Supplementary Information

Targeting RET solvent-front mutants with alkynyl nicotinamide-based inhibitors

Ujjwol Khatri[1,2#], Neetu Dayal[3#], Xueqing Hu[1,2#], Elizabeth Larocque[3], Nimishetti Naganna[3], Tao Shen[1,2], Xuan Liu[1,2], Frederick W. Holtsberg[4], M. Javad Aman[4], Herman O. Sintim[3,5*], Jie Wu[1,2,*]

[1]Department of Pathology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

[2]Peggy and Charles Stephenson Cancer Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA

[3]Department of Chemistry and Center for Drug Discovery, Purdue University, West Lafayette, IN 47907, USA

[4]KinaRx, Inc, 4 Research Court, Suite 350, Rockville, MD 20850, USA

Purdue Center for Cancer Research, Purdue University, West Lafayette, IN 47907, USA

[#]Equal contributing authors.

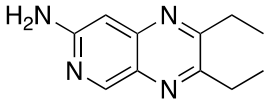
*Correspondence to: Herman Sintim, Department of Chemistry and Center for Drug Discovery, Purdue University, Room 123, 720 Clinic Drive, West Lafayette, IN 47907-2112, USA, Tel. 765-496-6078 email: hsintim@purdue.edu, or Jie Wu, University of Oklahoma Health Sciences Center, 975 NE 10th Street, BRC413, Oklahoma City, OK 73104, USA, Tel. 405-271-8001 ext. 31092, email: jie-wu@ouhsc.edu

Table of contents:

General Procedure for Sonogashira Coupling

A solution of bromo or iodo substrate (1 mmol), $Pd(PPh_3)_4$ (10 mol%), CuI (5 mol%) and triphenylphosphine (10 mg) in triethylamine (30 equiv) was de-oxygenated using steam of argon gas. Followed by addition of a de-oxygenated solution of alkyne substrate (0.95 mmol) in DMF (4 mL) slowly over a period of 10 min. Reaction was allowed to stir at 55 ºC for12 h. After completion, reaction was quenched by addition of NH_4Cl (5 mL) at room temperature and diluted with ethyl acetate (300 mL). The organic layer was extracted with water (2 × 50 mL) and washed with brine solution (1 × 50 mL). Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. Purified by flash column chromatography using dichloromethane/ methanol (97:3 to 95:5).

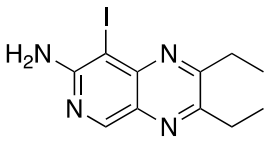
2,3-Diethylpyrido[3,4-b]pyrazin-7-amine



To a reaction vial, pyridine-2,4,5-triamine (150 mg, 0.797 mmol, 1 equiv.) and ethanol (10 mL) were added. Hexane-3,4-dione (90 mg, 0.645 mmol, 1 equiv.) was then added. The reaction was then allowed to stir for 2.5 hours at room temperature. An additional equivalent of hexane-3,4-dione was added 2.5 hours later. The reaction was then allowed to stir for an additional 1.5 hours. Upon reaction completion, crude mixture was concentrated under reduced pressure and purified via silica gel column chromatography.

Brown solid (80%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (d, J = 0.8 Hz, 1H), 6.63 (d, J = 0.8 Hz, 1H), 6.27 (s, 2H), 2.89 (dq, J = 14.7, 7.4 Hz, 4H), 1.25 (td, J = 7.4, 0.9 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.2, 159.5, 153.6, 152.1, 145.9, 130.4, 97.7, 28.1, 27.3, 12.0, 11.7. HRMS (ESI) m/z calcd for $C_{11}H_{15}N_4$ $[M + H]^+$ 203.1297, found 203.1301.

2,3-Diethyl-8-iodopyrido[3,4-*b*]pyrazin-7-amine

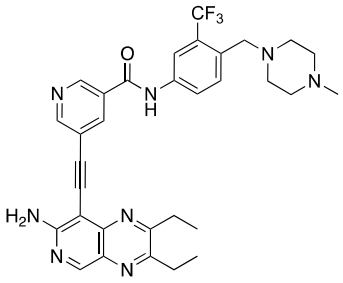


2,3-Diethylpyrido[3,4-*b*]pyrazin-7-amine (100 mg, 0.500 mmol, 1 equiv) were added to a reaction vial with methanol (5 mL). The reaction was then cooled to 0 °C and N-Iodosuccinimide (112 mg, 0.500 mol, 1.1 equiv) was slowly added over 10 minutes. The

reaction was then allowed to run at 0°C for 10 minutes. Crude mixture was concentrated down under reduced pressure and purified via column chromatography.

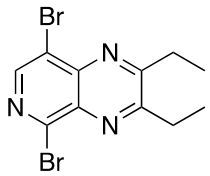
Off-white solid (64%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.73 (s, 1H), 6.49 (s, 2H), 2.99 (q, J = 7.3 Hz, 2H), 2.93 (q, J = 7.4 Hz, 2H), 1.34 (t, J = 7.2 Hz, 3H), 1.28 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.0, 159.1, 154.7, 151.8, 145.4, 131.1, 74.4, 27.9, 26.9, 11.9, 11.3. HRMS (ESI) m/z calcd for $C_{11}H_{14}IN_4$ $[M + H]^+$ 329.0263, found 329.0263.

5-((7-Amino-2,3-diethylpyrido[3,4-*b*]pyrazin-8-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSL507)



Synthesized by utilizing general procedure. Brown solid (63.1%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.05 (d, J = 2.2 Hz, 1H), 9.00 (d, J = 2.0 Hz, 1H), 8.88 (s, 1H), 8.58 (t, J = 2.1 Hz, 1H), 8.20 (d, J = 2.2 Hz, 1H), 8.04 (dd, J = 8.6, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.04 (s, 2H), 3.58 (s, 2H), 3.02 (q, J = 7.3 Hz, 2H), 2.94 (q, J = 7.4 Hz, 2H), 2.54 (s, 8H), 2.31 (s, 3H), 1.38 (t, J = 7.3 Hz, 3H), 1.29 (t, J = 7.4 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.2, 162.6, 160.3, 154.4, 154.2, 153.4, 147.7, 145.8, 138.4, 137.7, 132.6, 131.9, 130.4, 130.0, 128.1 (q, J = 30.2 Hz), 125.8 (q, J = 274.6 Hz), 124.0, 120.8, 117.7, 95.4, 90.6, 88.5, 57.6, 54.6, 52.2, 45.2, 28.1, 27.2, 11.8, 11.3. HRMS (ESI) m/z calcd for $C_{32}H_{34}F_3N_8O$ $[M + H]^+$ 603.2807, found 603.2814.

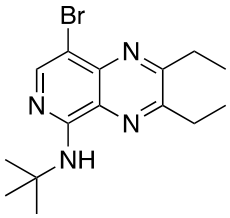
5,8-Dibromo-2,3-diethylpyrido[3,4-b]pyrazine



To a reaction vial, 2,5-dibromopyridine-3,4-diamine (300 mg, 0.123 mmol, 1 equiv.) and ethanol (10 mL) were added. Hexane-3,4-dione (256 mg, 2.25 mmol, 2 equiv.) was then added. The reaction was then allowed to stir at room temperature for 4 hours. After 4 hours, 2 more equivalents of hexane-3,4-dione was added. The reaction was then allowed to stir for 3 days.

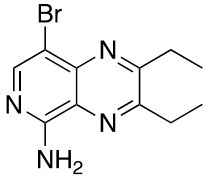
Upon reaction completion, crude mixture was concentrated under reduced pressure and purified *via* silica gel column chromatography. Off white solid (82%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.79 (s, 1H), 3.16 – 3.10 (m, 4H), 1.37 (td, J = 7.2, 2.7 Hz, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.7, 162.1, 146.8, 145.4, 142.4, 135.7, 120.3, 28.0, 27.7, 11.1, 11.0. HRMS (ESI) m/z calcd for $C_{11}H_{12}Br_2N_3$ $[M + H]^+$ 343.9398, found 343.9410.

8-Bromo-*N*-(*tert*-butyl)-2,3-diethylpyrido[3,4-*b*]pyrazin-5-amine



To a pressure tube fitted with a magnetic stir bar, 5,8-dibromo-2,3-diethylpyrido[3,4-b]pyrazine (153 mg, 0.443 mmol, 1 eq) in ethanol (3 mL) were added. Tert-butylamine (97.1 mg, 1.43 mmol, 3 eq) was then added and reaction was capped and moved to 110 ºC. The reaction was then allowed to run for 16 hours. Following 16 hours, 3 additional equivalents of tert-butylamine was then added. The reaction was run for 3 days. Crude product was then cooled to room temperature and concentrated under reduced pressure. Pure product was obtained via silica gel column chromatography. Yellow solid (14%);^{1}H NMR (500 MHz, DMSO) δ 8.23 (s, 1H), 6.83 (s, 1H), 2.68 (s, 4H), 2.66 (s, 4H), 1.50 (s, 10H); ^{13}C NMR (126 MHz, DMSO) δ 159.4, 155.1, 153.3, 146.1, 141.6, 127.5, 103.6, 51.7, 40.4, 40.3, 40.1, 39.9, 39.8, 39.6, 39.4, 28.8, 23.4, 22.8. HRMS (ESI) m/z calcd for $C_{15}H_{22}BrN_4$ $[M + H]^+$ 337.1028, found 337.1042.

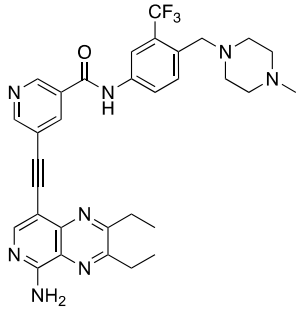
8-Bromo-2,3-diethylpyrido[3,4-b]pyrazin-5-amine



To a pressure tube fitted with a magnetic stir bar, 8-bromo-2,3-diethylpyrido[3,4-b]pyrazin-5-amine (163 mg, 0.445 mmol) in concentrated HCl (1.5 mL) were added. The reaction was capped and moved to 80 ºC and run for 2 hours. The reaction was then diluted with water (50 mL) and neutralized using saturated sodium bicarbonate solution. Product was then extracted with ethyl acetate (3x 100 mL). Organic layers were dried over anhydrous sodium sulfate,

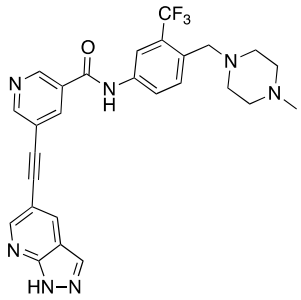
filtered, and concentrated under reduced pressure. Pure product was obtained via column chromatography. Yellow solid (68%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.16 (s, 1H), 7.14 (s, 2H), 3.01 (dq, J = 16.0, 7.3 Hz, 4H), 1.33 (q, J = 7.3 Hz, 6H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 162.5, 158.2, 156.6, 146.7, 141.7, 126.7, 103.8, 27.9, 27.2, 11.8, 11.7. HRMS (ESI) m/z calcd for $C_{11}H_{14}BrN_4$ $[M + H]^+$ 281.0402, found 281.0412.

5-((5-Amino-2,3-diethylpyrido[3,4-*b*]pyrazin-8-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSND15)



Synthesized by utilizing general procedure. Yellow solid (36%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.80 (s, 1H), 9.05 (d, J = 2.2 Hz, 1H), 8.89 (d, J = 2.0 Hz, 1H), 8.44 (t, J = 2.1 Hz, 1H), 8.33 (s, 1H), 8.20 (d, J = 2.3 Hz, 1H), 8.03 (dd, J = 8.4, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.53 (bs, 2H), 3.56 (s, 2H), 3.12 – 2.97 (m, 4H), 2.44 – 2.24 (m, 8H), 2.14 (s, 3H), 1.43 – 1.30 (m, 6H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 162.3, 159.0, 156.6, 153.9, 150.9, 147.8, 144.1, 138.3, 137.1, 133.0, 131.8, 130.3, 128.0 (q, J = 28.9 Hz), 125.8 (q, J = 270.9 Hz), 125.0, 124.0, 120.7, 117.7, 103.3, 90.9, 89.4, 57.9, 55.2, 53.1, 46.2, 27.9, 27.4, 11.7, 11.4. HRMS (ESI) m/z calcd for $C_{32}H_{34}F_3N_8O$ $[M + H]^+$ 603.2807, found 603.2805.

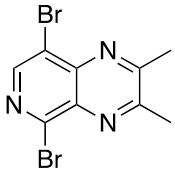
5-((1*H*-Pyrazolo[3,4-*b*]pyridin-5-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSND17)



Synthesized by utilizing general procedure. Off-white solid (53%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.82 (s, 1H), 9.09 (d, J = 2.1 Hz, 1H), 8.98 (d, J = 2.0 Hz, 1H), 8.74 (d, J = 2.0

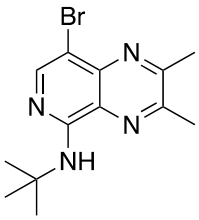
Hz, 1H), 8.54 (dd, *J* = 3.8, 2.2 Hz, 2H), 8.27 – 8.17 (m, 2H), 8.07 (dd, *J* = 8.5, 2.2 Hz, 1H), 7.72 (d, *J* = 8.5 Hz, 1H), 3.65 (s, 2H), 3.55-3.37 (bs, 8H), 2.69 (s, 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 163.9, 154.4, 151.6, 151.0, 148.6, 138.6, 137.9, 134.4, 133.9, 132.0, 130.2, 128.2 (q, *J* = 30.2 Hz), 125.7 (q, *J* = 274.6 Hz), 124.0, 121.0, 119.5, 117.8, 114.4, 111.5, 91.8, 86.8, 57.0, 53.3, 50.1, 42.9; HRMS (ESI) m/z calcd for $C_{30}H_{30}F_3N_8O$ $[M + H]^+$ 575.2494, found 575.2489.

