Electronic Supplementary Information (ESI)

Unexpectedly Rigid Short Peptide Foldamers in which NH-π and CH-π Interactions are Preserved in Solution

Luhan Zhai[1], Masayuki Nara[2], Yuko Otani[1], Tomohiko Ohwada[*,1]

[1] *Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan*
[2]*Deparment of Chemistry, College of Liberal Arts and Sciences, Tokyo Medical and Dental University, Ichikawa, Chiba 272-0827, Japan*

E-mail: ohwada@mol.f.u-tokyo.ac.jp

Table of Contents

1.Supporting graphics and Tables

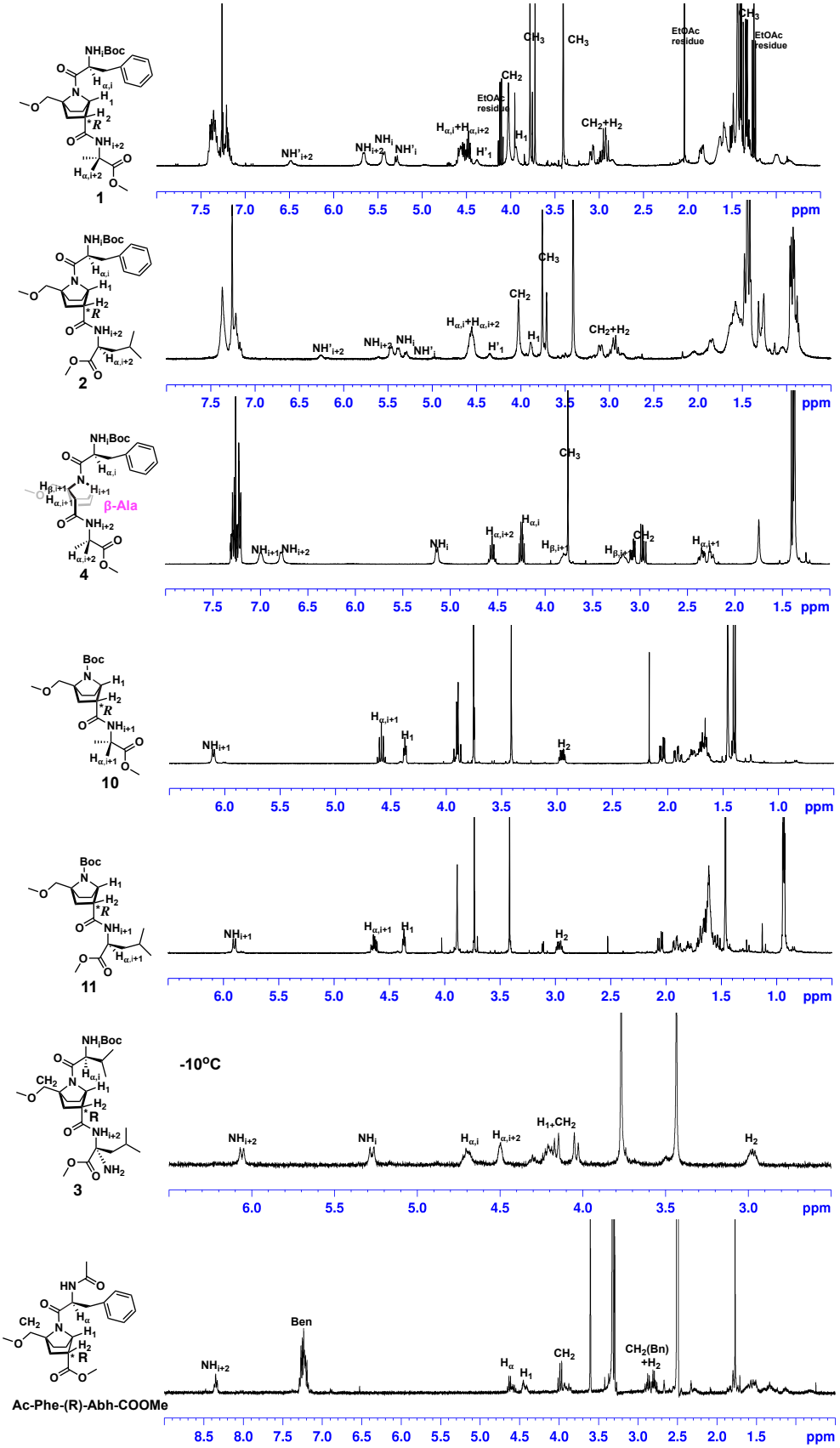


Figure S1, 1H-NMR of **peptide 1, 2, 4, 10** and **11** in $CDCl_3$ at 25°C, **peptide 3** in

$CDCl_3$ at -10°C and dipeptide Ac-Phe-(R)-Abh-COOMe in DMSO at 25°C. Tripeptides **1** and **2** have two sets of peaks. Tripeptide **4**, dipeptides **10**, **11** and Ac-Phe-(R)-Abh-COOMe have a single set of peak.

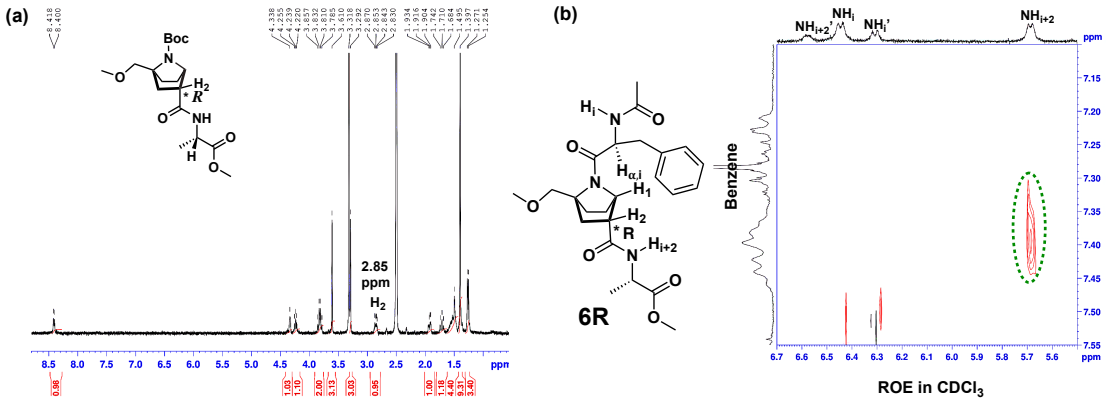


Figure S2, (a)^{1}H-NMR of Boc-(*R*)-Abh-Ala-OMe **10** in DMSO at 25 °C. (b) Enlarged ROE of **6R** in $CDCl_3$.

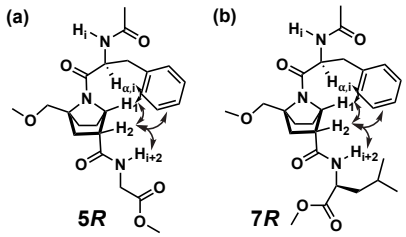


Figure S3. Key ROEs in the major component in DMSO. (a) Tripetide **5R** (b) Tripetide **7R**.

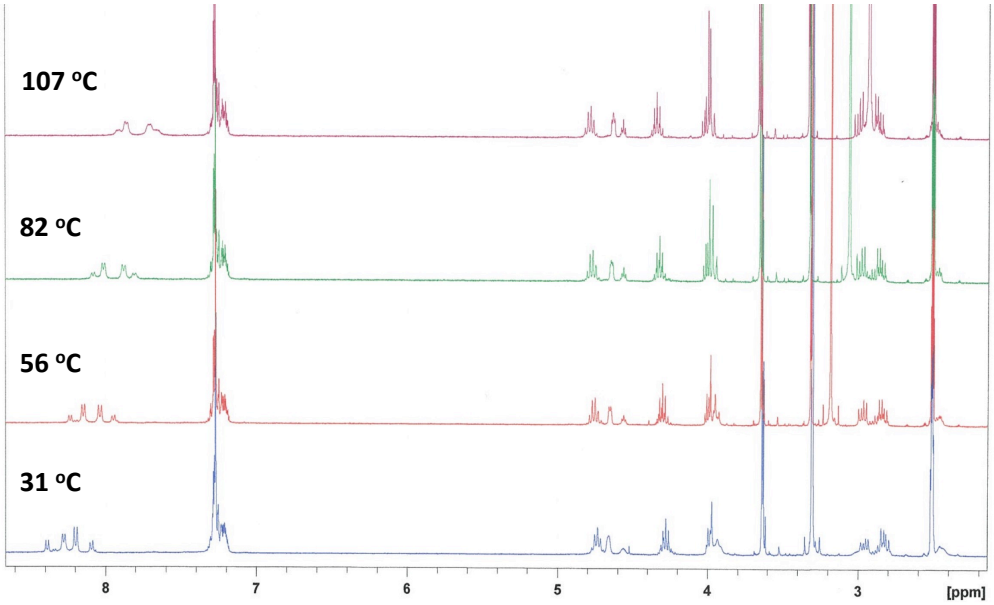


Figure S4, Variable-Temperature NMR of tripeptide **6R** in DMSO. The temperature is after calibration. As the temperature increases, the ratio of major to minor conformer didn't change obviously (around 2.3:1). Thus, the major and minor conformers are independent.

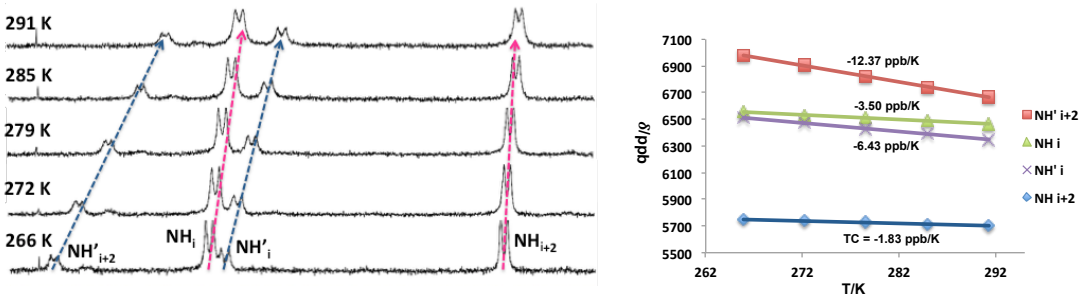


Figure S5. Temperature dependence of the NMR signals of **6R** in $CDCl_3$. Major component (folded form): NH_{i+2}: Ala-NH; NH_i: Phe-NH. Minor component (unfolded form): NH'_{i+2}: Ala-NH; NH_i': Phe-NH.

Table S1. Temperature Coefficient (ppb/K) of tripeptide 5R, 6R and 7R.

5R				
	Phe-NH major	Phe-NH minor	Gly-NH major	Gly-NH minor
In $CDCl_3$	-5.02	-10.28	**-4.09**	-13.97
In DMSO	-6.59	-5.80	**-4.14**	-5.13
In CD_3OH	-8.81	-8.23	**-6.19**	-5.77
6R				
	Phe-NH major	Phe-NH minor	Ala-NH major	Ala-NH minor
In $CDCl_3$	-3.50	-6.43	**-1.83**	-12.37
In DMSO	-6.30	-5.69	**-5.22**	-6.02
In CD_3OH	-8.56	-8.16	**-6.97**	-5.81
7R				
	Phe-NH major	Phe-NH minor	Leu-NH major	Leu-NH minor
In $CDCl_3$	-3.54	-7.35	**-1.20**	-10.28
In DMSO	-6.59	-5.93	**-5.36**	-6.37
In CD_3OH	N/A[a]	N/A[a]	N/A[a]	N/A[a]

[a] the separation of the peaks in CD_3OH is not good, so the data is not available.

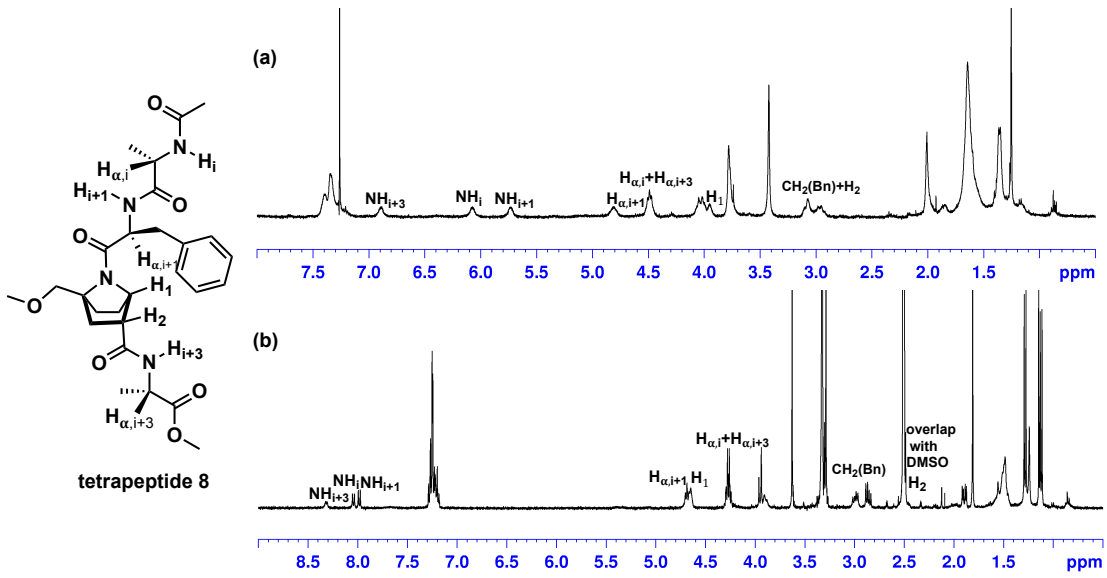


Figure S6, (a) ^{1}H-NMR of tetrapeptide **8** in $CDCl_3$ at 25°C. (b) ^{1}H-NMR of tetrapeptide **8** in DMSO at 25°C. They all show one set of peak.

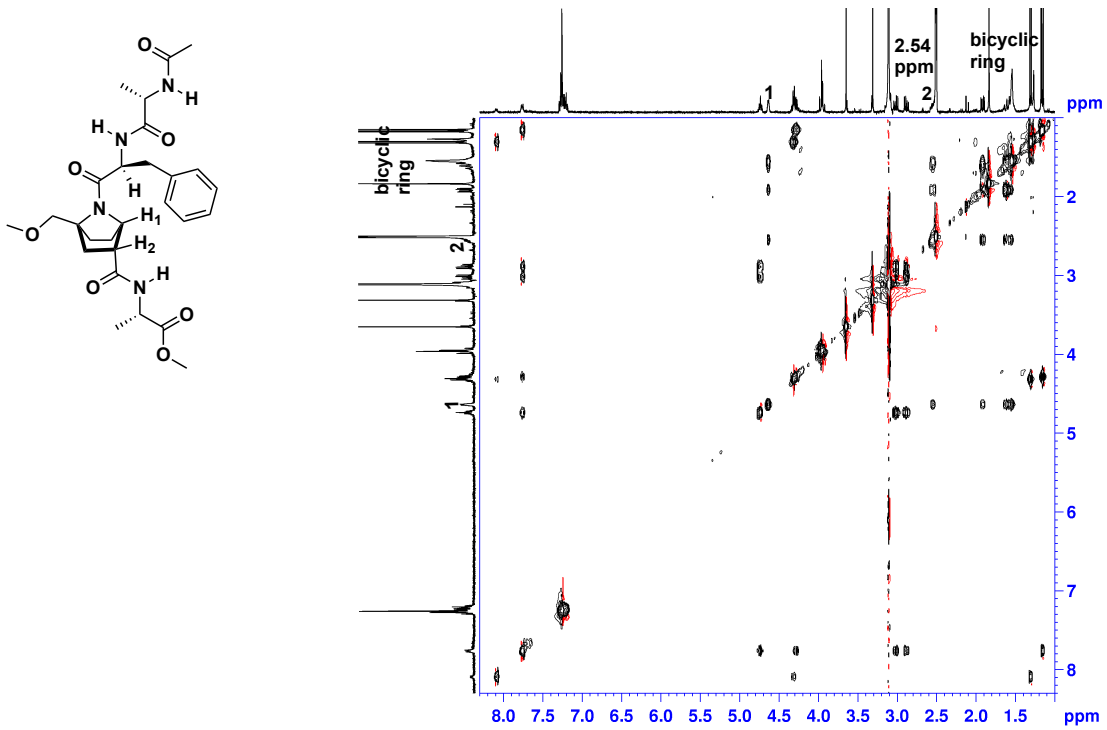


Figure S7, COSY of Ac-A-F-Abh-A-OMe **8** in DMSO at 60 °C.

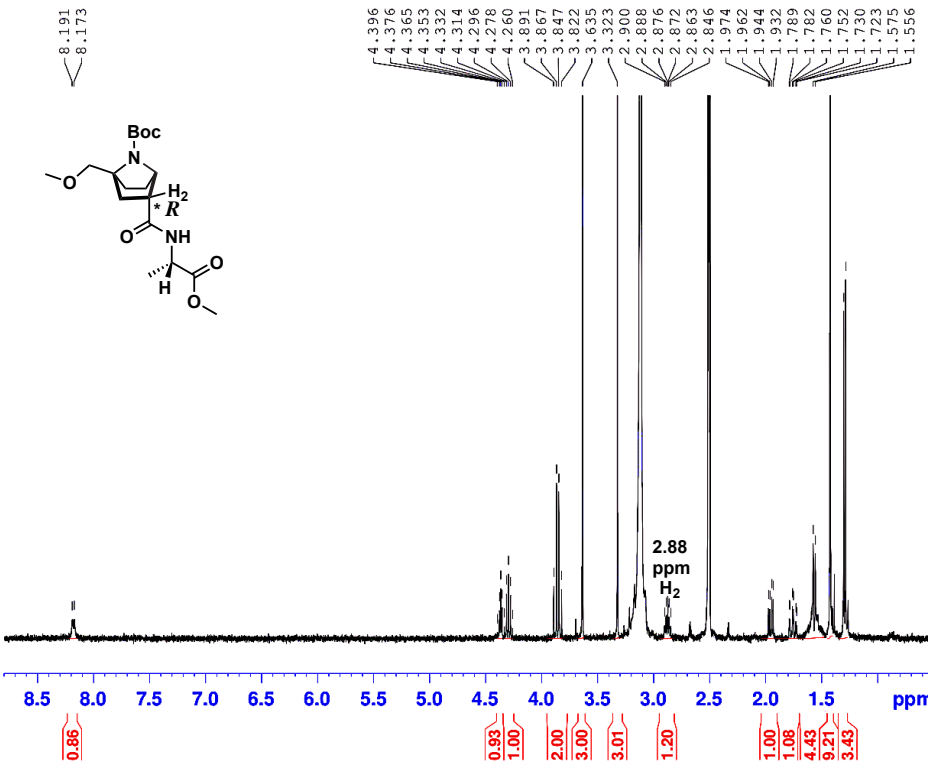
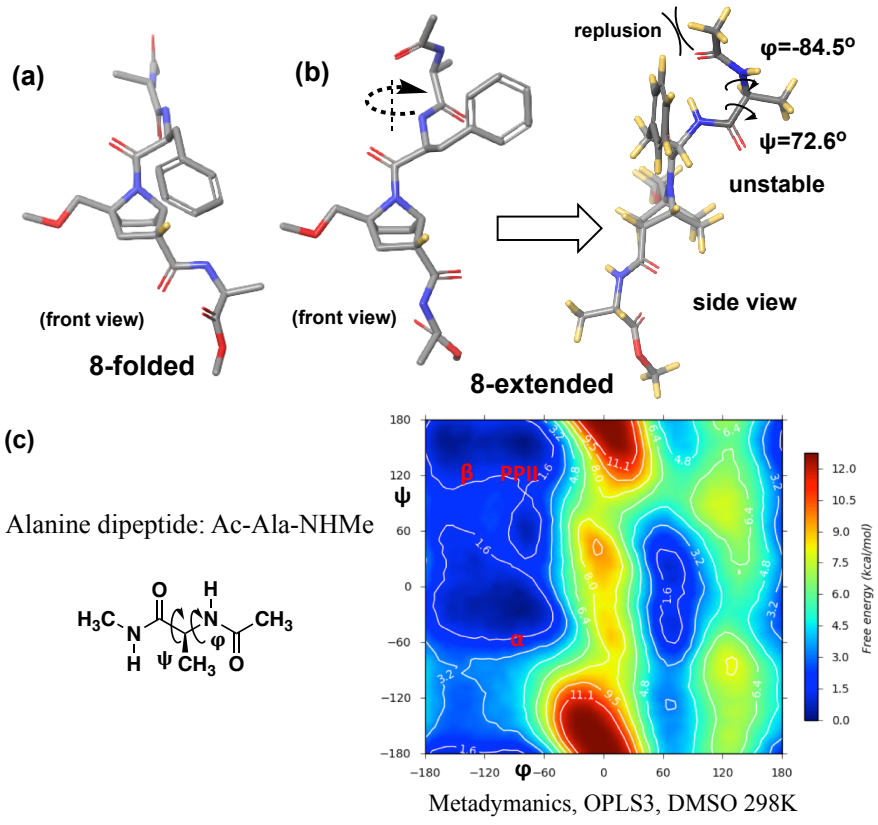


Figure S8, ^{1}H-NMR of Boc-(*R*)-Abh-Ala-OMe **10** in DMSO at 60 °C.



Metadymanics, OPLS3, DMSO 298K

Figure S9, (a) Folded structure of **8**. (b) Front view and side view of the unfolded structure of **8**. Carbon atom, grey; oxygen, red; nitrogen, blue; hydrogen, orange. (c) energy-landscape of Ala dipeptide.

