Electronic Supplementary Information

Synthesis of MUC1-Lipopeptide Chimeras

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Contents

General procedures

^{1}H NMR spectra were recorded using a Bruker DRX 300 or DRX 400 spectrometer at frequencies of 300 MHz and 400 MHz, respectively. ^{13}C NMR spectra were recorded on a Bruker DPX 300 MHz or DPX 400 spectrometer at a frequency of 75 MHz or 100 MHz, respectively. The spectra are reported as parts per million (ppm) downfield shift using trimethylsilane as the internal reference. The data are reported as chemical shift (δ), multiplicity, relative integral, coupling constant (*J* Hz) and assignment where possible. IR spectra were measured on a Bruker ALPHA-E FTIR spectrometer fitted with a ZnSe ATR accessory as a thin film. Optical rotations were obtained using a Perkin Elmer model 341 polarimeter at 20 °C and $[\alpha]_D$ values are reported in 10^{-1} deg cm^2 g^{-1}. Low resolution mass spectra were recorded on a Finnigan LCQ Deca ion trap mass spectrometer (ESI) operating in positive ion mode. High resolution ESI and MALDI-TOF mass spectra were measured on a Bruker–Daltonics Apex Ultra 7.0T fourier transform mass spectrometer (FTICR).

MALDI matrices:

Matrix A: 10mg/mL α-cyano-4-hydroxycinnamic acid (α-CHCA) water/acetonitrile (1:1 v/v) containing 0.1% vol. TFA.

Matrix B: 18mg/mL 2',4',6'-trihydroxyacetophenone (THAP) and 7 mg ammonium citrate in acetonitrile/water (1:1 v/v).

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. A Waters Sunfire 5 μm, 2.1 x 150 mm column (C-18) was used at a flow rate of 0.2 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) using a linear gradient of 0-25% B or 0-30% B over 30 min. Lipopeptide **7** and chimeras **18a-c** were analysed using a Waters Symmetry 5 μm, 2.1 x 150 mm column (C-4) at a flow rate of 0.2 mL min^{-1} using a mobile phase of 0.1% TFA in water/acetonitrile/isopropanol (8:1:1 v/v/v) (Solvent A) and 0.1% TFA in acetonitrile/isopropanol (1:1 v/v) (Solvent B). Results were analysed with Waters Empower software. Preparative and semi-preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array

detector or Waters 490E Programmable wavelength detector operating at 230 and 214 nm. Peptide **10a** and glycopeptides **10b** and **10c** were purified on a Waters Sunfire 5 μm (C-18) preparative column operating a flow rate of 7 mL min^{-1} using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). Lipopeptide chimeras **18a-c** were purified on a Vydac 5 μm (C-4) semi-preparative column operating a flow rate of 5 mL min^{-1} using a mobile phase of water/acetonitrile/isopropanol (8:1:1 v/v/v) (Solvent A) and acetonitrile/isopropanol (1:1 v/v) (Solvent B).

LC-MS was performed on a Thermo Separation Products: Spectra System consisting of P400 Pump and a UV6000LP Photodiode array detector on a Phenomenex Jupiter 5 μm, 2.1 x 150 mm column at a flow rate of 0.2 mL min^{-1} coupled to a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI) operating in positive mode. Separations involved a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B) using a linear gradient of 0-25% B or 0-30% B over 30 min.

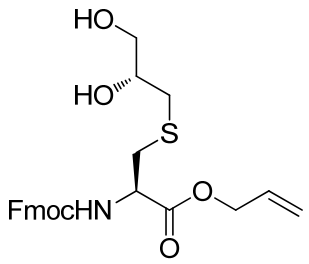
Materials

Analytical thin layer chromatography (TLC) was performed on commercially prepared silica plates (Merck Kieselgel 60 0.25 mm F254). Flash column chromatography was performed using 230-400 mesh Kieselgel 60 silica eluting with distilled solvents as described.

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem. DCM and methanol were distilled from calcium hydride. DMF was obtained as peptide synthesis grade from Merck or Labscan.

Synthesis of Pam_3CysOH (1):

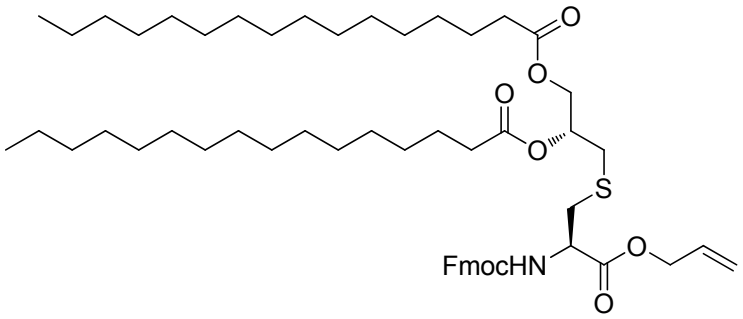
***N*-Fluorenylmethoxycarbonyl-*S*-[2,3-dihydroxy-(2R)-propyl]-(R)-cysteine allyl ester (4)**



A suspension of *N*-Fluorenylmethoxycarbonyl-(R)-cysteine allyl ester[1] (**2**, 1.0 g, 2.6 mmol) and powdered, activated 4Å MS in dry DMF (7 mL) was cooled in an ice bath under an atmosphere of argon. Cesium carbonate (850 mg, 2.6 mmol, 1.0 equiv.) and tetrabutyl-ammonium iodide (960 mg, 2.6 mmol, 1.0 equiv.) were added sequentially and the suspension was stirred for 10 minutes at 0 ^{o}C. A solution of (R)-1,2-bromopropane diol[2] (**3**, 610 mg, 3.9 mmol, 1.5 equiv.) in dry DMF (2 mL) was then added dropwise and the mixture was warmed to rt. The mixture was stirred for 1 h at rt, before being filtering through Celite® and eluting with ethyl acetate (50 mL). The solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography (SiO_2, toluene/ethyl acetate, 1:1 v/v). The mixture was then filtered on Celite®, eluting with ethyl acetate (50 mL) and concentrated *in vacuo*. The resulting residue was purified by flash chromatography (SiO_2, toluene/ethyl acetate 1:1 v/v) to afford **4** as a white solid (572 mg, 43%): R_f 0.2 (toluene/ethyl acetate, 1:1 v/v); mp 82.3 ^{o}C; $[\alpha]_D^{20}$ +2.5 (*c*, 0.6, $CHCl_3$); IR (ATR, Zn/Se): 3990, 3065, 1788, 1717, 1528, 1182 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 1H, *J* = 9.0 Hz, Fmoc-Ar), 7.61 (d, 2H, *J* = 6.0 Hz, Fmoc-Ar), 7.40 (t, 2H, *J* = 9.0 Hz, Fmoc-Ar), 7.34-7.26 (m, 2H, Fmoc-Ar), 6.01-5.75 (m, 2H, NH, C*H*=CH_2), 5.42-5.25 (m, 2H, CH=CH_2), 4.72-4.66 (m, 3H, Cys-CHα, OCH_2CH=CH_2), 4.55-4.41 (m, 2H, Fmoc-CH_2), 4.23 (t, 1H, *J* = 8.0 Hz, Fmoc-CH), 3.87-3.61 (m, 3H, *S*-glyceryl-CH_2, *S*-glyceryl-OCH_{2a}), 3.61-3.48 (m, 1H, *S*-glyceryl OCH_{2b}), 3.00 (br s, 1H, OH), 2.65-2.54 (2H, m, *S*-glyceryl-CH_2), 2.35 (br s, 1H, OH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4 (C=O), 156.0 (Fmoc C=O), 143.7, 143.6, 141.3 (Fmoc-ArCH), 131.2 (*C*H=CH_2), 127.7, 127.1, 125.1, 120.0 (Fmoc-ArC), 119.4 (CH=*C*H_2), 70.7 (*S*-glyceryl-CH_2), 67.3 (Fmoc-CH_2), 66.5 (O*C*H_2CH=CH_2), 65.1 (*S*-glyceryl CH_2), 54.0 (Cys-CH_2), 47.1

(Fmoc-CH), 36.4 (*S*-glyceryl-CH_2). *m/z* (ESI^+) 457.99 ($M+H^+$, 45%), 931.71 ($2M+Na^+$, 100%); HRMS Calcd for $C_{24}H_{27}NO_6SNa$: MNa^+, 480.1457, found MNa^+, 480.1453.

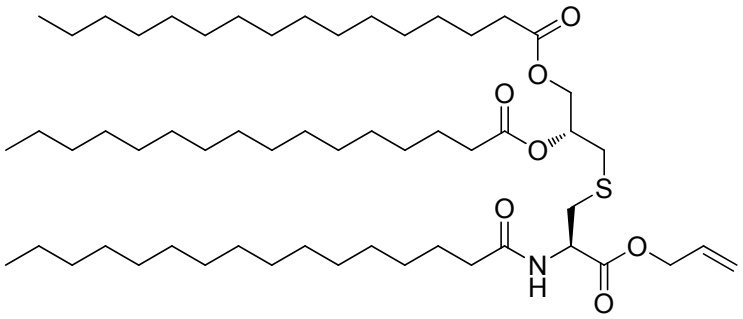
_N_-Fluorenylmethoxycarbonyl-_S_-[2,3-_bis_(palmitoyloxy)-(2R)-propyl]-(R)-cysteine allyl ester (5)



To a solution of *N*-Fluorenylmethoxycarbonyl-*S*-[2,3-dihydroxy-(2R)-propyl]-(R)-cysteine allyl ester **4** (500 mg, 1.1 mmol) in dry THF (13 mL) was added palmitic acid (860 mg, 3.4 mmol, 3.2 equiv.), *N*,*N*-dimethylaminopyridine (49 mg, 0.4 mmol, 0.4 equiv.) and *N*,*N'*-diisopropylcarbodiimide (640 μL, 4.1 mmol, 3.9 equiv.) under an atmosphere of nitrogen. The solution was stirred vigorously at rt for 5 h before quenching by the addition of glacial acetic acid (1 mL). The solvent was removed *in vacuo* and the residue purified by flash chromatography (SiO_2, toluene/ethyl acetate, 50:1 v/v) to afford **5** as a waxy solid (1.0 g, 91%): R_f 0.1 (toluene/ethyl acetate, 50:1 v/v); mp 63-64 ^oC; $[\alpha]_D^{20}$ +1.7 (*c*, 1.0, $CHCl_3$); IR (ATR, Zn/Se): 2921, 2851, 1737, 1168 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 7.76 (d, 2H, *J* = 9.1 Hz, Fmoc-Ar), 7.61 (d, 2H, *J* = 6.0 Hz, Fmoc-Ar), 7.40 (t, 2H, *J* = 9.0 Hz, Fmoc-Ar), 7.34-7.26 (m, 2H, Fmoc-Ar), 5.92 (m, 1H, C*H*=CH_2), 5.69 (d, 1H, $J_{NH\text{-}CH}$ = 9.0 Hz, Fmoc-NH), 5.39-5.29 (m, 2H, CH=CH_2), 5.16-5.11 (1H, m, *S*-glyceryl-CH), 4.69-4.66 (m, 3H, OCH_2CH=CH_2, Cys-CHα), 4.38-4.22 (m, 4H, Fmoc-CH_2, Fmoc-CH, *S*-glyceryl-OCH_{2a}), 4.18-4.13 (m, 1H, *S*-glyceryl-OCH_{2b}), 3.16-3.01 (m, 2H, Cys-CH_2), 2.75 (d, 2H, *J* = 6.0 Hz, *S*-glyceryl-CH_2), 2.38-2.27 (m, 4H, 2 × Pal-CH_2), 1.70-1.50 (m, 4H, 2 × Pal-CH_2), 1.25 (m, 48H, 24 × Pal-CH_2), 0.88 (t, 6H, *J* = 6.0 Hz, 2 × Pal-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.5, 173.2, 155.9 (C=O), 143.9, 141.5 (Fmoc-Ar), 131.4 (*C*H=CH_2), 127.9, 127.2, 125.3, 120.1 (Fmoc-Ar), 119.4 (CH=*C*H_2), 70.3 (*S*-glyceryl-CH), 67.5 (Fmoc-CH_2), 66.6 (O*C*H_2CH=CH_2), 63.6 (*S*-glyceryl-OCH_2), 54.0 (Cys-CHα), 47.3 (Fmoc-CH), 35.2 (Cys-CH_2), 34.4, 34.2 (Pal-CH_2), 33.3 (*S*-glyceryl-CH_2), 32.1, 29.9, 29.8, 29.7, 29.5, 29.3, 29.0

(Pal-CH_2), 25.0, 22.9 (Pal-CH_2), 14.3 (Pal-CH_3). *m/z* (ESI^+): 956.87 ($M+Na^+$, 100%). HRMS Calcd for $C_{56}H_{87}NO_8SNa$: MNa^+, 956.6050, found MNa^+, 956.6045.

