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Supporting Information

A Tripeptide Approach to the Solid-Phase Synthesis of Peptide Thioacids and N-Glycopeptides

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Author Contributions

M.S. Conceptualization: Lead; Data curation: Equal; Investigation: Lead; Methodology: Lead; Writing - Original Draft: Lead; Writing - Review & Editing: Equal
O.K. Investigation: Supporting
C.U. Data curation: Supporting; Resources: Supporting; Writing - Review & Editing: Supporting
V.W. Conceptualization: Lead; Data curation: Lead; Funding acquisition: Lead; Supervision: Lead; Writing - Original Draft: Equal; Writing - Review & Editing: Lead.

Supporting Information

1. General Methods

Technical solvents were distilled prior to use (petroleum ether, EtOAc, dichloromethane, methanol). Reagents for solid phase peptide synthesis were bought from *Carbolution Chemicals* (HBTU), *GL Biochem* (HOBt, anhydrous), *Iris Biotech* (DMF peptide grade; stored over molecular sieves 4 Å), *Merck* (2-chlorotriyl resin), *Sigma-Aldrich* (DIPEA: *BioSyn* 99.5%; Piperazine: *BioUltra*, anhydrous, > 99%). Palladium on charcoal was bought from *TCI*. When necessary, reactions were conducted under inert atmosphere (argon or nitrogen) using the *Schlenk* technique. Solvents were dried by common methods (dichloromethane: calcium hydride, diethyl ether: sodium, DMF peptide grade: molecular sieves 4 Å). Preparative flash column chromatography (FC) was performed with *Kieselgel 60 M* (0.04–0.063 mm) from *Machery-Nagel*. Eluent mixtures are given in terms of volume/volume ratios. Preparative RP-MPLC purification was performed on a *Reveleris X2* from *Büchi*. Mobile Phase: acetonitrile in water, flow rate: 30 mL min^{-1}. Stationary phase: *Reveleris C18* (12 g). Preparative RP-HPLC purification was performed on a LC-20 A from *Shimadzu*. Degaser: DGU-20A3, pumps: LC-20AT, autosampler: SIL-20A, column oven: CTO-20AC, photodiode array detector: SPD-M20A, controller: CBM-20A. Mobile phase: acetonitrile (0.1% formic acid) in water (0.1% formic acid), flow rate: 9 mL min^{-1}. Stationary phase: *Phenomenex Kinetex* C18 100 Å, AXIA (5 μm, 250 × 21.2 mm, column 1). LC-MS analysis was performed on a LCMS 2020 from *Shimadzu*. Degaser: DGU-20A3, pumps: LC-20AD, autosampler: SIL-20AT HT, column oven: CTO-20AC, UV-vis detector: SPD-20A, controller: CBM-20A. Mobile phase: acetonitrile (0.1% formic acid) in water (0.1% formic acid), flow rate: 0.4 mL min^{-1}. Stationary phase: *Machery-Nagel Nucleodur C18 Gravity* (3 μm, 125 × 4 mm, column 2) at 40 °C or *Phenomenex Kinetex C18 100 Å* (2.6 μm, 150 × 4.6 mm, column 3) at 40 °C. For analytical thin-layer chromatography (TLC), silica coated aluminum plates from *Merck* (*60 F_{254}*) were used. Spots were visualized by fluorescence quenching at 254 nm and/or by dipping in one of the following solutions followed by applying gentle heat: *Vanillin*: 6 g vanillin, 2.5 mL conc. H_2SO_4, 250 mL EtOH; *$KMnO_4$*: 0.1% $KMnO_4$ in $NaOH_{(aq.)}$ (1 M); *Mostain*: 10 g ammonium molybdophosphate, 0.2 g $Cerium^{IV}$ sulfate, 200 mL $H_2SO_{4(aq)}$ (10%); *Anisaldehyde*: 3.7 mL 4-anisaldehyd, 5 mL conc. H_2SO_4, 15 mL glacial acetic acid, 135 mL EtOH; *Ninhydrin*: 0.15 g ninhydrin, 0.75 mL AcOH, 150 mL acetone. NMR spectra were collected at 300 K on an *Avance III* 400 or 600 MHz spectrometer from *Bruker*. Chemical shifts δ are given in ppm, coupling constants *J* were measured in Hz. As internal reference the signal of non or partly deuterated solvent was used ($CDCl_3$: δ_H = 7.26, δ_C = 77.16, methanol-d4: δ_H = 3.31, δ_C = 49.00).[1] If necessary, two-dimensional spectra were collected (HSQC, COSY, HMBC, ROESY, NOESY). High-resolution mass spectrometry (ESI+) was performed either on a *micrOTOF II* spectrometer from *Bruker* or on a *LTQ Orbitrap Velos* from *Thermo Scientific*. Samples were dissolved in water, acetonitrile or mixtures of both. UV-Vis spectra were recorded on a *Cary 50* from *Varian*.

2. General Procedures

General procedure 1: Fmoc deprotection and peptide coupling in solution
Fmoc protected amino acid/peptide was dissolved in 20% piperidine (DMF) (0.01 mL μmol^{-1}) and stirred at room temperature for 15 minutes. The solvents were removed under reduced pressure and the residue was repeatedly co-evaporated with toluene and subsequently dried *in vacuo*.
For peptide coupling in solution, the carboxylic acid, HOBt and HBTU were dissolved/suspended in DMF or CH_2Cl_2 (4 mL mmol^{-1}) and DIPEA was added. The mixture was stirred at room temperature for approx. 1 min before the deprotected amine, dissolved in DMF or CH_2Cl_2 (8 mL mmol^{-1}), was added. The reaction mixture was typically stirred for 2 to 5 hours at room temperature.

General procedure 2: Thioesterification with PyBOP/DIPEA
Carboxylic acid, PyBOP and thiol was dissolved in dichloromethane (5 mL mmol^{-1}) and cooled to –15. DIPEA was slowly added over 30 minutes at that temperature and the reaction mixture was then stirred at room temperature for further 30 min. The solvents were removed under reduced pressure and the residue was purified by FC (silica).

General procedure 3: Palladium-catalyzed hydrogenolysis of benzyl esters
The benzyl ester was dissolved in EtOAc/MeOH (4:1, 30 mL mmol^{-1}) and pyridine (3 eq.) was added. Palladium (10% on carbon, wetted with ca. 55% water, 0.19 g mmol^{-1} benzyl ester) was added and the mixture was stirred under a hydrogen atmosphere until TLC indicated complete conversion of starting material (30–90 min). The reaction mixture was immediately filtered through a short plug of celite and the solvents were removed under reduced pressure. The residue was repeatedly co-evaporated with toluene to remove any residual pyridine.

General procedure 4: Palladium-catalyzed hydrogenolysis of glycosyl azides
The glycosyl azide was dissolved in MeOH (15 mL mmol^{-1}) and palladium (5% on carbon, wetted with ca. 55% water, 0.11 g mmol^{-1} azide) was added. The mixture was stirred under a hydrogen atmosphere until TLC indicated complete conversion and was then immediately filtered through a short plug of celite or a syringe filter (0.45 μm). In cases the solution was not colorless after filtration, it was stirred for further 5–15 min over activated carbon and was again filtered. The solvent was removed and the residual glycosyl amine was used without further purification.

General procedure 5: Fmoc-SPPS
Fmoc-SPPS was performed manually in a polypropylene syringe with PE frit installed at the outlet. During all steps the syringe was gently shaken. Preloaded dry 2-chlorotrityl polystyrene resin (*cf. general procedure GP8*) was swollen for at least three hours in DMF (10 mL g^{-1}) and afterwards washed with DMF (2 × 1 min, 10 mL g^{-1}). Fmoc deprotection was performed using piperazine (6 w/w% in 0.1 M HOBt (DMF), 5 + 15 min, 10 mL g^{-1})[2]. The deprotection solution was freshly prepared on a daily base and stored at 4 °C between couplings. Before the first coupling the resin was washed with DMF (5 × 1 min, 10 mL g^{-1}). Amino acids (5 eq., 5 eq. HBTU, 5 eq. HOBt, 7.5 eq. DIPEA) were preactivated for approx. 1 minute and coupled for 2 (natural a.a.) or 3 hours (tripeptide building blocks **18a–f**) in DMF (5–10 g mL^{-1}). After coupling, the resin was washed with DMF (5 × 1 min, 10 mL g^{-1}) and the procedure was repeated starting with Fmoc deprotection as mentioned above. In cases the Kaiser Test (*cf. general procedure GP11*) indicated incomplete coupling, the coupling was repeated using the same equivalents as mentioned above with a coupling time of 60 min. During synthesis of the six described decapeptides **21a**–**f** this was always the case for the coupling of Fmoc-Asn(Trt)-OH.
After the last coupling the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min). In cases SPPS had to be stopped the resin was washed with DMF (5 × 1 min) and CH_2Cl_2 (5 × 1 min) and stored at 4 °C overnight.

General procedure 6: One-pot deprotection/N-glycosylation reaction

Fully protected thioaspartic acid-containing peptide (1 eq.) was dissolved in TFA/TIS/H_2O (95:2.5:2.5; 0.2 mL mg^{-1}) and stirred at room temperature for 50–60 min. The solvents were immediately removed *in vacuo*. The residue was co-evaporated with toluene (3 ×) and further dried *in vacuo*.

Subsequently, the peptide thioacid was ligated with glycosyl amines via a slightly modified protocol of Garner and co-workers[3]: A solution of HOBt (2 eq.) and $Cu(OAc)_2 \cdot H_2O$ (1 eq.) in DMF (40 μL μmol^{-1} copper) was prepared by ultrasonication. Peptide thioacid (1 eq.) and glycosyl amine (4 eq.) were dissolved in DMF (40 μL μmol^{-1} peptide thioacid) and the copper solution was immediately added. The resulting mixture was stirred for 30 min at room temperature.

Water was added (0.25 volumes rel. to DMF) and the mixture was stirred for 15 to 30 minutes. The precipitate (CuS) was removed by centrifugation (1 min, 14000 rpm) and washed with DMF three times (3 × 1 min, 14000 rpm). The crude peptide in DMF/water was subjected to RP-HPLC (MeCN/H_2O + 0.1% FA) and subsequently lyophilized to yield a colorless, fluffy solid.

General procedure 7: Loading of 2-chlorotrityl PS resin

Resin loading of 2-chlorotrityl PS resin was performed as described by *Merck Novabiochem*[4] (method 2-3, p. 2.17): After swelling of the 2-chlorotrityl PS resin in dry CH_2Cl_2 (10 mL g^{-1}) for 60 min, carboxylic acid (1.2 eq.) and DIPEA (1.2 eq.) in dry dichloromethane (10 mL g^{-1}) were added. After shaking for 2–3 hours the resin was washed with CH_2Cl_2/MeOH/DIPEA (17:2:1, 3 × 1 min), dichloromethane (3 × 1 min), DMF (2 × 1 min) and CH_2Cl_2 (2 × 1 min). The resin was dried *in vacuo* over KOH or *Silica Gel Orange*.

General procedure 8: Determination of resin loading

Resin loading was determined using the absorbance of 1-((9*H*-fluoren-1-yl)methyl)piperidine at 301 nm. Dry resin (3–4 mg) was shaken in 5 mL 20% piperidine (DMF) for three hours. A dilution series was prepared and the absorbance compared to 20% piperidine (DMF) was measured at 301 nm. A straight calibration line was calculated using the relative concentration and absorbance. According to Beer-Lambert-Law, the concentration of the deprotection solution and thereby the resin loading (mmol g^{-1}) was calculated: $A = c \times d \times \varepsilon$ (A: absorbance, c: concentration [M], d: path length [cm], ε: molar attenuation coefficient at 301 nm, 7800 M^{-1} cm^{-1}). The resin loading was determined to be 0.59–0.88 mmol g^{-1}.

General procedure 9: Analytical resin cleavage

A small amount of pre-swollen (CH_2Cl_2) 2-chlorotrityl polystyrene resin beads were treated with TFA (0.5 mL, 0.5–1% in dichloromethane) for 1 min. The solution was subsequently neutralized with pyridine (10 μL) and the solvents were removed *in vacuo*. Water (2 mL) was added and the solution was immediately lyophilized. The residue was dissolved in MeCN/H_2O (250 μL, 1:1), filtered through a syringe filter (0.45 μm) and analyzed by LC-MS.

General procedure 10: Preparative resin cleavage

Peptide cleavage from 2-chlorotrityl PS resin was performed as described by *Merck Novabiochem*[4] (method 3-30, p. 3.30): The pre-swollen resin (CH_2Cl_2) was treated with TFA (1% in CH_2Cl_2, 2 min, 10 mL g^{-1}) and filtered in a solution of pyridine (10% in methanol, 2 mL g^{-1}). The acid treatment was repeated up to ten times. Subsequently, the resin was washed with dichloromethane (3 x 30 mL g^{-1}), methanol (3 × 30 mL g^{-1}), dichloromethane (2 × 30 mL g^{-1}) and methanol (3 × 30 mL g^{-1}). The combined filtrates were evaporated to dryness. Residual pyridine was removed by co-evaporation with toluene and the crude product was purified by FC (silica) or RP-MPLC.

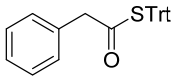
General procedure 11: Kaiser Test for free amino groups in SPPS

A small amount of resin was mixed with each one drop of solution A, B and C (A: 5 g ninhydrin in 100 mL ethanol; B: 2 mL of 0.1 mM $KCN_{(aq.)}$ in 98 mL pyridine; C: 80 g phenol in 20 mL ethanol). The mixture was heated to 100 °C for 5–10 minutes. Blue to brown beads indicated the presence of

remaining free amino groups whereas a yellow color indicated complete coupling and no free amino groups.

3. Synthesis and Evaluation of Thioester Model Compounds S1–S2

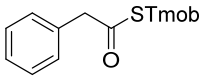
S-Trityl 2-phenylethanethioate (S1)



$C_{27}H_{22}OS$ [394.53 g mol^{-1}]

Phenylacetic acid (33 mg, 0.24 mmol), PyBOP (140 mg, 0.27 mmol) and tritylthiol (74 mg, 0.27 mmol) were dissolved in dichloromethane (1.2 mL) and DIPEA (95 μL, 0.54 mmol) was added. After 1 hour the solvent was removed by rotary evaporation and the crude product was purified by FC (silica, petroleum ether/EtOAc 20:1) to yield the desired thioester **S1** as colorless syrup (89 mg, 93%). **TLC**: R_f = 0.36 (petroleum ether/EtOAc 20:1); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.33 – 7.14 (m, 20H, H_{aryl} (superimposed by solvent)), 3.75 (s, 2H, CH_2); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 194.5 (COS), 143.8 (3 × $C_{quart,Ph}$), 133.7 ($C_{quart,Ph}$), 129.9 (6 × C_{aryl}), 129.5 (2 × C_{aryl}), 128.7 (2 × C_{aryl}), 127.9 (6 × C_{aryl}), 127.4 (C_{aryl}), 127.2 (3 × C_{aryl}), 70.8 ($\underline{C}Ph_3$), 50.6 (CH_2).

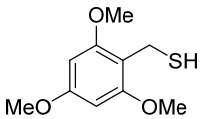
S-(2,4,6-trimethoxybenzyl) 2-phenylethanethioate (S2)



$C_{18}H_{20}O_4S$ [332.41 g mol^{-1}]

Phenylacetic acid (100 mg, 0.73 mmol) and TmobSH (**S3**) (314 mg, 1.47 mmol) were dissolved in dichloromethane (4 mL) and the mixture was cooled to 0 °C. DCC (167 mg, 0.81 mmol) and DMAP (9 mg, 0.07 mmol) were added. The mixture was allowed to warm to room temperature and was stirred for a total of 2 hours. The mixture was filtered and the solvent was removed by evaporation. The crude product was purified by FC (silica, petroleum ether/EtOAc 6:1) to yield the desired thioester **S2** as colorless solid (197 mg, 81%). **TLC**: R_f = 0.28 (petroleum ether/EtOAc 6:1); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.36 – 7.22 (m, 5 H, H_{aryl} (superimposed by solvent)), 6.09 (s, 2H, $H_{aryl,Tmob}$), 4.21 (s, 2H, CH_2S), 3.81 (s, 2H, CH_2CO), 3.79 (s, 3H, OMe), 3.77 (s, 6H, 2 × OMe); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 198.0 (CO), 160.9 ($C_{quart,OMe}$), 159.3 (2 × $C_{quart,OMe}$), 134.2 ($C_{quart,Ph}$), 129.7, 128.6, 127.3 (5 × C_{aryl}), 104.9 ($C_{quart,Tmob}$), 90.7 (2 × $C_{aryl,Tmob}$), 55.9 (2 × OMe), 55.5 (OMe), 50.4 ($\underline{C}H_2CO$), 22.6 (CH_2S); **HRMS:** m/z calcd. for $C_{18}H_{20}O_4SNa^+$: 355.0975 [*M*+Na]$^+$, found: 355.0967.

2,4,6-Trimethoxybenzyl thiol (TmobSH) (S3)



$C_{10}H_{14}O_3S$ [214.28 g mol^{-1}]

2,4,6-Trimethoxybenzaldehyde (25.0 g, 127 mmol) was suspended in ethanol (175 mL) and $NaBH_4$ (5.3 g, 140 mmol) was slowly added. The mixture was stirred for 30 minutes before the solvent was removed *in vacuo*. The residue was dissolved in water (175 mL) and was extracted with Et_2O (4 × 100 mL). The combined organic phases were washed with water (2 × 150 mL), dried ($MgSO_4$) and the solvent was removed by evaporation. The crude benzylic alcohol was further reacted to the thiol **S3** as descried by Vetter[5]. After FC (silica, petroleum ether/EtOAc 5:1) TmobSH (**S3**) was obtained as colorless solid (7.97 g, 68% over two steps). Spectroscopic data was in accordance with literature.

Stability of phenylacetic acid thioesters **S1** and **S2**.

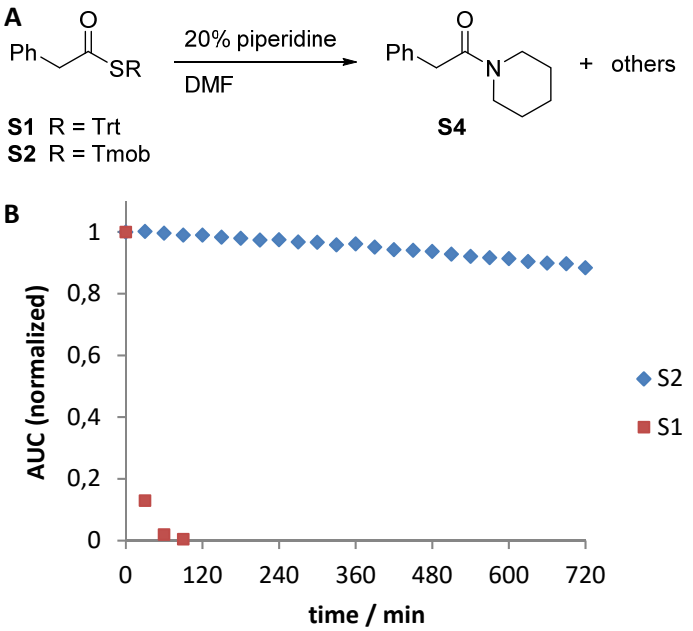
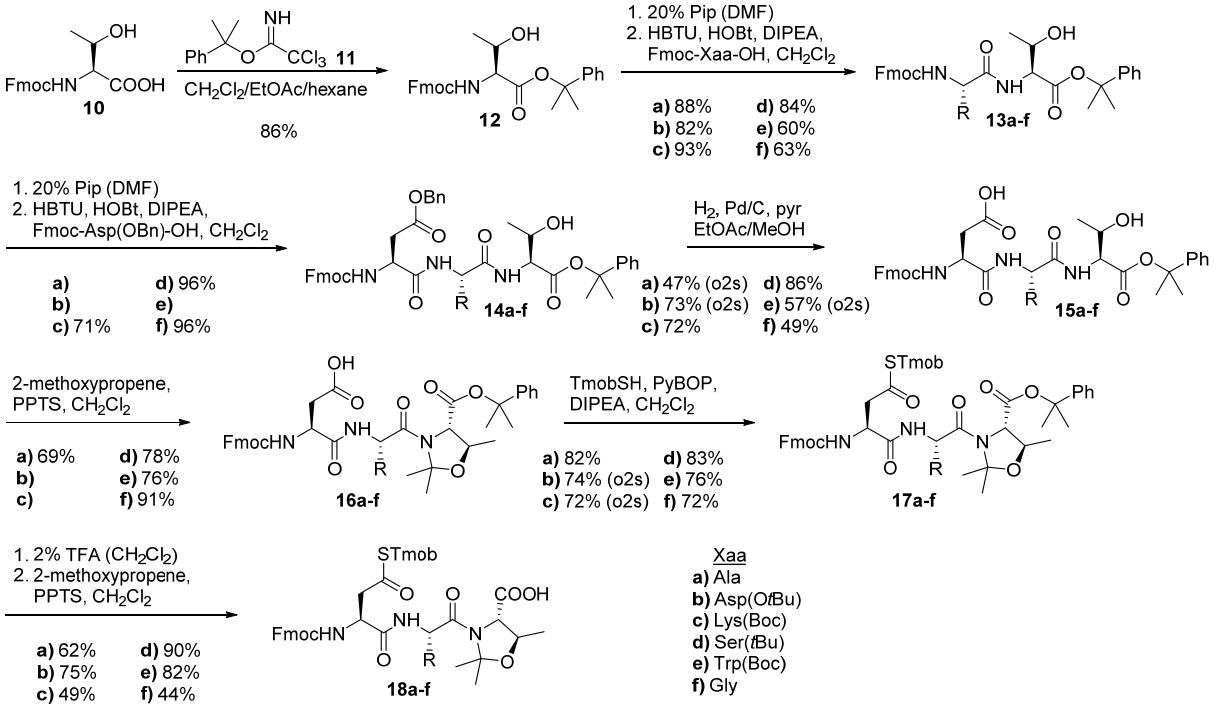


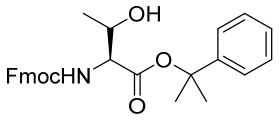
Figure S1. A Trityl **S1** or Tmob **S2** protected thioester was dissolved in 20% piperidine in DMF (final concentration of thioester: 10 μmol mL^{-1}) and the reaction mixture was analyzed by LC-MS every 30 minutes. **B** The decline of starting material **S1** or **S2** at 254 nm was plotted against time by integration of the corresponding peak (AUC: area under curve).

4. Synthesis of Tripeptide Building Blocks 18a–f



Scheme S1. Synthesis of the six aspartic thioacid-containing tripeptide building blocks **18a–f**. Xaa = **a**: Ala, **b**: Asp(O*t*Bu), **c**: Lys(Boc), **d**: Ser(*t*Bu), **e**: Trp(Boc), **f**: Gly.

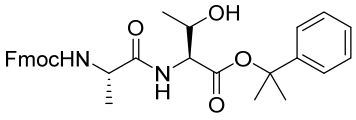
Fmoc-Thr(OH)-*O*-2-Ph*i*Pr (12)



$C_{28}H_{29}NO_5$ [459.54 g mol^{-1}]

Trichloroacetimidate **11** was prepared according to Wessel *et al*.[6] using 2-phenylpropan-2-ol (3.7 g, 27.2 mmol, 1 eq.), NaH (109 mg, 60% dispersion in mineral oil, 2.7 mmol, 0.1 eq) and trichloroacetonitrile (2.6 mL, 25.8 mmol, 0.95 eq.) in dry Et_2O (8.8 mL). The trichloroacetimidate was obtained as yellowish solution in hexane (~0.75 M) which was used for esterification without further purification. Therefore, Fmoc-Thr(OH)-OH (**10**) (6.15 g, 13.6 mmol, 0.5 eq.) was dissolved in EtOAc/CH_2Cl_2 (135 mL, 1:1) and the solution of imidate **11** was added. After 1.5 hours the solvents were evaporated under reduced pressure and the residue was dissolved in dichloromethane (200 mL). The organic layer was washed with $NaOH_{(aq.)}$ (2 × 650 mL, 0.2 M (removal of trichloroacetamide)), dried ($MgSO_4$) and filtered. After removal of the solvent, the brown syrup was purified by FC (silica, petroleum ether/EtOAc/toluene 7.5:2.5:1) to yield a faint yellow foam (5.341 g, 86%). **TLC**: R_f = 0.28 (petroleum ether/EtOAc/toluene 7.5:2.5:1; UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.77 (δ, *J* = 7.5 Hz, 2H, H_{aryl}), 7.63–7.55 (m, 2H, H_{aryl}), 7.44–7.22 (m, 9H, H_{aryl} (superimposed by solvent)), 5.52 (d, *J* = 9.1 Hz, 1H, NH), 4.54–4.36 (m, 3H, $CH_{2,Fmoc}$ & CH_β), 4.32 (d, *J* = 8.9 Hz, 1H, CH_α), 4.23 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 1.89 (d, *J* = 5.1 Hz, 1H, OH), 1.82 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.81 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.26 (d, *J* = 6.3 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 169.8 (CO_{Thr}), 156.9 (COONH), 145.0 ($C_{quart,Ph}$), 144.0, 143.9, 141.4 (4 × $C_{quart,Fmoc}$), 128.5, 127.9, 127.5, 127.2, 125.2, 124.4, 120.13, 120.11 (13 × C_{aryl}), 83.9 ($C_{quart,\mathit{Phi}Pr}$), 68.3 (CH_β), 67.3 ($CH_{2,Fmoc}$), 59.5 (CH_α), 47.3 (CH_{Fmoc}), 28.7, 28.5 (2 × $CH_{3,Ph\mathit{i}Pr}$), 20.0 ($CH_{3,Thr}$); **HRMS**: m/z calculated for $C_{28}H_{29}NO_5Na^+$: 482.19379 [*M*+Na]$^+$, found: 482.19171.

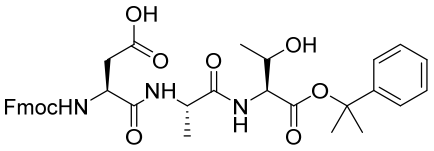
Fmoc-Ala-Thr(OH)-*O*-2-Ph*i*Pr (13a)



$C_{31}H_{34}N_2O_6$ [530.62 g mol^{-1}]

According to *general procedure 1*, Fmoc-Thr(OH)-*O*-2-Ph*i*Pr (**12**) (5.3 g, 11.54 mmol, 1 eq.) was deprotected with piperidine (115 mL, 20% in DMF) and reacted with Fmoc-Ala-OH (4.31 g, 13.85 mmol, 1.2 eq.) using HOBt (1.87g, 13.85 mmol, 1.2 eq), HBTU (5.25 g, 13.85 mmol, 1.2 eq.) and DIPEA (4.83 mL, 27.7 mmol, 2.4 eq.) in dichloromethane (150 mL) for 3.5 hours. The reaction mixture was extracted with sat. $NH_4Cl_{(aq.)}$ (200 mL), $NaOH_{(aq.)}$ (200 mL, 1 M) and H_2O (2 × 200 mL). After drying ($MgSO_4$) the solvent was removed under reduced pressure and the crude product was purified by FC (silica, petroleum ether/EtOAc 1:1). Dipeptide **13a** was obtained as colorless foam (5.566 g, 88%). **TLC**: R_f = 0.29 (petroleum ether/EtOAc 1:1; UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.75 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.57 (d, *J* = 7.4 Hz, 2H, H_{aryl}), 7.44–7.16 (m, 9H, H_{aryl} (superimposed by solvent)), 6.73 (d, *J* = 9.0 Hz, 1H, NH_{Thr}), 5.53 (d, *J* = 7.5 Hz, 1H, NH_{Ala}), 4.56 (dd, *J* = 9.0 Hz, 2.6 Hz, 1H, $CH_{\alpha,Thr}$), 4.43–4.33 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.33–4.24 (m, 1H, CH_{Ala}), 4.18 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 2.37 (br. s, 1H, OH), 1.80 (s, 3H, $CH_{3,Ph\textit{i}Pr}$), 1.78 (s, 3H, $CH_{3,Ph\textit{i}Pr}$), 1.38 (d, *J* = 6.9 Hz, 3H, $CH_{3,Ala}$), 1.19 (d, *J* = 6.9 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 173.0 (CO_{Ala}), 169.4 (CO_{Thr}), 156.1 (COONH), 145.0 ($C_{quart,Ph}$), 144.0, 141.4 (4 × $C_{quart,Fmoc}$), 128.5, 127.8, 127.4, 127.2, 125.2, 124.4, 120.1 (13 × C_{aryl}), 83.8 ($C_{quart,Ph\textit{i}Pr}$), 68.3 ($CH_{\beta,Thr}$), 67.3 ($CH_{2,Fmoc}$), 57.9 ($CH_{\alpha,Thr}$), 50.8 (CH_{Ala}), 47.2 (CH_{Fmoc}), 28.8, 28.4 (2 × $CH_{3,Ph\textit{i}Pr}$), 20.2 ($CH_{3,Thr}$), 18.9 ($CH_{3,Ala}$); **HRMS**: m/z calculated for $C_{31}H_{34}N_2O_6Na^+$: 553.23091 [*M*+Na]$^+$, found: 553.22961.

Fmoc-Asp(OH)-Ala-Thr(OH)-*O*-2-Ph*i*Pr (15a)



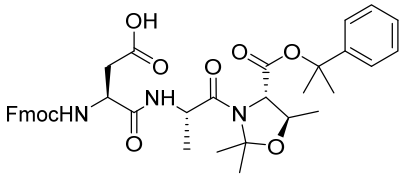
$C_{35}H_{39}N_3O_9$ [645.71 g mol^{-1}]

According to *general procedure 1*, Fmoc-Ala-Thr(OH)-*O*-2-Ph*i*Pr (**13a**) (1.92 g, 3.62 mmol, 1 eq.) was deprotected with piperidine (37 mL, 20% in DMF) and reacted with Fmoc-Asp(OBn)-OH (1.97 g, 4.42 mmol, 1.2 eq.) using HOBt (0.60 g, 4.42 mmol, 1.2 eq.), HBTU (1.68 g, 4.42 mmol, 1.2 eq.) and DIPEA (1.41 mL, 8.11 mmol, 2.4 eq.) in dichloromethane (30 mL) for 3.5 hours. The solvent was removed under reduced pressure and the residue was purified by FC (silica, petroleum ether/EtOAc 2:3) to yield dipeptide **14a** as colorless foam (1.93 g) with still minor impurities. It was used for forward reaction sequence without further purification.

According to *general procedure 3*, benzyl-protected tripeptide **14a** (1.89 g, 2.56 mmol) was hydrogenated for 30 minutes. The product was purified by FC (silica, dichloromethane/methanol 10:1) to yield tripeptide **15a** as off-white foam (1.10g, 47% over two steps). **TLC**: R_f = 0.34 (dichloromethane/methanol 10:1; UV/vanillin); **^{1}H NMR** (methanol-d4, 400 MHz): δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.66 – 7.58 (m, 2H, H_{aryl}), 7.40 – 7.32 (m, 4H, H_{aryl}), 7.31 – 7.24 (m, 4H, H_{aryl}), 7.21 – 7.15 (m, 1H, H_{aryl}), 4.53 (t, *J* = 7.1 Hz, 1H, CH_{Asp}), 4.49 (q, *J* = 7.1 Hz, 1H, CH_{Ala}), 4.40 – 4.27 (m, 4H, $CH_{2,Fmoc}$, $CH_{\alpha,Thr}$ & $CH_{\beta,Thr}$), 4.18 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 2.80 (dd, *J* = 16.5 Hz, 6.5 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.65 (dd, *J* = 16.4 Hz, 7.0 Hz, 1H, CH<u>H</u>'$_{Asp}$), 1.75 (s, 6H, 2 × $CH_{3,Ph\textit{i}Pr}$), 1.38 (d, *J* = 7.1 Hz, 3H, $CH_{3,Ala}$), 1.19 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** (methanol-d4, 101 MHz): δ [ppm] = 176.6 (COOH) 175.2 (CO_{Ala}), 173.7 (CO_{Asp}), 170.5 (CO_{Thr}), 158.4 (COONH), 146.7 ($C_{quart,Ph}$), 145.21, 145.16, 142.5 (4 × $C_{quart,Fmoc}$), 129.2, 128.8, 128.2, 127.9, 126.2, 125.5, 120.9 (13 × C_{aryl}), 84.5 ($C_{quart,Ph\textit{i}Pr}$), 68.4, 68.2 ($CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 60.3 ($CH_{\alpha,Thr}$), 53.4 (CH_{Asp}), 50.4 (CH_{Ala}), 48.3 (CH_{Fmoc}), 38.8 ($CH_{2,Asp}$), 29.05, 28.97 (2 ×

$CH_{3,Phi Pr}$), 20.6 ($CH_{3,Thr}$), 18.1 ($CH_{3,Ala}$); **HRMS:** m/z calcd. for $C_{35}H_{40}N_3O_9^+$: 646.27591 [*M*+H]$^+$, found: 646.27526.

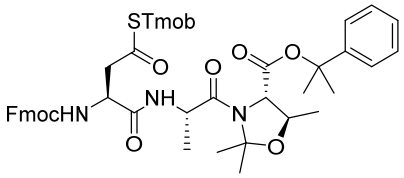
Fmoc-Asp(OH)-Ala-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (16a)



$C_{38}H_{43}N_3O_9$ [685.77 g mol^{-1}]

Threonine-containing tripeptide **15a** (1.90 g, 2.94 mmol, 1 eq.) was suspended in dry dichloromethane (41 mL) and 2-methoxypropene (2.95 mL, 58.83 mmol, 20 eq.), followed by MS 4 Å, was added. The mixture was cooled to 0 °C, PPTS (370 mg, 1.47 mmol, 0.5 eq.) was added and the reaction was allowed to warm to room temperature. After 3 hours the solution was washed with water (3 × 30 mL), was dried ($MgSO_4$) and the solvent was removed by evaporation. The crude product was purified by FC (silica, dichloromethane/methanol 20:1) to yield pseudoproline-protected tripeptide **16a** as colorless foam (1.39 g, 69%); **TLC**: R_f = 0.11 (dichloromethane/methanol 20:1; UV/vanillin); **HRMS:** m/z calcd. for $C_{38}H_{43}N_3O_9Na^+$: 708.2892 [*M*+Na]$^+$, found: 708.2890.

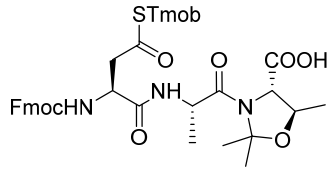
Fmoc-Asp(STmob)-Ala-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (17a)



$C_{48}H_{55}N_3O_{11}S$ [882.04 g mol^{-1}]

Tripeptide **16a** (1.38 g, 2,01 mmol, 1 eq.), TmobSH (473 mg, 2.21 mmol, 1.1 eq.) and PyBOP (1.05 g, 2.01 mmol, 1 eq.) were dissolved in dichloromethane (10.2 mL) and the solution was cooled to 0 °C. DIPEA (10.8 mL, 6.23 mmol, 3.1 eq.) was added dropwise at 0 °C and the reaction mixture was then allowed to warm to room temperature. After 40 minutes the mixture was diluted with dichloromethane (20 mL) and was washed with sat. $NH_4Cl_{(aq.)}$ (2 × 30 mL), sat. $NaHCO_{3(aq.)}$ (30 mL) and sat. $NaCl_{(aq.)}$ (30 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The crude product was purified by FC (silica, petroleum ether/EtOAc 2:1) to yield fully protected tripeptide **17a** as colorless foam (1.46 g, 82%). **TLC**: R_f = 0.18 (petroleum ether/EtOAc 2:1; UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = 7.80 – 7.72 (m, 2H, H_{aryl}), 7.60 (d, *J* = 7.2 Hz, 2H, H_{aryl}), 7.46 – 7.18 (m, 10H, H_{aryl} & NH_{Ala} (superimposed by solvent)), 6.08 (s, 2H, $H_{aryl,Tmob}$), 5.83 (d, *J* = 8.7 Hz, 1H, NH_{Asp}), 4.60 – 4.47 (m, 1H, $CH_{\alpha,Asp}$), 4.47 – 4.18 (m, 7H, $CH_{2,Fmoc}$, CH_{Fmoc}, $CH_{2,Tmob}$, $CH_{\alpha,Ala}$, $CH_{\beta,Thr}$), 4.01 (d, *J* = 6.1 Hz, 1H, $CH_{\alpha,Thr}$), 3.78 (s, 3H, OMe), 3.77 (s, 6H, 2 × OMe), 3.30 – 3.19 (m, 1H, C<u>H</u>H'$_{Asp}$), 2.87 (dd, *J* = 16.3, 4.6 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.81 (s, 3H, $CH_{3,Phi Pr}$), 1.79 (s, 3H, $CH_{3,Phi Pr}$), 1.66 (s, 3H, $CH_{3,pseudoproline}$), 1.54 (s, 3H, $CH_{3,pseudoproline}$), 1.50 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$), 1.35 (d, *J* = 6.4 Hz, 3H, $CH_{3,Ala}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 198.5 (COS), 170.1 (CO_{Ala}), 168.9 (CO_{Asp}), 168.3 (CO_{Thr}), 161.0 ($C_{quart,Tmob}$), 159.3 (2 × $C_{quart,Tmob}$), 156.0 (COONH), 144.4 ($C_{quart,Ph}$), 144.0, 143.8, 141.4 (4 × $C_{quart,Fmoc}$), 128.6, 128.4, 127.8, 127.7, 127.3, 125.3, 124.4, 120.1 (13 × C_{aryl}), 104.5 ($C_{quart,Tmob}$), 97.2 ($C_{quart,pseudoproline}$), 90.7 (2 × $C_{aryl,Tmob}$), 84.9 ($C_{quart,Phi Pr}$), 75.0 ($CH_{\beta,Thr}$), 67.6 ($CH_{2,Fmoc}$), 65.8 ($CH_{\alpha,Thr}$), 55.9 (2 × OMe), 55.5 (OMe), 52.0 ($CH_{\alpha,Asp}$), 48.7 ($CH_{\alpha,Ala}$), 47.3 (CH_{Fmoc}), 44.8 ($CH_{2,Asp}$), 27.9, 26.5 (2 × $CH_{3,Phi Pr}$), 23.9, 22.5 (2 × $CH_{3,pseudoproline}$), 20.4 ($CH_{3,Thr}$) 19.0 ($CH_{3,Ala}$); **HRMS:** m/z calcd. for $C_{48}H_{56}N_3O_{11}S^+$: 882.36301 [*M*+H]$^+$, found: 882.36107.

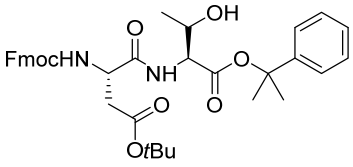
Fmoc-Asp(STmob)-Ala-Thr($\Psi^{Me,Me}$pro)-OH (18a)



$C_{39}H_{45}N_3O_{11}S$ [763.86 g mol^{-1}]

C-terminal protected tripeptide **17a** (5.00 g, 5.67 mmol) was dissolved in TFA (163 mL, 2% in dichloromethane) and stirred at room temperature for 15 min until TLC indicated complete disappearance of starting material. The reaction mixture was immediately diluted with toluene (160 mL) and the solvents were removed *in vacuo*. The residue was purified by FC (silica, dichloromethane/methanol 20:1→10:1→5:1) to yield a slightly brown solid which was subsequently dissolved in dry dichloromethane (80 mL). 2-Methoxypropene (5.43 mL, 56.7 mmol, 10 eq.) and PPTS (130 mg, 0.57 mmol, 0.1 eq.) were added and the mixture was stirred at room temperature for a total of 27 hours while adding further portions of 2-methoxypropene (10 eq.) after 2.5, 5 and 22 hours, respectively. The solution was diluted with dichloromethane (50 mL) and subsequently shaken with sat. $NaHCO_{3(aq.)}$ (50 mL). The colorless precipitate was filtered, washed with petroleum ether (600 mL) and dried *in vacuo* before being further purified by FC (silica, dichloromethane/methanol 10:1→8:1) to yield alanine-containing tripeptide building block **18a** as off-white solid (2.67 g, 62%). **TLC**: R_f = 0.29 (dichloromethane/methanol/toluene 10:1:1; UV/vanillin); **^{1}H NMR** (methanol-d4, 400 MHz, major conformer): δ [ppm] = 7.77 – 7.74 (m, 2H, H_{aryl}), 7.67 – 7.60 (m, 2H, H_{aryl}), 7.40 – 7.33 (m, 2H, H_{aryl}), 7.33 – 7.25 (m, 2H, H_{aryl}), 6.09 (s, 2H, $H_{aryl,Tmob}$), 4.66 (dd, *J* = 9.0 Hz, 4.3 Hz, 1H, CH_{Asp}), 4.47 (dd, *J* = 6.9 Hz, 6.8 Hz, 1H, CH_{Ala}), 4.42 – 4.29 (m, 2H, C<u>H</u>H'$_{Fmoc}$ & $CH_{\beta,Thr}$), 4.29 – 4.11 (m, 4H, CH<u>H</u>'$_{Fmoc}$, CH_{Fmoc} & $CH_{2,Tmob}$), 4.08 (d, *J* = 6.5 Hz, 1H, $CH_{\alpha,Thr}$), 3.72 (s, 3H, OMe), 3.70 (s, 6H, 2 × OMe), 3.06 (dd, *J* = 15.9 Hz, 4.3 Hz, 1H C<u>H</u>H'$_{Asp}$), 3.00 (dd, *J* = 15.9 Hz, 9.0 Hz, 1H CH<u>H</u>'$_{Asp}$), 1.59 (s, 3H, $CH_{3,pseudoproline}$), 1.55 (s, 3H, $CH_{3,pseudoproline}$), 1.43 (d, *J* = 6.0 Hz, 3H, $CH_{3,Thr}$), 1.28 (d, *J* = 6.7 Hz, 3H, $CH_{3,Ala}$); **^{13}C NMR** (methanol-d4, 101 MHz, major conformer): δ [ppm] = 198.4 (COS), 175.1 (COOH), 172.1, 172.0 (CO_{Asp} & CO_{Ala}), 162.5 ($C_{quart,Tmob}$), 160.5 (2 × $C_{quart,Tmob}$), 158.1 (COONH), 145.3, 145.2, 142.54, 142.50 (4 × $C_{quart,Fmoc}$), 128.8, 128.2, 126.4, 126.3, 120.9 (8 × $C_{aryl,Fmoc}$), 105.4 ($C_{quart,Tmob}$), 97.8 ($C_{quart,pseudoproline}$), 91.6 (2 × $C_{aryl,Tmob}$), 76.8 ($CH_{\beta,Thr}$), 68.4 ($CH_{\alpha,Thr}$), 68.1 ($CH_{2,Fmoc}$), 56.3 (2 × OMe), 55.7 (OMe), 53.2 (CH_{Asp}), 49.8 (CH_{Ala}), 48.3 (CH_{Fmoc}), 45.8 ($CH_{2,Asp}$), 26.7, 24.2 (2 × $CH_{3,pseudoproline}$), 23.1 ($CH_{2,Tmob}$), 20.3 ($CH_{3,Thr}$), 18.6 ($CH_{3,Ala}$); **HRMS:** m/z calcd. for $C_{39}H_{46}N_3O_{11}S^+$: 764.28476 [*M*+H]$^+$, found: 764.28256.

Fmoc-Asp(O*t*Bu)-Thr(OH)-*O*-2-Ph*i*Pr (13b)

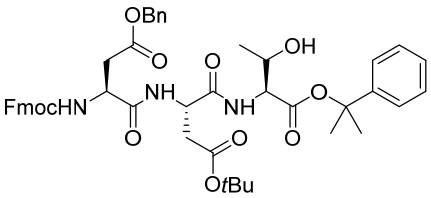


$C_{36}H_{42}N_2O_8$ [630.74 g mol^{-1}]

According to *general procedure 1*, threonine **10** (10.21 g, 22.2 mmol, 1 eq.) was deprotected with piperidine (220 mL, 20% in DMF) and subsequently reacted with Fmoc-Asp(O*t*Bu)-OH (10.97 g, 26.7 mmol, 1.2 eq.), HOBt (3.6 g, 26.7 mmol, 1.2 eq), HBTU (10.12 g, 26.7 mmol, 1.2 eq.) and DIPEA (9.3 mL, 53.3 mmol, 2.4 eq.) in dichloromethane (285 mL) for 4 hours. The reaction mixture was washed with sat. $NaHCO_{3(aq.)}$ (3 × 150 mL), H_2O (3 × 150 mL) and sat. $NaCl_{(aq.)}$ (150 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The product was purified by FC (silica, petroleum ether/EtOAc 3:1→2:1→1:1) to yield a colorless foam (11.52 g, 82%). **TLC**: R_f = 0.18 (petroleum ether/EtOAc 2:1; UV); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.78 – 7.72 (m,

2H, H_{aryl}), 7.61 – 7.53 (m, 2H, H_{aryl}), 7.43 – 7.20 (m, 9H, H_{aryl}), 7.16 (d, *J* = 8.8 Hz, 1H, NH_{Thr}), 6.01 (d, *J* = 8.7 Hz, 1H, NH_{Asp}), 4.67 – 4.58 (m, 1H, $CH_{\alpha,Asp}$), 4.55 (dd, *J* = 8.8, 2.7 Hz, 1H, $CH_{\alpha,Thr}$), 4.45 – 4.33 (m, 3H, $CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 4.21 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 2.96 (dd, *J* = 17.1, 4.7 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.64 (dd, *J* = 17.8, 5.6 Hz, 1H, CH<u>H'</u>$_{Asp}$), 2.14 (s, 1H, OH), 1.80 (s, 3H, $CH_{3,PhiPr}$), 1.78 (s, 3H, $CH_{3,PhiPr}$), 1.43 (s, 9H, 3 × $CH_{3,tBu}$), 1.23 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 171.3, 171.1 (CO_{Asp} & COO*t*Bu), 169.1 (CO_{Thr}), 156.1 (COONH), 145.1 ($C_{quart,Ph}$), 143.9, 143.8, 141.4 (4 × $C_{quart,Fmoc}$), 128.5, 127.9, 127.4, 127.23, 127.22, 125.20, 125.19, 124.4, 120.11, 120.10 (13 × C_{aryl}), 83.8 ($C_{quart,Ph\mathit{i}Pr}$), 82.1 ($C_{quart,tBu}$), 68.5 ($CH_{\beta,Thr}$), 67.5 ($CH_{2,Fmoc}$), 58.1 ($CH_{\alpha,Thr}$), 51.5 ($CH_{\alpha,Asp}$), 47.2 (CH_{Fmoc}), 37.7 ($CH_{2,Asp}$), 28.7, 28.4 (2 × $CH_{3,Ph\mathit{i}Pr}$), 28.2 (3 × $CH_{3,tBu}$), 20.1 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{36}H_{42}N_2O_8Na^+$: 653.2833 [*M*+Na]$^+$, found: 653.2828.

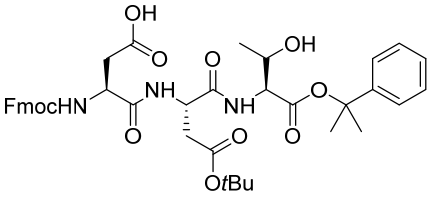
Fmoc-Asp(OBn)-Asp(O*t*Bu)-Thr(OH)-*O*-2-Ph*i*Pr (14b)



$C_{47}H_{53}N_3O_{11}$ [835.95 g mol^{-1}]

According to *general procedure 1*, dipeptide **13b** (11.49 g, 19.1 mmol, 1 eq.) was deprotected with piperidine (205 mL, 20% in DMF) and reacted with Fmoc-Asp(OBn)-OH (10.20 g, 22.9 mmol, 1.2 eq.), HOBt (3.09 g, 22.9 mmol, 1.2 eq), HBTU (8.68 g, 22.9 mmol, 1.2 eq.) and DIPEA (7.97 mL, 45.8 mmol, 2.4 eq.) in dichloromethane (245 mL) for 4 hours. The reaction mixture was washed with sat. $NaHCO_{3(aq.)}$ (3 × 100 mL) and H_2O (3 × 100 mL). After drying ($MgSO_4$) of the organic phase, the solvent was evaporated. The residue was purified by FC (silica, petroleum ether/EtOAc 3:1→2:1→3:2→1:1) to yield tripeptide **14b** as colorless foam (15.29 g, 96%). **TLC**: R_f = 0.19 (petroleum ether/EtOAc 3:2; UV); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.68 (d, *J* = 8.6 Hz, 1H, NH_{Asp1}), 7.58 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.44 – 7.24 (m, 13H, H_{aryl}), 7.21 – 7.10 (m, 2H, H_{aryl} & NH_{Thr}), 5.81 (d, *J* = 7.9 Hz, 1H, NH_{Asp2}), 5.09 (d, *J* = 12.3 Hz, 1H, C<u>H</u>H'$_{Bn}$), 4.99 (d, *J* = 12.3 Hz, 1H, C<u>H</u>H'$_{Bn}$), 4.80 (ddd, *J* = 8.7, 4.8, 4.8 Hz, 1H, $CH_{\alpha,Asp1}$), 4.60 (dd, *J* = 9.1, 2.8 Hz, 1H, $CH_{\alpha,Thr}$), 4.57 – 4.49 (m, 1H, $CH_{\alpha,Asp2}$), 4.49 – 4.31 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.21 (t, *J* = 6.9 Hz, 1H, CH_{Fmoc}), 3.08 – 2.95 (m, 2H, C<u>H</u>H'$_{Asp1}$ & C<u>H</u>H'$_{Asp2}$), 2.88 (dd, *J* = 17.2, 6.9 Hz, 1H, CH<u>H'</u>$_{Asp2}$), 2.58 (dd, *J* = 17.2, 5.3 Hz, 1H, CH<u>H'</u>$_{Asp1}$), 1.79 (s, 3H, $CH_{3,PhiPr}$), 1.78 (s, 3H, $CH_{3,PhiPr}$), 1.40 (s, 9H, 3 × $CH_{3,tBu}$), 1.21 (d, *J* = 6.5 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 171.9, 171.6 (CO_{Bn} & CO), 170.4, 170.2 (2 × CO), 168.9 (CO_{Thr}), 156.2 (COONH), 145.3 ($C_{quart,Ph\mathit{i}Pr}$), 143.9, 143.6, 141.44, 141.42 (4 × $C_{quart,Fmoc}$), 135.2 ($C_{quart,Bn}$), 128.7, 128.6, 128.5, 128.4, 127.92, 127.91, 127.25, 127.24, 125.2, 124.5, 120.1 (18 × C_{aryl}), 83.5 ($C_{quart,Ph\mathit{i}Pr}$), 82.0 ($C_{quart,tBu}$), 68.9 ($CH_{\beta,Thr}$), 67.7 ($CH_{2,Fmoc}$), 67.4 ($CH_{2,Bn}$), 58.3 ($CH_{\alpha,Thr}$), 51.7 ($CH_{\alpha,Asp2}$), 49.9 ($CH_{\alpha,Asp1}$), 47.2 (CH_{Fmoc}), 36.4, 36.3 ($CH_{2,Asp1}$ & $CH_{2,Asp2}$), 28.7, 28.5 (2 × $CH_{3,Ph\mathit{i}Pr}$), 28.1 (3 × $CH_{3,tBu}$), 20.1 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{47}H_{53}N_3O_{11}Na^+$: 858.3572 [*M*+Na]$^+$, found: 858.3573.

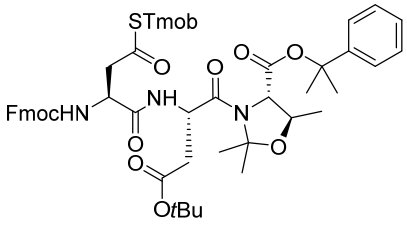
Fmoc-Asp(OH)-Asp(O*t*Bu)-Thr(OH)-*O*-2-Ph*i*Pr (15b)



$C_{40}H_{47}N_3O_{11}$ [745.83 g mol^{-1}]

Benzyl-protected tripeptide **14b** (9.96 g, 13.36 mmol) was hydrogenated for 90 min as described in *general procedure 3*. The crude peptide was purified by FC (silica, dichloromethane/methanol 15:1→10:1) to yield aspartic acid peptide **15b** as colorless foam (9.99 g, 73%). **TLC**: R_f = 0.32 (dichloromethane/methanol 10:1; UV/vanillin); **^{1}H NMR** (methanol-d4, 400 MHz): δ [ppm] = 7.78 (dt, *J* = 7.6, 0.9 Hz, 2H, H_{aryl}), 7.65 (d, *J* = 7.2 Hz, 2H, H_{aryl}), 7.42 – 7.33 (m, 4H, H_{aryl}), 7.34 – 7.23 (m, 4H, H_{aryl}), 7.23 – 7.14 (m, 1H, H_{aryl}), 4.80 (dd, *J* = 6.7, 5.9 Hz, 1H, $CH_{\alpha,Asp2}$), 4.52 (dd, *J* = 7.4, 5.9 Hz, 1H, $CH_{\alpha,Asp1}$), 4.41 – 4.26 (m, 4H, $CH_{\alpha,Thr}$, $CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 4.21 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 2.89 (dd, *J* = 16.9, 5.9 Hz, 1H, C<u>H</u>H'$_{Asp1}$), 2.80 (dd, *J* = 16.5, 5.8 Hz, 1H, C<u>H</u>H'$_{Asp2}$), 2.72 (dd, *J* = 16.8, 7.5 Hz, 1H, CH<u>H'</u>$_{Asp1}$), 2.69 (dd, *J* = 16.5, 6.9 Hz, 1H, CH<u>H'</u>$_{Asp2}$), 1.75 (s, 3H, $CH_{3,PhiPr}$), 1.75 (s, 3H, $CH_{3,PhiPr}$), 1.38 (s, 9H, 3 × $CH_{3,tBu}$), 1.18 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** (methanol-d4, 101 MHz): δ [ppm] = 174.1 (COOH), 173.3 (CO_{Asp1}), 172.9 (CO_{Asp2}), 171.7 (COO*t*Bu), 170.2 (CO_{Thr}), 158.4 (COONH), 146.7 ($C_{quart,Ph}$), 145.2, 145.1, 142.5 (4 × $C_{quart,Fmoc}$), 129.2, 128.8, 128.2, 127.9, 126.27, 126.25, 125.5, 120.9 (13 × C_{aryl}), 84.5 ($C_{quart,PhiPr}$), 82.5 ($C_{quart,tBu}$), 68.6, 68.3 ($CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 60.2 ($CH_{\alpha,Thr}$), 53.1 ($CH_{\alpha,Asp2}$), 51.3 ($CH_{\alpha,Asp1}$), 48.3 (CH_{Fmoc}), 37.8 ($CH_{2,Asp2}$), 36.8 ($CH_{2,Asp1}$), 29.1, 28.9 (2 × $CH_{3,PhiPr}$), 28.3 (3 × $CH_{3,tBu}$), 20.5 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{40}H_{47}N_3O_{11}Na^+$: 768.3103 [*M*+Na]$^+$, found: 768.3119.

Fmoc-Asp(STmob)-Asp(O*t*Bu)-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (17b)



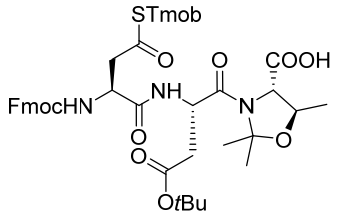
$C_{53}H_{63}N_3O_{13}S$ [982.16 g mol^{-1}]

Threonine-containing tripeptide **15b** (9.96 g, 13.36 mmol, 1 eq.) was suspended in dry dichloromethane (175 mL), 2-methoxypropene (12.8 mL, 133.6 mmol, 10 eq.) was added and the mixture was cooled to 0 °C prior to the addition of PPTS (335 mg, 1.34 mmol, 0.1 eq.). The solution was then allowed to warm to room temperature. After a total reaction time of 2, 4, 6.5 and 23 hours, respectively, further 2-methoxypropene (6.4 mL, 56.8 mmol, 5 eq.) and PPTS (168 mg, 0.67 mmol, 0.05 eq.) were added. After 27 hours, the solvent was removed and the residue was co-evaporated with toluene. The crude product was purified by FC (silica, dichloromethane/methanol 30:1→20:1→15:1→10:1) to yield a yellow foam which was used for forward conversion with still minor impurities.

Aspartic acid-containing tripeptide **16b** (10.49 g, 13.36 mmol, 1 eq.) was reacted with PyBOB (7.65 g, 14.69 mmol, 1.1 eq.), TmobSH (3.15 g, 14.69 mmol, 1.1 eq.) and DIPEA (3.80 g, 29.39 mmol, 2.2 eq.) according to *general procedure 2*. The product was purified by FC (silica, petroleum ether/EtOAc 3:1→2:1) to yield a colorless foam (9.25 g, 74% over two steps). **TLC**: R_f = 0.18 (petroleum ether/EtOAc 3:1; UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = 7.79 – 7.72 (m, 2H, H_{aryl}), 7.59 (d, *J* = 6.9 Hz, 2H, H_{aryl}), 7.44 – 7.20 (m, 9H, H_{aryl} (superimposed by solvent)), 7.07 (d, *J* = 7.8 Hz, 1H, NH_{Asp2}), 6.09 (s, 2H, $H_{aryl,Tmob}$), 5.75 (s, 1H, NH_{Asp1}), 4.68 (dd, *J* = 7.7, 5.2 Hz, 1H, $CH_{\alpha,Asp2}$), 4.65 (d, *J* = 5.6 Hz, 1H, $CH_{\alpha,Thr}$), 4.51 – 4.44 (m, 1H, $CH_{\alpha,Asp1}$), 4.44 – 4.31 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.30 – 4.18 (m, 2H, $CH_{2,Tmob}$), 3.77 (s, 3H, OMe), 3.77 (s, 6H, 2 × OMe), 3.13 (d, *J* = 18.6 Hz, 1H, C<u>H</u>H'$_{Asp1}$), 2.88 – 2.79 (m, 1H, CH<u>H'</u>$_{Asp1}$), 2.76 – 2.68 (m, 2H, $CH_{2,Asp2}$), 1.82 (s, 3H, $CH_{3,PhiPr}$), 1.78 (s, 3H, $CH_{3,PhiPr}$), 1.65 (s, 3H, $CH_{3,pseudoproline}$), 1.55 (d, *J* = 6.2 Hz, 3H, $CH_{3,Thr}$), 1.50 (s, 3H, $CH_{3,pseudoproline}$), 1.45 (s, 9H, 3 × $CH_{3,tBu}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 198.3 (COS), 169.6 (COO*t*Bu), 168.9 (CO_{Asp1}), 168.7, 168.5 (CO_{Asp2} & CO_{Thr}), 161.0 ($C_{quart,Tmob}$), 159.3 (2 × $C_{quart,Tmob}$), 155.8 (COONH), 144.7 ($C_{quart,Ph}$), 143.9, 143.8, 141.32, 141.30 (4 × $C_{quart,Fmoc}$), 128.5, 127.7, 127.4, 127.2, 125.2, 124.32, 124.27, 120.0 (13 × C_{aryl}), 104.5 ($C_{quart,Tmob}$), 97.0 ($C_{quart,pseudoproline}$), 90.6 ($C_{aryl,Tmob}$), 84.4 ($C_{quart,PhiPr}$), 81.3 ($C_{quart,tBu}$), 74.9 ($CH_{\beta,Thr}$), 67.4 ($CH_{2,Fmoc}$), 65.8 ($CH_{\alpha,Thr}$), 55.8 (2 × OMe), 55.3 (OMe), 51.7 ($CH_{\alpha,Asp1}$), 49.5 ($CH_{\alpha,Asp2}$), 47.2 (CH_{Fmoc}), 44.7 ($CH_{2,Asp1}$), 39.7 ($CH_{2,Asp2}$), 28.7 ($CH_{3,PhiPr}$), 28.1 (3 × $CH_{3,tBu}$), 27.6 ($CH_{3,PhiPr}$),

26.4 ($CH_{3,pseudoproline}$), 23.9 ($CH_{3,pseudoproline}$), 22.4 ($CH_{2,Tmob}$), 20.6 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{53}H_{63}N_3O_{13}SNa^+$: 1004.3974 [*M*+Na]$^+$, found: 1004.4005.

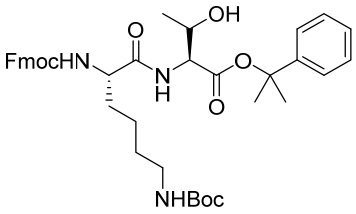
Fmoc-Asp(STmob)-Asp(O*t*Bu)-Thr($\Psi^{Me,Me}$pro)-OH (18b)



$C_{44}H_{53}N_3O_{13}S$ [863.98 g mol^{-1}]

C-terminal protected tripeptide **17b** (9.08 g, 9.24 mmol) was dissolved in dichloromethane (275 mL) and cooled to 0 °C before TFA (5.6 mL, 0.073 mmol) was added. The mixture was then allowed to warm to room temperature. After 35 minutes, TLC showed complete disappearance of starting material and the mixture was immediately diluted with toluene (190 mL). The solvents were removed *in vacuo* and the residue was co-evaporated with toluene two times to yield a brownish foam which was then dissolved in dry dichloromethane (130 mL). 2-Methoxypropene (7.03 mL, 63 mmol, 10 eq.) was added and the mixture was cooled to 0 °C prior to the addition of PPTS (245 mg, 0.63 mmol, 0.1 eq.). The mixture was allowed to warm to room temperature and after 4, 8 and 24 hours, respectively, further portions of 2-methoxypropene (7.03 mL, 63 mmol, 10 eq.) and PPTS (245 mg, 0.63 mmol, 0.1 eq.) were added. After a total of 27.5 hours, TLC showed complete conversion. Et_3N (1 mL) was added, the solvents were removed by evaporation and the residue was co-evaporated with toluene three times. The product was purified by FC (silica, dichloromethane/methanol 20:1→15:1→10:1) to obtain the aspartic acid-containing tripeptide building block **18b** as off-white foam (5.96 g, 75%). **TLC**: R_f = 0.37 (dichloromethane/methanol 10:1; UV/anisaldehyde); **HRMS:** m/z calcd. for $C_{44}H_{53}N_3O_{13}SNa^+$: 886.3191 [*M*+Na]$^+$, found: 886.3170.

Fmoc-Lys(Boc)-Thr(OH)-*O*-2-Ph*i*Pr (13c)

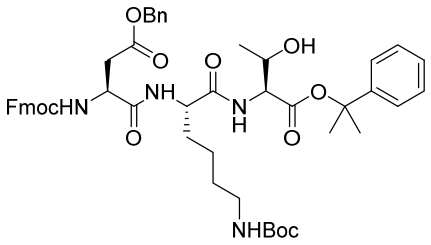


$C_{39}H_{49}N_3O_8$ [687.83 g mol^{-1}]

According to *general procedure 1*, Fmoc-Thr(OH)-*O*-2-Ph*i*Pr (**12**) (10.21 g, 22.2 mmol, 1 eq.) was deprotected with piperidine (220 mL, 20% in DMF) and reacted with Fmoc-Lys(Boc)-OH (12.50 g, 26.7 mmol, 1.2 eq.), HOBt (3.6 g, 26.7 mmol, 1.2 eq), HBTU (10.12 g, 26.7 mmol, 1.2 eq.) and DIPEA (9.3 mL, 53.3 mmol, 2.4 eq.) in dichloromethane (285 mL) for 4 hours. The reaction mixture was washed with sat. $NaHCO_{3(aq.)}$ (3 × 150 mL), H_2O (3 × 150 mL) and sat. $NaCl_{(aq.)}$ (150 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The product was purified by FC (silica, petroleum ether/EtOAc 1.5:1→1:1→1:2) to yield a colorless foam (14.15 g, 93%). **TLC**: R_f = 0.33 (petroleum ether/EtOAc 1:1; UV); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.77 – 7.72 (m, 2H, H_{aryl}), 7.63 – 7.51 (m, 2H, H_{aryl}), 7.44 – 7.13 (m, 9H, H_{aryl}), 6.89 (d, *J* = 8.8 Hz, 1H, NH_{Thr}), 5.66 (d, *J* = 8.0 Hz, 1H, NH_{Asp}), 4.82 – 4.59 (m, 1H, NH_{Boc}), 4.55 (dd, *J* = 8.8, 2.7 Hz, 1H, $CH_{\alpha,Thr}$), 4.44 – 4.30 (m, 3H, $CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 4.28 – 4.10 (m, 2H, $CH_{\alpha,Lys}$ & CH_{Fmoc}), 3.12 – 2.92 (m, 2H, $CH_{2,\varepsilon,Lys}$), 2.56 (br. s, 1H, OH), 1.89 – 1.52 (m, 8H, 2 × $CH_{3,PhiPr}$, C<u>H</u>H'$_{\beta,Lys}$, CH<u>H'</u>$_{\beta,Lys}$), 1.50 – 1.25 (m, 13H, 3 × $CH_{3,tBu}$, $CH_{2,\gamma,Lys}$, $CH_{2,\delta,Lys}$), 1.19 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 172.5, 169.4 (CO_{Lys} & CO_{Thr}), 156.4 (CO_{Fmoc} & CO_{Boc}), 145.1 ($C_{quart,Ph}$), 144.0, 143.9, 141.4 (4 × $C_{quart,Fmoc}$), 128.4,

127.8, 127.3, 127.2, 125.2, 125.22, 124.4, 120.07, 120.05 (13 × C_{aryl}), 83.8 ($C_{quart,Ph\mathit{i}Pr}$), 79.3 ($C_{quart,\mathit{t}Bu}$), 68.2 ($CH_{\beta,Thr}$), 67.3 $CH_{2,Fmoc}$), 58.1 ($CH_{\alpha,Thr}$), 54.9 ($CH_{\alpha,Lys}$), 47.2 (CH_{Fmoc}), 40.1 ($CH_{2,\varepsilon,Lys}$), 32.4 ($CH_{2,\beta,Lys}$), 29.5 ($CH_{2,\delta,Lys}$), 28.7 ($CH_{3,Ph\mathit{i}Pr}$), 28.6 (3 × $CH_{3,\mathit{t}Bu}$), 28.5 ($CH_{3,Ph\mathit{i}Pr}$), 22.5 ($CH_{2,\gamma,Lys}$), 20.2 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{39}H_{49}N_3O_8Na^+$: 710.34119 [*M*+Na]$^+$, found: 710.34204.

