Supporting Information

Expedient on-resin modification of a peptide C-terminus through a benzotriazole linker

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Content

Materials and Methods

All commercial materials were used without further purification. All solvents were reagent grade or HPLC grade from Fisher. Diethyl ether, dichloromethane, ethanol, methanol, DMF, and DMSO were obtained from a dry solvent system and used without further purification. Trifluoroacetic acid (TFA) and isoamyl nitrite were purchased from Acros . Poly(amidoamine) (PAMAM) dendrimers were purchased from Dendritech. Inc. Triisopropylsilane (TIS), *N, N*-diisopropylethylamine (DIPEA), *N*-methy morpholin (NMM), 4-amino-3-nitrobenzoic acid (ANB), piperidine, 3-mercaptopropionic acid (MPA) and Tin(II) chloride were purchased from Alfa Aesar. *o*-(1-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and amino acids were purchased from AK Scientific, Inc. Analytical thin-layer chromatography was pre-coated and detected at 254 nm by fluorescence detector. Water was purified using a Millipore MilliQ water purification system. Peptides were synthesized on a Rink amide resin(LS) 100-200 mesh 1% DVB crosslinking (0.3 mmol/g) from Advanced ChemTech Inc.

NMR spectra were obtained on a Joel 400 MHz and Varian 600 MHz spectrometer. ESI Mass spectra were taken on Bruker APEII. HRMS (FAB^+) were recorded with a JEOL JMS SX/SX 102A four sector mass spectrometer. MALDI-Mass spectra were recorded from Autoflex III MALDI-TOF system (Bruker Daltonics).

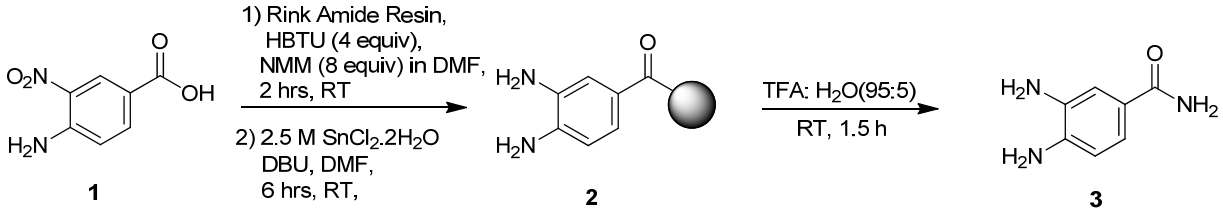
HPLC

Analytical HPLC was carried out in Agilent Model 1100 equipped and performed using a Pyramid C18 column (Nucleodex, 5.0 micron, ϕ4.6 x 250 mm) at a flow rate of 0.5 mL/min with a gradient from 0 to 80% of B in 40 min (eluent A: 0.1% TFA in H_2O, B: 0.1% TFA in CH_3CN), 25°C. Analytical injections were monitored at 220 nm.

Gel permeation chromatography (GPC) experiments were carried out in Agilent Model 1100 HPLC system (1100 and performed using TSK G2000sw column (ϕ7.5 x 300 mm, 10 μm size) at a flow rate of 1 mL/min with an isocratic in 20 min (eluent A: 0.1% TFA in H_2O), 25°C. Analytical injections were monitored at 214 nm.

Chiral experiments were performed using CHIRACEL OD-H (Chiral Technologies Europe, ϕ4.6 x 250 mm) columns at a flow rate of 1 mL/min with an isocratic in 30 min (eluent: *i*PrOH/n-hexane), 25°C. Analytical injections were monitored at 220 nm.

Scheme S1: Synthesis of on resin diaminobenzoic acid linker (*Dbz*)



The suspension of rink amide resin (0.3 mmol/g loading; 0.1 mmol) in DMF and swelled for 30 min, then the resin was treated with 20% piperidine stand for 5 min twice and then washed with DMF and coupled to 4-amino-3-nitrobenzoic acid (ANB) (4 equiv.), HBTU (4 equiv.) and NMM (8 equiv.) in DMF (3 mL). The reaction mixture was allowed to stirred for 2 h. The resin was washed with DCM (3 mL x 3) and DMF (3 mL x 3). The unreacted resin was capped by acetic anhydride. The supernatant was removed and the resin was washed with DMF (3 mL x 3) and DCM (3 mL x 3). The resulting resin-bound ANB was bubbled with N_2 for 10 min and then carried out with the dihydrate of tin (II) chloride (2.5 M) in the presence of DBU (2 mmol) in DMF during 6 h at room temperature. The resulting resin-bound *o*-aminoanilide was filtered off and then washed DMF (3 mL x 3) and DCM (3 mL x 3). The resin was dried under *vacuo* and treated with TFA: H_2O (95:5) to afford 95% yield. **^{1}H NMR** (400 MHz, DMSO) **:** δ 7.10 (s, 1H), 7.04 (d, J = 7.2 Hz, 1H), 6.47 (d, J = 8.1 Hz, 1H). **HRMS** calcd for $C_7H_{10}N_3O$ $(M+H)^+$ 152.0824, found 152.0821.

Loading Estimation of the First Amino Acid

The resin was treated with piperidine/DMF (1:4, v/v, 3 mL, 5 min × 3) and the combined deprotection solution (20% piperidine in DMF) made up to 10 mL with DMF. The solution was diluted 200-fold with DMF and the UV absorbance of the piperidine-fulvene adduct measured (λ = 301 nm, ε = 7800 $M^{-1}cm^{-1}$) to estimate the amount of amino acid loaded onto the resin.

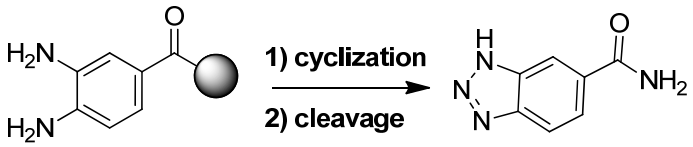
General procedure for solid phase peptide synthesis

The peptide synthesis was carried out using Fmoc-SPPS on rink amide resin (0.3 mmol/g, 0.05 mmol). The resin was treated with 20% piperidine (5 min x 2) washed with DMF and coupled with 4-amino-3-nitrobenzoic acid (4 equiv.) using HBTU (4 equiv.), NMM (8 equiv.) in DMF for 1 h. After the resin bound ANB was reduced with tin(II) chloride (2.5 M) in the presence of DBU (2 mmol) in DMF. The remaining peptide was synthesized on an automated PS3 peptide synthesizer using Fmoc-Xaa-OH (2 equiv.), NMM (4 equiv.) and HBTU (2 equiv.). The coupling was performed for 60 min and Fmoc-deprotection was achieved using 20% piperidine (10 min x 2). Coupling reactions were checked by Kaiser test.

General procedure for cyclization and cleavage of the benzotriazole linker

The resin-bound *Dbz* peptide was washed with DMF (3 mL x 3) and DCM (3 mL x 3), then treated with isoamyl nitrite (10 equiv.) in DMF (4 mL) for 90 min at room temperature. Unreacted isoamylnitrite was washed with DCM. The nucleophilic (4 equiv.) and DIPEA (8 equiv.) in DMF was bubbled with N_2 for 20 min and then immediately added into the activated peptidyl-resin. The reaction was allowed to shake for 2 h at room temperature. After completion of the reaction, the supernatant was removed and concentrate under *vacuo*. The resulting peptide derivative was deprotected with 20% piperidine in DMF and then TFA cocktail (TFA/TIS/H_2O (95:2.5:2.5) or TFA/phenol/water/thioanisole/EDT (82.5:5:5:5:2.5)). The peptide was precipitated in cold ether and collected by centrifugation. The supernatant was removed and the residue was dissolved in 30% acetonitrile/H_2O and lyophilized to afford pure product without further purification.

Table S1: Optimization of reaction conditions for synthesis of resin bounded benzotriazole (**17**)

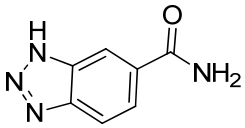


2 **17**

Entry	Reagent (equiv.)	Acid	Solvent	Time(h)	Conversion[a](%)
1	$NaNO_2$(10 equiv.)	AcOH	DMF	24	40
2	$NaNO_2$(10 equiv.)	Acetic acid/Con. HCl	DMF	24	15
3	$NaNO_2$(10 equiv.)	Con. HCl	DMF	24	30
4	**Isoamyl nitrite (10 equiv.)**	-	**DMF**	**1.5**	**99**
5	Isoamyl nitrite (5 equiv.)	-	DMF	1.5	85
6	Isoamyl nitrite (10 equiv.)	-	NMP	3	99
7	Isoamyl nitrite (10 equiv.)	-	DCM	1.5	99

Reaction conditions: Peptide (25 mg, 0.30 mmol/g) on solid support at room temperature. [a]Conversion to **17** was calculated from the absorbance at 220 nm using HPLC. The entry in bold represents the optimized reaction conditions for cyclization. Cleavage condition: TFA: H_2O (95:5) for 1.5 h at RT.

Synthesis of benzotriazole (17, *Bt*)



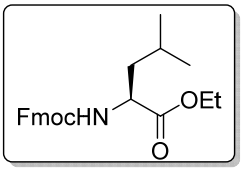
17

The resin-bound *Dbz* linker **3** (0.05 mmol) in DMF (4 mL) was added isoamyl nitrite (10 equiv.) at room temperature. The reaction mixture was allowed to shaken for 90 min at room temperature. The resin was filtered and then washed DCM (3 mL x 3). The benzotriazole was cleaved from resin using TFA: H_2O (95:5) to afford the compound **17.** The crude mixture was applied directly to flash chromatography (50-75% EtOAc/ hexanes) to yield 8 mg (92%) as a yellow solid. **^{1}H NMR** (400 MHz, DMSO) **:** δ 8.44 (s, 1H), 7.90 (dd, J = 16.2, 6.8 Hz, 2H). **HRMS** calcd for $C_7H_7N_4O$ $(M+H)^+$ 163.0614, found 163.0612.

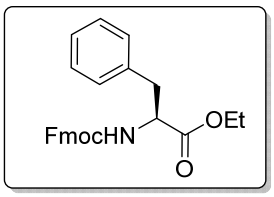
General procedure for nucleophilic substitution on benzotriazole resin

The resin-bound *Dbz* **2** (0.05 mmol, 157mg) was coupled with amino acid (4 equiv.) in the presence of HBTU (2 equiv.) and NMM (4 equiv.) in DMF for 1 h. The resin was washed with DMF (3 mL x 3) and DCM (3 mL x 3). After that resin bound *Dbz* amino acid was treated with a solution of isoamylnitrite (10 equiv.) in 4 mL of DMF for 90 min at room temperature. After washing with DCM (3 mL x 3), the resulting resin was shaken with a mixture of ethanol (10 equiv.) and DIPEA (8 equiv.) in DCM for 2 h at room temperature. The resin was washed with DCM (3 mL x 3), then the supernatant was concentrated under *vacuo* and washed through silica gel pad (ϕ 10 mm x 12 mm) to afford pure product.

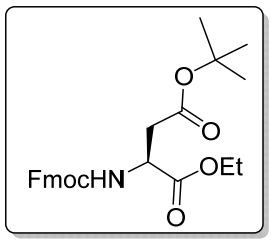
Fmoc-L-Leu-OEt (6a): R_f = 0.4 (Hexane: Ethyl acetate, 8:2). White solid (18 mg, 91%). **^{1}H NMR** (400 MHz, $CDCl_3$) **:** δ 7.76 (d, J = 7.5 Hz, 2H), 7.59 (dd, J = 6.8, 5.2 Hz, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 5.20 (d, J = 8.5 Hz, 1H), 4.40-4.38 (m, 3H), 4.24-4.16 (m, 3H), 1.71-1.60 (m, 2H), 1.56 -1.51 (m, 1H), 1.27 (t, J = 7.1 Hz, 3H), 0.95 (dd, J = 6.1, 3.4 Hz, 6H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 173.3, 156.1, 144.1, 143.9, 141.4, 127.8, 127.1, 125.2, 120.1, 120.1, 67.0, 61.5, 52.6, 47.3, 42.0, 24.8, 23.0, 22.0, 14.3. **HRMS (FAB)** calcd for $C_{23}H_{28}NO_4$ $(M+H)^+$ 382.2018, found 382.2016.



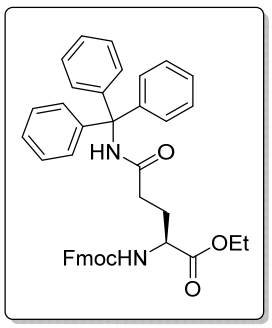
Fmoc-L-Phe-OEt (6b): R_f = 0.2 (Hexane: Ethyl acetate, 8:2). White solid (19 mg, 90%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 7.78 (d, J = 7.5 Hz, 2H), 7.58-7.55 (m, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.33-7.25 (m, 5H), 7.12 (d, J = 6.9 Hz, 2H), 5.30 (d, J = 7.4 Hz, 1H), 4.67-4.64 (m, 1H), 4.46-4.42 (m, 1H), 4.36-4.32 (m, 1H), 4.23-4.15 (m, 3H), 3.12 (t, J = 5.6 Hz, 2H), 1.24 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 171.6, 155.6, 144.0, 143.9, 141.4, 135.9, 129.5, 128.7, 127.8, 127.2, 127.2, 125.2, 125.2, 120.1, 120.1, 67.0, 61.7, 54.9, 47.3, 38.4, 14.2. **HRMS (FAB)** calcd for $C_{26}H_{26}NO_4$ $(M+H)^+$ 416.1862, found 416.1859.



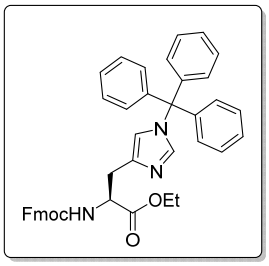
Fmoc-L-Asp(OtBu)-OEt (6c): R_f = 0.5 (Hexane: Ethyl acetate, 8:2). White solid (19 mg, 87%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 7.76 (d, J = 7.5 Hz, 2H), 7.61-7.59 (m, 2H), 7.39 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.4 Hz, 2H), 5.84 (d, J = 8 Hz, 1H), 4.60-4.58 (m, 1H), 4.44-4.40 (m, 1H), 4.36-4.32 (m, 1H), 4.26-4.21 (m, 3H), 2.97-2.92 (dd, J= 16.8, 4.7 Hz, 1H), 2.80-2.75 (dd, J= 16.8, 4.5 Hz, 1H), 1.45 (s, 9H), 1.27 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 171.0, 170.1, 156.1, 144.0, 143.9, 141.4, 127.8, 127.2, 125.3, 125.2, 120.1, 81.9, 67.3, 61.9, 50.7, 47.2, 37.9, 28.1, 14.2. **HRMS (FAB)** calcd for $C_{25}H_{30}NO_6$ $(M+H)^+$ 440.2073, found 440.2074.



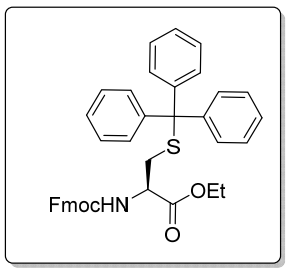
Fmoc-L-Gln(Trt)-OEt (6d): R_f = 0.4 (Hexane: Ethyl acetate, 6:4). White solid (27 mg, 86%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 7.77 (d, J = 7.5 Hz, 2H), 7.61 (d, J = 7.5 Hz, 2H), 7.43-7.38 (m, 2H), 7.34-7.23 (m, 17H), 5.66 (d, J = 8 Hz, 1H), 4.48-4.449 (m, 1H), 4.40-4.33 (m, 2H), 4.25-4.16 (m, 3H), 2.39-2.34 (m, 2H), 2.25-2.21 (m, 1H), 1.96-1.90 (m, 1H), 1.27 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.2, 171.0, 156.5, 144.8, 144.0, 143.8, 141.5, 128.8, 128.1, 127.9, 127.2, 127.2, 125.3, 120.1, 70.8, 67.1, 61.8, 53.8, 47.3, 31.7, 22.8, 14.3. **HRMS (FAB)** calcd for $C_{41}H_{39}N_2O_5$ $(M+H)^+$ 639.2859, found 639.2861.



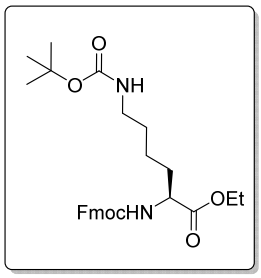
Fmoc-L-His(Trt)-OEt (6e): R_f = 0.3 (Hexane: Ethyl acetate, 6:4). White solid (26 mg, 82%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 7.75 (d, J = 7.5 Hz, 2H), 7.65-7.61 (m, 2H), 7.43-7.33 (m, 3H), 7.32-7.30 (m, 9H), 7.28-7.27 (m, 2H), 7.13-7.12 (m, 6H), 6.59 (m, 2H), 4.63-4.60 (m,1H), 4.39-4.25 (m, 3H), 4.13-4.06 (m, 2H), 3.10 (m, 2H), 1.17 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 171.7, 156.3, 144.2, 144.1, 142.4, 142.4, 141.3, 141.3, 139.0, 136.5, 129.9, 128.2, 127.2, 125.5, 125.4, 120.0, 119.7, 75.4, 67.3, 61.2, 54.4, 47.3, 29.8, 14.3. **HRMS (FAB)** calcd for $C_{42}H_{38}N_3O_4$ $(M+H)^+$ 648.2862, found 648.2859.



Fmoc- L-Cys(Trt)-OEt (6f): R_f = 0.5 (Hexane: Ethyl acetate, 8:2). White solid (26 mg, 84%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ 7.77 (d, J = 7.5 Hz, 2H), 7.63 (m, 2H), 7.42-7.38 (m, 8H), 7.33-7.19 (m, 11H), 5.32-5.28 (m, 1H), 4.39-4.26 (m, 3H), 4.21-4.17 (m, 3H), 2.66 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 170.6, 155.7, 144.4, 144.0, 143.9, 141.4, 141.4, 129.6, 128.1, 127.9, 127.8, 127.2, 127.0, 125.3, 125.3, 120.1, 67.2, 61.9, 53.1, 47.2, 29.8, 14.2. **HRMS (FAB)** calcd for $C_{39}H_{36}NO_4S$ $(M+H)^+$ 614.2365, found 614.2366.

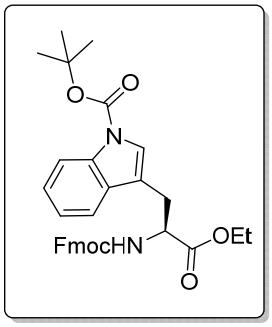


Fmoc-L-Lys(Boc)-OEt (6g): R_f = 0.4 (Hexane: Ethyl acetate, 8:2). White solid (22 mg, 88%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:**



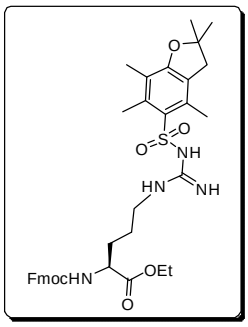
δ 7.76 (d, J = 7.4 Hz, 2H), 7.59 (dd, J = 7.1, 2.4 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.30 (t, J = 7.5 Hz, 2H), 5.45 (d, J = 7.6 Hz , 1H), 4.59 (m, 1H), 4.40-4.33 (m, 3H), 4.23-4.17 (m, 3H), 3.10 (m, 2H), 1.85-1.82 (m, 1H), 1.71-1.66 (m, 1H), 1.49-1.47 (m, 1H), 1.42 (s, 9H), 1.37-1.35 (m, 2H), 1.26 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.6, 156.2, 144.0, 143.9, 141.4, 127.8, 127.2, 125.2, 120.1, 79.2, 67.1, 61.6, 53.9, 47.3, 40.2, 32.3, 29.7, 28.5, 22.4, 14.3. **HRMS (FAB)** calcd for $C_{26}H_{26}NO_4$ $(M+H)^+$ 497.2652, found 497.2654

Fmoc-L-Trp(Boc)-OEt (6h): Rf = 0.4 (Hexane: Ethyl acetate, 8:2). White solid (24 mg, 86%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:**



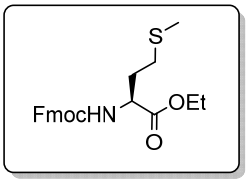
δ 8.13 (m, 1H), 7.76 (d, J = 7.5 Hz, 2H), 7.58-7.53 (m, 3H), 7.44-7.37 (m, 4H), 7.33-7.22 (m, 4H), 5.49 (d, J = 8.2 Hz,1H), 4.77-4.75 (m, 1H), 4.41-4.37 (m, 2H), 4.22-4.15 (m, 3H), 3.28 (m, 1H), 1.66 (m, 9H), 1.23 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 171.7, 155.8, 143.9, 141.4, 127.8, 127.2, 127.2, 125.3, 125.2, 124.7, 124.3, 122.8, 120.1, 119.0, 115.4, 115.1, 83.8, 67.3, 61.8, 54.3, 47.2, 28.3, 14.2. **HRMS (FAB)** calcd for $C_{33}H_{35}N_6O_2$ $(M+H)^+$ 555.2495, found 555.2498.

