Supporting Information

Synthesis and Biological Evaluation of an Indazole-Based Selective Protein Arginine Deiminase 4 (PAD4) Inhibitor

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General Experimental Methods: Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Reaction progress was monitored on TLC EMD 60 F_{254} 0.25 mm silica plates or by UV/MS on an Agilent 6120 Quadrupole HPLC/MSD and Agilent 1260 Infinity. Organic extracts were dried over $MgSO_4$ and then concentrated under reduced pressure. Flash-column chromatography was performed on Teledyne Isco automated chromatography system using either normal phase RediSep Rf silica gel cartridges or reverse phase RediSep Rf 50g C18 silica gel cartridges. Product yields are not optimized. Methanol-d_4, chloroform-*d*, and dimethylsulfoxide-d_6 were obtained from commercial sources and used as received. NMR chemical shifts are reported in ppm relative to $CDCl_3$ (7.26 ppm ^1H and 77.2 ppm ^{13}C), CD_3OD (3.31, 4.78 ppm ^1H and 49.0 ppm ^{13}C), or C_2D_6OS (2.50 ppm ^1H and 39.5 ppm ^{13}C). HRMS were obtained on an Agilent 6550 iFunnel QTOF LCMS. Enzymatic assays were carried out on a microtiter plate reader (Envision 2100, Perkin Elmer).

Detailed Synthetic Procedures

Preparation of Boc-protected Indole Intermediate 5[1]

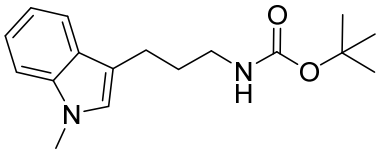
To a flame-dried, N_2 flushed round bottom flask equipped with a stir bar was added **1** (1.00 g, 5.31 mmol) in 50 mL of dry THF. The solution was stirred and cooled in an ice bath. To this solution was slowly added lithium aluminum hydride in THF (21.3 mL, 21.3 mmol). The mixture was warmed to rt and then refluxed for 2 h. After the reaction was complete, the reaction mixture was cooled to rt, and the reaction was quenched by addition of a H_2O/ice mixture followed by aqueous 15% NaOH and subsequently water. The mixture was stirred at rt for 1 h, at which point a salt had formed. The solid was filtered off, rinsed with diethyl ether, and the solvent was removed under reduced pressure. The crude product was used in the next step without further purification.

The crude amine (830 mg, 4.76 mmol) was dissolved in THF (20 mL) and cooled to 0 °C. Triethylamine (1.33 mL, 9.53 mmol) and Boc anhydride (1.14 g, 5.24 mmol) were added to the reaction mixture. The reaction mixture was stirred and allowed to warm to rt. After 1 h, the reaction mixture was washed with water, extracted with ethyl acetate, dried and concentrated. The residue was purified by column chromatography (silica gel, 0-100% ethyl acetate in hexane) to yield Boc-protected indole intermediate **3** (1.28 g, 97.9%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.51 (dt, *J* = 8.0, 1.0 Hz, 1H), 7.31 (d, *J* = 8.1 Hz, 1H), 7.06 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 7.01 (s, 1H), 6.98 (ddd, *J* = 8.0, 7.0, 1.1 Hz, 1H), 3.10 (t, *J* = 7.1 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 1.85 (p, *J* = 7.3 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 158.6, 138.2, 128.7, 122.8, 122.1, 119.3, 119.3, 115.8, 112.1, 79.8, 41.3, 31.6, 28.8, 23.4. LRMS (*m/z*): $[M+H]^+$ calcd for $C_{16}H_{22}N_2O_2$, 275.2; found 275.2.

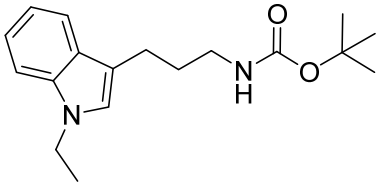
General Procedure A: Alkylation and Warhead Addition for the Preparation of Indole Intermediates 6a-6d.

N-Boc-protected indole intermediate **3** (1 equiv) was dissolved in dry DMF (0.25 M) and cooled to 0 °C in an ice bath. To this solution was added NaH (60 wt% mineral oil dispersion, 2 equiv), and the

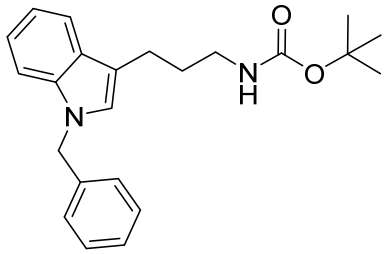
resulting suspension was stirred for 30 min to 1 h. Next, the reaction mixture was cooled to 0 °C, and the alkyl halide (3 equiv) was added dropwise, and the mixture was stirred for another 1-2 h. The reaction was quenched by addition of water. The mixture was extracted with ethyl acetate, and the combined organic extracts were washed with brine, dried, and concentrated. The crude product was purified by column chromatography (silica gel, gradient elution: 0-100% ethyl acetate in hexane) to afford intermediate **6**.



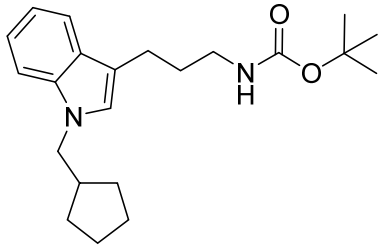
6a: General Procedure A employed using **5** (100 mg, 0.364 mmol) and CH_3I (68.7 μL, 1.09 mmol) to give **6a** (67 mg, 98%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.50 (d, *J* = 7.9 Hz, 1H), 7.26 (d, *J* = 8.2 Hz, 1H), 7.16 – 7.10 (m, 1H), 7.04 – 6.97 (m, 1H), 6.89 (s, 1H), 3.68 (s, 3H), 3.12 – 3.05 (m, 2H), 2.72 (t, *J* = 7.5 Hz, 2H), 1.83 (p, *J* = 7.3 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 158.6, 138.6, 129.2, 127.4, 122.3, 119.6, 119.4, 115.2, 110.0, 79.8, 41.2, 32.5, 31.7, 28.8, 23.2. LRMS (*m/z*): $[M+H]^+$ calc. for $C_{17}H_{24}N_2O_2$, 289.2; found 289.2.



6b: General Procedure A employed using **5** (90 mg, 0.328 mmol) and CH_2CH_3I (78.7 μL, 0.984 mmol) to give **6b** (90 mg, 91%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.51 (dt, *J* = 7.9, 0.9 Hz, 1H), 7.30 (d, *J* = 8.2 Hz, 1H), 7.11 (ddd, *J* = 8.2, 6.9, 1.2 Hz, 1H), 7.02 – 6.98 (m, 1H), 6.97 (s, 1H), 4.11 (q, *J* = 7.3 Hz, 2H), 3.09 (t, *J* = 6.7 Hz, 2H), 2.74 (t, *J* = 7.5 Hz, 2H), 1.85 (p, *J* = 7.3 Hz, 2H), 1.43 (s, 9H), 1.37 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 158.6, 137.6, 129.4, 125.7, 122.2, 119.7, 119.4, 115.4, 110.1, 79.8, 41.5, 41.4, 31.7, 28.8, 23.3, 15.9. LRMS (*m/z*): $[M+H]^+$ calc. for $C_{18}H_{26}N_2O_2$, 303.2; found 303.2.



6c: General Procedure A employed using **5** (90 mg, 0.328 mmol) and BnBr (117 μL, 0.984 mmol) to give **6c** (62 mg, 72%). The reaction mixture was heated to 55 °C for 1 h before workup. ^{1}H NMR (500 MHz, CD_3OD) δ 7.53 (d, *J* = 7.8 Hz, 1H), 7.26 – 7.16 (m, 4H), 7.11 – 7.04 (m, 3H), 7.03 – 6.97 (m, 2H), 5.25 (s, 2H), 3.10 (t, *J* = 7.0 Hz, 2H), 2.75 (t, *J* = 7.5 Hz, 2H), 1.91 – 1.80 (m, 2H), 1.43 (s, 9H). ^{13}C NMR (126 MHz, CD_3OD) δ 158.6, 139.8, 138.2, 129.6, 129.5, 128.3, 127.8, 126.9, 122.5, 119.8, 119.7, 115.9, 110.7, 79.8, 50.5, 41.2, 31.6, 28.8, 23.3. LRMS (*m/z*): $[M+H]^+$ calc. for $C_{23}H_{28}N_2O_2$, 365.2; obs. 365.2.

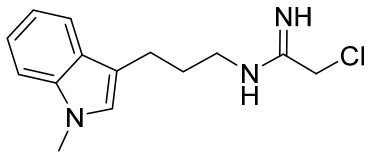


6d: General Procedure A employed using **5** (114 mg, 0.**416** mmol) and cyclopentylmethyl bromide (236 μL, 1.25 mmol) to give **6d** (100 mg, 94%). The product was contaminated with other impurities, which was removed in a subsequent purification. ^{1}H NMR (400 MHz, CD_3OD) δ 7.50 (dd, *J* = 7.9, 1.1 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.10 (ddd, *J* = 8.2, 6.9, 1.1 Hz, 1H), 7.03 – 6.96 (m, 1H), 6.94 (s, 1H), 3.93 (d, *J* = 7.5 Hz, 2H), 3.14 - 3.03 (m, 2H), 2.73 (t, *J* = 7.5 Hz, 2H), 2.36 (septet, *J* = 7.5 Hz, 1H), 1.90 – 1.78 (m, 2H), 1.71 – 1.56 (m, 4H), 1.47 – 1.56 (m, 2H), 1.43 (s, 9H), 1.18 – 1.32 (m, 2H). LRMS (*m/z*): $[M+H]^+$ calc. for $C_{22}H_{32}N_2O_2$, 357.2; found 357.2

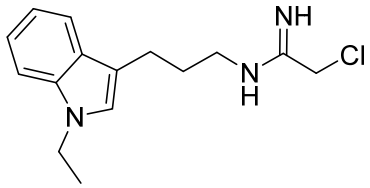
General procedure B: Preparation of Indole Inhibitors 7a-7d from intermediates 6a-6d.

Intermediate **6** was dissolved in CH_2Cl_2 (2.0 mL) and to this solution was added TFA (2.0 mL). The reaction mixture was stirred at rt for 1 h and then concentrated under reduced pressure and lyophilized overnight to give the TFA salt of the free amine. The crude TFA salt (1 equiv) was then dissolved in MeOH

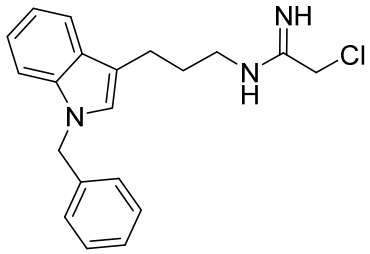
at a concentration of 0.10 M. Triethylamine (4.0 equiv) was added followed by ethyl-2-chloroacetimidate hydrochloride[2] (4.0 equiv). The resulting solution was stirred at rt for 1 h then concentrated under reduced pressure and purified by reverse phase chromatography (10-100% MeCN in H_2O + 0.1% TFA). After lyophilization of the desired fractions, indole inhibitors were isolated as TFA salts in 73-82% yield.



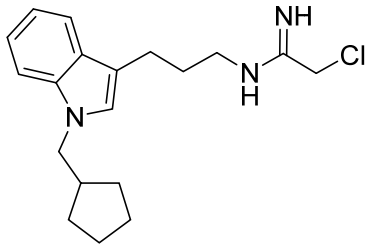
7a: General Procedure A employed using **6a** (60 mg, 0.198 mmol) to give **7a** (20 mg, 40%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.52 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.30 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.14 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 7.02 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 6.97 (s, 1H), 4.29 (s, 2H), 3.73 (s, 3H), 3.38 – 3.25 (m, 2H), 2.85 (t, *J* = 7.5 Hz, 2H), 2.06 (p, *J* = 7.2 Hz, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 164.5, 138.7, 129.0, 127.8, 122.5, 119.7, 119.5, 113.9, 110.2, 43.5, 40.0, 32.6, 29.0, 23.0. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{14}H_{18}ClN_3$, 264.1267; found 264.1266.



7b: General Procedure A employed using **6b** (90 mg, 0.285 mmol) to give **7b** (65 mg, 82%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.54 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.35 (dt, *J* = 8.2, 0.9 Hz, 1H), 7.15 (ddd, *J* = 8.2, 7.0, 1.1 Hz, 1H), 7.06 (s, 1H), 7.02 (ddd, *J* = 7.9, 7.0, 1.0 Hz, 1H), 4.32 (s, 2H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.34 (m, 2H), 2.88 (t, *J* = 7.3 Hz, 2H), 2.08 (p, *J* = 7.2 Hz, 2H), 1.40 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (126 MHz, CD_3OD) δ 164.5, 137.7, 129.2, 126.1, 122.5, 119.6, 119.6, 114.0, 110.4, 43.5, 41.6, 40.1, 29.0, 23.1, 15.9. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{15}H_{20}ClN_3$, 278.1424; found 278.1419.



7c: General Procedure A employed using **6c** (62 mg, 0.235 mmol) to give **7c** (58 mg, 73%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.57 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.32 – 7.25 (m, 3H), 7.25 – 7.18 (m, 1H), 7.15 – 7.08 (m, 4H), 7.04 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 5.33 (s, 2H), 4.32 (s, 2H), 3.34 (t, *J* = 7.0 Hz, 2H), 2.90 (t, *J* = 7.4 Hz, 2H), 2.09 (p, *J* = 7.2 Hz, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 164.6, 139.7, 138.3, 129.6, 129.3, 128.5, 127.9, 127.2, 122.7, 120.0, 119.7, 114.6, 111.0, 50.6, 43.6, 40.1, 28.9, 23.1. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{22}ClN_3$, 340.1580; found 340.1568.



7d: General Procedure A employed using **6d** (40 mg, 0.156 mmol) to give **7d** (38 mg, 73%). ^{1}H NMR (500 MHz, CD_3OD) δ 7.54 (dt, *J* = 7.9, 1.0 Hz, 1H), 7.36 (dt, *J* = 8.3, 0.9 Hz, 1H), 7.14 (ddd, *J* = 8.2, 7.0, 1.2 Hz, 1H), 7.05 (s, 1H), 7.04 – 6.98 (m, 1H), 4.33 (s, 2H), 4.03 (d, *J* = 7.6 Hz, 2H), 3.33 (t, *J* = 7.0 Hz, 2H), 2.88 (t, *J* = 7.3 Hz, 2H), 2.43 (septet, *J* = 7.6 Hz, 1H), 2.08 (p, *J* = 7.2 Hz, 2H), 1.74 – 1.63 (m, 4H), 1.63 – 1.53 (m, 2H), 1.37 – 1.25 (m, 2H). ^{13}C NMR (126 MHz, CD_3OD) δ 164.5, 138.2, 129.0, 127.0, 122.4, 119.6, 119.56, 113.7, 110.7, 51.7, 43.5, 42.3, 40.1, 31.4, 29.0, 25.9, 23.0. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{19}H_{26}ClN_3$, 332.1893; found 332.1888.

General Procedure C: Preparation of Indazole Inhibitors

The synthesis of **12a-12e** was prepared with the indazole assembled according to a reported literature protocol.[2] Boron trichloride dimethyl sulfide (1.1 equiv) was diluted with dichloroethane to a concentration of 0.50 M and cooled to 0 °C. The appropriate aniline **8a-8e** (1 equiv) was added dropwise and the solution was stirred at 0 °C for 15 min. 4-Chlorobutanenitrile (1.25 equiv) was added, followed by aluminum chloride (1.1 equiv), and the solution was allowed to warm to rt. After 15 min, the reaction mixture was refluxed overnight. The reaction mixture was cooled to rt, and 2M HCl was added. After addition, the reaction mixture was refluxed again for another 30 min. The reaction mixture was then cooled to rt, diluted with water, and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried, and concentrated to yield crude product **9a-9e**, which was used in the next step without further purification.

The crude intermediate **9a-9e** (1 equiv) was dissolved in conc. HCl at a concentration of 0.75 M and cooled to 0 °C. To this solution was added sodium nitrite dissolved in water (1.1 equiv, 3.6 M) dropwise. The reaction mixture was stirred at 0 °C for 1 h. To the reaction mixture was added $SnCl_2$ dissolved in conc. HCl (2.4 equiv, 3.4 M) dropwise, and the solution was stirred again at 0 °C for another 1-2 h. The reaction solution was extracted with CH_2Cl_2, the organic layer was washed with brine, dried, and concentrated to give the chloro indazole intermediates, which were used in the next step without further purification.

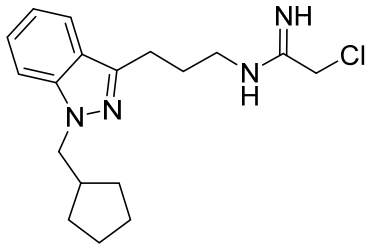
To a pressure tube containing chloro indazole intermediates (1 equiv) in DMF at a concentration of 0.50 M was added NaN_3 (1.2 equiv). The reaction mixture was heated to 50 °C and stirred overnight. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was separated and washed with water and brine three times, dried, and concentrated. The residue was purified by column chromatography using silica (0-40% ethyl acetate in hexanes) to give **10a-10e**.

Intermediate **10a-10e** (1 equiv) was then dissolved in dry DMF (0.25 M) and cooled to 0 °C in an ice bath. To this solution was added NaH (60 wt% mineral oil dispersion, 2 equiv), and the resulting suspension was stirred for 30 min to 1 h. Next, the reaction mixture was cooled to 0 °C, and the alkyl halide (3 equiv) was added dropwise, and the mixture was stirred for another 1-2 h. The reaction was quenched

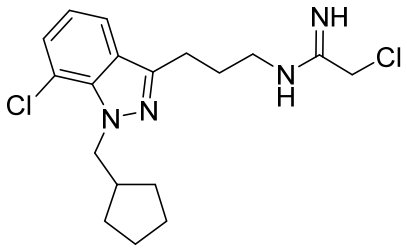
by addition of water. The mixture was extracted with ethyl acetate, and the combined organic extracts were washed with brine, dried, and concentrated. The crude product was purified by column chromatography (silica gel, gradient elution: 0-100% ethyl acetate in hexane to afford alkylated azido indazole intermediate **11a-11e**.

The alkylated azido indazole intermediate was then treated with $SnCl_2$ (2 equiv) in MeOH at a concentration of 0.20 M for 2 to 4 h at rt. The solvent was then evaporated to dryness, and the crude material was diluted with sat. $NaHCO_3$ and extracted with ethyl acetate. The organic extracts were washed sequentially with sat. $NaHCO_3$ and brine, dried, and concentrated to give the crude alkylated amino indazole intermediate, which was used in the next step without further purification.

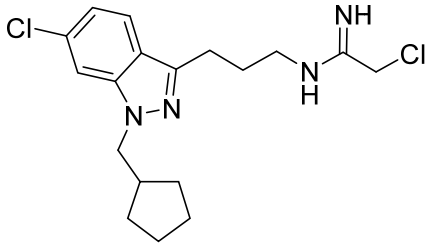
To a stirred solution of the corresponding alkylated amino indazole intermediate (1 equiv) in dry MeOH (0.1 M) was added triethylamine (5 equiv) followed by ethyl-2-chloroacetimidate (4 equiv). The reaction mixture was stirred at rt for 1-2 h. Solvent was evaporated under reduced pressure, and the crude product was purified by reverse phase column chromatography (10-100% MeCN in H_2O + 0.1% TFA) to give compound **12a-12e**.



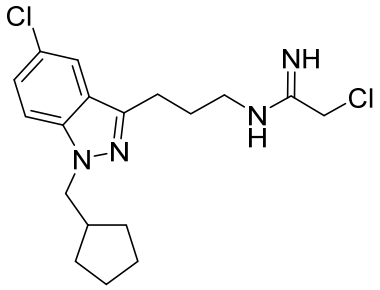
12a: General Procedure C was employed using **8a** (3.00 g, 32.2 mmol) to give **10a** (530 mg, 65% overall yield from **8a**) and a portion was taken on to give **12a** (18 mg, 66 % overall yield from **10a**). ^{1}H NMR (400 MHz, CD_3OD) δ 7.73 (dt, *J* = 8.2, 1.0 Hz, 1H), 7.53 (d, *J* = 8.4 Hz, 1H), 7.37 – 7.45 (m, 1H), 7.10 – 7.19 (m, 1H), 4.38 (s, 2H), 4.29 (d, *J* = 7.5 Hz, 2H), 3.40 (t, *J* = 7.1 Hz, 2H), 3.09 (t, *J* = 7.3 Hz, 2H), 2.59 – 2.44 (m, 1H), 2.17 (p, J = 7.2 Hz, 2H), 1.74 – 1.53 (m, 6H), 1.40 – 1.27 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 164.8, 144.6, 142.1, 128.0, 123.4, 121.4, 121.1, 110.7, 54.0, 43.3, 42.1, 40.2, 31.2, 27.7, 25.9, 24.3. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{25}ClN_4$, 333.1846; found 333.1853.



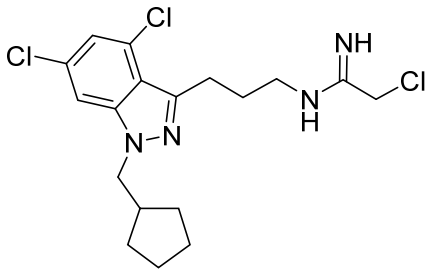
12b: General Procedure C was employed using **8b** (2.00 g, 15.7 mmol) to give **10b** (610 mg, 37% overall yield from **8b**) and a portion of **10b** was converted to give **12b** (30 mg, 39.7% overall yield from **10b**). ^{1}H NMR (400 MHz, CD_3OD) δ 7.70 (dt, *J* = 8.1, 0.6 Hz, 1H), 7.42 (dt, *J* = 7.3, 0.7 Hz, 1H), 7.10 (ddd, *J* = 8.0, 7.5, 0.5 Hz, 1H), 4.64 (d, *J* = 7.5 Hz, 2H), 4.38 (s, 2H), 3.41 (t, *J* = 7.1 Hz, 2H), 3.08 (t, *J* = 7.3 Hz, 2H), 2.49 (septet, *J* = 7.3 Hz, 1H), 2.17 (p, *J* = 7.2 Hz, 2H), 1.73 – 1.52 (m, 6H), 1.37 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 164.8, 145.2, 137.7, 129.3, 126.7, 122.2, 120.3, 116.9, 56.1, 43.3, 43.3, 40.1, 30.9, 27.4, 26.0, 24.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{24}Cl_2N_4$, 367.1456; found 367.1436.



12c: General Procedure C was employed using **8c** (4.00 g, 31.4 mmol) to give **10c** (240 mg, 30% overall yield from **8c**) and a portion was converted to give **12b** (23 mg, 46% overall yield from **10c**). ^{1}H NMR (400 MHz, CD_3OD) δ 7.71 (dt, *J* = 8.6, 0.6 Hz, 1H), 7.64 – 7.59 (m, 1H), 7.12 (ddd, *J* = 8.6, 1.7, 0.5 Hz, 1H), 4.38 (s, 2H), 4.26 (d, *J* = 7.5 Hz, 2H), 3.41 (t, *J* = 7.1 Hz, 2H), 3.07 (t, *J* = 7.3 Hz, 2H), 2.49 (septet, *J* = 7.5 Hz, 1H), 2.16 (p, *J* = 7.2 Hz, 2H), 1.76 – 1.52 (m, 6H), 1.39 – 1.25 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ 164.8, 145.1, 142.4, 134.3, 122.5, 122.2, 122.2, 110.4, 54.1, 43.3, 42.0, 40.1, 31.2, 27.5, 25.9, 24.2. HRMS (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{24}Cl_2N_4$, 367.1456; found 367.1458.

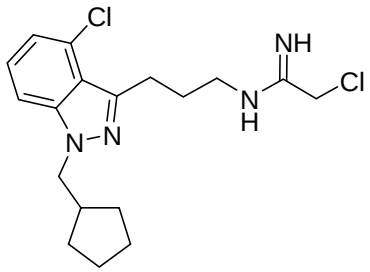


12d: General Procedure C was employed using **8d** (3.00 g, 29 mmol) to give **10d** (420 mg, 29% overall yield from **8d**) and a portion was converted to give **12d** (20 mg, 72.6% overall yield from **10d**). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.76 (dd, *J* = 1.9, 0.6 Hz, 1H), 7.60 – 7.51 (m, 1H), 7.37 (dd, *J* = 9.0, 1.9 Hz, 1H), 4.39 (s, 2H), 4.28 (d, *J* = 7.5 Hz, 2H), 3.40 (t, *J* = 7.1 Hz, 2H), 3.06 (t, *J* = 7.3 Hz, 2H), 2.50 (septet, *J* = 7.5 Hz, 1H), 2.16 (p, *J* = 7.2 Hz, 2H), 1.75 – 1.52 (m, 6H), 1.39 – 1.26 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ: 164.8, 144.3, 140.6, 128.3, 127.0, 124.3, 120.4, 112.2, 54.3, 43.3, 42.1, 40.1, 31.2, 27.6, 25.9, 24.2. HRMS (*m/z*): $[M+H]^+$ calc. for $C_{18}H_{24}Cl_2N_4$, 367.1456, found 367.1458.



12e: General Procedure C was employed using **8e** (3.00 g, 23.1 mmol) to give **10e** (180 mg, 19% overall yield from **8e**). All of **10e** was converted to give **12e** (56 mg, 57% overall yield from **10e**). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.62 (d, *J* = 1.5 Hz, 1H), 7.16 (d, *J* = 1.5 Hz, 1H), 4.39 (s, 2H), 4.26 (d, *J* = 7.5 Hz, 2H), 3.43 (t, *J* = 7.0 Hz, 2H), 3.26 (t, *J* = 7.3 Hz, 2H), 2.49 (septet, *J* = 7.6 Hz, 1H), 2.18 (p, *J* = 7.2 Hz, 2H), 1.76 – 1.52 (m, 6H), 1.33 (m, 2H). ^{13}C NMR (101 MHz, CD_3OD) δ: 164.8, 144.7, 143.4, 133.9, 128.6, 122.3, 119.6, 109.8, 54.4, 43.2, 41.9, 40.1, 31.2, 28.1, 25.9, 25.7. HRMS (*m/z*): $[M+H]^+$ calc. for $C_{18}H_{23}Cl_3N_4$, 401.1067, found 401.1058.

Procedure for the Preparation of 4-chloroindazole inhibitor 18



Silylation and subsequent acylation reaction was performed according to the published literature protocol.[3] To a flame-dried, N_2 flushed round bottom flask equipped with a stir bar was added **13** (3.26 g, 25.0 mmol) and TMEDA dissolved in THF (3.75 mL, 25.0 mmol, 1 equiv, 0.83 M), and the reaction solution was cooled to -75 °C. To this reaction mixture was added a solution of nBuLi in hexane (15.7 mL, 25.0 mmol, 1 equiv, 1.59 M in THF). The reaction mixture was stirred for 2 h and cooled again to -75 °C. A solution of Me_3SiCl (3.17 mL, 25.0 mmol, 1 equiv) dissolved in THF at a concentration of 2.50 M was added, and the reaction mixture was stirred at -75 °C to rt overnight. The reaction was quenched with sat. NH_4Cl, and the resulting mixture was extracted with Et_2O and concentrated under reduced pressure. The residue was purified by normal phase column chromatography using silica (100% hexane) to give the silylated intermediate **14** (4.00 g, 78.9%). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.33 (td, *J* = 8.1, 6.4 Hz, 1H), 7.18 (d, *J* = 7.9 Hz, 1H), 6.96 (t, *J* = 8.8 Hz, 1H), 0.42 (s, *J* = 2.3 Hz, 9H).

$AlCl_3$ (2.76 mg, 20.7 mmol) and anhydrous CH_2Cl_2 (40 mL) were added to a flame-dried, N_2 flushed round bottom flask. The solution was cooled to 0 °C. 4-Chlorobutanoyl chloride (2.32 mL, 20.7 mmol) was subsequently added and the reaction mixture was stirred at 0 °C. After 15 min, the reaction flask was cooled down to -80 °C, and a solution of silylated intermediate in CH_2Cl_2 (3.50 g, 17.3 mmol) was added. After stirring for 4 h at which time the temperature reached -10 °C, the mixture was quenched by adding saturated NH_4Cl. The organic phase was separated and the aqueous phase was washed with hexane. The organic extracts were combined and washed with $NaHCO_3$, dried, and concentrated. The mixture was purified by normal phase column chromatography using silica (0-10% ethyl acetate in hexane) to give

compound **15** (3.37 g, 83%). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.45 (m 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.23 – 7.14 (m, 1H), 3.66 (t, *J* = 6.5 Hz, 2H), 3.08 – 2.99 (m, 2H), 2.22 – 2.11 (m, 2H).

To a pressure tube containing **15** (3.37 g, 14.3 mmol) in DMF at a concentration of 0.70 M was added NaN_3 (1.12 g, 17.2 mmol). The reaction mixture was heated to 50 °C and stirred for 16 h. After completion, the reaction was quenched with water and extracted with ethyl acetate. The organic layer was separated and washed with water and brine, dried, and concentrated. The residue was purified by silica gel column chromatography (0-5% ethyl acetate in hexane) to give the corresponding azide (2.18 g, 63%). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.45 (m, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.19 (t, *J* = 8.7 Hz, 1H), 3.41 (t, *J* = 6.8 Hz, 2H), 2.96 (td, *J* = 7.0, 1.0 Hz, 2H), 1.97 (p, *J* = 6.9 Hz, 2H).

