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Supporting Information

A Fluorescent Kinase Inhibitor that Exhibits Diagnostic Changes in Emission upon Binding

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SUPPORTING INFORMATION

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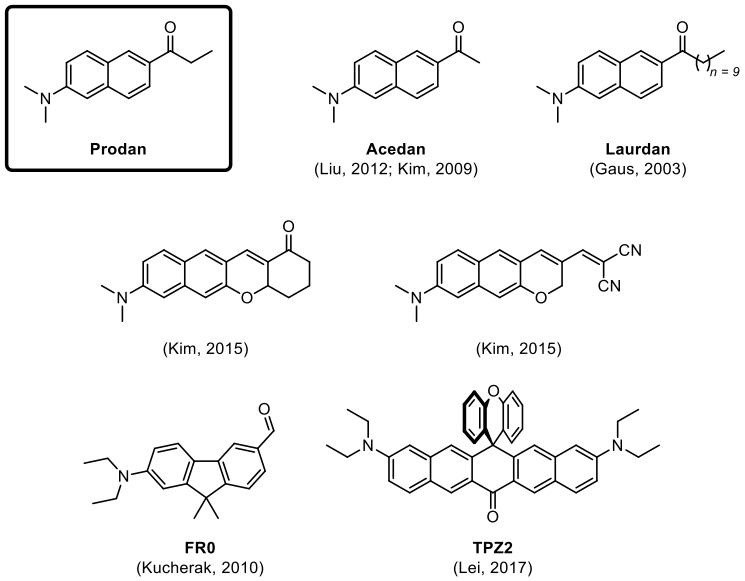


Figure S1: Examples of known Prodan derivatives that have found use as two-photon excitable probes.[1]

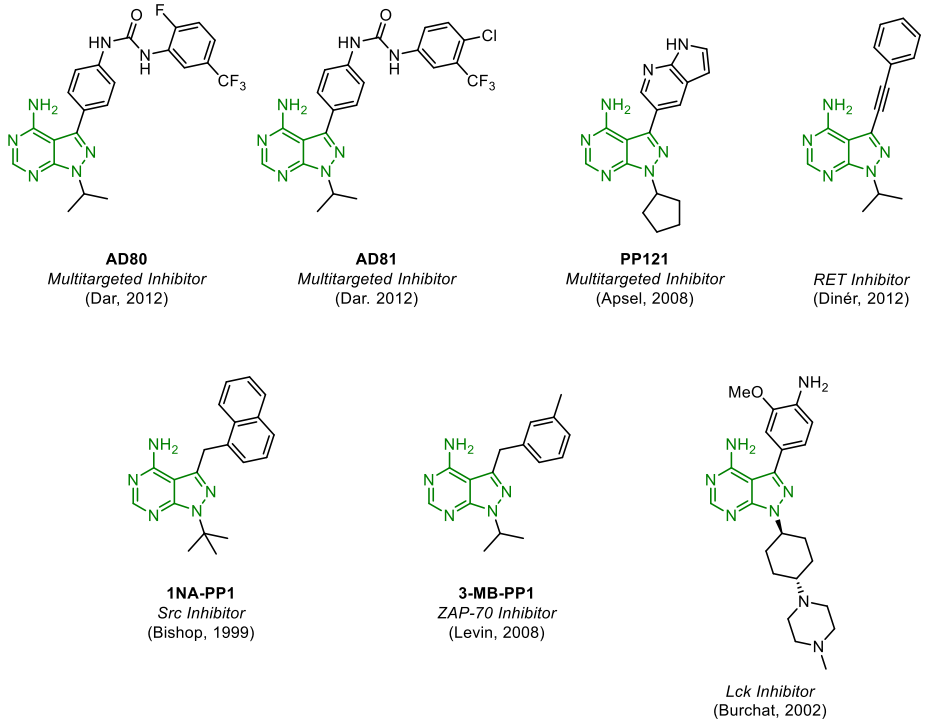


Figure S2: Examples of pyrazolo[3,4-*d*]pyrimidine containing kinase inhibitors that bind to the ATP-binding pocket (*Type I Kinase Inhibitors*).[2]

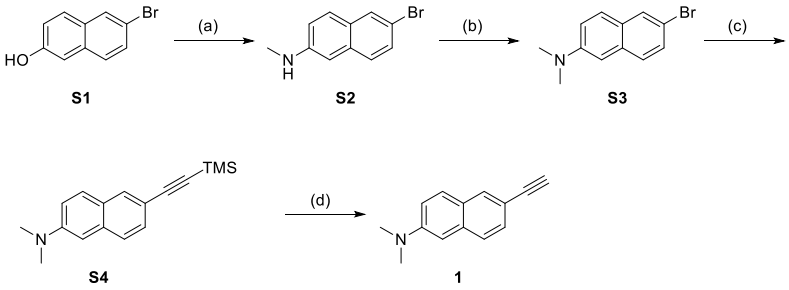
SUPPORTING INFORMATION

General Experimental Information for Synthesis and Compound Characterisation

General reagent and solvents for the synthesis of compounds were purchased from commercial sources and used as supplied, unless otherwise stated. When anhydrous THF was required, THF was distilled over Na/benzophenone. Those reactions that employed microwave irradiation were conducted using a Biotage Initiator™ reactor with fixed hold time. Purification by flash column chromatography was performed on a Biotage SP4 Flash® instrument using prefabricated silica columns. All NMR spectra (^{1}H and ^{13}C) were collected on a Varian 400 MHz spectrometer at 25 °C. Samples were dissolved (0.5 mL) in deuterated chloroform ($CDCl_3$). The residual solvent peaks specific to that to the deuterated solvent was used as an internal reference; $CDCl_3$: 7.26 ppm (^{1}H NMR) and 77.20 ppm (^{13}C NMR). NMR shifts were assigned using 2D NMR experiments (COSY, HSQC and HMBC). High Resolution Mass Spectra (HRMS) was performed on an Agilent 1290 Infinity LC system equipped with autosampler tandem to an Agilent 6520 Accurate Mass Q-TOF LC/MS with ESI ionisation source.

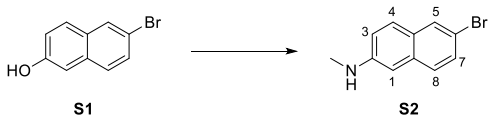
Detailed Synthetic Procedure and Characterisation of Compounds 1–6 and Prodan

Synthesis of Compound 1:



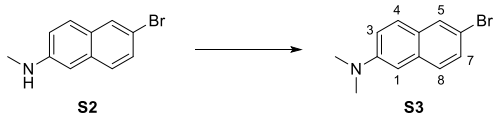
Reagents and conditions: (a) $Na_2S_2O_2$, NH_2CH_3 (40% in H_2O), H_2O, 140 °C, 4 days; (b) CH_3I, NaH, THF, 60 °C, 48 hours; (c) TMS-acetylene, $PdCl_2(PPh_3)_2$, CuI, dicyclohexylamine, MW 100 °C, 1 hour; (d) K_2CO_3, MeOH, 21 °C, 16 hours.

6-Bromo-2-methylaminonaphthalene (S2)



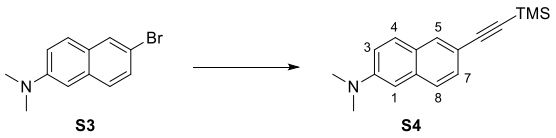
In a pressure vessel, a suspension of 6-bromo-2-naphthol **S1** (1.00 g, 4.48 mmol), $Na_2S_2O_5$ (1.64 g, 8.65 mmol) and aqueous methylamine (40%, 2.0 mL, 22.00 mmol) in H_2O (4 mL) was left to stir at 140 °C for 4 days. After cooling to 21 °C, the reaction mixture was dissolved in CH_2Cl_2 (50 mL). The resulting organic layer was washed with $NaHCO_3$ (5%, 30 mL × 3), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography (1:4 CH_2Cl_2/pentane) afforded the title compound **S2** (1.01 g, 95%, R_f = 0.16) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, *J* = 1.5 Hz, 1H, H-5), 7.52 (d, *J* = 6.2 Hz, 1H, H-4/H-8), 7.50 (d, *J* = 6.4 Hz, 1H, H-4/H-8), 7.42 (dd, *J* = 8.8, 2.0 Hz, 1H, H-7), 6.88 (dd, *J* = 8.9, 2.4 Hz, 1H, H-3), 6.74 (d, *J* = 2.4 Hz, 1H, H-1), 3.93 (br s, 1H, NH), 2.93 (s, 3H, NHC*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 147.3, 133.9, 129.7, 129.5, 128.5, 128.0, 127.7, 118.9, 115.0, 130.5, 30.7. Data is consistent with that previously reported.[3]

6-Bromo-2-dimethylaminonaphthalene (S3)



To a solution of 6-bromo-2-methylaminonaphthalene **S2** (1.01 g, 4.28 mmol) and CH_3I (2.67 mL, 42.80 mmol) in THF (80 mL) was added NaH (60%, 2.06 g, 51.36 mmol). After stirring at 60 °C for 48 h, the reaction mixture was cooled to 21 °C, the precipitate was then removed by vacuum filtration and excess solvent was removed. The resulting residue was dissolved in CH_2Cl_2 (50 mL). The resulting organic layer was washed with $NaHCO_3$ (5%, 30 mL × 3), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography (30% CH_2Cl_2 in pentane) afforded the title compound **S3** (925 mg, 86%, R_f = 0.17) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.86 (d, *J* = 1.9 Hz, 1H, H-5), 7.61 (d, *J* = 9.1 Hz, 1H, H-4), 7.54 (d, *J* = 8.8 Hz, 1H, H-8), 7.45 (dd, *J* = 8.8, 2.0 Hz, 1H, H-7), 7.17 (dd, *J* = 9.1, 2.6 Hz, 1H, H-3), 6.88 (d, *J* = 2.6 Hz, 1H, H-1), 3.06 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 148.8, 133.6, 129.5, 129.4, 127.94, 127.90, 127.8, 117.1, 115.1, 106.1, 40.8 (CH_3 × 2). Data is consistent with that previously reported.[3]

2-Dimethylamino-6-((trimethylsilyl)ethynyl)naphthalene (S4)



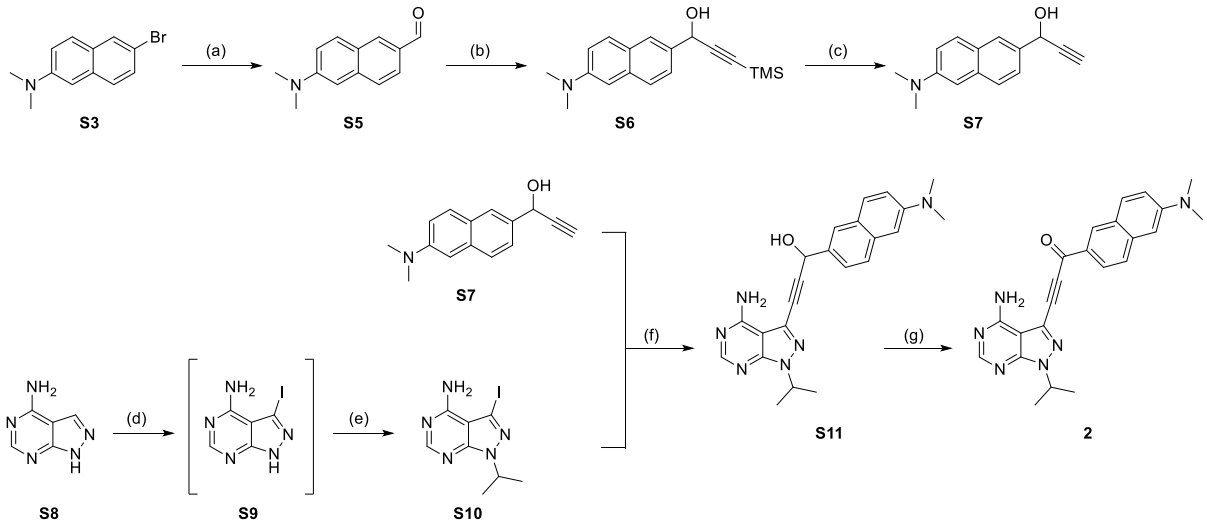
To a solution of 6-bromo-2-dimethylaminonaphthalene **S3** (819 mg, 3.27 mmol), $PdCl_2(PPh_3)_2$ (230 mg, 0.327 mmol, 10 mol%) and CuI (62 mg, 0.327 mmol, 10 mol%) in DMF (anhydrous, 7 mL) were added TMS-acetylene (2.26 mL, 16.37 mmol) and dicyclohexylamine (1.30 mL, 6.54 mmol) under nitrogen. The resulting reaction mixture was heated using microwave irradiation at 100 °C for 1 hour. After cooling to 21 °C, the reaction mixture was filtered through Celite, washing thoroughly with EtOAc. The filtrate was further diluted with H_2O (100 mL) and then extracted with EtOAc (3 × 40 mL). Combined organic phase was washed with $NaHCO_3$ (sat., 40 mL), brine (40 mL), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography (1:4 CH_2Cl_2/pentane) afforded the title compound **S4** (745 mg, 85%, R_f = 0.27) as a tan oil that solidified upon standing. The product was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, *J* = 0.7 Hz, 1H, H-5), 7.65 (d, *J* = 9.1 Hz, 1H, H-4), 7.57 (d, *J* = 8.6 Hz, 1H, H-8), 7.43 (dd, *J* = 8.5, 1.7 Hz, 1H, H-7), 7.14 (dd, *J* = 9.1, 2.6 Hz, 1H, H-3), 6.86 (d, *J* = 2.6 Hz, 1H, H-1), 3.06 (s, 6H, $N(CH_3)_2$), 0.31 (s, 9H, $Si(CH_3)_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 149.2, 134.8, 132.0, 129.1, 128.9, 126.1, 126.0, 116.6, 116.0, 106.5, 106.0, 92.9, 40.7 (CH_3 × 2), 0.3 (CH_3 × 3).

2-Dimethylamino-6-ethynlnaphthalene (1)

To a solution of 2-dimethylamino-6-((trimethylsilyl)ethynyl)naphthalene **S4** (1.13 g, 4.23 mmol) in MeOH (30 mL) was added K_2CO_3 (1.75 g, 12.70 mmol). After stirring at 21 °C for 16 hours, the reaction mixture was filtered, washing thoroughly with CH_2Cl_2 (*ca* 70 mL). The resulting filtrate was washed with brine (20 mL), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography (1:4 CH_2Cl_2/pentane) afforded the title compound **1** (711 mg, 86%, R_f = 0.26) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.87 (br s, 1H, H-5), 7.66 (d, *J* = 9.1 Hz, 1H, H-4), 7.57 (d, *J* = 8.6 Hz, 1H, H-8), 7.41 (dd, *J* = 8.6, 1.7 Hz, 1H, H-7), 7.16 (dd,

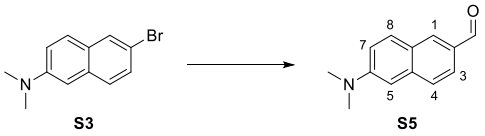
J = 9.1, 2.6 Hz, 1H, H-3), 6.86 (br s, 1H, H-1), 3.08 (s, 1H, C≡CH), 3.07 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 149.3, 134.9, 132.2, 129.1, 128.8, 126.3, 126.0, 116.7, 114.9, 106.0, 85.0, 76.2, 40.7 (CH_3 × 2).

Synthesis of Compound 2:



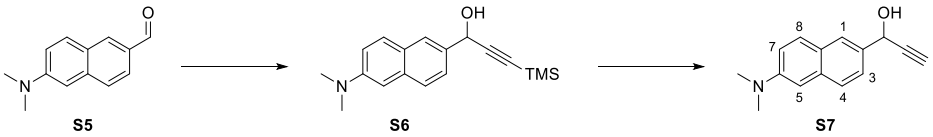
Reagents and conditions: (a) *n*-BuLi, THF, DMF, −78 °C, 5 hours; (b) TMS-acetylene, *n*-BuLi, THF, −78 °C, 4 hours, (c) K_2CO_3, MeOH, 21 °C, 12 hours; (d) NIS, DMF, MW 100 °C, 1 hour; (e) 2-iodopropane, Cs_2CO_3, 21 °C, 24 hours; (f) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, MW 100 °C, 1 hour; (g) CuI, DMAP, TEMPO, CH_3CN, O_2, 21 °C, 72 hours.

6-(Dimethylamino)naphthalene-2-carbaldehyde (S5)



A solution of 6-bromo-2-dimethylaminonaphthalene **S3** (1.37 g, 5.48 mmol) in THF (anhydrous, 12 mL) was cooled to −78 °C under nitrogen. To this was added *n*-BuLi (2.06 M solution in hexane, 3.2 mL, 6.58 mmol) in a dropwise manner. After stirring at −78 °C for 40 min, DMF (2.12 mL, 27.40 mmol) was slowly added. The reaction was monitored by TLC (5:95 EtOAc/cyclohexane) and after stirring at 0 °C for 5 hours, the reaction mixture was quenched with NH_4Cl (sat., 20 mL). The resulting aqueous phase was then extracted with Et_2O (3 × 20 mL). Combined organic layer was washed with brine (20 mL), dried ($MgSO_4$), filtered and excess solvent removed to afford the title compound **S5** (1.02 g, 93%) as a yellow solid. The product was used without further purification. ^1H NMR (400 MHz, $CDCl_3$): δ 10.01 (s, 1H, CHO), 8.15 (d, *J* = 1.4 Hz, 1H, H-1), 7.85–7.78 (m, 2H, H-4, H-8), 7.66 (d, *J* = 8.6 Hz, 1H, H-3), 7.18 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7), 6.88 (d, *J* = 2.6 Hz, 1H, H-5), 3.13 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 191.9, 150.8, 138.8, 134.9, 130.9, 130.8, 127.0, 125.2, 123.7, 116.4, 105.6, 40.5 (CH_3 × 2). Data is consistent with that previously reported.[4]

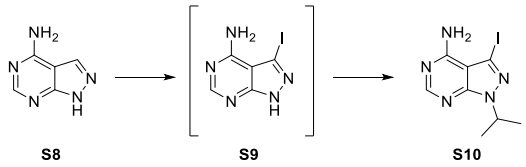
6-Dimethylamino-2-(prop-2-yn-1-ol)naphthalene (S7)



S5 **S6** **S7**

A solution of trimethylsilylacetylene (533 µL, 3.77 mmol) in THF (anhydrous, 7 mL) was cooled to −78 °C under nitrogen. To this was added *n*-BuLi (1.64 M solution in hexane, 2.3 mL, 3.77 mmol) in a dropwise manner. After stirring at −78 °C for 60 min, a solution of 6-(dimethylamino)naphthalene-2-carbaldehyde **S5** (500 mg, 2.51 mmol) in THF (3 mL) was added and the resulting reaction mixture was stirred for an additional 60 min at −78 °C. The reaction mixture was then warmed to 21 °C and continued to stir for 4 hours. The reaction mixture was then quenched with H_2O (15 mL) and extracted with EtOAc (3 × 20 mL). Combined organic layer was washed with brine (10 mL), dried ($MgSO_4$), filtered and excess solvent removed. The resulting residue was dissolved in MeOH (10 mL) to which K_2CO_3 (1.00 g, 7.53 mmol) was added. After stirring at 21 °C for 12 hours, the reaction mixture was filtered, washing thoroughly with CH_2Cl_2. The resulting filtrate was then washed with NH_4Cl (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered and excess solvent removed to afford the title compound **S7** (422 mg, 75%) as a brown oil that solidified upon standing. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.81 (d, *J* = 1.7 Hz, 1H, H-1), 7.67 (app. t, $J_{app.}$ = 6.8 Hz, 2H, H-4, H-8), 7.53 (dd, *J* = 8.6, 1.7 Hz, 1H, H-3), 7.16 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7), 6.92 (d, *J* = 2.6 Hz, 1H, H-5), 5.55 (d, *J* = 2.2 Hz, 1H, C*H*OH), 3.04 (s, 6H, $N(CH_3)_2$), 2.71 (d, *J* = 2.2 Hz, 1H, C≡CH), 2.66 (br s, 1H, OH). ^{13}C NMR (101 MHz, $CDCl_3$): δ 149.1, 135.0, 133.7, 129.1, 127.0, 126.4, 125.4, 125.0, 116.9, 106.5, 84.0, 74.8, 64.8, 41.0 (CH_3 × 2). Data is consistent with that previously reported.[5]

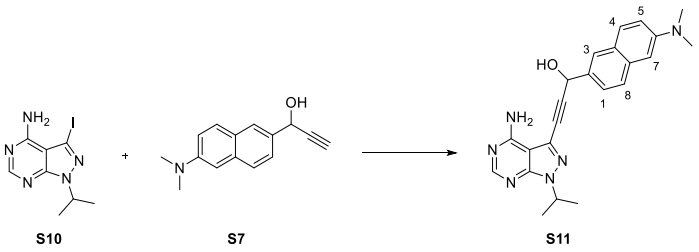
3-Iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (S10)



S8 **S9** **S10**

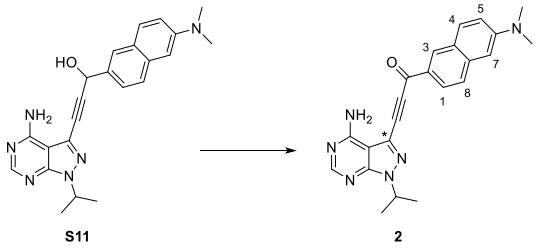
In a sealed 20 mL microwave vial, a suspension of 4-aminopyrazolo[3,4-*d*]pyrimidine **S8** (1.00g, 7.59 mmol) and NIS (2.56 g, 11.38 mmol) in DMF (anhydrous, 10 mL) was evacuated and backfilled with N_2 (× 3). The reaction mixture was then heated using microwave irradiation at 100 °C for 1 hour. Upon cooling to 21 °C, Cs_2CO_3 (2.93 g, 9.10 mmol) was added and the resulting reaction mixture was evacuated and backfilled with N_2 (× 3). Next, the reaction mixture was further cooled to 0 °C and 2-iodopropane (0.87 mL, 8.73 mmol) was added in a dropwise manner. After stirring at 21 °C for 24 hours the reaction mixture was filtered through Celite, washing thoroughly with $PhCH_3$ and excess solvent removed. Residual DMF was removed *via* azeotropic distillation using $PhCH_3$ (3 × 3 mL). The resulting tan solid was then dissolved in CH_2Cl_2, washed with H_2O (20 mL), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography (7:3 EtOAc/pentane) afforded the title compound **S10** (1.37 g, 60%, R_f = 0.31) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30 (s, 1H, Ar-H), 6.25 (br s, 2H, NH_2), 5.08 (sept, *J* = 6.7 Hz, 1H, C*H*$(CH_3)_2$), 1.54 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6, 155.7, 153.1, 104.1, 85.6, 49.8, 22.2 (CH_3 × 2). Data is consistent with that previously reported.[2b]

3-(4-Amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(6-(dimethylamino)naphthalen-2-yl)prop-2-yn-1-ol (S11)



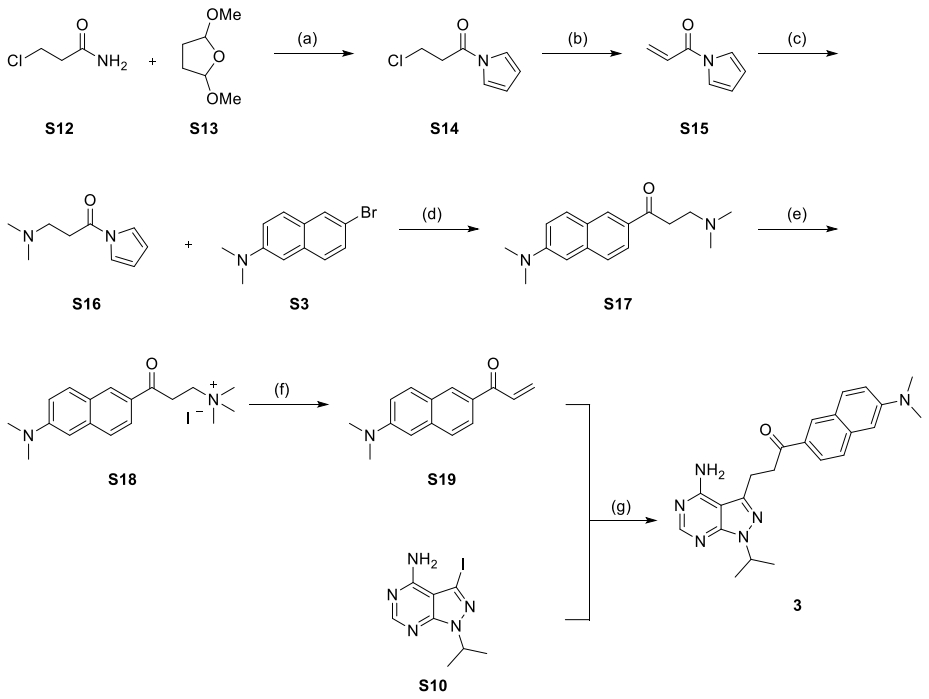
In a sealed microwave vial, a solution of 3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **S10** (404 mg, 1.33 mmol), 6-dimethylamino-2-(prop-2-yn-1-ol)naphthalene **S7** (600 mg, 2.67 mmol), $Pd(PPh_3)_4$ (307 mg, 0.266 mmol) and CuI (101 mg, 0.532 mmol) in DMF (6 mL) was evacuated and backfilled with N_2 (× 3). Next, Et_3N (556 µL, 3.99 mmol) was added and the reaction mixture was heated using microwave irradiation at 100 °C for 1 hour. Upon cooling to 21 °C, excess solvent was removed. The resulting residue was suspended in EtOAc (50 mL), washed with $NaHSO_3$ (sat. solution, 2 × 50 mL), dried ($MgSO_4$), filtered and excess solvent removed. Purification by column chromatography (1:40 MeOH/$CHCl_3$) afforded the title compound **S11** (512 mg, 96%, R_f = 0.17) as a tan solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.15 (s, 1H, Ar-H), 7.82 (s, 1H, H-3), 7.65–7.60 (m, 2H, H-4, H-8), 7.55 (dd, *J* = 8.6, 1.8 Hz, 1H, H-1), 7.10 (dd, *J* = 9.1, 2.6 Hz, 1H, H-5), 6.85 (d, *J* = 2.6 Hz, 1H, H-7), 6.03 (br s, 2H, NH_2), 5.82 (s, 1H, C*H*OH), 5.06 (sept, *J* = 6.7 Hz, 1H, C*H*(CH_3)$_2$), 3.01 (s, 6H, N(CH_3)$_2$), 1.51 (app. t, $J_{app.}$ = 6.3 Hz, 6H, CH(C*H*$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.6, 156.2, 152.4, 149.2, 135.1, 133.4, 129.1, 127.3, 126.4, 125.5, 125.4, 124.7, 116.9, 106.3, 102.2, 95.0, 78.5, 65.3, 49.6, 40.9 (CH_3 × 2), 22.1 (CH_3 × 2); HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{25}N_6O$ 401.2084; Found 401.2092.

3-(4-Amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(6-(dimethylamino)naphthalen-2-yl)prop-2-yn-1-one (2)



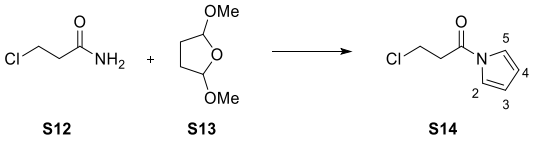
A suspension of 3-(4-amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(6-(dimethylamino)naphthalen-2-yl)prop-2-yn-1-ol **S11** (102 mg, 0.256 mmol) and CuI (5 mg, 0.0256 mmol) in CH_3CN (2 mL) was stirred at 21 °C for 10 min. To this, was added DMAP (3 mg, 0.0256 mmol) and TEMPO (0.5 mg, 0.00256 mmol). The resulting reaction mixture was left to stir under O_2 at 21 °C. After stirring for 72 hours, the reaction mixture was filtered, washing thoroughly with CH_3CN and excess solvent was removed. Purification by column chromatography (1:40 MeOH/$CHCl_3$) afforded the title compound **2** (15.1 mg, 15%, R_f = 0.31) as a dark yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.66 (d, *J* = 1.8 Hz, 1H, H-3), 8.40 (s, 1H, Ar-H), 8.06 (dd, *J* = 8.7, 1.8 Hz, 1H, H-1), 7.90 (d, *J* = 9.2 Hz, 1H, H-4), 7.65 (d, *J* = 8.7 Hz, 1H, H-8), 7.18 (dd, *J* = 9.2, 2.6 Hz, 1H, H-5), 6.88 (d, *J* = 2.6 Hz, 1H, H-7), 6.09 (s, 2H, NH_2), 5.21 (sept, *J* = 6.7 Hz, 1H, C*H*(CH_3)$_2$), 3.14 (s, 6H, N(CH_3)$_2$), 1.63 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3$)$_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 176.7, 157.6, 156.6, 153.1, 151.0, 138.9, 134.0, 131.5, 130.1, 126.6, 125.0, 124.6, 123.9, 116.4, 105.4, 92.4, 83.3, 50.2, 40.5 (CH_3 × 2), 22.2 (CH_3 × 2); HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{23}N_6O$ 399.1928; Found 399.1930. **This quaternary carbon was not identified in the ^{13}C NMR spectrum. It is thought that the absence of this carbon is the result of quadrupolar broadening.*

Synthesis of Compound 3:



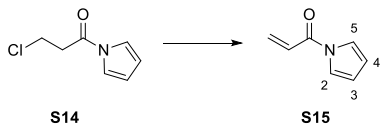
Reagents and conditions: (a) Ac_2O, 60 °C, 72 hours; (b) Et_3N, Et_2O, 21 °C, 23 hours; (c) 2,6-di-*tert*-butylphenol, $NH(CH_3)_2$ (2 M in THF), 65 °C, 22 hours; (d) *n*-BuLi, THF, −78 °C, 4 hours; (e) CH_3I, Et_2O, 21 °C, 48 hours; (f) $NaHCO_3$, 1:1 Et_2O/H_2O, 21 °C, 16 hours; (g) $Pd(OAc)_2$, PPh_3, Et_3N, 120 °C, 24 hours.