5,8-Dibromo-2,3-dimethylpyrido[3,4-b]pyrazine



To a reaction vial, 2,5-dibromopyridine-3,4-diamine (300 mg, 0.123 mmol, 1 equiv.) and ethanol (10 mL) were added. Biacetyl (130 mg, 2.25 mmol, 2 equiv.) was then added. The reaction was then allowed to stir at room temperature for 4 hours. After 4 hours 2 more equivalents of biacetyl were added. The reaction was then allowed to stir for 3 days. Upon reaction completion, crude mixture was concentrated under reduced pressure. Pure compound was obtained via column chromatography. Off-white solid (82%); ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 8.78 (s, 1H), 2.79 (s, 3H), 2.77 (s, 3H). ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 162.0, 159.3, 146.8, 145.1, 142.7, 135.9, 119.9, 23.7, 23.5. HRMS (ESI) m/z calcd for $C_9H_8Br_2N_3$ $[M + H]^+$ 315.9085, found 315.9096.

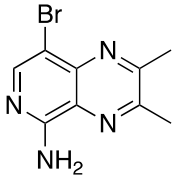
8-Bromo-N-(tert-butyl)-2,3-dimethylpyrido[3,4-b]pyrazin-5-amine



To a pressure tube fitted with a magnetic stir bar, 5,8-dibromo-2,3-dimethylpyrido[3,4-b]pyrazine (147 mg, 0.468 mmol, 1 eq) in ethanol (3 mL) was added. Tert-butylamine (106 mg, 1.4 mmol, 3 eq) were then added and reaction was capped and moved to 110 ºC. The reaction was then allowed to run for 16 hours. Following 16 hours, 3 additional equivalents of tert-butylamine was then added. The reaction was then run for 3 days. Crude product was then cooled to room temperature and concentrated under reduced pressure. Pure product was

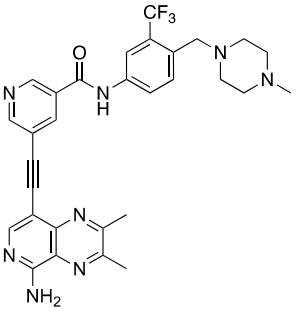
obtained via column chromatography. Yellow solid (16.6%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.23 (s, 1H), 6.83 (s, 1H), 2.68 (s, 3H), 2.66 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.4, 155.1, 153.3, 146.2, 141.6, 127.5, 103.6, 51.7, 28.8, 23.4, 22.8. HRMS (ESI) m/z calcd for $C_{13}H_{18}BrN_4$ $[M + H]^+$ 309.0715, found 309.0729.

8-Bromo-2,3-dimethylpyrido[3,4-b]pyrazin-5-amine



To a pressure tube fitted with a magnetic stir bar, 8-bromo-N-(tert-butyl)-2,3-dimethylpyrido[3,4-b]pyrazin-5-amine (88 mg, 0.285 mmol) in concentrated HCl (1 mL) were added. The reaction was capped and moved to 80 ºC and run for 2 hours. The reaction was then diluted with water (50 mL) and neutralized using saturated sodium bicarbonate solution. Product was then extracted with ethyl acetate (3x 100 mL). Organic layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. Pure product was obtained via column chromatography. Yellow solid (63%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.16 (s, 2H), 2.68 (s, 3H), 2.67 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.6, 163.1, 158.4, 151.4, 147.1, 132.0, 108.4, 28.5, 27.8. HRMS (ESI) m/z calcd for $C_9H_{10}BrN_4$ $[M + H]^+$ 253.0089, found 253.0097.

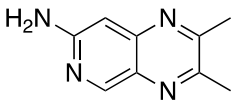
5-((5-Amino-2,3-dimethylpyrido[3,4-*b*]pyrazin-8-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSND16)



Synthesized by utilizing general procedure. Yellow solid (43%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.04 (d, J = 2.1 Hz, 1H), 8.92 (d, J = 2.0 Hz, 1H), 8.47 (t, J = 2.1 Hz, 1H), 8.33 (s, 1H), 8.21 (d, J = 2.2 Hz, 1H), 8.08 (dd, J = 8.4, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H),

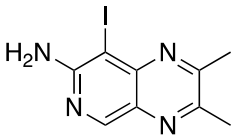
7.55 (s, 2H), 3.66 (s, 3H), 2.84 – 2.69 (m, 8H),2.72 (s, 3H), 2.68 (s, 3H);^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 159.4, 158.9, 154.1, 153.3, 151.35, 147.9, 144.3, 138.6, 137.2, 132.0, 131.9, 130.2, 128.4 (q, J = 27.7 Hz), 125.7 (q, J = 273.4 Hz), 125.2, 124.0, 120.6, 117.8, 103.0, 91.0, 89.1, 57.0, 53.3, 50.0, 42.8, 23.7, 23.0. HRMS (ESI) m/z calcd for $C_{30}H_{30}F_3N_8O$ [M + H]$^+$ 575.2494, found 575.2506.

2,3-Dimethylpyrido[3,4-*b*]pyrazin-7-amine



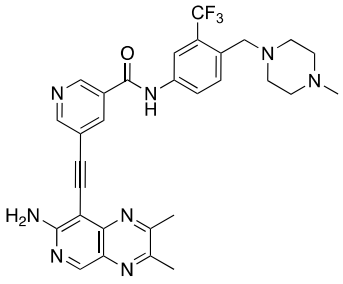
To a reaction vial, pyridine-2,4,5-triamine (80 mg, 0.645 mmol, 1 equiv.) and ethanol (5 mL) were added. Biacetyl (55.54 mg, 0.645 mmol, 1 equiv.) was then added. The reaction was then allowed to stir at room temperature for 2 hours. Upon reaction completion, crude mixture was concentrated under reduced pressure. Pure compound was obtained via column chromatography. Yellow solid (89%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.75 (s, 1H), 6.60 (s, 1H), 6.28 (s, 2H), 2.56 (s, 3H), 2.53 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 159.4, 159.2, 151.9, 150.3, 146.1, 130.6, 97.5, 23.7, 22.7. HRMS (ESI) m/z calcd for $C_9H_{11}N_4$ [M + H]$^+$ 175.0984, found 175.0983.

8-Iodo-2,3-dimethylpyrido[3,4-*b*]pyrazin-7-amine



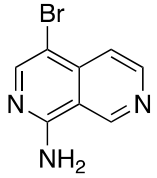
2,3-dimethylpyrido[3,4-b]pyrazin-7-amine (94.1 mg, 0.540 mmol, 1 equiv) were added to a reaction vial with methanol (10 mL). The reaction was then cooled to 0°C and N-Iodo succinimide (133.8 mg, 0.594 mol, 1.1 equiv) was slowly added over 10 minutes. The reaction was then allowed to run at 0°C for 15 minutes. Crude mixture was concentrated down under reduced pressure. Pure product was obtained via column chromatography. Brown solid (90.2%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.71 (s, 1H), 6.50 (s, 2H), 2.64 (s, 3H), 2.58 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 160.1, 159.1, 151.7, 151.3, 145.8, 131.3, 73.8, 23.7, 22.3. HRMS (ESI) m/z calcd for $C_9H_{10}IN_4$ [M + H]$^+$ 300.9950, found 300.9950.

5-((7-Amino-2,3-dimethylpyrido[3,4-*b*]pyrazin-8-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSL476)



In a 25 mL round bottom flask containing 8-iodo-2,3-dimethylpyrido[3,4-b]pyrazin-7-amine (1 mmol), alkyne substrate (1.1 mmol), $PdCl_2(PPh_3)_2$ (3 mol%), XPhos (2 mol%), Cs_2CO_3 (3 equiv) and CuI (1 mol%), anhydrous DMF (5 mL) and DIPEA (2.5 mL) was added under inert condition. Reaction mixture was allowed to stir at 60 °C for an overnight. After completion reaction mixture was concentrated and extracted with ethyl acetate. Organic layer was washed with brine solution (30 mL). Organic layer was passed through celite bad. Collected organic layer dried with sodium sulfate, concentrated and purified via silica gel column chromatography to yield the desired product. Yellow solid (57%); ^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 9.01 (dd, *J* = 7.2, 2.0 Hz, 2H), 8.80 (s, 1H), 8.59 (t, *J* = 2.1 Hz, 1H), 8.14 (d, *J* = 2.3 Hz, 1H), 7.95 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 3.66 (s, 2H), 2.74 (s, 3H), 2.64 (s, 3H), 2.56 (s, 8H), 2.33 (s, 3H); ^{13}C NMR (126 MHz, Methanol-*d*$_4$) δ 164.3, 160.1, 159.8, 153.5, 152.2, 151.4, 146.7, 145.8, 137.7, 137.5, 132.9, 131.2, 130.4, 130.2, 129.1 (q, *J* = 20.2 Hz), 127.5 (q, *J* = 274.6 Hz), 123.5, 121.0, 117.7, 94.9, 91.3, 86.8, 57.4, 54.5, 52.1, 44.4, 22.1, 21.1. HRMS (ESI) m/z calcd for $C_{30}H_{30}F_3N_8O$ $[M + H]^+$ 575.2494, found 575.2512.

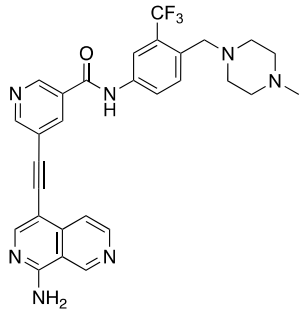
4-Bromo-2,7-naphthyridin-1-amine



To a solution of 2,7-naphthyridine-1-amine (1 g, 6.9 mmol, 1 equiv) in $CHCl_3$ (60 mL) at 0 ºC was added N-Bromo succinimide (1.23 g, 6.9 mmol, 1 equiv) slowly over a period of 10 min. The reaction was allowed to stir 1h, diluted the reaction mixture with $CHCl_3$ (200 mL) and washed with $NaHCO_3$ (5 × 200 mL) and brine solution (100 mL). Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The pure product was then obtained via flash column chromatography. Brown solid (68%); ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 9.52 (s, 1H), 8.71 (d, *J* = 5.8 Hz, 1H), 8.17 (s, 1H), 7.61 (d, *J* = 5.8

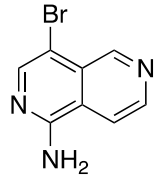
Hz, 1H), 7.58 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 158.0, 149.6, 149.0, 148.2, 139.0, 117.7, 114.2, 102.3. HRMS (ESI) m/z calcd for $C_8H_7BrN_3$ $[M + H]^+$ 225.9798, found 225.9798.

5-((1-Amino-2,7-naphthyridin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN721)



Synthesized by utilizing general procedure. Pale yellow solid (35%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.60 (s, 1H), 9.05 (d, J = 2.2 Hz, 1H), 8.99 (d, J = 2.1 Hz, 1H), 8.72 (dd, J = 5.7, 2.2 Hz, 1H), 8.55 (t, J = 2.2 Hz, 1H), 8.37 (d, J = 2.2 Hz, 1H), 8.20 (d, J = 2.3 Hz, 1H), 8.04 (dd, J = 8.5, 2.3 Hz, 1H), 7.99 – 7.90 (m, 3H), 7.71 (d, J = 8.6 Hz, 1H), 3.55 (s, 2H), 2.43 – 2.29 (m, 8H), 2.15 (d, J = 2.5 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 158.6, 154.0, 152.2, 149.6, 148.8, 148.0, 140.4, 138.3, 137.3, 132.9, 131.7, 130.2, 128.0 (q, J = 28.9 Hz), 125.8 (q, J = 274.6 Hz), 124.0, 120.2, 117.7, 117.5, 112.0, 100.8, 90.3, 89.9, 57.8, 55.1, 53.0, 46.0. HRMS (ESI) m/z calcd for $C_{29}H_{27}F_3N_7O$ $[M + H]^+$ 546.2224, found 546.2221.

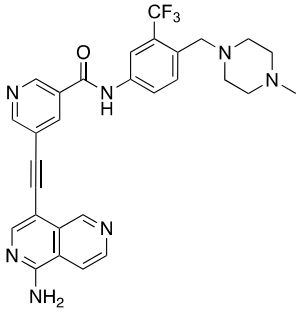
4-Bromo-2,6-naphthyridin-1-amine



To a solution of 2,6-naphthyridine-1-amine (1 g, 6.9 mmol, 1 equiv) in $CHCl_3$ (60 mL) at 0 ºC was added N-Bromo succinimide (1.23 g, 6.9 mmol, 1 equiv) slowly over a period of 10 min. The reaction was allowed to stir for 1h, diluted the reaction mixture with $CHCl_3$ (200 mL) and washed with $NaHCO_3$ (5 × 200 mL) and brine solution (100 mL). Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The pure product was then obtained via flash column chromatography. Brown solid (70%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.20 (d, J = 0.9 Hz, 1H), 8.68 (d, J = 5.7 Hz, 1H), 8.09 (dd, J = 5.8,

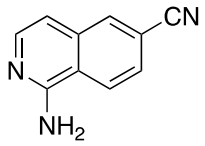
0.9 Hz, 1H), 8.08 (s, 1H), 7.42 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.0, 149.7, 145.2, 129.2, 122.4, 116.9, 101.8. HRMS (ESI) m/z calcd for $C_8H_7BrN_3$ $[M + H]^+$ 225.9798, found 225.9798.

