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

Supporting Information for

Helical Folding of α/β-Peptides Containing β-Amino Acids with an Eight-membered ring constraint

Woohyung Lee, Sunmi Kwon, Philjae Kang, Ilia A. Guzei, and Soo Hyuk Choi*

Department of Chemistry, Yonsei University, Seoul 120-749, Republic of Korea

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706, United States

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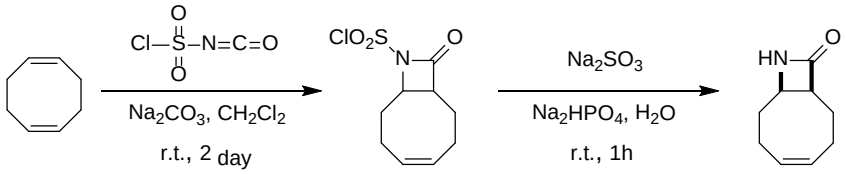
Synthesis

General

α-Amino acids and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDCI) were purchased from Chem-Impex International. Other reagents were purchased from Sigma-Aldrich, Alfa Aesar, Samchun Chemical, and TCI. Analytical thin-layer chromatography (TLC) was carried out on Pre-coated silica gel glass plate (Merck silica gel 60, F254, 0.25 mm). Silica gel 60 (230~240 mesh, Merck) was used for flash column chromatography. RP-HPLC analysis was performed on the Agilent 1260 infinity series with a UV detector and a C18 column. FT-IR spectra were recorded on Bruker Vertex 70 FT-IR spectrometer at 4000 cm^{-1} ~ 400 cm^{-1} of wave numbers. Mass spectra (MS) were acquired using an LTQ Orbitrap Spectrometer (ThermoFisher scientific Inc.).

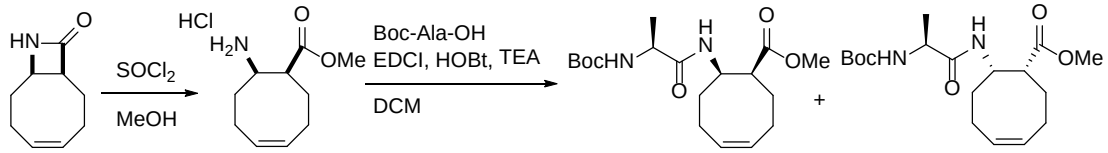
(±)-9-Azabicyclo[6.2.0]dec-4-en-10-one ((±)-1)



(±)-1

Racemic β-lactam **(±)-1** was synthesized using a modified procedure of a method reported previously in reference 6 of the main text. 1,5-Cyclooctadiene (65 mL, 531 mmol, 3 eq.), anhydrous sodium carbonate (2.8 g, 12.75 mmol, 0.15 eq.) were added to dichloromethane (65 mL) in 250 ml round-bottom flask and cooled to 0 °C under nitrogen gas. Chlorosulfonyl isocyanate (25 g, 15.38 mL, 177 mmol, 1 eq.) was added dropwisely to the reaction mixture with stirring over 30 min. After stirring at 0 °C for additional 1 hour, the mixture was stirred at rt for 2 days. The mixture was quenched with dropwise addition of water until bubbling ceased. The resulting solution was diluted with dichloromethane (25 mL) and added dropwisely to 1.67 M aqueous phosphate buffer solution (200 mL) containing 25 w/v % sodium sulfite (50 g) in a 500 mL Erlenmeyer flask with vigorous stirring. The organic layer was extracted with ethyl acetate (300 mL), washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The crude product was purified by flash column chromatography by a elution with 20 % ethyl acetate in hexanes, and then was recrystallized from a solution in ethyl acetate and hexanes to yield **1** as a white solid (11.0 g, 72.8 mmol, 41 %, yield over two steps): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.74~5.61 (m, 3H), 3.85 (m, 1H), 3.31 (m, 1H), 2.42 (m, 2H), 2.11~1.94 (m, 4H), 1.92~1.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.50, 131.03, 130.36, 54.06, 53.40, 30.69, 24.31, 23.81, 22.65; HRMS (ESI) *m/z* Calcd for $C_9H_{13}NO$ $[M + Na]^+$ 174.0889, found 174.0888, and $[M + H]^+$ 152.1069, found 152.1069.

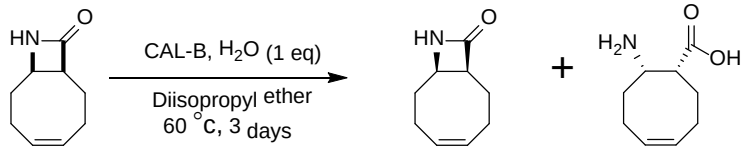
Boc-L-Ala-*cis*-ACOE-OMe (2a + 2b)



(±)-1 **(±)-10** **2a** **2b**

Racemic β-lactam **(±)-1** (1 g, 6.61 mmol, 1 eq.) was dissolved in methanol (20 mL) and cooled to 0 °C. Thionyl chloride (959 μL, 13.2 mmol, 2 eq.) was added dropwisely with stirring for 10 minutes at 0 °C, and the reaction mixture was stirred at rt for 12 hours. The solvent was evaporated off and methanol (20 mL) was added. Additional thionyl chloride (479 μL, 6.61 mmol, 1 eq.) was added with stirring for 10 minutes at 0 °C, and the reaction mixture was stirred at rt for 6 hours. The solvent was evaporated off, and a crude product was recrystallized from methanol and diethyl ether to yield a racemic mixture of β-amino esters **(±)-10** as a white solid quantitatively. Racemate **(±)-10** (1.45 g, 6.61 mmol, 1 eq.), EDCI (1.90 g, 9.92 mmol, 1.5 eq.), HOBt (1.16 g, 8.59 mmol, 1.3 eq.), triethylamine (1.02 mL, 7.27 mmol, 1.1 eq.) and Boc-L-Ala-OH (1.25 mg, 6.61 mmol, 1 eq.) were dissolved in dichloromethane (70 mL). The reaction mixture was stirred for a day at rt, diluted with excess amount of ethyl acetate, washed successively with aqueous 10% citric acid, aqueous saturated sodium carbonate, and brine. The organic layer was dried over magnesium sulfate and was concentrated in vacuo to give a crude product, which was purified by flash column chromatography by elution of 25 % ethyl acetate in hexanes to give a mixture of diastereomeric dipeptides **2a** and **2b** (2.18 g, 6.15 mmol, 93 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.63 (d, *J* = 8.5Hz, 1H), 5.73~5.66 (m, 2H), 4.93 (br, 1H), 4.48 (m, 1H), 4.10 (m, 1H), 3.71 (s, 3H), 2.80 (m, 1H), 2.44 (m, 1H), 2.27 (m, 1H), 2.17~2.00 (m, 3H), 1.91 (m, 1H), 1.78 (m, 2H), 1.45 (s, 9H), 1.33 (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.60, 171.27, 155.50, 130.55, 129.40, 80.03, 51.69, 50.12, 48.67, 47.27, 32.77, 28.32, 27.18, 24.40, 23.13, 18.19; HRMS (ESI) *m/z* Calcd for $C_{18}H_{30}N_2O_5$ $[M + Na]^+$ 377.2046, found 377.2047 and $[M + H]^+$ 355.2227, found 355.2228.

(1S,8R)-9-Azabicyclo[6.2.0]dec-4-en-10-one (11) and Methyl (1R, 2S)-8-aminocyclooc-4-enecarboxylic acid (12)



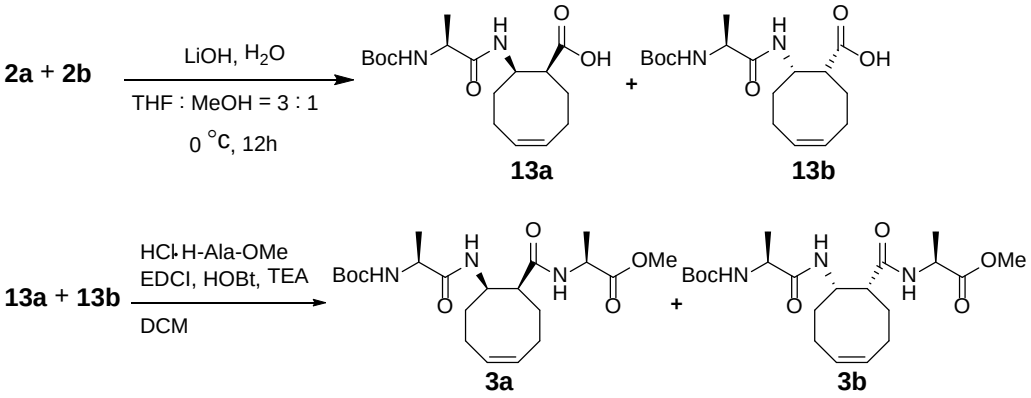
(±)-1 **11** **12**

Chiral resolution of racemic β-lactam **(±)-1** was carried out using a method reported previously in reference 6 of the main text. Racemic β-lactam **(±)-1** (1 g, 6.61 mmol, 1 eq.) and water (112 μL, 6.61

mmol, 1 eq.) were added to 250 mL evaporation flask containing diisopropyl ether (100 mL) and CAL-B (Lipase from *Candida Aantarctica*) (1.5 g). The reaction mixture was stirred at 60 °C for 3 days, filtered, and rinsed with hot diisopropyl ether. The filtrate was concentrated in vacuo and was recrystallized from a mixture in ethyl acetate and hexanes to yield chiral β-lactam **11** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.74~5.61 (m, 3H), 3.85 (m, 1H), 3.31 (m, 1H), 2.42 (m, 2H), 2.11~1.94 (m, 4H), 1.92~1.89 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 171.50, 131.03, 130.36, 54.06, 53.40, 30.69, 24.31, 23.81, 22.65; HRMS (ESI) Calcd for $C_9H_{13}NO$ $[M+Na]^+$ 174.0889, found 174.0888, $[M + H]^+$ 152.1069, found 152.1069.

The isolated mixture was pooled in hot water and filtered. The filtrate was concentrated in vacuo, and then was recrystallized from a solution in water and acetone to yield chiral β-amino acid **12** as a white solid (454 mg, 3.17 mmol, 48 %). ^{1}H NMR (400 MHz, D_2O) δ = 5.77~5.69 (m, 2H), 3.74 (m, 1H), 2.82 (m, 1H), 2.60 (m, 1H), 2.47 (m, 1H), 2.25~2.08 (m, 4H), 1.96 (m, 1H), 1.83 (m, 1H); ^{13}C NMR (100 MHz, D_2O) δ = 181.53, 129.54, 129.19, 52.09, 45.45, 29.40, 26.77, 24.26, 23.54; HRMS (ESI) *m/z* Calcd for $C_9H_{15}NO_2$, $[M + H]^+$ 170.1175, found 170.1175..

Boc-L-Ala-(1S,2R)-ACOE-L-Ala-OMe (3a) and Boc-L-Ala-(1R,2S)-ACOE-L-Ala-OMe (3b)



To a solution of a mixture of diastereomers **2a** and **2b** (1 g, 2.82 mmol, 1 eq.) in methanol (30 mL) and tetrahydrofuran (90 mL), an aqueous solution (30 mL) of lithium hydroxide (592 mg, 14.1 mmol, 5 eq.) was added at 0 °C. The reaction mixture was stirred for 12 hours at 0 °C. The solvent was evaporated off, diluted with ethyl acetate (50 mL), and acidified with aqueous 1 *N* HCl to pH 2. The mixture was extracted with ethyl acetate (150 mL). The combined organic layer was washed with brine, dried over magnesium sulfate, filtered, and concentrated to yield a diastereomeric mixture of carboxylic acids **13a** and **13b** quantitatively. The mixture of **13a** and **13b** (1 g, 2.94 mmol, 1 eq.), EDCI (844 mg, 4.41 mmol, 1.5 eq.), HOBt (516 mg, 3.82 mmol, 1.3 eq.), triethylamine (454 μL, 3.23 mmol, 1.1 eq.) and L-alanine methyl ester hydrochloride (410 mg, 2.94 mmol, 1 eq.) were dissolved in dichloromethane (30 mL). The reaction mixture was stirred at rt for 2 days, diluted with

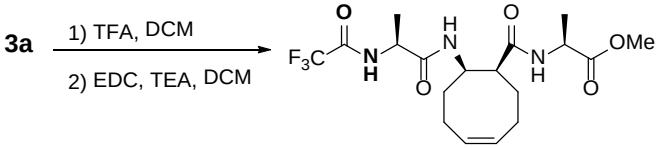
excess ethyl acetate, washed successively with aqueous 10% citric acid, aqueous saturated sodium carbonate, and brine. The organic layer was dried over magnesium sulfate and was concentrated in vacuo. Two diastereomers were isolated by flash column chromatography (20 % ethyl acetate in dichloromethane) to give **3a** (R_f = 0.6, 50 % ethyl acetate in dichloromethane, 587 mg, 1.38 mmol, 47 %) and **3b** (R_f = 0.35, 50 % ethyl acetate in dichloromethane, 540 mg, 1.27 mmol, 47 %). Tripeptide **3a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.61(d, *J* = 8.1 Hz,1H), 7.34 (d, *J* = 10.1 Hz, 1H), 5.78(m, 1H), 5.56 (m, 1H), 4.92 (d, *J* = 6.2 Hz, 1H), 4.71 (quintet, *J* = 7.7 Hz, 1H), 4.53 (m, 1H), 3.83 (qd, *J* = 6.8, 6.2 Hz, 1H), 3.77 (s, 3H), 3.00 (m, 1H), 2.69~2.32 (m, 4H), 1.90 (m, 1H), 1.74~1.54 (m, 3H), 1.46 (d, *J* = 7.5 Hz, 3H), 1.41(s, 9H), 1.33 (d, *J* = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.04, 173.61, 172.13, 155.99, 132.22, 125.85, 80.27, 52.64, 51.48, 50.67, 48.25, 46.98, 31.23, 28.45, 28.29, 25.33, 22.65, 17.43, 16.38; HRMS (ESI) *m/z* Calcd for $C_{21}H_{35}N_3O_6$ [M +Na]$^+$ 448.2418, found 448.2414 and [M + H]$^+$ 426.2598, found 426.2595. Tripeptide **3b**: ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (br, 1H), 6.98 (d, *J* = 8.9 Hz, 1H), 5.85 (m, 1H), 5.75 (m, 1H), 4.87 (d, *J* = 6.4 Hz, 1H), 4.54 (m, 1H), 4.47 (quintet, *J* = 7.8 Hz, 1H), 4.12 (m, 1H), 3.74 (s, 3H), 2.76 (m, 1H), 2.40~2.17 (m, 4H), 1.97~1.81 (m, 4H), 1.45 (s, 9H), 1.41 (d, 3H), 1.34 (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.82, 173.60, 172.49, 155.65, 130.98, 130.83, 80.38, 52.31, 50.50, 49.10, 48.66, 48.27, 33.66, 28.66, 28.31, 25.79, 22.68, 18.07, 17.65; HRMS (ESI) *m/z* Calcd for $C_{21}H_{35}N_3O_6$ [M + Na]$^+$ 448.2418, found 448.2414 and [M + H]$^+$ 426.2598, found 426.2595.

Boc-L-Ala-(1S,2R)-ACOE-OMe (2a)

Enantiomeric dipeptide **2a** was synthesized from chiral β-lactam **11** (1 g, 6.61 mmol, 1 eq) by methods described above. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.63 (d, *J* = 8.5 Hz, 1H), 5.73~5.66 (m, 2H), 4.93 (br, 1H), 4.48 (m, 1H), 4.10 (m, 1H), 3.71 (s, 3H), 2.80 (m, 1H), 2.44 (m, 1H), 2.27 (m, 1H), 2.17~2.00 (m, 3H), 1.91 (m, 1H), 1.78 (m, 2H), 1.45 (s, 9H), 1.33 (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 174.60, 171.27, 155.50, 130.55, 129.40, 80.03, 51.69, 50.12, 48.67, 47.27, 32.77, 28.32, 27.18, 24.40, 23.13, 18.19; HRMS (ESI) *m/z* Calcd for $C_{18}H_{30}N_2O_5$ [M + Na]$^+$ 377.2046 and found 377.2047, [M + H]$^+$ 355.2227, found 355.2228.

Boc-L-Ala-(1S,2R)-ACOE-OH (13a). Chiral acid **13a** was prepared from **2a** by saponification described above and used without purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 6.99 (d, 1H), 5.73 (m, 2H), 5.22 (m, 1H), 4.47 (m, 1H), 4.21 (m, 1H), 2.44 (m, 1H), 2.32 (m, 1H), 2.05 (m, 6H), 1.80 (m, 2H), 1.45 (s, 9H), 1.35 (d, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 177.86, 175.83, 172.47, 155.97, 130.65, 129.68, 80.42, 50.00, 48.95, 47.24, 32.59, 28.31, 27.27, 24.54, 22.93, 20.77, 17.90.

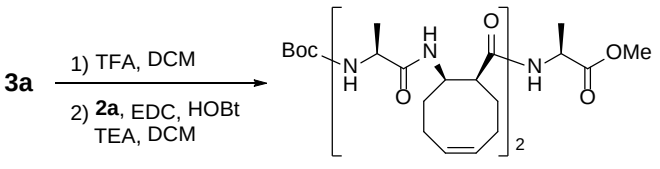
CF_3CO-L-Ala-(1S,2R)-ACOE-L-Ala-OMe (4)



4

Tripeptide **3a** (500 mg, 1.18 mmol, 1 eq.) in dichloromethane (2 mL) was treated with trifluoroacetic acid (2 mL). The mixture was stirred until the starting material spot disappeared on thin-layer chromatography. The reaction mixture was concentrated by a stream of nitrogen gas, diluted with dichloromethane (3 mL), and basified with triethylamine to pH ~8. EDCI (339 mg, 1.77 mmol, 1.5 eq.) and HOBt (306 mg, 1.53 mmol, 1.3 eq.) were added, and the reaction mixture was stirred at rt for a day. The resulting mixture was diluted with excess ethyl acetate, washed successively with aqueous 10% citric acid, aqueous saturated sodium bicarbonate, and brine. The organic layer was dried over magnesium sulfate, and concentrated in vacuo to give a crude product, which was purified by flash column chromatography by eluting 10 % ethyl acetate in dichloromethane to give *N*-trifluoroacetyl tripeptide **4** (134 mg, 0.32 mmol, 27 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.47 (d, *J* = 9.6 Hz, 1H), 7.39 (d, *J* = 5.1 Hz, 1H), 6.90 (d, *J* = 8.2 Hz, 1H), 5.68 (m, 1H), 5.59 (m, 1H), 4.71 (quintet, *J* = 7.7 Hz, 1H), 4.55 (m, 1H), 4.21 (quintet, *J* = 6.8 Hz, 1H), 3.78 (s, 3H), 2.97 (m, 1H), 2.66~2.50 (m, 3H), 2.32 (m, 1H), 1.94 (m, 1H), 1.74 (m, 2H), 1.60 (m, 1H), 1.50 (d, *J* = 7.0 Hz, 3H), 1.45 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.09, 173.29, 169.58, 132.01, 125.91, 52.81, 50.97, 50.94, 48.30, 47.46, 31.27, 28.36, 25.46, 22.72, 17.43, 16.49; HRMS (TOF MS ESI) *m/z* Calcd for $C_{18}H_{26}F_3N_3O_5$ $[M + Na]^+$ 444.1799, found 444.1799.

