Supporting Information

Site-Selective Solid-Phase Synthesis of a CCR5 Sulfopeptide Library to Interrogate HIV Binding and Entry

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1 General procedures

Unless otherwise stated, reactions were carried out under N_2 or Ar and standard syringe and cannulating techniques were applied for transferring reagents to the reaction flask. Reaction progress was monitored with aluminium backed TLC plates pre-coated with silica UV254 and visualized by UV at λ = 254 or 365 nm, vanillin, 5% H_2SO_4 or phosphomolybdic acid (PMA) dip. Silica gel 60 (particle size 0.040-0.065 mm) was used in flash chromatography with the ratio of solvents indicated in the experimental section. All solvents used were either analytical or HPLC grade. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 200 (200 MHz for ^{1}H), Bruker Avance 300 (300 MHz for ^{1}H and 75 MHz for ^{13}C), a Bruker Avance 400 (400 MHz for ^{1}H and 100 MHz for ^{13}C) or a Bruker Avance 500 (500 MHz for ^{1}H and 125 MHz for ^{13}C) spectrometer and referenced to residual solvent peaks. The data is reported as chemical shift (δ), multiplicity (app = apparent, s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublet, ddt = doublet of doublet of triplet, td = triplet of doublet, m = multiplet), coupling constant (J Hz) and relative integral.

Photochemical reactions were conducted in a Rayonet (model RPR-100) UV reactor containing 3654 Å ultraviolet (UV) lamps fitted with a suitable cover.

LC-MS analysis of peptides resulting from cleavage of resin bound **13-21** and sulfopeptides **2-9** was performed on a Shimadzu LC-MS 2020 instrument containing a LCM20A pump and a SPD-M20A photodiode array detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive and/or negative mode. Separations were performed on a Waters Sunfire^{TM} 5 μm, 2.1 × 150 mm column (C18) at a flow rate of 0.2 mL min^{-1} using ***Eluent A*** (solvent A: 0.1% formic acid in water, solvent B: 0.1% formic acid in acetonitrile) over a linear gradient from 0% to 100% solvent B. For sulfopeptides **2-9**, M denotes the molecular formula calculated when the sulfate esters of the sulfotyrosine residues are in the protonated (neutral) forms. Acidolysis of the Tyr sulfate ester moieties was observed in positive ion mode. These fragment ions are labeled in the spectra.

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column (heater at 30 ^{o}C) and a 2996 photodiode array detector. Peptides **2-9** were analyzed using a Waters SunFire^{TM} 5 μm, 2.1 x 150mm column (C18) operating at a flow rate of 0.2 mL min^{-1} using ***Eluent B*** (solvent A: 0.1 M NH_4OAc in water, solvent B: acetonitrile) with the ratio of solvents indicated in the experimental section.

Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvent Delivery System and a Waters 500 pump with a Waters 490E programmable wavelength detector operating at 230 and 280 nm. Sulfopeptides were purified on a Waters Xbridge^{TM} 5 μm (C18) 10 x 250 mm semi-preparative column operating at a flow rate of 4 mL min^{-1} over a linear gradient of ***Eluent B*** with the ratio of solvents indicated in the experimental section.

1.1 General procedures for SPPS

Solid-phase peptide synthesis was carried out in a polypropylene syringe with a Teflon filter, purchased from Torviq. Fmoc- and Boc-amino acids, PyBOP and HATU were purchased from GL Biochem (Shanghai) Ltd. *N,N*-Di*iso*propylethylamine (DIPEA), triethylamine (Et_3N) and 4-methylmorpholine (NMM) were purchased from Sigma-Aldrich.

1.1.1 Half-loading of amino acid onto Rink amide resin

Rink amide resin (100-200 mesh) (470 mg, 250 μmol, 1.0 equiv.) was allowed to swell in DMF (5 mL) for 10 min. The resin was drained and the Fmoc-group was removed by treatment with a solution of piperidine/DMF (2 × 5 mL, 2:8 v/v) for 3 min. The resin was then rinsed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL) and DMF (5 × 5 mL). A solution of Fmoc-protected amino acid (0.13 mmol, 0.5 equiv.),

PyBOP (68 mg, 0.13 mmol, 0.5 equiv.) and NMM (0.03 mL, 0.25 mmol, 1.0 equiv.) in DMF (2 mL) was added to the resin and the resulting mixture was shaken for 4 h. The resin was then drained and washed with DMF (5 x 5 mL). A solution of acetic anhydride/pyridine (5 mL, 1:9 v/v) was added to the resin and agitated for 5 min. The resin was drained and rinsed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL) and DMF (5 × 5 mL).

The loading efficiency was determined by the absorption of the fulvene-piperidine adduct at λ = 301 nm. The resin was treated with a solution of piperidine/DMF (5 mL, 2:8 v/v) twice for 5 min to remove the Fmoc-protecting group. The Fmoc-deprotection solutions were combined and diluted with a solution of piperidine/DMF (2:8 v/v) so that the maximum concentration of the fulvene-piperidine adduct was in the range of 12.5-25.0 μM. The UV absorbance of the resulting solution was measured at λ = 301 nm using piperidine/DMF (2:8 v/v) as a reference. The average of absorbance reading was used for calculating the percentage loading based on the Beer-Lambert law, where ε = 7800 $M^{-1}cm^{-1}$. The calculated percentage loading was 41% (0.17 mmol/g).

1.1.2 Full-loading of amino acid onto Rink amide resin

Rink amide resin (100-200 mesh) (19 mg, 10 μmol, 1.0 equiv.) was allowed to swell in DMF (2 mL) for 10 min. The resin was drained and the Fmoc-group was removed by treatment with a solution of piperidine/DMF (2 × 2 mL, 2:8 v/v) for 3 min. The resin was then rinsed with DMF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL) and DMF (5 × 2 mL). A solution of Fmoc-protected amino acid (40 μmol, 4.0 equiv.), PyBOP (21 mg, 40 μmol, 4.0 equiv.) and NMM (10 μl, 80 μmol, 8.0 equiv.) in DMF (0.5 mL) was added to the resin and the resulting mixture was shaken for 4 h. The resin was drained and washed with DMF (5 × 2 mL). A solution of acetic anhydride/pyridine (2 mL, 1:9 v/v) was added to the resin and agitated for 5 min. The resin was drained and rinsed with DMF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL) and DMF (5 × 2 mL).

The loading efficiency was determined by the absorption of the fulvene-piperidine adduct at λ = 301 nm. The resin was treated with a 20% solution of piperidine/DMF (5 mL) twice for 5 min to remove the Fmoc protecting group. The Fmoc-deprotection solutions were combined and diluted with a solution of piperidine/DMF (2:8 v/v) so that the maximum concentration of the fulvene-piperidine adduct was in the range of 1.25-2.5 μM. The UV absorbance of the resulting solution was measured at λ = 301 nm using piperidine/DMF (2:8 v/v) as a reference. The average of three absorbance reading was used for calculating the percentage of loading based on the Beer-Lambert law, where ε = 7800 $M^{-1}cm^{-1}$. The calculated loading was quantitative (0.41 mmol/g).

1.1.3 Fmoc-strategy peptide assembly (250 μmol of peptide)

Fmoc deprotection: A solution of piperidine/DMF (5 mL, 2:8 v/v) was added to the resin and shaken for 3 min. The resin was drained and the step was repeated once before rinsing the resin with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL) and DMF (5 × 5 mL).

Amino acid coupling: A solution of Fmoc-protected amino acid (1.00 mmol, 4.0 equiv.), PyBOP (520 mg, 1.00 mmol, 4.0 equiv.), and NMM (0.22 mL, 2.00 mmol, 8.0 equiv.) in DMF (2.5 mL) was added to the resin and the resulting mixture was shaken for 1 h. The resin was then drained and washed with DMF (5 × 5 mL). For the coupling of allyl- and *tert*-butyltrimethylsilyl (TBS)-protected tyrosine derivatives (**11** and **12**, respectively) to resin-bound peptides, a solution of amino acid **11** or **12** (0.38 mmol, 1.5 equiv.), HATU (140 mg, 0.38 mmol 1.5 equiv.) and DIPEA (0.13 mL, 0.75 mmol, 3.0 equiv.) in DMF (4 ml) was added to the resin. The resulting mixture was agitated at room temperature for 16 h. For the coupling of *o*-nitrobenzyl (*o*-Nb)-protected tyrosine **10**, a solution of **10** (270 mg, 0.50 mmol, 2.0 equiv.), PyBOP (260 mg, 0.50 mmol, 2.0 equiv.), HOBt (65 mg, 0.50 mmol, 2.0 equiv.) and NMM (0.11 mL, 1.00 mmol, 4.0 equiv.) in DMF (2.5 mL) was added to the resin. The resulting mixture was agitated at room

temperature for 2 h. The resin was subsequently washed with DMF (5 x 5 mL), CH_2Cl_2 (5 x 5 mL) and DMF (5 x 5 mL).

Capping: A solution of acetic anhydride/pyridine (5 mL, 1:9 v/v) was added to the resin and agitated for 5 min. The resin was drained and rinsed with DMF (5 × 5 mL), CH_2Cl_2 (5 × 5 mL) and DMF (5 × 5 mL).

1.2 General procedures for solid-phase deprotection reactions (25 μmol of peptide)

1.2.1 *o*-Nitrobenzyl *O*-tyrosine ether deprotection[1] (Deprotection condition A)

The photo-deprotection of resin-bound peptides (25 μmol) was carried out in DMF (20 mL) in a UV reactor (λ = 365 nm) for 24 h. The resin was then drained and washed with DMF (5 × 2 mL), CH_2Cl_2 (5 × 2 mL) and DMF (5 × 2 mL).

1.2.2 Allyl *O*-tyrosine ether deprotection (Deprotection condition B)

To the resin-bound peptide (25 μmol) was added a solution of tetrakis(triphenylphosphine)palladium(0) (29 mg, 25 μmol, 1.0 equiv.), triethylsilane (TES, 64 μL, 400 μmol, 16.0 equiv.) and acetic acid (AcOH, 23 μL, 400 μmol, 16.0 equiv.) in CH_2Cl_2 (2.0 mL) and the resulting mixture was agitated for 1 h. The resin was then drained and washed with CH_2Cl_2 (10 × 2 mL), DMF (10 × 2 mL) and CH_2Cl_2 (10 × 2 mL). The procedure was repeated once. For **16**, **18** and **20**: the procedure above was followed but the reaction was performed once for 30 min.

1.2.3 TBS *O*-tyrosine ether deprotection (Deprotection condition C)

A 1 M solution of tetrabutylammonium fluoride (400 μmol, 16.0 equiv.) in THF (0.4 mL) was concentrated and dried *in vacuo* for 1 h to remove the solvent and the resulting white solid was redissolved in CH_2Cl_2 (2.0 mL) containing AcOH (23 μL, 400 μmol, 16.0 equiv.). The resulting solution was added to peptide resin (25 μmol, 1.0 equiv.) and agitated for 3 h. The resin was drained and washed with CH_2Cl_2 (5 × 2 mL), DMF (5 × 2 mL) and CH_2Cl_2 (10 × 2 mL).

1.3 General procedures for solid-phase sulfation with imidazolium salt 1 (25 μmol of peptide)

A resin-bound peptide (25 μmol) containing side-chain deprotected tyrosine residues was allowed to swell in CH_2Cl_2 (5 mL) for 20 min. The resin was drained and washed with CH_2Cl_2 (10 × 2 mL) before a solution of imidazolium-sulfating reagent **1**[6] (8 equiv. per free phenol) and Et_3N (8 equiv. per free phenol) in CH_2Cl_2 (4 ml) was added and agitated for 16 h. The resin was then drained and washed with CH_2Cl_2 (5 × 2 mL), DMF (5 × 2 mL) and CH_2Cl_2 (10 × 2 mL). The above procedure was repeated once.

1.4 General procedure for cleaving peptide off the resin (25 μmol of peptide)

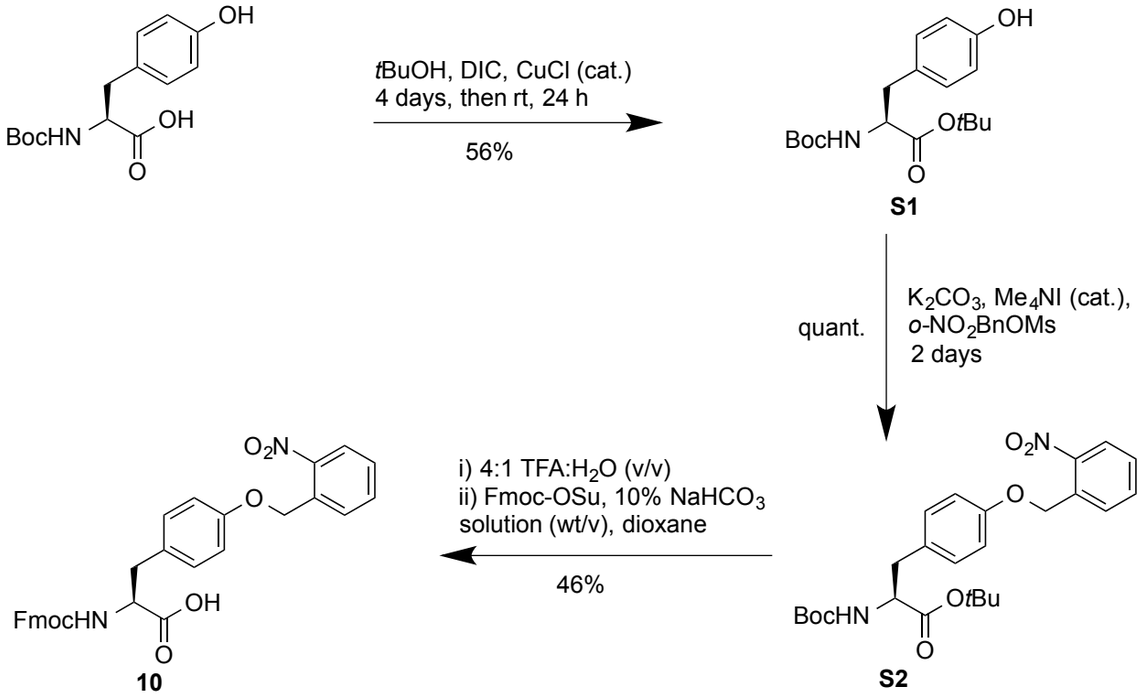
To the peptide resin was added a cleaving cocktail of trifluoroacetic acid (TFA)/tri*iso*propylsilane (TIS)/water (90:5:5 v/v/v, 5 mL) and the resulting mixture was agitated for 2 h. The resin was then drained and washed with the cocktail solution (3 x 2 mL). The combined cleaving and washing solutions were evaporated under reduced pressure. The crude mixture was resuspended in ice-cold Et_2O and centrifuged. The supernatant was carefully removed and the crude peptide solid was dried under high vacuum overnight.

1.5 General procedure for deprotection of trichloroethyl (TCE)-protected sulfopeptides

Deprotection of trichloroethyl (TCE)-protected sulfopeptides was performed using a slightly modified procedure to that described by Ali *et al.*[2] To a solution of the crude TCE-protected sulfopeptide (25 μmol) in water/MeOH (1:1, v/v, 8 mL) was added Et_3N (1.88 mmol, 260 μL) and $Pd(OH)_2$ (20 wt% on carbon, 5 mg) and the mixture was stirred under a hydrogen atmosphere for 4 h. The reaction mixture was filtered through a pad of reverse-phase silica and washed with methanol (5 × 5 mL). The filtrates were combined and evaporated under reduced pressure. The crude material was purified with preparative reverse-phase HPLC (***Eluent A***). Lyophilization of the appropriate fractions three times (to remove excess NH_4OAc) afforded the desired sulfopeptides.

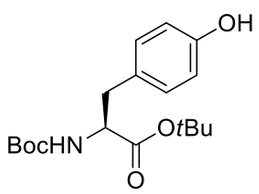
2 Synthesis of protected Tyr derivatives 10, 11 and 12

2.1 Synthesis of Fmoc-Tyr(*o*-Nb)-OH (10)



Scheme S1 Synthesis of Fmoc-Tyr(*o*-Nb)-OH (**10**)

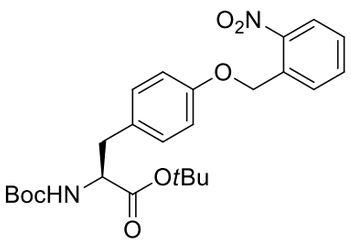
(*S*)-*tert*-Butyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-hydroxyphenyl)propanoate (S1)



A mixture of *t*BuOH (14.0 mL, 0.14 mol, 4.0 equiv.), *N*,*N*-diisopropylcarbodiimide (16.5 mL, 0.11 mol, 3.0 equiv.) and CuCl (70 mg, 0.71 mmol, 0.02 equiv.) was stirred for 4 days and then diluted with CH_2Cl_2 (300 mL). The resulting solution was added *via* cannula to a suspension of Boc-L-Tyr-OH (10.0 g, 36 mmol, 1.0 equiv.) in CH_2Cl_2 (45 mL) at 0 °C. The mixture was stirred for 24 h at room temperature before quenching with water (200 mL). The two layers were separated and the aqueous fraction was extracted with CH_2Cl_2 (3 × 100 mL). The organic fractions were combined and concentrated under reduced

pressure. The resulting mixture was redissolved in EtOAc (100 mL), dried over $MgSO_4$ and filtered. The organic solvent was removed under reduced pressure. The crude mixture was purified by gradient flash chromatography (from 7:1 to 2:1 v/v, hexane:EtOAc) to afford ester **S1** (6.70 g, 56%) as a white solid. ^{1}H NMR (300 MHz, $(CD_3)_2CO$) δ 8.18 (s, 1H), 7.08 (d, 2H, *J* = 8.4 Hz), 6.77 (d, 2H, *J* = 8.4 Hz), 5.87 (d, 1H, *J* = 7.5 Hz), 4.21 (q, 1H, *J* = 6.3 Hz), 3.01-2.84 (m, 2H), 1.41 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (75 MHz, $(CD_3)_2CO$) δ 172.1, 157.2, 156.2, 131.3, 129.0, 116.0, 81.6, 79.3, 57.0, 37.8, 28.7, 28.3; MS (ESI) *m/z* 337.8 $[M+H]^+$. These data are in agreement with those previously reported by Taleski *et al*.[3]

(*S*)-tert-Butyl 3-(4-(2-nitrobenzyloxy)phenyl)-2-(*tert*-butoxycarbonylamino)propanoate (S2)



To a solution of ester **S1** (1.00 g, 2.96 mmol, 1.0 equiv.) in DMF (30 mL) at 0 ^oC was added K_2CO_3 (818 mg, 5.92 mmol, 2.0 equiv.). The resulting mixture was stirred for 10 min followed by the addition of Bu_4NI (110 mg, 0.30 mmol) and a solution of 2-nitrobenzyl mesylate (1.37 g, 5.92 mmol, 2.0 equiv.) in DMF (10 mL). The reaction was stirred at 0 ^oC for 1 h and then at room temperature for 48 h. The reaction was quenched with water (100 mL) and the aqueous fraction was extracted with Et_2O (3 × 100 mL). The organic fractions were combined, dried and concentrated under reduced pressure. The crude oil was purified by gradient flash chromatography (from 12:1 to 5:1 v/v, hexane:EtOAc) to provide amino acid **S2** (1.40 g, quant.) as a pale yellow oil. $[\alpha]_D^{20}$ = +32.5^o (*c* 0.35, CH_2Cl_2); IR (film) 2976, 2934, 1709, 1509, 1340, 1293, 1149, 1051, 728; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.15 (dd, *J* = 8.2, 1.05 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 1H), 7.66 (t, *J* = 7.7, 1.02 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 1H), 7.10 (d, *J* = 8.6 Hz, 2H), 6.89 (d, *J* = 8.6 Hz, 2H), 5.46 (s, 2H), 4.98 (d, *J* = 4.98 Hz, 1H), 4.44-4.37 (m, 1H), 2.99 (app d, *J* = 5.2 Hz, 2H), 1.41 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 157.4, 155.3, 147.2, 134.2, 134.2, 131.0, 129.6, 128.8, 128.5, 125.2, 115.0, 82.2, 79.9, 67.1, 55.2, 37.9, 28.6, 28.2; MS (ESI+) *m/z* 495.0 $[M+Na]^+$; HRMS (ESI+) *m/z* calcd. for $C_{20}H_{28}F_3NO_8SNa$ $[M+Na]^+$ 495.2102, found 495.2102.

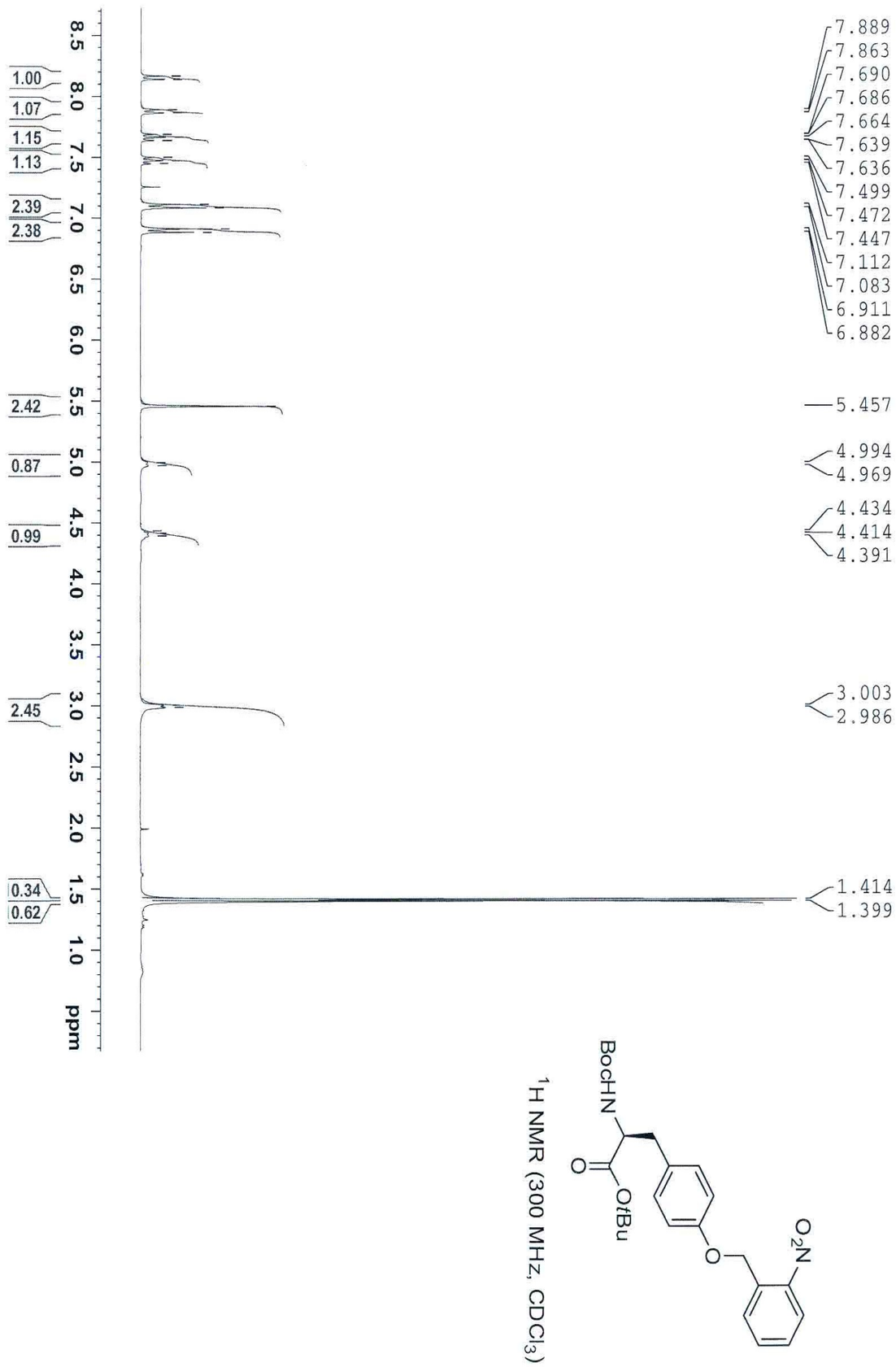


Figure 1. ^{1}H NMR spectrum of compound **S2**

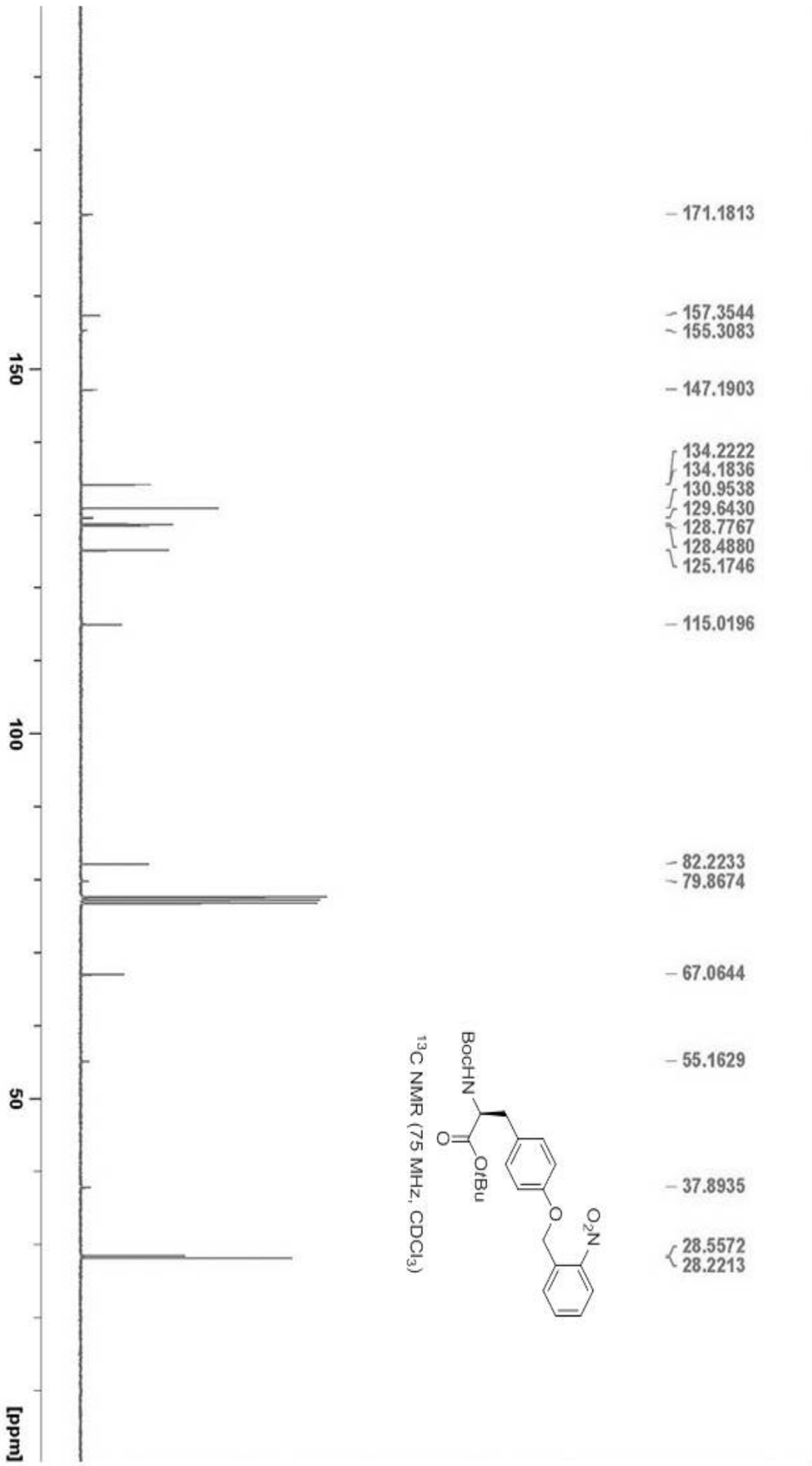
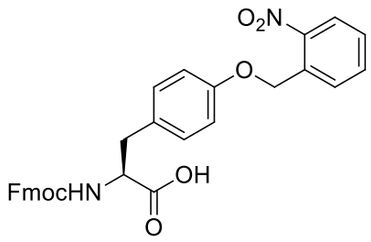