5-((1-Amino-2,6-naphthyridin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN722)



Synthesized by utilizing general procedure. Yellow solid (41%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.76 (s, 1H), 9.53 (s, 1H), 9.04 (dd, J = 8.1, 2.1 Hz, 2H), 8.69 (d, J = 5.7 Hz, 1H), 8.59 (t, J = 2.1 Hz, 1H), 8.32 (s, 1H), 8.20 (d, J = 2.2 Hz, 1H), 8.18 – 8.14 (m, 1H), 8.04 (dd, J = 8.5, 2.2 Hz, 1H), 7.79 (s, 2H), 7.73 (d, J = 8.5 Hz, 1H), 3.57 (s, 2H), 2.48 – 2.24 (m, 8H), 2.18 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 157.5, 154.1, 149.5, 149.4, 148.0, 145.2, 138.3, 137.4, 132.9, 131.8, 130.4, 130.3, 127.8, 125.8 (q, J = 274.6 Hz), 124.0, 120.1, 119.9, 117.7, 117.1, 100.6, 90.5, 89.6, 57.8, 55.0, 52.9, 45.9. HRMS (ESI) m/z calcd for $C_{29}H_{27}F_3N_7O$ $[M + H]^+$ 546.2224, found 546.2221.

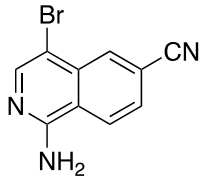
1-Aminoisoquinoline-6-carbonitrile



A solution of 1-aminoisoquinoline-6-carbonitrile (600 mg, 2.69 mmol, 1 equiv), $Pd(PPh_3)_4$ (932 mg, 0.81 mmol, 0.3 equiv), $Zn(CN)_2$ (630 mg, 5.38 mmol, 2 equiv) in DMF (12 mL) was de-oxygenated using steam of Argon gas. The reaction temperature was increased to 90 ºC and allowed to stir 12 h. The reaction was filtered and diluted with ethyl acetate (300 mL). The organic layer was washed with water (3 × 100 mL) and washed with brine (1 × 100 mL). Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The pure product was obtained by flash column chromatography. Brown solid (75%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.33 (d, J = 8.6 Hz, 1H), 8.30 (s, 1H), 7.91 (d, J = 5.7 Hz,

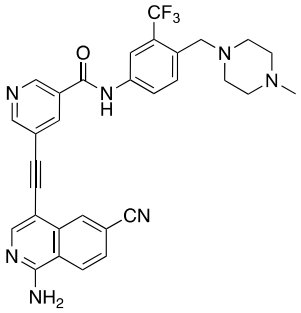
1H), 7.76 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.08 (s, 2H), 6.97 (d, *J* = 5.7 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.7, 144.2, 136.7, 132.8, 126.6, 126.0, 119.1, 118.6, 112.8, 109.6. HRMS (ESI) m/z calcd for $C_{10}H_8N_3$ $[M + H]^+$ 170.0713, found 170.0718.

1-Amino-4-bromoisoquinoline-6-carbonitrile



To a solution of 6-cyano isoquinolin-1-amine (320 mg, 1.88 mmol, 1 equiv) in $CHCl_3$ (20 mL) at 0 ºC was added N-bromosuccinimide (1.23 g, 6.9 mmol, 1 equiv) slowly over a period of 10 min. The reaction was allowed to stir for an hour. Reaction mixture was diluted with $CHCl_3$ (200 mL) and washed with $NaHCO_3$ (5 × 200 mL) and brine solution (100 mL). Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The pure product was then obtained via silica gel column chromatography. Yellow solid (70%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.40 (d, *J* = 8.5 Hz, 1H), 8.25 (s, 1H), 8.10 (s, 1H), 7.91 (d, *J* = 8.5 Hz, 1H), 7.39 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.6, 145.5, 134.7, 130.9, 128.1, 126.8, 120.3, 118.6, 114.5, 103.5. HRMS (ESI) m/z calcd for $C_{10}H_7BrN_3$ $[M + H]^+$ 247.9823, found 247.9824.

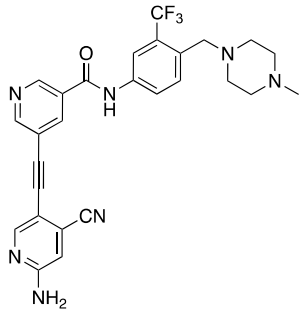
5-((1-Amino-6-cyanoisoquinolin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN804)



Synthesized by utilizing general procedure. Yellow solid (59 %); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 9.06 (dd, *J* = 8.7, 2.1 Hz, 2H), 8.60 (s, 1H), 8.59 (t, *J* = 2.2 Hz, 1H), 8.46 (d, *J* = 8.6 Hz, 1H), 8.33 (s, 1H), 8.21 (d, *J* = 2.2 Hz, 1H), 8.04 (d, *J* = 8.5 Hz, 1H), 7.93 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.77 (s, 2H), 7.73 (d, *J* = 8.5 Hz, 1H), 3.57 (s, 2H), 2.39 (s, 8H), 2.18 (s, 3H); NMR (126 MHz, DMSO-d_6) δ 164.1, 158.2, 154.3, 149.9, 148.0, 138.3, 137.5, 135.9, 132.9, 131.8, 130.4, 130.3, 128.1, 128.0 (q, *J* = 28.1 Hz), 126.5, 125.8 (q, *J* = 274.6 Hz), 124.0,

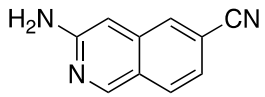
120.2, 118.8, 118.0, 117.7, 114.3, 102.0, 90.6, 90.0, 57.8, 55.1, 53.0, 46.0. HRMS (ESI) m/z calcd for $C_{31}H_{27}F_3N_7O$ $[M + H]^+$ 570.2222, found 570.2219.

5-((6-Amino-4-cyanopyridin-3-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN631)



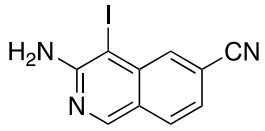
Synthesized by utilizing general procedure. Yellow solid (36%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 9.08 (d, J = 2.2 Hz, 1H), 8.88 (d, J = 2.0 Hz, 1H), 8.42 (t, J = 2.1 Hz, 1H), 8.39 (s, 1H), 8.18 (d, J = 2.3 Hz, 1H), 8.01 (dd, J = 8.5, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.15 (s, 2H), 6.86 (s, 1H), 3.56 (s, 2H), 2.38 (s, 8H), 2.18 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 159.7, 154.0, 153.5, 148.6, 138.2, 137.5, 132.9, 131.8, 130.3, 128.0, 125.8 (q, J = 274.6 Hz), 124.0, 122.5, 119.3, 117.7, 116.3, 111.1, 105.7, 90.0, 88.8, 57.8, 55.0, 52.9, 45.9. HRMS (ESI) m/z calcd for $C_{27}H_{25}F_3N_7O$ $[M + H]^+$ 520.2067, found 520.2068.

3-Aminoisoquinoline-6-carbonitrile [1]



Synthesized using literature procedure[1]

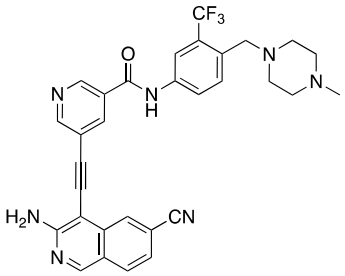
3-Amino-4-iodoisoquinoline-6-carbonitrile



To a solution of 6-cyano isoquinolin-3-amine (480 mg, 2.81 mmol, 1 equiv) in methanol (40 mL) at 0 ºC N-Iodo succinimide (696 mg, 3.09 mmol, 1.1 equiv) was added slowly over a period of 10 min. The reaction was allowed to stir for 15 min. After that reaction was concentrated and purified by flash column chromatography.

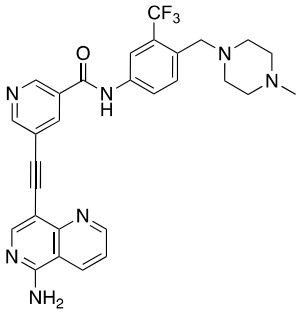
Yellow solid (72%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.91 (s, 1H), 8.02 – 7.99 (m, 2H), 7.50 (dd, J = 8.4, 1.4 Hz, 1H), 6.56 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 157.9, 153.2, 138.9, 133.9, 130.8, 124.3, 123.2, 119.0, 114.8, 71.6. HRMS (ESI) m/z calcd for $C_{10}H_7IN_3$ $[M + H]^+$ 295.9679, found 295.9681.

5-((3-Amino-6-cyanoisoquinolin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN742)



Synthesized by utilizing general procedure. Yellow solid (90%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.79 (s, 1H), 9.23 (d, J = 2.0 Hz, 1H), 9.04 (s, 2H), 8.75 (t, J = 2.1 Hz, 1H), 8.47 (s, 1H), 8.20 (d, J = 2.2 Hz, 1H), 8.08 (d, J = 8.3 Hz, 1H), 8.04 (dd, J = 8.5, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.53 (dd, J = 8.3, 1.5 Hz, 1H), 7.14 (s, 2H), 3.56 (s, 2H), 2.37 (s, 8H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 159.3, 154.7, 154.1, 147.8, 138.3, 137.9, 136.9, 132.9, 131.8, 130.7, 130.4, 128.8, 127.8 (q, J = 28.9 Hz), 125.8 (q, J = 273.4 Hz), 123.9, 123.6, 122.7, 120.2, 119.2, 117.6, 114.5, 96.7, 88.5, 87.6, 57.8, 55.1, 53.0, 46.1. HRMS (ESI) m/z calcd for $C_{31}H_{27}F_3N_7O$ $[M + H]^+$ 570.2224, found 570.2224.

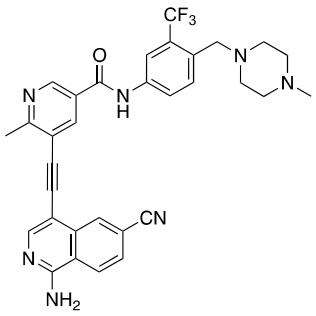
5-((5-Amino-1,6-naphthyridin-8-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN757)



Synthesized by utilizing general procedure. Yellow solid (21%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.08 – 9.01 (m, 2H), 8.90 (d, J = 2.0 Hz, 1H), 8.70 (dd, J = 8.4, 1.7 Hz, 1H), 8.48 (t, J = 2.1 Hz, 1H), 8.39 (s, 1H), 8.21 (d, J = 2.2 Hz, 1H), 8.04 (dd, J = 8.4, 2.2 Hz, 1H), 7.75 – 7.68 (m, 3H), 7.60 – 7.57 (m, 1H), 3.56 (s, 2H), 2.37 (s, 8H), 2.15 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 159.1, 154.8, 154.2, 152.0, 151.7, 147.9, 138.3, 137.2,

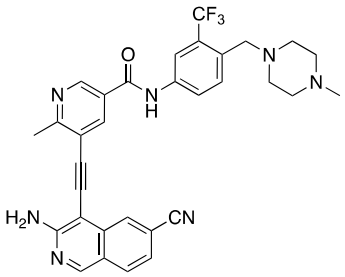
133.5, 132.9, 131.7, 130.1, 127.8, 125.8 (q, J = 274.6 Hz), 124.0, 122.0, 120.7, 117.7, 112.3, 104.5, 91.9, 89.0, 57.9, 55.1, 53.1, 46.1. HRMS (ESI) m/z calcd for $C_{29}H_{27}F_3N_7O$ $[M + H]^+$ 546.2224, found 546.2231.

5-((1-Amino-6-cyanoisoquinolin-4-yl)ethynyl)-6-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN805)



Synthesized by utilizing general procedure. Yellow solid (30%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.95 (d, J = 2.2 Hz, 1H), 8.55 (d, J = 2.2 Hz, 1H), 8.51 (s, 1H), 8.47 (d, J = 8.6 Hz, 1H), 8.33 (s, 1H), 8.20 (d, J = 2.2 Hz, 1H), 8.05 (dd, J = 8.6, 2.2 Hz, 1H), 7.93 (dd, J = 8.6, 1.7 Hz, 1H), 7.77 (s, 2H), 7.71 (d, J = 8.5 Hz, 1H), 3.57 (s, 2H), 2.81 (s, 3H), 2.41 (s, 8H), 2.21 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.1, 162.4, 158.1, 149.7, 147.4, 138.4, 138.0, 135.9, 132.7, 131.8, 130.2, 128.0, 127.9, 127.8 (q, J = 27.7 Hz), 126.6, 125.8 (q, J = 273.4 Hz), 123.9, 118.8, 118.0, 117.7, 114.2, 102.2, 92.7, 90.7, 57.8, 54.9, 52.8, 24.2. HRMS (ESI) m/z calcd for $C_{32}H_{29}F_3N_7O$ $[M + H]^+$ 584.2379, found 584.2375.