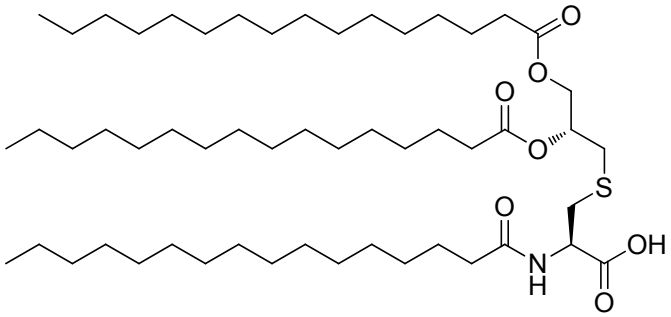
***S*-[2,3-*Bis*(palmitoyloxy)-(2R)-propyl]-*N*-palmitoyl-(R)-cysteine allyl ester (6).**



N-Fluorenylmethoxycarbonyl-*S*-[2,3-*bis*(palmitoyloxy)-(2R)-propyl]-(R)-cysteine allyl ester (**5**) (230 mg, 0.25 mmol) was dissolved in piperidine/DMF (1:1 v/v, 2.6 mL) and allowed to stir for 2 h at rt. The solvent was removed *in vacuo* and co-evaporated with toluene (3 x 5 mL). The resulting residue was re-dissolved in DCM/DMF (2:5 v/v, 2.6 mL) and treated with HOBt (70 mg, 0.43 mmol, 1.8 equiv.), palmitic acid (109 mg, 0.43 mmol, 1.8 equiv.) and *N,N'*-diisopropylcarbodiimide (70 µL, 0.43 mmol, 1.8 equiv.). After stirring at rt for 16 h, DCM (10 mL) was added and the mixture was washed with water (10 mL), saturated aqueous $NaHCO_3$ solution (10 mL) and brine (10 mL). The combined aqueous layers were then washed with DCM (3 × 10 mL) and the organic layers were combined, dried (Na_2SO_4), filtered and concentrated *in vacuo*. Crystallisation from $CHCl_3$/CH_3OH (1:5 v/v, 4.0 mL) afforded **6** as a white solid (170 mg, 72%): mp 61-62 ^oC. $[\alpha]_D^{20}$ +7.0 (*c*, 1.0, $CHCl_3$); IR (ATR Zn/Se): 2918, 2850, 1741, 1467 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.31 (t, 1H, *J* = 7.9 Hz, NH), 5.98-5.84 (m, 1H, *J* = 5.8 Hz, C*H*=CH_2), 5.38-5.25 (m, 2H, CH=C*H*$_2$), 5.16-5.09 (m, 1H, *S*-glyceryl-CH), 4.89-4.82 (m, 1H, Cys-CHα), 4.65 (d, 2H, *J* = 5.8 Hz, OC*H*$_2$CH=CH_2), 4.32 (dd, 1H, $J_{Ha\text{-}Hb}$ = 12.0 Hz, $J_{Ha\text{-}CH}$ = 3.6 Hz, *S*-glyceryl-OCH_{2a}), 4.13 (dd, 1H, $J_{Ha\text{-}Hb}$ = 12.0 Hz, $J_{Hb\text{-}CH}$ = 6.0 Hz, *S*-glyceryl-OCH_{2b}), 3.02 (m, 2H, *S*-glyceryl-CH_2), 2.72 (d, 2H, *J* = 6.5 Hz, Cys-CH_2), 2.33-2.22 (m, 6H, 3 × Pal-C(O)CH_2), 1.62-1.46 (m, 6H, 3 × Pal-CH_2), 1.41-1.22 (m, 72H, 36 × Pal-CH_2), 0.87 (t, 9H, *J* = 6.2 Hz, 3 × Pal-CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 173.1, 173.0, 170.5 (C=O), 131.3 (*C*H=CH_2), 119.2 (CH=*C*H_2), 70.3 (*S*-glyceryl-CH_2), 66.4 (O*C*H_2C=CH_2), 63.5 (*S*-glyceryl-OCH_2), 51.8 (Cys-CHα), 36.5, 35.0, 34.9, 34.3, 34.1, 33.2, 33.0, 31.9, 29.7, 29.5, 29.4, 29.3, 29.2, 25.5, 24.9,

22.7 (Cys-CH_2, *S*-glyceryl-CH_2, Pal-CH_2), 14.1 (Pal-CH_3). *m/z* (ESI+): 973.12 ($M+Na^+$, 35%); HRMS Calcd for $C_{57}H_{107}NO_7SNa$: MNa^+, 972.7667 found MNa^+, 972.6682.

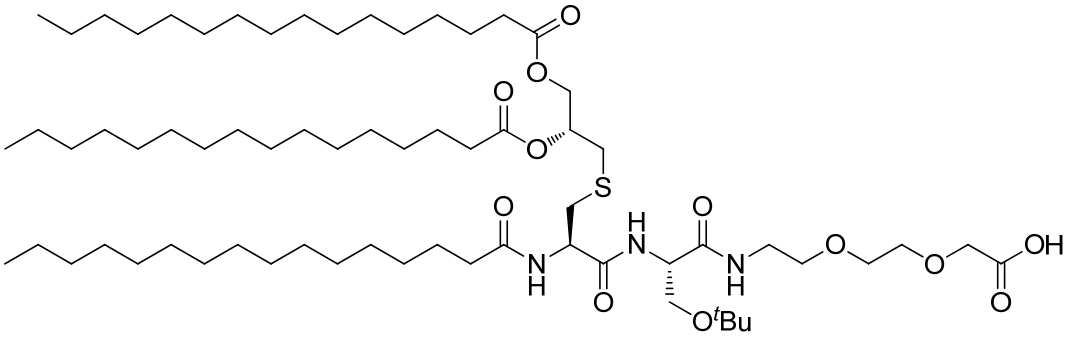
_S_-[2,3-_Bis_(palmitoloxy)-(2R)-propyl]-_N_-palmitoyl-(R)-cysteine (1)



S-[2,3-*Bis*(palmitoyloxy)-(2R)-propyl]-*N*-palmitoyl-(R)-cysteine allyl ester (**6**, 160 mg, 0.16 mmol) was dissolved in dry THF (4.8 mL) and placed under an atmosphere of argon. *N*-methylaniline (180 µL, 1.6 mmol, 10 equiv.) and tetrakis(triphenylphosphine)palladium(0) (10 mg, 0.016 mmol, 0.1 equiv.) were subsequently added and the solution stirred for 1 h at rt. The solvent was removed *in vacuo* and the resulting residue purified by gradient flash chromatography (SiO_2, DCM to 5% CH_3OH/DCM). The resulting waxy solid was lyophilized from *tert*-butyl alcohol to give **1** as white solid (142 mg, 0.16 mmol, quant.): R_f 0.2 (5% CH_3OH/DCM); mp 61-63 ^oC (lit. 66 ^oC).[3] ^1H NMR (300 MHz, $CDCl_3$): δ 6.58 (d, 1H, NH, $J_{NH,CH}$ = 6.3 Hz), 5.20-5.09 (m, 1H, *S*-glyceryl-CH), 4.75-4.64 (m, 1H, Cys-CHα), 4.37-4.28 (m, 1H, *S*-glyceryl-OCH_{2a}), 4.10 (dd, 1H, *S*-glyceryl-OCH_{2b}, $J_{Hb,Ha}$ = 12.0 Hz, $J_{Hb,CH}$ = 6.0 Hz), 3.18-2.97 (m, 2H, Cys-CH_2), 2.72 (d, 2H, *S*-glyceryl-OCH_2, $J_{CH2,CH}$ = 6.1 Hz), 2.35-2.23 (m, 6H, 3 x Pal-$C(O)CH_2$), 1.68-1.52 (m, 6H, 3 x Pal-CH_2), 1.35-1.17 (m, 72H, 36 x Pal-CH_2), 0.90-0.82 (m, 9H, 3 x Pal-CH_3). HRMS Calcd for $C_{54}H_{103}NO_7SNa$: MNa^+ 932.7353, found MNa^+ 932.7335. These data are in agreement with those reported by Boons and co-workers.[3]

Synthesis of $Pam_3CysSer(O^tBu)$triethylene glycolic acid (7)

***N*-Palmitoyl-*S*-[2,3-*bis*(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(O-*tert*-butyl)-(S)-serinyl-$NH(CH_2CH_2O)_2CH_2CO_2H$ (7)**



2-Chlorotrityl chloride resin (1 mmol/g loading; 100 mg, 0.1 mmol) was swollen in dry DCM (5.0 mL) for 30 minutes at rt. The resin was filtered and treated with a solution of Fmoc-PEG-OH (9 atoms, Novabiochem) (78 mg, 0.2 mmol, 2.0 equiv.) and *N,N*-diisopropylethylamine (70 µL, 0.4 mmol) in DMF/DCM (1:1 v/v, 1.0 mL) with shaking for 16 h at rt. The resin was washed with DMF (5 × 2 mL), DCM (5 × 2.0 mL) and DMF (5 × 2.0 mL) before being treated with a solution of DCM/CH_3OH/DIPEA (17:2:1 v/v/v, 3.0 mL) for 30 min with gentle agitation. The resin was filtered and washed with DMF (5 × 2.0 mL) and DCM (10 × 2.0 mL). The resin was dried *in vacuo* and split into two 50 µmol batches. The pre-loaded resin (50 µmol) was re-swollen in DCM (2.0 mL) for 30 min, washed with DMF (5 × 2.0 mL) and treated with a 10% piperidine/DMF solution (2 × 2.0 mL) for 3 min. The resin was then filtered and washed with DMF (5 × 2.0 mL), DCM (5 × 2.0 mL) and DMF (5 × 2.0 mL). The resin was treated with a solution of Fmoc-Ser(O^tBu)-OH (77 mg, 0.2 mmol, 4.0 equiv.), PyBOP (104 mg, 0.2 mmol, 4.0 equiv.) and NMM (44 uL, 0.4 mmol, 8.0 equiv.) and shaken for 2 h. The resin was filtered and washed with DMF (5 × 2.0 mL), DCM (5 × 2.0 mL), and DMF (5.0 mL) before treating with 2.0 mL of a 10% acetic anhydride/pyridine solution. The resin was filtered and washed with DMF (5 × 2.0 mL), DCM (5 × 2.0 mL) and DMF (5 × 2.0 mL) and treated with a 10% piperidine/DMF solution (2 × 2.0 mL) for 3 min. The resin was filtered and washed with DMF (5 × 2.0 mL), DCM (5 × 2.0 mL) and DMF (5 × 2.0 mL), before treating with a solution of **1** (75 mg, 83 µmol, 1.5 equiv.), 2-(1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) (32 mg, 83 µmol, 1.5 equiv.) and NMM (19 µL, 170 µmol, 3.0 equiv.) in DMF/DCM (5:2 v/v, 300 µL).

