Electronic Supporting Information (*ESI*)

Perfluoroalkylated Amphiphilic MUC1 Glycopeptide Antigens As Tools For Cancer Immunotherapy

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Contents

General:

DMF (amine-free, for peptide synthesis) and NMP were purchased from Roth, and Ac_2O in p.a. quality from Acros. 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). HPLC analyses were performed on a JASCO-HPLC system with Phenomenex Luna PFP(2) (250 × 4.6 mm, 5 μm column at a flow rate of 1 $mLmin^{-1}$. Preparative RP-HPLC separation was carried out on a JASCO-HPLC System with Phenomenex Luna PFP(2) (250 × 30 mm, 10 μm column at a flow rate of 20 $mLmin^{-1}$ or 10 $mLmin^{-1}$. ^1H, ^{13}C 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). HR-ESI-mass spectra were recorded on a Micromass Q TOF Ultima 3 spectrometer, and optical rotations were measured at 546 nm with a Perkin-Elmer polarimeter 241.

General Procedure 1: Solid-phase peptide synthesis

The synthesis was carried out in an Applied Biosystems ABI 433A peptide synthesiser (standard programme Fastmoc 0.1 mmol) using pre-loaded Fmoc-Pro-Trt-Tentagel S resin (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 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 T_N building block **7** (134 mg, 0.20 mmol, 2.0 eq. based on the loaded resin) was performed using HATU (1.2 eq. with respect to **7**), 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, the triethylene glycol spacer **6** (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.

Building block **4** (485 mg, 0.30 mmol, 3 eq. based on the loaded resin) was coupled manually in a Merrifield glass reactor after swelling the resin with CH_2Cl_2 for 30 min. Therefore, compound **4** was activated by HATU (1.2 eq. with respect to **4**), HOAt (1.2 eq.) and DIPEA (2.4 eq.) in a mixture of DMF/$CHCl_3$/dioxane (1:1:1). The reaction mixture was shaken 3 d, before the coupling solution was filtered and the resin was washed with DMF (5 × 10 cm^3). Unreacted amino groups were capped by treatment with a mixture of Ac_2O/pyridine (1:3), and the resin was washed two times with DMF (5 × 10 cm^3) and CH_2Cl_2 (5 × 10 cm^3). The peptide was detached from resin with simultaneous removal of all side chain protecting groups by shaking with TFA (10 cm^3), TIS (1.0 cm^3) and H_2O (1.0 cm^3) for 3 h. The solution was filtered, the resin was washed with TFA (5 × 10 cm^3) and the combined solutions were

concentrated in vacuo and co-evaporation with toluene (3 × 10 cm^3). The crude product was dissolved in MeOH/H_2O (80:20) and purified by F-SPE (GP 3). The product fraction was evaporated in vacuo and subjected to lyophilisation.

General Procedure 2: Deacetylation

The peptide was dissolved in 10 cm^3 of MeOH (HPLC grade). A fresh solution of NaOMe in MeOH (0.5 g Na in 25 cm^3 MeOH (HPLC grade)) was added drop wise until pH 9.5-10.0 was reached. The reaction mixture was stirred over night and neutralised with a few drops of HOAc. The solvent was removed in vacuo and the residue was purified by F-SPE. The product fraction was evaporated in vacuo and subjected to lyophilisation.

General Procedure 3: F-SPE

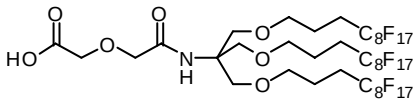
For fluorous solid-phase extraction (F-SPE) fluorous silica from Fluorous Technologies Inc. was utilised. The purification protocol is subdivided into three steps: (i) the crude product is loaded onto the silica gel using a minimum of organic solvent; (ii) a fluorophobic wash is performed and (iii) a fluorophilic eluation is conducted. To regenerate the silica gel, a final washing step with acetone is required.

Method 3a: (i) Loading: CH_2Cl_2, (ii) fluorophobic wash: MeOH/H_2O (v/v, 80:20), (iii) fluorophilic wash: acetone.

Method 3b: (i) Loading: MeOH/H_2O (80:20), (ii) fluorophobic wash: MeOH/H_2O (v/v, 80:20), (iii) fluorophilic wash: MeOH + 0.1% TFA.

***N*-((*N*-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-undecyloxy)methyl]aminomethane (4)**

(R_F-TRIS-NHCOCH$_2$OCH$_2$COOH)



A solution of diglycolic anhydride (97 mg, 0.8 mmol, 1.0 eq.) in 20 cm^3 abs. CH_2Cl_2 was cooled to 0 °C before a solution of the amine (1.25 g, 0.8 mmol) in 20 cm^3 abs. CH_2Cl_2 was added drop wise. The reaction mixture was stirred over night at room temperature and the solution was evaporated to dryness. The residue was re-suspended in EtOAc and washed with 1M HCl and water. The organic layer was dried over Na_2SO_4 and evaporated in vacuo. The crude product was purified by F-SPE (GP 3a) to give **5** as a colourless wax (1.0 g, 0.64 mmol, 80%). *ESI-MS (positive), (m/z):* 1640.22 ([M+Na]$^+$, calc.: 1640.11), 3257.41 ([2M+Na]$^+$, calc.: 3257.32). *^{1}H-NMR (300 MHz, $CDCl_3$), δ (ppm):* 4.15 (s, 2H, OCH_2COOH), 3.98 (s, 2H,

OCH_2CONH), 3.76 (s, 6H, C−CH_2O), 3.55 (t, 6H, OC$H_2$$CH_2$$CH_2$$R_F$, $J_{H,H}$ = 5.9 Hz), 2.34-2.16 (m, 6H, C$H_2$$R_F$), 1.91-1.81 (m, 6H, $CH_2$$CH_2$$R_F$). *^{13}C-NMR (75 MHz, CD_3OD), δ (ppm):* 171.4 (*C*OOH), 159.0 (*C*ONH), 70.2 (*C*H_2CO), 70.1 (CO*C*H_2), 70.7 (C*C*H_2O), 69.1 (O*C*$H_2$$CH_2$$CH_2$$R_F$), 61.1 (Cq(TRIS)), 28.7 (t, *C*$H_2$$R_F$, $J_{H,F}$ = 22.9 Hz), 21.6 (*C*$H_2$$CH_2$$R_F$). *^{19}F NMR (376.5 MHz, $CDCl_3$), δ (ppm):* -81.0 (t, 9F, CF_3, $J_{F,F}$ = 9.5 Hz), -114.5 (m, 6F), -122.0 (m, 18F), -122.9 (m, 6F), -123.6 (m, 6F), -126.3 (m, 6F).

