Supporting Information

Small-Molecule Ligands of Methyl-Lysine Binding Proteins: Optimization of Selectivity for L3MBTL3

Lindsey I. James,[1] Victoria K. Korboukh,[1⊥] Liubov Krichevsky,[2,3,4] Brandi M. Baughman,[1] J. Martin Herold,[1‡] Jacqueline L. Norris,[1] Jian Jin,[1] Dmitri B. Kireev,[1] William P. Janzen,[1] Cheryl H. Arrowsmith,[2,3,4] Stephen V. Frye[,1]*

[1]Center for Integrative Chemical Biology and Drug Discovery, Division of Chemical Biology and Medicinal Chemistry, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA. [2]Structural Genomics Consortium, University of Toronto, [3]Department of Medical Biophysics, University of Toronto, and [4]Princess Margaret Cancer Centre, 101 College Street, Toronto, Ontario, Canada, M5G 1L7

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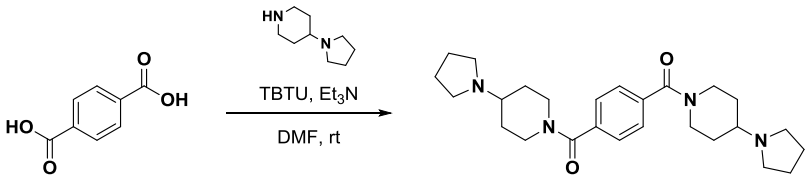
The synthesis of compounds **1**,[1] **3**,[2] **5**,[1] **37**,[1] **40**,[1] **57**,[3] **59**,[3] and **60**[3] has been reported previously.



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1,4-Bis(4-(pyrrolidin-1-yl)piperidinyl)benzamide (**5**): To a mixture of terephthalic acid (250 mg, 1.51 mmol) and TBTU (1.26 g, 3.91 mmol) in DMF (2 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (567 mg, 3.61 mmol) and triethylamine (1.26 mL, 9.03 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 640 mg (64%) of the TFA salt of **5** as a colorless solid. The characterization data (^{1}H NMR, HPLC, and ESI+) of the title compound match the reported.[1]

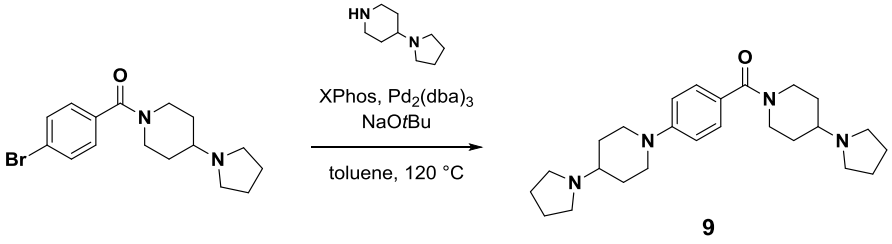
1,3-Bis(4-(pyrrolidin-1-yl)piperidinyl)benzamide (**4**): Compound **4** was prepared as a colorless solid from isophthalic acid by the same procedure as compound **5** (61% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63-7.54 (m, 3H), 7.50-7.47 (m, 1H), 4.87 – 4.62 (m, 2H), 3.97-3.75 (m, 2H), 3.75-3.54 (m, 4H), 3.45 (tt, *J* = 11.6, 3.8 Hz, 2H), 3.29 – 3.04 (m, 6H), 3.04 – 2.80 (m, 2H), 2.40 – 1.90 (m, 12H), 1.80 – 1.55 (m, 4H). HPLC: 99%, t_R 0.76 min. MS (ESI+): 439.3 $[M+H]^+$.

1,2-Bis(4-(pyrrolidin-1-yl)piperidinyl)benzamide (**6**): Compound **6** was prepared as a colorless solid from phthalic acid by the same procedure as compound **5** (76% yield). ^{1}H NMR (400 MHz, CD_3OD) δ 7.59-7.52 (m, 2H), 7.48-7.40 (m, 2H), 4.74 – 4.62 (m, 2H), 3.79-3.56 (m, 6H), 3.43 (tt, *J* = 11.7, 3.9 Hz, 2H), 3.29 – 3.06 (m, 6H), 2.88 (t, *J* = 13.0 Hz, 2H), 2.32 – 1.59 (m, 16H). HPLC: 99%, t_R 1.00 min. MS (ESI+): 439.3 $[M + H]^+$.

1,2,4-Tris(4-(pyrrolidin-1-yl)piperidinyl)benzamide (**7**): To a mixture of 1,2,4-benzenetricarboxylic acid (50 mg, 0.24 mmol) and TBTU (298 mg, 0.93 mmol) in DMF (2 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (132 mg, 0.86 mmol) and triethylamine (0.30 mL, 2.14 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts

were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 159 mg (70%) of the TFA salt of **7** as a colorless solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.62-7.52 (m, 2H), 7.50 (d, *J* = 1.2 Hz, 1H), 4.81 – 4.62 (m, 3H), 3.96-3.56 (m, 9H), 3.51-3.38 (m, 3H), 3.29 – 3.08 (m, 9H), 3.02 – 2.81 (m, 3H), 2.42 – 1.57 (m, 24H). HPLC: 99%, t_R 0.66 min. MS (ESI+): 619.4 $[M+H]^+$.

(4-(pyrrolidin-1-yl)piperidin-1-yl)(4-((4-(pyrrolidin-1-yl)piperidin-1-yl)sulfonyl)phenyl)methanone (**8**): To a solution of 4-(chlorosulfonyl)benzoic acid (2.0 g, 9.06 mmol) in dry CH_2Cl_2 at 0 °C was added 4-(1-pyrrolidinyl)piperidine (2.8 g, 18.13 mmol) and the mixture was stirred and allowed to warm up to room temperature overnight. The reaction mixture was filtered through celite and the solvent was removed by rotary evaporation. The crude mixture was purified by reverse phase HPLC in attempt to isolate 4-((4-(pyrrolidin-1-yl)piperidin-1-yl)sulfonyl)benzoic acid, and serendipitously a small amount of compound **8** was also recovered as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.93-7.88 (m, 2H), 7.70-7.65 (m, 2H), 4.82 – 4.71 (m, 1H), 3.98-3.90 (m, 2H), 3.82-3.56 (m, 5H), 3.45 (tt, *J* = 11.8, 4.0 Hz, 1H), 3.28 – 3.01 (m, 6H), 2.99 – 2.86 (m, 1H), 2.45 (td, *J* = 12.4, 2.4 Hz, 2H), 2.37 – 1.91 (m, 12H), 1.82 – 1.59 (m, 4H). HPLC: 99%, t_R 0.94 min. MS (ESI+): 475.3 $[M+H]^+$.



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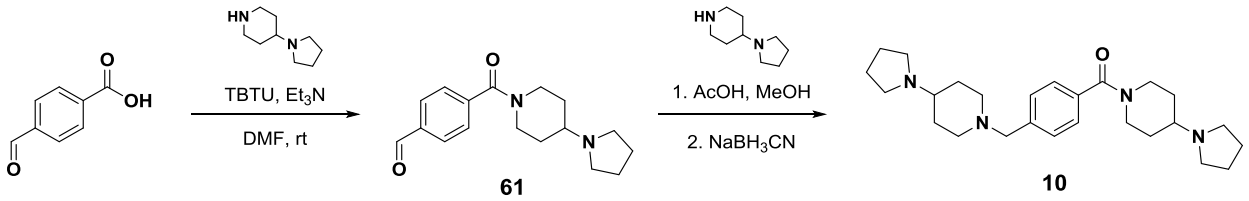
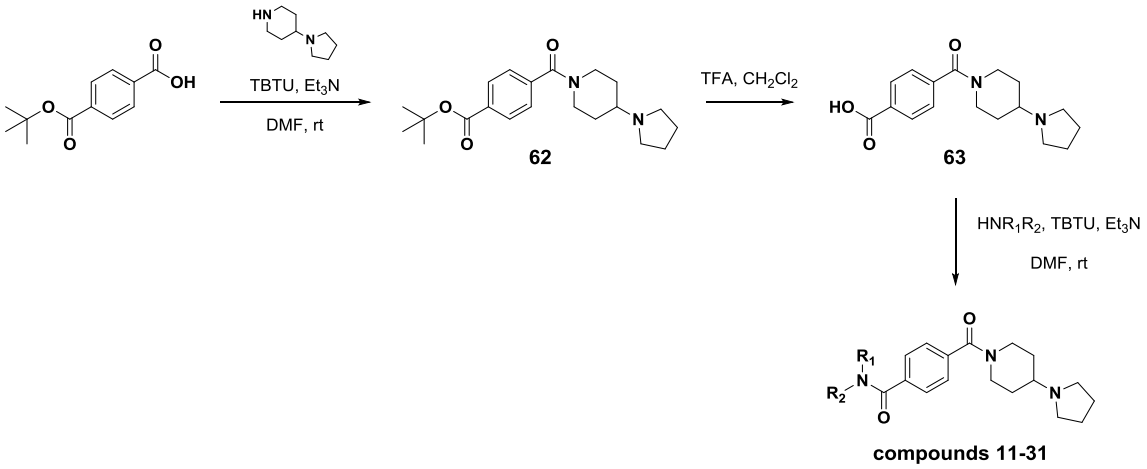
(4-(pyrrolidin-1-yl)piperidin-1-yl)(4-(4-(pyrrolidin-1-yl)piperidin-1-yl)phenyl)methanone (**9**): Compound **9** was prepared from 4-bromo-*N*-((4-(pyrrolidinyl)piperidinyl)benzamide, a previously reported compound.[3] A mixture of 4-bromo-*N*-((4-(pyrrolidinyl)piperidinyl)benzamide (150 mg, 0.45 mmol), 2-Dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (XPhos) (106 mg, 50 mol%), tris(dibenzylideneacetone)dipalladium(0) (102 mg, 25 mol%), and sodium *tert*-butoxide (64 mg, 0.67 mmol) in anhydrous toluene (3 mL) was added to a sealable reaction tube. The solution was degassed with N_2 for 20 minutes and 4-(1-pyrrolidinyl)piperidine (96 mg, 0.62 mmol) was added subsequently. The reaction tube was tightly sealed and the reaction was stirred at 120 °C for 15 hours. The reaction was cooled to room temperature, diluted with CH_2Cl_2, and filtered over a thick pad of celite which was subsequently washed with CH_2Cl_2. The CH_2Cl_2 was removed by rotary evaporation. The crude mixture was brought up in a small amount of a methanol-water mixture and filtered. The resulting filtrate was

purified by reverse phase HPLC and the solvents were removed to afford 237 mg (83%) of the TFA salt of **9** as a dark red solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.40-7.34 (m, 2H), 7.10-7.603 (m, 2H), 4.70 – 4.07 (bs, 2H), 4.03-3.93 (m, 2H), 3.75-3.58 (m, 4H), 3.42 (tt, *J* = 12.2, 4.3 Hz, 1H), 3.35 (tt, *J* = 12.2, 4.3 Hz, 1H), 3.26 – 2.95 (m, 6H), 2.90 (td, *J* = 13.4, 2.3 Hz, 2H), 2.29 – 1.93 (m, 12H), 1.81 (qd, *J* = 12.3, 4.1 Hz, 2H), 1.66 (qd, *J* = 12.4, 4.5 Hz, 2H). HPLC: 99%, t_R 1.43 min. MS (ESI+): 411.4 $[M+H]^+$.



4-(4-(pyrrolidin-1-yl)piperidine-1-carbonyl)benzaldehyde (**61**): To a mixture of 4-carboxybenzaldehyde (200 mg, 1.33 mmol) and TBTU (555 mg, 1.73 mmol) in DMF (2 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (247 mg, 1.60 mmol) and triethylamine (0.56 mL, 3.99 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was carried forward without further purification.

(4-(pyrrolidin-1-yl)piperidin-1-yl)(4-((4-(pyrrolidin-1-yl)piperidin-1-yl)methyl)phenyl)methanone (**10**): To a solution of **61** (102 mg, 0.25 mmol) in anhydrous methanol (1 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (157 mg, 1.02 mmol) and acetic acid (44 µL, 0.76 mmol) in anhydrous methanol (1.5 mL). The mixture was stirred at room temperature for 15 hours, after which $NaBH_3CN$ (64 mg, 1.02 mmol) was added and the reaction was stirred for 6 hours. The solvent was removed by rotary evaporation, redissolved in CH_2Cl_2, quenched with saturated aq. $NaHCO_3$ (10 mL), and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 65 mg (47% over 2 steps) of the TFA salt of **10** as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (d, *J* = 7.7 Hz, 2H), 7.54 (d, *J* = 7.7 Hz, 2H), 4.82 – 4.66 (m, 1H), 4.40 (s, 2H), 3.98-3.53 (m, 7H), 3.52-3.39 (m, 2H), 3.27 – 3.02 (m, 7H), 3.01 – 2.81 (m, 1H), 2.49 – 2.37 (m, 2H), 2.37 – 1.90 (m, 12H), 1.79 – 1.54 (m, 2H). HPLC: 99%, t_R 0.62 min. MS (ESI+): 425.4 $[M+H]^+$.



4-(*tert*-butoxycarbonyl)-(4-(pyrrolidin-1-yl)piperidinyl)benzamide (**62**): To a mixture of 4-(*tert*-butoxycarbonyl)benzoic acid (300 mg, 1.35 mmol) and TBTU (564 mg, 1.75 mmol) in DMF (2 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (250 mg, 1.62 mmol) and triethylamine (0.56 mL, 4.05 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by column chromatography on silica gel (0 – 20% MeOH (with 1% vol. of 7N NH_3)/CH_2Cl_2) to afford 411 mg (85%) of **62** as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 – 8.01 (m, 2H), 7.51 – 7.46 (m, 2H), 4.72 – 4.52 (m, 1H), 3.73 – 3.55 (m, 1H), 3.22 – 3.05 (m, 1H), 2.99 – 2.84 (m, 1H), 2.71 – 2.59 (m, 4H), 2.37 (tt, *J* = 11.0, 3.9 Hz, 1H), 2.18 – 2.03 (m, 1H), 1.99 – 1.87 (m, 1H), 1.87 – 1.79 (m, 4H), 1.61 (s, 9H), 1.56 – 1.36 (m, 2H). HPLC: 99%, t_R 3.82 min. MS (ESI+): 359.3 $[M+H]^+$.

4-carboxy-(4-(pyrrolidin-1-yl)piperidinyl)benzamide (**63**): To a solution of **62** in CH_2Cl_2 (10 mL) was added 2 mL of TFA and the reaction was stirred at room temperature until no starting material remained. After evaporation of the solvent to give the desired product as a TFA salt, the product was carried forward without further purification.

General procedure for the synthesis of compounds 11 – 31. To a mixture of **63** (1 eq) and TBTU (1.3 eq) in DMF (1 mL) was added a solution of the desired amine (1.2 eq) and triethylamine (6 eq) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were

removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford the desired product as a TFA salt. All compounds were synthesized in greater than 95% purity as confirmed by ^{1}H NMR and LCMS. See Supplementary Figure 7 for NMR spectra.

[1,4'-bipiperidin]-1'-yl(4-(4-(pyrrolidin-1-yl)piperidine-1-carbonyl)phenyl)methanone (**11**): Compound **11** was prepared as a TFA salt from **63** and 4-piperidinopiperidine to give a white solid (71%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.54 (s, 4H), 4.89 – 4.61 (m, 2H), 4.06 – 3.76 (m, 2H), 3.75-3.58 (m, 2H), 3.58 – 3.39 (m, 4H), 3.30 – 3.09 (m, 4H), 3.08 – 2.79 (m, 4H), 2.43 – 1.91 (m, 10H), 1.91 – 1.59 (m, 7H), 1.59 – 1.44 (m, 1H). HPLC: 99%, t_R 2.10 min. MS (ESI+): 453.4 $[M + H]^+$.

1,4-Bis(4-(pyrrolidin-1-yl)azepan-1-yl)benzamide (**32**): Compound **32** was prepared similarly to compound **5** from terephthalic acid and 4-(pyrrolidin-1-yl)azepane. The TFA salt of the desired product was obtained as colorless oil (63%). The NMR spectrum reveals that the compound exists as a pair of isomers. ^{1}H NMR (400 MHz, CD_3OD) δ 7.53 (s, 4H), 4.06 – 3.99 (m, 1H), 3.93 – 3.81 (m, 1H), 3.73 – 3.50 (m, 8H), 3.48 – 3.35 (m, 4H), 3.27 – 3.09 (m, 4H), 2.49 – 2.25 (m, 2H), 2.25 – 1.65 (m, 18H). HPLC: 99%, t_R 0.94 min. MS (ESI+): 467.4 $[M+H]^+$.

1,4-Bis((1R,5S)-8-(pyrrolidin-1-yl)-3-azabicyclo[3.2.1]octan-3-yl)benzamide (**33**): Compound **33** was prepared similarly to compound **5** from terephthalic acid and 3-(1-pyrrolidinyl)-8-azabicyclo[3.2.1]octane dihydrochloride. The TFA salt of the desired product was obtained as white solid (95%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.63 (s, 4H), 4.93 – 4.86 (m, 2H), 4.37 – 4.16 (m, 2H), 3.81 – 3.52 (m, 6H), 3.22 – 3.02 (m, 4H), 2.36 – 2.08 (m, 12H), 2.08 – 1.82 (m, 10H), 1.82 – 1.66 (m, 2H). HPLC: 99%, t_R 1.17 min. MS (ESI+): 491.4 $[M+H]^+$.

1,4-Bis(4-(azetidin-1-yl)piperidin-1-yl)benzamide (**34**): Compound **34** was prepared similarly to compound **5** from terephthalic acid and 4-(azetidin-1-yl)piperidine. The TFA salt of the desired product was obtained as white solid (42%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (s, 4H), 4.83 – 4.52 (m, 2H), 4.34 – 4.07 (m, 8H), 4.00 – 3.64 (m, 2H), 3.48 (tt, J = 11.5, 4.0 Hz, 2H), 3.28 – 2.79 (m, 4H), 2.71 – 2.56 (m, 2H), 2.44 – 2.30 (m, 2H), 2.28 – 1.85 (m, 4H), 1.56 – 1.23 (m, 4H). HPLC: 99%, t_R 0.57 min. MS (ESI+): 411.3 $[M+H]^+$.

1,4-Bis(4-(pyrrolidin-2-yl)piperidin-1-yl)benzamide (**35**): Compound **35** was prepared in two steps. First, 1,4-Bis(*tert*-butyl-2-(piperidin-4-yl)pyrrolidine-1-carboxylate)benzamide was prepared similarly to compound **5** from terephthalic acid and 2-piperidin-4-yl-pyrrolidine-1-carboxylic acid *tert*-butyl ester. Prior to purification, the crude reaction mixture was taken up in CH_2Cl_2 (10 mL) and TFA (2 mL) was

added to cleave the Boc protecting groups. Upon completion of the reaction, the crude mixture was purified by reverse phase HPLC to afford the desired product. The TFA salt of **35** was obtained as colorless solid (95% over 2 steps). ^{1}H NMR (400 MHz, CD_3OD) δ 7.51 (s, 4H), 4.79 – 4.57 (m, 2H), 3.88 – 3.64 (m, 2H), 3.36 – 3.24 (m, 6H), 3.23 – 3.07 (m, 2H), 2.99 – 2.79 (m, 2H), 2.36 – 2.18 (m, 2H), 2.15 – 1.97 (m, 4H), 1.97 – 1.83 (m, 4H), 1.82 – 1.61 (m, 4H), 1.53 – 1.24 (m, 4H). HPLC: 99%, t_R 1.23 min. MS (ESI+): 439.4 $[M+H]^+$.

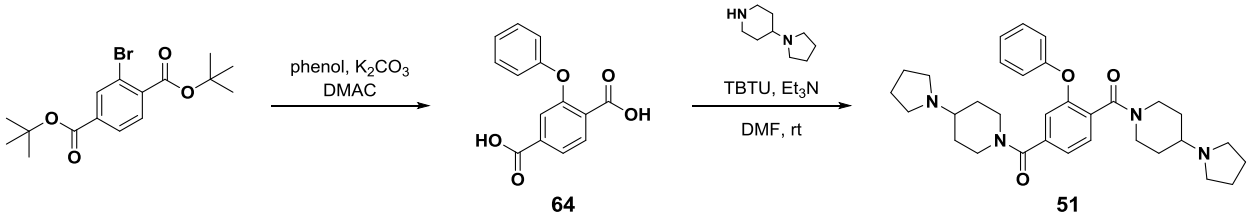
1,4-Bis(4-(1-methylpyrrolidin-2-yl)piperidin-1-yl)benzamide (**36**): To a solution of **35** (69 mg, 0.10 mmol) in anhydrous methanol (1 mL) was added formaldehyde (67 µL of a 36 weight % solution, 0.83 mmol) and acetic acid (18 µL, 0.31 mmol). The mixture was stirred at room temperature for 15 hours, after which $NaBH_3CN$ (64 mg, 1.02 mmol) was added and the reaction was stirred for 6 hours. The solvent was removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 54 mg (76%) of the TFA salt of **36** as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.52 (s, 4H), 4.81 – 4.65 (m, 2H), 3.91 – 3.69 (m, 2H), 3.63 (dt, *J* = 11.5, 6.8 Hz, 2H), 3.38 – 3.22 (m, 2H), 3.26 – 3.11 (m, 4H), 2.97 (s, 6H), 2.93 – 2.74 (m, 2H), 2.32 – 1.98 (m, 8H), 1.98 – 1.59 (m, 6H), 1.57 – 1.26 (m, 4H). HPLC: 99%, t_R 0.45 min. MS (ESI+): 467.4 $[M+H]^+$.

1,4-Bis(4-(dimethylamino)piperidin-1-yl)benzamide (**38**): Compound **38** was prepared similarly to compound **5** from terephthalic acid and *N*,*N*-dimethylpiperidin-4-amine hydrochloride. The TFA salt of the desired product was obtained as colorless oil (55%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.56 (s, 4H), 4.84 – 4.70 (m, 2H), 4.04 – 3.75 (m, 2H), 3.52 (tt, *J* = 12.0, 3.8 Hz, 2H), 3.29 – 3.11 (m, 2H), 3.04 – 2.80 (m, 2H), 2.90 (s, 12H), 2.39 – 1.91 (m, 4H), 1.88 – 1.55 (m, 4H). HPLC: 99%, t_R 0.70 min. MS (ESI+): 387.3 $[M+H]^+$.

General procedure for the synthesis of compounds 39 – 48. To a mixture of the desired diacid (1 eq) and TBTU (2.6 eq) in DMF (1 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (2.4 eq) and triethylamine (6 eq) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford the desired product as a TFA salt (with the exception of compounds **42** and **46** which were basified). All compounds were synthesized in greater than 95% purity as confirmed by ^{1}H NMR and LCMS. See supplementary Figure 8 for NMR spectra.

2-benzylamino-1,4-Bis(4-(pyrrolidin-1-yl)piperidin-1-yl)benzamide (**49**): To a solution of **42** (26 mg, 0.058 mmol) in anhydrous methanol (0.6 mL) was added benzaldehyde (18 µL, 0.18 mmol) and acetic acid (10 µL, 0.18 mmol). The mixture was stirred at room temperature under nitrogen for 15 hours, after which $NaBH_3CN$ (15 mg, 0.23 mmol) was added and the reaction was stirred for 6 hours. The solvent was removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC. The resulting product was basified with satured aq. $NaHCO_3$ and extracted with CH_2Cl_2 to afford 18 mg (57%) of the title compound as a pale yellow oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.37 – 7.27 (m, 4H), 7.25 – 7.19 (m, 1H), 7.17 (d, *J* = 7.7 Hz, 1H), 6.67 (dd, *J* = 7.7, 1.4 Hz, 1H), 6.52 (d, *J* = 1.4 Hz, 1H), 4.64 – 4.47 (m, 2H), 4.46 – 4.40 (m, 2H), 4.10 – 3.68 (m, 1H), 3.58 – 3.43 (m, 1H), 3.24 – 2.91 (m, 2H), 2.90 – 2.72 (m, 2H), 2.72 – 2.54 (m, 8H), 2.39 (tt, *J* = 11.0, 3.9 Hz, 1H), 2.28 (tt, *J* = 11.0, 3.9 Hz, 1H), 2.14 – 1.91 (m, 3H), 1.91 – 1.76 (m, 8H), 1.70 – 1.58 (m, 1H), 1.58 – 1.32 (m, 3H), 1.19 – 1.02 (m, 1H). HPLC: 99%, t_R 2.2 min. MS (ESI+): 544.4 $[M+H]^+$.

2-benzyl-1,4-Bis(4-(pyrrolidin-1-yl)piperidin-1-yl)benzamide (**50**): A mixture of compound **40** (60 mg, 0.12 mmol), benzylboronic acid pinacol ester (38 mg, 0.17 mmol), potassium carbonate (24 mg, 0.17 mmol), and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.035 mmol) in water (0.5 mL) and tetrahyrdofuran (2 mL) was heated by microwave irradiation to 110 °C for 40 min in a sealed tube. The crude reaction was diluted with CH_2Cl_2, filtered over celite, washed with saturated aq. $NaHCO_3$ (10 mL, 2x), and finally dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 62 mg (71%) of the TFA salt of **50** as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 – 7.10 (m, 8H), 4.83 – 4.62 (m, 2H), 4.18 – 3.94 (m, 2H), 3.92 – 3.74 (m, 1H), 3.73 – 3.33 (m, 7H), 3.29 – 3.02 (m, 6H), 2.96 – 2.82 (m, 1H), 2.68 – 2.52 (m, 1H), 2.38 – 1.38 (m, 16H). HPLC: 99%, t_R 2.3 min. MS (ESI+): 529.4 $[M+H]^+$.

