Electronic Supplementary Material (ESI) for ChemComm.

13-Helix folding of a β/γ-peptide manifold designed from a "minimal-constraint" blueprint

CLAIRE M. GRISON, SYLVIE ROBIN AND DAVID J. AITKEN

SUPPORTING INFORMATION

I. SYNTHESIS OF PEPTIDES 1-12

1. General Information

Boc–(1*S*,2*S*)-ACBC–OH was obtained according to the published procedure.[1] Boc–(1*S*,2*S*)-ACBC–OBn was obtained using the procedure described for the (1*R*,2*R*) enantiomer.[2] The γ^4-amino acids were purchased from PolyPeptide. Dichloromethane was dried over activated alumina, DMF was distilled from CaH_2. All other reagents and solvents were of commercial grade and were used without further purification. Flash chromatography was performed on an automated Combiflash station (Teledyne ISCO) with columns of 15−40 µm silica gel (SI60, Merck-Chimie SAS). Analytical thin-layer chromatography was performed with 0.25 mm commercial silica gel plates (EMD, Silica Gel $60F_{254}$). TLC plates were visualized by UV fluorescence at 254 nm then revealed using an ethanolic ninhydrin solution; retention factors (R_f) are given for such analyses. Routine nuclear magnetic resonance (NMR) data were acquired on Bruker spectrometers operating at 360, 400 or 600 MHz for ^{1}H and at 90 or 100 MHz for ^{13}C. Chemical shifts (δ) are reported in parts per million from tetramethylsilane. Multiplicities for ^{1}H NMR signals are designated as: s (singlet), d (doublet), t (triplet), bs (broad singlet) and m (multiplet). Coupling constants (*J*) are reported in hertz. High-resolution mass spectrometry (HRMS) data were recorded on a MicroTOF-Q (Bruker) instrument using positive mode electrospray ionization (ESI+). Fourier-transform infrared absorption spectroscopy (IR) was performed for solutions in $CDCl_3$ (10 mM) retained in a 0.2 mm path length NaCl solution cell with a $CDCl_3$ background; spectra were recorded on a Spectrum One (Perkin-Elmer) spectrometer. Maximum absorbances (ν_{max}) are reported for significant bands in cm^{-1}. Optical rotations were measured on a Specord 205 instrument (Analytik-Jena) using a 10 cm quartz cell; values for $[\alpha]_D^T$ were obtained with the D-line of sodium at the indicated temperature *T*, using solutions of concentration (c) in units of g·100 mL^{-1}.

2. Standard synthetic protocols used during the preparation of peptides ***1-12***

General procedure A for the hydrogenolytic cleavage of benzyl esters
To a solution of peptide-OBn (1 eq.) in CH_2Cl_2 (40 mL/mmol) was added 10 % Pd-C (10% w/w, with a minimum quantity of 50 mg). The black suspension was stirred under an H_2 atmosphere until the reaction was complete (TLC monitoring). The mixture was then filtered through a celite pad and washed through with CH_2Cl_2. The filtrate was concentrated under reduced pressure to afford peptide–OH which was directly used in the subsequent coupling reaction without further purification.

General procedure B for the cleavage of tert-butoxycarbonyl protecting goup
To a solution of Boc-peptide (1 eq.) in dry CH_2Cl_2 (25 mL/mmol) at rt under argon was added TFA (30 eq.). The yellowish mixture was stirred until the reaction was complete (TLC monitoring). The mixture was then concentrated under reduced pressure. Toluene was added to co-evaporate the residual TFA. The remaining TFA salt was used directly in the subsequent coupling reaction without further purification.

General procedure C for peptide coupling
To a solution of Boc–peptide–OH (1 eq.) in a mixture of CH_2Cl_2 and DMF (6 mL/mmol) was added DIPEA (2 eq.) followed by HATU (1.05 eq.). The resulting mixture was stirred for 10 min at rt and the solution became brownish. A solution of the appropriate TFA salt partner (1 eq.) and DIPEA (sufficient quantity to reach pH 9) in CH_2Cl_2 (6 mL/mmol) was prepared and added to the reaction mixture which was stirred for the specified time. The mixture was then concentrated under reduced pressure and the

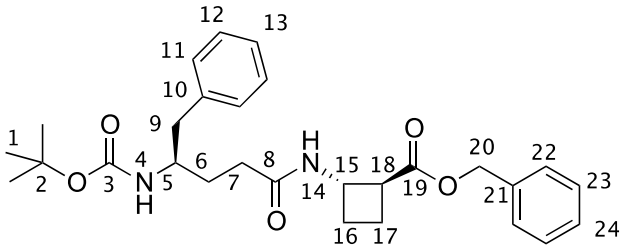
[1] V. Declerck and D. J. Aitken, *Amino Acids*, 2011, **41**, 587.
[2] C. M. Grison, S. Robin and D. J. Aitken, *Chem. Commun.*, 2015, **51**, 16233

crude product was taken up in EtOAc. This solution was washed successively with satd. aq. $NaHCO_3$, brine, 1 M aq. HCl, and brine. The organic solution was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was dried under vacuum then purified by flash chromatography.

*3. Preparation of peptides **1**-**12** and intermediate peptides **I**-**V***

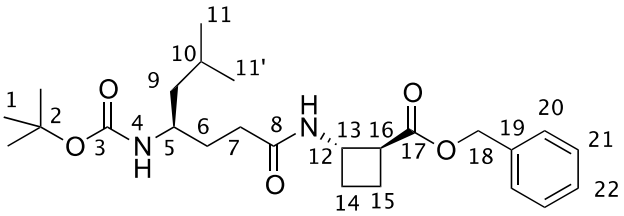
Peptides with γ^4-amino acids at N-terminal

Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (I)



Following the *general procedure B*, Boc–(1*S*,2*S*)-ACBC–OBn (252 mg, 1.17 mmol) was deprotected in 1.5 h to give the corresponding TFA salt, TFA·H_2N–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, a solution of this material and DIPEA (1.195 mL, 906 mg, 7.02 mmol) in CH_2Cl_2 (2 mL) was combined with a solution of Boc–(*R*)-γ^4-Phe–OH (342 mg, 1.17 mmol), DIPEA (400 μL, 302 mg, 2.34 mol) and HATU (461 mg, 1.23 mmol) in CH_2Cl_2/DMF (5 mL/2 mL) and left for 3 d. After work-up, chromatographic purification of the crude product (EtOAC/PE: gradient from 10/90 to 80/20) gave Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) as a sticky white solid (242 mg, 43%). R_f 0.15 (EtOAc/PE: 30/70); $[\alpha]_D^{26}$= –115 (*c.* 0.10, $CHCl_3$); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.39 (s, 9H, 9H-1), 1.56-1.65 (m, 1H, H-6), 1.70-1.82 (m, 1H, H-6'), 1.95-1.99 (m, 3H, H-16, 2H-17), 2.15-2.20 (m, 3H, 2H-7, H-16'), 2.65 (m, 2H, 2H-9), 3.13-3.15 (m, 1H, H-18), 3.81 (m, 1H, H-5), 4.55-4.57 (m, 1H, H-15), 4.79 (d, 1H, *J* = 7.5 Hz, H-4), 5.11 (s, 2H, 2H-20), 7.15 (m, 1H, H-14), 7.12-7.32 (10H, m, 2H-11, 2H-12, H-13, 2H-22, 2H-23, H-24); ^{13}C NMR (90 MHz, $CDCl_3$) δ 18.5 (C-6), 26.9 (C-7), 28.3 (C-1), 30.8 (C-17), 33.2 (C-16), 41.6 (C-9), 46.7 (C-18), 47.5 (C-15), 51.3 (C-5), 66.3 (C-20), 79.3 (C-2), 126.4-129.3 (C-11, C-12, C-13, C-22, C-23, C-24), 136.0 (C-10), 137.9 (C-21), 156.3 (C-3), 172.4 (C-8), 173.0 (C-19); IR ν_{max} 1454, 1505, 1603, 1670, 1697, 1725, 2870, 2981, 3031, 3066, 3087, 3302(br), 3436 cm^{-1}; HRMS (ESI): [M+Na]$^+$, theor. 503.2516 (calc. for $C_{28}H_{36}N_2NaO_5$), meas. 503.2536.

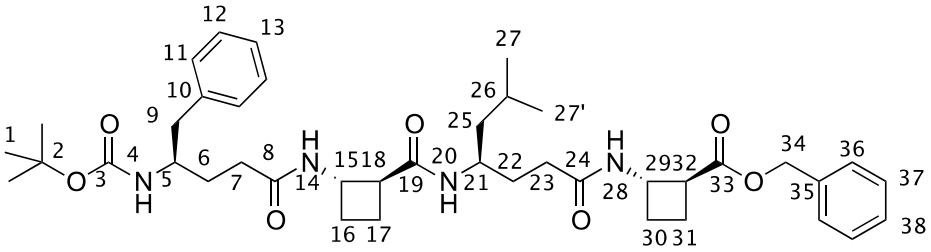
Boc–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (II)



Following the *general procedure B*, Boc–(1*S*,2*S*)-ACBC–OBn (170 mg, 0.55 mmol) was deprotected in 3 h to give the corresponding TFA salt, TFA·H_2N–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, a solution of this material and DIPEA (560 μL, 426 mg, 3.30 mmol) in CH_2Cl_2 (2 mL) was combined with

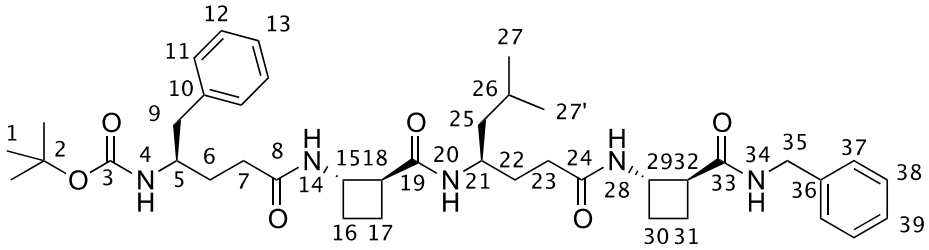
a solution of Boc–(*R*)-γ^4-Leu–OH (161 mg, 0.62 mmol), DIPEA (185 μL, 142 mg, 1.10 mol) and HATU (217 mg, 0.58 mmol) in CH_2Cl_2/DMF (3 mL/1 mL) and left for 3 d. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 80/20) gave Boc–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (**II**) as a sticky white solid (204 mg, 86%). R_f 0.20 (EtOAc/PE: 30/70); $[\alpha]_D^{21}$ = +16 (*c.* 0.35 in $CHCl_3$), $[\alpha]_D^{22}$ = +24 (*c.* 0.35 in CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80 (d, *J* = 6.9 Hz, 3H, 3H-11), 0.82 (d, *J* = 6.9 Hz, 3H, 3H-11'), 1.07 (m, 1H, H-9), 1.20 (m, 1H, H-9'), 1.43 (s, 9H, 9H-1), 1.56 (m, 3H, 2H-10, H-15), 1.69 (m, 1H, H-15'), 2.03 (m, 3H, 2H-6, H-7), 2.19 (m, 3H, H-7', 2H-14), 3.11 (m, 1H, H-16), 3.60 (m, 1H, H-5), 4.49 (d, *J* = 9.5 Hz, 1H, H-4), 4.56 (m, 1H, H-13), 5.09 (s, 2H, 2H-18), 7.31 (m, 5H, 2H-20, 2H-21, H-22), 7.41 (d, *J* = 7.9 Hz, 1H, H-12); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.5 (C-15), 21.8, 22.9 (C-11, C-11'), 24.8 (C-10), 26.5 (C-7), 28.4 (C-1), 32.7 (C-6), 32.9 (C-14), 44.7 (C-9), 46.9 (C-16), 47.1 (C-13), 48.2 (C-5), 66.4 (C-18), 79.5 (C-2), 128.1-128.4 (C-20, C-21, C-22), 135.8 (C-19), 156.8 (C-3), 172.9 (C-8), 173.5 (C-17); IR ν_{max} 1457, 1468, 1507, 1664, 1693, 1726, 2871, 2960, 3154, 3300(br), 3436 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 469.2673 (calc. for $C_{25}H_{38}N_2NaO_5$), meas. 469.2714.

Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (3)



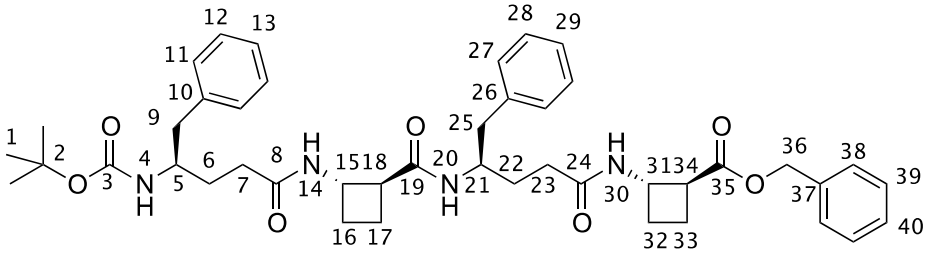
Following the *general procedure A*, Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) (245 mg, 0.51 mmol) was deprotected in 3 h to give the corresponding carboxylic acid, Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (**II**) (198 mg, 0.46 mmol) was deprotected in 2.5 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (155 μL, 116 mg, 0.90 mmol) and HATU (176 mg, 0.47 mmol) in CH_2Cl_2/DMF (2 mL/1 mL) and a solution of TFA·H_2N–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn and DIPEA (1.35 mL, 859 mg, 4.5 mmol) in CH_2Cl_2 (2 mL) for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0) gave Boc–γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)- γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (**3**) as a sticky white solid (225 mg, 71%). R_f 0.64 (EtOAc); $[\alpha]_D^{24}$= +16 (*c.* 0.50, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.76 (d, *J* = 6 Hz, 3H, 3H-27), 0.79 (d, *J* = 6 Hz, 3H, 3H-27'), 1.02-0.93 (m, 1H, H-26), 1.21-1.30 (m, 2H, 2H-25), 1.37-1.56 (m, 13H, 9H-1, 2H-6, 2H-22), 1.78-1.88 (m, 3H, H-16, 2H-23), 1.90-2.03 (m, 6H, 2H-7, H-17, H-30, 2H-31) 2.07-2.29 (m, 3H, H-16', H-17', H-30'), 2.64-2.69 (m, 1H, H-9), 2.76-2.89 (m, 2H, H-9', H-18), 3.17 (m, 1H, H-32), 3.8 (m, 1H, H-5), 3.9 (m, 1H, H-21), 4.41 (m, 1H, H-15), 4.52 (d, *J* = 7.7 Hz, 1H, H-4), 4.61 (m, 1H, H-29), 5.12 (s, 2H, 2H-34), 6.66 (d, *J* = 8.4 Hz, 1H, H-14), 7.11-7.37 (m, 10H, 2H-11, 2H-12, H-13, 2H-36, 2H-37, H-38), 7.52 (d, *J* = 8.4 Hz, 1H, H-20), 8.04 (d, *J* = 6.7 Hz, 1H, H-28); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.3 (C-6), 21.6, 22.6 (C-27, C27'), 24.8 (C-26), 25.3 (C-22), 26.5 (C-7), 28.2 (C-1), 30.7 (C-31), 32.5 (C-17), 33.3, 33.4 (C-16, C-30), 41.9 (C-9), 44.6 (C-25), 46.4 (C-21), 46.9 (C-32), 47.3 (C-29), 48.0 (C-15), 50.0 (C-5, C-18), 66.1 (C-34), 79.6 (C-2), 126.5, 127.8, 127.9, 128.3, 128.4, 129.1 (C-11, C-12, C-13, C-36, C-37, C-38), 135.1 (C-10), 135.9 (C-35), 156.1 (C-3), 172.5 (C-19), 172.7, 172.8 (C-8, C-24), 173.0 (C-33); IR ν_{max} 1507, 1545, 1658, 1700, 1725, 2870, 2958, 3031, 3067, 3279(br), 3342(br), 3434 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 741.4203 (calc. for $C_{41}H_{58}N_4NaO_7$), meas. 741.4201.

Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–NHBn (4)



Following the *general procedure A*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (**3**) (54 mg, 0.076 mmol) was deprotected in 3 h to give the corresponding carboxylic acid, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OH. Following the *general procedure C*, a solution of this material, DIPEA (25 μL, 19 mg, 0.152 mmol) and HATU (30 mg, 0.079 mmol) in CH_2Cl_2/DMF (1 mL/0.5 mL) was treated with benzylamine (12 μL, 12 mg, 0.114 mmol) and DIPEA (60 μL, 44 mg, 0.342 mmol) and left for 3 d. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0) gave Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–NHBn (**4**) as a sticky white foam (35 mg, 64%). R_f 0.60 (EtOAc); $[\alpha]_D^{24}$= +31 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.77 (d, *J* = 6.2 Hz, 3H, 3H-27), 0.78 (d, *J* = 6.3 Hz, 3H, 3H-27'), 1.06-1.14 (m, 1H, H-25), 1.20-1.30 (m, 3H, 2H-22, H-25'), 1.36-1.44 (m, 12H, 9H-1, 2H-6, H-26), 1.85-2.19 (m, 10H, H-16, H-17, 2H-23, 2H-30, 2H-31, 2H-7), 2.20-2.25 (m, 2H, H-16', H-17'), 2.63-2.66 (m, 1H, H-9), 2.72-2.76 (m, 2H, H-9', H-18), 3.05-3.06 (m, 1H, H-32), 3.75-3.82 (m, 1H, H-5), 3.89-3.96 (m, 1H, H-21), 4.35-4.37 (m, 1H, H-29), 4.42-4.43 (m, 1H, H-15), 4.45-4.46 (m, 2H, 2H-35), 4.57 (d, *J* = 9.4 Hz, 1H, H-4), 6.7 (d, *J* = 7.0 Hz, 1H, 14H), 7.09-7.34 (m, 11H, 2H-11, 2H-12, H-13, H-20, 2H-37, 2H-38, H-39), 8.29 (d, *J* = 5.7 Hz, 1H, H-28), 9.04 (m, 1H, H-34); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.9 (C-30), 18.4 (C-31), 22.1, 22.9 (C-27, C-27'), 24.4 (C-23), 24.9 (C-26), 25.7 (C-7), 28.4 (C-1), 29.1 (C-6), 29.7 (C-25), 30.6 (C-22), 32.5, 32.9 (C-16, C-17), 42.2 (C-9), 43.0 (C-35), 46.3 (C-21), 48.1, 48.2 (C-15, C-29), 49.7 (C-32), 50.0 (C-5), 50.2 (C-18), 79.8 (C-2), 126.7, 127.5, 128.4, 128.5, 129.3 (C-11, C-12, C-13, C-37, C-38, C-39), 137.2, 139.3 (C-10, C-36), 156.2 (C-3), 172.3, 172.5, 173.4 (C-8, C-19, C-24), 174.2 (C-33); IR ν_{max} 1442, 1455, 1467, 1509, 1548, 1648, 1699, 2870, 2930, 2959, 3031, 3067, 3281(br), 3333(br), 3434 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 740.4363 (calc. for $C_{41}H_{59}N_5NaO_6$), meas. 740.4361.

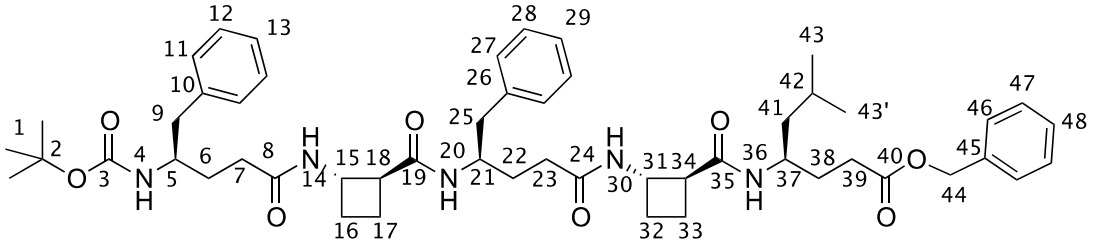
Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (III)



Following the *general procedure A*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) (360 mg, 0.75 mmol) was deprotected in 2 h to give the corresponding carboxylic acid, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) (335 mg, 0.69 mmol) was deprotected in 2 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (260 μL, 193 mg, 1.5 mmol) and HATU (293 mg, 0.78 mmol) CH_2Cl_2/DMF (2 mL/1 mL) and a solution of TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn and DIPEA (710 μL, 536 mg, 4.15 mmol) in CH_2Cl_2/DMF (2 mL/2 mL) for 18 h. After work-up, chromatographic purification

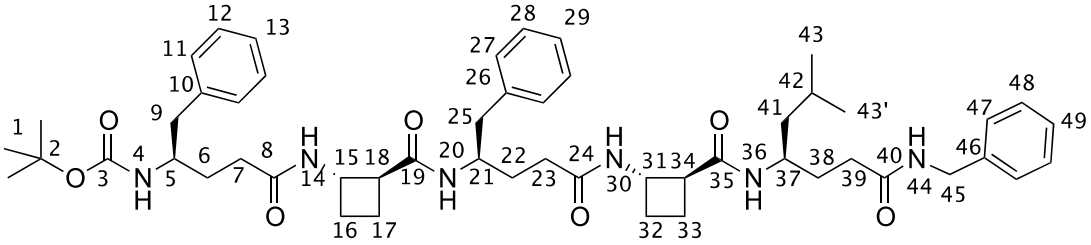
of the crude product (EtOAc/PE: gradient from 10/90 to 100/0) gave Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**III**) as a sticky white solid (450 mg, 79%). R_f 0.60 (EtOAc); $[\alpha]_D^{25}$= +8 (*c.* 0.50, CH_3OH); ^{1}H NMR (360 MHz, $CDCl_3$) δ 1.40 (s, 9H, 9H-1), 1.49-1.53 (m, 4H, 2H-6, 2H-22), 1.82-2.08 (m, 6H, H-16, 2H-17, H-32, 2H-33), 2.08-2.29 (m, 6H, 2H-7, H-16', 2H-23, H-32'), 2.61-2.81 (m, 5H, H-9, H-9', H-25, H-25', H-18), 3.09-3.20 (m, 1H, H-34), 3.80 (bs, 1H, H-5), 4.15 (bs, 1H, H-21), 4.31-4.35 (m, 1H, H-15), 4.56-4.63 (m, 2H, H-4, H-31), 5.11 (s, 2H, 2H-36), 6.90 (bs, 1H, H-14), 7.08-7.38 (m, 15H, 2H-11, 2H-12, H-13, 2H-27, 2H-28, H-29, 2H-38, 2H-39, H-40), 7.6 (d, *J* = 7.2 Hz, 1H, H-20), 7.91 (d, *J* = 5.4 Hz, 1H, H-30); ^{13}C NMR (90 MHz, $CDCl_3$) δ 17.4, 18.7 (C-17, C-33), 25.0 (C-16), 26.6 (C-32), 28.4 (C-1), 30.6, 31.8 (C-6, C-22), 32.6, 33.1 (C-7, C-23), 41.1 (C-25), 42.0 (C-9), 46.8 (C-34), 47.4 (C-31), 48.1 (C-15), 49.8 (C-18, C-21), 50.4 (C-5), 66.4 (C-36), 79.8 (C-2), 126.2, 126.7, 128.1, 128.2, 128.5, 129.1, 129.4, (C-11, C-12, C-13, C-27, C-28, C-29, C-38, C-39, C-40), 136.1, 137.4, 138.4 (C-10, C-26, C-37), 156.3 (C-3), 173.0, 173.2, 173.3 (C-8, C-19, C-24, C-35); IR ν_{max} 1444, 1455, 1509, 1544, 1659, 1699, 1724, 2869, 2955, 2981, 3031, 3067, 3086, 3300(br), 3335(br), 3434 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 775.4047 (calc. for $C_{44}H_{56}N_4NaO_7$), meas. 775.4035.

Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn (7)



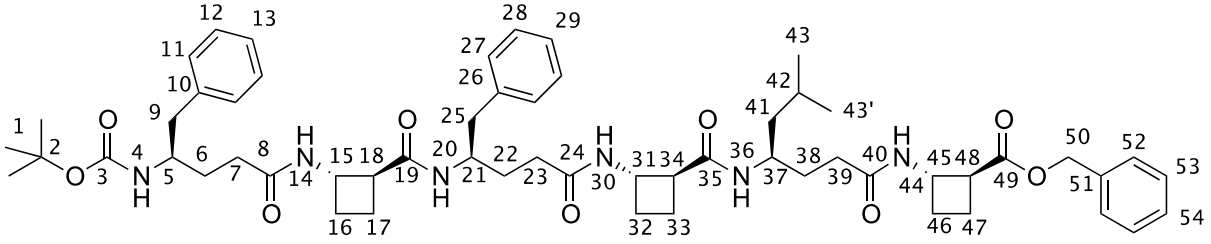
Following the *general procedure A*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**III**) (86 mg, 0.13 mmol) was deprotected in 5 h to give the corresponding carboxylic acid, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–(*R*)-γ⁴-Leu–OBn (110 mg, 0.30 mmol) was deprotected in 6 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ⁴-Leu–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (44 μL, 33 mg, 0.26 mmol) and HATU (53 mg, 0.14 mmol) in CH_2Cl_2/DMF (1 mL/1 mL) and a solution of TFA·H_2N–(*R*)-γ⁴-Leu–OBn, and DIPEA (135 μL, 101 mg, 0.78 mmol) in CH_2Cl_2 (2 mL) for 18 h. After work-up, chromatographic purification of the crude product (CH_3OH/CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn (**7**) as a sticky white solid (75 mg, 65%). R_f0.48 (EtOAc); $[\alpha]_D^{18}$ = +24 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.85 (d, *J* = 4.6 Hz, 3H, 3H-43), 0.86 (d, *J* = 4.6 Hz, 3H, 3H-43'), 1.18-1.22 (m, 2H, 2H-41), 1.30-1.45 (m, 13H, 9H-1, 2H-22, 2H-38), 1.45-1.49 (m, 2H, 2H-6), 1.56-1.59 (m, 1H, H-42),1.73-1.94 (m, 4H, H-7, H-17, H-23, H-32), 1.97-2.01 (m, 3H, H-32', 2H-33), 2.04-2.09 (m, 1H, H-17'), 2.14-2.20 (m, 3H, H-7', H-23', H-39), 2.22-2.31 (m, 3H, 2H-16, H-39'), 2.63-2.74 (m, 5H, 2H-9, H-18, 2H-25), 2.92-2.97 (m, 1H, H-34), 3.77-3.83 (m, 1H, H-5), 3.98-4.03 (m, 1H, H-37), 4.13-4.19 (m, 1H, H-21), 4.27-4.32 (m, 1H, H-31), 4.35-4.43 (m, 1H, H-15), 4.76 (d, *J* = 10.1 Hz, 1H, H-4), 5.11-5.12 (m, 2H, 2H-44), 6.86 (d, *J* = 9.7 Hz, 1H, H-14), 7.07-7.36 (m, 16H, 2H-11, 2H-12, H-13, H-20, 2H-27, 2H-28, H-29, 2H-46, 2H-47, H-48), 8.2 (d, *J* = 7.3 Hz, 1H, H-30), 8.33 (d, *J* = 8.3 Hz, 1H, H-36); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.9 (C-39), 18.0 (C-33), 22.2, 23.2 (C-43, C-43'), 24.4, 25.3, 26.7 (C-23, C-17, C-7), 24.9 (C-42), 28.5 (C-1), 31.0 (C-6), 32.4 (C-32), 32.8 (C-16), 41.5, 42.1 (C-9, C-25), 43.9 (C-22, C-38), 46.5 (C-37), 48.0, 48.1 (C-15, C-31), 48.9 (C-21), 49.9 (C-34), 50.0 (C-18), 50.2 (C-5), 66.1 (C-44), 79.6 (C-2), 126.1, 126.2, 126.5, 128.0, 128.1, 128.1, 128.2, 128.4, 128.5, 129.1, 129.2, 129.4 (C-11, C-12, C-13, C-27, C-28, C-29, C-46, C-47, C-48), 136.1, 137.5, 138.2 (C-10, C-26, C-45), 156.1 (C-3), 172.3 (C-19), 172.4 (C-35), 173.0 (C-40), 173.6, 173.8 (C-8, C-24); IR ν_{max} 1443, 1454, 1509, 1546, 1602, 1653, 1697, 1727, 2869, 2930, 2958, 3031, 3067, 3259(br), 3333(br), 3434 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 916.5200 (calc. for $C_{52}H_{71}N_5NaO_8$), meas. 916.5145.

Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–NHBn (8)



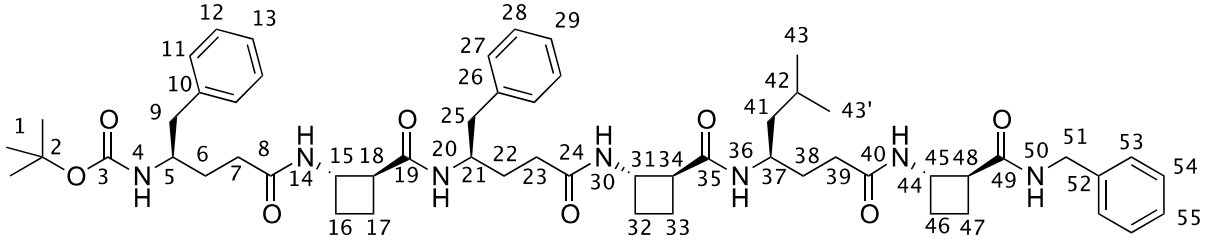
Following to the *general procedure A*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**III**) (141 mg, 0.18 mmol) was deprotected in 6 h to give the corresponding carboxylic acid, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–(*R*)-γ⁴-Leu–NHBn (73 mg, 0.21 mmol) was deprotected in 6 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ⁴-Leu–NHBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (60 μL, 46 mg, 0.36 mmol) and HATU (71 mg, 0.19 mmol) in CH_2Cl_2/DMF (1 mL/0.5 mL) and a solution of TFA·H_2N–(*R*)-γ⁴-Leu–NHBn and DIPEA (185 μL, 139 mg, 1.08 mmol) in CH_2Cl_2 (1 mL) for 18 h. After work-up, chromatographic purification of the crude product (CH_3OH / CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–NHBn (**8**) as a sticky white solid (60 mg, 45%). R_f 0.67 (MeOH/CH_2Cl_2: 10/90); $[\alpha]_D^{19}$ = +30 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.82 (d, *J* = 6.3 Hz, 3H, H-43), 0.86 (d, *J* = 6.4 Hz, 3H, H-43'), 1.12-1.19 (m, 4H, 2H-22, 2H-41), 1.28-1.34 (m, 1H, H-38), 1.43 (s, 9H, H-1), 1.46-1.48 (m, 3H, 2H-6, H-38'), 1.60-1.66 (m, 1H, H-42), 1.70-1.76 (m, 2H, H-17, H-33), 1.85-1.92 (m, 2H, H-16, H-32), 1.94-2.05 (m, 3H, H-7, H-17', H-23), 2.09-2.12 (m, 2H, H-23', H-32'), 2.18-2.23 (m, H-16', H-33'), 2.26-2.34 (m, 2H, H-7', H-39), 2.36-2.42 (m, 1H, H-25), 2.50-2.57 (m, 2H, H-25', H-39'), 2.61-2.69 (m, 1H, H-18), 2.71-2.78 (m, 1H, H-9), 2.74-2.78 (m, 1H, H-9'), 2.89-2.93 (m, 1H, H-34), 3.77-3.83 (m, 1H, H-5), 3.99-4.07 (m, 2H, H-37, H-21), 4.37-4.39 (m, 1H, H-15), 4.40-4.43 (m, 1H, H-31), 4.43-4.46 (m, 2H, H-45), 4.76 (d, *J* = 9.3 Hz, 1H, H-4), 6.59 (d, *J* = 9.5 Hz, 1H, H-20), 6.88 (d, *J* = 7.3 Hz, 1H, H-14), 7.00-7.37 (m, 15H, 2H-11, 2H-12, H-13, 2H-27, 2H-28, H-29, 2H-47, 2H-48, H-49), 7.85 (d, *J* = 9.4 Hz, 1H, H-36), 8.18 (d, *J* = 8.7 Hz, 1H, H-30), 8.5 (t, *J* = 5.0 Hz, 1H, H-44); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.7, 16.9 (C-33, C-17), 22.2, 23.2 (C-43, C-43'), 25.0 (C-42), 25.6, 26.0 (C-16, C-32), 28.5 (C-1), 29.7, 29.8, (C-22, C-41), 30.0 (C-38), 31.9 (C-23), 32.3 (C-7), 33.6 (C-39), 34.0 (C-6), 42.1 (C-9), 42.2 (C-25), 43.4 (C-45), 46.8 (C-37), 47.4 (C-21), 47.7 (C-15), 48.2 (C-31), 49.8, 49.9 (C34, C-5), 50.0 (C-18), 79.8 (C-2), 126.3, 126.6, 127.0, 127.9, 128.1, 128.4, 128.4, 128.4, 129.4, 129.4 (C-11, C-12, C-13, C-27, C-28, C-29, C-47, C48, C-49), 137.4, 137.8, 139.2 (C-10, C-26, C-46), 156.1 (C-3), 171.7 (C-19), 171.9 (C-24), 172.0 (C-8), 172.5 (C-35), 174.5 (C-40); IR ν_{max} 1443, 1454, 1510, 1551, 1604, 1649, 1697, 2870, 2931, 2958, 3031, 3066, 3086, 3317(br), 3434 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 915.5360 (calc. for $C_{52}H_{72}N_6NaO_7$), meas. 915.5312.

Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (11)



Following the *general procedure A*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) (48 mg, 0.10 mmol) was deprotected in 4 h to give the corresponding carboxylic acid, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (**3**) (65 mg, 0.09 mmol) was deprotected in 1 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (65 μL, 49 mg, 0.38 mmol) and HATU (39 mg, 0.11 mmol) in CH_2Cl_2/DMF (1 mL/1 mL) and a solution of TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn and DIPEA (200 μL, 151 mg, 1.17 mmol) in CH_2Cl_2 (2 mL) for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0 then CH_3OH/CH_2Cl_2: gradient from 0/100 to 20/80) to give Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (**11**) as a sticky white solid (30 mg, 30%). R_f 0.50 (MeOH/CH_2Cl_2: 10/90); $[\alpha]_D^{16}$ = +56 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.81 (d, *J* = 6.7 Hz, 3H, 3H-43), 0.83 (d, *J* = 6.7 Hz, 3H, 3H-43'), 1.05-1.08 (m, 2H, 2H-41), 1.24-1.30 (m, 3H, H-22, H-22', H-38), 1.43 (m, 10H, 9H-1, H-38'), 1.49-1.54 (m, 2H, 2H-6), 1.57-1.61 (m, 1H, H-42), 1.74-1.76 (m, 2H, H-17, H-33), 1.89-1.96 (m, 4H, H-16, H-32, H-46, H-47), 1.97-2.04 (m, 4H, H-7, H-17', H-23, H-47'), 2.11-2.19 (m, 4H, H-16', H-32', H-33', H-46'), 2.26-2.28 (m, 1H, H-7'), 2.55 (dd, *J* = 14.3 Hz, *J* = 7.9 Hz, 1H, H-25), 2.67-2.69 (m, 3H, H-9, H-18, H-25'), 2.75-2.77 (dd, *J* = 12.7 Hz, *J* = 5.7 Hz, 1H, H-9)', 2.90-2.94 (m, 1H, H-34), 3.16-3.21 (m, 1H, H-48), 3.82-3.85 (m, 1H, H-5), 4.00-4.02 (m, 1H, H-37), 4.10-4.14 (m, 1H, H-21), 4.38-4.44 (m, 2H, H-15, H-31), 4.60-4.64 (m, 1H, H-45), 4.71-4.73 (d, *J* = 9.6 Hz, 1H, H-4), 5.12 (d, *J* = 12.4 Hz, 1H, H-50), 5.16 (d, *J* = 12.4 Hz, 1H, H-50'), 6.64 (d, *J* = 10 Hz, 1H, H-20), 6.73 (d, *J* = 8.2 Hz, 1H, H-14), 7.86 (d, *J* = 7.4 Hz, 1H, H-36), 8.24 (d, *J* = 8.9 Hz, 1H, H-30), 8.39 (d, *J* = 6.7 Hz, 1H, H-44); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.9 (C-33), 16.9 (C-17), 18.7 (C-47), 22.0, 23.2 (C-43, C-43'), 24.9 (C-42), 25.9 (C-6), 26.5 (C-46), 28.4 (C-1), 29.7, 30.1 (C-38, C-23), 32.1, 32.4 (C-7, C-39), 33.5, 33.8 (C-16, C-32), 42.1 (C-9), 42.4 (C-25), 43.9 (C-41), 46.6 (C-37), 46.9 (C-48), 47.4 (C-45), 47.8 (C-15, C-21), 48.2 (C-31), 49.9, 50.0 (C-5, C-18, C-34), 66.2 (C-50), 79.8 (C-2), 126.4, 127.9, 128.1, 128.2, 128.4, 128.5, 129.4 (C-11, C-12, C-13, C-27, C-28, C-29, C-52, C-53, C-54), 136.0, 137.3, 137.7 (C-10, C-26, C-51), 156.2 (C-3), 171.8 (C-24), 172.0 (C-35), 172.1 (C-19), 172.7 (C-8), 173.4 (C-40), 173.6 (C-49); IR ν_{max} 1443, 1455, 1568, 1509, 1549, 1602, 1649 1651, 1697, 1724, 2869, 2957, 3030, 3066, 3318(br), 3433 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 1013.5727 (calc. for $C_{57}H_{78}N_6NaO_9$), meas. 1013.5722.

Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–NHBn (12)

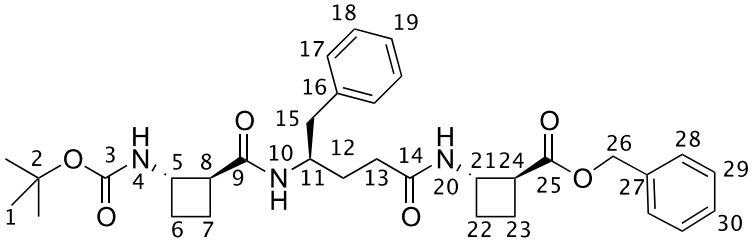


Following the *general procedure A*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (**11**) (70 mg, 0.070 mmol) was deprotected in 24 h to give the corresponding carboxylic acid, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OH. Following the *general procedure C*, Following the *general procedure C*, a solution of this material, DIPEA (20 μL, 13 mg, 0.112 mmol) and HATU (22 mg, 0.058 mmol) in a mixture of CH_2Cl_2 /DMF (1 mL/1 mL) was treated with benzylamine (9 μL, 9 mg, 0.084 mmol) and DIPEA (22 μL, 16 mg, 0.140 mmol) and left for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0 then CH_3OH/CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–NHBn (**12**) as a sticky yellow foam (25 mg, 50%). R_f 0.42 (MeOH/CH_2Cl_2: 10/90); $[\alpha]_D^{18}$= +46 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.82 (d, *J*

= 6.4 Hz, 3H, 3H-43), 0.82 (d, *J* = 6.4 Hz, 3H, 3H-43'), 1.13-1.17 (m, 1H, H-38), 1.28-1.32 (m, 2H, H-22, H-38'), 1.38-1.43 (m, 13H, 9H-1, 2H-6, 2H-41), 1.57-1.64 (m, 1H, H-42), 1.73-1.79 (m, 2H, H-17, H-33), 1.90-1.93 (m, 2H, H-16, H-32), 2.01-2.13 (m, 5H, H-17', H-23, H-39, H-46, H-47), 2.17-2.25 (m, 6H, H-7, H-16', H-23', H-32', H-33', H-39'), 2.29-2.31 (m, 3H, H-7', H-46', H-47'), 2.57-2.60 (m, 2H, 2H-25), 2.64-2.69 (m, 2H, H-9, H-18), 2.77 (dd, *J* = 13.9 Hz, *J* = 5.4 Hz, 1H, H-9'), 2.88-2.93 (m, 1H, H-34), 3.09-3.13 (m, 1H, H-48), 3.81-3.87 (m, 1H, H-5), 3.98-4.04 (m, 1H, H-37), 4.10-4.16 (m, 1H, H-21), 4.30-4.35 (m, 1H, H-45), 4.40-4.44 (m, 1H, H-15), 4.46-4.49 (m, 1H, H-31), 4.50-4.54 (m, 2H, 2H-51), 4.68 (d, *J* = 10.2 Hz, H-4), 6.72 (d, *J* = 9.8 Hz, 1H, H-20), 6.8 (d, *J* = 8.1 Hz, 1H, H-14), 7.04-7.37 (m, 15H, 2H-11, 2H-12, H-13, 2H-27, 2H-28, H-29, 2H-53, 2H-54, H-55), 7.64 (d, *J* = 9.3 Hz, 1H, H-36), 8.29 (d, *J* = 7.6 Hz, 1H, H-30), 8.54 (d, *J* = 6.5 Hz, 1H, H-44), 9.15 (t, *J* = 5.3 Hz, 1H, H-50); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.8 (C-33), 16.9 (C-17), 18.3, 24.3 (C-23, C-39), 22.4, 23.0 (C-43, C-43'), 25.0 (C-42), 25.8, 26.0 (C-16, C-32), 28.5 (C-1), 29.4 (C-22), 30.0 (C-6), 31.9 (C-7), 32.3 (C-41), 32.5, 32.8 (C-46, C-47), 42.1 (C-9), 42.7 (C-25), 43.0 (C-51), 44.8 (C-38), 46.0 (C-37), 47.8 (C-21), 48.1 (C-15), 48.1, 48.1 (C-31, C-45), 49.7 (C-47), 49.9, 49.9 (C-5, C-34), 50.1 (C-18), 79.8 (C-2), 126.4, 126.7, 126.7, 127.4, 128.2, 128.3, 128.4, 129.4, 129.4 (C-11, C-12, C-13, C-27, C-28, C-29, C-53, C-54, C-55), 137.3, 137.6 (C-10, C-26), 139.4 (C-52), 156.2 (C-3), 171.2 (C-19), 171.8 (C-24), 172.0 (C-8), 172.2 (C-35), 173.7 (C-49), 174.4 (C-40); IR ν_{max} 1443, 1455, 1509, 1554, 1603, 1648, 1696, 2870, 2930, 2958, 3032, 3067, 3086, 3310(br), 3433 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 1012.5882 (calc. for $C_{57}H_{79}N_7NaO_8$), meas. 1012.5820.

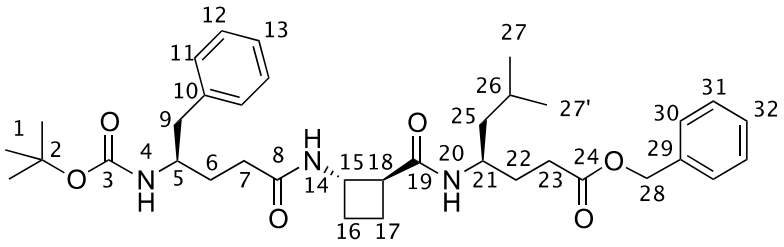
Peptides with tACBC at N-terminal

Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (IV)



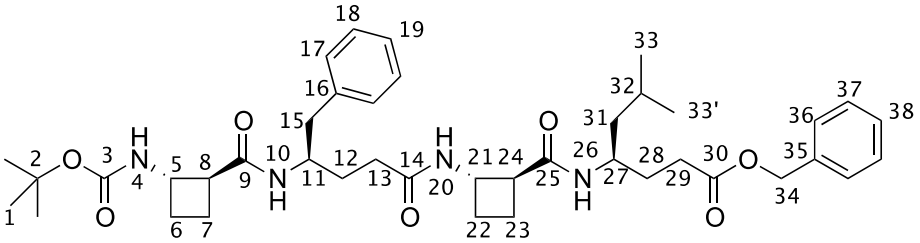
Following the *general procedure B*, Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) (240 mg, 0.50 mmol) was deprotected in 4 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, a solution of this material and DIPEA (515 μL, 387 mg, 3.00 mmol) in CH_2Cl_2 (2 mL) was combined with a solution of Boc–(1*S*,2*S*)-ACBC–OH (108 mg, 0.50 mmol), DIPEA (170 μL, 129 mg, 1.00 mmol) and HATU (197 mg, 0.53 mmol) in CH_2Cl_2/DMF (2 mL/1 mL) and left for 18 h. After work-up, chromatographic purification of the crude product (CH_3OH / CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (**IV**) as a sticky pale yellow solid (125 mg, 43%). R_f 0.77 (CH_3OH/CH_2Cl_2: 10/90); $[\alpha]_D^{29}$= +30 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.44 (s, 9H, 9H-1), 1.63-1.89 (m, 5H, 2H-6, H-7, 2H-12), 1.97-2.07 (m, 3H, H-7', H-22, H-23), 2.15-2.28 (m, 4H, H-22', H-23', 2H-13), 2.54-2.57 (m, 2H, 2H-15), 2.70-2.82 (m, 1H, H-8), 3.13-3.24 (m, 1H, H-24), 4.08-4.17 (m, 2H, H-5, H-11), 4.55-4.68 (m, 1H, H-21), 5.08 (d, *J* = 12.8 Hz, 1H, H-26), 5.17 (d, *J* = 12.8 Hz, 1H, H-26'), 5.19 (d, *J* = 5.6 Hz, 1H, H-4), 7.03-7.33 (m, 10H, 2H-17, 2H-18, H-19, 2H-28, 2H-29, H-30), 7.55 (d, *J* = 8.8 Hz, 1H, H-10), 7.77 (d, *J* = 8.1 Hz, 1H, H-20); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.3 (C-12), 24.9, 26.7 (C-22, C-23), 28.4 (C-1), 31.8, (C-6, C-7), 33.1 (C-13), 41.2 (C-15), 46.9 (C-24), 47.6 (C-21), 48.9, 49.6 (C-5, C-11), 50.0 (C-8), 66.3 (C-26), 80.4 (C-2), 126.2, 128.0, 128.1, 128.2, 128.5, 129.0, 129.3 (C-17, C-18, C-19, C-28, C-29, C-30), 136.1 (C-16), 138.1 (C-27), 156.1 (C-3), 172.6 (C-14), 173.1 (C-9), 173.8 (C-25); IR ν_{max} 1456, 1499, 1552, 1664, 1691, 1727, 2875, 2953, 2982, 3032, 3068, 3090, 3276(br), 3355(br), 3445 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 600.3044 (calc. for $C_{33}H_{43}N_3NaO_6$), meas. 600.3038.

Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (V)



Following the *general procedure A*, Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (**I**) (399 mg, 0.83 mmol) was deprotected in 5.5 h to give the corresponding carboxylic acid, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–OH. Following the *general procedure B*, Boc–(*R*)-γ^4-Leu–OBn (275 mg, 0.79 mmol) was deprotected in 5 h to give the corresponding TFA salt, TFA·H$_2$N–(*R*)-γ^4-Leu–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–OH, DIPEA (270 μL, 204 mg, 1.58 mmol) and HATU (311 mg, 0.83 mmol) in CH_2Cl_2/DMF (5 mL/1 mL) and a solution of TFA·H$_2$N–(*R*)-γ^4-*h*Leu–OBn and DIPEA (810 μL, 611 mg, 4.74 mmol) in CH_2Cl_2 (2 mL) for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0) gave Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (**V**) as a sticky white solid (340 mg, 69%). R_f 0.70 (CH_3OH/CH_2Cl_2: 10/90); $[\alpha]_D^{20}$= –9 (*c*. 0.50, CH_3OH)**;** ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.74 (d, *J* = 2.9 Hz, 3H, 3H-27), 0.77 (d, *J* = 3.0 Hz, 3H, 3H-27'), 1.11-1.13 (m, 11H, 9H-1, 2H-22), 1.41-1.51 (m, 4H, 2H-6, 2H-25), 1.57-1.66 (m, 1H, H-26), 1.73-1.85 (m, 4H, H-7, H-16, H-17, H-25'), 1.93-2.14 (m, 3H, H-7', H-16', H17'), 2.27-2.39 (m, 2H, 2H-23), 2.63-2.65 (m, 2H, 2H-9), 2.77-2.86 (m, 1H, H-18), 3.71 (bs, 1H, H-5), 3.81-3.91 (m, 1H, H-21), 4.18-4.25 (m, 1H, H-15), 4.58 (d, *J* = 9.3 Hz, 1H, H-4), 5.02 (m, 2H, 2H-28), 6.98 (d, *J* = 7.2 Hz, 1H, H-14), 7.03-7.28 (m, 10H, 2H-11, 2H-12, H-13, 2H-30, 2H-31, H-32), 7.78 (d, *J* = 8.7 Hz, 1H, H-20); ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 18.6, 24.5 (C-16, C-17), 22.1, 23.1 (C-27, C-27'), 24.9 (C-26), 28.3 (C-1), 30.8, 31.0 (C-6, C-23, C-25), 32.6 (C-7), 41.7 (C-9), 43.9 (C-22), 46.7 (C-21), 47.8 (C-15), 49.7 (C-18), 50.7 (C-5), 66.3 (C-28), 79.6 (C-2), 126.4, 126.9, 127.4, 128.1, 128.4, 128.4, 129.0, 129.2 (C-11, C-12, C-13, C-30, C-31, C-32), 135.8, 137.5 (C-10, C-29), 156.2 (C-3), 173.1, 173.7, 173.9 (C-8, C-19, C-24); IR ν_{max} 1455, 1506, 1563, 1603, 1645, 1694, 1727, 2839, 2870, 1946, 3031, 3067, 3267(br), 3435 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 644.3670 (calc. for $C_{36}H_{51}N_3NaO_6$), meas. 644.3680.

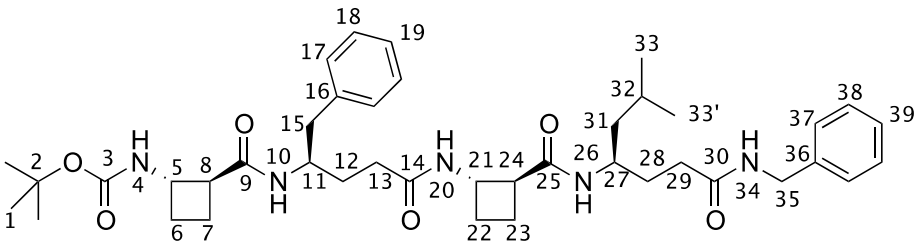
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (1)



Following the *general procedure A*, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (**IV**) (92 mg, 0.16 mmol) was deprotected in 3 h to give the corresponding carboxylic acid, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–(*R*)-γ^4-Leu–OBn (70 mg, 0.20 mmol) was deprotected in 1 h to give the corresponding TFA salt, TFA·H$_2$N–(*R*)-γ^4-Leu–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (45 μL, 33 mg, 0.28 mmol) and HATU (56 mg, 0.15 mmol) in CH_2Cl_2/DMF (2 mL/0.5 mL) and a solution of TFA·H$_2$N–(*R*)-γ^4-Leu–OBn and DIPEA (135 μL, 100 mg, 0.84 mmol) in CH_2Cl_2 (1 mL) for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE:

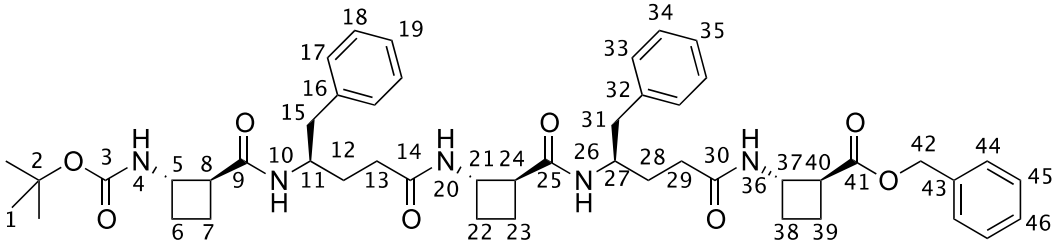
gradient from 10/90 to 100/0) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (**1**) as a sticky white solid (70 mg, 70%). R_f 0.64 (CH_3OH/CH_2Cl_2: 10/90); $[\alpha]_D^{18}$= +18 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.86 (d, *J* = 6.3 Hz, 3H, 3H-33), 0.88 (d, *J* = 6.3 Hz, 3H, 3H-33'), 1.19-1.23 (m, 1H, H-31), 1.37-1.42 (m, 10H, 9H-1, H-31'), 1.48-1.55 (m, 2H, 2H-12), 1.57-1.62 (m, 1H, H-32), 1.71-1.78 , (m, 3H, H-6, 2H-28), 1.84-1.97 (m, 6H, 2H-7, H-22, 2H-23, H-13), 2.10-2.21 (m, 3H, H-6', H-22', H-13'), 2.50-2.55 (m, 2H, 2H-29), 2.65-2.69 (m, 2H, H-8, H-15), 2.78 (dd, *J* = 13.8 Hz, 5.3 Hz, 1H, H-15'), 3.03-3.08 (m, 1H, H-24), 4.00-4.06 (m, 1H, H-27), 4.13-4.19 (m, 1H, H-11), 4.19-4.24 (m, 1H, H-5), 4.32-4.37 (m, 1H, H-21), 5.12-5.14 (m, 2H, 2H-34), 5.2 (d, *J* = 6.6 Hz, 1H, H-4), 6.75 (d, *J* = 9.0Hz, 1H, H-10), 7.10-7.39 (m, 10H, 2H-17, 2H-18, H-19, 2H-36, 2H-37, H-38), 7.88-7.89 (m, 1H, H-20), 8.25 (d, *J* = 8.3 Hz, 1H, H-26); ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.1, 18.1 (C-7, C-23), 22.1, 23.2 (C-33, C-33'), 24.4 (C-22), 25.0 (C-32), 25.3 (C-6), 28.3 (C-1), 30.9, 31.0 (C-28, C-29), 31.3 (C-12), 32.1 (C-13), 41.6 (C-15), 43.8 (C-31), 47.0 (C-27), 48.2 (C-21), 48.5, 48.7 (C-5, C-11), 49.4 (C-24), 50.2 (C-8), 66.2 (C-34), 80.3 (C-2), 126.4, 128.1, 128.2, 128.3, 128.5, 129.2, (C-17, C-18, C-19, C-36, C-37, C-38), 136.2, 137.8 (C-16, C-35), 155.8 (C-3), 172.9, 173.4, 173.6, 173.7 (C-9, C-14, C-25, C-30); IR ν_{max} 1455, 1499, 1564, 1649, 1693, 1729, 2871, 2959, 3032, 3068, 3265(br), 3348(br), 3445 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 741.4211 (calc. for $C_{41}H_{58}N_4NaO_7$), meas. 741.4203.

Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (2)



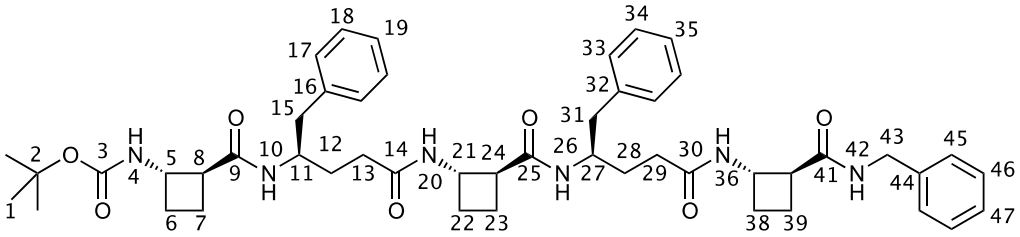
Following the *general procedure A*, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (**1**) (21 mg, 0.030 mmol) was deprotected in 8 h to give the corresponding carboxylic acid, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OH. Following the *general procedure C*, a solution of this material, DIPEA (8 μL, 6 mg, 0.052 mmol) and HATU (10 mg, 0.026 mmol) in CH_2Cl_2/DMF (1 mL/0.5 mL) was treated with benzylamine (7 μL, 7 mg, 0.063 mmol) and DIPEA (23 μL, 18 mg, 0.150 mmol) and left for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0 then CH_3OH/CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (**2**) as a sticky yellow foam (7 mg, 39%). R_f 0.68 (CH_3OH/CH_2Cl_2: 10/90); $[\alpha]_D$: insufficient solubility; ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.84 (d, *J* = 6.6 Hz, 3H, 3H-33), 0.86 (d, *J* = 6.6 Hz, 3H, 3H-33'), 1.11-1.15 (m, 4H, 2H-12, 2H-28), 1.18-1.31 (m, 1H, H-31), 1.42-1.45 (m, 10H, 9H-1, H-31'), 1.62-1.69 (m, 1H, H-32), 1.74-1.80 (m, 4H, H-6, H-7, H-13, H-23), 1.83-1.91 (m, 2H, H-7', H-22), 1.94-2.07 (m, 2H, H-13', H-22'), 2.10-2.14 (m, 1H, H-23'), 2.21-2.53 (m, 1H, H-6'), 2.31-2.36 (m, 1H, H-29), 2.42 (dd, *J* = 13.8 Hz, *J* = 7.0 Hz, 1H, H-15), 2.45-2.50 (m, 1H, H-29'), 2.52-2.56 (m, 1H, H-8), 2.62 (dd, *J* = 13.6 Hz, *J* = 4.1Hz, 1H, H-15'), 2.93-2.97 (m, 1H, H-24), 4.06 (bs, 2H, H-11, H-27), 4.23-4.28 (m, 1H, H-5), 4.44-4.52 (m, 3H, H-21, 2H-35), 5.06 (d, *J* = 6.8 Hz, 1H, H-4), 5.54 (d, *J* = 10.3 Hz, 1H, H-10), 6.99-7.40 (m, 10H, 2H-17, 2H-18, H-19, 2H-37, 2H-38, H-39), 7.65 (d, *J* = 9.2 Hz, 1H, H-26), 7.81 (d, *J* = 9.5 Hz, 1H, H-20), 8.29 (t, *J* = 5.6 Hz, 1H, H-34); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.7, 26.0 (C-13, C-23), 17.9 (C-7), 22.2, 23.2 (C-33, C-33'), 25.0 (C-32), 25.9 (C-6), 28.4 (C-1), 29.7, 30.0 (C-12, C-28), 31.7 (C-22), 33.6 (C-29), 41.6 (C-15), 43.4 (C-35), 44.3 (C-31), 46.7 (C-27), 47.3 (C-11), 48.2 (C-21), 48.8 (C-5), 49.7 (C-24), 50.7(C-8), 80.4 (C-2), 126.6, 126.9, 128.1, 128.3, 128.4, 129.5 (C-17, C-18, C-19, C-37, C-38, C-39), 137.1, 139.4 (C-16, C-36), 155.3 (C-3), 171.4, 171.8, 172.4 (C-9, C-14, C-25), 174.1 (C-30); IR ν_{max} 1443, 1455, 1499, 1511, 1554, 1603, 1650, 1699, 2869, 2929, 2958, 3030, 3065, 3260, 3332(br), 3431, 3443 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 740.4358 (calc. for $C_{41}H_{59}N_5NaO_6$), meas. 740.4350.

Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (5)



Following the *general procedure B*, Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**III**) (175 mg, 0.23 mmol) was deprotected in 2.5 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn. Following the *general procedure C*, a solution of this material and DIPEA (215 μL, 164 mg, 1.38 mmol) in CH_2Cl_2 (1 mL) was combined with a solution of Boc–(1*S*,2*S*)-ACBC–OH (50 mg, 0.23 mmol), DIPEA (75 μL, 53 mg, 0.46 mmol) and HATU (91 mg, 0.24 mmol) in CH_2Cl_2/DMF (1 mL/0.5 mL) and left for 3 d. After work-up, chromatographic purification of the crude product (CH_3OH / CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**5**) as a sticky pale yellow solid (85 mg, 44%). R_f 0.65 (CH_3OH/CH_2Cl_2: 10/90); $[\alpha]_D^{22}$= +32 (*c.* 0.50, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.36-1.44 (m, 13H, 9H-1, 2H-12, 2H-28), 1.68-1.71 (m, 1H, H-23), 1.81-1.84 (m, 1H, H-7), 1.87-1.94 (m, 3H, H-6, H-7', H-22), 1.99-2.07 (m, 4H, H-29, 2H-38, H-39), 2.08-2.25 (m, 6H, H-6', 2H-13, H-22', H-23', H-29'), 2.37-2.41 (m, 1H, H-38'), 2.56-2.62 (m, 3H, H-8, H-15, H-32), 2.69-2.74 (m, 2H, H-15', H-32'), 2.94-2.99 (m, 1H, H-24), 3.18-3.22 (m, 1H, H-40), 4.15 (bs, 1H, H-11), 4.20-4.23 (m, 1H, H-27), 4.29-4.32 (m, 1H, H-5), 4.49-4.52 (m, 1H, H-21), 4.60-4.62 (m, 1H, H-37), 5.12 (d, *J* = 12.8 Hz, 1H, H-42), 5.16 (d, *J* = 12.6 Hz, 1H, H-42'), 5.8 (d, *J* = 7.5 Hz, 1H, H-4), 6.28 (d, *J* = 9.4 Hz, 1H, H-10), 7.08-7.36 (m, 15H, 2H-17, 2H-18, H-19, 2H-34, 2H-35, H-36, 2H-44, 2H-45, H-46), 7.88 (d, *J* = 9.3 Hz, 1H, H-26), 7.99 (d, *J* = 9.6 Hz, 1H, H-20), 8.33 (d, *J* = 7.3 Hz, 1H, H-36); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.3 (C-23), 16.8 (C-7), 17.9 (C-39), 24.7 (C-6), 25.2 (C-22), 25.7 (C-29), 27.7 (C-1), 29.3 (C-12, C-28), 31.0 (C-13), 32.3 (C-6, C-22), 32.7 (C-38), 40.1 (C-32), 41.2 (C-15), 46.1 (C-40), 46.8 (C-37), 46.7 (C-11), 47.2 (C-21), 47.9 (C-5), 48.3 (C-24), 48.9 (C-8), 49.0 (C-27), 65.1 (C-42), 78.5 (C-2), 125.1, 125.4, 127.0, 127.1, 127.2, 127.3, 127.6, 128.5, 128.7 (C-17, C-18, C-19, C-33, C-34, C-35, C-44, C-45, C-46), 135.4, 137.2, 138.4 (C-16, C-32, C-43), 156.8 (C-3), 170.6 (C-14), 171.3 (C-25), 171.4 (C-9), 172.3 (C-30, C-41); IR ν_{max} 1443, 1455, 1497, 1512, 1555, 1603, 1650, 1698, 1724, 2870, 2980, 3031, 367, 3086, 3329(br), 3428 , 3443 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 872.4574 (calc. for $C_{49}H_{63}N_5NaO_8$), meas. 872.4528.

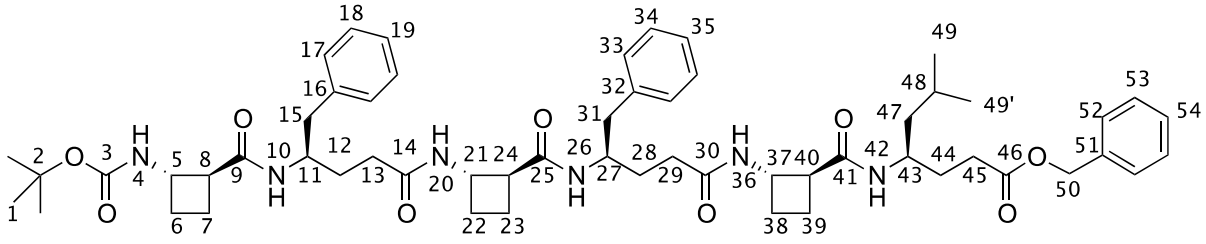
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–NHBn (6)



Following the *general procedure A*, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**5**) (26 mg, 0.030 mmol) was deprotected in 24 h to give the corresponding carboxylic acid, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure C*, a solution of this material, DIPEA (8 μL, 6 mg, 0.054 mmol) and HATU (11 mg, 0.029 mmol) in CH_2Cl_2/DMF (1 mL/0.5 mL) was treated with benzylamine (7 μL, 7 mg, 0.068 mmol) and DIPEA (25

μL, 19 mg, 0.162 mmol) and left for 3 d. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0 then CH_3OH/CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–NHBn (**6**) as a sticky yellow solid (17 mg, 74%). R_f 0.63 (CH_3OH/CH_2Cl_2: 10/90)**;** $[a]_D^{27}$= +20 (*c.* 0.50, CH_3OH); ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.27-1.43 (m, 13H, 9H-1, 2H-12, 2H-28), 1.67-1.72 (m, 1H, H-7), 1.83-1.90 (m, 2H, H-6, H-7'), 1.98-2.32 (m, 13H, H-6', H-13, H-13', H-22, H-22', 2H-23, H-29, H-29', H-38, H-38', H-39, H-39'), 2.47 (dd, *J* = 13.8 Hz, *J* = 7.3 Hz, 1H, H-32), 2.56 (dd, *J* = 13.6 Hz, *J* = 5.5 Hz, 1H, H-32'), 2.63-2.77 (m, 3H, H-8, 2H-15), 2.93-2.97 (m, 1H, H-24), 3.12-3.16 (m, 1H, H-40), 4.15 (m, 2H, H-11, H-27), 4.26-4.30 (m, 1H, H-5), 4.37-4.38 (m, 1H, H-43), 4.40-4.41 (m, 1H, H-37), 4.43-4.46 (m, 1H, H-21), 4.63 (dd, *J* = 15.3 Hz, *J* = 5.9 Hz, 1H, H-43'), 5.88 (bs, 1H, H-4), 6.42 (bs, 1H, H-10), 7.01-7.38 (m, 15H, 2H-17, 2H-18, H-19, 2H-34, 2H-35, H-36, 2H-45, 2H-46, H-47), 7.8 (d, *J* = 6.5 Hz, 1H, H-26), 8.06 (d, *J* = 8.9 Hz, 1H, H-20), 8.56 (bs, 1H, H-31), 9.16 (bs, 1H, H-42); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.7 (C-23), 17.6 (C-7), 18.0 (C-39), 24.6 (C-6), 25.8 (C-22), 26.1 (C-29), 28.4 (C-1), 28.5 (C-12), 29.7 (C-28), 29.7 (C-13), 30 (C-6), 31.2 (C-22), 32.7 (C-38), 41.7 (C-32), 42.0 (C-15), 43.0 (C-46), 47.4 (C-37), 48.1 (C-11), 48.2 (C-21), 48.5 (C-5), 48.9 (C-24), 49.5 (C-8), 49.7 (C-27), 70.5 (C-43), 80 (C-2), 126.0, 126.5, 126.8, 127.4, 128.0, 128.3, 128.4, 129.4, (C-17, C-18, C-19, C-33, C-34, C-35, C-45, C-46, C-47), 137.5, 138.5, 139.2 (C-16, C-32, C-44), 155.6 (C-3), 171.6, 172.1, 172.4, 173.8, 173.9 (C-9, C-14, C-25, C-30, C-41); IR ν_{max} 1443, 1455, 1497, 1512, 1554, 1603, 1650, 1698, 2858, 2930, 2956, 3031, 3066, 3320(br), 3429, 3443 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 871.4729 (calc. for $C_{49}H_{64}N_6NaO_7$), meas. 871.4577.