Boc-(L-Ala-(1S,2R)-ACOE)$_2$-L-Ala-OMe (5)

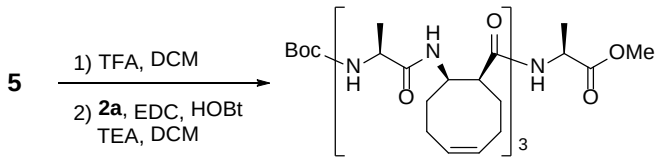


5

Tripeptide **3a** (500 mg, 1.18 mmol, 1 eq.) was dissolved in dichloromethane (2 mL) and treated with trifluoroacetic acid (2 mL). The mixture was stirred until the starting material spot disappeared on thin-layer chromatography. The reaction mixture was concentrated by a stream of nitrogen gas, diluted with dichloromethane (8 mL), basified with triethylamine to pH ~8, and then was transferred to a mixture of chiral acid **2a** (448 mg, 1.18 mmol, 1 eq.), EDCI (339 mg, 1.77 mmol, 1.5 eq.), and HOBt (306 mg, 1.53 mmol, 1.3 eq.) in dichloromethane (4 mL). The reaction mixture was stirred at rt for two days, and was diluted with excess ethyl acetate. The mixture was washed successively with

aqueous 10% citric acid, aqueous saturated sodium carbonate, and brine. The organic layer was dried over magnesium sulfate, concentrated in vacuo, and then was purified by flash column chromatography (20 % ethyl acetate in dichloromethane) to give pentamer **5** (673 mg, 1.04 mmol, 88 %) (R_f = 0.65, 50 % ethyl acetate in dichloromethane). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.26 (d, *J* = 8.2 Hz, 1H), 8.05 (d, *J* = 5.5 Hz, 1H), 7.63 (d, *J* = 9.6 Hz, 1H), 7.48 (d, *J* = 9.7 Hz, 1H), 5.68 (m, 2H), 5.55 (m, 2 H), 4.95 (d, *J* = 5.5 Hz, 1H), 4.71 (quintet, *J* = 7.7 Hz, 1H), 4.51 (m, 2H), 4.11 (qd, *J* = 5.5, 7.2 Hz, 1H), 3.95 (qd, *J* = 5.5, 6.9 Hz, 1H), 3.78 (s, 3H), 3.08~2.991 (m, 2H), 2.75~2.32 (m, 8H), 1.96~1.82 (m, 2H), 1.74~1.56 (m, 6H), 1.50 (d, *J* = 7.7 Hz, 3H), 1.44 (s, 9H), 1.41 (d, *J* = 7.2 Hz, 3H), 1.35 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.51, 175.03, 174.98, 173.51, 172.45, 156.02, 132.39, 125.83, 125.74, 80.34, 52.80, 52.40, 51.87, 51.07, 50.31, 48.51, 47.73, 47.19, 31.47, 31.09, 28.94, 28.65, 28.46, 25.40, 22.82, 22.66, 17.74, 16.67, 16.33; HRMS (ESI) *m/z* Calcd for $C_{33}H_{53}N_5O_8$ $[M + Na]^+$ 670.3786, found 670.3776 and $[M + H]^+$ 648.3966, found 648.3961.

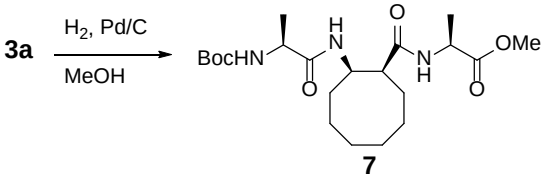
Boc-(L-Ala-(1S,2R)-ACOE)$_3$-L-Ala-OMe (6)



6

Heptamer **6** was synthesized from **2a** and **5** by a method analogous to that described for the synthesis of **5** above. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.71 (d, *J* = 5.2 Hz, 1H), 8.35 (d, *J* = 8.2 Hz, 1H), 7.97 (d, *J* = 5.2 Hz, 1H), 7.86 (d, *J* = 9.7 Hz, 1H), 7.74 (d, *J* = 9.8 Hz, 1H), 7.48 (d, *J* = 9.7 Hz, 1H), 5.67 (m, 3H), 5.55 (m, 3H), 4.90 (d, *J* = 5.5 Hz, 1H), 4.71 (quintet, *J* = 7.7 Hz, 1H), 4.50 (m, 3H), 4.24 (qd, *J* = 5.2, 7.5 Hz, 1H), 4.11 (qd, *J* = 5.2, 7.2 Hz, 1H), 3.93 (qd, *J* = 5.5, 6.9 Hz, 1H), 3.78 (s, 3H), 3.03 (m, 3H), 2.72~2.28 (m, 13H), 1.93~1.53 (m, 14H), 1.49 (d, *J* = 7.5 Hz, 3H), 1.42 (s, 9H), 1.40 (d, *J* = 7.2 Hz, 3H), 1.34 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.30, 175.07, 174.98, 174.69, 174.51, 173.34, 172.21, 155.79, 132.20, 132.13, 125.71, 125.58, 125.49, 80.18, 52.63, 52.42, 52.32, 51.69, 50.85, 50.52, 50.25, 48.30, 47.59, 46.97, 31.26, 30.97, 30.89, 28.75, 28.65, 28.51, 28.30, 25.53, 22.63, 22.44, 17.59, 16.61, 16.35, 16.15; HRMS (ESI) *m/z* Calcd for $C_{45}H_{71}N_7O_{10}$ $[M + Na]^+$ 892.5154, found 892.5142 and $[M + H]^+$ 870.5335, found 870.5327.

Boc-L-Ala-(1S,2R)-ACOC-L-Ala-OMe (7)



7

A mixture of **3a** (100 mg, 0.230 mmol) in methanol (20 mL) and 10% Pd/C (~50 mg) was charged with hydrogen gas (1.5 atm), and stirred overnight at rt. The resulting mixture was filtered through Celite pad. The filtrate was concentrated to give desired product **7** quantitatively. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.49 (d, *J* = 8.1 Hz, 1H), 7.38 (d, *J* = 9.6 Hz, 1H), 5.00 (d, *J* = 6.3 Hz, 1H), 4.67 (quintet, *J* = 7.7 Hz, 1H), 4.54 (m, 1H), 3.89 (qd, *J* = 6.3, 7.0 Hz, 1H), 3.67 (s, 3H), 2.52 (m, 1H), 2.04 (m, 1H), 1.92~1.701 (m, 8H), 1.59 (m, 1H), 1.52~1.34 (m, 17H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.09, 173.84, 171.99, 155.96, 80.20, 52.64, 51.36, 49.72, 48.32, 46.24, 31.35, 30.85, 28.30, 26.71, 25.73, 24.62, 23.79, 17.51, 16.28; HRMS (ESI) *m/z* Calcd for $C_{21}H_{37}N_3O_6$ $[M + Na]^+$ 450.2574, found 450.2568 and $[M + H]^+$ 428.2755, found 428.2752.

Boc-(L-Ala-(1S,2R)-ACOC)$_2$-L-Ala-OMe (8)

Pentapeptide **8** was synthesized from **5** by a method analogous to that described for the synthesis of **7** above. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.21 (d, *J* = 8.0 Hz, 1H), 7.90 (d, *J* = 5.5 Hz, 1H), 7.73 (d, *J* = 9.6 Hz, 1H), 7.49 (d, *J* = 9.6 Hz, 1H), 4.99 (d, *J* = 5.6 Hz, 1H), 4.65 (quintet, *J* = 7.7 Hz, 1H), 4.51 (m, 2H), 4.09 (qd, *J* = 5.5, 6.4 Hz, 1H), 4.00 (qd, *J* = 5.6, 7.4 Hz, 1H), 3.76 (s, 3H), 2.53 (m, 2H), 2.17~1.36 (m, 42H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.33, 174.96, 174.56, 173.63, 172.19, 155.79, 80.07, 52.56, 52.10, 51.53, 49.67, 49.13, 48.43, 46.74, 46.30, 31.34, 31.12, 30.95, 30.90, 28.29, 26.69, 26.63, 25.70, 25.66, 24.58, 24.50, 23.73, 23.68, 17.64, 16.52, 15.88; ; HRMS (ESI) *m/z* Calcd for $C_{33}H_{57}N_5O_8$ $[M + Na]^+$ 674.4099, found 674.4089 and $[M + H]^+$ 652.4279, found 652.4272.

Boc-(L-Ala-(1S,2R)-ACOC)$_3$-L-Ala-OMe (9)

Heptapeptide **9** was synthesized from **6** by a method analogous to that described for the synthesis of **7** above. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.71 (d, *J* = 5.2 Hz, 1H), 8.40 (d, *J* = 8.1 Hz, 1H), 7.94 (d, *J* = 9.7 Hz, 1H), 7.90 (d, 1H), 7.88 (d, 1H), 7.54 (d, *J* = 9.7 Hz, 1H), 4.98 (d, *J* = 5.5 Hz, 1H), 4.66 (quintet, *J* = 7.6 Hz, 1H), 4.52 (m, 3H), 4.24 (qd, 1H), 4.08 (qd, *J* = 5.2, 7.0 Hz, 1H), 4.00 (qd, *J* = 5.5, 6.8 Hz, 1H), 3.77 (s, 3H), 2.50 (m, 3H), 2.19~1.27 (m, 57H); ^{13}C NMR (100 MHz, $CDCl_3$) δ = 176.29, 174.85, 174.79, 174. 65, 173.64, 172.14, 155.75, 80.03, 52.56, 52.28, 51.23, 51.51, 49.63, 49.27, 49.21, 48.41, 46.84, 46.75, 46.32, 31.27, 31.11, 31.04, 30.97, 30.81, 28.31, 26.64, 25.70, 25.66, 24.77, 24.49, 23.72, 23.66, 17.70, 16.66, 16.36, 15.98; ; HRMS (ESI) *m/z* Calcd for $C_{45}H_{77}N_7O_{10}$ $[M + Na]^+$ 898.5624, found 898.5604, $[M + H]^+$ 876.5804, found 876.5794.

Circular dichroism experiments

Circular Dichroism spectra were measured by using JASCO-815 spectrometer at 298K. The spectra were obtained using 1-mm path length cell, wavelength range of 190 to 260 nm with 0.2 nm data interval, 1.0 nm bandwidth, and 200 nm/min scanning speed. CD data were acquired in methanol by the background from the sample spectrum and smoothened over 25 data points. Peptide concentrations were 0.3 mM. The final spectra were normalized for the number of residues, path length, and peptide concentration.

Two-dimensional NMR Experiments

Two-dimensional NMR spectra, such as Correlation Spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY), Nuclear Overhauser Effect Spectroscopy (NOESY) and Rotating frame Overhauser Effect Spectroscopy (ROESY), were recorded with Bruker Avance II (400 MHz) or Bruker DRX-500 (500 MHz) spectrometer equipped with cryogenic probe at 278 K in $CDCl_3$, pyridine-d_5, or CD_3OH. The TOCSY experiments were performed with mixing time of 60 ms. The NOESY and ROESY experiments were performed with mixing time of 300 to 400 ms.

Backbone Proton Chemical Shifts (in $CDCl_3$)

Tripeptide **3a**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.41	-	-	-	-
C-term	-	-	-	-	3.77
Ala 1	-	3.83	-	4.92	-
ACOE 2	-	2.63	4.53	7.34	-
Ala 3	-	4.71	-	7.61	-

Tripeptide **3b**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.41	-	-	-	-
C-term	-	-	-	-	3.77
Ala 1	-	4.12	-	4.87	-
ACOE 2	-	2.27	4.54	6.98	-
Ala 3	-	4.47	-	7.59	-

Tripeptide **4**:

	Hα	Hβ	HN	OMe
C-term	-	-	-	3.78
Ala 1	4.21	-	6.90	-
ACOE 2	2.63	4.55	7.39	-
Ala 3	4.71	-	7.47	-

Pentapeptide **5**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.44	-	-	-	-
C-term	-	-	-	-	3.78
Ala 1	-	3.95	-	4.95	-
ACOE 2	-	2.63	4.50	7.63	-
Ala 3	-	4.11	-	8.05	-
ACOE4	-	2.63	4.52	7.48	-
Ala5	-	4.71	-	8.26	-

heptapeptide **6**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.42	-	-	-	-
C-term	-	-	-	-	3.78
Ala 1	-	3.93	-	4.90	-
ACOE 2	-	2.61	4.50	7.74	-
Ala 3	-	4.11	-	7.97	-
ACOE4	-	2.61	4.52	7.86	-
Ala5	-	4.24	-	8.71	-
ACOE6	-	2.61	4.46	7.48	-
Ala7	-	4.71	-	8.35	-

Tripeptide **7**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.44	-	-	-	-
C-term	-	-	-	-	3.60
Ala 1	-	3.89	-	5.00	-
ACOE 2	-	2.52	4.54	7.38	-
Ala 3	-	4.67	-	7.49	-

Pentapeptide **8**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.46	-	-	-	-
C-term	-	-	-	-	3.76
Ala 1	-	4.00	-	4.99	-
ACOE 2	-	2.52	4.50	7.73	-
Ala 3	-	4.09	-	7.90	-
ACOE4	-	2.52	4.54	7.49	-
Ala5	-	4.65	-	8.21	-

heptapeptide **9**:

	Boc	Hα	Hβ	HN	OMe
N-term	1.43	-	-	-	-
C-term	-	-	-	-	3.77
Ala 1	-	4.08	-	4.98	-
ACOE 2	-	2.50	4.53	7.88	-
Ala 3	-	4.52	-	7.90	-
ACOE4	-	2.50	4.48	7.94	-
Ala5	-	4.24	-	8.71	-
ACOE6	-	2.50	4.50	7.54	-
Ala7	-	4.66	-	8.40	-

Characteristic NOEs observed for backbone protons

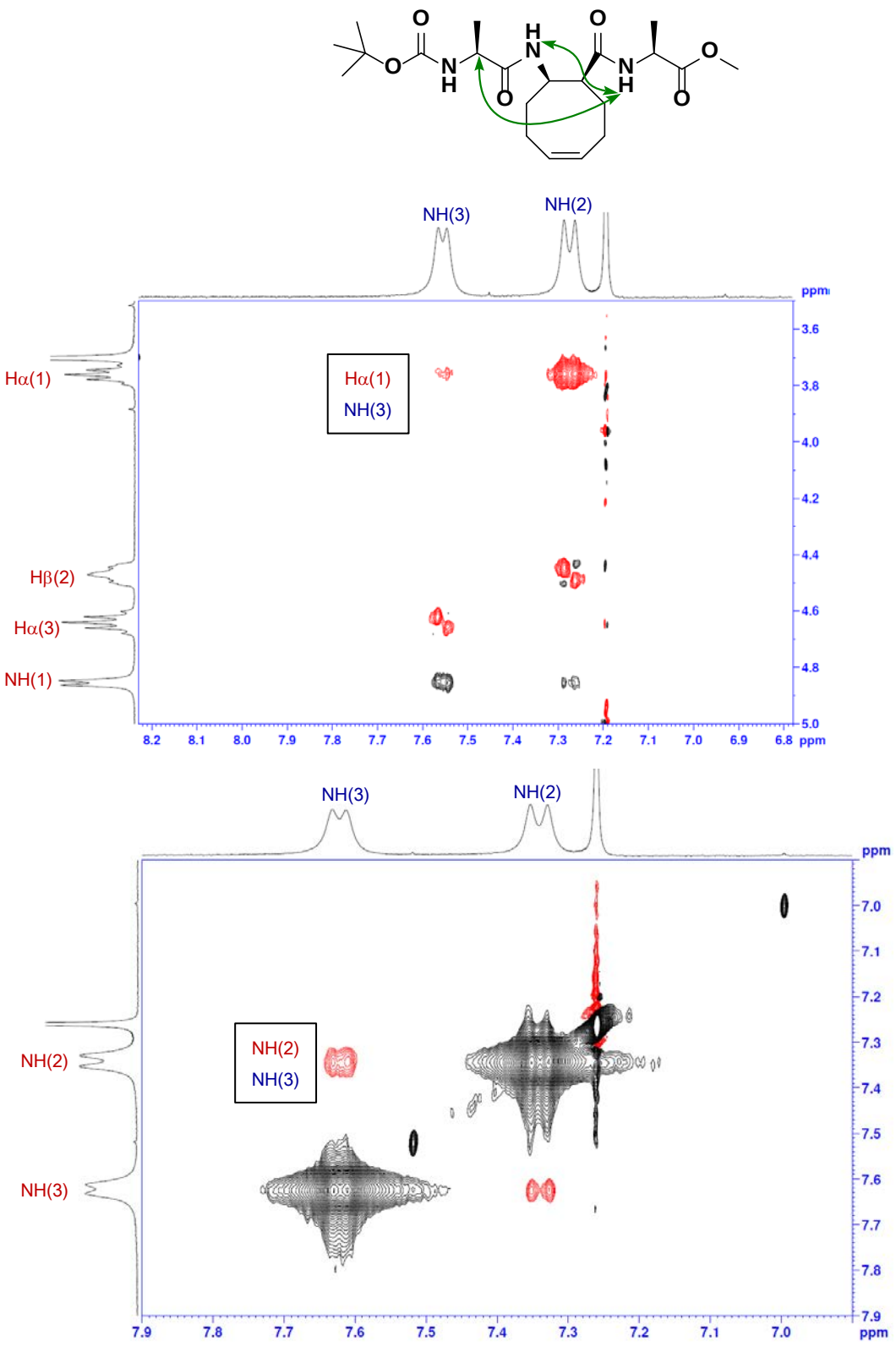


Figure S1: Characteristic NOEs observed for **3a** in $CDCl_3$. Solid arrows indicate characteristic NOEs for the 11/9-helix.

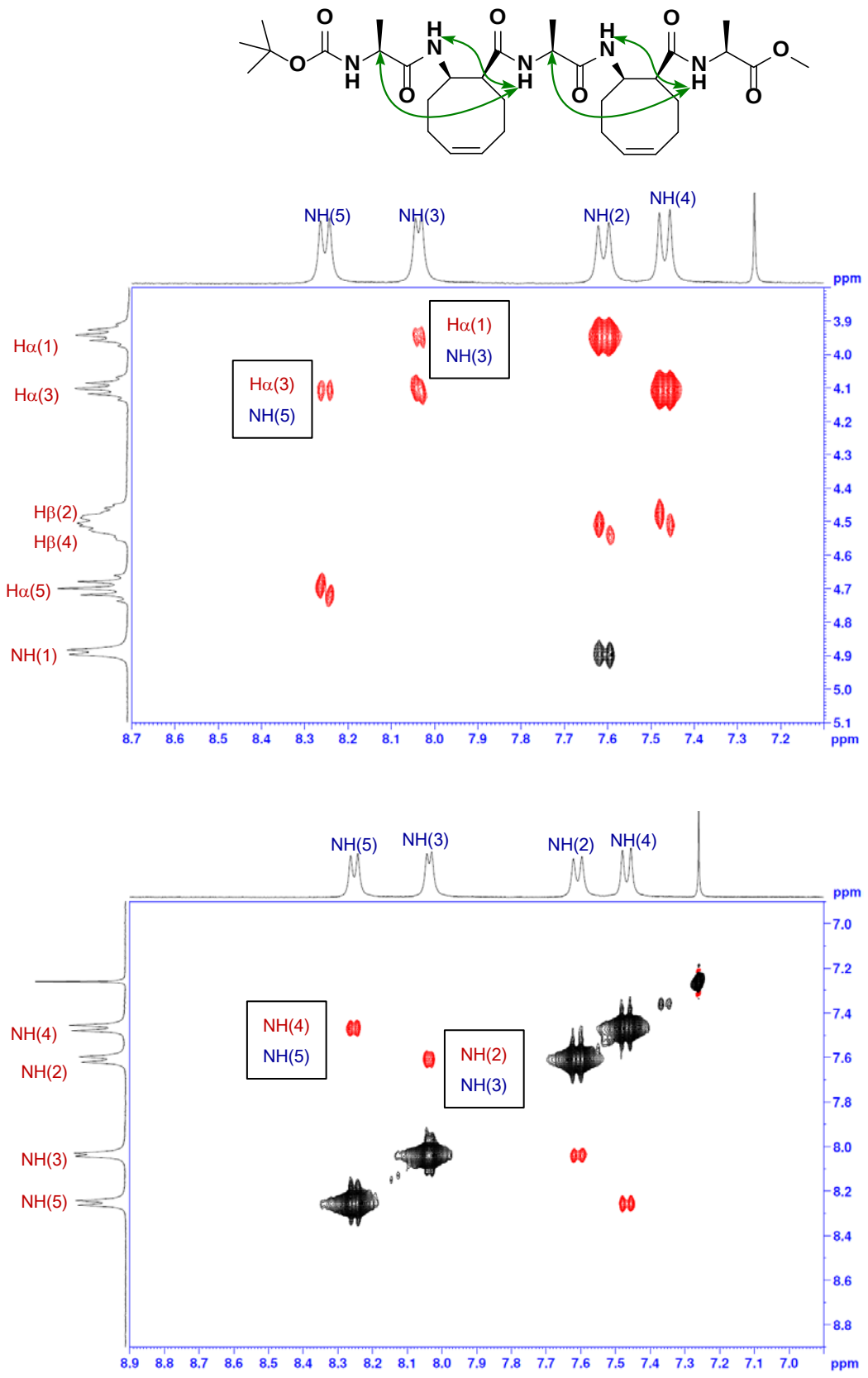


Figure S2: Characteristic NOEs observed for **5** in $CDCl_3$. Solid arrows indicate characteristic NOEs for the 11/9-helix.