Fmoc-L-Arg(Pbf)-OEt (6i): R_f = 0.4 (Hexane: Ethyl acetate, 5:1). White solid (26 mg, 78%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:**



δ 7.72 (d, J = 7.5 Hz, 2H), 7.54 (d, J = 7.0 Hz, 2H), 7.35 (t, J = 7.5 Hz, 2H), 7.26-7.23 (m, 2H), 5.73 (d, J = 4.8 Hz, 1H), 4.34-4.24 (m, 2H), 4.16-4.11 (m, 3H), 3.21 (m, 2H), 2.88 (s, 2H), 2.56 (s, 3H), 2.48 (s, 3H), 2.05 (s, 3H), 1.88-1.80 (m, 2H), 1.68-1.64 (m, 1H), 1.57-1.56 (m, 2H), 1.40 (s, 6H), 1.21 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.3, 158.8, 156.3, 143.9, 143.7, 141.3, 138.4, 132.3, 127.8, 127.2, 125.2, 124.7, 120.1, 117.6, 86.5, 67.2, 61.7, 47.2, 43.3, 38.7, 28.6, 22.7, 19.4, 18.0, 14.2, 12.5. **HRMS (FAB)** calcd for $C_{36}H_{45}N_4O_7S$ $(M+H)^+$ 677.3009, found 677.3007.

Fmoc-L-Met-OEt (6j) R_f = 0.4 (Hexane: Ethyl acetate, 5:1). White solid (18 mg, 90%). **^{1}H NMR** (400 MHz, $CDCl_3$)**:** δ

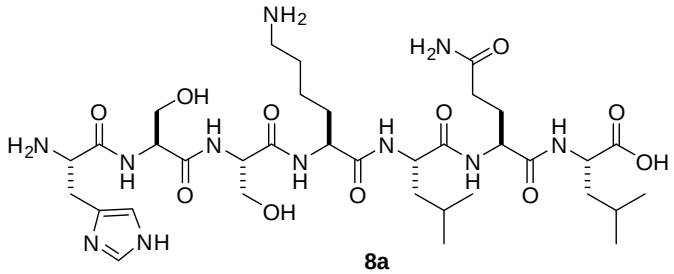


7.77 (d, J = 7.5 Hz, 2H), 7.60 (dd, J = 7.3, 2.6 Hz, 2H), 7.41 (t, J = 7.3 Hz, 2H), 7.32 (tt, J = 7.4, 1.3 Hz, 2H), 5.44 (d, J = 8.2 Hz, 1H), 4.51-4.46 (m, 1H), 4.43-4.41 (d, J = 7.0 Hz, 2H), 4.25-4.20 (m, 2H), 2.55-2.51 (m, 2H), 2.18-2.13 (m, 1H), 2.10 (s, 3H), 2.02-1.93 (m, 1H), 1.29 (t, J = 7.1 Hz, 3H); **^{13}C NMR** (100 MHz, $CDCl_3$): δ 172.0, 155.9, 143.8, 143.7, 141.3, 127.70, 127.0, 125.0, 120.0, 119.9, 67.0, 61.7, 53.2, 47.1, 32.0, 29.9, 15.5, 14.2. **HRMS (FAB)** calcd for $C_{22}H_{26}NO_4S$ $(M+H)^+$ 400.1583, found 400.1581.

General procedure for nucleophilic acyl substitution on peptide

Peptide was prepared according to Fmoc-strategy SPPS on rink amide resin (0.05 mmol), as outlined in the general procedures. The resin-bound *o*-aminoanilide peptide was washed with DMF, then treated with isoamyl nitrite (10 equiv.) in DMF at room temperature for 90 min. After the resin was filtered, washed sequentially with DCM (3 mL x 3). The nucleophiles (4 equiv.) and DIPEA (8 equiv.) in DMF was bubbled with N_2 for 20 min and then immediately added into the activated peptidyl-resin. After 2 h, the resin was washed with DMF (3 mL x 3) and the supernatant was concentrate under vacuo. Then, the resulting product was deprotected by using 20% piperidine in DMF for 30 min and TFA cocktail for 1.5 h. The peptide was precipitated in cold ether and collected by centrifugation. The supernatant was removed and the residue was dissolved in 30% acetonitrile/H_2O and lyophilized to afford pure product without further purification.

HSSKLQL (8a)



8a

Peptide **8a** was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. After the peptide-resin was activated and cleavage with ACN/H_2O (1:1) in the presence of DIPEA (8 equiv.) in DMF (4 mL) for 2 h at room temperature. The Fmoc was deprotected with 20% piperidine in DMF and global deprotection was effected using a solution of TFA/TIS/H_2O (95:2.5:2.5, 4 mL) affording pure peptide **8a** as a white solid after lyophilization (34 mg, 82% yield). **^{1}H NMR** (400 MHz, D_2O)**:** δ 8.77 (s, 1H), 7.51 (s, 1H), 4.66-4.63 (m, 1H), 4.60-4.58 (m, 1H), 4.46-4.44 (m, 3H), 4.39-4.31 (m, 2H), 3.97-3.92 (m, 4H), 3.50-3.48 (m, 2H), 3.06-3.02 (m, 2H), 2.55-2.52 (t, 1H), 2.18-2.14 (m, 1H), 2.06-2.02 (m, 1H), 1.90-1.87 (m, 1H), 1.75-1.72 (m, 9H), 1.50-1.47 (m, 2H), 0.99-0.93 (m, 12H). **ESI-FTMS** calcd for $C_{35}H_{61}N_{11}O_{11}$ $(M+H)^+$ 812.4630, found 812.4633.

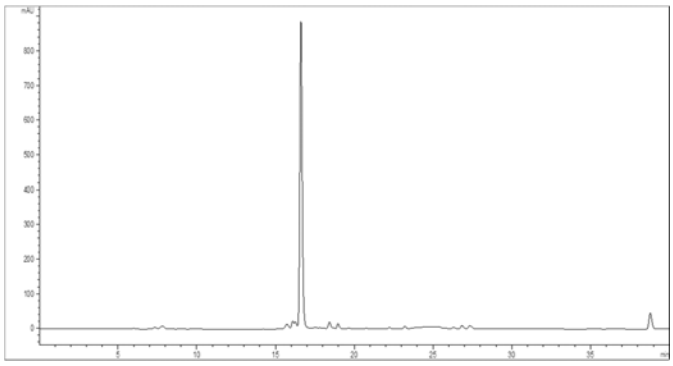
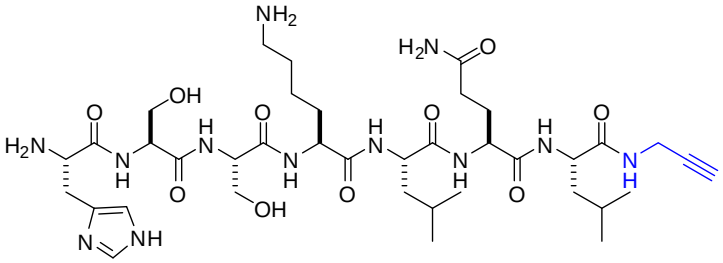


Figure S1. HPLC chromatogram of compound **8a** (linear gradient, 10 to 90% B over 40 min, 0.1% TFA, flow rate: 0.4 ml/min, λ = 220 nm)

HSSKLQL- propargyl amine (8b)



8b

Peptide **8b** was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. After the peptide-resin was activated and cleavage with propargyl amine (4 equiv.) in the presence of DIPEA (8 equiv.) in DMF(4 mL) for 2 h at room temperature. Then Fmoc was deprotected with 20% piperidine in DMF and global deprotection was effected using a solution of TFA/TIS/H_2O (95:2.5:2.5, 4 mL) affording pure peptide **8b** as a white solid after lyophilization (33 mg, 78% yield). **^{1}H NMR** (400 MHz, D_2O)**:** δ 8.77 (s, 1H), 7.52 (s, 1H), 4.66 (t, J = 5.8 Hz, 1H), 4.60 (t, J = 5.4 Hz, 1H), 4.46-4.37 (m, 5H), 4.04-3.93 (m, 6H), 3.51-3.48 (m, 2H), 3.05 (t, J = 7.5 Hz, 2H), 2.66-2.64 (m, 1H), 2.66-2.64 (m 2H), 2.16-2.13 (m, 1H), 2.07-2.03 (m, 1H), 1.93-1.46 (m, 10H), 1.52-1.46 (m, 2H), 0.98 (d, J = 5.4 Hz, 6H), 0.93 (d, J = 5.7 Hz, 6H). **MS(ESI):** calcd for $C_{38}H_{64}N_{12}O_{10}$ $(M+H)^+$ 849.4941, found 849.4944.

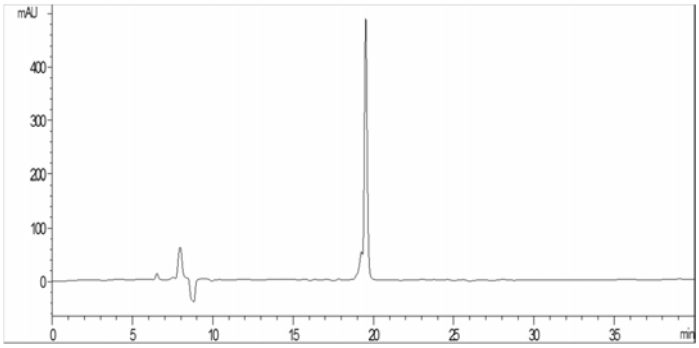
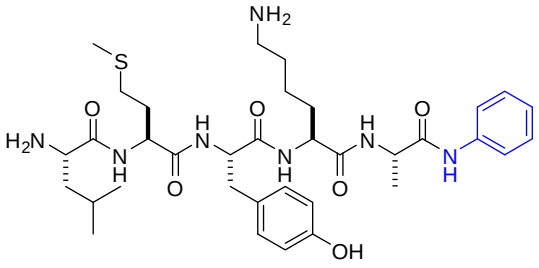


Figure S2. HPLC chromatogram of compound **8b** (linear gradient of 10 to 90% B over 40 min, 0.1% TFA, flow rate: 0.4 ml/min, λ = 220 nm)

LMYKA-aniline (8c)



8c

Peptide **8c** was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. After the peptide-resin was activated and cleavage with aniline (4 equiv.) in the presence of DIPEA (8 equiv.) in DMF (4 mL) for 5 h at room temperature. Then Fmoc was deprotected with 20% piperidine in DMF and global deprotection was effected using a solution of TFA/phenol/water/thioanisole/EDT (82.5:5:5:5:2.5, 4 mL) for 3 h affording pure peptide **8c** as a white solid after lyophilization (22 mg, 66% yield). **^{1}H NMR** (400 MHz, CD_3OD)**:** δ 7.54 (d, J = 8 Hz, 2H), 7.28 (t, J = 7.0 Hz, 2H), 7.09-7.02 (m, 3H), 6.68-6.65 (m, 2H), 4.52-4.42 (m, 3H), 4.35-4.33 (m, 2H), 3.92-3.90 (m, 1H), 3.12-3.10 (m, 1H), 3.00-2.83 (m, 6H), 2.65-2.62 (m, 1H), 2.51-2.45 (m, 2H), 2.04 (s, 3H), 1.65-1.63 (m, 8H), 1.45-1.38 (m, 6H), 0.95-0.94 (m, 6H). **MS(ESI):** calcd for $C_{38}H_{64}N_{12}O_{10}$ $(M+H)^+$ 700.3850, found 700.3851.

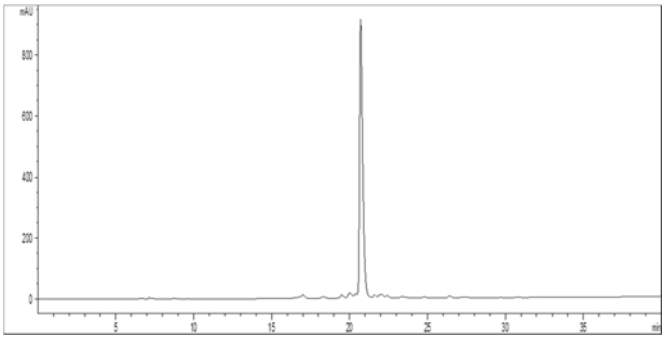
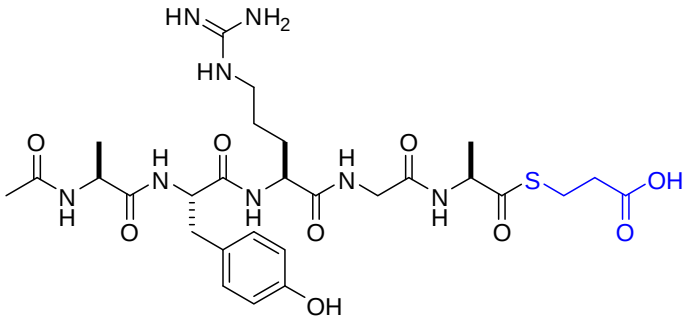


Figure S3. HPLC chromatogram of compound **8c** (linear gradient of 10 to 90% B over 40 min, 0.1% TFA flow rate: 0.4 ml/min, λ = 220 nm)

Ac-AYRGA-mercaptopropionic acid (8d)



8d

Peptide **8d** was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. The Fmoc was deprotected with 20% piperidine in DMF peptide-resin and then added 2 equvi. of acetic anhydride in DMF for 10 min. Thereafter, the peptide-resin was activated and cleavage with 3-mercaptopropionic acid (4 equiv.) in the presence of DIPEA (8 equiv.) in DMF (4 mL) for 2 h at room temperature. The global deprotection was effected using a solution of TFA/TIS/H_2O (95:2.5:2.5, 4 mL) affording pure peptide **8d** as a white solid after lyophilization (27 mg, 80% yield). **^{1}H NMR** (400 MHz, CD_3OD)**:** δ 7.03 (d, J = 8.5 Hz, 2H), 6.68 (d, J = 8.5 Hz, 2H), 4.49-4.44 (m, 2H), 4.30-4.27 (m, 1H), 4.20-4.1 (m, 1H), 3.87 (s, 2H), 3.29-3.28 (m, 1H), 3.25-3.21 (m, 2H), 3.04-3.01 (m, 3H), 2.91-2.84 (m, 1H), 2.54 (t, J = 7.0 Hz, 2H), 1.93 (s, 3H), 1.88-1.84 (m, 1H), 1.59-1.55 (m, 2H), 1.34 (d, J = 7.3 Hz, 3H), 1.23 (d, J = 7.2 Hz, 3H). **MS(ESI):** calcd for $C_{38}H_{64}N_{12}O_{10}$ $(M+H)^+$ 667.2868, found 667.2865.

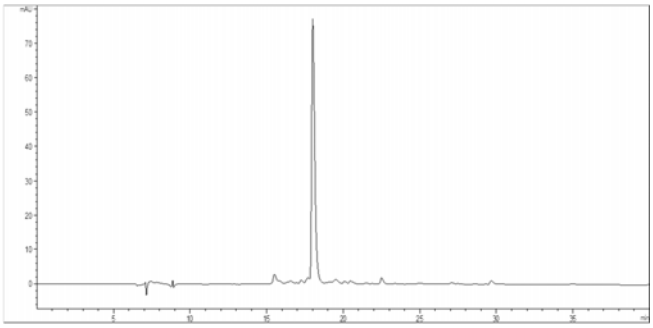
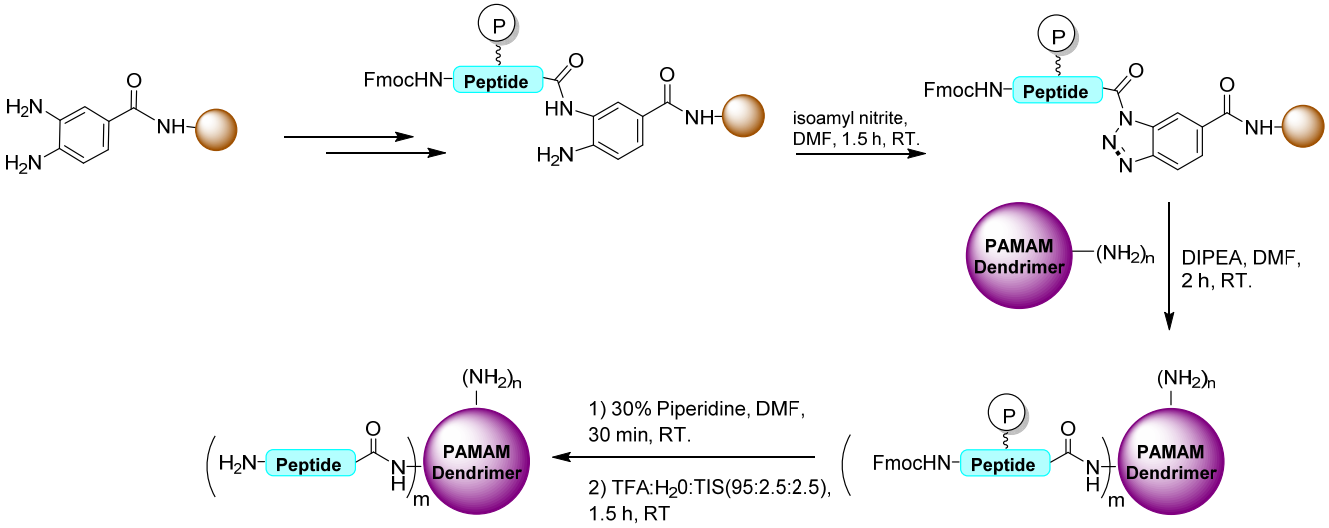


Figure S4. HPLC chromatogram of compound **8d** (linear gradient of 10 to 90% B over 40 min, 0.1% TFA, flow rate: 0.4 ml/min, λ = 220 nm).

Preparation of peptide decorated dendrimer

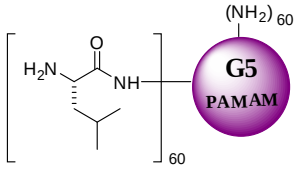
Scheme S2:



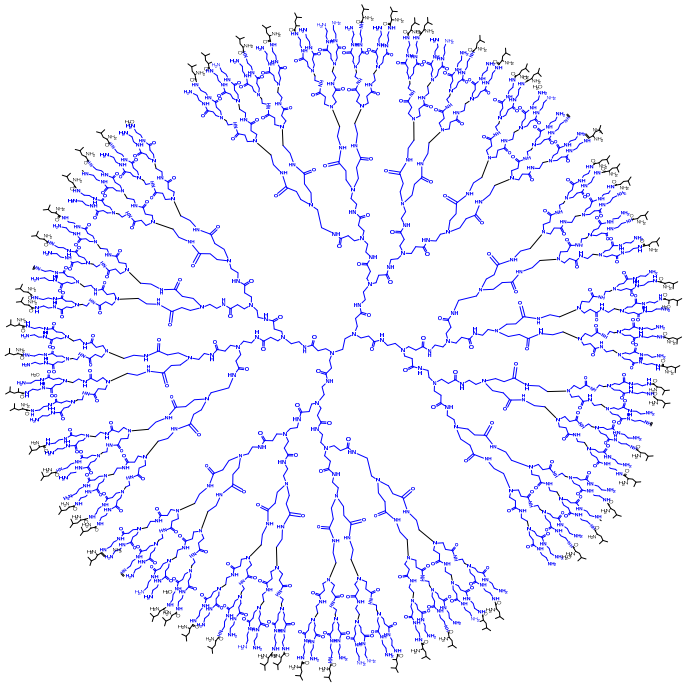
Peptide was prepared according to Fmoc-strategy SPPS on rink amide resin (0.2 mmol), as outlined in the general procedures. At the end of the synthesis, the resin-bound *o*-aminoanilide-peptide was washed with DMF and DCM, then treated with isoamyl nitrite (10 equiv.) at room temperature for 90 min. The resin was filtered, washed with DCM (3 mL x 3). The solvent DMF (4 ml) with DIPEA (8 equiv.) was bubbled with N_2 for 20 min. Then, it was treated with peptidyl resin and PAMAM dendrimer. After 2 h, the resin was washed with DMF and the solution was concentrate *in vacuo*. The resulting product was deprotected by using 20% piperidine in DMF for 30 min and then precipitated with cold ether (25 mL x 2) and collected by centrifugation. After that, the product was concentrate under *vacuo* and then treated with TFA/TIS/H_2O (95:2.5:2.5 v/v/v, 4 mL) was used to remove the protecting group at 25 °C for 1.5 h. Finally, the resulting peptide decorated dendrimer was allowed to precipitated with cold ether (25 mL x 2) and collected by centrifugation. The supernatant was removed and the residue was dissolved in 30% ACN/H_2O and lyophilized to afford pure product without further purification.