***N*-(3-Chloropropionyl)pyrrole (S14)**



To 2,5-dimethoxytetrahyrdrofuran **S13** (40 mL, 305.72 mmol) cooled to 0 °C was added acetic anhydride (8.23 mL, 87.1 mmol) under N_2. After stirring at 0 °C for 10 min, 3-chloropropionamide **S12** (9.08 g, 84.43 mmol) was added and the resulting reaction mixture was stirred at 60 °C for 72 hours. The reaction mixture was cooled to 21 °C and an aqueous solution (500 mL) of NaCl (100 g) and Na_2CO_3 (2 g) was added. The resulting solution was stirred vigorously for 15 min and then was allowed to settle. The aqueous layer was filtered by gravity filtration and the oily residue that remained in the filter paper was redissolved in 1:1 EtOAc/Hexane (500 mL). The organic layer was washed with brine (2 × 100 mL), dried (Na_2SO_4), filtered and excess solvent removed. Purification by flash column chromatography (1% Et_2O in pentane) afforded the title compound **S14** (2.47 g, 19%, R_f = 0.27) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (br s, 2H, H-2, H-5), 6.32 (app. t, $J_{app.}$ = 2.4 Hz, 2H, H-3, H-4), 3.92 (t, J = 6.8 Hz, 2H, $COCH_2CH_2Cl$), 3.32 (t, J = 6.8 Hz, 2H, $COCH_2CH_2Cl$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.4, 119.1 (CH × 2), 113.8 (CH × 2), 38.2, 37.7. Data is consistent with that previously reported.[6]

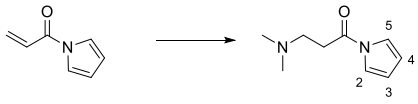
***N*-Acryloylpyrrole (S15)[6]**



S14 **S15**

To a solution of *N*-(3-chloropropionyl)pyrrole **S14** (2.43 g, 15.42 mmol) in Et_2O (20 mL), was added Et_3N (3.65 mL, 26.21 mmol). After stirring at 21 °C for 23 hours, the $Et_3N{\cdot}HCl$ was removed by vacuum filtration and excess solvent was removed to afford the title compound **S15** (1.75 g, 94%) as a colourless liquid. The product was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (app. t, $J_{app.}$ = 2.3 Hz, 2H, H-2, H-5), 6.87 (dd, *J* = 16.8, 10.4 Hz, 1H, COC*H*=CH_2), 6.67 (dd, *J* = 16.8, 1.6 Hz, 1H, COCH=CH_2), 6.34 (dd, *J* = 2.7, 2.0 Hz, 2H, H-3, H-4), 6.04 (dd, *J* = 10.4, 1.6 Hz, 1H, COCH=CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ 162.7, 133.2, 126.7, 119.4 (CH × 2), 113.7 (CH × 2). Data is consistent with that previously reported.[7]

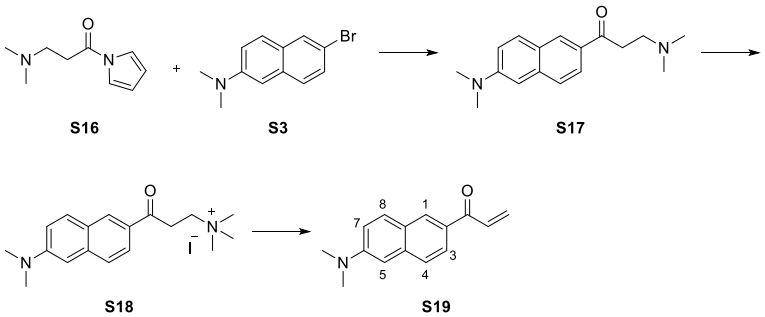
***N*-(3-(Dimethylamino)propionyl)pyrrole (S16)**



S15 **S16**

A solution of *N*-acryloylpyrrole **S15** (1.75 g, 14.45 mmol) and 2,6-di-*tert*-butylphenol (298 mg, 1.45 mmol) in a dimethylamine solution (2 M in THF, 7.30 mL), was allowed to stir under N_2 at 65 °C. After stirring for 22 hours, the reaction mixture was cooled to 21 °C. Excess solvent was removed to afford the title compound **S16** (2.38 g, 99%) as a colourless liquid, containing residual *N*-acryloylpyrrole **S15**. The material was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (br s, 2H, H-2, H-5), 6.22 (app. t, $J_{app.}$ = 2.3 Hz, 2H, H-3, H-4), 2.93 (t, *J* = 7.4 Hz, 2H, $N(CH_3)_2C\mathit{H}_2CH_2CO$), 2.71 (t, *J* = 7.4 Hz, 2H, $N(CH_3)_2CH_2C\mathit{H}_2CO$), 2.23 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 169.2, 118.8 (CH × 2), 113.0 (CH × 2), 54.2, 45.3 (CH_3 × 2), 32.9. Data is consistent with that previously reported.[6]

Acrylodan (S19)



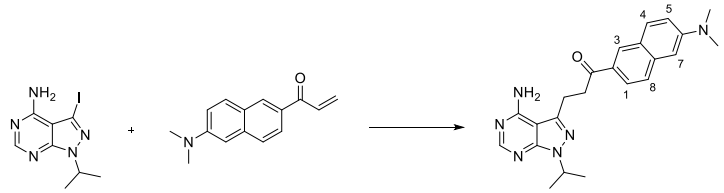
S16 **S3** **S17**

S18 **S19**

A solution of 6-bromo-2-dimethylaminonaphthalene **S3** (2.40 g, 9.63 mmol) in THF (anhydrous, 100 mL) was cooled to −78 °C under nitrogen. To this was added *n*-BuLi (2.03 M solution in hexane, 5.12 mL) in a dropwise manner. After stirring at −78 °C for 40 min, a solution of *N*-(3-(dimethylamino)propionyl)pyrrole **S16** (2.40 g, 14.44 mmol) in THF (50 mL) was slowly added and was allowed to stir at −78 °C for 3 hours. The reaction mixture was quenched with AcOH (50%, 1.20 mL) and left to stir at 21 °C for 16 hours. Next, the reaction mixture was poured into H_2O (600 mL) and extracted with 1:1 EtOAc/hexane (2 × 200 mL). The combined organic layer was washed with H_2O (2 × 200 mL), dried ($MgSO_4$), filtered and excess solvent removed. The resulting residue was then suspended in Et_2O (90 mL), to which CH_3I (690 µL, 11.07 mmol) was added and left to stir at 21 °C for 48 hours. The precipitate was collected by vacuum filtration, washing thoroughly with Et_2O. The precipitate was then suspended in Et_2O (600 mL) and H_2O (600 mL). To this, was added $NaHCO_3$ (16.3 g, 194 mmol) and the resulting reaction mixture was left to stir at 21 °C. After stirring for 16 hours, all of the solid material

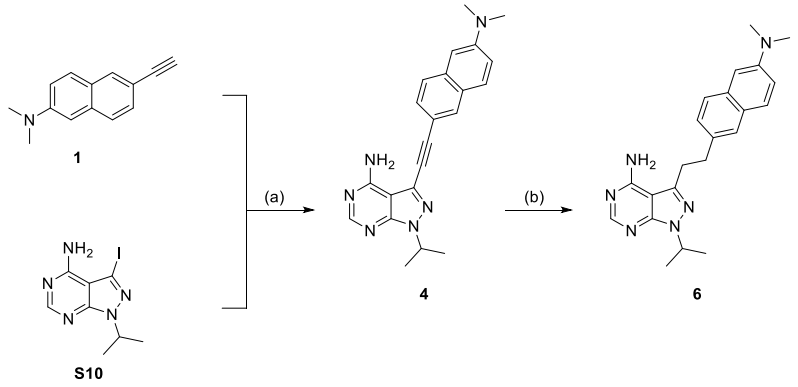
had dissolved. The two layers were allowed to separate and the aqueous phase was extracted with Et_2O (2 × 150 mL). Combined organic layer was dried ($MgSO_4$), filtered and excess solvent removed. Purification by recrystallisation (EtOH/H_2O) afforded the title compound **S19** (436 mg, 20%) as a bright yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (d, *J* = 1.8 Hz, 1H, H-1), 7.96 (dd, *J* = 8.7, 1.8 Hz, 1H, H-3), 7.80 (d, *J* = 9.1 Hz, 1H, H-8), 7.66 (d, *J* = 8.7 Hz, 1H, H-4), 7.34 (dd, *J* = 17.0, 10.5 Hz, 1H, COC*H*=CH_2), 7.17 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7), 6.88 (d, *J* = 2.6 Hz, 1H, H-5), 6.48 (dd, *J* = 17.0, 1.9 Hz, 1H, COCH=C*H*$_2$), 5.90 (dd, *J* = 10.5, 1.9 Hz, 1H, COCH=C*H*$_2$), 3.11 (s, 6H, $N(CH_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 190.1, 150.5, 137.9, 132.5, 130.9 (C, CH × 2), 129.0, 126.6, 125.24, 125.16, 116.4, 105.5, 40.6 (CH_3 × 2). Data is consistent with that previously reported.[6]

3-(4-Amino-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-yl)-1-(6-(dimethylamino)naphthalen-2-yl)propan-1-one (3)



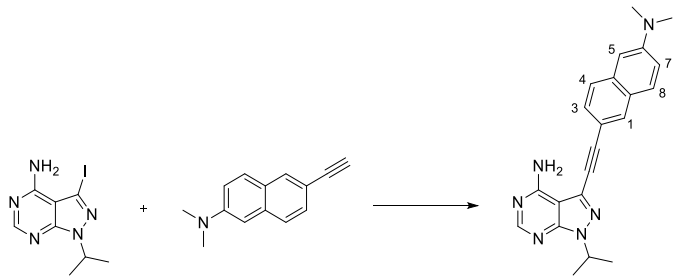
In a sealed vial, a suspension of 3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **S10** (119 mg, 0.393 mmol), acrylodan **S19** (177 mg, 0.785 mmol), $Pd(OAc)_2$ (18 mg, 0.079 mmol) and PPh_3 (4 mg, 0.079 mmol) in Et_3N (2.5 mL) was evacuated and backfilled with N_2 (× 5). The resulting reaction mixture was left to stir at 120 °C for 24 hours. After cooling to 21 °C, excess solvent was removed. Purification by flash column chromatography (1:40 MeOH/$CHCl_3$) afforded the title compound **3** (21 mg, 14%) as a green oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.35 (s, 1H, H-3), 8.27 (s, 1H, Ar-H), 7.90 (dd, *J* = 8.8, 1.8 Hz, 1H, H-1), 7.77 (d, *J* = 9.1 Hz, 1H, H-4), 7.60 (d, *J* = 8.8 Hz, 1H, H-8), 7.14 (dd, *J* = 9.1, 2.5 Hz, 1H, H-5), 6.83 (d, *J* = 2.5 Hz, 1H, H-7), 6.49 (br s, 2H, NH_2), 5.05 (sept, *J* = 6.7 Hz, 1H, C*H*$(CH_3)_2$), 3.62 (t, *J* = 6.4 Hz, 2H, CH_2CH_2CO), 3.40 (t, *J* = 6.4 Hz, 2H, CH_2CH_2CO), 3.09 (s, 6H, $N(CH_3)_2$), 1.51 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3)_2$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 199.5, 158.7, 155.3, 153.2, 150.5, 144.0, 138.1, 131.0, 130.6, 129.8, 126.4, 125.0, 124.5, 116.4, 105.3, 99.8, 48.5, 40.5, 38.4, 22.7, 22.1; HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{27}N_6O$ 403.2241; Found 403.2249.

Synthesis of Compounds 4 and 6:



Reagents and conditions: (a) $Pd(PPh_3)_4$, CuI, Et_3N, DMF, MW 100 °C, 1 hour; (b) $Pd(OH)_2$, MeOH, 21 °C.

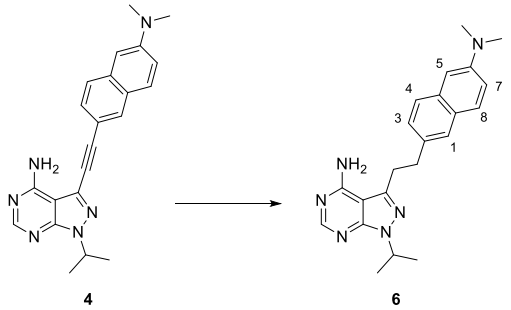
3-(2-(6-(Dimethylamino)naphthalen-2-yl)ethynyl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (4)



S10 **1** **4**

To a solution of 3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **S10** (362 mg, 1.19 mmol), 2-dimethylamino-6-ethynlnaphthalene **1** (467 mg, 2.39 mmol), $Pd(PPh_3)_4$ (275 mg, 0.238 mmol, 20 mol%) and CuI (91 mg, 0.476 mmol, 40 mol%) in DMF (anhydrous, 6 mL) was added Et_3N (498 µL, 3.57 mmol) under nitrogen. The resulting reaction mixture was heated using microwave irradiation at 100 °C for 1 hour. After cooling to 21 °C, excess solvent was removed under reduced pressure. The resulting crude residue was dissolved in EtOAc (50 mL) and washed with $NaHSO_3$ (sat., 2 × 30 mL), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography ($CHCl_3$) afforded the title compound **4** (386 mg, 87%, R_f = 0.10) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H, Ar-H), 7.95 (d, *J* = 1.0 Hz, 1H, H-1), 7.68 (d, *J* = 9.1 Hz, 1H, H-8), 7.62 (d, *J* = 8.7 Hz, 1H, H-4), 7.48 (dd, *J* = 8.5, 1.7 Hz, 1H, H-3), 7.17 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7), 6.87 (d, *J* = 2.5 Hz, 1H, H-5), 6.06 (br s, 2H, NH_2), 5.16 (sept, *J* = 6.8 Hz, 1H, C*H*$(CH_3)_2$), 3.09 (s, 6H, N$(CH_3)_2$), 1.59 (d, *J* = 6.8 Hz, 6H, CH(C*H*$_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.9, 156.1, 152.5, 149.6, 135.2, 132.1, 129.0, 128.5, 126.7, 126.5, 125.9, 116.8, 114.2, 105.9, 102.0, 95.7, 80.1, 49.5, 40.7 (CH_3 × 2), 22.2 (CH_3 × 2); HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{23}N_6$ 371.1983; Found 371.1979.

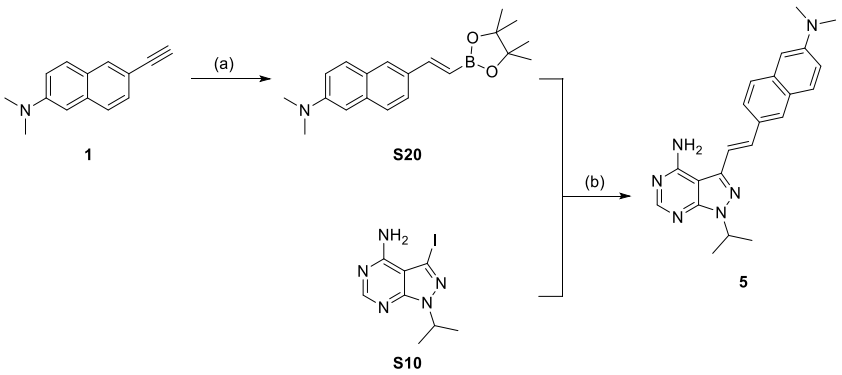
3-(2-(6-Dimethylamino)naphthalen-2-yl)ethyl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (6)



4 **6**

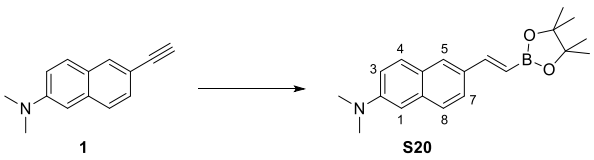
A suspension of 3-(2-(6-dimethylamino)naphthalen-2-yl)ethynyl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **4** (375 mg, 1.01 mmol) in MeOH (20 mL) was hydrogenated (1 atm.) on $Pd(OH)_2$ (150 mg) at 21 °C. The reaction was monitored by LCMS. Upon completion, $Pd(OH)_2$ was filtered off through a Celite plug using a sintered glass funnel and the filtrate was collected and concentrated *in vacuo*. Purification by flash column chromatography (1:40 MeOH/$CHCl_3$) afforded the title compound **6** (168 mg, 43%) as a yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 1H, Ar-H), 7.59 (app. t, $J_{app.}$ = 8.3 Hz, 2H, H-4, H-8), 7.48 (s, 1H, H-1), 7.18 (dd, *J* = 8.4, 1.8 Hz, 1H, H-3), 7.14 (dd, *J* = 9.0, 2.6 Hz, 1H, H-7), 6.88 (d, *J* = 2.6 Hz, 1H, H-5), 5.34 (br s, 2H, NH_2), 5.09 (sept, *J* = 6.7 Hz, 1H, C*H*$(CH_3)_2$), 3.31–3.23 (m, 2H, C*H*$_2$CH$_2$), 3.24–3.14 (m, 2H, CH$_2$C*H*$_2$), 3.02 (s, 6H, N$(CH_3)_2$), 1.55 (d, *J* = 6.7 Hz, 6H, CH(C*H*$_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 157.4, 153.9, 153.0, 148.5, 144.0, 134.4, 133.7, 128.3, 127.5, 127.0, 126.8, 126.4, 116.9, 106.6, 99.5, 48.7, 41.0 (CH_3 × 2), 35.5, 31.7, 22.1 (CH_3 × 2); HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{27}N_6$ 375.2292; Found 375.2300.

Synthesis of Compound 5:



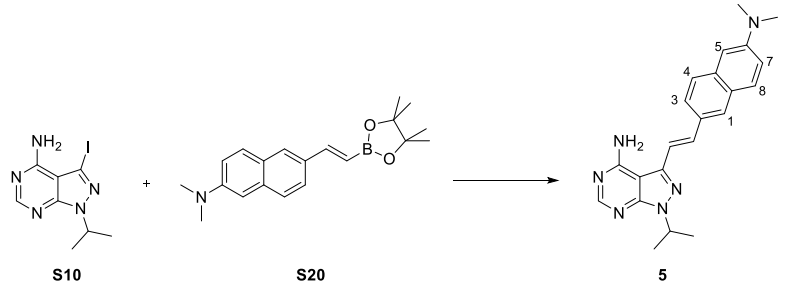
Reagents and conditions: (a) Bis(pinacolato)diboron, CuI, NaO^{t}Bu, Xantphos, THF, MeOH, 21 °C, 24 hours; (b) $Pd(PPh_3)_4$, K_2CO_3, 1,4-dioxane, H_2O, MW 150 °C, 30 min.

***(E)*-2-Dimethylamino-6-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)naphthalene (S20)**



In an oven dried microwave vial, CuCl (1.6 mg, 0.0.0157 mmol), NaO^{t}Bu (3 mg, 0.0313 mmol) and Xantphos (9 mg, 0.0157 mmol) were placed and dried in vaccum for 20 seconds, then backfilled with N_2 (× 5). To this, THF (anhydrous, 1 mL) was added. The resulting yellowish solution was left to stir at 21 °C for 2 hours under N_2. A solution of bis(pinacolato)diboron (146 mg, 0.574 mmol) in THF (1 mL) was then added. After stirring at 21 °C for 1 hour, 2-dimethylamino-6-ethynlnaphthalene **1** (102 mg, 0.522 mmol) was added, followed by MeOH (42 µL, 1.04 mmol). The flask was then sealed and the resulting reaction mixture was stirred at 21 °C for 24 hours. The reaction mixture was then filtered through a celite plug, washing thoroughly with Et_2O. Excess solvent was then removed *in vacuo*. Purification by flash column chromatography (1:4 CH_2Cl_2/pentane) afforded the title compound **S20** (73 mg, 43%, R_f = 0.27) as a brown solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73–7.56 (m, 4H, H-4, H-5, H-7, H-8), 7.52 (d, *J* = 18.4 Hz, 1H, C*H*=CH), 7.13 (dd, *J* = 9.1, 2.6 Hz, 1H, H-3), 6.88 (d, *J* = 2.6 Hz, 1H, H-1), 6.17 (d, *J* = 18.4 Hz, 1H, CH=C*H*), 3.06 (s, 6H, $N(CH_3)_2$), 1.33 (s, 12H, CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ 150.2, 149.2, 135.6, 131.5, 129.5, 128.2, 126.6, 126.5, 124.0, 116.5, 114.0[a], 106.4, 83.3 (C × 2), 40.9 (CH_3 × 2), 25.0 (CH_3 × 4); HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{27}BNO_2$ 324.2135; Found 324.2143. [a]*Detected via HSQC*

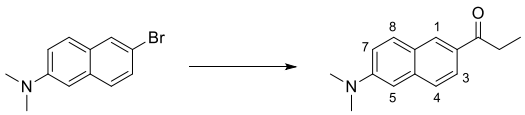
(*E*)-3-(2-(6-(Dimethylamino)naphthalen-2-yl)vinyl)-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (5)



A suspension of 3-iodo-1-isopropyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine **S10** (67 mg, 0.221 mmol), *(E)*-2-dimethylamino-6-(2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)vinyl)naphthalene **S20** (107 mg, 0.331 mmol), $Pd(PPh_3)_4$ (5 mg, 0.0044 mmol), K_2CO_3 (98 mg,

0.714 mmol) in 1,4-dioxane (1.2 mL) and H_2O (0.8 mL) was heated using microwave irradiation at 150 °C for 30 min. Upon cooling to 21 °C, the reaction mixture was extracted with EtOAc (3 × 10 mL). Combined organic layer was washed with brine (10 mL), dried ($MgSO_4$), filtered and excess solvent removed. Purification by flash column chromatography (1:40 MeOH/$CHCl_3$) afforded the title compound **5** (53 mg, 65%) as a dark yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.36 (s, 1H, Ar-H), 7.75 (s, 1H, H-1), 7.70 (d, *J* = 9.1 Hz, 1H, H-8), 7.65 (d, *J* = 1.1 Hz, 2H, H-3, H-4), 7.41 (d, *J* = 16.3 Hz, 1H, C*H*=CH), 7.29 (d, *J* = 16.3 Hz, 1H, CH=C*H*), 7.16 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7), 6.90 (d, *J* = 2.6 Hz, 1H, H-5), 5.78 (br s, 2H, NH_2), 5.16 (sept, *J* = 6.8 Hz, 1H, C*H*$(CH_3)_2$), 3.07 (s, 6H, N$(CH_3)_2$), 1.59 (d, *J* = 6.8 Hz, 6H, CH(C*H*$_3)_2$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 158.0, 155.2, 153.5, 149.2, 142.3, 135.4, 135.3, 129.9, 129.3, 127.6, 126.9, 126.6, 123.6, 117.5, 116.7, 106.3, 98.9, 49.0, 40.8 (CH_3 × 2), 22.2 (CH_3 × 2); HRMS (ESI/Q-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{25}N_6$ 373.2135; Found 373.2150.

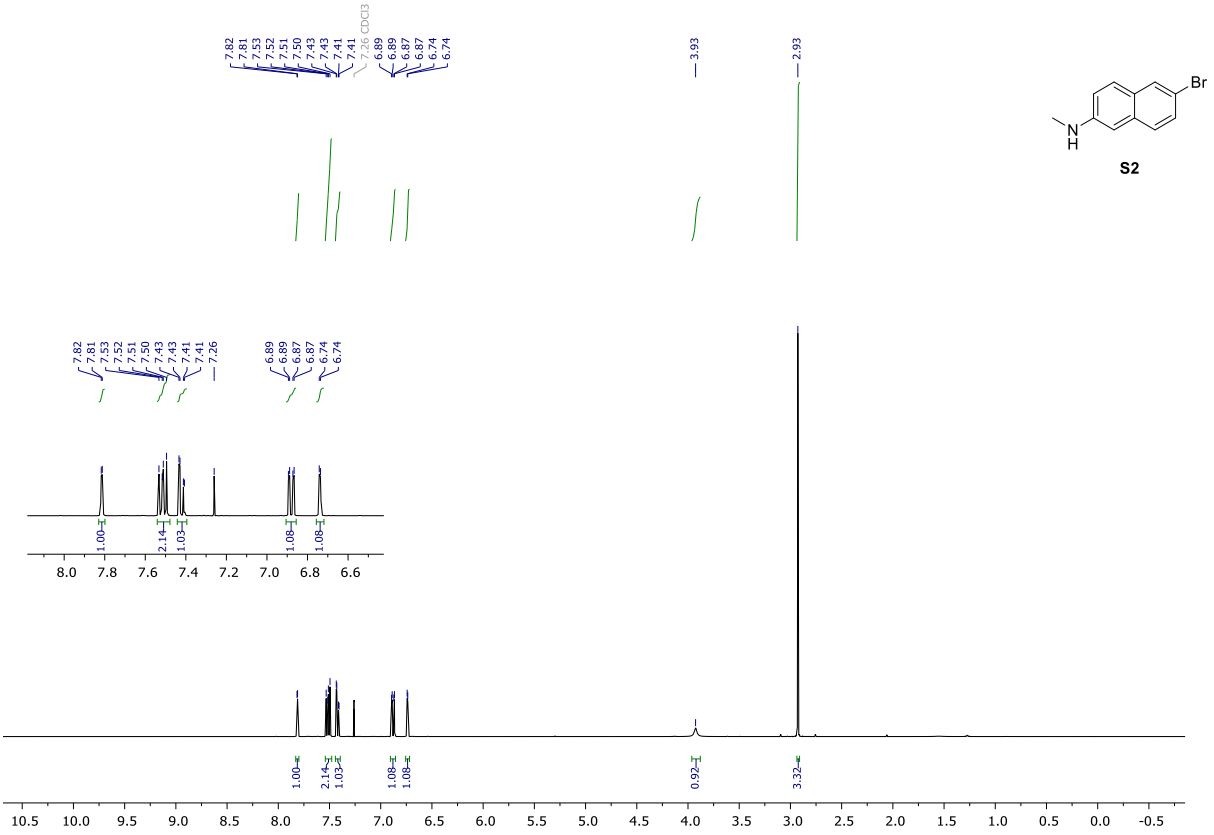
Prodan



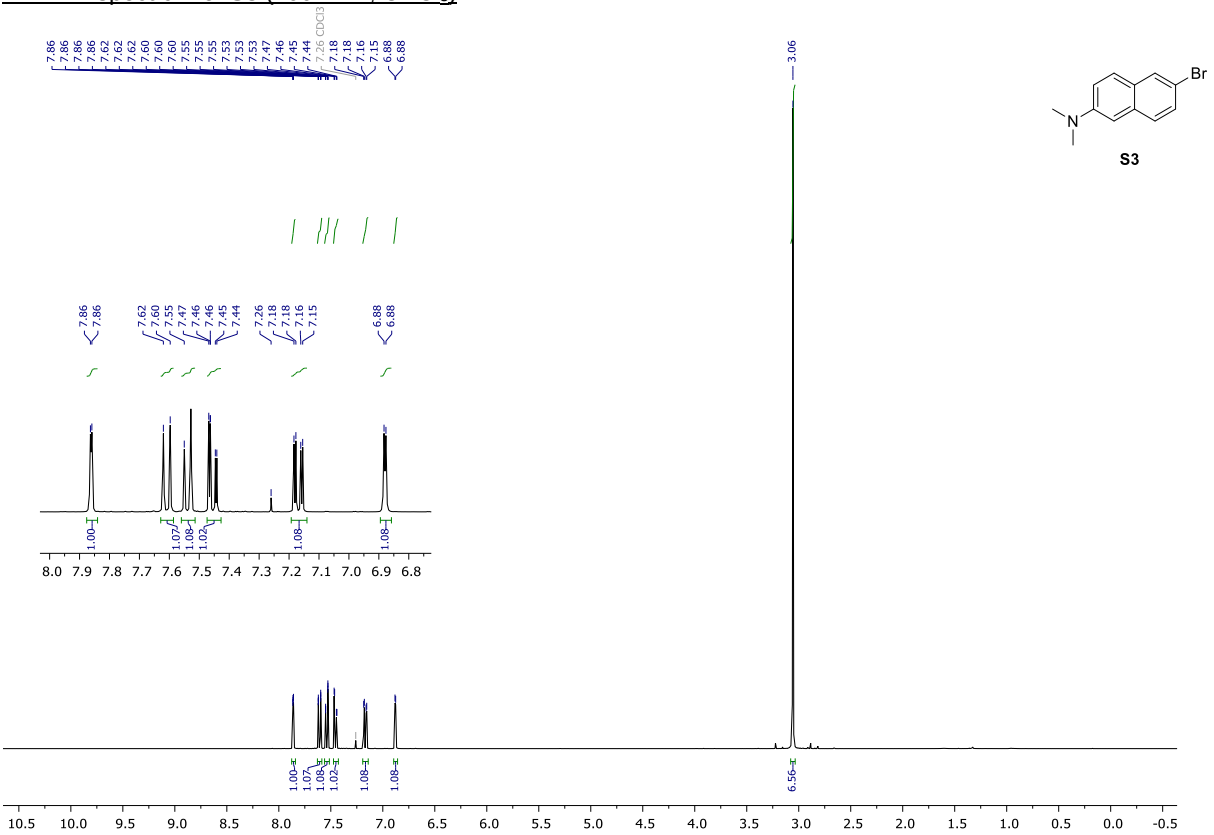
In an oved-dried microwave vial, a suspension of 6-bromo-2-dimethylaminonaphthalene **S3** (81 mg, 0.324 mmol), $Pd(OAc)_2$ (4 mg, 0.016 mmol) and DPPP (14 mg, 0.0324 mmol) in ethylene glycol (2 mL) was evacuated and backfilled with N_2 (× 5). To this, was added ethyl-1-propenyl ether (180 µL, 1.62 mmol) and triethylamine (113 µL, 0.810 mmol). The reaction mixture was then heated to 145 °C for 12 hours. After cooling to 21 °C, the reaction mixture was diluted with CH_2Cl_2 (3 mL) and aqueous HCl (5%, 2 mL) and left to stir for an additional 60 min at ambient temperature. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 × 10 mL). Combined organic phase was washed with brine (10 mL), dried ($MgSO_4$), filtered and excess solvent removed *in vacuo*. Purification by column chromatography (1:1 CH_2Cl_2/pentane) afforded the title compound **Prodan** (71 mg, 65%, R_f = 0.32) as a pale yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33 (d, *J* = 1.8 Hz, 1H, H-1), 7.94 (dd, *J* = 8.7, 1.8 Hz, 1H, H-3), 7.79 (d, *J* = 9.0 Hz, 1H, H-8), 7.64 (d, *J* = 8.7 Hz, 1H, H-4), 7.17 (dd, *J* = 9.1, 2.6 Hz, 1H, H-7), 6.87 (d, *J* = 2.6 Hz, 1H, H-5), 3.10 (s, 6H, N$(CH_3)_2$), 3. 08 (q, *J* = 7.3 Hz, 2H, C*H*$_2$CH$_3$), 1.27 (t, *J* = 7.3 Hz, 3H, CH_2C*H*$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ 200.5, 150.3, 137.7, 130.7, 130.6, 129.8, 126.3, 125.3, 124.7, 116.4, 105.5, 40.6 (CH_3 × 2), 30.3, 8.8. Data is consistent with the literature.[6]

^{1}H and ^{13}C NMR Spectra of Compounds 1–6 and Prodan

^{1}H NMR spectrum of **S2** (400 MHz, $CDCl_3$)

