Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Synthesis and conformational studies of peptido-squaramide foldable modules: a new class of turn-mimetic compounds.

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Supporting Information

General Methods

All reagents and solvents were commercially available and were used without further purification unless otherwise stated. All reactions sensitive to air or moisture were carried out under argon atmosphere in dry, freshly distilled solvents under anhydrous conditions. DMSO-d_6 and $CDCl_3$ (99.8 %D) were stored on molecular sieves (3 Å). ^1H and ^{13}C NMR spectra were recorded at 600, 300 and 75 MHz at 23 ºC unless otherwise specified. Chemicals shifts are reported as parts per million (δ) referenced to the residual deuterium lock solvents. The coupling constants values *J* are given in Hz. NMR peak assignments were performed by COSY, HSQC, HMBC, and NOESY experiments. Concentration dependence experiments were run recording spectra of the studied compounds at seven different concentrations (0.5- 100 mM). The temperature coefficients were determined by recording spectra of 1-2 mM solution of those compounds at seven different temperatures between 313-237 K. IR spectra were recorded on (FT-IR) Bruker IFS66 spectrophotometer. HRMS data were obtained on a MicroMass Autospec 3000 double focusing magnetic sector mass spectrometer operating at 3000 m/z with a cone voltage of 4V. Samples were introduced through an electrospray module (ESI).

Molecular Modeling. Molecular models for compound **1** were generated using the MMFF94 force field as implemented in MacSpartan 10[1]. The initial geometries were then subjected to a conformational search on 52488 structures generated from all the rotatable bonds. The geometry of the two different conformers of minimum energy were then subjected to full geometry optimization at the RB3LYP/6-31G* level of approximation.

Synthesis of the Compounds

3-(Butylamino)-4-ethoxycyclobut-3-ene-1,2-dione (5)

5 was prepared as described previously[2].

2-((2-(Butylamino)-3,4-dioxocyclobut-1-en-1-yl)amino)acetic acid (9)

5 (1.0 g, 5.07 mmol) in EtOH (20 ml) was added dropwise to a stirred solution of glycine (0.375 g, 5.0 mmol) and sodium ethoxide (0.375 g, 5.5 mmol) in EtOH (150 mL). The mixture was led to stand for 12 h at room temperature. Then, the solvent was removed and the residue suspended in Et_2O (50 mL) and filtered. The solid was dissolved in H_2O (5 mL) and HCl 37% was added until pH =2 to form a precipitate. The

[1] ***Spartan'10*** Wavefunction, Inc. Irvine, CA

[2] M. C. Rotger, M. N. Piña, A. Frontera, G. Martorell, P. Ballester, P. M. Deyà, A. Costa, *J. Org. Chem*., 2004, **69**, 2302-2308.

product was filtered and washed with H_2O at 0ºC to afford **9** (0.851 g 3.76 mmol) as a white solid. Yield 75%. Mp: 217 ºC with descomp; ^{1}H RMN (DMSO-d_6) δ: 12.87 (1H, s), 7.52 (2H, s), 4.24 (2H, s), 4.47 (2H, s), 4.71 (2H, m), 1.30 (2H, m), 0.86 (3H, t, *J*=7.5Hz). ^{13}C RMN (DMSO-d_6) δ: 186.17, 185.6, 174.4, (171.1, 170.8), 47.7, 46.1, 35.9, 22.2, 16.7. IR(KBr): 3168, 2959, 1655, 1570 cm^{-1}. HRMS (ESI): m/z calc. for $C_{20}H_{28}N_4O_8Na$: 475.1805, found 475.1815.

3-((2-(Butylamino)-3,4-dioxocyclobut-1-en-1-yl)amino)propanoic acid (10)

10 was prepared and purified as described for **9** starting from **5** (591 mg, 3.0 mmol), β-alanine (270 mg, 3.0 mmol) and sodium ethoxide (225 mg, 3.3 mmol). The desired product **10** (563 mg, 2.34 mmol) was obtained as white solid. Yield = 78%. Mp: 210-211 ºC. ^{1}H RMN (DMSO-d_6) δ: 12.37 (1H, s), 7.40 (2H, s), 3.67 (2H, q, *J*=6Hz), 3.46 (2H, m), 1.47 (2H, m), 1.30 (2H, m), 0.87 (3H, t, *J*=8.1Hz). ^{13}C RMN (DMSO-d_6) δ: 185.4, 175.9, 171.0, 170.6, 46.0, 38.6, 35.9, 22.1, 16.7. IR (KBr): 3164, 2958, 1647, 1583 cm^{-1}. HRMS (ESI): m/z calc. for $C_{22}H_{32}N_4O_8Na$: 503.2118, found 503.2138.

4-((2-(Butylamino)-3,4-dioxocyclobut-1-en-1-yl)amino)butanoic acid (11)

11 was prepared and purified as described for **9** starting from **5** (296 mg, 1.5 mmol), γ-aminobutiric acid (157 mg, 1.5 mmol) and sodium ethoxide (102 mg, 1.5 mmol). The desired product **11** (337 mg, 1.32 mmol) was obtained as white solid. Yield = 87%. Mp: 195 ºC with descomp. ^{1}H RMN (DMSO-d_6) δ: 12.13 (0.6H, s), 7.30 (0.6, s), 3.46 (4H, m), 2.24 (2H, t, *J*=6Hz), 1.72 (2H, m), 1.48 (2H, m), 0.87 (3H, t, *J*=7.5Hz). ^{13}C RMN (DMSO-d_6) δ: 185.5, 177.2, 170.9, 46.1, 45.8, 36.0, 33.7, 29.4, 22.2, 16.7. IR (KBr): 3165, 2958, 1640, 1584 cm^{-1}. HRMS (ESI): m/z calc. for $C_{24}H_{36}N_4O_8Na$: 531.2431, found 531.2441.

(S)-Ethyl 2-(2-((2-(butylamino)-3,4-dioxocyclobut-1-en-1-yl)amino)acetamido)-3-phenylpropanoate (1)

9 (500 mg, 2.2 mmol) and phenylalanine methylester (710 mg, 3.96 mmol) were mixed in a three neck round bottom flask and dissolved in DMF (30 mL). Then, DIPEA (2.3 g, 17.8 mmol) was added to the solution. Finally HBTU (2.5 g, 6.6 mmol) in DMF (20 mL) were added dropwise under inert atmosphere. The reaction mixture was covered with a foil and stirred for 15 h. The solvent was removed and the residue suspended in

CH_2Cl_2 (50 mL) was filtered. The solution was washed with HCl 3N (3 x 30 mL), H_2O (30 mL), NaCl sat (30 mL) and dried with Na_2SO_4. The solvent was removed and the solid obtained was digested in Et_2O (3 x 20 ml) obtaining **1** (552 mg, 1.42 mmol) as a pale brown solid. Yield 65%. Mp: 138-139 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.73 (1H, s), 7.13 (2H, d, *J*=5.7Hz), 4.67 (1H, q, *J*=6.3Hz), 4.14 (2H, s), 3.70 (3H, s), 3.64 (2H, m), 3.15/3.04 (2H, m), 1.62 (2H, m), 1.39 (2H, m), 0.94 (3H, t, *J*=7.2Hz). ^{13}C RMN ($CDCl_3$) δ: 185.8, 184.8, 174.3, 172.2, 168.9, 138.6, 131.8, 131.2, 129.7, 56.7, 55.1, 49.6, 47.2, 40.2, 35.8, 22.3, 16.4. IR (KBr): 3305, 3234, 2955, 1659, 1586 cm^{-1}. HRMS (ESI): m/z calc. for $C_{40}H_{50}N_6O_{10}Na$: 797.3486, found 797.3473.

(S)-Ethyl 2-(3-((2-(butylamino)-3,4-dioxocyclobut-1-en-1-yl)amino)propanamido)-3-phenylpropanoate (2)

2 was prepared and purified as described for **1** starting from **10** (500 mg, 2.0 mmol) phenylalanine methyl ester (365 mg, 2.0 mmol). The desired product **2** (434 mg, 1.08 mmol) was obtained as white solid. Yield = 54%. Mp: 202-203 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.13 (2H, d, *J*=5.7Hz), 6.52 (2H, s), 6.38 (0.8H, s), 4.82 (1H, q, *J*=5.7Hz), 3.93 (1H, s), 3.78 (1H, s), 3.77 (3H, s), 3.66 (2H, q, *J*=5.7Hz), 3.18/3.13 (2H, m), 2.55 (2H, m), 1.45 (2H, m), 0.98 (3H, t, *J*=7.5Hz). ^{13}C RMN (DMSO-d_6) δ: 185.4, 175.1, 173.5, 170.9, 170.6, 140.2, 132.2, 131.4, 129.7, 56.8, 54.9, 46.0, 39.9, 35.9, 22.1, 16.6. IR (KBr): 3302, 3166, 2958, 1650, 1567 cm^{-1}. HRMS (ESI): m/z calc. for $C_{21}H_{27}N_3O_5Na$: 424.1848, found 424.1848.

(S)-Ethyl 2-(4-((2-(butylamino)-3,4-dioxocyclobut-1-en-1-yl)amino)butanamido)-3-phenylpropanoate (3)

3 was prepared and purified as described for **1** starting from **11** (509 mg, 2.0 mmol) phenylalanine methyl ester (663 mg, 3.7 mmol). The desired product **3** (556 mg, 1.34 mmol) was obtained as white solid. Yield = 67%. Mp: 152-153 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.13 (2H, d, *J*=5.7Hz), 6.41 (1H, s), 6.24 (1H, s), 4.86 (1H, q, *J*=6.3Hz), 3.79 (3H, s), 3.74 (2H, q, *J*=6Hz), 3.44 (2H, s), 3.17/3.12 (2H,m), 2.33 (2H, m), 1.90 (2H, m), 1.67 (2H, m), 1.45 (2H, m), 0.99 (3H, t, *J*=7.2Hz). ^{13}C RMN ($CDCl_3$) δ: 185.3, 175.5, 171.1, 170.3, 138.7, 131.8, 131.3, 129.8, 56.24, 55.1, 47.1, 46.1, 40.5, 35.8, 29.8, 22.3, 16.4. IR (KBr): 3305, 3167, 2956, 1645, 1581 cm^{-1}. HRMS (ESI): m/z calc. for $C_{44}H_{58}N_6O_{10}Na$: 853.4112, encontrada: 853.4136.

