Electronic supplementary information

A multi-ligation strategy for the synthesis of heterofunctionalized glycosylated scaffolds

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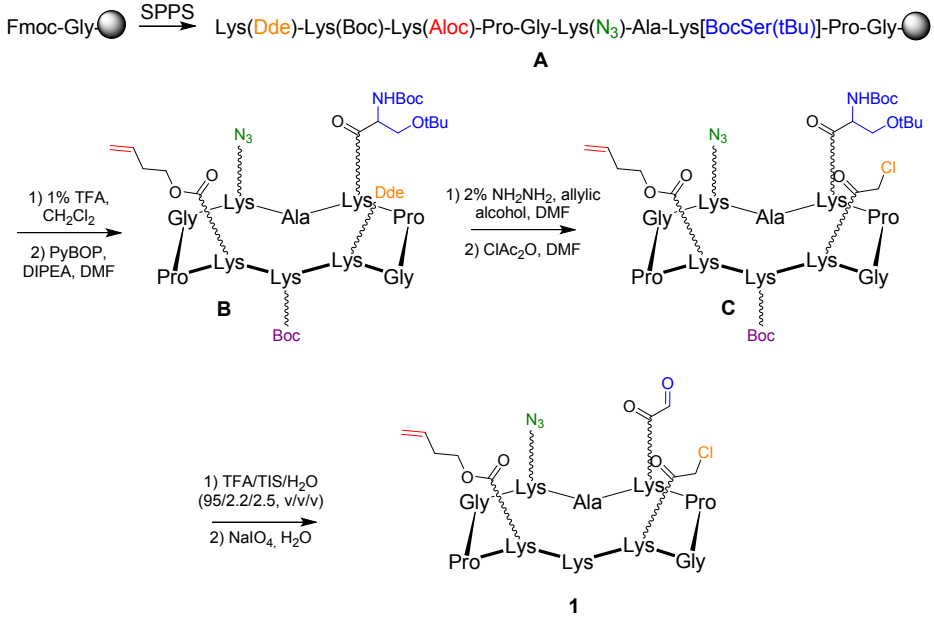
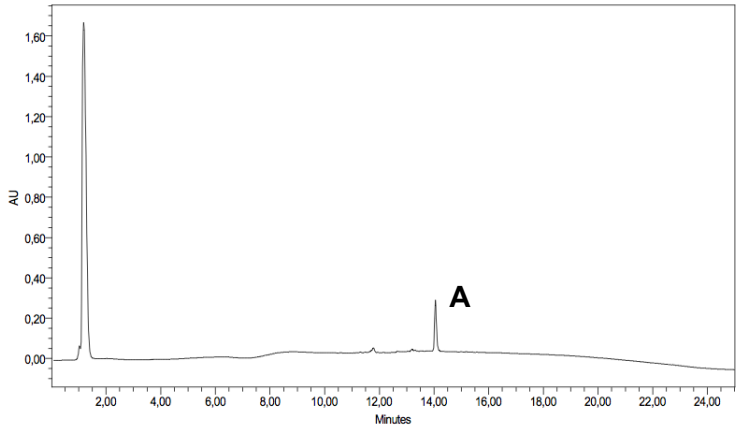
General details

All chemical reagents were purchased from Aldrich (Saint Quentin Fallavier, France) or Acros (Noisy-Le-Grand, France) and were used without further purification. Protected amino acids and Fmoc-Gly-Sasrin resin were obtained from Advanced ChemTech Europe (Brussels, Belgium), Bachem Biochimie SARL (Voisins-Les-Bretonneux, France) and France Biochem S.A. (Meudon, France). All amino-acids belong to the L-series. PyBOP was purchased from France Biochem. Analytical RP-HPLC was performed on Waters system equipped with a Waters 600 controller and a Waters 2487 Dual Absorbance Detector. Analysis was carried out at 1.0 mL.min^{-1} (EC 125/3 nucleosil 300-5 C_{18}) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (solvent A: 0.09% CF_3CO_2H in H_2O; solvent B: 0.09% CF_3CO_2H in 90% acetonitrile). Purifications were carried out at 22.0 mL.min^{-1} (VP 250/21 nucleosil 100-7 C_{18}) with UV monitoring at 214 nm and 250 nm using a linear A–B gradient (buffer A: 0.09% CF_3CO_2H in H_2O; buffer B: 0.09% CF_3CO_2H in 90% acetonitrile). ESI mass spectra of peptides and glycopeptides were measured on an Esquire 3000 spectrometer from Bruker or on an Acquity UPLC/MS system from Waters equipped with a SQ Detector 2. For **21** and **22**, HPLC analyses were performed on a Waters RP-HPLC system with dual wavelength detector on a Nucleosil C_{18} column (Macherey Nagel, 250 x 4.6 mm, 5 µm) at 1 mL.min^{-1} using a linear A–B gradient (solvent A: 50 mM triethylammonium acetate (TEAAc) buffer containing 5% acetonitrile; solvent B, acetonitrile containing 5% water. They were purified on a µ-Bondapak C_{18} column (Macherey-Nagel Nucleosil, 10 x 250 mm, 7 µm) with similar gradients at a flow rate of 4 mL.min^{-1} on a Gilson HPLC system with dual wavelength detector and a fraction collector (prep Fc). MALDI-ToF mass spectrum for compound **21** was performed on an Autoflex Bruker using hydropiccolinic acid (HPA, 45 mg; ammonium citrate 4 mg in 500 µL H_2O/CH_3CN) as matrix. Quantification was performed at 260 nm using CARY 400 Scan UV-Visible Spectrometer; ε (181100 M^{-1}.cm^{-1}) was estimated according to the nearest neighbor model.

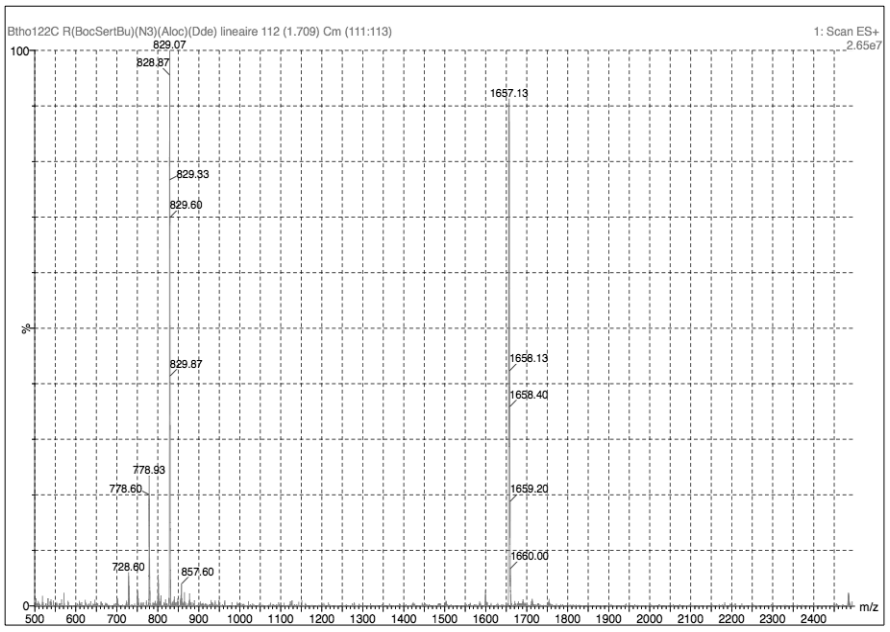


Synthetic scheme for compound **1**.

Compound A. The linear protected peptide was synthesized manually by solid-phase peptide synthesis (SPPS) using the standard 9-fluorenylmethoxycarbonyl/tertiobutyl (Fmoc/*t*Bu) protection strategy and the Fmoc-Gly-SASRIN resin. The peptide was cleaved with a solution of 1% TFA in CH_2Cl_2, precipitated in diethyl ether and was used without further purification. Analytical RP-HPLC: R_t = 14.1 min (C_{18}, 214 nm, 5-100% B in 25 min).