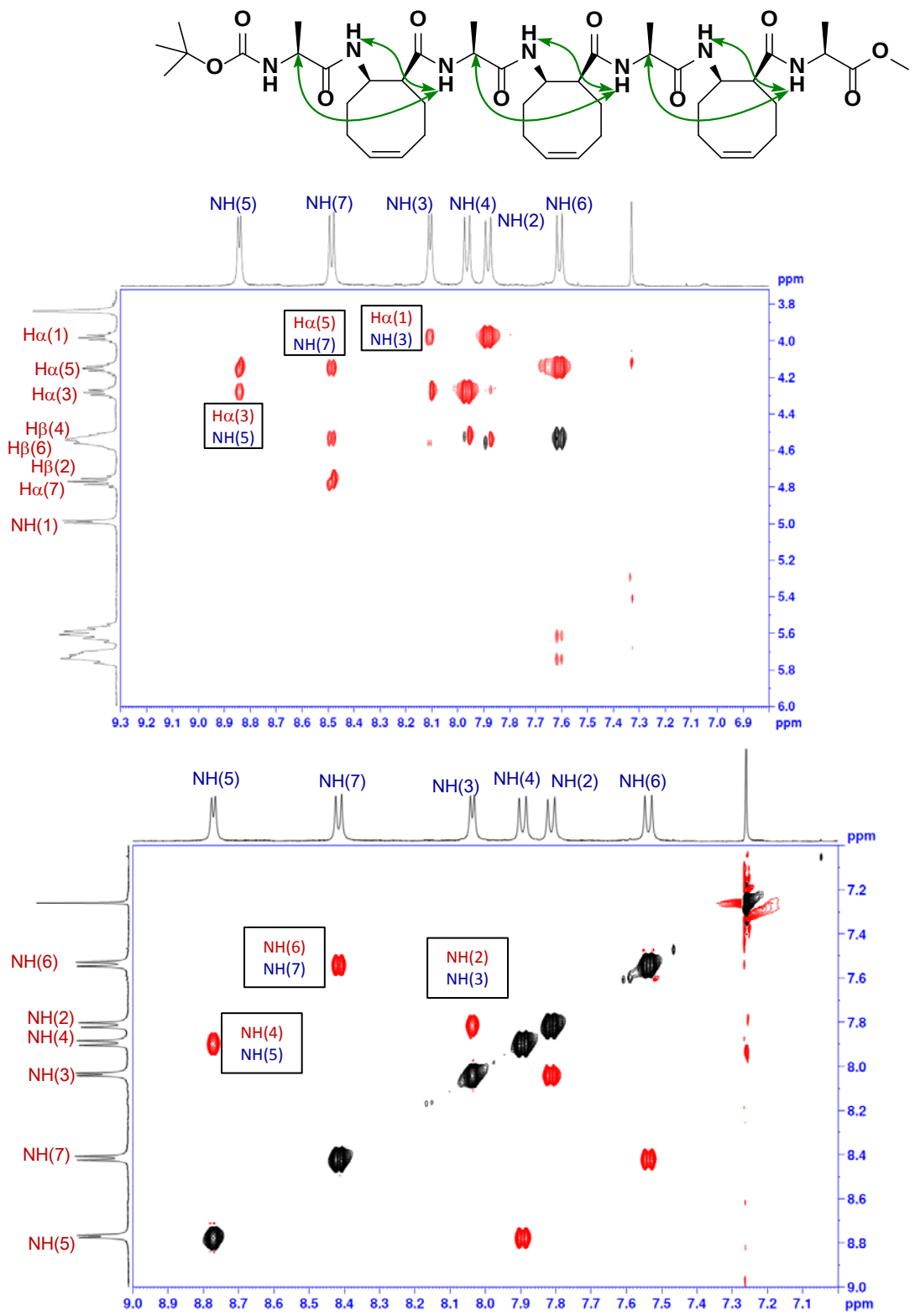


Figure S3: Characteristic NOEs observed for **6** in $CDCl_3$. Solid arrows indicate characteristic NOEs for the 11/9-helix.

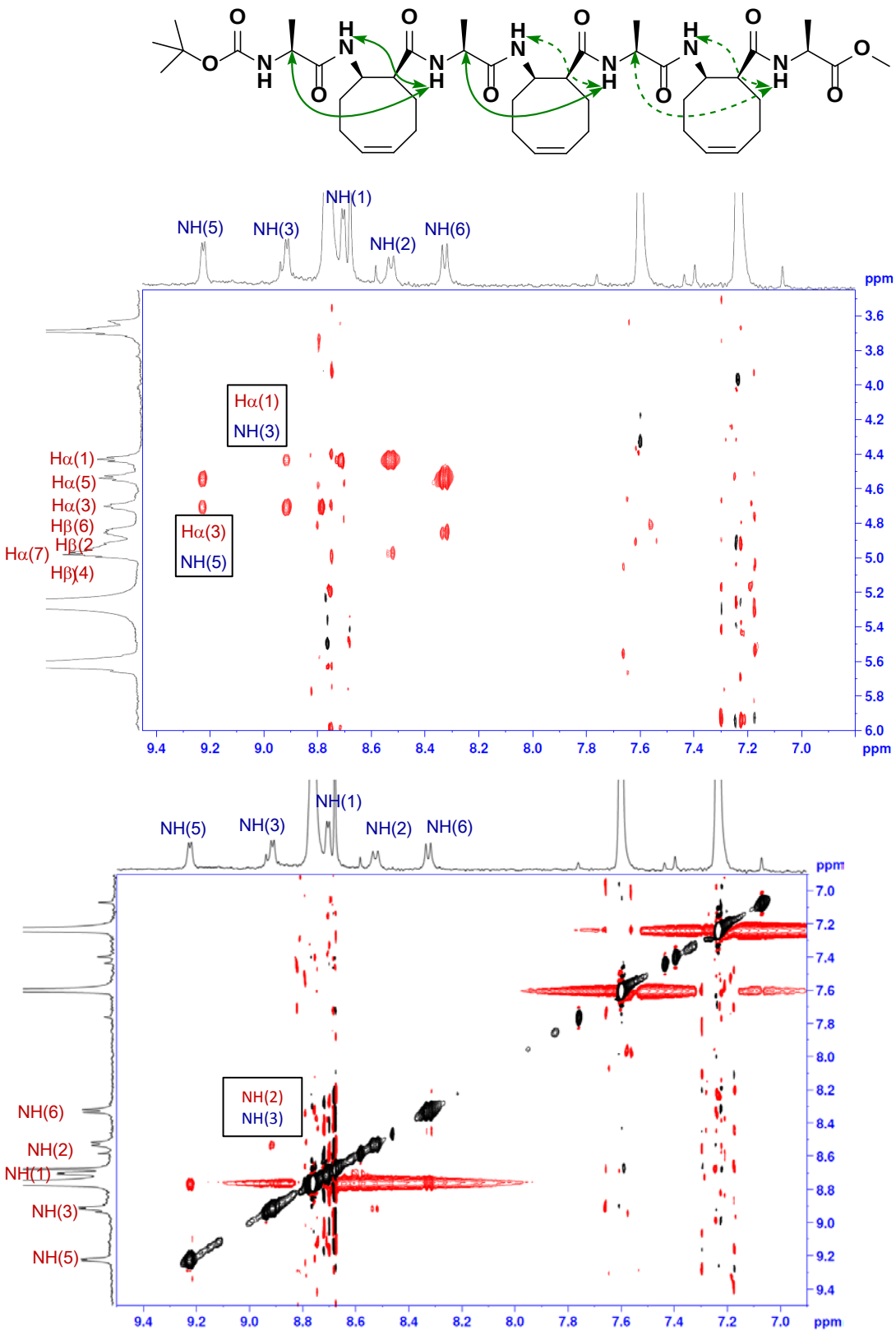


Figure S4: Characteristic NOEs observed for **6** in pyridine-d_5. Solid arrows indicate characteristic NOEs for the 11/9-helix. Dashed arrows indicate ambiguous NOEs that were not assigned because of signal overlap of δNH(4) and δNH(7) with solvent peaks.

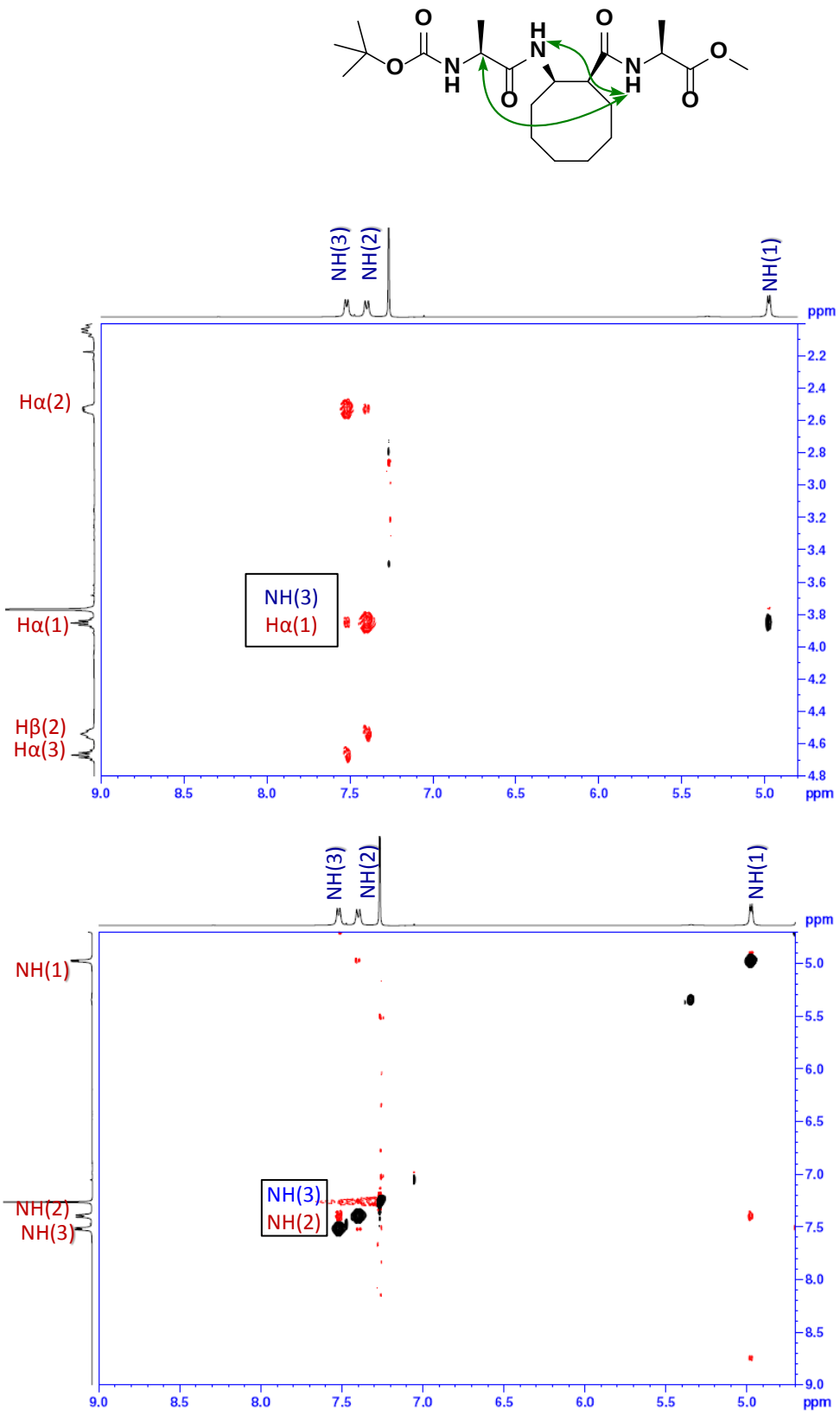


Figure S5: Characteristic NOEs observed for **7** in $CDCl_3$. Solid arrows indicate characteristic NOEs for the 11/9-helix.

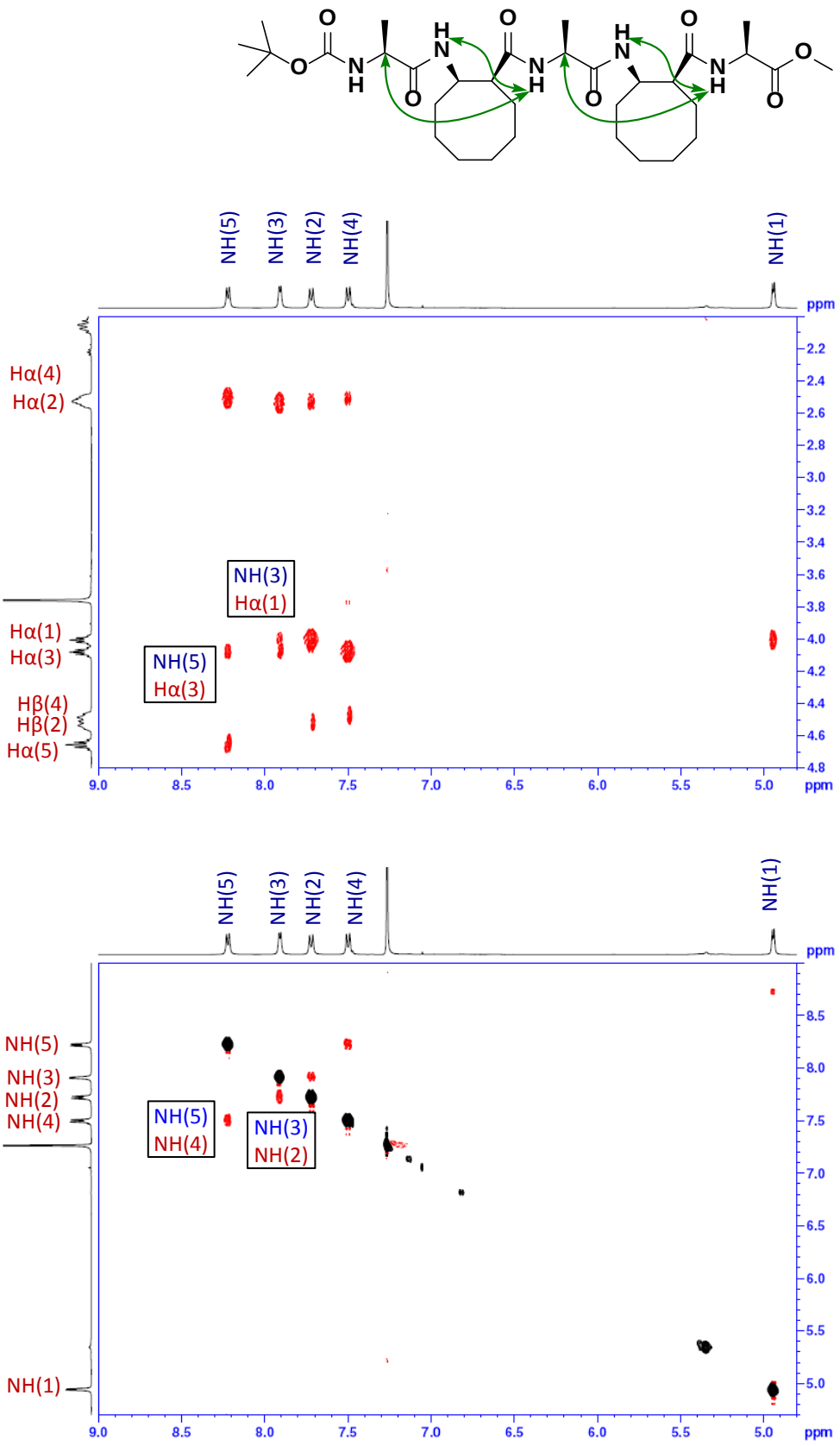


Figure S6: Characteristic NOEs observed for **8** in $CDCl_3$. Solid arrows indicate characteristic NOEs for the 11/9-helix.

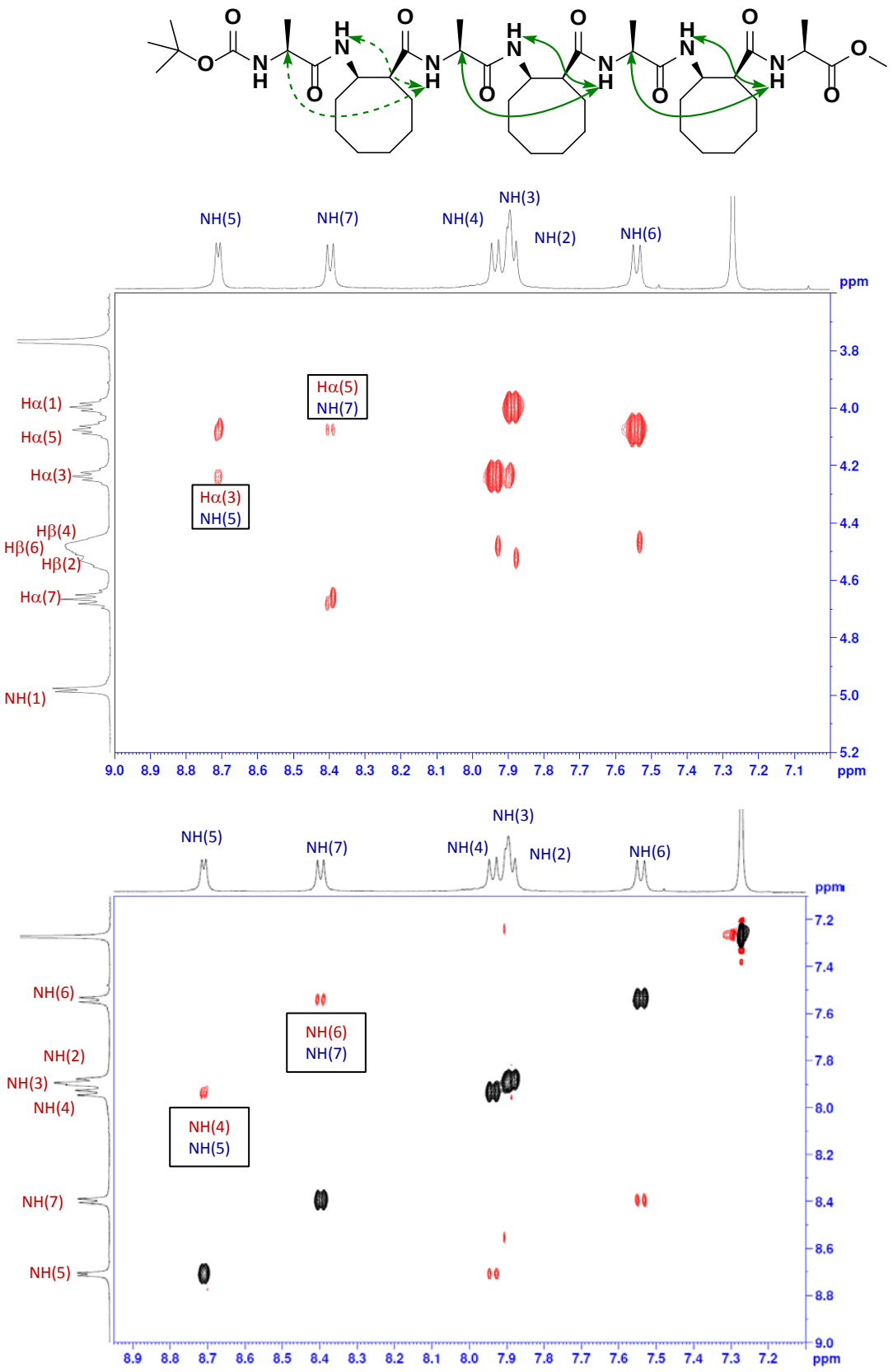


Figure S7: Characteristic NOEs observed for **9** in $CDCl_3$. Solid arrows indicate characteristic NOEs for the 11/9-helix. Dashed arrows indicate ambiguous NOEs that were not assigned because of overlap of crosspeaks.

