Electronic Supplementary Information (ESI)

Peptide-based Short Single β-Strand Mimics, without Hydrogen Bonding or Aggregation

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Table of Contents

1. Supporting graphics **page 3**

2. Experimental Section of Synthesis **page 9**

3. NMR measurements **page 22**

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

4. Circular Dichroism **page 38**

5. Calculations **page 39**

1. Supporting graphics

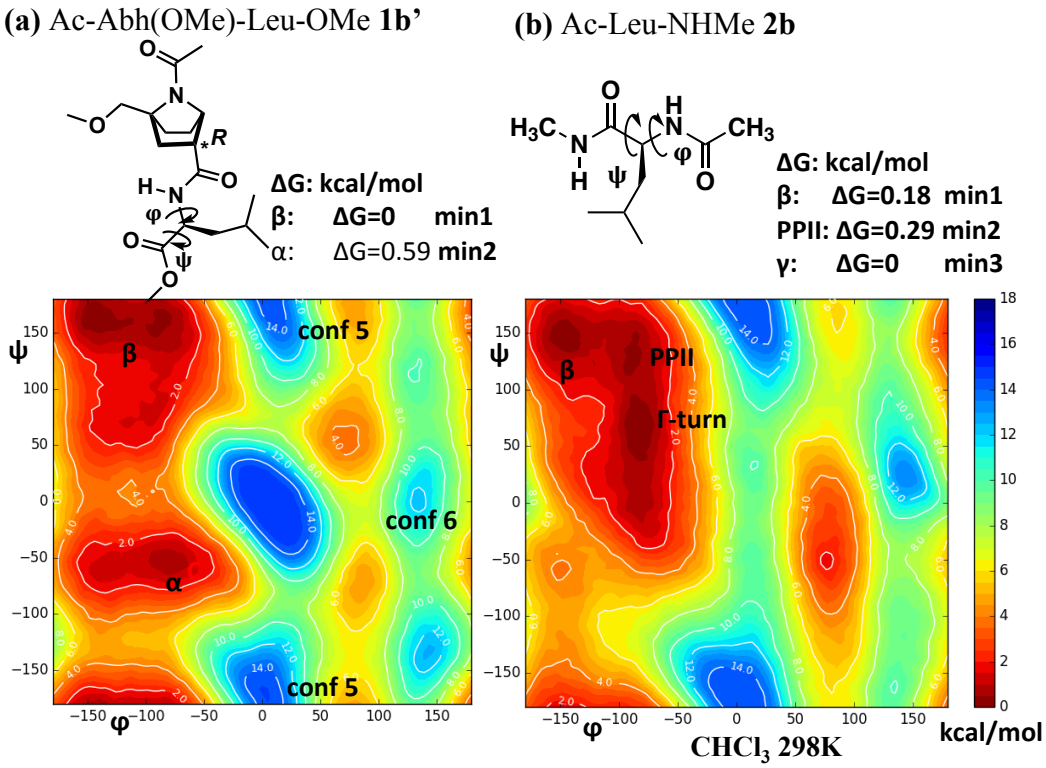


Figure S1. Energy landscape of 1b' and 2b.

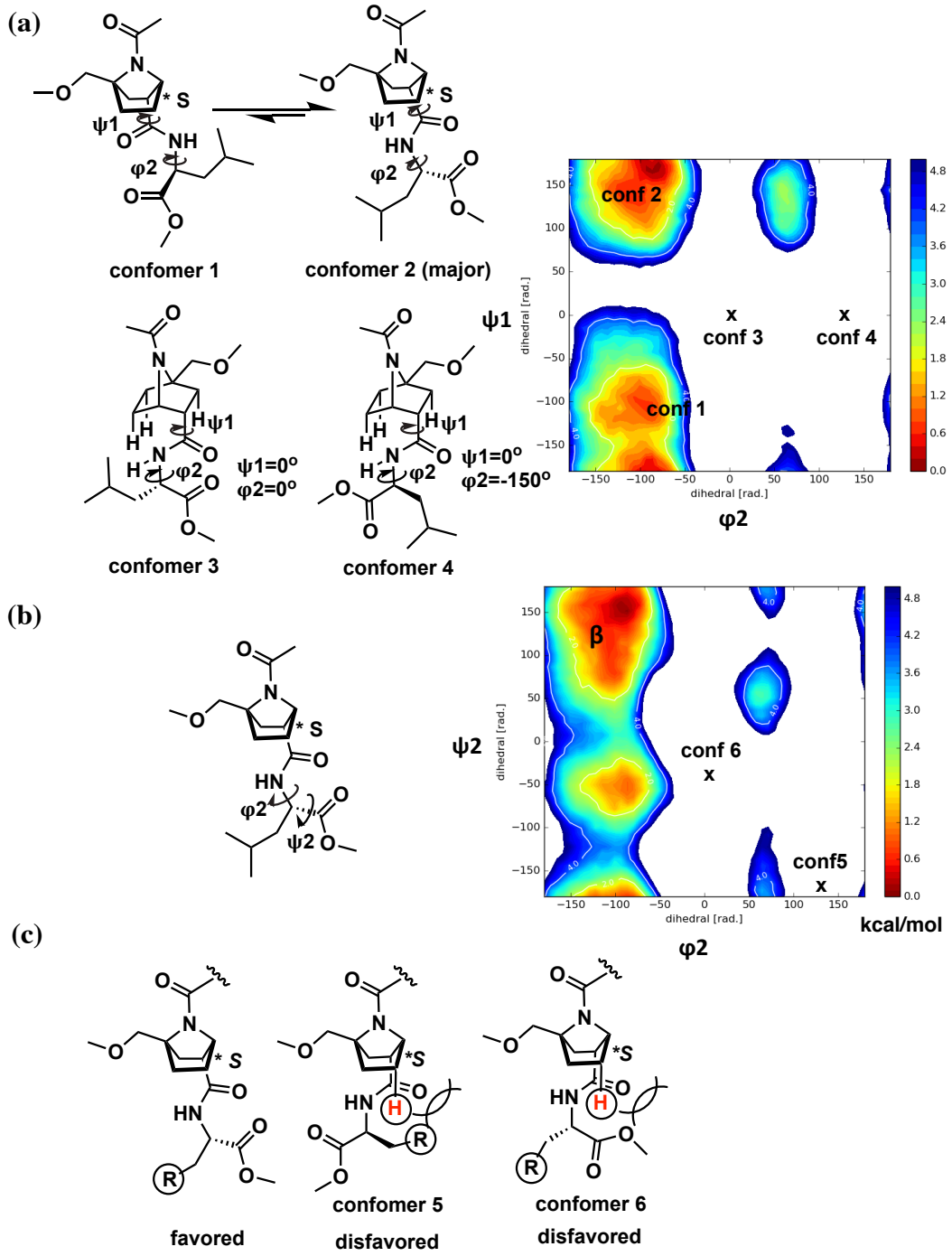


Figure S2. Energy landscape of Ac-*(S)*-Abh(OMe)-Leu-OMe 3b' in terms of (a), ψ_1 and φ_2. (b), ψ_2 and φ_2. (c) three possible dipeptide conformations.

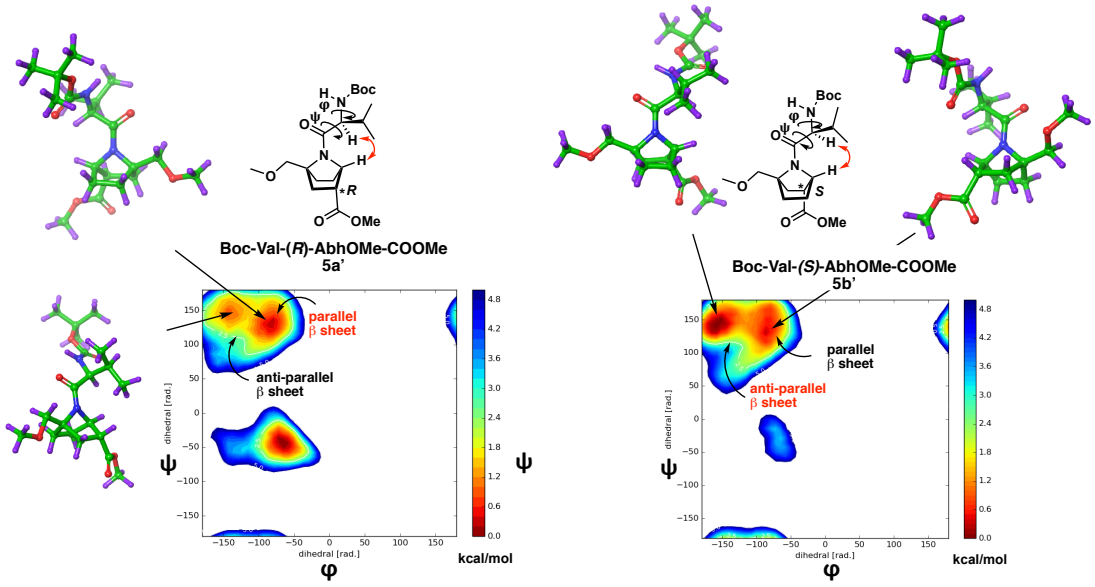


Figure S3-1. Energy landscape of 5a' and 5b'.

Energy landscape of Boc-Val-(*R*)-AbhOMe-CO_2Me **5a'** and Boc-Val-(*S*)-AbhOMe-CO_2Me **5b'** in chloroform at 298K. The red arrows show the proximity between the α-proton of Val and bridgehead proton of Abh amino acid.

We examined the conformation of α-amino acids coupled with (*R*)- or (*S*)-Abh amino acid on the N-terminal side. We conducted metadynamics simulations of **Boc-Val-(*R*)-AbhOMe-COOMe 5a'** and **Boc-Val-(*S*)-AbhOMe-COOMe 5b'** (Supporting graphics Figure S3, Figure S3-2 with a different energy level). The results suggested that **5a'** and **5b'** both favor β-strand conformations, but **5a'** favors parallel β-strand conformation to a greater extent (ϕ=-120°, ψ=115°), while **5b'** favors anti-parallel β-strand conformation to a greater extent (ϕ=-140°, ψ=135°). Experimentally, this is supported by the magnitudes of the ^3J coupling constants: the ^3J coupling constants of the Val residue in **5a** in chloroform at 25°C and 0 °C (9.2 Hz, 9.2 Hz) are a little smaller than those of **5b** in chloroform at 25°C and 0 °C (9.5 Hz, 9.4 Hz) (Main text, Figure 4). Thus, (*R*)/(*S*)-Abh amino acids can both enforce β-strand conformation on the α-amino acid at the N-terminal side. The NMR study indicated that the bridgehead proton is close to the α-proton of the α-amino acid, due to the characteristic upward-projecting direction of the bridgehead proton, and this proximity would influence the main chain rotation of the adjacent α-amino acid.

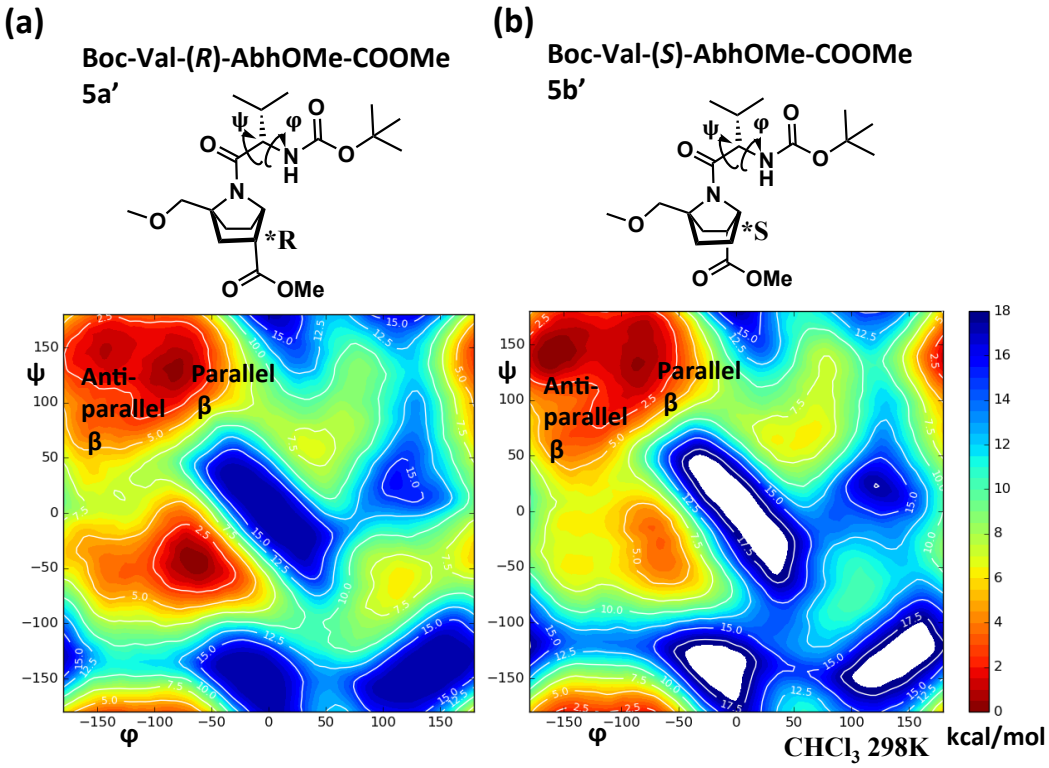


Figure S3-2. Energy landscape of 5a' and 5b'. With a different Energy Scale.

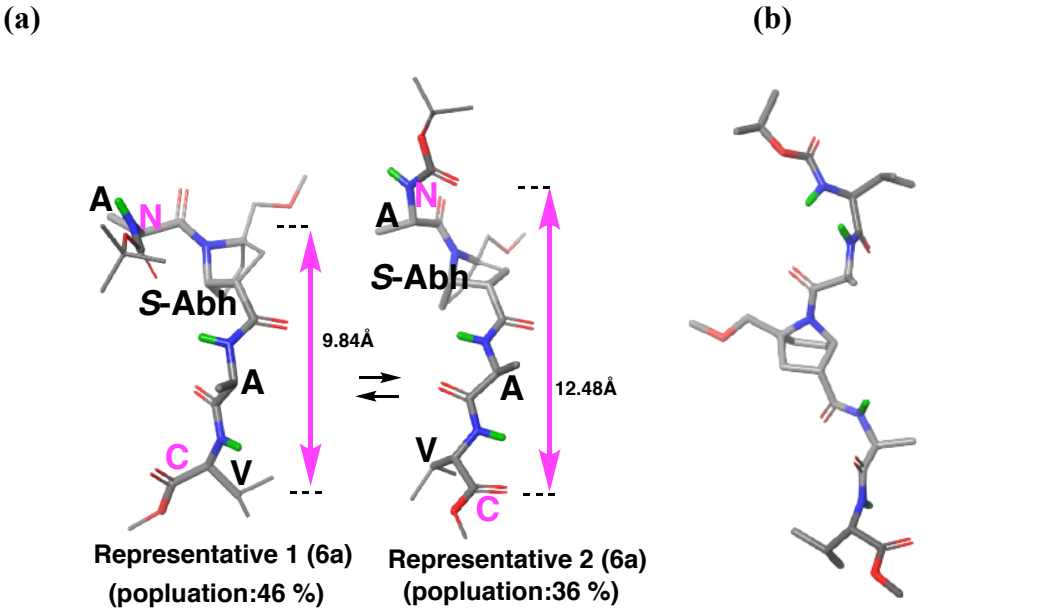


Figure S4. (a) Representative structures of 6a (in equilibrium) from molecular dynamics calculation in chloroform. (b) The most populated structure (also extended structure) of 7a (50%) from molecular dynamics calculation in methanol.

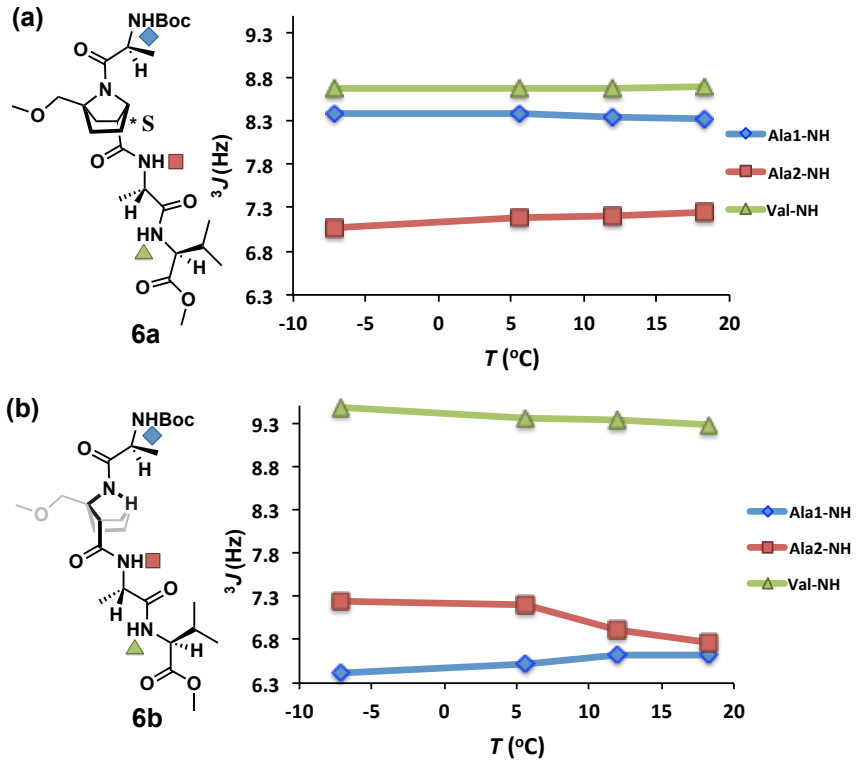


Figure S5, Temperature dependency of ^3J coupling constants in chloroform ($CDCl_3$-d_1). (a) tetrapeptide 7a (b) tetrapeptide 6b. The concentration of the sample is 60 mM.

