Supporting Information
Organic Letters

"Substituted Imidazo[1,2-a]pyridines as β-Strand Peptidomimetics"

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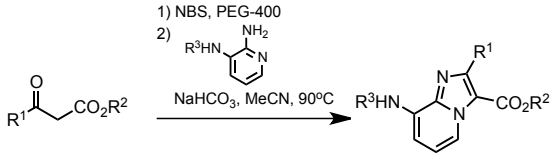
EXPERIMENTAL PROCEDURES

General Techniques. Unless stated otherwise, reactions were performed in flame-dried glassware under a positive pressure of argon or nitrogen gas using dry solvents. Commercial grade reagents and solvents were used without further purification except where noted. Diethyl ether, toluene, dimethylformamide dichloromethane, and tetrahydrofuran were purified by a Glass Contour column-based solvent purification system. Other anhydrous solvents were purchased directly from chemical suppliers. Thin-layer chromatography (TLC) was performed using silica gel 60 F254 pre-coated plates (0.25 mm). Flash chromatography was performed using silica gel (60 μm particle size). The purity of all compounds was judged by TLC analysis (single spot/two solvent systems) using a UV lamp, CAM (ceric ammonium molybdate), ninhydrin, or basic $KMnO_4$ stain(s) for detection purposes. 1D and 2D NMR spectra were recorded on a 400 or 500 MHz spectrometer. Proton chemical shifts are reported as δ values relative to residual signals from deuterated solvents ($CDCl_3$ or DMSO-d_6).



General Procedure for the Synthesis of IP Scaffolds from β-ketoesters. A mixture of *β*-ketoester (0.50 mmol) and solid *N*-bromosuccinimide (0.55 mmol) in PEG-400 (2 mL) was stirred at room temperature until TLC indicated consumption of the starting material (30-90 min). The reaction mixture was diluted with water and extracted with Et_2O. The combined organic layers were washed with water and brine, dried over Na_2SO_4, then filtered and concentrated. The crude residue was then dissolved in 2.5 mL MeCN and treated with either 2,3-diaminopyridine or N^2-amino-N^3-benzyloxycarbonylaminopyridine (0.50 mmol) and $NaHCO_3$

(0.55 mmol). The reaction mixture was heated to 90 °C in a sealed tube and strirred 15 h. The solution was then cooled to rt, diluted with EtOAc, filtered through a pad of celite, and concentrated. Purification by flash chromatography over silica gel (EtOAc/hexanes eluent) afforded the desired imidazo[1,2-a]pyridines.

Methyl 8-amino-2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (2a). Yield: 53%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 6.8 Hz, 1H), 6.78 (t, *J* = 7.2 Hz, 1H), 6.54 (d, *J* = 7.5 Hz, 1H), 4.50 (s, 2H), 3.94 (s, 3H), 2.69 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.1, 151.1, 140.3, 135.1, 118.4, 114.8, 113.7, 106.5, 51.4, 16.7; HRMS (ESI-TOF) *m*/*z* $[M+H]^+$ calcd for $C_{10}H_{12}N_3O_2$ 206.0924, found 206.0933.

Ethyl 8-amino-2-phenylimidazo[1,2-*a*]pyridine-3-carboxylate (2b). Yield: 55%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (d, *J* = 6.9 Hz, 1H), 7.76 – 7.71 (m, 2H), 7.52 – 7.35 (m, 3H), 6.83 (t, *J* = 7.2 Hz, 1H), 6.56 (d, *J* = 7.5 Hz, 1H), 4.63 (s, 2H), 4.27 (q, *J* = 7.2 Hz, 2H), 1.19 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.4, 152.2, 140.7, 135.9, 135.0, 130.3, 128.7, 127.8, 118.5, 115.4, 113.2, 106.3, 60.5, 14.2; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{16}H_{16}N_3O_2$ 282.1237, found 282.1250.

Methyl 8-(((benzyloxy)carbonyl)amino)-2-methylimidazo[1,2-*a*]pyridine-3-carboxylate (3a). Yield: 80%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 6.9 Hz, 1H), 8.18 (s, 1H), 8.05 (d, *J* = 6.7 Hz, 1H), 7.46 – 7.31 (m, 5H), 6.96 (t, *J* = 7.3 Hz, 1H), 5.26 (s, 2H), 3.96 (s, 3H), 2.68 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.8, 153.3, 151.4, 139.8, 135.8, 128.7, 128.5, 128.3, 126.8, 121.8, 114.4, 114.1, 111.9, 67.5, 51.5, 16.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{18}H_{18}N_3O_4$ 340.1292, found 340.1291.

Ethyl 8-(((benzyloxy)carbonyl)amino)-2-phenylimidazo[1,2-*a*]pyridine-3-carboxylate (3b). Yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.03 (dd, *J* = 7.0, 0.9 Hz, 1H), 8.22 (bs, 1H),

8.10 (d, J = 7.3 Hz, 1H), 7.78 – 7.69 (m, 2H), 7.51 – 7.30 (m, 8H), 7.02 (t, J = 7.3 Hz, 1H), 5.27 (s, 2H), 4.31 (q, J = 7.1 Hz, 2H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.2, 153.4, 152.3, 140.2, 135.9, 134.39, 130.3, 129.0, 128.8, 128.6, 128.5, 127.9, 127.6, 122.2, 115.0, 113.6, 112.0, 67.6, 60.8, 14.2. HRMS (ESI-TOF) (*m/z*) $[M+H]^+$ calcd for $C_{24}H_{22}N_3O_4$, 416.1605, found 416.1605.

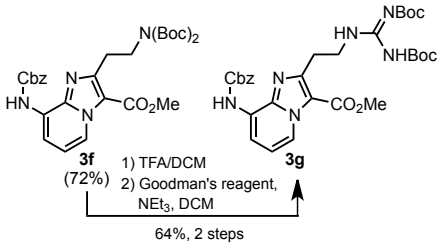
Methyl 8-(((benzyloxy)carbonyl)amino)-2-(but-3-en-1-yl)imidazo[1,2-*a*]pyridine-3-carboxylate (3c). Yield: 78%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (d, J = 6.9 Hz, 1H), 8.10 (s, 1H), 8.03 (d, J = 6.9 Hz, 1H), 7.47 – 7.30 (m, 5H), 6.94 (t, J = 7.3 Hz, 1H), 5.91 (ddt, J = 16.9, 10.2, 6.6 Hz, 1H), 5.25 (s, 2H), 5.07 (ddd, J = 17.1, 3.4, 1.6 Hz, 1H), 4.98 (ddt, J = 10.2, 2.0, 1.1 Hz, 1H), 3.95 (s, 3H), 3.19 – 3.09 (m, 2H), 2.51 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6, 154.6, 153.2, 139.8, 138.00, 137.9, 135.6, 128.7, 128.4, 126.9, 121.8, 115.0, 114.3, 113.6, 111.7, 67.4, 51.5, 33.3, 29.4; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{21}H_{22}N_3O_4$ 380.1605, found 380.1614.

Methyl 8-(((benzyloxy)carbonyl)amino)-2-(((4-methoxybenzyl)oxy)methyl)imidazo[1,2-*a*]pyridine-3-carboxylate (3d). Yield: 39%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.93 (d, J = 7.0 Hz, 1H), 8.21 (s, 1H), 8.08 (d, J = 7.0 Hz, 1H), 7.48 – 7.28 (m, 10H), 7.00 (t, J = 7.3 Hz, 1H), 5.26 (s, 2H), 4.95 (s, 2H), 4.71 (s, 2H), 3.87 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.1, 153.2, 149.8, 140.2, 137.9, 135.7, 128.6, 128.4, 128.3, 128.2, 128.1, 127.7, 127.5, 121.7, 115.0, 114.2, 111.9, 73.1, 67.4, 65.7, 51.6; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{25}H_{24}N_3O_5$ 446.1711, found 446.1702.

Ethyl 8-(((benzyloxy)carbonyl)amino)-2-(4-(methoxymethoxy)phenyl)imidazo[1,2-*a*]pyridine-3-carboxylate (3e). Yield: 75%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.00 (dd, J = 6.9, 0.9

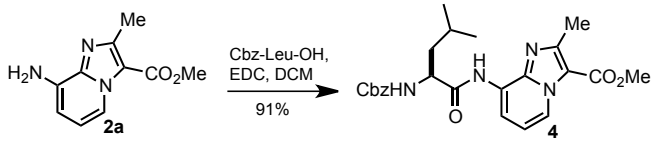
Hz, 1H), 8.23 (s, 1H), 8.07 (d, *J* = 7.7 Hz, 1H), 7.72 (d, *J* = 8.7 Hz, 2H), 7.50 – 7.28 (m, 4H), 7.10 (m, 2H), 6.99 (t, *J* = 7.4 Hz, 1H), 5.25 (s, 2H), 5.23 (s, 2H) 4.33 (q, *J* = 7.1 Hz, 2H), 3.50 (s, 3H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.0, 157.8, 153.2, 139.9, 135.7, 131.6, 131.5, 128.6, 128.4, 128.2, 127.2, 122.0, 116.1, 115.4, 114.6, 113.0, 111.8, 94.3, 67.4, 60.6, 56.0, 14.1; HRMS (ESI-TOF) (*m/z*) $[M+H]^+$ calcd for $C_{26}H_{26}N_3O_6$, 476.1822, found 476.1827.

Methyl 8-(((benzyloxy)carbonyl)amino)-2-(2-(bis(*tert*-butoxycarbonyl)amino)ethyl)imidazo[1,2-*a*]pyridine-3-carboxylate (3f). Yield: 72%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 6.9 Hz, 1H), 8.12 (s, 1H), 8.02 (d, *J* = 6.1 Hz, 1H), 7.49 – 7.31 (m, 5H), 6.95 (t, *J* = 7.3 Hz, 1H), 5.27 (s, 2H), 4.02 (t, *J* = 7.0 Hz, 2H), 3.96 (s, 3H), 3.36 (t, *J* = 7.0 Hz, 2H), 1.42 (s, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 161.6, 153.3, 152.7, 152.2, 140.1, 135.9, 128.8, 128.1, 128.5, 127.1, 121.8, 114.6, 114.2, 111.7, 82.2, 67.6, 51.7, 45.8, 29.6, 28.1; HRMS (ESI-TOF) (*m/z*) $[M+H]^+$ calcd for $C_{29}H_{37}N_4O_8$, 569.2606, found 569.2615.



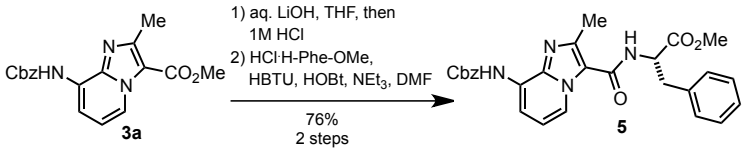
Methyl 8-(((benzyloxy)carbonyl)amino)-2-(2-(2,3-bis(*tert*-butoxycarbonyl)guanidino)ethyl)imidazo[1,2-*a*]pyridine-3-carboxylate (3g). A solution of **3f** (57 mg, 0.10 mmol) in 25% TFA in DCM (4 mL) was stirred for 2 h at rt. The reaction was diluted with EtOAc and concentrated under reduced pressure. This dilution/evaporation sequence

was repeated two more times to remove excess TFA and the crude material was dried under high vacuum. The crude TFA salt was dissolved in DCM (4 mL) and treated with NEt_3 (40 mg, 0.4 mmol) and 1,3-di-Boc-2-(trifluoromethyl sulfonyl)guanidine (47 mg, 0.12 mmol). The mixture was stirred for 3 h at rt. The mixture was then concentrated under reduced pressure and the residue was purified by silica gel flash chromatography (10% EtOAc/hexanes eluent) to give **3g** as white solid. (39 mg, 64% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.49 (s, 1H), 9.40 (s, 1H), 8.96 (d, J = 6.9 Hz, 1H), 8.32 (s, 1H), 8.08 (d, J = 7.6 Hz, 1H), 7.46 – 7.30 (m, 5H), 6.98 (t, J = 7.3 Hz, 1H), 5.26 (s, 2H), 3.94 (s, 3H), 3.92 – 3.84 (m, 2H), 3.33 (t, J = 6.0 Hz, 1H), 1.50 (s, 9H), 1.38 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 163.6, 161.4, 155.8, 153.5, 152.8, 152.2, 140.0, 135.8, 128.6, 128.4, 128.3, 127.2, 121.8, 114.6, 113.6, 112.8, 82.8, 79.3, 67.3, 51.5, 39.6, 28.7, 28.3, 27.9; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{30}H_{38}N_6O_8$ 611.2824, found 611.2821.



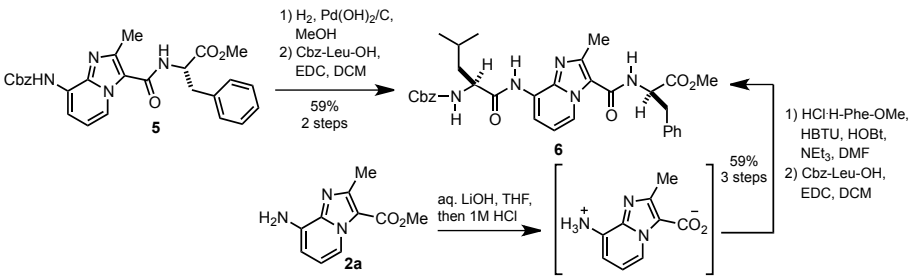
Cbz-Leu-IP(Me)-OMe (4). To a solution of Cbz-Leu-OH (265 mg, 1.00 mmol) and **2a** (103 mg, 0.50 mmol) in DCM (10 mL) was added EDC·HCl (192 mg, 1.00 mmol) at rt. After stirring 24 h at rt, the solvent was removed under reduced pressure and the residue was purified by silica gel flash chromatography (20% EtOAc/hexanes eluent) to give **4** as a pale yellow form (207 mg, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.93 (d, J = 6.9 Hz, 1H), 8.29 (d, J = 7.7 Hz, 1H), 7.39 – 7.22 (m, 5H), 6.90 (t, J = 7.3 Hz, 1H), 5.54 (d, J = 7.9 Hz, 1H), 5.13 (s, 2H), 4.59 – 4.48 (m, 1H), 3.96 (s, 3H), 2.67 (s, 3H), 1.83 – 1.75 (m, 2H), 1.69 – 1.61 (m, 1H), 0.96 (d, J =

5.9 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 161.8, 156.5, 151.4, 140.0, 136.4, 128.7, 128.4, 128.3, 126.4, 123.0, 114.7, 114.5, 114.0, 67.4, 54.8, 51.7, 41.8, 25.0, 23.3, 22.0, 16.5; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{24}H_{29}N_4O_5$ 453.2133, found 453.2151.



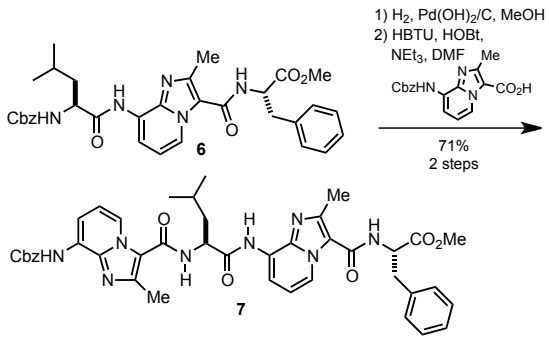
Cbz-IP(Me)-Phe-OMe (5). A solution of **3a** (154 mg, 0.45 mmol) and $LiOH \cdot H_2O$ (38 mg, 0.90 mmol) in 6 mL of 1:1 THF:H_2O was stirred for 17 h at rt. The reaction was quenched with 1M aq. HCl (0.45 mL, 0.45 mmol) and concentrated. The hydrolyzed intermediate was dried under high vacuum to remove excess water. The crude mixture was dissolved in DMF (6 mL) and treated with NEt_3 (184 mg, 1.80 mmol), H-Phe-OMe · HCl (117 mg, 0.54 mmol), HBTU (206 mg, 0.54 mmol), and HOBt (12 mg, 0.09 mmol). The reaction was stirred for 24 h at rt. The solvent was evaporated under reduced pressure and the residue was diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, and filtered. The organic layers were concentrated under reduced pressure and the residue was purified by silica gel flash chromatography (30% EtOAc/hexanes eluent) to give **5** as pale yellow foam (167 mg, 76% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.97 (d, *J* = 7.0 Hz, 1H), 8.00 (s, 1H), 7.96 (d, *J* = 7.1 Hz, 1H), 7.45 – 7.33 (m, 4H), 7.33 – 7.23 (m, 4H), 7.13 (dd, *J* = 7.7, 1.6 Hz, 2H), 6.88 (t, *J* = 7.3 Hz, 1H), 6.22 (d, *J* = 7.2 Hz, 1H), 5.24 (s, 2H), 5.14 – 5.06 (m, 1H), 3.81 (s, 3H), 3.33 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.24 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.44 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 160.7, 153.2, 144.8, 139.1, 135.7, 135.6, 129.3, 128.7, 128.6, 128.4, 128.3, 127.4, 126.5, 121.9, 116.3, 113.9,

111.2, 67.3, 53.2, 52.6, 37.3, 16.2; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{27}H_{27}N_4O_5$ 487.1976, found 487.1974.



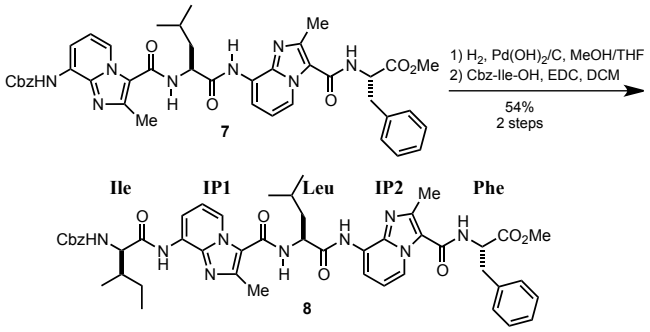
Cbz-Leu-IP(Me)-Phe-OMe (6). A mixture of **5** (107 mg, 0.22 mmol) and a catalytic amount of 10% $Pd(OH)_2/C$ (0.2 g/mmol, 44 mg) in MeOH (6 mL) was stirred under H_2 atmosphere (balloon) for 1 h at rt. The crude mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude amine was dissolved in DCM (6 mL) and treated with Cbz-Leu-OH (117 mg, 0.44 mmol) and EDC·HCl (85 mg, 0.44 mmol). After stirring 24 h at rt, the mixture was concentrated and the residue was purified by silica gel flash chromatography (30% EtOAc/hexanes) to give **6** as pale yellow solid. (78 mg, 59% over 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 9.00 (d, *J* = 6.9 Hz, 1H), 8.23 (d, *J* = 7.6 Hz, 1H), 7.42 – 7.22 (m, 8H), 7.19 – 7.08 (m, 2H), 6.85 (t, *J* = 7.3 Hz, 1H), 6.27 (d, *J* = 7.1 Hz, 1H), 5.43 (d, *J* = 8.0 Hz, 1H), 5.13 (s, 2H), 5.11 – 5.05 (m, 1H), 4.53 – 4.44 (m, 1H), 3.81 (s, 3H), 3.34 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.24 (dd, *J* = 13.9, 5.7 Hz, 1H), 2.45 (s, 3H), 1.79 – 1.70 (m, 2H), 1.68 – 1.60 (m, 1H), 0.99 – 0.92 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.6, 160.6, 156.2, 144.8, 139.3, 136.1, 135.6, 129.3, 128.7, 128.5, 128.2, 128.0, 127.4, 126.0, 122.9, 116.3, 113.8, 113.6, 67.2, 54.4, 53.2, 52.6, 41.6, 37.7, 24.8, 23.0, 21.8, 16.1; HRMS (ESI-TOF) *m/z* $[M + H]^+$ calcd for $C_{33}H_{38}N_5O_6$ 600.2817, found 600.2808.

Alternatively, **6** could be prepared from **2a** in improved overall yield using the following procedure: A solution of **2a** (205 mg, 1.00 mmol) and $LiOH \cdot H_2O$ (126 mg, 3.00 mmol) in 8 mL of 1:1 THF:H_2O was stirred for 17 h at rt. The reaction was quenched with 2 mL of 1M aq. HCl and concentrated under reduced pressure. The hydrolyzed intermediate dried under high vacuum to remove excess water. The crude zwitterion was dissolved in DMF (10 mL) and treated with NEt_3 (405 mg, 4.00 mmol), $HCl \cdot H_2N$-Phe-OMe (216 mg, 1.00 mmol), HBTU (455 mg, 1.20 mmol), and HOBt (27 mg, 0.20 mmol). The reaction was stirred for 24 h at rt. The reaction was concentrated and the remaining residue was diluted with EtOAc, washed with water and brine, and dried over $MgSO_4$. The organic layers were concentrated under reduced pressure and the residue was purified by passage through a silica gel pad (50% EtOAc/hexanes eluent) to give the amide intermediate. To a solution of this amide in 10 mL of DCM was added Cbz-Leu-OH (530 mg, 2.00 mmol) and EDC·HCl (383 mg, 2.00 mmol) at rt. After stirring 24 h at rt, the reaction was concentrated, and the residue was purified by silica gel flash chromatography (30% EtOAc/hexanes eluent) to give **6** as pale yellow solid (353 mg, 59% over 3 steps).