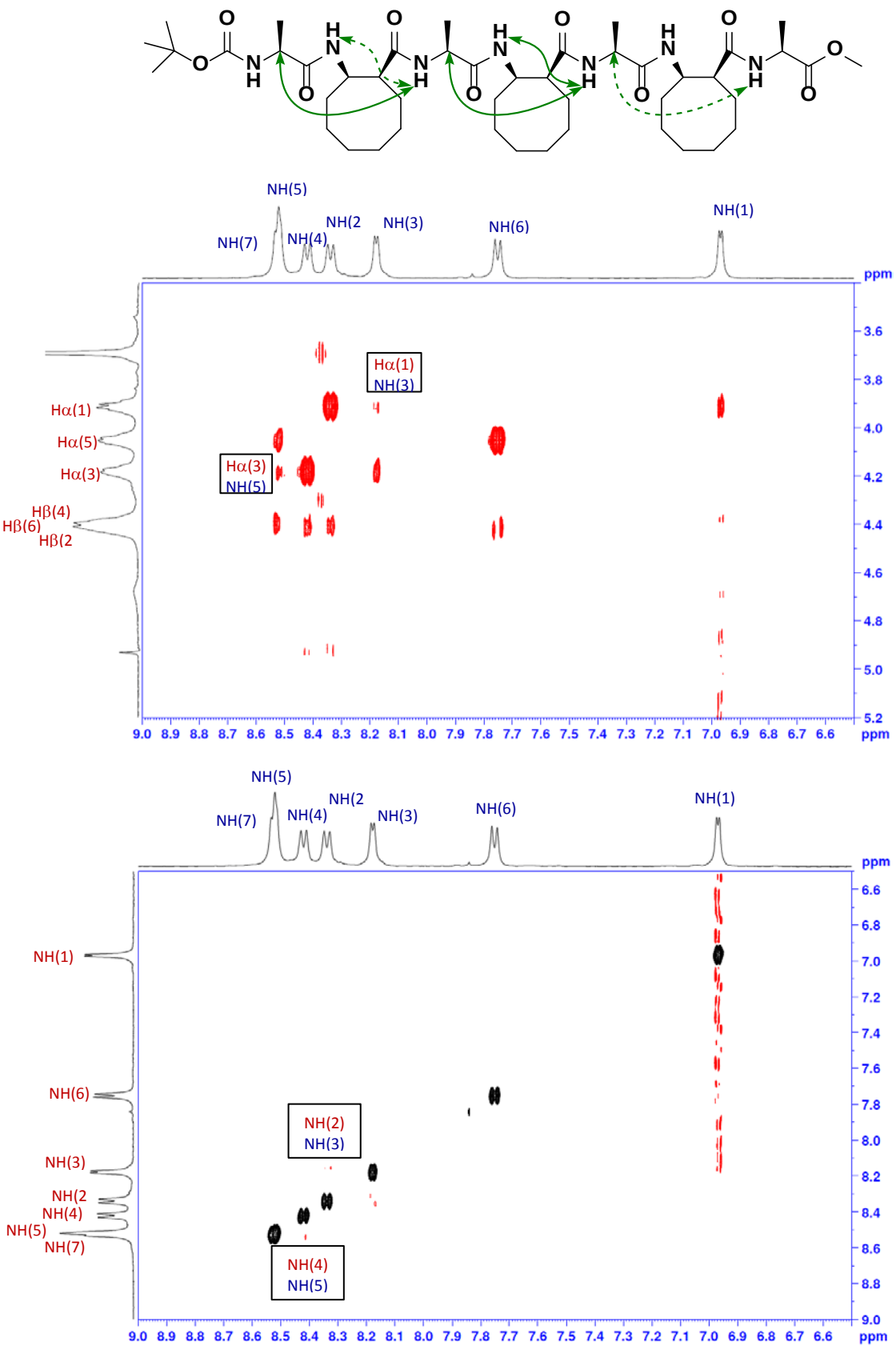


Figure S8: Characteristic NOEs observed for **6** in CD_3OH. Solid arrows indicate characteristic NOEs for the 11/9-helix. Dashed arrows indicate ambiguous NOEs that were not assigned because of overlap of crosspeaks.

X-ray Crystallographic Experiments

• α/β-Peptide trimer (4)

A specimen of suitable size and quality was coated with Paratone oil and mounted onto cryoloop. Reflection data were collected on a Bruker D8 Venture PHOTON 100 area detector diffractometer, with Mo Kα radiation (λ = 0.71073 Å). The full sphere of reflection data were collected as ω scan frames with 0.5°/frame and an exposure time of 10 s/frame. Cell parameters were determined and refined by APEX2 program.[S1] Data reduction was performed using SAINT software.[S2] The data were corrected for Lorentz and polarization effects. An empirical absorption correction was applied using the SADABS program.[S3] The structure was solved by direct methods and all nonhydrogen atoms were subjected to anisotropic refinement by full-matrix least-squares on F2 by using the SHELXTL/PC package.[S4] Hydrogen atoms were placed at their geometrically calculated positions and refined riding on the corresponding carbon atoms with isotropic thermal parameters.

Table S1. Crystal data and structural refinement for **4**.

Empirical formula	$C_{18}H_{28}F_3N_3O_6$
Formula weight	439.43
Temperature/K	138
Crystal system	monoclinic
Space group	C2
a/Å	17.5956(13)
b/Å	7.1768(4)
c/Å	18.6281(12)
α/°	90
β/°	114.020(4)
γ/°	90
Volume/Å^3	2148.7(2)
Z	4
ρ_{calc}mg/mm^3	1.358
m/mm^{-1}	0.118
F(000)	928.0
Crystal size/mm^3	0.1 × 0.1 × 0.1
2Θ range for data collection	5.07 to 54.18°
Index ranges	-22 ≤ h ≤ 22, -8 ≤ k ≤ 9, -23 ≤ l ≤ 23
Reflections collected	30981
Independent reflections	4578[R(int) = 0.0281]
Data/restraints/parameters	4578/1/282
Goodness-of-fit on F^2	1.086
Final R indexes [I>=2σ (I)]	R_1 = 0.0315, wR_2 = 0.0670
Final R indexes [all data]	R_1 = 0.0368, wR_2 = 0.0713
Largest diff. peak/hole / e Å^{-3}	0.16/-0.16

• **α/β-Peptide trimers (3a + *ent*-3a)**

Data Collection

A colorless crystal with approximate dimensions 0.276 x 0.234 x 0.116 mm^3 was selected under oil under ambient conditions and attached to the tip of a MiTeGen MicroMount©. The crystal was mounted in a stream of cold nitrogen at 100(1) K and centered in the X-ray beam by using a video camera. The crystal evaluation and data collection were performed on a Bruker SMART APEXII diffractometer with Cu K_α (λ = 1.54178 Å) radiation and the diffractometer to crystal distance of 4.03 cm. The initial cell constants were obtained from three series of ω scans at different starting angles. Each series consisted of 41 frames collected at intervals of 0.6º in a 25º range about ω with the exposure time of 3 seconds per frame. The reflections were successfully indexed by an automated indexing routine built in the APEXII program. The final cell constants were calculated from a set of 9940 strong reflections from the actual data collection. The data were collected by using the full sphere data collection routine to survey the reciprocal space to the extent of a full sphere to a resolution of 0.82 Å. A total of 22130 data were harvested by collecting 19 sets of frames with 0.6º scans in ω and φ with an exposure time 5-10 sec per frame. These highly redundant datasets were corrected for Lorentz and polarization effects. The absorption correction was based on fitting a function to the empirical transmission surface as sampled by multiple equivalent measurements.[S1]

Structure Solution and Refinement

The systematic absences in the diffraction data were uniquely consistent for the space group $P2_1/c$ that yielded chemically reasonable and computationally stable results of refinement.[S5] A successful solution by the direct methods provided most non-hydrogen atoms from the *E*-map. The remaining non-hydrogen atoms were located in an alternating series of least-squares cycles and difference Fourier maps. All non-hydrogen atoms were refined with anisotropic displacement coefficients. All hydrogen atoms were included in the structure factor calculation at idealized positions and were allowed to ride on the neighboring atoms with relative isotropic displacement coefficients. There is also one molecule of solvent chloroform in the asymmetric unit. There are four chiral centers in the molecule: C6(S), C9(R), C16(S), C18(S), but both stereoisomers are present in the lattice. The final least-squares refinement of 325 parameters against 5154 data resulted in residuals *R* (based on F^2 for $I \geq 2\sigma$) and *wR* (based on F^2 for all data) of 0.0367 and 0.0947, respectively.

Table S2. Crystal data and structural refinement for a racemic mixture, **3a** + ***ent*-3a**.

Empirical formula	$C_{22}H_{36}Cl_3N_3O_6$
Formula weight	544.89
Temperature/K	99.98
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	9.2941(10)
b/Å	14.081(3)
c/Å	21.205(3)
α/°	90
β/°	96.268(6)
γ/°	90
Volume/Å^3	2758.5(8)
Z	4
ρ_{calc}mg/mm^3	1.312
m/mm^{-1}	3.344
F(000)	1152.0
Crystal size/mm^3	0.276 × 0.234 × 0.116
2Θ range for data collection	7.55 to 139.55°
Index ranges	$-11 \le h \le 11$, $-16 \le k \le 14$, $-25 \le l \le 25$
Reflections collected	43289
Independent reflections	5154[R(int) = 0.0277]
Data/restraints/parameters	5154/3/325
Goodness-of-fit on F^2	1.037
Final R indexes [I>=2σ (I)]	R_1 = 0.0367, wR_2 = 0.0920
Final R indexes [all data]	R_1 = 0.0405, wR_2 = 0.0947
Largest diff. peak/hole / e Å^{-3}	0.83/-0.68

• α/β-Peptide trimers (7 + *ent*-7)

A specimen of suitable size and quality was coated with Paratone oil and mounted onto cryoloop. Reflection data were collected on a Bruker D8 Venture PHOTON 100 area detector diffractometer, with Mo Kα radiation (λ = 0.71073 Å). The full sphere of reflection data were collected as ω scan frames with 0.5°/frame and an exposure time of 10 s/frame. Cell parameters were determined and refined by APEX2 program.[S1] Data reduction was performed using SAINT software.[S2] The data were corrected for Lorentz and polarization effects. An empirical absorption correction was applied using the SADABS program.[S3] The structure was solved by direct methods and all nonhydrogen atoms were subjected to anisotropic refinement by full-matrix least-squares on F2 by using the SHELXTL/PC package.[S4] Hydrogen atoms were placed at their geometrically calculated positions and refined riding on the corresponding carbon atoms with isotropic thermal parameters.

Table S3. Crystal data and structural refinement for a racemic mixture, **7** + ***ent*-7**.

Empirical formula	$C_{22}H_{38}Cl_3N_3O_6$
Formula weight	546.90
Temperature/K	150(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	9.5371(7)
b/Å	14.1962(10)
c/Å	21.2896(15)
α/°	90
β/°	97.626(2)
γ/°	90
Volume/Å^3	2856.9(4)
Z	4
ρ_{calc}mg/mm^3	1.271
m/mm^{-1}	0.359
F(000)	1160.0
Crystal size/mm^3	0.1 × 0.1 × 0.1
2Θ range for data collection	3.86 to 52.19°
Index ranges	$-11 \le h \le 11$, $-17 \le k \le 17$, $-26 \le l \le 26$
Reflections collected	46007
Independent reflections	5661[R(int) = 0.0611]
Data/restraints/parameters	5661/52/356
Goodness-of-fit on F^2	1.024
Final R indexes [I>=2σ (I)]	R_1 = 0.0527, wR_2 = 0.129
Final R indexes [all data]	R_1 = 0.0827, wR_2 = 0.1473
Largest diff. peak/hole / e Å^{-3}	0.65/-0.53

References

[S1] APEX2, version 2012.2-0, Data collection software, Bruker AXS, Inc., Madison, WI, 2011.

[S2] SAINT, version 6.0, Data integration software, Bruker AXS Inc., Madison, WI, 2011.

[S3] G. M. Sheldrick, version 2.05 SADABS, Program for absorption correction with the Bruker SMART system, Universitat Gottingen, Germany, 2011.

[S4] G. M. Sheldrick, SHELXL-93: Program for the refinement of crystal structures; Universitat Gottingen: Germany, 2004.

[S5] Sheldrick, G. M. (2008) SHELXL. *Acta Cryst.* **A64**, 112-122.

Copies of NMR spectra

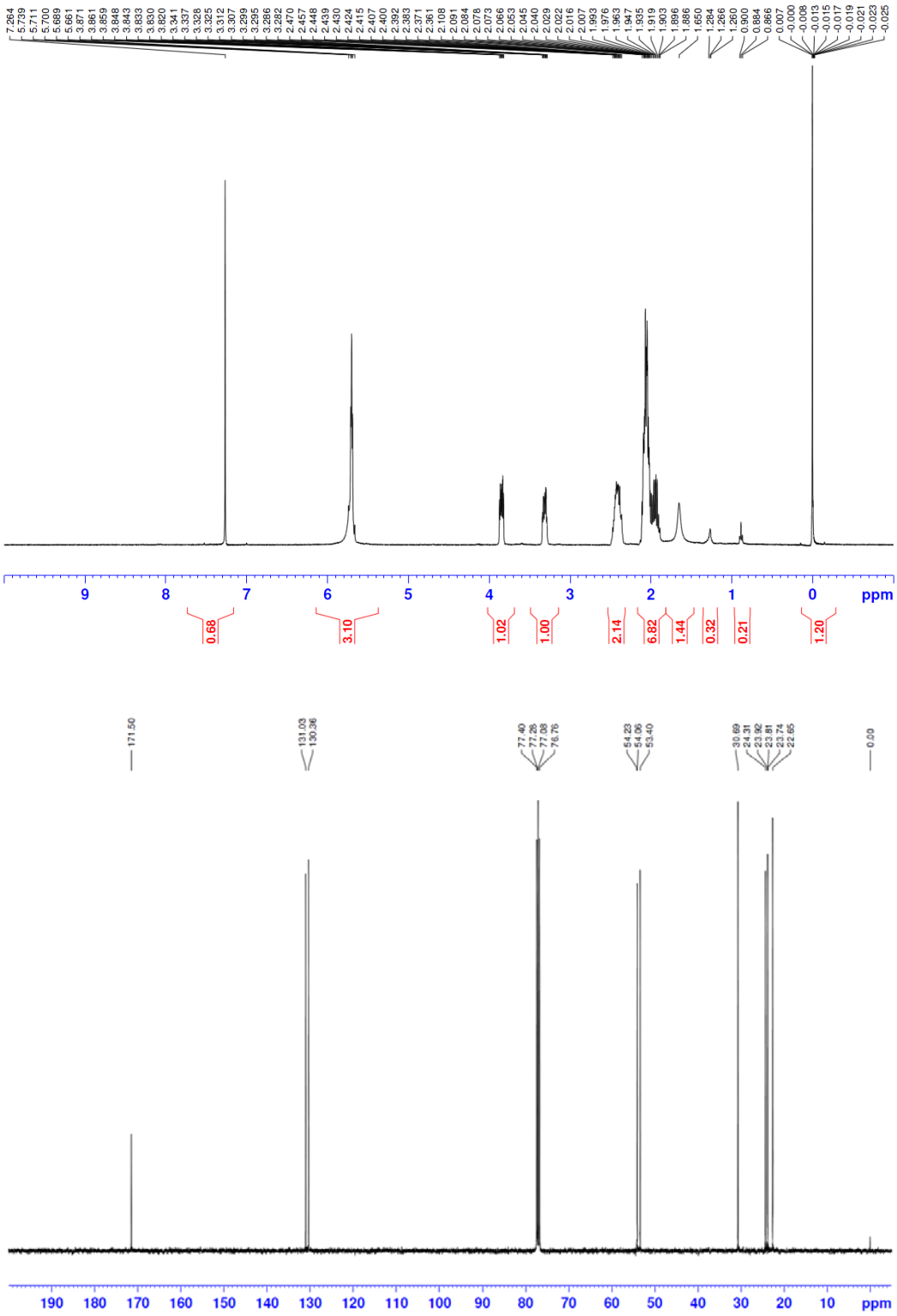


Figure S9. H-NMR and C-NMR spectra for **11** in $CDCl_3$.

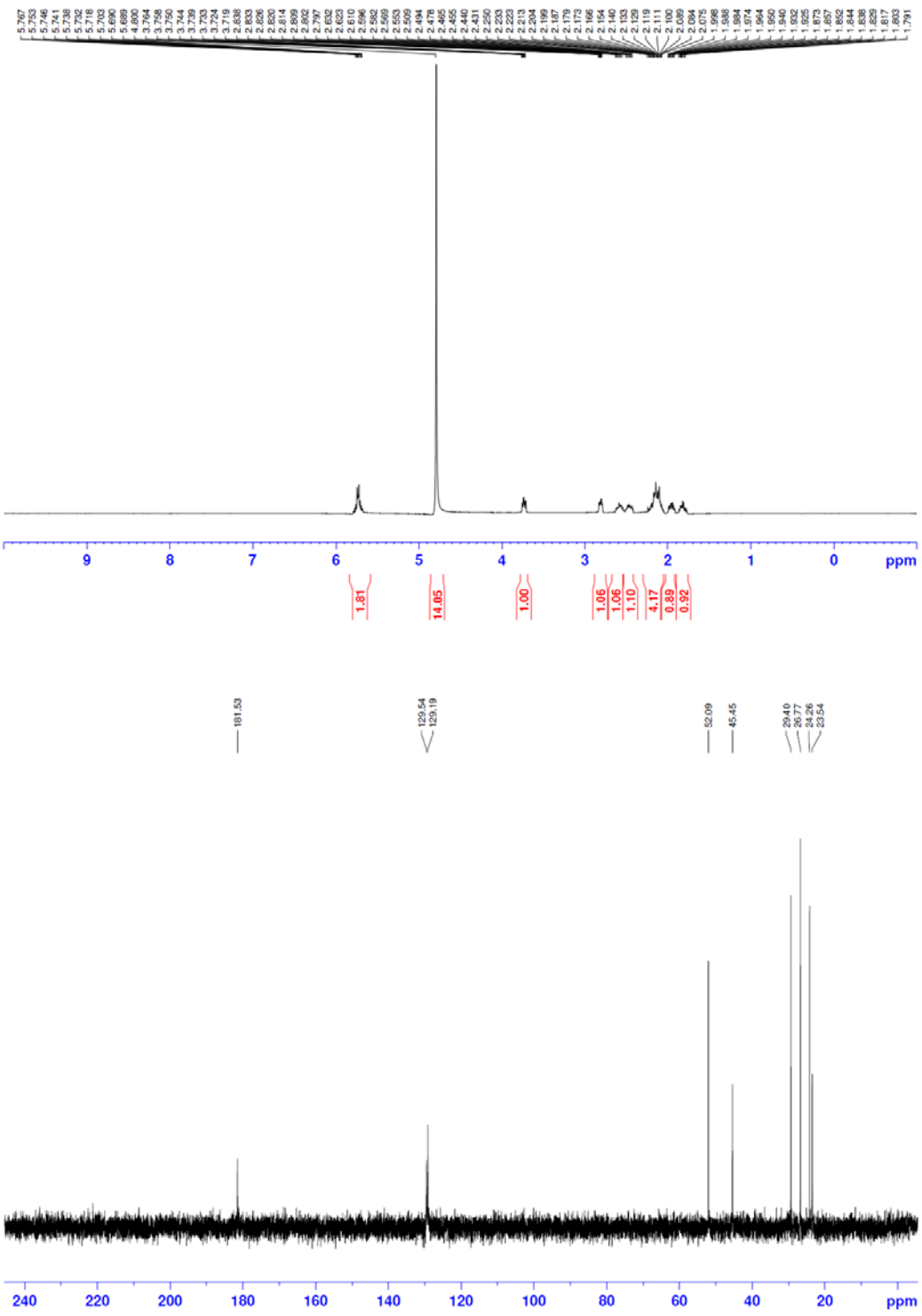


Figure S10. H-NMR and C-NMR spectra for **12** in CD_3OD.