_N_-((_N_-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-hepta-decafluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-_N_-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-L-threonyl-L-seryl-L-alanyl-L-proline (5) (R_F-TRIS-NHCOCH_2OCH_2CONH(CH_2CH_2O)$_3$CH_2CH_2CONH-Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-OH)

The synthesis followed GP 1 and GP 3b. Yield: 44 mg (17 μmol), 17% (based on the loaded resin), colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 31.3 min, λ = 212 nm). $[\alpha]_D^{23}$ = -31.45 (c = 0.90, MeOH/TFA (1%)). *HR-ESI-MS (positive, m/z)* calc. for $C_{86}H_{102}F_{51}N_{13}O_{22}$: 2638.6433 ($[M+H]^+$, calc.: 2638.6526). *ESI-MS (positive, m/z)*: 2638.65 ($[M+H]^+$, calc.: 2638.65), 1320.41 ($[M+2H]^{2+}$, calc.: 1319.83), 878.49 ($[M+3H]^{3+}$, calc.: 880.22). *^{1}H NMR (400 MHz, CD_3OD/TFA-d_1(1%), COSY, HSQC), δ (ppm):* 8.78 (d, 1H, $H_ε$, $J_{Hε, Hδ}$ = 1.40 Hz), 7.43 (d, 1H, $H_δ$, $J_{Hδ,Hε}$ = 0.90 Hz), 4.70-4.62 (m, 2H, $H_α$ {4.68}, $A_{2α}$ {4.62, d, $J_{Aα, Aβ}$ = 7.02 Hz }), 4.46-4.38 (m, 3H, $S_{1α}$ {4.45}, $V_α$ {4.43}, $T_{1α}$ {4.39}), 4.29-4.23 (m, 4H, $P_{1-2α}$ {4.26}, $T_{1β}$ {4.25}, $A_{1α}$ {4.25}), 4.05 (s, 2H, OCH_2CO), 4.01 (s, 2H, OCH_2CONH), 3.98 (s, 2H, $G_{1,2α}$), 3.85-3.53 (m, 24H, $S_{1β}$ {3.84, 3.79}, $P_{1-2δ}$ {3.78, 3.72, 3.68, 3.65}, 3.76 {s, 6H, C−CH_2O}, CH_2O-spacer {3.79, 3.78, 3.64, 3.63, 3.55, 3.55}, 3.54 {t, 6H, OC$H_2$$CH_2$$CH_2$$R_F$, $J_{H,H}$ = 6.08 Hz}), 3.43 (t, 2H, CH_2NH-spacer, $J_{CH2,CH2}$ = 5.61 Hz), 3.21 (dd, 1H, $H_{βa}$, $J_{Hβ,Hα}$ = 6.94 Hz, $J_{Hβa,Hβb}$ = 15.46 Hz), $H_{βa}$ {3.20}, 2.69 (t, 2H, CH_2CO-Spacer, $J_{CH2,CH2}$ = 6.10 Hz), 2.30-2.14 (m, 7H, C$H_2$$R_F$ {m, 2.30-2.19}, $V_β$ {2.16}), 2.07-1.97 (m, 8H, $P_{1-2γ}$ {2.02, 2.02}, $P_{1-2β}$ {1.98, 1.98}), 1.90-1.83 (m, 6H, $CH_2$$CH_2$$R_F$), 1.36 (d, 2H, $A_{1β}$, $J_{Aβ,Aα}$ = 7.25 Hz), 1.36 (d, 2H, $A_{2β}$, $J_{Aβ,Aα}$ = 6.99 Hz), 1.19 (d, 3H, $T_γ$, $J_{Tγ,Tβ}$ = 6.40 Hz), 0.99 (t, 6H, $V_γ$, $J_{Vγ,Vβ}$ = 6.23 Hz). *^{13}C NMR (400 MHz, CD_3OD/TFA-d_1(1%), COSY, HSQC), δ (ppm):* 175.6, 175.5, 175.5, 174.0, 173.6, 173.3, 172.7, 172.5, 172.4, 172.0, 171.9, 171.7 (C=O), 135.2 (H_{C2}), 129.5 (H_{C5}), 119.2 (H_{C4}), 71.9 (*C*H_2CO), 71.6 (CO*C*H_2), 70.9 (O*C*$H_2$$CH_2$$CH_2$$R_F$), 70.1 (C*C*H_2O), 71.8, 71.6, 71.0, 71.0, 68.5, 68.1 (CH_2-spacer), 68.5 ($T_β$), 63.2 ($S_β$), 62.2 ($T_α$), 61.4, 61.4 ($P_{1-2α}$), 60.3 ($V_α$), 56.9

(S_α), 53.9 (H_α), 51.5 ($A_{1\alpha}$), 48.9 ($A_{2\alpha}$), 49.4, 49.3, 48.6, 48.4 ($P_{1\text{-}2\delta}$), 43.7 (G_α), 40.1 (CH_2NH -spacer), 31.8 (V_β), 31.0, 31.6 ($P_{1\text{-}2\beta}$), 28.9 (t, CH_2R_F, $J_{C,F}$ = 21.97 Hz), 28.8 (CH_2CO-spacer), 28.1 ($H_{\beta a}$), 27.1 ($H_{\beta b}$), 26.0, 26.0 ($P_{1\text{-}2\gamma}$), 21.8 ($CH_2CH_2R_F$), 20.0 (T_γ), 20.0 ($V_{\gamma a}$), 19.0 ($V_{\gamma b}$), 17.2, 17.0 ($A_{1\text{-}2\beta}$). *^{19}F NMR (376.5 MHz, CD_3OD/TFA-d_1(1%)), δ (ppm):* -83.2 (t, 9F, CF_3, $J_{F,F}$ = 9.5 Hz), -116.7 (m, 6F), -123.6 (m, 18F), -124.6 (m, 6F), -125.2 (m, 6F), -128.1 (m, 6F).