Figure 2. ^{13}C NMR spectrum of compound **S2**

(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((2-nitrobenzyl)oxy)phenyl)propanoic acid (10)



Ester **S2** (850 mg, 1.80 mmol, 1.0 equiv.) was added to a solution of TFA/water (15 mL, 4:1 v/v). The reaction was stirred for 3 h before the solvent was removed under reduced pressure. The resulting white solid was suspended in 10% aqueous $NaHCO_3$ solution (18 mL, w/v) followed by the addition of a solution of Fmoc-OSu (730 mg, 2.20 mmol, 1.2 equiv.) in dioxane (9 mL). The reaction was stirred for 20 h before being quenched with 1 M HCl solution and acidified to pH 2. The resulting mixture was extracted with EtOAc (3 × 50 mL). The organic fractions were combined and dried over $MgSO_4$. The solution was filtered, evaporated and dry-loaded onto silica gel. The compound was purified by gradient flash chromatography (from 83:17:2 to 50:50:2 v/v/v, hexane:EtOAc:AcOH) to afford the amino acid **10** (460 mg, 46%) as a white solid. $[\alpha]_D^{20}$ = +29.0^o (*c* 0.10, DMF); IR (film) 3341, 1694, 1531, 1510, 1448, 1341, 1239, 1051; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.2 Hz, 1H), 7.85 (app d, *J* = 7.4 Hz, 1H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.64 (t, *J* = 7.3, 1H), 7.54 (t, *J* = 6.2 Hz, 2H), 7.46 (t, *J* = 7.7 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.29 (t, *J* = 7.4 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H), 6.9 (d, *J* = 8.3 Hz, 2H), 5.44 (s, 2H), 5.17 (d, *J* = 8.1 Hz, NH), 4.70-4.64 (m, 1H), 4.46 (dd, *J* = 10.5, 7.1 Hz, 1H), 4.36 (dd, *J* = 10.4, 6.5 Hz, 1H), 4.18 (t, *J* = 6.8 Hz, 1H), 3.16 (dd, *J* = 13.7, 4.8 Hz, 1H), 3.06 (dd, *J* = 14.1, 5.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.0, 157.5, 155.9, 147.0, 143.9, 143.8, 141.5, 134.1, 134.0, 130.7, 128.6, 128.5, 128.4, 127.9, 127.2, 126.1, 125.9, 120.2, 115.2, 67.2, 67.0, 54.8, 47.2, 37.1; HRMS (ESI+) *m/z* calcd. for $C_{37}H_{26}N_2O_7Na^+$ $[M+Na]^+$ 561.1632, found 561.1632.

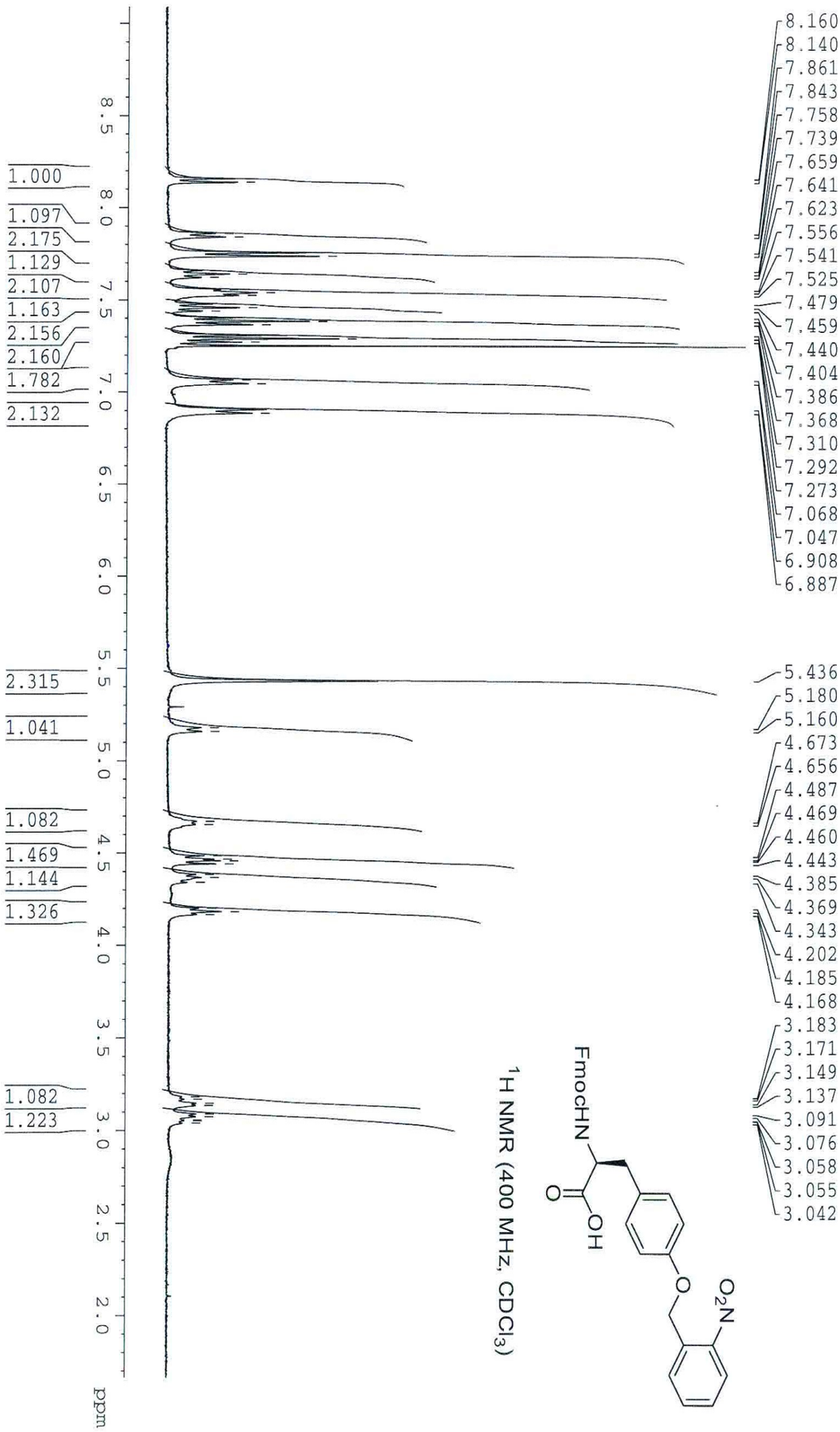


Figure 3. ^{1}H NMR spectrum of compound **10**

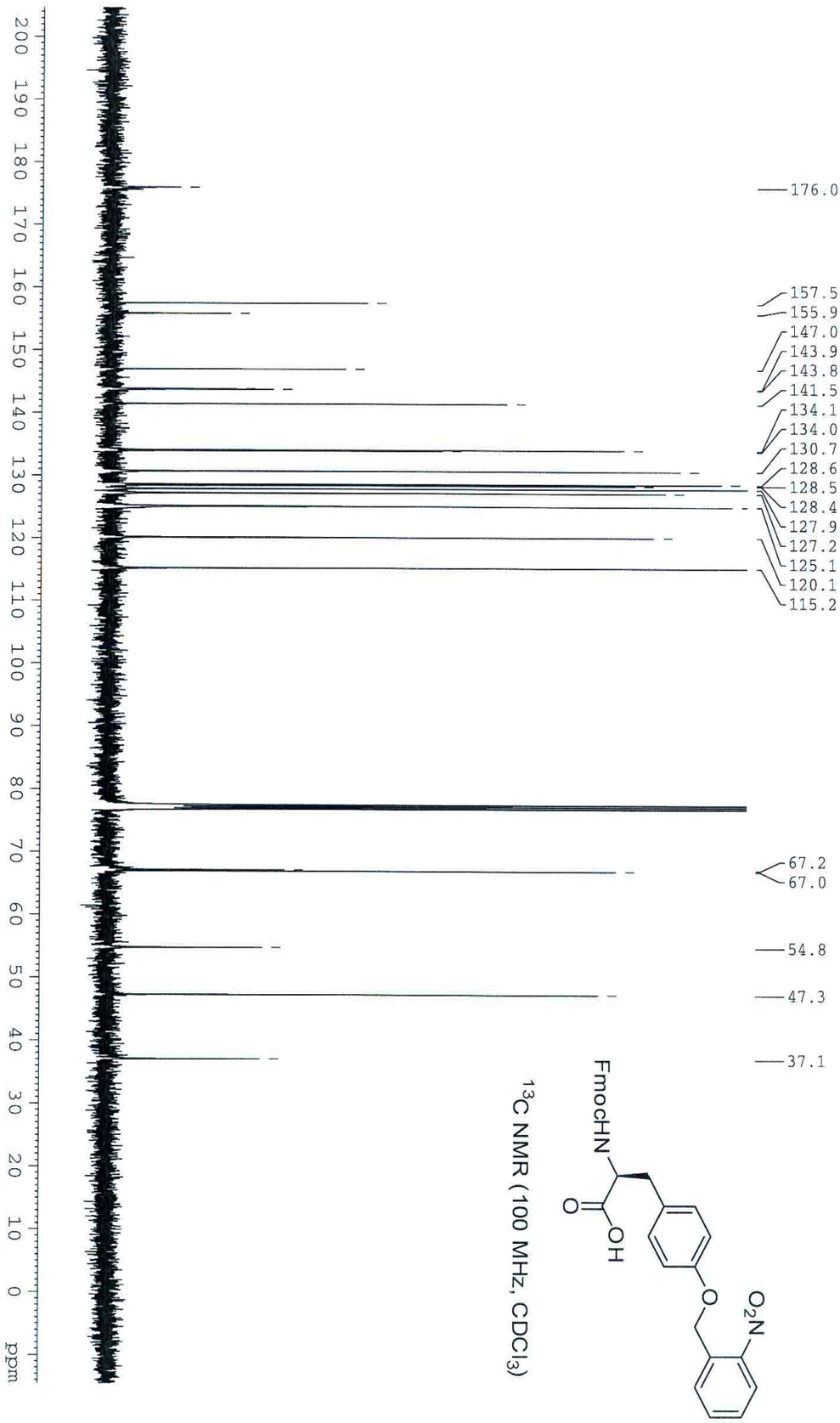
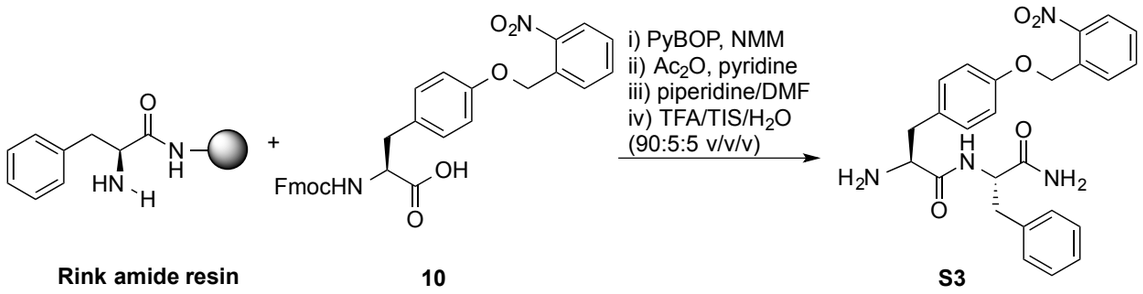


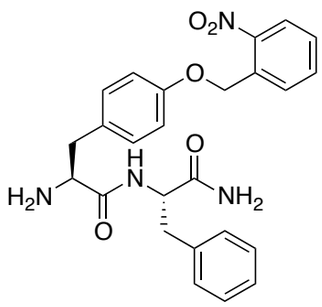
Figure 4. ^{13}C NMR spectrum of compound **10**

2.1.1 Analysis of the enantiomeric purity of Fmoc-Tyr(*o*-Nb)-OH (10)



Scheme 2 Synthesis of H-Tyr(*o*-Nb)-Phe-NH_2 (**S3**) dipeptide using Fmoc-strategy SPPS.

(*S*)-2-amino-*N*-((*S*)-1-amino-1-oxo-3-phenylpropan-2-yl)-3-(4-((2-nitrobenzyl)oxy)phenyl)propanamide (S3)



Dipeptide **S3** was synthesized on Rink Amide resin (10 μmol) according to Fmoc-strategy SPPS described in the general procedures (1.1.2 and 1.1.3) and analyzed *via* ^{1}H and ^{13}C NMR spectroscopy. The NMR spectra are consistent with the presence of a single diastereomer (4.0 mg, quant.), thus indicating that amino acid **S3** was prepared as a single enantiomer without racemization (see Figures 5 and 6). $[\alpha]_D^{20}$ = +1.34° (*c* 0.05, MeOH); IR (film) 3322, 3198, 2941, 1679, 1554, 1526, 1512, 1206, 1180, 1136; ^{1}H NMR (500 MHz, CD_3OD) δ 8.12 (d, *J* = 8.2 Hz, 1H), 7.83 (d, *J* = 7.70 Hz, 1H), 7.72 (t, *J* = 7.6 Hz, 1H), 7.56 (t, *J* = 7.6 Hz, 1H), 7.30-7.26 (m, 4H), 7.22-7.19 (m, 1H), 7.19 (d, *J* = 8.5 Hz, 2H), 6.97 (d, *J* = 8.5 Hz, 2H), 5.45 (s, 2H), 4.65 (dd, *J* = 8.20, 6.30 Hz, 1H), 3.93 (t, *J* = 6.38 Hz, 1H), 3.17-3.11 (m, 4H), 2.98-2.89 (m, 4H); ^{13}C NMR (125 MHz, CD_3OD) δ 175.3, 159.4, 149.1, 138.2, 134.8, 134.3, 131.8, 130.3, 130.2, 129.9, 129.5,127.9, 125.9, 116.4, 68.1, 55.9, 54.8, 39.1, 38.2; HRMS (ESI+) *m/z* calcd. for $C_{25}H_{26}N_4O_5Na^+$ $[M+Na]^+$ 485.1795, found 485.1795.

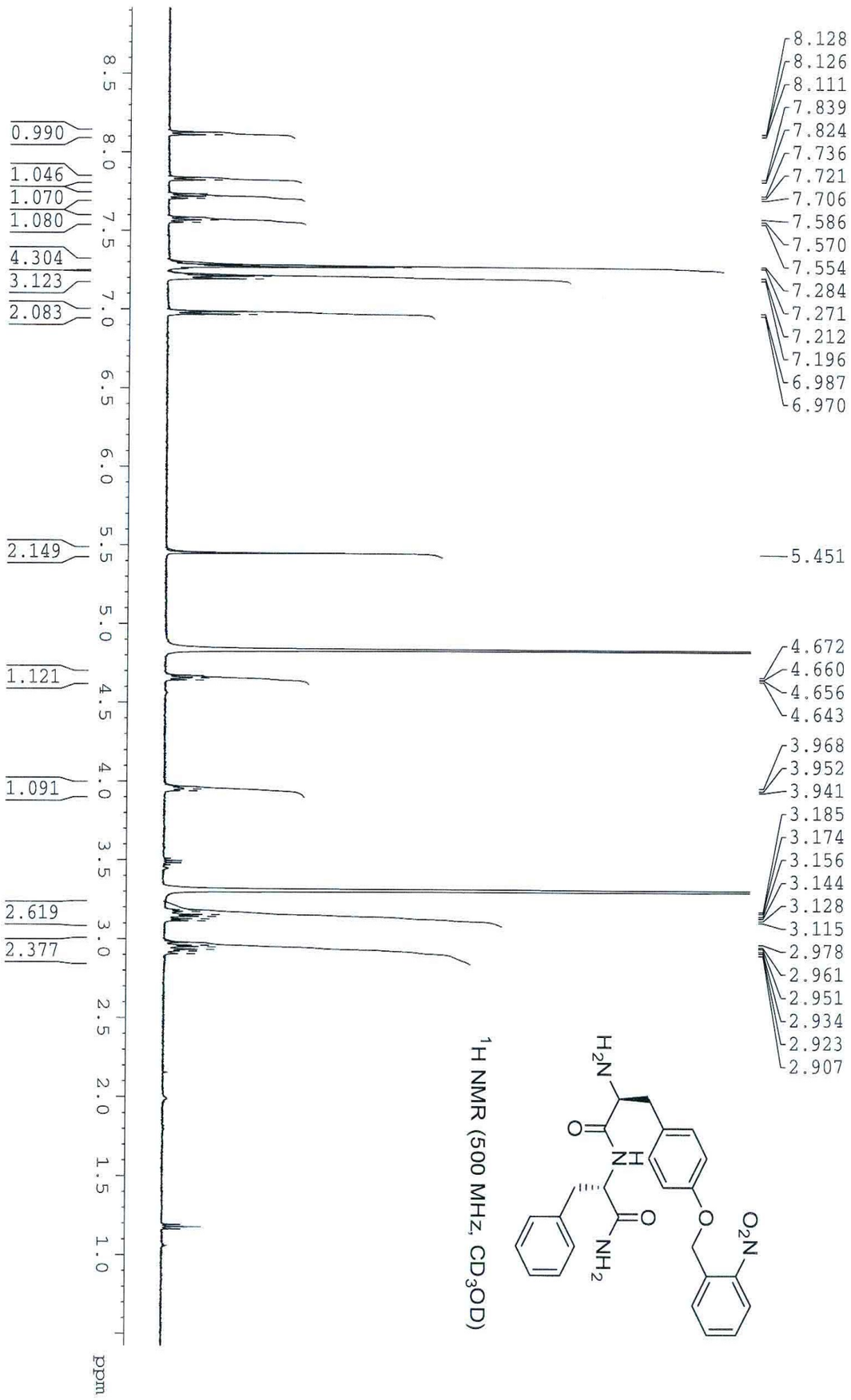


Figure 5. ^{1}H NMR spectrum of compound **S3**

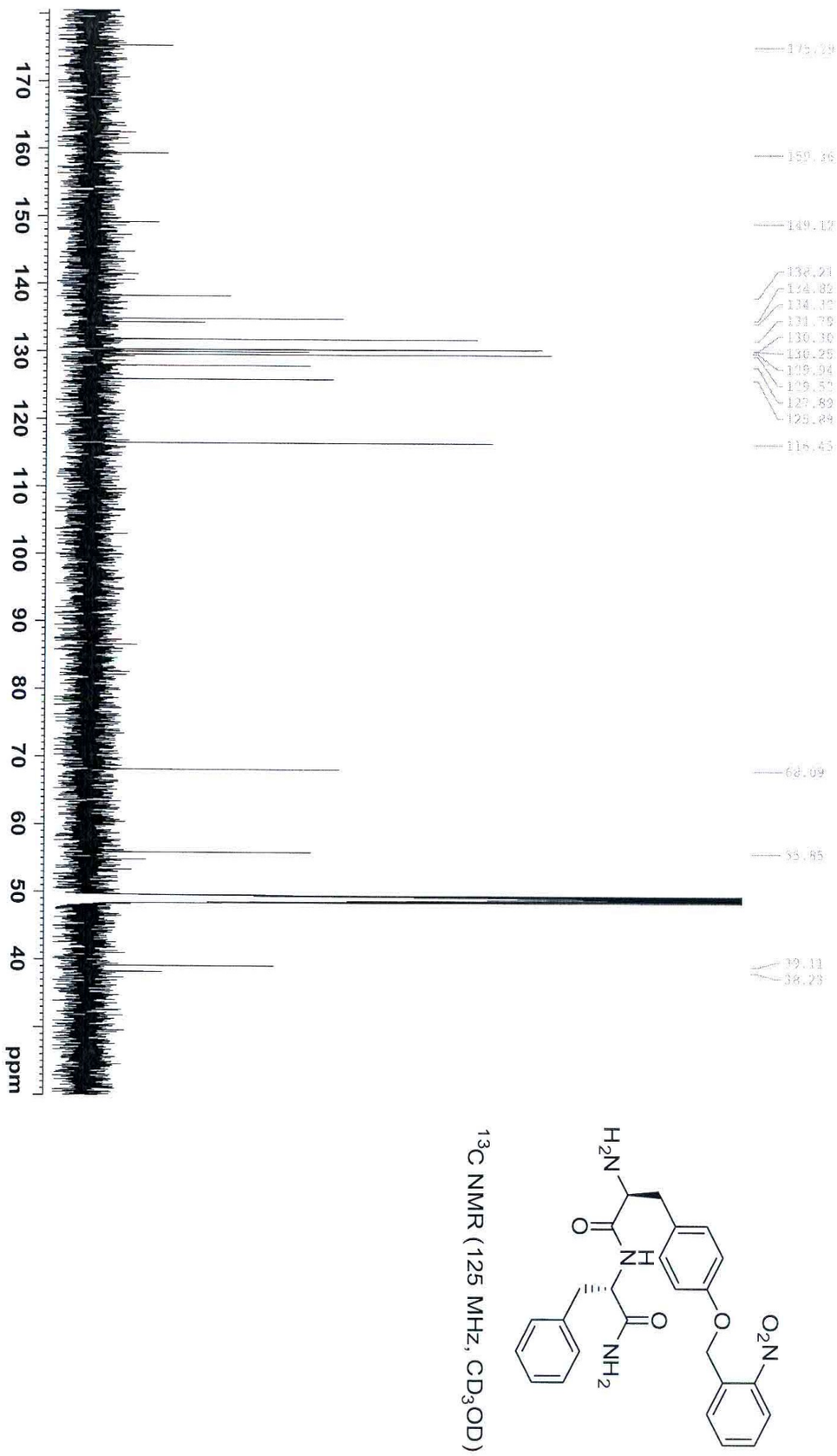
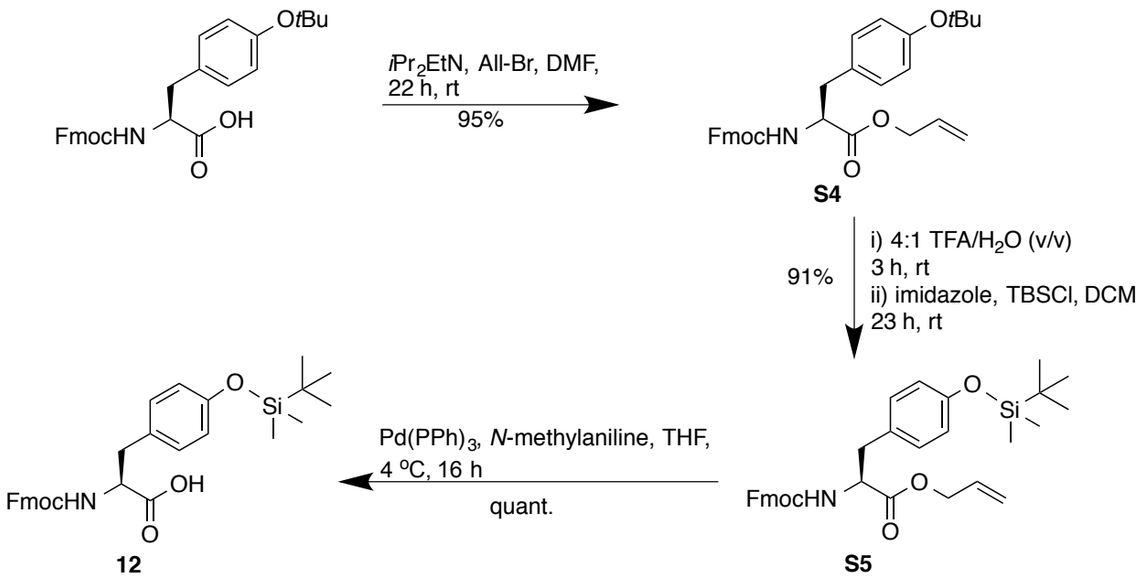