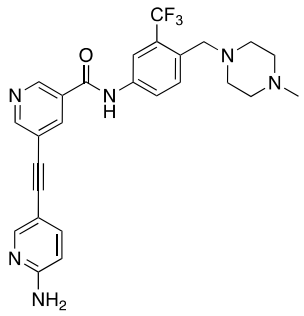
5-((3-Amino-6-cyanoisoquinolin-4-yl)ethynyl)-6-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN806)



Synthesized by utilizing general procedure. Yellow solid (35%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.72 (s, 1H), 9.06 (s, 1H), 8.96 (d, J = 2.2 Hz, 1H), 8.73 (d, J = 2.3 Hz, 1H), 8.33 (s, 1H), 8.20 (d, J = 2.2 Hz, 1H), 8.11 (d, J = 8.3 Hz, 1H), 8.04 (dd, J = 8.5, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 7.55 (dd, J = 8.3, 1.5 Hz, 1H), 7.07 (s, 2H), 3.56 (s, 2H), 2.86 (s, 3H), 2.37 (s, 8H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 162.3, 159.1, 154.0, 147.4, 138.8,

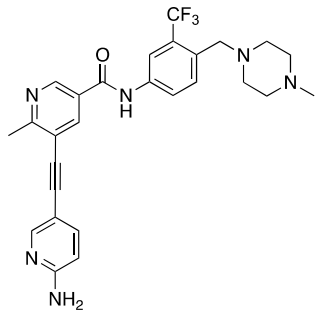
138.3, 136.9, 132.8, 131.8, 130.9, 128.8, 128.6, 128.1, 128.0 (q, J = 28.9 Hz), 125.87 (q, J = 274.6 Hz), 123.9, 123.5, 122.7, 119.2, 119.0, 117.6, 114.5, 96.8, 90.3, 88.8, 57.9, 55.1, 53.1, 46.1, 24.4. HRMS (ESI) m/z calcd for $C_{32}H_{29}F_3N_7O$ $[M + H]^+$ 584.2379, found 584.2374.

5-((6-Aminopyridin-3-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN815)



Synthesized by utilizing general procedure. Off-white solid (59%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.71 (s, 1H), 9.01 (d, J = 2.2 Hz, 1H), 8.85 (d, J = 2.0 Hz, 1H), 8.41 (t, J = 2.1 Hz, 1H), 8.18 (d, J = 2.2 Hz, 1H), 8.17 (dd, J = 2.4, 0.8 Hz, 1H), 8.02 (dd, J = 8.5, 2.2 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.54 (dd, J = 8.6, 2.3 Hz, 1H), 6.53 (s, 2H), 6.47 (dd, J = 8.6, 0.8 Hz, 1H), 3.56 (s, 2H), 2.38 (s, 8H), 2.17 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 160.1, 153.9, 152.1, 147.9, 139.9, 138.3, 137.2, 132.9, 131.8, 130.2, 128.0 (q, J = 28.9 Hz), 125.8 (q, J = 273.4 Hz), 123.6, 120.26, 117.6, 108.1, 105.5, 93.0, 85.7, 57.8, 55.0, 52.9, 46.0. HRMS (ESI) m/z calcd for $C_{26}H_{26}F_3N_6O$ $[M + H]^+$ 495.2115, found 495.2111.

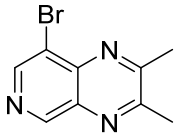
5-((6-Aminopyridin-3-yl)ethynyl)-6-methyl-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN816)



Synthesized by utilizing general procedure. Off-white solid (53%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.91 (d, J = 2.3 Hz, 1H), 8.36 (d, J = 2.3 Hz, 1H), 8.18 (d, J = 2.2 Hz, 2H), 8.02 (dd, J = 8.6, 2.2 Hz, 1H), 7.70 (d, J = 8.5 Hz, 1H), 7.55 (dd, J = 8.6, 2.4 Hz, 1H), 6.52 (s, 2H), 6.48 (d, J = 8.6 Hz, 1H), 3.55 (d, J = 1.7 Hz, 2H), 2.70 (s, 3H), 2.36 (d, J = 19.9

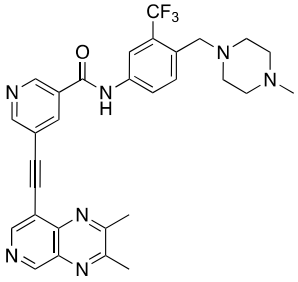
Hz, 8H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.9, 162.4, 160.0, 151.9, 147.3, 139.8, 138.3, 137.5, 132.8, 131.7, 128.0, 127.8, 125.8 (q, J = 274.6 Hz), 123.9, 118.9, 117.7, 108.2, 105.8, 95.7, 85.9, 57.8, 55.1, 53.0, 46.0, 24.0. HRMS (ESI) m/z calcd for $C_{27}H_{28}F_3N_6O$ $[M + H]^+$ 509.2271, found 509.2266.

8-Bromo-2,3-dimethylpyrido[3,4-*b*]pyrazine



To a reaction vial, 5-bromopyridine-3,4-diamine (100 mg, 0.532 mmol, 1 equiv.) and ethanol (10 mL) were added. Biacetyl (45.8 mg, 0.532 mmol, 1 equiv.) was then added. The reaction was then allowed to stir at room temperature for 2 hours. Upon reaction completion, crude mixture was concentrated under reduced pressure. Pure compound was obtained via column chromatography. Brown solid (92%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.90 (s, 1H), 2.73 (s, 3H), 2.71 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 161.3, 158.3, 152.4, 147.8, 141.4, 137.2, 119.3, 23.9, 23.2. HRMS (ESI) m/z calcd for $C_9H_9BrN_3$ $[M + H]^+$ 237.9980, found 237.9980.

5-((2,3-Dimethylpyrido[3,4-*b*]pyrazin-8-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSL211)



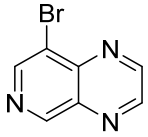
Synthesized by utilizing general procedure. Off-white solid (24%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.78 (s, 1H), 9.36 (s, 1H), 9.13 (d, J = 2.2 Hz, 1H), 9.06 – 8.94 (m, 2H), 8.58 (t, J = 2.1 Hz, 1H), 8.19 (d, J = 2.2 Hz, 1H), 8.02 (dd, J = 8.5, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 3.56 (s, 2H), 2.80 (s, 3H), 2.75 (s, 3H), 2.37 (s, 8H), 2.15 (s, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 161.0, 158.0, 154.6, 153.5, 150.2, 149.1, 143.4, 138.2, 138.0, 135.8, 133.0, 131.8, 130.3, 128.0 (q, J = 274.6 Hz), 125.8 (q, J = 274.6 Hz), 124.0, 119.2, 117.7, 116.0, 93.1, 88.2, 57.8, 55.1, 53.1, 46.1, 24.1, 23.5. HRMS (ESI) m/z calcd for $C_{30}H_{29}F_3N_7O$ $[M + H]^+$ 560.2385, found 560.2401.

5-((5-Aminopyrimidin-2-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSL468)

To a vial with 5-bromopyrimidin-2-amine (70 mg, 0.402 mmol, 1 equiv), $Pd(PPh_3)_2Cl_2$ (10 mol %), CuI, PPh_3 anhydrous TEA (1.5 mL) was added. The solution was then degassed with a stream of argon gas for 10 minutes. A solution of 5-ethynyl-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (197.1 mg, 0.482 mmol, 1.2 equiv) in anhydrous degasses DMF (3 mL) was then slowly added over the course of 10 minutes. Once addition was complete, it was then moved to 55°C and allowed to run overnight. The reaction was then cooled to room temperature and concentrated under reduced pressure. Pure product was then obtained via column chromatography.

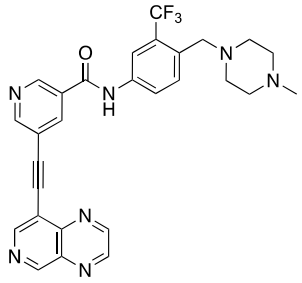
Off white solid (30.2%).^{1}H NMR (500 MHz, DMSO-d_6) δ 10.77 (s, 1H), 9.05 (d, J = 2.1 Hz, 1H), 8.87 (d, J = 2.0 Hz, 1H), 8.47 (s, 2H), 8.44 (t, J = 2.1 Hz, 1H), 8.19 (d, J = 2.2 Hz, 1H), 8.03 (dd, J = 8.6, 2.2 Hz, 1H), 7.71 (d, J = 8.5 Hz, 1H), 7.25 (s, 2H), 3.57 (s, 2H), 2.41 (s, 8H), 2.21 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 163.8, 154.8, 148.9, 148.9, 139.8, 138.3, 134.9, 132.5, 131.9, 130.2, 128.1, 127.8 (q, J = 28.7 Hz), 125.7 (q, J = 272.5 Hz), 123.9, 119.8, 118.9, 117.7, 85.6, 80.1, 57.4, 54.2, 51.6, 44.6. HRMS (ESI) m/z calcd for $C_{25}H_{25}F_3N_7O$ $[M + H]^+$ 496.2072, found 496.2075.

8-Bromopyrido[3,4-*b*]pyrazine



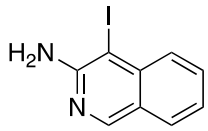
To a reaction vial, 5-bromopyridine-3,4-diamine (250 mg, 1.33 mmol, 1 equiv.) and ethanol (10 mL) were added. Oxalaldehyde (77 mg, 1.33 mmol, 1 equiv.) was then added. The reaction was then allowed to stir at room temperature for 6 hours. Upon reaction completion, crude mixture was concentrated under reduced pressure. Pure compound was obtained via column chromatography. Off white solid (85%) ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.48 (s, 1H), 9.27 (d, J = 1.8 Hz, 1H), 9.17 (d, J = 1.7 Hz, 1H), 9.12 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 154.2, 151.6, 149.1, 148.9, 143.0, 138.9, 120.2. HRMS (ESI) m/z calcd for $C_7H_5BrN_3$ $[M + H]^+$ 209.9667, found 209.9667.

***N*-(4-((4-Methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)-5-(pyrido[3,4-*b*]pyrazin-8-ylethynyl)nicotinamide**



Synthesized by utilizing general procedure. Brown solid (16%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.81 (s, 1H), 9.55 (s, 1H), 9.30 (d, J = 1.8 Hz, 1H), 9.20 (d, J = 1.7 Hz, 1H), 9.16 (s, 1H), 9.14 (d, J = 2.2 Hz, 1H), 9.04 (d, J = 2.0 Hz, 1H), 8.61 (t, J = 2.1 Hz, 1H), 8.20 (d, J = 2.3 Hz, 1H), 8.04 (dd, J = 8.5, 2.2 Hz, 1H), 7.72 (d, J = 8.5 Hz, 1H), 3.57 (s, 2H), 2.40 (s, 8H), 2.19 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 163.7, 155.1, 154.5, 151.4, 151.0, 149.3, 149.1, 145.0, 138.2, 138.1, 137.4, 132.9, 131.8, 130.3, 128.0 (q, J = 28.9 Hz), 125.8 (q, J = 274.6 Hz), 124.0, 119.1, 117.7, 116.9, 93.7, 87.7, 57.8, 55.0, 52.9, 45.9. HRMS (ESI) m/z calcd for $C_{28}H_{25}F_3N_7O$ $[M + H]^+$ 532.2072, found 532.2091.

4-Iodoisoquinolin-3-amine



To a solution of isoquinolin-3-amine (500 mg, 3.47 mmol, 1 equiv) in methanol (30 mL) at 0 ºC was added N-Iodo succinimide (858 mg, 3.8 mmol, 1.2 equiv) slowly over a period of 10 min. The reaction was allowed to stir for 15 min., concentrated. The product was purified by flash column chromatography.

Brown solid (68%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.78 (s, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.62 (d, J = 1.0 Hz, 1H), 7.62 – 7.60 (m, 1H), 7.26 (ddd, J = 8.0, 4.7, 3.2 Hz, 1H), 6.18 (s, 2H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.5, 152.6, 139.6, 132.6, 128.9, 128.1, 123.1, 72.8. HRMS (ESI) m/z calcd for $C_9H_8IN_2$ $[M + H]^+$ 270.9727, found 270.9728.

