Total Synthesis and Biological Evaluation of Phidianidines A and B Uncovers Unique Pharmacological Profiles at CNS Targets

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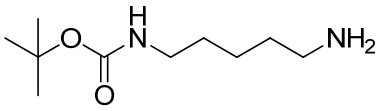
General Experimental

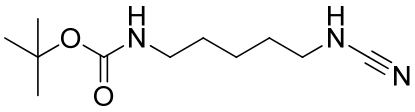
All reagents were purchased from Sigma-Aldrich Corp., Matrix Scientific, Fisher Scientific, and Ark Pharm Inc., and were used without purification. Analytical thin-layer chromatography (TLC) was performed on 250 µm silica gel plates from Sorbent Technologies. Visualization was accomplished via UV light, and/or the use of ninhydrin, I_2, 2,4-dinitrophenylhydrazine, and potassium permanganate solutions followed by application of heat. Chromatography was performed using Silica Gel 60 (230-400 mesh) from Sorbent Technologies or Silica RediSep Rf flash columns on a CombiFlash Rf automated flash chromatography system. All ^{1}H and ^{13}C NMR spectra were recorded on Bruker AV-400 (400 MHz), DRX-500 (500 MHz) and AV-600 (600 MHz) instruments. Chemical shifts are reported in ppm relative to residual solvent peaks as an internal standard set to δ 7.26 and δ 77.16 ($CDCl_3$), δ 3.31 and δ 49.00 (CD_3OD), and δ 2.50 and δ 39.52 (d_6-DMSO). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, p = pentet br = broad, dd=doublet of doublets, dq=doublet of quartets, td = triplet of doublets, pd = pentet of doublets, m = multiplet), coupling constant (Hz), integration. Low resolution mass spectra (LCMS) were obtained on an Agilent 1200 LCMS with electrospray ionization. High resolution mass spectra (HRMS) were recorded on a Waters Qtof-API-US plus Acquity system with ES as the ion source. Analytical high pressure liquid chromatography (HPLC) was performed on an Agilent 1200 analytical LCMS with UV detection at 214 nm and 254 nm along with ELSD detection.

Experimental Procedures



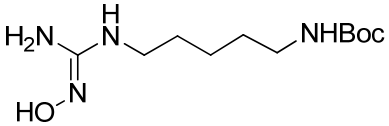
Tert-butyl (5-aminopentyl)carbamate (10).

Di-tert-butyl dicarbonate (2.8 mL, 12.23 mmol) in 50 mL of 9:1 dioxane/water was added to a solution of 1,5-diaminopentane **9** (5 g, 48.93 mmol) in 50 mL of 9:1 dioxane/water over a period of 3.0 hours. The solution was stirred at room temperature overnight and concentrated and the residue was taken up in 50 mL of water. The precipitated N,N'-di-Boc-1,5-diaminopentane was removed by filtration through a fritted glass funnel, and the filtrate was extracted with DCM (50 mLx4). The combined organic extracts were washed by water (20 mLx2), then dried by magnesium sulfate. Removing the solvent to yielding **(10)** 2.14 g (22% by 1,5-diaminopentane, 80% by di-tert-butyl dicarbonate) as a colorless oil that solidified upon drying. ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm)): 4.68 (s, 1H) 3.05 (s, 2H), 2.63 (q, *J* = 7.40 Hz, 2H), 1.38 (m, 15H), 1.28 (m, 2H), 1.13 (s, 1H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)): 155.9, 79.8, 41.9, 40.3, 33.3, 29.8, 28.3, 23.9; HRMS (TOF, ES+) $C_{10}H_{22}N_2O_2$ $[M+H]^+$ m/z 203.1760, measured 203.1758.



Tert-butyl (5-cyanamidopentyl)carbamate (11).

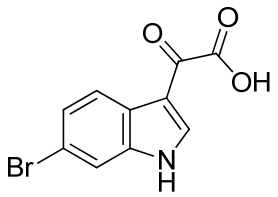
Tert-butyl (5-aminopentyl)carbamate **10** (1.52 g, 7.51 mmol) was dissolved in 20 mL THF and sodium carbonate (2.38 g, 22.5 mmol) was added. The resulting suspension was cooled to 0^{o}C. Cyanogen bromide (3.15 g, 30.05 mmol) was added as a solution in 20 mL chloroform via addition funnel over the course of 30 minutes. The solution was stirred 12 hours and monitored by thin layer chromatography. Upon completion, the solution was transferred to a separatory funnel and extracted 3X with 25 mL DCM. The combined organic layers were dried over magnesium sulfate, filtered and concentrated under reduced pressure to yield a colorless oil. The product was purified on silica (1:1 ethyl acetate:hexanes), yielded 1.32 g (75%) of **11** as a colorless solid. ^{1}H NMR (400.1 MHz, CD_3OD, δ (ppm)): 4.67 (s, 1 H), 4.58 (t, *J* = 5.10 Hz, 1H), 3.04 (m, 4H), 1.59 (m, 2H) 1.47 (m, 2H) 1.40 (m, 12H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)): 156.2, 116.7, 79.2, 45.8, 40.0, 29.5, 29.0, 28.6, 23.1 HRMS (TOF, ES+) $C_{11}H_{21}N_3O_2$ $[M+H]^+$ m/z 228.1712, measured 228.1710.



Tert-butyl (5-(2-hydroxyguanidino)pentyl)carbamate (12).

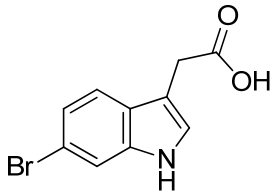
Tert-butyl (5-cyanamidopentyl)carbamate **11** (657 mg, 2.89 mmol) was dissolved in absolute ethanol. Sodium bicarbonate (485 mg, 5.78 mmol) and hydroxylamine HCl (221 mg, 3.18 mmol) were added and

the reaction was stirred at 25°C for 4 hours. Product formation was monitored by thin layer chromatography (80:18:2 $CHCl_3$:MeOH:NH_4OH) visualized with I_2 stain. Upon consumption of starting material, the reaction mixture was concentrated and purified on silica ($CHCl_3$ to 80:18:2 $CHCl_3$:MeOH:NH_4OH) and isolated **12** as a colorless solid 641 mg (85%). ^{1}H NMR (400.1 MHz, $CDCl_3$) δ (ppm)): 5.75 (br m, 3H) 5.17 (S, 1H) 2.99 (m, 4H), 1.36 (m, 13H), 1.27 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm)):156.5, 156.1, 78.9, 57.7, 49.9, 41.9, 41.2, 40.3, 29.5, 29.1, 28.3, 23.9, 18.1; HRMS (TOF, ES+) $C_{11}H_{24}N_4O_3$ $[M+H]^+$ m/z 261.1927, measured 261.1926.



2-(6-bromo-1H-indol-3-yl)-2-oxoacetic acid (16).

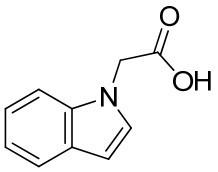
6-Bromoindole **15** (1 g, 5.1 mmol) was dissolved in 20 mL diethyl ether. Oxalyl chloride (890 uL, 10.2 mmol) was added and the reaction was stirred 45 minutes at 25°C. H_2O (500 uL, 25.5 mmol) was added, followed by 50 mL diethyl ether. The reaction was stirred 30 minutes and filtered via bukner funnel. The solid was washed with diethyl ether and dried under high vacuum. Obtained 1.37 g (99.9%) **16** as a yellow solid. ^{1}H NMR (400.1 MHz, d_6-DMSO, δ (ppm)): 8.47 (d, *J* = 3.25, 1H), 8.12 (d, *J* = 8.50, 1H), 7.75 (d, *J* = 1.50, 1H), 7.42 (dd, *J* =1.50, 8.50, 1H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)):181.1, 165.3, 139.1, 137.9, 125.9, 125.0, 123.1, 116.5, 115.7, 112.6; HRMS (TOF, ES+) $C_{10}H_6BrNO_3$ $[M+H]^+$ m/z 267.9609, measured [M ^{79}Br+H], [M ^{81}Br+H] 267.9610, 269.9600.



2-(6-bromo-1H-indol-3-yl)acetic acid (6).

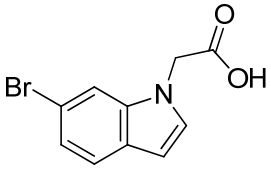
A microwave vial equipped with stir bar was charged with **16** (914 mg, 3.41 mmol) and dissolved in 17 mL 2-ethoxyethanol. Hydrazine hydrate (829 uL, 17.05 mmol) was added and the vial was sealed and heated in a microwave reactor at 80°C for 15 minutes. The vial was then unsealed and solid NaOMe (1.84 g, 34.1 mmol) was added. The vial was again sealed and heated in the microwave at 160°C for 1 hour. The vial contents were transferred to a 250 mL separatory funnel, acidified with 1N HCl (25 mL) and partitioned between ethyl acetate and H_2O. The aqueous phase was extracted 3X and the combined organic phase was dried over magnesium sulfate. The product was purified on silica (1:1 EtOAc+1%AcOH:Hex), yielded 717 mg (80%) of **6** as a beige solid. ^{1}H NMR (400.1 MHz, CD_3OD, δ (ppm)): 7.51 (s, 1H), 7.45 (d, *J* = 8.5 Hz, 1H), 7.16 (s, 1H), 7.13 (dd, *J* =1.56, 8.50 Hz, 1H), 3.72 (s, 1H); ^{13}C NMR

(150 MHz, CD_3OD, δ (ppm)): 174.7, 137.3, 126.1, 124.1, 121.5, 119.5, 114.3, 113.6, 107.8, 30.3; HRMS (TOF, ES+) $C_{10}H_8BrNO_2$ $[M+H]^+$ m/z 253.9817, measured [M ^{79}Br+H], [M ^{81}Br+H] 253.9815, 255.9792.



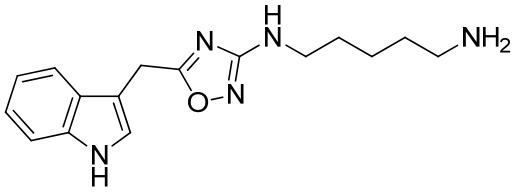
2-(1*H*-indol-1-yl)acetic acid (25).

A microwave vial equipped with stir bar was charged with indole **23** (1.0 g, 5.70 mmol) and dissolved in 15 mL acetonitrile. 2-Bromoethylacetate (11.41 mmol), K_2CO_3 (11.41 mmol), and KI (50 mg, cat) were added and the vial was sealed and heated in a microwave reactor at 160°C for 20 minutes. The mixture was transferred to a 250 mL separatory funnel and partitioned between ethyl acetate and H_2O. The aqueous phase was extracted 3x with 25 mL ethyl acetate and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The crude product was dissolved in dioxane (10 mL) and treated with 1M NaOH (10 mL). The reaction was stirred at 25°C for 3 hours and again transferred to a separatory funnel. The mixture was acidified with 2N HCl (20 mL) and extracted 3x with 25 mL ethyl acetate. The combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The product was purified on silica (1:1 EtOAc+1%AcOH:Hex), yielded 987 mg (76% over two steps) **25** as a beige solid. ^{1}H NMR (400.1 MHz, d_6-DMSO, δ (ppm)): 7.55 (d, *J* = 7.80 Hz, 1H), 7.38 (d, *J* = 8.0Hz, 1H), 7.33 (d, *J* = 3.25 Hz, 1H), 7.12 (t, *J* =7.56 Hz, 1H), 7.029 (t, *J* = 7.56 Hz, 1H), 6.44 (d, *J* = 3.25 Hz, 1H) 5.00 (s, 2H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)): 170.8, 136.7, 130.0, 128.4, 121.5, 120.6, 119.4, 110.1, 101.3, 47.4; HRMS (TOF, ES+) $C_{10}H_8BrNO_2$ $[M+H]^+$ m/z 176.0712, measured 176.0712.



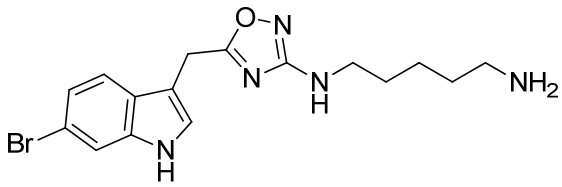
2-(6-bromo-1H-indol-1-yl)acetic acid (24).

The procedure for **25** was followed, utilizing 6-Bromoindole as starting material. ^{1}H NMR (400.1 MHz, d_6-DMSO, δ (ppm)): 7.70 (s, 1H), 7.51 (d, *J* = 8.5 Hz, 1H), 7.36 (d, *J* = 3.25 Hz, 1H), 7.17 (dd, *J* =1.56, 8.50 Hz, 1H), 6.84 (d, *J* = 3.25 Hz, 1H), 5.05 (s, 2H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)): 170.7, 137.7, 131.1, 127.5, 122.45, 114.5, 113.2, 101.7, 47.5; HRMS (TOF, ES+) $C_{10}H_8BrNO_2$ $[M+H]^+$ m/z 253.9817, measured [M ^{79}Br+H], [M ^{81}Br+H] 253.9815, 255.9792.



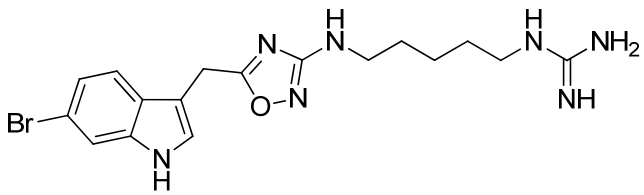
(5-((1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)pentane-1,5-diamine (14).

A flask was charged with **7** (105 mg, 0.599 mmol), HATU (227.7 mg, 0.599 mmol), DIEA (174 uL, 0.658 mmol) and DCM (2 mL). The solution was stirred for 30 minutes and transferring via pipette, was added dropwise to a stirred solution of **12** (171 mg, 0.658 mmol) in DCM (2 mL). The reaction was stirred 1 hour and concentrated under reduced pressure. The crude mixture was dissolved in DCE (5 mL). The flask was fitted with a reflux condenser and the reaction was heated to 100^{o}C for 1 hour. The reaction mixture was cooled and a 1:1 solution of DCM:TFA (5 mL) was added. The flask was heated to 50^{o}C for 3 hours, monitoring reaction progress by LCMS. The mixture was then transferred to a separatory funnel and partitioned between water and DCM. The mixture was extracted 3x with 25 mL DCM and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The product was purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10), obtained 78 mg (45%) of **14** as a brown glassy solid. ^{1}H NMR (400.1 MHz, CD_3OD, δ (ppm)): 7.53 (d, *J* = 8.25 Hz, 1H), 7.36 (d, *J* = 8.25 Hz, 1H), 7.21 (s, 1H), 7.11 (t, *J* = 7.50 Hz, 1H), 7.01 (t, *J* = 7.50, 1H), 4.22 (s, 2H), 3.16 (t, *J* = 7.0, 2H), 2.89 (m, 2H), 1.65 (m, 4H), 1.44 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)): 177.7, 168.6, 126.6, 123.2, 121.2, 118.6, 117.7, 110.9, 106.6, 42.1, 39.1, 28.1, 26.6, 23.1, 22.5; HRMS (TOF, ES+) $C_{16}H_{21}N_5O$ $[M+H]^+$ m/z 300.1824, measured 300.1823.



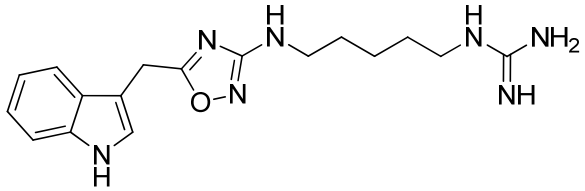
5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)pentane-1,5-diamine (13).

A flask was charged with **6** (105 mg, 0.599 mmol), HATU (227.7 mg, 0.599 mmol), DIEA (174 uL, 0.658 mmol) and DCM (2 mL). The solution was stirred for 30 minutes and transferring via pipette, was added dropwise to a stirred solution of **12** (171 mg, 0.658 mmol) in DCM (2 mL). The reaction was stirred 1 hour and concentrated under reduced pressure. The crude mixture was dissolved in DCE (5 mL). The flask was fitted with a reflux condenser and the reaction was heated to 100^{o}C for 1 hour. The reaction mixture was cooled and a 1:1 solution of DCM:TFA (5 mL) was added. The flask was heated to 50^{o}C for 3 hours, monitoring reaction progress by LCMS. The mixture was then transferred to a separatory funnel and partitioned between water and DCM. The mixture was extracted 3x with 25 mL DCM and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The product was purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10), obtained 119 mg (74%) of **13** as a brown glassy solid. ^{1}H NMR (400.1 MHz, CD_3OD, δ (ppm)): 7.51 (s, 1H), 7.44 (d, *J* = 8.50 Hz, 1H), 7.22 (s, 1H), 7.12 (d, *J* = 8.50 Hz, 1H), 4.18 (s, 2H), 3.16 (t, *J* = 7.0 Hz, 2H), 2.88 (t, *J* = 7.50 Hz, 2H), 1.66 (m, 4H), 1.42 (m, 2H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)):176.7, 168.5, 137.0, 125.7, 125.2, 121.4, 120.1, 114.2, 113.9, 107.2, 45.2, 42.1, 27.9, 26.5, 23.2, 22.5; HRMS (TOF, ES+) $C_{16}H_{20}BrN_5O$ $[M+H]^+$ m/z 378.0929, measured [M ^{79}Br+H], [M ^{81}Br+H] 378.0926, 380.0911.



Phidianidine A (4).

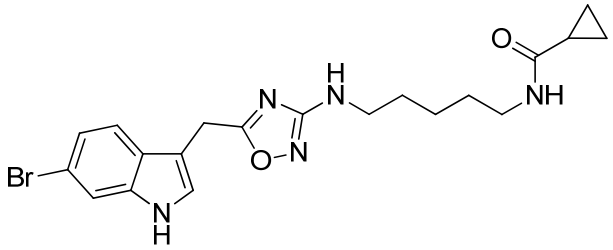
A 50 mL round bottom flask was charged with **13** (20 mg, 0.0528 mmol), DIEA (20 uL), N,N'-Bis(Boc)-1*H*-pyrazole-1-carboxamidine (17.69 mg, 0.058 mmol), and DCM (5 mL). The reaction was stirred 30 minutes, monitoring progress by LCMS. After completion, a 1:1 solution of DCM:TFA (5 mL) was added and the reaction was stirred 8 hours. The mixture was then transferred to a separatory funnel, washed with water and extracted 3 times with DCM. The combined organic phase was dried over magnesium sulfate before being concentrated under reduced pressure. The brown solid was then purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10), obtained 20 mg (90%) of **4** as a glassy golden brown solid. ^{1}H NMR (600 MHz, d_6-DMSO, δ (ppm)): 11.18 (s, 1H), 7.56 (d, *J* = 1.80 Hz, 1H), 7.47 (d, *J* = 8.50 Hz, 1H), 7.34 (d, *J* = 2.50, 1H), 7.13 (dd, *J* = 8.50, 1.78, 1H), 6.72 (t, *J* = 5.85 Hz, 1H), 4.2 (s, 2H), 3.00 (dq, *J* = 33.1, 7.0 Hz, 4H) 1.46 (m, 4H), 1.27 (m, 2H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)): 177.1, 168.9, 157.1, 137.4, 126.1, 125.7, 122.0, 120.6, 114.5, 114.4, 107.8, 42.6, 41.0, 28.54, 28.53, 23.8, 22.9; HRMS (TOF, ES+) $C_{17}H_{22}BrN_7O$ $[M+H]^+$ m/z 420.1147, measured [M ^{79}Br+H], [M ^{81}Br+H] 420.1148, 422.1132.



Phidianidine B (5).

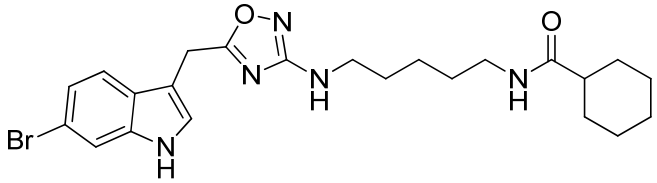
A 50 mL round bottom flask was charged with **13** (20 mg, 0.0668 mmol), DIEA (20 uL), N,N'-Bis(Boc)-1*H*-pyrazole-1-carboxamidine (23.0 mg, 0.073 mmol), and DCM (5 mL). The reaction was stirred 30 minutes, monitoring progress by LCMS. After completion, a 1:1 solution of DCM:TFA (5 mL) was added and the reaction was stirred 8 hours. The mixture was then transferred to a separatory funnel, washed with water and extracted 3 times with DCM. The combined organic phase was dried over magnesium sulfate before being concentrated under reduced pressure. The brown solid was then purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10), obtained 20 mg (78%) **5** as a glassy brown solid. ^{1}H NMR (400 MHz, d_6-DMSO, δ (ppm)): 11.0, 7.51 (d, *J* = 7.85 Hz, 1H), 7.37 (d, *J* = 8.10 Hz, 1H) 7.31 (d, *J* = 2.45 Hz, 1H), 7.08-6.99 (dt, *J* = 58.2, 8.0 Hz, 2H), 6.71 (br t, *J* = 5.30 Hz, 1H), 4.19 (s, 2H), 3.07-3.00 (m, 4H), 1.50-1.43 (m, 4H), 1.29-1.27 (m, 2H); ^{13}C NMR (100 MHz, d_6-DMSO, δ (ppm)):177.3, 168.9, 157.1, 136.5, 127.2, 124.5, 121.6, 119.0, 118.7, 111.9, 107.3, 42.7, 41.0,

28.57, 28.55, 23.8, 23.1; HRMS (TOF, ES+) $C_{17}H_{23}N_7O$ $[M+H]^+$ m/z 342.2042, measured 342.2041.



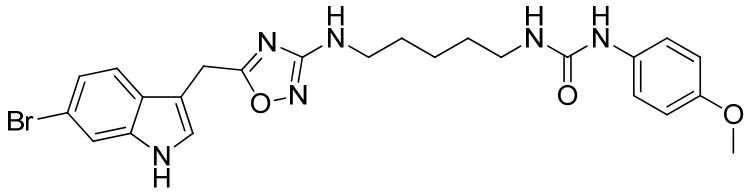
N-(5-((5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)cyclopropanecarboxamide (17).