The stable folded structure (**8-folded**) and unstable unfolded structure (**8-extended**, though experimentally undetectable) of tetrapeptide **8** also can be obtained by MD calculations, followed by structure optimization by DFT calculations (M06-2X/6-31G(d), SMD = DMSO), and are shown in Figure S9a and 9b, respectively. One possible reason for the instability of the unfolded structure is that in the model alanine dipeptide (Ac-Ala-NHMe), there are three energetically favorable conformations in equilibrium, i.e., β-strand (e.g. antiparallel, φ = -139°, ψ = -135°; parallel, φ = -119°, ψ = 113°), poly proline II helix (PPII) (-75°, 145°) and α-helix (-58°, -47°) (Figure S9c). We checked the φ and ψ dihedral angles of the N-terminal Ac-Ala moiety of **8** of the unfolded structure (Figure S9b), and obtained φ = -84.5° and ψ = 72.6° (Figure S9b). These values are outside the range of energetically favorable conformations, making the unfolded conformation less stable. The unfolded structure may also involve steric repulsion between the benzene ring and the acetyl group.

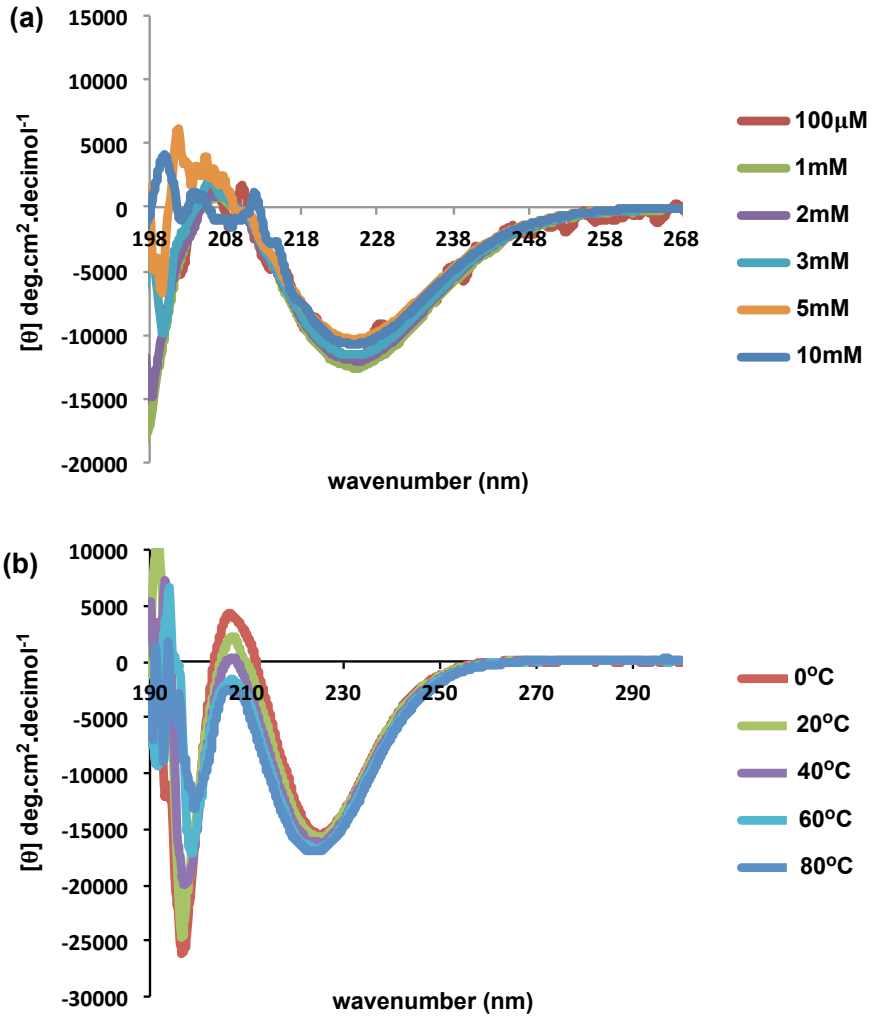


Figure S10, (a) CD spectra of concentration dependency of Ac-Ala-Phe-(*R*)-Abh-Ala-OMe **8**(100 μM to 10mM) in MeOH at 20 °C. (b) CD spectra of temperature dependency of Ac-Ala-Phe-(*R*)-Abh-Ala-OMe **8**(1mM) in MeOH.

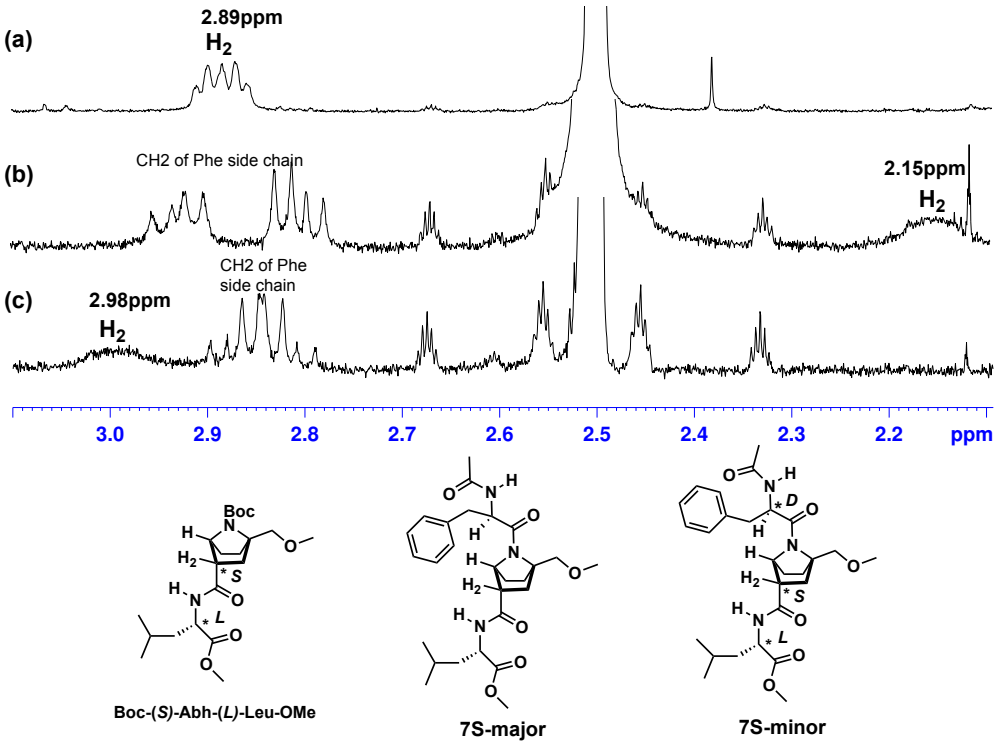


Figure S11, (a) ^{1}H-NMR of Boc-(*S*)-Abh-(*L*)-Leu-OMe in DMSO at 25 °C. **(b)** ^{1}H-NMR of **7S-major (**Ac-(*D*)-Phe-(*S*)-Abh-(*L*)-Leu-OMe**)** in DMSO at 25 °C. **(c)** ^{1}H-NMR of **7S-minor** in DMSO at 25 °C.

Comparing with the α-proton (H_2) signal (2.89 ppm) of a reference dipeptide Boc-(*S*)-Abh-Leu-OMe, the α-proton (H_2) signal of the major conformer (**7S-major**) is shifted to 2.15 ppm in DMSO, suggesting the existence of CH···π interaction, while the minor conformer (**7S-minor**) shows almost no shielding (at 2.98 ppm), suggesting the absence of CH···π interaction. Therefore, **7S-major** takes side chain folded conformation and **7S-minor** takes unfolded extended conformation.

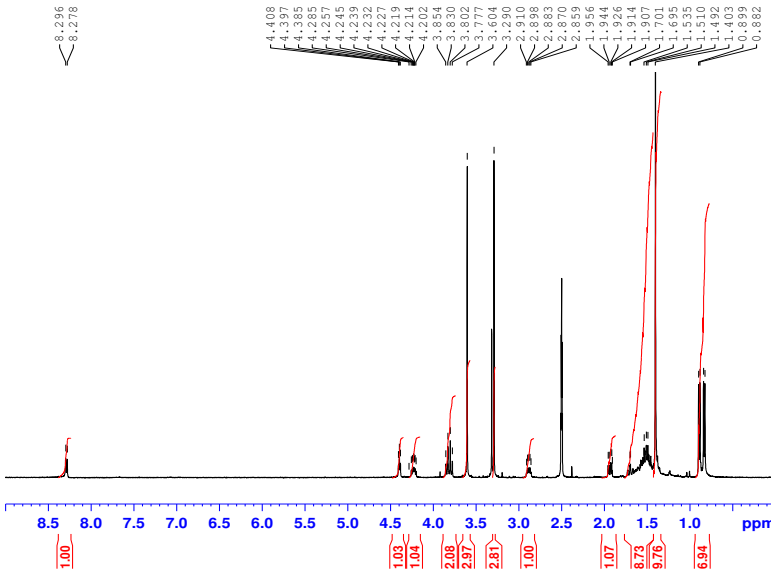


Figure S12, ^{1}H-NMR of Boc-(*S*)-Abh-(*L*)-Leu-OMe in DMSO at 25 °C.

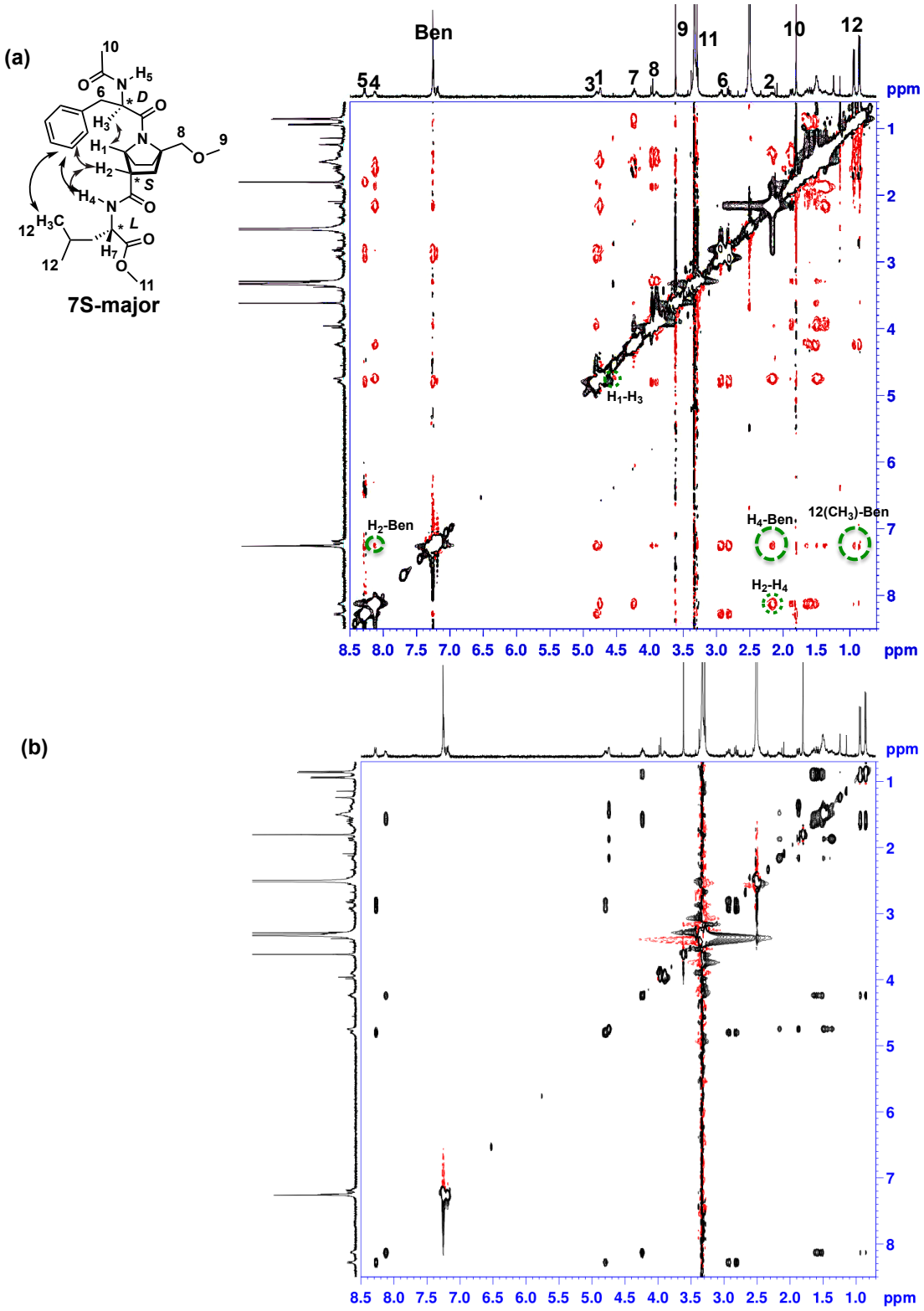


Figure S13, (a) ROE of **7S-major** and (b) COSY of **7S-major** in DMSO at 25 °C.

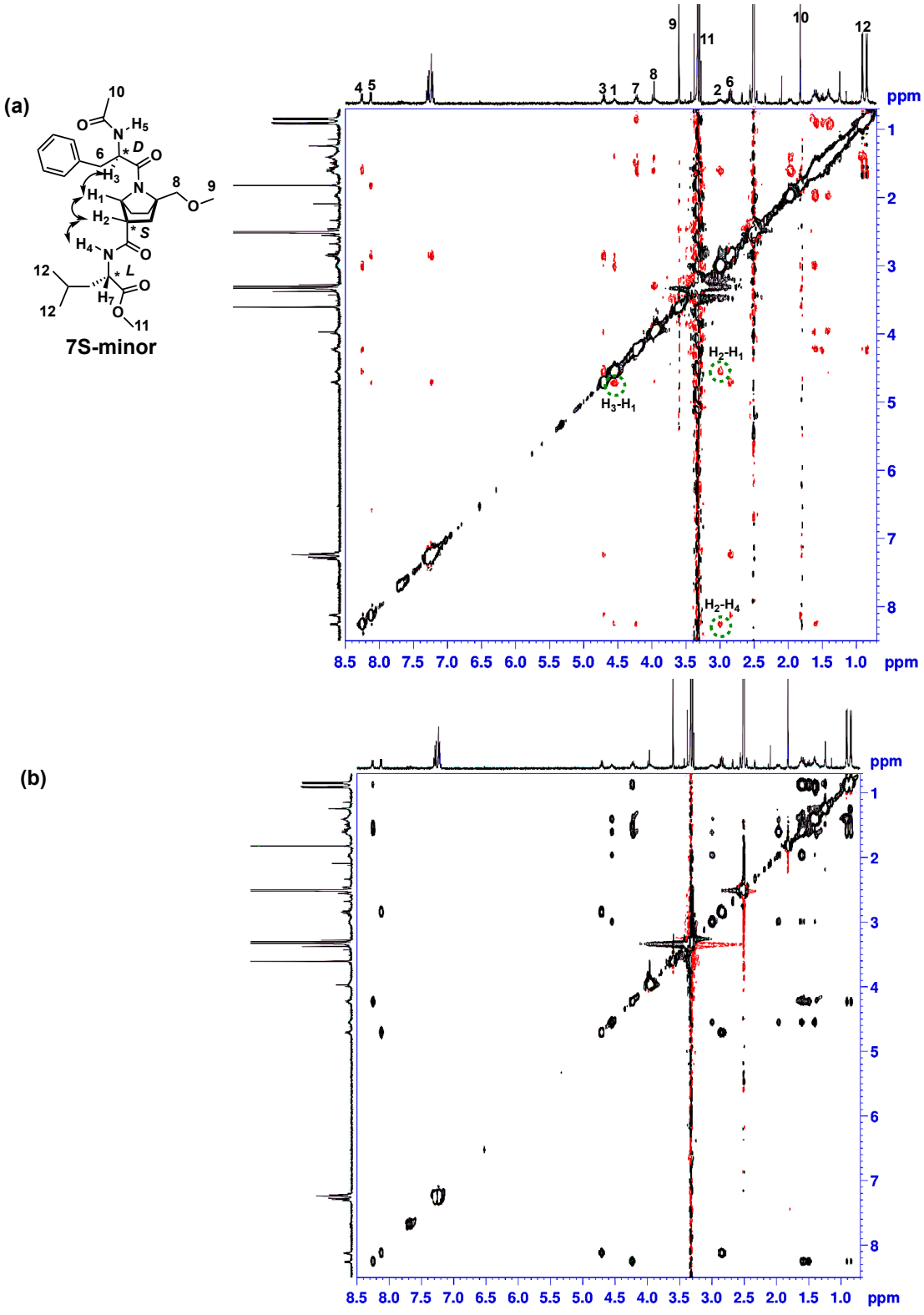


Figure S14, (a) ROE of **7S-minor** and (b) COSY of **7S-minor** in DMSO at 25 ºC.

7S(Ac-(*D*)-Phe-(*S*)-Abh-(*L*)-Leu-OMe)

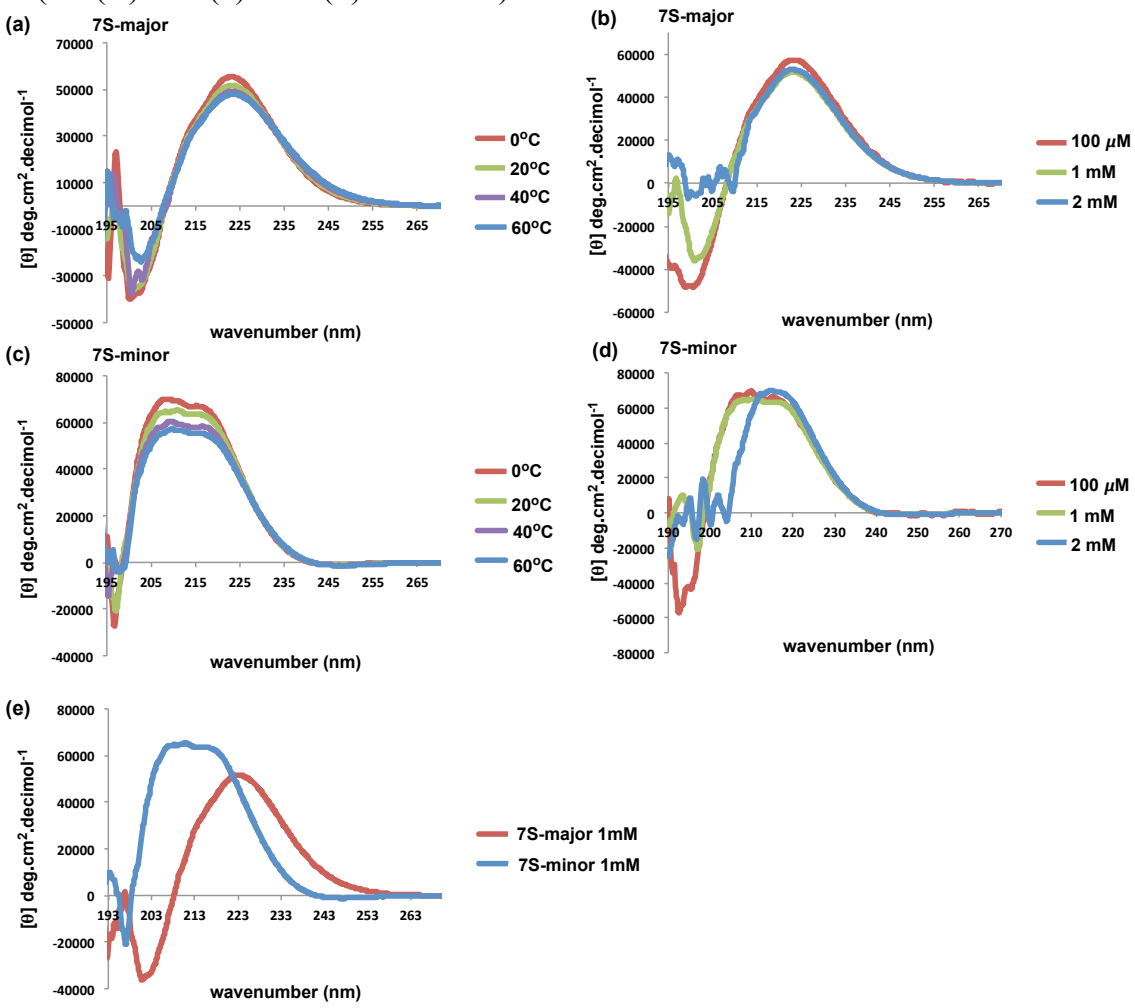


Figure S15, (a) temperature-dependent CD of **7S-major**, (b) concentration-dependent CD of **7S-major**, (c) temperature-dependent CD of **7S-minor**, (d) concentration-dependent CD of **7S-minor**, and (e) comparison of **7S-major** and **7S-minor** (at 20°C), in MeOH.

Temperature-dependency and concentration-dependency of CD spectra of **7S**-major are examined, and we found no significant dependency, suggesting no substantial intermolecular interactions occur and the structural consistency. This is also true for **7S**-minor. In Figure S15e, in comparing the CD spectra of **7S**-major and **7S**-minor, we find a clear difference. Thus, it suggests that **7S**-major and **7S**-minor also have different conformations.