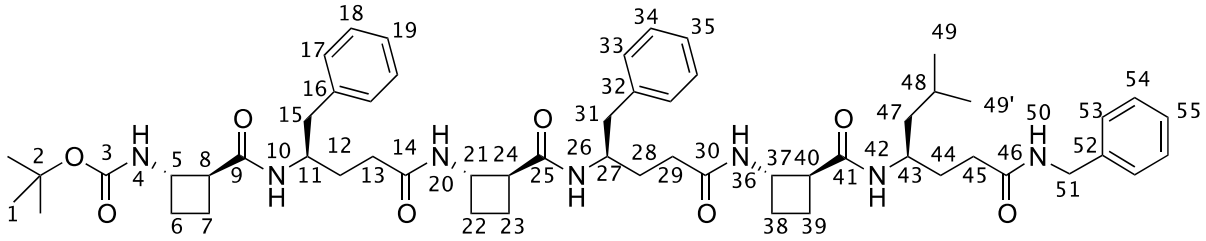
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn (9)



Following the *general procedure A*, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (**IV**) (125 mg, 0.22 mmol) was deprotected in 3 h to give the corresponding carboxylic acid, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH. Following the *general procedure B*, Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn (**V**) (186 mg, 0.30 mmol) was deprotected in 1 h to give the corresponding TFA salt, TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn. Following the *general procedure C*, the coupling reaction was performed between a solution of Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OH, DIPEA (75 μL, 57 mg, 0.44 mmol) and HATU (87 mg, 0.23 mmol) in CH_2Cl_2/DMF (2 mL/1 mL) and a solution of TFA·H_2N–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn and DIPEA (375 μL, 234 mg, 2.20 mmol) in CH_2Cl_2 (2 mL) for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn (**9**) as a sticky white solid (114 mg, 52%). R_f 0.56 (CH_3OH/CH_2Cl_2: 10/90); $[a]_D^{27}$= +36 (*c*. 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$) δ 0.79 (d, *J* = 6.1 Hz, 6H, 3H-49, 3H-49'), 1.11-1.16 (m, 1H, H-47), 1.29-1.37 (m, 14H, 9H-1, 2H-12, 2H-28, H-47'), 1.51-1.60 (m, 2H, H-23, H-48), 1.65-1.85 (m, 6H, H-6, 2H-7, H-38, 2H-44), 1.87-1.94 (m, 3H, H-22, H-38', H-39), 1.98-2.18 (m, 8H, H-6', 2H-13, H-22', H-23', 2H-29, H-39'), 2.44-2.51 (m, 3H, H-31, 2H-45), 2.55-2.62 (m, 3H, 2H-15, H-31'), 2.67-2.71 (m, 1H, H-8), 2.84-2.89 (m, 2H, H-24, H-40), 3.90 (bs, 1H, H-43), 4.00 (bs, 1H, H-11), 4.09 (bs, 1H, H-27), 4.16-4.22 (m, 2H, H-5, H-37), 4.33-4.38 (m, 1H, H-21), 5.04 (m, 2H, H-50), 6.40 (d, *J* = 7.9 Hz, 1H, H-4), 6.91 (d, *J* = 9.4 Hz, 1H, H-10), 6.98-7.28 (m, 15H, 2H-17, 2H-18, H-19, 2H-33, 2H-34, H-35, 2H-52, 2H-53, H-54), 7.74 (d, *J* = 9 .9 Hz, 1H, H-26), 8.04 (d, *J* = 9.9 Hz, 1H, H-20), 8.29 (d, *J* = 8.8 Hz, 1H, H-42), 8.39 (d, *J* = 6.6 Hz, 1H, H-36); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.2 (C-23), 17.4 (C-7), 18.1 (C-39), 22.1, 23 .1 (C-49, C-49'), 24.1

(C-22), 24.8 (C-48), 25.7 (C-6), 28.4 (C-1), 29.7 (C-12 or C-28, C-38), 30.8, 30.9 (C-44 or C-28, C-45), 31.5 (C-24), 31.7 (C-29), 32.7 (C-13), 41.4 (C-31), 42.0 (C-15), 43.8 (C-47), 46.4 (C-43), 47.2 (C-11), 47.8 (C-37), 47.9 (C-21), 48.6 (C-27), 48.7 (C-5), 49.2 (C-8), 49.9 (C-40), 66.0 (C-50), 79.6 (C-2), 125.8, 126.2, 127.9, 128.0, 128.1, 128.4, 129.2, 129.3 (C-17, C-18, C-19, C-33, C-34, C-35, C-52, C-53, C-54), 136.0, 137.6, 138.7 (C-16, C-32, C-51), 155.5 (C-3), 171.5 (C-14), 172.0 (C-9, C-25), 173.1 (C-41), 173.7 (C-30), 173.9 (C-46); IR ν_{max} 1455, 1498, 1514, 1558, 1602, 1649, 1698, 1735, 2876, 2932, 2959, 3028, 3068, 3330(br), 3429, 3446 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 1013.5722 (calc. for $C_{57}H_{78}N_6NaO_9$), meas. 1013.5743.

Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (10)

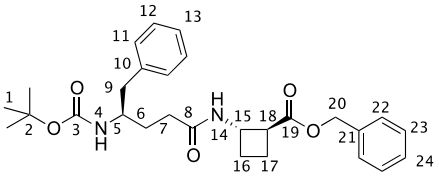


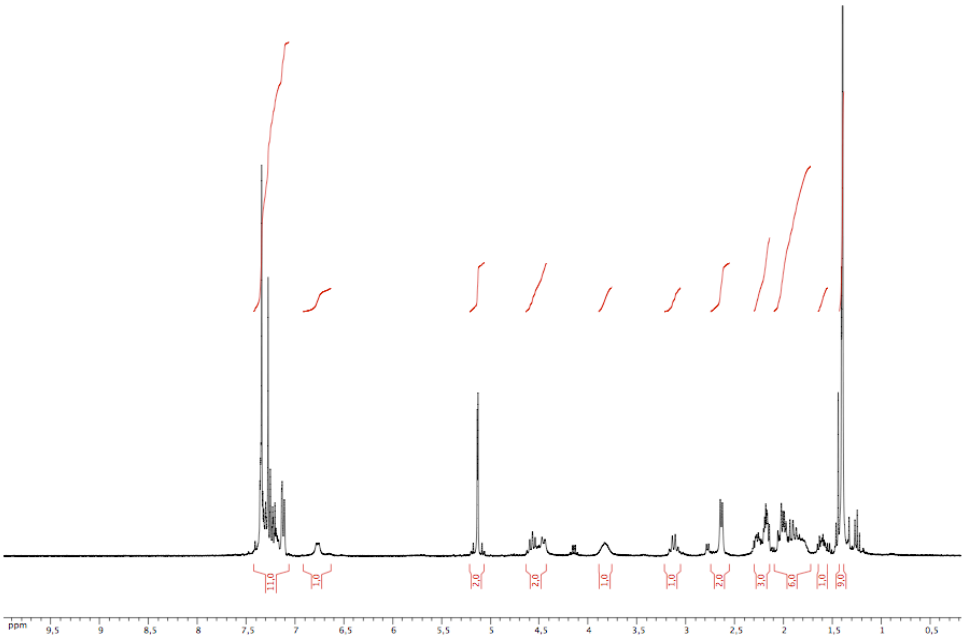
Following the *general procedure A*, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (**9**) (70 mg, 0.07 mmol) was deprotected in 24 h to give the corresponding carboxylic acid, Boc–(1*S*,2*S*)-ACBC–(*R*)-γ ^{4}-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OH. Following the *general procedure C*, a solution of this material, DIPEA (15 μL, 11 mg, 0.088 mmol) and HATU (17 mg, 0.046 mmol) in CH_2Cl_2/DMF (1 mL/1 mL) was treated with benzylamine (5 μL, 5 mg, 0.046 mmol, 1.05 eq) and DIPEA (15 μL, 11 mg, 0.88 mmol) and left for 18 h. After work-up, chromatographic purification of the crude product (EtOAc/PE: gradient from 10/90 to 100/0 then CH_3OH/CH_2Cl_2: gradient from 0/100 to 10/90) gave Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (**10**) as a sticky pale yellow solid (17 mg, 38%). R_f 0.43 (CH_3OH/CH_2Cl_2: 10/90); $[\alpha]_D^{20}$= +44 (*c.* 0.50, $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$/DMSO-d_6: 5/1) δ 0.76 (d, *J* = 6.4 Hz, 3H, 3H-49), 0.79 (d, *J* = 6.4 Hz, 3H, 3H-49'), 1.06-1.13 (m, 3H, 2H-28, H-47), 1.24-1.31 (m, 3H, 2H-12, H-47'), 1.35 (s, 9H, 9H-1), 1.43-1.50 (m, 1H, H-44), 1.53-1.79 (m, 5H, H-6, H-7, H-23, H-39, H-48), 1.80-2.12 (m, 14H, H-6', H-7', 2H-13, 2H-22, H-23', 2H-29, 2H-38, 2H-39, H-44), 2.37 (dd, *J* = 13.5 Hz, *J* = 7.7 Hz, 1H, H-31), 2.44-2.51 (m, 4H, H-15, H-31', 2H-45), 2.59-2.65 (m, 2H, H-8, H-15'), 2.79-2.84 (m, 2H, H-24, H-40), 3.94 (bs, 1H, H-43), 4.03 (bs, 2H, H-11, H-27), 4.12-4.17 (m, 1H, H-5), 4.29-4.36 (m, 4H, H-21, H-37, 2H-51), 5.90 (d, *J* = 8.0 Hz, 1H, H-4), 6.29 (d, *J* = 9.9 Hz, 1H, H-10), 6.95-7.29 (m, 15H, 2H-16, 2H-17, H-18, 2H-33, 2H-34, H-35, 2H-53, 2H-54, H-55), 7.49 (d, *J* = 9.8 Hz, 1H, H-26), 7.83 (d, *J* = 9.0 Hz, 1H, H-42), 7.99 (d, *J* = 9.6 Hz, 1H, H-20),8.26 (d, *J* = 8.7 Hz, 1H, H-36), 8.41 (t, *J* = 5.7 Hz, 1H, H-50); ^{13}C NMR (100 MHz, $CDCl_3$/DMSO-d_6: 5/1) δ 15.6, 15.9 (C-23, C-39), 17.4 (C-7), 22.3, 23.1 (C-49, C-49'), 24.8 (C-48), 25.6, 25.6 (C-22, C-38), 28.4 (C-1), 29.5, 29.5, 29.6 (C-6, C-12, C-28), 31.6, 31.8 (C-13, C-29), 33.4 (C-45), 33.6 (C-44), 41.7 (C-15), 42.1 (C-31), 43.2 (C-51), 44.0 (C-47), 46.7 (C-43), 46.9 (C-11), 47.5 (C-27), 47.8 (C-21, C-37), 48.0 (C-5), 48.9, 49.3 (C-24, C-40), 50.1 (C-8), 79.9 (C-2), 126.0, 126.4, 126.8 , 127.8, 127.9, 128.1, 128.3 129.4 (C-17, C-18, C-19, C-33, C-34, C-35, C-53, C-54, C-55), 137.3, 138.2, 139.2 (C-16, C-32, C-52), 155.5 (C-3), 171.5 (C-14), 171.6 (C-25), 172.0 (C-9), 172.1 (C-30), 172.4 (C-41), 174.3 (C-46); IR ν_{max} 1443, 1455, 1497, 1512, 1557, 1647, 1698, 2870, 2930, 2958, 3030, 3067, 3086, 3311(br), 3429, 3443 cm^{-1}; HRMS (ESI): $[M+Na]^+$, theor. 1012.5882 (calc. for $C_{57}H_{79}N_7NaO_8$), meas. 1012.5841.

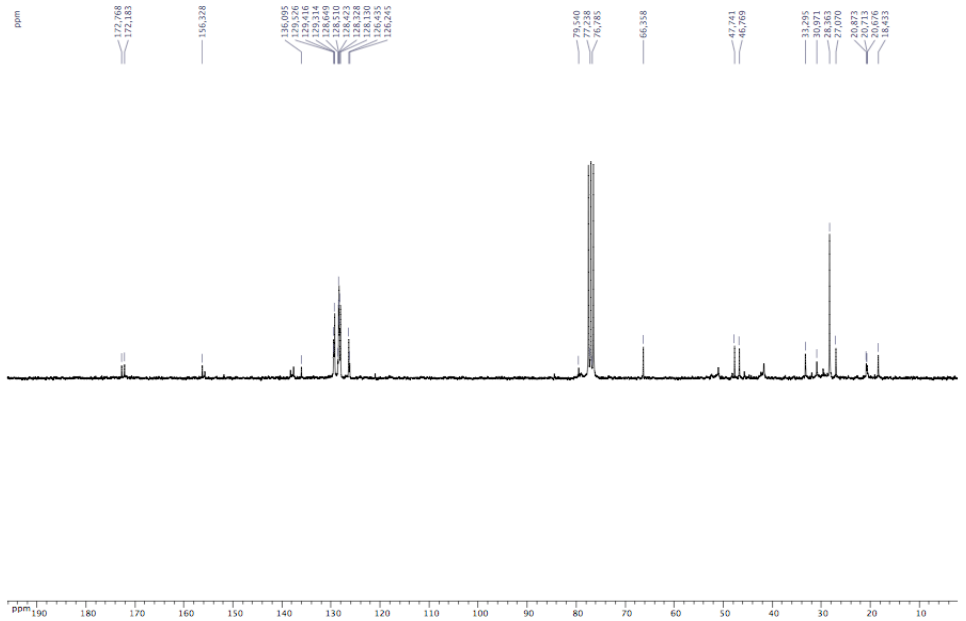
II. NMR SPECTROSCOPIC ANALYSIS

1. *^{1}H and ^{13}C NMR spectra of peptides **1-12** and intermediate peptides **I-V***

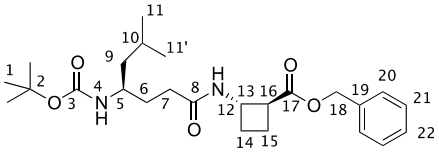
Boc–(*R*)-γ^{4}-Phe–(1*S*,2*S*)-ACBC–OBn (I)

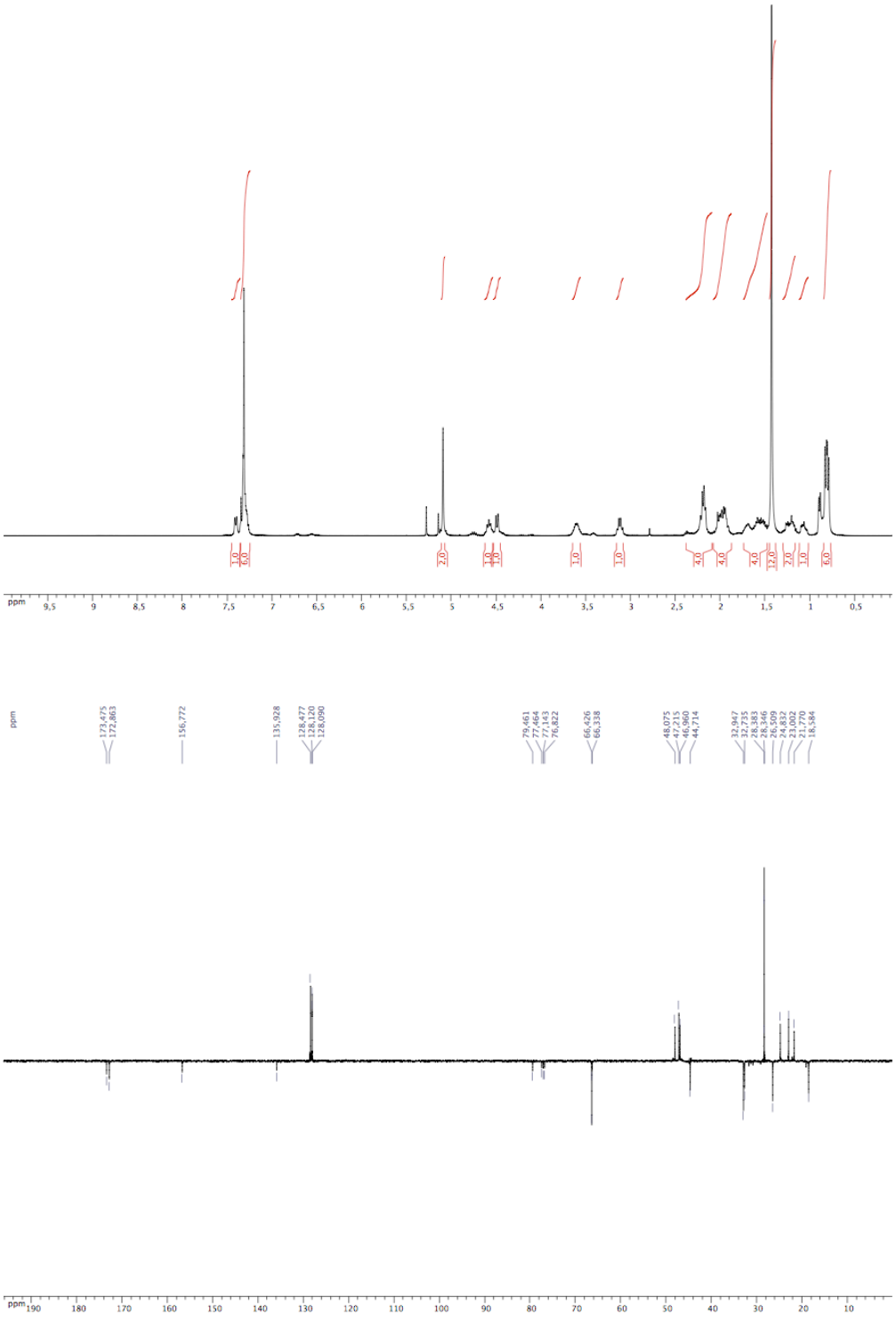




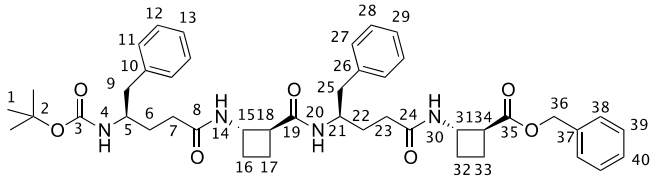


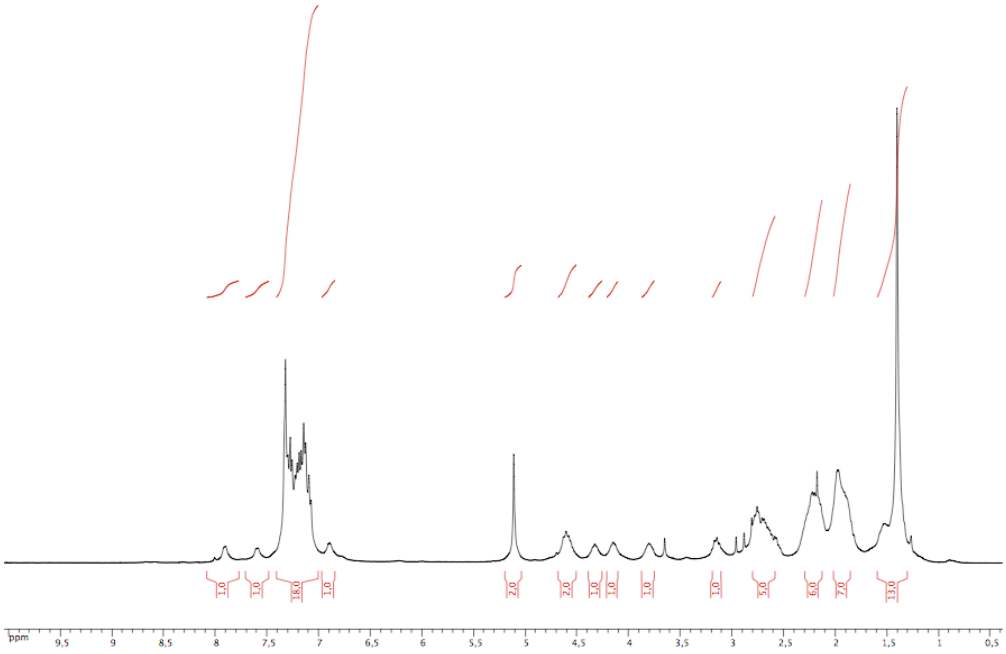
Boc–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (II)

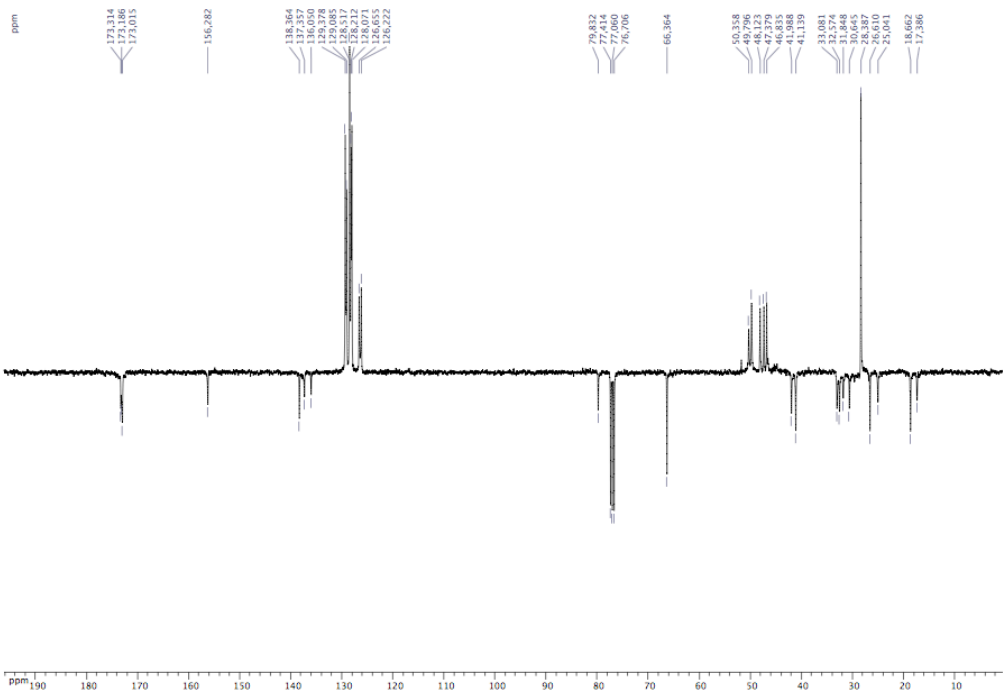




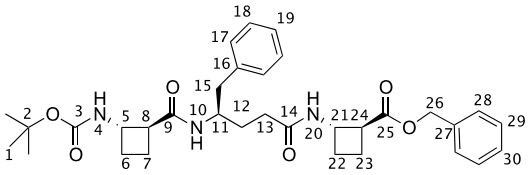
Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (III)

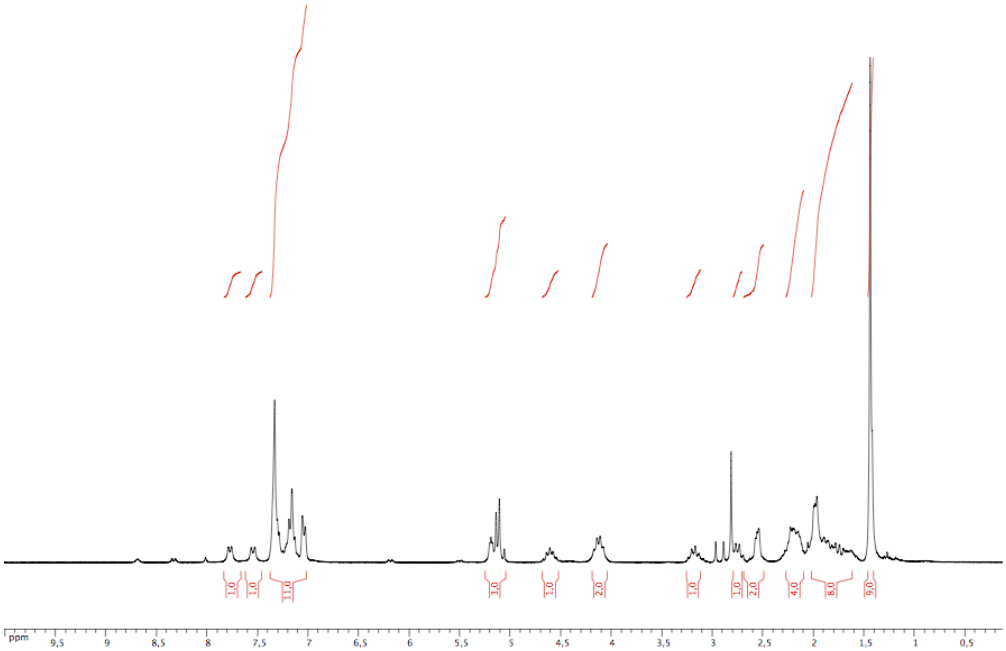


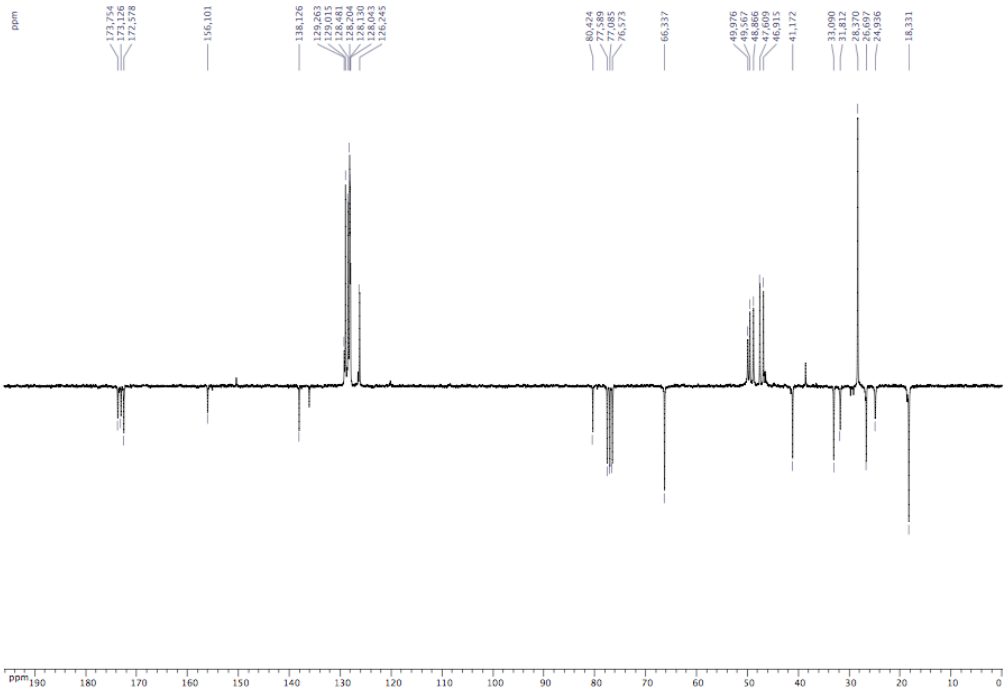




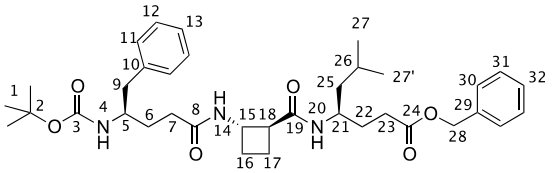
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (IV)

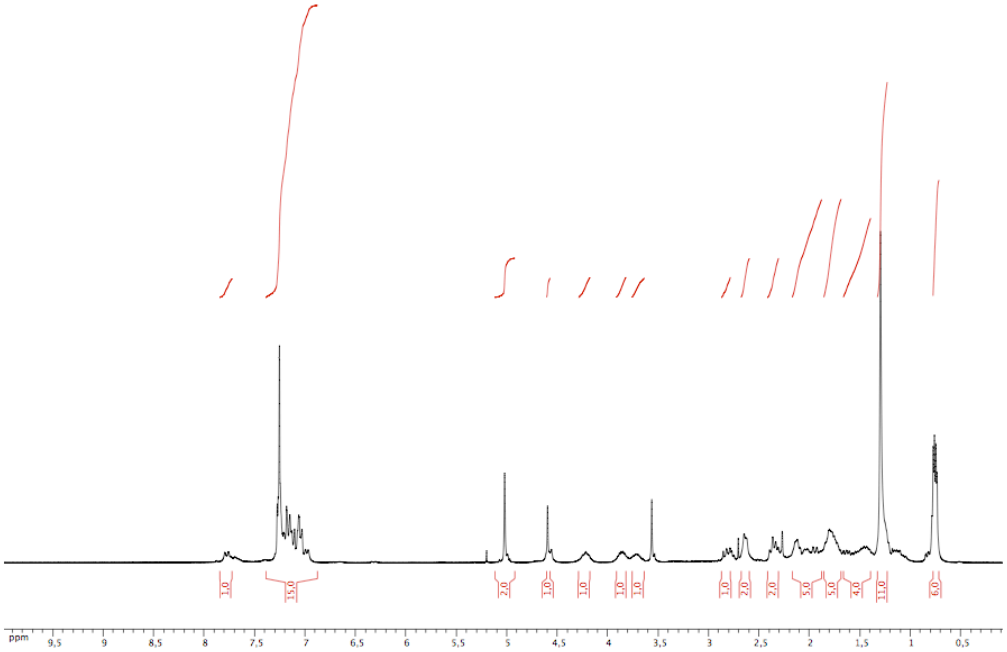


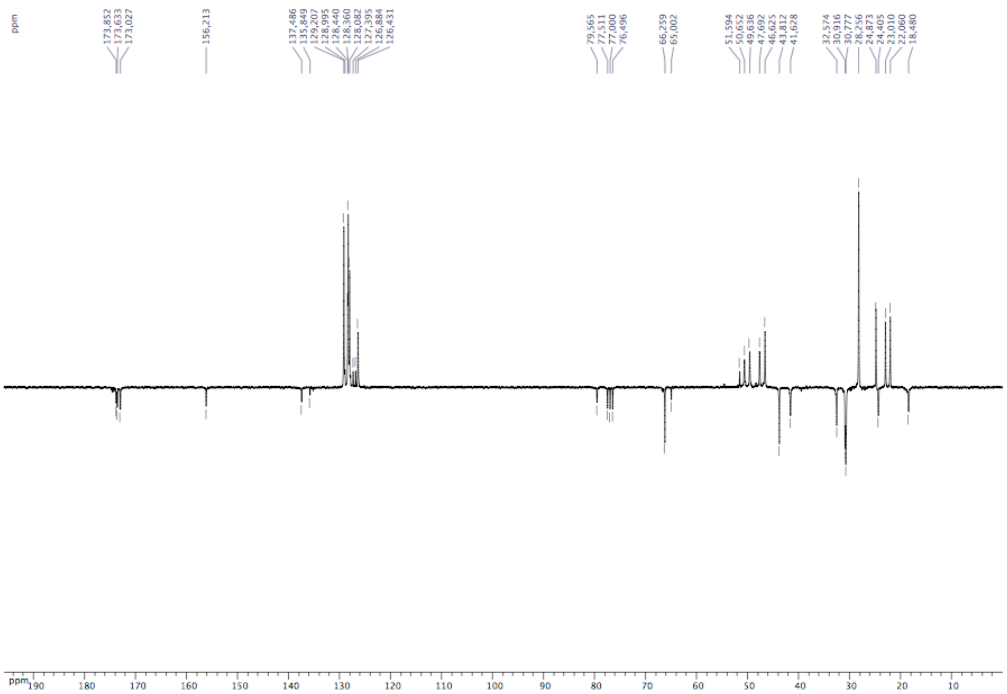




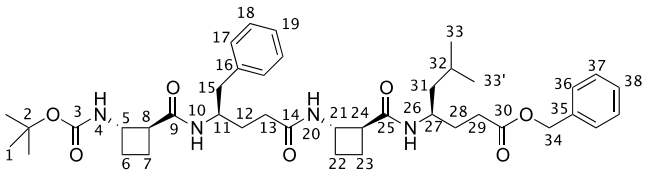
Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (V)