In the VT-NMR of **6a** and **6b**, the temperature-dependent change of ^3J coupling constants of the α-amino acid residues is small over a 30-degree temperature range, particularly for **6a**. The weak temperature dependency is consistent with the idea that **6a** takes conformationally biased structures.

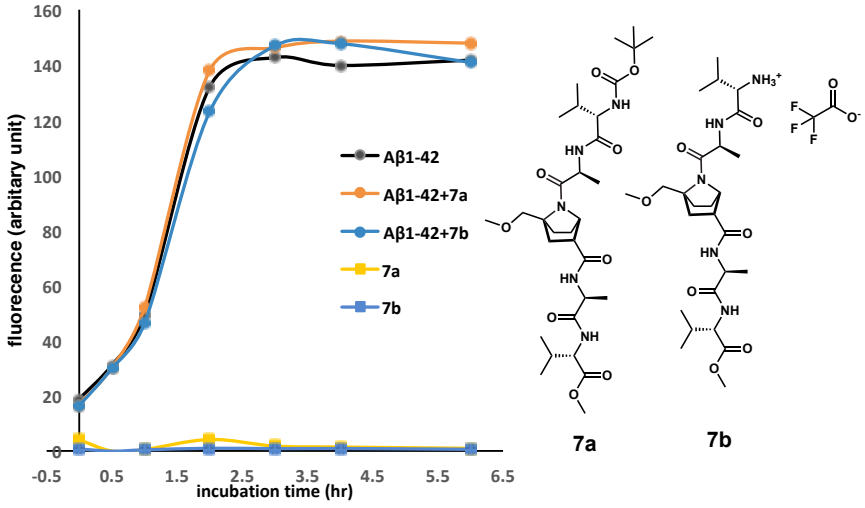


Figure S6. Effect of pentapetides 7a and 7b on aggregation of amyloid-β_{42}. Thioflavin T fluorescence assay. The concentration of amyloid-β_{42} is 22 μM and the concentration of 7a and 7b is 50 μM, respectively.

Amyloid assay method

Synthetic Aβ(1–42) peptides were purchased from Peptide Institute, Inc.. Aβ fibrillization assay was conducted as previously reported.[1,2] Briefly, synthetic Aβ(1–42) was solubilized at a concentration of 22 μM without or with pentapeptides **7a** and **7b** at a concentration of 50 μM and incubated at 37 °C. At the same time, only pentapeptides **7a** or **7b** were also incubated. Following incubation for the indicated times, samples were mixed with 3 μM Thioflavin T (Tokyo Chemical) in 0.1 M glycine-NaOH (pH 8.5) and the fluorescence were measured to monitor fibril formation (Ex = 433 nm and Em = 484 nm). [1,2]

Table S1. Chemical Shift Values of α-Protons for 1a, 1b, 1c, 3b, 4b, 5a, 5b and 5c.[a]

	Proton	δ (obs)[b]	δ (random coil)[3]
1a	Ala(CHα)	4.59 ppm	4.35 ppm
1b	Leu(CHα)	4.64 ppm	4.17 ppm
1c	Ile(CHα)	4.60 ppm	3.95 ppm
3b	Leu(CHα)	4.61 ppm	4.17 ppm
4b	D-Leu(CHα)	4.65 ppm	N/A
5a	Val(CHα)	4.17 ppm	3.95 ppm
	Leu(CHα)	4.67 ppm	4.17 ppm
5b	Val(CHα)	4.14 ppm	3.95 ppm
	Leu(CHα)	4.56 ppm	4.17 ppm
5c	Leu(CHα)	4.44 ppm	4.17 ppm
	D-Leu(CHα)	4.59 ppm	N/A

[a] the α-Proton of α-amino acid residues for **6a** and **7a** is overlapped form ^{1}H-NMR, thus the chemical shift of α-Proton is not available.

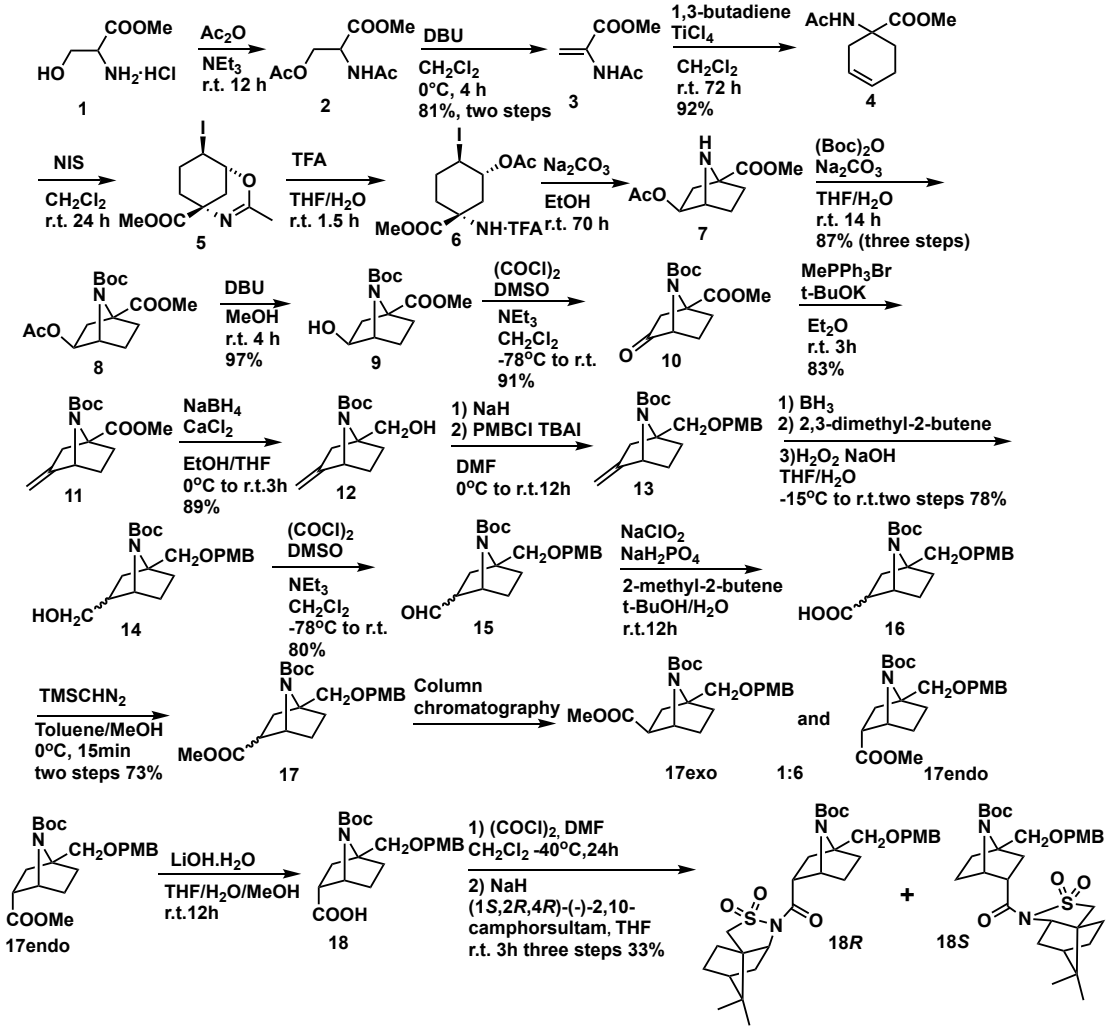
[b] the chemical shift of α-Proton is detected in d_1-$CDCl_3$ at room temperature. In d_3-CD_3OH, the big water peak from d_3-CD_3OH is close and overlapped with α-Proton. Thus the chemical shift of α-Proton in d_3-CD_3OH is not available.

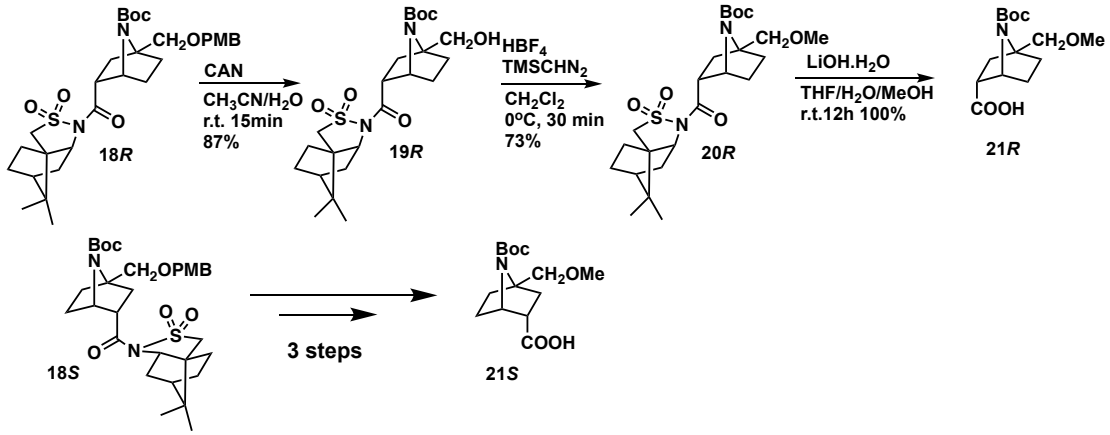
2. Experiment section of Synthesis

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 Senshu Pak Pegasil Silica SP-100 (250 mm x 20 mm) for a normal phase column, and Mightysil RP-18 GP Aqua (250 mm x 10 mm) for a reverse phase column. Flow rate: 5.0 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

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 in Figure S1.

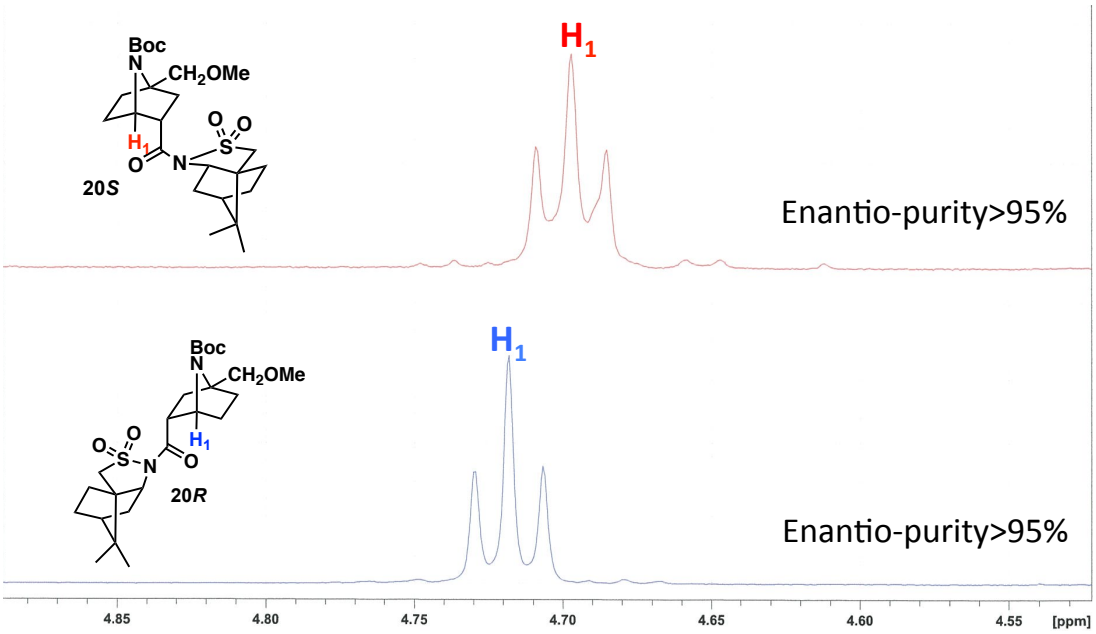
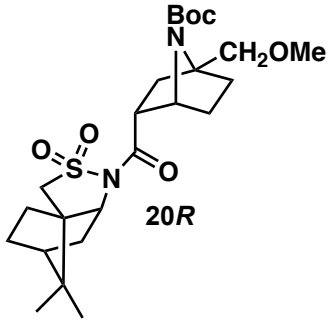
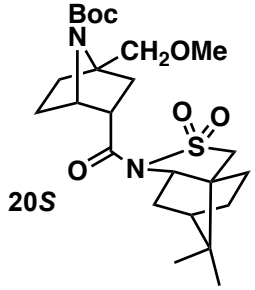


Figure S7



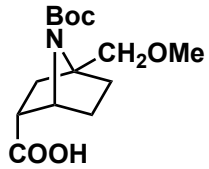
20*R*

^{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).



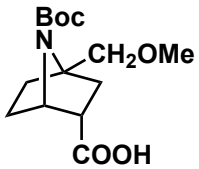
20*S*

^{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).



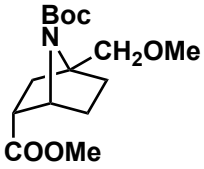
21*R*

^{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.



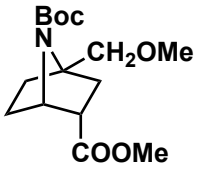
21*S*

^{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.



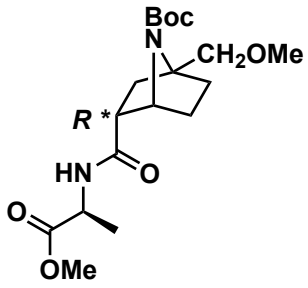
22*R*

^{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.



22*S*

^{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.

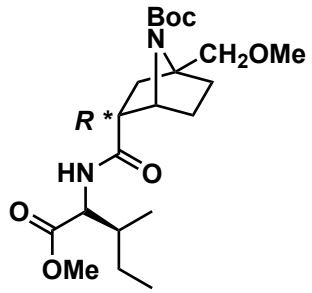


Boc-*R*-Abh(OMe)-Ala-OMe **(1a)**

To a solution of **21*R*** (32 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) were added NH_2-Ala-OMe.HCl (24 mg, 0.17 mmol), CDMT (39 mg, 0.22 mmol), DMAP (0.02 mmol, 2 mg) and NMM (60 μl, 0.55 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)-Ala-OMe **(1a)** (29 mg, 71%), as a colorless oil.

^{1}H NMR ($CDCl_3$, 400MHz) δ 6.104 (1H, d, J=7.4Hz), 4.622-4.551 (1H, m), 4.385-4.362 (1H, m), 3.930-3.869 (2H, m), 3.753 (3H, s), 3.413 (3H, s), 2.980-2.929 (1H, m), 2.071-2.028 (1H, m), 1.941-1.615 (5H, m), 1.458 (9H, s), 1.398 (3H, d, J=7.16Hz)
^{13}C-NMR (100 MHz, $CDCl_3$): 173.66, 170.97, 155.33, 80.34, 73.96, 68.67, 61.25, 59.49, 52.69, 48.28, 46.44, 35.84, 33.21, 28.52, 24.09, 18.67.

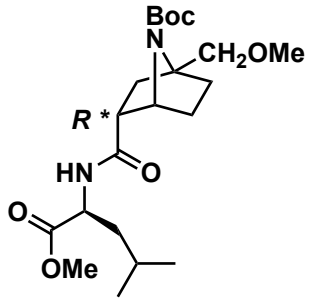
HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{18}H_{30}N_2NaO_6^+$, 393.1996. Found: 322.2020.



Boc-*R*-Abh(OMe)-Ile-OMe **(1c)**

To a solution of **21*R*** (49 mg, 0.17 mmol) in CH_2Cl_2 (2 mL) were added NH_2-Ile-OMe.HCl (46 mg, 0.26 mmol), CDMT (60 mg, 0.34 mmol), DMAP (2 mg, 0.02 mmol) and NMM (93 μl, 0.85 mmol) at rt. The reaction mixture was 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 = 1/1) to afford Boc-Abh(OMe)-Ile-OMe **(1c)** (46.7 mg, 71%), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.081 (1H, d, J=8.4Hz), 4.614-4.580 (1H, m), 4.377-4.354 (1H, m), 3.867 (2H, s), 3.721 (3H, s), 3.397 (3H, s), 2.990-2.940 (1H, m), 2.056-1.638 (7H, m), 1.451 (9H, s), 1.164-0.868 (8H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 172.68, 171.16, 155.37, 80.38, 73.85, 68.62, 61.28, 59.42, 56.50, 54.86, 52.27, 46.68, 37.96, 35.82, 33.25, 28.49, 25.31, 15.62, 11.60. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{36}N_2NaO_6^+$, 435.2466. Found: 435.2481.

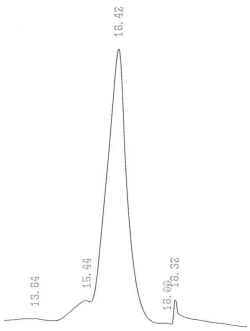


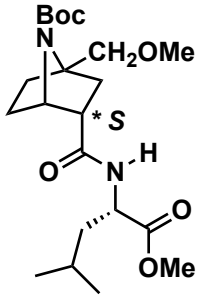
Boc-*R*-Abh(OMe)-Leu-OMe **(1b)**

To a solution of **21*R*** (96 mg, 0.33 mmol) in CH_2Cl_2 (3 mL) were added NH_2-Leu-OMe.HCl (91 mg, 0.50 mmol), CDMT (116 mg, 0.66 mmol), DMAP (4 mg, 0.03 mmol) and NMM (187 μl, 1.70 mmol) at rt. The reaction mixture was 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 = 1/1) to afford Boc-Abh(OMe)-Leu-OMe **(1b)** (102.3 mg, 76%), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.903 (1H, d, J=8.4Hz), 4.667-4.612 (1H, m), 4.381-4.358 (1H, m), 3.887 (2H, s), 3.733 (3H, s), 3.414 (3H, s), 2.992-2.937 (1H, m), 2.074-1.511 (9H, m), 1.464 (9H, s), 0.946-0.925 (6H, m) ^{13}C-NMR(100 MHz,

$CDCl_3$): 173.75, 171.23, 155.44, 80.38, 73.87, 68.66, 61.28, 59.45, 54.83, 50.87, 46.58, 35.82, 33.26, 28.50, 25.03, 23.98, 22.94, 21.94. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{36}N_2NaO_6^+$, 435.2466. Found: 435.2467. HPLC (Hexane: IPA = 6:1, 215 nm): t_R 16.42 min, 92% purity.