This material was then dissolved in DME (9.00 mL). To this solution was added hydrazine monohydrate (9.00 mL, 186 mmol) dropwise at rt. The reaction mixture was refluxed at 80 °C for 17 h. The mixture was cooled down to rt and the solvent was removed under reduced pressure. The resulting suspension was purified by normal phase column chromatography using silica (0-40% ethyl acetate in hexane) to give azido indazole intermediate **16** (650 mg, 30.6 %). ^{1}H NMR (400 MHz, CD_3OD) δ: 7.41 (d, *J* = 8.4 Hz, 1H), 7.29 (t, *J* = 7.9 Hz, 1H), 7.10 (d, *J* = 7.3 Hz, 1H), 3.40 (t, *J* = 6.7 Hz, 2H), 3.25 (t, *J* = 7.6 Hz, 2H), 2.08 (p, *J* = 7.0 Hz, 2H). LRMS (*m/z*): $[M+H]^+$ calc. for $C_{10}H_{10}ClN_5$, 236.1; found 236.1.

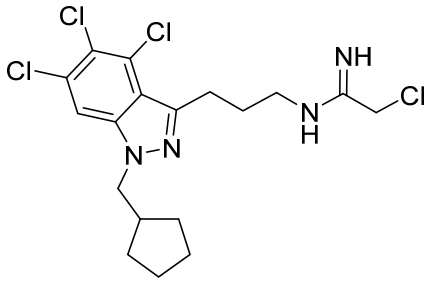
Intermediate **16** (310 mg, 1.32 mmol) was dissolved in dry DMF (5 mL) and cooled to 0 °C in an ice bath. To this solution was added NaH (60 wt% mineral oil dispersion, 60.5 mg, 2,64 mmol), and the resulting suspension was stirred for 1 h. Next, the reaction mixture was cooled to 0 °C, and cyclopentylmethyl bromide (486 uL, 3.96 mmol) was added dropwise, and the mixture was stirred for another 2 h. The reaction was quenched by addition of water. The mixture was extracted with ethyl acetate, and the combined organic extracts were washed with brine, dried, and concentrated. The crude product was purified by column chromatography (silica gel, gradient elution: 0-100% ethyl acetate in hexane to afford alkylated azido indazole intermediate **17** (250 mg, 60%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.46 (d, *J* = 8.5 Hz, 1H), 7.30 (dd, *J* = 8.6, 7.3 Hz, 1H), 7.10 (d, *J* = 7.4 Hz, 1H), 4.27 (d, *J* = 7.5 Hz, 2H), 3.40 (t, *J* = 6.7

Hz, 2H), 3.25 (t, *J* = 7.5 Hz, 2H), 2.52 (septet, *J* = 7.5 Hz, 1H), 2.12 – 2.02 (m, 2H), 1.78 – 1.48 (m, 6H), 1.27-1.40 (m, 2H). LRMS (*m/z*): $[M+H]^+$ calc. for $C_{16}H_{20}ClN_5$, 318.1; found 318.1.

Intermediate **17** (250 mg, 0.787 mmol) was then treated with $SnCl_2$ (355 mg, 1.57 mmol) in MeOH (8.00 mL) for 2.5 h at rt. The solvent was then evaporated to dryness and the crude material was diluted with sat. $NaHCO_3$ and extracted with ethyl acetate. The organic extracts were washed sequentially with sat. $NaHCO_3$, brine, dried, and concentrated to give the crude alkylated amino indazole intermediates which was used in the next step without further purification.

To a stirred solution of the corresponding alkylated amino indazole intermediate (70 mg, 0.24 mmol) in 2 mL of dry MeOH was added triethylamine (167 uL, 1.20 mmol) followed by ethyl-2-chloroacetimidate (117 mg, 0.96 mmol). The reaction mixture was stirred at rt for 2 h. The solvent was evaporated under reduced pressure, and the crude product was purified by reverse phase column chromatography (10-100% MeCN in H_2O + 0.1% TFA) to give compound **18** (54 mg, 61%). ^1H NMR (400 MHz, CD_3OD) δ: 7.49 (d, *J* = 8.5 Hz, 1H), 7.37 – 7.28 (m, 1H), 7.12 (d, *J* = 7.4 Hz, 1H), 4.42 (s, 2H), 4.29 (d, *J* = 7.5 Hz, 2H), 3.46 (t, *J* = 7.0 Hz, 2H), 3.30 (t, *J* = 7.4 Hz, 2H), 2.52 (septet, *J* = 7.5 Hz, 1H), 2.21 (p, *J* = 7.2 Hz, 2H), 1.76 – 1.51 (m, 6H), 1.33 (m, 2H). ^{13}C NMR (151 MHz, CD_3OD) δ: 163.3, 142.8, 142.1, 127.1, 126.3, 120.4, 119.2, 108.4, 52.8, 41.8, 40.5, 38.7, 29.8, 26.9, 24.5, 24.4. HRMS (*m/z*): $[M+H]^+$ calc. for $C_{18}H_{24}Cl_2N_4$, 367.1456, found 367.1443.

Procedure for the Preparation of 4,5,6-trichloroindazole inhibitor 24



Conversion of trichloroaniline **19** to trichlorofluorobenzene **20** was accomplished in analogous manner to a literature procedure.[4] To a round bottom flask equipped with a stir bar and immersed in an ice bath was added **19** (5.00 g, 25.5 mmol), water (7.50 mL) and conc. HCl (7.50 mL). To this reaction mixture was added dropwise $NaNO_2$ (2.20 g, 31.8 mmol) in 5 mL of water. The reaction mixture was stirred for 30 min, and then 48 % aq. HBF_4 (7.80 mL, 12.7 mmol) was added, and stirred was continued for another 90 min. The precipitate was collected by vacuum filtration and washed with cold water and chilled ether, and dried under vaccum to afford diazonium tetrafluoroborate salt. The diazonium tetrafluoroborate salt was then transferred into a three-neck round bottom decomposition flask that was equipped with a N_2 inlet and a condenser connected to another round bottom receiving flask containing 20% aq. NaOH and cooled in dry ice/acetone bath. The decomposition flask was then slowly heated with an open flame under nitrogen and with water running through the condenser. After all the diazonium tetrafluoroborate salt had decomposed, the residue in the decomposition flask was washed with 10% aq. NaOH and extracted with CH_2Cl_2. The receiving flask, adaptor, and condenser were washed with CH_2Cl_2. The combined organic extracts were washed with 10% aq. NaOH and water, dried, and concentrated. The residue was purified by silica gel column chromatography (100% hexane) to afford **20** (3.52 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 7.8 Hz, 1H). ^{19}F NMR (376 MHz, $CDCl_3$) δ -112.04.

Ortho-directed metalation and subsequent acylation was performed according to a literature procedure.[5] To a solution of **20** (3.52 mg, 17.6 mmol) in anhydrous THF (18 mL) at -78 °C was added LDA (10 mL, 18.5 mmol) dropwise. Upon complete addition, the reaction mixture was stirred for 2 h at -78 °C. Then $ZnCl_2$ (2.41 g, 17.6 mmol) was added dropwise maintaining the internal temperature below -55 °C.

The resulting solution was stirred for another 4 h until it warmed up to -10 °C, at which time CuCl in THF (1.05 g, 10.6 mmol) and 4-chlorobutanoyl chloride (3.95 mL, 35.3 mmol) were added. The reaction mixture was allowed to warm to rt and stirred overnight. The reaction was quenched with sat. NH_4Cl and extracted with ethyl acetate. The crude product is isolated by silica gel column chromatography (0-10% ethyl acetate in hexane) to give **21** (920 mg, 17%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.59 (d, *J* = 8.6 Hz, 1H), 3.67 (t, *J* = 6.4 Hz, 2H), 3.06 (td, *J* = 6.9, 0.9 Hz, 2H), 2.17 (p, *J* = 6.7 Hz, 2H). ^{19}F NMR (376 MHz, CD_3OD) δ -117.39.

To a pressure tube containing **21** (380 mg, 1.25 mmol) in DMF (2.00 mL) was added NaN_3 (97.5 mg, 1.50 mmol). The reaction mixture was heated to 50 °C and stirred overnight. After completion, the reaction was quenched with water and extracted with ethyl acetate. The organic layer was separated and washed with water three times, dried, and concentrated. The residue was purified by column chromatography using silica (0-5% ethyl acetate in hexane) to give the corresponding azide (140 mg, 36%). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.53 (d, *J* = 8.6 Hz, 1H), 3.40 (t, *J* = 6.7 Hz, 2H), 2.99 – 2.94 (m, 2H), 1.96 (p, *J* = 6.9 Hz, 2H). ^{19}F NMR (376 MHz, CD_3OD) δ -117.36.

This material (130 mg, 0.419 mmol) was then dissolved in DME (3.00 mL). To this solution was added hydrazine monohydrate (406 μL, 8.37 mmol) dropwise at room temperature. The reaction mixture was refluxed at 80 °C overnight. The mixture was cooled down to rt and the solvent was removed under reduced pressure. The resulting suspension was purified by normal phase column chromatography using silica (0-40% ethyl acetate in hexane) to give azido indazole intermediate **22** (60 mg, 47%). LRMS (*m/z*): $[M+H]^+$ calc. for $C_{10}H_8Cl_3N_5$, 304.0; found 304.0.

Intermediate **22** (60 mg, 0.197 mmol) was dissolved in dry DMF (3 mL) and cooled to 0 °C in an ice bath. To this solution was added NaH (60 wt% mineral oil dispersion, 9.70 mg, 0.42 mmol), and the resulting suspension was stirred for 1 h. Next, the reaction mixture was cooled to 0 °C, and cyclopentylmethyl bromide (73 uL, 0.60 mmol) was added dropwise, and the mixture was stirred for another 2 h. The reaction was quenched by addition of water. The mixture was extracted with ethyl acetate, and the combined organic extracts were washed with brine, dried, and concentrated. The crude product was

purified by column chromatography (silica gel, gradient elution: 0-100% ethyl acetate in hexane) to afford alkylated azido indazole intermediate **23** (40 mg, 53%).

Indazole intermediate **23** (40 mg, 0.103 mmol) was then treated with $SnCl_2$ (58.5 mg, 0.310 mmol) in MeOH at a concentration of 0.20 M overnight for complete reduction. The solvent was then evaporated to dryness and the crude material was diluted with sat. $NaHCO_3$ and extracted with ethyl acetate. The organic extracts were washed sequentially with sat. $NaHCO_3$, brine, dried, and concentrated to give the crude amino indazole intermediate, which was used in the next step without further purification. To a stirred solution of the intermediate (40 mg, 0.11 mmol) in dry MeOH (1 mL) was added triethylamine (77.3 uL, 0.55 mmol) followed by ethyl-2-chloroacetimidate (53.9 uL, 0.44 mmol). The reaction mixture was stirred at rt for 2 h. The solvent was evaporated under reduced pressure, and the crude product was purified by reverse phase column chromatography (10-100% MeCN in H_2O + 0.1% TFA) to give compound **24** (34 mg, 70%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.86 (s, 1H), 4.38 (s, 2H), 4.27 (d, *J* = 7.5 Hz, 2H), 3.44 (t, *J* = 7.1 Hz, 2H), 3.27 (m, 2H), 2.49 (septet, *J* = 7.4 Hz, 1H), 2.19 (p, *J* = 7.3 Hz, 2H), 1.74 – 1.57 (m, 6H), 1.36 – 1.27 (m, 2H). ^{13}C NMR (151 MHz, CD_3OD) δ (ppm): 164.8, 144.6, 140.9, 132.9, 127.1, 124.4, 120.6, 111.4, 54.4, 43.2, 41.9, 40.2, 31.2, 28.0, 25.9, 25.8. HRMS (*m/z*): $[M+H]^+$ calc. for $C_{18}H_{22}Cl_4N_4$, 435.0677, found 435.0685.

IC_{50} Determination for Inhibitor 7a-7d, 12a Against PAD4. The IC_{50} values of the unsubstituted indole and indazole inhibitors against PAD4 were determined following literature procedures.[6,7] As provided in more detail below, reaction volumes of 25 μL were used in 96-well microtiter plates. Inhibitor dilutions in DMSO (0.625 μL, with 2-fold dilutions) were added to each well followed by enzyme solution (23.75 μL, 100 nM in assay) (diluted in assay buffer containing 100 mM Tris-HCl pH 7.6, 50 mM NaCl, 10 mM $CaCl_2$, 0.01% Triton X-100, and 2.0 mM freshly added DTT). The contents of the assay plate were gently mixed and then the plate was incubated at 37 °C for 15 min. At this time, 24.4 μL of this solution was added to wells containing *N*-benzoylated arginine ethyl ester hydrochloride (BAEE) in DMSO (0.625 μL, 10 mM in assay). This solution was gently mixed and incubated at 37 °C for another fifteen min. At this time, the enzymatic reaction was quenched by transferring 20 μL of the assay mix to 70 μL of COLDER developing solution[9] (1:3 ratio of COLDER A solution and COLDER B solution) plated in Corning® 96-well half area plate. This plate was incubated at 95 °C for 25 min and then cooled for 10 min at rt. Residual enzymatic activity was measured at each inhibitor concentration by reading the absorbance at 540 nm on a spectrophotometric plate reader. The data collected were fit according to the equation $FA = \frac{1}{1+\frac{[I]}{IC_{50}}}$ (where [I] = inhibitor concentration and IC_{50} = concentration of [I]at half-maximal inhibition) using Prism v6 (GraphPad). Assays were run in duplicate using the same inhibitor stock solutions.

***k_{inact}/K_i* Determination for Inhibitor 12b-12f, 18, and 24 Against PAD1,2,3,4.** The k_{inact}/K_i values of the unsubstituted and Cl-substituted indazole inhibitors against PAD1, PAD2, PAD3, and PAD4 were determined following literature procedures.[6,7] Inhibitor dilutions in DMSO (4 μL, with 2-fold dilutions) were added to each well of a starter plate followed by 155 μL of enzyme solution (diluted in assay buffer containing 100 mM Tris-HCl pH 7.6, 50 mM NaCl, 10 mM $CaCl_2$, 0.01% Triton X-100, and 2.0 mM freshly added DTT). The contents of the starter plate were gently mixed and then the plate was incubated at 37 °C. Final enzyme concentration in the assay was 200 nM for PAD1 and PAD2, 400 nM for PAD3, and 100 nM for PAD4. At each time point (2.5, 5, 10, 15, 20, and 30 min), aliquots from the starter plate (24.4 μL) were

taken and added to an assay plate containing BAEE in DMSO (0.625 μL, 10 mM in assay). Assay plates corresponding to each time point were incubated at 37 °C for 15 min. At this time, the enzymatic reaction was quenched by transferring 20 μL of the assay mix to 70 μL of COLDER developing solution[9] (1:3 ratio of COLDER A solution and COLDER B solution) plated in Corning® 96-well half area plate. This plate was incubated at 95 °C for 25 min and then cooled for 10 min at rt. Residual enzymatic activity was measured at each inhibitor concentration as a function of time by reading the absorbance at 540 nm on a spectrophotometric plate reader. The data collected were used to calculate k_{obs} values for each inhibitor concentration via a nonlinear regression according to the equation $v = v_o e^{-kt}$(where v = velocity, v_o= initial velocity, t = time (s)) using Prism v7 (GraphPad). When k_{obs} varied linearly with[I], $k_{\mathrm{inact}}/K_{\mathrm{i}}$was determined by linear regression analysis according to the equation $k_{\mathrm{obs}} = k_{\mathrm{inact}}[\mathrm{I}]/ K_{\mathrm{i}}$. (where k_{inact}= maximal rate of inactivation and K_{i}= concentration of I that yields half-maximal inactivation). When k_{obs} varied hyperbolically with [I], nonlinear regression was performed to determine $k_{\mathrm{inact}}/K_{\mathrm{i}}$ according to the following equation $k_{\mathrm{obs}} = \frac{k_{\mathrm{inact}}[\mathrm{I}]}{[\mathrm{I}]} + K_{\mathrm{i}}\left(1 + \frac{[\mathrm{S}]}{K_{\mathrm{M}}}\right)$. Assays were run in duplicate using the same inhibitor stock solutions.

HL-60 Cell Culture and Differentiation

HL-60 cells (American Type Culture Collection, CCL-240™) were grown in RPMI 1640 medium (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum. To induce differentiation into mature granulocytes, HL-60 cells were seeded at a density of 3 x 10^5 cells/mL in RPMI 1640 medium supplemented with 1.25% DMSO. PAD4 overexpression in differentiated HL-60 derived granulocytes was monitored after 1, 3, and 5 days by western blot (Figure 1). Subsequent PAD4 inhibition assays were performed with HL-60 derived granulocytes obtained following 3 days of treatment with 1.25% DMSO.

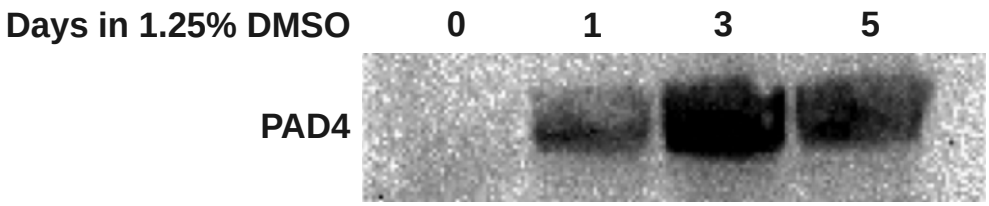


Figure 1. Western blot detection of PAD4 expression in differentiated HL-60 cell lysate

PAD4 Inhibition Assays

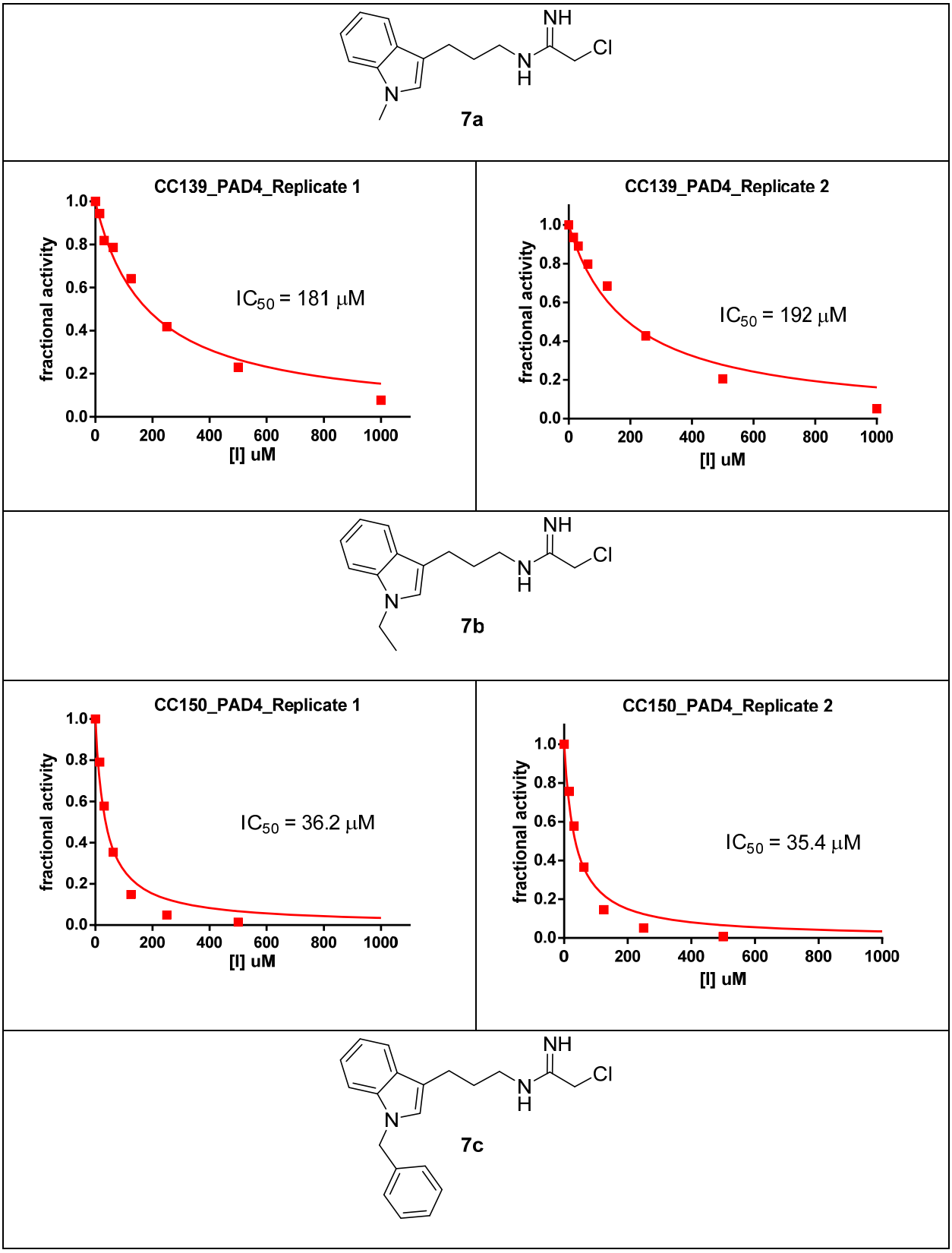
HL-60 derived granulocytes were harvested by centrifugation (300 x g, 5 min), re-suspended in Locke's solution (10 mM HEPES pH 7.5, 150 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 0.1% glucose), and seeded into 12-well plates at a density of 1 x 10^6 cells/mL (2 mL total). 5 mM stocks of Cl-amidine and compound **24** were prepared in anhydrous DMSO. After 10 min, the cells were treated with 5 or 10 μM of Cl-amidine or compound **24** for 15 min at 37 °C. Cells that were not treated with either inhibitor were incubated with the same amount of DMSO used in the 10 μM inhibitor treatments. Next, the cells were treated with 5 μM of calcium ionophore A23187 (Millipore Sigma) or an equivalent amount of DMSO and incubated for an additional 15 min at 37 °C. After stimulation with the calcium ionophore, the cells were harvested by centrifugation (300 x g, 5 min), washed with Dulbecco's phosphate buffered saline (DPBS), re-pelleted by

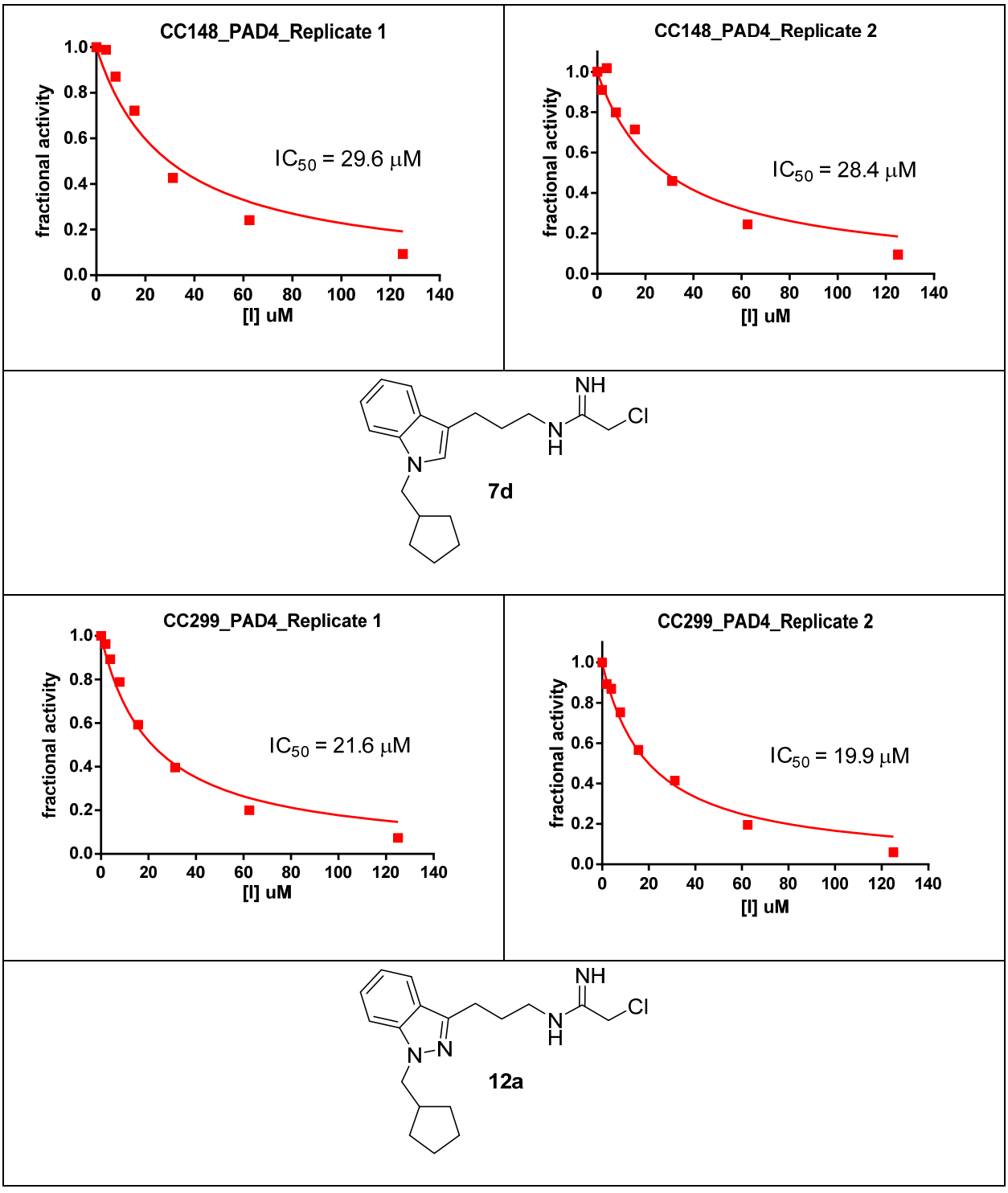
centrifugation and lysed in RIPA buffer (Thermo Fisher Scientific) for 15 min. The cell lysates were clarified by centrifugation (15,000 x g, 15 min) and boiled in SDS-containing gel loading buffer prior to analysis by western blot.

Western Blot Analysis

For western blot analysis, denatured cellular lysates were separated on Any kD™ Mini-PROTEAN® TGX™ Precast Protein Gels (Bio-Rad) and transferred onto PVDF membranes using the iBlot® blotting system (Thermo Fisher Scientific). Blots treated with primary rabbit-derived antibodies Anti-Histone H4 (ab10158, Abcam) and Anti-Histone H4 Citrulline 3 (07-596, Millipore Sigma) were blocked at room temperature for 1 h with 3% bovine serum albumin (BSA) in Tris-buffered saline containing 0.1% Tween 20 (TBST). Blots treated with a primary antibody against rabbit-derived β-actin (4967, Cell Signaling Technology) or mouse-derived PAD4 (049H5, BioLegend) were blocked at room temperature for 1 h in 5% nonfat dry milk. The primary antibodies were diluted in each indicated blocking buffer in the following manner: Anti-Histone H4 (1:500), Anti-Histone H4 Citrulline 3 (1:300), Anti β-actin (1:1000), and Anti PAD4 (1:500) prior to overnight incubation with the membranes at 4 °C. Next, the blots were washed with TBST (3 x 5 min) and incubated with an HRP-conjugated anti-rabbit IgG antibody (7074, Cell Signaling Technology) or anti-mouse IgG antibody (7076, Cell Signaling Technology) diluted 1:3,000 in 5% nonfat dry milk for 1 h at room temperature. Next, the blots were washed in TBST (3 x 5 min) and the HRP signal was developed using Clarity™ Western ECL Substrates (Bio-Rad). The blots were imaged by using a ChemiDoc® imaging system (Bio-Rad).

Table S1. IC_{50} of Unsubstituted Indole and Indazole Inhibitors.