A 10 mL round bottom flask was charged with **13** (10 mg, 0.026 mmol), cyclopropanecarbonyl chloride (5 uL, 0.053 mmol), triethylamine (10 uL) and DCM (2 mL). The flask was stirred for 3 hours, monitoring by LCMS. After 3 hours, water (3 mL) and DCM (2 mL) were added and the biphasic solution was stirred vigorously before being passed through an Isolute phase separator. The resulting organic layer was then concentrated and purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10). Isolated 10 mg (85%) of **17** as an off white solid. ^{1}H NMR (600.1 MHz, CD_3OD, δ (ppm)): 7.51 (d, *J* = 1.70, 1H), 7.45 (d, *J* = 8.50 Hz, 1H), 7.22 (s, 1H), 7.126 (dd, *J* = 8.50, 1.70, 1H), 4.19 (s, 2H), 3.13 (dt, *J* = 17.83, 7.13 Hz, 4H), 1.58 (m, 2H), 1.50 (m, 3H), 1.36 (m, 2H), 0.080 (m, 2H), 0.693 (m, 2H) ; ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)): 177.4, 174.9, 168.6, 137.4, 125.7, 124.2, 121.7, 119.3, 114.7, 113.8, 107.2, 42.4, 38.9, 28.7, 28.3, 23.7, 22.4, 13.3, 5.6; HRMS (TOF, ES+) $C_{20}H_{24}BrN_5O_2$ $[M+H]^+$ m/z 446.1192, measured [M ^{79}Br+H], [M ^{81}Br+H] 446.1196, 448.1181.



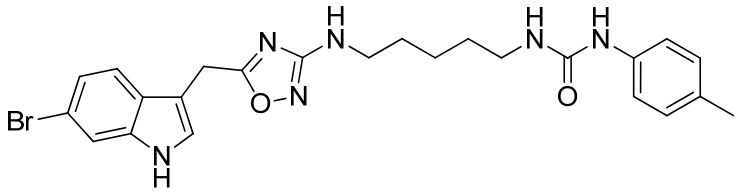
N-(5-((5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)cyclohexanecarboxamide (18).

A 10 mL round bottom flask was charged with **13** (10 mg, 0.026 mmol), cyclohexanecarbonylchloride (5 uL mg, 0.057 mmol), triethylamine (10 uL) and DCM (2 mL). The flask was stirred for 3 hours, monitoring by LCMS. After 3 hours, water (3 mL) and DCM (2 mL) were added and the biphasic solution was stirred vigorously before being passed through an Isolute phase separator. The resulting organic layer was then concentrated and purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10). Isolated 9 mg (70%) of **25** as an off white solid. ^{1}H NMR 600.1 MHz, CD_3OD, δ (ppm)):7.54 (d, *J* = 1.70 Hz, 1H), 7.48 (d, *J* = 8.50 Hz, 1H), 7.25 (s, 1H), 7.157 (dd, *J* = 8.50, 1.70, 1H), 4.21 (s, 2H), 3.15 (m, 4H), 2.14 (m, 2H), 1.79 (m, 4H), 1.69 (m, 1H), 1.61 (dp, *J* = 56.7, 7.33, 4H), 1.46-1.21 (m, 8H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)) 177.7, 177.4, 168.6, 137.4, 125.7, 124.2, 121.7, 119.3, 114.7, 113.8, 107.2, 45.1, 45.1, 42.4, 38.7, 38.5, 29.3, 28.6, 28.3, 25.4, 25.3, 23.6, 22.4; HRMS (TOF, ES+) $C_{23}H_{30}BrN_5O_2$ $[M+H]^+$ m/z 488.1661, measured [M ^{79}Br+H], [M ^{81}Br+H] 488.1664, 490.1648.



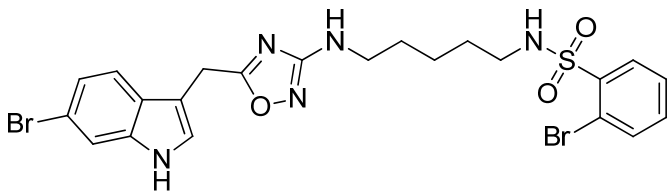
1-(5-((5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)-3-(4-methoxyphenyl)urea (21).

A 10 mL round bottom flask was charged with **13** (10 mg, 0.026 mmol), 4-methoxyphenylisocyanate (5 uL, 0.057 mmol), triethylamine (10 uL) and DCM (2 mL). The flask was stirred for 3 hours, monitoring by LCMS. After 3 hours, water (3 mL) and DCM (2 mL) were added and the biphasic solution was stirred vigorously before being passed through an Isolute phase separator. The resulting organic layer was then concentrated and purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10). Isolated 10 mg (73%) of **21** as a brown solid. ^{1}H NMR (600.1 MHz, d_6-DMSO, δ (ppm)): 11.16 (s, 1H), 8.17 (s, 1H), 7.50 (d, *J* = 1.70 Hz, 1H), 7.47 (d, *J* = 8.50 Hz, 1H), 7.35 (d, *J* = 2.50 Hz, 1H), 7.27 (dd, *J* = 8.90, 0.87 Hz, 2H), 7.14 (dd, 8.50, 1.85 Hz, 1H), 6.78 (m, 3H), 4.20 (s, 2H), 3.68 (s, 3H), 3.01 (m, 4H),1.50 (m, 2H), 1.40 (m, 2H), 1.27 (m, 2H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)): 177.1, 168.9, 168.9, 155.8, 155.7, 154.2, 137.4, 134.1, 134.0, 126.2, 125.7, 123.5, 122.0, 120.6, 119.7, 119.6, 114.5, 114.4, 114.2, 107.8, 55.5, 42.8, 42.7, 29.9, 28.7, 24.1, 22.9, ; HRMS (TOF, ES+) $C_{24}H_{27}BrN_6O_3$ $[M+H]^+$ m/z 527.1406, measured [M ^{79}Br+H], [M ^{81}Br+H] 527.1405,529.1391.



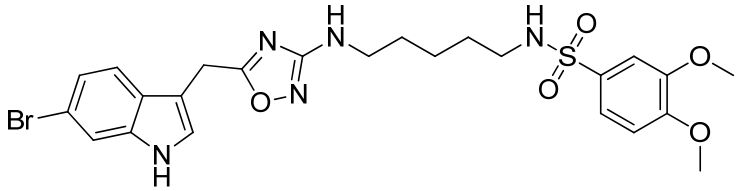
1-(5-((5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)-3-(p-tolyl)urea (22).

A 10 mL round bottom flask was charged with **13** (10 mg, 0.026 mmol), p-Tolylisocyanate (5 uL, 0.057 mmol), triethylamine (10 uL) and DCM (2 mL). The flask was stirred for 3 hours, monitoring by LCMS. After 3 hours, water (3 mL) and DCM (2 mL) were added and the biphasic solution was stirred vigorously before being passed through an Isolute phase separator. The resulting organic layer was then concentrated and purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10). Isolated 8 mg (60%) of **22** as a brown solid. ^{1}H NMR (600.1 MHz, d_6-DMSO, δ (ppm)): 11.16 (s, 1H), 8.17 (s, 1H), 7.50 (d, *J* = 1.70 Hz, 1H), 7.47 (d, *J* = 8.50 Hz, 1H), 7.35 (d, *J* = 2.50 Hz, 1H), 7.27 (dd, *J* = 8.90, 0.87 Hz, 2H), 7.14 (dd, 8.50, 1.85 Hz, 1H), 6.78 (m, 3H), 4.20 (s, 2H), 3.01 (m, 4H), 2.68 (s, 3H), 1.50 (m, 2H), 1.40 (m, 2H), 1.27 (m, 2H); ^{13}C NMR (150 MHz, d_6-DMSO, δ (ppm)): 177.1, 168.9, 168.9, 155.8, 155.7, 154.2, 137.4, 134.1, 134.0, 126.2, 125.7, 1235, 122.0, 120.6, 119.7, 119.6, 114.5, 114.4, 114.2, 107.8, 45.5, 42.8, 42.7, 29.9, 28.7, 24.1, 22.9, HRMS (TOF, ES+) $C_{24}H_{27}BrN_6O_2$ $[M+H]^+$ m/z 510.1441, measured [M ^{79}Br+H], [M ^{81}Br+H] 510.1445, 512.1458.



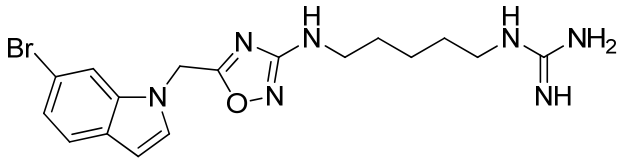
2-bromo-N-(5-((5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)benzenesulfonamide (19).

A 10 mL round bottom flask was charged with **13** (10 mg, 0.026 mmol), 2-bromophenylsulfonylchloride (10 mg, 0.057 mmol), triethylamine (10 uL) and DCM (2 mL). The flask was stirred for 3 hours, monitoring by LCMS. After 3 hours, water (3 mL) and DCM (2 mL) were added and the biphasic solution was stirred vigorously before being passed through an Isolute phase separator. The resulting organic layer was then concentrated and purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10). Isolated 9 mg (58%) of **19** as an off white glassy solid. ^{1}H NMR (600.1 MHz, CD_3OD, δ (ppm)): 8.08 (dd, 7.83, *J* = 1.70 Hz, 1H, 7.75 (dd, *J* = 7.83, 1.15 Hz, 1H), 7.54 (d, *J* = 1.70 Hz, 1H), 7.49 (td, *J* = 7.50, 1.50), 7.48 (d, *J* = 8.50 Hz, 1H), 7.42 (dt, *J* = 7.75, 1.75 Hz, 1H), 7.26 (s, 1H), 7.16 (dd, 8.50, 1.75), 4.60 (s, 2H), 4.22 (s, 2H), 3.06-2.90 (dt, *J* = 92.7, 7.0 Hz, 4H), 1.49-1.42 (m, 4H), 1.31 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)): 177.5, 168.5, 139.6, 137.4, 135.0, 133.3, 130.8, 127.4, 125.7, 124.3, 121.8, 119.4, 114.7, 113.8, 107.2, 42.3, 28.7, 28.1, 23.3, 22.4; HRMS (TOF, ES+) $C_{22}H_{23}Br_2N_5O_3S$ $[M+H]^+$ m/z 595.9967, measured 595.9966.



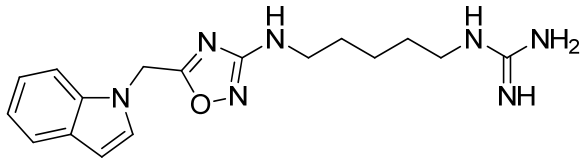
N-(5-((5-((6-bromo-1H-indol-3-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)-3,4-dimethoxybenzenesulfonamide (20).

A 10 mL round bottom flask was charged with **13** (10 mg, 0.026 mmol), 3,4-dimethoxyphenylsulfonylchloride (10 mg, 0.057 mmol), triethylamine (10 uL) and DCM (2 mL). The flask was stirred for 3 hours, monitoring by LCMS. After 3 hours, water (3 mL) and DCM (2 mL) were added and the biphasic solution was stirred vigorously before being passed through an Isolute phase separator. The resulting organic layer was then concentrated and purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10). Isolated 10 mg (66%) **20** as an off white glassy solid. ^{1}H NMR (600.1 MHz, CD_3OD, δ (ppm)): 7.54 (s, 1H), 7.47 (d, *J* = 8.50 Hz, 1H), 7.45 (dd, *J* =8.50, 2.20 Hz, 1H), 7.36 (d, *J* = 2.20 Hz, 1H), 7.25 (s, 1H), 7.15 (dd, *J* = 8.50, 1.70 Hz, 1H), 7.05 (d, *J* = 8.50 Hz, 1H), 4.22 (s, 2H), 3.88 (d, *J* = 5.35 Hz, 6H), 3.09 (t, *J* = 7.0 Hz, 4H), 2.88 (t, *J* = 7.0 Hz, 4H), 1.54-1.44 (m, 4H), 1.33 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)): 177.5, 168.5, 152.5, 149.1, 137.4, 1319, 125.7, 124.2, 121.8, 120.5, 119.3, 114.7, 113.8, 110.5, 109.4, 107.2, 55.19, 55.14, 42.4, 42.3, 28.6, 28.1, 23.4, 22.4; HRMS (TOF, ES+) $C_{24}H_{28}BrN_5O_5S$ $[M+H]^+$ m/z 578.1073, measured [M ^{79}Br+H], [M ^{81}Br+H] 578.1074, 580.1061.



1-(5-((5-((6-bromo-1H-indol-1-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)guanidine (26).

A flask was charged with **24** (100 mg, 0.393 mmol), HATU (150 mg, 0.393 mmol), DIEA (70 uL, 0.393 mmol) and DCM (2 mL). The solution as stirred for 30 minutes and transferring via pipette, added dropwise to a stirred solution of **12** (112 mg, 0.432 mmol) in DCM (2 mL). The reaction was stirred 1 hour and concentrated under reduced pressure. The crude mixture was dissolved in DCE (5 mL). The flask was fitted with a reflux condenser and the reaction was heated to 100^{o}C for 1 hour. The reaction mixture was cooled and a 1:1 solution of DCM:TFA (5 mL) was added. The flask was heated to 50^{o}C for 3 hours, monitoring reaction progress by LCMS. The mixture was then transferred to a separatory funnel and partitioned between water and DCM. The mixture was extracted 3x with 25 mL DCM and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The crude mixture was dissolved in DCM and N,N'-Bis(Boc)-1*H*-pyrazole-1-carboxamidine (134 mg, 0.432 mmol) was added. The reaction was stirred 1 hour and a 1:1 solution of DCM:TFA (10 mL) was added. The reaction was heated to 80^{o}C for 3 hours. The mixture was then transferred to a separatory funnel and partitioned between water and DCM. The mixture was extracted 3x with 25 mL DCM and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The product was purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10), obtained 112 mg (67%) as a bright red glassy solid. ^{1}H NMR (600.1 MHz, CD_3OD, δ (ppm)): 7.58 (s, 1H), 7.43 (d, *J* = 8.5 Hz, 1H), 7.25 (d, *J* = 3.25 Hz, 1H), 7.14 (dd, *J* = 8.50, 1.56 Hz, 1H), 6.49 (d, *J* = 3.25 Hz, 1H), 5.46 (s, 2H), 3.10 (m, 4H), 1.55 (m, 6H), 1.35 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)):157.9, 167.0, 135.8, 124.1, 122.6, 120.2, 117.7, 113.1, 112.2, 105.6, 40.5, 37.5, 26.5, 25.1, 21.6, 20.8; HRMS (TOF, ES+) $C_{17}H_{22}BrN_7O$ $[M+H]^+$ m/z 420.1147, measured [M ^{79}Br+H], [M ^{81}Br+H] 420.1148, 422.1126.



1-(5-((5-((1H-indol-1-yl)methyl)-1,2,4-oxadiazol-3-yl)amino)pentyl)guanidine (27).

A flask was charged with **24** (100 mg, 0.292 mmol), HATU (111 mg, 0.292 mmol), DIEA (101 uL, 0.584mmol) and DCM (2 mL). The solution as stirred for 30 minutes and transferring via pipette, added dropwise to a stirred solution of **12** (112 mg, 0.432 mmol) in DCM (2 mL). The reaction was stirred 1 hour and concentrated under reduced pressure. The crude mixture was dissolved in DCE (5 mL). The flask was fitted with a reflux condenser and the reaction was heated to 100^{o}C for 1 hour. The reaction mixture was cooled and a 1:1 solution of DCM:TFA (5 mL) was added. The flask was heated to 50^{o}C for 3 hours, monitoring reaction progress by LCMS. The mixture was then transferred to a separatory funnel

and partitioned between water and DCM. The mixture was extracted 3x with 25 mL DCM and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The crude mixture was dissolved in DCM and N,N'-Bis(Boc)-1*H*-pyrazole-1-carboxamidine (100 mg, 0.321 mmol) was added. The reaction was stirred 1 hour and a 1:1 solution of DCM:TFA (10 mL) was added. The reaction was heated to 80^{o}C for 3 hours. The mixture was then transferred to a separatory funnel and partitioned between water and DCM. The mixture was extracted 3x with 25 mL DCM and the combined organic phase was dried over magnesium sulfate and concentrated under reduced pressure. The product was purified by reverse phase HPLC using acetonitrile and 0.1% TFA/water (gradient: 10:90 to 90:10), obtained 32 mg (32%) as a beige glassy solid. ^{1}H NMR (600.1 MHz, CD_3OD, δ (ppm)): 7.61 (d, *J* = 7.80 Hz, 1H), 7.44 (d, *J* = 8.0Hz, 1H), 7.36 (d, *J* = 3.25 Hz, 1H), 7.22 (t, *J* =7.56 Hz, 1H), 7.11 (t, *J* = 7.56 Hz, 1H), 6.55 (d, *J* = 3.25 Hz, 1H) 5.58 (s, 2H); 3.14 (m, 4H), 1.59 (m, 4H), 1.40 (m, 2H); ^{13}C NMR (150 MHz, CD_3OD, δ (ppm)):174.1, 168.8, 157.1, 157.0, 436.3, 128.9, 128.6, 121.7, 120.7, 119.7, 109.4, 102.1, 42.4, 41.2, 40.9, 28.2, 28.1, 23.5; HRMS (TOF, ES+) $C_{17}H_{23}N_7O$ $[M+H]^+$ m/z 342.2042, measured 342.2042.

NMR Shift Correlation Tables:

Tables formulated to match Phidianidines A & B isolation NMR data tables by Carbone et al.

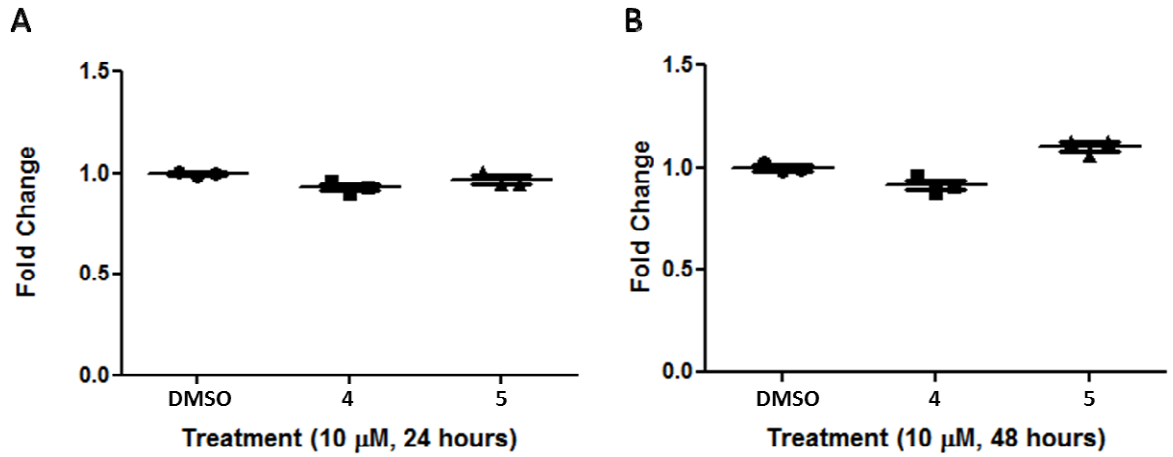
1H

Natural PA-A	Synthetic PA-A	Natural PA-B	Synthetic PA-B
11.15	11.18	11.00	11.0
7.35	7.34	7.31	7.31
7.53	7.47	7.50	7.51
7.13	7.13	6.99	6.99
7.56	7.56	7.09	7.08
4.2	4.20	7.36	7.37
6.72	6.72	4.20	4.19
2.99	3.00	6.71	6.71
1.36-1.56	1.40-1.52	3.00	3.01
1.28	1.27	1.36-1.56	1.42-1.52
1.36-1.56	1.40-1.52	1.28	1.28
3.02	3.00	1.36-1.56	1.42-1.52
7.41	7.40	3.05	3.01
		7.43	7.49

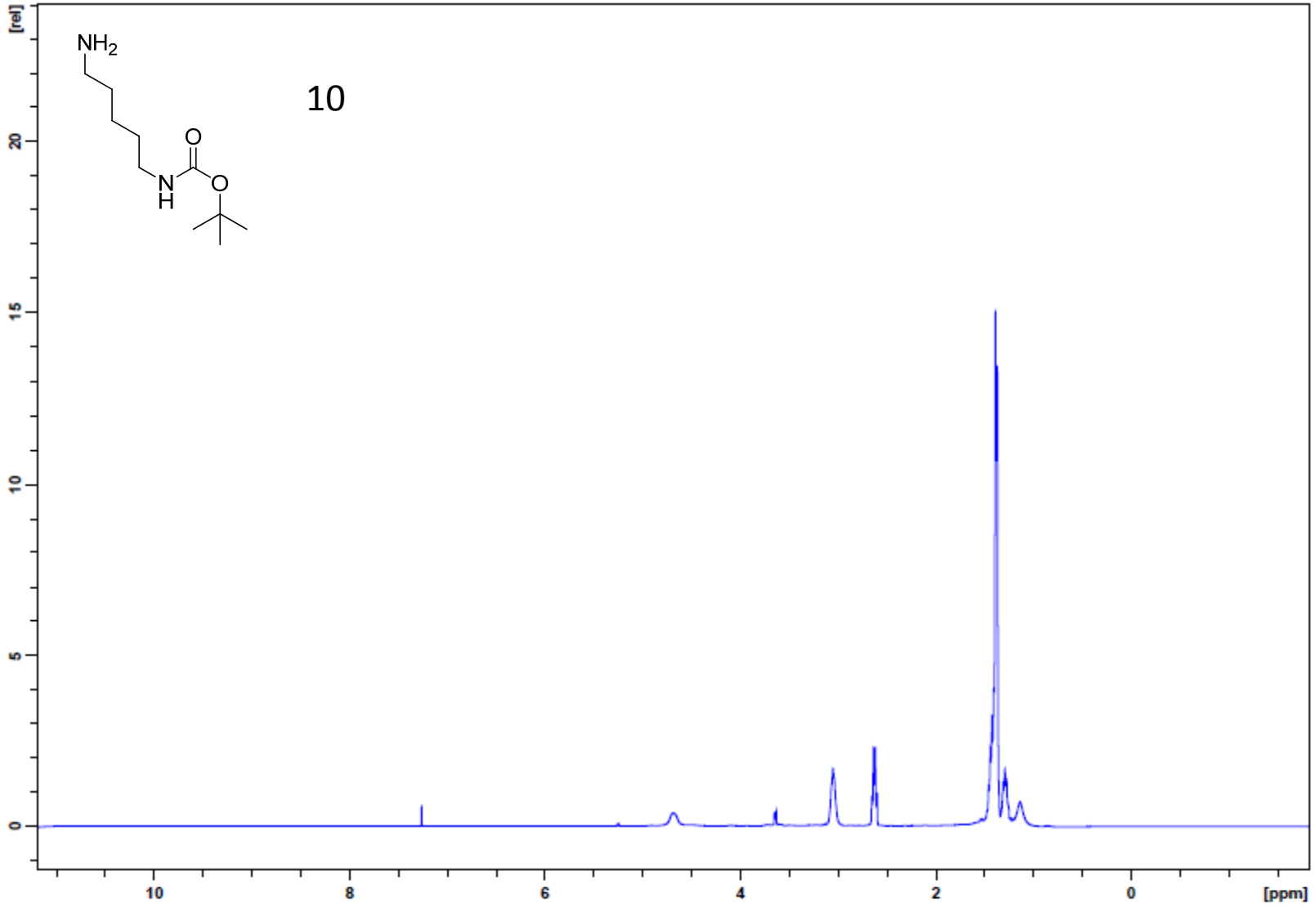
13C

Natural PA-A	Synthetic PA-A	Natural PA-B	Synthetic PA-B
125.0	125.7	125.3	124.50
106.8	107.8	103.9	107.30
125.2	126.1	126.7	127.20
120.0	120.6	117.3	118.70
121.3	122.0	117.6	121.60
113.5	114.4	120.2	119.00
114.1	114.5	111.5	111.90
137.0	137.4	136.1	136.50
22.5	22.9	22.7	23.10
168.5	168.9	168.5	168.90
176.7	177.1	176.9	177.30
40.5	41	40.7	41.00
28.1	28.5	28.1	28.57
23.4	23.8	23.4	23.80
28.1	28.54	28.1	28.55
41.9	42.6	42.3	42.70
156.6	157.1	156.6	157.10