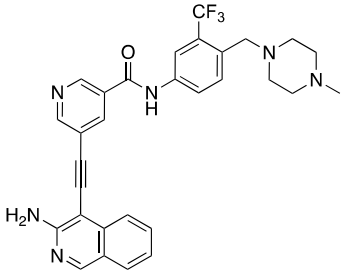
5-((1-amino-6-fluoroisoquinolin-4-yl)ethynyl)-N-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN692)

A solution of Bromo compound 1a (134 mg, 0.56 mmol, 1.5 equiv), $Pd(PPh_3)_4$ (10 mol%), CuI (5 mol%) and Triphenylphosphine (10 mg) in Triethylamine (1.5 mL) was de-oxygenated

using steam of Argon gas. A de-oxygenated solution of alkyne 1b (150 mg, 0.37 mmol, 1 equiv) in DMF (4 mL) was added slowly over a period of 10 min to the solution and the reaction temperature was increased to 55 ºC and allowed to stir 12 h. The reaction was diluted with ethyl acetate (300 mL). The organic layer was washed with water (5 × 50 mL), and brine (1 × 50 mL). Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated in vacuo. The pure product HSN692 was obtained by flash column chromatography. Yield = 51 %.; TLC R_f = 0.2 (10 % MeOH/CH_2Cl_2)

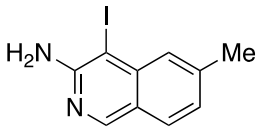
^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 10.82 (s, 1H), 9.15 (d, *J* = 2.1 Hz, 1H), 9.03 – 8.99 (m, 1H), 8.60 (t, *J* = 2.1 Hz, 1H), 8.23 – 8.18 (m, 2H), 8.03 (dd, *J* = 8.4, 2.3 Hz, 2H), 7.71 (m, 3H), 7.48 (s, 3H), 3.56 (s, 2H), 2.39 (s, 8H), 2.18 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 163.58, 149.68, 139.32, 138.23, 137.35, 134.83, 133.01, 131.82, 131.14, 130.32, 128.82, 128.05, 127.81, 125.82, 123.99, 123.65, 117.68, 113.73, 79.80, 76.89, 57.83, 55.04, 52.91, 45.92; HRMS (ESI$^+$): calcd. for $C_{30}H_{27}F_4N_6O$ (MH^+) 563.2177, found 563.2182

5-((3-Aminoisoquinolin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN700)



Synthesized by utilizing general procedure. Yellow solid (61%); ^{1}H NMR (500 MHz, DMSO-*d*$_6$) δ 10.79 (s, 1H), 9.14 (d, *J* = 2.0 Hz, 1H), 9.03 (d, *J* = 2.2 Hz, 1H), 8.91 (d, *J* = 0.7 Hz, 1H), 8.67 (t, *J* = 2.1 Hz, 1H), 8.20 (d, *J* = 2.2 Hz, 1H), 8.08 – 7.98 (m, 2H), 7.90 (d, *J* = 8.1 Hz, 1H), 7.73 (d, *J* = 8.5 Hz, 1H), 7.66 (ddd, *J* = 8.3, 6.8, 1.3 Hz, 1H), 7.28 (ddd, *J* = 8.0, 6.8, 1.1 Hz, 1H), 6.78 (s, 2H), 3.58 (s, 2H), 2.42 (s, 8H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, DMSO-*d*$_6$) δ 164.3, 158.4, 154.3, 153.7, 147.6, 138.3, 137.9, 137.6, 132.8, 132.2, 131.8, 130.3, 129.1, 128.0 (q, *J* = 55.4 Hz), 125.84 (q, *J* = 275.9 Hz), 124.0, 123.1, 122.8, 122.3, 120.5, 117.7, 95.8, 89.0, 88.5, 57.7, 54.9, 52.7, 45.7. HRMS (ESI) m/z calcd for $C_{30}H_{28}F_3N_6O$ $[M + H]^+$ 545.2271, found 545.2271.

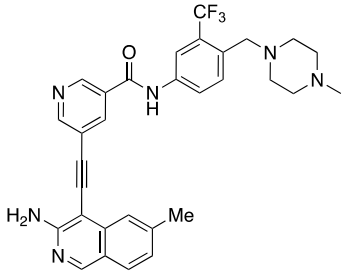
4-Iodo-6-methylisoquinolin-3-amine:



To a solution of 6-methylisoquinolin-3-amine (1 g, 6.3 mmol, 1 equiv) in methanol (40 mL) at 0 ºC was added N-Iodo succinimide (1.56 mg, 6.96 mmol, 1.1 equiv) slowly over a period of 10 min. The reaction was allowed to stir for 15 min, concentrated. The reaction mixture was purified by flash column chromatography.

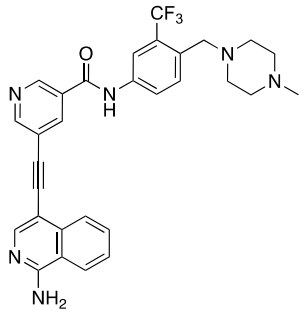
Brown solid (67%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.69 (s, 1H), 7.72 (d, J = 8.2 Hz, 1H), 7.40 (s, 1H), 7.10 (dd, J = 8.2, 1.5 Hz, 1H), 6.11 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 156.5, 152.1, 142.7, 139.8, 128.8, 127.0, 125.3, 122.4, 22.3. HRMS (ESI) m/z calcd for $C_{10}H_{10}IN_2$ $[M + H]^+$ 284.9883, found 284.9883.

5-((3-Amino-6-methylisoquinolin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN744)



Synthesized by utilizing general procedure. Yellow solid (76%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.84 (s, 1H), 9.14 (d, J = 2.0 Hz, 1H), 9.04 (d, J = 2.2 Hz, 1H), 8.82 (s, 1H), 8.70 (s, 1H), 8.23 (d, J = 2.2 Hz, 1H), 8.06 (dd, J = 8.5, 2.2 Hz, 1H), 7.86 – 7.66 (m, 3H), 7.11 (dd, J = 8.3, 1.6 Hz, 1H), 6.70 (s, 2H), 3.58 (s, 2H), 2.46 – 2.37 (m, 8H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.3, 158.6, 154.3, 153.2, 147.6, 142.4, 138.4, 138.2, 137.6, 132.8, 131.8, 130.3, 129.0, 128.0 (q, J = 28.9 Hz), 125.8 (q, J = 273.4 Hz), 125.4, 124.0, 121.7, 120.8, 120.6, 117.7, 95.7, 89.2, 88.2, 57.7, 54.9, 52.6, 45.6, 22.3. HRMS (ESI) m/z calcd for $C_{32}H_{30}F_3N_6O$ $[M + H]^+$ 559.2427, found 559.2431.

5-((1-Aminoisoquinolin-4-yl)ethynyl)-*N*-(4-((4-methylpiperazin-1-yl)methyl)-3-(trifluoromethyl)phenyl)nicotinamide (HSN789)



Synthesized by utilizing general procedure. Yellow solid (50%); ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.75 (s, 1H), 9.03 (d, J = 2.2 Hz, 1H), 8.99 (d, J = 2.0 Hz, 1H), 8.53 (t, J = 2.1 Hz, 1H), 8.29 (d, J = 8.3 Hz, 1H), 8.20 (s, 2H), 8.15 (d, J = 7.8 Hz, 1H), 8.04 (dd, J = 8.5, 2.2 Hz, 1H), 7.80 (t, J = 7.3 Hz, 1H), 7.73 (d, J = 8.5 Hz, 1H), 7.61 – 7.55 (m, 1H), 7.47 (d, J = 16.5 Hz, 3H), 3.57 (s, 2H), 2.38 (s, 8H), 2.17 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6) δ 164.0, 158.4, 154.0, 148.2, 147.8, 138.3, 137.2, 136.3, 134.8, 132.9, 131.8, 131.6, 130.3, 128.8, 126.8, 125.8 (q, J = 274.6 Hz), 124.9, 124.0, 120.4, 117.6, 116.6, 102.3, 91.5, 89.8, 57.8, 55.1, 53.0, 46.0. HRMS (ESI) m/z calcd for $C_{30}H_{28}F_3N_6O$ $[M + H]^+$ 545.2271, found 545.2268.

Synthesis and characterization of compounds HSN608, 632, 648, 649, 670, 650 and 690 reported in reference 2.

Synthesis and characterization of compounds HSN431, 576 and 580 reported in references 3 and 4.

References:

1. Kemp, Mark Ian et al. Preparation of heterocyclic carbonitrile, carboxamide, and urea derivatives for pharmaceutical use. PCT Int. Appl., 2017103614, 22 Jun **2017**.
2. Wang, M., Naganna, N. and Sintim, H.O. Identification of nicotinamide aminonaphthyridine compounds as potent RET kinase inhibitors and antitumor activities against RET rearranged lung adenocarcinoma. *Bioorganic chemistry*, **2019**, *90*, 103052.
3. Larocque, E.A., Naganna, N., Opoku-Temeng, C., Lambrecht, A.M. and Sintim, H.O. Alkynylnicotinamide-Based Compounds as ABL1 Inhibitors with Potent Activities against Drug-Resistant CML Harboring ABL1 (T315I) Mutant Kinase. *ChemMedChem*, **2018**, *13*, 1172-1180.
4. Naganna, N., Opoku-Temeng, C., Choi, E.Y., Larocque, E., Chang, E.T., Carter-Cooper, B.A., Wang, M., Torregrosa-Allen, S.E., Elzey, B.D., Lapidus, R.G. and

Sintim, H.O. Amino alkynylisoquinoline and alkynylnaphthyridine compounds potently inhibit acute myeloid leukemia proliferation in mice. *EBioMedicine*, **2019**, *40*, 231-239.

Table S1. HPLC Purity

Compound[*method*]	**Retention Time**	**% Purity area**
HSL507[d]	19.8	95
HSND_15[a]	18.8	99
HSND_17[a]	19.5	95
HSND16[d]	16.8	95
HSL476[d]	15.2	95
HSN721[a]	14.9	95
HSN722[a]	18.1	97
HSN804[a]	15.9	95
HSN631[a]	14.9	96
HSN650[a]	17.5	95
HSN742[d]	14.6	95
HSN757[a]	14.8	95
HSN805[a]	17.7	95
HSN806[d]	18.7	95
HSN815[a]	14.9	95
HSN816[d]	14.8	95
HSL211[d]	14.0	95
HSL468[c]	13.0	95
HSL212[a]	16.7	96
HSN431[a]	19.9	99
HSN576[a]	19.9	98
HSN580[a]	21.4	100
HSN692[a]	19.3	98
HSN700[a]	17.5	97
HSN744[a]	19.4	98
HSN789[a]	16.6	95

[a]UV detection wavelength 280 nm, Agilent Eclipse instrument; C18 column 5C_{18}-MS-II COSMOSIL (4.6ID × 250 mm); method: 0 → 5 min 50% B, 5 → 8 min 50 to 90% B, 8 →30 min 90% B (A: 0.1% NH_4OH in H_2O, B: MeOH), 25 °C.

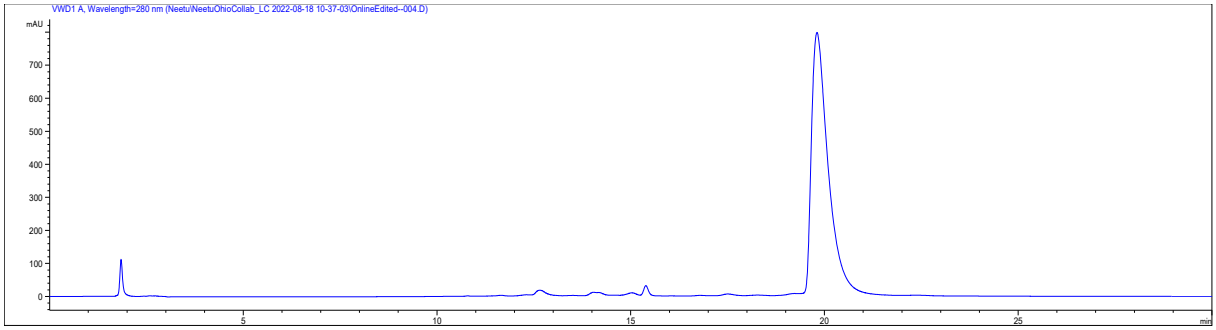
[b]UV detection wavelength 250 nm, Agilent Eclipse instrument; C18 column 5C_{18}-MS-II COSMOSIL (4.6ID × 250 mm); method: 0 → 10 min 50% B, 10 → 12 min 50 to 90% B, 12 →19 min 90% B, 19 → 20 min 90 to 50% B (A: 0.1% NH_4OH in H_2O, B: MeOH), 25 °C.

[c]UV detection wavelength 250 nm, Agilent Eclipse instrument; C18 column 5C_{18}-MS-II COSMOSIL (4.6ID × 250 mm); method: 0 → 10 min 50% B, 10 → 12 min 50 to 90% B, 12 →19 min 90% B, 19 → 20 min 90 to 50% B (A: 0.1% NH_4OH in H_2O, B: MeOH), 25 °C.

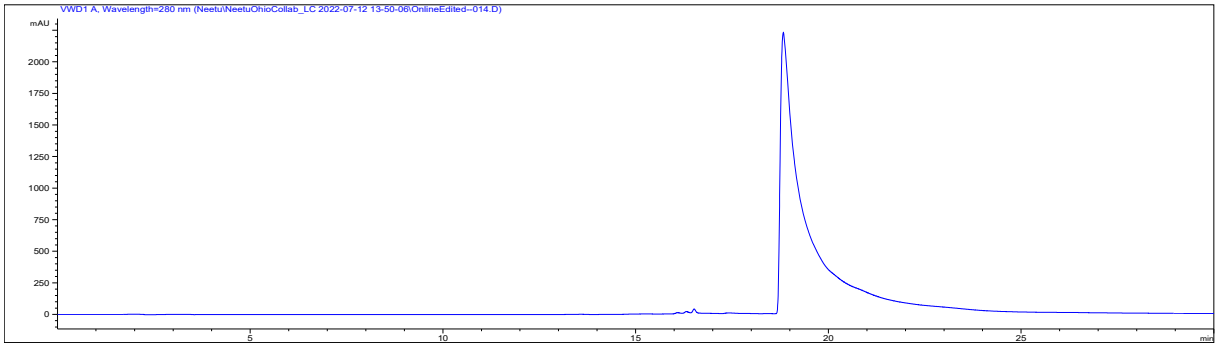
[d]UV detection wavelength 280 nm, Agilent Eclipse instrument; C18 column 5C_{18}-MS-II COSMOSIL (4.6ID × 250 mm); method: 0 → 5 min 50% B, 5 → 8 min 50 to 100% B, 8 →30 min 100% B (A: 0.1% NH_4OH in H_2O, B: MeOH), 25 °C.