CC148_PAD4_Replicate 1
fractional activity
IC50 = 29.6 μM
[I] uM
CC148_PAD4_Replicate 2
fractional activity
IC50 = 28.4 μM
[I] uM
NH
Cl
N
H
N
7d
CC299_PAD4_Replicate 1
fractional activity
IC50 = 21.6 μM
[I] uM
CC299_PAD4_Replicate 2
fractional activity
IC50 = 19.9 μM
[I] uM
NH
Cl
N
H
N-N
12a

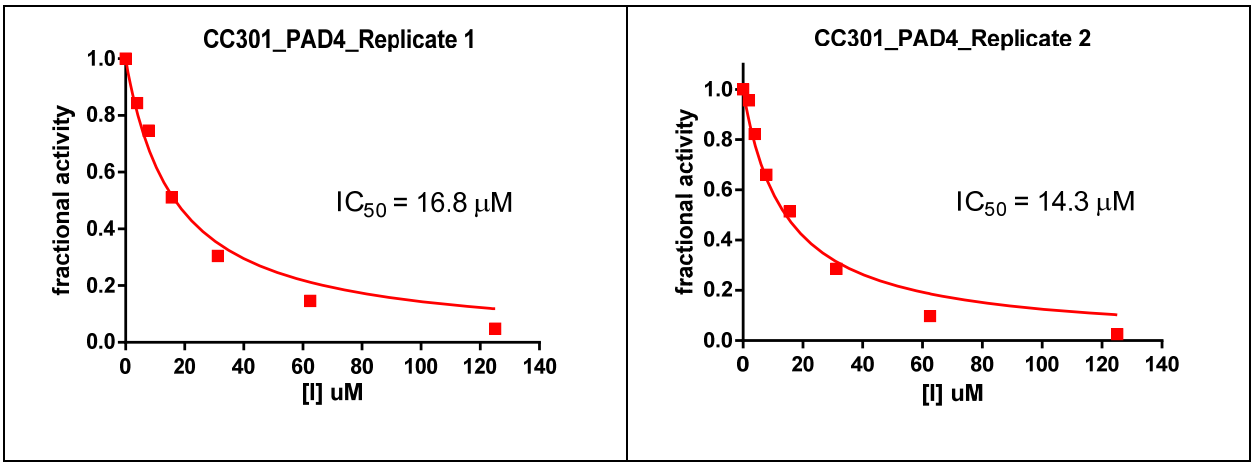
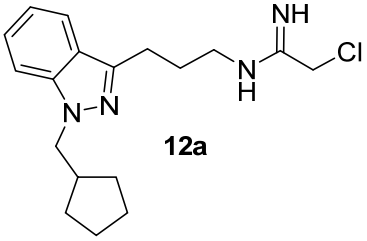
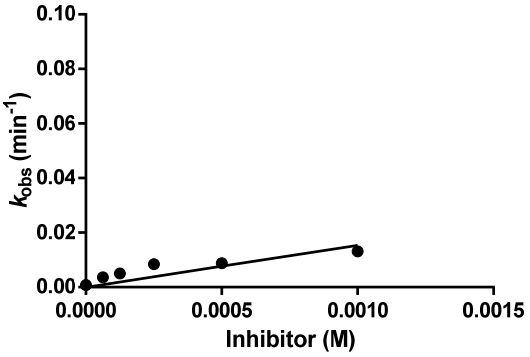

CC301_PAD4_Replicate 1
fractional activity
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
[I] uM
IC50 = 16.8 μM
CC301_PAD4_Replicate 2
fractional activity
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
120
140
[I] uM
IC50 = 14.3 μM

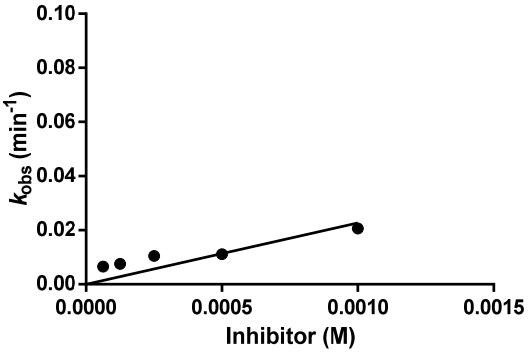
Table S2. k_{inact}/K_i of Inhibitors.



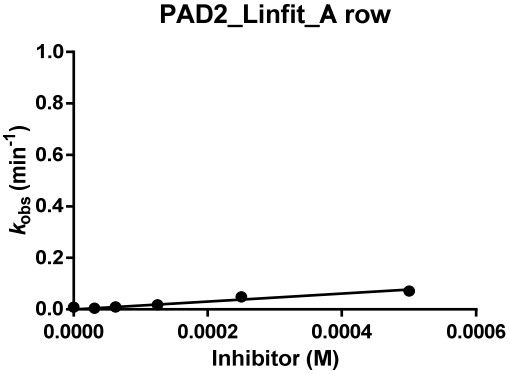
12a



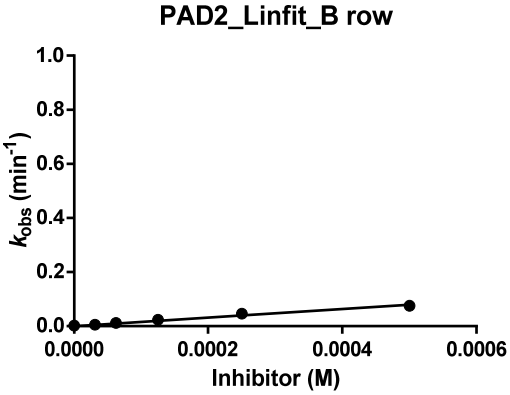
k_{inact} / K_i = 15.37 ± 2.625 (min^{-1}M^{-1})



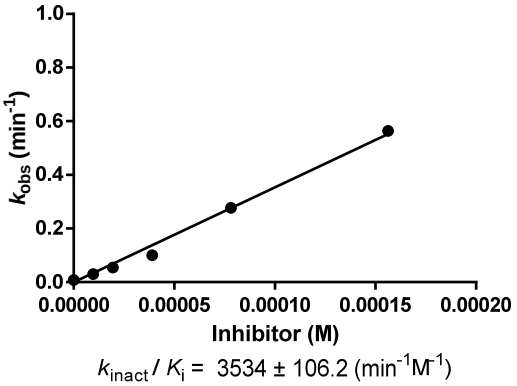
k_{inact} / K_i = 22.68 ± 3.993 (min^{-1}M^{-1})

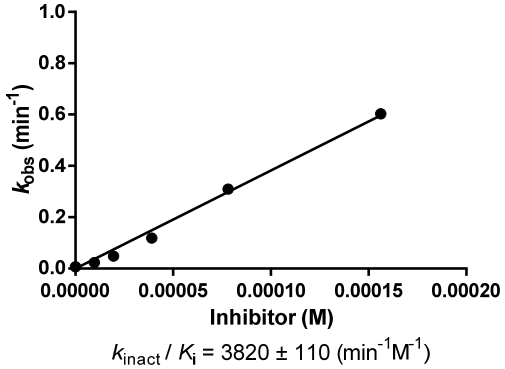


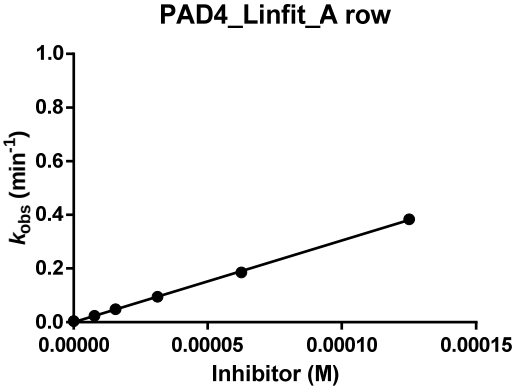
k_{inact} / K_i = 153.3 ± 11.38 (min^{-1}M^{-1})

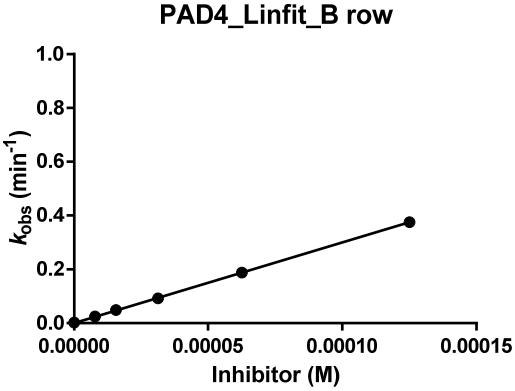


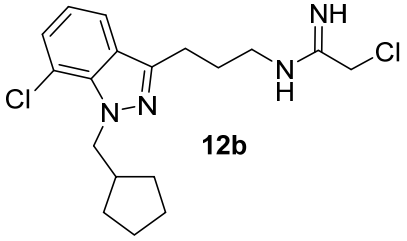
k_{inact} / K_i = 158.8 ± 6.769 (min^{-1}M^{-1})


PAD3_Linfit_A row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00005
0.00010
0.00015
0.00020
Inhibitor (M)
k_{inact} / K_i = 3534 ± 106.2 (min^{-1}M^{-1})


PAD3_Linfit_B row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00005
0.00010
0.00015
0.00020
Inhibitor (M)
k_{inact} / K_i = 3820 ± 110 (min^{-1}M^{-1})

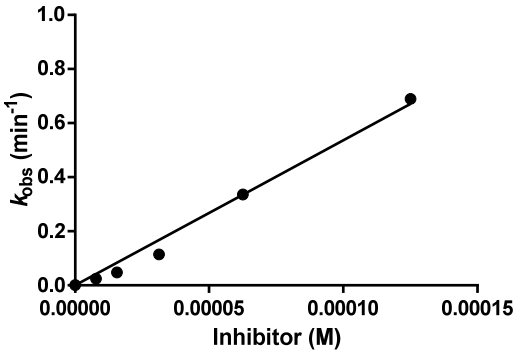

PAD4_Linfit_A row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00005
0.00010
0.00015
Inhibitor (M)
k_{inact} / K_i = 3047 ± 20.46 (min^{-1}M^{-1})


PAD4_Linfit_B row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00005
0.00010
0.00015
Inhibitor (M)
k_{inact} / K_i = 3003 ± 10.07 (min^{-1}M^{-1})

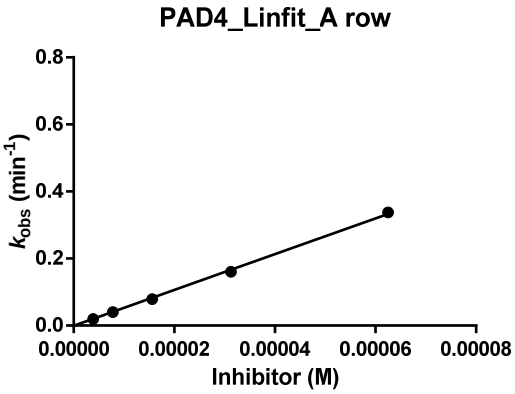

NH
Cl
N
H
Cl
N–N

12b

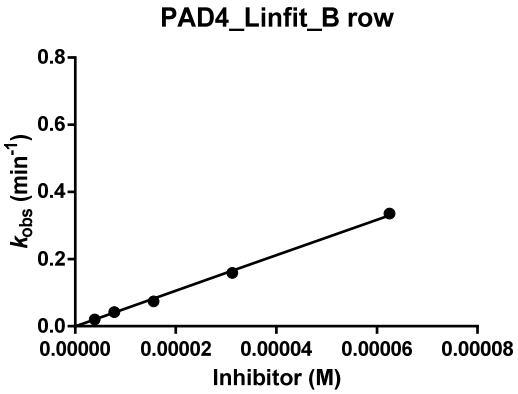
k_{inact} / K_i = 5460 ± 224.6 (min^{-1}M^{-1})



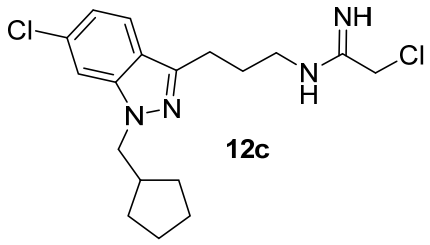
k_{inact} / K_i = 5369 ± 213 (min^{-1}M^{-1})



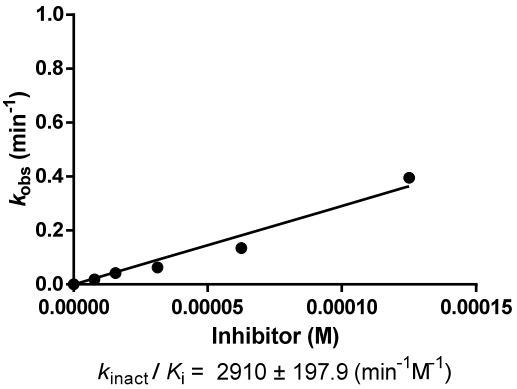
k_{inact} / K_i = 5332 ± 58.06 (min^{-1}M^{-1})



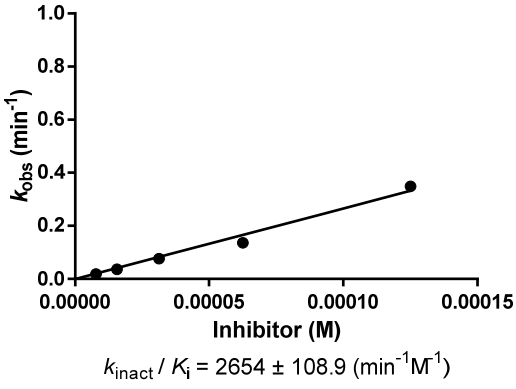
k_{inact} / K_i = 5287 ± 72.99 (min^{-1}M^{-1})



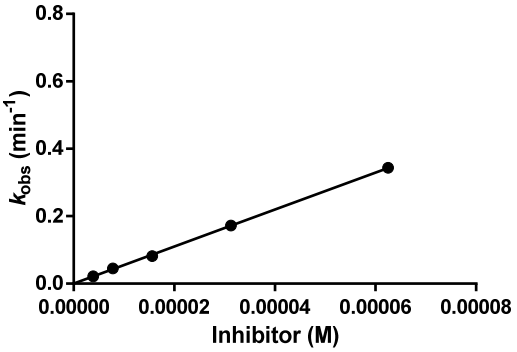
12c



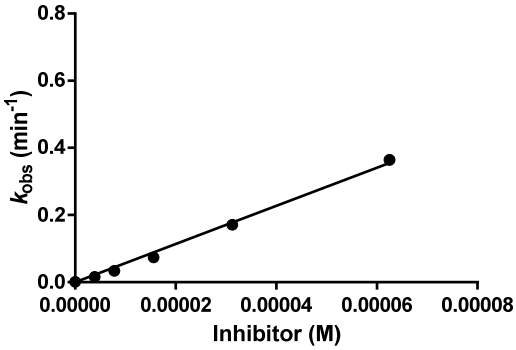
k_{inact} / K_i = 2910 ± 197.9 (min^{-1}M^{-1})



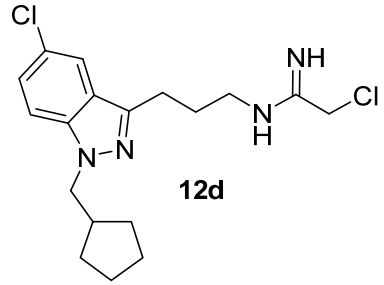
k_{inact} / K_i = 2654 ± 108.9 (min^{-1}M^{-1})



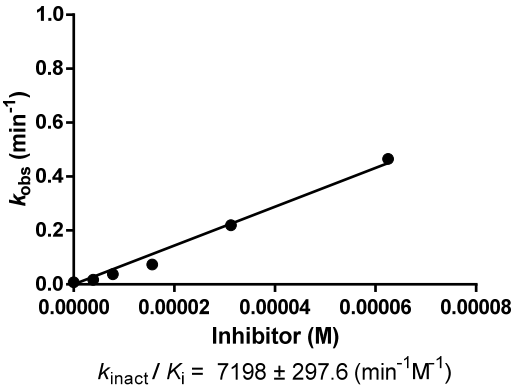
k_{inact} / K_i = 5495 ± 37.15 (min^{-1}M^{-1})

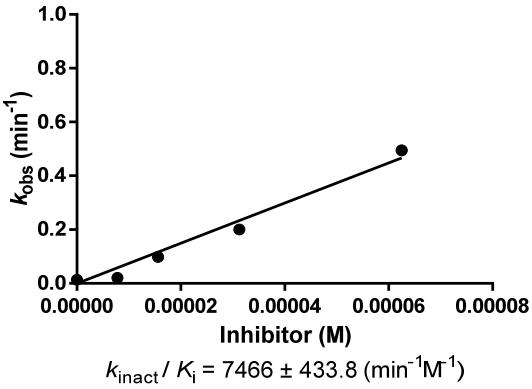


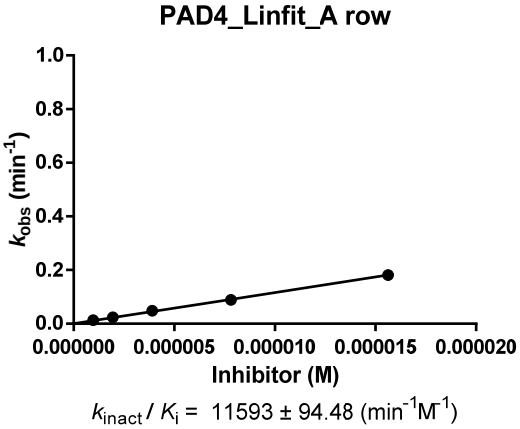
k_{inact} / K_i = 5684 ± 140.3 (min^{-1}M^{-1})

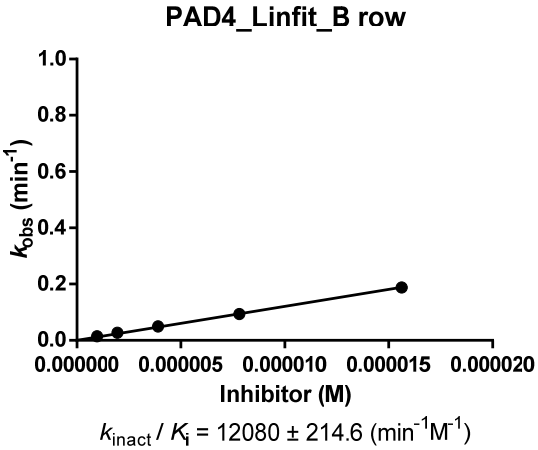


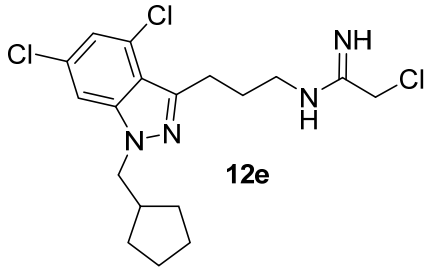
12d

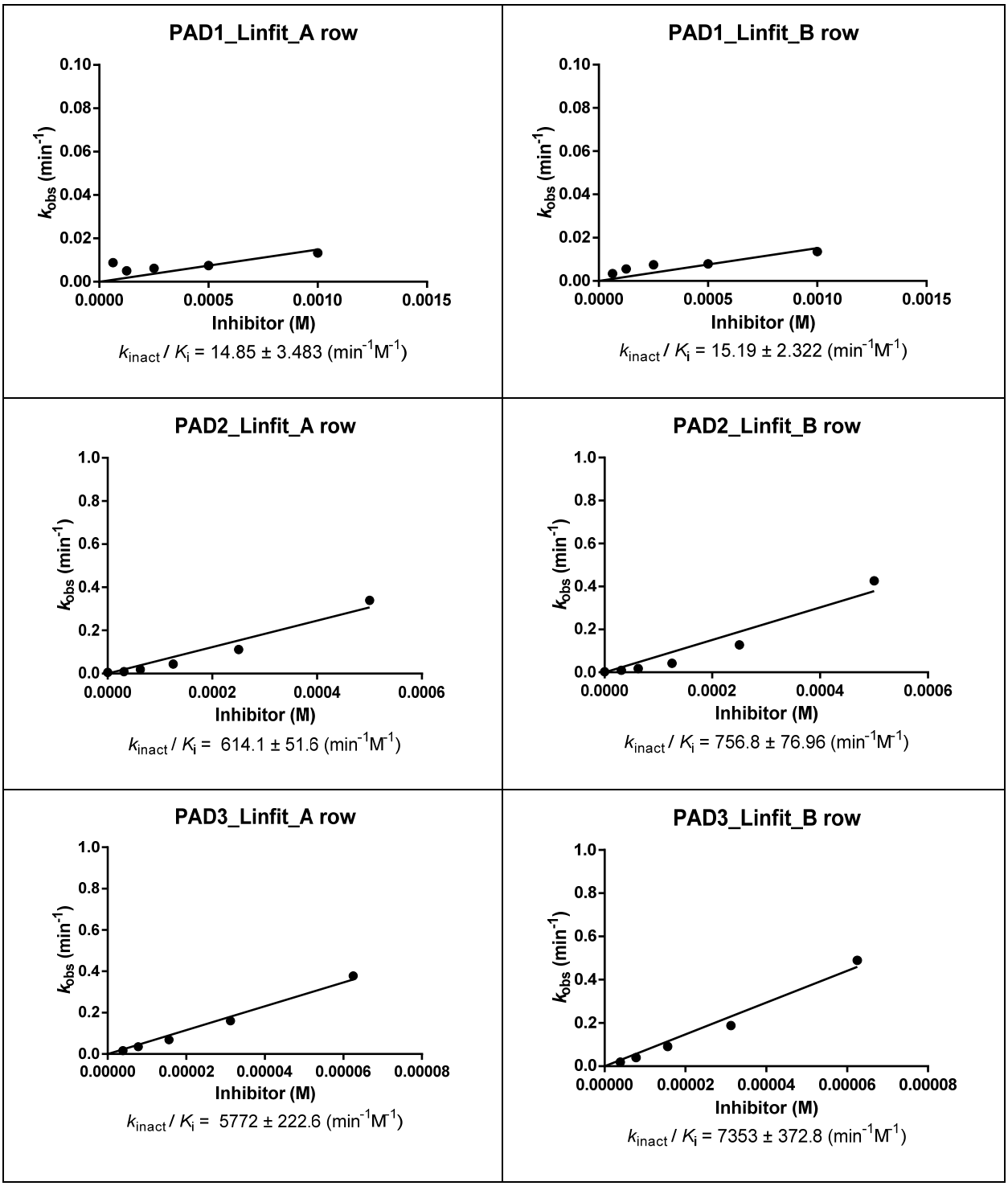

PAD3_Linfit_A row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00002
0.00004
0.00006
0.00008
Inhibitor (M)
k_{inact} / K_i = 7198 ± 297.6 (min^{-1}M^{-1})

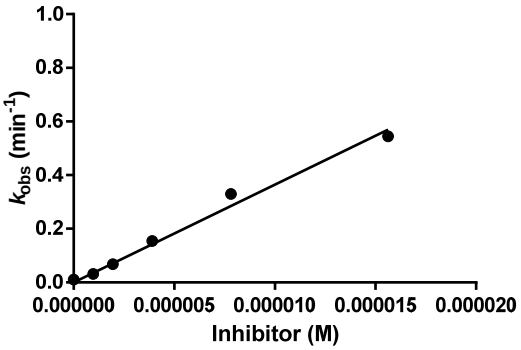

PAD3_Linfit_B row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00002
0.00004
0.00006
0.00008
Inhibitor (M)
k_{inact} / K_i = 7466 ± 433.8 (min^{-1}M^{-1})


PAD4_Linfit_A row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.000000
0.000005
0.000010
0.000015
0.000020
Inhibitor (M)
k_{inact} / K_i = 11593 ± 94.48 (min^{-1}M^{-1})

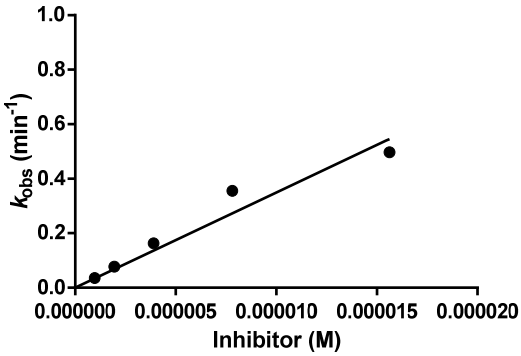

PAD4_Linfit_B row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.000000
0.000005
0.000010
0.000015
0.000020
Inhibitor (M)
k_{inact} / K_i = 12080 ± 214.6 (min^{-1}M^{-1})


Cl
Cl
NH
Cl
N
H
N
N
12e

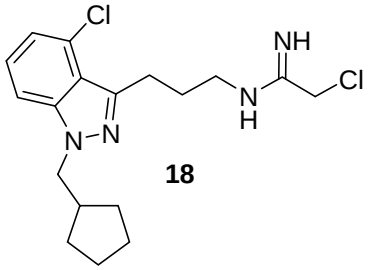

PAD1_Linfit_A row
0.10
0.08
0.06
0.04
0.02
0.00
k_{obs} (min^{-1})
0.0000
0.0005
0.0010
0.0015
Inhibitor (M)
k_{inact} / K_i = 14.85 ± 3.483 (min^{-1}M^{-1})
PAD1_Linfit_B row
0.10
0.08
0.06
0.04
0.02
0.00
k_{obs} (min^{-1})
0.0000
0.0005
0.0010
0.0015
Inhibitor (M)
k_{inact} / K_i = 15.19 ± 2.322 (min^{-1}M^{-1})
PAD2_Linfit_A row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.0000
0.0002
0.0004
0.0006
Inhibitor (M)
k_{inact} / K_i = 614.1 ± 51.6 (min^{-1}M^{-1})
PAD2_Linfit_B row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.0000
0.0002
0.0004
0.0006
Inhibitor (M)
k_{inact} / K_i = 756.8 ± 76.96 (min^{-1}M^{-1})
PAD3_Linfit_A row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00002
0.00004
0.00006
0.00008
Inhibitor (M)
k_{inact} / K_i = 5772 ± 222.6 (min^{-1}M^{-1})
PAD3_Linfit_B row
1.0
0.8
0.6
0.4
0.2
0.0
k_{obs} (min^{-1})
0.00000
0.00002
0.00004
0.00006
0.00008
Inhibitor (M)
k_{inact} / K_i = 7353 ± 372.8 (min^{-1}M^{-1})



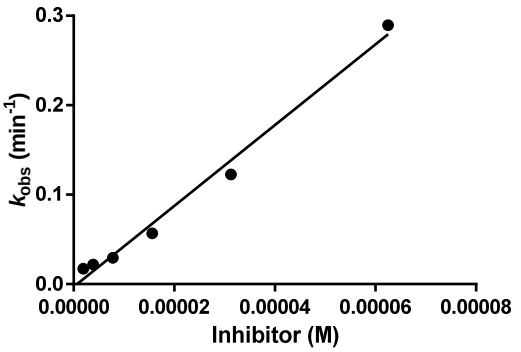
k_{inact} / K_i = 36435 ± 1332 (min^{-1}M^{-1})



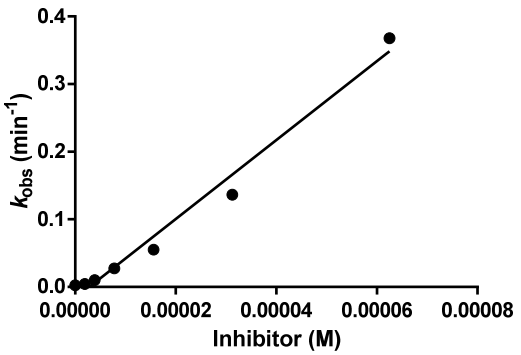
k_{inact} / K_i = 34928 ± 2474 (min^{-1}M^{-1})