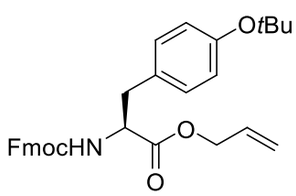
Figure 6. ^{13}C NMR spectrum of compound **S3**

2.2 Synthesis of Fmoc-Tyr(TBS)-OH (6)



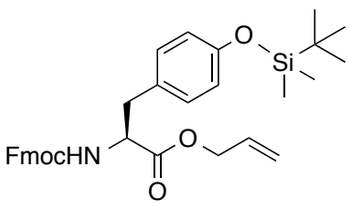
Scheme 3 Synthesis of Fmoc-Tyr(TBS)-OH (**12**)

(*S*)-allyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-(*tert*-butoxy)phenyl)propanoate (S4)



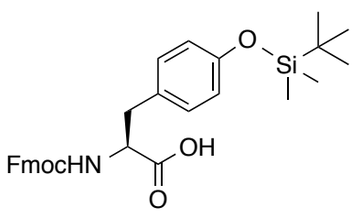
To a solution of Fmoc-Tyr(*t*Bu)-OH (2.50 g, 5.4 mmol, 1.0 equiv.) in DMF (5 mL) at 0 °C was added DIPEA dropwise (1.90 mL, 10.9 mmol, 2.0 equiv.) followed by allyl bromide (0.92 mL, 11.0 mmol, 2.0 equiv.). The reaction mixture was stirred for 16 h at room temperature then quenched with water (100 mL) and extracted with EtOAc (5 × 20 mL). The organic fractions were combined, dried with $MgSO_4$, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (80:20 v/v, hexane:EtOAc) to afford the desired allyl ester **S4** (2.45 g, 90%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (d, 2H, *J* = 7.4 Hz), 7.56 (d, 2H, *J* = 7.4 Hz), 7.40 (t, 2H, *J* = 7.4 Hz), 7.30 (t, 2H, *J* = 7.4 Hz), 7.00 (d, 2H, *J* = 7.7 Hz), 6.89 (d, 2H, *J* = 7.7 Hz), 5.85 (ddt, 1H, *J* = 16.5, 10.8, 5.4 Hz), 5.32-5.22 (m, 3H, NH), 4.69-4.31 (m, 5H), 4.20 (t, 1H, *J* = 6.8 Hz), 3.07 (app s, 2H), 1.31 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.2, 155.5, 154.5, 143.7, 141.3, 131.4, 130.4, 129.8, 127.7, 127.0, 125.0, 124.1, 119.9, 119.0, 78.4, 66.9, 66.0, 54.9, 47.2, 37.7, 28.8; MS (ESI+) 499.93 $[M+H]^+$. These data are in agreement with those previously reported by Taleski *et al*.[3]

(*S*)-allyl 2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)propanoate (S5)



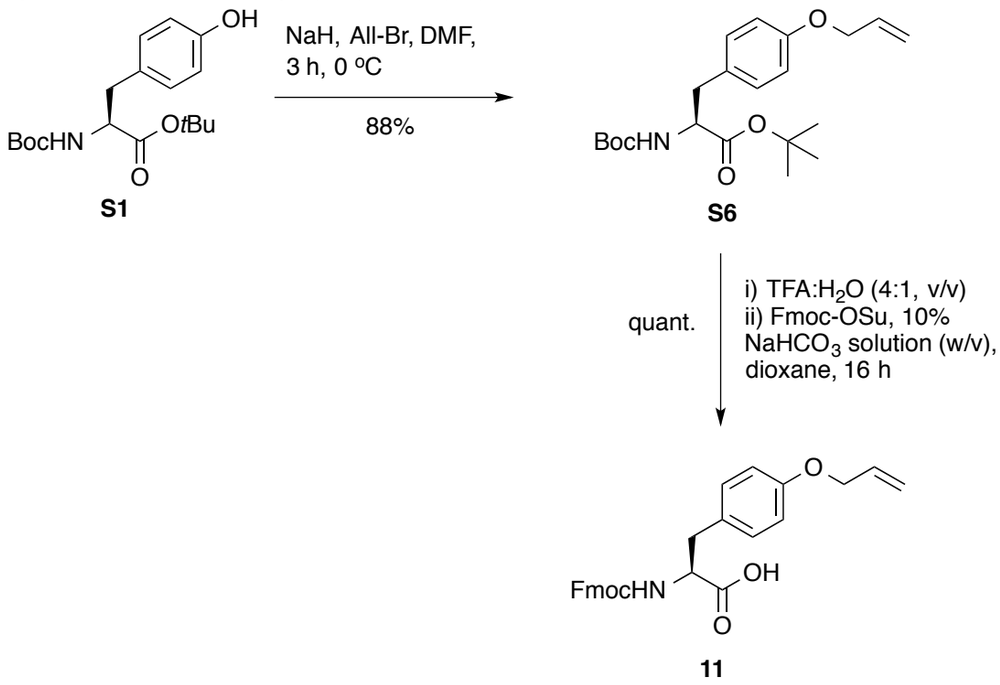
The allyl ester **S4** (1.25 g, 2.3 mmol, 1.0 equiv.) was added to a solution of TFA and water (10 mL, 4:1 v/v). The reaction was stirred for 3 h before the solvent was removed under reduced pressure. The resulting orange solid was redissolved in CH_2Cl_2 (12 mL) followed by the addition of imidazole (490 mg, 7.2 mmol, 3.2 equiv.) at 0 ^oC. After 10 min, TBSCl (850 mg, 5.6 mmol, 2.5 equiv.) was added at 0 ^oC and the reaction mixture was warmed to room temperature and stirred for 23 h. The reaction was quenched with water (200 mL) and extracted with CH_2Cl_2 (3 x 200 mL). The organic fractions were combined, dried with Na_2SO_4 and the solvent was removed under reduced pressure. The crude mixture was purified by gradient flash chromatography (from 5:1 to 2:1 v/v, hexane:EtOAc) to afford the allyl ester **S5** (1.25 g, 91%) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J* = 7.5 Hz), 7.55 (d, 2H, *J* = 4.5 Hz), 7.38 (t, 2H, *J* = 7.5 Hz), 7.29 (t, 2H, *J* = 7.5 Hz), 6.95 (d, 2H, *J* = 8.0 Hz), 6.73 (d, 2H, *J* = 8.6 Hz), 5.92-5.78 (m, 1H), 5.32-5.22 (m, 3H), 4.67-4.30 (m, 5H), 4.19 (t, 1H, *J* = 6.9 Hz) 3.05 (s, 2H), 0.96 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4, 155.7, 154.9, 143.9, 141.5, 131.6, 130.5, 128.4, 127.8, 127.2, 125.2, 120.3, 120.1, 119.2, 67.1, 66.2, 55.1, 47.3, 37.7, 25.8, 18.3, -4.3; MS (ESI+) 1136.91 $[2M+Na]^+$. These data are in agreement with those previously reported by Taleski *et al*.[3]

(*S*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)amino)-3-(4-((*tert*-butyldimethylsilyl)oxy)phenyl)propanoic acid (12)



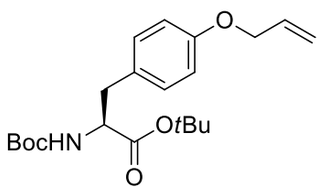
To a solution of the allyl ester **S5** (470 mg, 0.84 mmol, 1.0 equiv.) in THF (20 mL) was added *N*-methylaniline (0.92 mL, 8.50 mmol, 10.0 equiv.) and $Pd(PPh_3)_4$ (12 mg, 0.01 mmol, 0.1 equiv.). The reaction mixture was stirred at room temperature for 1 h and then left at 4 ^oC for 16 h. The solvent was removed under reduced pressure and the crude mixture was purified by gradient flash chromatography (90:10:2 to 67:33:2 v/v/v, hexane:EtOAc:AcOH) to afford the carboxylic acid **12** (430 mg, quant.) as a cream-coloured solid. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J* = 7.3 Hz), 7.54 (d, 2H, *J* = 4.8 Hz), 7.41-7.26 (m, 4H), 6.99 (d, 2H, *J* = 8.0 Hz), 6.76 (d, 2H, *J* = 8.0 Hz), 5.17 (d, 1H, *J* = 8.0 Hz), 4.65 (q, 1H, *J* = 5.9 Hz), 4.46-4.33 (m, 2H), 4.20 (t, 1H, *J* = 6.6 Hz), 3.17-3.00 (m, 2H), 0.96 (s, 9H), 0.17 (s, 6H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 176.1, 155.7, 154.9, 143.6, 141.3, 130.3, 128.0, 127.7, 127.1, 125.0, 120.2, 120.0, 67.1, 54.6, 47.1, 37.0, 25.7, 18.2, -4.4; MS (ESI+) *m/z* 517.9 $[M+H]^+$; HRMS (ESI+) *m/z* calcd. for $C_{30}H_{35}NO_5SiNa^+$ $[M+Na]^+$ 540.2177, found 540.2178. These data are in agreement with those previously reported by Taleski *et al*.[3]

2.3 Synthesis of Fmoc-Tyr(All)-OH (11)



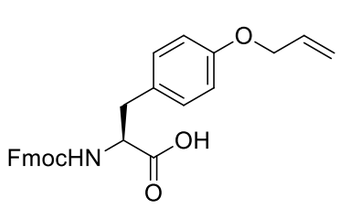
Scheme 4 Synthesis of Fmoc-Tyr(All)-OH (**11**)

***tert*-Butyl 3-(4-(allyloxy)phenyl)-2-(*tert*-butoxycarbonylamino)propanoate (S6)**



To a cooled suspension of NaH (72 mg of 60% wt suspension, 1.80 mmol) in DMF (1.5 mL) at 0 °C was added dropwise a solution of compound **S1** (550 mg, 1.60 mmol) in DMF (3 mL), and the resulting mixture was stirred at 0 °C for 3 h. Allyl bromide (0.26 mL, 3.10 mmol) was then added and stirring was continued for a further 3 h. The mixture was diluted with brine (20 mL), extracted with Et_2O (5 x 20 mL), then dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by column chromatography (9:1 v/v, hexane:EtOAc) to afford the desired allyl ether **S6** (540 mg, 88%) as a colorless liquid. ^{1}H NMR (300 MHz , $CDCl_3$) δ 7.07 (d, 2H, *J* = 8.4 Hz), 6.83 (d, 2H, *J* = 8.8 Hz), 6.04 (1H, ddt, *J* = 17.2, 10.6, 5.3 Hz), 5.40 (dd, 1H, *J* = 17.2, 1.8 Hz), 5.27 (dd, 1H, *J* = 10.6, 1.5 Hz), 4.97 (d, 1H, *J* = 8.4 Hz), 4.51 (td, 2H, *J* = 1.6, 5.2 Hz), 4.55-4.35 (m, 1H), 2.99 (d, 2H, *J* = 6.2 Hz), 1.42 (s, 9H), 1.41 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 157.5, 155.1, 133.3, 130.5, 128.6, 117.5, 114.6, 81.9, 79.6, 68.8, 54.9, 37.6, 28.3, 28.0; MS (ESI+) *m/z* 377.8 $[M+H]^+$. These data are in agreement with those previously reported by Taleski *et al*.[3]

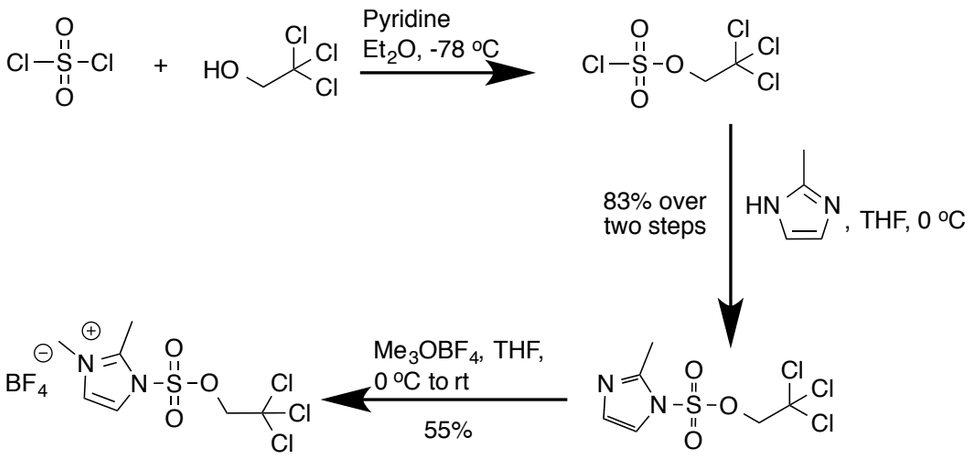
2-([(9H-fluoren-9-yl)methoxy]carbonylamino)-3-(4-(allyloxy)phenyl)propanoic acid (11)



Compound **S6** (400 mg, 1.10 mmol) was added to a solution of TFA/water (4:1 v/v, 10 mL) and the mixture was stirred for 3 h then concentrated under reduced pressure. The residue was suspended in 10%

aqueous Na_2CO_3 (7.6 mL, w/v) solution and treated with a solution of Fmoc-OSu (710 mg, 2.10 mmol) in dioxane (3.8 mL). The reaction mixture was stirred for 16 h at room temperature. The mixture was then diluted with H_2O (50 mL) and washed with Et_2O (2 x 50 mL). The aqueous layer was acidified to pH 3 (1.0 M HCl) and extracted with EtOAc (2 x 50 mL). The combined EtOAc fractions were then dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by column chromatography (92.5:7.5:2 v/v/v, CH_2Cl_2:MeOH:AcOH) to afford the amino acid **11** (620 mg, quant.) as a white solid.^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, 2H, *J* = 7.4 Hz), 7.57-7.53 (m, 2H), 7.40 (t, 2H, *J* = 7.3 Hz), 7.28 (t, 2H, *J* = 7.4 Hz), 7.05 (d, 2H, *J* = 7.7 Hz), 6.84 (d, 2H, *J* = 7.8 Hz), 6.08-5.97 (m, 1H), 5.40-5.18 (m, 3H), 4.65-4.30 (m, 5H), 4.18 (t, 1H, *J* = 6.3 Hz), 3.15-3.00 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.8, 157.8, 155.8, 143.7, 141.3, 133.2, 130.3, 127.7, 127.5, 127.1, 125.0, 120.0, 117.7, 114.9, 68.8, 67.1, 54.7, 47.1, 36.9; MS (ESI+) *m/z* 443.8 $[M+H]^+$; HRMS (ESI+) *m/z* calcd. for $C_{27}H_{25}NO_5Na^+$ $[M+Na]^+$ 466.1625, found 466.1626. These data are in agreement with those previously reported by Taleski *et al.*[3] and Loffet *et al.*[4]

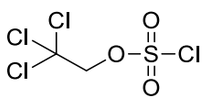
3 Synthesis of Trichloroethyl sulfating reagent 1



Scheme 5 Synthesis of imidazolium sulfating reagent **1**[5]

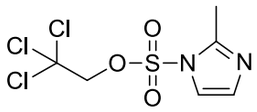
Imidazolium sulfating reagent **1 was synthesized using the conditions previously described by Taylor and co-workers.*[5]

2,2,2-trichloroethyl sulfochloridate (S7)



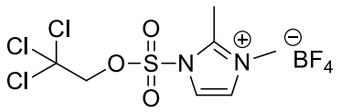
Sulfuryl chloride (7.2 mL, 89 mmol, 1.0 equiv.) was added dropwise to a solution of pyridine (7.0 mL, 89 mmol, 1.0 equiv.) and trichloroethanol (8.6 mL, 89 mmol, 1.0 equiv.) in Et_2O (100 mL) at -78 °C. The reaction was stirred for 1 h at -78 °C and then warmed to room temperature for 3 h. The reaction mixture was filtered and the filtrate was washed with ice-cold Et_2O (100 mL) and dried with $MgSO_4$. The solvent was evaporated to afford **S7** (19.0 g, 86%) as a colorless oil. The crude oil was used directly in the next reaction. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.92 (s, 2H).

2,2,2-trichloroethyl 2-methyl-1*H*-imidazole-1-sulfonate (S8)



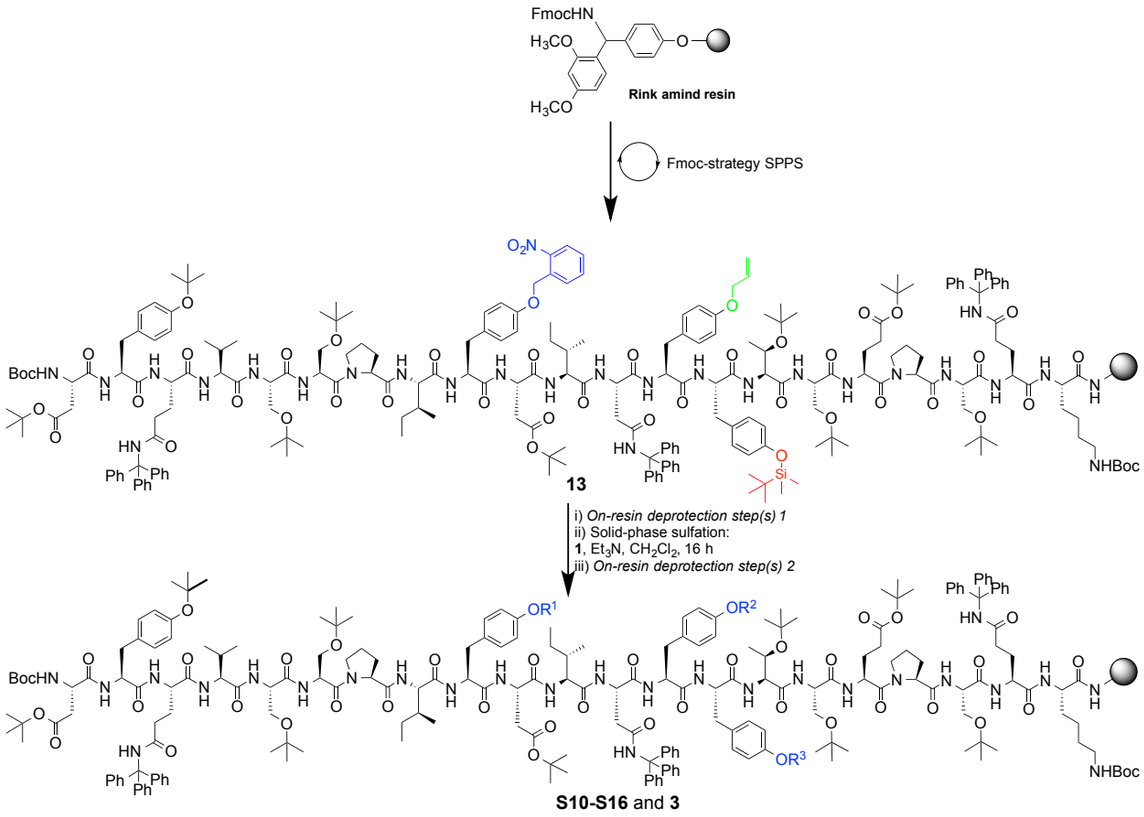
A solution of **S7** (5.0 g, 20 mmol, 1.0 equiv.) in THF (50 mL) was added dropwise to a solution of 2-methylimidazole (5.9 g, 72 mmol, 3.6 equiv.) in THF (40 mL) at 0 ^{o}C. The reaction was stirred for 1 h then warmed to room temperature over 1 h. The reaction mixture was filtered and washed with THF (50 mL). The filtrate was concentrated and purified by flash chromatography (3:1 v/v, hexane:EtOAc) to afford **S8** (5.0 g, 85 %) as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (app s, 1H), 6.96 (app s, 1H), 4.65 (s, 2H), 2.68 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 146.4, 128.1, 120.0, 91.7, 79.9, 14.8; MS (ESI+) 292.7 [M+H^{+}]. These data are in agreement with those previously reported by Taylor and co-workers.[5]

2,3-Dimethyl-1-(2,2,2-trichloroethoxysulfonyl)-1H-imidazol-3-ium tetrafluoroborate (1)



A solution of sulfonate **S8** (2.0 g, 6.8 mmol, 1.0 equiv.) was added dropwise to a suspension of $(Me)_3OBF_4$ (1.0 g, 6.8 mmol, 1.0 equiv.) in THF (20 mL) at 0 ^{o}C. The resulting mixture was warmed to room temperature and stirred overnight. After 24 h, the mixture was concentrated under reduced pressure then triturated with CH_2Cl_2/Et_2O (v/v, 1:4) to obtain the desired imidazolium salt **1** (1.5 g, 55%) as a white solid. m.p. = 142-144 °C; IR (film) 1432, 1232, 1208, 1058 cm^{-1}; ^{1}H NMR (400 MHz, MeOD) δ 8.11 (app s, 1H), 7.77 (app s, 1H), 5.36 (s, 2H), 3.94 (s, 3H), 2.94 (s, 3H); ^{13}C NMR (100 MHz, MeOD) δ 150.0, 124.9, 122.1 93.0, 83.4, 36.7, 11.8; MS (ESI+) m/z 306.7 [M-BF_4]$^{+}$. These data are in agreement with those previously reported by Taylor and co-workers.[5]

4 Synthesis of peptide 3 and sulfopeptides (S9-S15) *via* Fmoc-Strategy SPPS



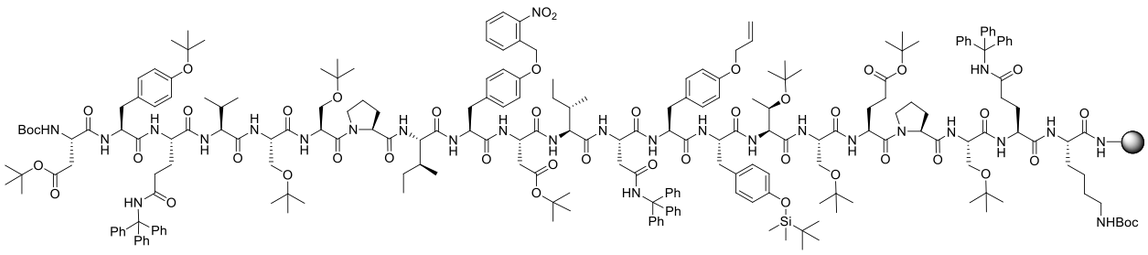
Peptide	*On resin deprotection step(s) 1**	*On resin deprotection step(s) 2**	R^1 (Tyr-10)	R^2 (Tyr-14)	R^3 (Tyr-15)
S10	A, B, C	-	SO_3TCE	SO_3TCE	SO_3TCE
3	A, B	-	H	H	H
S11	A	B	SO_3TCE	H	H
S12	B	A	H	SO_3TCE	H
S13	C	A, B	H	H	SO_3TCE
S14	A, B	-	SO_3TCE	SO_3TCE	H
S15	A, C	B	SO_3TCE	H	SO_3TCE
S16	B, C	A	H	SO_3TCE	SO_3TCE

* Deprotection conditions are described in Section 1.2.

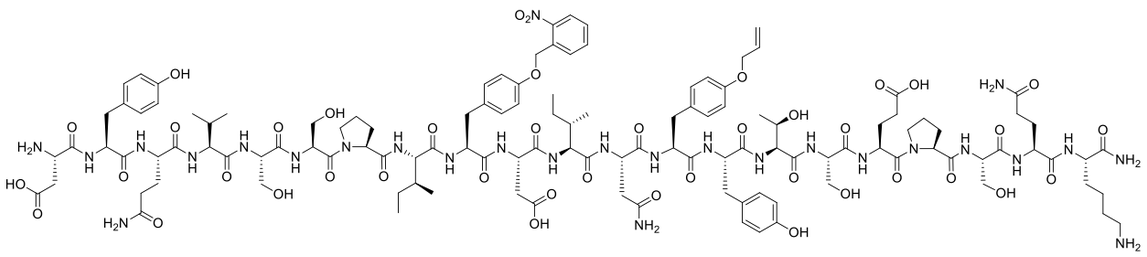
Scheme 6 Divergent solid-phase sulfation strategy employing three orthogonal protecting groups.

4.1 Synthesis of resin-bound and side chain protected CCR5 N-terminal fragment (2-22)

Boc-Asp(O*t*Bu)-Tyr(*t*Bu)-Gln(Trt)-Val-Ser(*t*Bu)-Ser(*t*Bu)-Pro-Ile-Tyr(O*o*-Nb)-Asp(O*t*Bu)-Ile-Asn(Trt)-Tyr(OAll)-Tyr(OTBS)-Thr(*t*Bu)-Ser(*t*Bu)-Glu(O*t*Bu)-Pro-Ser(*t*Bu)-Gln(Trt)-Lys(Boc)-Rink amide resin (13)

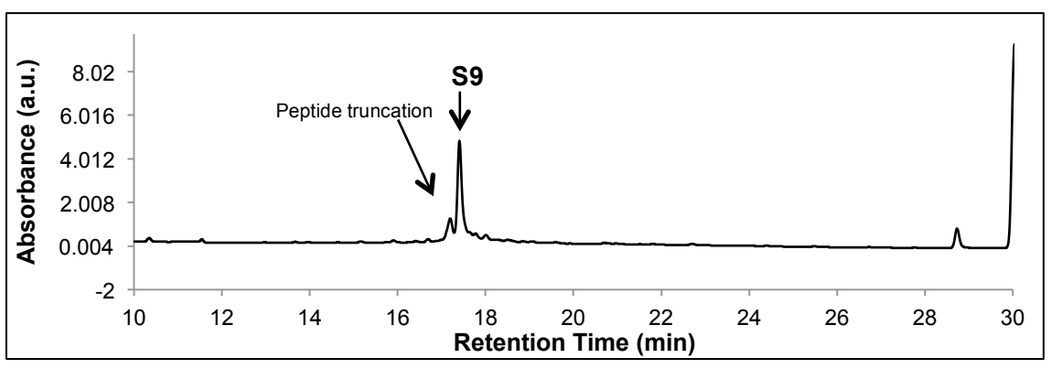


Resin bound peptide **13** was prepared by Fmoc-strategy SPPS on Rink amide resin outlined in the general procedures (1.1). The resin was split into eight 12.5 µmol batches (based on the original resin loading) for selective deprotection followed by solid-phase sulfation to afford protected resin-bound sulfoforms **14-21**. A minicleavage of resin-bound **13** led to peptide **S9** where the *o*-Nb and allyl ether protecting groups remained intact but all other side chain protecting groups including the TBS ether were cleaved.