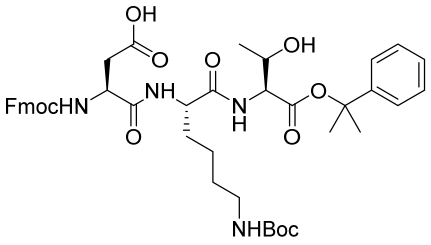
Fmoc-Asp(OBn)-Lys(Boc)-Thr(OH)-*O*-2-Ph*i*Pr (14c)



$C_{50}H_{60}N_4O_{11}$ [893.05 g mol^{-1}]

According to *general procedure 1*, dipeptide **13c** (14.11 g, 20.5 mmol, 1 eq.) was deprotected with piperidine (205 mL, 20% in DMF) and reacted with Fmoc-Asp(OBn)-OH (10.98 g, 24.6 mmol, 1.2 eq.), HOBt (3.33 g, 24.6 mmol, 1.2 eq), HBTU (9.35 g, 24.6 mmol, 1.2 eq.) and DIPEA (8.6 mL, 49.3 mmol, 2.4 eq.) in dichloromethane (260 mL) for 4 hours. The reaction mixture was washed with sat. $NaHCO_{3(aq.)}$ (3 × 100 mL) and H_2O (3 × 100 mL). After drying ($MgSO_4$) of the organic phase, the solvent was removed under reduced pressure. The residue was purified by FC twice (silica, petroleum ether/EtOAc 1:1→1:2) and was then recrystallized (280 mL, heptane/toluene/EtOAc 10:1:3) to yield a colorless powder (13.09 g, 71%). **TLC**: R_f = 0.31 (petroleum ether/EtOAc 1:1.5; UV); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ[ppm] = 7.79 – 7.70 (m, 2H, H_{aryl}), 7.57 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.43 – 7.27 (m, 13H, H_{aryl}), 7.24 – 7.18 (m, 1H, H_{aryl}), 7.04 – 6.95 (m, 1H, NH_{Lys}), 6.84 (d, *J* = 8.9 Hz, 1H, NH_{Thr}), 5.94 (br. s, 1H, NH_{Asp}), 5.14 – 5.03 (m, 2H, $CH_{2,Bn}$), 4.78 – 4.65 (m, 1H, NH_{Boc}), 4.63 – 4.55 (m, 1H, $CH_{\alpha,Asp}$), 4.52 (dd, *J* = 8.9, 2.7 Hz, 1H, $CH_{\alpha,Thr}$), 4.48 – 4.31 (m, 4H, $CH_{2,Fmoc}$, $CH_{\alpha,Lys}$ & $CH_{\beta,Thr}$), 4.20 (t, *J* = 6.9 Hz, 1H, CH_{Fmoc}), 3.09 – 2.91 (m, 3H, $CH_{2,\varepsilon,Lys}$ & C<u>H</u>H'$_{Asp}$), 2.80 (dd, *J* = 17.0, 6.0 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.95 – 1.83 (m, 1H, C<u>H</u>H'$_{\beta,Lys}$), 1.79 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.78 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.71 – 1.62 (m, 1H, CH<u>H'</u>$_{\beta,Lys}$), 1.47 – 1.24 (m, 13H, 3 × $CH_{3,\mathit{t}Bu}$, $CH_{2,\gamma,Lys}$ & $CH_{2,\delta,Lys}$), 1.19 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ[ppm] = 171.8 (CO), 171.6 (CO_{Bn}), 170.6 (CO), 169.3 (CO_{Thr}), 156.4, 156.3 (2 × COONH), 145.2 ($C_{quart,Ph\mathit{i}Pr}$), 143.8, 143.7, 141.4 (4 × $C_{quart,Fmoc}$), 135.4 ($C_{quart,Bn}$), 128.7, 128.6, 128.5, 128.4, 127.9, 127.4, 127.3, 125.2, 124.5, 120.2 (18 × C_{aryl}), 83.7 ($C_{quart,Ph\mathit{i}Pr}$), 79.3 ($C_{quart,\mathit{t}Bu}$), 68.4 ($CH_{\beta,Thr}$), 67.6 ($CH_{2,Fmoc}$), 67.2 ($CH_{2,Bn}$), 58.1 ($CH_{\alpha,Thr}$), 53.4 ($CH_{\alpha,Lys}$), 51.4 ($CH_{\alpha,Asp}$), 47.2 (CH_{Fmoc}), 40.1 ($CH_{2,\varepsilon,Lys}$), 36.4 ($CH_{2,Asp}$), 31.5 ($CH_{2,\beta,Lys}$), 29.4 ($CH_{2,\delta,Lys}$), 28.8 ($CH_{3,Ph\mathit{i}Pr}$), 28.6 (3 × $CH_{3,\mathit{t}Bu}$), 28.5 ($CH_{3,Ph\mathit{i}Pr}$), 22.6 ($CH_{2,\gamma,Lys}$), 20.3 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{50}H_{60}N_4O_{11}Na^+$: 915.41508 [*M*+Na]$^+$, found: 915.41632.

Fmoc-Asp(OH)-Lys(Boc)-Thr(OH)-*O*-2-Ph*i*Pr (15c)

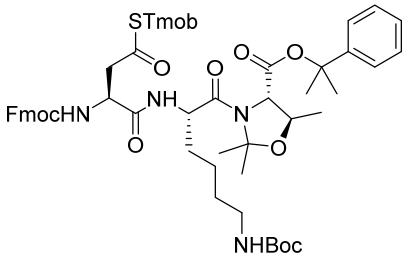


$C_{43}H_{54}N_4O_{11}$ [802.92 g mol^{-1}]

Benzyl-protected tripeptide **14c** was hydrogenated for 90 minutes as described in general procedure 3. The crude product was purified by FC (silica, dichloromethane/methanol 15:1→10:1) to yield tripeptide **15c** as colorless foam (8.43 g, 72%). **TLC**: R_f = 0.27 (dichloromethane/methanol 10:1; UV/vanillin); **^{1}H NMR** (methanol-d4, 400 MHz): δ[ppm] = 8.11 (d, *J* = 7.8 Hz, 1H, NH_{Lys}), 7.92 (d, *J* =

8.6 Hz, 1H, NH_{Thr}), 7.78 (dt, *J* = 7.5, 0.9 Hz, 2H, H_{aryl}), 7.69 – 7.60 (m, 2H, H_{aryl}), 7.42 – 7.34 (m, 4H, H_{aryl}), 7.33 – 7.25 (m, 4H, H_{aryl}), 7.22 – 7.16 (m, 1H, H_{aryl}), 4.54 (dd, *J* = 7.7, 5.7 Hz, 1H, $CH_{\alpha,Asp}$), 4.48 – 4.41 (m, 1H, $CH_{\alpha,Lys}$), 4.41 – 4.28 (m, 4H, $CH_{\alpha,Thr,}$ $CH_{\beta,Thr}$, $CH_{2,Fmoc}$), 4.22 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 3.01 – 2.90 (m, 2H, $CH_{2,\varepsilon,Lys}$), 2.87 (dd, *J* = 16.9, 5.7 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.72 (dd, *J* = 16.8, 7.7 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.93 – 1.80 (m, 1H, C<u>H</u>H'$_{\beta,Lys}$), 1.76 (s, 3H, $CH_{3,Ph/Pr}$), 1.75 (s, 3H, $CH_{3,Ph/Pr}$), 1.73 – 1.61 (m, 1H, CH<u>H'</u>$_{\beta,Lys}$), 1.47 – 1.27 (m, 13H, 3 × $CH_{3,tBu}$, $CH_{2,\gamma,Lys}$, $CH_{2,\delta,Lys}$), 1.19 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** (methanol-d4, 101 MHz): δ [ppm] = 174.4, 174.0, 173.4 (COOH, CO_{Asp} & CO_{Lys}), 170.4 (CO_{Thr}), 158.4, 158.3 (CO_{Fmoc} & CO_{Boc}), 146.7 ($C_{quart,Ph}$), 145.22, 145.17, 142.5 (4 × $C_{quart,Fmoc}$), 129.2, 128.8, 128.2, 127.9, 126.3, 126.2, 125.5, 120.9 (13 × C_{aryl}), 84.5 ($C_{quart,Ph/Pr}$), 79.8 ($C_{quart,tBu}$), 68.4 ($CH_{\beta,Thr}$), 68.3 ($CH_{2,Fmoc}$), 60.1 ($CH_{\alpha,Thr}$), 54.6 ($CH_{\alpha,Lys}$), 53.0 ($CH_{\alpha,Asp}$), 48.3 (CH_{Fmoc}), 41.1 ($CH_{2,\varepsilon,Lys}$), 36.9 ($CH_{2,Asp}$), 32.7 ($CH_{2,\beta,Lys}$), 30.3 ($CH_{2,\delta,Lys}$), 29.1, 29.0 (2 × $CH_{3,Ph/Pr}$), 28.8 (3 × $CH_{3,tBu}$), 24.0 ($CH_{2,\gamma,Lys}$), 20.6 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{43}H_{54}N_4O_{11}Na^+$: 825.36813 [*M*+Na]$^+$, found: 825.36902.

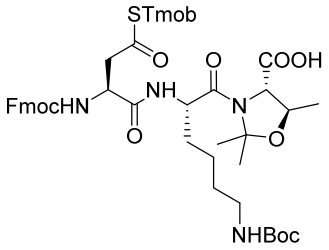
Fmoc-Asp(STmob)-Lys(Boc)-Thr(Ψ^{Me,Me}pro)-*O*-2-Ph*i*Pr (17c)



$C_{56}H_{70}N_4O_{13}S$ [1039.25 g mol^{-1}]

Tripeptide **15c** (8.40 g, 10.48 mmol, 1 eq.) was suspended in dry dichloromethane (150 mL) and 2-methoxypropene (10.0 mL, 104.8 mmol, 10 eq.) was added. PPTS was added (263 mg, 1.05 mmol, 0.1 eq.) at 0 °C and the mixture was then allowed to warm to room temperature. After two and four hours, respectively, further portions of 2-methoxypropene (5.0 mL, 52.4 mmol, 5 eq.) and PPTS (132 mg, 0.53 mmol, 0.05 eq.) were added. After a total of 65 hours, TLC showed complete conversion and the solvents were removed by evaporation. The residue was co-evaporated with toluene two times and the crude product was purified by FC (silica, dichloromethane/methanol 1:0→20:1→10:1) to yield a yellow foam of pseudoproline-protected tripeptide **16c** with still minor impurities (7.40 g). It was used for forward conversion without further purification.
Tripeptide **16c** (7.37 g, 8.75 mmol, 1 eq.) was thioesterified as described in *general procedure 2* using PyBOP (5.01 g, 9.62 mmol, 1.1 eq.), TmobSH (2.06 g, 9.62 mmol, 1.1 eq.) and DIPEA (3.35 mL, 19.25 mmol, 2.2 eq.) in dichloromethane (150 mL) at –15 °C. The product was purified by FC (silica, petroleum ether/EtOAc 2:1→3:2) to yield a colorless foam (6.59 g, 72% over two steps). **TLC**: R_f = 0.17 (petroleum ether/EtOAc 2:1; UV/vanillin); **HRMS:** m/z calcd. for $C_{56}H_{70}N_4O_{13}SNa^+$: 1061.45523 [*M*+Na]$^+$, found: 1061.45569.

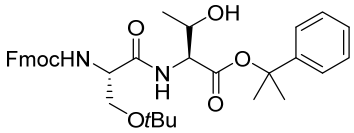
Fmoc-Asp(STmob)-Lys(Boc)-Thr(Ψ^{Me,Me}pro)-OH (18c)



$C_{47}H_{60}N_4O_{13}S$ [921.07 g mol^{-1}]

C-terminal protected tripeptide **17c** (6.54 g, 6.30 mmol) was dissolved in dichloromethane (184 mL) and cooled to 0 °C before TFA (3.8 mL, 0.049 mmol, final concentration: 2% in dichloromethane) was added. The mixture was then allowed to warm to room temperature until TLC showed complete disappearance of the starting material (35 min). The mixture was immediately diluted with toluene (190 mL), the solvents were removed *in vacuo* and the residue was co-evaporated with toluene two times to yield brownish foam. Next, the foam was dissolved in dry dichloromethane (85 mL) and 2-methoxypropene (6.02 mL, 63 mmol, 10 eq.) was added. PPTS (158 mg, 0.63 mmol, 0.1 eq.) was added at 0 °C and the mixture was then allowed to warm to room temperature. After a total of 23 hours, TLC showed complete conversion. Et_3N (0.5 mL) was added, the solvents were removed by evaporation and the crude product was purified by FC twice (silica, dichloromethane/methanol 20:1→15:1→10:1, EtOAc + 2% AcOH). The combined product fractions were washed with sat. $NaHCO_{3(aq.)}$ (2 ×), water (2 ×), citric $acid_{(aq.)}$ (5%, 3 ×) water (2 ×) and sat. $NaCl_{(aq.)}$ (1 ×), was dried ($MgSO_4$) and the solvent was removed by evaporation. Tripeptide building block **18c** was obtained as off-white solid (2.86 g, 49%). **TLC**: R_f = 0.33 (EtOAc + 2% AcOH; UV); **HRMS:** m/z calcd. for $C_{47}H_{60}N_4O_{13}SNa^+$: 943.37698 [*M*+Na]$^+$, found: 943.37671.

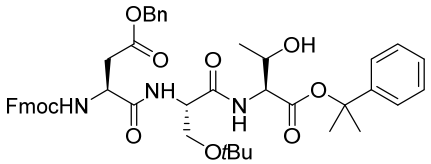
Fmoc-Ser(*t*Bu)-Thr(OH)-*O*-2-Ph*i*Pr (13d)



$C_{35}H_{42}N_2O_7$ [602.73 g mol^{-1}]

According to *general procedure 1*, Fmoc-Thr(OH)-*O*-2-Ph*i*Pr **12** (11.71 g, 25.5 mmol, 1 eq.) was deprotected with piperidine (255 mL, 20% in DMF) and reacted with Fmoc-Ser(*t*Bu)-OH (11.74 g, 30.6 mmol, 1.2 eq.) using HOBt (4.14 g, 30.6 mmol, 1.2 eq), HBTU (11.61 g, 30.6 mmol, 1.2 eq.) and DIPEA (10.66 mL, 61.2 mmol, 2.4 eq.) in dichloromethane (320 mL) for 2 hours. The reaction mixture was extracted with sat. $NH_4Cl_{(aq.)}$ (3 × 75 mL), sat. $NaHCO_{3(aq.)}$ (2 × 75 mL) and H_2O (75 mL) and afterwards dried ($MgSO_4$). The solvent was removed under reduced pressure and the crude product was purified by FC (silica, petroleum ether/EtOAc 3:1→2.5:1→2:1). The serine dipeptide **13d** was obtained as colorless foam (12.831 g, 84%). **TLC**: R_f = 0.32 (petroleum ether/EtOAc 3:1; UV/$KMnO_4$); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.65 – 7.53 (m, 2H, H_{aryl}), 7.50 – 7.15 (m, 10H, H_{aryl} & NH_{Thr}), 5.76 (d, *J* = 6.4 Hz, 1H, NH_{Ser}), 4.56 (dd, *J* = 8.8, 2.4 Hz, 1H, $CH_{\alpha,Thr}$), 4.44 – 4.28 (m, 4H, $CH_{2,Fmoc}$, $CH_{\beta,Thr}$ & $CH_{\alpha,Ser}$), 4.23 (t, *J* = 7.3 Hz, 1H, CH_{Fmoc}) 3.85 (dd, *J* = 8.6, 3.7 Hz, 1H, C<u>H</u>H'$_{Ser}$), 3.42 (dd, *J* = 8.07, 8.0 Hz, 1H, CH<u>H'</u>$_{Ser}$), 2.02 (br. s, 1H, OH), 1.80 (s, 6H, 2 × $CH_{3,Phi Pr}$), 1.23 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$), 1.18 (s, 9H, *t*Bu); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 171.0 (CO_{Ser}), 169.3 (CO_{Thr}), 156.2 (COONH), 145.1 ($C_{quart,Phi Pr}$), 144.0, 143.9, 141.4 (4 × $C_{quart,Fmoc}$), 128.5, 128.3, 127.8, 127.4, 127.2, 125.3, 125.2, 124.4, 120.1 (13 × C_{aryl}), 83.7 ($C_{quart,Phi Pr}$), 74.4 ($C_{quart,tBu}$), 68.2 ($CH_{\beta,Thr}$), 67.3 ($CH_{2,Fmoc}$), 61.9 ($CH_{2,Ser}$), 57.9 ($CH_{\alpha,Thr}$), 54.8 ($CH_{\alpha,Ser}$), 47.3 (CH_{Fmoc}), 28.6, 28.5 (2 × $CH_{3,Phi Pr}$), 27.5 (3 × $CH_{3,tBu}$), 20.0 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{35}H_{42}N_2O_7Na^+$: 625.2884 [*M*+Na]$^+$, found: 625.2887.

Fmoc-Asp(OBn)-Ser(*t*Bu)-Thr(OH)-*O*-2-Ph*i*Pr (14d)

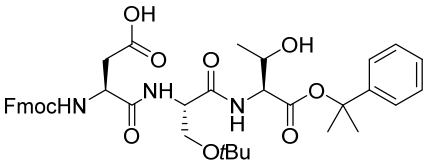


$C_{46}H_{53}N_3O_{10}$ [807.94 g mol^{-1}]

According to *general procedure 1*, serine dipeptide **13d** (12.8 g, 21.25 mmol, 1 eq.) was first deprotected with piperidine (210 mL, 20% in DMF) and then reacted with Fmoc-Asp(OBn)-OH

(11.36 g, 25.5 mmol, 1.2 eq.), HOBt (3.45 g, 25.5 mmol, 1.2 eq.), HBTU (9.67 g, 25.5 mmol, 1.2 eq.) and DIPEA (8.88 mL, 51 mmol, 2.4 eq.) in dichloromethane (270 mL). After four hours, the reaction mixture was washed with sat. $NH_4Cl_{(aq.)}$ (2 × 75 mL), sat. $NaHCO_{3(aq.)}$ (2 × 75 mL) and H_2O (75 mL). The organic layer was dried ($MgSO_4$) and the solvent was removed *in vacuo*. The crude product was purified by FC (silica, petroleum ether/EtOAc 1:1→1:1.5) to yield a colorless foam (16.153 g, 96%). **TLC**: R_f = 0.36 (petroleum ether/EtOAc 1:1; UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.58 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.47 – 7.22 (m, 15H, 13 × H_{aryl}, NH_{Asp} & NH_{Thr}), 7.22 – 7.14 (m, 1H, H_{aryl}), 5.86 (d, *J* = 8.2 Hz, 1H, NH_{Ser}), 5.11 (d, *J* = 12.3 Hz, 1H, C<u>H</u>H'$_{Bn}$), 4.98 (d, *J* = 12.3 Hz, 1H, CH<u>H</u>'$_{Bn}$), 4.67 – 4.48 (m, 3H, $CH_{\alpha,Asp}$, $CH_{\alpha,Ser}$ & $CH_{\alpha,Thr}$), 4.48 – 4.30 (m, 3H, $CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 4.21 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 3.89 (dd, *J* = 8.7, 3.2 Hz, 1H, C<u>H</u>H'$_{Ser}$), 3.42 (dd, *J* = 8.6, 5.9 Hz, 1H, CH<u>H</u>'$_{Ser}$), 3.00 (dd, *J* = 17.4, 4.3 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.86 (dd, *J* = 17.3, 7.4 Hz, 1H, CH<u>H</u>'$_{Asp}$), 2.25 (br. s, 1H, OH), 1.79 (s, 3H, $CH_{3,Phi Pr}$), 1.78 (s, 3H, $CH_{3,Phi Pr}$), 1.22 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$), 1.16 (s, 9H, 3 × $CH_{3,tBu}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 171.9 (CO_{Bn}), 170.4, 170.2 (CO_{Asp} & CO_{Ser}), 169.1 (CO_{Thr}), 156.1 (COONH), 145.2 ($C_{quart,Phi Pr}$), 143.8, 143.7, 141.4 (4 × $C_{quart,Fmoc}$), 135.4 ($C_{quart,Bn}$), 128.7, 128.5, 128.4, 127.9, 127.3, 127.2, 125.2, 125.1, 124.5, 120.15, 120.13 (18 × C_{aryl}), 83.5 ($C_{quart,Phi Pr}$), 74.1 ($C_{quart,tBu}$), 68.5 ($CH_{\beta,Thr}$), 67.6 ($CH_{2,Fmoc}$), 67.2 ($CH_{2,Bn}$), 61.2 ($CH_{2,Ser}$), 58.0 ($CH_{\alpha,Thr}$), 53.8 ($CH_{\alpha,Asp}$), 51.4 ($CH_{\alpha,Ser}$), 47.2 (CH_{Fmoc}), 36.6 ($CH_{2,Asp}$), 28.7, 28.4 (2 × $CH_{3,Phi Pr}$), 27.5 (3 × $CH_{3,tBu}$), 20.0 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{46}H_{53}N_3O_{10}Na^+$: 830.3623 [*M*+Na]$^+$, found: 830.3632.

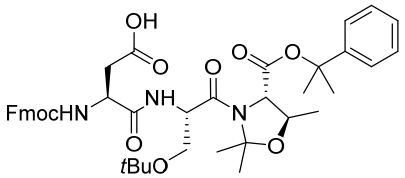
Fmoc-Asp(OH)-Ser(*t*Bu)-Thr(OH)-*O*-2-Ph*i*Pr (15d)



$C_{39}H_{47}N_3O_{10}$ [717.82 g mol^{-1}]

According to *general procedure 3*, serine tripeptide **14d** (16.15 g, 20.0 mmol) was hydrogenated for 85 minutes. After purification by FC (silica, dichloromethane/methanol 10:1) the peptide acid **15d** was obtained as colorless solid (12.284 g, 86%). **TLC**: R_f = 0.29 (dichloromethane/methanol 10:1; UV); **^{1}H NMR** (methanol-d4, 400 MHz): δ [ppm] = 7.78 (dt, *J* = 7.6, 1.0 Hz, 2H, H_{aryl}), 7.64 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.41 – 7.34 (m, 4H, H_{aryl}), 7.33 – 7.24 (m, 4H, H_{aryl}), 7.22 – 7.13 (m, 1H, H_{aryl}), 4.58 (dd, *J* = 7.7, 5.6 Hz, 1H, $CH_{\alpha,Asp}$), 4.51 (dd, *J* = 5.7, 4.2 Hz, 1H, $CH_{\alpha,Ser}$), 4.45 – 4.28 (m, 4H, $CH_{\alpha,Thr}$, $CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 4.21 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 3.73 (dd, *J* = 9.0, 4.3 Hz, 1H, C<u>H</u>H'$_{Ser}$), 3.54 (dd, *J* = 9.0, 5.8 Hz, 1H, CH<u>H</u>'$_{Ser}$), 2.90 (dd, *J* = 16.9, 5.6 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.73 (dd, *J* = 16.9, 7.7 Hz, 1H, CH<u>H</u>'$_{Asp}$), 1.76 (s, 3H, $CH_{3,Phi Pr}$), 1.75 (s, 3H, $CH_{3,Phi Pr}$), 1.19 (d, *J* = 6.5 Hz, 3H, $CH_{3,Thr}$), 1.12 (s, 9H, 3 × $CH_{3,tBu}$); **^{13}C NMR** (methanol-d4, 101 MHz): δ [ppm] = 174.1 (COOH), 173.3 (CO_{Asp}), 172.5 (CO_{Ser}), 170.2 (CO_{Thr}), 158.4 (COONH), 146.7 ($C_{quart,Ph}$), 145.1, 142.5 (4 × $C_{quart,Fmoc}$), 129.2, 128.8, 128.2, 127.9, 126.3, 126.2, 125.5, 120.9 (13 × C_{aryl}), 84.5 ($C_{quart,Phi Pr}$), 74.9 ($C_{quart,tBu}$), 68.5 ($CH_{\beta,Thr}$), 68.3 ($CH_{2,Fmoc}$), 62.5 ($CH_{2,Ser}$), 59.9 ($CH_{\alpha,Thr}$), 55.0 ($CH_{\alpha,Ser}$), 53.0 ($CH_{\alpha,Asp}$), 48.3 (CH_{Fmoc}) 36.7 ($CH_{2,Asp}$), 29.2, 28.9 (2 × $CH_{3,Phi Pr}$), 27.6 (3 × $CH_{3,tBu}$), 20.7 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{39}H_{47}N_3O_{10}Na^+$: 740.3154 [*M*+Na]$^+$, found: 740.3163.

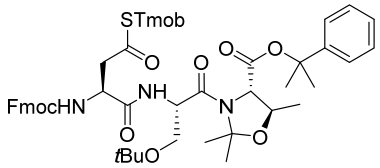
Fmoc-Asp(OH)-Ser(*t*Bu)-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (16d)



$C_{42}H_{51}N_3O_{10}$ [757.88 g mol^{-1}]

Tripeptide **15d** (12.0 g, 17.0 mmol, 1 eq.) was dissolved in dry dichloromethane (240 mL) and cooled to 0 °C. PPTS (430 mg, 0.17 mmol, 0.1 eq.) was added and subsequently 2-methoxypropene (8.15 mL, 85 mmol, 5 eq.) was added over a period of two hours at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for one hour. Then, another portion of 2-methoxypropene (8.15 mL, 85 mmol, 5 eq.) was added within two hours at 0 °C. After stirring at room temperature for 17 hours, one last portion of 2-methoxypropene (8.15 mL, 85 mmol, 5 eq.) was added over two hours at 0 °C. The mixture was allowed to warm to room temperature and was stirred for one hour before Et_3N (0.5 mL) was added. The solvents were removed *in vacuo* and the crude product was purified by FC (silica, petroleum ether/EtOAc 1:1 + 1% AcOH). The combined product fractions were washed with sat. $NaHCO_{3(aq.)}$ (3 × 100 mL), citric $acid_{(aq.)}$ (5%, 3 × 100 mL), water (100 mL) and sat. $NH_4Cl_{(aq.)}$ (100 mL). After drying of the organic phase ($MgSO_4$), the solvents were removed *in vacuo* to yield the backbone-protected tripeptide **16d** as colorless foam (9.98 g, 78%). **TLC**: R_f = 0.34 (petroleum ether/EtOAc 1:1 + 1% AcOH; vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = δ 7.73 (d, *J* = 7.6 Hz, 2H, H_{aryl}), 7.61 – 7.53 (m, 2H, H_{aryl}), 7.50 (d, *J* = 7.6 Hz, 1H, NH_{Ser}), 7.41 – 7.16 (m, 9H, H_{aryl}), 6.06 (d, *J* = 8.9 Hz, 1H, NH_{Asp}), 4.70 – 4.45 (m, 3H, $CH_{\alpha,Asp}$, $CH_{\alpha,Ser}$ & $CH_{\alpha,Thr}$), 4.43 – 4.23 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.20 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 3.63 – 3.55 (m, 1H, C<u>H</u>H'$_{Ser}$), 3.34 (dd, *J* = 9.9, 8.0 Hz, 1H, CH<u>H</u>'$_{Ser}$), 2.94 (dd, *J* = 17.3, 5.2 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.66 (dd, *J* = 17.2, 5.4 Hz, 1H, CH<u>H</u>'$_{Asp}$), 1.81 (s, 3H, $CH_{3,Ph/Pr}$), 1.78 (s, 3H, $CH_{3,Ph/Pr}$), 1.68 (s, 3H, $CH_{3,pseudoproline}$), 1.53 – 1.44 (m, 6H, $CH_{3,pseudoproline}$ & $CH_{3,Thr}$), 1.14 (s, 9H, 3 × $CH_{3,tBu}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 174.4 (COOH), 170.0 (CO_{Asp}), 169.3 (CO_{Ser}), 168.7 (CO_{Thr}), 156.0 (COONH), 144.7 ($C_{quart,Ph}$), 144.0, 143.9, 141.4 (4 × $C_{quart,Fmoc}$), 128.6, 127.8, 127.5, 127.2, 125.3, 124.3, 120.0 (13 × C_{aryl}), 97.4 ($C_{quart,pseudoproline}$), 84.7 ($C_{quart,Ph/Pr}$), 74.9 ($CH_{\beta,Thr}$), 73.9 ($C_{quart,tBu}$), 67.5 ($CH_{2,Fmoc}$), 66.1 ($CH_{\alpha,Thr}$), 63.5 ($CH_{2,Ser}$), 52.9 ($CH_{\alpha,Ser}$), 51.2 ($CH_{\alpha,Asp}$), 47.2 (CH_{Fmoc}), 36.6 ($CH_{2,Asp}$), 28.7, 27.7 (2 × $CH_{3,Ph/Pr}$) 27.5 (3 × $CH_{3,tBu}$), 26.6, 24.0 (2 × $CH_{3,pseudoproline}$), 20.8 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{42}H_{51}N_3O_{10}Na^+$: 780.3467 $[M+Na]^+$, found: 780.3474.

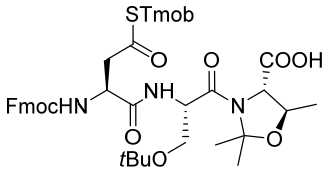
Fmoc-Asp(STmob)-Ser(*t*Bu)-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (17d)



$C_{52}H_{63}N_3O_{12}S$ [954.15 g mol^{-1}]

According to *general procedure 2*, tripeptide **16d** (9.94 g, 13.13 mmol, 1 eq.) was reacted with PyBOP (7.51 g, 14.44 mmol, 1.1 eq.), TmobSH (3.09 g, 14.44 mmol, 1.1 eq.) and DIPEA (5.03 mL, 28.87 mmol, 2.2 eq.) in dichloromethane (60 mL) at –15 °C. Purification by FC (silica, petroleum ether/EtOAc 3:1→2.5:1→2:1) yielded peptide thioester **17d** as colorless foam (10.424 g, 83%). **TLC**: R_f = 0.42 (petroleum ether/EtOAc 2:1; vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.60 (d, *J* = 7.2 Hz, 2H, H_{aryl}), 7.44 – 7.15 (m, 10H, 9 × Haryl, NH_{Ser}), 6.08 (s, 2H, 2 × $H_{aryl,Tmob}$), 5.77 (d, *J* = 8.7 Hz, 1H, NH_{Asp}), 4.58 (d, *J* = 6.0 Hz, 1H, $CH_{\alpha,Thr}$), 4.55 – 4.46 (m, 2H, $CH_{\alpha,Asp}$ & $CH_{\alpha,Ser}$), 4.44 – 4.34 (m, 2H, $CH_{2,Fmoc}$), 4.33 – 4.18 (m, 4H, $CH_{2,Tmob}$, CH_{Fmoc} & $CH_{\beta,Thr}$), 3.78 (s, 3H, OMe), 3.78 (s, 6H, 2 × OMe), 3.63 (dd, *J* = 8.1, 4.5 Hz, 1H, C<u>H</u>H'$_{Ser}$), 3.35 (dd, *J* = 9.7, 8.0 Hz, 1H, CH<u>H</u>'$_{Ser}$), 3.21 (dd, *J* = 16.4, 5.4 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.84 (dd, *J* = 16.6, 5.1 Hz, 1H, CH<u>H</u>'$_{Asp}$), 1.81 (s, 3H, $CH_{3,Ph/Pr}$), 1.79 (s, 3H, $CH_{3,Ph/Pr}$), 1.72 (s, 3H, $CH_{3,pseudoproline}$), 1.54 (s, 3H, $CH_{3,pseudoproline}$), 1.50 (d, *J* = 6.1 Hz, 3H, $CH_{3,Thr}$), 1.18 (s, 9H, 3 × $CH_{3,tBu}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 198.4 (COS), 169.1, 169.0, 168.6 (CO_{Asp}, CO_{Ser} & CO_{Thr}), 161.0 ($C_{quart,Tmob}$), 159.3 (2 × $C_{quart,Tmob}$), 155.9 (COONH), 144.8 ($C_{quart,Ph}$), 144.0, 143.82, 141.38 (4 × $C_{quart,Fmoc}$), 128.55, 127.81, 127.48, 127.26, 125.32, 124.33, 120.06 (13 × C_{aryl}), 104.5 ($C_{quart,Tmob}$), 97.2 ($C_{quart,pseudoproline}$), 90.7 (2 × $C_{aryl,Tmob}$), 84.5 ($C_{quart,Ph/Pr}$), 75.0 ($CH_{\beta,Thr}$), 73.9 ($C_{quart,tBu}$), 67.5 ($CH_{2,Fmoc}$), 66.0 ($CH_{\alpha,Thr}$), 63.7 ($CH_{2,Ser}$), 55.9 (2 × OMe), 55.4 (OMe), 52.8 ($CH_{\alpha,Ser}$), 51.9 ($CH_{\alpha,Asp}$) 47.2 (CH_{Fmoc}), 44.8 ($CH_{2,Asp}$), 28.5, 27.9 (2 × $CH_{3,Ph/Pr}$), 27.5 (3 × $CH_{3,tBu}$), 26.6, 24.1 (2 × $CH_{3,pseudoproline}$), 22.5 ($CH_{2,Tmob}$), 20.8 ($CH_{3,Thr}$).

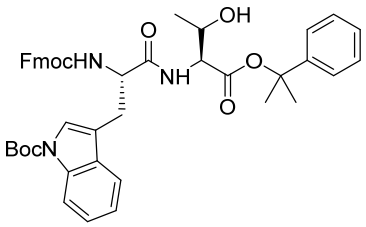
Fmoc-Asp(STmob)-Ser(*t*Bu)-Thr($\Psi^{Me,Me}$pro)-OH (18d)



$C_{43}H_{53}N_3O_{12}S$ [835.97 g mol^{-1}]

2-Phenyl*iso*propyl-protected tripeptide **17d** (10.38 g, 10.9 mmol) was dissolved in TFA (313 mL, 2% in dichloromethane) at 0 °C. The reaction mixture was allowed to warm to room temperature and was stirred for 55 minutes until TLC indicated complete disappearance of the starting material. The solution was immediately diluted with toluene (313 mL), the solvents were removed *in vacuo* and the residue was co-evaporated with toluene. The brown foam was dissolved in dry dichloromethane (150 mL) and cooled to 0 °C. PPTS (274 mg, 1.09 mmol, 0.1 eq.) was added, followed by 2-methoxypropene (10.4 mL, 109 mmol, 10 eq., over 6 hours). The solution was then allowed to warm to room temperature and was stirred for further 24 hours. Et_3N (150 µL, 1.09 mmol, 0.1 eq) was added, the solvents were removed and the residue was co-evaporated with toluene two times. The crude product was purified by FC twice (silica, EtOAc→EtOAc + 0.5% AcOH→ EtOAc + 1% AcOH). The combined product fractions were washed with sat. $NaHCO_{3(aq.)}$ (2 × 100 mL), citric $acid_{(aq.)}$ (5%, 3 × 100 mL), water (2 × 100 mL) and sat. $NH_4Cl_{(aq.)}$ (100 mL). After drying of the organic phase ($MgSO_4$) the solvents were removed *in vacuo* to yield tripeptide building block **18d** as faint yellow foam (7.80 g, 90%). **TLC**: R_f = 0.29 (EtOAc + 1% AcOH; UV); **HRMS:** m/z calcd. for $C_{43}H_{53}N_3O_{12}SNa^+$: 858.3242 [*M*+Na]$^+$, found: 858.3248.

Fmoc-Trp(Boc)-Thr(OH)-*O*-2-Ph*i*Pr (13e)

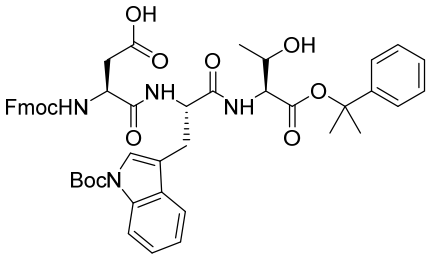


$C_{44}H_{47}N_3O_8$ [745.87 g mol^{-1}]

Dipeptide **13e** was synthesized according to *general procedure 1*, by deprotecting Fmoc-Thr(OH)-*O*-2-Ph*i*Pr (**12**) (1.00 g, 2.18 mmol, 1 eq.) with piperidine (44 mL, 20% in DMF) and subsequent coupling with Fmoc-Trp(Boc)-OH (1.38 g, 2.61 mmol, 1.2 eq.) using HBTU (0.99 g, 2.61 mmol, 1.2 eq.), HOBt (0.35 g, 2.61 mmol, 1.2 eq.) and DIPEA (0.91 mL, 5.22 mmol, 2.4 eq.) in dichloromethane (55 mL). After stirring at room temperature for 4.5 hours, the solvent was removed by evaporation and the residue was purified by FC (silica, petroleum ether/EtOAc 2:1→1.5:1). Trp-dipeptide **13e** was obtained as colorless foam (974 mg, 60%). **TLC**: R_f = 0.23 (petroleum ether/EtOAc 2:1, UV/anisaldehyde); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 8.11 (d, *J* = 8.4 Hz, 1H, H_{aryl}), 7.74 (d, *J* = 7.6 Hz, 2H, H_{aryl}), 7.58 (d, *J* = 7.8 Hz, 1H, H_{aryl}), 7.50 (d, *J* = 9.3 Hz, 3H, H_{aryl}), 7.42 – 7.14 (m, 11H, H_{aryl} (superimposed by solvent)), 6.84 – 6.72 (m, 1H, NH_{Thr}), 5.61 – 5.51 (m, 1H, NH_{Trp}), 4.70 – 4.57 (m, 1H, $CH_{\alpha,Trp}$), 4.53 (dd, *J* = 8.8, 2.7 Hz, 1H, $CH_{\alpha,Thr}$), 4.41 – 4.23 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.20 – 4.06 (m, 1H, CH_{Fmoc}), 3.31 – 3.11 (m, 2H, $CH_{2,Trp}$), 2.29 (s, 1H, OH), 1.78 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.75 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.61 (s, 9H, 3 × $CH_{3,tBu}$), 1.11 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 171.6 (CO_{Trp}), 169.1 (CO_{Thr}), 156.2 ($COONH_{Fmoc}$), 149.6 ($COONH_{Boc}$), 145.0 ($C_{quart,Ph}$), 143.9, 143.8, 141.36, 141.35 (4 × $C_{quart,Fmoc}$), 135.6, 130.4 (2 × $C_{quart,Trp}$), 128.5, 127.8, 127.4, 127.2, 125.23, 125.21, 124.74, 124.72, 124.4, 122.9, 120.1, 120.0, 119.1, 115.5 (18 × C_{aryl}) 115.3 ($C_{quart,Trp}$), 83.9 ($C_{quart,tBu}$), 83.8 ($C_{quart,Ph\mathit{i}Pr}$), 68.3 ($C_{\beta,Thr}$), 67.5 ($CH_{2,Fmoc}$), 58.1 ($CH_{\alpha,Thr}$), 55.2 ($CH_{\alpha,Trp}$), 47.1 (CH_{Fmoc}), 28.9, 28.3, 28.1 ($CH_{2,Trp}$, 3 ×

$CH_{3,tBu}$, 2 × $CH_{3,PhiPr}$), 20.1 ($CH_{3,Thr}$); **HRMS**: m/z calcd. for $C_{44}H_{47}N_3O_8Na^+$: 768.32554 [*M*+Na]$^+$, found: 768.32534.

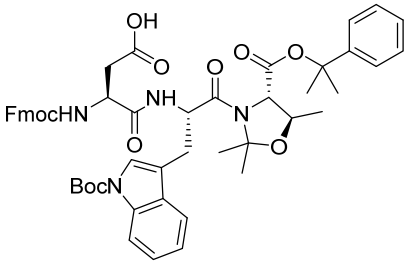
Fmoc-Asp(OH)-Trp(Boc)-Thr(OH)-*O*-2-Ph*i*Pr (15e)



$C_{48}H_{52}N_4O_{11}$ [860.96 g mol^{-1}]

Dipeptide **13e** (934 mg, 1.25 mmol, 1 eq.) was deprotected with piperidine (12.5 mL, 20% in DMF) and coupled with Fmoc-Asp(OBn)-OH (670 mg, 1.50 mmol, 1.2 eq.), HOBt (203 mg, 1.50 mmol, 1.2 eq.), HBTU (570 mg, 1.50 mmol, 1.2 eq.) and DIPEA (523 mL, 3.01 mmol, 2.4 eq.) in dichloromethane (16 mL) according to *general procedure 1*. After stirring for 4 hours, the solvent was removed *in vacuo* and the residue was purified by FC (silica, petroleum ether/EtOAc 2:1→1:1) to obtain a colorless foam with still minor impurities (847 mg).
The crude tripeptide **14e** was subsequently hydrogenated for 40 minutes according to *general procedure 3*. The product was purified by FC (dichloromethane/methanol 15:1→10:1) to yield a colorless foam (615 mg, 57% over two steps). **TLC:** R_f = 0.35 (dichloromethane/methanol 10:1; UV/anisaldehyde); **^{1}H NMR** ($CDCl_3$, 600 MHz): δ [ppm] = 8.00 (br. s, 1H, H_{aryl}), 7.72 (d, *J* = 7.6 Hz, 2H, H_{aryl}), 7.55 – 7.40 (m, 4H, H_{aryl}), 7.39 – 7.32 (m, 2H, H_{aryl}), 7.34 – 7.11 (m, 10H, 9 × H_{aryl} & NH_{Trp} (superimposed by solvent)), 7.09 (d, *J* = 9.7 Hz, 1H, NH_{Thr}), 5.92 (d, *J* = 8.4 Hz, 1H, NH_{Asp}), 4.80 (dd, *J* = 7.5, 7.3 Hz, 1H, $CH_{\alpha,Trp}$), 4.52 (dd, *J* = 9.1, 3.1 Hz, 1H, $CH_{\alpha,Thr}$), 4.52 – 4.46 (m, 1H, $CH_{\alpha,Asp}$), 4.34 – 4.20 (m, 3H, $CH_{\beta,Thr}$ & $CH_{2,Fmoc}$), 4.11 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 3.23 (dd, *J* = 15.0, 6.1 Hz, 1H, C<u>H</u>H'$_{Trp}$), 3.18 (dd, *J* = 15.1, 7.2 Hz, 1H, CH<u>H</u>'$_{Trp}$), 2.78 (dd, *J* = 16.6, 4.8 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.69 (dd, *J* = 16.8, 7.4 Hz, 1H, CH<u>H</u>'$_{Asp}$), 1.72 (s, 3H, $CH_{3,PhiPr}$), 1.71 (s, 3H, $CH_{3,PhiPr}$), 1.58 (s, 9H, 3 × $CH_{3,tBu}$), 1.06 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 151 MHz): δ [ppm] = 173.8 (COOH), 171.4 (CO_{Trp}), 170.9 (CO_{Asp}), 169.2 (CO_{Thr}), 156.2 ($COONH_{Fmoc}$), 149.9 ($COONH_{Boc}$), 145.0 ($C_{quart,Ph}$), 143.8, 143.6, 141.4 (4 × $C_{quart,Fmoc}$), 135.4, 130.4 (2 × $C_{quart,Trp}$), 128.6, 127.9, 127.4, 127.2, 125.2, 124.7, 124.53, 124.47, 122.8, 120.1, 119.1 (17 × C_{aryl}), 115.6 ($C_{quart,Trp}$), 115.4 (C_{aryl}), 83.95, 83.90 ($C_{quart,PhiPr}$ & $C_{quart,tBu}$), 68.6 ($CH_{\beta,Thr}$), 67.5 ($CH_{2,Fmoc}$), 58.2 ($CH_{\alpha,Thr}$), 53.8 ($CH_{\alpha,Trp}$), 51.5 ($CH_{\alpha,Asp}$), 47.1 (CH_{Fmoc}), 36.4 ($CH_{2,Asp}$), 28.8 ($CH_{3,PhiPr}$), 28.3 (3 × $CH_{3,tBu}$), 28.1 ($CH_{3,PhiPr}$), 26.8 ($CH_{2,Trp}$), 19.8 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{48}H_{52}N_4O_{11}Na^+$: 883.3525 [*M*+Na]$^+$, found: 883.3550.

Fmoc-Asp(OH)-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (16e)

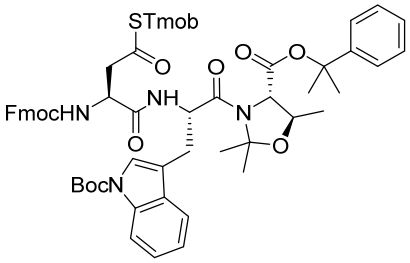


$C_{51}H_{56}N_4O_{11}$ [901.03 g mol^{-1}]

Threonine-tripeptide **15e** (560 mg, 0.65 mmol, 1 eq.) was dissolved in dry dichloromethane (10 mL) and PPTS (16 mg, 0.065 mmol, 0.1 eq) was added. The solution was cooled to 0 °C and 2-methoxypropene (0.62 mL, 6.5 mmol, 10 eq.) was added dropwise. The mixture was then allowed to

warm to room temperature. After 6 hours, further portions of PPTS (16 mg, 0.065 mmol, 0.1 eq) and 2-methoxypropene (0.62 mL, 6.5 mmol, 10 eq.) were added at 0 °C. The mixture was again allowed to warm to room temperature and was stirred for a total of 22 hours. Triethylamine (27 μL, 0.13 mmol, 0.2 eq.) was added and the solvent was removed *in vacuo*. Residual pyridine and triethylamine was removed by repeated co-evaporation with toluene. The crude product was purified by FC (silica, dichloromethane/methanol 15:1) to yield the desired product **16e** as colorless foam (439 mg, 75%). **TLC**: R_f = 0.36 (dichloromethane/methanol 15:1; UV/anisaldehyde); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = 8.09 (d, *J* = 7.9 Hz, 1H, H_{aryl}), 7.88 (d, *J* = 7.7 Hz, 1H, H_{aryl}), 7.73 (d, *J* = 7.7 Hz, 3H, 2 × H_{aryl} & NH_{Trp}), 7.63 – 7.49 (m, 2H, H_{aryl}), 7.41 – 7.07 (m, 12H, H_{aryl} (superimposed by solvent)), 6.48 (d, *J* = 9.3 Hz, 1H, NH_{Asp}), 4.68 (s, 2H, $CH_{\alpha,Asp}$ & $CH_{\alpha,Trp}$), 4.50 – 4.25 (m, 2H, $CH_{2,Fmoc}$), 4.19 (t, *J* = 7.2 Hz, 1H, CH_{Fmoc}), 3.87 (dq, *J* = 6.0 Hz, 1H, $CH_{\alpha,Thr}$), 3.29 (d, *J* = 13.7 Hz, 1H, C<u>H</u>H'$_{Asp}$), 3.18 (d, *J* = 17.8 Hz, 1H, C<u>H</u>H'$_{Trp}$), 2.93 – 2.86 (m, 1H, CH<u>H'</u>$_{Asp}$), 2.84 (d, *J* = 5.7 Hz, 1H, $CH_{\beta,Thr}$), 2.76 (dd, *J* = 16.8, 5.4 Hz, 1H, CH<u>H'</u>$_{Trp}$), 1.73 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.64 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.59 (s, 9H, 3 × $CH_{3,\mathit{t}Bu}$), 1.50 (s, 3H, $CH_{3,pseudoproline}$), 1.35 (s, 3H, $CH_{3,pseudoproline}$), 0.70 (d, *J* = 6.1 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 174.8 (COOH), 170.2, 169.7 (CO_{Asp} & CO_{Trp}), 168.0 (CO_{Thr}), 156.1 ($COONH_{Fmoc}$), 149.3 ($COONH_{Boc}$), 144.6 ($C_{quart,Ph}$), 144.0, 141.4 (4 × $C_{quart,Fmoc}$), 135.4, 130.0 (2 × $C_{quart,Trp}$), 128.5, 127.8, 127.5, 127.2, 125.3, 125.1, 124.4, 124.2, 123.3, 120.0, 119.3, 115.5 (18 × C_{aryl}), 114.8 ($C_{quart,Trp}$), 97.4 ($C_{quart,pseudoproline}$), 84.6 ($C_{quart,Ph\mathit{i}Pr}$), 83.8 ($C_{quart,\mathit{t}Bu}$), 74.2 ($CH_{\beta,Thr}$), 67.5 ($CH_{2,Fmoc}$), 65.7 ($CH_{\alpha,Thr}$), 53.2 ($CH_{\alpha,Trp}$), 51.5 ($CH_{\alpha,Asp}$), 47.2 (CH_{Fmoc}), 36.4 ($CH_{2,Asp}$), 30.4 ($CH_{2,Trp}$), 28.9 ($CH_{3,Ph\mathit{i}Pr}$), 28.2 (3 × $CH_{3,\mathit{t}Bu}$), 27.2 ($CH_{3,Ph\mathit{i}Pr}$), 26.4, 23.8 (2 × $CH_{3,pseudoproline}$), 19.9 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{51}H_{57}N_4O_{11}^+$: 901.4018 [*M*+H]$^+$, found: 901.4014.

Fmoc-Asp(STmob)-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (17e)

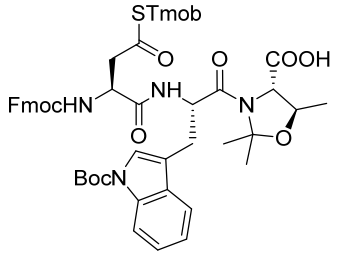


$C_{61}H_{68}N_4O_{13}S$ [1097.29 g mol^{-1}]

Thioesterification of aspartic acid **16e** (399 mg, 0.44 mmol, 1eq.) was achieved according to *general procedure 2*, using TmobSH (104 mg, 0.49 mmol, 1.1 eq.), PyBOP (253 mg, 0.49 mmol, 1.1 eq.) and DIPEA (170 μL, 0.49 mmol, 2.2 eq.) in dichloromethane (2.2 mL) at –15 °C. After removal of the solvent, the crude product was purified by FC (silica, petroleum ether/EtOAc 2:1) to yield a colorless foam (367 mg, 76%). **TLC**: R_f = 0.31 (petroleum ether/EtOAc 2:1; UV/anisaldehyde); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = 8.20 (d, *J* = 8.2 Hz, 1H, H_{aryl}), 7.98 (d, *J* = 7.6 Hz, 1H, H_{aryl}), 7.77 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.67 – 7.55 (m, 2H, H_{aryl}), 7.46 – 7.11 (m, 13H, 12 × H_{aryl} & NH_{Trp} (superimposed by solvent)), 6.06 (s, 2H, $H_{aryl,Tmob}$), 5.82 (d, *J* = 8.7 Hz, 1H, NH_{Asp}), 4.71 – 4.61 (m, 1H, $CH_{\alpha,Trp}$), 4.56 (s, 1H, $CH_{\alpha,Asp}$), 4.47 – 4.35 (m, 2H, $CH_{2,Fmoc}$), 4.33 – 4.19 (m, 3H, $CH_{2,Tmob}$ & CH_{Fmoc}), 3.90 (dq, *J* = 6.0, 5.9 Hz, 1H, $CH_{\beta,Thr}$), 3.76 (s, 3H, OMe), 3.75 (s, 6H, 2 × OMe), 3.40 – 3.21 (m, 2H, C<u>H</u>H'$_{Asp}$ & C<u>H</u>H'$_{Trp}$), 3.01 – 2.89 (m, 2H, CH<u>H'</u>$_{Asp}$ & CH<u>H'</u>$_{Trp}$), 2.86 (d, *J* = 5.6 Hz, 1H, $CH_{\alpha,Thr}$), 1.74 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.66 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.65 (s, 9H, 3 × $CH_{3,\mathit{t}Bu}$), 1.57 (s, 3H, $CH_{3,pseudoproline}$), 1.40 (s, 3H, $CH_{3,pseudoproline}$), 0.77 (d, *J* = 6.1 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 198.5 (COS), 169.2 (CO_{Trp}), 169.0 (CO_{Asp}), 168.4 (CO_{Thr}), 161.0 ($C_{quart,Tmob}$), 159.3 (2 × $C_{quart,Tmob}$), 156.0 ($COONH_{Fmoc}$), 149.5 ($COONH_{Boc}$), 144.7 ($C_{quart,Ph}$), 144.0, 143.8, 141.4 (4 × $C_{quart,Fmoc}$), 135.6, 130.4 (2 × $C_{quart,Trp}$), 128.5, 127.8, 127.4, 127.3, 125.3, 125.4, 124.4, 124.2, 123.4, 120.1, 119.5 (17 × C_{aryl}) 115.5, 115.4 (C_{aryl} & $C_{quart,Trp}$), 104.6 ($C_{quart,Tmob}$), 97.2 ($C_{quart,pseudoproline}$), 90.6 (2 × $C_{aryl,Tmob}$), 84.4 ($C_{quart,PhiPr}$), 83.8 ($C_{quart,\mathit{t}Bu}$), 74.2 ($CH_{\beta,Thr}$), 67.6 ($CH_{2,Fmoc}$), 65.6 ($CH_{\alpha,Thr}$), 55.9 (2 × OMe), 55.4 (OMe), 52.9 ($CH_{\alpha,Trp}$), 51.9 ($CH_{\alpha,Asp}$), 47.2 (CH_{Fmoc}), 44.8 ($CH_{2,Asp}$), 30.4 ($CH_{2,Trp}$), 28.9 ($CH_{3,Ph\mathit{i}Pr}$), 28.3 (3 × $CH_{3,\mathit{t}Bu}$), 27.3 ($CH_{3,Ph\mathit{i}Pr}$),

26.5, 23.8 (2 × $CH_{3,pseudoproline}$), 22.5 ($CH_{2,Tmob}$), 20.0 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{61}H_{69}N_4O_{13}S^+$: 1097.4576 [*M*+H]$^+$, found: 1097.4558.

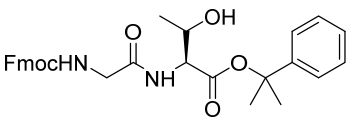
Fmoc-Asp(STmob)-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-OH (18e)



$C_{52}H_{58}N_4O_{13}S$ [979.11 g mol^{-1}]

Phenyl*iso*propyl ester **17e** (327 mg, 0.30 mmol, 1 eq.) was dissolved in dichloromethane (8.4 mL) and TFA (170 µL, final concentration: 2% in dichloromethane) was added dropwise. After 30 minutes, TLC indicated complete conversion and the solution was diluted with toluene (10 mL). The solvents were removed by rotary evaporation and the residue was co-evaporated with toluene three times. The crude product was dissolved in dry dichloromethane (4 mL), the solution was cooled to 0 °C and PPTS (7.5 mg, 0.03 mmol, 0.1 eq) was added. 2-methoxypropene (290 µL, 2.98 mmol, 10 eq.) was added dropwise and the reaction mixture was allowed to warm to room temperature. After 3 hours, further portions of PPTS (7.5 mg, 0.03 mmol, 0.1 eq) and 2-methoxypropene (290 µL, 2.98 mmol, 10 eq.) were added at 0 °C and the mixture was stirred at room temperature for another hour until TLC indicated complete conversion. Triethylamine (16 µL, 0.2 eq.) was added and the solvent was removed *in vacuo*. The crude product **18e** was purified by FC (silica, dichloromethane/methanol 20:1 → 15:1) to yield an off-white foam (239 g, 82%). **TLC**: R_f = 0.49 (dichloromethane/methanol 10:1, UV/anisaldehyde); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 8.17 (d, *J* = 8.1 Hz, 1H, H_{aryl}), 7.76 (d, *J* = 7.8 Hz, 1H, H_{aryl}), 7.72 (d, *J* = 7.6 Hz, 2H, H_{aryl}), 7.59 – 7.50 (m, 2H, H_{aryl}), 7.50 – 7.41 (m, 2H, H_{aryl} & NH_{Trp}), 7.40 – 7.18 (m, 6H, H_{aryl} (superimposed by solvent)), 6.29 (d, *J* = 8.7 Hz, 1H, NH_{Asp}), 6.01 (s, 2H, $H_{aryl,Tmob}$), 4.96 – 4.84 (m, 1H, $CH_{\alpha,Trp}$), 4.81 – 4.64 (m, 1H, $CH_{\alpha,Asp}$), 4.40 – 4.13 (m, 5H, $CH_{2,Fmoc}$, $CH_{2,Tmob}$ & CH_{Fmoc}), 4.08 (dq, *J* = 6.1, 6.1 Hz, 1H, $CH_{\beta,Thr}$), 3.73 (s, 3H, OMe), 3.69 (s, 6H, 2 × OMe), 3.37 – 3.20 (m, 2H, $CH_{\alpha,Thr}$ & $C\underline{H}H'_{Asp}$), 3.20 – 3.05 (m, 2H, $CH_{2,Trp}$), 2.99 (dd, *J* = 16.7, 5.5 Hz, 1H, $CH\underline{H}'_{Asp}$), 1.63 (s, 9H, 3 × $CH_{3,tBu}$), 1.44 (s, 3H, $CH_{3,pseudoproline}$), 1.38 (s, 3H, $CH_{3,pseudoproline}$), 0.85 (d, *J* = 6.1 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 198.6 (COS), 172.5 (COOH), 171.1 (CO_{Asp}), 168.6 (CO_{Trp}), 161.0 ($C_{quart,Tmob}$), 159.3 (2 × $C_{quart,Tmob}$), 156.1 ($COONH_{Fmoc}$), 149.5 ($COONH_{Boc}$), 144.0, 143.8, 141.3 (4 × $C_{quart,Fmoc}$), 135.6, 130.2 (2 × $C_{quart,Trp}$), 127.8, 127.3, 125.4 125.3, 124.9, 124.7, 123.2, 120.0, 119.3 (12 × $C_{aryl,Tmob}$), 115.5, 114.8 (C_{aryl} & $C_{quart,Trp}$), 104.5 ($C_{quart,Tmob}$), 96.9 ($C_{quart,pseudoproline}$), 90.6 (2 × $C_{aryl,Tmob}$), 83.8 ($C_{quart,tBu}$), 74.5 ($CH_{\alpha,Thr}$), 67.7 ($CH_{2,Fmoc}$), 65.9 ($CH_{\beta,Thr}$), 55.9 (2 × OMe), 55.4 (OMe), 52.5 ($CH_{\alpha,Trp}$), 51.9 ($CH_{\alpha,Asp}$), 47.5 (CH_{Fmoc}), 45.0 ($CH_{2,Asp}$), 30.0 ($CH_{2,Trp}$), 28.3 (3 × $CH_{3,tBu}$), 26.1, 23.7 (2 × $CH_{3,pseudoproline}$), 22.5 ($CH_{2,Tmob}$), 19.8 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{52}H_{58}N_4O_{13}SNa^+$: 1001.3613 [*M*+Na]$^+$, found: 1001.3602.

Fmoc-Gly-Thr(OH)-*O*-2-Ph*i*Pr (13f)

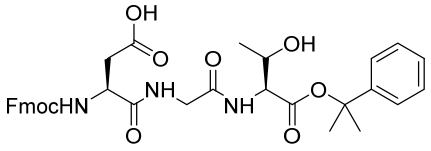


$C_{30}H_{32}N_2O_6$ [516.59 g mol^{-1}]

Dipeptide **13f** was synthesized according to *general procedure 1*, by deprotecting Fmoc-Thr(OH)-*O*-2-Ph*i*Pr (**12**) (14.37 g, 31.28 mmol, 1 eq.) with piperidine (315 mL, 20% in DMF) and subsequent coupling with Fmoc-Gly-OH (11.16 g, 37.54 mmol, 1.2 eq.) using HBTU (12.24 g, 37.54 mmol, 1.2 eq.),

HOBt (5.07 g, 37.54 mmol, 1.2 eq.) and DIPEA (13.08 mL, 75.08 mmol, 2.4 eq.) in dichloromethane (400 mL). After stirring at room temperature for 2.5 hours, the solvent was removed by evaporation and the residue was dissolved in EtOAc (600 mL). The organic phase was washed with sat. $NaHCO_{3(aq.)}$ (3 × 150 mL), water (2 × 150 mL), $NaOH_{(aq.)}$ (150 mL, 1 μ), water (1 × 150 mL) and sat. $NaCl_{(aq.)}$ (3 × 150 mL). After drying ($MgSO_4$), the solvent was removed and the residue was purified by FC (silica, petroleum ether/EtOAc 1:2→1:3). Glycine dipeptide **13f** was obtained as colorless foam (10.12 g, 63%). **TLC**: R_f = 0.26 (petroleum ether/EtOAc 1:2, UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz): δ [ppm] = 7.75 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.57 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.43 – 7.20 (m, 9H, H_{aryl} (superimposed by solvent)), 6.64 (d, *J* = 8.9 Hz, 1H, NH_{Thr}), 5.45 (t, *J* = 5.7 Hz, 1H, NH_{Gly}), 4.59 (dd, *J* = 8.9, 2.5 Hz, 1H, $CH_{\alpha,Thr}$), 4.45 – 4.34 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.20 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 3.90 (d, *J* = 5.4 Hz, 2H, $CH_{2,Gly}$), 2.05 (br. s, 1H, OH), 1.80 (s, 3H, $CH_{3,Ph/Pr}$), 1.79 (s, 3H, $CH_{3,Ph/Pr}$), 1.21 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz): δ [ppm] = 169.6, 169.5 (CO_{Gly} & CO_{Thr}), 156.7 (COONH), 145.0 ($C_{quart,PhiPr}$), 143.9, 141.4 (4 × $C_{quart,Fmoc}$), 128.5, 127.9, 127.5, 127.3, 125.22, 125.20, 124.4, 120.1 (13 × C_{aryl}), 84.0 ($C_{quart,PhiPr}$), 68.4 ($CH_{\beta,Thr}$), 67.4 ($CH_{2,Fmoc}$), 57.8 ($CH_{\alpha,Thr}$), 47.2 (CH_{Fmoc}), 44.5 ($CH_{2,Gly}$), 28.7, 28.4 (2 × $CH_{3,Ph}$), 20.3 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{30}H_{32}N_2O_6Na^+$: 539.21526 [*M*+Na]$^+$, found: 539.21539.

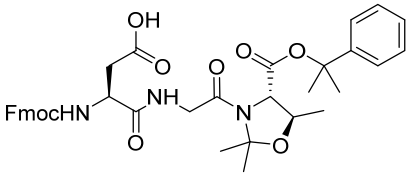
Fmoc-Asp(OH)-Gly-Thr(OH)-*O*-2-Ph*i*Pr (15f)



$C_{34}H_{37}N_3O_9$ [631.68 g mol^{-1}]

The dipeptide **13f** (10.12 g, 19.60 mmol, 1 eq.) was deprotected with piperidine (195 mL, 20% in DMF) and coupled with Fmoc-Asp(OBn)-OH (10.48 g, 23.52 mmol, 1.2 eq.), HOBt (3.18 g, 23.52 mmol, 1.2 eq.), HBTU (8.92 g, 23.52 mmol, 1.2 eq.) and DIPEA (8.20 mL, 47.05 mmol, 2.4 eq.) in dichloromethane (250 mL) according to *general procedure 1*. After stirring at r.t. for 4 hours, the reaction mixture was diluted with dichloromethane (150 mL) and was washed with water (2 × 200 mL), sat. $NaHCO_{3(aq.)}$ (2 × 200 mL), water (200 mL) and sat. $NaCl_{(aq.)}$ (200 mL). After drying ($MgSO_4$), the solvent was removed *in vacuo* and the residue was purified by FC (silica, petroleum ether/EtOAc 1:2→2:5) to obtain a colorless foam of **14f** with still minor impurities (6.62 g).
The crude tripeptide **14f** was subsequently hydrogenated for one hour according to *general procedure 3*. The product was purified by FC (dichloromethane/methanol 15:1→10:1) to yield a colorless foam of **15f** (6.08 g, 49% over two steps). **TLC**: R_f = 0.12 (dichloromethane/methanol 10:1; UV/mostain); **^{1}H NMR** (methanol-d4, 400 MHz): δ [ppm] = 7.82 – 7.75 (m, 2H, H_{aryl}), 7.65 (t, *J* = 7.1 Hz, 2H, H_{aryl}), 7.42 – 7.34 (m, 4H, H_{aryl}), 7.34 – 7.23 (m, 4H, H_{aryl}), 7.21 – 7.14 (m, 1H, H_{aryl}), 4.50 – 4.42 (m, 2H, CH_{Asp} & $CH_{\alpha,Thr}$), 4.41 – 4.30 (m, 3H, $CH_{2,Fmoc}$ & $CH_{\beta,Thr}$), 4.20 (t, *J* = 6.8 Hz, 1H, CH_{Fmoc}), 4.03 (d, *J* = 17.0 Hz, 1H, C<u>H</u>H'$_{Gly}$), 3.82 (d, *J* = 17.0 Hz, 1H, CH<u>H'</u>$_{Gly}$), 2.89 (dd, *J* = 16.9, 5.5 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.75 (dd, *J* = 16.9, 7.5 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.75 (s, 3H, $CH_{3,Ph/Pr}$), 1.74 (s, 3H, $CH_{3,Ph/Pr}$), 1.18 (d, *J* = 6.4 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** (methanol-d4, 101 MHz): δ [ppm] = 174.4, 174.0, 172.0, 170.6 (4 × CO), 158.6 (COONH), 146.7, 145.22, 145.15 (4 × $C_{quart,Fmoc}$), 142.6 ($C_{quart,Ph}$), 129.2, 128.8, 128.2, 128.0, 126.3, 125.5, 120.9 (13 × C_{aryl}), 84.6 ($C_{quart,PhiPr}$), 68.7 ($CH_{\beta,Thr}$), 68.3 ($CH_{2,Fmoc}$), 60.0 ($CH_{\alpha,Thr}$), 53.2 (CH_{Asp}), 48.3 (CH_{Fmoc}), 43.7 ($CH_{2,Gly}$), 36.9 ($CH_{2,Asp}$), 29.2, 28.8 (2 × $CH_{3,Ph/Pr}$), 20.4 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{34}H_{37}N_3O_9Na^+$: 654.24220 [*M*+Na]$^+$, found: 654.24258.