The resin was shaken for 24 h, before being filtered and washed with DMF (5 × 2.0 mL), DCM (5 × 2.0 mL), and DMF (5.0 mL). The resin was washed thoroughly with DCM (10 × 2.0 mL) and treated with a 30 vol. % hexafluoroisopropanol/DCM solution (2.0 mL) at rt for 2 h. The resin was filtered, washed with DCM (5 × 2.0 mL) and the filtrate evaporated to dryness. The resulting residue was purified by flash chromatography (SiO_2, 5% methanol/DCM) to afford a waxy solid. Lyophilisation from *tert*-butyl alcohol afforded the lipopeptide **7** as a colourless solid (56 mg, 95%): R_f 0.4 (CH_3OH/DCM, 1:9 v/v); mp 52 ^oC. $[\alpha]_D^{20}$ +7.0 (*c*, 0.7 in $CHCl_3$); IR (ATR Zn/Se): 3295, 2956, 2910, 2850, 1741, 1638, 1545, 1468, 1365, 1244, 1196, 1163, 1114 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 5.15-5.09 (m, 1H, *S*-glyceryl-CH), 4.63-4.58 (m, 1H, Cys-CHα), 4.51-4.49 (m, 1H, *S*-glyceryl-OCH_{2a}), 4.34-4.27 (m, 1H, Ser-CHα), 4.14-4.08 (m, 3H, *S*-glyceryl-OCH_{2b}, PEG-CH_2CO_2H), 3.65-3.30 (m, 10H, Ser-CH_2, 4 x PEG-CH_2), 2.97-2.71 (m, 4H, Cys-CH_2, *S*-glyceryl-CH_2), 2.30-2.20 (m, 6H, 3 × Pal-C(O)CH_2), 1.70-1.50 (m, 6H, 3 × Pal-CH_2), 1.32-1.19 (m, 72H, 36 × Pal-CH_2), 1.30 (s, 9H, *tert*-Bu CH_3), 0.85-0.82 (m, 9H, 3 x Pal-CH_3). *m/z* (ESI^+): 1221.40 ($M+Na^+$, 100%); HRMS Calcd for $C_{67}H_{127}N_3O_{12}SNa$: MNa^+ 1220.9038, found MNa^+ 1220.9061.

General procedure for the solid-phase peptide synthesis (SPPS) of peptide (10a) (20 μmol scale) and glycopeptides (10b and 10c) (25 μmol scale):

Resin loading: 2-chlorotrityl chloride resin (Novabiochem) was swollen in dry DCM (5 mL) for 30 min. A solution of Fmoc-His(Trt)-OH (31 mg, 50 μmol, 2.0 equiv.) and DIPEA (70 μL, 0.4 mmol) in DMF/DCM (1:1 v/v, 1.0 mL) was added and the resin shaken at rt for 16 h. The resin was filtered and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL). The resin was treated with a solution of DCM/CH_3OH/DIPEA (17:1:1 v/v/v, 3 mL) for 1 h, filtered and washed with DMF (5 × 3 mL), DCM (5 × 3 mL) and DMF (5 × 3 mL).

Fmoc Deprotection: Pre-loaded 2-chlorotrityl chloride resin was initially swollen in DMF (5 mL) for 30 min. A solution of piperidine/DMF (1:9 v/v, 5 mL) was added to the resin which was shaken for 3 min and the procedure repeated. The resin was subsequently washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

Unglycosylated Amino Acid Coupling: A solution of protected amino acid (100 μmol), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (52 mg, 100 μmol, 4.0 equiv.) and *N*-methylmorpholine (NMM) (22 μL, 200 μmol, 4.0

equiv.) in DMF (1 mL) was added to the resin and shaken. After 1 h the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

For **10b** and **10c**: *Coupling of Glycosylamino Acids* **13-16***:* A solution of **13, 14, 15,** or **16** (30 μmol, 1.2 equiv.), 2-(1H-7-azabenzotriazole-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate (HATU) (11 mg, 30 μmol, 1.2 equiv.) and NMM (6.6 μL, 60 μmol, 2.4 equiv.) in DMF (0.7 mL) was added to the resin and shaken. After 18 h the resin was washed with DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

Capping: Acetic anhydride/pyridine (1:9 v/v, 2 mL) was added to the resin and shaken. After 3 min the resin was washed DMF (5 × 3 mL), DCM (5 × 3 mL), and DMF (5 × 3 mL).

The above steps were repeated in an iterative fashion to assemble the desired peptide/glycopeptide on the resin.

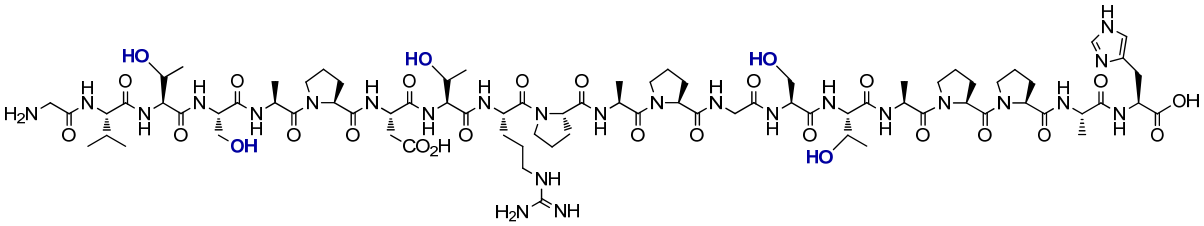
Resin cleavage: The resin was washed thoroughly with DCM (20 × 3 mL) and treated with a solution of TFA/TIS/thioanisole/water (85:5:5:5 v/v/v/v, 2 mL) and shaken for 2 h at rt. The resin was filtered and the filtrate was evaporated to dryness. At this stage, peptide **18a** was purified by preparative HPLC.

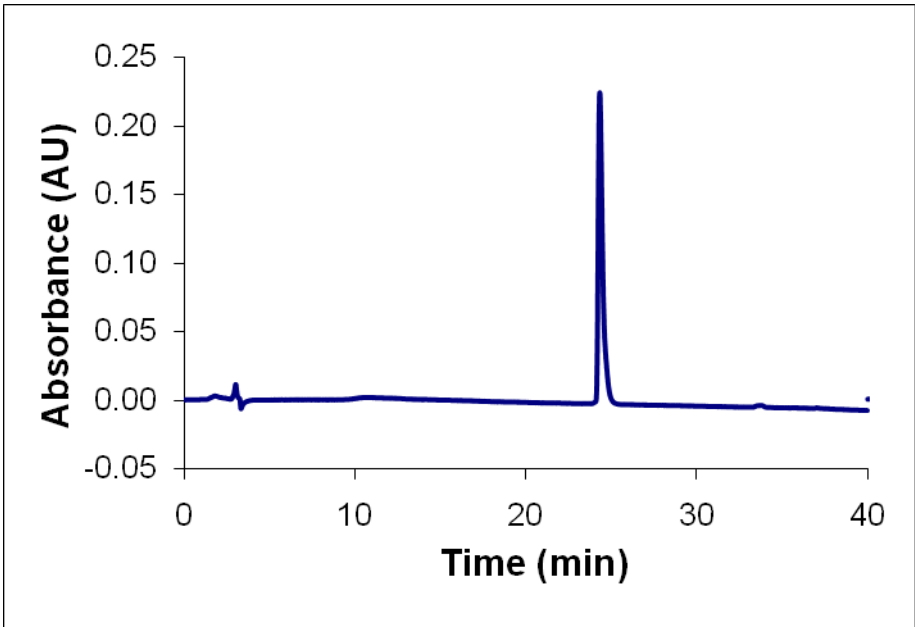
De-O-acetylation of glycopeptides **18b** *and* **18c**: Acetylated glycopeptides were suspended in Milli-Q water (approx. 6 mL) and filtered through a 5 μm membrane filter to remove insoluble material. The filtrate was treated with hydrazine hydrate (300 μL) and gently agitated at rt for 2 h. Glycopeptides **18b** an **18c** were then purified by reverse phase preparative HPLC.

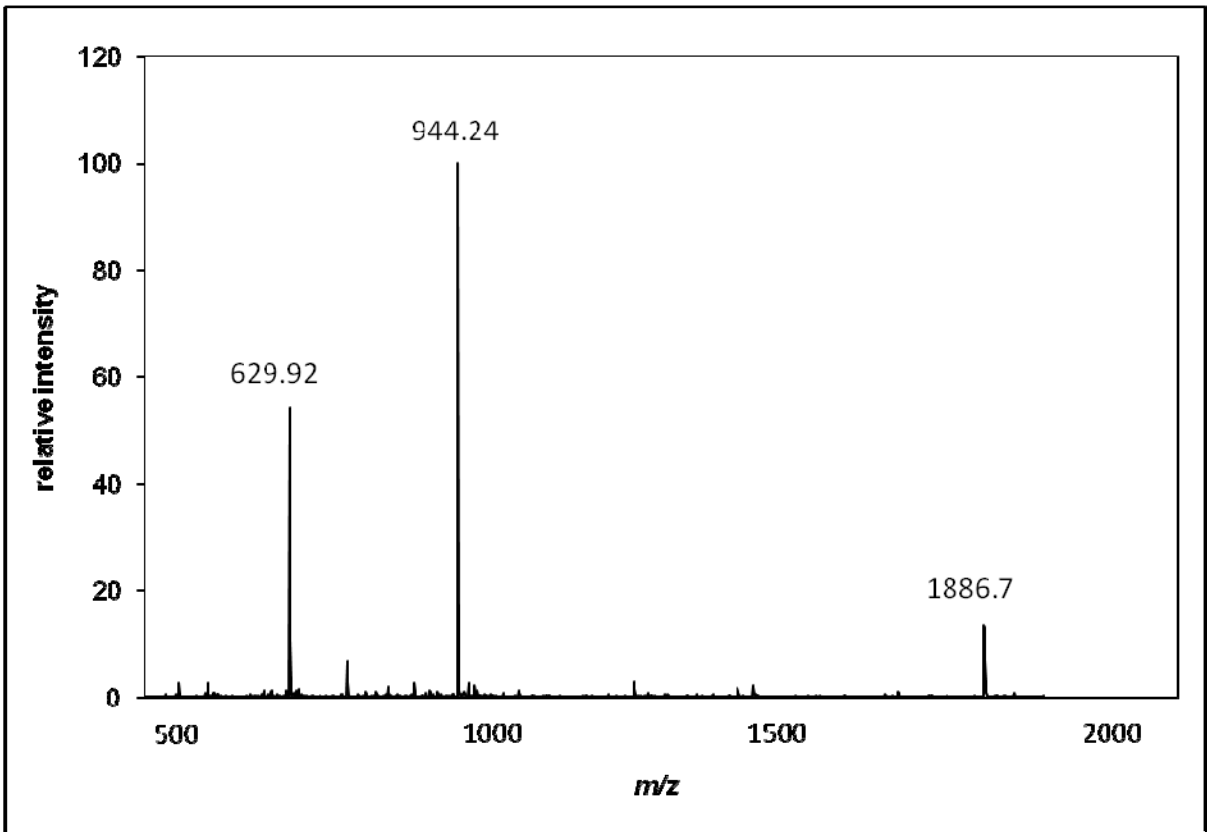
Analytical Data for MUC1 peptide (10a) and glycopeptides (10b and 10c).

Peptide 10a: H-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-His-OH

Peptide **10a** was prepared according to the Fmoc-strategy SPPS method outlined in the general procedures and purified by preparative reverse phase HPLC (0 to 30% B over 60 min) affording **10a** as a white solid following lyophilisation (17 mg, 45% yield based on the original 20 μmol resin loading).