S9

A)



B)

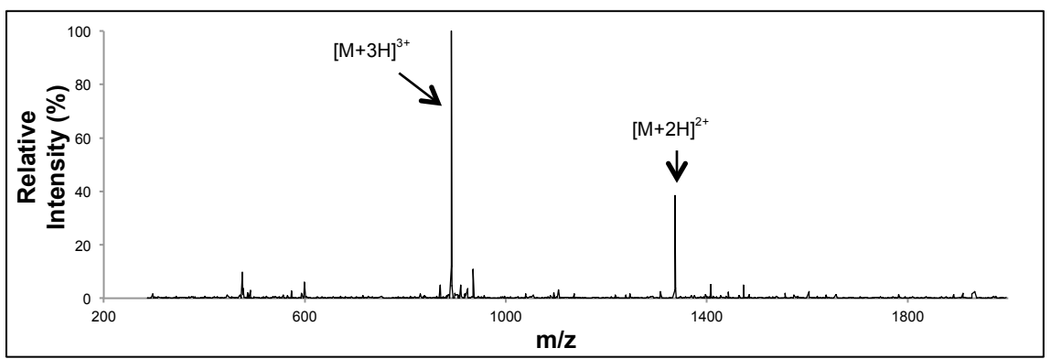
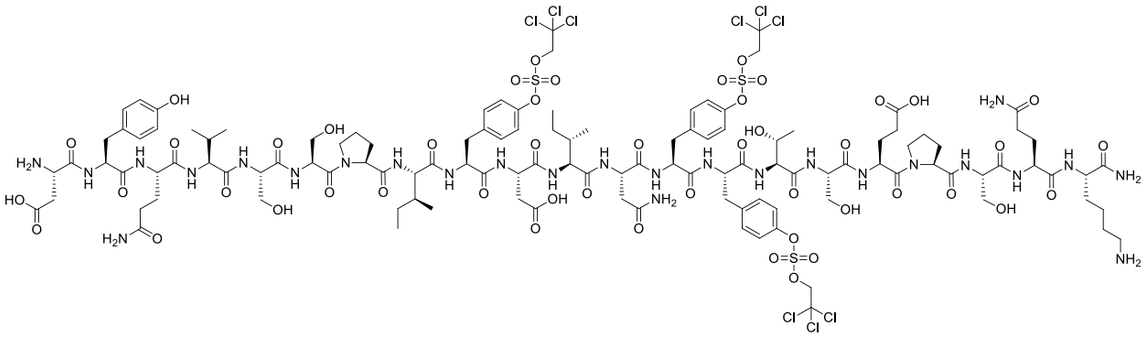


Figure 7. A) LC-MS analysis of crude **S9** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S9**: Calculated mass $[M+2H]^{2+}$: 1336.6, $[M+3H]^{3+}$: 891.4; Mass found 1337.1 $[M+2H]^{2+}$, 891.7 $[M+3H]^{3+}$.

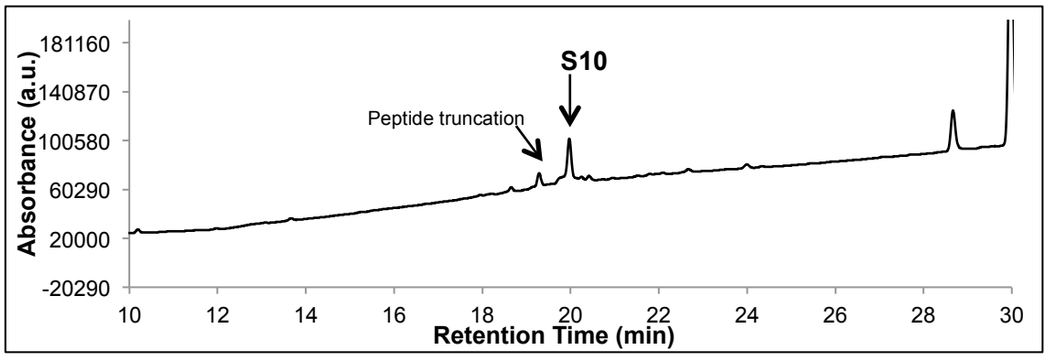
4.2 Synthesis of trichloroethyl sulfoester-derived N-terminal CCR5 peptides

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OSO_3TCE)-Asp-Ile-Asn-Tyr(OSO_3TCE)-Tyr(OSO_3TCE)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (S10)



The *o*-nitrobenzyl, allyl and TBS protecting groups of resin bound peptide **13** were deprotected according to deprotection conditions A, B and C. The peptide was then triply sulfated on resin with **1** (general procedure 1.3) followed by acidic deprotection and cleavage of the peptide from the resin to provide the crude TCE-protected sulfoester-derived peptide **S10** (general procedure 1.4).

A)



B)

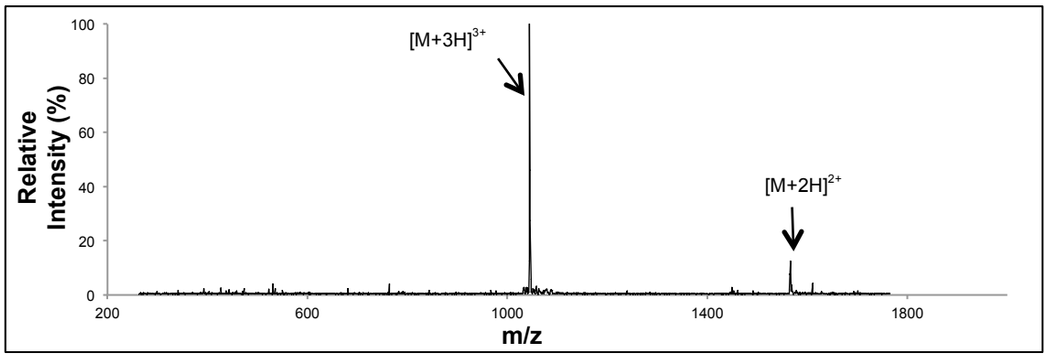
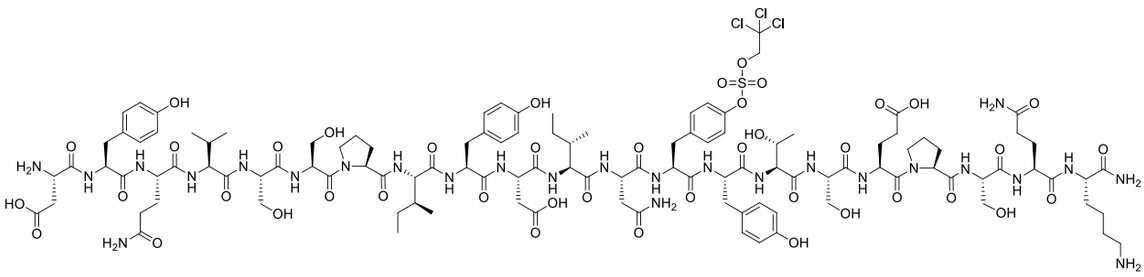
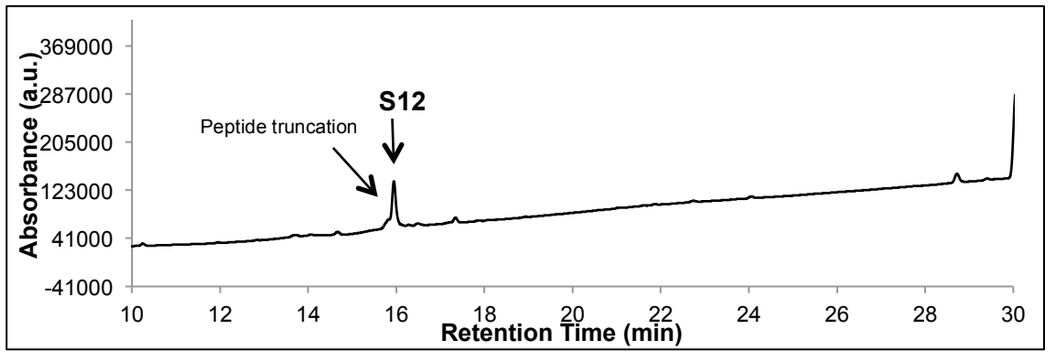


Figure 8. A) LC-MS of crude **S10** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S10**: Calculated mass $[M+2H]^{2+}$:1565.9, $[M+3H]^{3+}$: 1044.3; Mass found 1565.3 $[M+2H]^{2+}$, 1044.5 $[M+3H]^{3+}$.

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr(OSO_3TCE)-Tyr(OH)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (S12)



The allyl protecting group of resin bound peptide **13** was deprotected according to deprotection conditions B. The peptide was then sulfated on resin with **1** (general procedure 1.3). The *o*-nitrobenzyl protecting group was subsequently removed according to deprotection conditions A followed by acidic deprotection and cleavage of the peptide from the resin to provide the crude TCE-protected sulfoester-derived peptide **S12** (general procedure 1.4).



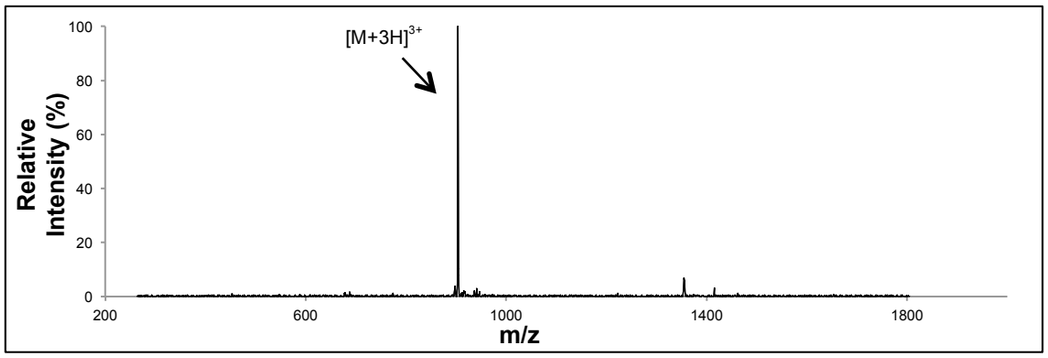
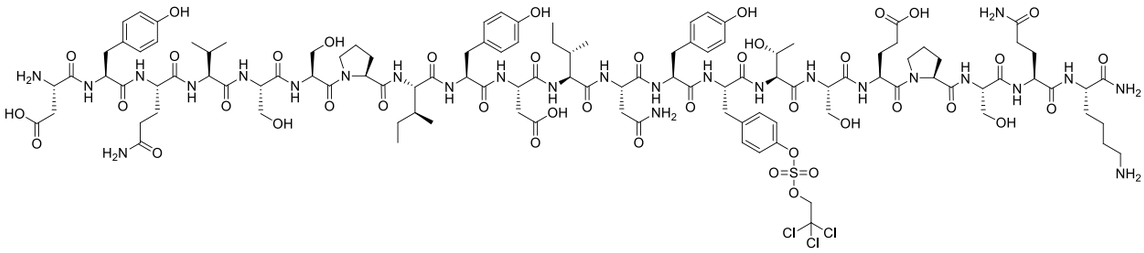
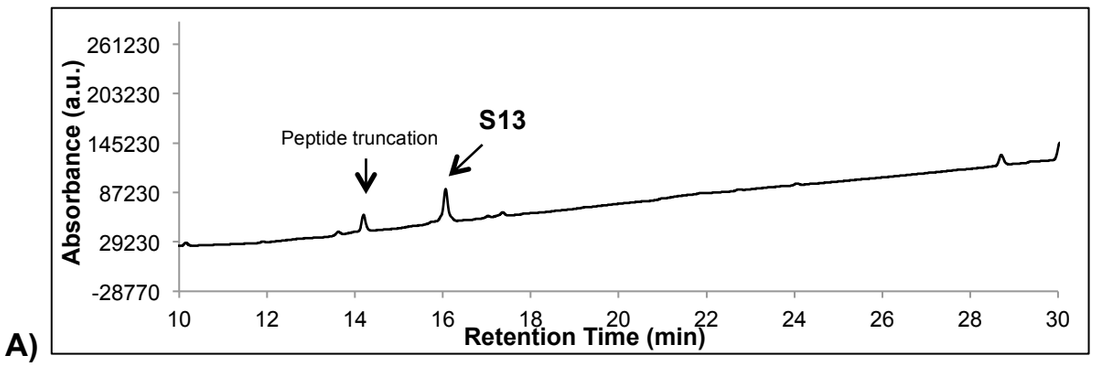


Figure 9. A) LC-MS of **S12** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S12**: Calculated mass $[M+3H]^{3+}$: 903.0; Mass found: 903.8 $[M+3H]^{3+}$.

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr(OH)-Tyr(OSO_3TCE)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (S13)



The TBS protecting group of resin bound peptide **13** was deprotected according to deprotection conditions C. The peptide was then sulfated on resin with **1** (general procedure 1.3). The *o*-nitrobenzyl and allyl protecting groups were removed according to deprotection conditions A and B followed by acidic deprotection and cleavage of the peptide from the resin to provide the crude TCE-protected sulfoester-derived peptide **S13** (general procedure 1.4)



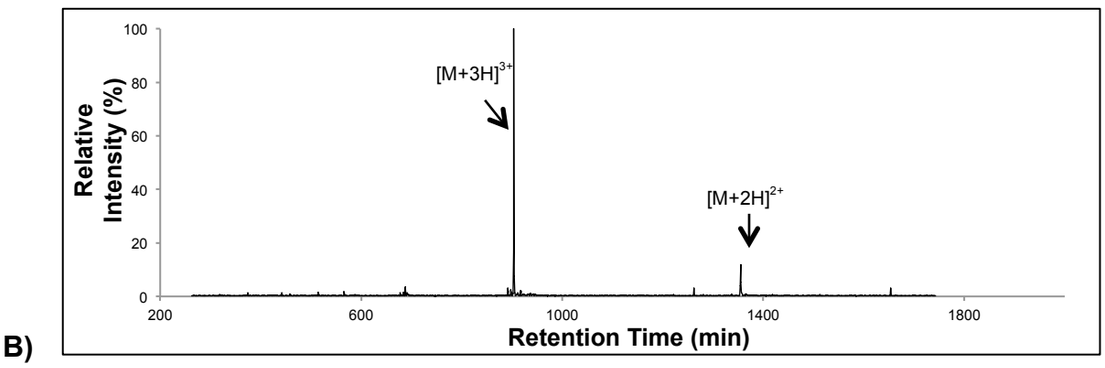
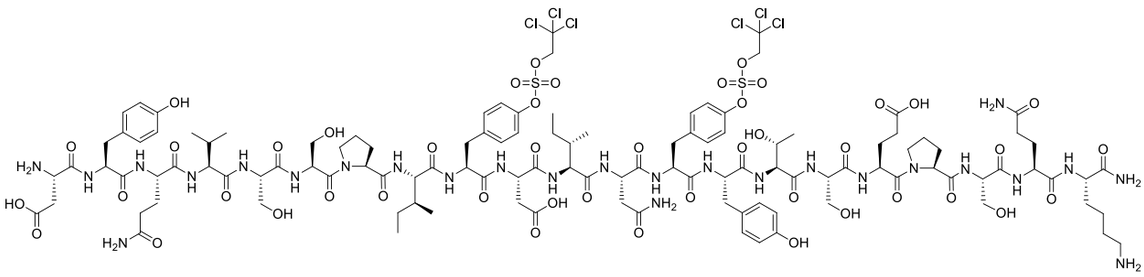
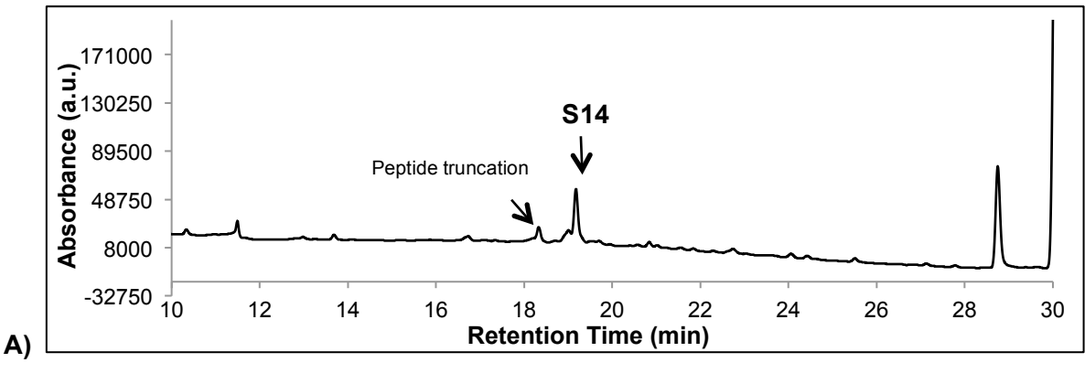


Figure 10. A) LC-MS of **S13** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S13**: Calculated mass $[M+2H]^{2+}$: 1354.0, $[M+3H]^{3+}$: 903.0; Mass found 1355.2 $[M+2H]^{2+}$, 903.7 $[M+3H]^{3+}$.

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OSO$_3$TCE)-Asp-Ile-Asn-Tyr(OSO$_3$TCE)-Tyr(OH)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH$_2$ (S14)



The *o*-nitrobenzyl and allyl protecting groups of resin bound peptide **13** were deprotected according to deprotection conditions A and B. The peptide was then doubly sulfated on resin with **1** (general procedure 1.3) followed by acidic deprotection and cleavage of the peptide from the resin to provide the crude TCE-protected sulfoester-derived peptide **S14** (general procedure 1.4).



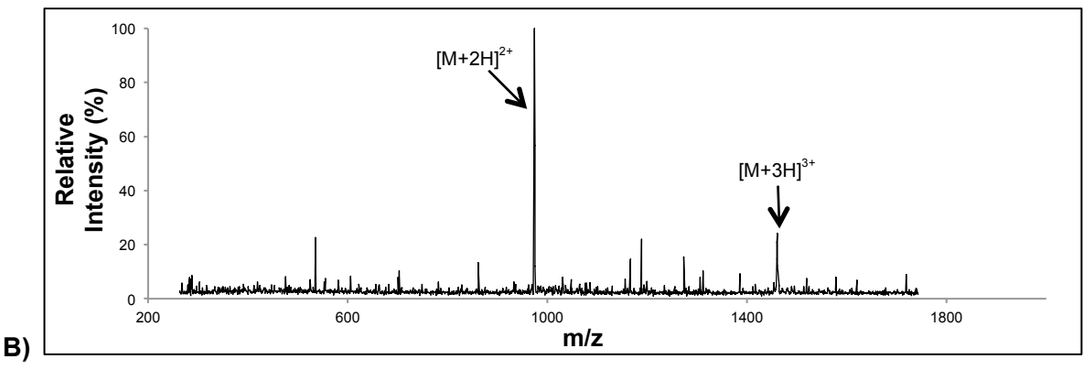
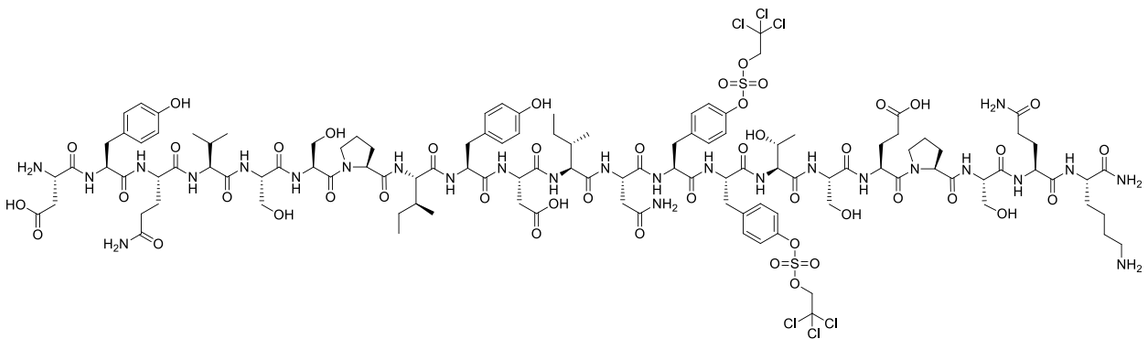
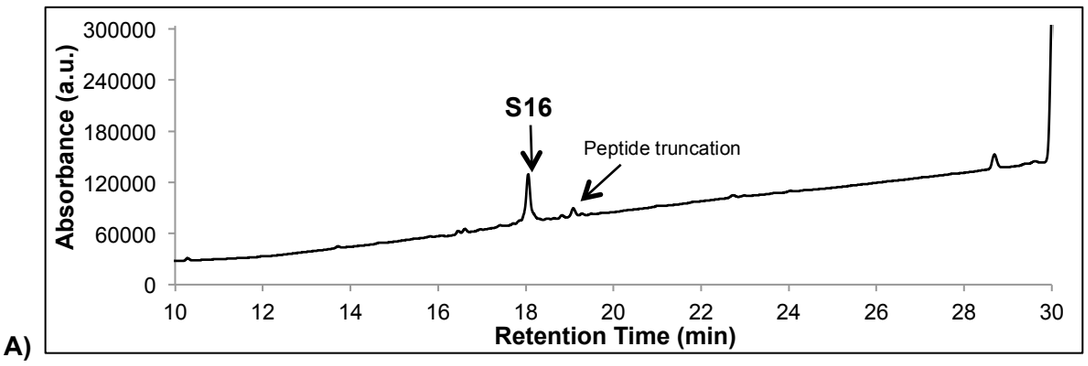


Figure 11. A) LC-MS of crude **S14** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S14**: Calculated mass $[M+2H]^{2+}$: 1460.0, $[M+3H]^{3+}$: 973.6; Mass found 1460.3 $[M+2H]^{2+}$, 974.0 $[M+3H]^{3+}$.

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr(OSO_3TCE)-Tyr(OSO_3TCE)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (S16)



The allyl and TBS protecting groups of resin bound peptide **13** were deprotected according to deprotection conditions B and C. The peptide was then doubly sulfated on resin with **1** (general procedure 1.3). The *o*-nitrobenzyl protecting group was subsequently removed according to deprotection conditions A followed by acidic deprotection and cleavage of the peptide from the resin to provide the crude TCE-protected sulfoester-derived peptide **S16** (general procedure 1.4).



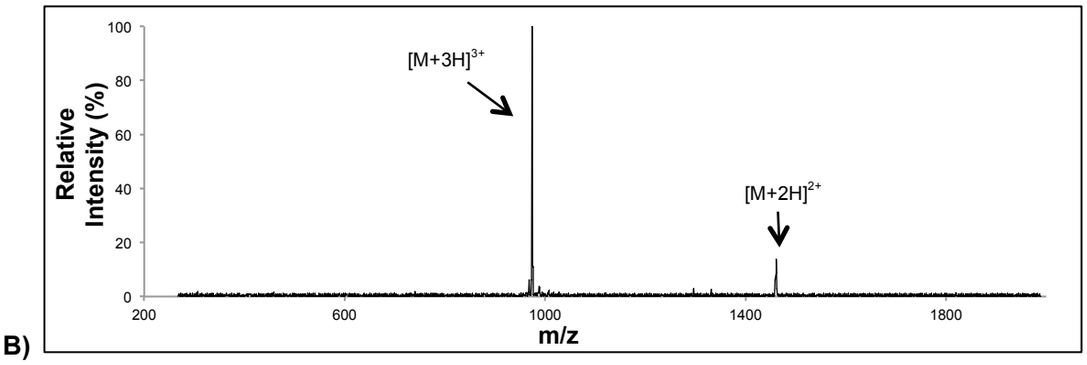
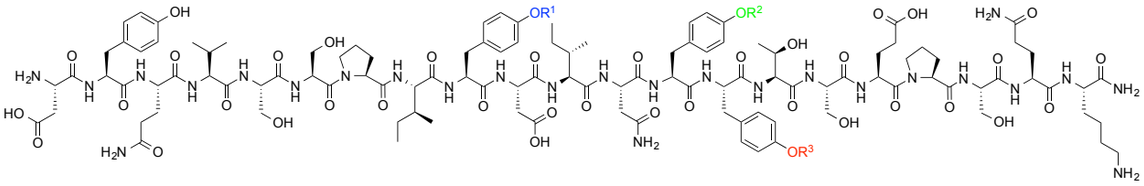


Figure 12. A) LC-MS of crude **S16** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S16**: Calculated mass $[M+2H]^{2+}$:1460.0, $[M+3H]^{3+}$: 973.7; Mass found 1460.6 $[M+2H]^{2+}$, 974.4 $[M+3H]^{3+}$.