HPLC Chromatogram

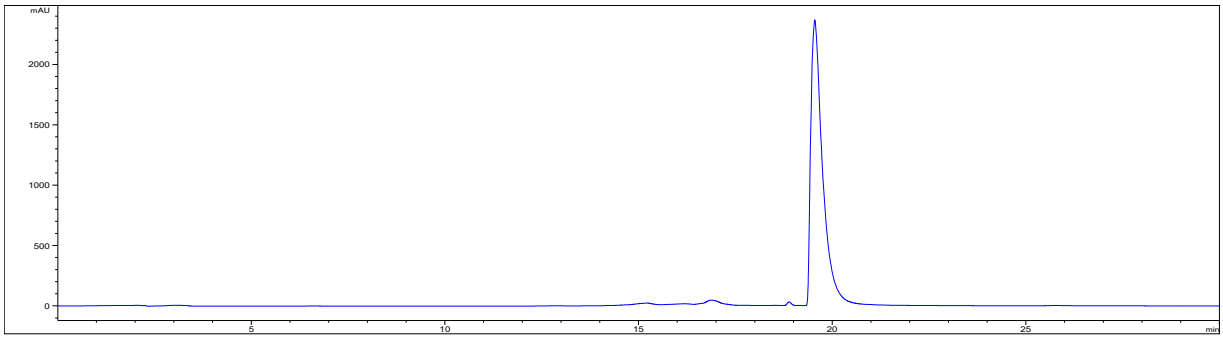
Compound HSL507



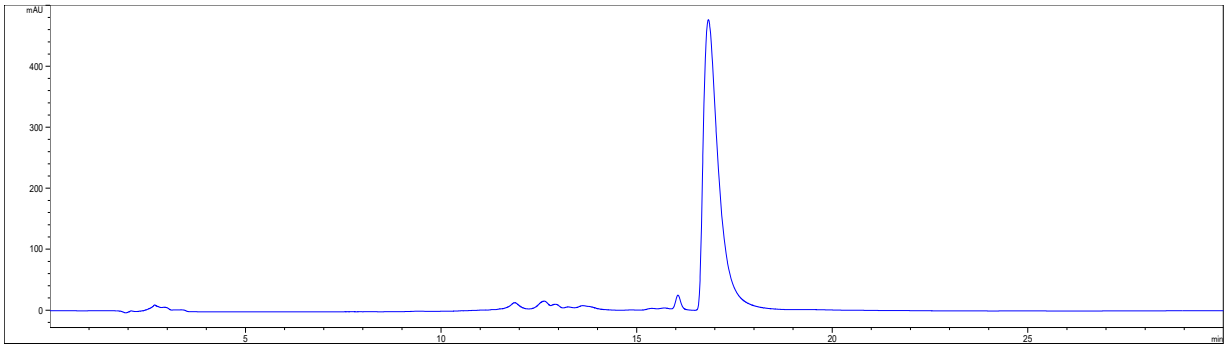
Compound HSND15



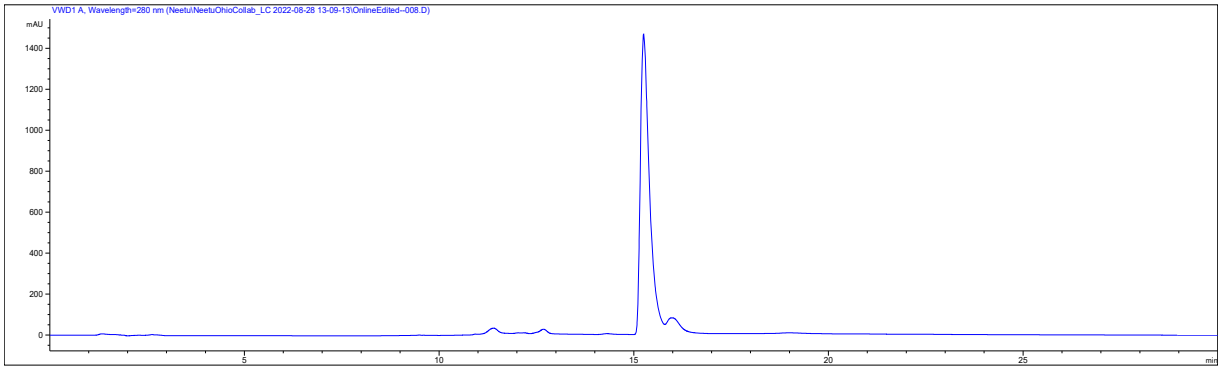
Compound HSND17



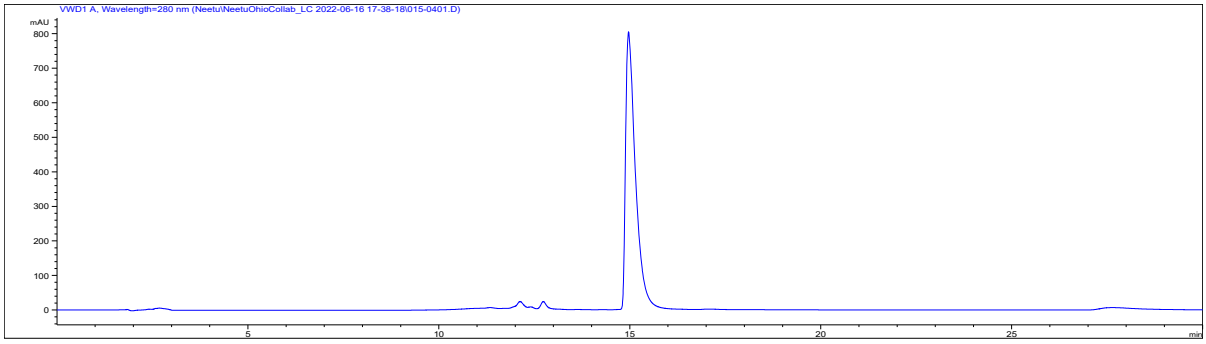
Compound HSND16



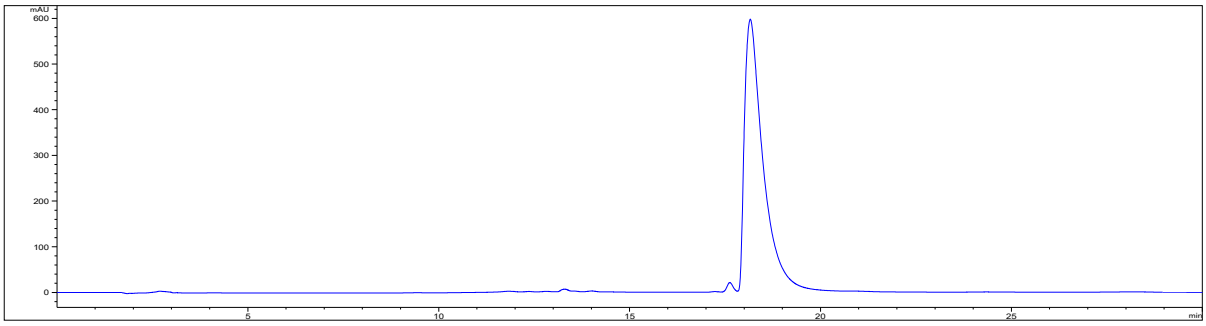
Compound HSL476



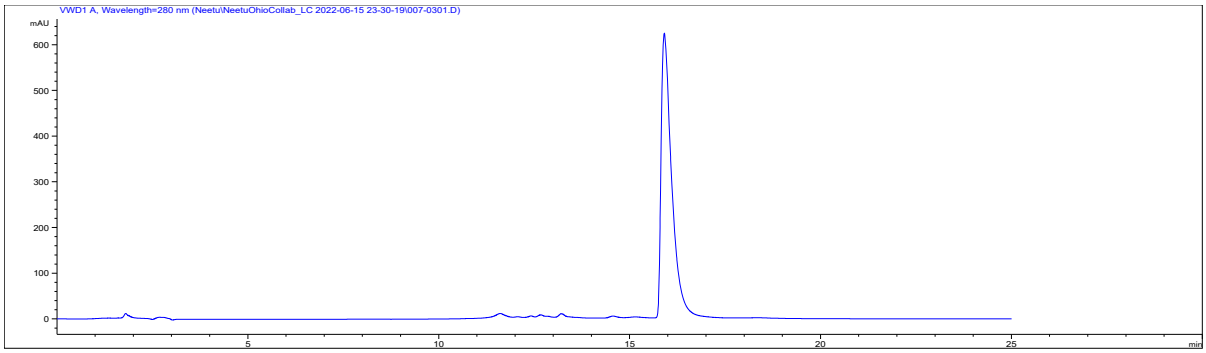
Compound HSN721



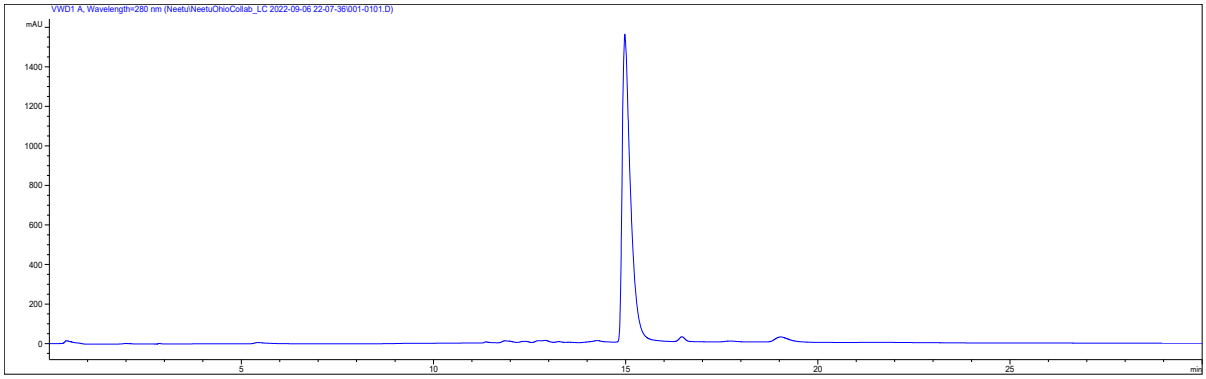
Compound HSN722



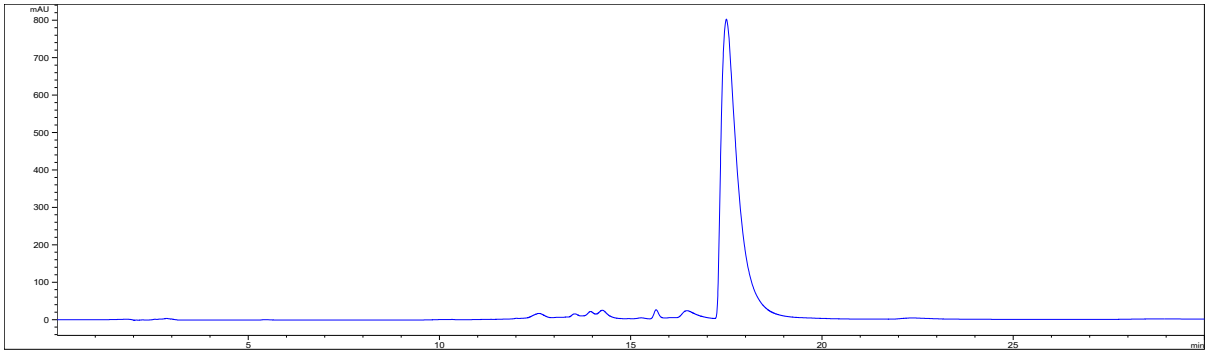
Compound HSN804



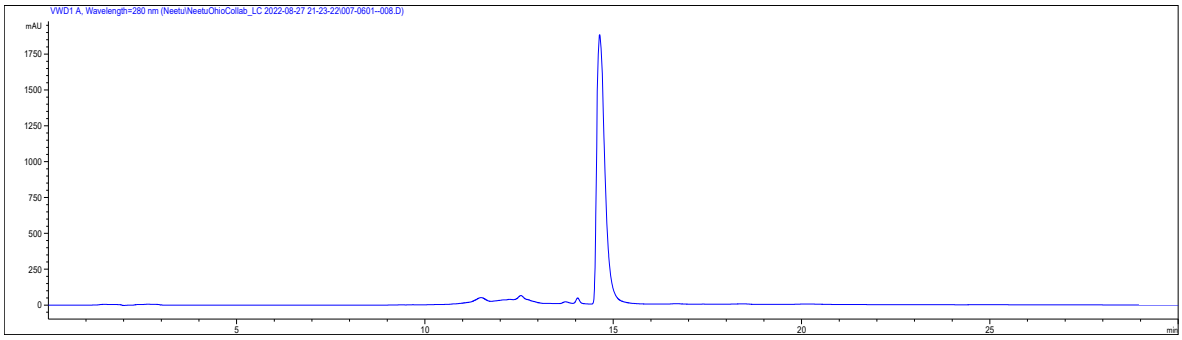