Phenyl (1-((2-(tert-butoxycarbonyl)amino)ethyl)aminoethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (12)

12 was prepared the coupling procedure described for **1** starting from N-tert-Buthyl-aminoethyl-carbamate (361 mg, 2.26 mmol) and N-[(benzyloxy)carbonyl]leucine (500 mg, 1.88 mmol) obtaining **12** (517 mg, 1.27 mmol) as a pale brown solid. Yield 67%. Mp: 129-130 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.35 (5H, s), 6.61 (1H, s), 5.11 (2H, m), 4.91 (1H, s), 4.14 (1H, q, *J*=3.9Hz), 3.33 (2H, m), 3.26 (2H, m), 1.66 (2H, m), 1.48 (1H, m), 1.44 (9H, s), 0.94 (6H, d, *J*=6.6Hz). ^{13}C RMN (DMSO-d_6) δ: 175.5, 159.1, 140.2, 131.2, 131.5, 130.8, 80.8, 68.5, 56.3, 31.4, 27.4, 26.1, 24.6. IR (KBr): 3350, 3305, 2963, 1686, 1656, 1535 cm^{-1}. HRMS (ESI): m/z calc. for $C_{21}H_{33}N_3O_5Na$: 430.2318, found 430.2320.

(S)-Benzyl (1-((2-((2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)amino)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (13)

12 (100 mg, 0.25 mmol) was dissolved in CH_2Cl_2/TFA 9:1 v:v (7 mL) and stirred for 2 h at room temperature. Then, the solvent was removed and the trifluoroacetate salt of the amine was used without further purification. This was dissolved in ethanol (1 mL) with DIPEA (200µL, 2 .0 mmol). This solution was diluted with diethyl ether (17 mL) and added dropwise to a solution of diethyl squarate (51 mg, 0.3 mmol) in ethanol (2 mL). The reaction was stirred for 48 h. The solvent was removed and the residue was suspended in dichloromethane (20 ml), washed with HCl 3 M (3 x 15 mL), NaCl sat (15 mL) and dried with Na_2SO_4. The solvent was removed and the product was purified by flash column chromatography using as eluent mixtures of ethyl acetate-dichloromethane to afford **13** as a white solid (90 mg, 0.21 mmol). Yield 84%. Mp: 47-48 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.33 (5H, s), 7.13 (0.7H, s), 7.98 (0.7H, s), 6.62 (0.3H, s), 6.15 (0.3H, s), 5.66 (0.7, s), 5.11 (2H, m), 4.73 (2H, q, *J*=7.2Hz), 4.14 (1H, m), 3.77 (0.7H, m), 3.53 (2H, m), 3.43 (1.3H, m), 1.67 (2H, m), 1.51 (1H, m), 1.44 (1H, t, *J*=6.6Hz), 0.92 (6H, t, *J*=4.8Hz). ^{13}C RMN ($CDCl_3$) δ: 180.6, 176.2, 175.0, 159.2, 138.9, 131.9, 130.6, 72.8, 69.8, 56.9, 47.1, 43.8, 42.4, 27.5, 25.7, 24.4, 18.5. IR (KBr): 3297, 2957, 1706, 1609, 1532 cm^{-1}. HRMS (ESI): m/z calc. for $C_{22}H_{29}N_3O_6Na$: 454.1954, found 454.1943.

(S)-tert-Butyl (2-((1-(cyclohexylamino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)carbamate (14)

14 was prepared following the coupling procedure described for **1** starting from N-tert-butoxy-glycine (177 mg, 1.01 mmol) and L-Phenylalanine-cyclohexylamide (250 mg, 1.01 mmol) to afford **14** as a white solid (302 mg, 0.75 mmol). Yield 74 %. Mp: 171-172 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.26-7.22 (5H, m), 6.60 (1H, s), 5.46 (1H, s), 5.01 (1H, s), 4.56 (1H, q, *J*=5.7Hz), 3.77 (2H, d, *J*=5.7Hz), 3.65 (2H, m), 3.18-2.94 (2H, m), 1.56 (4H, m), 1.44 (9H, s), 1.28 (4H, m), 1.01 (2H, m). ^{13}C RMN (DMSO-d_6) δ: 172.6, 172.0, 158.9, 140.7, 139.4, 131.1, 129.4, 81.2, 56.8, 50.6, 46.4, 35.3, 31.3, 28.3, 27.7, 27.6. IR (KBr): 3275, 2931, 1680, 1639, 1546 cm^{-1}. HRMS (ESI): m/z calc. for $C_{22}H_{33}N_3O_4Na$: 426.2369, found 426.2373.

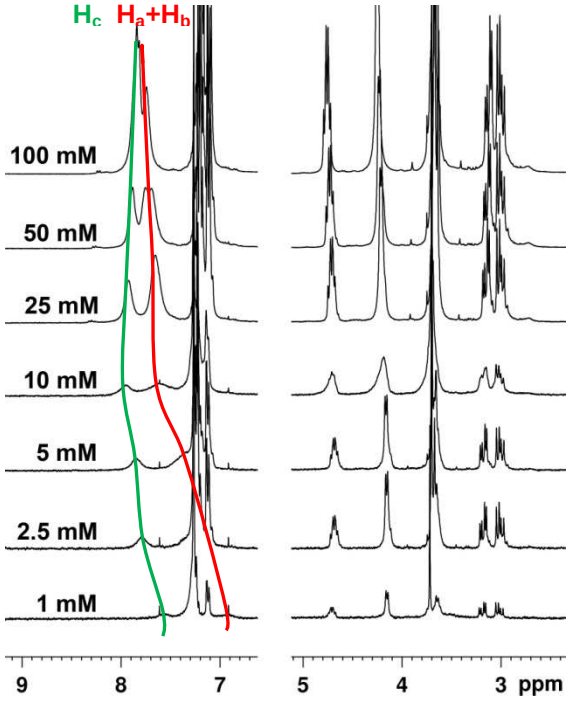
(S)-tert-Butyl (4-((2-((1-(cyclohexylamino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)amino)-4-oxobutyl)carbamate (15)

14 was deprotected as described for **12** (200 mg, 0.2 mmol). The trifluoroacetate salt of the amine was used without farther purification and coupled with 4-(tert-butoxycarbonylamino)butanoic acid (102 mg, 0.5 mmol) following the coupling procedure described for **1** to obtain **15** (178 mg, 0.36 mmol) as a white solid. Yield 73%. Mp:180-181 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.26/7.22 (5H, m), 6.87 (1H, d, *J*=7.2Hz), 6.74 (1H, s), 5.69 (1H, d, *J*=6.6Hz) 4.56 (1H, q, *J*=6.6Hz), 3.89 (2H, m), 3.65 (1H, m), 3.14 (2H, m), 3.02/3.13 (2H, m), 2.26 (3H, t, *J*=6Hz), 1.80 (2H, m), 1.62 (4H, m), 1.44 (9H, s), 1.25 (4H, m), 0.98 (2H, m). ^{13}C RMN (DMSO-d_6) δ: 175.5, 172.7, 171.8, 158.7, 140.9, 132.3, 131.2, 129.3, 80.6, 57.0, 50.7, 45.2, 35.5, 31.4, 28.9, 28.3, 27.7. IR (KBr): 3330, 2931, 28544, 1688, 1636, 1537 cm^{-1}. HRMS (ESI): m/z calc. for $C_{26}H_{40}N_4O_5Na$: 511.2896, found 511.2879.

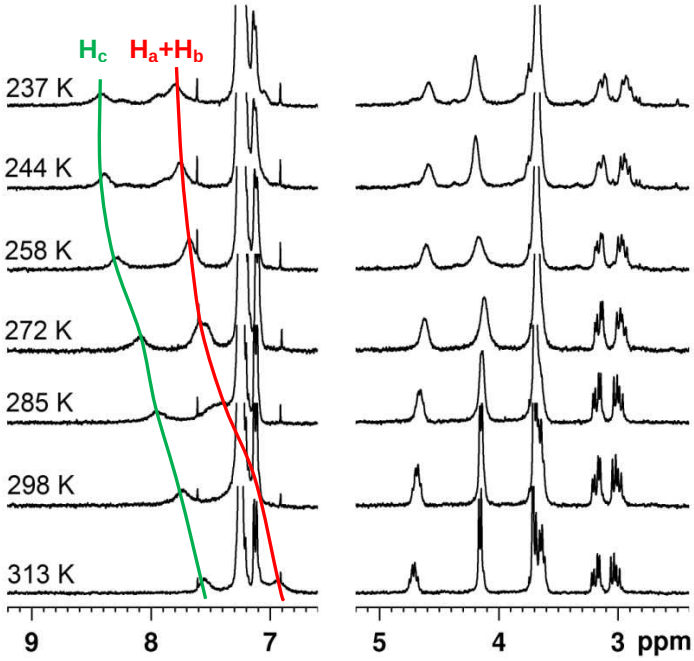
Benzyl ((S)-1-((2-((2-((4-((2-(((S)-1-(cyclohexylamino)-1-oxo-3-phenylpropan-2-yl)amino)-2-oxoethyl)amino)-4-oxobutyl)amino)-3,4-dioxocyclobut-1-en-1-yl)amino)ethyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate (4)