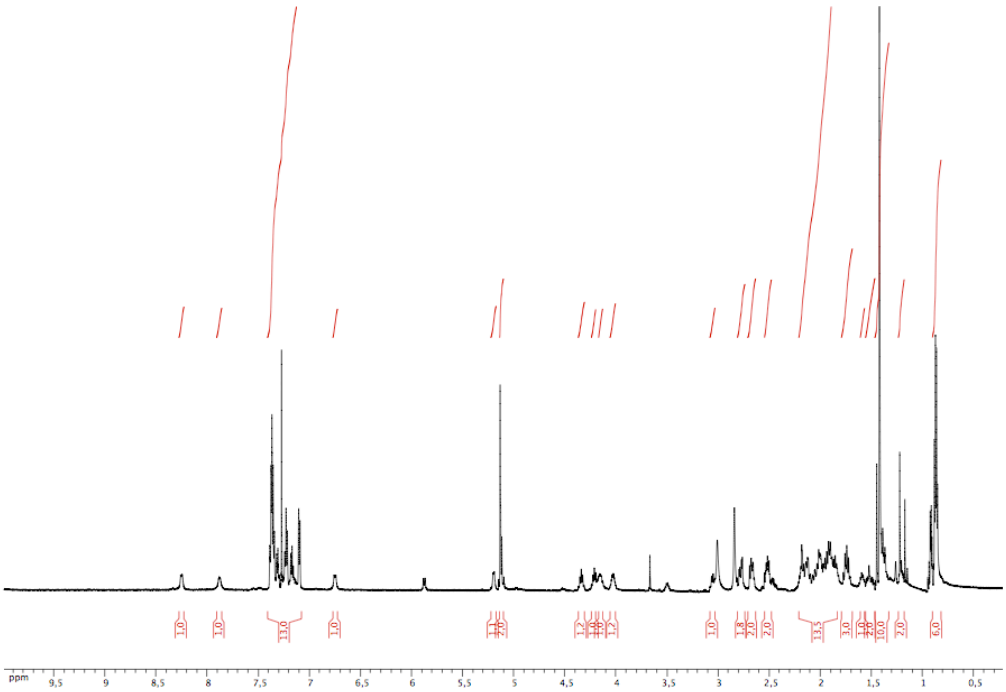


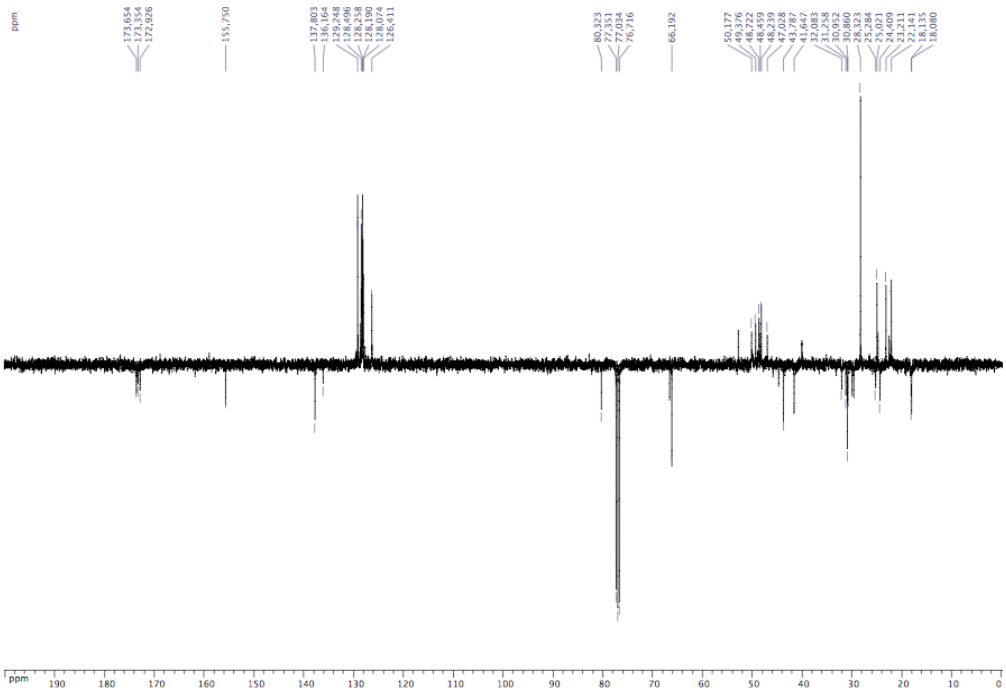




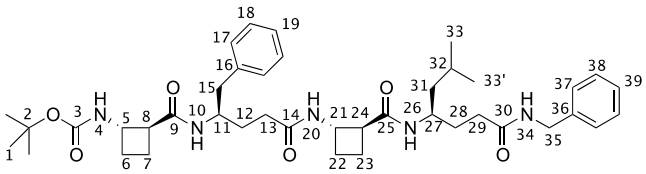
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (1)

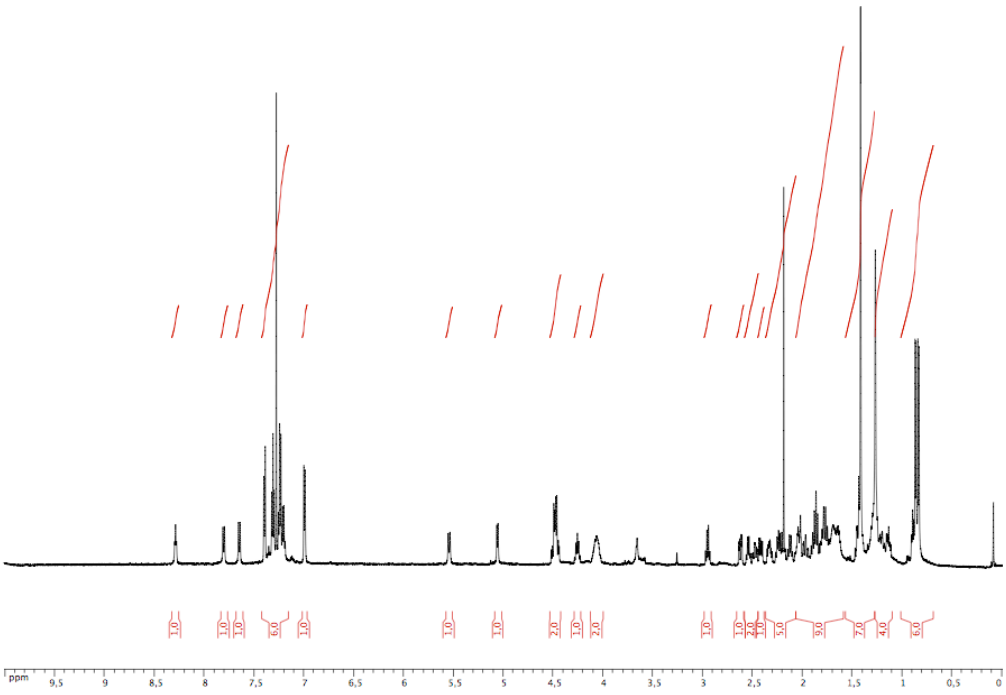


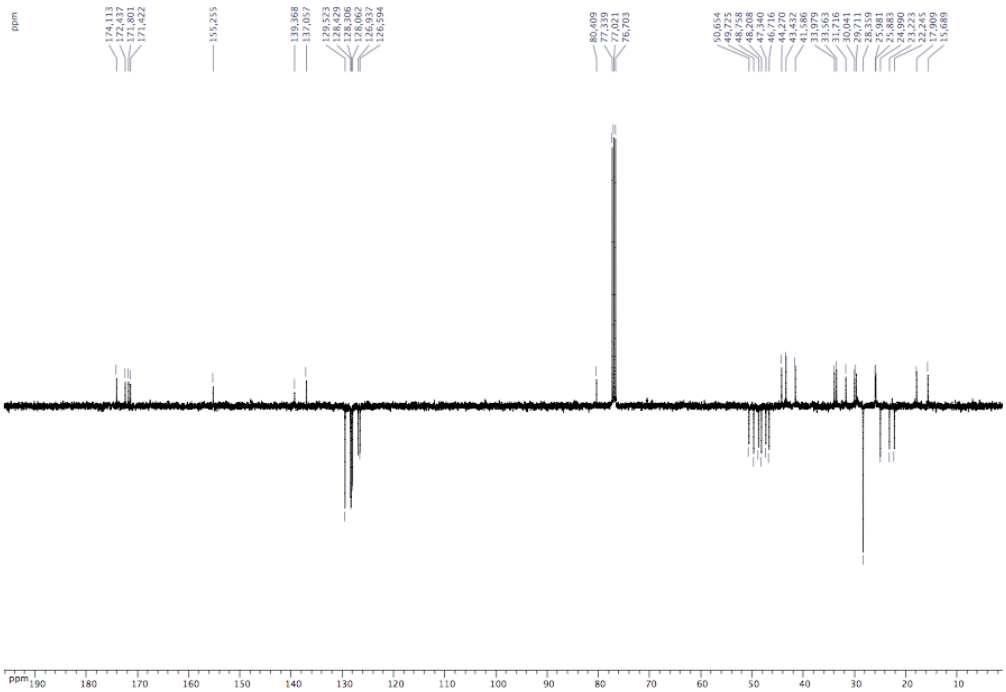




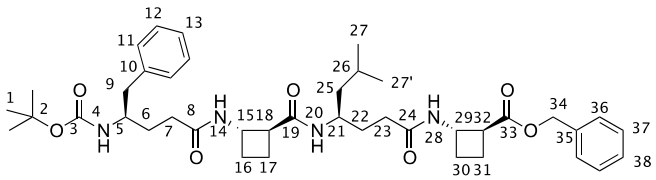
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (2)

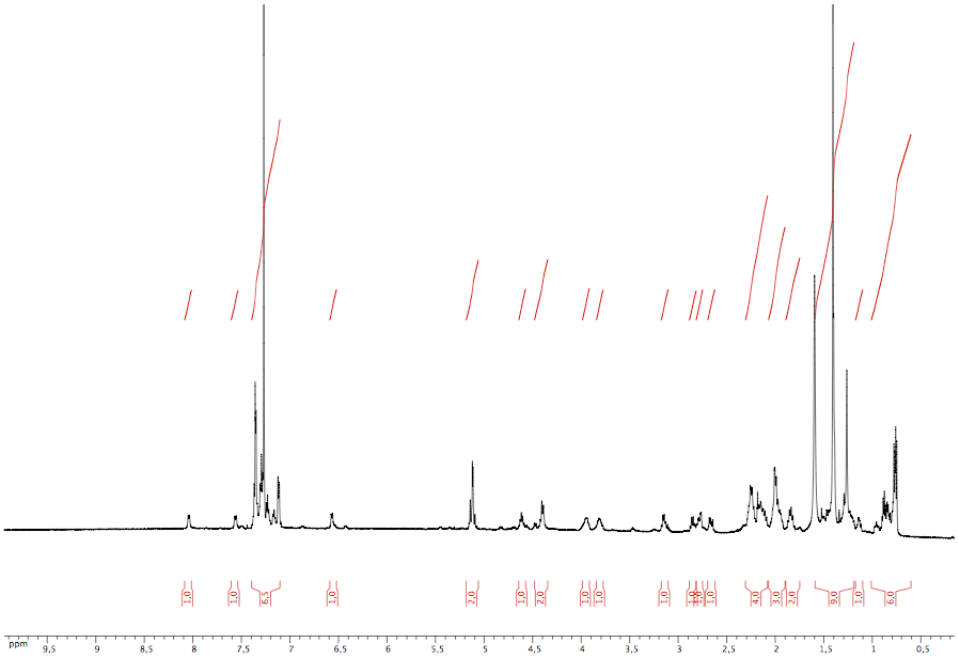


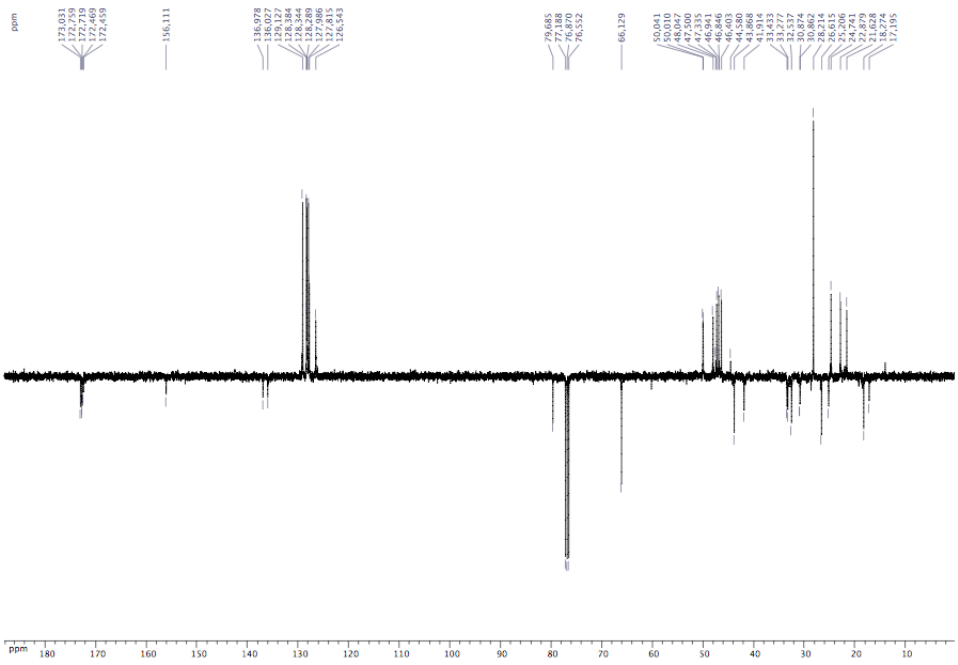




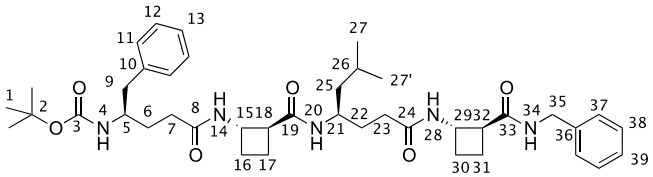
Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (3)

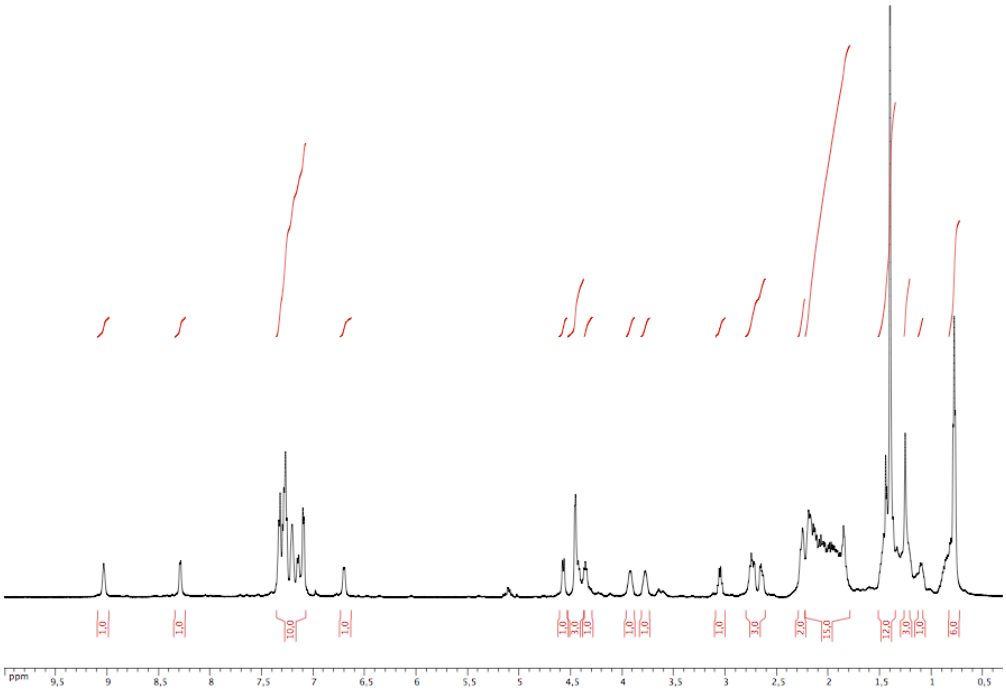


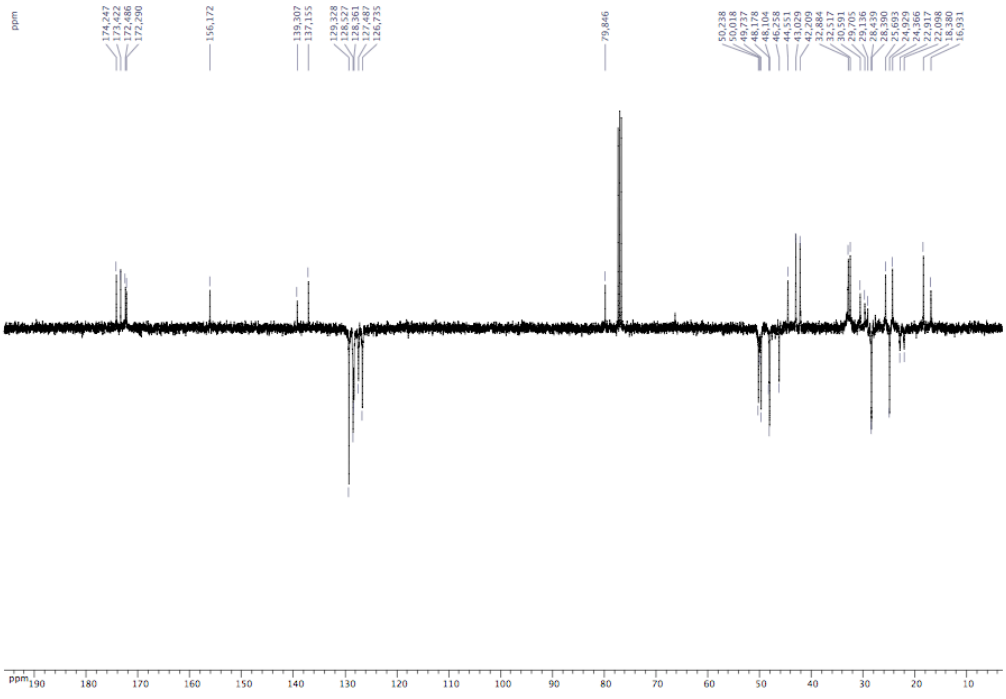




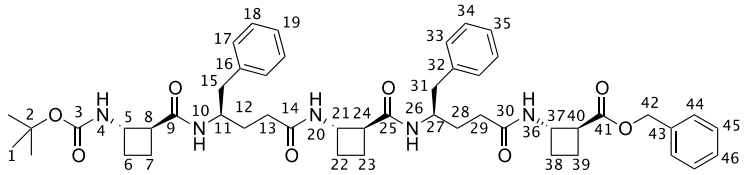
Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–NHBn (4)

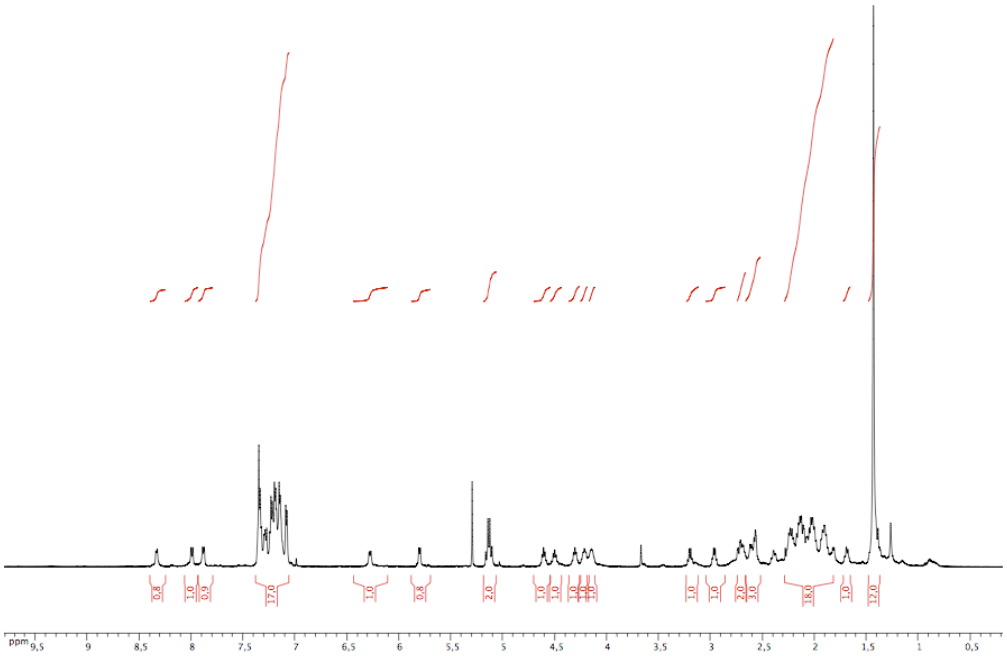


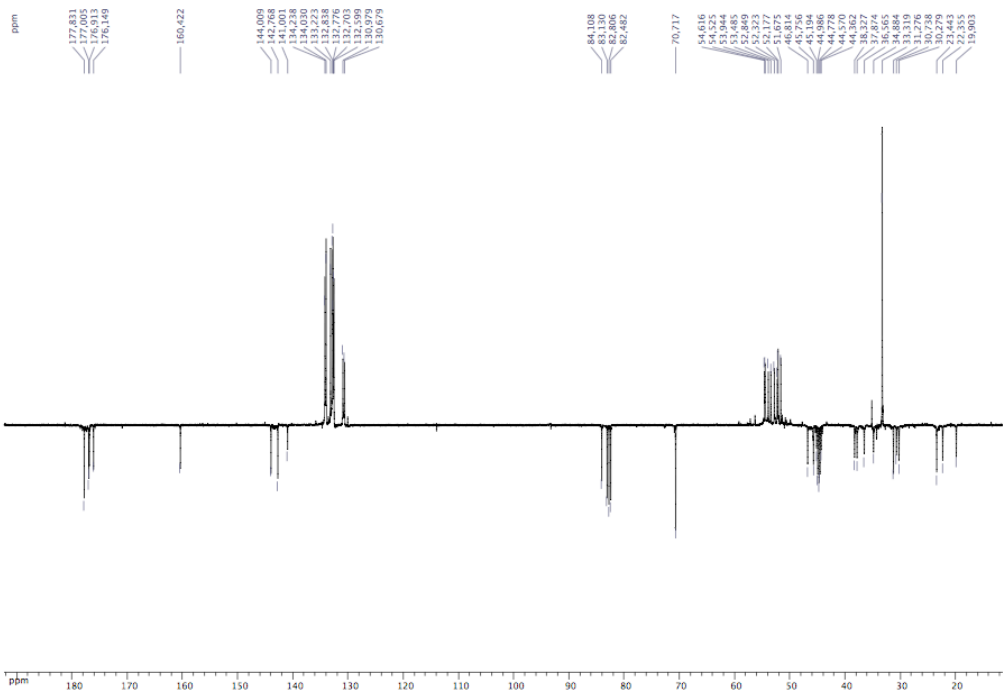




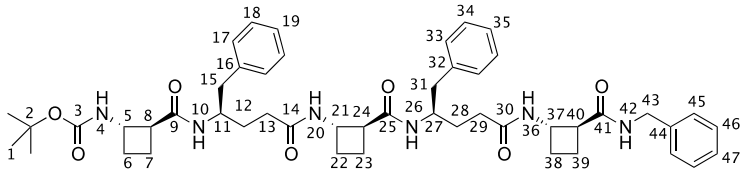
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–OBn (5)

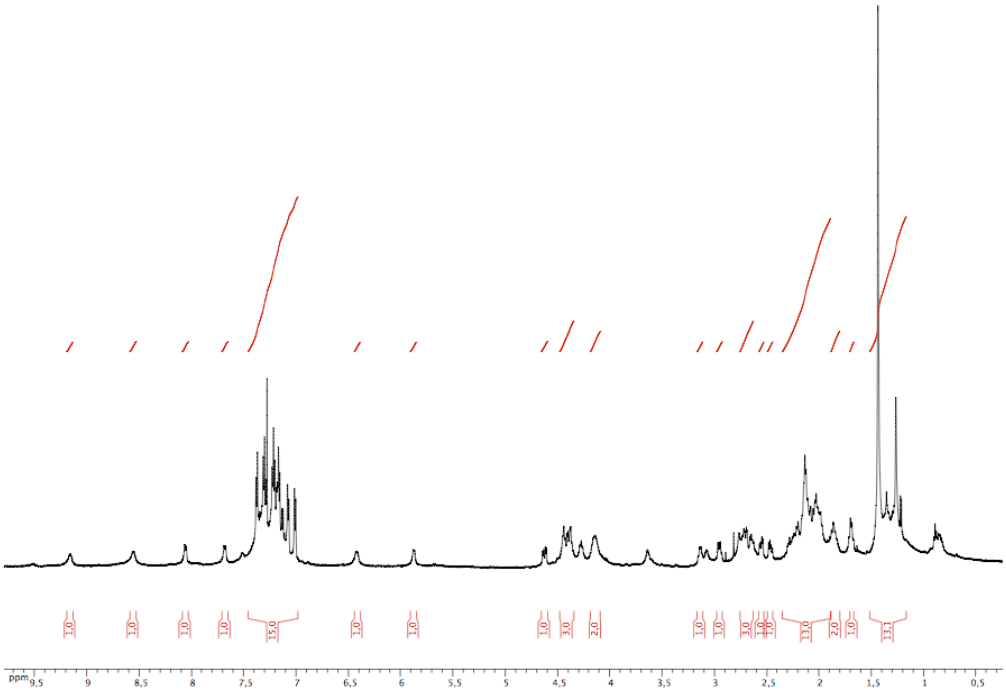


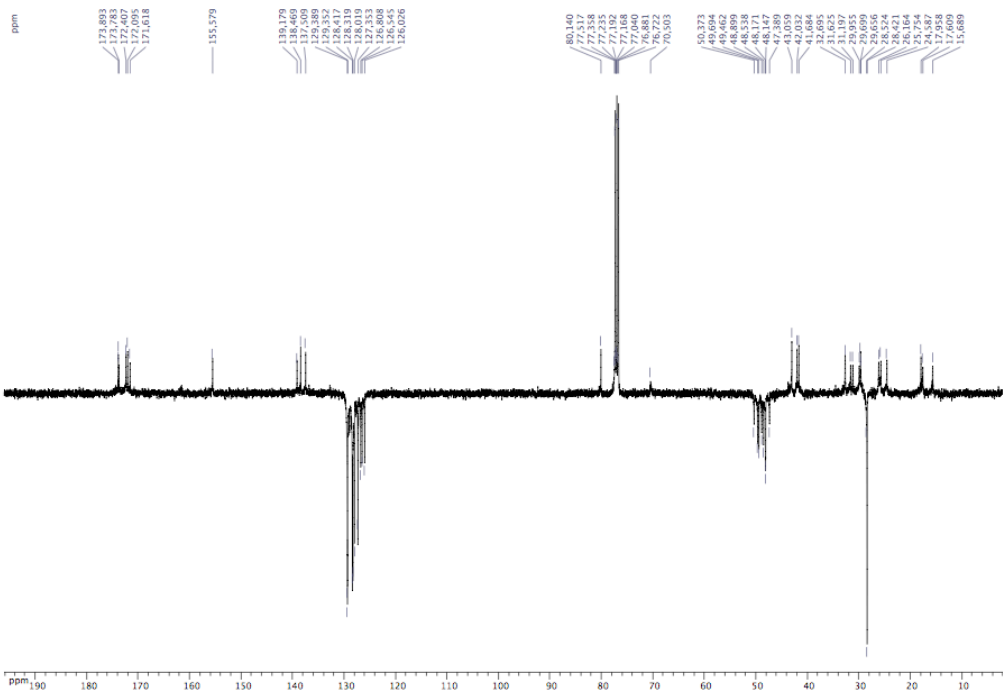




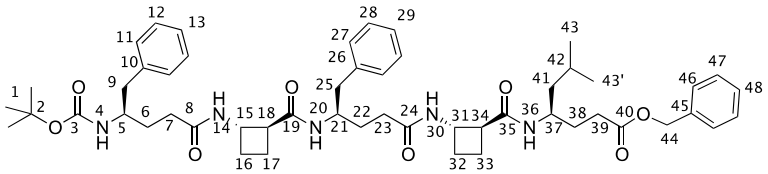
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–NHBn (6)

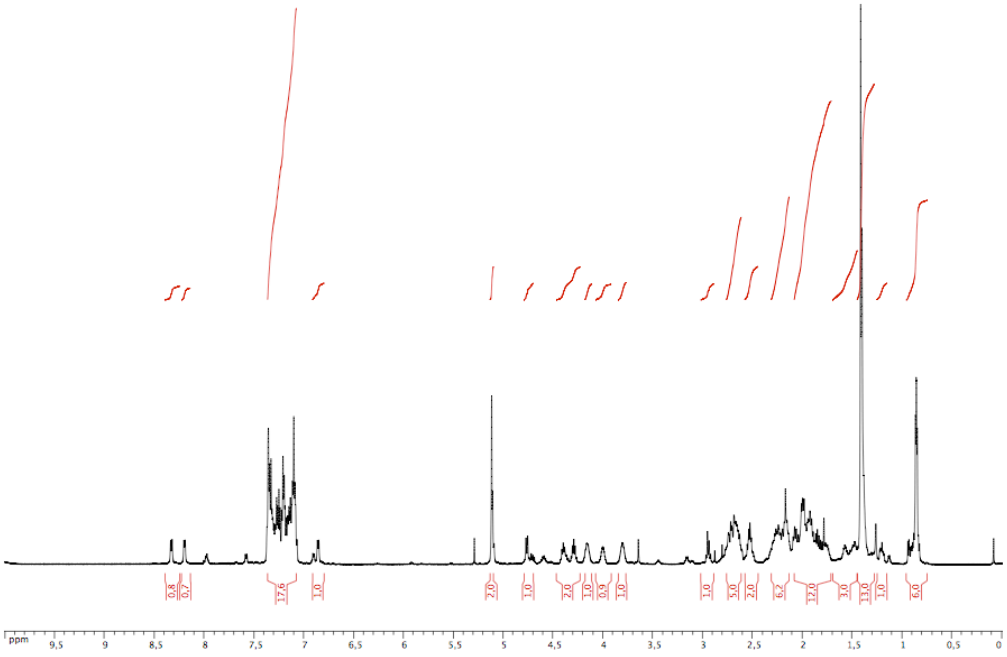


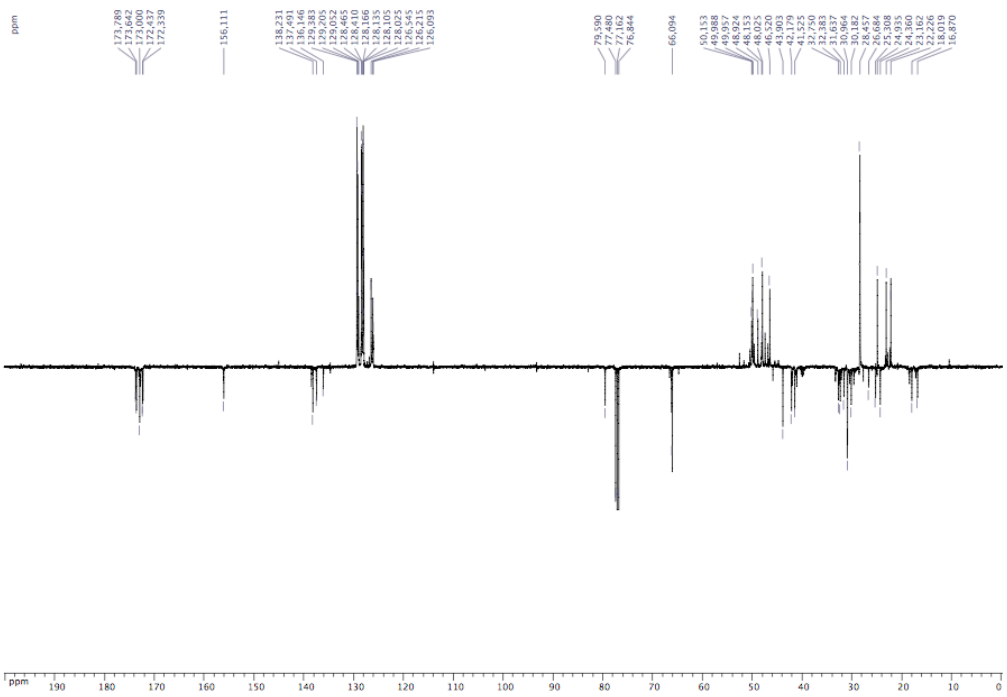




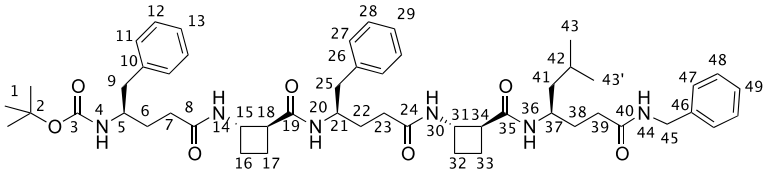
Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (7)