PDI value was obtained from Gel Permeation chromatogram(GPC) instrumentation. Conditions: preparation of compound **11a-f** (1 mg/ml in H_2O); TSK G2000SW column; elute: 0.1 % TFA in H_2O; follow rate: 1 ml/min; detector: UV detector @ 214nm. A typical combined SEC of a reference G0-G7 PAMAM dendrimer having MW from 517 to 116,000 dalton.

(G:5)-*dendric*- PAMAM- (Leu)$_{60}$ (8e)



8e



White solid (11 mg, 79% yield). **^{1}H NMR** (400 MHz, D_2O)**:** δ 3.95-3.91 (m, 60H), 3.64-3.30 (m, 905H), 2.82-2.76 (m, 312H), 1.70-1.59 (m, 195H), 0.91-0.90 (m, 362H). PDI = 1.07.

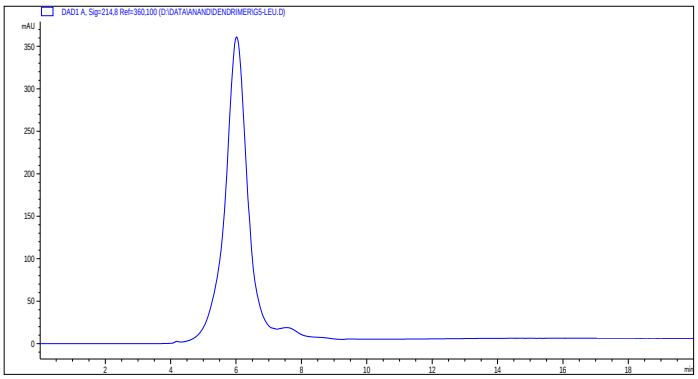
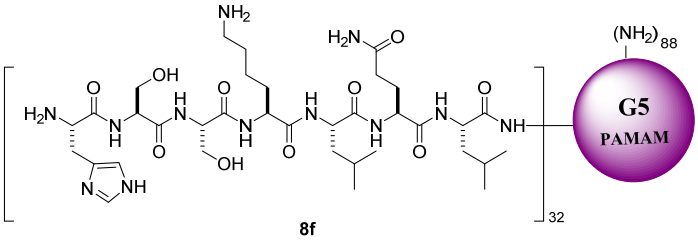
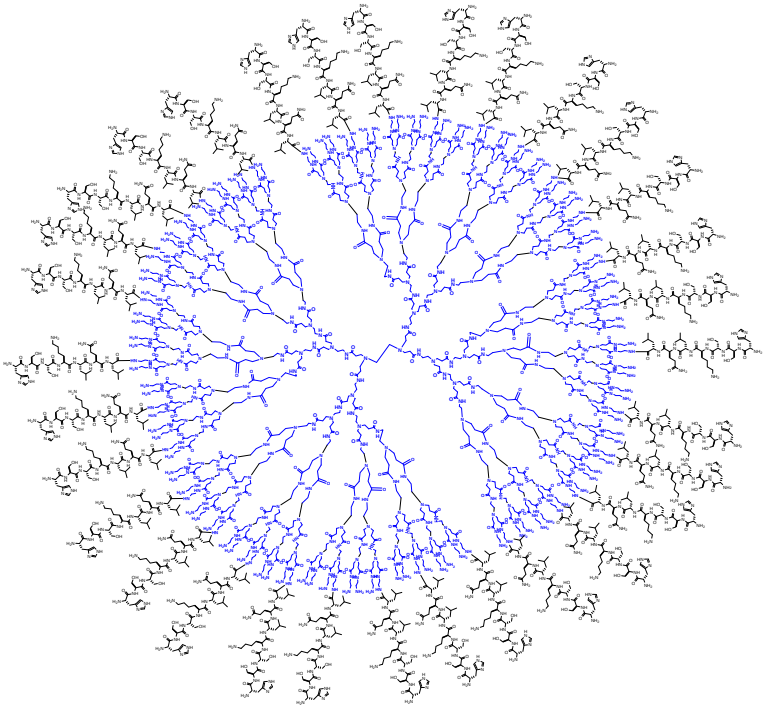


Figure S5. GPC chromatogram of peptide decorated dendrimer **8e** (isocratic of 0.1% TFA in DIW over 30 min, flow rate: 1.0 ml/min, λ = 214 nm)

(G:5)-*dendric*-PAMAM-(LQLKSSH)$_{32}$ (8f)



8f



$C_{2382}H_{4416}N_{858}O_{572}$
Exact Mass: 54198.2840

White solid (12 mg, 70 %). **^{1}H NMR** (400 MHz, D_2O)**:** δ 8.77 (s, 32H), 7.52 (s, 32H), 4.38-4.37 (m, 212H), 3.94 (m, 210H), 3.72-3.22 (m, 1739H), 3.05 (m,130H), 2.88-2.70 (m, 529H), 2.41(m, 103H), 1.82-1.37 (m, 410H), 0.98-0.93 (m, 388H). PDI = 1.10.

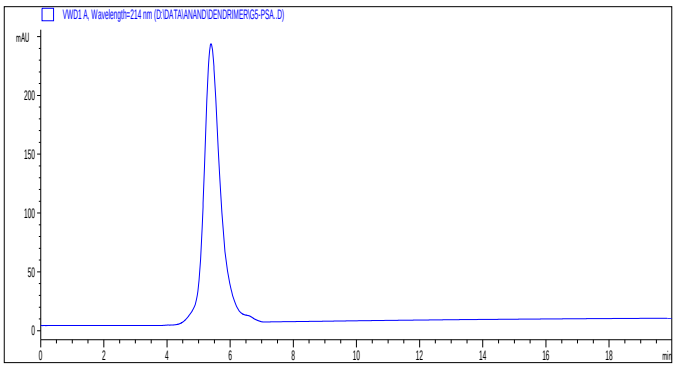


Figure S6. GPC chromatogram of peptide decorated dendrimer **8f** (isocratic of 0.1% TFA in DIW over 30 min, flow rate: 1.0 mL/min, λ = 214 nm)

Gel Permeation Chromatography

The experiments were conducted at 25°C at a flow rate of 1.0 mL/min with 0.1% TFA in DIW as eluent and detected at 214 nm. The results of filtration were followed with the figures that (**8a**) (9.323 min), ((G:5)-*dendri*-PAMAM -$(NH_2)_{128}$) (6.774 min), (**8e**) (6.016 min) and (**8f**) (5.395 min) are discovered.

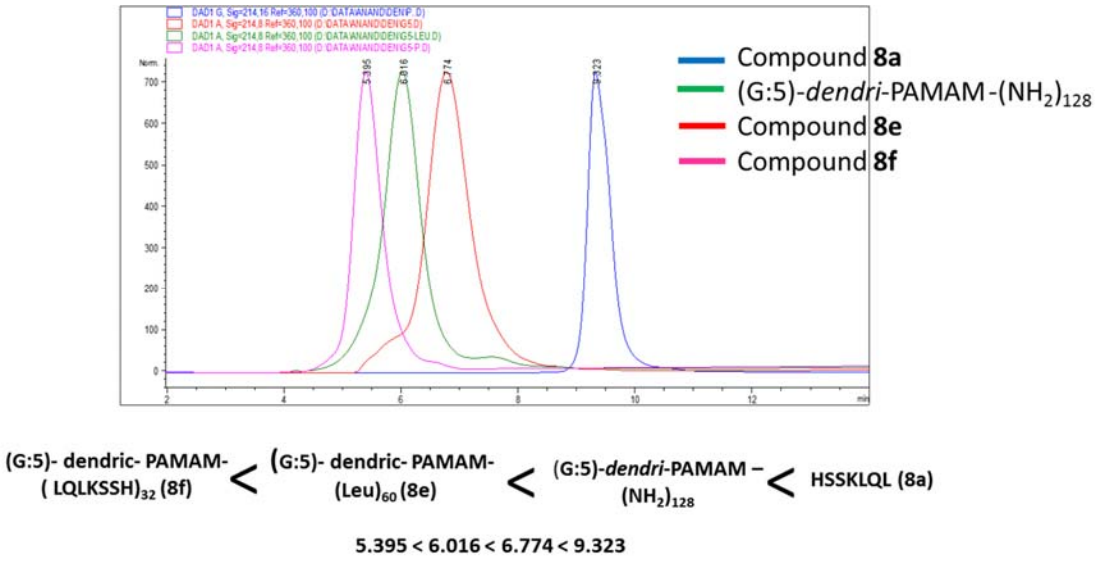


Figure S7. GPC chromatogram of compound **8a**, (G:5)-*dendri*-PAMAM -$(NH_2)_{128,}$ **8e** and **8f**.

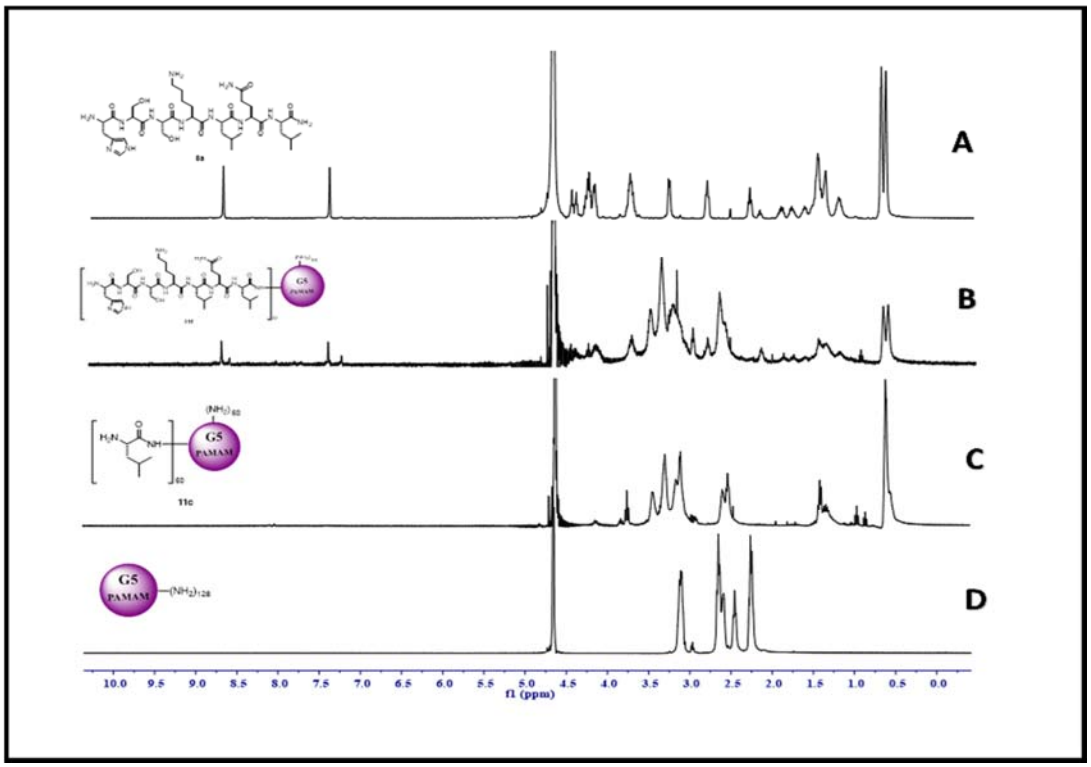
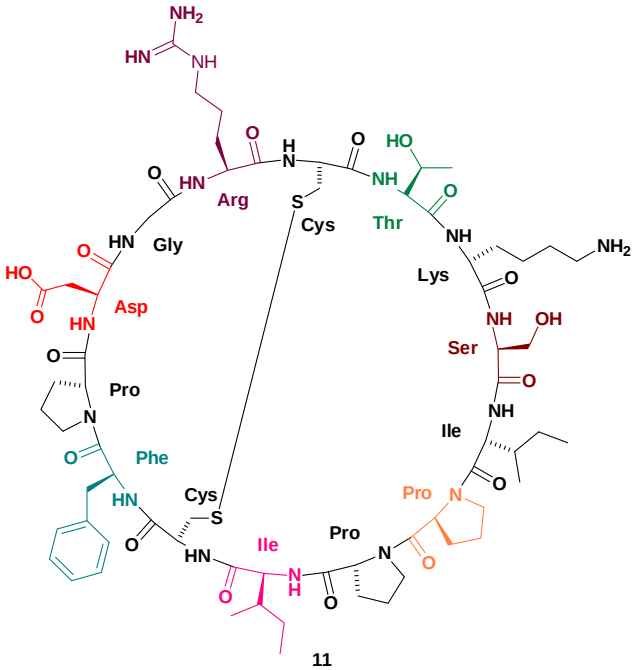


Figure S8. ^{1}H NMR Spectrum of Compound **8a, 8f, 8e** and (G:5)-*dendri*-PAMAM -$(NH_2)_{128}$

Preparation of cyclic peptide



The linear SFTI-1 peptide was prepared according to Fmoc-strategy SPPS on rink amide resin (0.05 mmol), as outlined in the general procedures. At the end of the synthesis, the Fmoc protected peptide-resin was deprotected with 20% piperidine in DMF and washed with DMF (3 mL x 3). The free N-terminal amine resin-bound *o*-aminoanilide-peptide is activated with isoamyl nitrite (10 equiv.) at room temperature for 90 min. The resin was filtered, washed with DCM (3 mL x 3). The on resin N-terminal amine is reacted with C-terminal acid and cleaved from resin in the presence of DIPEA (4 equiv.) in DMF (3 mL) for 5 h at room temperature. Then global deprotection was effected using a solution of TFA/TIS/H_2O (95:2.5:2.5, 4 mL) affording the oxidized SFTI-1 peptide was acquired **11** as a white solid after lyophilization (12 mg, 42% yield). **^{1}H NMR** (600 MHz, D_2O)**:** δ 8.23 (s, 1H), 7.51-7.15 (m, 5H), 4.58-4.24 (m, 6H), 4.23-4.05 (m, 2H), 4.06-3.57 (m, 8H), 3.56-3.27 (m, 4H), 3.17 (m, 3H), 2.94 (m, 5H), 2.24 (m, 4H), 2.17-1.78 (m, 10H), 1.75 (m, 4H), 1.57-1.34 (m, 4H), 1.29-1.04 (m, 4H), 1.02-0.66 (m, 8H). Mass (MALDI, m/z) calcd for $C_{67}H_{105}N_{18}O_{18}S_2$ $(M+H)^+$ 1513.7, found 1513.6.

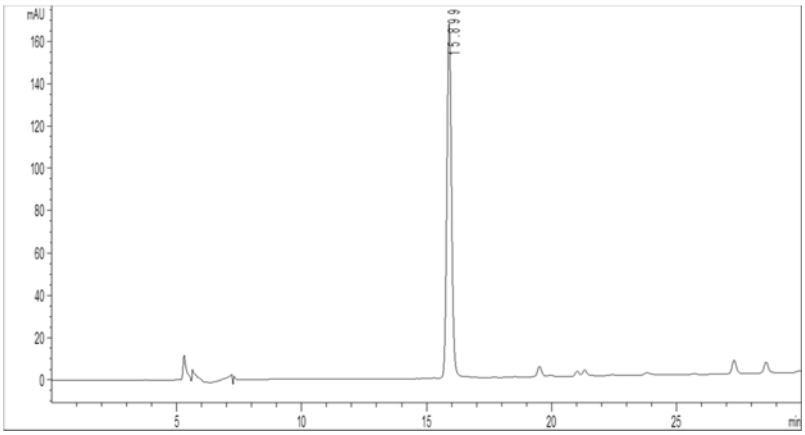


Figure S9. HPLC chromatogram of compound **11** (linear gradient of 10 to 90% B over 30 min, 0.1% TFA, flow rate: 0.5 ml/min, λ = 220 nm)

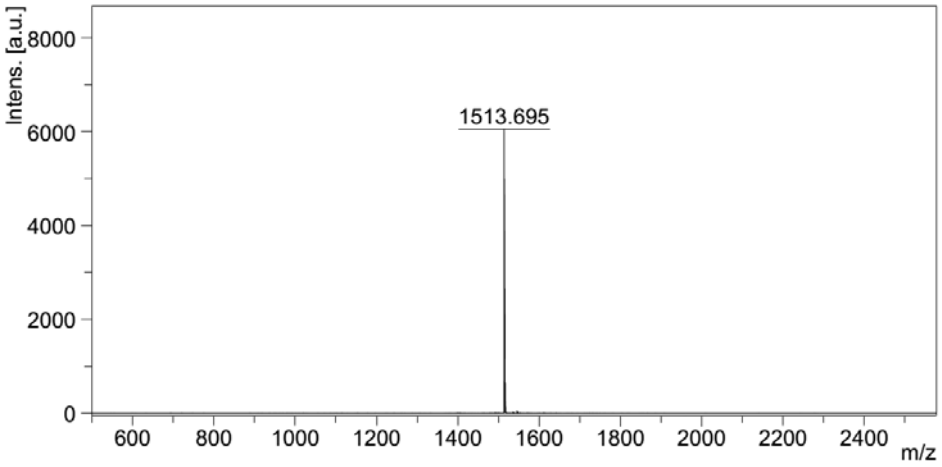


Figure S10. MALDI-TOF MS spectrum of cyclic peptide **11**

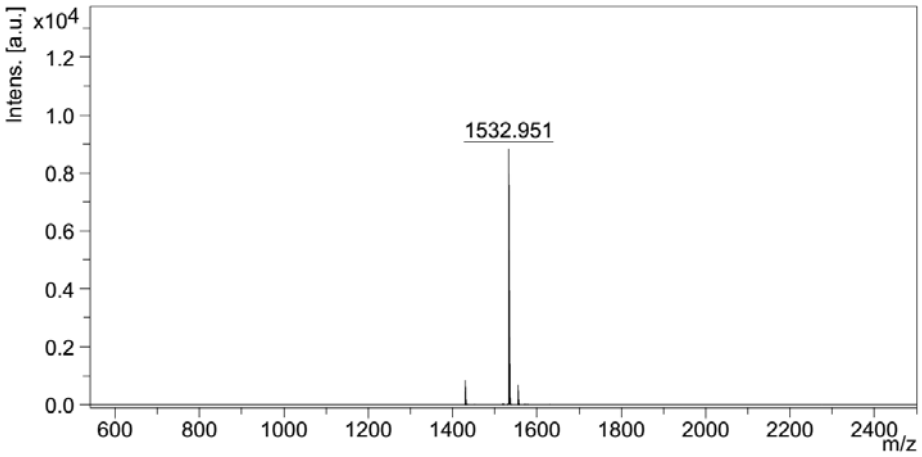
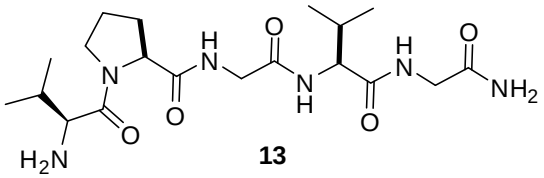


Figure S11. MALDI-TOF MS spectrum of linear peptide SFTI-1.

Synthesis of VPGVG (13)

Peptide was prepared according to the Fmoc-strategy SPPS on rink amide resin (0.1 mmol/g loading; 0.78 mmol). The peptide was synthesized on an automated PS3 peptide synthesizer using Fmoc-Xaa-OH (2 equiv.), NMM (4 equiv.) and HBTU (2 equiv.). The coupling was performed for 60 min and Fmoc-deprotection was achieved using 20% piperidine (10 min x 2). After washing the resin, the cleavage was carried out by treating the resin with cleavage cocktail TFA: H_2O (95:5) for 1.5 h. Then the solution was precipitation with cold Et_2O and centrifugation the crude peptides can be obtained. After lyophilisation, the desired peptide was afford in 86 % yield. **^{1}H NMR** (400 MHz, D_2O)**:** δ 4.39- 4.35 (m, 1H), 4.06-4.02 (m, 2H), 3.85-3.77 (m, 4H), 3.68-3.50 (m, 2H), 2.21-1.81 (m, 6H), 0.98 (d, J = 7.0 Hz, 3H), 0.88 (d, J = 6.9 Hz, 3H), 0.82 (dd, J = 6.8, 5.3 Hz, 6H). **ESI MS** calcd for $C_{19}H_{35}N_6O_5$ $(M+H)^+$ 427.2, found 427.2.



