H, $J_{H6b,H6a}$ = 11.06 Hz, $J_{H6b,H5}$ = 7.25 Hz, 6b-H), 3.83–3.73 (m, 2H, 3-H, 5'-H), 2.15, 2.11, 2.11, 2.03, 2.02, 2.00 (6s, 18H, 6×CH$_3$), 1.44 (s, 9H, *t*Bu), 1.27 (d, 3H, $J_{T\gamma,T\beta}$ = 5.65 Hz, T$^\gamma$). *^{13}C-NMR (100.6 MHz, CDCl$_3$, DEPT, HMQC), δ (ppm):* 170.7 (CO-NHAc), 170.4, 170.3, 170.2, 170.1, 169.7 (CO), 156.6 (CO-carbamate), 143.9 (C1a-, C8a-Fmoc), 141.6 (C4a-, C5a-Fmoc), 128.1 (C3-, C6-Fmoc), 127.3 (C2-, C7-Fmoc), 125.1 (C1-, C8-Fmoc), 120.3 (C4-, C5-Fmoc), 100.5 (d, $J_{C1',F}$ = 10.38 Hz, C1'), 100.3 (C1), 88.85 (d, $J_{C3',F}$ = 194.33 Hz, C3'), 83.5 (Cq-*t*Bu), 77.4 (T$^\beta$), 73.7 (C3), 70.2 (d, $J_{C5',F}$ = 5.95 Hz, C5'), 70.0 (d, $J_{C3',F}$ = 19.26 Hz, C2'), 69.2 (C4), 68.2 (C5), 67.2 (CH$_2$-Fmoc), 66.9 (d, $J_{C4',F}$ = 16.68 Hz, C4'), 63.2 (C6), 61.2 (d, $J_{C6',F}$ = 2.28 Hz, C6'), 59.3 (T$^\alpha$), 48.6 (C2), 47.4 (CH-Fmoc), 28.3 (CH$_3$-*t*Bu), 21.0, 20.9, 20.9, 20.9, 20.9, 20.9 (6×CH$_3$-Ac), 18.8 (T$^\gamma$). *^{19}F NMR (376.5 MHz, CDCl$_3$), δ (ppm):* -200.4 (ddd, $J_{F,H3'}$ = 47.62 Hz, $J_{F,H4'}$ = 11.12 Hz, $J_{F,H2'}$ = 4.41 Hz).

To Fmoc-Thr-(β-3F-Ac$_3$Gal-(1-3)-α-Ac$_2$GalNAc)-O*t*Bu (276 mg, 0.28 mmol), dissolved in water (0.6 mL) was added TFA (6.0 mL), and the solution was stirred for 2.5 h at ambient temperature. The mixture was co-evaporated with toluene (5×30 mL) and CH$_2$Cl$_2$ (3×30 mL), and the residue was purified by flash chromatography (SiO$_2$, EtOAc/MeOH/AcOH, 9:1:0.1) to afford Fmoc-Thr-(β-3F-Ac$_3$Gal-(1-3)-α-Ac$_2$GalNAc)-OH **9** (225 mg, 0.25 mmol, 87%) as a colorless amorphous solid. R$_f$ = 0.11 (EtOAc/MeOH/AcOH, 9:1:0.1). *Analytical RP-HPLC*

(Luna C18, gradient **B**, t_R = 19.4 min, λ = 264 nm). *HR-ESI-MS (positive, m/z)* calc. for $C_{43}H_{51}FN_2O_{19}$: 941.2979 ($[M+Na]^+$, calc.: 941.2968). *ESI-MS (positive), (m/z):* 941.30 ($[M+Na]^+$, calc.: 941.30).

Amino-4,7,10-trioxadodecanylamido-*N*-L-prolyl-L-Alanyl-L-histidyl-L-glycyl-L-valyl-*O*-(2-acetamido-2-deoxy-3-*O*-[3-deoxy-3-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-prolin (10)

The synthesis was carried out in an Applied Biosystems ABI 433A peptide synthesiser (standard program Fastmoc 0.1 mmol) using pre-loaded Fmoc-Pro-Trt-Tentagel S resin (417 mg, 0.10 mmol, loading 0.24 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 coupling of the amino acids (1 mmol or 10 eq. based on the loaded resin) was 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 Ac_2O (0.5 M), DIPEA (0.125 M), and HOBt (0.015 M) in NMP (10 min vortex).

Coupling of the protected TF building block **9** (152 mg, 0.165 mmol, 1.65 eq. based on the loaded resin) was performed using HATU (1.2 eq. with respect to **9**), HOAt (1.2 eq.) and NMM (2.4 eq.) for activation (8 h vortex). After coupling of the remaining five amino acids by the standard procedure, a triethylene glycol spacer[S10] (1 mmol, 10 eq. based on the loaded resin) was coupled using HBTU (1 mmol), HOBt (1 mmol) and DIPEA (2 mmol) in DMF (20−30 min vortex) and the *N*-terminal Fmoc group was removed by piperidine (20 %) in NMP. The peptide was detached from resin with simultaneous removal of all side chain protecting groups by shaking with TFA (10 mL), TIS (1 mL) and H_2O (1 mL) for 2 h. The solution was filtered, the resin was washed with TFA (2×10 mL) and the combined solutions were concentrated in vacuo, H_2O was added (10 mL) and the crude glycopeptide was subjected to lyophilisation.