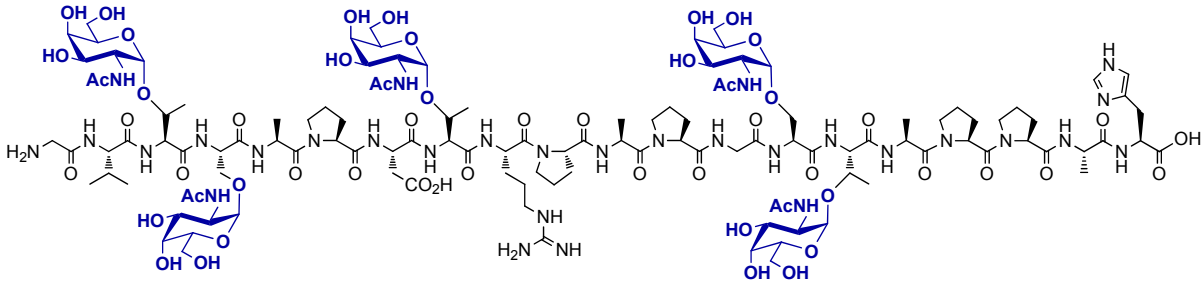




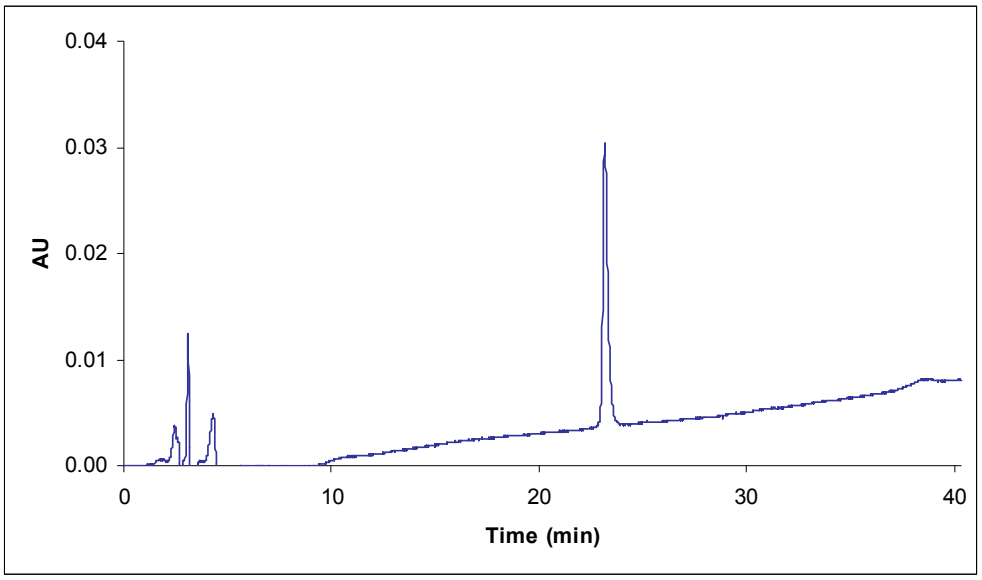


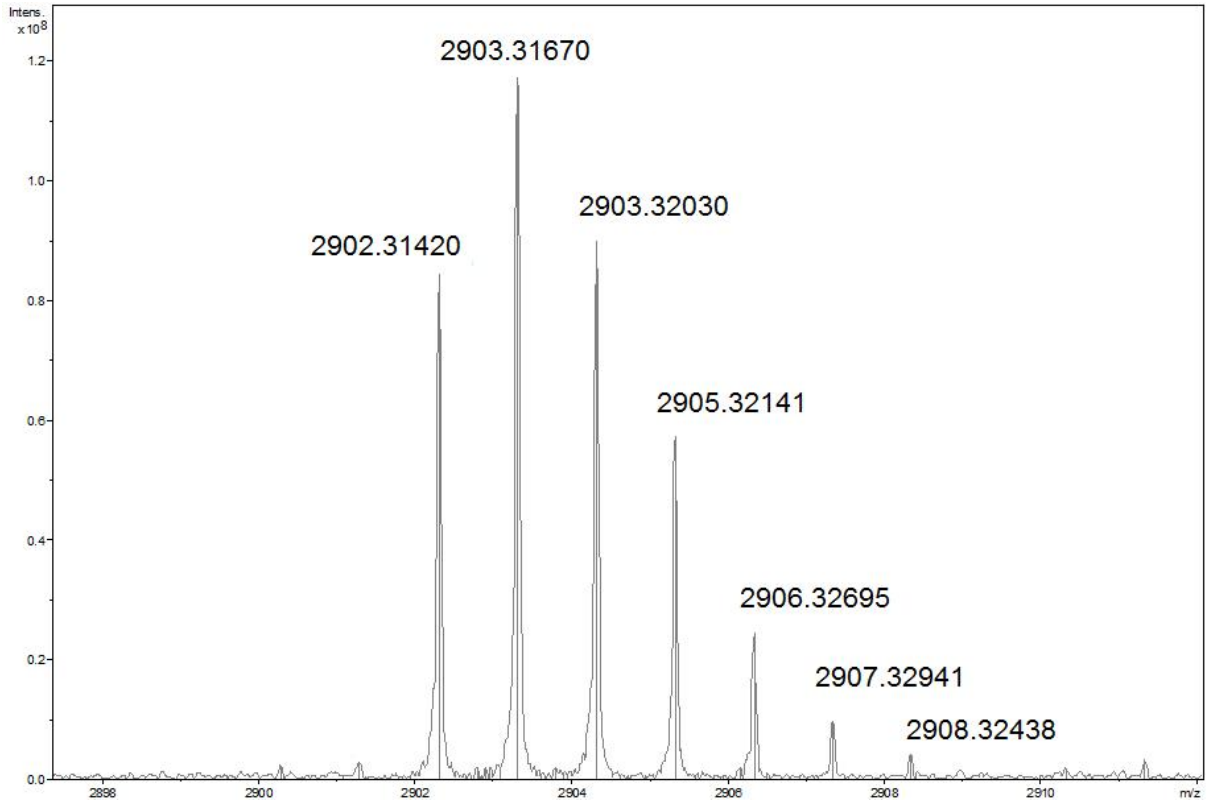
Analytical HPLC: R_t 24.3 min (0-30% B over 40 min, λ = 230 nm); MS (ESI^+) *m/z* 629.92 [$(M+3H)^{3+}$, 55%], 944.23 [$(M+2H)^{2+}$, 100%], 1886.78 [$M+H^+$, 20%)].

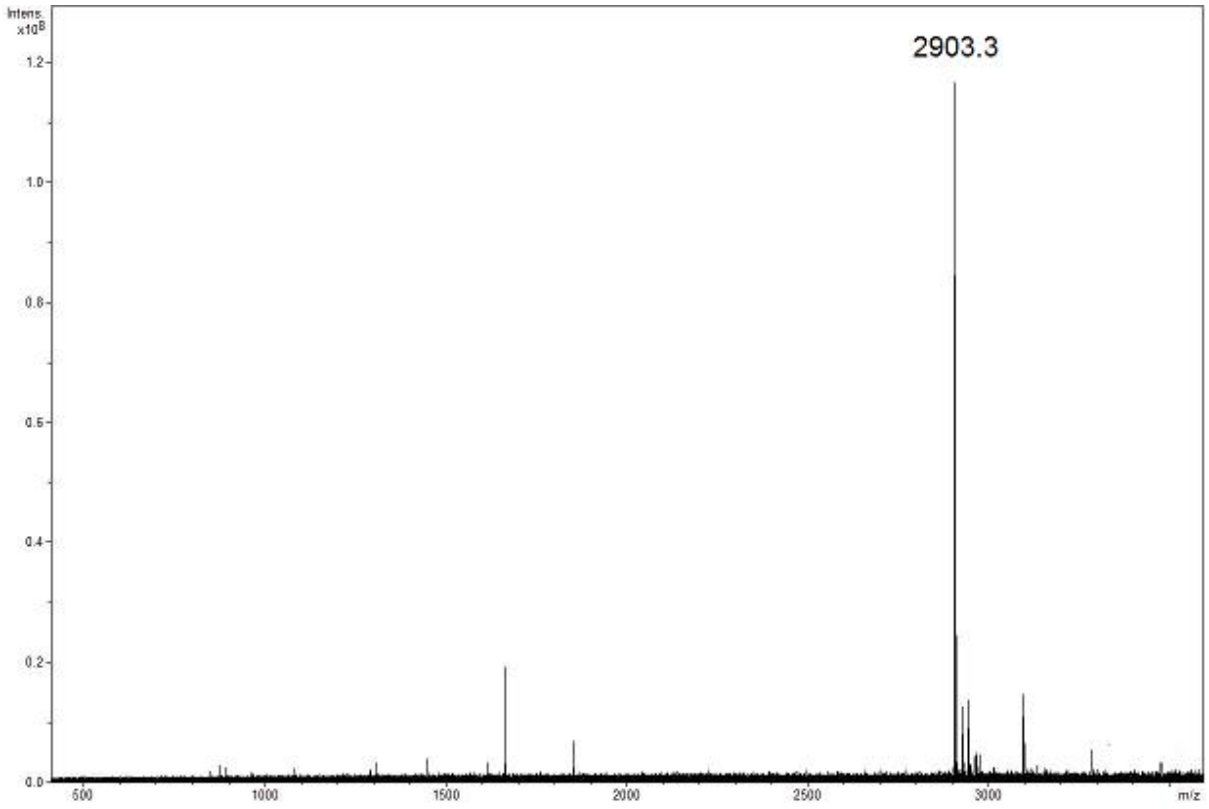
Glycopeptide 10b: H-Gly-Val-Thr(α-GalNAc)-Ser(α-GalNAc)-Ala-Pro-Asp-Thr(α-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser(α-GalNAc)-Thr(α-GalNAc)-Ala-Pro-Pro-Ala-His-OH



Glycopeptide **10b** was prepared according to the Fmoc-strategy SPPS method outlined in the general procedures and purified by preparative reverse phase HPLC (0 to 25% B over 60 min) affording **10b** as a white solid following lyophilisation (16 mg, 22% yield based on the original 25 μmol resin loading).

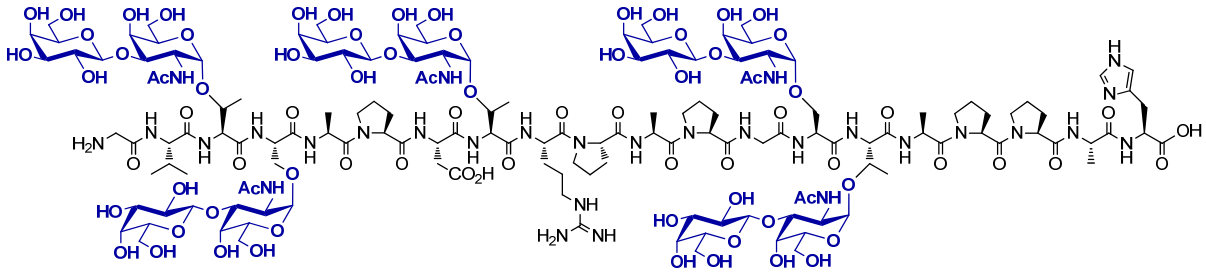



Intens.
x10^8
2903.31670
2903.32030
2902.31420
2905.32141
2906.32695
2907.32941
2908.32438
m/z