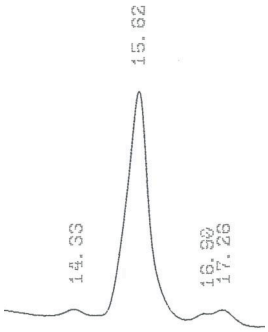


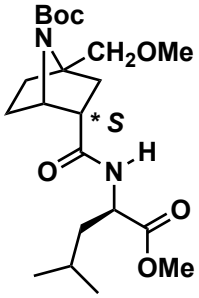


Boc-*S*-Abh(OMe)-Leu-OMe **(3b)**

To a solution of **21*S*** (30 mg, 0.11 mmol) in CH_2Cl_2 (1.5 mL) were added NH_2-Leu-OMe.HCl (24 mg, 0.13 mmol), CDMT (39 mg, 0.22 mmol), DMAP (2 mg, 0.02 mmol) and NMM (60 μl, 0.55 mmol) at rt. The reaction mixture was 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 = 1/1) to afford Boc-*S*-Abh(OMe)-Leu-OMe **(3b)** (31 mg, 71 %), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.781 (1H, d, J=8.4Hz), 4.635-4.580 (1H, m), 4.437-4.415 (1H, m), 3.935-3.870 (2H, m), 3.729 (3H, s), 3.416 (3H, s), 2.984-2.930 (1H, m), 2.078-1.510 (9H, m), 1.460 (9H, s), 0.952-0.931 (6H, m) ^{13}C-NMR(100 MHz, $CDCl_3$): 173.57, 171.39, 155.37, 80.32, 73.99, 68.69, 61.20, 59.49, 52.44, 51.03, 46.41, 35.91, 33.21, 28.54, 25.06, 24.14, 22.94, 22.01. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{36}N_2NaO_6^+$, 435.2466. Found: 435.2460. HPLC (Hexane: IPA = 6:1, 215 nm): t_R 15.62 min, 94% purity.



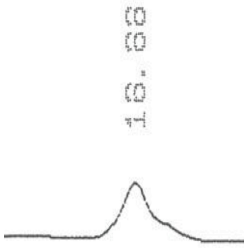


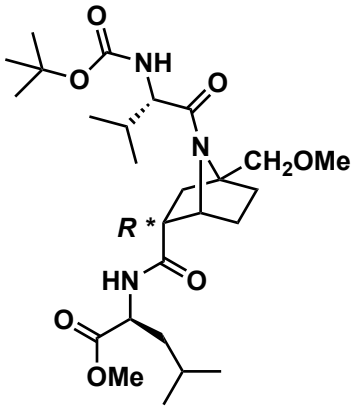
Boc-*S*-Abh(OMe)-D-Leu-OMe **(4b)**

To a solution of **21*S*** (30 mg, 0.11 mmol) in CH_2Cl_2 (1.5 mL) were added NH_2-D-Leu-OMe.HCl (24 mg, 0.13 mmol), CDMT (39 mg, 0.22 mmol), DMAP (2 mg, 0.02 mmol) and NMM (60 μl, 0.55 mmol) at rt. The reaction mixture was 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 = 1/1) to afford Boc-*S*-Abh(OMe)-Leu-OMe **(4b)** (25 mg, 58 %) as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.823 (1H, d, J=8.4Hz), 4.673-4.618 (1H, m), 4.380-4.357 (1H, m), 3.893 (2H, m), 3.737 (3H, s), 3.418 (3H, s), 2.989-2.938 (1H, m), 2.074-1.623 (9H, m), 1.468 (9H, s), 0.949-0.930 (6H, m) ^{13}C-NMR(100 MHz, $CDCl_3$): 173.70, 171.20, 155.38, 80.39, 73.91, 68.66, 61.30, 59.48, 52.49, 50.89, 46.65, 41.75, 35.88, 33.28, 28.53, 25.06, 24.02, 22.96, 22.00. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{21}H_{36}N_2NaO_6^+$, 435.2466. Found: 435.2475. HPLC (Hexane: IPA = 6:1, 215 nm): t_R 16.66 min, >95% purity.

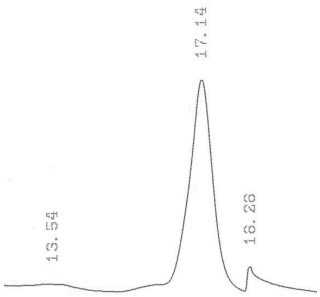




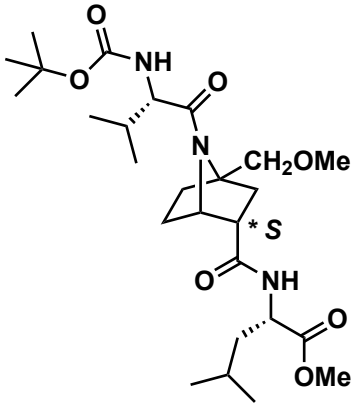
Boc-Val-*R*-Abh(OMe)-Leu-OMe **(5a)**

To a solution of **1b** (73 mg, 0.18 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-Val-OH (59 mg, 0.27 mmol), CDMT (63 mg, 0.36 mmol), DMAP (2 mg, 0.02 mmol) 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/Acetone = 2/1) to afford Boc-Val-*R*-Abh(OMe)-Leu-OMe **(5a)** (9 mg, 10 %), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.904 (1H, d, J=8.5Hz), 5.163 (1H, d, J=9.2Hz), 4.700-4.642 (1H, m), 4.521 (1H, brs), 4.294-4.051 (3H, m), 3.746 (3H, s), 3.418 (3H, s), 2.950-2.922 (1H, m), 2.079-1.595 (9H, m), 1.423 (9H, s), 0.990-0.911 (13H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 173.84, 170.58, 169.86, 155.94, 79.70, 72.58, 69.34, 60.30,



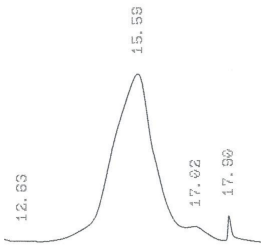
59.44, 52.57, 50.91, 47.37, 41.77, 32.20, 29.85, 28.47, 27.84, 25.03, 22.99, 21.94, 19.79, 17.68. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{26}H_{45}N_3NaO_7^+$, 534.3150. Found: 534.3180. HPLC (Hexane: IPA = 6:1, 215 nm): t_R 17.14 min, >95% purity.

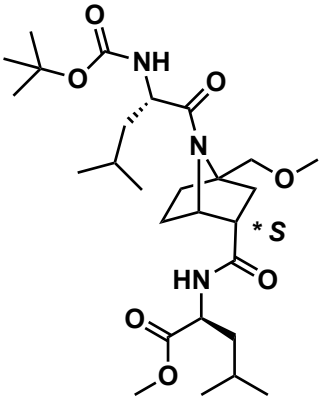


Boc-Val-*S*-Abh(OMe)-Leu-OMe **(5b)**

To a solution of **3b** (31 mg, 0.18 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-Val-OH (26 mg, 0.12 mmol), CDMT (28 mg, 0.16 mmol), DMAP (2 mg, 0.02 mmol) 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/Acetone = 2/1) to afford Boc-Val-*S*-Abh(OMe)-Leu-OMe **(5b)** (12 mg, 31 %), as a colorless oil.

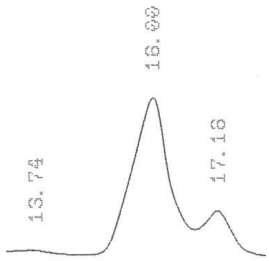
^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.206 (1H, d, J=8.0Hz), 5.163 (1H, d, J=9.5Hz), 4.708 (1H, brs), 4.590-4.535 (1H, brs), 4.154-4.039 (3H, m), 3.735 (3H, s), 3.408 (3H, s), 2.925-2.873 (1H, m), 2.177-1.544 (9H, m), 1.429 (9H, s), 0.956-0.840 (13H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 173.56, 170.77, 170.22, 156.24, 79.89, 73.97, 69.67, 59.48, 58.42, 53.95, 52.41, 51.10, 41.18, 33.55, 29.85, 28.50, 27.84, 24.95, 23.79, 21.79, 19.67, 18.12. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{26}H_{45}N_3NaO_7^+$, 534.3150. Found: 534.3177. HPLC (Hexane: IPA = 6:1, 215 nm): t_R 15.59 min, >95% purity.



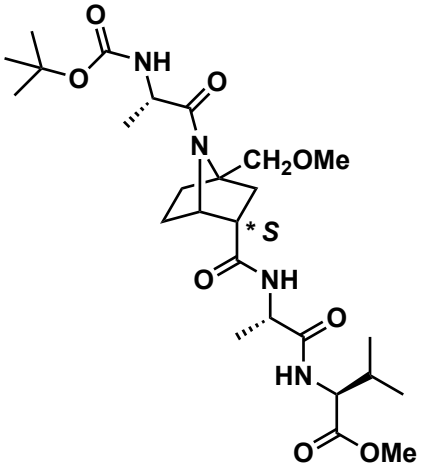


Boc-Leu-*S*-Abh(OMe)-D-Leu-OMe **(5c)**

To a solution of **4b** (25 mg, 0.06 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-Leu-OH (21 mg, 0.09 mmol), CDMT (21 mg, 0.12 mmol), DMAP (2 mg, 0.02 mmol) 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/Acetone = 2/1) to afford Boc-Leu-*S*-Abh(OMe)-D-Leu-OMe **(5c)** (4 mg, 13 %), as a colorless oil.



^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.276 (1H, d, J=8.3Hz), 5.045 (1H, d, J=9.4Hz), 4.694 (1H, brs), 4.624-4.569 (1H, m), 4.469-4.409 (1H, m), 4.133-4.022 (2H, m), 3.716 (3H, s), 3.409 (3H, s), 2.899-2.862 (1H, m), 2.134-2.091 (1H, m), 1.904-1.643 (9H, m), 1.445 (9H, s), 0.975-0.923 (14H, m). HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{27}H_{47}N_3NaO_7^+$, 548.3306. Found: 548.3315. HPLC (Hexane: IPA = 6:1, 215 nm): t_R 16.00 min, 90% purity.



Boc-Ala-*S*-Abh(OMe)-Ala-Val-OMe **(6a)**

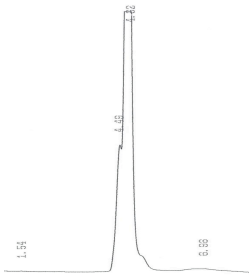
To a solution of **22*S*** (80 mg, 0.27 mmol) in CH_2Cl_2 (1 mL) was added TFA (1.0 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature, and stirred for 20 min. The reaction was then quenched with 2 M aqueous solution of NaOH. The resulting solution was extracted with EtOAc (3 times). 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-Ala-OH (78 mg, 0.41 mmol), CDMT (95 mg, 0.54 mmol), DMAP (4 mg, 0.03 mmol) and NMM (148 μl, 1.35 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-Ala-*S*-Abh(OMe)-OMe** (54 mg, 55 %), as a colorless oil.

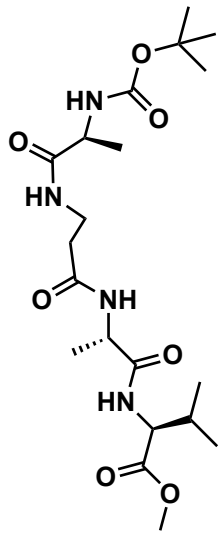
^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.370 (1H, d, J=7.68Hz), 4.509 (1H, brs), 4.420-4.384 (1H, m), 4.092-4.038 (2H, m), 3.701 (3H, s), 3.417 (3H, s), 3.104-3.075 (1H, m), 2.118-1.547 (6H, m), 1.422 (9H, s), 1.302 (3H, d, J=6.88Hz) ^{13}C-NMR(100 MHz, $CDCl_3$): 172.25, 169.45, 155.14, 79.61, 73.53, 69.26, 59.38, 52.11, 48.37, 46.43, 35.28, 32.93, 28.42, 19.86. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{18}H_{30}N_2NaO_6^+$, 393.1996. Found: 393.2024.

To a solution of **Boc-Ala-*S*-Abh(OMe)-OMe** (53 mg, 0.14 mmol) in THF (3 mL) was added a solution of $LiOH.H_2O$ (12 mg, 8.46 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 **Boc-Ala-*S*-Abh(OMe)-OH** (50 mg, 100%) was afforded, which was used in the next reaction without further purification.

To a solution of Boc-Ala-Val-OMe (78 mg, 0.21 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 (3 mL) were added Boc-Ala-*S*-Abh(OMe)-OH (50 mg), CDMT (49 mg, 0.28 mmol), DMAP (3 mg, 0.02 mmol) and NMM (66 µl, 0.6 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) to afford **Boc-Ala-*S*-Abh(OMe)-Ala-Val-OMe (6a)** (57 mg, 75 %), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.662 (1H, d, J=7.4Hz), 6.616 (1H, d, J=8.7Hz), 5.387 (1H, d, J=8.4Hz), 4.614 (1H, brs), 4.507-4.449 (3H, m), 4.063-4.018 (2H, m), 3.731 (3H, s), 3.406 (3H, s), 2.970-2.919 (1H, m), 2.175-1.703 (7H, m), 1.417 (9H, s), 1.381 (3H, d, J=7.04Hz), 1.302 (3H, d, J=6.88Hz), 0.932-0.899 (6H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 172.33, 172.28, 170.80, 170.24, 155.53, 80.02, 73.63, 69.69, 60.80, 59.43, 57.41, 52.32, 49.34, 48.27, 45.44, 34.29, 33.19, 31.33, 28.51, 24.18, 19.79, 19.01, 17.97, 17.83. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{26}H_{44}N_4NaO_8^+$, 563.3051. Found: 563.3046. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 4.62 min, >95% purity.



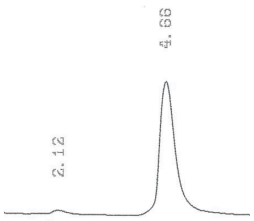


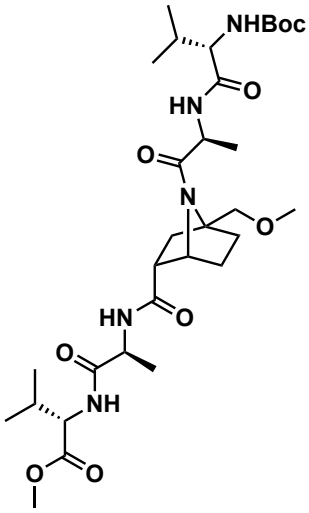
Boc-Ala-β-Ala-Ala-Val-OMe **(6b)**

To a solution of Boc-Ala-Val-OMe (51 mg, 0.17 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 (3 mL) were added Boc-Ala-β-Ala-OH (29 mg, 0.11), CDMT (39 mg, 0.22 mmol), DMAP (3 mg, 0.02 mmol) and NMM (60 µl, 0.55 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 Boc-Ala-β-Ala-Ala-Val-OMe **(6b)** (20 mg, 41 %), as white powder.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.532 (1H, brs), 6.976 (1H, d, J=6.6Hz), 6.807 (1H, d, J=9.1Hz), 5.201 (1H, d, J=6.5Hz), 4.633-4.598 (1H, m), 4.477-4.441 (1H, m), 4.071-4.037 (1H, m), 3.788 (1H, brs), 3.747 (3H, s), 3.214 (1H, brs), 2.525-2.454 (1H, m), 2.309-2.277 (1H, m), 2.169-2.123 (1H, m), 1.423 (9H, s), 1.332-1.247 (6H, m), 0.936-0.854 (6H, m) ^{13}C-NMR(100 MHz, $CDCl_3$): 172.31, 172.28, 170.79, 170.26, 155.52, 80.02, 69.69, 59.43, 57.40, 49.34, 48.26, 31.34, 28.51, 19.76, 19.01, 17.96, 17.83. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{20}H_{36}N_4NaO_7^+$, 467.2476. Found: 467.2470. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 4.66 min, >95% purity.





Boc-Val-Ala-*R*-Abh(OMe)-Ala-Val-OMe **(7a)**

To a solution of **22*R*** (40 mg, 0.13 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-Val-Ala-OH (57 mg, 0.2 mmol), CDMT (46 mg, 0.26 mmol), DMAP (2 mg) and NMM (71 μl, 0.65 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-Val-Ala-*R*-Abh(OMe)-OMe** (30 mg, 49 %), as a colorless oil.

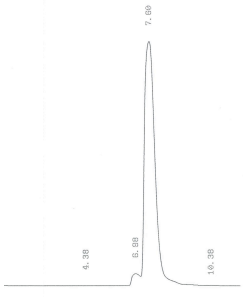
^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.826 (1H, d, J=6.4Hz), 5.009 (1H, d, J=8.4Hz), 4.657-4.591 (1H, m), 4.535 (1H, brs) 4.109-3.948 (3H, m), 3.705 (3H, s), 3.405 (3H,

s), 3.052-2.998 (1H, m), 2.160-2.047 (7H, m), 1.424 (9H, s), 1.346 (3H, d, J=6.8Hz), 0.936-0.849 (6H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 172.19, 170.82, 170.67, 169.05, 155.86, 79.98, 73.48, 69.44, 59.80, 59.62, 59.40, 52.22, 47.27, 45.70, 35.83, 32.36, 31.05, 28.37, 19.63, 19.42, 17.42. HRMS (ESI, $[M+H]^+$): Calcd. for $C_{23}H_{40}N_3O_7^+$, 470.2861. Found: 470.2887.

To a solution of **Boc-Val-Ala-*R*-Abh(OMe)-OMe** (30 mg, 0.06 mmol) in THF (2 mL) was added a solution of $LiOH.H_2O$ (5 mg, 0.13 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 **Boc-Val-Ala-*R*-Abh(OMe)-OH** (30 mg, 100%) was afforded, which was used in the next reaction without further purification.