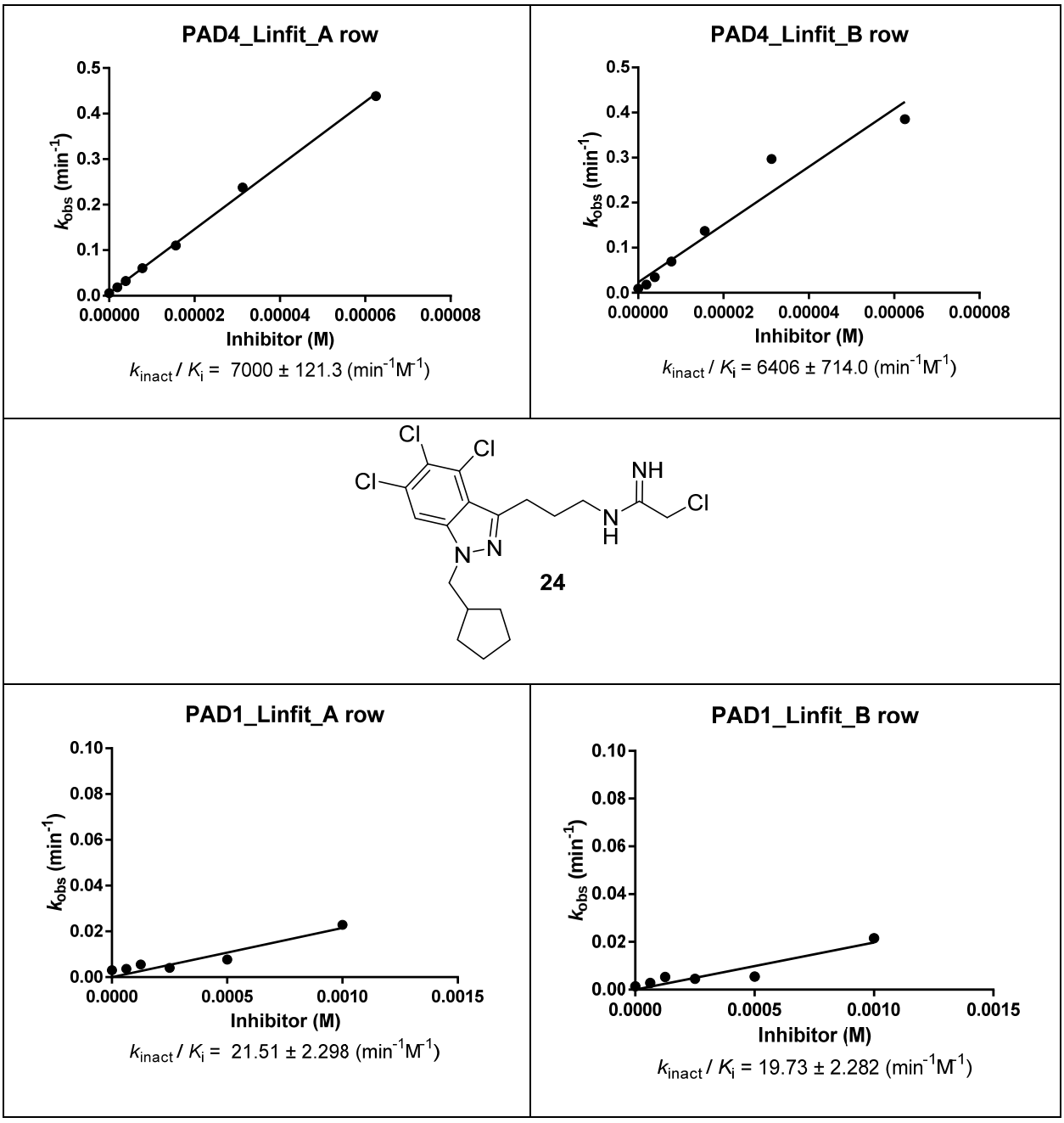
18

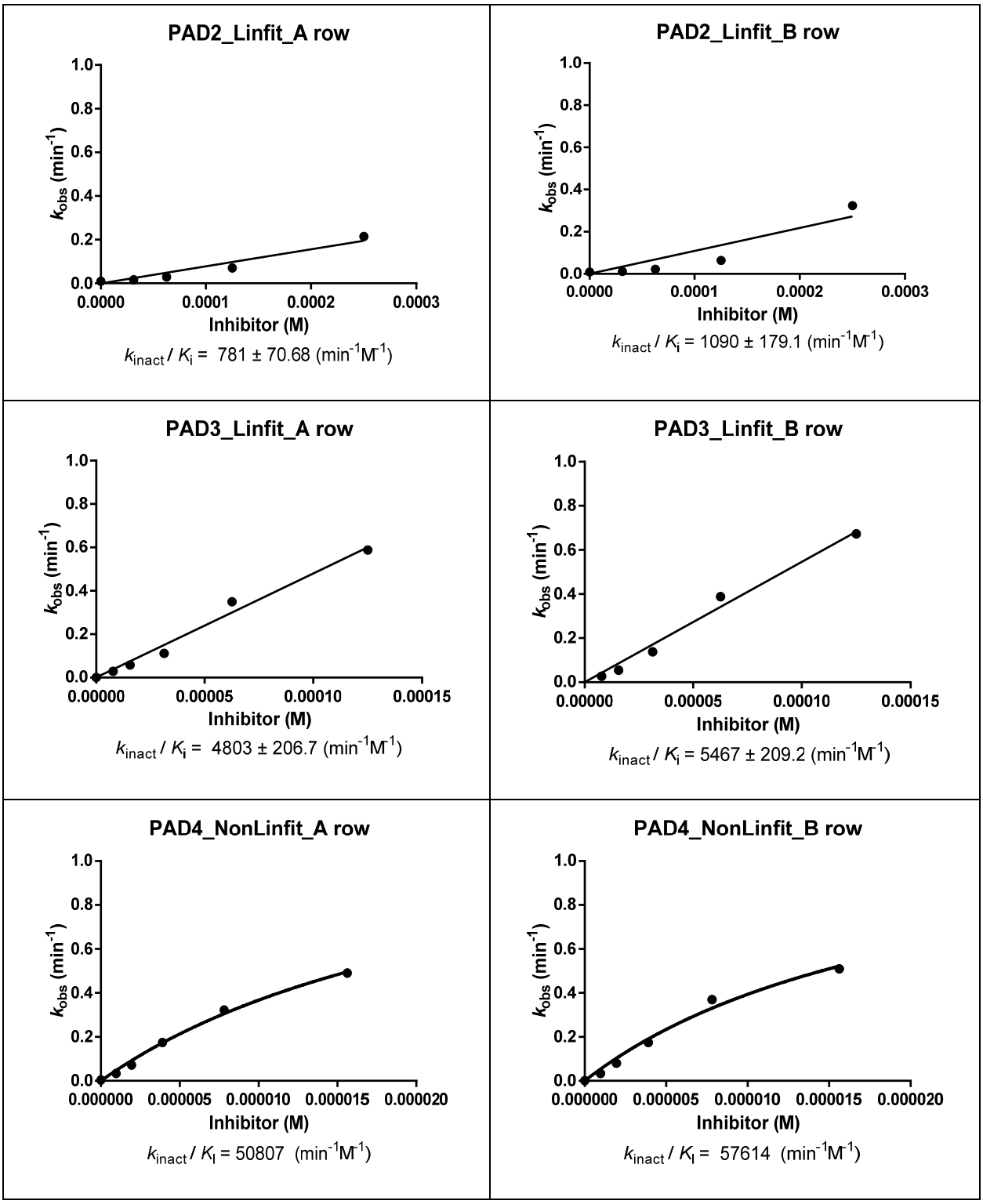


k_{inact} / K_i = 4521 ± 207.2 (min^{-1}M^{-1})

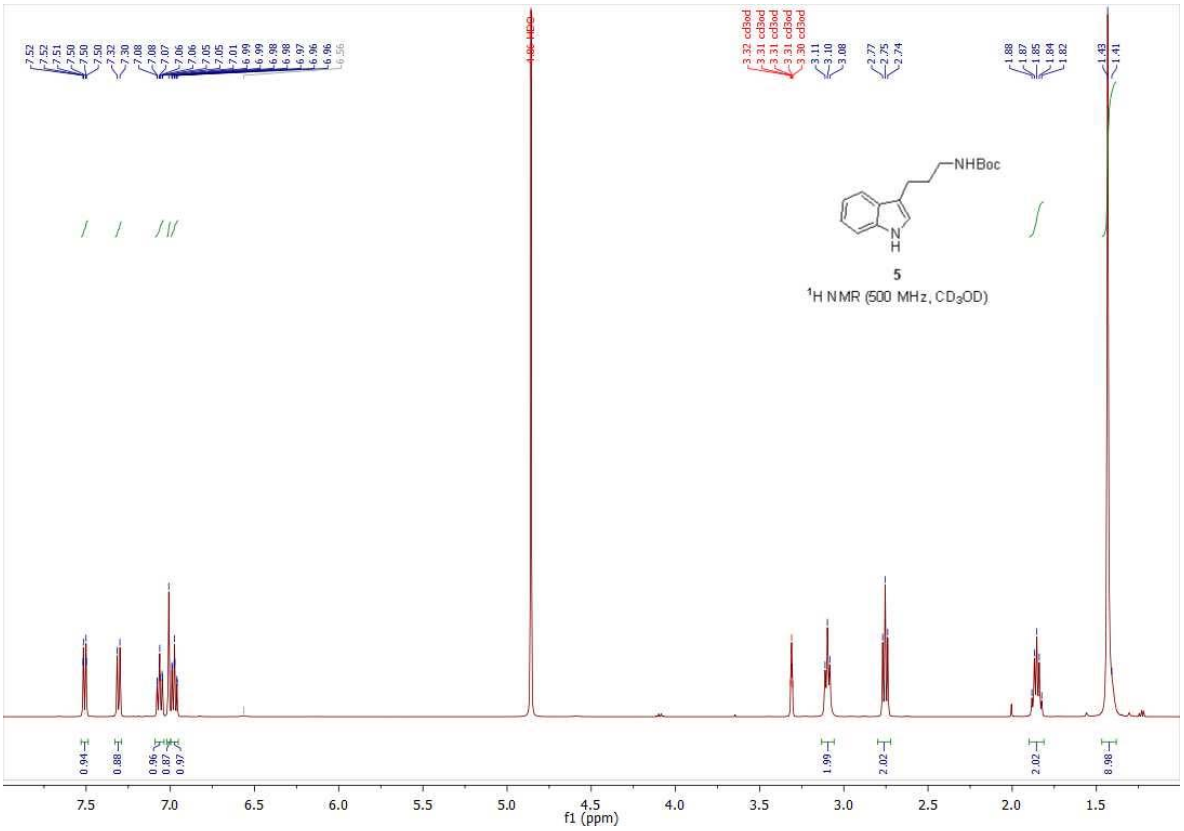


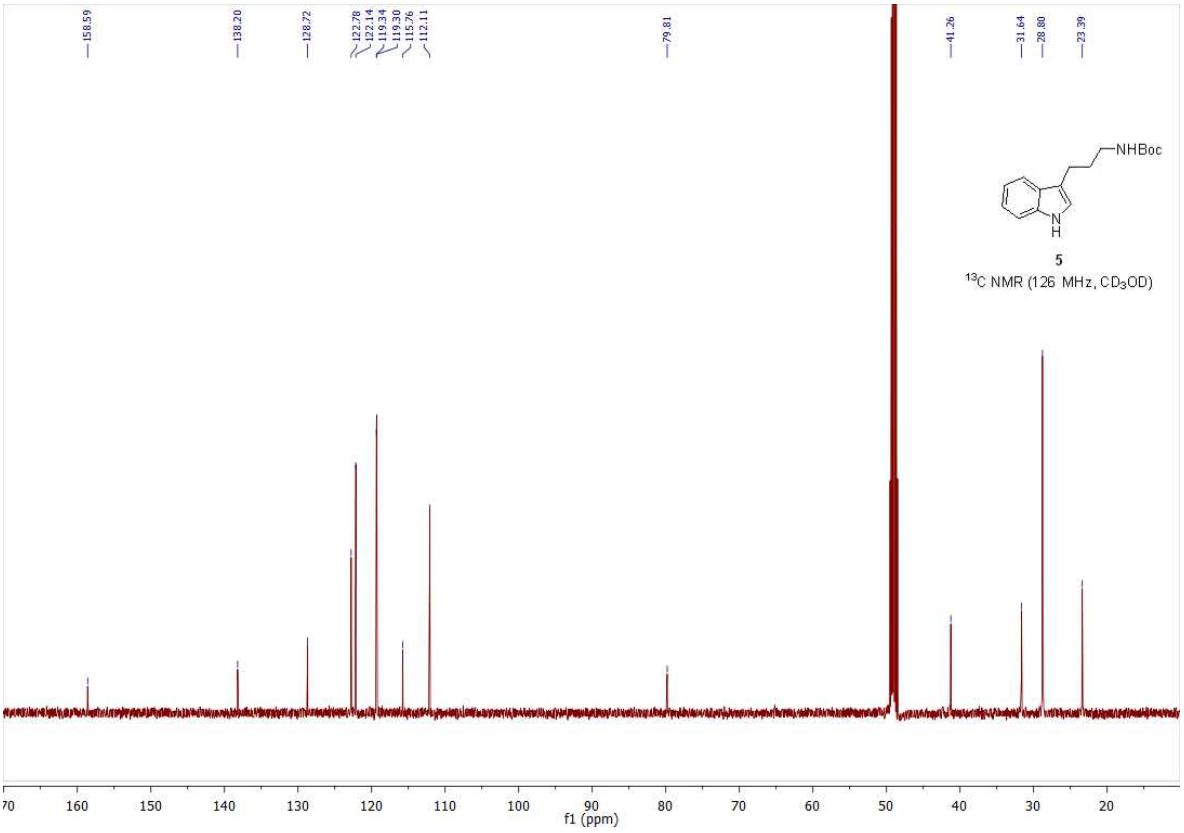
k_{inact} / K_i = 5835 ± 372.0 (min^{-1}M^{-1})

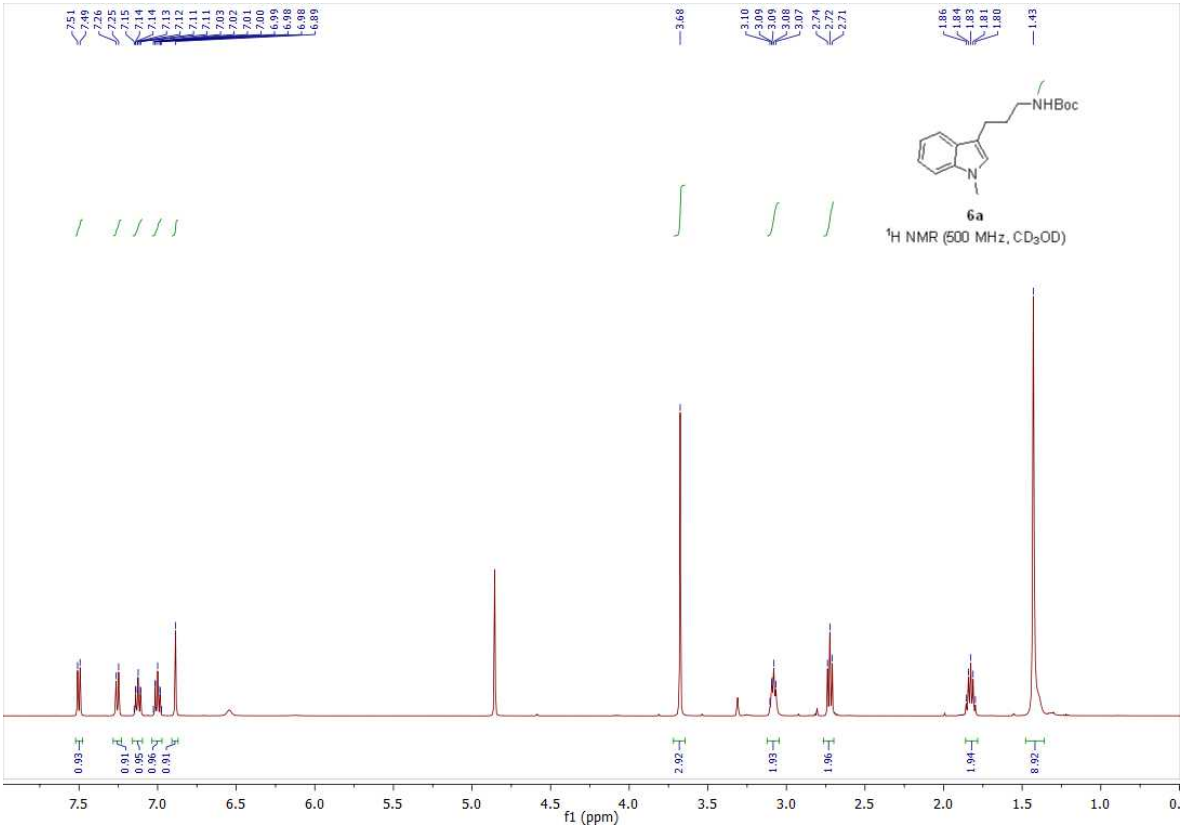

PAD4_Linfit_A row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 7000 ± 121.3 (min^{-1}M^{-1})
PAD4_Linfit_B row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 6406 ± 714.0 (min^{-1}M^{-1})
Cl
Cl
Cl
NH
N
H
Cl
N–N
24
PAD1_Linfit_A row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 21.51 ± 2.298 (min^{-1}M^{-1})
PAD1_Linfit_B row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 19.73 ± 2.282 (min^{-1}M^{-1})

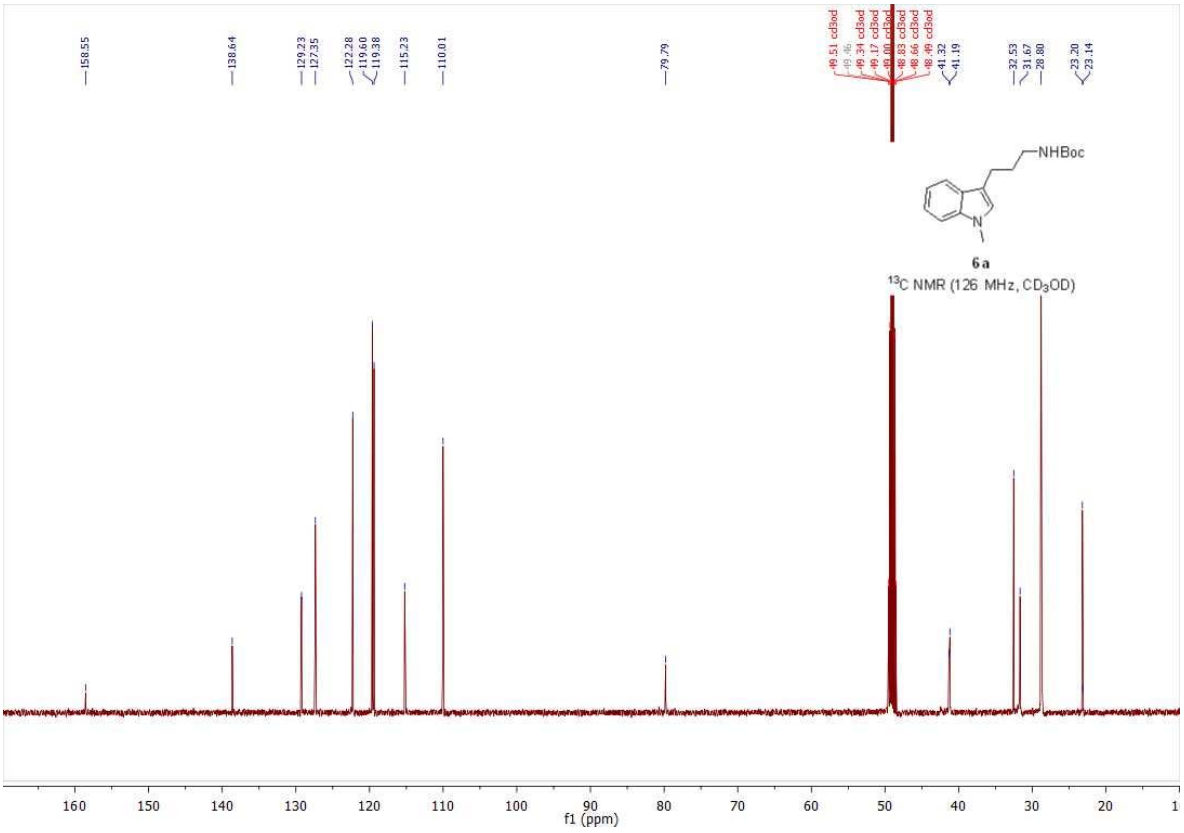

PAD2_Linfit_A row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 781 ± 70.68 (min^{-1}M^{-1})
PAD2_Linfit_B row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 1090 ± 179.1 (min^{-1}M^{-1})
PAD3_Linfit_A row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 4803 ± 206.7 (min^{-1}M^{-1})
PAD3_Linfit_B row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 5467 ± 209.2 (min^{-1}M^{-1})
PAD4_NonLinfit_A row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 50807 (min^{-1}M^{-1})
PAD4_NonLinfit_B row
k_{obs} (min^{-1})
Inhibitor (M)
k_{inact} / K_i = 57614 (min^{-1}M^{-1})

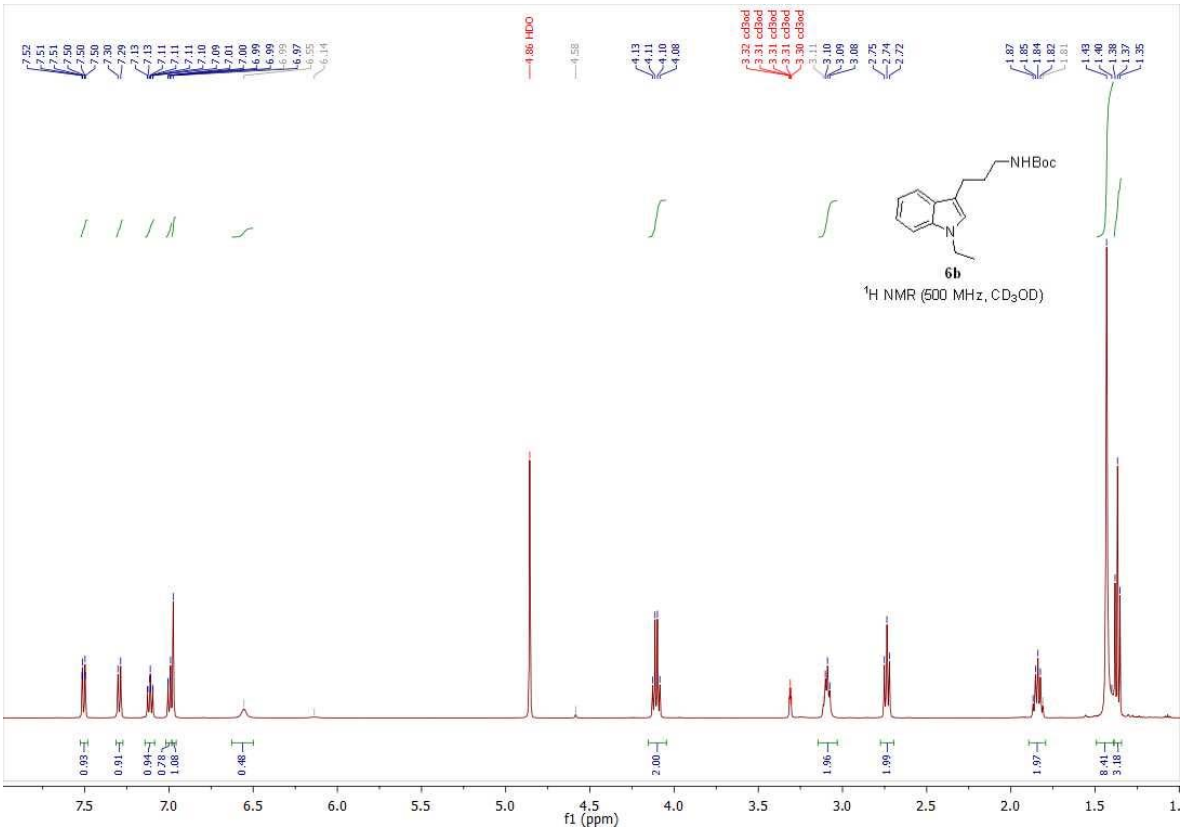
^{1}H NMR and ^{13}C NMR

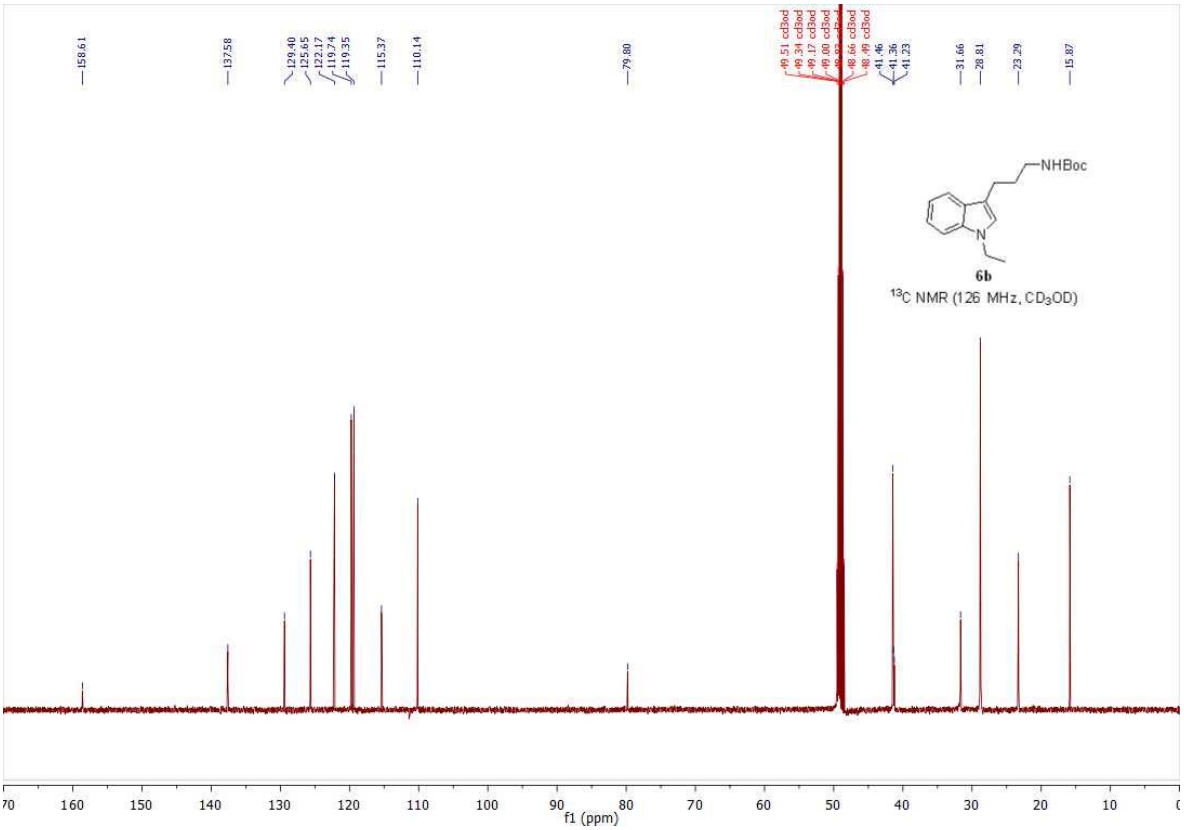


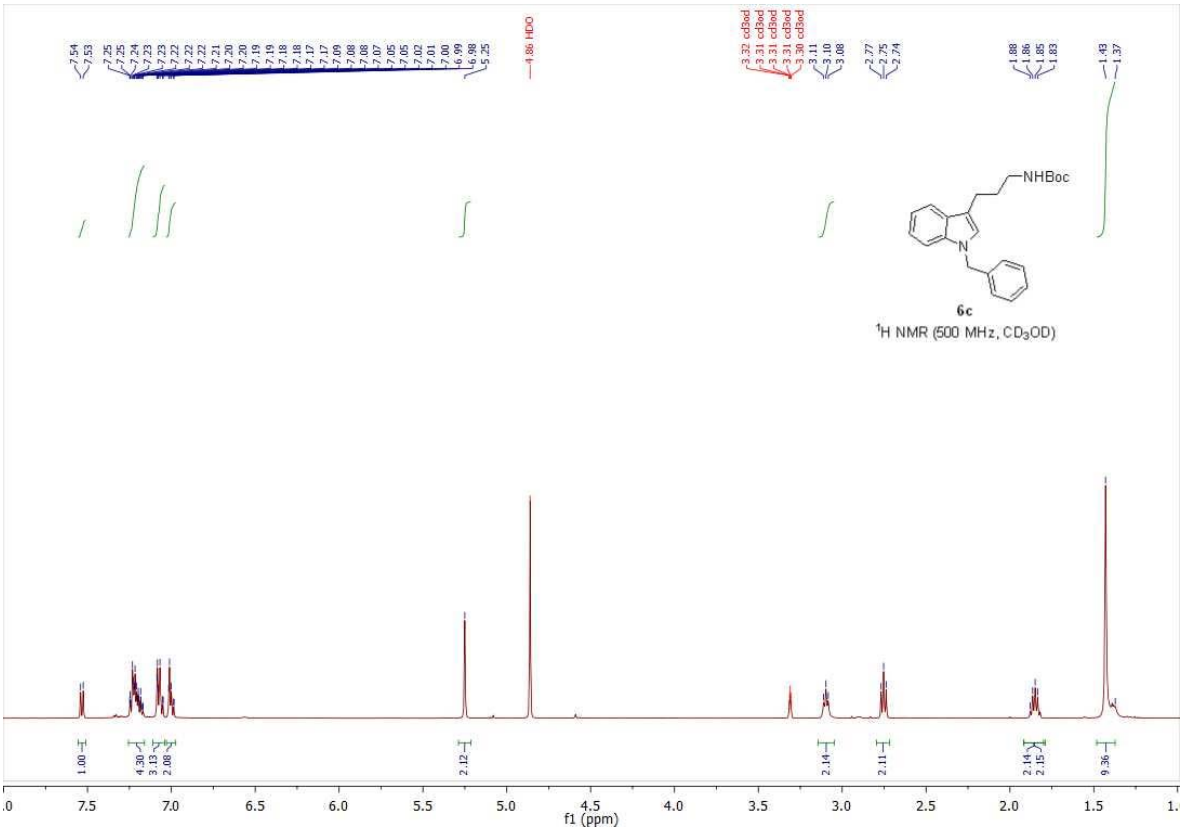

158.59
138.20
128.72
122.78
122.14
119.34
119.30
115.76
112.11
79.81
41.26
31.64
28.80
23.39
NHBoc
N
H
5
13C NMR (126 MHz, CD3OD)
f1 (ppm)