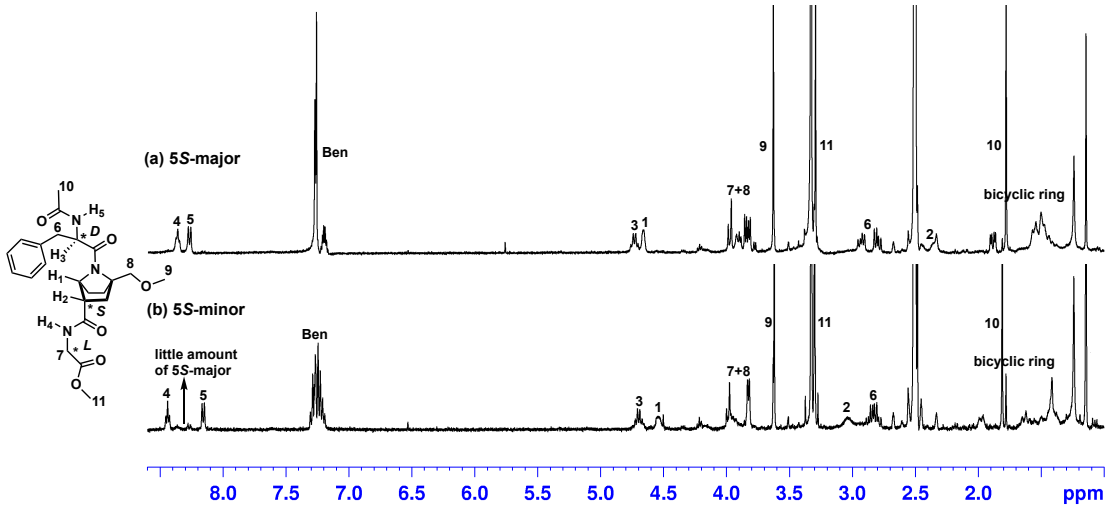


Figure S16, (a) ^{1}H-NMR of **5S-major** in DMSO at 35 °C. **(b)** ^{1}H-NMR of **5S-minor** in DMSO at 35 °C.

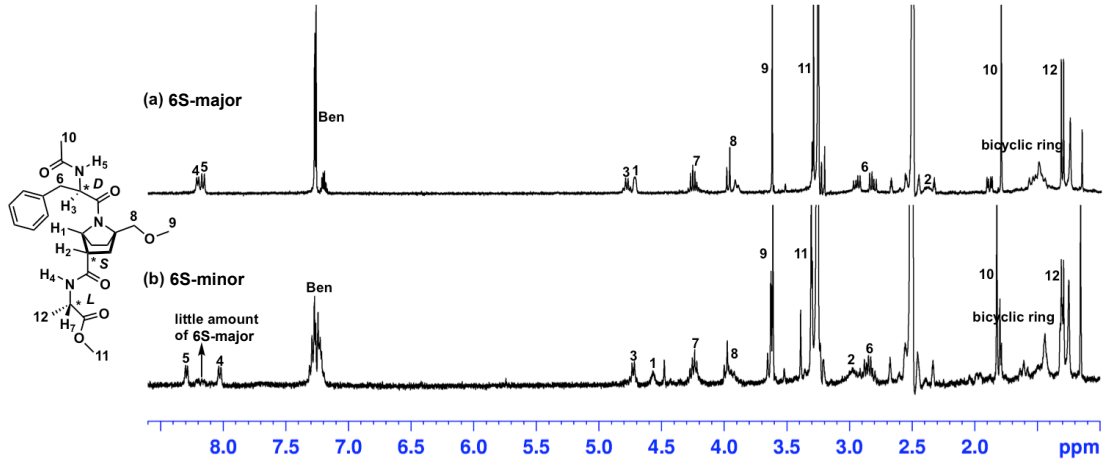


Figure S17, (a) ^{1}H-NMR of **6S-major** in DMSO at 35 °C. **(b)** ^{1}H-NMR of **6S-minor** in DMSO at 35 °C.

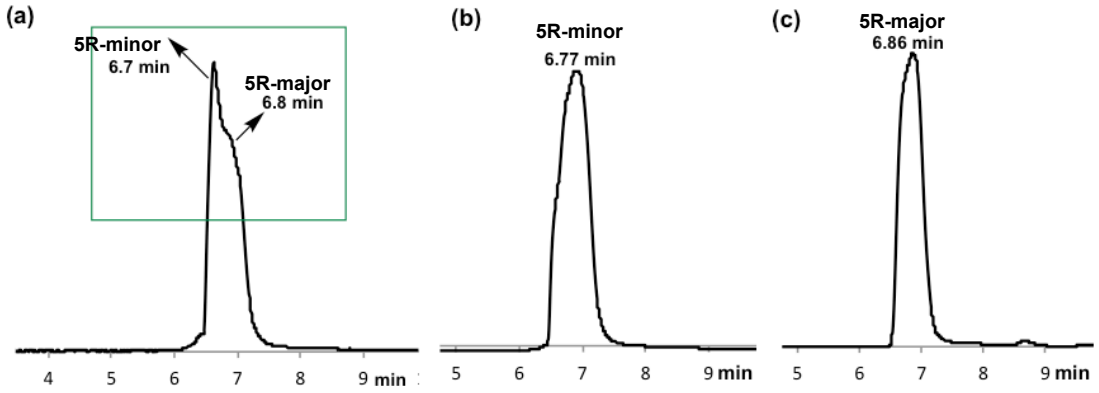


Figure S18. Reverse-phase HPLC (CH_3CN 100%, 256 nm) **(a)** tripeptide **5**(minor+major) **(b)** isolated tripeptide **5-minor** and **(c) 5-major**.

5R(Ac-(*L*)-Phe-(*R*)-Abh-Gly-OMe)

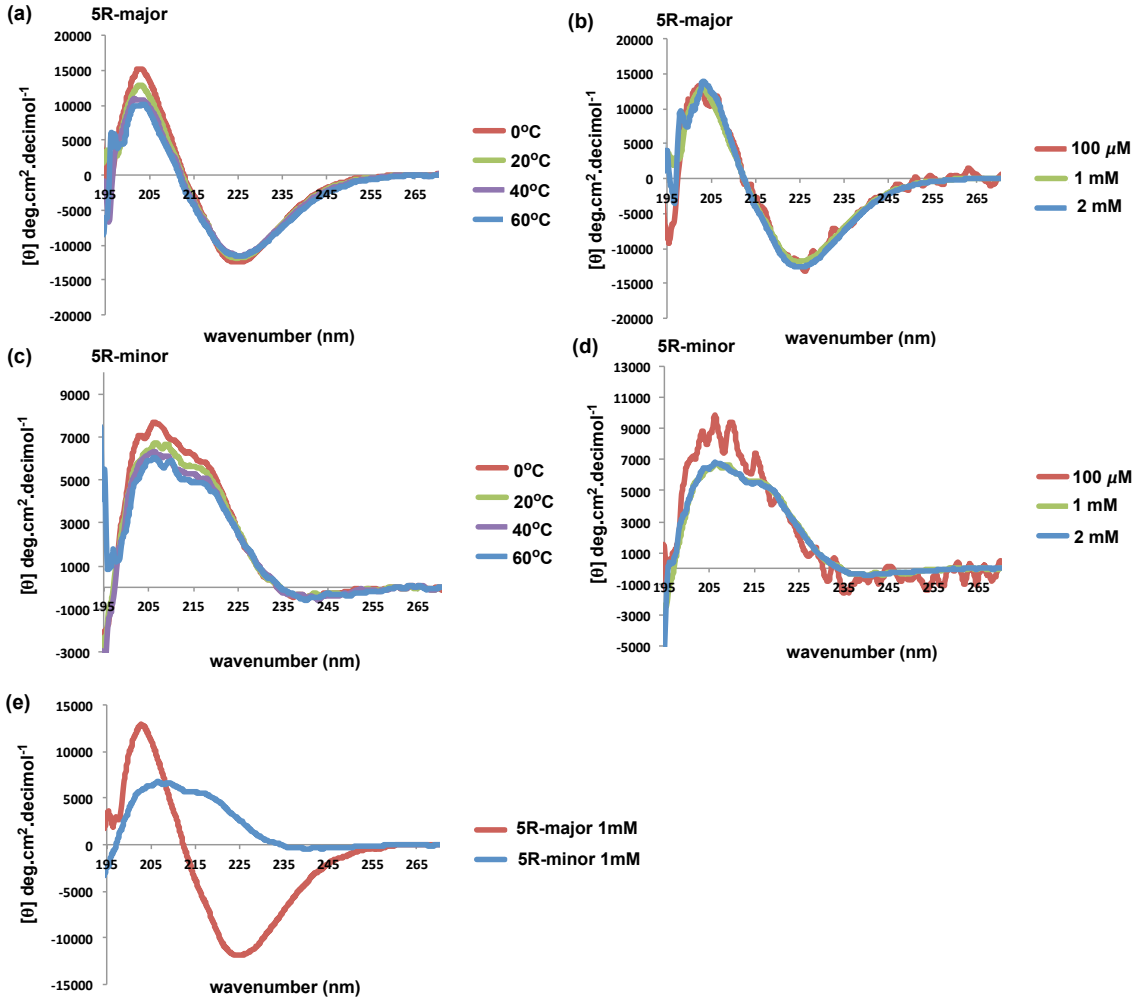


Figure S19, (a) temperature-dependent CD of **5R-major**, (b) concentration-dependent CD of **5R-major**, (c) temperature-dependent CD of **5R-minor**, (d) concentration-dependent CD of **5R-minor**, and (e) comparison of **5R-major** and **5R-minor** (at 20°C), in MeOH.

There is no significant temperature-dependency and concentration-dependency of the CD spectra of **5R**-major, suggesting the no substantial intermolecular interactions occur and the structure is consistent. This is also true for **5R**-minor. In Figure S19e, comparing the CD spectra of **5R**-major and **5R**-minor, we found that they have a clear difference in the shape and intensity in a similar manner to those of **7S**-major and **7S**-minor. Thus, it suggests that **5R**-major and **5R**-minor have totally different conformations.

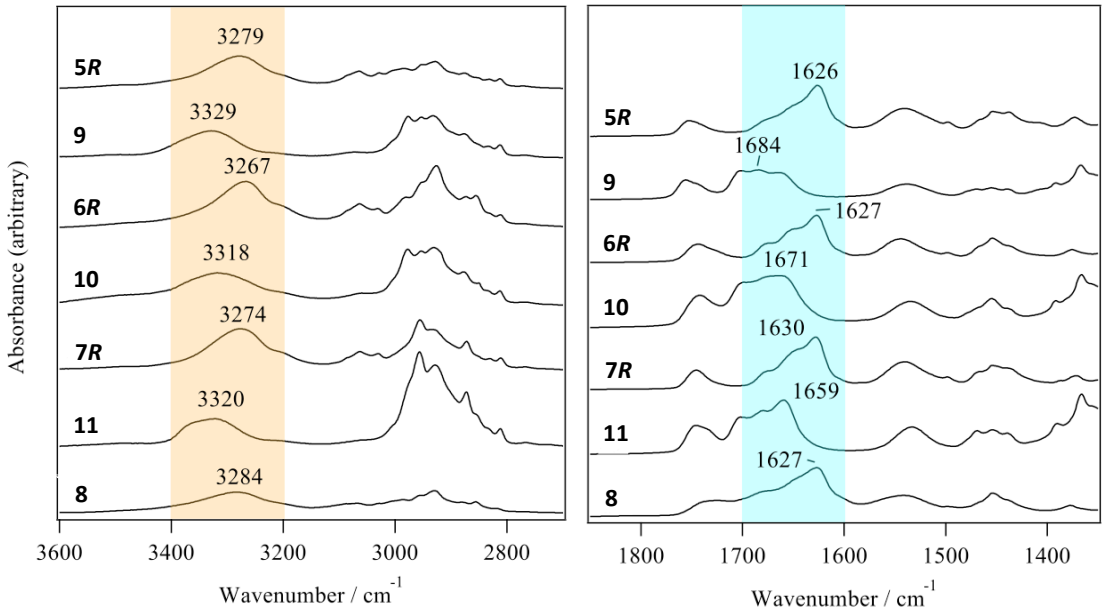


Figure S20. ATR-FTIR spectra in the dry film state over the range of 4000-1000 cm^{-1}.

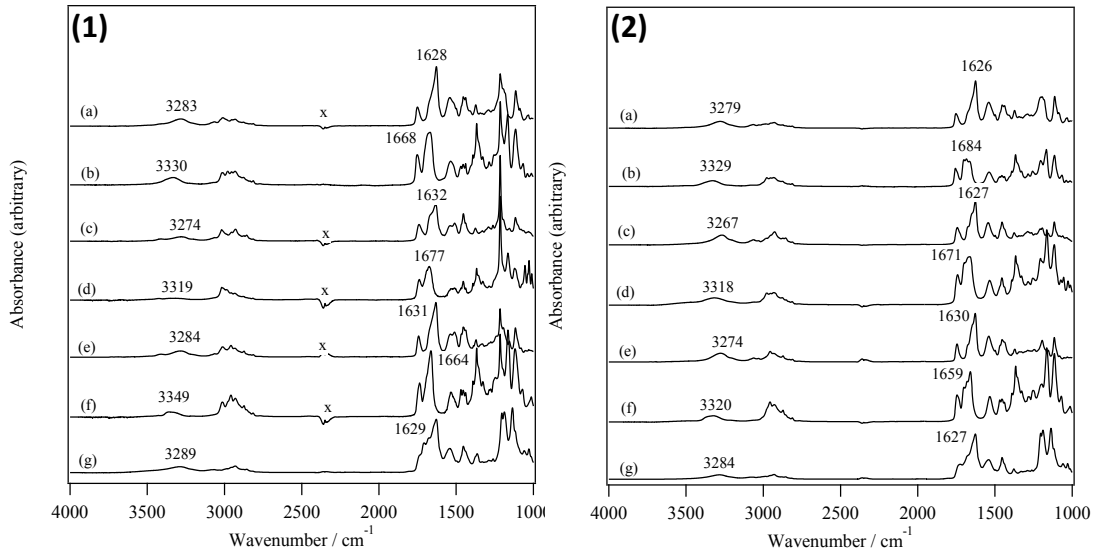


Figure S21. **(1),** Full ATR-FTIR spectra of (a) tripeptide **5R**, (b) dipeptide **9**, (c) tripeptide **6R**, (d) dipeptide **10**, (e) tripeptide **7R**, (f) dipeptide **11** and (g) tetrapeptide **8** in $CHCl_3$ solution over the range of 4000-1000 cm^{-1}. x: band due to CO_2. **(2),** Full ATR-FTIR spectra of (a) **5R**, (b) **9**, (c) **6R**, (d) **10**, (e) **7R**, (f) **11** and (g) **8** in the dry film state over the range of 4000-1000 cm^{-1}.

Fig. S20 and S21 show the ATR-FTIR spectra of a series of peptides in the dry film state over the range of 4000-1000 cm^{-1}. As far as the amide A and amide I regions are concerned, the spectral profiles are similar to the corresponding profiles obtained in $CHCl_3$ solution, although slight downshifts of the amide A and amide I bands were observed due to intermolecular interactions. We also confirmed that amide A moved together with amide I in the dry film state.

2. 2D-NMR spectra

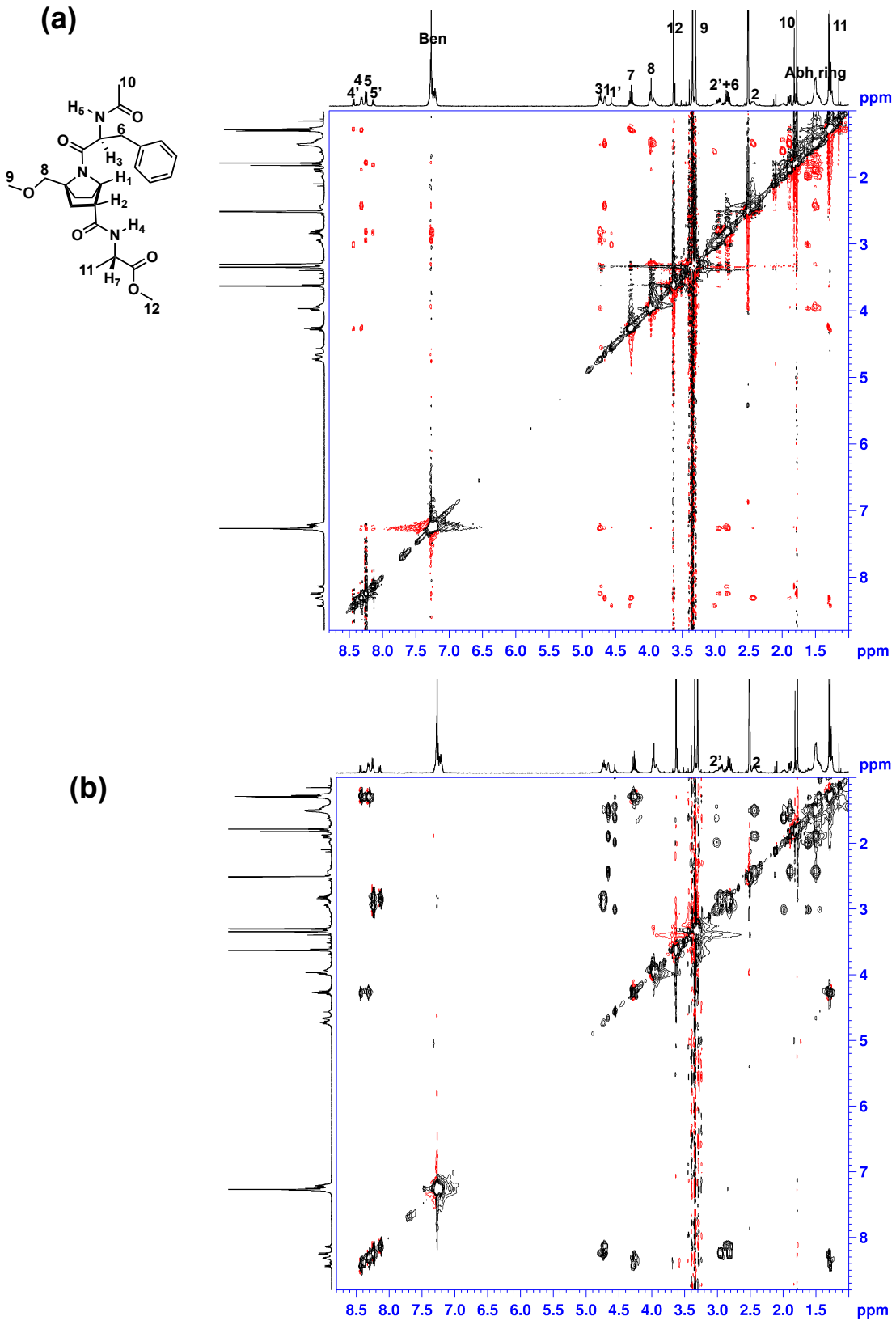


Figure S22, (a) ROE of Ac-F-Abh-A-OMe 6R and (b) COSY of Ac-F-Abh-A-OMe 6R in DMSO at 25 °C.

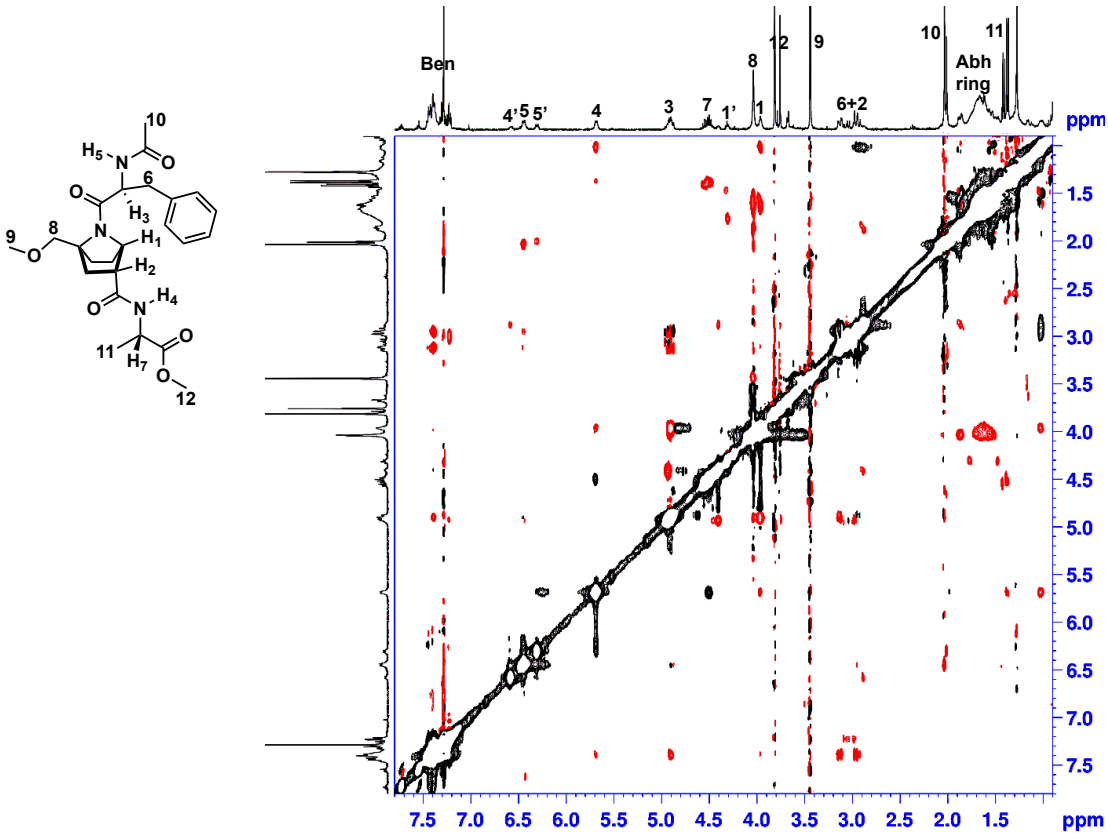


Figure S23, ROE of Ac-F-Abh-A-OMe 6R in $CDCl_3$ at 25 °C.