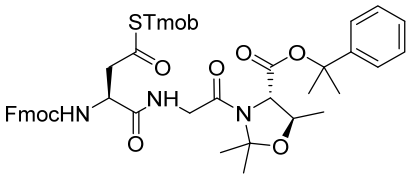
Fmoc-Asp(OH)-Gly-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (16f)



$C_{37}H_{41}N_3O_9$ [671.75 g mol^{-1}]

For backbone protection, threonine-containing tripeptide **15f** (6.21 g, 9.84 mmol, 1 eq.) was dissolved in dry dichloromethane (135 mL) and MS 4 Å was added. 2-methoxypropene (9.39 mL, 98.4 mmol, 10 eq.) was added and the mixture was cooled to 0 °C before PPTS (1.26 g, 4.92 mmol, 0.5 eq.) was added. The mixture was then allowed to warm to room temperature. After a total reaction time of 2.25 and 3.5 hours, further portions of 2-methoxypropene (4.70 mL, 49.2 mmol, 5 eq.) and PPTS (0.63 g, 2.46 mmol, 0.25 eq.) were added. After four hours of total reaction time, the mixture was diluted with dichloromethane (100 mL) and extracted with water (3 × 150 mL) and sat. $NH_4Cl_{(aq.)}$ (150 mL). The organic phase was dried ($MgSO_4$) and the solvent was removed by evaporation. The residue was purified by FC (silica, dichloromethane/methanol 15:1→10:1) and residual traces of pyridine were removed by repeated co-evaporation with toluene. The pseudoproline-protected tripeptide **16f** was obtained as colorless foam (5.99 g, 91%). **TLC**: R_f = 0.22 (dichloromethane/methanol 10:1; UV/mostain); **^{1}H NMR** (methanol-d4, 400 MHz, major conformer): δ [ppm] = 7.78 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.65 (d, *J* = 7.6 Hz, 2H, H_{aryl}), 7.44 – 7.15 (m, 9H, H_{aryl}), 4.59 (t, *J* = 6.8 Hz, 1H, CH_{Asp}), 4.43 – 4.29 (m, 4H, $CH_{2,Fmoc}$, $CH_{\alpha,Thr}$ & $CH_{\beta,Thr}$), 4.23 (t, *J* = 7.0 Hz, 1H, CH_{Fmoc}), 3.97 (d, *J* = 16.5 Hz, 1H, C<u>H</u>H'$_{Gly}$), 3.48 (d, *J* = 16.5 Hz, 1H, CH<u>H'</u>$_{Gly}$), 2.88 (dd, *J* = 16.7, 5.5 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.70 (dd, *J* = 16.7, 7.8 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.83 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.79 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.62 (s, 3H, $CH_{3,pseudoproline}$), 1.49 (d, *J* = 6.0 Hz, 3H, $CH_{3,Thr}$), 1.45 (s, 3H, $CH_{3,pseudoproline}$); **^{13}C NMR** (methanol-d4, 101 MHz, major conformer): δ [ppm] = 174.2 (COOH), 173.5 (CO_{Asp}), 170.2 (CO_{Thr}), 167.8 (CO_{Gly}), 158.3 (COONH), 145.9 ($C_{quart,Ph}$), 145.24, 145.22, 142.6 (4 × $C_{quart,Fmoc}$), 129.5, 128.8, 128.5, 128.2, 126.3, 125.5, 120.9 (13 × C_{aryl}), 98.4 ($C_{quart,pseudoproline}$), 85.6 ($C_{quart,Ph\mathit{i}Pr}$), 76.7 ($CH_{\beta,Thr}$), 68.2 ($CH_{2,Fmoc}$), 66.4 ($CH_{\alpha,Thr}$), 53.0 (CH_{Asp}), 48.5 (CH_{Fmoc} (superimposed by solvent)), 43.8 ($CH_{2,Gly}$), 37.2 ($CH_{2,Asp}$), 29.2 ($CH_{3,Ph\mathit{i}Pr}$), 28.0 ($CH_{3,Ph\mathit{i}Pr}$), 27.3 ($CH_{3,pseudoproline}$), 24.3 ($CH_{3,pseudoproline}$), 20.6 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{37}H_{41}N_3O_9Na^+$: 694.27350 [*M*+Na]$^+$, found: 694.27388.

Fmoc-Asp(STmob)-Gly-Thr($\Psi^{Me,Me}$pro)-*O*-2-Ph*i*Pr (17f)

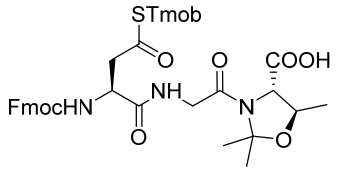


$C_{47}H_{53}N_3O_{11}S$ [868.01 g mol^{-1}]

Thioesterification of aspartic acid **16f** (5.97 g, 8.89 mmol, 1eq.) was achieved according to *general procedure 2* using TmobSH (2.09 g, 9.78 mmol, 1.1 eq.), PyBOP (5.09 g, 9.78 mmol, 1.1 eq.) and DIPEA (3.41 mL, 19.57 mmol, 2.2 eq.) in dichloromethane (45 mL) at –15 °C. After removal of the solvent, the crude product was purified by FC (silica, petroleum ether/EtOAc/toluene 1:1:1) to yield a colorless foam (5.57 g, 72%). **TLC**: R_f = 0.23 (petroleum ether/EtOAc/toluene 1:1:1; UV/vanillin); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ [ppm] = 7.76 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.61 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.45 – 7.21 (m, 9H (superimposed by solvent)), 7.22 – 7.13 (m, 1H, NH_{Gly}), 6.09 (s, 2H, $H_{aryl,Tmob}$), 5.86 (d, *J* = 8.6 Hz, 1H, NH_{Asp}), 4.71 – 4.58 (m, 1H, CH_{Asp}), 4.50 – 4.35 (m, 2H, $CH_{2,Fmoc}$), 4.35 – 4.18 (m, 4H, $CH_{2,Tmob}$, $CH_{\beta,Thr}$ & CH_{Fmoc}), 4.08 – 3.98 (m, 2H, C<u>H</u>H'$_{Gly}$ & $CH_{\alpha,Thr}$), 3.79 (s, 3H, OMe), 3.78 (s, 6H, 2 × OMe), 3.50 (dd, *J* = 16.9, 3.4 Hz, 1H, CH<u>H'</u>$_{Gly}$), 3.29 (dd, *J* = 16.7, 4.4 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.99 (dd, *J* = 16.7, 5.6 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.85 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.83 (s, 3H, $CH_{3,Ph\mathit{i}Pr}$), 1.70 (s, 3H,

$CH_{3,pseudoproline}$), 1.54 – 1.49 (m, 6H, $CH_{3,pseudoproline}$ & $CH_{3,Thr}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 198.5 (COS), 169.9 (CO_{Asp}), 168.4 (CO_{Thr}), 165.0 (CO_{Gly}), 160.9 ($C_{quart,Tmob}$), 159.2 (2 × $C_{quart,Tmob}$), 156.0 (COONH), 144.1 ($C_{quart,Ph\mathit{i}Pr}$), 143.8, 143.7, 141.3 (4 × $C_{quart,Fmoc}$), 128.6, 127.7, 127.2, 125.2, 124.3, 120.0 (13 × C_{aryl}), 104.3 ($C_{quart,Tmob}$), 97.6 ($C_{quart,pseudoproline}$), 90.5 ($C_{aryl,Tmob}$), 84.5 ($C_{quart,Ph}$), 75.4 ($CH_{\beta,Thr}$), 67.4 ($CH_{2,Fmoc}$), 65.3 ($CH_{\alpha,Thr}$), 55.8 (2 × OMe), 55.3 (OMe), 51.7 (CH_{Asp}), 47.1 (CH_{Fmoc}), 44.7 ($CH_{2,Asp}$), 42.8 ($CH_{2,Gly}$), 28.6, 27.8 (2 × $CH_{3,Ph\mathit{i}Pr}$), 26.9, 23.9 (2 × $CH_{3,pseudoproline}$), 22.4 ($CH_{2,Tmob}$), 20.3 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{47}H_{53}N_3O_{11}SNa^+$: 890.32930 [*M*+Na]$^+$, found: 890.32952.

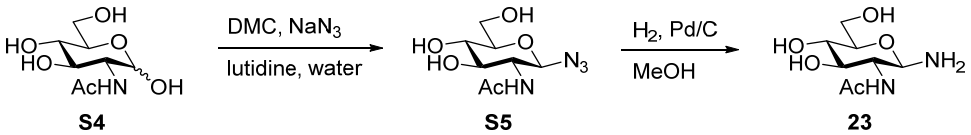
Fmoc-Asp(STmob)-Gly-Thr($\Psi^{Me,Me}$pro)-OH (18f)



$C_{38}H_{43}N_3O_{11}S$ [749.83 g mol^{-1}]

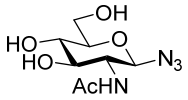
For C-terminal deprotection, tripeptide **17f** (5.44 g, 6.27 mmol, 1 eq.) was dissolved in dichloromethane (176 mL), tri*iso*propylsilane (6.42 mL, 31.35 mmol, 5 eq.) was added and the solution was cooled to 0 °C. TFA (3.6 mL, final concentration: 2% in dichloromethane) was added and the mixture was allowed to warm to room temperature. After 35 minutes, TLC indicated complete consumption of starting material. Saturated $NaHCO_{3(aq.)}$ (60 mL) and water (100 mL) was immediately added and the colorless precipitate was filtered and dried by lyophylization. Further product was isolated from the filtrate which was acidified by adding sat. citric $acid_{(aq.)}$ and extracted with dichloromethane (8 × 100 mL). After removal of the solvent, the combined dry products were dissolved in dry dichloromethane (44 mL) and MS 4 Å was added. The solution was cooled to 0 °C and 2-methoxypropene (3.0 mL, 31.35 mmol, 5 eq.), followed by PPTS (400 mg, 1.59 mmol, 0.25 eq.) were added. After 30 and 60 minutes, respectively, further portions of 2-methoxypropene (1.5 mL, 15.68 mmol, 2.5 eq.), followed by PPTS (200 mg, 0.80 mmol, 0.13 eq.) were added. After a total reaction time of 100 minutes, the reaction mixture was diluted with dichloromethane (50 mL) and extracted with water (2 × 50 mL) and sat. $NH_4Cl_{(aq.)}$. The organic layer was dried ($MgSO_4$) and the crude product was purified by FC (silica, dichloromethane/methanol 15:1→10:1). Further purification was achieved by RP-MPLC (50–60% acetonitrile in water, 0.1% FA) to yield glycine tripeptide building block **18f** as colorless solid (2.06 g, 44%). **TLC**: R_f = 0.10 (dichloromethane/methanol 10:1, UV/anisaldehyde); **^{1}H NMR** (methanol-d4, 400 MHz, major conformer): δ [ppm] = 7.78 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.69 – 7.59 (m, 2H, H_{aryl}), 7.37 (t, *J* = 7.5 Hz, 2H, H_{aryl}), 7.29 (tdd, *J* = 7.5 Hz, 4.0 Hz, 1.1 Hz, 2H, H_{aryl}), 6.09 (s, 2H, $H_{aryl,Tmob}$), 4.67 (dd, *J* = 9.0 Hz, 4.5 Hz, 1H, CH_{Asp}), 4.43 – 4.31 (m, 3H, $CH_{\alpha,Thr}$, $CH_{\beta,Thr}$ & $C\underline{H}H'_{Fmoc}$), 4.27 (dd, *J* = 10.5 Hz, 7.3 Hz, 1H, $CH\underline{H}'_{Fmoc}$), 4.23 – 4.12 (m, 3H, $CH_{2,Tmob}$ & CH_{Fmoc}), 3.97 (d, *J* = 16.5 Hz, 1H, $C\underline{H}H'_{Gly}$), 3.80 – 3.66 (m, 10H, 3 × OMe & $CH\underline{H}'_{Gly}$), 3.10 (dd, *J* = 15.9 Hz, 4.5 Hz, 1H, $C\underline{H}H'_{Asp}$), 2.95 (dd, *J* = 15.8 Hz, 9.0 Hz, 1H, $CH\underline{H}'_{Asp}$), 1.64 (s, 3H, $CH_{3,pseudoproline}$), 1.52 (s, 3H, $CH_{3,pseudoproline}$), 1.46 (d, *J* = 5.9 Hz, 3H, $CH_{3,Thr}$); **^{13}C NMR** (methanol-d4, 101 MHz, major conformer): δ [ppm] = 198.5 (COS), 173.2, 173.0 (COOH & CO_{Asp}), 167.9 (CO_{Gly}), 162.5 ($C_{quart,Tmob}$), 160.5 (2 × $C_{quart,Tmob}$), 158.2 (COONH), 145.3, 145.2, 142.6, 142.5 (4 × $C_{quart,Fmoc}$), 128.8, 128.2, 126.4, 126.3, 120.9 (8 × $C_{aryl,Fmoc}$), 105.4 ($C_{quart,Tmob}$), 98.4 ($C_{quart,pseudoproline}$), 91.6 (2 × $C_{aryl,Tmob}$), 77.0 ($CH_{\beta,Thr}$), 68.3 ($CH_{2,Fmoc}$), 66.0 ($CH_{\alpha,Thr}$), 56.3 (2 × OMe), 55.7 (OMe), 53.2 (CH_{Asp}), 48.5 (CH_{Fmoc} (superimposed by solvent)), 45.9 ($CH_{2,Asp}$), 43.6 ($CH_{2,Gly}$) 27.4, 24.3 (2 × $CH_{3,pseudoproline}$), 23.1 ($CH_{2,Tmob}$), 20.5 ($CH_{3,Thr}$); **HRMS:** m/z calcd. for $C_{38}H_{43}N_3O_{11}SNa^+$: 772.25105 [*M*+Na]$^+$, found: 772.25149.

5. Synthesis of Carbohydrate Derivatives 23, 25 and 26



Scheme S2. Synthesis of glycosyl amine **23**.

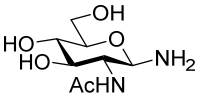
2-Acetamido-2-deoxy-β-D-glucopyranosyl azide (S5)



$C_8H_{14}N_4O_5$ [246.22 g mol^{-1}]

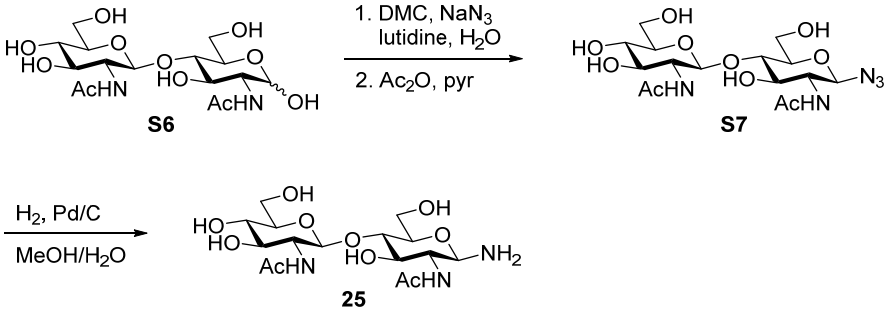
$GlcNAcN_3$ **S5** was synthesized according to Shoda and co-workers.[7]

2-Acetamido-2-deoxy-β-D-glucopyranosyl amine (23)



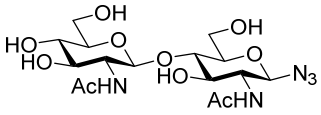
$C_8H_{16}N_2O_5$ [220.25 g mol^{-1}]

Glycosyl azide **S5** (150 mg, 0.41 mmol) was hydrogenolyzed according to *general procedure 4*. Glycosyl amine **23** was obtained as colorless solid. (133 mg, quant.). Spectroscopic data was in accordance with literature.[3]



Scheme S3. Synthesis of glycosyl amine **25**.

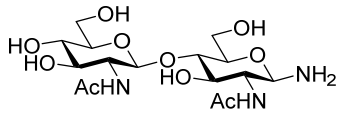
2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-3,6-di-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide (S7)



$C_{16}H_{27}N_5O_{10}$ [449.42 g mol^{-1}]

Chitobiosyl azide **S7** was synthesized according to Shoda and co-workers.[7]

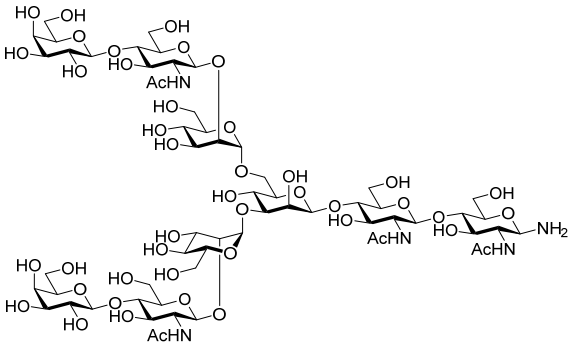
2-Acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-2-acetamido-2-deoxy-β-D-glucopyranosyl amine (**25**)



$C_{16}H_{29}N_3O_{10}$ [423.42 g mol^{-1}]

Chitobiosyl azide **S7** (20 mg, 0.0445 mmol) was dissolved in methanol/water (1:1, 1 mL) and palladium (2 mg, 5% on carbon, wetted with ca. 55% water) was added. The mixture was stirred under a hydrogen atmosphere for 30 minutes until TLC showed complete conversion. The solution was filtered through a syringe filter (0.45 μm) and the solvent was removed under reduced pressure. Residual water was removed by lyophylization. The desired chitobiose **25** was obtained as colorless fluffy solid. Due to its known instability[8] it was used without further purification and identification (quantitative yield).

Nonasaccharide glycosyl amine 26

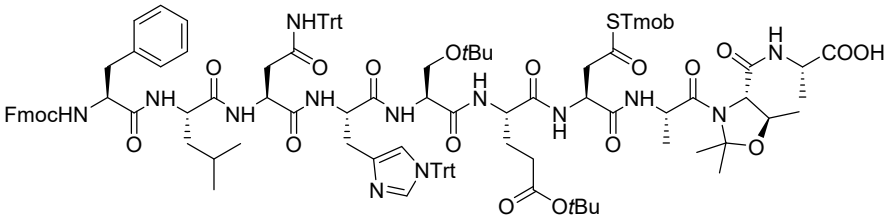


$C_{62}H_{105}N_5O_{45}$ [1640.51 g mol^{-1}]

Complex glycosyl amine **26** was synthesized starting from the corresponding glycosyl azide[9] as described by C. Unverzagt and co-workers.[10]

6. Fmoc-SPPS of Decapeptides 21a–f

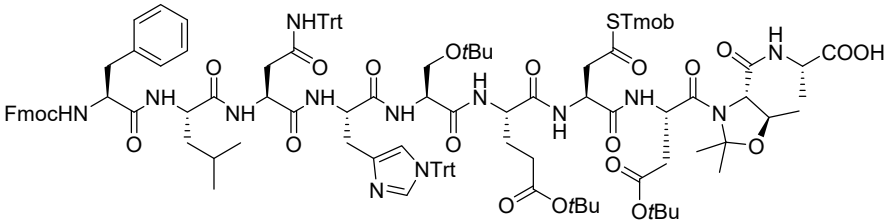
Fmoc-Phe-Leu-Asn(Trt)-His(Trt)-Ser(*t*Bu)-Glu(O*t*Bu)-Asp(STmob)-Ala-Thr($\Psi^{Me,Me}$pro)-Ala-OH (21a)



$C_{121}H_{139}N_{13}O_{22}S$ [2159.57 g mol^{-1}]

Ala-decapeptide **21a** was synthesized on solid-phase according to *general procedure 5* (250 mg resin, 0.883 g mmol^{-1}) and as depicted in Scheme 3. Analytical resin cleavage was conducted at the penta- and decapeptide stage as described in general procedure 9. The ratio of desired peptide to aspartimide was > 99:1 at the penta- (**19a**/**20a**) and 96:4 at the decapeptide (**21a**/**22a**) stage as determined by integration of the corresponding peaks in the LC-MS chromatograms (254 nm). Preparative resin cleavage was performed according to *general procedure 10*. Purification by FC (silica, dichloromethane/methanol 15:1, UV) yielded decapeptide **21a** as colorless solid (280 mg, 59%). **TLC** R_f = 0.34 (dichloromethane/methanol 15:1); **HRMS**: m/z calculated for $C_{121}H_{140}N_{13}O_{22}SNa^{2+}$: 1091.4938 [*M*+H+Na]$^{2+}$, found: 1091.4956.

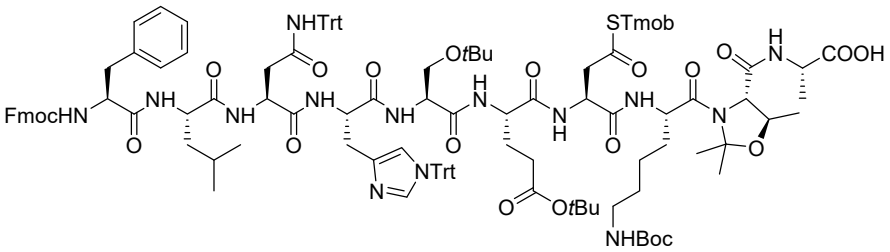
Fmoc-Phe-Leu-Asn(Trt)-His(Trt)-Ser(*t*Bu)-Glu(O*t*Bu)-Asp(STmob)-Asp(O*t*Bu)-Thr($\Psi^{Me,Me}$pro)-Ala-OH (21b)



$C_{126}H_{147}N_{13}O_{24}S$ [2259.69 g mol^{-1}]

Asp-decapeptide **21b** was synthesized on solid-phase according to *general procedure 5* (50 mg resin, 0.590 g mmol^{-1}) and as depicted in Scheme 3. Analytical resin cleavage was conducted at the penta- and decapeptide stage as described in general procedure 9. The ratio of desired peptide to aspartimide was 99:2 at the penta- (**19b**/**20b**) and 89:11 at the decapeptide (**21b**/**22b**) stage as determined by integration of the corresponding peaks in the LC-MS chromatograms (254 nm). Preparative resin cleavage was performed according to *general procedure 10*. Purification by FC (silica, dichloromethane/methanol 20:1→15:1, UV/mostain) yielded decapeptide **21b** as colorless solid (44 mg, 66%). **TLC** R_f = 0.27 (dichloromethane/methanol 15:1); **HRMS**: m/z calculated for $C_{126}H_{149}N_{13}O_{24}S^{2+}$: 1130.5291 [*M*+2H]$^{2+}$, found: 1130.5288.

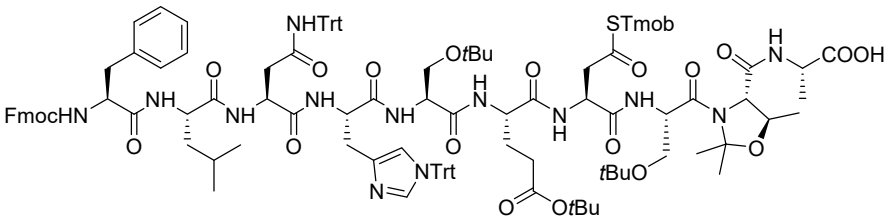
Fmoc-Phe-Leu-Asn(Trt)-His(Trt)-Ser(*t*Bu)-Glu(O*t*Bu)-Asp(STmob)-Lys(Boc)-Thr($\Psi^{Me,Me}$pro)-Ala-OH (21c)



$C_{129}H_{154}N_{14}O_{24}S$ [2316.79 g mol^{-1}]

Lys-decapeptide **21c** was synthesized on solid-phase according to *general procedure 5* (350 mg resin, 0.590 g mmol^{-1}) and as depicted in Scheme 3. Analytical resin cleavage was conducted at the penta- and decapeptide stage as described in *general procedure 9*. The ratio of desired peptide to aspartimide was > 99:1 at the penta- (**19c**/**20c**) and 99:1 at the decapeptide (**21c**/**22c**) stage as determined by integration of the corresponding peaks in the LC-MS chromatograms (254 nm). Preparative resin cleavage was performed according to *general procedure 10*. Purification by FC (silica, dichloromethane/methanol 12:1) yielded decapeptide **21c** as colorless solid (343 mg, 72%). **TLC** R_f = 0.19 (dichloromethane/methanol 15:1, UV); **HRMS**: m/z calculated for $C_{129}H_{155}N_{14}O_{24}SNa^{2+}$: 1170.0490 [*M*+H+Na]$^{2+}$, found: 1170.0510.

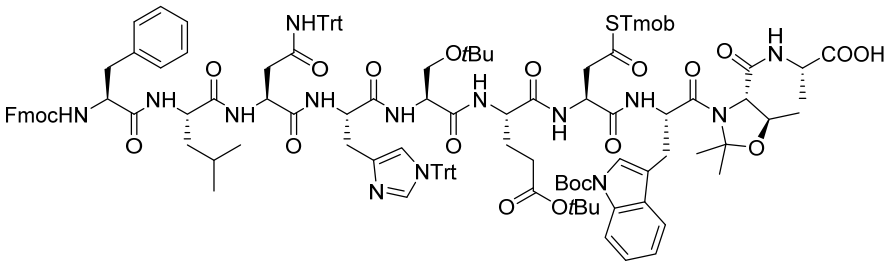
Fmoc-Phe-Leu-Asn(Trt)-His(Trt)-Ser(*t*Bu)-Glu(O*t*Bu)-Asp(STmob)-Ser(*t*Bu)-Thr($\Psi^{Me,Me}$pro)-Ala-OH (21d)



$C_{125}H_{147}N_{13}O_{23}S$ [2231.68 g mol^{-1}]

Lys-decapeptide **21d** was synthesized on solid-phase according to *general procedure 5* (50 mg resin, 0.590 g mmol^{-1}) and as depicted in Scheme 3. Analytical resin cleavage was conducted at the penta- and decapeptide stage as described in *general procedure 9*. The ratio of desired peptide to aspartimide was > 99:1 at the penta- (**19d**/**20d**) and 95:5 at the decapeptide (**21d**/**22d**) stage as determined by integration of the corresponding peaks in the LC-MS chromatograms (254 nm). Preparative resin cleavage was performed according to *general procedure 10*. Purification by FC (silica, dichloromethane/methanol 15:1) yielded decapeptide **21d** as colorless solid (41 mg, 62%). **TLC** R_f = 0.37 (dichloromethane/methanol 15:1, UV); **HRMS**: m/z calculated for $C_{125}H_{149}N_{13}O_{23}S^{2+}$: 1116.5316 [*M*+2H]$^{2+}$, found: 1116.5313.

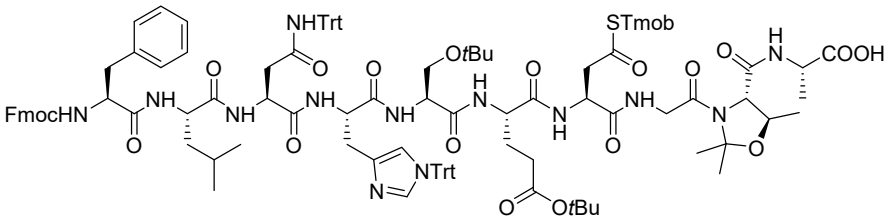
Fmoc-Phe-Leu-Asn(Trt)-His(Trt)-Ser(*t*Bu)-Glu(O*t*Bu)-Asp(STmob)-Trp(Boc)-Thr($\Psi^{Me,Me}$pro)-Ala-OH (21e)



$C_{134}H_{152}N_{14}O_{24}S$ [2374.82 g mol^{-1}]

Lys-decapeptide **21e** was synthesized on solid-phase according to *general procedure 5* (350 mg resin, 0.590 g $mmol^{-1}$) and as depicted in Scheme 3. Analytical resin cleavage was conducted at the penta- and decapeptide stage as described in *general procedure 9*. The ratio of desired peptide to aspartimide was > 99:1 at the penta- (**19e**/**20e**) and 94:6 at the decapeptide (**21e**/**22e**) stage as determined by integration of the corresponding peaks in the LC-MS chromatograms (254 nm). Preparative resin cleavage was performed according to *general procedure 10*. Purification by FC (silica, dichloromethane/methanol 20:1, UV) yielded decapeptide **21e** as colorless solid (253 mg, 52%). **TLC** R_f = 0.18 (dichloromethane/methanol 20:1); **HRMS**: m/z calculated for $C_{134}H_{153}N_{14}O_{24}SNa^{2+}$: 1199.0412 $[M+H+Na]^{2+}$, found: 1199.0455.

Fmoc-Phe-Leu-Asn(Trt)-His(Trt)-Ser(*t*Bu)-Glu(O*t*Bu)-Asp(STmob)-Gly-Thr($\Psi^{Me,Me}$pro)-Ala-OH (21f)

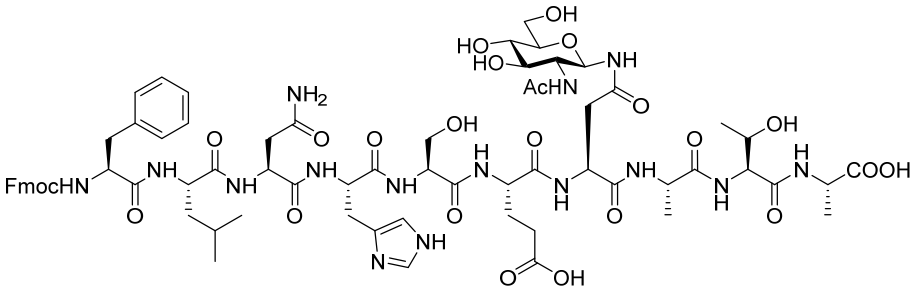


$C_{120}H_{137}N_{13}O_{22}S$ [2145.55 g mol^{-1}]

Gly-decapeptide **ms254** was synthesized on solid-phase according to *general procedure 5* (250 mg resin, 0.883 g $mmol^{-1}$) and as depicted in Scheme 3. Analytical resin cleavage was conducted at the penta- and decapeptide stage as described in *general procedure 9*. The ratio of desired peptide to aspartimide was 96:4 at the penta- (**19f**/**20f**) and 12:88 at the decapeptide (**21f**/**22f**) stage as determined by integration of the corresponding peaks in the LC-MS chromatograms (254 nm).

7. Synthesis of N-Glycopeptides 24a–e, 27a and 28a

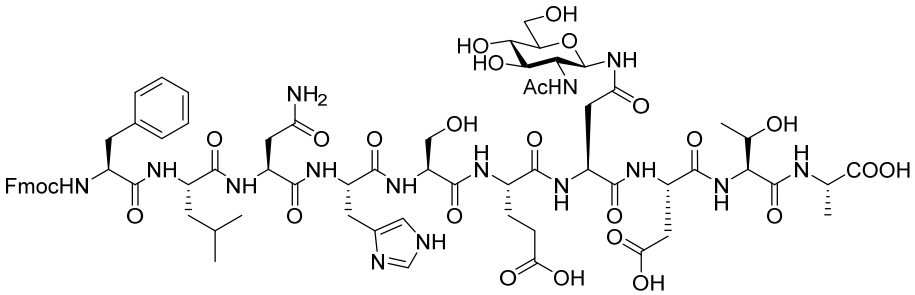
Ala-Glycopeptide 24a



$C_{70}H_{93}N_{15}O_{24}$ [1528.60 g mol^{-1}]

Glycopeptide **24a** was synthesized according to *general procedure 6* starting from decapeptide **21a** (10.79 mg, 0.005 mmol). RP-HPLC purification (30–40 in 8 min, 40% for 7 min, R_t = 14.9 min, column 1), followed by lyophilization yielded glycopeptide **24a** as colorless fluffy solid (4.45 mg, 58%). **HRMS**: m/z calcd. for $C_{70}H_{94}N_{15}O_{24}^+$ [*M*+H]$^+$: 1528.6591, found: 1528.6558.

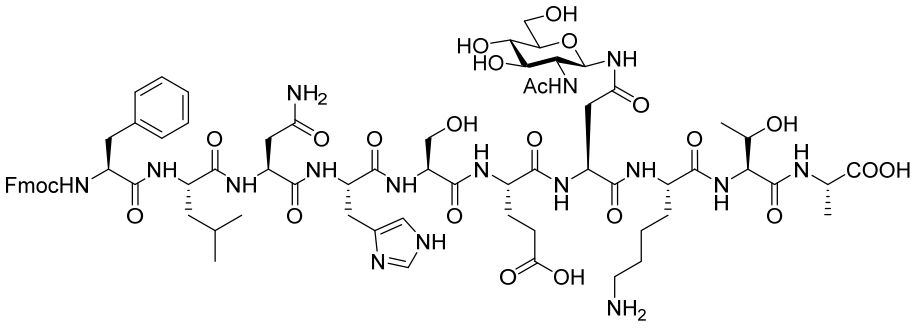
Asp-Glycopeptide 24b



$C_{71}H_{93}N_{15}O_{26}$ [1572.60 g mol^{-1}]

Glycopeptide **24b** was synthesized according to *general procedure 6* starting from decapeptide **21b** (11.30 mg, 0.005 mmol). RP-HPLC purification (32–36% in 8 min, 36% for 2 min, 36–40% in 10 min, R_t = 19.6 min, column 1), followed by lyophilization yielded glycopeptide **24b** as colorless fluffy solid (4.09 mg, 52%). **HRMS**: m/z calcd. for $C_{71}H_{94}N_{15}O_{26}^+$ [*M*+H]$^+$: 1572.6489, found: 1572.6490.

Lys-Glycopeptide 24c

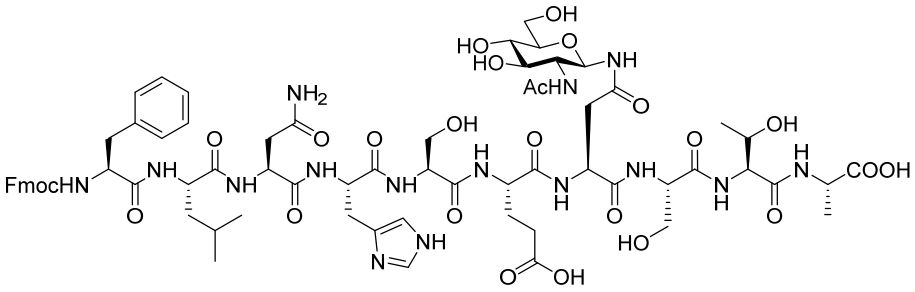


$C_{73}H_{100}N_{16}O_{24}$ [1585.69 g mol^{-1}]

Glycopeptide **24c** was synthesized according to *general procedure 6* starting from decapeptide **21c** (11.58 mg, 0.005 mmol). RP-HPLC purification (30% for 5 min, 30–40% in 15 min, R_t = 19.0 min,

column 1), followed by lyophilization yielded glycopeptide **24c** as colorless fluffy solid (4.81 mg, 61%). **HRMS**: m/z calcd. for $C_{73}H_{101}N_{16}O_{24}^+$ [*M*+H]$^+$: 1585.7169, found: 1585.7160.

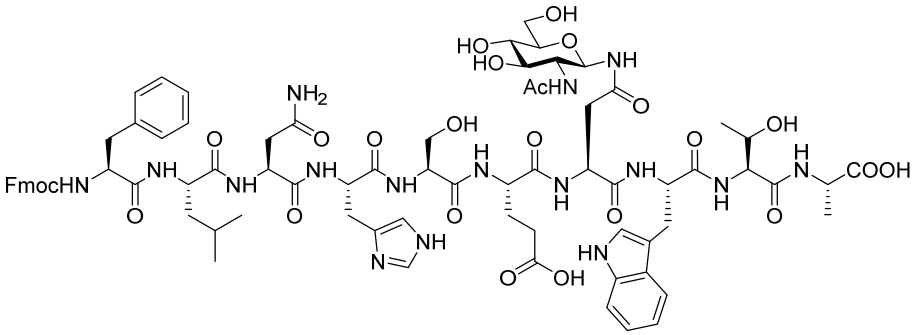
Ser-Glycopeptide 24d



$C_{70}H_{93}N_{15}O_{25}$ [1544.59 g mol^{-1}]

Glycopeptide **24d** was synthesized according to *general procedure 6* starting from decapeptide **21d** (11.16 mg, 0.005 mmol). RP-HPLC purification (32–36% in 8 min, 36% for 2 min, 36–40% in 10 min, R_t = 19.1 min, column 1), followed by lyophilization yielded glycopeptide **24d** as colorless fluffy solid (3.97 mg, 51%). **HRMS**: m/z calcd. for $C_{70}H_{94}N_{15}O_{25}^+$ [*M*+H]$^+$: 1544.6540, found: 1544.6554.

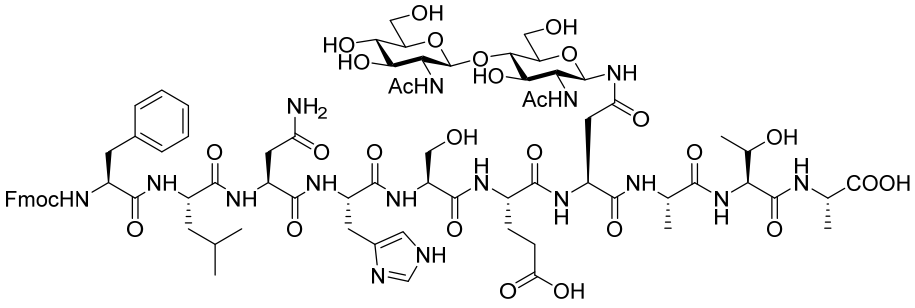
Trp-Glycopeptide 24e



$C_{78}H_{98}N_{16}O_{24}$ [1643.73 g mol^{-1}]

Glycopeptide **24e** was synthesized according to *general procedure 6* starting from decapeptide **21e** (11.87 mg, 0.005 mmol). RP-HPLC purification (35–39% in 8 min, 39% for 2 min, 39–43% in 10 min, R_t = 18.4 min, column 1), followed by lyophilization yielded glycopeptide **24e** as colorless fluffy solid (6.32 mg, 77%). **HRMS**: m/z calcd. for $C_{78}H_{99}N_{16}O_{24}^+$ [*M*+H]$^+$: 1643.7013, found: 1643.7008.

Glycopeptide 27a

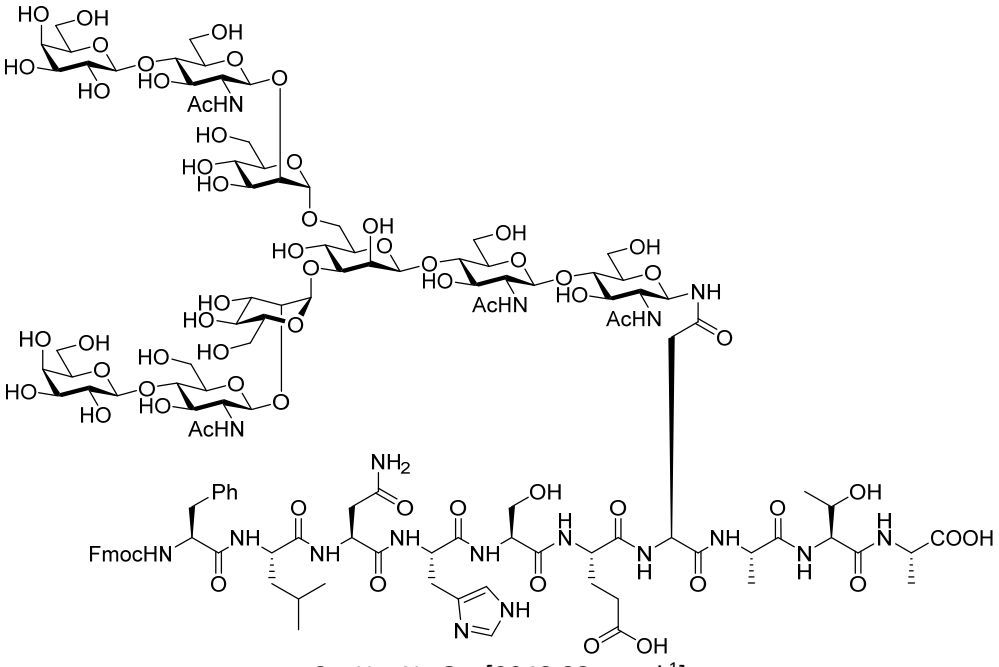


$C_{78}H_{106}N_{16}O_{29}$ [1731.79 g mol^{-1}]

Glycopeptide **27a** was synthesized according to *general procedure 6* starting from decapeptide **21a** (10.79 mg, 0.005 mmol). RP-HPLC purification (32–36% in 8 min, 36% for 2 min, 36–40% in 10 min,

R_t = 19.1 min, column 1), followed by lyophilization yielded glycopeptide **27a** as colorless fluffy solid (4.83 mg, 56%). **HRMS**: m/z calcd. for $C_{78}H_{107}N_{16}O_{29}^+$ [*M*+H]$^+$: 1731.7384, found: 1731.7389.

Glycopeptide 28a

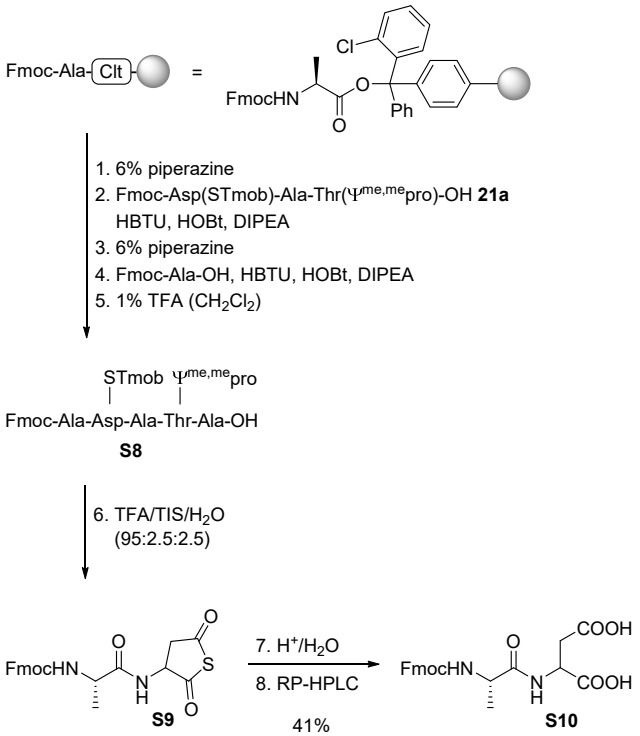


$C_{124}H_{182}N_{18}O_{64}$ [2948.88 g mol^{-1}]

Complex glycopeptide **28a** was synthesized according to *general procedure 6* starting from decapeptide **21a** (1.65 mg, 0.76 μmol) using DMSO as solvent. RP-HPLC purification (35–55% in 30 min, R_t = 9.7 min, column 1), followed by lyophilization yielded glycopeptide **28a** as colorless fluffy solid (0.48 mg, 21%). **HRMS**: m/z calcd. for $C_{124}H_{183}N_{18}O_{64}Na^{2+}$ [*M*+H+Na]$^+$: 1486.0769, found: 1486.0836.

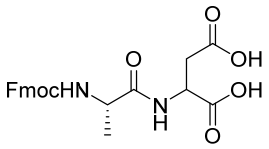
8. Peptide Cleavage Using Thioacids (CUT)
8.1 Racemization during CUT

During CUT and subsequent hydrolysis of aspartic thioacid-containing peptides a shortened peptide with a C-terminal aspartic acid is released (Figure 3A). To analyze whether racemization of the C-terminal aspartic acid had occurred, a short model peptide **S8** was synthesized (Scheme S4). Subsequent CUT and hydrolysis yielded dipeptide **S10** which was analyzed by NMR spectroscopy and compared to authentic samples of Fmoc-Ala-L-Asp(OH)-OH (**S17**) and Fmoc-Ala-D-Asp(OH)-OH (**S14**). ^{1}H and ^{13}C NMR spectroscopy of dipeptide **S10** revealed only one set of signals belonging to the non-racemized LL-dipeptide **S17** (spectra on page 95–103).



Scheme S4. CUT and subsequent hydrolysis of pentapeptide **S8** to dipeptide **S10**.

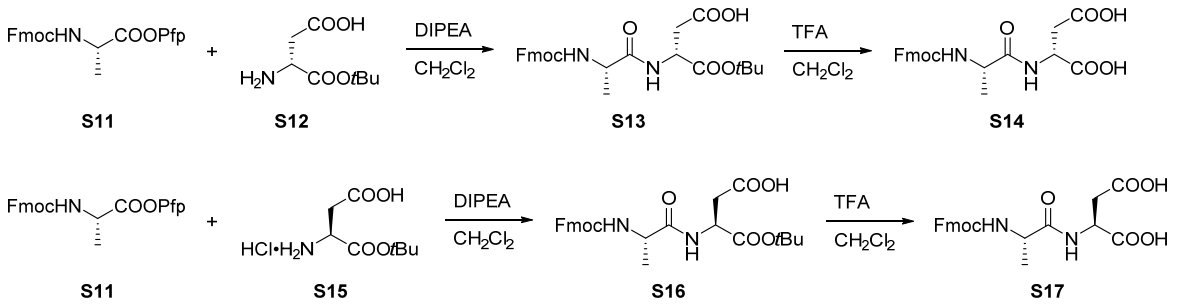
Fmoc-Ala-Asp-OH (S10)



$C_{22}H_{22}N_2O_7$ [426.43 g mol^{-1}]

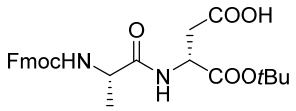
Pentapeptide **S8** was synthesized on solid-phase according to *general procedure 5* (50 mg resin, 0.590 g mmol^{-1}). Preparative resin cleavage of the pentapeptide Fmoc-Ala-Asp(STmob)-Ala-Thr(Ψ^{me,me}pro)-Ala-OH **S8** was performed according to *general procedure 10*. To remove residual pyridine, the peptide was dissolved in dichloromethane (6 mL) and the solution was washed with aqueous citric acid (5%, 3 × 5 mL) and sat. $NH_4Cl_{(aq.)}$ (1 × 3 mL). The organic layer was dried ($MgSO_4$). After removal of the solvent, the pentapeptide **S8** was used for CUT without further purification. Peptide **S8** was dissolved in TFA/TIS/H_2O (95:2.5:2.5, 1.67 mL) and stirred at room temperature for 20 hours. The solvents were removed by rotary evaporation and the residue was repeatedly co-evaporated with toluene. The thioanhydride **S9** was then dissolved in 1% $FA_{(aq.)}$/DMF (1:1, 55 mL). After 6 hours, the solvent was removed *in vacuo* and the residue was purified by RP-HPLC (35–55% in

20 min, R_t = 16.0 min, column 1). Dipeptide **S10** was obtained as colorless, fluffy solid (6.28 mg, 41%). The absence of racemic product could be confirmed by ^{1}H and ^{13}C NMR. Therefore, **S10** was mixed with either authentic Fmoc-Ala-L-Asp-OH (**S17**) giving rise to a single set of signals or authentic Fmoc-Ala-D-Asp-OH (**S14**) giving rise to two sets of signals. **^{1}H NMR** (methanol-d4, 600 MHz): δ[ppm] = 7.79 (d, *J* = 7.5, 2H, H_{aryl}), 7.68 (d, *J* = 7.5, 1H, H_{aryl}), 7.65 (d, *J* = 7.6 Hz, 1H, H_{aryl}), 7.39 (t, *J* = 7.5, 2H, H_{aryl}), 7.31 (tt, *J* = 7.4, 1.3 Hz, 2H, H_{aryl}), 4.73 (t, *J* = 5.8 Hz, 1H, $CH_{\alpha,Asp}$), 4.41 – 4.28 (m, 2H, $CH_{2,Fmoc}$), 4.27 – 4.15 (m, 2H, $CH_{\alpha,Ala}$ & CH_{Fmoc}), 2.84 (d, *J* = 5.7 Hz, 2H, $CH_{2,Asp}$), 1.36 (d, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (methanol-d4, 151 MHz): δ[ppm] = 175.29 (CO_{Ala}), 174.12, 173.94 (CO_{Asp} & COOH), 158.24 (COONH), 145.42, 145.18, 142.57 (4 × $C_{quart,Fmoc}$), 128.77, 128.76, 128.19, 128.17, 126.31, 126.23, 120.89 (8 × C_{aryl}), 68.05 ($CH_{2,Fmoc}$), 51.90 ($CH_{\alpha,Ala}$), 50.16 ($CH_{\alpha,Asp}$), 48.38 (CH_{Fmoc}), 36.96 ($CH_{2,Asp}$), 18.23 ($CH_{3,Ala}$); **HRMS**: m/z calcd. for $C_{22}H_{22}N_2O_7Na^+$ [*M*+Na]$^+$: 449.1319, found: 449.1314.



Scheme S5. Synthesis of authentic Fmoc-Ala-D-Asp-OH (**S14**) and Fmoc-Ala-L-Asp-OH (**S17**).

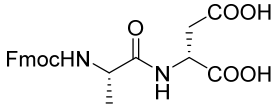
Fmoc-Ala-D-Asp(OH)-O*t*Bu (S13)



$C_{26}H_{30}N_2O_7$ [482.53 g mol^{-1}]

Fmoc-Ala-OPfp **S11** (300 mg, 0.63 mmol) was dissolved in dichloromethane (2.5 mL) and DIPEA (220 µL, 1.26 mmol) was added. A suspension of H-D-Asp(OH)-O*t*Bu **S12** in dichloromethane (2.5 mL) was prepared and subsequently added to the alanine solution. The mixture was stirred for 45 minutes at room temperature and was then diluted with dichloromethane (5 mL). The solution was extracted with $HCl_{(aq.)}$ (5 × 5 mL, 1 M) and dried ($MgSO_4$). After removal of the solvent, the crude product was purified by FC (silica, dichloromethane/methanol 20:1→10:1) to yield dipeptide **S13** as colorless foam (292 mg, 96%). **TLC** R_f = 0.34 (dichloromethane/methanol 10:1); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ[ppm] = 7.75 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.61 – 7.52 (m, 2H, H_{aryl}), 7.38 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.27 (m, 3H, NH_{Asp} & H_{aryl}), 5.68 (d, *J* = 7.8 Hz, 1H, NH_{Ala}), 4.70 (dt, *J* = 8.4, 4.8 Hz, 1H, $CH_{\alpha,Asp}$), 4.44 – 4.31 (m, 3H, $CH_{\alpha,Ala}$ & $CH_{2,Fmoc}$), 4.20 (t, *J* = 7.1 Hz, 1H, CH_{Fmoc}), 3.00 (dd, *J* = 16.9, 5.0 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.85 (dd, *J* = 16.7, 3.6 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.42 (s, 9H, 3 × $CH_{3,tBu}$), 1.37 (d, *J* = 7.0 Hz, 3H, $CH_{3,Ala}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ [ppm] = 174.3 (COOH), 172.6 (CO_{Ala}), 169.5 (CO_{Asp}), 156.3 (COONH), 143.8, 141.43, 141.41 (4 × $C_{quart,Fmoc}$), 127.9, 127.2, 125.2, 120.1 (8 × C_{aryl}), 83.1 ($C_{quart,tBu}$), 67.5 ($CH_{2,Fmoc}$), 50.5 ($CH_{\alpha,Ala}$), 49.4 ($CH_{\alpha,Asp}$), 47.2 (CH_{Fmoc}), 36.2 ($CH_{2,Asp}$), 28.0 (3 × $CH_{3,tBu}$), 19.0 ($CH_{3,Ala}$); **HRMS**: m/z calcd. for $C_{26}H_{30}N_2O_7Na^+$ [*M*+Na]$^+$: 505.1945, found: 505.1947.

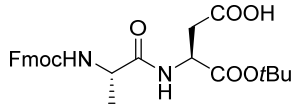
Fmoc-Ala-D-Asp(OH)-OH (S14)



$C_{22}H_{22}N_2O_7$ [426.43 g mol^{-1}]

Dipeptide **S13** (15 mg, 31 μmol) was dissolved in TFA (60% in dichloromethane, 3 mL) and stirred at room temperature for 60 minutes. The solvents were removed *in vacuo* and the residue was co-evaporated with toluene. The desired dipeptide **S14** was obtained as colorless solid (12.8 mg, 96%). **^{1}H NMR** (methanol-d4, 600 MHz): δ[ppm] = 7.79 (d, *J* = 7.2 Hz, 2H, H_{aryl}), 7.68 (d, *J* = 7.5 Hz, 1H, H_{aryl}), (d, *J* = 7.5 Hz, 1H, H_{aryl}), 7.38 (t, *J* = 7.5 Hz, 2H, H_{aryl}), 7.31 (tt, *J* = 7.6, 1.5 Hz, 2H, H_{aryl}), 4.73 (t, *J* = 5.6 Hz, 1H, $CH_{\alpha,Asp}$), 4.40 – 4.29 (m, 2H, $CH_{2,Fmoc}$), 4.26 – 4.15 (m, *J* = 7.0 Hz, 2H, $CH_{\alpha,Ala}$ & CH_{Fmoc}), 2.84 (d, *J* = 5.7 Hz, 2H, $CH_{2,Asp}$), 1.35 (d, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (methanol-d4, 151 MHz): δ[ppm] = 175.23 (CO_{Ala}), 174.05, 173.80 (CO_{Asp} & COOH), 158.25 (COONH), 145.43, 145.16, 142.59, 142.56 (4 × $C_{quart,Fmoc}$), 128.77, 128.76, 128.18, 128.16, 126.32, 126.21, 120.89 (8 × C_{aryl}), 68.07 ($CH_{2,Fmoc}$), 52.04 ($CH_{\alpha,Ala}$), 50.15 ($CH_{\alpha,Asp}$), 48.37 (CH_{Fmoc}), 36.84 ($CH_{2,Asp}$), 18.25 ($CH_{3,Ala}$); **HRMS**: m/z calcd. for $C_{22}H_{22}N_2O_7Na^+$ [*M*+Na]$^+$: 449.1319, found: 449.1315.

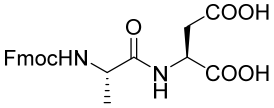
Fmoc-Ala-L-Asp(OH)-O*t*Bu (S16)



$C_{26}H_{30}N_2O_7$ [482.53 g mol^{-1}]

Fmoc-Ala-OPfp **S11** (100 mg, 0.21 mmol) was suspended in dichloromethane (0.8 mL) and DIPEA (110 μL, 0.63 mmol) was added. A suspension of HCl·H-L-Asp(OH)-O*t*Bu **S15** in dichloromethane (0.8 mL) was prepared and subsequently added to the alanine solution. The mixture was stirred for 45 minutes at room temperature. The solvent was removed by rotary evaporation and the crude product was purified by FC (silica, dichloromethane/methanol 15:1→10:1) to yield dipeptide **S16** as colorless foam (88 mg, 87%). **TLC** R_f = 0.29 (dichloromethane/methanol 10:1); **^{1}H NMR** ($CDCl_3$, 400 MHz, major conformer): δ[ppm] = 7.73 (d, *J* = 7.5 Hz, 2H, H_{aryl}), 7.56 (t, *J* = 7.0 Hz, 2H, H_{aryl}), 7.42 – 7.33 (m, 3H, H_{aryl} & NH_{Asp}), 7.32 – 7.27 (m, 2H, H_{aryl}), 5.84 (d, *J* = 8.4 Hz, 1H, NH_{Ala}), 4.78 (ddd, *J* = 8.6, 4.4, 4.1 Hz, 1H, $CH_{\alpha,Asp}$), 4.53 (dq, *J* = 8.1, 7.5 Hz, 1H, $CH_{\alpha,Ala}$), 4.43 – 4.26 (m, 2H, $CH_{2,Fmoc}$), 4.17 (t, *J* = 7.2 Hz, 1H, CH_{Fmoc}), 3.06 (dd, *J* = 17.6, 3.3 Hz, 1H, C<u>H</u>H'$_{Asp}$), 2.75 (dd, *J* = 17.5, 3.8 Hz, 1H, CH<u>H'</u>$_{Asp}$), 1.45 (s, 9H, 3 × $CH_{3,tBu}$), 1.37 (d, *J* = 7.0 Hz, 3H, $CH_{3,Ala}$); **^{13}C NMR** ($CDCl_3$, 101 MHz, major conformer): δ[ppm] = 174.3, 172.9, 169.5 (3 × CO), 156.5 (COONH), 143.9, 143.8, 141.4 (4 × $C_{quart,Fmoc}$), 127.9, 127.2, 125.2, 120.1 (8 × C_{aryl}), 82.7 ($C_{quart,tBu}$), 67.6 ($CH_{2,Fmoc}$), 50.3 ($CH_{\alpha,Ala}$), 48.8 ($CH_{\alpha,Asp}$), 47.1 (CH_{Fmoc}), 36.4 ($CH_{2,Asp}$), 28.0 (3 × $CH_{3,tBu}$), 19.1 ($CH_{3,Ala}$); **HRMS**: m/z calcd. for $C_{26}H_{30}N_2O_7Na^+$ [*M*+Na]$^+$: 505.1945, found: 505.1943.

Fmoc-Ala-L-Asp(OH)-OH (S17)

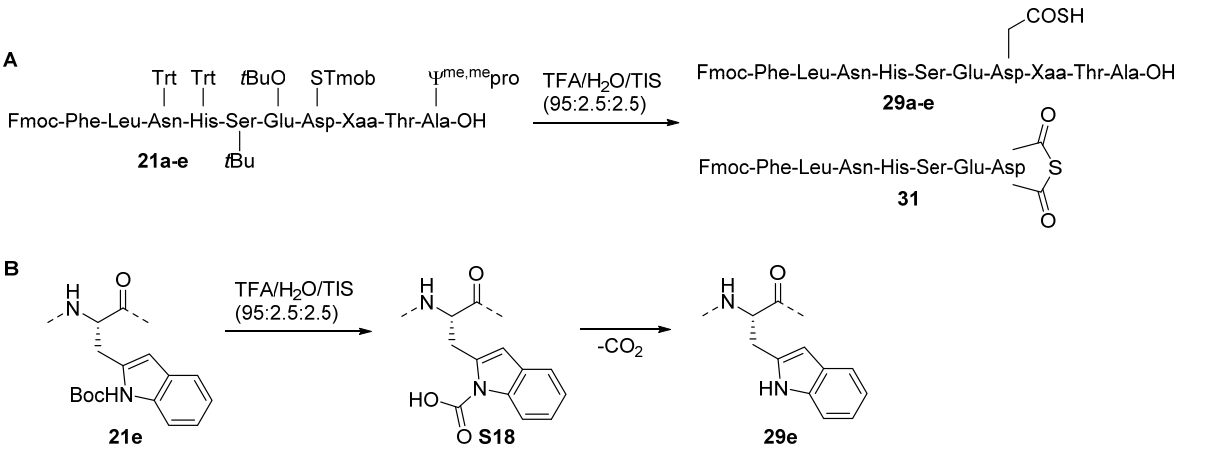


$C_{22}H_{22}N_2O_7$ [426.43 g mol^{-1}]

Dipeptide **S16** (15 mg, 31 μmol) was dissolved in TFA (60% in dichloromethane, 3 mL) and stirred at room temperature for 60 minutes. The solvents were removed *in vacuo* and the residue was co-evaporated with toluene. The desired dipeptide **S17** was obtained as colorless solid (12.1 mg, 91%). **^{1}H NMR** (methanol-d4, 600 MHz): δ[ppm] = 7.78 (d, *J* = 7.5, 2H, H_{aryl}), 7.68 (d, *J* = 7.6, 1H, H_{aryl}), 7.65 (d, *J* = 7.7 Hz, 1H, H_{aryl}), 7.38 (t, *J* = 7.4, 2H, H_{aryl}), 7.31 (tt, *J* = 7.5, 1.2 Hz, 2H, H_{aryl}), 4.73 (t, *J* = 5.7 Hz, 1H, $CH_{\alpha,Asp}$), 4.40 – 4.28 (m, 2H, $CH_{2,Fmoc}$), 4.26 – 4.16 (m, 2H, $CH_{\alpha,Ala}$ & CH_{Fmoc}), 2.84 (d, *J* = 5.7 Hz, 2H, $CH_{2,Asp}$), 1.36 (d, *J* = 7.2 Hz, 3H, CH_3); **^{13}C NMR** (methanol-d4, 151 MHz): δ [ppm] = 175.30 (CO_{Ala}), 174.09, 173.87 (CO_{Asp} & COOH), 158.23 (COONH), 145.40, 145.17, 142.56 (4 × $C_{quart,Fmoc}$), 128.77, 128.75, 128.19, 128.16, 126.30, 126.22, 120.89 (8 × C_{aryl}), 68.05 ($CH_{2,Fmoc}$), 51.89 ($CH_{\alpha,Ala}$), 50.12 ($CH_{\alpha,Asp}$), 48.37 (CH_{Fmoc}), 36.88 ($CH_{2,Asp}$), 18.22 ($CH_{3,Ala}$); **HRMS**: m/z calcd. for $C_{22}H_{22}N_2O_7Na^+$ [*M*+Na]$^+$: 449.1319, found: 449.1319.

8.2 Sequence Dependency of CUT

To study the influence of amino acid *n* + 1 (rel. to Asp) on CUT, the decapeptides **21a–e** were treated with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) and the reaction mixture was analyzed by LC-MS after 60, 90 and 120 minutes, respectively (Scheme S6A). The ratio of fully deprotected decapeptide **29a–e** to thioanhydride **31** was determined by integration of the corresponding peaks in the LC-MS chromatogram (254 nm). Mean values of two independent experiments are given in Table S1 (chromatograms can be found on page S105*ff.*).



Scheme S6. A Global deprotection of the decapeptides **21a–e**. **B** Deprotection of Trp-decapeptide **21e** to decapeptide **29e** via the partially stable carbamic acid **S18**.

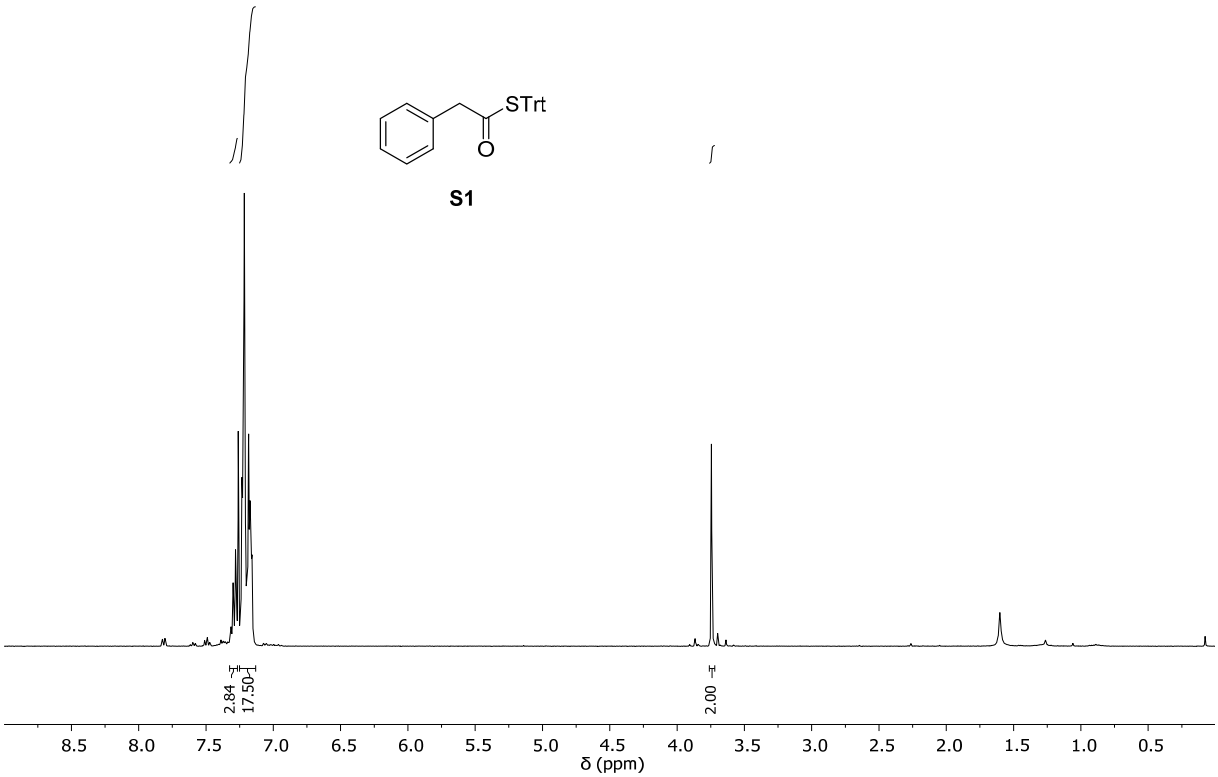
Table S1. Ratio of fully deprotected decapeptide **29a–e** to thioanhydride **31**. The decapeptides **21a–e** (typically 5 mg) were treated with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) for 60, 90 or 120 min, respectively, and the reaction mixture was analyzed by LC-MS. The ratios given are determined by integration of the corresponding peaks in the LC-MS chromatogram (254 nm).

			ratio 29a–e/31		
	starting material	**measurement**	**60 min**	**90 min**	**120 min**
1	**21a**	1	4.56:1	3.00:1	1.94:1
		2	4.56:1	2.70:1	1.78:1
		average	4.56:1	2.85:1	1.86:1
2	**21b**	1	8.09:1	4.56:1	3.35:1
		2	6.69:1	4.00:1	2.85:1
		average	7.39:1	4.28:1	3.10:1
3	**21c**	1	7.33:1	4.26:1	2.85:1
		2	6.69:1	4.56:1	3.17:1
		average	7.01:1	4.41:1	3.01:1
4	**21d**	1	4.26:1	2.57:1	1.78:1
		2	4.00:1	2.45:1	1.70:1
		average	4.13:1	2.51:1	1.74:1
5	**21e**	1	3.35:1[1]	1.86:1[1]	1.17:1[1]
		2	3.17:1[1]	1.86:1[1]	1.17:1[1]
		average	3.26:1[1]	1.86:1[1]	1.17:1[1]

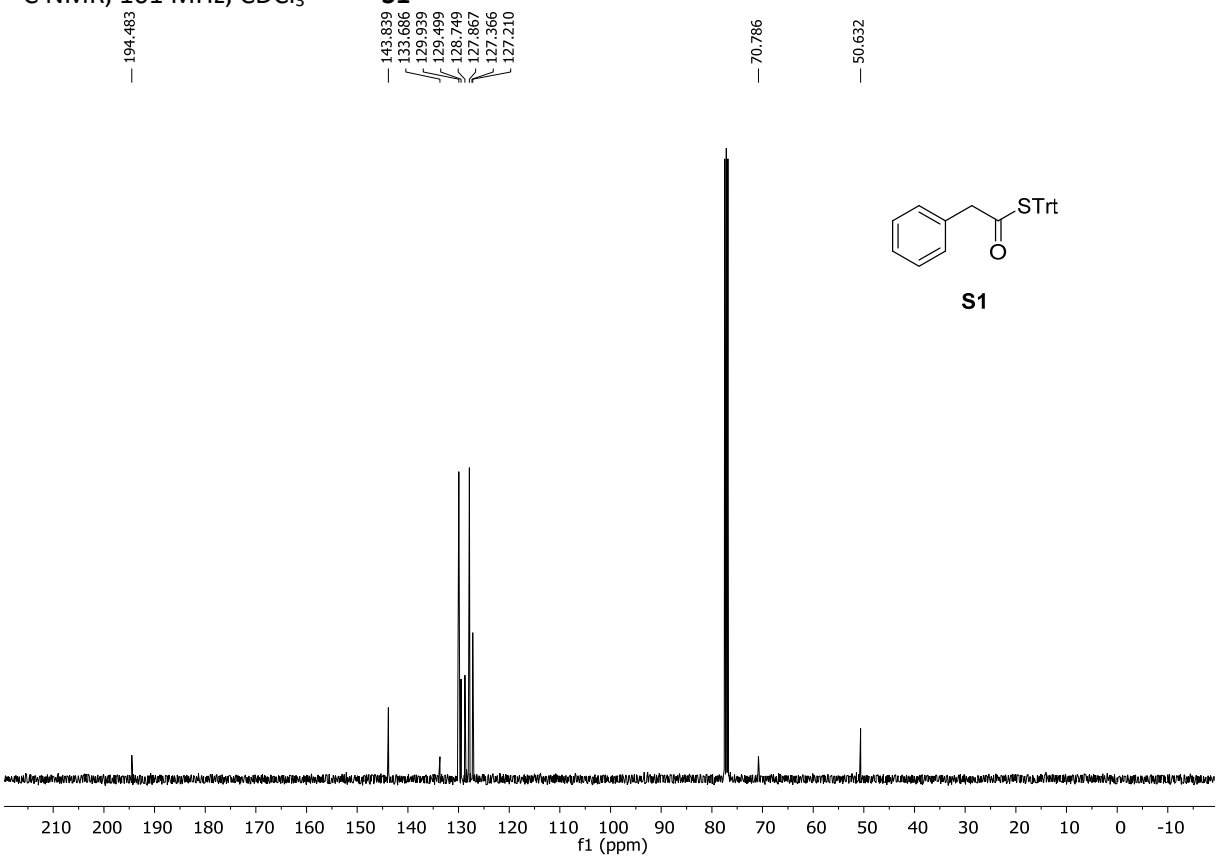
[1] The amount of **29e** was calculated by summing up the integrals of **29e** and transient carbamic acid **S18** (Scheme S6B).

