Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Total Synthesis of Myxoprincomide, a Secondary Metabolite from *Myxococcus xanthus*

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Supporting Information

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General Information

All air and moisture sensitive reactions were carried out in dried glassware (> 100 °C) under nitrogen or argon atmosphere. THF was dried over sodium and distilled before use. The products were purified by automated column chromatography on silica columns (RediSep Rf, Teledyne Isco) or C18 columns (Telos). Mixtures of ethyl acetate (EtOAc), petroleum ether (PE, 40–60 °C fraction), cyclohexane, dichloromethane (DCM), methanol (MeOH) or acetonitrile (MeCN) and water were generally used as eluents. Analytical TLC was performed on pre-coated silica gel plates (Machery-Nagel, Polygram Sil G/UV_{254}). Detection was accomplished with UV light (254 nm), $KMnO_4$ solution, ninhydrin solution or ceric ammonium sulfate solution. Compounds were occasionally freeze-dried with a Christ lyophiliser.

Melting points were detected with a MEL-TEMP II (Laboratory devices) apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV400 [400 MHz (^{1}H) and 100 MHz (^{13}C)] or a Bruker AV500 [500 MHz (^{1}H) and 125 MHz (^{13}C)] spectrometer. For some rotamer containing samples the NMR spectra were measured at 373 K. Chemical shifts are reported in ppm relative to TMS or internal solvent signal. Mass spectra were recorded with a Finnigan MAT 95 spectrometer (quadrupole) using the CI technique. Optical rotations were measured with a Perkin-Elmer polarimeter (model 241 or 341) in a thermostated (20 °C ± 0.1 °C) cuvette, using a sodium vapour lamp (λ = 589 nm) as radiation source. The concentrations are given in g per 100 mL. LCMS analyses were accomplished on a Shimadzu (LC-10At, autoinjector SCL-6B, mass spectrometer LCMS-2020) with a Phenomenex Luna C18(2) column (50 x 4.6 mm, grain size 3 µm).

General Procedures (GP)

GP1: Boc-deprotection

The Boc-protected amine was dissolved in a HCl solution in 1,4-dioxane (4 M, 10 eq.) and the mixture was stirred until full consumption of the starting material. After solvent evaporation the crude amine hydrochloride salt was used in the next step without further purification.

GP2: Peptide coupling with TBTU

TBTU (1.05 eq.) and DIPEA (2.2 eq.) were added to a mixture of amine hydrochloride salt (1.0 eq.) and carboxylic acid component (1.05 eq.) in DMF (0.1 M) at 0 °C. The reaction mixture was warmed to room temperature overnight and thereafter hydrolyzed with aqueous HCl solution (1M). The mixture was extracted three times with chloroform and the combined organics were washed with saturated $NaHCO_3$ solution and dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography.

Synthesis of Compounds

tert-Butyl ((2*S*)-1-cyano-1-hydroxy-3-methylbutan-2-yl)carbamate (2)

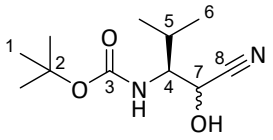
Reduction:

$LiAlH_4$ (288 mg, 7.58 mmol, 1.1 eq.) was added to a solution of Boc-L-valine-*N*-methoxy-*N*-methylamide (1.79 g, 6.89 mmol, 1.0 eq.) in THF (19 mL) at –35 °C and the resulting mixture was stirred at 0 °C for 1 hour. The reaction mixture was hydrolyzed carefully with aqueous $KHSO_4$ solution (1 M, 15 mL) and aqueous Rochelle salt solution (saturated, 15 mL). The suspension was stirred for another 30 minutes and thereafter extracted three times with ethyl acetate. The combined organic phases were washed with saturated Rochelle salt solution and dried over Na_2SO_4. After filtration, the solvent was evaporated to afford the corresponding aldehyde (1.39 g, 6.89 mmol, 100 %), which was used in the next step without further purification.

Cyanation:

Sodium bisulfite (935 mg, 8.99 mmol, 1.3 eq.) in water (10.8 mL) was added to a solution of the previously prepared aldehyde (1.39 g, 6.89 mmol, 1.0 eq.) in MeOH (10.8 mL) at 0 °C. The resulting mixture was stirred for 2.5 h and thereafter treated with NaCN (441 mg, 8.99 mmol, 1.3 eq.) in water (10.8 mL). After addition of ethyl acetate (10.8 mL) the reaction mixture was stirred at room temperature overnight before adding ethyl acetate to the solution. The phases were then separated

and the organic phase was dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, PE:EtOAc 100:0 → 50:50) to afford cyanohydrine **2** (1.39 g, 6.08 mmol, 88 %, *dr* 53:47) as a colorless resin. $\mathbf{R_f}$ = 0.40 (SiO_2, PE:EtOAc 60:40).



2

Diastereomer A:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.99 (d, $^3J_{6,5}$ = 6.9 Hz, 3 H, 6-H), 1.02 (d, $^3J_{6',5}$ = 6.9 Hz, 3 H, 6-H'), 1.46 (s, 9 H, 1-H), 1.86 (dsept, $^3J_{5,4}$ = 7.7 Hz, $^3J_{5,6}$ = 6.9 Hz, 1 H, 5-H), 3.66 (ddd, $^3J_{4,5}$ = $^3J_{5,NH}$ = 7.7 Hz, $^3J_{4,7}$ = 2.6 Hz, 1 H, 4-H), 4.63 (m, 1 H, 7-H), 4.83 (m, 1 H, N-H), 5.05 (bs, 1 H, O-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 18.8 (q, C-6), 19.6 (q, C-6'), 28.2 (q, C-1), 29.6 (d, C-5), 60.6 (d, C-4), 65.5 (d, C-7), 81.5 (s, C-2), 118.1 (s, C-8), 158.1 (s, C-3).

Diastereomer B:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.04 (d, $^3J_{6,5}$ = 6.7 Hz, 3 H, 6-H), 1.02 (d, $^3J_{6',5}$ = 6.7 Hz, 3 H, 6-H'), 1.48 (s, 9 H, 1-H), 2.29 (m, 1 H, 5-H), 3.26 (m, 1 H, 4-H), 4.63 (m, 1 H, 7-H), 5.02 – 5.09 (m, 2 H, N-H, O-H).
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 18.6 (q, C-6), 19.7 (q, C-6'), 27.7 (d, C-5), 28.2 (q, C-1), 60.2 (d, C-4), 63.7 (d, C-7), 81.1 (s, C-2), 118.9 (s, C-8), 157.3 (s, C-3).
HRMS (CI) calculated for $C_{11}H_{21}N_2O_3$ $[M+H]^+$: 229.1547, found: 229.1550.

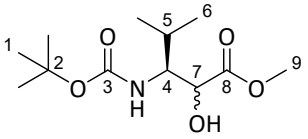
Methyl (3*S*)-3-((*tert*-butoxycarbonyl)amino)-2-hydroxy-4-methylpentanoate (4)

Boc-deprotection and esterification:

Acetyl chloride (6.70 mL, 7.39 g, 94.0 mmol, 10 eq.) was added dropwise to a solution of **2** (2.15 g, 9.42 mmol, 1.0 eq.) in methanol (22 mL) at 0 °C. The reaction mixture was stirred at 50 °C for 18 hours and thereafter concentrated in vacuo. The residue was then suspended in acetonitrile and filtrated through a pad of Celite in order to remove the newly formed ammonium chloride. After solvent evaporation, the crude methyl ester hydrochloride **3** (1.73 g, 8.75 mmol, 93 %) was obtained and used in the next step without further purification.

Boc-protection:

Sodium bicarbonate (1.58 g, 18.8 mmol, 2.2 eq.) was added to a solution of the previously synthesized methylester **3** (1.69 g, 8.53 mmol, 1.0 eq.) in water (4.3 mL) at 0 °C. Subsequently, a solution of Boc_2O (1.95 g, 8.95 mmol, 1.05 eq.) in THF (4.3 mL) was added dropwise to the reddish solution and the reaction mixture was then stirred at room temperature overnight. The mixture was hydrolyzed with aqueous HCl solution (1 M) and the aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and thereafter filtrated. After solvent removal, the crude product was purified by flash chromatography (SiO_2, PE:EtOAc 100:0 → 85:15) to afford methylester **4** (1.76 g, 6.74 mmol, 79 %) as a white solid. $\mathbf{R_f}$ **(4A)** = 0.46 (SiO_2, PE:EtOAc 50:50); $\mathbf{R_f}$ **(4B)** = 0.38 (SiO_2, PE:EtOAc 50:50).



4

Diastereomer A:

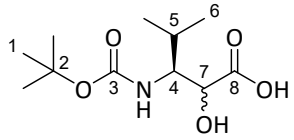
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.98 (d, $^3J_{6,5}$ = 6.7 Hz, 3 H, 6-H), 1.02 (d, $^3J_{6',5}$ = 6.7 Hz, 3 H, 6-H'), 1.40 (s, 9 H, 1-H), 1.87 (dsept, $^3J_{5,4}$ = 9.7 Hz, $^3J_{5,6}$ = 6.7 Hz, 1 H, 5-H), 3.11 (d, $^3J_{OH,7}$ = 4.7 Hz, 1 H, O-H), 3.67 (ddd, $^3J_{4,5}$ = $^3J_{4,NH}$ = 9.7 Hz, $^3J_{4,7}$ = 1.2 Hz, 1 H, 4-H), 3.78 (s, 3 H, 9-H), 4.35 (dd, $^3J_{7,OH}$ = 4.7 Hz, $^3J_{7,4}$ = 1.2 Hz, 1 H, 7-H), 4.72 (d, $^3J_{NH,4}$ = 9.7 Hz, 1 H, N-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 19.4 (q, C-6), 19.7 (q, C-6'), 28.2 (q, C-1), 30.2 (d, C-5), 52.7 (q, C-9), 58.5 (d, C-4), 70.4 (d, C-7), 79.3 (s, C-2), 155.5 (s, C-3), 174.8 (s, C-8).

Diastereomer B:

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 0.94 (d, $^3J_{6,5}$ = 6.7 Hz, 3 H, 6-H), 1.44 (s, 9 H, 1-H), 1.89 (m, 1 H, 5-H), 3.18 (bs, 1 H, O-H), 3.75 (m, 1 H, 4-H), 3.79 (s, 3 H, 9-H), 4.27 (d, $^3J_{7,4}$ = 3.6 Hz, 1 H, 7-H), 4.76 (d, $^3J_{NH,4}$ = 9.3 Hz, 1 H, N-H). **^{13}C-NMR (100 MHz, $CDCl_3$):** δ = 18.3 (q, C-6), 20.1 (q, C-6'), 28.3 (q, C-1), 28.8 (d, C-5), 52.6 (q, C-9), 58.4 (d, C-4), 72.6 (d, C-7), 79.6 (s, C-2), 156.1 (s, C-3), 173.9 (s, C-8).
HRMS (CI) calculated for $C_{12}H_{24}NO_5$ $[M+H]^+$: 262.1649, found: 262.1652.

(3*S*)-3-((*tert*-Butoxycarbonyl)amino)-2-hydroxy-4-methylpentanoic acid (5)

A solution of lithium hydroxide hydrate (281 mg, 6.69 mmol, 1.1 eq.) in water (6.0 mL) was added dropwise to a solution of **4** (1.59 g, 6.08 mmol, 1.0 eq.) in THF (60 mL) at 0 °C. The reaction mixture was warmed to room temperature overnight before acidifying it with aqueous HCl solution (1 M). The resulting mixture was extracted three times with ethyl acetate and the combined organics were dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, PE:EtOAc 100:0 → 50:50) to afford carboxylic acid **5** (1.51 g, 6.08 mmol, 100 %) as a white solid. $\mathbf{R_f}$ = 0.13 (SiO_2, PE:EtOAc 60:40).



5

Diastereomer A:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.84 (d, $^3J_{6,5}$ = 6.9 Hz, 3 H, 6-H), 0.87 (d, $^3J_{6',5}$ = 6.9 Hz, 3 H, 6'-H), 1.39 (s, 9 H, 1-H), 1.95 (septd, $^3J_{5,6}$ = 6.9 Hz, $^3J_{5,4}$ = 5.7 Hz, 1 H, 5-H), 3.64 (ddd, $^3J_{4,NH}$ = 9.7 Hz, $^3J_{4,7}$ = 6.6 Hz, $^3J_{4,5}$ = 5.7 Hz, 1 H, 4-H), 3.95 (d, $^3J_{7,4}$ = 6.6 Hz, 1 H, 7-H), 5.70 – 6.00 (m, 2 H, N-H, O-H). The signal of the carboxylic proton could not be detected. **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 17.0 (q, C-6), 19.6 (q, C-6'), 27.7 (d, C-5), 27.8 (q, C-1), 57.1 (d, C-4), 71.4 (d, C-7), 77.3 (s, C-2), 155.0 (s, C-3), 173.7 (s, C-8).

Diastereomer B:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.89 (d, $^3J_{6,5}$ = 6.6 Hz, 3 H, 6-H), 0.93 (d, $^3J_{6',5}$ = 6.9 Hz, 3 H, 6'-H), 1.38 (s, 9 H, 1-H), 1.82 (m, 1 H, 5-H), 3.56 (ddd, $^3J_{4,NH}$ = 10.4 Hz, $^3J_{4,5}$ = 8.5 Hz, $^3J_{4,7}$ = 2.5 Hz, 1 H, 4-H), 4.13 (d, $^3J_{7,4}$ = 2.5 Hz, 1 H, 7-H), 5.70 – 6.00 (m, 2 H, N-H, O-H). The signal of the carboxylic proton could not be detected. **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 18.6 (q, C-6), 19.2 (q, C-6'), 27.8 (q, C-1), 29.6 (d, C-5), 57.9 (d, C-4), 69.9 (d, C-7), 77.3 (s, C-2), 155.0 (s, C-3), 173.6 (s, C-8).
HRMS (CI) calculated for $C_{11}H_{22}NO_5$ $[M+H]^+$: 248.1492, found: 248.1492.

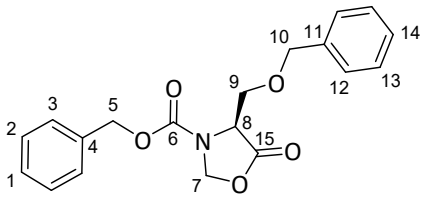
Benzyl (*S*)-4-((benzyloxy)methyl)-5-oxooxazolidine-3-carboxylate

The oxazolidine was prepared according to a modified literature procedure.[1]

Paraformaldehyde (1.13 g, 37.5 mmol, 5.9 eq.) and *p*-toluenesulfonic acid monohydrate (61.0 mg, 318 µmol, 5 mol%) were added successively to a solution of Cbz-L-Ser(Bzl)-OH (2.10 g, 6.37 mmol, 1.0 eq.) in toluene (120 mL) and the reaction mixture was heated under reflux for 75 minutes. The

[1] Y. Luo, G. Evindar, D. Fishlock and G. A. Lajoie, *Tetrahedron Lett*. **2001**, *42*, 3807–3809.

resulting mixture was washed successively with saturated $NaHCO_3$ solution and saturated NaCl solution before drying it over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, PE:EtOAc 100:0 → 60:40) to afford the oxazolidinone (1.84 g, 5.38 mmol, 84 %) as a white solid. $\mathbf{R_f}$ = 0.47 (SiO_2, PE:EtOAc 50:50); $[\alpha]_D^{20}$ = + 115.5 ($CHCl_3$, c = 1.0); **m.p.** 56 – 58 °C.

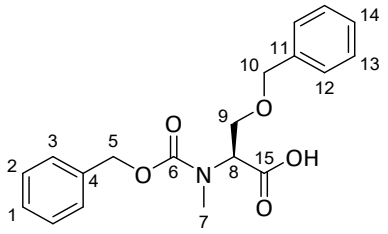


^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 3.85 (dd, $^2J_{9a,9b}$ = 10.4 Hz, $^3J_{9a,8}$ = 2.2 Hz, 1 H, 9-H_a), 3.94 (dd, $^2J_{9b,9a}$ = 10.4 Hz, $^3J_{9b,8}$ = 2.8 Hz, 1 H, 9-H_b), 4.47 (m, 1 H, 8-H), 4.50 (s, 2 H, 10-H), 5.15 (d, $^2J_{5a,5b}$ = 12.6 Hz, 1 H, 5-H_a), 5.18 (d, $^2J_{5b,5a}$ = 12.6 Hz, 1 H, 5-H_b), 5.23 (dd, $^2J_{7a,7b}$ = 4.0 Hz, $^4J_{7a,8}$ = 0.6 Hz, 1 H, 7-H_a), 5.49 (d, $^2J_{7b,7a}$ = 4.0 Hz, 1 H, 7-H_b), 7.23 – 7.38 (m, 10 H, 1-H, 2-H, 3-H, 12-H, 13-H, 14-H). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 55.5 (d, C-8), 66.4 (t, C-5), 67.4 (t, C-9), 72.3 (t, C-10), 77.8 (t, C-7), 126.7 (d, C-3), 127.0 (d, C-12), 127.1 (d, C-1), 127.5 (d, C-14), 127.7 (d, C-13), 127.9 (d, C-2), 135.6 (s, C-4), 137.3 (s, C-11), 151.7 (s, C-6), 170.4 (s, C-15).
HRMS (CI) calculated for $C_{19}H_{20}NO_5$ $[M+H]^+$: 342.1336, found: 342.1345.

***O*-Benzyl-*N*-((benzyloxy)carbonyl)-*N*-methyl-L-serine**

The *N*-methylserine derivative was prepared according to a modified literature procedure.[2]

Triethylsilane (3.35 mL, 2.43 g, 20.9 mmol, 4.0 eq.) was added dropwise to a solution of benzyl (*S*)-4-((benzyloxy)methyl)-5-oxooxazolidine-3-carboxylate (1.78 g, 5.22 mmol, 1.0 eq.) in $CHCl_3$:TFA (1:1, 8.7 mL) at 0 °C. The reaction mixture was warmed to room temperature overnight and stirred for one additional day. After solvent evaporation, the crude product was purified by column chromatography (SiO_2, PE:EtOAc 100:0 → 50:50) to afford the carboxylic acid (1.55 g, 4.50 mmol, 86 %) as a colorless syrup. $\mathbf{R_f}$ = 0.23 (SiO_2, PE:EE 70:30); $[\alpha]_D^{20}$ = – 6.8 ($CHCl_3$, c = 1.0).



^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 2.91 (s, 3 H, 7-H), 3.87 (m, 2 H, 9-H), 4.49 (d, $^2J_{10a,10b}$ = 12.2 Hz, 1 H, 10-H_a), 4.54 (d, $^2J_{10b,10a}$ = 12.2 Hz, 1 H, 10-H_b), 4.80 (dd, $^3J_{8,9a}$ = 6.6 Hz, $^3J_{8,9b}$ = 6.0 Hz, 1 H, 8-H), 5.11 (s, 2 H, 5-H), 7.26 – 7.37 (m, 10 H, 1-H, 2-H, 3-H, 12-H, 13-H, 14-H), 12.50 (bs, 1 H, COO-H). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 32.3 (q, C-7), 58.5 (d, C-8), 66.0 (t, C-5), 67.0 (t, C-9), 71.8 (t, C-10), 126.8 (d, C-3), 126.9 (d, C-12), 126.9 (d, C-1), 127.2 (d, C-14), 127.7 (d, C-13), 127.8 (d, C-2), 136.5 (s, C-4), 137.7 (s, C-11), 155.5 (s, C-6), 169.9 (s, C-15).
HRMS (CI) calculated for $C_{19}H_{22}NO_5$ $[M+H]^+$: 344.1492, found: 344.1486.

[2] Y. Luo, G. Evindar, D. Fishlock and G. A. Lajoie, *Tetrahedron Lett*. **2001**, *42*, 3807–3809.

Benzyl N_β-*tert*-butoxycarbonyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (8)

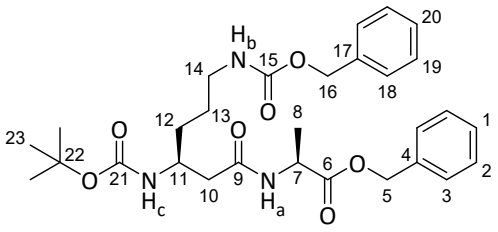
N_β-Boc-N_ε-Cbz-L-β-lysine **6** was prepared according to a literature procedure.[3]

Arndt-Eistert-homologation:

A mixture of diethylether (59 mL) and aqueous KOH solution (40 wt%, 13 mL) was cooled to –10 °C and slowly treated with *N*-nitroso-*N*-methylurea (2.70 g, 26.2 mmol, 2.0 eq.). After complete addition the mixture was stirred for another 30 minutes until all solid was dissolved. Subsequently, water (5-10 mL) was added and the emulsion was partially freezed with a cooling bath. The diethyl ether phase was then washed with water to afford the diazomethane solution. Meanwhile, N_β-Boc-N_ε-Cbz-L-β-lysine **6** (5.00 g, 13.1 mmol, 1.0 eq.) was dissolved in THF (72 mL) and the resulting solution was cooled to –20 °C. Subsequently, triethylamine (2.19 mL, 1.59 g, 15.7 mmol, 1.2 eq.) and ethyl chloroformate (1.32 mL, 1.49 g, 13.8 mmol, 1.05 eq.) were added slowly to the carboxylic acid solution and the mixture was stirred for 30 minutes. The resulting suspension was then cooled to –78 °C and the previously prepared diazomethane solution was added dropwise. After complete addition, the reaction mixture was warmed to room temperature overnight. The suspension was hydrolyzed with water and thereafter extracted twice with diethyl ether. The combined organic phases were then washed with saturated aqueous $NaHCO_3$ solution and dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, PE:EtOAc 100:0 → 50:50) to afford the diazoketone **7** (4.97 g, 12.7 mmol, 97 %) as a yellow resin.

Wolff-rearrangement:

Benzyl L-alaninate hydrochloride (2.03 g, 5.68 mmol, 2.6 eq.) was treated with a semisaturated Na_2CO_3 solution (50 mL) and the aqueous phase was extracted three times with diethyl ether. The combined organics were dried over Na_2SO_4 and the solvent was removed *in vacuo* to afford the free amine as a yellow oil. The free amine was added to a solution of diazoketone **7** (854 mg, 2.19 mmol, 1.0 eq.) in THF (44 mL). The resulting mixture was then cooled to –25 °C before adding triethylamine (868 µL, 631 mg, 6.23 mmol, 2.85 eq.) and silver benzoate (65.1 mg, 284 µmol, 0.13 eq.). The resulting suspension was warmed to room temperature overnight under exclusion of light and was then concentrated *in vacuo*. The obtained residue was diluted with ethyl acetate and washed with aqueous HCl solution (1 M). The aqueous phase was extracted three times with ethyl acetate and the combined organics thereafter dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, PE:EtOAc 70:30 → 0:100) to afford dipeptide **8** (995 mg, 1.84 mmol, 84 %) as a white solid. $\mathbf{R_f}$ = 0.47 (SiO_2, EtOAc); $[\alpha]_D^{20}$ = – 23.9 (MeOH, c = 1.0); **m.p.** 126 – 127 °C.



8

^{1}H-NMR (400 MHz, DMSO-d_6): δ = 1.26 – 1.39 (m, 4 H, 12-H, 13-H), 1.27 (d, $^3J_{8,7}$ = 7.3 Hz, 3 H, 8-H), 1.36 (s, 9 H, 23-H), 2.20 (dd, $^2J_{10a,10b}$ = 14.3 Hz, $^3J_{10a,11}$ = 7.7 Hz, 1 H, 10-H_a), 2.25 (dd, $^2J_{10b,10a}$ = 14.3 Hz, $^3J_{10b,11}$ = 6.2 Hz, 1 H, 10-H_b), 2.93 (m, 2 H, 14-H), 3.72 (m, 1 H, 11-H), 4.28 (qd, $^3J_{7,8}$ = 7.3 Hz, $^3J_{7,NHa}$ = 6.9 Hz, 1 H,

[3] G. Pattenden, T. Thompson, *Chem. Commun*. **2001**, 717–718.

7-H), 4.99 (s, 2 H, 16-H), 5.10 (s, 2 H, 5-H), 6.62 (d, $^3J_{NHc,11}$ = 8.7 Hz, 1 H, N-H$_c$), 7.19 (dd, $^3J_{NHb,14a}$ = 5.4 Hz, $^3J_{NHb,14b}$ = 5.1 Hz, 1 H, N-H$_b$), 7.27 – 7.39 (m, 10 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H), 8.30 (d, $^3J_{NHa,7}$ = 6.9 Hz, 1 H, N-H$_a$). **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ = 16.8 (q, C-8), 26.2 (t, C-13), 28.2 (q, C-23), 31.4 (t, C-12), 40.3 (t, C-14), 40.9 (t, C-10), 47.5 (d, C-11), 47.6 (d, C-7), 65.1 (t, C-16), 65.8 (t, C-5), 77.4 (s, C-22), 127.7 (d, C-3/C-18), 127.7 (d, C-3/C-18), 127.7 (d, C-1/C-20), 128.0 (d, C-1/C-20), 128.3 (d, C-2/C-19), 128.4 (d, C-2/C-19), 136.0 (s, C-4), 137.3 (s, C-17), 155.0 (s, C-21), 156.0 (s, C-15), 170.2 (s, C-9), 172.5 (s, C-6).
HRMS (CI) calculated for $C_{29}H_{40}N_3O_7$ [M+H]$^+$: 542.2861, found: 542.2870.

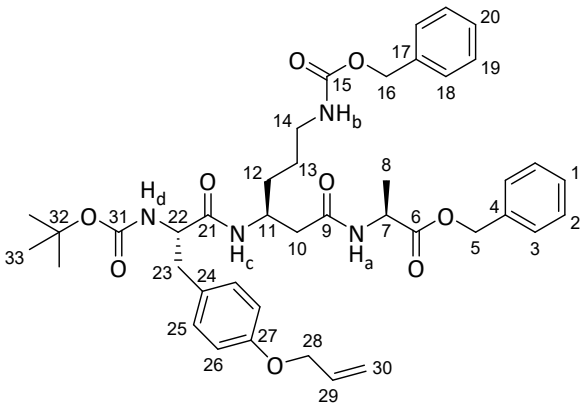
Benzyl *N*-*tert*-butoxycarbonyl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (9)

Boc-deprotection:

Acetyl chloride (5.40 mL, 5.96 g, 76.0 mmol, 10.0 eq.) was added dropwise to a solution of methanol (3.38 mL, 2.67 g, 83.0 mmol, 11.0 eq.) in 1,4-dioxane (20 mL) at 0 °C. The mixture was stirred at room temperature for 15 minutes before pouring it to dipeptide **8** (4.11 g, 7.59 mmol, 1.0 eq.). The resulting solution was stirred at room temperature for 1 hour and the solvent was removed *in vacuo* to afford the amine hydrochloride salt (3.63 g, 7.59 mmol, 100 %) which was used in the next step without further purification.

Peptide coupling:

Boc-L-Tyr(allyl)-OH (2.44 g, 7.59 mmol, 1.0 eq.) was dissolved in THF (55 mL) and cooled to –20 °C before slowly adding *N*-methylmorpholine (1.05 mL, 957 mg, 9.46 mmol, 1.25 eq.) and isobutyl chloroformate (1.05 mL, 1.09 g, 7.97 mmol, 1.05 eq.).The resulting suspension was stirred at – 20 °C for 15 minutes and a mixture of the previously prepared amine hydrochloride salt (3.63 g, 7.59 mmol, 1.0 eq.) and *N*-methylmorpholine (1.05 mL, 957 mg, 9.46 mmol, 1.25 eq.) in THF (21 mL) was added dropwise. The reaction mixture was warmed to room temperature overnight and thereafter diluted with chloroform. After washing the solution with aqueous HCl solution (1 M), the mixture was extracted three times with chloroform and the combined organics were washed with saturated $NaHCO_3$ solution and dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by column chromatography (SiO_2, DCM:MeOH 100:0 → 95:5) to afford tripeptide **9** (5.38 g, 7.22 mmol, 95 % over 2 steps) as a white solid. **R$_f$** = 0.33 (SiO_2, DCM:MeOH 95:5); $[\alpha]_D^{20}$ = – 14.2 ($CHCl_3$, c = 1.0); **m.p.** 153 – 155 °C.



9

^{1}H-NMR (400 MHz, DMSO-d$_6$): δ = 1.27 (d, $^3J_{8,7}$ = 7.3 Hz, 3 H, 8-H), 1.29 (s, 9 H, 33-H), 1.20 – 1.45 (m, 4 H, 12-H, 13-H), 2.21 (d, $^3J_{10,11}$ = 6.4 Hz, 2 H, 10-H), 2.65 (dd, $^2J_{23a,23b}$ = 13.5 Hz, $^3J_{23a,22}$ = 9.9 Hz, 1 H, 23-H$_a$), 2.84 (dd, $^2J_{23b,23a}$ = 13.5 Hz, $^3J_{23,22}$ = 4.5 Hz, 1 H, 23-H$_b$), 2.95 (m, 2 H, 14-H), 3.95 – 4.10 (m, 2 H, 11-H, 22-H), 4.28 (qd, $^3J_{7,8}$ = 7.3 Hz, $^3J_{7,NHa}$ = 6.9 Hz, 1 H, 7-H), 4.50 (dt, $^3J_{28,29}$ = 5.1 Hz, $^4J_{28,30}$ = 1.5 Hz, 2 H, 28-H), 4.99 (s, 2 H, 16-H), 5.07 (d, $^2J_{5a,5b}$ = 12.7 Hz, 1 H, 5-H$_a$), 5.11 (d, $^2J_{5b,5a}$ = 12.7 Hz, 1 H, 5-H$_b$), 5.23 (ddt, $^3J_{30a,29}$ = 10.5 Hz, $^2J_{30a,30b}$ = 1.6 Hz, $^4J_{30a,28}$ = 1.5 Hz, 1 H, 30-H$_a$), 5.37 (ddt, $^3J_{30b,29}$ = 17.2 Hz, $^2J_{30b,30a}$ =

1.6 Hz, $^4J_{30b,28}$ = 1.5 Hz, 1 H, 30-H$_b$), 6.01 (ddt, $^3J_{29,30b}$ = 17.2 Hz, $^3J_{29,30a}$ = 10.5 Hz, $^3J_{29,28}$ = 5.1 Hz, 1 H, 29-H), 6.80 – 6.85 (m, 3 H, 25-H, N-H$_d$), 7.11 – 7.20 (m, 3 H, 26-H, N-H$_b$), 7.26 – 7.38 (m, 10 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H), 7.73 (d, $^3J_{NHc,11}$ = 8.7 Hz, 1 H, N-H$_c$), 8.33 (d, $^3J_{NHa,7}$ = 6.9 Hz, 1 H, N-H$_a$). **^{13}C-NMR (100 MHz, DMSO-d$_6$):** δ = 16.9 (q, C-8), 26.0 (t, C-12), 28.1 (q, C-33), 31.0 (t, C-13), 36.6 (t, C-23), 40.2 (t, C-14), 40.2 (t, C-10), 45.8 (d, C-11), 47.6 (d, C-7), 56.2 (d, C-22), 65.1 (t, C-16), 65.8 (t, C-5), 68.1 (t, C-28), 77.9 (s, C-32), 114.2 (d, C-25), 117.2 (t, C-30), 127.6 (d, C-3/C-18), 127.6 (d, C-3/C-18), 127.7 (d, C-1/C-20), 128.0 (d, C-1/C-20), 128.3 (d, C-2/C-19), 128.4 (d, C-2/C-19), 130.2 (d, C-26), 130.3 (s, C-24), 133.9 (d, C-29), 136.0 (s, C-4), 137.3 (s, C-17), 155.1 (s, C-31), 156.0 (s, C-15), 156.6 (s, C-27), 170.1 (s, C-9), 171.0 (s, C-21), 172.5 (s, C-6).
HRMS (ESI) calculated for $C_{41}H_{53}N_4O_9$ $[M+H]^+$: 745.3807, found: 745.3779.