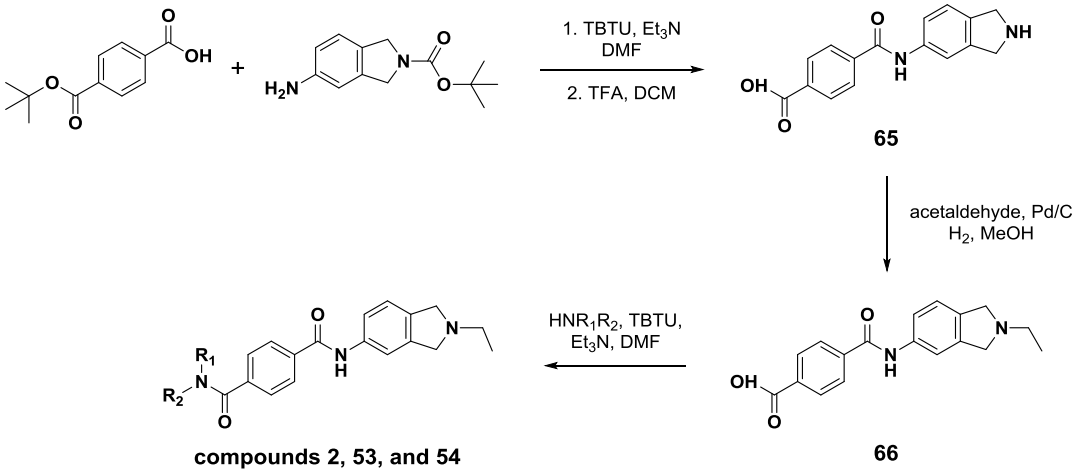


2-(phenoxy)terephthalic acid (64): A mixture of di-tert-butyl 2-bromoterephthalate (142 mg, 0.40 mmol) and potassium carbonate (38 mg, 0.40 mmol) in dimethylacetamide (0.5 mL) was heated at reflux overnight. The reaction was concentrated and determined to be a mixture of **64** and 2-bromoterephthalic acid. The crude mixture was purified by reverse phase HPLC (with a gradient of 10%

MeOH in H_2O with 0.1% TFA to 90% MeOH in H_2O with 0.1% TFA over 25 min) to afford 51 mg (51%) of **64** as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.94 (d, *J* = 8.1 Hz, 1H), 7.82 (dd, *J* = 8.1, 1.5 Hz, 1H), 7.52 (d, *J* = 1.4 Hz, 1H), 7.42 – 7.35 (m, 2H), 7.18 – 7.13 (m, 1H), 7.03 – 6.98 (m, 2H). HPLC: 99%, t_R 4.6 min. MS (ESI-): 257.1.4 $[M-H]^-$.

2-phenoxy-1,4-Bis(4-(pyrrolidin-1-yl)piperidin-1-yl)benzamide (**51**): To a mixture of **64** (44 mg, 0.17 mmol) and TBTU (76 mg, 0.24 mmol) in DMF (0.5 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (34 mg, 0.22 mmol) and triethylamine (0.12 mL, 0.85 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 56 mg (44%) of the TFA salt of **51** as a light tan solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.56 – 7.37 (m, 3H), 7.31 – 7.18 (m, 2H), 7.11 (dd, *J* = 27.0, 8.0 Hz, 2H), 6.89 (d, *J* = 18.4 Hz, 1H), 4.85 – 4.55 (m, 2H), 3.97 – 3.75 (m, 2H), 3.74-3.53 (m, 4H), 3.49 – 3.36 (m, 2H), 3.28 – 3.02 (m, 6H), 2.97 – 2.74 (m, 2H), 2.38 – 1.88 (m, 12H), 1.79 – 1.38 (m, 4H). HPLC: 99%, t_R 2.08 min. MS (ESI+): 531.4 $[M + H]^+$.

2-phenyl-1,4-Bis(4-(pyrrolidin-1-yl)piperidin-1-yl)benzamide (**52**): A mixture of compound **40** (60 mg, 0.12 mmol), phenylboronic acid (21 mg, 0.17 mmol), potassium carbonate (24 mg, 0.17 mmol), and tetrakis(triphenylphosphine)palladium(0) (40 mg, 0.035 mmol) in water (0.5 mL) and tetrahyrdofuran (2 mL) was heated by microwave irradiation to 110 °C for 40 min in a sealed tube. The crude reaction was diluted with CH_2Cl_2, filtered over celite, washed with saturated aq. $NaHCO_3$ (10 mL, 2x), and finally dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 51 mg (59%) of the TFA salt of **52** as a colorless oil. The NMR spectrum reveals that the compound exists as a pair of isomers in about a 1:1 ratio. ^{1}H NMR (400 MHz, CD_3OD) δ 7.59 – 7.40 (m, 8H), 4.83 – 4.63 (m, 2H), 4.04 – 3.81 (m, 2H), 3.75 – 3.51 (m, 3H), 3.50 – 3.36 (m, 3H), 3.28 – 2.85 (m, 7H), 2.79 – 2.69 (m, 0.5H), 2.48 – 1.84 (m, 13H), 1.80 – 1.51 (m, 3H), 1.50 – 1.37 (m, 0.5H), 1.15 – 1.02 (m, 0.5H), 0.17 – 0.05 (m, 1H). HPLC: 99%, t_R 2.2 min. MS (ESI+): 515.4 $[M+H]^+$.



4-(isoindolin-5-ylcarbamoyl)benzoic acid (**65**): To a mixture of 4-(*tert*-butoxycarbonyl)benzoic acid (198 mg, 0.89 mmol) and TBTU (400 mg, 1.25 mmol) in DMF (1 mL) was added a solution of *tert*-butyl 5-aminoisoindoline-2-carboxylate (250 mg, 1.07 mmol) and triethylamine (0.37 mL, 2.67 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation. The crude product was then deprotected in CH_2Cl_2 (5 mL) and TFA (2 mL) and the reaction was stirred at room temperature overnight. After evaporation of the solvent the crude mixture was purified by reverse phase HPLC to afford 257 mg (73% over 2 steps) of the TFA salt of **65** as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 – 8.14 (m, 2H), 8.03 – 7.99 (m, 2H), 7.97 – 7.94 (m, 1H), 7.67 – 7.63 (m, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 4.65 (s, 2H), 4.62 (s, 2H). HPLC: 99%, t_R 2.7 min. MS (ESI+): 283.1 $[M+H]^+$.

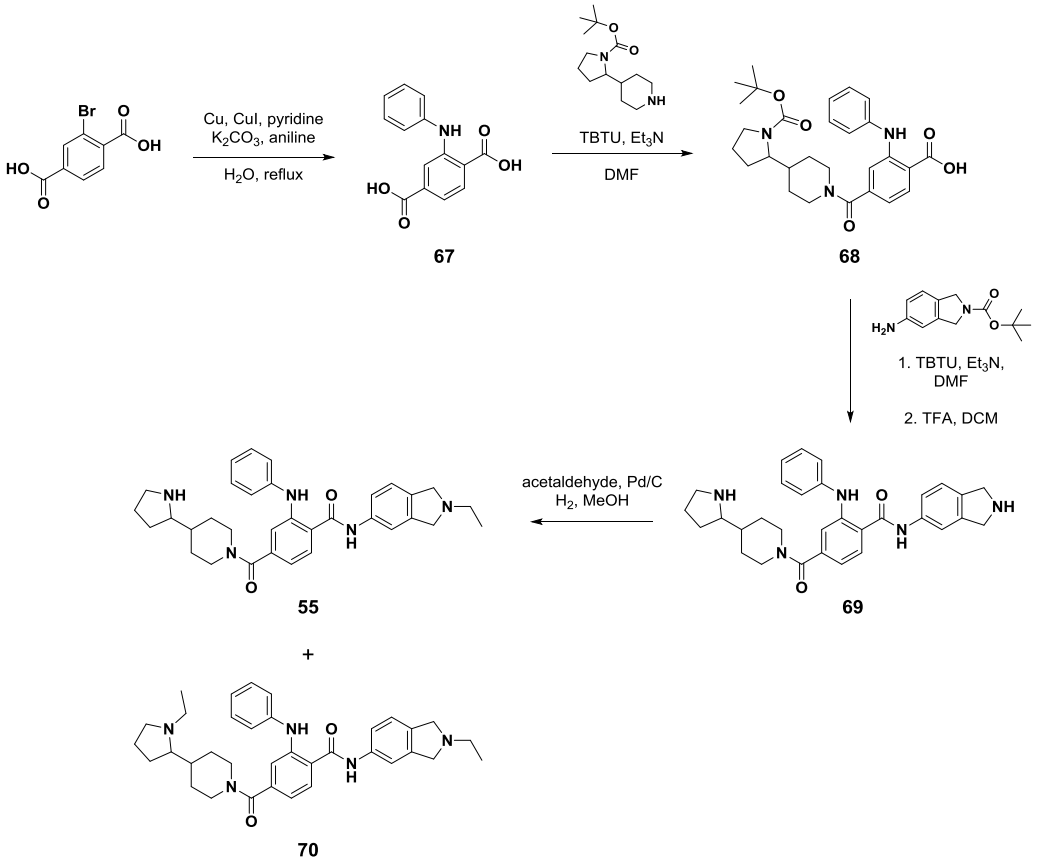
4-((2-ethylisoindolin-5-yl)carbamoyl)benzoic acid (**66**): A mixture of **65** (52 mg, 0.13 mmol) and Pd/C (5.2 mg, 10% wt) in anhydrous MeOH (5 mL) under hydrogen atmosphere was stirred for 20 min, after which acetaldehyde (15 µl, 0.26 mmol) was added. The reaction was stirred at room temperature for 2 hours. The resulting solution was diluted with MeOH, filtered over celite, and the solvents were removed by rotary evaporation. The crude mixture was purified by reverse phase HPLC to afford 36 mg (65%) of the TFA salt of **66** as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18 – 8.14 (m, 2H), 8.03 – 7.99 (m, 2H), 7.98 – 7.96 (m, 1H), 7.67 – 7.63 (m, 1H), 7.42 (d, *J* = 8.3 Hz, 1H), 4.79 – 4.53 (bs, 4H), 3.51 (q, *J* = 7.3 Hz, 2H), 1.44 (t, *J* = 7.3 Hz, 3H). HPLC: 99%, t_R 3.0 min. MS (ESI+): 311.1 $[M+H]^+$.

***N*-(2-ethylisoindolin-5-yl)-4-(4-(pyrrolidin-1-yl)piperidine-1-carbonyl)benzamide** (**53**): To a mixture of **66** (17 mg, 0.040 mmol) and TBTU (34 mg, 0.10 mmol) in DMF (0.5 mL) was added a solution of 4-(1-pyrrolidinyl)piperidine (15 mg, 0.096 mmol) and triethylamine (33 µL, 0.24 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 18 mg (67%) of **53** as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.06 – 8.02 (m, 2H), 7.96 – 7.94 (m, 1H), 7.66 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.60 – 7.57 (m, 2H), 7.42 (d, *J* = 8.3 Hz, 1H), 4.97 – 4.74 (m, 3H), 4.65 – 4.47 (m, 2H), 3.95 – 3.76 (m, 1H), 3.76 – 3.58 (m, 2H), 3.51 (q, *J* = 7.3 Hz, 2H), 3.49 – 3.41 (m, 1H), 3.29 – 3.09 (m, 3H), 3.03 – 2.85 (m, 1H), 2.40 – 1.94 (m, 6H), 1.80 – 1.57 (m, 2H), 1.44 (t, *J* = 7.3 Hz, 3H). HPLC: 99%, t_R 1.9 min. MS (ESI+): 447.3 $[M+H]^+$.

***N*-(2-ethylisoindolin-5-yl)-4-((1R,5S)-8-(pyrrolidin-1-yl)-3-azabicyclo[3.2.1]octane-3-carbonyl)benzamide** (**54**): Compound **54** was prepared similarly to compound **53** from **66** and (1*R*,5*S*)-8-(pyrrolidin-1-yl)-3-azabicyclo[3.2.1]octane dihydrochloride. The TFA salt of the desired product was obtained as colorless oil (51%). ^{1}H NMR (400 MHz, CD_3OD) δ 8.07 – 8.03 (m, 2H), 7.97 – 7.94 (m, 1H), 7.69 – 7.63 (m, 3H), 7.42 (d, *J* = 8.3 Hz, 1H), 4.98 – 4.86 (m, 3H), 4.65 – 4.47 (m, 2H), 4.32 – 4.15 (m, 1H), 3.80 – 3.69 (m, 1H), 3.69 – 3.55 (m, 2H), 3.51 (q, *J* = 7.2 Hz, 2H), 3.21 – 3.04 (m, 2H), 2.36 – 2.08 (m, 6H), 2.07 – 1.94 (m, 3H), 1.94 – 1.70 (m, 3H), 1.44 (t, *J* = 7.3 Hz, 3H). HPLC: 99%, t_R 2.2 min. MS (ESI+): 473.4 $[M+H]^+$.

***N*-(2-ethylisoindolin-5-yl)-4-(4-(pyrrolidin-2-yl)piperidine-1-carbonyl)benzamide** (**2**): To a mixture of **66** (22 mg, 0.052 mmol) and TBTU (43 mg, 0.14 mmol) in DMF (0.5 mL) was added a solution of *tert*-butyl 2-(piperidin-4-yl)pyrrolidine-1-carboxylate (32 mg, 0.13 mmol) and triethylamine (44 µL, 0.31 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation. The crude product was then deprotected in CH_2Cl_2 (5 mL) and TFA (1 mL) and the reaction was stirred at room temperature for 3 hours. After evaporation of the solvent the crude mixture was purified by reverse phase HPLC to afford 28 mg (80% over 2 steps) of the TFA salt of **2** as a colorless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 8.05 – 8.00 (m, 2H), 7.96 – 7.94 (m, 1H), 7.66 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.59 – 7.54 (m, 2H), 7.42 (d, *J* = 8.3 Hz, 1H), 5.00 – 4.87 (m, 2H), 4.78 – 4.67 (m, 1H), 4.65

– 4.45 (m, 2H), 3.83 – 3.68 (m, 1H), 3.51 (q, *J* = 7.3 Hz, 2H), 3.35 – 3.32 (m, 2H), 3.30 – 3.25 (m, 1H), 3.24 – 3.10 (m, 1H), 2.99 – 2.83 (m, 1H), 2.35 – 2.18 (m, 1H), 2.17 – 1.84 (m, 4H), 1.84 – 1.63 (m, 2H), 1.44 (t, *J* = 7.3 Hz, 3H), 1.53 – 1.27 (m, 2H). HPLC: 99%, t_R 2.3 min. MS (ESI+): 447.3 $[M+H]^+$.

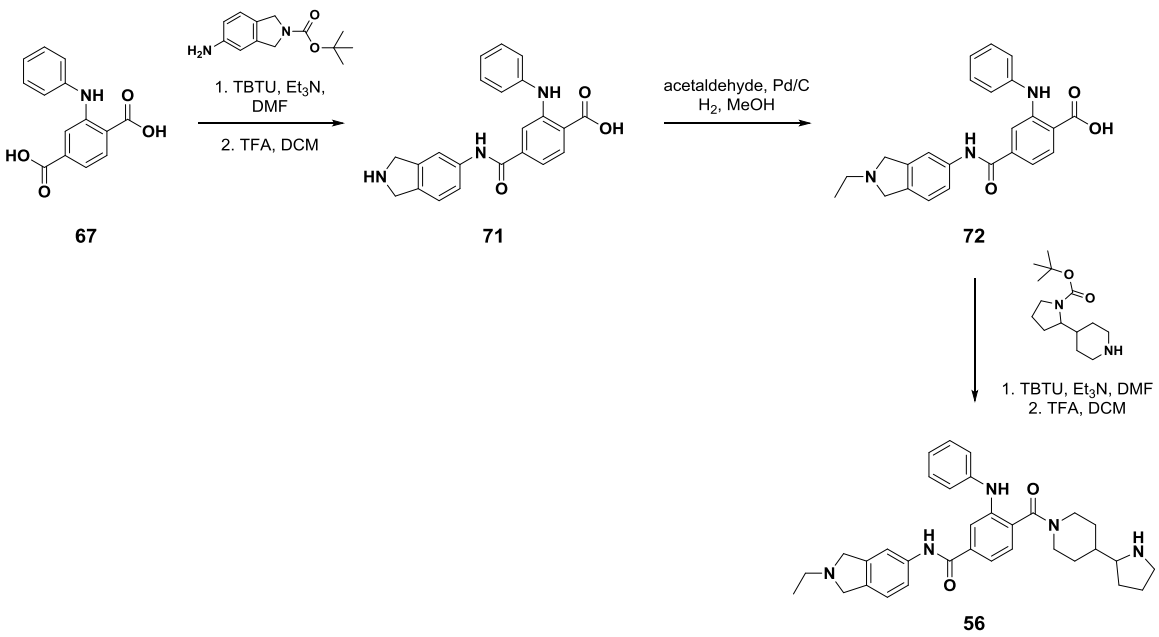


2-(phenylamino)terephthalic acid (**67**): Compound **67** was prepared as reported previously.[1] The crude product was carried forward without purification.

4-(4-(1-(*tert*-butoxycarbonyl)pyrrolidin-2-yl)piperidine-1-carbonyl)-2-(phenylamino)benzoic acid (**68**): To a mixture of **67** (150 mg, 0.58 mmol) and TBTU (240 mg, 0.76 mmol) in DMF (1 mL) was added a solution of *tert*-butyl 2-(piperidin-4-yl)pyrrolidine-1-carboxylate (160 mg, 0.64 mmol) and triethylamine (240 µL, 1.7 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation and the crude product was purified by column chromatography on silica gel (0 – 20% MeOH/CH_2Cl_2). Compound **68** was obtained with slight impurities and was used in the next step without further purification.

***N*-(isoindolin-5-yl)-2-(phenylamino)-4-(4-(pyrrolidin-2-yl)piperidine-1-carbonyl)benzamide** (**69**): To a mixture of **68** (74 mg, 0.15 mmol) and TBTU (63 mg, 0.20 mmol) in DMF (0.5 mL) was added a solution of *tert*-butyl 5-aminoisoindoline-2-carboxylate (42 mg, 0.18 mmol) and triethylamine (63 µL, 0.45 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation. The crude product was then deprotected in CH_2Cl_2 (5 mL) and TFA (1 mL) and the reaction was stirred at room temperature overnight. After evaporation of the solvent the crude mixture was purified by reverse phase HPLC to afford 75 mg (17% over 4 steps) of the TFA salt of **2** as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.89 (s, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.61 (dd, *J* = 8.3, 1.8 Hz, 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 7.36 – 7.30 (m, 2H), 7.23 (s, 1H), 7.22 – 7.17 (m, 2H), 7.08 – 7.02 (m, 1H), 6.86 (dd, *J* = 8.0, 1.4 Hz, 1H), 4.70 – 4.64 (m, 1H), 4.63 (s, 2H), 4.61 (s, 2H), 3.90 – 3.73 (m, 1H), 3.35 – 3.20 (m, 3H), 3.19 – 3.06 (m, 1H), 2.93 – 2.77 (m, 1H), 2.31 – 2.16 (m, 1H), 2.16 – 1.96 (m, 2H), 1.96 – 1.63 (m, 4H), 1.45 – 1.16 (m, 2H). HPLC: 99%, t_R 3.1 min. MS (ESI+): 510.4 $[M+H]^+$.

***N*-(2-ethylisoindolin-5-yl)-2-(phenylamino)-4-(4-(pyrrolidin-2-yl)piperidine-1-carbonyl)benzamide** (**55**): A mixture of **69** (66 mg, 0.089 mmol) and Pd/C (6.6 mg, 10% wt) in anhydrous MeOH (2 mL) under hydrogen atmosphere was stirred for 20 min, after which acetaldehyde (11 µl, 0.19 mmol) was added. The reaction was stirred at room temperature for 4 hours. The resulting solution was diluted with MeOH, filtered over celite, and the solvents were removed by rotary evaporation. A crude mixture containing compounds **55** and **70** was purified by reverse phase HPLC to afford 22 mg (33%) of the TFA salt of **55** as a pure light brown solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 – 7.87 (m, 1H), 7.81 (d, *J* = 8.0 Hz, 1H), 7.62 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.39 (d, *J* = 8.3 Hz, 1H), 7.36 – 7.29 (m, 2H), 7.23 (s, 1H), 7.21 – 7.16 (m, 2H), 7.08 – 7.02 (m, 1H), 6.86 (dd, *J* = 8.0, 1.5 Hz, 1H), 5.00 – 4.74 (m, 2H), 4.71 – 4.61 (m, 1H), 4.61 – 4.41 (m, 2H), 3.90 – 3.73 (m, 1H), 3.50 (q, *J* = 7.2 Hz, 2H), 3.36 – 3.20 (m, 3H), 3.19 – 3.06 (m, 1H), 2.93 – 2.76 (m, 1H), 2.31 – 2.16 (m, 1H), 2.16 – 1.96 (m, 2H), 1.96 – 1.62 (m, 4H), 1.43 (t, *J* = 7.2 Hz, 3H), 1.38 – 1.15 (m, 2H). HPLC: 99%, t_R 3.1 min. MS (ESI+): 538.3 $[M+H]^+$.



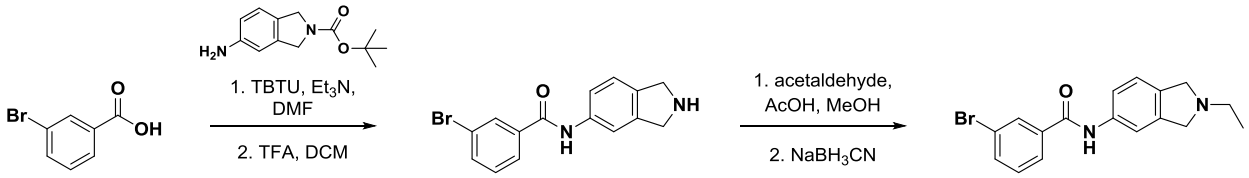
67 71 72 56

4-(isoindolin-5-ylcarbamoyl)-2-(phenylamino)benzoic acid (**71**): To a mixture of **67** (150 mg, 0.58 mmol) and TBTU (240 mg, 0.76 mmol) in DMF (1 mL) was added a solution of *tert*-butyl 5-aminoisoindoline-2-carboxylate (150 mg, 0.64 mmol) and triethylamine (240 µL, 1.7 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation. The crude product was then deprotected in CH_2Cl_2 (5 mL) and TFA (1 mL) and the reaction was stirred at room temperature overnight. After evaporation of the solvent the crude mixture was purified by reverse phase HPLC to afford 118 mg (42% over 3 steps) of the TFA salt of **71** as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, *J* = 8.3 Hz, 1H), 7.89 – 7.86 (m, 1H), 7.74 (d, *J* = 1.7 Hz, 1H), 7.58 (dd, *J* = 8.2, 1.9 Hz, 1H), 7.41 – 7.36 (m, 3H), 7.32 – 7.27 (m, 2H), 7.20 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.15 – 7.10 (m, 1H), 4.62 (s, 2H), 4.60 (s, 2H). HPLC: 99%, t_R 4.1 min. MS (ESI+): 374.2 $[M+H]^+$.

4-((2-ethylisoindolin-5-yl)carbamoyl)-2-(phenylamino)benzoic acid (**72**): A mixture of **71** (96 mg, 0.20 mmol) and Pd/C (9.6 mg, 10% wt) in anhydrous MeOH (5 mL) under hydrogen atmosphere was stirred for 20 min, after which acetaldehyde (17 µl, 0.3 mmol) was added. The reaction was stirred at room temperature overnight. The resulting solution was diluted with MeOH, filtered over celite, and the solvents were removed by rotary evaporation. The crude mixture was purified by reverse phase HPLC to afford 28 mg (28%) of the TFA salt of **72** as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (d, *J* = 8.2 Hz, 1H), 7.90 – 7.86 (m, 1H), 7.74 (d, *J* = 1.6 Hz, 1H), 7.59 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.41 – 7.36 (m, 3H),

7.32 – 7.27 (m, 2H), 7.20 (dd, *J* = 8.2, 1.7 Hz, 1H), 7.15 – 7.10 (m, 1H), 4.80 – 4.32 (m, 4H), 3.50 (q, *J* = 7.3 Hz, 2H), 1.43 (t, *J* = 7.3 Hz, 3H). HPLC: 99%, t_R 4.1 min. MS (ESI+): 402.2 $[M+H]^+$.

***N*-(2-ethylisoindolin-5-yl)-3-(phenylamino)-4-(4-(pyrrolidin-2-yl)piperidine-1-carbonyl)benzamide** (**56**): To a mixture of **72** (28 mg, 0.055 mmol) and TBTU (23 mg, 0.072 mmol) in DMF (0.5 mL) was added a solution of *tert*-butyl 2-(piperidin-4-yl)pyrrolidine-1-carboxylate (17 mg, 0.066 mmol) and triethylamine (23 µL, 0.17 mmol) in DMF (0.5 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation. The crude product was then deprotected in CH_2Cl_2 (5 mL) and TFA (1 mL) and the reaction was stirred at room temperature overnight. After evaporation of the solvent the crude mixture was purified by reverse phase HPLC to afford 32 mg (76%) of the TFA salt of **56** as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.91 – 7.87 (m, 1H), 7.79 (d, *J* = 1.6 Hz, 1H), 7.61 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.49 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.42 – 7.36 (m, 2H), 7.31 – 7.25 (m, 2H), 7.13 – 7.08 (m, 2H), 6.96 (tt, *J* = 7.4, 1.2 Hz, 1H), 5.00 – 4.88 (m, 2H), 4.74 – 4.41 (m, 3H), 3.93 – 3.61 (m, 1H), 3.50 (q, *J* = 7.3 Hz, 2H), 3.30 – 3.25 (m, 2H), 3.23 – 2.98 (m, 2H), 2.92 – 2.63 (m, 1H), 2.28 – 1.91 (m, 3H), 1.91 – 1.55 (m, 4H), 1.43 (t, *J* = 7.3 Hz, 3H), 1.39 – 1.17 (m, 2H). HPLC: 99%, t_R 2.9 min. MS (ESI+): 538.4 $[M+H]^+$. HRMS calculated for $C_{33}H_{39}N_5O_2$ + H: 538.32; found: 538.3163 $[M+H]^+$. HRMS calculated for $C_{33}H_{39}N_5O_2$ - H: 536.30; found: 536.3040 $[M+H]^+$.