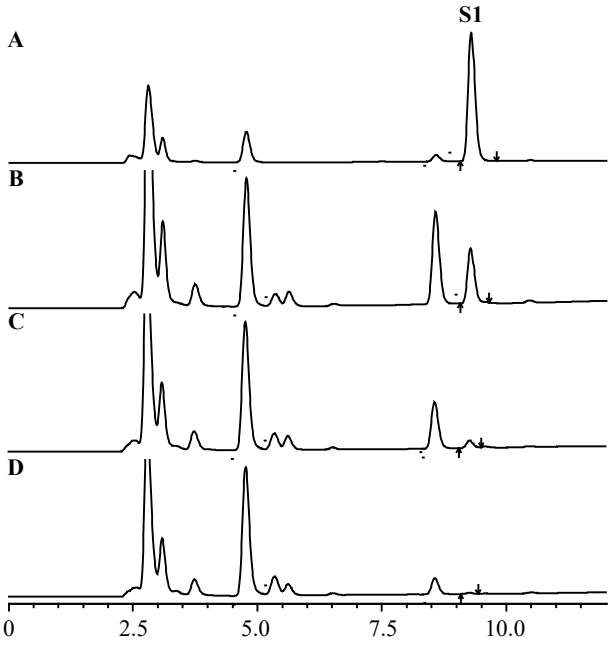
9. Analytical Data (NMR Spectra, LC-MS & HPLC Chromatograms)
9.1 Thioester Model Compounds

^{1}H NMR, 400 MHz, $CDCl_3$ **S1**



^{13}C NMR, 101 MHz, $CDCl_3$ **S1**

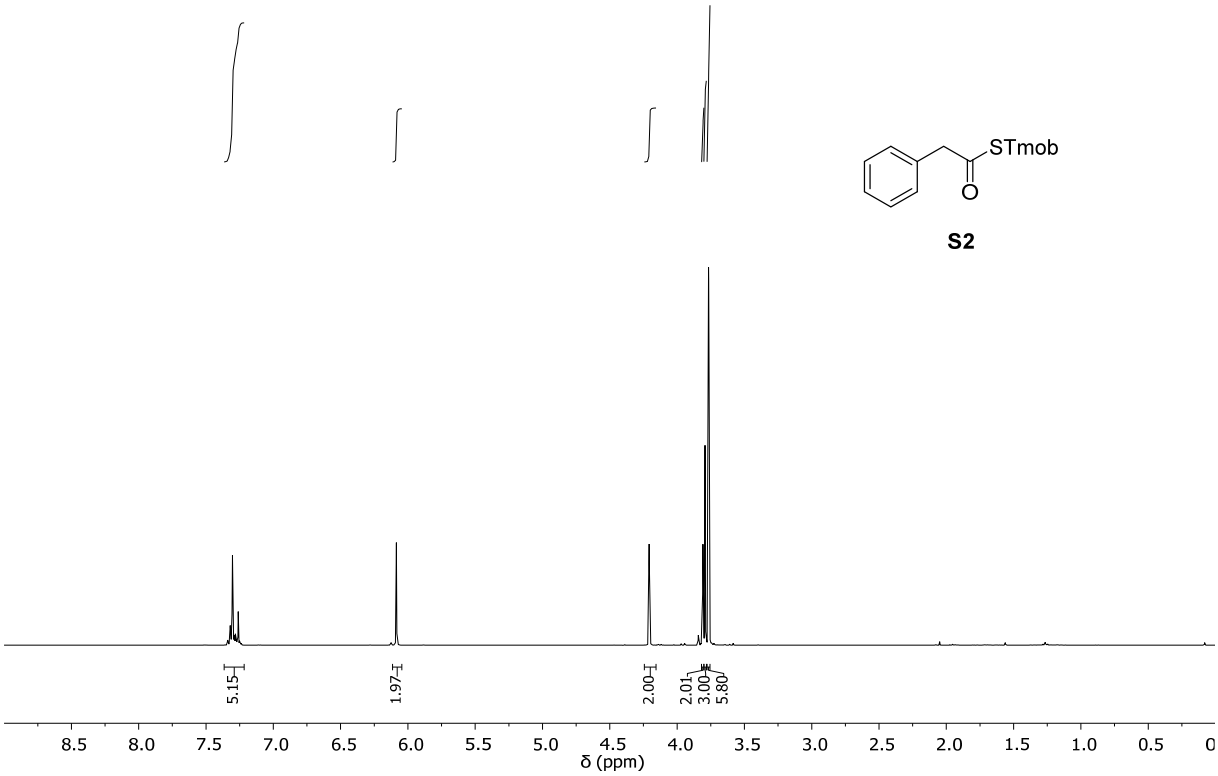




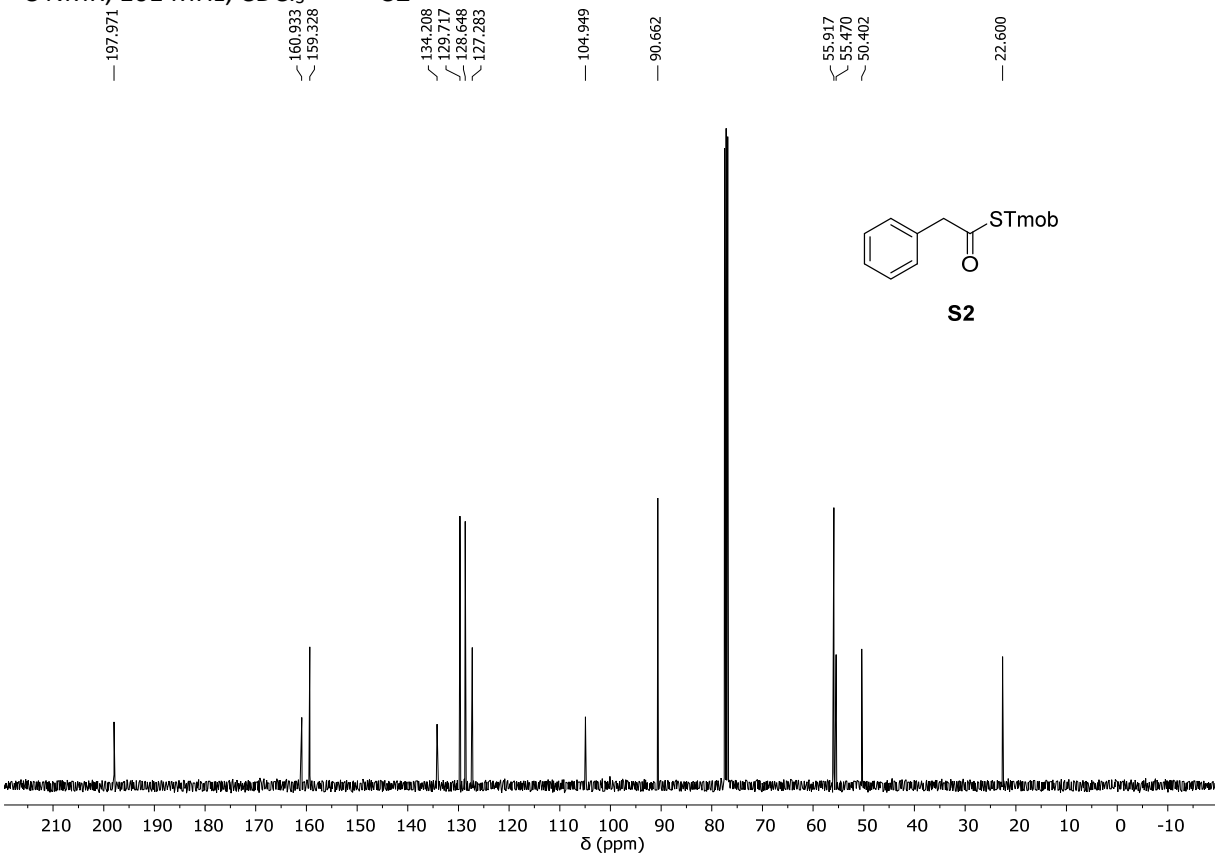
Time [min]	Norm. integral of **S1** (254 nm)
0	1.00
30	0.13
60	0.02
90	0.005

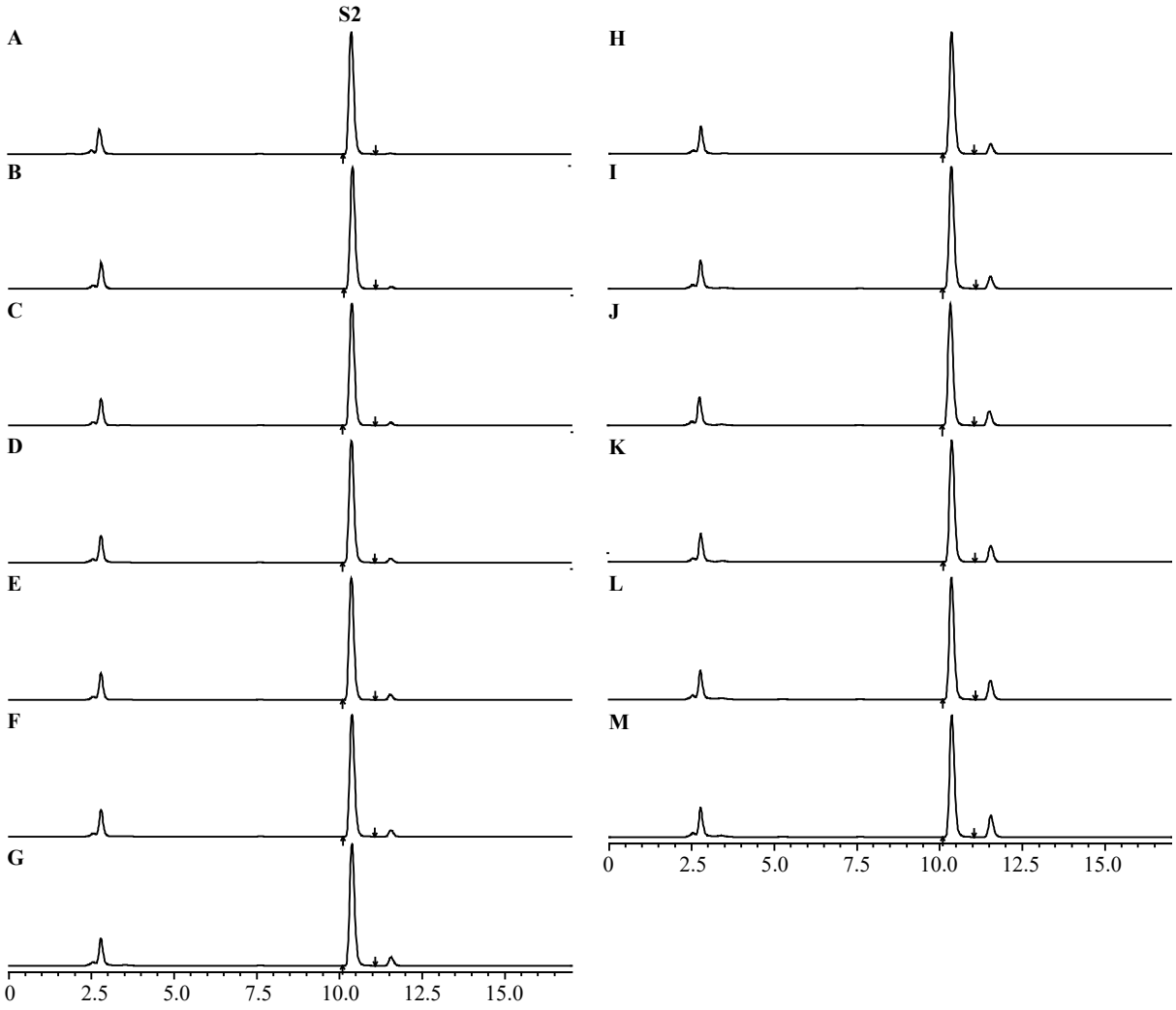
Figure S2. Thioester **S1** was treated with 20% piperidine in DMF. The reaction mixture was analyzed by LC-MS (254 nm) after **A** 0 min, **B** 30 min, **C** 60 min and **D** 90 min (80–100% in 10 min, R_t(**S1**) = 9.3 min, column 2).

^{1}H NMR, 400 MHz, $CDCl_3$ **S2**



^{13}C NMR, 101 MHz, $CDCl_3$ **S2**





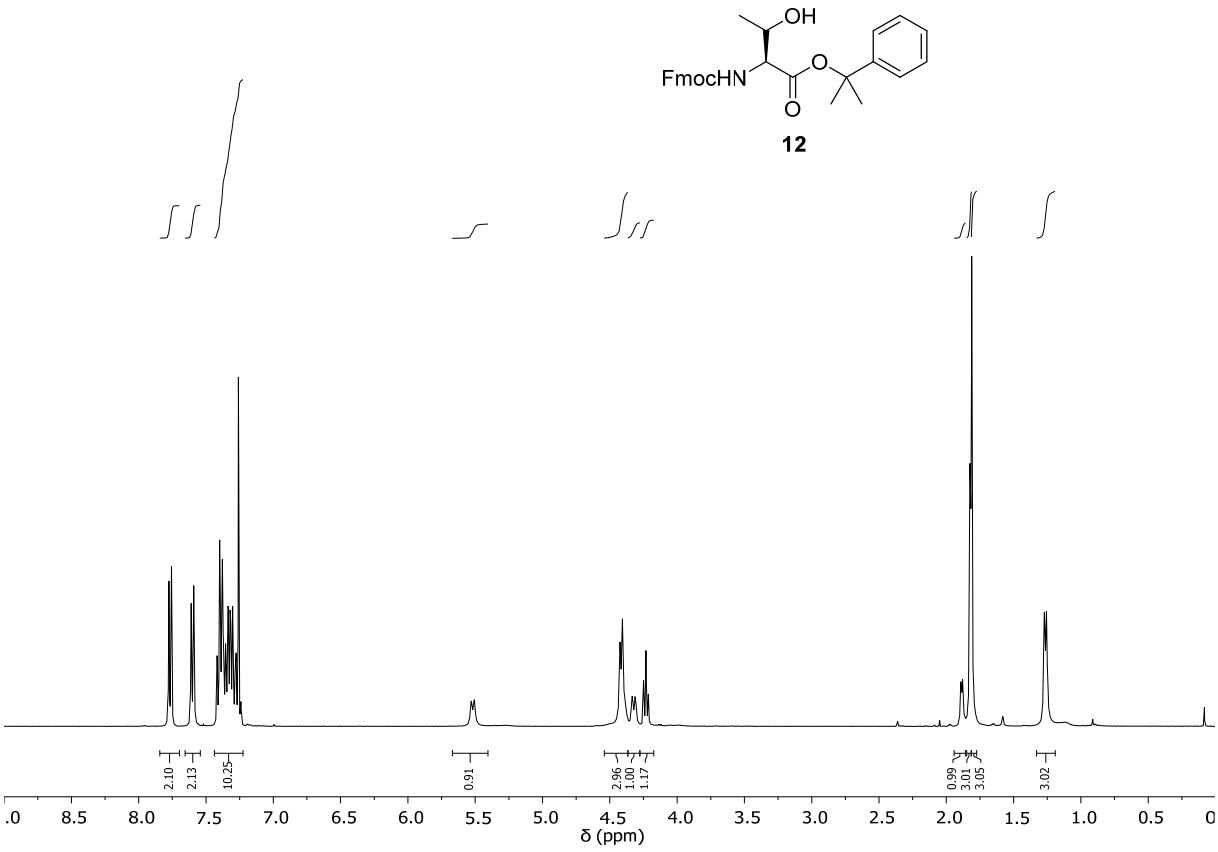
Time [min]	Norm. integral of **S2** (254 nm)
0	1.00
30	1.00
60	1.00
90	0.99
120	0.99
150	0.98
180	0.98
210	0.97
240	0.97
270	0.97
300	0.97
330	0.96
360	0.96

Time [min]	Norm. integral of **S2** (254 nm)
390	0.95
420	0.94
450	0.94
480	0.94
510	0.93
540	0.92
570	0.92
600	0.91
630	0.90
660	0.90
690	0.90
720	0.88

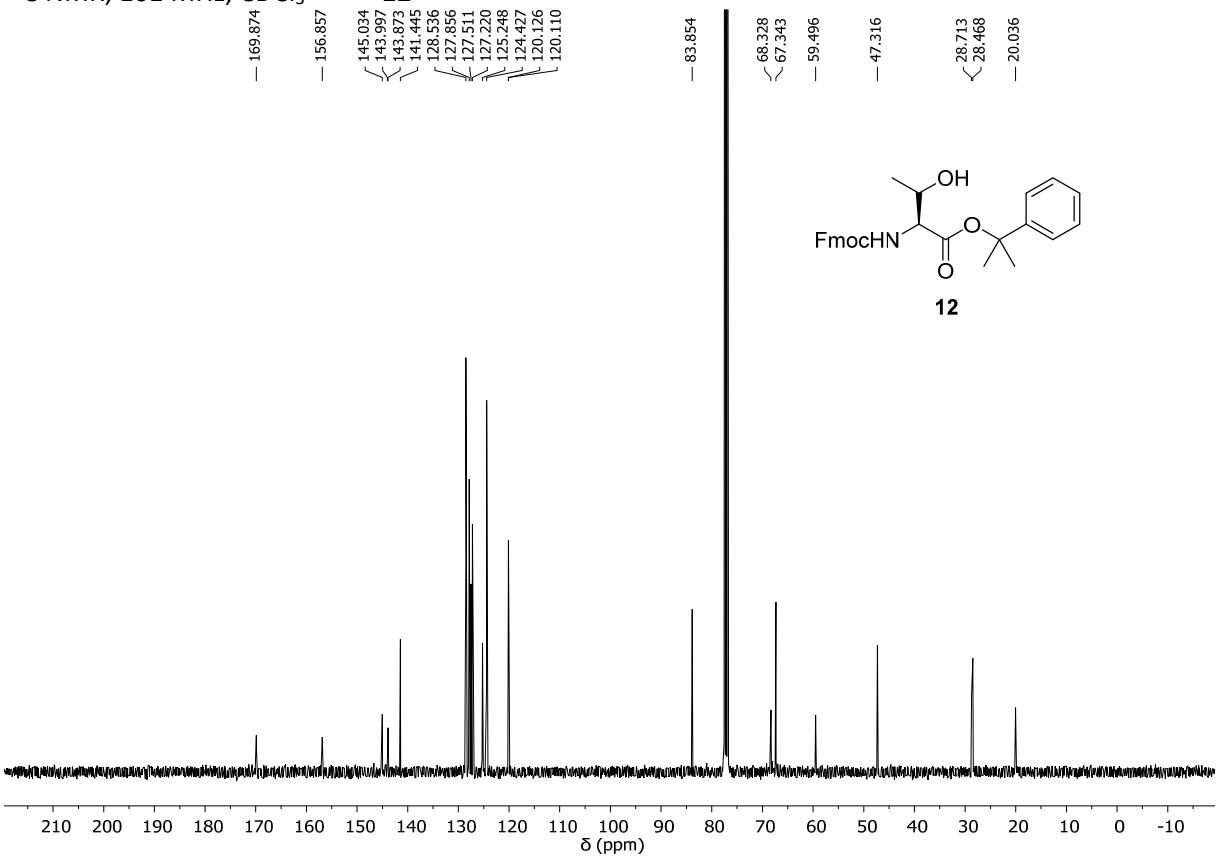
Figure S3. Thioester **S2** was treated with 20% piperidine in DMF. The reaction mixture was analyzed by LC-MS (254 nm) after **A** 0 h, **B** 1 h, **C** 2 h, **D** 3 h, **E** 4 h, **F** 5 h, **G** 6 h, **H** 7 h, **I** 8 h, **J** 9 h, **K** 10 h, **L** 11 h and **M** 12 h (60–100% in 15 min, R_t (**S2**) = 10.4 min, column 2).

9.2 Tripeptide Building Blocks

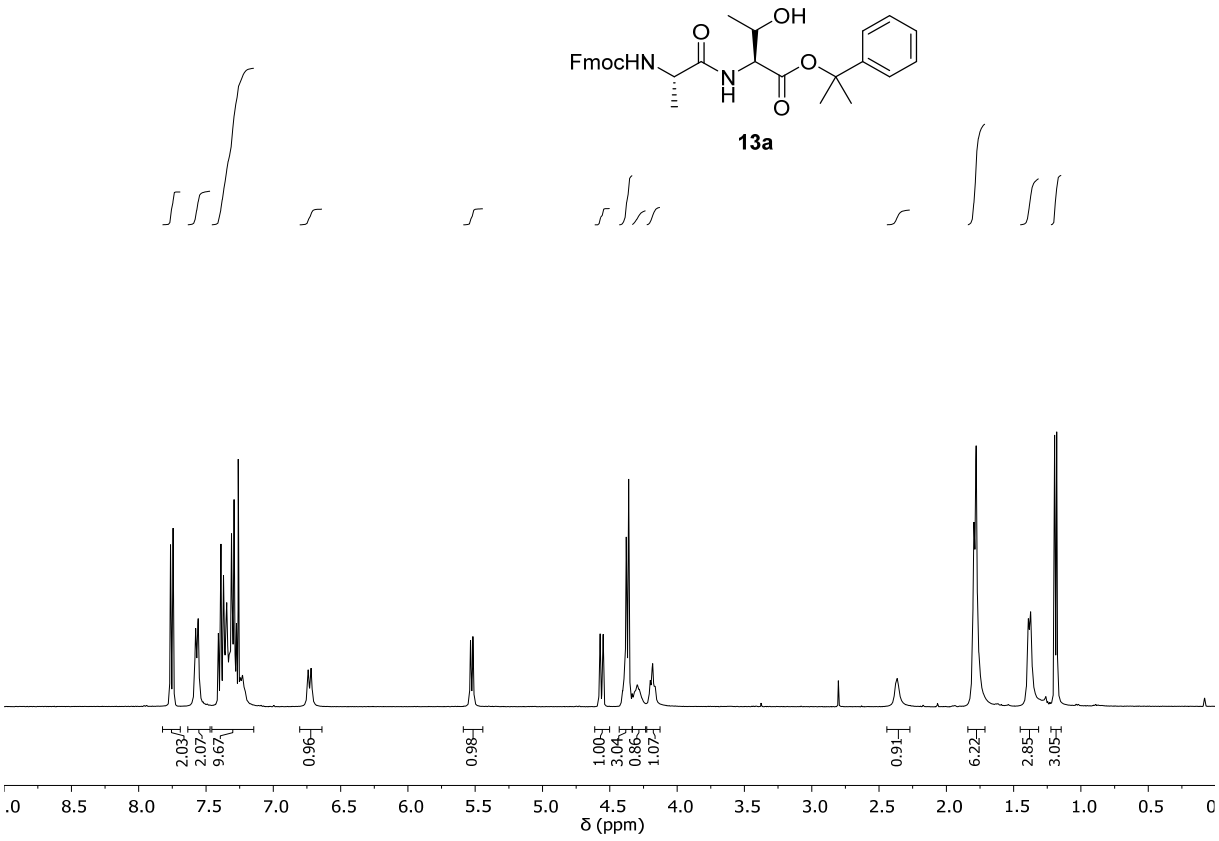
^{1}H NMR, 400 MHz, $CDCl_3$ **12**



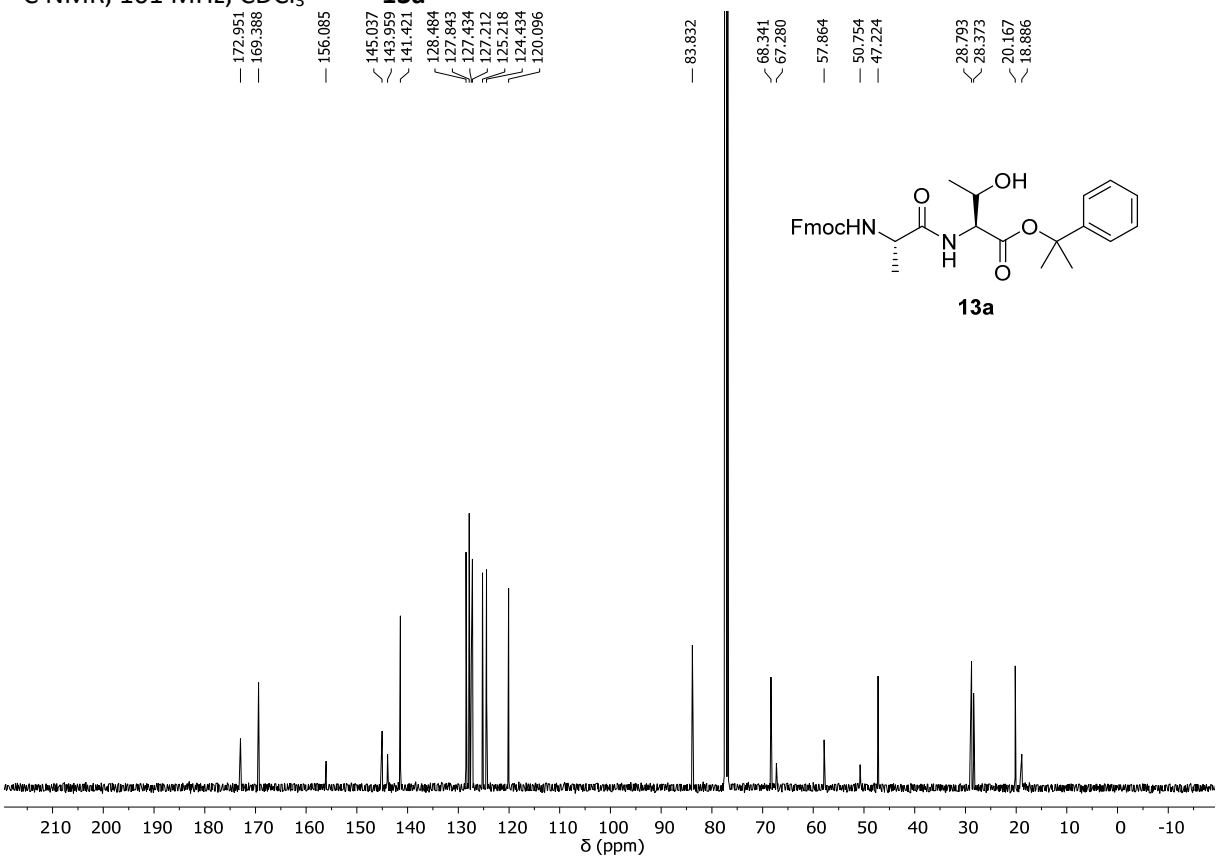
^{13}C NMR, 101 MHz, $CDCl_3$ **12**



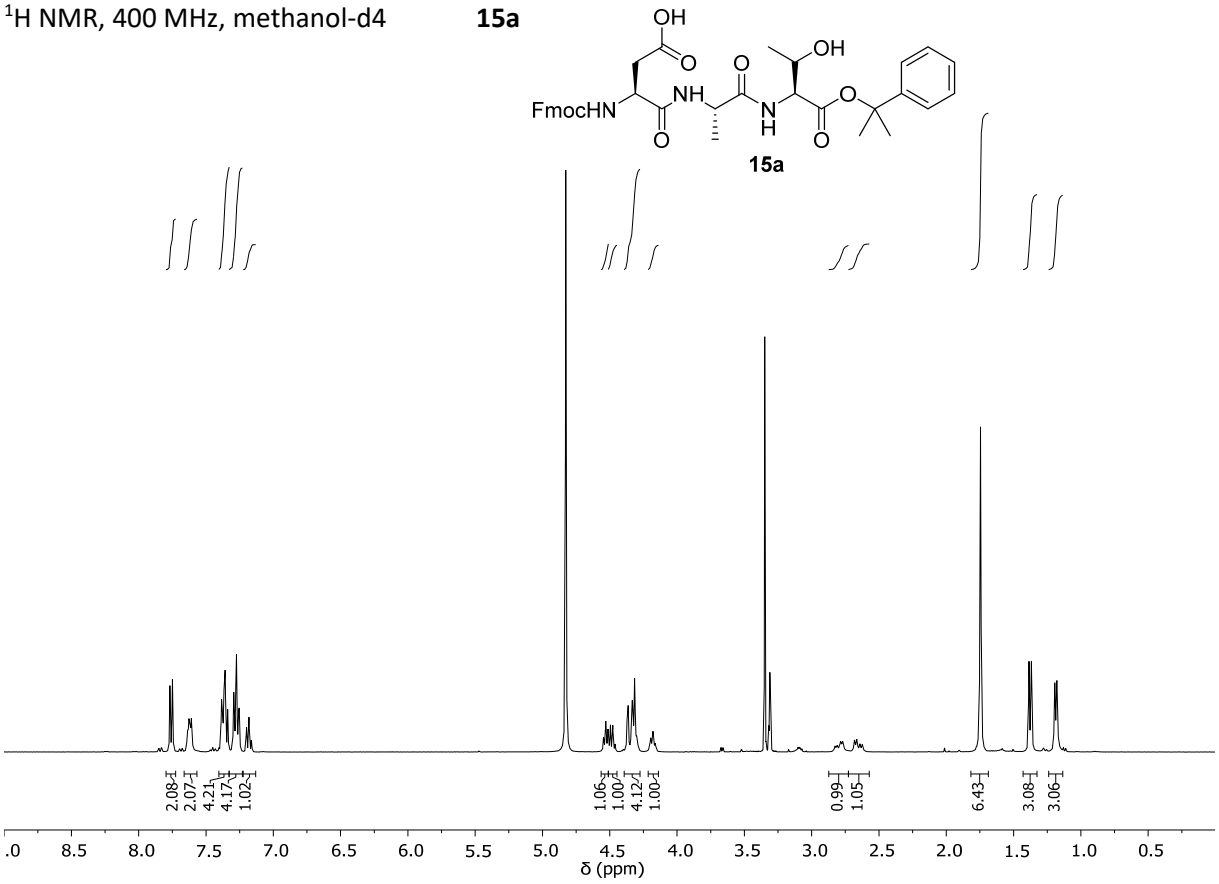
^{1}H NMR, 400 MHz, $CDCl_3$ **13a**



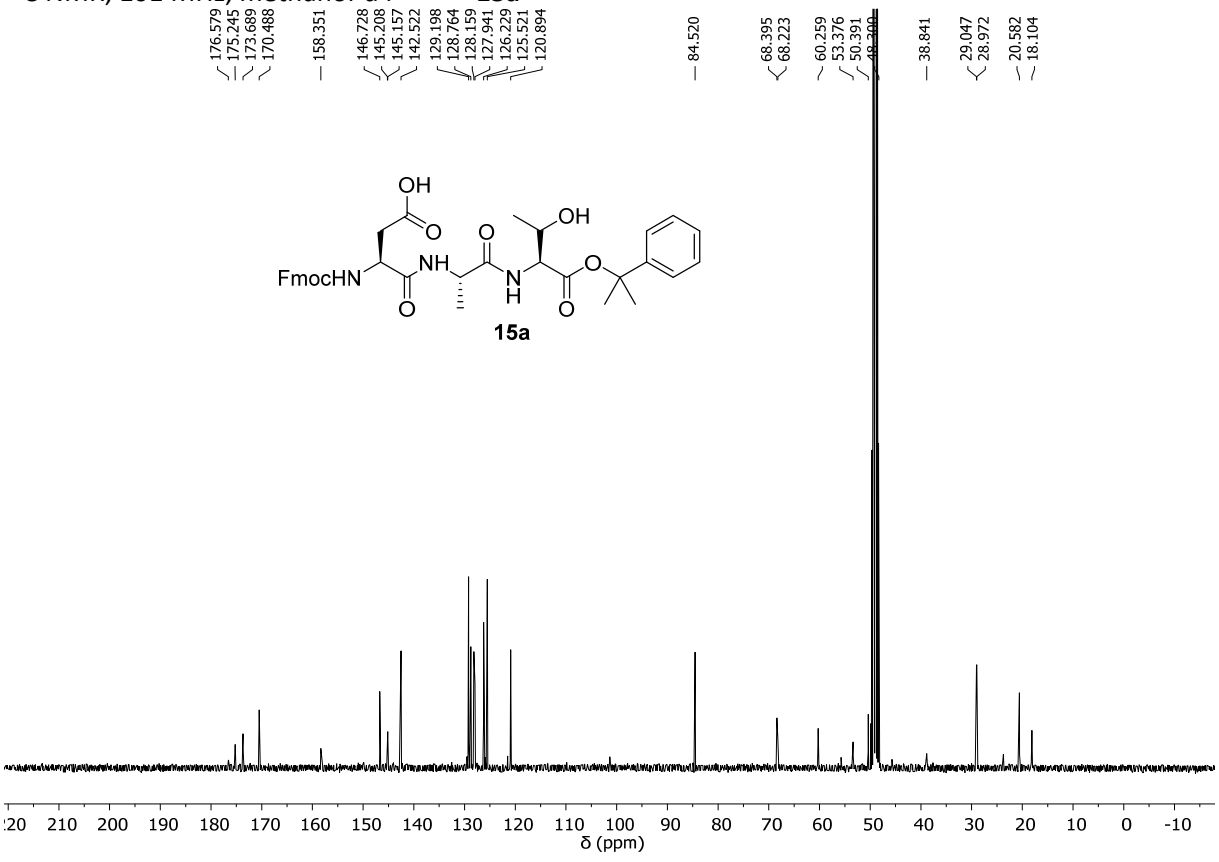
^{13}C NMR, 101 MHz, $CDCl_3$ **13a**



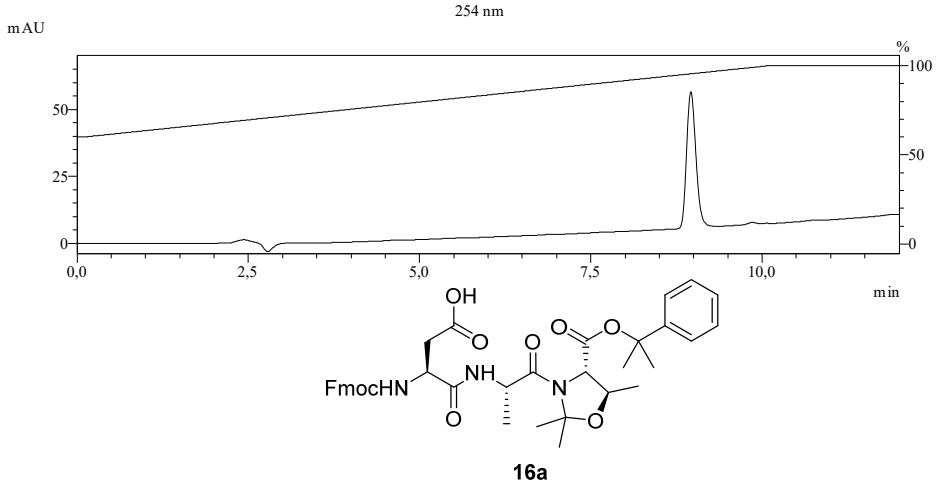
^{1}H NMR, 400 MHz, methanol-d4 **15a**



^{13}C NMR, 101 MHz, methanol-d4 **15a**

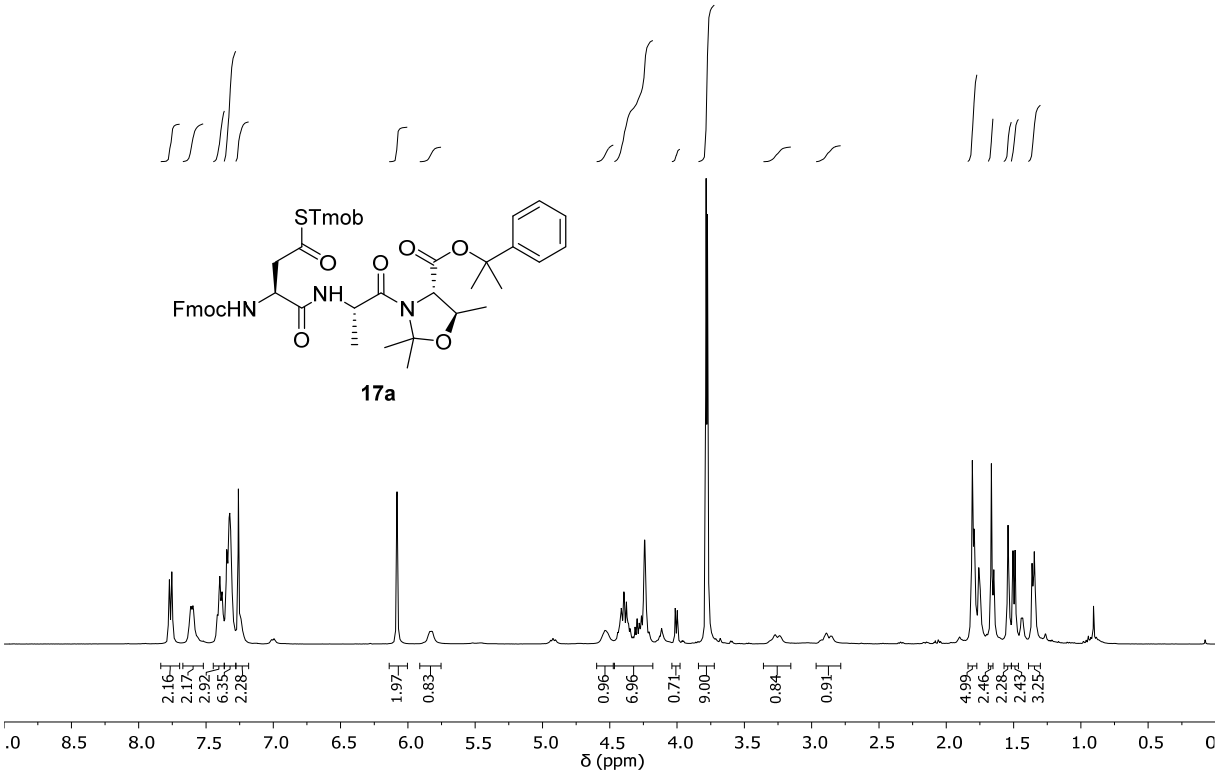


LC-MS chromatogram (254 nm) of purified tripeptide **16a** (60–100% in 10 min, R_t = 9.0 min, column 2).

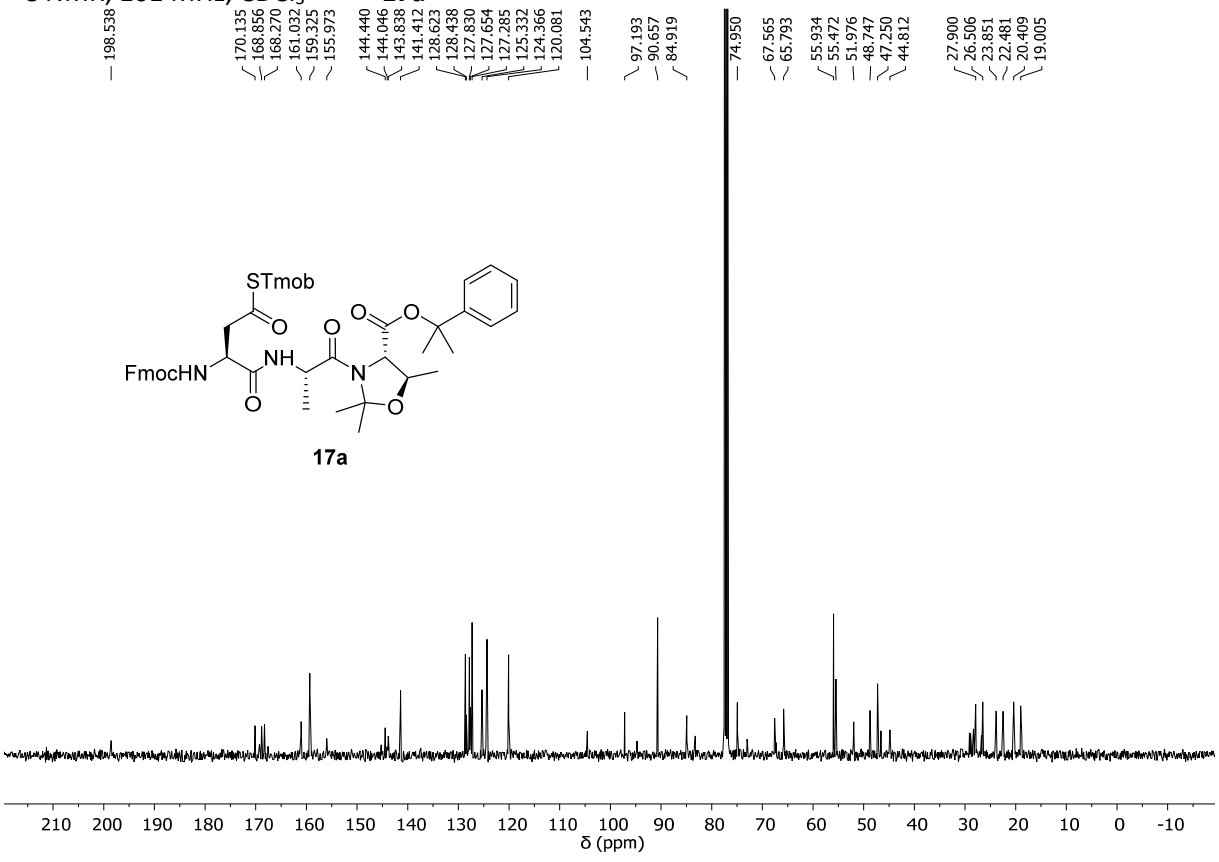


16a

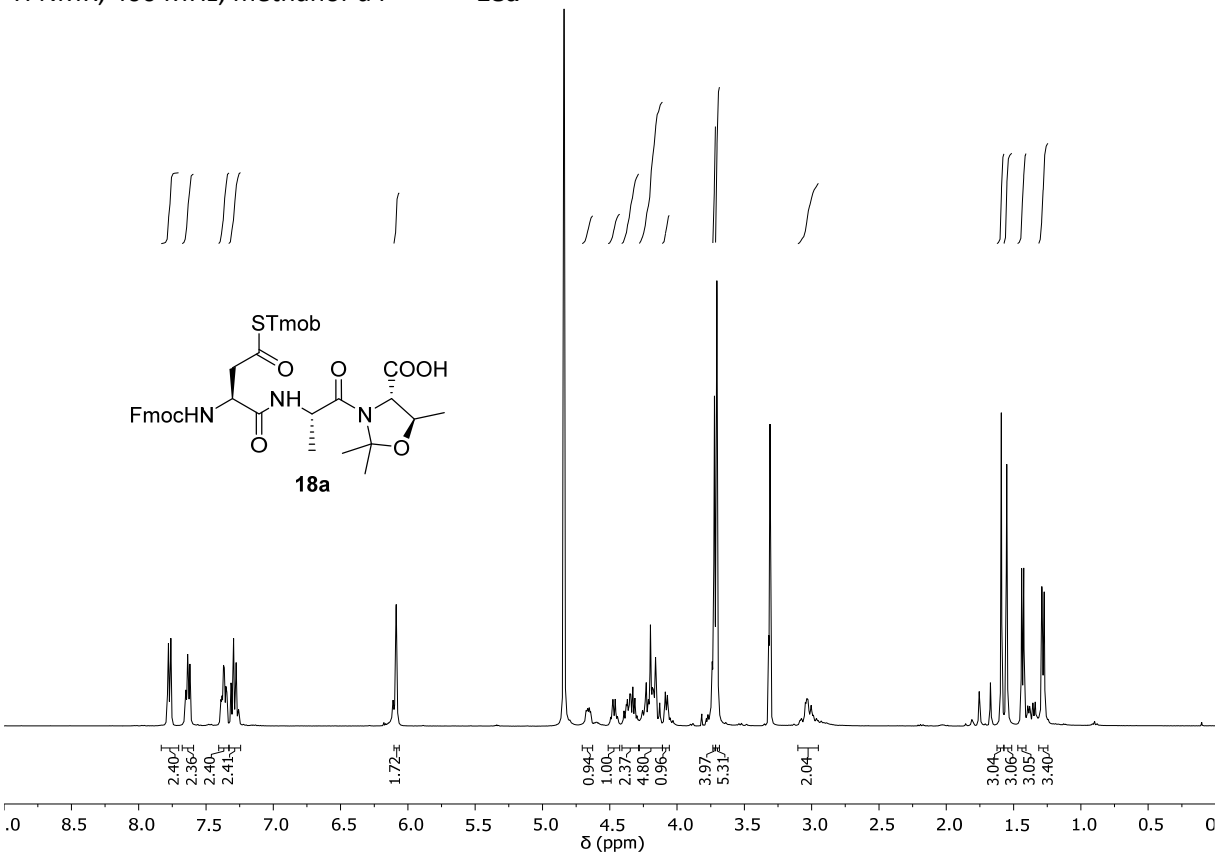
^{1}H NMR, 400 MHz, $CDCl_3$ **17a**



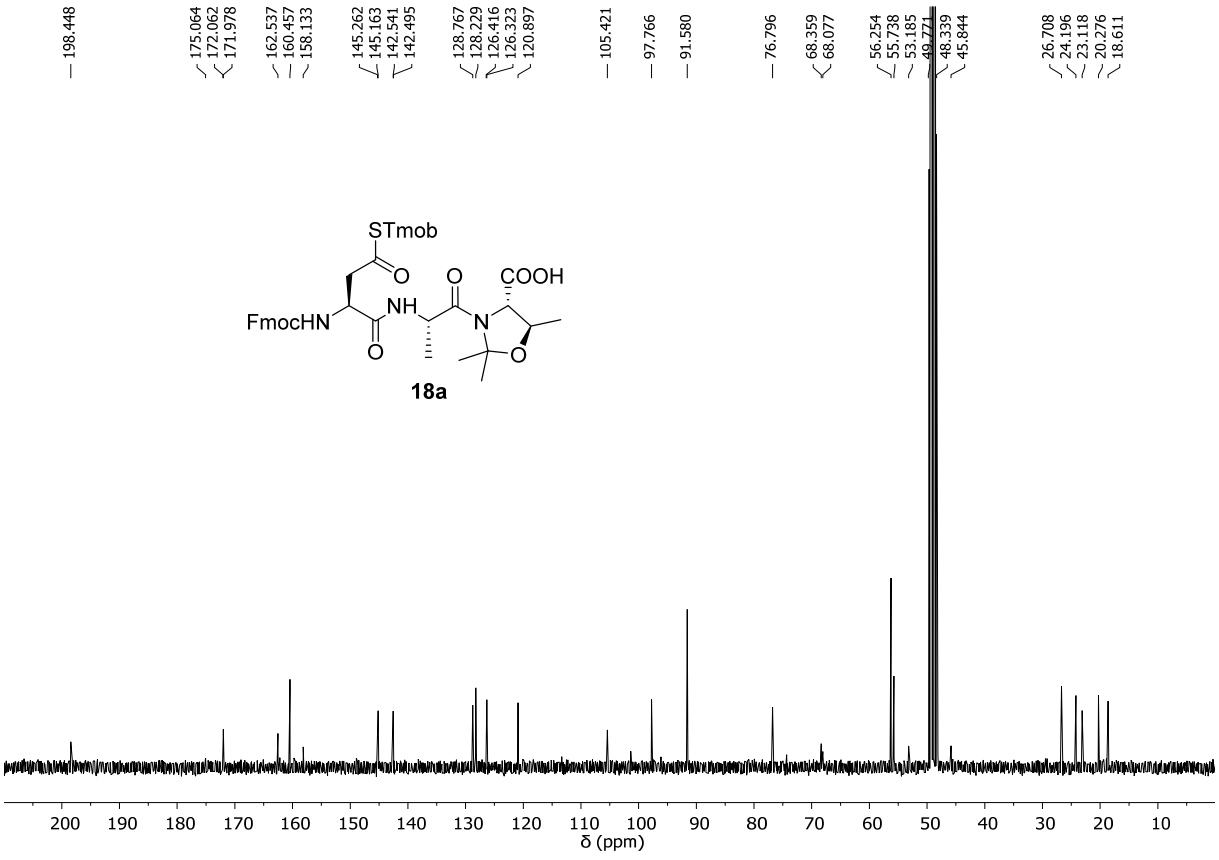
^{13}C NMR, 101 MHz, $CDCl_3$ **17a**



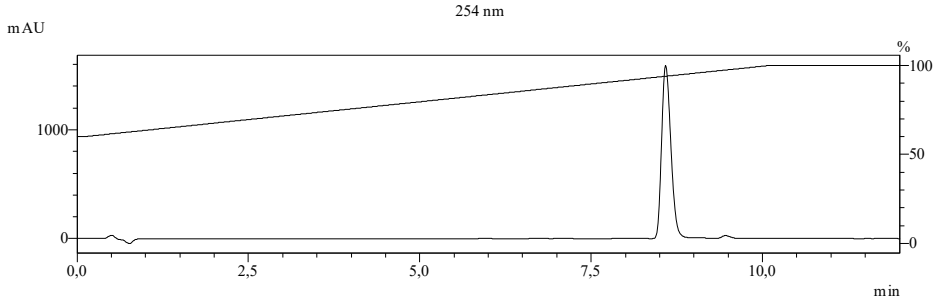
^{1}H NMR, 400 MHz, methanol-d4 **18a**

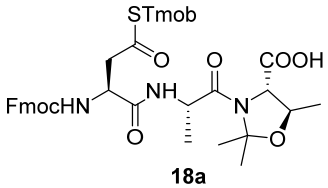


^{13}C NMR, 101 MHz, methanol-d4 **18a**



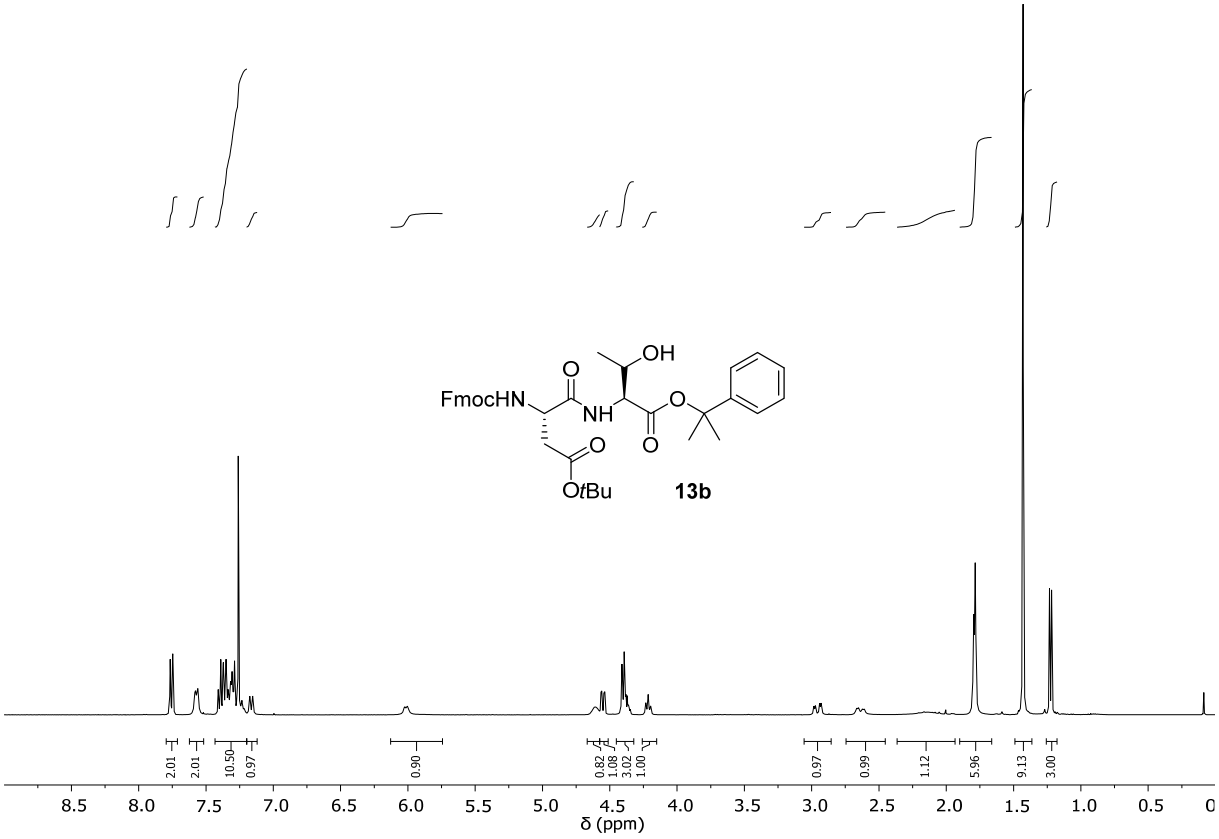
LC-MS chromatogram (254 nm) of purified tripeptide **18a** (60–100% in 10 min, R_t = 8.6 min, column 2).