Compound HSN631



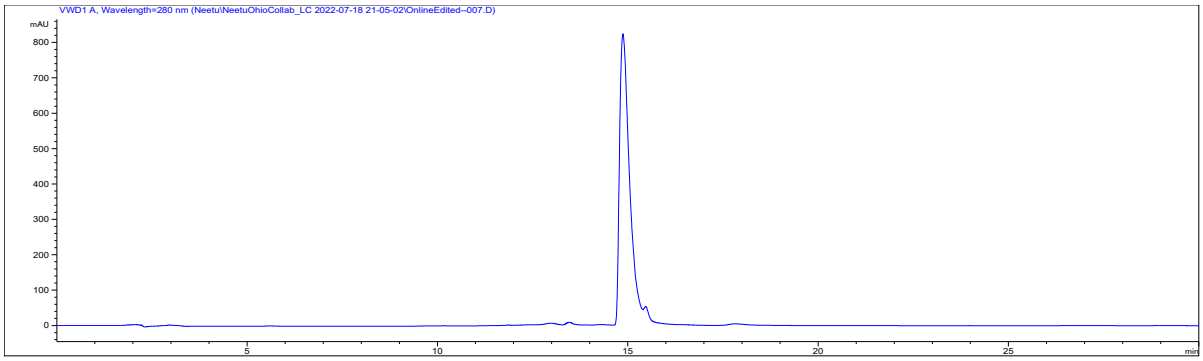
Compound HSN650



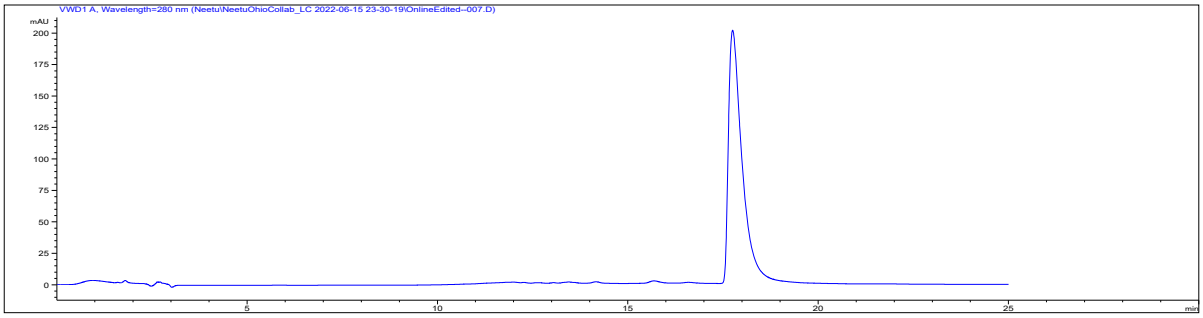
Compound HSN742



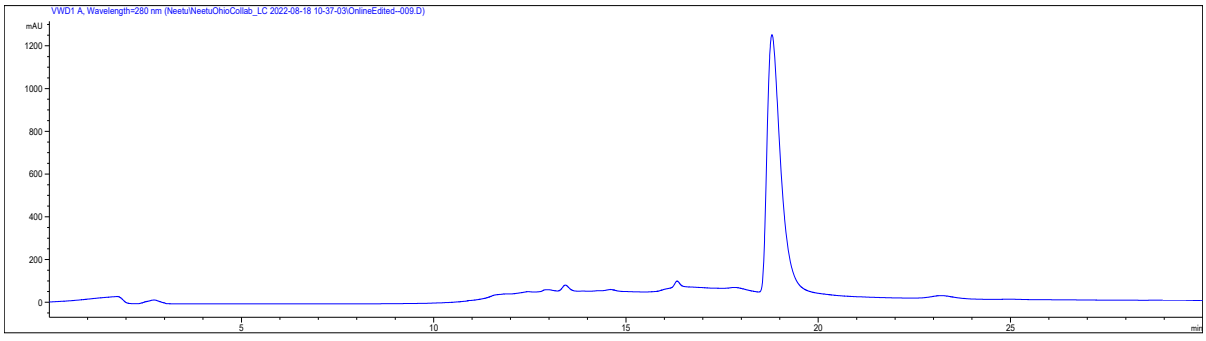
Compound HSN757



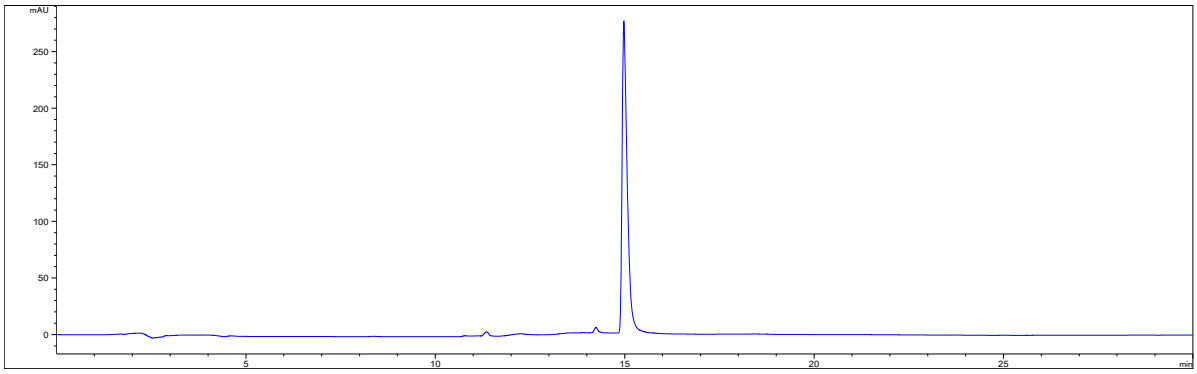
Compound HSN805



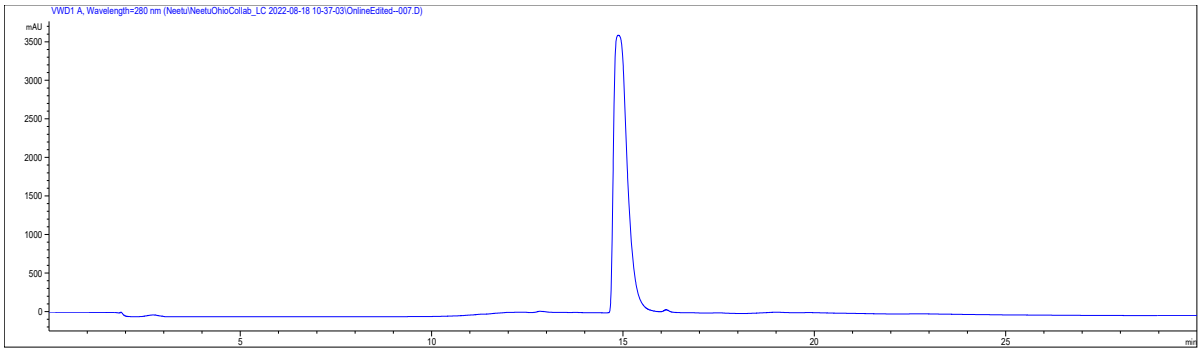
Compound HSN806



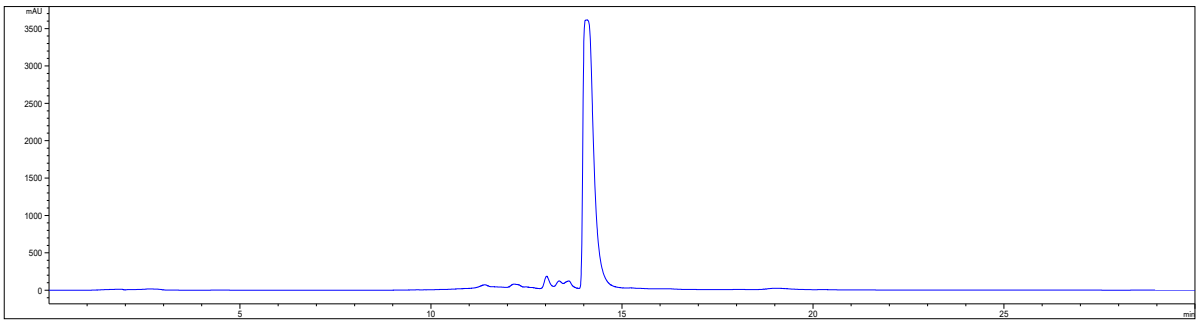
Compound HSN815



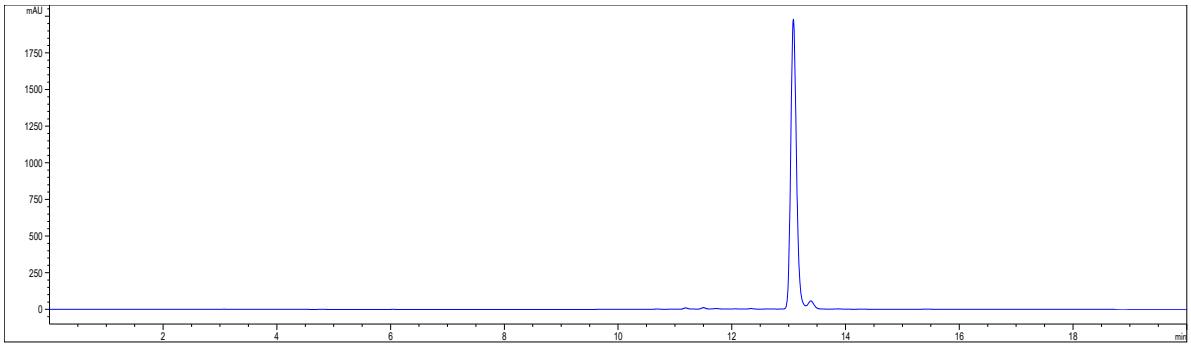
Compound HSN816



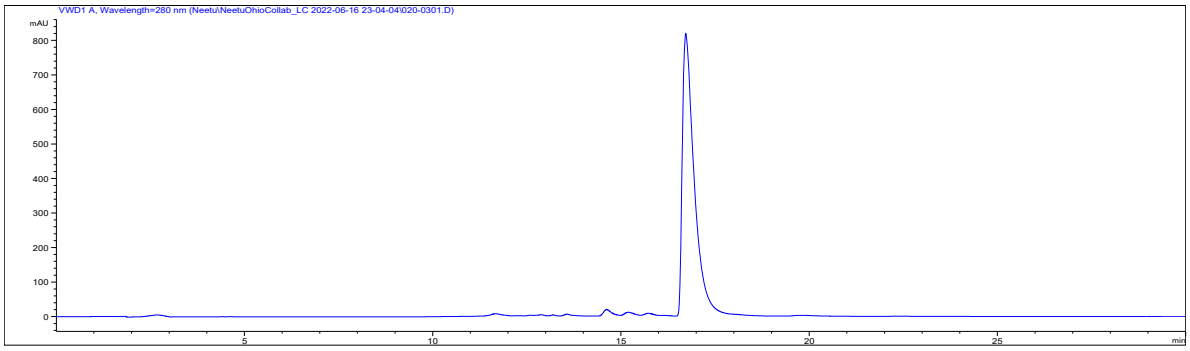
Compound HSL211