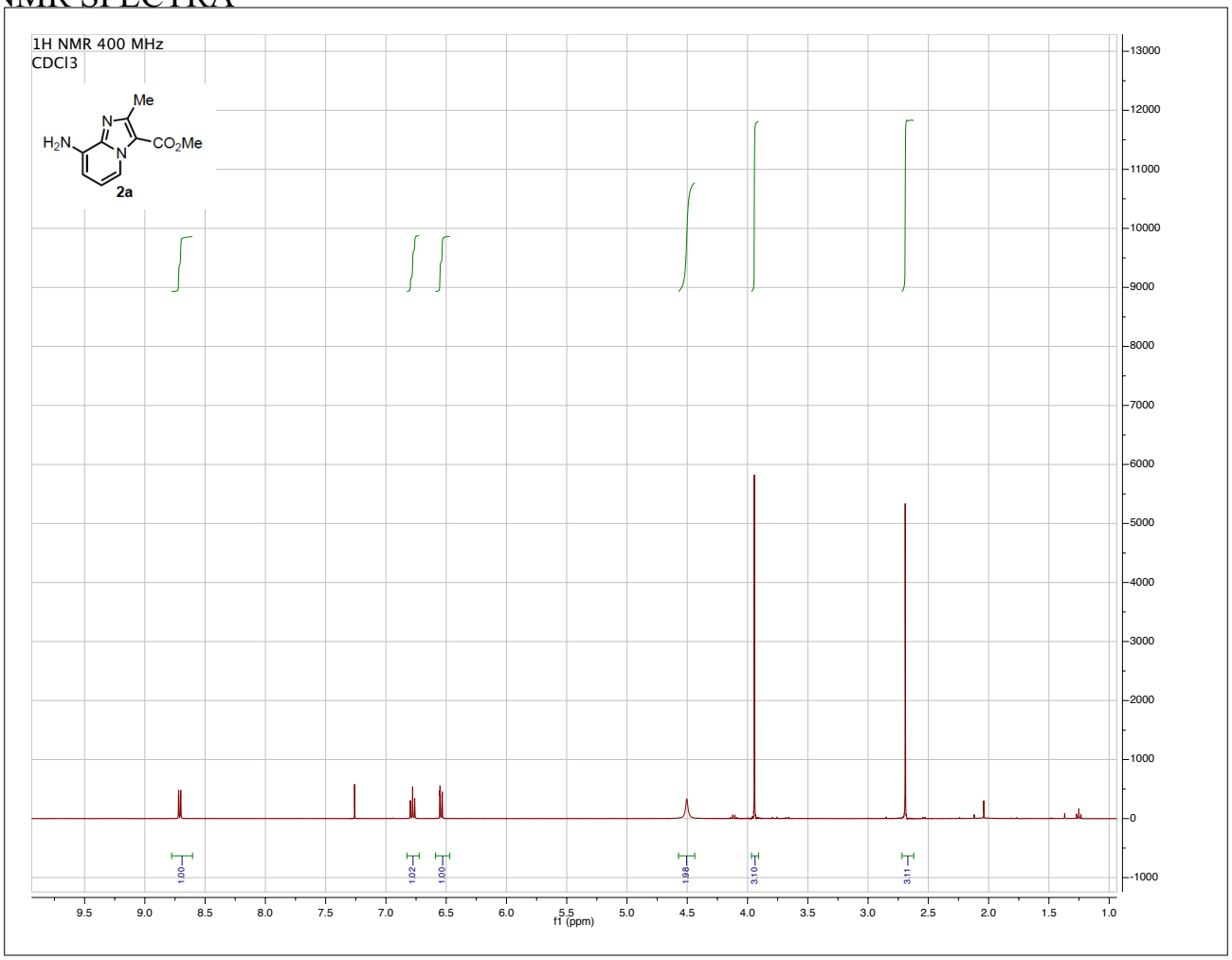
Cbz-IP1(Me)-Leu-IP2(Me)-Phe-OMe (7). A mixture of **6** (60 mg, 0.10 mmol) and a catalytic amount of 10% $Pd(OH)_2/C$ (20 mg) in MeOH (5 mL) was stirred under H_2 atmosphere

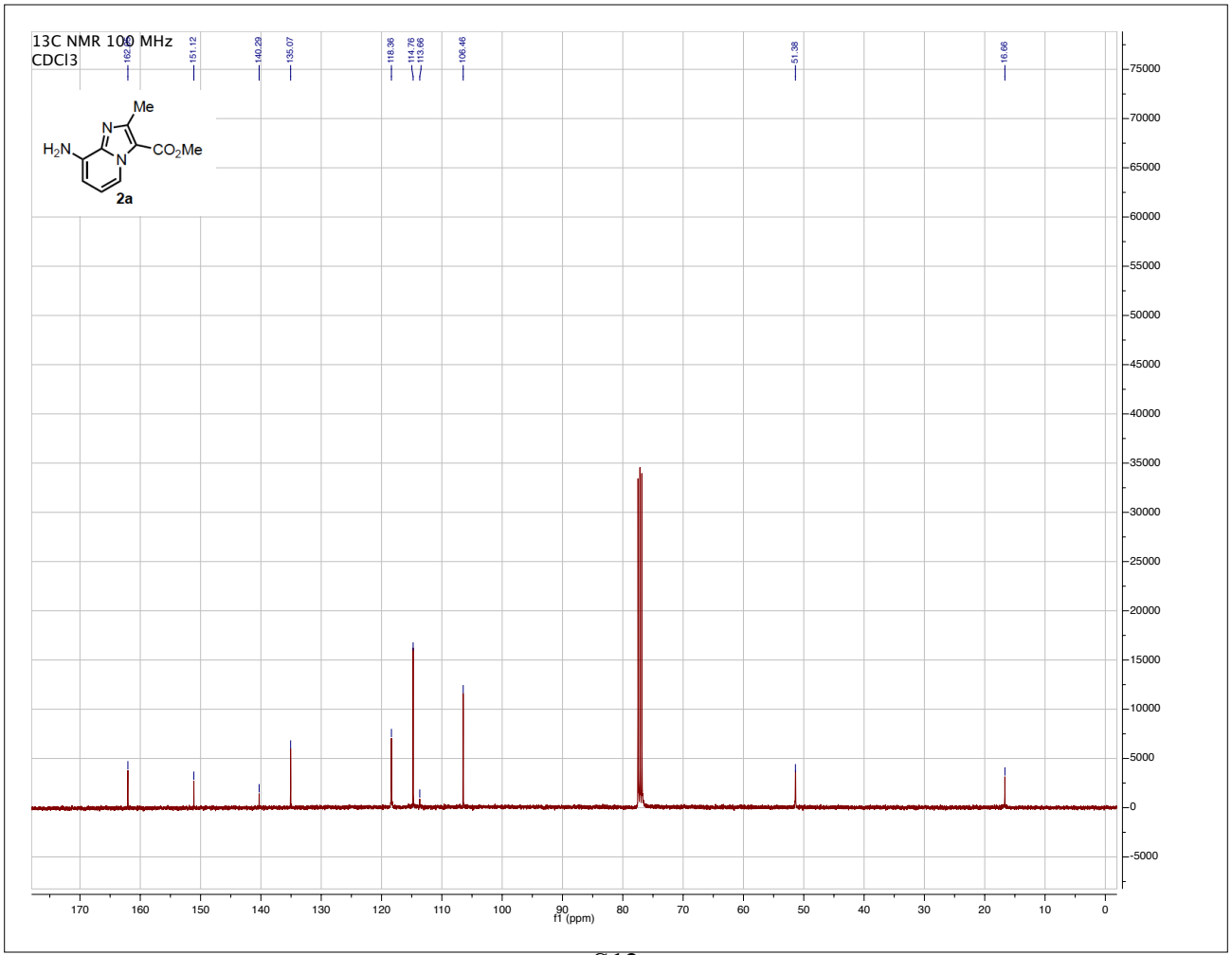
(balloon) for 1 h at rt. The crude mixture was filtered through celite and the filtrate was concentrated to give the crude amine. Separately, a solution of **3a** (40 mg, 0.12 mmol) and $LiOH \cdot H_2O$ (10 mg, 0.24 mmol) in 3 mL of 1:1 THF:H_2O was stirred for 17 h at rt. The reaction was neutralized with 120 μL of 1M aq. HCl and concentrated. The hydrolyzed intermediate was dried under high vacuum. Both the crude carboxylic acid and amine from above crude materials were dissolved in DMF (8 mL) and treated with NEt_3 (47 mg, 0.48 mmol), HBTU (53 mg, 0.14 mmol), and HOBt (3.2 mg, 23 μmol). The reaction was stirred for 24 h at rt. The mixture was concentrated and the remaining residue diluted with EtOAc, washed with water and brine, and dried over $MgSO_4$. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel flash chromatography (50% EtOAc/hexanes eluent) to give **7** as pale yellow solid. (55 mg, 71% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.34 (s, 1H), 9.03 (dd, *J* = 6.9, 1.0 Hz, 1H), 9.02 (dd, *J* = 6.9, 1.0 Hz, 1H), 8.25 (d, *J* = 7.3 Hz, 1H), 8.04 (s, 1H), 7.97 (d, *J* = 6.8 Hz, 1H), 7.45 – 7.26 (m, 8H), 7.13 (dd, *J* = 7.6, 1.8 Hz, 2H), 6.88 (t, *J* = 7.2 Hz, 1H), 6.87 (t, *J* = 7.6 Hz, 1H), 6.35 (d, *J* = 6.4 Hz, 1H), 6.24 (d, *J* = 7.3 Hz, 1H), 5.25 (s, 2H), 5.09 (dd, *J* = 12.8, 5.6 Hz, 1H), 5.01 (dd, *J* = 11.0, 5.9 Hz, 1H), 3.82 (s, 3H), 3.34 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.24 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.77 (s, 3H), 2.46 (s, 3H), 1.91 – 1.75 (m, 3H), 1.04 – 1.01 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.4, 161.3, 160.6, 153.2, 144.8, 144.7. 139.3, 139.2, 135.7, 135.6, 129.3, 128.7, 128.6, 128.4, 128.3, 127.4, 126.5, 126.0, 123.0, 122.0, 116.4, 116.3, 113.9, 113.8, 113.7, 111.3, 67.3, 53.2, 52.7, 52.6, 41.6, 37.7, 25.1, 23.1, 22.0, 16.5, 16.2; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{42}H_{45}N_8O_7$ 773.3406, found 773.3401.

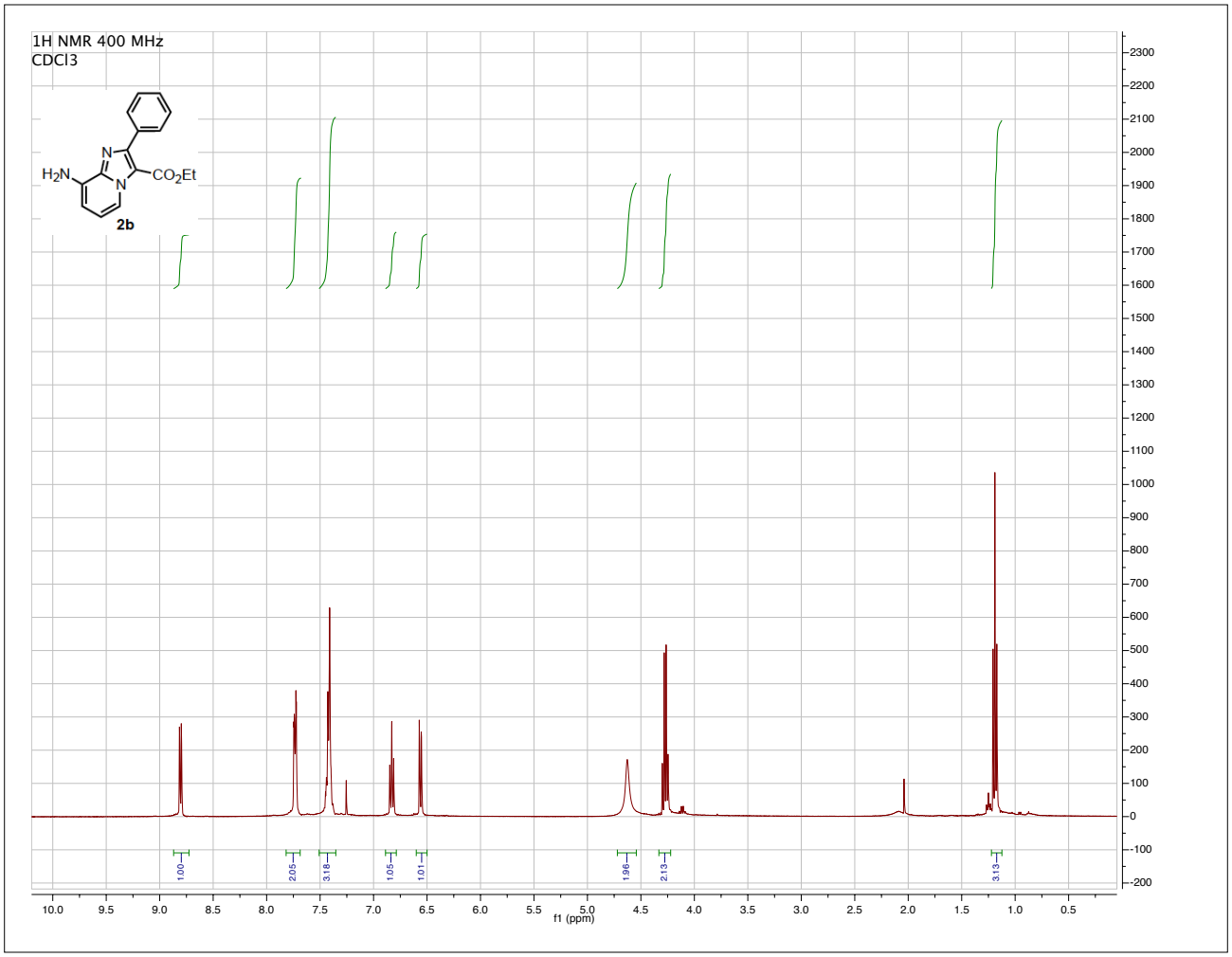


Cbz-Ile-IP1(Me)-Leu-IP2(Me)-Phe-OMe (8). A mixture of **7** (24.5 mg, 0.032 mmol) and a catalytic amount of 10% $Pd(OH)_2/C$ (6 mg) in 6 mL of 2:1 MeOH:THF was stirred under H_2 atmosphere (balloon) for 1 h at rt. The crude mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The crude amine was dissolved in DCM (6 mL) and treated with Cbz-Ile-OH (17 mg, 64 μmol) and EDC·HCl (12 mg, 64 μmol). After stirring 24 h at rt, the solvent was evaporated, and the residue was purified by silica gel flash chromatography (2:1:1 EtOAc:hexanes:$CHCl_3$ eluent) to give **8** as pale yellow solid. (15 mg, 54% for 2 steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.37 (s, 1H), 9.09 (s, 1H), 9.05 (d, *J* = 6.9 Hz, 1H), 9.02 (d, *J* = 6.9 Hz, 1H), 8.26 (d, *J* = 8.0 Hz, 1H), 8.24 (d, *J* = 7.8 Hz, 1H), 7.42 – 7.23 (m, 8H), 7.13 (d, *J* = 6.1 Hz, 2H), 6.87 (t, *J* = 7.5 Hz, 1H), 6.85 (t, *J* = 7.6 Hz, 1H), 6.45 (d, *J* = 7.8 Hz, 1H), 6.26 (d, *J* = 7.2 Hz, 1H), 5.50 (d, *J* = 8.7 Hz, 1H), 5.14 (s, 2H), 5.09 (dd, *J* = 12.7, 5.7 Hz, 1H), 5.04 – 4.96 (m, 1H), 4.51 – 4.26 (m, 1H), 3.81 (s, 3H), 3.33 (dd, *J* = 13.9, 5.6 Hz, 1H), 3.24 (dd, *J* = 13.9, 5.6 Hz, 1H), 2.77 (s, 3H), 2.46 (s, 3H), 2.02 – 1.95 (m, 1H), 1.95 – 1.88 (m, 1H), 1.86 – 1.77 (m, 2H), 1.61 – 1.51 (m, 1H), 1.22 – 1.14 (m, 1H), 1.05 – 0.86 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.1, 171.4, 170.8, 161.2, 160.6, 144.8, 144.7, 139.3, 139.2, 135.6, 129.3, 128.7, 128.5, 128.2, 128.1, 127.4, 126.0, 125.9, 123.1, 123.0, 116.5, 116.3, 116.0, 113.8, 113.7, 113.5, 67.2, 60.45, 53.2, 52.6, 52.5, 41.6, 37.7, 29.9, 25.1, 24.6, 23.1, 22.0, 16.5, 16.2, 15.6, 11.4; HRMS (ESI-TOF) *m*/*z* $[M + H]^+$ calcd for $C_{48}H_{56}N_9O_8$ 886.4246, found 886.4227.