Half of the obtained peptide was dissolved in 30 mL of MeOH (HPLC grade). A fresh solution of NaOMe in MeOH (0.5 g Na in 25 mL MeOH (HPLC grade)) was added drop wise until pH 9.0 was reached. The reaction mixture was stirred over night and neutralised with a few drops of HOAc. The solvent was removed in vacuo, H_2O was added (10 mL) and the residue was subjected to lyophilization. Purification by preparative RP-HPLC (Luna C18, gradient **C**) afforded glycopeptide **10** (61 mg, 0.025 mmol, 50%) as a colorless amorphous solid. *Analytical RP-HPLC* (Luna C18, gradient **C**, R_t = 9.2 min, λ = 214 nm). $[\alpha]_D^{23}$ = -112.2 (c = 1, H_2O). *HR-ESI-MS (positive, m/z)* calc. for $C_{103}H_{166}FN_{27}O_{41}$: 1229.0948 ($[M+2H]^{2+}$, calc.: 1229.0937). *ESI-MS (positive), (m/z):* 1229.65 ($[M+2H]^{2+}$, calc.: 1229.09), 820.09 ($[M+3H]^{3+}$, calc.: 819.72). *MALDI-TOF-MS (dhb, positive) (m/z)*: 2458.48 ($[M+H]^+$, calc.: 2458.57). *^{1}H-NMR (400 MHz, $CDCl_3$, COSY, HSQC), δ (ppm):* 8.51 (d, 1H, $J_{H\varepsilon,H\delta}$ = 1.30 Hz, H_ε), 7.20 (d, 1H, $J_{H\delta,H\varepsilon}$ = 1.04 Hz, H_δ), 4.84 (d, 1H, $J_{H1,H2}$ = 3.67 Hz, 1-H), 4.65 – 4.00 (m, 25H, D_α {4.60}, H_α {4.57}, $T_{T*\alpha}$ {4.53}, R_α {4.52}, $A_{3\alpha}$ {4.48}, $A_{2\alpha}$ {4.42}, $S_{2\alpha}$ {4.41}, 1'-H {4.37}, $S_{1\alpha}$ {4.36}, $A_{4\alpha}$ {4.35}, $P_{1\text{-}5\alpha}$ {4.33, 4.32, 4.28, 4.26, 4.24}, $T_{2\alpha}$ {4.24}, $T_{1\alpha}$ {4.20}, V_α {4.20}, $T_{T*\beta}$ {4.20}, $A_{1\alpha}$ {4.13}, $T_{1\beta}$ {4.10}, 2-H {4.10}, $T_{2\beta}$ {4.07}, 2'-H {4.06}), 3.98–3.45 (m, 37H, $G_{1\alpha}$ {3.96}, 5-H {3.93},3-H {3.90}, $G_{2\alpha}$ {3.78}, $S_{2\beta}$ {3.77}, $S_{1\beta}$ {3.66}, 6a/b'-H {3.66}, 4`-H {3.65}, 6a/b-H {3.64}, $CH_2$-spacer {3.64}, 4×$CH_2O$-spacer {3.64–3.52}, 4-H {3.60}, $P_{1\text{-}5\delta}$ {3.69, 3.67, 3.64, 3.57, 3.50}, 5`-H {3.52}), 3.19 (dd, 1H, $J_{H\beta a,H\beta b}$ = 15.52 Hz, $J_{H\beta a,H\alpha}$ = 5.49 Hz, $H_{\beta a}$), 3.14–3.02 (m, 7H, CH_2-spacer {3.11}, R_δ {3.08}, $H_{\beta b}$ {3.07},), 2.91–2.72 (m, 2H, $D_{\beta a}$, $D_{\beta b}$), 2.70–2.48 (m, 2H, CH_2-spacer), 2.29–2.07 (m, 6H, $P_{1\text{-}3\beta}$ {2.27, 2.18, 2.09}), 2.03–1.67 (m, 19H, $P_{1\text{-}5\gamma}$ {1.96-1.80}, V_β {1.96}, $P_{4,5\beta}$ {1.92, 1.76}, Ac-NH (s, 1.87},

$R_{\beta a}$ {1.69}), 1.67–1.48 (m, 3H, $R_{\beta b}$ {1.59}, Rγ {1.54}), 1.31–1.18 (m, 12H, $A_{2\beta}$, {1.29}, $A_{3\beta}$ {1.26}, $A_{4\beta}$ {1.22}, {1.21, d, $J_{A\beta,A\alpha}$ = 7.20 Hz, $A_{1\beta}$}), 1.15 (d, 3H, $J_{T*\gamma,T\beta}$ = 6.27 Hz, $T_{T*\gamma}$), 1.10–1.02 (m, 6H, $T_{2\gamma}$, $T_{1\gamma}$), 0.85 (t, 6H, $J_{V\gamma,V\beta}$ = 6.96 Hz, Vγ). *^{13}C-NMR (100.6 MHz, $CDCl_3$, DEPT, HMQC), δ (ppm):* 175.9, 174.9, 174.4, 174.0, 173.9, 173.7, 173.6, 173.5, 173.1, 172.6, 172.5, 172.4, 172.0, 171.9, 171.5, 171.3, 171.2, 171.2, 171.1, 170.9, 170.7 (CO), 156.7 (C=NH), 133.4 ($H_{C\gamma}$), 128.4 ($H_{C\varepsilon}$), 117.2 ($H_{C\delta}$), 103.8 (d, $J_{C1',F}$ = 11.68 Hz, C1'), 99.1 (C1), 92.8 (d, $J_{C3',F}$ = 182.83 Hz, C3`), 77.4 (C3), 77.0 ($T_{T*\beta}$), 73.7 (d, $J_{C5',F}$ = 6.24 Hz, C5`), 70.9 (C5), 69.6, 69.5, 69.4, 69.4 (CH_2-spacer), 68.8 (C4), 67.0 ($T_{1\beta}$), 67.0 ($T_{2\beta}$), 66.7 (d, $J_{C2',F}$ = 16.05 Hz,C2`), 66.3 (CH_2-spacer), 60.8 (C6'), 60.5, 60.0, 60.0, 59.4, 59.3 ($P_{1\text{-}5\alpha}$), 60.5 (C6), 60.2, 60.1 ($S_{1\beta}$, $S_{2\beta}$), 58.9 (V_α), 58.9 ($T_{1\alpha}$), 58.7 ($T_{2\alpha}$), 57.0 ($T_{T*\alpha}$), 55.5 ($S_{1\alpha}$), 54.9 ($S_{2\alpha}$), 52.3 (H_α), 51.1 (R_α), 50.1 (D_α), 48.2 (C2), 48.2 ($A_{1\alpha}$), 48.0 ($A_{2\alpha}$), 47.7 ($A_{3\alpha}$), 47.7 ($A_{4\alpha}$), 48.0, 47.8, 47.7, 47.6, 47.4 ($P_{1\text{-}5\delta}$), 42.4 ($G_{2\alpha}$), 42.3 ($G_{1\alpha}$), 40.5 (R_δ), 39.0 (CH_2-spacer), 35.0 (D_β), 34.0 (CH_2-spacer), 30.2 (V_β), 29.6, 29.3, 29.3, 29.2, 28.7 ($P_{1\text{-}5\beta}$), 27.4 (R_β), 26.2 (H_β), 24.7, 24.6, 24.5, 24.3, 24.0 ($P_{1\text{-}5\gamma}$), 23.9 (R_γ), 22.3 (CH_3-AcNH), 18.8 ($T_{1\gamma}$), 18.7 ($T_{2\gamma}$), 18.5 ($V_{\gamma a}$), 18.3 ($T_{T*\gamma}$), 17.8 ($V_{\gamma b}$), 16.3, 15.2, 15.1 ($A_{1\text{-}4\beta}$). *^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm):* -199.2 (ddd, $J_{F,3'\text{-}H}$ = 48.12 Hz, $J_{F,4'\text{-}H}$ = 12.86 Hz, $J_{F,2'\text{-}H}$ = 5.62 Hz, 4'-F).