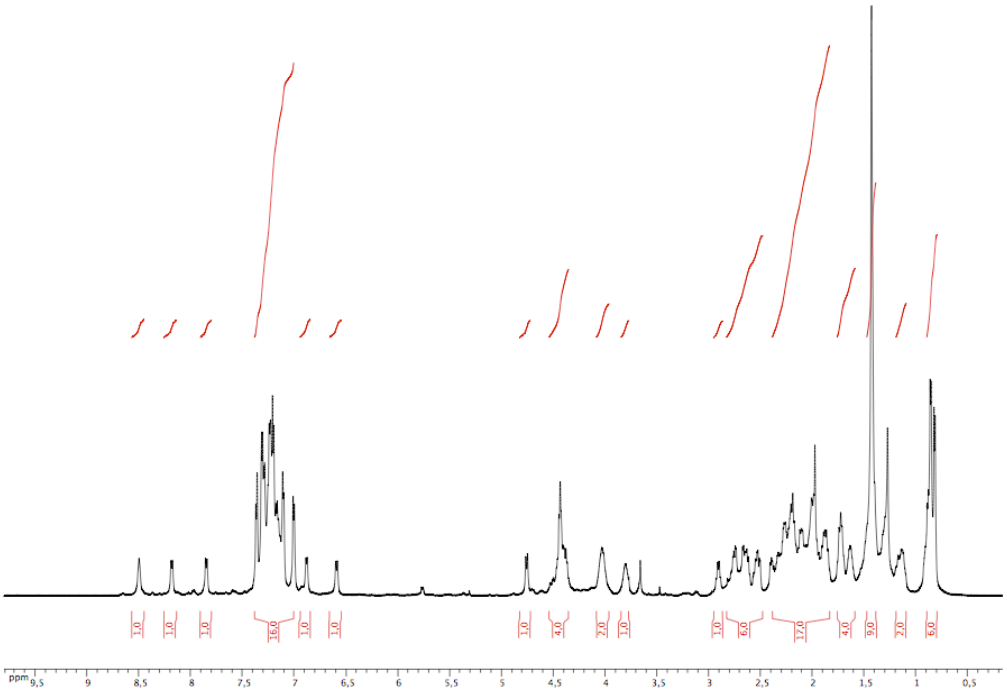


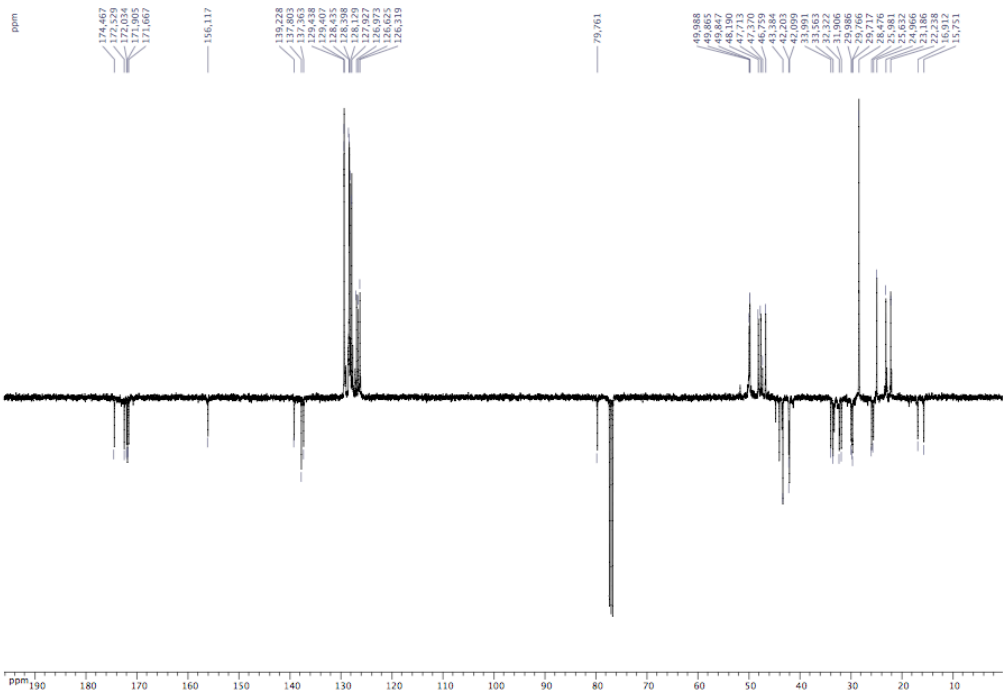




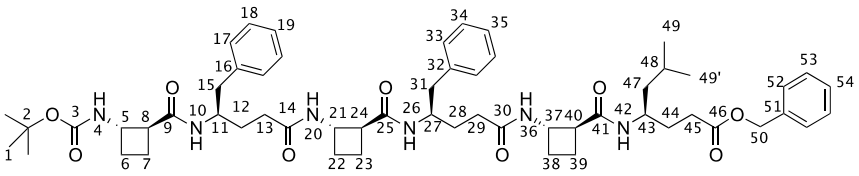
Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–NHBn (8)

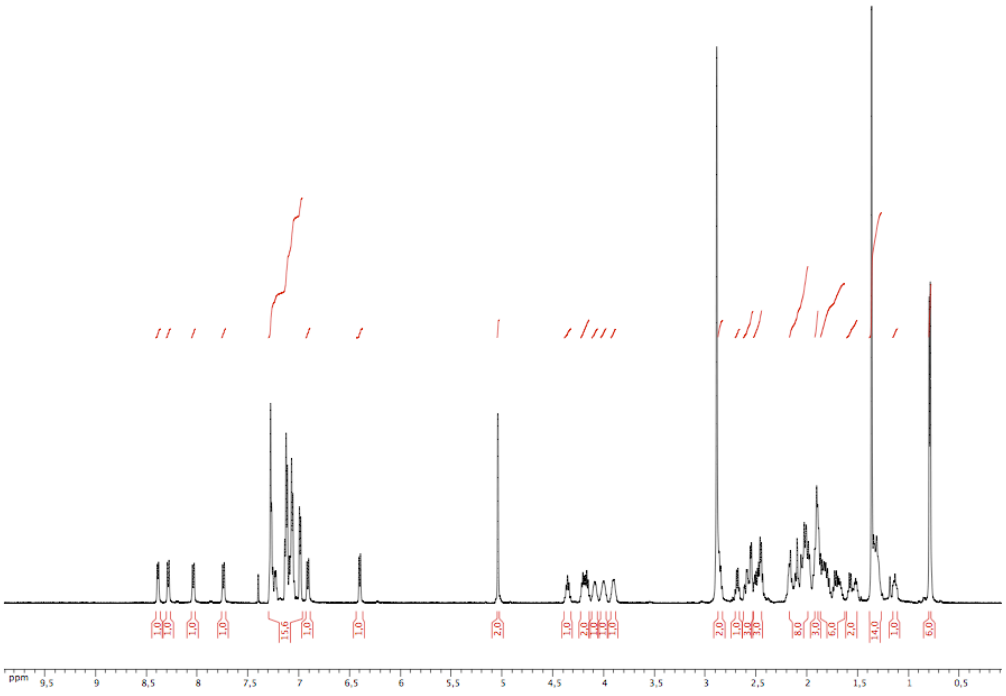


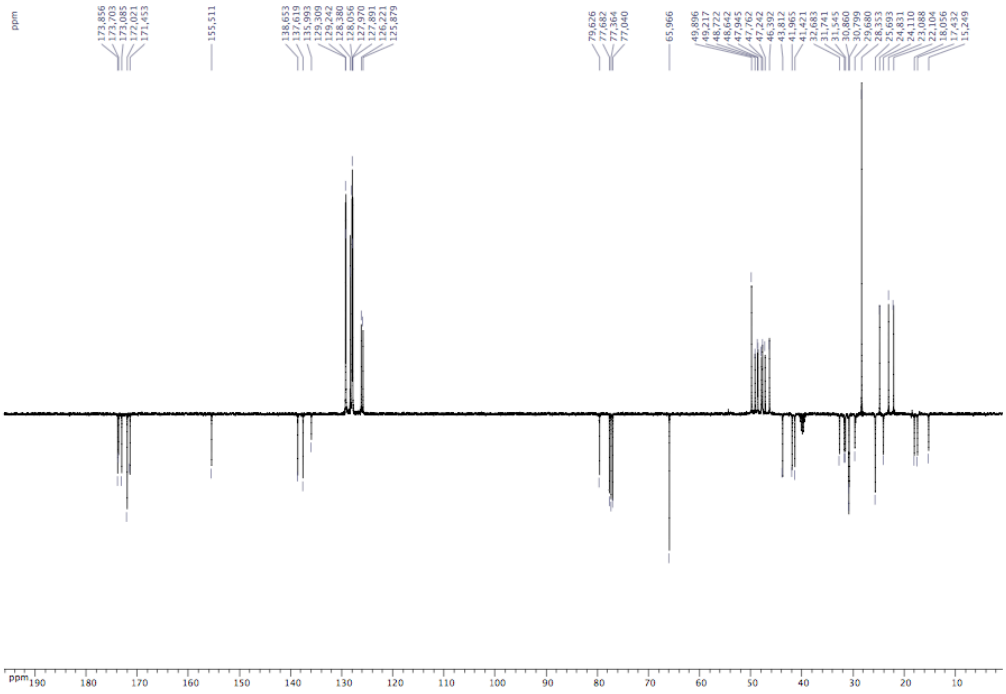




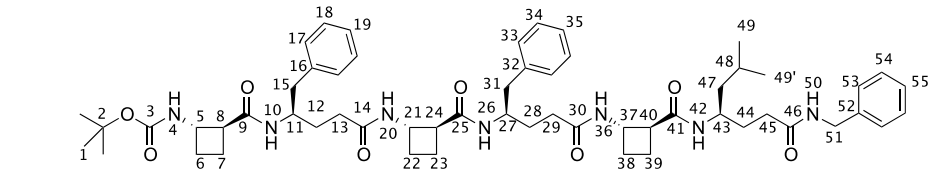
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (9)

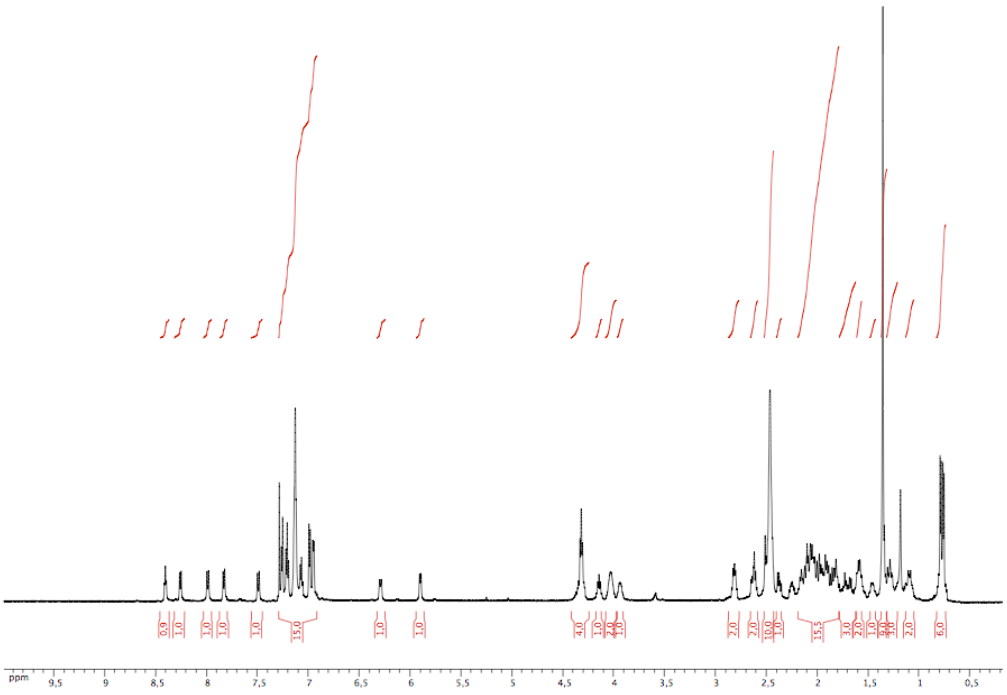


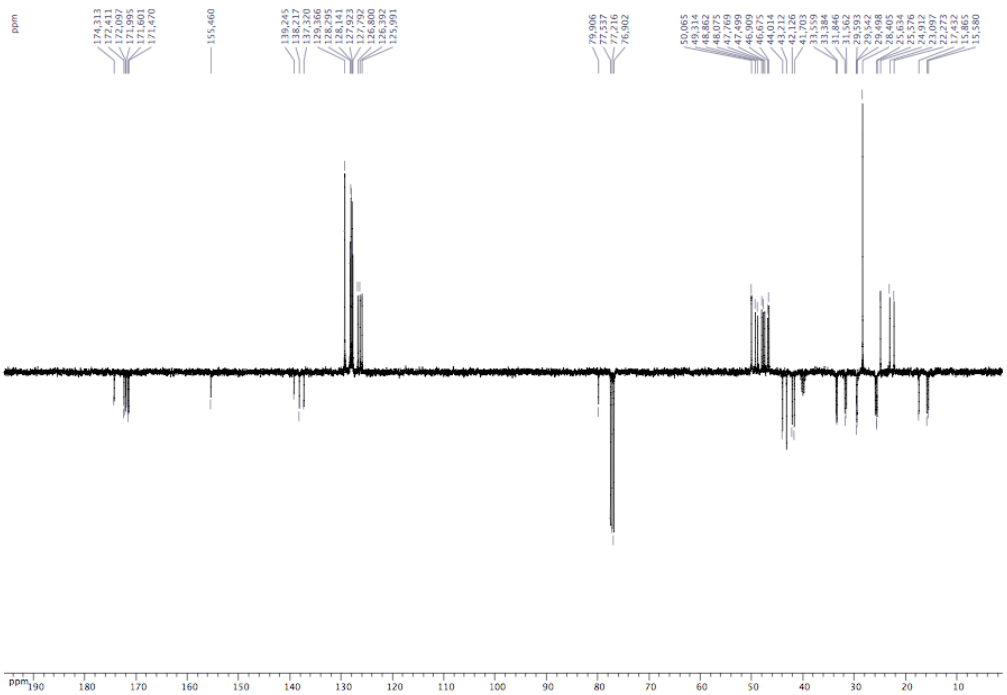




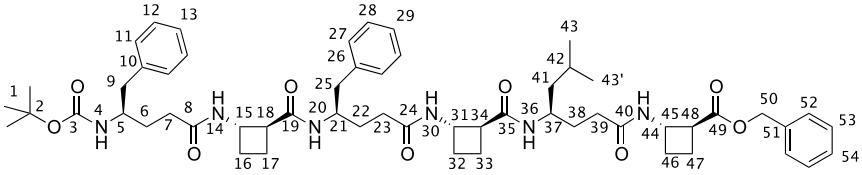
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (10)

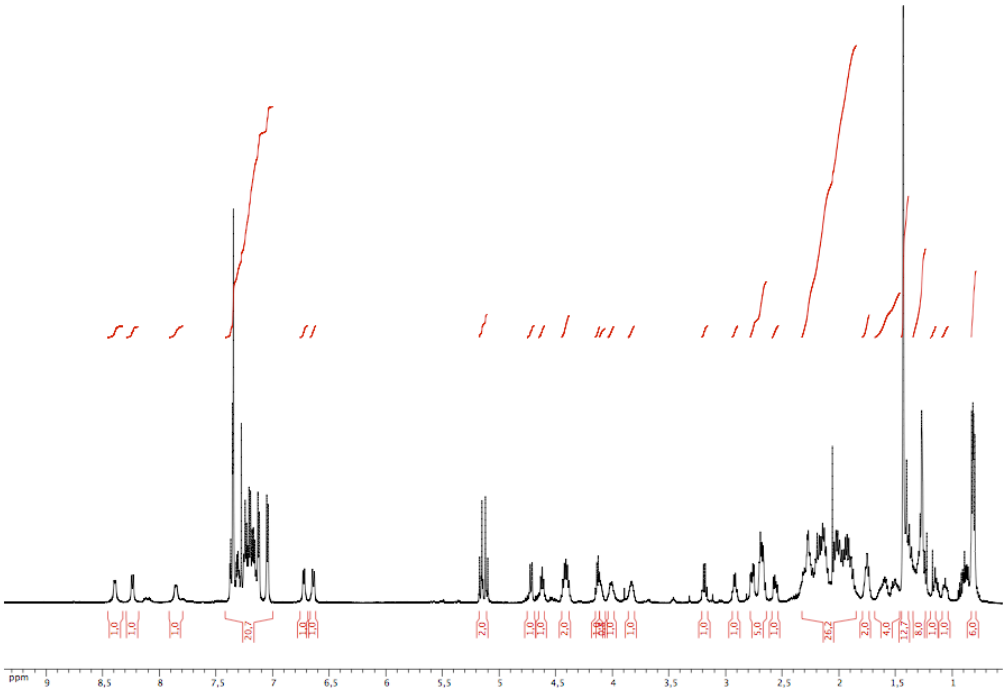


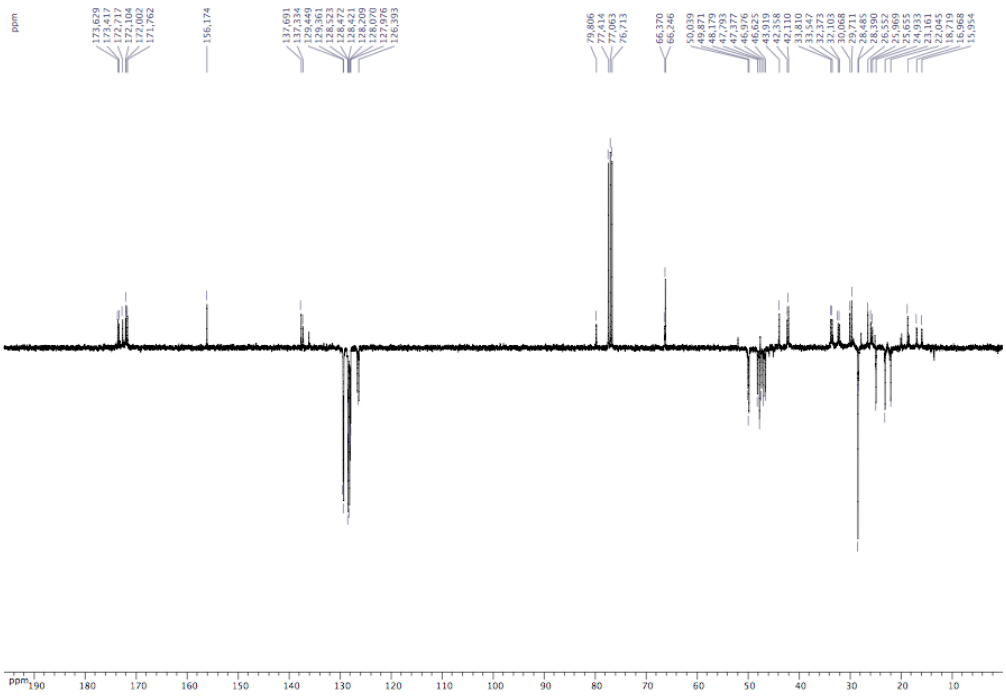




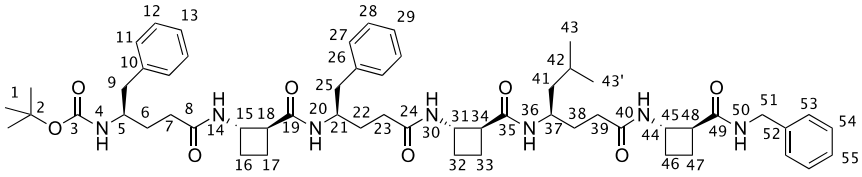
Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–OBn (11)

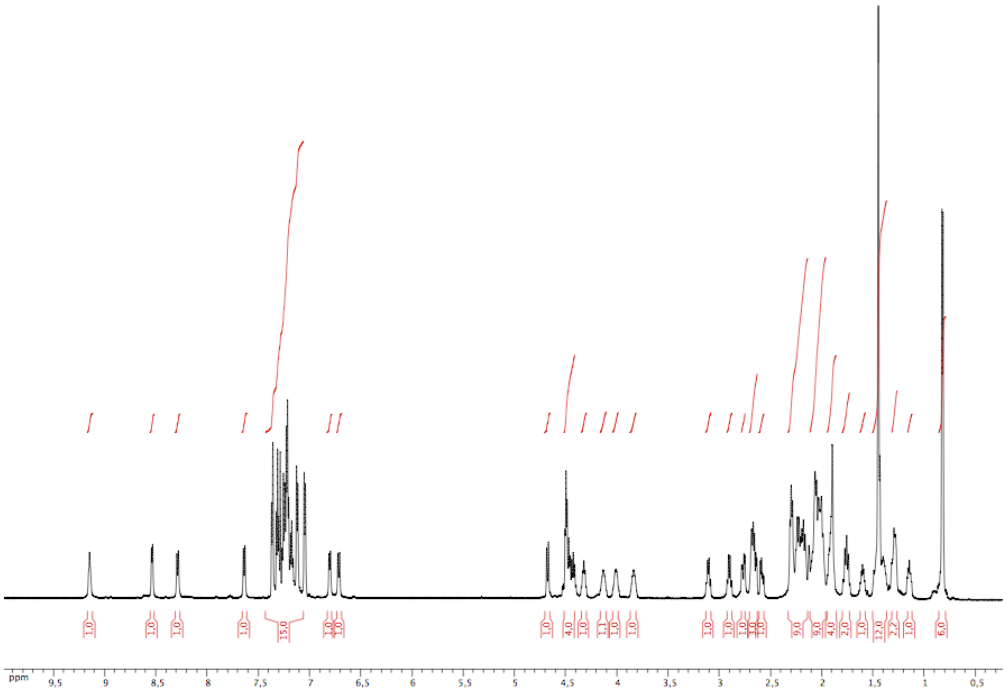


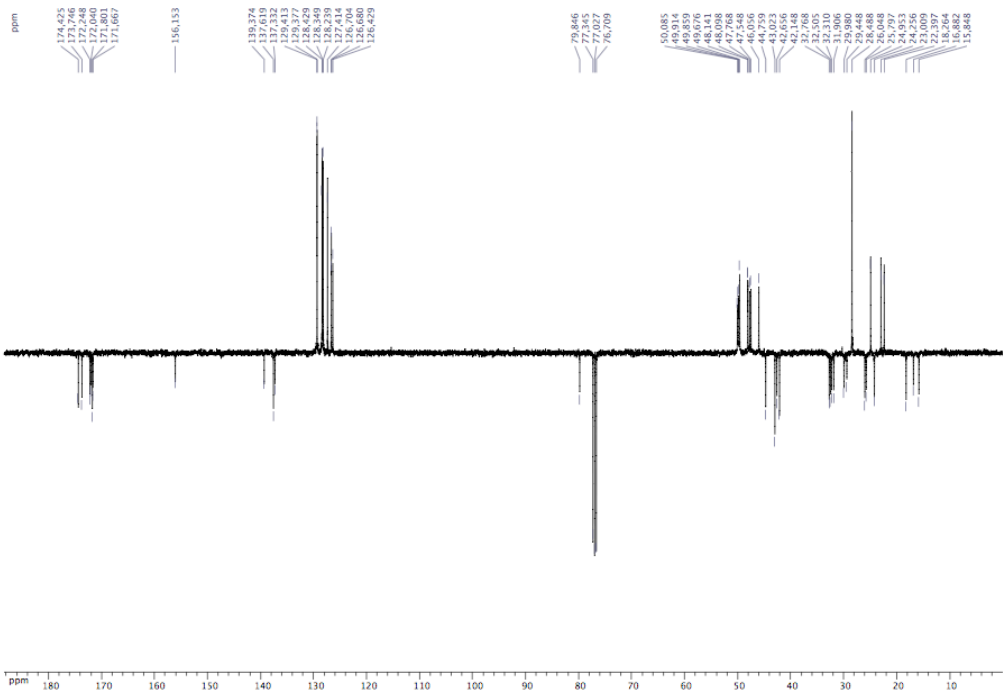




Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–NHBn (12)



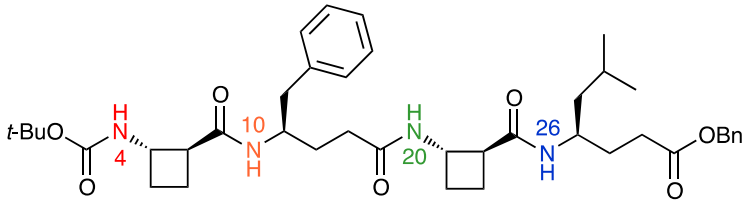




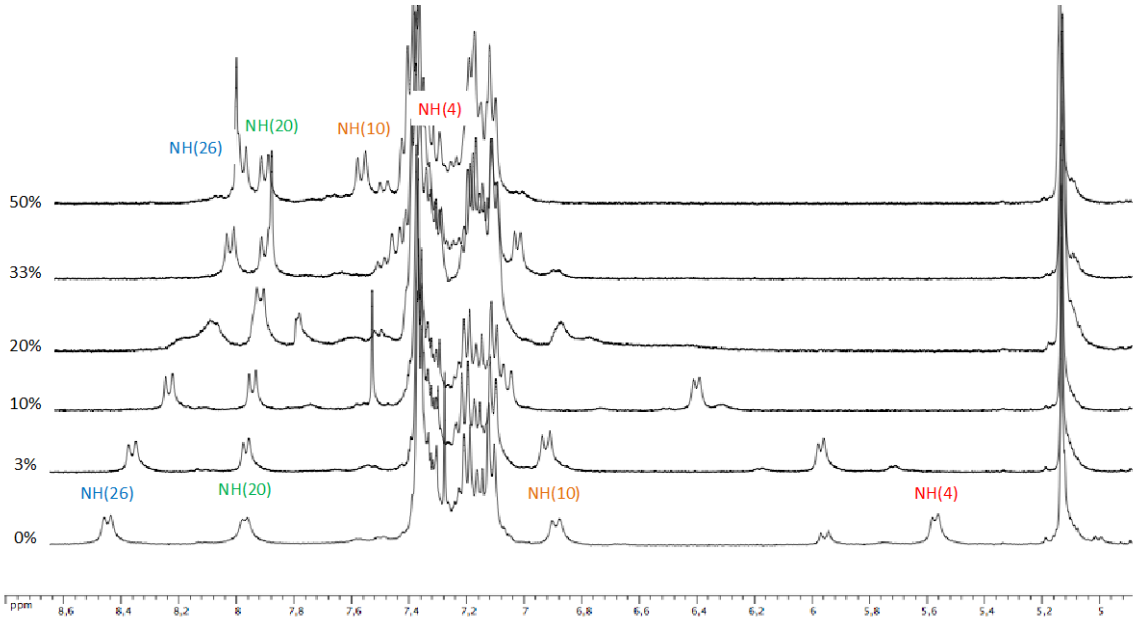
2. *DMSO-d_6 titrations*

^{1}H NMR spectra were recorded at 300 K on a Bruker 600 MHz spectrometer. Samples were dissolved in $CDCl_3$ (600 µL) to give solutions of concentration 10 mM. Aliquots of DMSO-d_6 (20 µL, 40 µL, 60 µL, 80 µL and 100 µL) were added successively to the NMR tube followed (after each addition) by rapid manual agitation then re-recording of the ^{1}H spectra.