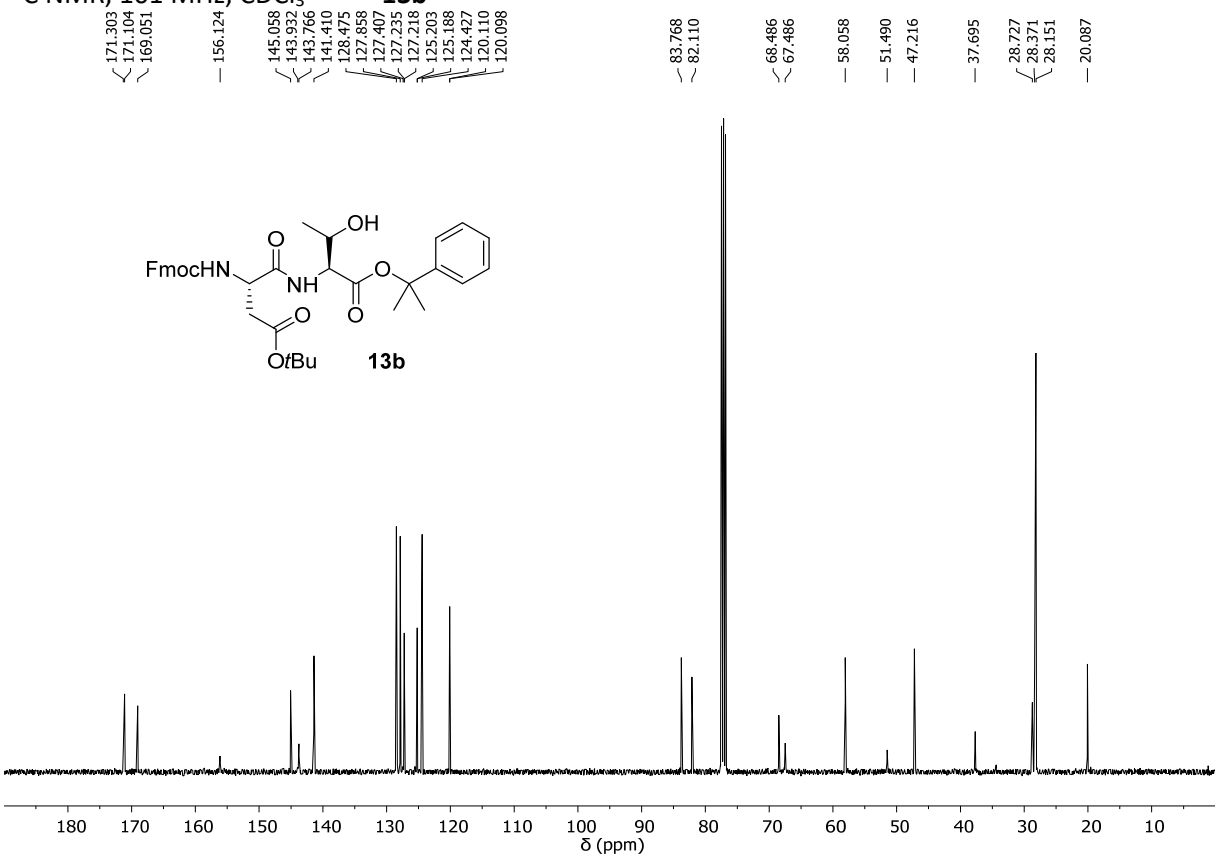


18a

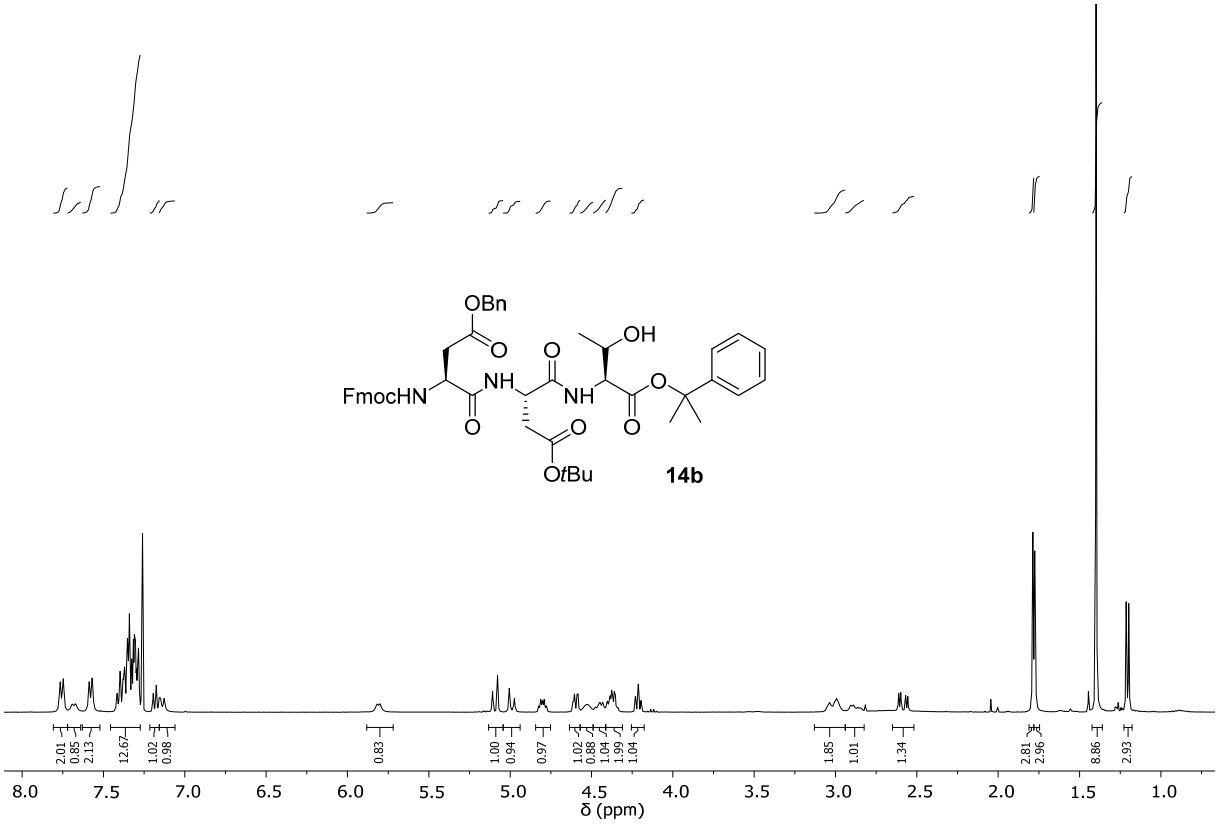
^{1}H NMR, 400 MHz, $CDCl_3$ **13b**



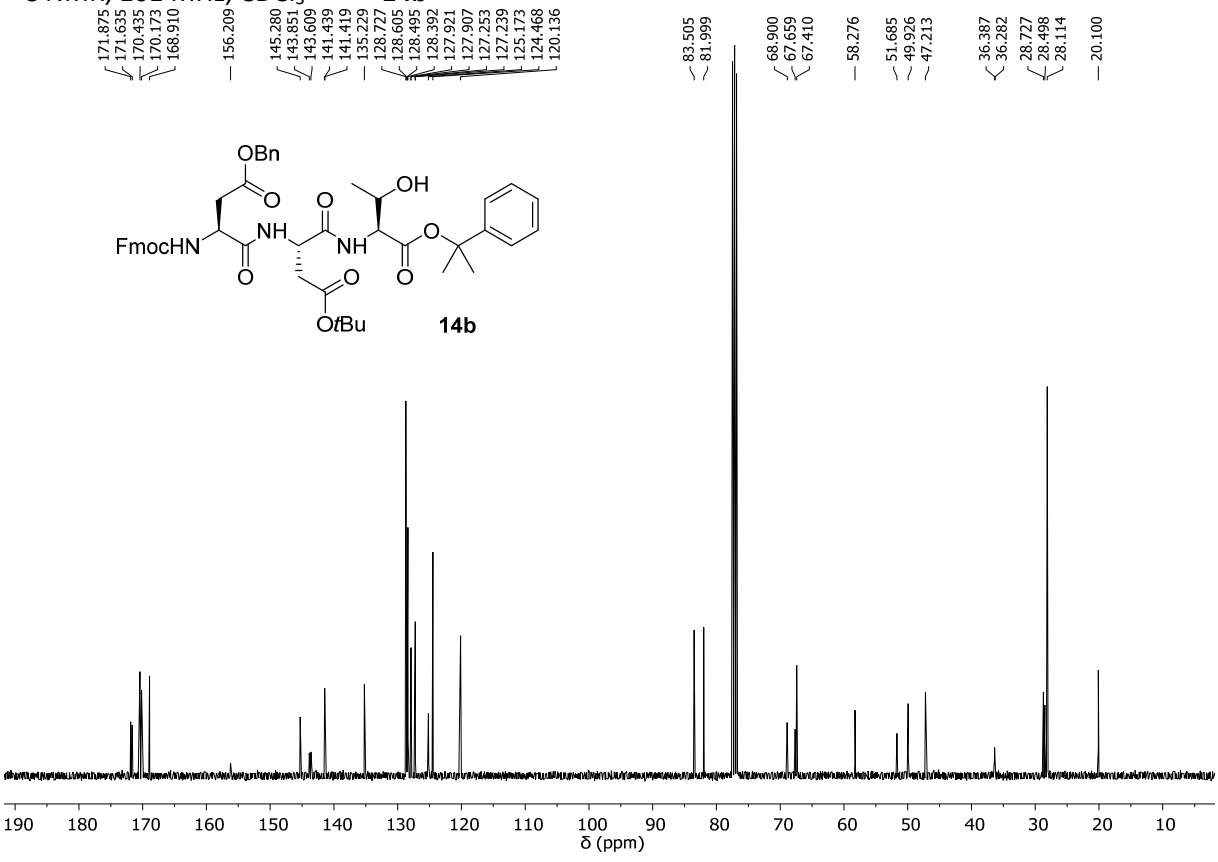
^{13}C NMR, 101 MHz, $CDCl_3$ **13b**



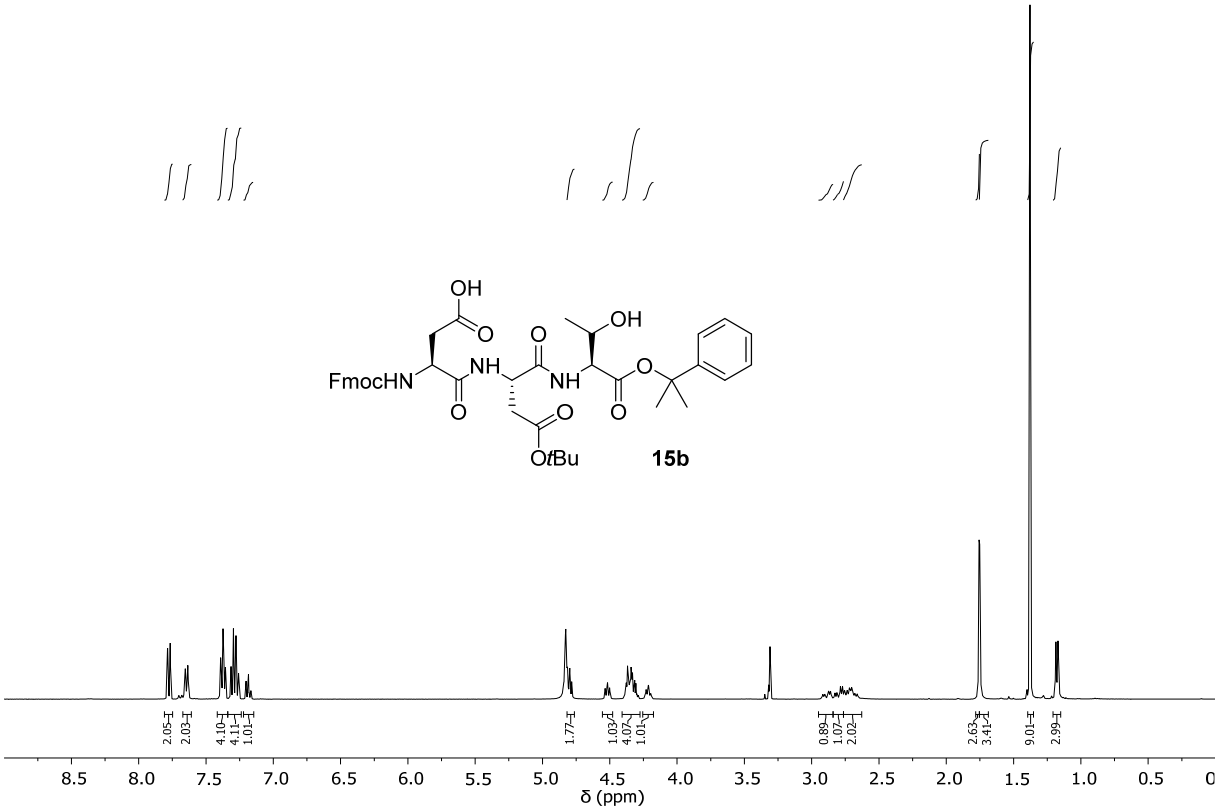
^{1}H NMR, 400 MHz, $CDCl_3$ **14b**



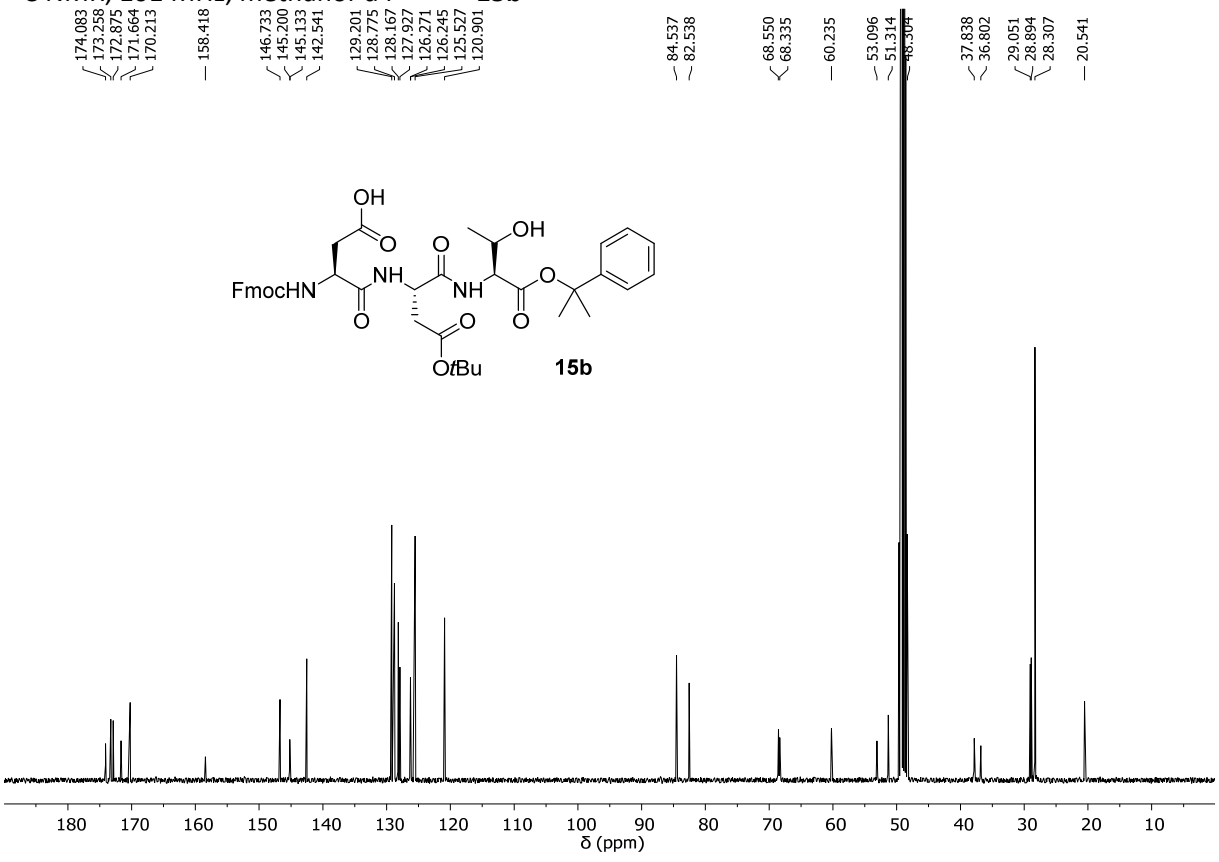
^{13}C NMR, 101 MHz, $CDCl_3$ **14b**



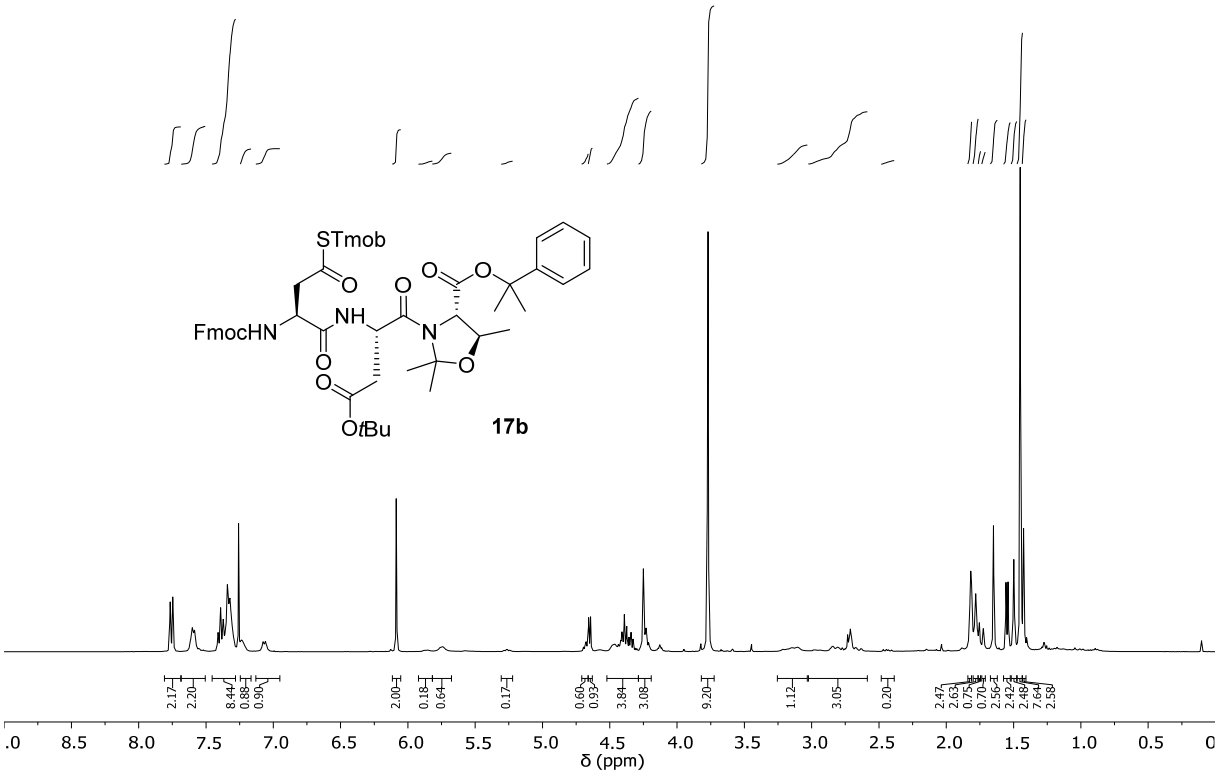
^{1}H NMR, 400 MHz, methanol-d4 **15b**



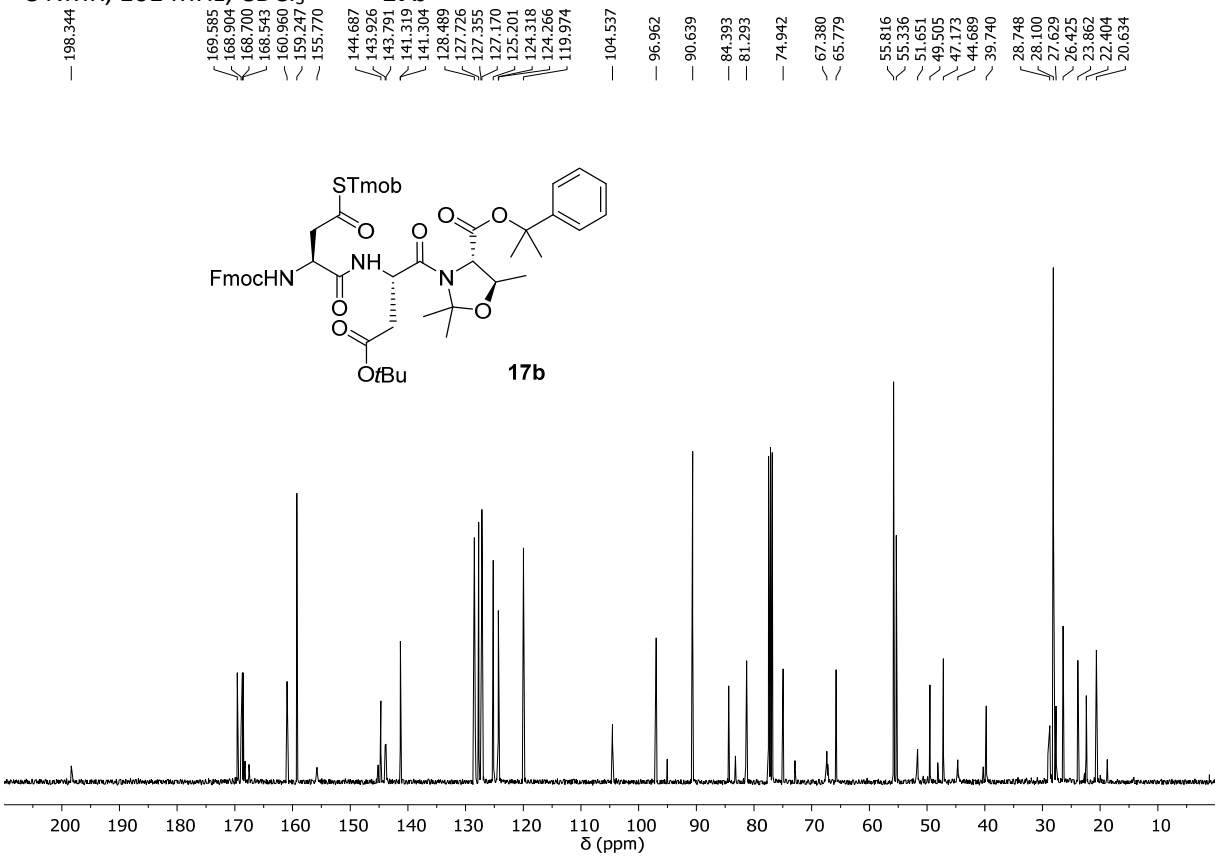
^{13}C NMR, 101 MHz, methanol-d4 **15b**



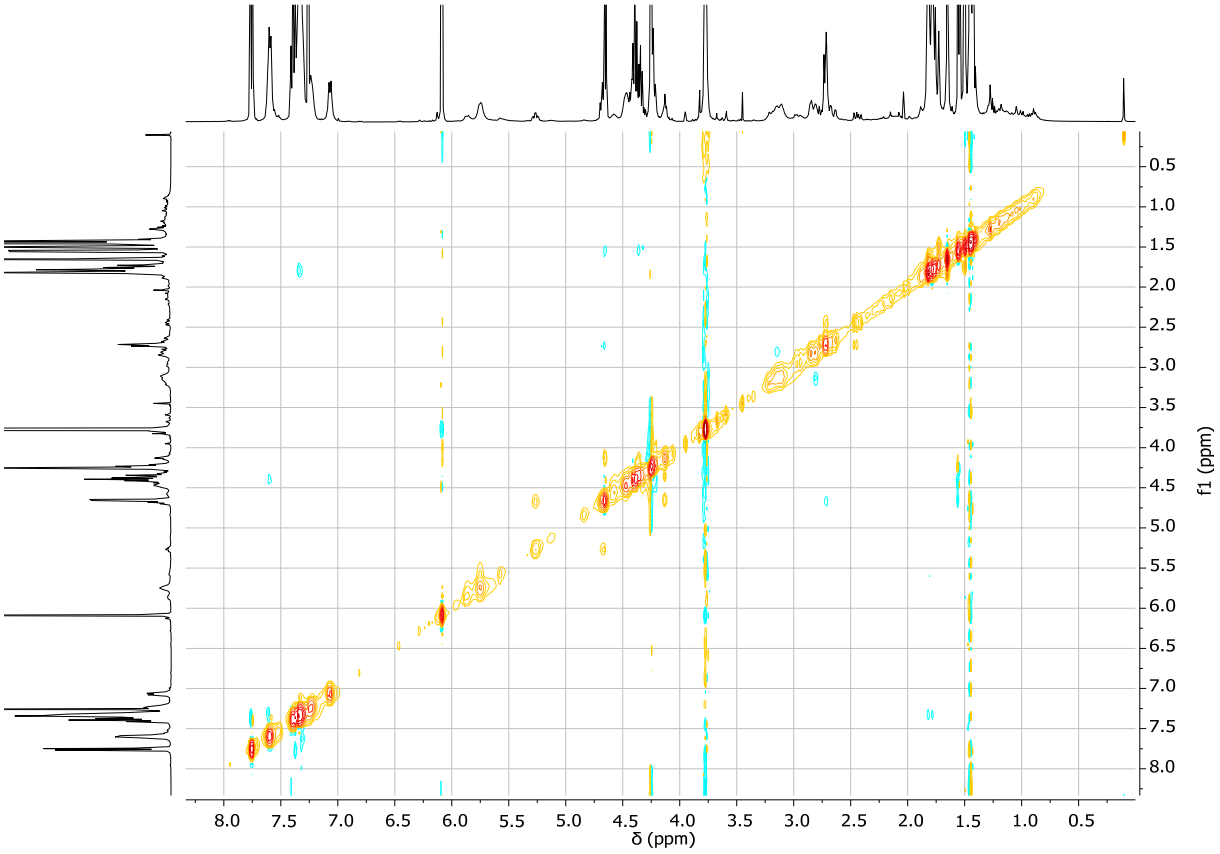
^{1}H NMR, 400 MHz, $CDCl_3$ **17b**



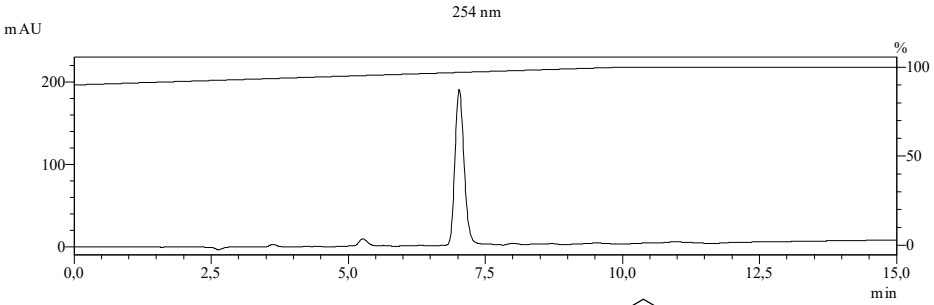
^{13}C NMR, 101 MHz, $CDCl_3$ **17b**

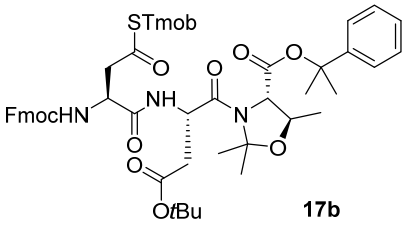


^{1}H-^{1}H ROESY NMR, 400 MHz, $CDCl_3$ **17b**



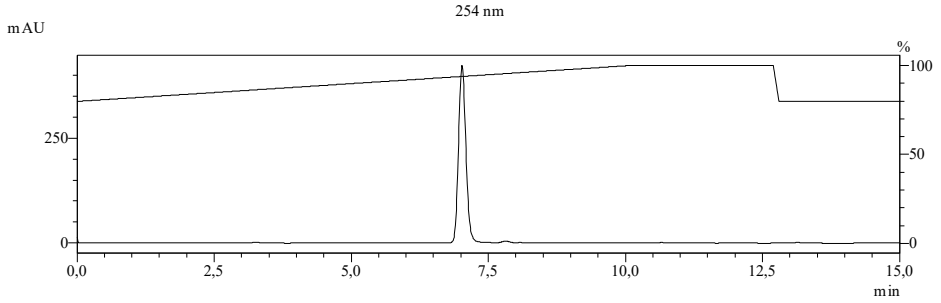
LC-MS chromatogram (254 nm) of purified tripeptide **17b** (90–100% in 10 min, R_t = 7.0 min, column 3).

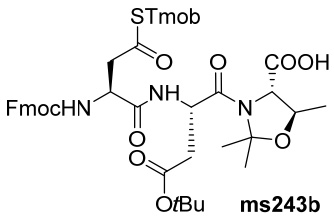




17b

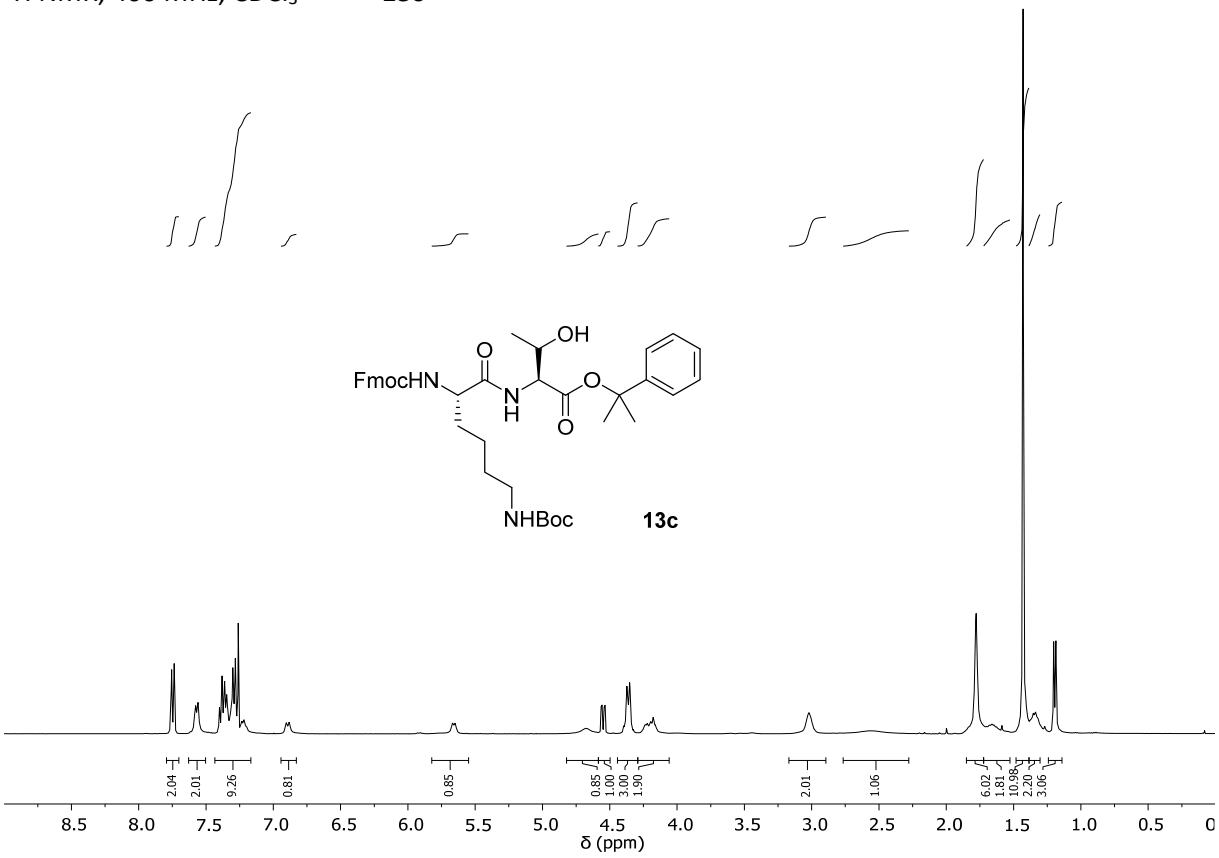
LC-MS chromatogram (254 nm) of purified tripeptide **18b** (80–100% in 10 min, R_t = 7.0 min, column 3).



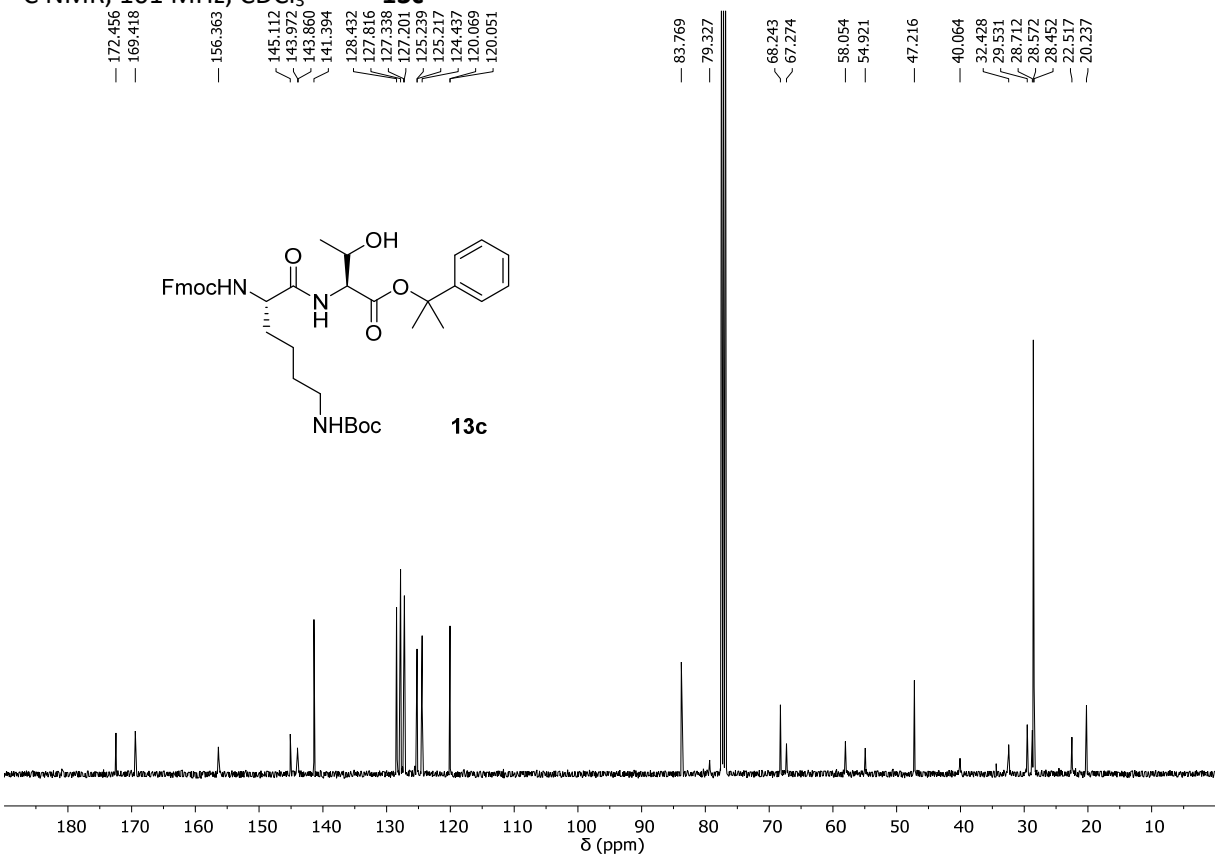


ms243b

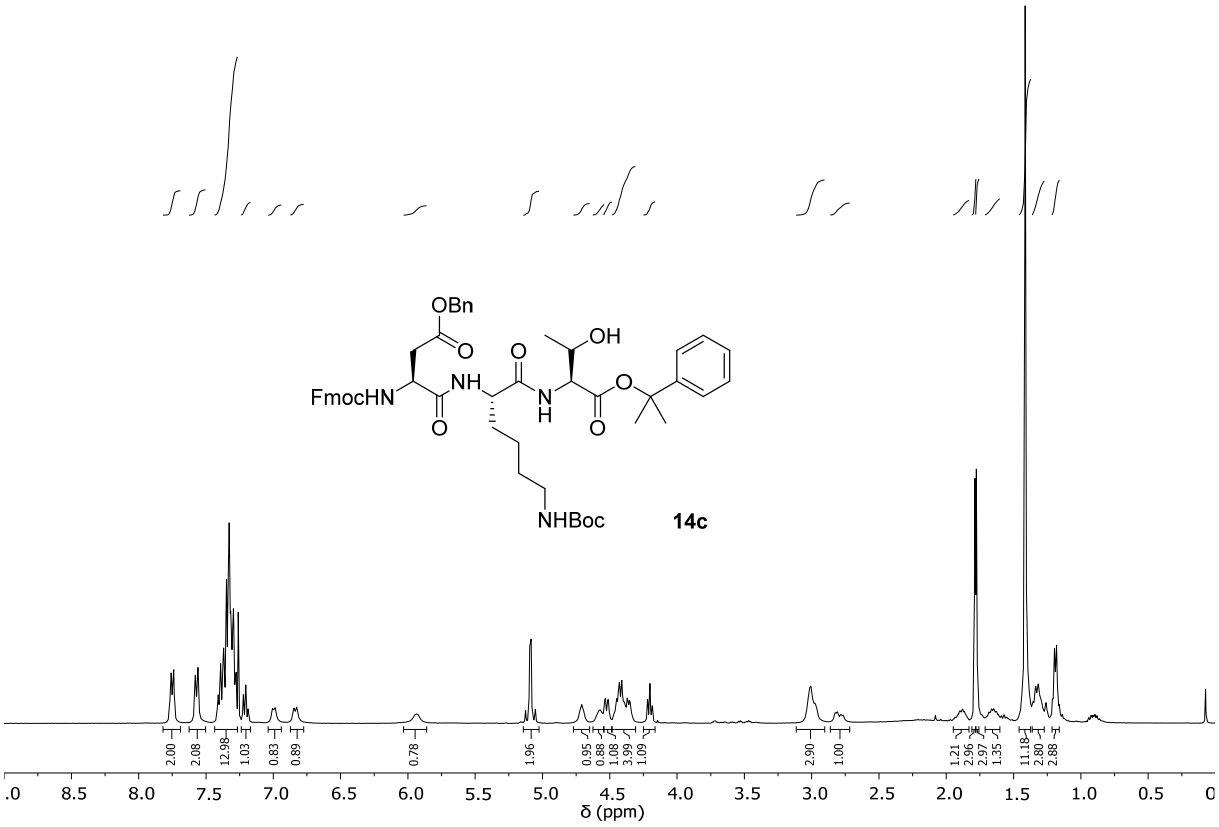
^{1}H NMR, 400 MHz, $CDCl_3$ **13c**



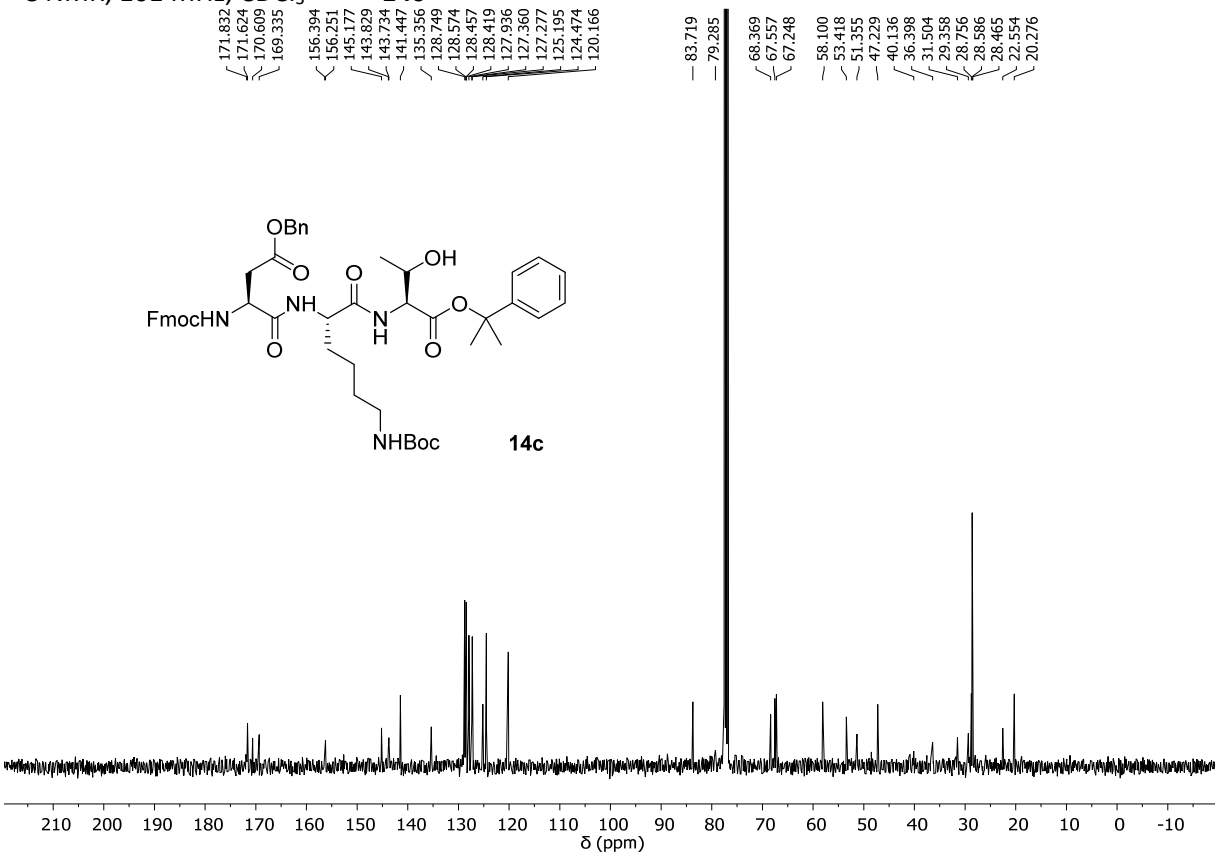
^{13}C NMR, 101 MHz, $CDCl_3$ **13c**



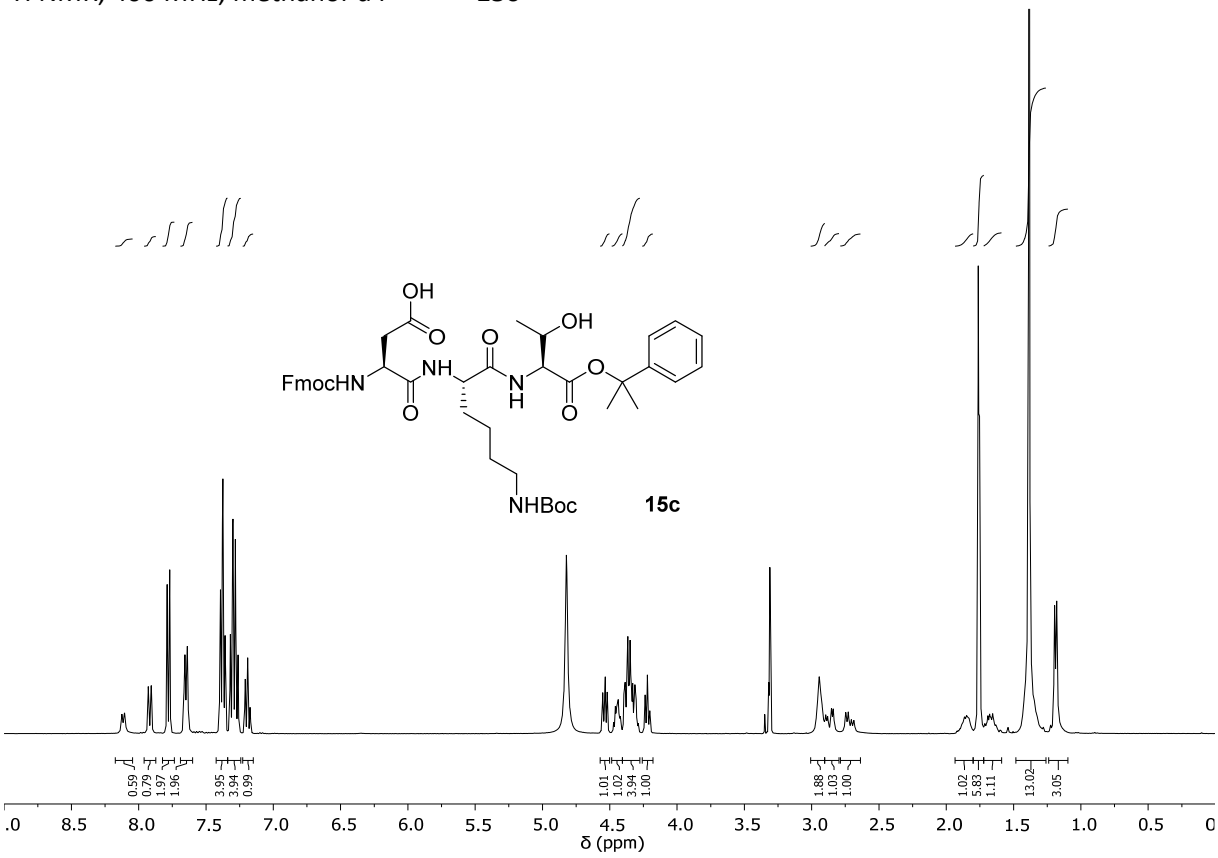
^{1}H NMR, 400 MHz, $CDCl_3$ **14c**



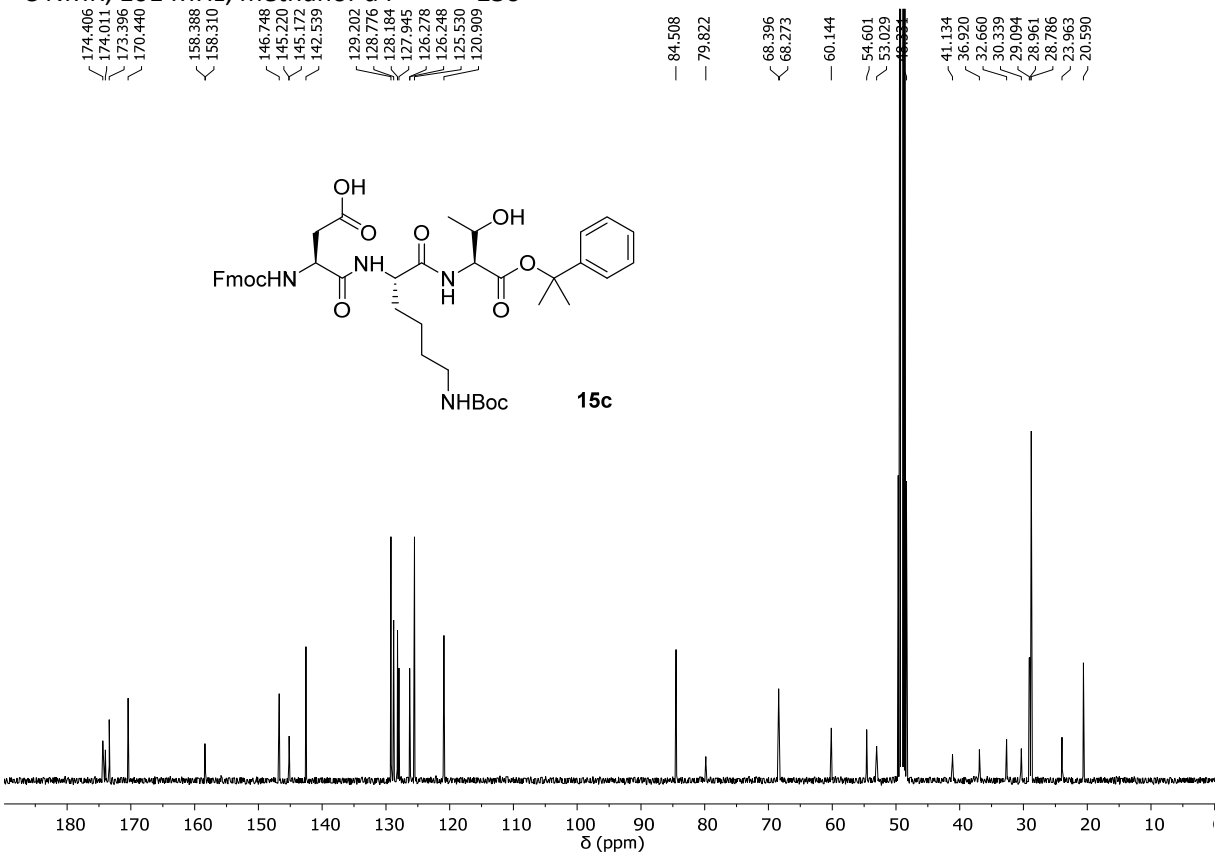
^{13}C NMR, 101 MHz, $CDCl_3$ **14c**



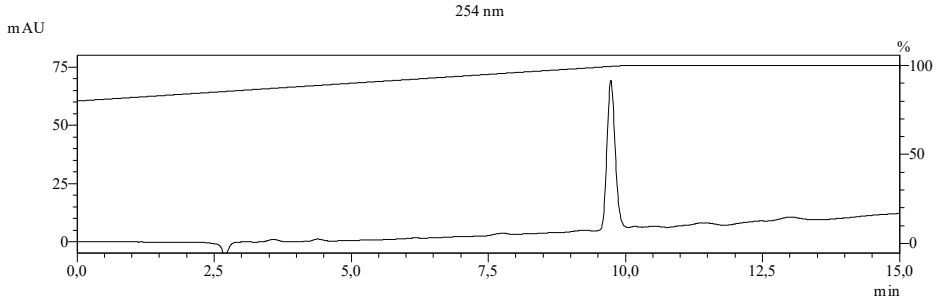
^{1}H NMR, 400 MHz, methanol-d4 **15c**

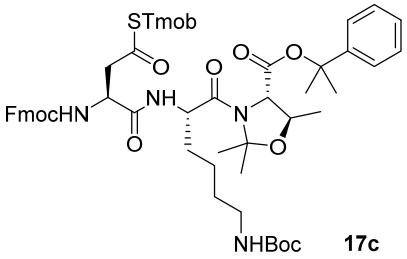


^{13}C NMR, 101 MHz, methanol-d4 **15c**



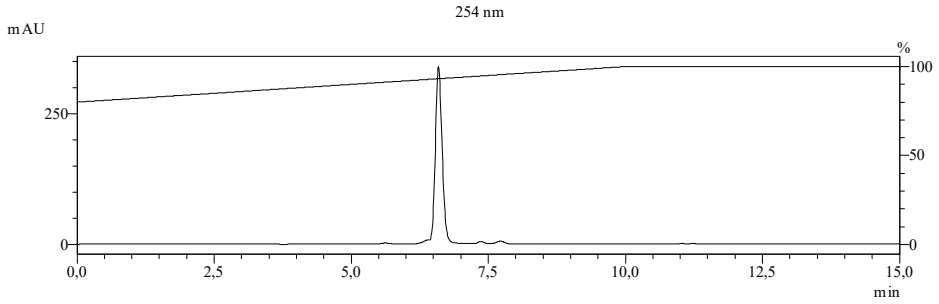
LC-MS chromatogram (254 nm) of purified tripeptide **17c** (80–100% in 10 min, R_t = 9.7 min, column 3).

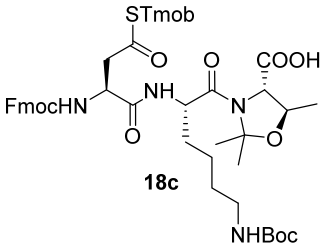




17c

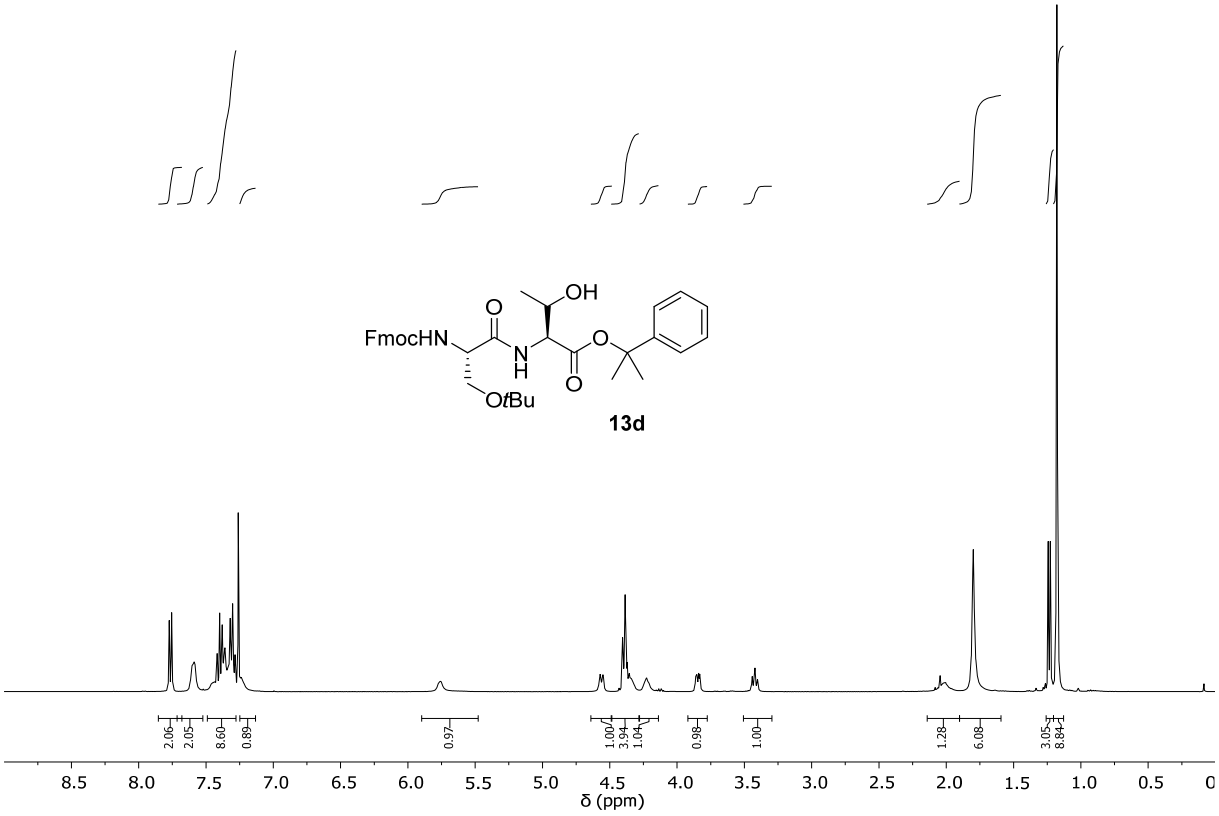
LC-MS chromatogram (254 nm) of purified tripeptide **18c** (80–100% in 10 min, R_t = 6.6 min, column 5).



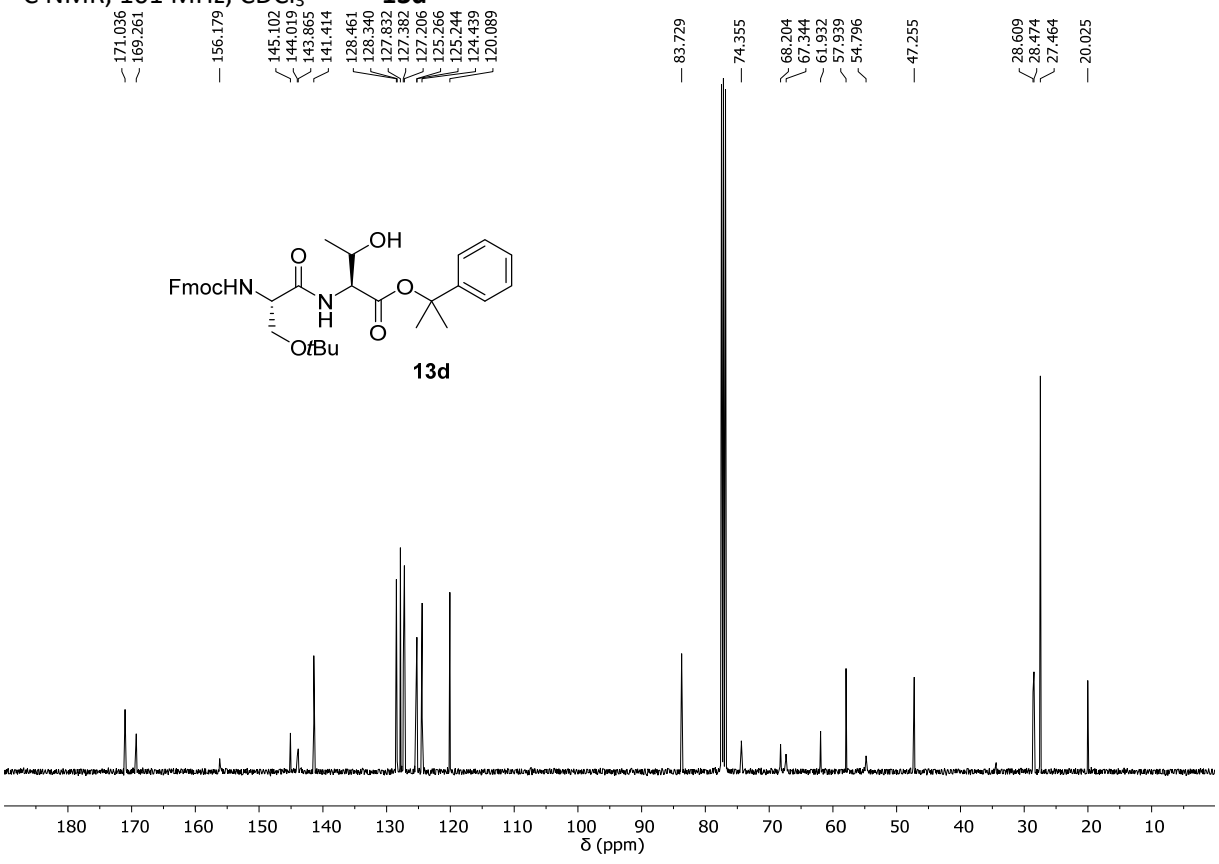


18c

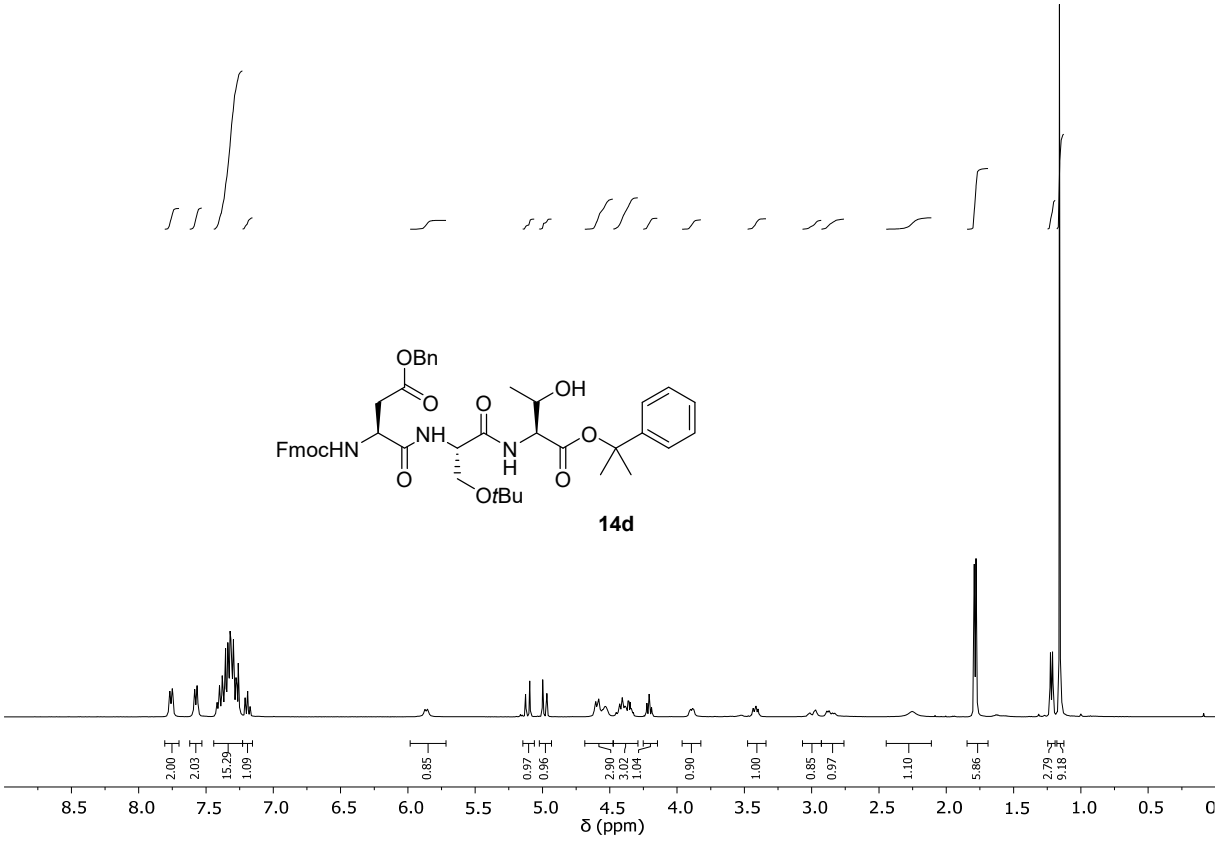
^{1}H NMR, 400 MHz, $CDCl_3$ **13d**



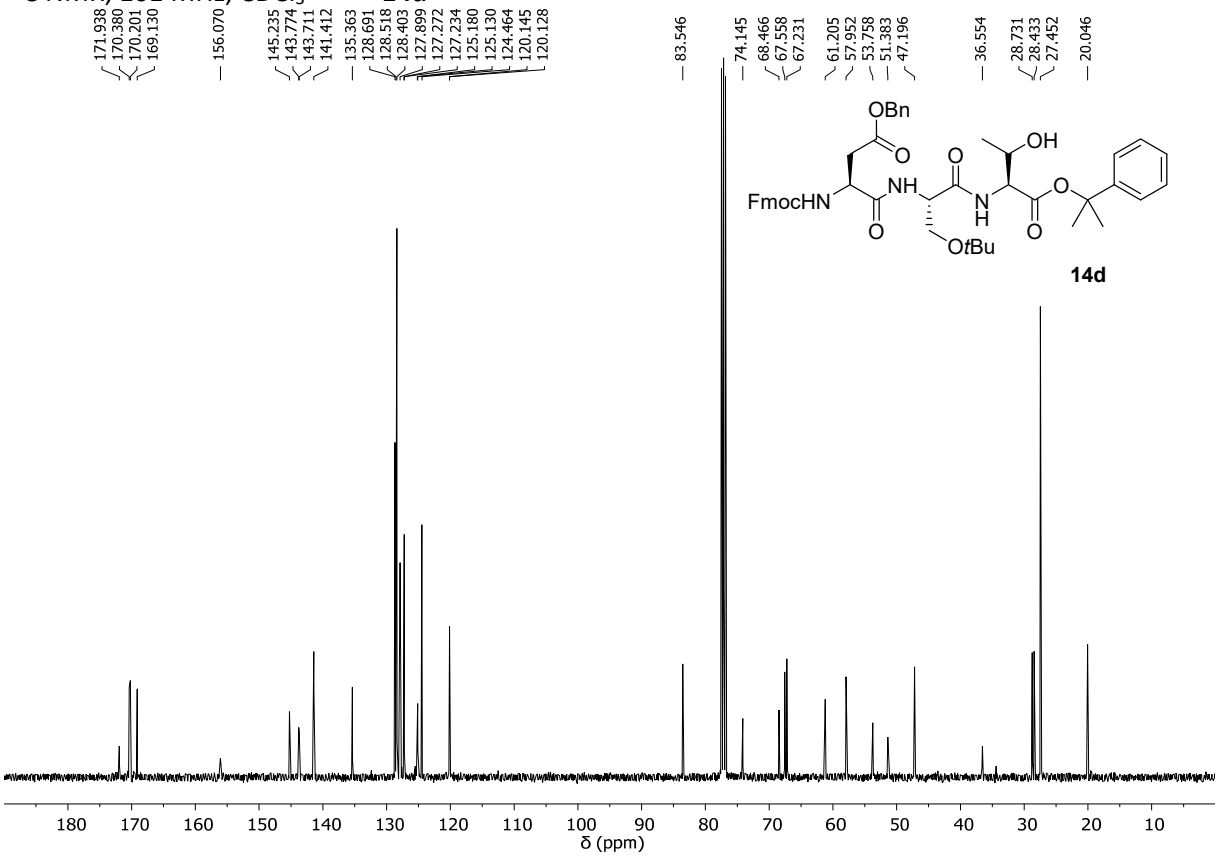
^{13}C NMR, 101 MHz, $CDCl_3$ **13d**



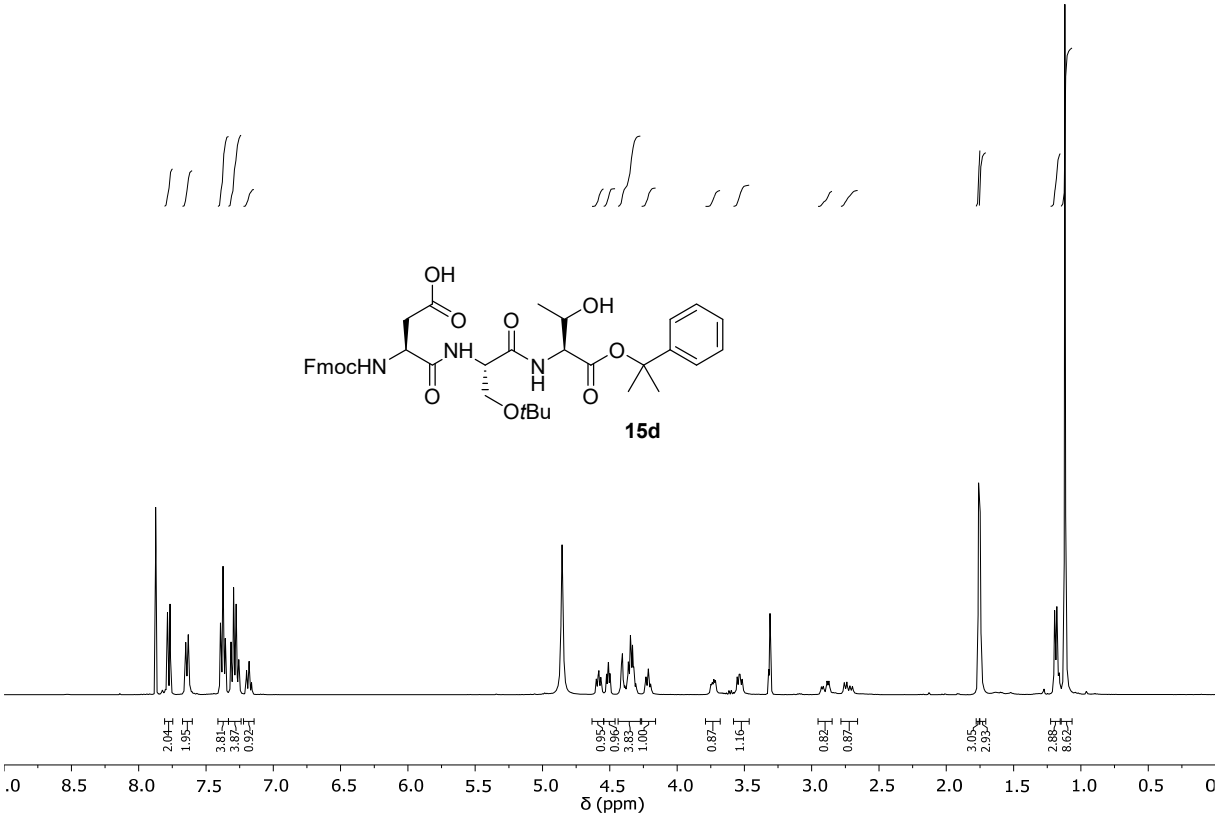
^{1}H NMR, 400 MHz, $CDCl_3$ **14d**



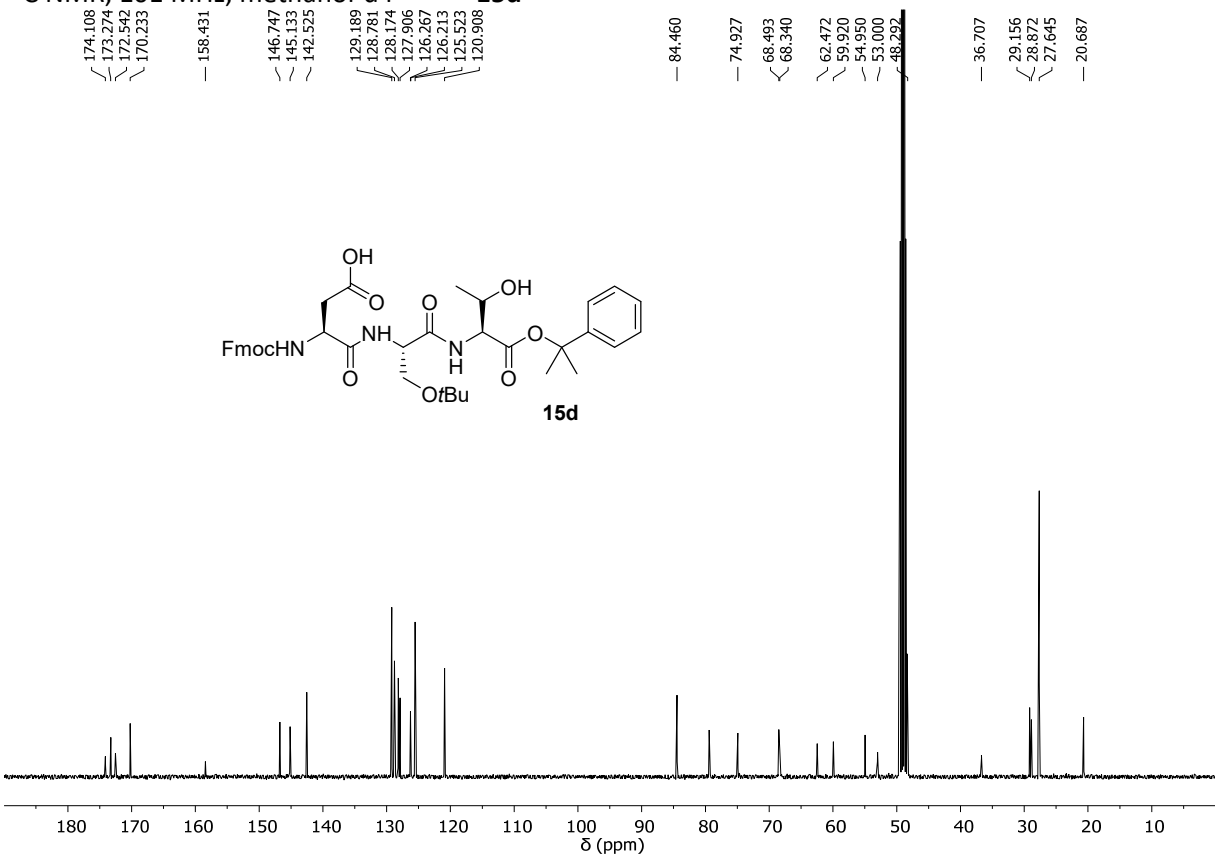
^{13}C NMR, 101 MHz, $CDCl_3$ **14d**



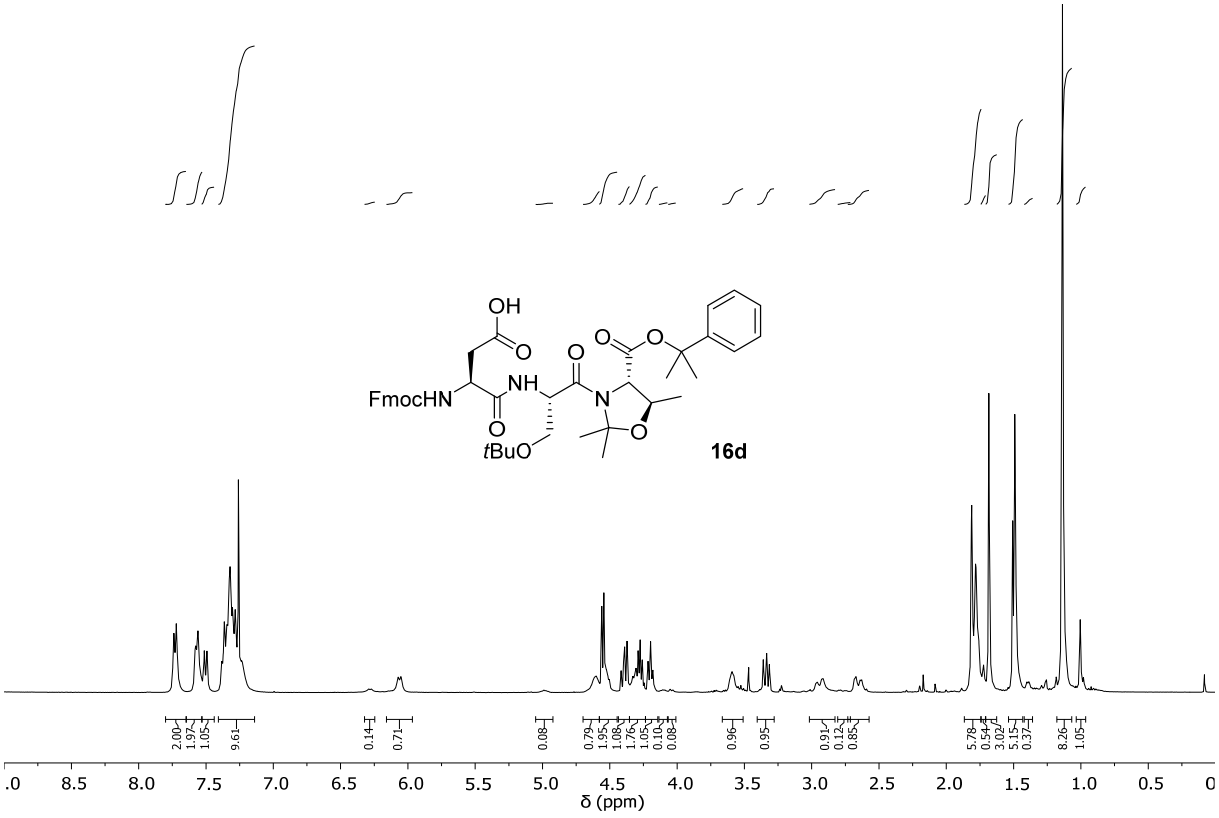
^{1}H NMR, 400 MHz, methanol-d4 **15d**



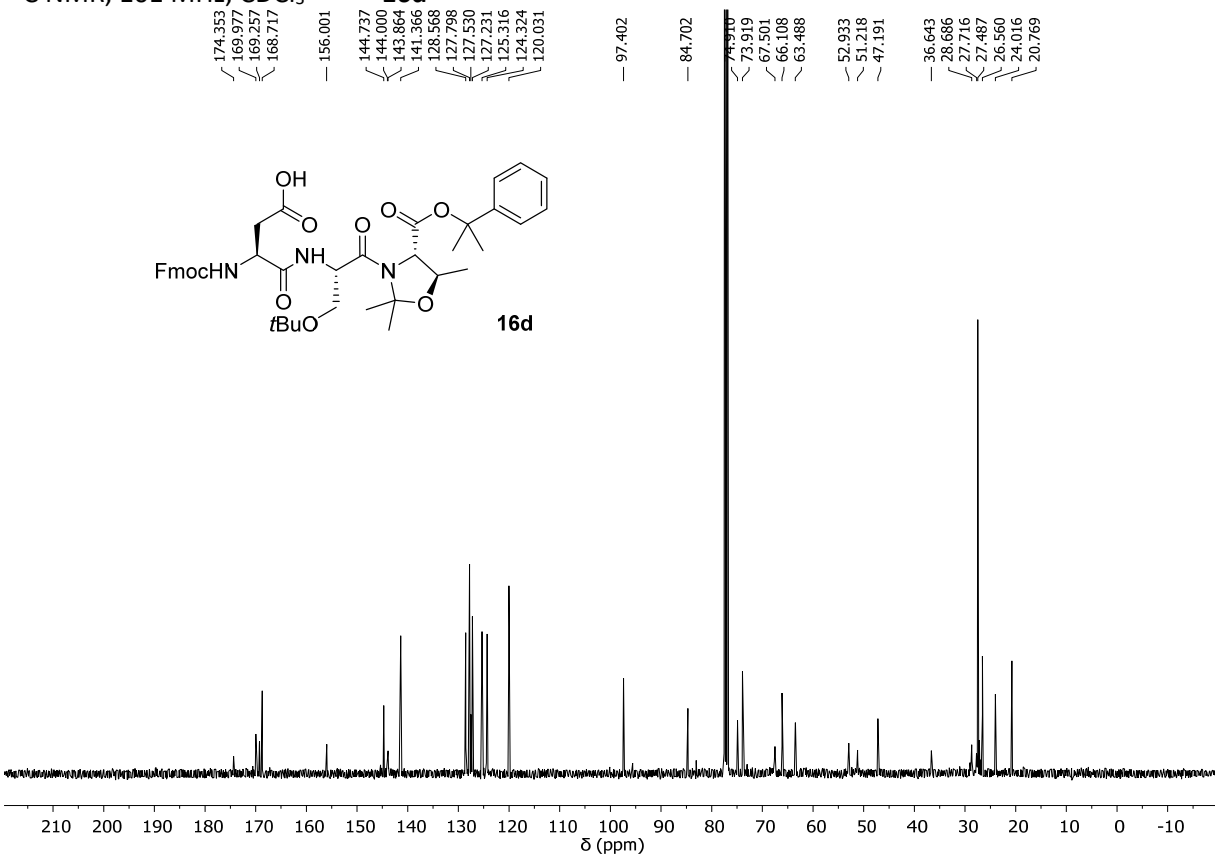
^{13}C NMR, 101 MHz, methanol-d4 **15d**