Assay Results: WST-1 Cell Proliferation

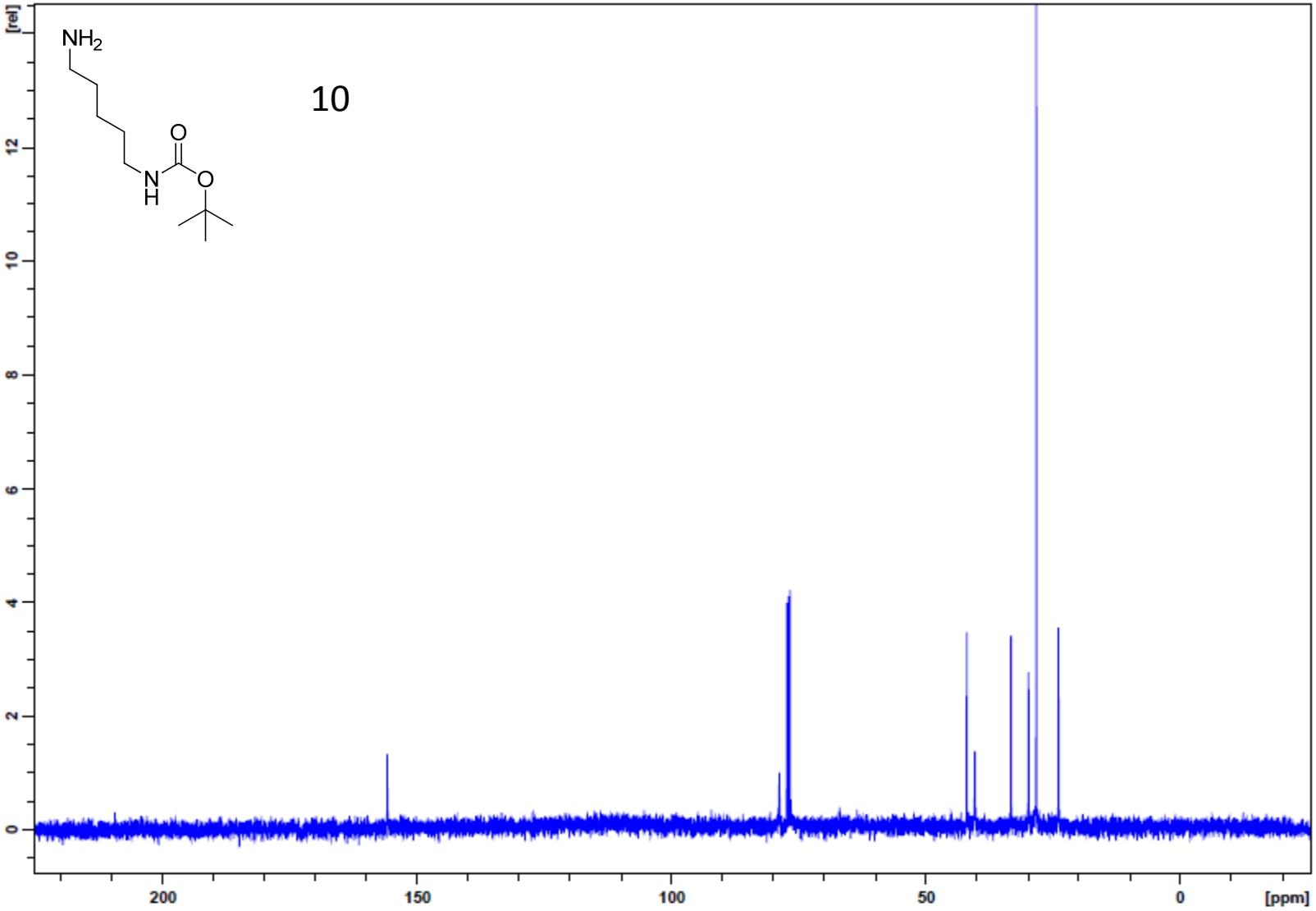


400 MHz, $CDCl_3$

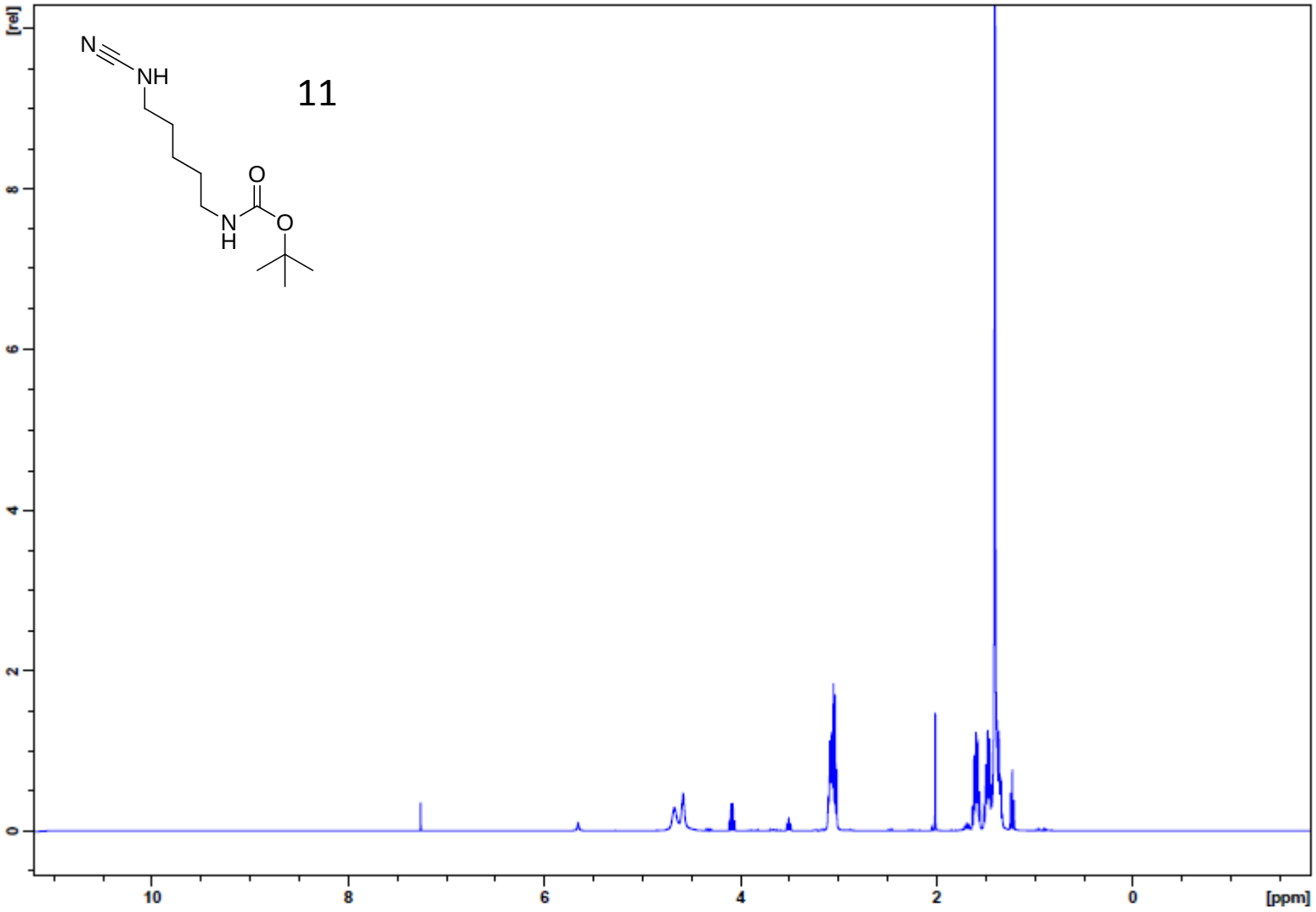


10

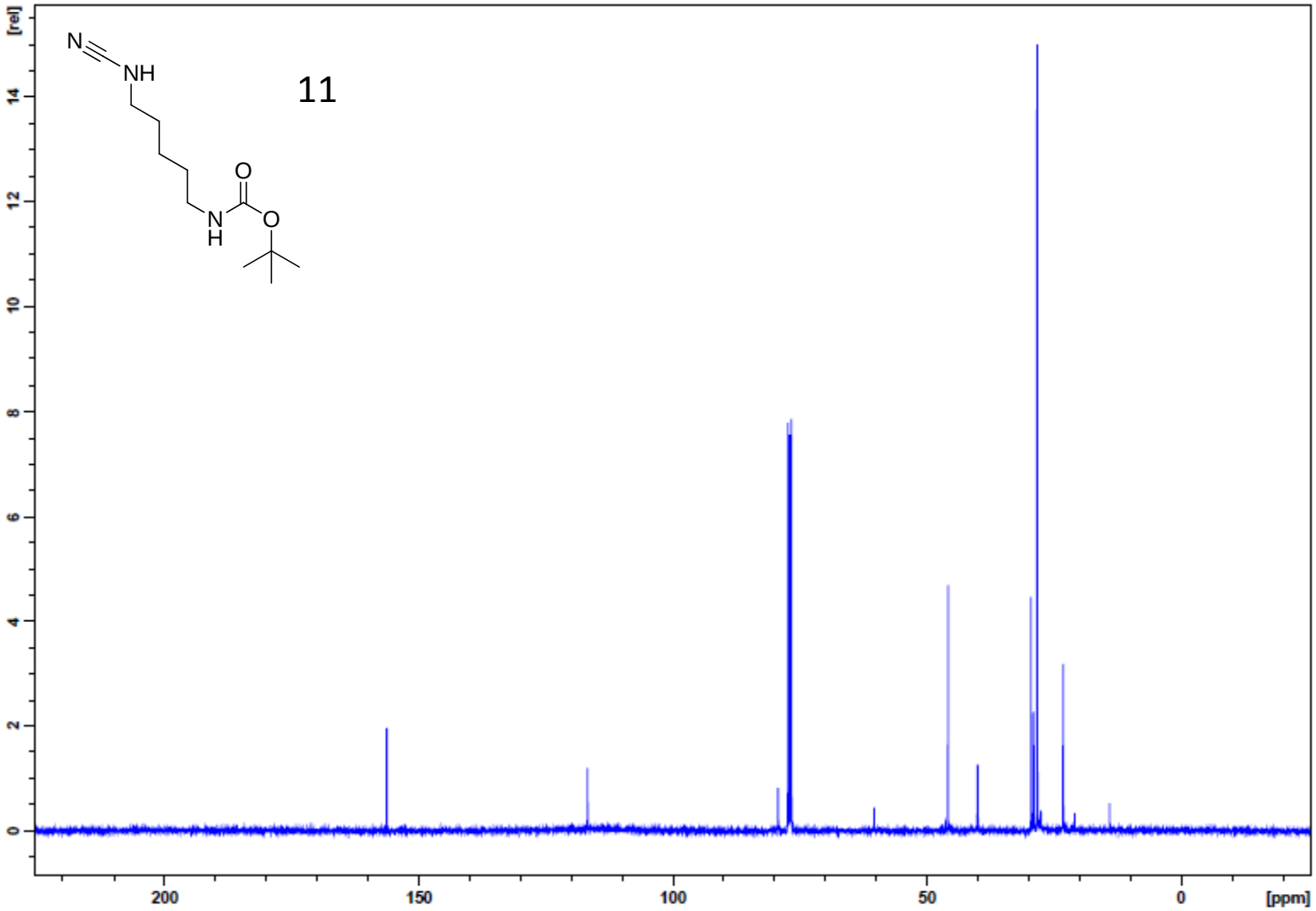
100 MHz, $CDCl_3$



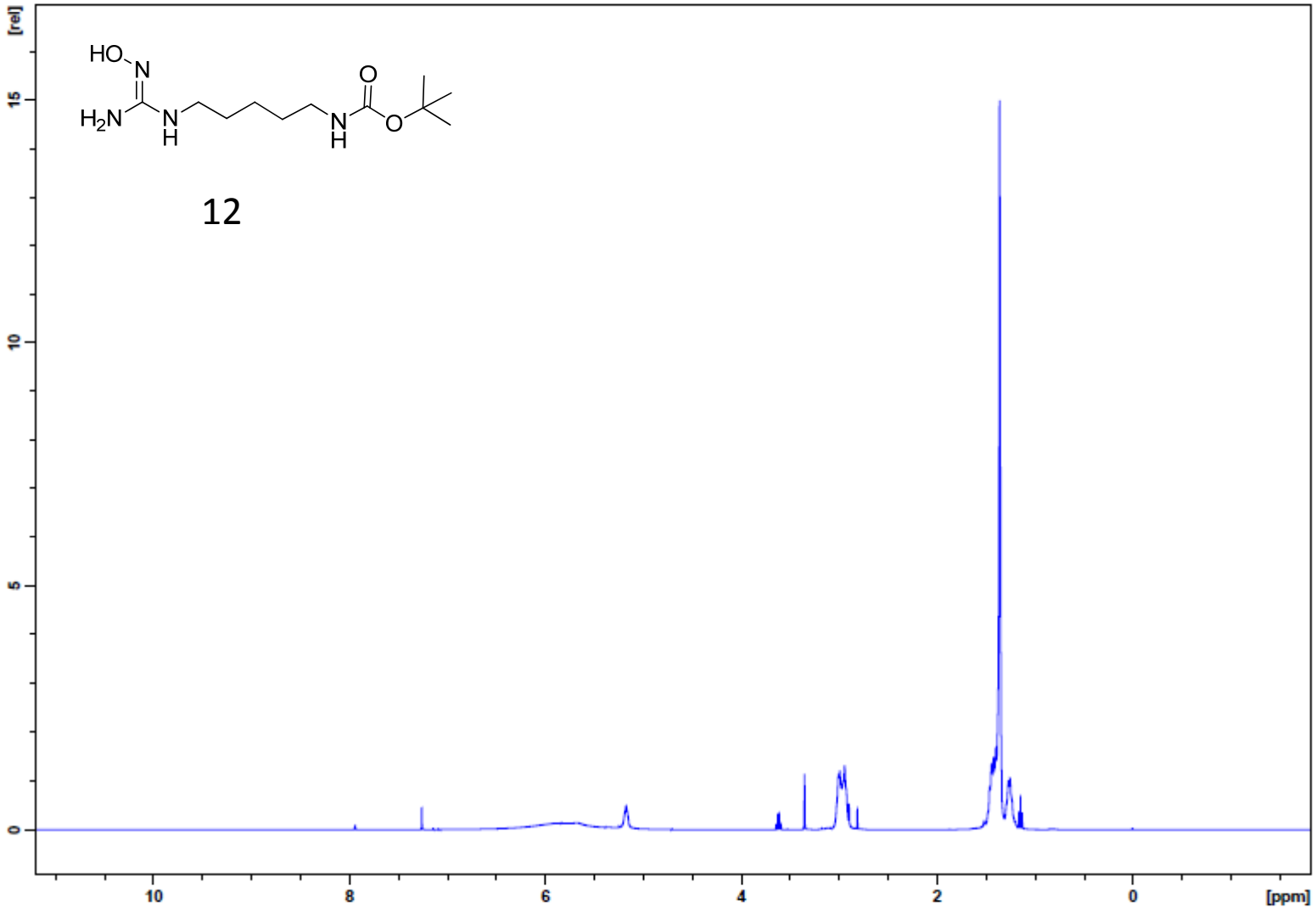
400 MHz, CD_3OD



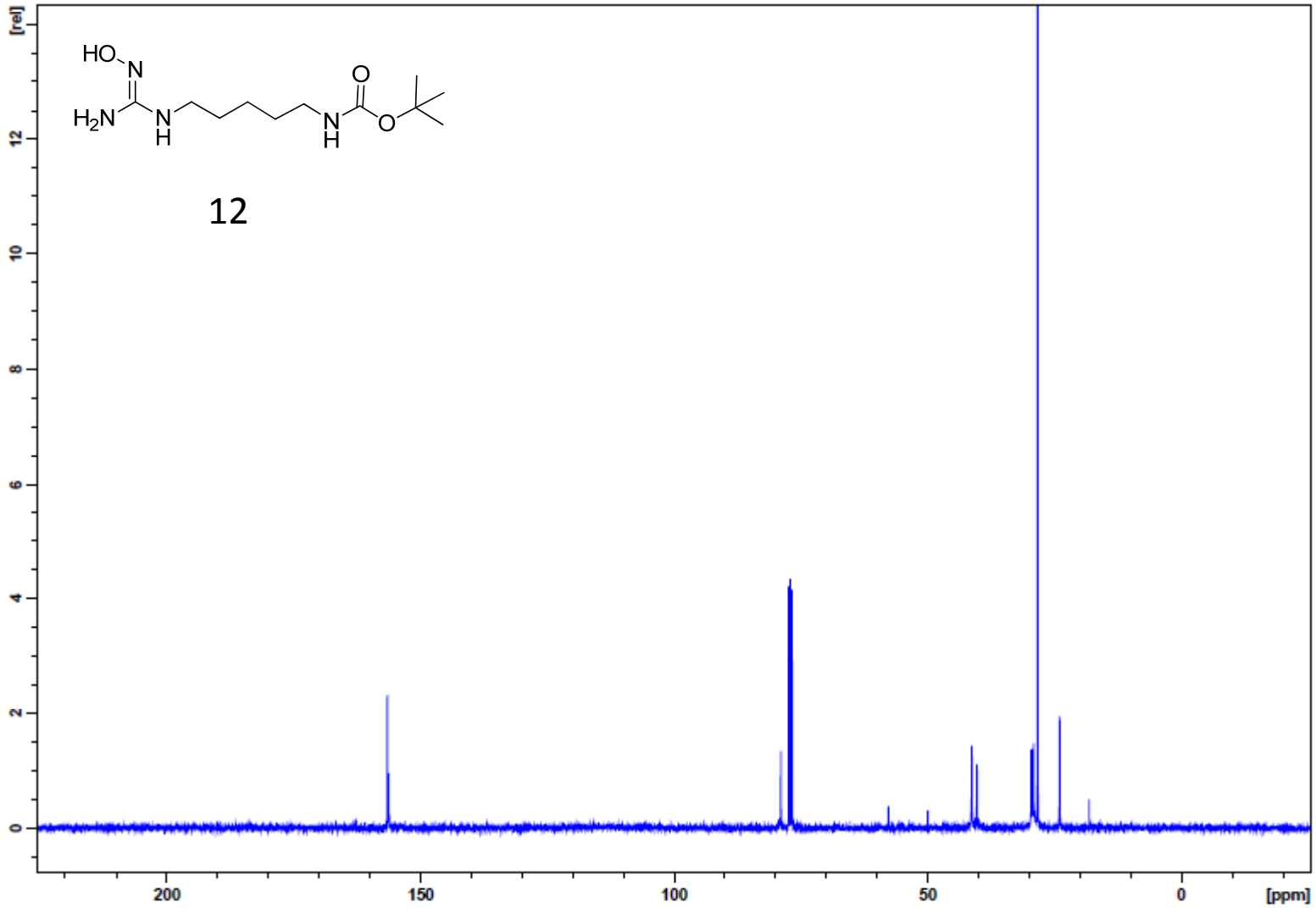
100 MHz, CD_3OD



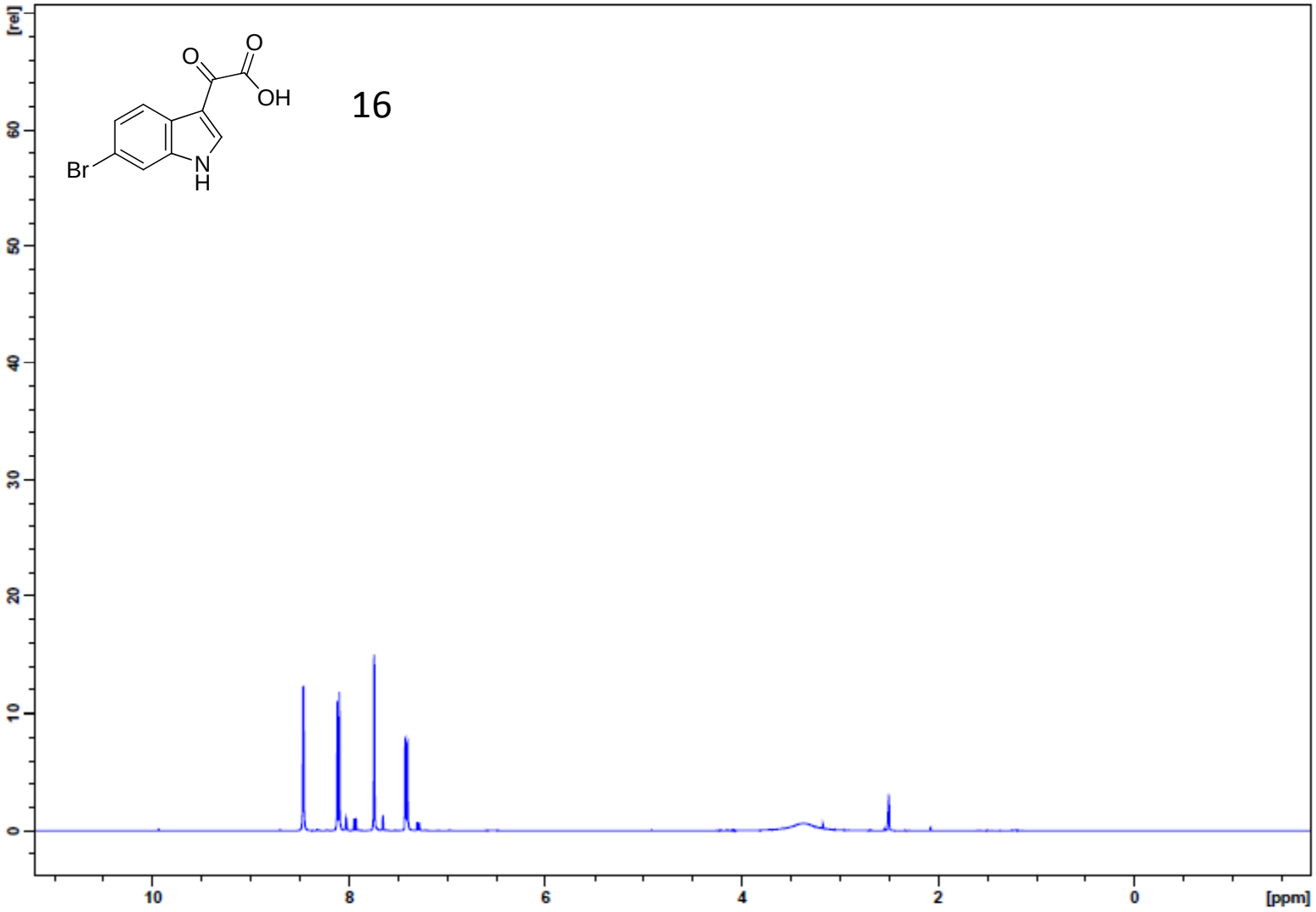
400 MHz, $CDCl_3$