To a solution of Boc-Ala-Val-OMe (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 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-Val-Ala-*R*-Abh(OMe)-OH** (30 mg), 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) to afford **Boc-Val-Ala-*R*-Abh(OMe)-Ala-Val-OMe (7a)** (32 mg, 84 %), as a colorless oil.

^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.928 (1H, brs), 6.803 (2H, d, J=8.8Hz), 5.191 (1H, d, J=8.4Hz), 4.712-4.661 (1H, m), 4.597-4.526 (2H, m), 4.508-4.473 (1H, m), 4.070-3.987 (3H, m), 3.729 (3H, s), 3.403 (3H, s), 2.959-2.906 (1H, m), 2.173-1.714 (8H, m), 1.416 (9H, s), 1.354-1.315 (6H, m), 0.923-0.849 (12H, m). ^{13}C-NMR(100 MHz, $CDCl_3$): 172.40, 172.35, 172.31, 171.70, 155.97, 79.92, 77.36, 69.67, 60.38, 59.78, 59.32, 57.38, 53.97, 52.35, 49.09, 47.14, 32.44, 31.29, 29.40, 28.43, 19.62, 19.46, 19.06, 18.16, 17.80, 17.57. HRMS (ESI, $[M+Na]^+$): Calcd. for $C_{31}H_{53}N_5NaO_9^+$, 622.3735. Found: 622.3754. Reverse-phase HPLC (CH_3CN 100%, 215 nm): t_R 7.60 min, >95% purity.



3. NMR measurements

Dry samples were dissolved in organic solvents and aqueous solvents and the final concentrations were 2-3 mg in 700µl NMR solvents.

NMR experiments were performed on a Bruker Avance 400MHz NMR spectrometer. The NMR data were processed with Bruker TopSpin 2.1. The $^{3}J(H_N,H_\alpha)$ coupling constants were determined with the amide proton doublets by deconvolution of the peaks by fitting a Lorentz function to the peaks. About Lorentz fitting, a representative fit for AbhOMe-Leu-OMe (**1b**) in $CHCl_3$ at 298K is shown in Figure S2. This process was repeated for all samples.

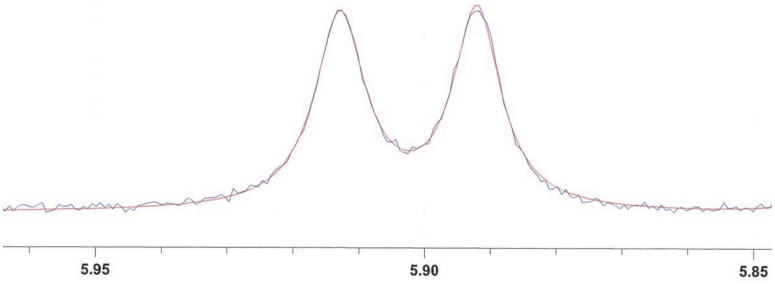
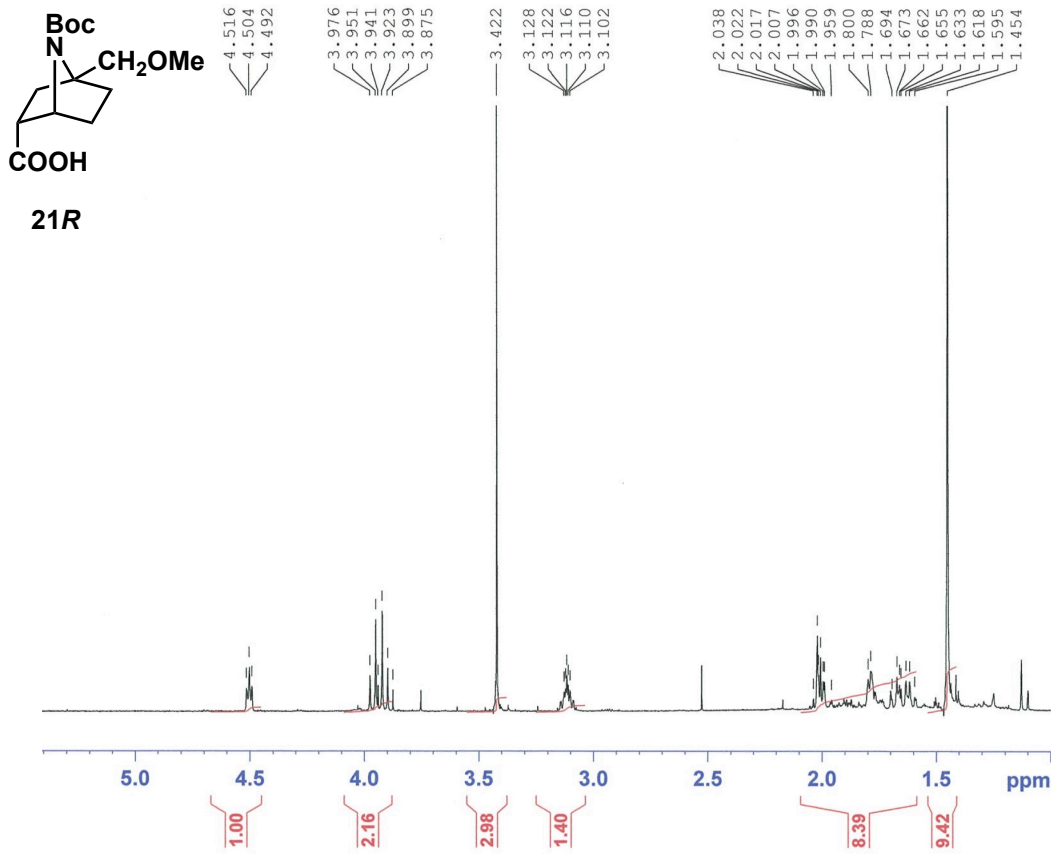
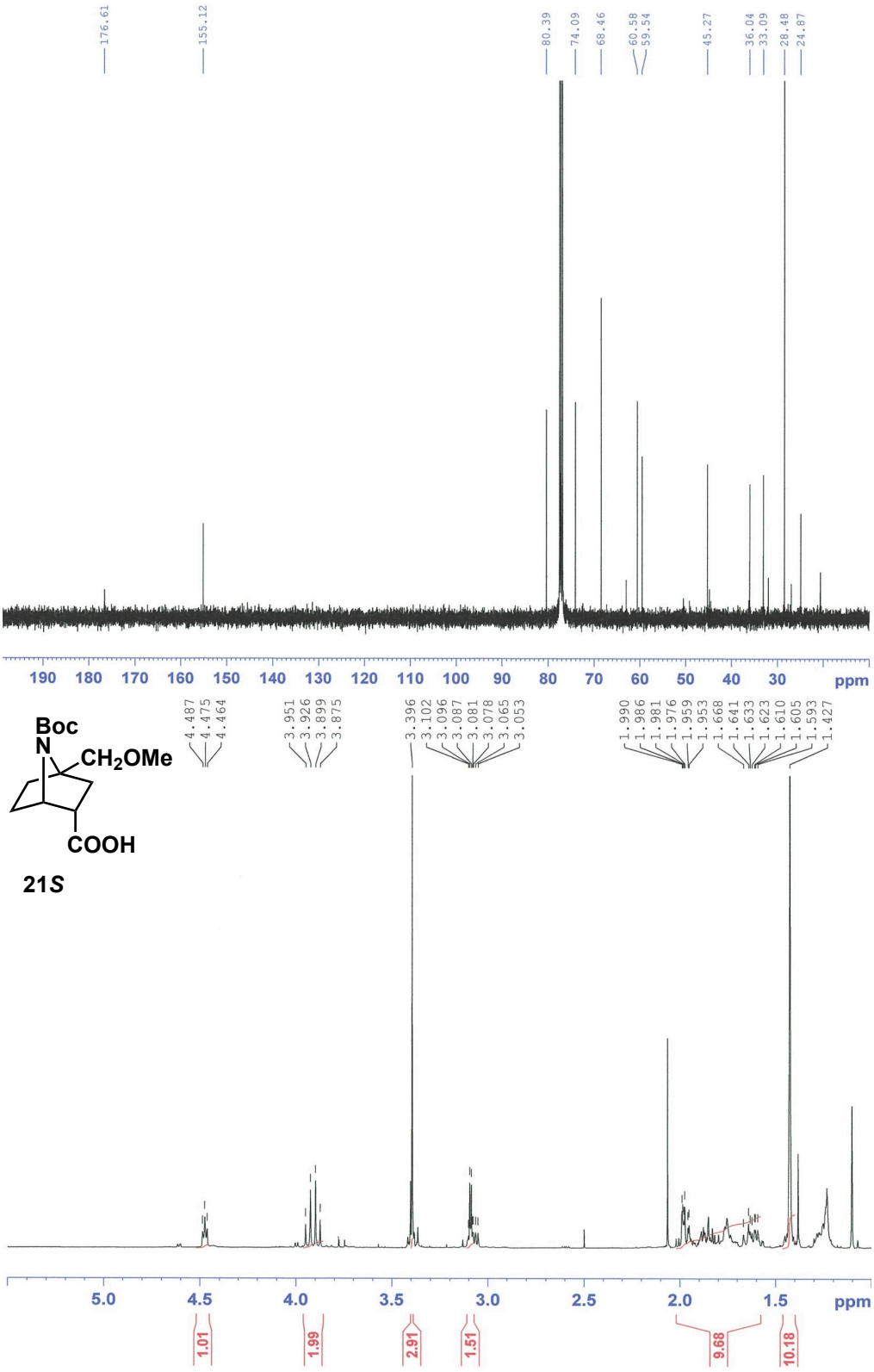


Figure S8. Deconvolution spectra of AbhOMe-Leu-OMe (1b) in $CHCl_3$ at 298K: blue, original line; red, deconvolution line.