4.3 Synthesis of CCR5(2-22) N-terminal fragments 2-9

Peptide **S9-S16**

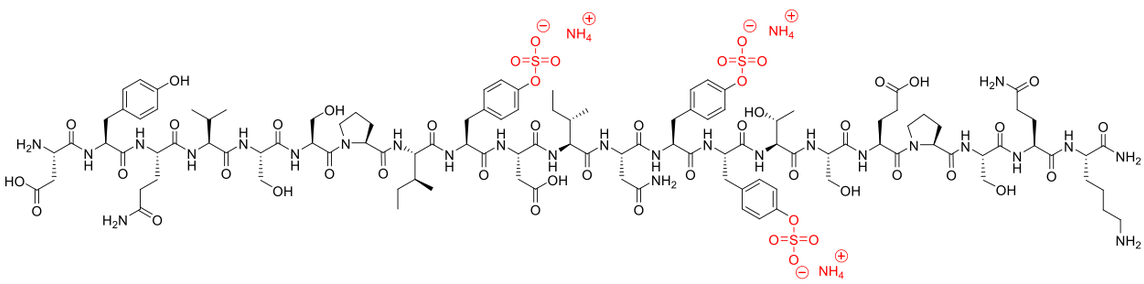
H_2, $Pd(OH)_2/C$, Et_3N,
water/MeOH (1:1, v/v), H_2, 4 h;
RP-HPLC purification (0.1 M
NH_4OAc-MeCN)



Sulfopeptide **2-9**

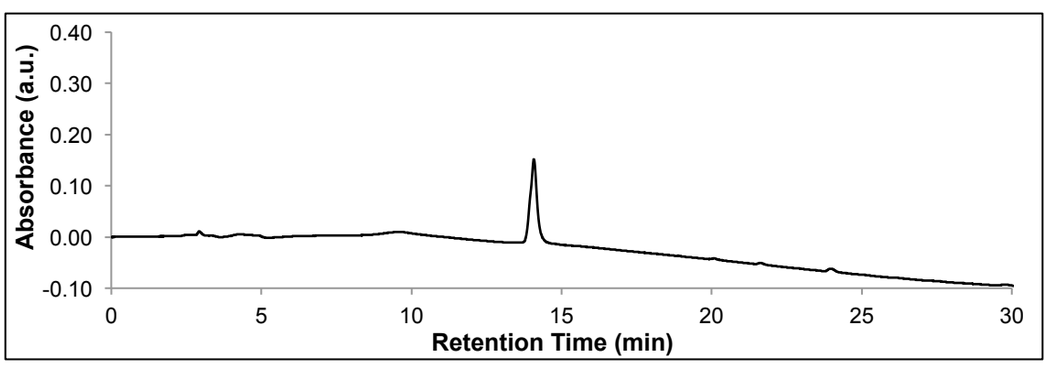
Sulfopeptide	R^1 (Tyr-10)	R^2 (Tyr-14)	R^3 (Tyr-15)	Yield
2	$SO_3^-NH_4^+$	$SO_3^-NH_4^+$	$SO_3^-NH_4^+$	28%
3	H	H	H	60%
4	$SO_3^-NH_4^+$	H	H	42%
5	H	$SO_3^-NH_4^+$	H	37%
6	H	H	$SO_3^-NH_4^+$	31%
7	$SO_3^-NH_4^+$	$SO_3^-NH_4^+$	H	29%
8	$SO_3^-NH_4^+$	H	$SO_3^-NH_4^+$	20%
9	H	$SO_3^-NH_4^+$	$SO_3^-NH_4^+$	34%

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr($SO_3^-NH_4^+$)-Asp-Ile-Asn-Tyr($SO_3^-NH_4^+$)-Tyr($SO_3^-NH_4^+$)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (2)

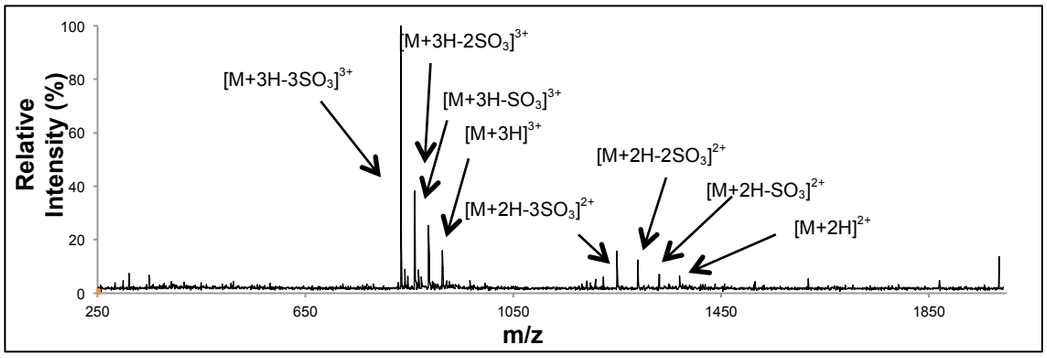


The crude TCE-protected sulfoester-derived peptide **S10** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by preparative reverse-phase HPLC (0 to 30% B over 60 min; ***Eluent B***) afforded pure sulfopeptide **2** as a white solid after lyophilization (9.8 mg, 28%).

A)



B)



C)

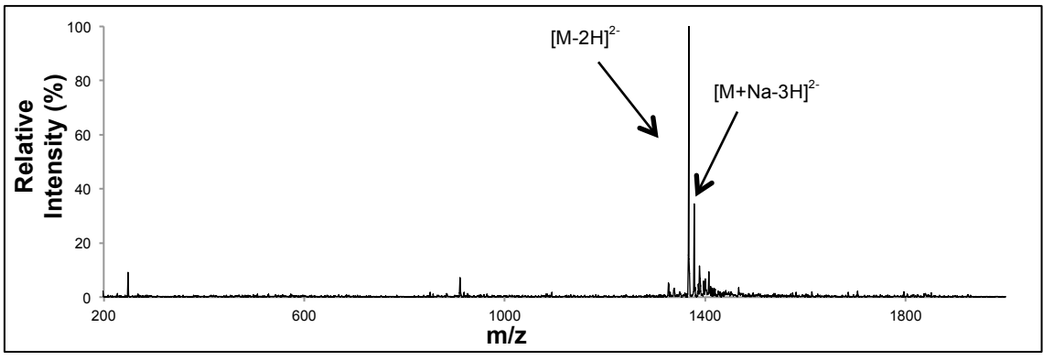


Figure 13. A) Analytical HPLC of sulfopeptide **2**: R_t 14.2 min (0-100% B over 30 min, ***Eluent B***, λ = 230 nm); **B)** Mass spectrum (ESI+) of sulfopeptide **2**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{48}S_3$ [M]: 2736.0, $[M+2H]^{2+}$: 1369.0, $[M+3H]^{3+}$: 913.0; Mass found 1369.6 $[M+2H]^{2+}$, 913.7 $[M+3H]^{3+}$; C) Mass spectrum (ESI-) of sulfopeptide **2**: Calculated for $[M-2H]^{2-}$: 1367.0, $[M+Na-3H]^{2-}$: 1378.0; Mass found 1367.2 $[M-2H]^{2-}$, 1378.0 $[M+Na-3H]^{2-}$.

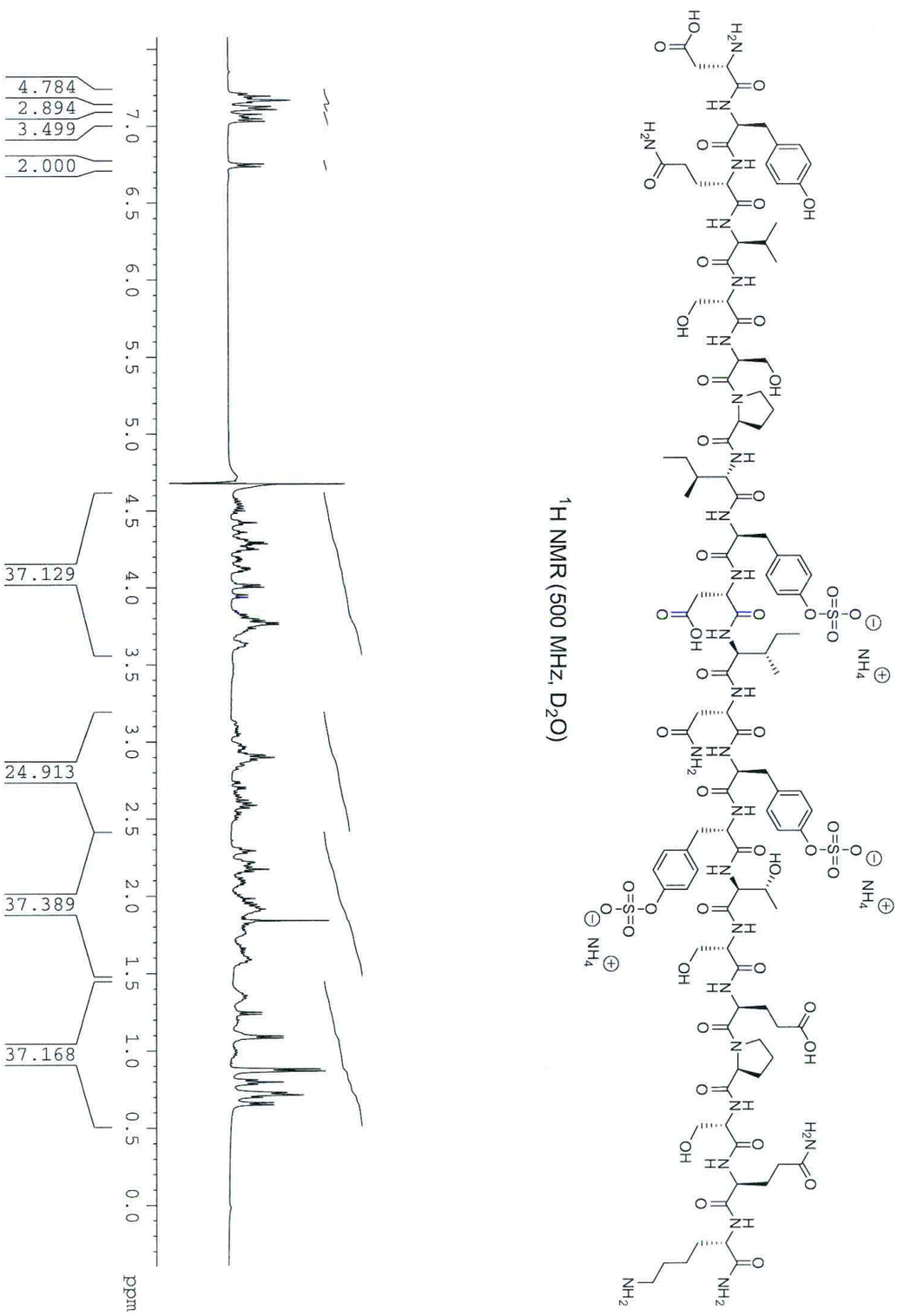
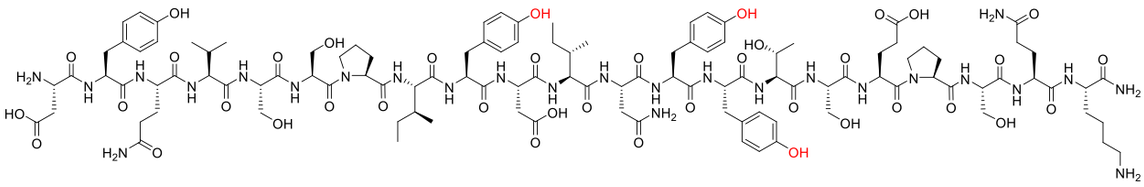


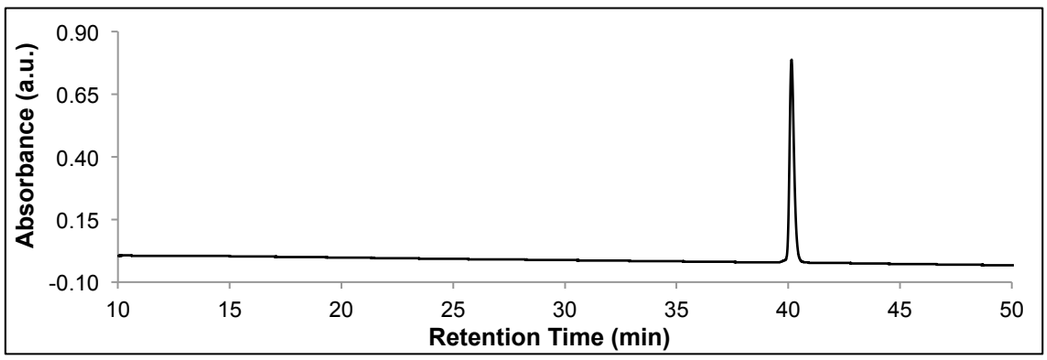
Figure 14. ^{1}H NMR spectrum of sulfopeptide **2** in D_2O with water suppression

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr(OH)-Tyr(OH)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (3)



Removal of the *o*-nitrobenzyl and allyl protecting groups (deprotection conditions A and B), followed by acidic deprotection and cleavage of the peptide **13** from the resin provided the crude peptide (general procedure 1.4). Purification by preparative reverse-phase HPLC (0 to 40% B over 60 min; ***Eluent B***) afforded pure peptide **3** as a white solid after lyophilization (18.7 mg, 60%).

A)



B)

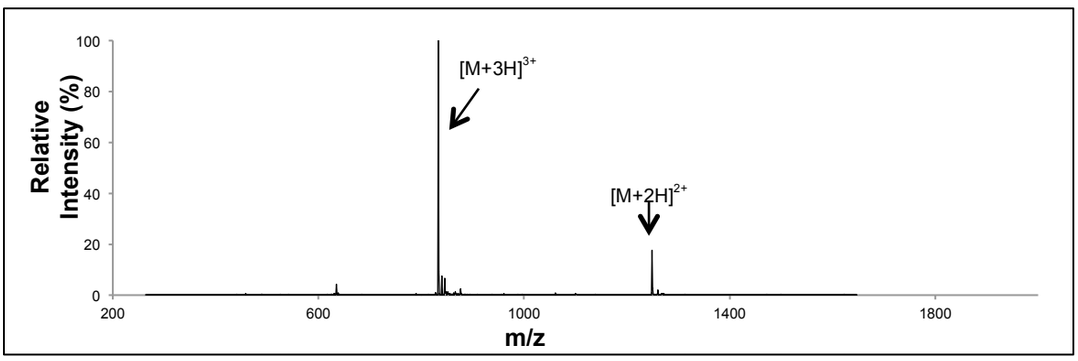
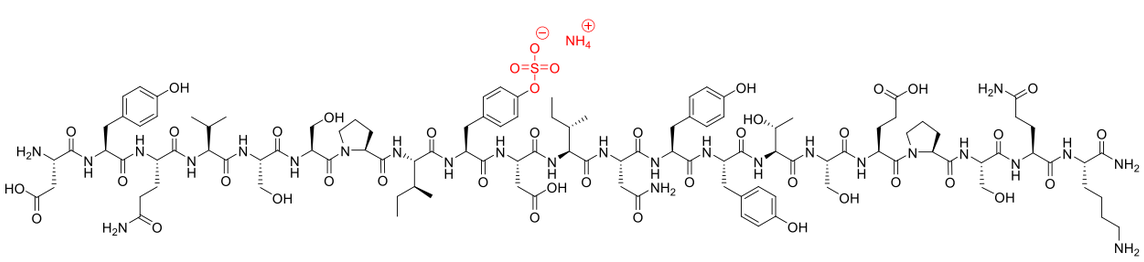


Figure 15. A) Analytical HPLC of peptide **3**: R_t 40.1 min (0-40% B over 60 min, ***Eluent B***, λ = 230 nm); **B)** Mass spectrum (ESI+) of peptide **3**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{39}$ [M]: 2496.2, $[M+2H]^{2+}$: 1249.1, $[M+3H]^{3+}$: 833.1; Mass found 1249.2 $[M+2H]^{2+}$, 833.2 $[M+3H]^{3+}$.

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr($OSO_3^-NH_4^+$)-Asp-Ile-Asn-Tyr(OH)-Tyr(OH)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (4)



The crude TCE-protected sulfoester-derived peptide **S11** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by

preparative reverse-phase HPLC (0 to 40% B over 60 min; ***Eluent B***) afforded pure sulfopeptide **4** as a white solid after lyophilization (13.6 mg, 42%).

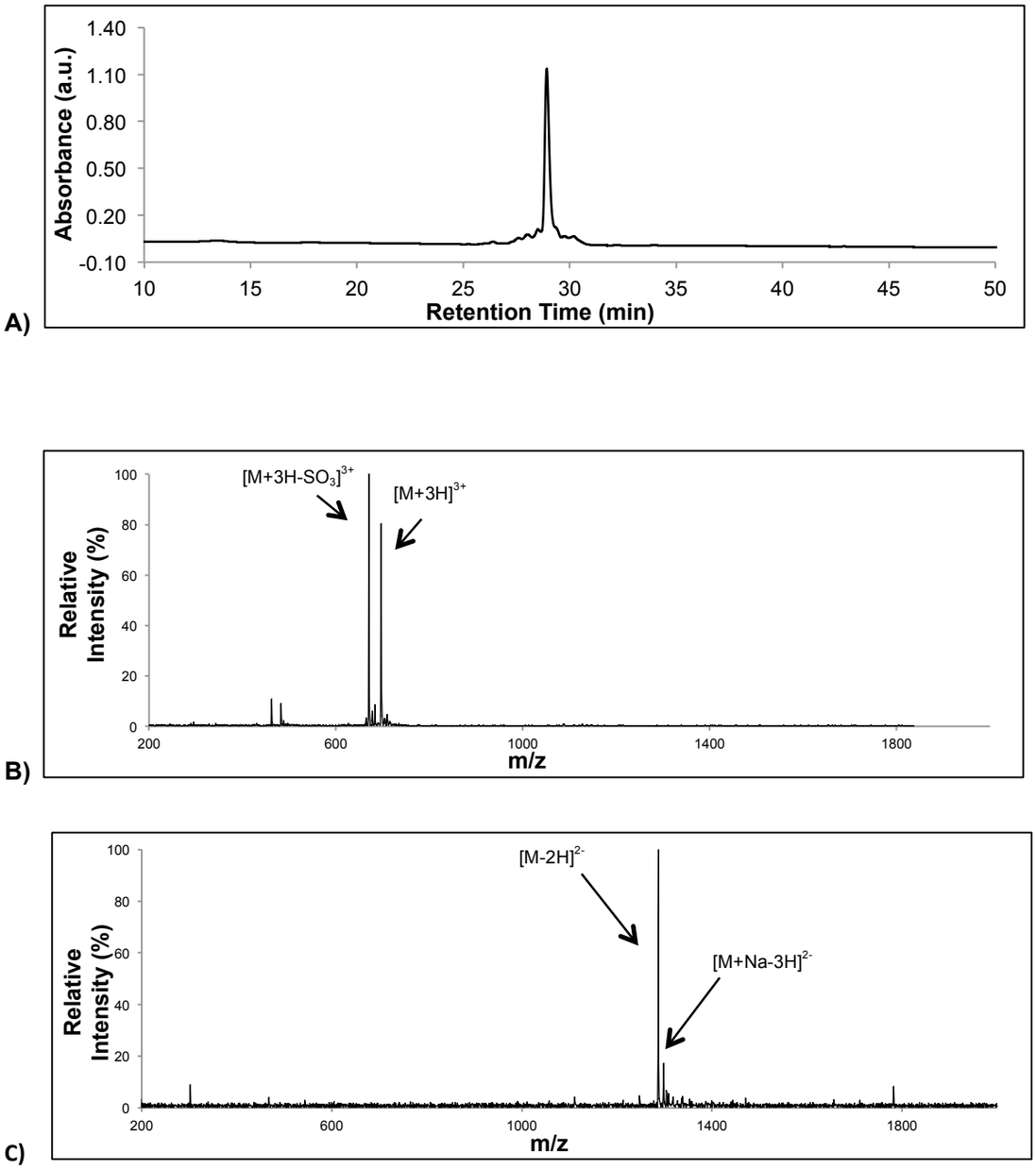


Figure 16. A) Analytical HPLC of sulfopeptide **4**: R_t 28.9 min (0-40% B over 60 min, ***Eluent B***, λ = 230 nm); **B)** Mass spectrum (ESI+) of sulfopeptide **4**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{42}S$ [M]: 2576.1, $[M+3H]^{3+}$: 859.7; Mass found 860.3 $[M+3H]^{3+}$; C) Mass spectrum (ESI-) of sulfopeptide **4**: Calculated for $[M-2H]^{2-}$: 1287.0, $[M+Na-3H]^{2-}$:1298.0; Mass found 1287.3 $[M-2H]^{2-}$, 1298.2 $[M+Na-3H]^{2-}$.

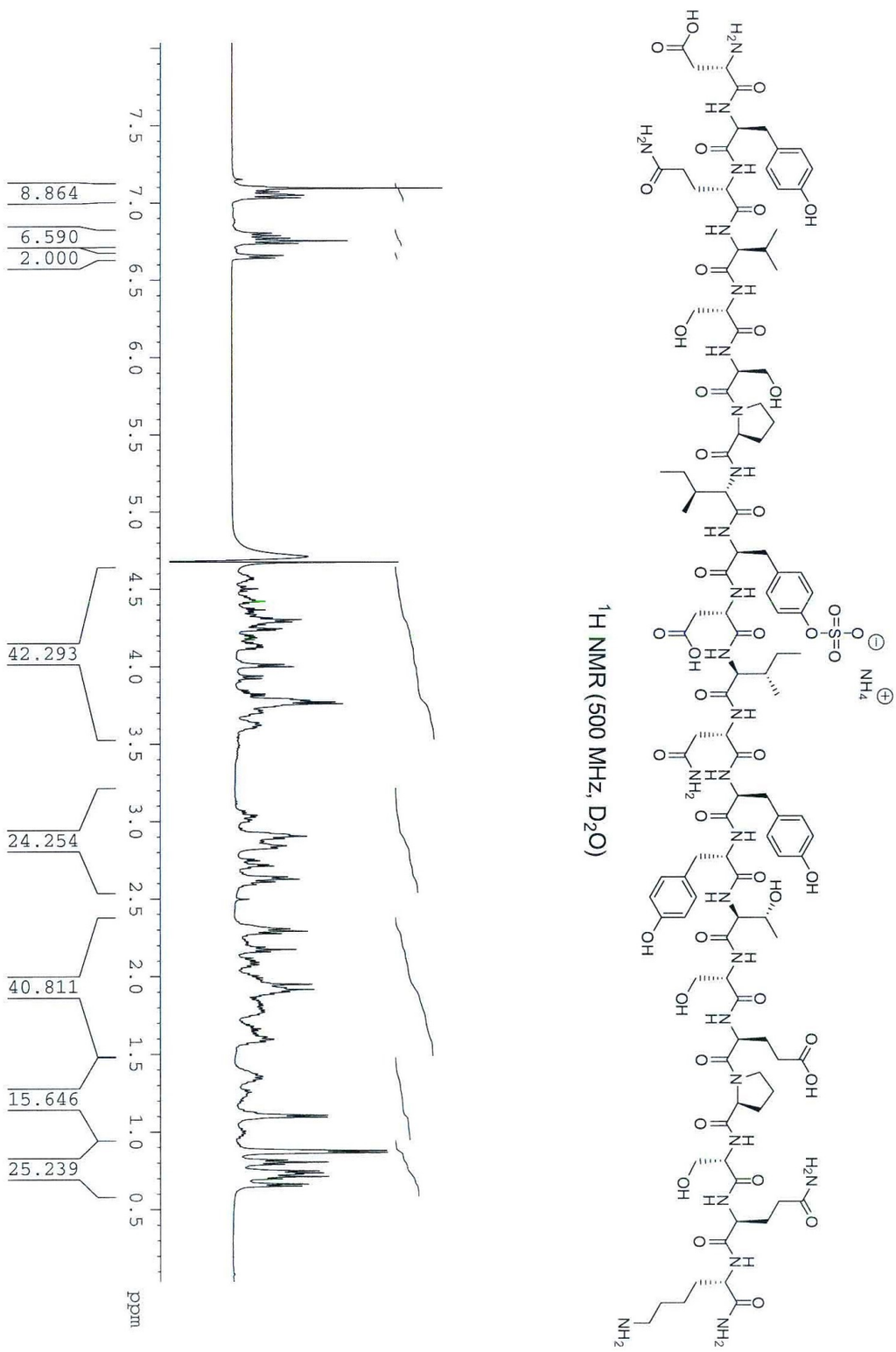
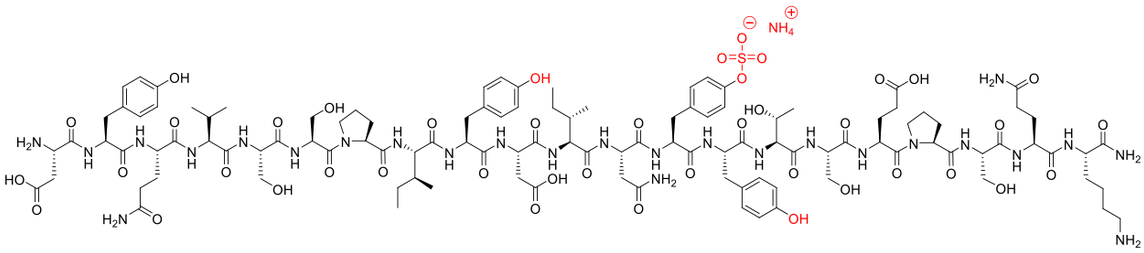


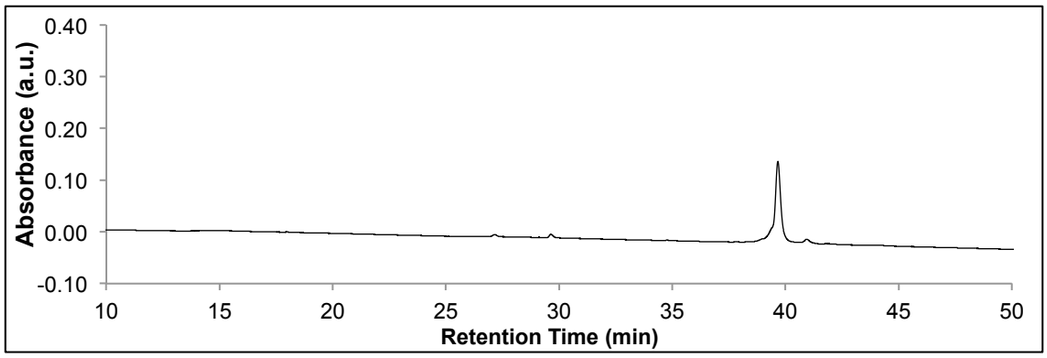
Figure 17. ^{1}H NMR spectrum of sulfopeptide **4** in D_2O with water suppression

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr($SO_3^-NH_4^+$)-Tyr(OH)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (5)

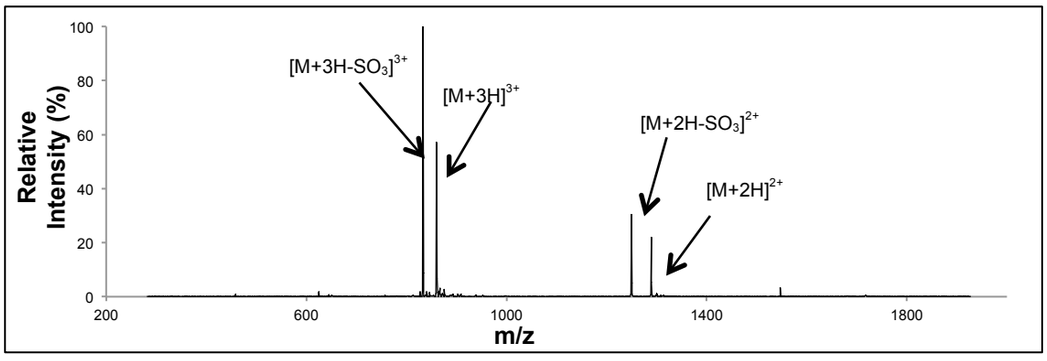


The crude TCE-protected sulfoester-derived peptide **S12** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by preparative reverse-phase HPLC (0 to 40% B over 60 min; ***Eluent B***) afforded pure sulfopeptide **5** as a white solid after lyophilization (12.0 mg, 38%).