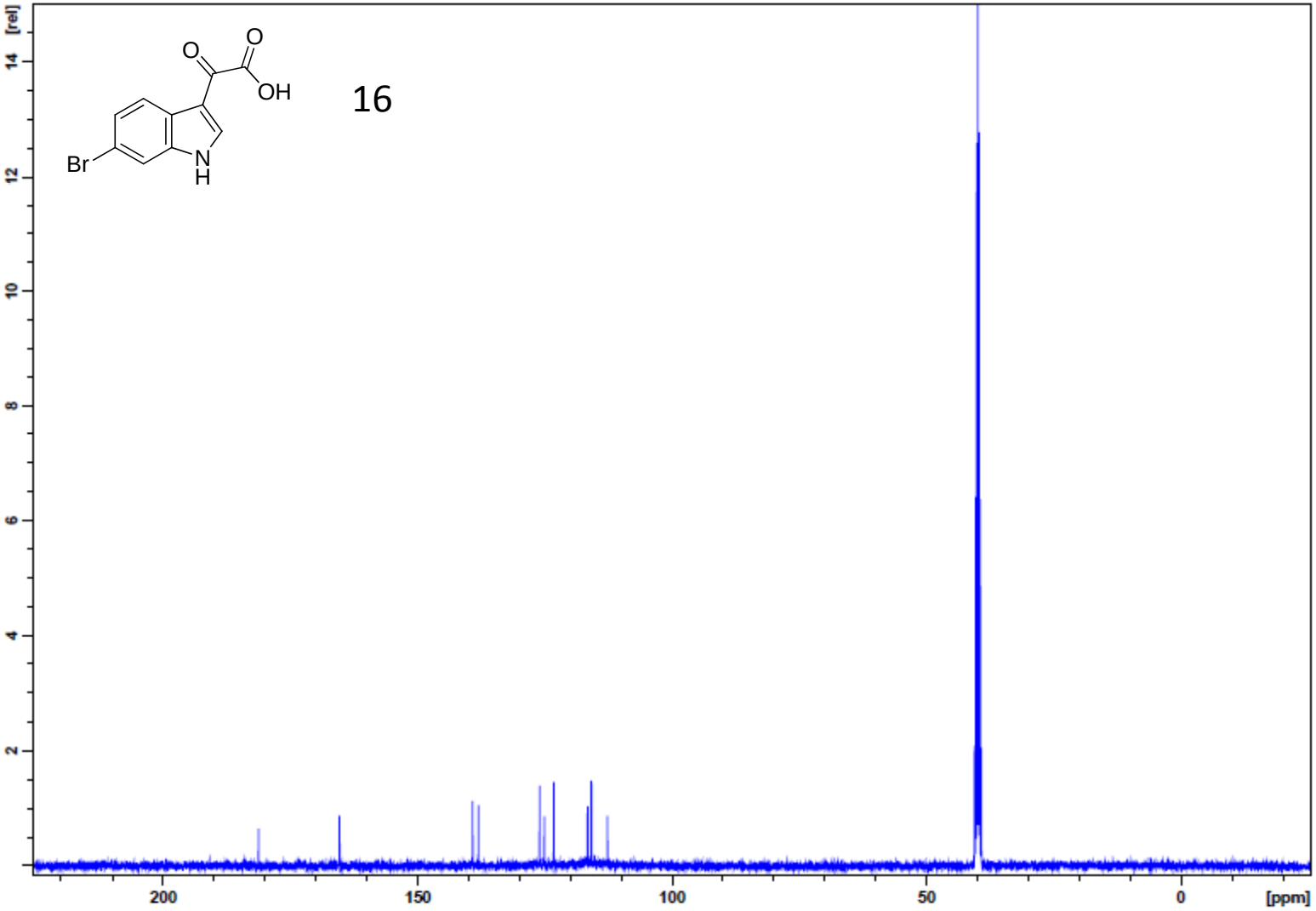
100 MHz, $CDCl_3$



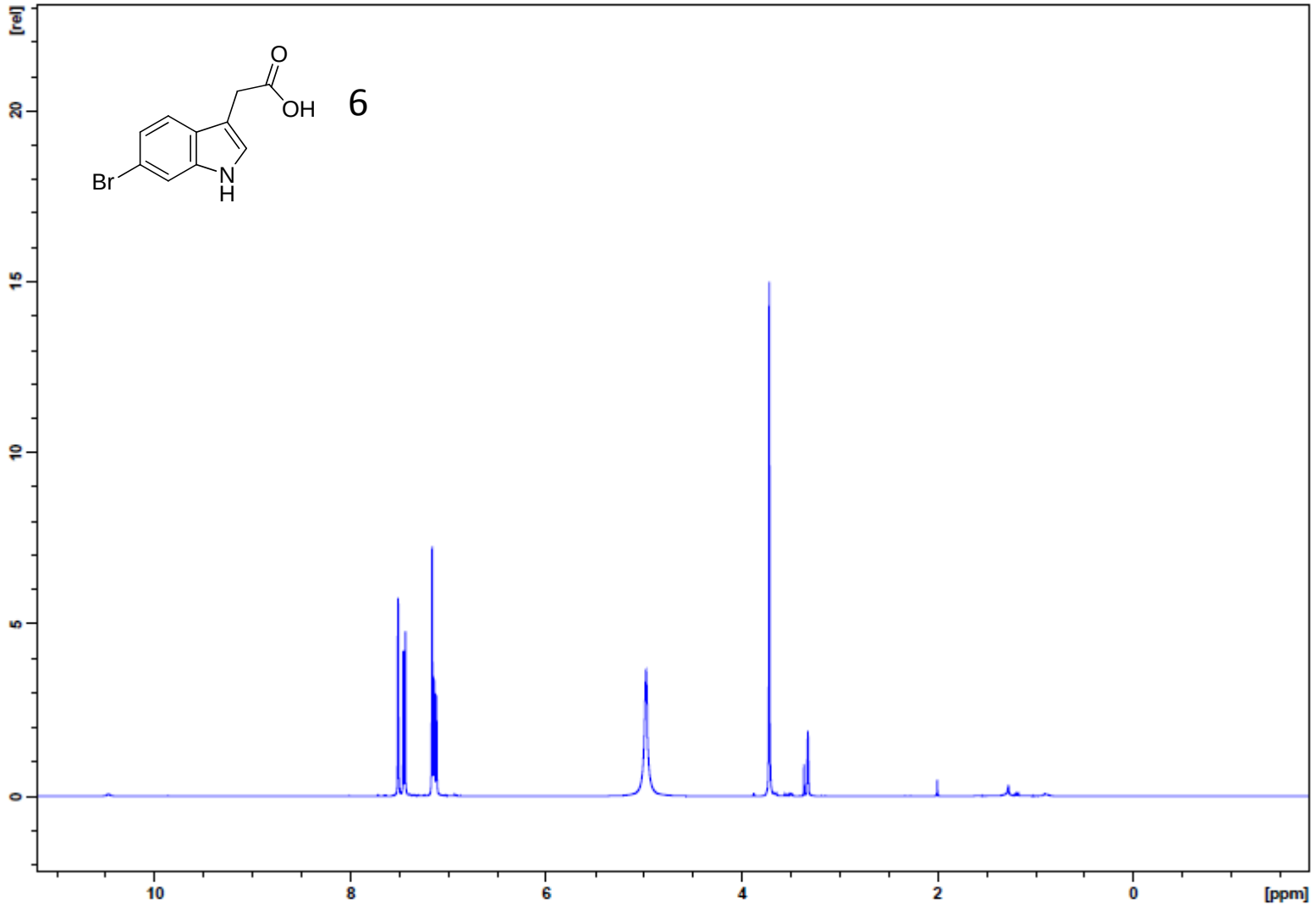
400 MHz, d_6-DMSO



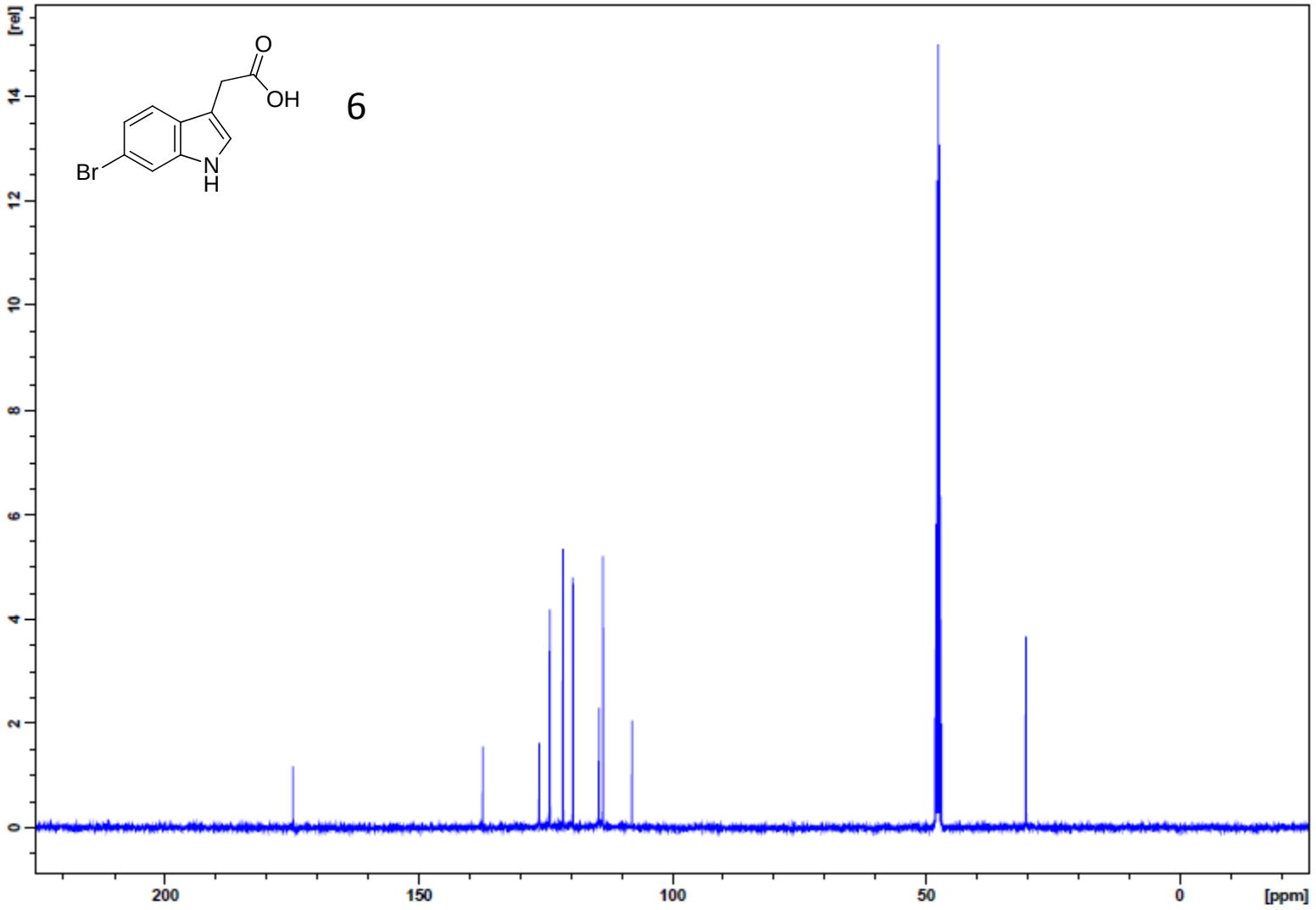
100 MHz, d_6-DMSO



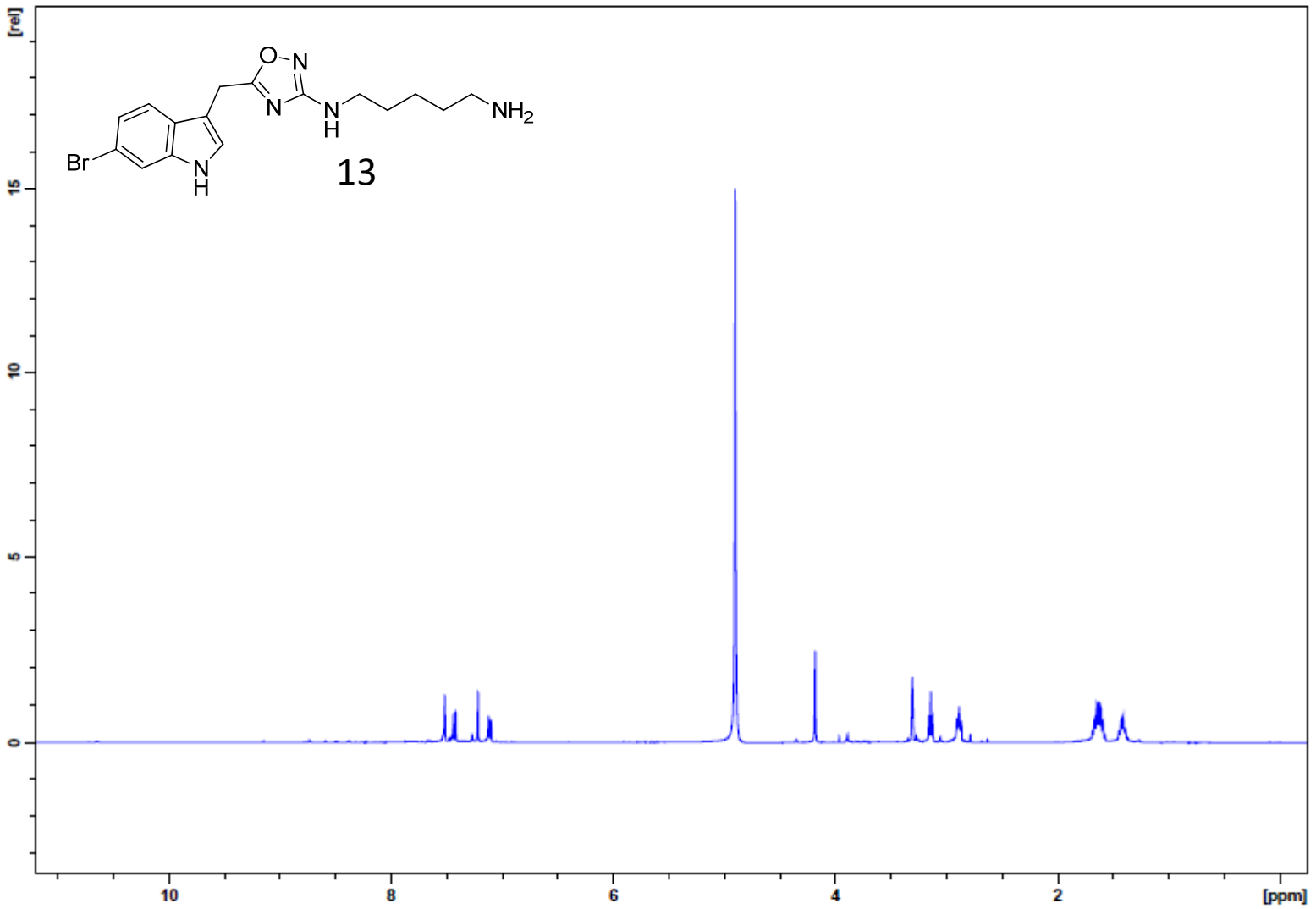
400 MHz, CD_3OD



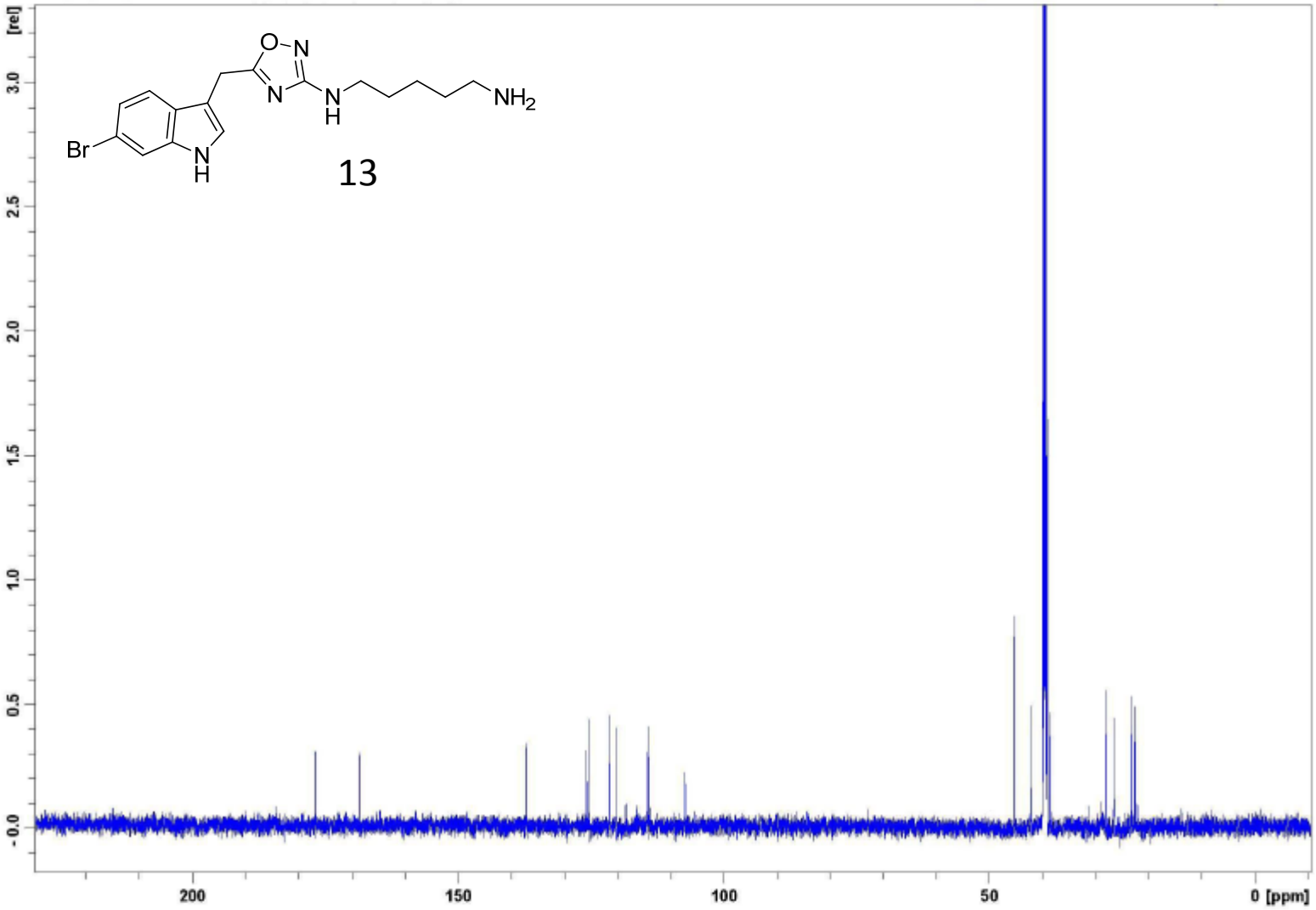
100 MHz, CD_3OD



400 MHz, CD_3OD

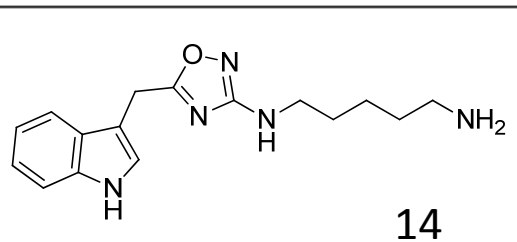


150 MHz, DMSO



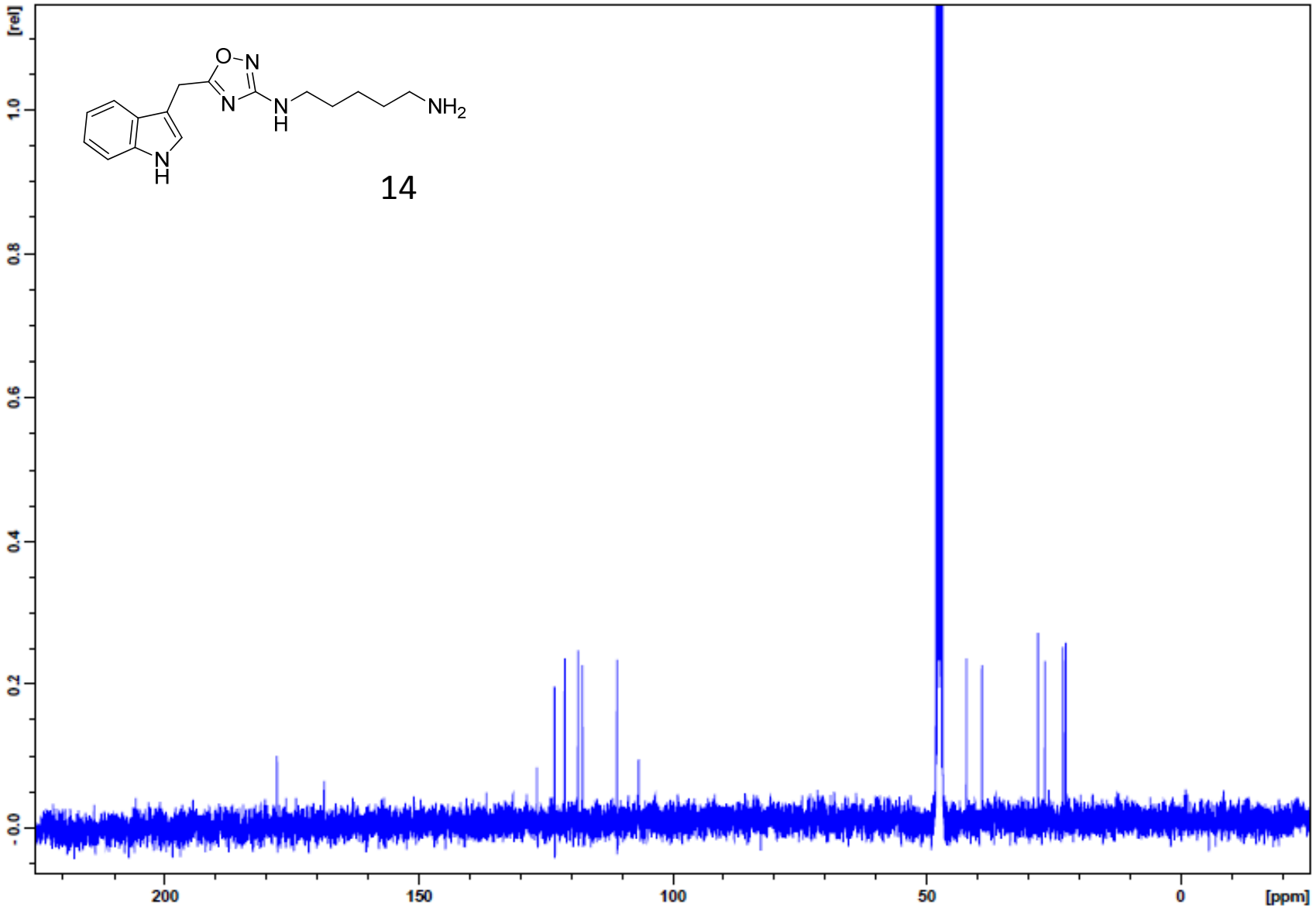