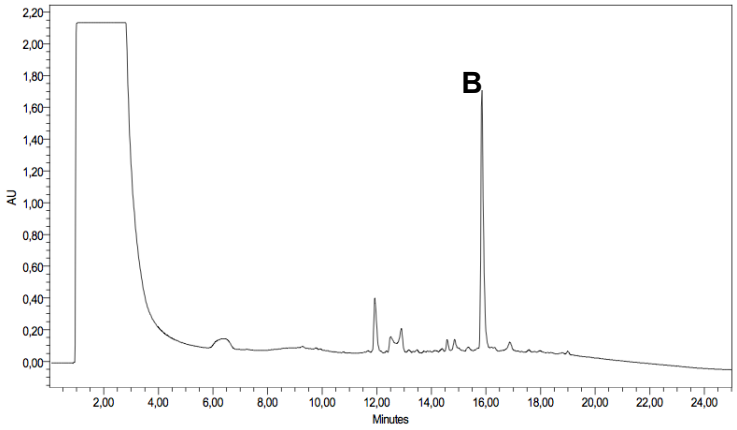


RP-HPLC profile of crude compound **A**.

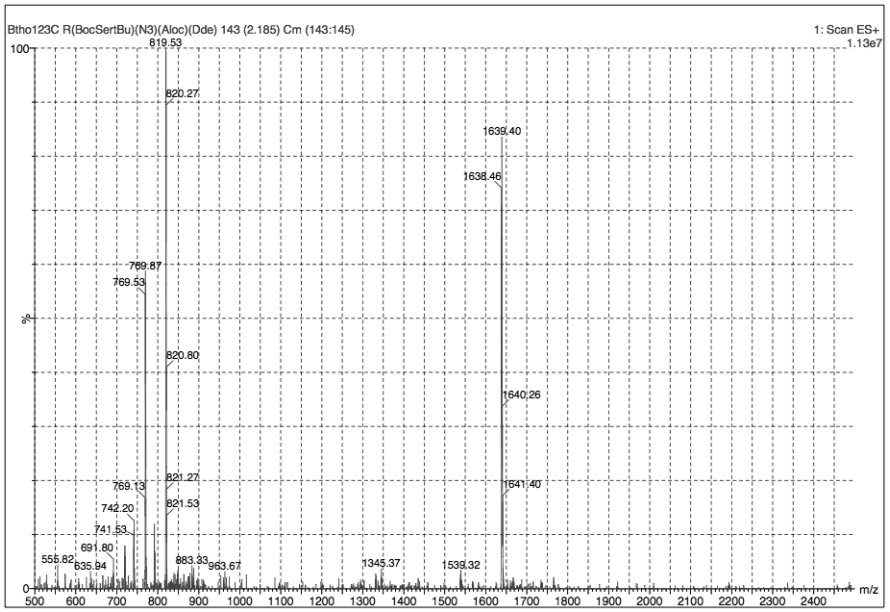


ESI$^+$-MS: *m/z* calcd for $C_{78}H_{131}N_{18}O_{21}$: 1656.9; found: 1657.1 $[M+H]^+$.

Compound B. Linear peptide **A** (370 mg, 0.22 mmol) was dissolved in DMF (0.5 mM). PyBOP (139.5 mg, 0.27 mmol) was added and the pH value was adjusted to 8 by addition of DIPEA. The solution was stirred at room temperature for 1 h and the solvent was removed under reduced pressure. The residue was dissolved in a minimum of CH_2Cl_2 and diethyl ether was added for precipitation. Peptide **B** was obtained as a white powder and was used in the next step without further purification. Analytical RP-HPLC: R_t = 15.8 min (C_{18}, 214 nm, 5-100% B in 25 min).

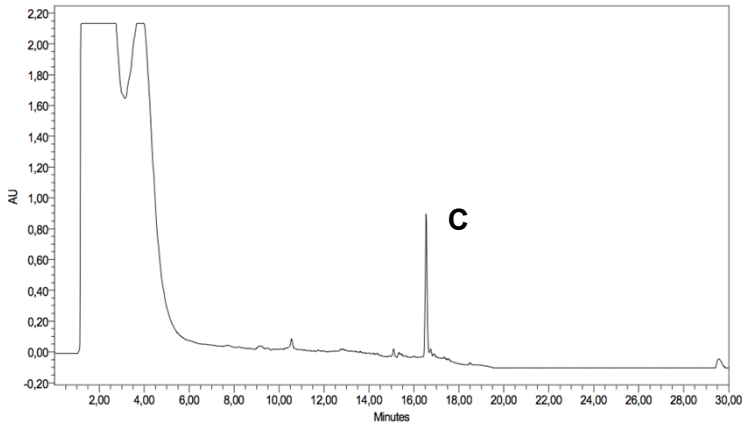


RP-HPLC profile of crude compound **B**.

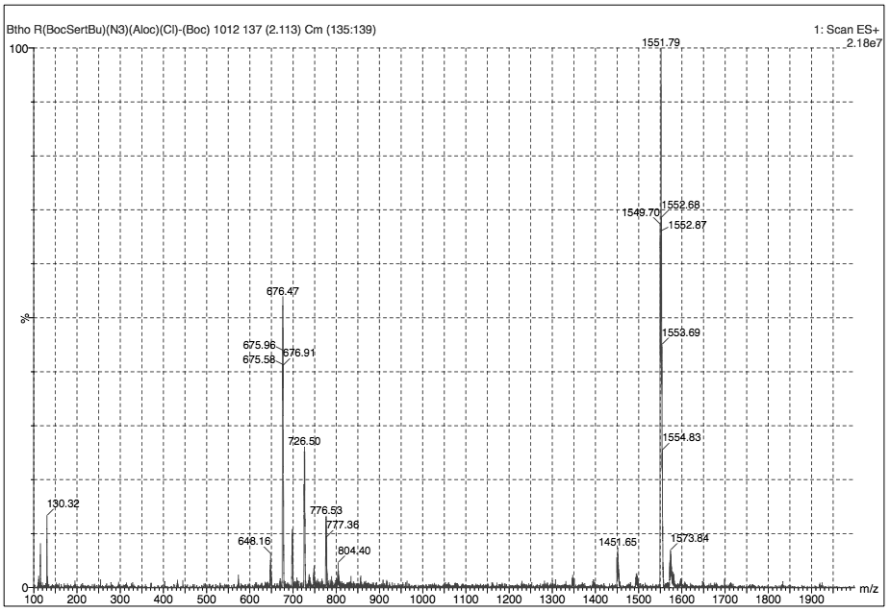


ESI$^+$-MS: *m/z* calcd for $C_{78}H_{129}N_{18}O_{20}$: 1638.9; found: 1639.4 $[M+H]^+$.

Compound C. Compound **B** (126 mg, 0.077 mmol) was dissolved in dry DMF (6.1 mL), allyl alchool (14 µL, 0.38 mmol) and hydrazine monohydrate (126 µL, 0.077 mmol) were added. The solution was stirred at room temperature. Anlytical HPLC confirmed the complete conversion of **B** into **C** within 30 minutes. After evaporation, the product was precipitated in diethyl ether to give **C** as a white powder. Analytical RP-HPLC: R_t = 16.5 min (C_{18}, 214 nm, 5-100% B in 25 min).

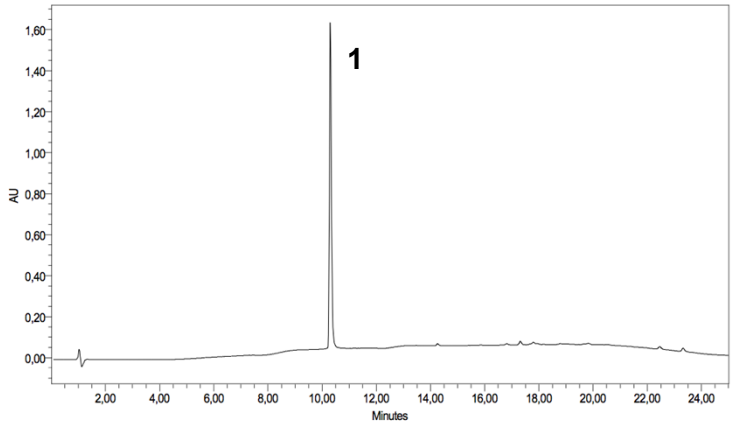


RP-HPLC profile of crude compound **C**.