A)



B)



C)

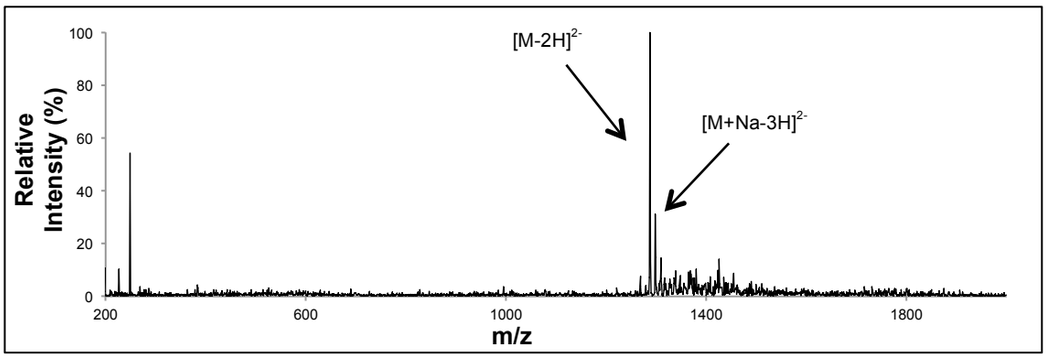


Figure 18. A) Analytical HPLC of sulfopeptide **5**: R_t 39.6 min (0-40% B over 60 min, ***Eluent B***, $\lambda = 230$ nm); **B)** Mass spectrum (ESI+) of sulfopeptide **5**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{42}S$ [M]: 2576.1, $[M+2H]^{2+}$: 1289.1, $[M+3H]^{3+}$: 859.7; Mass found 1289.2 $[M+2H]^{2+}$, 859.8 $[M+3H]^{3+}$; C) Mass spectrum (ESI-) of sulfopeptide **5**: Calculated for $[M-2H]^{2-}$: 1287.0, $[M+Na-3H]^{3-}$: 1298.0; Mass found 1287.2 $[M-2H]^{2-}$, 1297.8 $[M+Na-3H]^{2-}$.

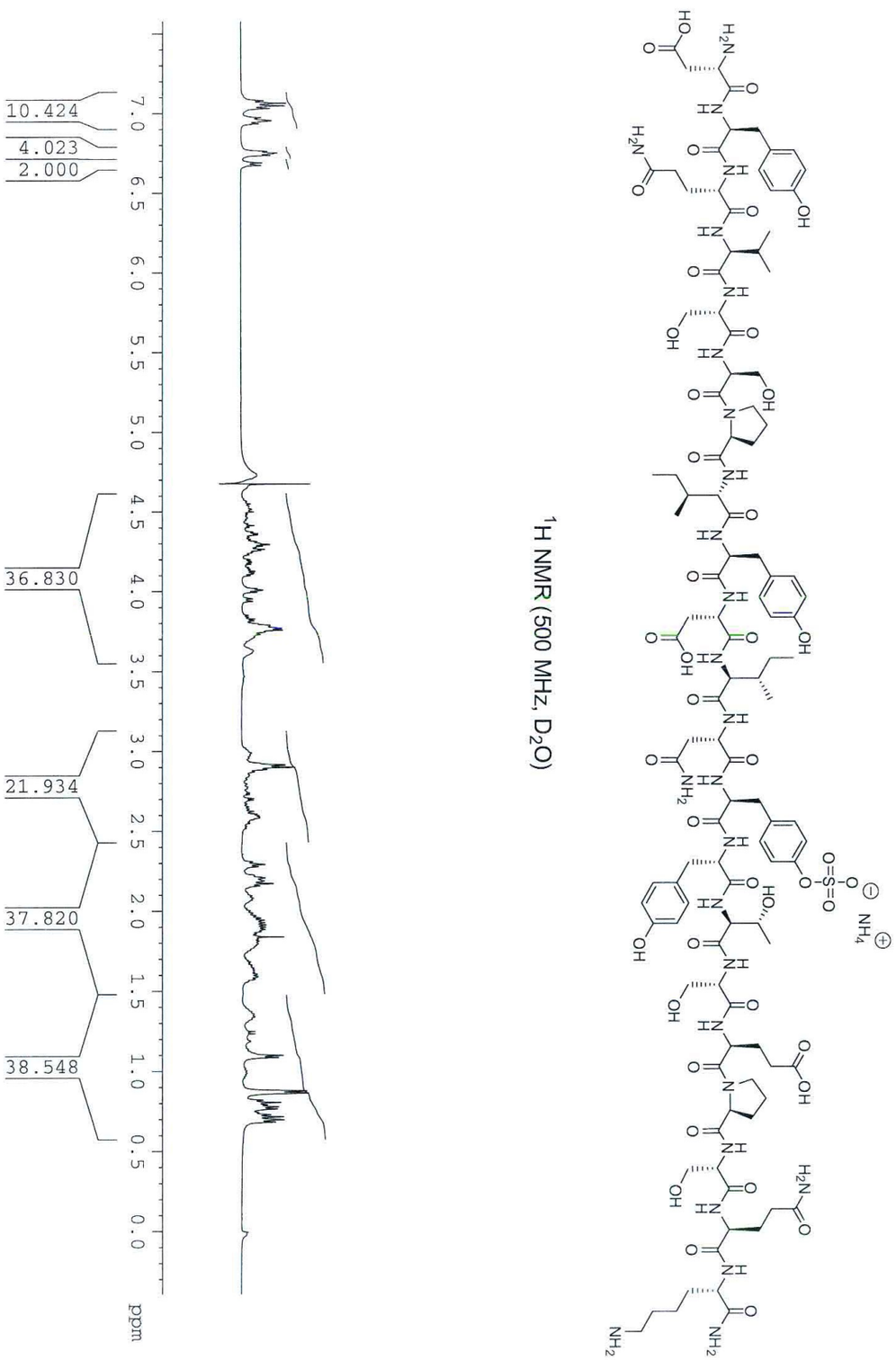
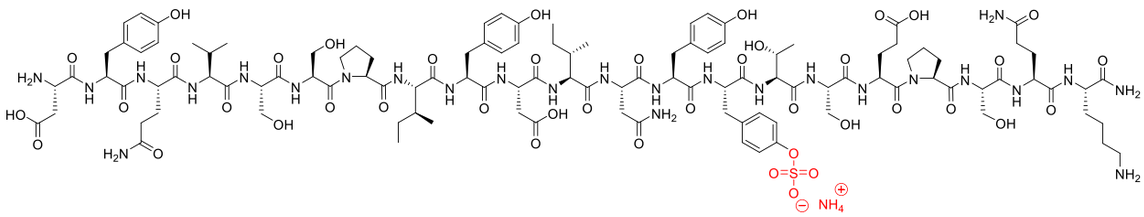


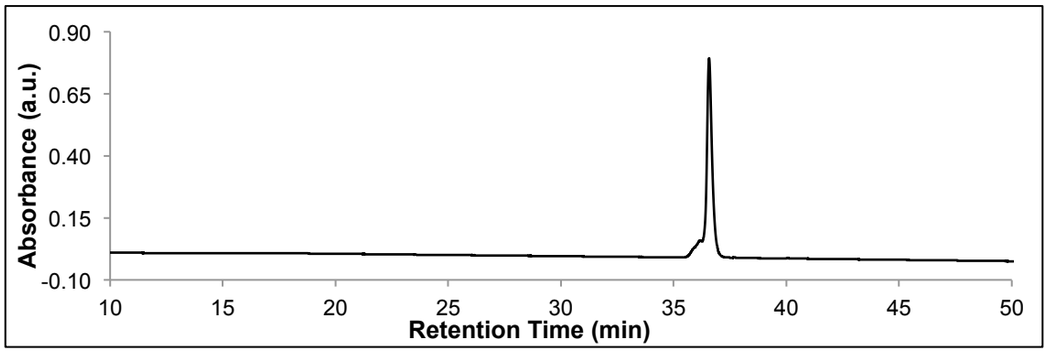
Figure 19. ^{1}H NMR spectrum of sulfopeptide **5** in D_2O with water suppression

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr(OH)-Tyr($SO_3^-NH_4^+$)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (6)

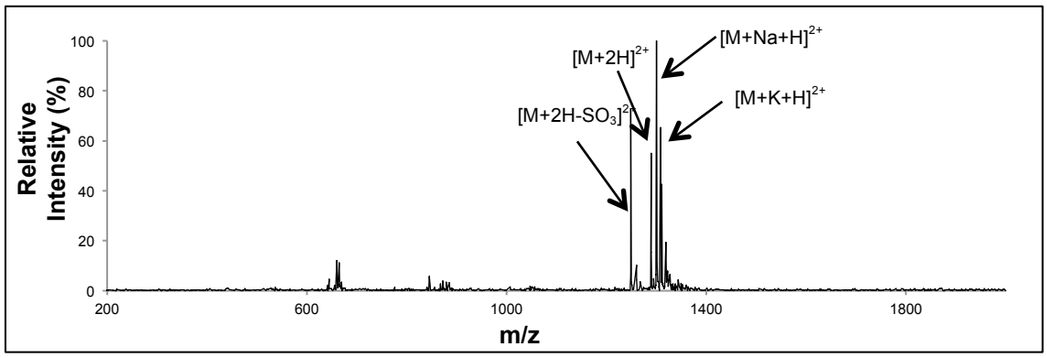


The crude TCE-protected sulfoester-derived peptide **S13** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by preparative reverse-phase HPLC (0 to 40%B over 60 min; ***Eluent B***) afforded pure sulfopeptide **6** as a white solid after lyophilization (10.0 mg, 31%).

A)



B)



C)

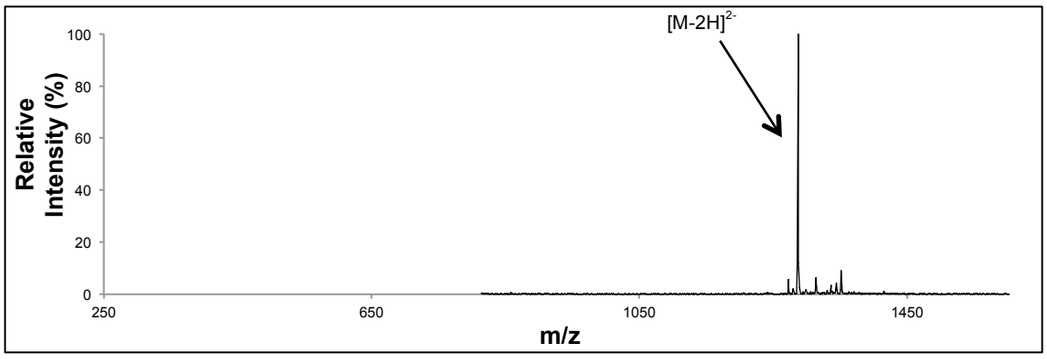


Figure 20. A) Analytical HPLC of sulfopeptide **6**: R_t 36.5 min (0-40% B over 60 min, ***Eluent B***, λ = 230 nm); **B)** Mass spectrum (ESI+) of sulfopeptide **6**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{42}S$ [M]: 2576.1, $[M+2H]^{2+}$: 1289.1, $[M+Na+H]^{2+}$: 1300.1, $[M+K+H]^{2+}$: 1308.0; Mass found 1289.2 $[M+2H]^{2+}$, 1300.1 $[M+Na+H]^{2+}$, 1380.1 $[M+K+H]^{2+}$; **C)** Mass spectrum (ESI-) of sulfopeptide **6**: Calculated for $[M-2H]^{2-}$: 1287.0; Mass found 1287.2 $[M-2H]^{2-}$.

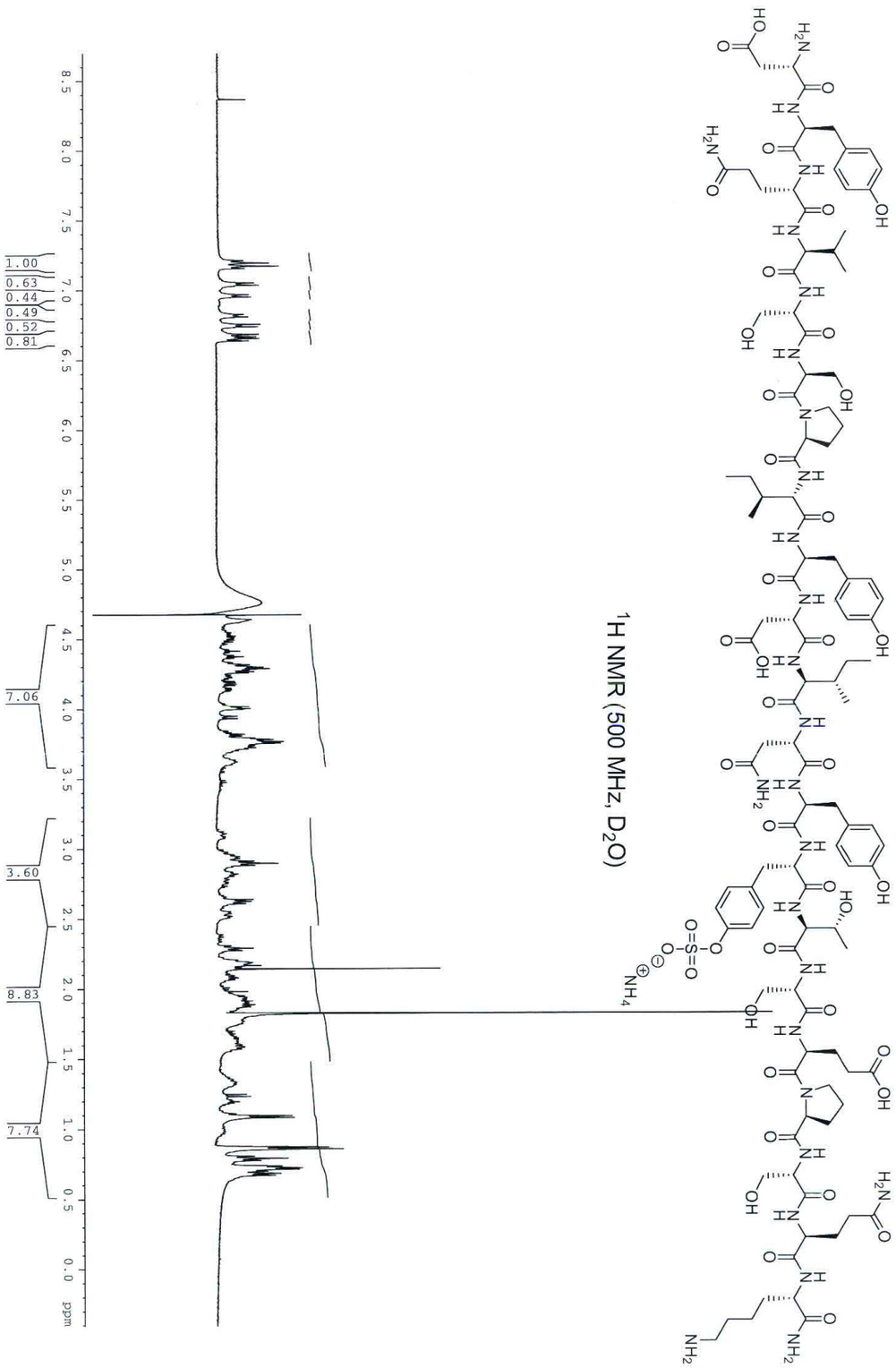
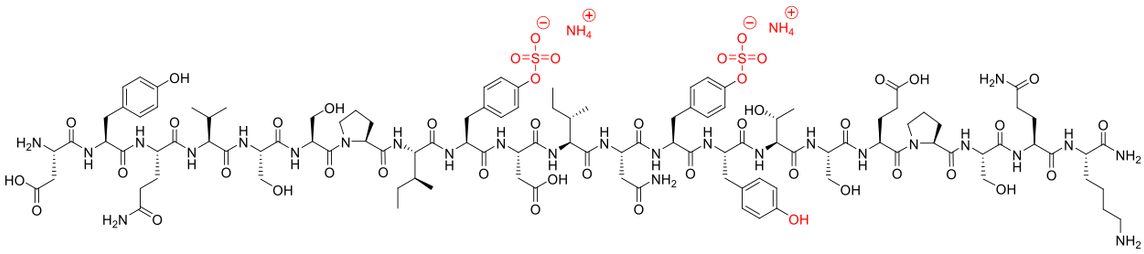


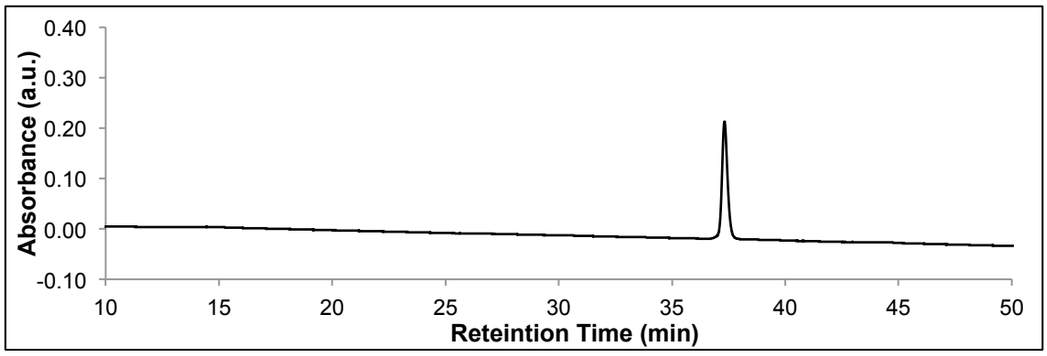
Figure 21. ^{1}H NMR spectrum of sulfopeptide **6** in D_2O with water suppression

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr($SO_3^-NH_4^+$)-Asp-Ile-Asn-Tyr($SO_3^-NH_4^+$)-Tyr(OH)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (7)

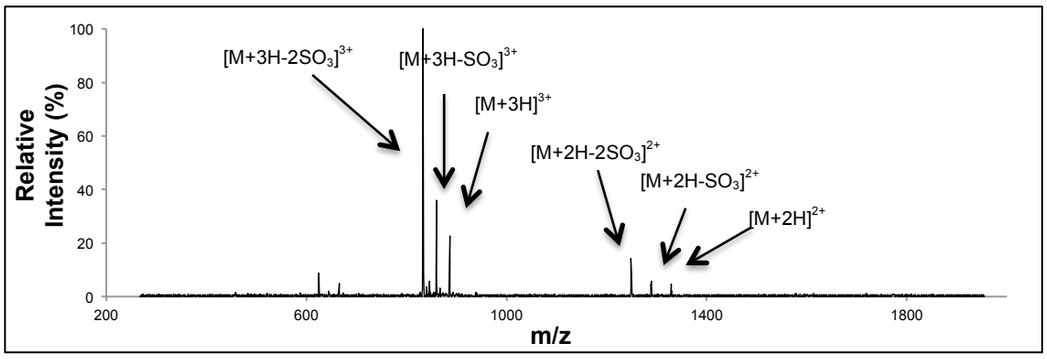


The crude TCE-protected sulfoester-derived peptide **S14** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by preparative reverse-phase HPLC (0 to 40% B over 60 min; ***Eluent B***) afforded pure sulfopeptide **7** as a white solid after lyophilization (9.7 mg, 29%).

A)



B)



C)

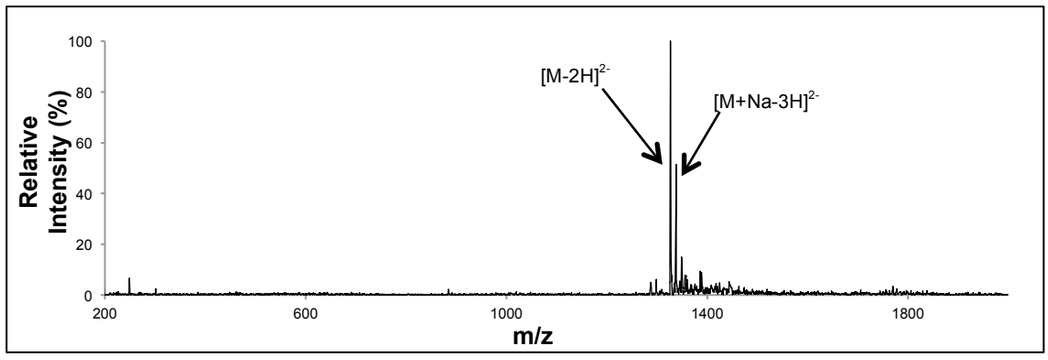


Figure 22. A) Analytical HPLC of sulfopeptide **7**: R_t 37.2 min (0-40% B over 60 min, ***Eluent B***, λ = 230 nm); **B)** Mass spectrum of sulfopeptide **7**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{45}S_2$ [M]: 2656.1, $[M+2H]^{2+}$: 1329.0, $[M+3H]^{3+}$: 886.4; Mass found 1329.1 $[M+2H]^{2+}$, 886.5 $[M+3H]^{3+}$; C) Mass spectrum (ESI-) of sulfopeptide **7**: Calculated for $[M-2H]^{2-}$: 1327.0, $[M+Na-3H]^{2-}$: 1338.0; Mass found 1327.2 $[M-2H]^{2-}$, 1338.2 $[M+Na-3H]^{2-}$.