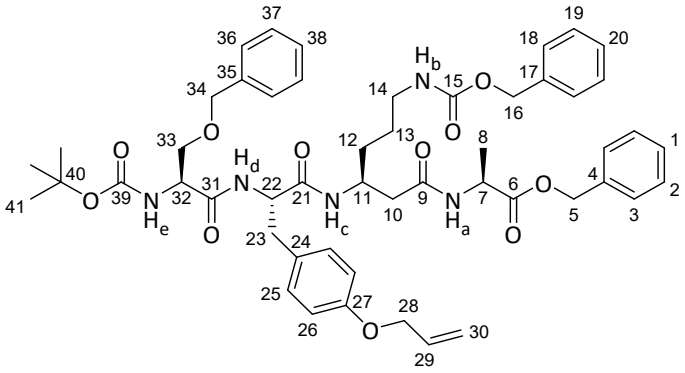
Benzyl *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-*N*$_\varepsilon$-benzyloxycarbonyl-L-β-lysyl-L-alaninate (10)

Boc-deprotection:

Tripeptide **9** (122 mg, 164 µmol, 1.0 eq.) was reacted with HCl according to **GP1** for 1 hour to afford the amine hydrochloride (106 mg, 164 µmol, 100 %) as a yellowish resin and the crude product was used in the next step without further purification.

Peptide coupling:

TBTU (38.7 mg, 121 µmol, 1.05 eq.), DIPEA (44.1 µL, 32.6 mg, 252 µmol, 2.2 eq.), the previously synthesized amine hydrochloride (74.0 mg, 115 µmol, 1.0 eq.) and Boc-L-Ser(Bzl)-OH (33.9 mg, 115 µmol, 1.0 eq.) were reacted according to **GP2** and the crude product was purified by column chromatography (C18, H_2O:MeCN 100:0 → 0:100) to afford tetrapeptide **10** (105 mg, 114 µmol, 99 %) as a yellowish solid. **R$_f$** = 0.28 (SiO_2, DCM:MeOH 95:5); $[\alpha]_D^{20}$ = – 19.9 ($CHCl_3$, c = 1.0); **m.p.** 164 – 165 °C.



10

^{1}H-NMR (500 MHz, DMSO-d$_6$): δ = 1.24 – 1.42 (m, 4 H, 12-H, 13-H), 1.27 (d, $^3J_{8,7}$ = 7.6 Hz, 3 H, 8-H), 1.37 (s, 9 H, 41-H), 2.15 (dd, $^2J_{10a,10b}$ = 14.5 Hz, $^3J_{10a,11}$ = 6.0 Hz, 1 H, 10-H$_a$), 2.19 (dd, $^2J_{10b,10a}$ = 14.5 Hz, $^3J_{10b,11}$ = 7.6 Hz, 1 H, 10-H$_b$), 2.71 (dd, $^2J_{23a,23b}$ = 13.6 Hz, $^3J_{23a,22}$ = 8.2 Hz, 1 H, 23-H$_a$), 2.88 (dd, $^2J_{23b,23a}$ = 13.6 Hz, $^3J_{23b,22}$ = 5.0 Hz, 1 H, 23-H$_b$), 2.92 (m, 2 H, 14-H), 3.47 (dd, $^2J_{33a,33b}$ = 10.1 Hz, $^3J_{33a,32}$ = 7.6 Hz, 1 H, 33-H$_a$), 3.51 (dd, $^2J_{33b,33a}$ = 10.1 Hz, $^3J_{33b,32}$ = 4.4 Hz, 1 H, 33-H$_b$), 4.02 (m, 1 H, 11-H), 4.19 (ddd, $^3J_{32,NHe}$ = 8.5 Hz, $^3J_{32,33a}$ = 7.6 Hz, $^3J_{32,33b}$ = 4.7 Hz, 1 H, 32-H), 4.29 (qd, $^3J_{7,8}$ = 7.6 Hz, $^3J_{7,NHa}$ = 6.9 Hz, 1 H, 7-H), 4.40 – 4.45 (m, 3 H, 22-H, 34-H), 4.46 (ddd, $^3J_{28,29}$ = 5.0 Hz, $^4J_{28,30b}$ = 1.9 Hz, $^4J_{28,30a}$ = 1.6 Hz, 2 H, 28-H), 4.98 (s, 2 H, 16-H), 5.07 (d, $^2J_{5a,5b}$ = 12.6 Hz, 1 H, 5-H$_a$), 5.10 (d, $^2J_{5b,5a}$ = 12.6 Hz, 1 H, 5-H$_b$), 5.22 (dtd, $^3J_{30a,29}$ = 10.7 Hz, $^4J_{30a,29}$ = $^2J_{30a,30b}$ = 1.6 Hz, 1 H, 30-H$_a$), 5.36 (dtd, $^3J_{30b,29}$ = 17.0 Hz, $^4J_{30b,28}$ = 1.9 Hz, $^2J_{30b,30a}$ = 1.6 Hz, 1 H, 30-H$_b$), 6.01 (ddt, $^3J_{29,30b}$ = 17.0 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.0 Hz, 1 H, 29-H), 6.76 (d, $^3J_{25,26}$ = 8.5 Hz, 2 H, 25-H), 6.95 (d, $^3J_{NHe,32}$ = 8.5 Hz, 1 H, N-H$_e$), 7.08 (d, $^3J_{26,25}$ = 8.5 Hz, 2 H, 26-H), 7.17 (t, $^3J_{NHb,14}$ = 5.6 Hz, 1 H, N-H$_b$), 7.25 – 7.38 (m, 15 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H), 7.84 (d, $^3J_{NHc,11}$ = 8.5 Hz, 1 H, N-H$_c$), 7.87 (d, $^3J_{NHd,22}$ = 8.2 Hz, 1 H, N-H$_d$), 8.35 (d, $^3J_{NHd,22}$ = 6.9 Hz, 1 H, N-H$_a$). **^{13}C-NMR (125 MHz, DMSO-d$_6$):** δ = 16.9 (q, C-8), 26.0 (t, C-13), 28.1 (q, C-41), 31.0 (t, C-12), 37.1 (t, C-23), 45.9

(d, C-11), 47.6 (d, C-7), 54.0 (d, C-22), 54.6 (d, C-32), 65.1 (t, C-16), 65.8 (t, C-5), 68.0 (t, C-28), 70.0 (t, C-33), 71.9 (t, C-34), 78.4 (s, C-40), 114.1 (d, C-25), 117.2 (t, C-30), 127.4 (d, C-38), 127.5 (d, C-36), 127.7 (d, C-3), 127.7 (d, C-18), 127.7 (d, C-1), 128.0 (d, C-20), 128.1 (d, C-37), 128.3 (d, C-2), 128.4 (d, C-19), 129.5 (s, C-24), 130.3 (d, C-26), 133.8 (d, C-29), 136.0 (s, C-4), 137.2 (s, C-17), 138.2 (s, C-35), 155.2 (s, C-39), 156.0 (s, C-15), 156.7 (s, C-27), 169.3 (s, C-31), 169.9 (s, C-9), 169.9 (s, C-21), 172.5 (s, C-6). The signals of C-10 and C-14 are located under the DMSO-signal.
HRMS (ESI) calculated for $C_{51}H_{64}N_5O_{11}$ $[M+H]^+$: 922.4597, found: 922.4629.

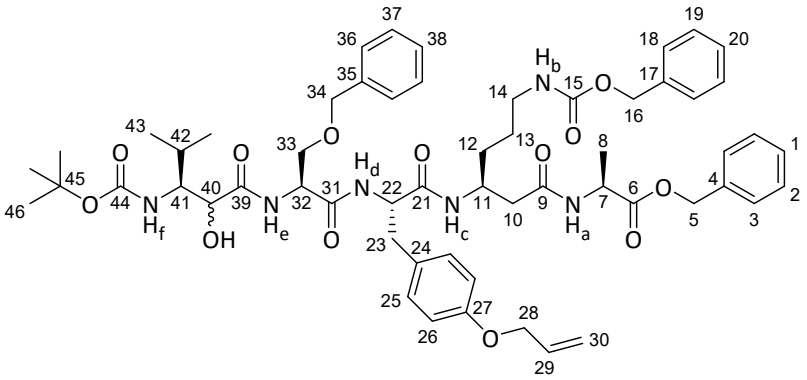
Benzyl 3-*tert*-butoxycarbonylamino-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (11)

Boc-deprotection:

Tetrapeptide **10** (100 mg, 108 µmol, 1.0 eq.) was reacted with HCl according to **GP1** for 1 hour to afford the amine hydrochloride (89.0 mg, 108 µmol, 100 %) as a yellowish resin and the crude product was used in the next step without further purification.

Peptide coupling:

DIPEA (20.4 µL, 15.1 mg, 117 µmol, 1.2 eq.) was added to a solution of the previously synthesized amine hydrochloride (80.0 mg, 97.0 µmol, 1.0 eq.) in DMF (970 µL) at 0 °C and the resulting mixture was stirred for 5 minutes. Subsequently, carboxylic acid **5** (24.1 mg, 97.0 µmol, 1.0 eq.), HOBt (15.7 mg, 102 µmol, 1.05 eq.) and EDC·HCl (19.6 mg, 102 µmol, 1.05 eq.) were added and the reaction mixture was warmed to room temperature overnight. The mixture was first diluted with chloroform, then washed with aqueous HCl solution (1M) and the aqueous phase was extracted three times with chloroform. The combined organic phases were washed with saturated $NaHCO_3$ solution and dried over Na_2SO_4. After filtration, the solvent was evaporated and the crude product was purified by flash chromatography (C18, H_2O:MeCN 100:0 → 0:100) to afford pentapeptide **11** (92.0 mg, 88.0 µmol, 90 %) as a white solid. $\mathbf{R_f}$ = 0.09 (SiO_2, DCM:MeOH 95:5).



11

Diastereomer A:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.80 (d, $^3J_{43,42}$ = 6.6 Hz, 3 H, 43-H), 0.81 (d, $^3J_{43',42}$ = 6.6 Hz, 3 H, 43-H'), 1.25 – 1.50 (m, 4 H, 12-H, 13-H), 1.29 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.39 (s, 9 H, 46-H), 1.84 (m, 1 H, 42-H), 2.20 (dd, $^2J_{10a,10b}$ = 14.4 Hz, $^3J_{10a,11}$ = 6.6 Hz, 1 H, 10-H_a), 2.25 (dd, $^2J_{10b,10a}$ = 14.4 Hz, $^3J_{10b,11}$ = 5.7 Hz, 1 H, 10-H_b), 2.78 (dd, $^2J_{23a,23b}$ = 13.8 Hz, $^3J_{23a,22}$ = 8.2 Hz, 1 H, 23-H_a), 2.94 – 3.00 (m, 3 H, 23-H_b, 14-H), 3.54 – 3.70 (m, 3 H, 33-H, 41-H), 3.97 – 4.08 (m, 2 H, 11-H, 40-H), 4.35 (qd, $^3J_{7,8}$ = $^3J_{7,NHa}$ = 7.2 Hz, 1 H, 7-H), 4.43 – 4.56 (m, 6 H, 22-H, 28-H, 32-H, 34-H), 5.01 (s, 2 H, 16-H), 5.09 (d, $^2J_{5a,5b}$ = 12.9 Hz, 1 H, 5-H_a), 5.13 (d, $^2J_{5b,5a}$ = 12.9 Hz, 1 H, 5-H_b), 5.22 (m, 1 H, 30-H_a), 5.36 (m, 1 H, 30-H_b), 5.55 (d, $^3J_{OH,40}$ = 4.7 Hz, 1 H, O-H), 5.81 (bs, 1 H, N-H_f), 6.01 (ddt, $^3J_{29,30b}$ = 17.6 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.3 Hz, 1 H, 29-H), 6.72 (bs, 1 H, N-H_b), 6.79 (d, $^3J_{25,26}$ = 8.2 Hz, 2 H, 25-H), 7.10 (d, $^3J_{26,25}$ = 8.2 Hz, 2 H, 26-H), 7.23 – 7.39 (m, 15 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H), 7.47 (d, $^3J_{NHc,11}$ = 8.2 Hz, 1 H, N-H_c), 7.59 (d, $^3J_{NHe,32}$ = 7.9 Hz, 1 H, N-H_e), 7.84 (d, $^3J_{NHd,22}$ = 7.9 Hz, 1 H, N-H_d), 7.99 (d, $^3J_{NHa,7}$ = 7.2 Hz, 1 H, N-H_a).

^{13}C-NMR (125 MHz, DMSO-d_6, 373 K): δ = 16.4 (q, C-8), 17.8 (q, C-43'), 19.9 (q, C-43), 25.5 (t, C-13), 27.8 (q, C-46), 27.8 (d, C-42), 30.6 (t, C-12), 36.4 (t, C-23), 45.7 (d, C-11), 47.2 (d, C-7), 51.7 (d, C-32), 54.0 (d, C-22), 57.5 (d, C-41), 64.7 (t, C-16), 65.4 (t, C-5), 68.0 (t, C-28), 69.6 (t, C-33), 71.9 (t, C-34), 73.1 (d, C-40), 77.3 (s, C-45), 114.1 (d, C-25), 116.5 (t, C-30), 126.8, 126.9, 127.0, 127.1, 127.4, 127.6, 127.6, 127.8, 127.9 (9d, C-1, C-2, C-3, C-18, C-19, C-20, C-36, C-37, C-38), 129.4 (s, C-24), 129.6 (d, C-26), 133.5 (d, C-29), 135.6 (s, C-4), 136.9 (s, C-17), 137.7 (s, C-35), 155.6 (s, C-15), 156.5 (s, C-27), 168.5 (s, C-31), 169.4 (s, C-21), 169.5 (s, C-9), 171.2 (s, C-39), 171.8 (s, C-6). The signals of C-10 and C-14 are located under the DMSO-signal. The signal of C-44 could not be detected.

Diastereomer B (selected signals):

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.85 (d, $^{3}J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.92 (d, $^{3}J_{43',42}$ = 6.6 Hz, 3 H, 43-H'), 1.33 (s, 9 H, 46-H), 5.32 (d, $^{3}J_{OH,40}$ = 7.2 Hz, 1 H, O-H), 5.68 (bs, 1 H, N-H_f), 7.09 (d, $^{3}J_{26,25}$ = 8.2 Hz, 1 H, 26-H), 7.45 (d, $^{3}J_{NHc,11}$ = 8.5 Hz, 1 H, N-H_c), 7.70 (d, $^{3}J_{NHe,32}$ = 7.5 Hz, 1 H, N-H_e), 7.80 (d, $^{3}J_{NHd,22}$ = 7.5 Hz, 1 H, N-H_d), 7.97 (d, $^{3}J_{NHa,7}$ = 7.2 Hz, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 18.3 (q, C-43), 19.4 (q, C-43'), 27.7 (q, C-46), 29.2 (d, C-42), 36.5 (t, C-23), 52.0 (d, C-32), 54.0 (d, C-22), 57.7 (d, C-41), 67.9 (t, C-28), 69.5 (t, C-33), 70.7 (d, C-40), 77.2 (s, C-45), 116.5 (t, C-30), 129.3 (s, C-24), 129.6 (d, C-26), 137.8 (s, C-35), 168.5 (s, C-31), 169.3 (s, C-21), 169.5 (s, C-9), 172.0 (s, C-39).
HRMS (ESI) calculated for $C_{57}H_{75}N_6O_{13}$ $[M+H]^+$: 1051.5387, found: 1051.5342.

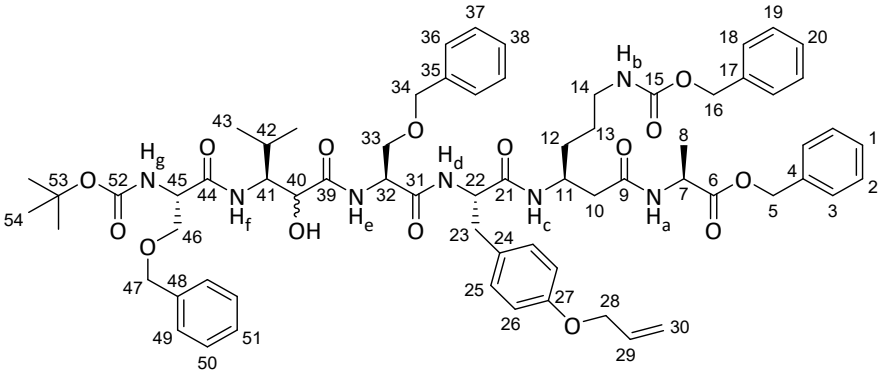
Benzyl 3-(*N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (12)

Boc-deprotection:

Pentapeptide **11** (1.13 g, 1.08 mmol, 1.0 eq.) was reacted with HCl according to **GP1** for 90 min to afford the amine hydrochloride (1.02 g, 1.08 mmol, 100 %) as a yellowish resin which was used in the next step without further purification.

Peptide coupling:

TBTU (16.7 mg, 52.0 µmol, 1.05 eq.), DIPEA (19.0 µL, 14.1 mg, 109 µmol, 2.2 eq), the previously synthesized amine hydrochloride (47.0 mg, 49.0 µmol, 1.0 eq.) and Boc-L-Ser(Bzl)-OH (14.6 mg, 49.0 µmol, 1.0 eq.) were reacted according to **GP2** and the crude product was purified by column chromatography (SiO_2, DCM:MeOH 100:0 → 95:5) to afford hexapeptide **12** (53.0 mg, 43.0 µmol, 87 %) as a white solid. $\mathbf{R_f}$ = 0.18 (SiO_2, DCM:MeOH 95:5).



12

Diastereomer A:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.80 (d, $^{3}J_{43,42}$ = 6.6 Hz, 3 H, 43-H), 0.83 (d, $^{3}J_{43',42}$ = 6.6 Hz, 3 H, 43-H'), 1.27 – 1.53 (m, 4 H, 12-H, 13-H), 1.30 (d, $^{3}J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.41 (s, 9 H, 54-H), 1.89 (m, 1 H, 42-H), 2.21 (m, 1 H, 10-H_a), 2.26 (dd, $^{2}J_{10b,10a}$ = 14.4 Hz, $^{3}J_{10b,11}$ = 6.0 Hz, 1 H, 10-H_b), 2.79 (dd, $^{2}J_{23a,23b}$ = 14.1 Hz, $^{3}J_{23a,22}$ = 8.2 Hz, 1 H, 23-H_a), 2.95 – 3.02 (m, 3 H, 14-H, 23-H_b), 3.50 – 3.73 (m, 4 H, 33-H, 46-H), 4.00 – 4.06 (m, 3 H, 11-H, 40-H, 41-H), 4.22 (m, 1 H, 45-H), 4.36 (qd, $^{3}J_{7,8}$ = $^{3}J_{7,NHa}$ = 7.2 Hz, 1 H, 7-H),

4.40 – 4.56 (m, 8 H, 22-H, 28-H, 32-H, 34-H, 47-H), 5.02 (s, 2 H, 16-H), 5.09 (d, $^2J_{5a,5b}$ = 12.9 Hz, 1 H, 5-H_a), 5.13 (d, $^2J_{5b,5a}$ = 12.9 Hz, 1 H, 5-H_b), 5.22 (m, 1 H, 30-H_a), 5.35 (m, 1 H, 30-H_b), 5.60 (d, $^3J_{OH,41}$ = 4.1 Hz, 1 H, O-H), 6.01 (ddt, $^3J_{29,30b}$ = 17.3 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.3 Hz, 1 H, 29-H), 6.50 (bs, 1 H, N-H_g), 6.67 (bs, 1 H, N-H_b), 6.79 (d, $^3J_{25,26}$ = 8.8 Hz, 2 H, 25-H), 7.10 (d, $^3J_{26,25}$ = 8.8 Hz, 2 H, 26-H), 7.22 (m, 1 H, N-H_f), 7.24 – 7.38 (m, 20 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H, 49-H, 50-H, 51-H), 7.44 (d, $^3J_{NHc,11}$ = 7.9 Hz, 1 H, N-H_c), 7.62 (d, $^3J_{NHe,32}$ = 8.2 Hz, 1 H, N-H_e), 7.79 (d, $^3J_{NHd,22}$ = 7.2 Hz, 1 H, N-H_d), 7.94 (m, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 16.4 (q, C-8), 17.8 (q, C-43'), 19.9 (q, C-43), 25.4 (t, C-13), 27.6 (d, C-42), 27.7 (q, C-54), 30.6 (t, C-12), 36.4 (t, C-23), 45.8 (d, C-11), 47.2 (d, C-7), 51.8 (d, C-32), 54.0 (d, C-22), 54.5 (d, C-45), 55.7 (d, C-41), 64.7 (t, C-16), 65.3 (t, C-5), 68.0 (t, C-28), 69.5 (t, C-46), 69.6 (t, C-33), 71.8 (t, C-34/C-47), 71.9 (t, C-34/C-47), 72.9 (d, C-40), 78.0 (s, C-53), 114.1 (d, C-25), 116.4 (t, C-30), 126.7, 126.8, 126.8, 126.9, 127.0, 127.0, 127.3, 127.5, 127.6, 127.7, 127.8 (12d, C-1, C-2, C-3, C-18, C-19, C-20, C-36, C-37, C-38, C-49, C-50, C-51), 129.4 (s, C-24), 129.6 (d, C-26), 133.5 (d, C-29), 135.6 (s, C-4), 136.9 (s, C-17), 137.7 (s, C-35/C-48), 137.9 (s, C-35/C-48), 154.8 (s, C-52), 155.5 (s, C-15), 156.5 (s, C-27), 168.4 (s, C-31), 169.2 (s, C-44), 169.3 (s, C-21), 169.5 (s, C-9), 171.2 (s, C-39), 171.8 (s, C-6). The signals of C-10 and C-14 are located under the DMSO-signal.

Diastereomer B (selected signals):

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.84 (d, $^3J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.93 (d, $^3J_{43',42}$ = 6.6 Hz, 3 H, 43-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.40 (s, 9 H, 54-H), 2.83 (dd, $^2J_{23a,23b}$ = 14.1 Hz, $^3J_{23a,22}$ = 7.9 Hz, 1 H, 23-H_a), 3.90 (ddd, $^3J_{41,NHf}$ = 9.7 Hz, $^3J_{41,42}$ = 7.9 Hz, $^3J_{41,40}$ = 2.5 Hz, 1 H, 41-H), 4.09 (dd, $^3J_{40,OH}$ = 6.0 Hz, $^3J_{40,41}$ = 2.5 Hz, 1 H, 40-H), 4.35 (qd, $^3J_{7,8}$ = $^3J_{7,NHa}$ = 7.2 Hz, 1 H, 7-H), 5.50 (d, $^3J_{OH,40}$ = 6.0 Hz, 1 H, O-H), 6.47 (bs, 1 H, N-H_g), 6.78 (d, $^3J_{25,26}$ = 8.8 Hz, 2 H, 25-H), 7.09 (d, $^3J_{26,25}$ = 8.8 Hz, 2 H, 26-H), 7.48 (d, $^3J_{NHc,11}$ = 7.9 Hz, 1 H, N-H_c), 7.64 (d, $^3J_{NHe,32}$ = 8.2 Hz, 1 H, N-H_e), 7.70 (d, $^3J_{NHd,22}$ = 7.2 Hz, 1 H, N-H_d).
^{13}C-NMR (125 MHz, DMSO-d_6, 373 K): δ = 18.3 (q, C-43), 19.4 (q, C-43'), 25.4 (t, C-13), 27.7 (q, C-54), 29.0 (d, C-42), 36.2 (t, C-23), 45.7 (d, C-11), 52.6 (d, C-32), 54.0 (d, C-22), 54.5 (d, C-45), 56.1 (d, C-41), 69.3 (t, C-33), 70.5 (d, C-40), 71.8 (t, C-34/C-47), 71.9 (t, C-34/C-47), 78.1 (s, C-53), 116.4 (t, C-30), 129.5 (s, C-24), 129.5 (d, C-26), 135.6 (s, C-4), 137.8 (s, C-35/C-48), 156.5 (s, C-27), 168.4 (s, C-31), 169.4 (s, C-9), 172.4 (s, C-39).
HRMS (ESI) calculated for $C_{67}H_{86}N_7O_{15}$ $[M+H]^+$: 1228.6176, found: 1228.6233.

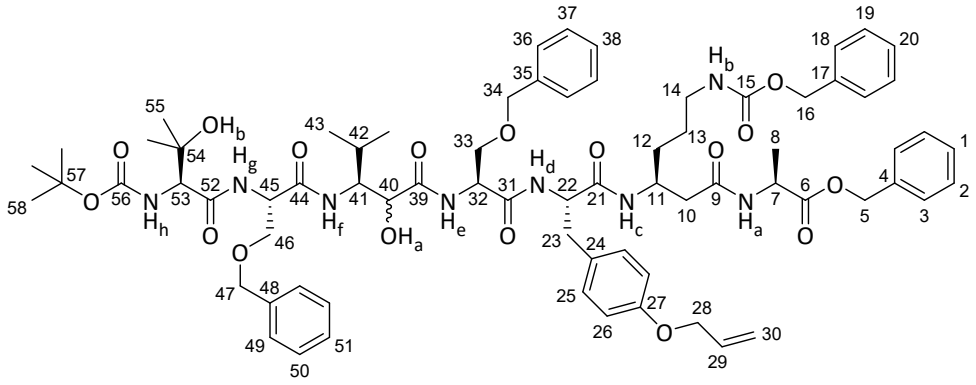
Benzyl 3-(*N*-*tert*-butoxycarbonyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methyl-pentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (13)

Boc-deprotection:

Hexapeptide **12** (42.5 mg, 35.0 µmol, 1.0 eq.) was reacted with HCl according to **GP1** for 1 hour to afford the amine hydrochloride (39.0 mg, 35.0 µmol, 100 %) as a yellowish resin which was used in the next step without further purification.

Peptide coupling:

TBTU (11.8 mg, 37.0 µmol, 1.05 eq.), DIPEA (13.5 µL, 10.0 mg, 77.0 µmol, 2.2 eq), the previously synthesized amine hydrochloride (39.5 mg, 35.0 µmol, 1.0 eq.) and Boc-β-hydroxy-L-valine (8.6 mg, 37.0 µmol, 1.05 eq.) were reacted according to **GP2** and the crude product was purified by column chromatography (C18, H_2O:MeCN 100:0 → 0:99) to afford heptapeptide **13** (34.0 mg, 25.0 µmol, 72 %) as a white solid. $\mathbf{R_f}$ = 0.15 (SiO_2, DCM:MeOH 95:5).



13

Diastereomer A:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.79 (d, $^3J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.83 (d, $^3J_{43',42}$ = 6.9 Hz, 3 H, 43-H'), 1.13 (s, 3 H, 55-H), 1.19 (s, 3 H, 55-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.35 – 1.53 (m, 4 H, 12-H, 13-H), 1.40 (s, 9 H, 58-H), 1.91 (m, 1 H, 42-H), 2.21 (m, 1 H, 10-H_a), 2.26 (m, 1 H, 10-H_b), 2.79 (dd, $^2J_{23a,23b}$ = 14.1 Hz, $^3J_{23a,22}$ = 8.2 Hz, 1 H, 23-H_a), 2.95 – 3.02 (m, 3 H, 14-H, 23-H_b), 3.59 – 3.73 (m, 4 H, 33-H, 46-H), 4.00 – 4.07 (m, 4 H, 11-H, 40-H, 41-H, 53-H), 4.36 (qd, $^3J_{7,8}$ = $^3J_{7,NHa}$ = 7.2 Hz, 1 H, 7-H), 4.40 – 4.61 (m, 10 H, 22-H, 28-H, 32-H, 34-H, 45-H, 47-H, O-H_b), 5.02 (s, 2 H, 16-H), 5.09 (d, $^2J_{5a,5b}$ = 12.6 Hz, 1 H, 5-H_a), 5.13 (d, $^2J_{5b,5a}$ = 12.6 Hz, 1 H, 5-H_b), 5.22 (m, 1 H, 30-H_a), 5.35 (m, 1 H, 30-H_b), 5.55 (d, $^3J_{OH,41}$ = 5.0 Hz, 1 H, O-H_a), 6.01 (ddt, $^3J_{29,30b}$ = 17.3 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.3 Hz, 1 H, 29-H), 6.11 (d, $^3J_{NHh,53}$ = 6.9 Hz, 1 H, N-H_h), 6.67 (bs, 1 H, N-H_b), 6.79 (d, $^3J_{25,26}$ = 8.8 Hz, 2 H, 25-H), 7.10 (d, $^3J_{26,25}$ = 8.8 Hz, 2 H, 26-H), 7.23 – 7.38 (m, 21 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H, 49-H, 50-H, 51-H, N-H_f), 7.44 (d, $^3J_{NHc,11}$ = 8.5 Hz, 1 H, N-H_c), 7.59 (d, $^3J_{NHe,32}$ = 7.9 Hz, 1 H, N-H_e), 7.76 – 7.82 (m, 2 H, N-H_d, N-H_g), 7.95 (d, $^3J_{NHa,7}$ = 7.2 Hz, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 16.4 (q, C-8), 17.9 (q, C-43'), 19.8 (q, C-43), 25.0 (q, C-55), 25.4 (t, C-13), 26.8 (q, C-55'), 27.4 (d, C-42), 27.7 (q, C-58), 30.6 (t, C-12), 36.4 (t, C-23), 45.8 (d, C-11), 47.2 (d, C-7), 51.8 (d, C-32), 52.7 (d, C-45), 54.0 (d, C-22), 55.9 (d, C-41), 61.9 (d, C-53), 64.7 (t, C-16), 65.3 (t, C-5), 68.0 (t, C-28), 69.5 (t, C-46), 69.5 (t, C-33), 70.7 (s, C-54), 71.9 (t, C-34/C-47), 71.9 (t, C-34/C-47), 73.0 (d, C-40), 78.0 (s, C-57), 114.1 (d, C-25), 116.4 (t, C-30), 126.7, 126.8, 126.8, 126.9, 126.9, 127.0, 127.3, 127.5, 127.5, 127.7, 127.8 (12d, C-1, C-2, C-3, C-18, C-19, C-20, C-36, C-37, C-38, C-49, C-50, C-51), 129.4 (s, C-24), 129.6 (d, C-26), 133.5 (d, C-29), 135.6 (s, C-4), 136.9 (s, C-17), 137.7 (s, C-35/C-48), 137.7 (s, C-35/C-48), 154.8 (s, C-56), 155.5 (s, C-15), 156.5 (s, C-27), 168.4 (s, C-31), 168.8 (s, C-44), 169.3 (s, C-21), 169.5 (s, C-9), 170.0 (s, C-52), 171.1 (s, C-39), 171.7 (s, C-6). The signals for C-10 and C-14 are located under the DMSO-signal.