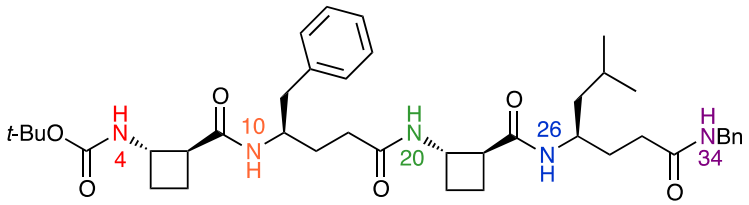
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (1)



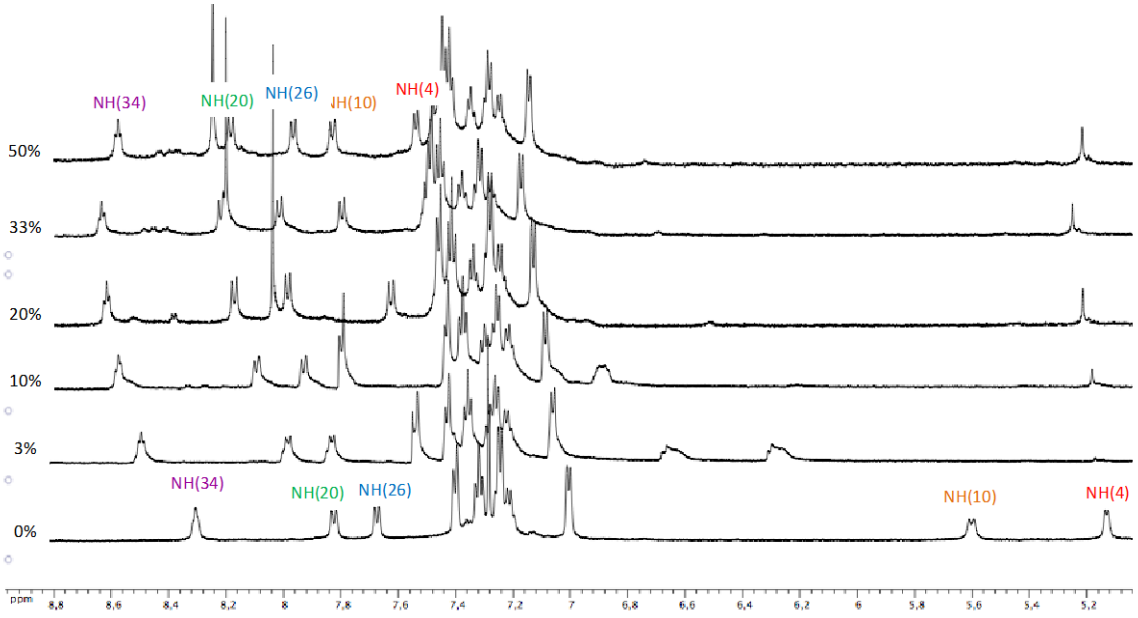
DMSO-d_6 (%. v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	5.57	5.95	6.36	6.87	7.00	7.24	1.67
NH(10)	6.89	6.93	-	-	7.44	7.56	0.67
NH(20)	7.95	7.95	7.93	7.90	7.90	7.90	-0.05
NH(26)	8.43	8.36	8.24	8.08	8.03	7.98	-0.45



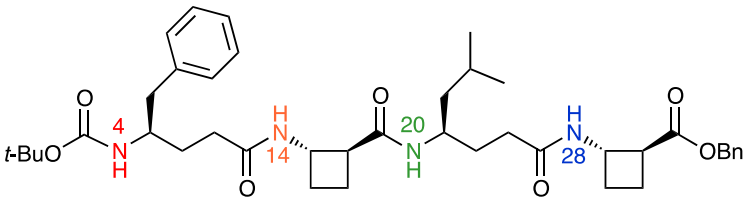
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–NHBn (2)



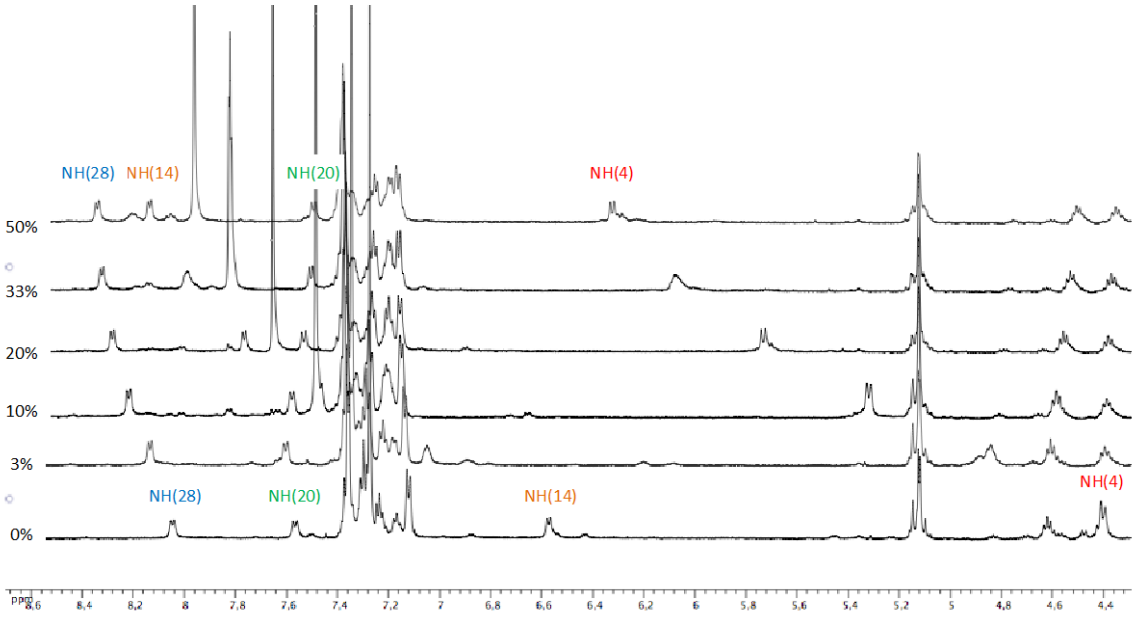
DMSO-d_6 (%, v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	5.13	6.28	6.91	-	-	7.47	2.34
NH(10)	5.58	6.67	-	7.62	7.96	7.79	2.21
NH(26)	7.67	7.83	7.92	7.89	7.95	8.02	0.35
NH(20)	7.83	7.98	8.01	8.17	-	8.19	0.36
NH(34)	8.31	8.50	8.57	8.61	8.56	8.63	0.32



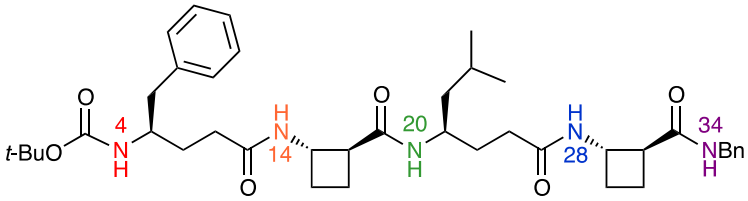
Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (3)



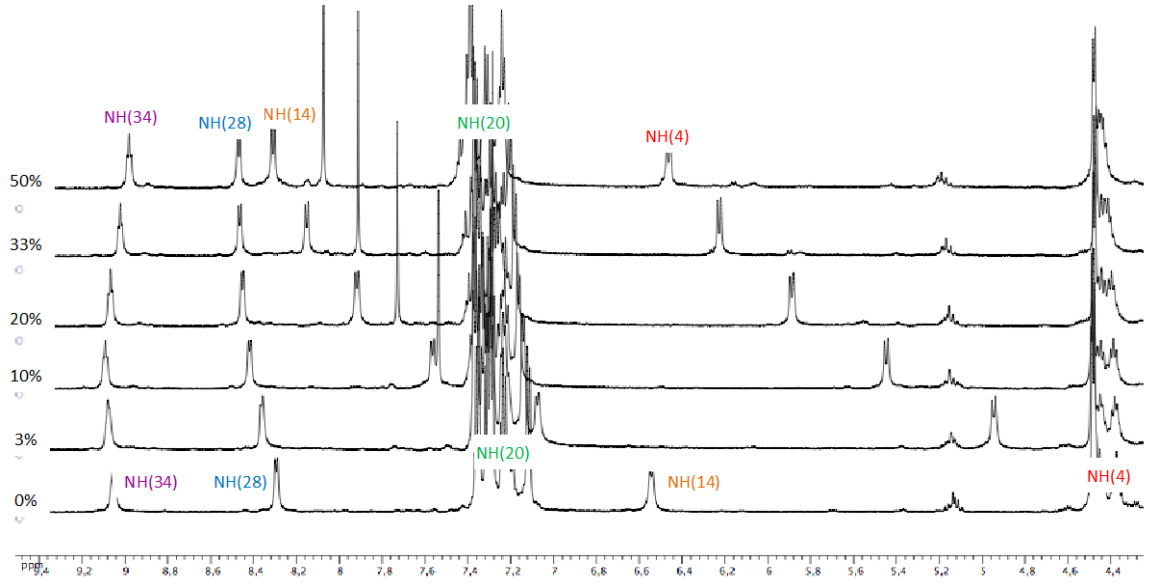
DMSO-d_6 (%, v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	4.40	4.84	5.31	5.73	6.07	6.32	1.92
NH(14)	6.57	7.05	7.48	7.76	7.99	8.14	1.57
NH(20)	7.56	7.61	7.58	7.53	7.50	7.49	-0.07
NH(28)	8.04	8.13	8.22	8.28	8.33	8.34	0.3



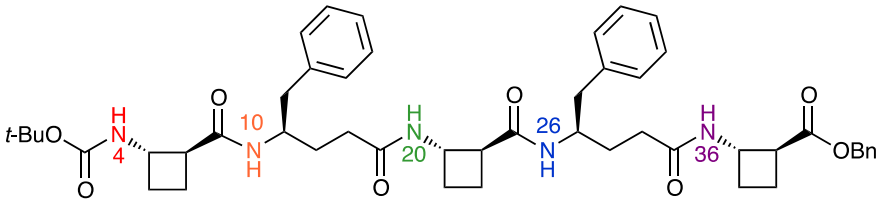
Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–NHBn (4)



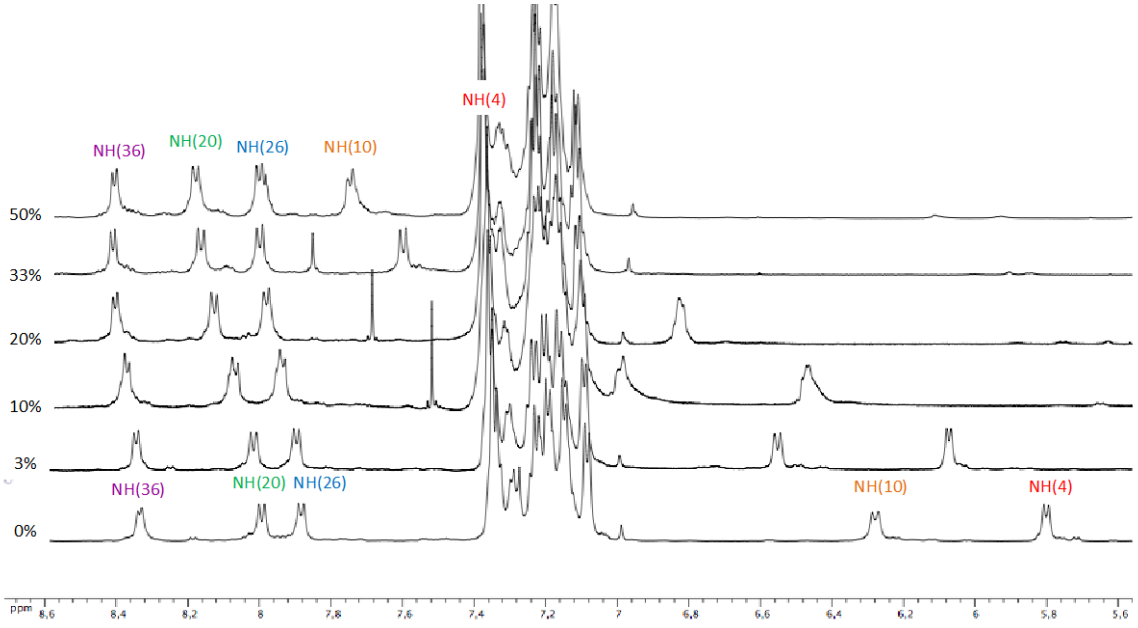
DMSO-d_6 (%, v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	4.48	4.95	5.43	5.89	6.22	6.47	1.99
NH(14)	6.54	7.06	7.56	7.91	8.14	8.31	1.77
NH(20)	7.13	-	-	-	-	7.25	0.12
NH(28)	8.30	8.37	8.42	8.45	8.46	8.47	0.17
NH(34)	9.05	9.08	9.09	9.06	9.02	8.98	0.07



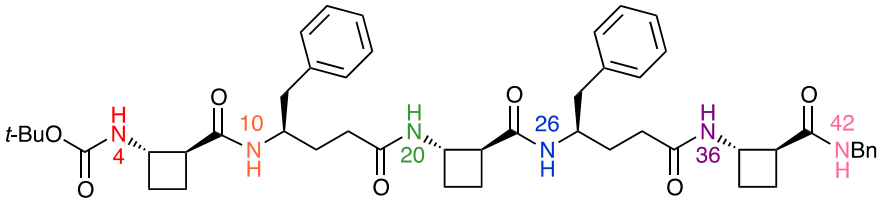
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–OBn (5)



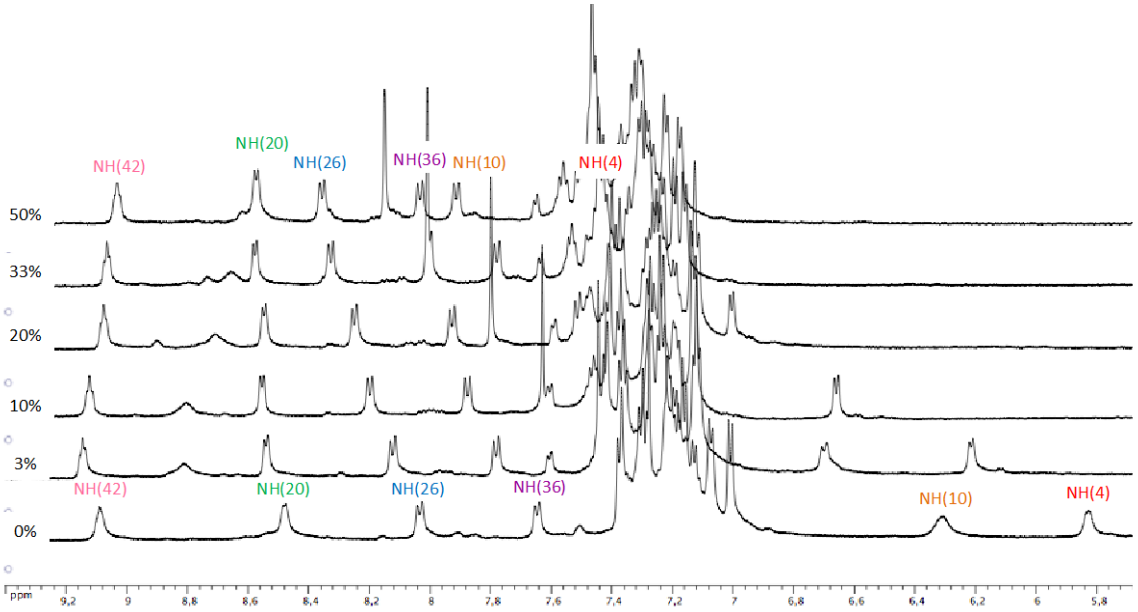
DMSO-d_6 (%, v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	5.8	6.07	6.46	6.82	7.19	7.31	1.51
NH(10)	6.27	6.55	6.98	7.30	7.60	7.73	1.46
NH(26)	8.00	8.01	8.07	8.11	8.17	8.17	0.17
NH(20)	7.88	7.89	7.93	7.97	7.99	7.99	0.11
NH(36)	8.34	8.35	8.37	8.39	8.40	8.40	0.06



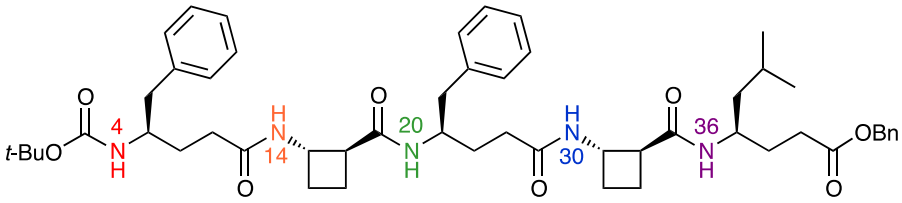
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–NHBn (6)



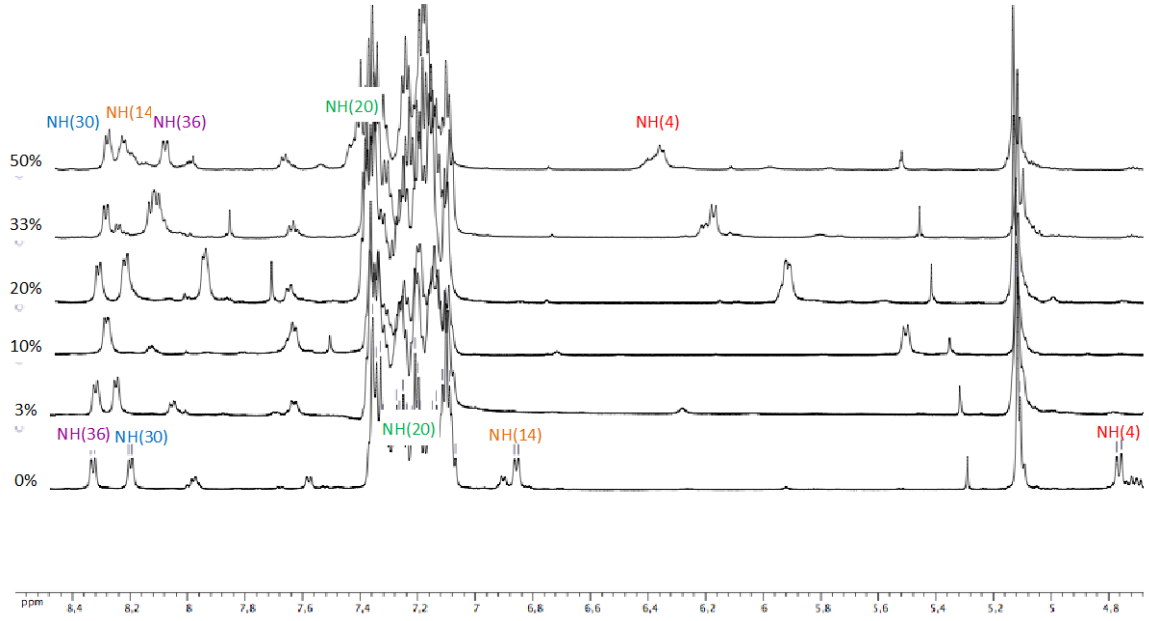
DMSO-d_6 (%. v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	5.83	6.21	6.66	7.00	-	7.26	1.43
NH(10)	6.3	6.69	-	7.51	7.79	7.91	1.61
NH(36)	7.65	7.78	7.88	7.92	7.99	8.02	0.37
NH(26)	8.03	8.12	8.19	8.24	8.33	8.35	0.32
NH(20)	8.49	8.54	8.55	8.55	8.58	8.58	0.09
NH(42)	9.08	9.14	9.12	9.08	9.06	9.03	-0.05



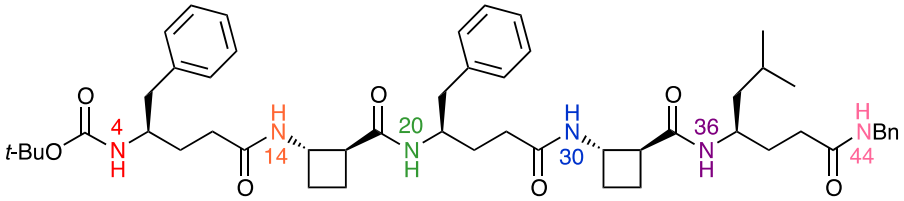
Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–OBn (7)



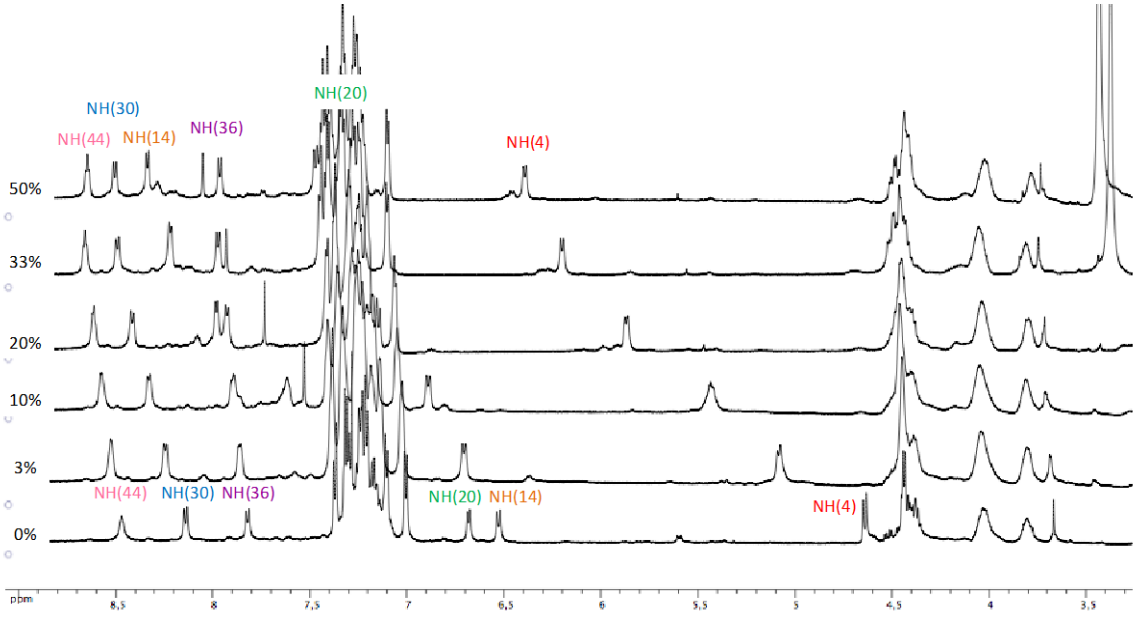
DMSO-d_6 (%, v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	4.76	5.08	5.49	5.92	6.18	6.36	1.6
NH(14)	6.84	7.22	7.64	7.95	8.10	8.22	1.38
NH(20)	7.12	7.15	7.17	7.23	7.31	7.42	0.3
NH(30)	8.20	8.25	8.27	8.31	8.27	8.27	0.07
NH(36)	8.32	8.32	8.27	8.23	8.11	8.08	-0.24



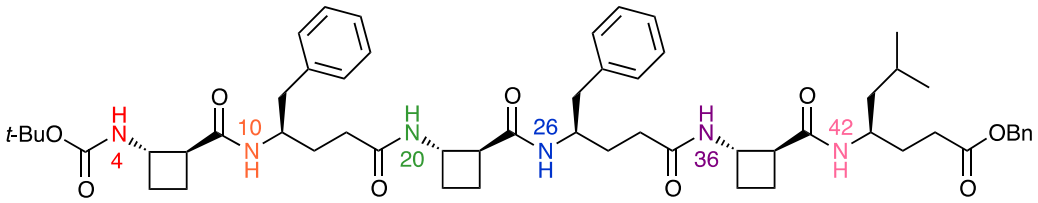
Boc–γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–NHBn (8)



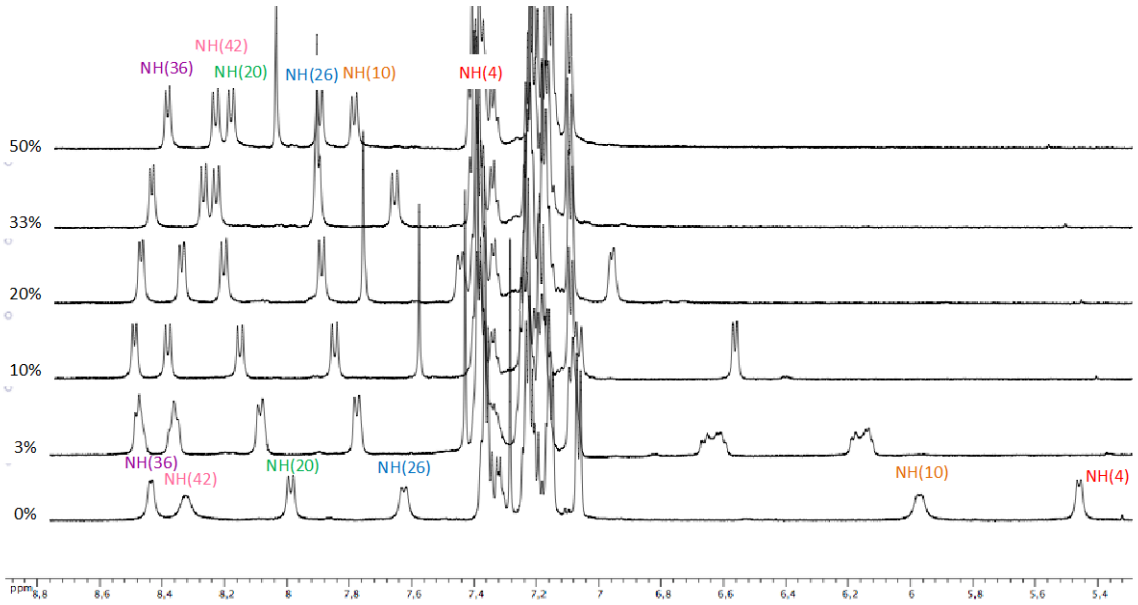
DMSO-d_6 (%. v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	4.63	5.08	5.43	5.86	6.20	6.41	1.78
NH(14)	6.69	7.05	7.62	7.92	8.19	8.36	1.67
NH(20)	6.53	6.70	6.88	7.08	7.20	7.25	0.72
NH(30)	8.13	8.24	8.33	8.41	8.49	8.49	0.36
NH(36)	7.81	7.87	7.89	7.93	7.97	7.97	0.16
NH(44)	8.47	8.52	8.57	8.61	8.64	8.64	0.17



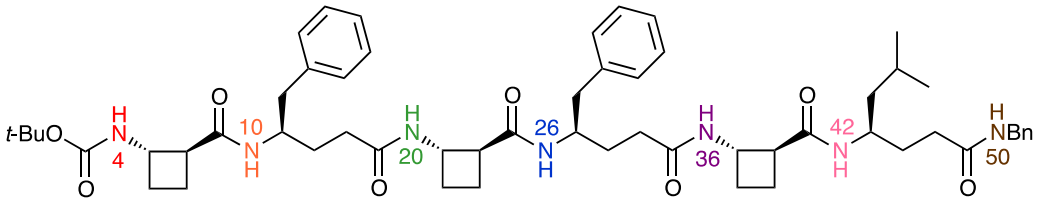
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–OBn (9)



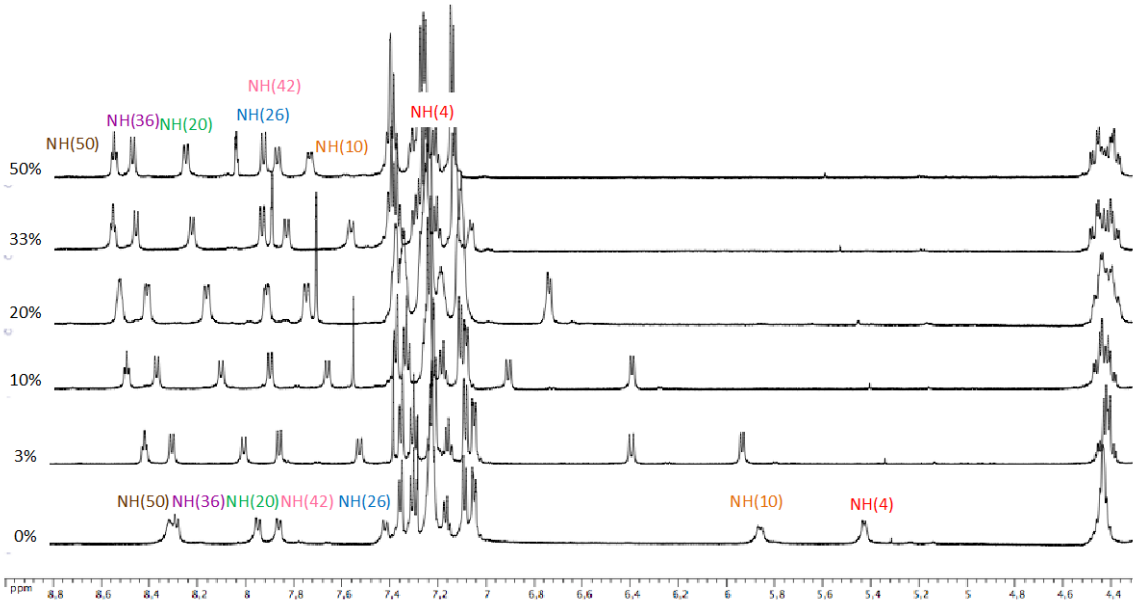
DMSO-d_6 (%. v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	5.83	6.14	6.55	6.94	7.20	7.38	1.55
NH(10)	6.38	6.62	7.05	7.43	7.64	7.78	1.4
NH(26)	7.70	7.77	7.84	7.88	7.89	7.89	0.19
NH(20)	8.06	8.07	8.14	8.19	8.21	8.17	0.11
NH(42)	8.34	8.35	8.38	8.33	8.26	8.24	-0.1
NH(36)	8.47	8.47	8.47	8.46	8.42	8.38	-0.09



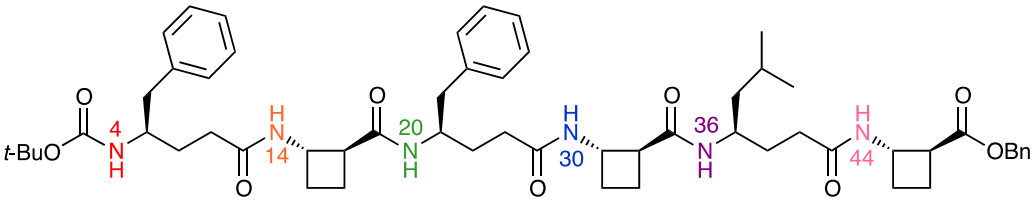
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–NHBn (10)



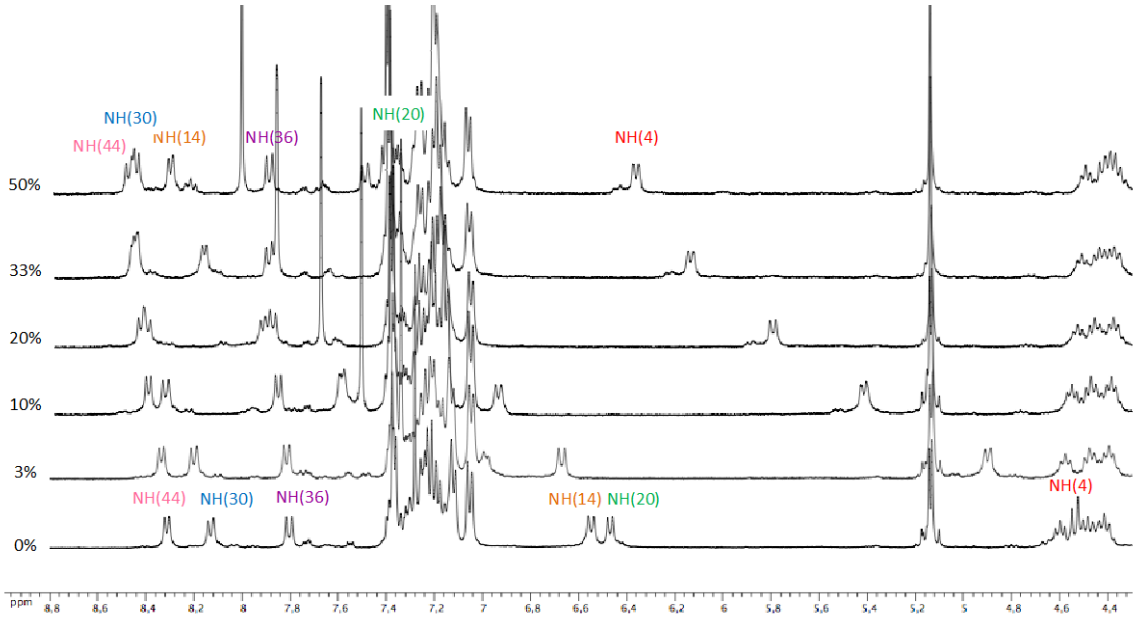
DMSO-d_6 (%. v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	5.43	5.93	6.39	6.73	7.06	7.30	1.87
NH(10)	5.86	6.39	6.91	-	7.56	7.73	1.87
NH(26)	7.42	7.52	7.65	7.74	7.83	7.87	0.45
NH(42)	7.86	7.86	7.90	7.92	7.93	7.93	0.07
NH(20)	7.95	8.00	8.10	8.16	8.22	8.25	0.3
NH(36)	8.28	8.30	8.37	8.41	8.45	8.47	0.19
NH(50)	8.32	8.41	8.49	8.52	8.55	8.55	0.23



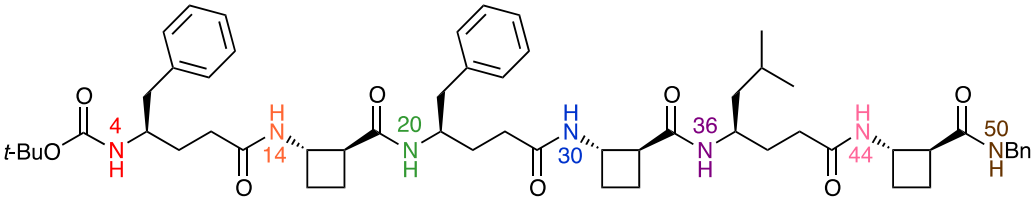
Boc–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-Leu–(1*S*,2*S*)-ACBC–OBn (11)



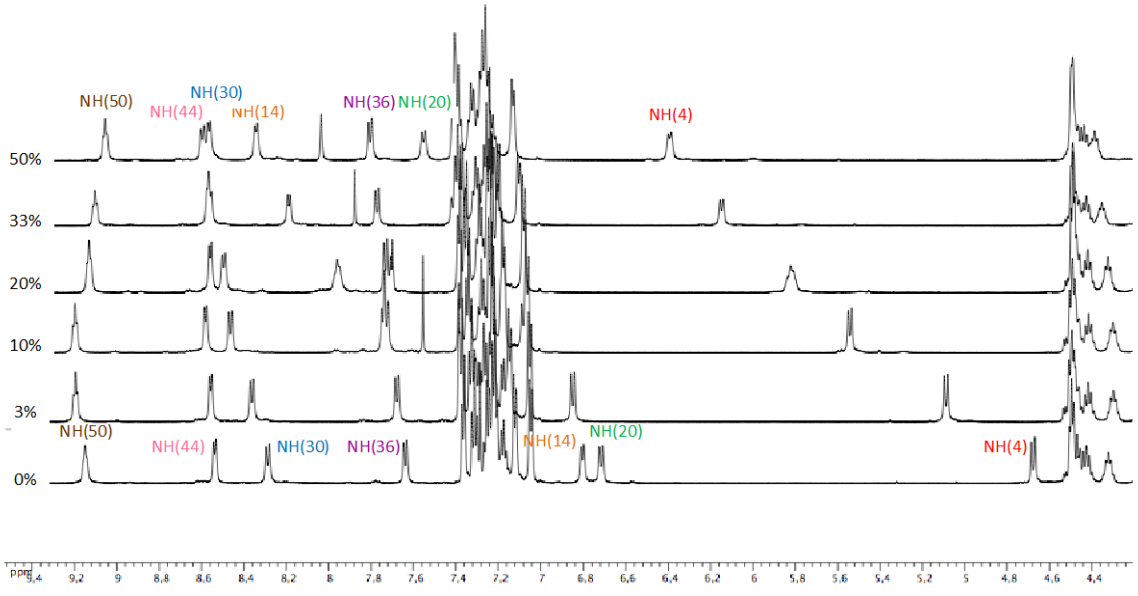
DMSO-d_6 (%, v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	4.54	4.90	5.41	5.79	6.13	6.36	1.82
NH(14)	6.55	6.99	7.59	7.92	8.16	8.30	1.84
NH(20)	6.46	6.67	6.94	-	-	7.49	0.94
NH(36)	7.80	7.81	7.85	7.87	7.89	7.89	0.09
NH(30)	8.13	8.20	8.32	8.39	8.44	8.47	0.34
NH(44)	8.31	8.33	8.39	8.42	8.44	8.44	0.13



Boc–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Phe–(1*S*,2*S*)-ACBC–(*R*)-γ^4-Leu–(1*S*,2*S*)-ACBC–NHBn (12)



DMSO-d_6 (%. v/v)							
NH	**0%**	**3%**	**10%**	**20%**	**33%**	**50%**	**Δδ**
NH(4)	4.68	5.09	5.55	5.81	6.15	6.39	1.71
NH(14)	6.80	-	7.75	7.95	8.19	8.36	1.65
NH(20)	6.71	6.85	7.09	-	-	7.57	0.77
NH(36)	7.64	7.68	7.74	7.74	7.78	7.82	0.18
NH(30)	8.28	8.36	8.47	8.49	8.56	8.61	0.33
NH(44)	8.53	8.56	8.59	8.56	8.57	8.58	0.05
NH(50)	9.15	9.19	9.21	9.13	9.10	9.07	-0.08



3. *ROESY correlations*

ROESY spectra were recorded at 300 K on a Bruker 600 MHz spectrometer. Samples were prepared in $CDCl_3$ at a concentration of 10 mM. The pulse sequence was roesyph. ROESY experiments employed a pulse spinlock of 200 ms. All experiments were performed by collecting 6492 points in f1 and 512 points in f2.