13

400 MHz, CD_3OD



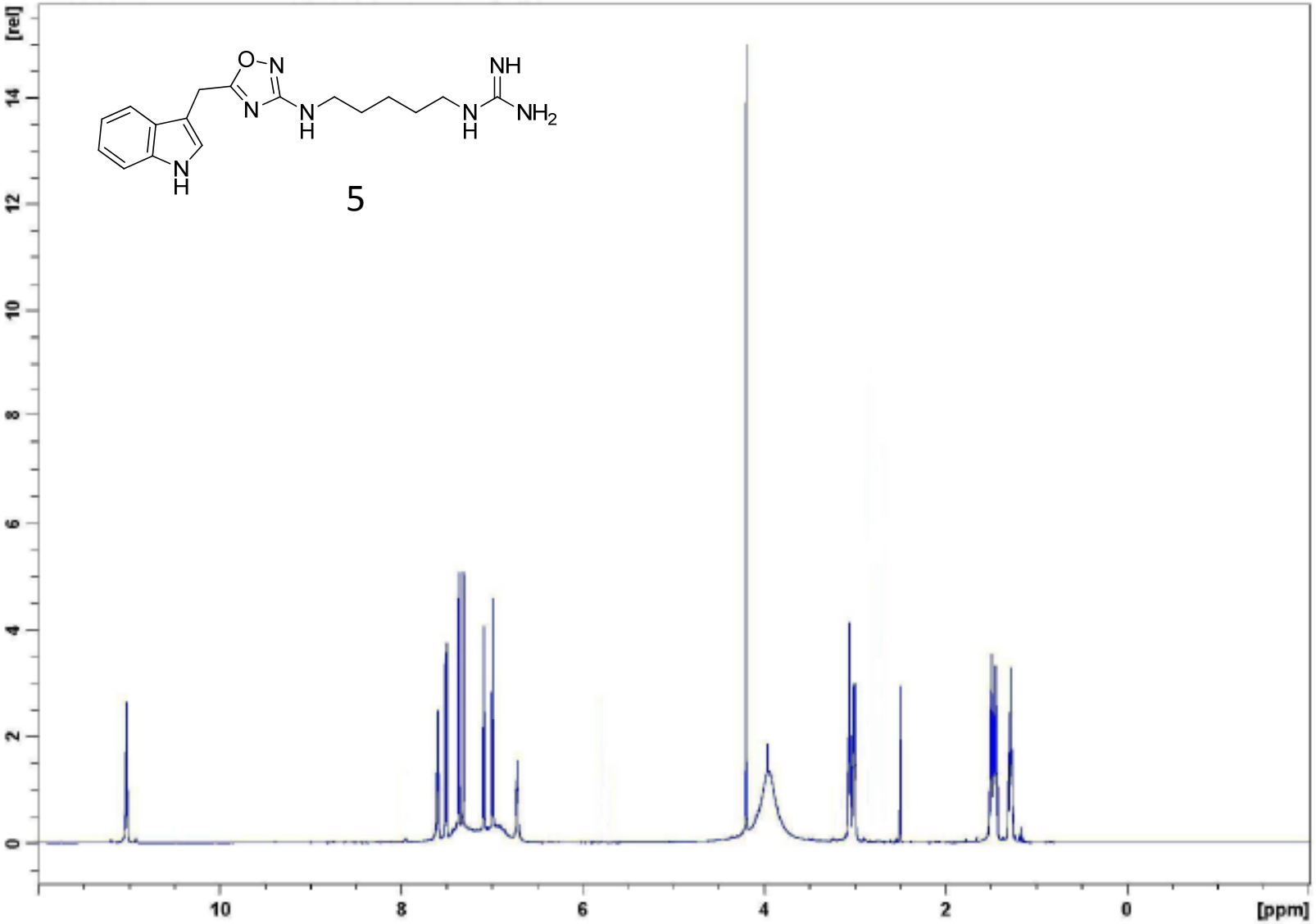
14

100 MHz, CD_3OD



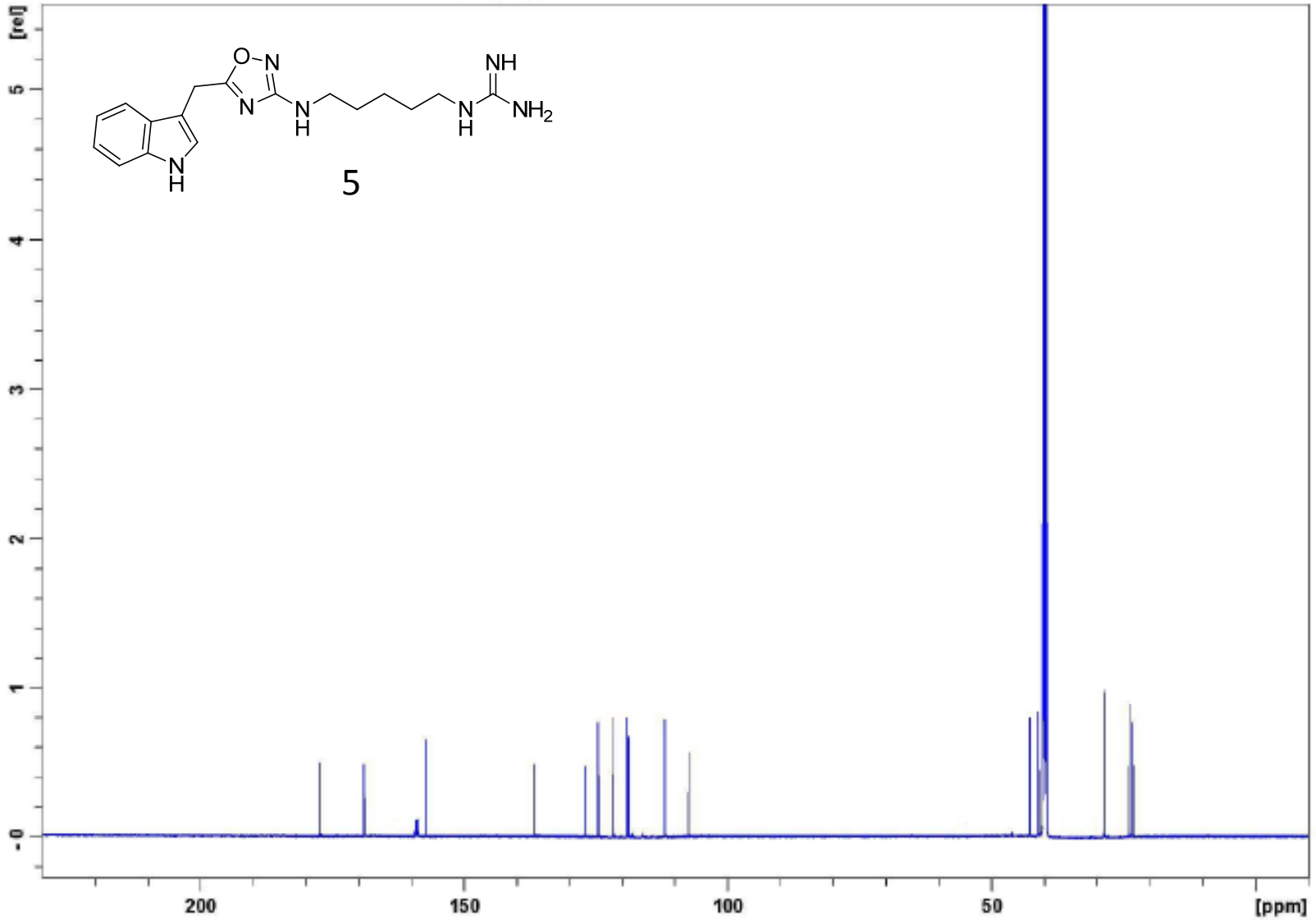
14

400 MHz, d_6-DMSO



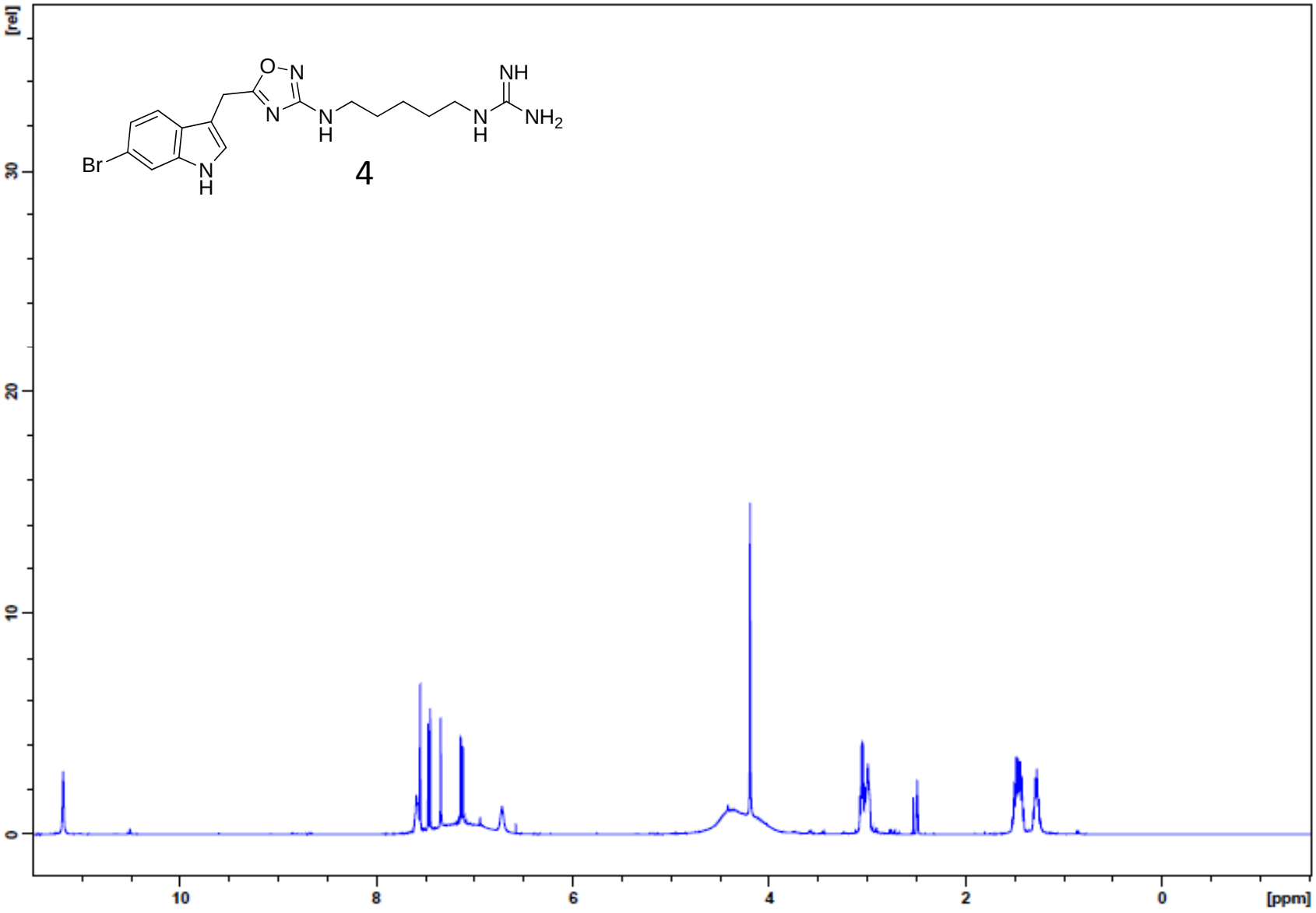
5

100 MHz, d_6-DMSO

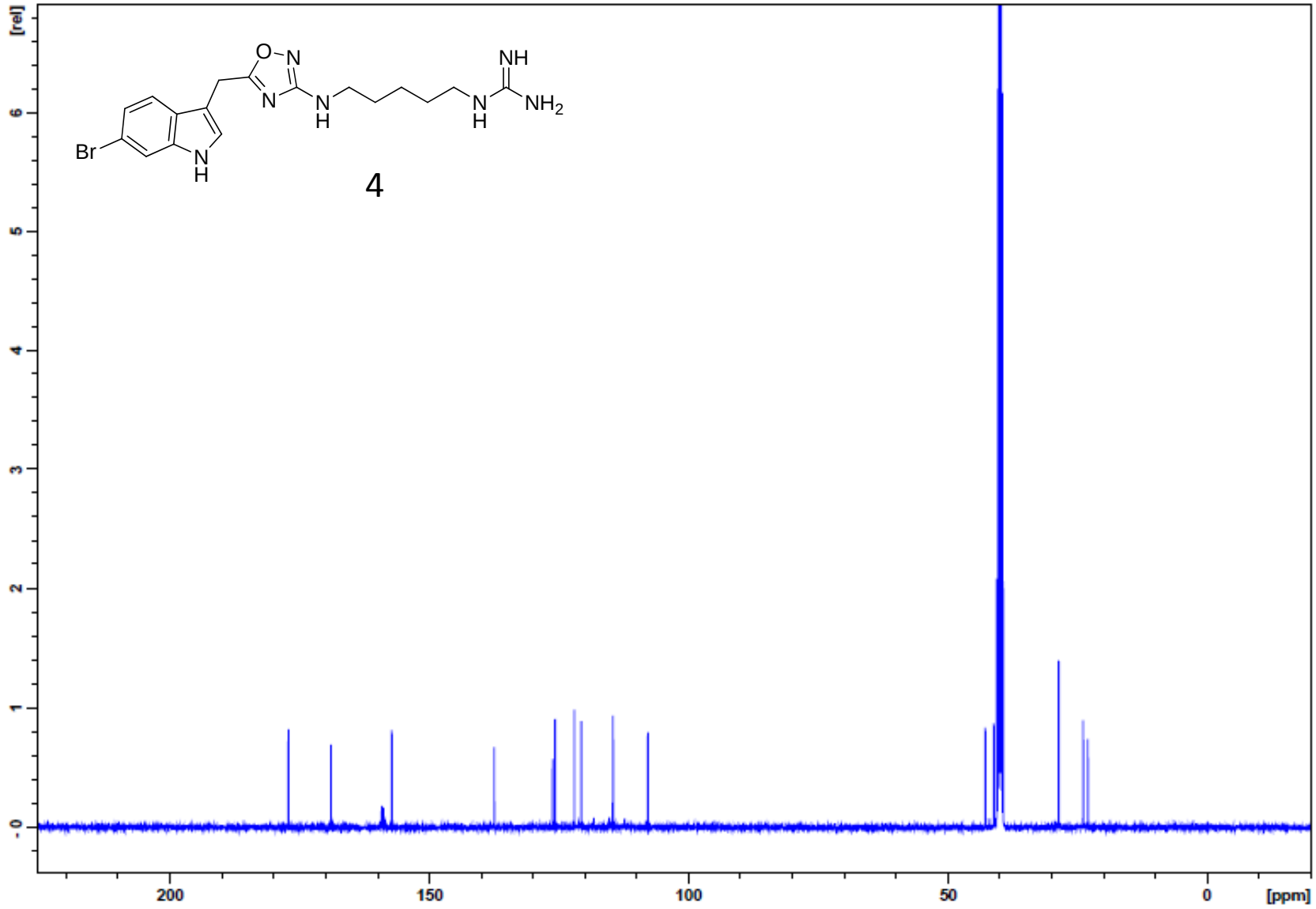


5

600 MHz, d_6-DMSO

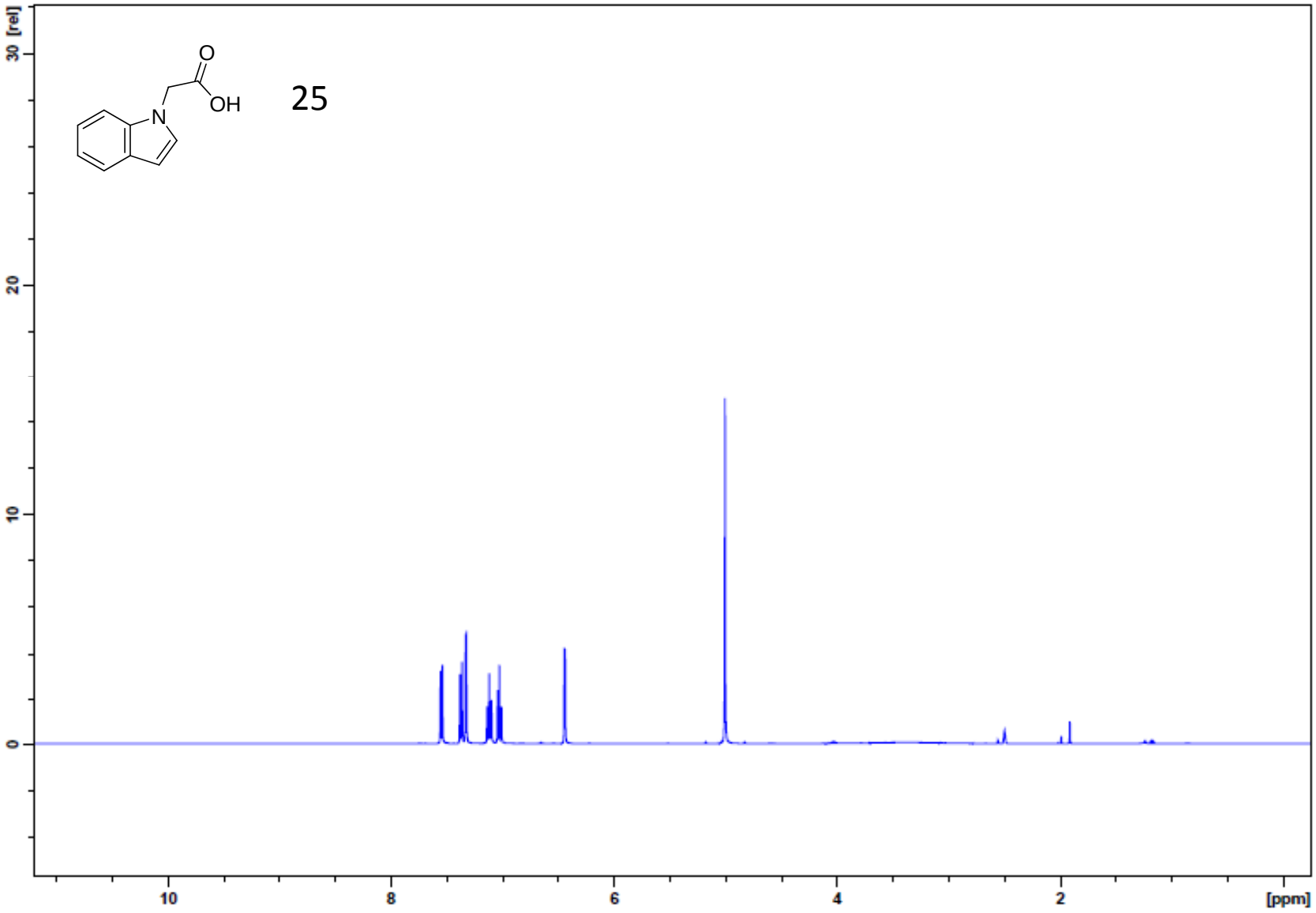


600 MHz, d_6-DMSO

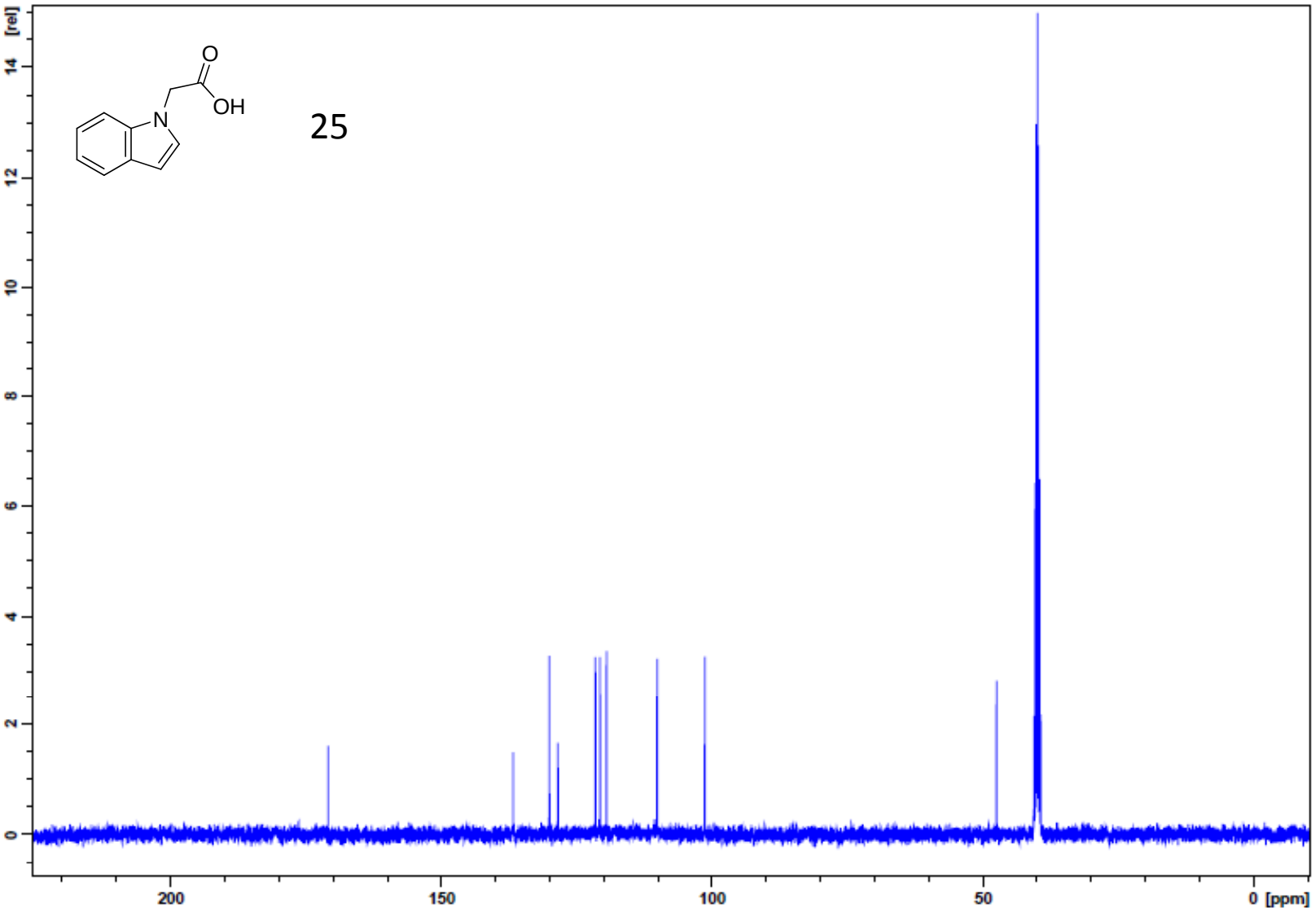