Diastereomer B (selected signals):

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.84 (d, $^3J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.92 (d, $^3J_{43',42}$ = 6.6 Hz, 3 H, 43-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.40 (s, 9 H, 58-H), 1.86 (m, 1 H, 42-H), 2.83 (dd, $^2J_{23a,23b}$ = 14.1 Hz, $^3J_{23a,22}$ = 7.9 Hz, 1 H, 23-H_a), 3.90 (ddd, $^3J_{41,NHf}$ = 9.7 Hz, $^3J_{41,42}$ = 7.9 Hz, $^3J_{41,40}$ = 2.8 Hz, 1 H, 41-H), 4.10 (dd, $^3J_{40,OHa}$ = 6.0 Hz, $^3J_{40,41}$ = 2.8 Hz, 1 H, 40-H), 4.35 (qd, $^3J_{7,8}$ = $^3J_{7,NHa}$ = 7.2 Hz, 1 H, 7-H), 5.31 (d, $^3J_{OH,40}$ = 6.0 Hz, 1 H, O-H_a), 6.05 (m, 1 H, N-H_h), 6.77 (d, $^3J_{25,26}$ = 8.8 Hz, 2 H, 25-H), 7.08 (d, $^3J_{26,25}$ = 8.8 Hz, 2 H, 26-H), 7.28 (m, 1 H, N-H_f), 7.65 (d, $^3J_{NHe,32}$ = 6.9 Hz, 1 H, N-H_e), 7.71 (d, $^3J_{NHd,22}$ = 7.5 Hz, 1 H, N-H_d), 7.92 (d, $^3J_{NHa,7}$ = 7.2 Hz, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 18.4 (q, C-43), 19.3 (q, C-43'), 24.9 (q, C-55), 25.4 (t, C-13), 26.9 (q, C-55'), 28.8 (d, C-42), 36.2 (t, C-23), 45.7 (d, C-11), 52.7 (d, C-32), 52.8 (d, C-45), 54.0 (d, C-22), 56.4 (d, C-41), 69.3 (t, C-33), 69.5 (t, C-46), 70.6 (d, C-40), 71.9 (t, C-34/C-47), 116.4 (t, C-30), 129.4 (s, C-24), 129.5 (d, C-26), 137.7 (s, C-35/C-48), 156.5 (s, C-27), 169.4 (s, C-9), 170.0 (s, C-52), 172.3 (s, C-39).

HRMS (ESI) calculated for $C_{72}H_{94}N_8O_{17}$ $[M+H]^+$: 1343.6810, found: 1343.6794.

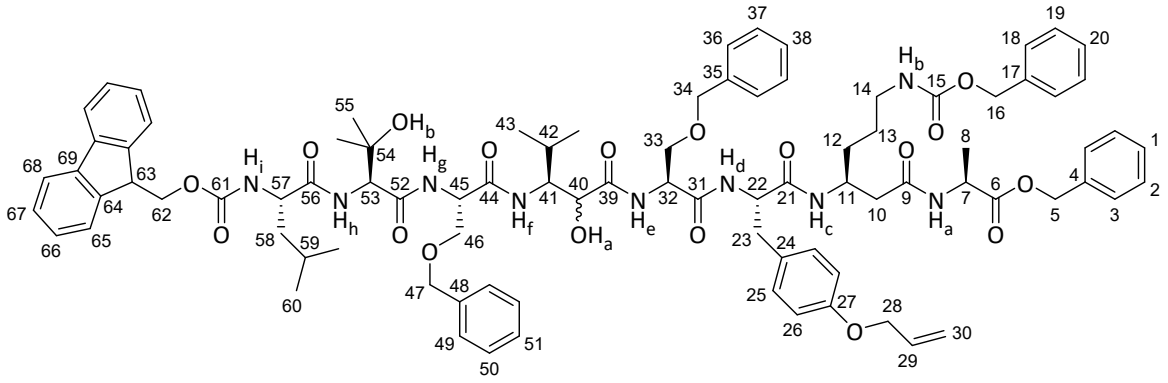
Benzyl 3-(*N*-Fluorenylmethoxycarbonyl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (14)

Boc-deprotection:

Heptapeptide **13** (136 mg, 101 µmol, 1.0 eq.) was reacted with HCl according to **GP1** for 1 hour to afford the amine hydrochloride (125 mg, 101 µmol, 100 %) as a yellowish solid which was used in the next step without further purification.

Peptide coupling:

TBTU (7.1 mg, 22.0 µmol, 1.05 eq.), DIPEA (8.1 µL, 6.0 mg, 46.0 µmol, 2.2 eq), the previously synthesized amine hydrochloride (26.1 mg, 21.0 µmol, 1.0 eq.) and Fmoc-L-Leu-OH (7.8 mg, 22.0 µmol, 1.05 eq.) were reacted according to **GP2** and the crude product was purified by column chromatography (C18, H_2O:MeCN 100:0 → 0:100) to afford octapeptide **14** (26.3 mg, 17.0 µmol, 79 %) as a white solid. $\mathbf{R_f}$ = 0.18 (SiO_2, DCM:MeOH 95:5).



14

Diastereomer A:

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.78 (d, $^3J_{43,42}$ = 6.6 Hz, 3 H, 43-H), 0.82 (d, $^3J_{43',42}$ = 6.9 Hz, 3 H, 43-H'), 0.87 (d, $^3J_{60,59}$ = 6.6 Hz, 3 H, 60-H), 0.90 (d, $^3J_{60',59}$ = 6.6 Hz, 3 H, 60-H'), 1.13 (s, 3 H, 55-H), 1.17 (s, 3 H, 55-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.35 – 1.50 (m, 4 H, 12-H, 13-H), 1.53 (m, 2 H, 58-H), 1.65 (m, 1 H, 59-H), 1.92 (m, 1 H, 42-H), 2.24 (m, 2 H, 10-H), 2.79 (dd, $^2J_{23a,23b}$ = 14.1 Hz, $^3J_{23a,22}$ = 8.2 Hz, 1 H, 23-H_a), 2.95 – 3.02 (m, 3 H, 14-H, 23-H_b), 3.55 – 3.75 (m, 4 H, 33-H, 46-H), 3.99 – 4.06 (m, 3 H, 11-H, 40-H, 41-H), 4.12 (dt, $^3J_{57,NHi}$ = 7.9 Hz, $^3J_{57,58}$ = 7.5 Hz, 1 H, 57-H), 4.22 (t, $^3J_{63,62}$ = 7.2 Hz, 1 H, 63-H), 4.28 – 4.38 (m, 3 H, 7-H, 62-H), 4.42 (m, 1 H, 53-H), 4.45 – 4.50 (m, 7 H, 22-H, 28-H, 34-H, 47-H), 4.50 – 4.57 (m, 2 H, 32-H, 45-H), 4.61 (bs, 1 H, O-H_b), 5.02 (s, 2 H, 16-H), 5.09 (d, $^2J_{5a,5b}$ = 12.6 Hz, 1 H, 5-H_a), 5.13 (d, $^2J_{5b,5a}$ = 12.6 Hz, 1 H, 5-H_b), 5.21 (m, 1 H, 30-H_a), 5.34 (m, 1 H, 30-H_b), 5.51 (d, $^3J_{OH,41}$ = 5.0 Hz, 1 H, O-H_a), 6.00 (ddt, $^3J_{29,30b}$ = 17.3 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.3 Hz, 1 H, 29-H), 6.65 (bs, 1 H, N-H_b), 6.78 (m, 2 H, 25-H), 7.09 (m, 2 H, 26-H), 7.15 – 7.45 (m, 28 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H, 49-H, 50-H, 51-H, 66-H, 67-H, N-H_c, N-H_f, N-H_h, N-H_i), 7.58 (d, $^3J_{NHe,32}$ = 8.2 Hz, 1 H, N-H_e), 7.66 (m, 2 H, 65-H), 7.77 (d, $^3J_{NHd,22}$ = 7.5 Hz, 1 H, N-H_d), 7.80 – 7.86 (m, 3 H, 68-H, N-H_g), 7.93 (d, $^3J_{NHa,7}$ = 7.2 Hz, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 16.4 (q, C-8), 17.9 (q, C-43'), 19.8 (q, C-43), 21.0 (q, C-60), 22.3 (q, C-60'), 23.8 (d, C-59), 24.8 (q, C-55), 25.4 (t, C-13), 27.0 (q, C-55'), 27.4 (d, C-42), 30.6 (t, C-12), 36.4 (t, C-23), 45.8 (d, C-11), 46.5 (d, C-63), 47.2 (d, C-7), 51.8 (d, C-32), 52.9 (d, C-45), 53.3 (d, C-57), 54.0 (d, C-22), 55.9 (d, C-41), 59.5 (d, C-53), 64.7 (t, C-16), 65.3 (t, C-5), 65.3 (t, C-62), 67.9 (t, C-28), 69.4 (t, C-46), 69.4 (t, C-33), 71.1 (s, C-54), 71.9, 71.9 (2t, C-34, C-47), 73.1 (d, C-40), 114.1 (d, C-25), 116.4 (t, C-30), 119.4 (d, C-68), 124.5 (d, C-65), 126.4, 126.4, 126.7, 126.7, 126.8, 126.9, 126.9, 126.9, 127.0, 127.3, 127.5, 127.5, 127.7, 127.8 (14d, C-1, C-2, C-3, C-18, C-19, C-20, C-36, C-37, C-38, C-49, C-50, C-51, C-66, C-67), 129.4 (s, C-24), 129.6 (d, C-26), 133.4 (d, C-29), 135.6 (s, C-4), 136.9 (s, C-17), 137.7 (s, C-35/C-48), 137.7 (s, C-35/C-48), 140.3 (s, C-69), 143.3 (s, C-64), 155.3 (s, C-61), 155.5

(s, C-15), 156.5 (s, C-27), 168.4 (s, C-31), 168.8 (s, C-44), 169.3 (s, C-21), 169.3 (s, C-52), 169.4 (s, C-9), 171.1 (s, C-39), 171.6 (s, C-56), 171.7 (s, C-6). The signals of C-10, C-14 and C-58 are located under the DMSO-signal.

Diastereomer B (selected signals):

^{1}H-NMR (500 MHz, DMSO-d$_6$, 373 K): δ = 0.84 (d, $^3J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.86 (d, $^3J_{60,59}$ = 6.6 Hz, 3 H, 60-H), 0.89 (d, $^3J_{60',59}$ = 6.6 Hz, 3 H, 60-H'), 0.92 (d, $^3J_{43',42}$ = 6.6 Hz, 3 H, 43-H'), 1.29 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.86 (m, 1 H, 42-H), 2.82 (dd, $^2J_{23a,23b}$ = 15.4 Hz, $^3J_{23a,22}$ = 7.9 Hz, 1 H, 23-H$_a$), 3.90 (ddd, $^3J_{41,NHf}$ = 10.1 Hz, $^3J_{41,42}$ = 7.9 Hz, $^3J_{41,40}$ = 2.8 Hz, 1 H, 41-H), 4.09 (m, 1 H, 40-H), 4.22 (t, $^3J_{63,62}$ = 6.6 Hz, 1 H, 63-H), 4.42 (m, 1 H, 32-H), 5.01 (s, 2 H, 16-H), 5.22 (m, 1 H, O-H$_a$), 7.65 (m, 1 H, N-H$_e$), 7.69 (m, 1 H, N-H$_d$), 7.90 (d, $^3J_{NHa,7}$ = 7.2 Hz, 1 H, N-H$_a$). **^{13}C-NMR (125 MHz, DMSO-d$_6$, 373 K):** δ = 18.3 (q, C-43), 19.3 (q, C-43'), 24.7 (q, C-55), 25.4 (t, C-13), 27.1 (q, C-55'), 28.8 (d, C-42), 36.3 (t, C-23), 45.7 (d, C-11), 52.6 (d, C-32), 53.3 (d, C-57), 56.4 (d, C-41), 59.5 (d, C-53), 69.3 (t, C-33), 69.5 (t, C-46), 70.6 (d, C-40), 71.9 (t, C-34/C-47), 116.4 (t, C-30), 119.4 (d, C-68), 129.4 (s, C-24), 129.5 (d, C-26), 137.6 (s, C-35/C-48), 137.7 (s, C-35/C-48), 140.3 (s, C-69), 143.4 (s, C-64), 156.5 (s, C-27), 168.8 (s, C-44), 169.4 (s, C-9), 171.5 (s, C-56), 172.2 (s, C-39).
HRMS (ESI) calculated for $C_{88}H_{107}N_9O_{18}$ $[M+H]^+$: 1578.7807, found: 1578.7818.

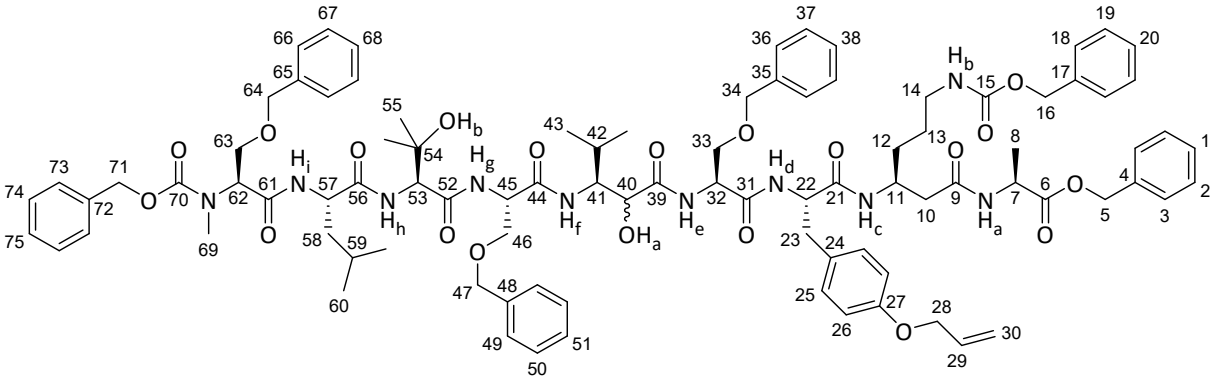
Benzyl 3-(*N*-Benzyloxycarbonyl-*N*-methyl-*O*-benzyl-L-seryl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-*N*$_ε$-benzyloxycarbonyl-L-β-lysyl-L-alaninate (15)

Fmoc-deprotection:

Diethyl amine (420 µL, 294 mg, 4.02 mmol, 30 eq.) was added to a solution of octapeptide **135** (212 mg, 134 µmol, 1.0 eq.) in DMF (1.35 mL) and the resulting mixture was stirred at room temperature for 60 min. After solvent removal, the crude product was obtained and used in the next step without further purification.

Peptide coupling:

TBTU (47.3 mg, 147 µmol, 1.1 eq.), DIPEA (28.1 µL, 20.8 mg, 162 µmol, 1.2 eq), the previously synthesized amine and Cbz-*N*-Me-Ser(Bzl)-OH (48.3 mg, 141 µmol, 1.05 eq.) were reacted according to **GP2** and the crude product was purified by column chromatography (SiO_2, cyclohexane:EtOAc 80:20, then DCM:MeOH 100:0 → 95:5) to afford nonapeptide **15** (197 mg, 117 µmol, 87 %) as a white solid. **R$_f$** = 0.21 (SiO_2, DCM:MeOH 96:4).



15

Diastereomer A:

^{1}H-NMR (500 MHz, DMSO-d$_6$, 373 K): δ = 0.79 (d, $^3J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.80 – 0.87 (m, 9 H, 43-H', 60-H, 60-H'), 1.12 (s, 3 H, 55-H), 1.17 (s, 3 H, 55-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.35 – 1.50 (m, 4 H, 12-H, 13-H), 1.50 – 1.65 (m, 3 H, 58-H, 59-H), 1.92 (m, 1 H, 42-H), 2.23 (m, 2 H, 10-H), 2.79 (dd, $^2J_{23a,23b}$ = 14.1 Hz, $^3J_{23a,22}$ = 7.9 Hz, 1 H, 23-H$_a$), 2.89 (s, 3 H, 69-H), 2.94 – 3.04 (m, 3 H, 14-H, 23-H$_b$), 3.57 – 3.75

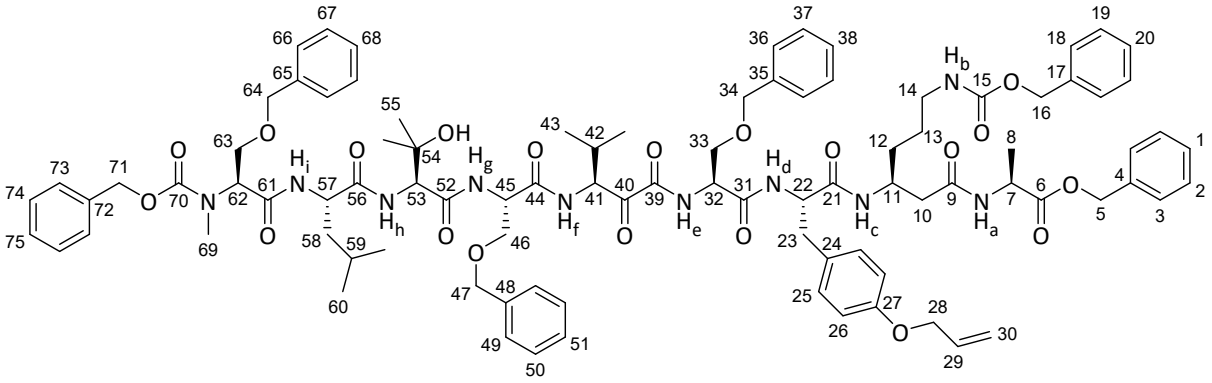
(m, 4 H, 33-H, 46-H), 3.78 (m, 1 H, 63-H_a), 3.86 (m, 1 H, 63-H_b), 4.00 – 4.06 (m, 3 H, 11-H, 40-H, 41-H), 4.31 – 4.44 (m, 3 H, 7-H, 53-H, 57-H), 4.44 – 4.61 (m, 12 H, 22-H, 28-H, 32-H, 34-H, 45-H, 47-H, 64-H, O-H_b), 4.85 (dd, $^3J_{62,63a}$ = 7.9 Hz, $^3J_{62,63b}$ = 5.3 Hz, 1 H, 62-H), 5.02 (s, 2 H, 16-H), 5.09 (d, $^2J_{5a,5b}$ = 12.6 Hz, 1 H, 5-H_a), 5.11 (s, 2 H, 71-H), 5.13 (d, $^2J_{5b,5a}$ = 12.6 Hz, 1 H, 5-H_b), 5.21 (m, 1 H, 30-H_a), 5.35 (m, 1 H, 30-H_b), 5.52 (d, $^3J_{OHa,40}$ = 5.0 Hz, 1 H, O-H_a), 6.00 (ddt, $^3J_{29,30b}$ = 17.3 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.0 Hz, 1 H, 29-H), 6.64 (bs, 1 H, N-H_b), 6.78 (m, 2 H, 25-H), 7.09 (m, 2 H, 26-H), 7.20 – 7.36 (m, 31 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H, 49-H, 50-H, 51-H, 66-H, 67-H, 68-H, 73-H, 74-H, 75-H, N-H_f), 7.39 – 7.49 (m, 2 H, N-H_h, N-H_c), 7.58 (d, $^3J_{NHe,32}$ = 7.8 Hz, 1 H, N-H_e), 7.78 (d, $^3J_{NHd,22}$ = 8.2 Hz, 1 H, N-H_d), 7.81 (d, $^3J_{NHg,45}$ = 7.9 Hz, 1 H, N-H_g), 7.86 (d, $^3J_{NHi,57}$ = 6.6 Hz, 1 H, N-H_i), 7.93 (d, $^3J_{NHa,7}$ = 7.5 Hz, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 16.4 (q, C-8), 17.9 (q, C-43'), 19.8 (q, C-43), 21.1 (q, C-60), 22.3 (q, C-60'), 23.8 (d, C-59), 24.8 (q, C-55), 25.4 (t, C-13), 27.0 (q, C-55'), 27.4 (d, C-42), 30.4 (q, C-69), 30.6 (t, C-12), 36.4 (t, C-23), 45.8 (d, C-11), 47.2 (d, C-7), 51.2 (d, C-57), 51.8 (d, C-32), 52.9 (d, C-45), 54.0 (d, C-22), 55.9 (d, C-41), 58.1 (d, C-62), 59.6 (d, C-53), 64.7 (t, C-16), 65.3 (t, C-5), 66.0 (t, C-71), 66.9 (t, C-63), 68.0 (t, C-28), 69.4 (t, C-46), 69.4 (t, C-33), 71.0 (s, C-54), 71.8 (t, C-64), 71.9, 71.9 (2t, C-34, C-47), 73.1 (d, C-40), 114.1 (d, C-25), 116.4 (t, C-30), 126.7, 126.7, 126.7, 126.8, 126.8, 126.9, 126.9, 126.9, 127.0, 127.1, 127.3, 127.5, 127.5, 127.6, 127.7, 127.7, 127.8 (18d, C-1, C-2, C-3, C-18, C-19, C-20, C-36, C-37, C-38, C-49, C-50, C-51, C-66, C-67, C-68, C-73, C-74, C-75), 129.4 (s, C-24), 129.6 (d, C-26), 133.5 (d, C-29), 135.6 (s, C-4), 136.4 (s, C-72), 136.9 (s, C-17), 137.6 (s, C-35/C-48/C-65), 137.7 (s, C-35/C-48/C-65), 137.7 (s, C-35/C-48/C-65), 155.5 (s, C-15), 155.5 (s, C-70), 156.5 (s, C-27), 168.1 (s, C-61), 168.4 (s, C-31), 168.8 (s, C-44), 169.3 (s, C-21), 169.3 (s, C-52), 169.4 (s, C-9), 171.0 (s, C-56), 171.1 (s, C-39), 171.7 (s, C-6). The signals of C-10, C-14 and C-58 are located under the DMSO-signal.

Diastereomer B (selected signals):

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.92 (d, $^3J_{43',42}$ = 6.9 Hz, 3 H, 43-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.87 (m, 1 H, 42-H), 2.82 (dd, $^2J_{23a,23b}$ = 15.4 Hz, $^3J_{23a,22}$ = 7.9 Hz, 1 H, 23-H_a), 2.89 (s, 3 H, 69-H), 3.91 (ddd, $^3J_{41,NHf}$ = 8.5 Hz, $^3J_{41,42}$ = 7.9 Hz, $^3J_{41,40}$ = 2.8 Hz, 1 H, 41-H), 4.10 (dd, $^3J_{40,OHa}$ = 6.0 Hz, $^3J_{40,41}$ = 2.8 Hz, 1 H, 40-H), 5.01 (s, 2 H, 16-H), 5.23 (m, 1 H, O-H_a), 7.66 (d, $^3J_{NHe,32}$ = 7.2 Hz, 1 H, N-H_e), 7.70 (d, $^3J_{NHd,22}$ = 8.2 Hz, 1 H, N-H_d), 7.81 (d, $^3J_{NHg,45}$ = 7.5 Hz, 1 H, N-H_g), 7.84 (d, $^3J_{NHi,57}$ = 7.5 Hz, 1 H, N-H_i), 7.90 (d, $^3J_{NHa,7}$ = 6.3 Hz, 1 H, N-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 18.3 (q, C-43), 19.3 (q, C-43'), 24.8 (q, C-55), 25.4 (t, C-13), 27.0 (q, C-55'), 28.8 (d, C-42), 36.3 (t, C-23), 45.7 (d, C-11), 51.2 (d, C-57), 52.6 (d, C-32), 56.4 (d, C-41), 59.7 (d, C-53), 67.9 (t, C-28), 69.3 (t, C-33), 69.5 (t, C-46), 70.6 (d, C-40), 71.0 (s, C-54), 71.9 (t, C-34/C-47), 116.4 (t, C-30), 129.4 (s, C-24), 129.5 (d, C-26), 156.5 (s, C-27), 168.8 (s, C-44), 169.4 (s, C-9), 171.1 (s, C-56), 172.2 (s, C-39).
HRMS (ESI) calculated for $C_{92}H_{117}N_{10}O_{20}$ $[M+H]^+$: 1681.8440, found: 1681.8455.

Benzyl 3-(*N*-Benzyloxycarbonyl-*N*-methyl-*O*-benzyl-L-seryl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-4-methyl-2-oxopentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (16)

DMP (6.7 mg, 16.0 µmol, 1.2 eq.) was added to a solution of nonapeptide **15** (22.2 mg, 13.0 µmol, 1.0 eq.) in DMSO (132 µL) and the resulting mixture was stirred at room temperature for 2 hours. The crude mixture was purified by flash chromatography (C18, H_2O:MeCN 100:0 → 0:100) to afford ketoamide **16** (20.1 mg, 12.0 µmol, 91 %) as a white lyophilisate. $\mathbf{R_f}$ = 0.26 (SiO_2, DCM:MeOH 95:5); $[\alpha]_D^{20}$ = – 24 ($CHCl_3$, c = 0.5).



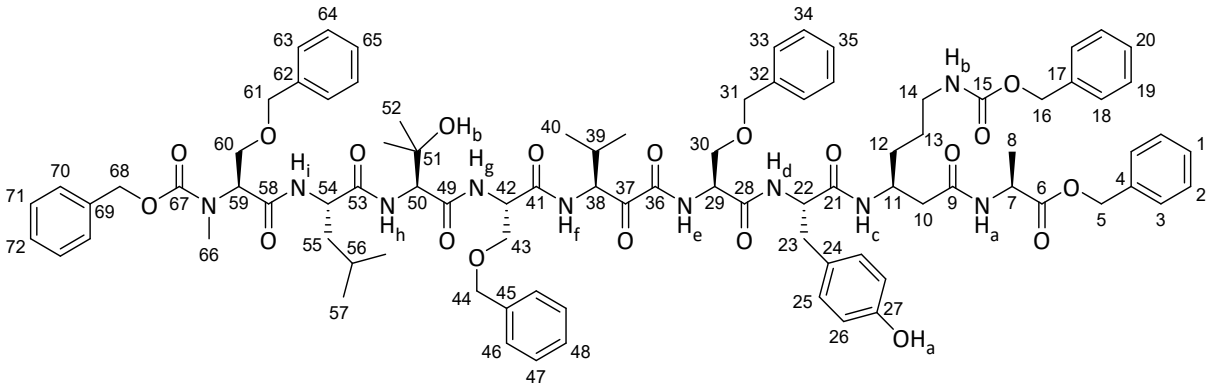
16

^{1}H-NMR (500 MHz, DMSO-d$_6$, 373 K): δ = 0.80 (d, $^3J_{43,42}$ = 6.9 Hz, 3 H, 43-H), 0.83 (d, $^3J_{60,59}$ = 6.3 Hz, 3 H, 60-H), 0.86 (d, $^3J_{60',59}$ = 6.6 Hz, 3 H, 60-H'), 0.88 (d, $^3J_{43',42}$ = 6.9 Hz, 3 H, 43-H'), 1.14 (s, 3 H, 55-H), 1.18 (s, 3 H, 55-H'), 1.30 (d, $^3J_{8,7}$ = 7.5 Hz, 3 H, 8-H), 1.35 – 1.50 (m, 4 H, 12-H, 13-H), 1.54 (m, 2 H, 58-H), 1.60 (m, 1 H, 59-H), 2.19 (m, 1 H, 42-H), 2.23 (dd, $^2J_{10a,10b}$ = 14.4 Hz, $^3J_{10a,11}$ = 6.0 Hz, 1 H, 10-H$_a$), 2.26 (dd, $^2J_{10b,10a}$ = 14.4 Hz, $^3J_{10b,11}$ = 6.0 Hz, 1 H, 10-H$_b$), 2.79 (dd, $^2J_{23a,23b}$ = 14.1 Hz, $^3J_{23a,22}$ = 8.2 Hz, 1 H, 23-H$_a$), 2.89 (s, 3 H, 69-H), 2.94 – 3.01 (m, 3 H, 14-H, 23-H$_b$), 3.64 – 3.75 (m, 4 H, 33-H, 46-H), 3.78 (dd, $^2J_{63a,63b}$ = 10.7 Hz, $^3J_{63a,62}$ = 8.8 Hz, 1 H, 63-H$_a$), 3.87 (dd, $^2J_{63b,63a}$ = 10.7 Hz, $^3J_{63b,62}$ = 5.2 Hz, 1 H, 63-H$_b$), 4.04 (m, 1 H, 11-H), 4.36 (qd, $^3J_{7,8}$ = 7.5 Hz, $^3J_{7,NHa}$ = 6.6 Hz, 1 H, 7-H), 4.35 – 4.45 (m, 2 H, 53-H, 57-H), 4.45 – 4.57 (m, 10 H, 22-H, 28-H, 34-H, 47-H, 64-H, O-H), 4.60 (dt, $^3J_{32,NHe}$ = 7.7 Hz, $^3J_{32,33}$ = 6.3 Hz, 1 H, 32-H), 4.65 (dt, $^3J_{45,NHg}$ = 8.2 Hz, $^3J_{45,46}$ = 5.0 Hz, 1 H, 45-H), 4.85 (dd, $^3J_{62,63a}$ = 8.8 Hz, $^3J_{62,63b}$ = 5.2 Hz, 1 H, 62-H), 5.02 (s, 2 H, 16-H), 5.09 (d, $^2J_{5a,5b}$ = 12.9 Hz, 1 H, 5-H$_a$), 5.11 (s, 2 H, 71-H), 5.13 (d, $^2J_{5b,5a}$ = 12.9 Hz, 1 H, 5-H$_b$), 5.15 (dd, $^3J_{41,NHf}$ = 8.2 Hz, $^3J_{41,42}$ = 5.3 Hz, 1 H, 41-H), 5.21 (m, 1 H, 30-H$_a$), 5.35 (m, 1 H, 30-H$_b$), 6.01 (ddt, $^3J_{29,30b}$ = 17.3 Hz, $^3J_{29,30a}$ = 10.7 Hz, $^3J_{29,28}$ = 5.3 Hz, 1 H, 29-H), 6.66 (bs, 1 H, N-H$_b$), 6.79 (d, $^3J_{25,26}$ = 8.5 Hz, 2 H, 25-H), 7.10 (d, $^3J_{26,25}$ = 8.5 Hz, 2 H, 26-H), 7.23 – 7.37 (m, 30 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 36-H, 37-H, 38-H, 49-H, 50-H, 51-H, 66-H, 67-H, 68-H, 73-H, 74-H, 75-H), 7.43 (d, $^3J_{NHh,53}$ = 8.8 Hz, 1 H, N-H$_h$), 7.46 (d, $^3J_{NHc,11}$ = 7.9 Hz, 1 H, N-H$_c$), 7.75 (d, $^3J_{NHf,41}$ = 8.2 Hz, 1 H, N-H$_f$), 7.84 (d, $^3J_{NHg,45}$ = 8.2 Hz, 1 H, N-H$_g$), 7.86 (d, $^3J_{NHi,57}$ = 6.6 Hz, 1 H, N-H$_i$), 7.90 (d, $^3J_{NHd,22}$ = 7.5 Hz, 1 H, N-H$_d$), 7.93 (d, $^3J_{NHa,7}$ = 6.6 Hz, 1 H, N-H$_a$), 8.18 (d, $^3J_{NHe,32}$ = 7.7 Hz, 1 H, N-H$_e$). **^{13}C-NMR (125 MHz, DMSO-d$_6$, 373 K):** δ = 16.4 (q, C-8), 17.0 (q, C-43), 18.7 (q, C-43'), 21.1 (q, C-60), 22.3 (q, C-60'), 23.8 (d, C-59), 24.7 (q, C-55), 25.4 (t, C-13), 27.1 (q, C-55'), 28.7 (d, C-42), 30.4 (q, C-69), 30.6 (t, C-12), 36.5 (t, C-23), 45.8 (d, C-11), 47.2 (d, C-7), 51.2 (d, C-57), 52.4 (d, C-32), 52.4 (d, C-45), 54.0 (d, C-22), 57.9 (d, C-41), 58.1 (d, C-62), 59.5 (d, C-53), 64.7 (t, C-16), 65.3 (t, C-5), 66.0 (t, C-71), 66.9 (t, C-63), 68.0 (t, C-28), 69.1 (t, C-33), 69.4 (t, C-46), 71.0 (s, C-54), 71.8 (t, C-64), 71.8 (t, C-47), 71.9 (t, C-34), 114.1 (d, C-25), 116.4 (t, C-30), 126.7, 126.8, 126.8, 126.9, 126.9, 126.9, 127.0, 127.0, 127.1, 127.3, 127.5, 127.5, 127.6, 127.7, 127.7, 127.8 (18d, C-1, C-2, C-3, C-18, C-19, C-20, C-36, C-37, C-38, C-49, C-50, C-51, C-66, C-67, C-68, C-73, C-74, C-75), 129.3 (s, C-24), 129.6 (d, C-26), 133.5 (d, C-29), 135.6 (s, C-4), 136.4 (s, C-72), 136.9 (s, C-17), 137.6 (s, C-35), 137.6 (s, C-48), 137.6 (s, C-65), 155.5 (s, C-15), 155.5 (s, C-70), 156.5 (s, C-27), 160.2 (s, C-39), 167.5 (s, C-31), 168.1 (s, C-61), 169.3 (s, C-44), 169.3 (s, C-21), 169.3 (s, C-52), 169.4 (s, C-9), 171.0 (s, C-56), 171.7 (s, C-6), 195.8 (s, C-40). The signals of C-10, C-14 and C-58 are located under the DMSO-signal.
HRMS (ESI) calculated for $C_{92}H_{115}N_{10}O_{20}$ [M+H]$^+$: 1679.8284, found: 1679.8260.