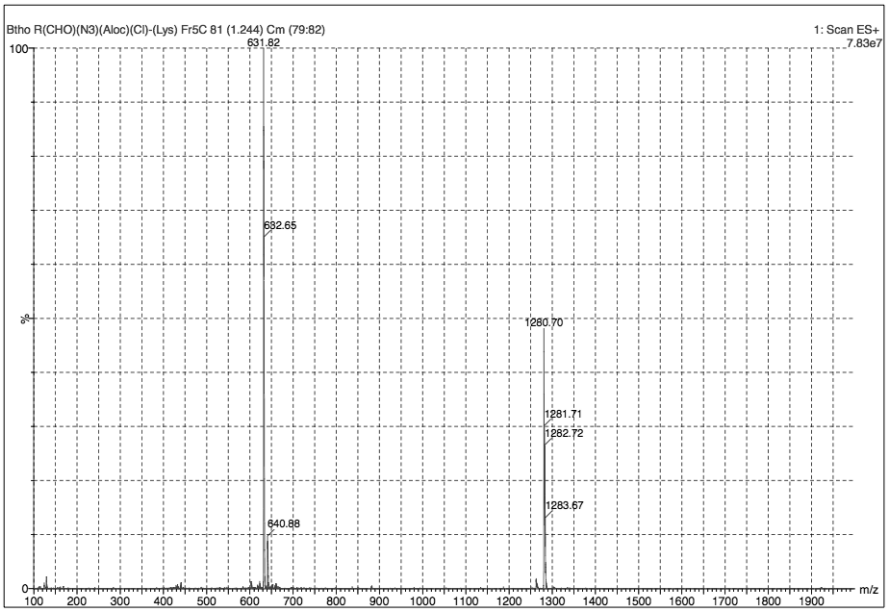


ESI$^+$-MS: *m/z* calcd for $C_{70}H_{118}N_{18}O_{19}Cl$: 1549.8; found: 1549.7 $[M+H]^+$.

Compound 1. The crude compound **C** (20.6 mg, 13.3 µmol) was treated with a cocktail of TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 5 mL) and stirred for 1 h at room temperature. After evaporation, the cyclodecapeptide was dissolved in water (10^{-2}M) and $NaIO_4$ (28.4 mg, 0.13 mmol) was added. The solution was stirred at room temperature for 30 min and the product was directly purified by RP-HPLC, affording pure aldehyde-containing cyclodecapeptide **1** as a white powder after freeze-drying. Analytical RP-HPLC: R_t = 10.3 min (C_{18}, 214 nm, 5-100% B in 25 min).



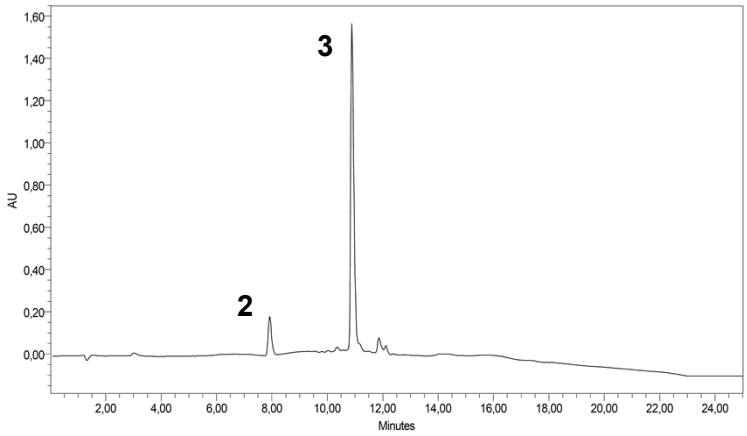
RP-HPLC profile of purified compound **1**.



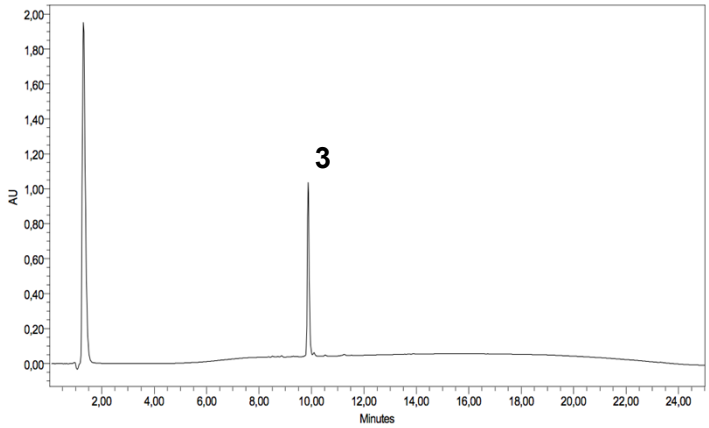
ESI$^+$-MS: *m/z* calcd for $C_{55}H_{91}N_{17}O_{16}Cl$ (hydrated aldehyde): 1280.6; found: 1280.7 $[M+H]^+$.

Stepwise synthesis of 9.

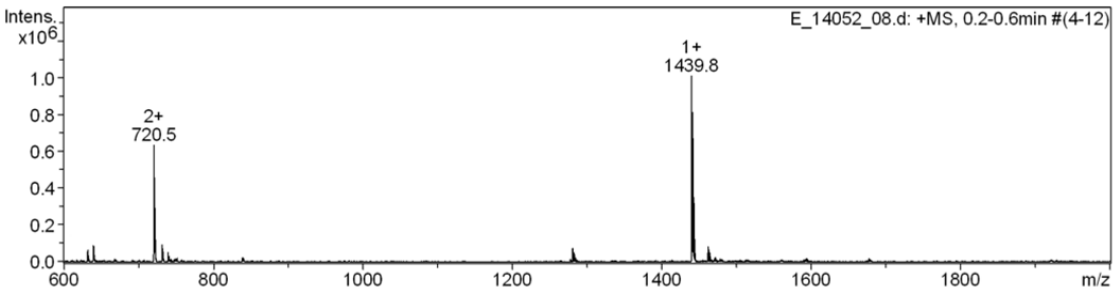
Compound 3. Cyclopeptide **1** (9.6 mg, 7.6 µmol) and β-Glc aminooxy **2** (1.8 mg, 9.1 µmol) were dissolved in 0.1% TFA in H_2O (10 mM). After stirring for 30 min at room temperature, analytical HPLC indicated complete conversion. The crude mixture was directly purified by preparative HPLC without additional treatment to give, after freeze-drying compound **3** as a flocculent powder. Yield: 87% (9.5 mg); analytical RP-HPLC: R_t = 9.9 min (C_{18}, 214 nm, 5-100% B in 25 min).



RP-HPLC profile of crude compound **3**.

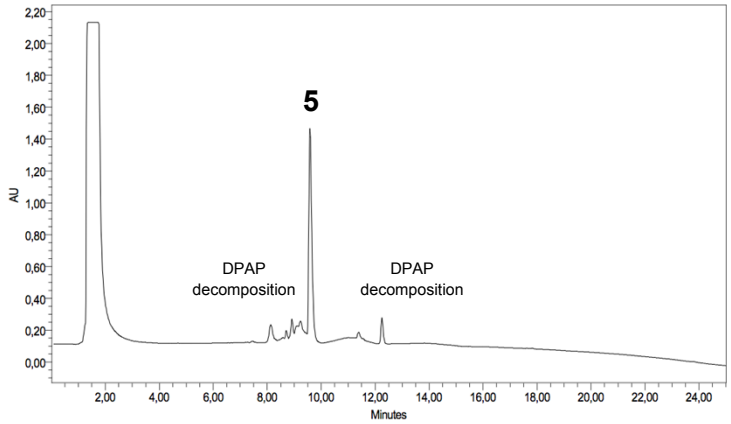


RP-HPLC profile of purified compound **3**.