4

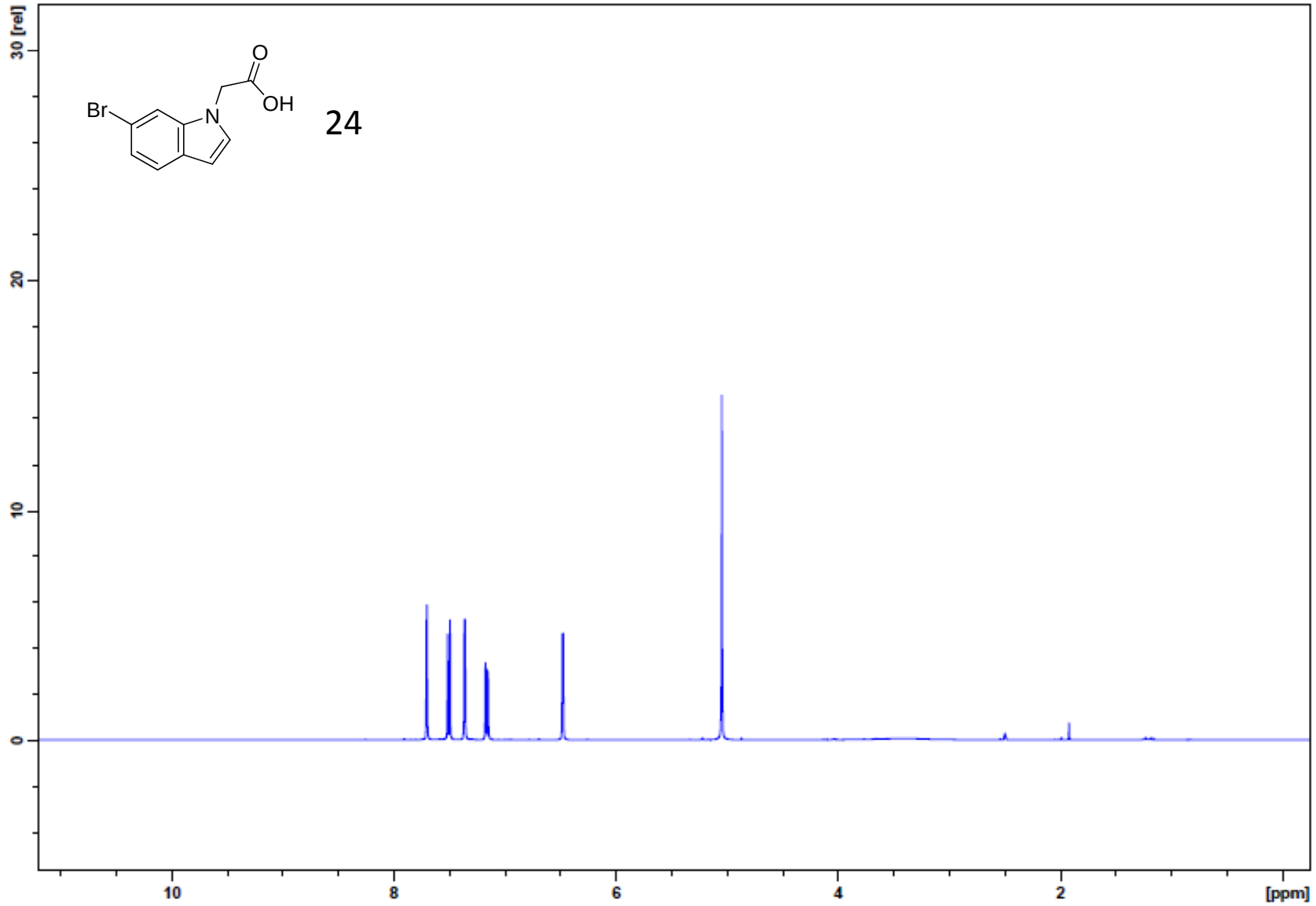
400 MHz, d_6-DMSO



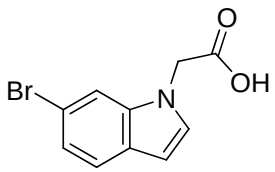
400 MHz, d_6-DMSO



400 MHz, d_6-DMSO

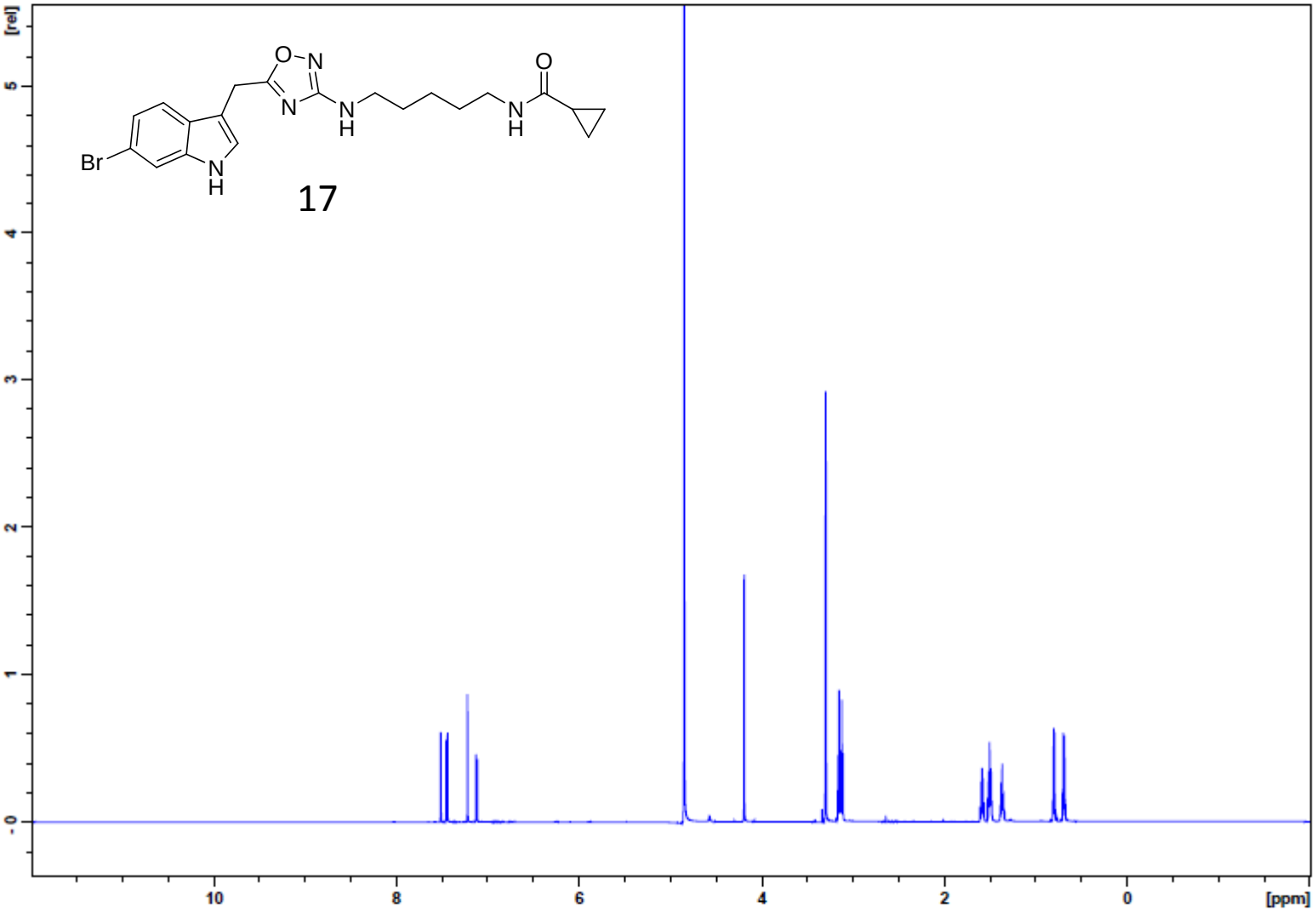


100 MHz, d_6-DMSO



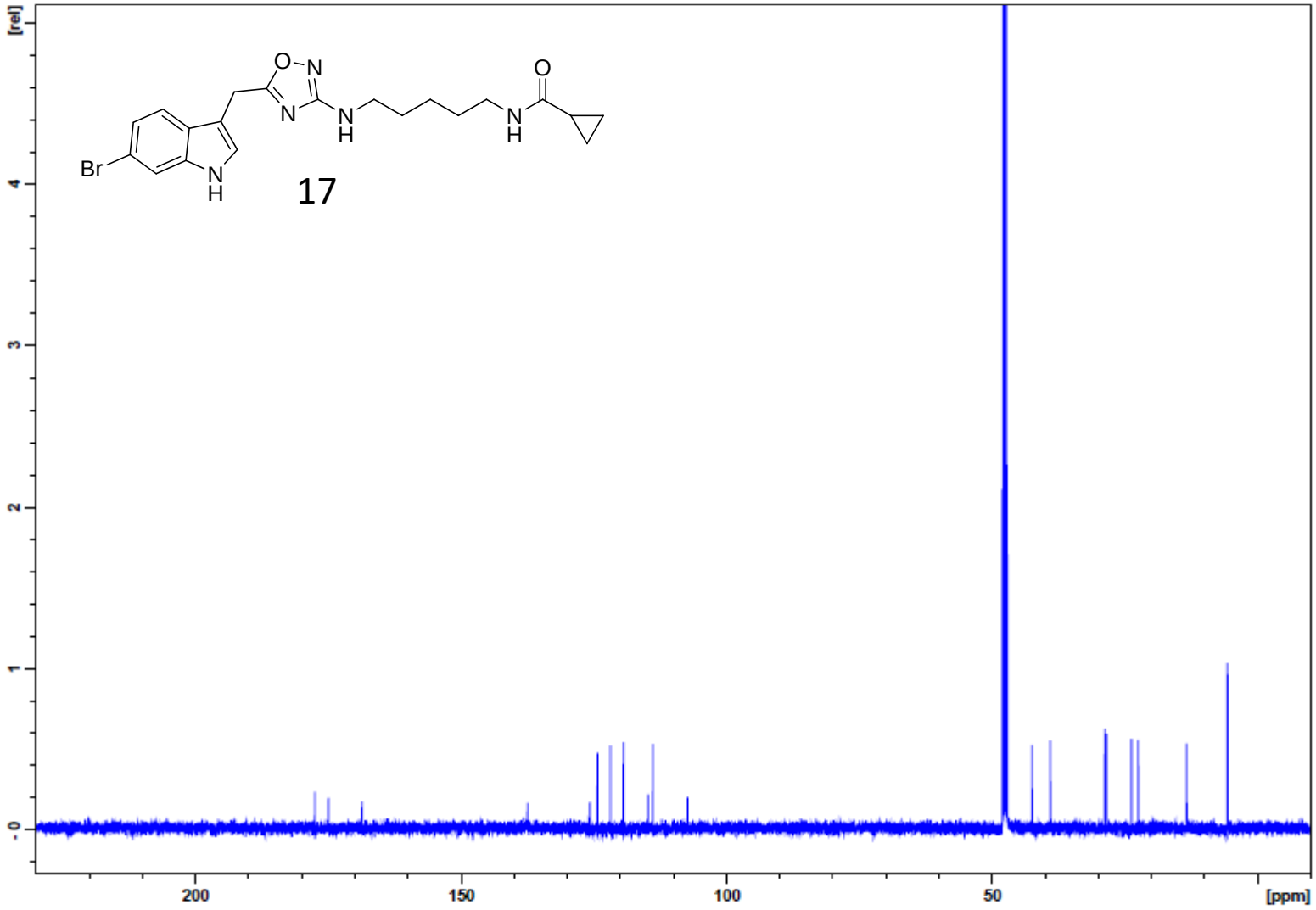
24

600 MHz, CD_3OD



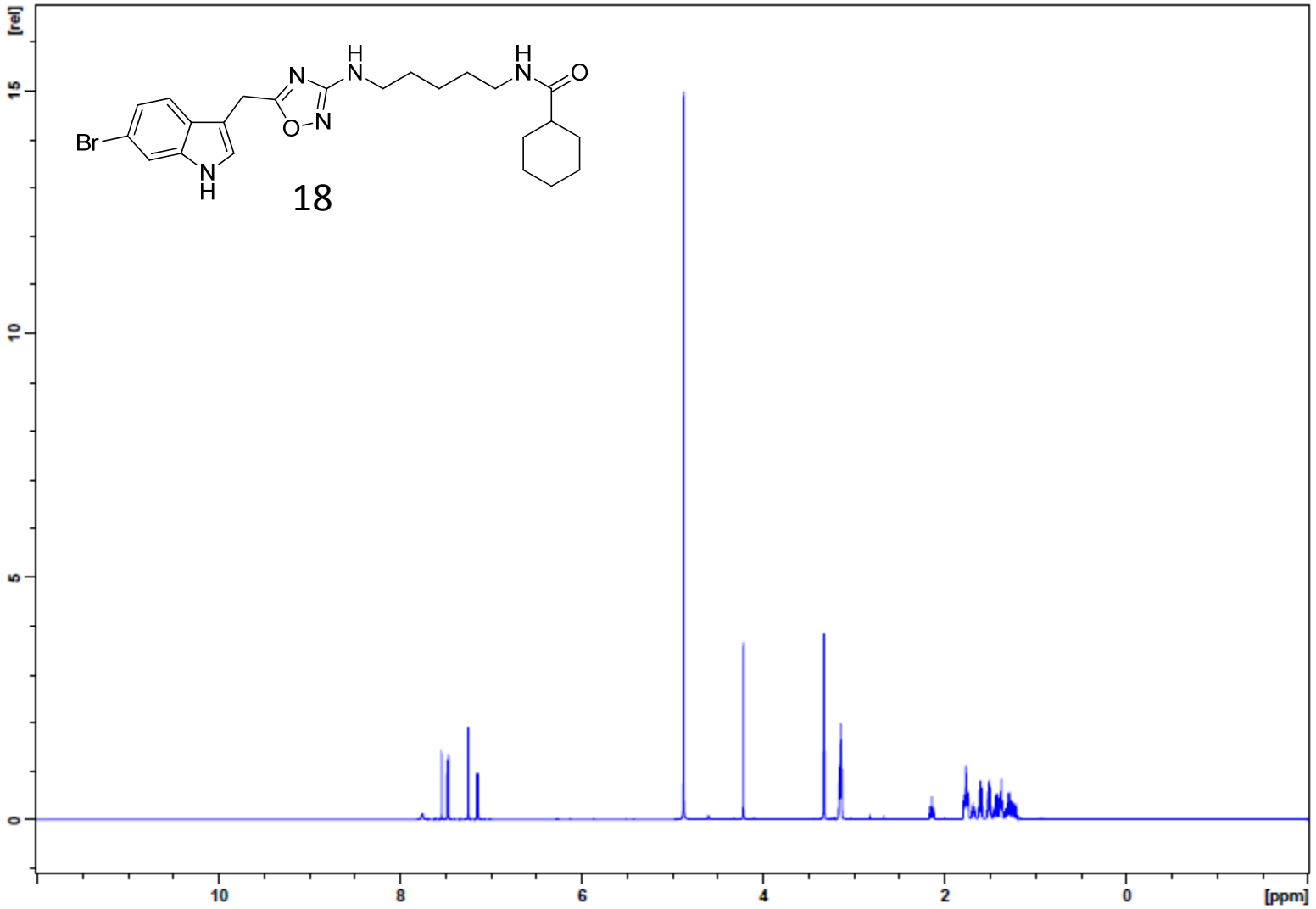
17

600 MHz, CD_3OD

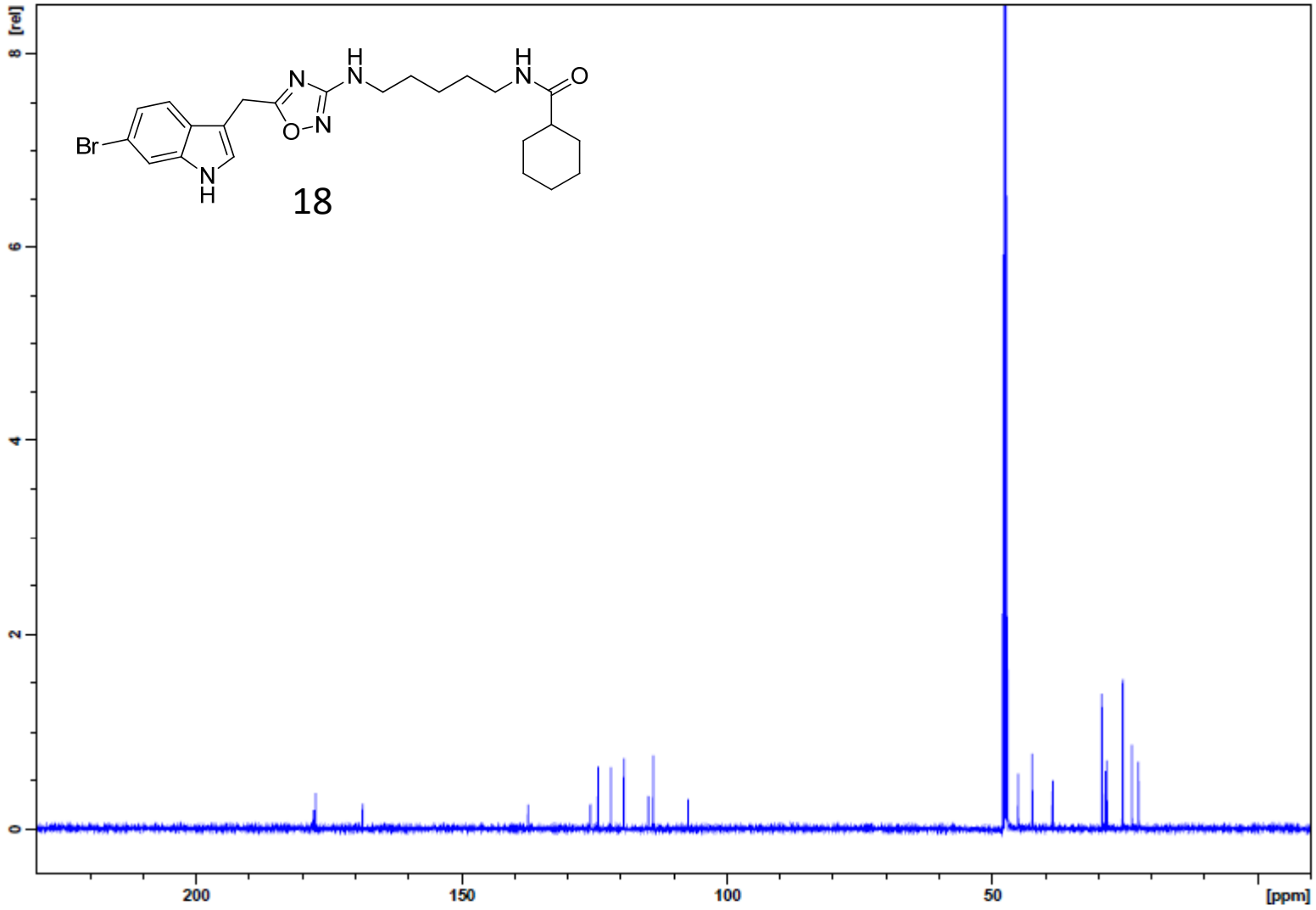


17

600 MHz, CD_3OD