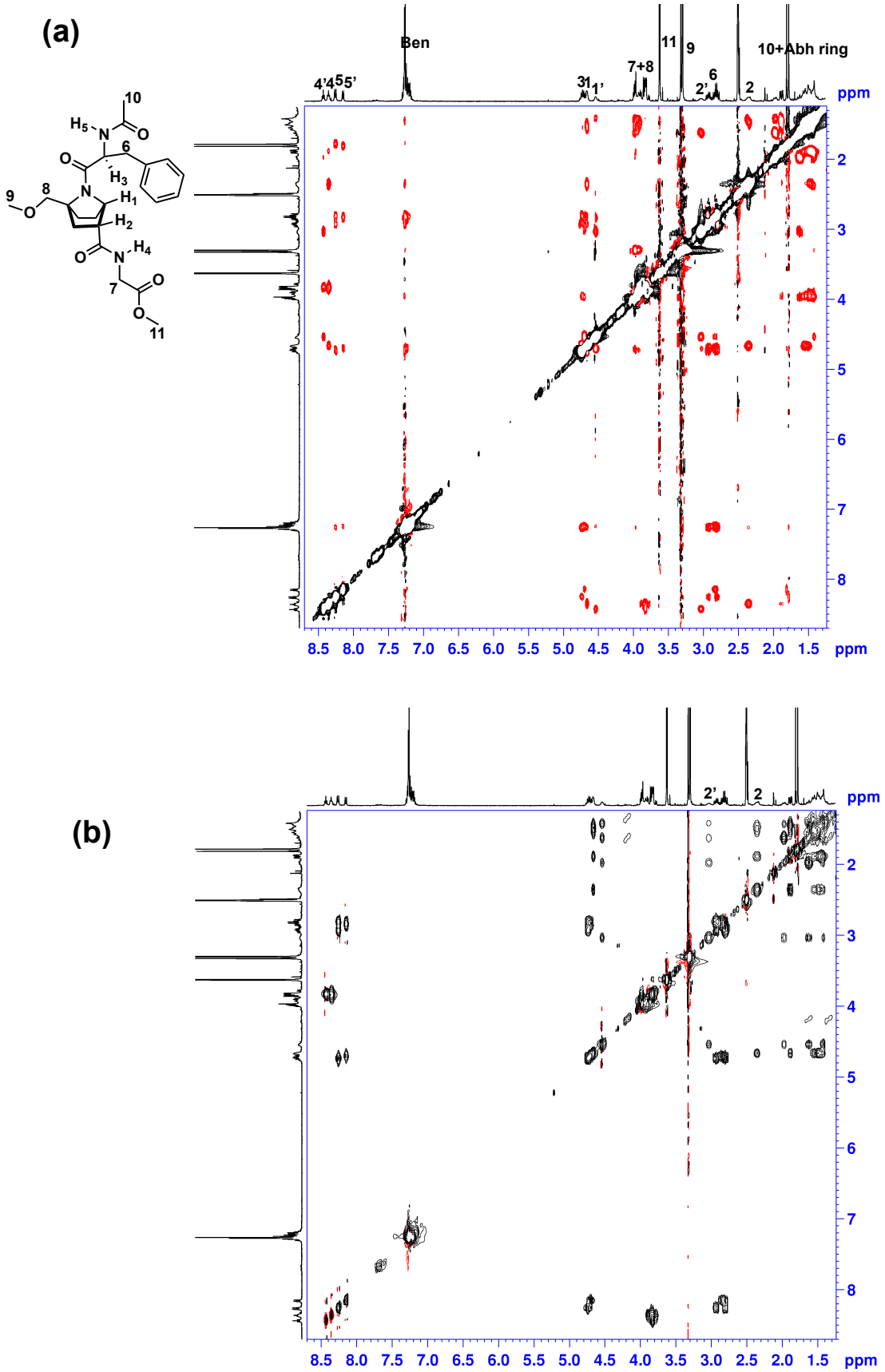


Figure S24, (a) ROE of Ac-F-Abh-G-OMe 5R and (b) COSY of Ac-F-Abh-G-OMe 5R in DMSO at 25 ºC.

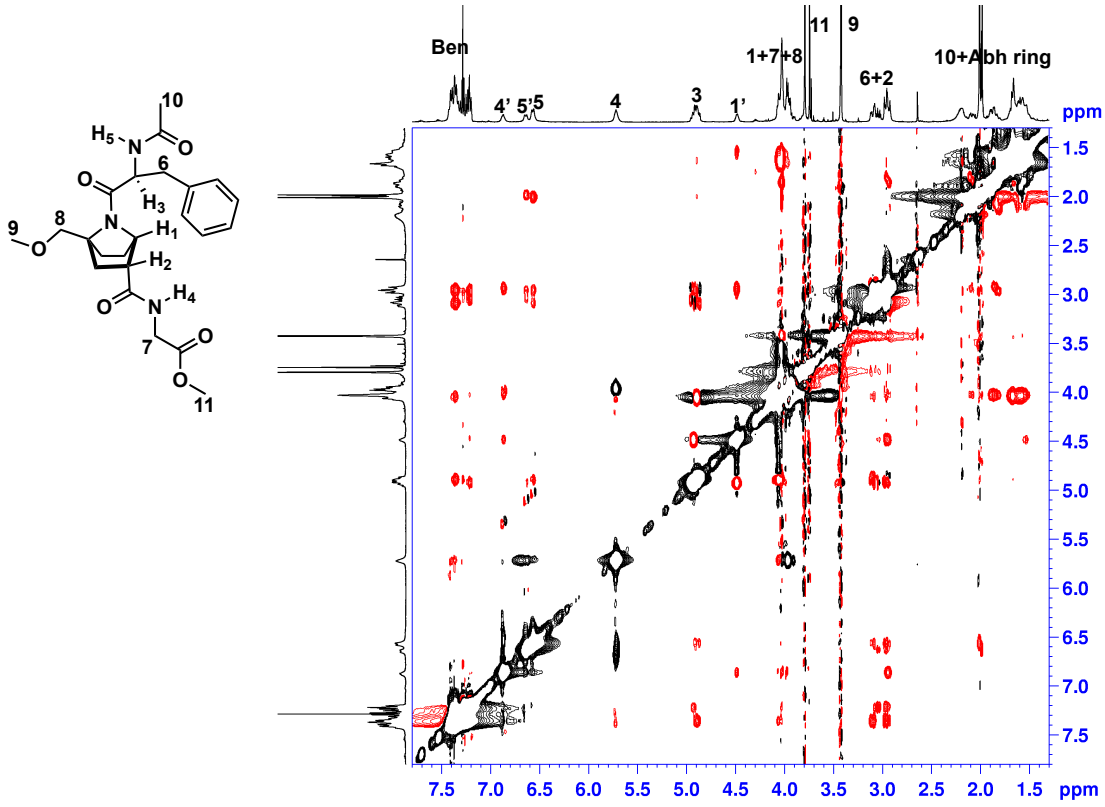


Figure S25, ROE of Ac-F-Abh-G-OMe 5R in $CDCl_3$ at 25 °C.

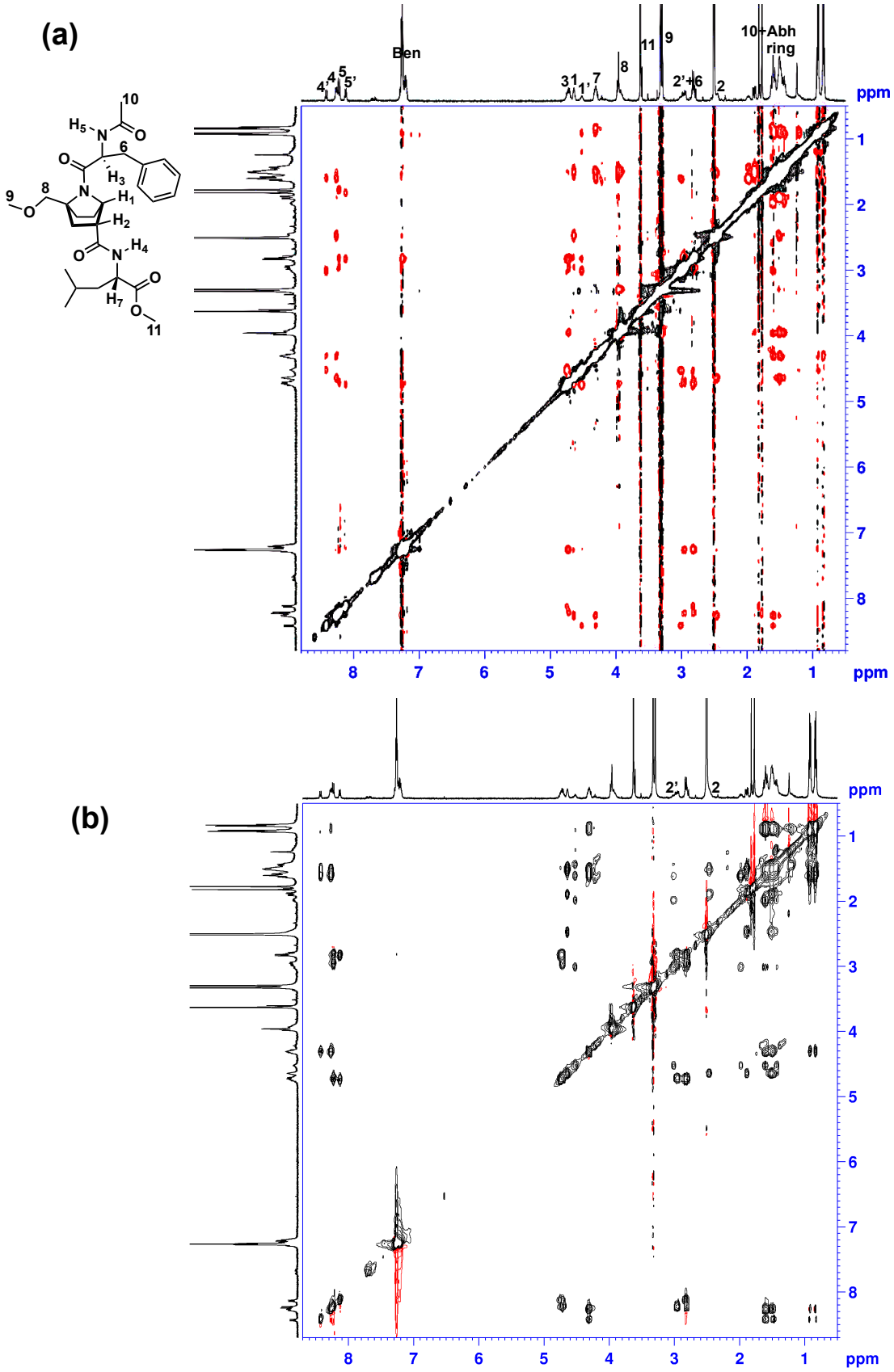


Figure S26, (a) ROE of Ac-F-Abh-L-OMe 7R and (b) COSY of Ac-F-Abh-L-OMe 7R in DMSO at 25 ºC.

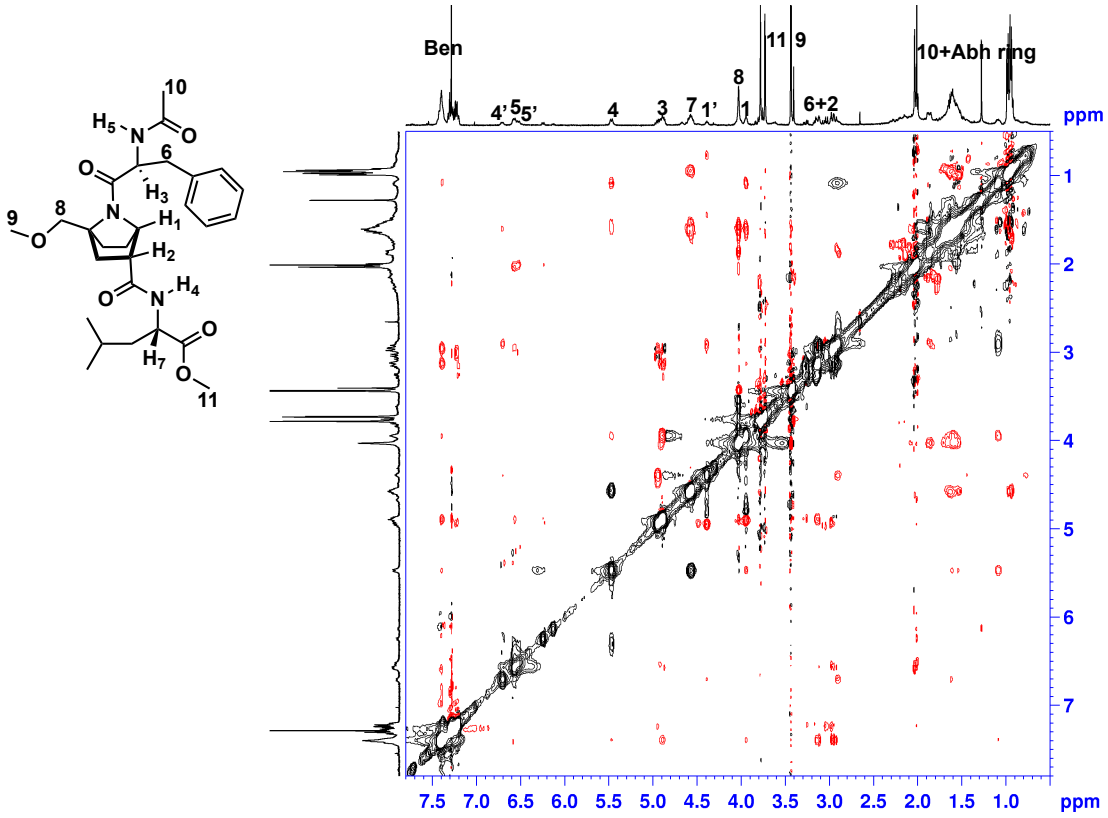


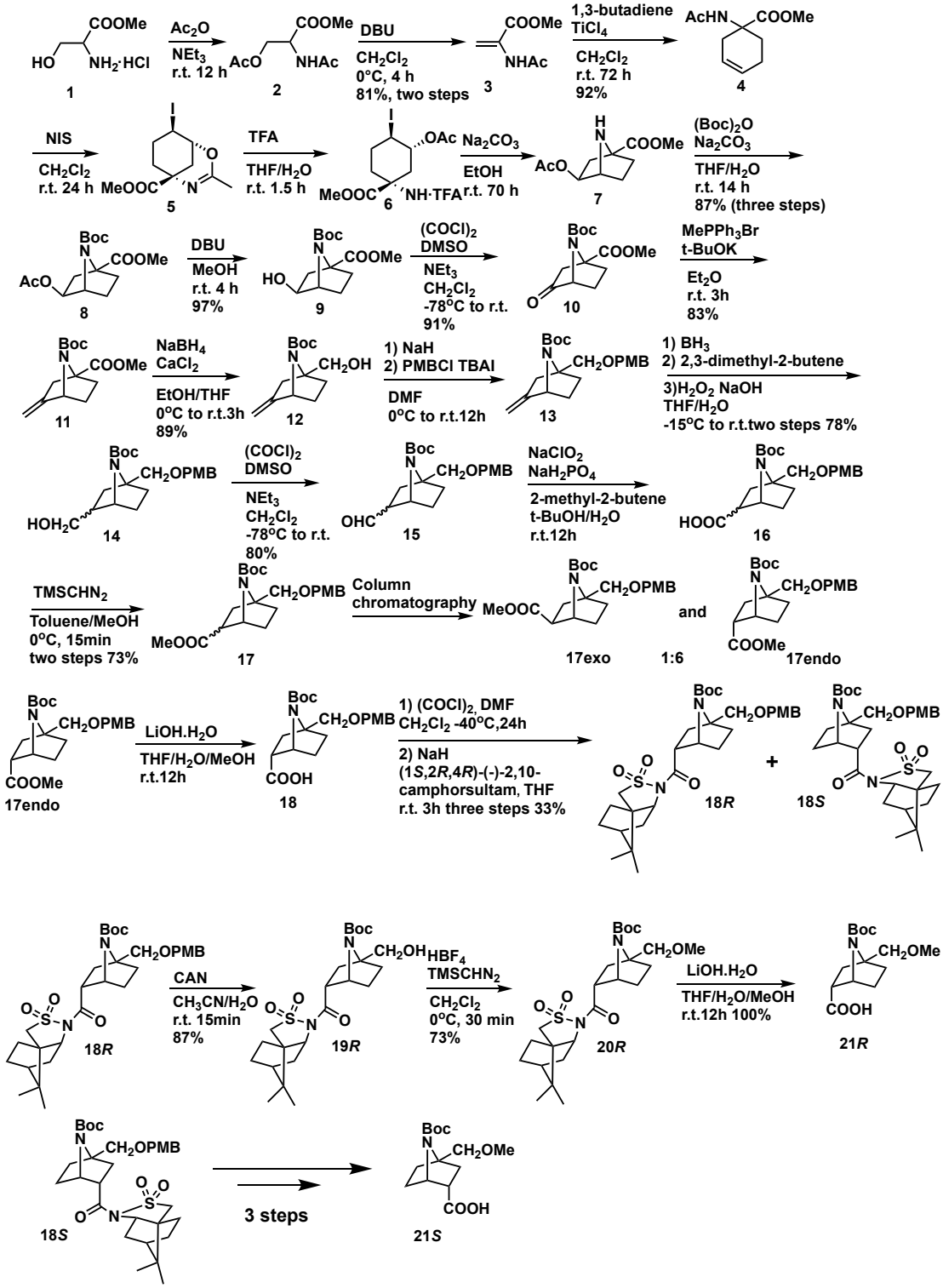
Figure S27, ROE of Ac-F-Abh-L-OMe 7R in $CDCl_3$ at 25 °C.

3. Experiment section of Synthesis

General methods

Open column chromatography was carried out on silica gel (silica gel 60N (100-210 μm), Kanto Chemicals, Japan). All the NMR experiments were recorded on a Bruker Avance 400 NMR spectrometer. ^{1}H-NMR and ^{13}C-NMR chemical shifts (δ) were calibrated with the solvent peak and are shown in ppm. Coupling constants are given in Hz. Mass spectra were recorded on a Bruker micrOTOF-05. HPLC data were obtained using a Hitachi instrument with Mightysil RP-18 GP Aqua (250 mm x 10 mm) for a reverse phase column. Flow rate: 2.0 ml/min.

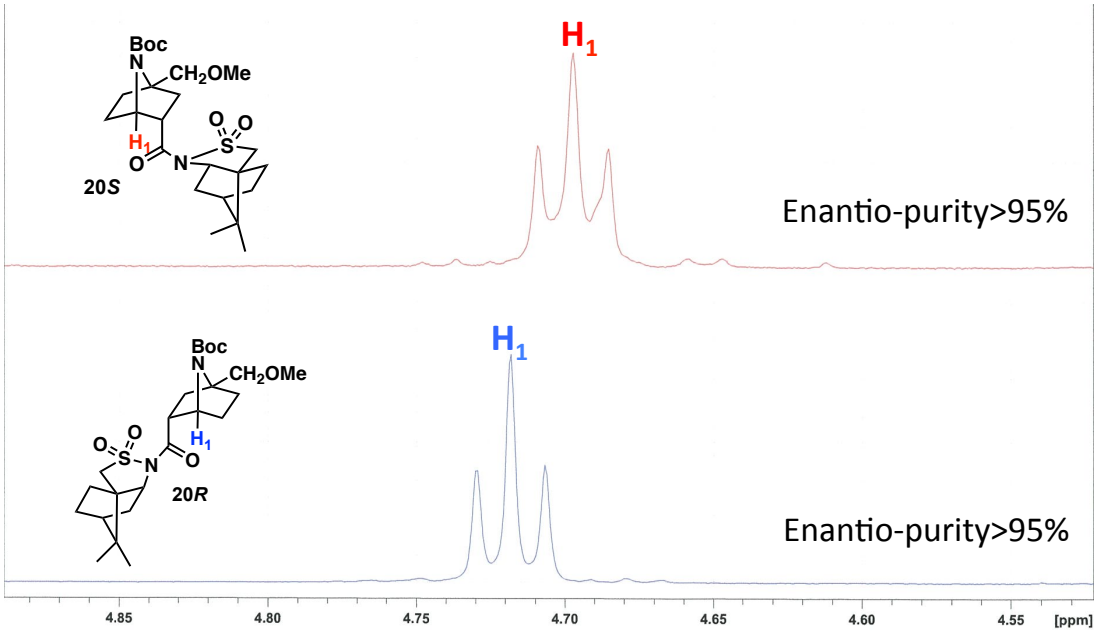
Scheme 1

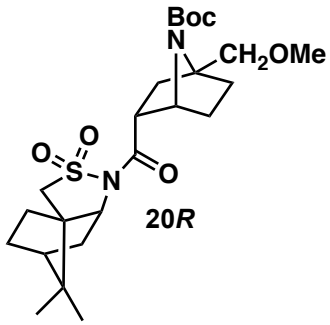


The starting material, **21*R*** and **21*S*** were synthesized according to our previously published route. (Hosoya, M., Otani, Y., Kawahata, M., Yamaguchi, K., Ohwada, T. Water-Stable Helical Structure of Tertiary Amides of Bicyclic β-Amino Acid Bearing 7-Azabicyclo[2.2.1]Heptane. Full Control of Amide Cis-Trans Equilibrium by Bridgehead Substitution. *J. Am. Chem. Soc.* **132**, 14780–14789 (2010).)

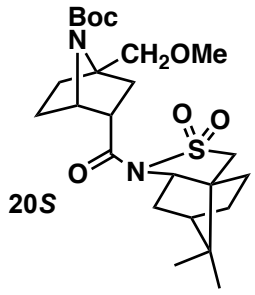
Enantio-purity check[1]

The R/S enantio-separation was achieved by coupling (1*S,* 2*R,* 4*R*)-(-)-2, 10-camphorsultam to the C-terminal of Abh amino acid, followed by column chromatography or normal phase HPLC. From ^{1}H-NMR, the chemical shift of bicyclic β-amino acid enantiomers is different. The chemical shift difference of **20*R*/20*S*** is shown below.