15 was deprotected as described for **12** (112 mg, 0.23 mmol). The trifluoroacetate salt of the free amine of **15** was used without farther purification and dissolved in borax buffer 0.1 M, pH = 9 (10 mL). To this solution, **13** (100 mg, 0.23 mmol) in EtOH (10 mL) was added dropwise. The reaction was stirred for 72 h. The EtOH was removed under reduced pressure and from the aqueous phase an organic residue was separated by decantation. This was suspended in ethyl acetate (15 ml) and filtered to yield **4** as a pale brown solid 40 mg

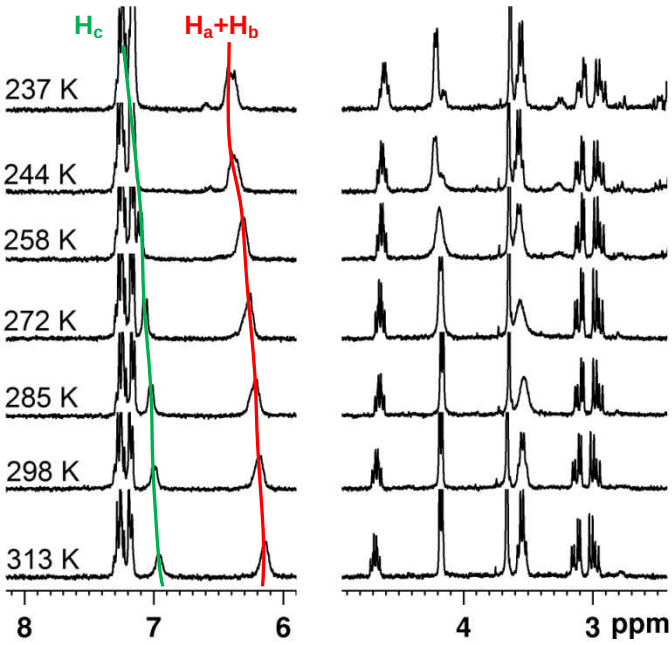
(0.05 mmol). Yield 22%. Mp:142-143 ºC. ^{1}H RMN ($CDCl_3$) δ: 7.94 (1.5H, s), 7.73 (1.5H, s), 7.49 (1.5H, s), 7.23 (2H, s), 6.78 (0.7H, s), 6.41 (0.2H, s), 6.13 (0.8H, s), 5.11 (1H, d, *J*=6Hz), 5.05 (1H, d, *J*=6Hz), 4.66 (1H, s), 4.31 (1H, s), 3.91 (2H, s), 3.71 (4H, m), 3.62 (1H, s), 3.52 (1H, s), 3.39 (1H, s), 3.08 (2H, m), 2.37 (2H, s), 1.97 (2H, s), 1.68 (8H, m), 1.09 (2H, m), 0.92 (8H, m). ^{13}C RMN (DMSO-d_6) δ: 185.5, 175.9, 175.3, 172.7, 171.8, 171.0, 159.1, 140.8, 140.2, 132.3, 131.1, 129.4, 68.5, 57.0, 56.3, 50.6, 46.0, 45.2, 41.2, 35.3, 30.1, 28.3, 27.7, 27.6, 27.3, 26.1, 24.6. IR (KBr): 3293, 2933, 1656, 1593, 1542 cm^{-1}. HRMS (ESI): m/z calc. for $C_{41}H_{55}N_7O_8Na$: 796.4010, found 796.4006.



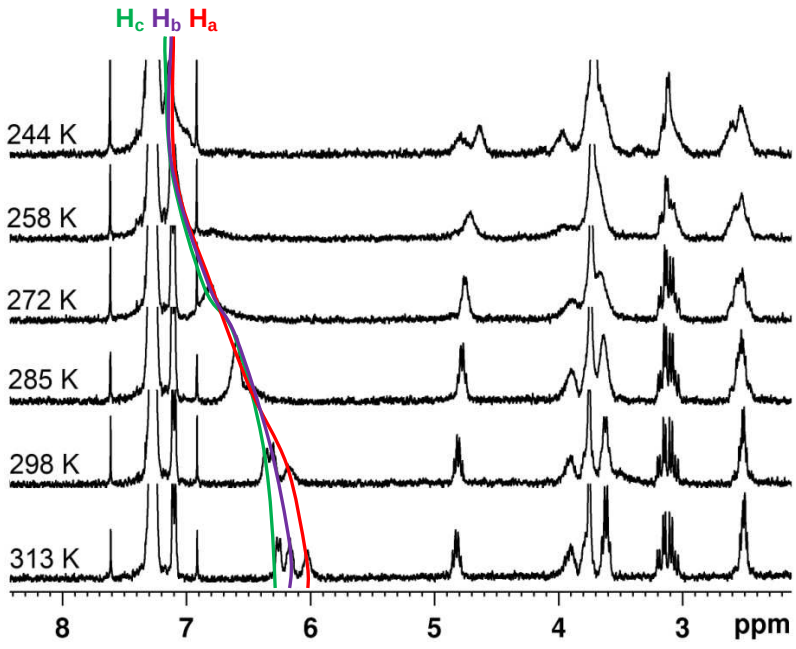
S1a: Variable-concentration ^{1}H-NMR spectra of **1** in $CDCl_3$.



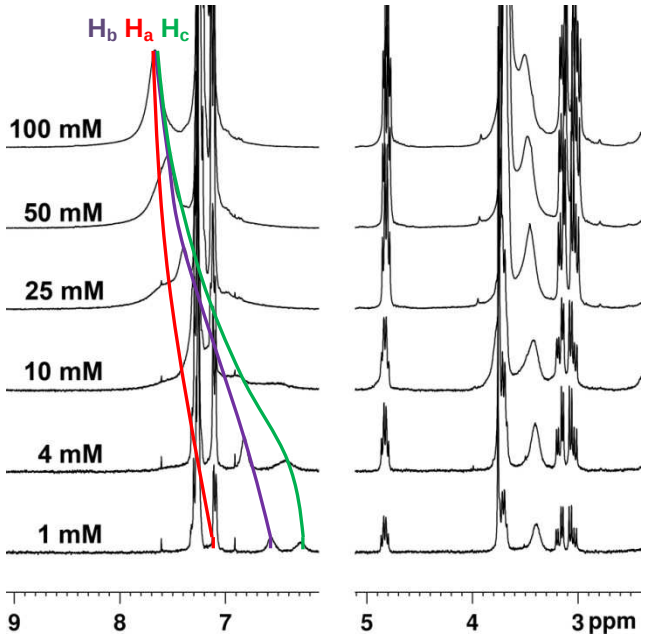
S1b: Variable-temperature ^{1}H-NMR spectra of **1** (2mM) in $CDCl_3$.



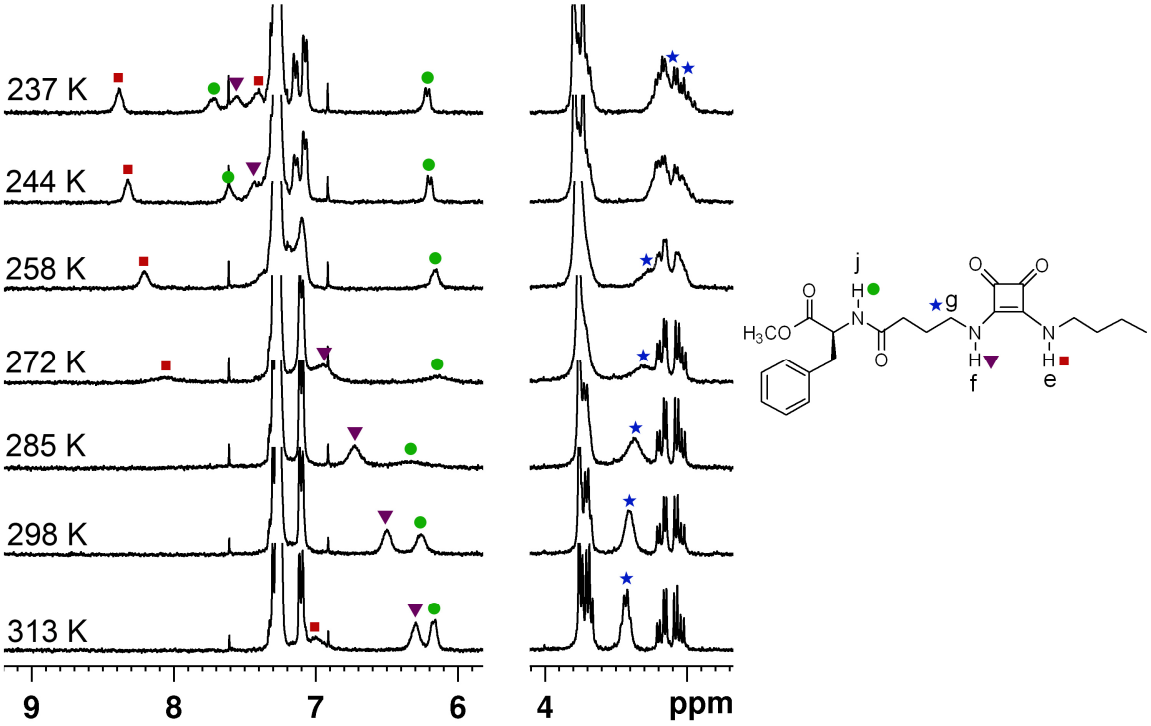
S1c: Variable-temperature ^{1}H-NMR spectra of **1** (2mM) in CD_3CN.