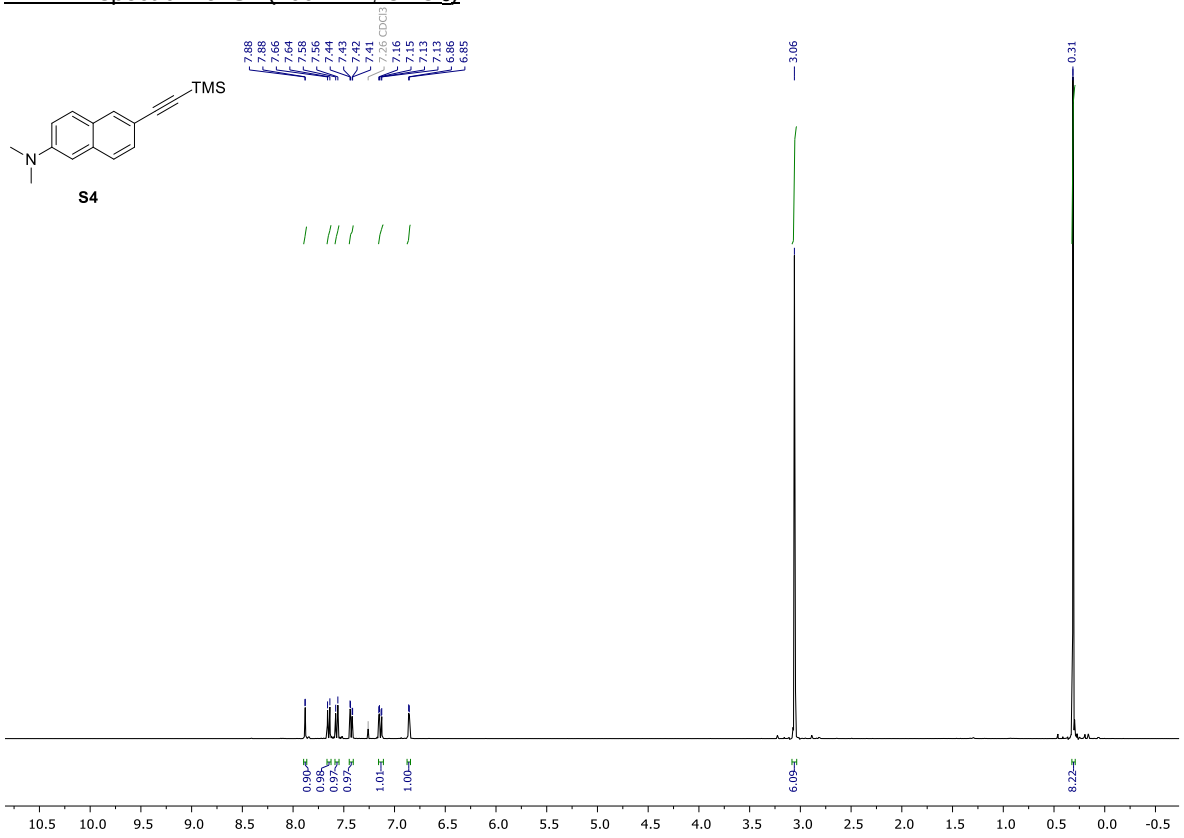


^{1}H NMR spectrum of **S3** (400 MHz, $CDCl_3$)

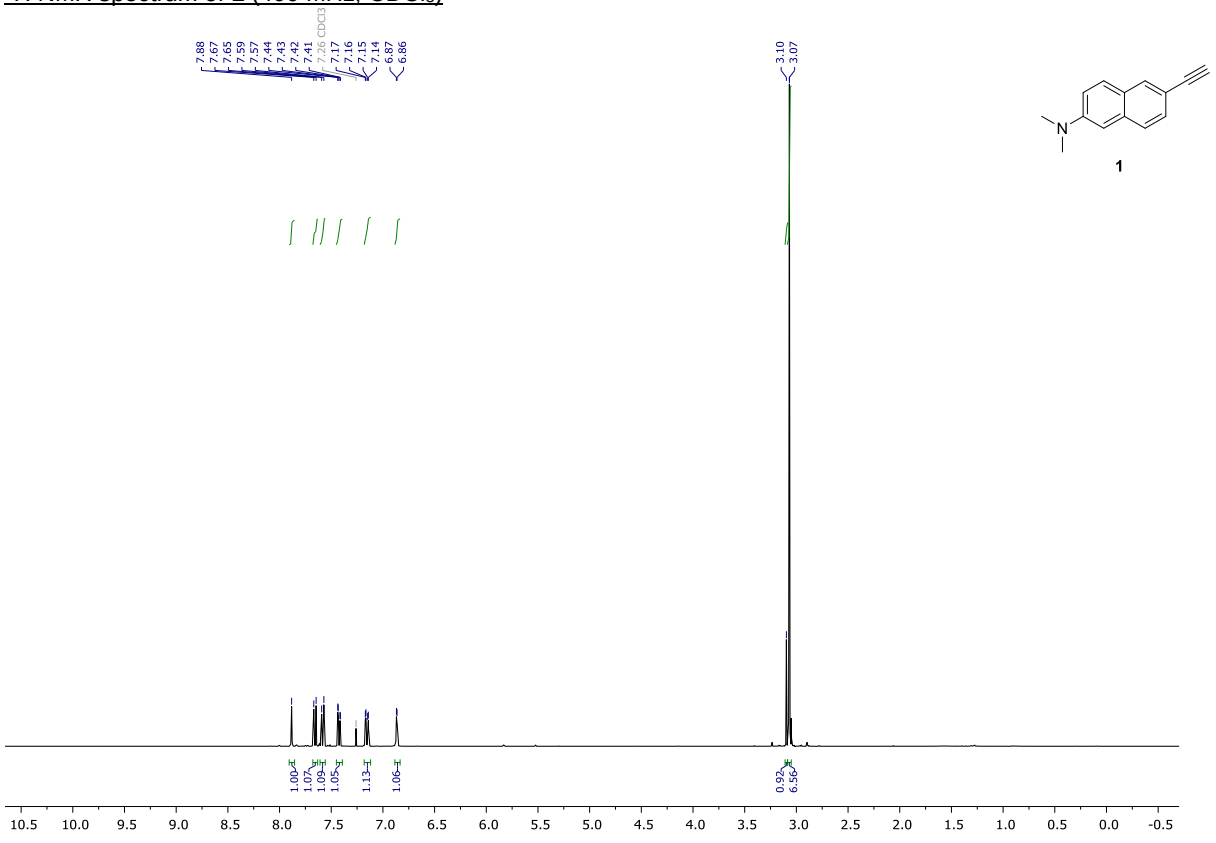


SUPPORTING INFORMATION

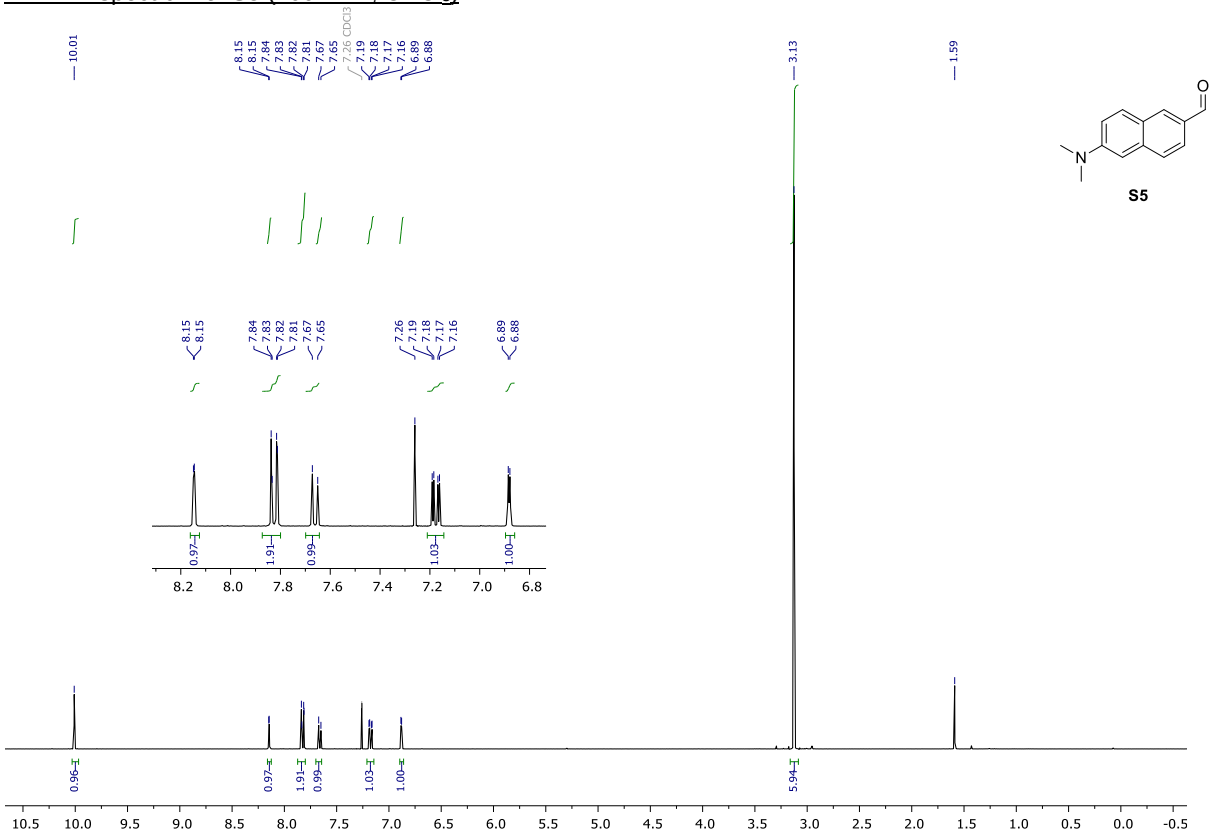
^{1}H NMR spectrum of **S4** (400 MHz, $CDCl_3$)



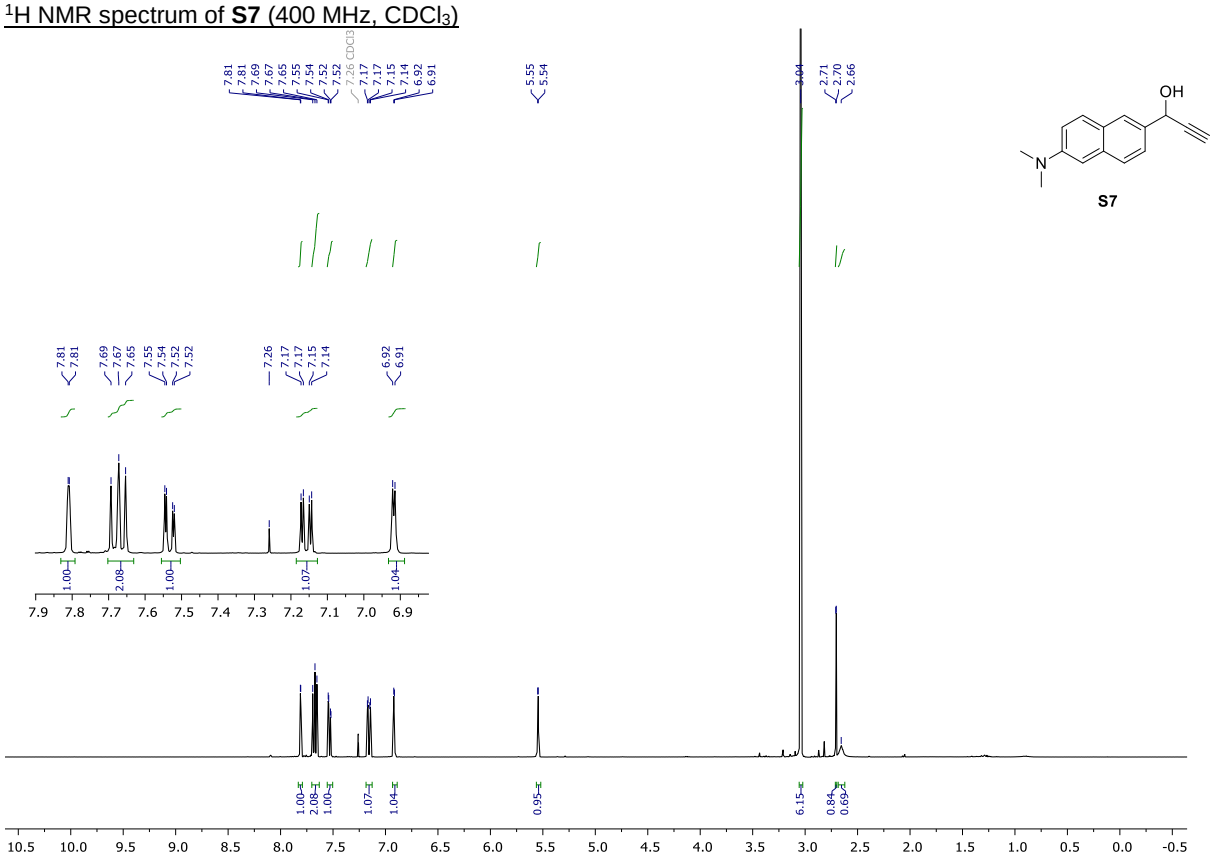
^{1}H NMR spectrum of **1** (400 MHz, $CDCl_3$)



^{1}H NMR spectrum of **S5** (400 MHz, $CDCl_3$)

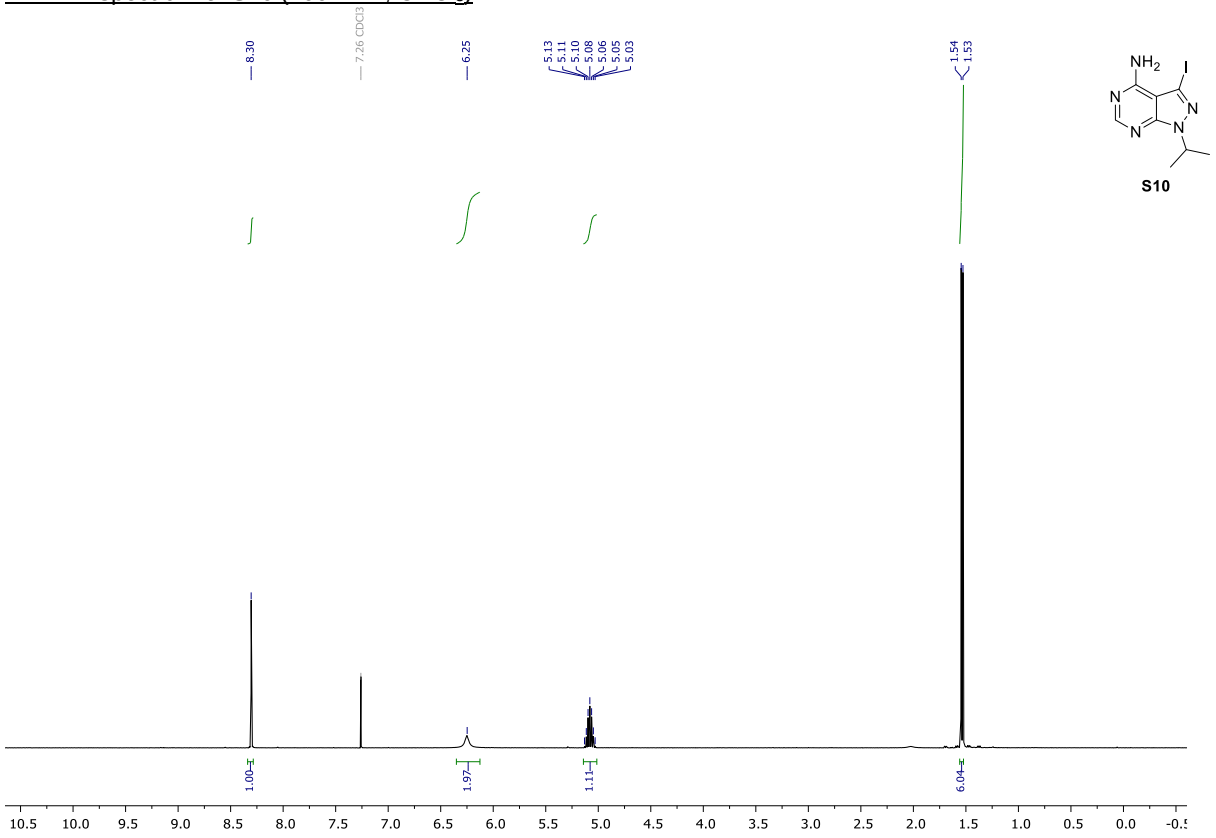


^{1}H NMR spectrum of **S7** (400 MHz, $CDCl_3$)

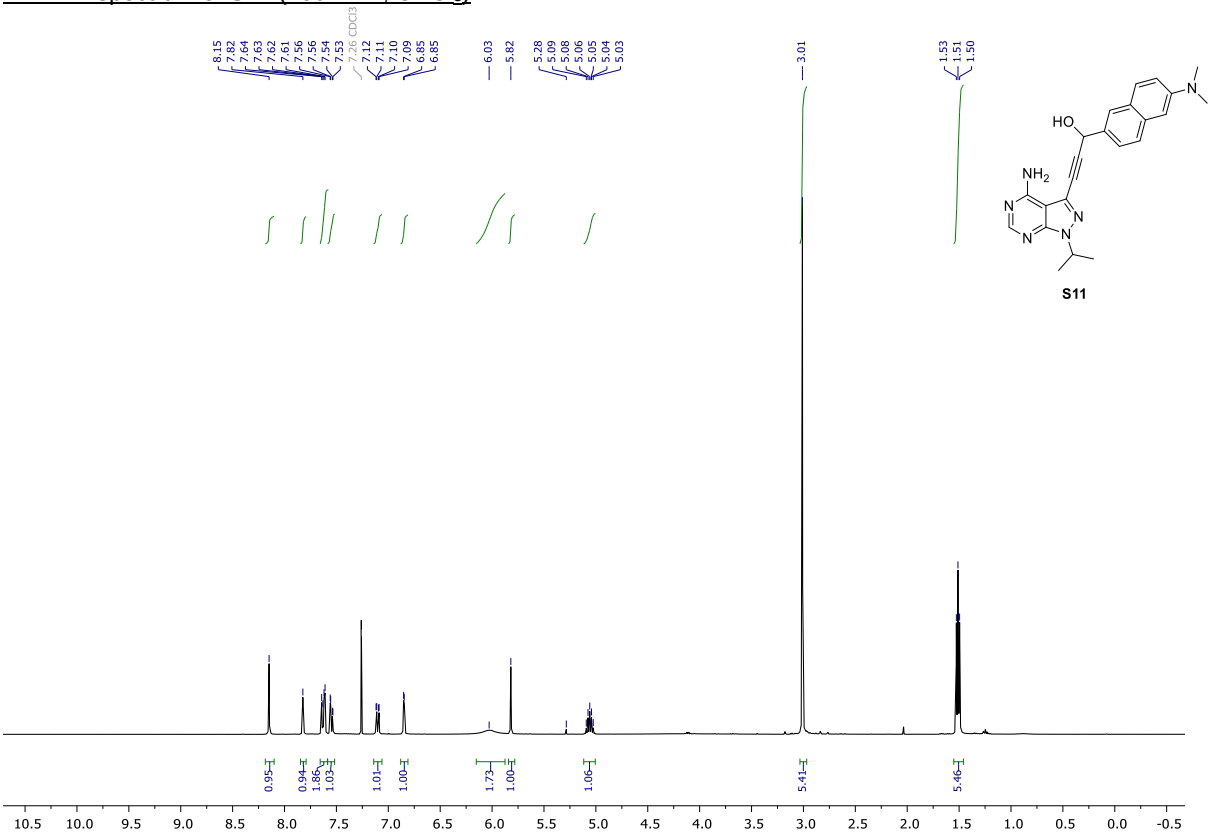


SUPPORTING INFORMATION

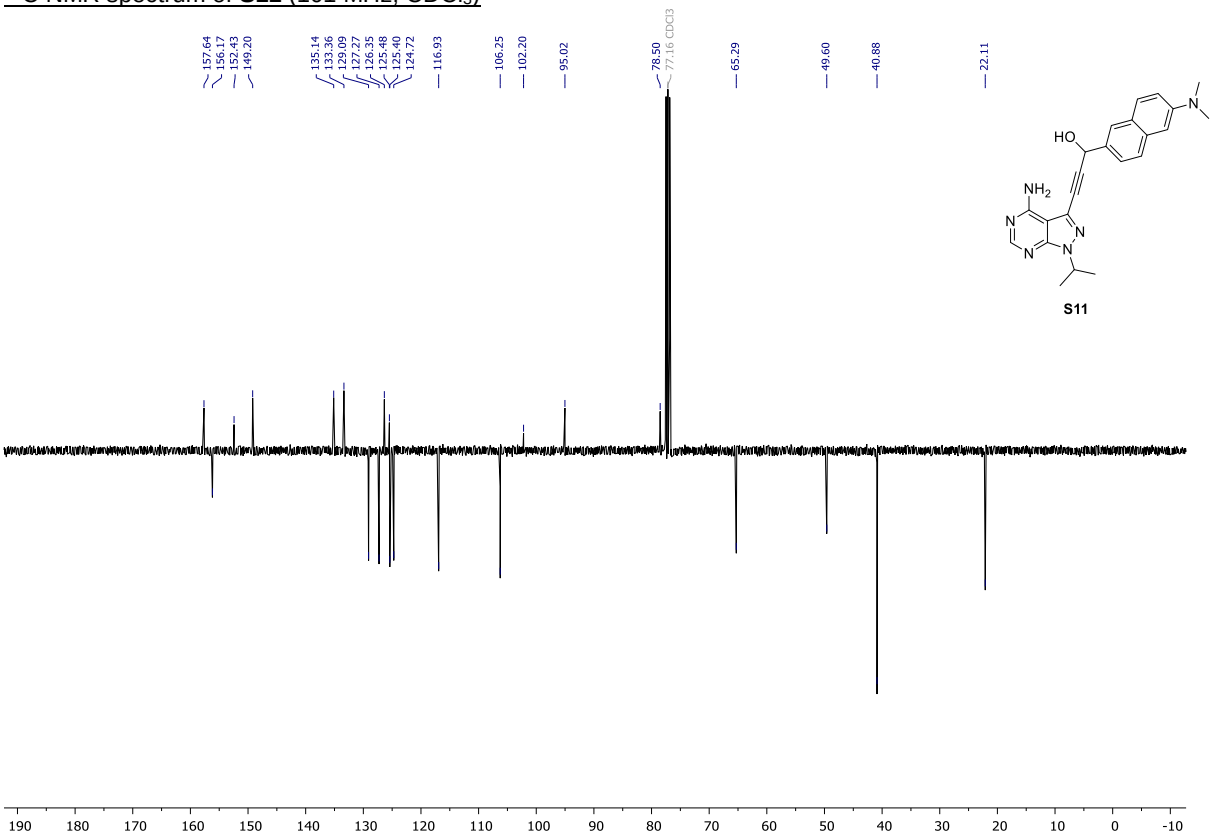
^{1}H NMR spectrum of **S10** (400 MHz, $CDCl_3$)



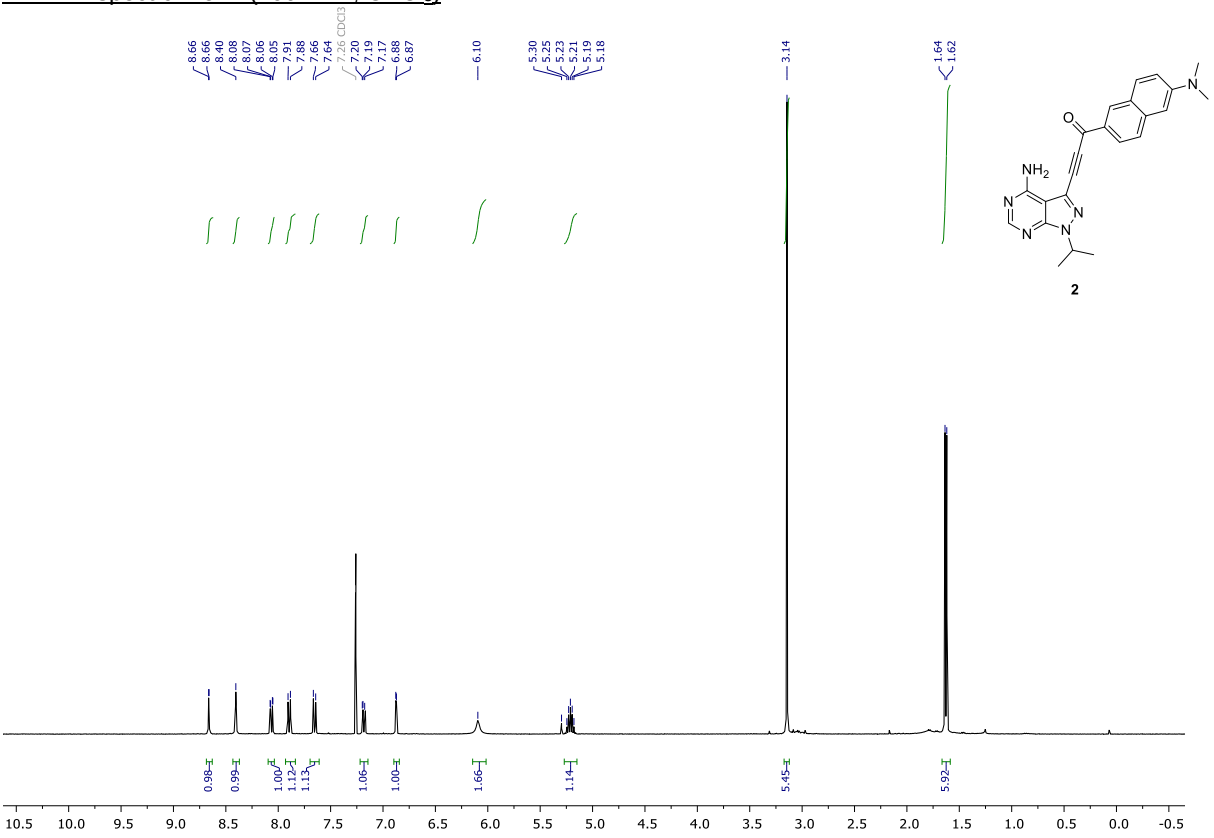
^{1}H NMR spectrum of **S11** (400 MHz, $CDCl_3$)



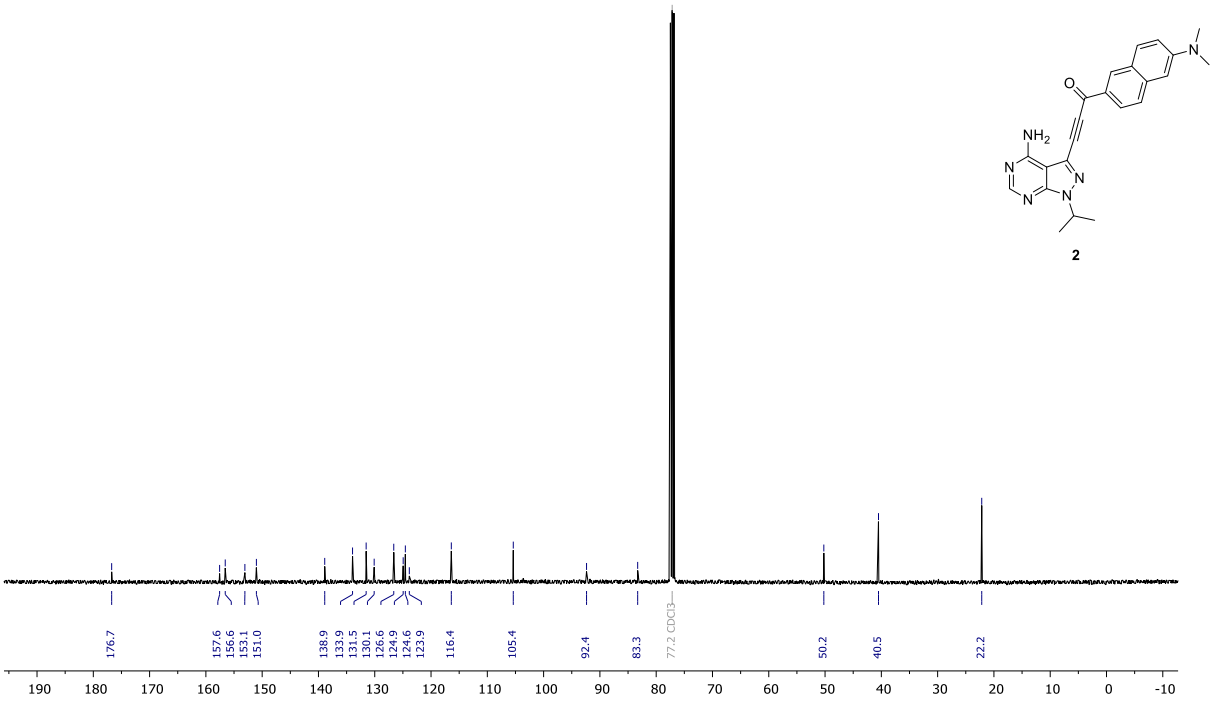
^{13}C NMR spectrum of **S11** (101 MHz, $CDCl_3$)



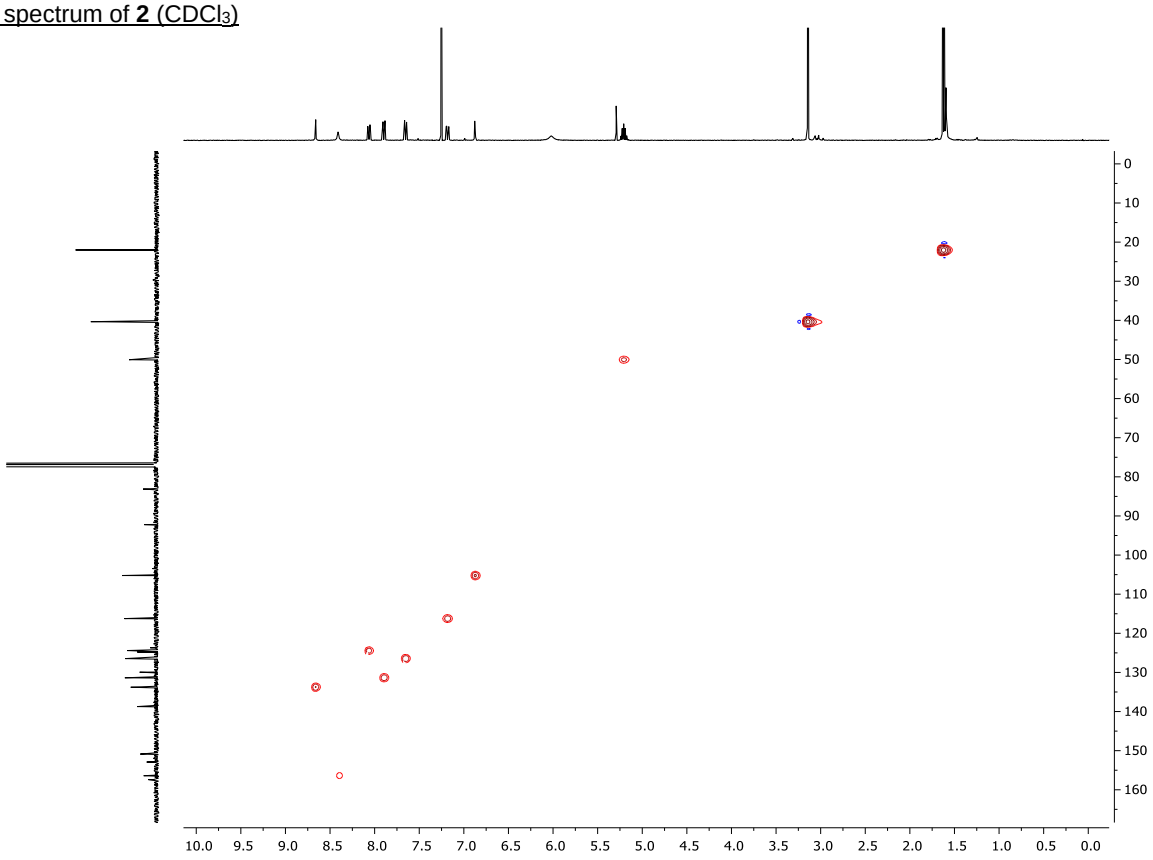
^{1}H NMR spectrum of **2** (400 MHz, $CDCl_3$)