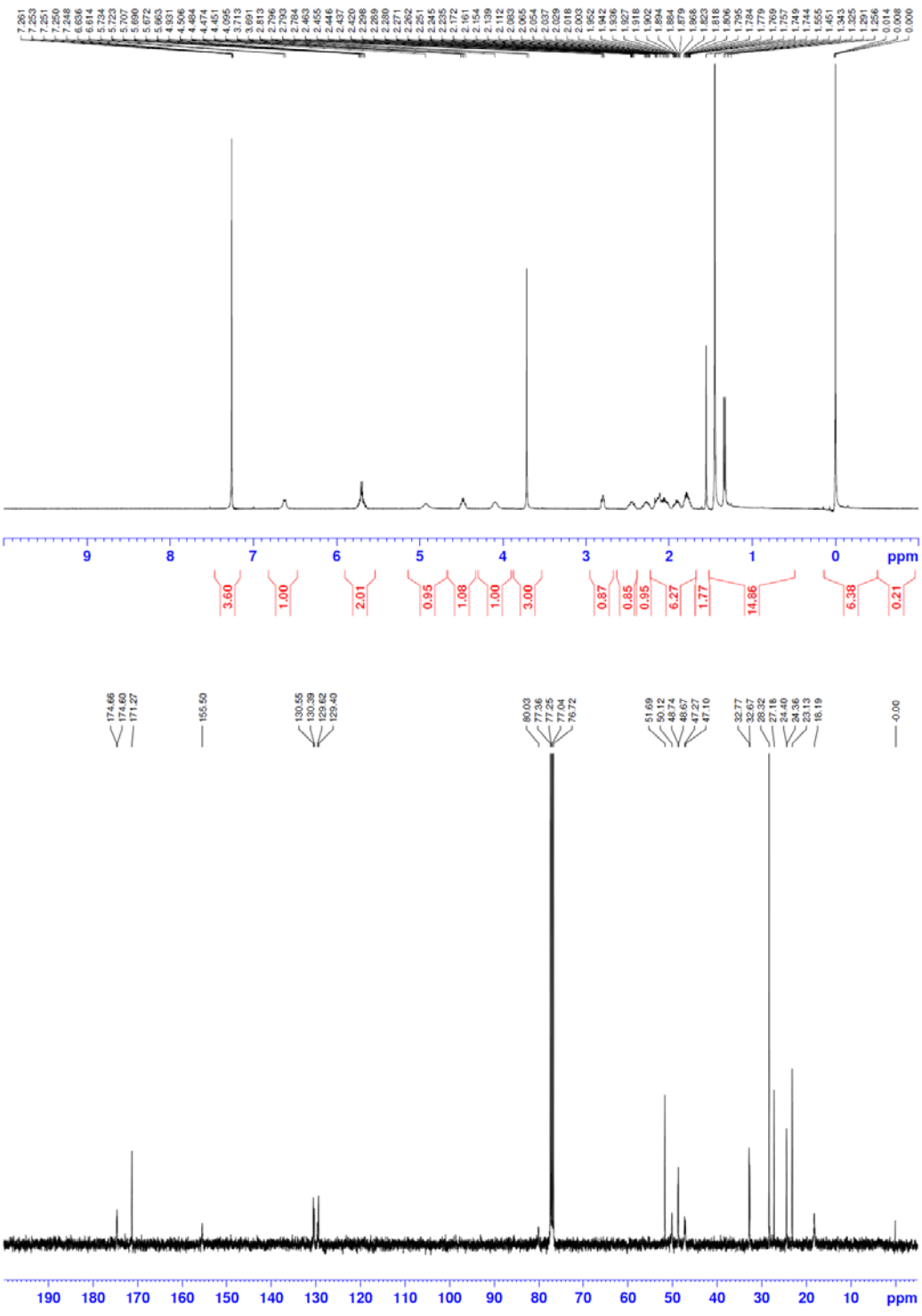


Figure S11. H-NMR and C-NMR spectra for **2a** in $CDCl_3$.

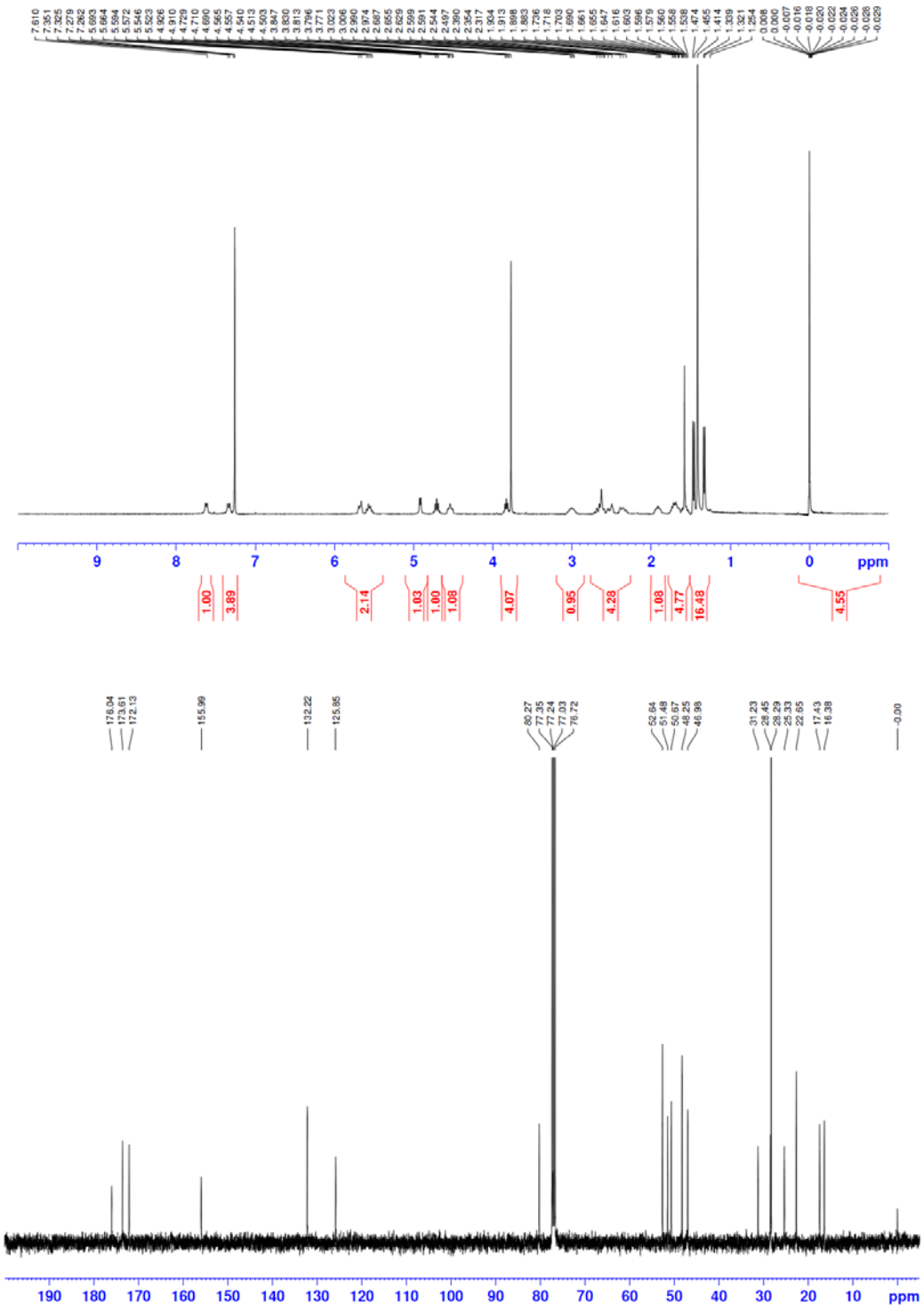


Figure S12. H-NMR and C-NMR spectra for **3a** in $CDCl_3$.

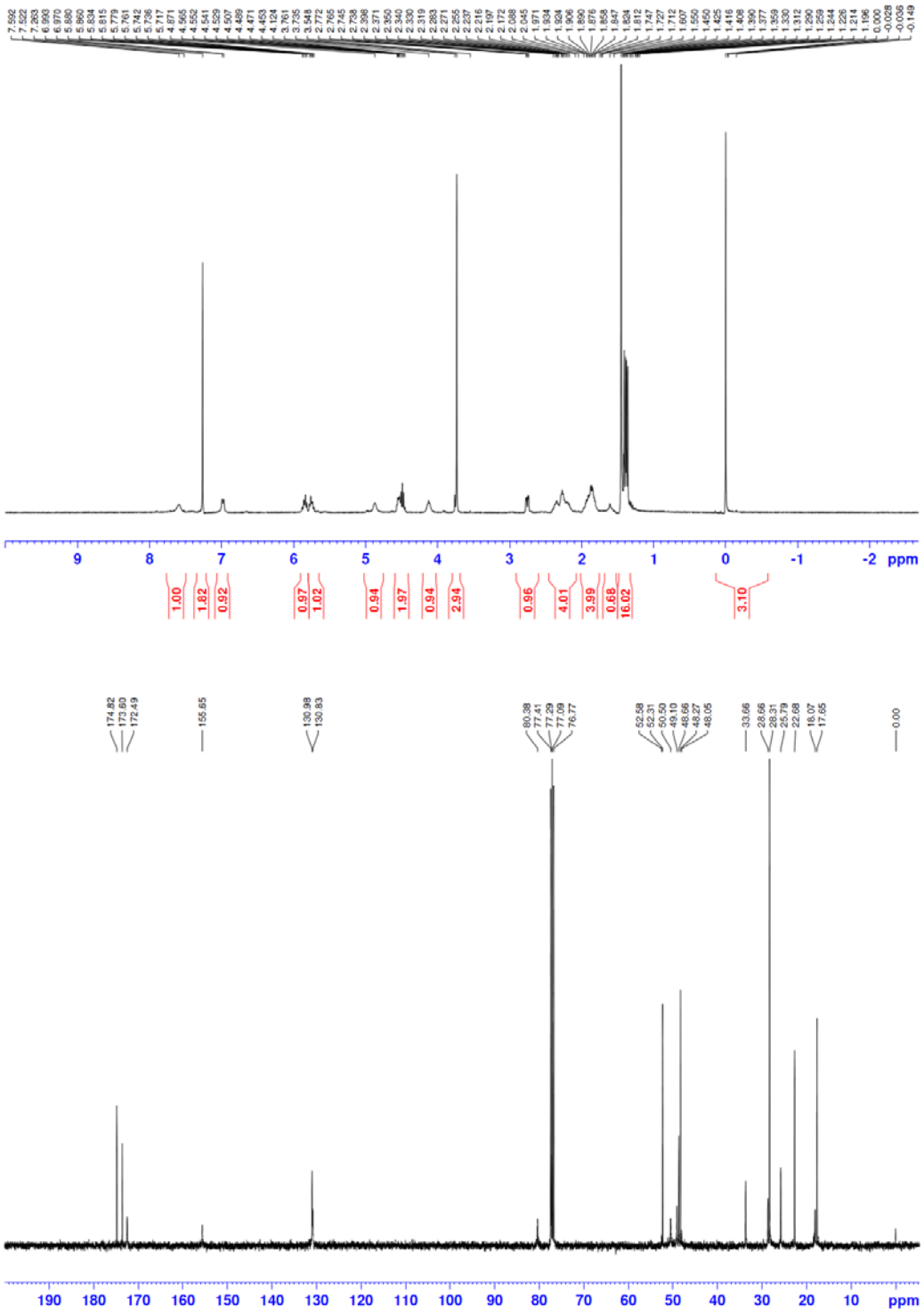


Figure S12. H-NMR and C-NMR spectra for **3b** in $CDCl_3$.

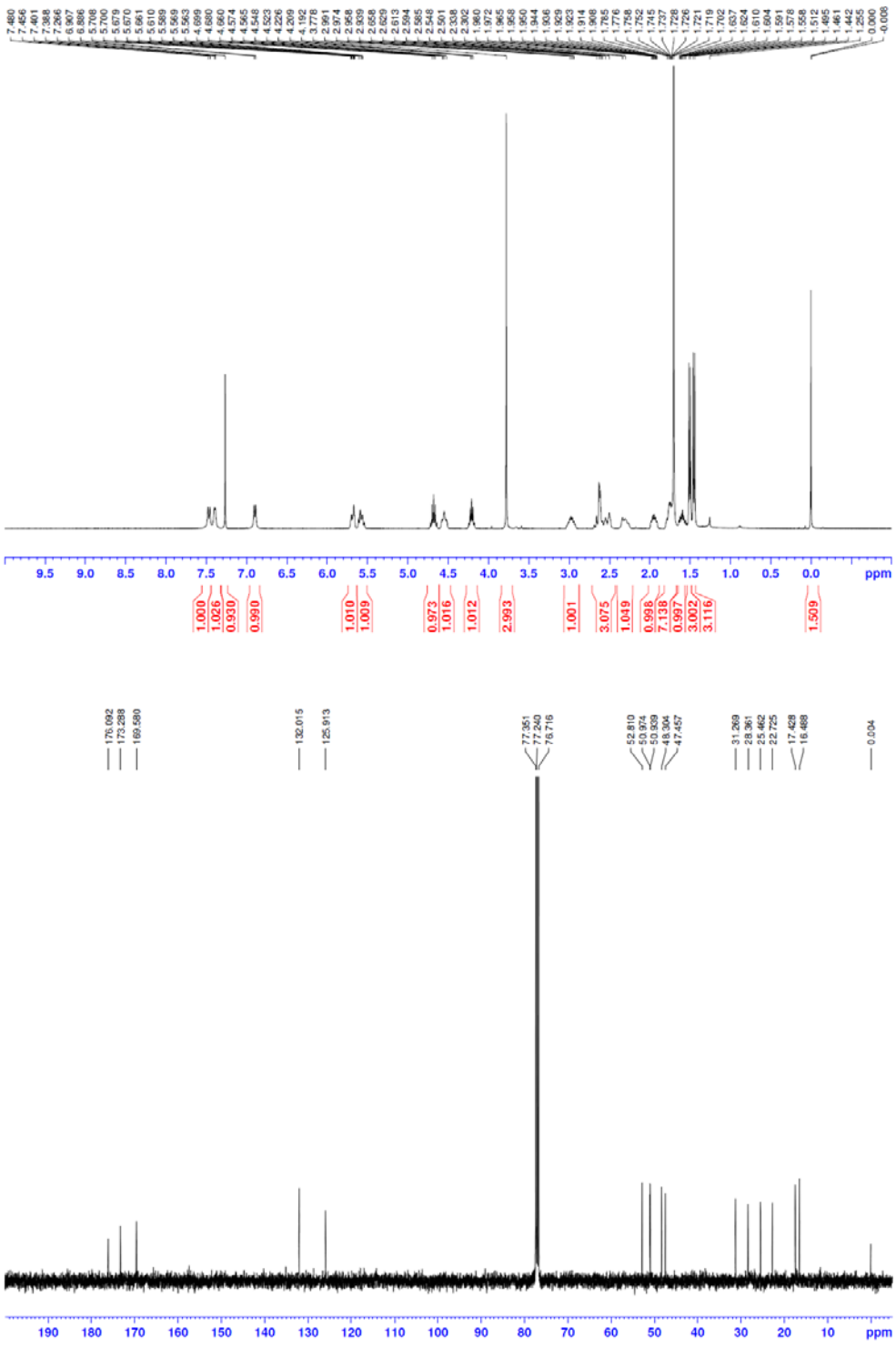


Figure S13. H-NMR and C-NMR spectra for **4** in $CDCl_3$.

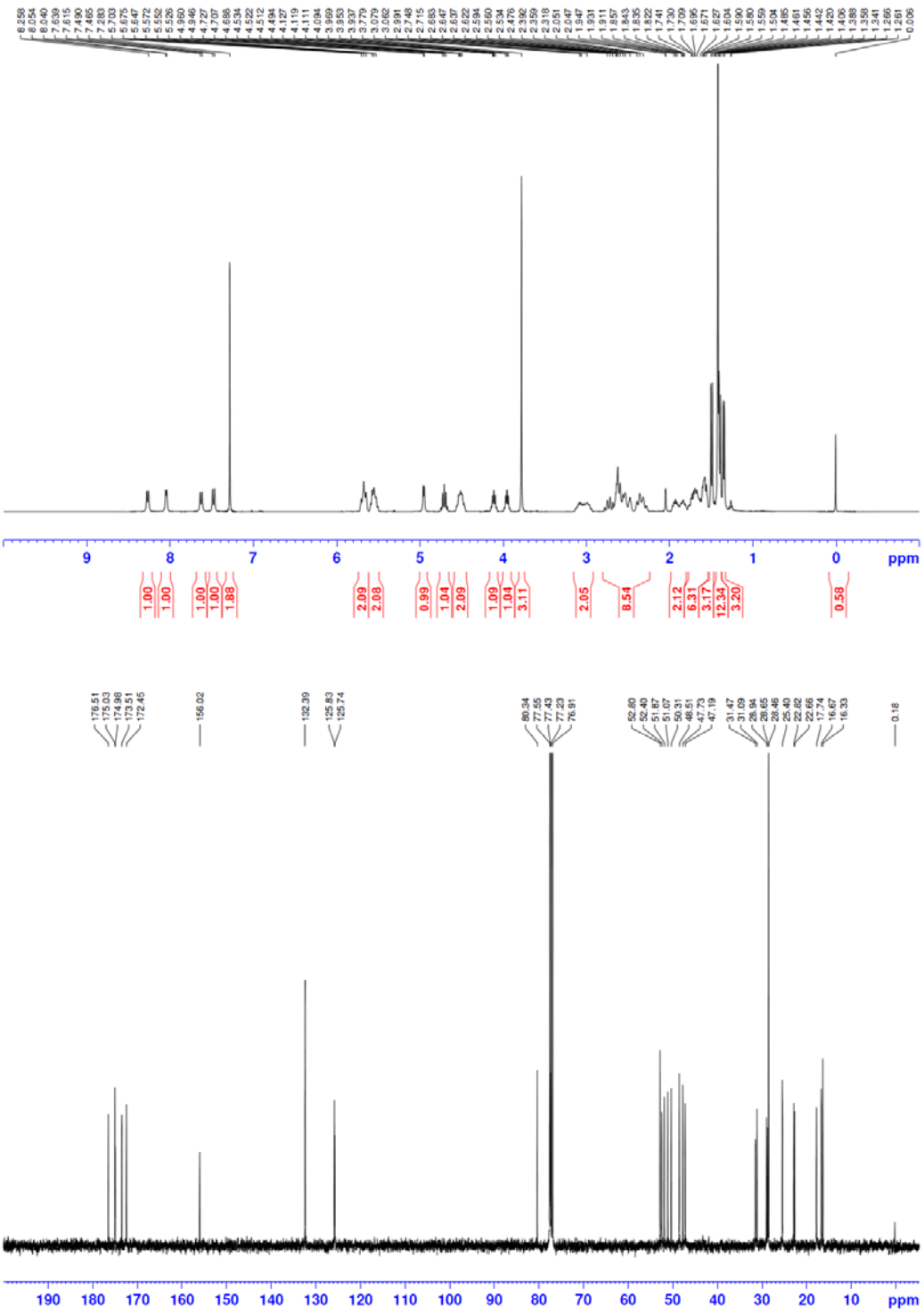


Figure S14. H-NMR and C-NMR spectra for **5** in $CDCl_3$.