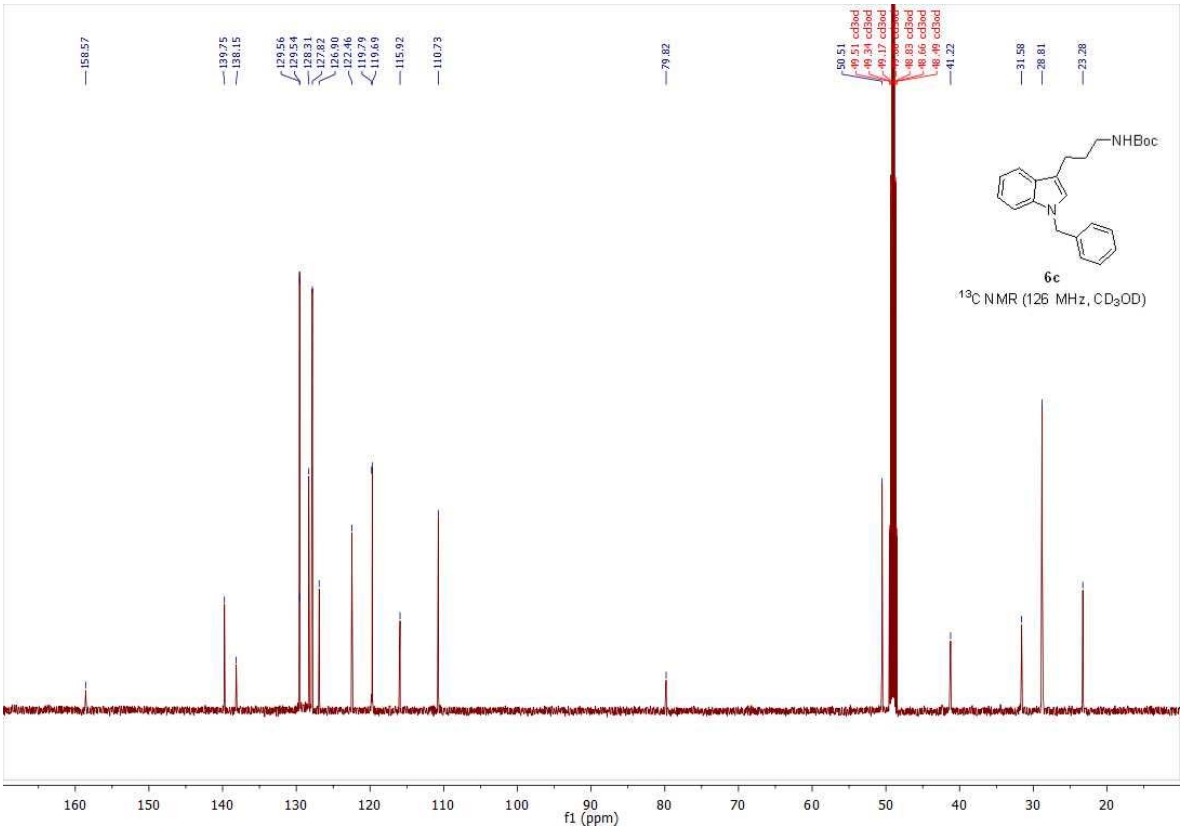

7.51
7.49
7.26
7.25
7.15
7.14
7.14
7.13
7.12
7.11
7.11
7.03
7.02
7.01
7.00
6.99
6.98
6.98
6.89
3.68
3.10
3.09
3.09
3.08
3.07
2.74
2.72
2.71
1.86
1.84
1.83
1.81
1.80
1.43
NHBoc
N
6a
1H NMR (500 MHz, CD3OD)
0.93
0.91
0.95
0.96
0.91
2.92
1.93
1.96
1.94
8.92
f1 (ppm)

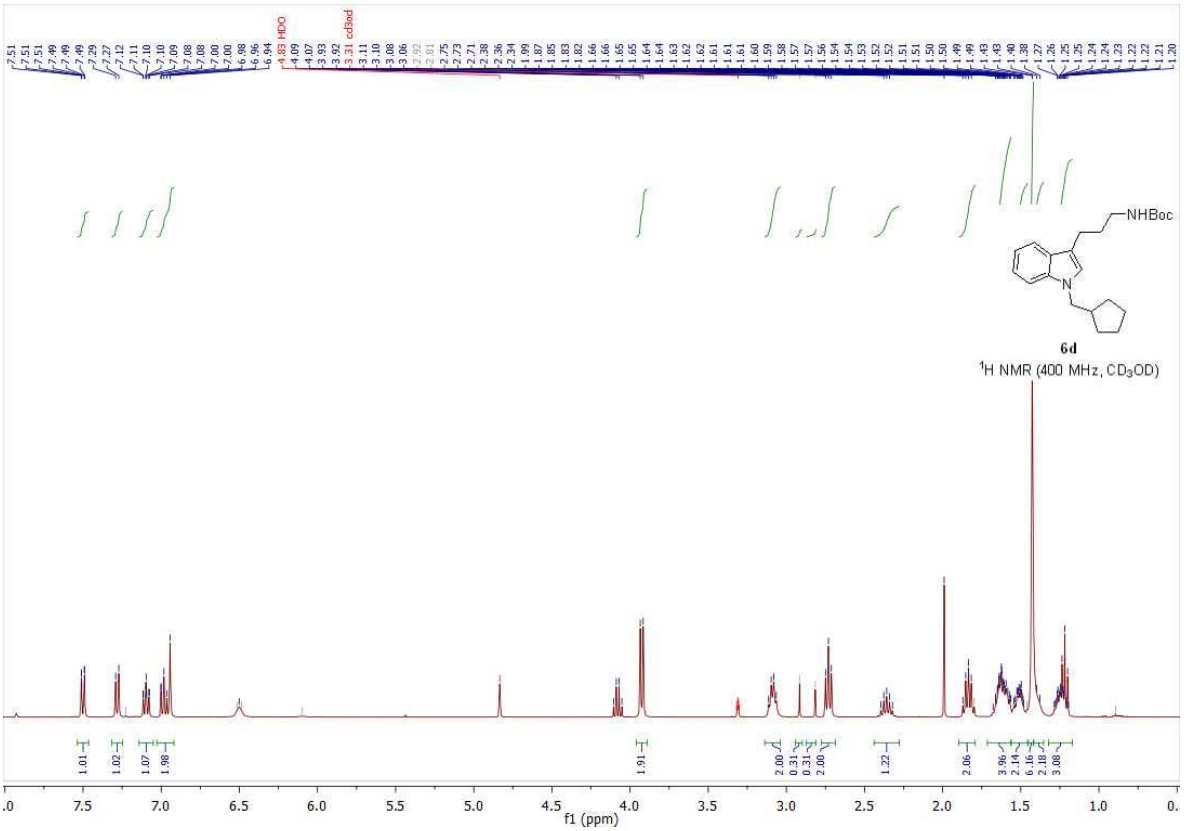

158.55
138.64
129.23
127.35
122.28
119.60
119.38
115.23
110.01
79.79
49.51 cd3od
49.36
49.21 cd3od
49.07 cd3od
48.93 cd3od
48.80 cd3od
48.66 cd3od
48.49 cd3od
41.32
41.19
32.53
31.67
28.80
23.20
23.14
NHBoc
N
6a
13C NMR (126 MHz, CD3OD)
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
1
f1 (ppm)

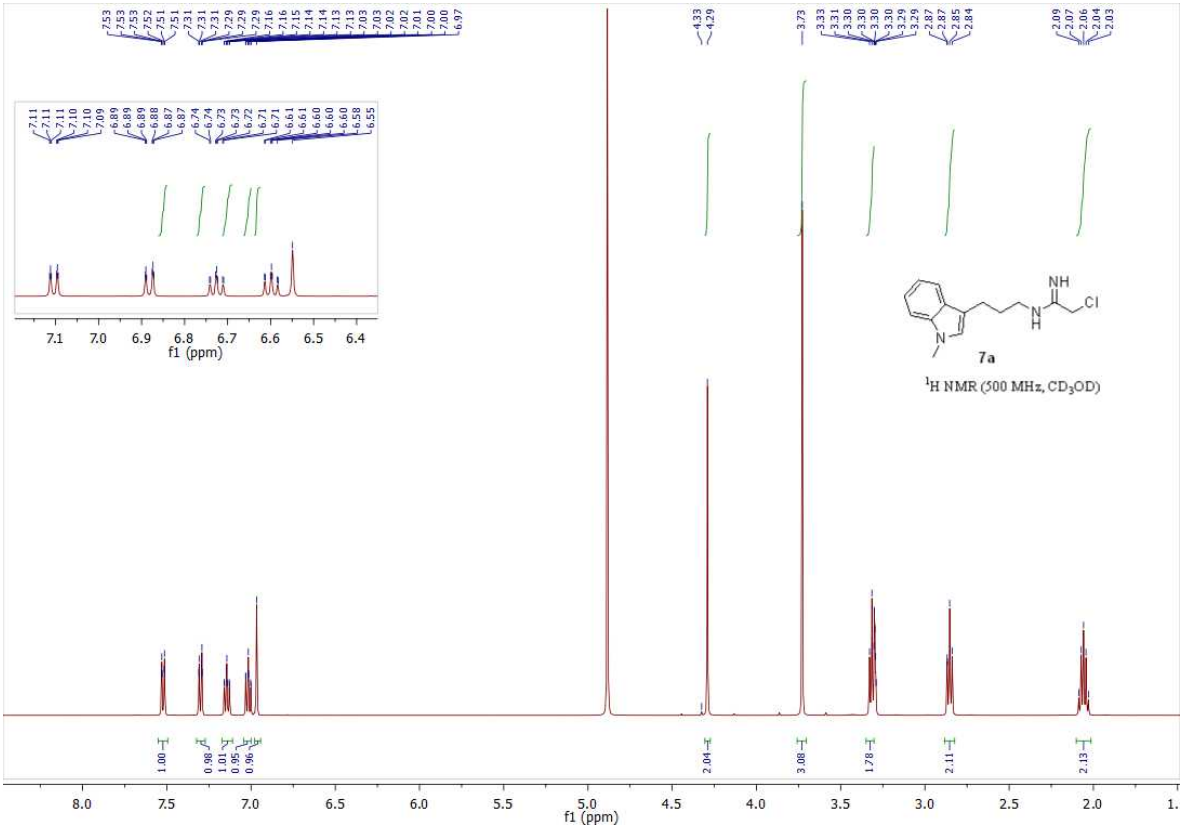

NHBoc
6b
^{1}H NMR (500 MHz, CD_3OD)
f1 (ppm)

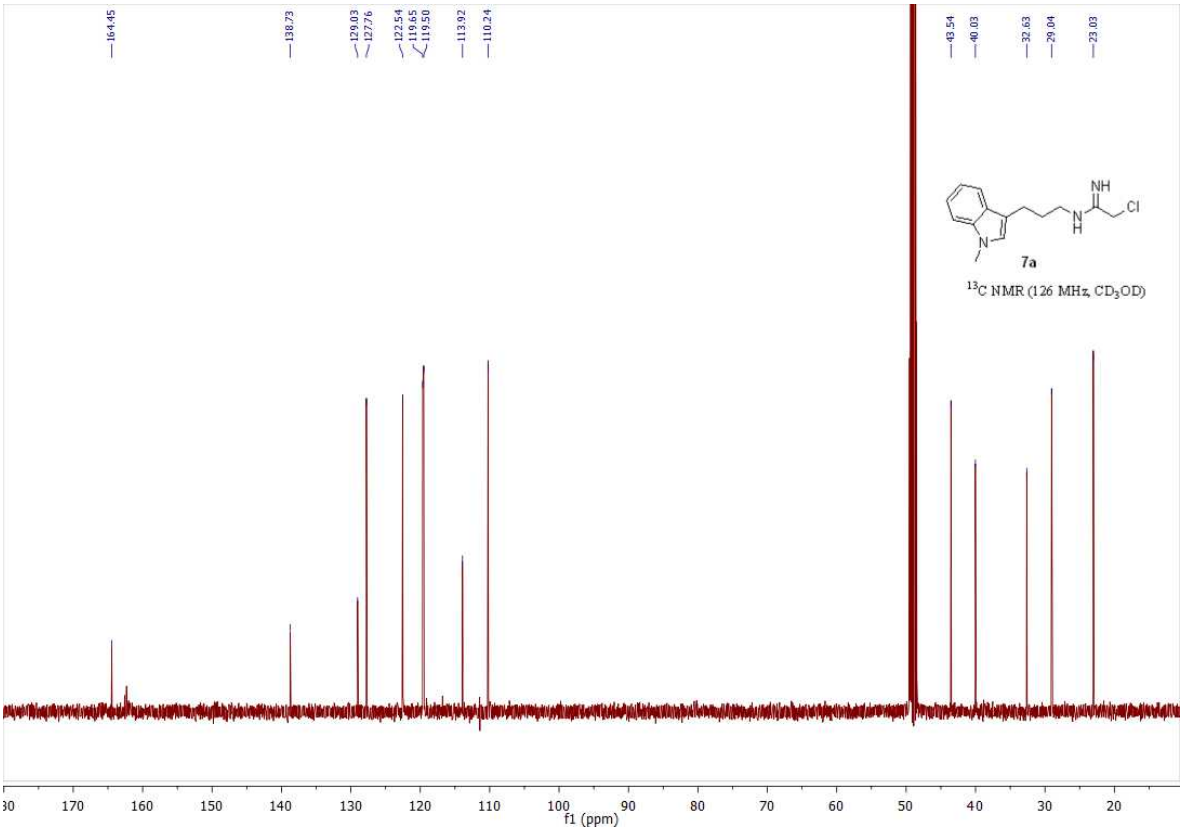

NHBoc
6b
^{13}C NMR (126 MHz, CD_3OD)
f1 (ppm)

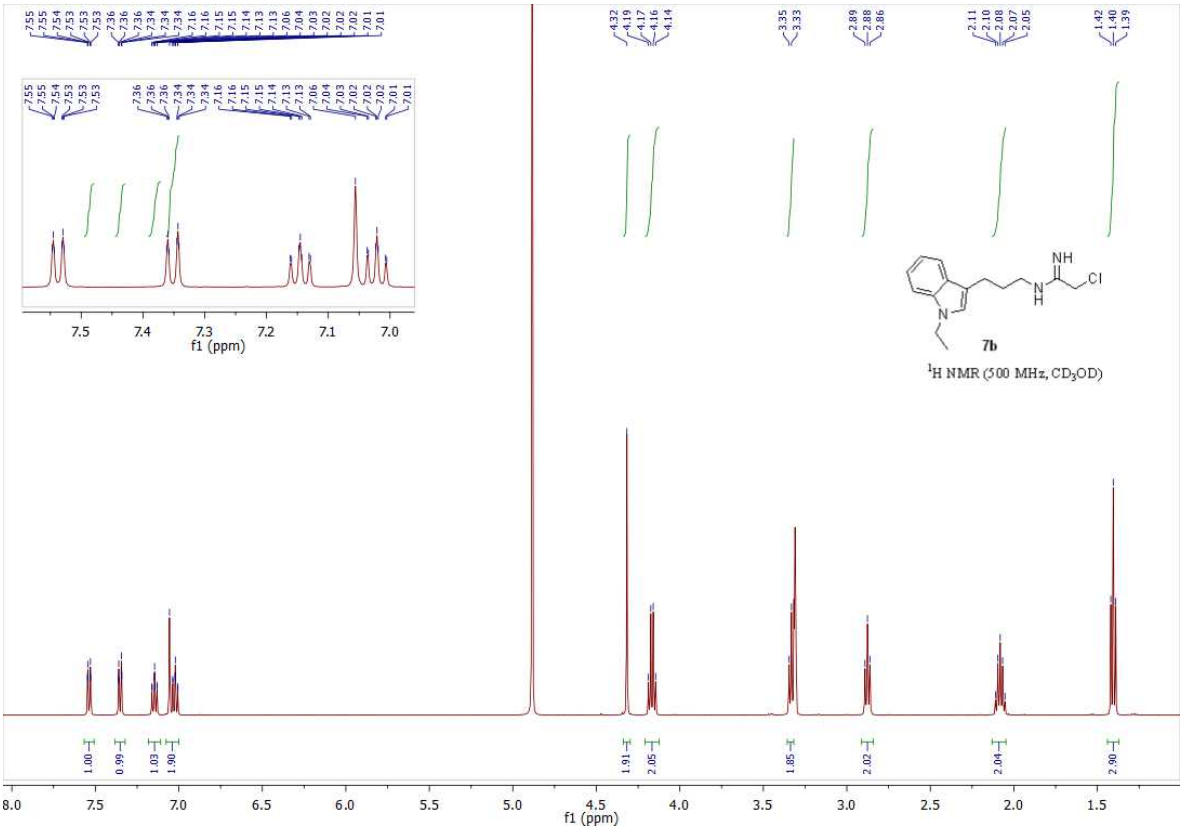

NHBoc
6c
^{1}H NMR (500 MHz, CD$_3$OD)
f1 (ppm)

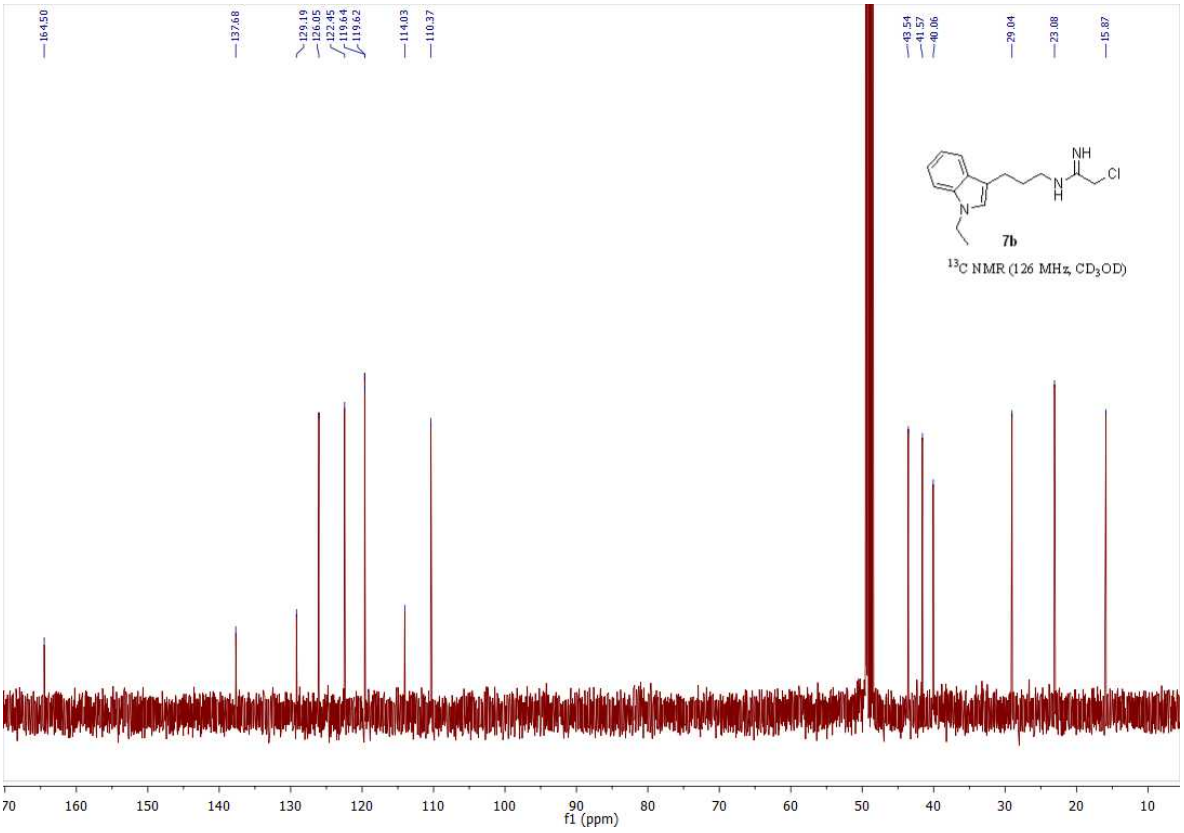

NHBoc
6c
^{13}C NMR (126 MHz, CD$_3$OD)
f1 (ppm)

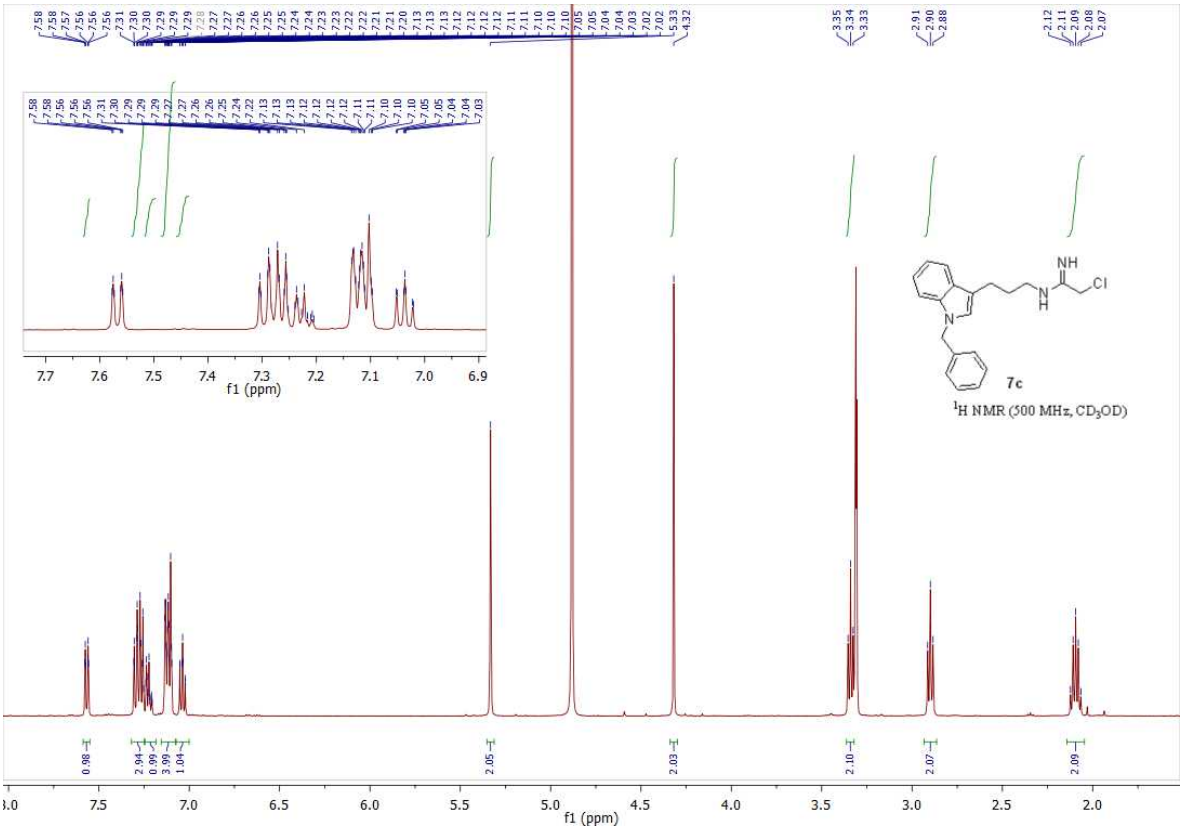

NHBoc
6d
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

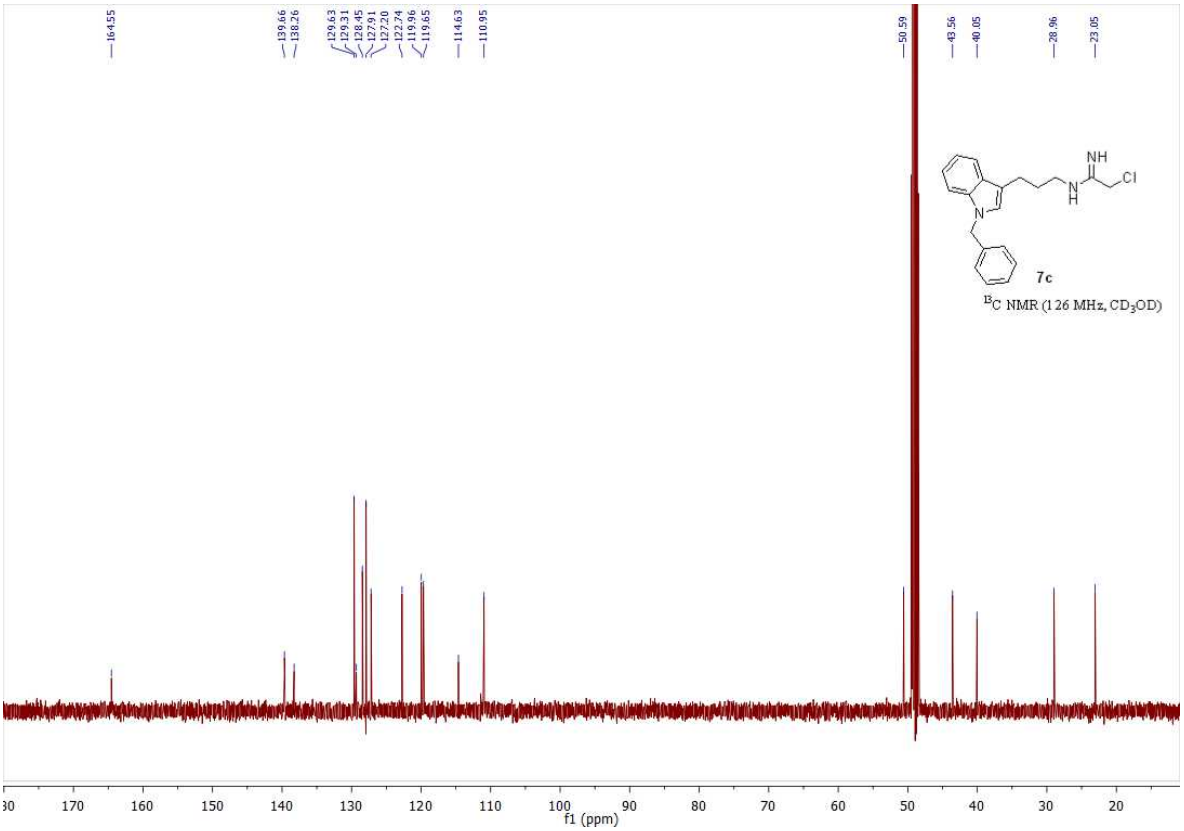

NH
Cl
7a
^{1}H NMR (500 MHz, CD_3OD)
f1 (ppm)

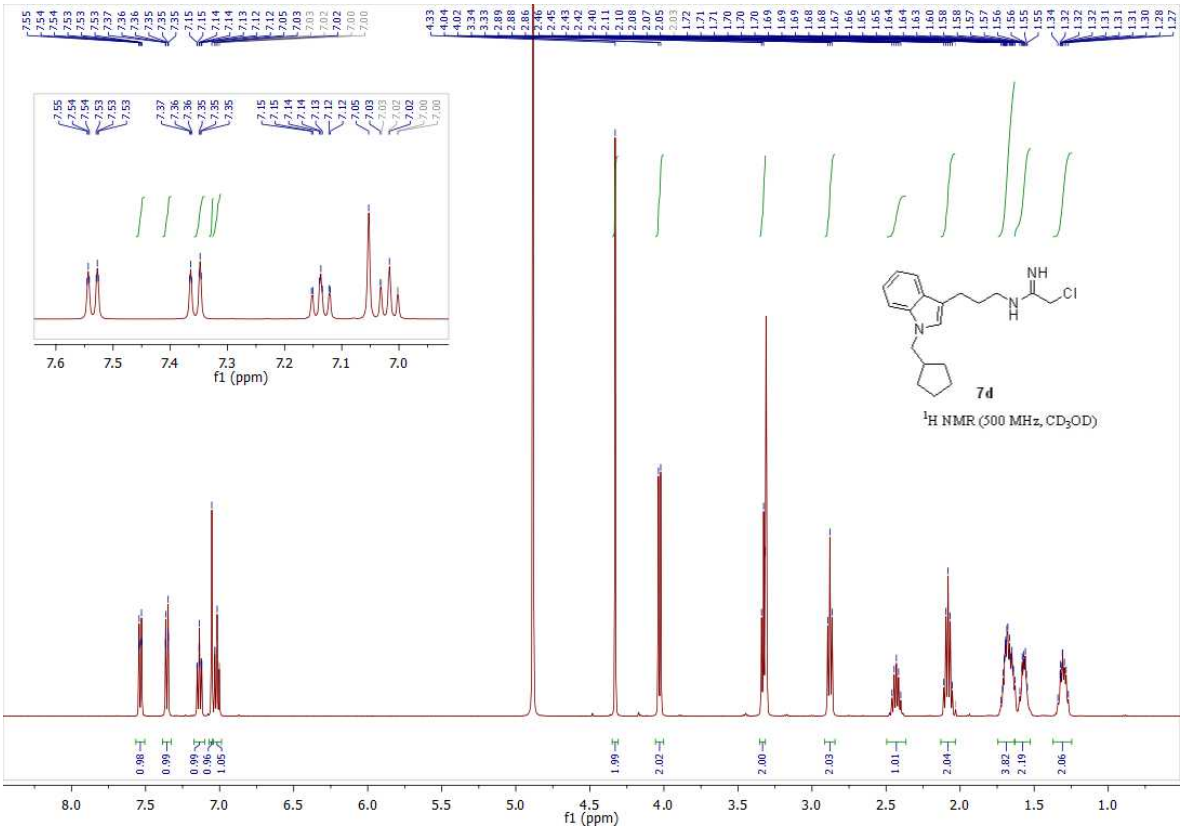

164.45
138.73
129.03
127.76
122.54
119.65
119.50
113.92
110.24
49.54
40.03
32.63
29.04
23.03
NH
Cl
N
H
N
7a
^{13}C NMR (126 MHz, CD$_3$OD)
f1 (ppm)