BSA-conjugate (11)

To a solution of glycopeptide **10** (20 mg, 8.138 µmol) in a mixture of EtOH and water (5 mL, 1:1) was added 3,4-diethoxy-3-cyclobutene-1,3-dione (1.26 µL, 8.545 µmol). After adjusting the pH value to 8.0 by adding sat. aq. $NaHCO_3$ (15 µL) the mixture was stirred at ambient temperature for 3 h, neutralized with diluted HOAc and subjected to lyophilisation. Purification by preparative RP-HPLC (Luna C18, gradient **D)** afforded the glycopeptide monoamide (6.8 mg, 2.63 µmol, 31%) as a colorless amorphous solid. *Analytical RP-HPLC* (Luna C18, gradient **D**, R_t = 22.2 min, λ = 214 nm). $[\alpha]_D^{23}$ = -92.7 (c = 1.00, H_2O). *HR-ESI-MS (positive, m/z)* calc. for $C_{109}H_{170}FN_{27}O_{44}$: 1291.0972 ($[M+2H]^{2+}$, calc.: 1291.1018). *ESI-MS (positive), (m/z):* 1291.05 ($[M+2H]^{2+}$, calc.: 1291.10), 868.40 ($[M+2H+Na]^{3+}$, calc.: 868.39). *MALDI-TOF-MS (dhb, positive) (m/z)*: 2582.80 ($[M+H]^+$, calc.: 2582.67). *^{1}H-NMR (400 MHz, $CDCl_3$, COSY, HSQC), δ (ppm):* 8.50 (d, 1H, $J_{H\varepsilon,H\delta}$ = 1.36 Hz, H_ε), 7.20 (d, 1H, $J_{H\delta,H\varepsilon}$ = 1.09 Hz, H_δ), 4.83 (d, 1H, $J_{H1,H2}$ = 3.96 Hz, 1-H), 4.70–4.00 (m, 27H, CH_2O-squarate {4.62}, D_α {4.60}, H_α {4.57}, $T_{T*\alpha}$ {4.54}, R_α {4.51}, $A_{3\alpha}$ {4.48}, 3'-H {4.40}, $A_{2\alpha}$ {4.41}, $S_{2\alpha}$ {4.41}, 1'-H {4.36}, $A_{4\alpha}$ {4.35}, $S_{1\alpha}$ {4.35}, $P_{1\text{-}5\alpha}$ {4.35, 4.31, 4.27, 4.20, 4.17}, $T_{1\alpha}$ {4.23}, $T_{T*\beta}$ {4.22}, V_α {4.19}, $T_{2\alpha}$ {4.19}, $A_{1\alpha}$ {4.13}, 2-H {4.11} $T_{1\beta}$ {4.10}, $T_{2\beta}$ {4.06}, 2'-H {4.06}), 3.97–3.41 (m, 41H, $G_{1\alpha}$ {3.93}, 5-H {3.92}, 3-H {3.90}, $G_{2\alpha}$ {3.80}, $S_{2\beta}$ {3.77}, $S_{1\beta}$ {3.69}, 6a/b'-H {3.66}, CH_2-spacer {3.68}, 4`-H {3.66}, $CH_2$-spacer {3.64}, 6a/b-H {3.61}, 4×$CH_2O$-spacer {3.61–3.48}, 4-H {3.60}, $P_{1\text{-}5\delta}$ {3.75, 3.70, 3.57, 3.53, 3.50}, 3.52 {$CH_2$-spacer}, 5`-H {3.52}), 3.20 (dd, 1H, $J_{H\beta a,H\beta b}$ = 15.52 Hz, $J_{H\beta a,H\alpha}$ = 5.57 Hz, $H_{\beta a}$), 3.13–3.01 (m, 3H, R_δ {3.09}, $H_{\beta b}$ {3.08}), 2.88–2.69 (m, 2H, $D_{\beta a}$, $D_{\beta b}$), 2.67–2.47 (m, 2H, CH_2-spacer), 2.26–2.07 (m, 5H, $P_{1\text{-}5\beta a}$ {2.25, 2.23, 2.19, 2.15, 2.07}), 2.03–1.66 (m, 20H, $P_{1\text{-}5\gamma}$ {1.97–1.86}, V_β {1.95}, $P_{1\text{-}5\beta b}$ {1.95, 1.91, 1.85 1.82, 1.79}, Ac-NH (s, 1.87}, $R_{\beta a}$ {1.68}), 1.67–1.48 (m, 3H, $R_{\beta b}$ {1.59}, Rγ {1.55}), 1.31 (t, 3H, $J_{CH3,CH2}$ = 6.97 Hz, H_3C-CH_2-squarate), 1.29–1.18 (m, 12H, $A_{2\beta}$, {1.29}, $A_{3\beta}$ {1.25}, $A_{4\beta}$ {1.22}, {1.21, d, $J_{A\beta,A\alpha}$ = 7.21 Hz, $A_{1\beta}$}), 1.14 (d, 3H, $J_{T*\gamma,T\beta}$ = 6.32 Hz, $T_{T*\gamma}$), 1.11–1.03 (m, 6H, $T_{2\gamma}$, $T_{1\gamma}$), 0.84 (t, 6H, $J_{V\gamma,V\beta}$ = 6.86 Hz, Vγ). *^{13}C-NMR (100.6 MHz, $CDCl_3$, DEPT, HMQC), δ (ppm):* 175.8, 174.9, 174.8, 174.4, 173.9, 173.7, 173.6, 173.5, 173.1, 172.7, 172.6, 172.5 172.4, 172.1, 172.0, 171.9, 171.5, 171.5, 171.3, 171.2, 171.1, 170.9, 170.9 (CO), 156.7 (C=NH), 133.5 ($H_{C\gamma}$), 128.2 ($H_{C\varepsilon}$), 117.0 ($H_{C\delta}$), 103.8 (C1'), 99.0 (C1), 90.9 (d, $J_{C\text{-}3',F}$ = 186.62 Hz, C3`), 77.3 (C3), 76.9 ($T_{T*\beta}$), 73.7 (C5`), 70.9 (C5), 70.5 (CH_2-squarate), 69.6 (CH_2-spacer), 69.2 (C4`), 68.7 ($T_{2\beta}$), 68.1 (C4), 67.0 (C2`), 66.9 ($T_{1\beta}$), 66.1 (CH_2-spacer), 61.2, 61.1 ($S_{1\beta}$, $S_{2\beta}$), 61.1 (C6), 60.8, 60.6, 60.1, 59.7, 59.3 ($P_{1\text{-}5\alpha}$), 60.6 (C6'), 59.0 ($T_{1\alpha}$), 58.9 ($T_{2\alpha}$), 58.8 (V_α), 56.9 ($T_{T*\alpha}$), 55.5

($S_{1\alpha}$), 55.0 ($S_{2\alpha}$), 52.2 (H_α), 51.1 (R_α), 50.2 (D_α), 49.6 (C2), 48.4 ($A_{1\alpha}$), 47.7 ($A_{2\alpha}$), 47.6 ($A_{3\alpha}$), 47.4 ($A_{4\alpha}$), 48.0, 47.8, 47.7, 47.6, 47.4 ($P_{1\text{-}5\delta}$), 43.8 (CH_2-spacer), 42.3 ($G_{2\alpha}$), 42.3 ($G_{1\alpha}$), 40.5 (R_δ), 35.3 (D_β), 34.0 (CH_2-spacer), 30.2 (V_β), 29.8, 29.6, 29.3, 29.0, 28.8 ($P_{1\text{-}5\beta}$), 27.6 (R_β), 26.2 (H_β), 24.1, 24.6, 24.5, 24.4, 24.3 ($P_{1\text{-}5\gamma}$), 23.9 (R_γ), 22.4 (CH_3-AcNH), 18.8 ($T_{1\gamma}$), 18.7 ($T_{2\gamma}$), 18.5 ($V_{\gamma a}$), 18.3 ($T_{T^*\gamma}$), 17.8 ($V_{\gamma b}$), 16.3, 15.2, 15.1 ($A_{1\text{-}4\beta}$), 15.0 (H_3C-CH_2-squarate).
^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm): -199.2 (ddd, $J_{F,3'\text{-}H}$ = 47.83 Hz, $J_{F,4'\text{-}H}$ = 13.27 Hz, $J_{F,2'\text{-}H}$ = 6.14 Hz, 4'-F).
The squarate monoamide (2.0 mg, 0.775 µmol) was dissolved in a phosphate buffer (pH 9.5, 130 mg Na_2HPO_4, 2 mL H_2O) and treated with BSA (2.0 mg, 0.031 µmol). The mixture was stirred for 5 d at ambient temperature, before it was subjected to dialysis (membrane 30 kDa) with deionized water (3×50 mL). Lyophilisation yielded the BSA conjugate (5.1 mg, 0.076 µmol) as a colorless fluffy solid. *MALDI-TOF-MS (sinapic acid, positive) (m/z)*: 79470.12.