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

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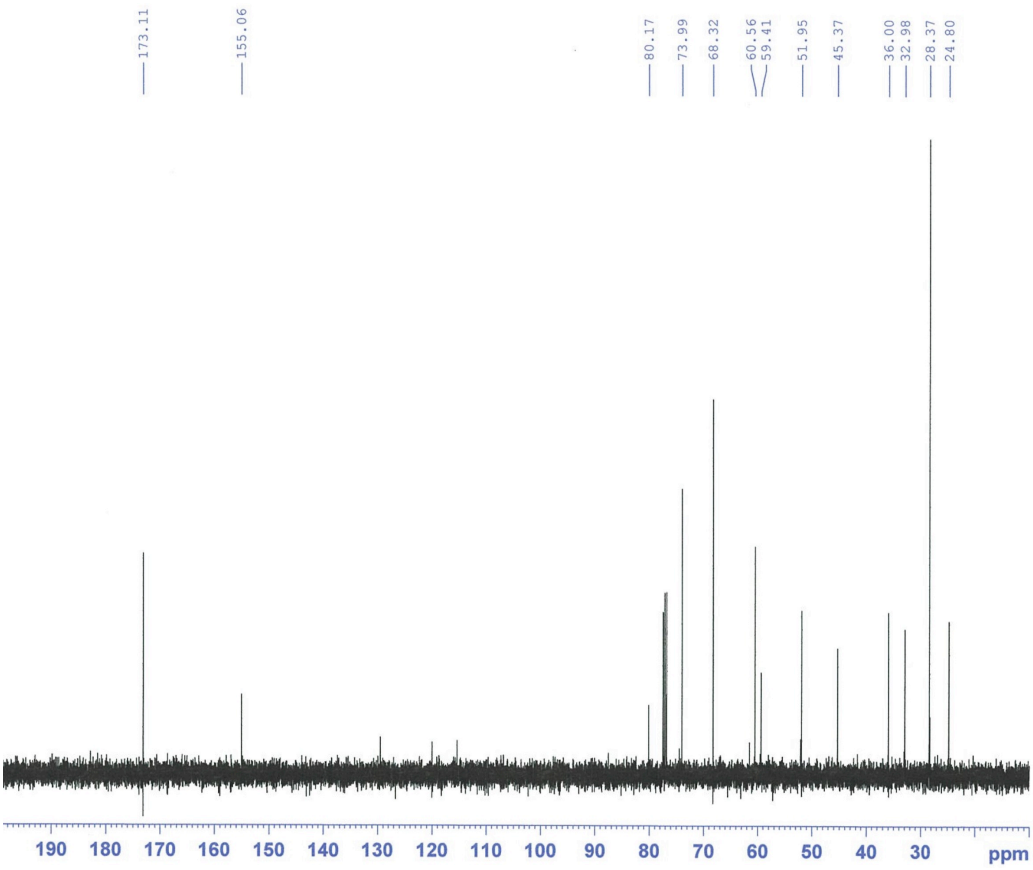
74.09
68.46
60.58
59.54
45.27
36.04
33.09
28.48
24.87
ppm
4.487
4.475
4.464
3.951
3.926
3.899
3.875
3.396
3.102
3.096
3.087
3.081
3.078
3.065
3.053
1.990
1.986
1.981
1.976
1.959
1.953
1.668
1.641
1.633
1.623
1.610
1.605
1.593
1.427
Boc
N
CH2OMe
COOH
21S
ppm
1.01
1.99
2.91
1.51
9.68
10.18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68.32
60.56
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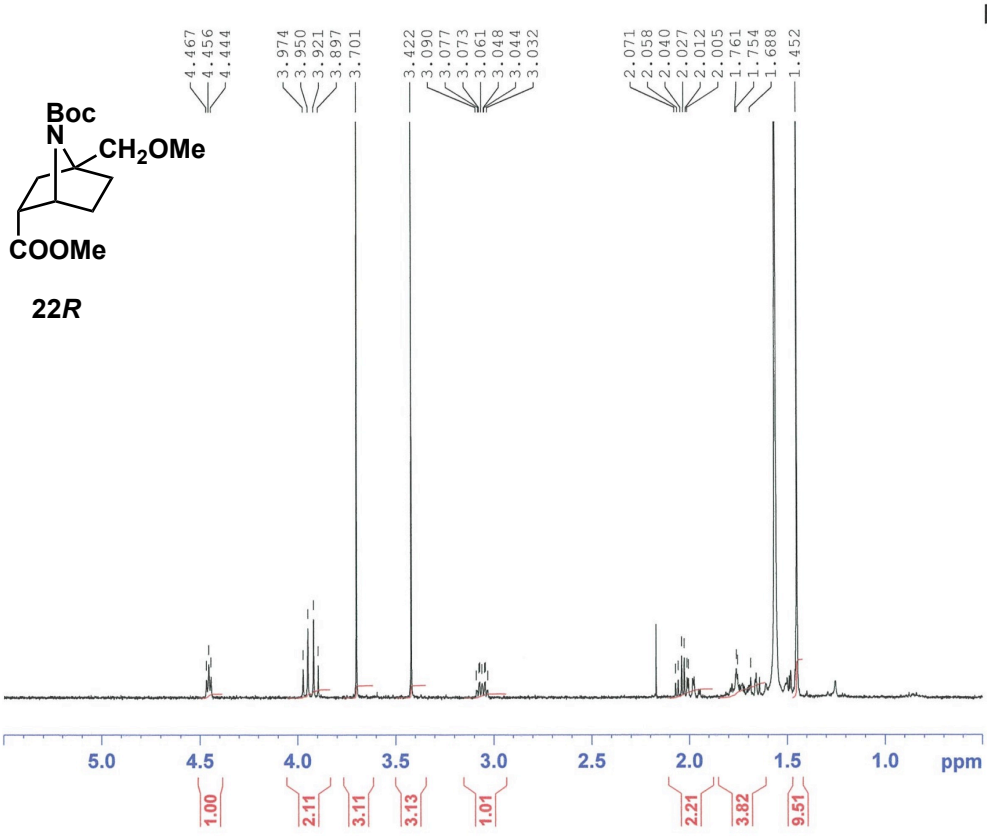
51.95
45.37
36.00
32.98
28.37
24.80
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm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3.701
3.422
3.090
3.077
3.073
3.061
3.048
3.044
3.032
2.071
2.058
2.040
2.027
2.012
2.005
1.761
1.754
1.688
1.452
Boc
N
CH2OMe
COOMe
22R
5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 ppm
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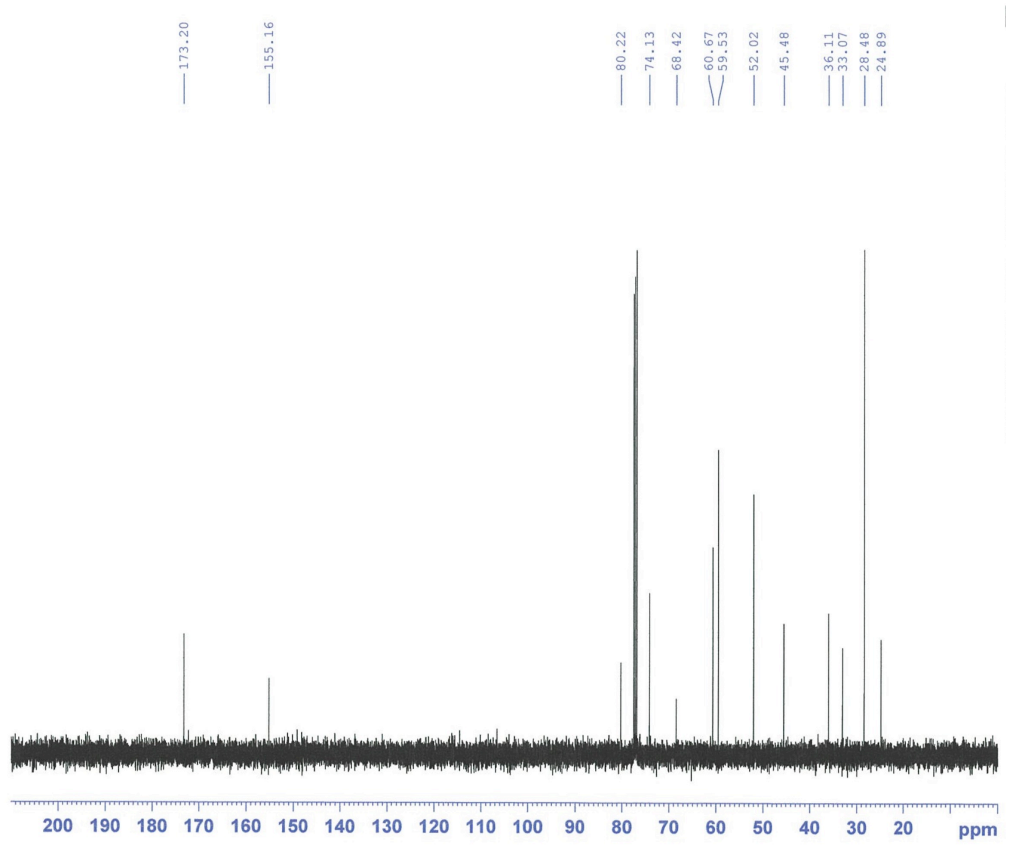
2.11
3.11
3.13
1.01
2.21
3.82
9.51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74.13
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60.67
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52.02
45.48
36.11
33.07
28.48
24.89
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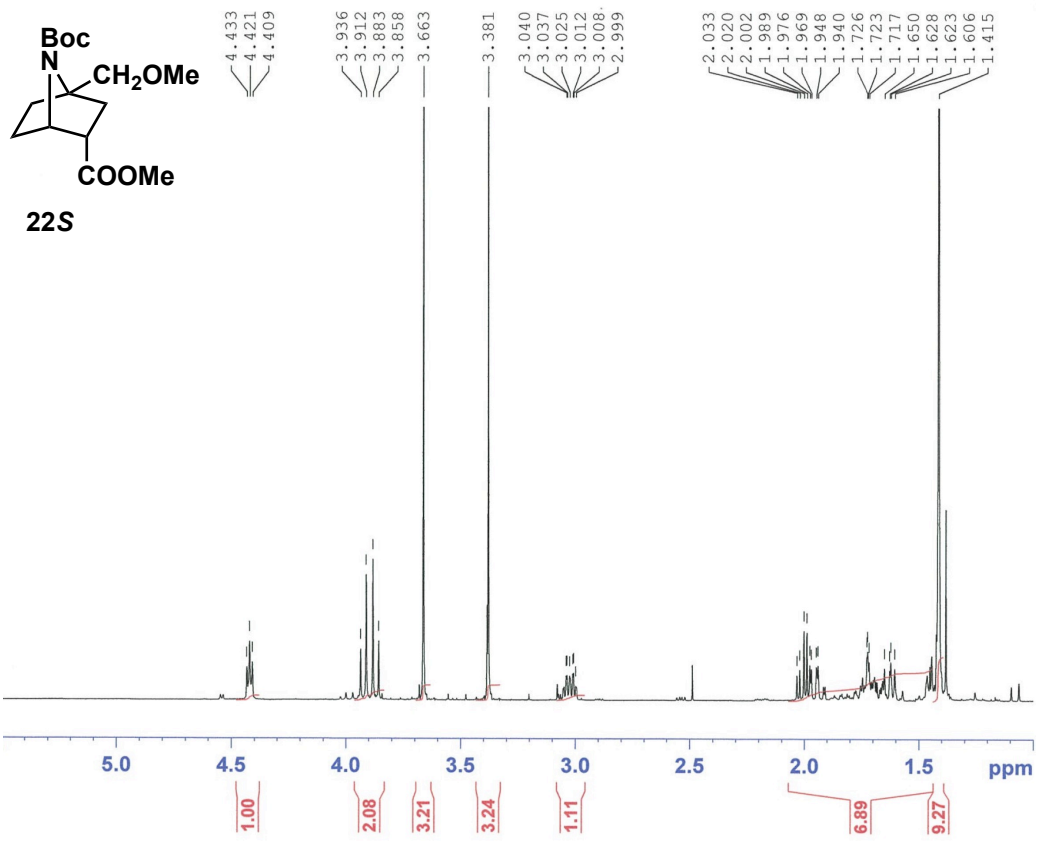
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CH2OMe
COOMe
22S
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4.409
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3.912
3.883
3.858
3.663
3.381
3.040
3.037
3.025
3.012
3.008
2.999
2.033
2.020
2.002
1.989
1.976
1.969
1.948
1.940
1.726
1.723
1.717
1.650
1.628
1.623
1.606
1.415
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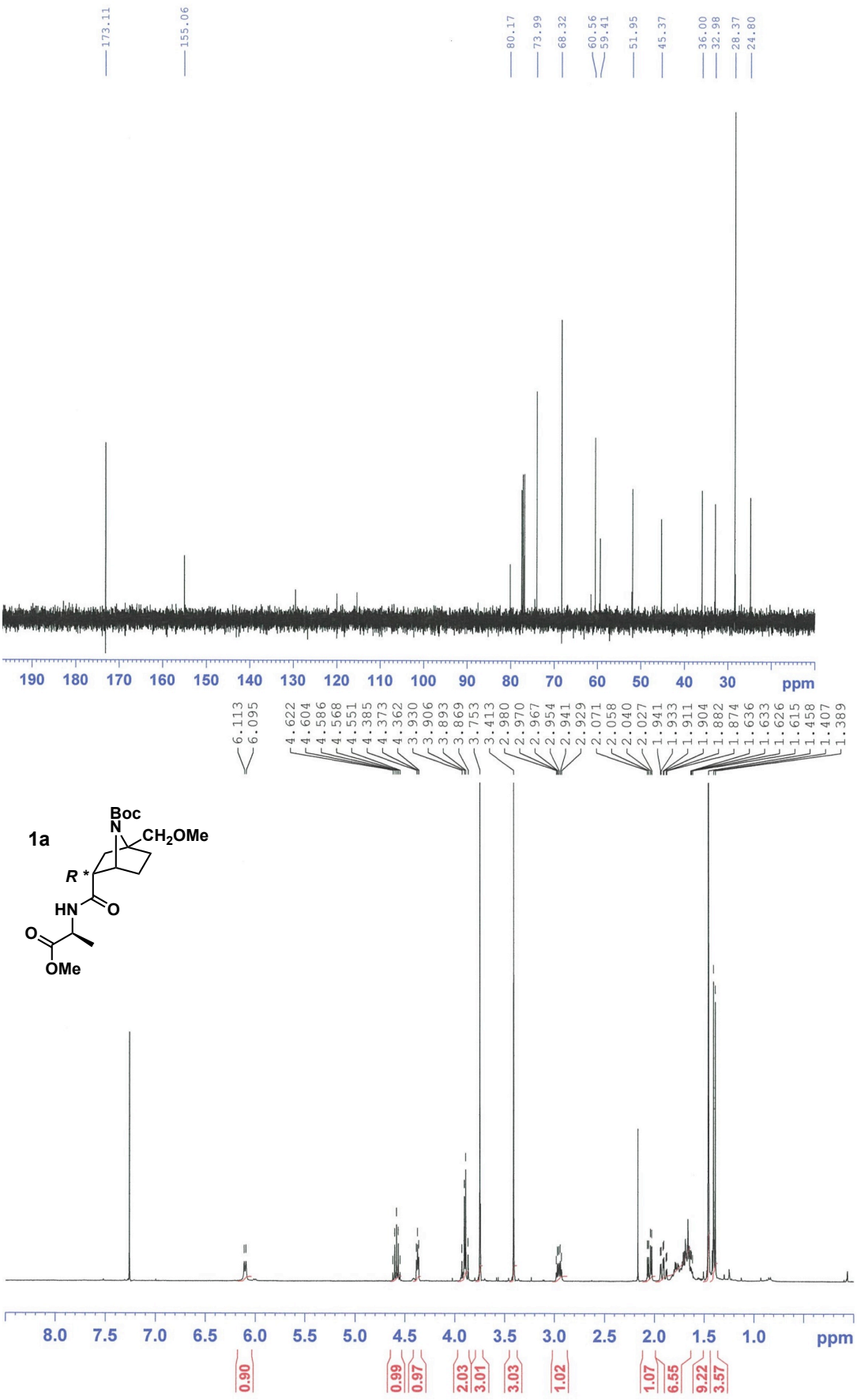
2.08
3.21
3.24
1.11
6.89
9.27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68.32
60.56
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36.00
32.98
28.37
24.80
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 ppm
6.113
6.095
4.622
4.604
4.586
4.568
4.551
4.385
4.373
4.362
3.930
3.906
3.893
3.869
3.753
3.413
2.980
2.970
2.967
2.954
2.941
2.929
2.071
2.058
2.040
2.027
1.941
1.933
1.911
1.904
1.882
1.874
1.636
1.633
1.626
1.615
1.458
1.407
1.389
1a
Boc
N
CH2OMe
R*
HN
O
O
O
OMe
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
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0.97
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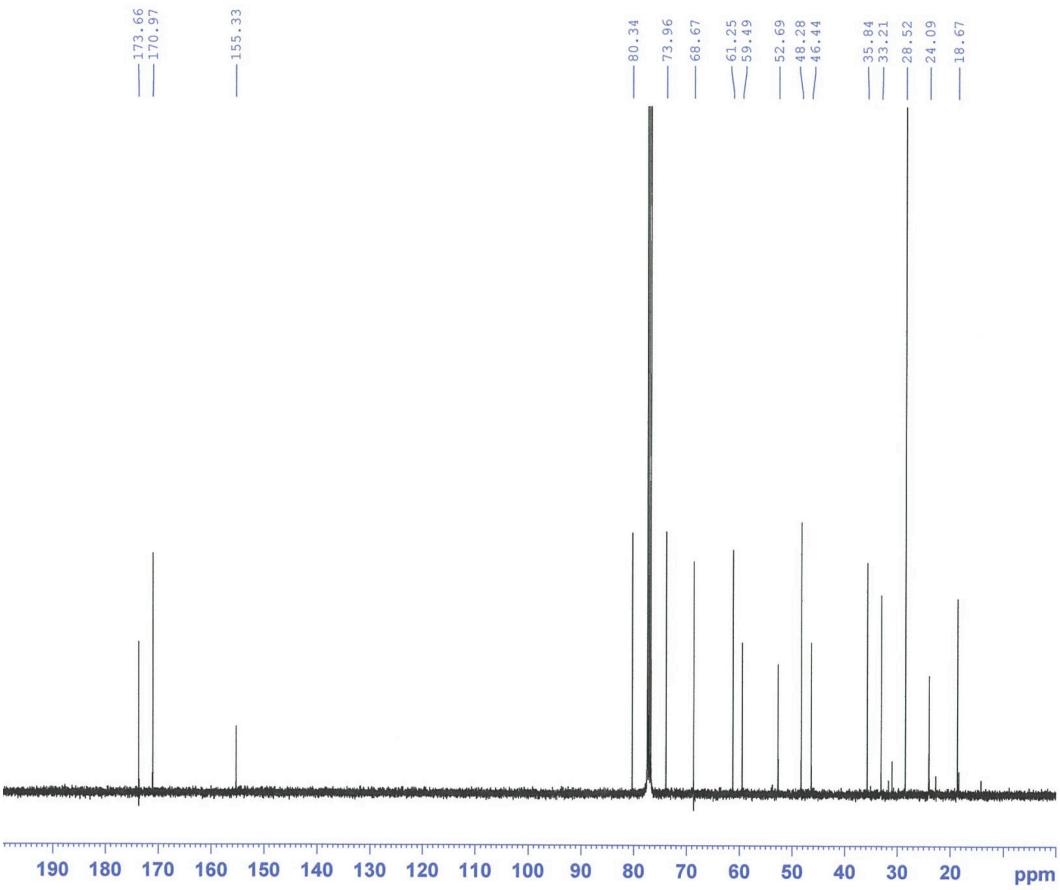
3.03
1.02
1.07
6.55
9.22
3.57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18.67
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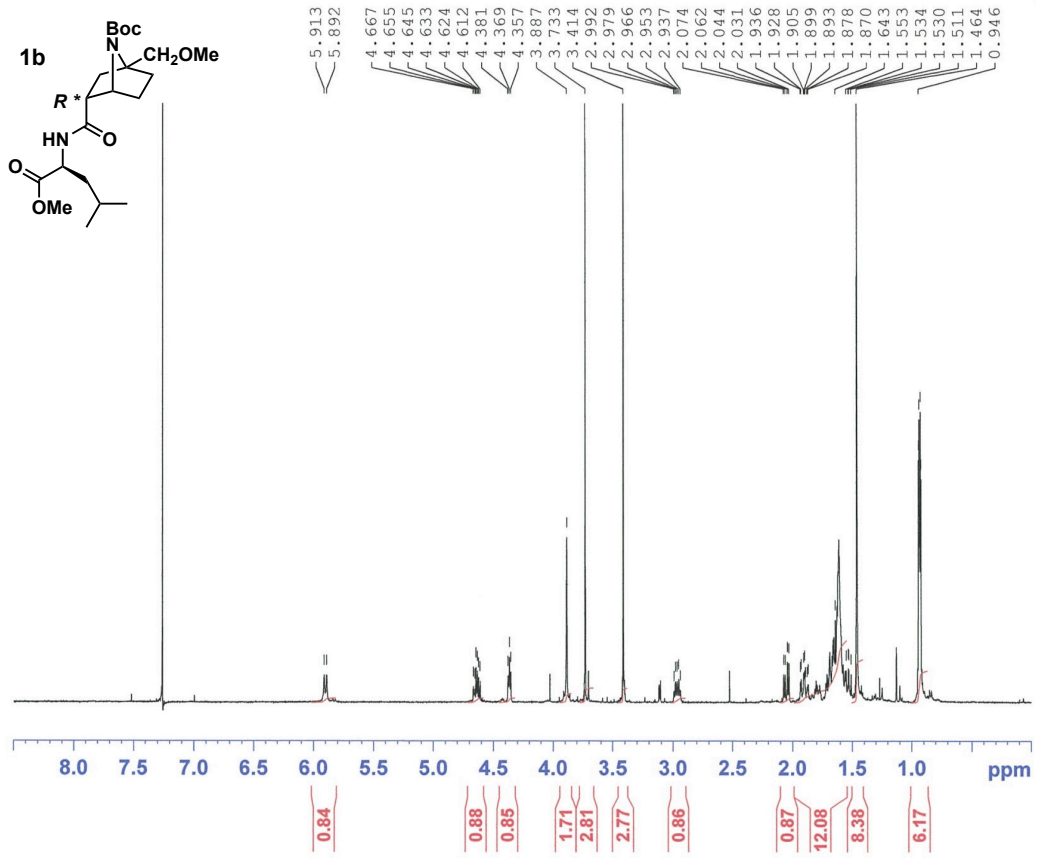
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R *
HN
O
O
OMe
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4.655
4.645
4.633
4.624
4.612
4.381
4.369
4.357
3.887
3.733
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2.966
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2.937
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2.062
2.044
2.031
1.936
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1.905
1.899
1.893
1.878
1.870
1.643
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1.530
1.511
1.464
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0.86
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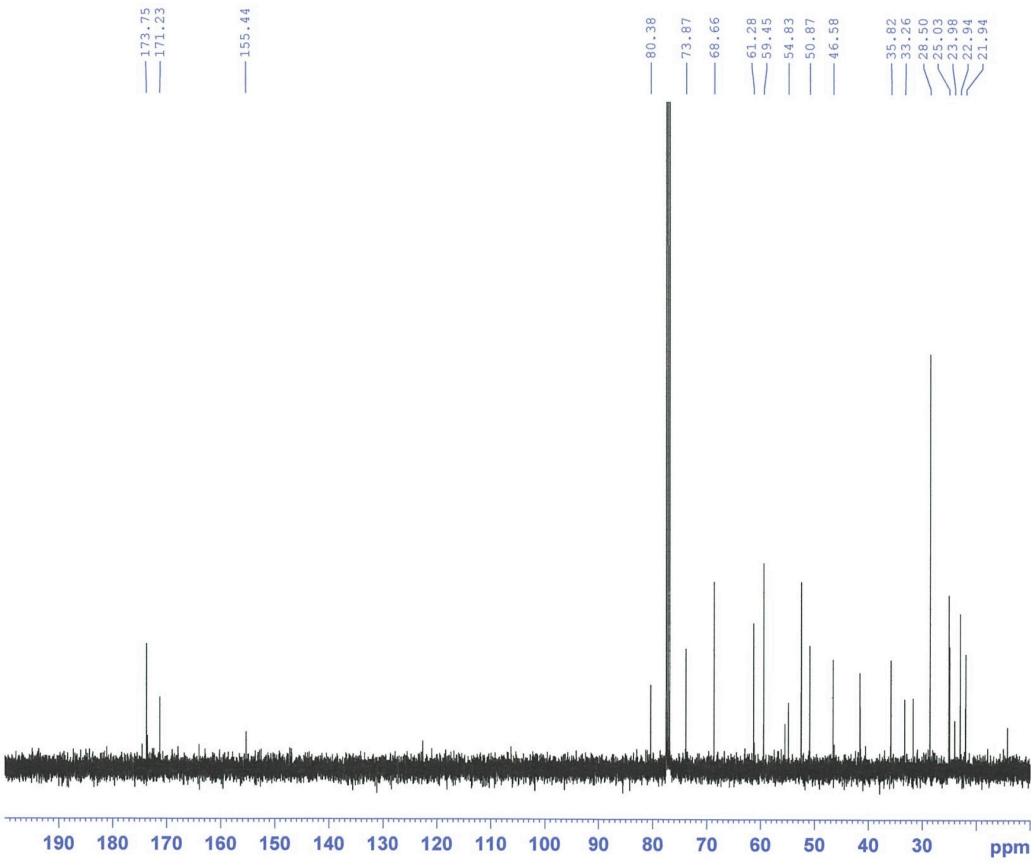
8.38
6.17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50.87
46.58
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28.50