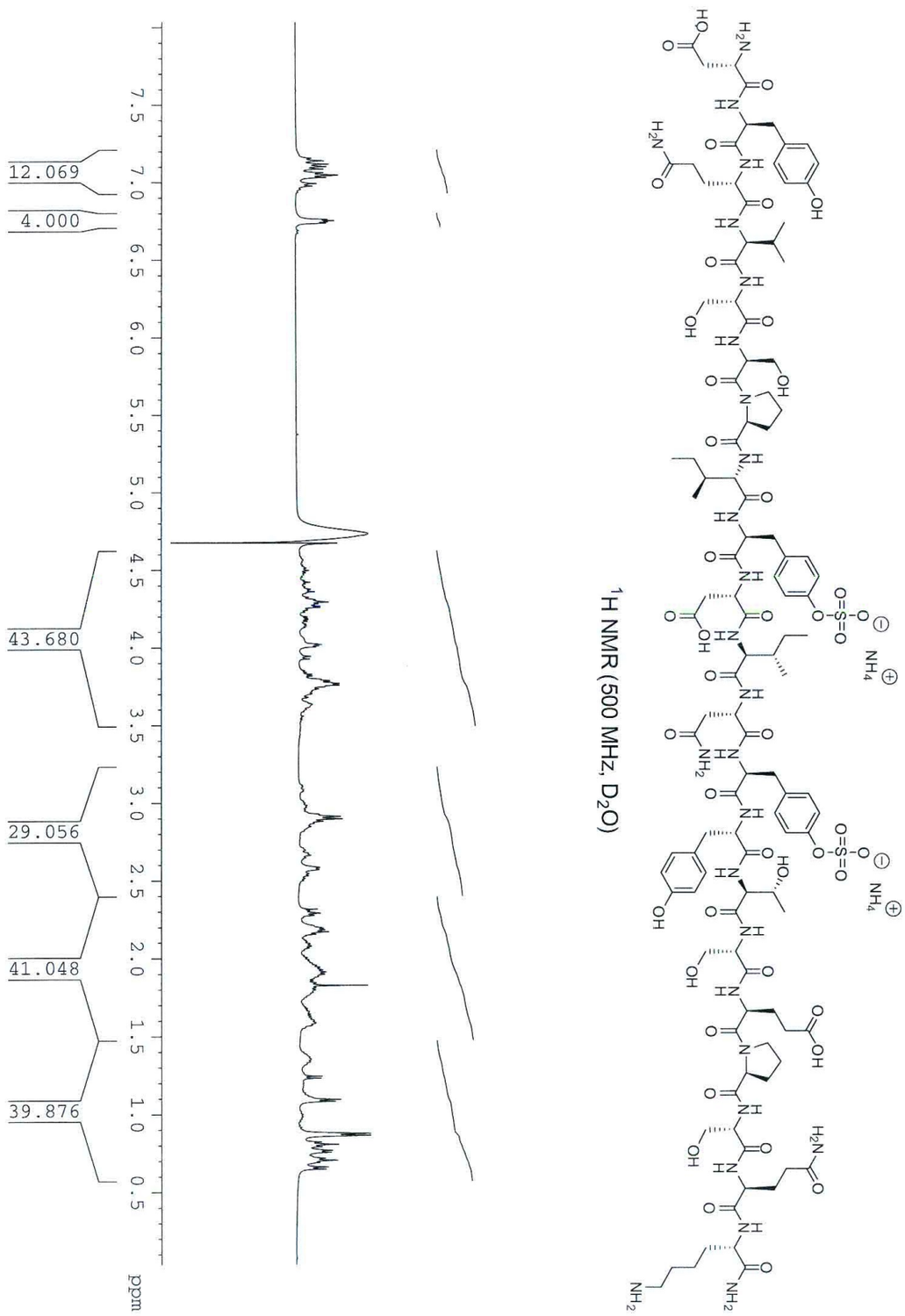
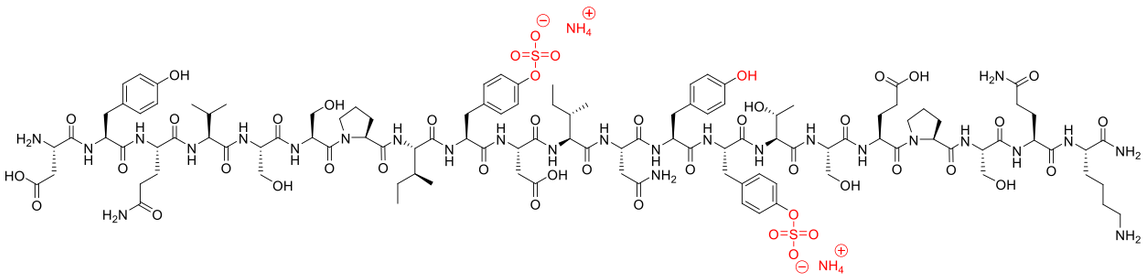


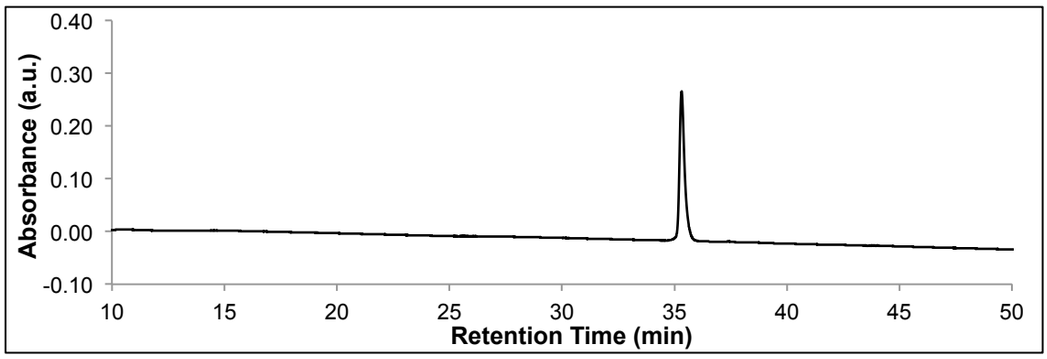
Figure 23. ^{1}H NMR spectrum of sulfopeptide **7** in D_2O with water suppression

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OSO3$^-$NH4$^+$)-Asp-Ile-Asn-Tyr(OH)-Tyr(SO3$^-$NH4$^+$)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH2 (8)

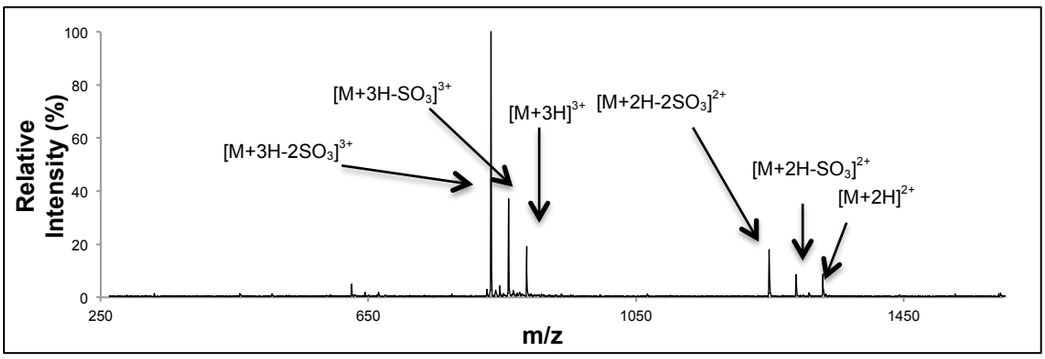


The crude TCE-protected sulfoester-derived peptide **S15** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by preparative reverse-phase HPLC (0 to 40%B over 60 min; Eluent B) afforded pure sulfopeptide **8** as a white solid after lyophilization (6.7 mg, 20%).

A)



B)



C)

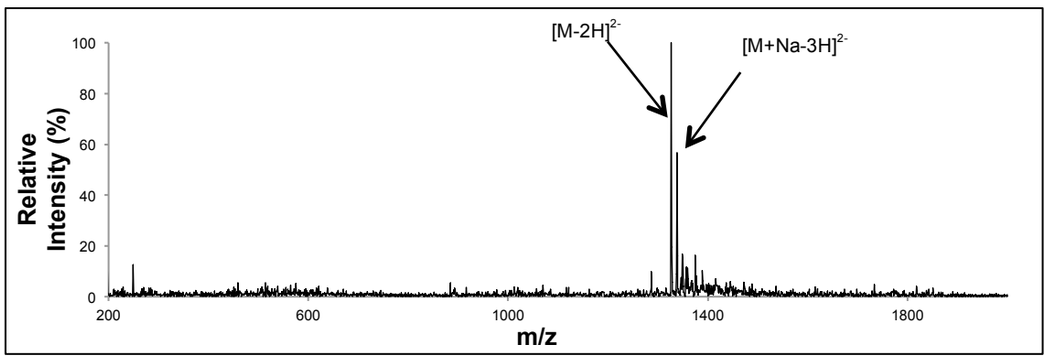


Figure 24. A) Analytical HPLC of sulfopeptide **8**: R_t 35.2 min (0-40% B over 60 min, ***Eluent B***, $\lambda = 230$ nm); B) Mass spectrum (ESI+) of sulfopeptide **8**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{45}S_2$ [M]: 2656.1, $[M+2H]^{2+}$: 1329.0, $[M+3H]^{3+}$: 886.4; Mass found 1329.1 $[M+2H]^{2+}$, 886.6 $[M+3H]^{3+}$; C) Mass spectrum (ESI-) of sulfopeptide **8**: Calculated for $[M-2H]^{2-}$: 1327.0, $[M+Na-3H]^{2-}$: 1338.0; Mass found 1327.1 $[M-2H]^{2-}$, 1338.0 $[M+Na-3H]^{2-}$.

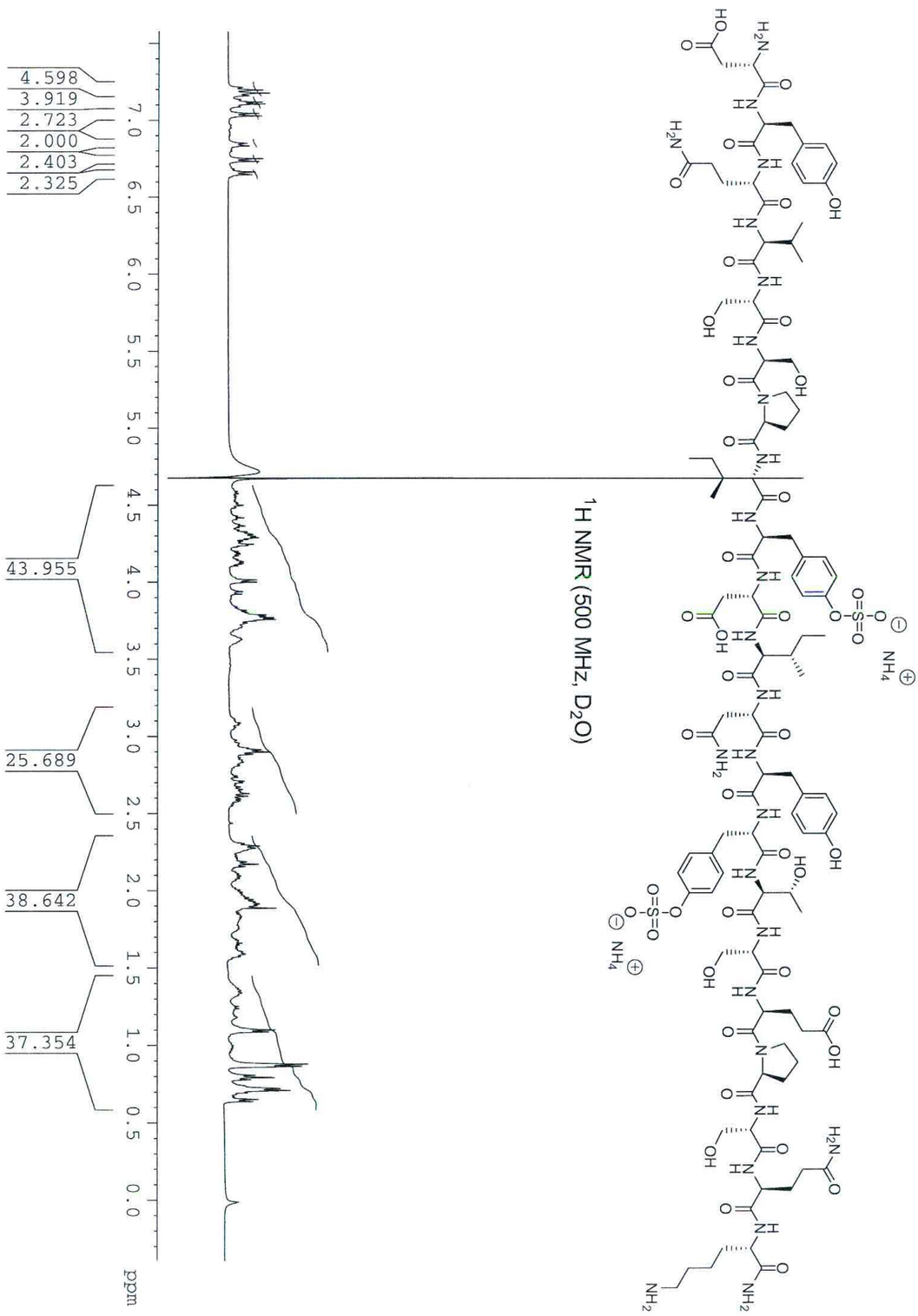
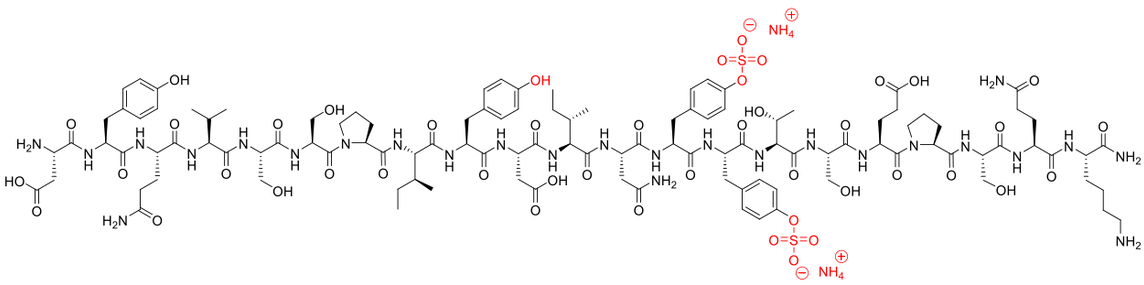


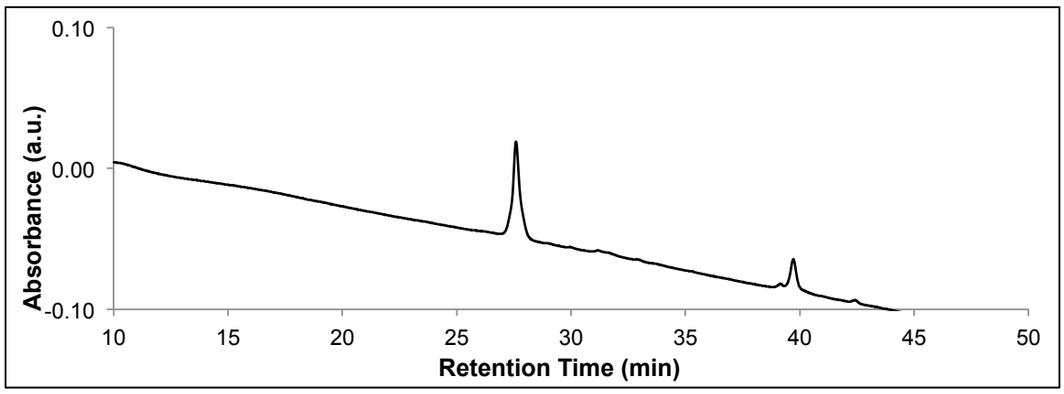
Figure 25. ^{1}H NMR spectrum of sulfopeptide **8** in D_2O with water suppression

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OH)-Asp-Ile-Asn-Tyr($SO_3^-NH_4^+$)-Tyr($SO_3^-NH_4^+$)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (9)

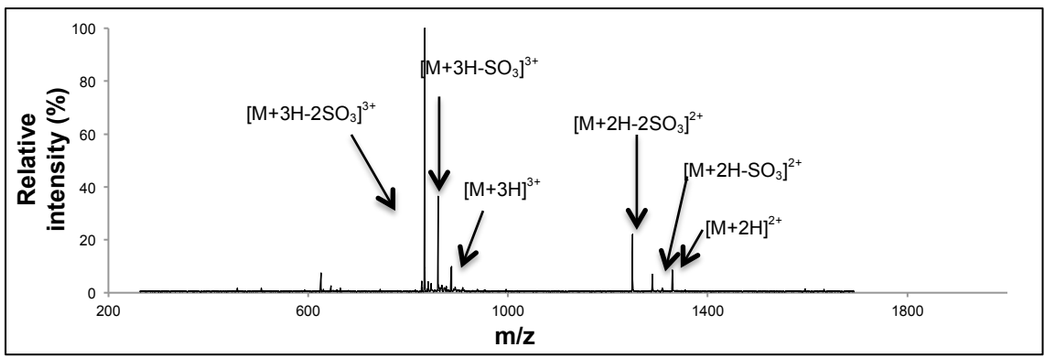


The crude TCE-protected sulfoester-derived peptide **S16** was subjected to hydrogenation catalyzed by $Pd(OH)_2$ on activated carbon to provide the crude sulfopeptide (general procedure 1.5.1). Purification by preparative reverse-phase HPLC (0 to 40% B over 60 min; ***Eluent B***) afforded pure peptide **9** as a white solid after lyophilization (11.4 mg, 34%).

A)



B)



C)

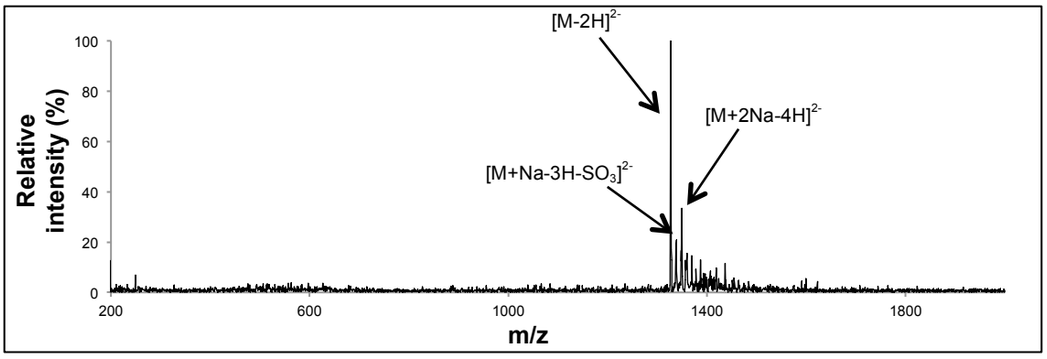


Figure 26. A) Analytical HPLC: R_t 27.4 min (0-40% B over 60 min, ***Eluent B***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **9**: Calculated for protonated (neutral) $C_{112}H_{162}N_{26}O_{45}S_2$ [M]: 2656.1 $[M+2H]^{2+}$: 1329.0, $[M+3H]^{3+}$: 886.4; Mass found 1329.2 $[M+2H]^{2+}$, 886.6 $[M+3H]^{3+}$; **C)** Mass spectrum (ESI-) of **9**:

Calculated for $[M-2H]^{2-}$: 1327.0, $[M+Na-3H]^{2-}$: 1338.0, $[M+2Na-4H]^{2-}$: 1349.0; Mass found 1327.2 $[M-2H]^{2-}$, 1337.6 $[M+Na-3H]^{2-}$, $[M+2Na-4H]^{2-}$: 1349.2.

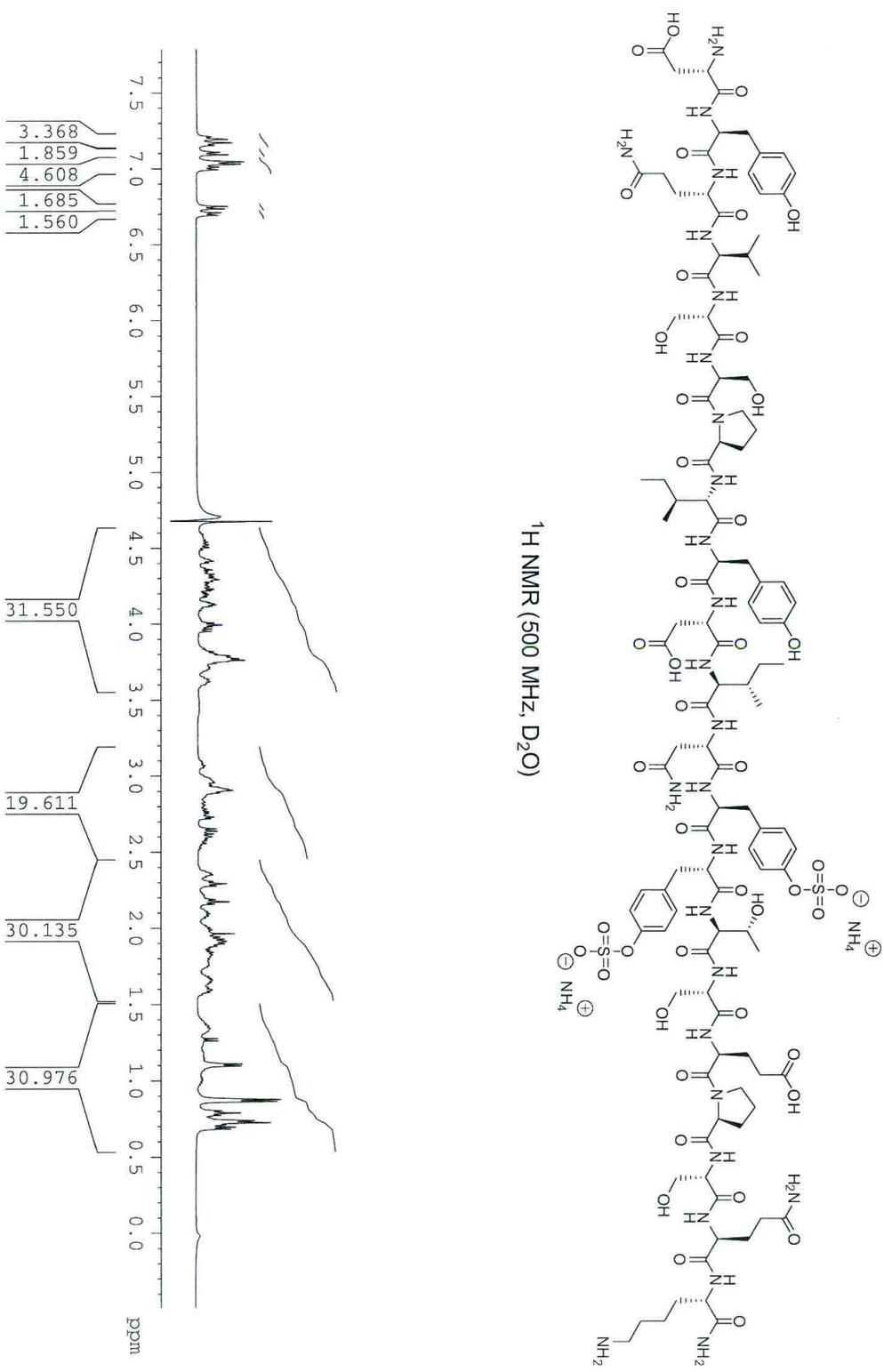
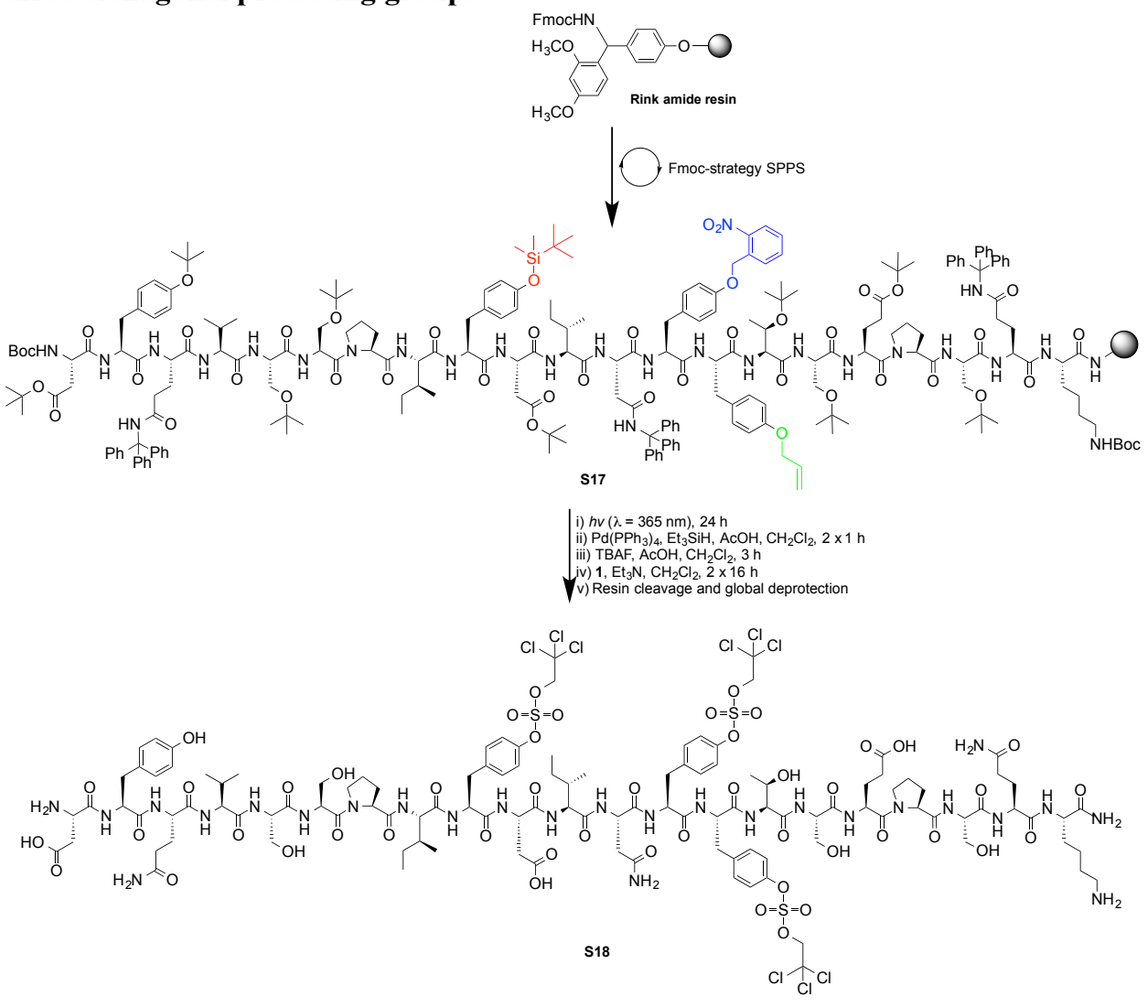
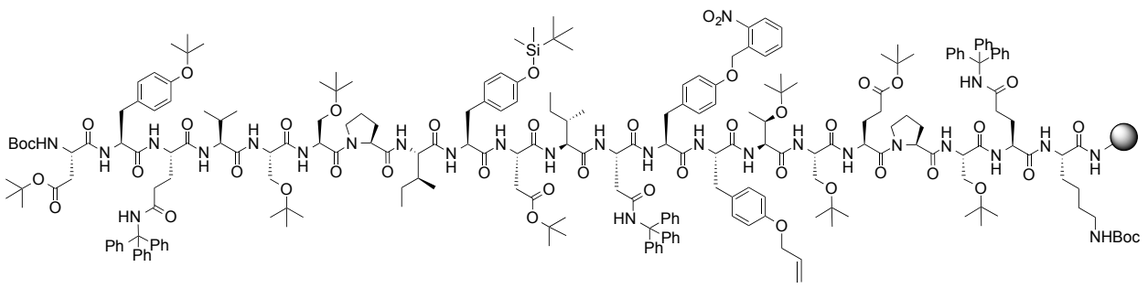


Figure 27. ^{1}H NMR spectrum of sulfopeptide **9** in D_2O with water suppression

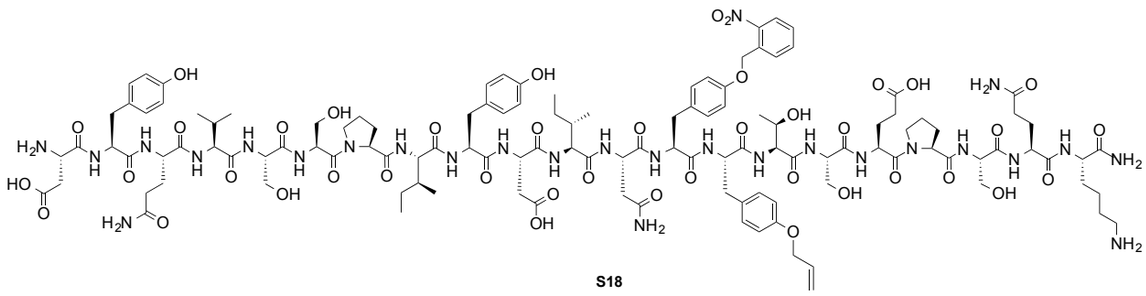
4.4 Synthesis of CCR5 N-terminal fragments with variations in the location of the three orthogonal protecting groups

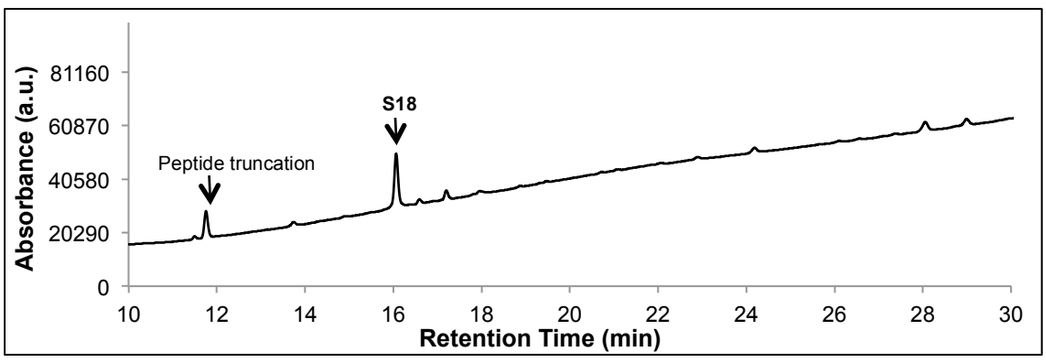


Boc-Asp(O*t*Bu)-Tyr(*t*Bu)-Gln(Trt)-Val-Ser(*t*Bu)-Ser(*t*Bu)-Pro-Ile-Tyr(OTBS)-Asp(O*t*Bu)-Ile-Asn(Trt)-Tyr(O*o*-Nb)-Tyr(OAll)-Thr(*t*Bu)-Ser(*t*Bu)-Glu(O*t*Bu)-Pro-Ser(*t*Bu)-Gln(Trt)-Lys(Boc)-Rink amide resin



Resin bound peptide **S17** (12.5 µmol) was prepared by Fmoc-strategy SPPS on Rink amide resin outlined in the general procedures (1.1). A minicleavage of resin-bound **S17** led to peptide **S18** where the *o*-Nb and allyl ether protecting groups remained intact but all other side chain protecting groups including the TBS ether were cleaved.