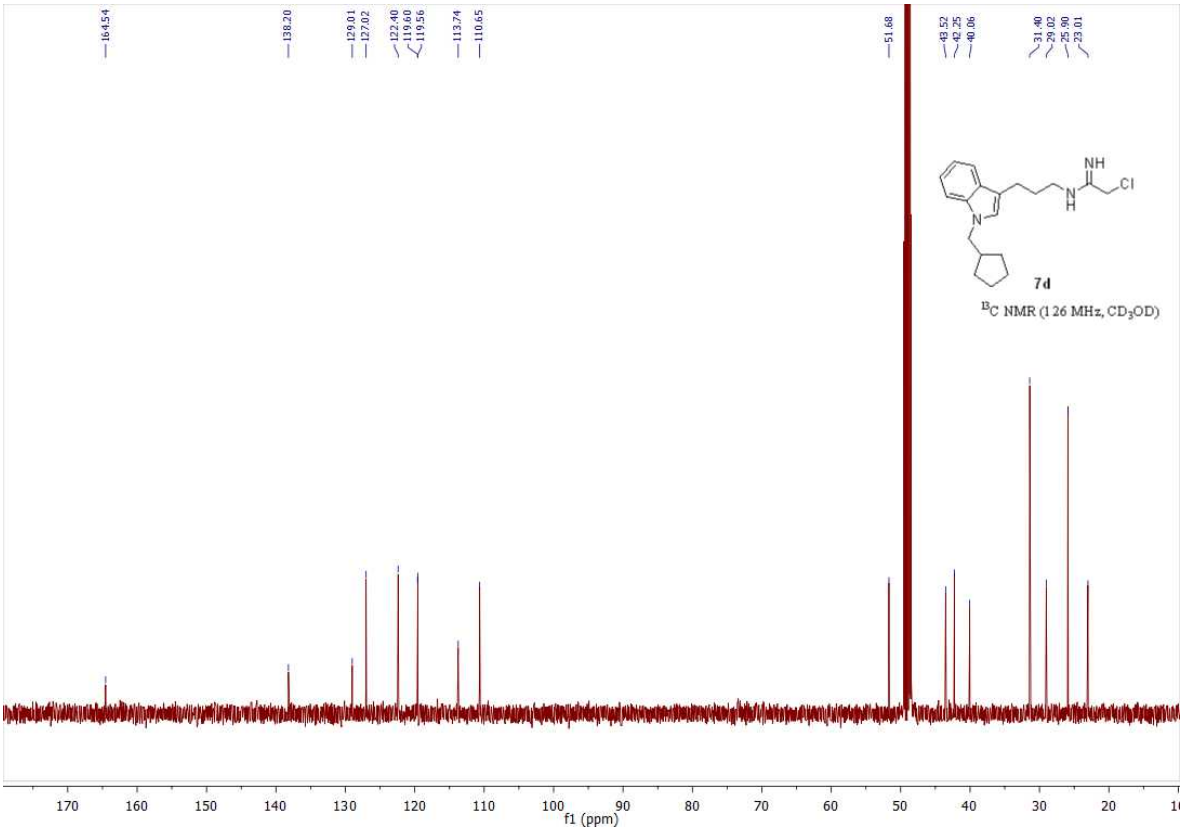

7.55
7.54
7.53
7.36
7.34
7.16
7.15
7.14
7.13
7.06
7.04
7.03
7.02
7.01
4.32
4.19
4.17
4.16
4.14
3.35
3.33
2.89
2.88
2.86
2.11
2.10
2.08
2.07
2.05
1.42
1.40
1.39
NH
Cl
N
H
N
7b
^{1}H NMR (500 MHz, CD$_3$OD)
1.00
0.99
1.03
1.90
1.91
2.05
1.85
2.02
2.04
2.90
f1 (ppm)

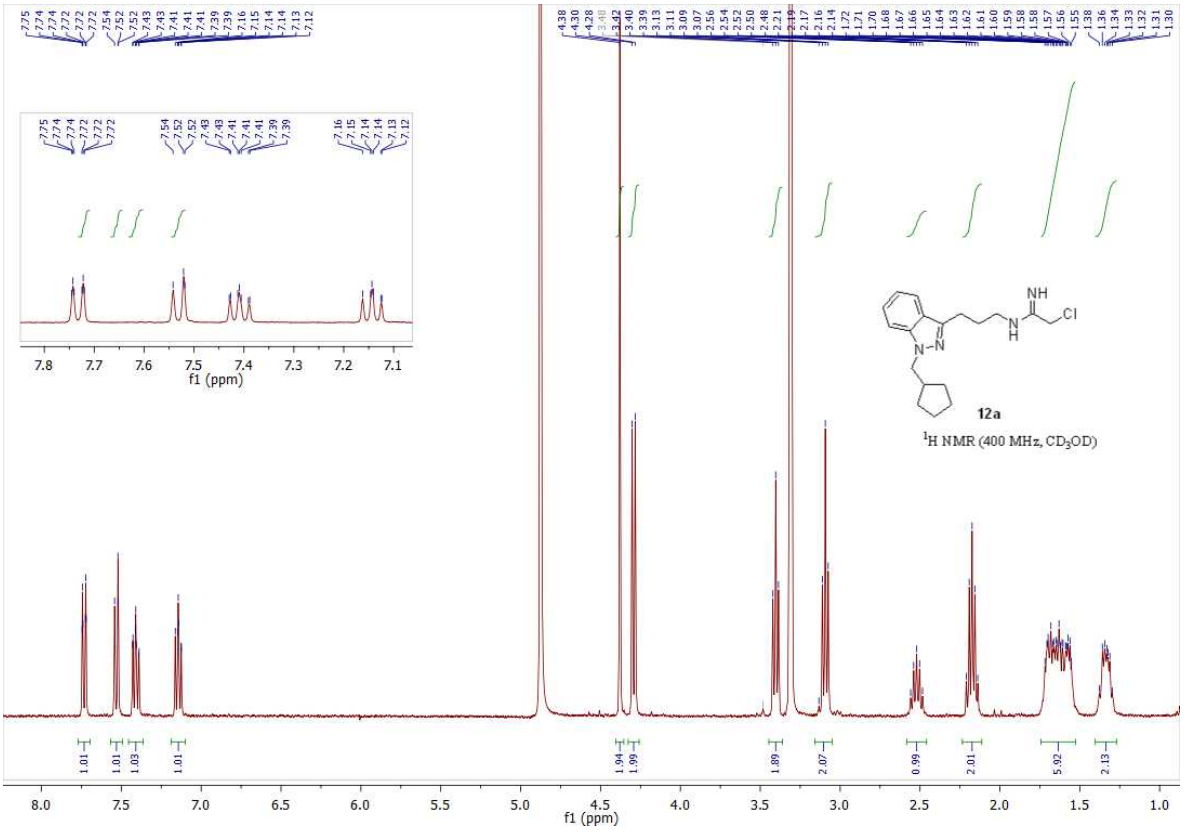

164.50
137.68
129.19
126.05
122.45
119.64
119.62
114.03
110.37
43.54
41.57
40.06
29.04
23.08
15.87
NH
N
H
N
Cl
7b
^{13}C NMR (126 MHz, CD$_3$OD)
f1 (ppm)

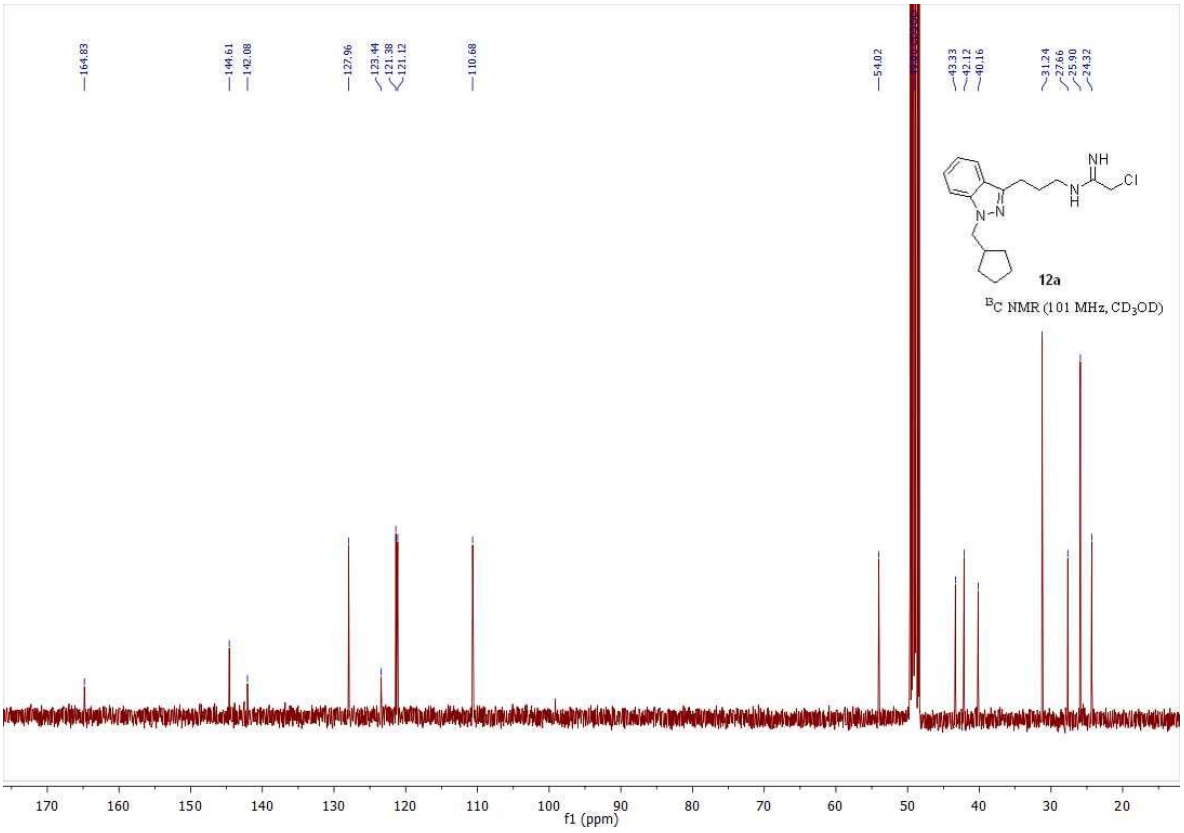

7.58
7.56
7.31
7.30
7.29
7.27
7.26
7.25
7.24
7.22
7.13
7.12
7.11
7.10
7.05
7.04
7.03
7.02
5.33
4.32
3.35
3.34
3.33
2.91
2.90
2.88
2.12
2.11
2.09
2.08
2.07
NH
N
H
N
Cl
7c
^{1}H NMR (500 MHz, CD$_3$OD)
0.98
2.94
0.99
3.99
1.04
2.05
2.03
2.10
2.07
2.09
f1 (ppm)

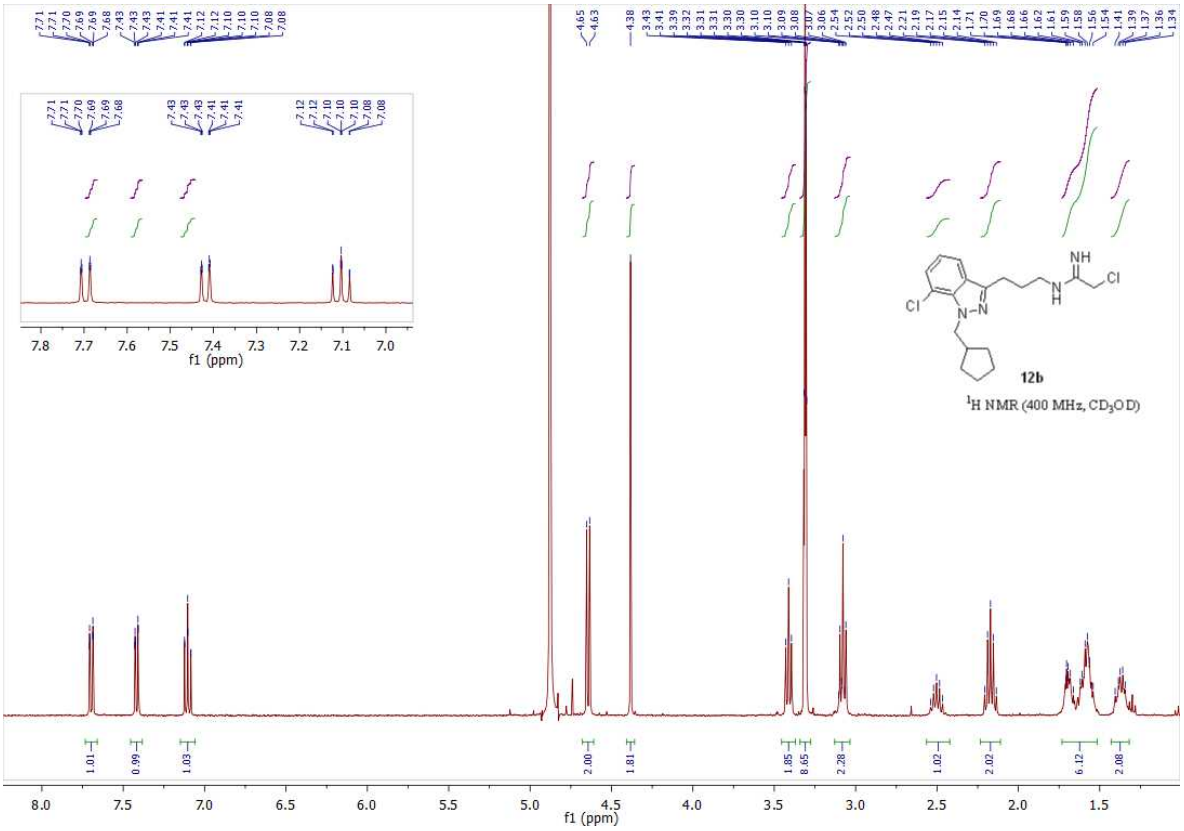

164.55
139.66
138.26
129.63
129.31
128.45
127.91
127.30
122.74
119.96
119.65
114.63
110.95
50.59
43.56
40.05
28.96
23.05
NH
N
H
N
Cl
7c
^{13}C NMR (126 MHz, CD$_3$OD)
f1 (ppm)

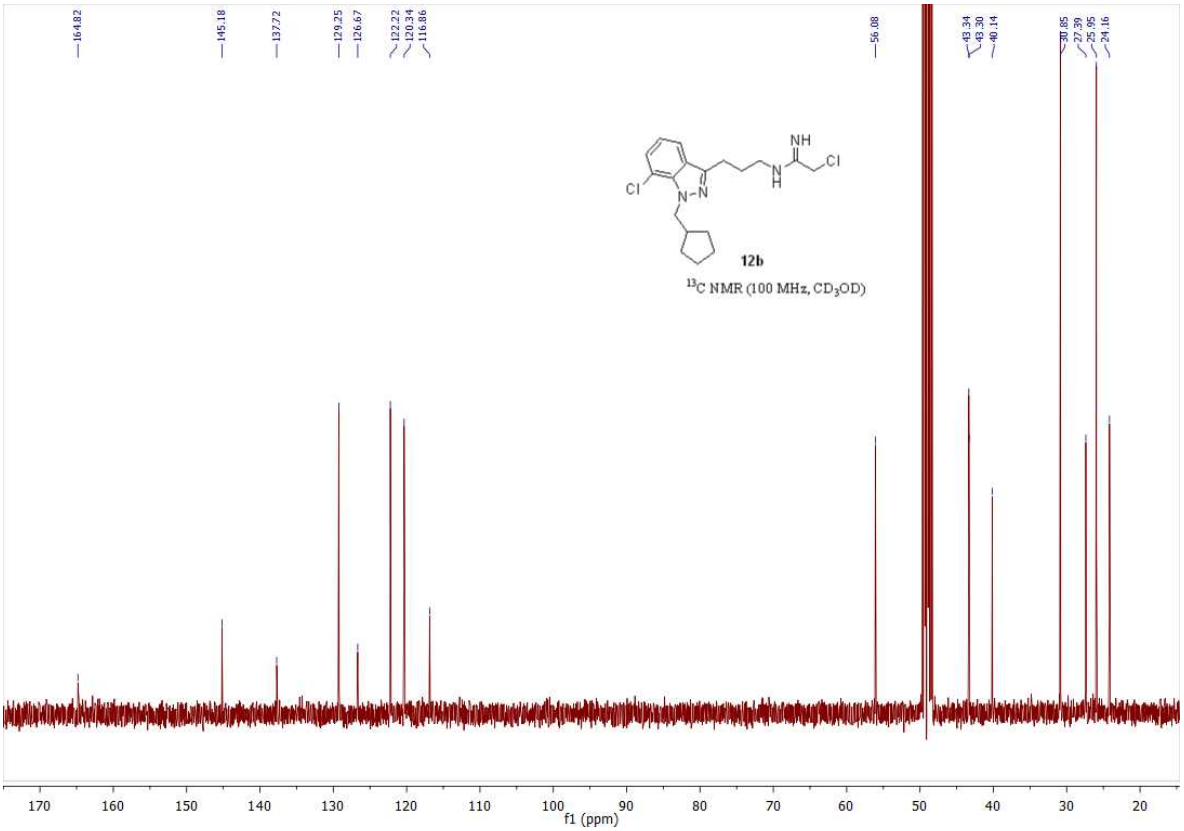

f1 (ppm)
NH
N
H
N
Cl
7d
^{1}H NMR (500 MHz, CD$_3$OD)
f1 (ppm)

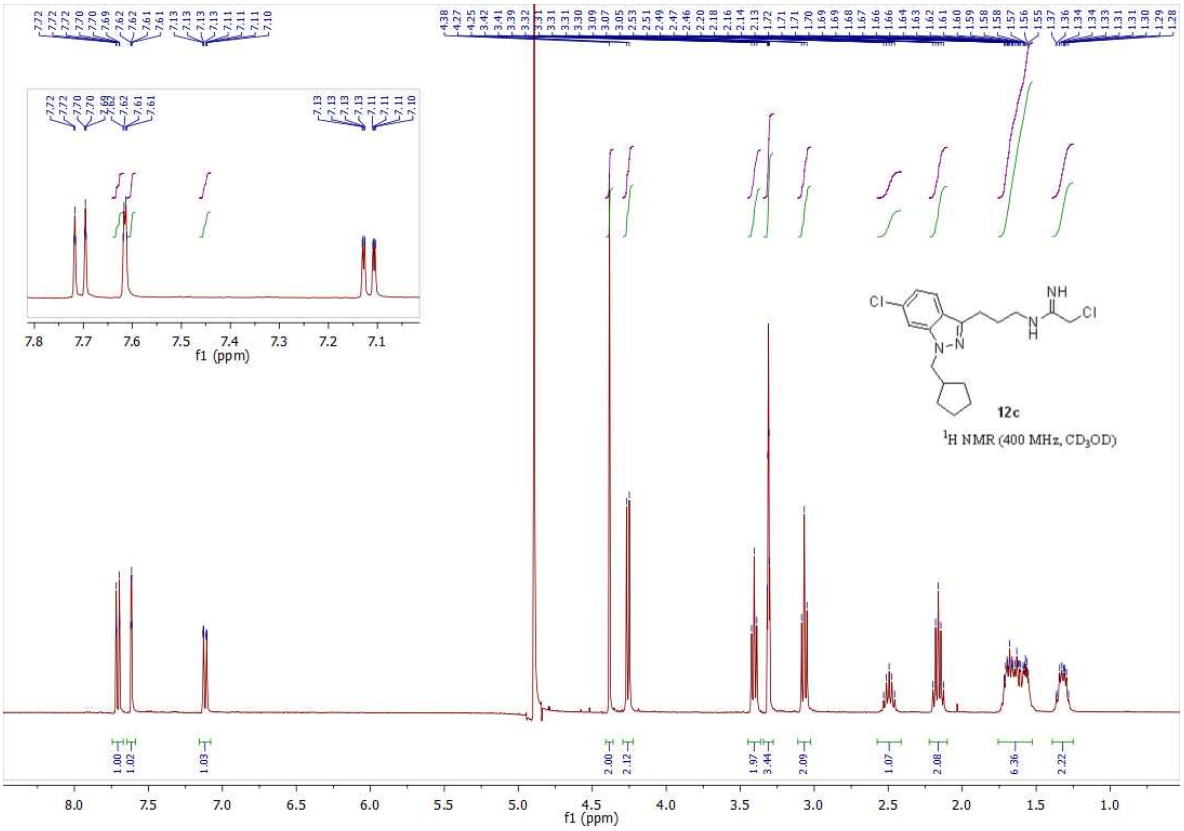

164.54
138.20
129.01
127.02
122.40
119.60
119.56
113.74
110.65
51.68
49.52
42.25
40.86
31.40
29.02
25.90
23.01
NH
Cl
N
H
N
7d
^{13}C NMR (126 MHz, CD$_3$OD)
f1 (ppm)

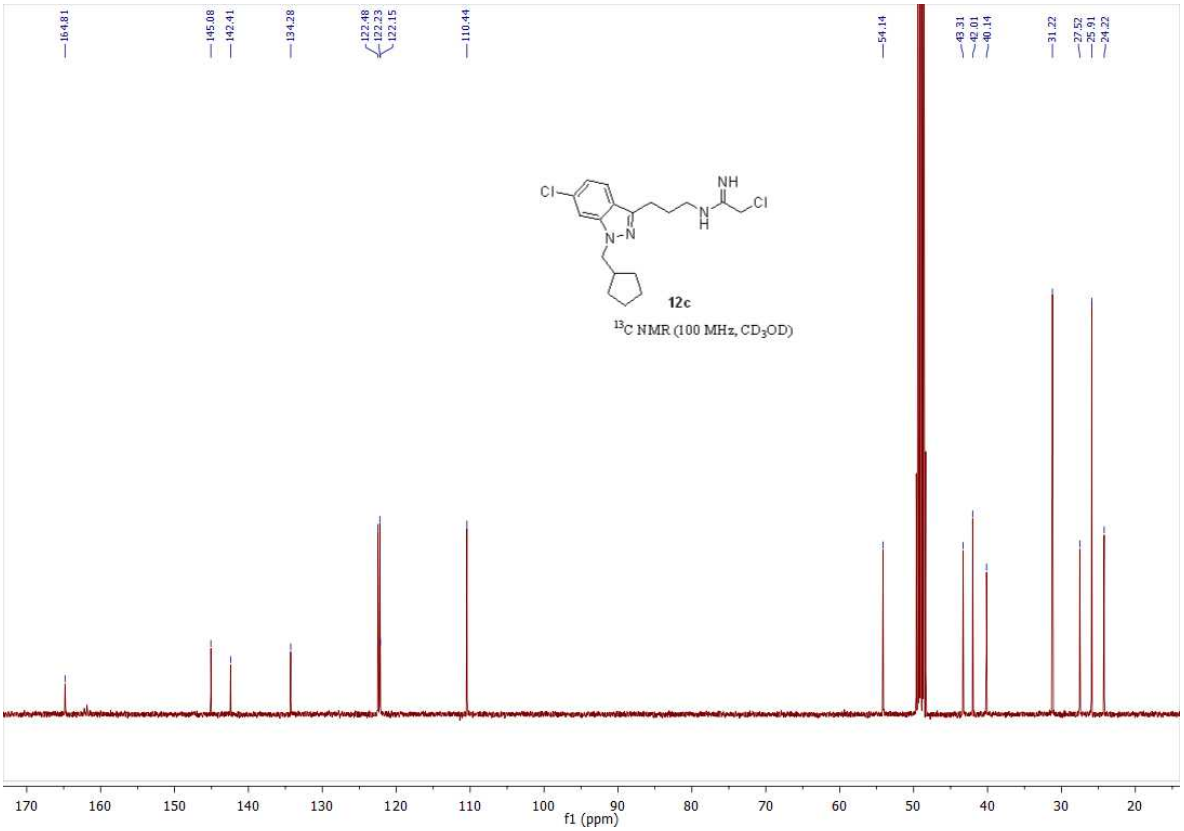

NH
Cl
N
H
N-N
12a
^{1}H NMR (400 MHz, CD$_3$OD)
f1 (ppm)

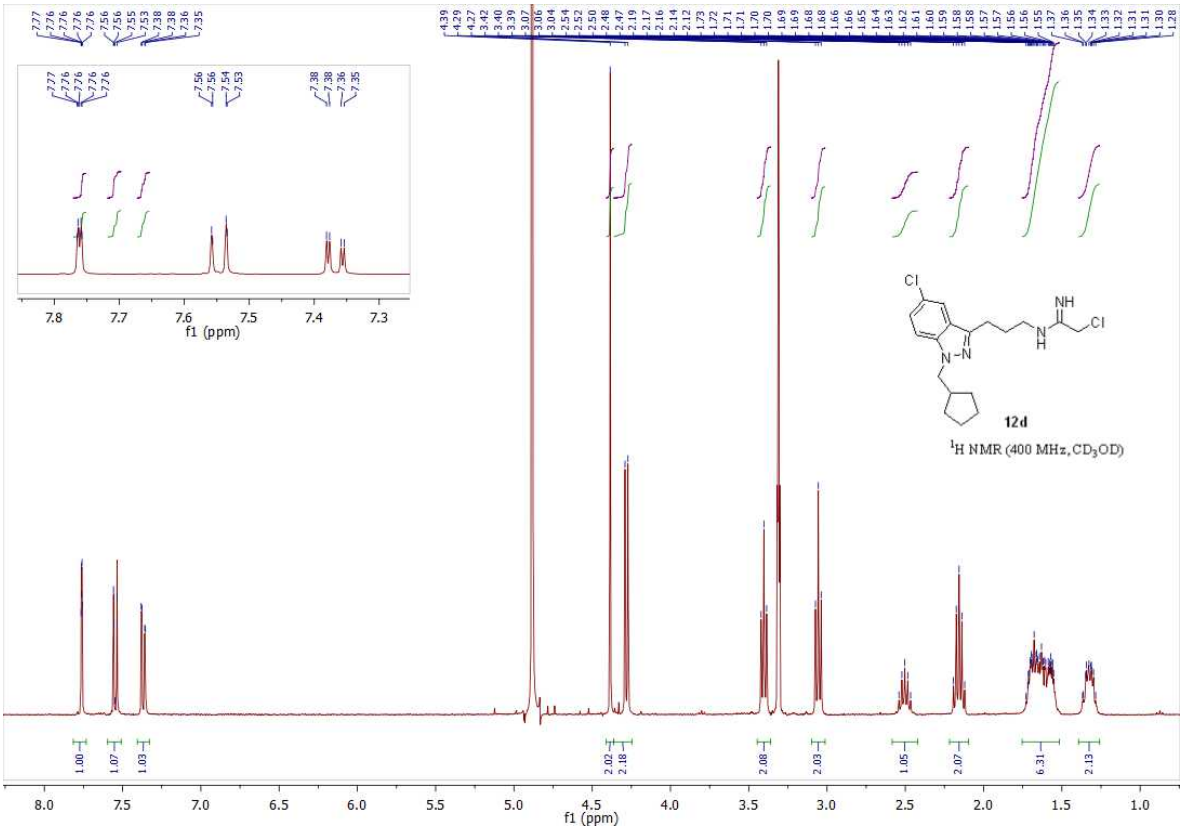

164.83
144.61
142.08
127.96
123.44
121.38
121.12
110.68
54.02
43.33
42.12
40.16
31.24
27.86
25.90
24.32
NH
Cl
N-N
12a
^{13}C NMR (101 MHz, CD$_3$OD)
f1 (ppm)

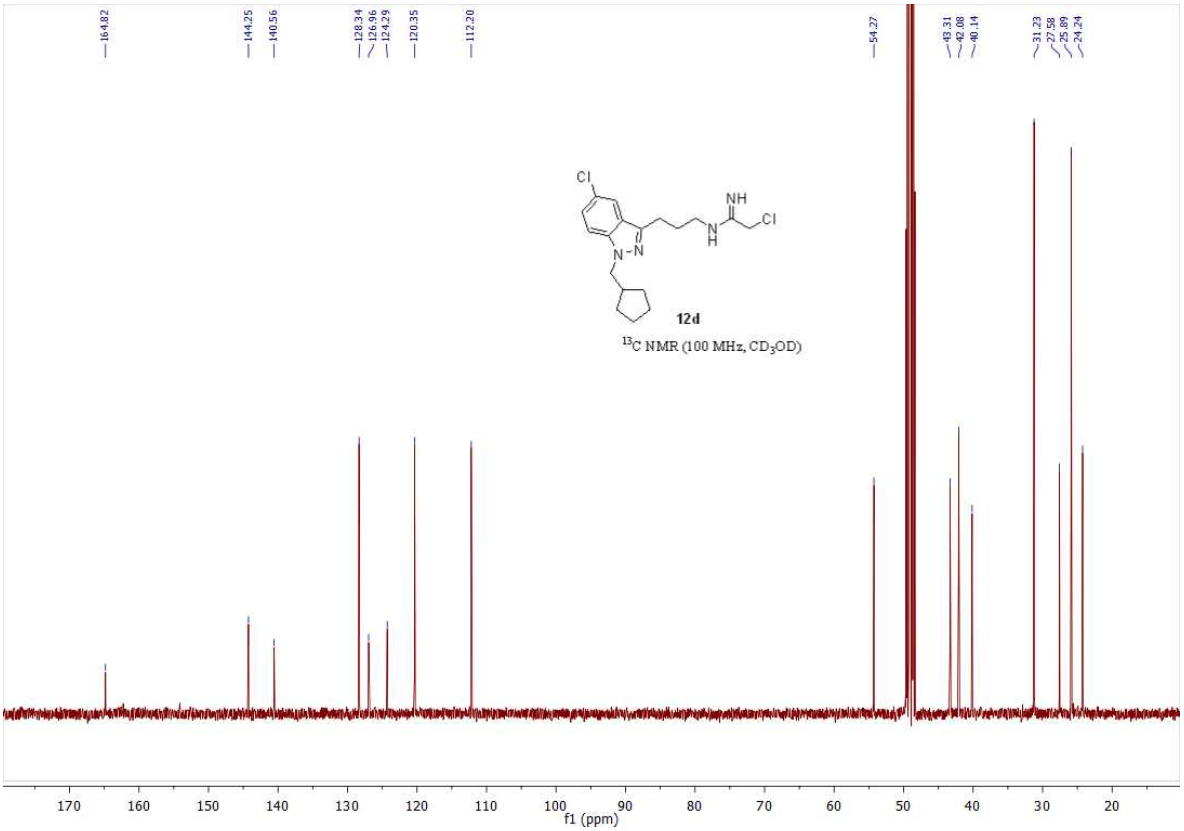

7.71
7.71
7.70
7.69
7.69
7.68
7.43
7.43
7.41
7.41
7.12
7.10
7.10
7.08
7.08
4.65
4.63
4.38
3.43
3.41
3.39
3.32
3.31
3.31
3.30
3.30
3.10
3.09
3.08
3.07
3.06
2.54
2.52
2.50
2.48
2.27
2.21
2.19
2.17
2.15
2.14
1.71
1.70
1.69
1.68
1.66
1.62
1.61
1.59
1.58
1.56
1.54
1.41
1.39
1.37
1.36
1.34
NH
Cl
Cl
N-N
12b
^{1}H NMR (400 MHz, CD$_3$OD)
f1 (ppm)
1.01
0.99
1.03
2.00
1.81
1.85
8.65
2.28
1.02
2.02
6.12
2.08