25.03
23.98
22.94
21.94
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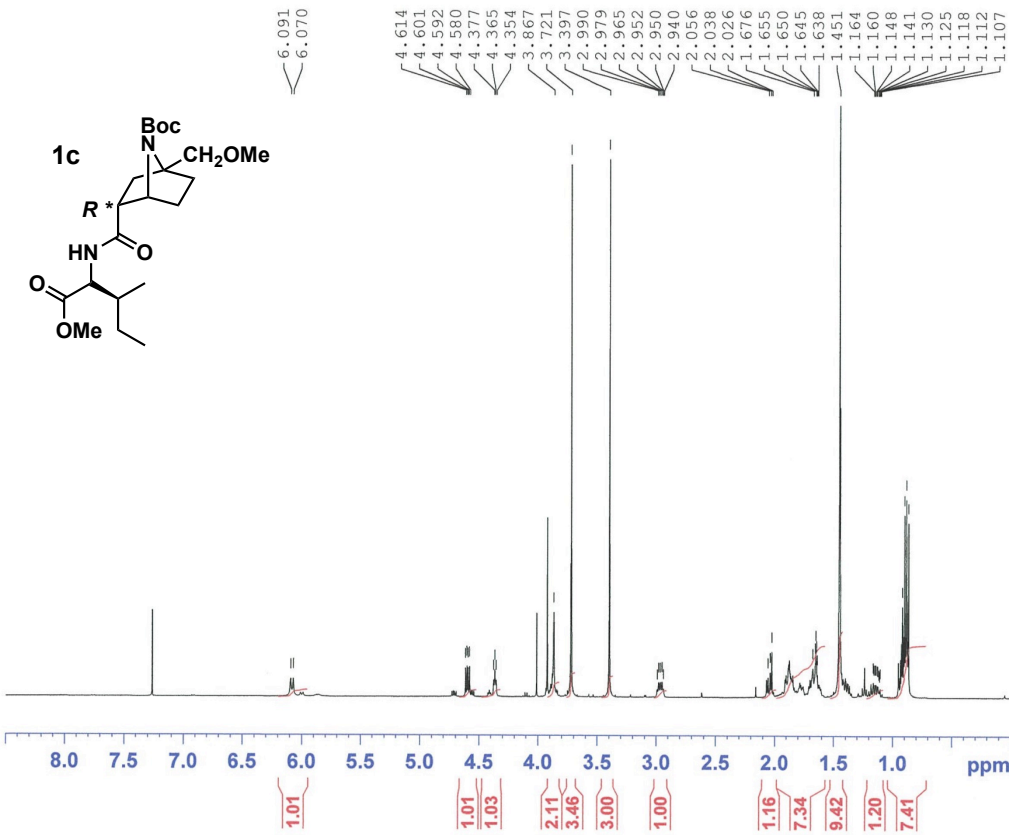
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2.950
2.940
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2.038
2.026
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1.645
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1.160
1.148
1.141
1.130
1.125
1.118
1.112
1.107
1c
Boc
N
CH2OMe
R *
HN
O
O
OMe
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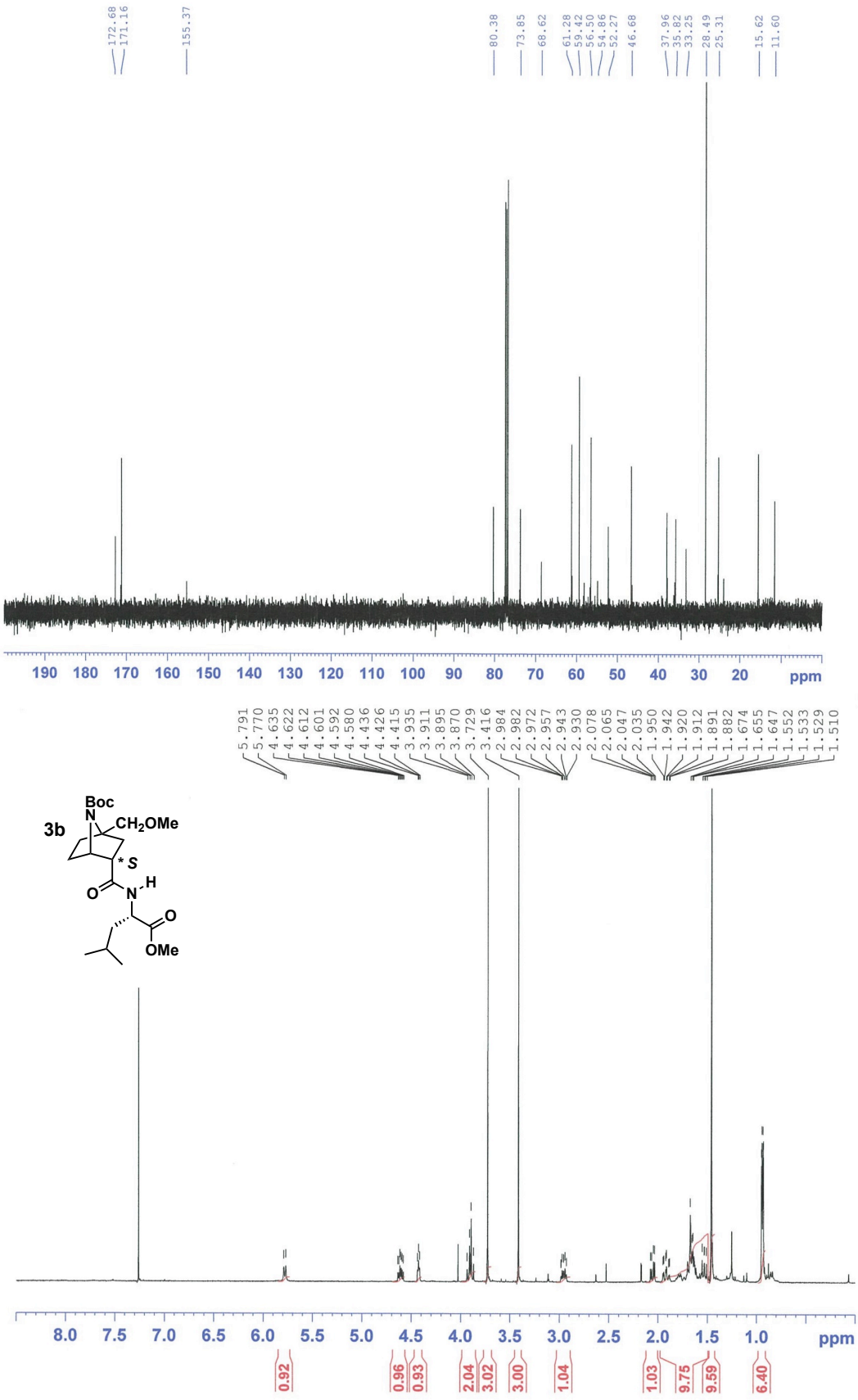
7.41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37.96
35.82
33.25
28.49
25.31
15.62
11.60
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
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5.770
4.635
4.622
4.612
4.601
4.592
4.580
4.436
4.426
4.415
3.935
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2.930
2.078
2.065
2.047
2.035
1.950
1.942
1.920
1.912
1.891
1.882
1.674
1.655
1.647
1.552
1.533
1.529
1.510
Boc
N
CH2OMe
3b
*S
O
N
H
O
OMe
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
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1.04
1.03
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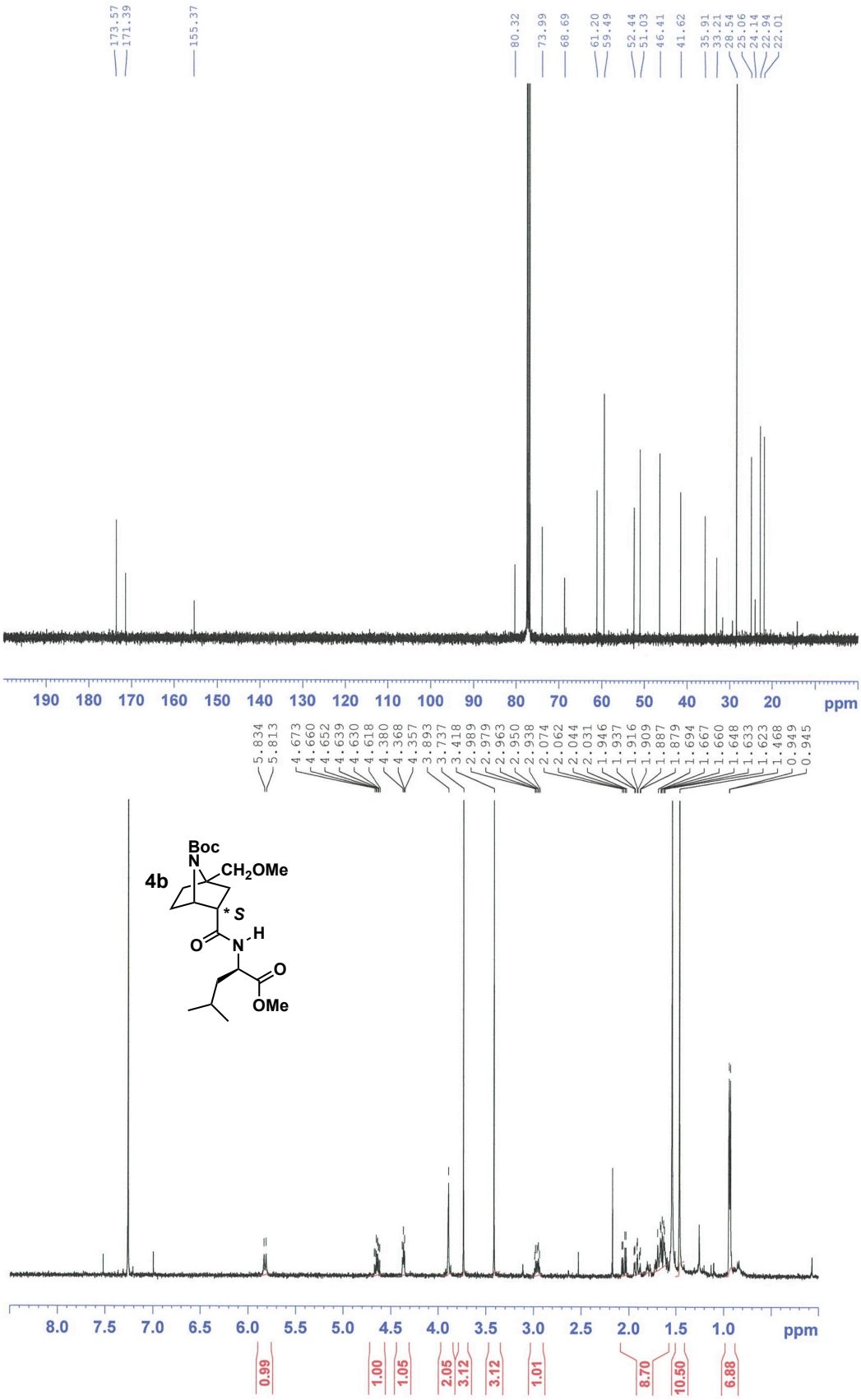
9.59
6.40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41.62
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33.21
28.54
25.06
24.14
22.94
22.01
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
5.834
5.813
4.673
4.660
4.652
4.639
4.630
4.618
4.380
4.368
4.357
3.893
3.737
3.418
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2.979
2.963
2.950
2.938
2.074
2.062
2.044
2.031
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1.916
1.909
1.887
1.879
1.694
1.667
1.660
1.648
1.633
1.623
1.468
0.949
0.945
Boc
N
CH2OMe
4b
*S
O
N
H
O
OMe
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
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2.05
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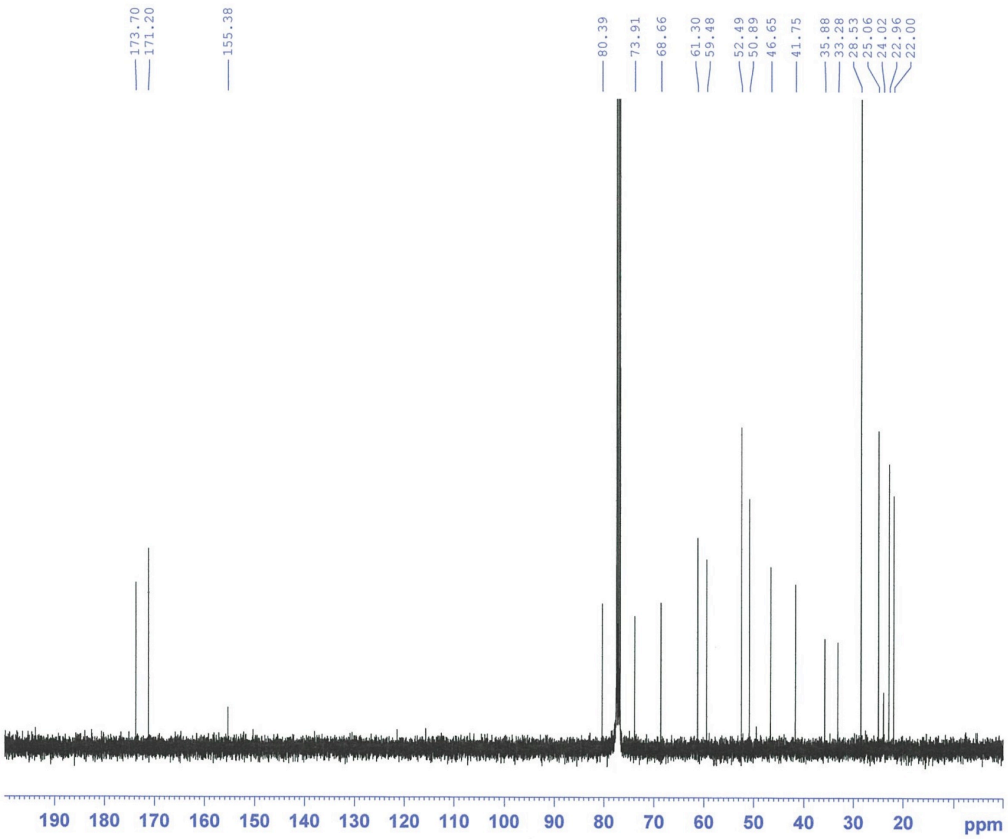
3.12
1.01
8.70
10.50
6.88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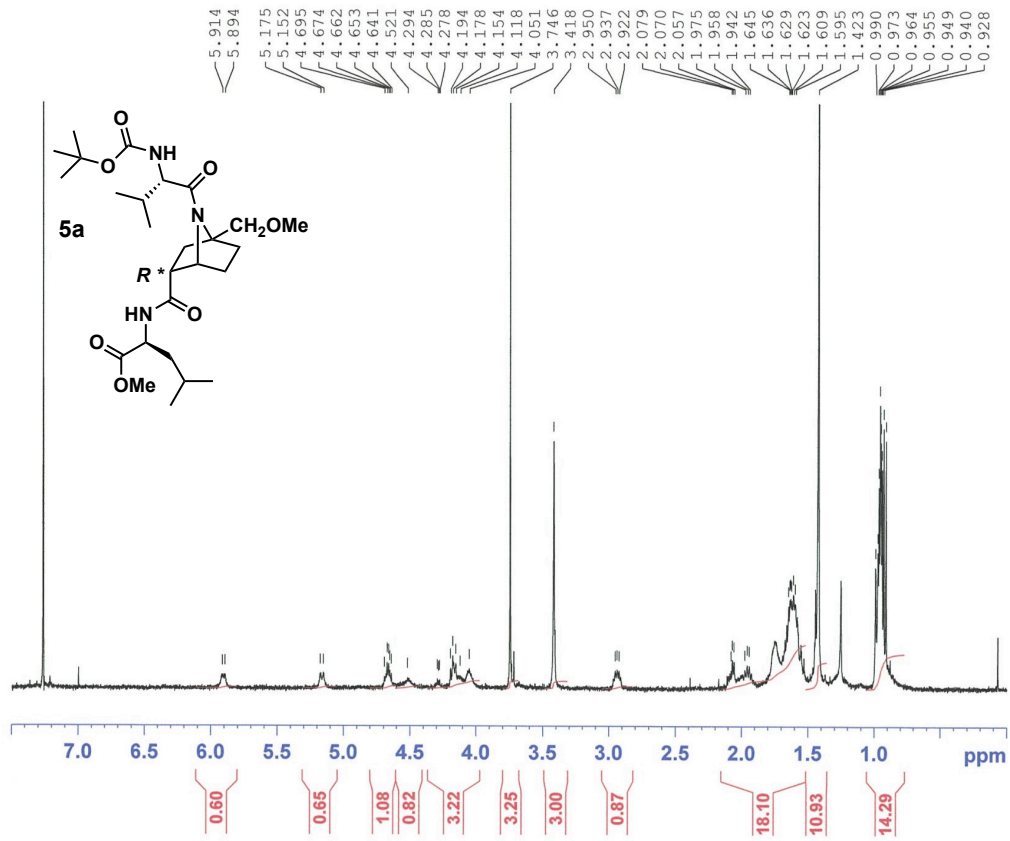
59.48
52.49
50.89
46.65
41.75
35.88
33.28
28.53
25.06
24.02
22.96
22.00
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm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4.278
4.194
4.178
4.154
4.118
4.051
3.746
3.418
2.950
2.937
2.922
2.079
2.070
2.057
1.975
1.958
1.942
1.645
1.636
1.629
1.623
1.609
1.595
1.423
0.990
0.973
0.964
0.955
0.949
0.940
0.928
5a
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
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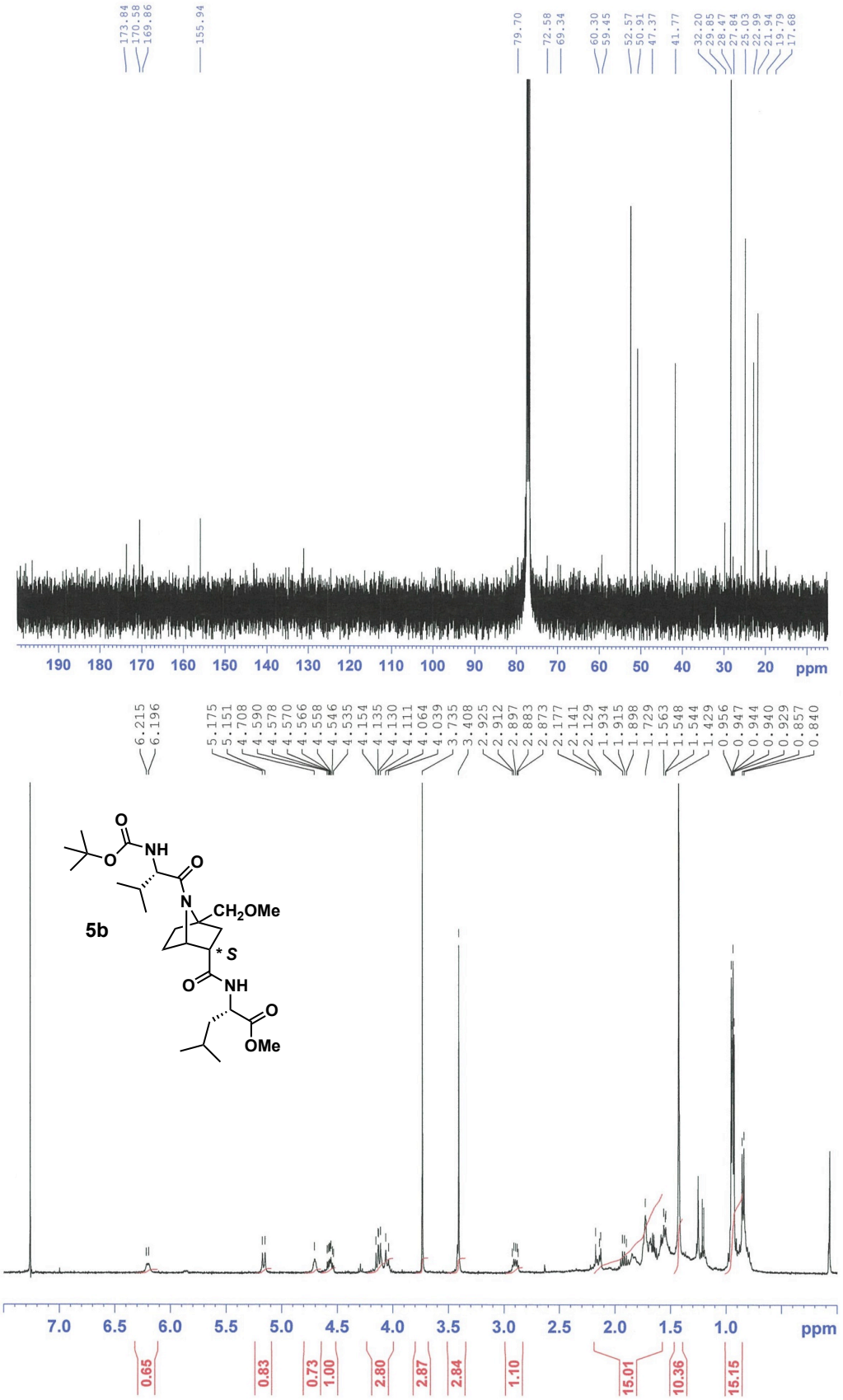
3.25
3.00
0.87
18.10
10.93
14.29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72.58
69.34
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41.77
32.20
29.85
28.47
27.84
25.03
22.99
21.94
19.79
17.68
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
6.215
6.196
5.175
5.151
4.708
4.590
4.578
4.570
4.566
4.558
4.546
4.535
4.154
4.135
4.130
4.111
4.064
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1.934
1.915
1.898
1.729
1.563
1.548
1.544
1.429
0.956
0.947
0.944
0.940
0.929
0.857
0.840
5b
CH2OMe
* S
OMe
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
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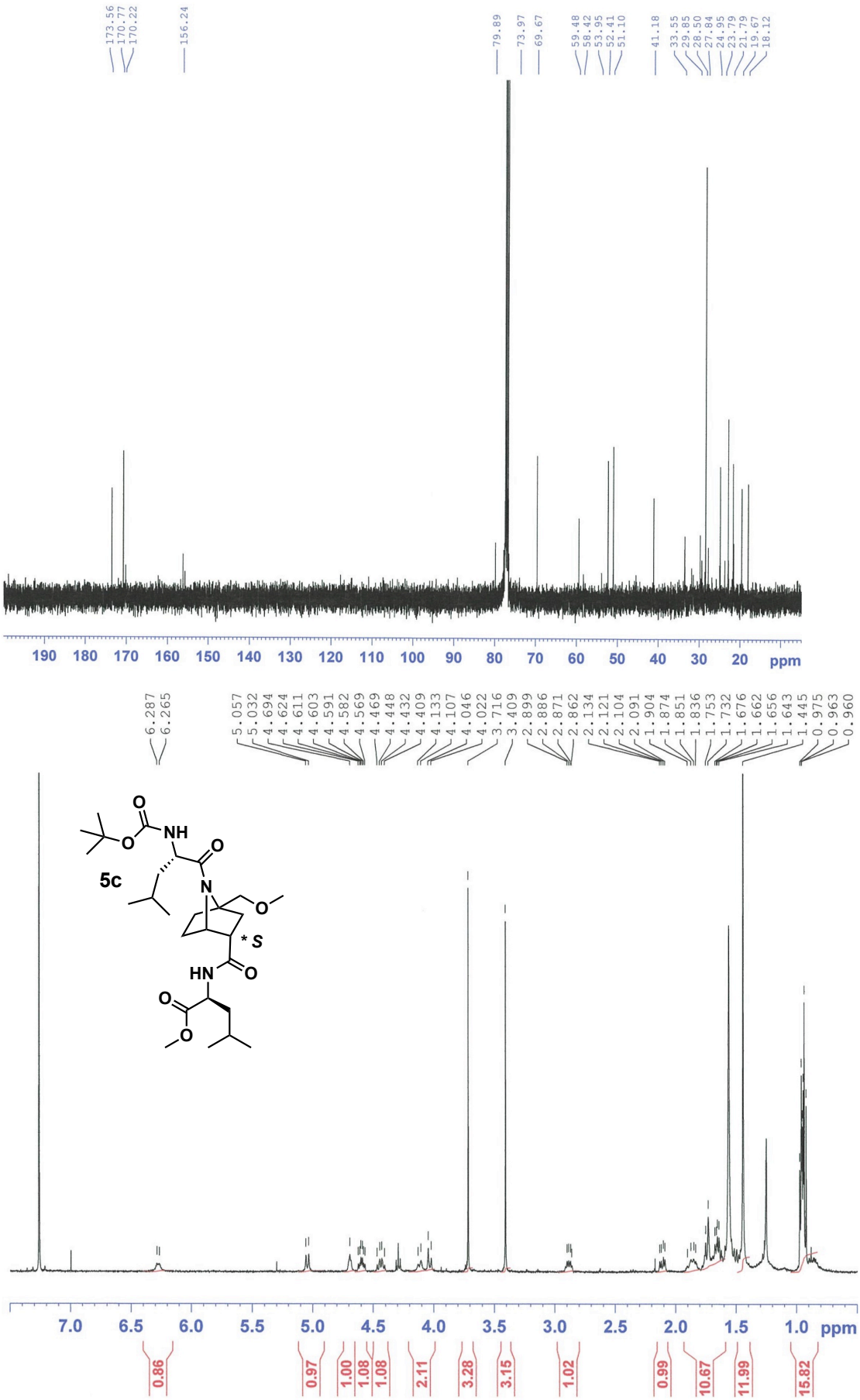
2.80
2.87
2.84
1.10
15.01
10.36
15.15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79.89
73.97
69.67
59.48
58.42
53.95
52.41
51.10
41.18
33.55
29.85
28.50
27.84
24.95