1D NMR SPECTRA



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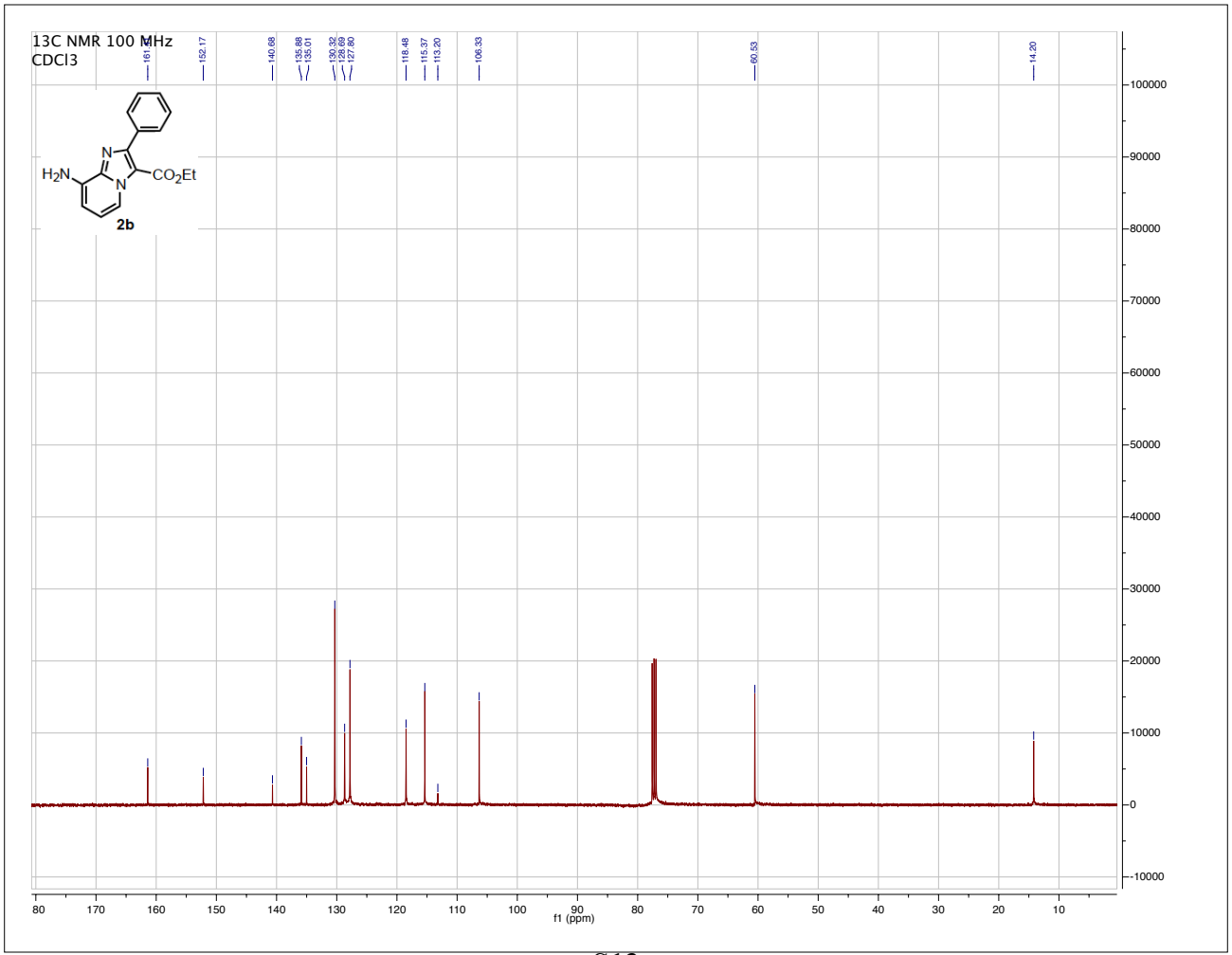
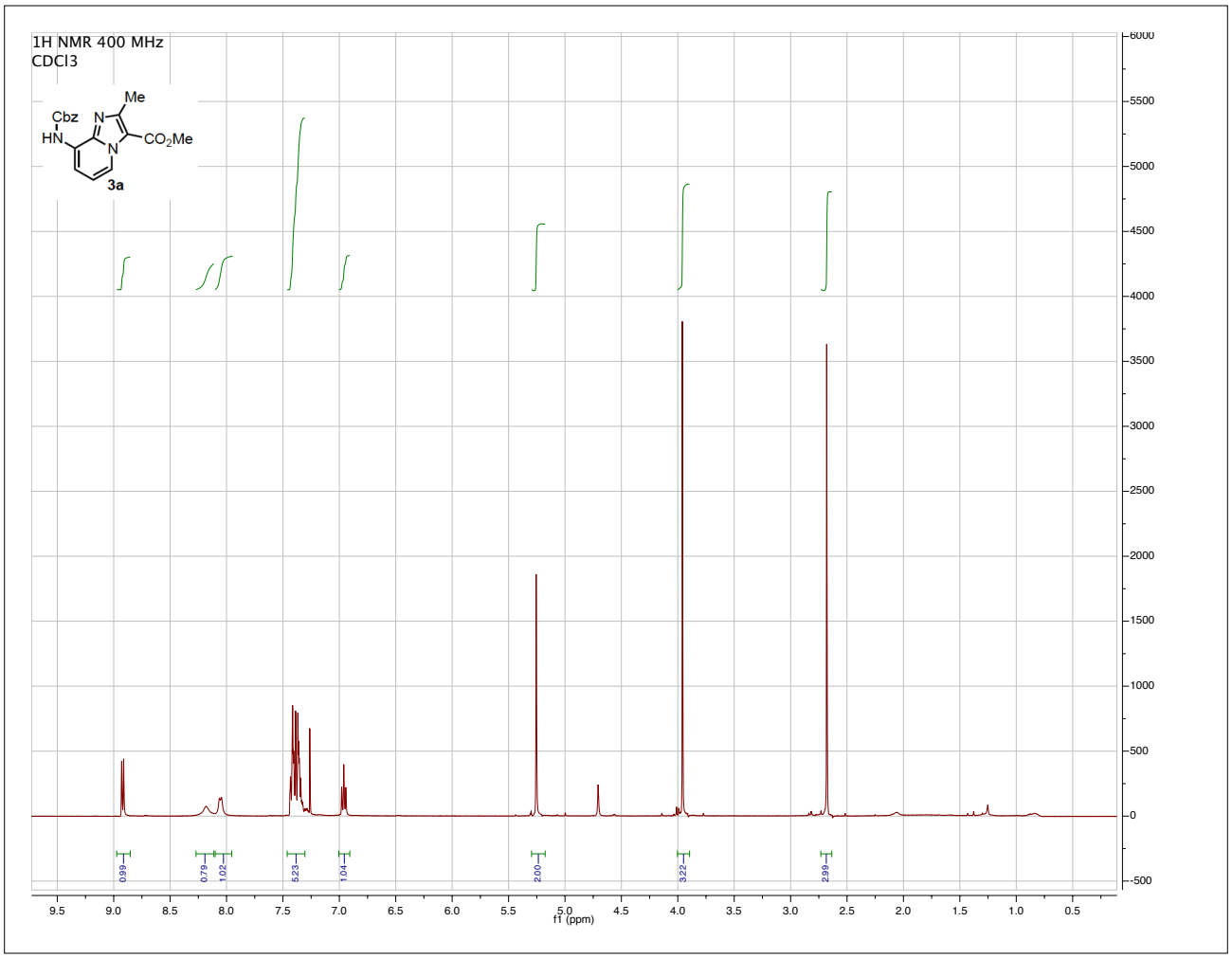
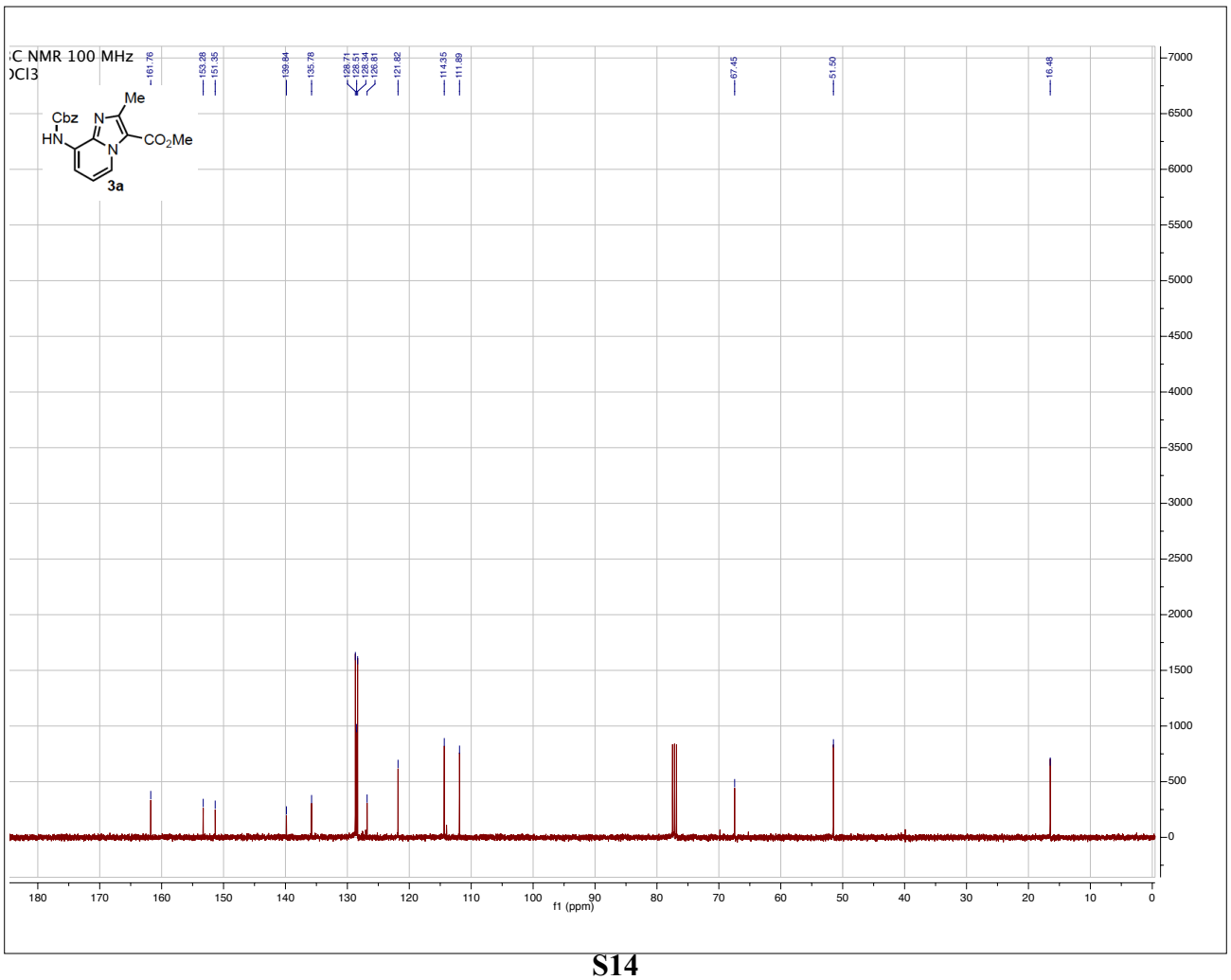
CDCl3
H2N
CO2Et
2b
1.00
2.05
3.18
1.05
1.01
1.96
2.13
3.13
f1 (ppm)


13C NMR 100 MHz
CDCl3
161
152.17
140.68
135.88
135.01
130.32
128.69
127.80
118.48
115.37
113.20
106.33
60.53
14.20
H2N
CO2Et
2b
f1 (ppm)


1H NMR 400 MHz
CDCl3
Me
Cbz
HN
N
N
CO2Me
3a
0.99
0.79
1.02
5.23
1.04
2.00
3.22
2.99
f1 (ppm)


C NMR 100 MHz
DCl3
Me
Cbz
HN
N
N
CO2Me
3a
161.76
153.28
151.35
139.84
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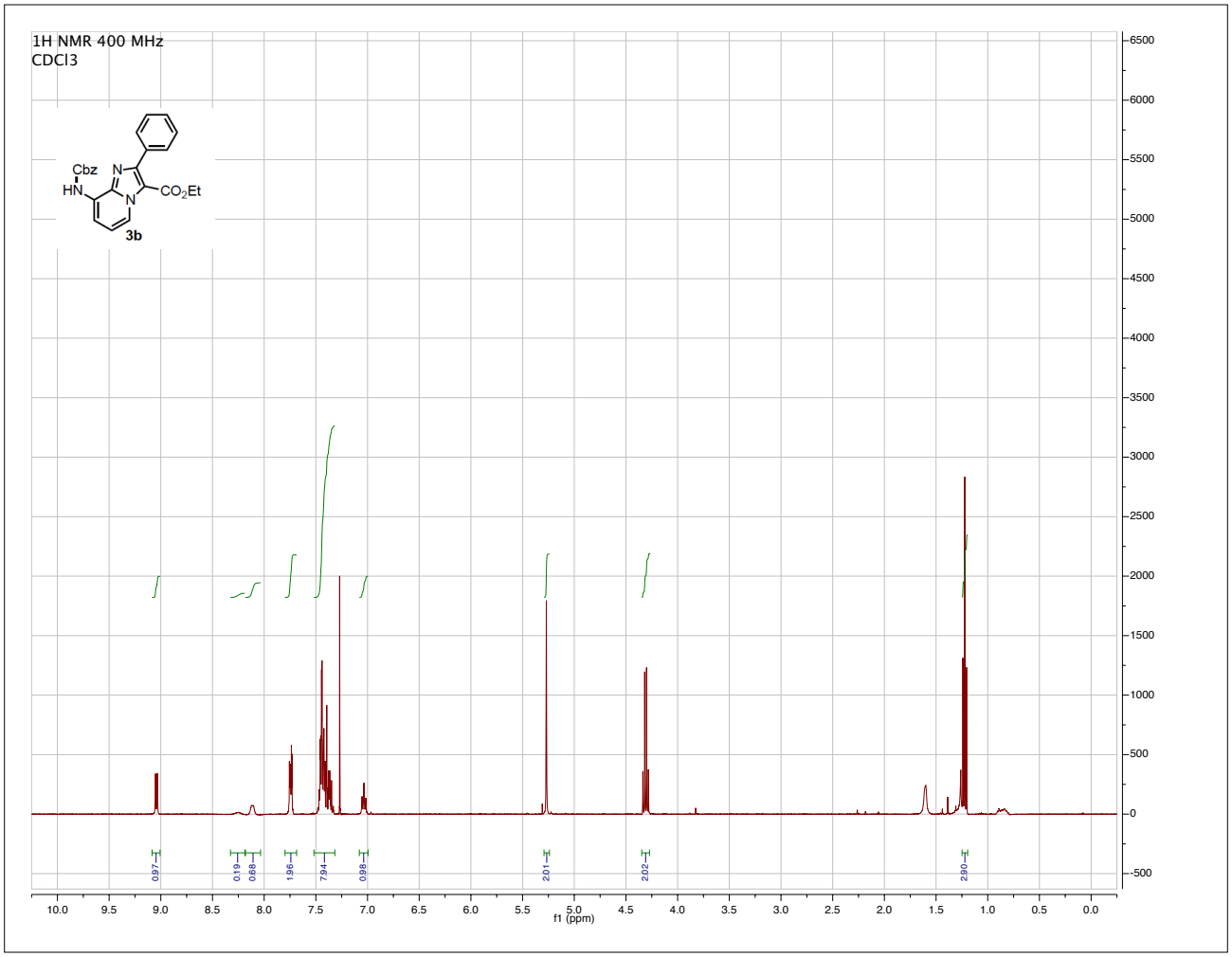
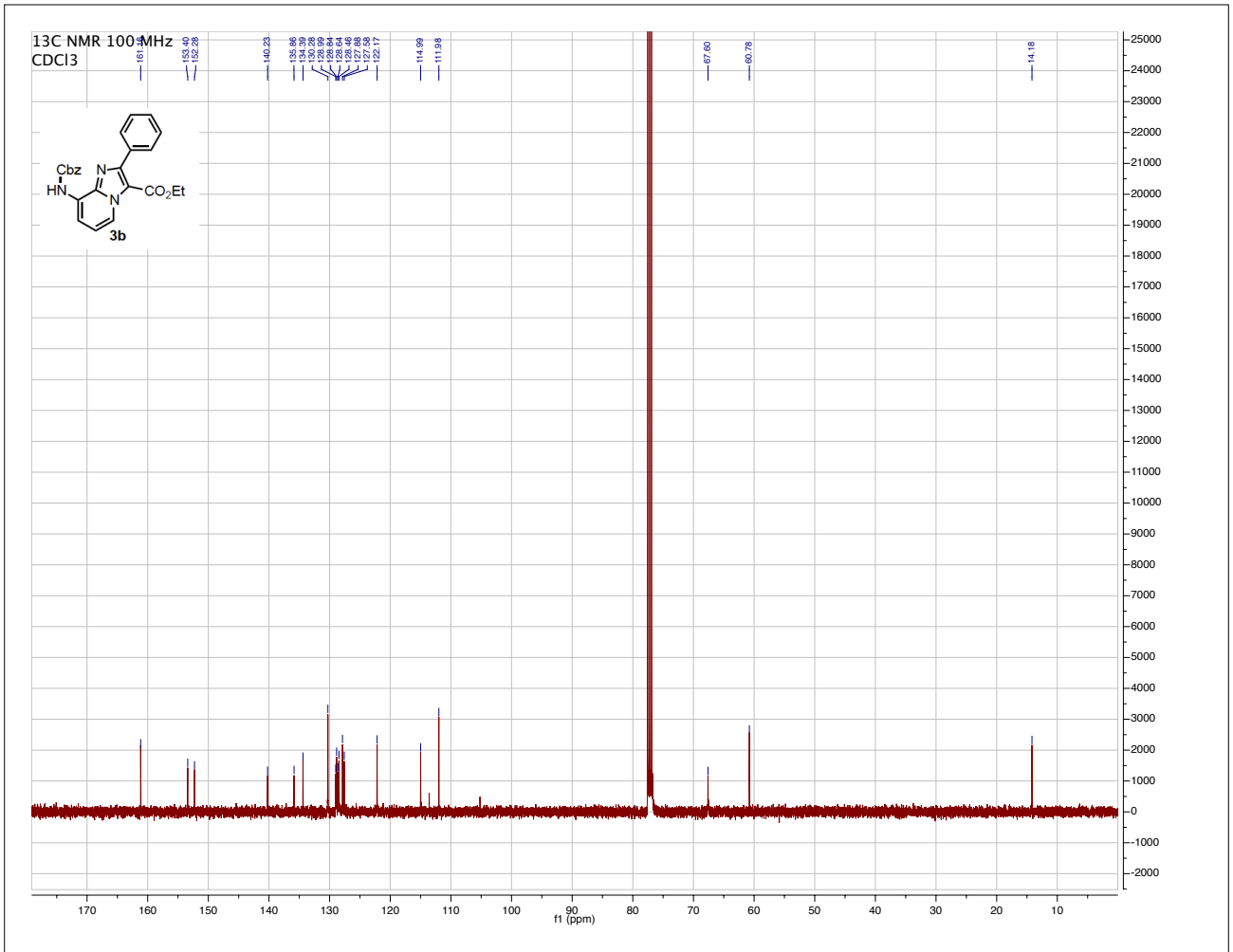
111.89
67.45
51.50
16.48
f1 (ppm)