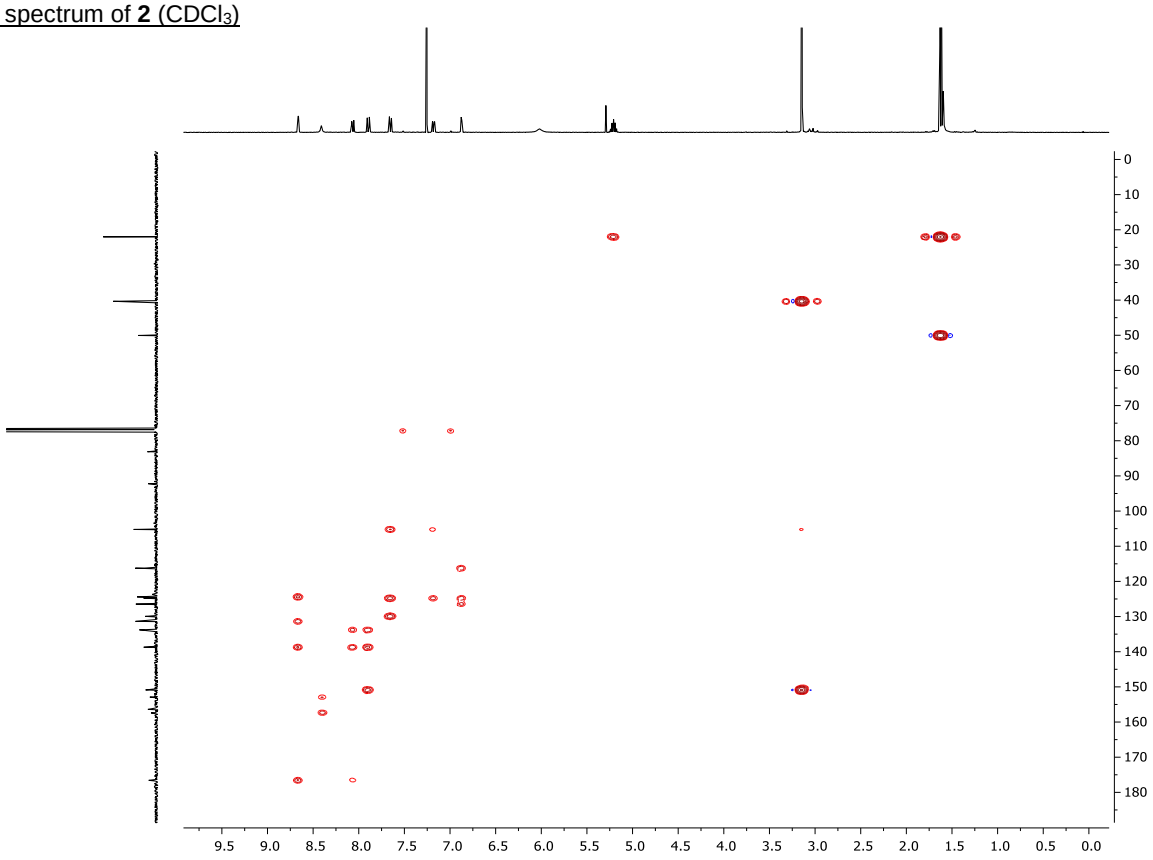
^{13}C NMR spectrum of **2** (101 MHz, $CDCl_3$)



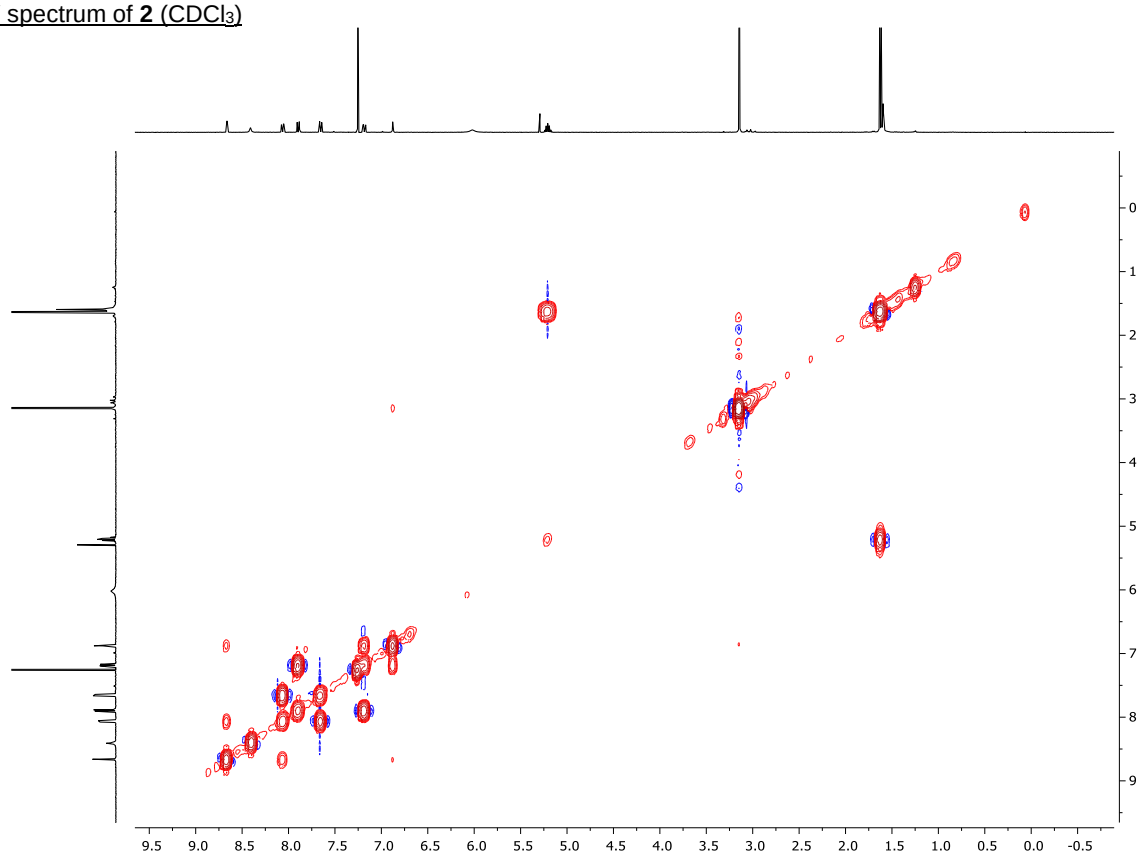
HSQC spectrum of **2** ($CDCl_3$)



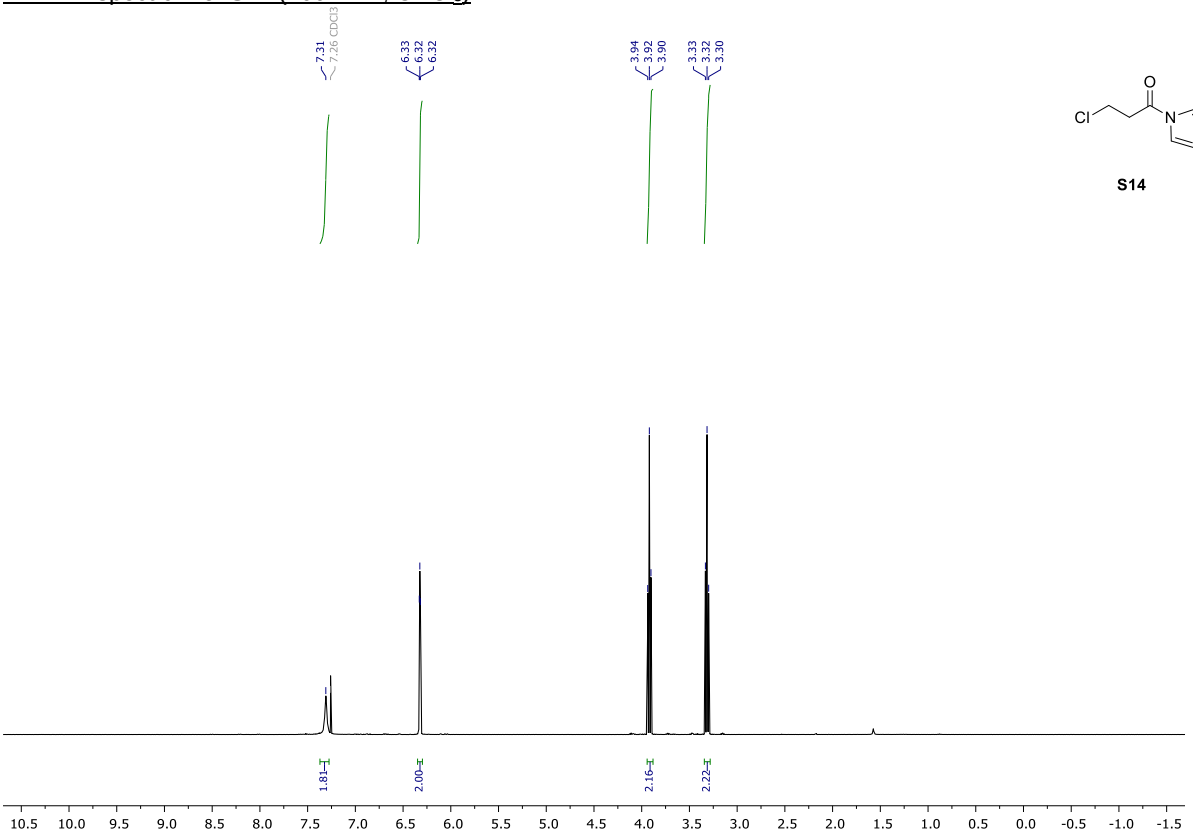
HMBC spectrum of **2** ($CDCl_3$)



COSY spectrum of **2** ($CDCl_3$)

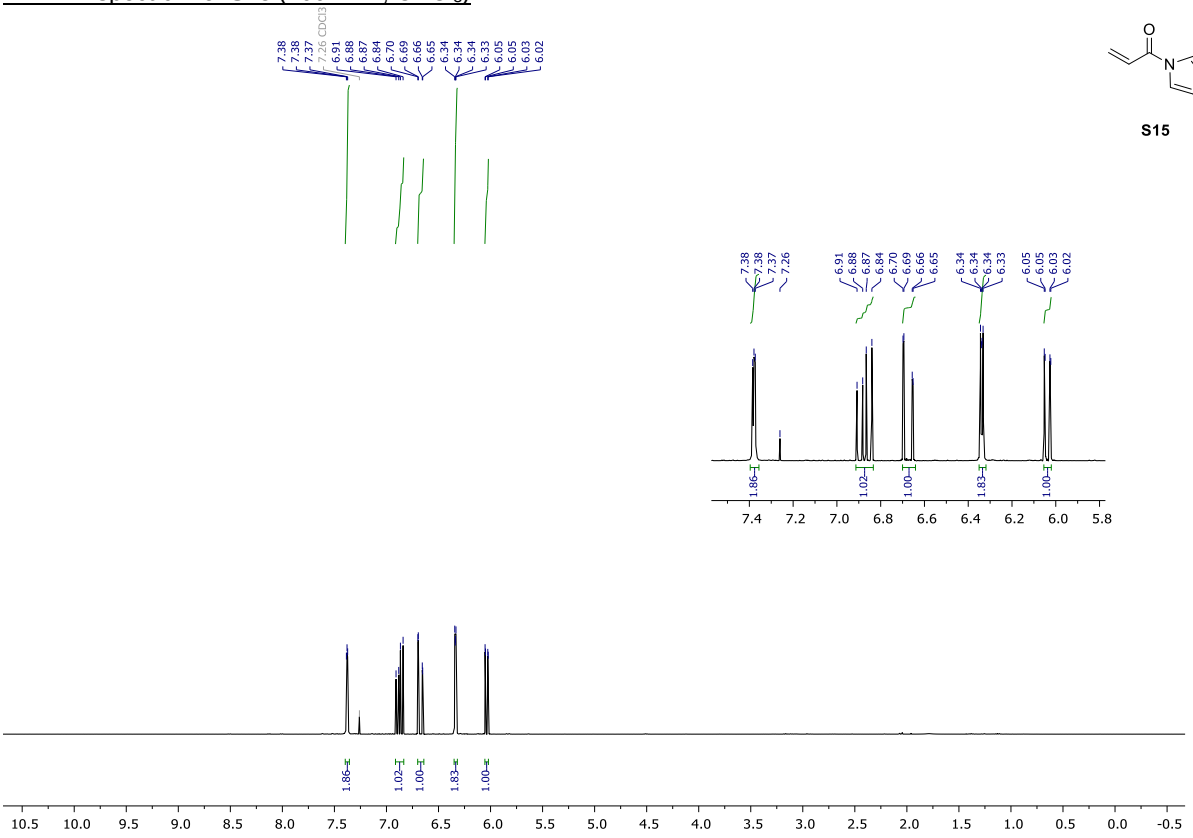


^{1}H NMR spectrum of **S14** (400 MHz, $CDCl_3$)



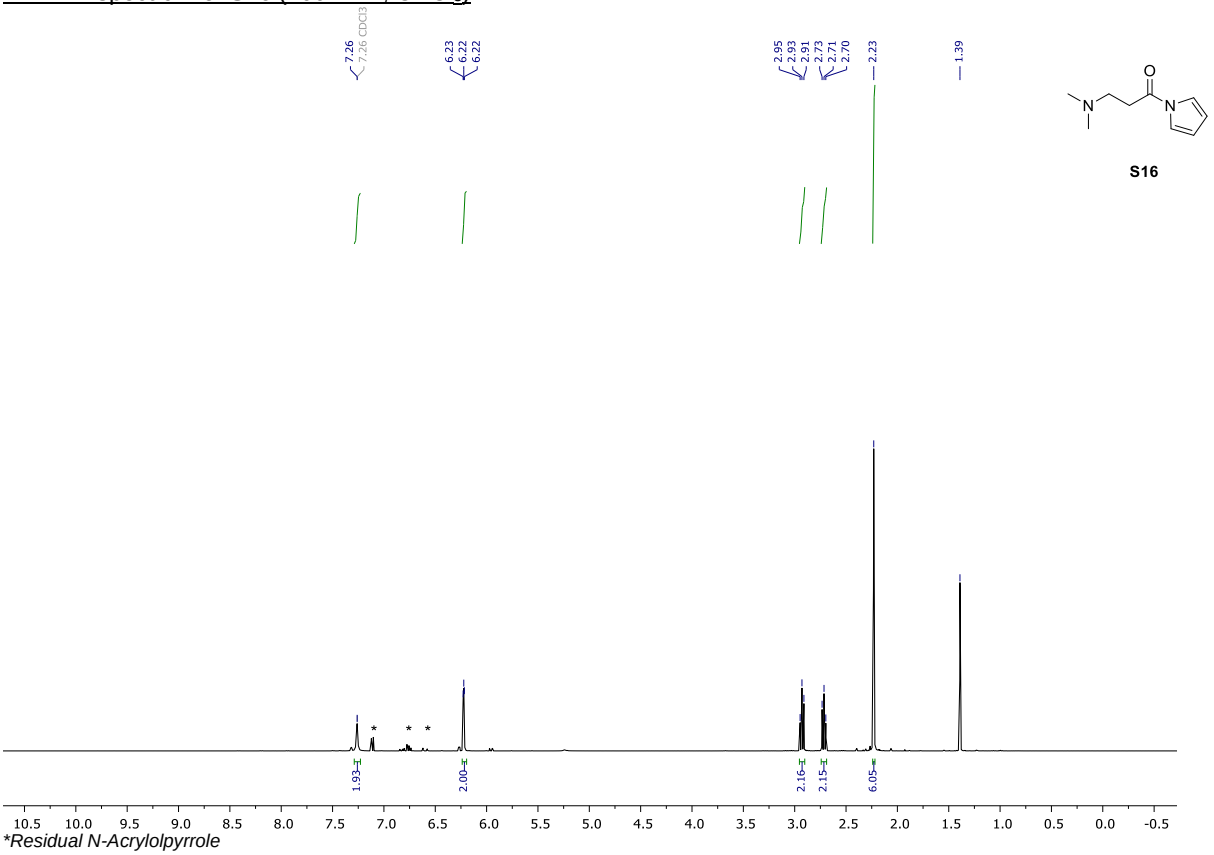
S14

^{1}H NMR spectrum of **S15** (400 MHz, $CDCl_3$)



S15

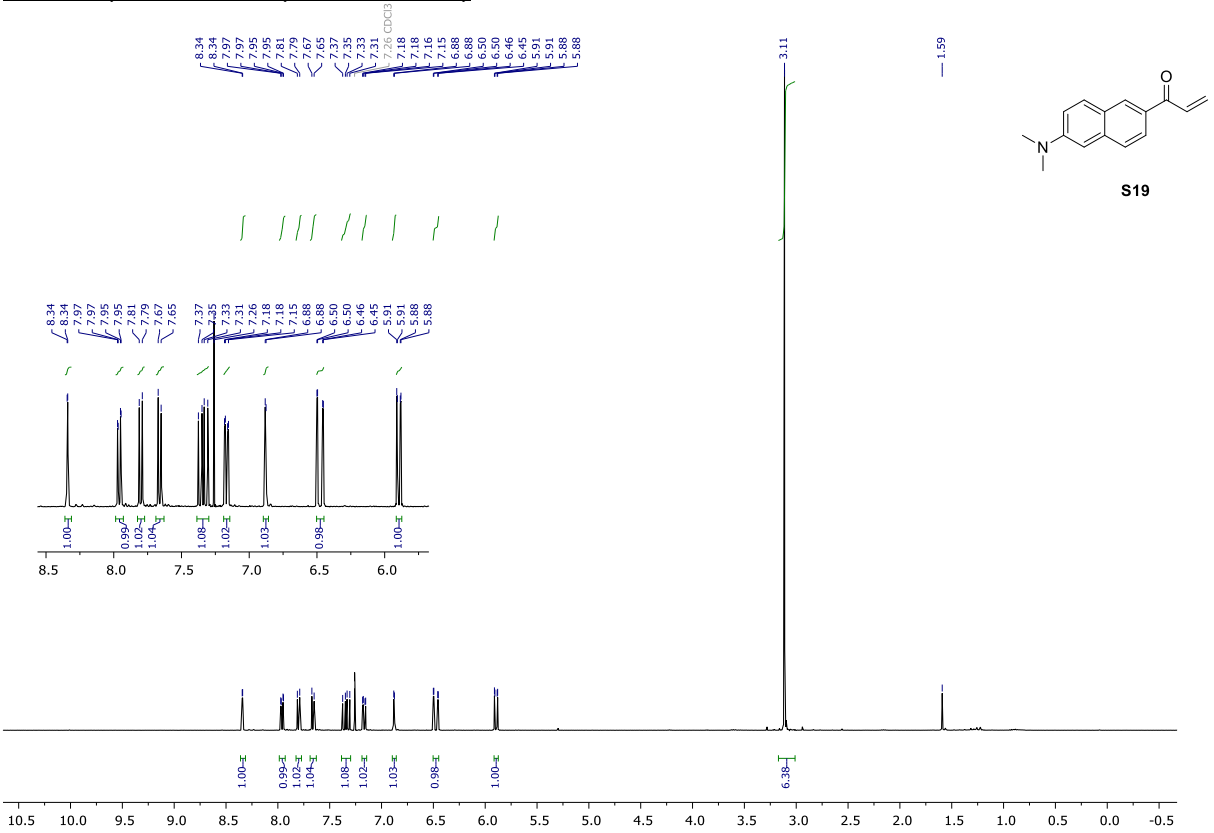
^{1}H NMR spectrum of **S16** (400 MHz, $CDCl_3$)



S16

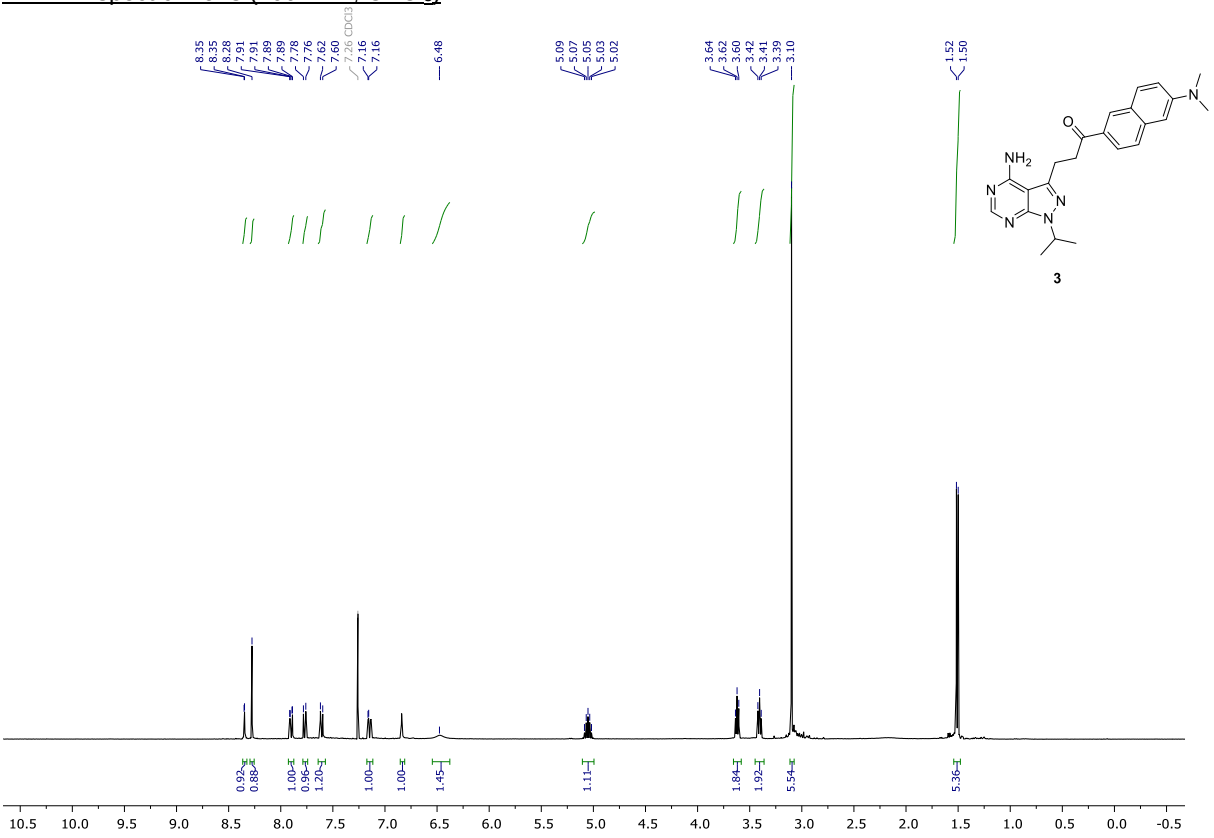
**Residual N-Acrylolpyrrole*

^{1}H NMR spectrum of **S19** (400 MHz, $CDCl_3$)

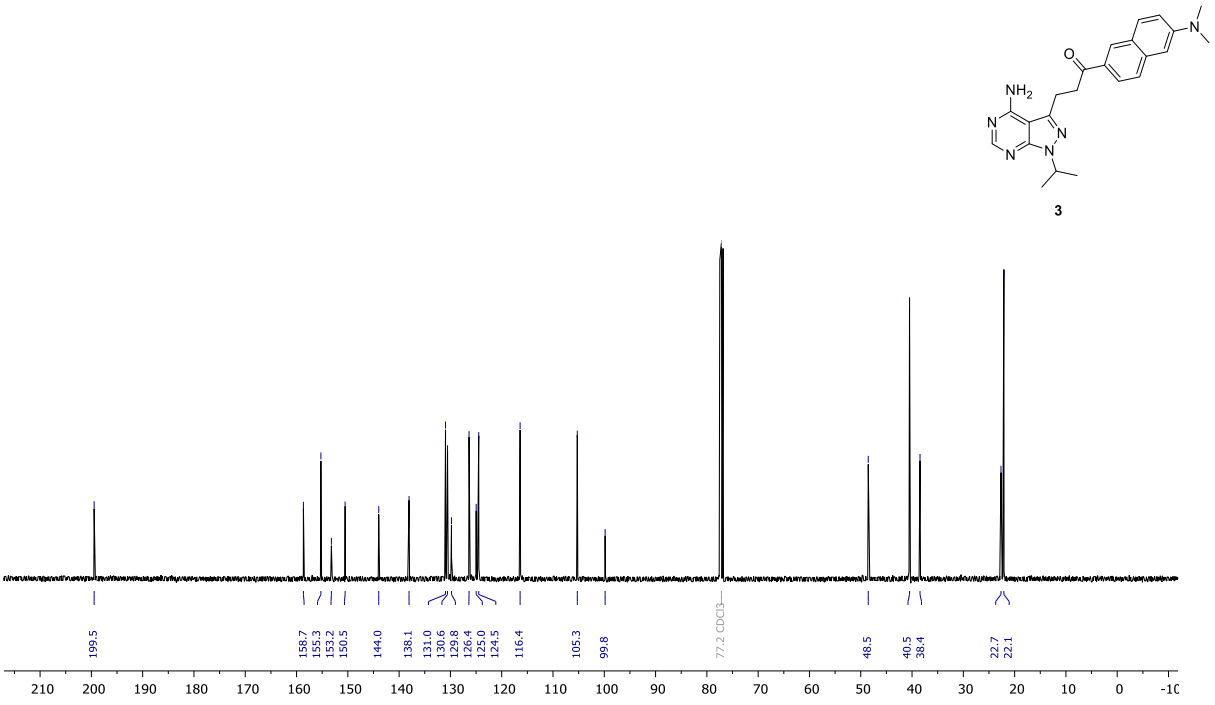


S19

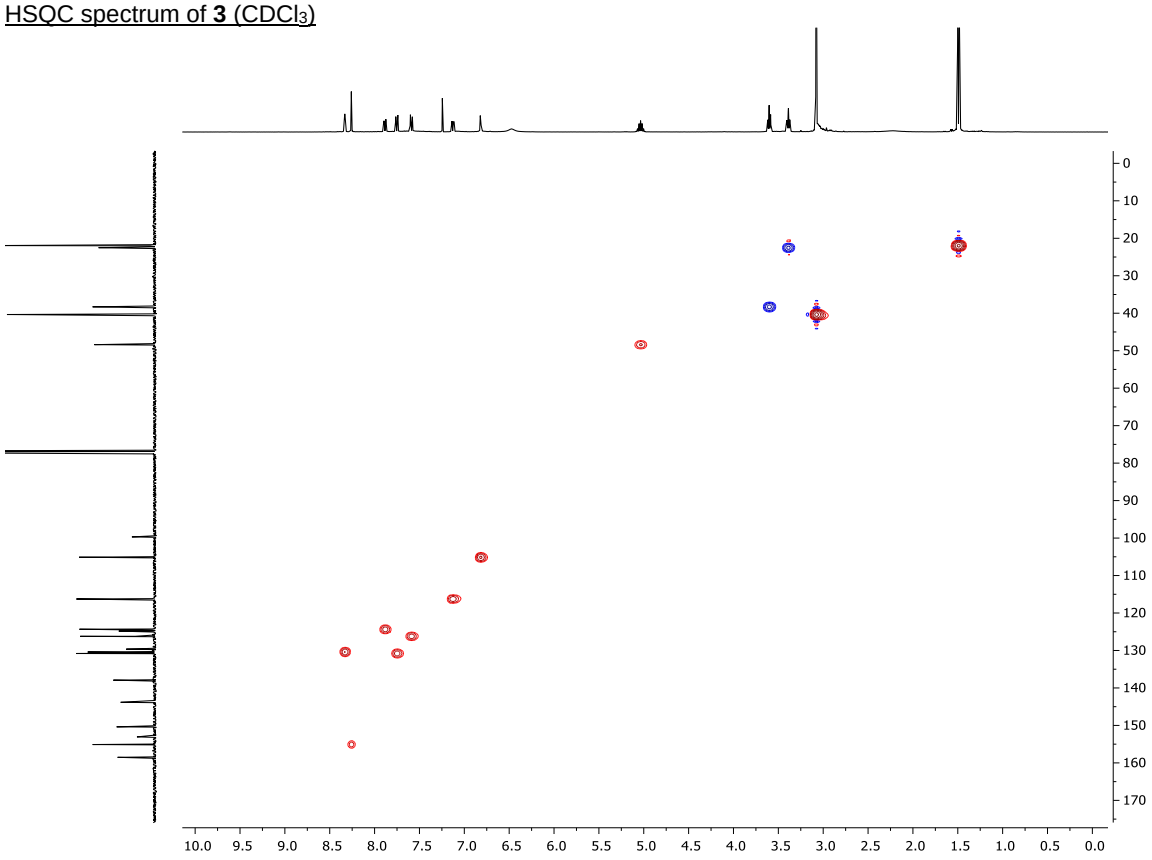
^{1}H NMR spectrum of **3** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **3** (101 MHz, $CDCl_3$)

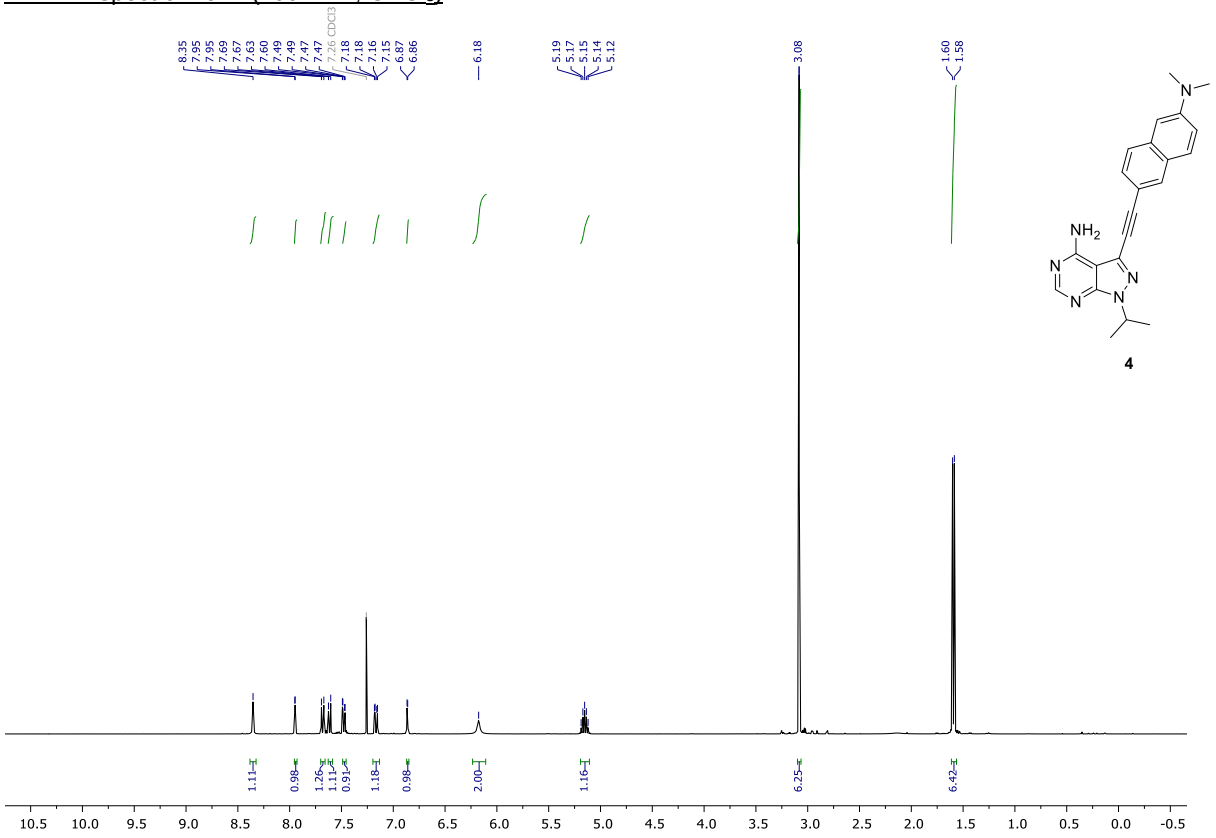


HSQC spectrum of **3** ($CDCl_3$)