23.79
21.79
19.67
18.12
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm
6.287
6.265
5.057
5.032
4.694
4.624
4.611
4.603
4.591
4.582
4.569
4.469
4.448
4.432
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2.104
2.091
1.904
1.874
1.851
1.836
1.753
1.732
1.676
1.662
1.656
1.643
1.445
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0.963
0.960
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NH
O
N
O
* S
HN
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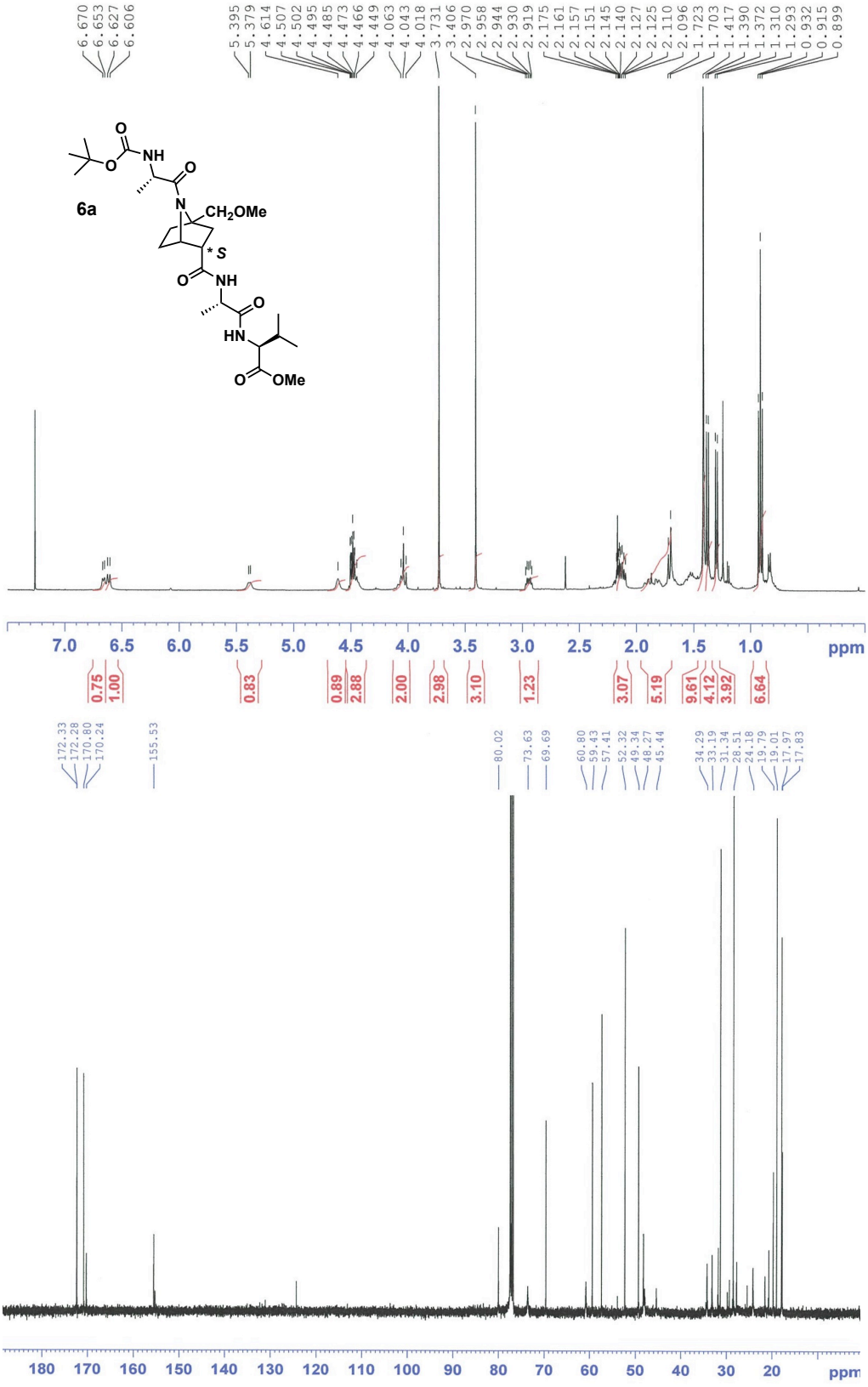
15.82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4.018
3.731
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2.930
2.919
2.175
2.161
2.157
2.151
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2.125
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2.096
1.723
1.703
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1.390
1.372
1.310
1.293
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0.915
0.899
6a
CH2OMe
*S
OMe
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
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0.89
2.88
2.00
2.98
3.10
1.23
3.07
5.19
9.61
4.12
3.92
6.64
172.33
172.28
170.80
170.24
155.53
80.02
73.63
69.69
60.80
59.43
57.41
52.32
49.34
48.27
45.44
34.29
33.19
31.34
28.51
24.18
19.79
19.01
17.97
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160
150
140
130
120
110
100
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80
70
60
50
40
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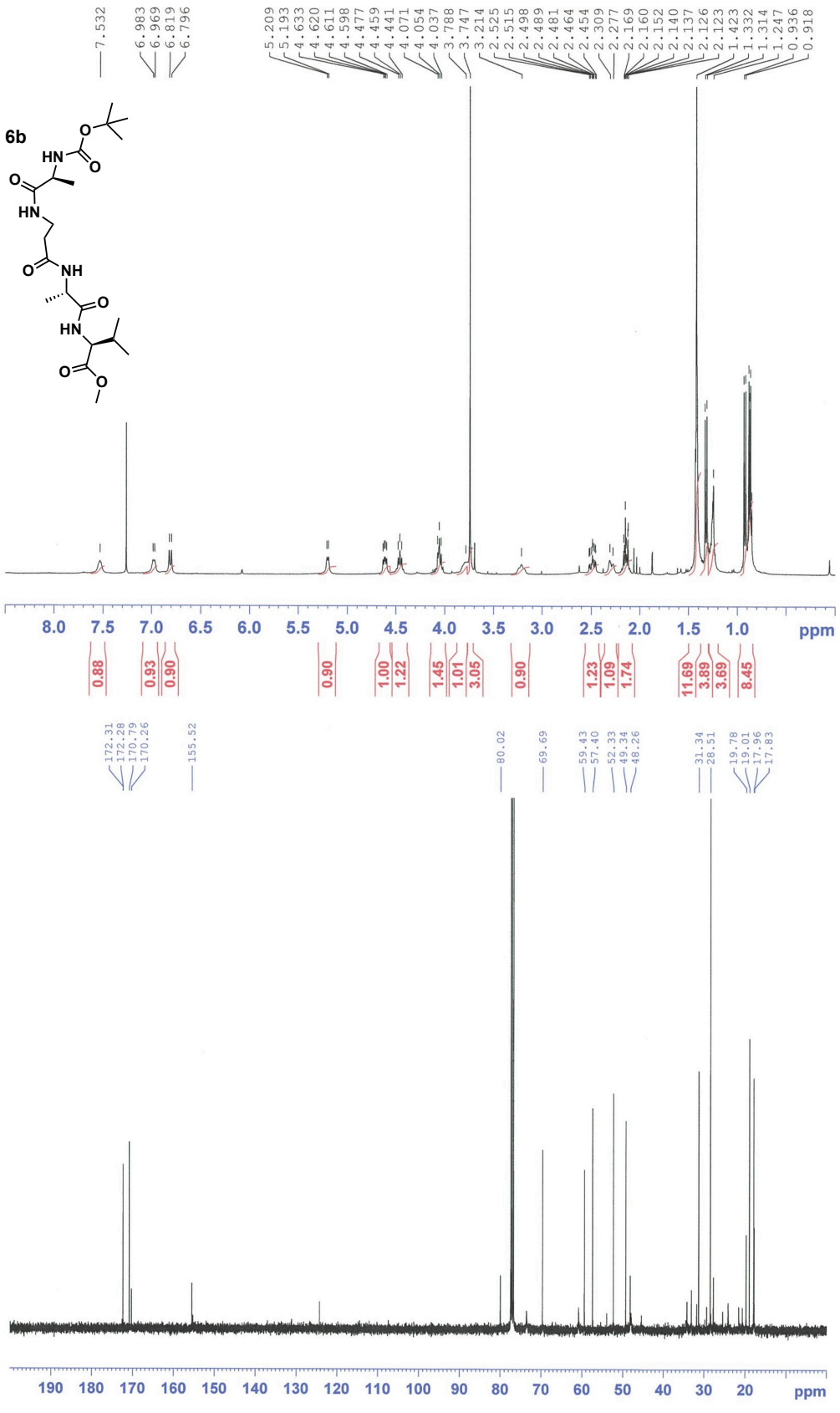
ppm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6.819
6.796
5.209
5.193
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4.620
4.611
4.598
4.477
4.459
4.441
4.071
4.054
4.037
3.788
3.747
3.214
2.525
2.515
2.498
2.489
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2.464
2.454
2.309
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2.160
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1.247
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ppm
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0.90
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1.09
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11.69
3.89
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8.45
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170.26
155.52
80.02
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28.51
19.78
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17.96
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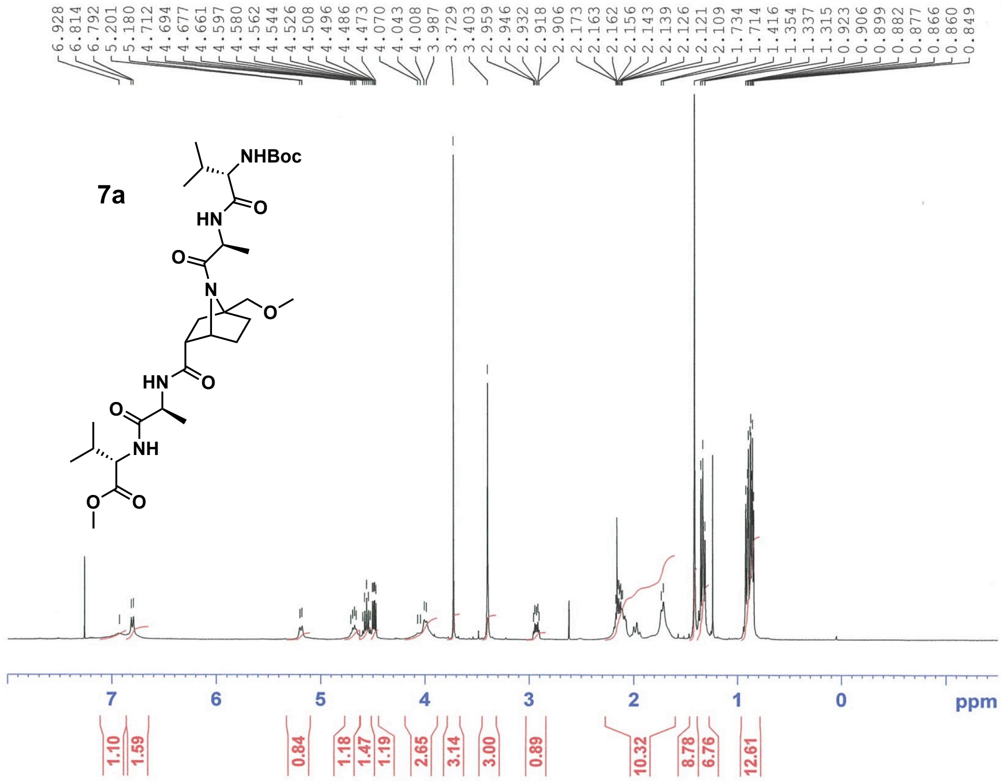
ppm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4.526
4.508
4.496
4.486
4.473
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4.043
4.008
3.987
3.729
3.403
2.959
2.946
2.932
2.918
2.906
2.173
2.163
2.162
2.156
2.143
2.139
2.126
2.121
2.109
1.734
1.714
1.416
1.354
1.337
1.315
0.923
0.906
0.899
0.882
0.877
0.866
0.860
0.849
7a
NHBoc
HN
O
N
O
HN
NH
ppm
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1.59
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1.18
1.47
1.19
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3.14
3.00
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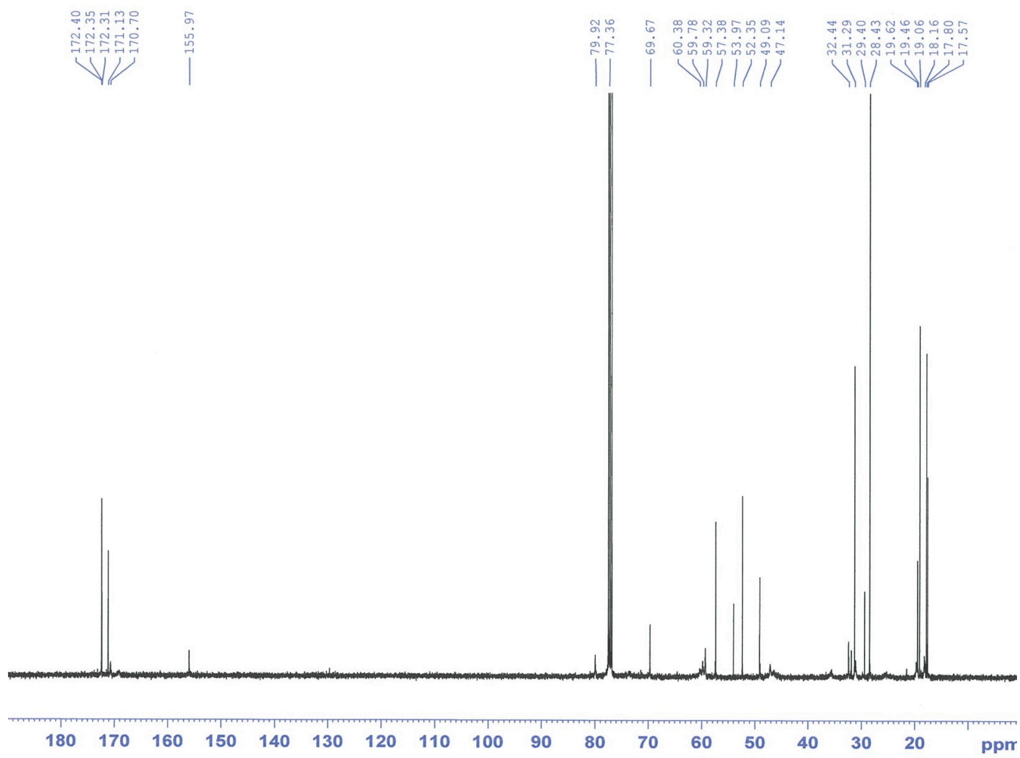
10.32
8.78
6.76
12.61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79.92
77.36
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59.78
59.32
57.38
53.97
52.35
49.09
47.14
32.44
31.29
29.40
28.43
19.62
19.46
19.06
18.16
17.80
17.57
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20
ppm