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; 50 µL/well). The diluted serum is added and increasingly further diluted (1:2 or 1:3). Then 10 µL of the fluorinated glycopeptide **10** (c = 100µg/mL) is added and incubated for 60 minutes.
Coating: The BSA-conjugates used as vaccines were dissolved in a phosphate buffer (0.1 M $Na_2HPO_4 \cdot H_2O$, pH = 9.3; *c* = 5 µg/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 tree 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 µg/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 µL/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 µL/well ABTS/H_2O_2 solution (c(ABTS) = 1 mg/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 λ = 410 nm. As a negative control, the ELISA test was performed without coating by the antigen conjugate.

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. 2)

concentration [µg/ml]	1/10.000	1/30.000	1/90.000	1/180.000
MUC1(20)6Thr-3'F-TF	1.1967	0.7808	0.3684	0.1643
MUC1(20)6Thr-TF	1.0874	0.7260	0.4339	0.1924
positive control	1.1996	1.0170	0.7071	0.3241
negative control	0.0560	0.0574	0.0570	0.0532

Data for Fig. 3)

concentration [µg/ml]	1/5.000	1/15.000	1/45.000	1/135.000
MUC1(20)6Thr-3'F-TF	0.7076	0.2698	0.1539	0.0876
MUC1(20)6Thr-6',6F-TF	1.0086	0.4589	0.2300	0.1300
MUC1(20)6Thr-TF	1.2310	0.6080	0.3397	0.1420
positive control	1.3518	0.9745	0.5135	0.2724
negative control	0.0563	0.0590	0.0558	0.0563

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Spectra of disaccharide **8**