1H NMR 400 MHz
CDCl3
Cbz
HN
N
N
CO2Et
3b
0.97
0.19
0.68
1.96
7.94
0.98
2.01
2.02
2.90
f1 (ppm)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153.40
152.28
140.23
135.86
134.39
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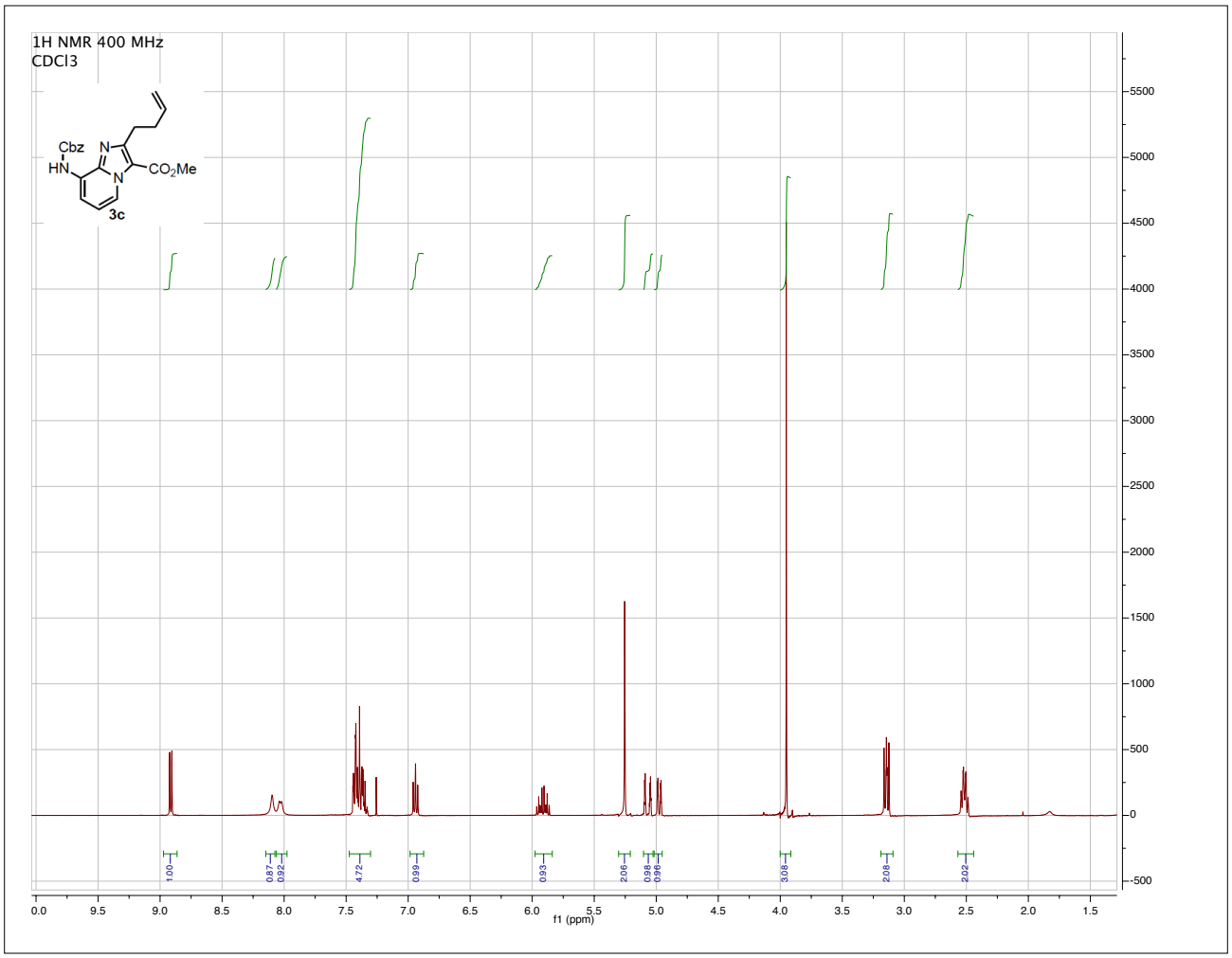
129.89
128.84
128.64
128.46
127.88
127.58
122.17
114.99
111.98
67.60
60.78
14.18
Cbz
HN
N
N
CO2Et
3b
f1 (ppm)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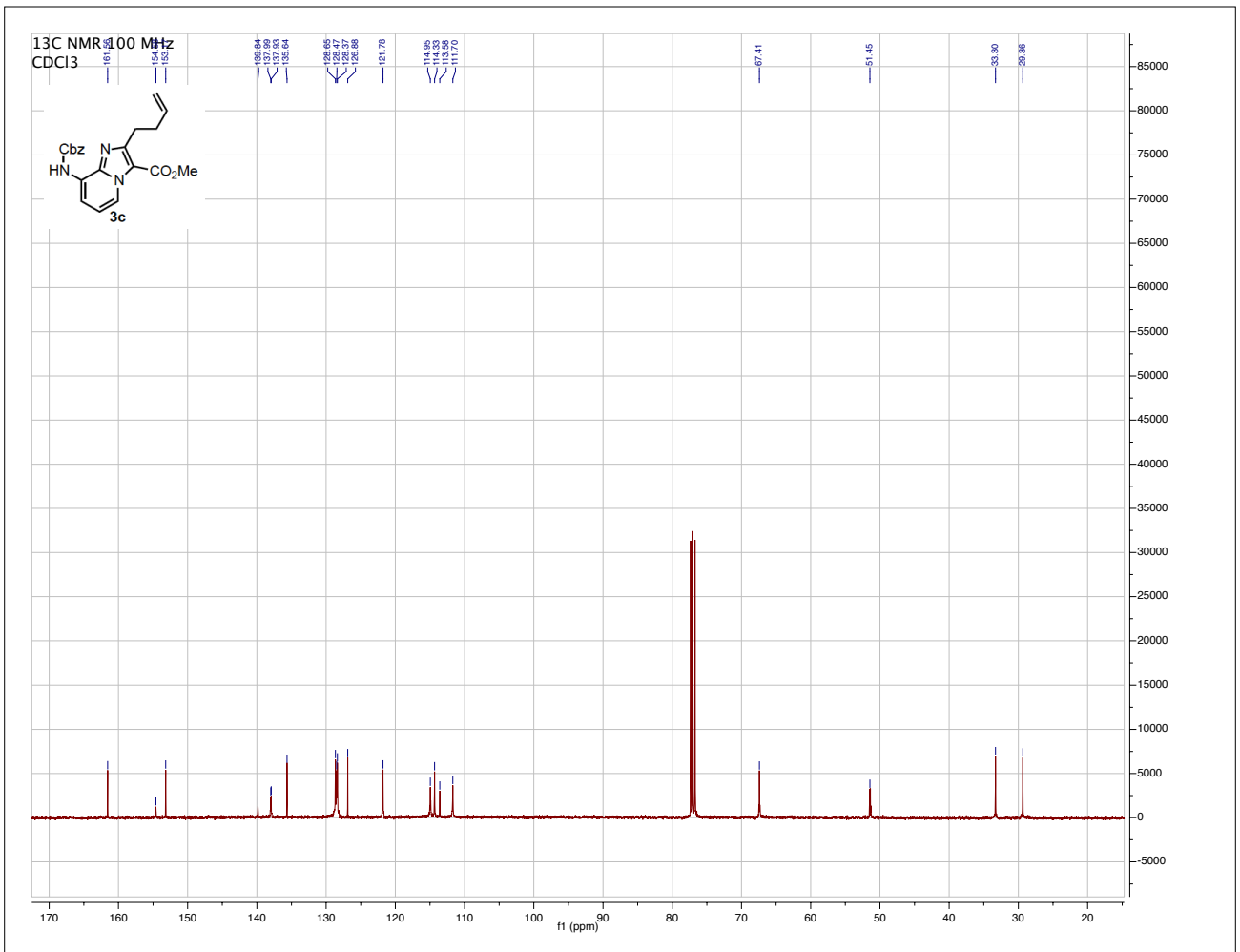
CDCl3
Cbz
HN
N
N
CO2Me
3c
1.00
0.87
0.92
4.72
0.99
0.93
2.06
0.98
0.96
3.08
2.08
2.02
f1 (ppm)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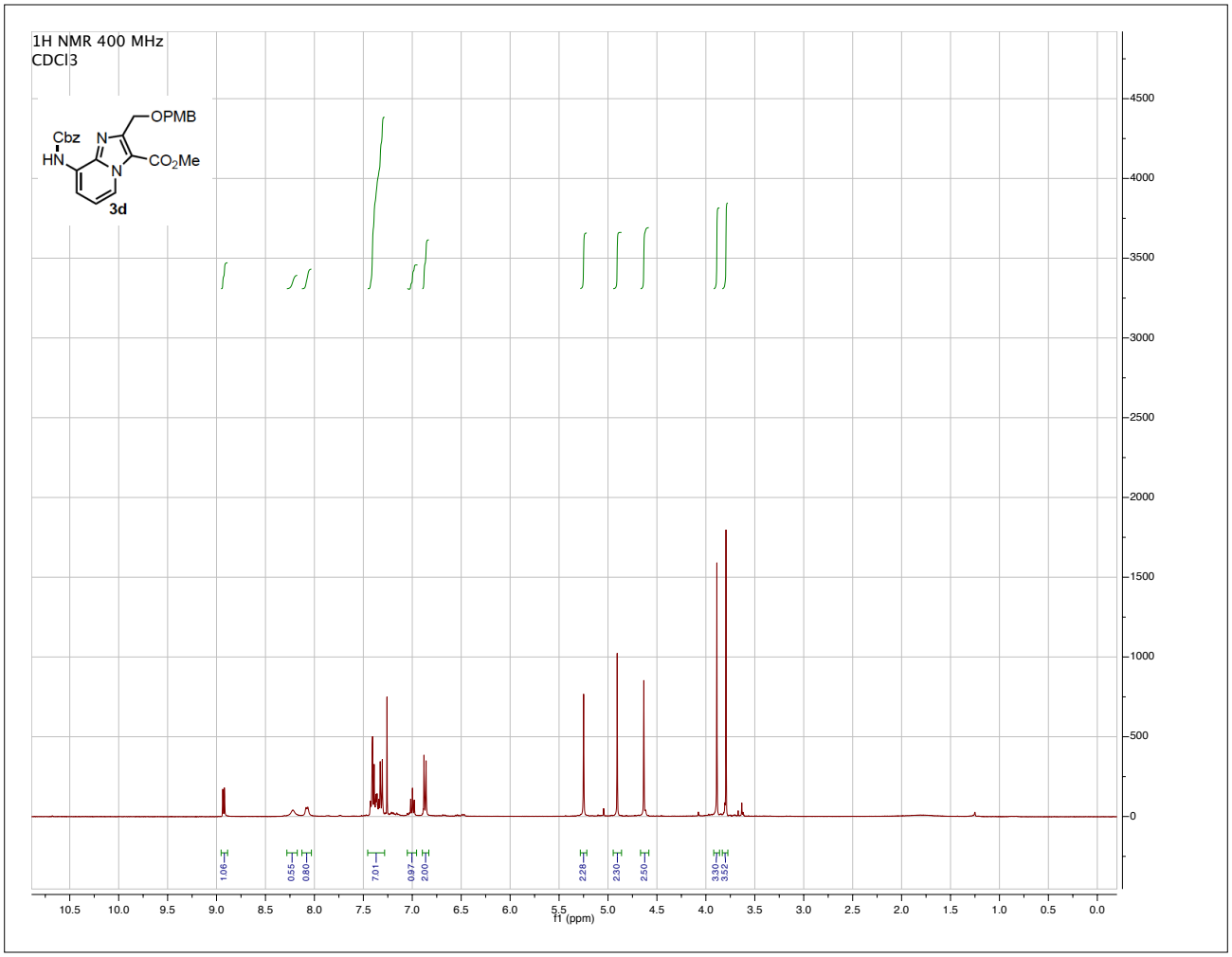
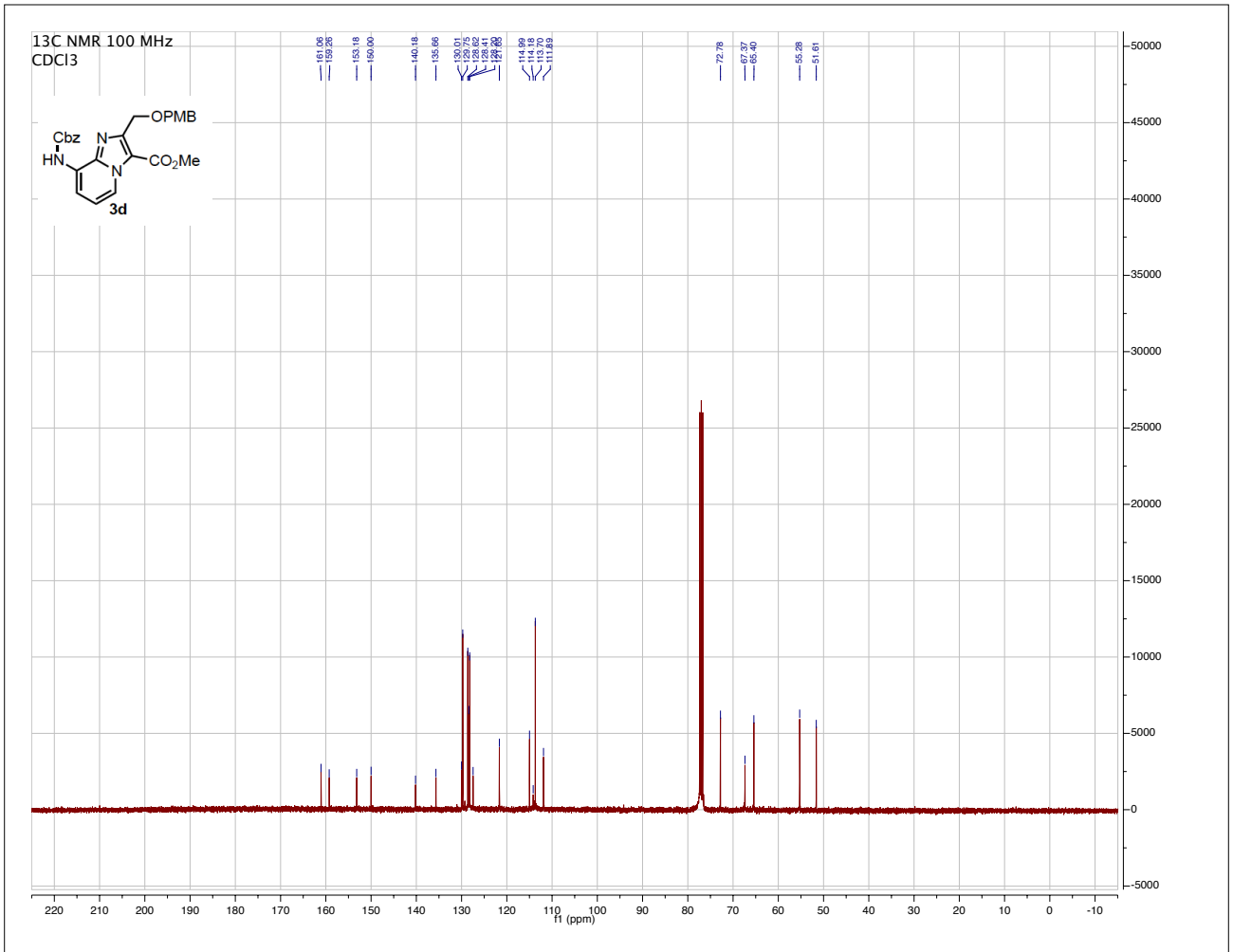
CDCl3
Cbz
HN
N
N
CO2Me
3c
161.95
154.11
153.
139.84
137.89
137.93
135.64
128.65
128.47
128.37
126.88
121.78
114.95
114.33
113.58
111.70
67.41
51.45
33.30
29.36
f1 (ppm)


1H NMR 400 MHz
CDCl3
OPMB
Cbz
HN
N
N
CO2Me
3d
1.06
0.55
0.80
7.01
0.97
2.00
2.28
2.30
2.50
3.30
3.52
f1 (ppm)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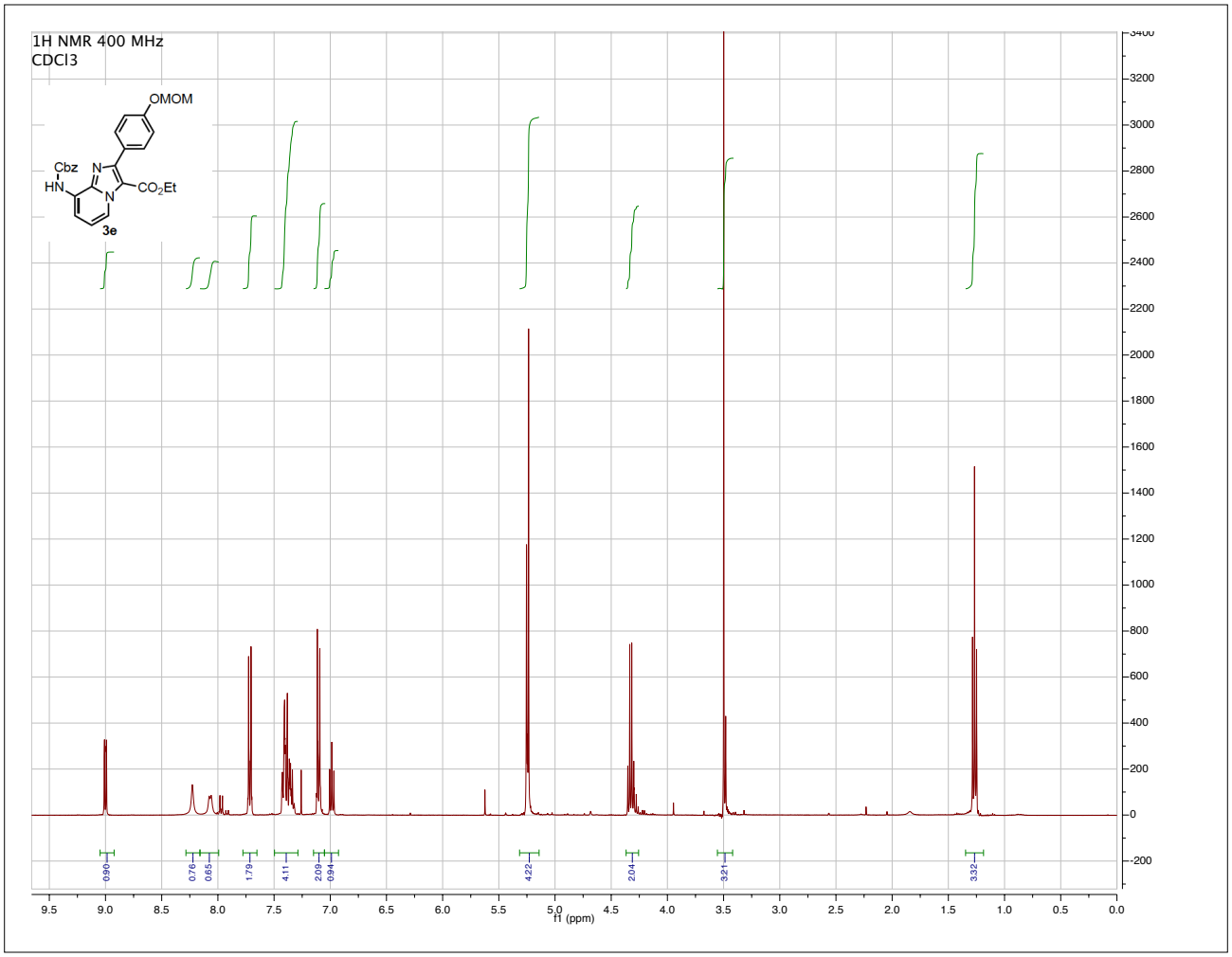
CDCl3
OPMB
Cbz
HN
N
N
CO2Me
3d
161.06
159.26
153.18
150.00
140.18
135.66
130.01
129.75
128.82
128.41
114.99
114.45
113.70
111.89
72.78
67.37
65.40
55.28
51.61
f1 (ppm)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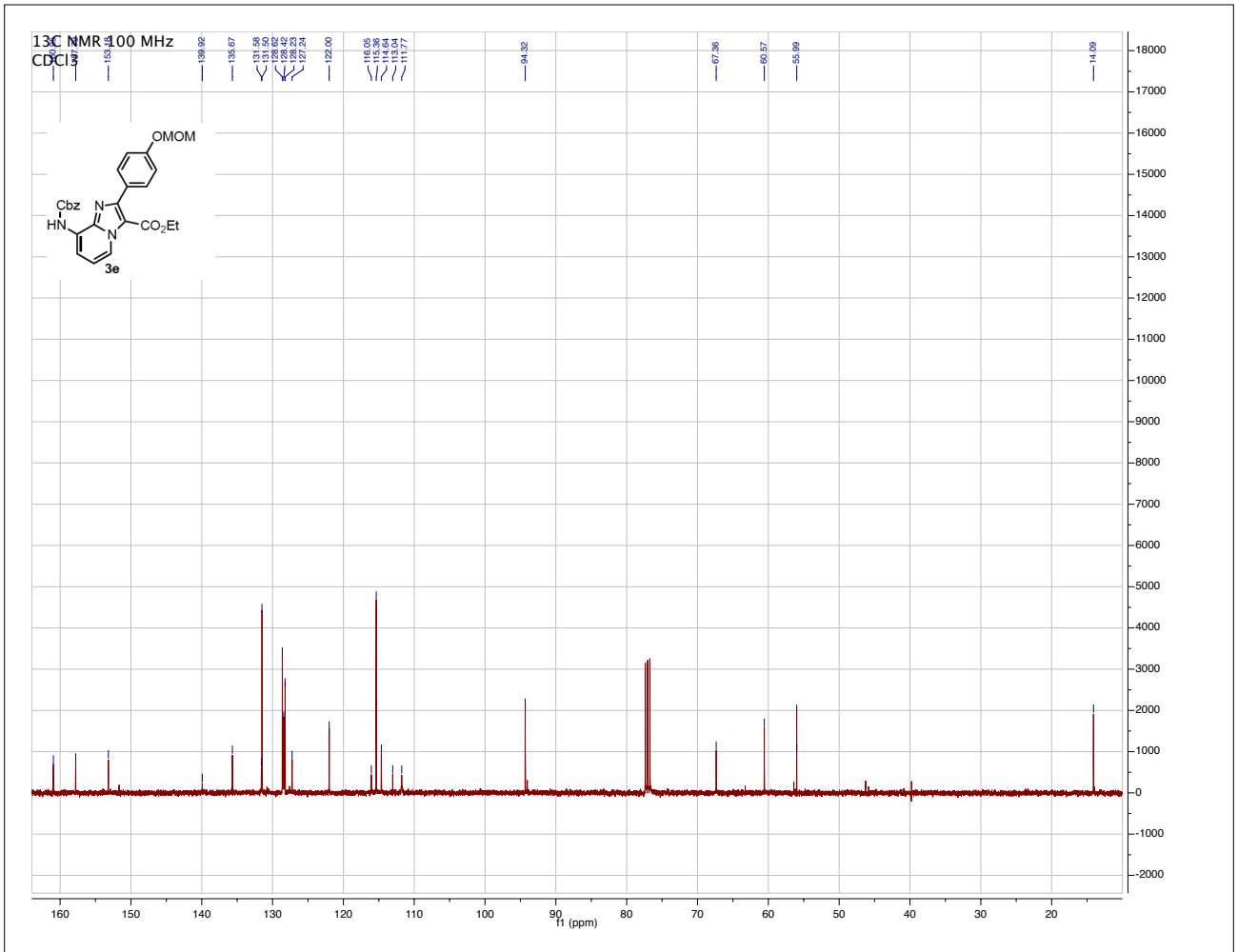
CDCl3
OMOM
Cbz
HN
N
N
CO2Et
3e
0.90
0.76
0.65
1.79
4.11
2.09
0.94
4.22
2.04
3.21
3.32
f1 (ppm)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CDCl3
153.47
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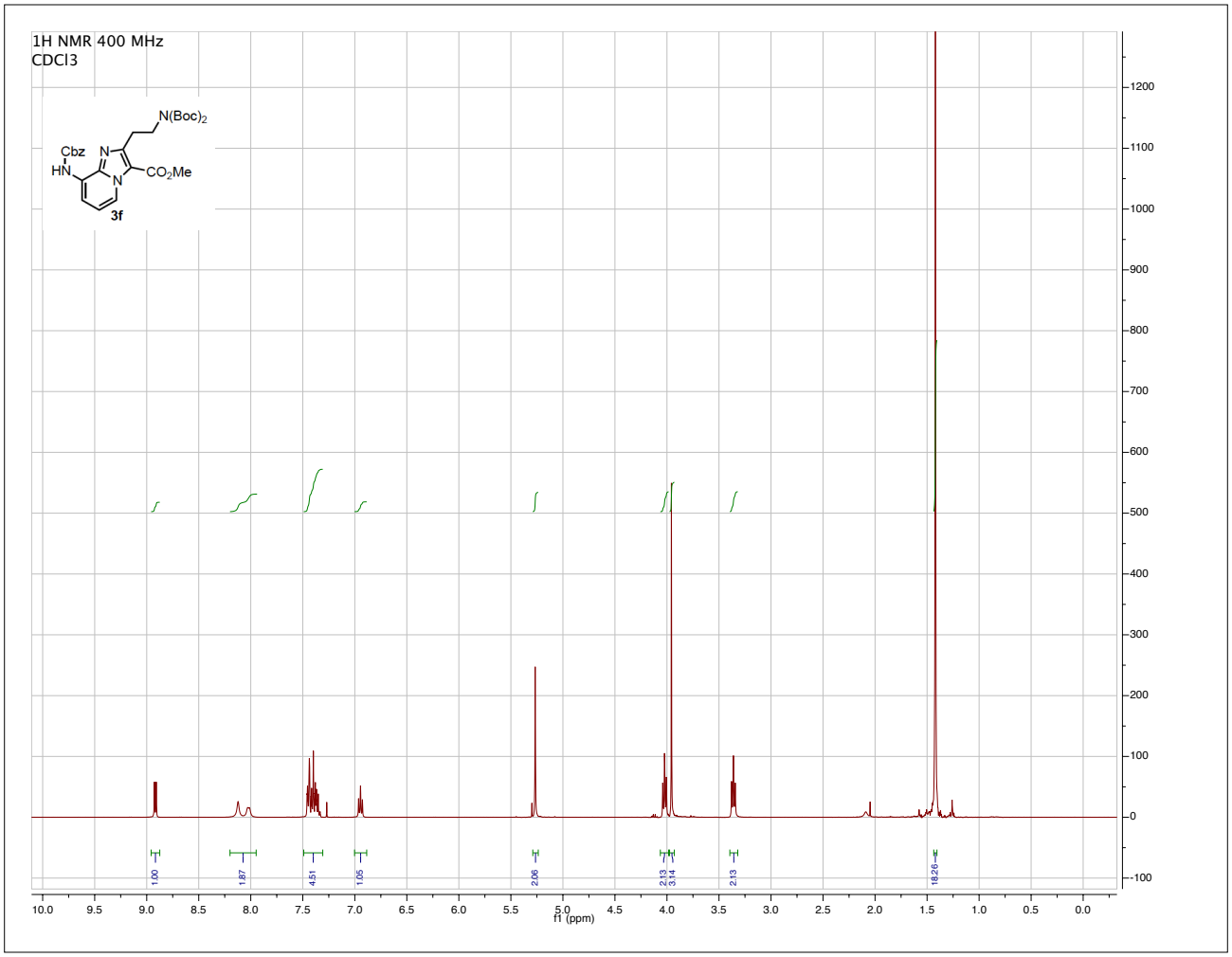
135.67
131.58
131.50
128.62
128.42
128.23
127.24
122.00
116.05
115.86
114.34
113.04
111.77
94.82
67.36
60.57
55.99
14.09
OMOM
Cbz
HN
N
N
CO2Et
3e
f1 (ppm)