4. 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}).

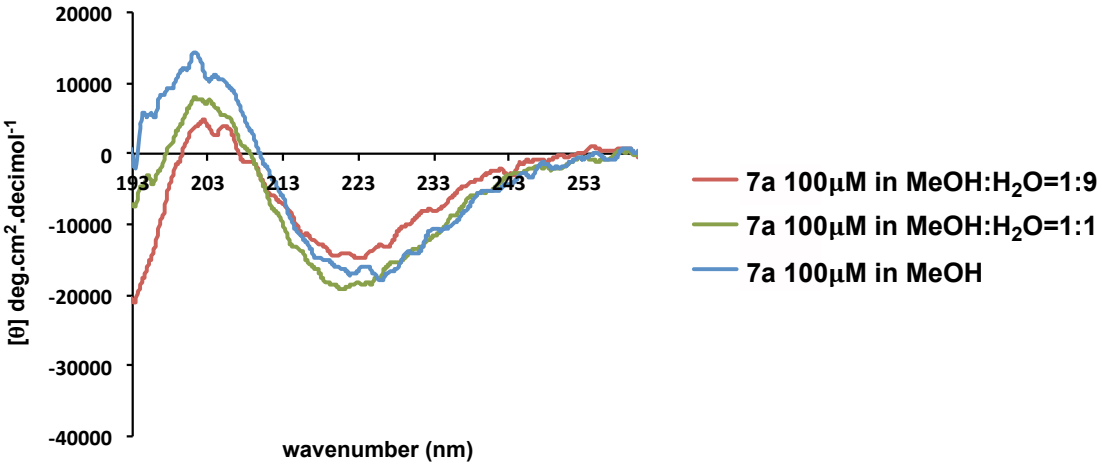

Figure S9, solvent effect of **7a** (100μM) in MeOH, MeOH:H_2O=1:1, and MeOH:H_2O=1:9 at 20^{o}C.

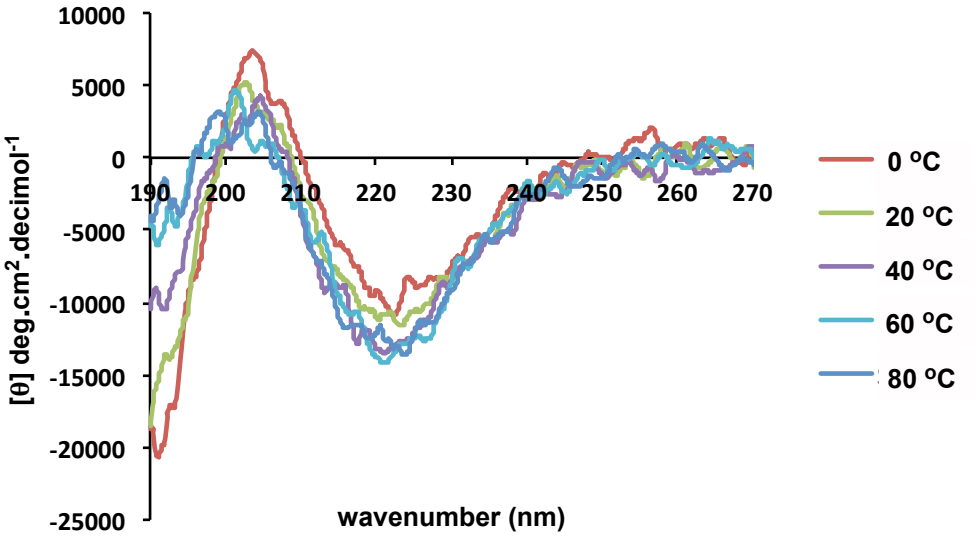

Figure S10, temperature-dependence of **7a** (100μM) in MeOH:H_2O=1:9 at 0-80 ^{o}C.

As the temperature increases from 0 to 80 ^{o}C, the CD spectrum of **7a** shows little change in ellipticity, reflecting **7a** takes stable conformation in water environment.

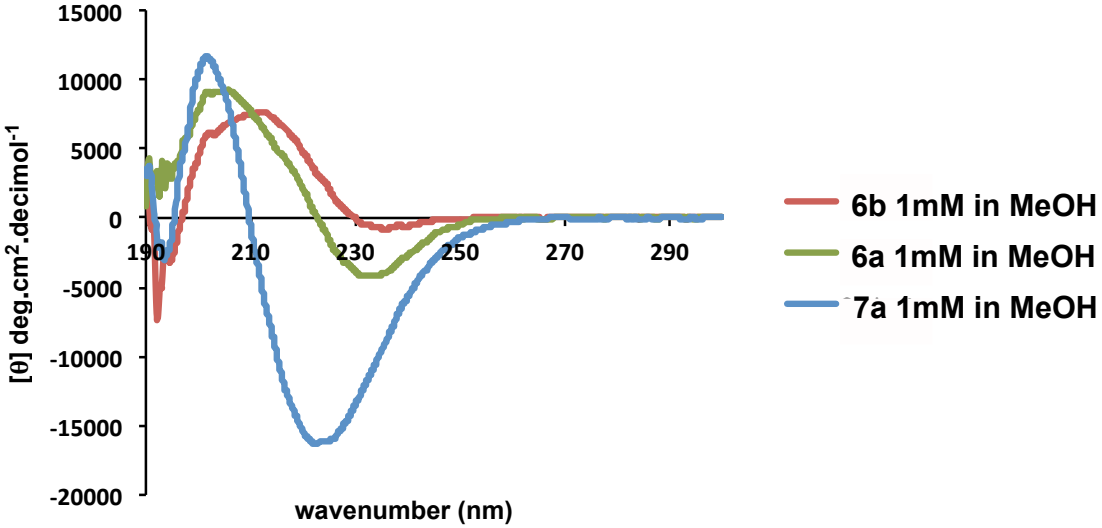

Figure S11, CD spectrum of **6a, 6b** and **7a** (1mM) in MeOH at 20 °C.

The spectrum for **7a** shows one band of positive ellipticity ≈ 202 nm and one minima of negative ellipticity ≈ 224 nm. The spectrum for β conformation has in general a positive band between 195-200 nm and a negative band between 216-218nm.[4] Therefore, **7a** adopts β configuration. For **6a**, **6b** and **7a**, one positive band is detected between 200-210nm in methanol, while the intensity of negative band become weaker from **7a** to **6a**, which may suggest the population of β conformation is reducing. And in **6b**, the negative band is almost disappeared, thus **6b** don't take β conformation.

5. Calculations

Metadynamics simulation

The metadynamic calculation we used in this work is Desmond metadynamics with OPLS3 force field. The cubic solvent model is built in chloroform. The box size calculation method is buffer. The distance of the solvent box is 10 Å each and the angle is 90° each. About the simulation, collective variable (CV) is defined as dihedral angles. One is φ and the other is ψ. The ensemble class is NPT. Meta-dynamics parameters (size of the Gaussians) are set to height as 0.03 kcal/mol, torsion angle as 5° and interval as 0.09 ps. The total simulation time is 20 ns and the trajectory of recording interval is 6.0 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 calculation

Energy minimum of β conformation of 1b': B3LYP/6-31G(d), scrf=(smd, solvent=chloroform)

NIMG=1 (15.9252 cm^{-1})

Zero-point correction= 0.477232 (Hartree/Particle)

Thermal correction to Energy= 0.504828

Thermal correction to Enthalpy= 0.505772

Thermal correction to Gibbs Free Energy= 0.416318

Sum of electronic and zero-point Energies= -1189.174013

Sum of electronic and thermal Energies= -1189.146417

Sum of electronic and thermal Enthalpies= -1189.145473

Sum of electronic and thermal Free Energies= -1189.234927

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
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2	6	0	-1.903972	-1.531539	0.556062
3	6	0	-3.054805	0.379301	0.313284
4	6	0	-2.778869	0.357624	1.737169
5	1	0	-2.674329	-1.562599	2.627156
6	1	0	-1.774057	-2.613703	0.565299
7	1	0	-3.737921	0.407432	2.252672
8	1	0	-2.219581	1.219068	2.102342
9	6	0	-1.695487	0.586941	-0.517156
10	1	0	-1.148649	1.416137	-0.068293
11	1	0	-1.871715	0.762744	-1.578300
12	6	0	-0.966567	-0.742456	-0.271260
13	1	0	-0.779757	-1.285204	-1.197980
14	7	0	-3.162604	-1.033814	0.004618
15	6	0	-4.140050	1.304516	-0.097635
16	1	0	-4.443789	1.291337	-1.144442
17	1	0	-4.985502	1.044453	0.539387
18	8	0	-3.678215	2.637428	0.272481
19	6	0	-4.333264	3.603003	-0.529625
20	1	0	-4.151977	4.591631	-0.107919
21	1	0	-5.408067	3.448621	-0.624375
22	1	0	-1.156232	-0.781328	2.575046
23	6	0	-3.958549	-3.225958	-0.530197
24	6	0	-4.013498	-1.722231	-0.777976
25	8	0	-4.926653	-1.166793	-1.397203
26	1	0	-3.541454	-3.713195	-1.411465
27	1	0	-3.906008	3.660226	-1.530705
28	1	0	-5.004947	-3.529969	-0.504178
29	1	0	-3.514287	-3.631933	0.378713
30	6	0	0.446277	-0.632489	0.243743
31	8	0	0.761331	-1.457955	1.076737
32	7	0	1.308692	0.153578	-0.411127
33	1	0	0.942795	0.611276	-1.233721
34	6	0	2.796384	0.207379	-0.079870
35	1	0	2.789976	0.023128	0.994494
36	6	0	3.253918	1.658483	-0.328718
37	8	0	2.586382	2.595495	-0.799039
38	8	0	4.390848	1.813703	0.255999
39	6	0	5.009772	3.143344	0.455778
40	1	0	5.624225	3.302977	1.341943
41	1	0	5.640308	3.423144	-0.388180

42	1	0	4.172070	3.836532	0.379051
43	6	0	3.540271	-0.837692	-0.848600
44	1	0	2.868877	-1.690925	-0.945460
45	6	0	4.851859	-1.358989	-0.174768
46	1	0	5.506929	-0.507641	0.009951
47	1	0	3.645203	-0.555679	-1.896320
48	6	0	4.762463	-2.026255	1.144679
49	1	0	4.155458	-2.927856	1.062921
50	1	0	5.738913	-2.237103	1.580817
51	1	0	4.232878	-1.401699	1.864073
52	6	0	5.550856	-2.295922	-1.217835
53	1	0	4.911474	-3.146143	-1.455127
54	1	0	5.662902	-1.739839	-2.148572
55	1	0	6.534407	-2.620959	-0.878342

Energy minimum of α conformation of 1b': B3LYP/6-31G(d), scrf=(smd, solvent=chloroform)

NIMG=1 (11.1106 cm^{-1})

Zero-point correction= 0.477124 (Hartree/Particle)

Thermal correction to Energy= 0.504782

Thermal correction to Enthalpy= 0.505726

Thermal correction to Gibbs Free Energy= 0.415323

Sum of electronic and zero-point Energies= -1189.174609

Sum of electronic and thermal Energies= -1189.146951

Sum of electronic and thermal Enthalpies= -1189.146007

Sum of electronic and thermal Free Energies= -1189.236411

Standard orientation:

Center Number	Atomic Number	Atomic Type	Coordinates (Angstroms) X	Y	Z
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4	6	0	2.822890	-0.292606	1.673900
5	1	0	2.390632	1.657242	2.517839
6	1	0	0.928141	2.307547	0.609166
7	1	0	3.768943	-0.182349	2.202547
8	1	0	2.408098	-1.261707	1.959331
9	6	0	1.758722	-0.778678	-0.579546
10	1	0	1.535289	-1.792822	-0.242328
11	1	0	1.935565	-0.833420	-1.655404
12	6	0	0.654504	0.265163	-0.270364
13	1	0	0.326607	0.731469	-1.200638
14	7	0	2.705029	1.220640	-0.041354
15	6	0	4.360459	-0.767234	-0.358477
16	1	0	4.412862	-0.717145	-1.448793
17	1	0	5.178613	-0.162162	0.038043
18	8	0	4.494045	-2.113878	0.088585
19	6	0	5.691326	-2.738533	-0.341500
20	1	0	5.703764	-2.857673	-1.425299
21	1	0	5.758501	-3.730641	0.104694
22	1	0	1.046065	0.555863	2.665412
23	6	0	2.832527	3.608189	-0.696032
24	6	0	3.366915	2.181081	-0.728463
25	8	0	4.391583	1.958606	-1.365362
26	1	0	2.624884	3.924004	0.327937

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    28          1           0        1.925480    3.688127   -1.294733
    29          1           0        3.575820    4.291088   -1.110108
    30          6           0       -0.590463   -0.338602    0.420644
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    34          6           0       -3.092187   -0.360217    0.392932
    35          1           0       -3.175404   -0.035290    1.430177
    36          6           0       -3.377202   -1.893142    0.174250
    37          8           0       -3.508349   -2.606949    1.105266
    38          8           0       -3.411786   -2.224407   -1.124041
    39          6           0       -3.844667   -3.517213   -1.519695
    40          1           0       -4.832690   -3.739433   -1.110892
    41          1           0       -3.920222   -3.559365   -2.606635
    42          1           0       -3.149147   -4.290592   -1.191938
    43          6           0       -4.067154    0.482714   -0.450793
    44          1           0       -3.789048    0.369860   -1.498706
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    46          1           0       -3.356748    2.555858    0.150410
    47          1           0       -5.072340    0.066327   -0.516941
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    50          1           0       -4.145238    3.147759   -1.941260
    51          1           0       -5.634852    2.311023   -1.662335
    52          6           0       -5.093925    2.050684    1.326481
    53          1           0       -6.117294    1.819586    1.030833
    54          1           0       -4.806452    1.396740    2.153858
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References

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[4] N. Greenfield, and G. D. Fasman, *Biochemistry.* 1969, **8**, 4108-4116.