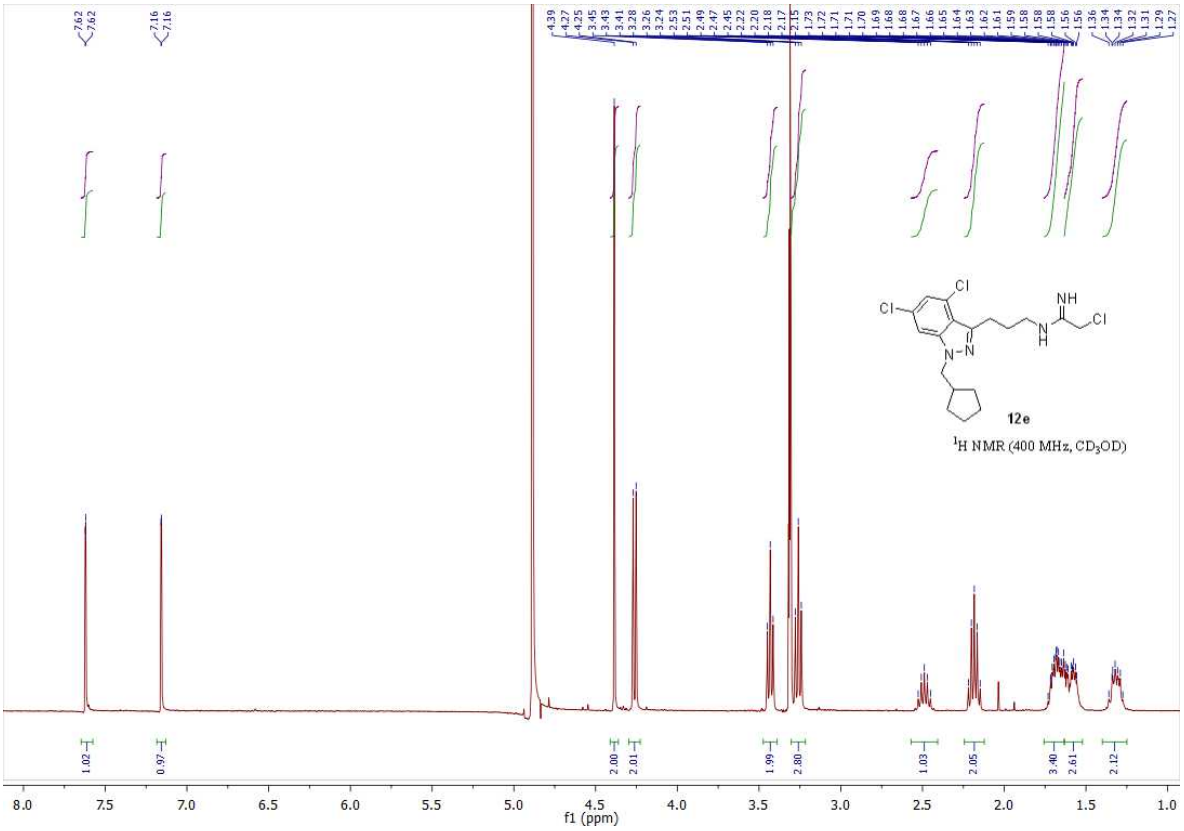

164.82
145.18
137.72
129.25
126.67
122.22
120.34
116.86
56.08
43.34
43.30
40.14
30.85
27.39
25.95
24.16
NH
Cl
Cl
N-N
12b
13C NMR (100 MHz, CD3OD)
f1 (ppm)

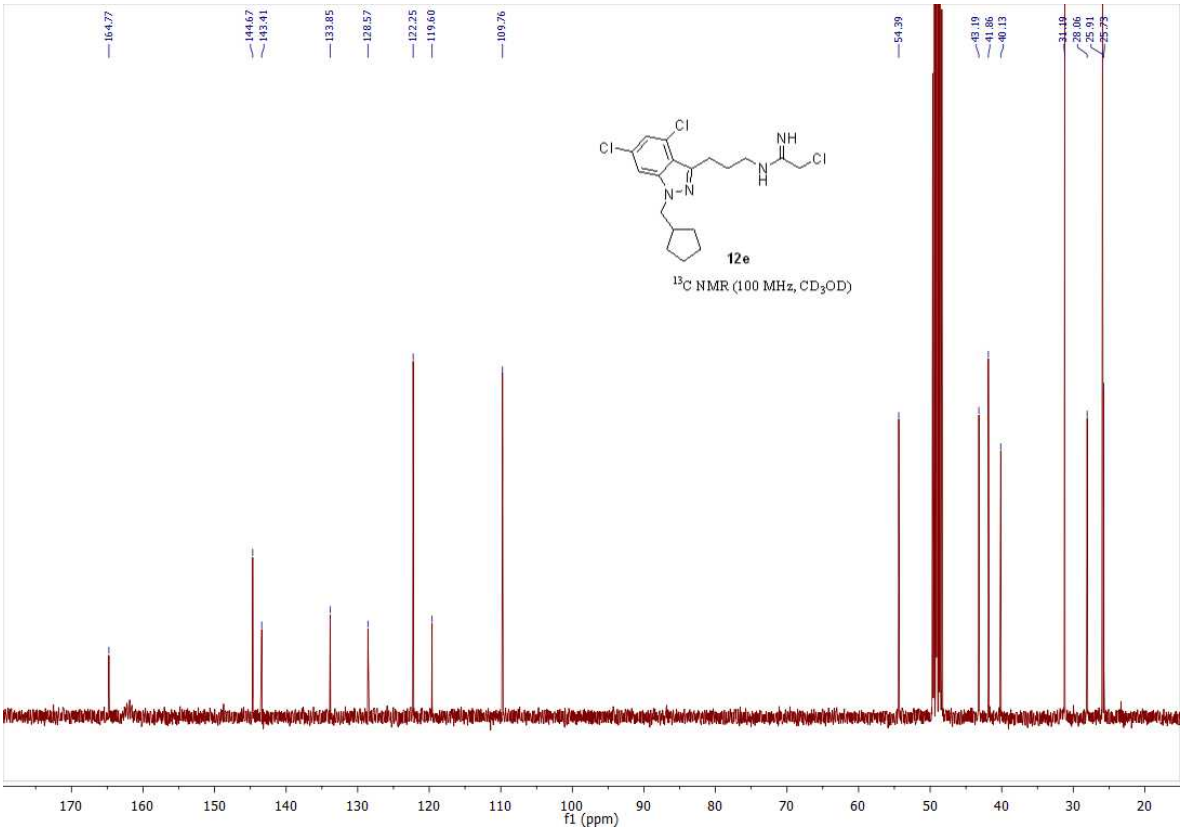

7.72
7.72
7.72
7.70
7.70
7.69
7.62
7.62
7.61
7.61
7.13
7.13
7.13
7.11
7.11
7.11
7.10
4.38
4.27
4.25
3.42
3.41
3.39
3.32
3.31
3.31
3.31
3.30
3.09
3.07
3.05
2.53
2.51
2.49
2.47
2.46
2.20
2.18
2.16
2.14
2.13
1.72
1.71
1.71
1.70
1.69
1.69
1.68
1.67
1.66
1.66
1.64
1.63
1.62
1.61
1.60
1.59
1.58
1.58
1.57
1.56
1.55
1.37
1.36
1.34
1.34
1.33
1.31
1.30
1.29
1.28
NH
Cl
Cl
N-N
12c
1H NMR (400 MHz, CD3OD)
f1 (ppm)
1.00
1.02
1.03
2.00
2.12
1.97
3.44
2.09
1.07
2.08
6.36
2.22

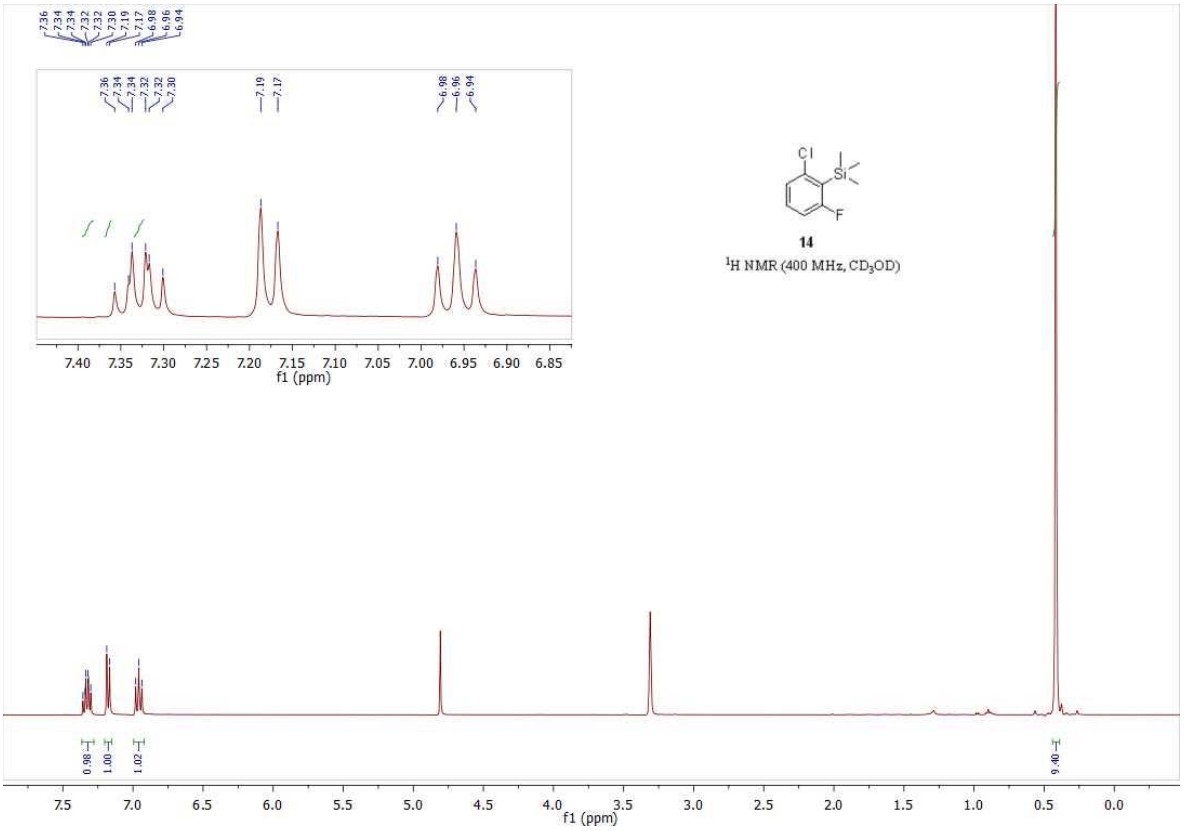

12c
^{13}C NMR (100 MHz, CD_3OD)
f1 (ppm)

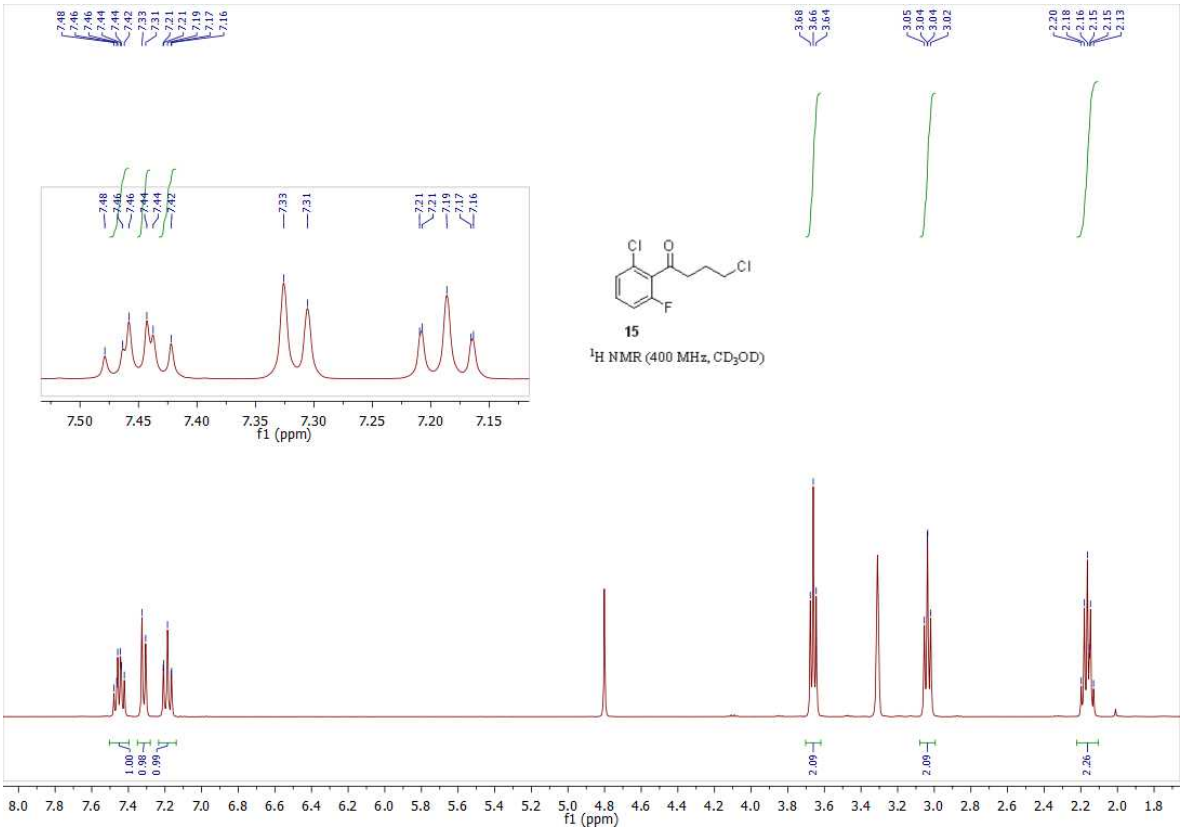

12d
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

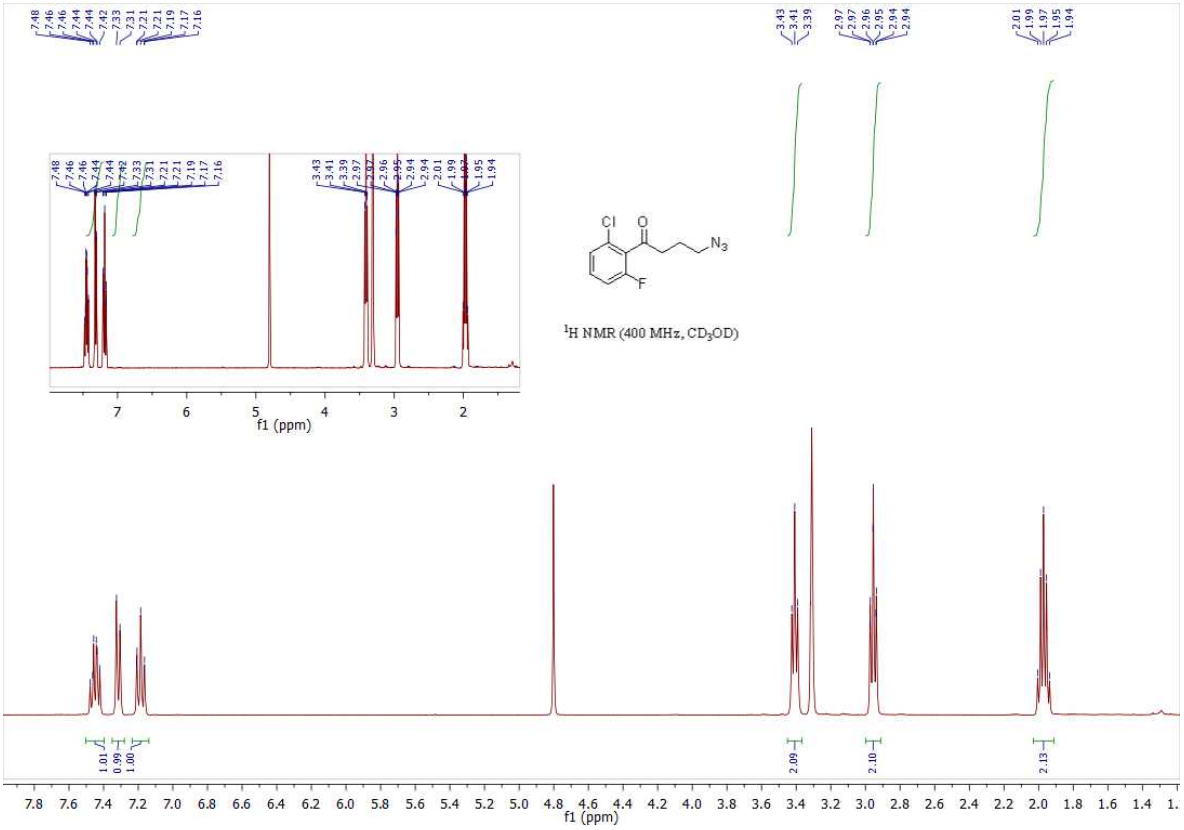

164.82
144.25
140.56
128.34
126.96
124.29
120.35
112.20
54.27
43.31
42.08
40.14
31.23
27.58
25.89
24.24
Cl
NH
N
N
H
Cl
12d
^{13}C NMR (100 MHz, CD$_3$OD)
f1 (ppm)

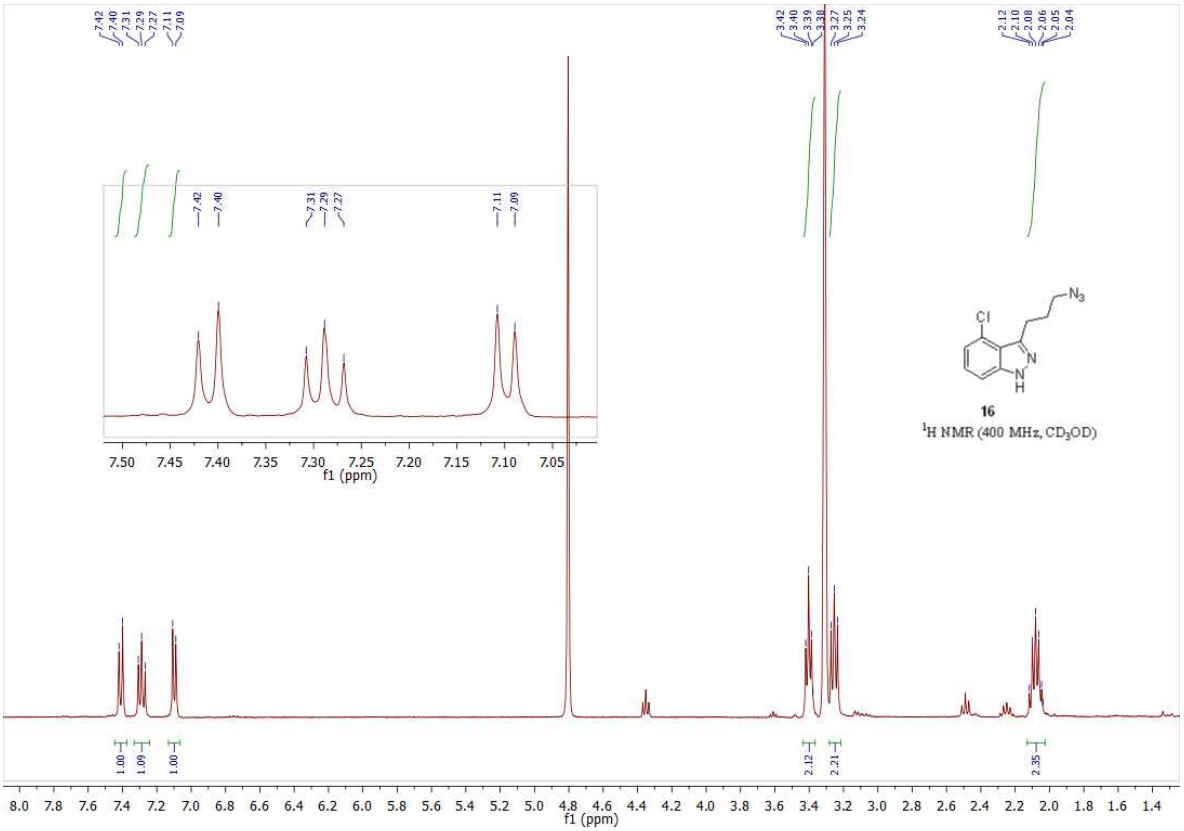

7.62
7.62
7.16
7.16
Cl
Cl
NH
N
N
H
Cl
12e
^{1}H NMR (400 MHz, CD$_3$OD)
1.02
0.97
2.00
2.01
1.99
2.80
1.03
2.05
3.40
2.61
2.12
f1 (ppm)

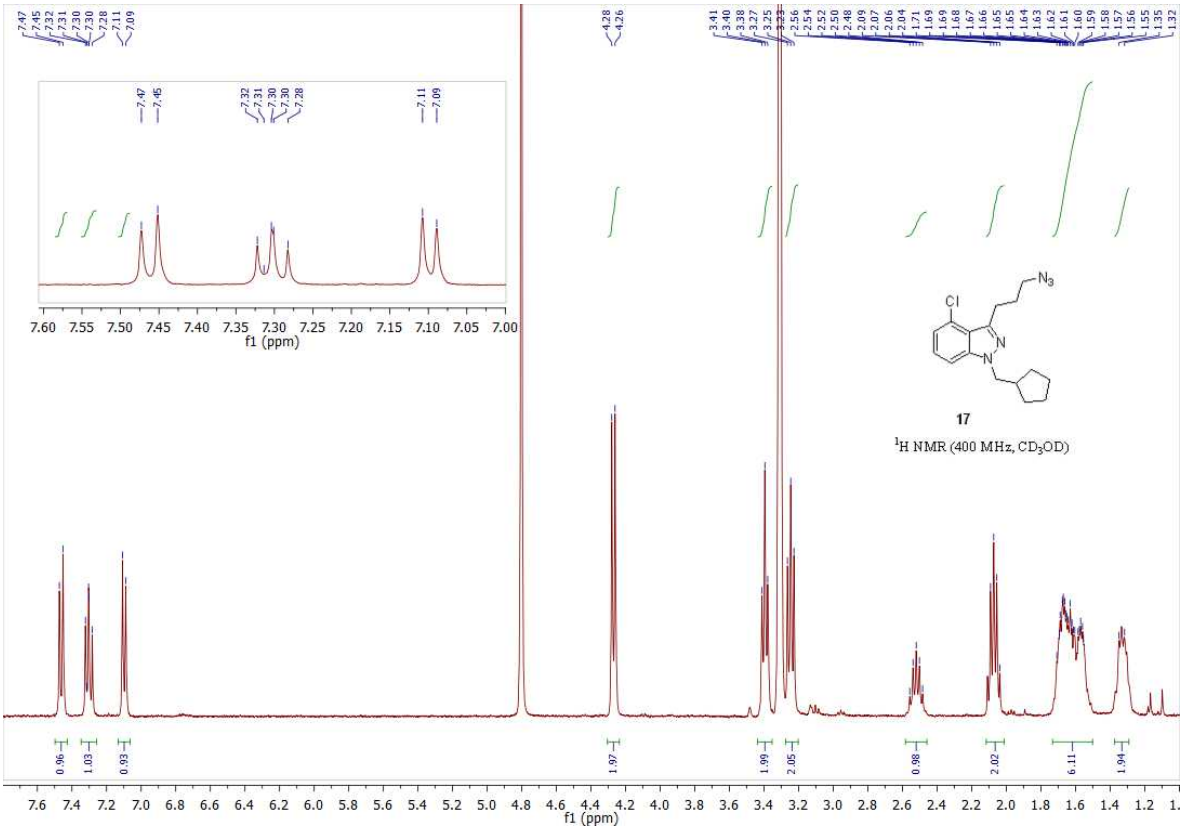

164.77
144.67
143.41
133.85
128.57
122.25
119.60
109.76
54.39
43.19
41.86
40.13
31.19
28.06
25.91
25.73
Cl
Cl
NH
N
H
N
N
Cl
12e
^{13}C NMR (100 MHz, CD_3OD)
f1 (ppm)

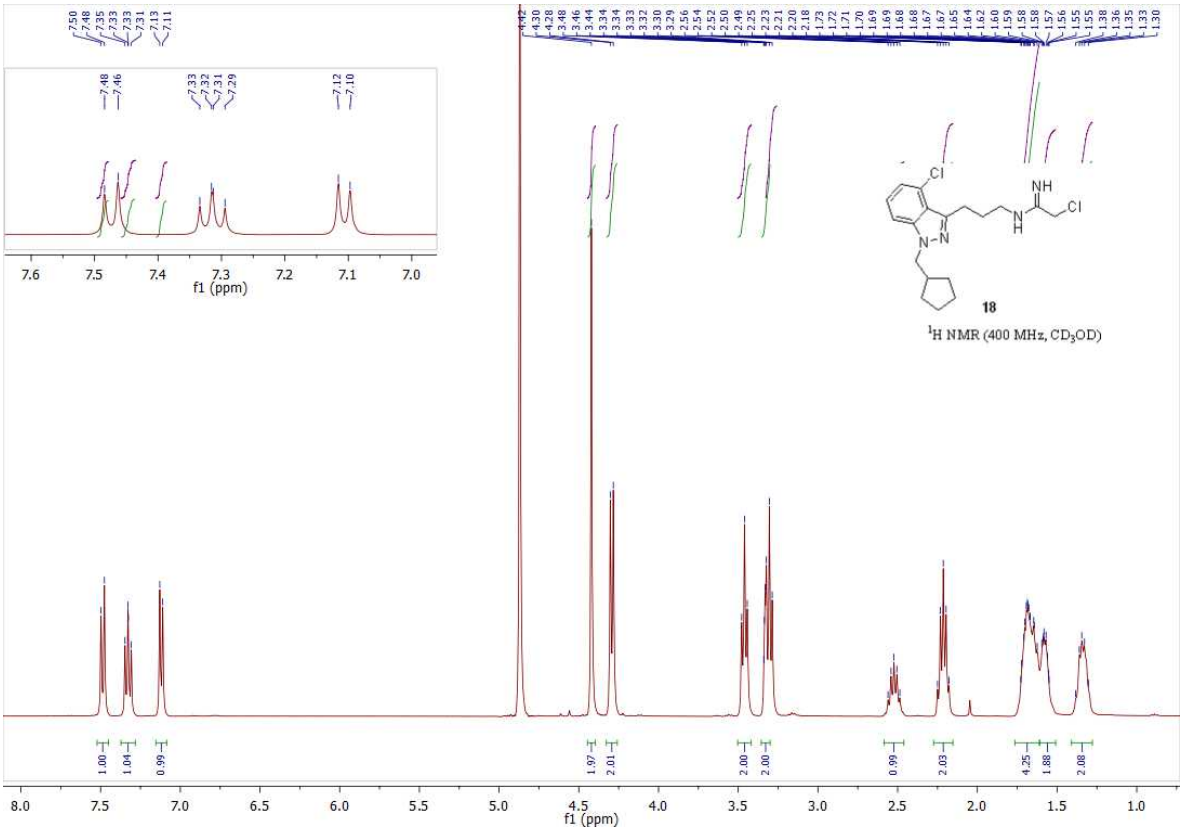

7.36
7.34
7.34
7.32
7.32
7.30
7.19
7.17
6.98
6.96
6.94
Cl
Si
F
14
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)
0.98
1.00
1.02
9.40