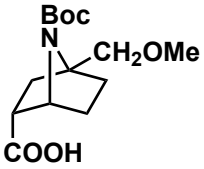




^{1}H NMR ($CDCl_3$, 400MHz) δ 4.730-4.707 (1H, m), 4.118-4.064 (1H, m), 4.023-3.998 (1H, m), 3.895-3.871 (1H, m), 3.856-3.824 (1H, m), 3.612-3.559 (1H, m), 3.468-3.429 (1H, m) 3.385 (3H, s), 2.067-1.611 (12H, m), 1.434 (9H, s), 1.114 (3H, s), 0.938 (3H, s).

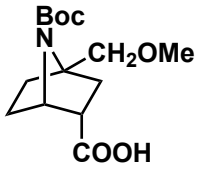


^{1}H NMR ($CDCl_3$, 400MHz) δ 4.709-4.686 (1H, m), 4.039-4.015 (1H, m), 3.896-3.864 (2H, m), 3.649-3.583 (1H, m), 3.514-3.442 (2H, m), 3.409 (3H, s), 2.251-1.611 (12H, m), 1.454 (9H, s), 1.127 (3H, s), 0.962 (3H, s).



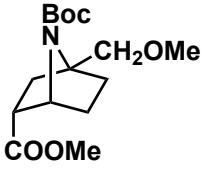
21R

^{1}H NMR ($CDCl_3$, 400MHz) δ 4.516-4.493 (1H, m), 3.976-3.9951 (2H, m), 3.422 (3H, s), 3.128-3.102 (1H, m), 2.038-1.454 (6H, m), 1.454 (9H, s) ^{13}C-NMR (100 MHz, $CDCl_3$): 176.61, 155.12, 80.390, 74.09, 68.46, 60.58, 59.54, 45.27, 36.04, 33.09, 28.48, 24.87. HRMS (ESI, $[M-H]^-$): Calcd. for $C_{14}H_{22}NO_5^-$, 284.1503. Found: 284.1517.



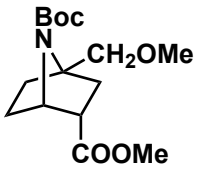
21S

^{1}H NMR ($CDCl_3$, 400MHz) δ 4.488-4.464 (1H, m), 3.951-3.875 (2H, m), 3.396 (3H, s), 3.102-3.053 (1H, m), 1.990-1.593 (6H, m), 1.427 (9H, s) ^{13}C-NMR (100 MHz, $CDCl_3$): 177.634, 155.056, 80.376, 74.009, 68.374, 62.889, 59.427, 45.432, 35.910, 33.007, 28.40, 24.79. HRMS (ESI, $[M-H]^-$): Calcd. for $C_{14}H_{22}NO_5^-$, 284.1503. Found: 284.1498.



22R

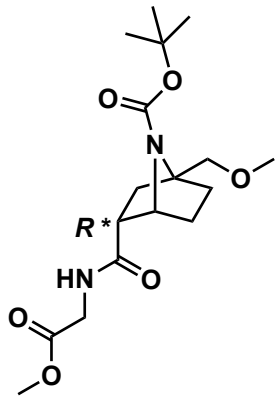
^{1}H NMR ($CDCl_3$, 400MHz) δ 4.467-4.444 (1H, m), 3.974-3.897 (2H, m), 3.701 (3H, s), 3.422 (3H, s), 3.090-3.032 (1H, m), 2.071-1.688 (6H, m), 1.452 (9H, s) ^{13}C-NMR (100 MHz, $CDCl_3$): 173.20, 155.16, 80.22, 74.13, 68.42, 60.67, 59.53, 52.02, 45.48, 36.11, 33.07, 28.48, 24.89. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{15}H_{25}NNaO_5^+$, 322.1625. Found: 322.1638.



22S

^{1}H NMR ($CDCl_3$, 400MHz) δ 4.433-4.409 (1H, m), 3.936-3.858 (2H, m), 3.663 (3H, s), 3.381 (3H, s), 3.040-2.999 (1H, m), 2.033-1.606 (6H, m), 1.415 (9H, s) ^{13}C-NMR (100 MHz, $CDCl_3$): 173.106, 155.060, 80.165, 73.994, 68.320, 60.560, 59.412,

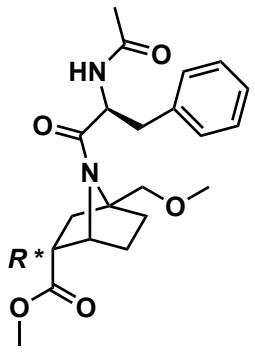
51.948, 45.375, 36.002, 32.976, 28.374, 24.795. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{15}H_{25}NNaO_5^+$, 322.1625. Found: 322.1615.



Boc-Abh(OMe)-Gly-OMe

To a solution of 21*R* (50 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) were added NH_2-Gly-OMe.HCl (33 mg, 0.26 mmol), CDMT (63 mg, 0.36 mmol), DMAP (2 mg) and NMM (99 μl, 0.9 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/1) to afford Boc-Abh(OMe)-Gly-OMe (48 mg, 77%), as colorless oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 6.044-6.020 (1H, m), 4.421-4.402 (1H, m), 4.061-4.042 (2H, m), 3.944-3.873 (2H, m), 3.764 (3H, s), 3.418 (3H, s), 3.013-2.959 (1H, m), 2.106-1.690 (6H, m), 1.458 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 171.72, 170.51, 155.34, 80.35, 73.98, 68.67, 61.23, 59.50, 52.57, 46.43, 41.48, 35.91, 33.24, 28.52, 24.17. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{17}H_{28}N_2NaO_6^+$, 379.1840. Found: 379.1850.

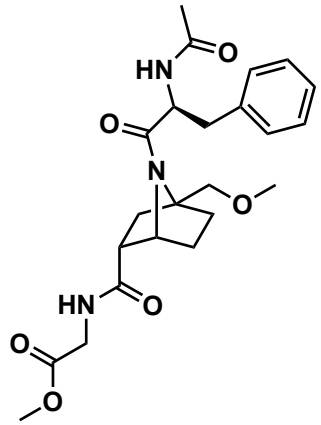


Ac-Phe-Abh(OMe)-OMe

To a solution of 22*R* (30 mg, 0.1 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude

amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) were added Ac-Phe-OH (31 mg, 0.15 mmol), CDMT (35 mg, 0.2 mmol), DMAP (2 mg) and NMM (55 µl, 0.5 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/1) to afford Ac-Phe-Abh(OMe)-OMe (24 mg, 62 %), as colorless oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.278-7.176 (5H, m), 6.572 (1H, d, J=8.08Hz), 4.908-4.850 (1H, m), 4.414-4.271 (1H, m), 4.001 (2H, brs), 3.642 (3H, d), 3.401 (3H, s), 2.999-2.965 (3H, m), 1.965 (3H, s), 1.888-1.428 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.28, 169.91, 169.71, 136.52, 129.74, 128.67, 127.22, 73.69, 69.19, 60.36, 59.44, 53.94, 52.83, 52.05, 44.62, 39.96, 31.69, 31.03, 29.37. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{28}N_2NaO_5^+$, 411.1890. Found: 411.1873.

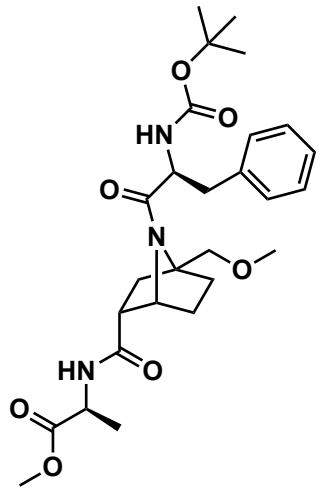


Ac-Phe-Abh(OMe)-Gly-OMe

To a solution of Ac-Phe-Abh(OMe)-OMe (24 mg, 0.06 mmol) in THF (2 mL) was added a solution of $LiOH.H_2O$ (5 mg, 0.12 mmol) in H_2O (1 mL) at rt. MeOH (0.5 mL) was added to the reaction mixture and the mixture was stirred for 12 h at rt. The reaction mixture was poured into 5% aqueous solution of $KHSO_4$, and the whole was extracted with $CHCl_3$ (×3 times). The combined organic phase was washed with brine, dried over Na_2SO_4, and the solvent was evaporated. Compound Ac-Phe-Abh(OMe)-OH (24 mg, 100%) was afforded, which was used in the next reaction without further purification.

To a solution of crude carboxyl acid in CH_2Cl_2 (2 mL) were added NH_2-Gly-OMe.HCl (11 mg, 0.09), CDMT (21 mg, 0.12 mmol), DMAP (2 mg) and NMM (33 µl, 0.3 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (EtOAc:MeOH=20:1) to afford **Ac-Phe-Abh(OMe)-Gly-OMe** (19 mg, 71 %), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.385-7.173 (5H, m), 6.835 (0.3H, s), 6.560 (0.3H, d, J=8.44Hz), 6.515 (0.7H, d, J=7.52Hz), 5.675 (0.7H, s), 4.916-4.839 (1H, m), 4.4531 (0.4H, brs), 4.041-3.920 (4.6H, m), 3.772-3.706 (3H, m), 3.412-3.398 (3H, m), 3.100-2.899 (3H, m), 2.151-1.438 (9H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 170.91, 170.74, 170.46, 170.32, 169.56, 169.53, 167.86, 167.13, 137.34, 136.29, 130.21, 129.53, 128.82, 128.63, 127.48, 127.21, 73.71, 73.59, 69.64, 69.52, 61.43, 61.32, 59.40, 53.96, 53.10, 52.82, 52.55, 52.39, 48.03, 45.44, 41.41, 41.38, 40.46, 39.40, 35.49, 34.11, 33.24, 31.95, 31.86, 29.39, 23.38, 23.28. HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{32}N_3O_6^+$, 446.2286. Found: 446.2294. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 7.06 min, >95% purity.

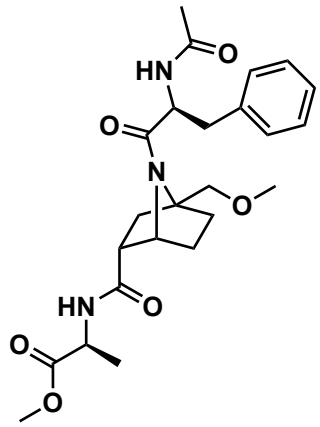


Boc-Phe-Abh(OMe)-Ala-OMe

To a solution of Boc-*R*-Abh(OMe)-Ala-OMe[1] (28 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) were added Boc-Phe-OH (32 mg, 0.12 mmol), CDMT (28 mg, 0.16 mmol), DMAP (2 mg) and NMM (44 μl, 0.4 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 1/1) to afford Boc-Phe-Abh(OMe)-Ala-OMe (29 mg, 69 %), as colorless oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.396-7.191 (5H, m), 6.489 (0.3H, d, J=5.88Hz), 5.661 (0.7H, d, J=6.04Hz), 5.433 (0.7H, d, J=6.96Hz), 5.292 (0.3H, d, J=0.8Hz), 4.592-4.459 (2H, m), 4.0256 (2H, s), 3.954 (1H, s), 3.782-3.726 (3H, m), 3.409-3.405 (3H, m), 3.105-2.896 (3H, m), 1.864-1.371 (6H, m), 1.409 (9H, s), 1.335 (3H, d, J=7.12Hz). ^{13}C-NMR (100 MHz, $CDCl_3$): 173.49, 173.41, 170.16, 169.99, 168.44, 167.41, 155.68, 155.24, 137.65, 136.72, 130.20, 129.61, 128.85, 128.56, 127.54,

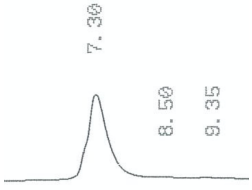
127.05, 80.30, 80.01, 73.82, 69.41, 69.31, 61.42, 59.45, 54.51, 54.12, 52.68, 52.56, 48.31, 48.08, 45.59, 40.92, 39.81, 35.36, 34.28, 33.34, 32.12, 28.49, 28.48, 27.93, 25.19, 18.90, 18.46. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{27}H_{39}N_3NaO_7^+$, 540.2680. Found: 540.2696.

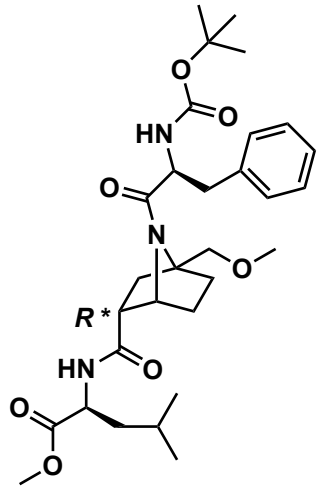


Ac-Phe-Abh(OMe)-Ala-OMe

To a solution of Boc-Phe-Abh(OMe)-Ala-OMe (28 mg, 0.05 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) was added Ac_2O (24 μl, 0.25 mmol) and Et_3N (21 μl, 0.15 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. The solution was concentrated, and the residue was purified with column chromatography (EtOAc/MeOH = 20/1) to afford Ac-Phe-Abh(OMe)-Ala-OMe (20 mg, 87%) as a colorless oil.

^{1}H-NMR (400 MHz, DMSO) δ 8.435 (0.3H, d, J=7.12Hz), 8.316 (0.7H, d, J=6.6Hz), 8.248 (0.7H, d, J=8.2Hz), 8.141 (0.3H, d, J=7.44Hz), 7.281-7.188 (5H, m), 4.743-4.706 (1H, m), 4.660 (0.7H, brs), 4.564 (0.3H, brs), 4.283-4.248 (1H, m), 3.998-3.931 (2H, m), 3.630-3.608 (3H, m), 3.304-3.297 (3H, m), 2.942-2.786 (2.3H, m), 2.434 (0.7H, m), 1.995-1.492 (9H, m), 1.299-1.264 (3H, m). ^{13}C-NMR (100 MHz, DMSO): 173.18, 173.07, 170.20, 170.12, 169.15, 168.79, 168.12, 168.01, 137.49, 137.31, 129.50, 129.46, 128.09, 128.03, 126.48, 126.38, 79.31, 78.99, 78.65, 74.11, 68.39, 68.23, 59.97, 58.69, 52.15, 51.85, 47.81, 47.73, 44.99, 37.88, 35.15, 34.56, 32.13, 32.06, 30.98, 29.61, 22.39, 22.28, 17.05, 16.61. HRMS (ESI, $[M+H]^+$): Calcd. for $C_{24}H_{34}N_3O_6^+$, 460.2442. Found: 460.2464. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 7.30 min, >95% purity.

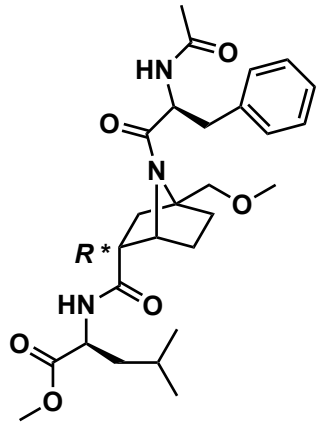




Boc-Phe-Abh(OMe)-Leu-OMe

To a solution of Boc-*R*-Abh(OMe)-Leu-OMe[1] (117 mg, 0.29 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) were added Boc-Phe-OH (117 mg, 0.44 mmol), CDMT (102 mg, 0.58 mmol), DMAP (2 mg) and NMM (96 μl, 0.87 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 2/1) to afford Boc-Phe-Abh(OMe)-Leu-OMe (68 mg, 42 %), as colorless oil.

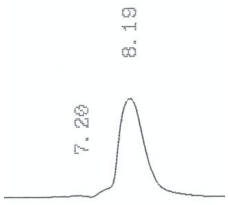
^{1}H NMR ($CDCl_3$, 400MHz) δ 7.371-7.155 (5H, m), 6.255 (0.2H, brs), 5.608 (0.1H, brs), 5.459 (0.7H, brs), 5.385 (0.7H, d, J=6.48 Hz), 5.298 (0.3H, d, J=8.2 Hz), 4.573-4.553 (2H, m), 4.026-3.886 (3H, m), 3.758-3.710 (3H, m), 3.410 (3H, s), 3.119-2.900 (3H, m), 2.062-1.257 (9H, m), 1.443 (9H, s), 0.961-0.880 (6H, m). ^{13}C-NMR (100 MHz, $CDCl_3$): 173.55, 173.47, 170.34, 170.23, 168.40, 167.36, 155.68, 155.21, 137.65, 136.82, 130.20, 129.82, 129.59, 129.53, 128.79, 128.49, 128.23, 127.50, 127.01, 126.62, 80.24, 79.94, 73.64, 69.37, 69.26, 62.85, 61.31, 59.38, 54.47, 52.43, 52.33, 50.86, 50.56, 45.81, 41.97, 41.41, 40.88, 36.19, 35.27, 33.16, 32.05, 28.45, 27.94, 24.96, 24.89, 22.95, 22.93, 21.89, 21.85. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{30}H_{45}N_3NaO_7^+$, 582.3150. Found: 582.3152.

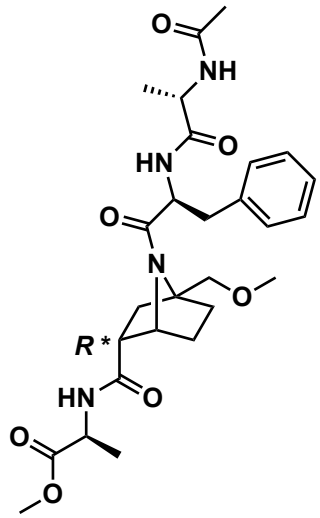


Ac-Phe-Abh(OMe)-Leu-OMe

To a solution of Boc-Phe-Abh(OMe)-Leu-OMe (20 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (1 mL) was added Ac_2O (19 μl, 0.2 mmol) and Et_3N (17 μl, 0.12 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. The solution was concentrated, and the residue was purified with column chromatography (EtOAc/MeOH = 20/1) to afford Ac-Phe-Abh(OMe)-Leu-OMe (16 mg, 89%) as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.379-7.192 (5H, m), 6.581 (0.3H, d, J=6.12 Hz), 6.514 (0.7H, d, J=7.08 Hz), 6.428 (0.3H, d, J=5.28 Hz), 5.411 (0.7H, d, J=8.0 Hz), 4.935-4.853 (1H, m), 4.580-4.529 (1H, m), 4.349 (0.3H, brs), 4.008 (2H, s), 3.902 (0.7H, s), 3.760-3.710 (3H, m), 3.417-3.411 (3H, m), 3.138-2.897 (3H, m), 2.016-1.253 (11H, m), 0.967-0.914 (7H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 173.78, 173.62, 170.44, 170.36, 170.17, 169.69, 169.02, 167.02, 137.39, 136.20, 130.27, 129.64, 129.52, 128.91, 128.69, 128.64, 127.72, 127.28, 127.25, 127.22, 73.59, 73.40, 72.88, 70.76, 69.68, 69.60, 69.48, 61.38, 59.60, 59.41, 53.44, 53.27, 52.48, 52.42, 52.37, 50.94, 50.64, 45.73, 44.94, 42.02, 41.37, 40.62, 39.49, 35.29, 33.13, 31.87, 31.40, 25.11, 25.03, 24.96, 23.45, 23.34, 23.06, 22.98. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{27}H_{39}N_3NaO_6^+$, 524.2731. Found: 524.2733. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 8.19 min, >95% purity.

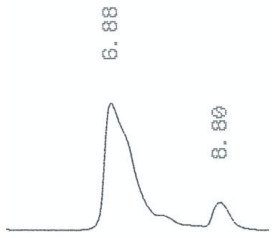


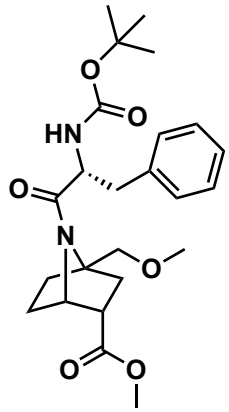


Ac-Ala-Phe-Abh(OMe)-Ala-OMe

To a solution of Boc-Phe-Abh(OMe)-Ala-OMe (10 mg, 0.02 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). The combined organic layer was dried over Na_2SO_4, and filtered. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (1 mL) were added Ac-Ala-OH (4 mg, 0.03 mmol), CDMT (7 mg, 0.04 mmol), DMAP (1 mg) and NMM (11 μl, 0.1 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (EtOAc/MeOH = 20/1.5) to afford Ac-Ala-Phe-Abh(OMe)-Ala-OMe (9 mg, 85 %), as colorless oil.

^{1}H-NMR (400 MHz, CD_3OD) δ 7.315-7.234 (5H, m), 4.596 (1H, s), 4.539 (1H, brs), 4.391-4.287 (2H, m), 4.025-3.954 (2H, m), 3.722 (3H, s), 3.383 (3H, s), 3.081-2.955 (2H, m), 2.083-2.066 (1H, m), 1.962 (3H, s), 1.946-1.571 (6H, m), 1.371 (3H, d, J=7.28Hz), 1.277 (3H, d, J=7.2Hz). ^{13}C-NMR(100 MHz, CD_3OD): 174.68, 174.61, 173.16, 172.90, 169.25, 138.10, 130.87, 129.53, 128.03, 75.16, 70.41, 69.61, 62.08, 59.52, 56.05, 54.43, 52.75, 50.29, 46.67, 39.34, 36.19, 33.01, 29.53, 17.80, 17.38. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{27}H_{38}N_4NaO_7^+$, 553.2633. Found: 553.2639. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 6.88 min, >95% purity.