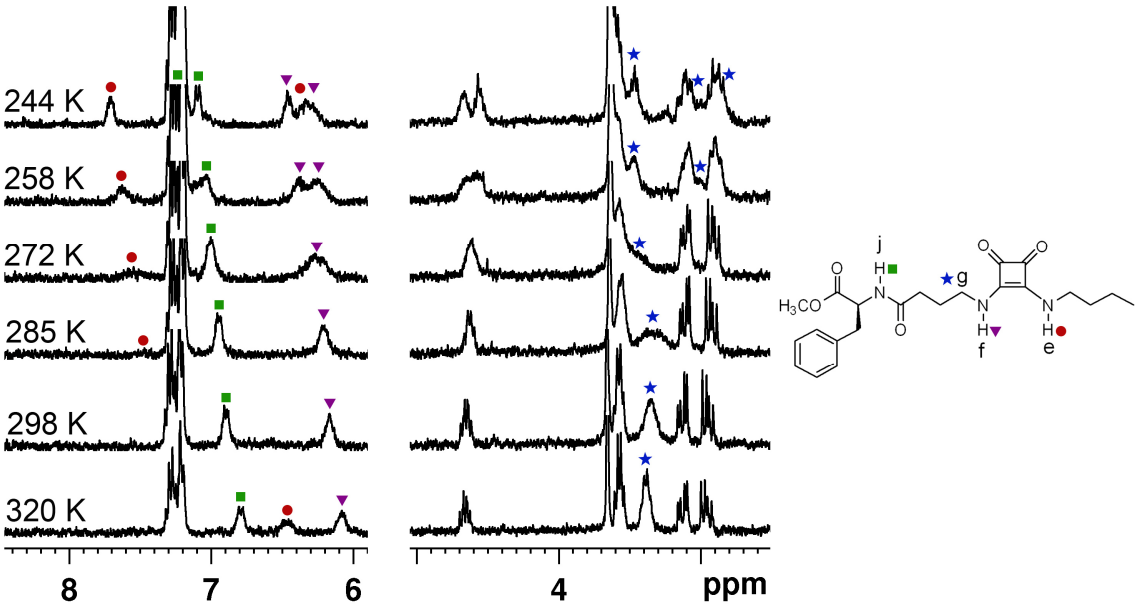
S2a: Variable-temperature ^{1}H-NMR spectra of **2** (1 mM) in $CDCl_3$



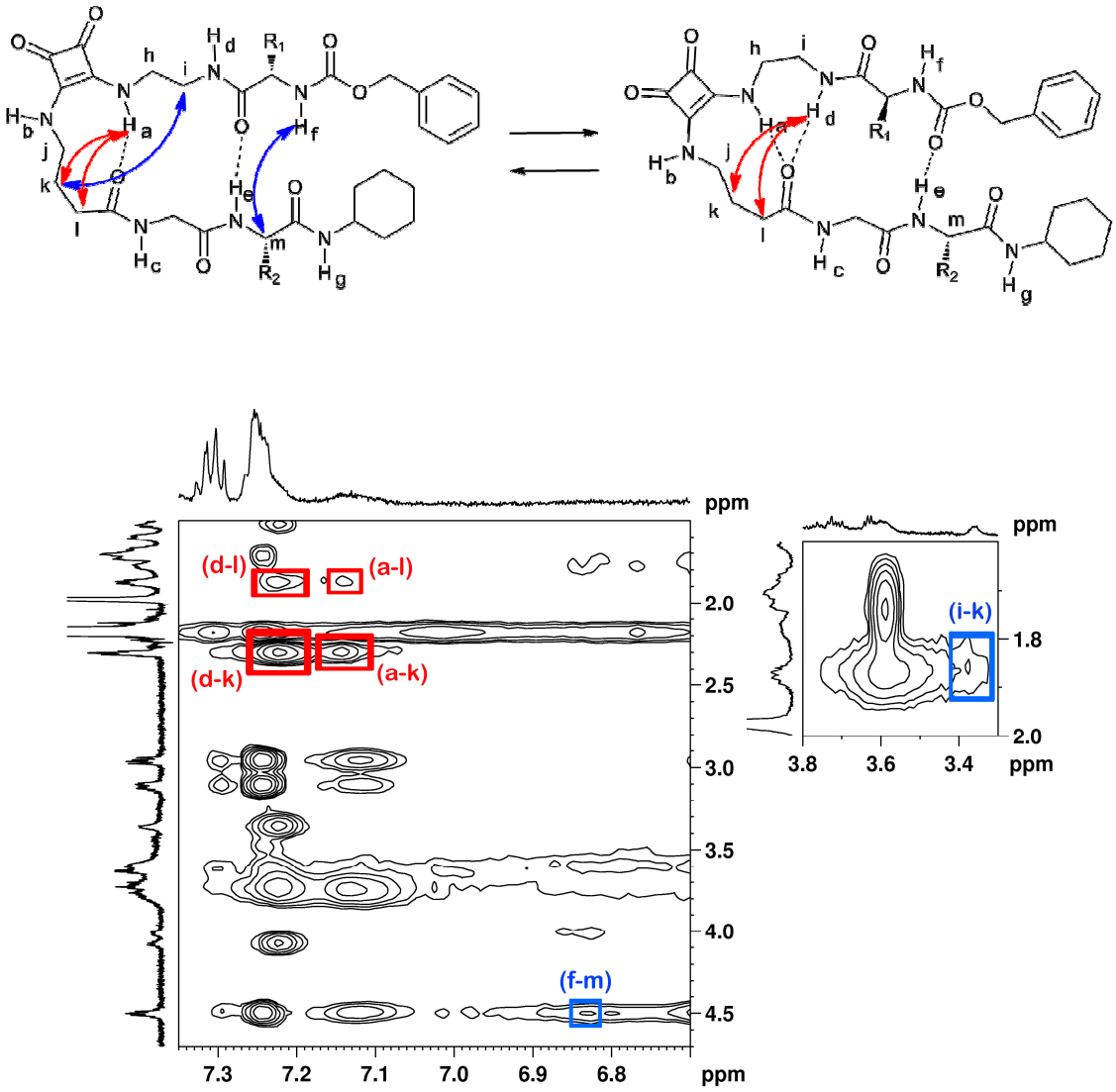
S3a: Variable-concentration ^{1}H-NMR spectra of **3** in $CDCl_3$



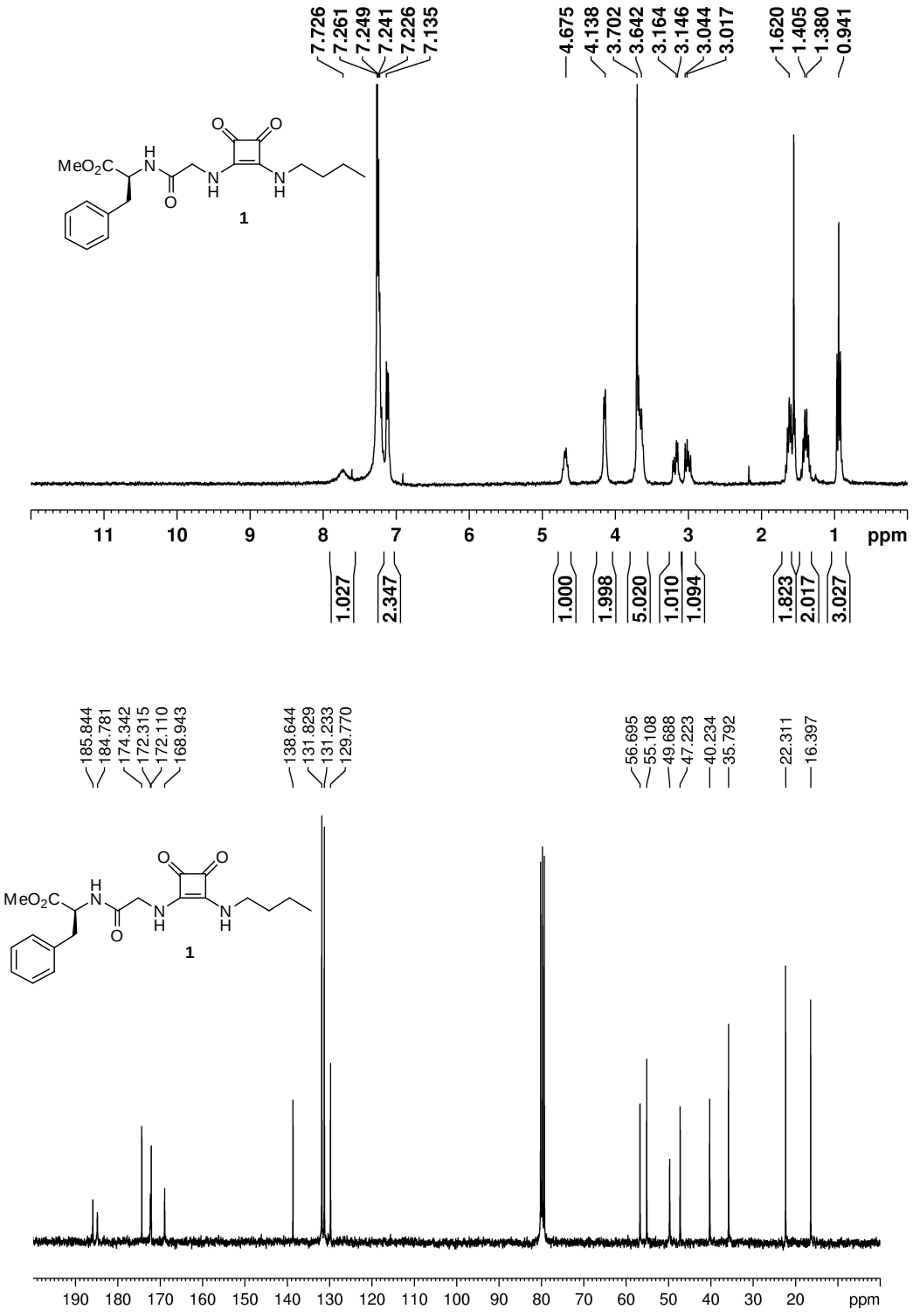
S3b: Variable-temperature ^{1}H-NMR spectra of **3** in $CDCl_3$