73 **58**

3-bromo-*N*-(isoindolin-5-yl)benzamide (**73**): To a mixture of 3-bromobenzoic acid (180 mg, 0.90 mmol) and TBTU (431 mg, 1.34 mmol) in DMF (2 mL) was added a solution of *tert*-butyl 5-aminoisoindoline-2-carboxylate (252 mg, 1.06 mmol) and triethylamine (0.4 mL, 2.7 mmol) in DMF (1 mL). The mixture was stirred at room temperature for 15 hours. The reaction was quenched by the addition of saturated aq. $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (15 mL, 3x). The combined organic extracts were washed with sat. aq. $NaHCO_3$ and dried over Na_2SO_4. After filtration, the solvents were removed by rotary evaporation. The crude product was then deprotected in CH_2Cl_2 (5 mL) and TFA (1 mL) and the reaction was stirred at room temperature overnight. After evaporation of the solvent the crude mixture was

purified by reverse phase HPLC to afford 309 mg (80% over 2 steps) of the TFA salt of **73** as a white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (t, *J* = 1.8 Hz, 1H), 7.94 – 7.88 (m, 2H), 7.76 (ddd, *J* = 8.0, 2.0, 1.0 Hz, 1H), 7.66 – 7.61 (m, 1H), 7.45 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.5 Hz, 1H), 4.64 (s, 2H), 4.61 (s, 2H). HPLC: 99%, t_R 3.5 min. MS (ESI+): 317.0 + 319.0 $[M+H]^+$.

3-bromo-*N*-(2-ethylisoindolin-5-yl)benzamide (**58**): To a solution of **73** (80 mg, 0.19 mmol) in anhydrous methanol (2 mL) was added acetaldehyde (31 µL, 0.56 mmol) and acetic acid (53 µL, 0.93 mmol) in anhydrous methanol (1.5 mL). The mixture was stirred at room temperature for 15 hours, after which $NaBH_3CN$ (64 mg, 1.02 mmol) was added and the reaction was stirred for an additional 2 hours. The solvent was removed by rotary evaporation and the crude mixture was purified by reverse phase HPLC to afford 22 mg (26%) of the TFA salt of **58** as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.11 (t, *J* = 1.9 Hz, 1H), 7.95 – 7.93 (m, 1H), 7.91 (ddd, *J* = 7.8, 1.8, 1.0 Hz, 1H), 7.76 (ddd, *J* = 8.0, 1.9, 1.0 Hz, 1H), 7.67 – 7.62 (m, 1H), 7.45 (t, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 8.3 Hz, 1H), 4.82 (m, 4H), 3.51 (q, *J* = 7.3 Hz, 2H), 1.44 (t, *J* = 7.3 Hz, 3H). HPLC: 99%, t_R 3.5 min. MS (ESI+): 345.1 + 347.1 $[M+H]^+$.

Protein Expression and Purification. L3MBTL1 and L3MBTL3 were purified essentially as described previously.[2, 4] Briefly, cell pellets from 2 L cultures expressing His-tagged proteins were lysed with BugBuster protein extraction reagent (EMD Millipore, Darmstadt, Germany) containing 20 mM imidazole. The cell lysate was clarified by centrifugation and loaded onto a 5 mL HisTrap HP column (GE Healthcare, Piscataway, NJ) equilibrated with binding and wash buffer (50 mM sodium phosphate buffer pH 7.2, 500 mM NaCl, 30 mM imidazole) using an ÄKTA FPLC (GE Healthcare, Piscataway, NJ) at 1 mL/min. His-tagged protein was eluted using a linear gradient of elution buffer (50 mM sodium phosphate buffer pH 7.2, 500 mM NaCl, 500 mM imidazole) over 20 column volumes. Fractions containing the desired protein were confirmed by SDS-PAGE, pooled and loaded at 2 mL/min onto a HiLoad 26/60 Superdex 200 prep grade size exclusion column (GE Healthcare, Piscataway, NJ) using an ÄKTA FPLC. A constant flow of 2 mL/min size exclusion buffer (25 mM Tris·HCl pH 8.0, 250 mM NaCl, 1 mM EDTA, 2 mM DTT, 0.02% Tween 20) was used to elute proteins. Fractions containing the desired protein were identified by SDS-PAGE, pooled and subjected to simultaneous concentration and buffer exchange using an Amicon Ultra-15 centrifugal filter unit (Millipore, Billerica, MA) and storage buffer (20 mM Tris·HCl pH 8.0, 150 mM NaCl and 2 mM DTT for L3MBTL1 and 20 mM Tris HCl, pH 8.0, 250 mM NaCl and 2 mM DTT for L3MBTL3).

A pET28-mhl vector containing the coding region for residues 1-455 of SFMBT1 (reference sequence AAH14614) was transformed into BL21 Codon Plus cells (Agilent Technologies, Santa Clara, CA). A 2 L

culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. SFMBT1 protein was purified essentially as described above, except for the use of a buffer consisting of 25 mM Tris HCl pH 7.5, 250 mM NaCl, and 1 mM DTT for size exclusion chromatography and protein storage.

A pET28-mhl vector containing the coding region for residues 130-566 of MBTD1 (reference sequence NP_060113) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. MBTD1 protein was purified essentially as described for SFMBT1.

A pET28-mhl vector containing the coding region for residues 1485-1611 of 53BP1 (reference sequence (NP_001135452) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. 53BP1 protein was purified essentially as described above except for the use of a buffer consisting of 25 mM Tris HCl pH 7.5, 150 mM NaCl, and 2 mM DTT for size exclusion chromatography and protein storage.

A pET28 vector containing the coding region for residues 121-286 of UHRF1 (reference sequence (NP_001041666) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. UHRF1 protein was purified exactly as described for 53BP1.

A pET28 vector containing the coding region for residues 8-62 of CBX7 (reference sequence (NP_783640) was transformed into BL21 Rosetta DE3 pLysS cells (EMD Millipore). A 2 L culture was grown to mid log phase at 37 °C then the temperature was lowered to 18 °C and protein expression was induced by addition of 0.5 mM IPTG. Expression was allowed to continue overnight. CBX7 protein was purified exactly as described for 53BP1.

The identity of all expression constructs was verified by DNA sequencing and all proteins were at least 95% pure as determined by Coomassie staining. Protein concentration was determined by absorbance at 280 nm using the Edelhoch method. L3MBTL4 and L3MBTL1 L361F were provided by the Structural Genomics Consortium. PHF23 and JARID1A proteins were provided by Greg Wang (UNC).

CellTiter-Glo Luminescent Cell Viability Assay. The effect of UNC1215, UNC1021, and UNC1679 on cell viability was determined using a modified CellTiter-Glo™ ATP detection system (Promega #7573). Ten point, 1:3 dilution curves of compounds starting at 100 µM final concentration were diluted to 5X final concentration in PBS (vehicle control) and then 5 µL were added to 384-well white, clear bottom tissue culture plates (Corning #3707) with a Multimek automated liquid handling device (Nanoscreen, Charleston, SC). Twenty microliters of low passage, subconfluent HEK293T/17 cells (ATCC CRL-11268) grown in Dulbecco's Modified Eagle's Medium without phenol red (Gibco #31053) and supplemented with 10% Fetal Bovine Serum (Gibco #26140) were immediately added at a density of 5,000 cells per well using a Multidrop 384 (Titertek). Cell plates were incubated for 24 hours at 37°C and 5% $C0_2$, and then lysed with 25 microliters of CellTiter-Glo™ reagent. Luminescence was read on an Envision platereader (Perkin Elmer) after 15 minutes at room temperature in dim light.

Experimental Procedures for M_1 and M_2 Assays. Protocols of the M_1 Ca^{2+} mobilization assay are available from the NIMH PDSP website: http://pdsp.med.unc.edu/UNC-CH%20Protocol%20Book.pdf.

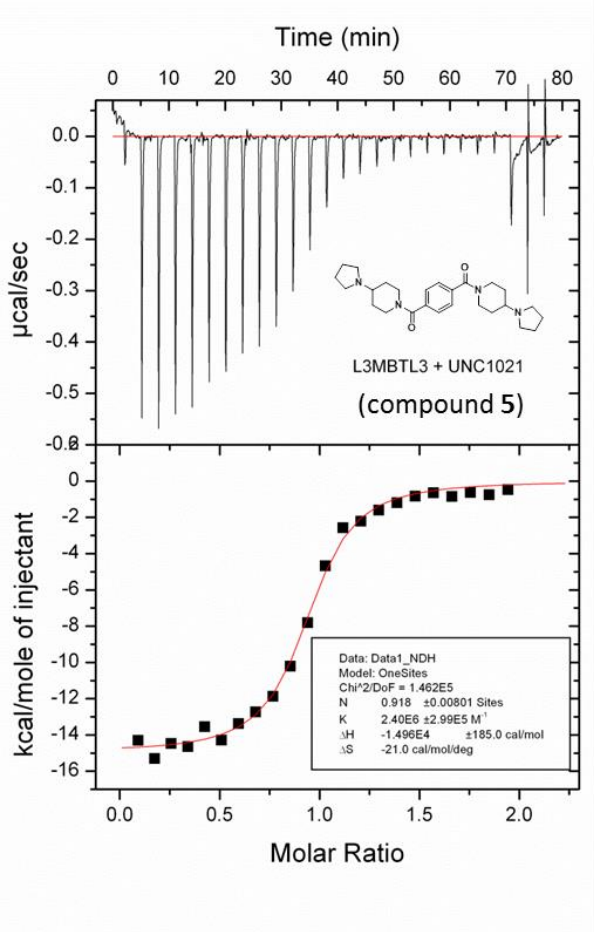
Protocols of M_2 cAMP biosensor assays: HEK293T cells co-transfected with the cAMP biosensor GloSensor-22F (Promega) and hM_2 were seeded (20,000 cells/40 µL/well) into poly-L-Lysine coated 384-well white, clear-bottom, tissue culture plates in DMEM with 1% dialyzed FBS for overnight. Before the assay, media was removed and cells were loaded with 4 mM luciferin (20 µL/well) in drug buffer (1x HBSS, 20 mM HEPES, pH 7.4) for 90 min at 37°C. For agonist activity, cells were first treated with UNC1679 for 10 min, followed by addition of 200 nM isoproterenol for another 10 min. For antagonist activity, cells were first treated with UNC1679 for 10 min, followed by 100 nM acetylcholine for 10 min, then 200 nM isoproterenol for 10 min. Luminescence per well per second was read on a Wallac TriLux microbeta plate counter. Data were normalized to the isoproterenol response (0%) and the maximal atropine-induced inhibition thereof (0%) and regressed using the sigmoidal dose-response function built into GraphPad Prism 5.0.

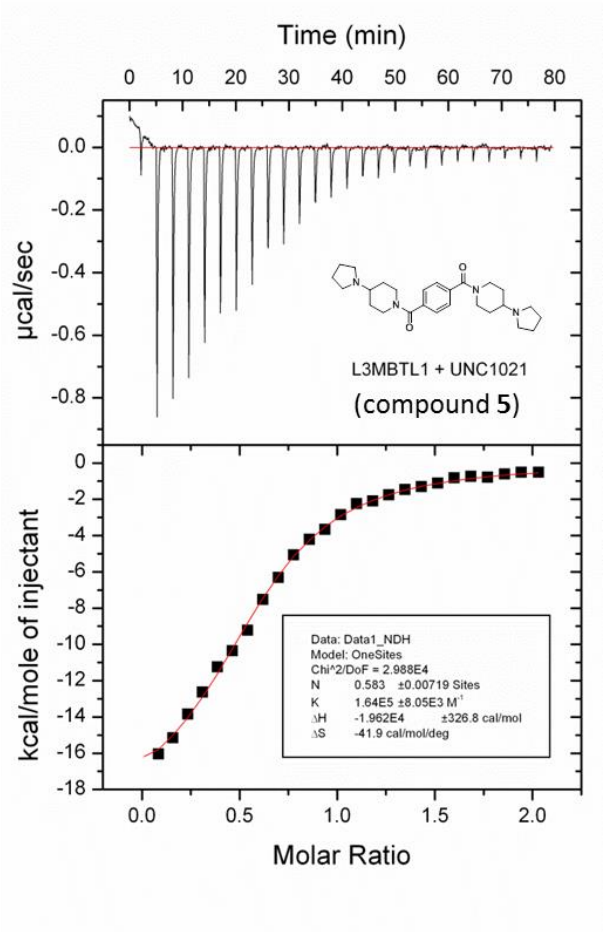
Supplementary Table 1. AlphaScreen proteins and corresponding peptide substrates.

Protein	Peptide	Peptide sequence	Protein final concentration in 10 µL	Peptide final concentration in 10 µL
L3MBTL1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-COOH	100 nM	150 nM
L3MBTL3	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH	100 nM	150 nM
L3MBTL4	H2AK36Me1	Biotin-AHA-GRVHRLLRK(Me1)GNYSER-COOH	100 nM	150 nM
MBTD1	H4K20Me1	Biotin-AHA-KGGAKRHRK(Me1)VLRDNIQ-COOH	100 nM	150 nM
SFMBT1	H3K9Me1	Biotin-AHA-ARTKQTARK(Me1)STGGKA-COOH	100 nM	150 nM
CBX7-Flag	H3K9Me3	ARTKQTARK(Me3)STGGKAPRKQL-K(Biotin)-NH2	100 nM	150 nM
53BP1	H4K20Me2	Biotin-AHX-KGGAKRHRK(Me2)VLRDNIQ-OH	100 nM	150 nM
UHRF1	H3K9Me3	Biotin-AHA-ARTKQTARK(Me3)STGGKA-COOH	100 nM	150 nM
PHF23	H3K4Me3	NH2-ARTK(Me3)QTARKSTGGKAPRKQYT-K(Biotin)	60 nM	100 nM
JARID1A	H3K4Me3	NH2-ARTK(Me3)QTARKSTGGKAPRKQYT-K(Biotin)	10 nM	30 nM

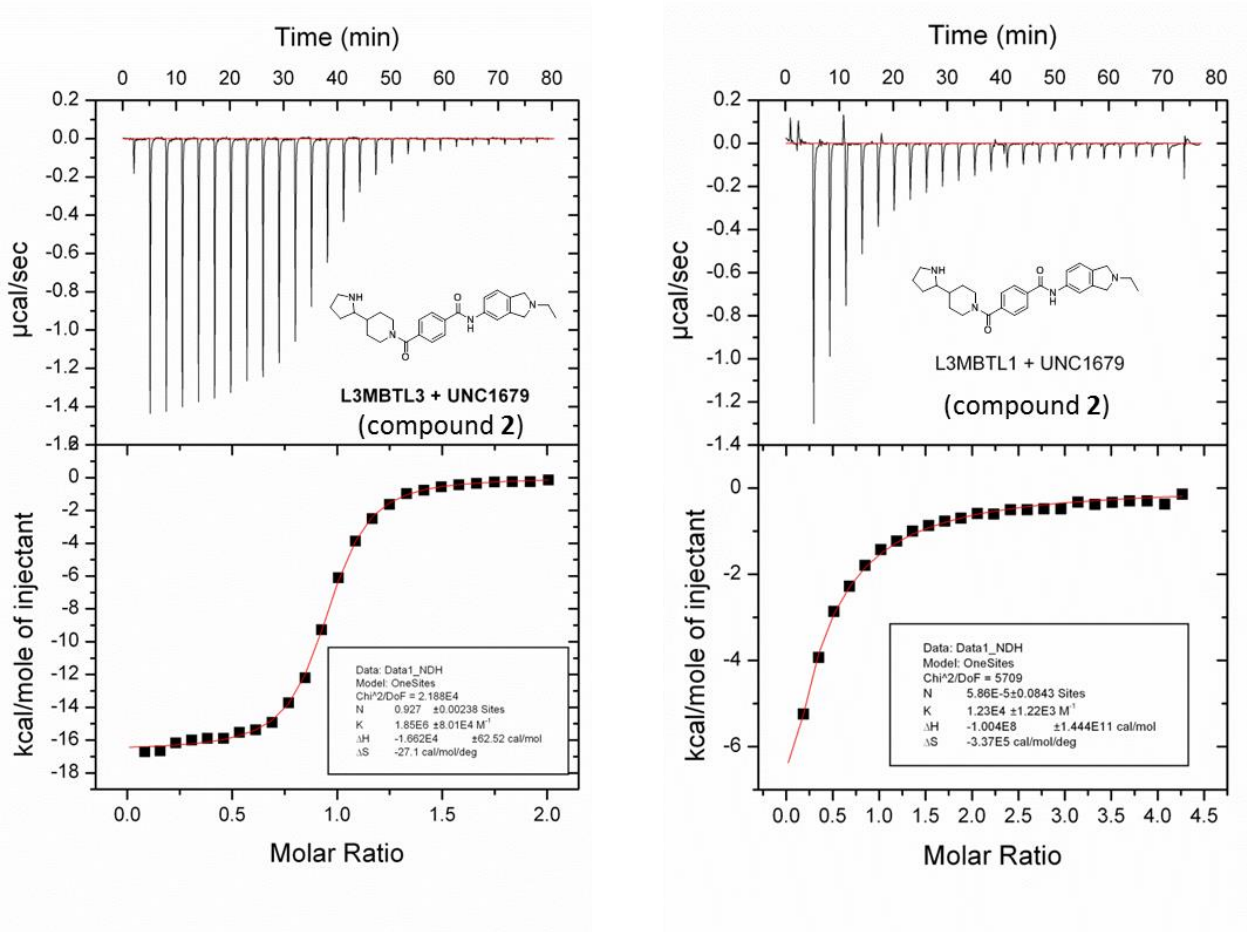
Supplementary Figure 1. Representative ITC binding curves.

a) Compound **5** is a potent inhibitor of L3MBTL3 with an average K_d of 0.39 ± 0.044 µM (left), but it has weaker affinity for L3MBTL1 (average K_d of 6.2 ± 1.9 µM).

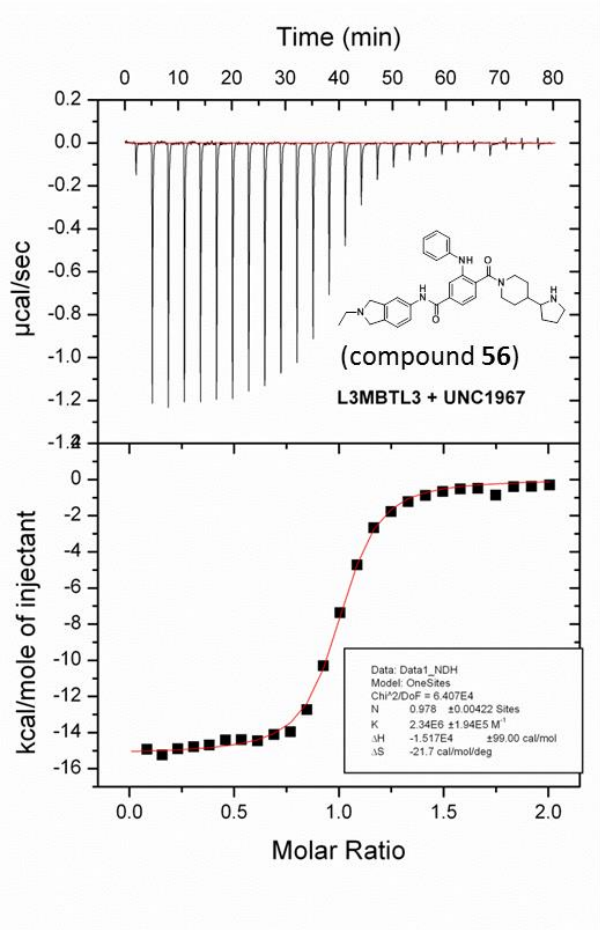


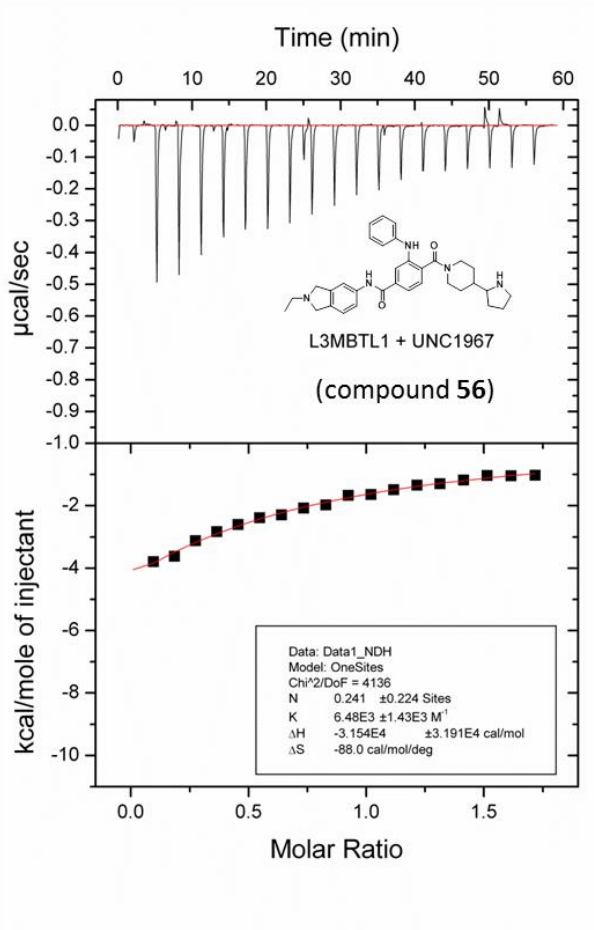


b) Compound **2** is a potent inhibitor of L3MBTL3 with an average K_d of 0.47 ± 0.14 µM (left), and demonstrates selectivity over L3MBTL1 (average K_d of 68 ± 18 µM).

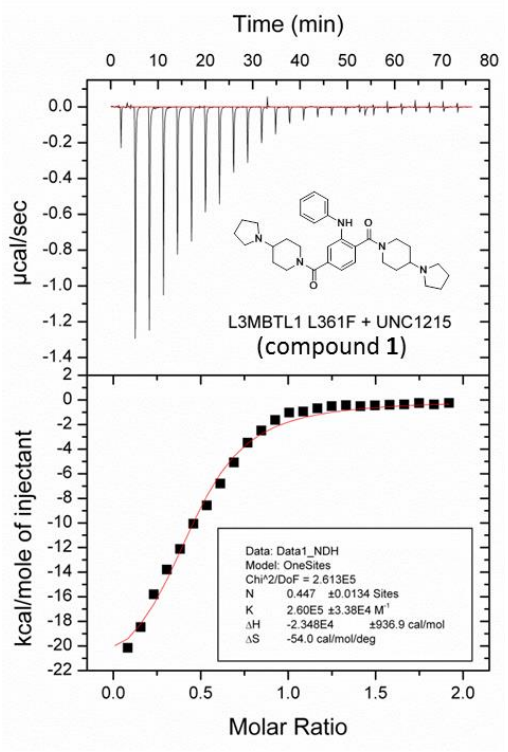


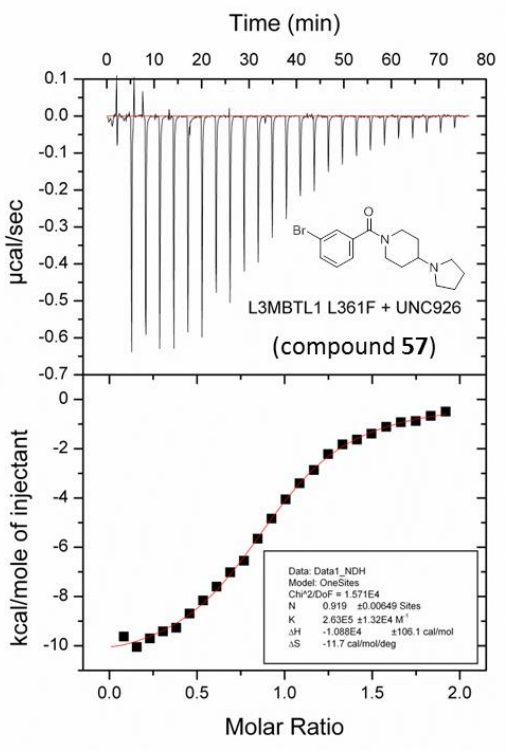
c) Compound **56** is a potent inhibitor of L3MBTL3 with an average K_d of 0.35 ± 0.087 µM (left), and demonstrates selectivity over L3MBTL1 (average K_d of 132 ± 32 µM).