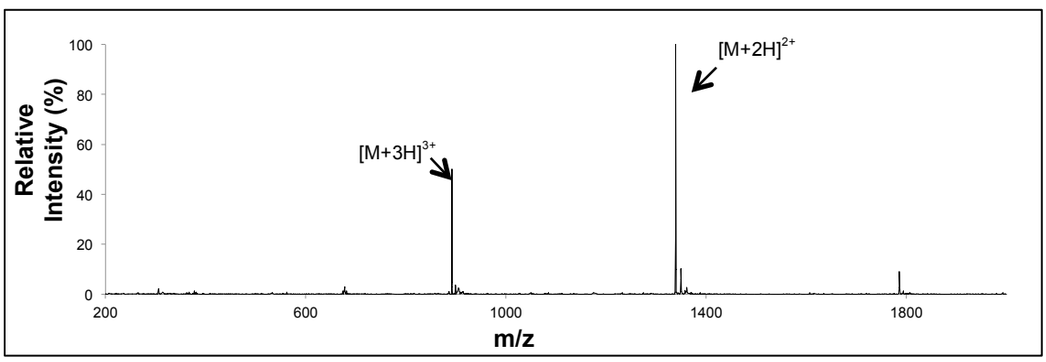
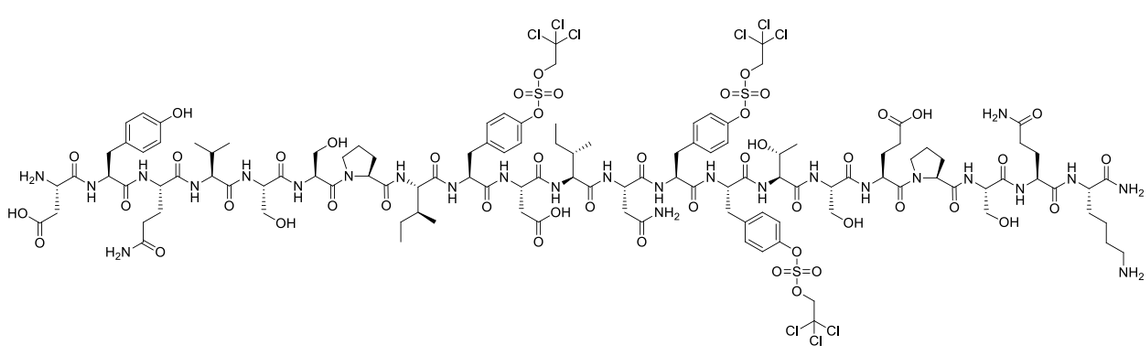


Figure 28 A) LC-MS analysis of crude **S18** (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S18**: Calculated mass $[M+2H]^{2+}$: 1336.6, $[M+3H]^{3+}$: 891.4; Mass found 1338.8 $[M+2H]^{2+}$, 891.8 $[M+3H]^{3+}$.

H-Asp-Tyr-Gln-Val-Ser-Ser-Pro-Ile-Tyr(OSO_3TCE)-Asp-Ile-Asn-Tyr(OSO_3TCE)-Tyr(OSO_3TCE)-Thr-Ser-Glu-Pro-Ser-Gln-Lys-NH_2 (S10)



The *o*-nitrobenzyl, allyl and TBS protecting groups of resin bound peptide **S17** were deprotected according to deprotection conditions A, B and C. The peptide was then triply sulfated on resin with **1** (general procedure 1.3) followed by acidic deprotection and cleavage of the peptide from the resin to provide the crude TCE-protected sulfoester-derived peptide **S10** (general procedure 1.4). Purification by

preparative reverse-phase HPLC (20 to 70% B over 40 min; ***Eluent A***) afforded pure peptide **S10** as a white solid after lyophilization (5.8 mg, 15%).

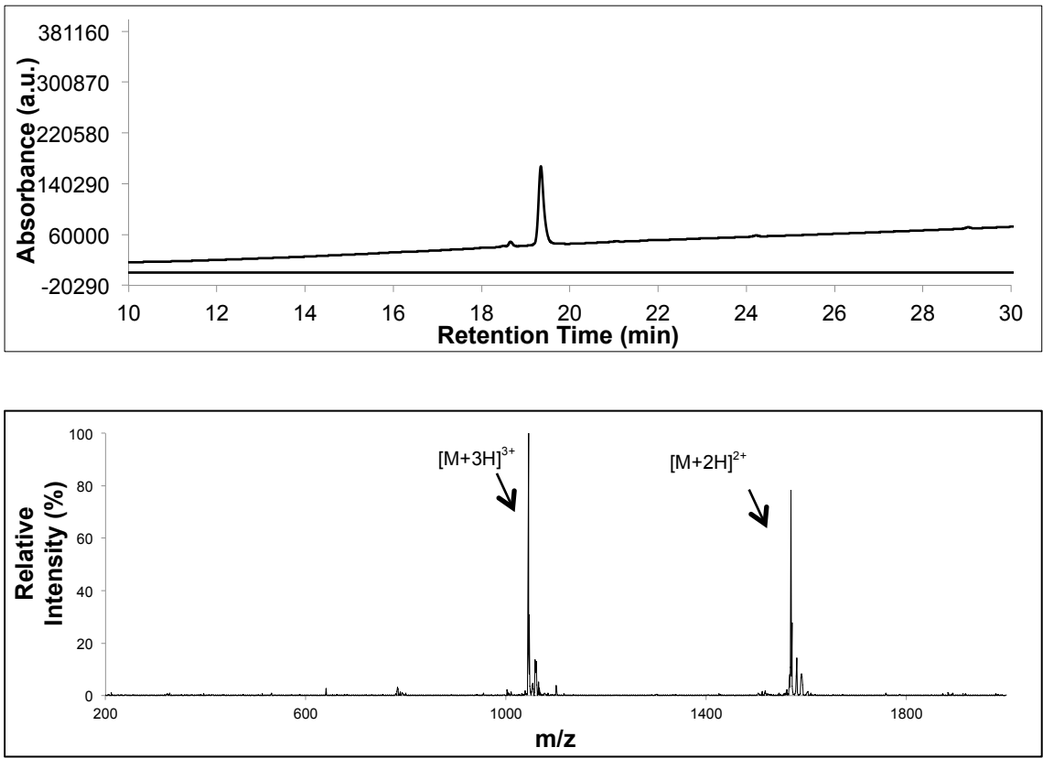


Figure 29 A) LC-MS of purified **S10:** R_t 19.3 min (0-100% B over 30 min, ***Eluent A***, λ = 230 nm); **B)** Mass spectrum (ESI+) of **S10**: Calculated mass $[M+2H]^{2+}$:1565.9, $[M+3H]^{3+}$: 1044.3; Mass found 1568.9 $[M+2H]^{2+}$, 1044.7 $[M+3H]^{3+}$.

5 Molecular modeling of the interactions between CCR5 sulfopeptides and gp120

Env structural models were developed using the crystal structure of CD4-bound YU2 gp120 containing V3 docked with the nuclear magnetic resonance (NMR) structure of an N-terminus peptide of CCR5 (^{7}SPIsYDINsYY^{15}), which was kindly provided by P. D. Kwong.[6] Tyrosines 10, 14 and 15 of the CCR5 N-terminus peptide were modified using the Mutate Protein protocol that we have described previously.[7] Briefly, tyrosine (Tyr) residues were modified to sulfotyrosine (Tys) and harmonic constraints were applied prior to optimization using the Steepest Decent protocol. Steepest Decent incorporates multiple sequential cycles of conjugate gradient energy minimization against a probability density function that applies spatial constraints derived from the original template and from amino acid-specific properties.[8]

Electrostatic interactions:

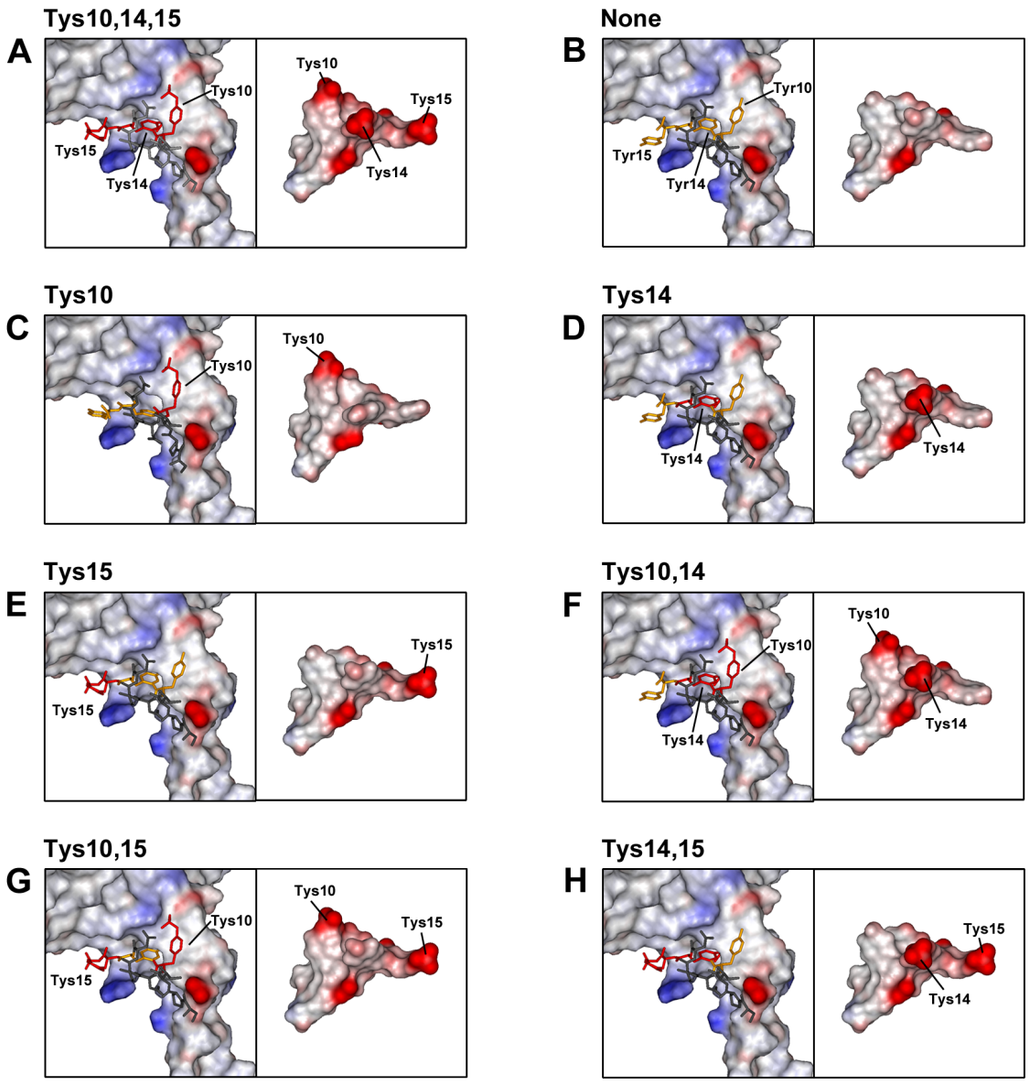


Figure 30 Electrostatic interactions between YU2 gp120 and N-terminal CCR5(7-15) sulfoforms. Three-dimensional model of the YU2 gp120 V3 loop region (left panel, protein molecular surface colored according to charge) docked to the triply sulfated (A), unsulfated (B), Tys10 alone (C), Tys14 alone (D), Tys15 alone (E) or doubly sulfated forms (F, G, H) of the CCR5 N-terminus(7-15) (left panel, protein stick models; right panel, 180^0 view of the CCR5 N-terminus peptide protein molecular surface colored according to charge).

Intermolecular interactions:

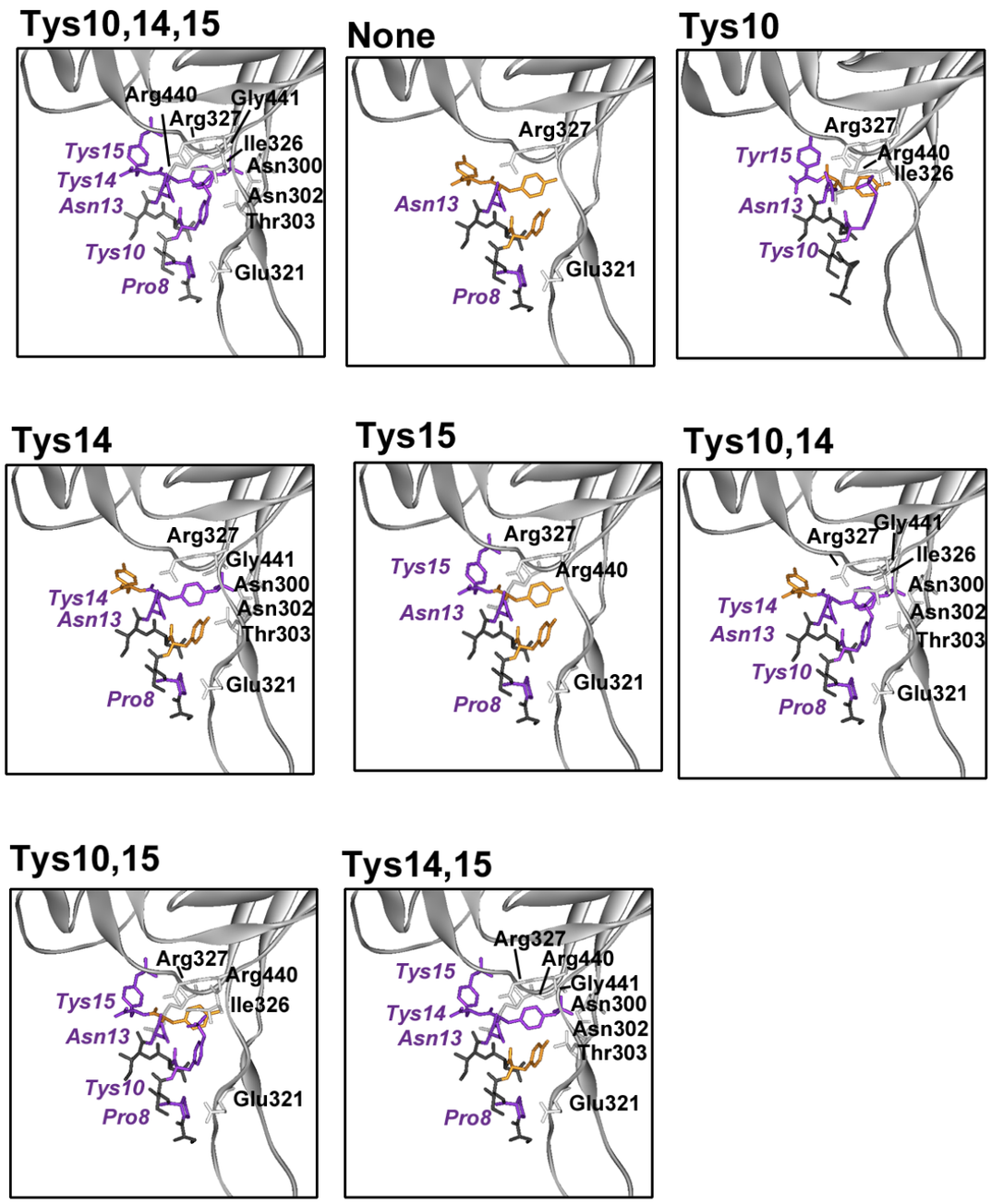


Figure 31 Predicted intermolecular hydrogen-bond potential between YU2 gp120 and N-terminal CCR5(7-15) sulfoforms. Ribbon diagrams of gp120 YU2 (grey ribbon) modelled with the differentially sulfated N-terminal CCR5 peptides (stick representation). Amino acids in gp120 predicted to form hydrogen bonds with the CCR5 N-terminal peptides are shown as light grey stick models. Amino acids in the CCR5 N-terminal peptides predicted to interact with gp120 are shown as purple stick models. CCR5 N-terminal amino acids and unsulfated Tyr residues that were not predicted to form hydrogen bonds with gp120 are shown in dark grey and orange, respectively.

Notes on binding interactions from molecular modeling:

Tys at position 10 appears to promote interactions with the descending strand of the gp120 V3 loop (Ile326, Arg327), while the presence of a Tys at position 14 promotes interactions with both the

ascending (Asn300, Asn302, Thr303), and the descending strand of the V3 loop. These results may explain why the presence of Tys14 alone or in combination with other sulfation events has the greatest effect on peptide binding. Tys at position 15 is oriented away from the V3 loop and appears to be important for interactions with the C3 region of gp120 (Arg440, Gy441).

CCR5 N-term Sulfoform		gp120 amino acid [atom]	Distance (Å)	N-term amino acid [atom]
Tys10,14,15	1	ARG 327 [NH1]	2.95	TYS 10 [O2]
	2	ILE 326 [N]	2.78	TYS 10 [O3]
	3	ARG 327 [NH2]	3.20	ASN 13 [OD1]
	4	ARG 327 [NH1]	3.43	ASN 13 [OD1]
	5	GLY 441 [N]	2.87	TYS 14 [O1]
	6	ASN 300 [ND2]	3.27	TYS 14 [O2]
	7	ASN 302 [N]	2.86	TYS 14 [O2]
	8	ASN 302 [ND2]	2.78	TYS 14 [O2]
	9	THR 303 [N]	3.73	TYS 14 [O2]
	10	ARG 440 [N]	3.62	TYS 15 [O1]
	11	GLU 321 [OE2]	3.10	PRO 8 [N]
None	1	ARG 327 [NH1]	3.43	ASN 13 [OD1]
	2	ARG 327 [NH2]	3.20	ASN 13 [OD1]
	3	GLU 321 [OE2]	3.10	PRO 8 [N]
Tys10	1	ILE 326 [N]	3.21	TYS 10 [O3]
	2	ARG 327 [N]	3.89	TYS 10 [O3]
	3	ARG 440 [NH2]	3.28	ASP 11 [OD1]
	4	ARG 327 [NH1]	3.14	ASN 13 [OD1]
	5	ARG 327 [NH2]	2.79	ASN 13 [OD1]
Tys14	1	ARG 327 [NH1]	3.43	ASN 13 [OD1]
	2	ARG 327 [NH2]	3.20	ASN 13 [OD1]
	3	GLY 441 [N]	2.87	TYS 14 [O1]
	4	ASN 300 [ND2]	3.26	TYS 14 [O2]
	5	ASN 302 [N]	2.87	TYS 14 [O2]
	6	ASN 302 [ND2]	2.78	TYS 14 [O2]
	7	THR 303 [N]	3.73	TYS 14 [O2]
	8	GLU 321 [OE2]	3.10	PRO 8 [N]
Tys15	1	ARG 327 [NH2]	3.20	ASN 13 [OD1]

	2	ARG 327 [NH1]	3.43	ASN 13 [OD1]
	3	ARG 440 [N]	3.62	TYS 15 [O1]
	4	GLU 321 [OE2]	3.10	PRO 8 [N]
Tys10,14	1	ARG 327 [NH1]	2.95	TYS 10 [O2]
	2	ILE 326 [N]	2.78	TYS 10 [O3]
	3	ARG 327 [NH1]	3.43	ASN 13 [OD1]
	4	ARG 327 [NH2]	3.20	ASN 13 [OD1]
	5	GLY 441 [N]	2.87	TYS 14 [O1]
	6	ASN 300 [ND2]	3.26	TYS 14 [O2]
	7	ASN 302 [N]	2.87	TYS 14 [O2]
	8	ASN 302 [ND2]	2.78	TYS 14 [O2]
	9	THR 303 [N]	3.73	TYS 14 [O2]
	10	GLU 321 [OE2]	3.10	PRO 8 [N]
Tys10,15	1	ARG 327 [NH1]	2.94	TYS 10 [O2]
	2	ILE 326 [N]	2.78	TYS 10 [O3]
	3	ARG 327 [NH2]	3.20	ASN 13 [OD1]
	4	ARG 327 [NH1]	3.44	ASN 13 [OD1]
	5	ARG 440 [N]	3.61	TYS 15 [O1]
	6	GLU 321 [OE2]	3.09	PRO 8 [N]
Tys14,15	1	ARG 327 [NH2]	3.20	ASN 13 [OD1]
	2	ARG 327 [NH1]	3.42	ASN 13 [OD1]
	3	GLY 441 [N]	2.87	TYS 14 [O1]
	4	ASN 300 [ND2]	3.27	TYS 14 [O2]
	5	ASN 302 [N]	2.87	TYS 14 [O2]
	6	ASN 302 [ND2]	2.78	TYS 14 [O2]
	7	THR 303 [N]	3.72	TYS 14 [O2]
	8	ARG 440 [N]	3.63	TYS 15 [O1]
	9	GLU 321 [OE2]	3.11	PRO 8 [N]

Table 1 Potential intermolecular hydrogen bond/ionic interaction partners between N-terminal CCR5 sulfoforms and gp120 YU2. Intermolecular hydrogen bond/ionic interaction potential was

determined using the Protein interfaces, surfaces and assemblies (PISA) service at the European Bioinformatics Institute (http://www.ebi.ac.uk/pdbe/prot_int/pistart.html).

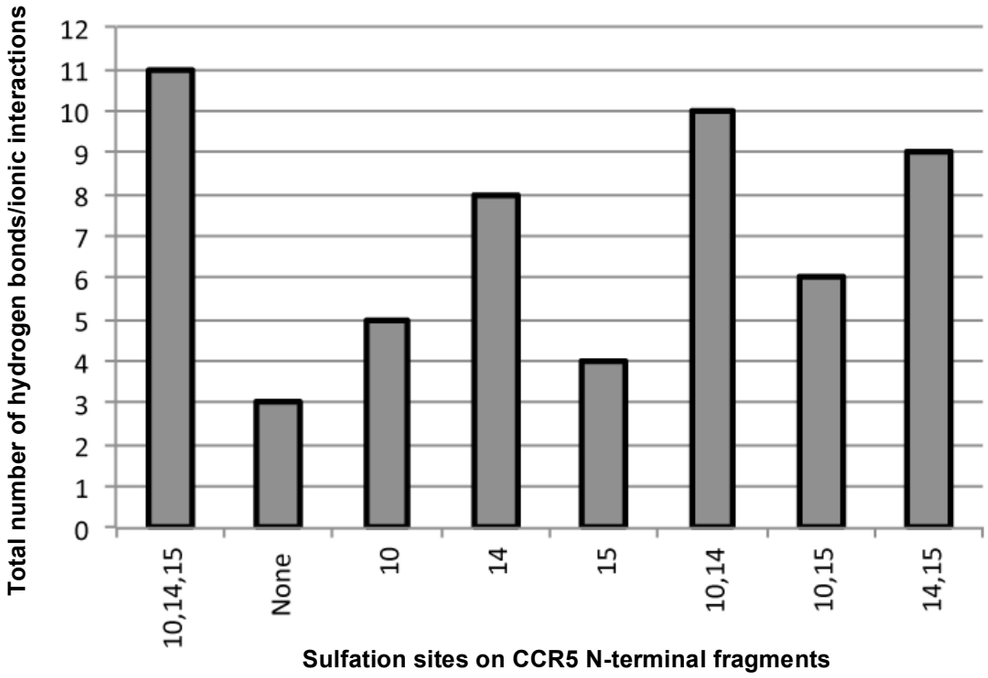


Figure 32 Graphical representation of the total number of potential hydrogen bonds/ionic interactions between differentially sulfated N-terminal CCR5 peptides and gp120.

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