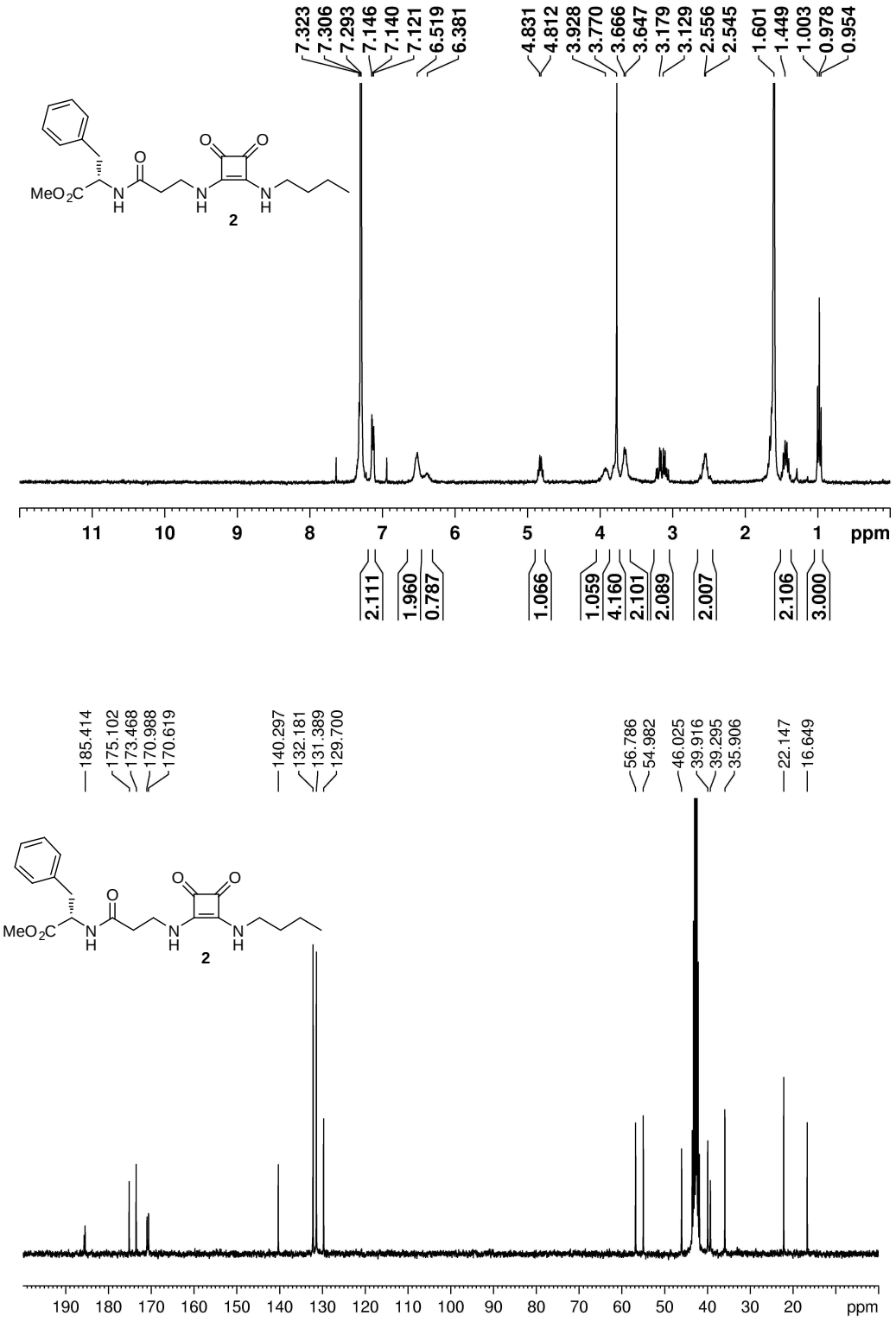
S3c: Variable-temperature ^{1}H-NMR spectra of **3** in CD_3CN



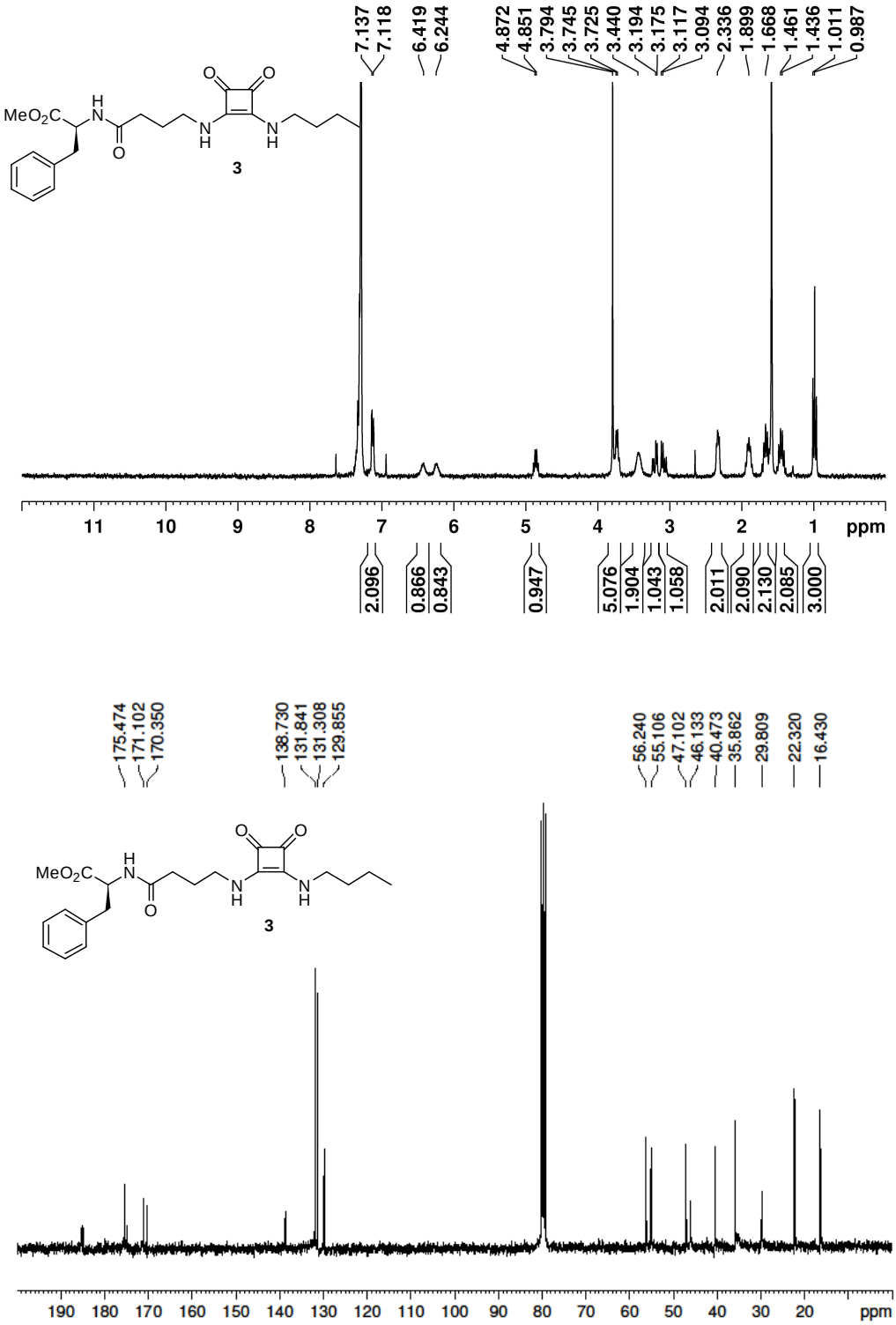
S4: Selected fragments of NOESY spectrum of **4** (1 mM) in CD_3CN at 298 K



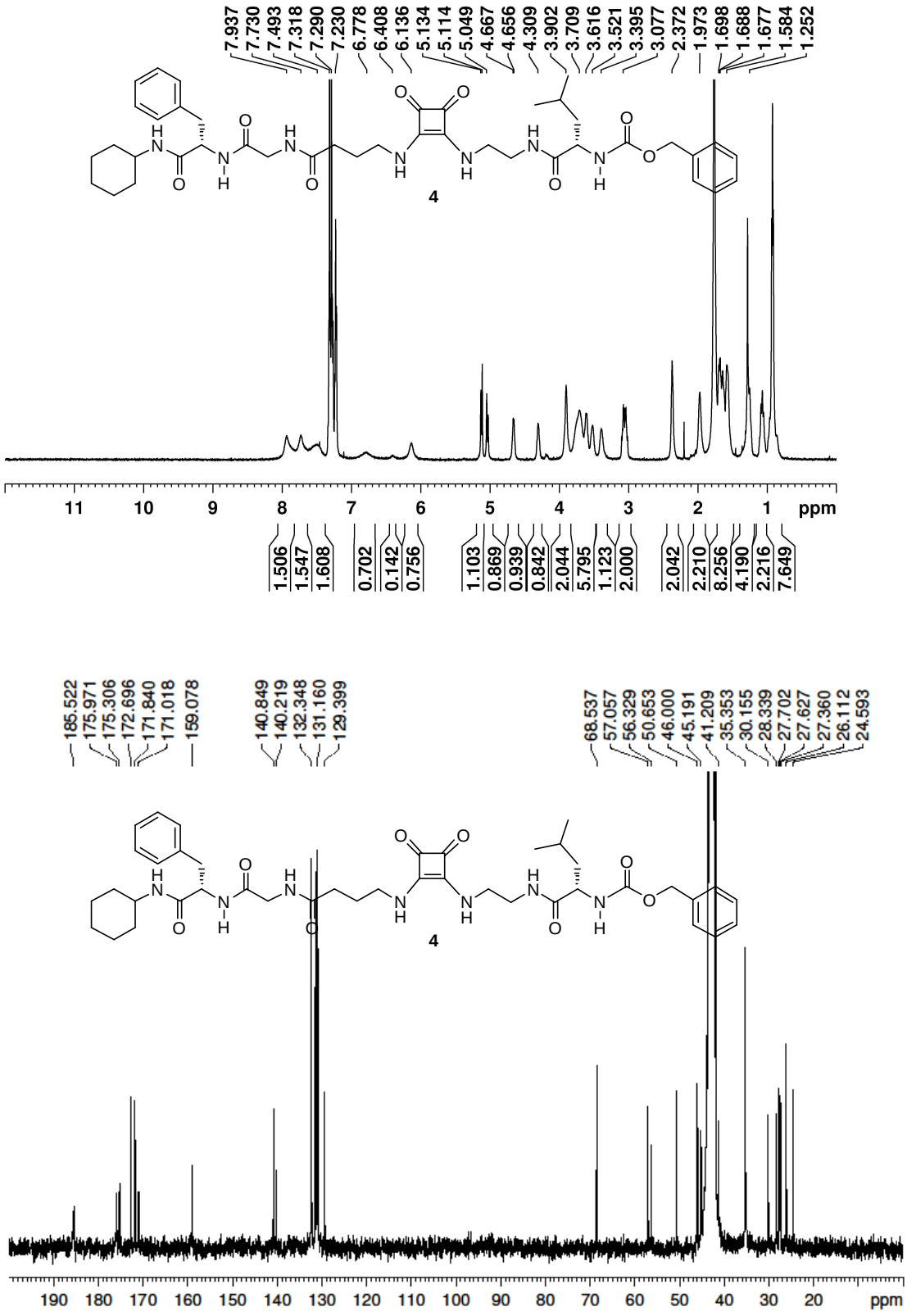
S5: ^{1}H-NMR and ^{13}C NMR spectra of **1**