13

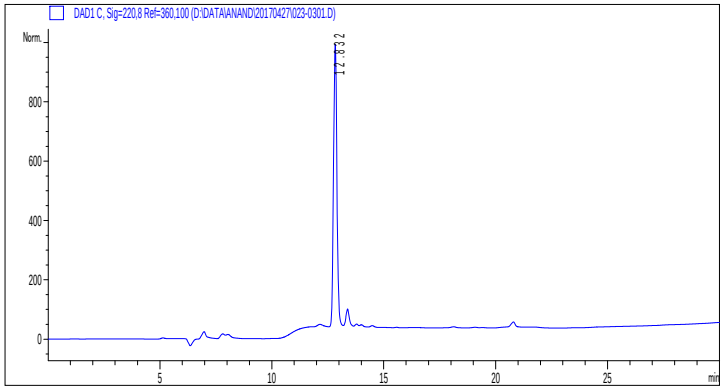


Figure S12. HPLC chromatogram of compound **13** (linear gradient of 10 to 90% B over 30 min, 0.1% TFA, flow rate: 0.4 ml/min, λ = 220 nm)

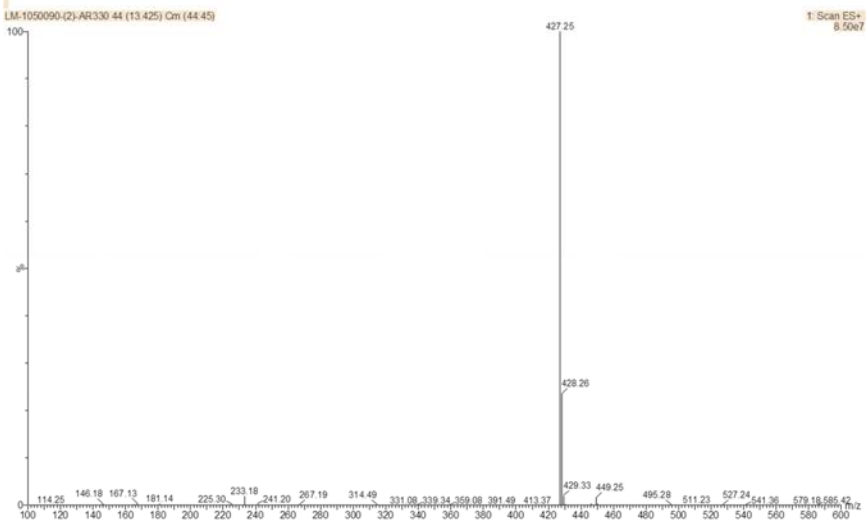
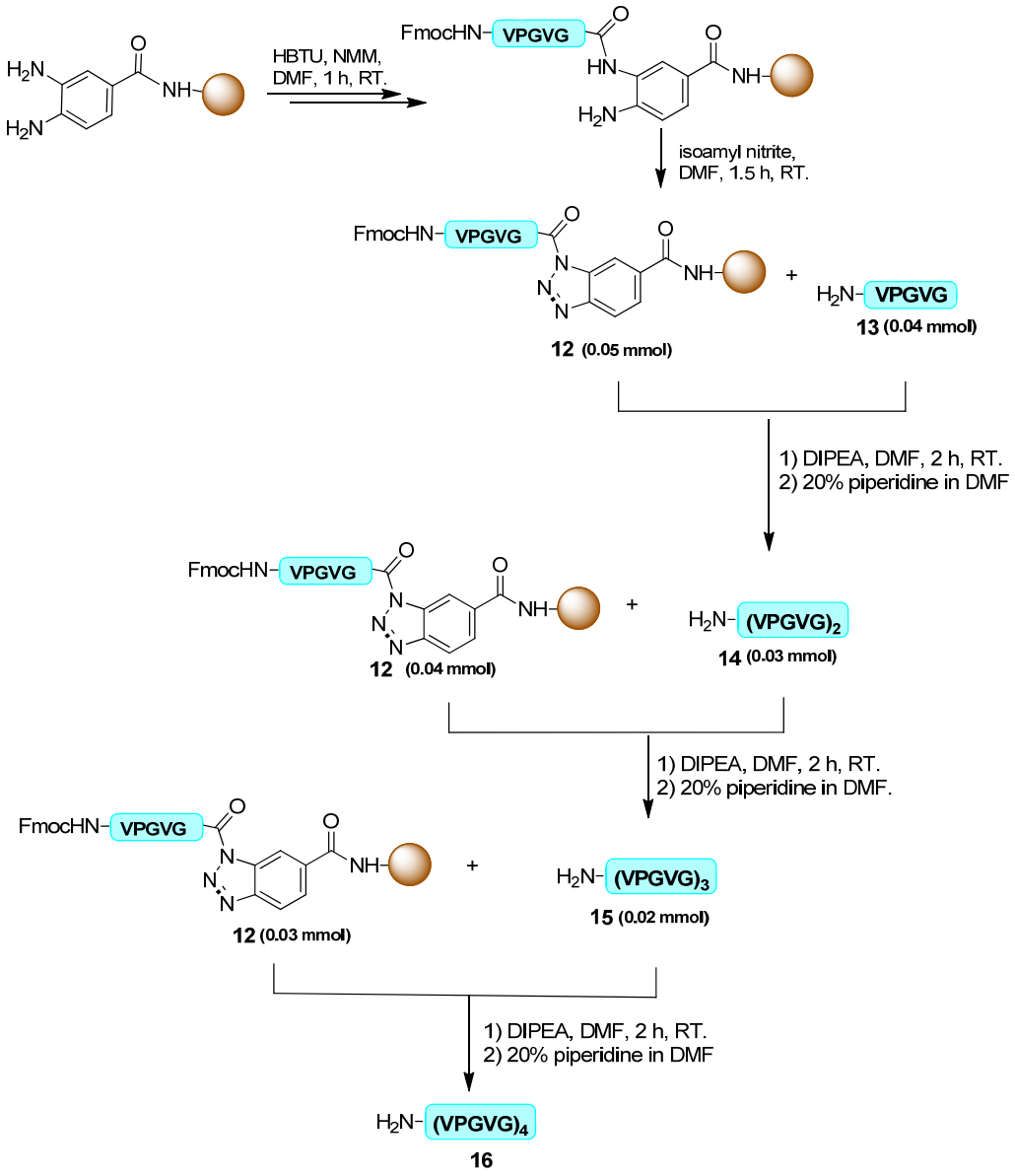


Figure S13. ESI-MS spectrum of compound **13**

General procedure for preparation of elastin peptide

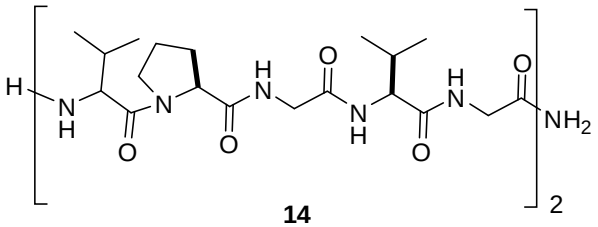
Scheme S3:



Peptide was prepared according to Fmoc-strategy SPPS on rink amide resin, as outlined in the general procedures. The resin-bound *o*-aminoanilide peptide was washed with DMF, then treated with isoamyl nitrite (10 equiv.) in DMF at room temperature for 90 min. After the resin was filtered, washed sequentially with DCM (3 mL x 3). The peptide nucleophile and DIPEA (4 equiv.) in DMF was bubbled with N_2 for 20 min and then immediately added into the activated peptidyl-resin. After 2 h, the resin was washed with DMF (3 mL x 3) and the supernatant was concentrate. The resulting product was deprotected by using 20% piperidine in DMF for 30 min, thereafter completely removing the DMF. The peptide was precipitated in cold ether and collected by centrifugation. The supernatant was removed and the residue was dissolved in 30% acetonitrile/H_2O and lyophilized to afford pure product without further purification.

$(VPGVG)_2$ (14)

Peptide **7g** (0.05 mmol) was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. After the peptide-resin was activated and cleavage with compound **13** (17 mg, 0.04 mmol) in the presence of DIPEA (4 equiv.) in DMF for 2 h at room temperature. Then Fmoc was deprotected with 20% piperidine in DMF affording pure peptide **14** as a white solid after lyophilization (31 mg, 92% yield). **^{1}H NMR** (400 MHz, D_2O)**:** δ 4.38-4.27 (m, 3H), 4.06-4.01 (m, 3H), 3.85-3.65 (m, 9H), 3.78-3.50 (m, 3H), 2.26-1.77 (m, 12H), 0.99 (d, J = 7.0 Hz, 3H). 0.92-0.78 (m, 21H). Mass (MALDI, m/z) calcd for $C_{38}H_{66}N_{11}O_{10}$ $(M+H)^+$ 836.4, found 836.5.



14

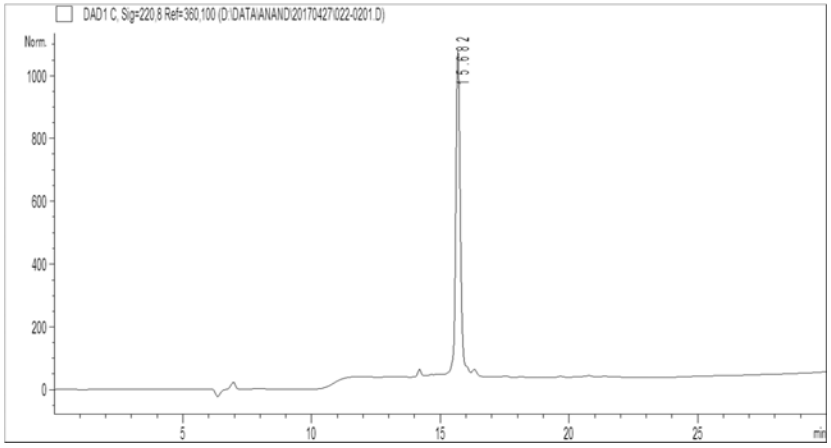


Figure S14. HPLC chromatogram of compound **14** (linear gradient of 10 to 90% B over 30 min, 0.1% TFA, flow rate: 0.5 ml/min, λ = 220 nm)

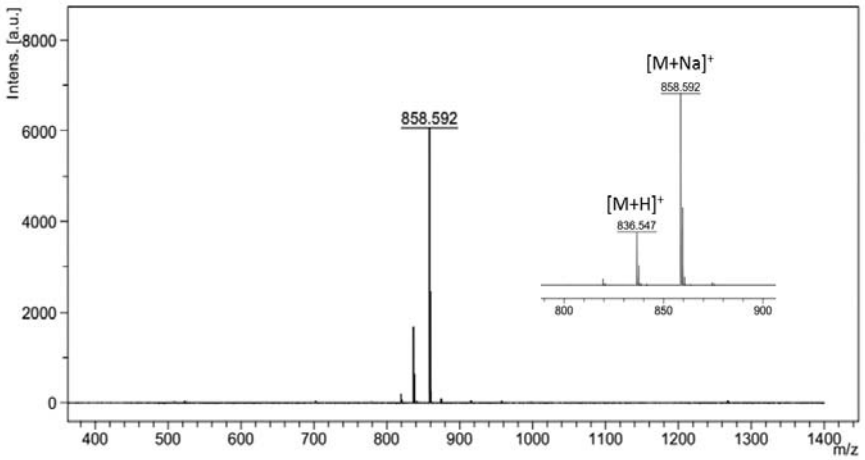
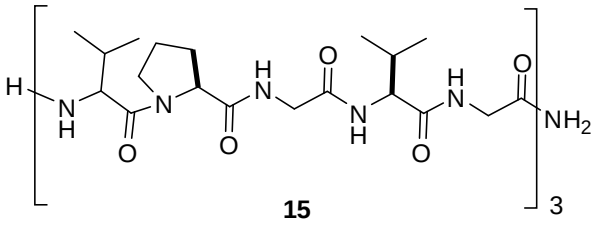


Figure S15. MALDI-TOF MS spectrum of compound **14**

$(VPGVG)_3$ (15)

Peptide **7g** (0.04 mmol) was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. After the peptide-resin was activated and cleavage with compound **14** (25 mg, 0.03 mmol) in the presence of DIPEA (4 equiv.) in DMF for 2 h at room temperature. Then Fmoc was deprotected with 20% piperidine in DMF affording pure peptide **15** as a white solid after lyophilization (35 mg, 93% yield). **^{1}H NMR** (400 MHz, D_2O)**:** δ 4.38-4.27 (m, 4H), 4.06-400 (m, 4H), 3.84-3.77 (m, 12H), 3.66-3.50 (m, 4H), 2.21-1.78 (m, 18H), 0.99 (d, J = 7.0 Hz, 3H). 0.92-0.79 (m, 33H). Mass (MALDI, m/z) calcd for $C_{57}H_{96}N_{16}NaO_{15}$ $(M+Na)^+$ 1267.7, found 1267.5.



15

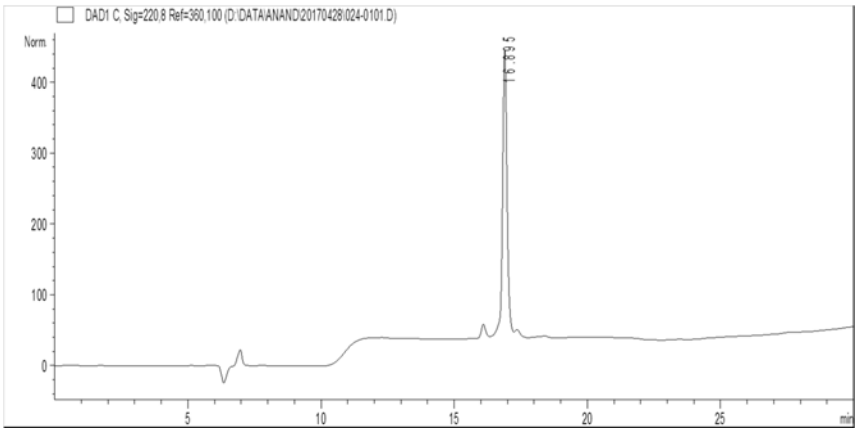


Figure S16. HPLC chromatogram of compound **15** (linear gradient of 10 to 90% B over 0 min, 0.1% TFA, flow rate: 0.5 ml/min, λ = 220 nm)

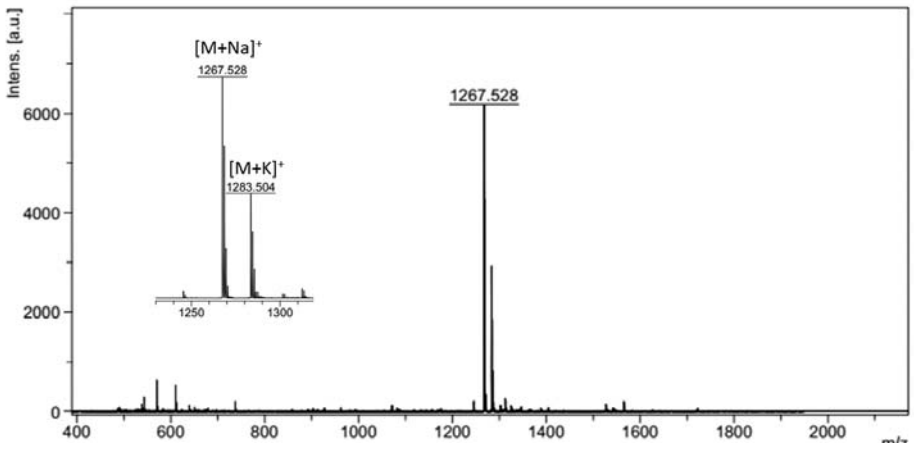
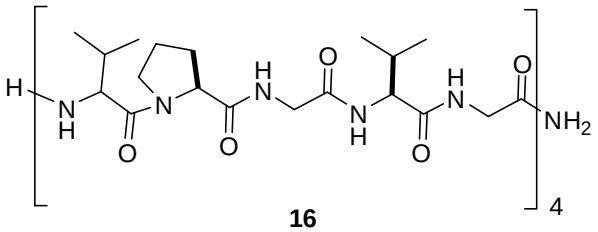


Figure S17. MALDI-TOF MS spectrum of compound **15**

$(VPGVG)_4$ (16)

Peptide **7g** (0.03 mmol) was prepared according to Fmoc-strategy SPPS, as outlined in the general procedures. After the peptide-resin was activated and cleavage with compound **15** (24 mg, 0.02 mmol) in the presence of DIPEA (4 equiv.) in DMF for 2 h at room temperature. Then Fmoc was deprotected with 20% piperidine in DMF affording pure peptide **16** as a white solid after lyophilization (30 mg, 90% yield). **^{1}H NMR** (400 MHz, D_2O)**:** δ 4.36-4.28 (m, 6H), 4.06-4.01 (m, 5H), 3.84-3.72 (m, 20H), 3.65-3.50 (m, 5H), 2.24-1.78 (m, 24H), 0.99 (d, J = 7.0 Hz, 3H), 0.91-0.71 (m, 45H). Mass (MALDI, m/z) calcd for $C_{76}H_{127}N_{21}NaO_{20}$ $(M+Na)^+$ 1676.9, found 1676.6.



16

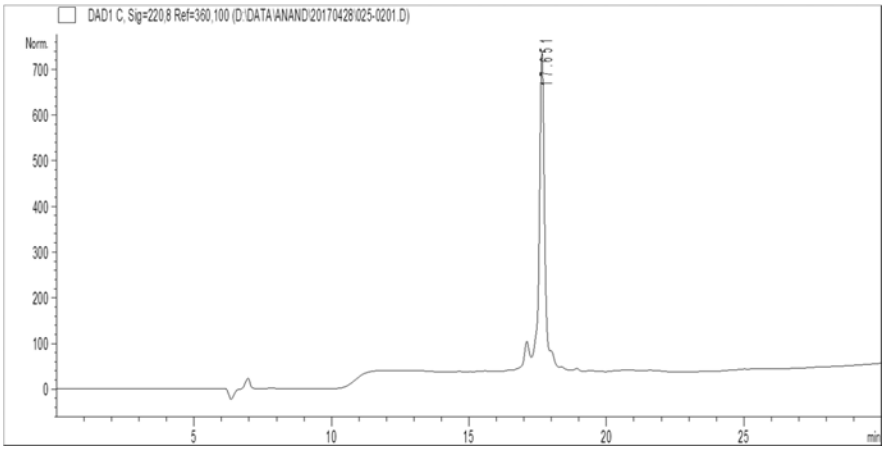


Figure S18. HPLC chromatogram of compound **16** (linear gradient of 10 to 90% B over 30 min, 0.1% TFA, flow rate: 0.5 ml/min, λ = 220 nm).