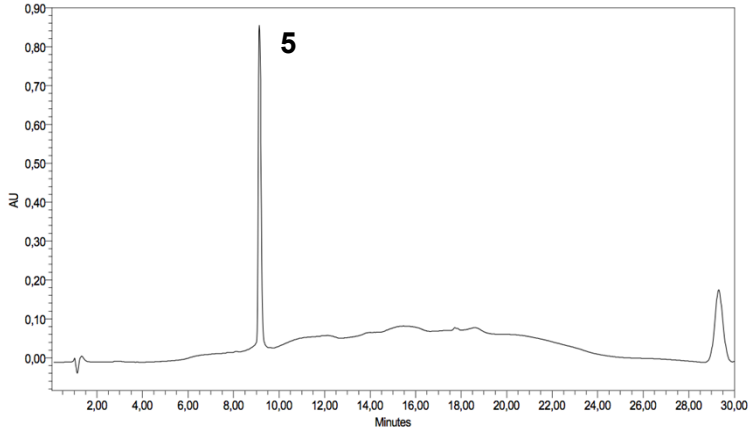


ESI$^+$-MS: *m/z* calcd for $C_{61}H_{100}N_{18}O_{20}Cl$: 1439.7; found: 1439.8 $[M+H]^+$.

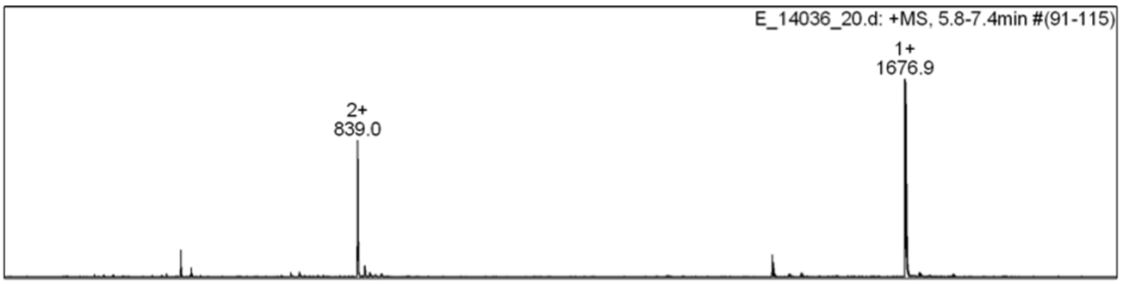
Compound 5. Glycopeptide **3** (9.5 mg, 6.6 µmol) and β-GlcNAc thiol **4** (4.7 mg, 19.8 µmol) were dissolved in a mixture DMF/H_2O (2:1, 1.8 mL) and DPAP (0.5 mg, 2 mmol) was added. The solution was irradiated at 365 nm for 30 min and directly purified to obtain compound **5** as a white foam. Yield: 67% (7.4 mg); analytical RP-HPLC: R_t = 9.1 min (C_{18}, 214 nm, 5-100% B in 25 min).



RP-HPLC profile of crude compound **5**.

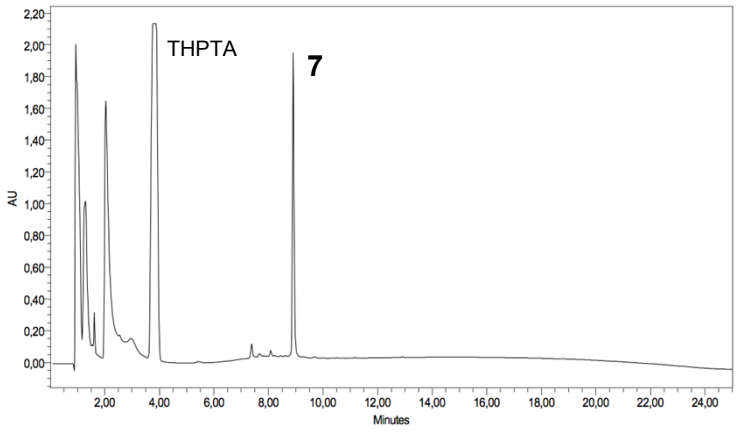


RP-HPLC profile of purified compound **5**.

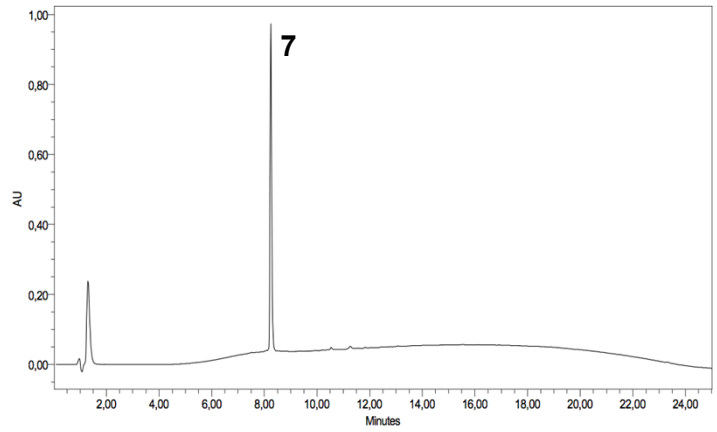


ESI$^+$-MS: *m/z* calcd for $C_{69}H_{115}N_{19}O_{25}SCl$: 1676.8; found: 1676.9 $[M+H]^+$.

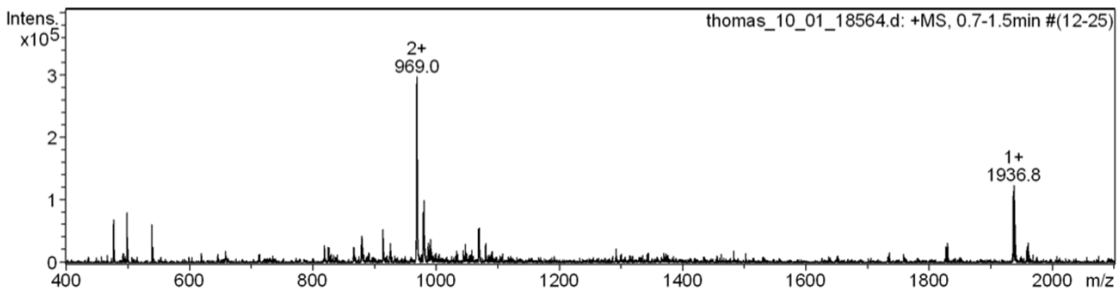
Compound 7. To a degassed solution of **5** (7.4 mg, 4.4 µmol) and α-GalNAc propargyl **6** (2.4 mg, 11.1 µmol) in DMF (1.0 mL), $CuSO_4$ (1.1 mg, 4.4 µmol) in PBS buffer (100 mM, 500 µL) then THPTA (9.5 mg, 22 µmol) and sodium ascorbate (6.1 mg, 30.8 µmol) in PBS buffer (100 mM, 500 µL) were added. The reaction was stirred at room temperature under argon and analytical HPLC indicated complete reaction after 1 h. Chelex resin was then added to remove excess of copper, the reaction mixture was filtered and directly purified by RP-HPLC affording pure compound **7** as a white powder. Yield: 85% (7.2 mg); analytical RP-HPLC: R_t = 8.3 min (C_{18}, 214 nm, 5-100% B in 25 min).



RP-HPLC profile of crude compound **7**.

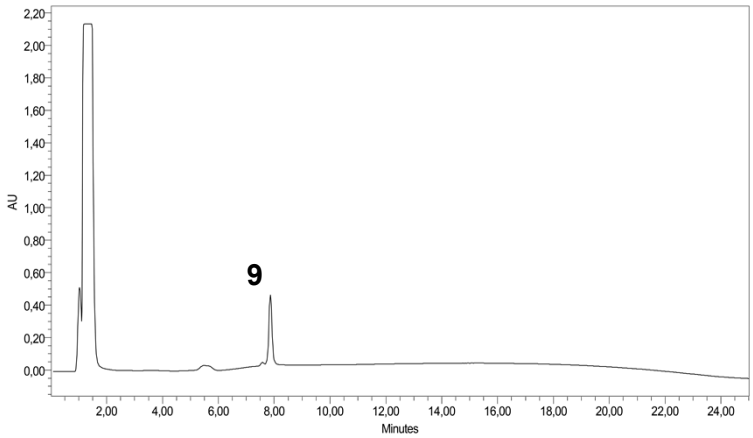


RP-HPLC profile of purified compound **7**.