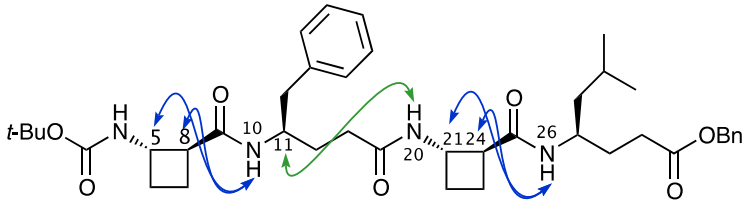
Known[2] ROESY correlations representative of the 8H-membered ring (C8): ⟷

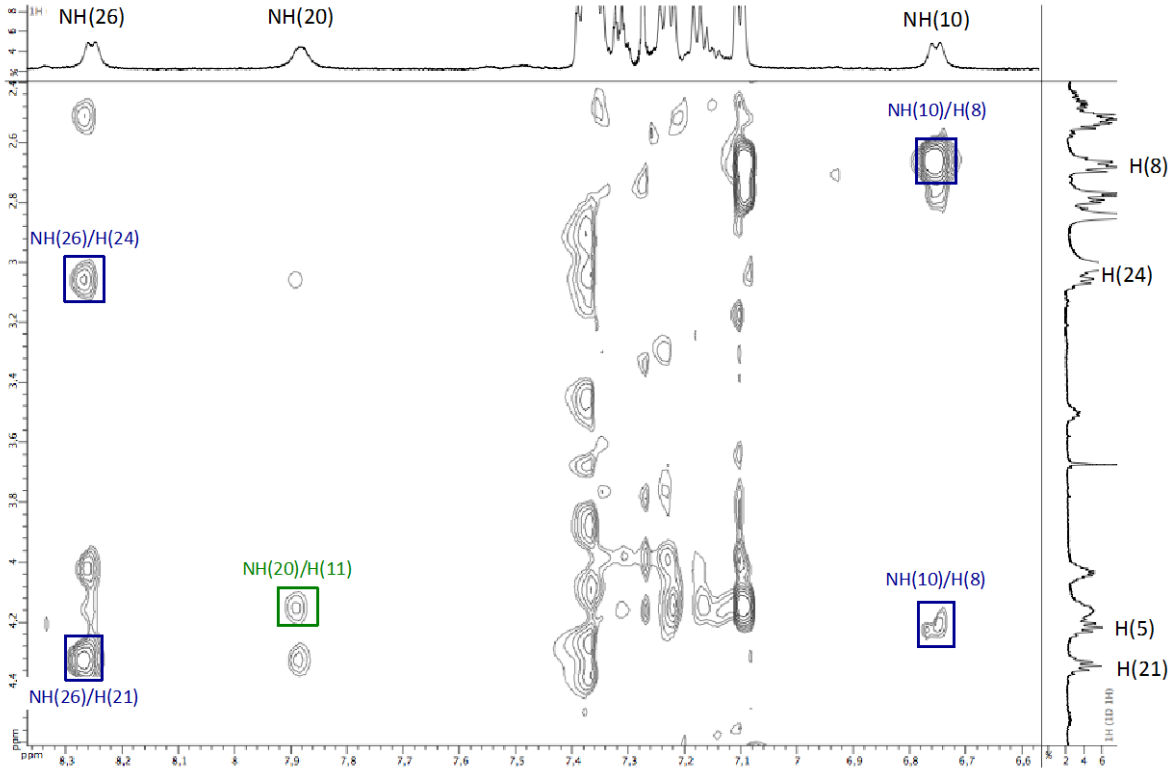
Known[2] ROESY correlations representative of the 9H-membered ring (C9): ⟷

Known[3] ROESY correlations representative of the 13H-membered ring (C13): ⟷

New ROESY correlations representative of the 13H-membered ring (C13): ⟷

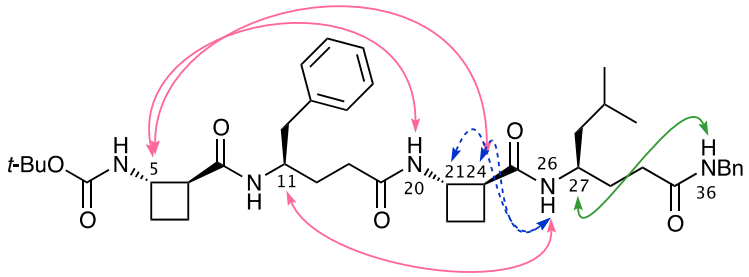
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–OBn (+) - (1)

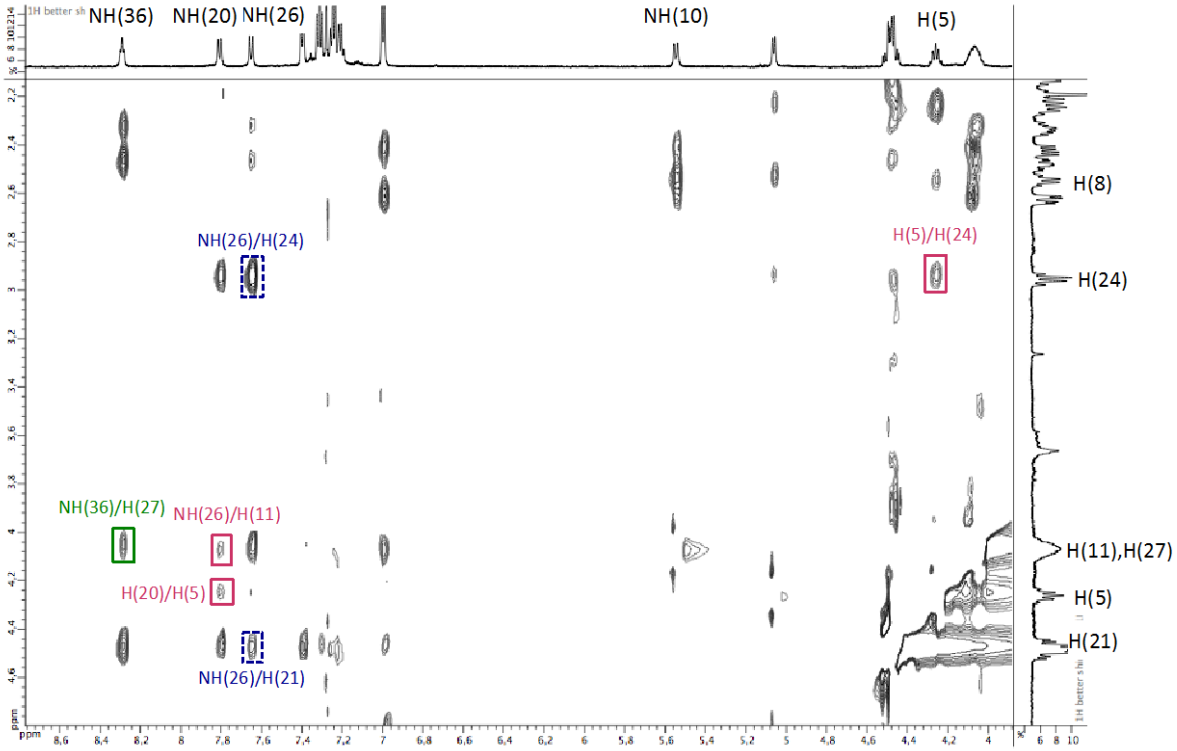




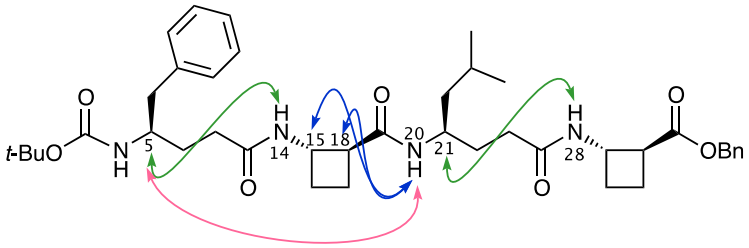
[3] L. Guo, A. M. Almeida, W. Zhang, A. G. Reidenbach, S. H. Choi, I. A. Guzei and S. H. Gellman, J. Am. Chem. Soc., 2010, 132, 7868.

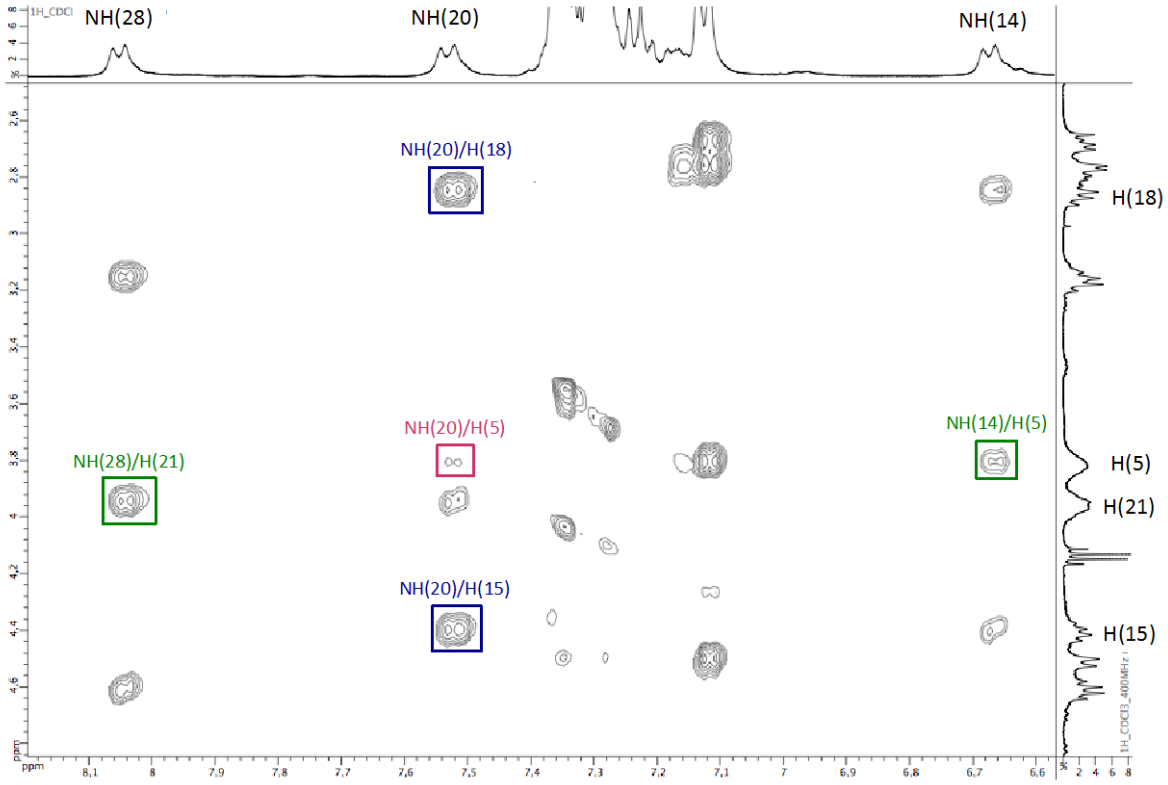
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–NHBn - (2)



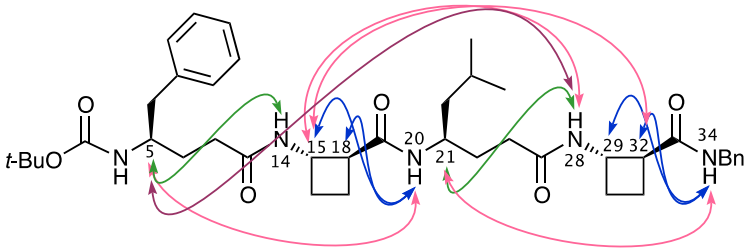


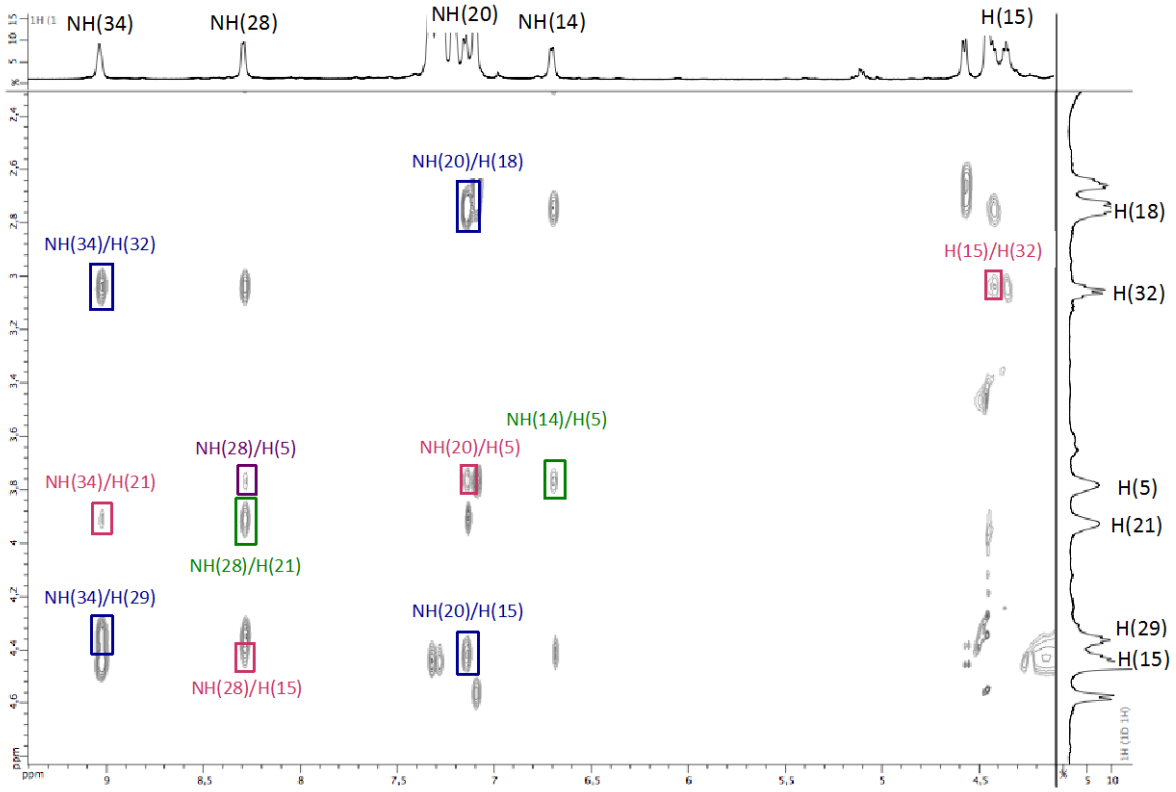
Boc–γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–(1*S*,2*S*)-ACBC–OBn (+) - (3)



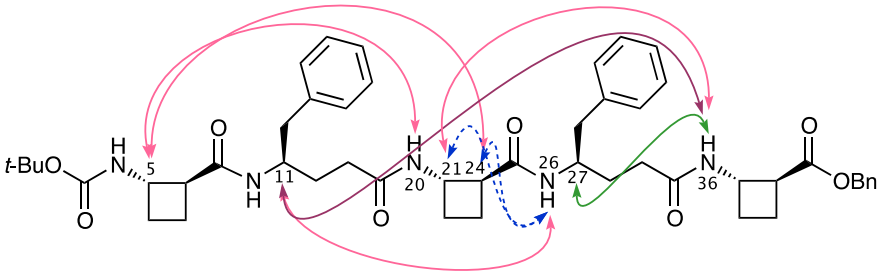


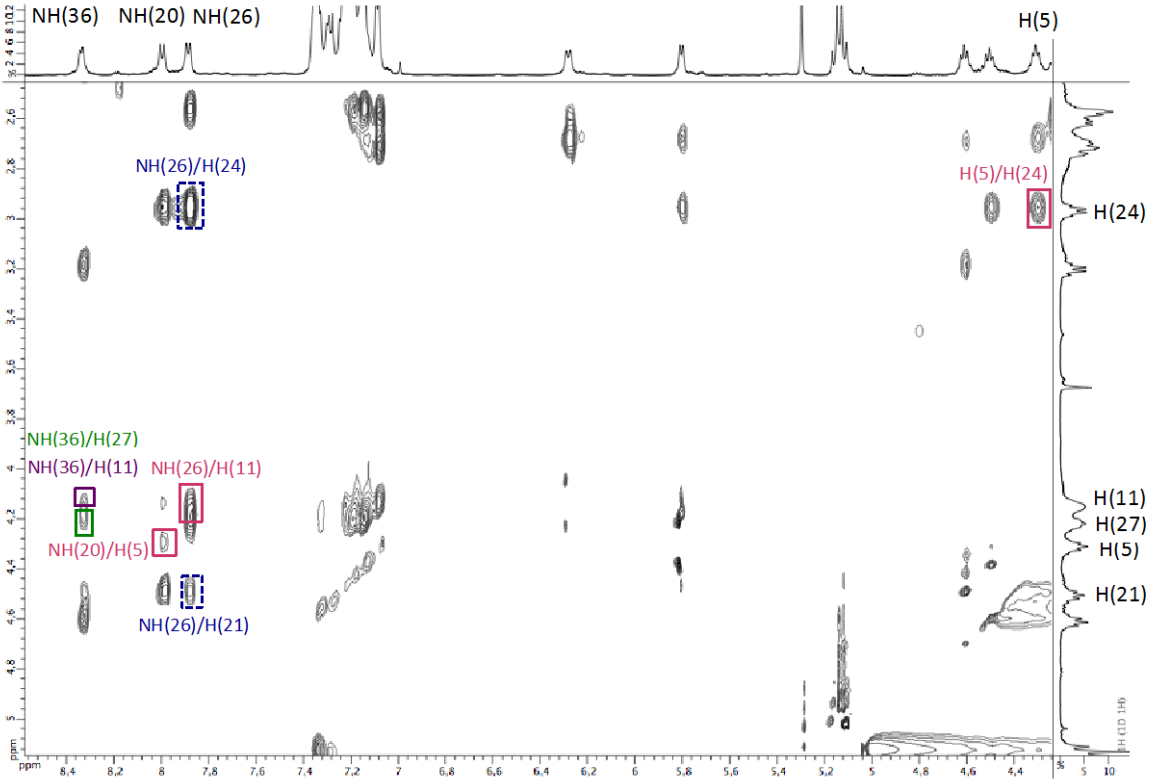
Boc–γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–(1*S*,2*S*)-ACBC–NHBn (+) - (4)



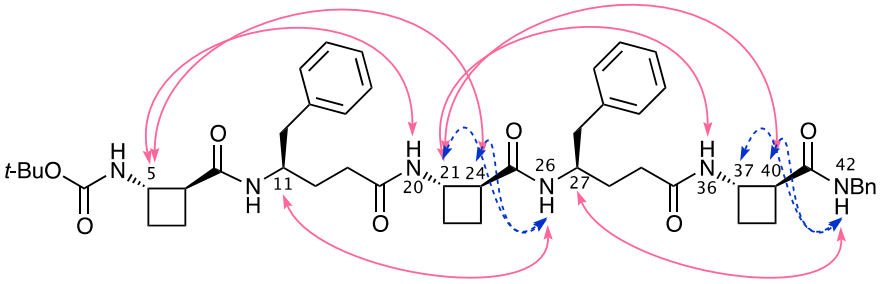


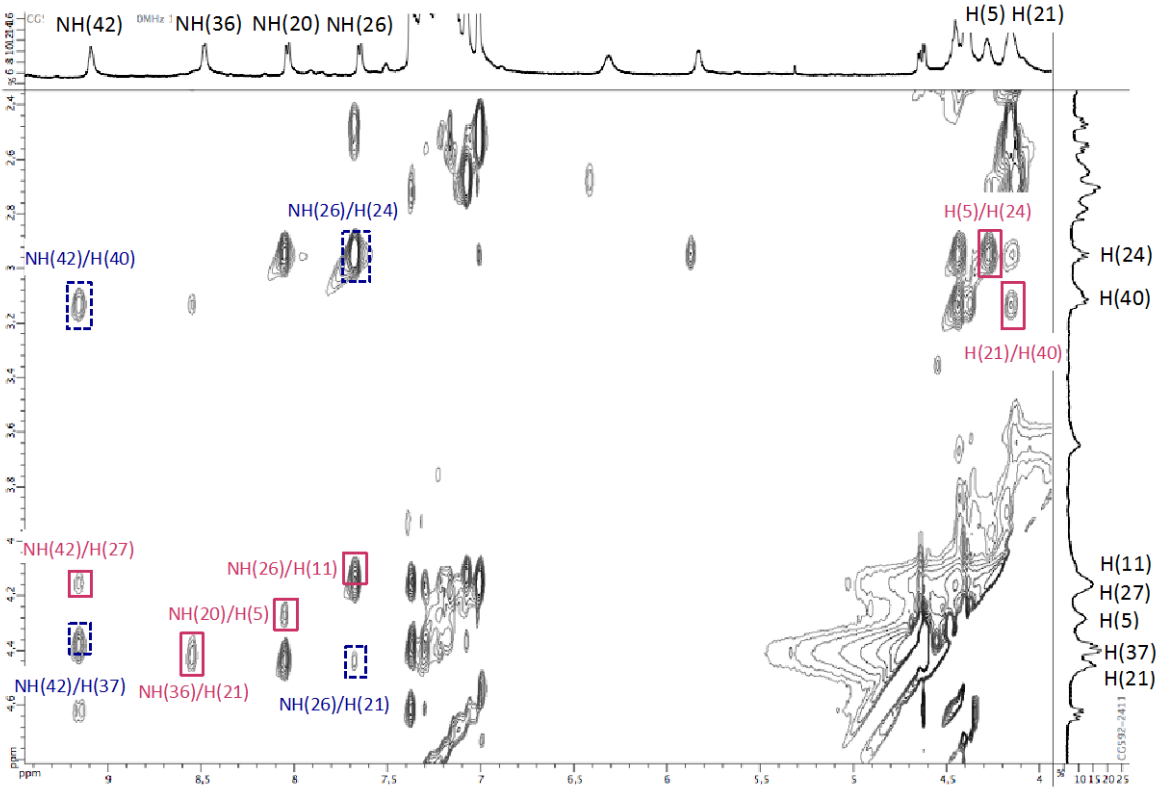
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–OBn (+) - (5)



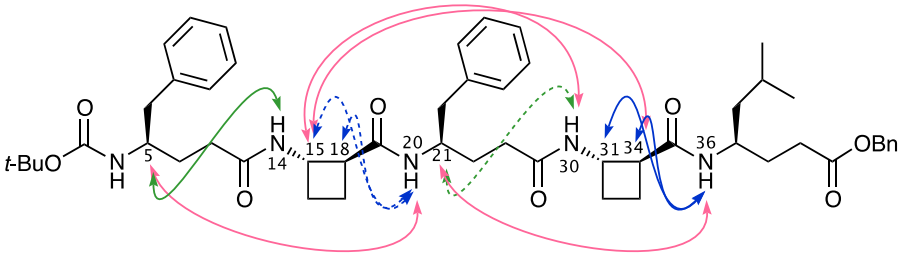


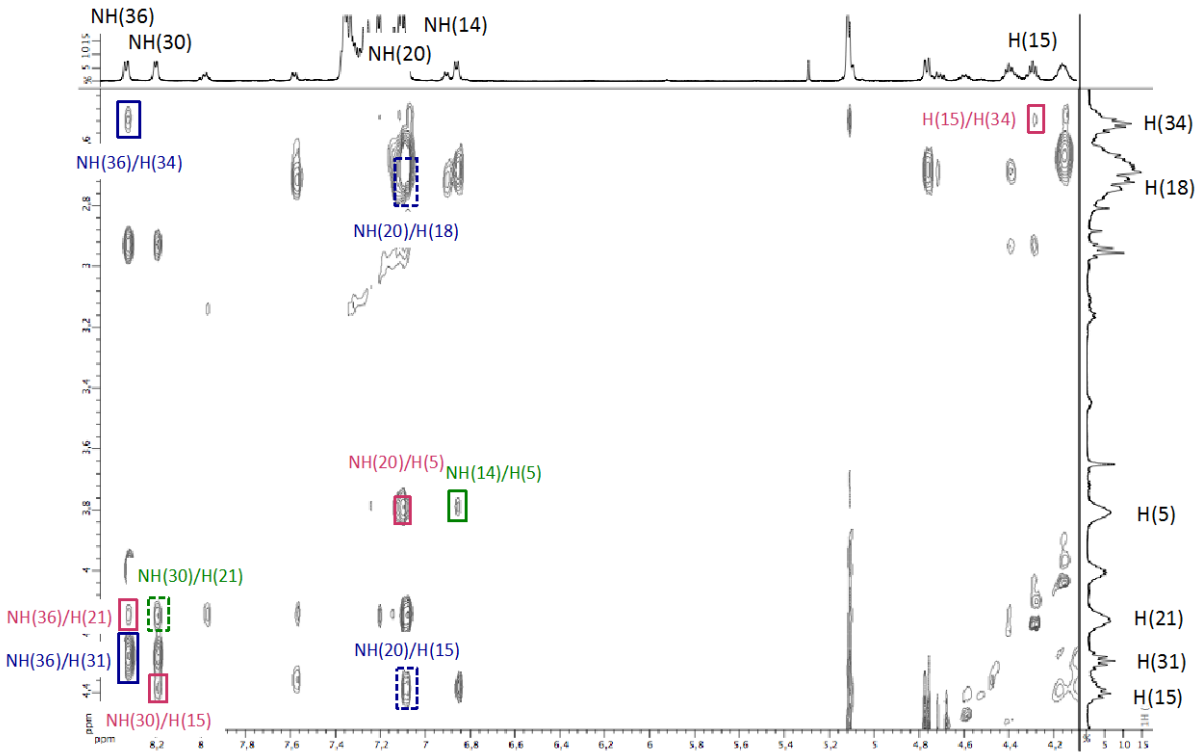
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–NHBn (+) - (6)



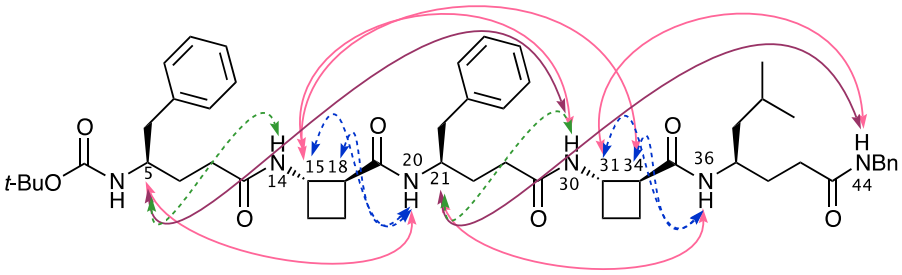


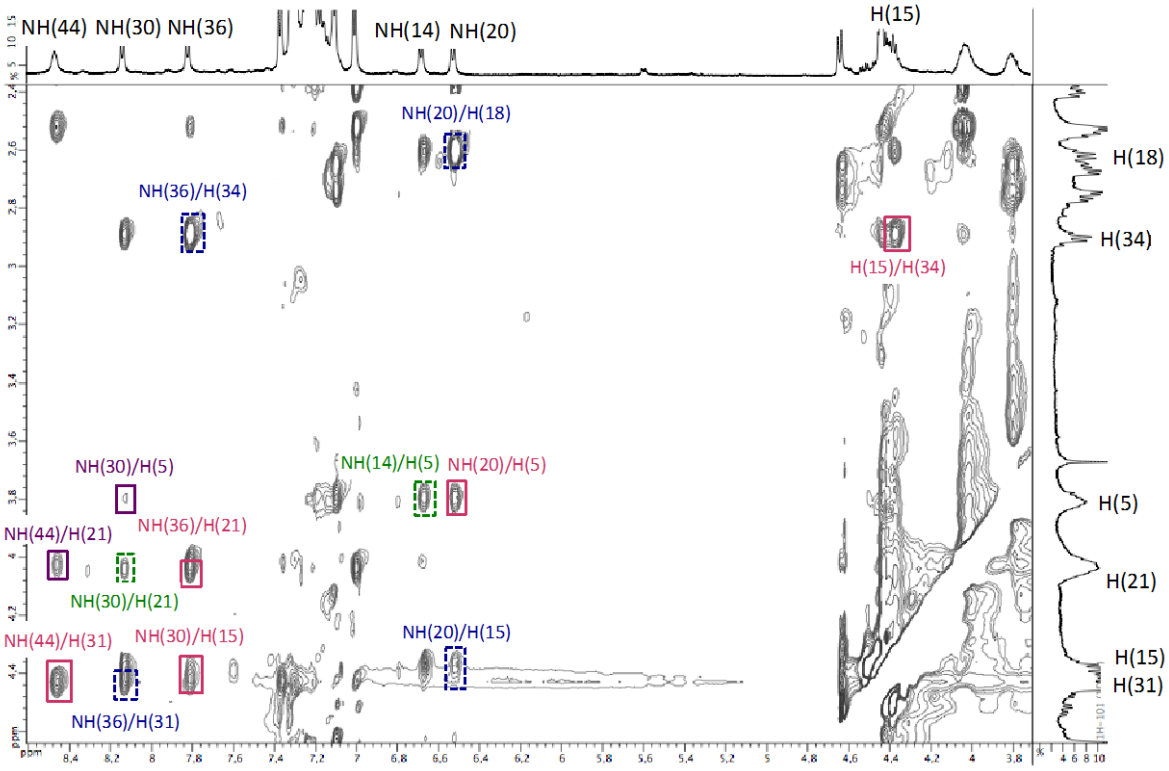
Boc–γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–OBn (+) - (7)