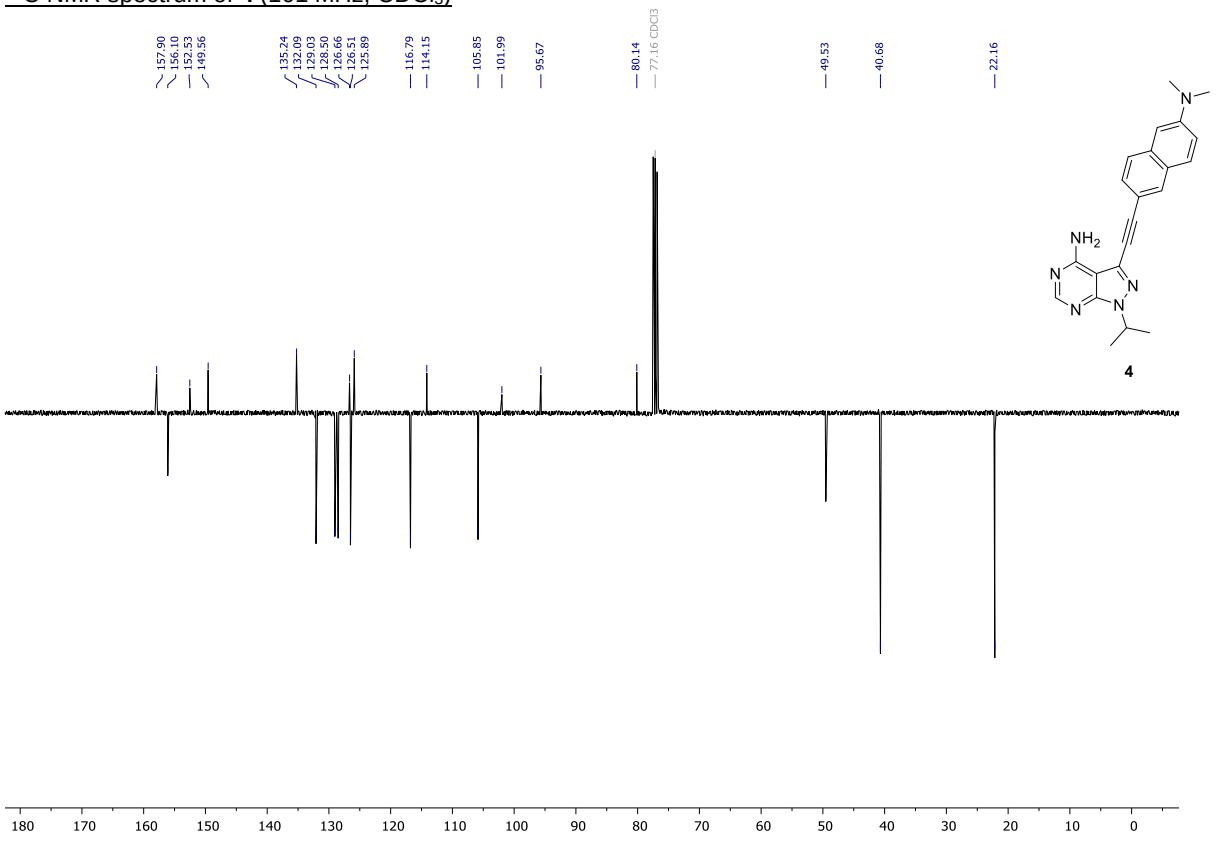


SUPPORTING INFORMATION

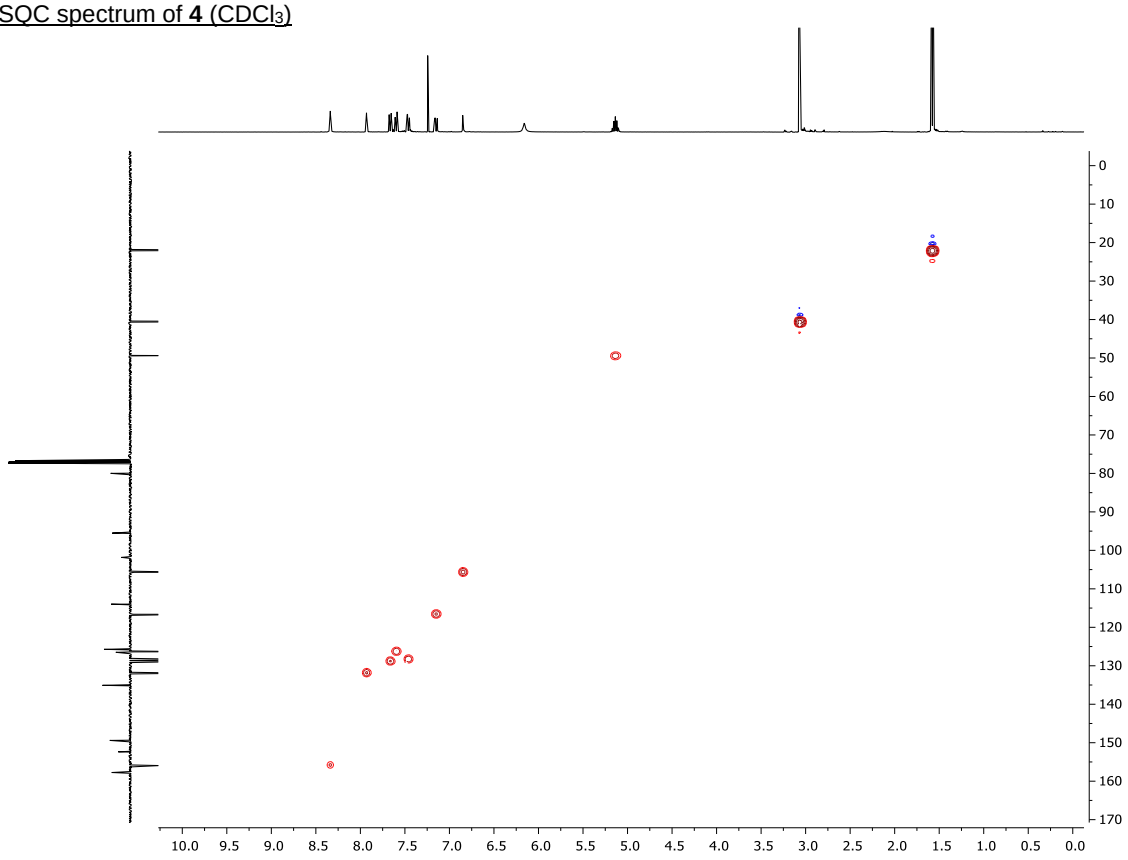
^{1}H NMR spectrum of **4** (400 MHz, $CDCl_3$)



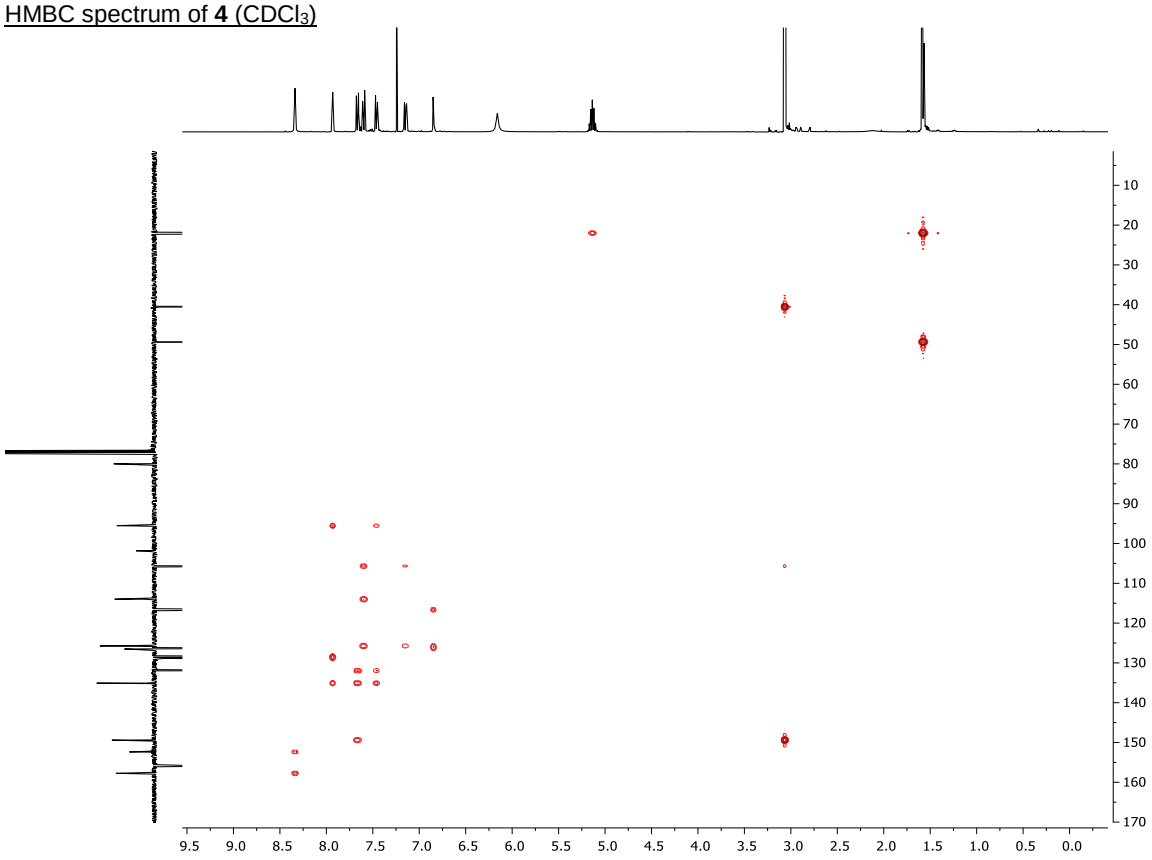
^{13}C NMR spectrum of **4** (101 MHz, $CDCl_3$)



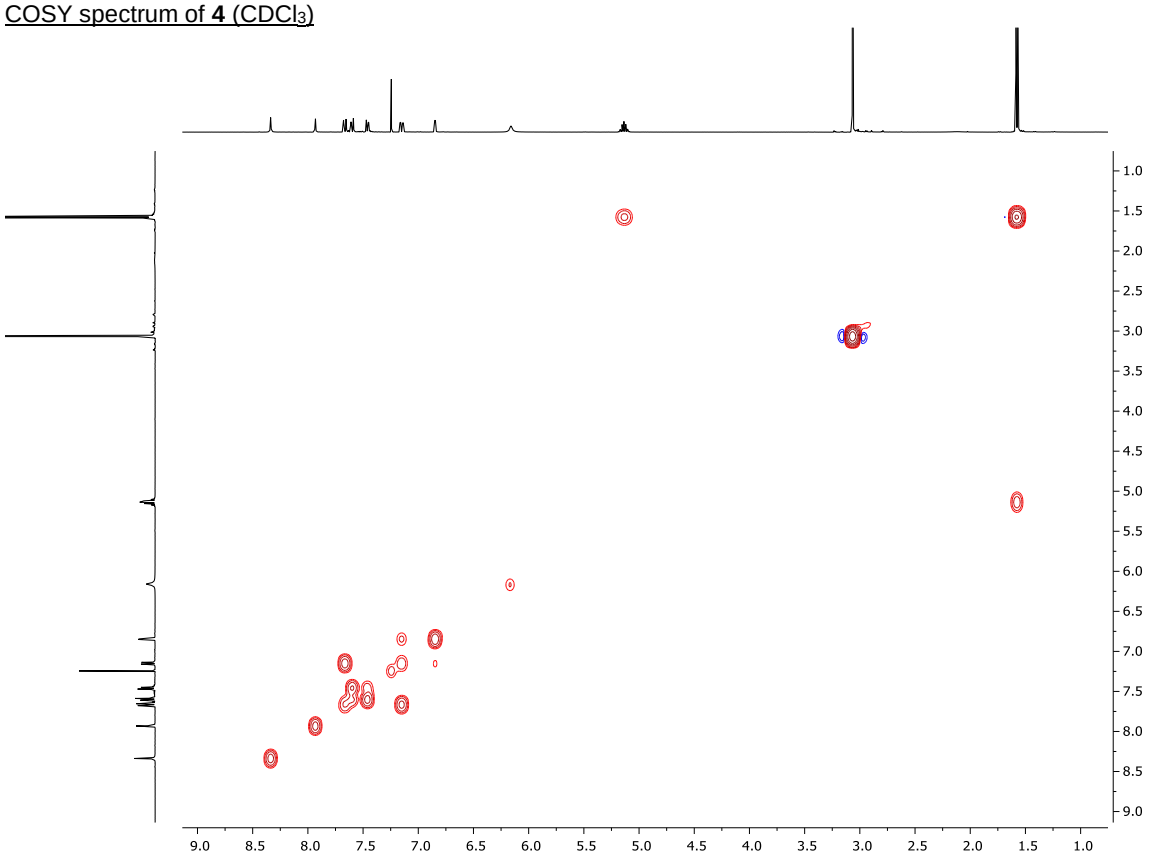
HSQC spectrum of **4** ($CDCl_3$)



HMBC spectrum of **4** ($CDCl_3$)

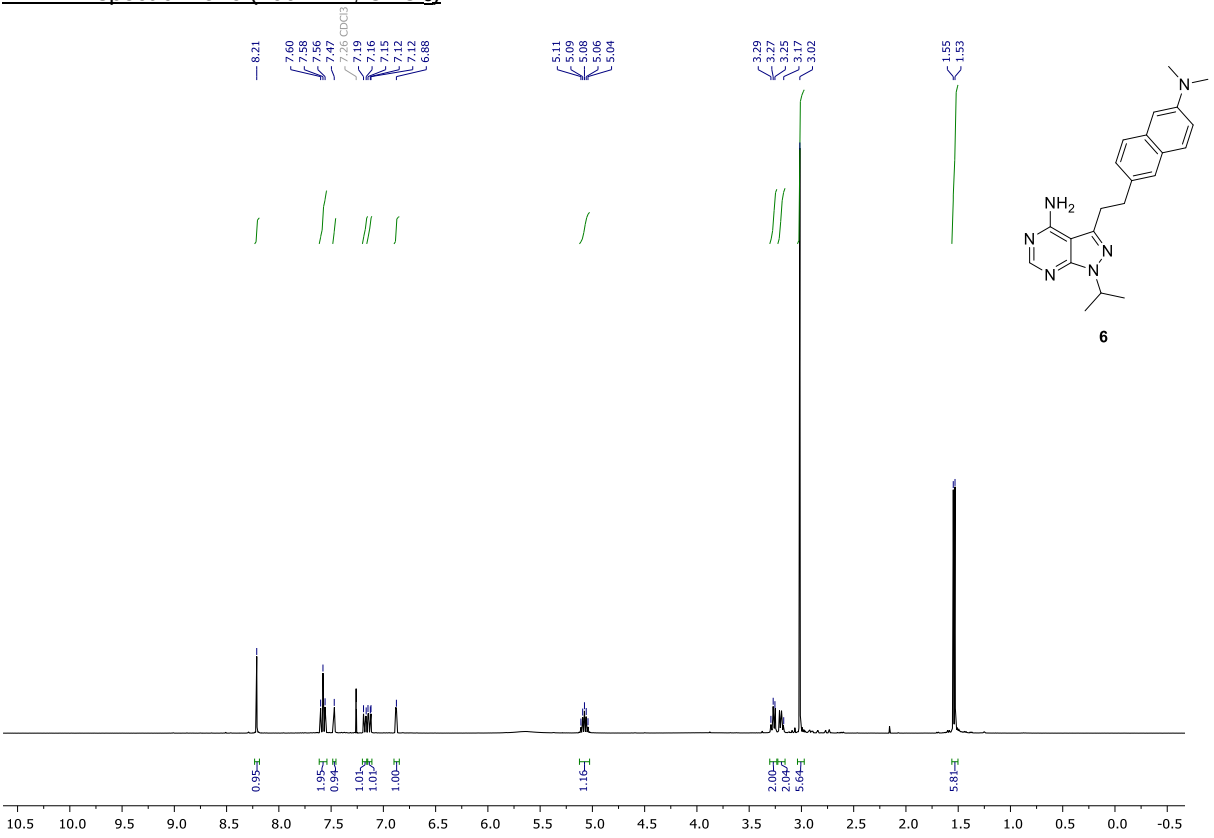


COSY spectrum of **4** ($CDCl_3$)

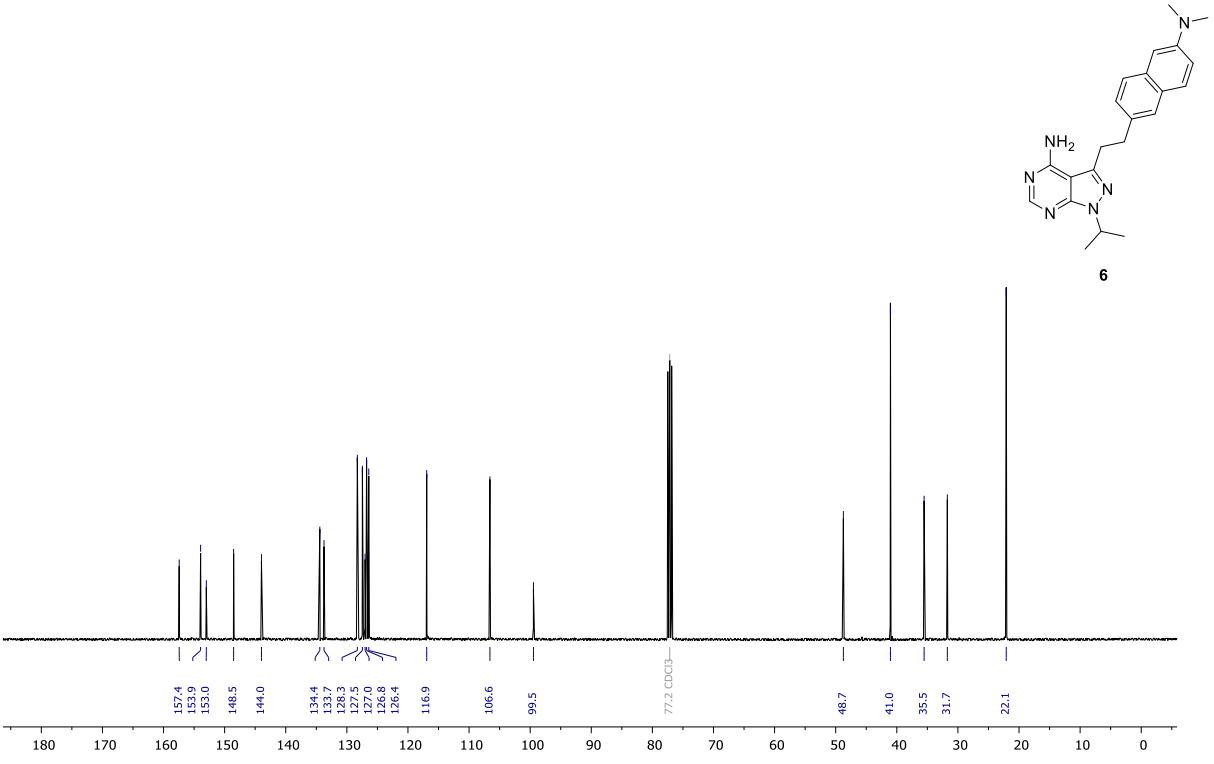


SUPPORTING INFORMATION

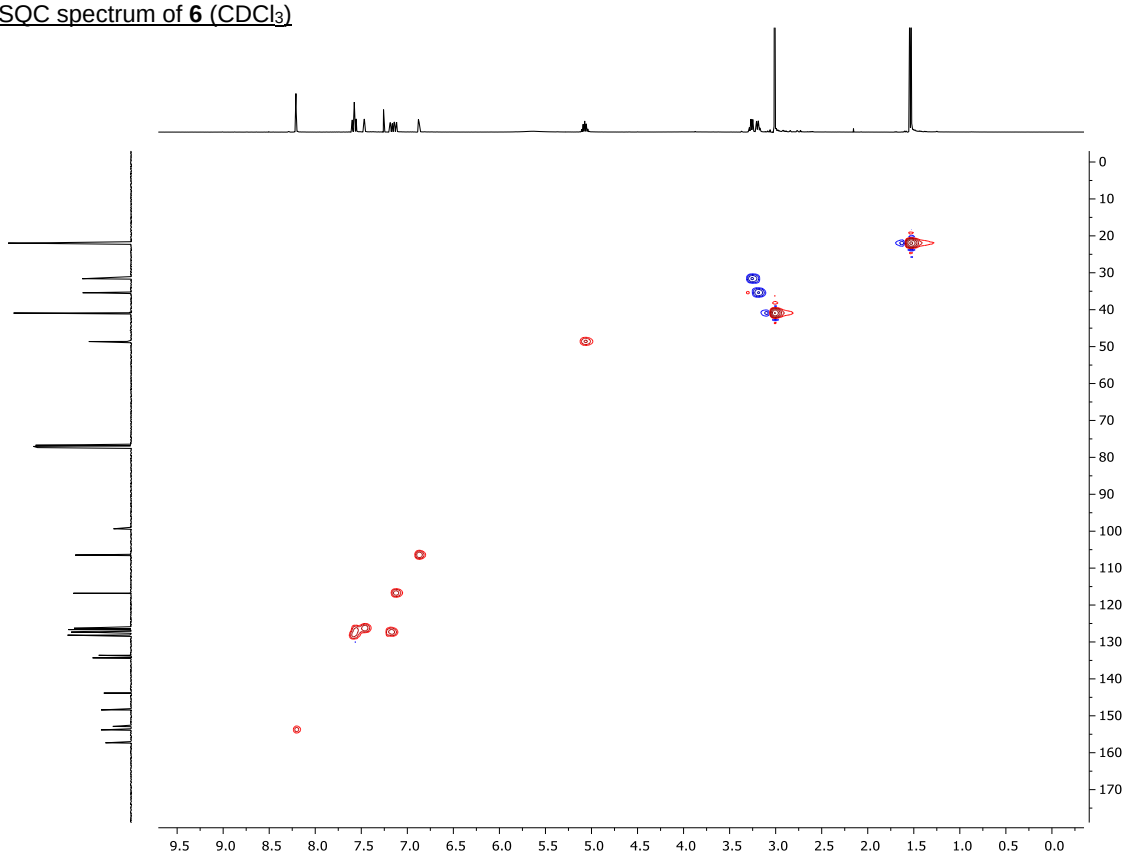
^{1}H NMR spectrum of **6** (400 MHz, $CDCl_3$)



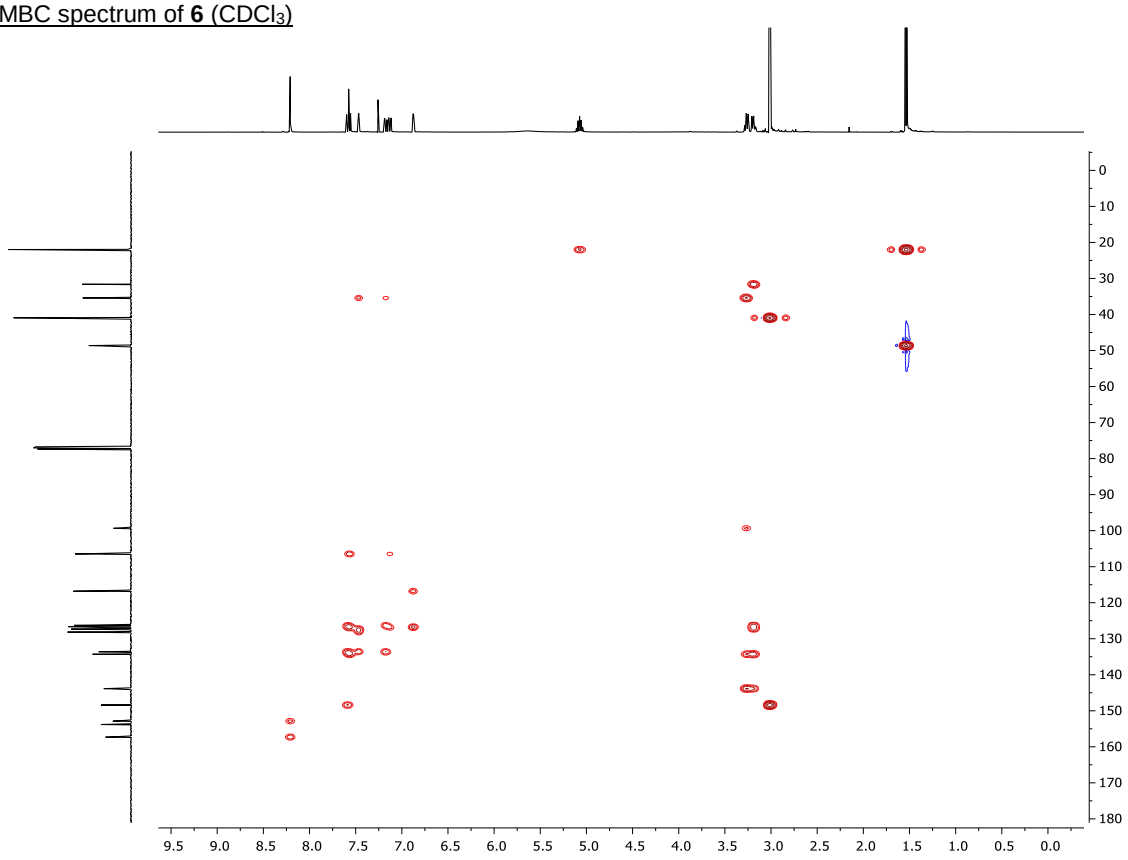
^{13}C NMR spectrum of **6** (101 MHz, $CDCl_3$)



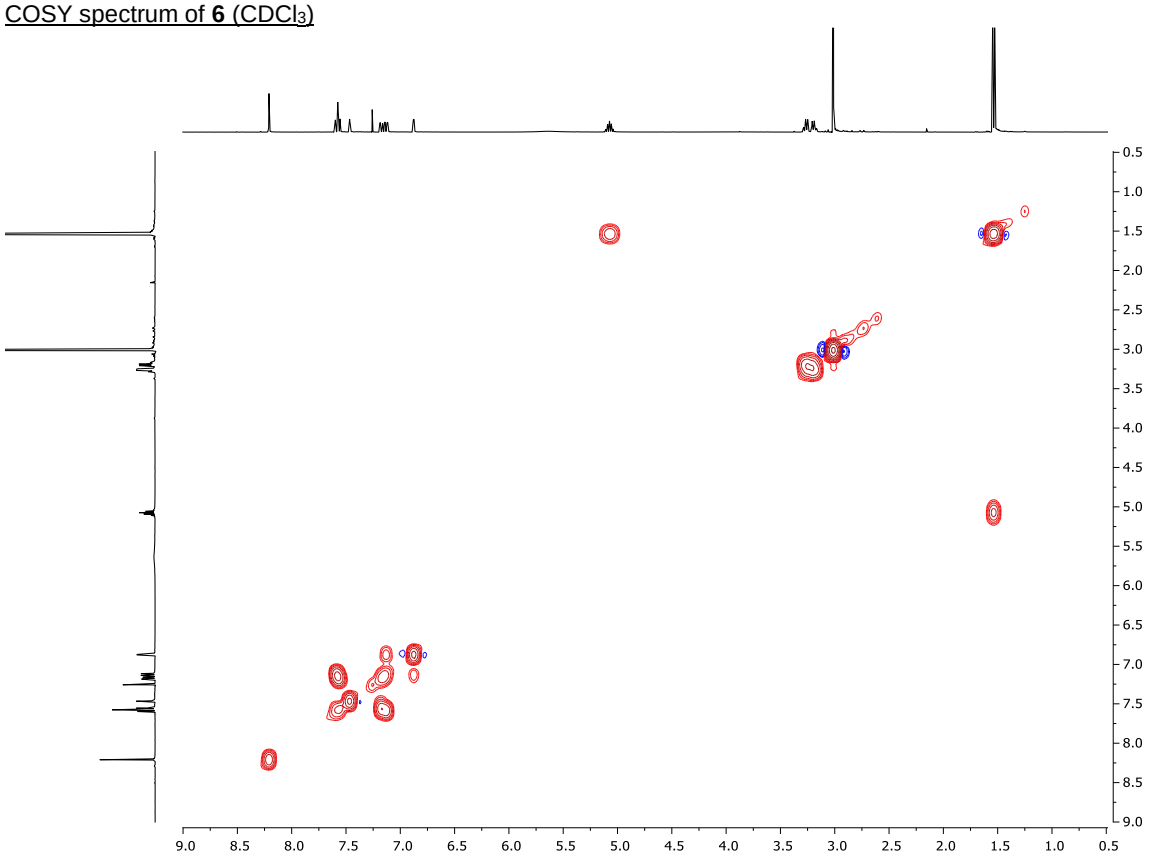
HSQC spectrum of **6** ($CDCl_3$)