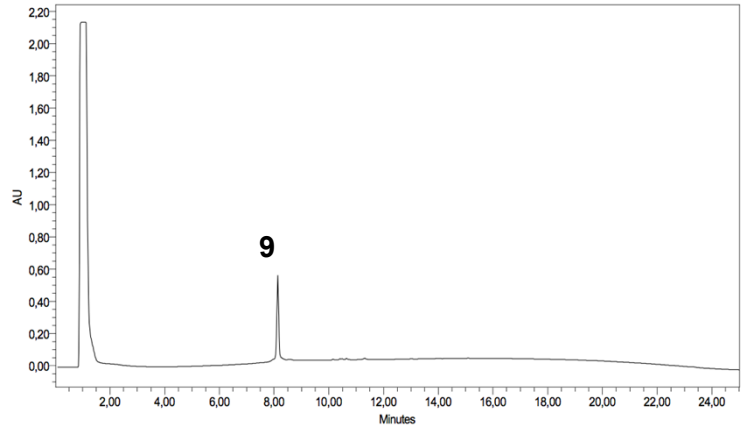


ESI$^+$-MS: *m/z* calcd for $C_{80}H_{132}N_{20}O_{31}SCl$: 1935.9; found: 1936.8 $[M+H]^+$.

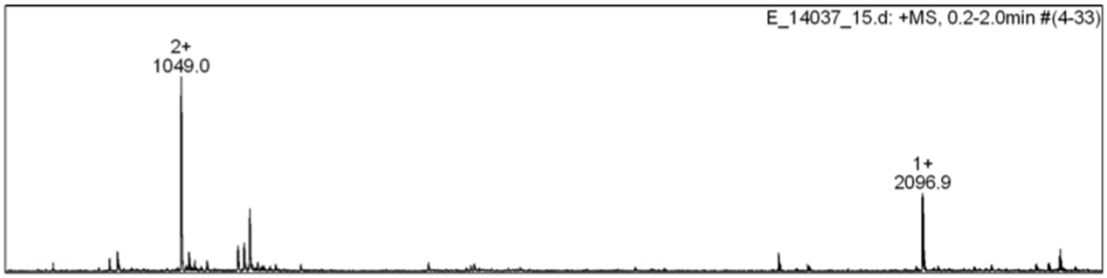
Compound 9. Compound **7** (7.2 mg, 3.7 μmol) and α-Man thiol **8** (3.6 mg, 18.5 mmol) were dissolved in a mixture DMF/H_2O (1:1, 1.6 mL), then KI (12.2 mg, 74.1 μmol) and DIPEA (35.4 μL, 0.21 mmol) were added. The suspension was left stirring for 1 h. The reaction mixture was directly purified by RP-HPLC affording **9** as a white powder. Yield: 84% (6.5 mg); analytical RP-HPLC: R_t = 8.1 min (C_{18}, 214 nm, 5-100% B in 25 min).



RP-HPLC profile of crude compound **9**.



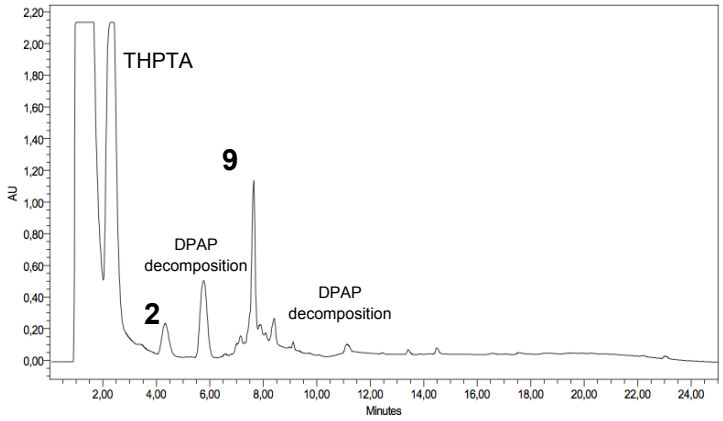
RP-HPLC profile of purified compound **9**.



ESI$^+$-MS: *m/z* calcd for $C_{86}H_{143}N_{20}O_{36}S_2$: 2096.9; found: 2096.9 $[M+H]^+$.

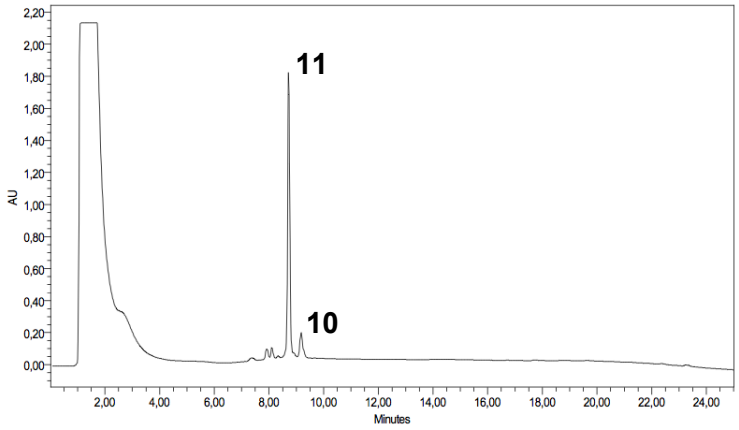
One-pot synthesis of 9.

Cyclopeptide **1** (2 mg, 1.6 µmol) and β-Glc aminooxy **2** (0.36 mg, 1.9 µmol) were dissolved in 0.1% TFA in H_2O (200 µL). After stirring for 30 min at room temperature, analytical HPLC indicated complete conversion. To this crude mixture, β-GlcNAc thiol **4** (0.55 mg, 2.3 µmol) and DPAP (0.06 mg, 0.23mmol) dissolved in DMF (400 µL) were added. The solution was irradiated at 365 nm for 30 min. To this crude mixture, α-GalNAc propargyl **6** (0.68 mg, 3.1 µmol) and $CuSO_4$ (0.39 mg, 1.6 µmol) in PBS (100 mM, 100 µL) were added followed by a solution of THPTA (3.4 mg, 7.8 µmol) and sodium ascorbate (2.2 mg, 10.9 µmol) in PBS (100 mM, 100 µL). The reaction was stirred at room temperature under argon and analytical HPLC indicated complete reaction after 1 h. To the crude mixture, α-Man thiol **8** (1.5 mg, 7.8 mmol), KI (5.1 mg, 31.2 µmol) and DIPEA (14.9 µL, 85.9 µmol) were added. The solution was stirred for 1 h and was finally purified by semi-preparative HPLC to provide **9** as a white powder. Overall yield: 47% (1.5 mg).

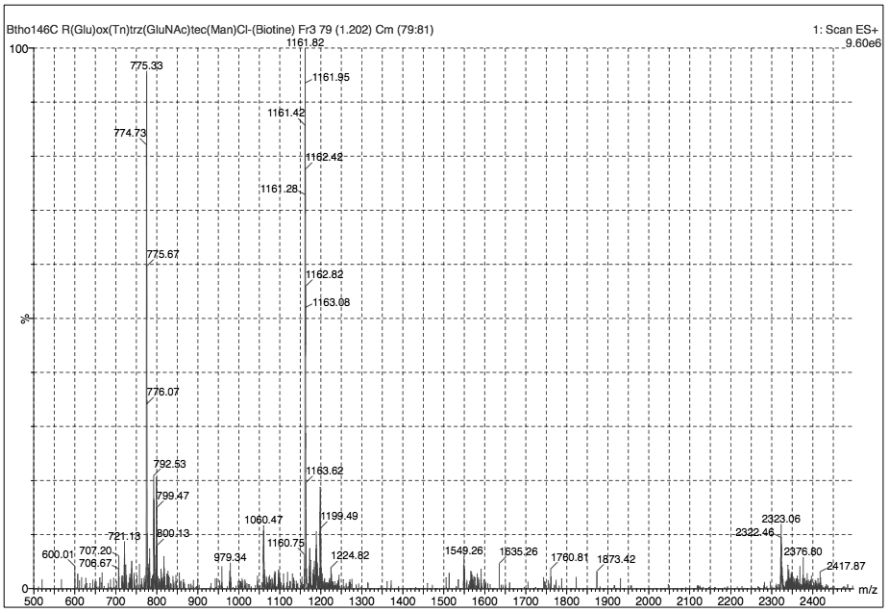


RP-HPLC profile of crude compound **9**.