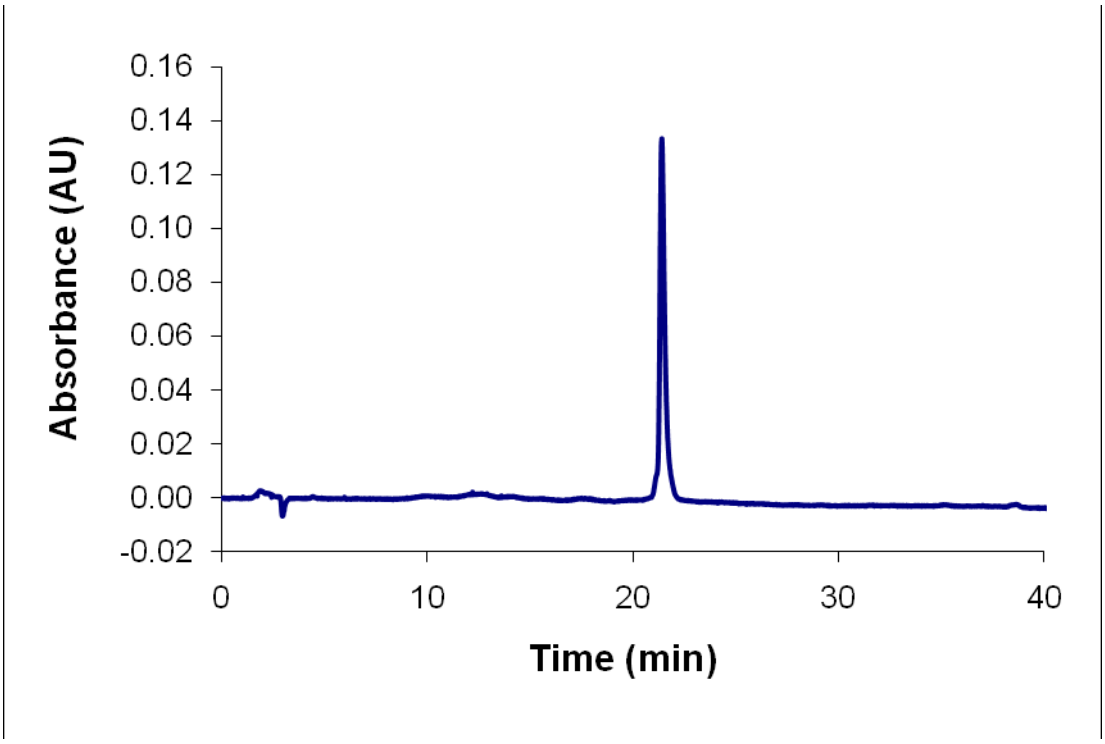

Intens.
x10^8
2903.3
m/z

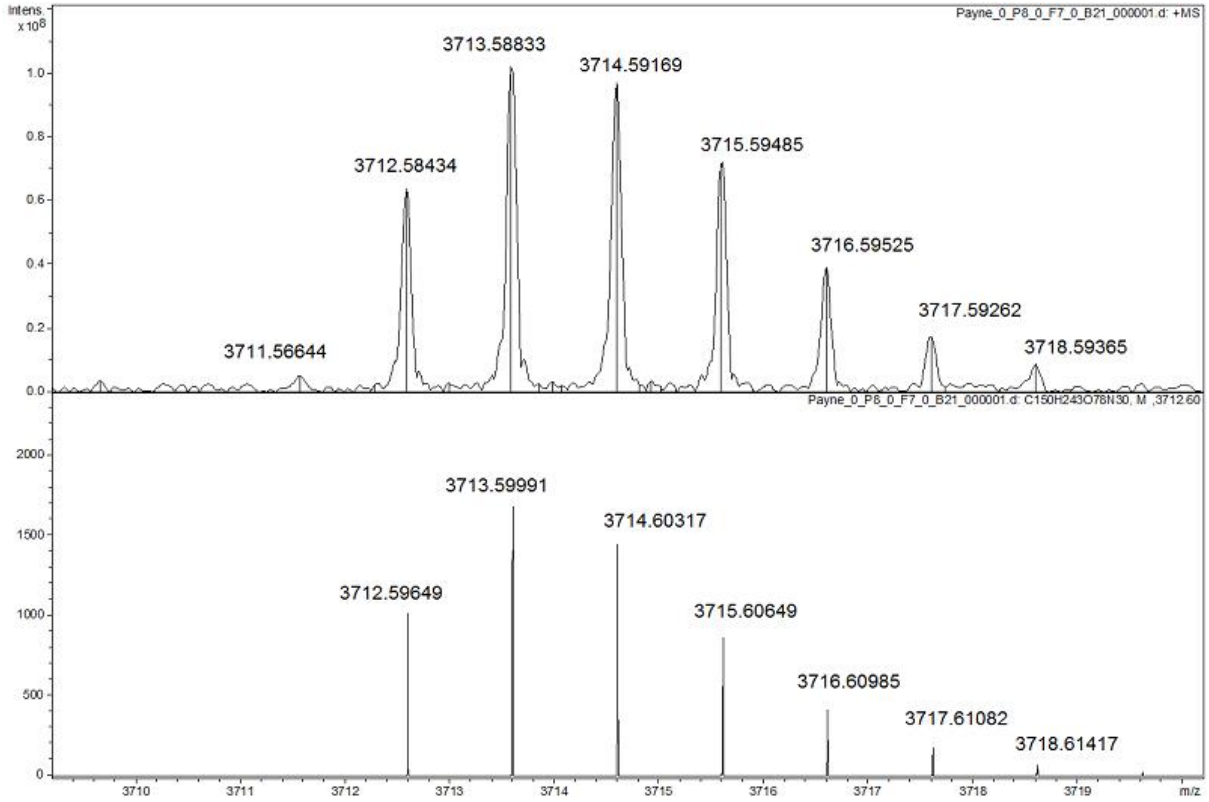
Analytical HPLC: R_t 23.0 min (0-25% B over 40 min, λ = 230 nm). MS (ESI^+): *m/z* 1452.2 [$(M+2H)^{2+}$ 100%]; 968.4 [$(M+3H)^{3+}$ 48%]; MALDI-TOF HRMS: Calcd for $C_{120}H_{193}N_{30}O_{53}$: MH^+, 2903.3230 found MH^+, 2903.3167.

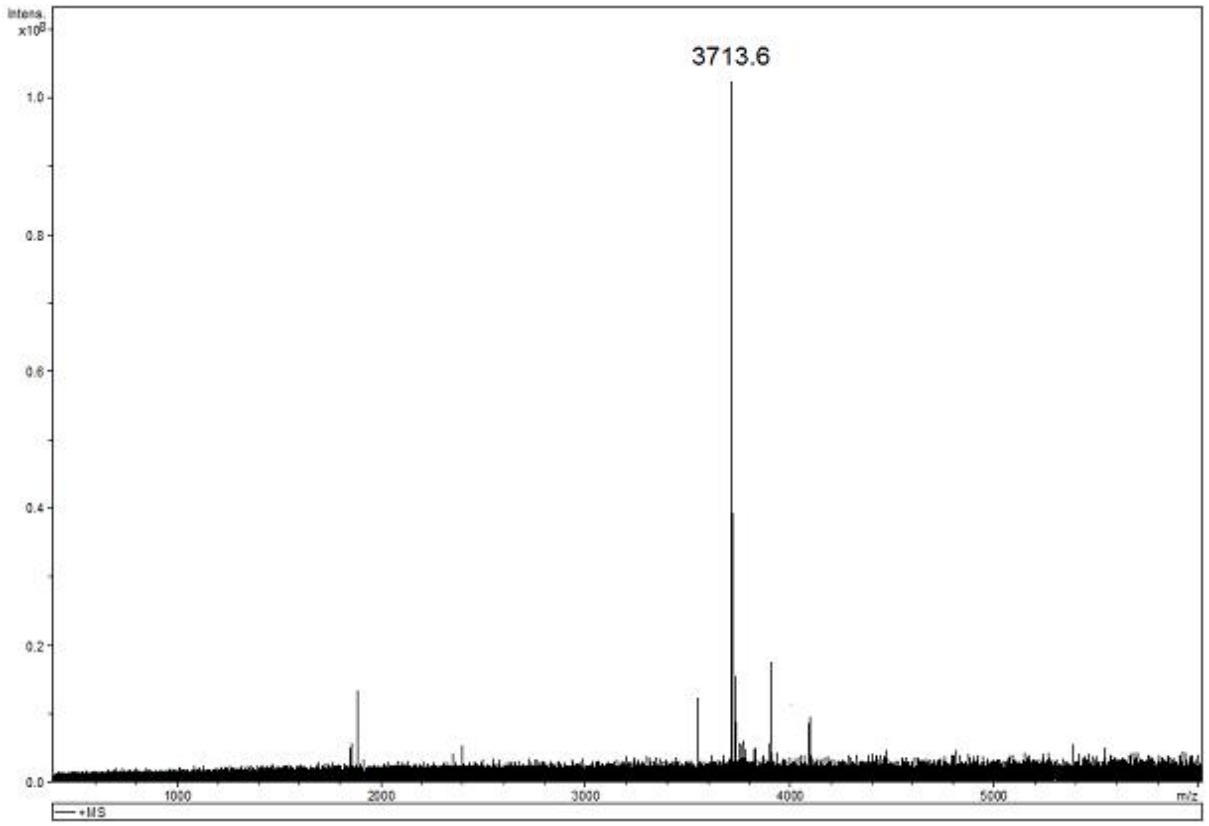
Glycopeptide 10c: H-Gly-Val-Thr(βGal-(1→3)-α-GalNAc)-Ser(βGal-(1→3)-α-GalNAc)-Ala-Pro-Asp-Thr(βGal-(1→3)-α-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser(βGal-(1→3)-α-GalNAc)-Thr(βGal-(1→3)-α-GalNAc)-Ala-Pro-Pro-Ala-His-OH



Glycopeptide **10c** was prepared according to the Fmoc-strategy SPPS method outlined in the general procedures and purified by preparative reverse phase HPLC (0 to 25% B over 60 min) affording **10c** as a white solid following lyophilisation (13 mg, 14% yield based on the original 25 μmol resin loading).




Intens.
x10^8
Payne_0_P8_0_F7_0_B21_000001.d: +MS
3713.58833
3714.59169
3712.58434
3715.59485
3716.59525
3717.59262
3711.56644
3718.59365
Payne_0_P8_0_F7_0_B21_000001.d: C150H243O78N30, M ,3712.60
3713.59991
3714.60317
3712.59649
3715.60649
3716.60985
3717.61082
3718.61417
m/z


Intens.
x10^8
3713.6
m/z
+MS

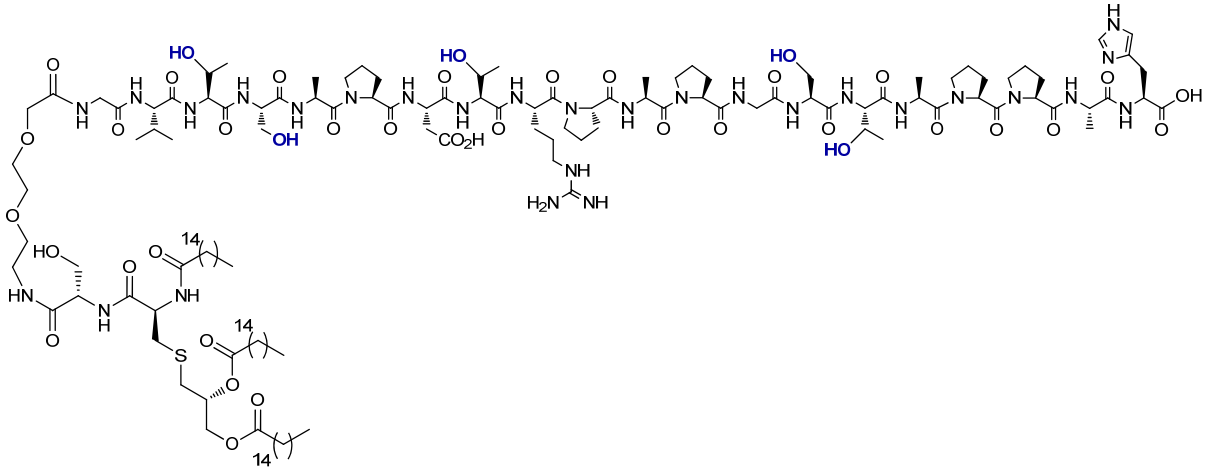
Analytical HPLC: R_t 21.7 min (0-25% B over 40 min, λ = 230 nm). MS (ESI^+) *m/z* 1857.04 [$(M+2H)^{2+}$, 30%)], 1238.66 [$(M+3H)^{3+}$, 100%]; MALDI-TOF HRMS: Calcd for $C_{150}H_{243}N_{30}O_{78}$: MH^+, 3713.5999 found MH^+ 3713.5883.