1H NMR 400 MHz
CDCl3
N(Boc)2
Cbz
HN
CO2Me
3f
1.00
1.87
4.51
1.05
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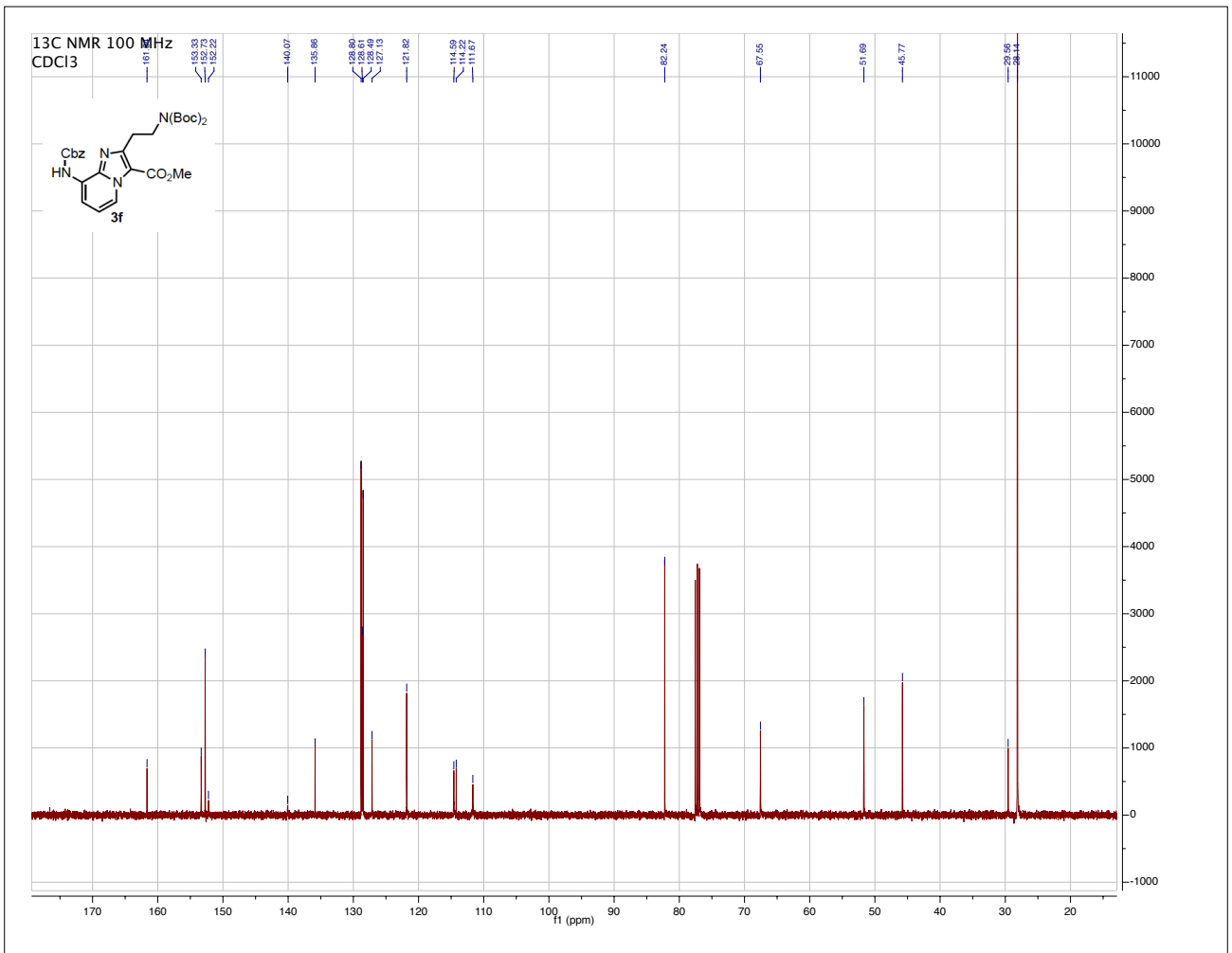
2.13
3.14
2.13
18.26
f1 (ppm)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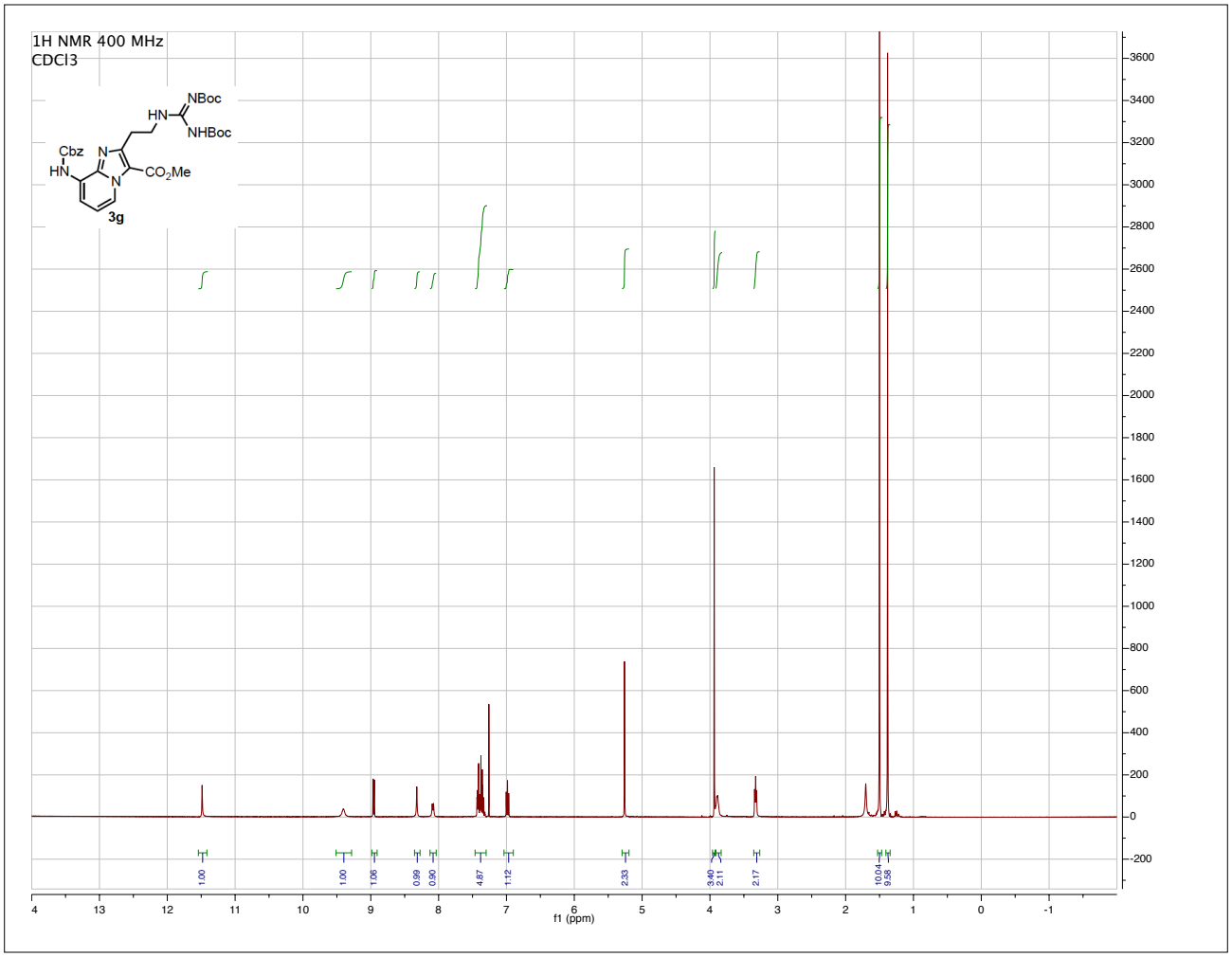
N(Boc)2
Cbz
HN
CO2Me
3f
161.86
153.33
152.73
152.49
152.22
140.07
135.86
128.80
128.61
128.49
127.13
121.82
114.59
114.22
111.67
82.24
67.55
51.69
45.77
29.56
28.14
f1 (ppm)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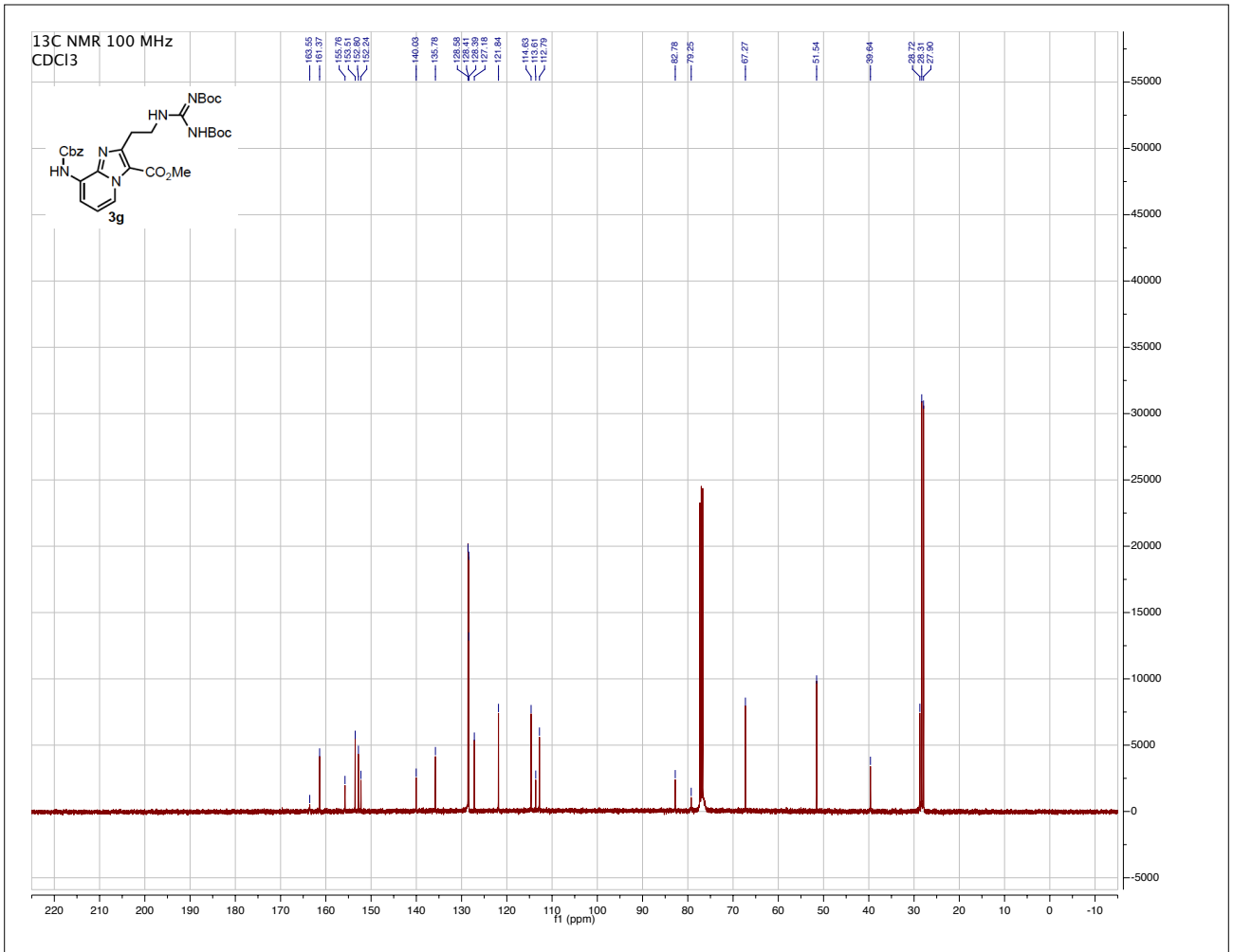
NBoc
HN
NHBoc
Cbz
HN
N
N
CO2Me
3g
f1 (ppm)