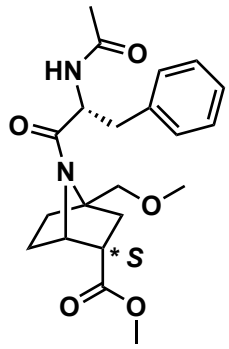




Boc-*D*-Phe-*S*-Abh(OMe)-OMe

To a solution of 22*S* (81 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of crude amine in CH_2Cl_2 (2 mL) were added Boc-*D*-Phe-OH (108 mg, 0.4 mmol), CDMT (95 mg, 0.54 mmol), DMAP (2 mg) and NMM (148 μl, 1.35 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 12 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (Hexane/EtOAc = 3/1-2/1) to afford Boc-*D*-Phe-*S*-Abh(OMe)-OMe (54 mg, 45 %), as colorless oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 7.270-7.190 (5H, m), 5.276 (1H, d, J=8.8Hz), 4.590-4.532 (1H, m), 4.281 (1H, brs), 4.017-4.012 (2H, m), 3.639 (3H, d), 3.401 (3H, s), 3.015-2.903 (3H, m), 1.895-1.249 (6H, m), 1.409 (9H, s). ^{13}C-NMR (100 MHz, $CDCl_3$): 172.36, 168.08, 155.17, 136.85, 129.73, 128.59, 127.04, 79.83, 74.09, 68.96, 59.43, 55.44, 54.10, 52.04, 51.98, 44.71, 40.43, 35.87, 31.68, 28.42. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{24}H_{34}N_2NaO_6^+$, 469.2309. Found: 469.2317.

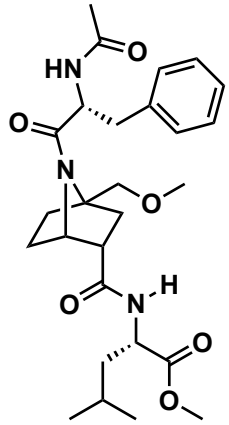


Ac-*D*-Phe-*S*-Abh(OMe)-OMe

To a solution of Boc-*D*-Phe-*S*-Abh(OMe)-OMe (54 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The solvent was evaporated to afford crude amine, which was used in the next reaction without further purification. To a solution of

crude amine in CH_2Cl_2 (2 mL) was added Ac_2O (75 μl, 0.6 mmol) and Et_3N (50 μl, 0.36 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for overnight. The solution was concentrated, and the residue was purified with column chromatography (EtOAc/MeOH = 20/1) to afford Ac-*D*-Phe-*S*-Abh(OMe)-OMe (41 mg, 89%) as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.287-7.187 (5H, m), 6.467 (1H, brs), 4.922-4.844 (1H, m), 4.301 (1H, brs), 4.046-4.025 (2H, m), 3.655 (3H, d), 3.424 (3H, s), 3.042-2.942 (3H, m), 2.174-1.585 (6H, m), 1.978 (3H, s). ^{13}C-NMR(100 MHz, $CDCl_3$): 172.27, 169.70, 167.63, 136.54, 129.74, 128.66, 127.21, 73.70, 69.18, 60.36, 59.46, 53.95, 52.84, 52.05, 44.62, 39.96, 35.80, 31.68, 26.27. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{28}N_2NaO_5^+$, 411.1890. Found: 411.1894.



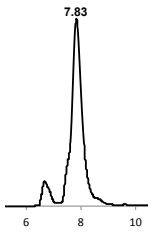
Ac-*D*-Phe-*S*-Abh(OMe)-Leu-OMe

To a solution of Ac-*D*-Phe-*S*-Abh(OMe)-OMe (41 mg, 0.11 mmol) in THF (2 mL) was added a solution of $LiOH.H_2O$ (9 mg, 0.21 mmol) in H_2O (1 mL) at rt. MeOH (0.5 mL) was added to the reaction mixture and the mixture was stirred for 12 h at rt. The reaction mixture was poured into 5% aqueous solution of $KHSO_4$, and the whole was extracted with $CHCl_3$ (×3 times). The combined organic phase was washed with brine, dried over Na_2SO_4, and the solvent was evaporated. Compound Ac-*D*-Phe-*S*-Abh(OMe)-OH (40 mg, 100%) was afforded, which was used in the next reaction without further purification.

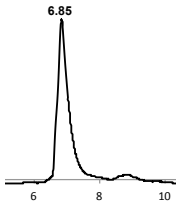
To a solution of crude carboxyl acid in CH_2Cl_2 (2 mL) were added NH_2-Leu-OMe.HCl (29 mg, 0.16), CDMT (37 mg, 0.21 mmol), DMAP (2 mg) and NMM (58 μl, 0.53 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 24 h. The reaction mixture was diluted with Et_2O, washed with 1M aqueous solution of HCl and saturated aqueous solution of $NaHCO_3$, and the organic layer was dried over Na_2SO_4. The solution was concentrated, and the residue was purified with column chromatography (EtOAc:MeOH=20:1) to afford Ac-*D*-Phe-*S*-Abh(OMe)-Leu-OMe (42 mg (fold conformation 39 mg and unfold conformation 3mg), 80 %), as a colorless oil.

Fold conformation: ^{1}H-NMR (400 MHz, DMSO) δ 8.269 (1H, d, J=7.2Hz), 8.119 (1H, d, J=5.6Hz), 7.269-7.175 (5H, m), 4.800-4.782 (1H, m), 4.739 (1H, brs), 4.260-4.203 (1H, m), 3.977-3.876 (2H, m), 3.609 (3H, s), 3.282 (3H, s), 2.934-2.778 (2H, m), 2.149 (1H, brs), 1.885-1.356 (9H, m), 1.844 (3H, s), 0.889 (6H, dd, ^{1}J=26.8Hz,

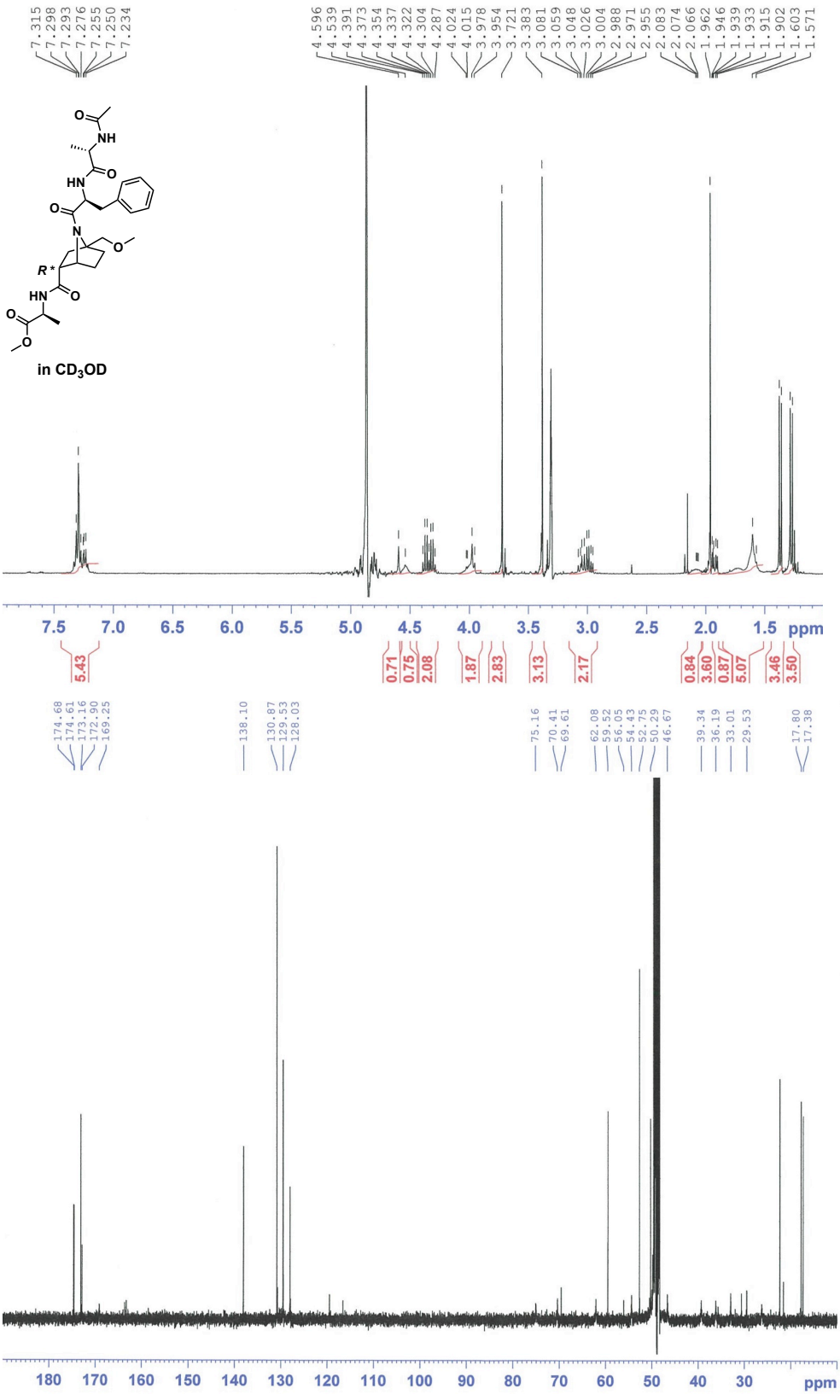
^{2}J=6.4Hz). ^{13}C-NMR (100 MHz, DMSO): 173.02, 170.43, 168.70, 167.72, 137.31, 129.62, 127.92, 126.31, 74.07, 68.46, 68.09, 59.71, 58.61, 52.09, 51.71, 50.49, 44.83, 38.09, 35.10, 31.93, 24.87, 24.29, 22.75, 22.24, 21.12. HRMS (ESI, $[M+Na]^{+}$): Calcd. for $C_{27}H_{39}N_3NaO_6^{+}$, 524.2731. Found: 524.2742. Reverse-phase HPLC (CH_3CN 100%, 256 nm): t_R 7.83 min, 91% purity.

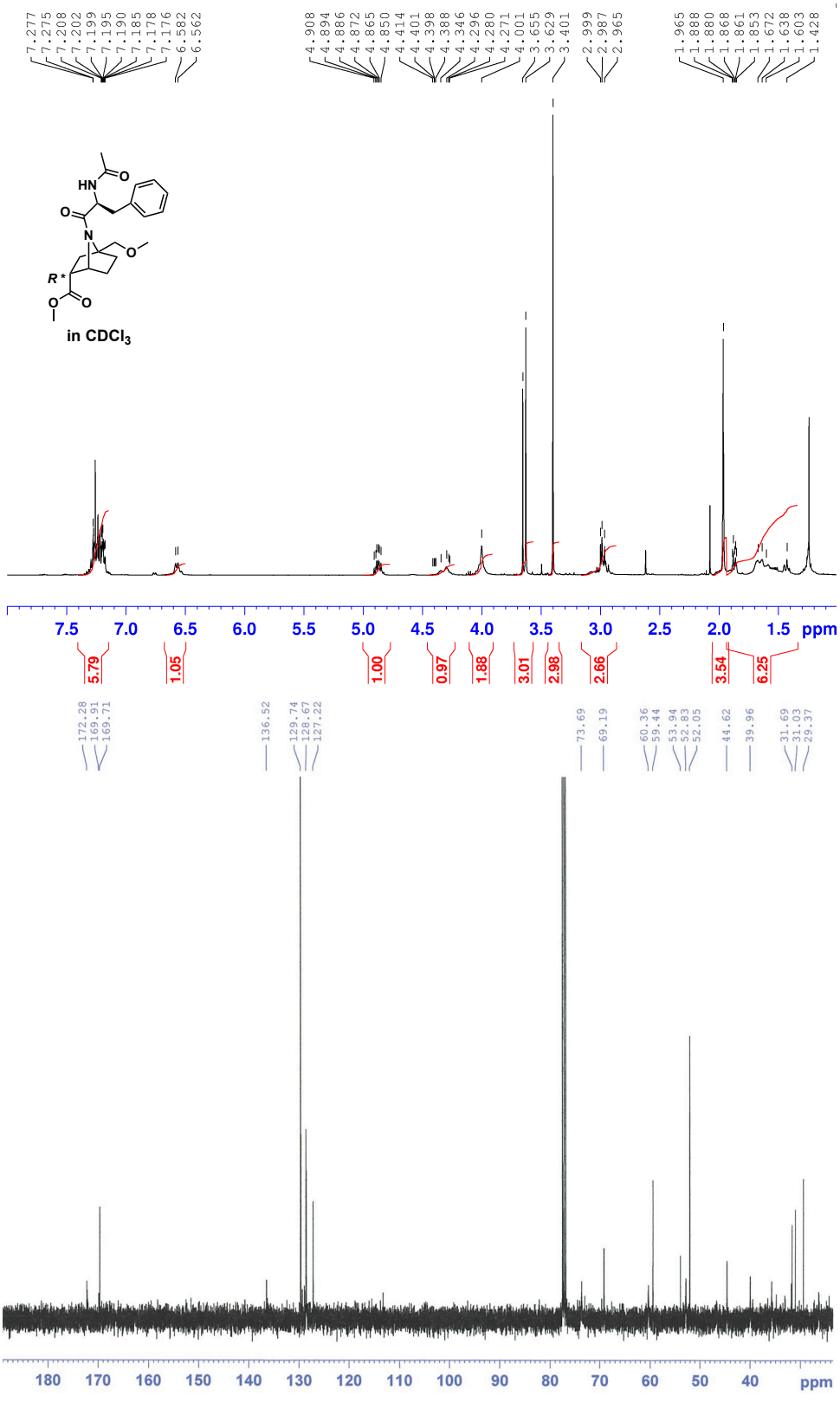


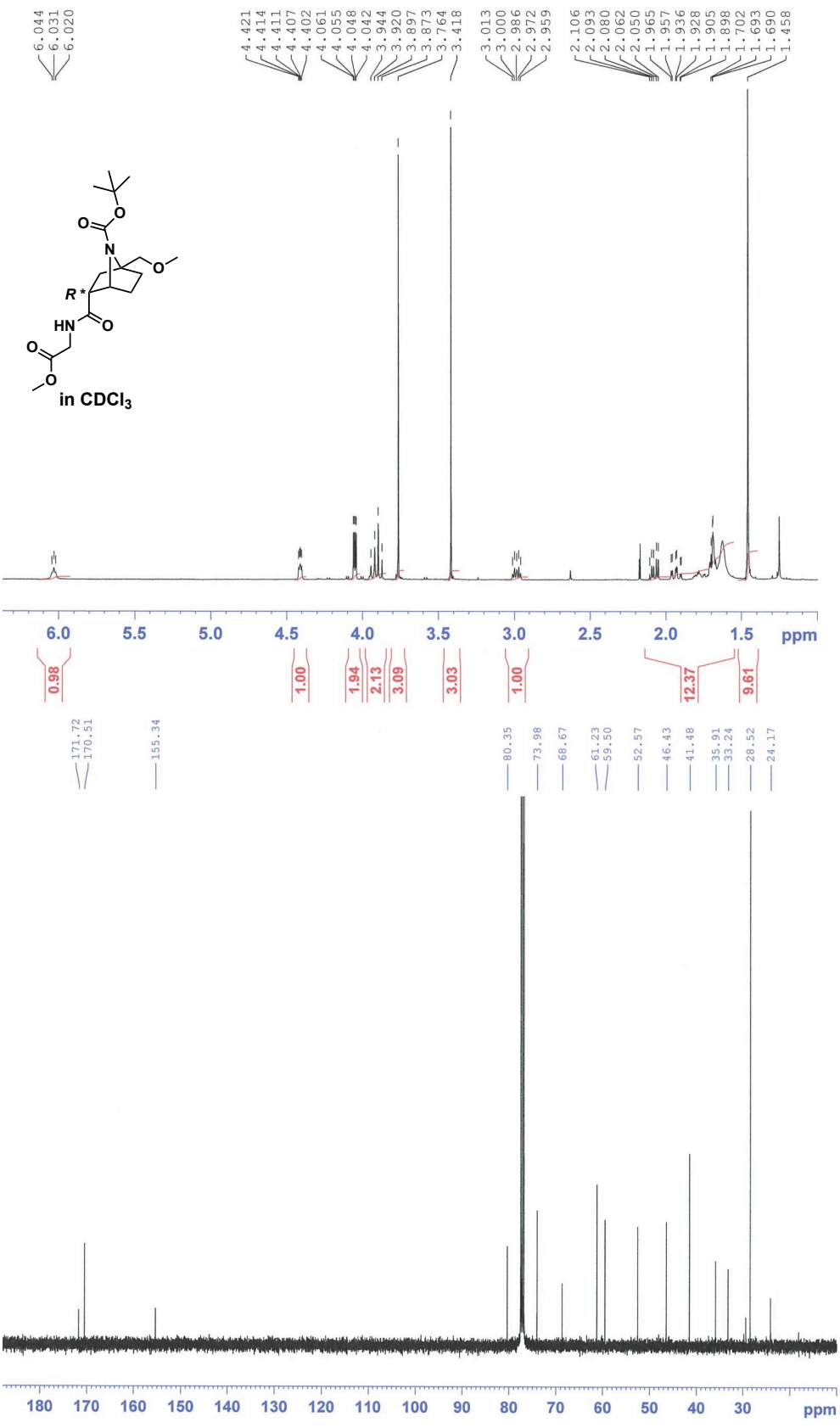
Unfold conformation: ^{1}H-NMR (400 MHz, DMSO) δ 8.256 (1H, d, J=6.8Hz), (1H, d, J=7.6Hz), 7.310-7.197 (5H, m), 4.734-4.679 (1H, m), 4.557 (1H, brs), 4.257-4.202 (1H, m), 3.992-3.866 (2H, m), 3.600 (3H, s), 3.297 (3H, s), 2.980 (1H, brs), 2.897-2.789 (2H, m), 1.984-1.365 (9H, m), 1.819 (3H, s), 0.874 (6H, dd, ^{1}J=19.2Hz, ^{2}J=6.4Hz). HRMS (ESI, $[M+Na]^{+}$): Calcd. for $C_{27}H_{39}N_3NaO_6^{+}$, 524.2731. Found: 524.2745. Reverse-phase HPLC (CH_3CN 100%, 256 nm): t_R 6.85 min, >95% purity.