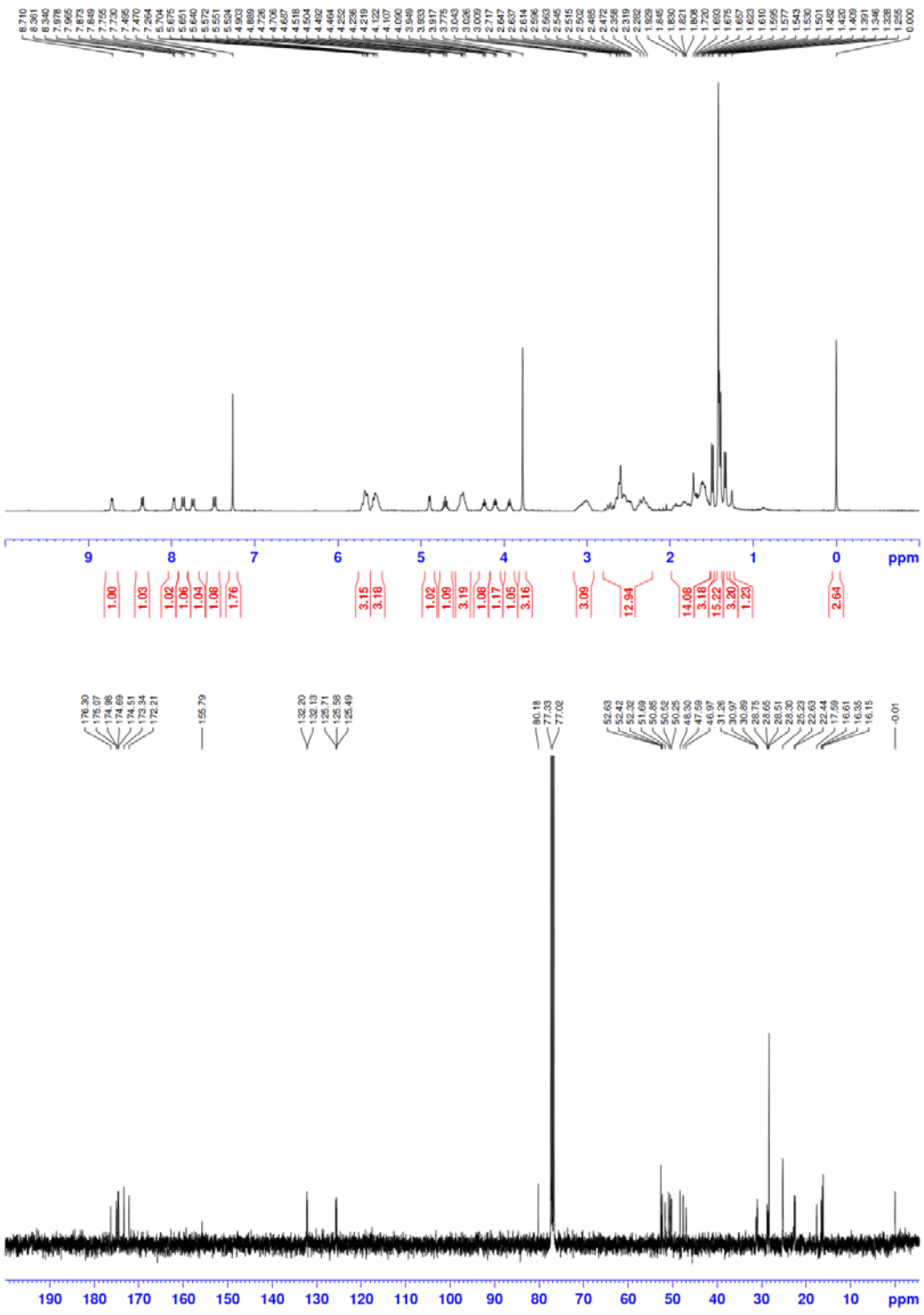


Figure S15. H-NMR and C-NMR spectra for **6** in $CDCl_3$.

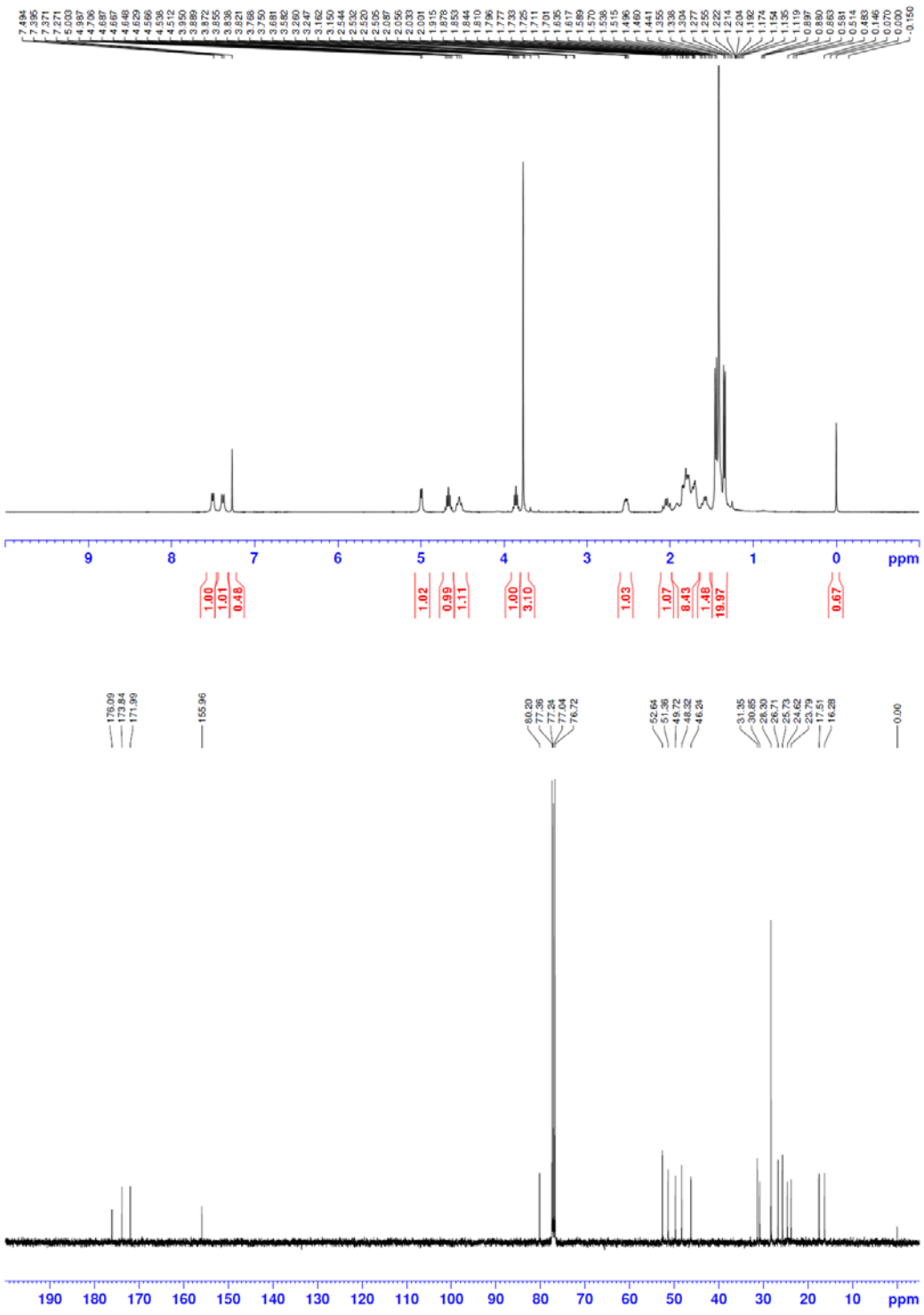


Figure S16. H-NMR and C-NMR spectra for **7** in $CDCl_3$.

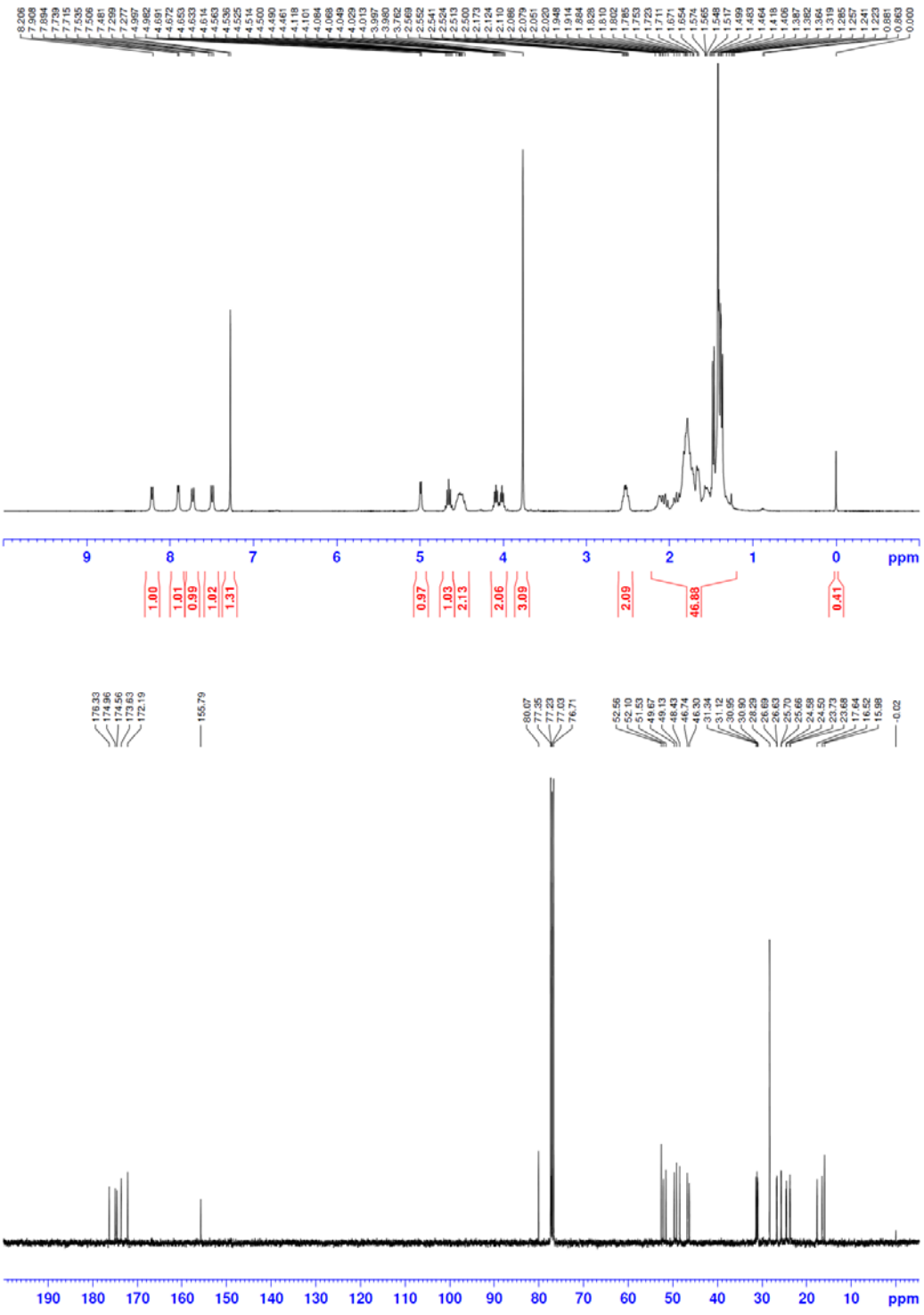


Figure S17. H-NMR and C-NMR spectra for **8** in $CDCl_3$.

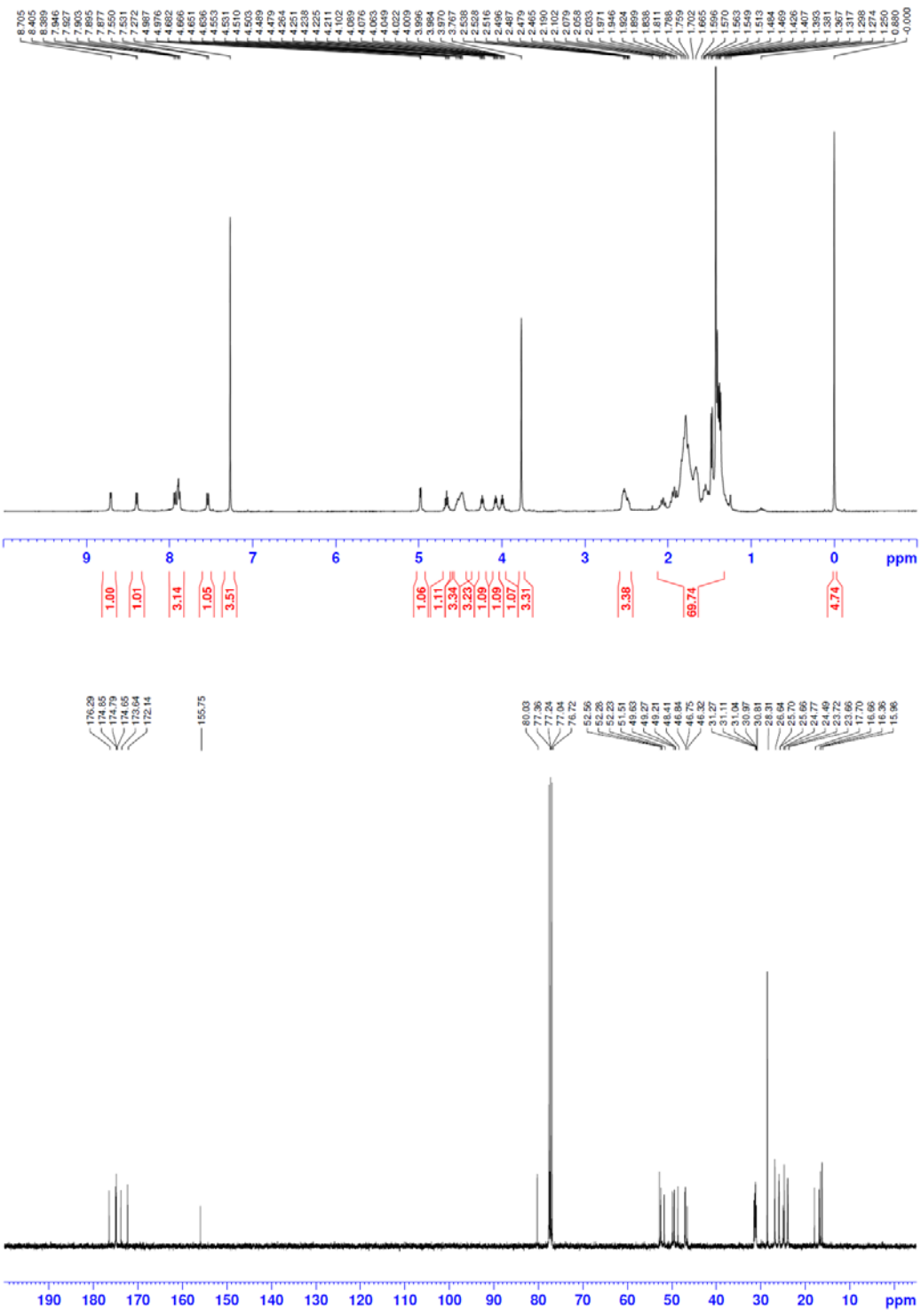


Figure S18. H-NMR and C-NMR spectra for **9** in $CDCl_3$.

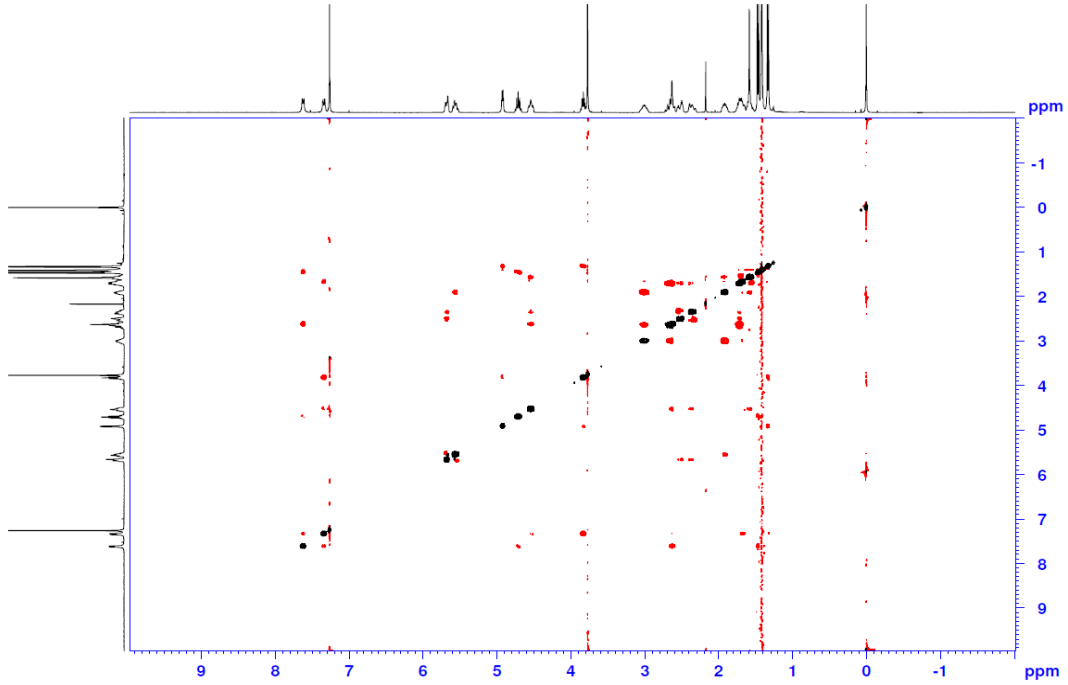


Figure S19. NOESY spectrum for **3a** in $CDCl_3$.

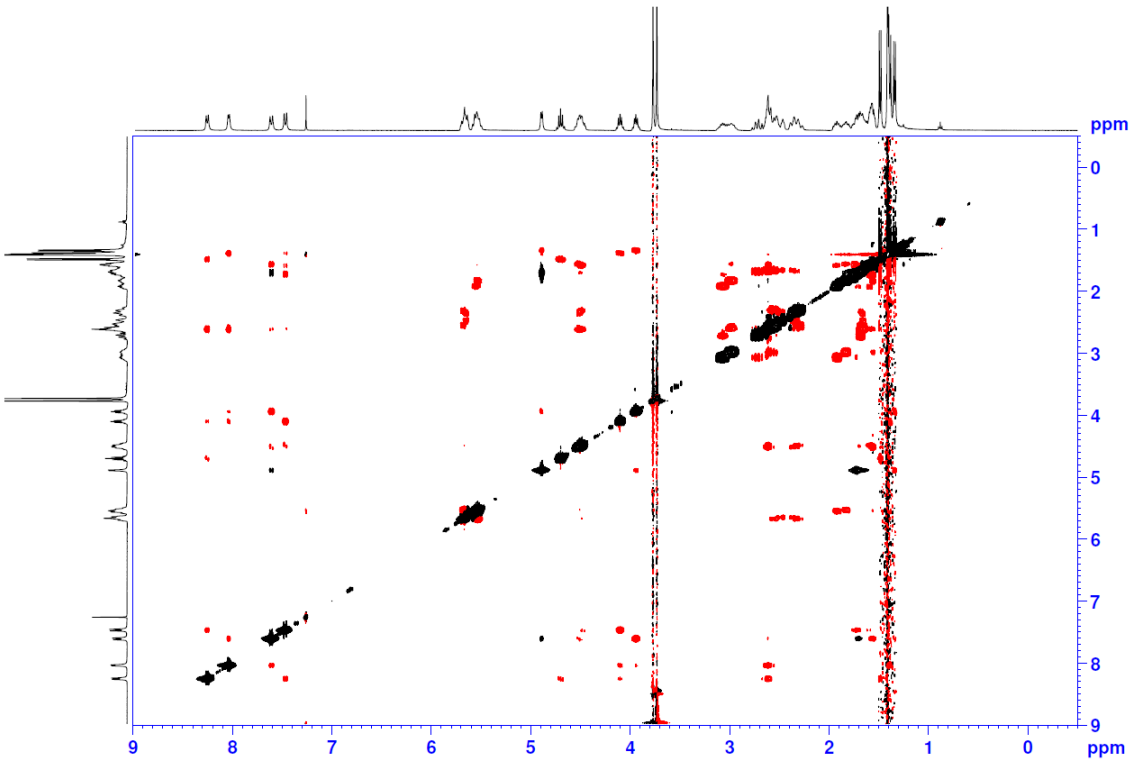


Figure S20. NOESY spectrum for **5** in $CDCl_3$.