13C NMR 100 MHz
CDCl3
NBoc
HN
NHBoc
Cbz
HN
N
N
CO2Me
3g
163.55
161.37
155.76
155.51
152.80
152.24
140.63
135.78
128.58
128.41
128.39
127.18
121.84
114.63
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82.78
79.25
67.27
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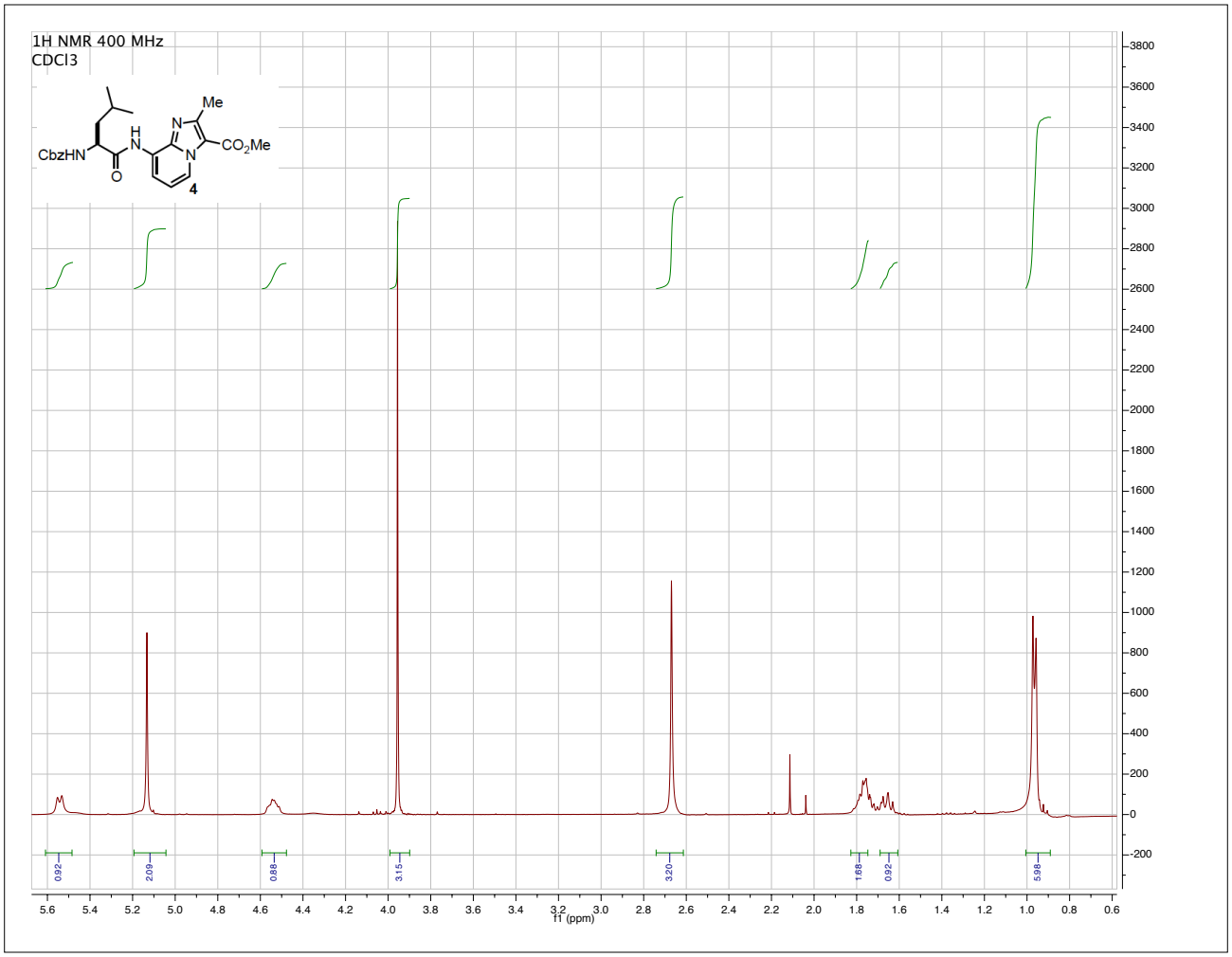
28.22
28.31
27.80
f1 (ppm)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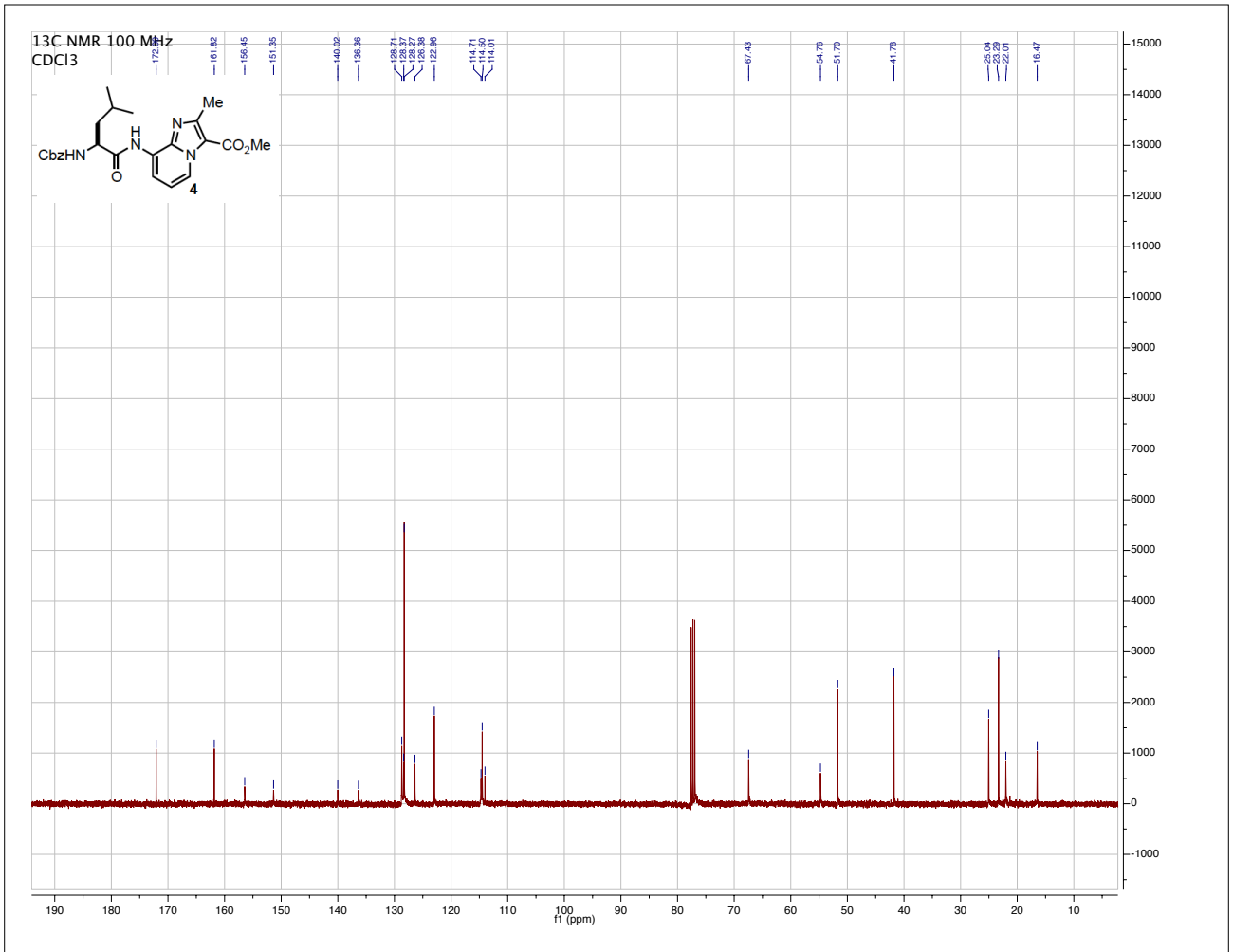
CDCl3
Me
CbzHN
CO2Me
4
f1 (ppm)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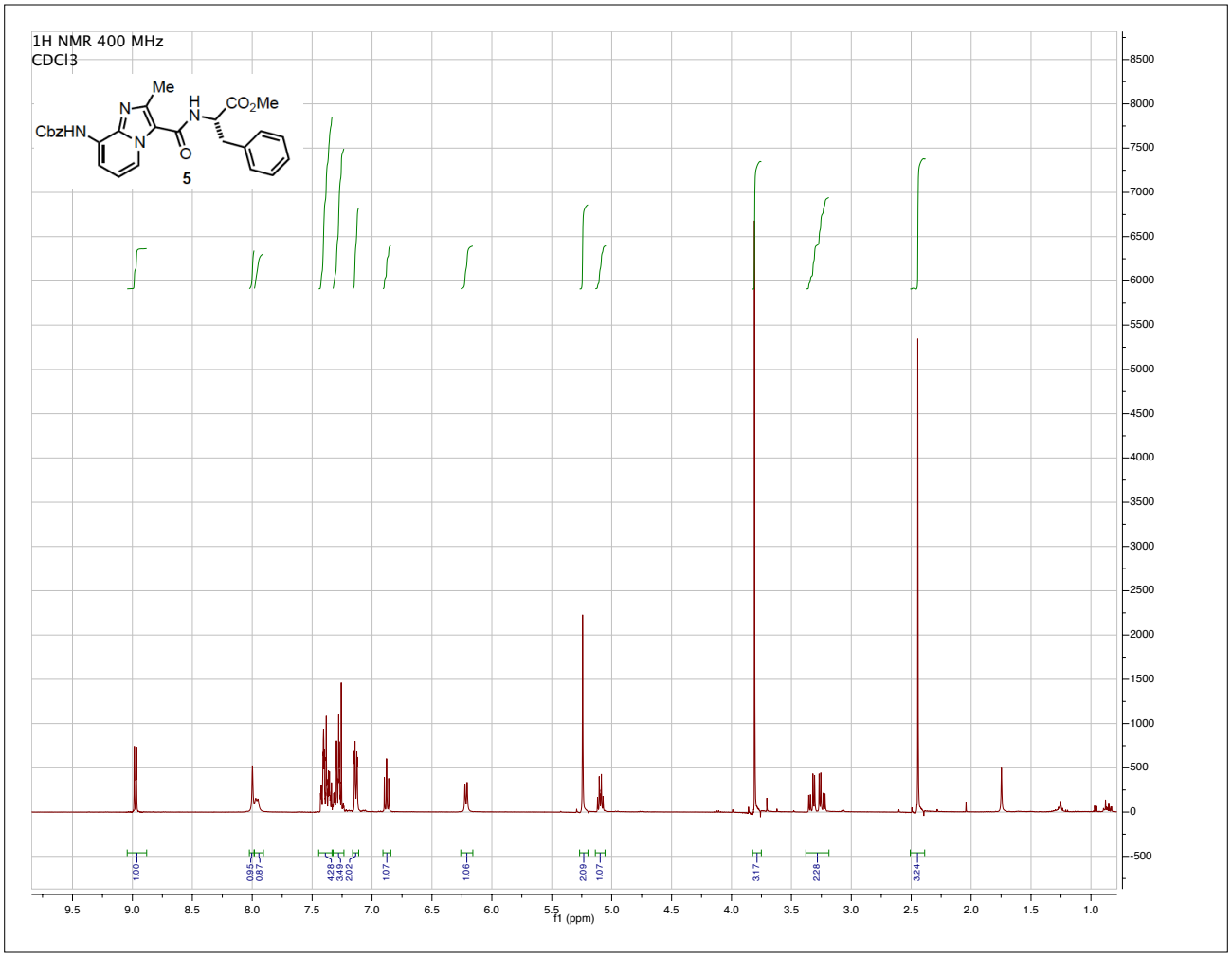
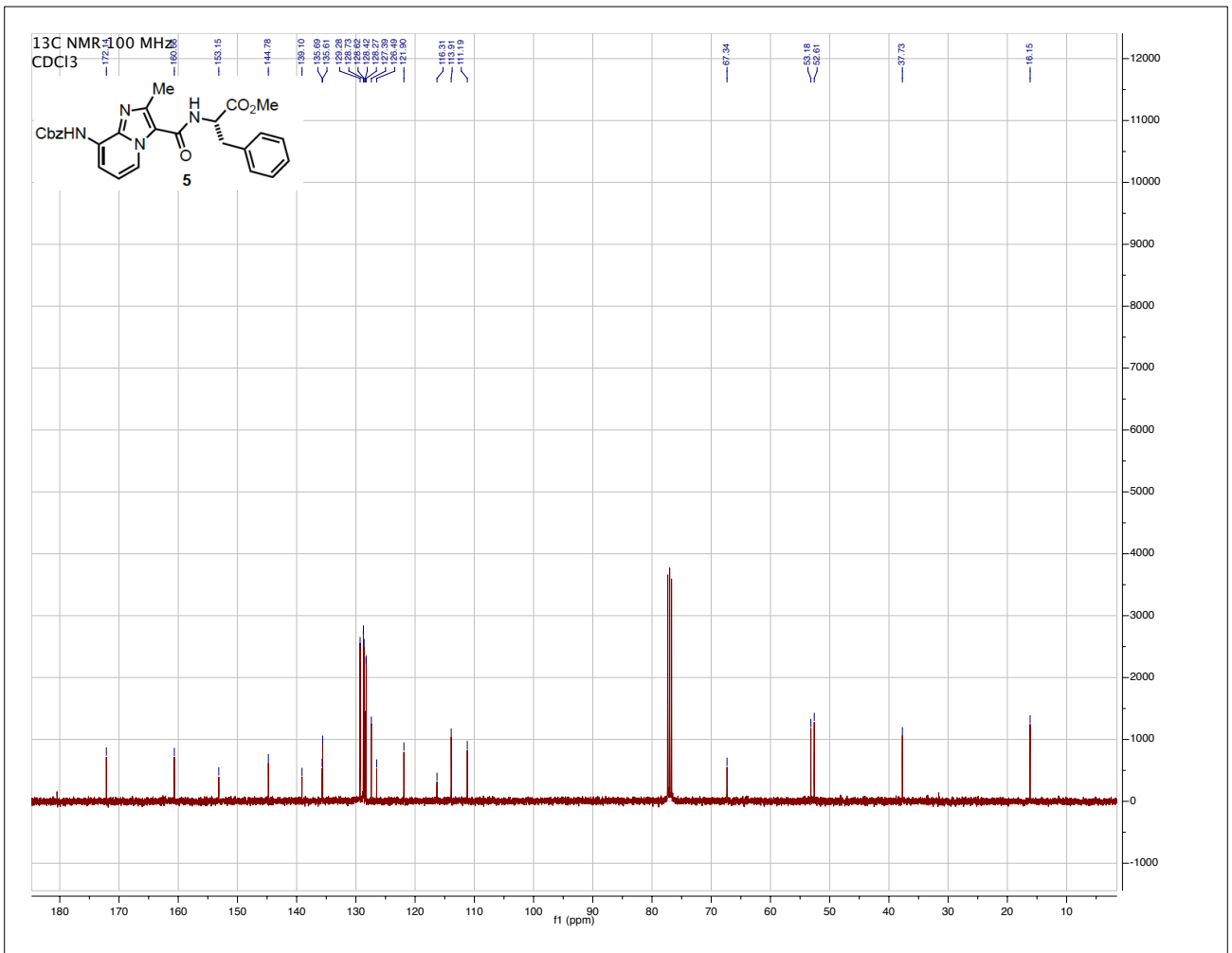
CDCl3
Me
CbzHN
CO2Me
4
f1 (ppm)


1H NMR 400 MHz
CDCl3
Me
N
H
N
CO2Me
CbzHN
N
O
5
1.00
0.95
0.87
4.28
3.49
2.02
1.07
1.06
2.09
1.07
3.17
2.28
3.24
f1 (ppm)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Me
N
H
N
CO2Me
CbzHN
N
O
5
172.41
160.88
153.15
144.78
139.10
135.69
135.61
129.28
128.82
128.62
128.42
128.27
127.98
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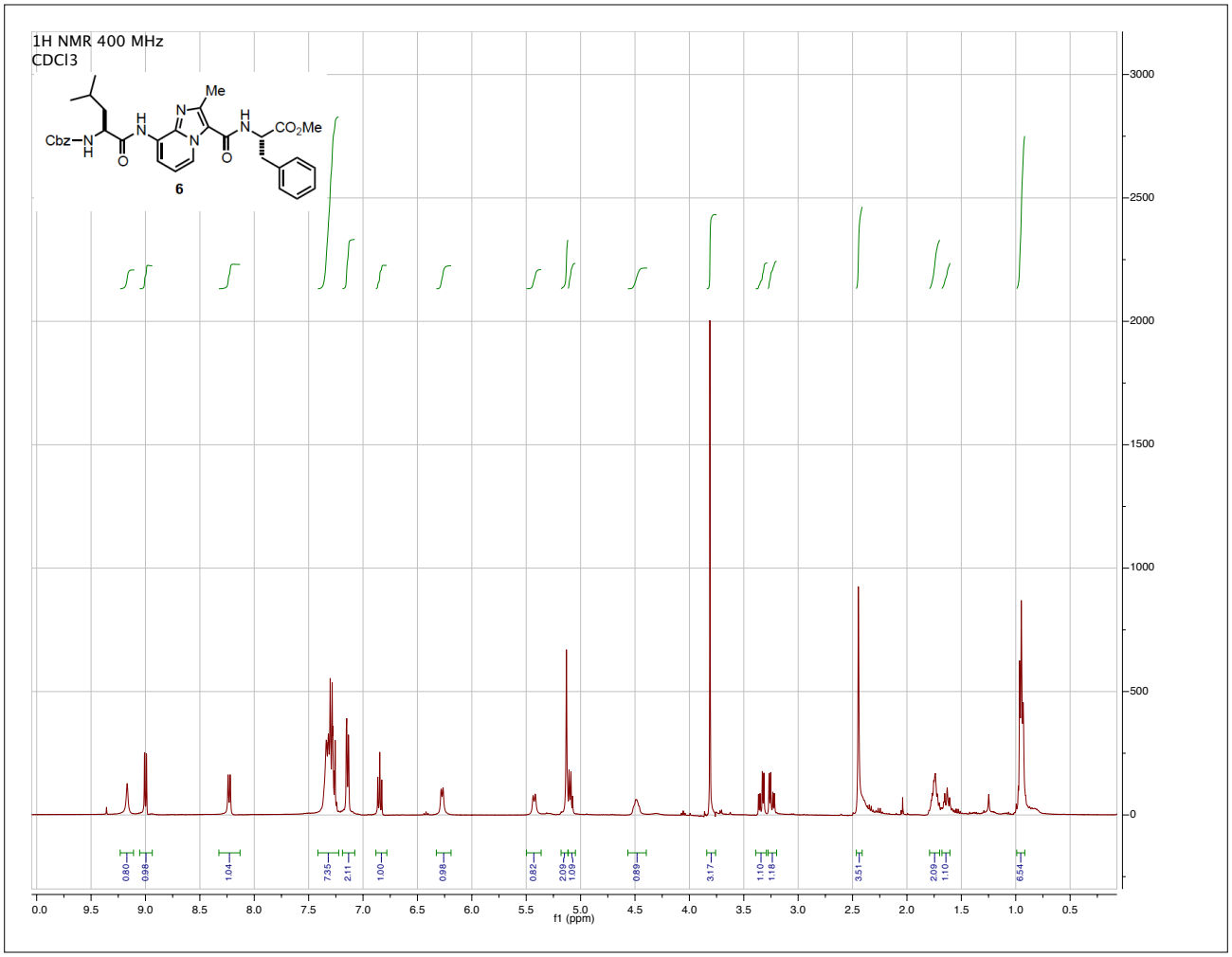
116.91
115.31
111.19
67.34
53.18
52.61
37.73
16.15
f1 (ppm)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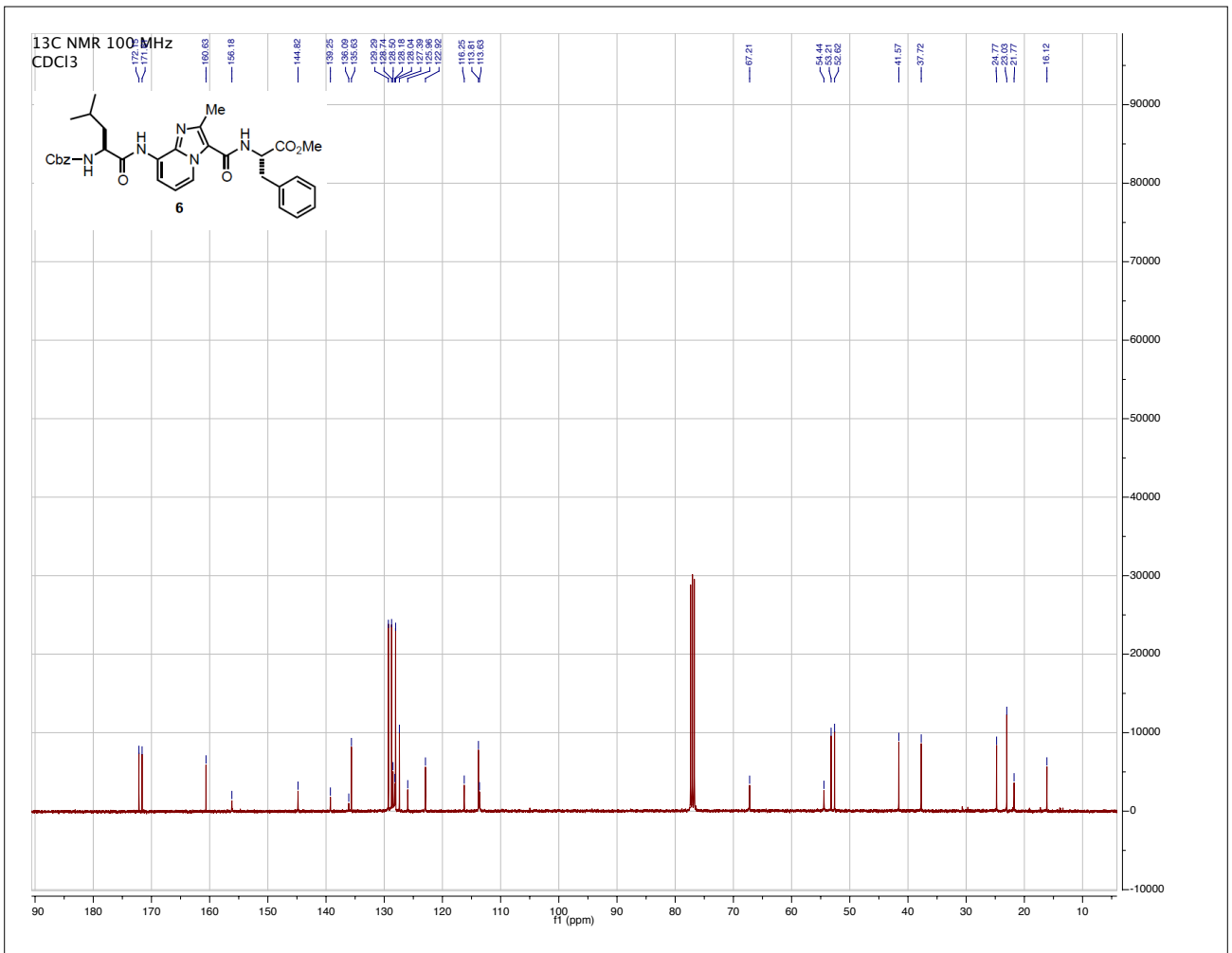
CDCl3
Me
Cbz–N
H
H
N
N
N
H
CO2Me
O
O
6
0.80
0.98
1.04
7.35
2.11
1.00
0.98
0.82
2.09
1.09
0.89
3.17
1.10
1.18
3.51
2.09
1.10
6.54
f1 (ppm)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CDCl3
172.34
171.
160.83
156.18
144.82
139.25
136.09
135.63
129.29
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128.18
128.04
127.39
125.96
122.92
116.25
113.81
113.63
67.21
54.44
53.21
52.62
41.57
37.72
24.77
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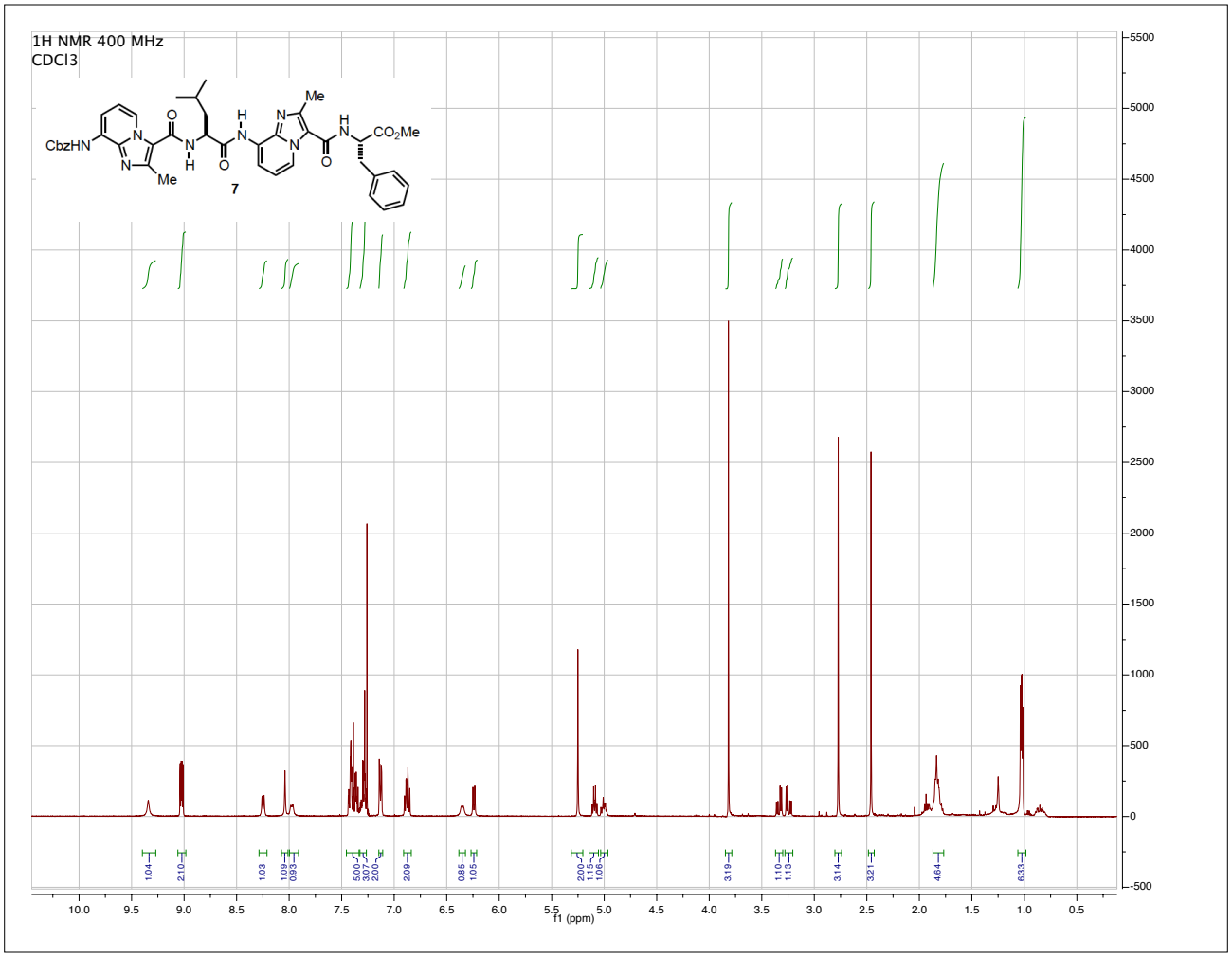
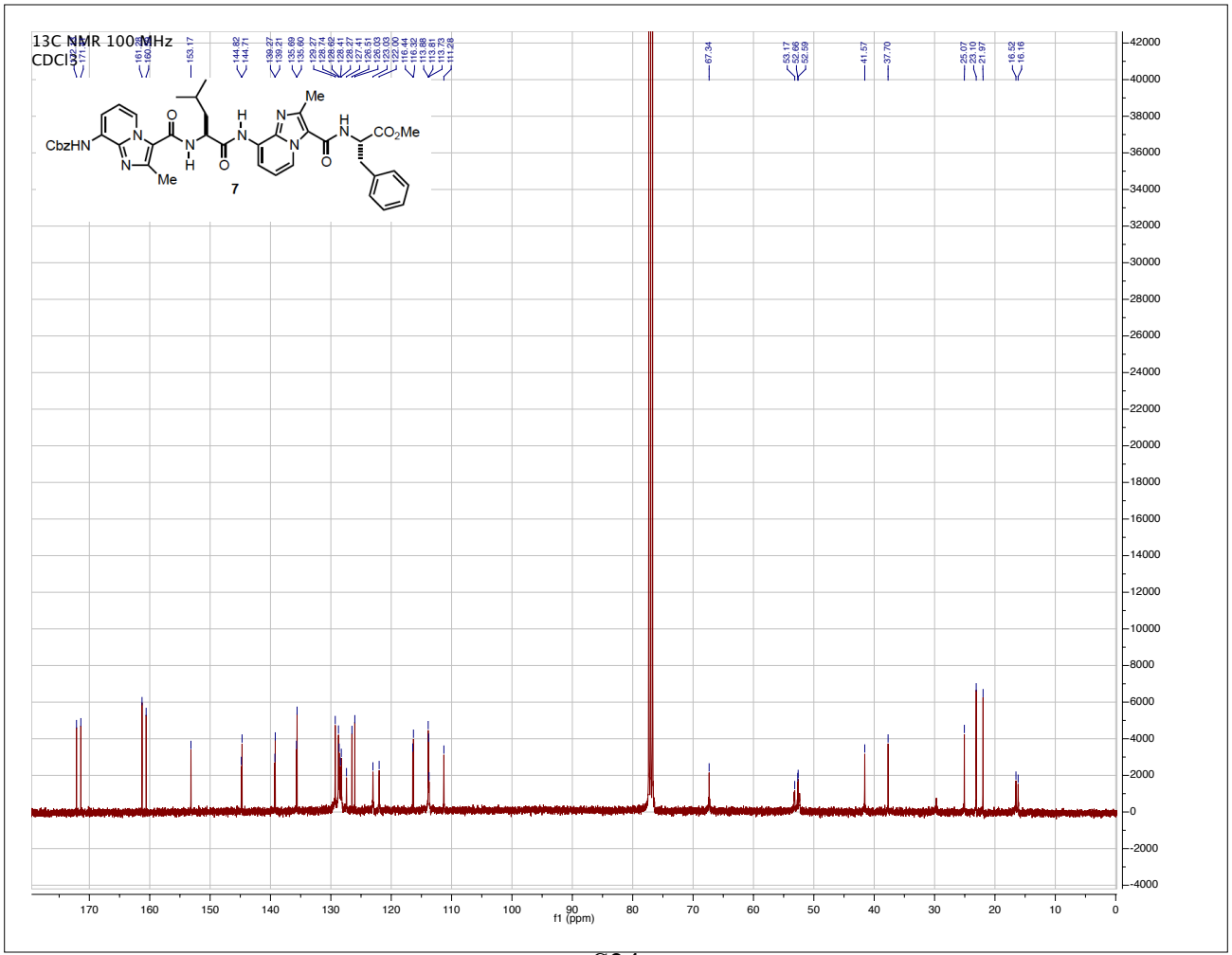
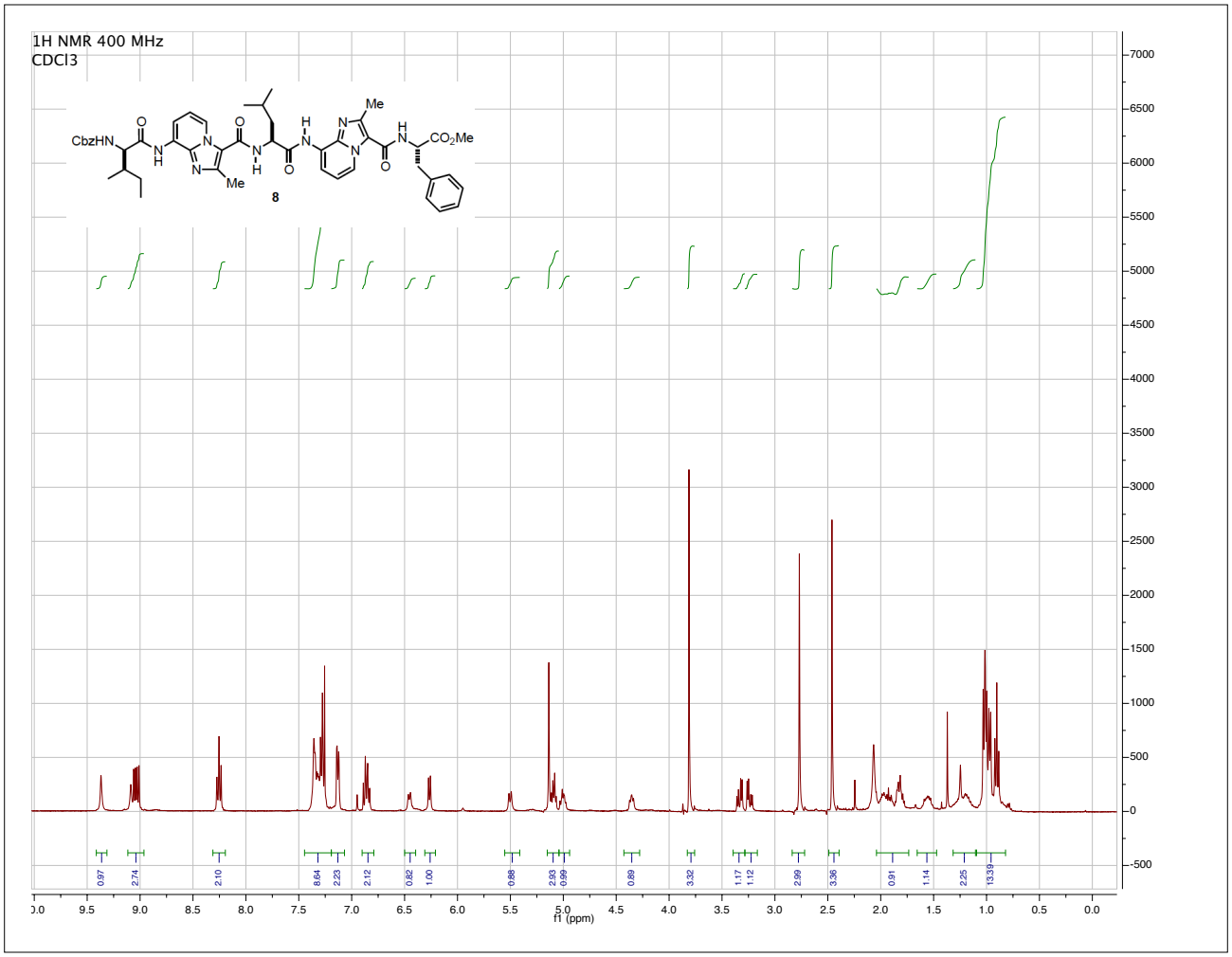
N
N
H
CO2Me
O
O
6
f1 (ppm)