Compound 11. Compound **9** (2.0 mg, 0.95 μmol) was dissolved in dry DMF (1 mL) and Biotin-OSu **11** (0.48 mg, 1.4 μmol) was added to the solution. The pH was adjusted at 8 with DIPEA and the solution was stirred at room temperature. After 30 min, the DMF was evaporated under reduced pressure and the crude mixture was purified by semi-preparative RP-HPLC. Yield: 90% (1.9 mg); analytical RP-HPLC: R_t = 8.7 min (C_{18}, 214 nm, 5-100% B in 25 min).

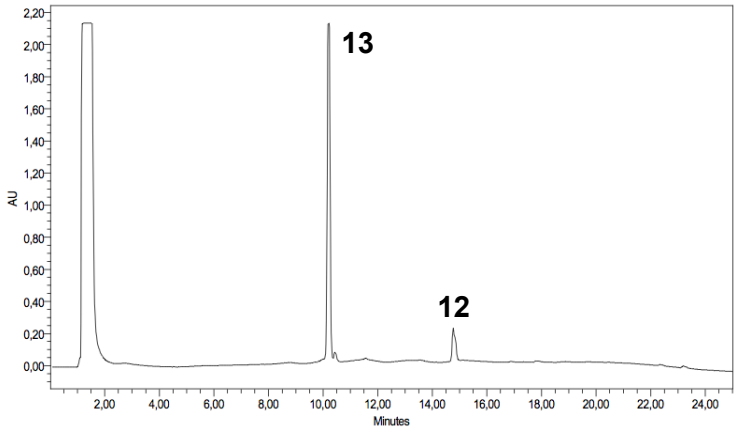


RP-HPLC profile of crude compound **11**.

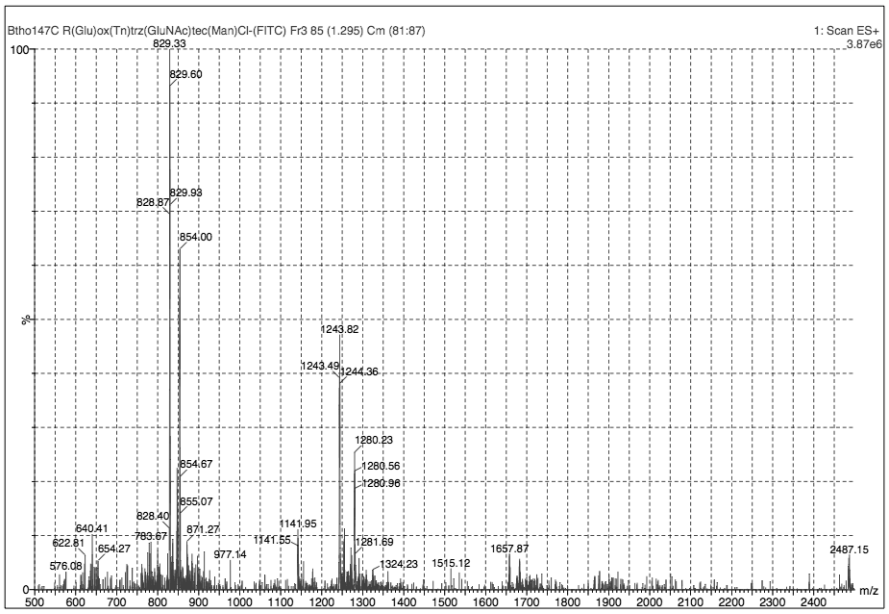


ESI$^+$-MS: *m/z* calcd for $C_{96}H_{157}N_{22}O_{38}S_3$: 2323.0; found: 2323.0 $[M+H]^+$.

Compound 13. Compound **9** (3.5 mg, 1.7 µmol) was dissolved in dry DMF (1.7 mL) and FITC **12** (0.9 mg, 2.5 µmol) was added to the solution. The pH was adjusted at 8 with DIPEA and the solution was stirred at room temperature. After 30 min, the DMF was evaporated under reduced pressure and the crude mixture was purified by RP-HPLC. Yield: 85% (3.5 mg); analytical RP-HPLC: R_t = 10.2 min (C_{18}, 214 nm, 5-100% B in 25 min).

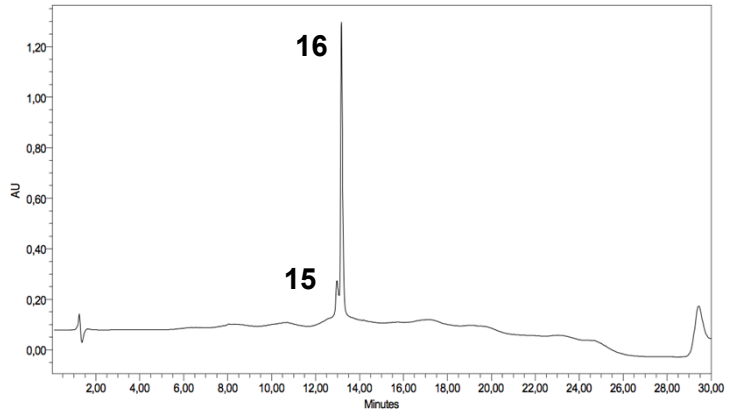


RP-HPLC profile of crude compound **13**.

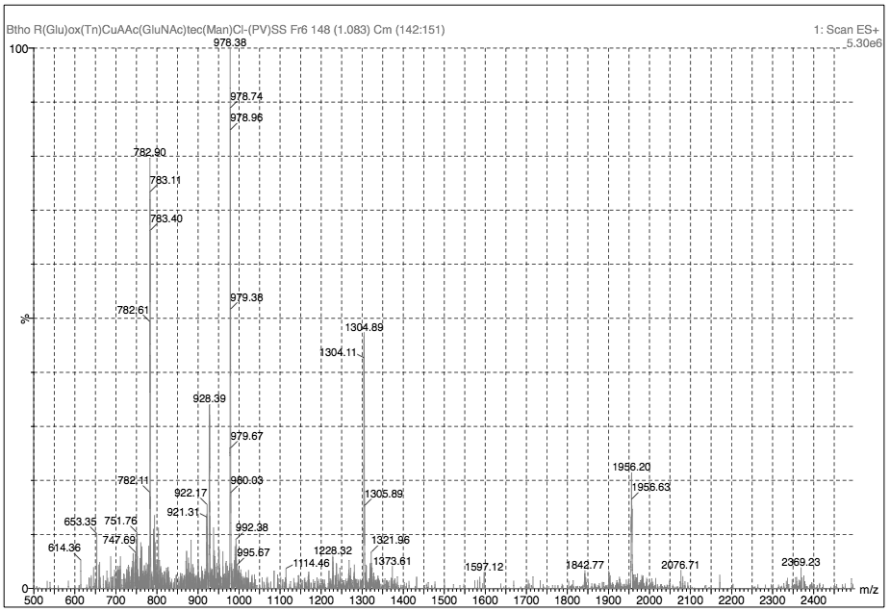


ESI$^+$-MS: *m/z* calcd for $C_{107}H_{154}N_{21}O_{41}S_3$: 2487.0; found: 2487.1 $[M+H]^+$.