Benzyl 3-(*N*-Benzyloxycarbonyl-*N*-methyl-*O*-benzyl-L-seryl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-4-methyl-2-oxopentanoyl-*O*-benzyl-L-seryl-L-tyrosyl-*N*$_ε$-benzyloxycarbonyl-L-β-lysyl-L-alaninate (17)

Quinaldic acid (0.44 mg, 2.51 µmol, 21 mol%) and CpRu(MeCN)$_3$PF$_6$ (1.3 mg, 2.99 µmol, 25 mol%) were added to a suspension of ketoamide **16** (20.1 mg, 12.0 µmol, 1.0 eq.) in methanol (179 µL) and the resulting mixture was heated under microwave irradiation (150 W, 50 °C) for 60 min. Subsequently, the mixture was treated with DMSO (4.25 µL) and stirred at room temperature overnight. After solvent removal, the crude product was purified by flash chromatography (C18, H$_2$O:MeCN 100:0 → 0:100) to

afford the deprotected phenol **17** (14.2 mg, 8.66 µmol, 72 %) as a white lyophilisate. $[\alpha]_D^{20}$ = – 12.3 ($CHCl_3$, c = 0.5).

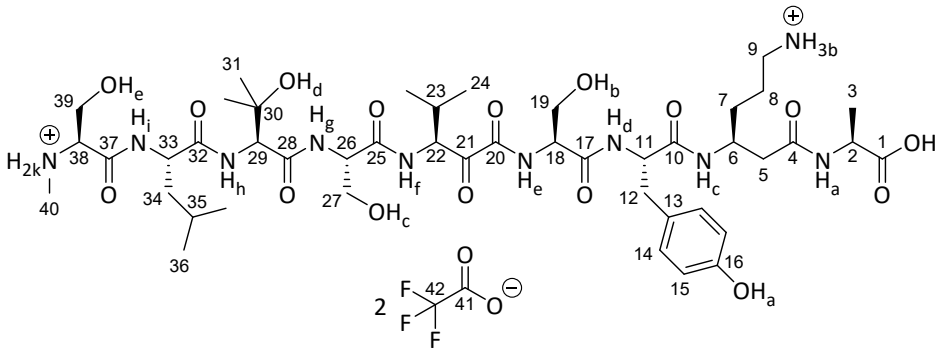


17

^{1}H-NMR (500 MHz, DMSO-d_6, 373 K): δ = 0.80 (d, $^3J_{40,39}$ = 6.9 Hz, 3 H, 40-H), 0.83 (d, $^3J_{57,56}$ = 6.3 Hz, 3 H, 57-H), 0.86 (d, $^3J_{57',56}$ = 6.4 Hz, 3 H, 57-H'), 0.88 (d, $^3J_{40',39}$ = 6.7 Hz, 3 H, 40-H'), 1.13 (s, 3 H, 52-H), 1.18 (s, 3 H, 52-H'), 1.30 (d, $^3J_{8,7}$ = 7.2 Hz, 3 H, 8-H), 1.35 – 1.50 (m, 4 H, 12-H, 13-H), 1.54 (m, 2 H, 55-H), 1.60 (m, 1 H, 56-H), 2.19 (m, 1 H, 39-H), 2.22 (dd, $^2J_{10a,10b}$ = 14.5 Hz, $^3J_{10a,11}$ = 7.2 Hz, 1 H, 10-H_a), 2.27 (dd, $^2J_{10b,10a}$ = 14.5 Hz, $^3J_{10b,11}$ = 6.0 Hz, 1 H, 10-H_b), 2.74 (dd, $^2J_{23a,23b}$ = 14.0 Hz, $^3J_{23a,22}$ = 7.9 Hz, 1 H, 23-H_a), 2.89 (s, 3 H, 66-H), 2.93 (dd, $^2J_{23b,23a}$ = 14.0 Hz, $^3J_{23b,22}$ = 5.8 Hz, 1 H, 23-H_b), 2.98 (m, 2 H, 14-H), 3.64 – 3.75 (m, 4 H, 30-H, 43-H), 3.78 (dd, $^2J_{60a,60b}$ = 10.5 Hz, $^3J_{60a,59}$ = 8.9 Hz, 1 H, 60-H_a), 3.87 (dd, $^2J_{60b,60a}$ = 10.7 Hz, $^3J_{60b,59}$ = 5.2 Hz, 1 H, 60-H_b), 4.04 (m, 1 H, 11-H), 4.33 – 4.40 (m, 2 H, 7-H, 54-H), 4.40 – 4.45 (m, 2 H, 22-H, 50-H), 4.45 – 4.57 (m, 7 H, 31-H, 44-H, 61-H, O-H_b), 4.59 (m, 1 H, 29-H), 4.65 (m, 1 H, 42-H), 4.85 (dd, $^3J_{59,60a}$ = 8.9 Hz, $^3J_{59,60b}$ = 5.2 Hz, 1 H, 59-H), 5.02 (s, 2 H, 16-H), 5.07 – 5.20 (m, 5 H, 5-H_a, 5-H_b, 38-H, 68-H), 6.62 – 6.66 (m, 3 H, 25-H, N-H_b), 6.98 (m, 2 H, 26-H), 7.20 – 7.37 (m, 30 H, 1-H, 2-H, 3-H, 18-H, 19-H, 20-H, 33-H, 34-H, 35-H, 46-H, 47-H, 48-H, 63-H, 64-H, 65-H, 70-H, 71-H, 72-H), 7.40 – 7.48 (m, 2 H, N-H_c, N-H_h), 7.75 (d, $^3J_{NHf,38}$ = 7.8 Hz, 1 H, N-H_f), 7.79 – 7.89 (m, 1 H, N-H_d, N-H_g, N-H_i), 7.93 (d, $^3J_{NHa,7}$ = 6.9 Hz, 1 H, N-H_a), 8.18 (d, $^3J_{NHe,29}$ = 8.2 Hz, 1 H, N-H_e), 8.73 (s, 1 H, O-H_a). **^{13}C-NMR (125 MHz, DMSO-d_6, 373 K):** δ = 16.4 (q, C-8), 17.0 (q, C-40), 18.7 (q, C-40'), 21.1 (q, C-57), 22.3 (q, C-57'), 23.8 (d, C-56), 24.7 (q, C-52), 25.4 (t, C-13), 27.1 (q, C-52'), 28.8 (d, C-39), 30.4 (q, C-66), 30.6 (t, C-12), 36.5 (t, C-23), 45.8 (d, C-11), 47.2 (d, C-7), 51.2 (d, C-54), 52.4 (d, C-29), 52.4 (d, C-42), 54.2 (d, C-22), 57.8 (d, C-38), 58.1 (d, C-59), 59.5 (d, C-50), 64.7 (t, C-16), 65.3 (t, C-5), 66.0 (t, C-68), 66.9 (t, C-60), 69.1 (t, C-30), 69.4 (t, C-43), 71.0 (s, C-51), 71.8 (t, C-61), 71.8 (t, C-44), 71.9 (t, C-31), 114.6 (d, C-25), 126.7, 126.7, 126.8, 126.8, 126.9, 126.9, 127.0, 127.0, 127.0, 127.0, 127.5, 127.6, 127.6, 127.7, 127.7, 127.8 (18d, C-1, C-2, C-3, C-18, C-19, C-20, C-33, C-34, C-35, C-46, C-47, C-48, C-63, C-64, C-65, C-70, C-71, C-72), 127.3 (s, C-24), 129.4 (d, C-26), 135.6 (s, C-4), 136.4 (s, C-69), 136.9 (s, C-17), 137.6 (s, C-32), 137.6 (s, C-45), 137.6 (s, C-62), 155.4 (s, C-27), 155.5 (s, C-15), 155.5 (s, C-67), 160.1 (s, C-36), 167.5 (s, C-28), 168.1 (s, C-58), 169.3 (s, C-41), 169.3 (s, C-21), 169.4 (s, C-49), 169.4 (s, C-9), 171.0 (s, C-53), 171.7 (s, C-6), 195.8 (s, C-37). The signals of C-10, C-14 and C-55 are located under the DMSO-signal.
HRMS (ESI) calculated for $C_{89}H_{111}N_{10}O_{20}$ $[M+H]^+$: 1639.7971, found: 1639.7908.

Myxoprincomide

Palladium on charcoal (27.2 mg, 10 wt% Pd) was added to a solution of compound **17** (27.2 mg, 16.6 µmol, 1.0 eq.) in a mixture of ethyl acetate (1.36 mL), chloroform (408 µL) and methanol (2.7 mL) and the resulting dark suspension was stirred at room temperature under hydrogen atmosphere (1 atm) for 3 hours. The crude mixture was filtered through a syringe filter before the solvent was evaporated. After preparative HPLC (phenomenex Luna 5 µm, H_2O + 0.05 % TFA:MeCN 100:0 → 0:100) Myxoprincomide (13.6 mg, 13.5 µmol, 81 %) was obtained as a white hygroscopic lyophilisate. $[\alpha]_D^{20}$ = – 33.9 (MeOH, c = 0.2).



Major rotamer:

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.80 (d, $^3J_{24,23}$ = 6.9 Hz, 3 H, 24-H), 0.87 (d, $^3J_{36,35}$ = 6.3 Hz, 3 H, 36-H), 0.88 (d, $^3J_{24',23}$ = 7.0 Hz, 3 H, 24-H'), 0.90 (d, $^3J_{36',35}$ = 6.7 Hz, 3 H, 36-H'), 1.12 (s, 3 H, 31-H), 1.14 (s, 3 H, 31-H'), 1.25 (d, $^3J_{3,2}$ = 7.3 Hz, 3 H, 3-H), 1.36 (m, 1 H, 7-H_a), 1.45 – 1.56 (m, 5 H, 8-H, 7-H_b, 34-H), 1.63 (m, 1 H, 35-H), 2.17 (m, 1 H, 23-H), 2.21 (d, $^3J_{5,6}$ = 6.9 Hz, 2 H, 5-H), 2.53 (s, 3 H, 40-H), 2.68 (dd, $^2J_{12a,12b}$ = 14.2 Hz, $^3J_{12a,11}$ = 8.8 Hz, 1 H, 12-H_a), 2.75 (m, 2 H, 9-H), 2.90 (dd, $^2J_{12b,12a}$ = 14.2 Hz, $^3J_{12b,11}$ = 5.0 Hz, 1 H, 12-H_b), 3.54 – 3.64 (m, 3 H, 19-H, 27-H_a), 3.65 – 3.76 (m, 2 H, 27-H_b, 39-H_a), 3.82 (m, 1 H, 39-H_b), 3.87 (m, 1 H, 38-H), 4.04 (m, 1 H, 6-H), 4.19 (dq, $^3J_{2,NHa}$ = $^3J_{2,3}$ = 7.3 Hz, 1 H, 2-H), 4.28 – 4.36 (m, 2 H, 11-H, 18-H), 4.39 (m, 1 H, 26-H), 4.44 (d, $^3J_{29,NHh}$ = 9.1 Hz, 1 H, 29-H), 4.49 (m, 1 H, 33-H), 4.92 (m, 1 H, O-H_c), 5.01 (s, 1 H, O-H_d), 5.06 (m, 1 H, O-H_b), 5.14 (dd, $^3J_{22,NHf}$ = 7.9 Hz, $^3J_{22,23}$ = 5.4 Hz, 1 H, 22-H), 5.49 (m, 1 H, O-H_e), 6.63 (m, 2 H, 14-H), 6.99 (m, 2 H, 15-H), 7.65 (bs, 3 H, N-H_b), 7.82 (d, $^3J_{NHc,6}$ = 8.5 Hz, 1 H, N-H_c), 7.92 (d, $^3J_{NHh,29}$ = 9.1 Hz, 1 H, N-H_h), 7.97 (d, $^3J_{NHg,26}$ = 8.2 Hz, 1 H, N-H_g), 8.02 (d, $^3J_{NHf,22}$ = 7.9 Hz, 1 H, N-H_f), 8.13 (d, $^3J_{NHd,11}$ = 7.9 Hz, 1 H, N-H_d), 8.17 (d, $^3J_{NHa,2}$ = 7.3 Hz, 1 H, N-H_a), 8.30 (d, $^3J_{NHe,18}$ = 8.2 Hz, 1 H, N-H_e), 8.71 (d, $^3J_{NHi,33}$ = 8.2 Hz, 1 H, N-H_i), 8.81 (bs, 2 H, N-H_k), 9.14 (s, 1 H, O-H_a), 12.45 (bs, 1 H, COO-H). **^{13}C-NMR (125 MHz, DMSO-d_6):** δ = 17.2 (q, C-3), 17.7 (q, C-24), 19.4 (q, C-24'), 21.3 (q, C-36), 23.2 (q, C-36'), 23.7 (t, C-8), 24.2 (d, C-35), 24.6 (q, C-31), 27.9 (q, C-31'), 29.2 (d, C-23), 30.5 (t, C-7), 31.4 (q, C-40), 36.8 (t, C-12), 38.7 (t, C-9), 40.4 (t, C-5), 40.4 (t, C-34), 45.5 (d, C-6), 47.4 (d, C-2), 51.5 (d, C-33), 54.7 (d, C-11), 54.9 (d, C-18/C-26), 55.1 (d, C-18/C-26), 57.9 (d, C-22), 59.6 (t, C-39), 59.7 (d, C-29), 61.5 (t, C-27), 61.6 (t, C-19), 62.0 (d, C-38), 71.8 (s, C-30), 114.9 (d, C-14), 127.6 (s, C-13), 130.1 (d, C-15), 155.8 (s, C-16), 158.0 (q, $^2J_{41,F}$ = 31.2 Hz, C-41), 160.2 (s, C-20), 165.9 (s, C-37), 168.6 (s, C-17), 169.5 (s, C-28), 169.6 (s, C-4), 170.3 (s, C-10), 170.5 (s, C-25), 171.4 (s, C-32), 174.2 (s, C-1), 196.6 (s, C-21). The signal of C-42 could not be detected.

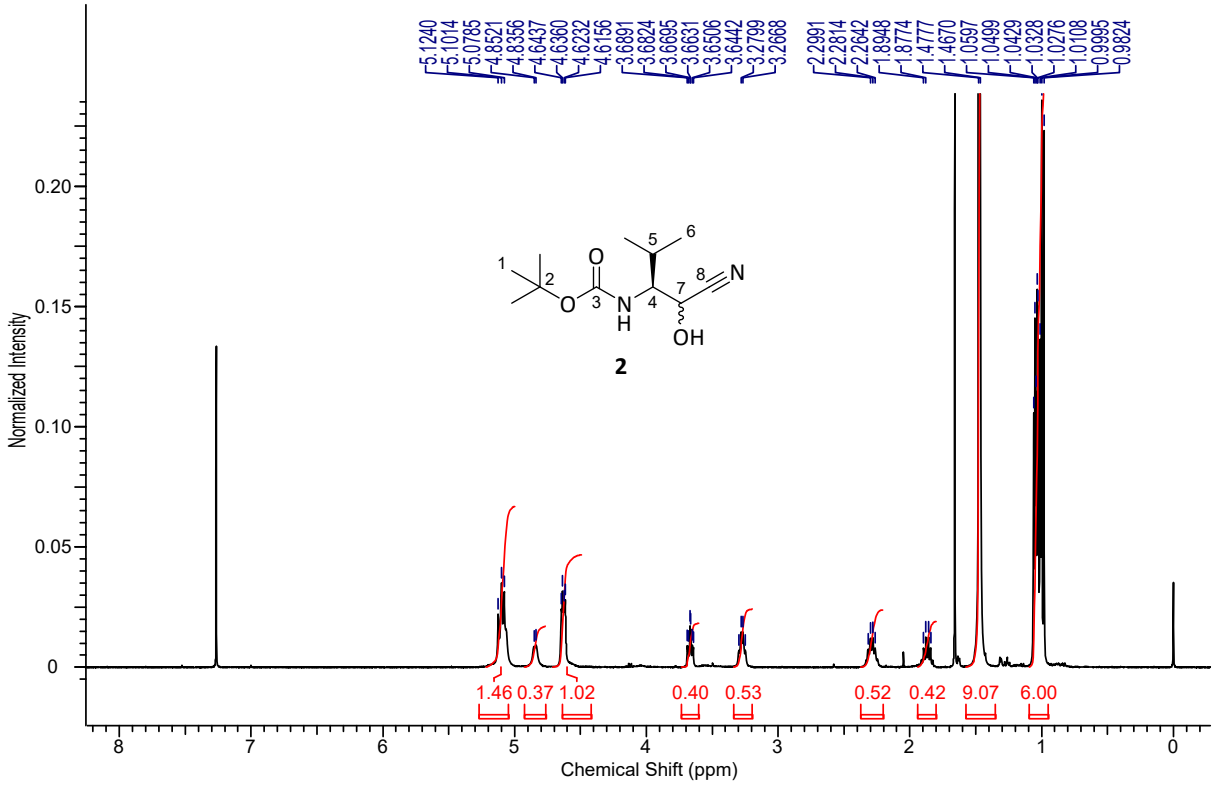
Minor rotamer:

^{1}H-NMR (500 MHz, DMSO-d_6): δ = 0.72 (d, $^3J_{24,23}$ = 6.9 Hz, 3 H, 24-H), 0.78 (d, $^3J_{36,35}$ = 6.9 Hz, 3 H, 36-H), 0.84 (d, $^3J_{36',35}$ = 7.6 Hz, 3 H, 36-H'), 1.08 (s, 3 H, 31-H), 5.00 (s, 1 H, O-H_d), 5.53 (m, 1 H, O-H_e), 6.65 (m, 2 H, 14-H), 7.02 (m, 2 H, 15-H), 8.68 (d, $^3J_{NHi,33}$ = 7.3 Hz, 1 H, N-H_i), 9.18 (s, 1 H, O-H_a).
HRMS (ESI) calculated for $C_{45}H_{75}N_{10}O_{16}$ $[M+H]^+$: 1011.5357, found: 1011.5364.

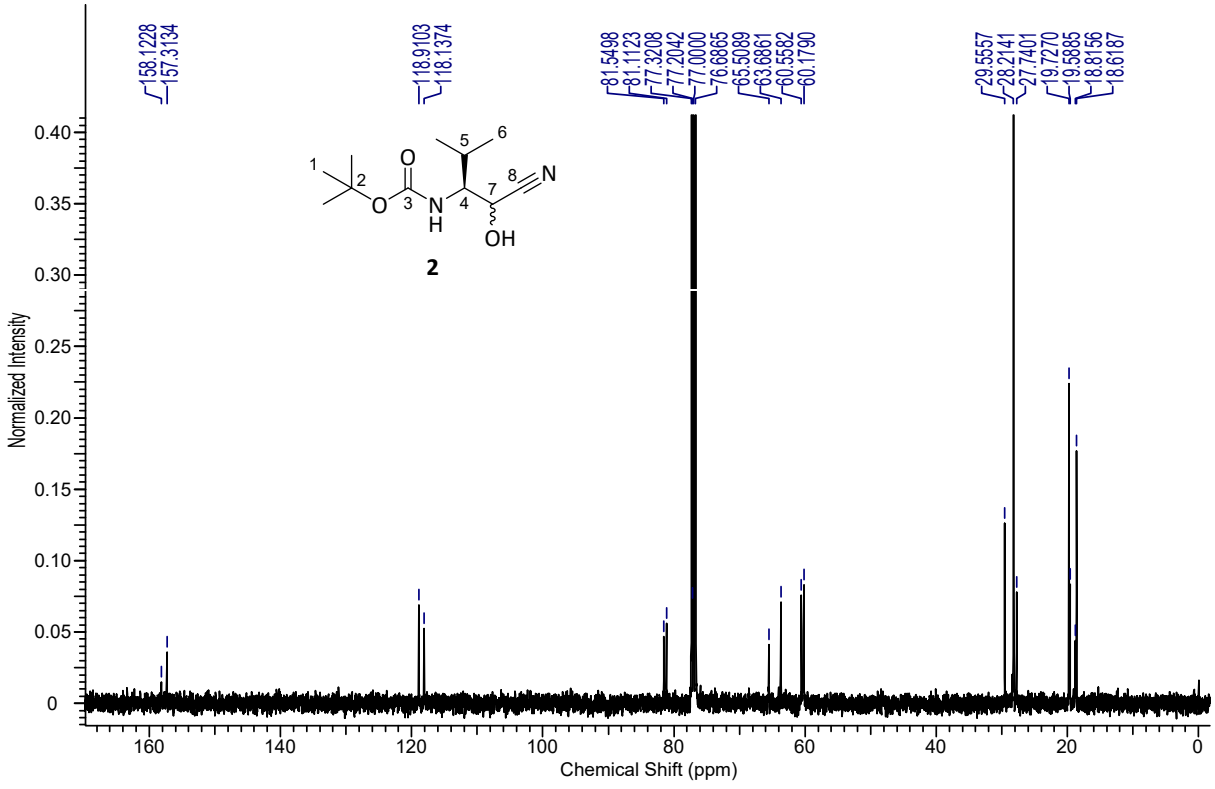
NMR spectra

tert-Butyl ((*2S*)-1-cyano-1-hydroxy-3-methylbutan-2-yl)carbamate

400 MHz, $CDCl_3$

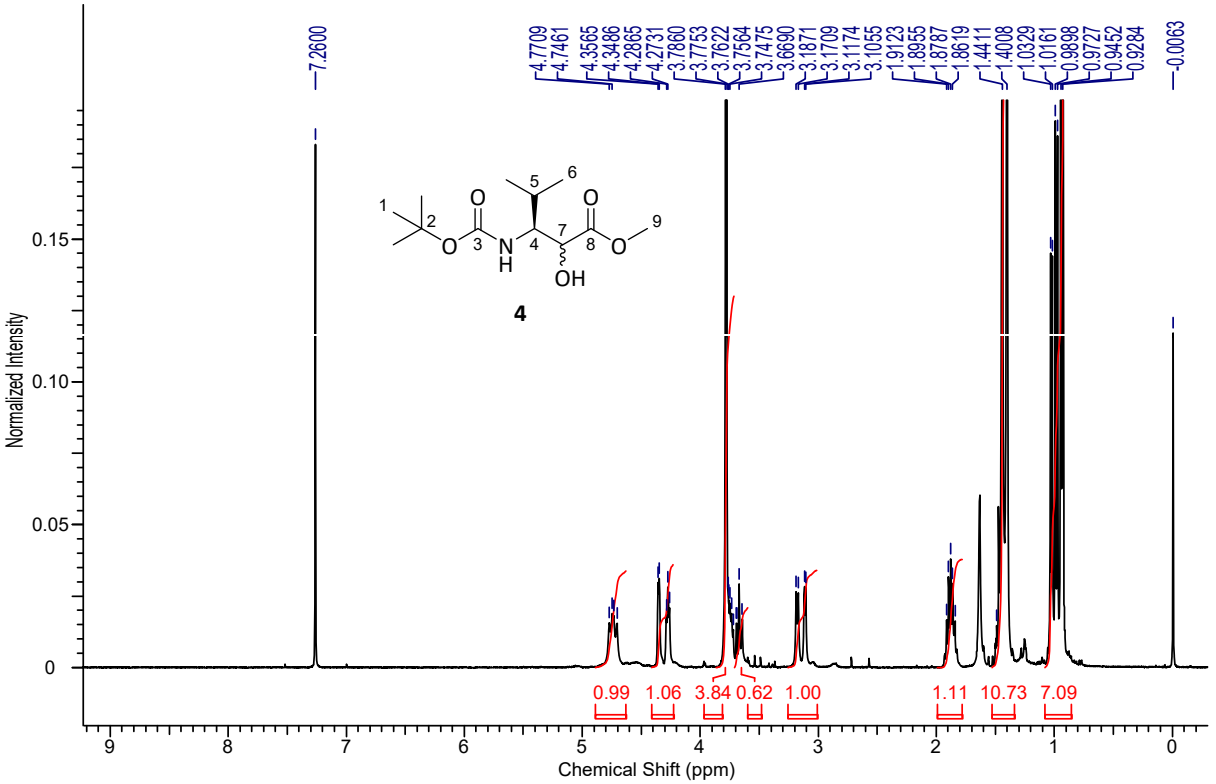


100 MHz, $CDCl_3$

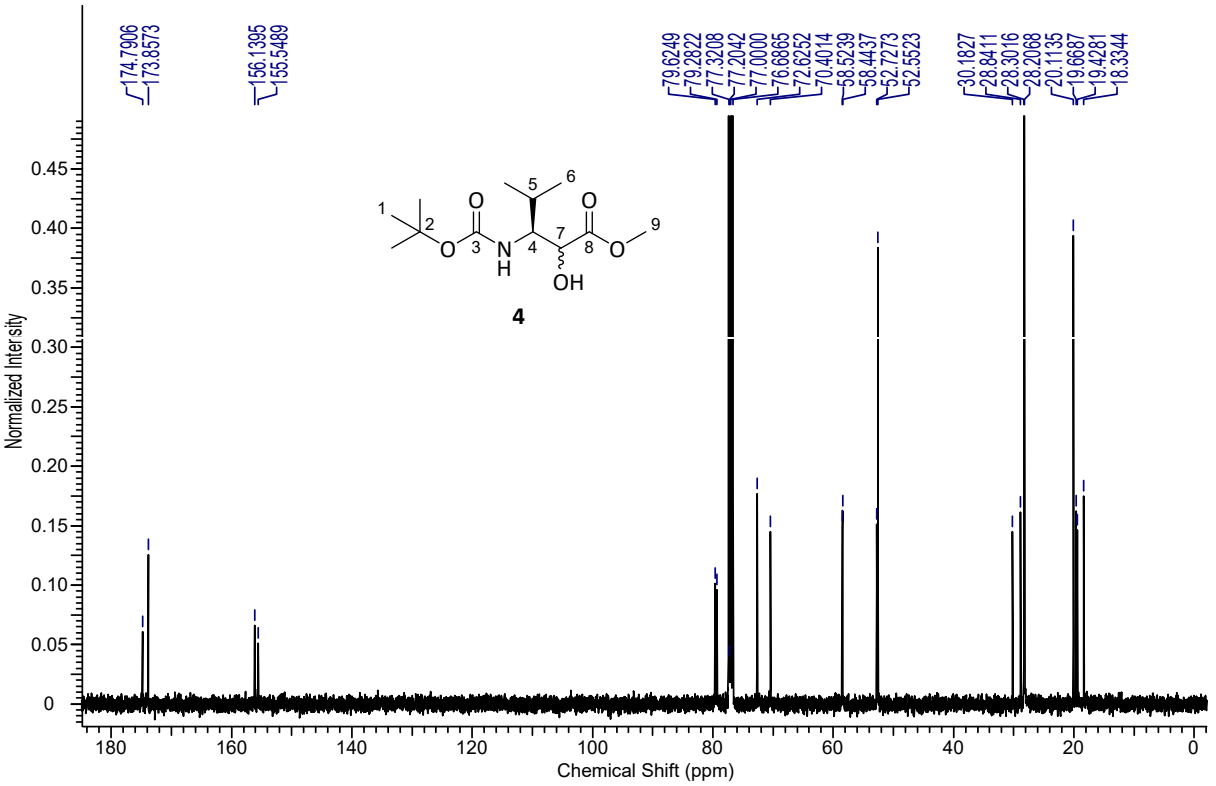


Methyl (3*S*)-3-((*tert*-butoxycarbonyl)amino)-2-hydroxy-4-methylpentanoate (4)