General procedure for the synthesis of MUC1-lipopeptide chimeras (18a-c)

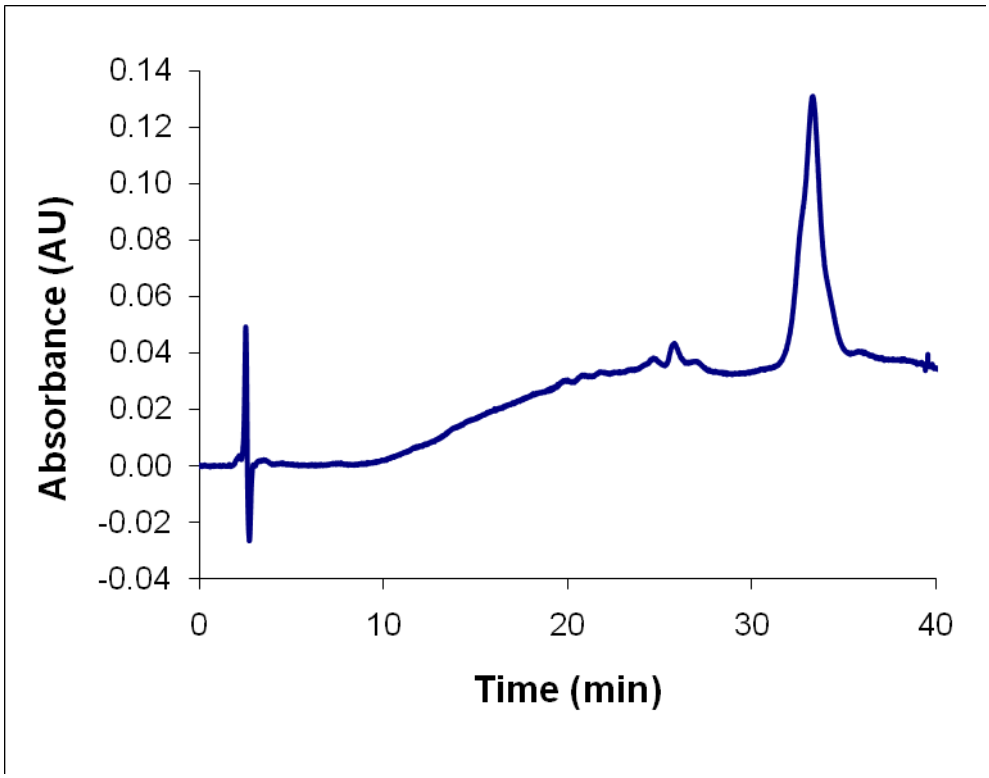
1.0 μmol scale: To a solution of **7** (1.2 mg, 1.0 μmol) in dry DCM (100 μL) was added pentafluorophenol (10 μL of a 20 mg mL^{-1} solution, 1.0 μmol, 1.0 equiv.) and *N,N'*-diisopropylcarbodiimide (10 μL of a 20 μL mL^{-1} solution, 1.0 μmol, 1.0 equiv.). The solution was placed under and atmosphere of argon and gently agitated for 1 h at rt. TLC analysis (5% methanol/DCM) showed consumption of starting material (R_f 0.2) and formation of product (R_f 0.5). The solvent was gently evaporated under a stream of argon. A solution of peptide **10a** or glycopeptides **10b** or **10c** (1.2 μmol, 1.2 equiv.), *N,N*-diisopropylethylamine (10 μL of a 24 μL mL^{-1} solution in DMF, 2.4 μmol, 2.4 equiv.) and 1-hydroxybenzotriazole (10 μL of a 12 mg mL^{-1} solution in DMF, 1.2 μmol, 1.2 equiv.) in dry DMF (100 μL) was then added and the solution was gently agitated under an argon atmosphere for 16 h at rt. The solvent was removed *in vacuo* and a mixture of TFA/TIS (9:1 v/v, 1 mL) was added. The solution was stirred for 1 h at rt before concentrating *in vacuo*. Purification by semi-preparative, reverse-phase HPLC (C-4) followed by lyophilisation afforded the desired MUC1-lipopeptide chimeras (**18a-c**).

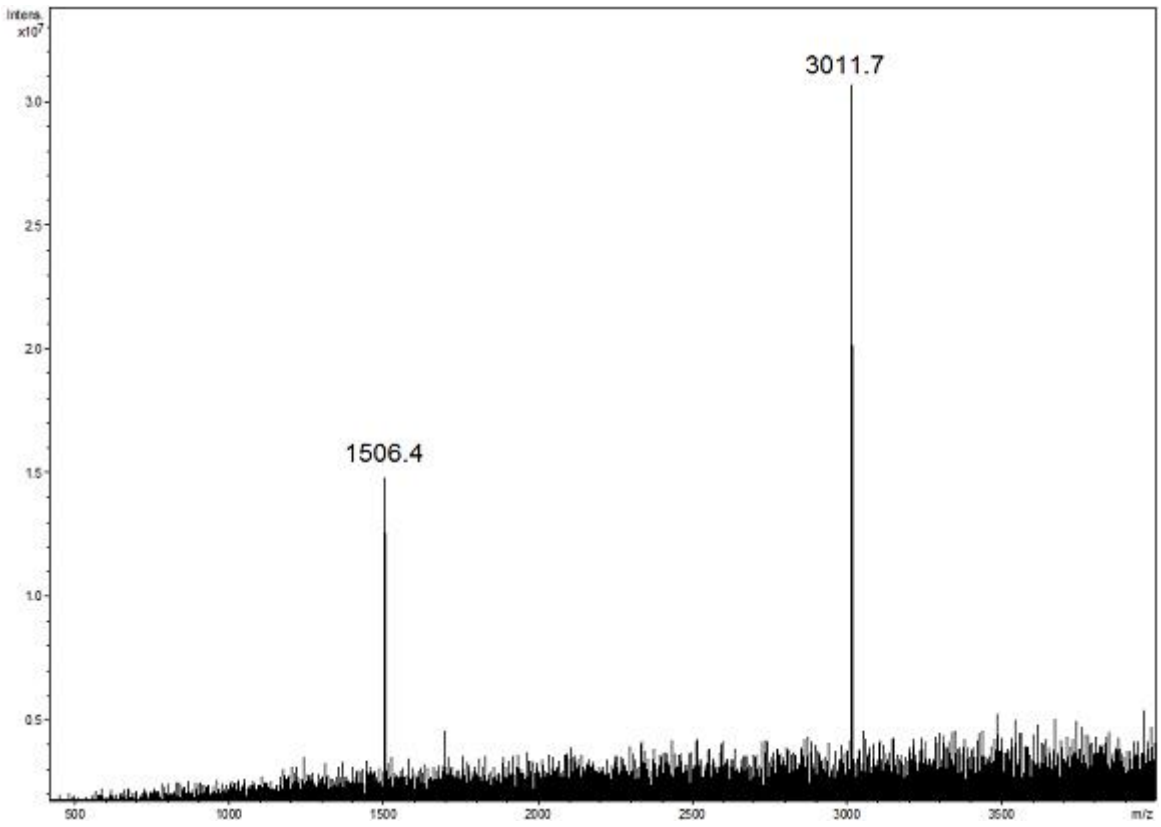
Analytical data for MUC1-lipopeptide chimeras (18a-c).

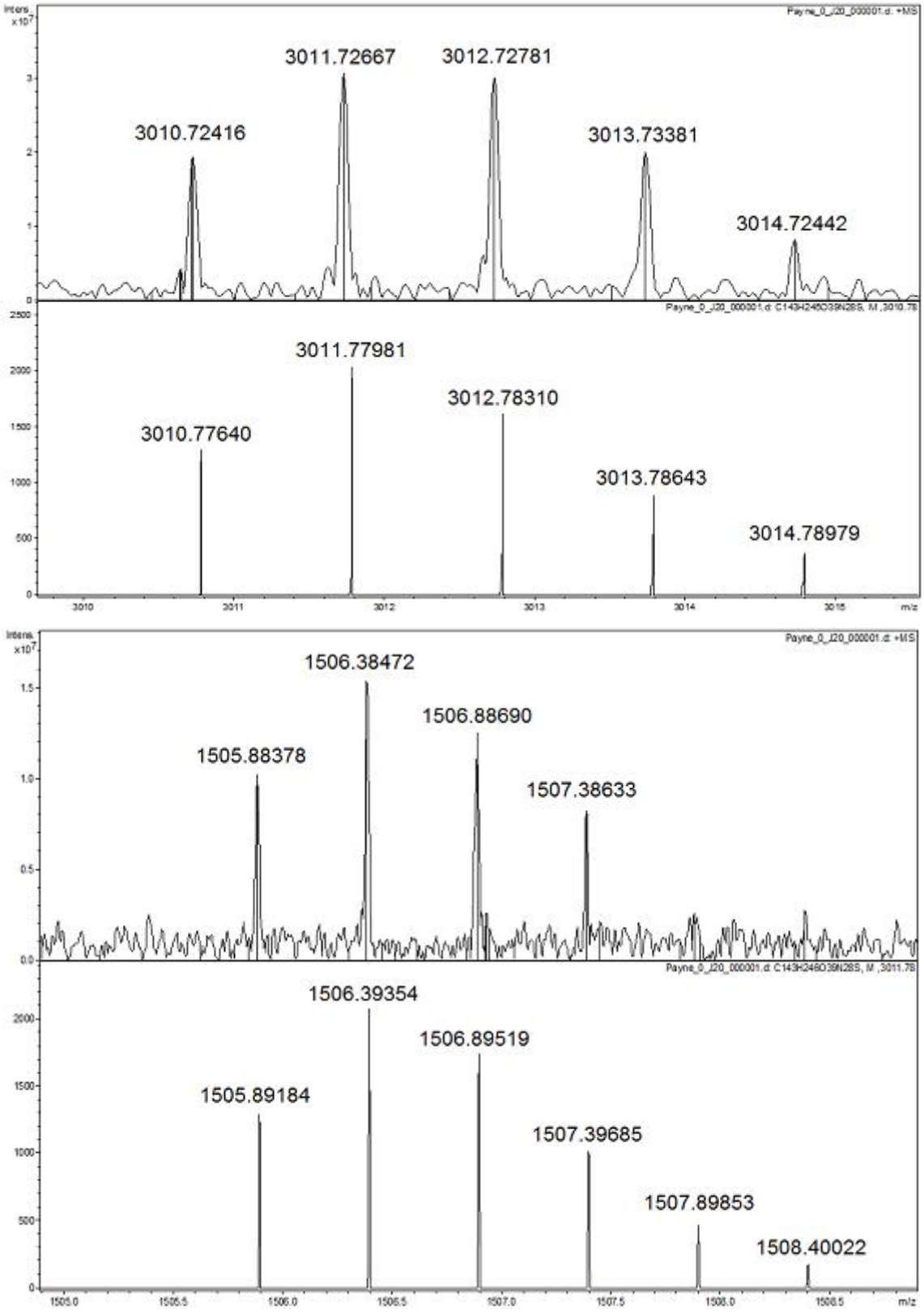
Peptide-lipopeptide chimera 18a: *S*-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-*N*-palmitoyl-(R)-cysteine-Ser- $NH(CH_2CH_2O)_2CH_2$-Gly-Val-Thr-Ser-Ala-Pro-Asp-Thr-Arg-Pro-Ala-Pro-Gly-Ser-Thr-Ala-Pro-Pro-Ala-His-OH



Chimera **18a** (1.7 μmol scale) was prepared according to the general procedure for the synthesis of MUC1-lipopeptide chimeras outlined above and purified by reverse phase semi-preparative HPLC (C-4) (0 to 100% B over 40 min) to afford **18a** as a white solid following lyophilisation (4.5 mg, 90%).