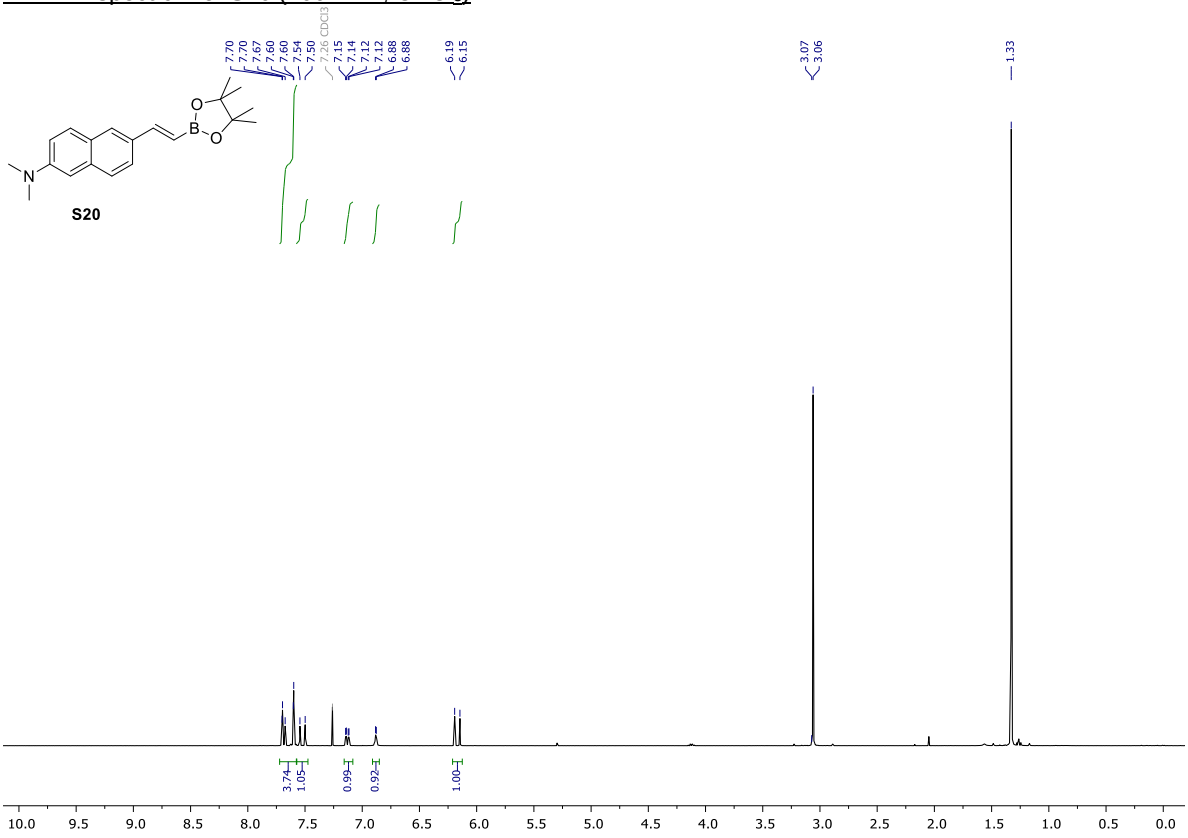
HMBC spectrum of **6** ($CDCl_3$)



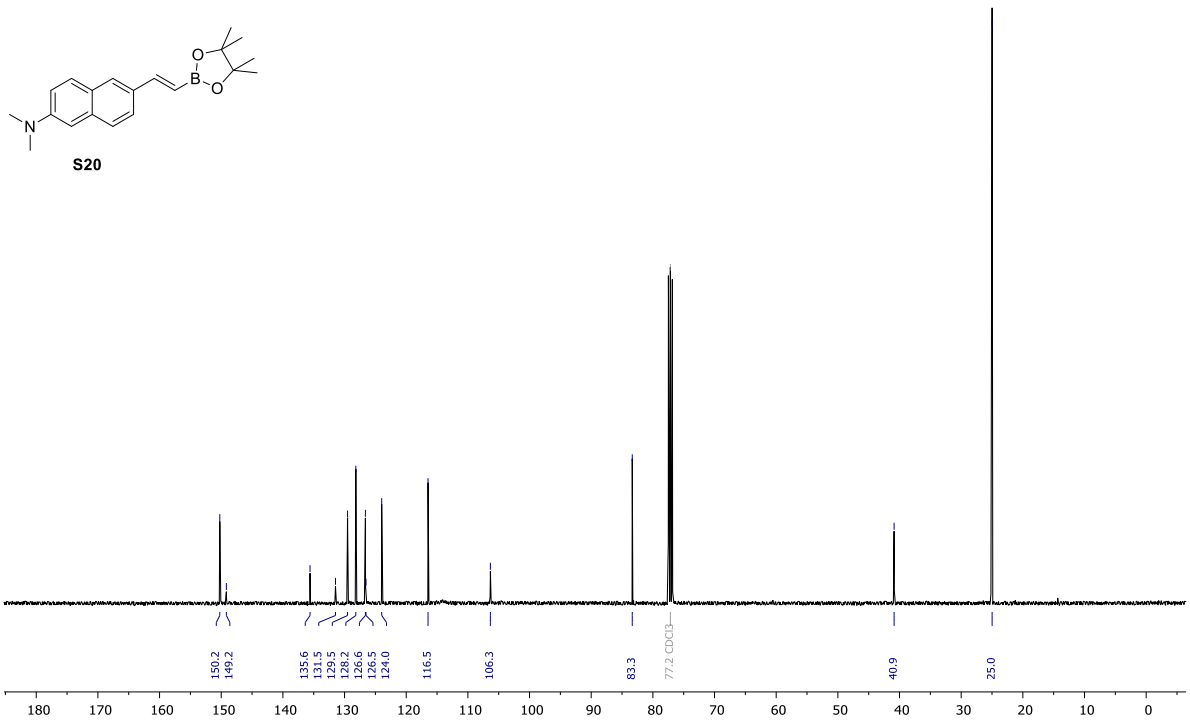
COSY spectrum of **6** ($CDCl_3$)



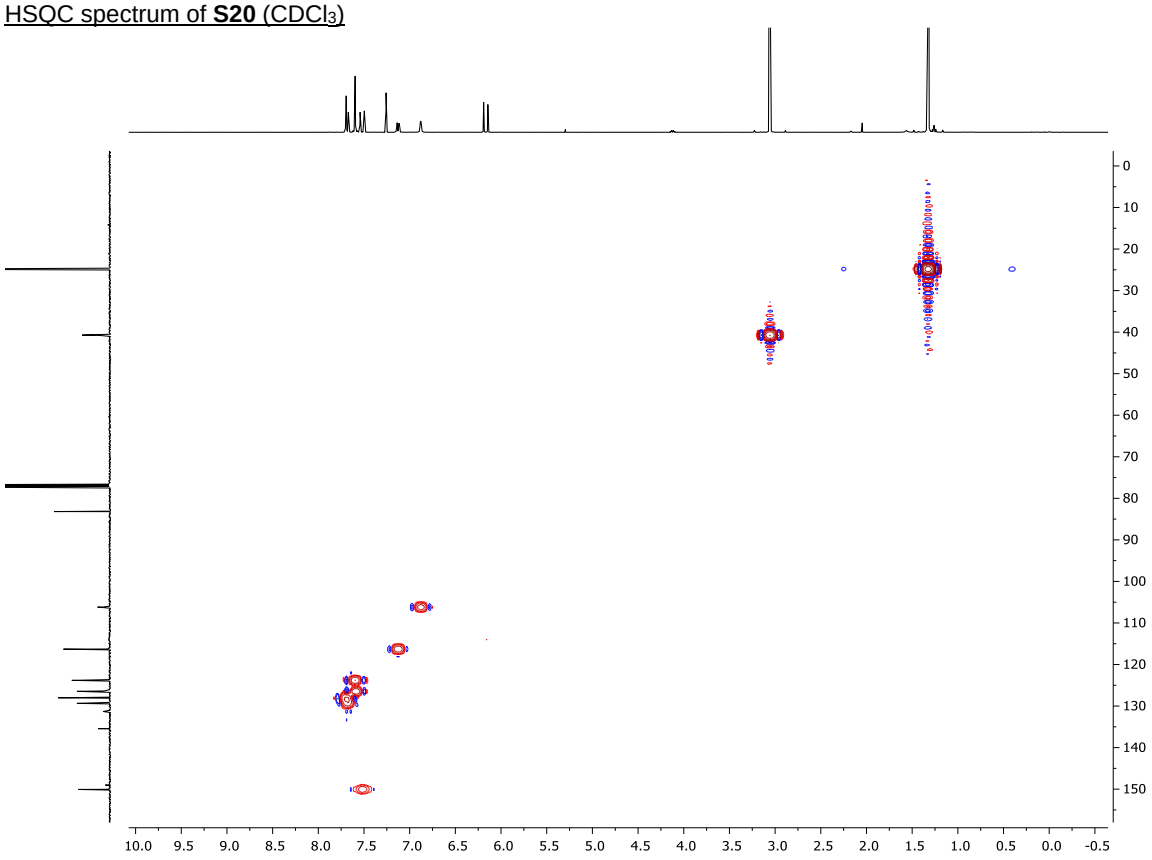
^{1}H NMR spectrum of **S20** (400 MHz, $CDCl_3$)



^{13}C NMR spectrum of **S20** (101 MHz, $CDCl_3$)

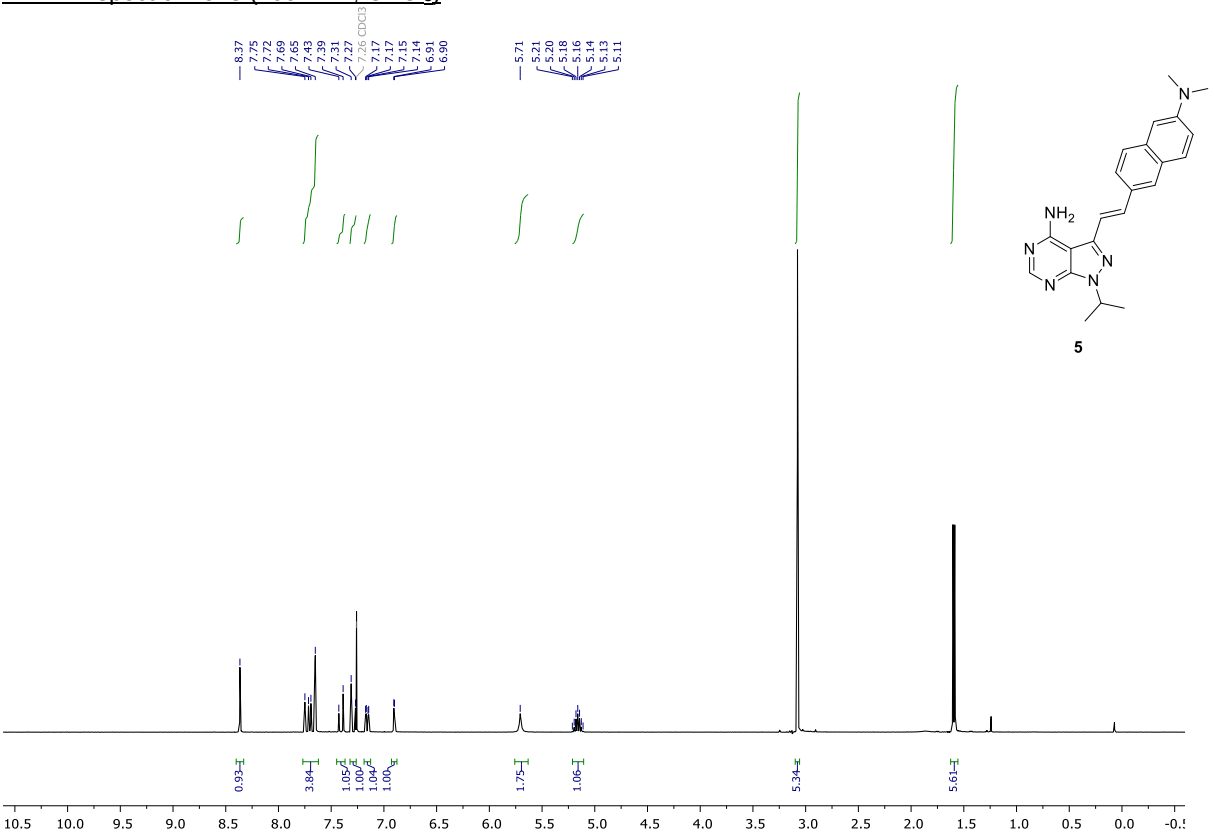


HSQC spectrum of **S20** ($CDCl_3$)

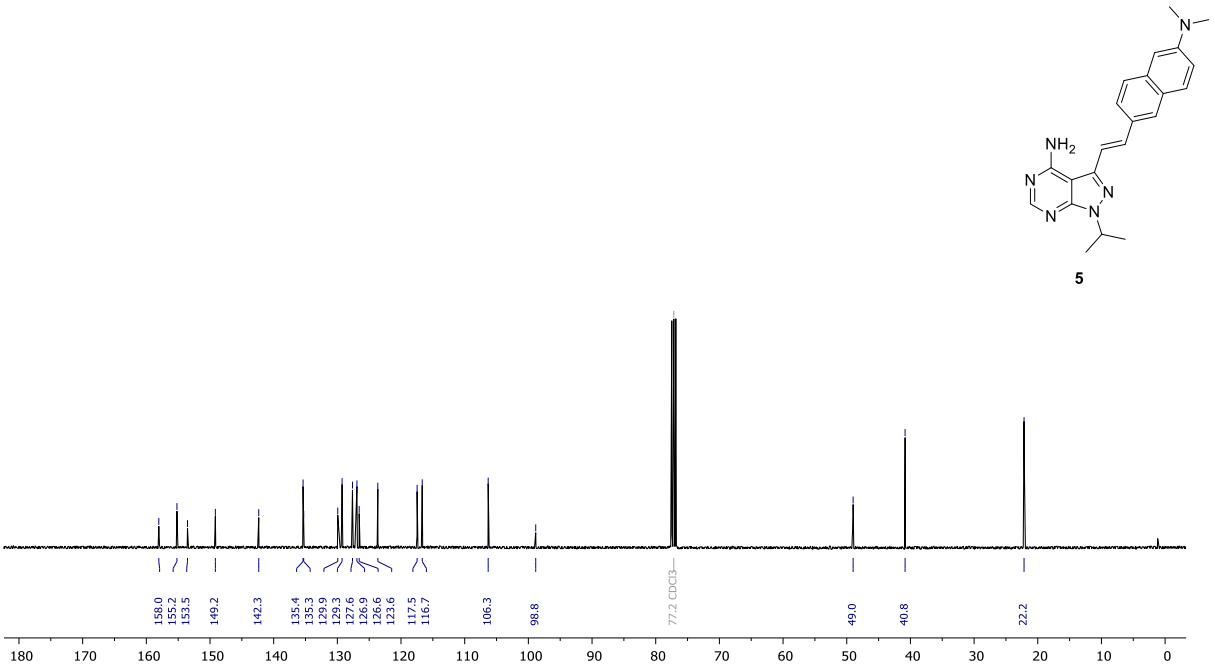


SUPPORTING INFORMATION

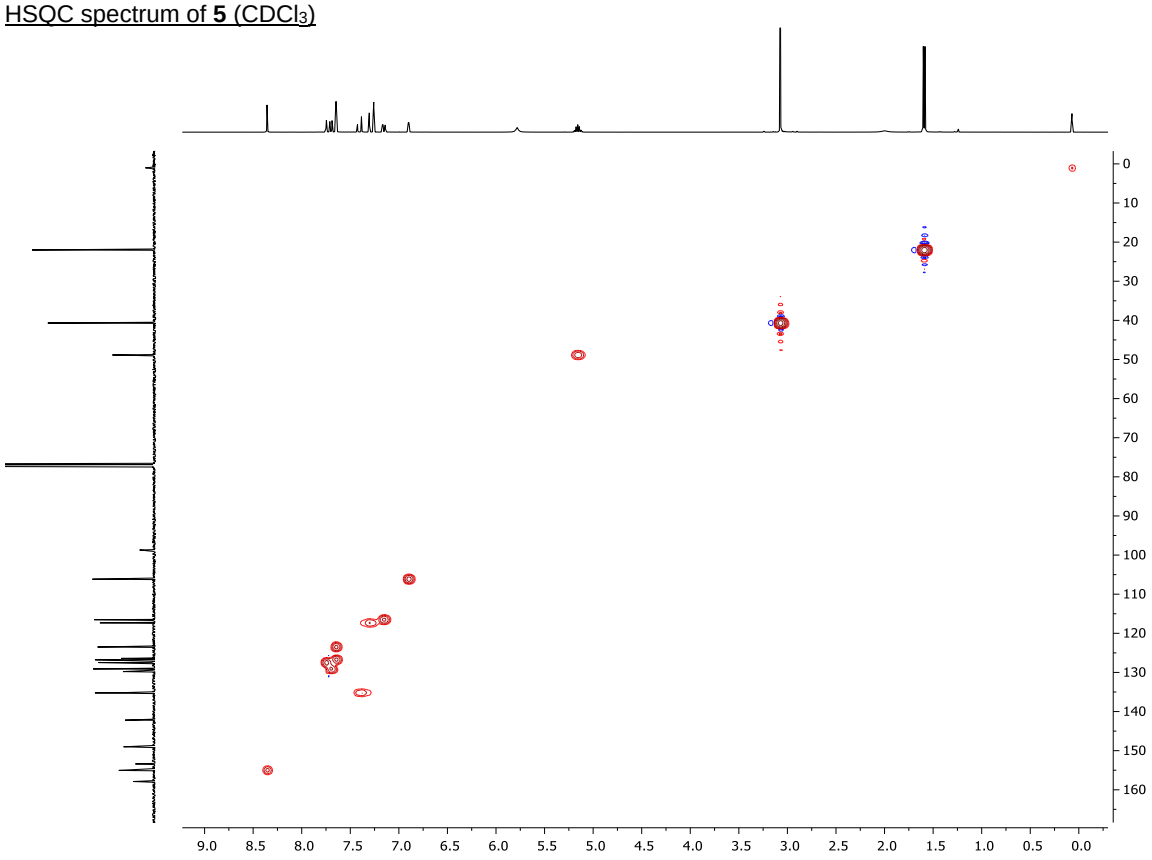
^{1}H NMR spectrum of **5** (400 MHz, $CDCl_3$)



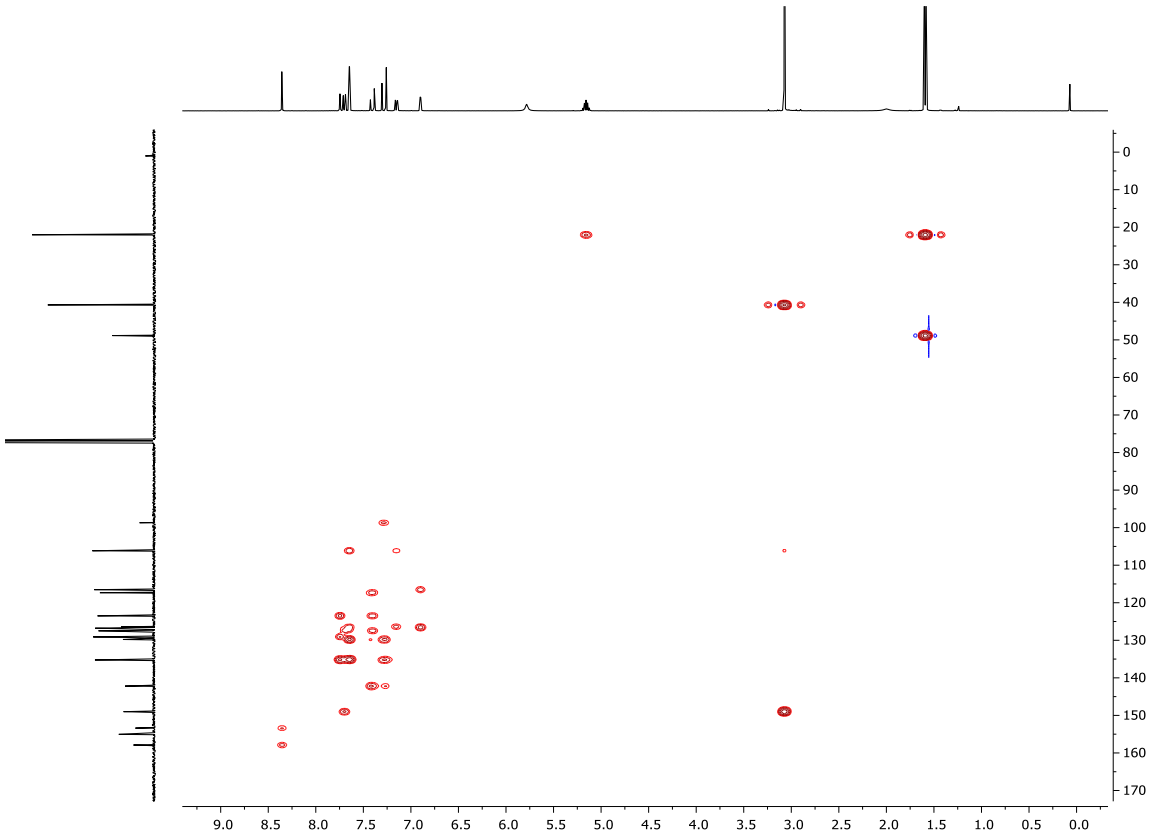
^{13}C NMR spectrum of **5** (101 MHz, $CDCl_3$)



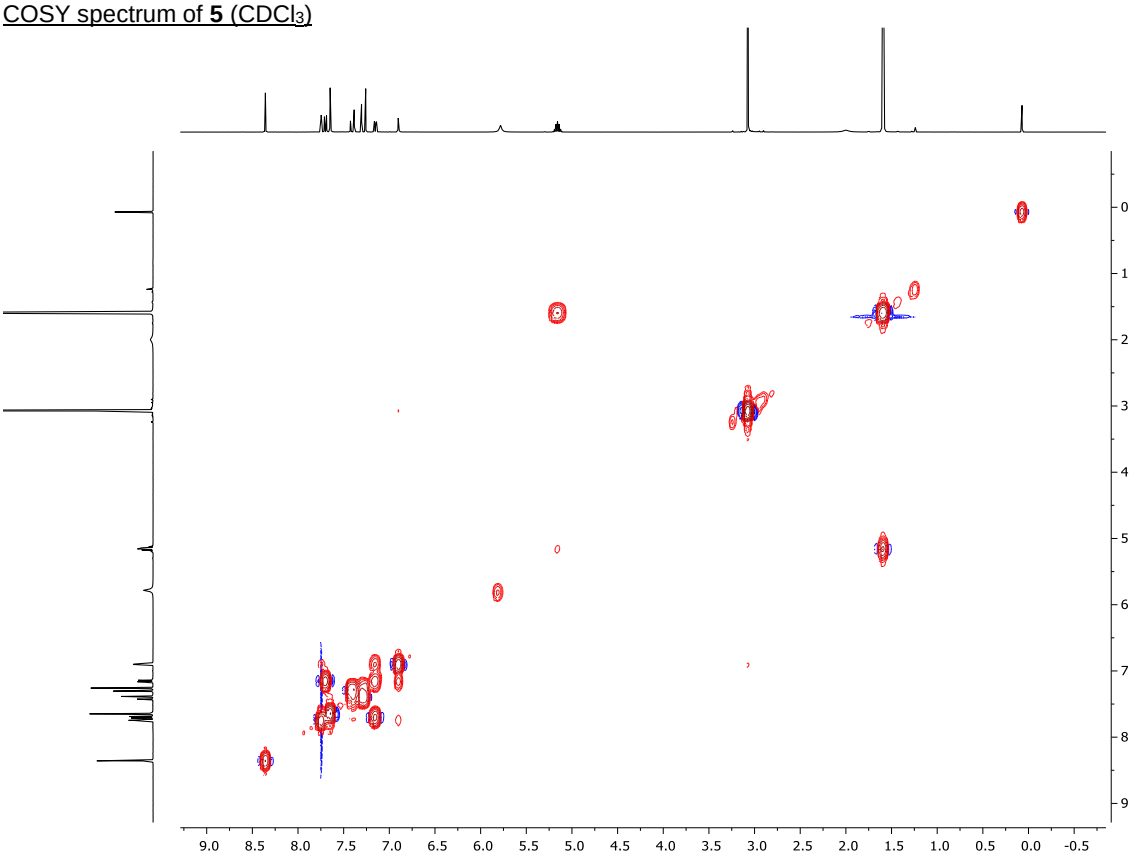
HSQC spectrum of **5** ($CDCl_3$)



HMBC spectrum of **5** ($CDCl_3$)

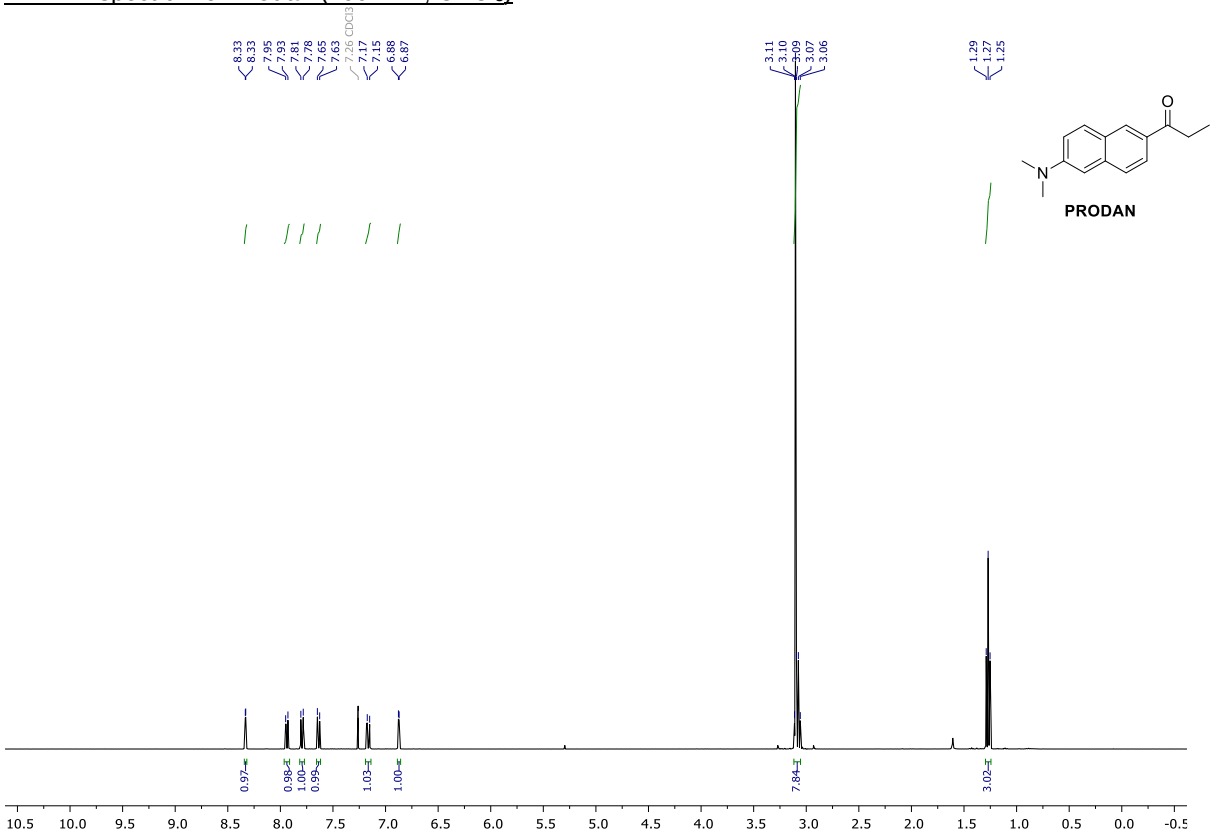


COSY spectrum of **5** ($CDCl_3$)



SUPPORTING INFORMATION

^{1}H NMR spectrum of **Prodan** (400 MHz, $CDCl_3$)



SUPPORTING INFORMATION

Photophysical Characterisation of Compounds 1–6 and Prodan

All solvents used for photophysical characterisation were of spectroscopic grade. Ground state absorption spectra were collected using a Varian CaryBio 50 UV/vis spectrophotometer. All steady-state fluorescent measurements were recorded on a SPEX Fluorolog-3 spectrofluorometer (JY Hariba). Samples were recorded in a macro quartz cuvette (light path = 10 × 10 mm) or for the titration experiments, in a reduced volume cuvette (v = 45 µL, light path = 3 × 3 mm). For **Prodan** and compounds **1**–**6** fluorescence quantum yields were determined using 9,10-diphenylanthracene (DPA) in cyclohexane (Φ_F = 0.97) as a reference. For compound **2**, fluorescence quantum yields were determined using Rhodamine 6G in EtOH (Φ_F = 0.94) as a reference. All quantum yield values were corrected for the solvent refractive index (cyclohexane = 1.4262, EtOH = 1.3614, toluene = 1.4969, acetonitrile = 1.3441, 1% DMSO in 10 mM phosphate buffer at pH 7.4 = 1.335).

Fluorescence lifetimes were determined using a time correlated single photon counting (TC-SPC) setup. The excitation light (λ_{exc} = 377 nm or λ_{exc} = 405 nm) was provided at a repetition rate of 10 MHz by either a 377 nm diode laser (LDH-P-C-375), or a 405 nm diode laser (LDH-P-C-405) powered by a PDL 800B pulsed diode driver (Picoquant, GmgH Germany). The emitted photons were collected at the magic angle (54.7°) at around the respective emission maxima by a thermoelectrically cooled microchannel plate photomultiplier tube (R3809U-50, Hamamatsu). The signal was digitalised using a multi-channel analyser with 2048 channels (SPC-300, Edinburgh Analytical Instruments) and to ensure good statistics 10,000 counts were recorded in the top channel. The measured fluorescence decays were fitted using the program FluoFit Pro version 4.6.6 (PicoQuant GmbH, Germany) after deconvolution of the data with the instrument response function (IRF) with FWHM~60 ps or FWHM~100 ps for 377 nm and 405 nm laser diose, respectively.

The average lifetimes were calculated according to the following equation:

$$\tau_{aver} = \sum \frac{A_i \times \tau_i}{\sum A_i}$$

where τ_{aver} is the average lifetime, τ_i are the lifetimes of the individual decays, and A_i are the amplitudes of the individual lifetimes.