400 MHz, $CDCl_3$

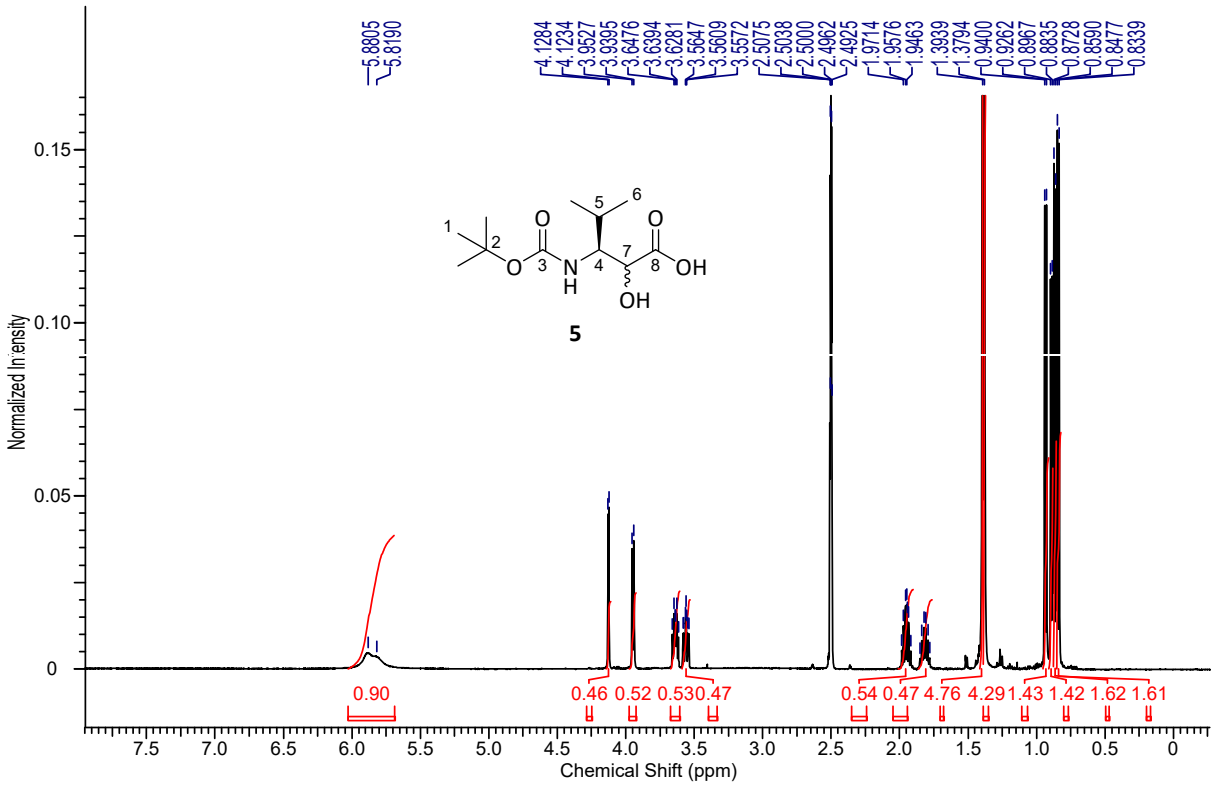


100 MHz, $CDCl_3$

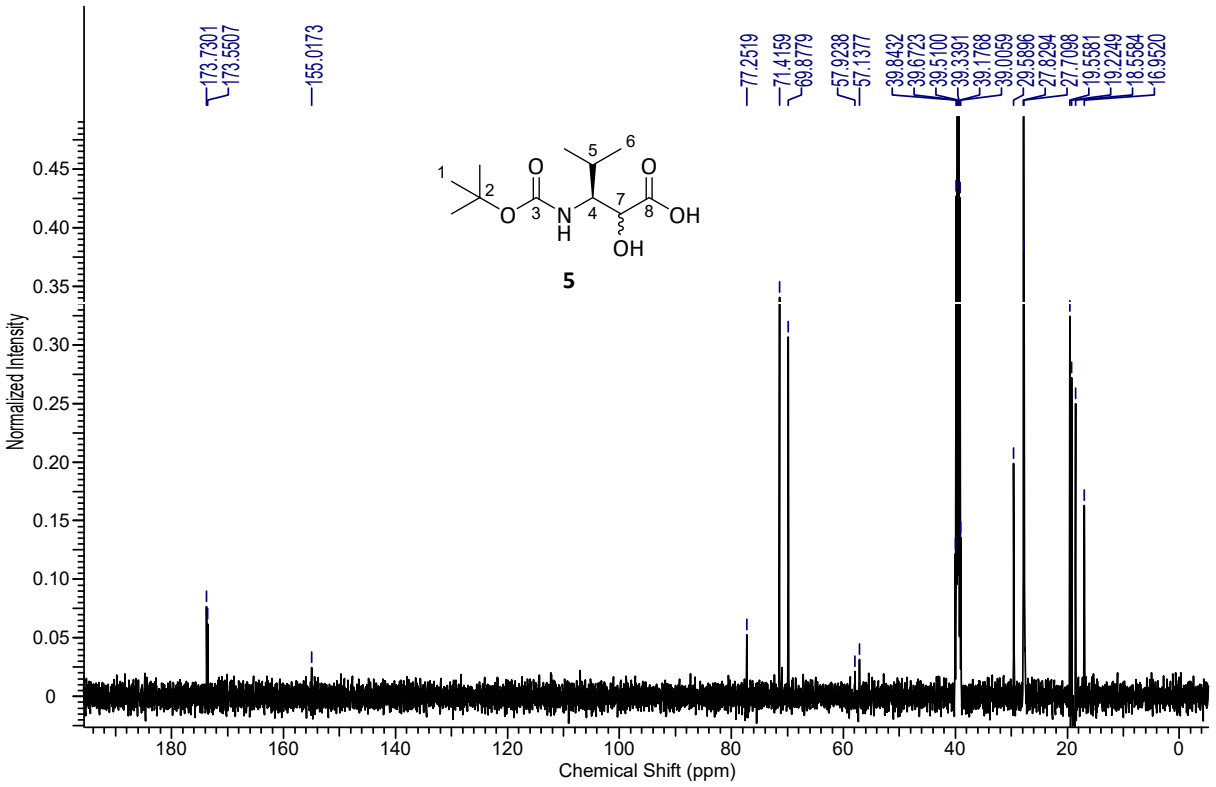


(3*S*)-3-((*tert*-Butoxycarbonyl)amino)-2-hydroxy-4-methylpentanoic acid (5)

500 MHz, DMSO-d_6, 373 K

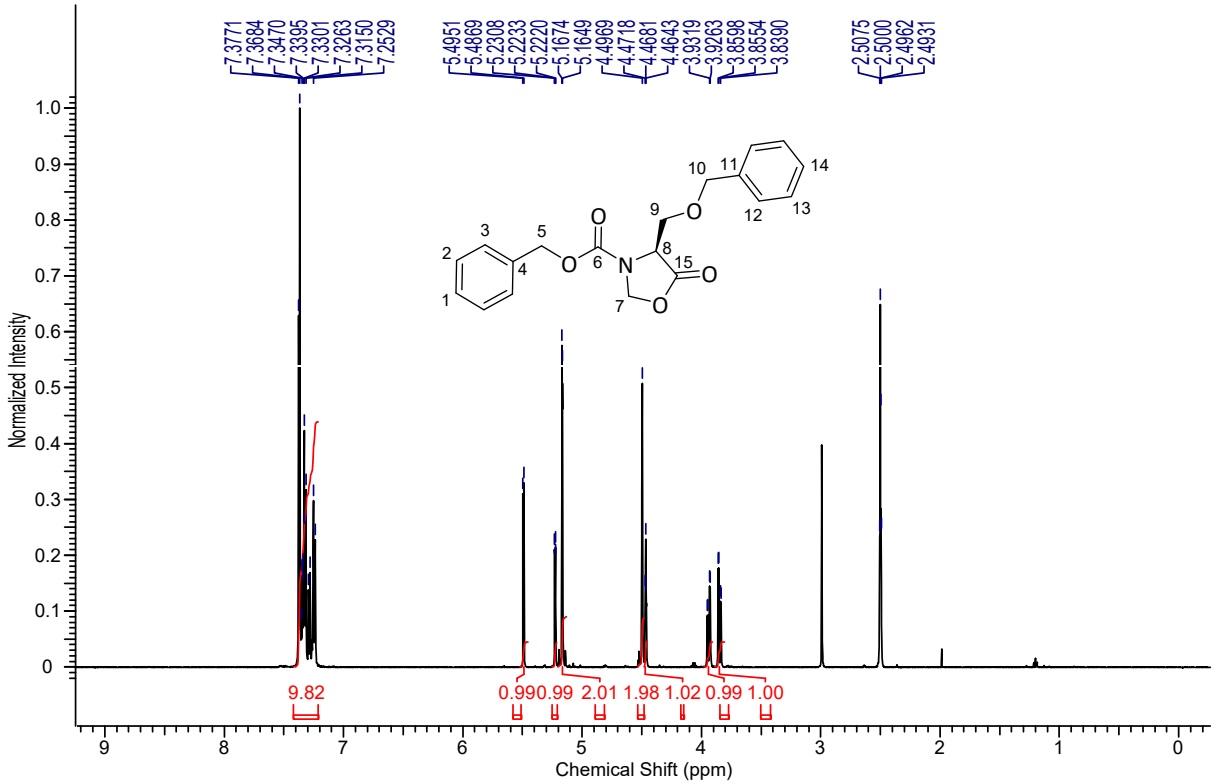


125 MHz, DMSO-d_6, 373 K

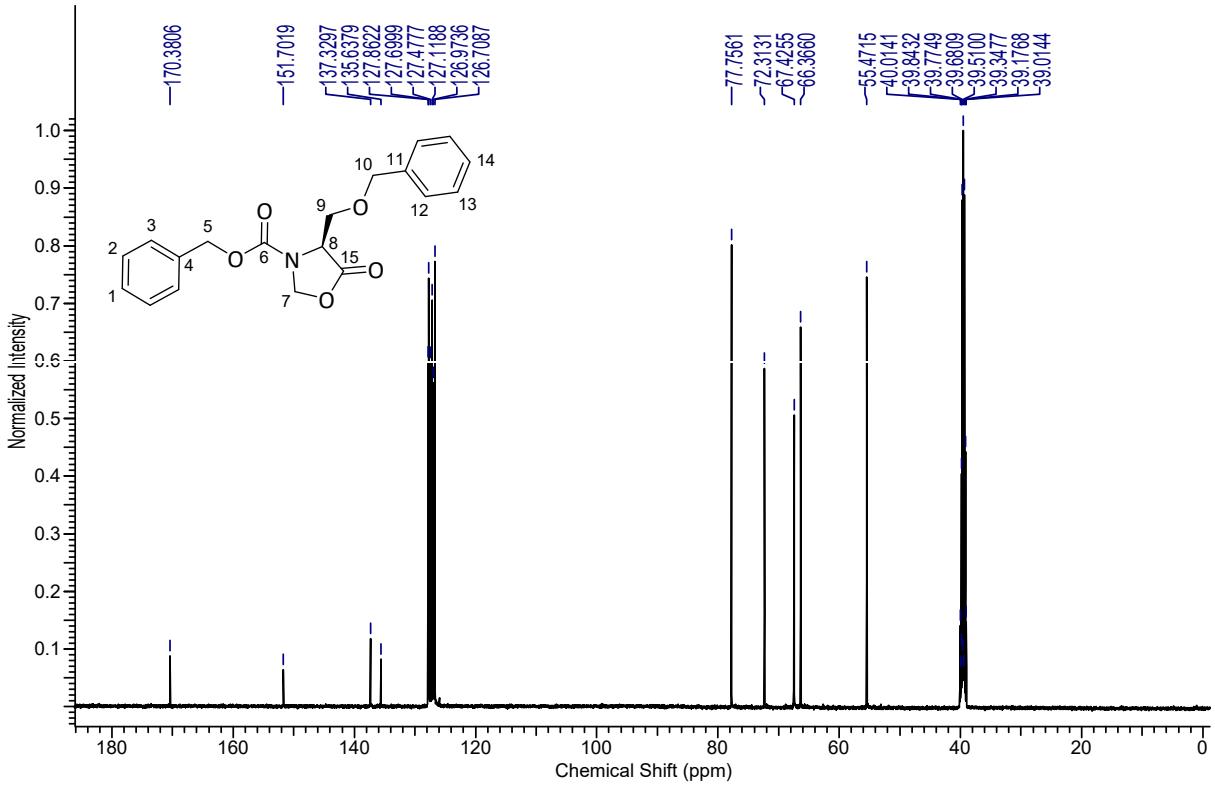


Benzyl (*S*)-4-((benzyloxy)methyl)-5-oxooxazolidine-3-carboxylate

500 MHz, DMSO-d_6, 373 K

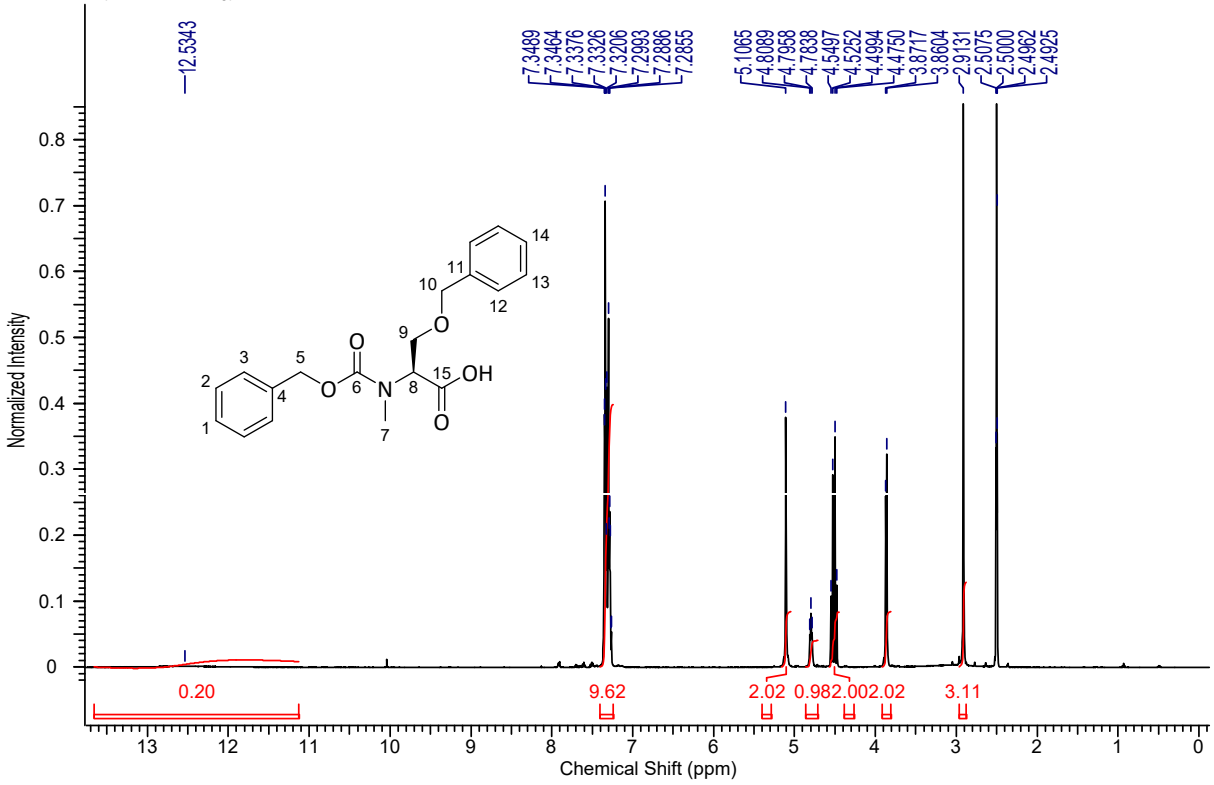


125 MHz, DMSO-d_6, 373 K

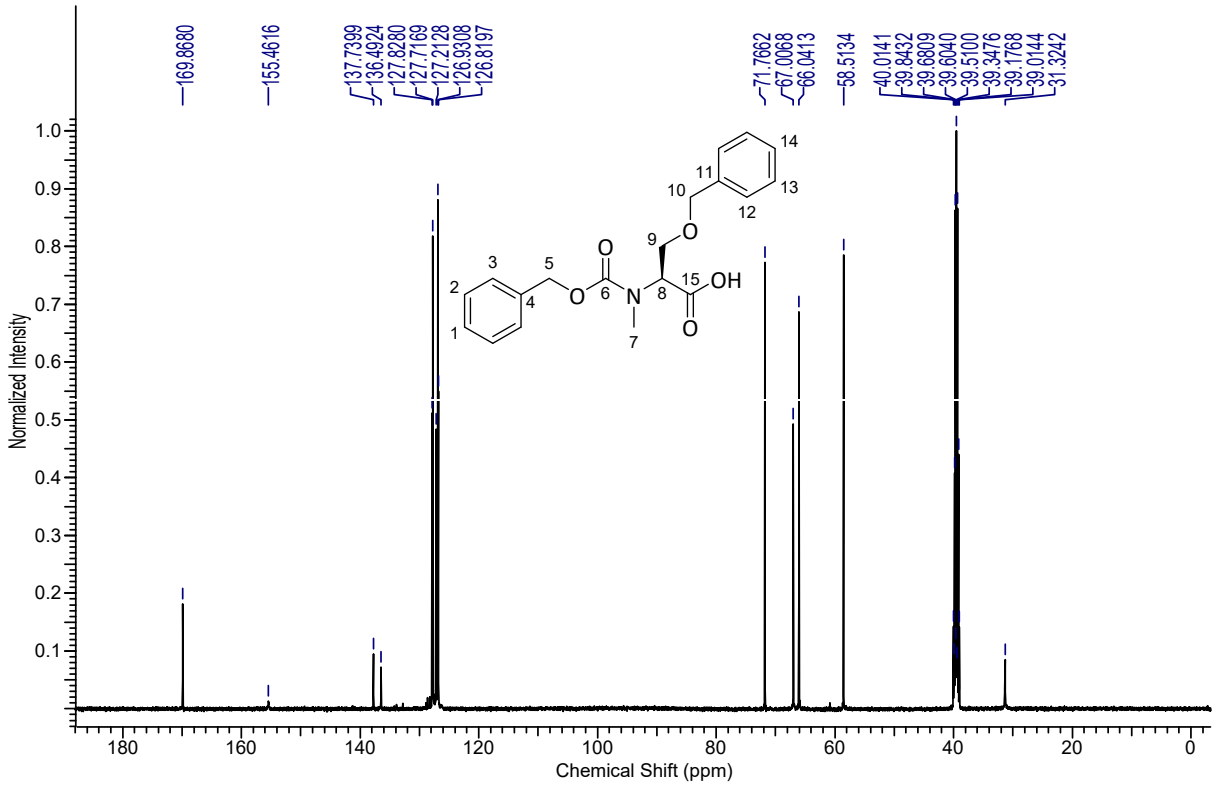


***O*-Benzyl-*N*-((benzyloxy)carbonyl)-*N*-methyl-L-serine**

500 MHz, DMSO-d_6, 373 K

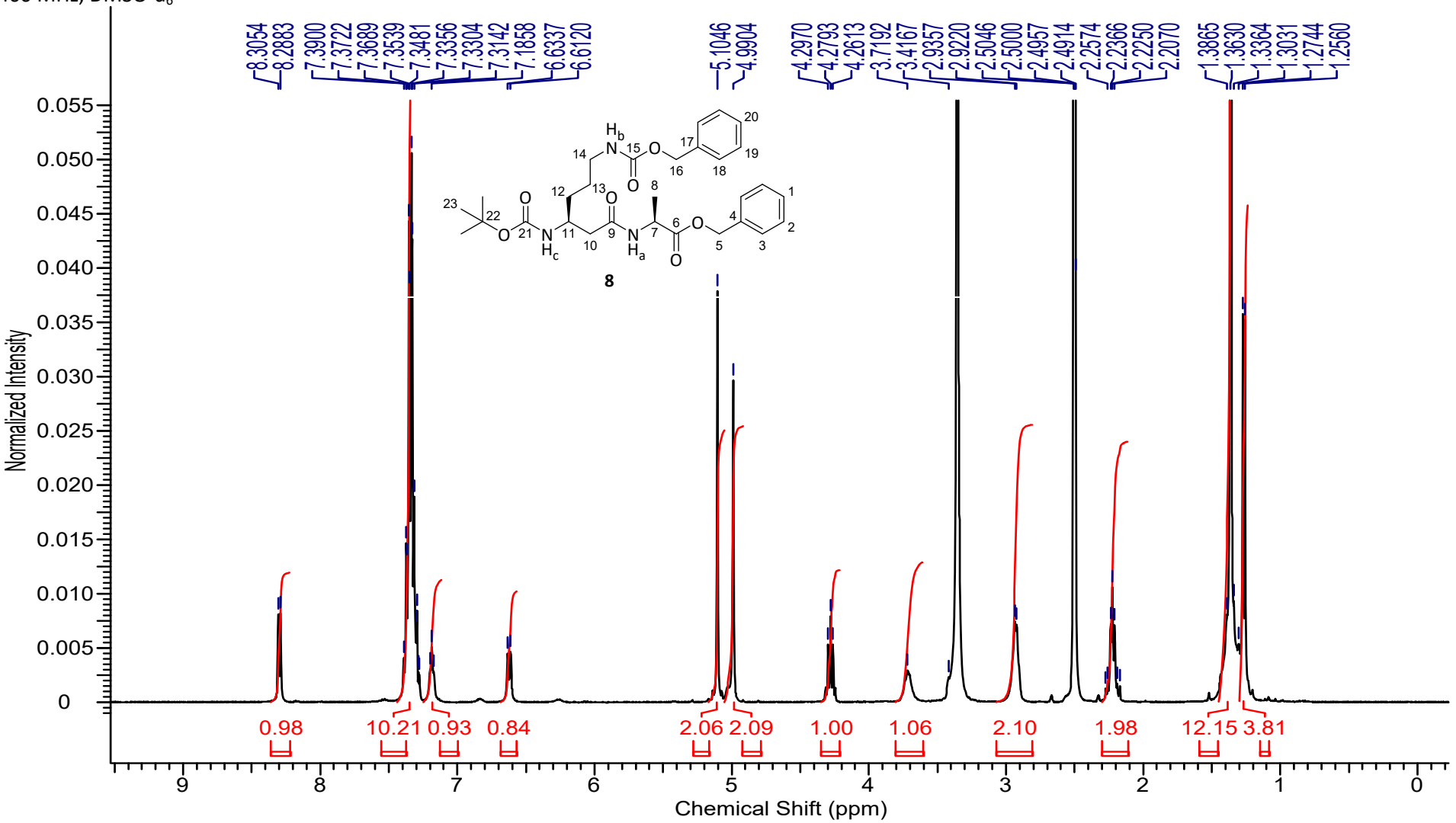


125 MHz, DMSO-d_6, 373 K



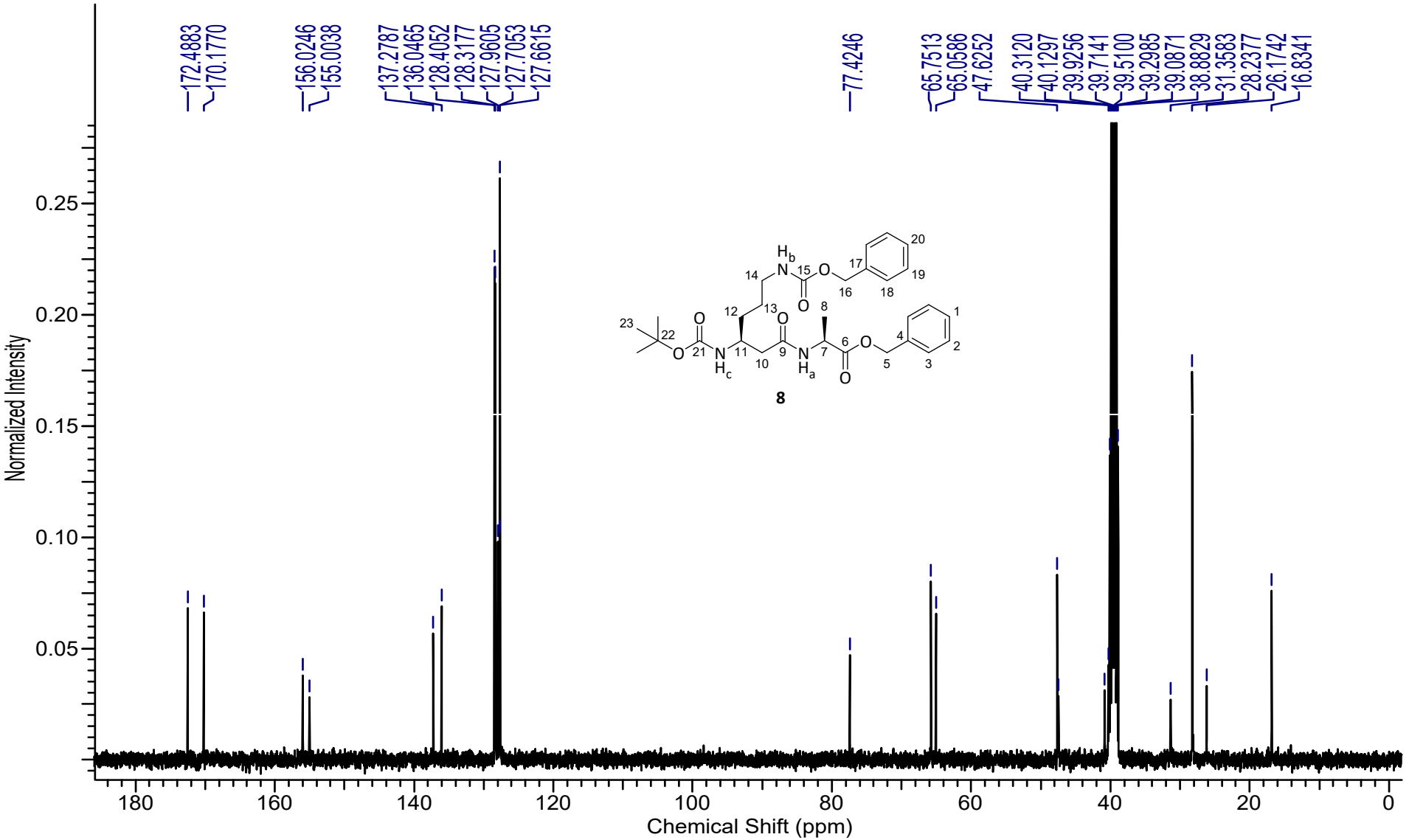
Benzyl N_{β}-*tert*-butoxycarbonyl-N_{ε}-benzyloxycarbonyl-L-β-lysyl-L-alaninate (8)

400 MHz, DMSO-d_6



8

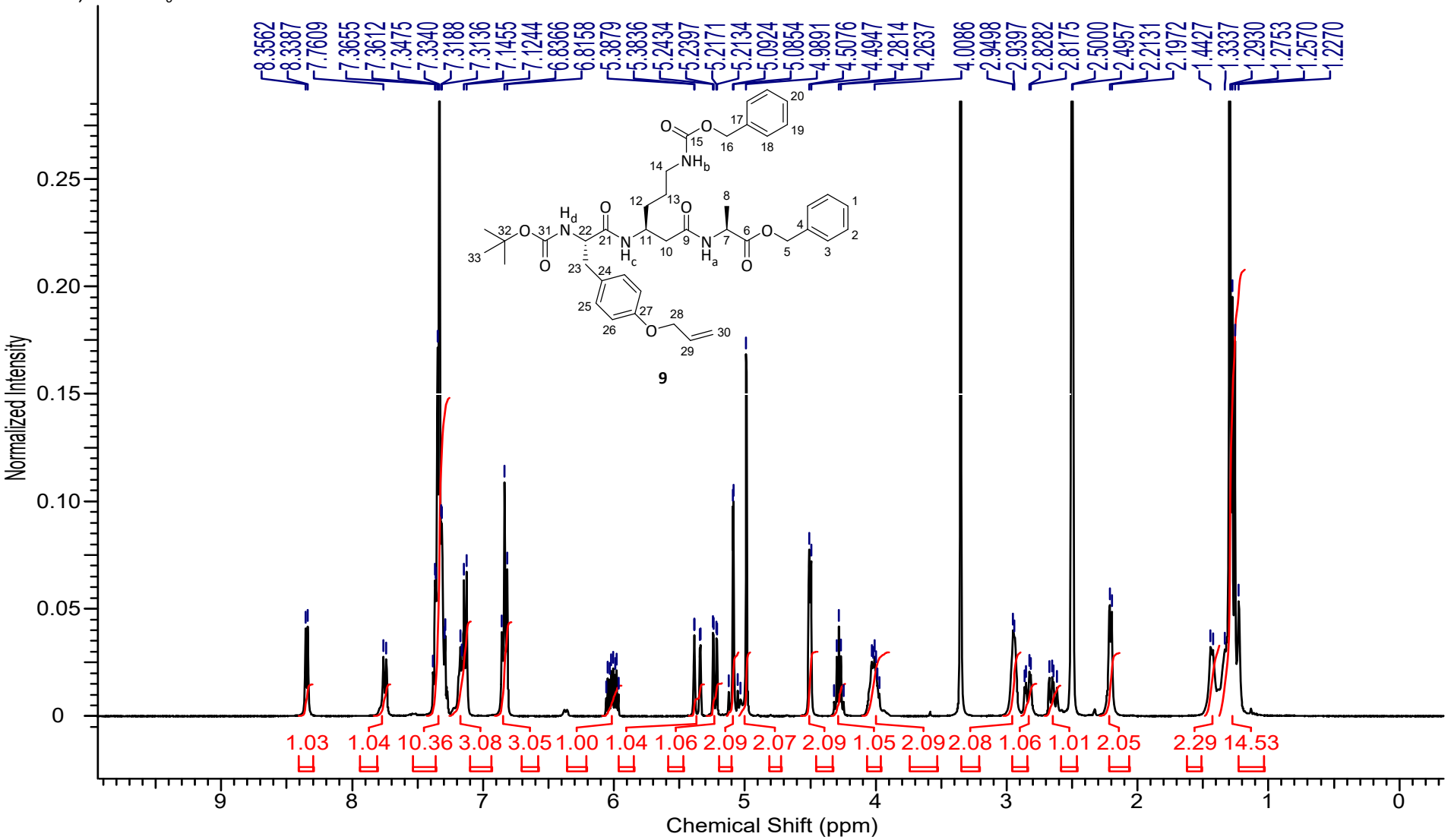
100 MHz, DMSO-d_6



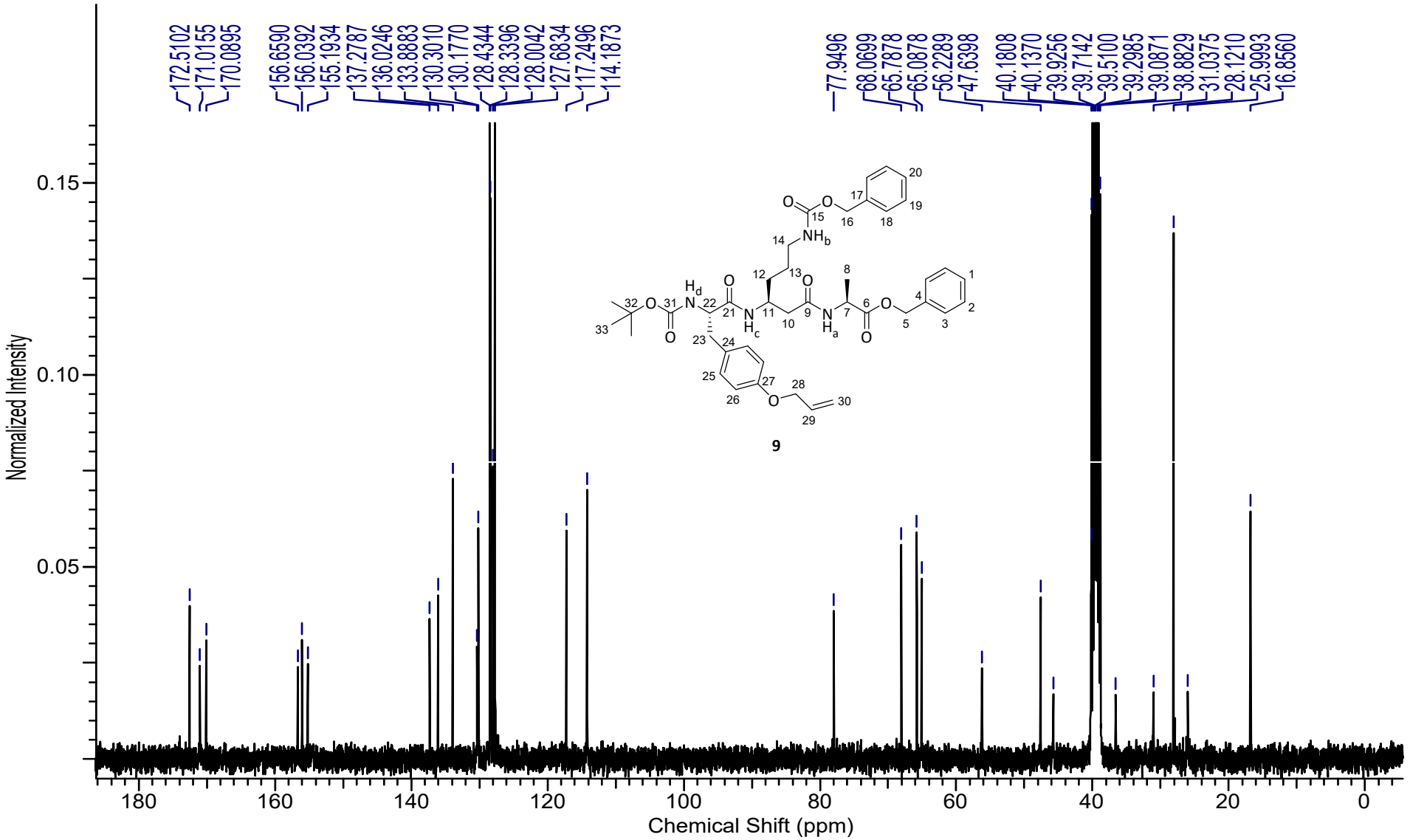
8

Benzyl *N*-*tert*-butoxycarbonyl-*O*-allyl-L-tyrosyl-N_{ε}-benzyloxycarbonyl-L-β-lysyl-L-alaninate (9)