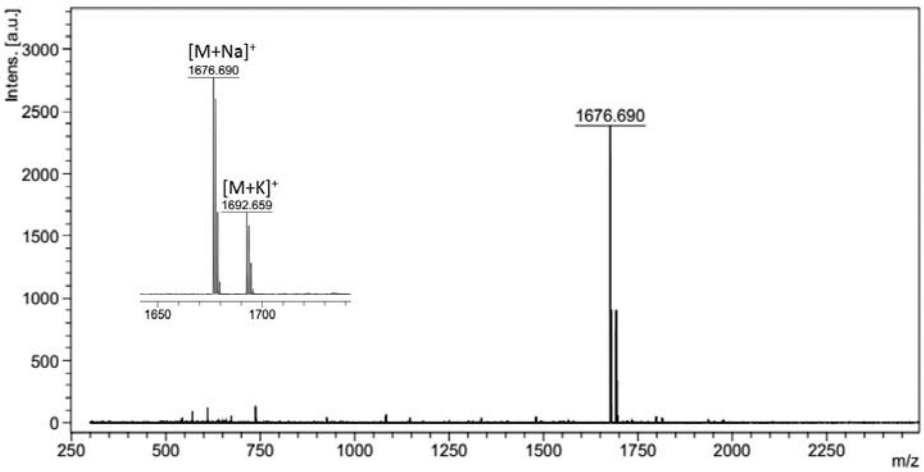


Figure S19. MALDI-TOF MS spectrum of compound **16**

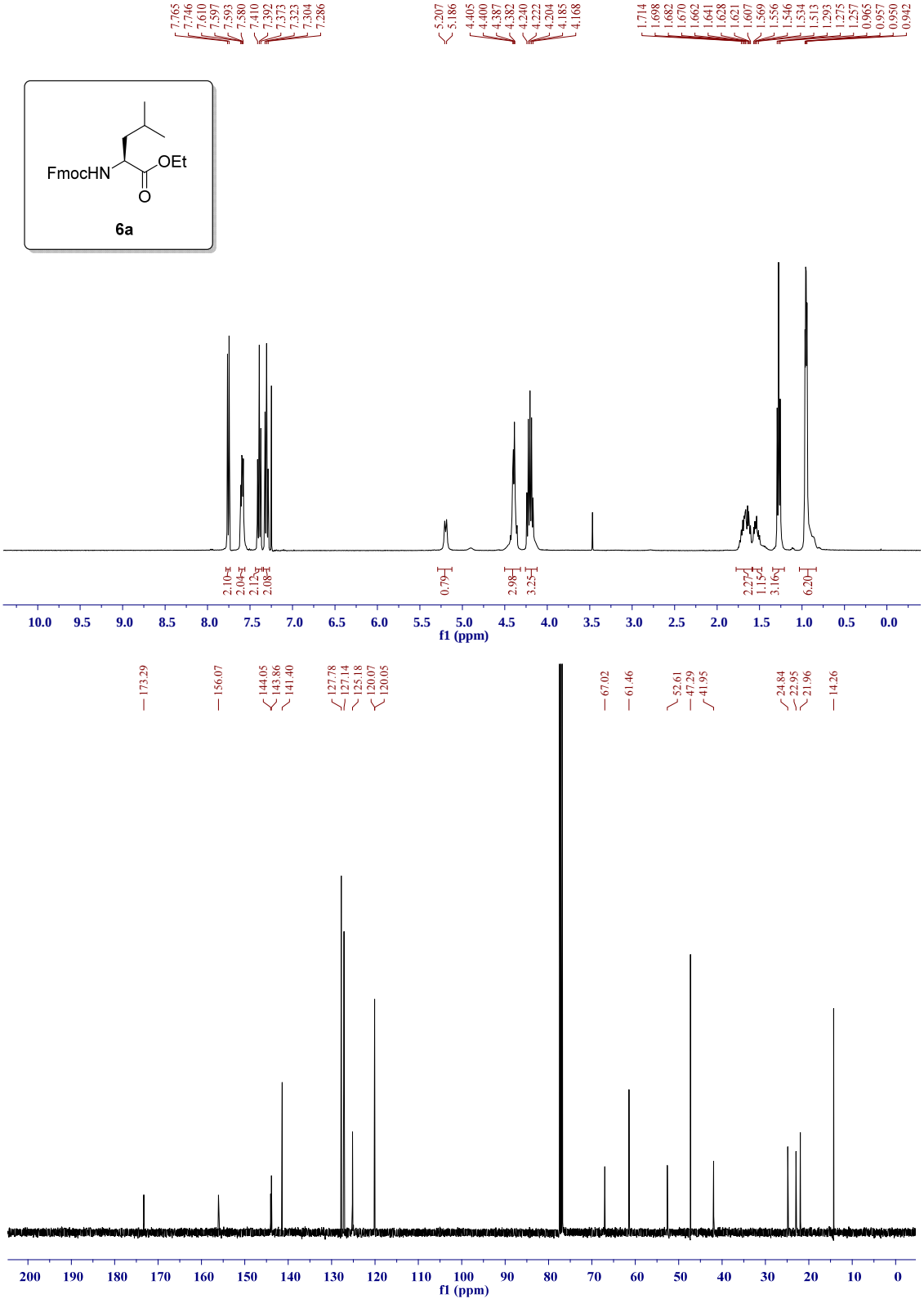


Figure S20. ^{1}H and ^{13}C NMR spectrum of compound **6a**

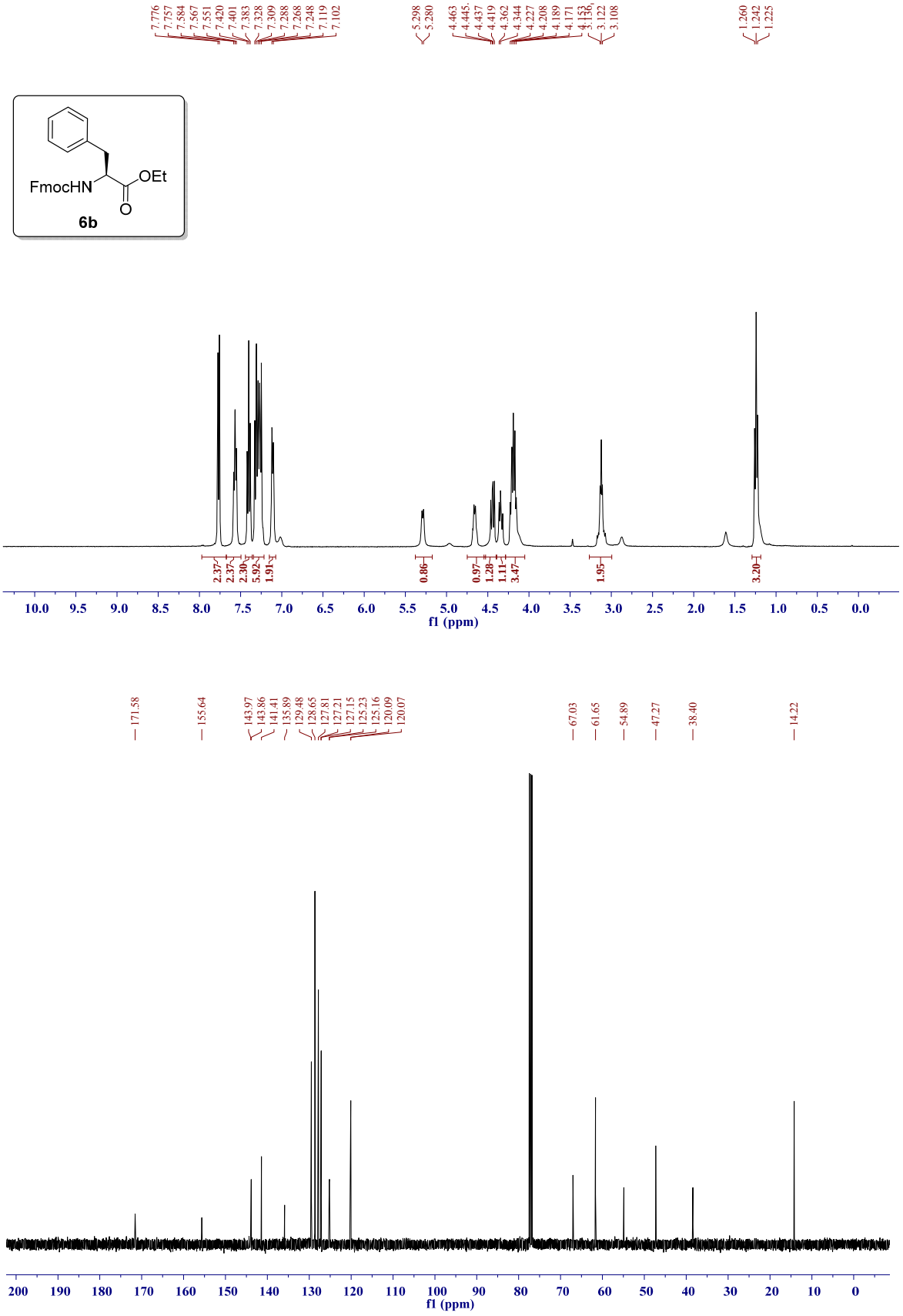


Figure S21. ^{1}H and ^{13}C NMR spectrum of compound **6b**

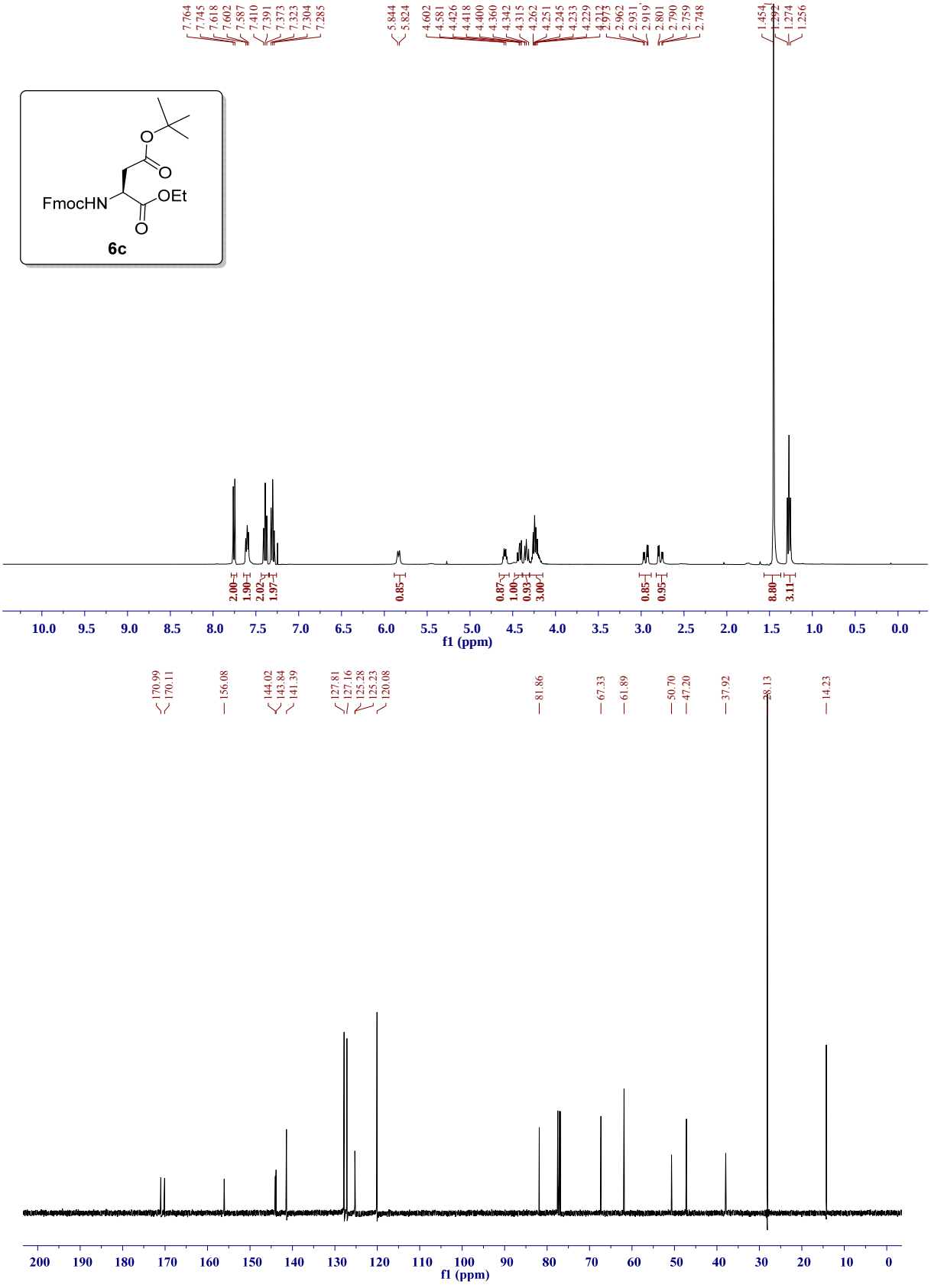


Figure S22. ^{1}H and ^{13}C NMR spectrum of compound **6c**

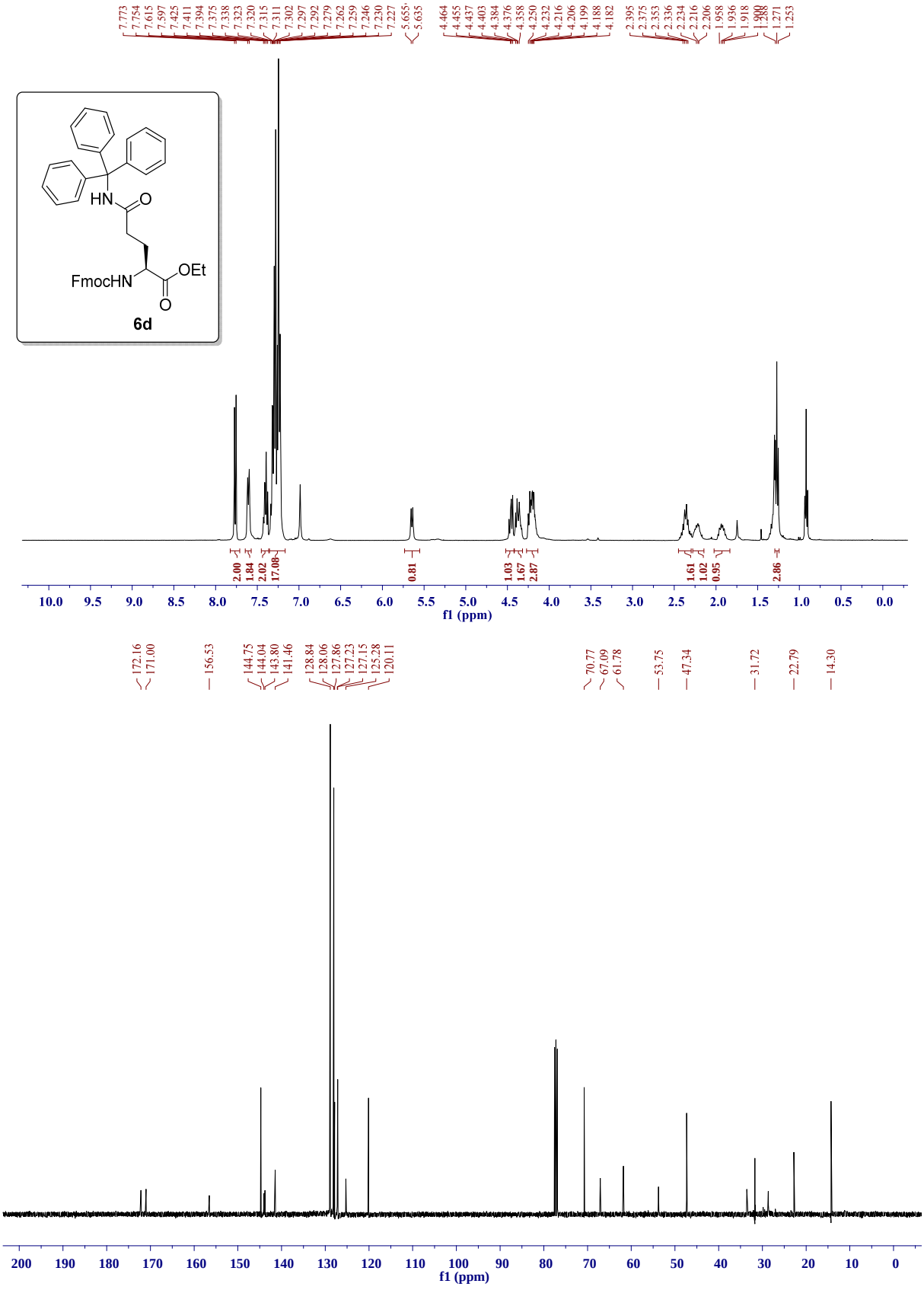


Figure S23. ^{1}H and ^{13}C NMR spectrum of compound **6d**

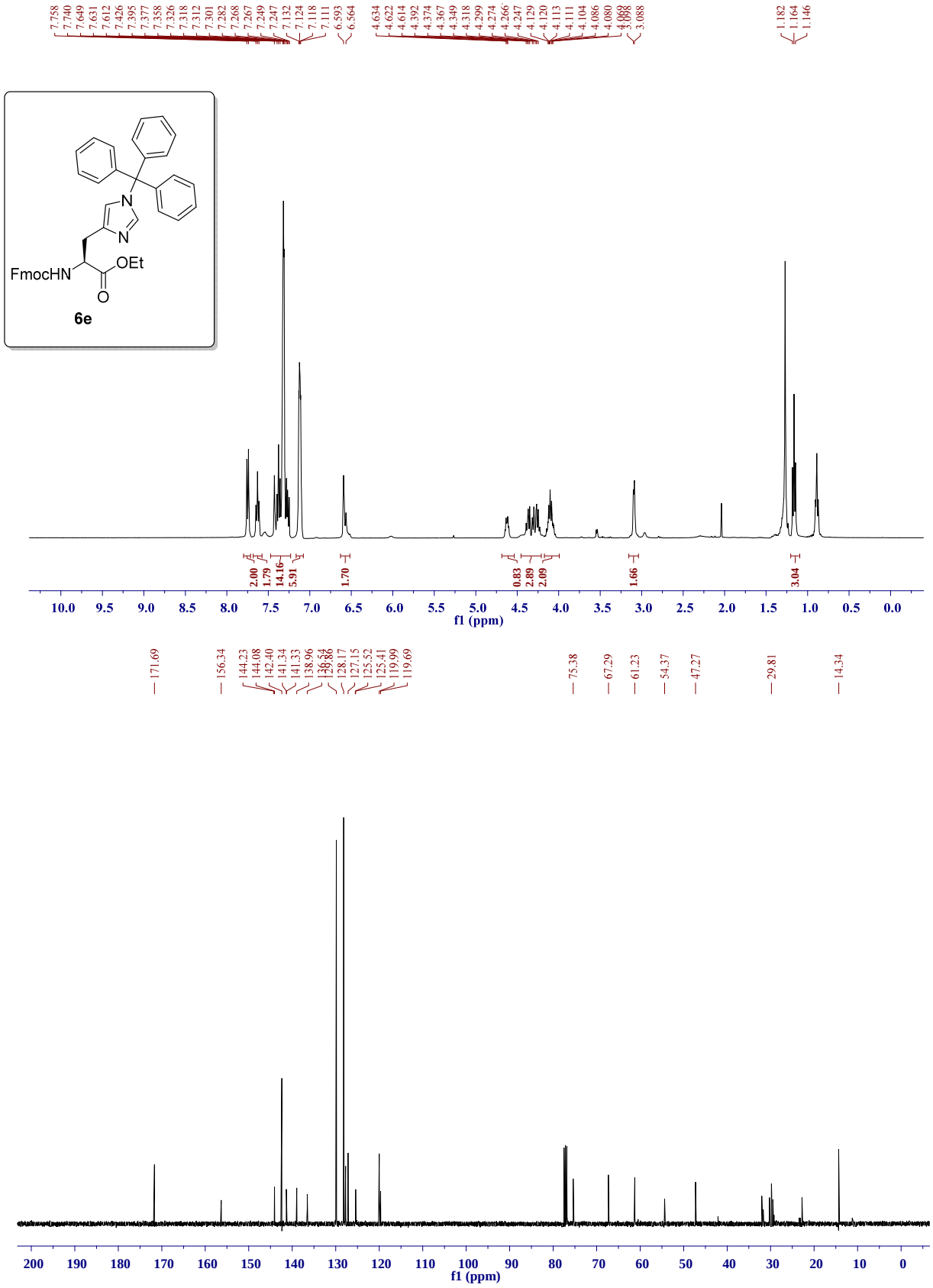


Figure S24. ^{1}H and ^{13}C NMR spectrum of compound **6e**

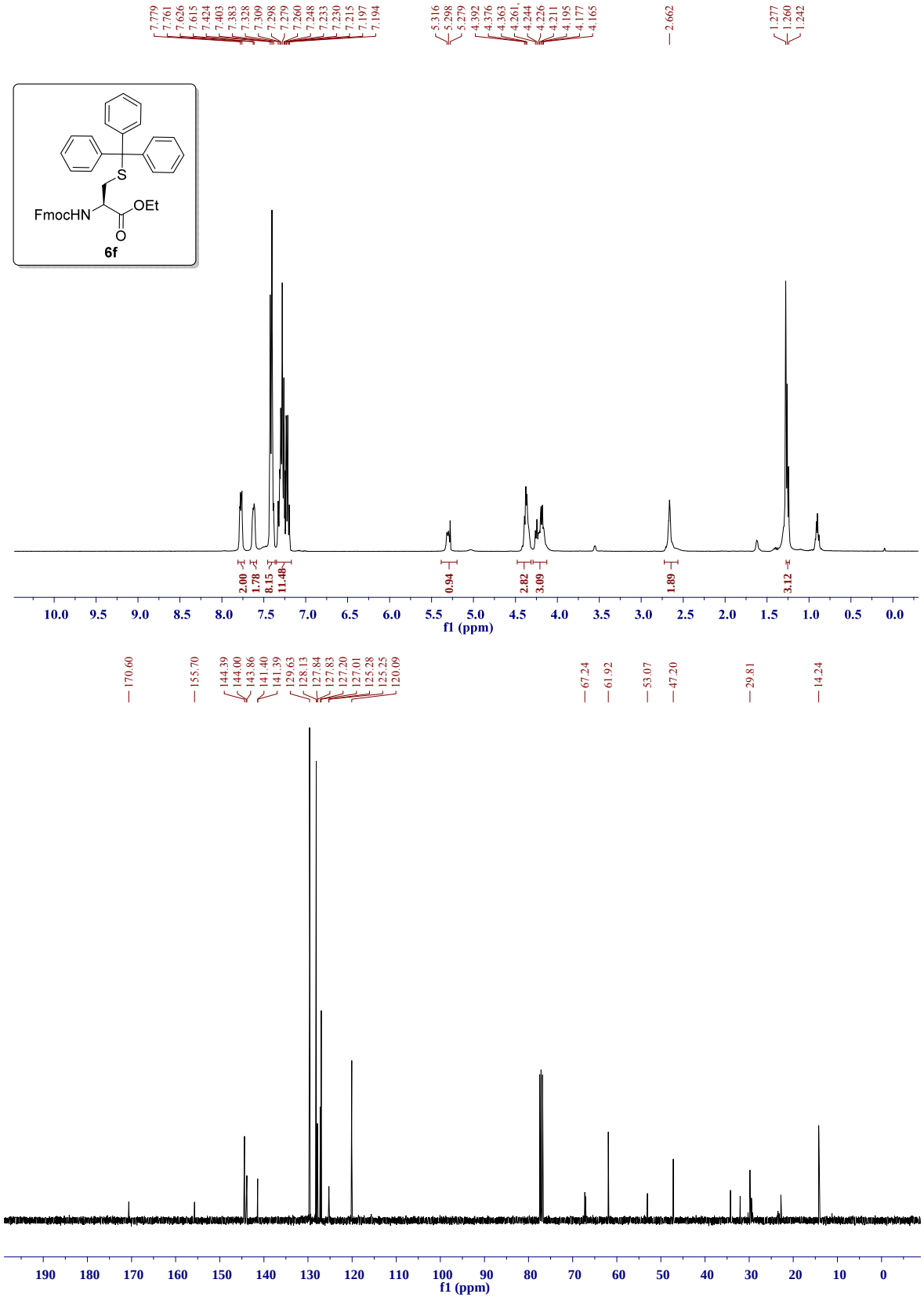


Figure S25. ^{1}H and ^{13}C NMR spectrum of compound **6f**

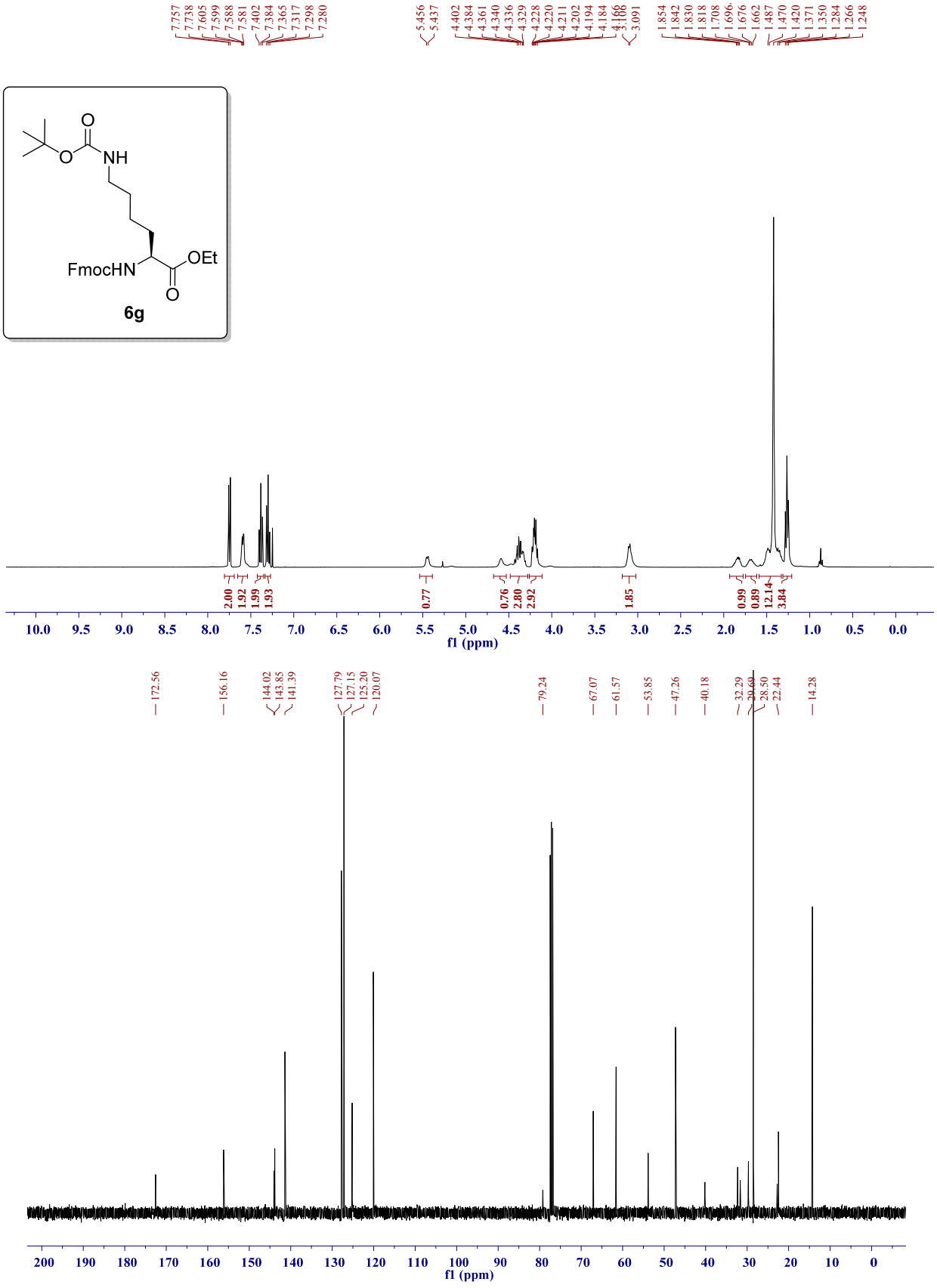


Figure S26. ^{1}H and ^{13}C NMR spectrum of compound **6g**

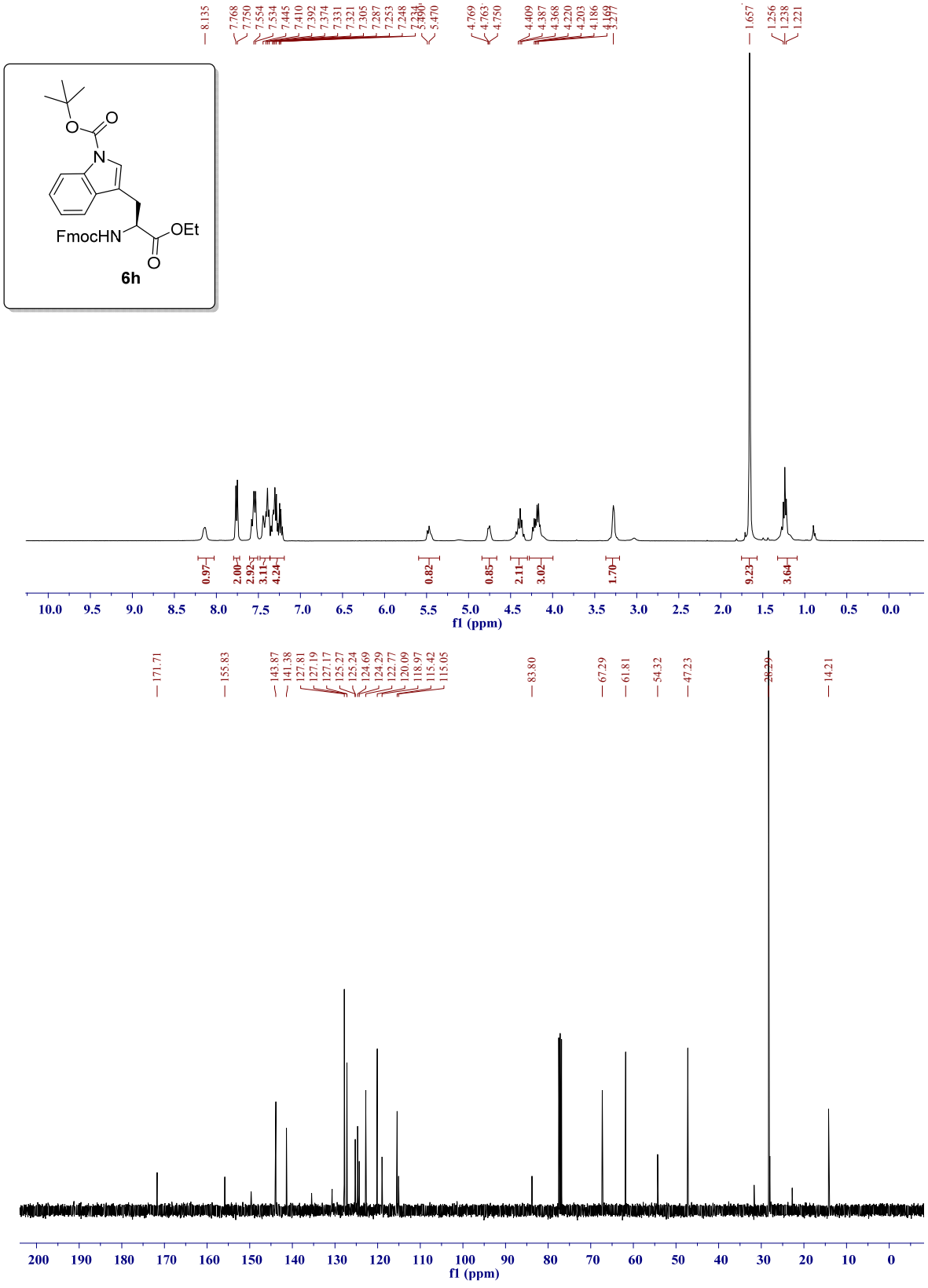


Figure S27. ^{1}H and ^{13}C NMR spectrum of compound **6h**

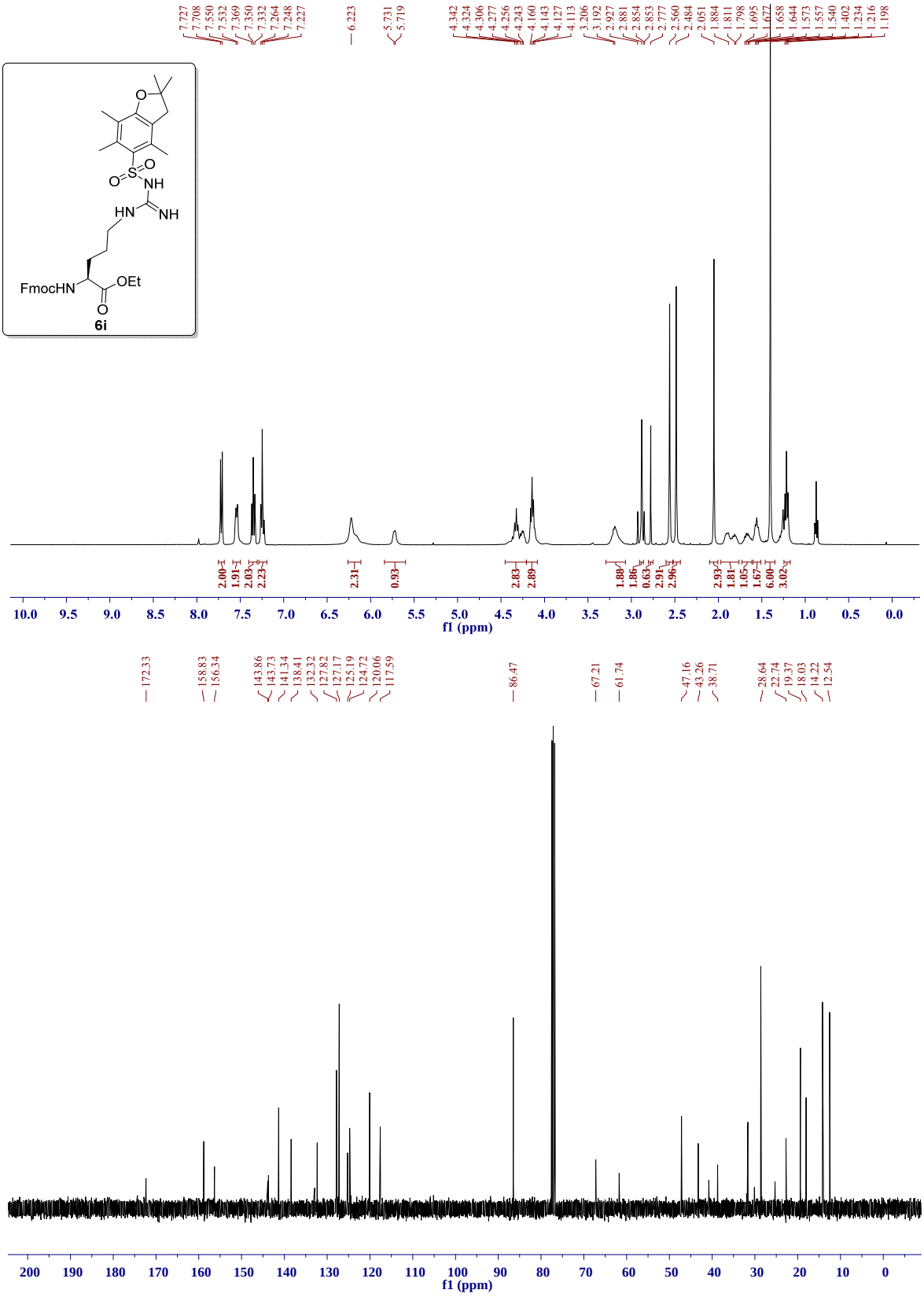


Figure S28. ^{1}H and ^{13}C NMR spectrum of compound **6i**

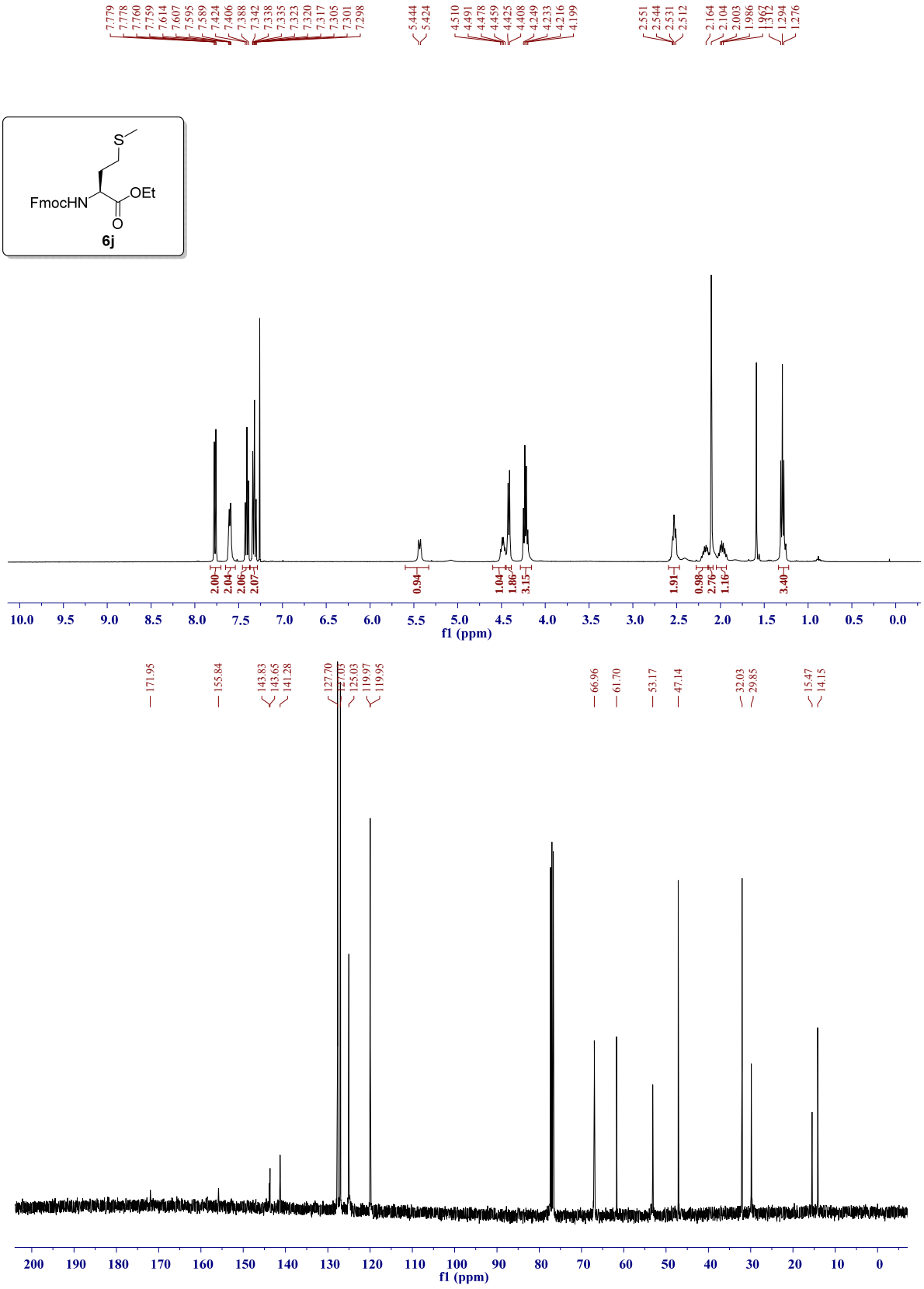


Figure S29. ^{1}H and ^{13}C NMR spectrum of compound **6j**