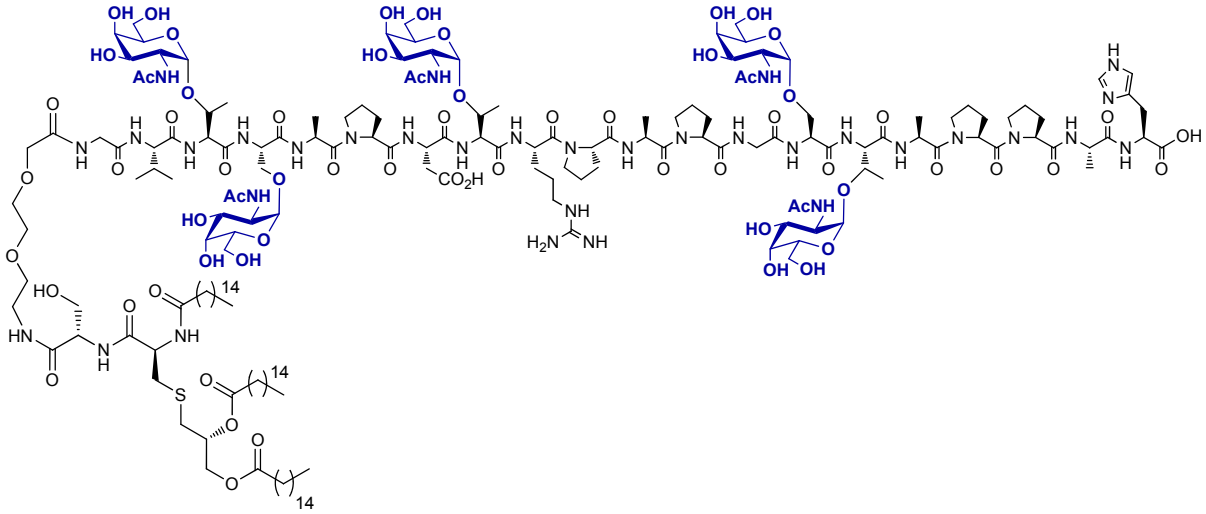

Absorbance (AU)
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
0
10
20
30
40
Time (min)


Intens.
x10^7
3.0
2.5
2.0
1.5
1.0
0.5
3011.7
1506.4
500
1000
1500
2000
2500
3000
3500
m/z

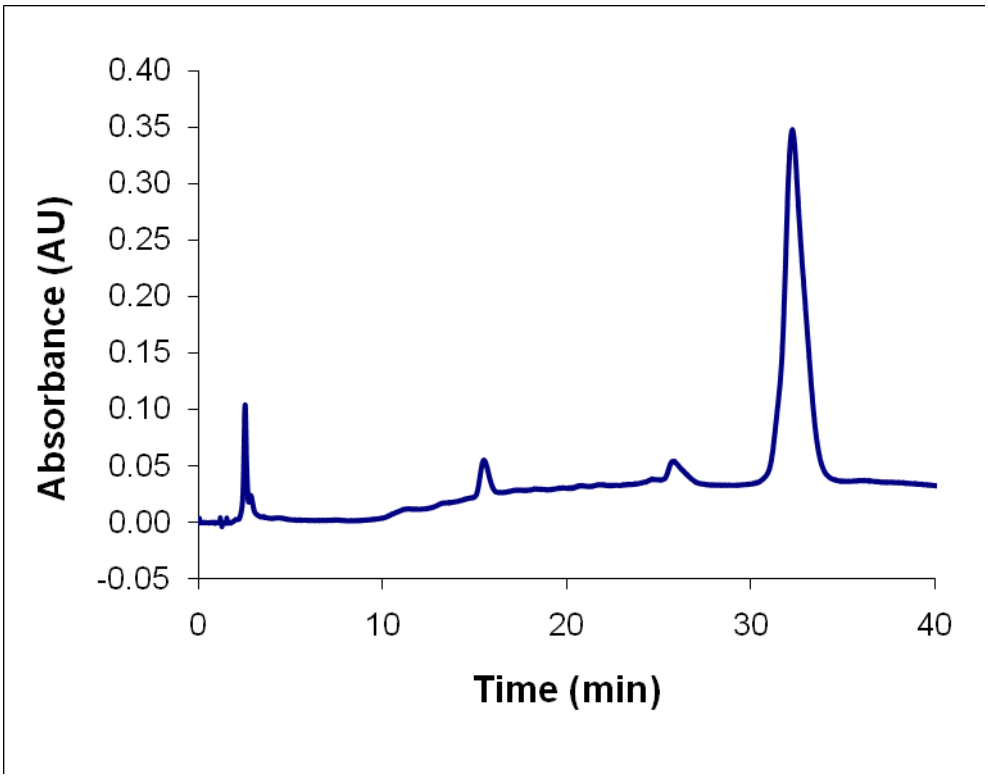


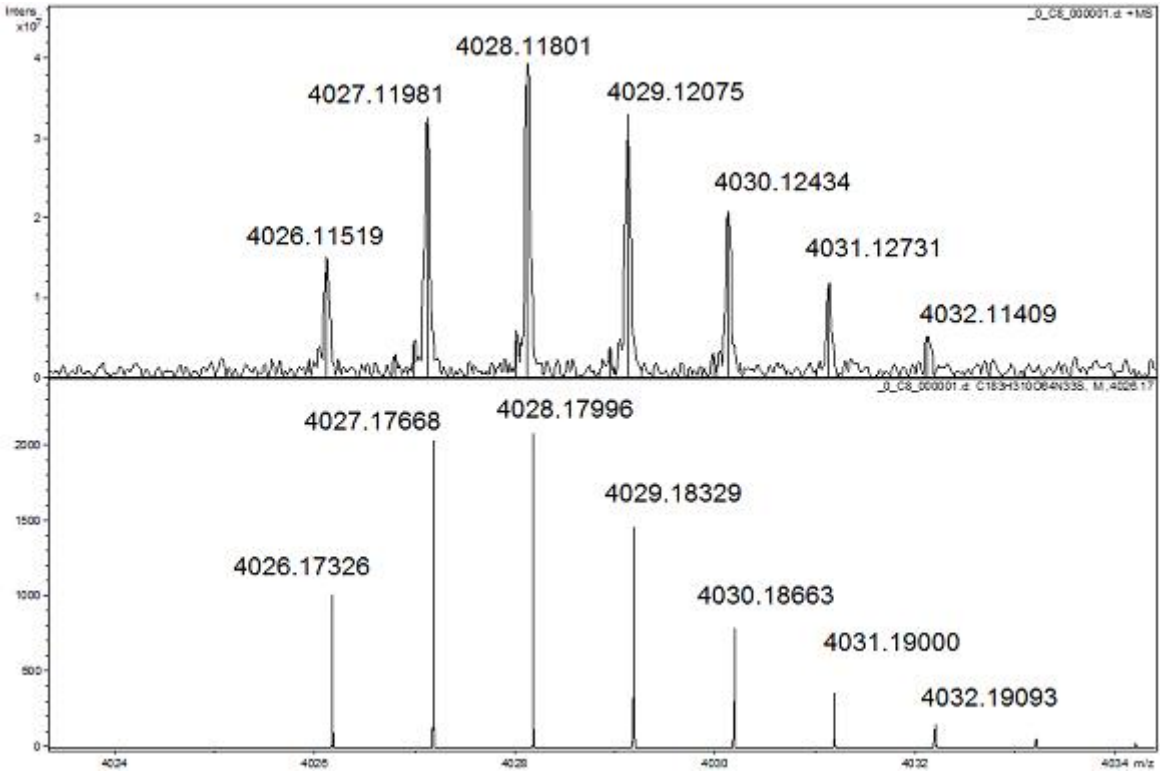
Analytical HPLC: R_t 33.3 min (0-100% B over 40 min, λ = 214 nm); MALDI-TOF HRMS Calcd for $C_{143}H_{245}N_{28}O_{39}S$: MH^+, 3011.7798 found MH^+, 3011.7267.

Glycopeptide-lipopeptide chimera 18b: *S*-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-*N*-palmitoyl-(R)-cysteine-Ser-NH($CH_2CH_2O)_2CH_2$-Gly-Val-Thr(α-GalNAc)-Ser(α-GalNAc)-Ala-Pro-Asp-Thr(α-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser(α-GalNAc)-Thr(α-GalNAc)-Ala-Pro-Pro-Ala-His-OH



Chimera **18b** (1.0 μmol scale) was prepared according to the general procedure for the synthesis of MUC1-lipopeptide chimeras outlined above and purified by reverse phase semi-preparative HPLC (C-4) (0 to 100% B over 40 min) to afford **18b** as a white solid following lyophilisation (3.2 mg, 79%).


Absorbance (AU)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
0
10
20
30
40
Time (min)


4028.11801
4027.11981
4029.12075
4030.12434
4026.11519
4031.12731
4032.11409
4027.17668
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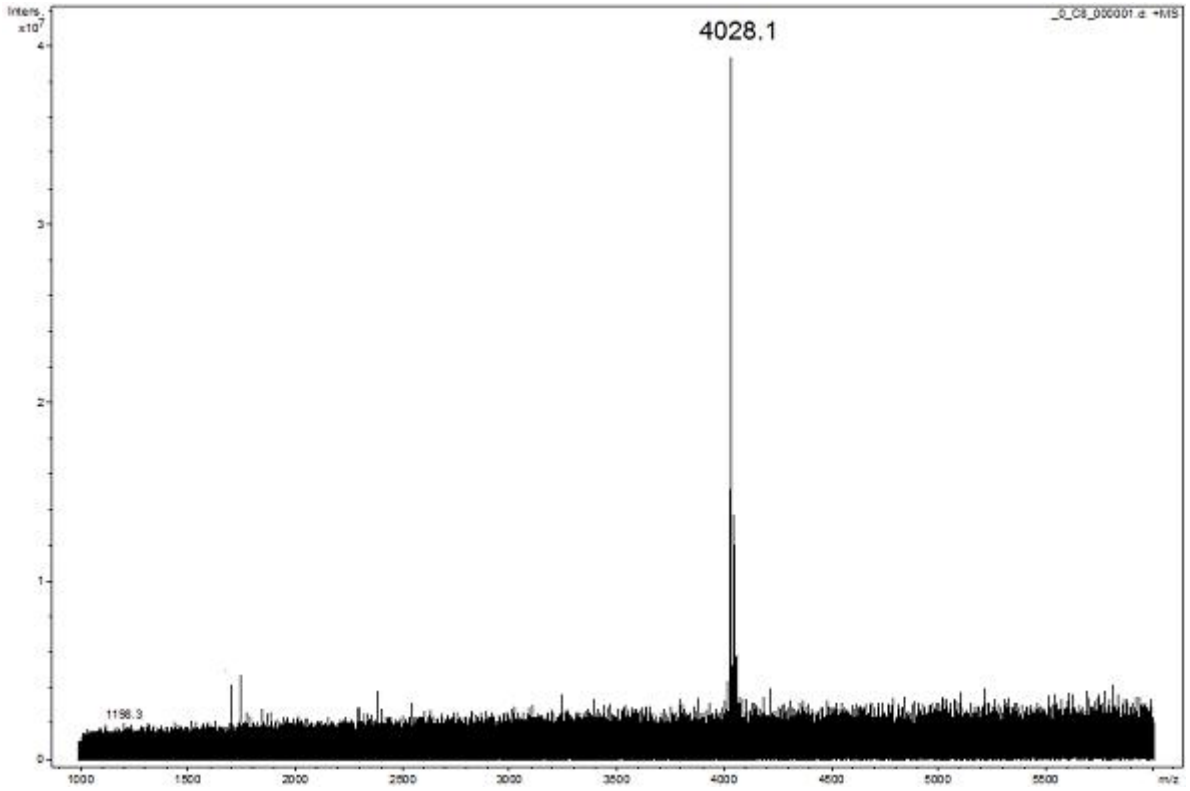
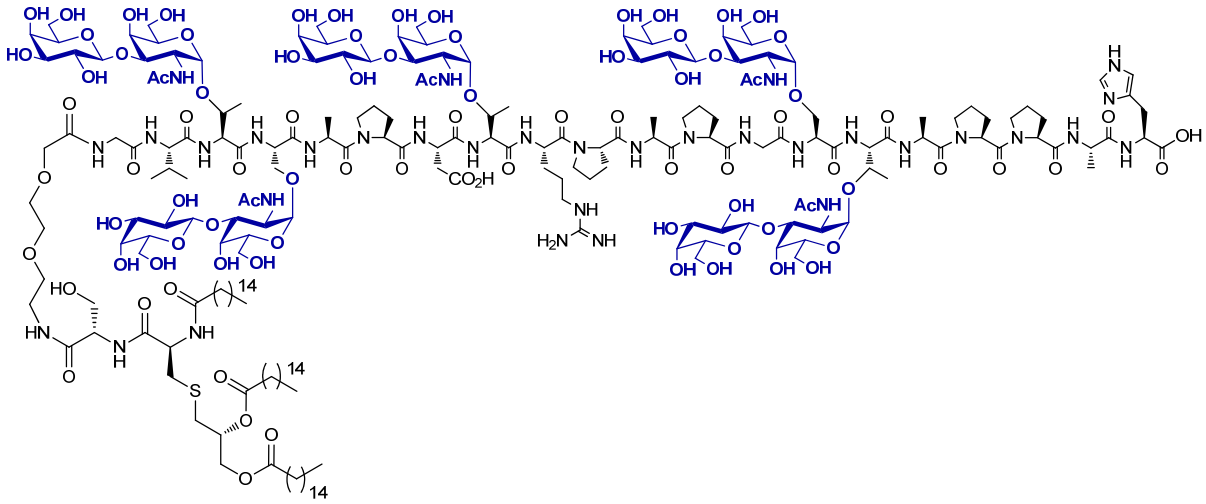
4029.18329
4026.17326
4030.18663
4031.19000
4032.19093