400 MHz, DMSO-d_6



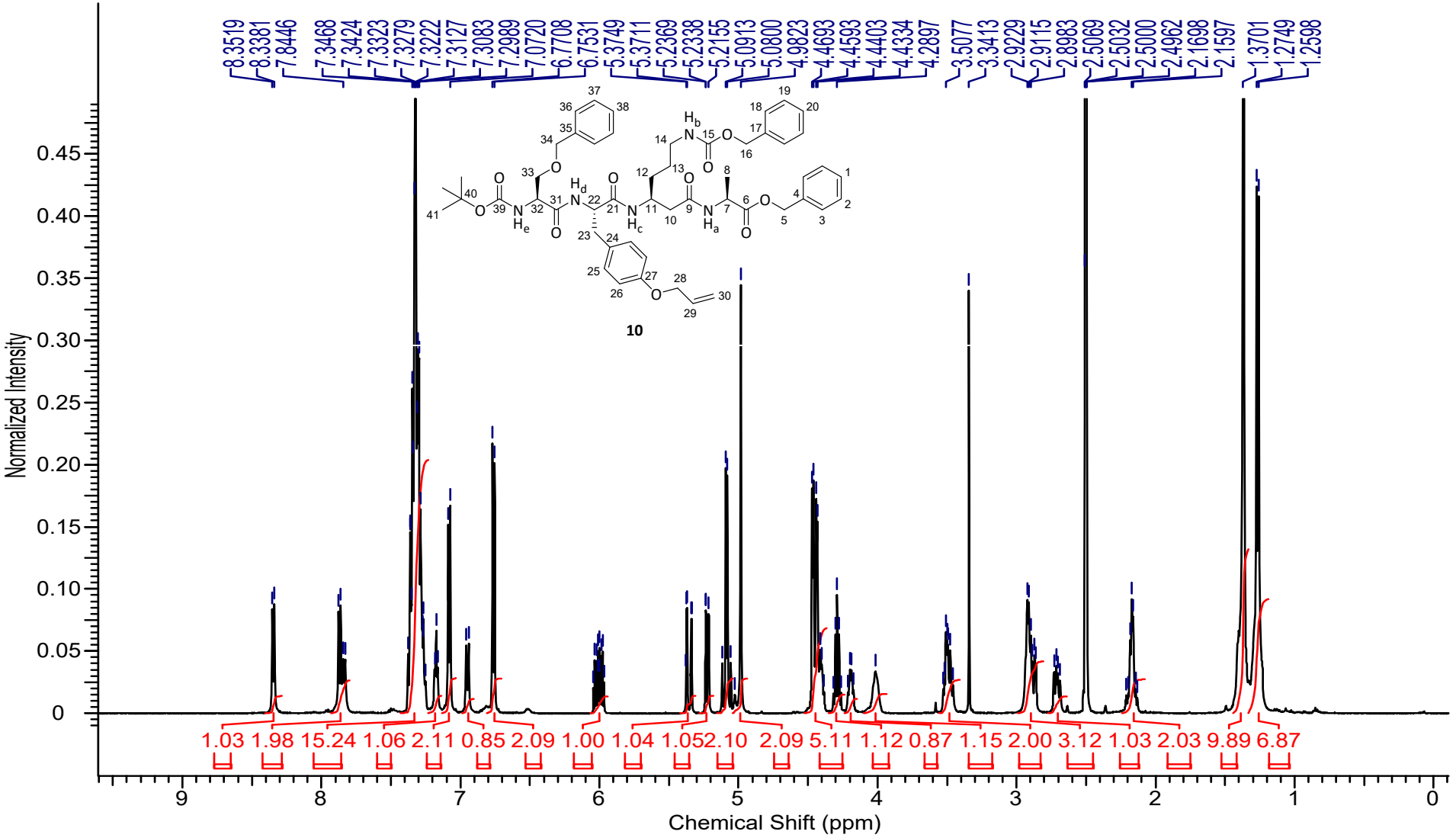
100 MHz, DMSO-d_6



9

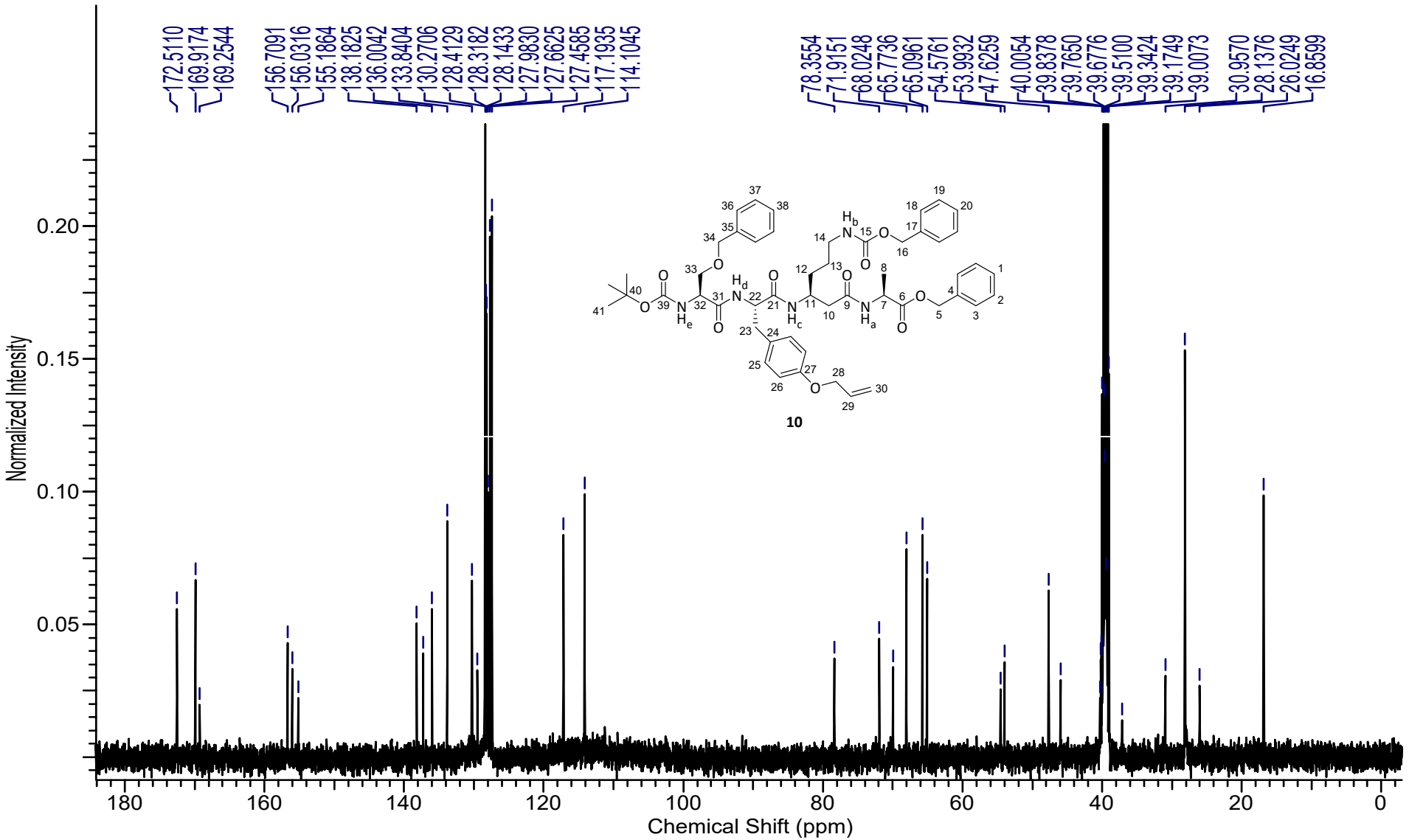
Benzyl *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N$_\varepsilon$-benzyloxycarbonyl-L-β-lysyl-L-alaninate (10)

500 MHz, DMSO-d_6



10

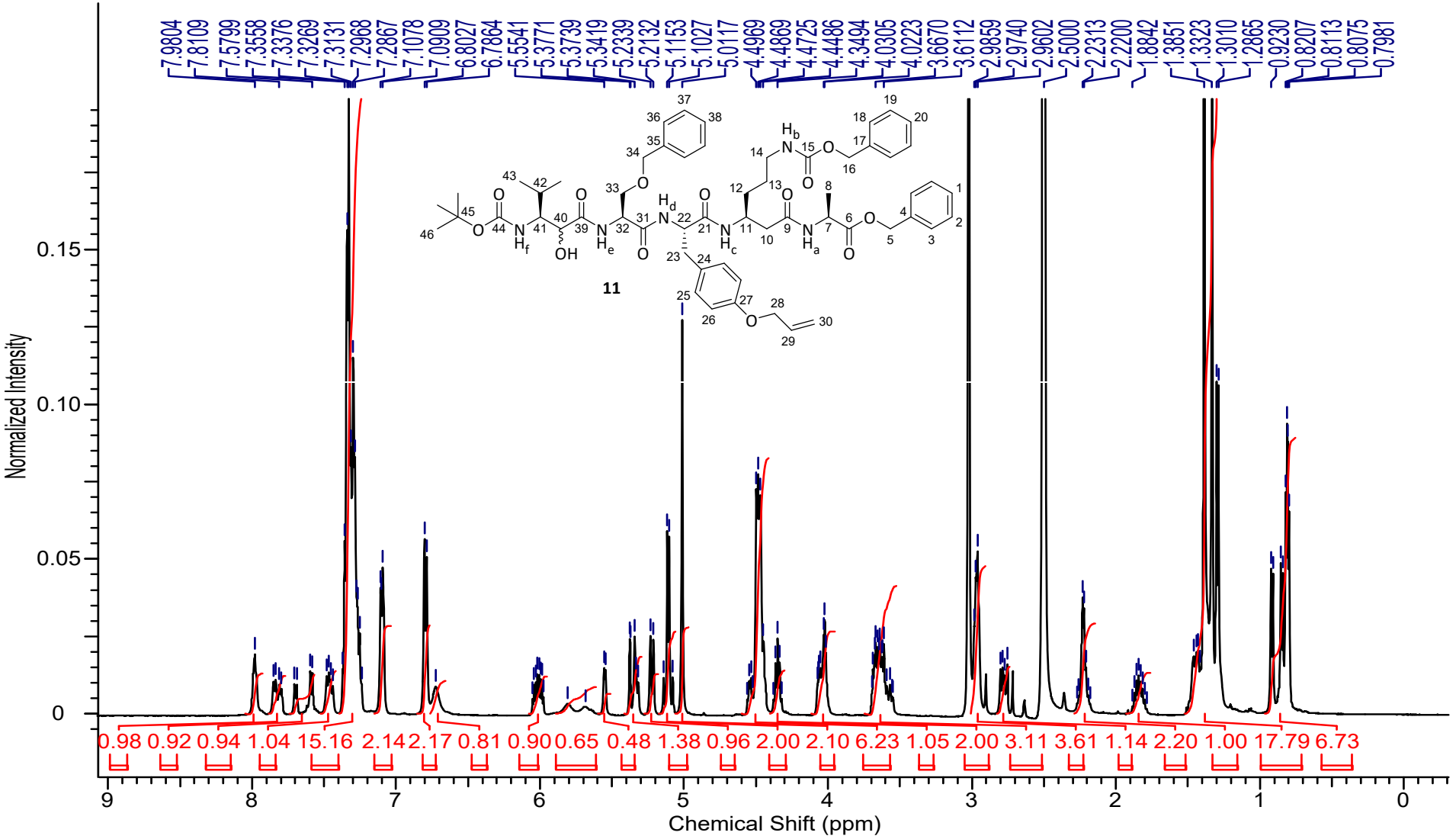
125 MHz, DMSO-d_6



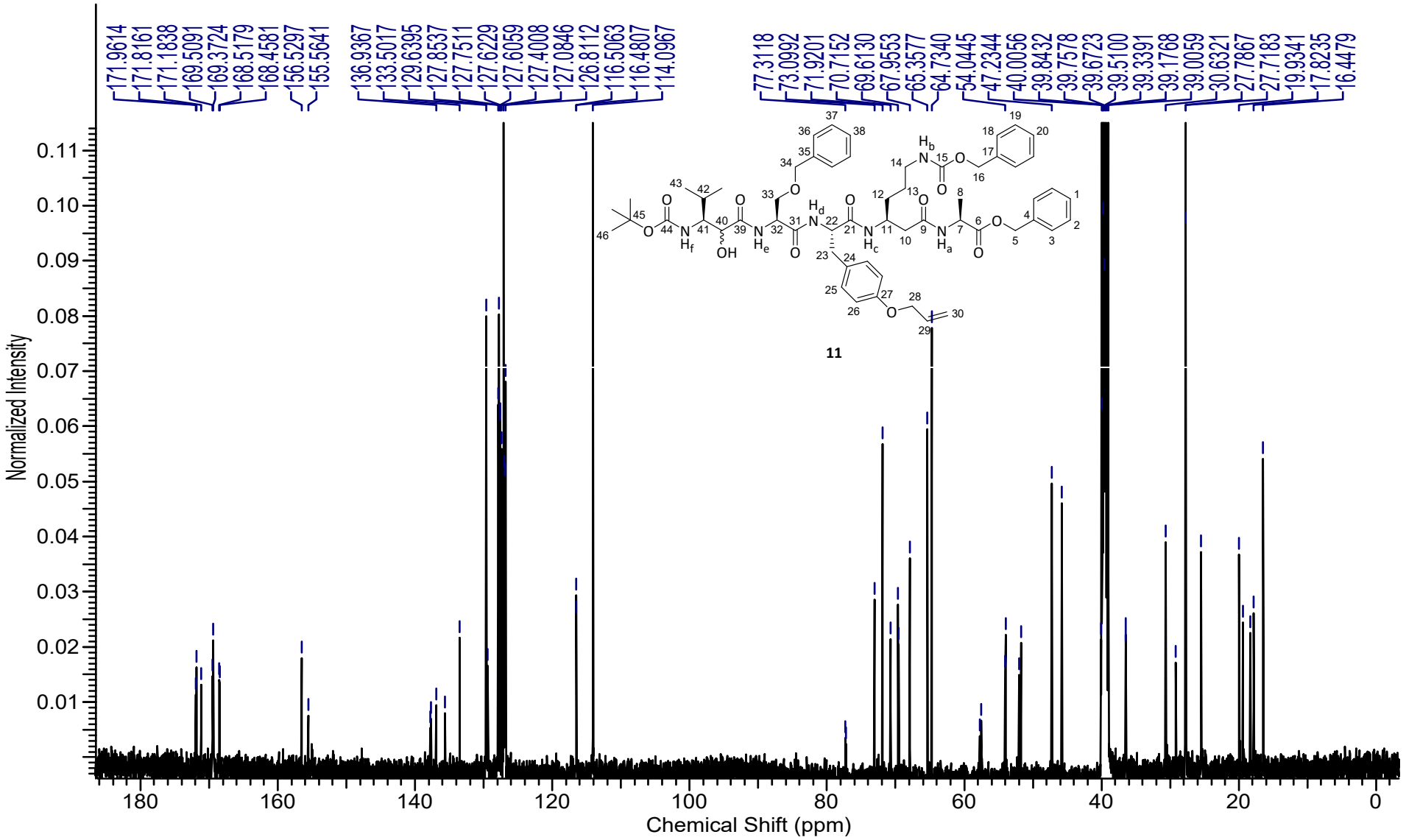
10

Benzyl 3-*tert*-butoxycarbonylamino-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N$_\varepsilon$-benzyloxycarbonyl-L-β-lysyl-L-alaninate (11)

500 MHz, DMSO-d$_6$, 373 K



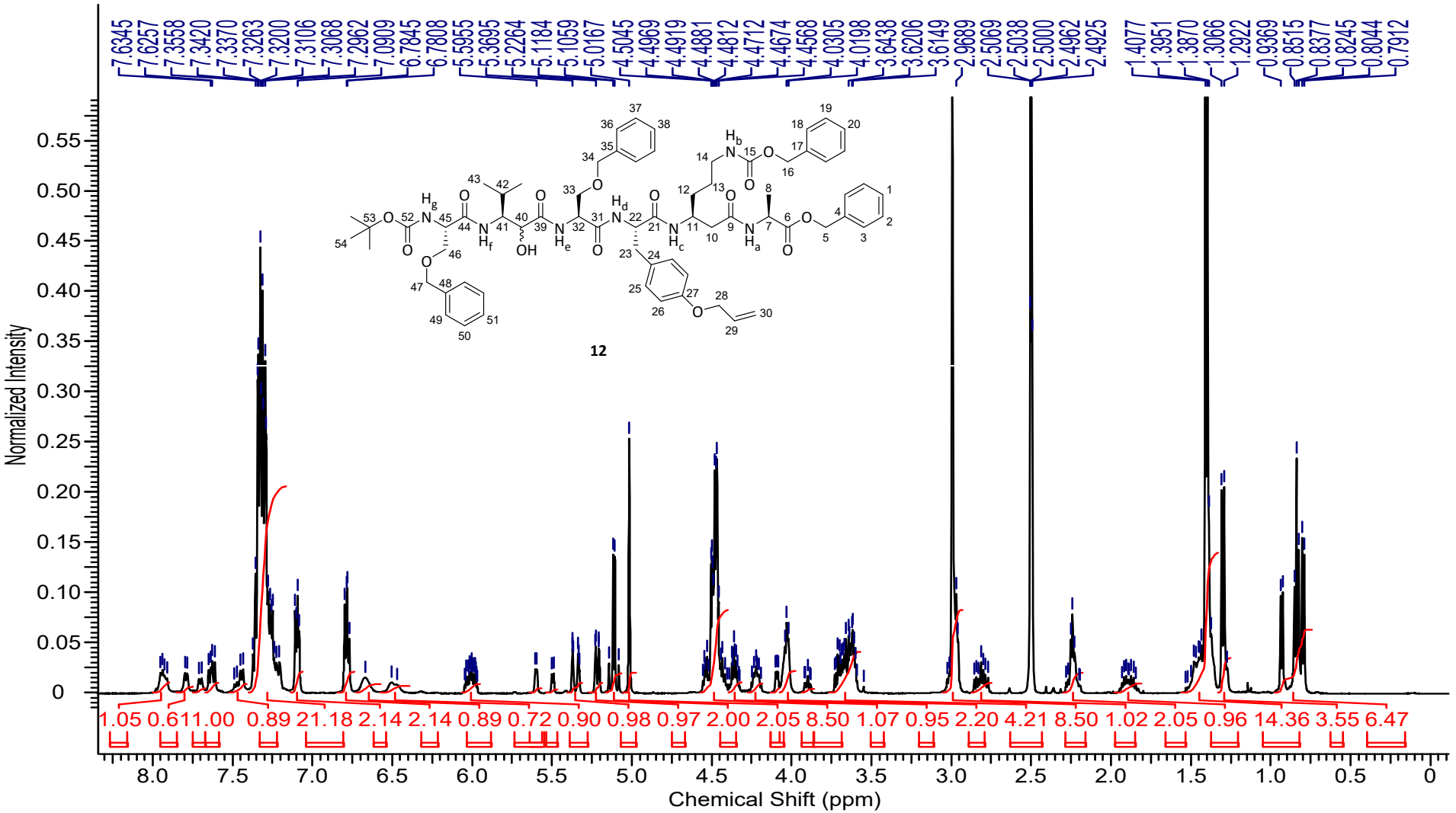
125 MHz, DMSO-d_6, 373 K



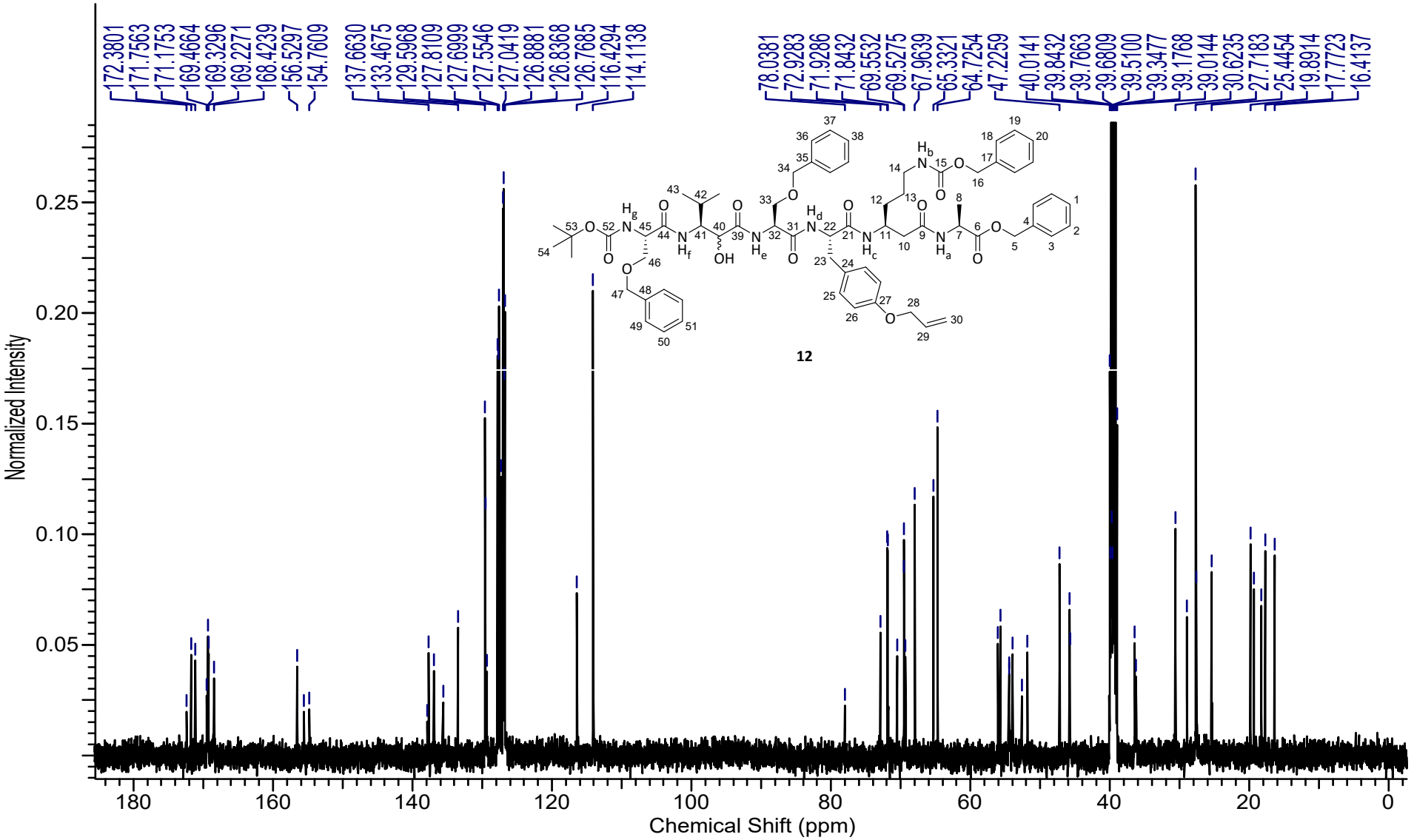
11

Benzyl 3-(*N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_{ε}-benzyloxycarbonyl-L-β-lysyl-L-alaninate (12)

500 MHz, DMSO-d_6, 373 K



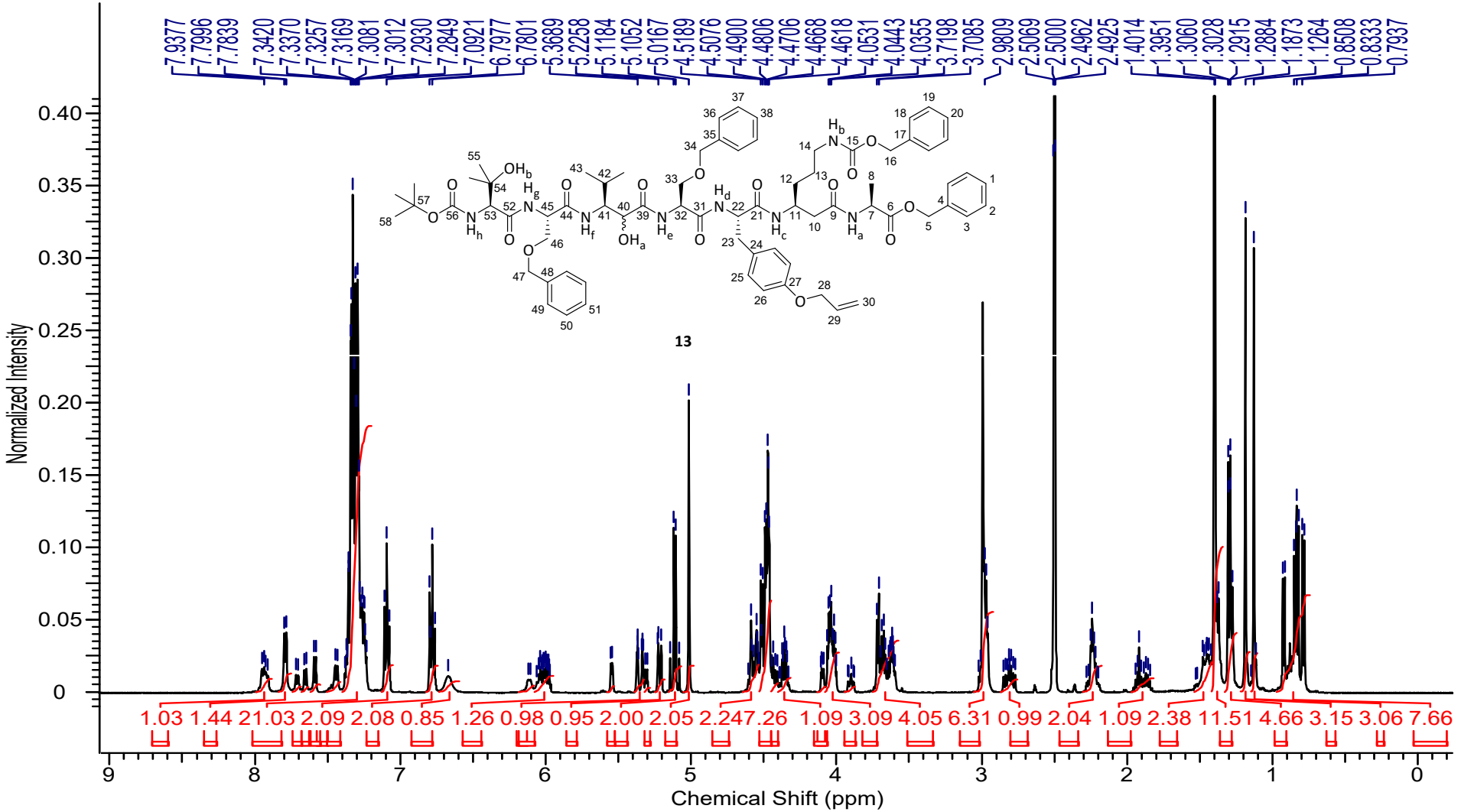
125 MHz, DMSO-d_6, 373 K



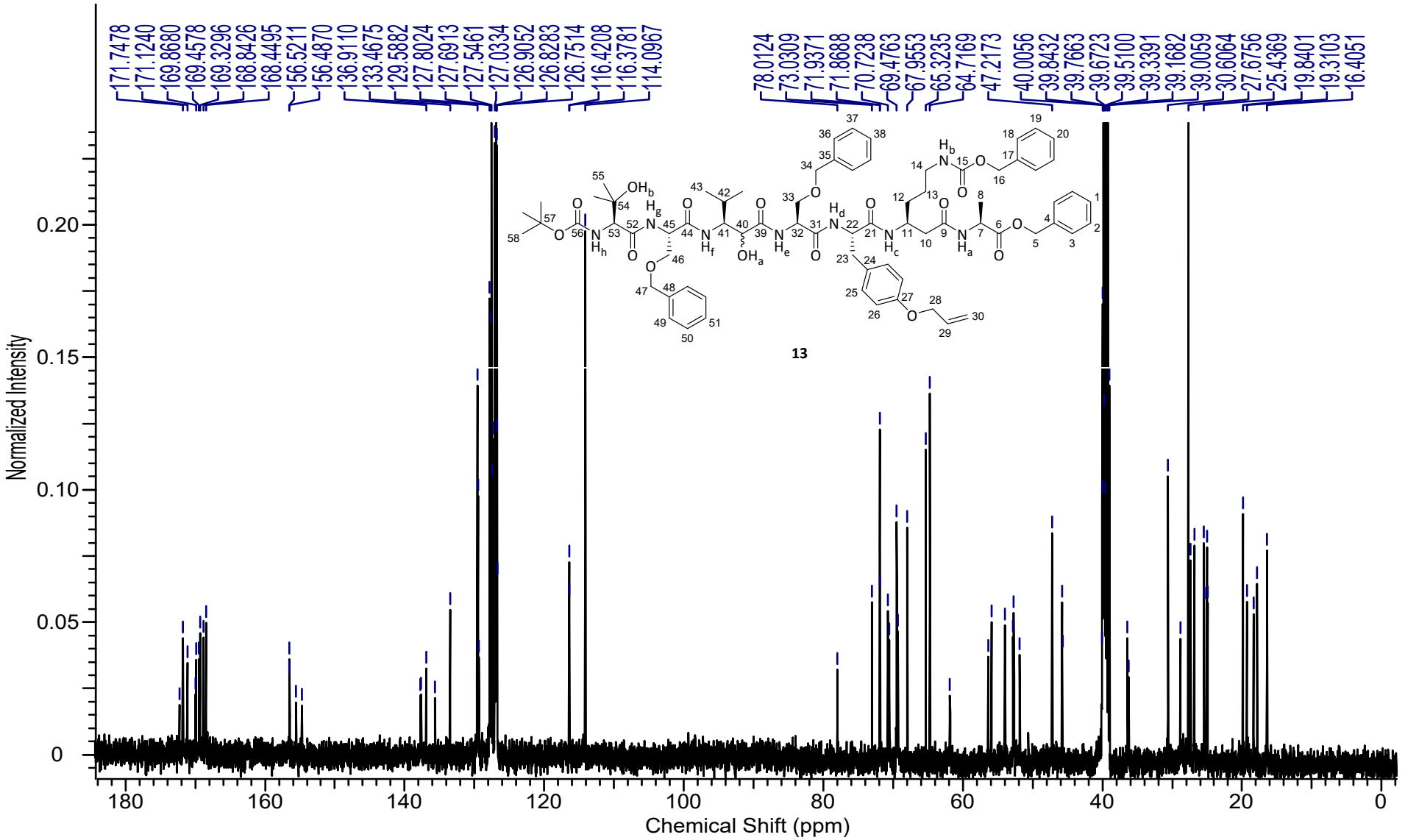
12

Benzyl 3-(*N*-*tert*-butoxycarbonyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_{ε}-benzyloxycarbonyl-L-β-lysyl-L-alaninate (13)