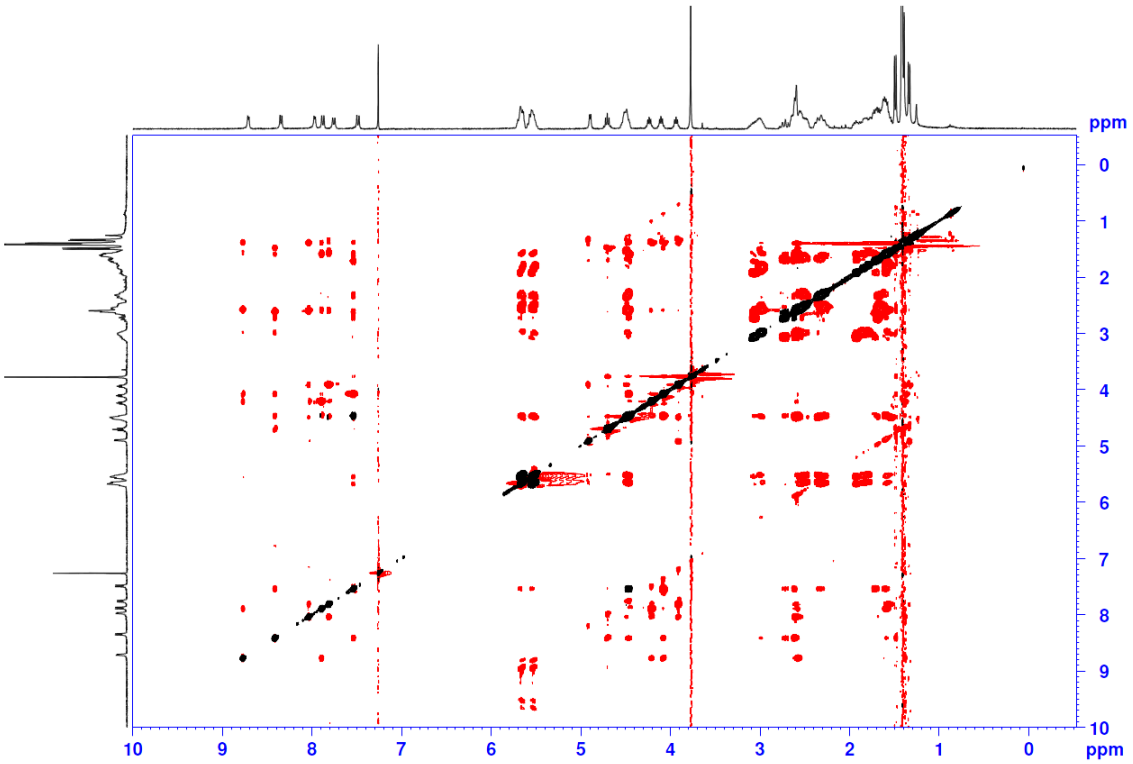


Figure S21. ROESY spectrum for **6** in $CDCl_3$.

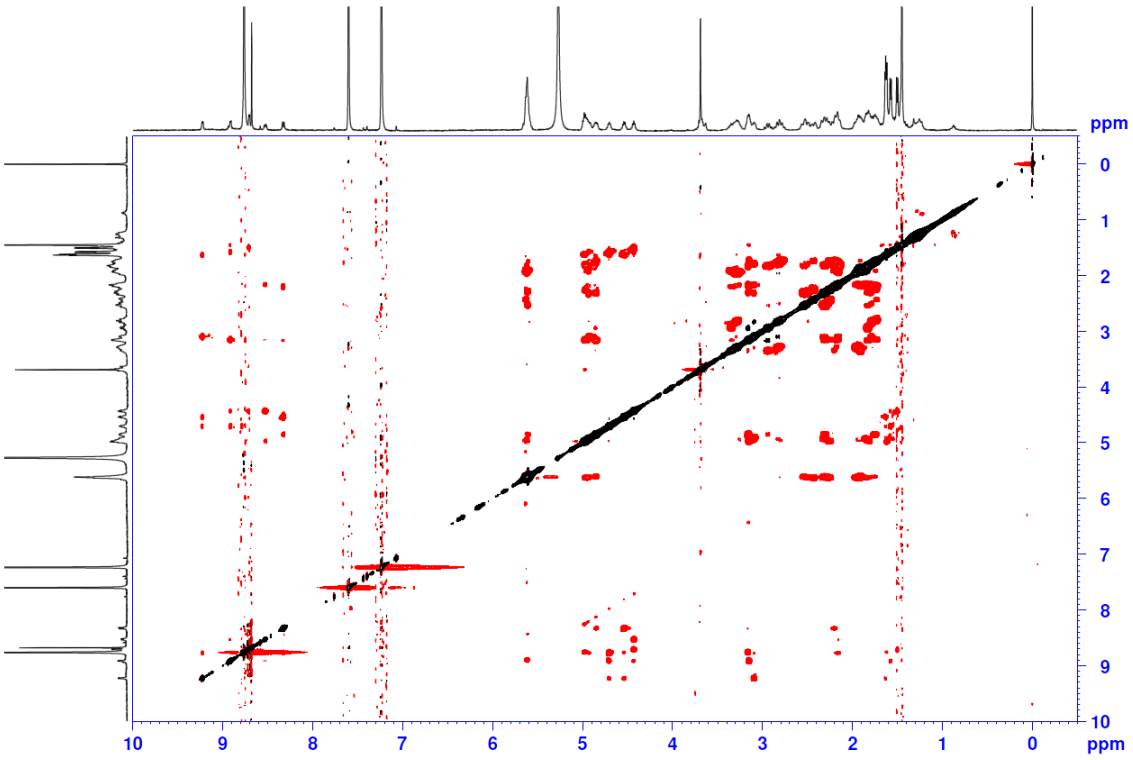


Figure S22. ROESY spectrum for **6** in pyridine-d_5.

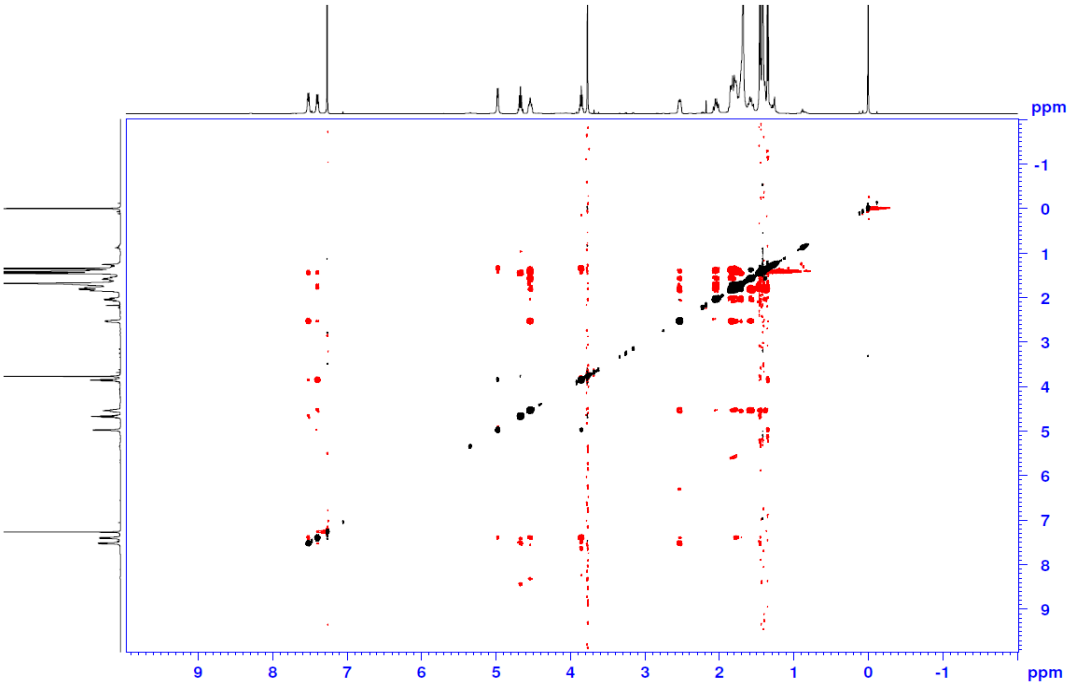


Figure S23. ROESY spectrum for **7** in $CDCl_3$.

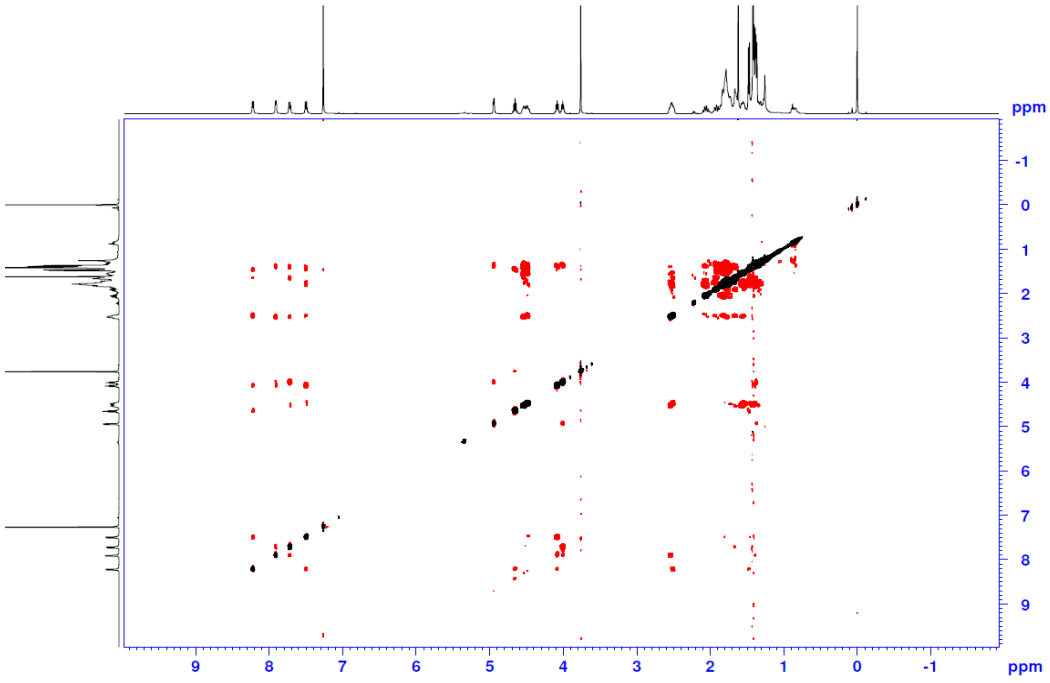


Figure S24. ROESY spectrum for **8** in $CDCl_3$.

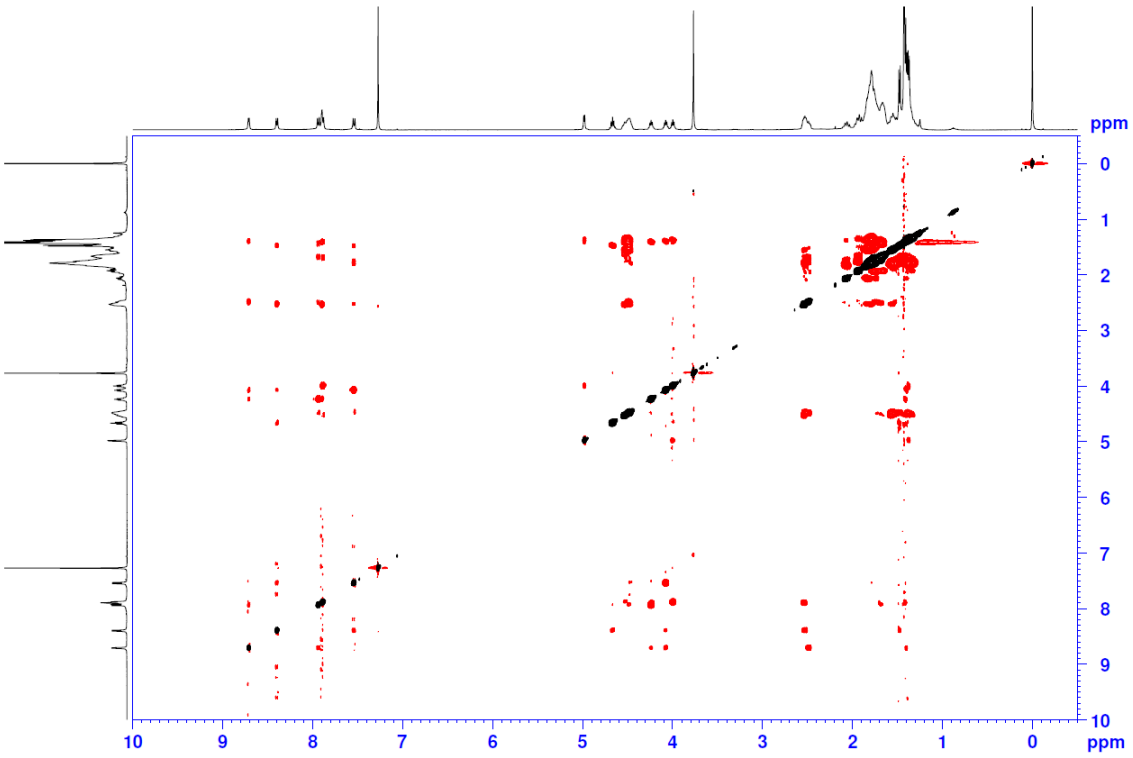


Figure S25. ROESY spectrum for **9** in $CDCl_3$.

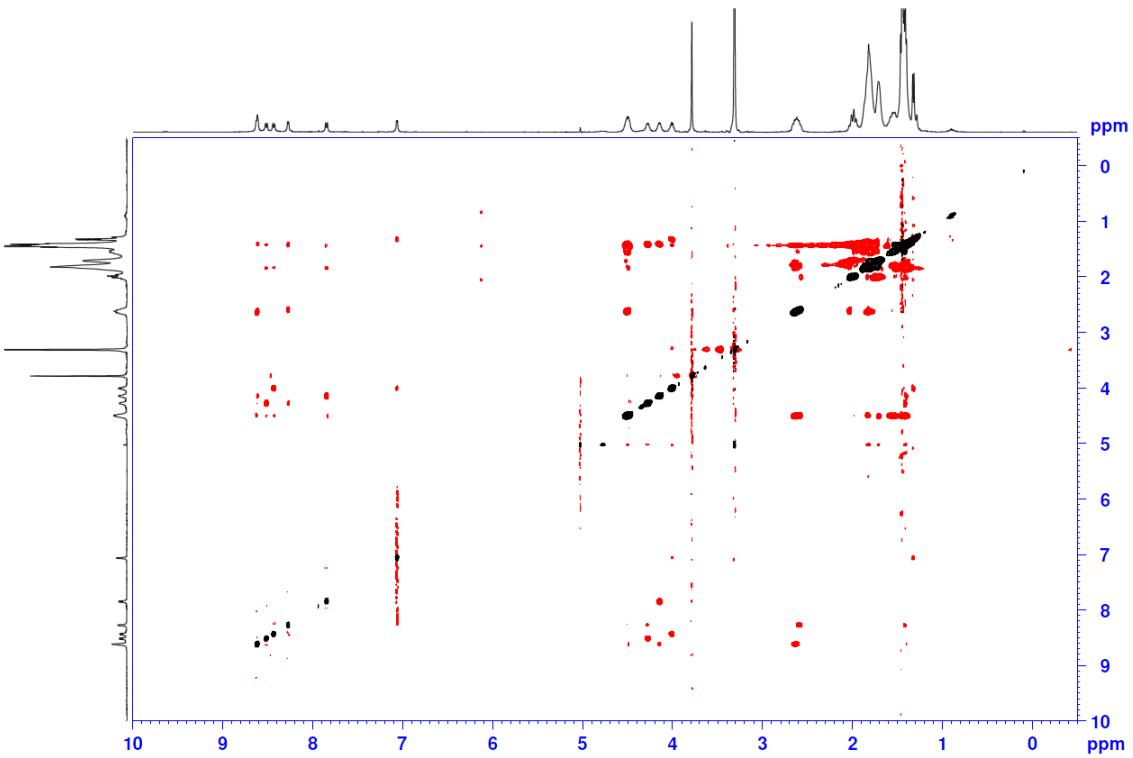


Figure S26. ROESY spectrum for **9** in pyridine-d_5.

Copies of HRMS data

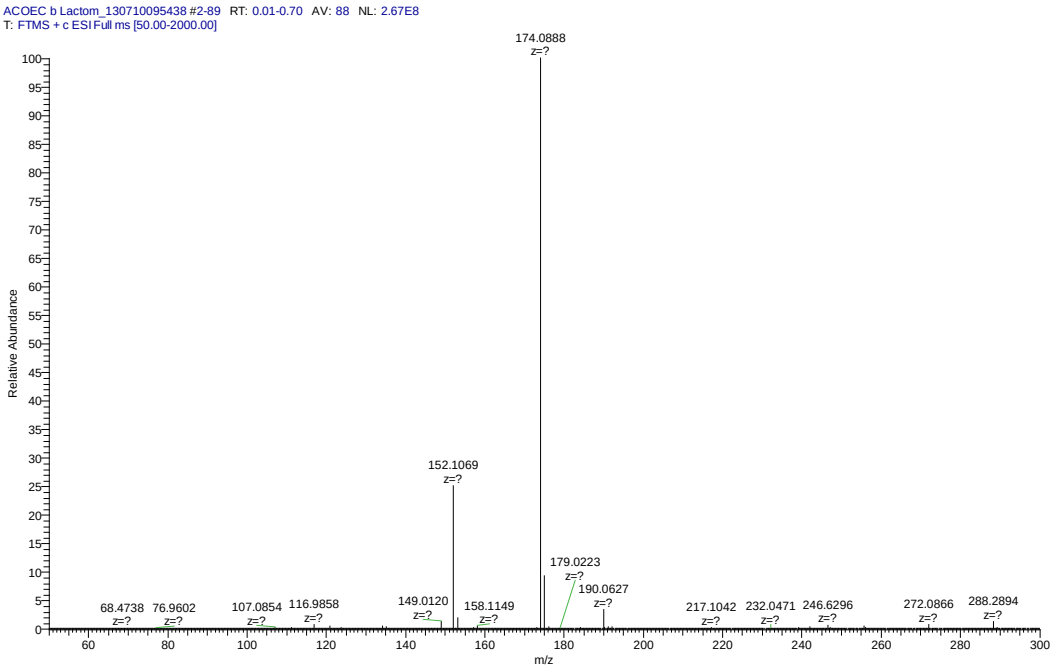


Figure S27. HRMS data for **11**.

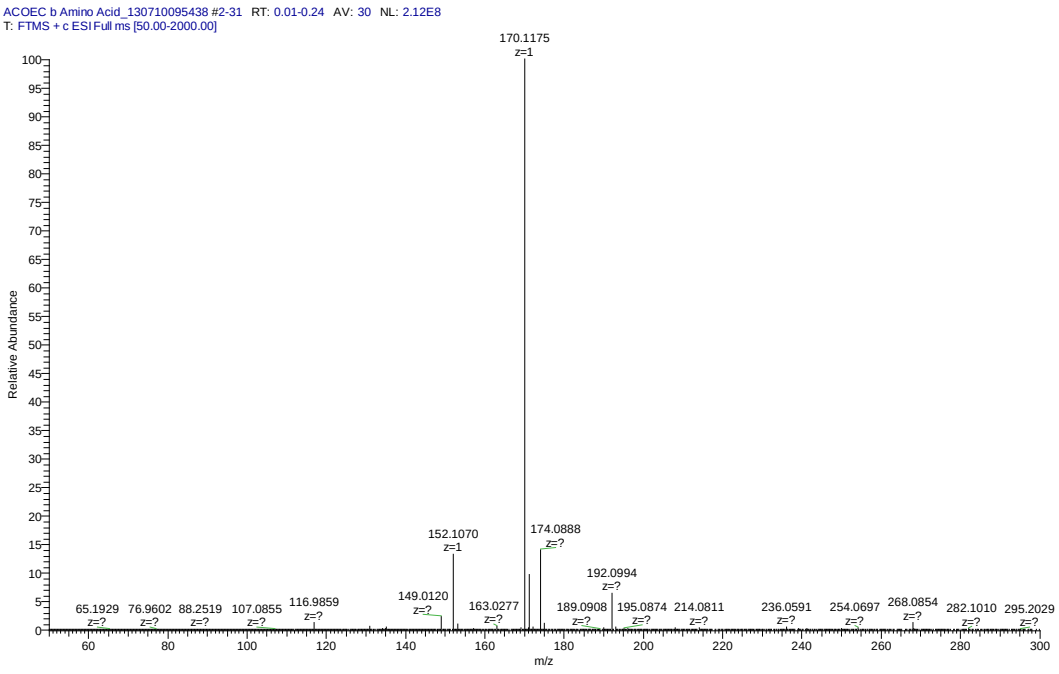


Figure S28. HRMS data for **12**.