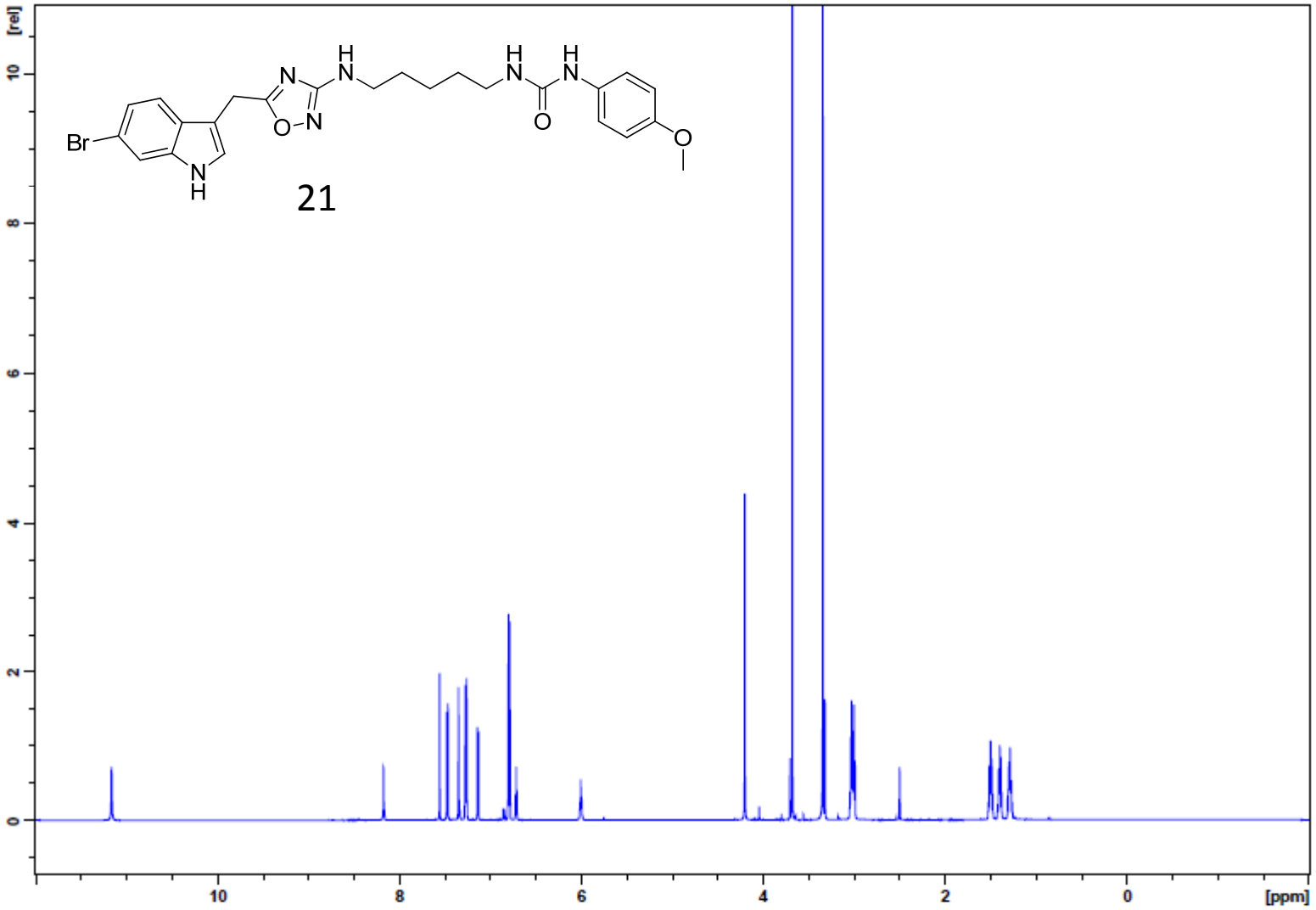


600 MHz, CD_3OD



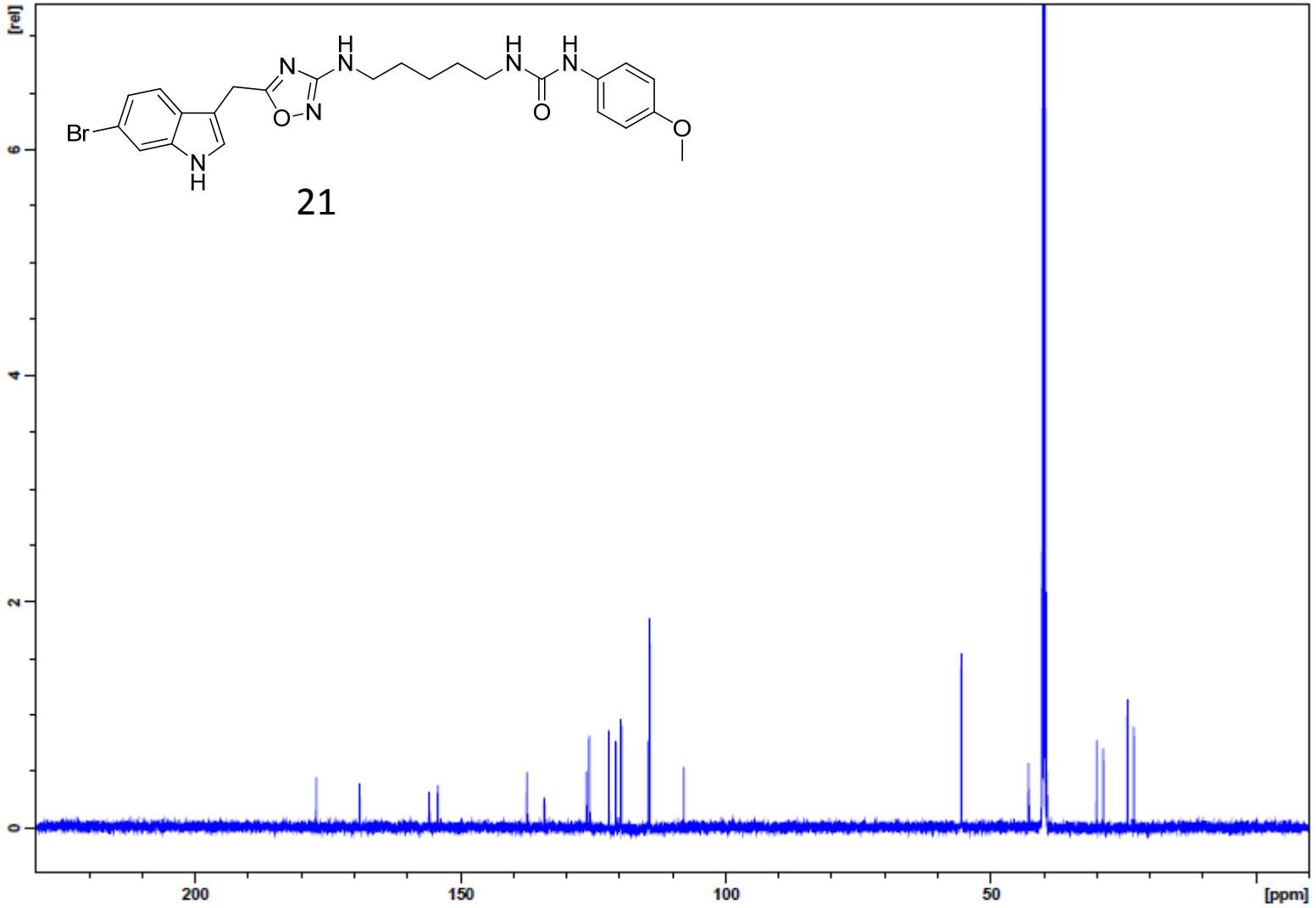
18

600 MHz, d_6-DMSO



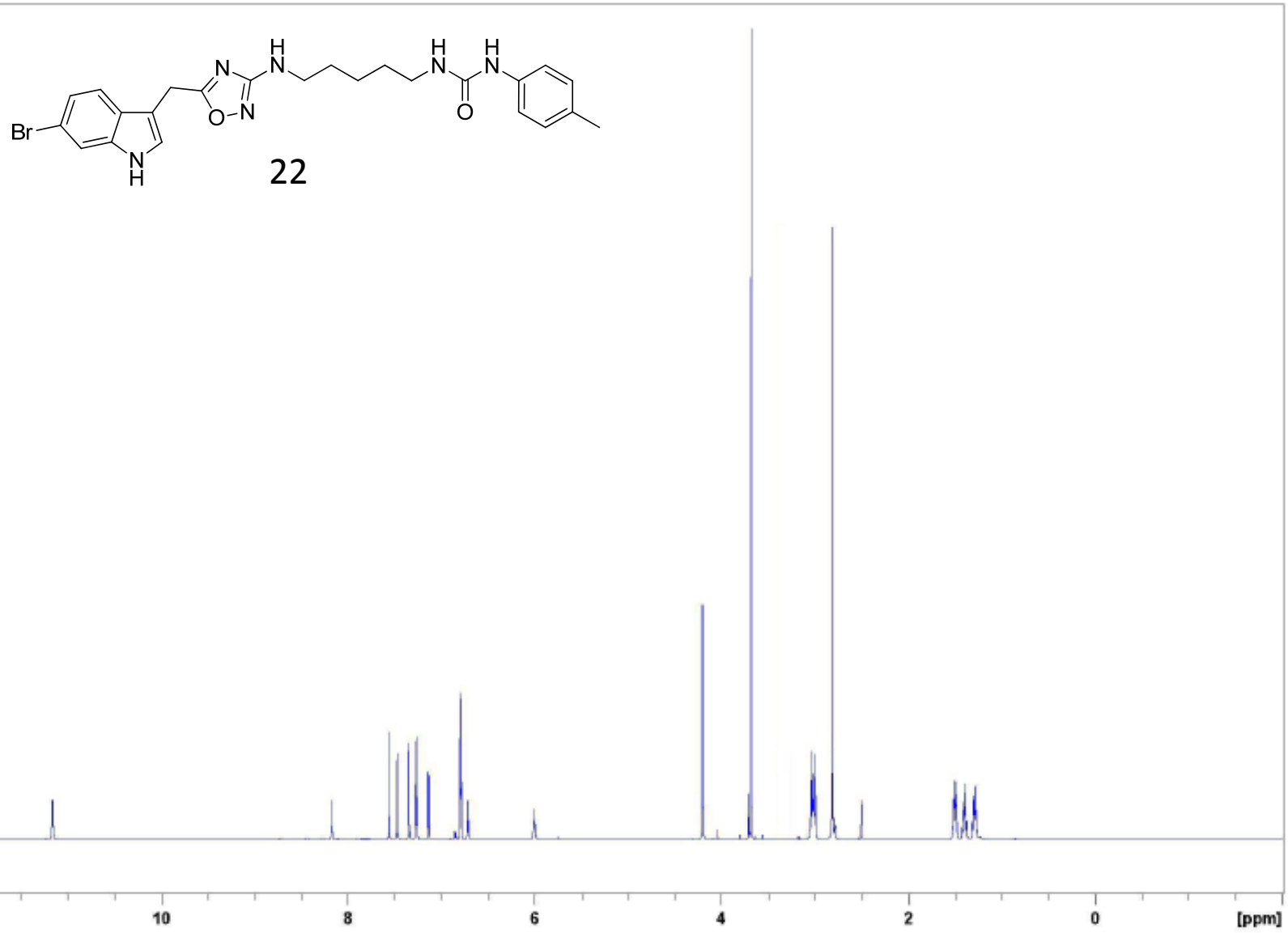
21

150 MHz, d_6-DMSO



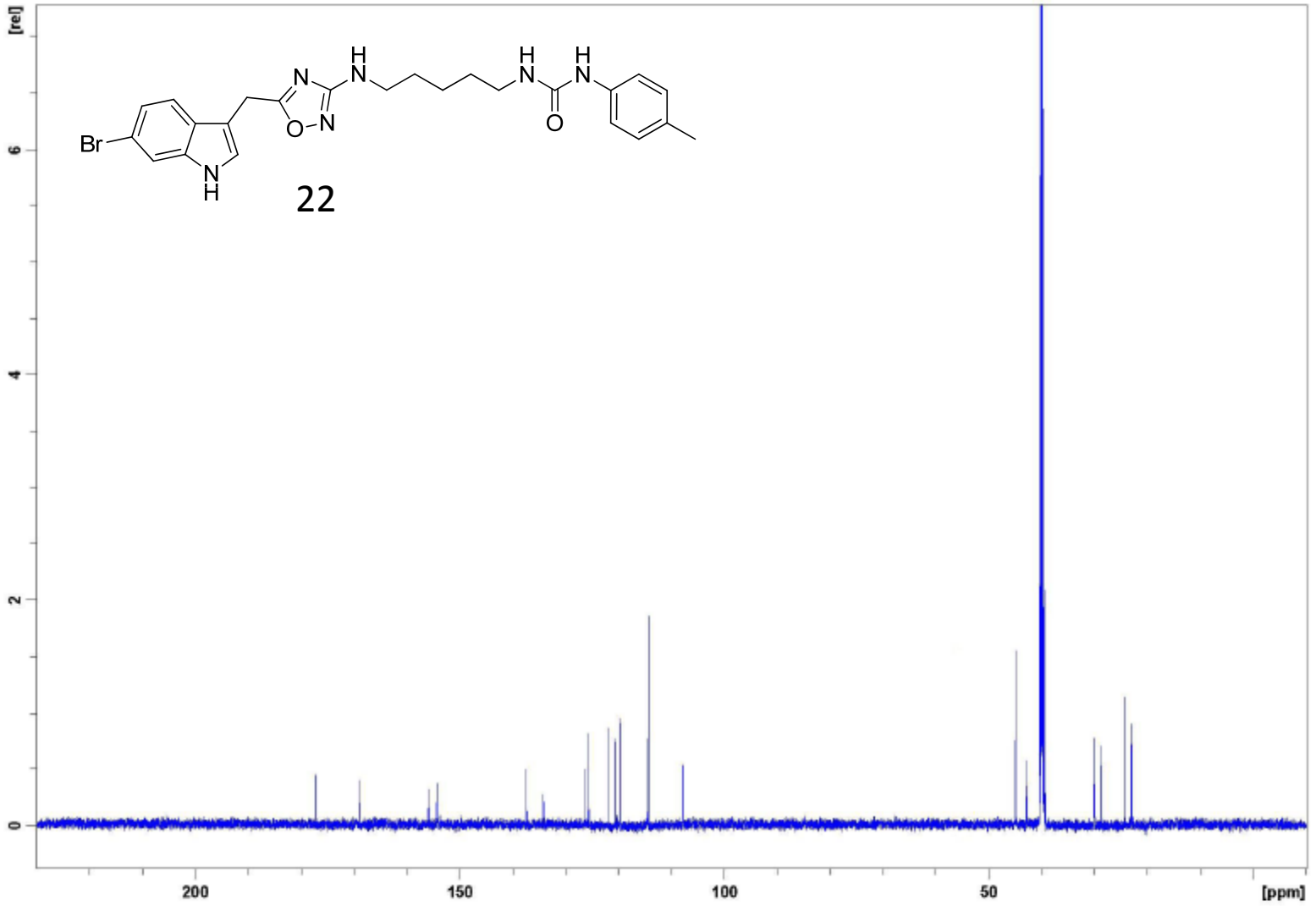
21

600 MHz, d_6-DMSO

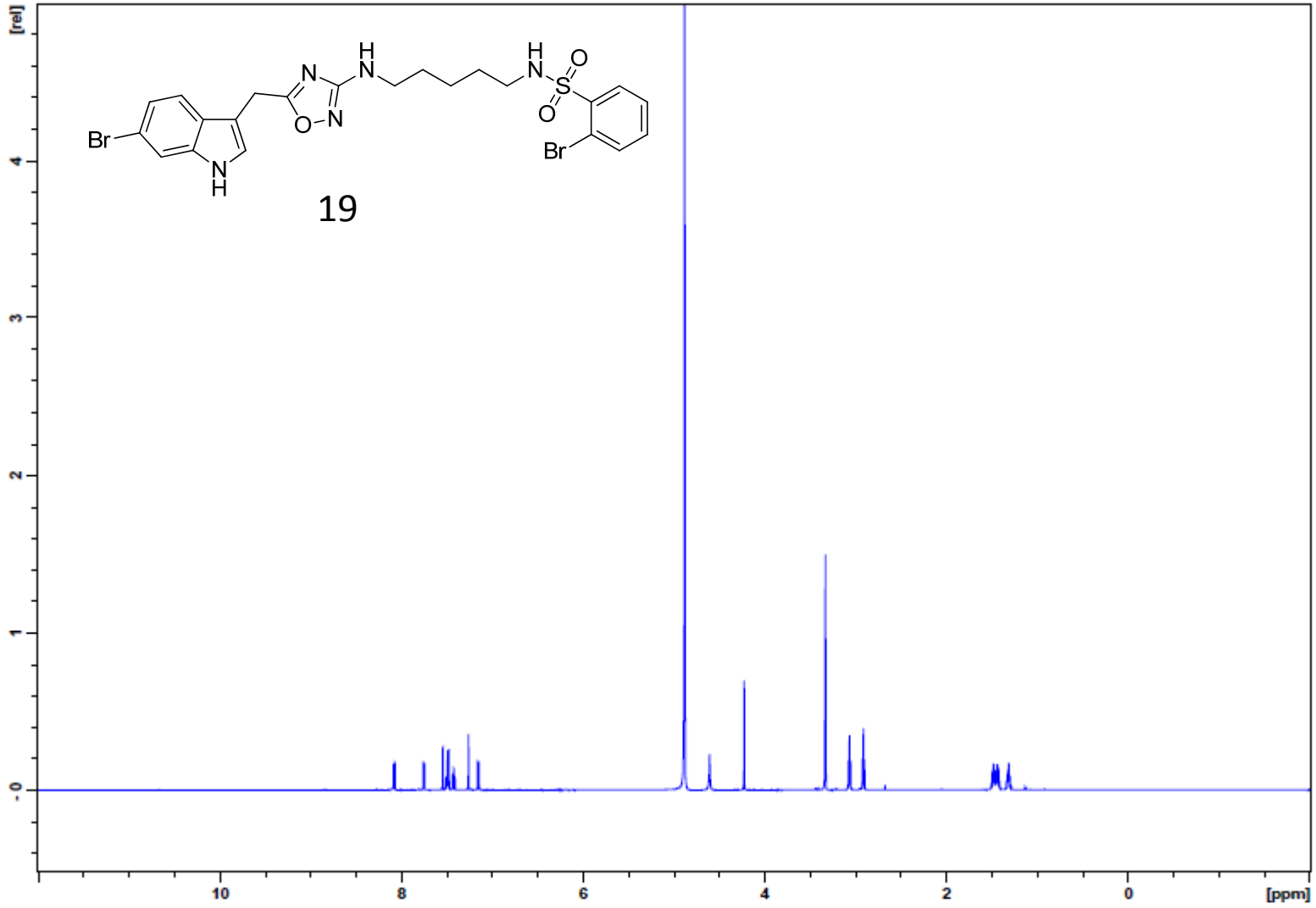


22

150 MHz, d_6-DMSO

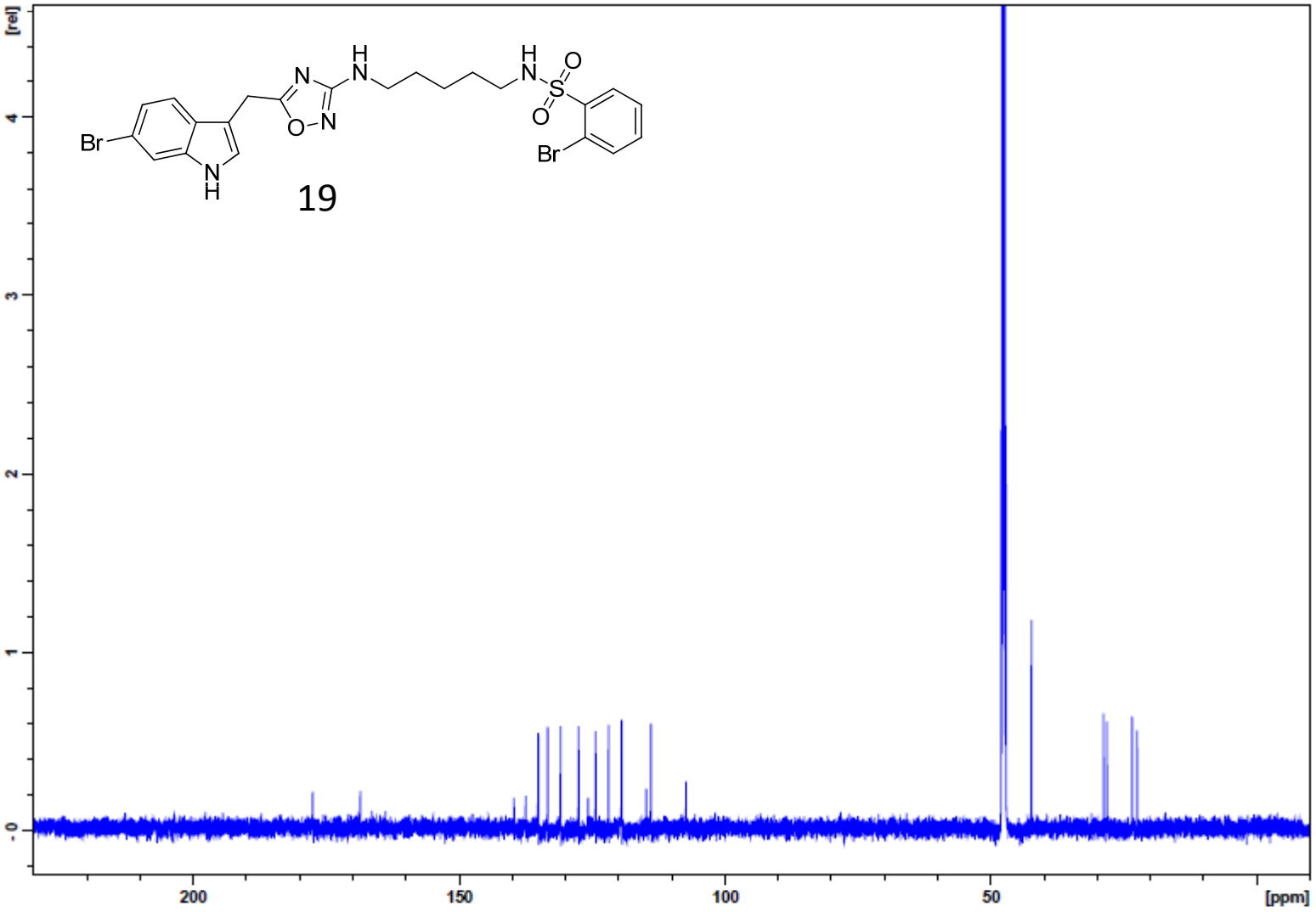


600 MHz, CD_3OD