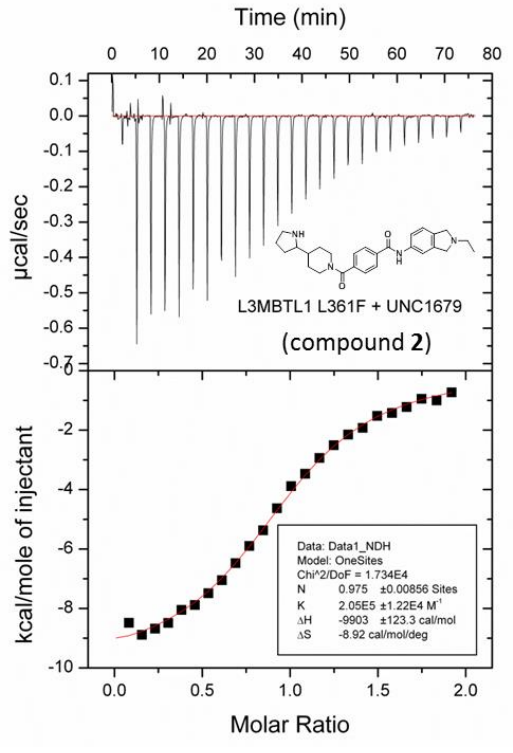




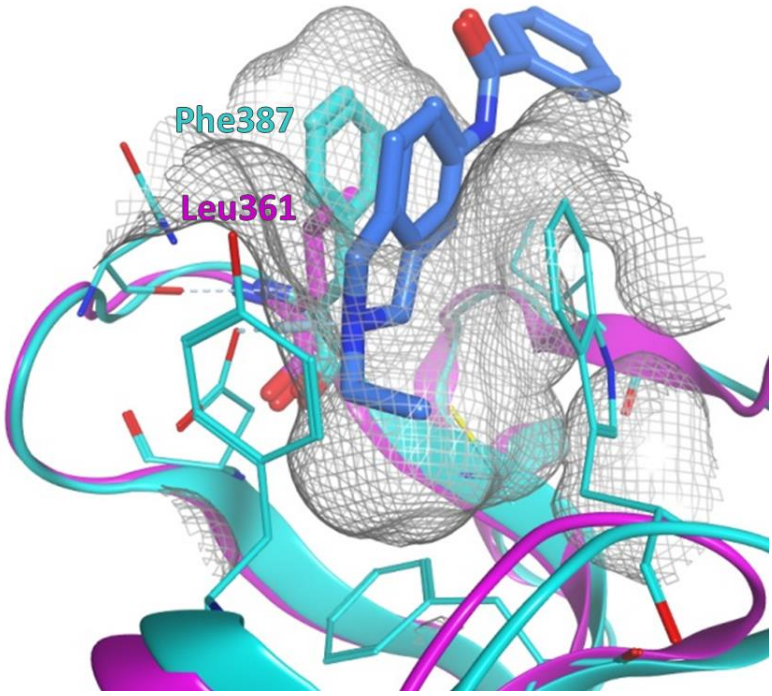
d) Compounds **1** and **57** bind an L3MBTL1 L361F mutant (K_d = 3.2 ± 0.92 µM and K_d = 3.8 ± 0.057 µM, respectively) similarly to wildtype L3MBTL1, whereas compound **2** binds L3MBTL1 L361F (K_d = 5.6 ± 0.99 µM) significantly better than wildtype L3MBTL1.



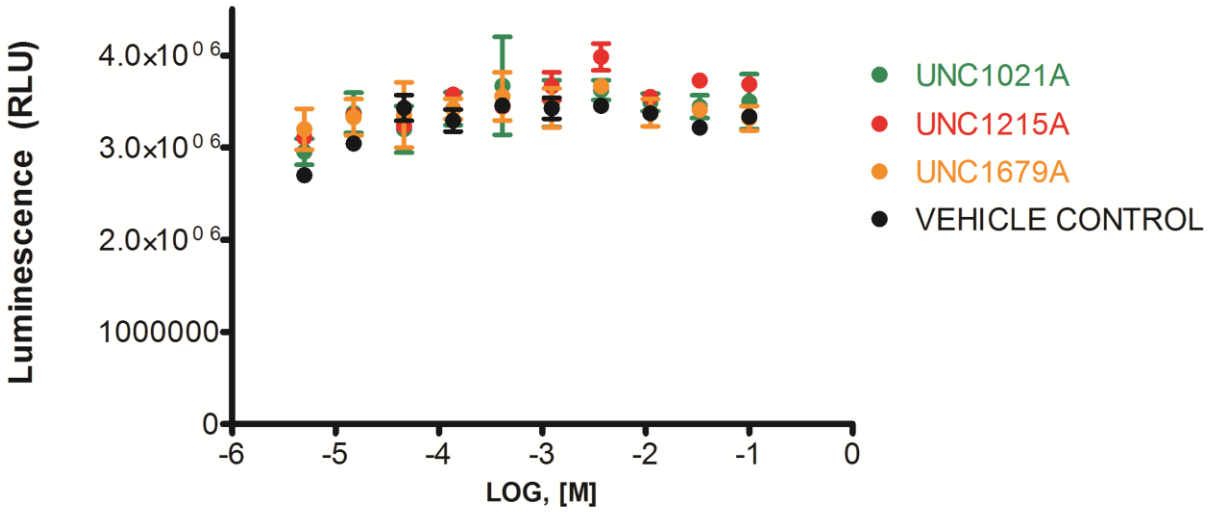




Supplementary Figure 2. Model of compound 58 (blue) in complex with L3MBTL3 (cyan; PDB: 3UT1) and L3MBTL1 (magenta; PDB: 3P8H). The ligand is likely to have an extra π-π interaction with Phe387 of L3MBTL3 (Leu361 in L3MBTL1) resulting in improved affinity for L3MBTL3 over L3MBTL1.

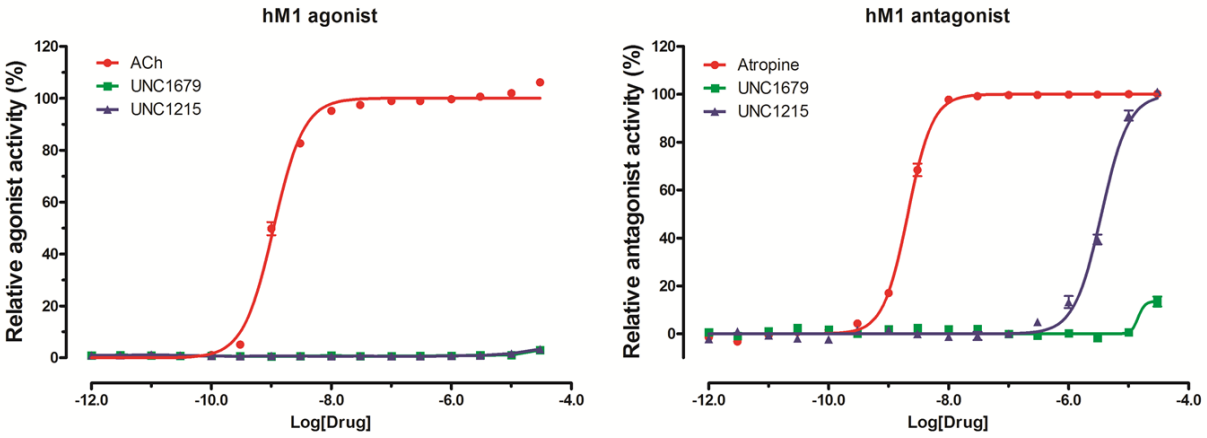


Supplementary Figure 3. CellTiter-Glo Luminescent Cell Viability Assay. Compounds **1** (UNC1215), **2** (UNC1679), and **5** (UNC1021) show no toxicity up to 100 µM.

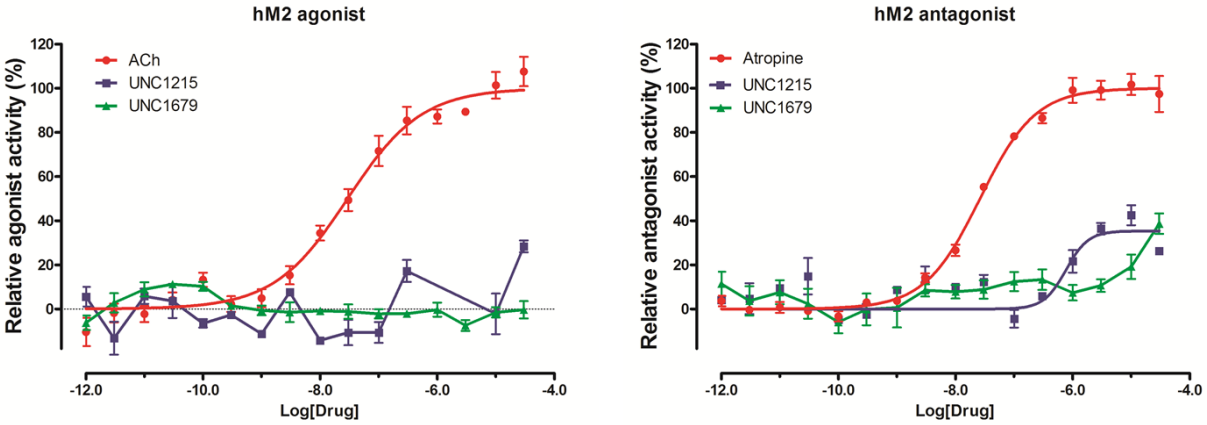


Supplementary Figure 4. M_1 and M_2 functional assays.

a) UNC1679 (**2**, green) had no agonist or antagonist activity at M_1 while UNC1215 (**1**, blue) shows weak antagonist activity at M_1. The agonist activity of UNC1679 (**2**) was measured against 10 nM acetylcholine (hM_1). Atropine serves as an antagonist control.

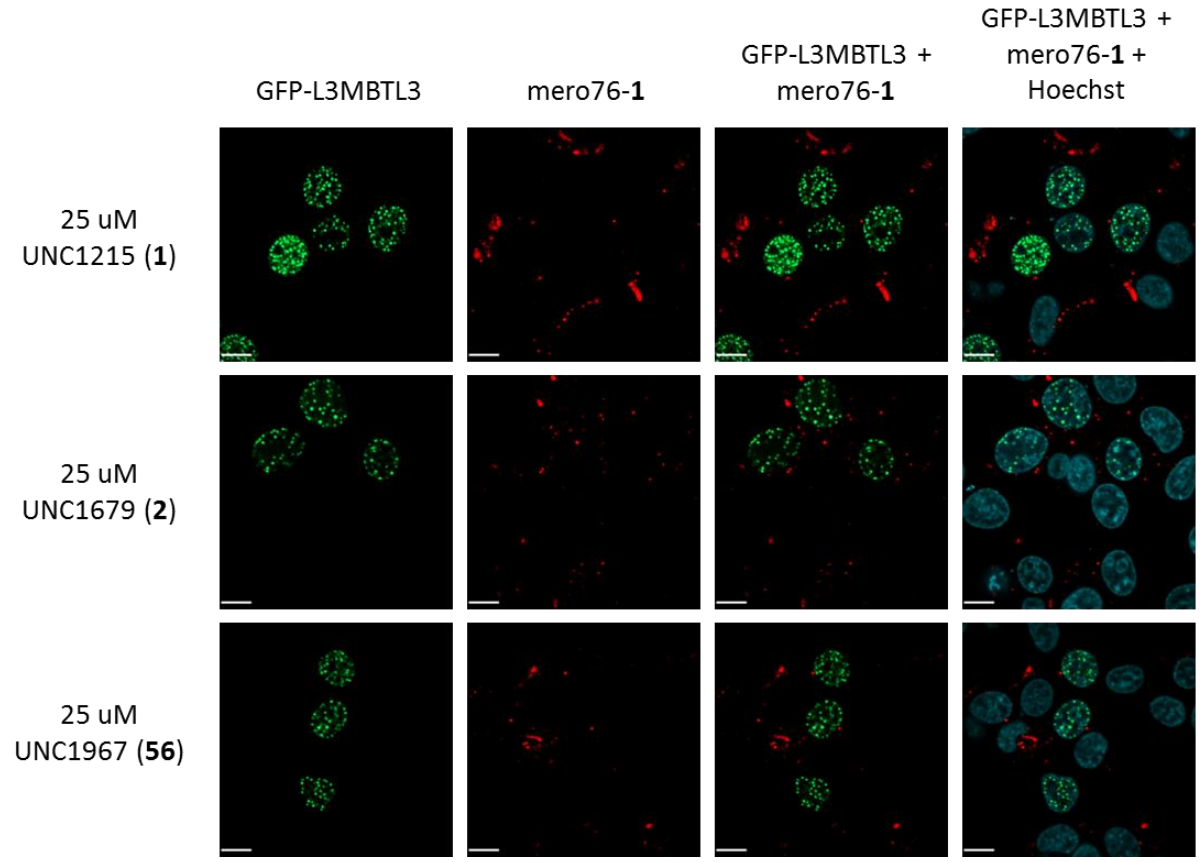


b) UNC1679 (**2**, green) had no agonist activity and weak functional antagonist activity at M_2, similarly to UNC1215 (**1**, blue). The agonist activity of UNC1679 (**2**) was measured against 100 nM acetylcholine (hM_2). Atropine serves as an antagonist control.

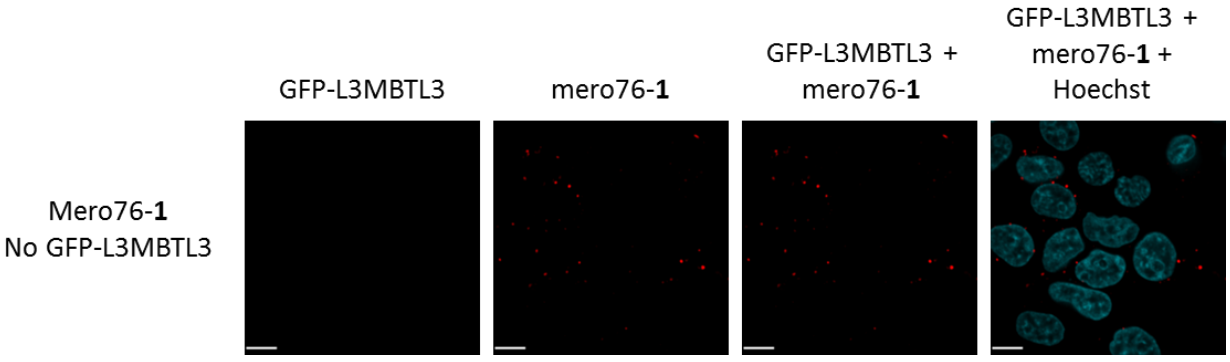


Supplementary Figure 5. UNC1679 (2) and UNC1967 (56) are active in cells.

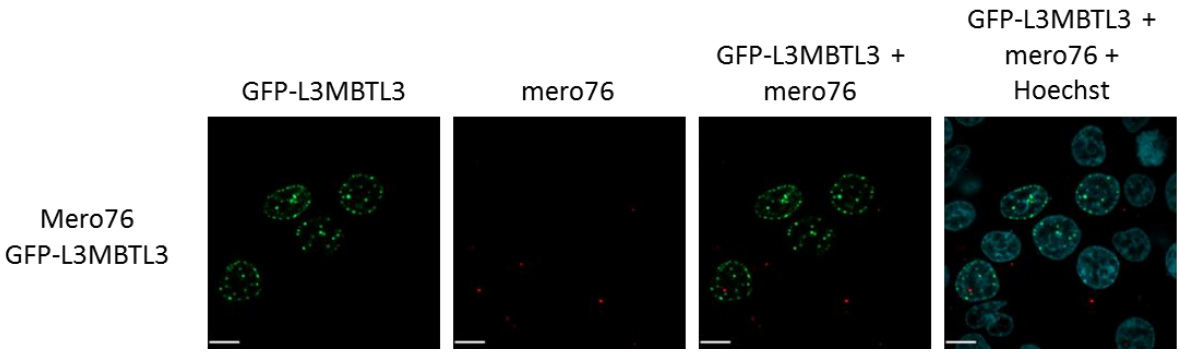
a) Treatment with 25 µM of free, untagged UNC1215 (**1**, 1st row), UNC1679 (**2**, 2nd row), or UNC1967 (**56**, 3rd row) in HEK293 cells fully displace mero76-**1** from GFP-L3MBTL3 (red is mero76-1; green is GFP-L3MBTL3; blue is Hoechst).



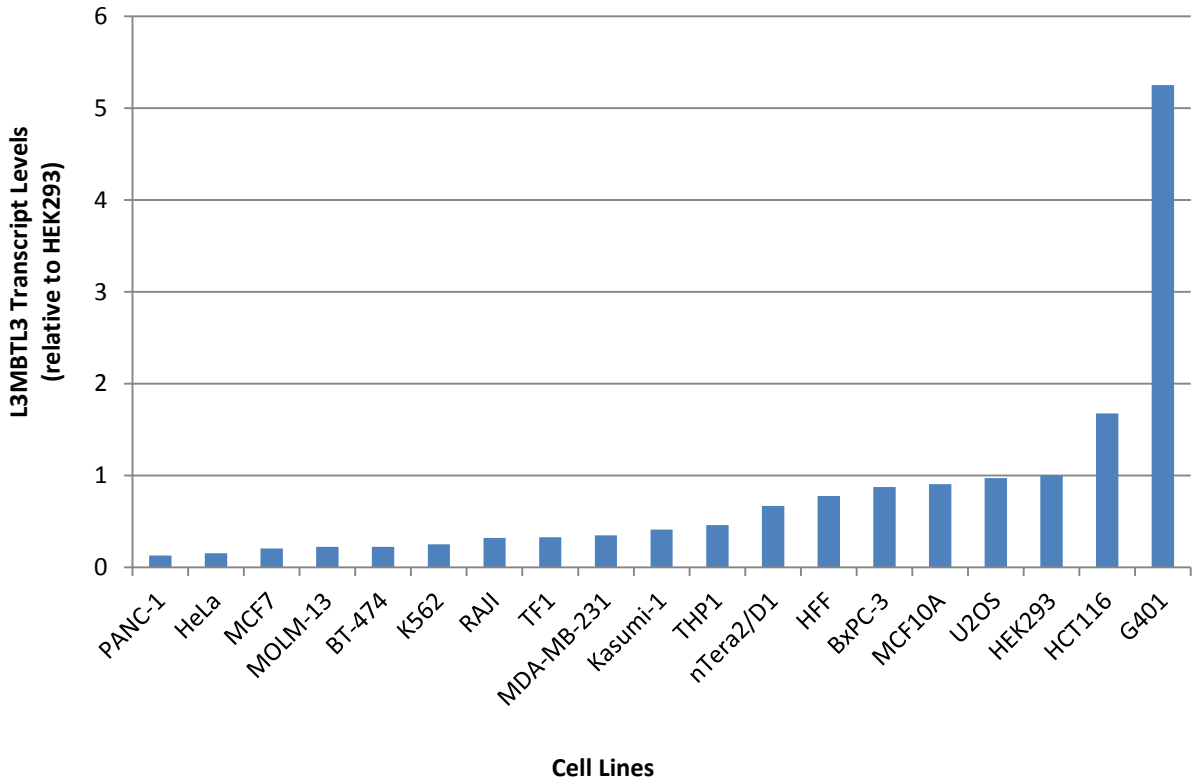
b) Mero76-**1** does not form nuclear foci in the absence of GFP-L3MBTL3.



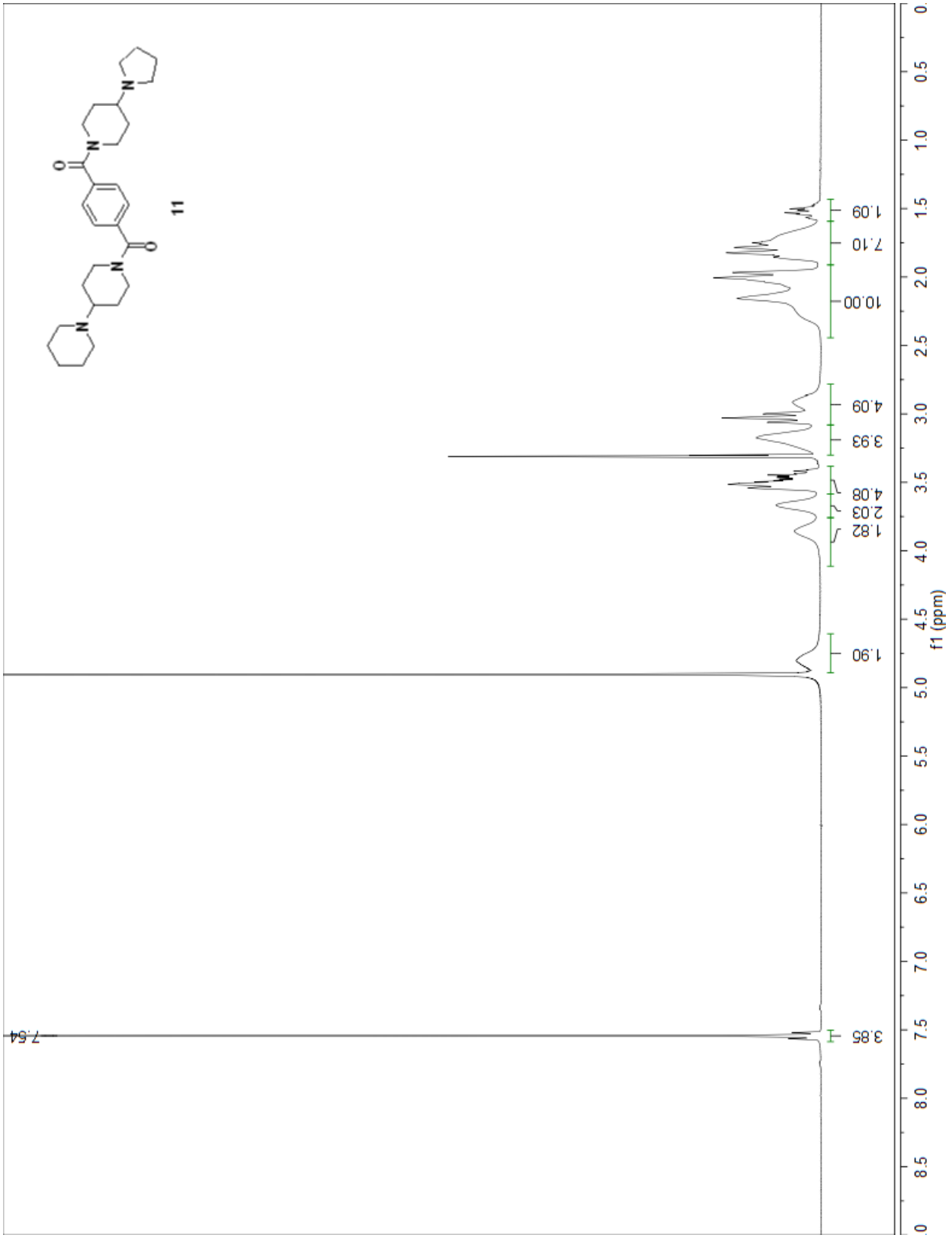
c) Mero76 alone does not form nuclear foci and therefore does not contribute to L3MBTL3 binding.

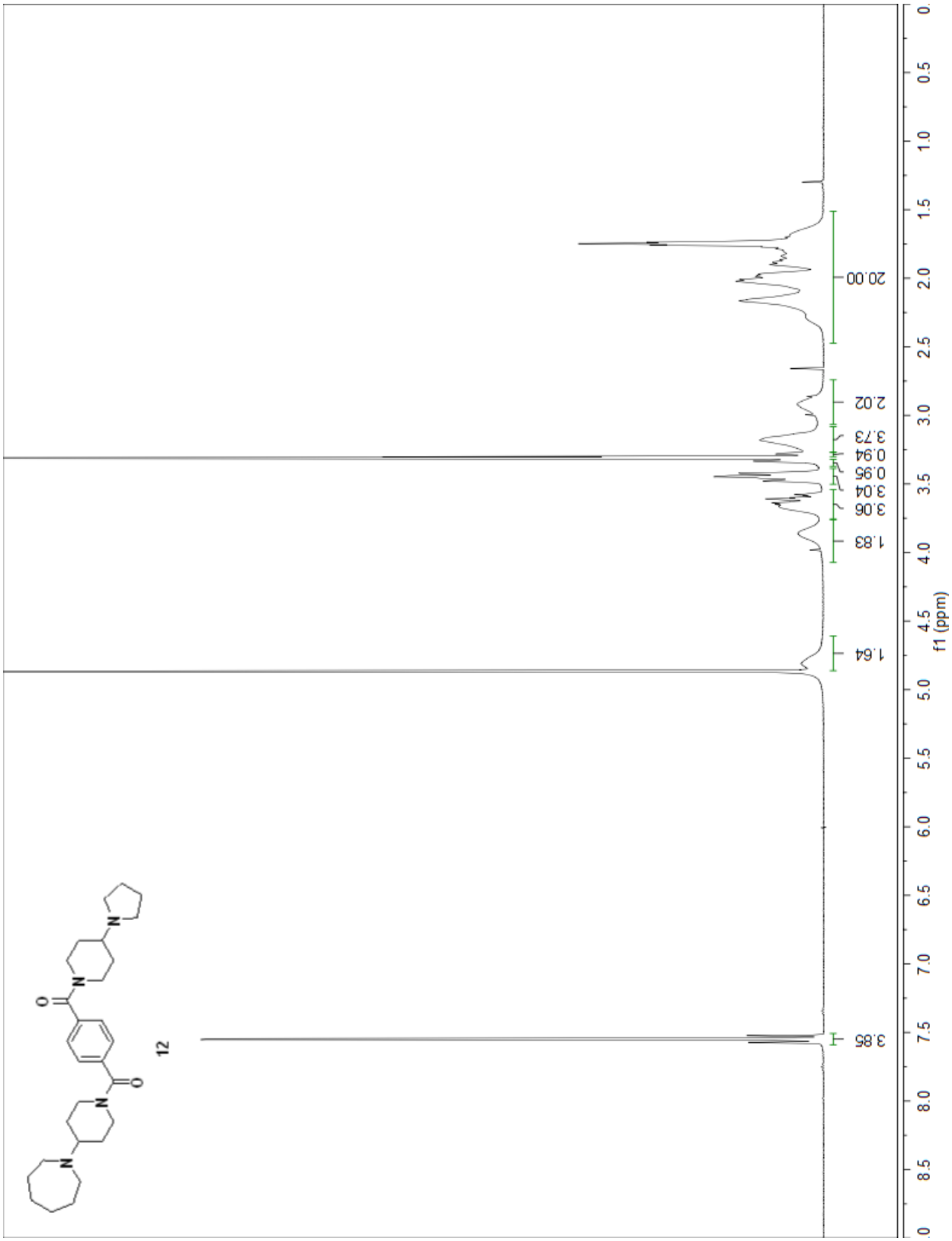


Supplementary Figure 6. L3MBTL3 mRNA levels in various cell lines. G-401 cells were identified as having higher levels (by greater than 5-fold) of endogenous L3MBTL3 than other cell lines.