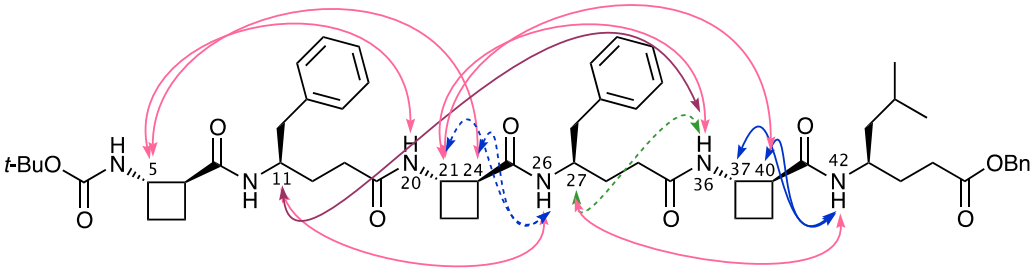


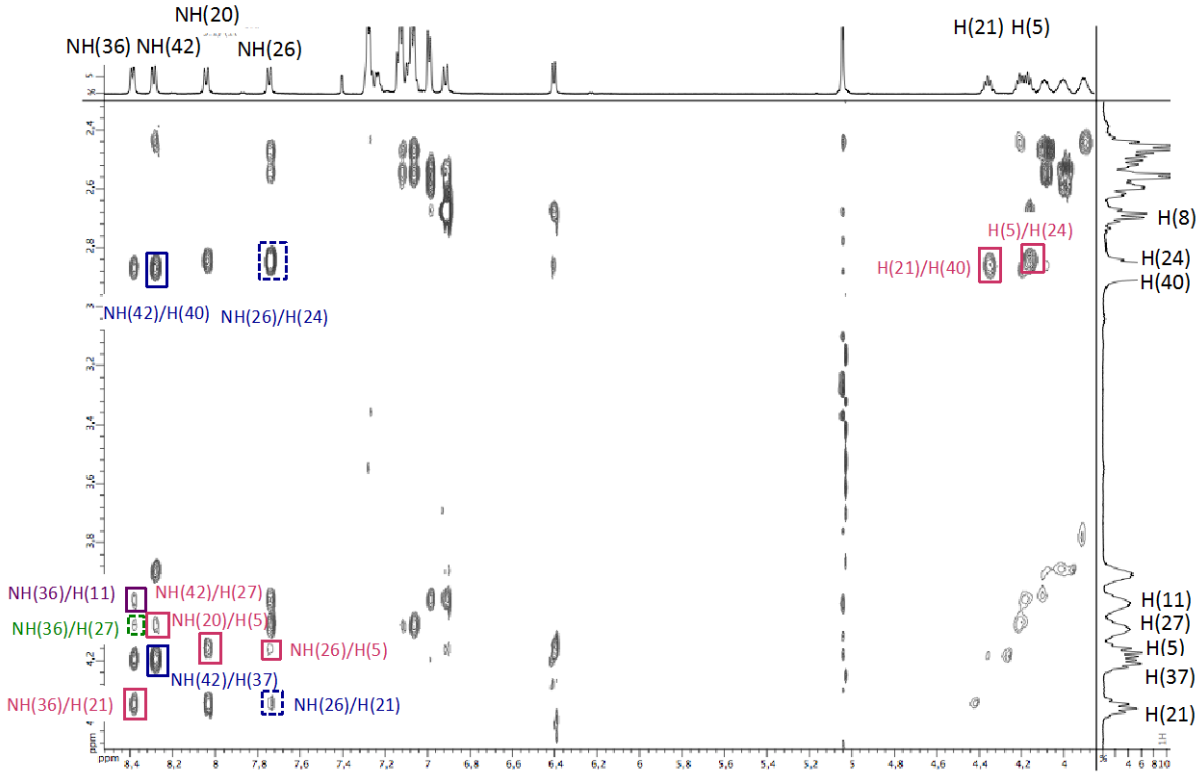
Boc–γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–NHBn (+) - (8)



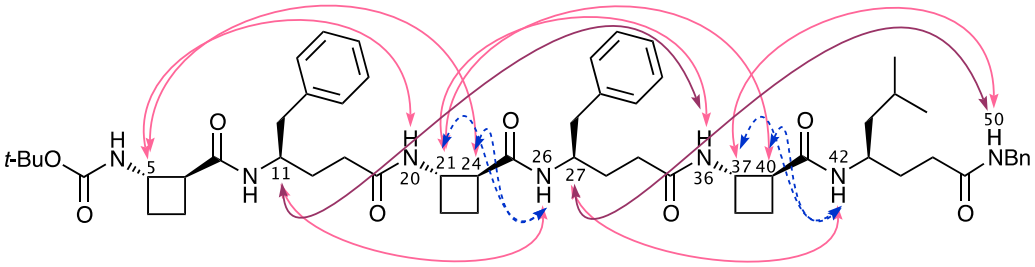


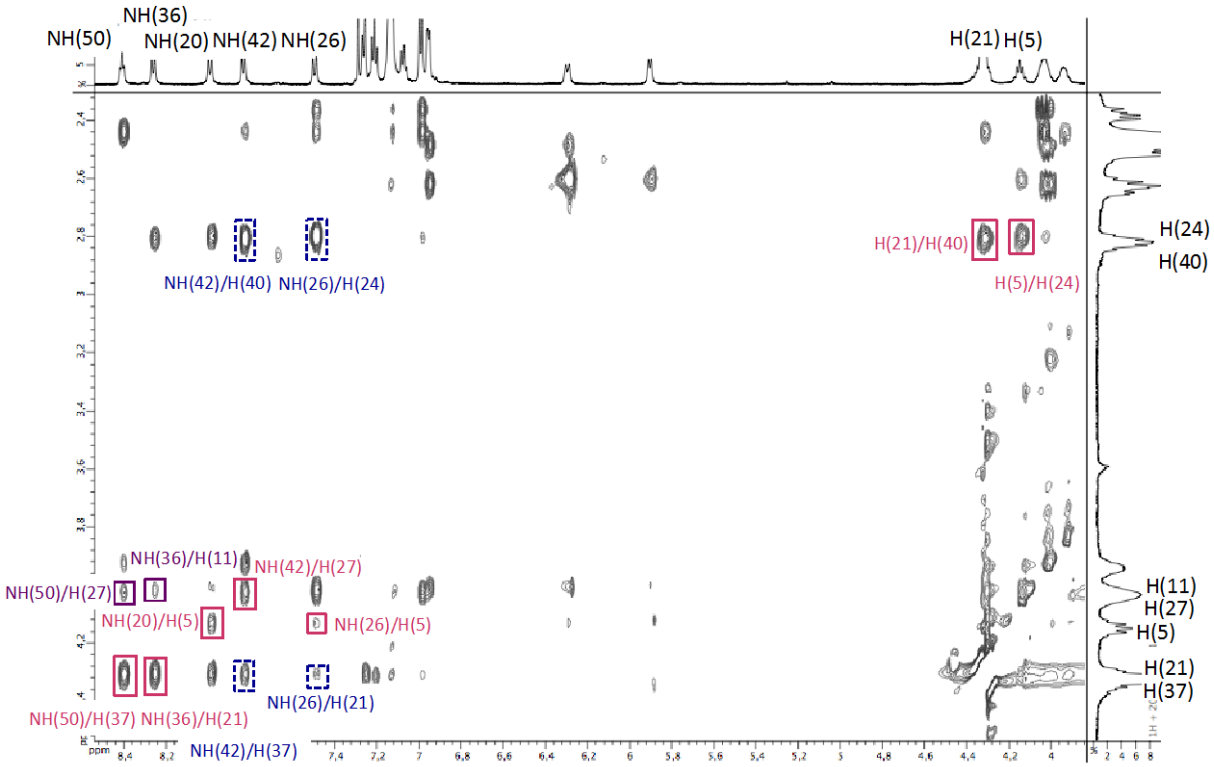
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–OBn (+) - (9)



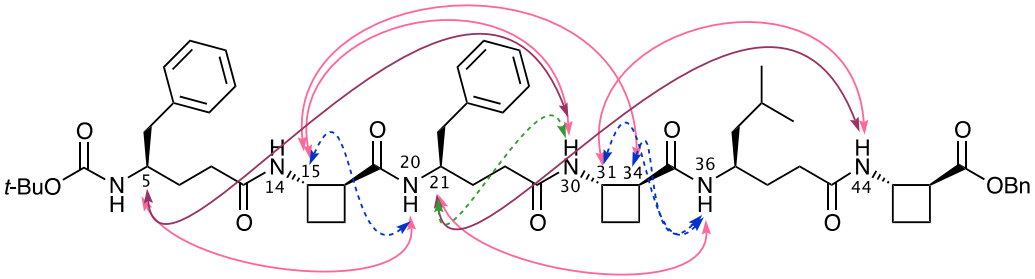


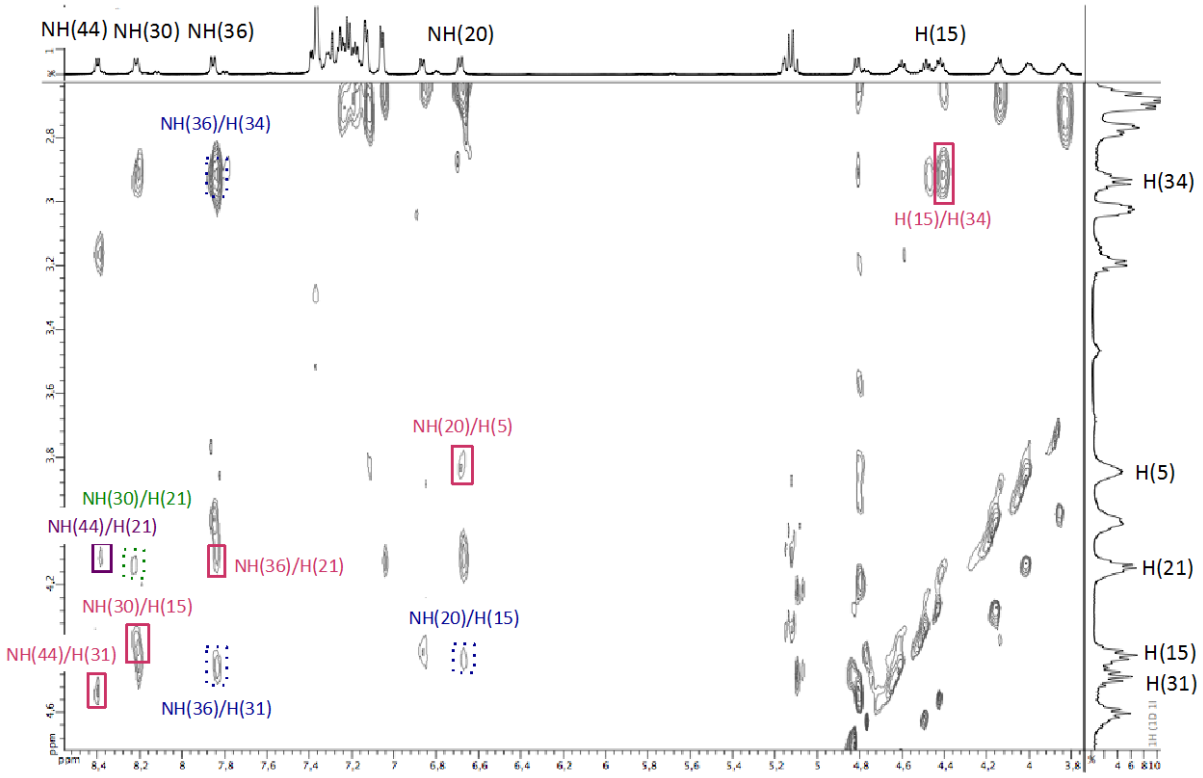
Boc–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–NHBn (+) - (10)



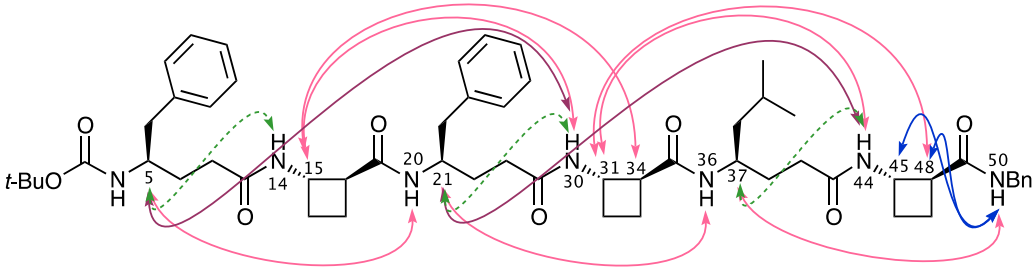


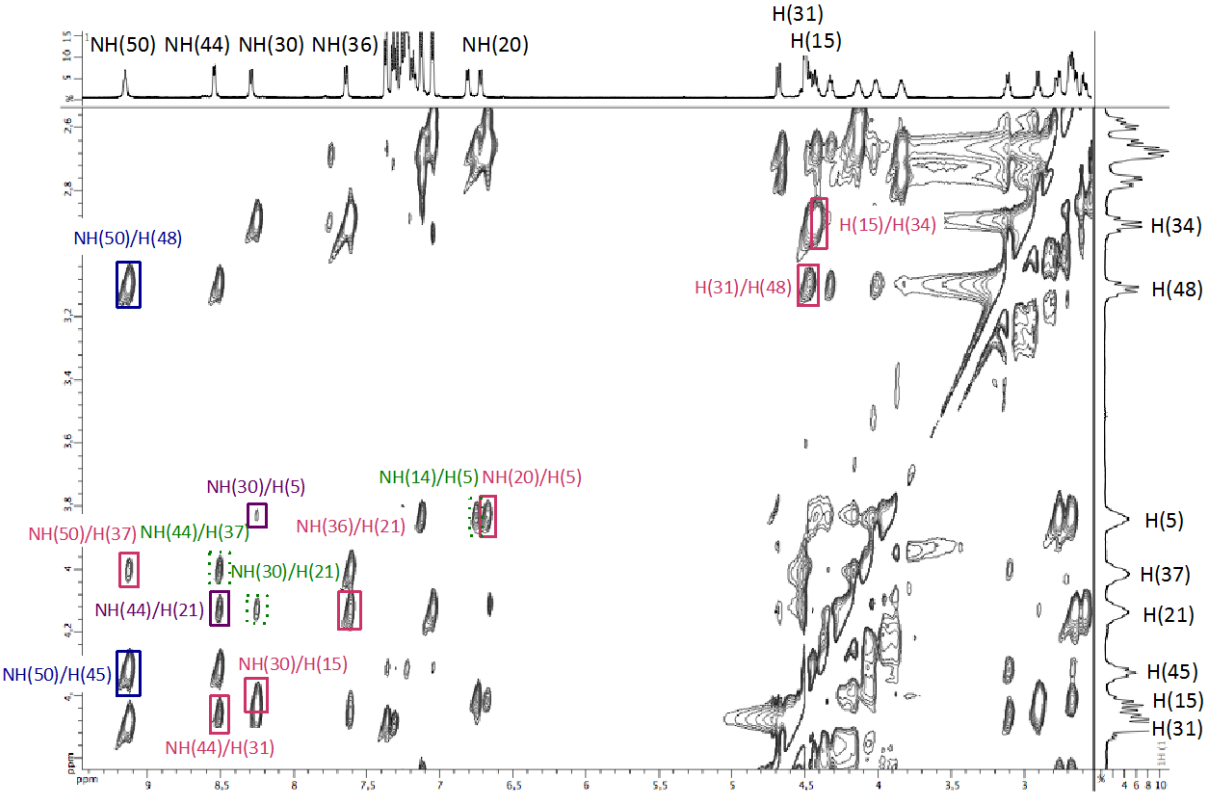
Boc–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–(1*S*,2*S*)-ACBC–OBn (+) -(11)





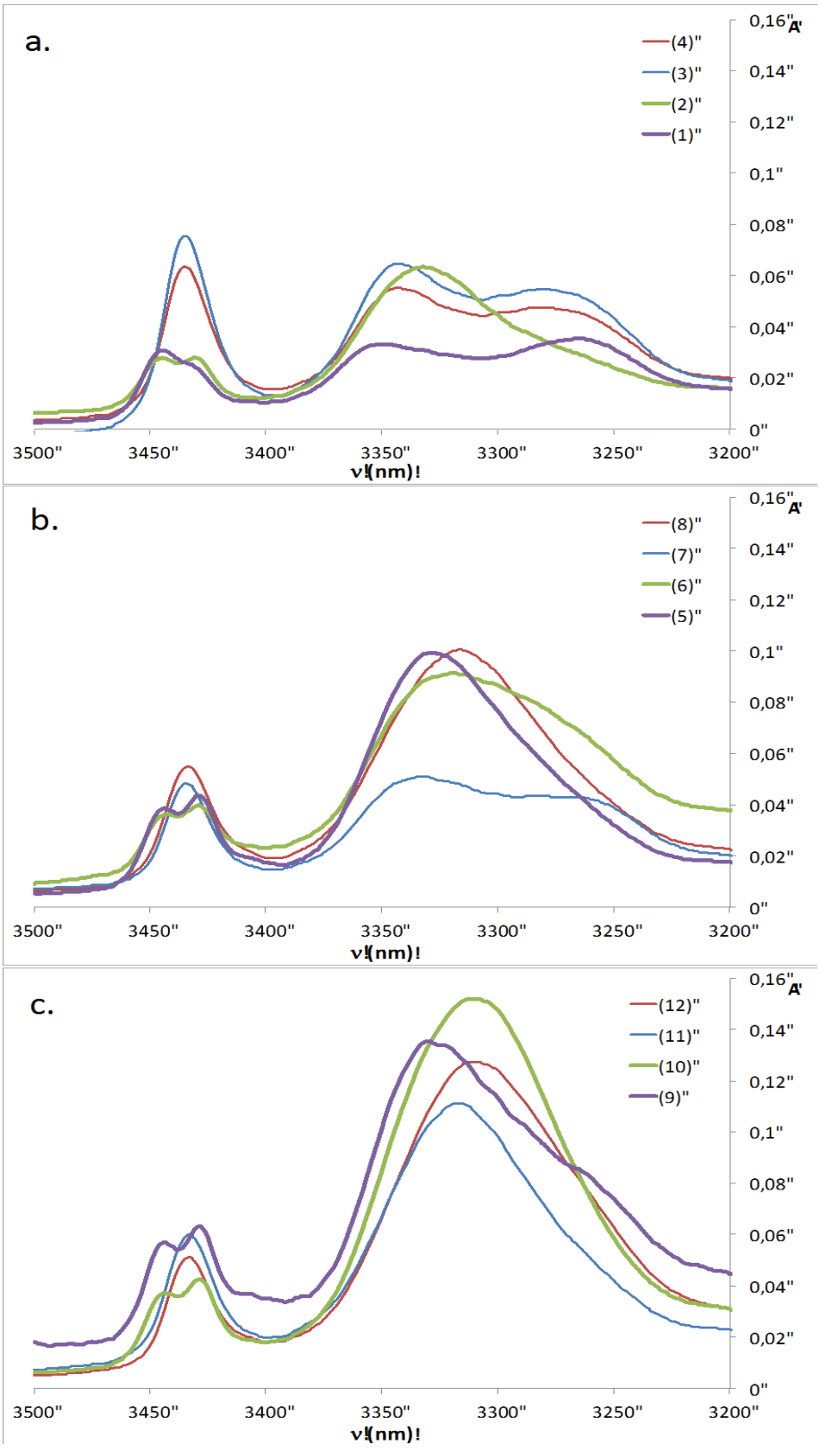
Boc–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Phe–(1*S*,2*S*)-ACBC–(*R*)-γ⁴-*h*Leu–(1*S*,2*S*)-ACBC–NHBn (+) - (12)





III. INFRARED SPECTRA

Infrared spectra were recorded in chloroform solutions at a concentration of 10 mM.



IV. CIRCULAR DICHROISM

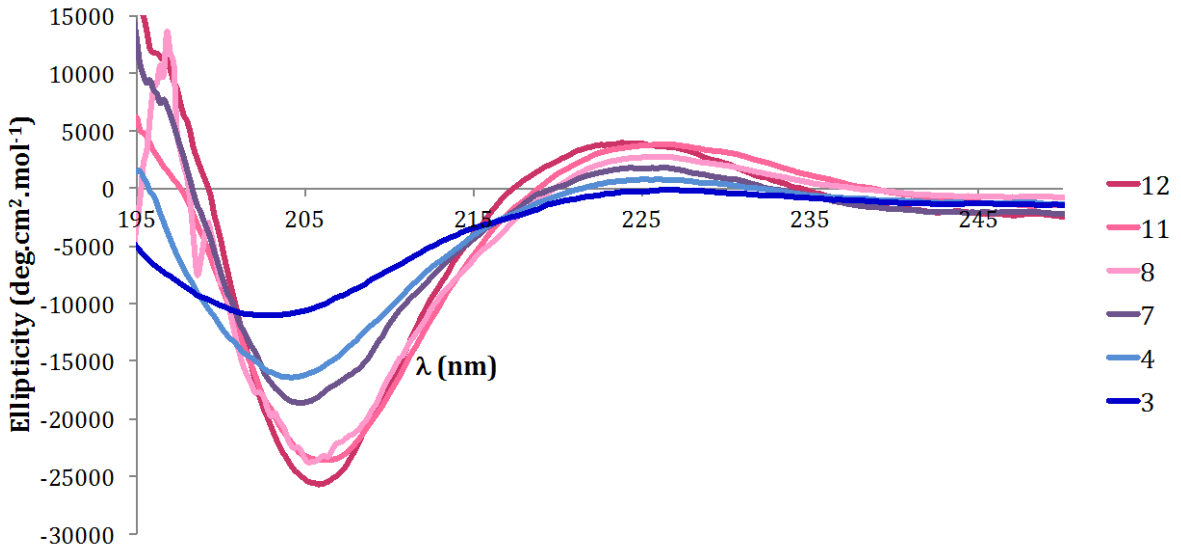
The far-UV circular dichroism spectra were recorded for solutions in MeOH (0.20 mM) in 1 mm sample cells at 20 °C.

The observed ellipticity, θ_{obs}, was converted into mean residue molar ellipticity, [Θ], ($deg \cdot cm^2 \cdot dmol^{-1}$) according to the equation:

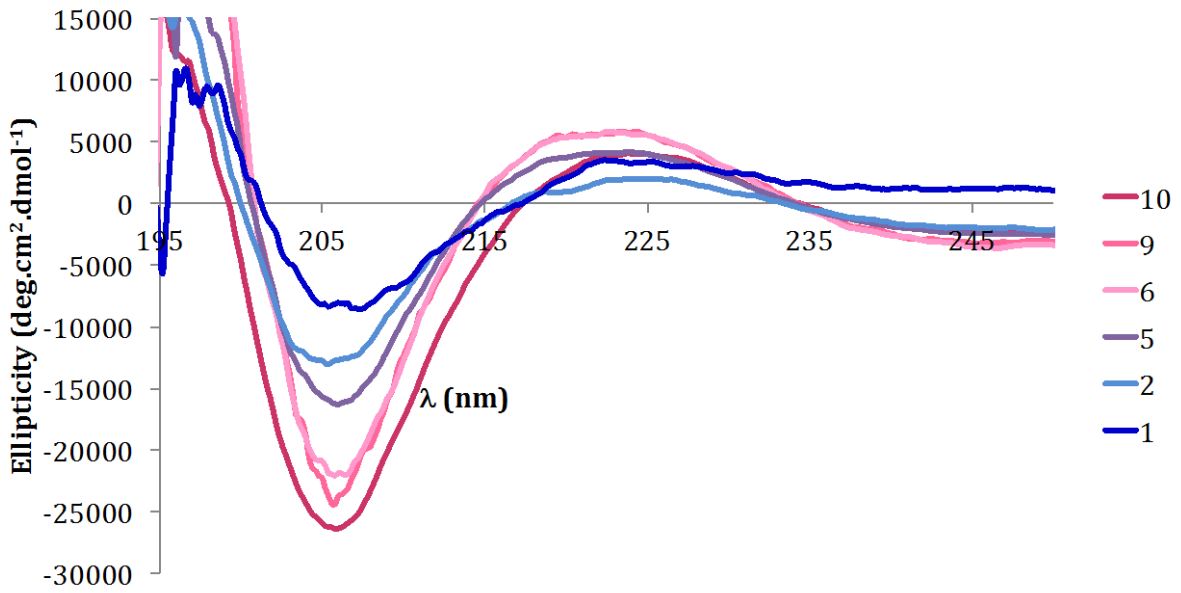
$$[\Theta] = \frac{\theta_{obs}}{10 \times c \times n \times l}$$

θ_{obs}: observed ellipticity (mdeg),
c: concentration ($mol \cdot L^{-1}$),
n: number of residues,
l: cell path length (cm).

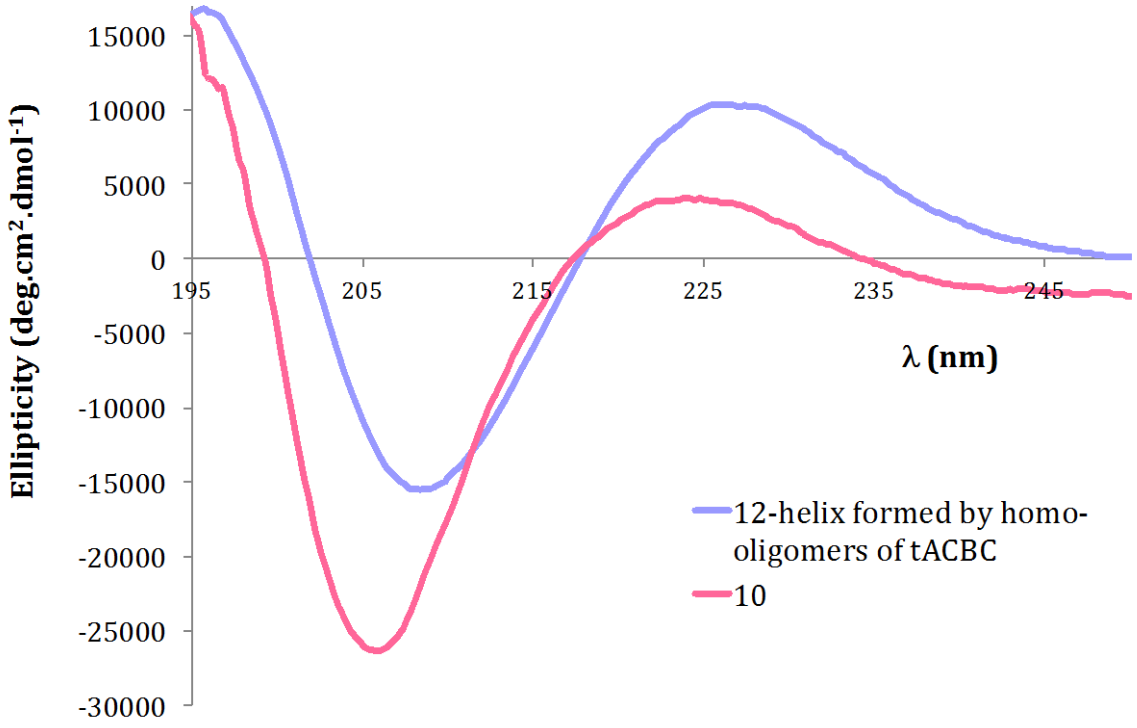
Peptides with γ^4-amino acids at N-terminal



Peptides with tACBC at N-terminal



Comparison of the CD spectra of the β-peptide 12-helix and the β/γ-peptide 13-helix



V. MOLECULAR MODELLING

1. Conformations obtained from a hybrid MCMM calculation

A hybrid Monte Carlo Molecular Mechanics (MCMM) conformational search in a chloroform medium was carried out on each of the twelve peptides **1**-**12** using Macromodel 04 software from Schrödinger and the MMFF force field without restraints.
For each peptide, 10 000 conformers were generated by MCMM. From this collection, low energy conformers (< 20 $kJ.mol^{-1}$ of relative energy) were retained. Different types of conformations were observed and were sorted according to the hydrogen-bonded ring systems they displayed:

- 9/8-ribbon type conformers, composed of alternating 9- and 8-membered H-bonded rings,
- 13-helix conformers, composed only of successive 13-membered H-bonded rings,
- "mixed" conformers, composed of various combinations of 8-, 9-, 13- and 18-membered H-bonded rings.

The conformers are identified and their relative abundances indicated in the table below.

For conformer identification, the following notation system is used:
Discreet, successive rings are separated by the symbol '-'.
The 13-membered H-bonded rings which implicate a carbonyl oxygen that is bifurcated between two amide hydrogens form two rings, wherein the larger ring includes the smaller; the combined system is noted with the symbol ','.

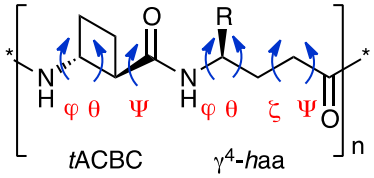
9/8-ribbon conformers are highlighted thus; 13-helix conformers are highlighted thus.

Conformations	**Abundance of each conformer family** (number of conformers of that family/total number of conformers < 20 kJ.mol^{-1}; expressed as %)	**Conformations**	**Abundance of each conformer family** (number of conformers of that family/total number of conformers < 20 kJ.mol^{-1}; expressed as %)
Peptide **1** (135 conformers < 20 kJ.mol^{-1})		Peptide **2** (88 conformers < 20 kJ.mol^{-1})	
13-13	42	13-13-13	10
8-9,13	14	8-9,13-8	19
8-9-8	**44**	8-9-8,13	7
		8-9-8-9	**44**
Peptide **3** (276 conformers < 20 kJ.mol^{-1})		Peptide **4** (389 conformers < 20 kJ.mol^{-1})	
13-9	5	13-13-13	2
9-8-9	**95**	13-9,13	3
		13-13-8	15
		13-9-8	6
		9-8-9,13	6
		9-8-9-8	**68**
Peptide **5** (100 conformers < 20 kJ.mol^{-1})		Peptide **6** (101 conformers < 20 kJ.mol^{-1})	
13-13-13	**84**	13-13-13-13	34
13-13-9	16	13-13-9,13	5
		13-13-13-8	**35**
		13-13-9-8	12
		8-9-13-13	14
Peptide **7** (66 conformers < 20 kJ.mol^{-1})		Peptide **8** (134 conformers < 20 kJ.mol^{-1})	
13-13-13	**59**	13-13-13-13	**100**
9-13-13	34		
13-13-8	6		
Peptide **9** (75 conformers < 20 kJ.mol^{-1})		Peptide **10** (99 conformers < 20 kJ.mol^{-1})	
13-13-13-13	**65**	13-13-13-13-13	**92**
13-13-13-8	19	8-18-8-18	5
13-13-9-8	9	13-13-13-8-18	3
8-13-13-13	7		
Peptide **11** (98 conformers < 20 kJ.mol^{-1})		Peptide **12** (127 conformers < 20 kJ.mol^{-1})	
13-13-13-13	**75**	13-13-13-13-13	**86**
13-13-13-9	25	13-13-13-13-8	7
		9-13-13-13-13	7

2. *DFT optimization of 13-helices*

The geometries of the 13-helix conformers of hexapeptides **9**-**12** obtained in the above MCMM search were each optimized by DFT using GAUSSIAN 09 and the B3LYP/6-311G(d,p) basis set in a chloroform medium. Images are presented in the manuscript (Figure 4).

The dihedral angles of the backbone residues of the 13-helix conformers are presented in the table below. Dihedral angles are defined conventionally, as shown in the illustration.



13-helix conformer	residue	φ	θ	ζ	ψ
Peptide 9	*t*ACBC-1	–104.2	99.9		–111.8
	γ⁴-Phe-2	–128.2	58.4	61.9	–131.3
	*t*ACBC-3	–103.9	100.1		–103.2
	γ⁴-Phe-4	–121.2	49.9	62.4	–139.0
	*t*ACBC-5	–99.8	101.7		–100.2
	γ⁴-Leu-6	–106.9	60.7	67.0	–158.4
Peptide 10	*t*ACBC-1	–105.7	99.2		–113.4
	γ⁴-Phe-2	–124.0	62.1	56.7	–134.8
	*t*ACBC-3	–101.7	100.7		–98.4
	γ⁴-Phe-4	–129.0	49.5	60.8	–130.8
	*t*ACBC-5	–107.6	102.5		–96.3
	γ⁴-Leu-6	–106.3	61.1	179.3	122.6
Peptide 11	γ⁴-Phe-1	–112.8	55.4	62.3	–149.9
	*t*ACBC-2	–110.8	104.4		–94.0
	γ⁴-Phe-3	–127.9	44.0	56.0	–134.5
	*t*ACBC-4	–93.6	105.8		–106.9
	γ⁴-Leu-5	–142.4	53.6	58.7	–120.6
	*t*ACBC-6	–108.9	103.1		–65.2
Peptide 12	γ⁴-Phe-1	–117.4	53.3	60.7	143.4
	*t*ACBC-2	–105.9	105.8		–96.7
	γ⁴-Phe-3	–133.0	45.7	56.2	–130.2
	*t*ACBC-4	–95.9	104.6		–113.0
	γ⁴-Leu-5	–127.7	51.5	59.7	–127.8
	*t*ACBC-6	–109.4	104.5		–102.4

3. *Superimposition of oligo-Ala and peptide **10***

An α-helix of a C- and N-capped oligo-Ala octapeptide (Boc-AAAAAAAA-OBn) was generated by using Macromodel 04 software from Schrödinger with the appropriate constraints. The geometry was optimized by DFT using GAUSSIAN 09 and the B3LYP/6-311G(d,p) basis set in a chloroform medium. The backbones of the α-helix of oligo-Ala and peptide **10** were superimposed using γ⁴-amino acids C(α) for alignment using PyMOL 1.3. Images are presented in the manuscript (Figure 5).