1H NMR 400 MHz
CDCl3
CbzHN
Me
CO2Me
7
f1 (ppm)


13C NMR 100 MHz
CDCl3
CbzHN
Me
CO2Me
7
f1 (ppm)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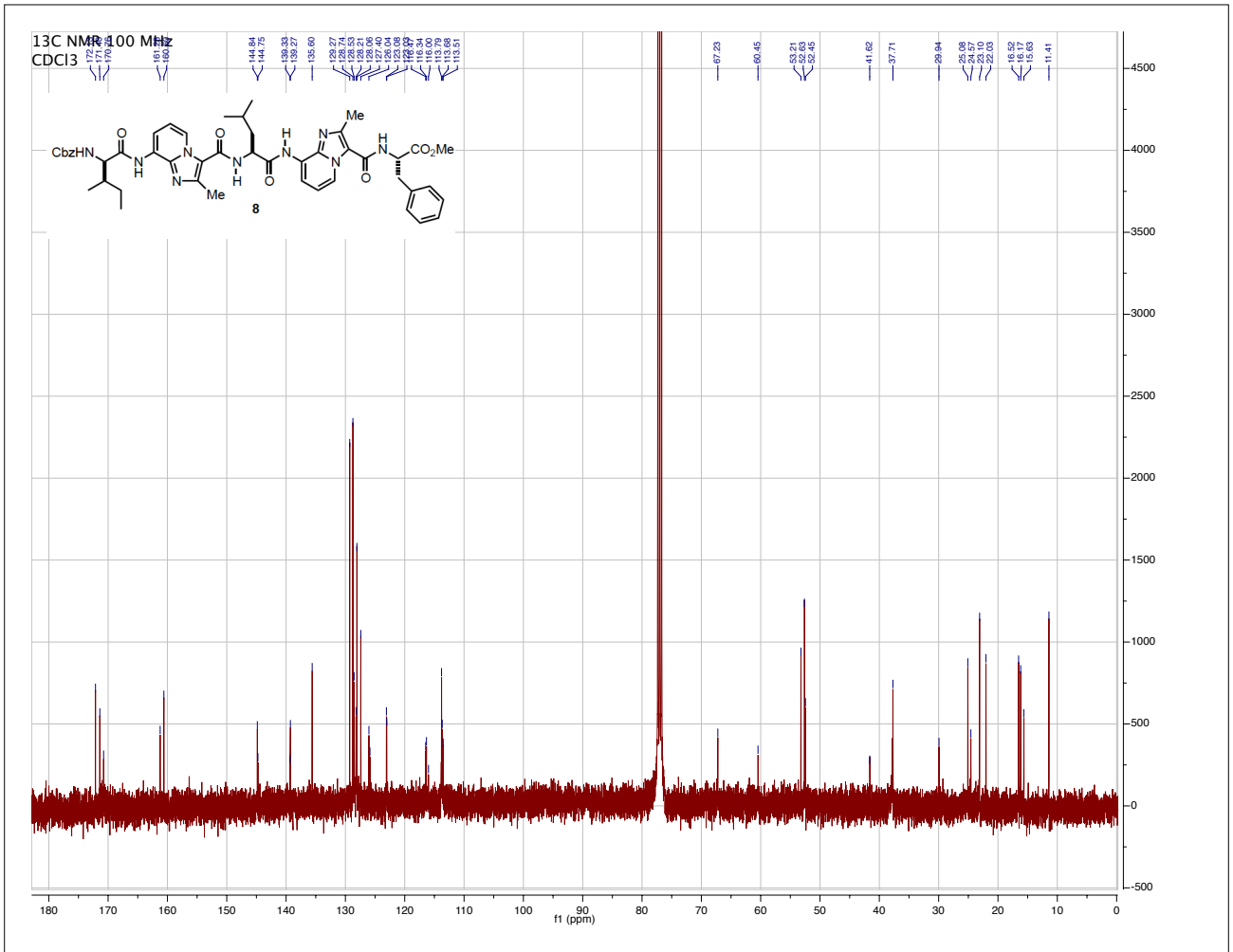
CDCl3
CbzHN
Me
CO2Me
8
f1 (ppm)

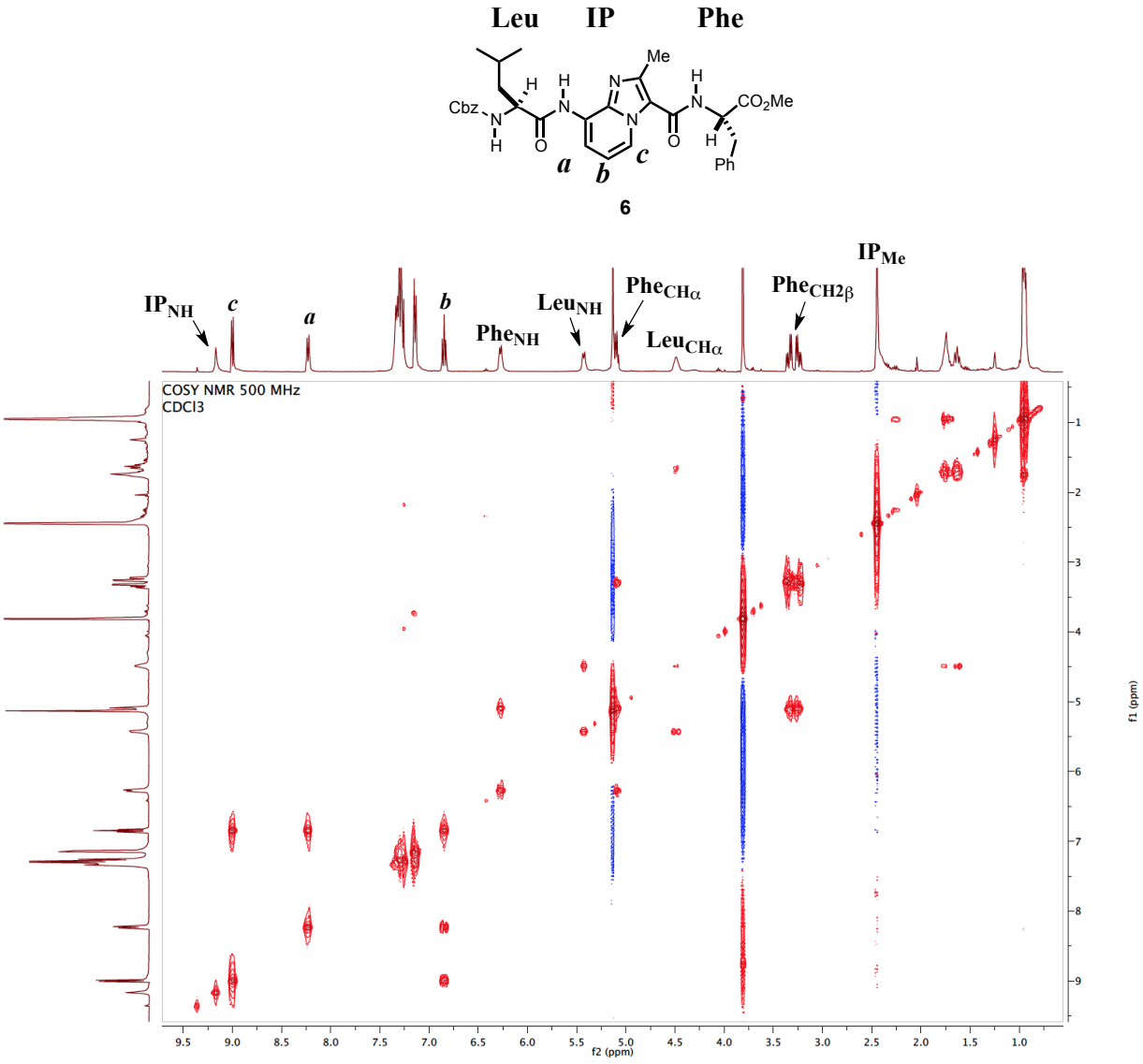

13C NMR 100 MHz
CDCl3
CbzHN
Me
CO2Me
8
f1 (ppm)

2D NMR SPECTRA

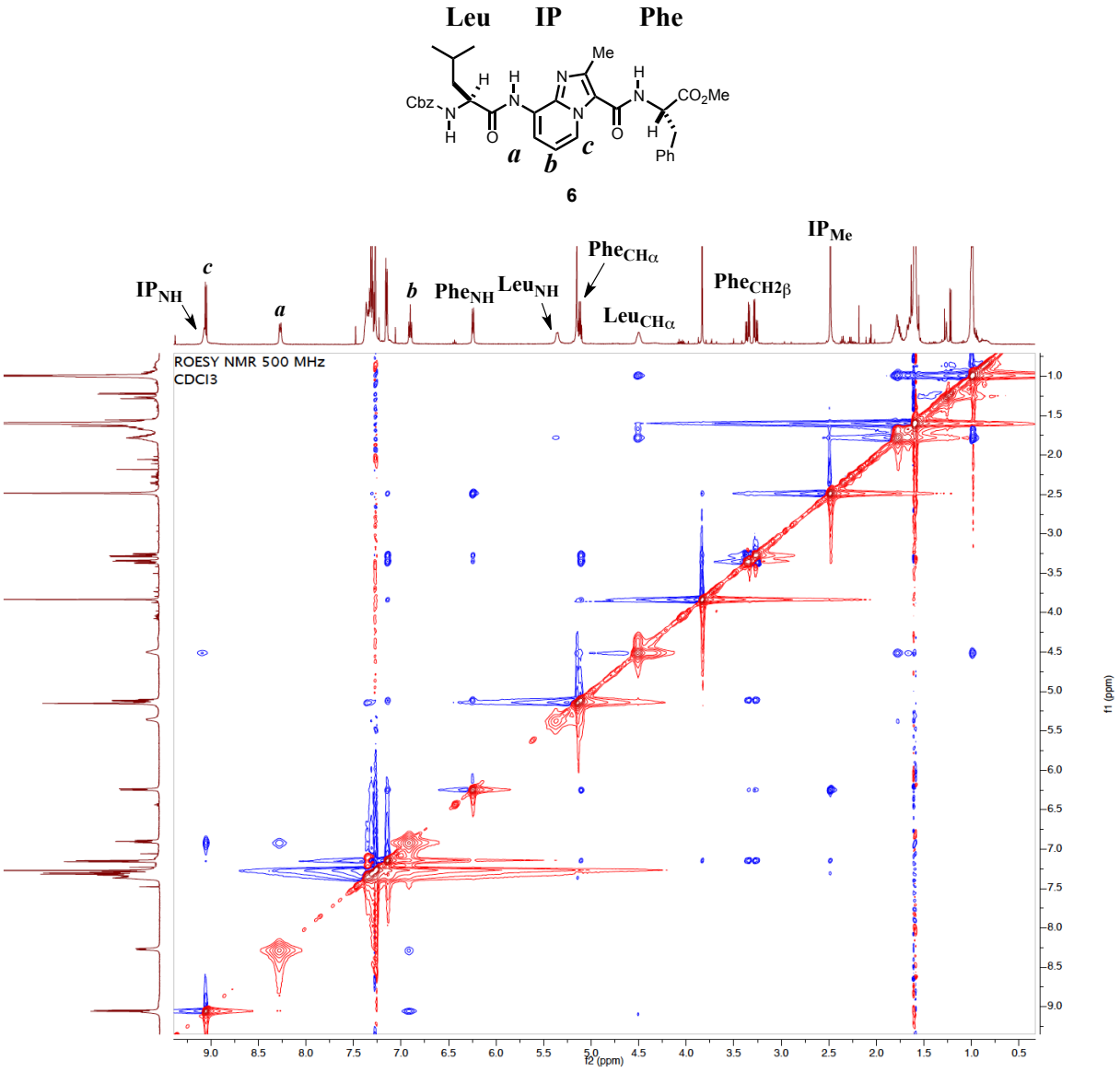
General. COSY spectra were recorded on an Agilent Technologies 400 MHz NMR at 298 K by collecting 2048 complex data points in the t_2 domain by averaging 32 scans and 256 increments in the t_1 domain. The original FIDs were zero-filled to give a final matrix of 1024 by 1024 real data points. A 0° sine-bell window function was applied in both dimensions. COSY data were obtained with 5 mM compound in $CDCl_3$.

ROESY spectra were recorded on an Agilent Technologies 500 MHz NMR at 298 K by collecting 4096 complex data points in the t_2 domain by averaging 32 scans and 512 increments in the t_1 domain with a mixing time of 300 ms. The original FIDs were zero-filled to give a final matrix of 2048 by 2084 real data points. A 90° sine-square window function was applied in both dimensions. ROESY data were obtained with 1 mM compound in $CDCl_3$. All the data were processed and analyzed using MestreNova 7.1 software.