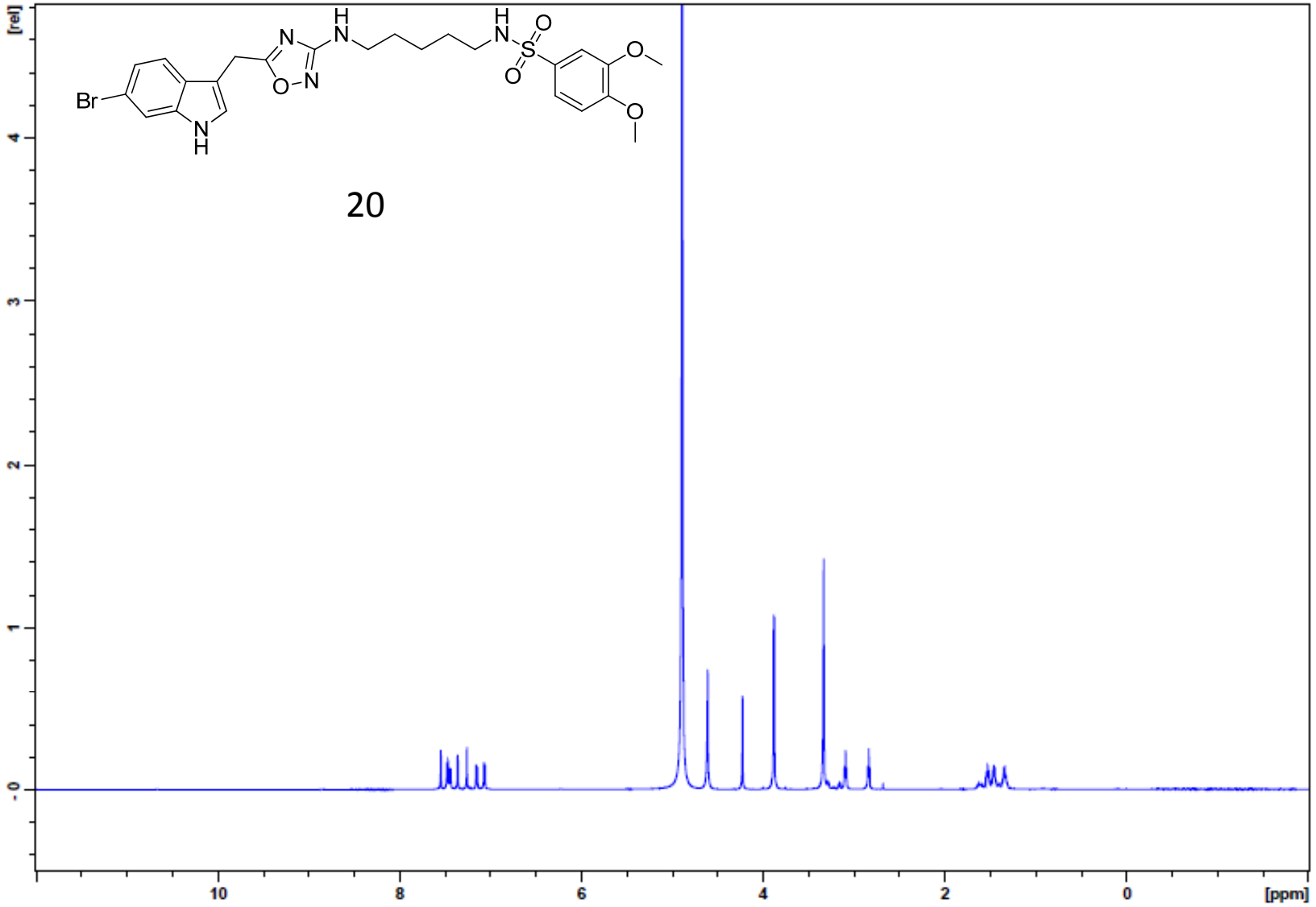
19

150 MHz, CD_3OD



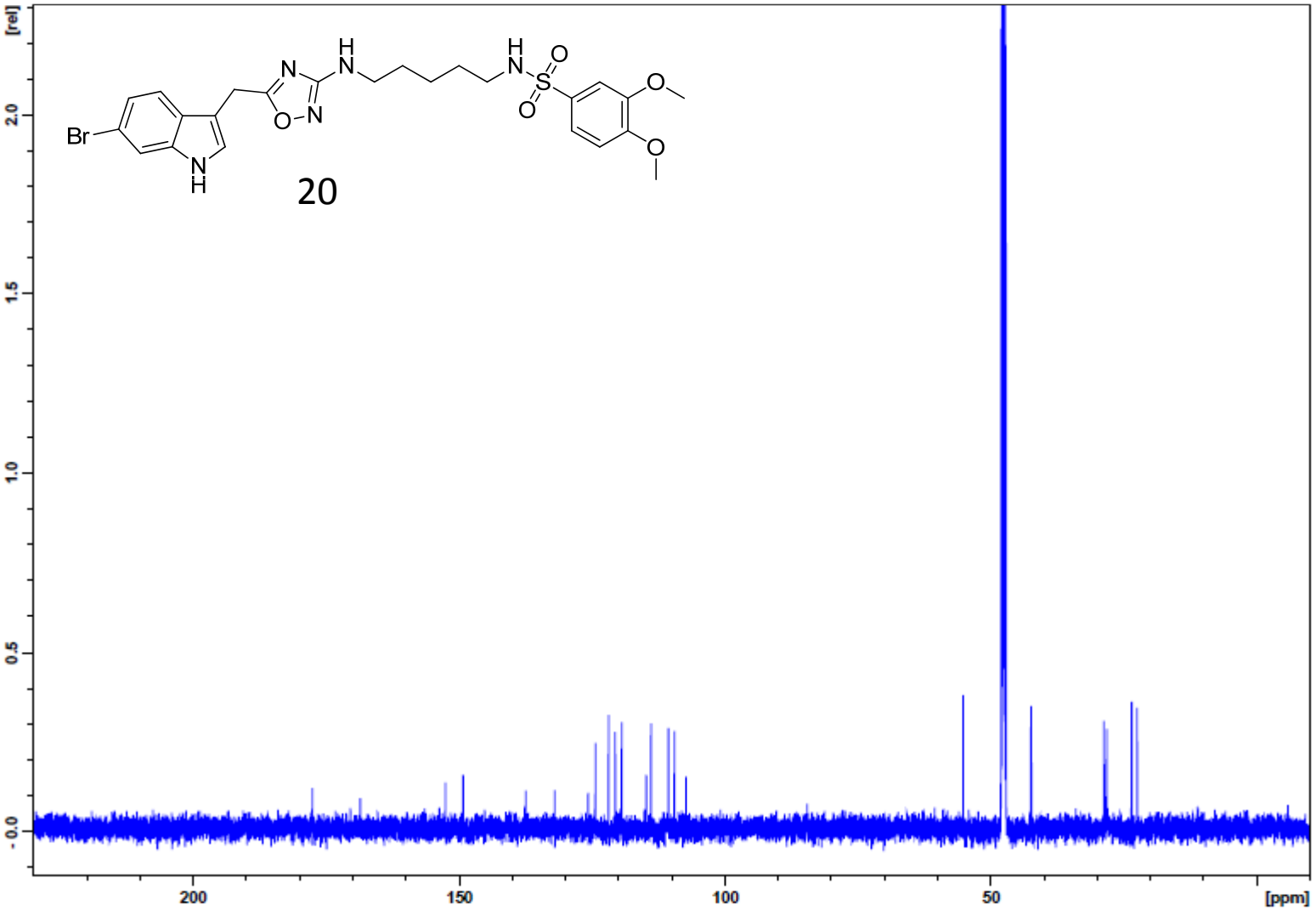
19

600 MHz, CD_3OD



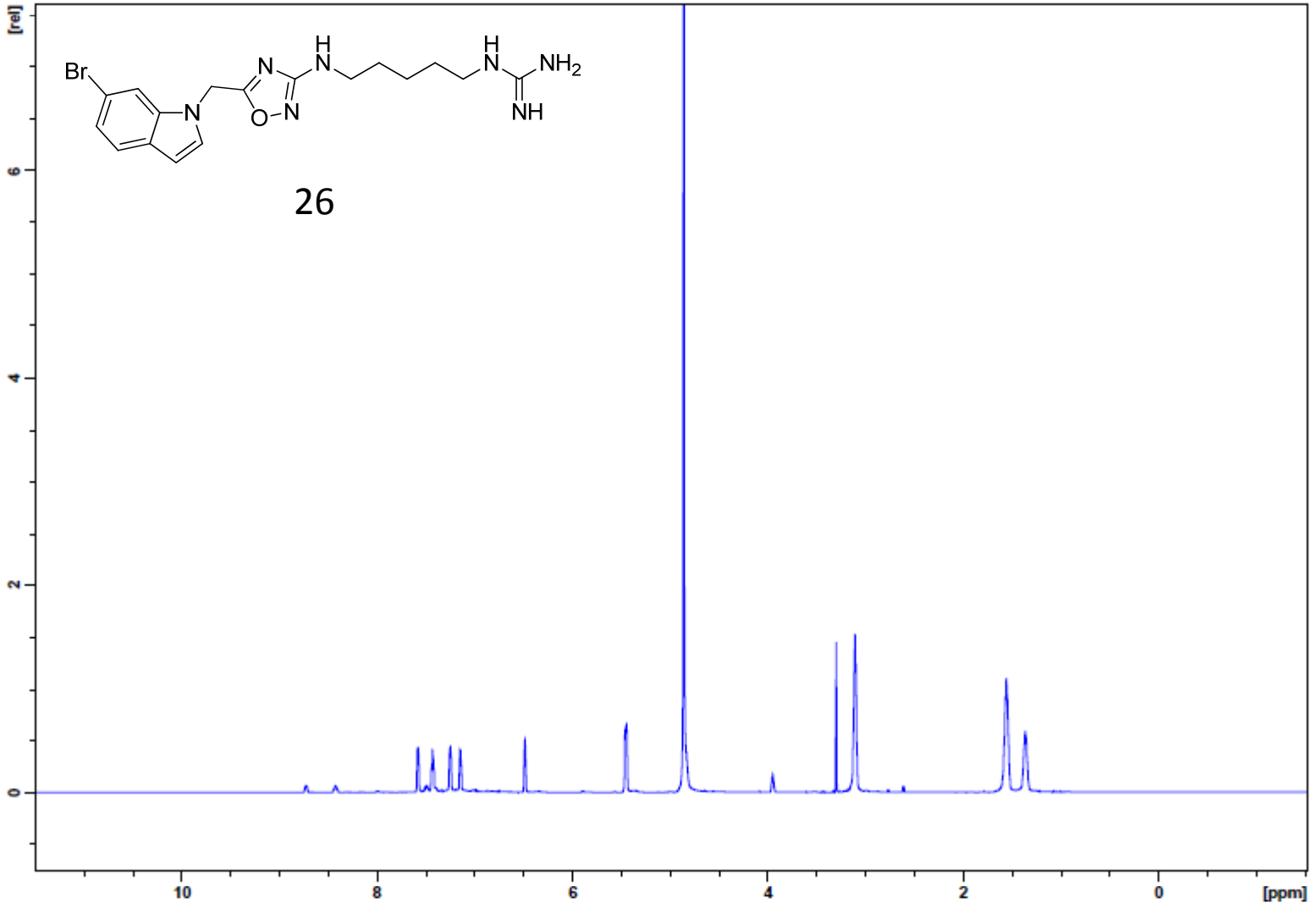
20

150 MHz, CD_3OD

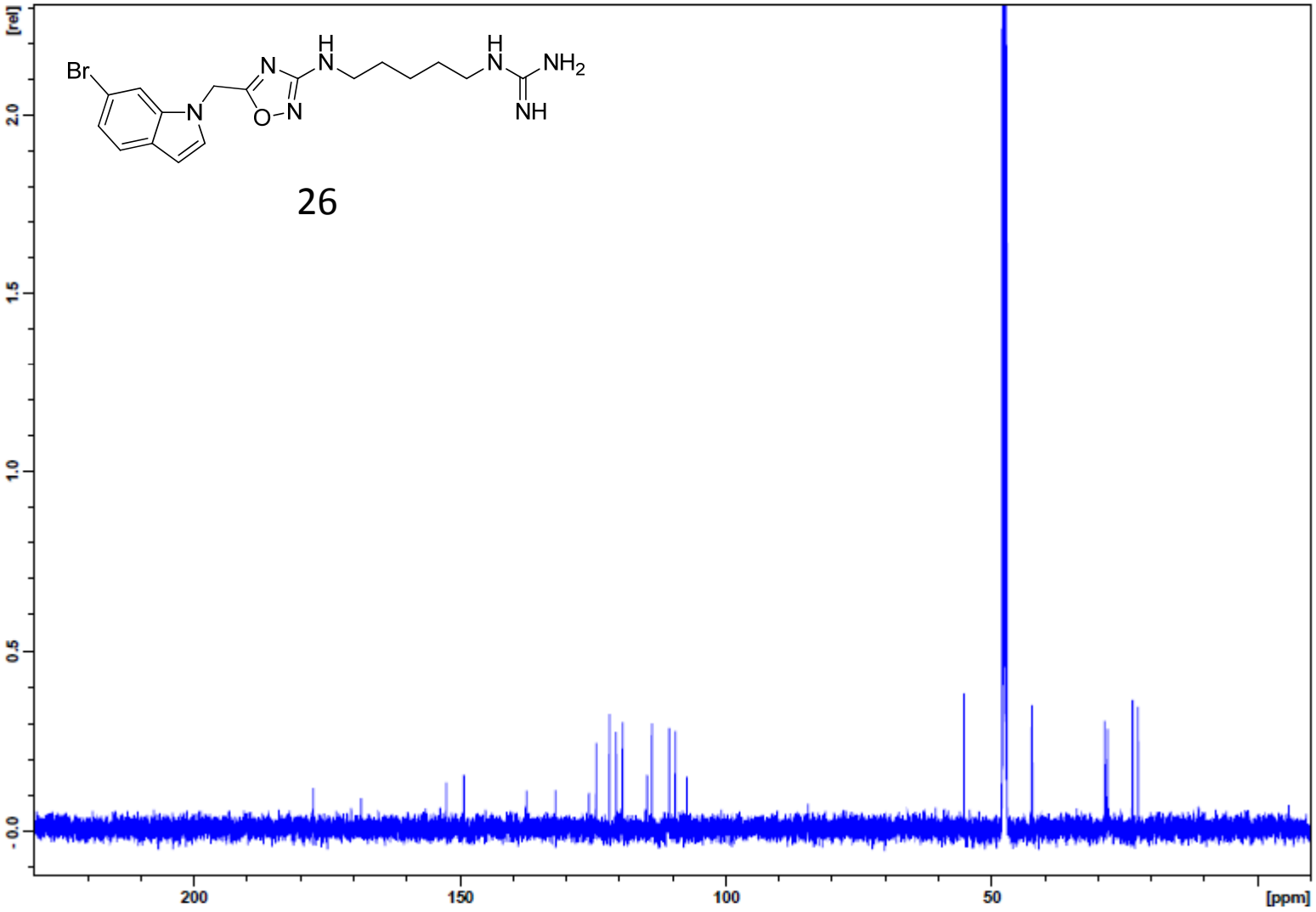


20

600 MHz, CD_3OD

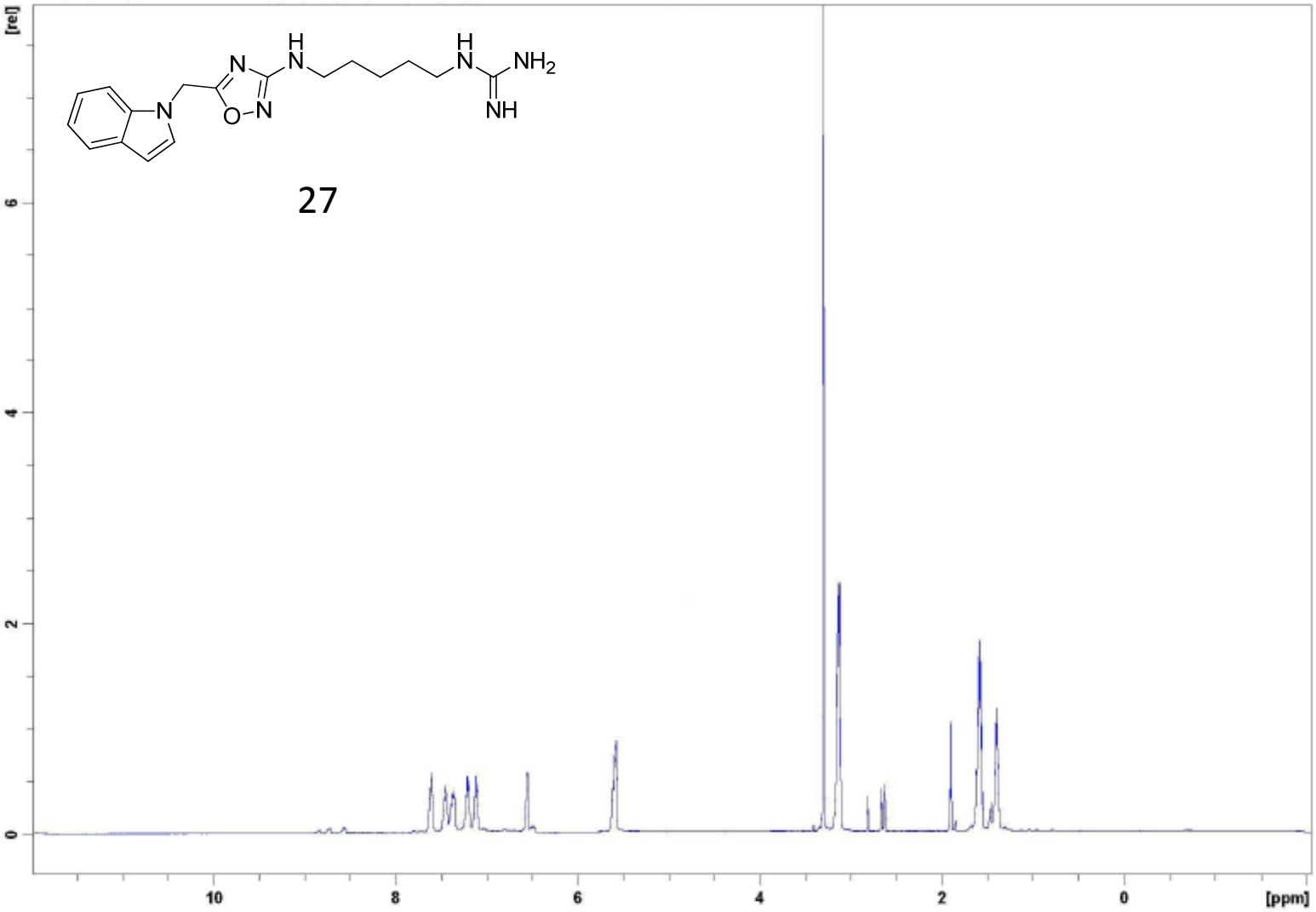


150 MHz, CD_3OD



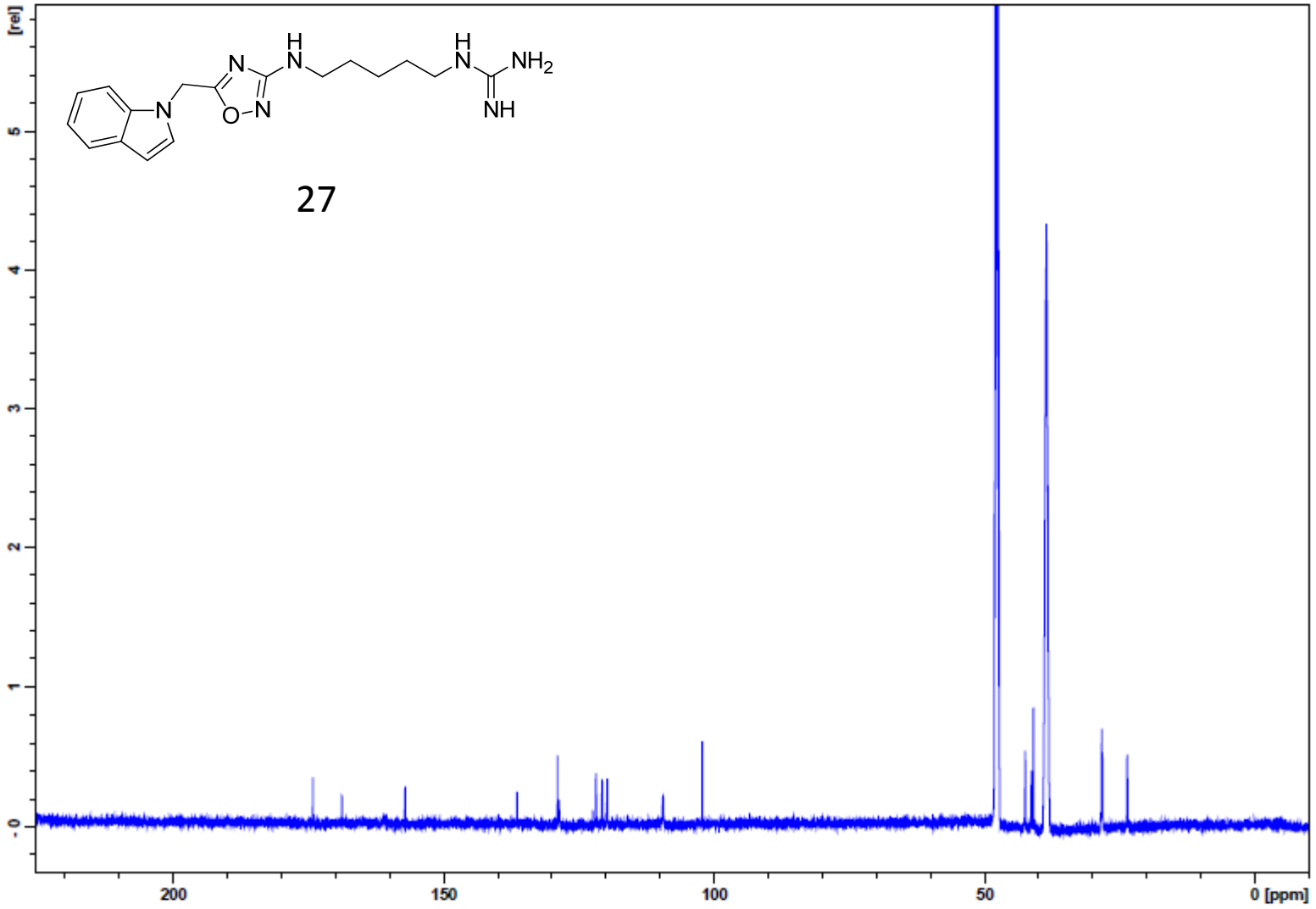
26

600 MHz, CD_3OD



27

150 MHz, CD_3OD



27