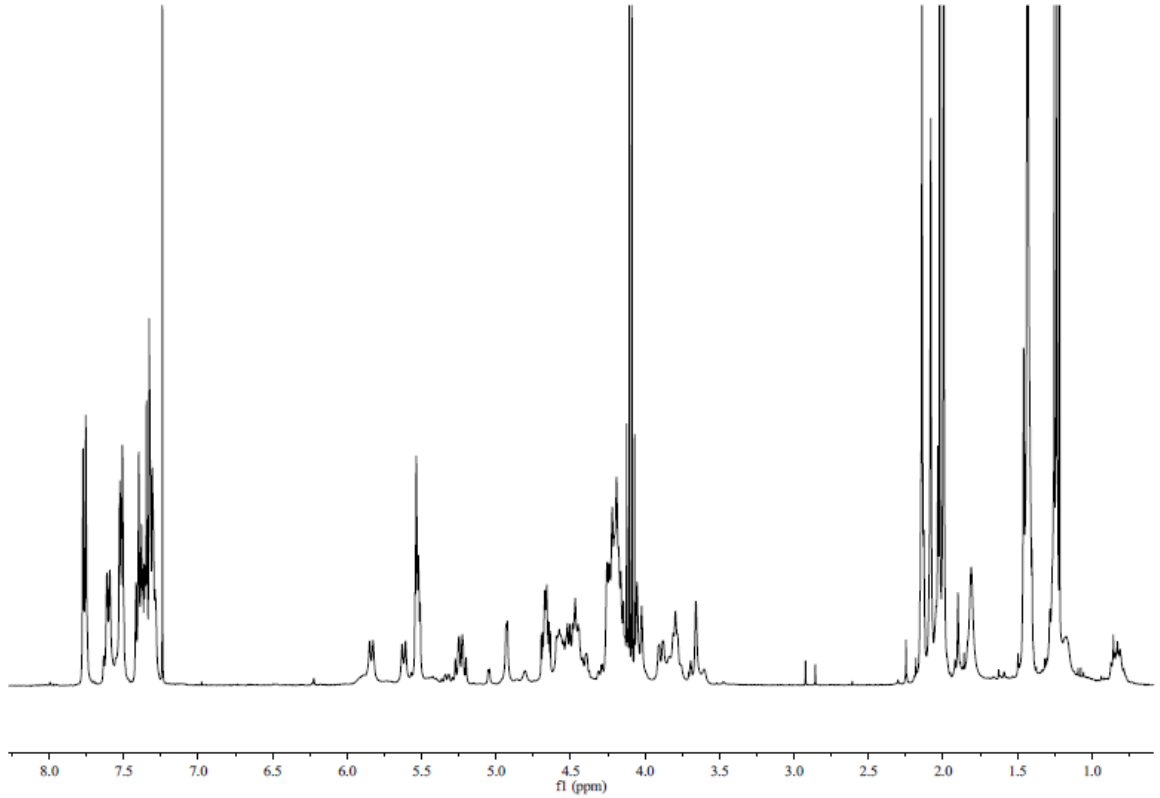

Fig. 1: ^{1}H-NMR (300 MHz, $CDCl_3$) spectra of **8**

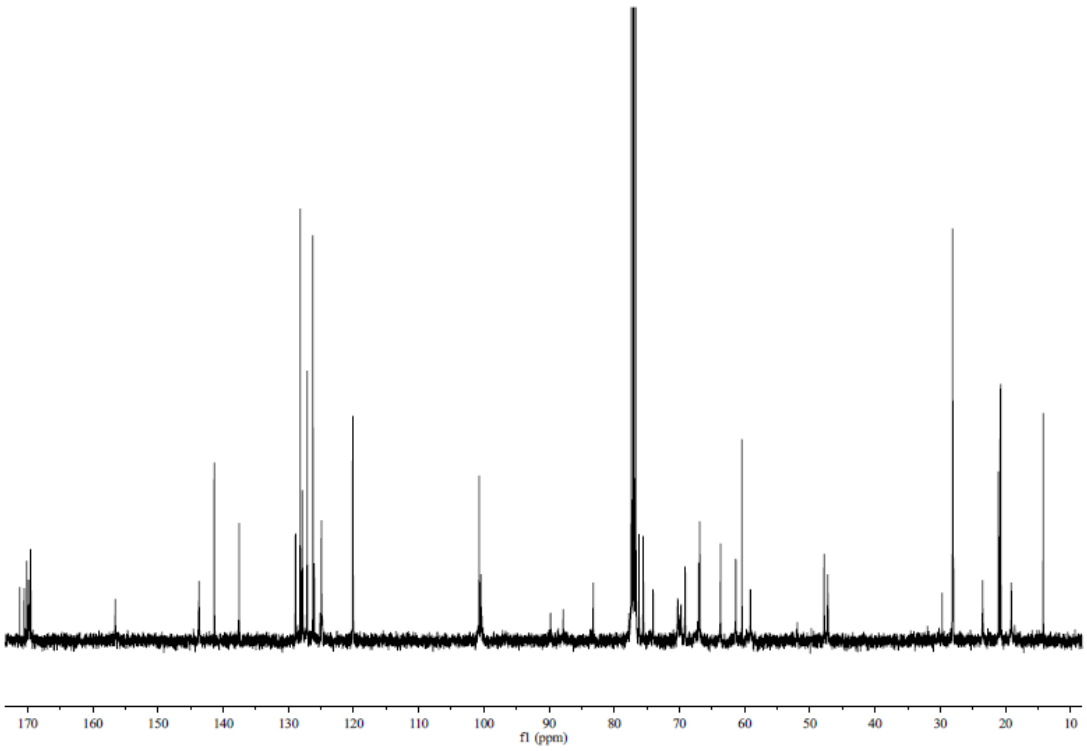

Fig. 2: ^{13}C-NMR (75 MHz, $CDCl_3$) spectra of **8**

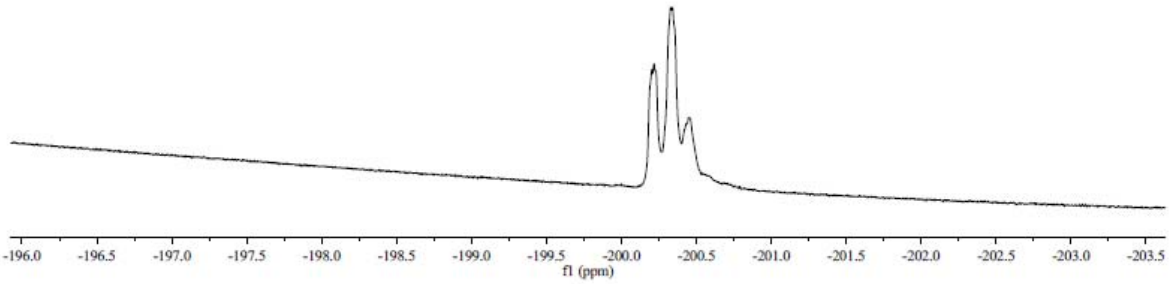

Fig. 3: ^{19}F-NMR (376.5 MHz, $CDCl_3$) spectra of **8**

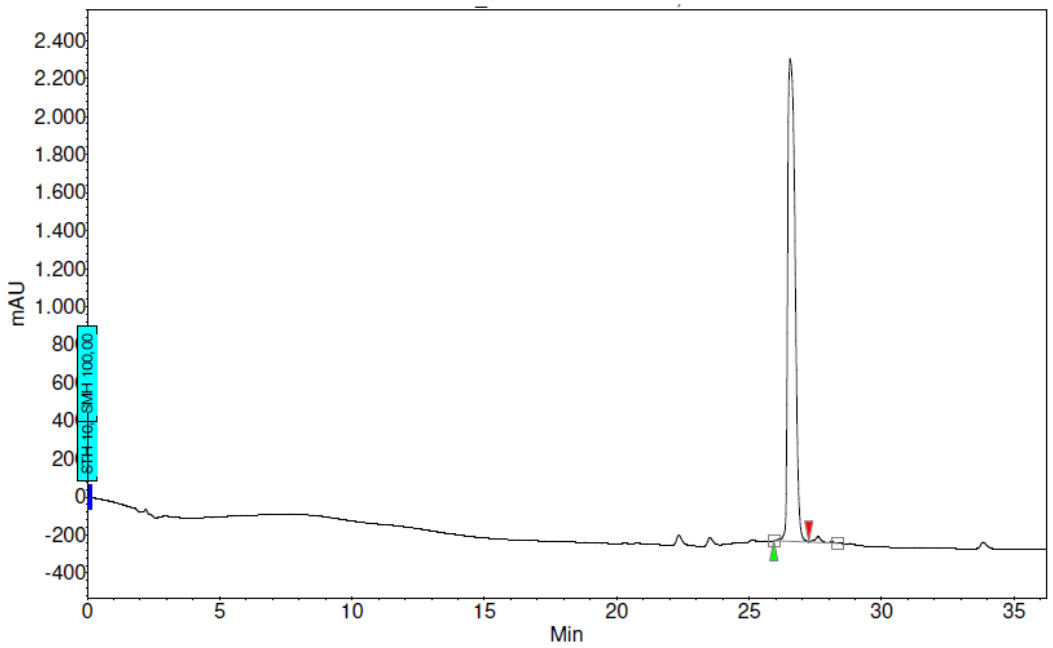