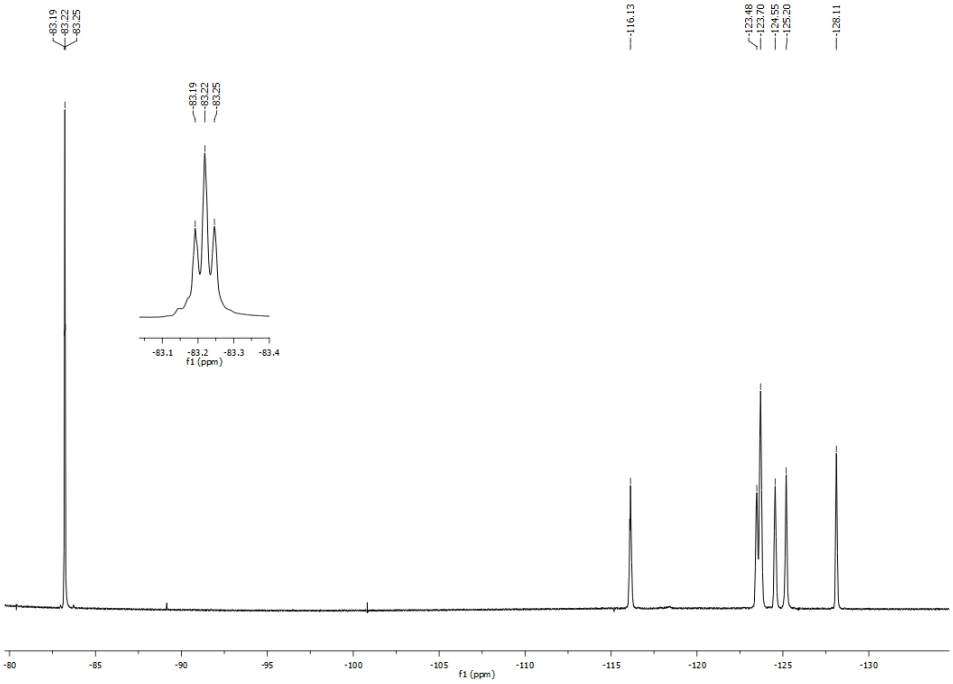


Fig. Suppl. 1: ^{19}F NMR spectra of compound **5**.

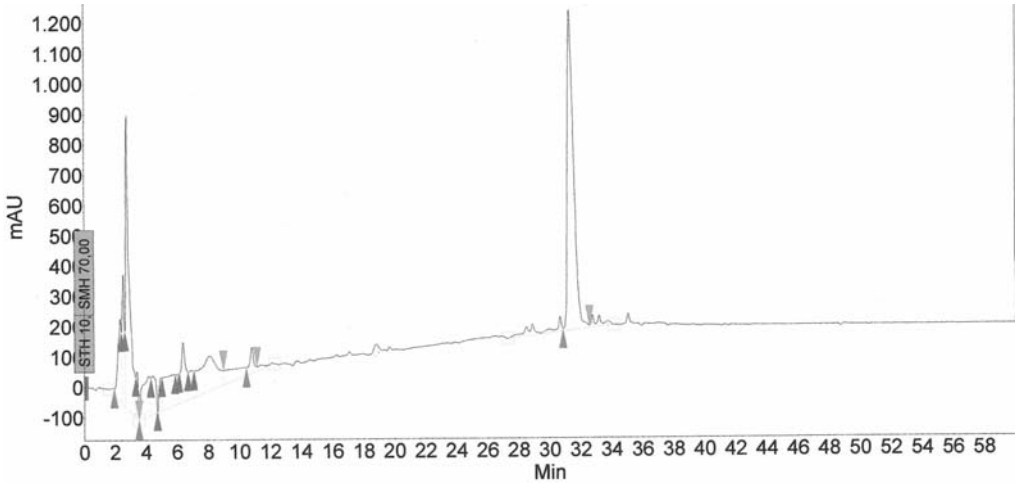


Fig. Suppl. 2: Analytical RP-HPLC chromatogram of compound **5**.

_N_-((_N_-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-_N_-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-_O_-(2-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-proline (8a)

(R_F-TRIS-NHCOCH$_2$OCH$_2$CONH(CH$_2$CH$_2$O)$_3$CH$_2$CH$_2$CONH-Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr(α-Ac$_3$GalNAc)-Ala-Pro-OH)

The synthesis followed GP 1 and GP 3b. Yield: 63 mg (17 μmol), 17% (based on the loaded resin), colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 26.8 min, λ = 212 nm). *HR-ESI-MS (positive, m/z)* calc. for $C_{144}H_{191}F_{51}N_{28}O_{46}$: 2009.6433 (([M+2H]$^{2+}$, calc.: 2009.6405). *MALDI-TOF-MS (dhb, positive, m/z):* 4020.50 ([M+H]$^+$, calc.: 4018.27).

_N_-((_N_-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-_N_-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-_O_-(2-acetamido-3,4,6-tri-_O_-acetyl-2-deoxy-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 (8b)

(R_F-TRIS-NHCOCH$_2$OCH$_2$CONH(CH$_2$CH$_2$O)$_3$CH$_2$CH$_2$CONH-Pro-Ala-His-Gly-Val-Thr(α-Ac$_3$GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-OH)

The synthesis followed GP 1 and GP 3b. Yield: 110 mg (27 μmol), 27% (based on the loaded resin), colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 26.4 min, λ = 212 nm). $[\alpha]_D^{23}$ = -37.77 (c = 1.00, MeOH/TFA (1%)). *HR-ESI-MS (positive, m/z)* calc. for $C_{144}H_{191}F_{51}N_{28}O_{46}$: 2009.6482 ([M+2H]$^{2+}$, calc.: 2009.6405). *MALDI-TOF-MS (dhb, positive, m/z):* 4019.66 ([M+H]$^+$, calc.: 4018.27). *ESI-MS (positive, m/z):* 2009.44 ([M+2H]$^{2+}$, calc.: 2009.64), 1340.33 ([M+3H]$^{3+}$, calc.: 1340.10). *^{1}H NMR (400 MHz, CD$_3$OD/TFA-d$_1$(1%), COSY, HSQC), δ (ppm):* 8.79 (d, 1H, $H_ε$, $J_{Hε,\ Hδ}$ = 1.34 Hz), 7.44 (d, 1H, $H_δ$, $J_{Hδ,Hε}$ = 0.90 Hz), 5.36 (d, 1H, H-4, $J_{H3,H4}$ = 2.62 Hz), 5.08 (dd, 1H, H-3, $J_{H3,H4}$ = 3.21, $J_{H3,H2}$ = 11.38 Hz), 5.11 (d, 1H, H1, $J_{H1,H2}$ = 3.69 Hz), 4.72-4.20 (m, 29H, $H_α$ {4.70}, $R_α$ {4.68}, $D_α$ {4.62}, T*$_α$ {4.65}, $A_{3α}$ {4.60}, $A_{2α}$ {4.59}, $A_{4α}$ {4.56}, $S_{1α}$ {4.52}, $S_{2α}$ {4.46}, $P_{1\text{-}5α}$ {4.45, 4.41, 4.37, 4.36, 4.34}, $T_{1α}$ {4.35}, $T_{2α}$ {4.34}, $V_α$ {4.41}, T*$_β$ {4.32}, $T_{2β}$ {4.29}, $T_{1β}$ {4.27}, $A_{1α}$ {4.25}), H2 {4.38}, CH_2O-spacer