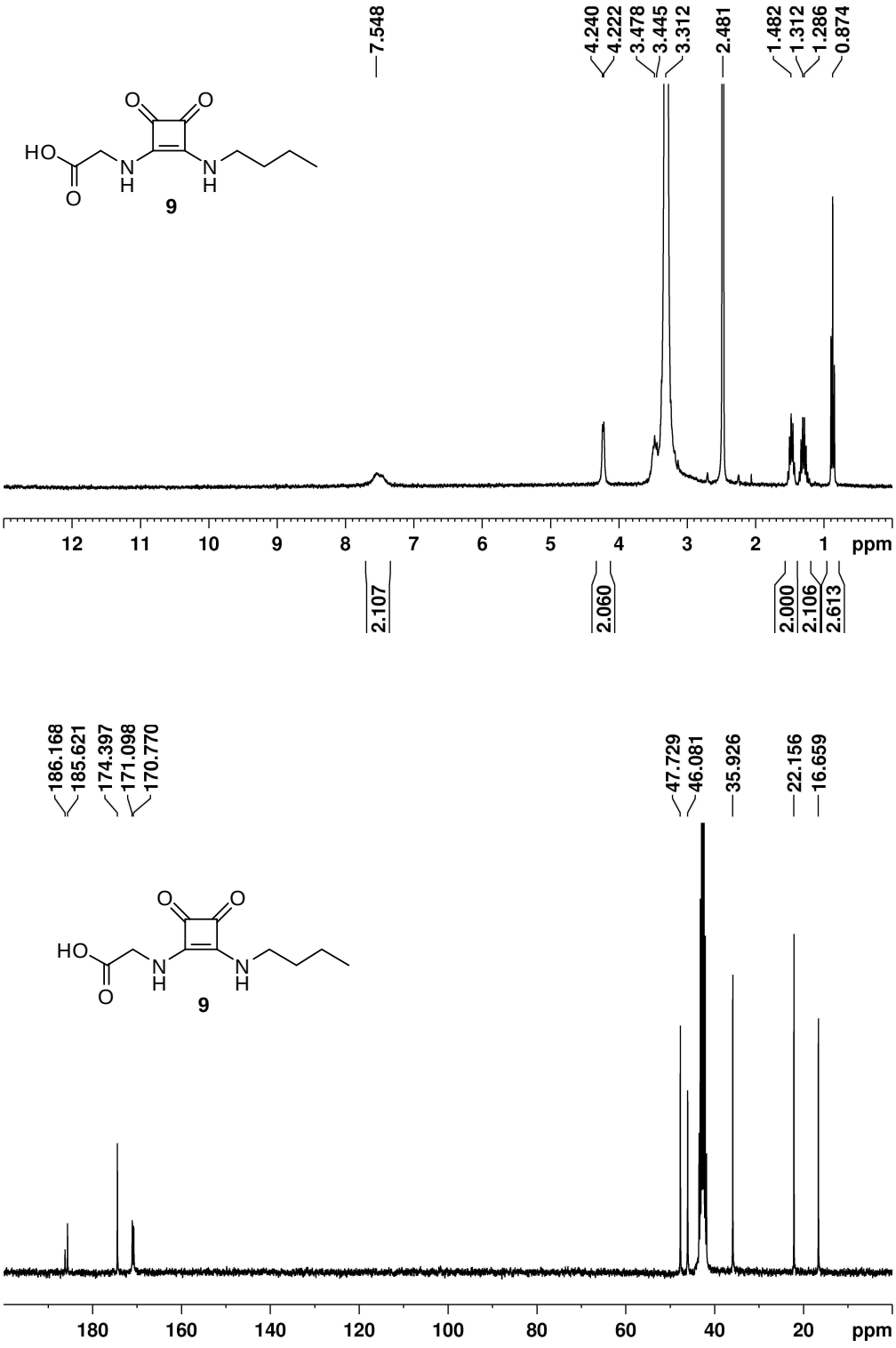
S6: ^{1}H-NMR and ^{13}C NMR spectra of **2**



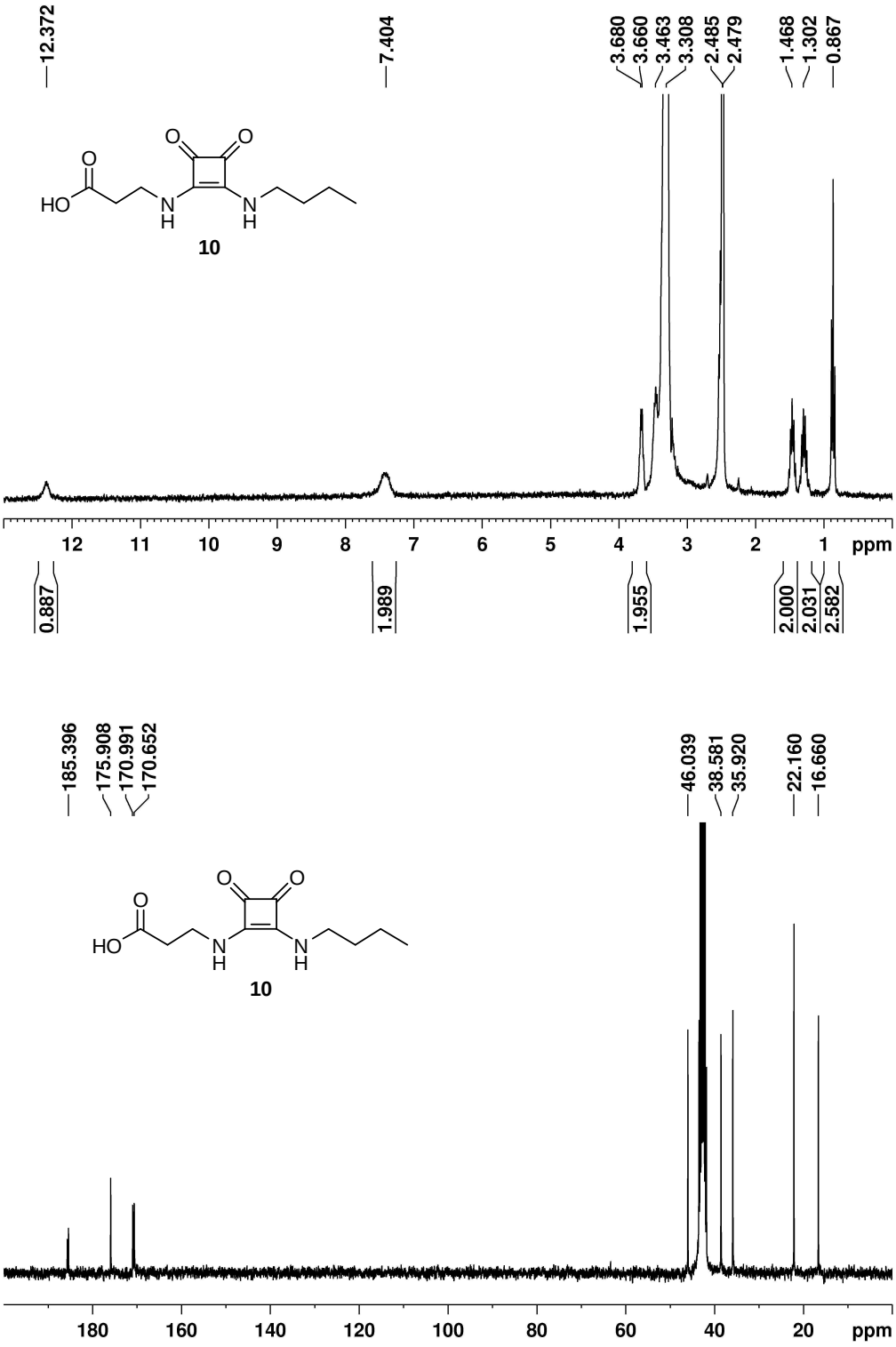
S7: ^{1}H-NMR and ^{13}C NMR spectra of **3**