Absorption and Emission Spectra of Compounds 1–6 and Prodan

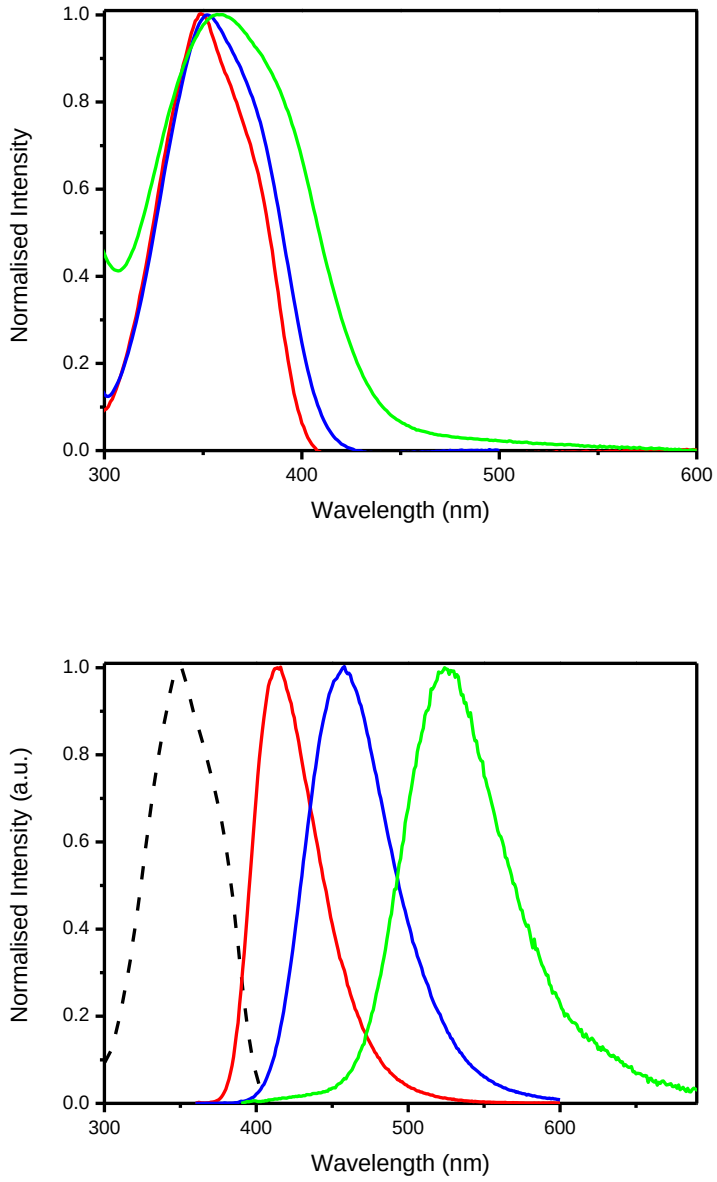


Figure S3. (*Top*) Absorption spectra of **Prodan** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of **Prodan** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green). Absorption spectrum is that recorded in toluene.

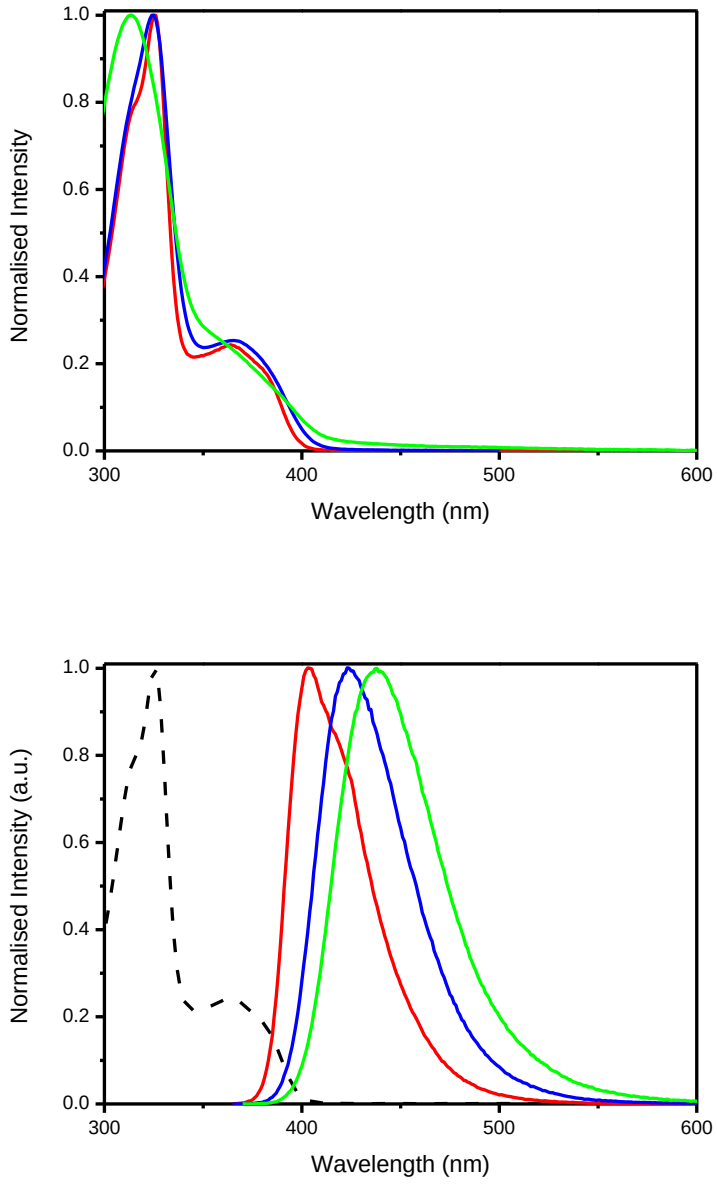


Figure S4. (*Top*) Absorption spectra of compound **1** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of compound **1** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green). Absorption spectrum is that recorded in toluene.

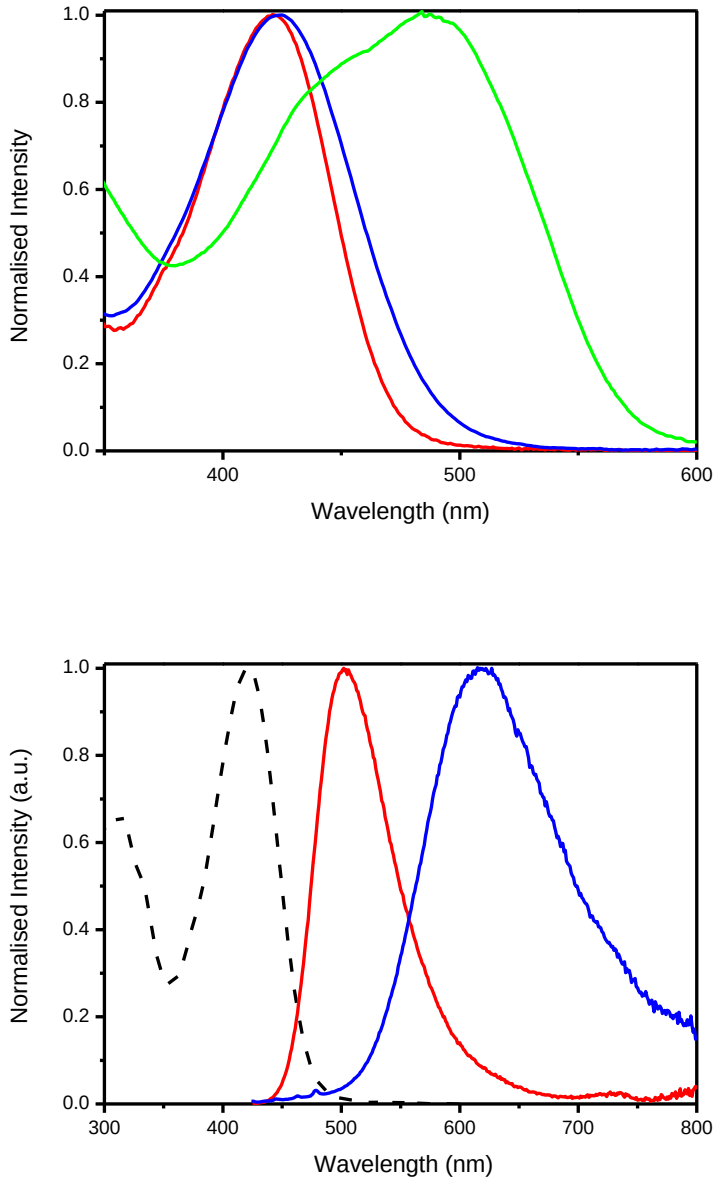


Figure S5. (*Top*) Absorption spectra of compound **2** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of compound **2** in toluene (red) and acetonitrile (blue). Absorption spectrum is that recorded in toluene. Emission intensity of compound **2** in 1% DMSO in 10 mM phosphate buffer at pH 7.4 was too low to obtain a spectrum.

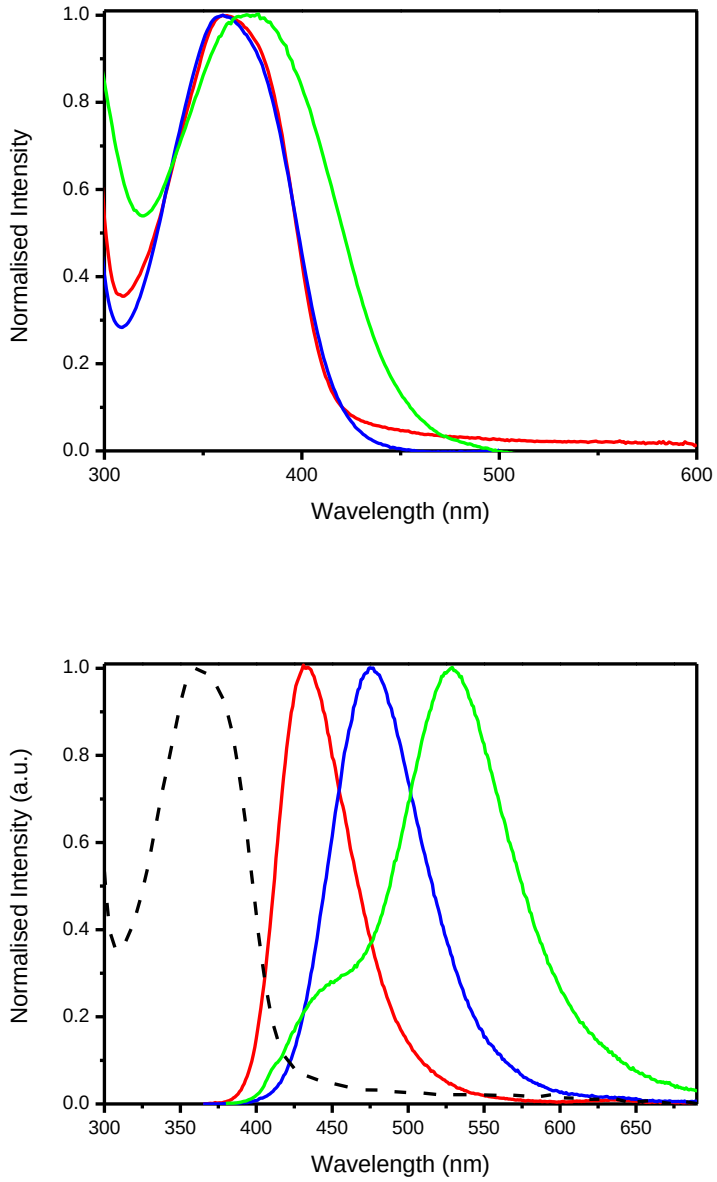


Figure S6. (*Top*) Absorption spectra of compound **3** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of compound **3** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green). Absorption spectrum is that recorded in toluene. Shoulder at 440 nm in 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green) is ascribed to DMSO impurity.

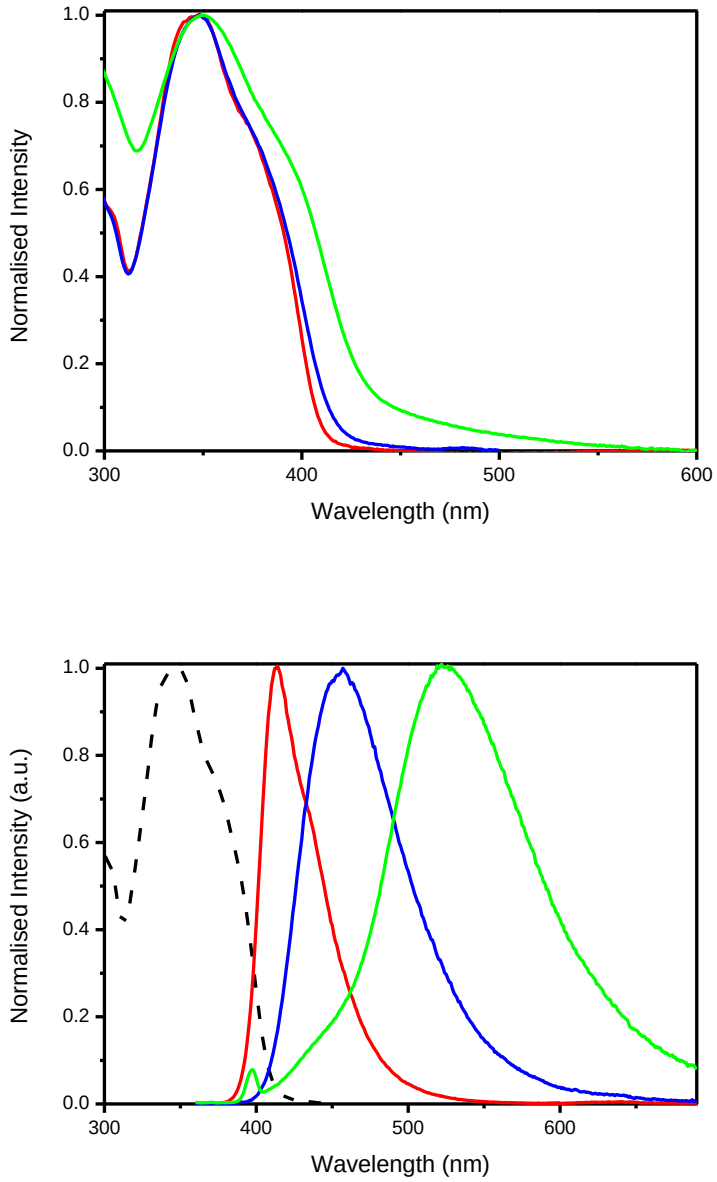


Figure S7. (*Top*) Absorption spectra of compound **4** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of compound **4** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green). Absorption spectrum is that recorded in toluene. Minor peak at 395 nm in 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green) originates from Raman Scattering.

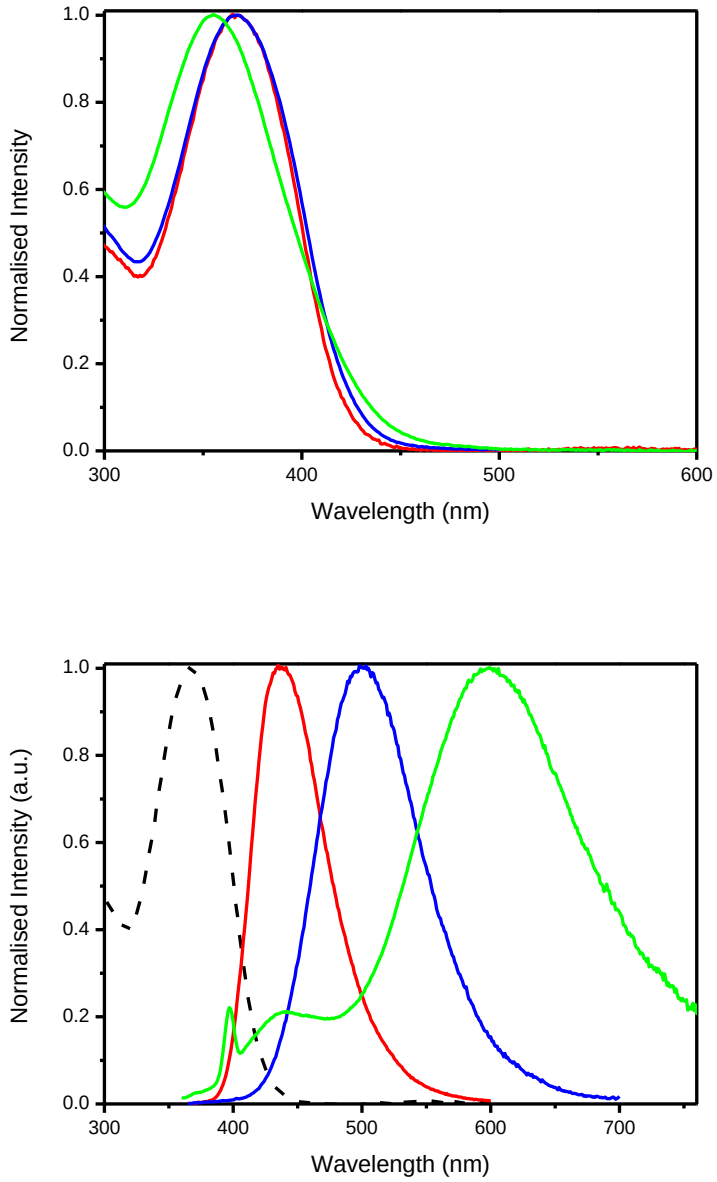


Figure S8. (*Top*) Absorption spectra of compound **5** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of compound **5** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green). Absorption spectrum is that recorded in toluene. Emission band at 440 nm in 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green) is ascribed to DMSO impurity. Minor peak at 395 nm in 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green) originates from Raman Scattering.

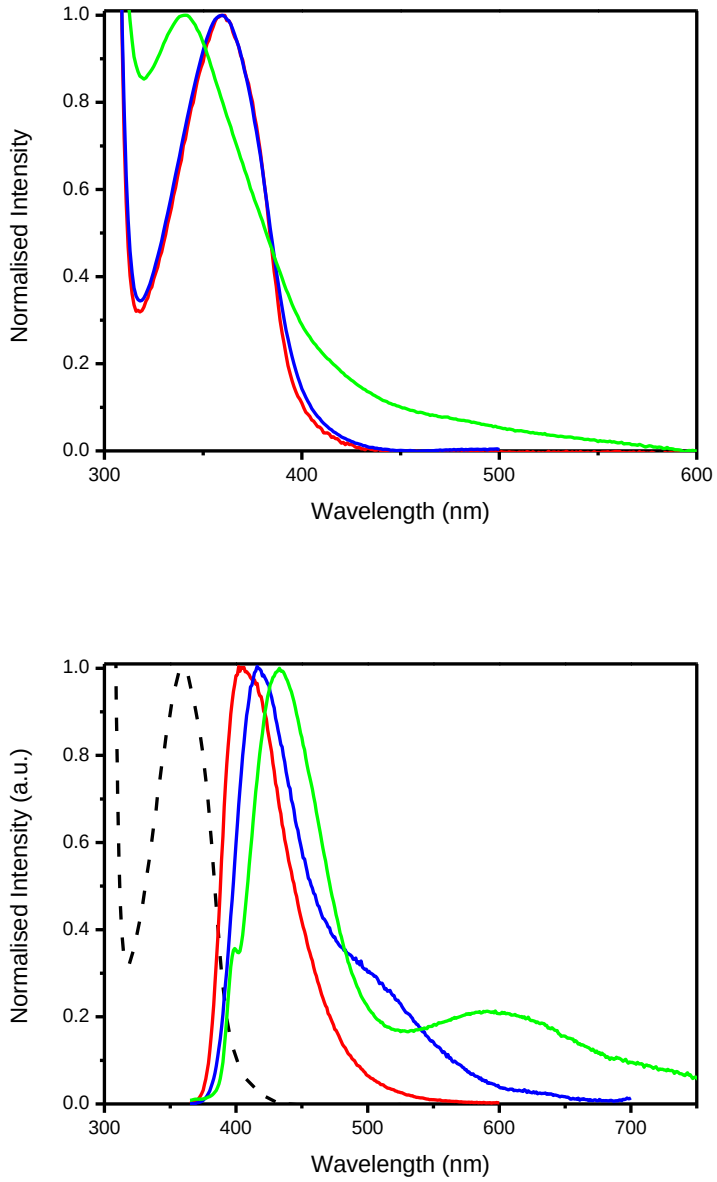


Figure S9. (*Top*) Absorption spectra of compound **6** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green); (*Bottom*) Absorption (dashed) and emission (solid) spectra of compound **6** in toluene (red), acetonitrile (blue) and 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green). Absorption spectrum is that recorded in toluene. Minor peak at 395 nm in 1% DMSO in 10 mM phosphate buffer at pH 7.4 (green) originates from Raman Scattering.

SUPPORTING INFORMATION

Table S1. Detailed spectroscopic data for **Prodan** and compounds **1**–**6**.

Compound	Solvent	λ_{abs} (nm) [a]	ε_{max} (M^{-1} cm^{-1})	λ_{em} (nm) [b]	Stokes Shift	Φ_F [c]	Brightness ($\varepsilon \times \Phi_F$)	τ_{aver} (ns) [d]
Prodan[e]	AQ	358	18127[f]	525	167	0.19	3444	1.0
	ACN	352	*nd*	457	105	0.86	*nd*	3.4
	Tol	350	18899	414	64	0.57	10772	2.1
1	AQ[e]	314, 370[g]	6022[f]	437	123, 67	0.57	3433	9.3
	ACN[h]	325, 366[g]	*nd*	424	99, 58	0.59	*nd*	7.1
	Tol[h]	325, 366[g]	5500	403	78, 37	0.66	3630	6.0
2[i]	AQ[j]	482	20926[f]	628	146	0.001	21	*nd*[k]
	ACN[l]	424	*nd*	619	195	0.02	*nd*	0.13
	Tol[l]	422	26060	504	82	0.55	14333	3.4
3	AQ	375	13832[f]	528	153	0.06	830	0.77
	ACN	360	*nd*	475	115	0.67	*nd*	2.9
	Tol	360	16080	432	72	0.74	11899	2.0
4	AQ	350	27268[f]	523	173	0.03	818	0.38
	ACN	348	*nd*	456	108	0.71	*nd*	2.4
	Tol	349	29419	413	64	0.71	20887	2.9
5	AQ[e]	356	29686[f]	599	243	0.006	178	0.088
	ACN[h]	367	*nd*	500	133	0.98	*nd*	2.3
	Tol[h]	367	26057	437	70	0.97	25275	1.6
6	AQ[e]	341	3522[f]	433, 597[m]	92, 256	0.005	18	0.051
	ACN[h]	360	*nd*	417	57	0.51	*nd*	6.6
	Tol[h]	359	3637	404	45	0.54	1964	5.3

AQ = 1% DMSO in 10 mM phosphate buffer (pH 7.4). *nd* = not determined. [a] Wavelength of the absorption maximum. [b] Wavelength of the emission maximum. [c] Fluorescence quantum yields were determined by taking 1,9-diphenylanthracene (DPA) in cyclohexane (Φ_F = 0.97) as a reference. [d] Average fluorescence lifetime. See ESI, Table S1 for complete lifetime data. An excitation wavelength of 377 nm was used for all compounds, with the exception of compound **2**, for which an excitation wavelength of 405 nm was used. [e] Excitation wavelength was 350 nm. [f] Due to poor solubility in aqueous solution at high concentrations, the molar absorption coefficient was determined in DMSO. [g] Shoulder. [h] Excitation wavelength was 360 nm. [i] Fluorescence quantum yields were determined by taking Rhodamine 6G in EtOH(Φ_F = 0.94) as a reference. [j] Excitation wavelength was 480 nm. [k] Lifetime was too short to be resolved in the SPC experiment. [l] Excitation wavelength was 470 nm. [m] Emission tentatively ascribed to aggregation.

Table S2. Detailed fitting parameters of the fluorescence lifetimes of **Prodan** and compounds **1**–**6**.[a]

Compound	Solvent	λ_{em} (nm)[b]	τ_1 (ns)	A_1 (%)	τ_2 (ns)	A_2 (%)	τ_3 (ns)	A_3 (%)	χ^2
Prodan	AQ	525	1.9	25	0.68	75			1.00
	Acetonitrile	457	3.4	100					1.11
		457	4.3	16	3.2	84			1.02
	Toluene	430	2.2	100					1.26
		430	3.6	6	2.0	94			1.00
1	AQ	440	11.0	83	0.68	17			1.08
	Acetonitrile	424	7.5	92	2.2	8			1.04
	Toluene	420	2.3	20	6.9	80			1.00
2[c]	AQ		*nd*						*nd*
	Acetonitrile	620	1.3	1	0.31	25	0.058	74	1.25
	Toluene	520	3.4	100					1.11
3	AQ	530	1.9	17	0.54	83			1.10
	Acetonitrile	456	3.3	85	0.68	15			1.00
	Toluene	435	2.4	66	1.3	34			1.06
4	AQ	520	3.7	2	0.80	24	0.16	74	1.00
	Acetonitrile	456	2.4	100					1.06
	Toluene	414	2.9	100					1.00
5	AQ	599	0.20	17.8	0.059	82	2.0	0.2	1.00
	Acetonitrile	500	2.3	100					1.25
		500	2.5	70	1.8	30			1.01
	Toluene	440	1.6	100					1.08
6	AQ	433	3.8	0.06	0.95	1.40	0.015	98	1.87
	Acetonitrile	450	9.5	61	2.1	39			1.15
	Toluene	410	8.3	58	1.3	42			1.00

AQ = 1% DMSO in 10 mM phosphate buffer (pH 7.4). *nd* = not determined due to a low count rate. [a] Excitation wavelength was 377 nm. [b] Emission wavelength. [c] Excitation wavelength was 405 nm.

Biochemical Analysis

Kinome Screen

Compounds **3** and **4** were tested in duplicate at 1 µM concentration and with an ATP concentration of 10 µM. The screen was performed by Eurofins Pharma Discovery Services in which the Diversity Pre-Set Panel supplemented with Blk(m), Fgr(h), Hck(h), Lck(h), Src(1–530)(h) and Yes(h) was used.

IC_{50} Determination

Kinase inhibition and IC_{50} values against Aurora-A, Blk and Lck for compound **4** were determined using the KinaseProfiler™ service of Eurofins Pharma Discovery Services UK Limited. The kinase was incubated with compound **4** in assay buffer containing substrate, 10 mM magnesium acetate and [γ-^{33}P-ATP]. The reaction was initiated by the addition of the Mg/ATP mixture. Following incubation at 21 °C, the reaction was stopped by the addition of a 3% phosphoric acid solution. An aliquot of the reaction was then spotted onto a filtermat and washed in phosphoric acid followed by a rinse in methanol prior to drying and scintillation counting. Results were expressed in relation to controls containing DMSO only in place of compound **4**. The ATP concentration in each assay was within 15 µM of the determined apparent K_m for ATP.

SUPPORTING INFORMATION

Table S3. Kinome Selectivity for compounds **3** and **4**.

	% of Remaining Kinase Activity (at 1 µM)	
Kinase	**4**	**3**
Abl(h)	79	105
ALK(h)	95	100
AMPKα1(h)	93	102
ASK1(h)	103	105
Aurora-A(h)	13	82
Blk(m)	15	86
CaMKI(h)	64	111
CDK1/cyclinB(h)	101	100
CDK2/cyclinA(h)	112	113
CDK6/cyclinD3(h)	110	102
CDK7/cyclinH/MAT1(h)	109	105
CDK9/cyclin T1(h)	86	105
CHK1(h)	118	102
CK1γ1(h)	102	104
CK2α2(h)	94	83
c-RAF(h)	90	99
DRAK1(h)	90	89
eEF-2K(h)	116	96
EGFR(h)	88	85
EphA5(h)	103	96
EphB4(h)	110	95
Fgr(h)	59	88
Fyn(h)	85	141
GSK3β(h)	104	107
Hck(h)	33	64
IGF-1R(h)	78	85
IKKα(h)	105	100
IRAK4(h)	102	108
JAK2(h)	101	110
KDR(h)	76	102
Lck(h)	12	68
LOK(h)	20	87
Lyn(h)	33	96
MAPKAP-K2(h)	109	103

20–50% remaining kinase activity

≤15% remaining kinase activity

Table S3 (*continued*)**.** Kinome Selectivity for compounds **3** and **4**.

	% of Remaining Kinase Activity (at 1 µM)	
Kinase	**4**	**3**
MKK7β(h)	96	99
MLK1(h)	52	91
Mnk2(h)	108	99
MSK2(h)	78	96
MST1(h)	63	97
mTOR(h)	67	133
NEK2(h)	80	98
p70S6K(h)	91	99
PAK2(h)	101	111
PDGFRβ(h)	117	100
Pim-1(h)	102	101
PKA(h)	79	75
PKBα(h)	103	101
PKCα(h)	96	100
PKCθ(h)	108	96
PKG1α(h)	99	93
Plk3(h)	102	100
PRAK(h)	104	89
ROCK-I(h)	108	101
Rse(h)	115	105
Rsk1(h)	116	123
SAPK2a(h)	106	111
Src(1-530)(h)	45	63
SRPK1(h)	119	107
TAK1(h)	82	104
Yes(h)	71	97
PI3 Kinase (p110β/p85α)(h)	98	101
PI3 Kinase (p120γ)(h)	88	96
PI3 Kinase (p110δ/p85α)(h)	97	97
PI3 Kinase (p110α/p85α)(h)	125	129

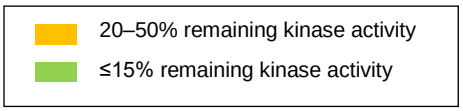


Table S4. Selectivity profile of compound **4** and ist corresponding inhibitory effects.

Kinase	Family	KA (%)[a]	IC_{50} (nM)[b]
Aurora-A	MISC	13	222
Blk	TK	15	554
LCK	TK	12	124

MISC = miscellaneous. TK = tyrosine kinase. [a] KA = percentage of remaining kinase activity. [b] Values are the average of two measurements with a standard error ≤ 5%.

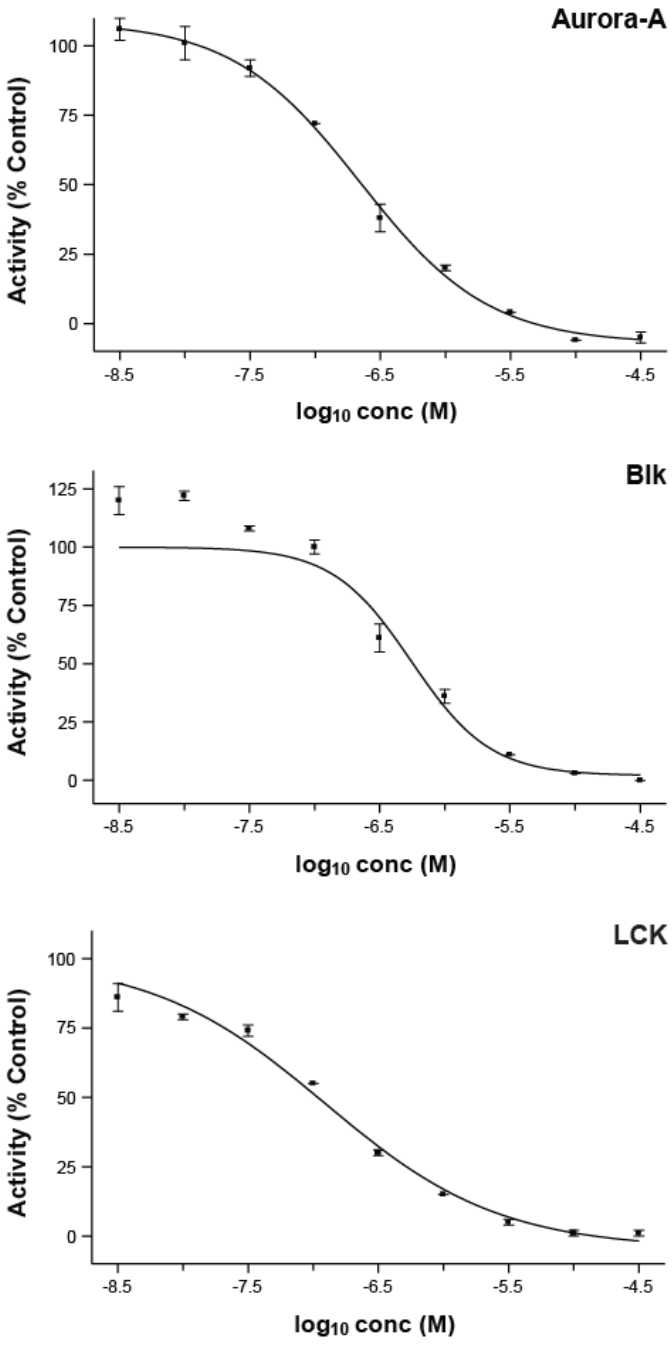


Figure S10. IC_{50} data for compound **4** against Aurora-A, Blk and LCK.