{4.11, 4.06, 4.02, 3.97}, 4.05 (s, 2H, OC*H*$_2$CO), 4.01 (s, 2H, OC*H*$_2$CONH), 3.97-3.53 (m, 22H, $G_{1,2\alpha}$ {3.79, 3.74}, $S_{1,2\beta}$ {3.90, 3.80}, H5 {4.05}, $P_{1\text{-}5\delta}$ {3.83, 3.80, 3.68, 3.65, 3.63}), 3.76 (s, 6H, C−CH_2O), CH_2O-spacer {3.67, 3.63}, H6a,b {4.09}, H4 {4.27}, $H_{\beta b}$ {3.39}), 3.55 (t, 6H, OC*H*$_2$$CH_2CH_2R_F$, $J_{H,H}$ = 6.03 Hz), 3.43 (t, 2H, CH_2NH-spacer, $J_{CH2,CH2}$ = 5.60 Hz), 3.21 (dd, 1H, $H_{\beta a}$, $J_{H\beta,H\alpha}$ = 7.66 Hz, $J_{H\beta a,H\beta b}$ = 15.41 Hz), R_δ {3.21}), $D_{\beta a}$ {2.92}, 2.69 (dd, $D_{\beta b}$, $J_{D\beta,D\alpha}$ = 6.01 Hz, $J_{D\beta a,D\beta b}$ = 11.92 Hz), 2.29-2.18 (m, 8H, C*H*$_2$$R_F$ {2.27, 2.22, 2.18}, CH_2CO-spacer {2.22}, V_β {2.13}), $P_{1\text{-}5\gamma}$ {2.13-2.02}, $P_{1\text{-}5\beta}$ {2.27-1.92}), CH_3Ac {2.14, 2.00, 1.93}, CH_3AcNH {s, 2.00}, 1.90-1.83 (m, 6H, *CH*$_2$$CH_2R_F$), $R_{\beta a}$ {1.87}, $R_{\beta b}$ {1.73}, R_γ {1.69}), 1.44-1.35 (m, 12H, {1.42, d, $A_{4\beta}$, $J_{A\beta,A\alpha}$ = 7.04 Hz}, $A_{1\text{-}3\beta}$ {1.37, 1.36, 1.36}), T^*_γ {1.28}, $T_{2\gamma}$ {1.28}, $T_{1\gamma}$ {1.20}, 1.00 (d, 3H, $V_{\gamma a}$, $J_{V\gamma,V\beta}$ = 6.61 Hz), 0.99 (d, 3H, $V_{\gamma b}$, $J_{V\beta,V\gamma}$ = 6.73 Hz). *^{13}C NMR (400 MHz, CD_3OD/TFA-d_1(1%), COSY, HSQC), δ (ppm):* 175.5, 175.4, 175.3, 174.7, 174.7, 174.5, 174.4, 174.3, 174.4, 173.9, 173.8, 173.7, 173.5, 173.3, 173.2, 173.1, 172.8, 172.7, 172.4, 172.4, 172.3, 172.2, 172.1, 172.0, 171.9 (C=O, C=O-acetyl), 157.9 (*C*ONHR_F), 156.6 (C=NH), 134.6 (H_β), 129.2 (H_ε), 119.0 (H_δ), 100.9 (C1), 79.1 (T^*_β), 71.7 (CO*C*H_2), 71.5 (*C*H_2CO), 70.8 (O*C*$H_2CH_2CH_2R_F$), 70.5 (C3), 69.1 (C4), 70.0 (C*C*H_2O), 68.4 ($T_{1,2\beta}$), 70.7 (C5), 43.9, 43.8, 43.6, 43.5 (CH_2-spacer), 63.6 (C6), 62.2 (Cq_{TRIS}), 61.4 (V_α), 60.3 ($T_{1,2\alpha}$), 62.6, 62.5, 62.5, 62.5 ($P_{1\text{-}5\alpha}$), 58.0 (T^*_α), 57.2 ($S_{1\alpha}$), 56.5 ($S_{2\alpha}$), 63.3, 63.2 ($S_{1\beta}$, $S_{2\beta}$), 53.7 (H_α), 52.1 (R_α), 48.6 (D_α), 48.9 (C2), 51.3 ($A_{1\alpha}$), 48.9, 48.7, 48.6 ($A_{2\text{-}4\alpha}$), 49.0, 49.0, 48.8, 48.5, 48.5 ($P_{1\text{-}5\delta}$), 49.0, 48.5 (CH_2-spacer), 43.9, 43.8 ($G_{1,2\alpha}$), 42.2 (R_δ), 39.9 (CH_2NH-spacer), 35.9 ($D_{\beta b}$), 35.9 ($D_{\beta a}$), 31.6 (V_β), 30.6, 30.6, 30.5, 30.3 ($P_{1\text{-}5\beta}$), 28.8 (t, *C*H_2R_F, $J_{C,F}$ = 21.85 Hz), 29.0, 29.0 ($R_{\beta a,b}$), 28.8 (CH_2CO-spacer), 28.0 ($H_{\beta a}$), 27.9 ($H_{\beta b}$), 26.2, 26.2, 26.0, 26.0 ($P_{1\text{-}5\gamma}$), 25.7 (R_γ), 23.2 (CH_3-AcNH), 21.7 (*C*$H_2CH_2R_F$), 19.3 ($T_{2\gamma}$), 20.2 ($T_{1\gamma}$), 19.7 ($V_{\gamma a}$), 19.3 (T^*_γ), 19.0 ($V_{\gamma b}$), 17.0, 16.9, 16.7, 16.7 ($A_{1\text{-}4\beta}$). *^{19}F NMR (376.5 MHz, CD_3OD/TFA-d_1(1%)), δ (ppm):* -82.1 (m, 9F, CF_3), -114.7 (m, 6F), -122.5 (m, 18F), -123.5 (m, 6F), -123.9 (m, 6F), -127.0 (m, 6F).