Supplementary Figure 7. ^{1}**H NMR spectra of compounds 11-31 (Table 3).**

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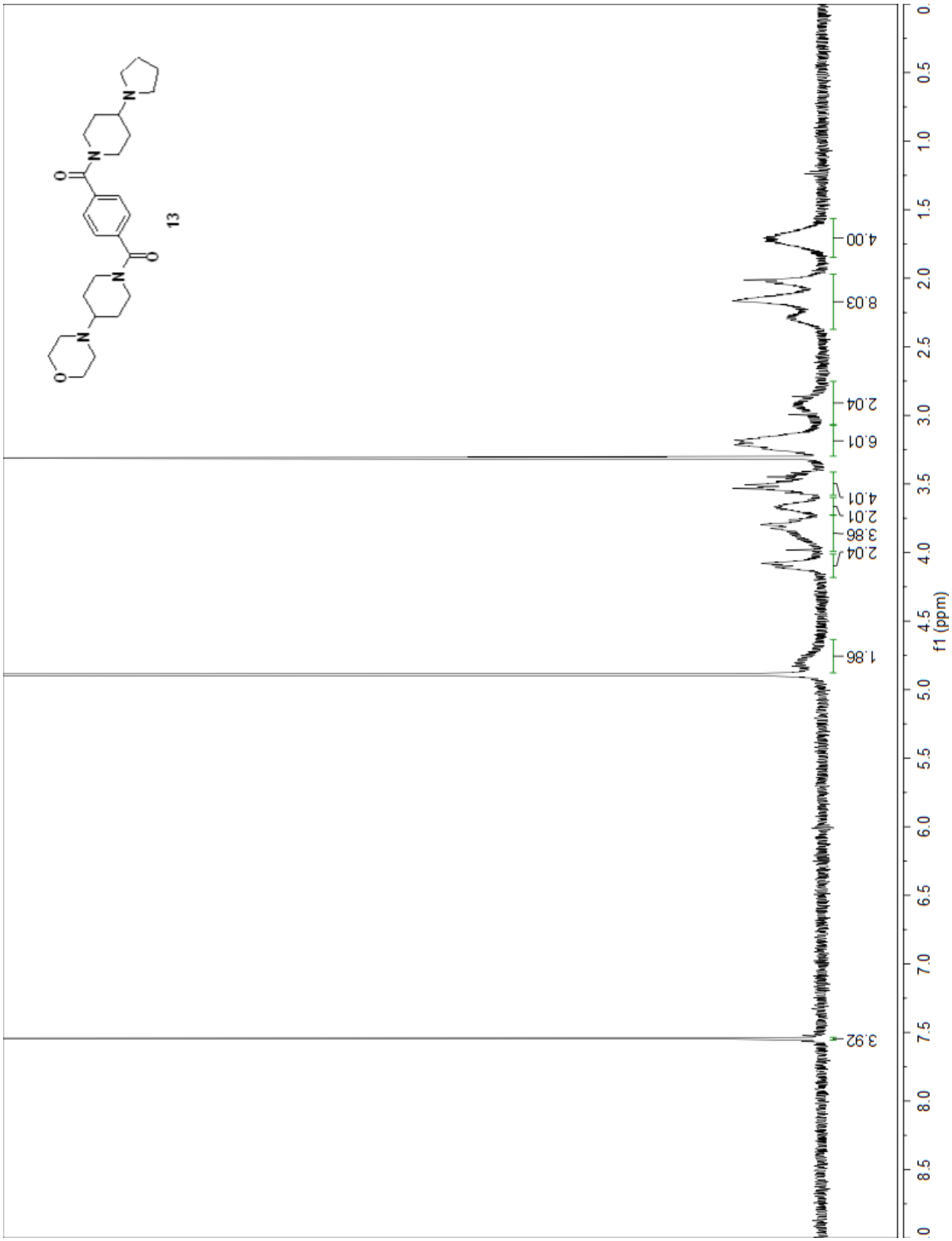
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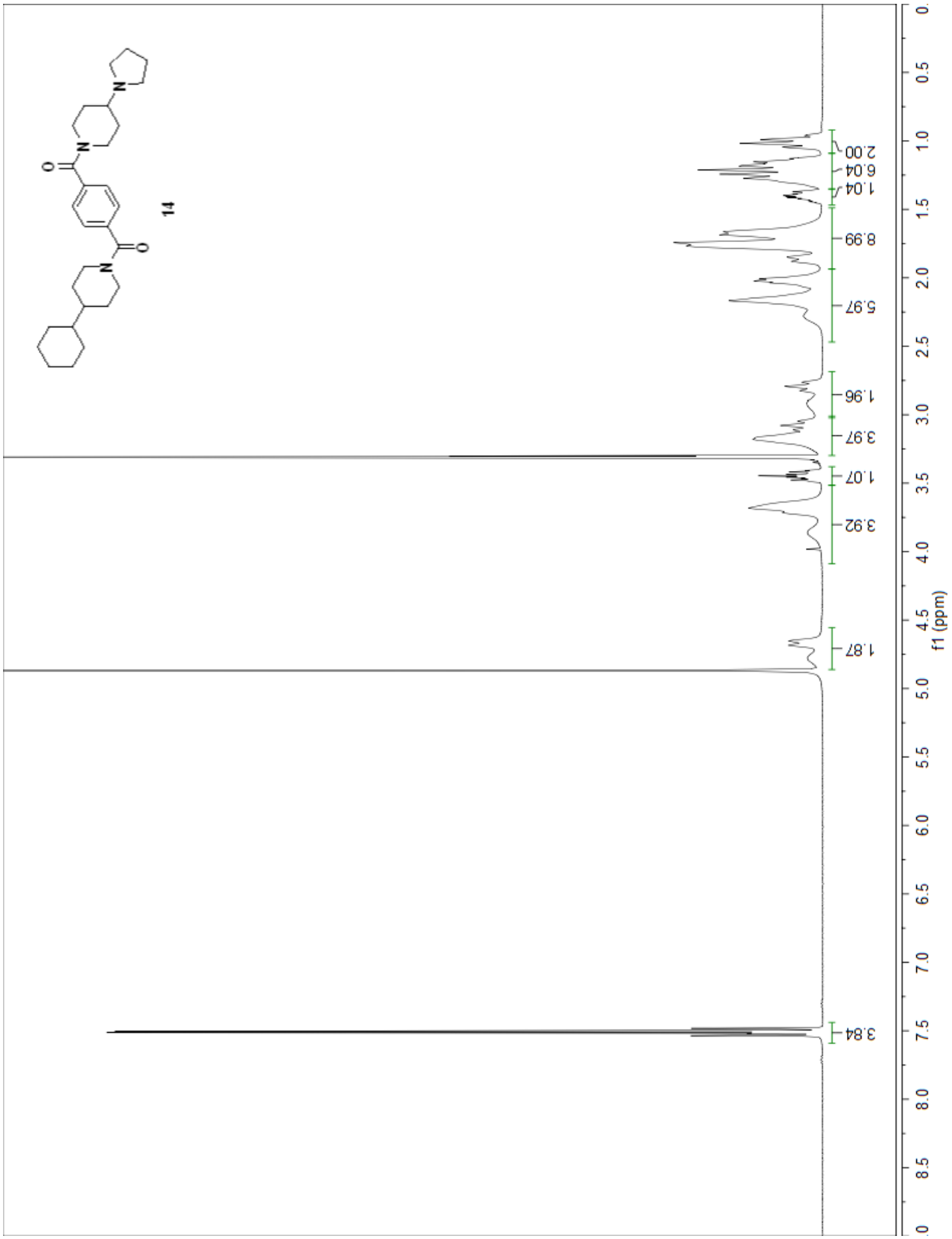
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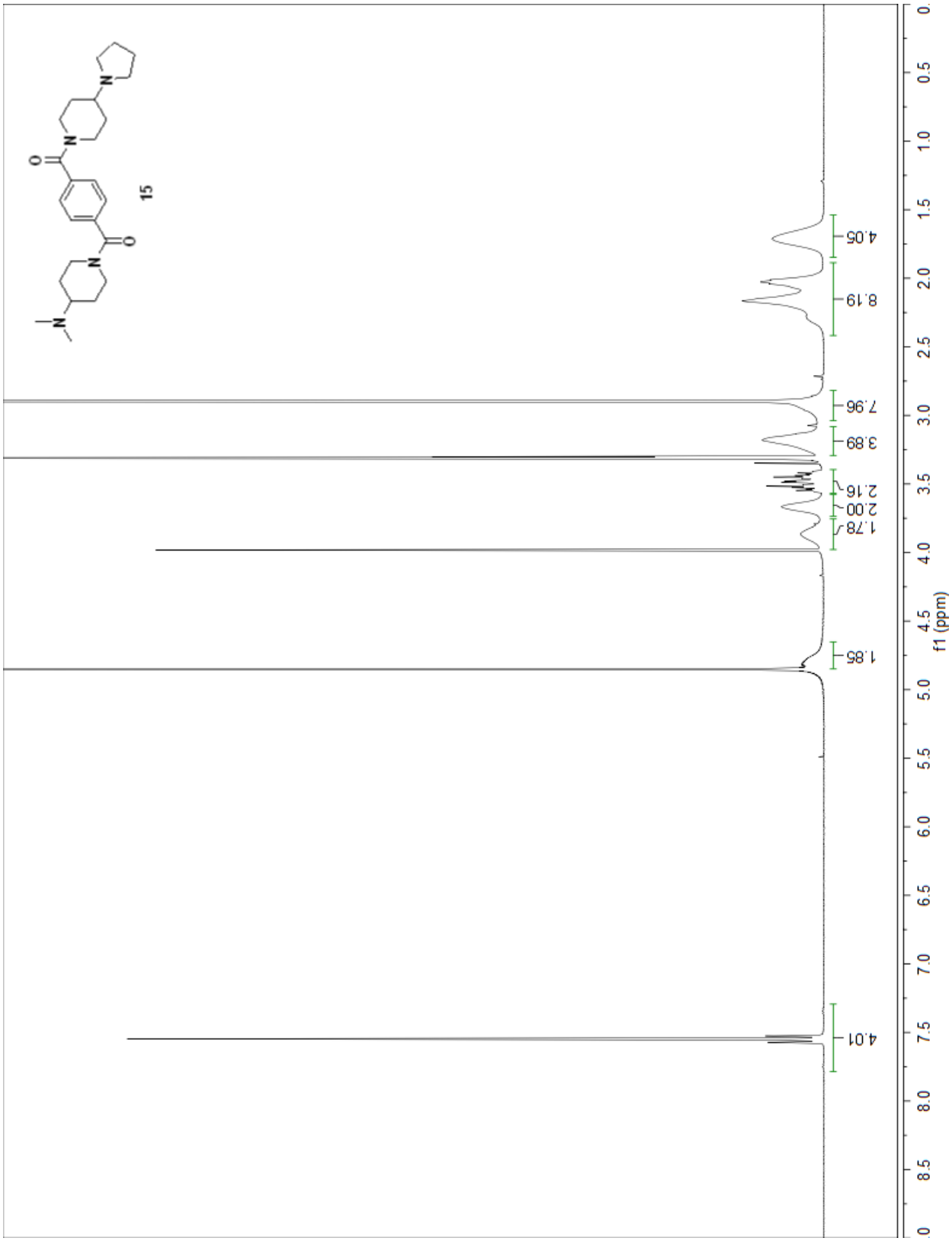
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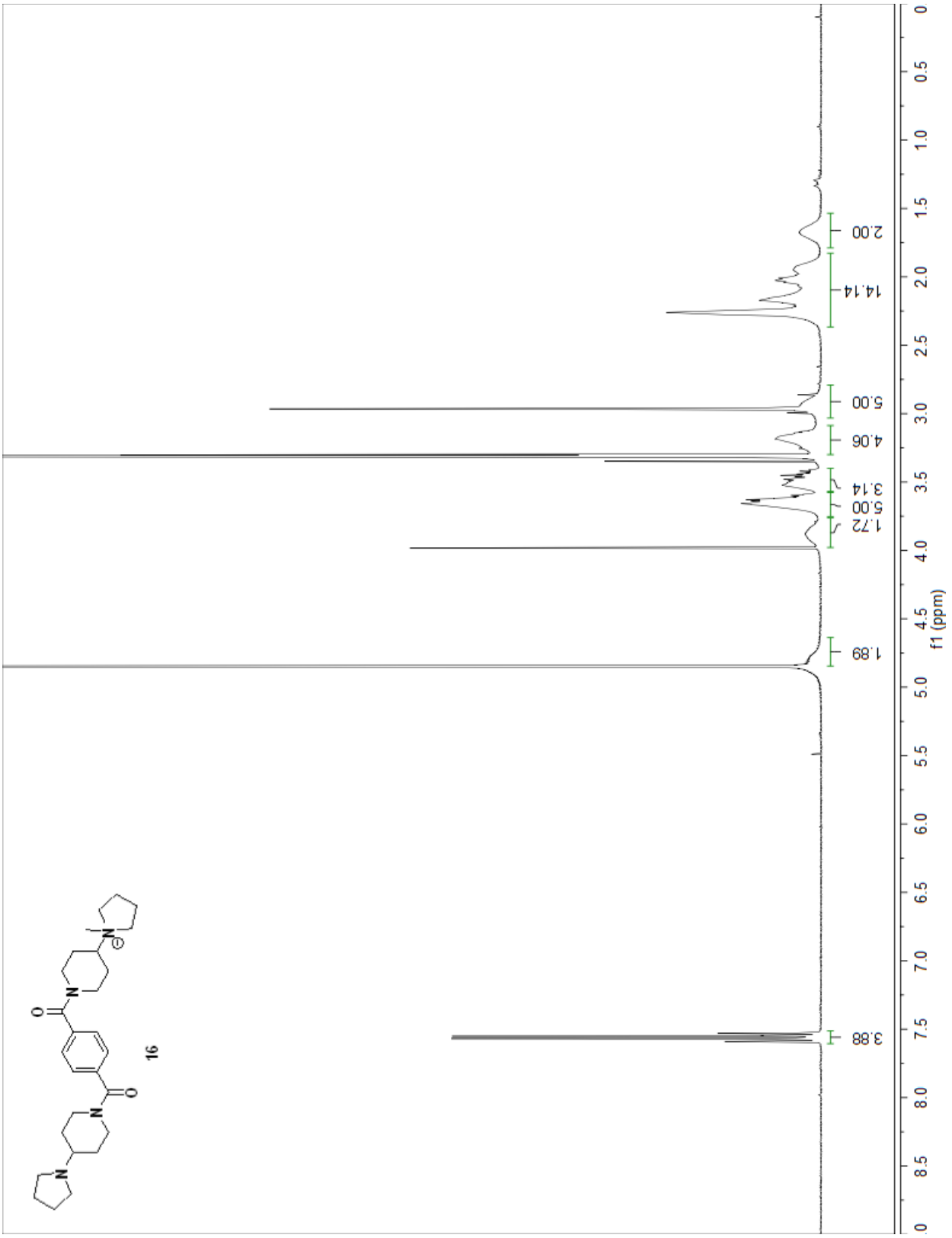
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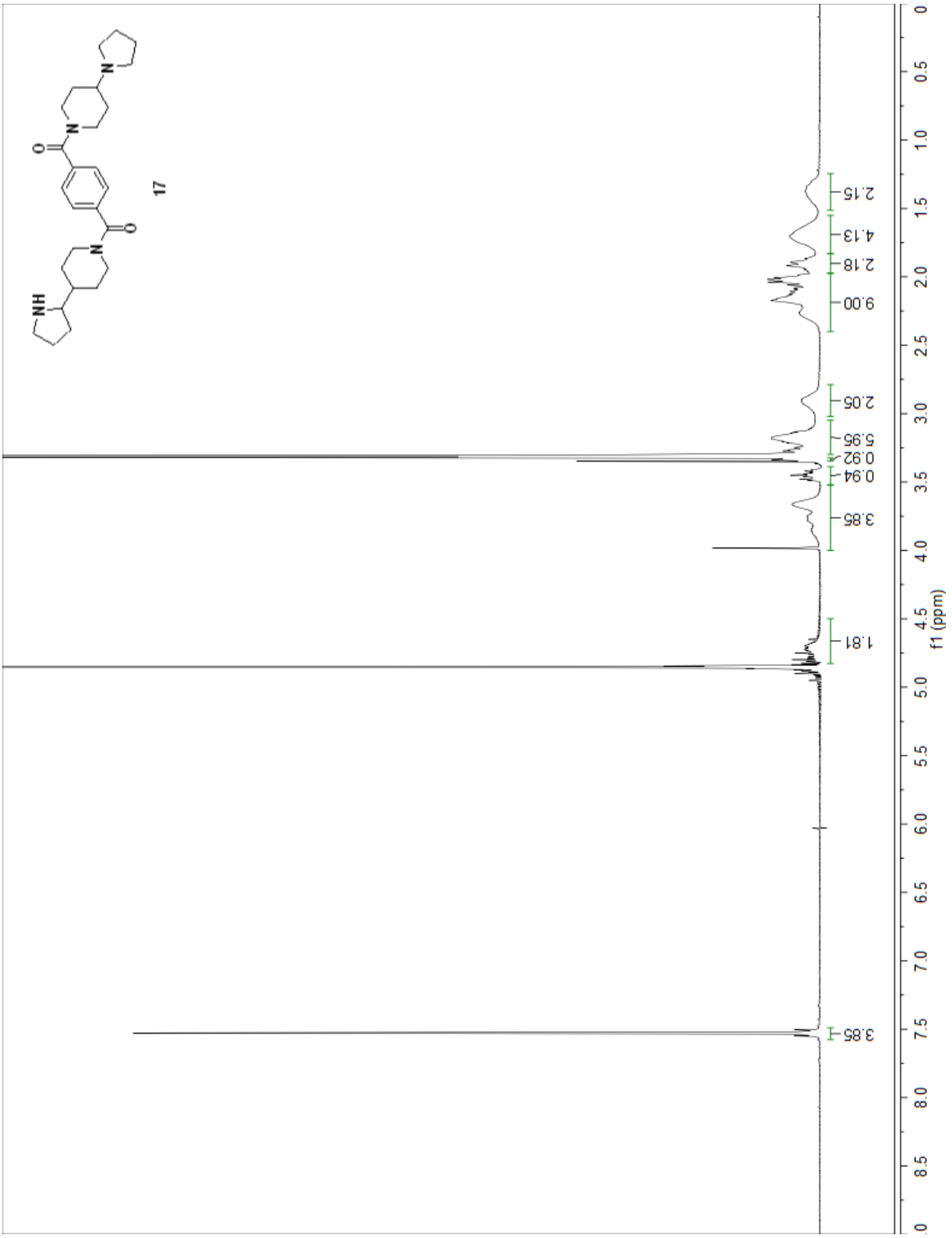
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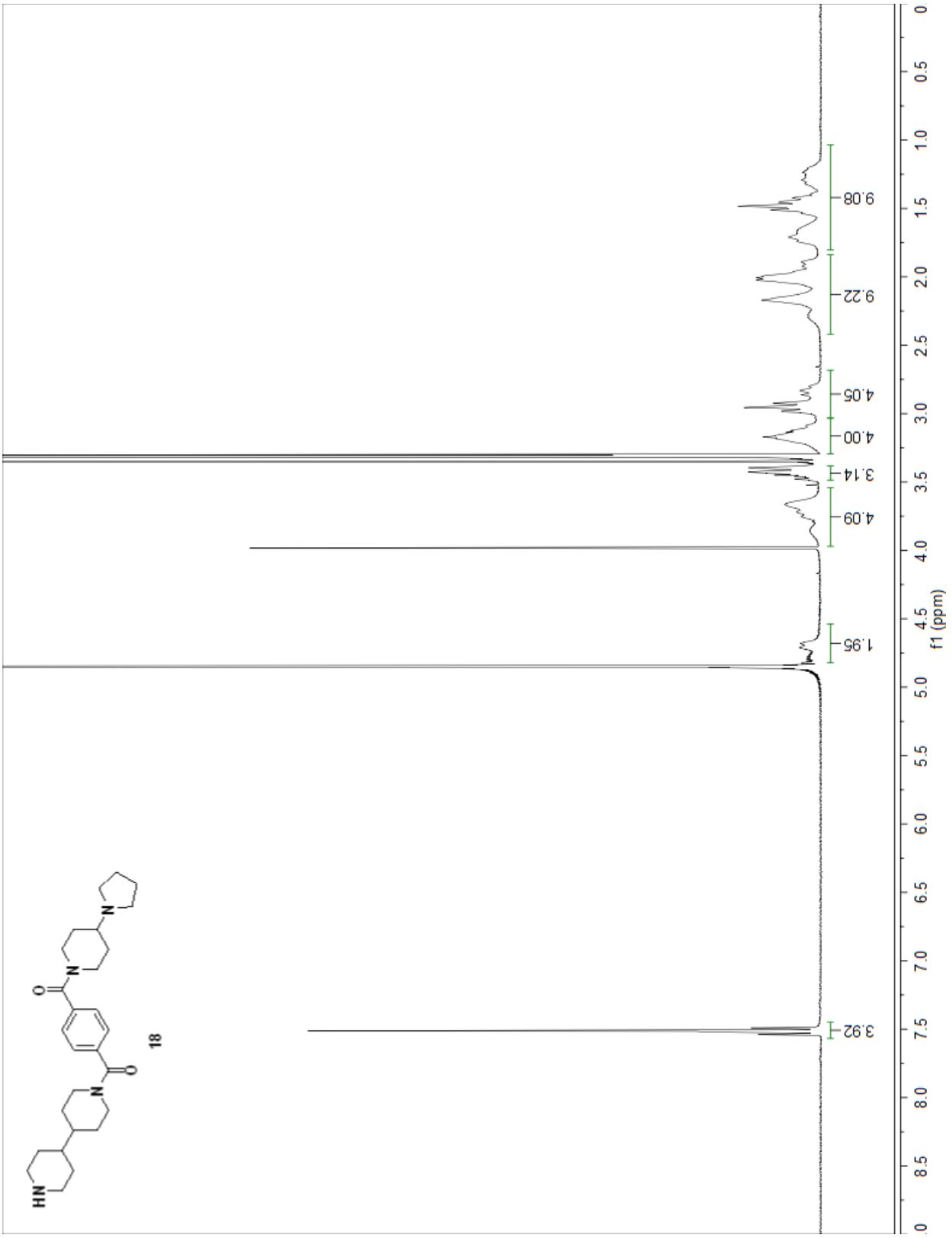
f1 (ppm)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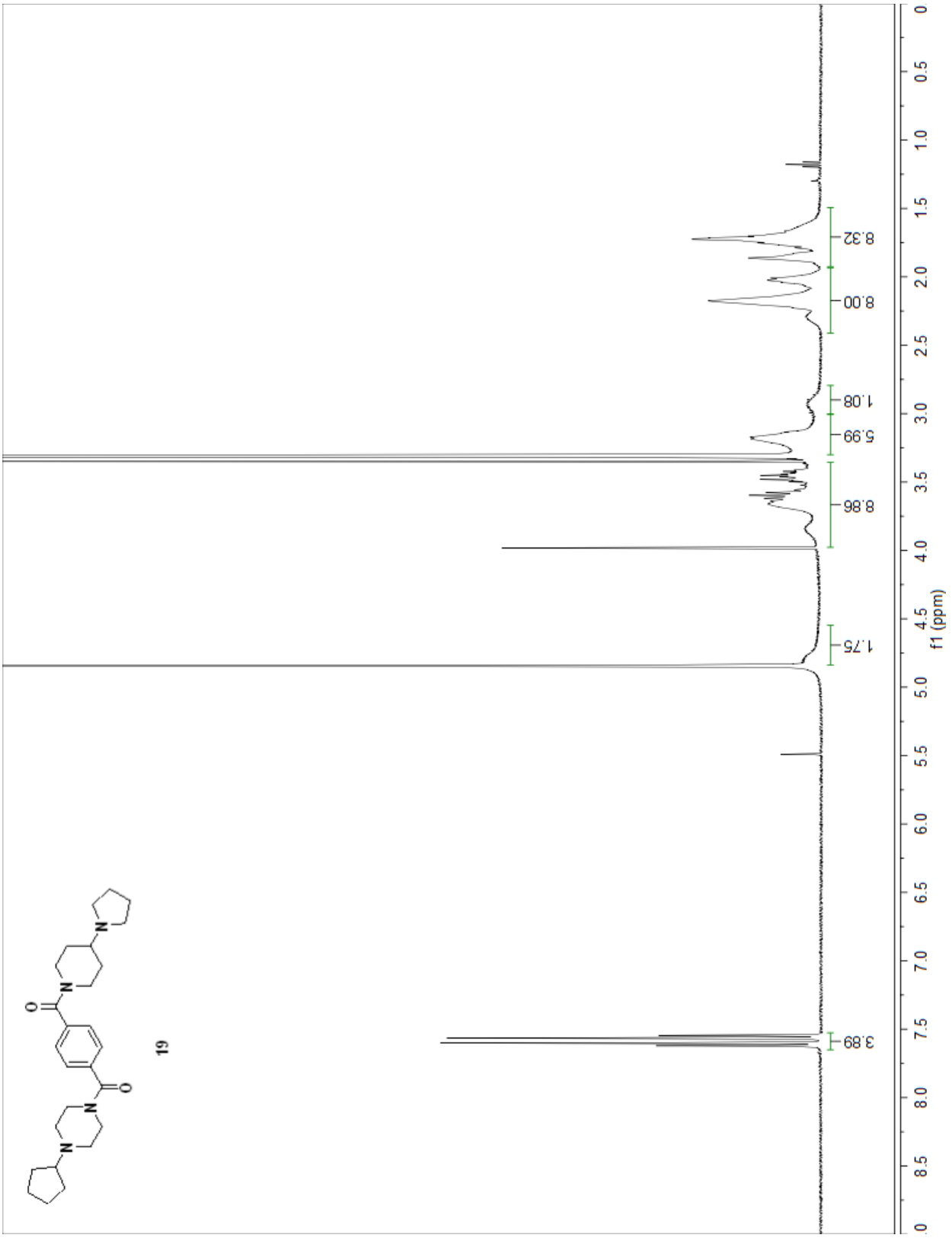