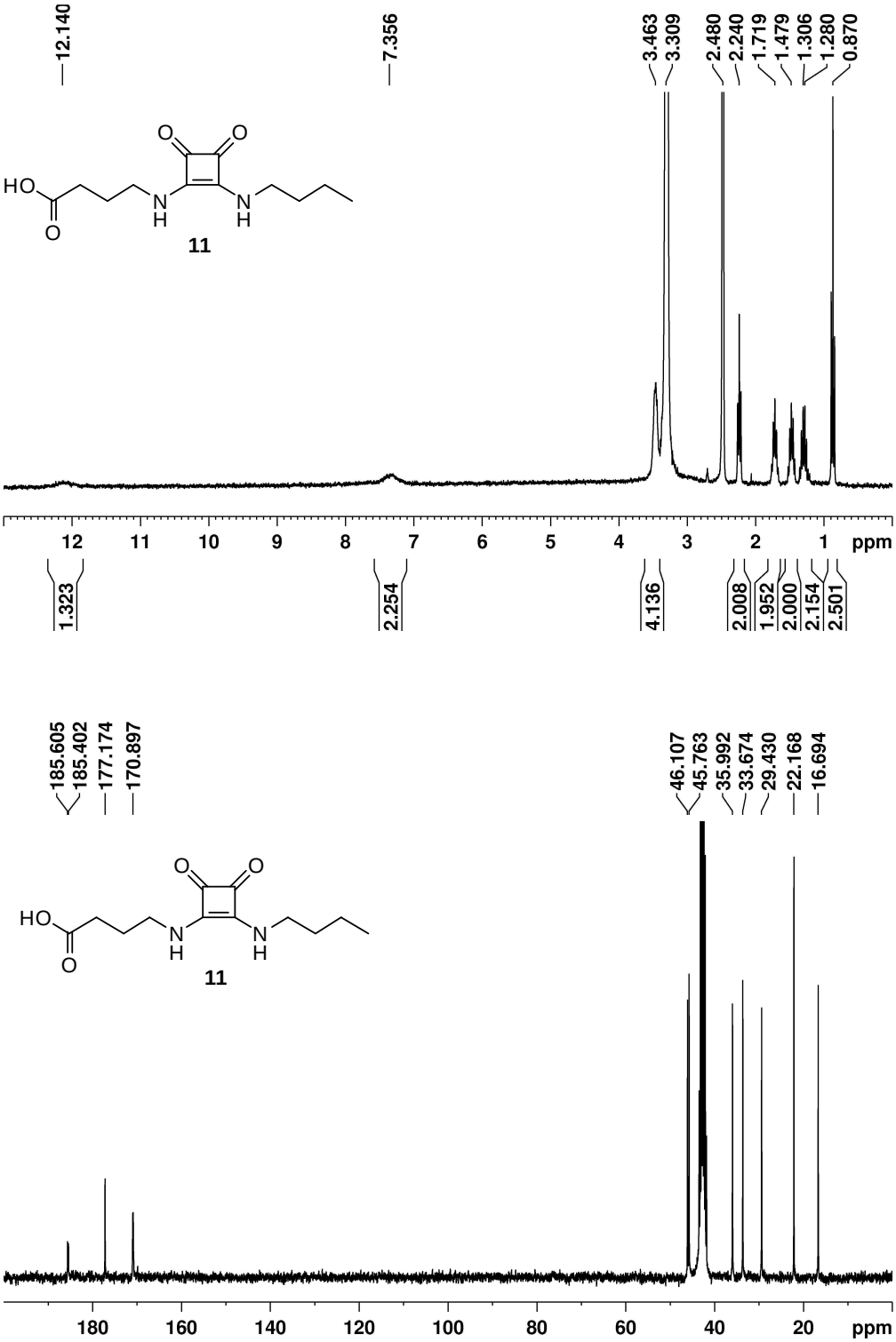
S8: ^{1}H-NMR and ^{13}C NMR spectra of **4**



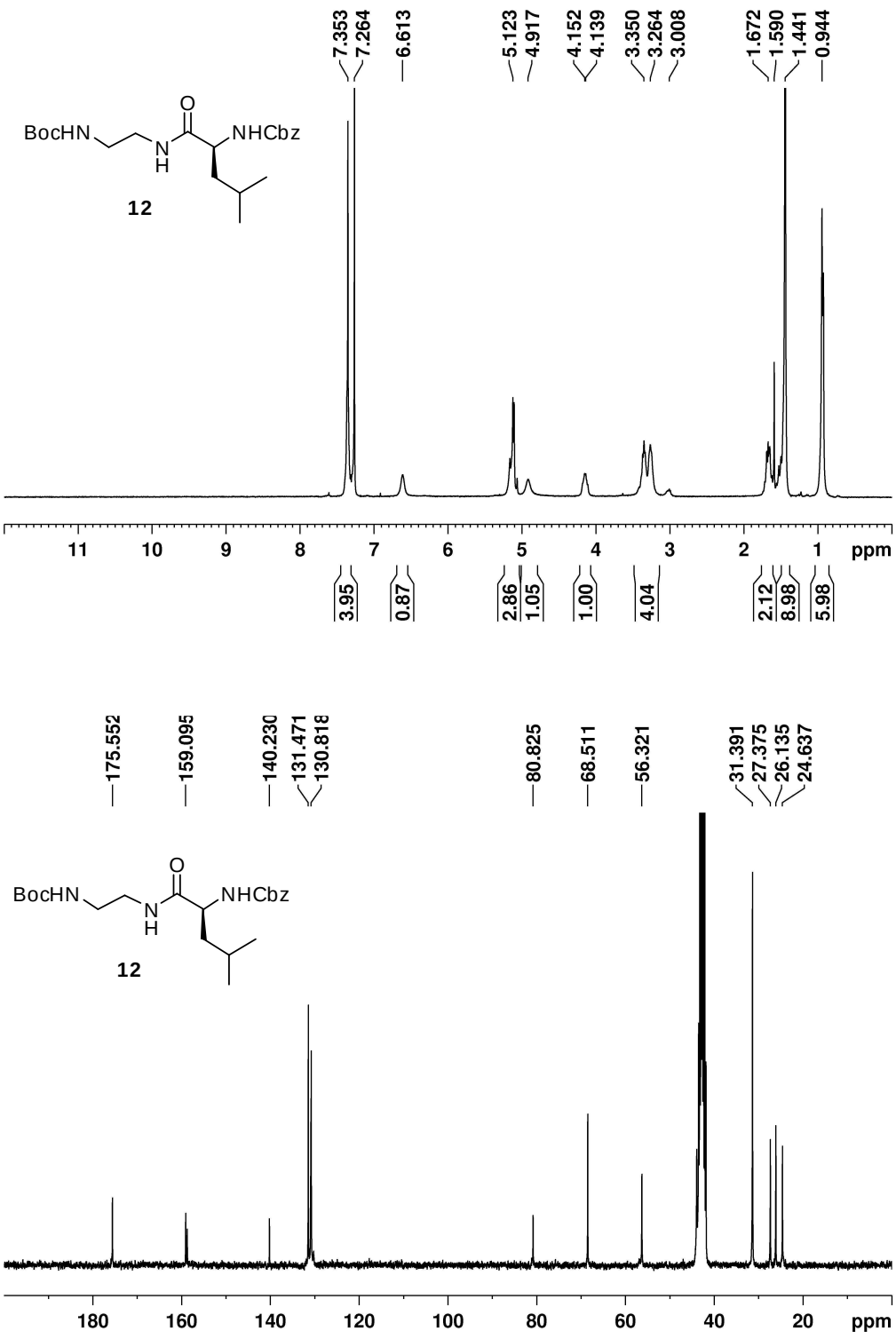
S9: ^{1}H-NMR and ^{13}C NMR spectra of **9**



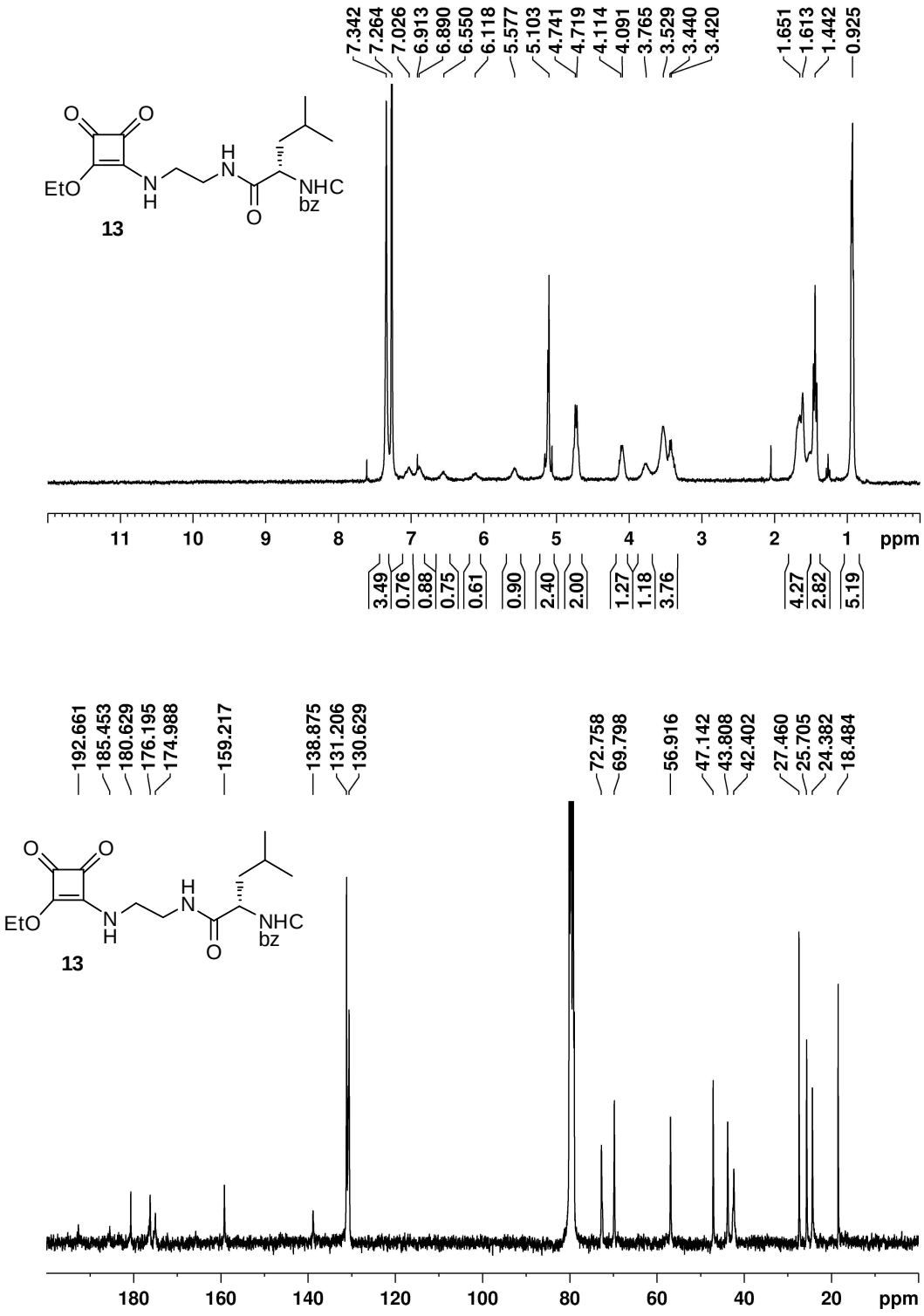
S10: ^{1}H-NMR and ^{13}C NMR spectra of **10**