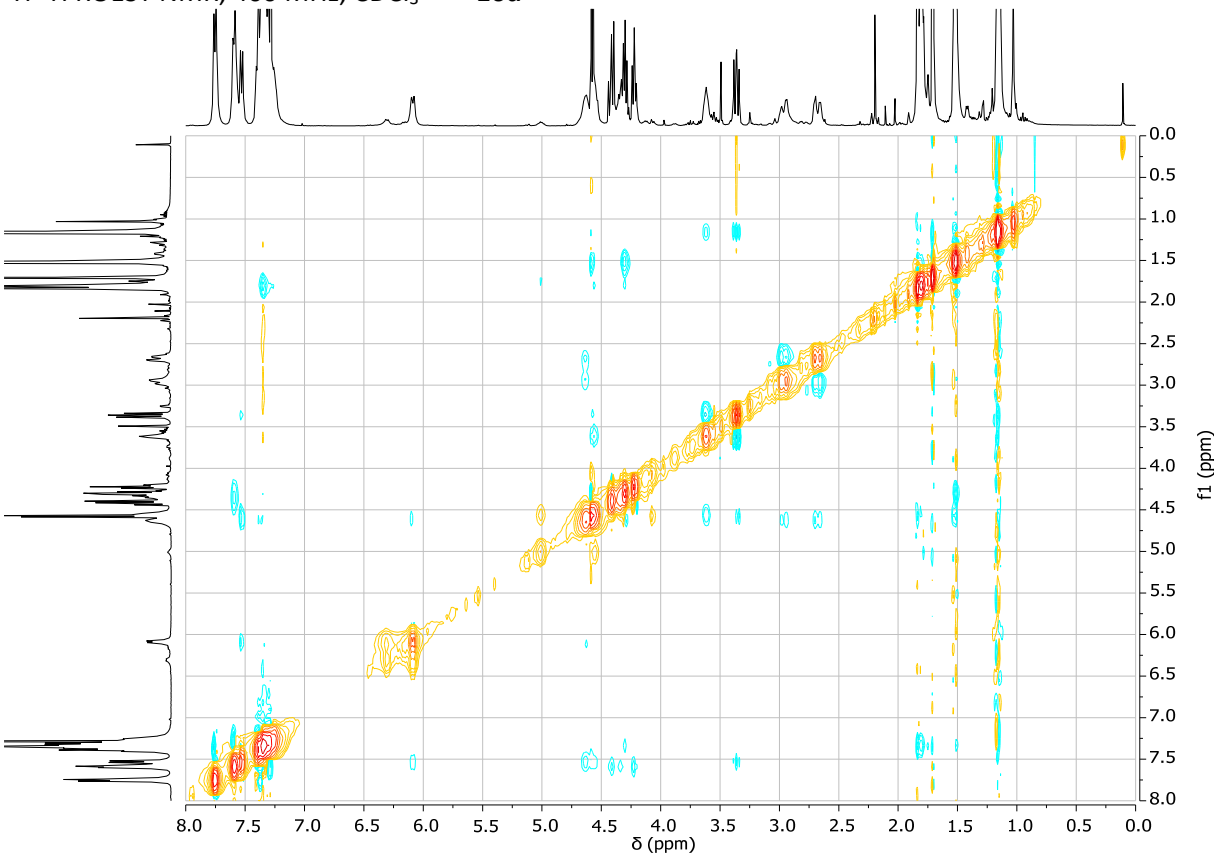
^{1}H NMR, 400 MHz, $CDCl_3$ **16d**

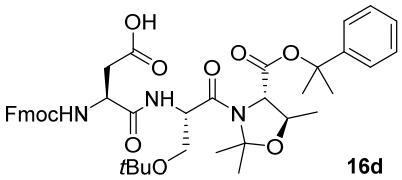


^{13}C NMR, 101 MHz, $CDCl_3$ **16d**



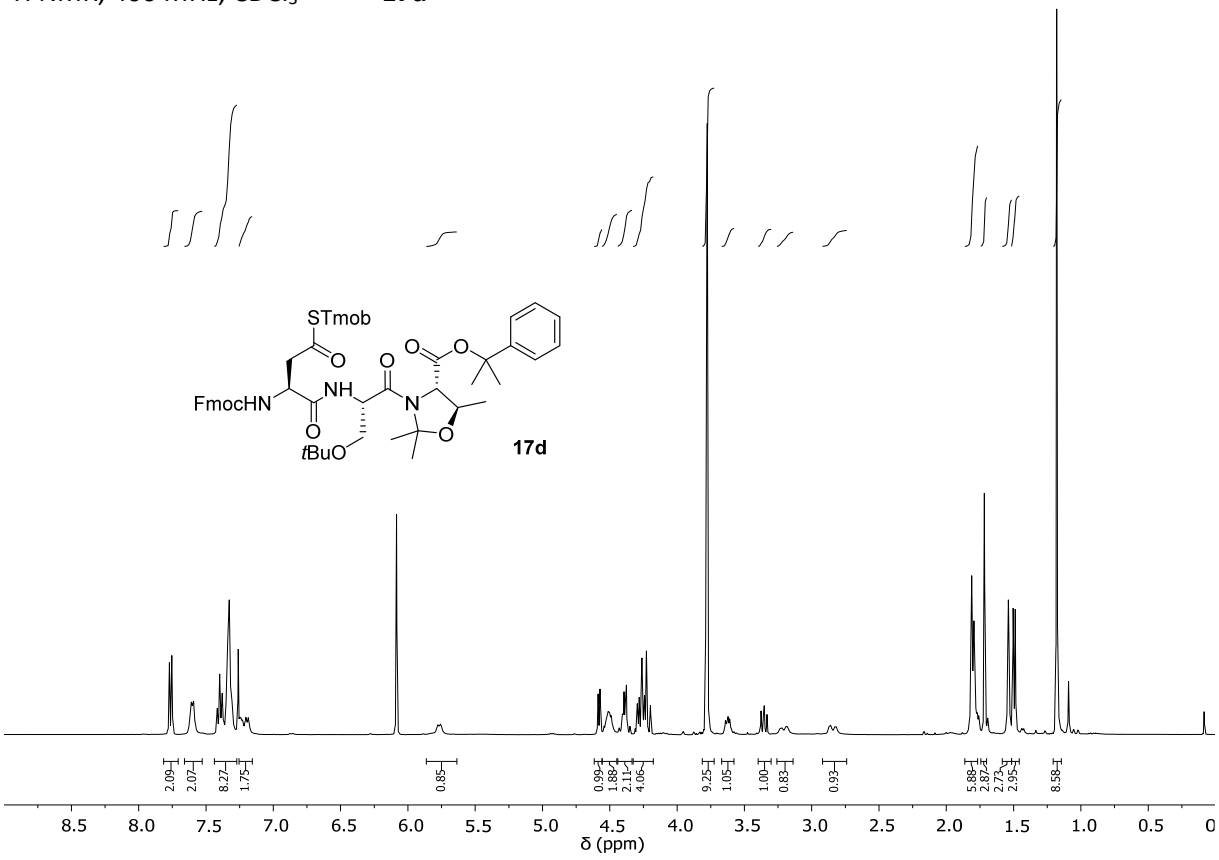
^{1}H-^{1}H ROESY NMR, 400 MHz, $CDCl_3$ **16d**



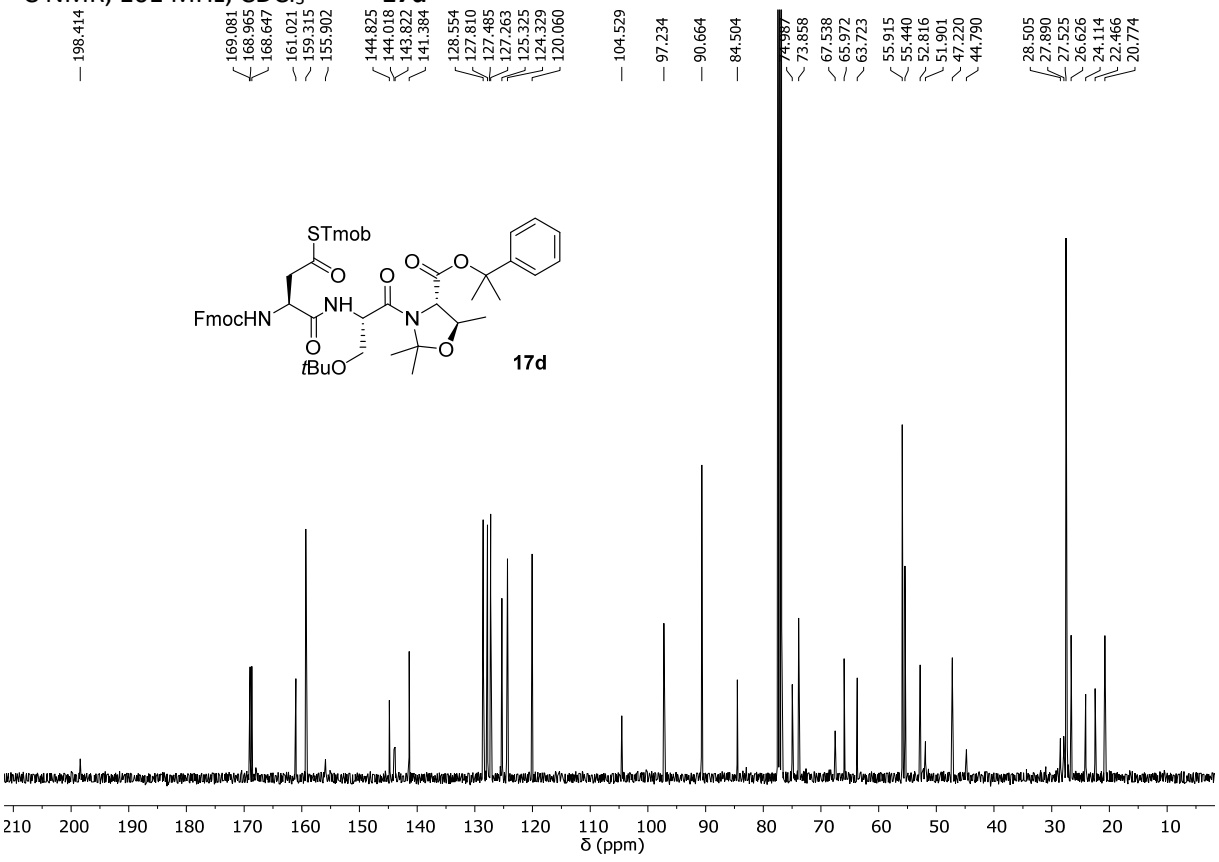


16d

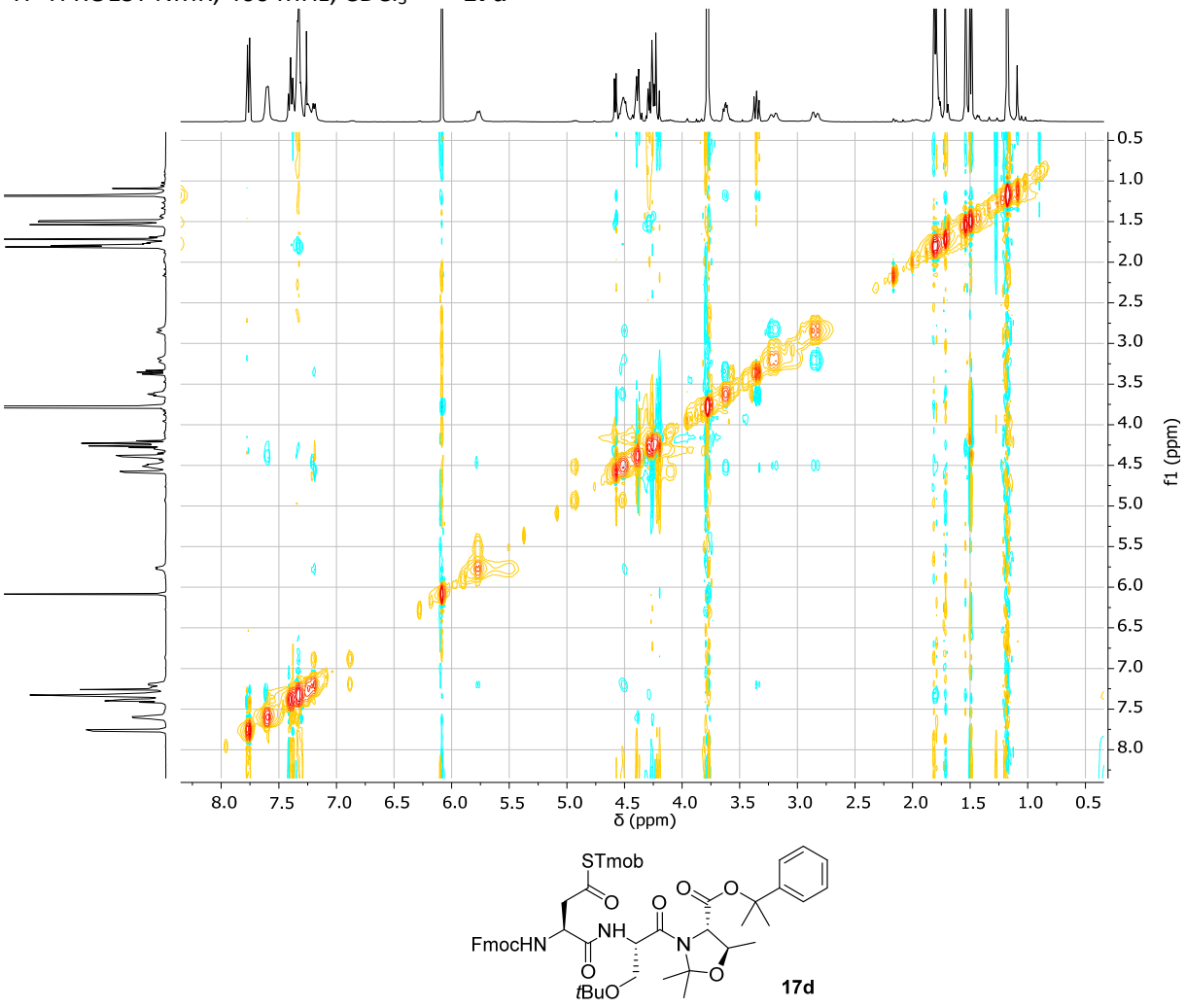
^{1}H NMR, 400 MHz, $CDCl_3$ **17d**



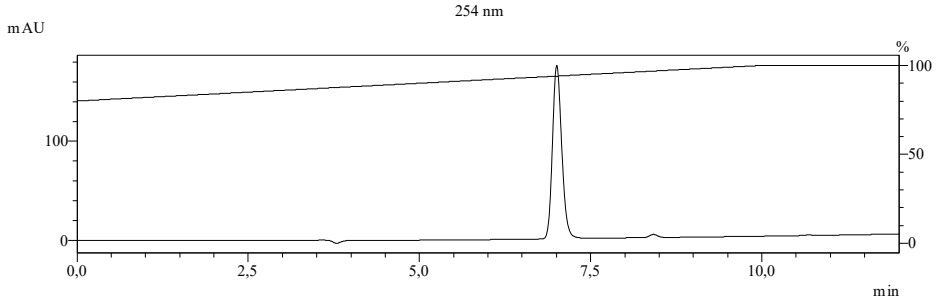
^{13}C NMR, 101 MHz, $CDCl_3$ **17d**

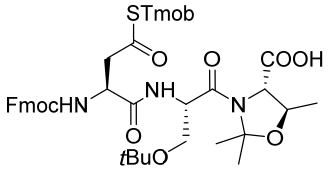


^{1}H-^{1}H ROESY NMR, 400 MHz, $CDCl_3$ **17d**



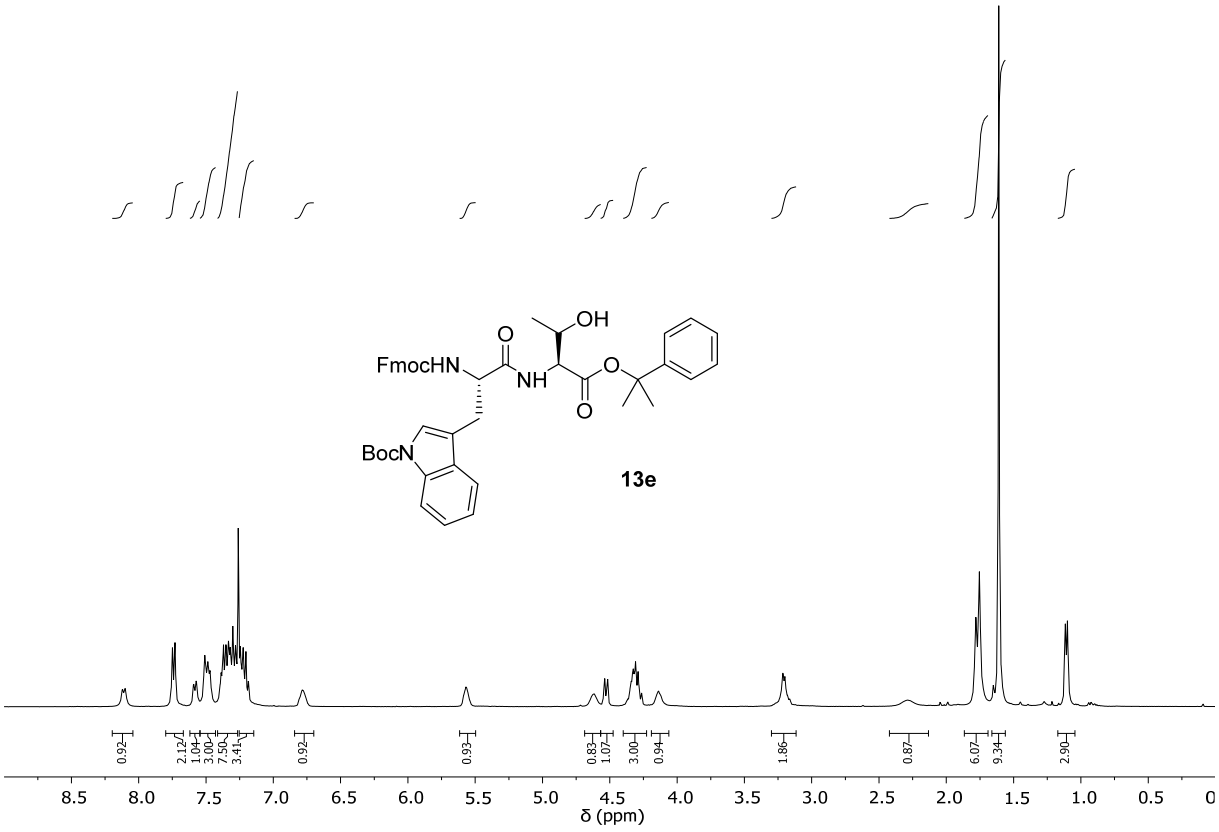
LC-MS chromatogram (254 nm) of purified tripeptide **18d** (80–100% in 10 min, R_t = 7.0 min, column 3).



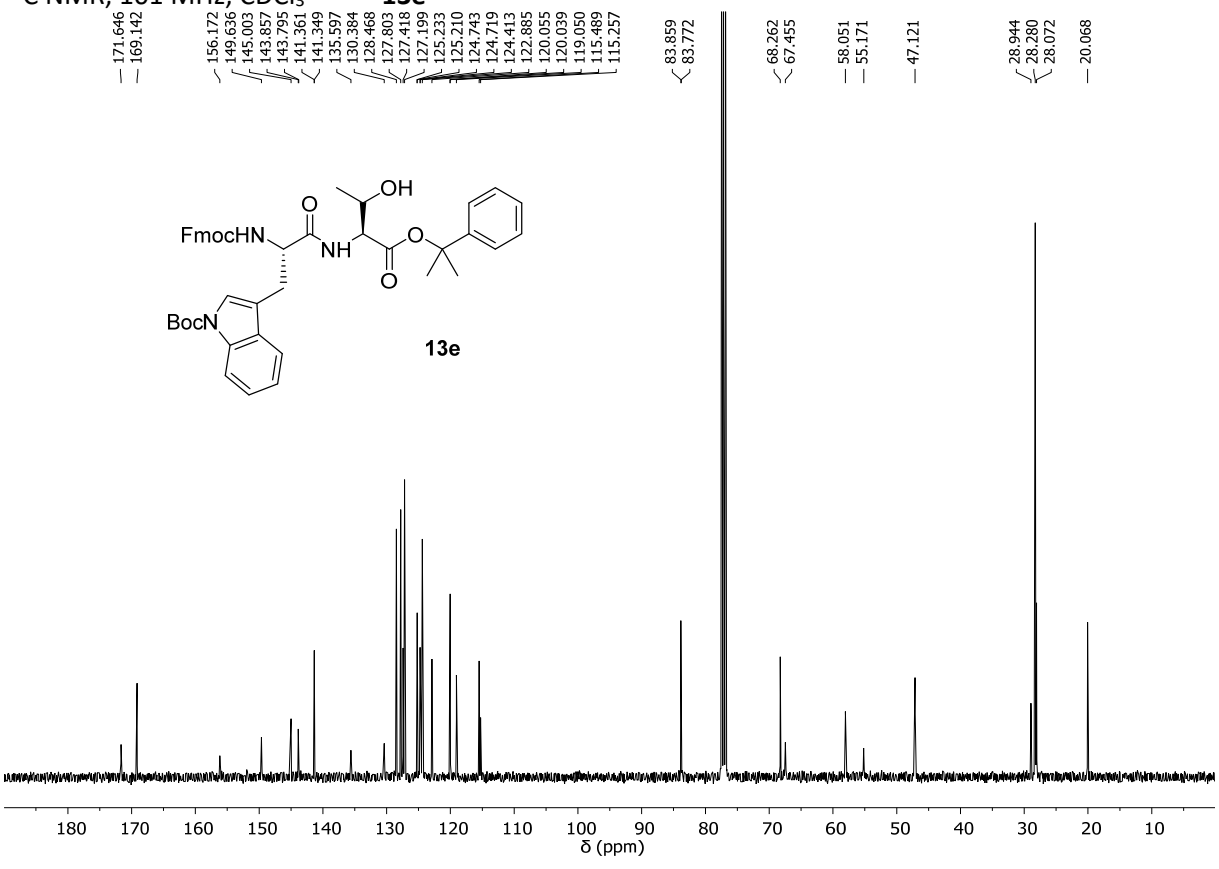


18d

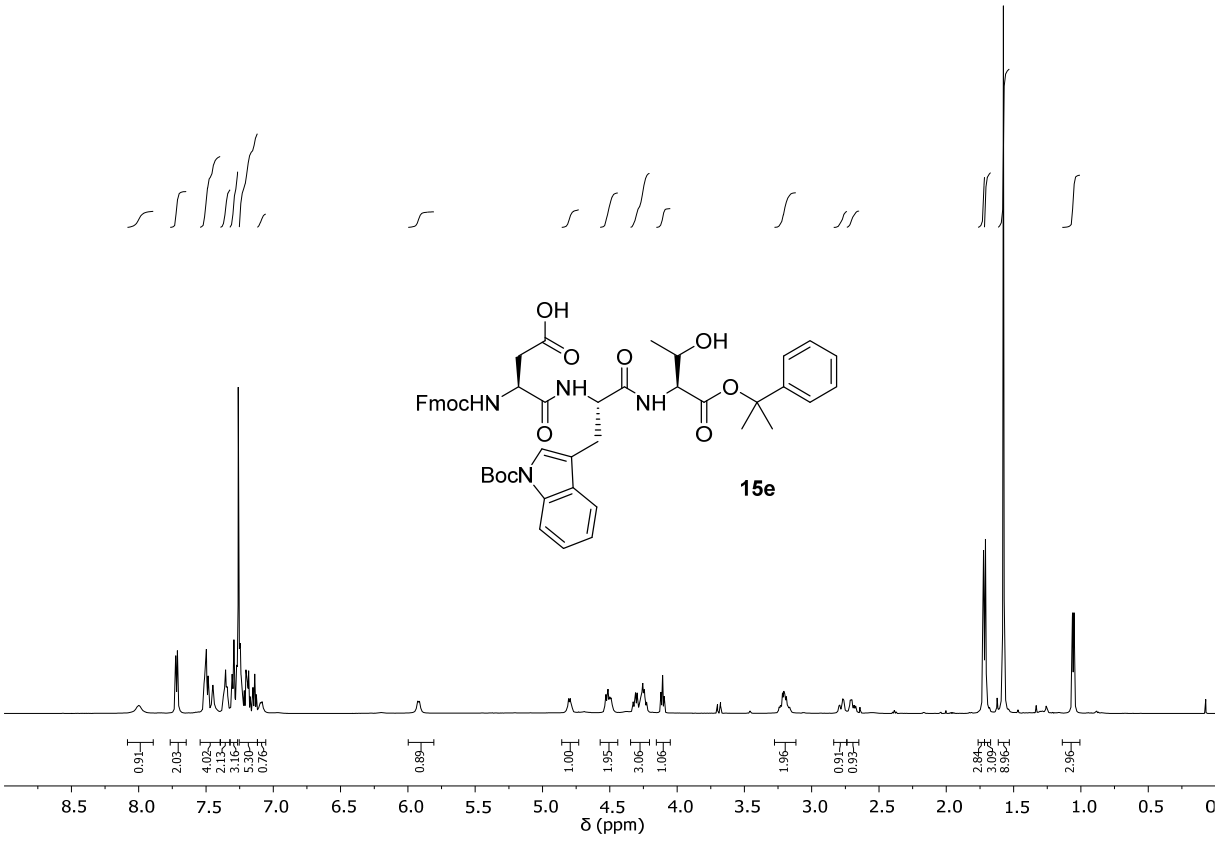
^{1}H NMR, 400 MHz, $CDCl_3$ **13e**



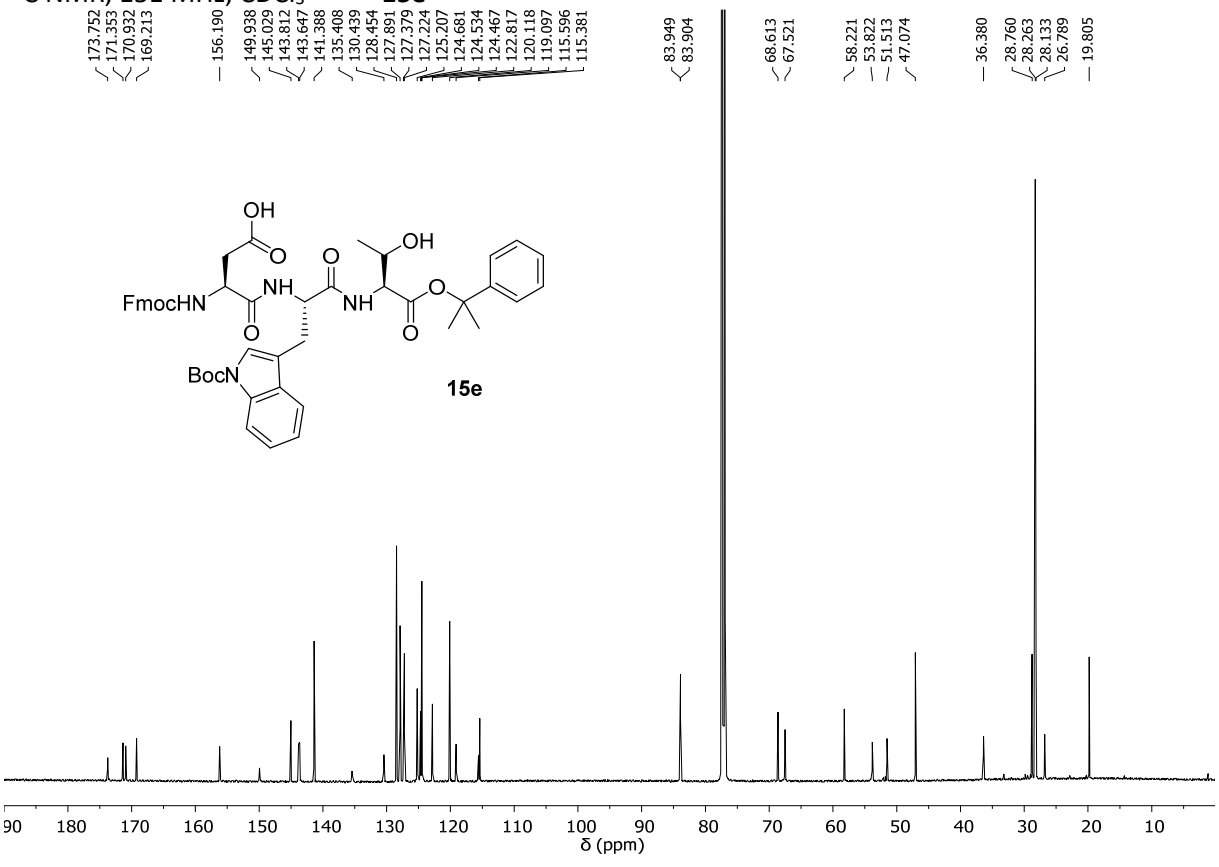
^{13}C NMR, 101 MHz, $CDCl_3$ **13e**



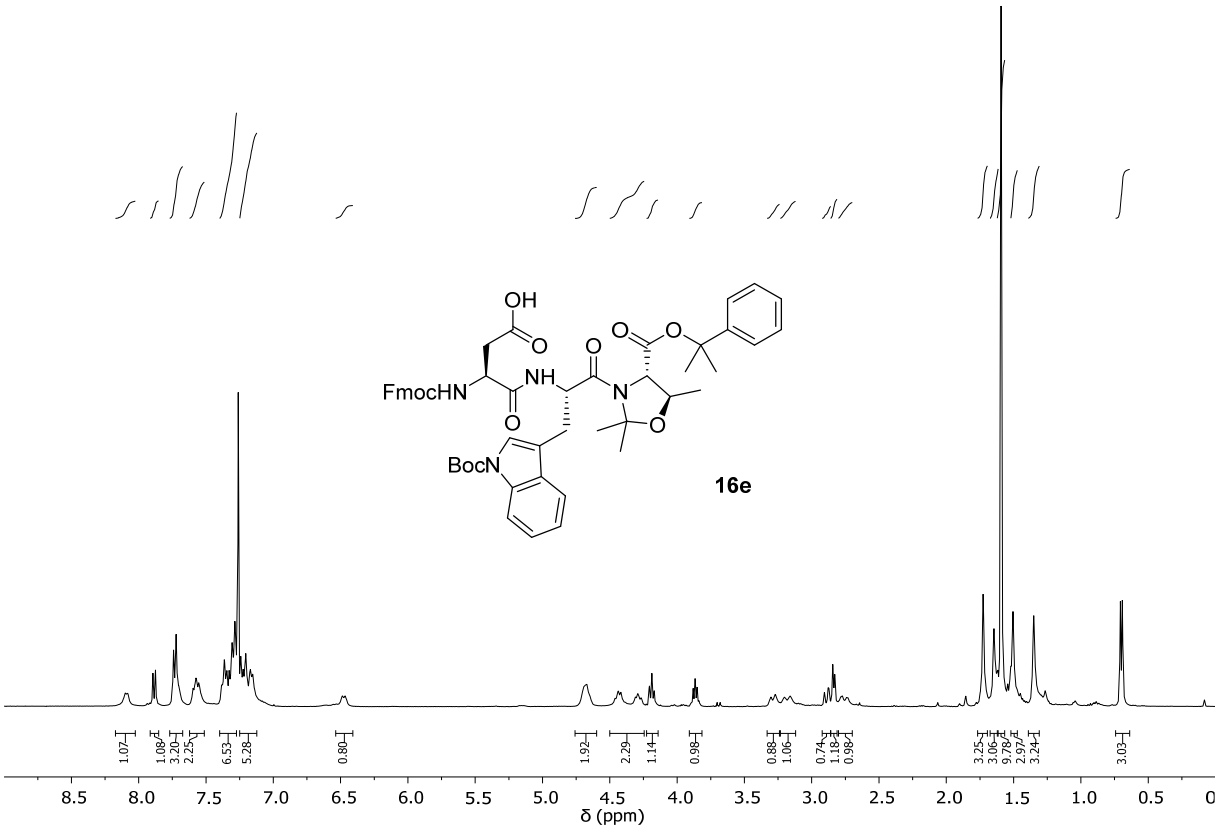
^{1}H NMR, 600 MHz, $CDCl_3$ **15e**



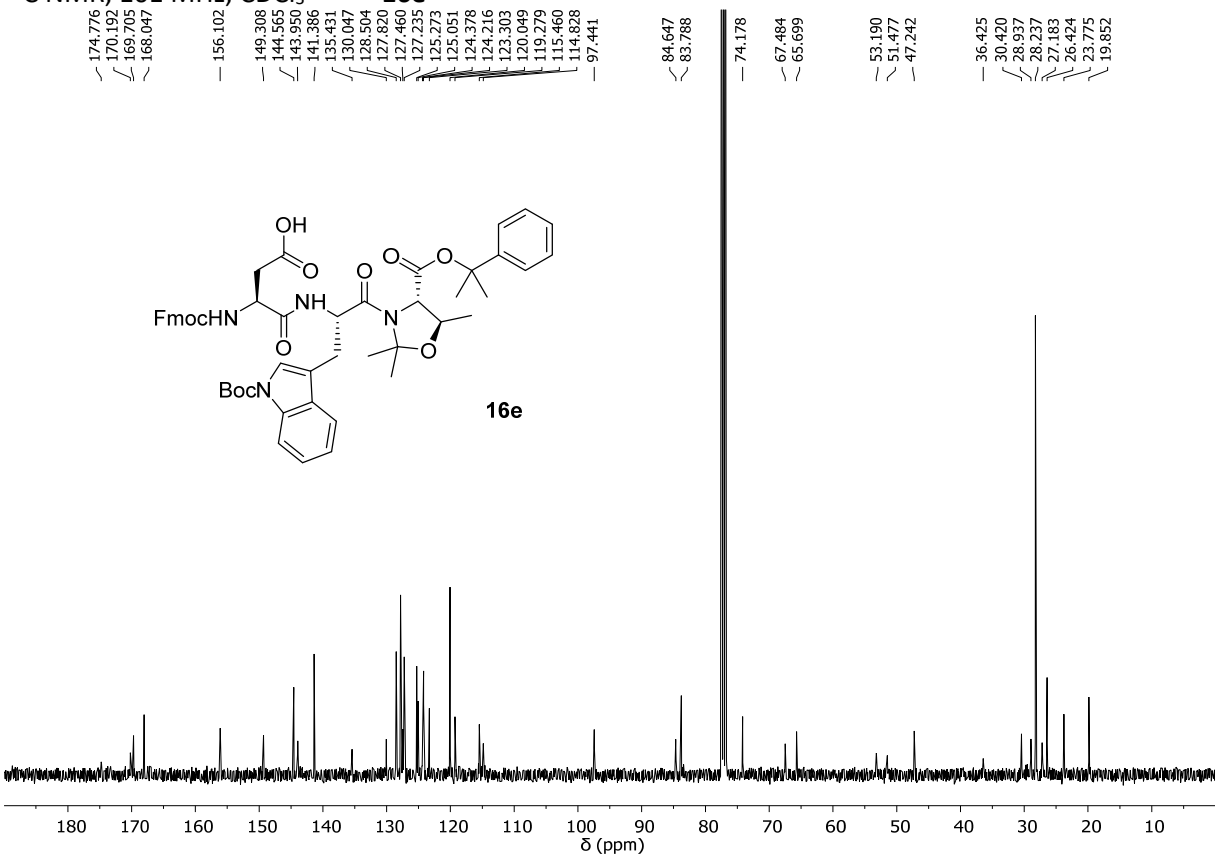
^{13}C NMR, 151 MHz, $CDCl_3$ **15e**



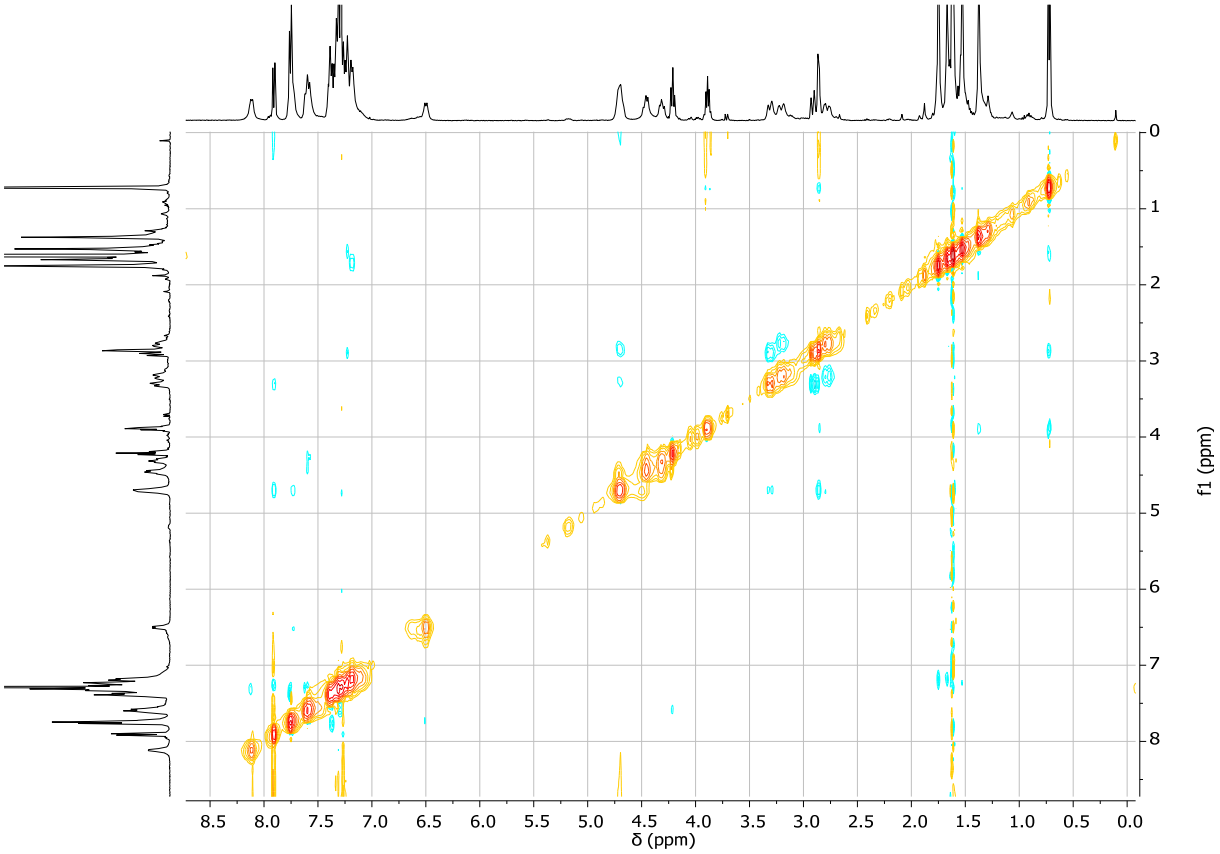
^{1}H NMR, 400 MHz, $CDCl_3$ **16e**

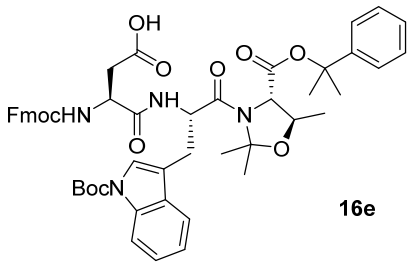


^{13}C NMR, 101 MHz, $CDCl_3$ **16e**



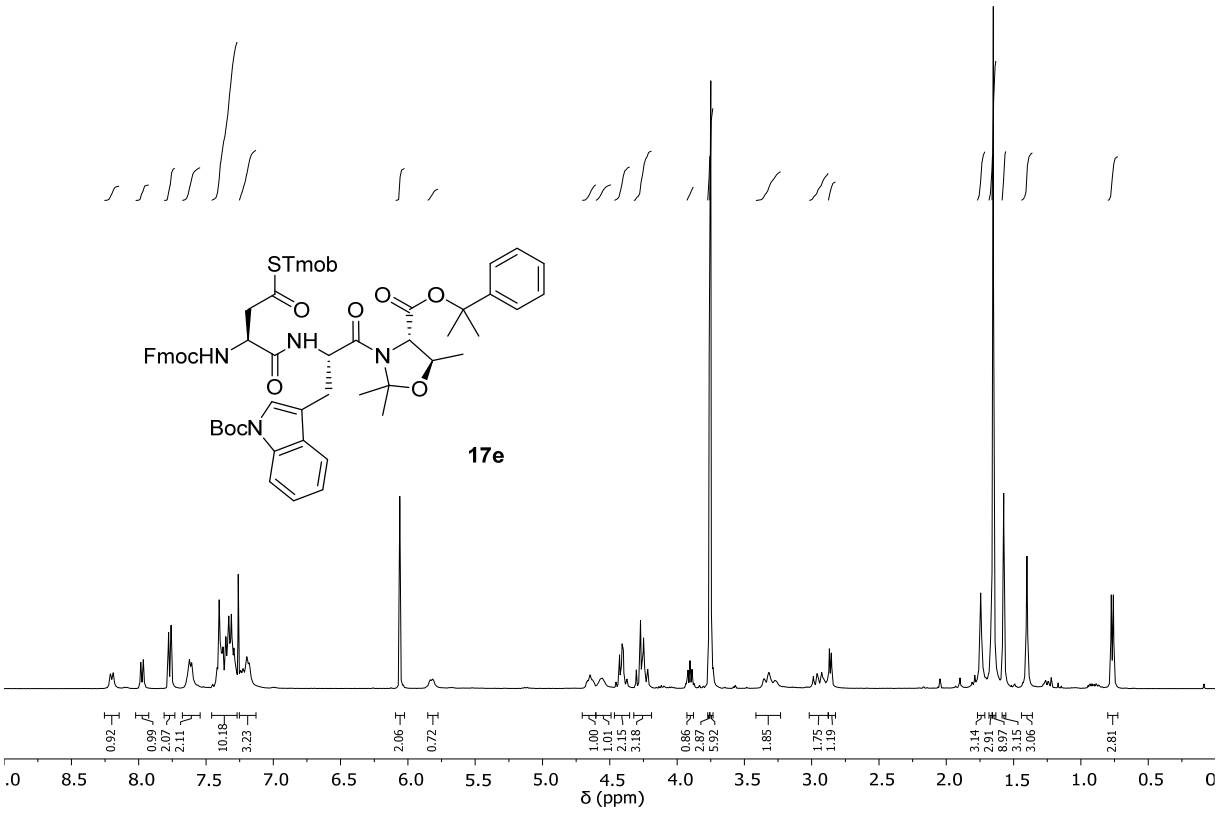
^{1}H-^{1}H ROESY NMR, 400 MHz, $CDCl_3$ **16e**



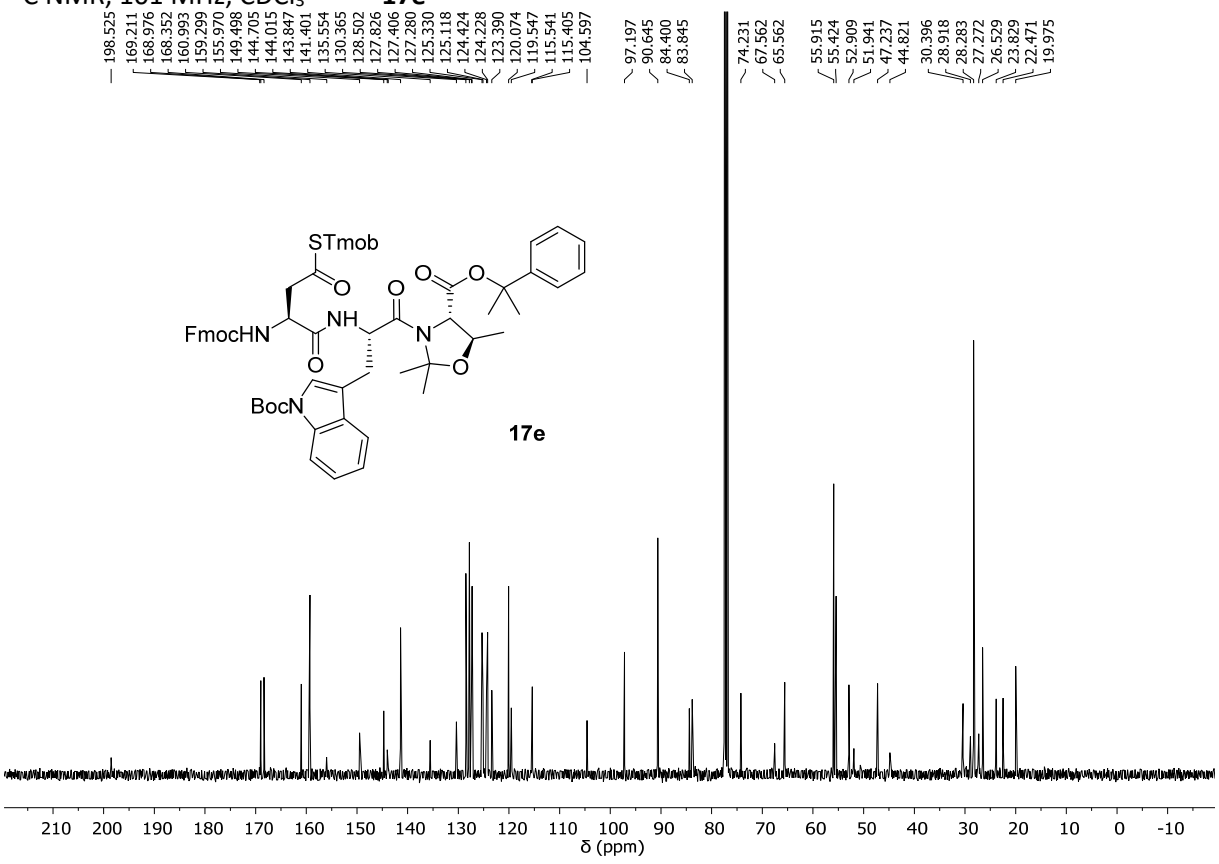


16e

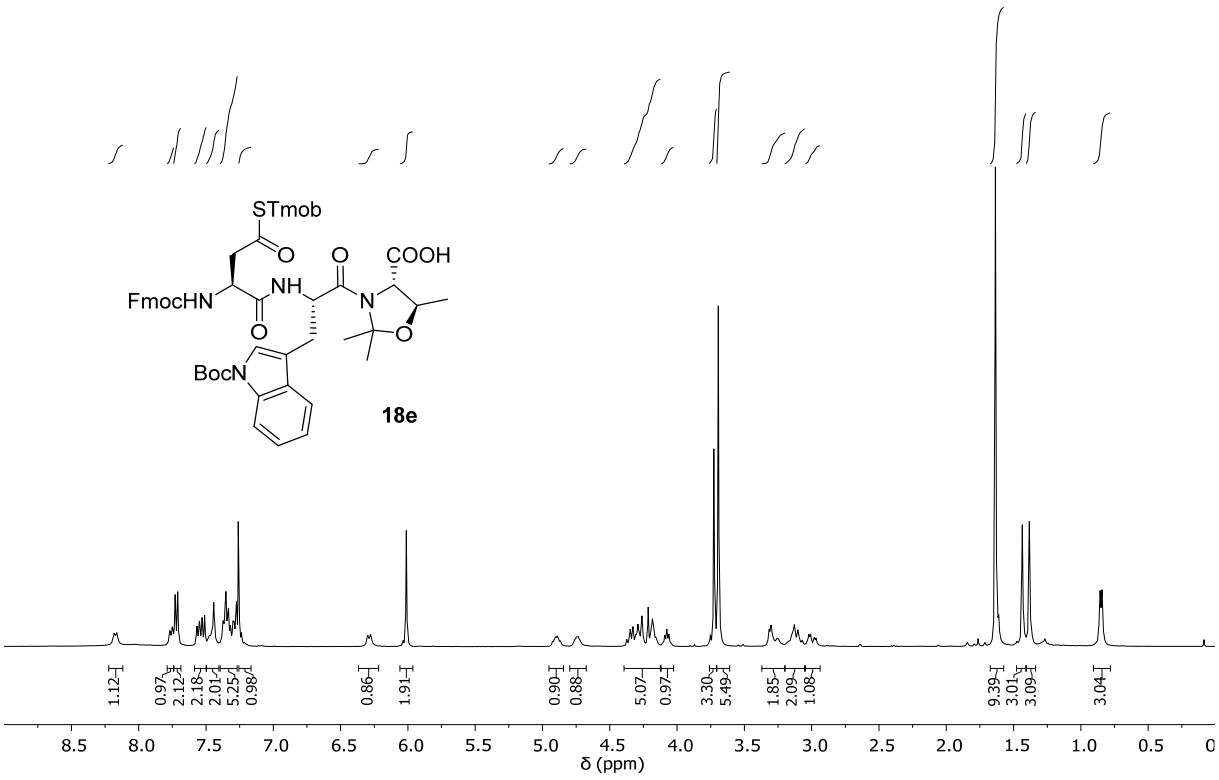
^{1}H NMR, 400 MHz, $CDCl_3$ **17e**



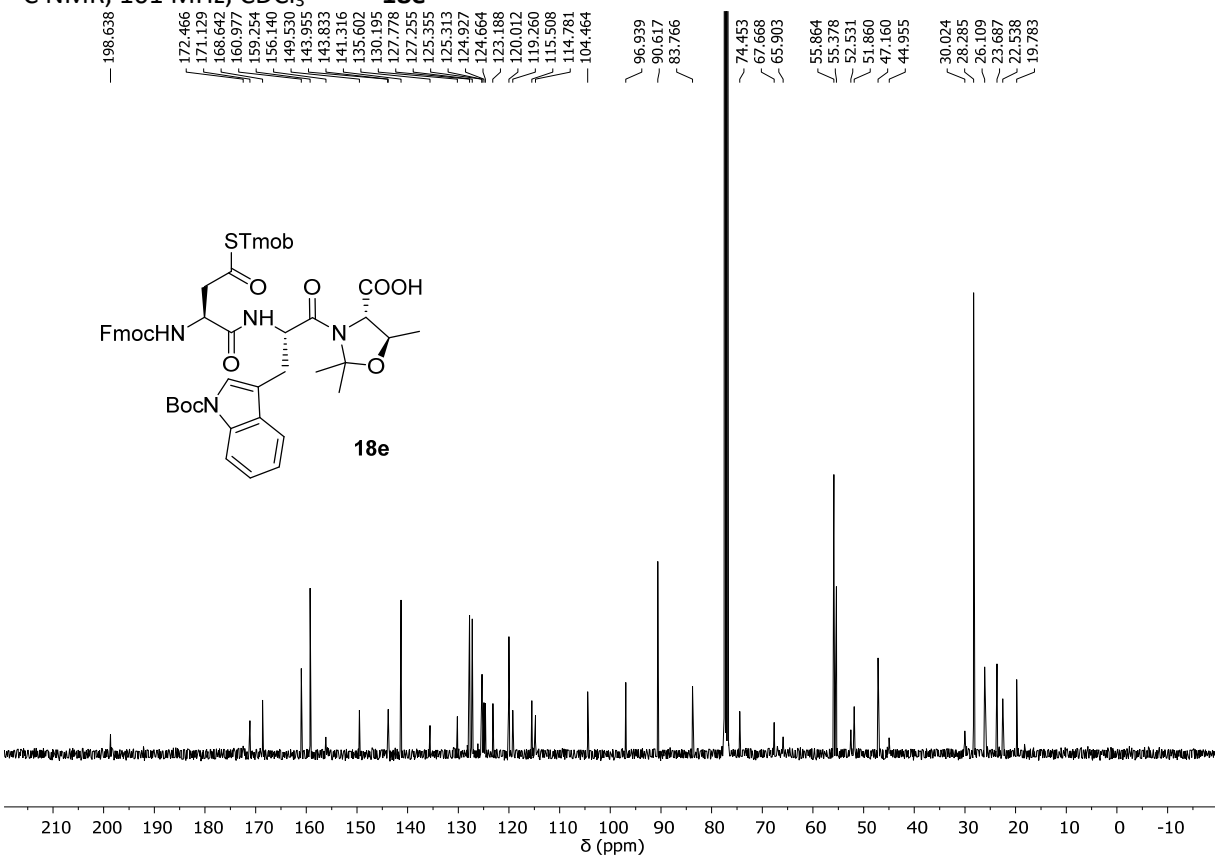
^{13}C NMR, 101 MHz, $CDCl_3$ **17e**



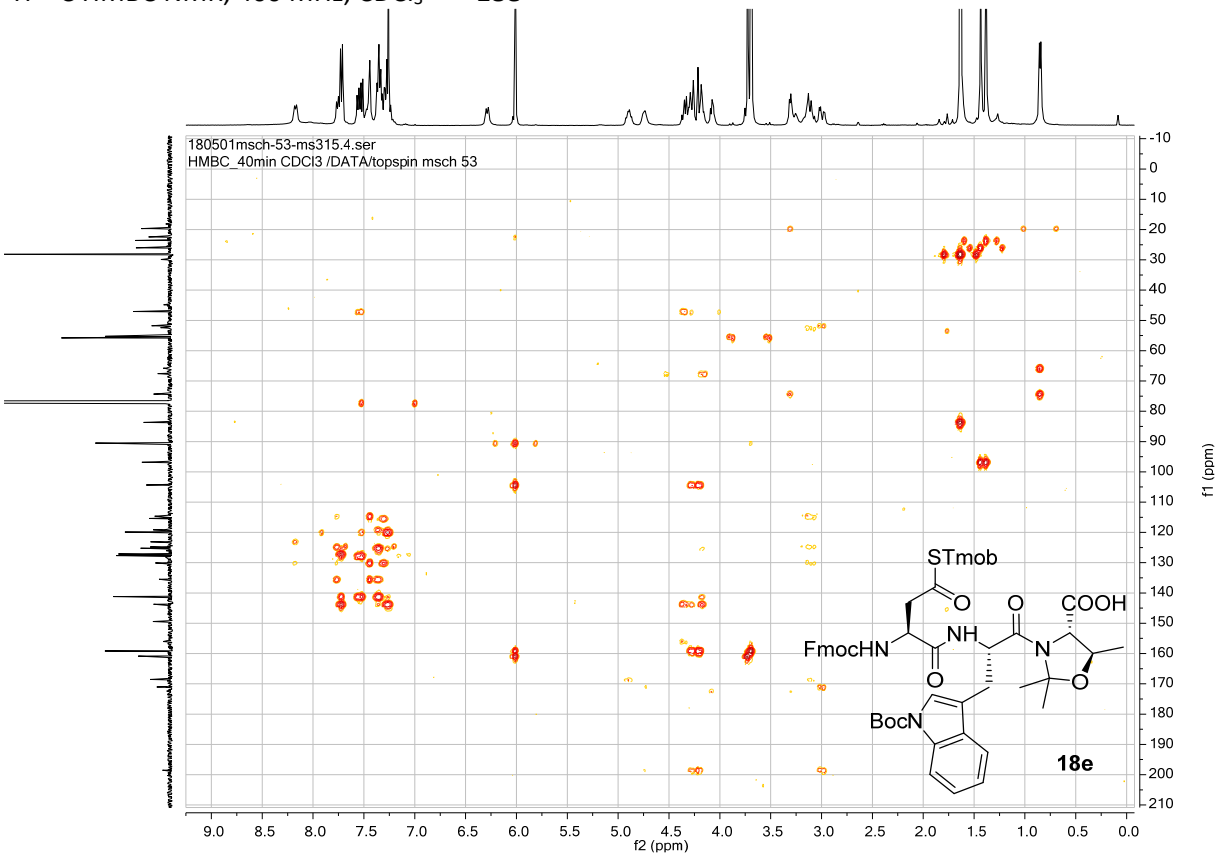
^{1}H NMR, 400 MHz, $CDCl_3$ **18e**



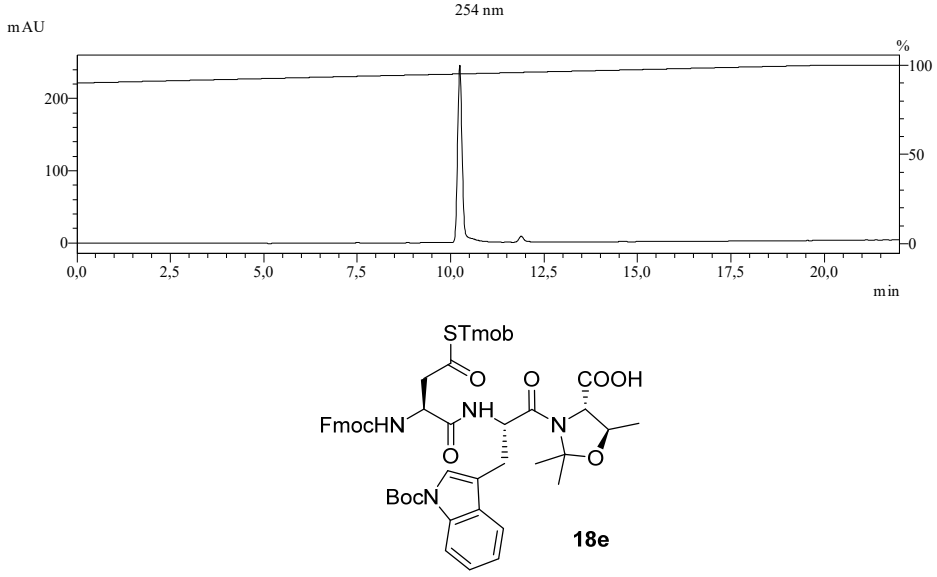
^{13}C NMR, 101 MHz, $CDCl_3$ **18e**



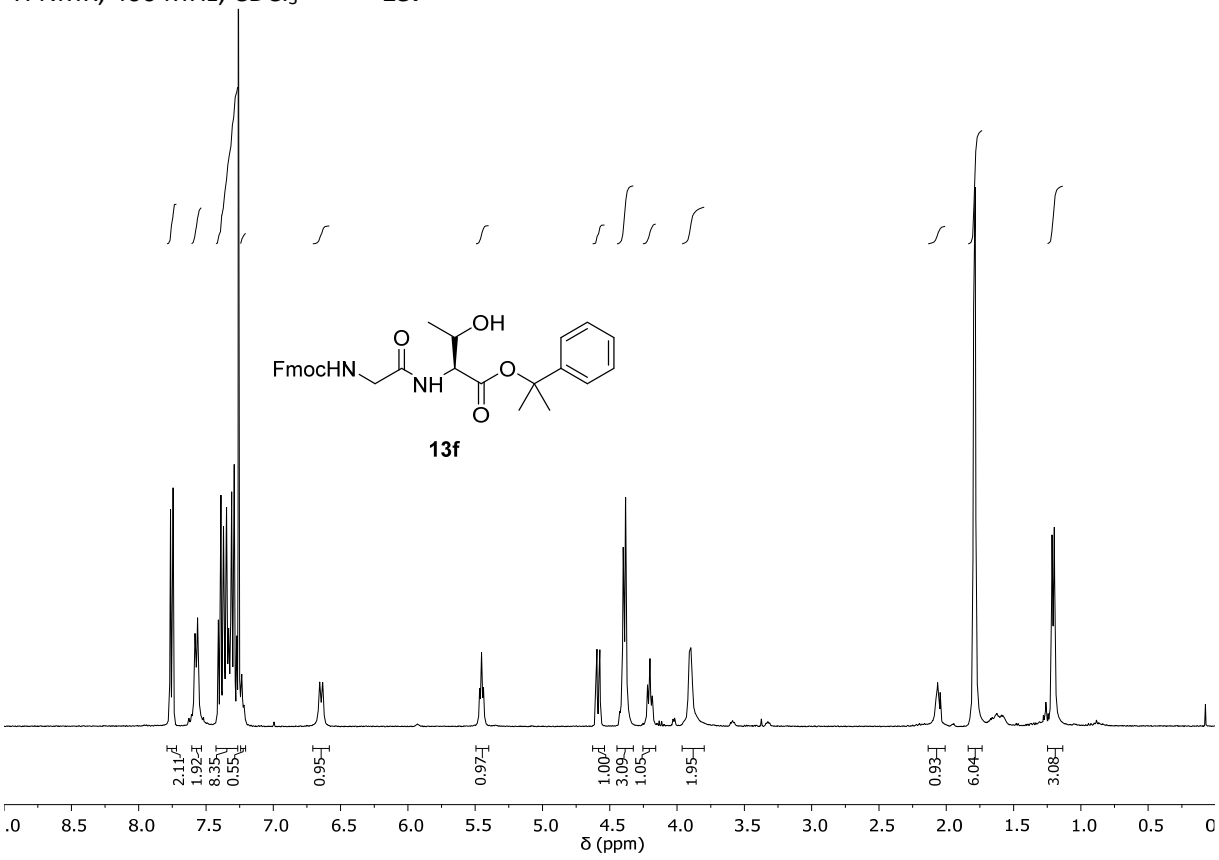
^{1}H-^{13}C HMBC NMR, 400 MHz, $CDCl_3$ **18e**



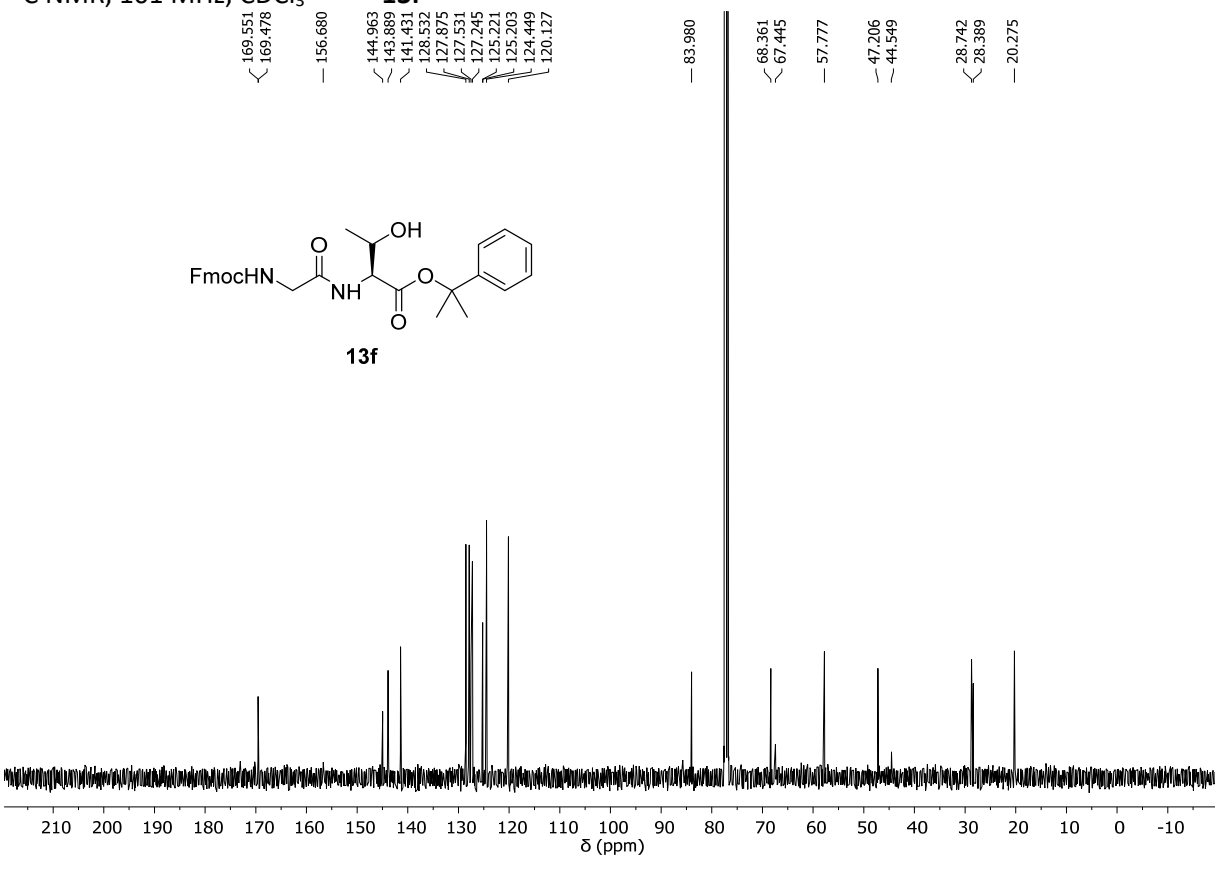
RP-HPLC chromatogram (254 nm) of purified tripeptide **18e** (90–100% in 20 min, R_t = 10.3 min, column 1).