500 MHz, DMSO-d_6, 373 K



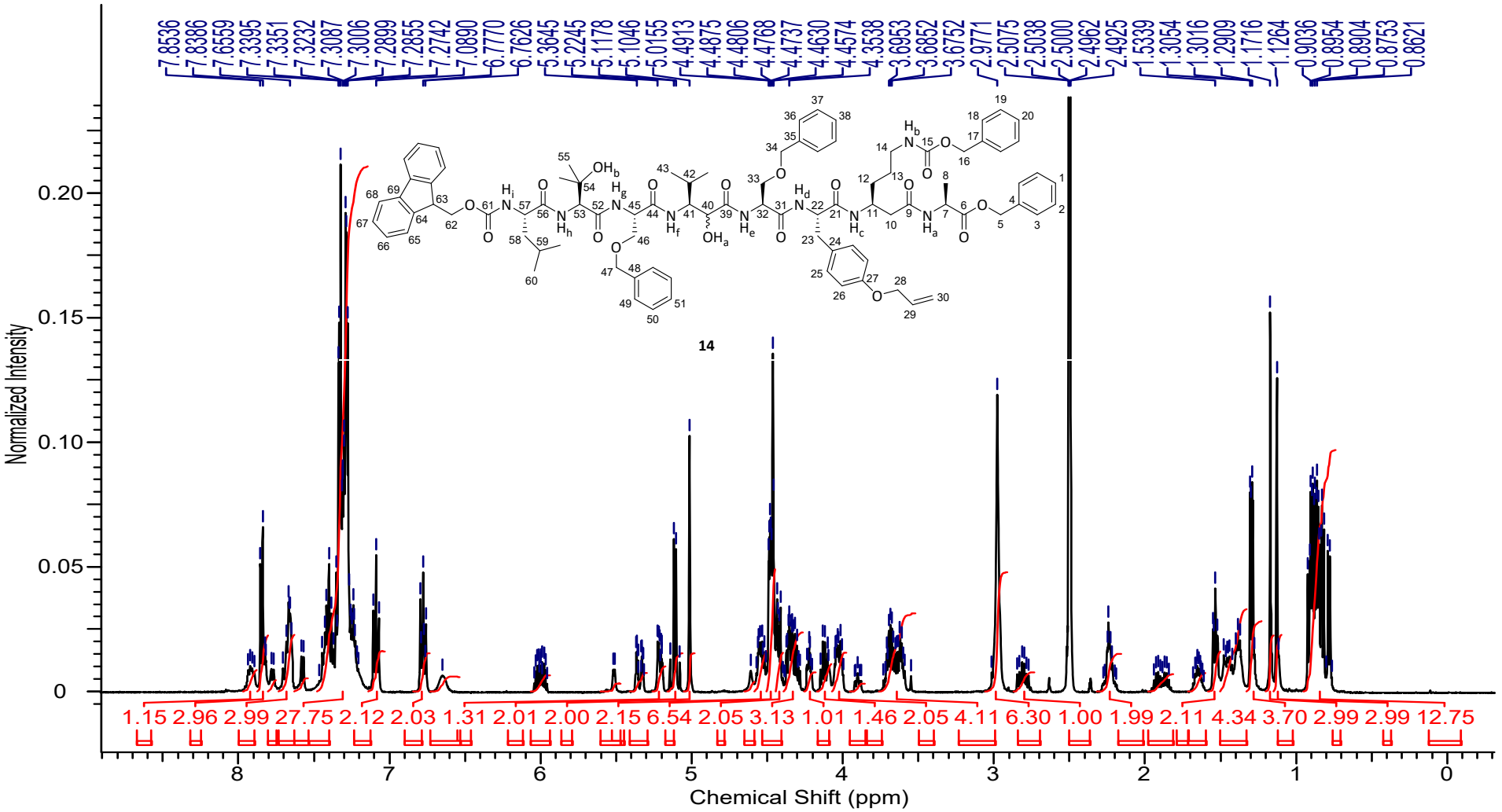
125 MHz, DMSO-d_6, 373 K



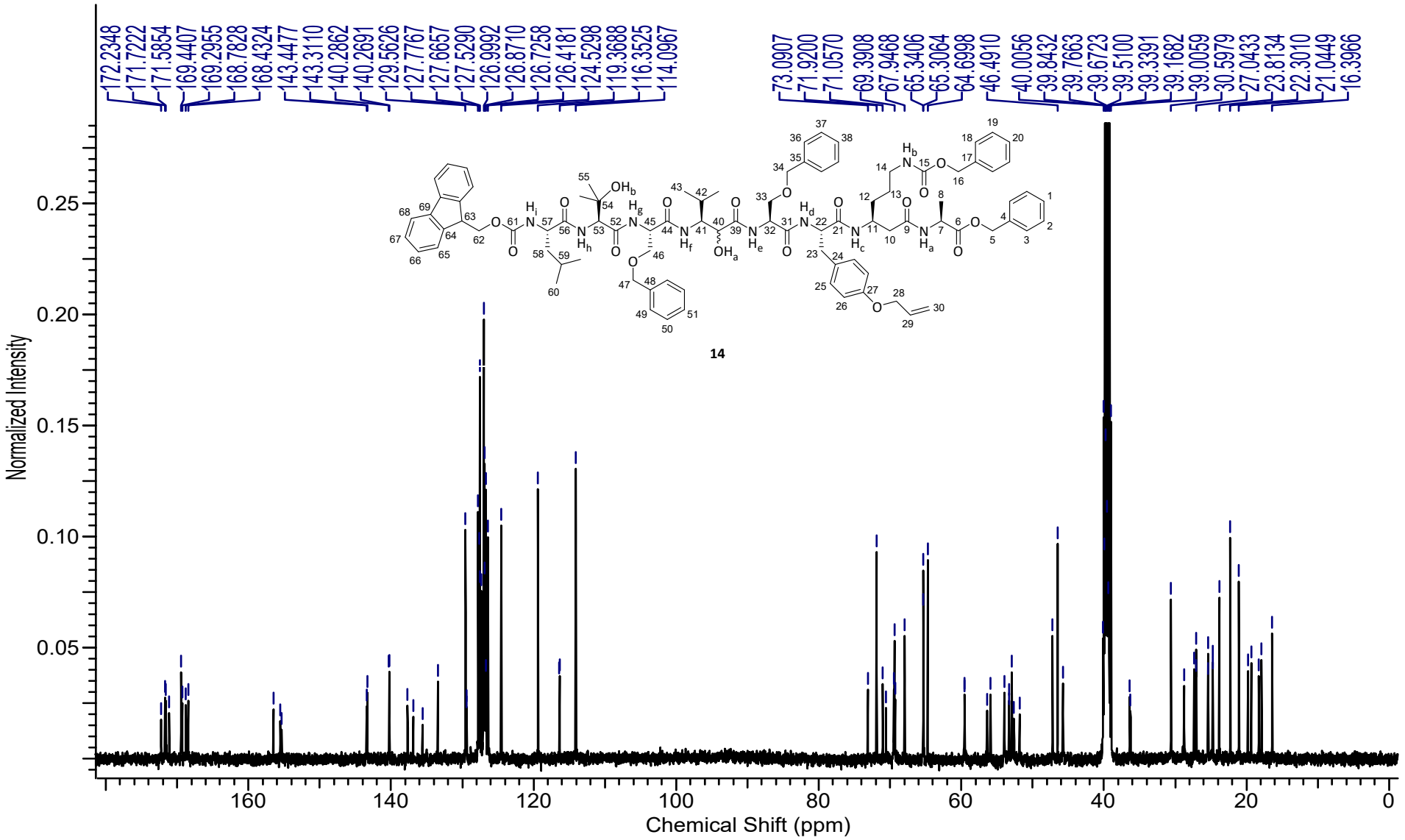
13

Benzyl 3-(*N*-Fluorenylmethoxycarbonyl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_{ε}-benzyloxycarbonyl-L-β-lysyl-L-alaninate (14)

500 MHz, DMSO-d_6, 373 K

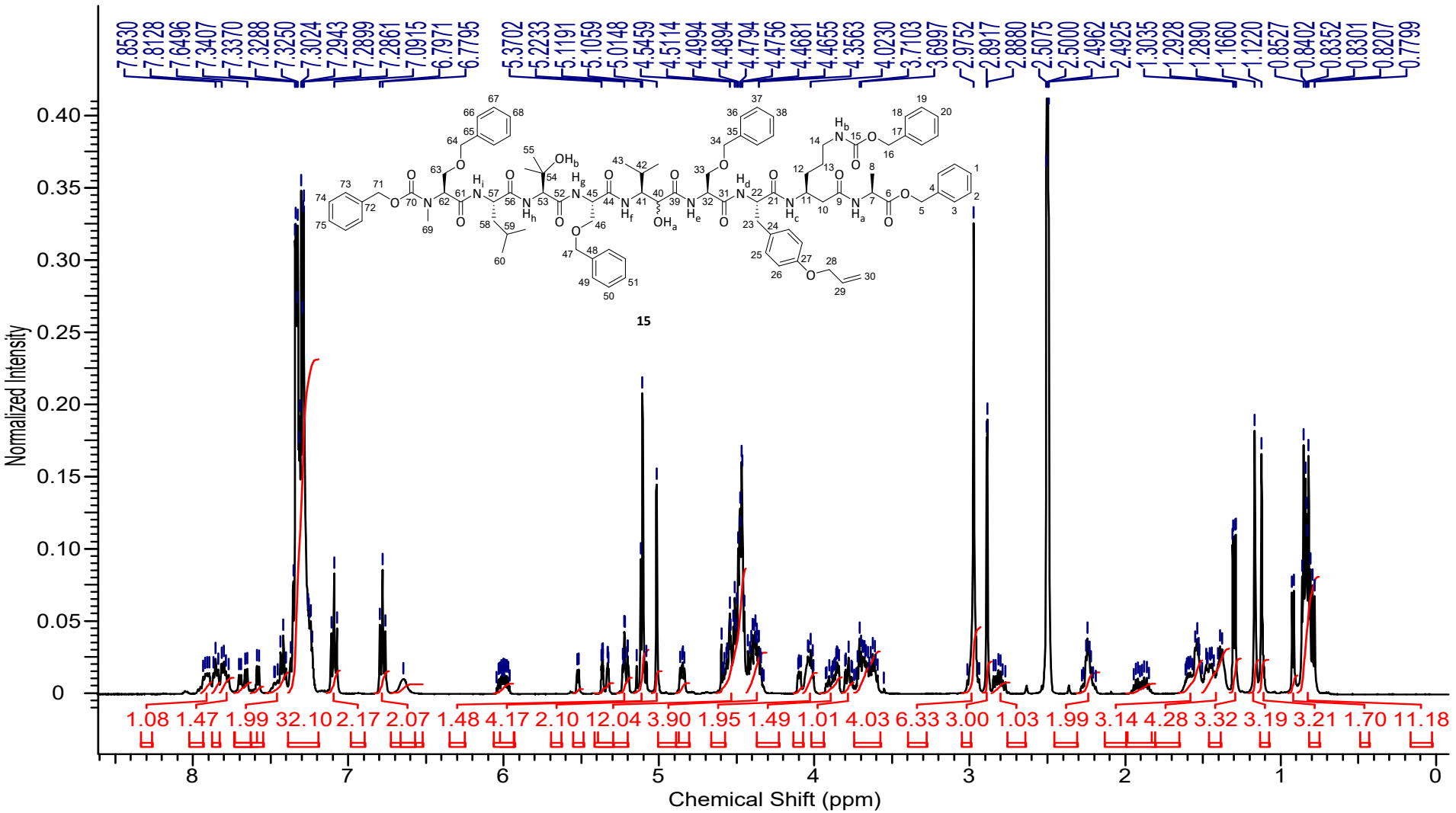


125 MHz, DMSO-d_6, 373 K

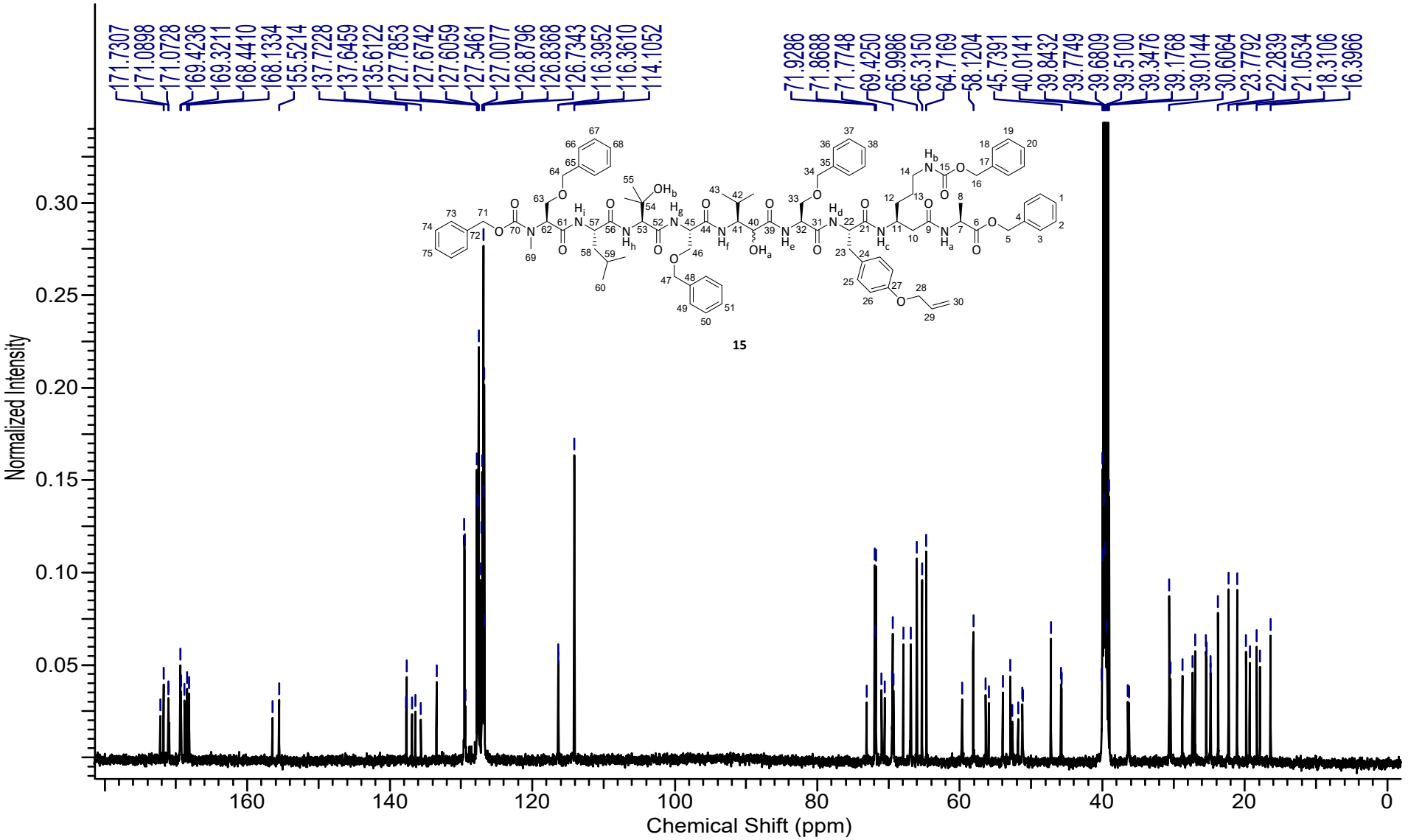


Benzyl 3-(*N*-Benzyloxycarbonyl-*N*-methyl-*O*-benzyl-L-seryl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-2-hydroxy-4-methylpentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (15)

500 MHz, DMSO-d_6, 373 K



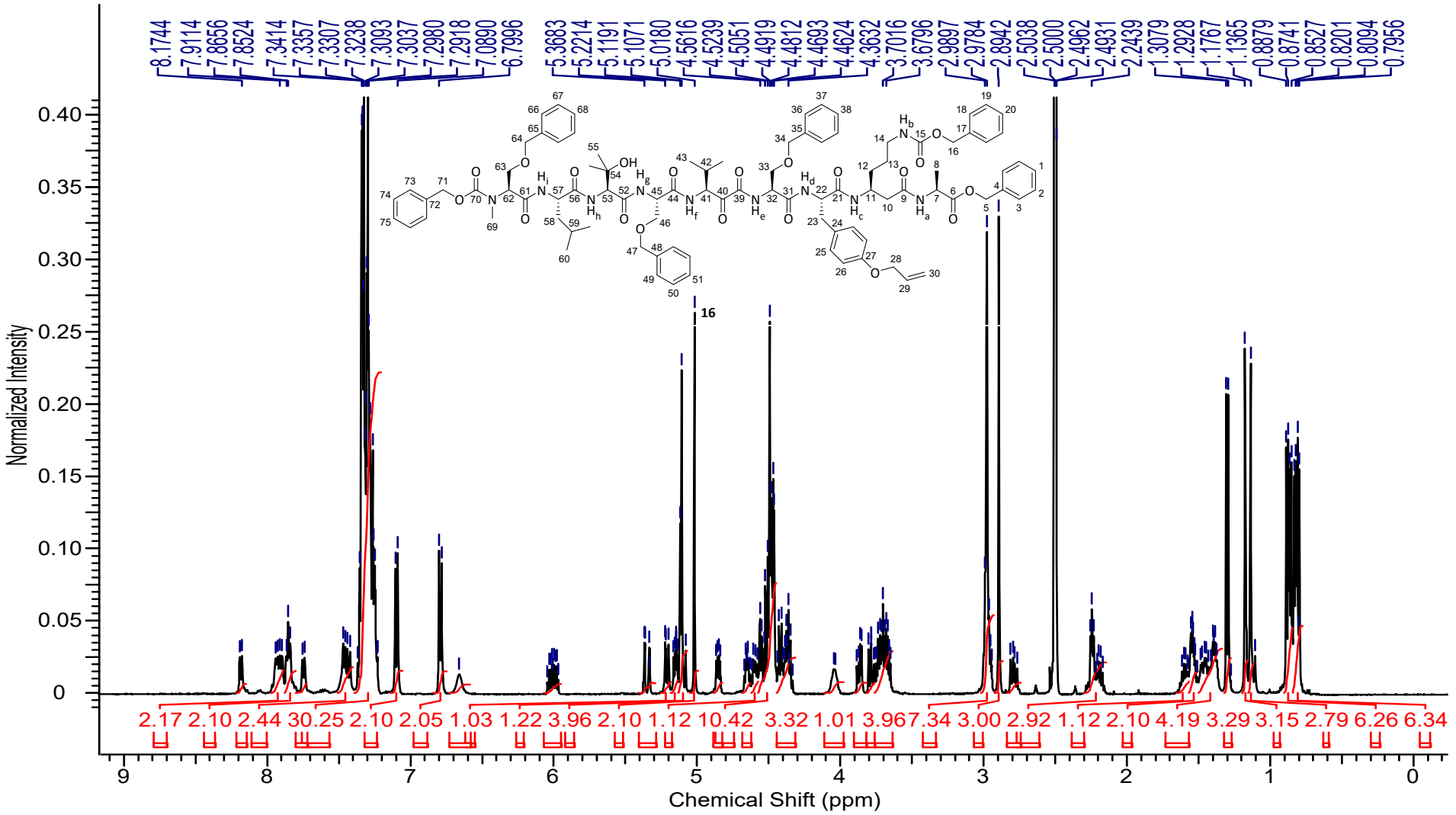
125 MHz, DMSO-d_6, 373 K



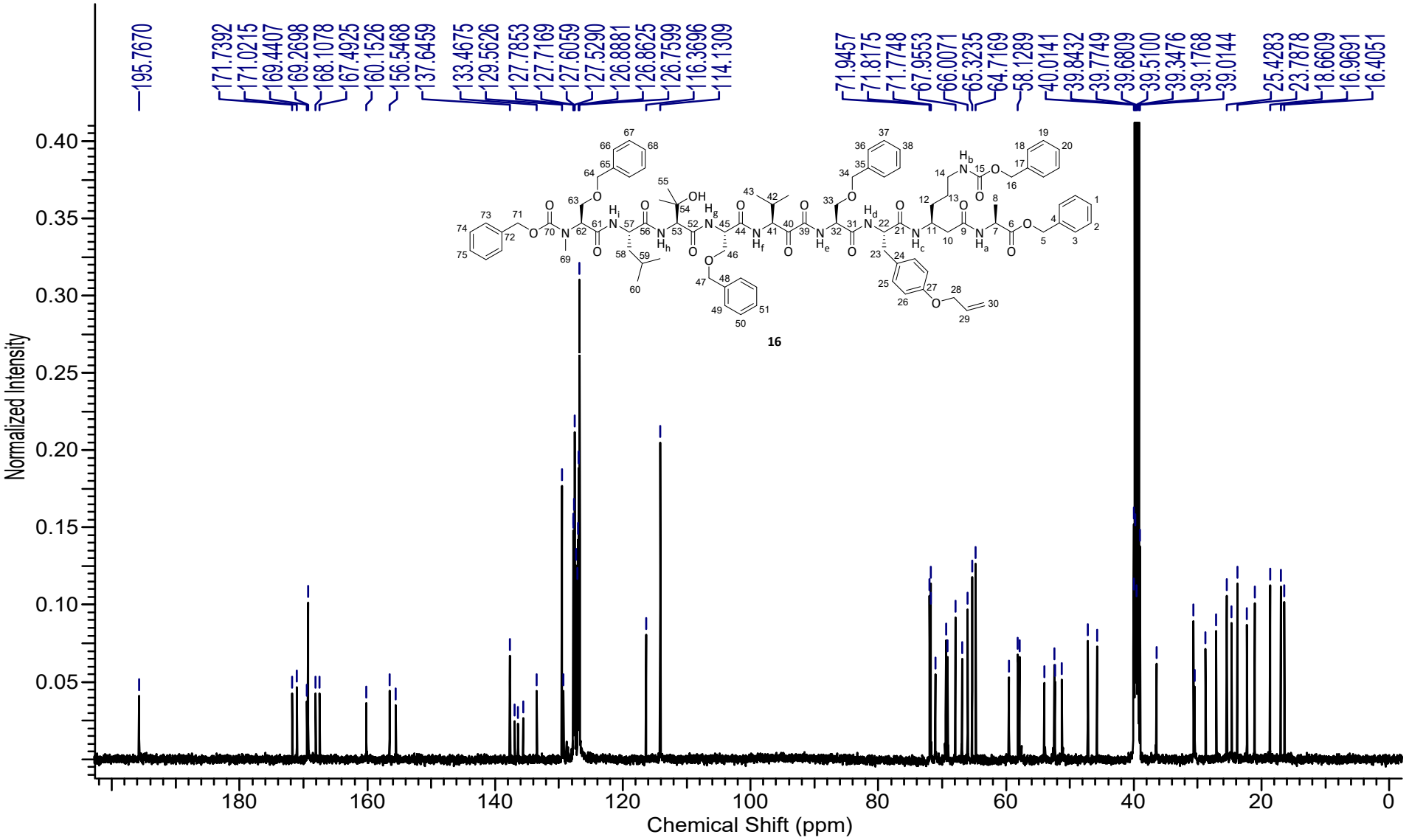
15

Benzyl 3-(*N*-Benzyloxycarbonyl-*N*-methyl-*O*-benzyl-L-seryl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-4-methyl-2-oxopentanoyl-*O*-benzyl-L-seryl-*O*-allyl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (16)

500 MHz, DMSO-d_6, 373 K



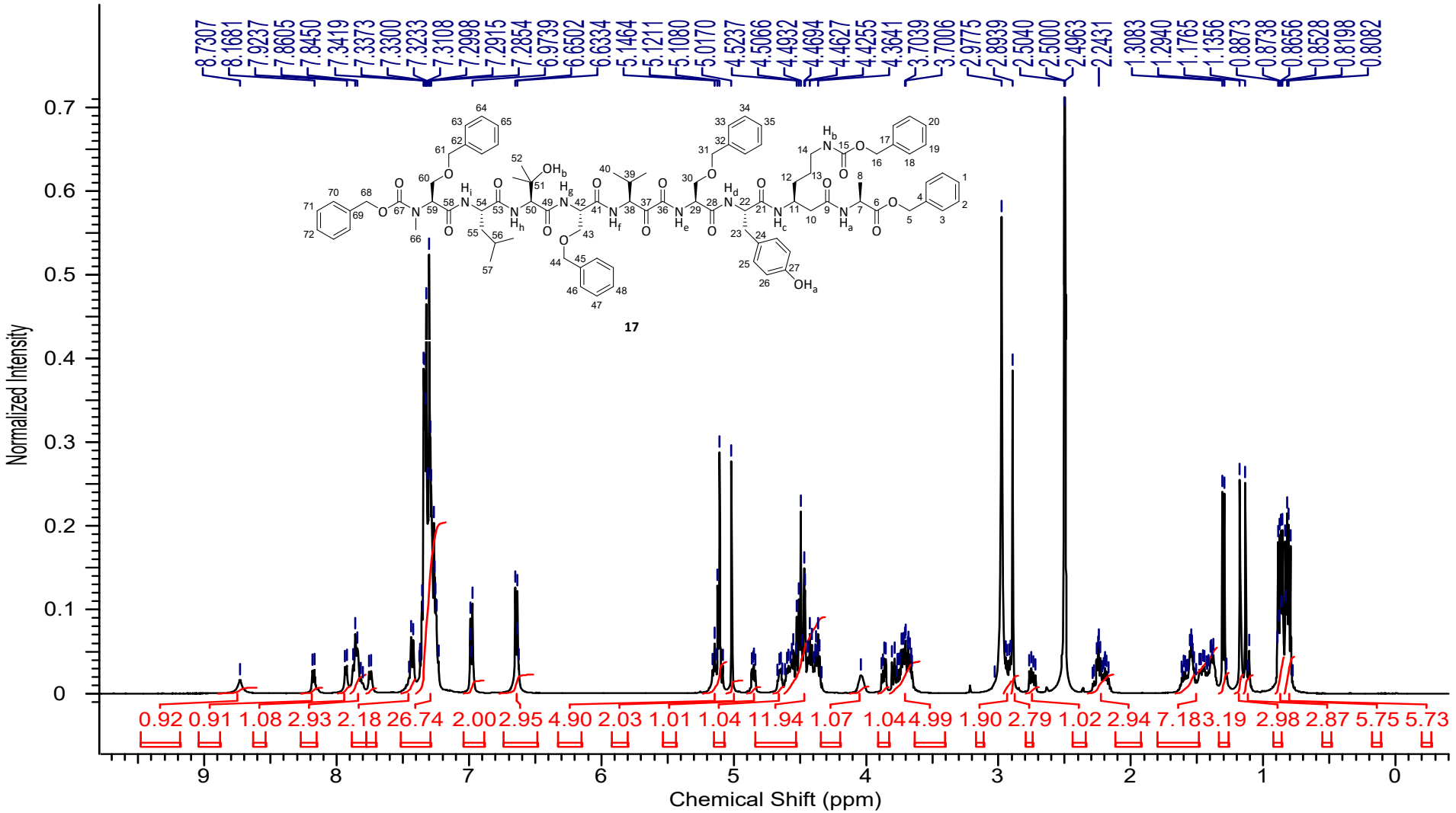
125 MHz, DMSO-d_6, 373 K



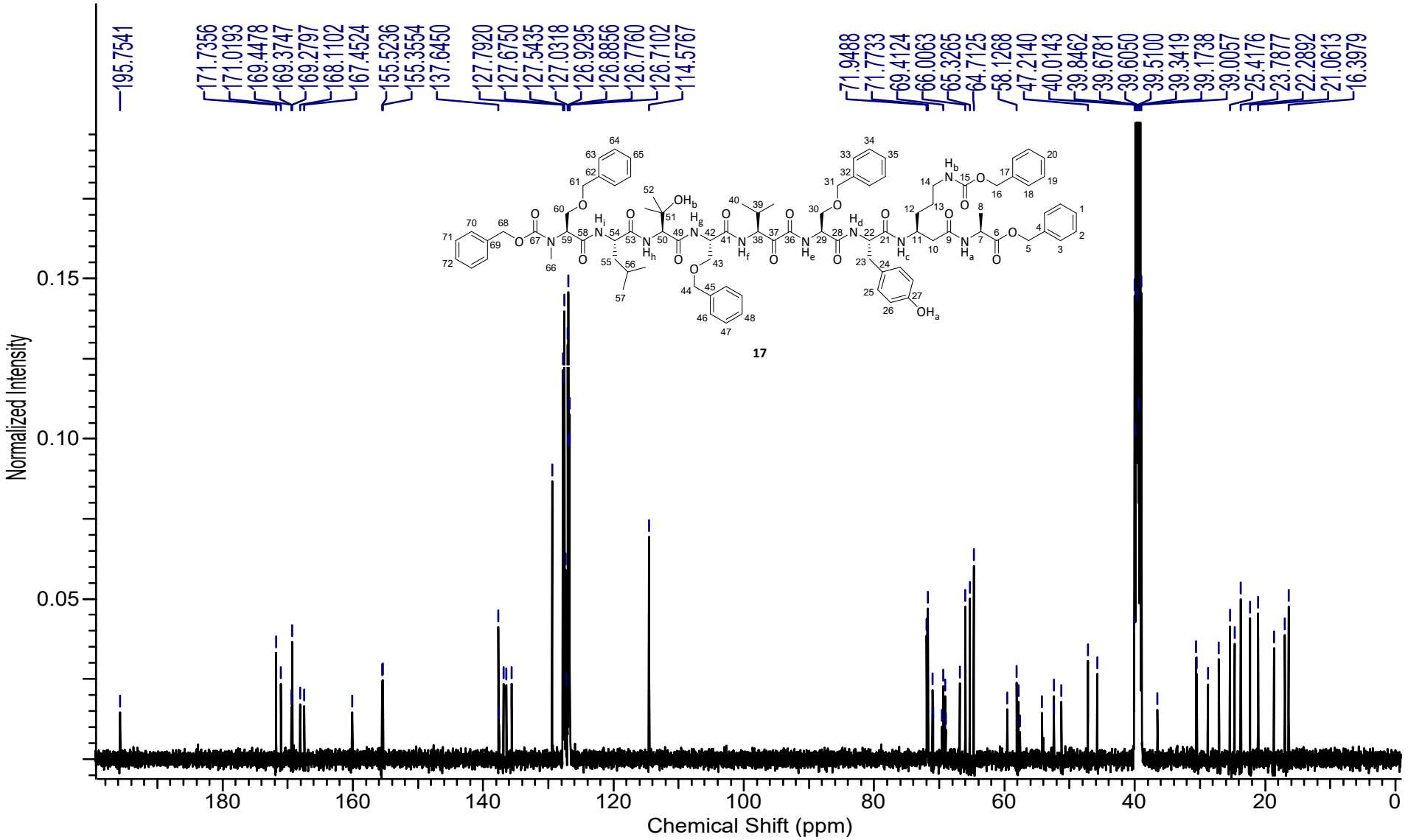
16

Benzyl 3-(*N*-Benzyloxycarbonyl-*N*-methyl-*O*-benzyl-L-seryl-L-leucyl-β-hydroxy-L-valyl-*O*-benzyl-L-serylamino)-4-methyl-2-oxopentanoyl-*O*-benzyl-L-seryl-L-tyrosyl-N_ε-benzyloxycarbonyl-L-β-lysyl-L-alaninate (17)