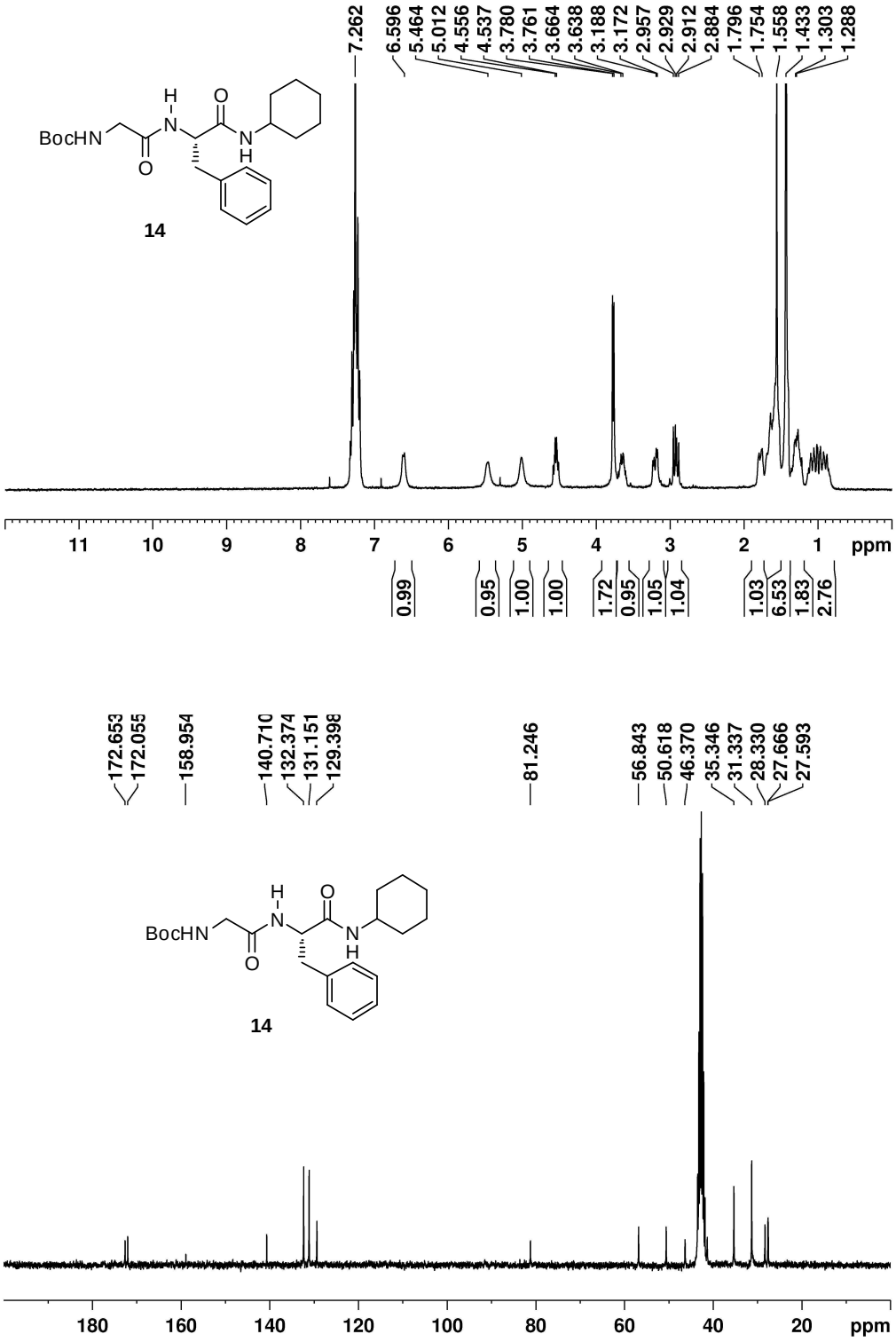
S11: ^{1}H-NMR and ^{13}C NMR spectra of **11**



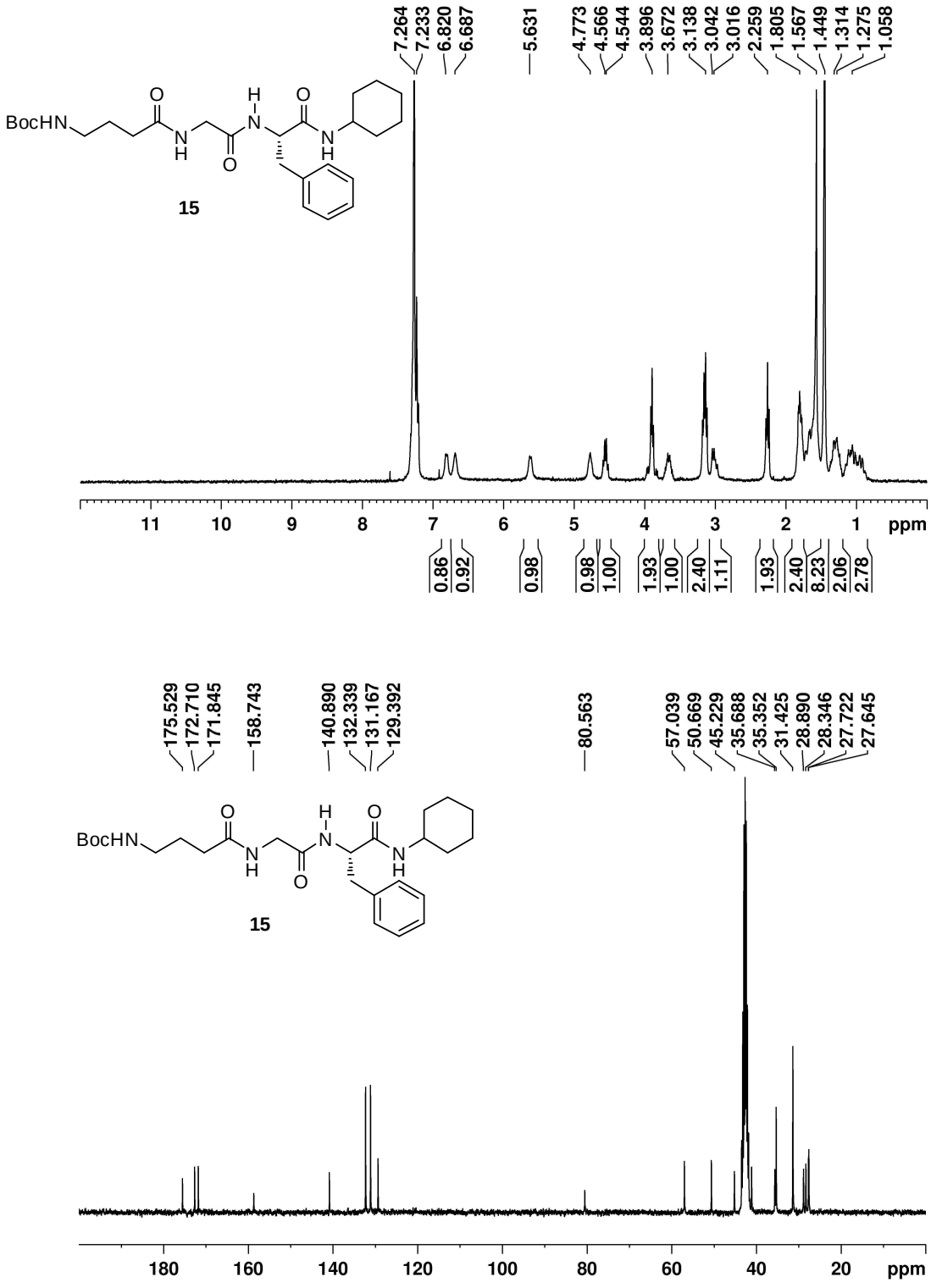
S12: ^{1}H-NMR and ^{13}C NMR spectra of **12**



S13: ^{1}H-NMR and ^{13}C NMR spectra of **13**



S14: ^{1}H-NMR and ^{13}C NMR spectra of **14**



S15: ^{1}H-NMR and ^{13}C NMR spectra of **15**