Compound 16. Compound **9** (3.4 mg, 1.6 µmol) was dissolved in dry DMF (600 µL) and BocCys(NPys)OSu **14** (1.5 mg, 3.2 µmol) was added. The pH was adjusted at 8 with DIPEA and the solution was stirred at room temperature. After 1 h, the DMF was evaporated under reduced pressure and the crude mixture was precipitated in diethyl ether. Analytical RP-HPLC: R_t = 12.9 min (C_{18}, 214 nm, 5-100% B in 25 min); ESI$^+$-MS: *m/z* calcd for $C_{99}H_{158}N_{23}O_{41}S_4$: 2454.0; found: 2453.7 $[M+H]^+$. The crude compound was subsequently treated with a solution of TFA/CH_2Cl_2 (1/1, 1 mL). After 30 min, the solution was evaporated and the residue purified by semi-preparative RP-HPLC. Yield: 93% (3.7 mg); analytical RP-HPLC: R_t = 9.4 min (C_{18}, 214 nm, 5-100% B in 25 min); ESI$^+$-MS: *m/z* calcd for $C_{94}H_{150}N_{23}O_{39}S_4$: 2353.9; found: 2353.0 $[M+H]^+$. This compound (3.7 mg, 1.5 µmol) and the PV peptide **15** (2.8 mg, 1.6 µmol) were dissolved separately in a mixture of sodium acetate buffer 25 mM pH 5 and DMF (1/1, 1.2 mL). Both solutions were degassed under reduced pressure, mixed together and the reaction mixture was stirred at room temperature under argon atmosphere. After 1 h, the crude yellow mixture was purified by semi-preparative RP-HPLC to obtain compound **16** as a pure lyophilized powder. Yield: 85% (4.9 mg); analytical RP-HPLC: R_t = 13.2 min (C_{18}, 214 nm, 5-100% B in 25 min).

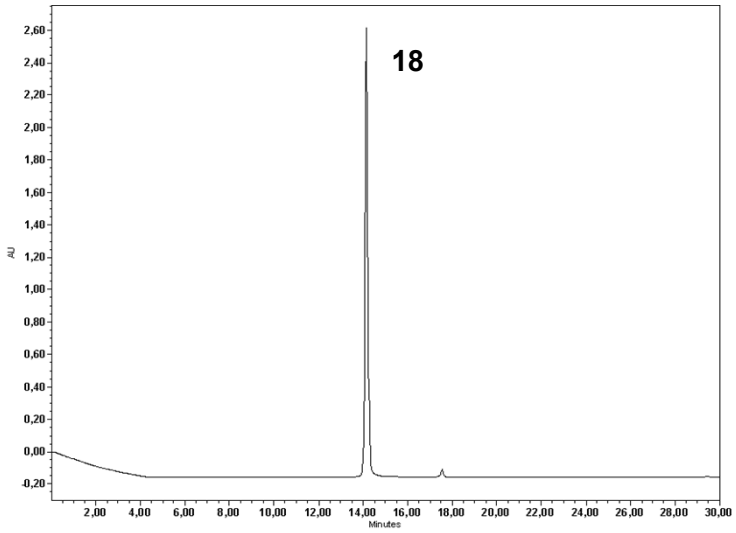


RP-HPLC profile of crude compound **16**.

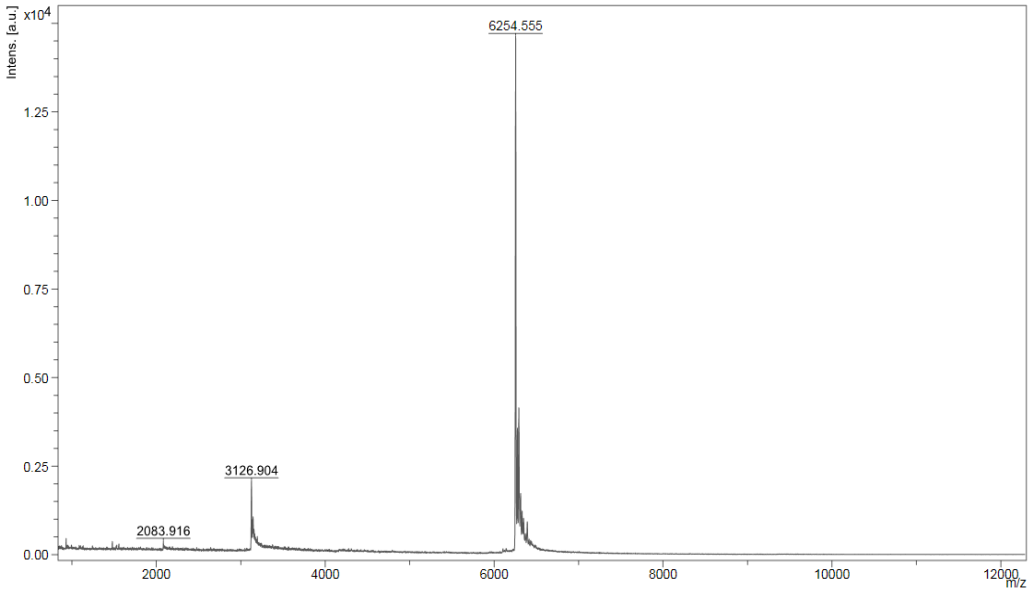


ESI$^+$-MS: *m/z* calcd for $C_{171}H_{272}N_{40}O_{56}S_4$: 3911.9; found: 1956.6 $[M+2H]^{2+}$; 1304.9 $[M+3H]^{3+}$; 978.9 $[M+4H]^{4+}$; 783.4 $[M+5H]^{5+}$.

Compound 18. 5'diol oligonucleotide ($^{5'}$X TCC ATG ACG TTC CTG ACG TT$^{3'}$ with X = $(CH_2)_4CH(OH)CH_2OH$) was prepared on an ABI 3400 DNA synthesizer (Applied Biosystems) by using standard β-cyanoethyl nucleoside phosphoramidite chemistry at 1 µM scale. 5' diol functionality was introduced using acetal protected diol phophoramidite.[1] 5' diol Oligonucleotide (0.426 µmol; 42%) was obtained after RP-HPLC and treatment with 80 % aqueous acetic acid solution for 1 h at room temperature. 5'-diol ODN (0.25 µmol) was subjected to periodate oxidation with a 0.02 M aqueous sodium-*m*-periodate solution (300 µL) to obtain **18** after desalting on NAP10 cartridge. Yield: 80% (0.2 µmol); analytical RP-HPLC: R_t = 14.2 min (C_{18}, 260 nm, 0-30% B in 20 min).



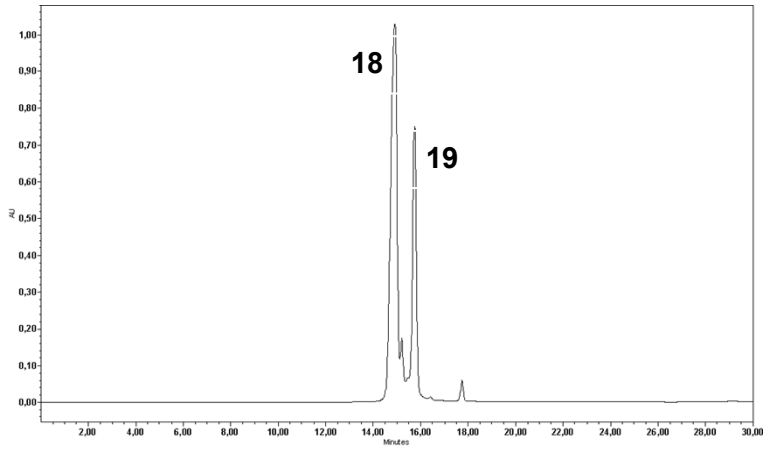
RP-HPLC profile of purified compound **18**.