500 MHz, DMSO-d_6, 373 K



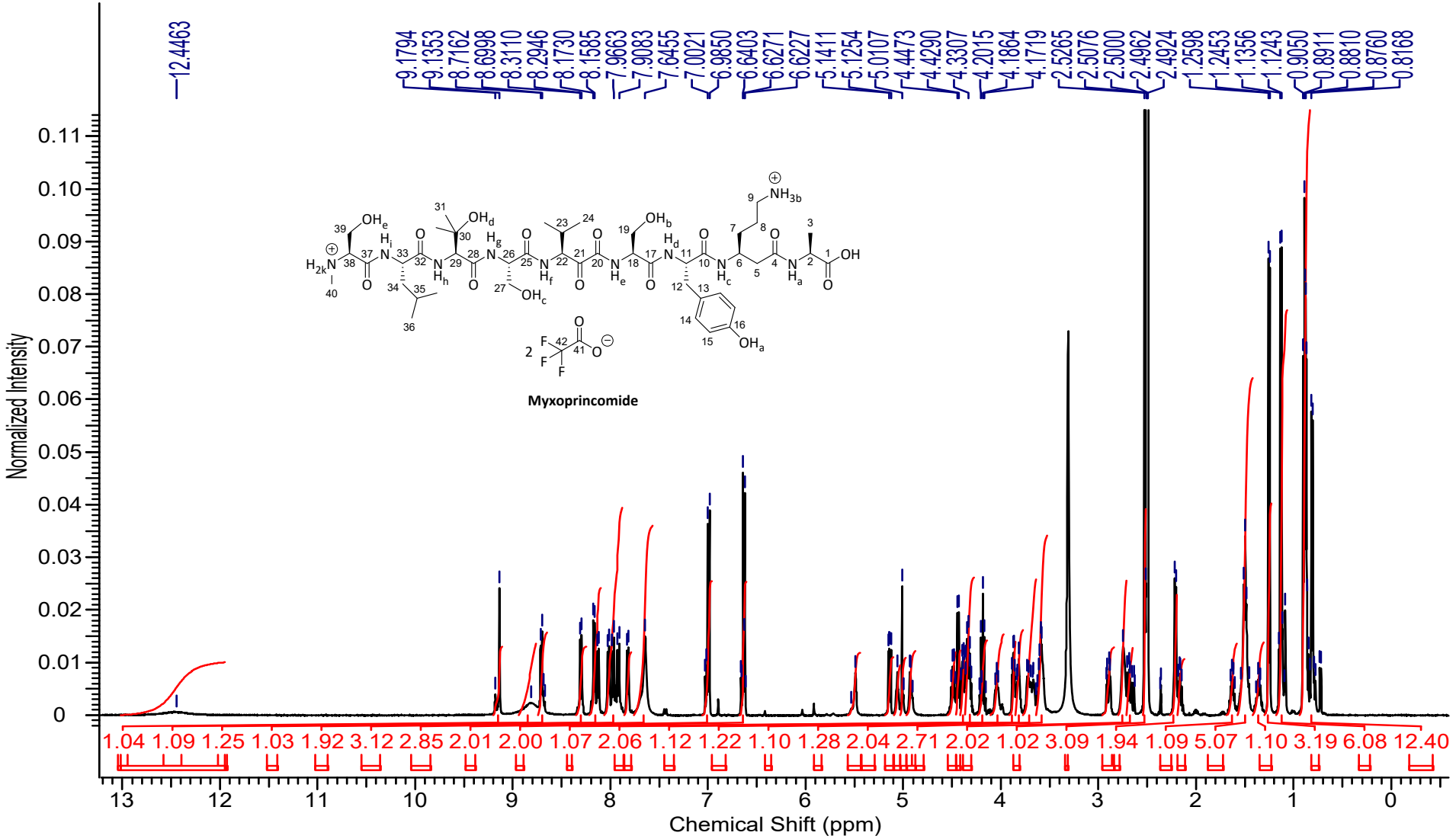
125 MHz, DMSO-d_6, 373 K



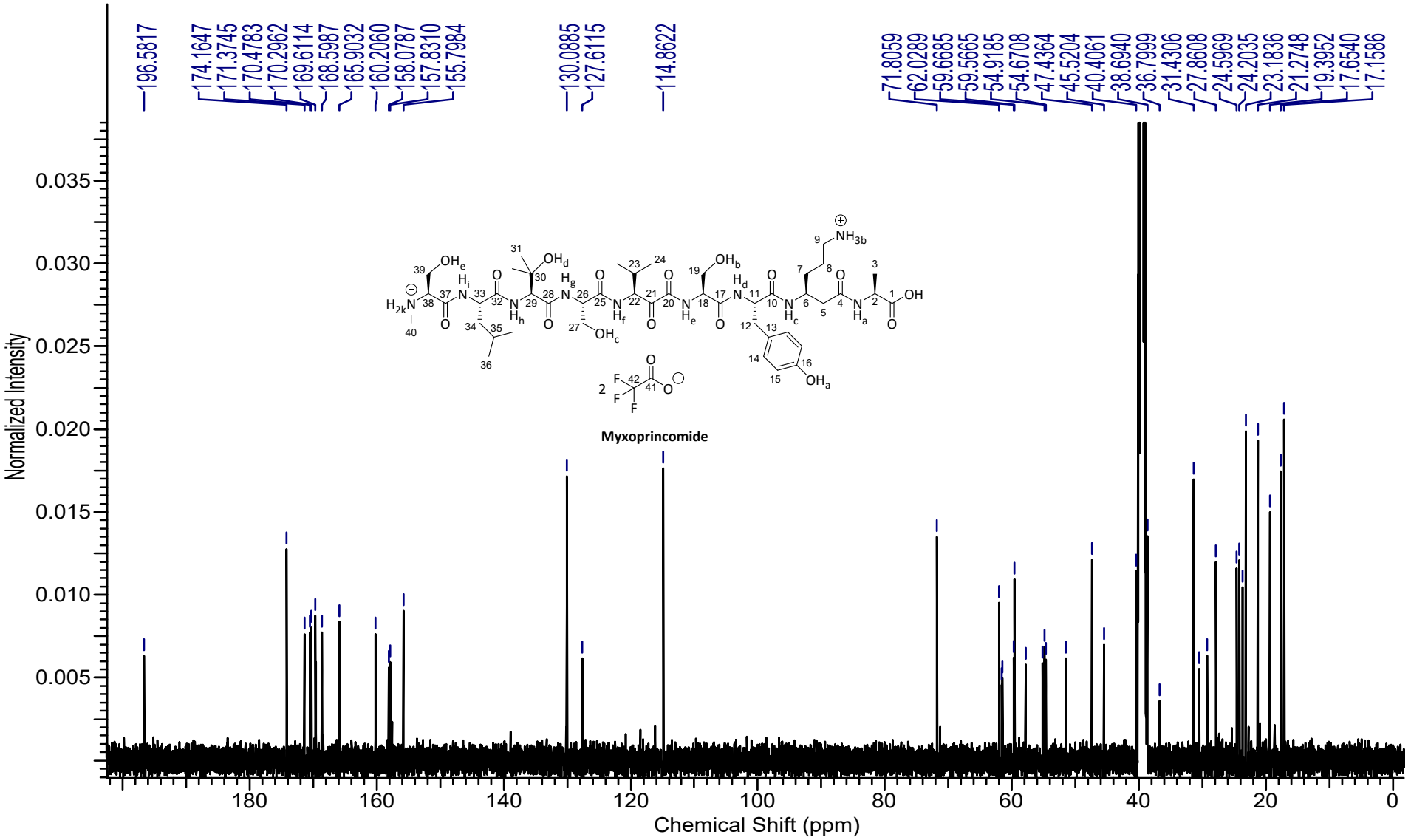
17

Myxoprincomide

500 MHz, DMSO-d_6



125 MHz, DMSO-d_6



Myxoprincomide

NMR Comparison of synthetic and isolated[4] Myxoprincomide

Table 1: ^{1}H-NMR comparison

Amino acid	No.	δ (isolated) [ppm]	δ (synthetic) [ppm]	Δδ [ppm]
NmeSer	2	3.86	3.87	0.01
	3	3.82	3.82	0.00
		3.69	3.72	0.03
	NH_2^+	8.83	8.81	– 0.02
		8.74	8.81	0.07
	Nme	2.51	2.53	0.02
	OH	5.50	5.49	– 0.01
Leu	2	4.48	4.49	0.01
	3	1.48	1.49	0.01
	4	1.63	1.63	0.00
	5	0.87	0.87	0.00
	6	0.89	0.90	0.01
	NH	8.70	8.71	0.01
OH-Val	2	4.43	4.44	0.01
	4	1.13	1.14	0.01
	5	1.08	1.12	0.04
	NH	7.96	7.92	– 0.04
	OH	5.05	5.01	– 0.04
Ser1	2	4.38	4.39	0.01
	3	3.68	3.68	0.00
		3.56	3.59	0.03
	OH	4.96	4.92	– 0.04
	NH	8.00	7.97	– 0.03
2-oxo-β-Leu	3	5.20	5.14	– 0.06
	4	2.15	2.17	0.02
	5	0.87	0.88	0.01
	6	0.78	0.80	0.02
	NH	8.01	8.02	0.01
Ser2	2	4.31	4.34	0.03
	3	3.64	3.59	– 0.05
		3.58	3.59	0.01
	OH	5.10	5.06	– 0.04
	NH	8.32	8.30	– 0.02
Tyr	2	4.35	4.34	– 0.01
	3	2.88	2.90	0.02
		2.67	2.68	0.01

[4] N. S. Cortina, D. Krug, A. Plaza, O. Revermann, R. Müller, *Angew. Chem*. **2012**, *124*, 836–841; *Angew. Chem. Int. Ed.* **2012**, *51*, 811–816.

	2', 6'	6.99	6.99	0.00
	3', 5'	6.62	6.63	0.01
	OH	9.16	9.14	– 0.02
	NH	8.08	8.13	0.05
β-Lys	2	2.20	2.21	0.01
	3	4.03	4.04	0.01
	4	1.48	1.48	0.00
		1.34	1.36	0.02
	5	1.48	1.49	0.01
		1.34	1.46	0.12
	6	2.73	2.75	0.02
	NH_3^+	7.61	7.65	0.04
	NH	7.88	7.82	– 0.06
Ala	2	4.18	4.19	0.01
	3	1.25	1.25	0.00
	NH	8.21	8.17	– 0.04

Table 2: ^{13}C-NMR comparison

Amino acid	No.	δ (isolated) [ppm]	δ (synthetic) [ppm]	Δδ [ppm]
NmeSer	1	165.4	165.9	0.5
	2	61.5	62.0	0.5
	3	59.2	59.6	0.4
	Nme	31.1	31.4	0.3
Leu	1	171.1	171.4	0.3
	2	51.1	51.5	0.4
	3	40.1	40.4	0.3
	4	23.9	24.2	0.3
	5	21.1	21.3	0.2
	6	22.8	23.2	0.4
OH-Val	1	169.4	169.5	0.1
	2	59.3	59.7	0.4
	3	71.3	71.8	0.5
	4	27.7	27.9	0.2
	5	24.5	24.6	0.1
Ser1	1	169.9	170.5	0.6
	2	54.4	54.9	0.5
	3	61.4	61.5	0.1
2-oxo-β-Leu	1	159.8	160.2	0.4
	2	196.4	196.6	0.2
	3	57.2	57.9	0.7
	4	29.0	29.2	0.2
	5	18.9	19.4	0.5
	6	17.3	17.7	0.4

Ser2	1	168.3	168.6	0.3
	2	54.8	55.1	0.3
	3	61.1	61.6	0.5
Tyr	1	170.0	170.3	0.3
	2	54.3	54.7	0.4
	3	36.6	36.8	0.2
	1'	127.2	127.6	0.4
	2', 6'	129.9	130.1	0.2
	3', 5'	114.4	114.9	0.5
	4'	155.3	155.8	0.5
β-Lys	1	169.4	169.6	0.2
	2	40.1	40.4	0.3
	3	45.1	45.5	0.4
	4	30.2	30.5	0.3
	5	23.5	23.7	0.2
	6	38.3	38.7	0.4
Ala	1	173.9	174.2	0.3
	2	47.1	47.4	0.3
	3	16.8	17.2	0.4

HPLC/MS Comparison of synthetic and isolated Myxoprincomide

Large scale fermentation

Producer: *Myxococcus xanthus* A2 pCKA2c506 (Red book No°90)

The Myxoprincomide producer strain *M. xanthus* A2 pCKA2c506 was grown first on a CTT agar plate (10 g Casitone (Difco), 10 ml 0.8 M $MgSO_4$, 10 mL 1 M Tris-HCl pH 8.0, 1 mL K_2HPO_4 pH 7.6 and distilled water up to 1 L, pH adjusted to 7.6, agar 15 g/L). Cell mass was inoculated in 100 mL CTT and the culture was incubated for 5 days at 30°C, 180 rpm. The 100 mL liquid culture was then diluted 1:200 v/v at 12 L scale (6x 2 L in 5 L shake flasks) in CTT medium (10 g Casitone (Difco), 10 ml 0.8 M $MgSO_4$, 10 ml 1 M Tris-HCl pH 8.0, 1 ml K_2HPO_4 pH 7.6 and distilled water up to 1 L, pH adjusted to 7.6) and the culture was incubated for 72 hours, 30°C, 180 rpm before the addition of 2% XAD-16 resin. After XAD introduction, the fermentation was carried out for additional 24 hours before harvesting: XAD-16 resin was separated from the cells and medium by decantation and amounted after lyophilization 26.0 g.

Purification of Myxoprincomide from the optimized producer strain *M. xanthus* A2 c506

The XAD-16 resin was extracted with 900 mL methanol (3x 300 mL, each 300 mL liquid extraction carried out for 4 hours). The methanolic extract was concentrated *in vacuo* to a volume of approx. 100 mL; the methanol extract was then defatted with cyclohexane. The methanol partition was dried *in vacuo*. The dried residue (6302 mg) was suspended in 50 mL distilled water and liquid-liquid partitioned with chloroform (1:1 v:v). The chloroform was removed and the remaining water phase was partitioned with ethylacetate (1:1 v:v). The water phase was lyophilized at –80°C. The dried residue was redissolved in 4 ml methanol. The mixture was centrifuged and the supernatant was fractionated on a Sephadex LH-20 column using methanol as the eluent. Aliquots from fractions were obtained and analyzed by LC-MS. Fractions in which acceptable amounts of Myxoprincomide were identified, pooled, dried *in vacuo* and re-dissolved in 5 mL methanol. 1 mL of methanolic solution was

purified by HPLC (XBridge® BEH Amide OBD™ Prep Column, 130 Å, 5 µM, 10 mm x 250 mm, DAD at 220 nm and 280 nm) using a H_2O-acetonitrile fluid containing 0.1% v/v formic acid. Elution was carried out as follows: 5% ACN (0–2 min), 5 – 95% ACN linear gradient (2-40 min). Myxoprincomide (2.63 mg, t_r = 2.7-3.0 min) was collected and dried *in vacuo* at –80 °C.

Analysis of synthetic Myxoprincomide MK 715-7 and isolated Myxoprincomide from *Myxococcus xanthus* A2 pCKc506 via LC-HRMS

Both compounds, synthetic and isolated Myxoprincomide, were dissolved in methanol to a concentration of 10 µM and analyzed by HPLC-HRESI-DAD-MS on a Bruker maXis 4G mass spectrometer coupled with a Dionex Ultimate 3000 RSLC system using a BEH C18 column (100 × 2.1 mm, 1.7 µm, Waters, Germany) with a gradient of 5–95% acetonitrile (ACN) + 0.1% formic acid (FA) in H_2O + 0.1% FA at 0.6 mL/min and 45°C over 18 min with UV detection by a diode array detector at 200–600 nm. Mass spectra were acquired from 150 to 2000 m/z at 2 Hz. For MS^2 measurements, the AutoMS^2 acquisition was ramped from 1- 4 Hz and CID Energy is ramped from 20 eV for 100 m/z to 35 eV for 1000 m/z. The detection was performed in the positive MS mode. The plugin for Chromeleon Xpress (Dionex) was used for operation of UltiMate 3000 LC System. HyStar (Bruker Daltonic) was used to operate on maXis 4G speed MS system. HPLC-MS mass spectra were analyzed with DataAnalysis 4.4 (Bruker Daltonic).

Figure 1: extracted ion chromatogram of synthetic Myxoprincomide MK715-7 $[M+2H]^{2+}$= 506.2691

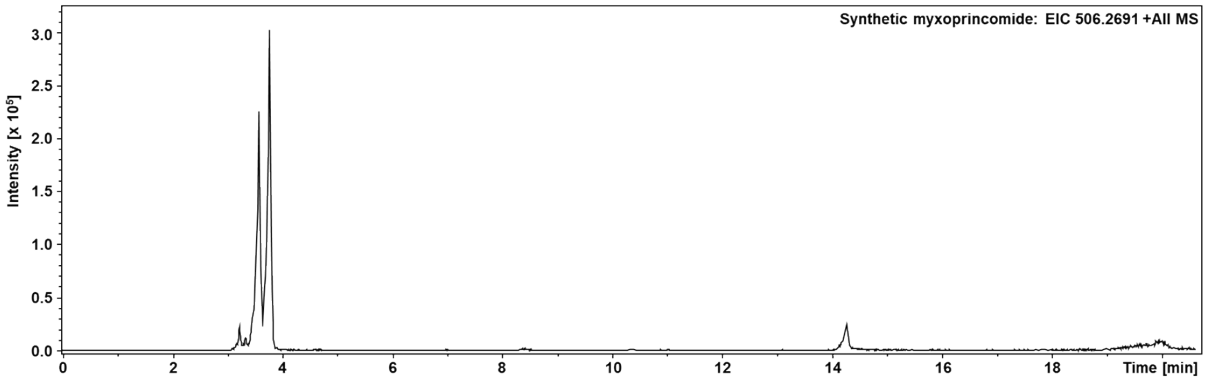


Figure 2: extracted ion chromatogram of isolated Myxoprincomide from *Myxococcus xanthus* A2 pCKc506 $[M+2H]^{2+}$= 506.2691

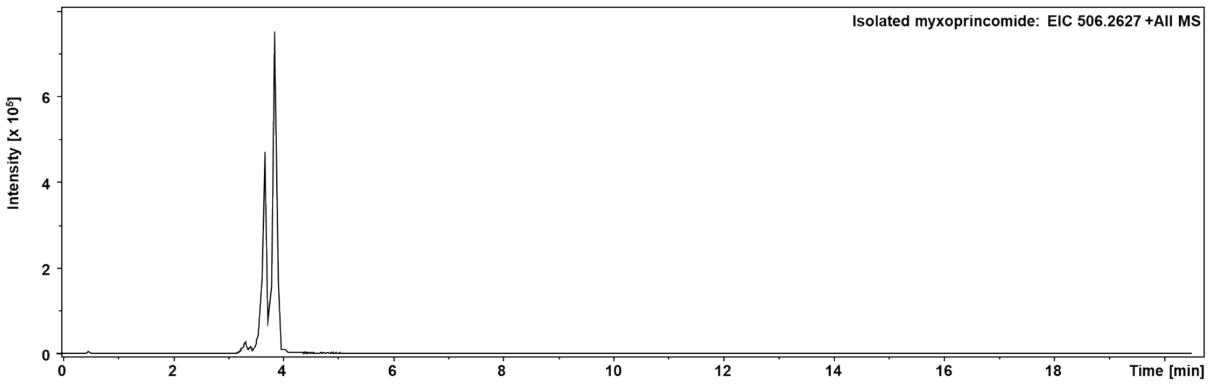


Figure 3: MS^2 fragmentation pattern of parent ion $[M+2H]^{2+}$= 506.2691 (marked with blue rhombus) from synthetic Myxoprincomide MK715-7

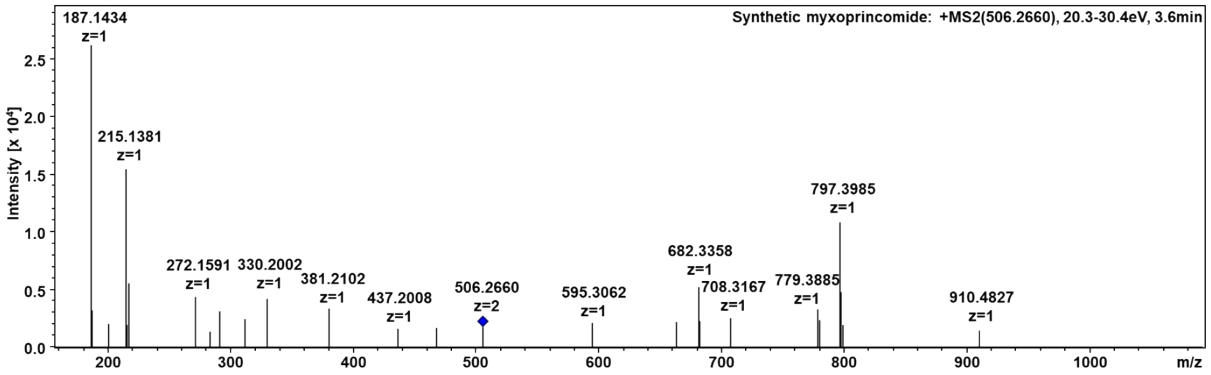


Figure 4: MS^2 fragmentation pattern of parent ion $[M+2H]^{2+}$= 506.2660 (marked with blue rhombus) from isolated Myxoprincomide from *Myxococcus xanthus* A2 pCKc506.

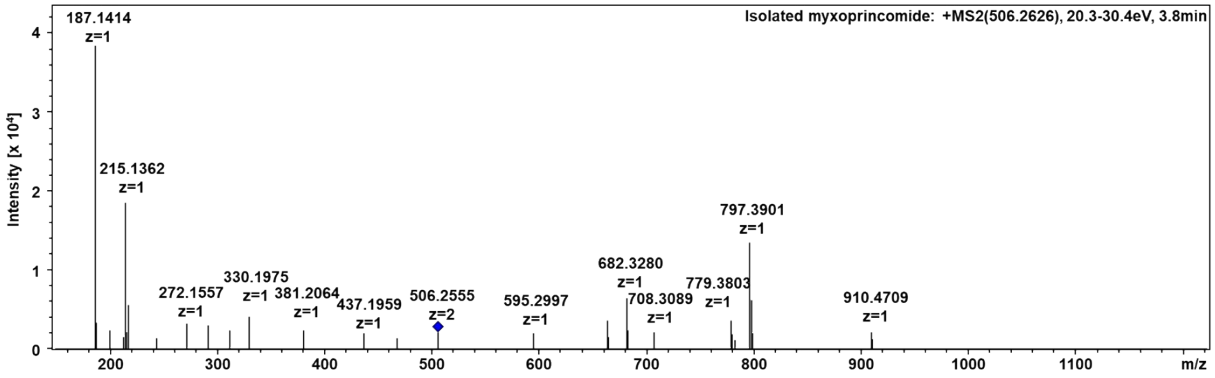


Table 3: Annotated MS^2: fragmented ions of synthetic Myxoprincomide MK715-7

MW (fragment) [Da]	**Fragment**
910.4827	$M+H^+$ - N-MeSer
797.3985	$M+H^+$ - 2 H_2O - N-MeSer
779.3885	$M+H^+$ - H_2O - N-MeSerLeu
682.3358	$M+H^+$ - N-MeSerLeuOxVal
664.3260	$M+H^+$ - H_2O - N-MeSerLeuOxVal
595.3062	$M+H^+$ - H_2O - N-MeSerLeuOxValSer
506.2660	$M+2H^{2+}$
381.2102	PheLysAla + $2H^+$
330.2002	N-MeSerLeuOxVal + H^+
272.1591	N-MeSerLeuOxVal – CH_4N
215.1381	N-MeSer-Leu + H^+

187.1434	N-MeSer-Leu - CO + H^+

Table 4: Annotated MS^2: fragmented ions of Myxoprincomide from *Myxococcus xanthus* A2 pCKc506

MW (fragment) [Da]	**Fragment**
910.4709	$M+H^+$ - N-MeSer
797.3901	$M+H^+$ - 2 H_2O - N-MeSer
779.3803	$M+H^+$ - H_2O - N-MeSerLeu
682.3280	$M+H^+$ - N-MeSerLeuOxVal
664.3258	$M+H^+$ - H_2O - N-MeSerLeuOxVal
595.2997	$M+H^+$ - H_2O - N-MeSerLeuOxValSer
506.2555	$M+2H^{2+}$
381.2064	PheLysAla + $2H^+$
330.1975	N-MeSerLeuOxVal + H^+
272.1557	N-MeSerLeuOxVal – CH_4N
215.1362	N-MeSer-Leu + H^+
187.1414	N-MeSer-Leu - CO + H^+

UHPLC Separation of Myxoprincomide

UHPLC-MS measurements

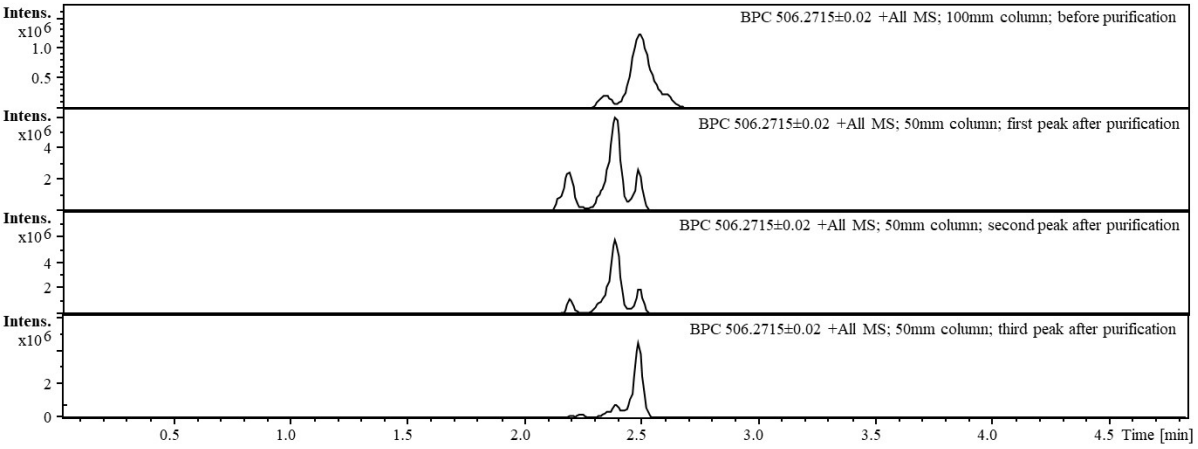
A Bruker Daltonics maXis 4G ultra high resolution time of flight mass spectrometer coupled to a Dionex Ultimate 3000 SL system was used for high resolution measurements, which is equipped with an ESI source and operated under following MS settings: capillary voltage 4000 V, end plate off-set -500 V, nebulizer gas pressure 1 bar, dry gas flow rate 5 L/min, dry gas temperature 200 °C, mass scan range *m/z* 150–2500. Co-injection of a 1 mM sodium hydroxide in 1:1 water:isopropanol + 0.1% formic acid mix leads to sodium formate cluster formation in the ion source, which masses of the first isotope signal are used for calibration in Quadratic + HPC mode. MS/MS measurements are carried out in CID fragmentation mode using a collision energy of 5 eV. Separation is achieved by a Waters Acquity BEH C18 column (50 or 100 x 2.1 mm, 1.7 µm) with a mobile phase consisting of A) 0.1 % formic acid in ddH_2O and B) 0.1 % formic acid in acetonitrile at a flow rate of 0.6 mL/min at 45 °C. Before entering the mass spectrometer the flow is split to 75 µL/min. LC-MS data are examined via DataAnalysis 4.4.

Separation attempts of the Myxoprincomide isomers

The three isomers that can be observed on reverse phase chromatography should be separated by semipreparative workup using a Thermo Scientific ISQ EM single quadrupole MS system armed with a HESI ion source in combination with a Thermo Fisher Scientific Dionex ultimate 3000 series system. Separation was reached via a Waters XBridge Peptide BEH C18 OBD prep column (250 x 10 mm, 5 µm)

together with following 30 min gradient with (A) ddH_2O with 0.1% formic acid and (B) acetonitrile with 0.1% formic acid: 0-2 min hold at 5% B, 2-2.5 min increasing to 10% B, 2.5-25 min increasing to 20% B, 25-25.5 min increasing to 95% B, hold for 2 min, 27.5-28.0 decreasing to 5% B and reequilibration at 5% B for 2 min. Thermostat was set to 45 °C, flow rate to 5 mL/min and single ion monitoring was adjusted for detection of the target mass at *m/z* 506.27 for $[M+2H]^{2+}$ and *m/z* 1011.53 for $[M+H]^{+}$ with a source CID voltage of 10 V.

Figure 5: Base peak chromatograms (BPCs) of Myxoprincomide before purification (upper chromatogram, measured on UHR-TOF device on a 100 mm column) and after purification (lower 3 chromatograms, measured on UHR-TOF device on a 50 mm column); isomerization can be observed for both before and after purification of the 3 individual peaks.



Even after purification of each of the three isomer peaks, LC-HRMS measurements uncover repeated conversion into the same three isomers, whereby the latest eluting isomer seems to be comparably most stable (Figure 5).