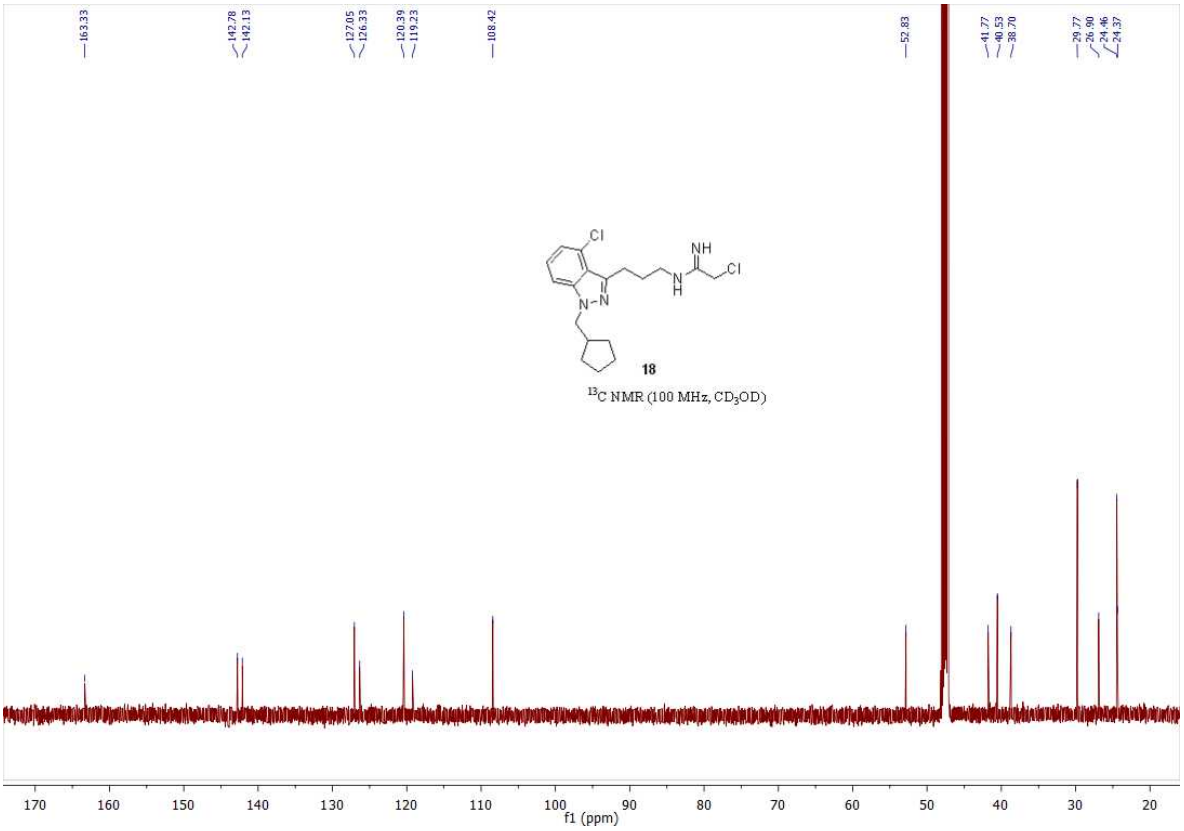

15
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

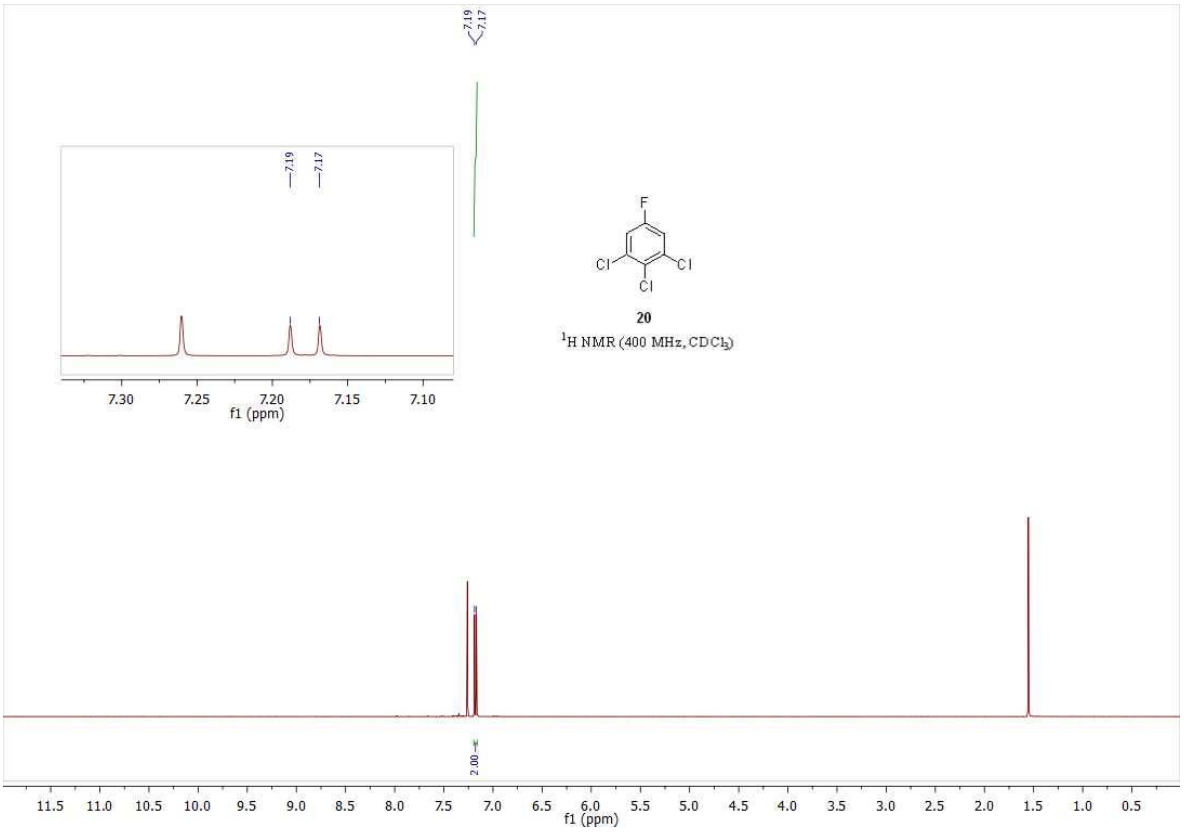

^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

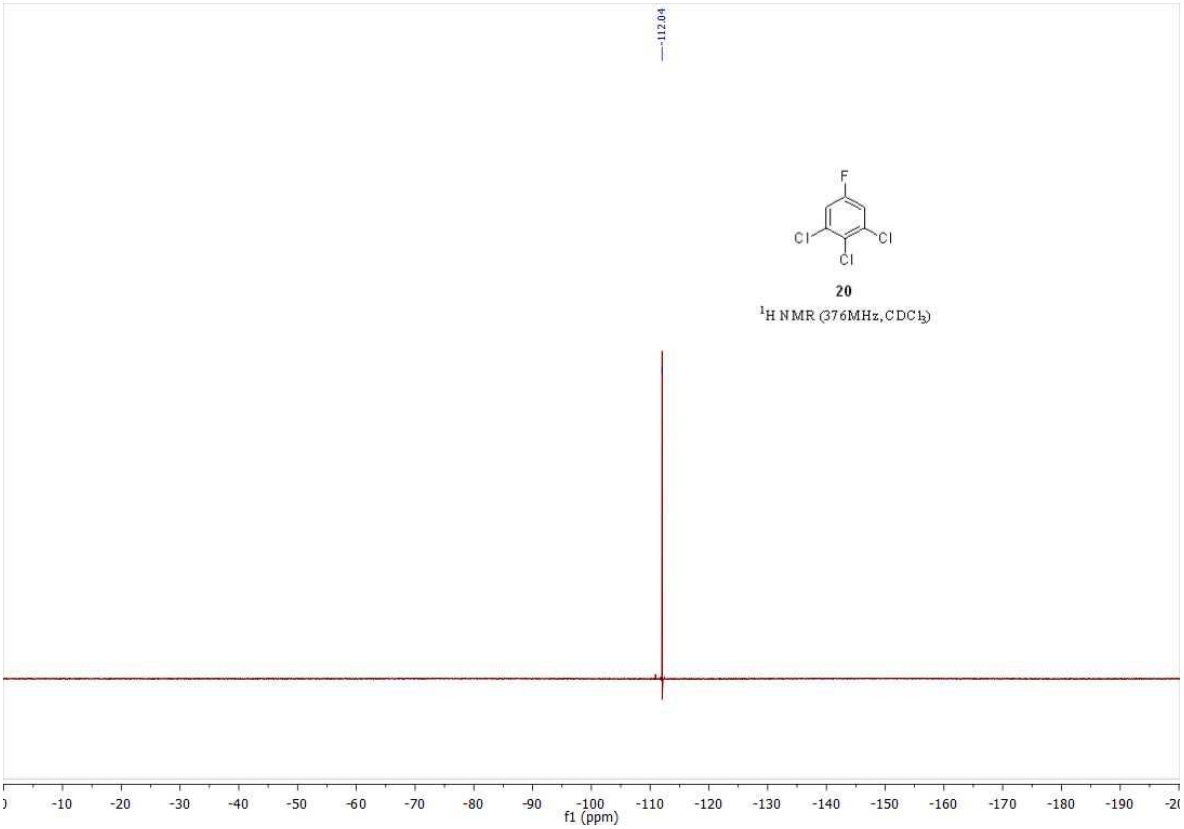

16
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

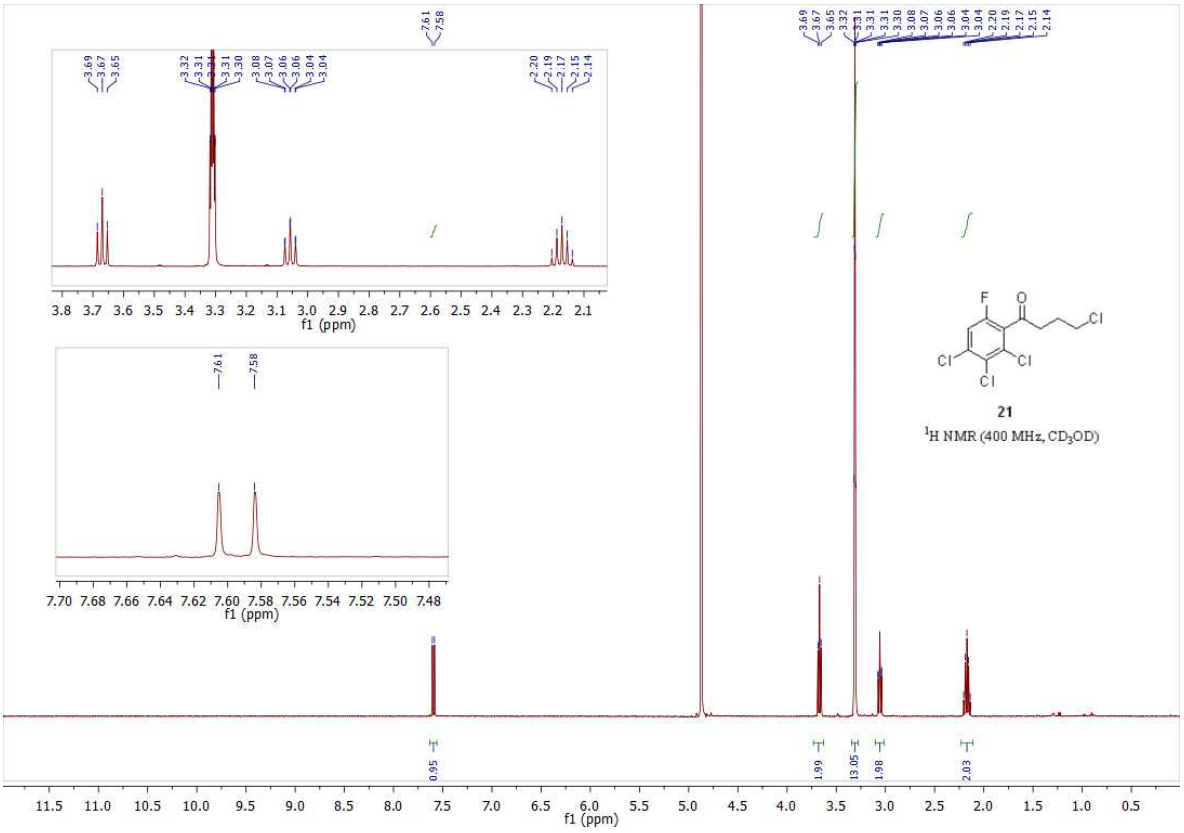

17
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

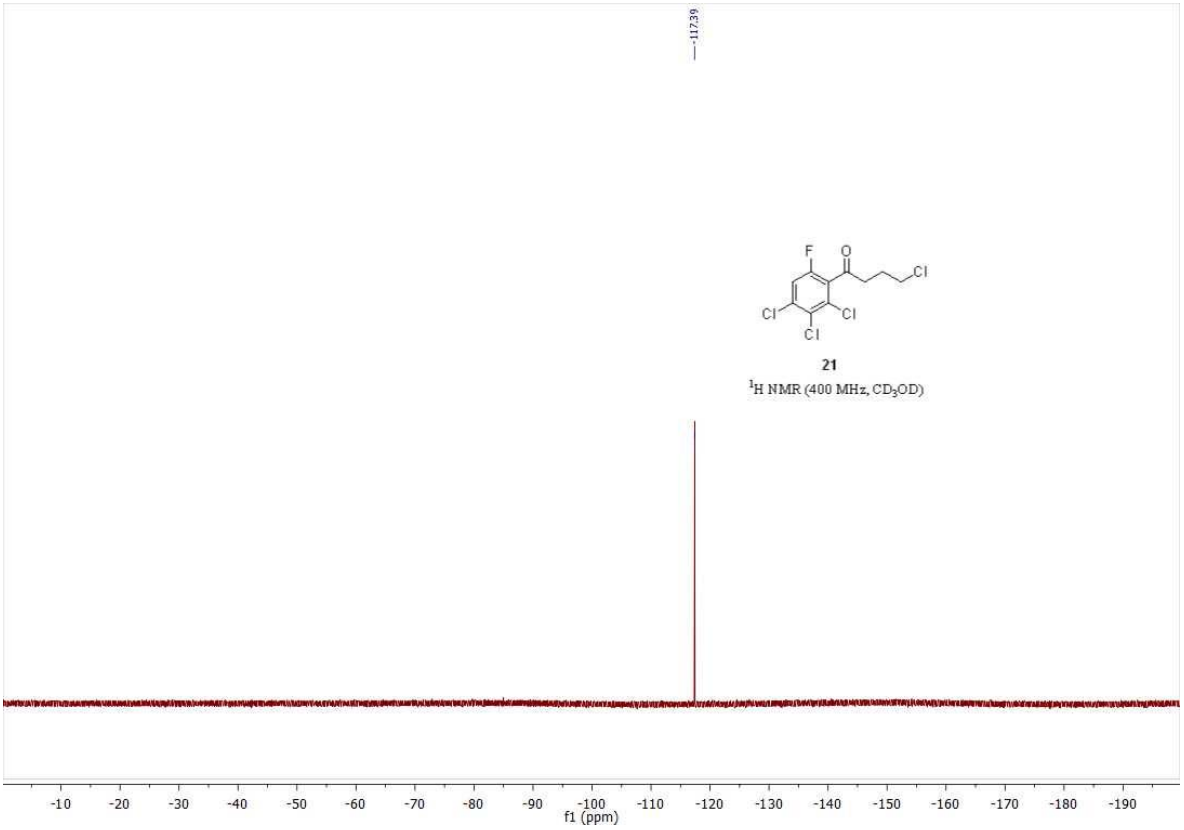

18
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)

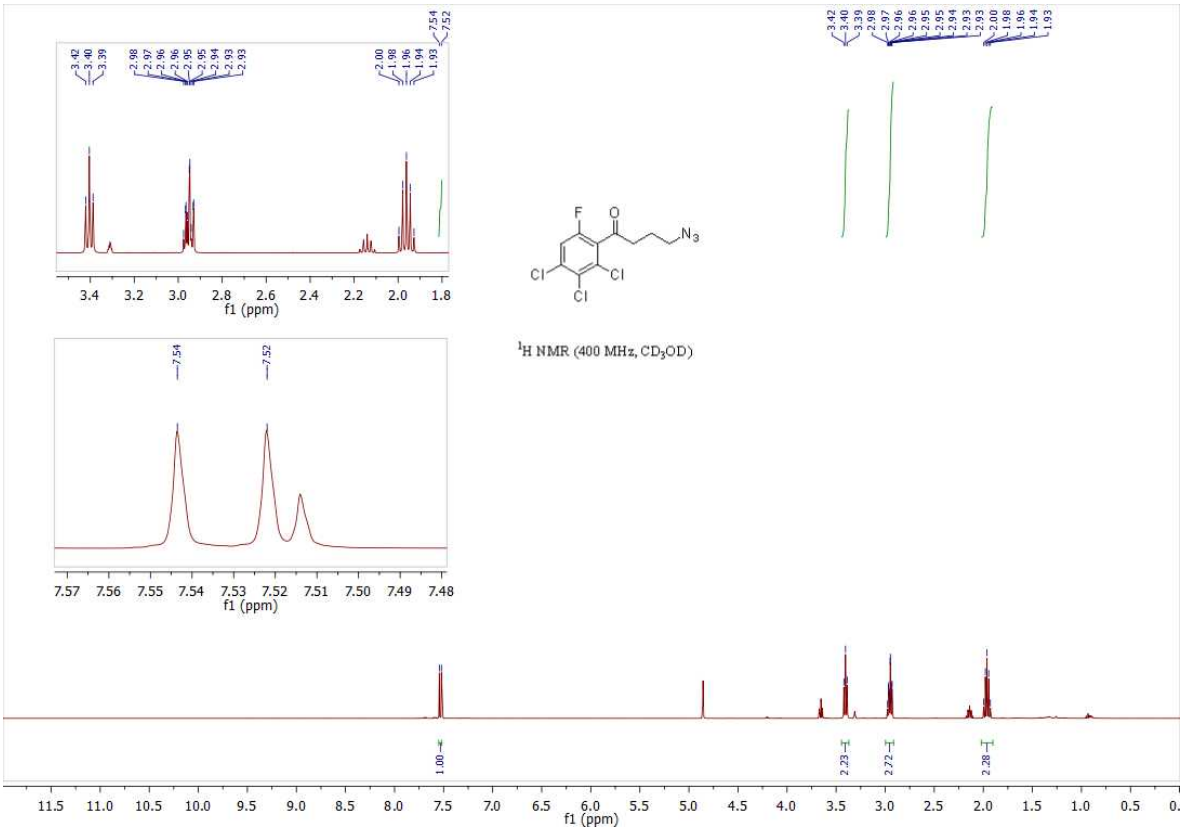

18
^{13}C NMR (100 MHz, CD_3OD)
f1 (ppm)

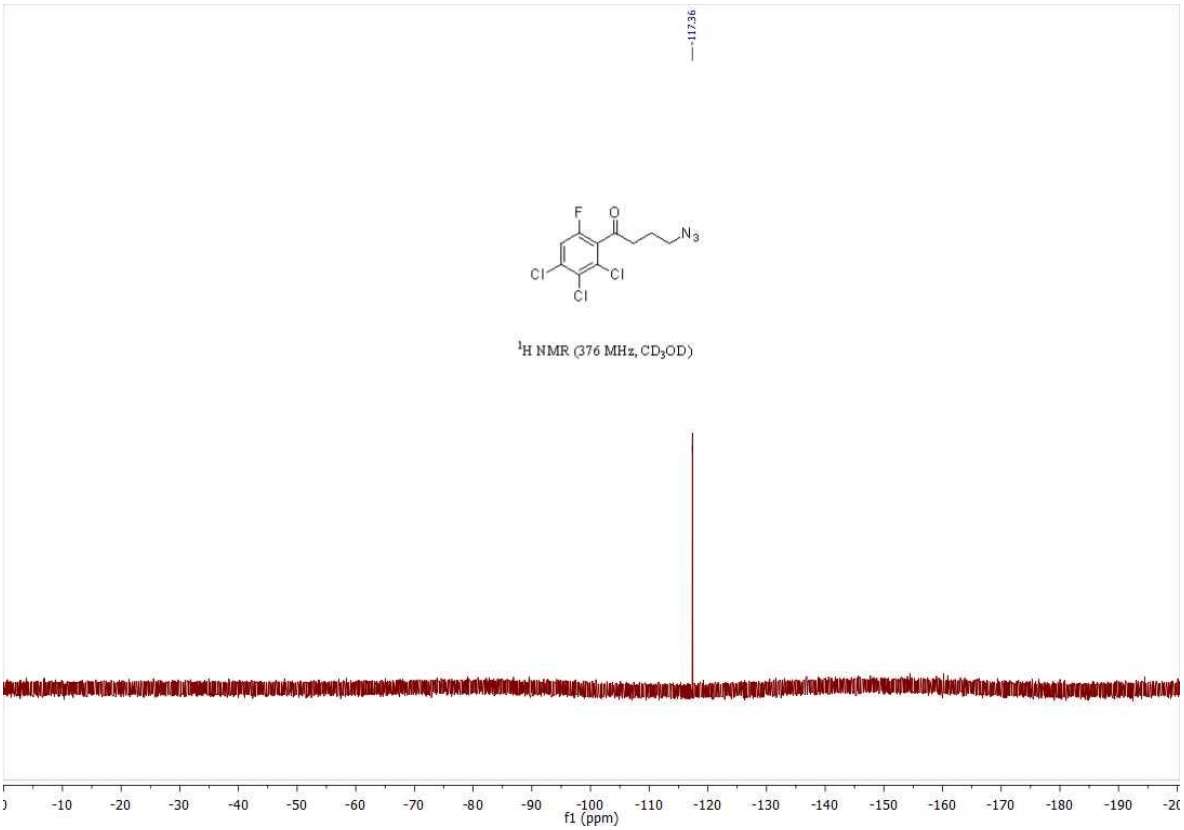

7.19
7.17
F
Cl
Cl
Cl
20
^{1}H NMR (400 MHz, $CDCl_3$)
7.30
7.25
7.20
7.15
7.10
f1 (ppm)
2.00
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

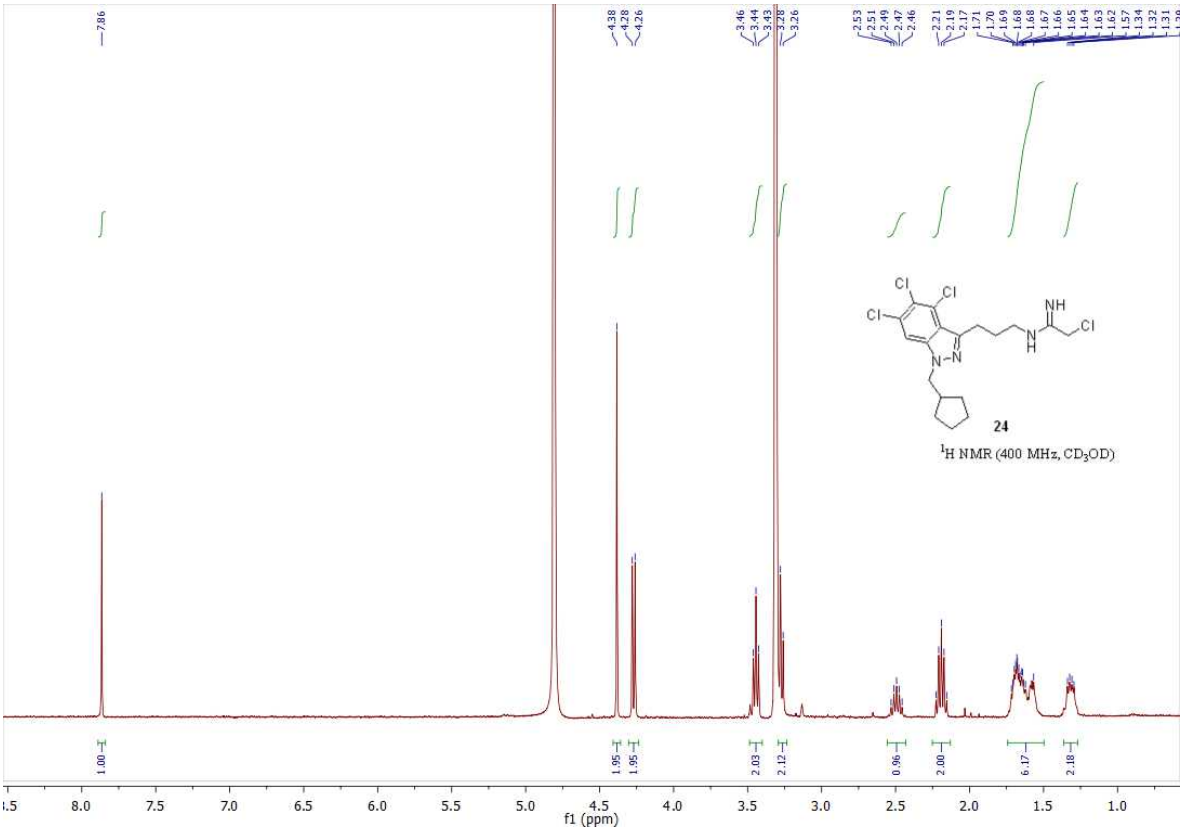

-112.04
F
Cl
Cl
Cl
20
^{1}H NMR (376MHz, $CDCl_3$)
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
-110
-120
-130
-140
-150
-160
-170
-180
-190
f1 (ppm)

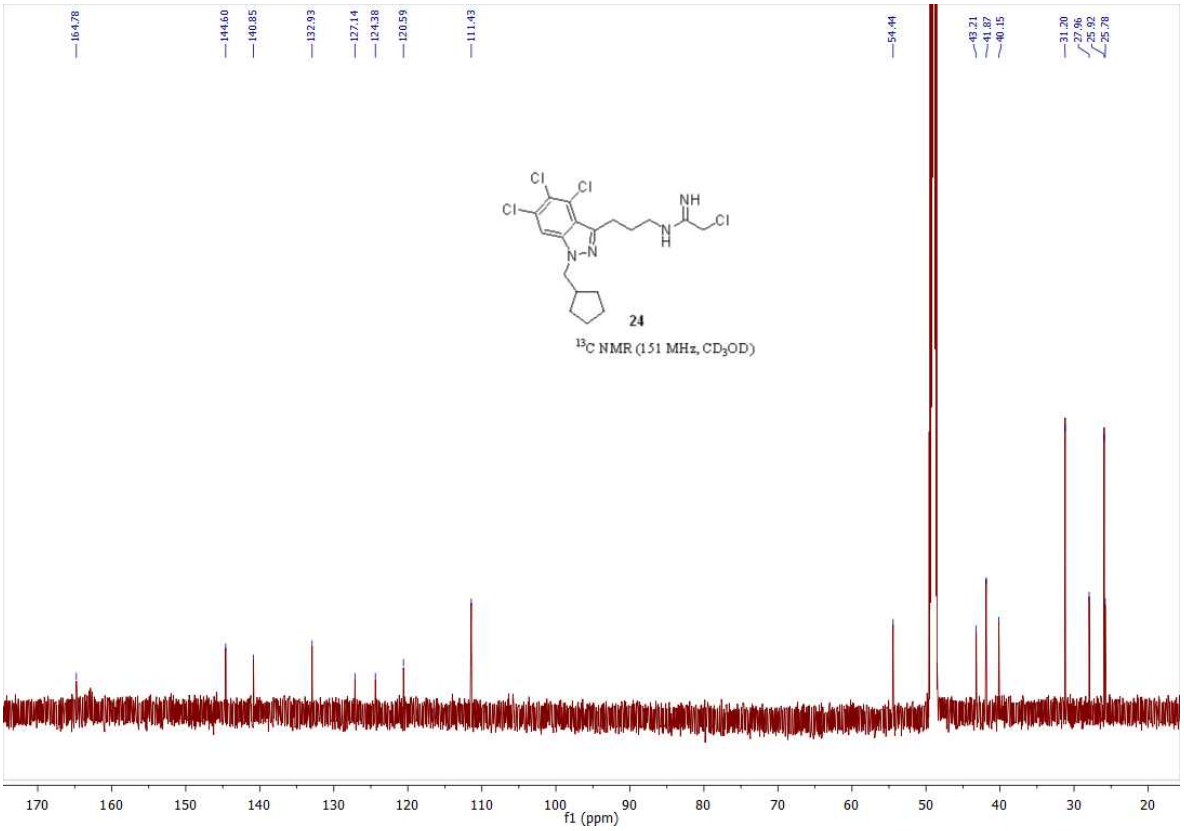

F
O
Cl
Cl
Cl
Cl
21
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)


-117.39
F
O
Cl
Cl
Cl
Cl
21
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)


F
O
N3
Cl
Cl
Cl
1H NMR (400 MHz, CD3OD)
f1 (ppm)


-117.36
F
O
N3
Cl
Cl
Cl
1H NMR (376 MHz, CD3OD)
f1 (ppm)


24
^{1}H NMR (400 MHz, CD_3OD)
f1 (ppm)


24
^{13}C NMR (151 MHz, CD_3OD)
f1 (ppm)

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