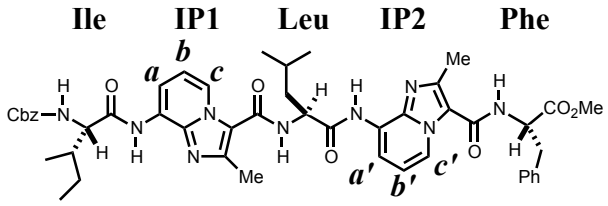
COSY spectrum of compound 6 in $CDCl_3$ (400 MHz):



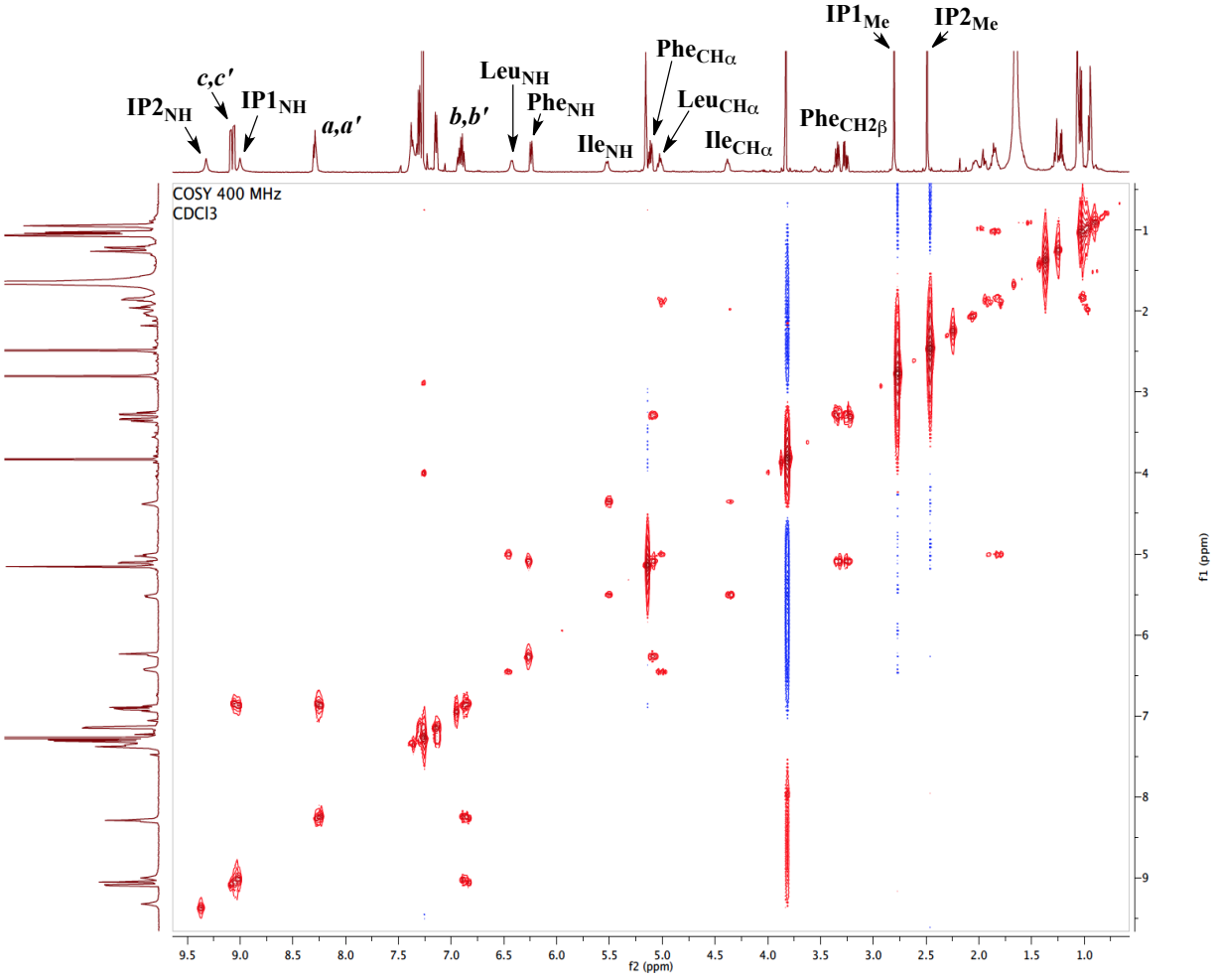
ROESY spectrum of compound 6 in $CDCl_3$ (500 MHz):



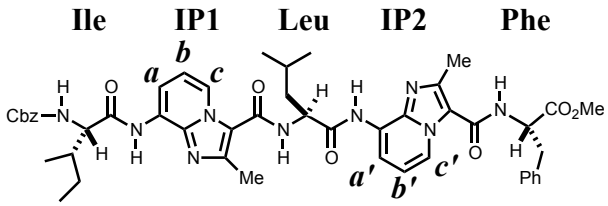
COSY spectrum of compound 8 in $CDCl_3$ (500 MHz):



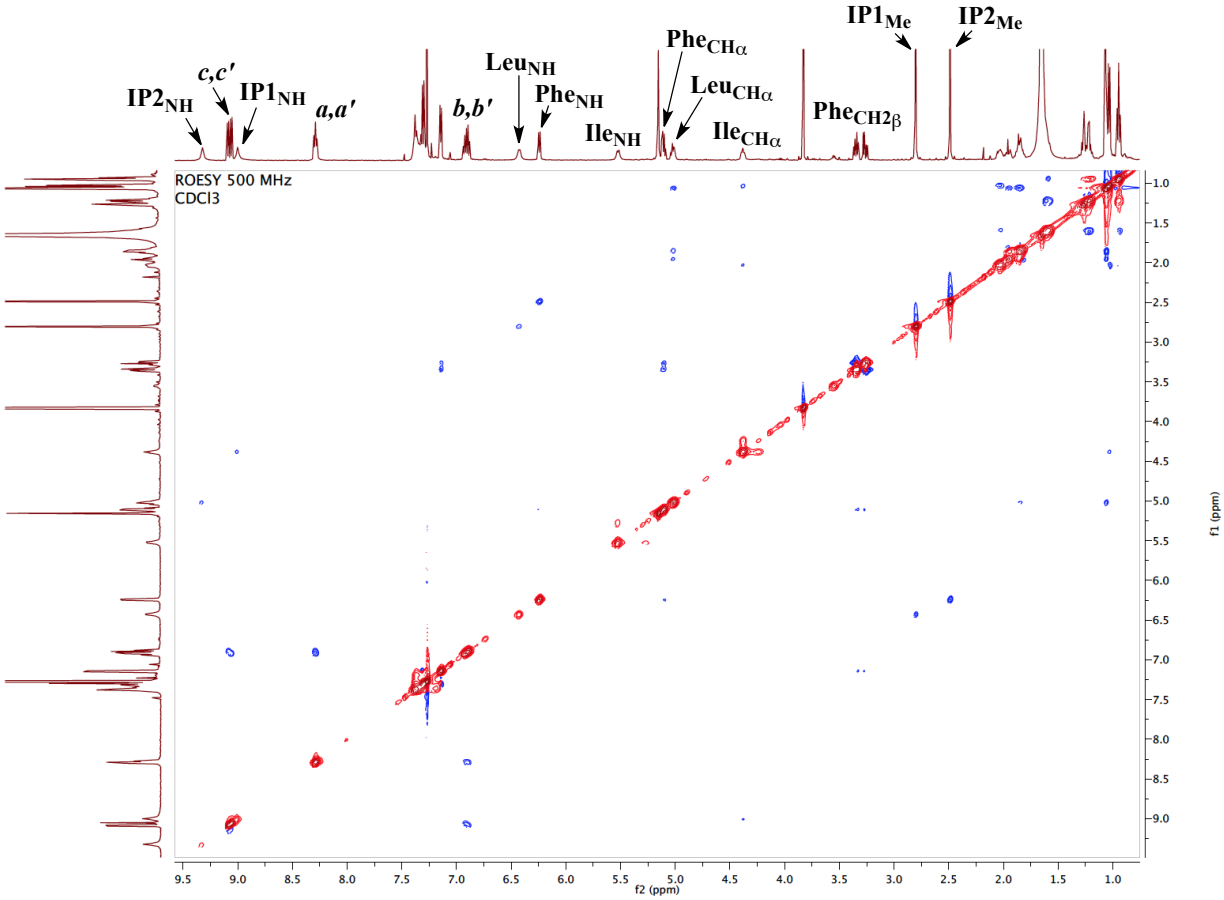
8



ROSEY spectrum of compound 8 in $CDCl_3$ (500 MHz):

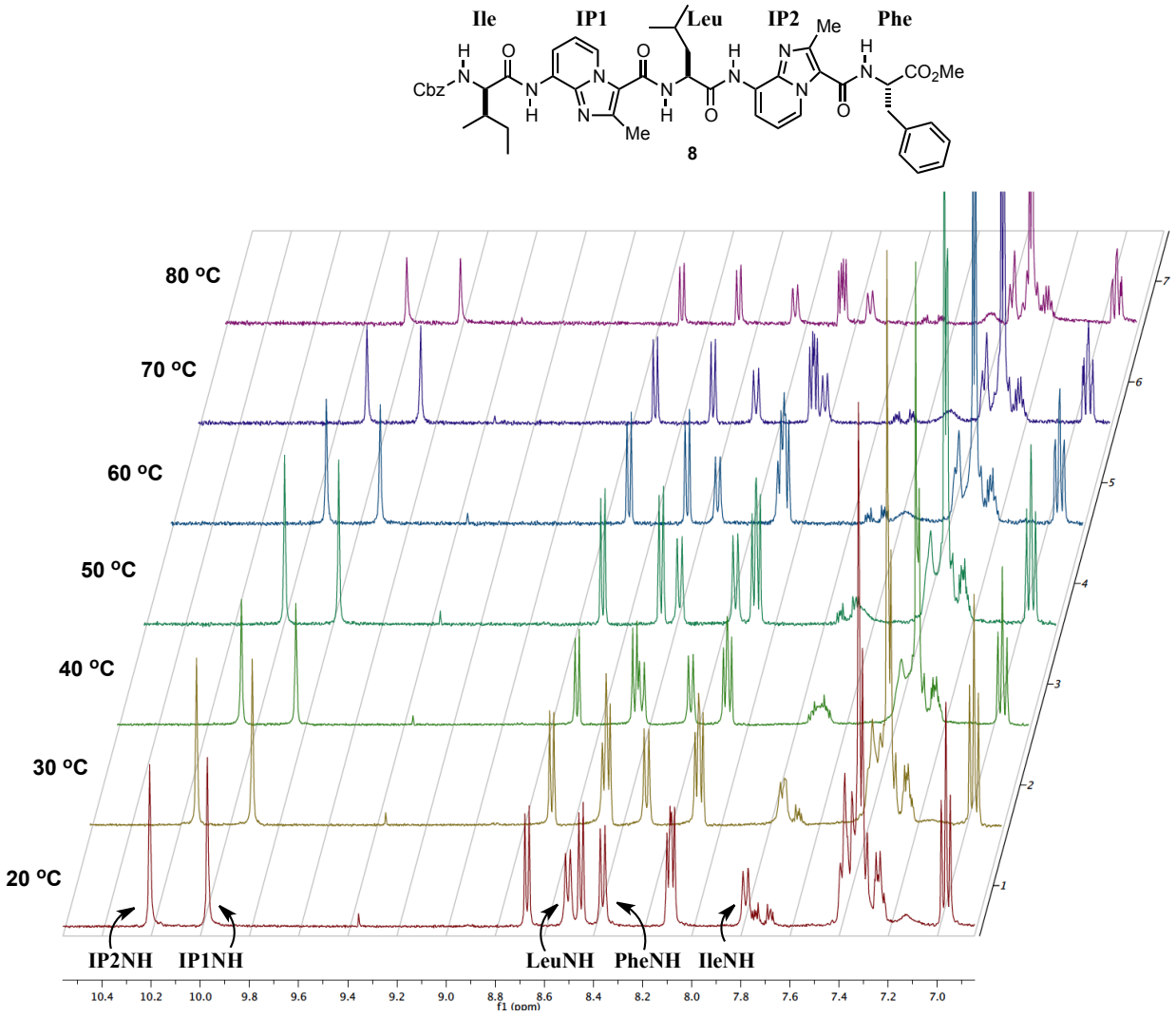


8

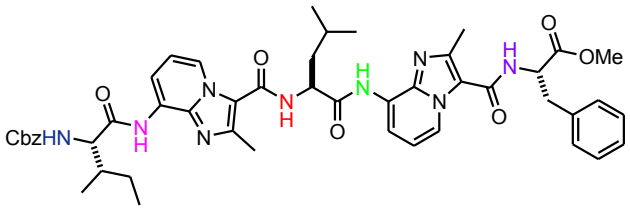


δΔ/δT EXPERIMENTS

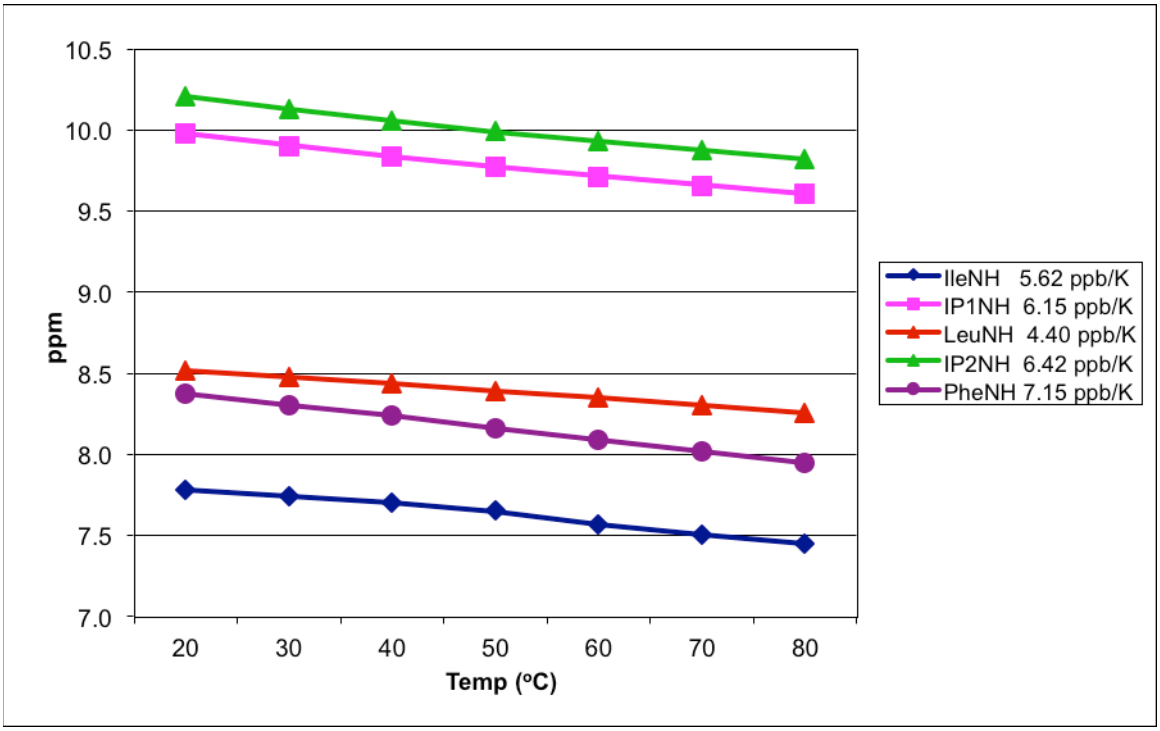
Variable temperature ^{1}H NMR spectra at 400 MHz (in DMSO-d_6):



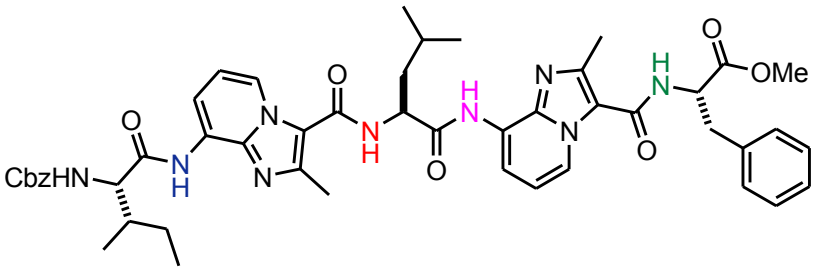
Tabular data for δΔ/δT experiments:



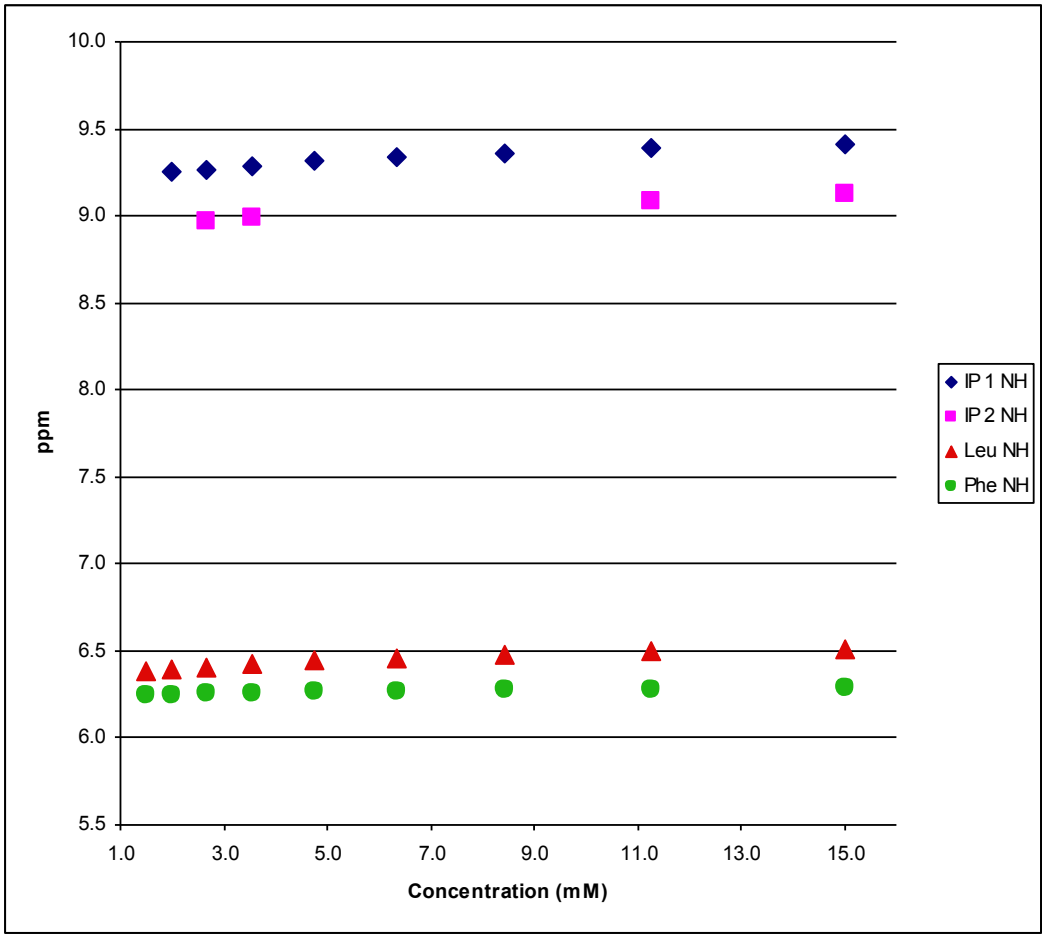
Proton	δ (ppm)						
	20 °C	30 °C	40 °C	50 °C	60 °C	70 °C	80 °C
IleNH	7.781	7.740	7.699	7.649	7.569	7.501	7.444
IP1NH	9.972	9.899	9.831	9.768	9.709	9.654	9.603
LeuNH	8.513	8.474	8.433	8.390	8.345	8.298	8.249
IP2NH	10.206	10.126	10.054	9.988	9.928	9.873	9.821
PheNH	8.372	8.304	8.234	8.162	8.090	8.018	7.943



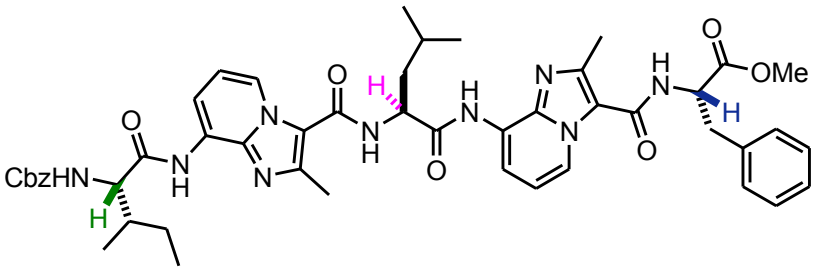
Concentration Dependence of amide NH chemical shifts of ck-05-066 @ 20 °C



Proton	δ (ppm) @ concentration								
	1.50 mM	2.00 mM	2.67 mM	3.56 mM	4.75 mM	6.33 mM	8.44 mM	11.25 mM	15.00 mM
IP 1 NH	-	9.256	9.267	9.290	9.317	9.336	9.359	9.386	9.414
IP 2 NH	-	-	8.969	8.990	-	-	-	9.089	9.126
Leu NH	6.385	6.395	6.408	6.428	6.447	6.460	6.476	6.496	6.512
Phe NH	6.244	6.249	6.253	6.257	6.263	6.269	6.275	6.283	6.292



Concentration Dependence of α-proton chemical shifts of ck-05-066 @ 20 °C



Proton	δ (ppm) @ concentration								
	1.50 mM	2.00 mM	2.67 mM	3.56 mM	4.75 mM	6.33 mM	8.44 mM	11.25 mM	15.00 mM
Phe α	5.097	5.099	5.100	5.101	5.101	5.102	5.101	5.101	5.100
Leu α	4.985	4.988	4.990	4.994	4.997	4.998	4.999	5.001	5.002
Ile α	4.374	4.375	4.372	4.369	4.368	4.367	4.363	4.362	4.360