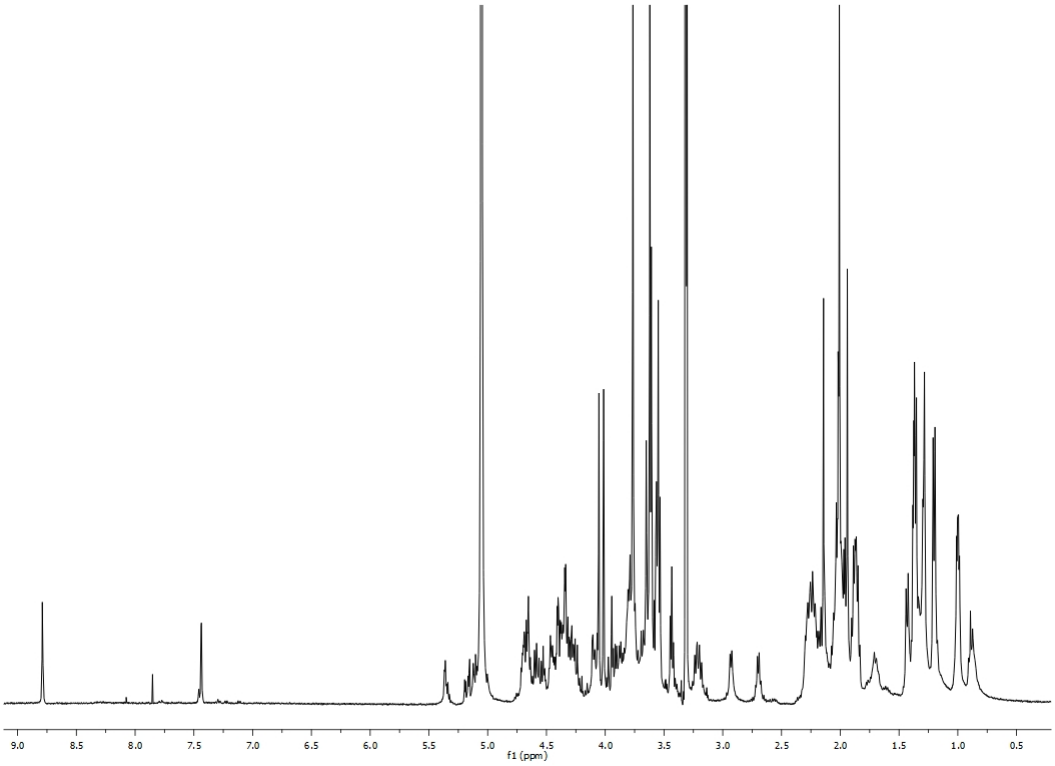


Fig. Suppl. 3: ^{1}H NMR (400 MHz, CD_3OD/TFA-d_1(1%)) spectra of **8b**.

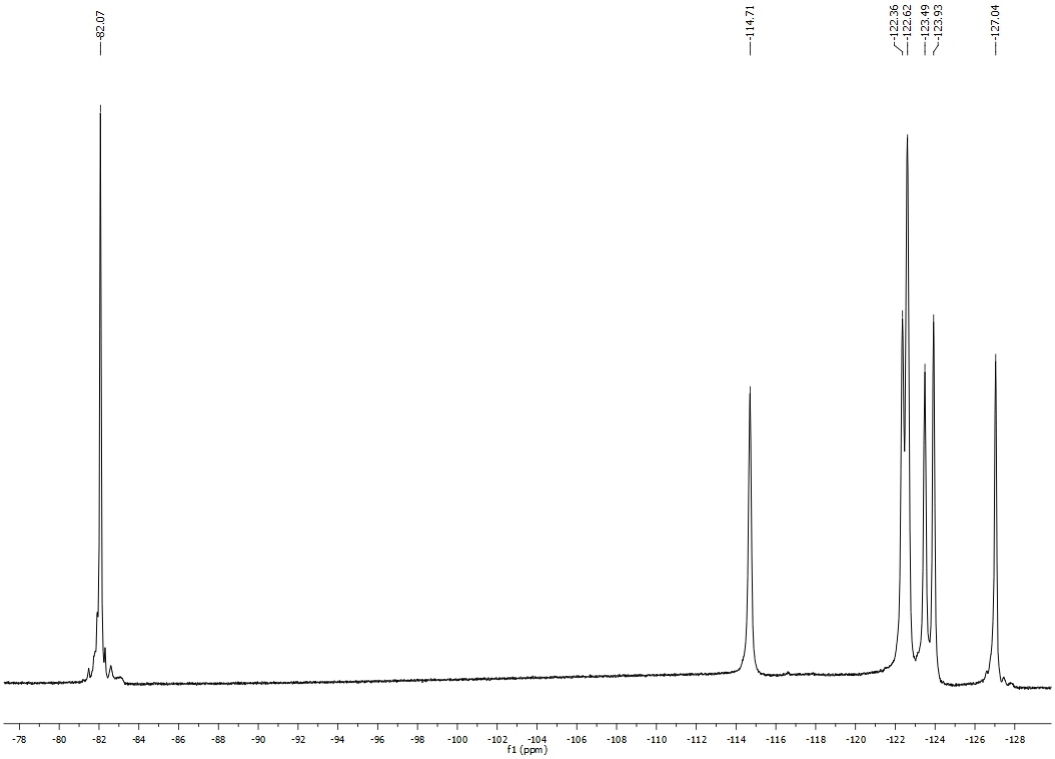


Fig. Suppl. 4: ^{19}F NMR Spectra of compound **8b**.

***N*-((*N*-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-hepta-decafluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-*N*-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-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-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-proline (8c)**

(R_F-TRIS-NHCOCH$_2$OCH$_2$CONH(CH$_2$CH$_2$O)$_3$CH$_2$CH$_2$CONH-Pro-Ala-His-Gly-Val--Thr(α-Ac$_3$GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr(α-Ac$_3$GalNAc)-Ala-Pro-OH)

The synthesis followed GP 1 and GP3b. Yield: 22 mg (5 μmol), 5% (based on the loaded resin), colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 27.4 min, λ = 212 nm). *HR-ESI-MS (positive, m/z)* calc. for $C_{158}H_{210}F_{51}N_{29}O_{54}$: 2174.1917 ($[M+2H]^{2+}$, calc.: 2174.1960). *MALDI-TOF-MS (dhb, positive, m/z):* 4348.28 ($[M+H]^+$, calc.: 4347.38). *ESI-MS (positive, m/z):* 2174.23 ($[M+2H]^{2+}$, calc.: 2174.20), 1457.18 ($[M+Na+2H]^{3+}$, calc.: 1457.13).