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8.32
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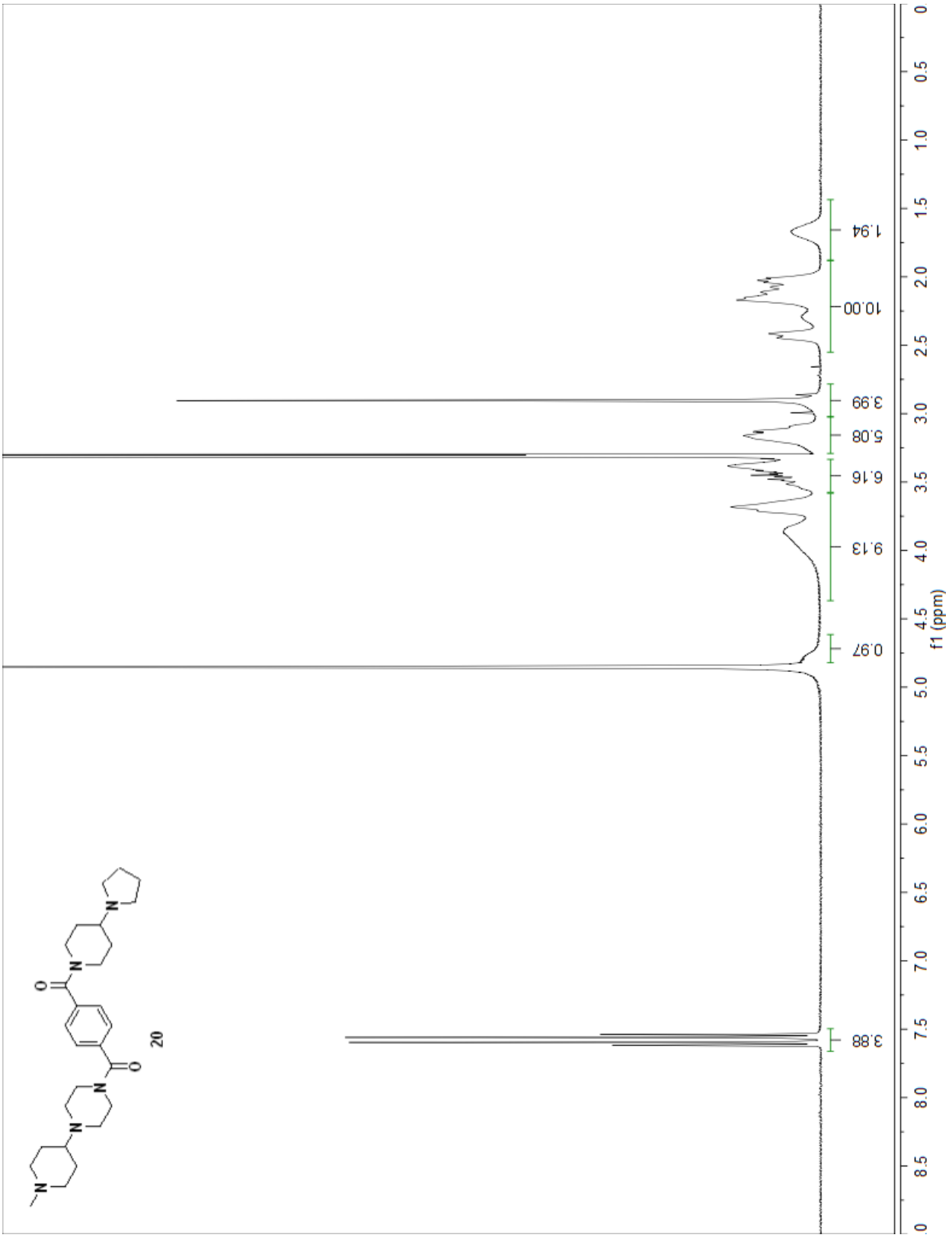
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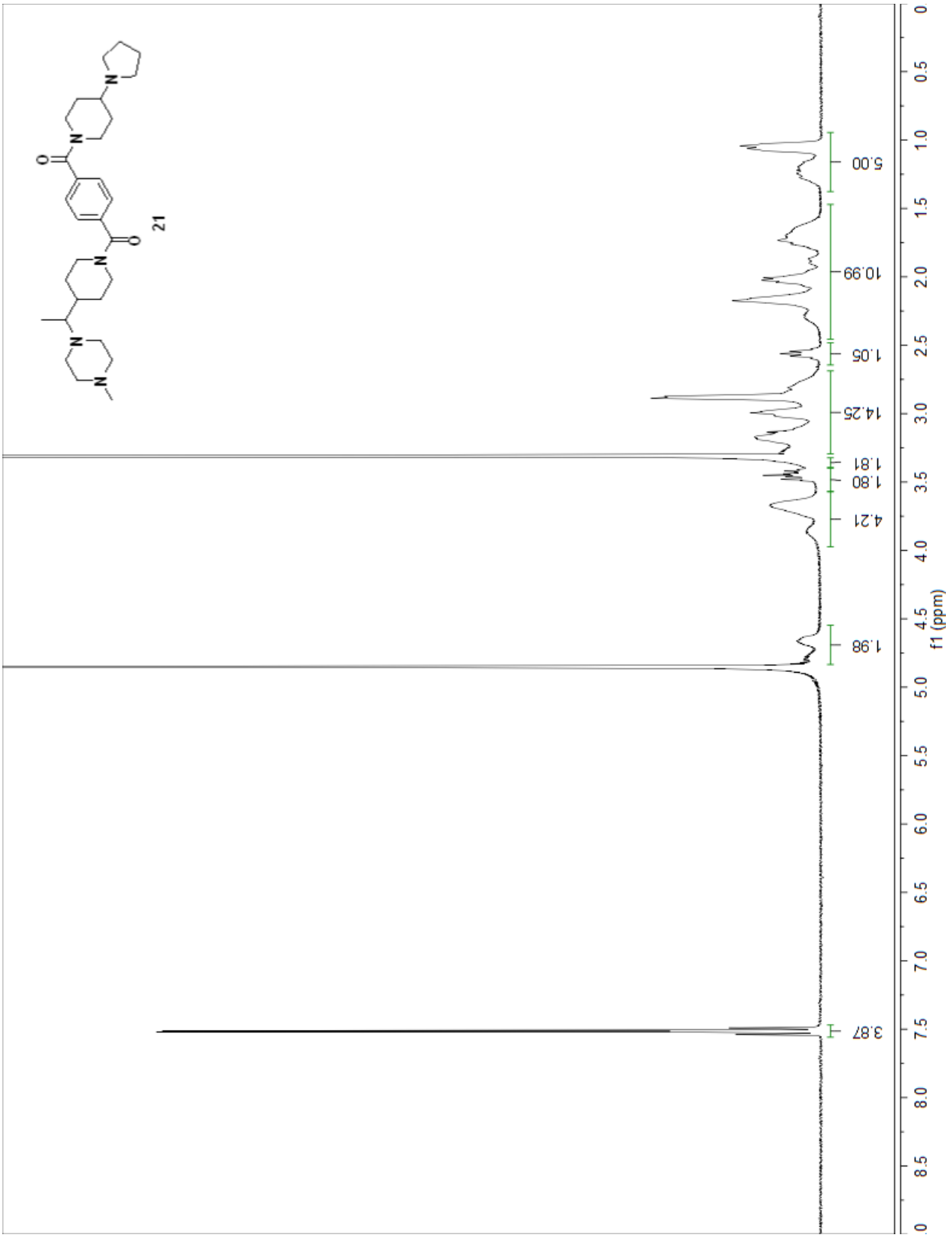
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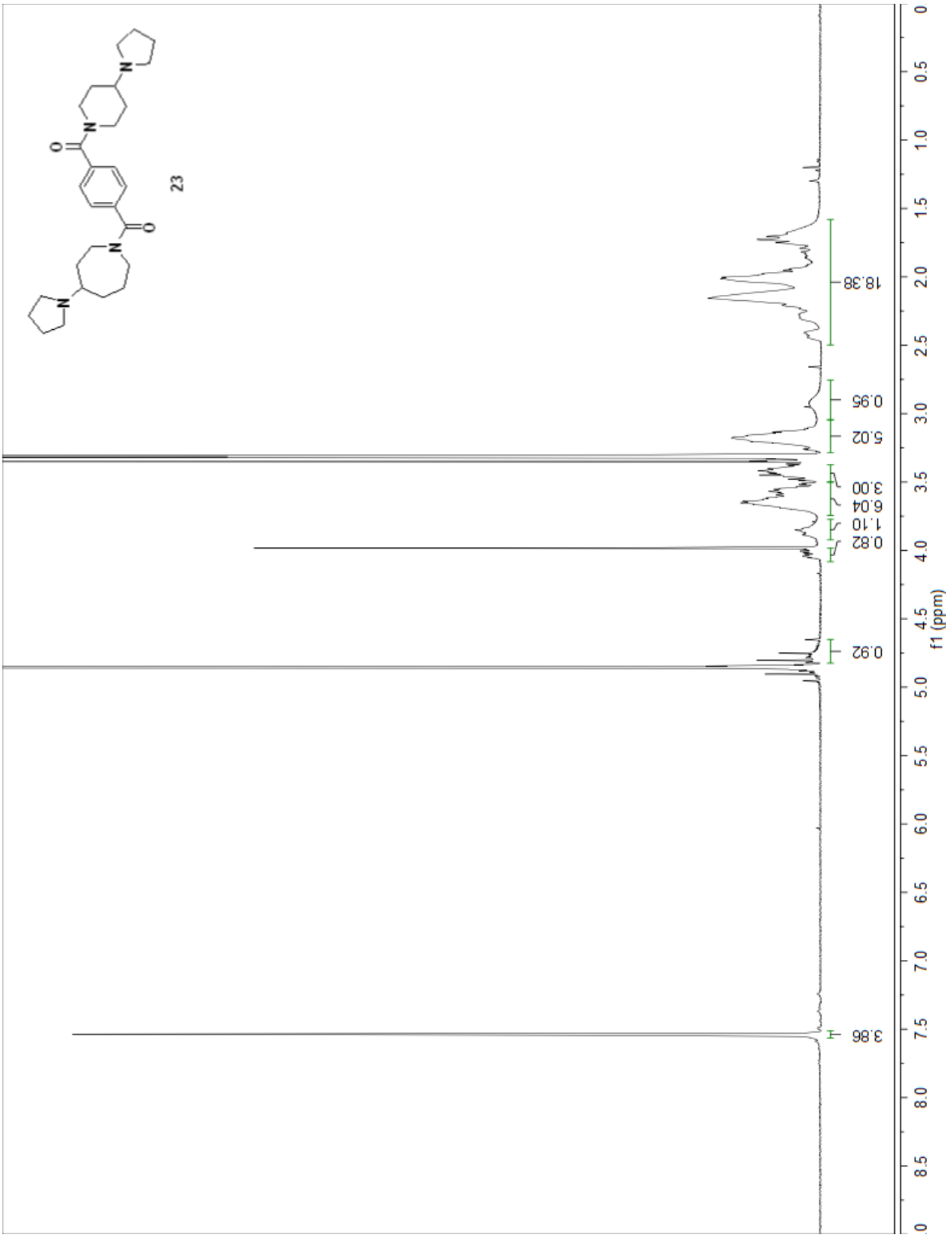
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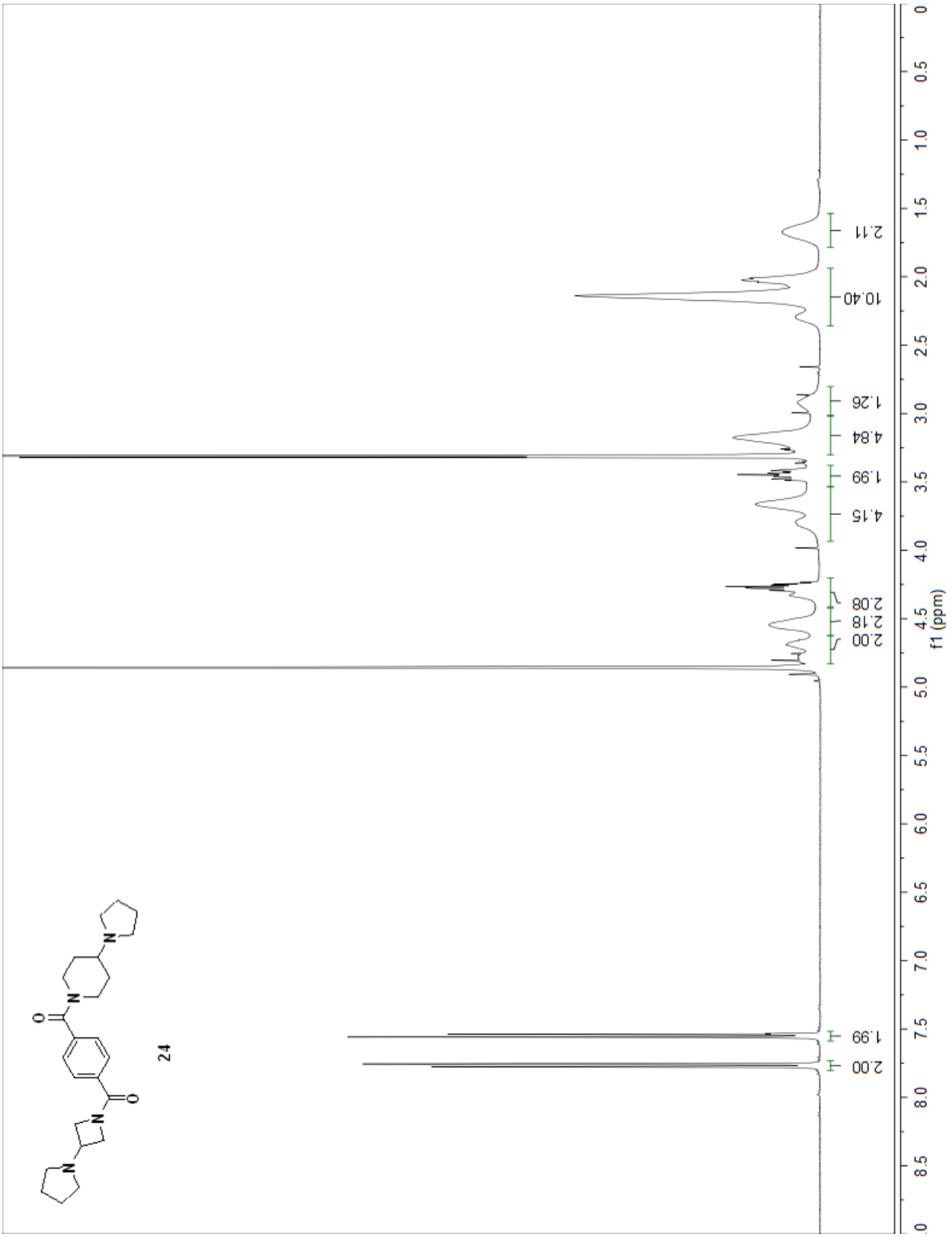
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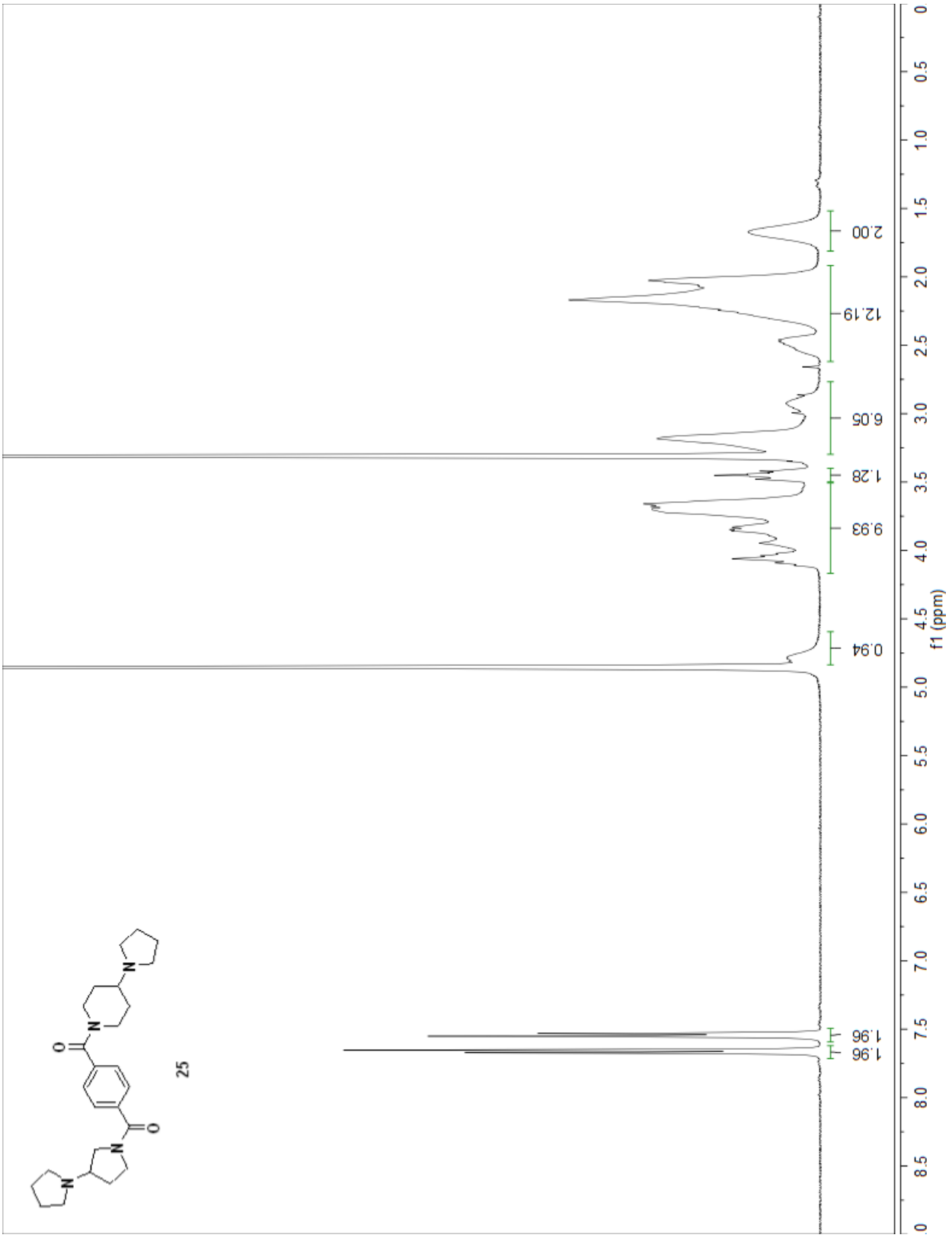
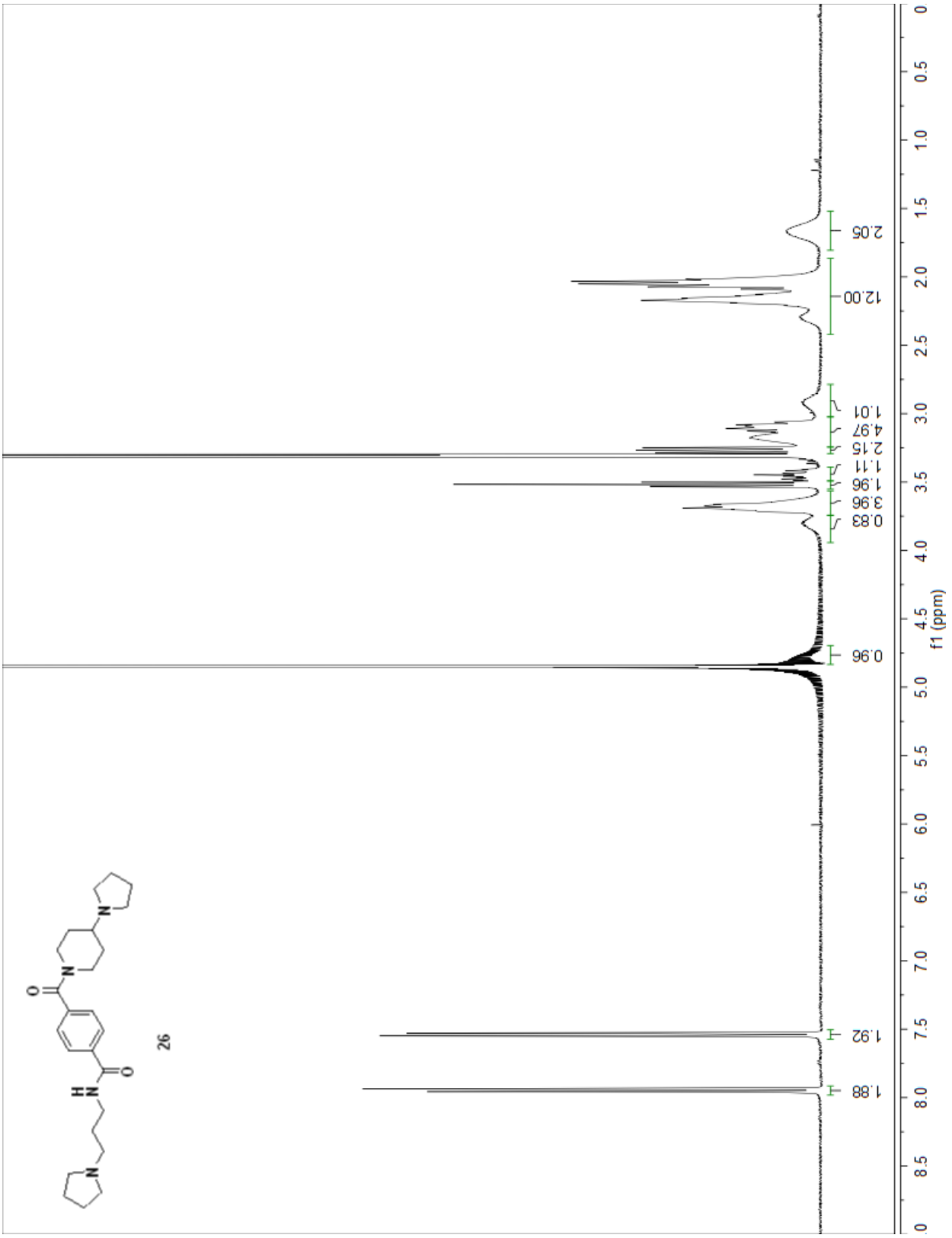
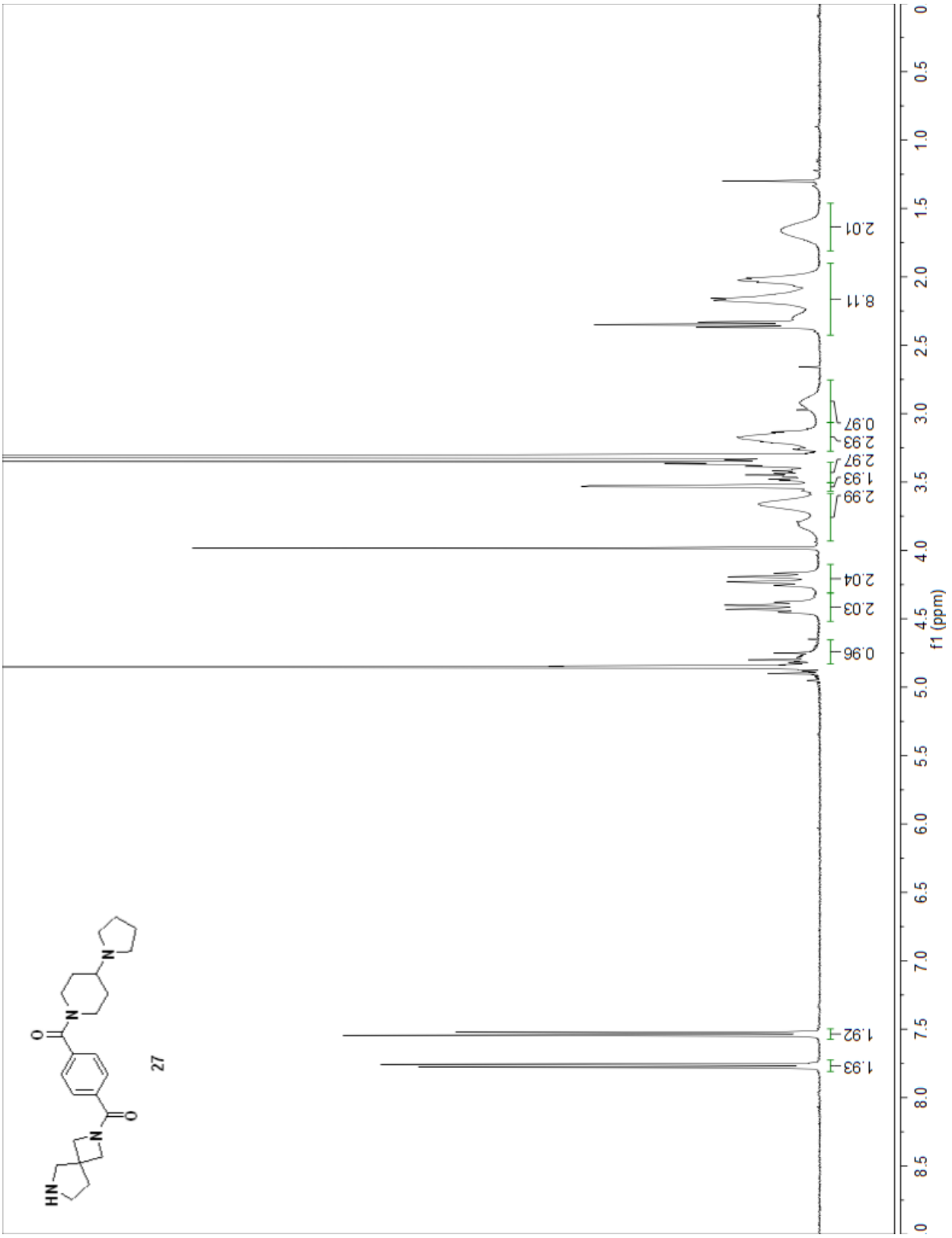
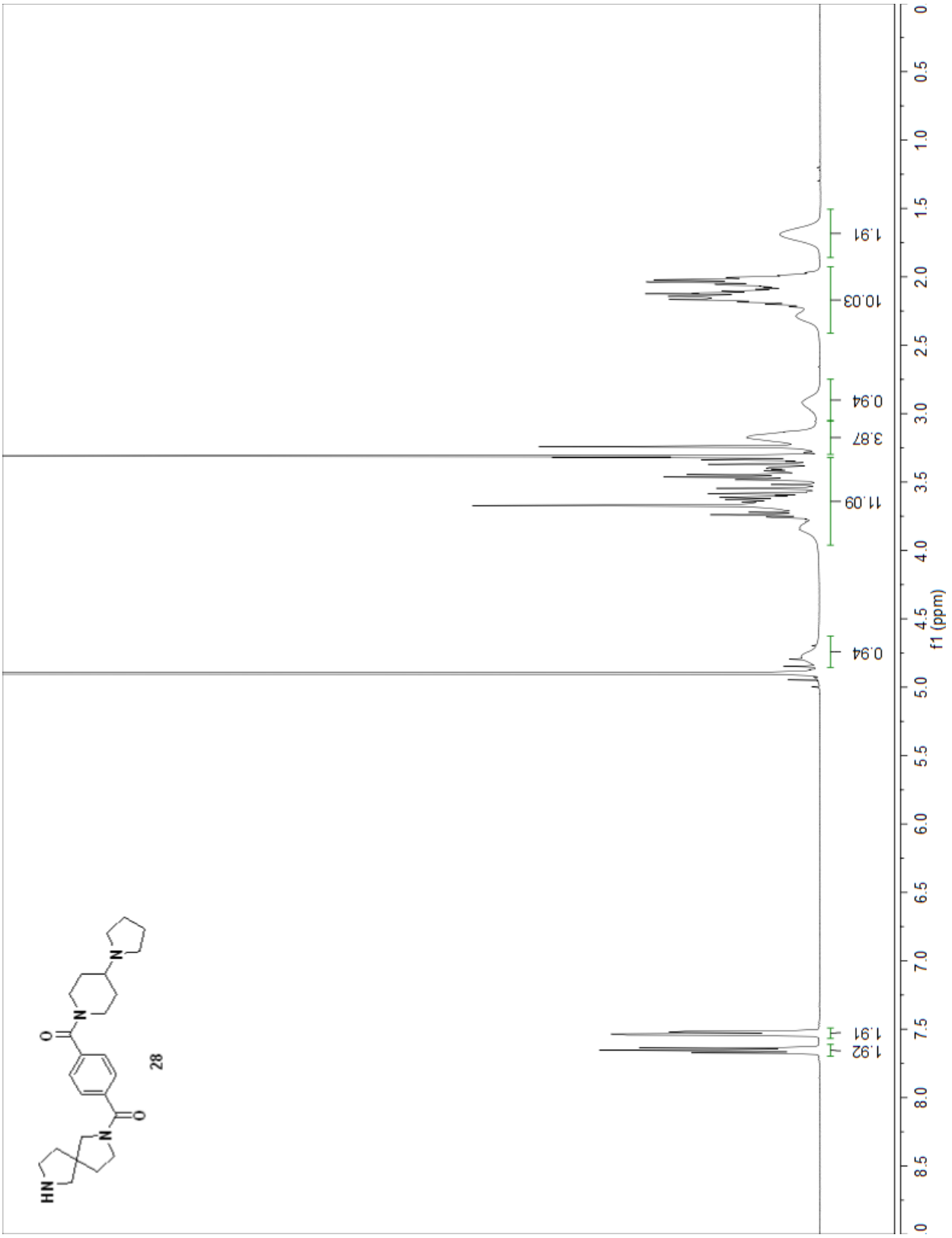
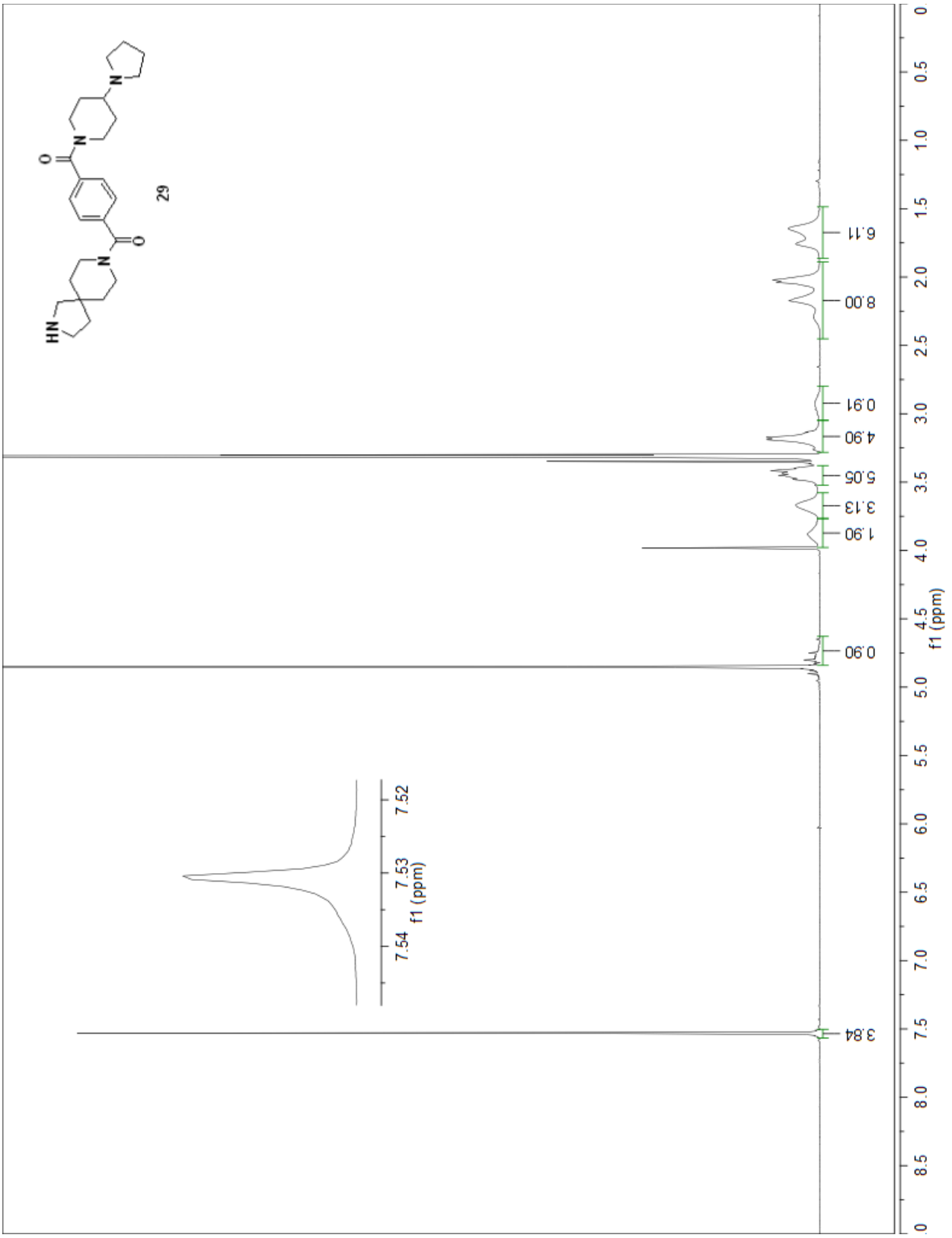
2.00
f1 (ppm)