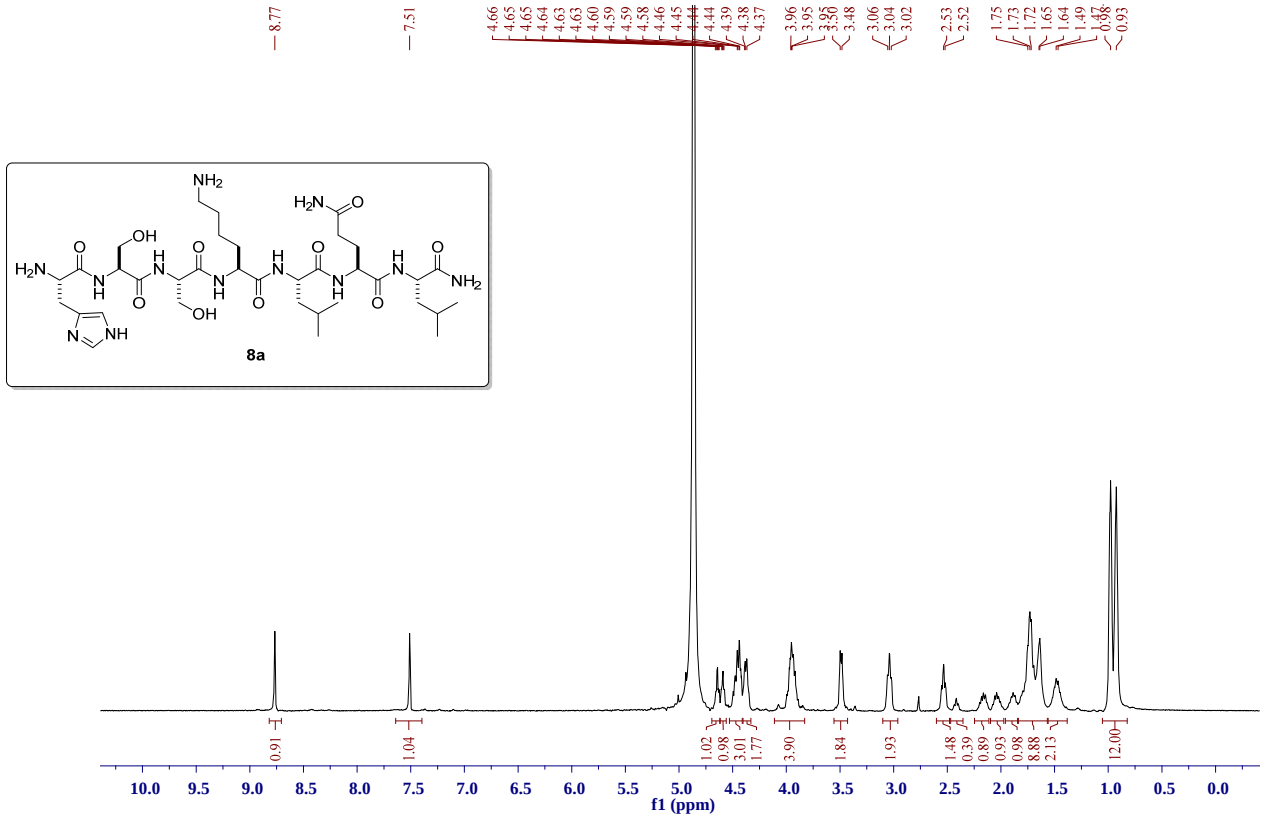


Figure S30. ^{1}H NMR spectrum of compound **8a**

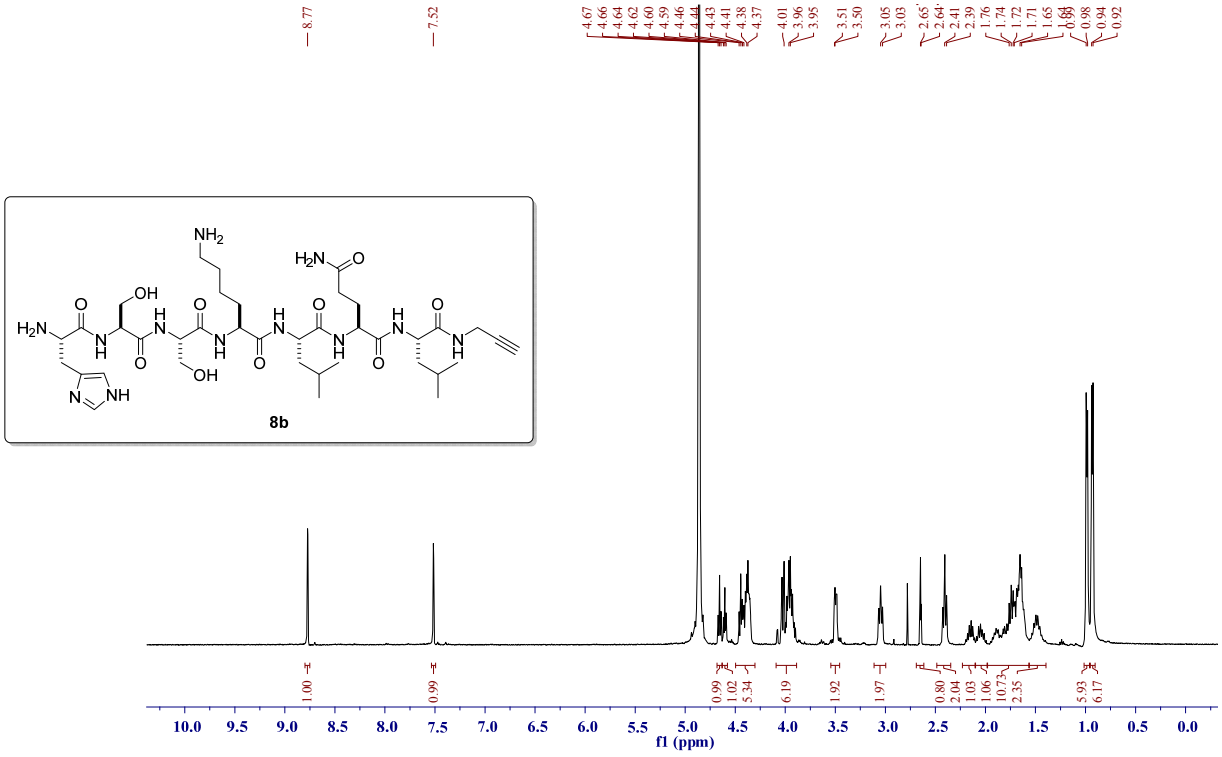


Figure S31. ^{1}H NMR spectrum of compound **8b**

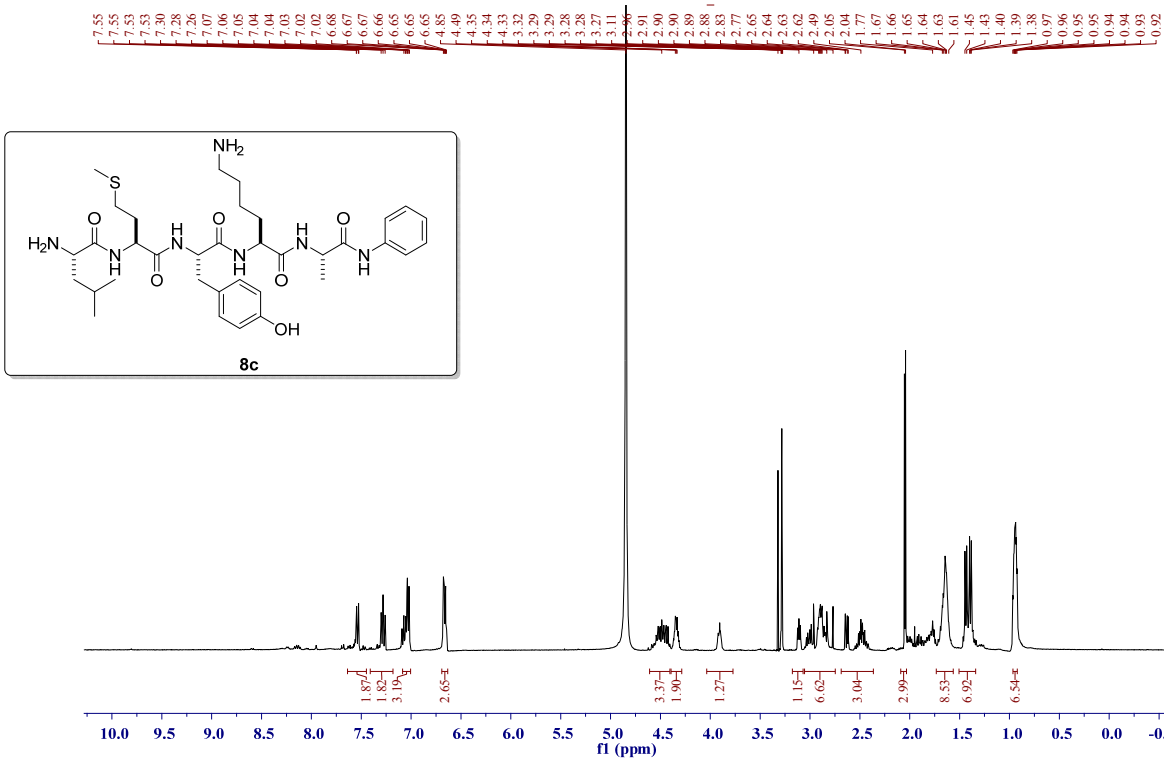


Figure S32. ^{1}H NMR spectrum of compound **8c**

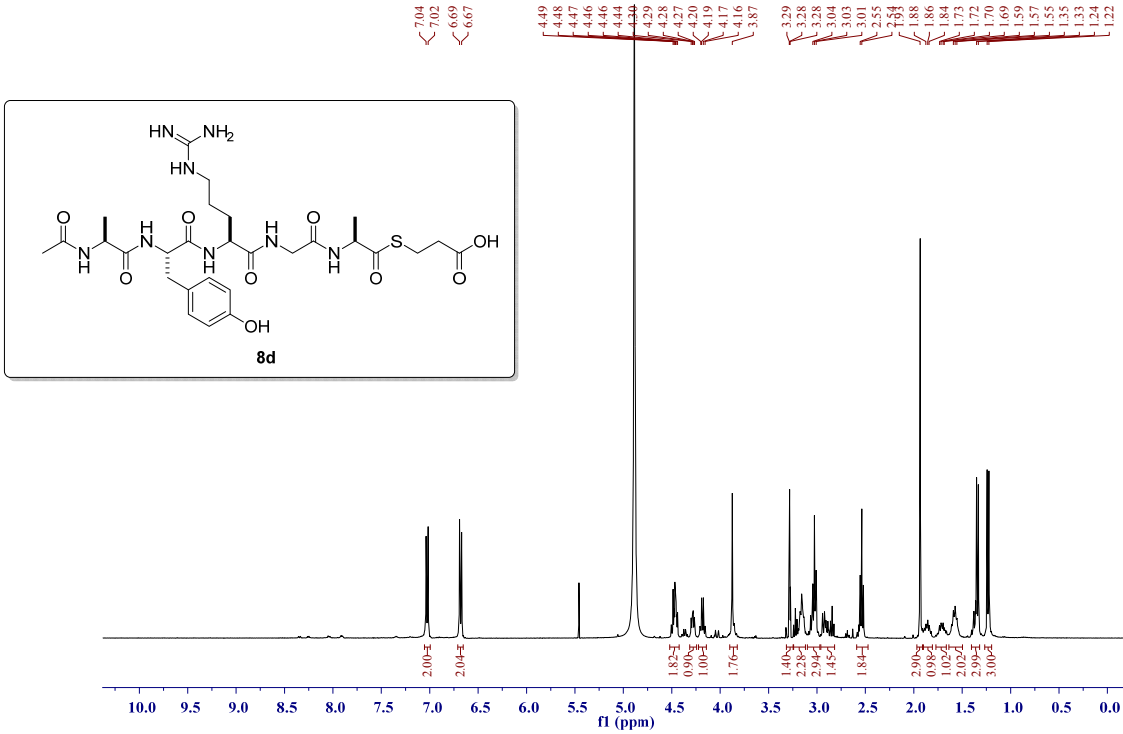


Figure S33. ^{1}H NMR spectrum of compound **8d**

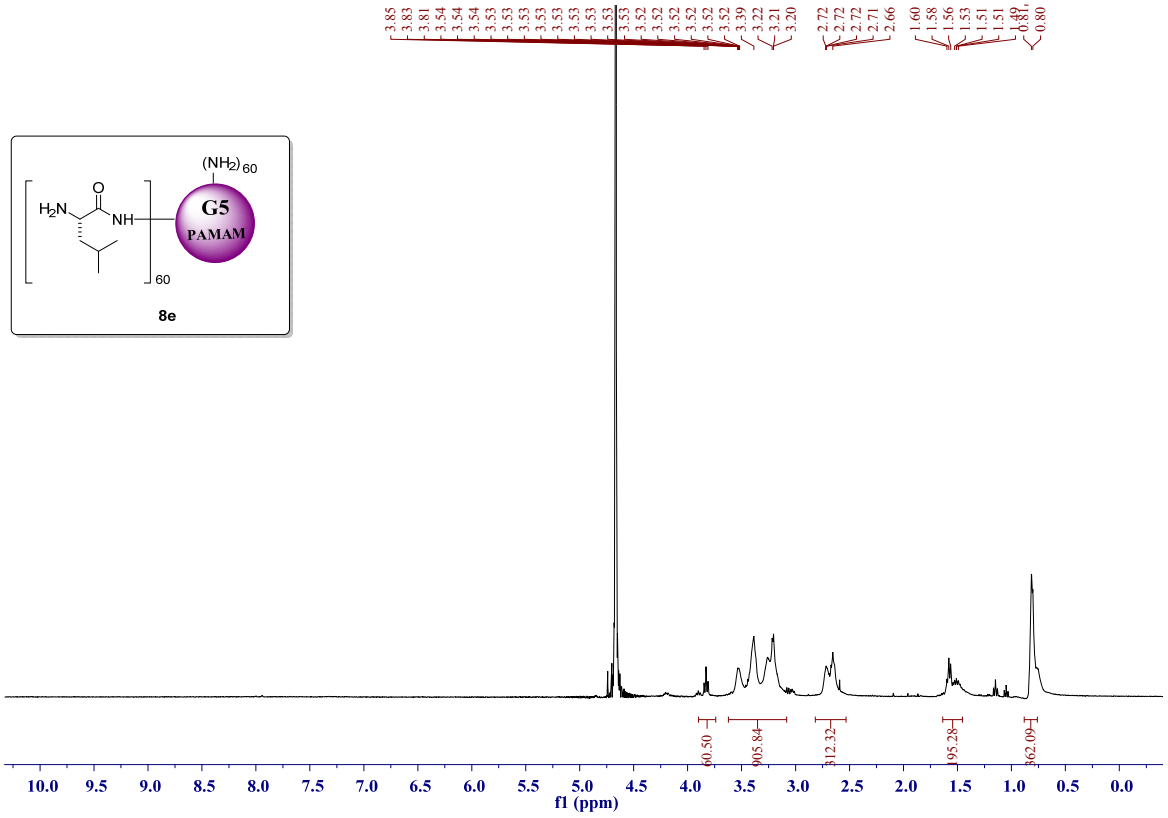


Figure S34. ^{1}H NMR spectrum of compound **8e**

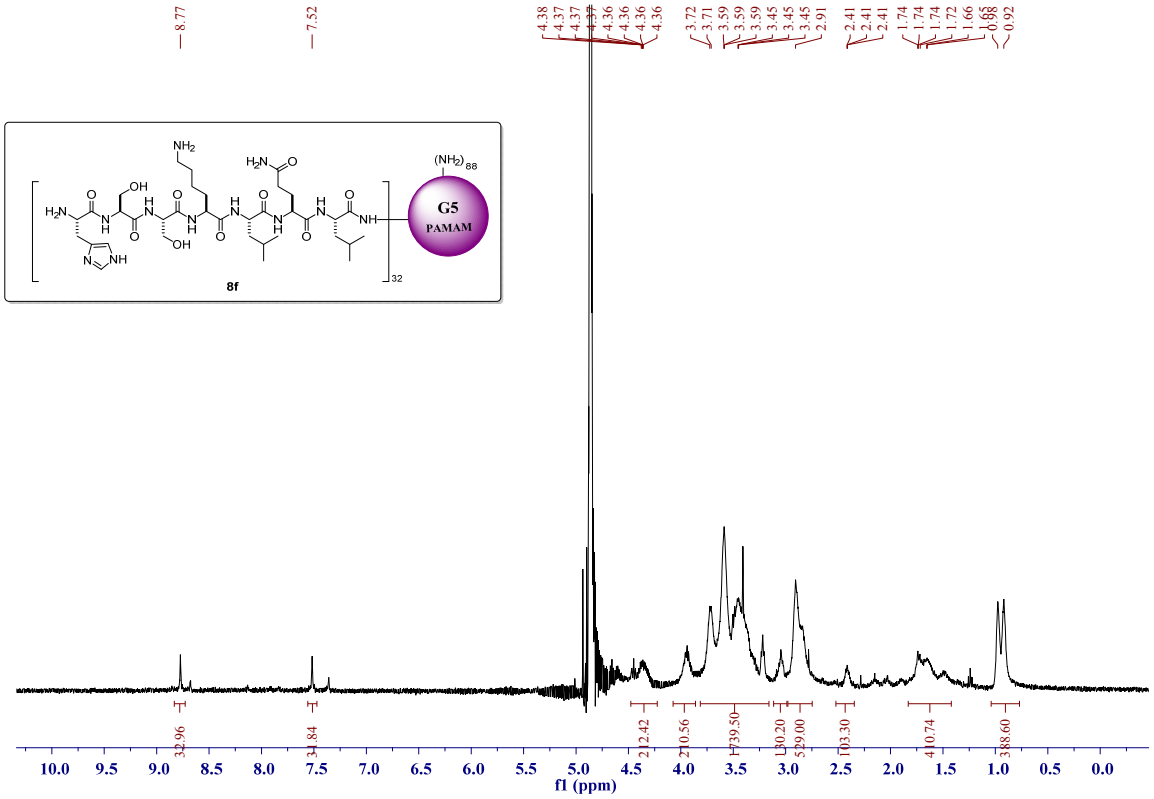


Figure S35. ^{1}H NMR spectrum of compound **8f**

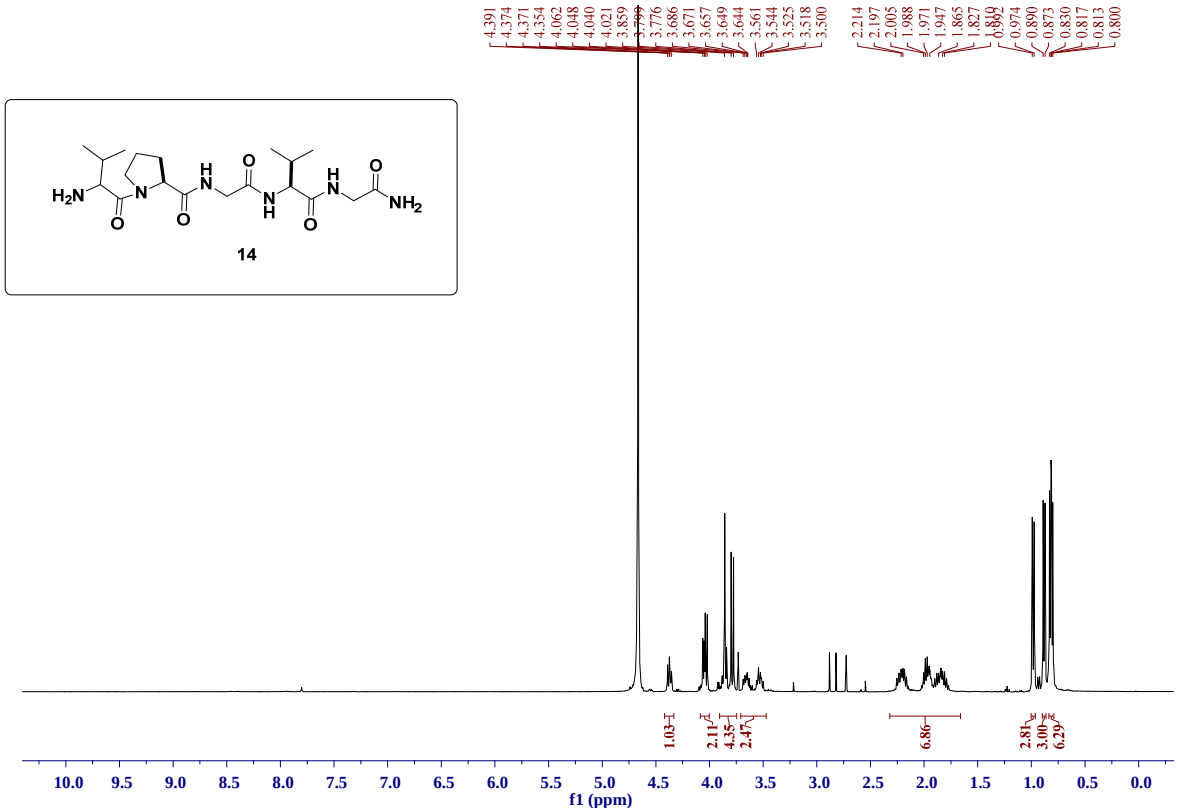


Figure S36. ^{1}H NMR spectrum of compound **14**

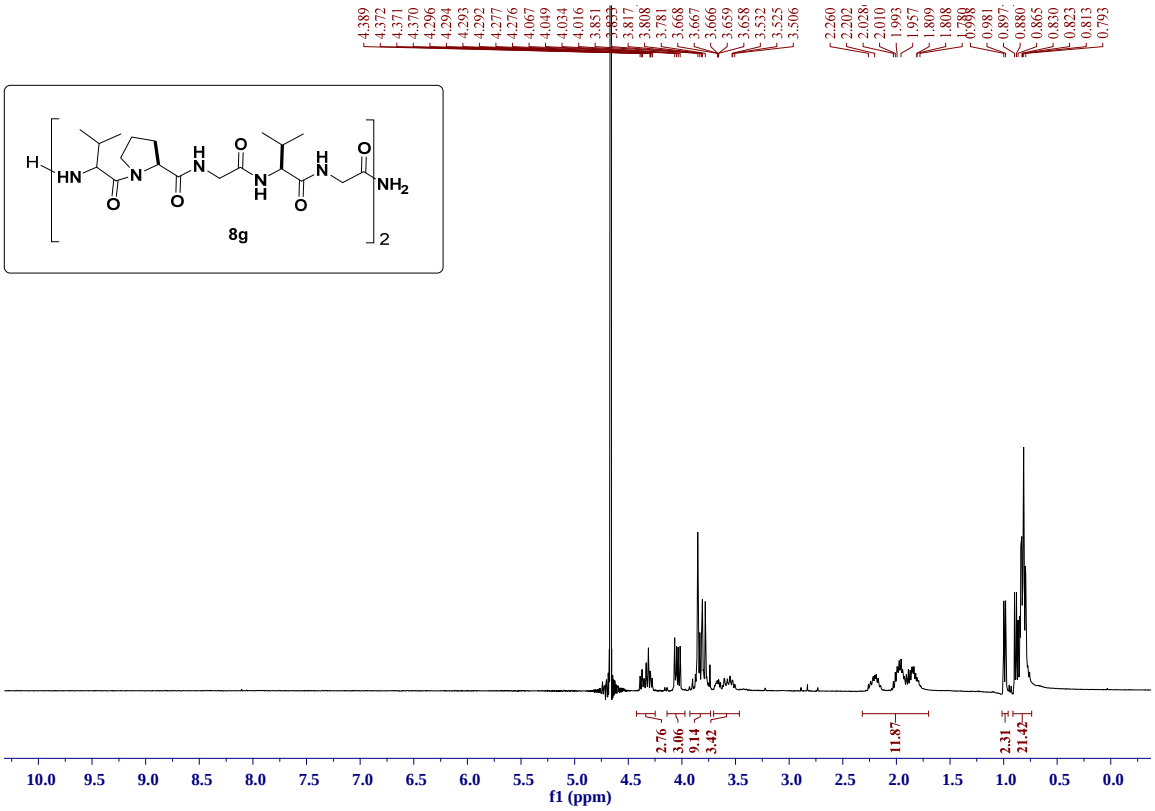


Figure S37. ^{1}H NMR spectrum of compound **8g**

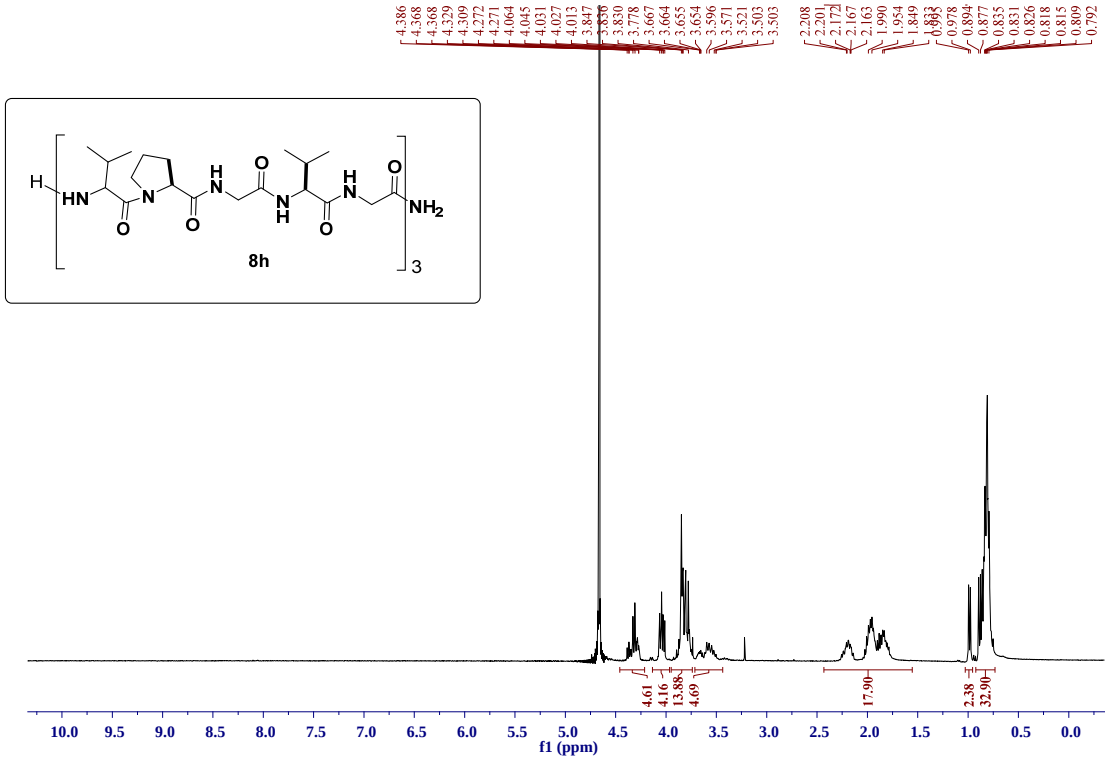


Figure S38. [1]H NMR spectrum of compound **8h**

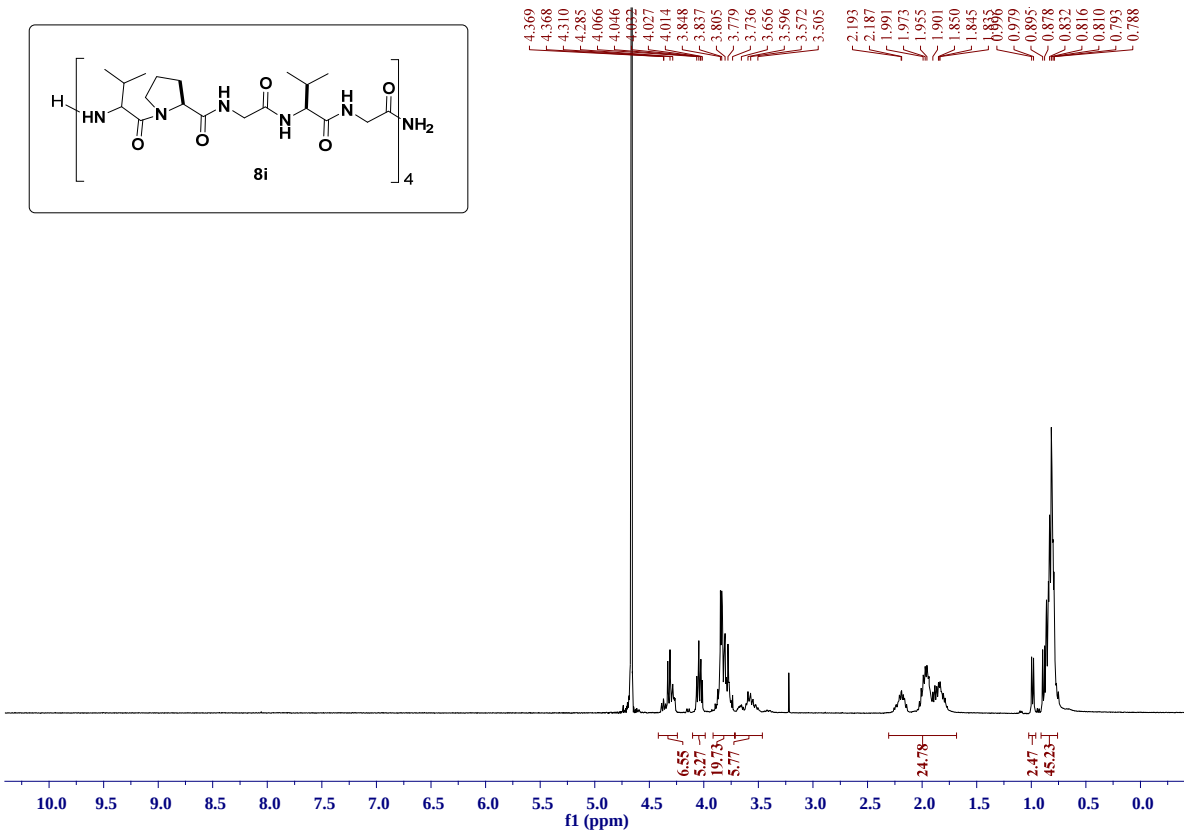


Figure S39. [1]H NMR spectrum of compound **8i**

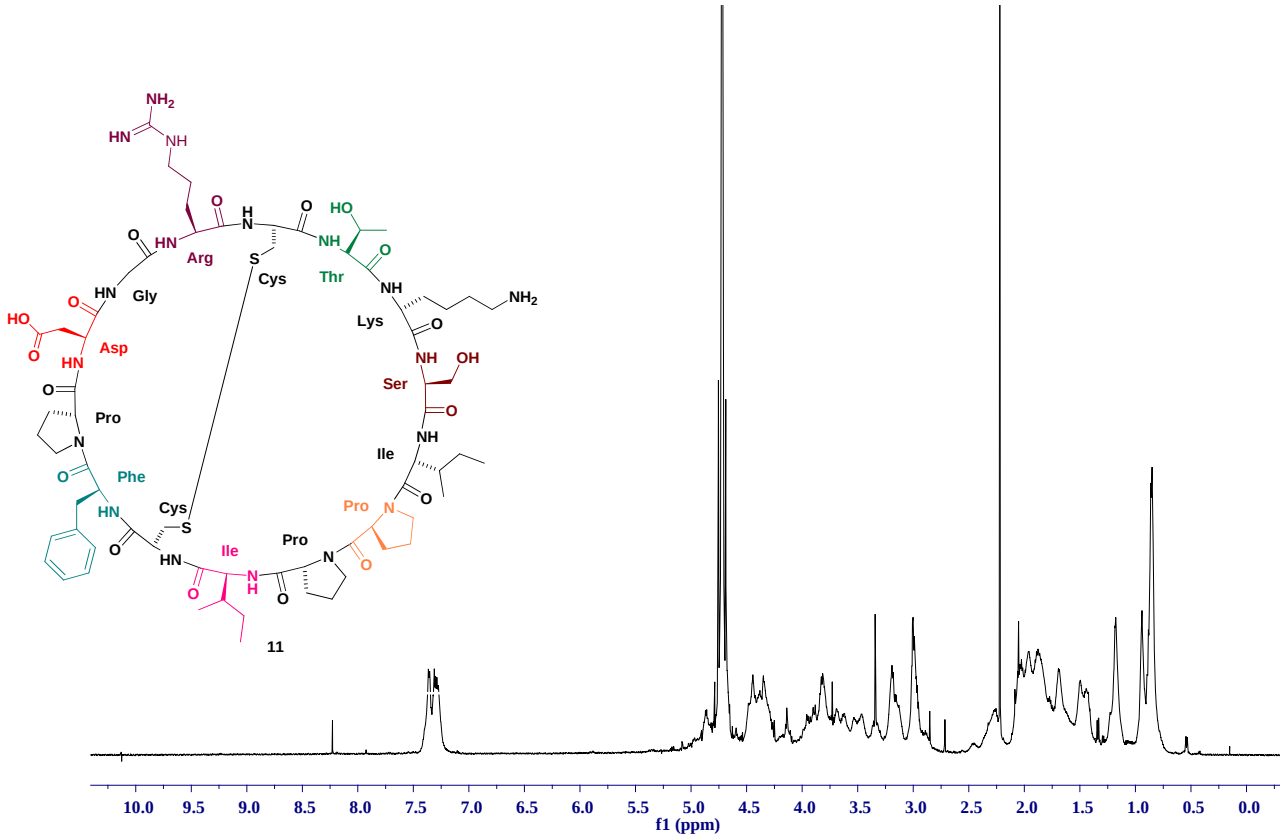


Figure S40. ^{1}H NMR (600 MHz) spectrum of cyclic peptide **11**

Chiral HPLC chromatogram

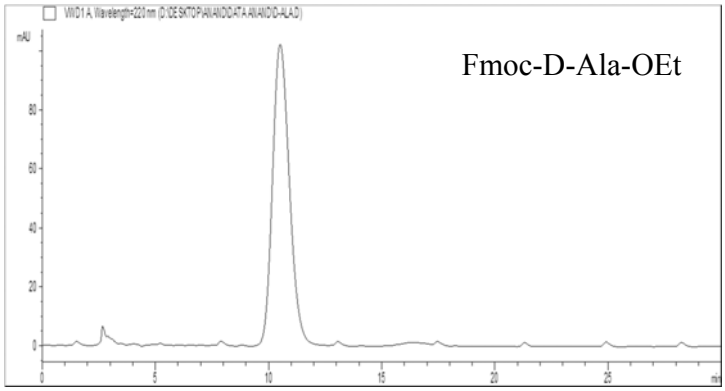