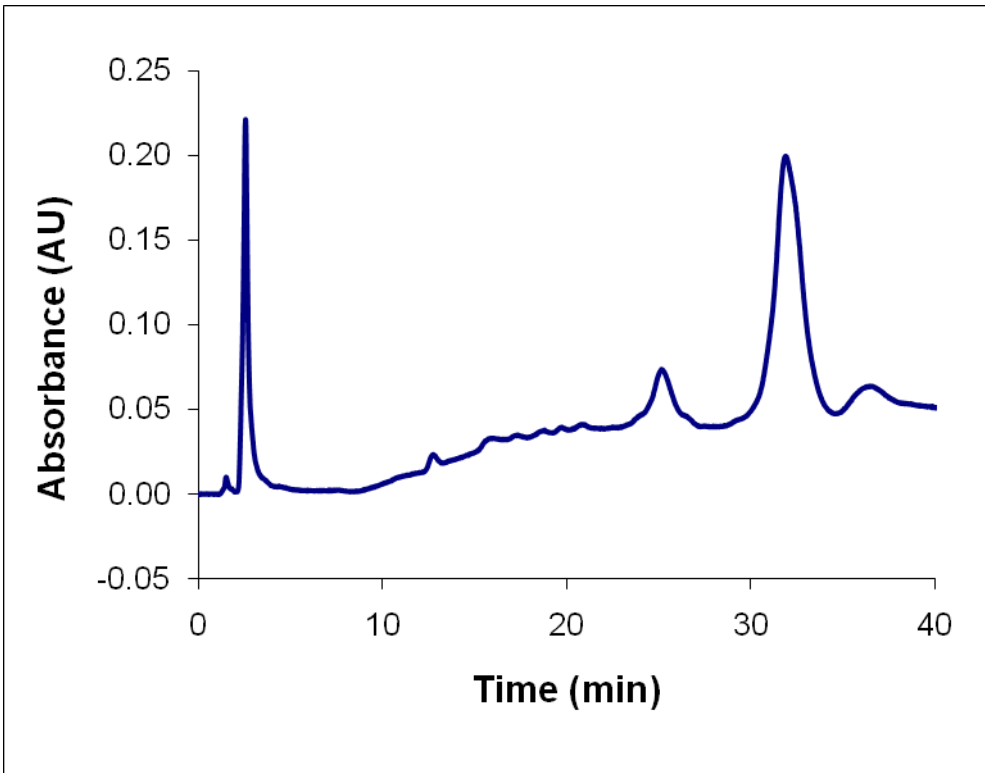


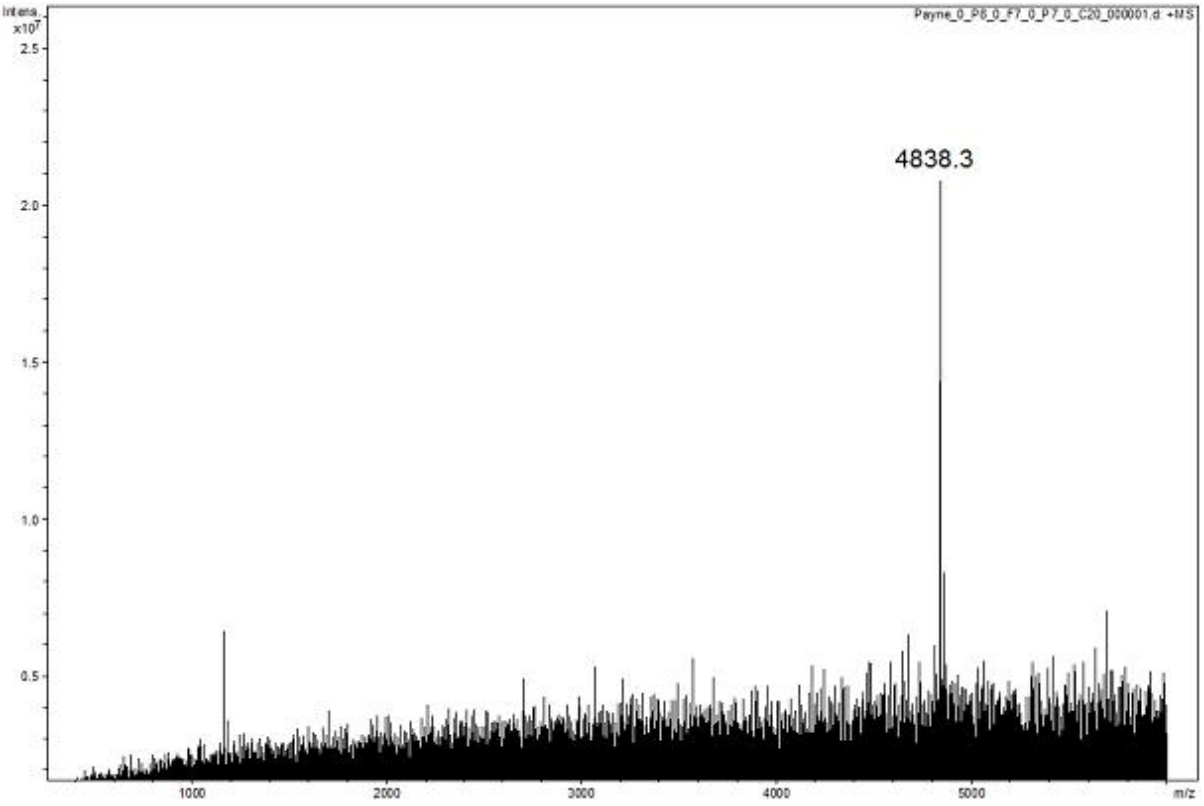
Analytical HPLC: R_t 32.2 min (0-100% B over 40 min, λ = 214 nm); MALDI-TOF HRMS Calcd for $C_{183}H_{310}N_{33}O_{64}S$: MH^+, 4028.1710 found MH^+ 4028.1180.

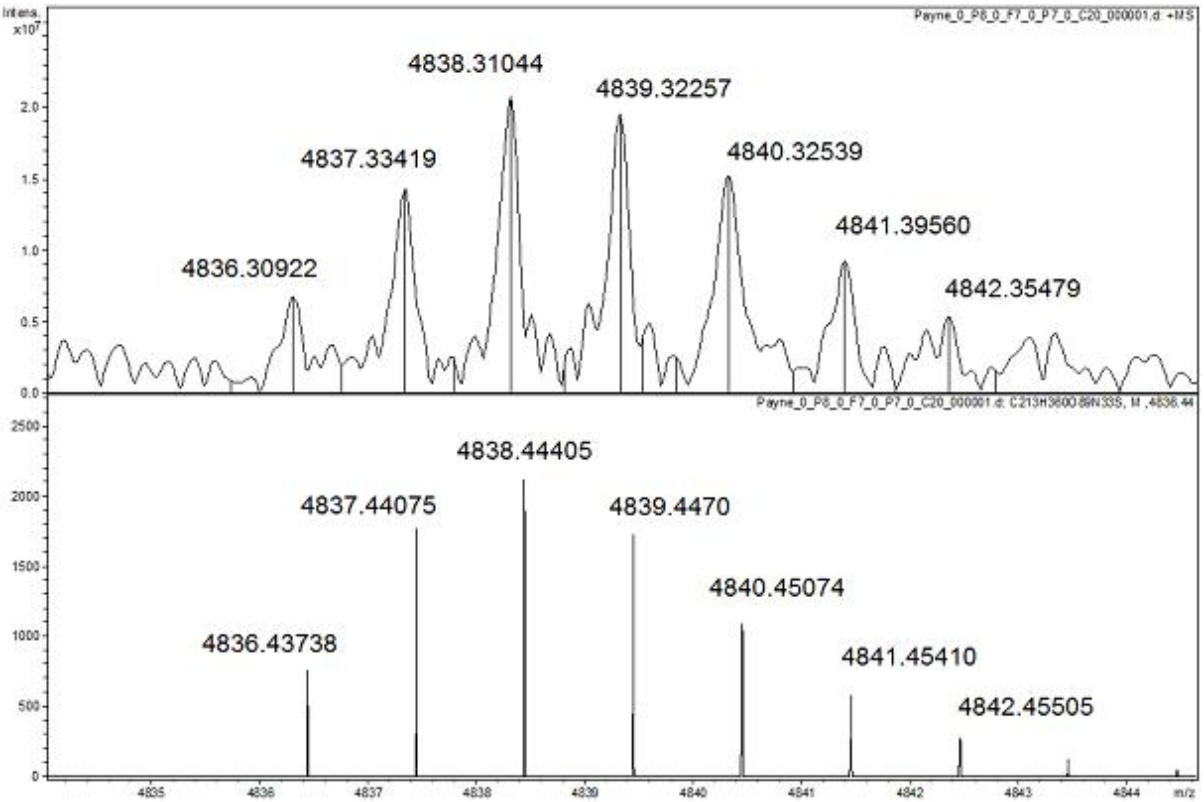
Glycopeptide-lipopeptide chimera 18c: *S*-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-*N*-palmitoyl-(R)-cysteine-Ser- NH($CH_2CH_2O)_2CH_2$-Gly-Val-Thr(βGal-(1→3)-α-GalNAc)-Ser(βGal-(1→3)-α-GalNAc)-Ala-Pro-Asp-Thr(βGal-(1→3)-α-GalNAc)-Arg-Pro-Ala-Pro-Gly-Ser(βGal-(1→3)-α-GalNAc)-Thr(βGal-(1→3)-α-GalNAc)-Ala-Pro-Pro-Ala-His-OH



Chimera **18c** (0.8 μmol scale) was prepared according to the general procedure for the synthesis of MUC1-lipopeptide chimeras outlined above and purified by reverse phase semi-preparative HPLC (C-4) (0 to 100% B over 40 min) to afford **18a** as a white solid following lyophilisation (2.7 mg, 72%).




Intens.
x10^7
2.5
2.0
1.5
1.0
0.5
4838.3
1000
2000
3000
4000
5000
m/z


Intens.
x10^7
2.0
1.5
1.0
0.5
0.0
4838.31044
4839.32257
4837.33419
4840.32539
4841.39560
4836.30922
4842.35479
2500
2000
1500
1000
500
0
4838.44405
4837.44075
4839.4470
4840.45074
4836.43738
4841.45410
4842.45505
4835
4836
4837
4838
4839
4840
4841
4842
4843
4844
m/z

Analytical HPLC: R_t 31.9 min (0-100% B over 40 min, λ = 214 nm); MALDI-TOF HRMS: Calcd for $C_{213}H_{360}N_{33}O_{89}S$: MH^+, 4838.4441 found MH^+, 4838.3104.

References

1. E. Lukovic, J. –A. Gonzalez-Vera, B. Imperiali, B. *J. Am. Chem. Soc.* 2008, **38**, 12821

2. B. N. Thomas, C. M. Lindeman, R. C. Corcoran, C. L. Cotant, J. E. Kirsch, P. J. Persichini, *J. Am. Chem. Soc.* 2002, **124***,* 1227. (b) S. E. Schaus, B. D. Brandes, J. F. Larrow, M. Tokunaga, K. B. Hansen, A. E. Gould, M. E. Furrow, E. N. Jacobsen, *J. Am. Chem. Soc.* 2002, **124**, 1307.

3. F. Reichel, A. M. Roelofsen, H. P. M. Guerts, T. I. Hämäläinen, M. C. Feiters, G. –J. Boons, *J. Am. Chem. Soc.* 1999, **121***,* 7987.