Fig. 4: Analytical RP-HPLC chromatogram of **8**

RP-HPLC chromatogram of disaccharide **9**

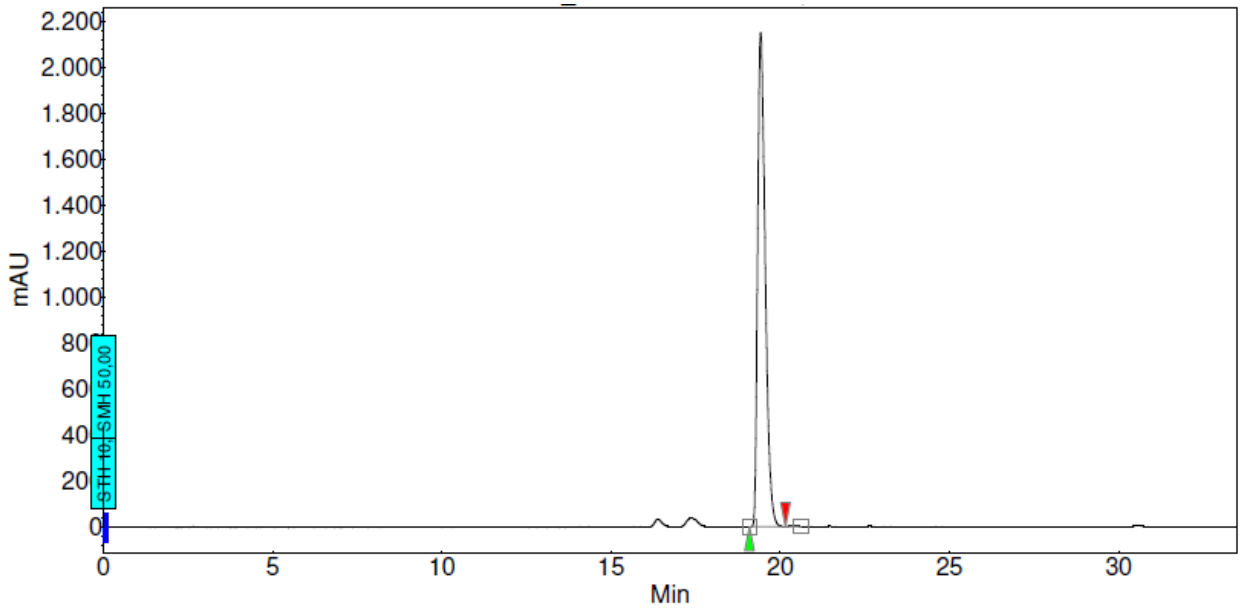

Fig. 5: Analytical RP-HPLC chromatogram of **9**

Spectra of glycopeptide **10**

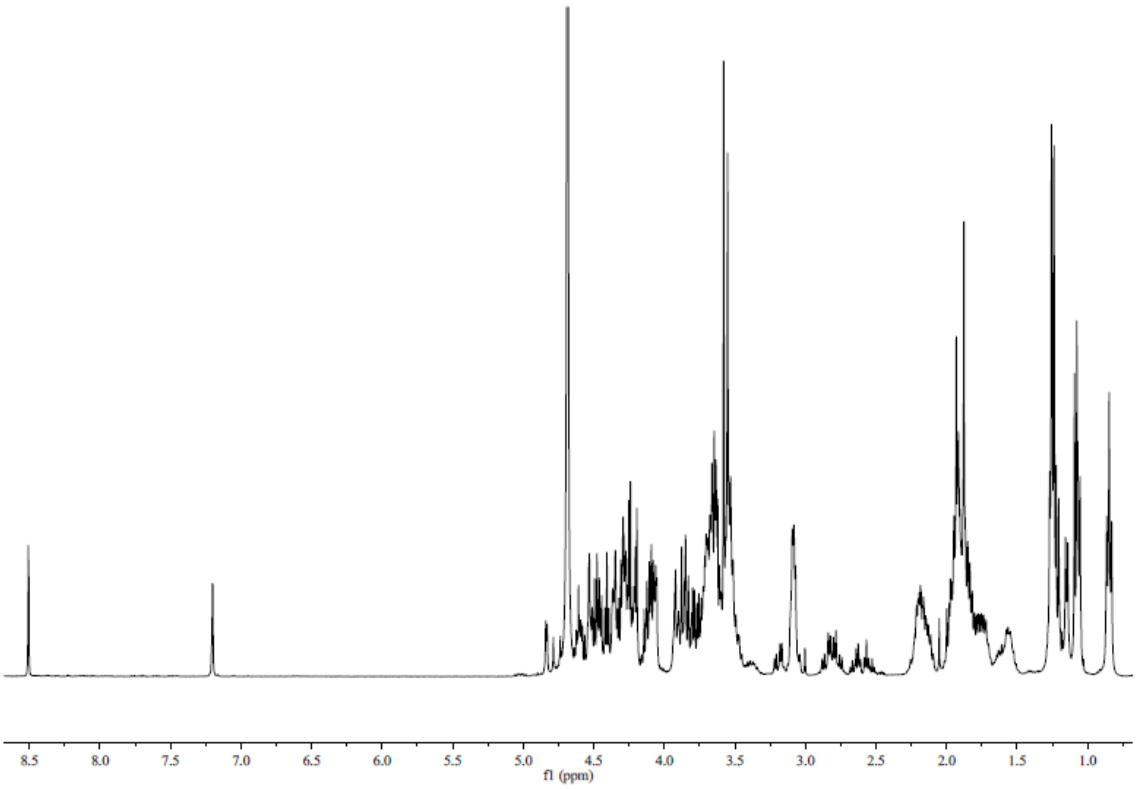

Fig. 6: ^{1}H-NMR (300 MHz, $CDCl_3$) spectra of **10**

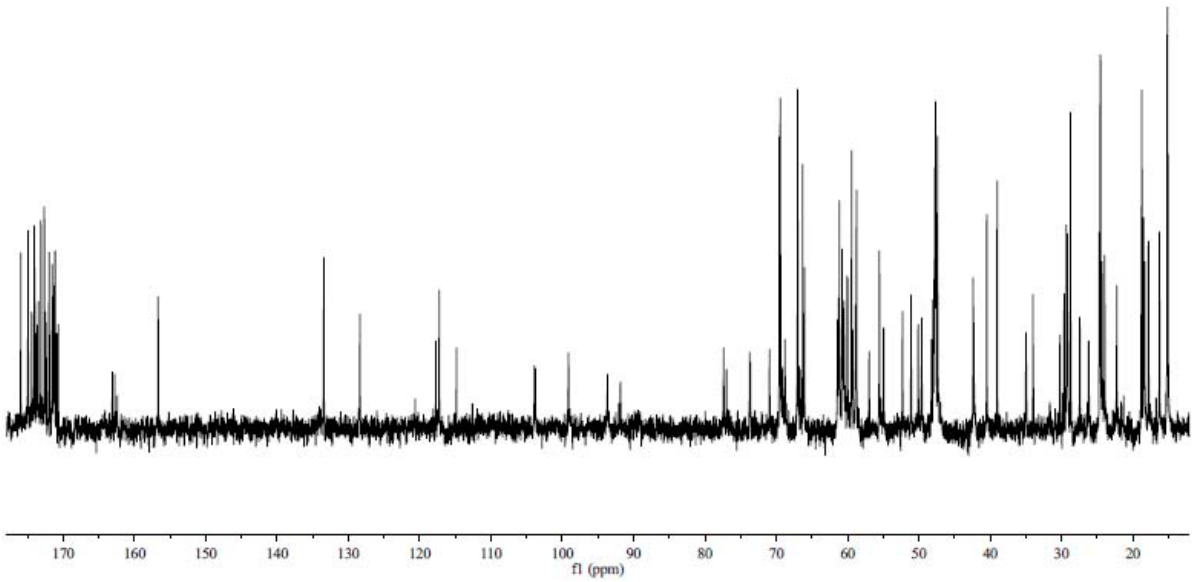