***N*-((*N*-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-hepta-deca-fluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-*N*-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-*O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-proline (9a)**

(R_F-TRIS-NHCOCH$_2$OCH$_2$CONH(CH$_2$CH$_2$O)$_3$CH$_2$CH$_2$CONH-Pro-Ala-His-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr(α-GalNAc)-Ala-Pro-OH)

The synthesis followed GP2 and GP 3b. Amounts: 30 mg (7.5 μmol) **8a**. Yield: 17 mg (4.4 μmol), 58%, colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 26.6 min, λ = 212 nm). *HR-ESI-MS (positive, m/z)* calc. for $C_{138}H_{185}F_{51}N_{28}O_{43}$: 1968.6061 ($[M+2Na]^{2+}$, calc.: 1968.6066). *MALDI-TOF-MS (dhb, positive, m/z):* 3892.82 ($[M+H]^+$, calc.: 3892.24).

***N*-((*N*-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-hepta-decafluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-*N*-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-*O*-(2-acetamido-2-deoxy-α-D-galacto-**

pyranosyl)-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 (9b) (R_F-TRIS-NHCOCH$_2$OCH$_2$CONH(CH$_2$CH$_2$O)$_3$CH$_2$CH$_2$CONH-Pro-Ala-His-Gly-Val-Thr(α-GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-OH)

The synthesis followed GP 2 and GP 3b. Amounts: 39 mg (9.7 µmol) **8b**.Yield: 33 mg (8.5 µmol), 87%, colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 27.5 min, λ = 212 nm). $[\alpha]_D^{23}$ = -31.58 (c = 1.00, MeOH/TFA (1%)). *HR-ESI-MS (positive, m/z)* calc. for $C_{138}H_{185}F_{51}N_{28}O_{43}$: 1946.6218 ($[M+2Na]^{2+}$, calc.: 1946.6246). *ESI-MS (positive, m/z):* 1957.60 ($[M+2H]^{2+}$, calc.: 1957.62), 1313.07 ($[M+Na+2H]^{3+}$, calc.: 1312.74), 990.56 ($[M+3Na+H]^{4+}$, calc.: 990.30). *MALDI-TOF-MS (dhb, positive, m/z):* 3893.49 ($[M+H]^+$, calc.: 3891.23). *^{1}H-NMR (400 MHz, CD$_3$OD/TFA-d$_1$(1%), COSY, HSQC), δ (ppm):* 8.76 (d, 1H, $H_ε$, $J_{Hε, Hδ}$ = 1.38 Hz), 7.43 {$H_δ$}, 5.03 {H1}, 4.71-4.21 (m, 21H, $H_α$ {4.70}, $R_α$ {4.70}, $D_α$ {4.63}, T*$_α$ {4.63}, $A_{3α}$ {4.61}, $A_{2α}$ {4.59}, $A_{4α}$ {4.57}, $S_{1α}$ {4.52}, $S_{2α}$ {4.46}, $P_{1-5α}$ {4.45, 4.42, 4.40, 4.36, 4.31}, $T_{1α}$ {4.36}, $T_{2α}$ {4.31}, $V_α$ {4.42}, T*$_β$ {4.29}, $T_{2β}$ {4.29}, $T_{1β}$ {4.28}, $A_{1α}$ {4.24}), 4.18 (dd, 1H, H2, $J_{H2,H1}$ = 3.75 Hz, $J_{H2,H3}$ = 10.91 Hz), 4.05 (s, 2H, OC*H*$_2$CO), 4.01 (s, 2H, OC*H*$_2$CONH), 3.99-3.40 (m, 30H, $S_{1,2β}$ {3.94, 3.94}, CH_2O-spacer {3.92, 3.87, 3.85, 3.81}, H5 {3.89}, $P_{1-5δ}$ {3.89, 3.85, 3.81, 3.76, 3.74}), H3 {3.84}, $G_{1,2α}$ {3.79, 3.75}, 3.76 (s, 6H, C−CH_2O), CH_2O-spacer {3.63, 3.61}, H6a,b {3.73}, H4 {3.62}, $H_{βb}$ {3.40}), 3.55 (t, 6H, OC*H*$_2$CH$_2$CH$_2$R$_F$, $J_{H,H}$ = 6.02 Hz), 3.44 (t, 2H, CH_2NH-spacer, $J_{CH2,CH2}$ = 5.64 Hz), 3.21 (dd, 1H, $H_{βa}$, $J_{Hβ,Hα}$ = 7.20 Hz, $J_{Hβa,Hβb}$ = 14.75 Hz), $R_δ$ {3.21}), $D_{βa}$ {2.93}, $D_{βb}$ {2.69}, 2.29 -2.12 (m, 8H, C*H*$_2$R$_F$ {2.27, 2.26, 2.21}, CH_2CO-spacer {2.22}, $V_β$ {2.15}), $P_{1-5γ}$ {2.14-1.99}, $P_{1-5β}$ {2.02-1.93}), CH_3AcNH {s, 2.02}, 1.90-1.83 (m, 6H, *CH*$_2$CH$_2$R$_F$), $R_{βa,b}$ {1.72}, $R_γ$ {1.69}), 1.43 -1.33 (m, 12H, $A_{1-4β}$ {1.42, 1.41, 1.36, 1.36}), 1.27 (d, 3H, T*$_γ$, $J_{Tγ,Tβ}$ = 6.04 Hz), $T_{2γ}$ {1.20}, 1.20 (d, 6H, $T_{1γ}$, $J_{Tγ,Tβ}$ = 6.54 Hz), 1.00 (d, 3H, $V_{γa}$, $J_{Vγ,Vβ}$ = 6.70 Hz), 0.99 (d, 3H, $V_{γb}$, $J_{Vβ,Vγ}$ = 6.62 Hz). *^{13}C-NMR (400 MHz, CD$_3$OD/TFA-d$_1$(1%), COSY, HSQC), δ (ppm):* 176.5, 176.4, 176.3, 175.6, 175.5, 175.1, 174.5, 174.4, 174.3, 174.0, 173.8, 173.4, 173.3, 173.0, 172.6, 172.3, 172.2, 172.1, 172.0, 171.7, 171.3, 171.2, 171.1, 170.7, 170.6 (C=O, C=O-acetyl), 158.1 (*C*ONHR$_F$), 156.5 (C=NH), 135.2 ($H_β$), 129.2 ($H_ε$), 118.7 ($H_δ$), 100.4 (C1), 77.9 (T*$_β$), 100.4 (C1), 71.6 (CO*C*H$_2$), 71.4 (*C*H$_2$CO), 70.7 (O*C*H$_2$CH$_2$CH$_2$R$_F$), 70.3 (C3), 69.9 (C4), 69.9 (C*C*H$_2$O), 68.6 ($T_{1β}$), 68.4 ($T_{2β}$), 70.3 (C5), 63.0, 63.0, 63.0, 63.0 (CH_2-spacer), 62.9 (C6), 61.9 (Cq_{TRIS}), 61.4 ($V_α$), 60.7 ($T_{2α}$), 60.4, 60.3, 60.5, 59.8 ($P_{1-5α}$), 60.3 ($T_{1α}$), 57.9 (T*$_α$), 57.2 ($S_{1α}$), 56.6 ($S_{2α}$), 54.6, 54.4 ($S_{1β}$, $S_{2β}$), 53.6 ($H_α$), 52.1 ($R_α$), 51.8 ($D_α$), 51.4 (C2), 51.4 ($A_{1α}$), 49.1, 48.7, 48.7 ($A_{2-4α}$), 49.0, 48.9, 48.8, 48.7, 48.5 ($P_{1-5δ}$), 48.7, 48.5 (CH_2-spacer), 43.6, 43.6 ($G_{1,2α}$), 42.1