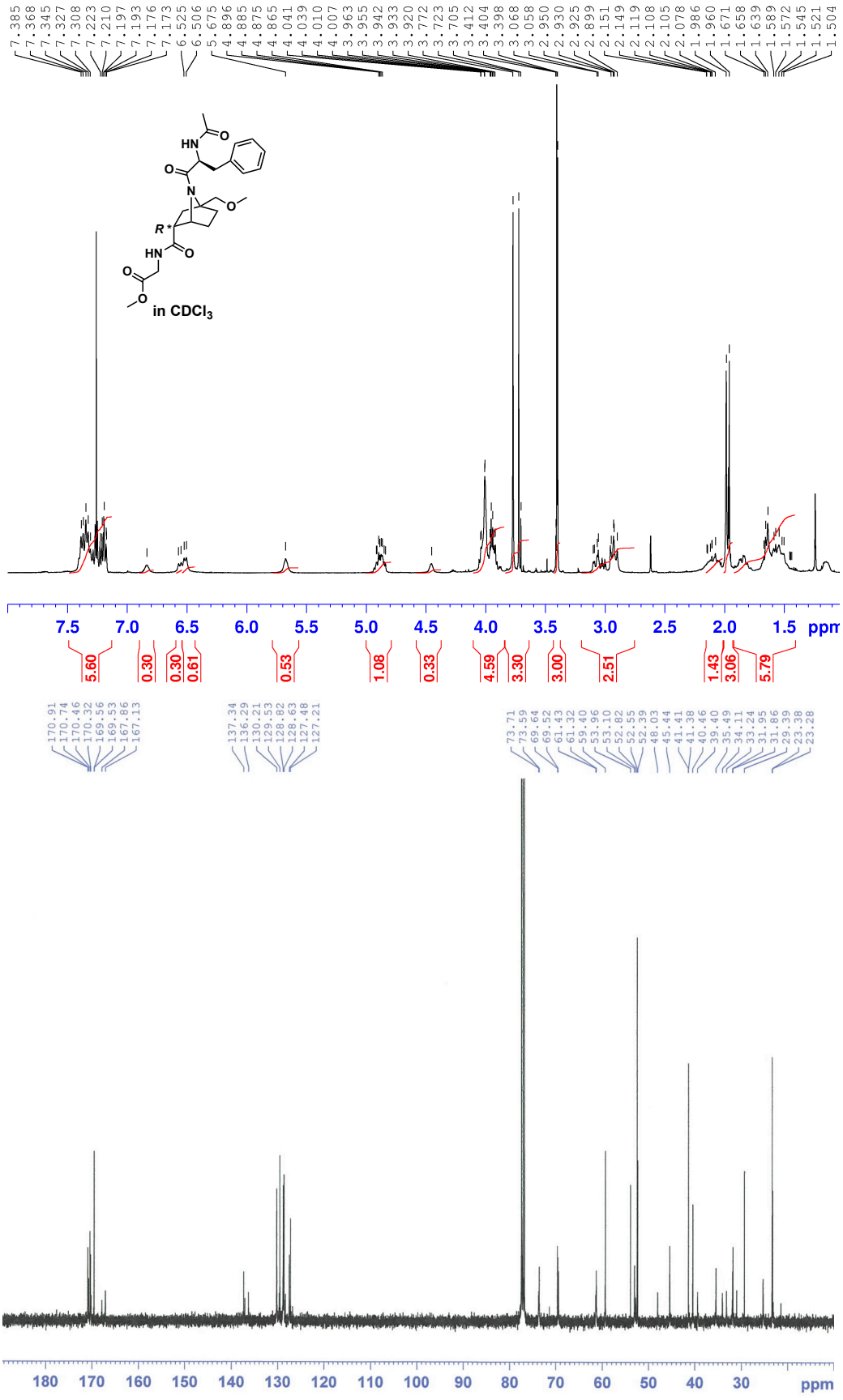


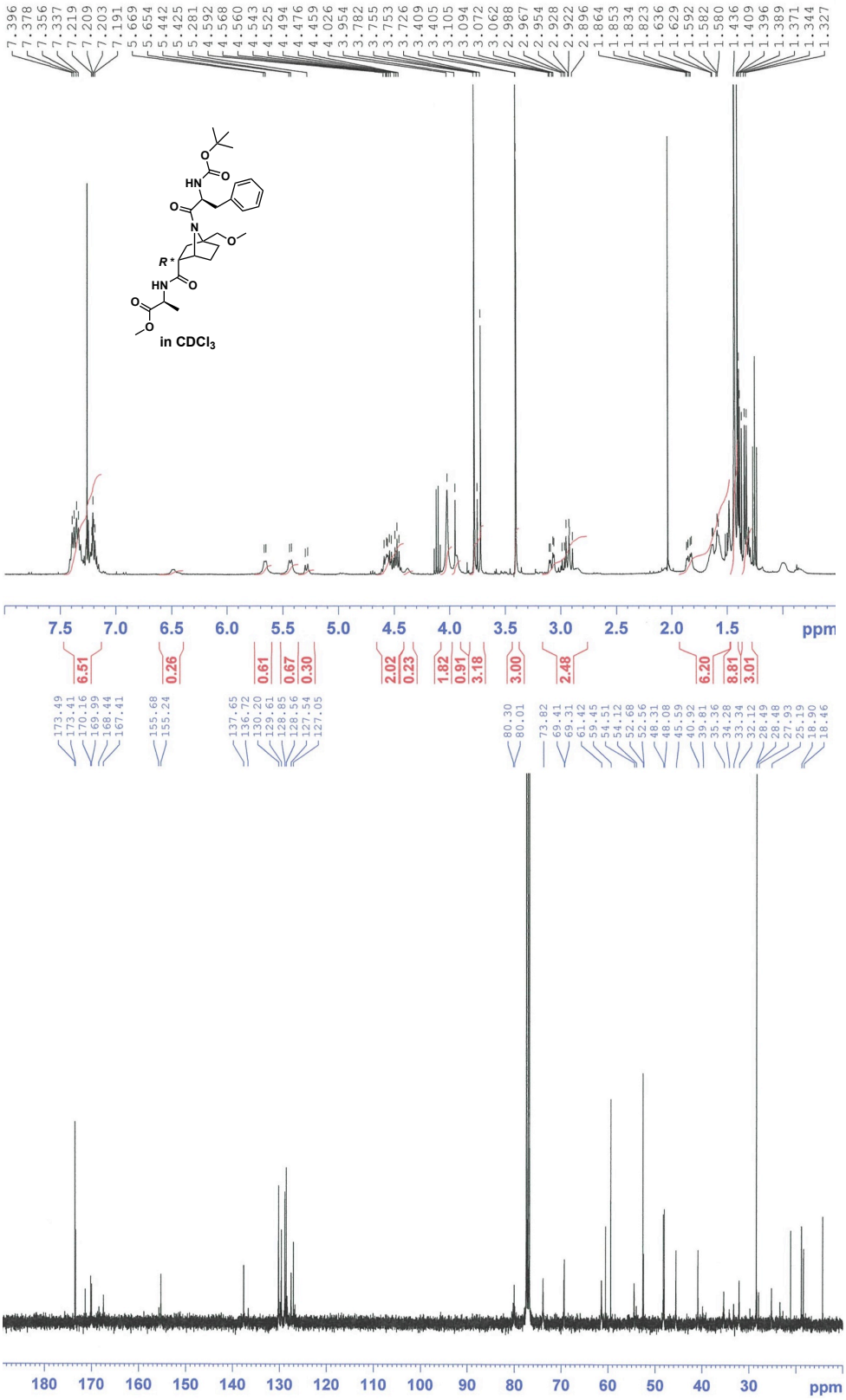
4. ^{1}H and ^{13}C-NMR charts of synthesized compounds

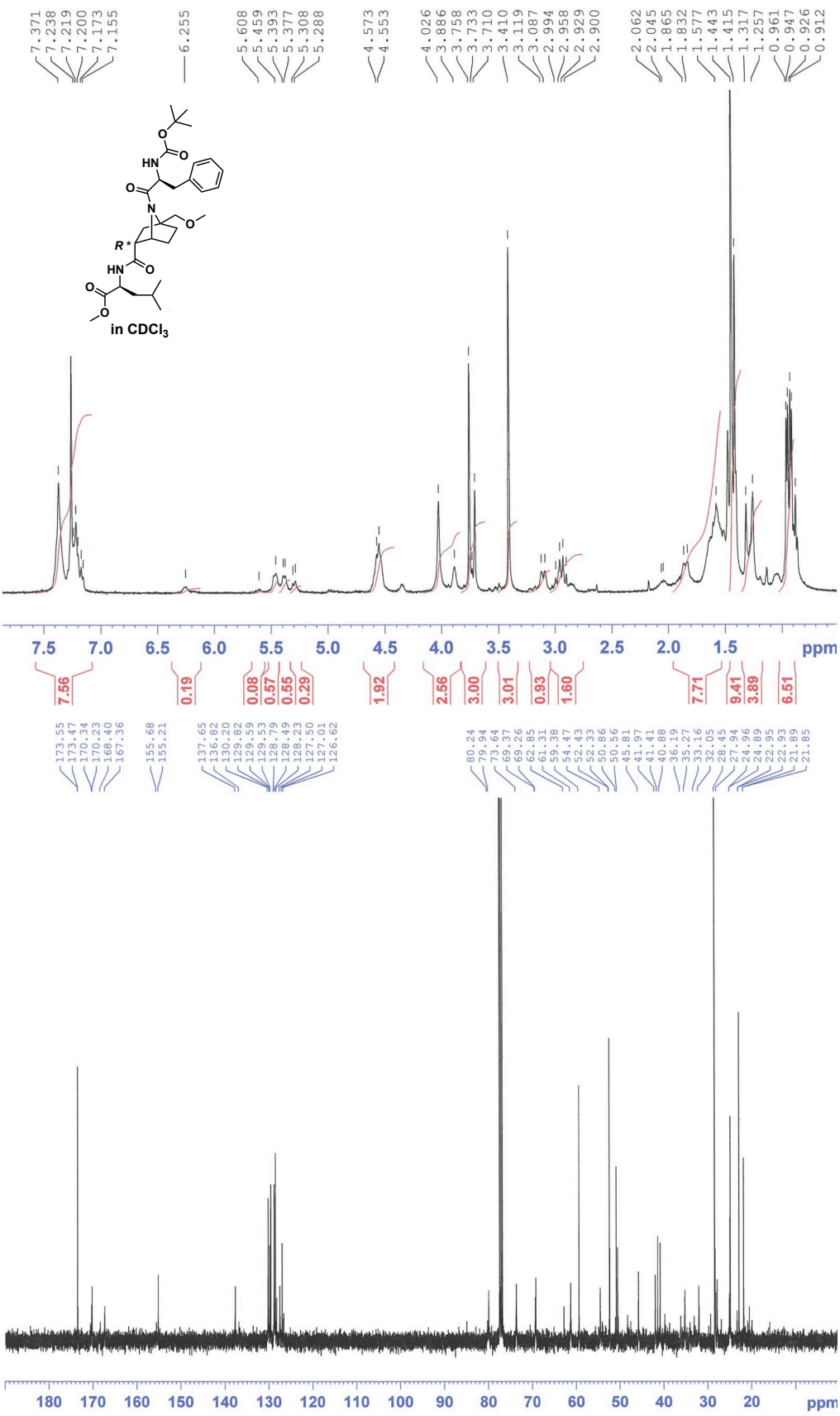


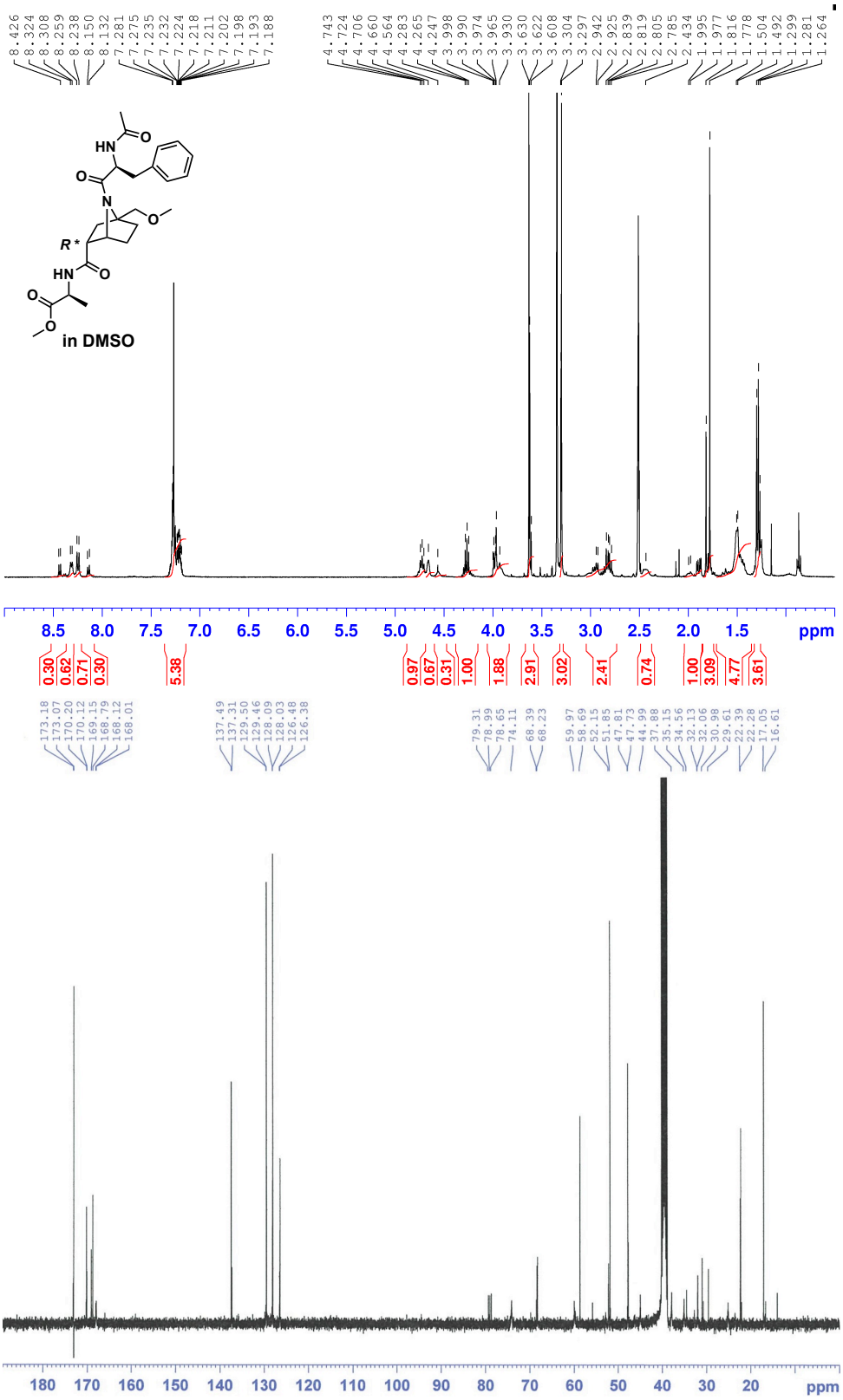

7.277
7.275
7.208
7.202
7.199
7.195
7.190
7.185
7.178
7.176
6.582
6.562
4.908
4.894
4.886
4.872
4.865
4.850
4.414
4.401
4.398
4.388
4.346
4.296
4.280
4.271
4.001
3.655
3.629
3.401
2.999
2.987
2.965
1.965
1.888
1.880
1.868
1.861
1.853
1.672
1.638
1.603
1.428
HN
O
O
N
O
R*
O
O
in CDCl3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
5.79
1.05
1.00
0.97
1.88
3.01
2.98
2.66
3.54
6.25
172.28
169.91
169.71
136.52
129.74
128.67
127.22
73.69
69.19
60.36
59.44
53.94
52.83
52.05
44.62
39.96
31.69
31.03
29.37
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm

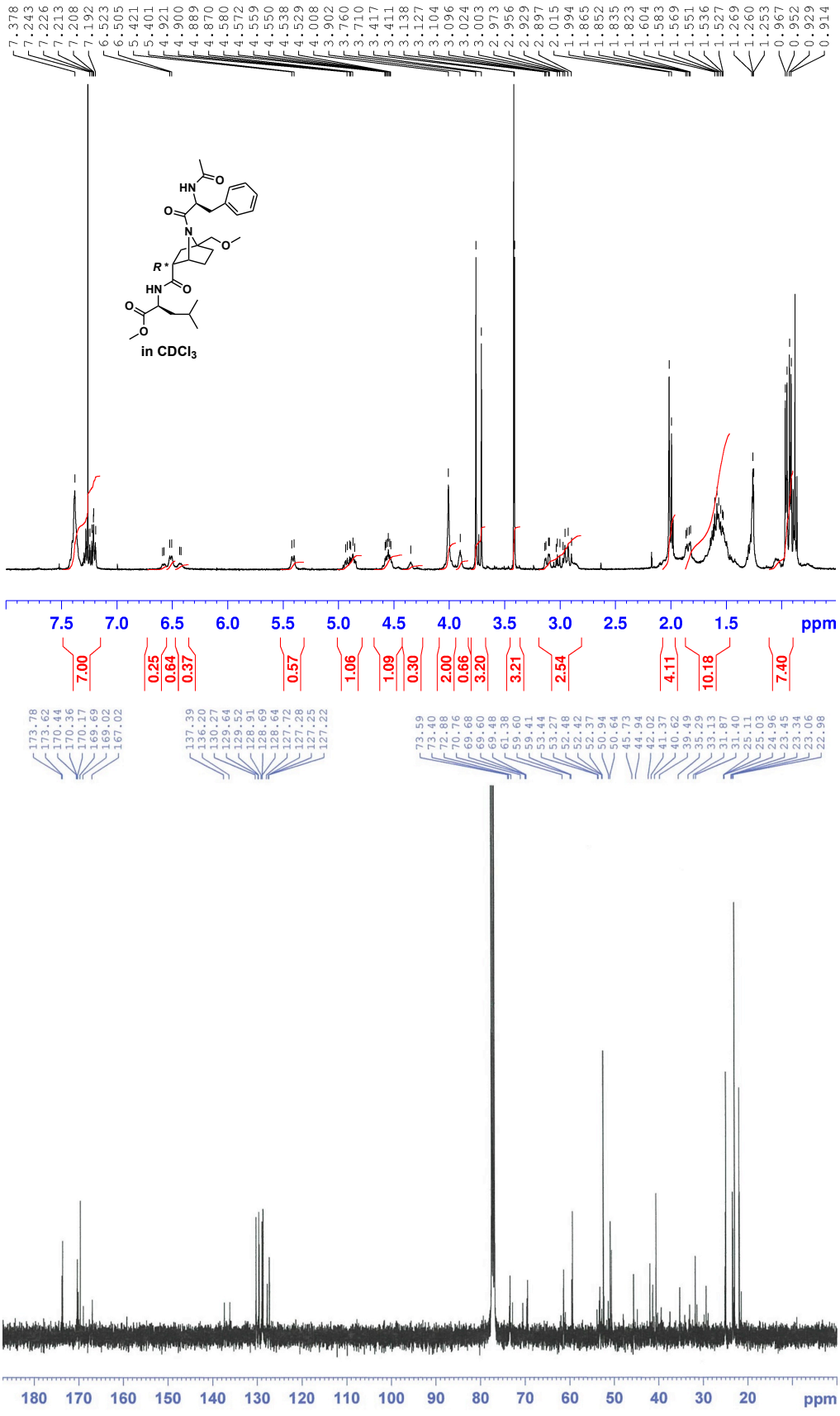

6.044
6.031
6.020
4.421
4.414
4.411
4.407
4.402
4.061
4.055
4.048
4.042
3.944
3.920
3.897
3.873
3.764
3.418
3.013
3.000
2.986
2.972
2.959
2.106
2.093
2.080
2.062
2.050
1.965
1.957
1.936
1.928
1.905
1.898
1.702
1.693
1.690
1.458
R*
HN
in CDCl3
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
0.98
1.00
1.94
2.13
3.09
3.03
1.00
12.37
9.61
171.72
170.51
155.34
80.35
73.98
68.67
61.23
59.50
52.57
46.43
41.48
35.91
33.24
28.52
24.17
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