Fig. 7: ^{13}C-NMR (75 MHz, $CDCl_3$) spectra of **10**

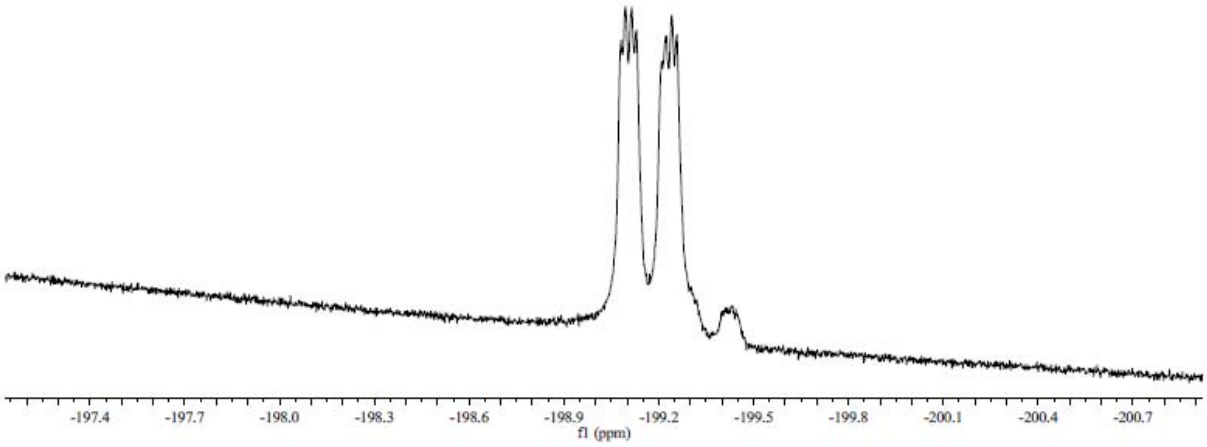

Fig. 8: ^{19}F-NMR (376.5 MHz, $CDCl_3$) spectra of **10**

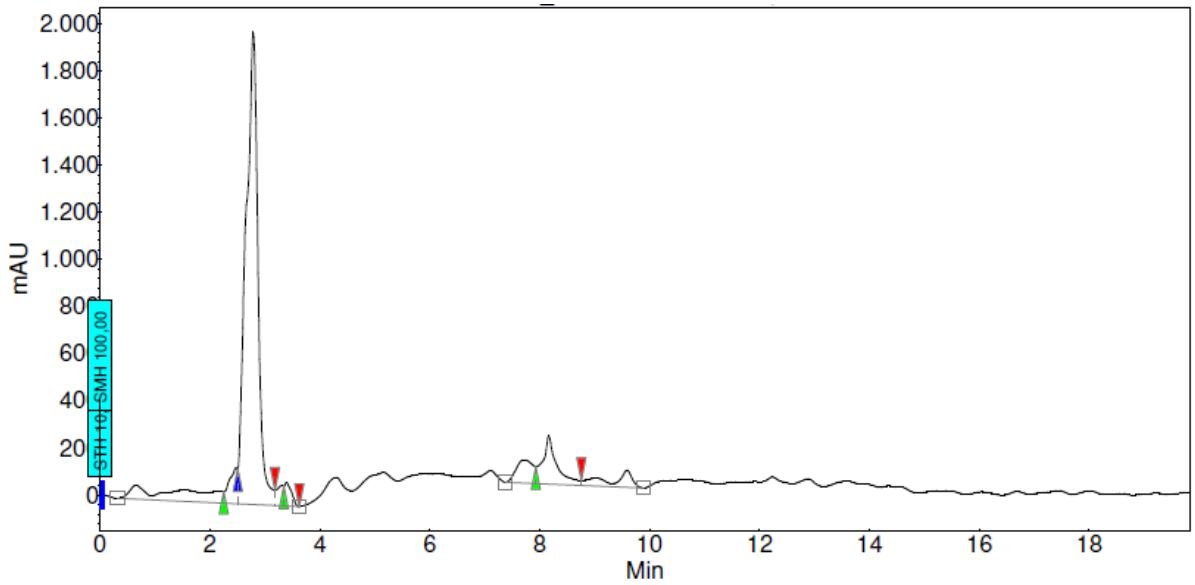

Fig. 9: Analytical RP-HPLC chromatogram of **10**

Spectra of MUC1-squarate monoamide

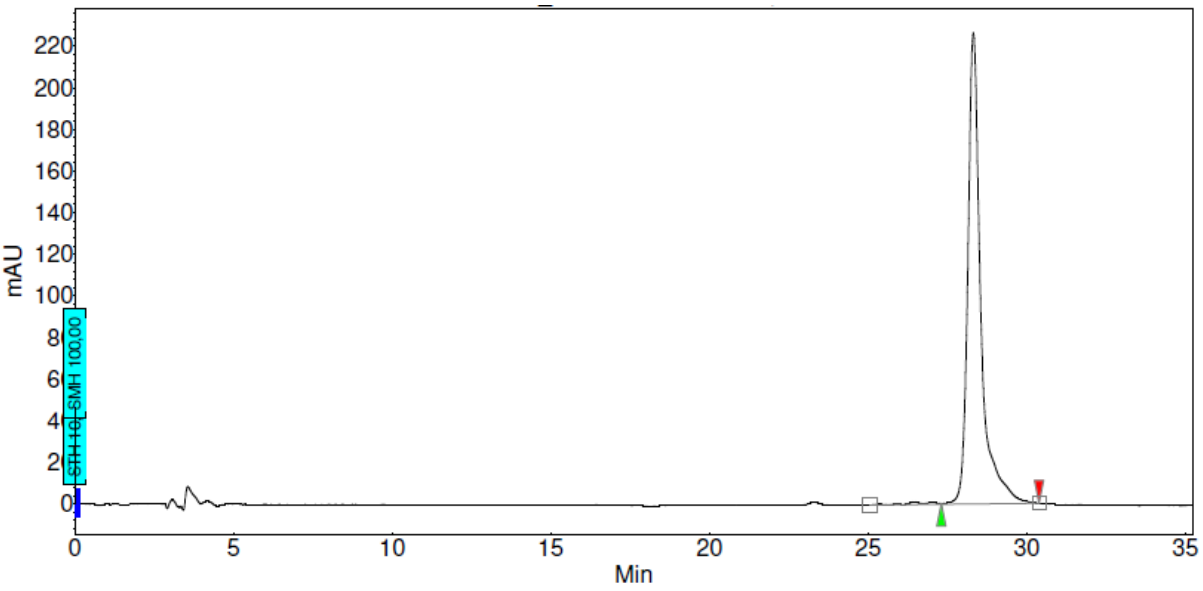