(R_δ), 39.7 (CH_2NH-spacer), 35.8 ($D_{\beta b}$), 35.7 ($D_{\beta a}$), 31.2 (V_β), 30.4, 30.3, 30.2 ($P_{1\text{-}5\beta}$), 30.3 (CH_2R_F), 29.1 ($R_{\beta a,b}$), 28.6 (CH_2CO-spacer), 27.7 ($H_{\beta a}$), 27.7 ($H_{\beta b}$), 26.1, 26.0, 25.9, 25.8 ($P_{1\text{-}5\gamma}$), 25.7 (R_γ), 23.2 (CH_3-AcNH), 21.5 ($CH_2CH_2R_F$), 20.1 ($T_{2\gamma}$), 20.0 ($T_{1\gamma}$), 19.7 ($V_{\gamma a}$), 19.3 (T^*_γ), 18.9 ($V_{\gamma b}$), 16.9, 16.8, 16.6, 16.6 ($A_{1\text{-}4\beta}$). ^{19}F NMR (376.5 MHz, *CD_3OD/TFA-d_1(1%)*), δ (ppm): -84.1 (m, 9F, CF_3), -117.1 (m, 6F), -124.5 (m, 18F), -125.5 (m, 6F), -126.1 (m, 6F), -129.0 (m, 6F).

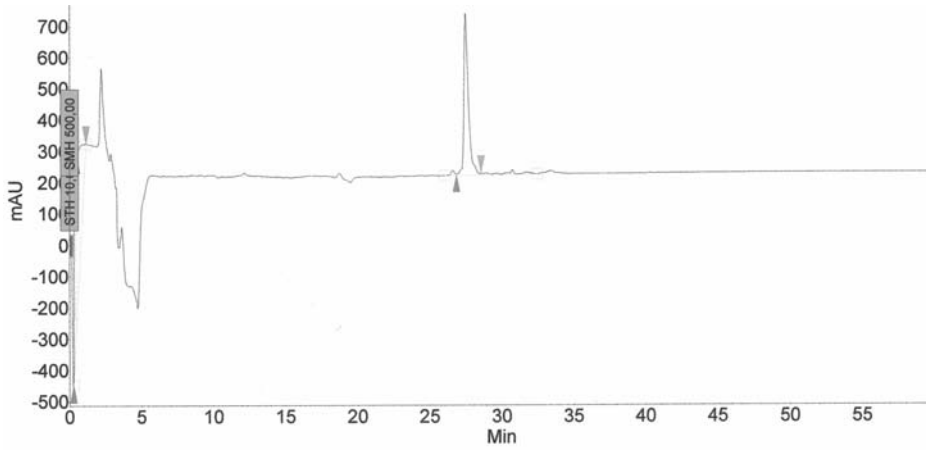


Fig. Suppl. 5: Analytical RP-HPLC chromatogram of compound **9b**.

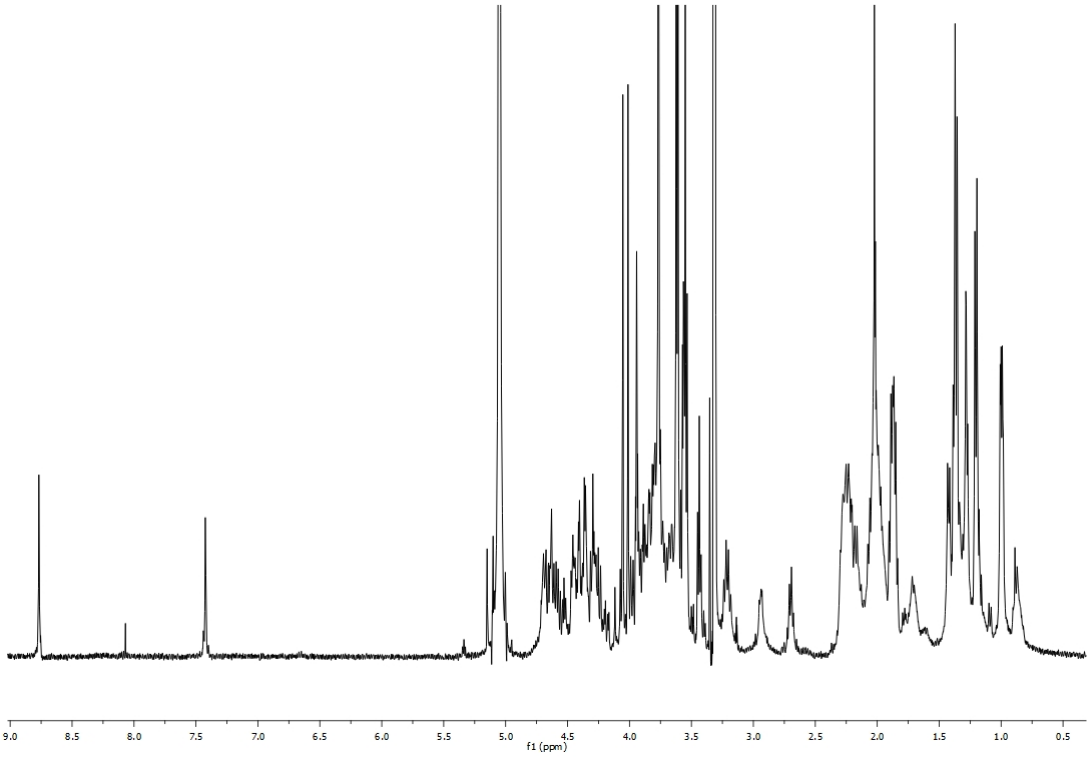


Fig. Suppl. 6: ^1H NMR (400 MHz, CD_3OD/TFA-d_1(1%)) spectra of **9b**.