Figure S41. Chiral HPLC chromatogram of Fmoc-D-Ala-OEt (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm). Synthesis of Fmoc-D-Ala-OEt using general procedure for nucleophilic acyl substitution on benzotriazole resin.

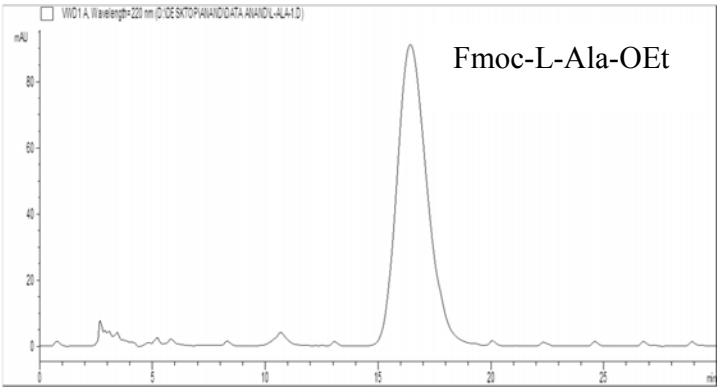


Figure S42. Chiral HPLC chromatogram of Fmoc-L-Ala-OEt (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm). Synthesis of Fmoc-L-Ala-OEt using general procedure for nucleophilic acyl substitution on benzotriazole resin.

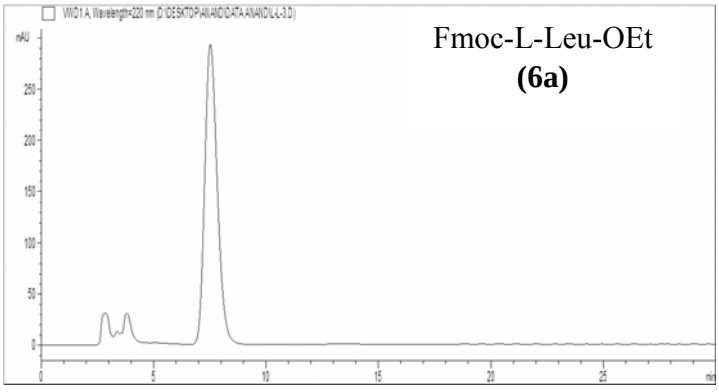


Figure S43. Chiral HPLC chromatogram of compound **6a** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm).

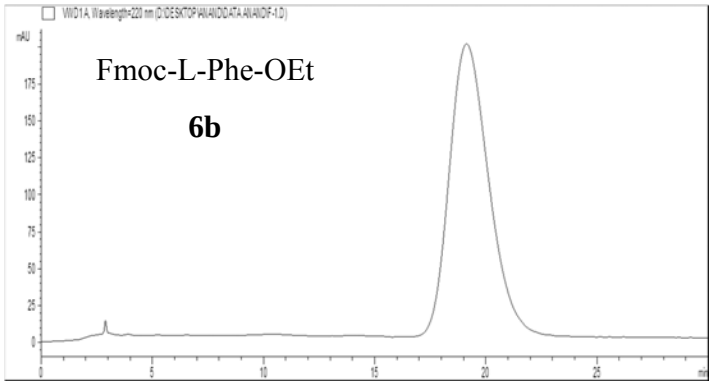


Figure S44. Chiral HPLC chromatogram of compound **6b** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

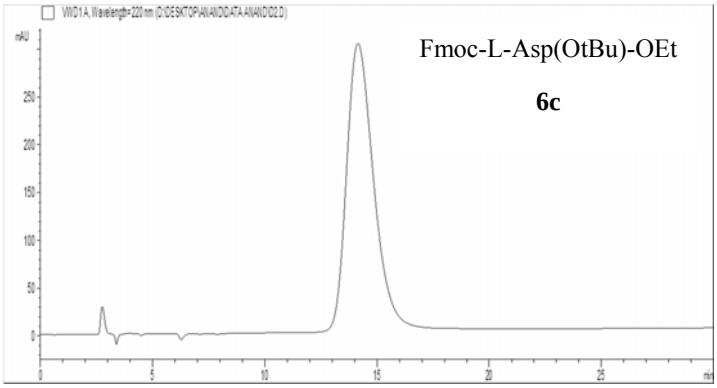


Figure S45. Chiral HPLC chromatogram of compound **6c** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

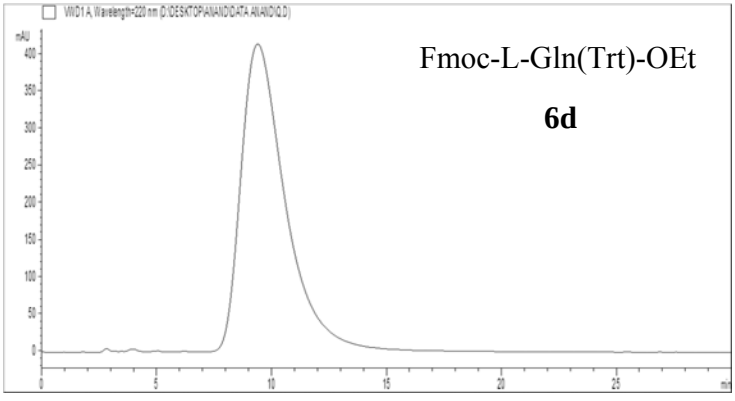


Figure S46. Chiral HPLC chromatogram of compound **6d** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

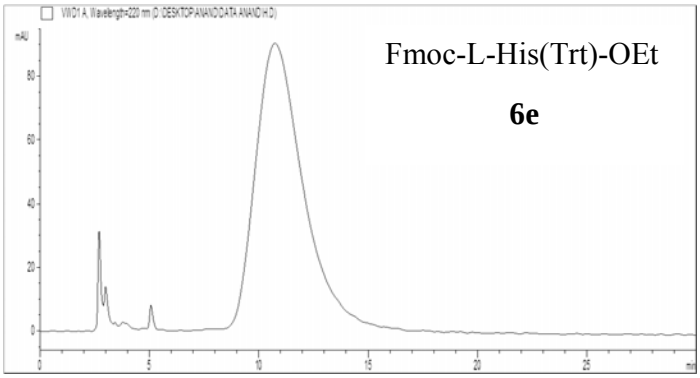