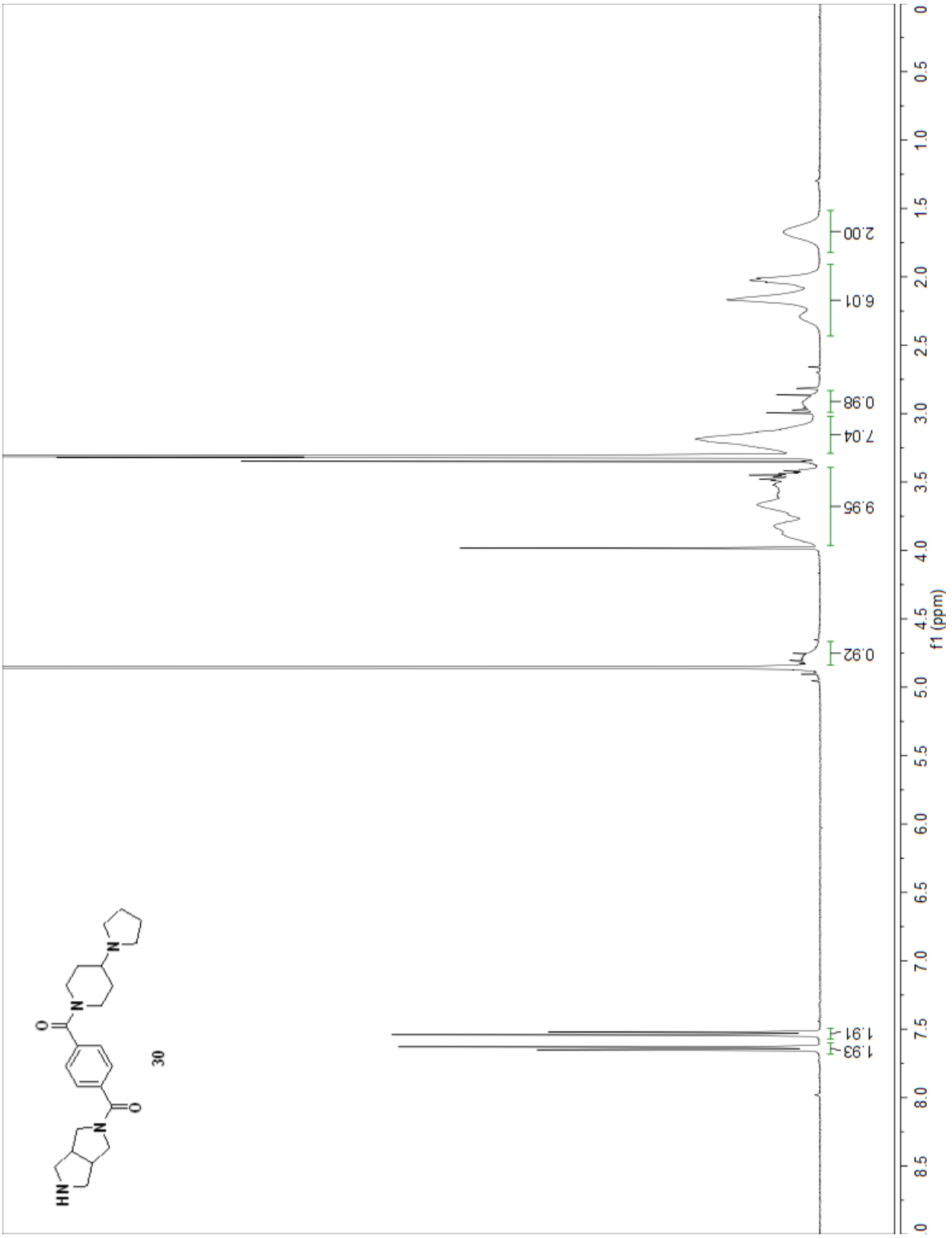

25
1.96
1.96
0.94
9.93
1.28
6.05
12.19
2.00
f1 (ppm)

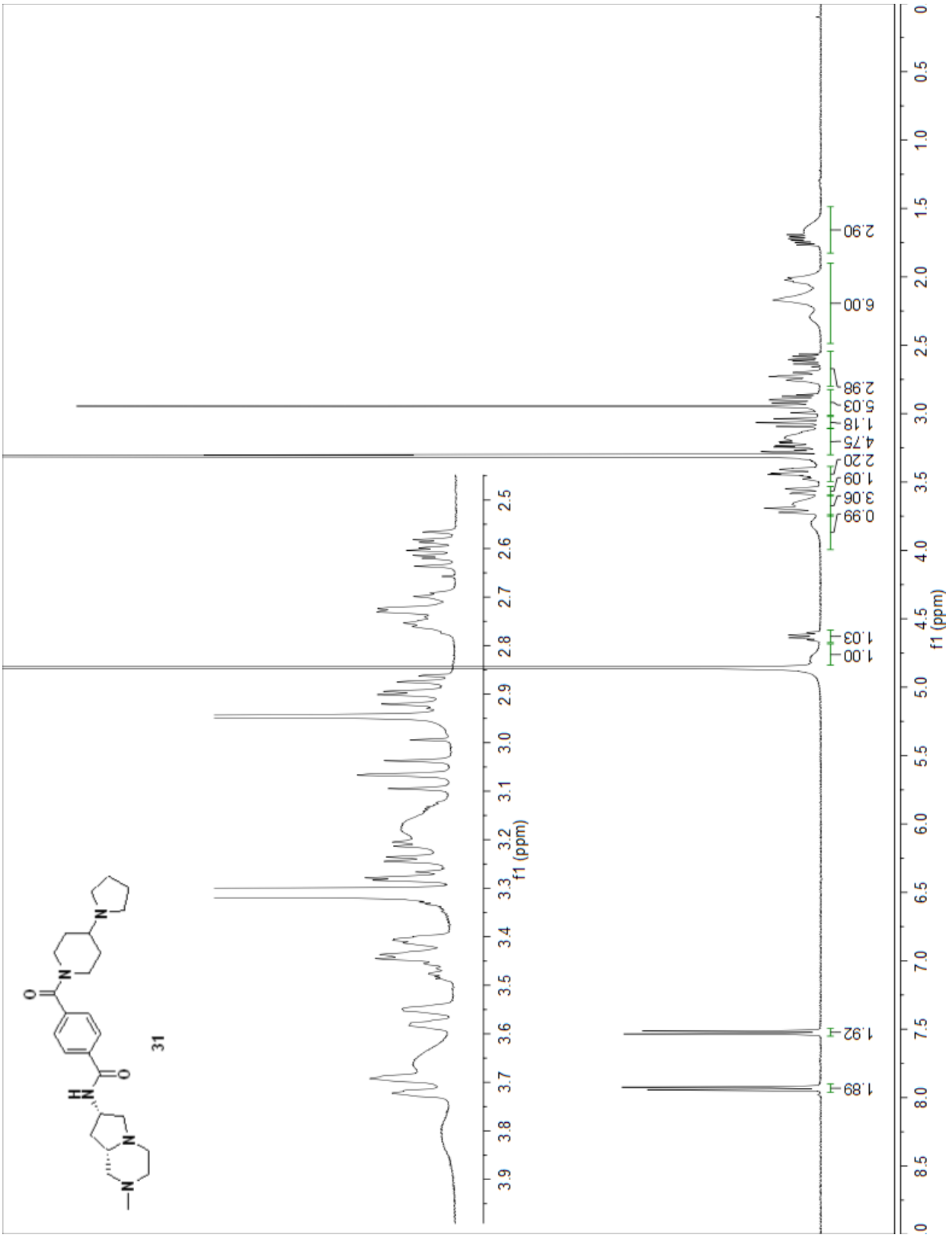

26
1.88
1.92
0.96
0.83
3.96
1.96
1.11
2.15
4.97
1.01
12.00
2.05
f1 (ppm)


27
1.93
1.92
0.96
2.03
2.04
2.99
1.93
2.97
2.93
0.97
8.11
2.01
f1 (ppm)

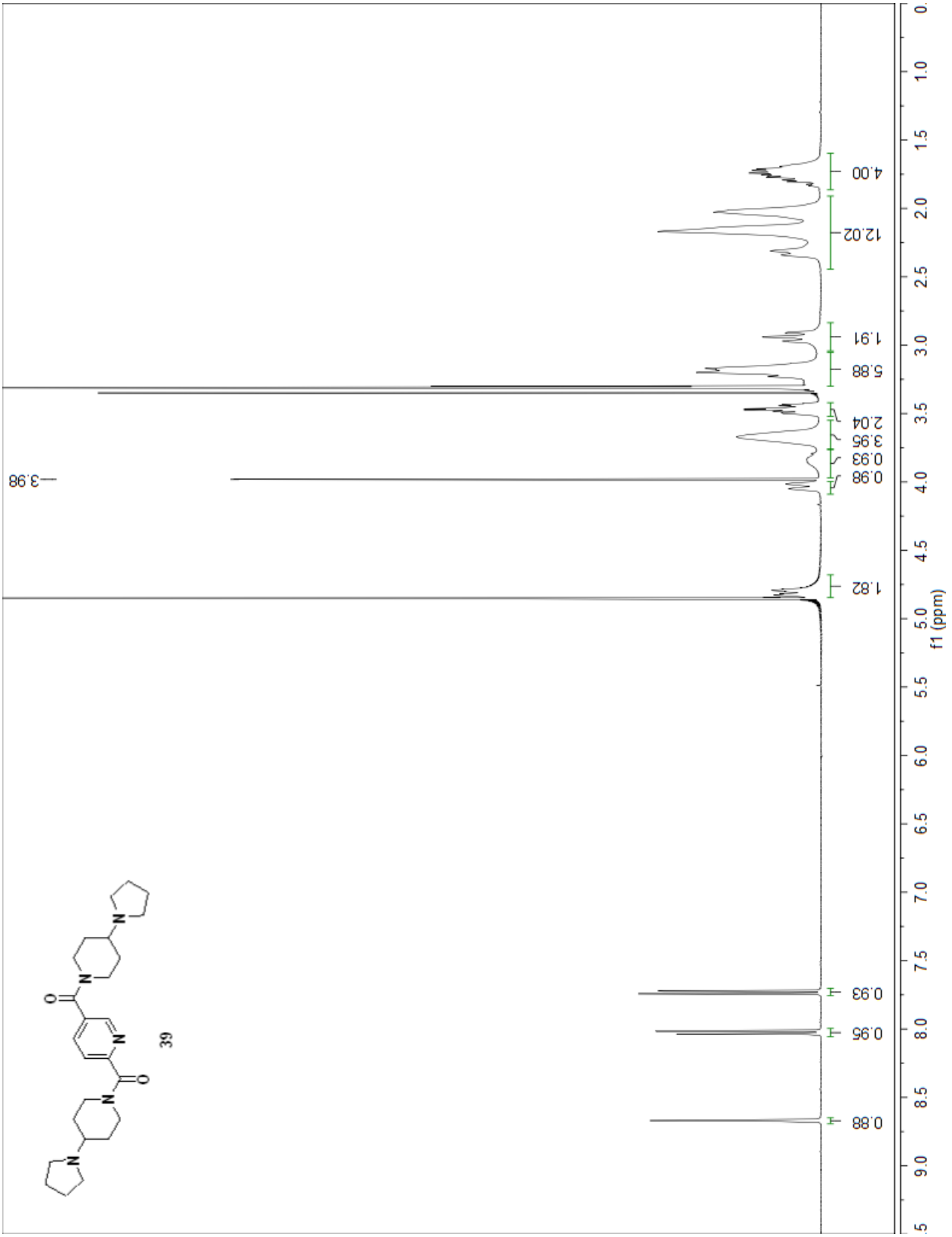

28
1.92
1.91
0.94
11.09
3.87
0.94
10.03
1.91
f1 (ppm)

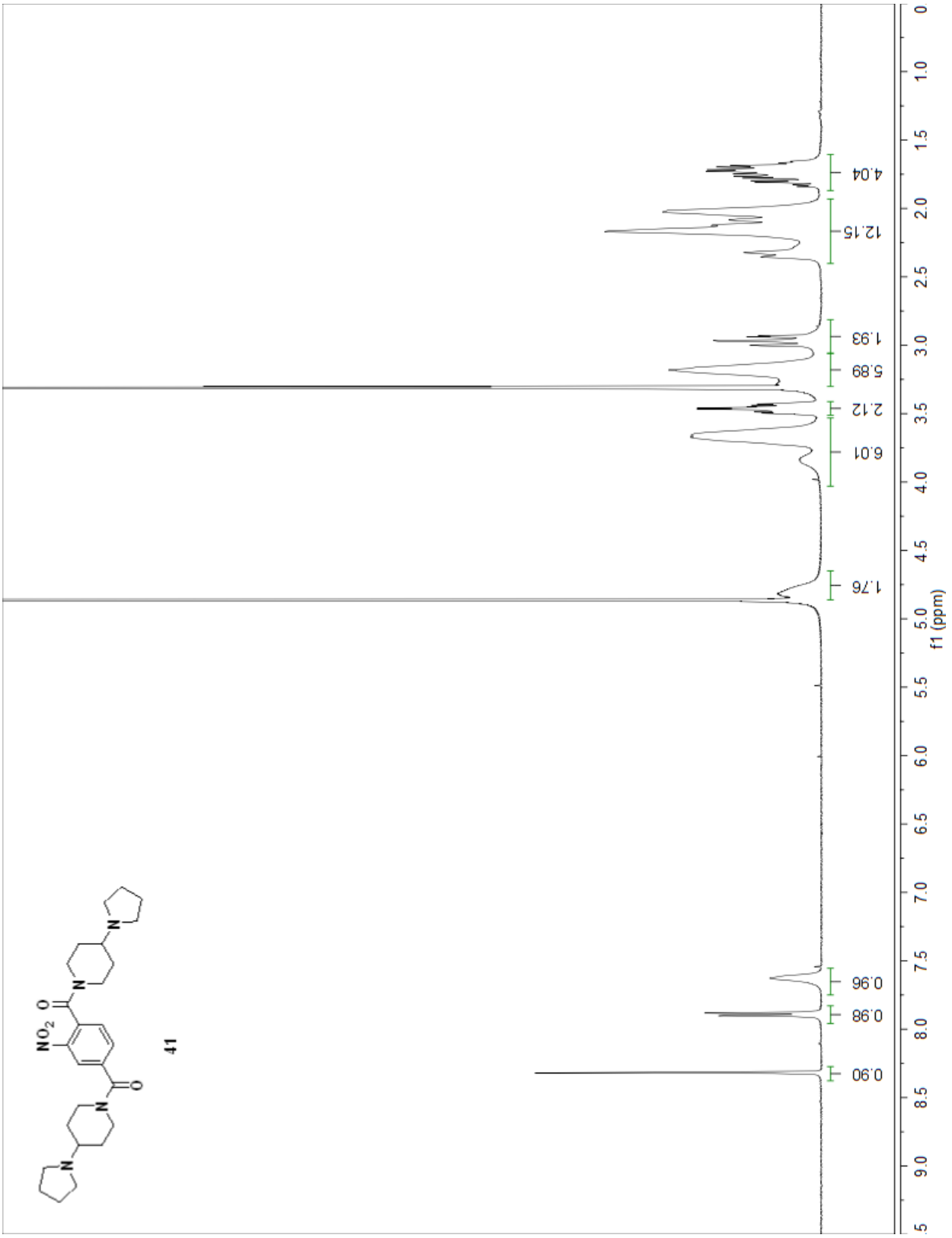

29
HN
O
N
3.84
0.90
1.90
3.13
5.05
4.90
0.91
8.00
6.11
7.54
7.53
7.52
f1 (ppm)
f1 (ppm)

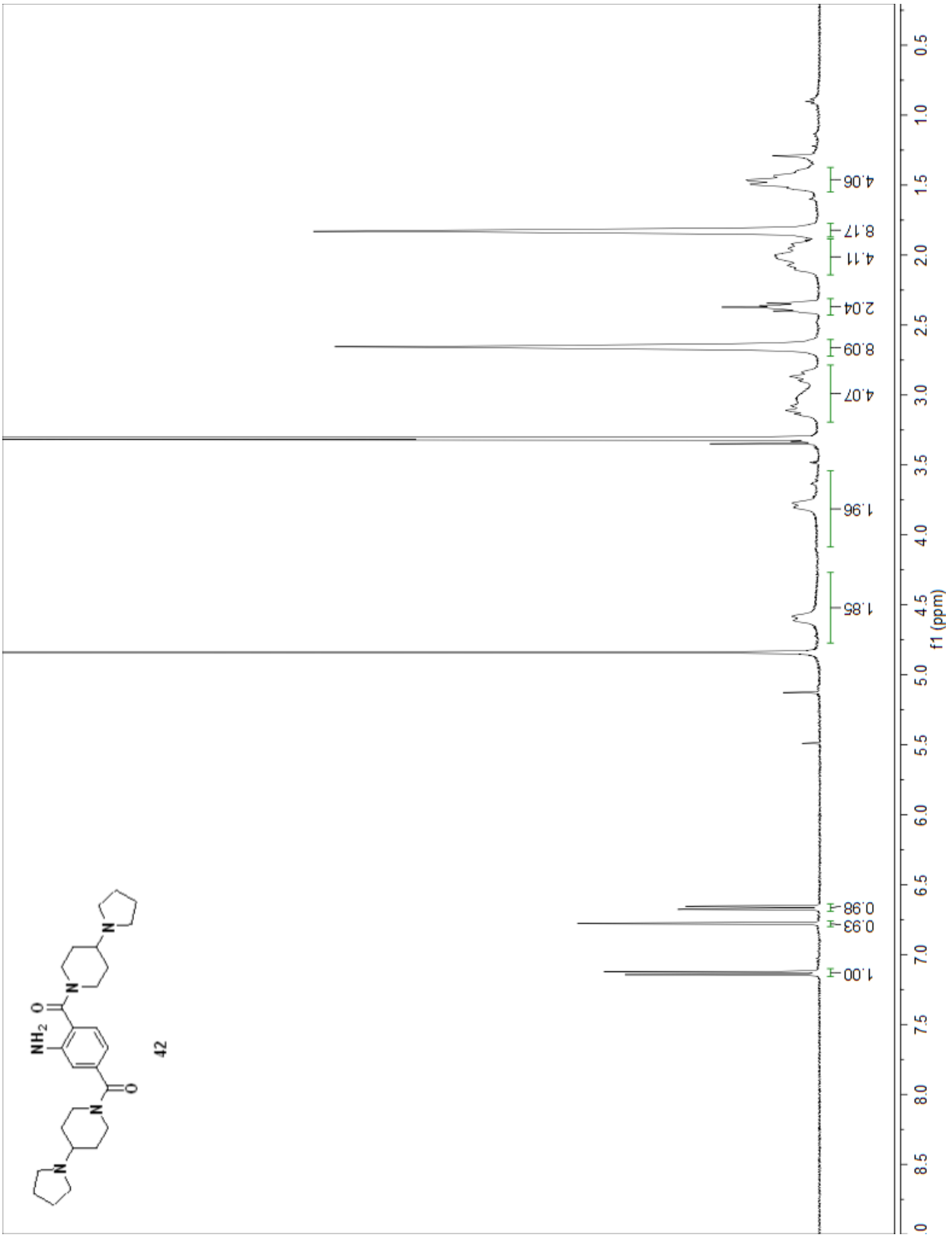

30
1.93
1.91
0.92
9.95
7.04
0.98
6.01
2.00
f1 (ppm)