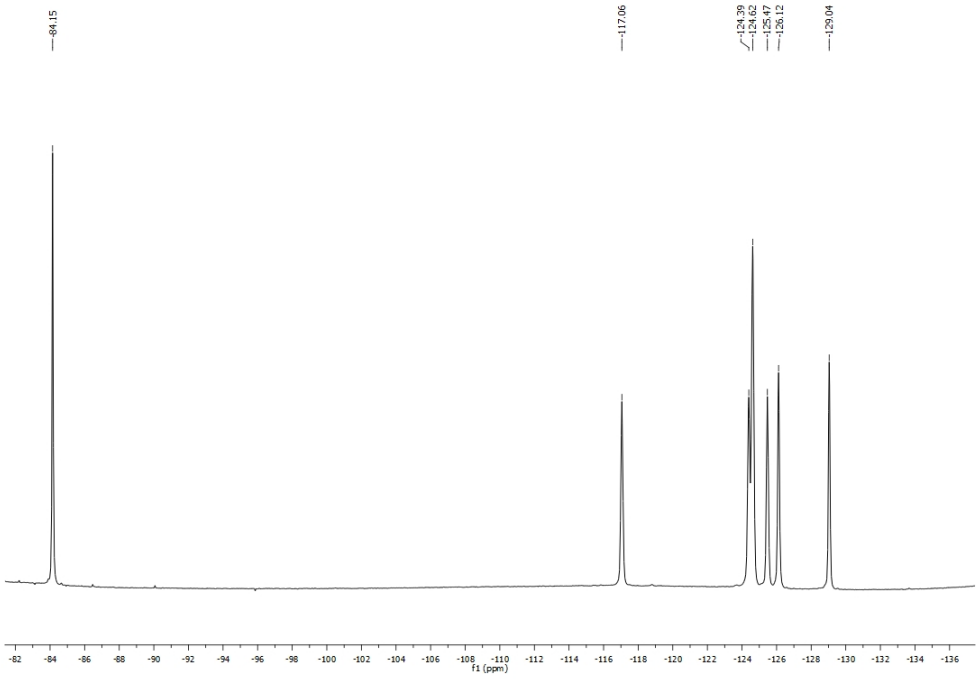


Fig. Suppl. 7: ^{19}F NMR Spectra of compound **9b**.

***N*-((*N*-3-2-oxoethoxy)-2-oxopropanyl)-tris[(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-hepta-decafluoro-undecyloxy)-methyl]aminomethane)-amido-4,7,10-trioxa-dodecanylamido-*N*-L-prolyl-L-alanyl-L-histidyl-L-glycyl-L-valyl-*O*-(2-acetamido-2-deoxy-α-D-galacto-pyranosyl)-L-threonyl-L-seryl-L-alanyl-L-prolyl-L-aspartyl-L-threonyl-L-arginyl-L-prolyl-L-alanyl-L-prolyl-L-glycyl-L-seryl-*O*-(2-acetamido-deoxy-α-D-galactopyranosyl)-L-threonyl-L-alanyl-L-proline (9c)**

(R_F-TRIS-NHCOCH$_2$OCH$_2$CONH(CH$_2$CH$_2$O)$_3$CH$_2$CH$_2$CONH-Pro-Ala-His-Gly-Val--Thr(α-GalNAc)-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr(α-GalNAc)-Ala-Pro-OH)

The synthesis followed GP 2 and GP 3b. Amounts: 10 mg (2.3 μmol) **8c**.Yield: 8 mg (1.9 μmol), 83%, colourless amorphous solid. *Analytical RP-HPLC* (Luna PFP, MeOH/H_2O + 0.1% TFA, 80:20 → 100:0, 30 min, R_t = 26.2 min, λ = 212 nm). *HR-ESI-MS (positive, m/z)* calc. for $C_{146}H_{198}F_{51}N_{29}O_{48}$: 2059.1594 ($[M+H+Na]^{2+}$, calc.: 2059.1553).
MALDI-TOF-MS (dhb, positive, m/z): 4099.23 ($[M+H]^+$, calc.: 4094.31).

ELISA Protocol:

Coating: The amphiphilic glycoconjugates were dissolved in a phosphate buffer (0.1 M $Na_2HPO_4 \bullet H_2O$, pH = 9.3; *c* = 5 µg/mL) and transferred to the wells of a PS-microtitre plate (Immuno-Plate F96 MaxiSorp, Nunc, Wiesbaden, Germany; 50 µL/well). 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.

Titration: To a solution of 1% BSA in PBS (50 µL) in the first well were added 50 µL of diluted serum (1:25 in 1% BSA/PBS). After careful mixing 50 µL of this solution were transferred to the subsequent well and the procedure was repeated down the plate yielding serial half log dilutions from 1:1000 to 1:2.048.000. 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.

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 of Fig. 1 a)

dilution	**1/1.000**	***1/2.000***	***1/4.000***	***1/8.000***	***1/16.000***	***1/32.000***	***1/64.000***	***1/128.000***	***1/256.000***	***1/512.000***	***1/1.024.000***	***1/2.048.000***
mouse	1,6524	1,7442	1,5667	1,5099	1,0253	0,7071	0,5531	0,3921	0,1716	0,1056	0,0859	0,0684
SM3	0,8804	0,8173	0,8260	0,6856	0,4885	0,2281	0,1465	0,1190	0,0799	0,0665	0,0589	0,0548
negative	0,0586	0,0539	0,0596	0,0584	0,0585	0,0580	0,0586	0,0564	0,0490	0,0599	0,0570	0,0592

Data of Fig. 1 b)

dilution	**1/1.000**	***1/2.000***	***1/4.000***	***1/8.000***	***1/16.000***	***1/32.000***	***1/64.000***	***1/128.000***	***1/256.000***	***1/512.000***	***1/1.024.000***	***1/2.048.000***
9a	1,3632	1,0750	1,0844	0,6647	0,3887	0,2513	0,1505	0,1038	0,0831	0,0730	0,0626	0,0610
9b	1,6524	1,7442	1,5667	1,5099	1,0253	0,7071	0,5531	0,3921	0,1716	0,1056	0,0859	0,0684
9c	1,4160	1,2989	0,9224	0,6645	0,3908	0,2311	0,1417	0,0914	0,0754	0,0649	0,0603	0,0665
negative	0,0539	0,0596	0,0584	0,0585	0,0580	0,0586	0,0564	0,0490	0,0599	0,0570	0,0592	0,0539