Figure S47. Chiral HPLC chromatogram of compound **6e** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

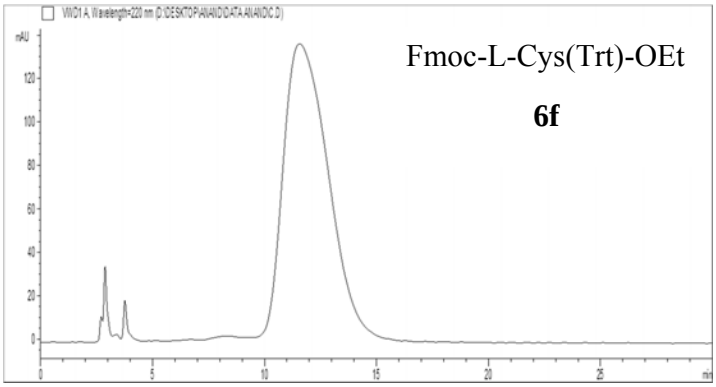


Figure S48. Chiral HPLC chromatogram of compound **6f** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

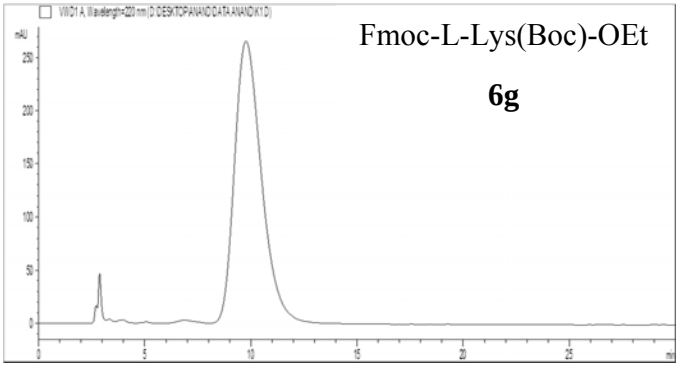


Figure S49. Chiral HPLC chromatogram of compound **6g** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

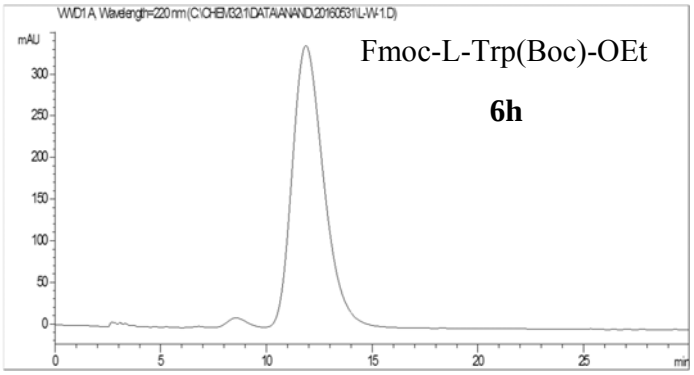


Figure S50. Chiral HPLC chromatogram of compound **6h** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

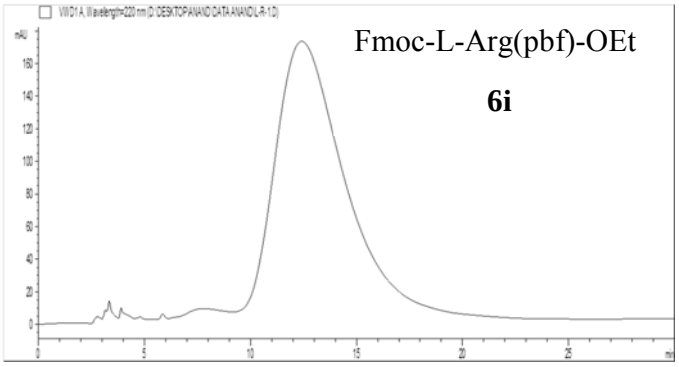


Figure S51. Chiral HPLC chromatogram of compound **6i** (isocratic of 40:60 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

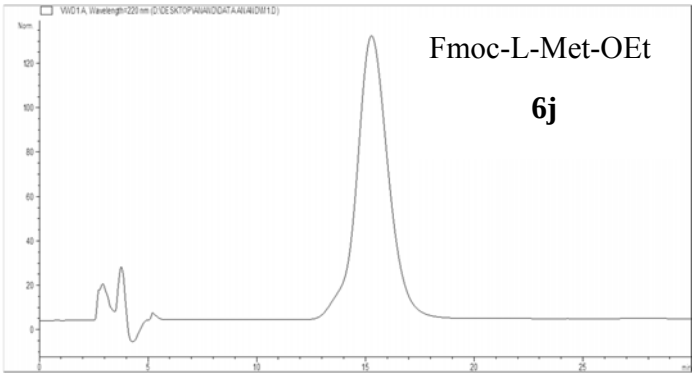


Figure S52. Chiral HPLC chromatogram of compound **6j** (isocratic of 60:40 *n*- hexane/*i*PrOH; flow rate: 1.0 mL/min, λ = 220 nm)

References

[1] S. Krupkova, P. Funk, M. Soural, J. Hlavac, *ACS Combinatorial Science* **2013**, *15*, 20-28.

[2] A. R. Katritzky, S. A. Belyakov, D. O. Tymoshenko, *Journal of Combinatorial Chemistry* **1999**, *1*, 173-176.