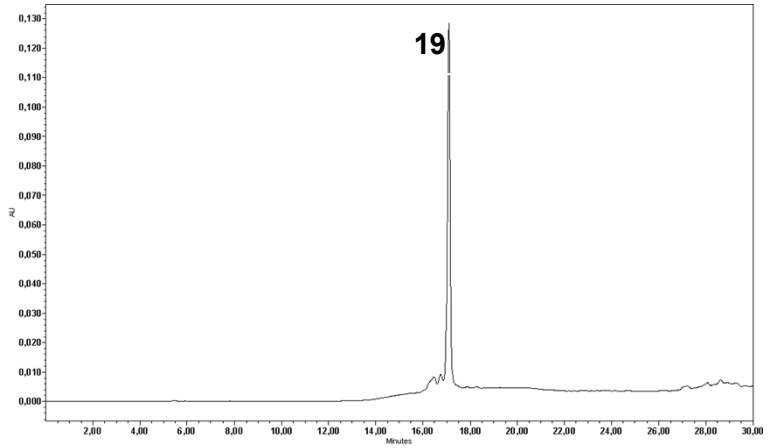
MALDI-Tof MS (-): *m/z* calcd for $C_{200}H_{261}N_{67}O_{127}P_{20}$: 6255.05; found: 6254.55 $[M-H]^-$.

[1] O. P.Edupuganti, Y. Singh, E. Defrancq, P. Dumy, *Chem. Eur. J.* **2004**, *10,* 5988.

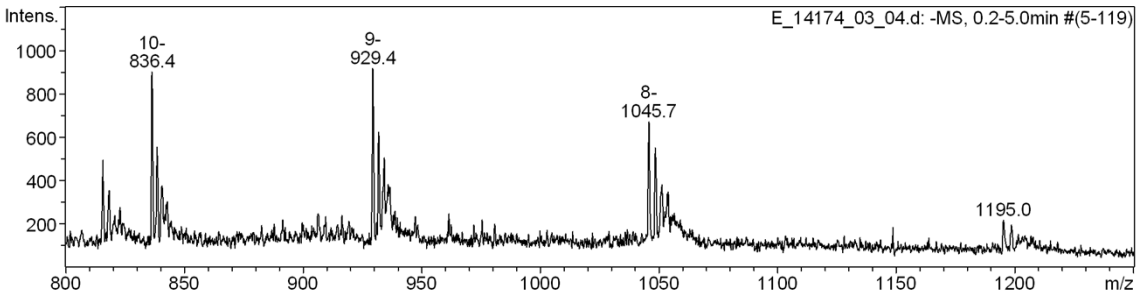
Compound 19. Compound **9** (5.7 mg, 2.7 µmol) was dissolved in dry DMF (1.1 mL), then BocAoa-OSu **17** (1.5 eq, 1.2 mg, 4.1 µmol) was added and the pH was adjusted at 8 using DIPEA. After 1 h analytical HPLC indicated complete reaction coupling. The DMF was evaporated under reduced pressure and the crude mixture was precipitated in diethyl ether and used without further purification. Analytical RP-HPLC: R_t = 11.9 min (C_{18}, 214 nm, 5-100% B in 25 min); ESI$^+$-MS: *m/z* calcd for $C_{93}H_{154}N_{21}O_{40}S_2$: 2270.0; found: 2270.1 $[M+H]^+$. The crude compound was subsequently deprotected using a solution of TFA (60% in CH_2Cl_2, 1.5 mL) within 30 min. The resulting aminooxylated compound was purified by RP-HPLC. Yield: 93% (5.7 mg); analytical RP-HPLC: R_t = 8.2 min (C_{18}, 214 nm, 5-100% B in 25 min); ESI$^+$-MS: *m/z* calcd for $C_{88}H_{146}N_{21}O_{38}S_2$: 2270.0; found: 2169.6 $[M+H]^+$. To a solution of **18** (0.2 µmol) in ammonium acetate buffer (200 µL, 0.4 M, pH 4.6) was added this aminoxylated cyclopeptide (0.069 µmol, 0.15 mg) solubilized in water. The reaction was stirred at room temperature during 4 h and monitored by RP-HPLC. The conjugate **19** was obtained after RP-HPLC purification. Yield: 65% (0.045 µmol); analytical RP-HPLC: R_t = 15.7 min (C_{18}, 260 nm, 0-30% B in 20 min).



RP-HPLC profile of crude compound **19**.

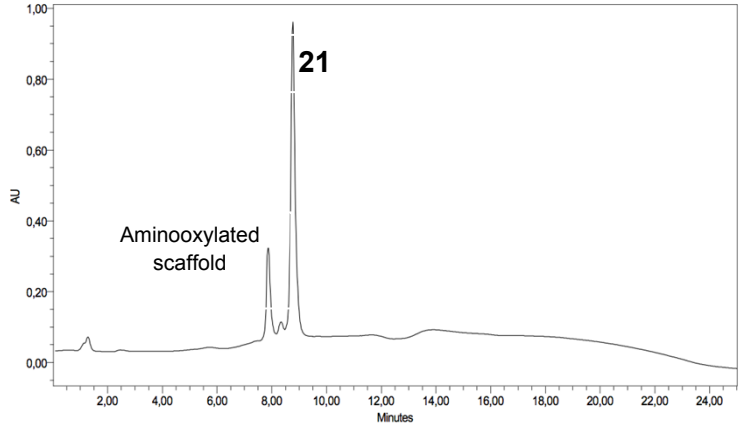


RP-HPLC profile of purified compound **19**.

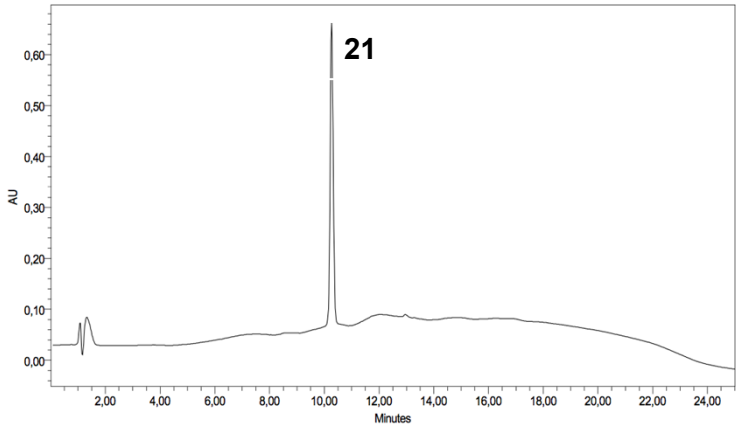


ESI$^+$-MS: *m/z* calcd for $C_{287}H_{398}N_{88}O_{163}P_{20}S_2$: 8372.0; found: 8372.9.

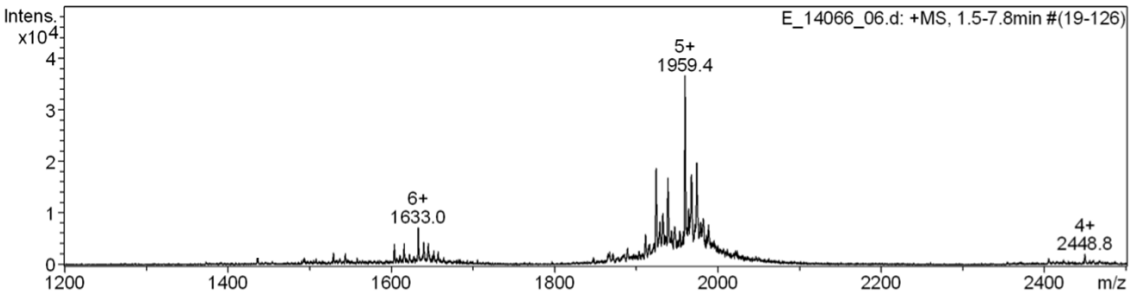
Compound 21. Compound **20** (0.53 mg, 0.45 µmol) was dissolved in 0.1% TFA in water (10 mM) and the previous aminooxylated compound (5.8 mg, 2.7 µmol) was added. The solution was stirred for 1 h at 37°C and purified by RP-HPLC to obtained compound **21**. Yield: 87% (3.8 mg); analytical RP-HPLC: R_t = 10.3 min (C_{18}, 214 nm, 5-100% B in 25 min).



RP-HPLC profile of crude compound **21**.



RP-HPLC profile of purified compound **24**.



ESI$^+$-MS: *m/z* calcd for $C_{404}H_{651}N_{98}O_{166}S_8$: 9793.3; found: 9792.8.