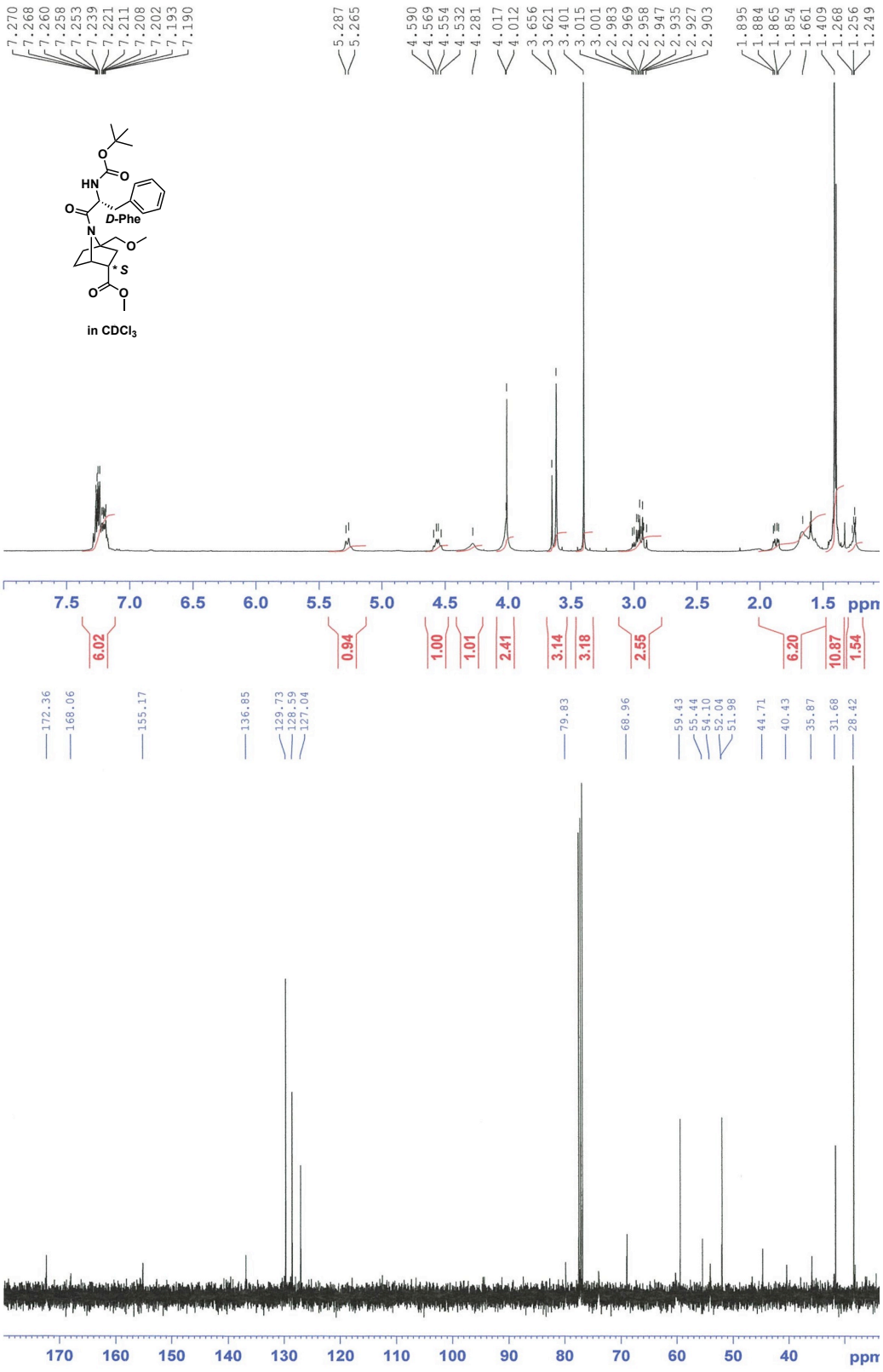

in CDCl3
ppm

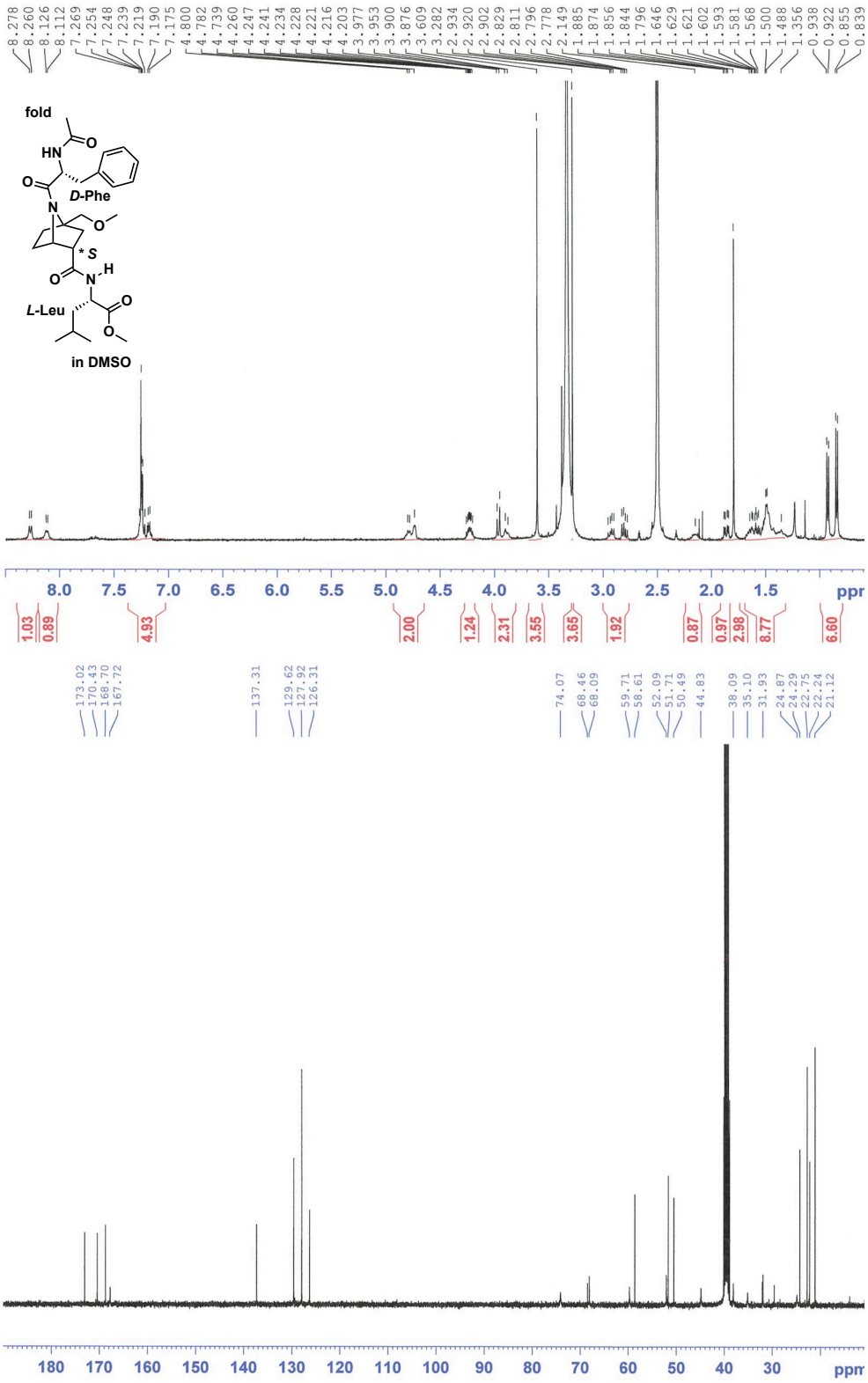

in CDCl3

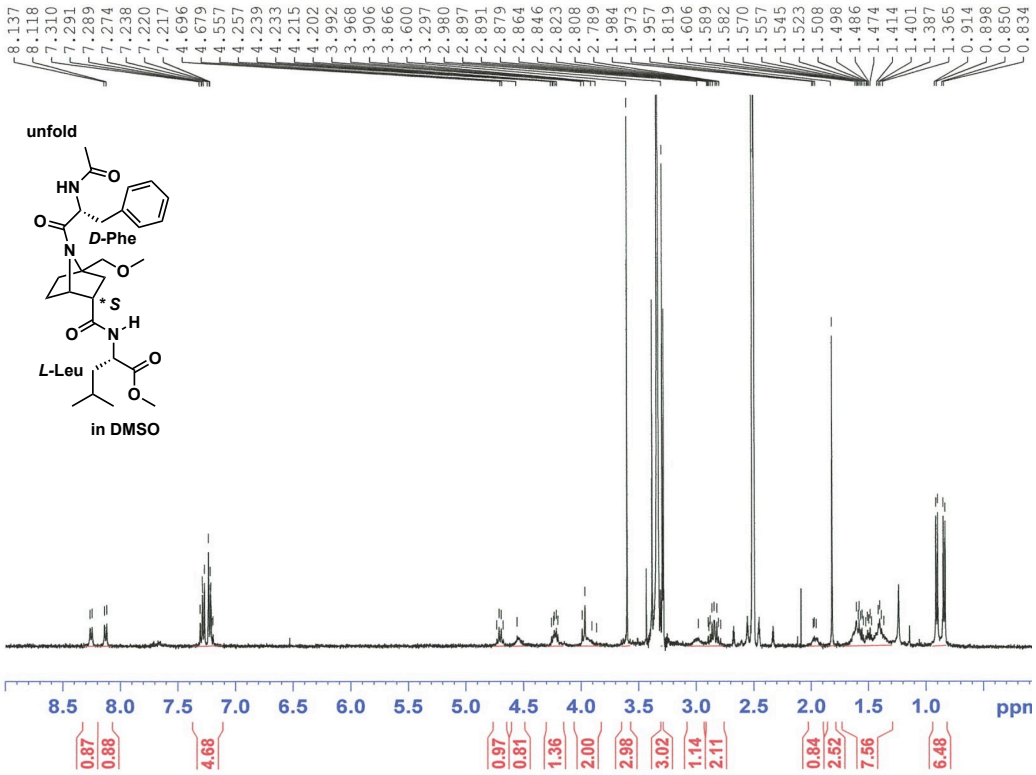

7.371
7.238
7.219
7.200
7.173
7.155
6.255
5.608
5.459
5.393
5.377
5.308
5.288
4.573
4.553
4.026
3.886
3.758
3.733
3.710
3.410
3.119
3.087
2.994
2.958
2.929
2.900
2.062
2.045
1.865
1.832
1.577
1.443
1.415
1.317
1.257
0.961
0.947
0.926
0.912
in CDCl3
ppm
7.56
0.19
0.08
0.57
0.55
0.29
1.92
2.56
3.00
3.01
0.93
1.60
7.71
9.41
3.89
6.51
ppm


8.426
8.324
8.308
8.259
8.238
8.150
8.132
7.281
7.275
7.235
7.232
7.224
7.218
7.211
7.202
7.198
7.193
7.188
4.743
4.724
4.706
4.660
4.564
4.283
4.265
4.247
3.998
3.990
3.974
3.965
3.930
3.630
3.622
3.608
3.304
3.297
2.942
2.925
2.839
2.819
2.805
2.785
2.434
1.995
1.977
1.816
1.778
1.504
1.492
1.299
1.281
1.264
HN
O
O
N
O
R*
HN
O
O
O
in DMSO
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
0.30
0.62
0.71
0.30
5.38
0.97
0.67
0.31
1.00
1.88
2.91
3.02
2.41
0.74
1.00
3.09
4.77
3.61
173.18
173.07
170.20
170.12
169.15
168.79
168.12
168.01
137.49
137.31
129.50
129.46
128.09
128.03
126.48
126.38
79.31
78.99
78.65
74.11
68.39
68.23
59.97
58.69
52.15
51.85
47.73
44.99
37.88
35.15
34.56
32.13
32.06
30.98
29.61
22.39
22.28
17.05
16.61
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


in CDCl3
ppm
ppm


7.270
7.268
7.260
7.258
7.253
7.239
7.221
7.211
7.208
7.202
7.193
7.190
5.287
5.265
4.590
4.569
4.554
4.532
4.281
4.017
4.012
3.656
3.621
3.401
3.015
3.001
2.983
2.969
2.958
2.947
2.935
2.927
2.903
1.895
1.884
1.865
1.854
1.661
1.409
1.268
1.256
1.249
HN
O
O
N
D-Phe
O
* S
O
O
in CDCl3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
6.02
0.94
1.00
1.01
2.41
3.14
3.18
2.55
6.20
10.87
1.54
172.36
168.06
155.17
136.85
129.73
128.59
127.04
79.83
68.96
59.43
55.44
54.10
52.04
51.98
44.71
40.43
35.87
31.68
28.42
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm


8.278
8.260
8.126
8.112
7.269
7.254
7.248
7.239
7.219
7.190
7.175
4.800
4.782
4.739
4.260
4.247
4.241
4.234
4.228
4.221
4.216
4.203
3.977
3.953
3.900
3.876
3.609
3.282
2.934
2.920
2.902
2.829
2.811
2.796
2.778
2.149
1.885
1.874
1.856
1.844
1.796
1.646
1.629
1.621
1.602
1.593
1.581
1.568
1.500
1.488
1.356
0.938
0.922
0.855
0.839
fold
HN
O
O
D-Phe
N
O
* S
O
N H
L-Leu
O
O
in DMSO
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.03
0.89
4.93
2.00
1.24
2.31
3.55
3.65
1.92
0.87
0.97
2.98
8.77
6.60
173.02
170.43
168.70
167.72
137.31
129.62
127.92
126.31
74.07
68.46
68.09
59.71
58.61
52.09
51.71
50.49
44.83
38.09
35.10
31.93
24.87
24.29
22.75
22.24
21.12
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm


8.137
8.118
7.310
7.291
7.289
7.274
7.238
7.220
7.217
4.696
4.679
4.557
4.257
4.239
4.233
4.215
4.202
3.992
3.968
3.906
3.866
3.600
3.297
2.980
2.897
2.891
2.879
2.864
2.846
2.823
2.808
2.789
1.984
1.973
1.957
1.819
1.606
1.589
1.582
1.570
1.557
1.545
1.523
1.508
1.498
1.486
1.474
1.414
1.401
1.387
1.365
0.914
0.898
0.850
0.834
unfold
HN
O
O
D-Phe
N
O
* S
O
N
H
L-Leu
O
O
in DMSO
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
0.87
0.88
4.68
0.97
0.81
1.36
2.00
2.98
3.02
1.14
2.11
0.84
2.52
7.56
6.48

5. Circular Dichroism

The CD spectra were obtained in a 1mm path length quartz cell with scanning speed of 100nm/min and scan range of 190-300nm. The values are expressed in terms of [θ] the total molar ellipticity (deg.cm^2.dmol^{-1}).

6. FTIR measurements

Attenuated total reflection (ATR)-FTIR measurements were carried out for peptides at room temperature on a Perkin-Elmer Spectrum 100 Fourier-transform infrared spectrometer equipped with an ATR unit and an MCT detector at 2 cm^{-1} resolution. Dry air gas was constantly pumped into the ATR unit of the spectrometer to suppress water vapor. Approximately 0.020 ml of a sample in $CHCl_3$ solution was placed on a Diamond/ZnSe 1-reflection top–plate (Perkin-Elmer). To suppress the evaporation of the solvent as much as possible, a cover glass covered on the drop of sample. We checked the evaporation of $CHCl_3$ solvent by monitoring the intensity of 741 cm^{-1} due to C-Cl stretching mode. Interferograms from 30 scans were averaged to obtain one spectrum for the series of measurements of samples in solution. Interferograms from 200 scans were averaged to obtain one spectrum for the series of measurements of samples in dry film, after the solvent has completely evaporated. The treatment and analyses of ATR-FTIR spectra have been performed by using Igor Pro 6.3 (WaveMetrix Lake Oswago) as described previously.[2]

7. Calculations

Molecular dynamics

The Molecular dynamics calculation we used in this work is Desmond molecular dynamics with OPLS3 force field. The cubic solvent model is built in DMSO. The box size calculation method is buffer. The distance of the solvent box is 10 Å each and the angle is 90^o each. The ensemble class is NPT. The total simulation time is 100 ns and the trajectory of recording interval is 30 ps. The approximate number of frames is 3333 and the temperature is set to 298K. Thermostat method is Nose-Hoover chain. Relaxation time is 2ps and coupling style is isotropic.

DFT calculations

8-fold : M06-2x/6-31G(d), scrf=(smd, solvent=DMSO)

```
NIMG=1 (3.7654 cm-1)
Zero-point correction=                           0.648088 (Hartree/Particle)
 Thermal correction to Energy=                    0.687569
```

```
 Thermal correction to Enthalpy=                  0.688513
 Thermal correction to Gibbs Free Energy=         0.568648
 Sum of electronic and zero-point Energies=          -1796.055021
 Sum of electronic and thermal Energies=             -1796.015540
 Sum of electronic and thermal Enthalpies=           -1796.014596
 Sum of electronic and thermal Free Energies=        -1796.134461
Standard orientation:
 ---------------------------------------------------------------------
 Center     Atomic      Atomic             Coordinates (Angstroms)
 Number     Number       Type              X           Y           Z
 ---------------------------------------------------------------------
      1          7           0        3.382719   -1.668009   -0.015042
      2          6           0        4.305815   -2.756825    0.033893
      3          6           0        5.739978   -2.220671   -0.403890
      4          8           0        5.962775   -1.038891   -0.841321
      5          6           0        3.782214   -3.935602   -0.792142
      6          1           0        4.350268   -3.140143    1.053289
      7          6           0       -0.016940   -0.997202    2.199669
      8          6           0        0.333431   -0.452981    0.721409
      9          6           0        0.313341    1.374120    2.004689
     10          6           0        0.000162    0.300172    2.988874
     11          1           0       -0.986406   -1.490885    2.266518
     12          1           0        0.024931   -1.123561   -0.080530
     13          1           0       -0.920108    0.439445    3.556286
     14          1           0        0.737034    0.155082    3.778814
     15          6           0        1.785460    1.353553    1.512509
     16          1           0        2.434219    1.244492    2.381585
     17          1           0        2.119184    2.322789    1.141955
     18          6           0        1.838302    0.091730    0.637304
     19          1           0        2.064389    0.335511   -0.400736
     20          7           0       -0.316944    0.853765    0.796748
     21          6           0       -0.148327    2.775605    2.427043
     22          1           0       -0.091083    3.497987    1.612722
     23          1           0       -1.203131    2.846794    2.692484
     24          8           0        0.798867    3.195587    3.436516
     25          1           0        0.788295   -1.671381    2.491746
     26          7           0       -3.287198    0.378279   -1.078336
     27          6           0       -1.829179    0.618451   -1.230970
     28          6           0       -1.331479    1.297387    0.035493
     29          8           0       -2.036008    2.240511    0.435205
     30          6           0       -1.681833    1.646864   -2.350828
     31          1           0       -3.895156    1.100201   -1.438139
     32          1           0       -1.272569   -0.287130   -1.472411
     33          1           0       -2.362280    2.443108   -2.049011
     34          1           0       -0.317775    4.857109    4.065123
     35          1           0        1.293424    4.926147    4.519114
     36          6           0        0.671926    4.609486    3.681462
     37          1           0        0.946307    5.103434    2.749385
     38          6           0        2.948655   -0.909556    1.016624
     39          8           0        3.362945   -1.110627    2.181187
     40          1           0        3.028996   -1.558892   -0.954679
     41          1           0        2.803410   -4.277848   -0.456092
     42          6           0       -3.833780   -0.713582   -0.465754
     43          8           0       -3.118548   -1.587649    0.017803
     44          1           0       -2.047870    1.272517   -3.306852
     45          6           0       -5.307166   -0.837712   -0.401073
     46          1           0       -5.636567   -0.576525   -1.406768
     47          6           0        2.091868    3.604381   -3.049570
     48          6           0        0.931287    4.380828   -2.763143
     49          6           0       -0.248957    3.698294   -2.469054
     50          6           0       -0.324740    2.280343   -2.582881
```

51	6	0	0.780111	1.602650	-3.058242
52	6	0	1.927472	2.245627	-3.379192
53	1	0	2.983124	4.139399	-3.342788
54	1	0	0.962695	5.459215	-2.812956
55	1	0	-1.133502	4.202671	-2.108949
56	1	0	0.787176	0.522680	-3.047925
57	1	0	2.868698	1.789486	-3.648128
58	8	0	6.696341	-3.124820	-0.223274
59	6	0	8.045209	-2.891829	-0.651492
60	1	0	8.339477	-1.902513	-1.001886
61	1	0	8.727294	-3.302026	0.093144
62	1	0	8.140613	-3.512347	-1.542517
63	1	0	3.563260	-3.560227	-1.791744
64	1	0	4.468303	-4.776237	-0.895884
65	7	0	-5.505444	-2.253155	-0.116363
66	1	0	-4.634178	-2.697910	0.134838
67	6	0	-6.587742	-2.993837	-0.281890
68	8	0	-7.639247	-2.634484	-0.853490
69	6	0	-6.541453	-4.447432	0.223900
70	1	0	-6.620318	-5.116952	-0.632591
71	1	0	-5.627464	-4.720662	0.751408
72	1	0	-7.387146	-4.673384	0.873429
73	6	0	-6.083095	0.007224	0.639544
74	1	0	-5.861579	0.994493	0.233914
75	1	0	-7.169633	-0.048779	0.573589
76	1	0	-5.756503	-0.285244	1.637438

8-unfold : M06-2x/6-31G(d), scrf=(smd, solvent=DMSO)

NIMG=1 (12.5072 cm^{-1})

Zero-point correction= 0.648659 (Hartree/Particle)

Thermal correction to Energy= 0.687658

Thermal correction to Enthalpy= 0.688602

Thermal correction to Gibbs Free Energy= 0.572214

Sum of electronic and zero-point Energies= -1796.053135

Sum of electronic and thermal Energies= -1796.014136

Sum of electronic and thermal Enthalpies= -1796.013192

Sum of electronic and thermal Free Energies= -1796.129580

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
1	7	0	4.756197	-1.188996	-0.720354
2	6	0	6.004182	-1.811467	-0.459375
3	6	0	7.003751	-0.932134	0.360967
4	8	0	6.846143	0.285836	0.484290
5	6	0	6.611353	-2.409131	-1.732478
6	1	0	5.843676	-2.641405	0.228780
7	6	0	1.387762	0.707343	1.647853
8	6	0	1.187828	-0.190099	0.405257
9	6	0	1.529497	1.939161	-0.474107
10	6	0	1.731387	2.119122	1.069815
11	1	0	0.584525	0.545839	2.366788
12	1	0	0.630328	-1.115535	0.549730
13	1	0	0.932401	2.785547	1.394611
14	1	0	2.707049	2.401668	1.465382
15	6	0	2.687811	1.042267	-0.981801
16	1	0	3.557677	1.470056	-0.483273

17	1	0	2.701522	1.176121	-2.063449
18	6	0	2.383042	-0.393987	-0.537920
19	1	0	2.014140	-0.921367	-1.417612
20	7	0	0.517485	0.889407	-0.383186
21	6	0	1.321859	3.273104	-1.178840
22	1	0	0.877327	3.225881	-2.172987
23	1	0	0.492494	3.780282	-0.686003
24	8	0	2.574622	4.004858	-1.086814
25	1	0	2.193644	0.361004	2.294906
26	7	0	-3.022903	0.576233	0.087028
27	6	0	-1.827320	-0.104765	-0.331494
28	6	0	-0.820368	0.977083	-0.713726
29	8	0	-1.279563	1.920881	-1.379200
30	6	0	-2.225797	-1.087796	-1.503159
31	1	0	-3.591531	0.896436	-0.683855
32	1	0	-1.343470	-0.712337	0.433255
33	1	0	-1.379990	-1.693445	-1.828588
34	1	0	3.363019	5.843141	-1.374934
35	1	0	2.481473	5.069881	-2.660954
36	6	0	2.444992	5.298599	-1.595893
37	1	0	1.502820	5.823032	-1.436342
38	6	0	3.639221	-1.192792	0.038388
39	8	0	3.590887	-1.730000	1.182365
40	1	0	4.634296	-0.817998	-1.651793
41	1	0	6.846083	-1.529605	-2.332011
42	6	0	-3.338179	0.772081	1.335535
43	8	0	-2.558046	0.551397	2.294834
44	1	0	-2.540181	-0.576183	-2.412834
45	6	0	-4.710587	1.401514	1.593041
46	1	0	-4.710426	2.349204	1.054510
47	6	0	-5.021927	-4.208368	-0.417330
48	6	0	-5.479377	-3.000121	-0.937246
49	6	0	-4.640753	-1.966974	-1.291811
50	6	0	-3.252550	-2.104081	-1.024715
51	6	0	-2.897507	-3.273942	-0.340920
52	6	0	-3.740796	-4.280432	-0.043588
53	1	0	-5.686704	-5.051898	-0.303061
54	1	0	-6.517639	-2.890204	-1.213644
55	1	0	-4.975887	-1.091680	-1.828569
56	1	0	-1.837924	-3.340270	-0.142903
57	1	0	-3.363900	-5.170256	0.438577
58	8	0	7.929932	-1.711684	1.030394
59	6	0	8.590235	-1.126099	2.060890
60	1	0	8.806466	-1.890268	2.807515
61	1	0	9.545343	-0.637847	1.867362
62	1	0	8.021519	-0.398240	2.639766
63	1	0	7.573352	-2.907571	-1.613495
64	1	0	5.967238	-3.159154	-2.191472
65	7	0	-5.860895	0.623853	1.167839
66	1	0	-6.349152	0.107669	1.885563
67	6	0	-6.447335	0.718926	-0.055776
68	8	0	-5.949941	1.428246	-0.926941
69	6	0	-7.747033	0.005753	-0.293786
70	1	0	-8.601118	0.586336	0.054956
71	1	0	-7.853107	-0.264625	-1.344360
72	1	0	-7.795246	-0.965052	0.199554
73	6	0	-4.980067	1.760203	3.056458
74	1	0	-4.116291	2.262321	3.492151
75	1	0	-5.881103	2.344481	3.243405
76	1	0	-5.054131	0.848485	3.649184

8. Reference

(1) L. Zhai, Y. Otani, Y. Hori, T. Tomita and T. Ohwada, *Chem. Commun.* , 2020, **56**, 1573–1576.

(2) M. Nara, H. Morii and M. Tanokura, Springer Nature, 2019, 127-134.

(3) E. van Lenthe and E. Baerends, *J Comput Chem*, 2003, **24**, 1142–1156.