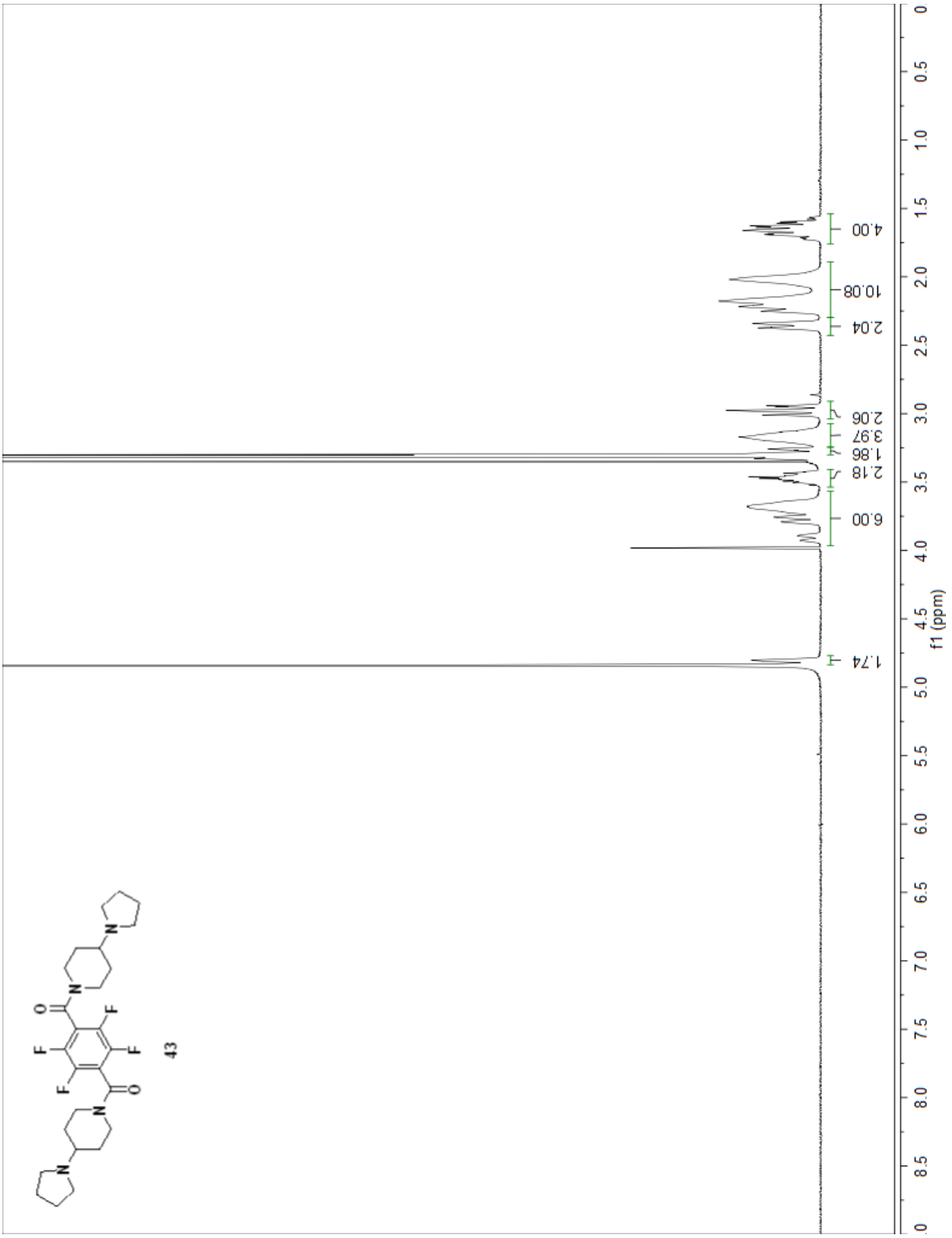

31
f1 (ppm)
f1 (ppm)
1.92
1.89
1.00
1.03
0.99
3.06
1.09
2.20
4.75
1.18
5.03
2.98
6.00
2.90

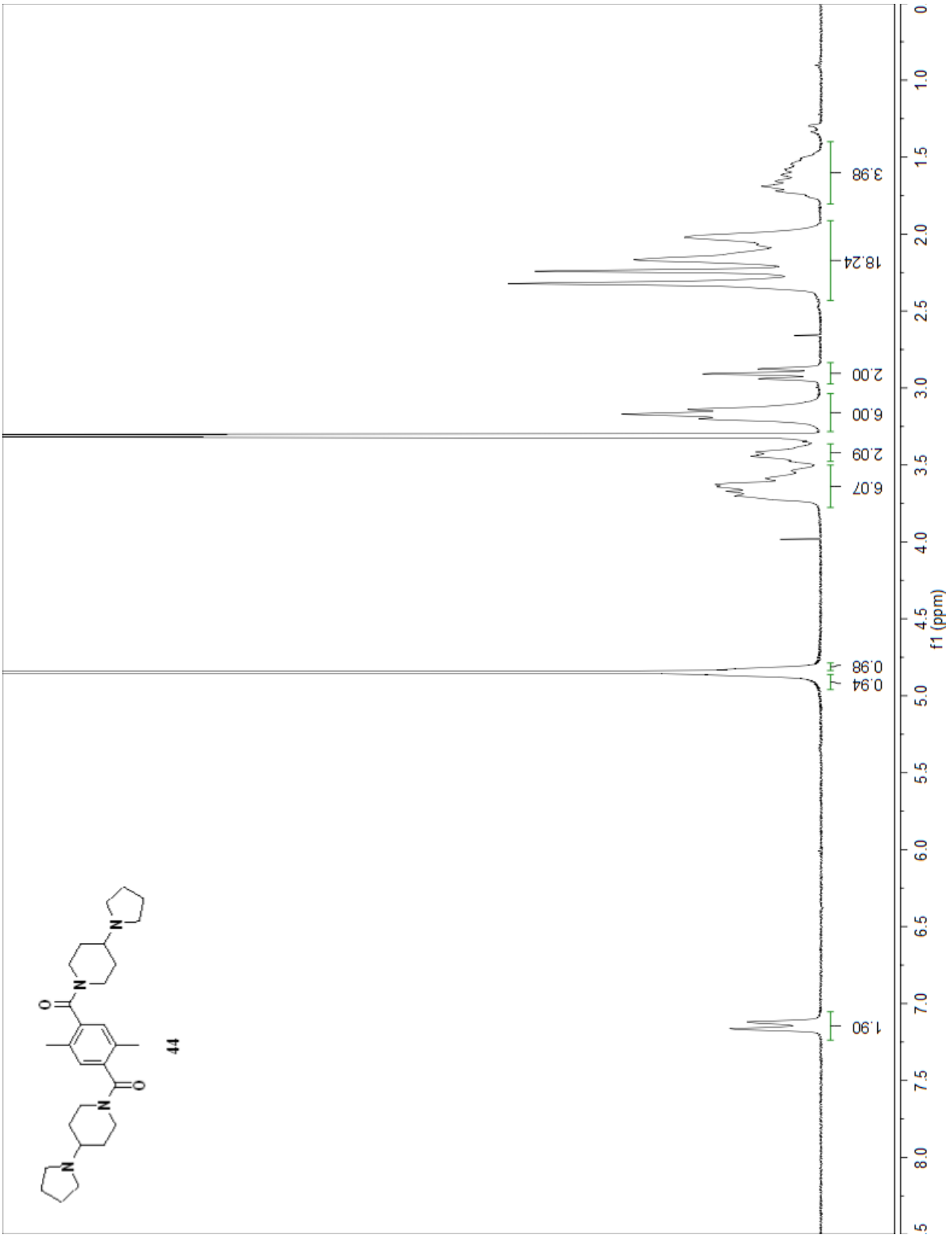
Supplementary Figure 8. ^{1}**H NMR spectra of compounds 39, 41 - 48 (Table 5).**

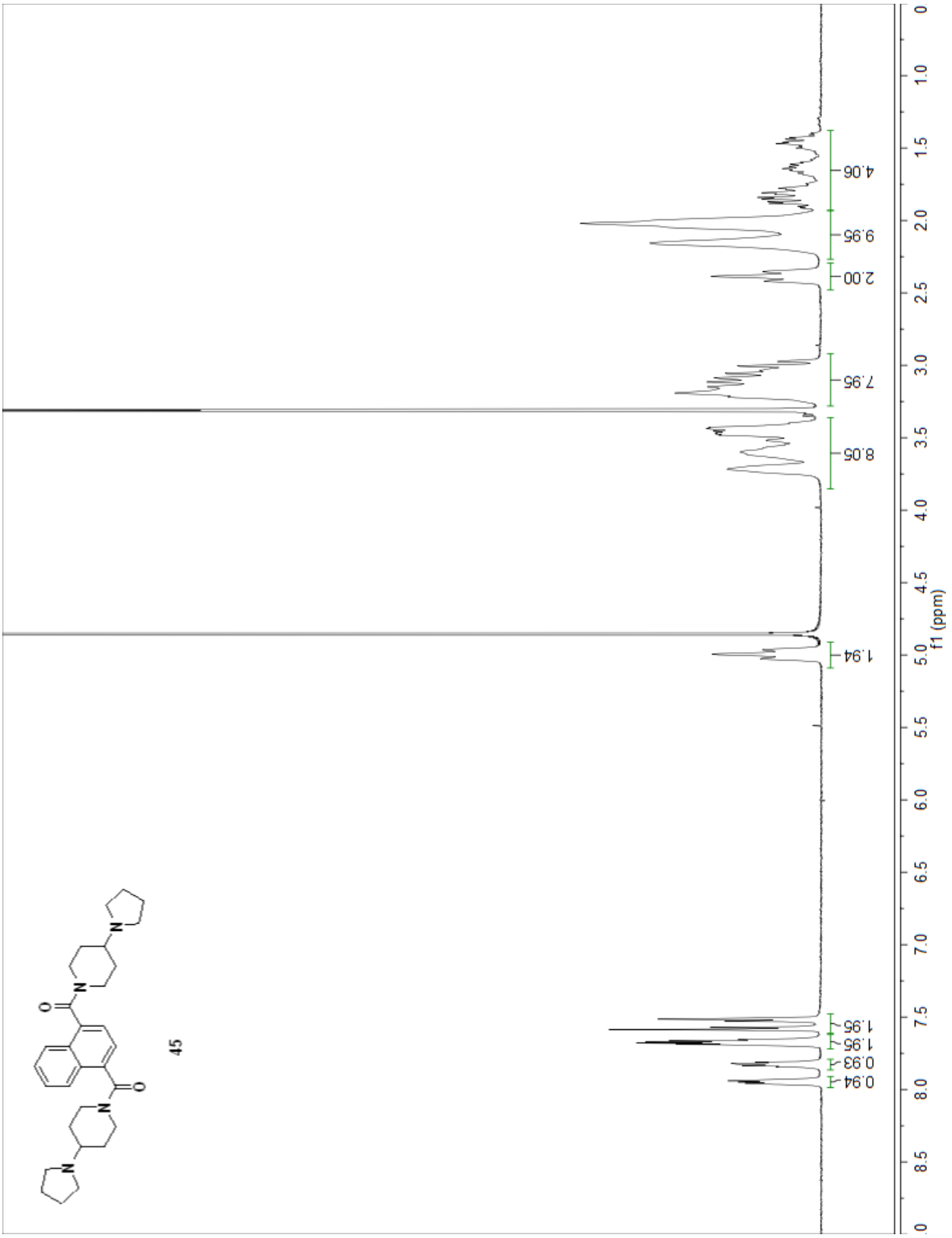


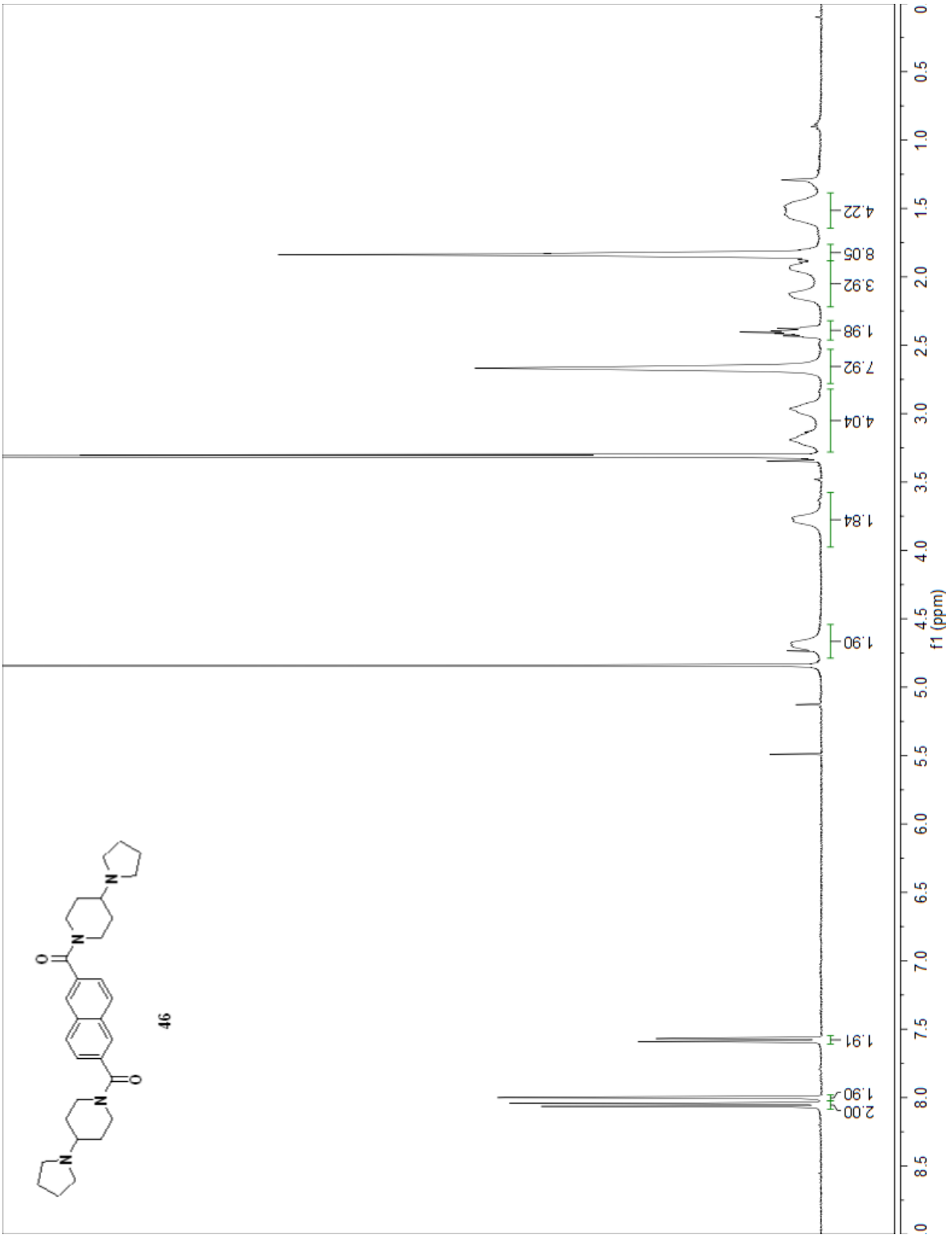

41
0.90
0.98
0.96
1.76
6.01
2.12
5.89
1.93
12.15
4.04
f1 (ppm)

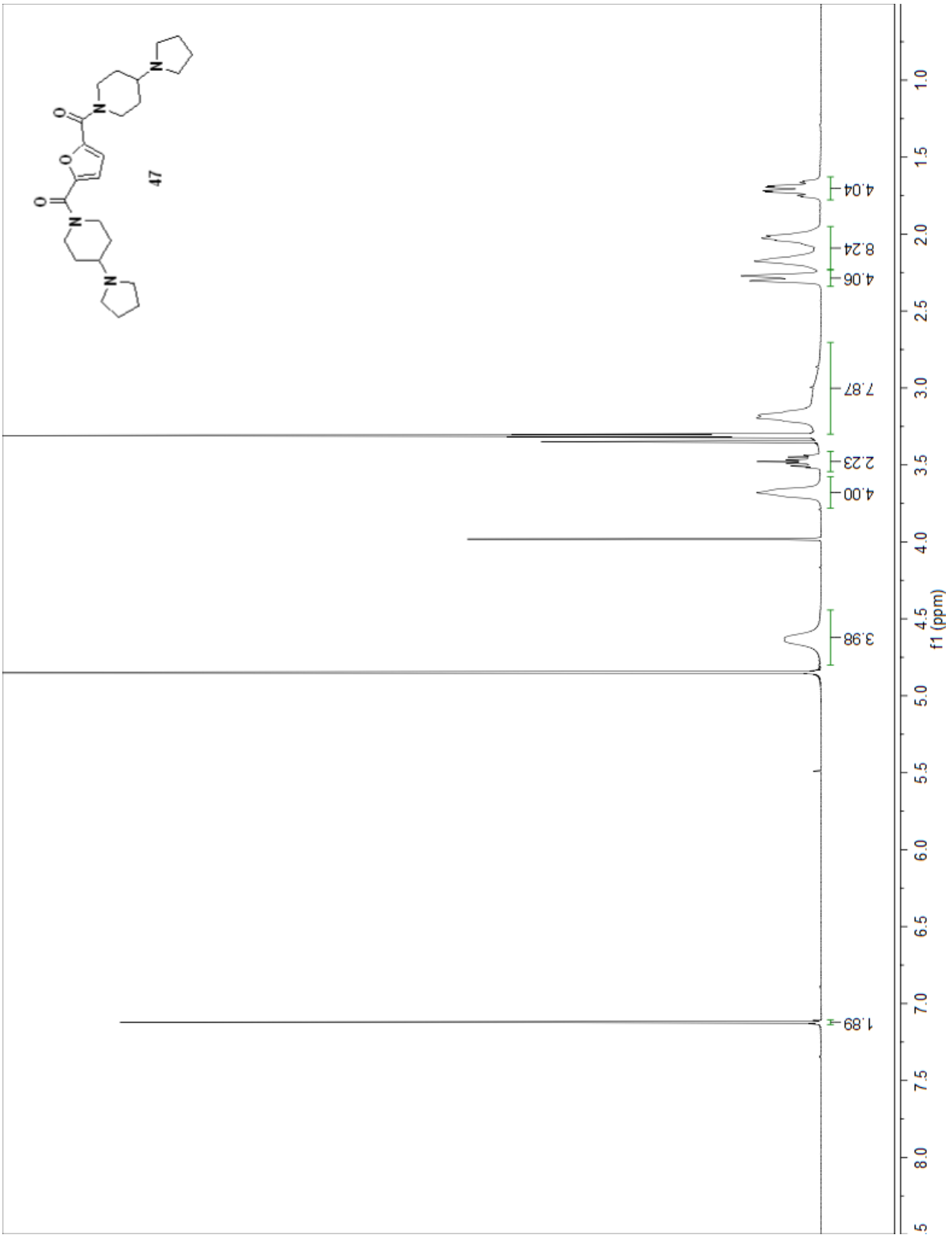

42
1.00
0.93
0.98
1.85
1.96
4.07
8.09
2.04
4.11
8.17
4.06
f1 (ppm)

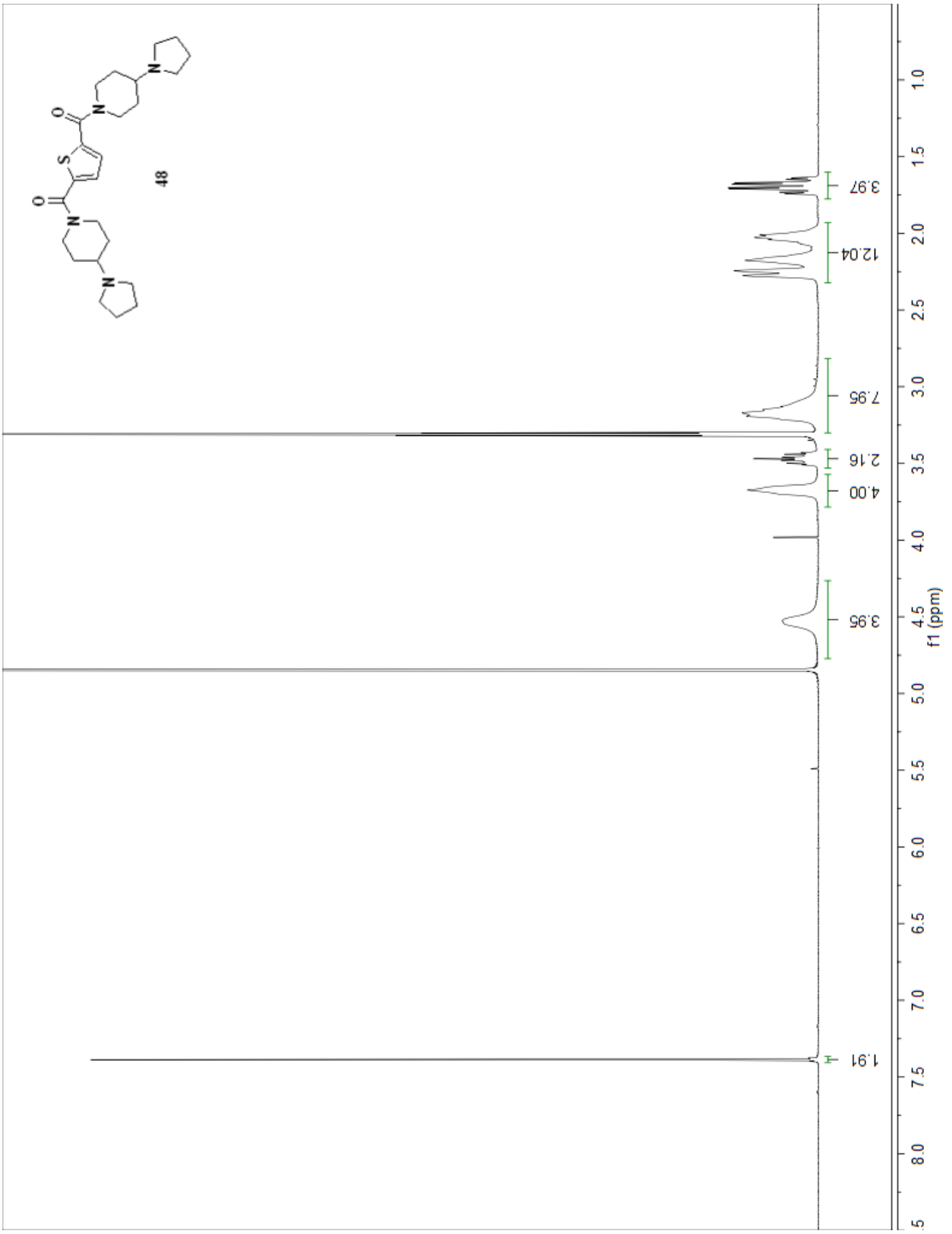

43
1.74
6.00
2.18
1.86
3.97
2.06
2.04
10.08
4.00
f1 (ppm)


44
1.90
0.94
0.98
6.07
2.09
6.00
2.00
18.24
3.98
f1 (ppm)


45
0.94
0.93
1.95
1.95
1.94
8.05
7.95
2.00
9.95
4.06
f1 (ppm)


46
1.91
2.00
1.90
1.90
1.84
4.04
7.92
1.98
3.92
8.05
4.22
f1 (ppm)


47
4.04
8.24
4.06
7.87
2.23
4.00
3.98
1.89
f1 (ppm)


48
1.91
3.95
4.00
2.16
7.95
12.04
3.97
f1 (ppm)

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