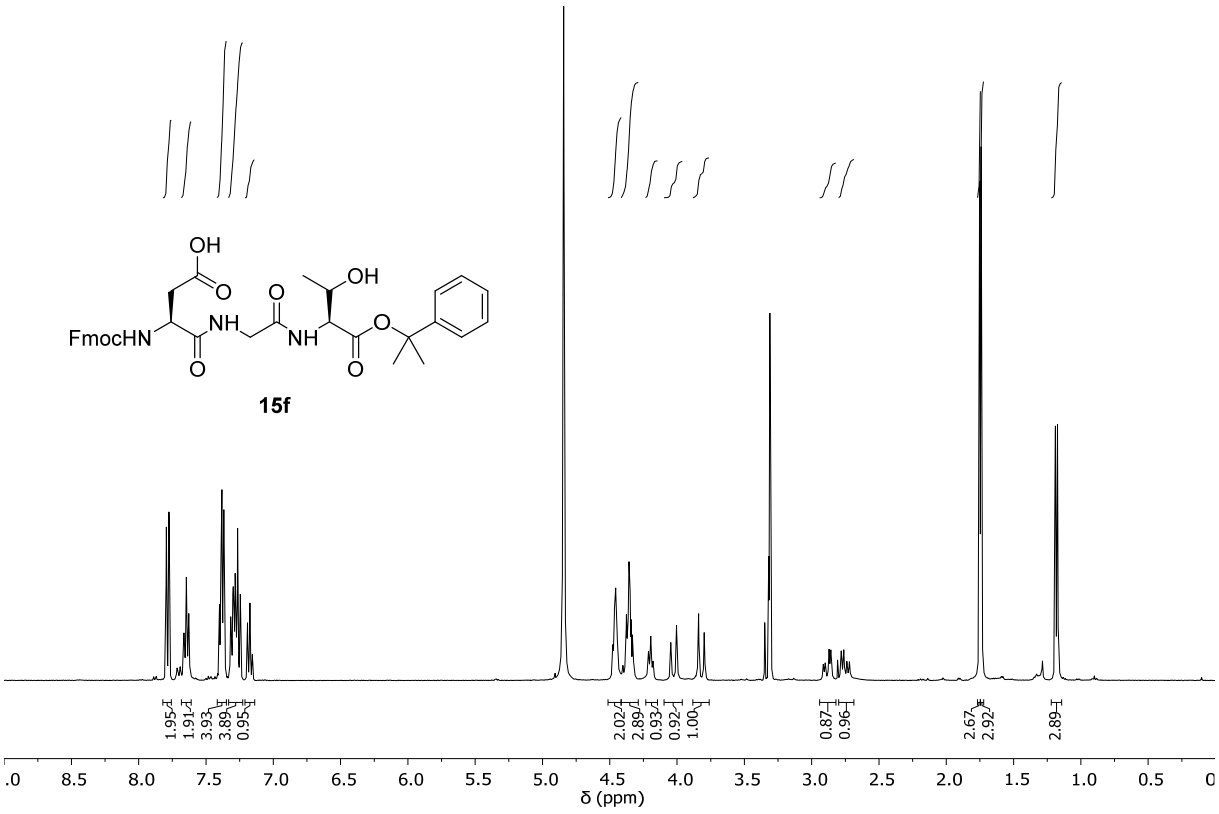
^{1}H NMR, 400 MHz, $CDCl_3$ **13f**



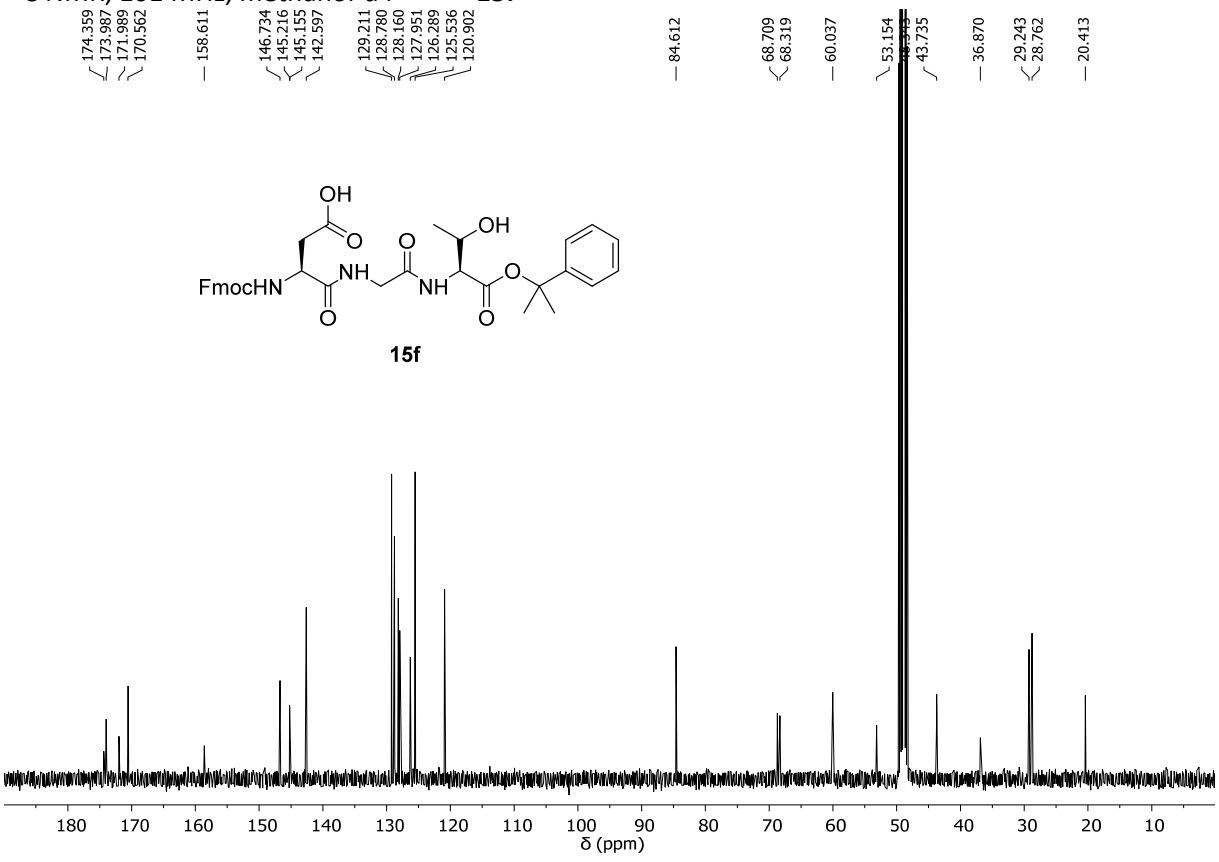
^{13}C NMR, 101 MHz, $CDCl_3$ **13f**



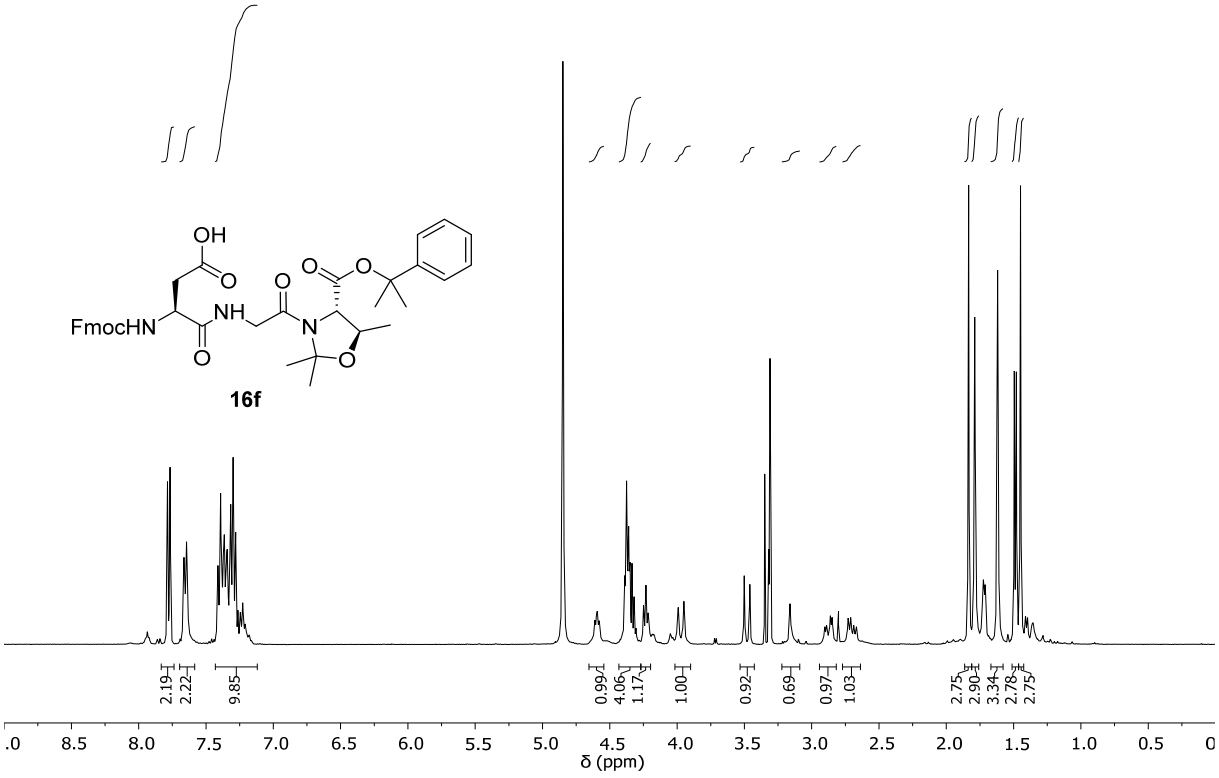
^{1}H NMR, 400 MHz, methanol-d4 **15f**



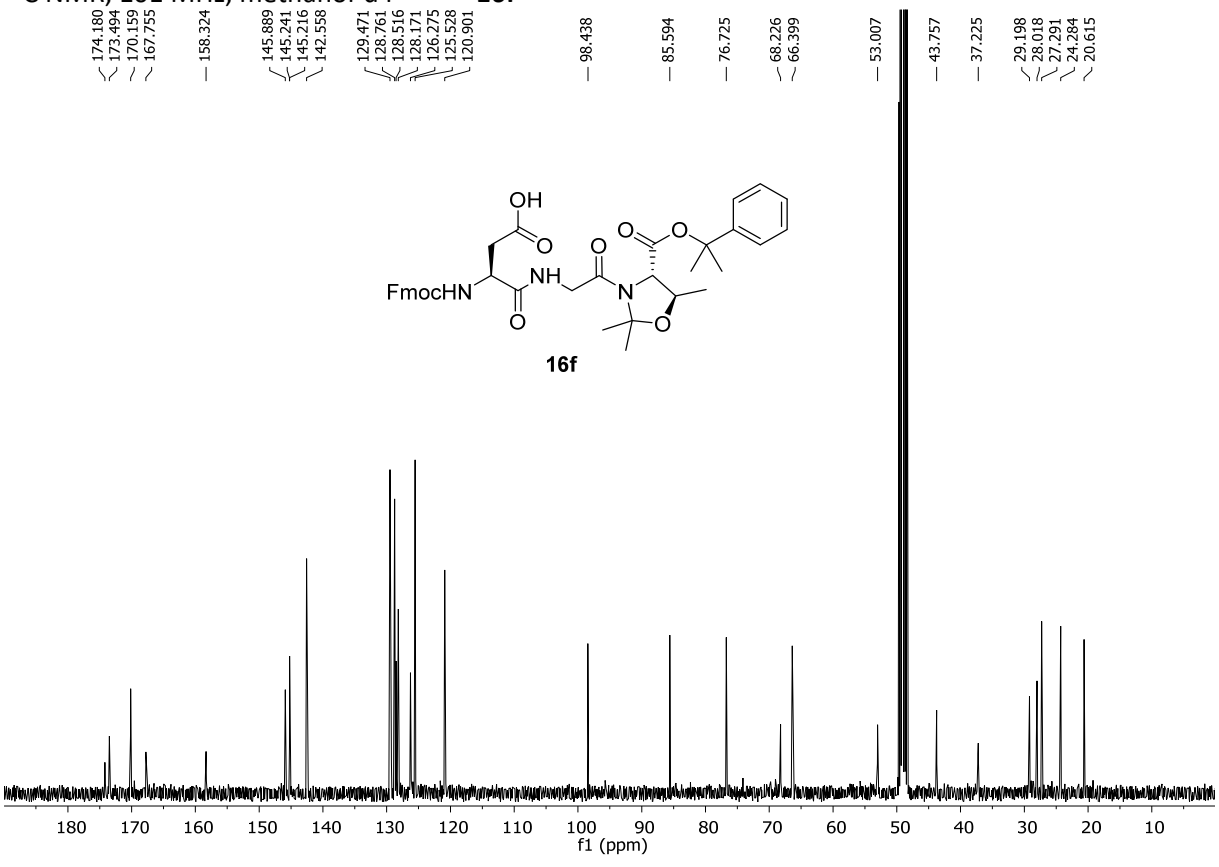
^{13}C NMR, 101 MHz, methanol-d4 **15f**



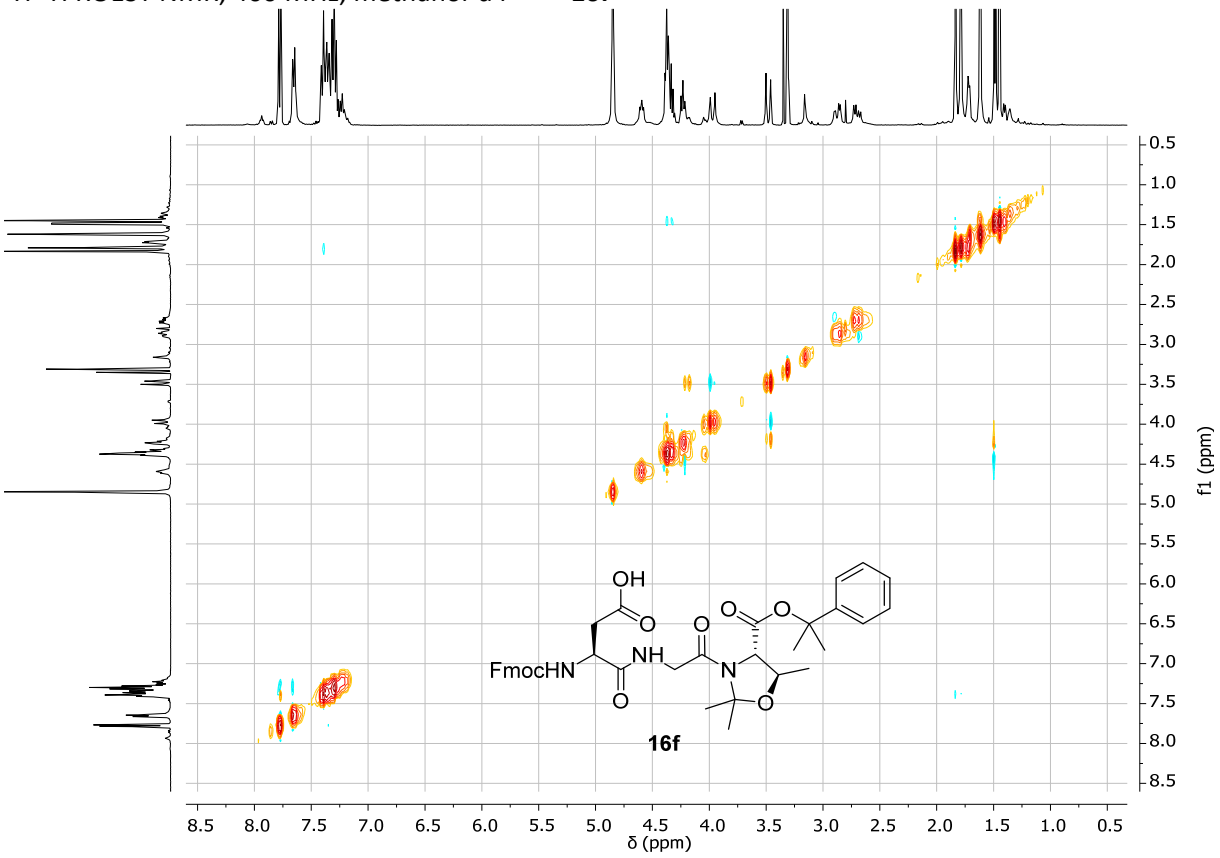
^{1}H NMR, 400 MHz, methanol-d4 **16f**



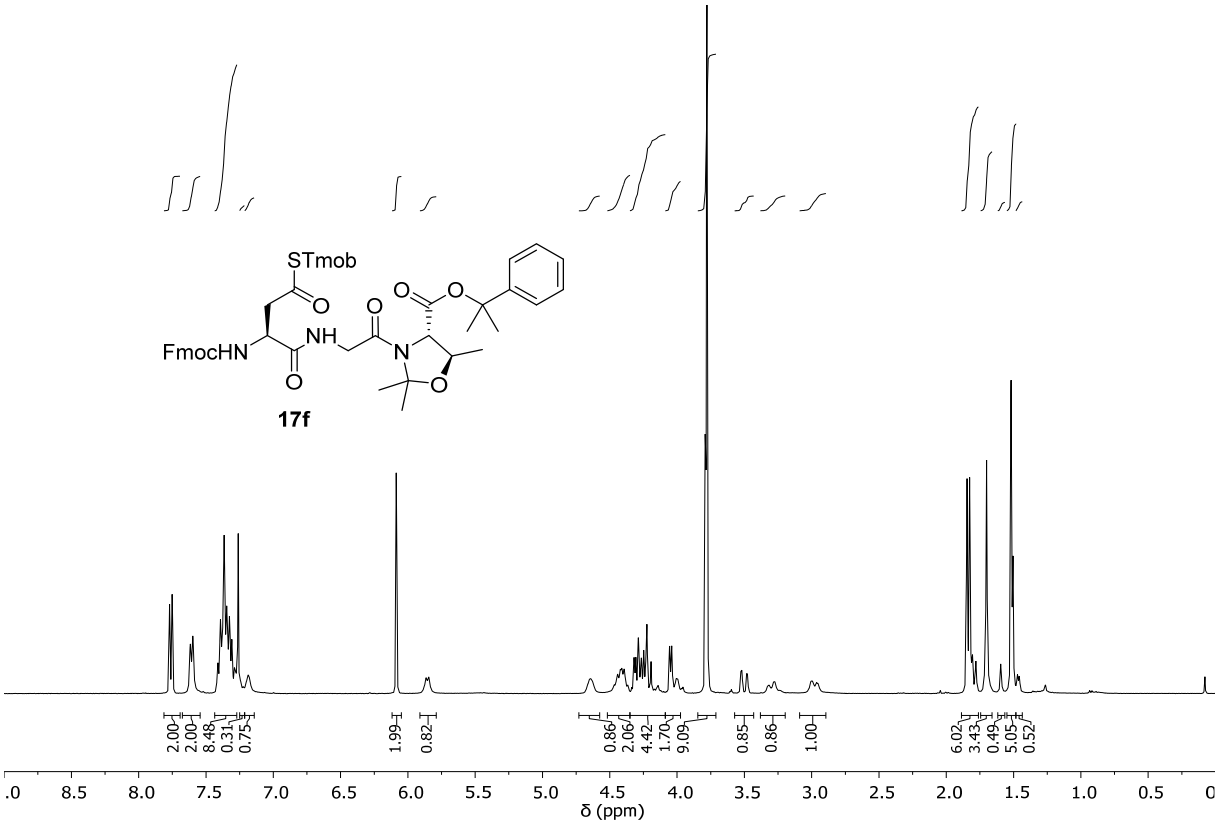
^{13}C NMR, 101 MHz, methanol-d4 **16f**



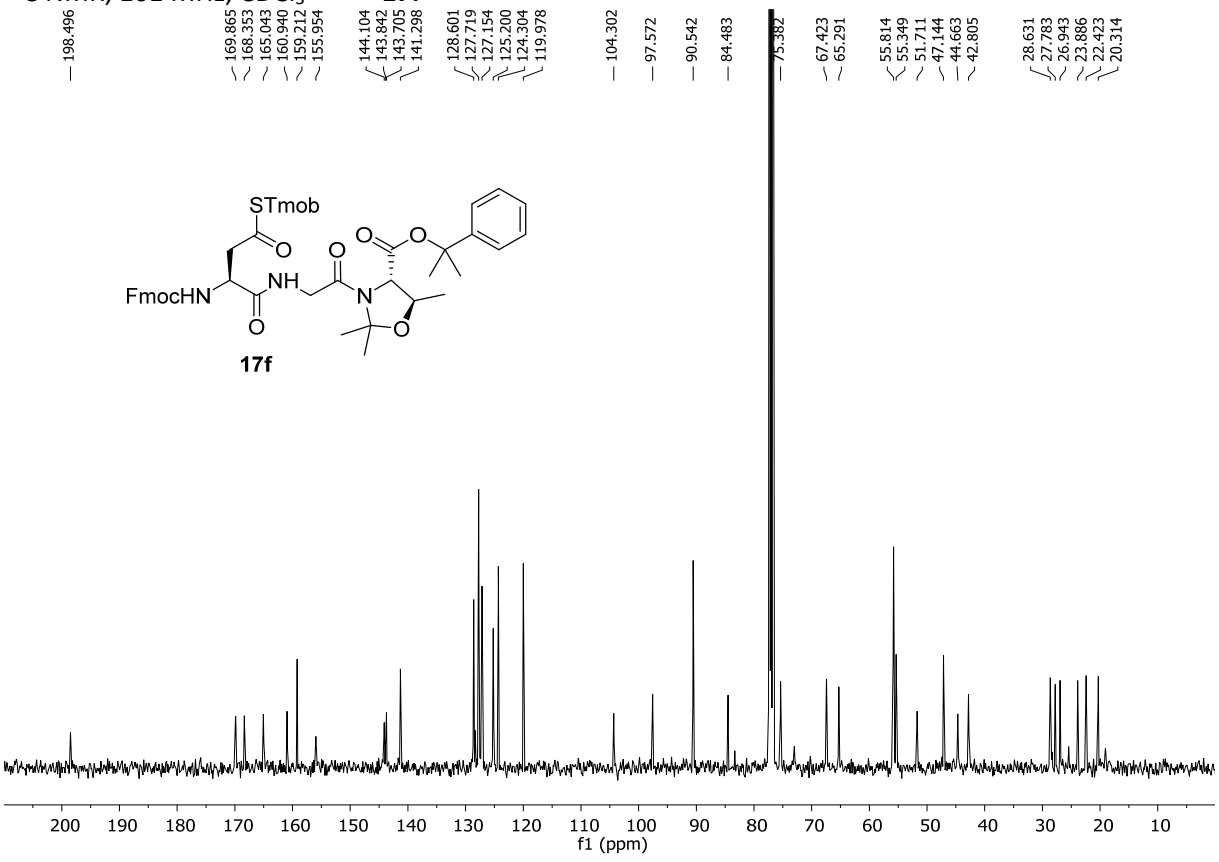
^{1}H-^{1}H ROESY NMR, 400 MHz, methanol-d4 **16f**



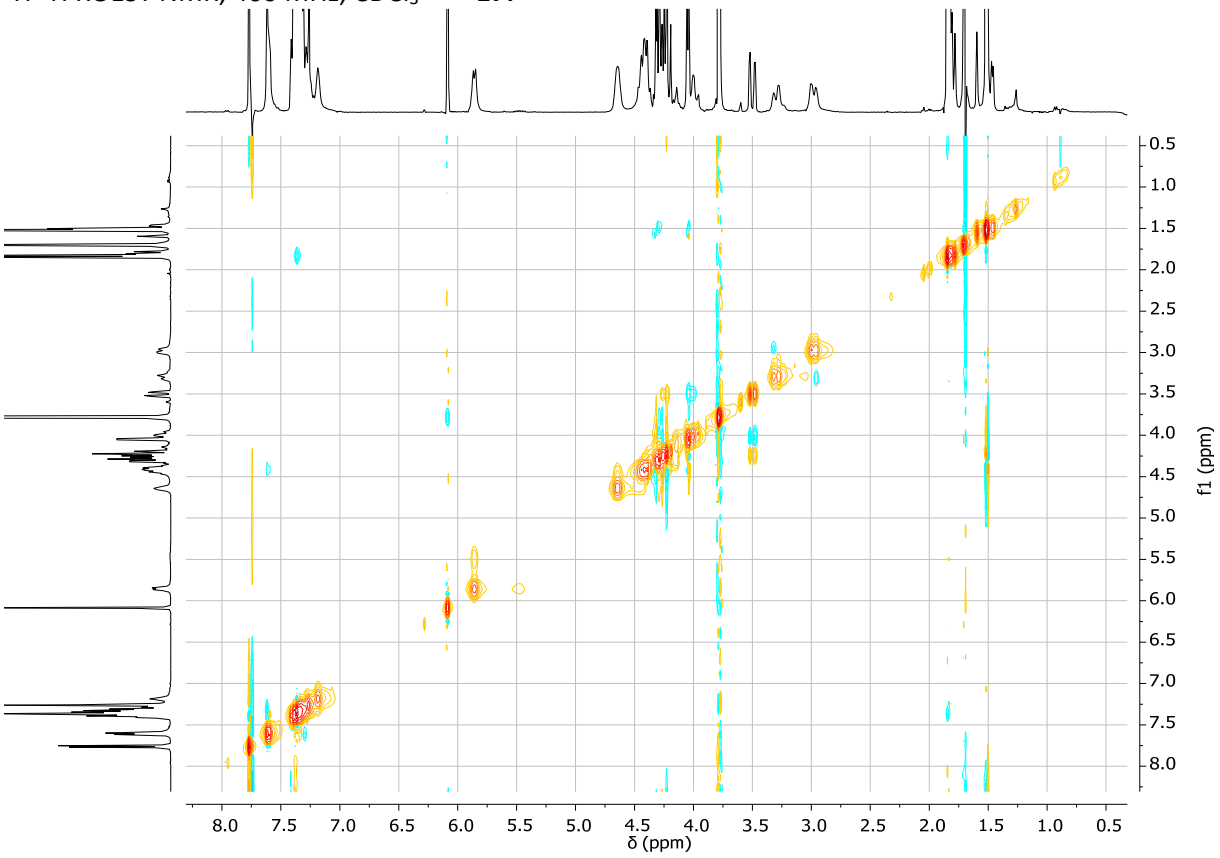
^{1}H NMR, 400 MHz, $CDCl_3$ **17f**

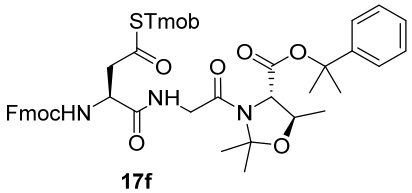


^{13}C NMR, 101 MHz, $CDCl_3$ **17f**



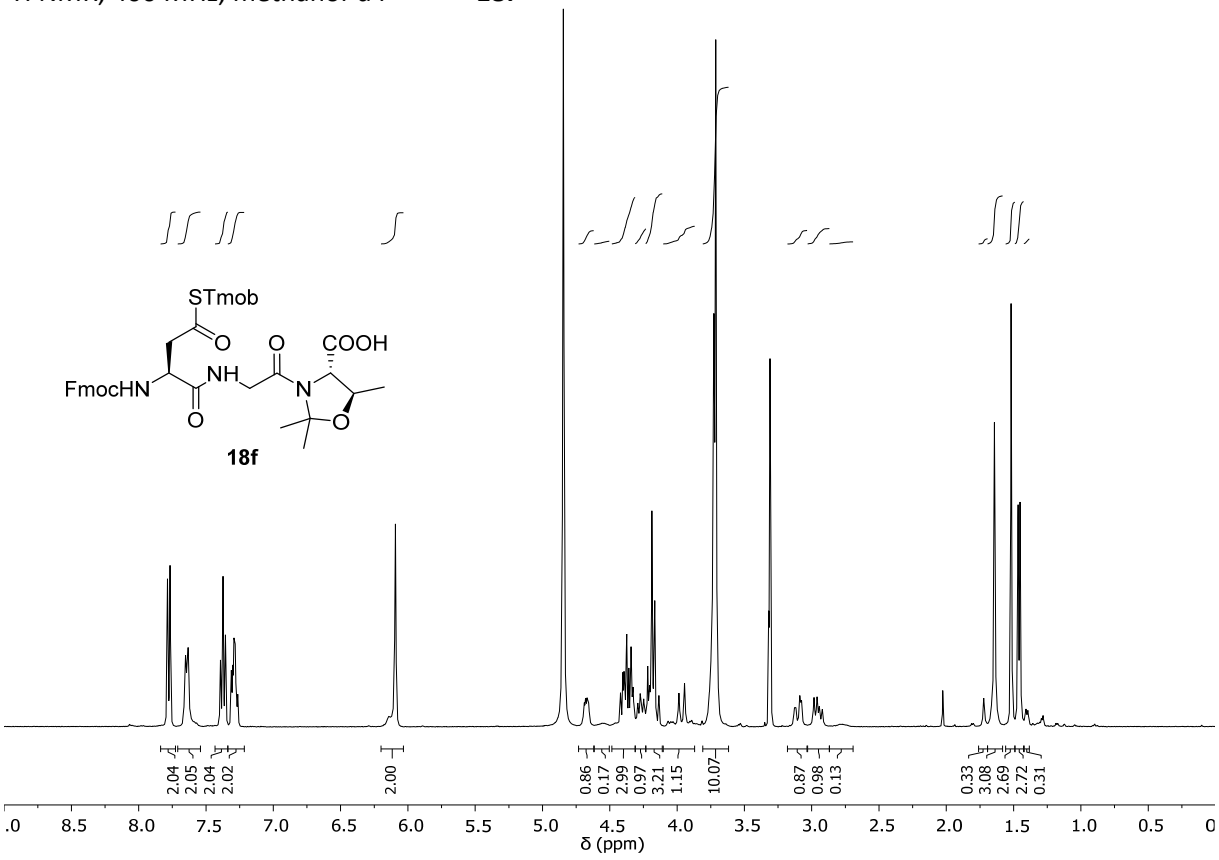
^{1}H-^{1}H ROESY NMR, 400 MHz, $CDCl_3$ **17f**



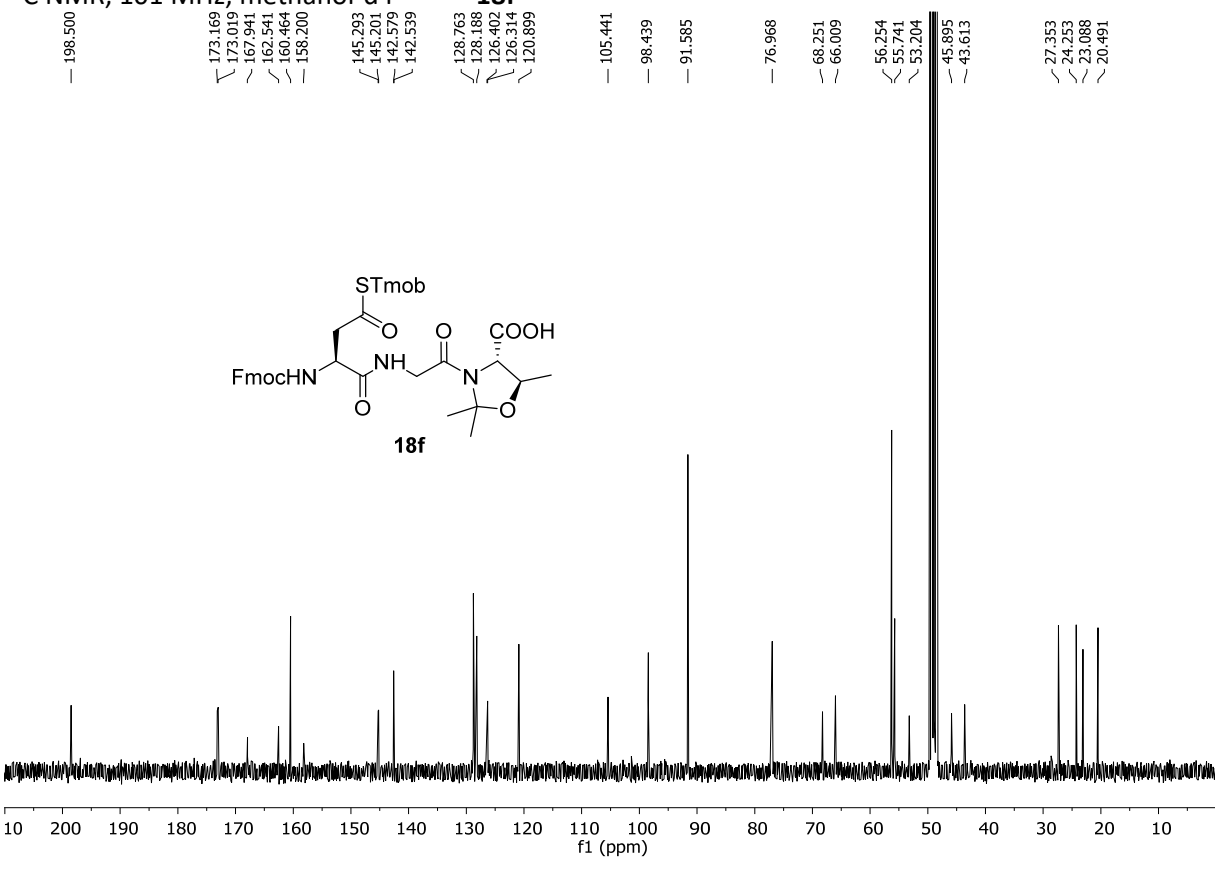


17f

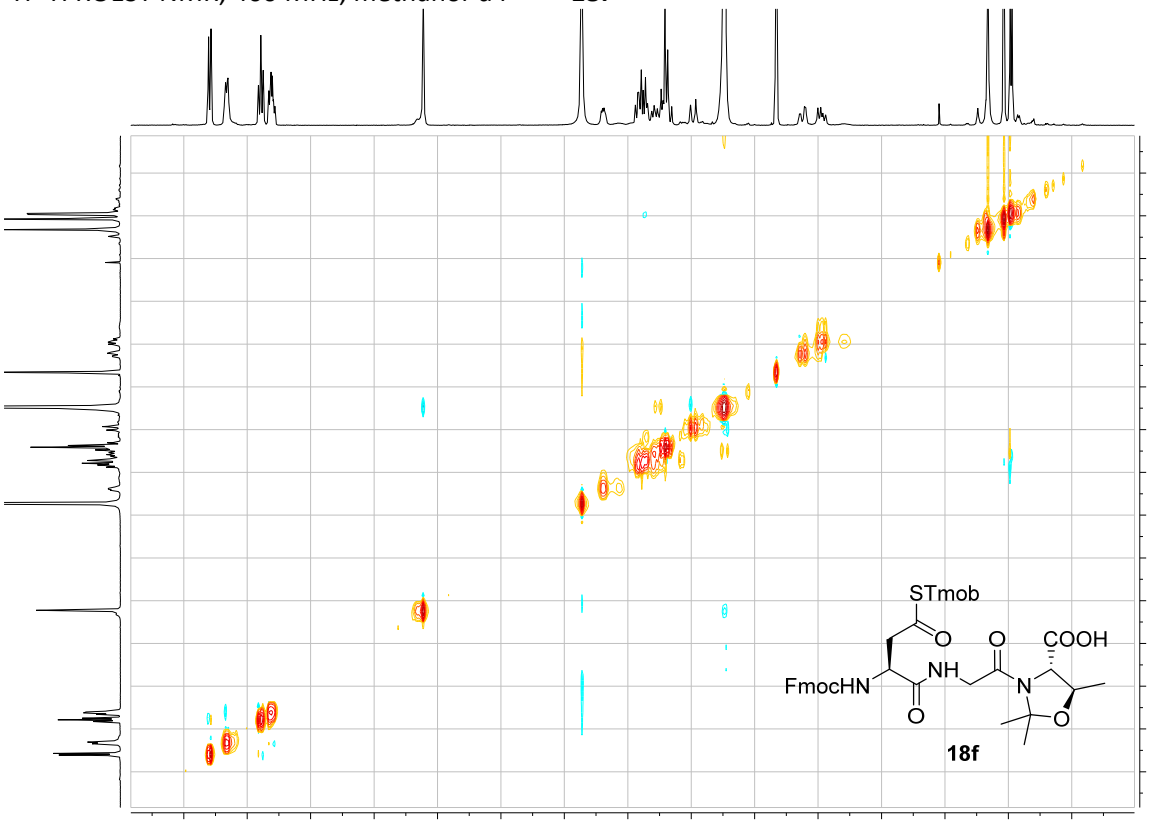
^{1}H NMR, 400 MHz, methanol-d4 **18f**



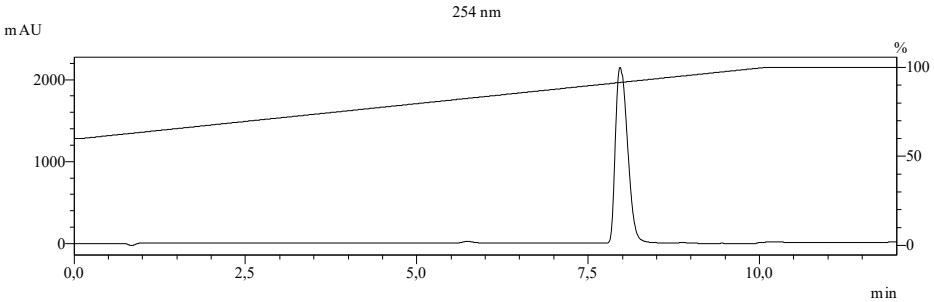
^{13}C NMR, 101 MHz, methanol-d4 **18f**

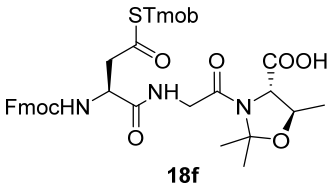


^{1}H-^{1}H ROESY NMR, 400 MHz, methanol-d4 **18f**



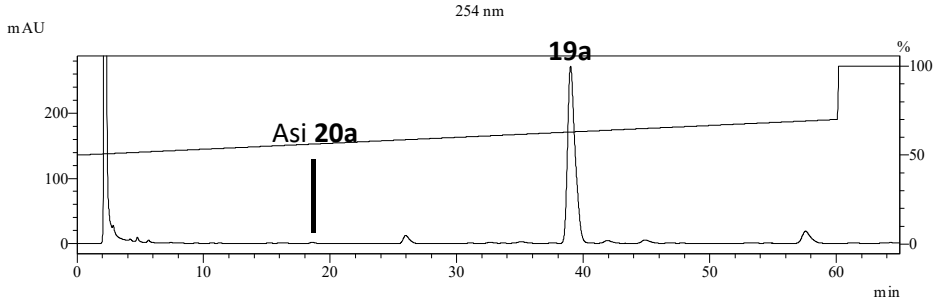
LC-MS chromatogram (254 nm) of purified tripeptide **18f** (60–100% in 10 min, R_t = 8.0 min, column 2).



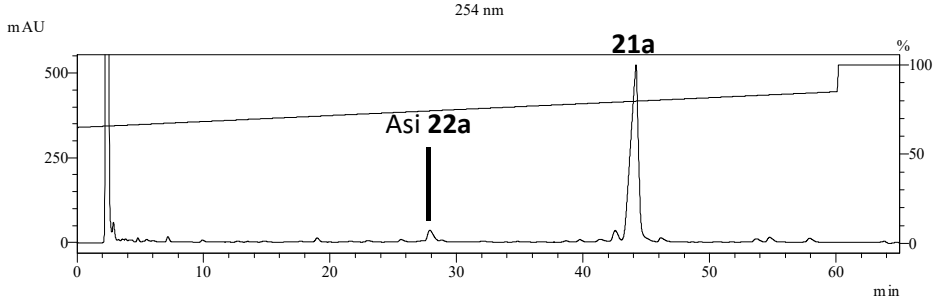


9.3 Penta- and Decapeptides

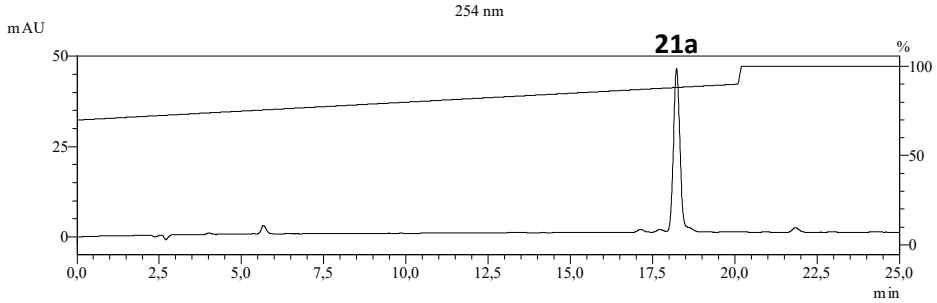
LC-MS chromatogram (254 nm) of Ala-pentapeptide **19a** after analytical resin cleavage (50–70% in 60 min, R_t = 38.9 min, column 2).



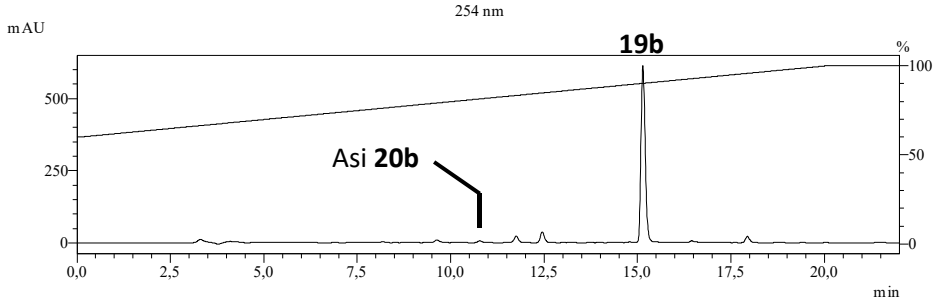
LC-MS chromatogram (254 nm) of Ala-decapeptide **21a** after analytical resin cleavage (65–85% in 60 min, R_t = 44.2 min, column 2).



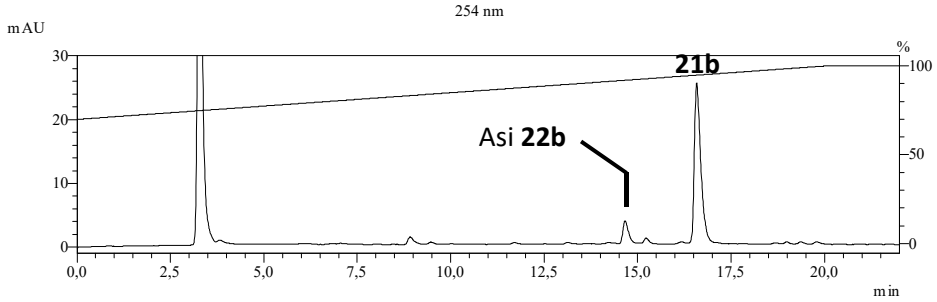
LC-MS chromatogram (254 nm) of purified Ala-decapeptide **21a** (70–90% in 20 min, R_t = 18.2 min, column 2).



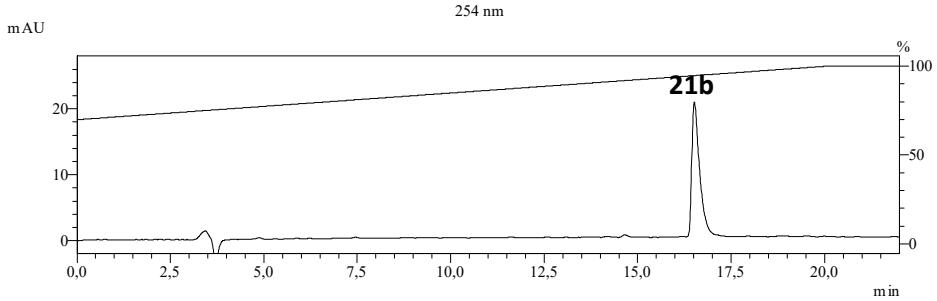
LC-MS chromatogram (254 nm) of Asp-pentapeptide **19b** after analytical resin cleavage (60–100% in 20 min, R_t = 15.1 min, column 3).



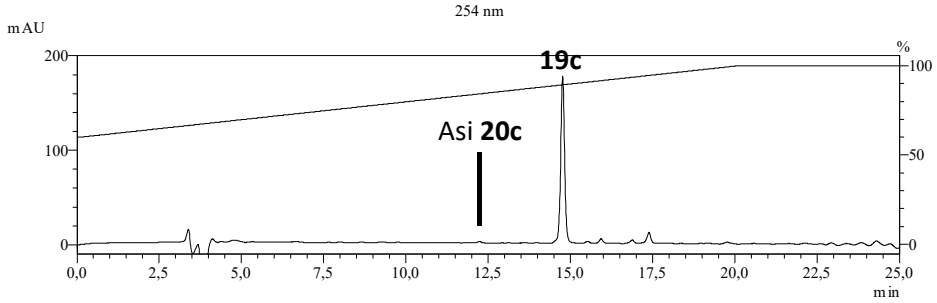
LC-MS chromatogram (254 nm) of Asp-decapeptide **21b** after analytical resin cleavage (70–100% in 20 min, R_t = 16.6 min, column 3).



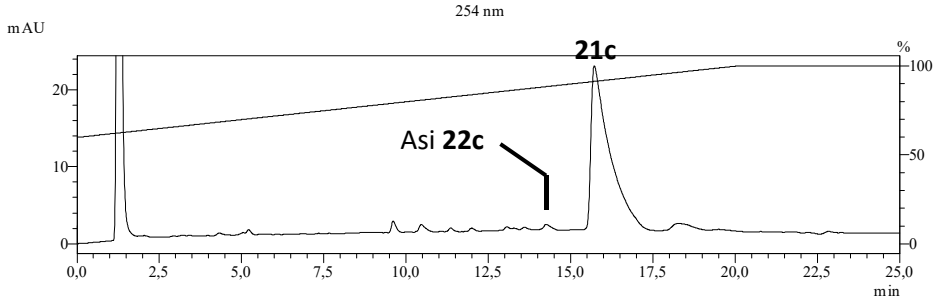
LC-MS chromatogram (254 nm) of purified Asp-decapeptide **21b** (70–100% in 20 min, R_t = 16.5 min, column 3).



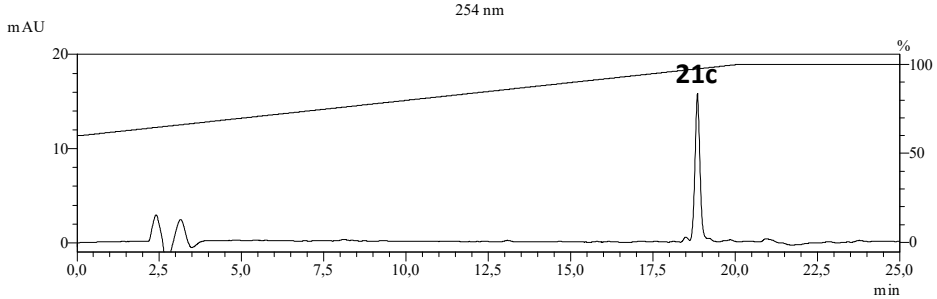
LC-MS chromatogram (254 nm) of Lys-pentapeptide **19c** after analytical resin cleavage (60–100% in 20 min, R_t = 14.8 min, column 2).



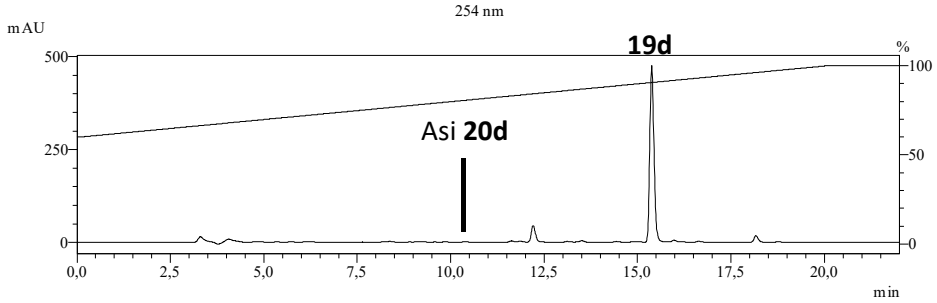
LC-MS chromatogram (254 nm) of Lys-decapeptide **21c** after analytical resin cleavage (60–100% in 20 min, R_t = 15.7 min, column 2).



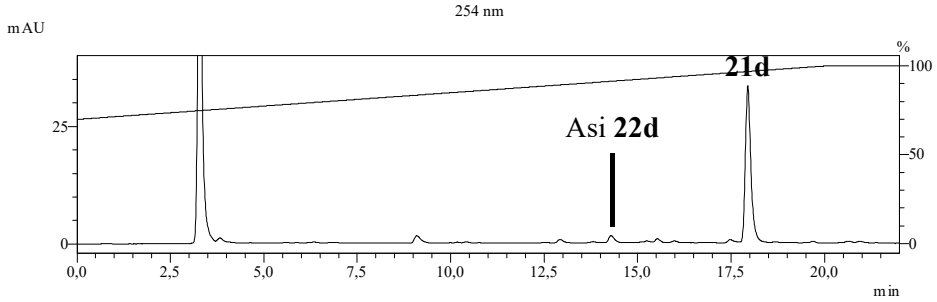
LC-MS chromatogram (254 nm) of purified Lys-decapeptide **21c** (60–100% in 20 min, R_t = 18.9 min, column 2).



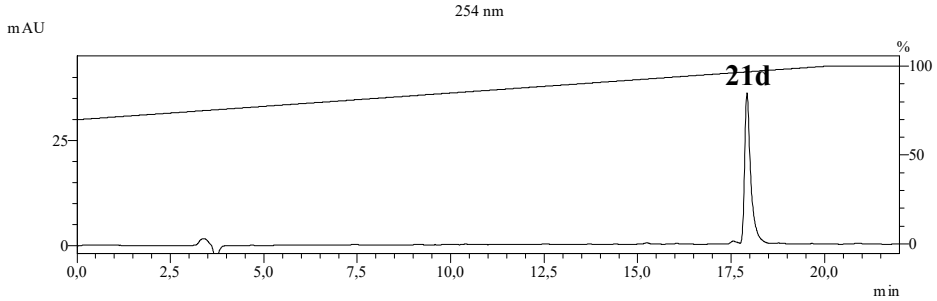
LC-MS chromatogram (254 nm) of Ser-pentapeptide **19d** after analytical resin cleavage (60–100% in 20 min, R_t = 15.4 min, column 3).



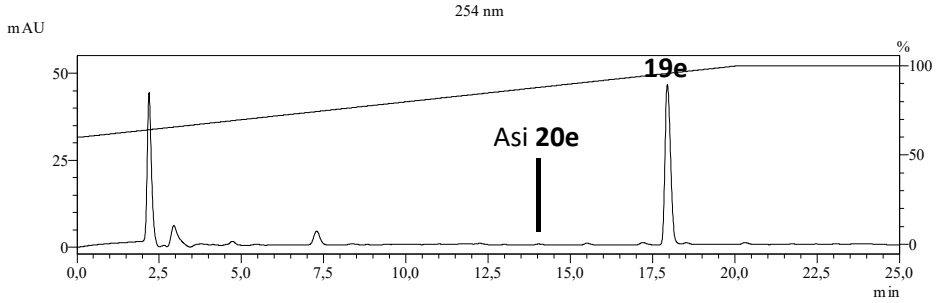
LC-MS chromatogram (254 nm) of Ser-decapeptide **21d** after analytical resin cleavage (70–100% in 20 min, R_t = 18.0 min, column 3).



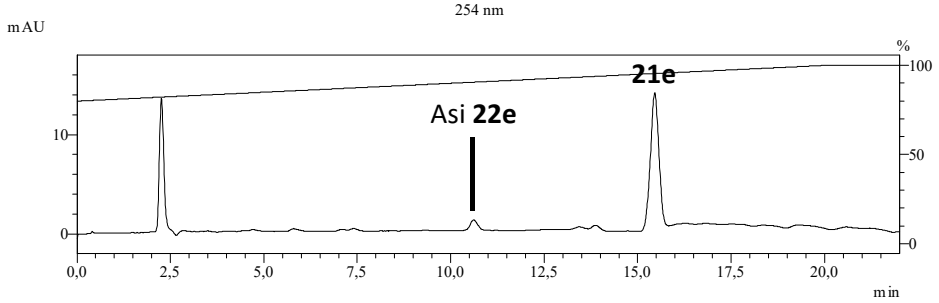
LC-MS chromatogram (254 nm) of purified Ser-decapeptide **21d** (70–100% in 20 min, R_t = 17.9 min, column 3).



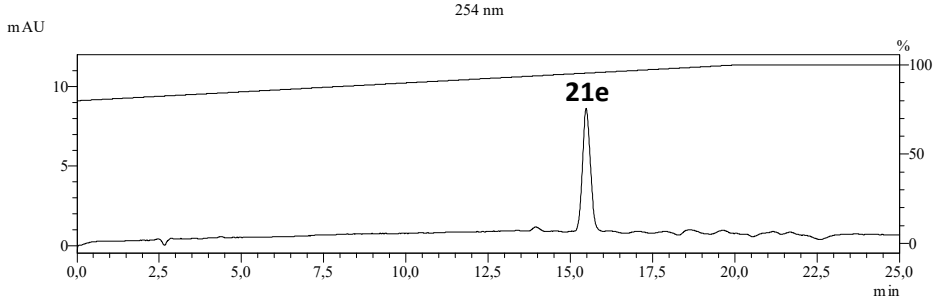
LC-MS chromatogram (254 nm) of Trp-pentapeptide **19e** after analytical resin cleavage (60–100% in 20 min, R_t = 18.0 min, column 2).



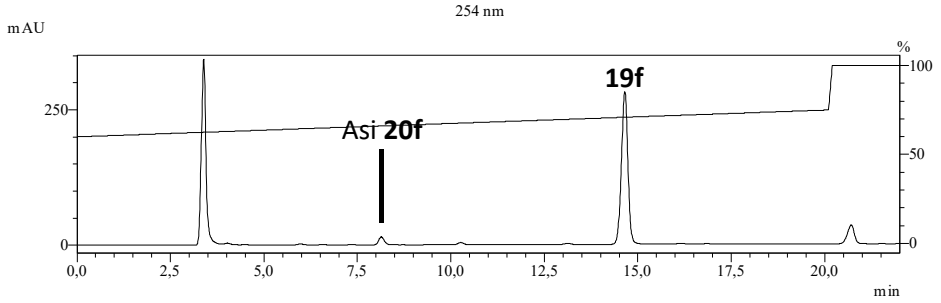
LC-MS chromatogram (254 nm) of Trp-decapeptide **21e** after analytical resin cleavage (80–100% in 20 min, R_t = 15.5 min, column 2).



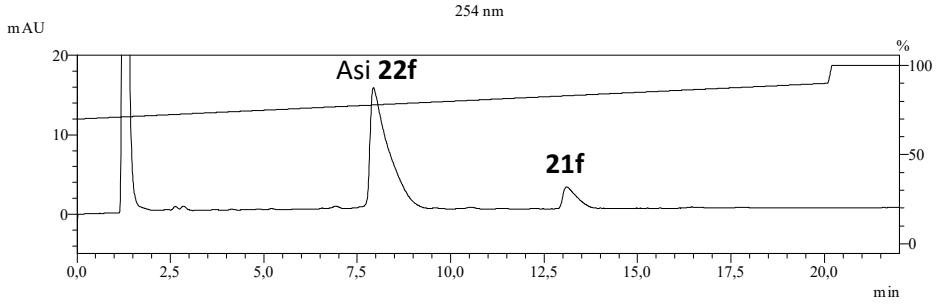
LC-MS chromatogram (254 nm) of purified Trp-decapeptide **21e** (80–100% in 20 min, R_t = 15.5 min, column 2).



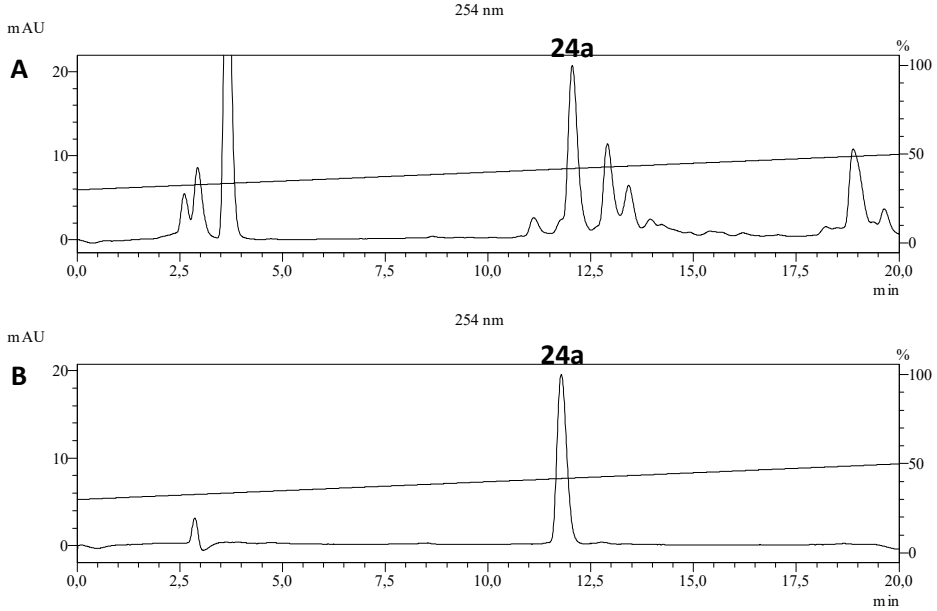
LC-MS chromatogram (254 nm) of Gly-pentapeptide **19f** after analytical resin cleavage (60–75% in 20 min, R_t = 14.7 min, column 2).



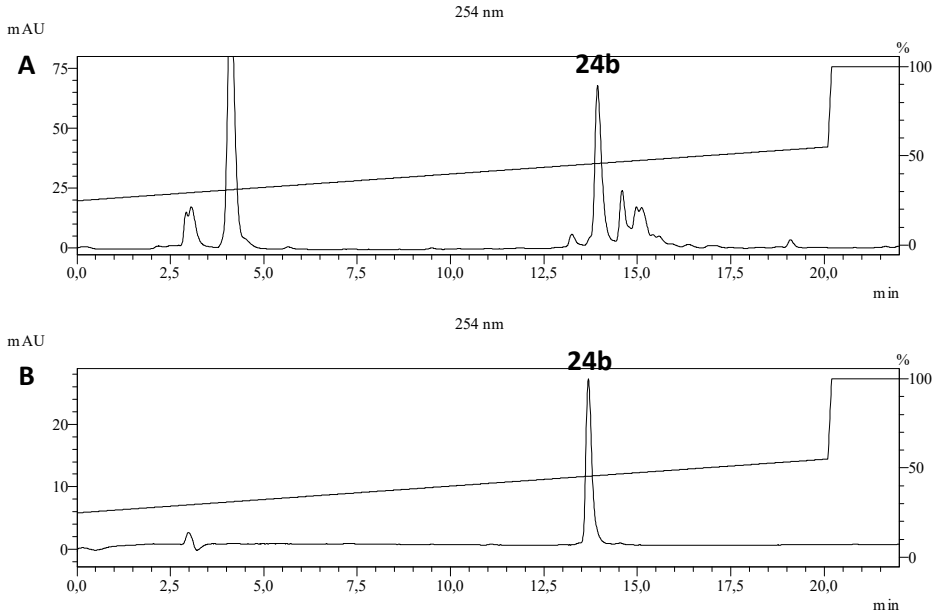
LC-MS chromatogram (254 nm) of Gly-decapeptide **21f** after analytical resin cleavage (70–90% in 20 min, R_t = 13.1 min, column 2).



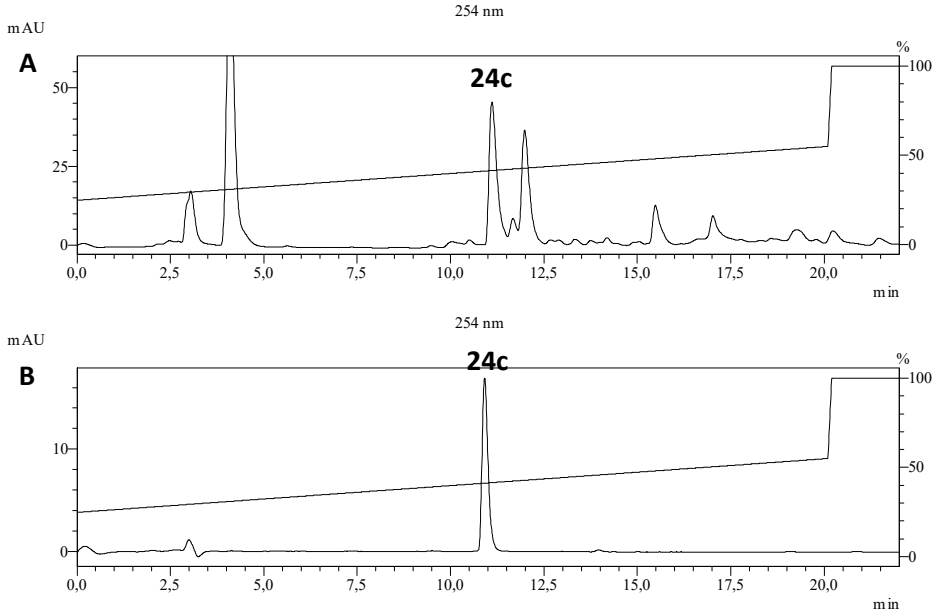
9.4 N-Glycopeptides



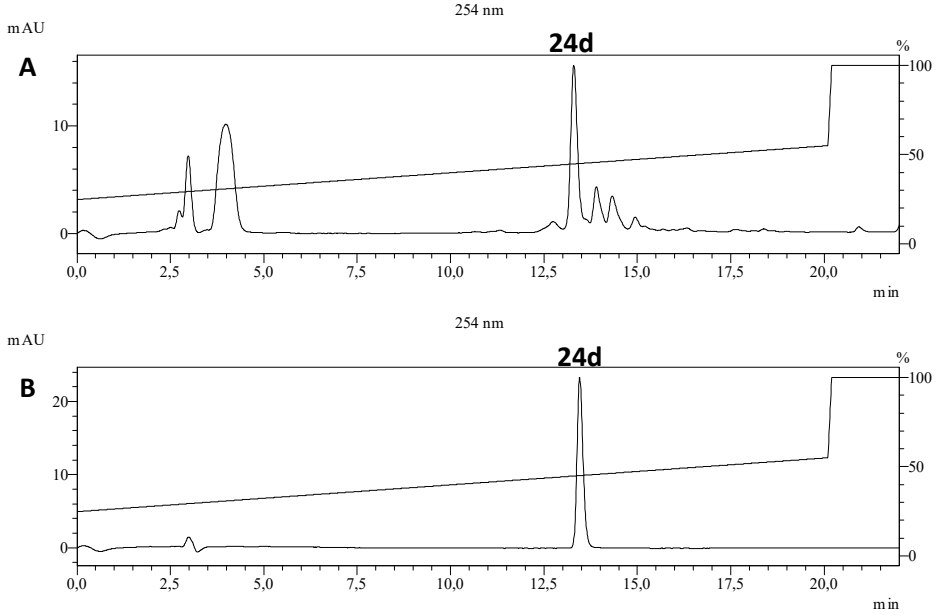
LC-MS chromatogram (254 nm) **A** before and **B** after HPLC purification of Ala-glycopeptide **24a** (30–50% in 20 min, R_t = 11.8 min, column 2).



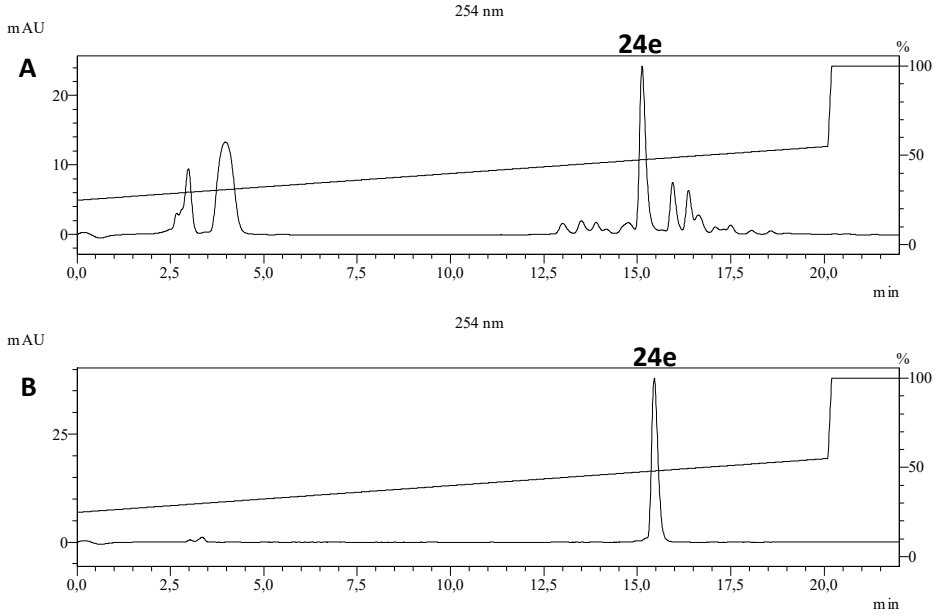
LC-MS chromatogram (254 nm) **A** before and **B** after HPLC purification of Asp-glycopeptide **24b** (25–55% in 20 min, R_t = 13.7 min, column 2).



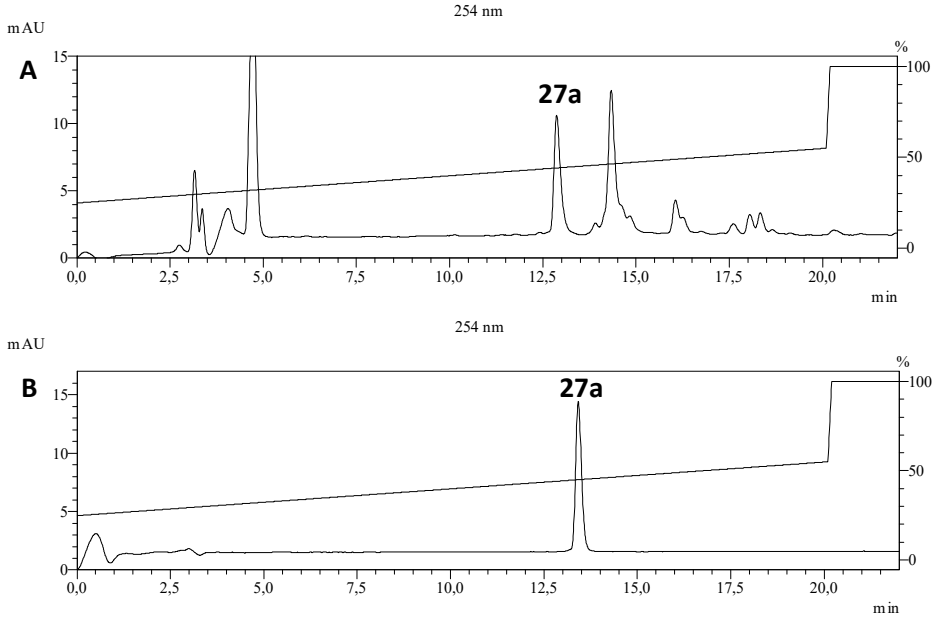
LC-MS chromatogram (254 nm) **A** before and **B** after HPLC purification of Lys-glycopeptide **24c** (25–55% in 20 min, R_t = 10.9 min, column 2).



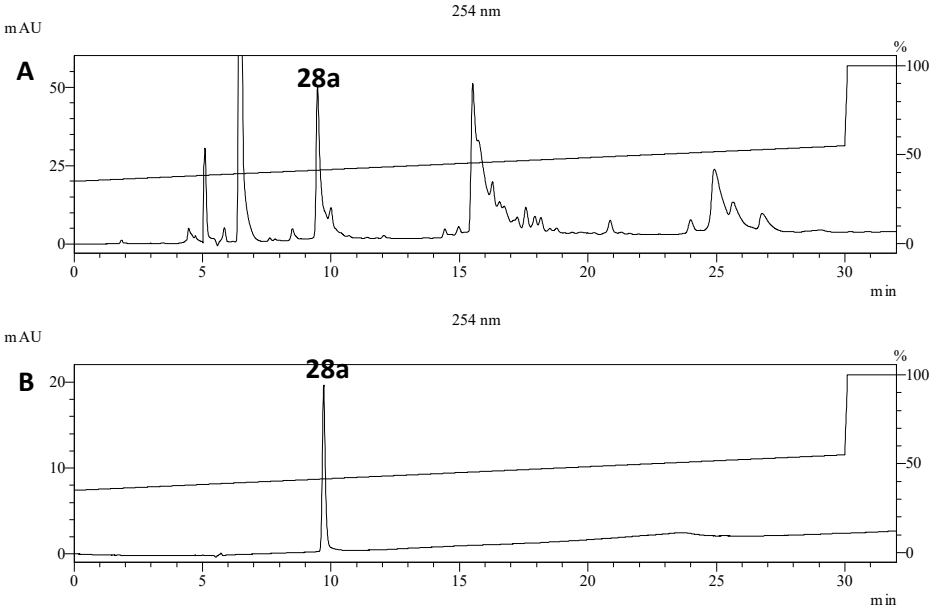
LC-MS chromatogram (254 nm) **A** before and **B** after HPLC purification of Ser-glycopeptide **24d** (25–55% in 20 min, R_t = 13.5 min, column 2).