Titration Experiments

Titration experiments with the protein (LCK or JAK2) were conducted by administering aliquots of a stock solution of compound **4** to a reduced cuvette containing the protein. In order to eliminate the effects of dilution during titrations, the titrant solution also contained the protein (*i.e.* the host) at the same concentration as the receiving host solution. An emission spectrum was recorded 15 minutes after each addition.

For the displacement titration experiment with **TC-S7003** (*see Figure S12*), to a reduced cuvette containing LCK, compound **4** was first titrated with the protein until a 1:1 ratio was achieved. Next, aliquots of a stock solution of TC-S7003 was administered to the cuvette containing 1:1 compound **4**:LCK. Again, in order to eliminate the effects of dilution during the titrations, the titrant solution also contained both the protein (*i.e.* the host) and compound **4** at the same concentration as the receiving host solution. An emission spectrum was recorded 15 minutes after each addition.

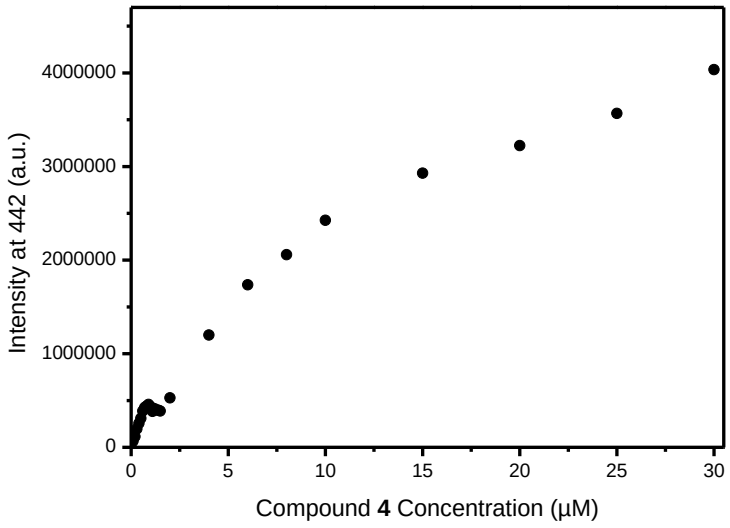
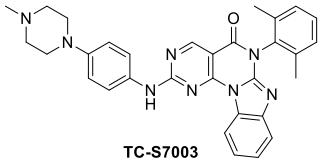
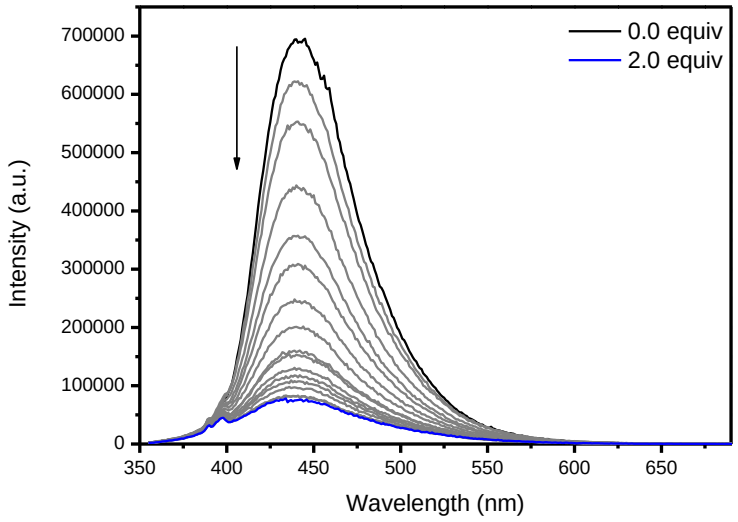


Figure S11. Fluorescence titration of LCK (1 μM) with compound **4**. Monitoring the changes in fluorescence intensity at 442 nm upon increasing concentration of compound **4** (0–30 μM).





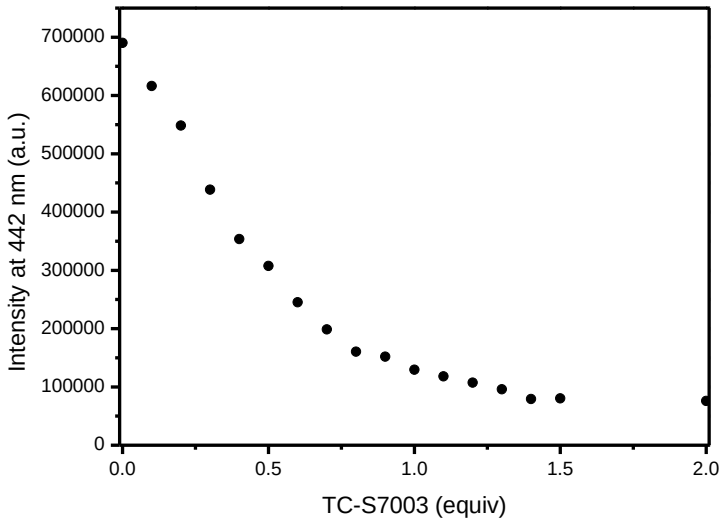


Figure S12. Displacement fluorescence titration, displacing compound **4** from the ATP-binding site of LCK with the ATP-competitive inhibitor **TC-S7003**. (*Top*) Changes in the emission spectrum of compound **4** upon the addition of **TC-S7003** to 1:1 compound **4**:LCK (1 μM); (*Bottom*) Changes in fluorescence intensity at 442 nm upon increasing concentration of ATP-competitive LCK inhibitor **TC-S7003** (0–2.0 equiv).

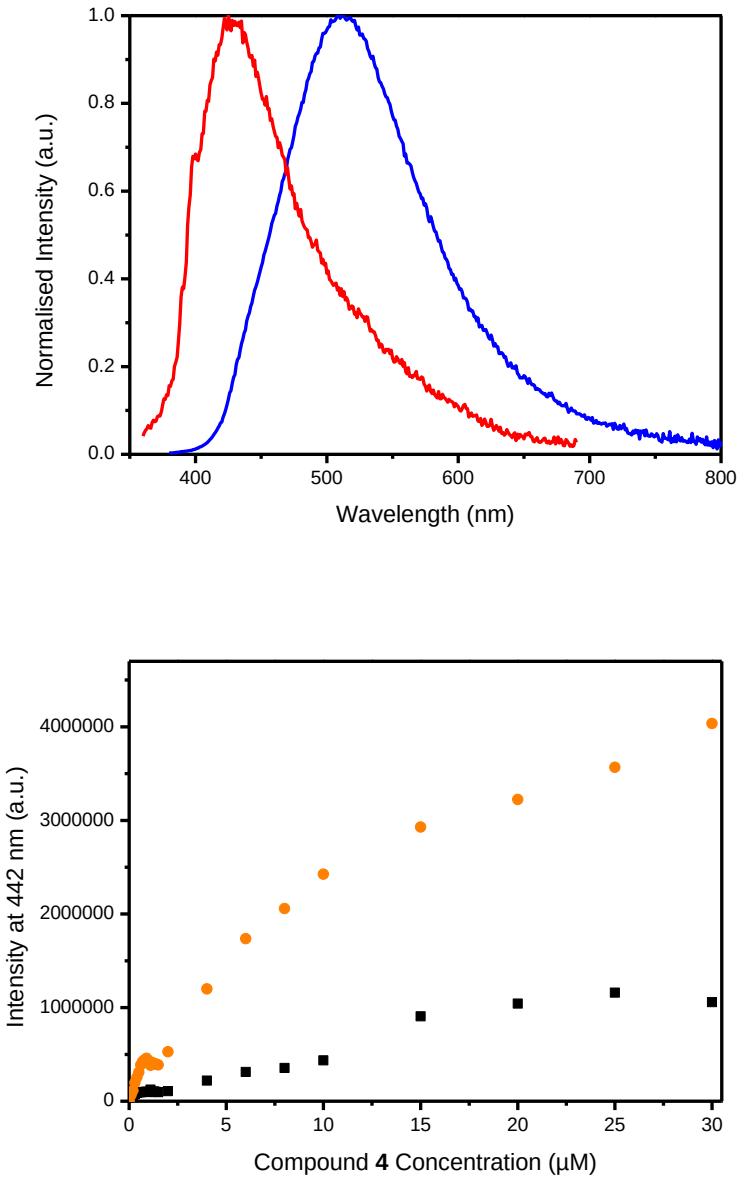


Figure S13. Fluorescence titration of JAK2 (1 μM) with compound **4**. (*Top*) Emission spectra of compound **4** (blue) and 1:1 compound **4**:JAK2 (red) in tris buffer at pH 7.5; (*Bottom*) Changes in fluorescence intensity at 442 nm upon increasing concentration of compound **4** (0–30 μM) are shown in black. Binding isotherm for LCK is shown in orange for comparison.

SUPPORTING INFORMATION

Microscopy and Flow Cytometry Experiments

Cell Lines

Confocal microscopy and flow cytometry studies employed the use of Jurkat, J.CaM1.6, K562 and A498 cell lines. The J.CaM1.6 cell line is a mutant derivative of the Jurkat cell line and expresses a truncated, non-functional version of LCK. Cells were cultured in RPMI cell culture medium supplemented with 10% FCS, 1% penicillin/streptomycin, 1% non-essential amino acids and 1% L-glutamine. Cells were regularly tested for mycoplasma. Cells were incubated at 37 °C in 5% v/v CO_2, under humidified conditions.

Transfection with LCK Plasmid

Human wild-type LCK (Genscript Corp., USA) was subcloned into a pcDNA3.1 vector.

A498 Cell Line for Microscopy. A498 cells were plated onto glass-bottom dishes. After seeding for 24 hours, cells were transfected with the LCK plasmid, using Lipofectamine 2000 (Thermo) according to the manufacturer's protocol, for 24 hours. Next, cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature and then permeabilised in 0.1% saponin and washed with PBS/0.05% saponin (× 3).

K562 Cell Line for Flow Cytometry. K562 cells were electroporated using a Gene Pulser II (BioRad) in electroporation buffer (BioRad) with a plasmid expressing wild-type untagged LCK for 24 hours. Transfection efficiency was evaluated by flow cytometry.

Microscopy

Jurkat cells were plated onto Cell-Tak (Fisher Scientific) coated (3.5 µg/cm^2) glass-bottom dishes. After seeding for 1 hour, cells were incubated with compound **4** (5 nM–5 µM) for 15 minutes at 37 °C. Cells were then washed with PBS (×3), fixed with 3% paraformaldehyde for 15 minutes at room temperature, washed again with PBS (× 3) and then permeabilised in 0.1% saponin.

For the co-localisation studies in A498 cells, following transfection with the LCK plasmid, cells were incubated compound **4** (0.5 µM) for 15 minutes at 37 °C. Next, cells were washed with PBS (× 3), fixed with 3% paraformaldehyde for 15 minutes at room temperature, washed again with PBS (× 3) and then permeabilised in pre-cooled methanol for 4 minutes and washed with PBS (× 3). Next, cells were stained with anti-LCK-AlexaFluor647 (BioLegend) for 30 minutes at room temperature in PBS/0.05% saponin.

Images were acquired using either a Zeiss Axio Observer (Colibri 7) or a Zeiss LSM880 microscope and a 63× oil objective (NA = 1.4). Compound **4** was excited at 385 nm and anti-LCK-AlexaFluor647 was excited at 633 nm. Images were collected, processed and exported using Zen 2.3 edition.

Flow Cytometry

Jurkat and J.CaM1.6 cells were washed with PBS (× 2) prior to fixation with BD Cytofix-Cytoperm for 15 minutes at room temperature. Next, cells were stained with anti-LCK-AlexaFluor647 for 15 minutes at room temperature in BD Perm/Wash buffer, then were washed an additional two times.

24 hours following electroporation of the LCK plasmid, K562 cells were incubated with compound **4** (0.5 µM) for 15 minutes at 37 °C. Cells were then washed with PBS (× 3) prior to fixation with BD Cytofix-Cytoperm for 15 minutes at room temperature. Next, cells were stained using anti-LCK-AlexaFluor647 for 15 minutes at room temperature in BD Perm/Wash buffer, then were washed an additional two times.

SUPPORTING INFORMATION

Analysis of was performed on a FACSCanto (BD Biosciences) or a FACS LSRFortessa (BD Biosciences) and the data processed using FLowJo V10. For compound **4**, either excitation wavelength was at 405 nm, with the emitted light collected at 450 / 40 nm or excitation wavelength was at 355 nm, with the emitted light collected at 379 / 28 nm. For AlexaFluor647, excitation wavelength was at 633 nm, with the emitted light collected at 660 / 20 nm.

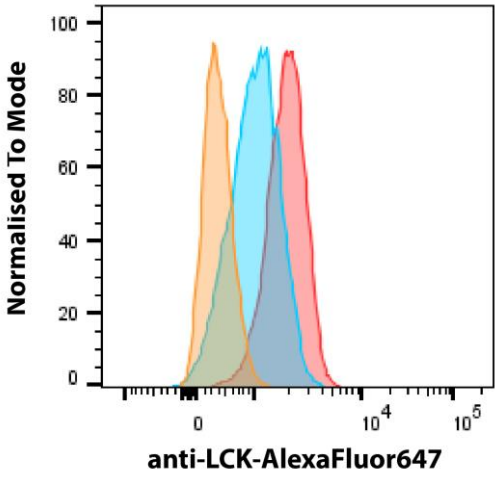


Figure S14. Analysis of endogenous LCK in Jurkat (*red*, LCK functional) and J.CaM1.6 (*blue*, LCK non-functional) cells using flow cytometry. Cells were stained with anti-LCK-AF647 for 15 minutes at room temperature. The orange band is the unstained control (*i.e.* not treated with anti-LCK-AF647). While LCK is not active in the J.CaM1.6 cells, this experiment shows that the anti-LCK-AF647 still recognises the LCK fragment.

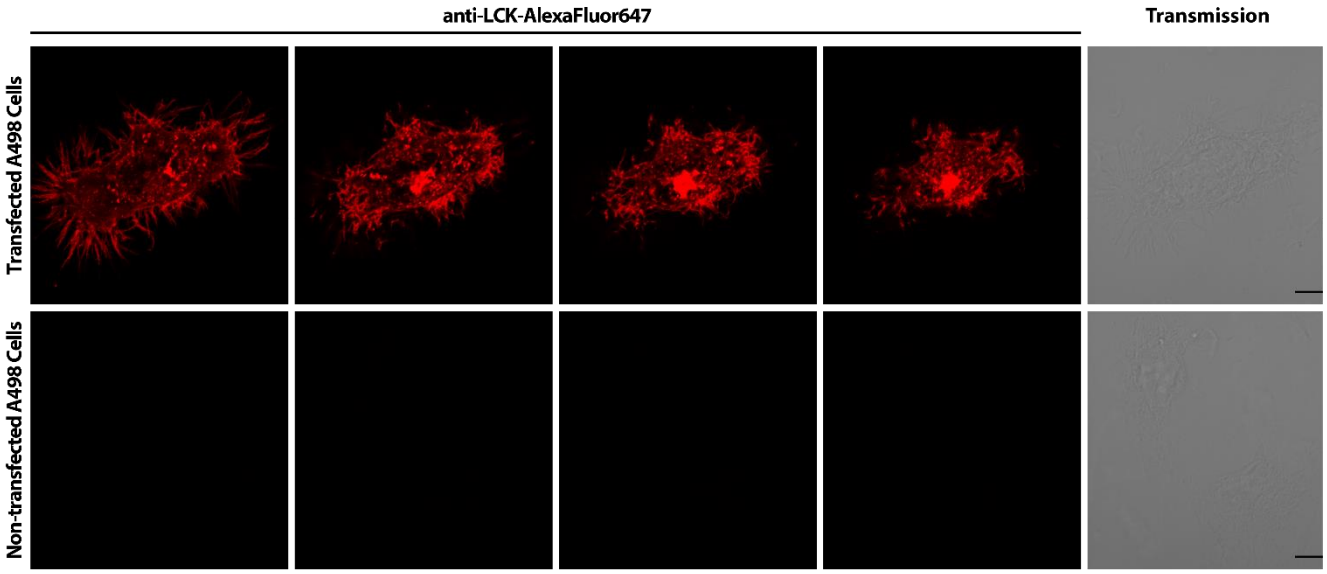


Figure S15. Microscopy images of (*top*) A498 cells transfected with plasmid expressing wild-type untagged LCK; and (*bottom*) non-transfected A498 cells. For both transfected and non-transfected samples, cells were stained with anti-LCK-AF647 for 30 minutes at room temperature. Images shown are a z-stack at 1 µm thick. Scale bar: 10 µm.

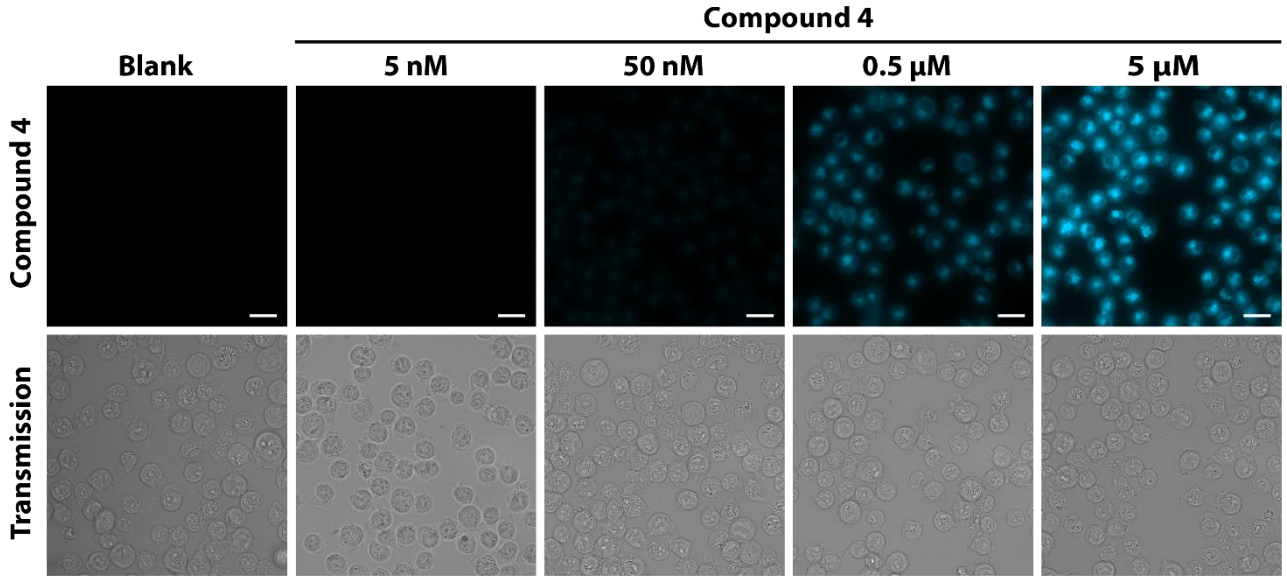


Figure S16. Images of Jurkat cells incubated for 15 minutes with compound **4** at 5 nM–5 µM. Scale bar: 25 µm.

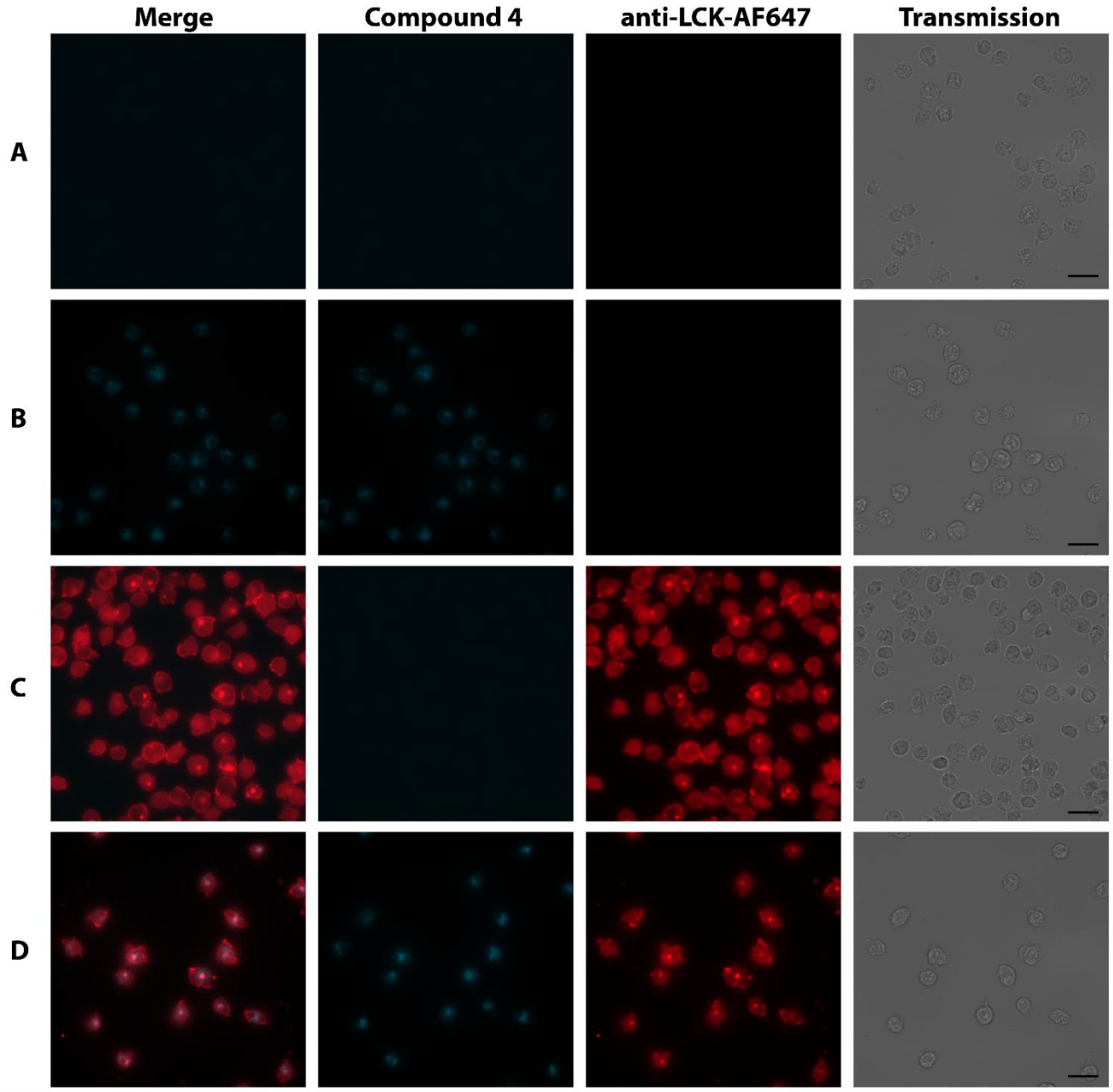


Figure S17. Images of Jurkat cells co-stained with anti-LCK-AF647 and compound **4** (0.5 µM). (A) Untreated cells; (B) Cells treated only with compound **4**; (C) Cells treated only with anti-LCK-AF647; (D) Cells treated with both compound **4** and anti-LCK-AF647. Scale bar: 25 µm

Multiphoton Live Cell Imaging

Cell Line

Multiphoton microscopy studies employed the use of the human lung cancer cell line, HTB-177. Cells were maintained in exponential growth as monolayers in RPMI-1640 cell culture medium (without phenol red) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine. Cells were incubated at 37 °C in 5% v/v CO_2, under humidified conditions.

Microscopy

Cells were plated in 60 × 15 mm petri dishes. Cells were dosed with 0.1–10 μM solutions of compound **4** in RPMI-1640 cell medium (without phenol red and supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin and 1% L-glutamine) 5 minutes before the images were taken. Images were acquired on a ZEISS LSM710 NLO MP/Confocal microscope housed at the Centre of Cellular Imaging at Gothenburg University, using a 20× water-immersion objective lens. Samples were excited at 700 nm.

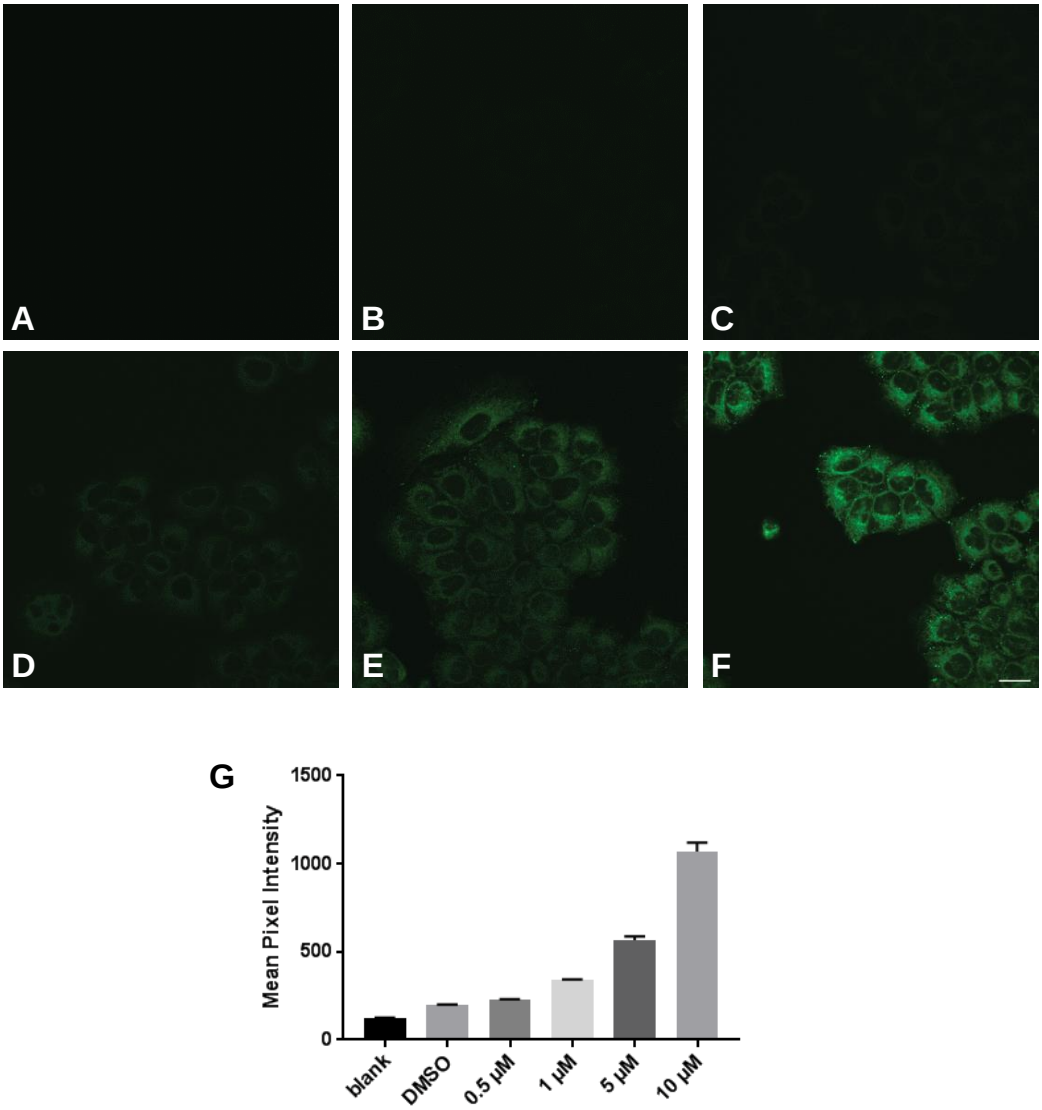


Figure S18. Quantification of fluorescence signal in HTB-177 cells incubated for 5 mins with increasing concentration of compound **4**; (A) 0 µM, cells in the absence of compound **4** and DMSO, (B) 0 µM cells in the absence of compound **4** but with 1% DMSO, (C) 0.5 µM, (D) 1 µM, (E) 5 µM; and (F) 10 µM. Scale bar: 20 µm. (G) Mean pixel intensity (grey values) in response to increasing concentration of compound **4**. Data reported as mean ± SE.

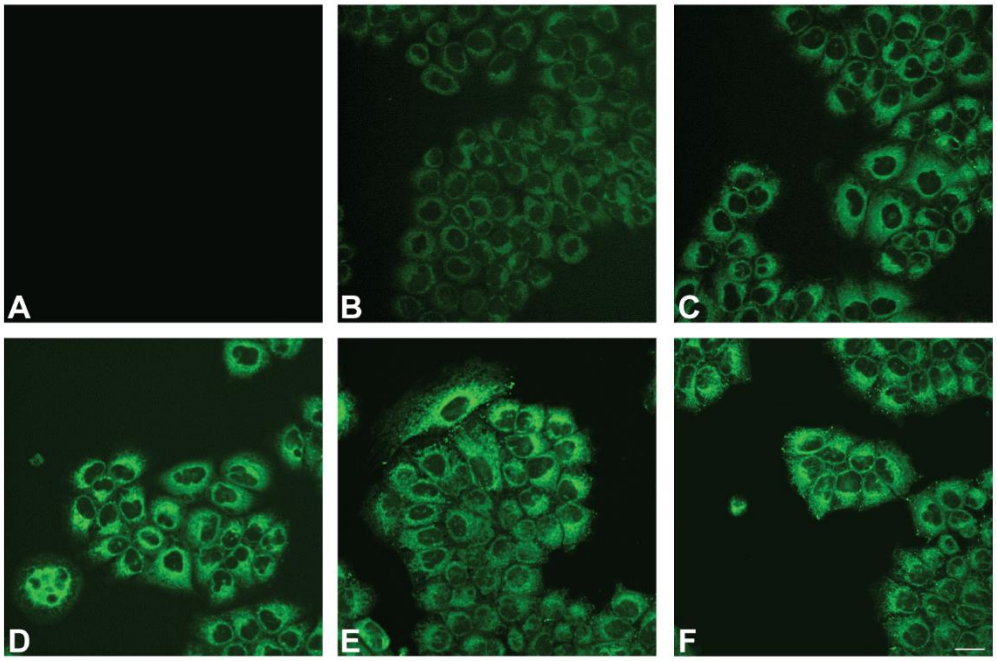


Figure S19. Two-photon microscopy images of HTB-177 cells incubated for 5 mins with compound **4** at (A) 0 µM, (B) 0.1 µM, (C) 0.5 µM, (D) 1 µM, (E) 5 µM and (F) 10 µM. Scale bar: 20 µm. Samples were excited at 700 nm. Brightness is not representative of the dose response. For dose response curve see ESI, Figure S18.

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Author Contributions

C.L.F. conducted all the experimental work regarding the synthesis, photophysical characterisation, titration and multiphoton experiments. C.L.F. wrote the manuscript. P.A.S. performed all the work for the microscopy and flow cytometry experiments. All authors analysed the experimental data and discussed the results. M.G. and J.A. directed the project.