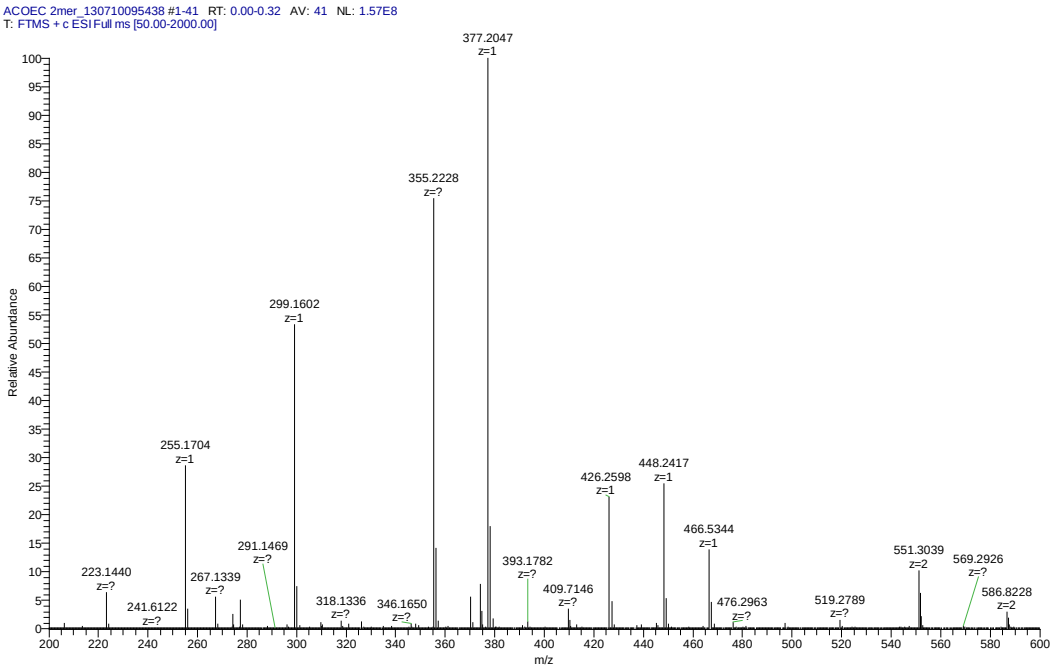


Figure S29. HRMS data for **2a**.

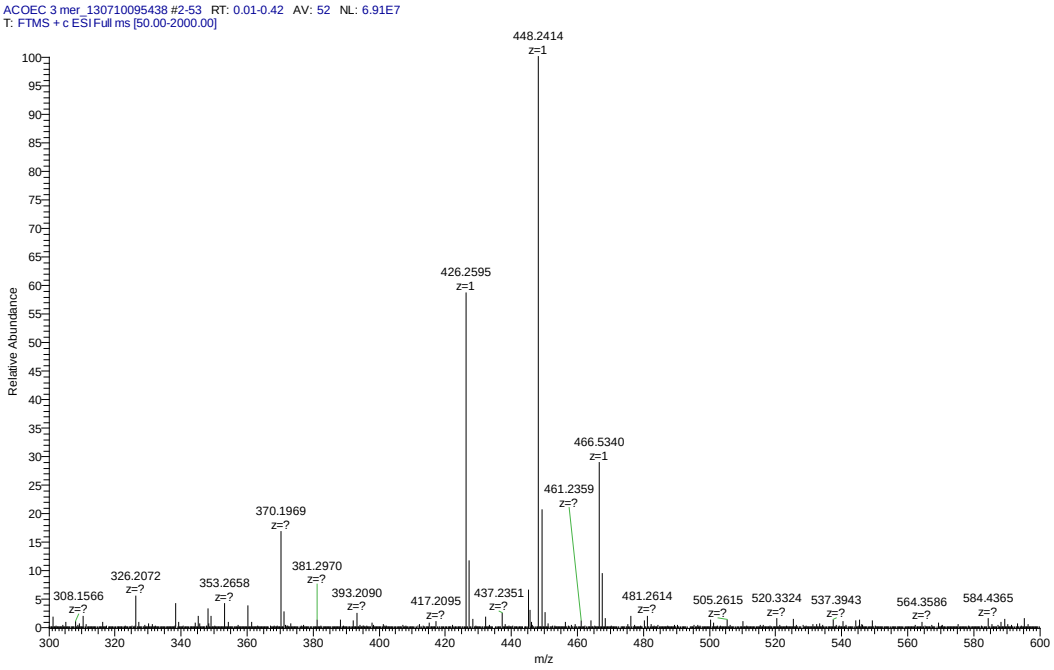


Figure S30. HRMS data for **3a**.

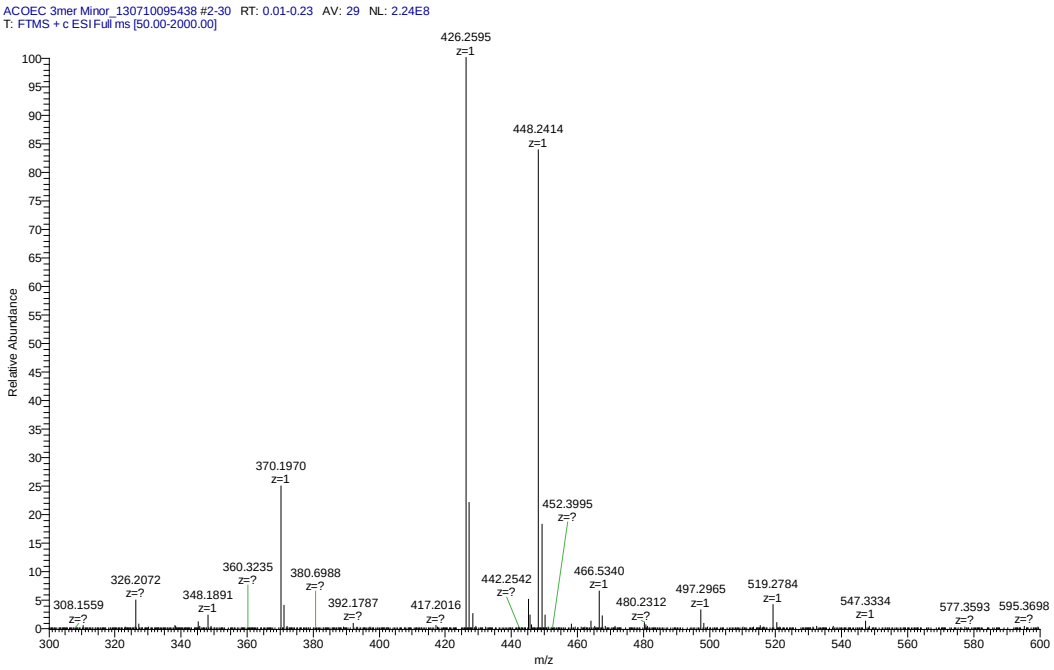


Figure S31. HRMS data for **3b**.

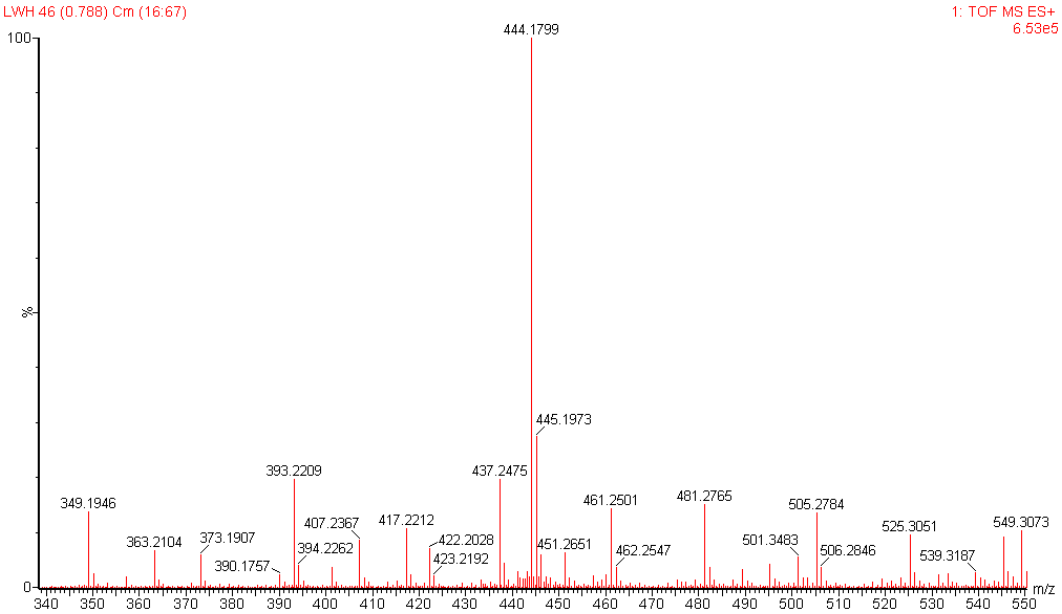


Figure S32. HRMS data for **4**.

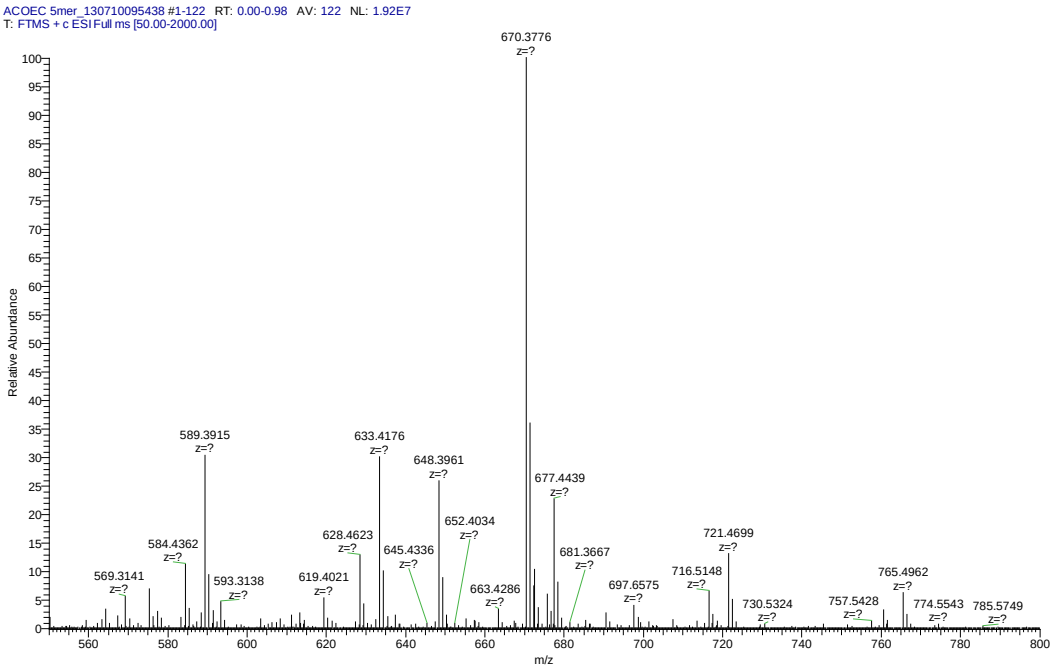


Figure S33. HRMS data for **5**.

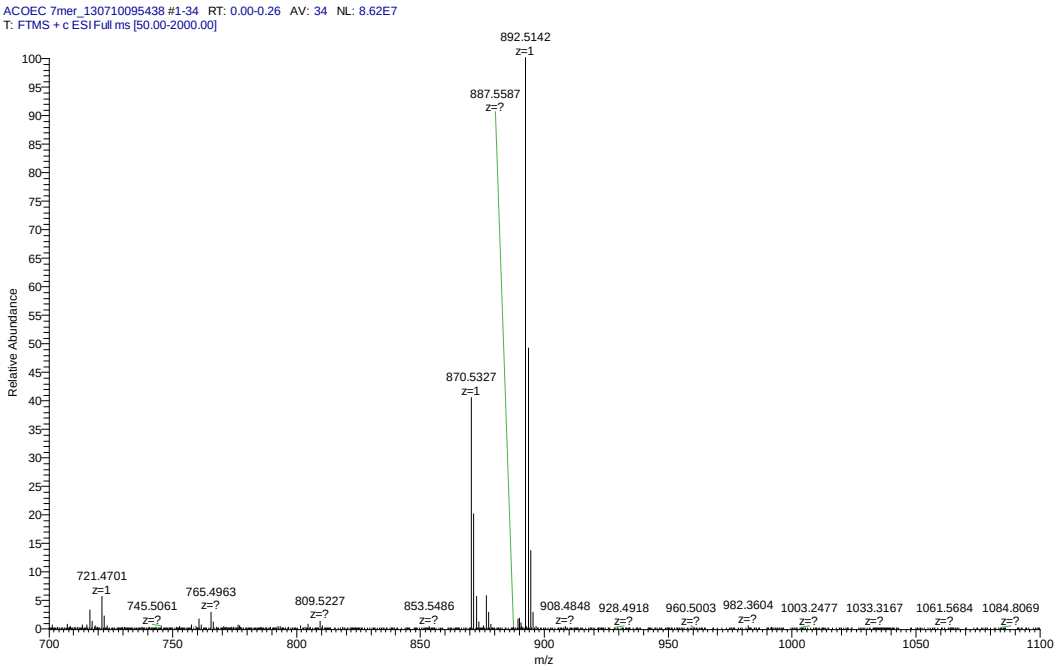


Figure S34. HRMS data for **6**.

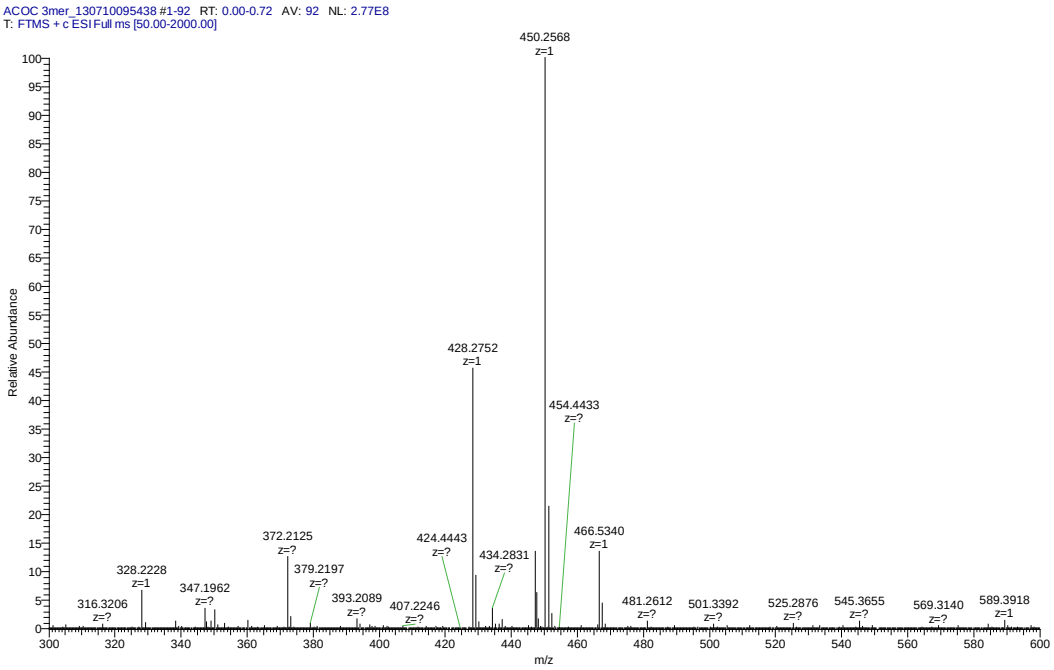


Figure S35. HRMS data for **7**.

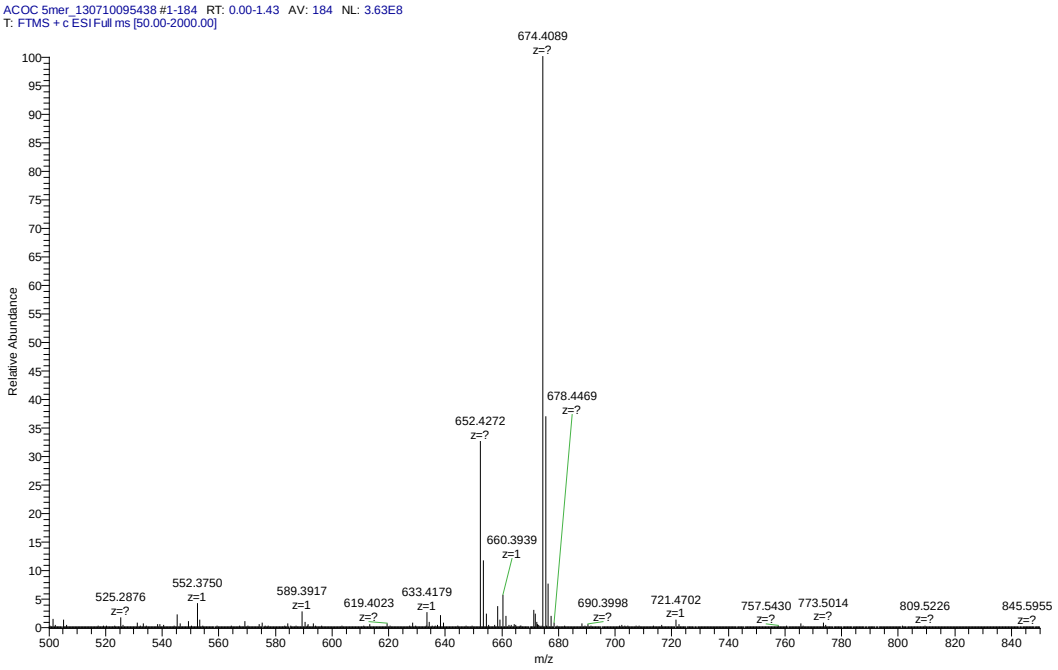


Figure S36. HRMS data for **8**.

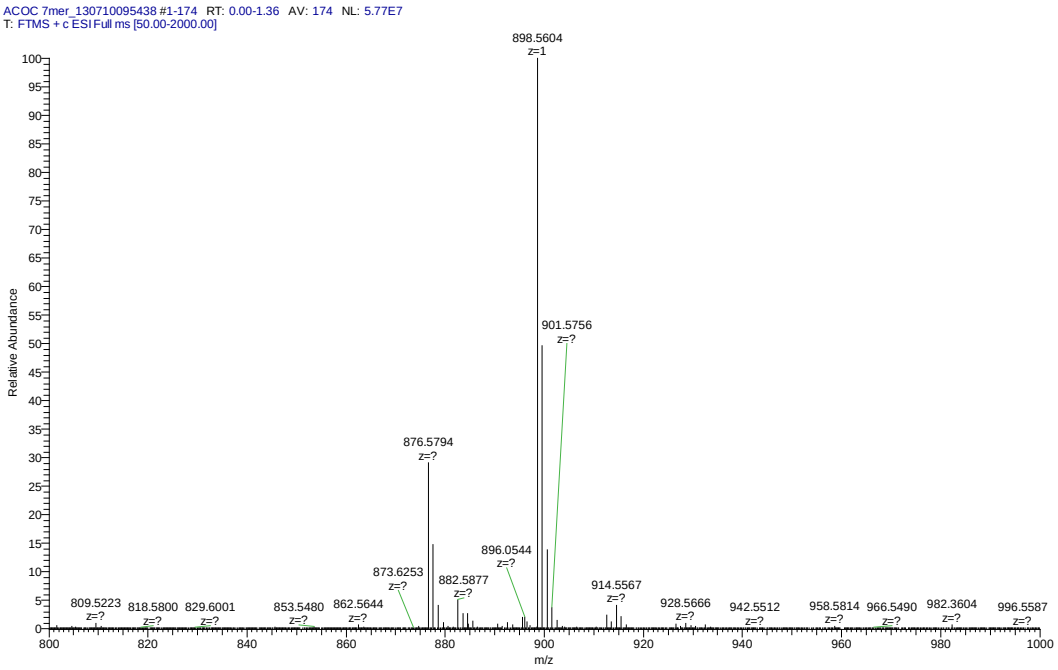


Figure S37. HRMS data for **9**.