LC-MS chromatogram (254 nm) **A** before and **B** after HPLC purification of Trp-glycopeptide **24e** (25–55% in 20 min, R_t = 15.5 min, column 2).



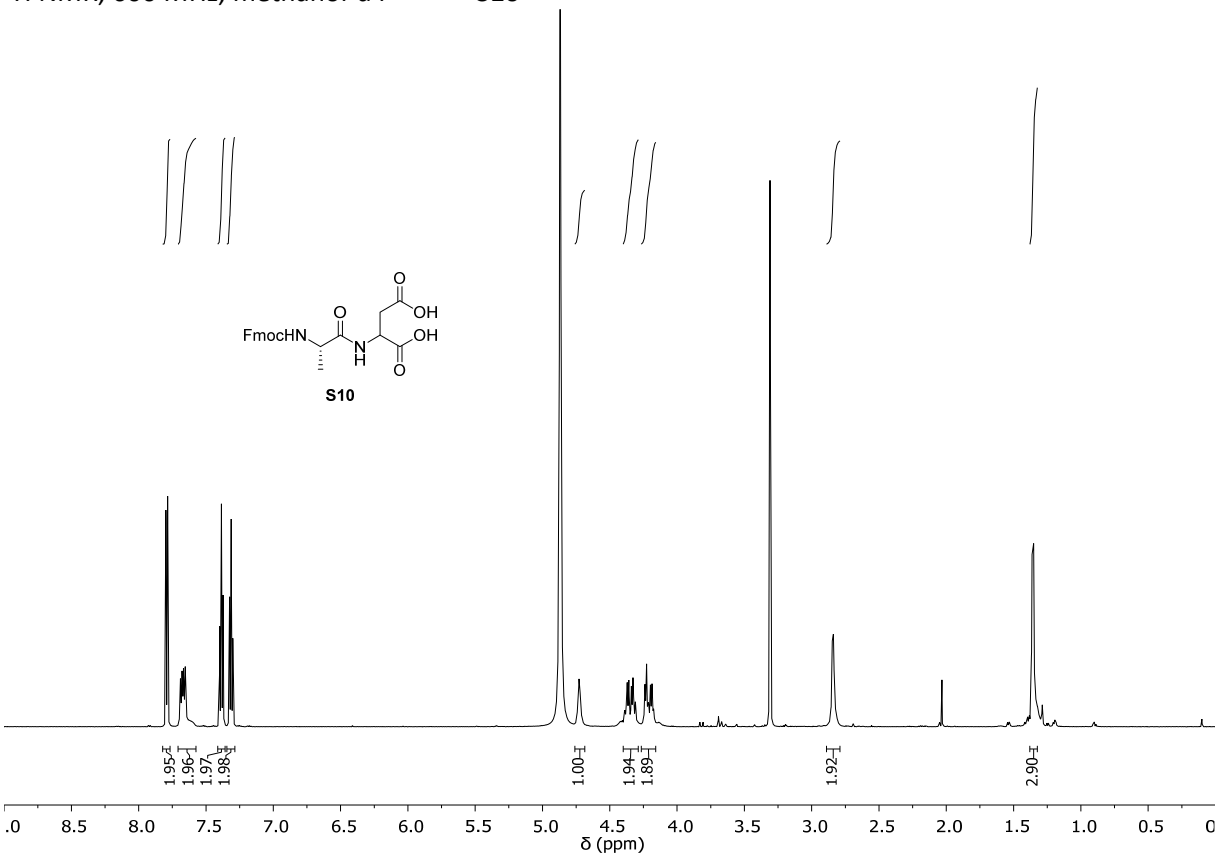
LC-MS chromatogram (254 nm) **A** before and **B** after HPLC purification of Ala-glycopeptide **27a** (25–55% in 20 min, R_t = 13.4 min, column 2).



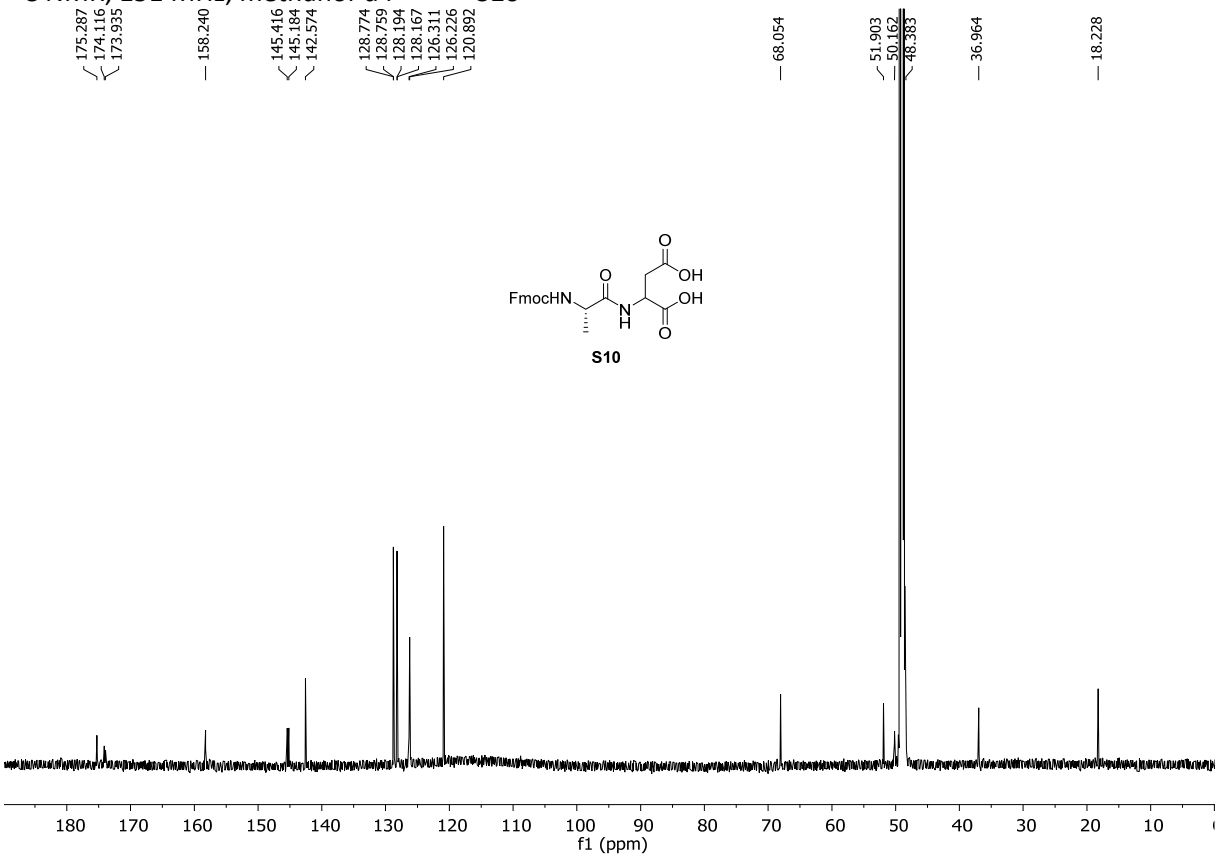
RP-HPLC chromatogram (254 nm) **A** before and **B** after HPLC purification of Ala-glycopeptide **28a** (35–55% in 30 min, R_t = 9.7 min, column 1).

9.5 Peptide Cleavage Using Thioacids (CUT)
9.5.1 Racemization during CUT

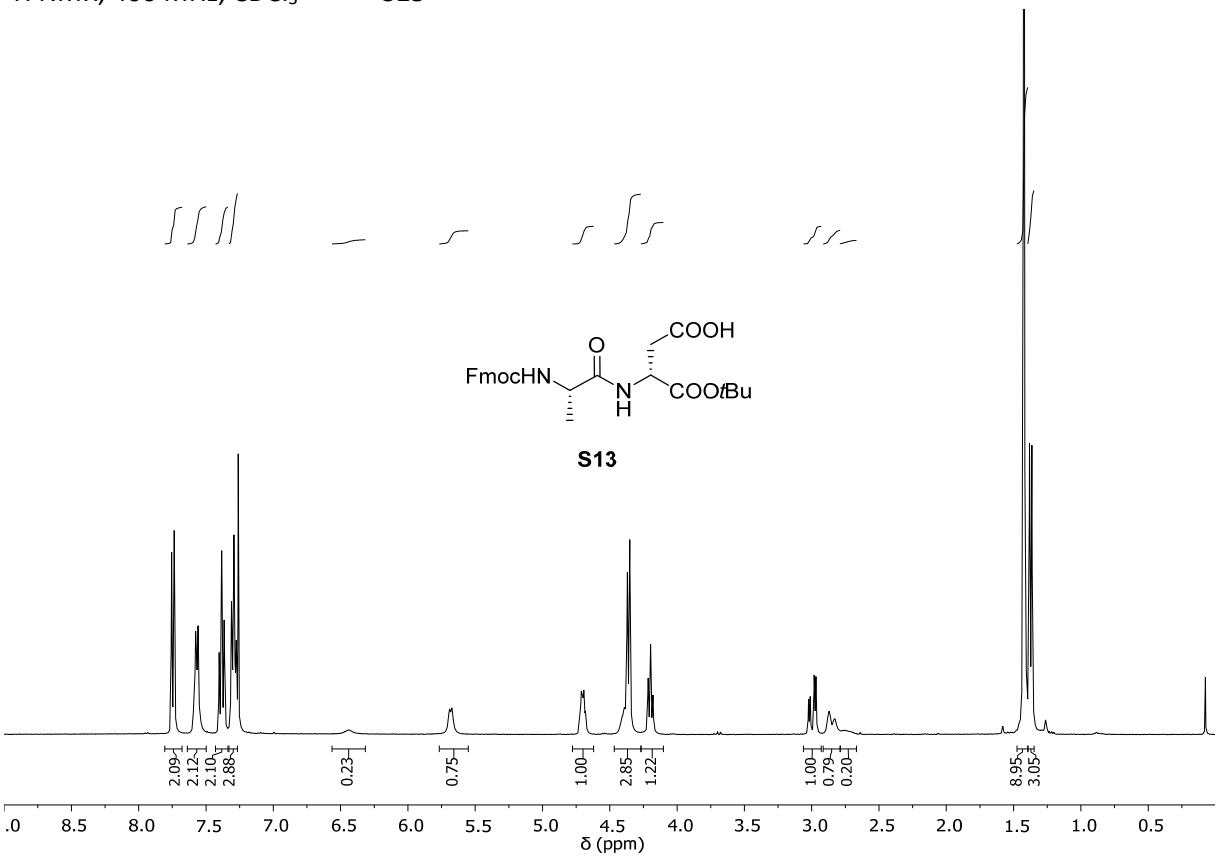
^{1}H NMR, 600 MHz, methanol-d4 **S10**



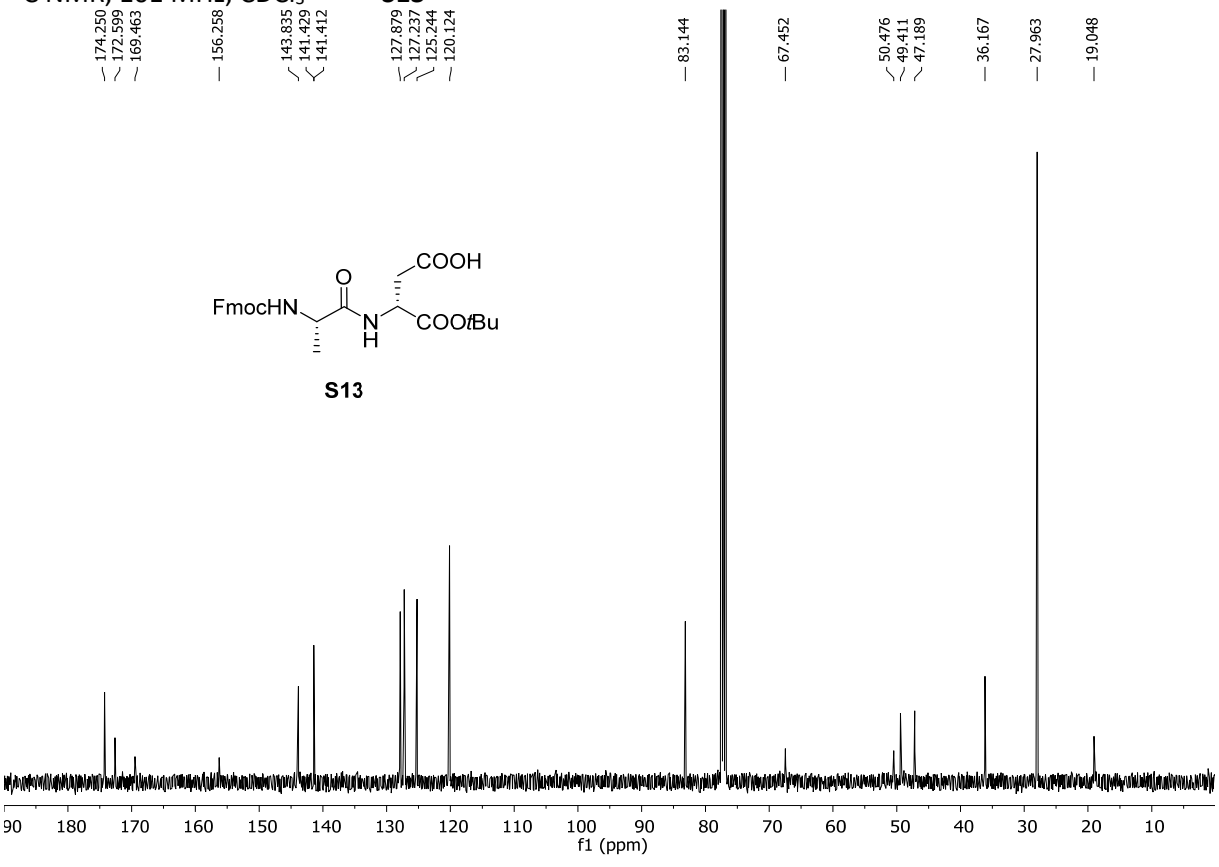
^{13}C NMR, 151 MHz, methanol-d4 **S10**



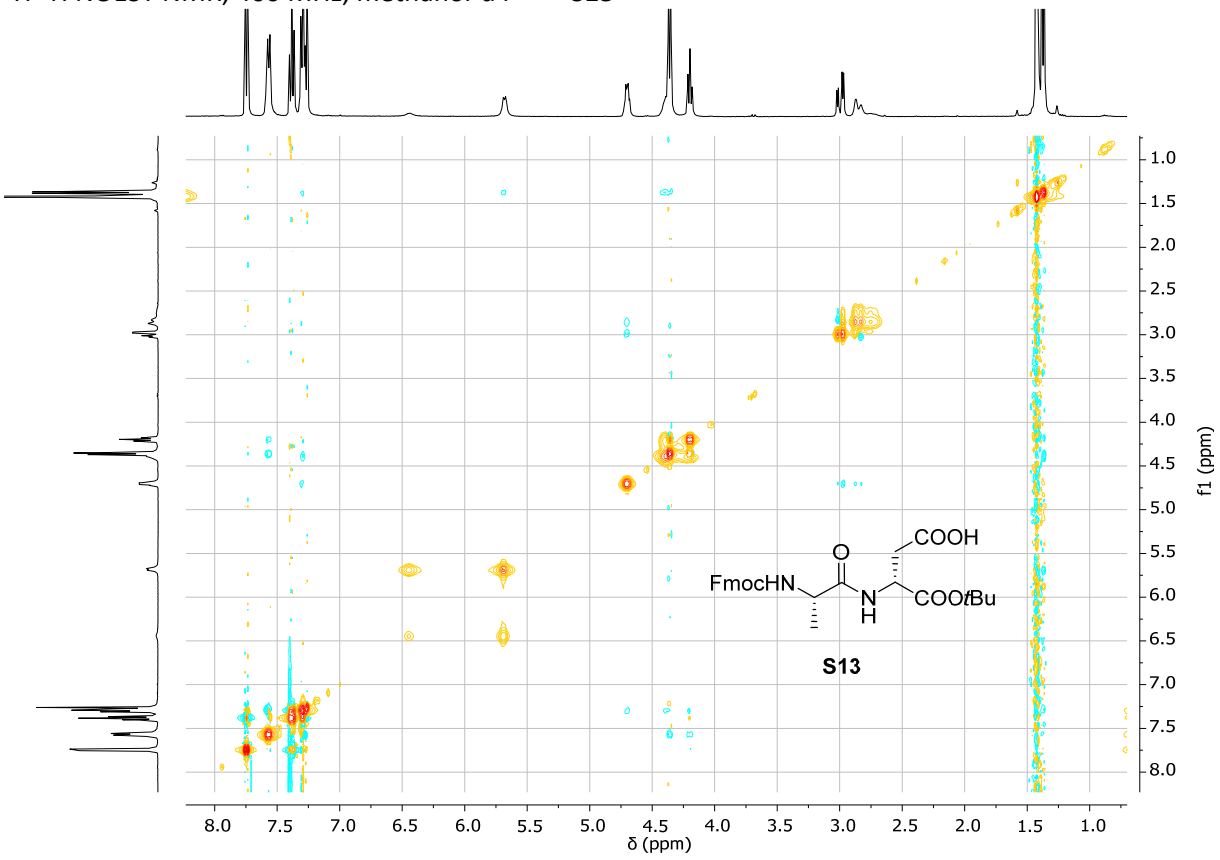
^{1}H NMR, 400 MHz, $CDCl_3$ **S13**



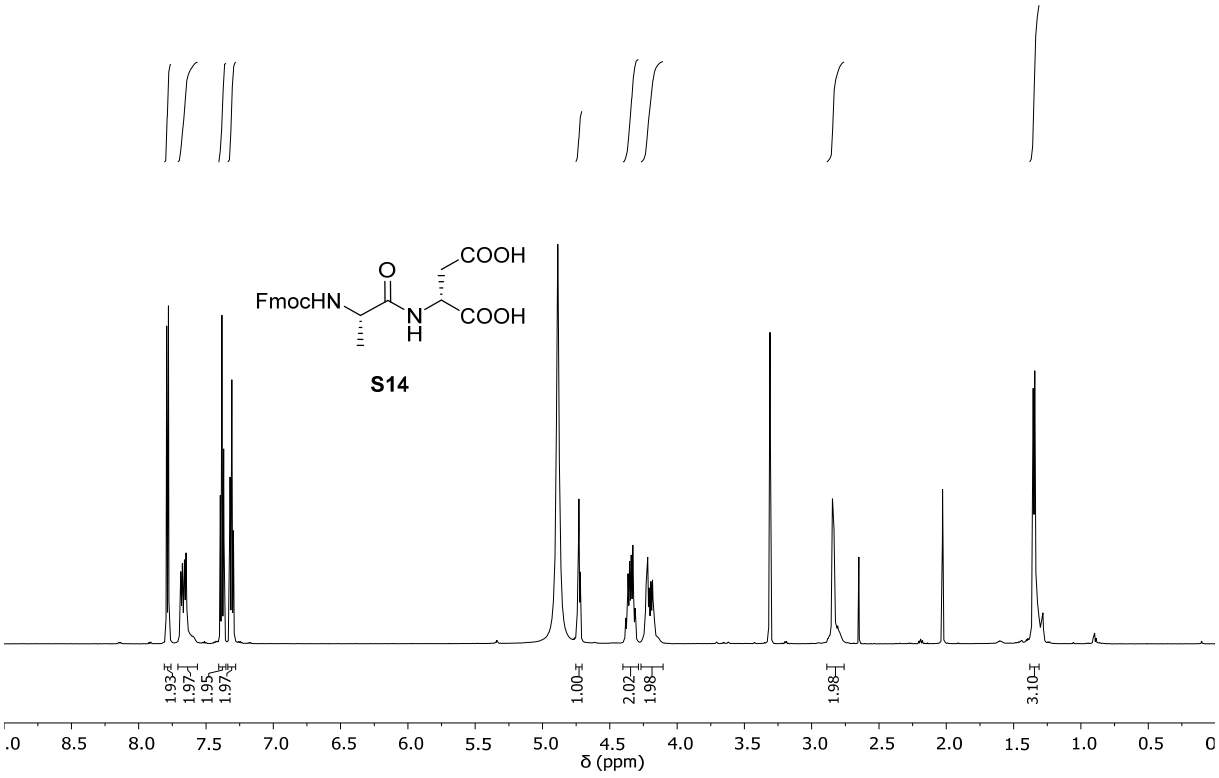
^{13}C NMR, 101 MHz, $CDCl_3$ **S13**



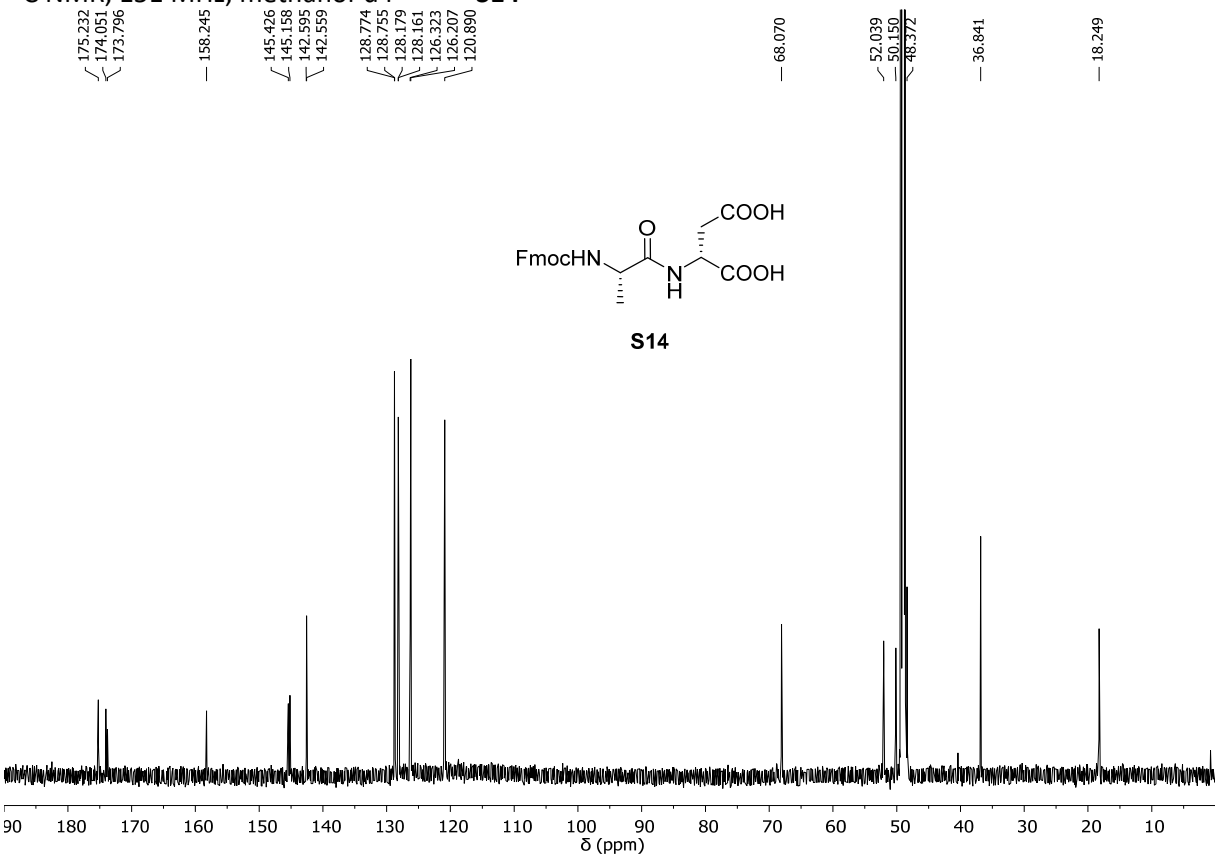
^{1}H-^{1}H NOESY NMR, 400 MHz, methanol-d4 **S13**



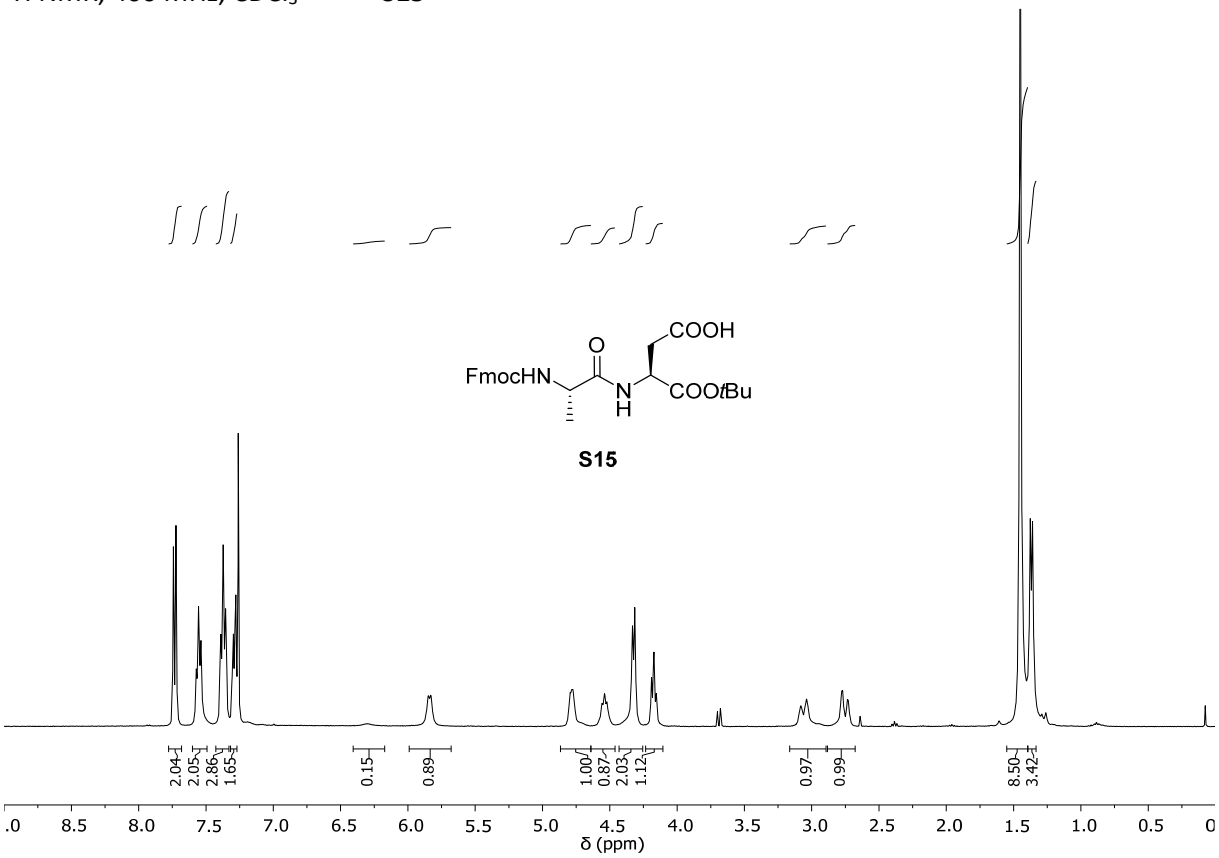
^{1}H NMR, 600 MHz, methanol-d4 **S14**



^{13}C NMR, 151 MHz, methanol-d4 **S14**

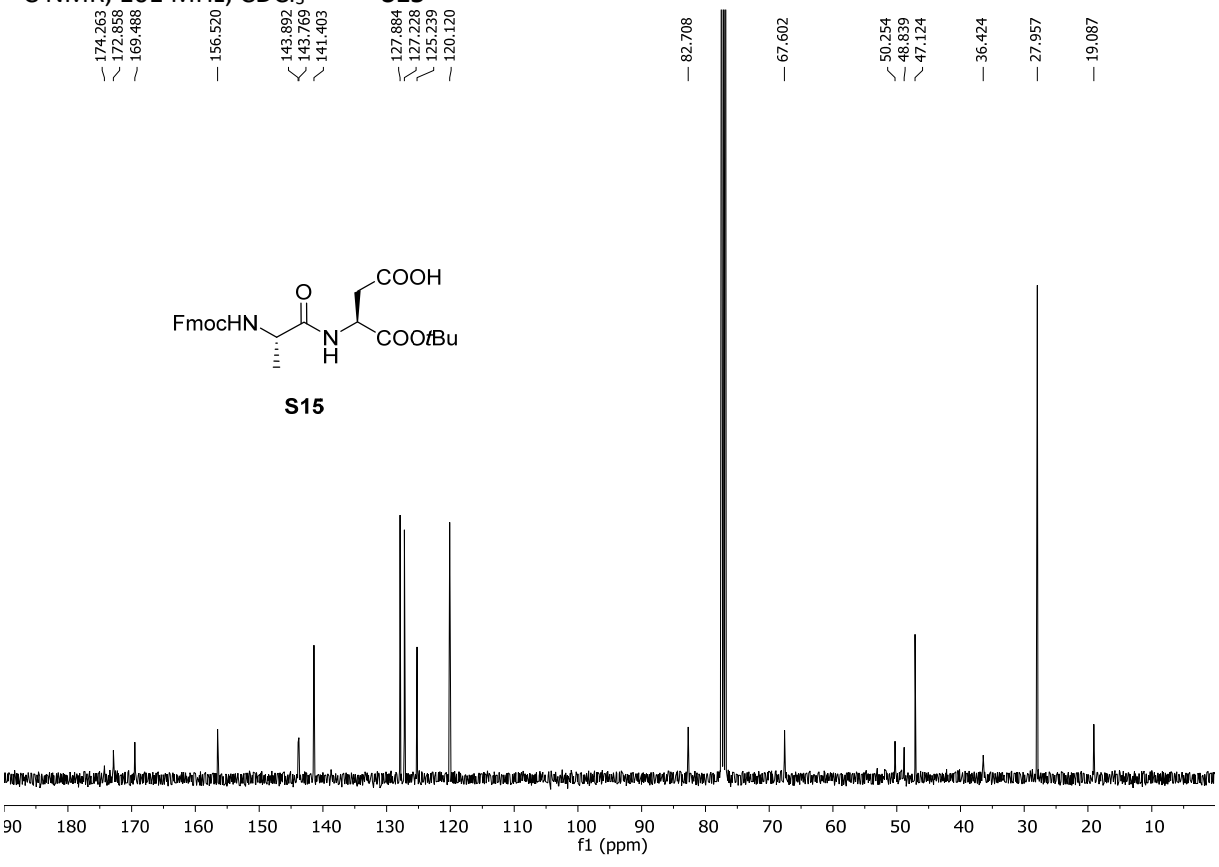


^{1}H NMR, 400 MHz, $CDCl_3$ **S15**



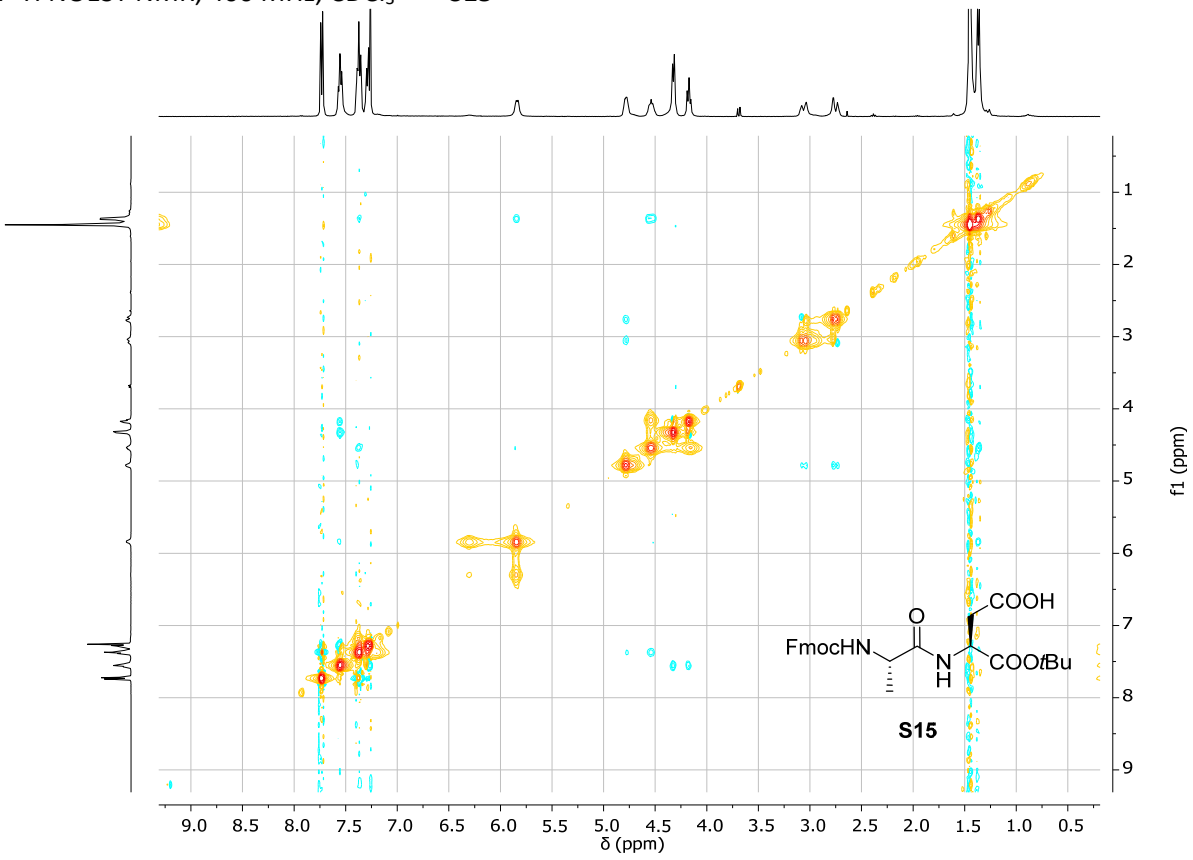
S15

^{13}C NMR, 101 MHz, $CDCl_3$ **S15**

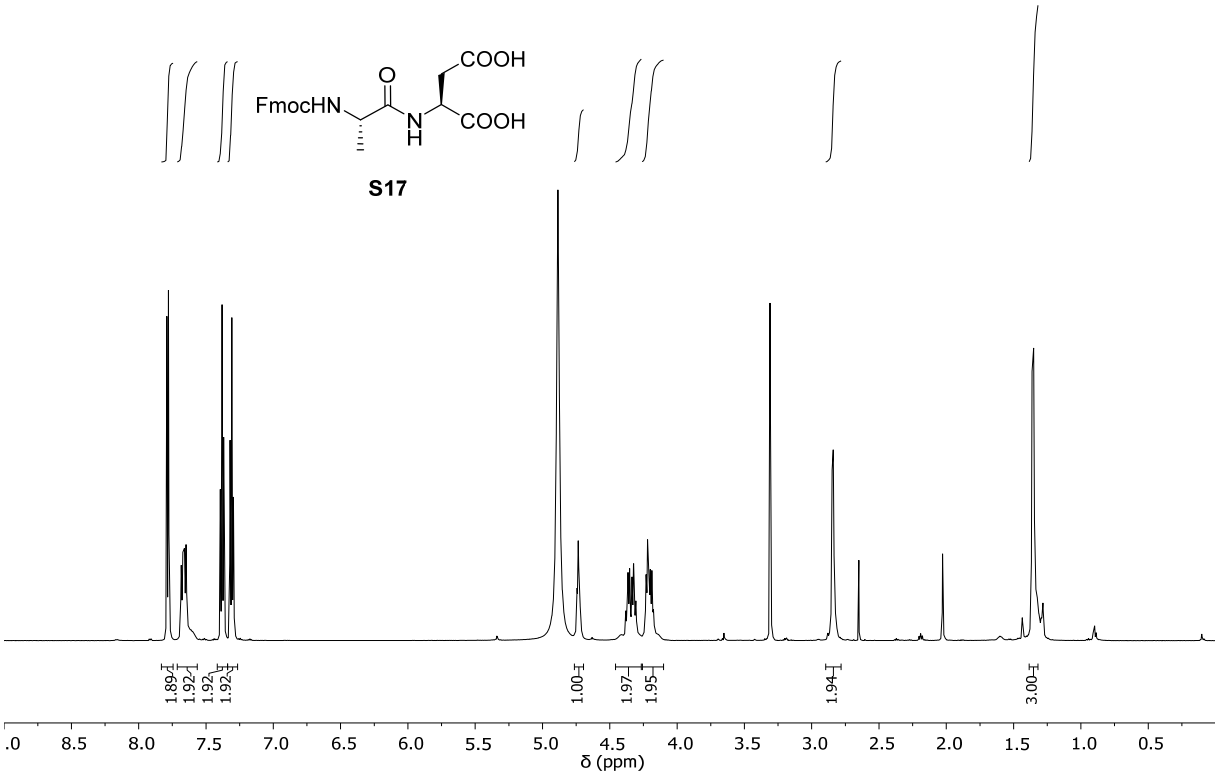


S15

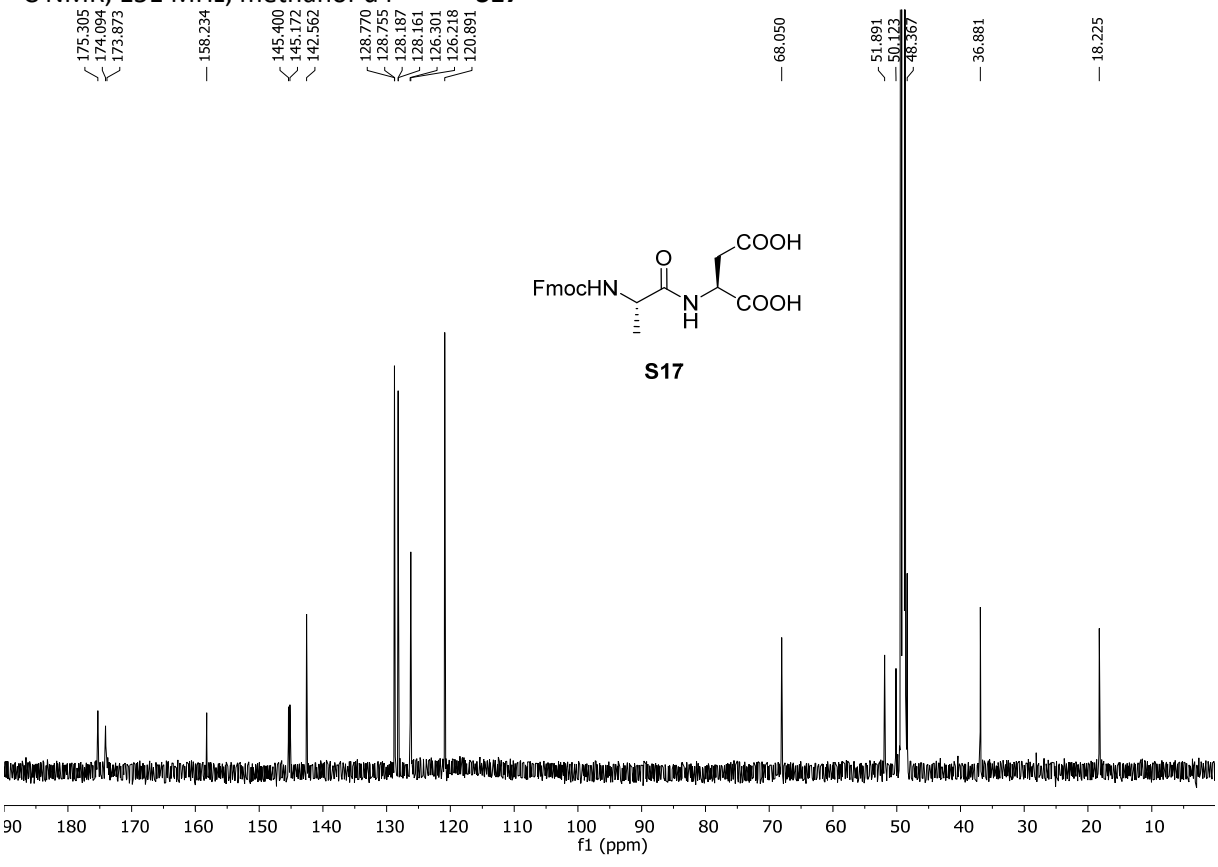
^{1}H-^{1}H NOESY NMR, 400 MHz, $CDCl_3$ **S15**



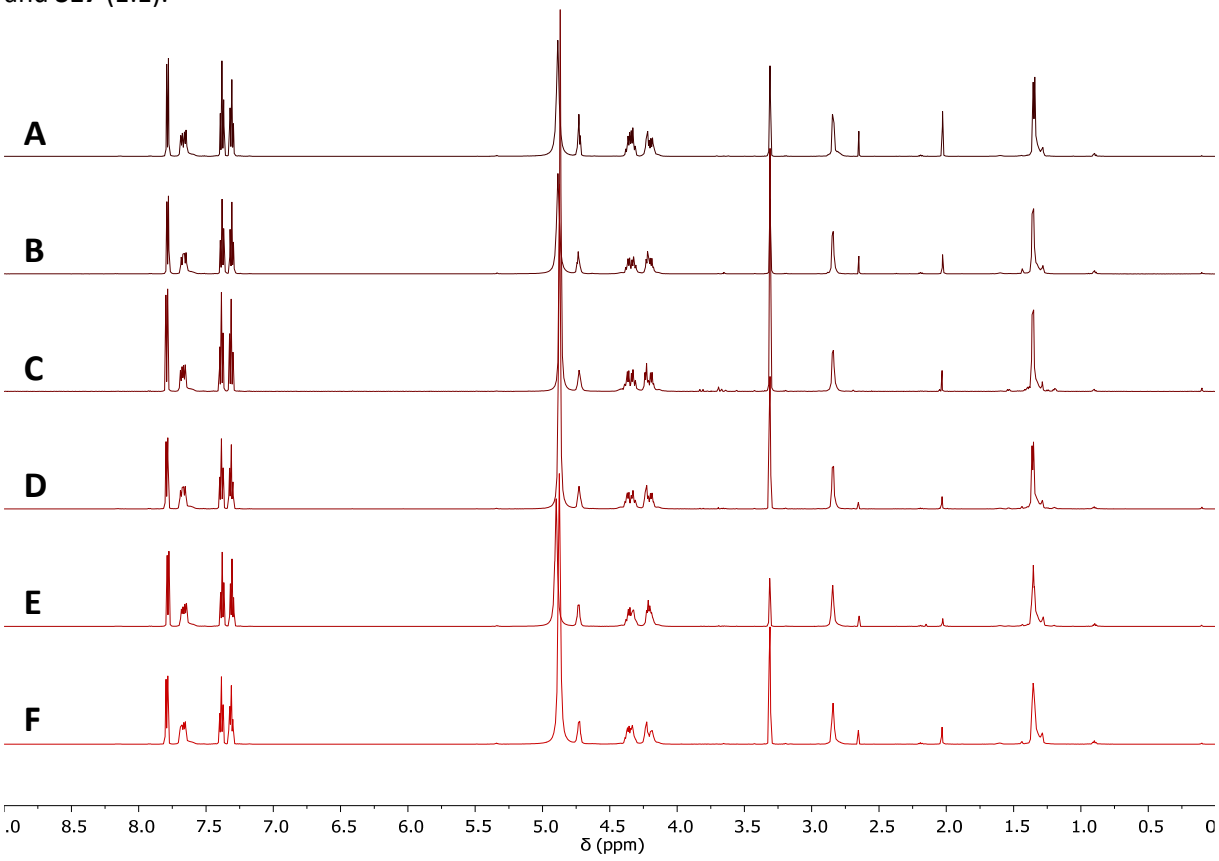
^{1}H NMR, 600 MHz, methanol-d4 **S17**

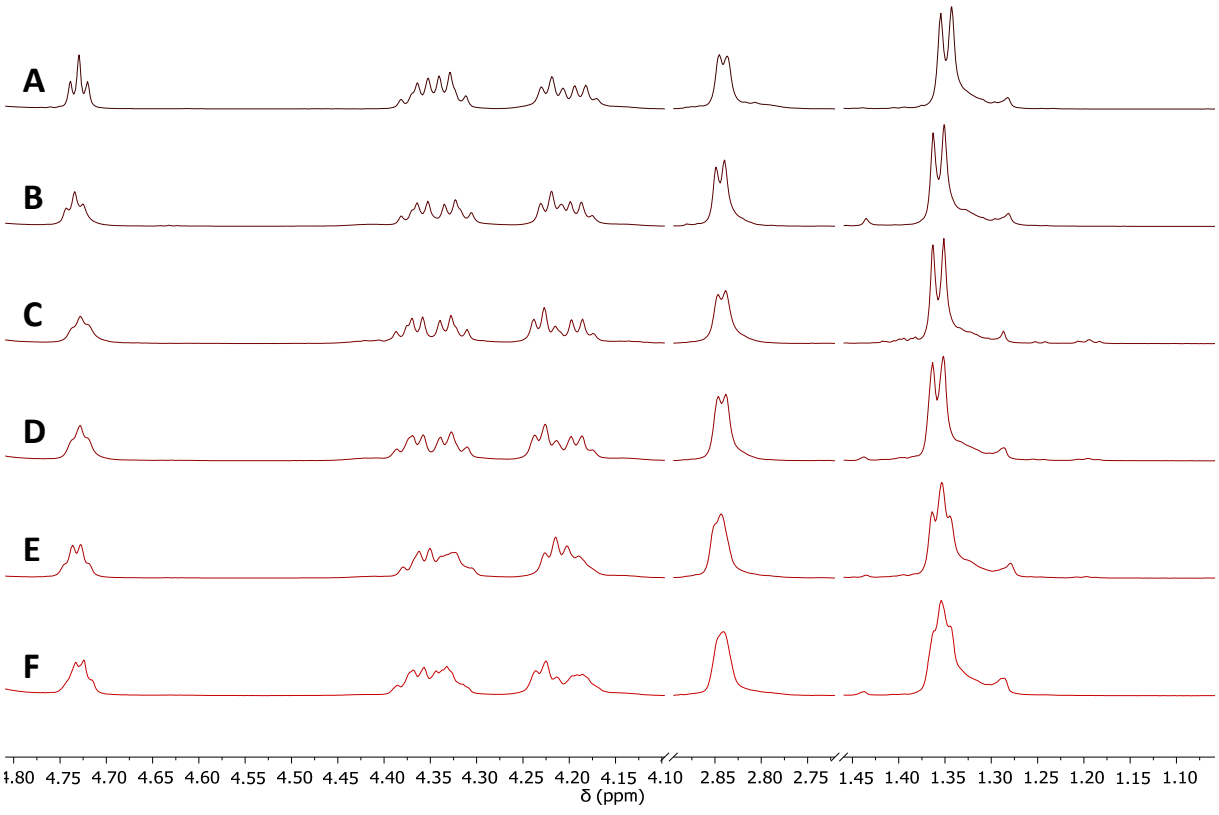


^{13}C NMR, 151 MHz, methanol-d4 **S17**

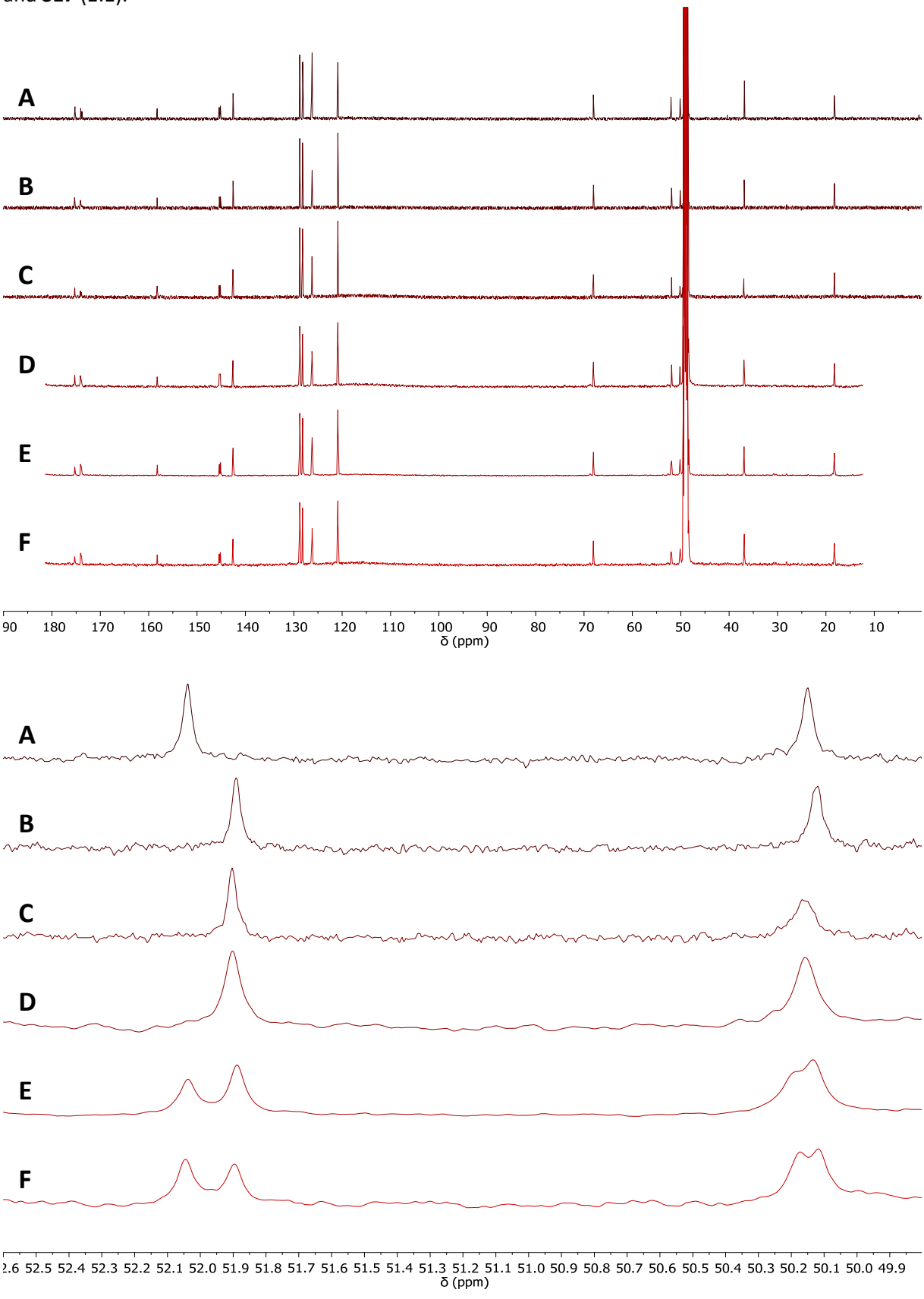


^{1}H NMR, 600 MHz, methanol-d4 Stacked spectra of (A) **S14** (LD-isomer), (B) **S17** (LL-isomer), (C) **S10**, (D) mixture of **S10**and **S17** (1:1), (E) mixture of **S10** and, **S17** and **S14** (1:1:1), (F) mixture of **S14** and **S17** (1:1).

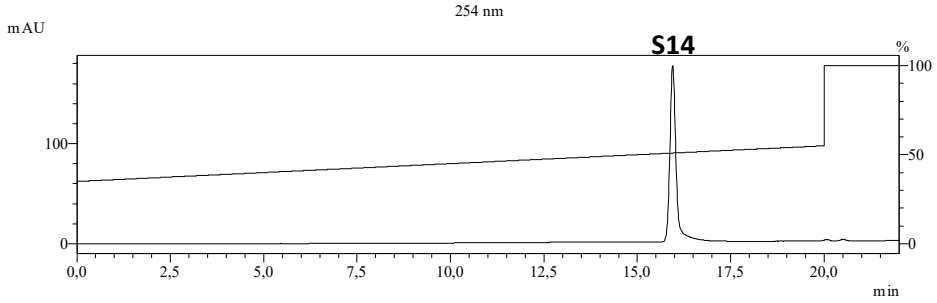




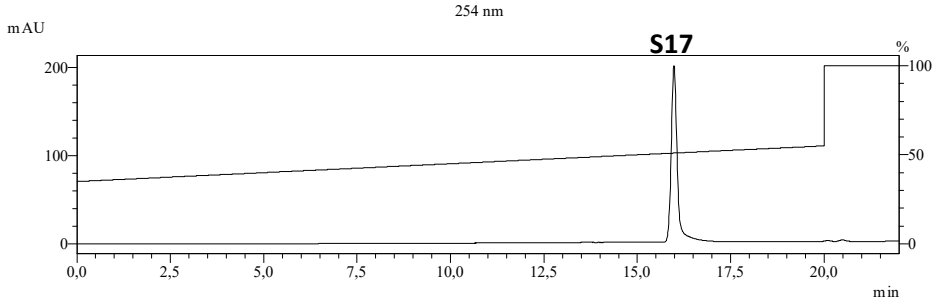
^{13}C NMR, 151 MHz, methanol-d4 Stacked spectra of (A) **S14** (LD-isomer), (B) **S17** (LL-isomer), (C) **S10**, (D) mixture of **S10**and **S17** (1:1), (E) mixture of **S10** and, **S17** and **S14** (1:1:1), (F) mixture of **S14** and **S17** (1:1).



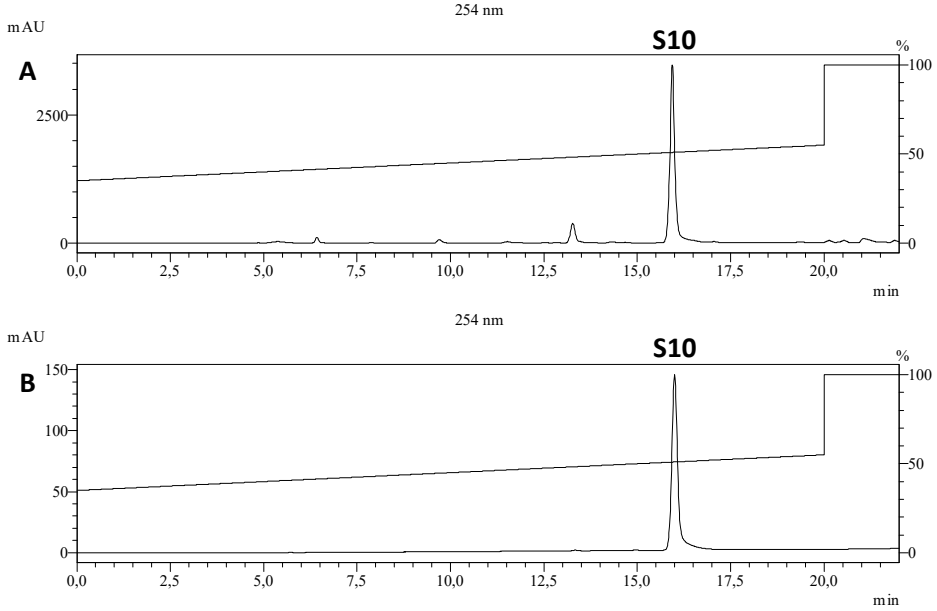
RP-HPLC chromatogram (254 nm) of Fmoc-Ala-D-Asp-OH (**S14**) (35–55% in 20 min, R_t = 16.0 min, column 1).



RP-HPLC chromatogram (254 nm) of Fmoc-Ala-L-Asp-OH (**S17**) (35–55% in 20 min, R_t = 16.0 min, column 1).

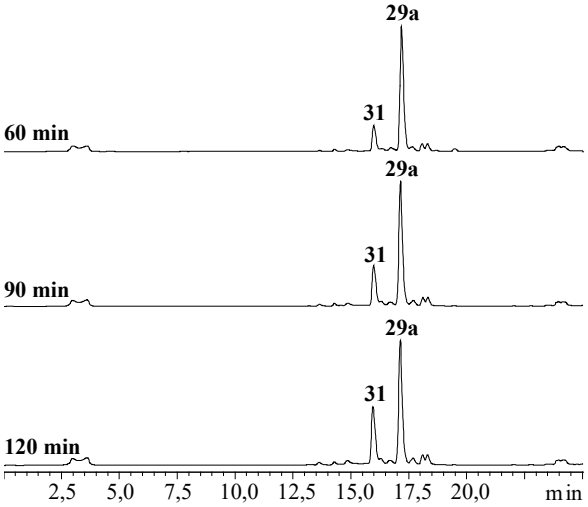


RP-HPLC chromatogram (254 nm) **A** before and **B** after HPLC purification of Fmoc-Ala-L-Asp-OH (**S10**) (35–55% in 20 min, R_t = 16.0 min, column 1).

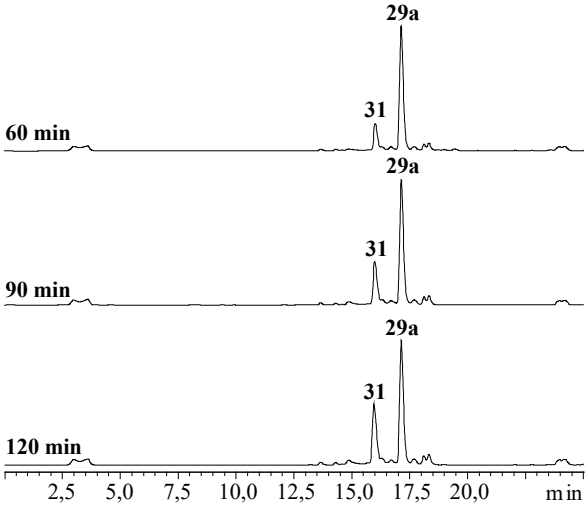


9.5.1 Sequence Dependency of CUT

LC-MS chromatograms (254 nm) for the treatment of Ala-decapeptide **21a** with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) for the times given (25–55% in 20 min, R_t = 16.0, 17.1 min, column 2).

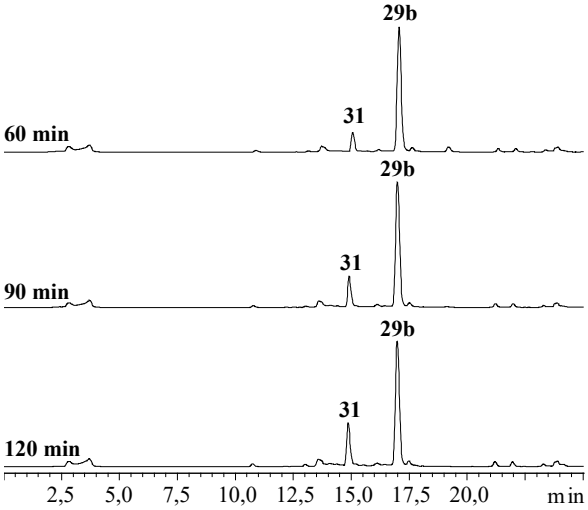


experiment 1

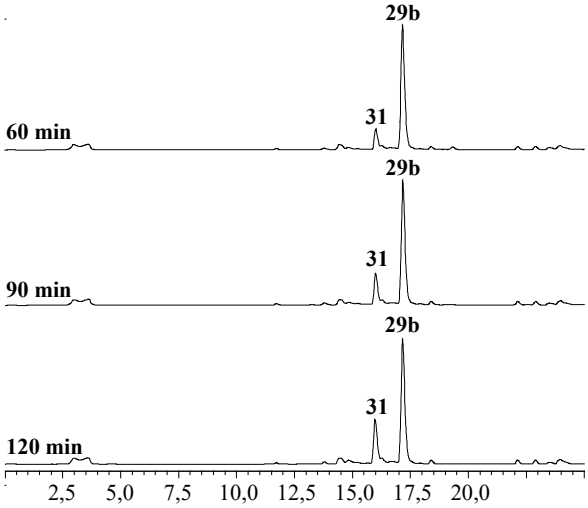


experiment 2

LC-MS chromatograms (254 nm) for the treatment of Asp-decapeptide **21b** with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) for the times given (25–55% in 20 min, R_t = 14.9, 17.0 min, column 3 (left) or 25–55% in 20 min, R_t = 16.0, 17.2 min, column 2 (right)).

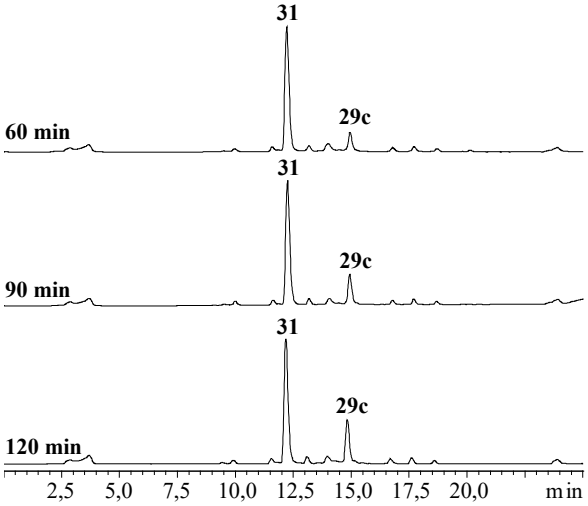


experiment 1

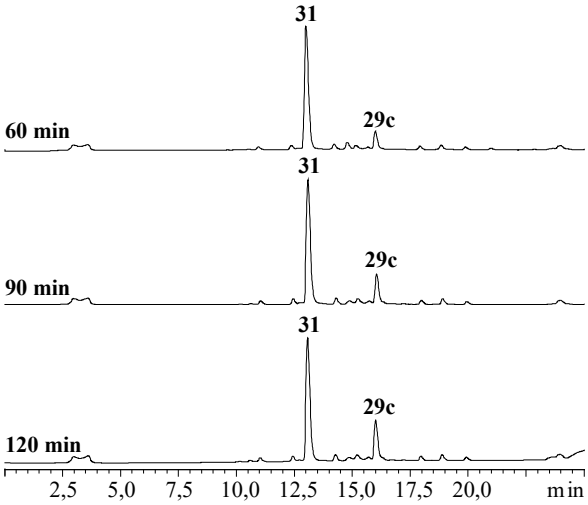


experiment 2

LC-MS chromatograms (254 nm) for the treatment of Lys-decapeptide **21c** with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) for the times given (25–55% in 20 min, R_t = 12.2, 14.9 min, column 3 (left) or 25–55% in 20 min, R_t = 13.1, 16.0 min, column 2 (right)).

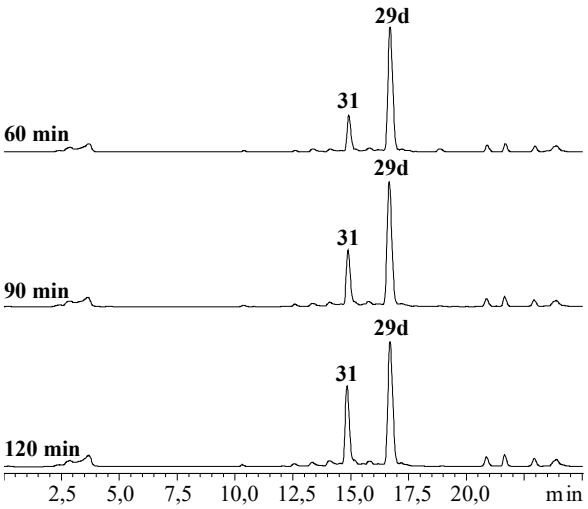


experiment 1

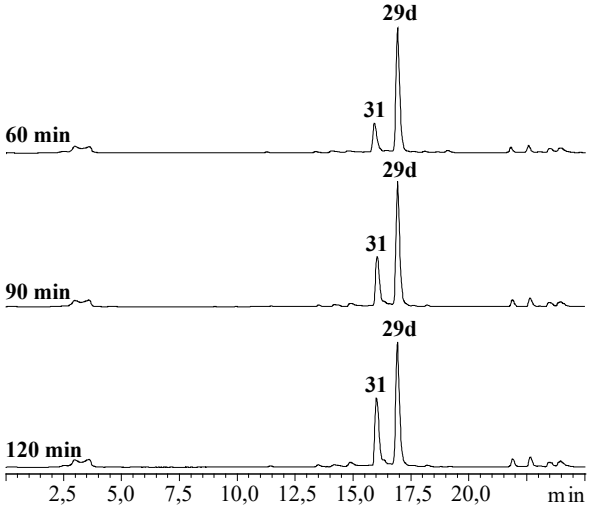


experiment 2

LC-MS chromatograms (254 nm) for the treatment of Ser-decapeptide **21d** with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) for the times given (25–55% in 20 min, R_t = 14.9, 16.7 min, column 3 (left) or 25–55% in 20 min, R_t = 16.0, 16.9 min, column 2 (right)).

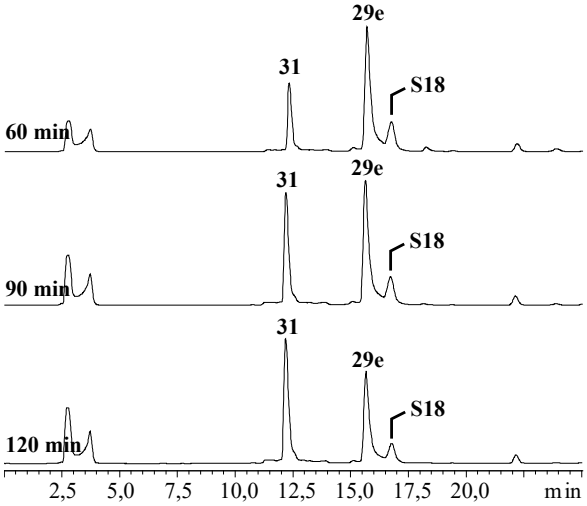


experiment 1

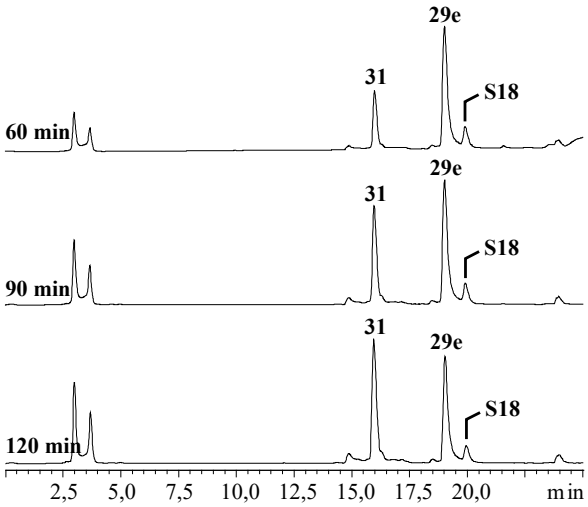


experiment 2

LC-MS chromatograms (254 nm) for the treatment of Trp-decapeptide **21e** with TFA/TIS/H_2O (95:2.5:2.5, 0.2 mL mg^{-1}) for the times given (30–60% in 20 min, R_t = 12.5, 15.7, 16.7 min, column 2 (left) or 25–55% in 20 min, R_t = 16.0, 19.0, 19.9 min, column 2 (right)).



experiment 1



experiment 2

10. References

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