Fig. 10: Analytical RP-HPLC chromatogram of squarate monoamide

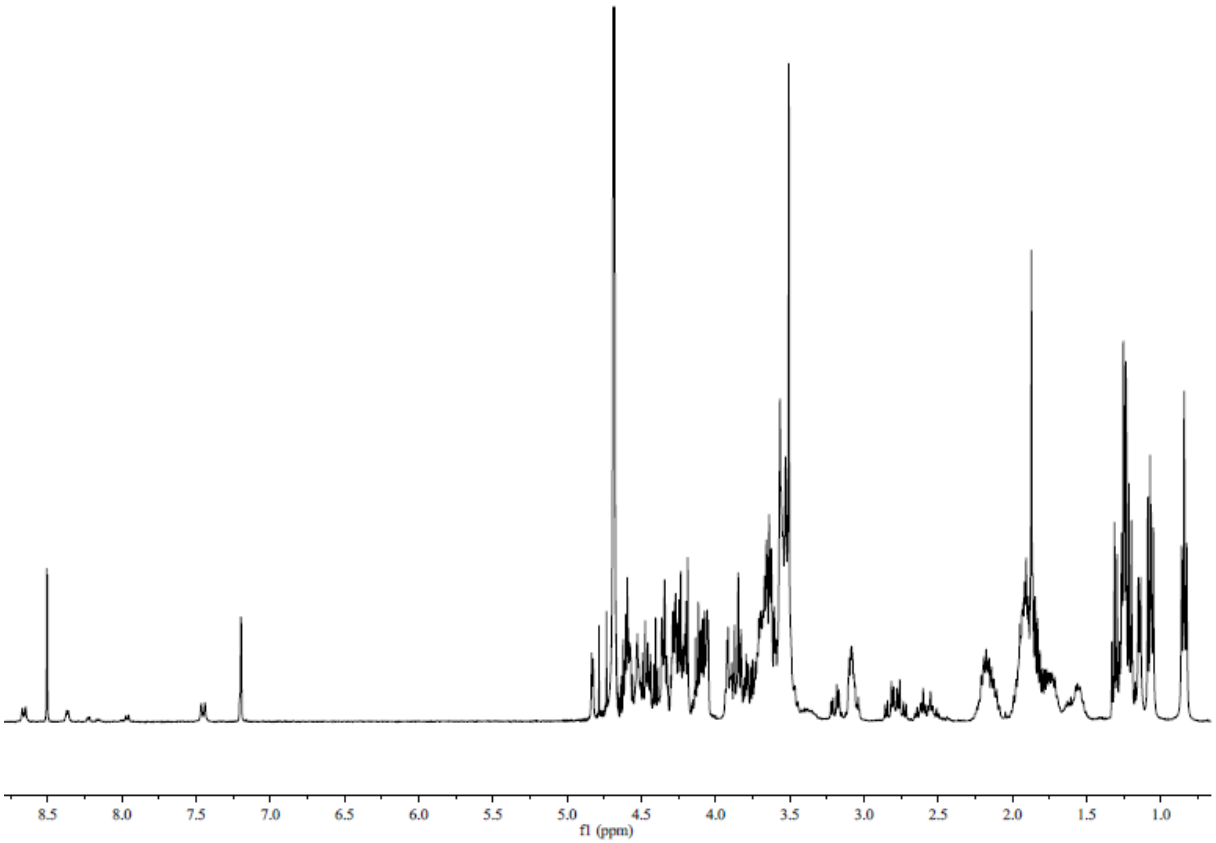

Fig. 11: ^{1}H-NMR (300 MHz, $CDCl_3$) spectra of squarate monoamide

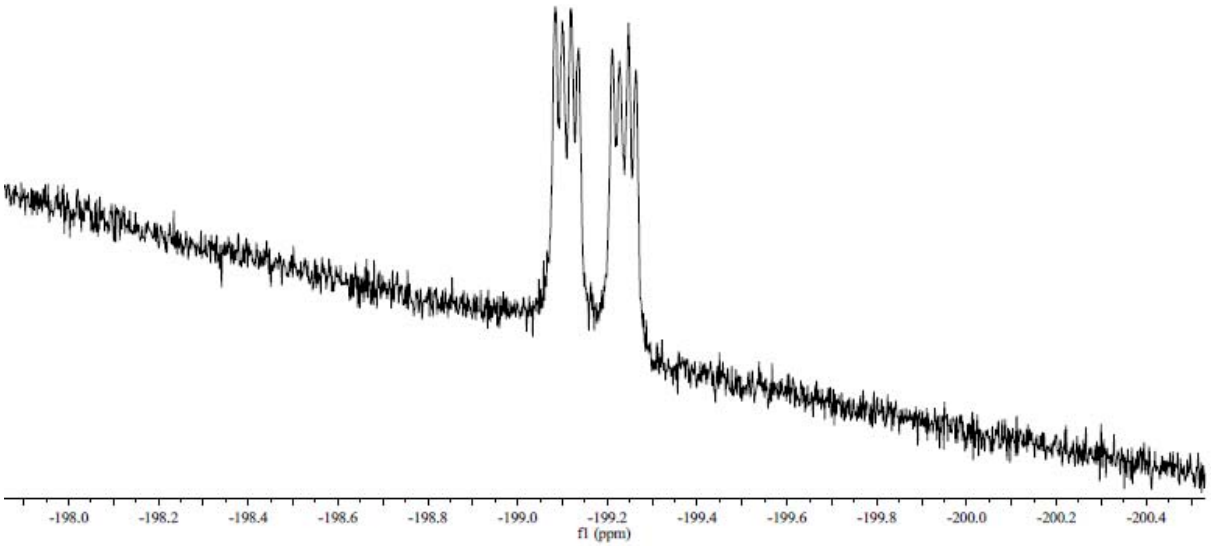

Fig. 12: ^{19}F-NMR (376.5 MHz, $CDCl_3$) spectra of squarate

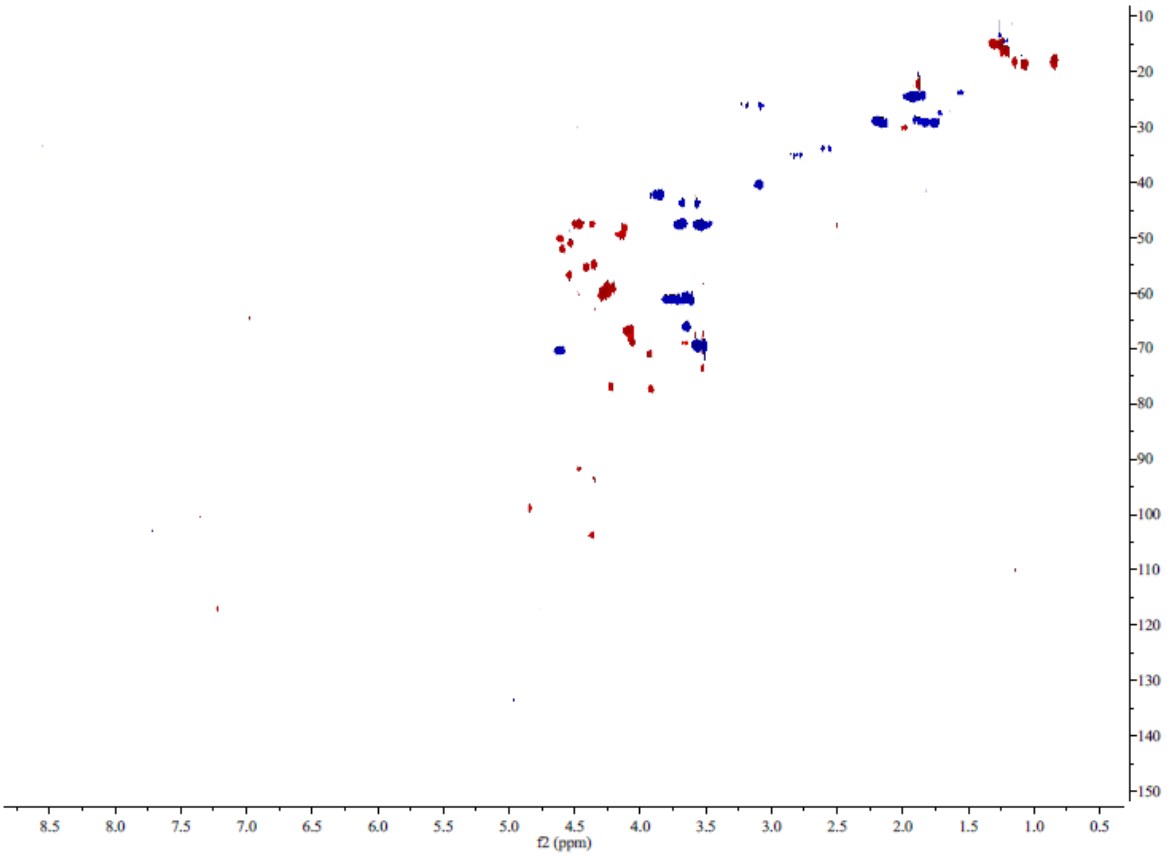

Fig. 13: HSQC of squarate