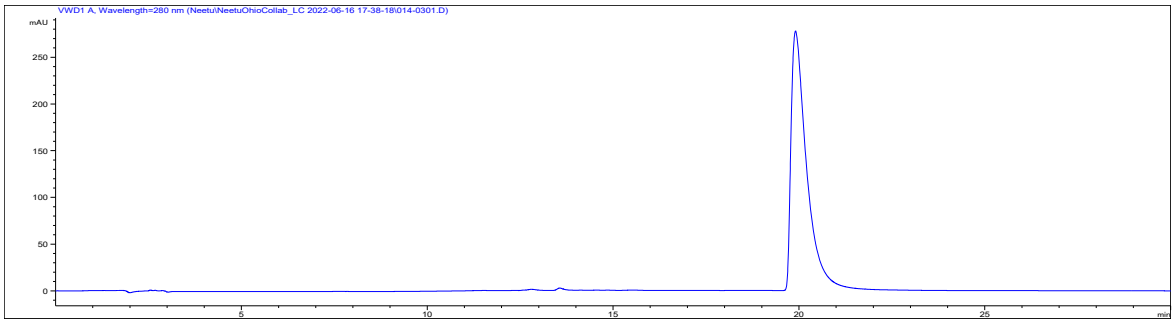
Compound HSL468



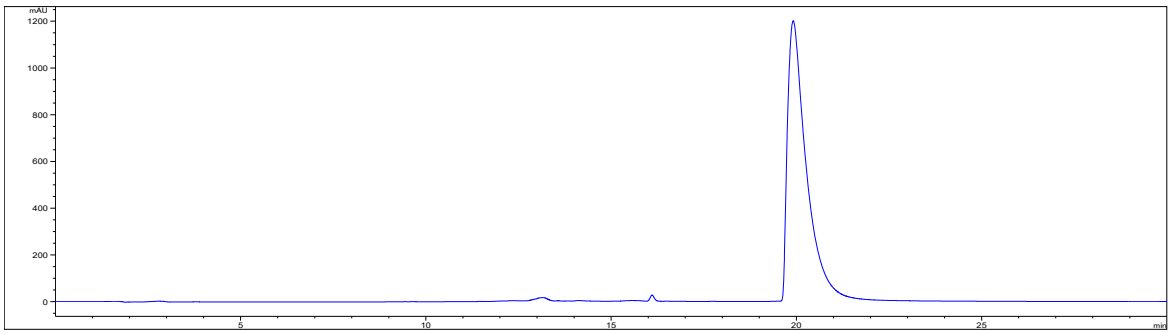
Compound HSL212



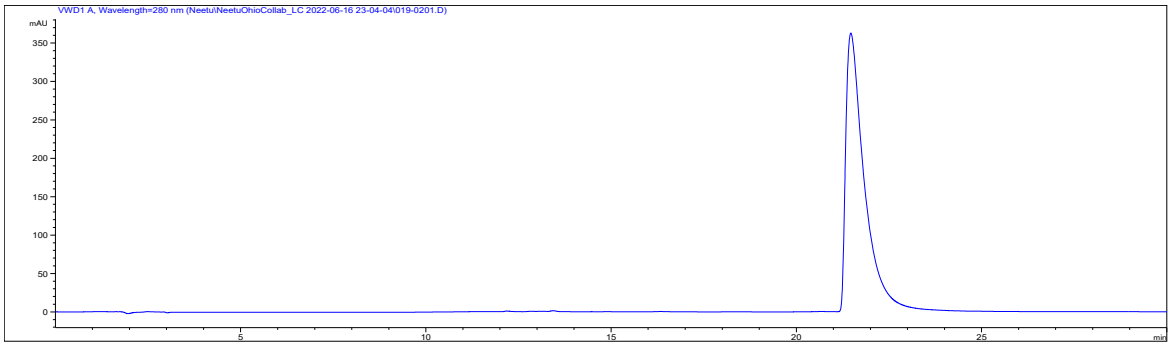
Compound HSN431



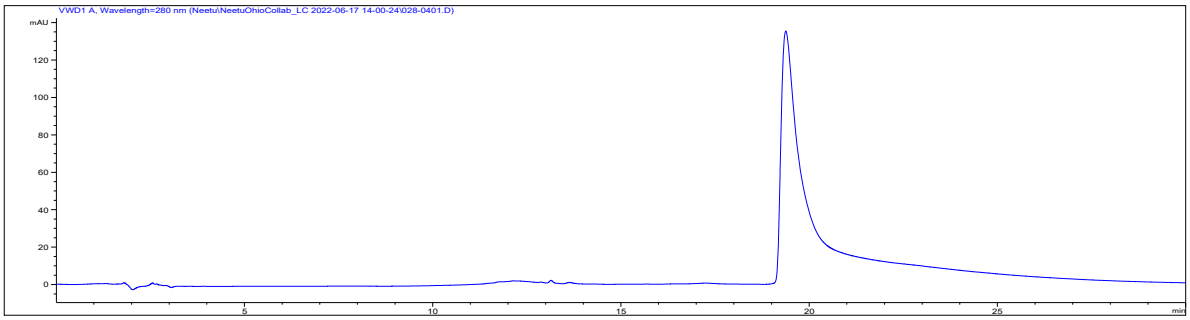
Compound HSN576



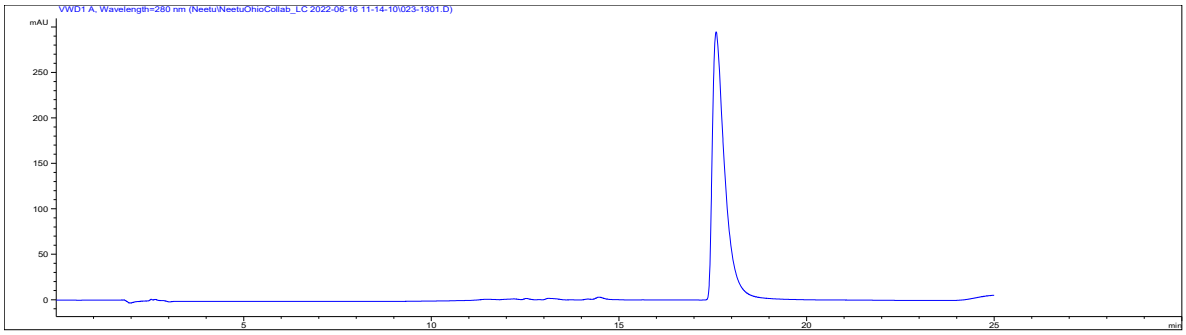
Compound HSN580



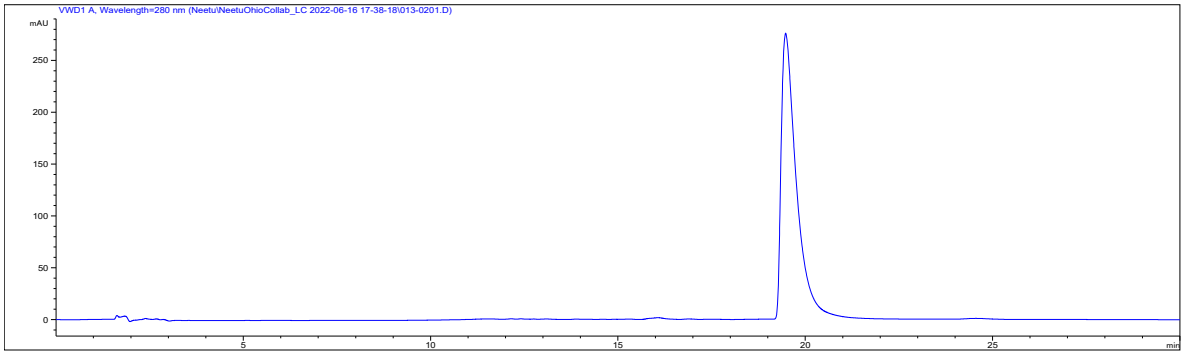
Compound HSN692



Compound HSN700



Compound HSN744



Compound HSN789

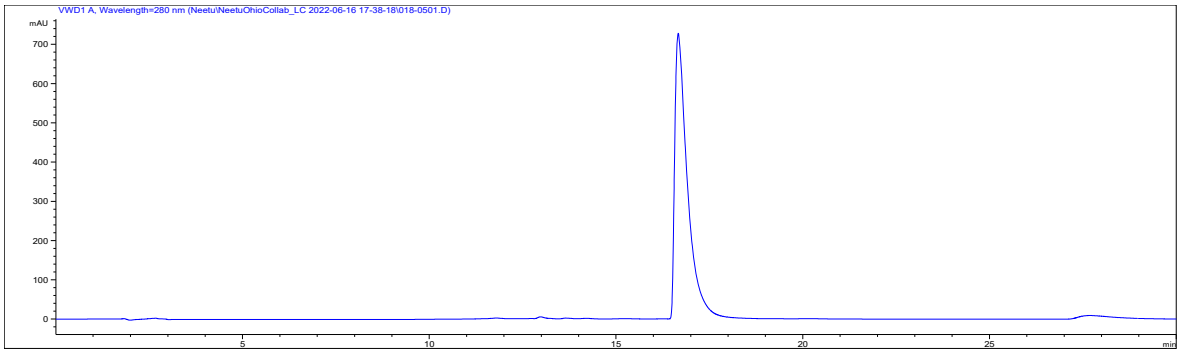
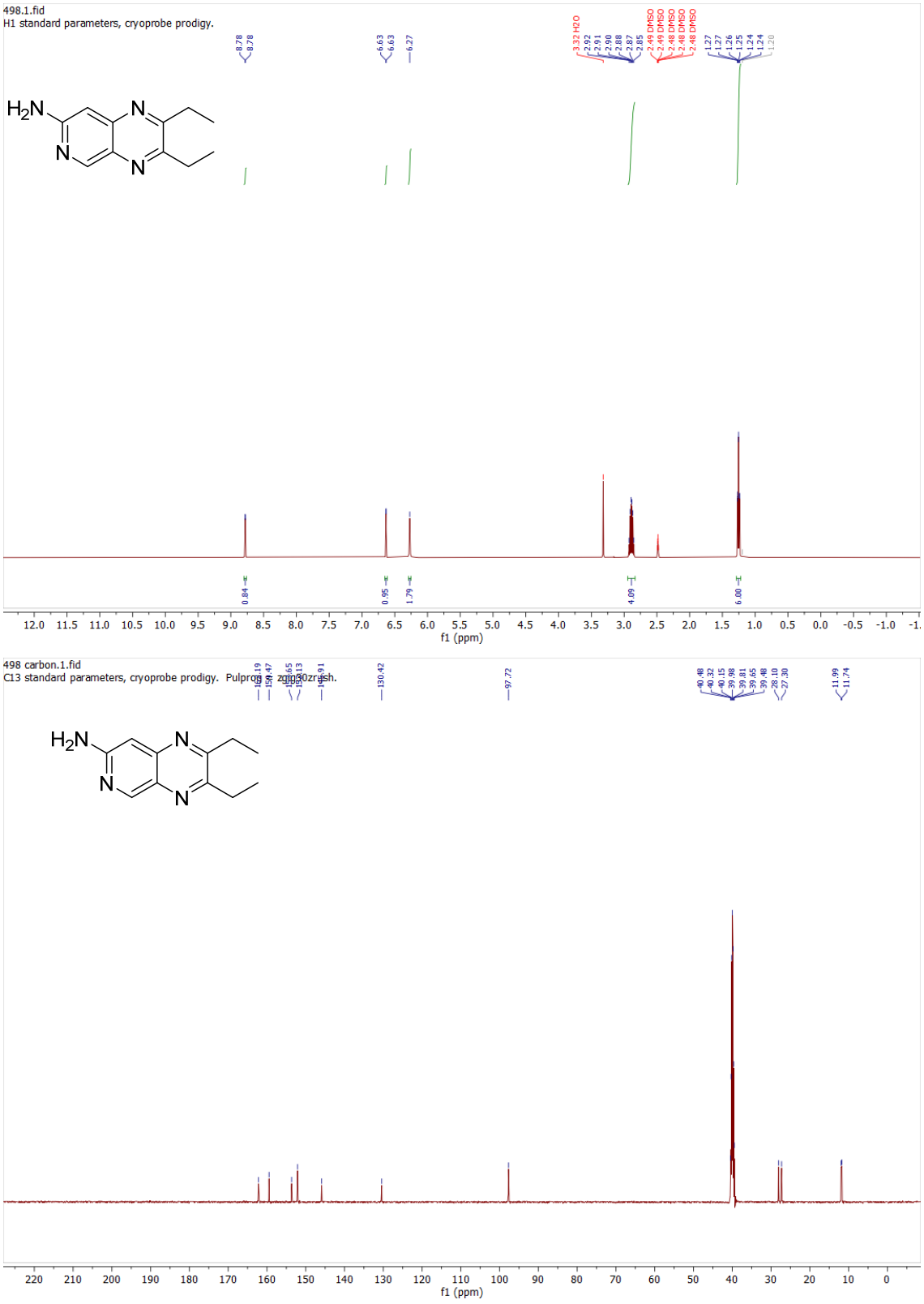
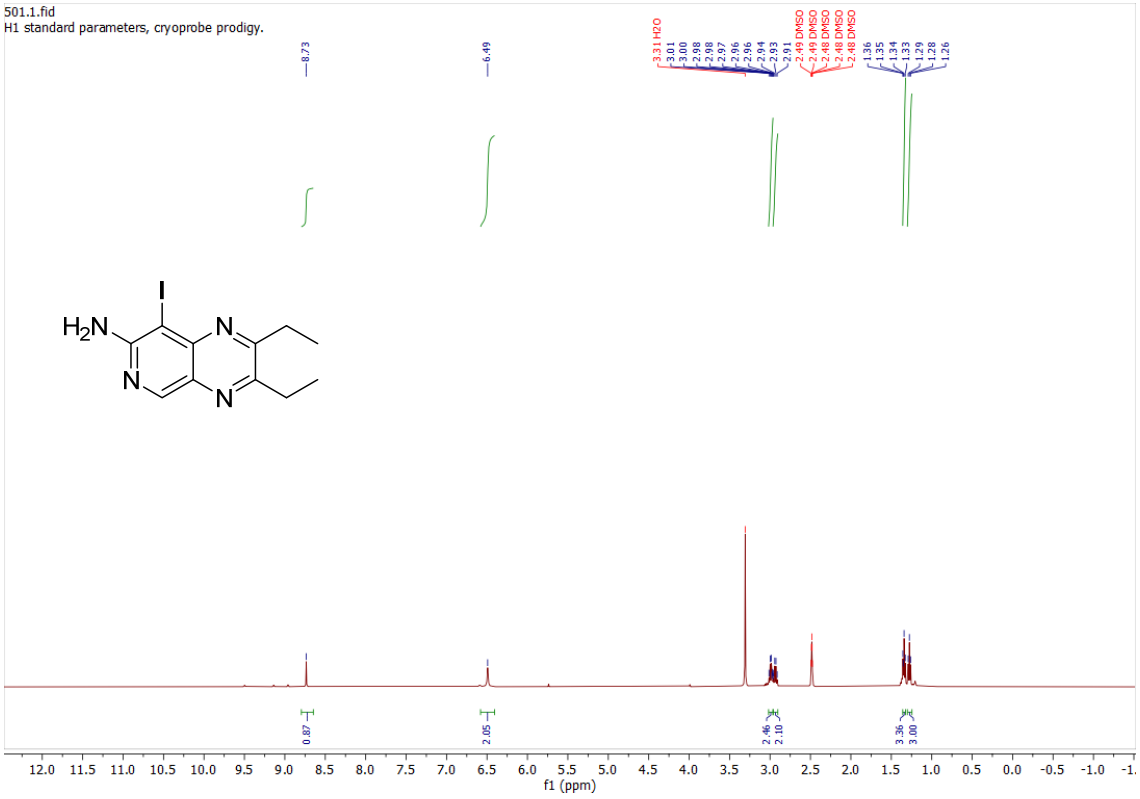
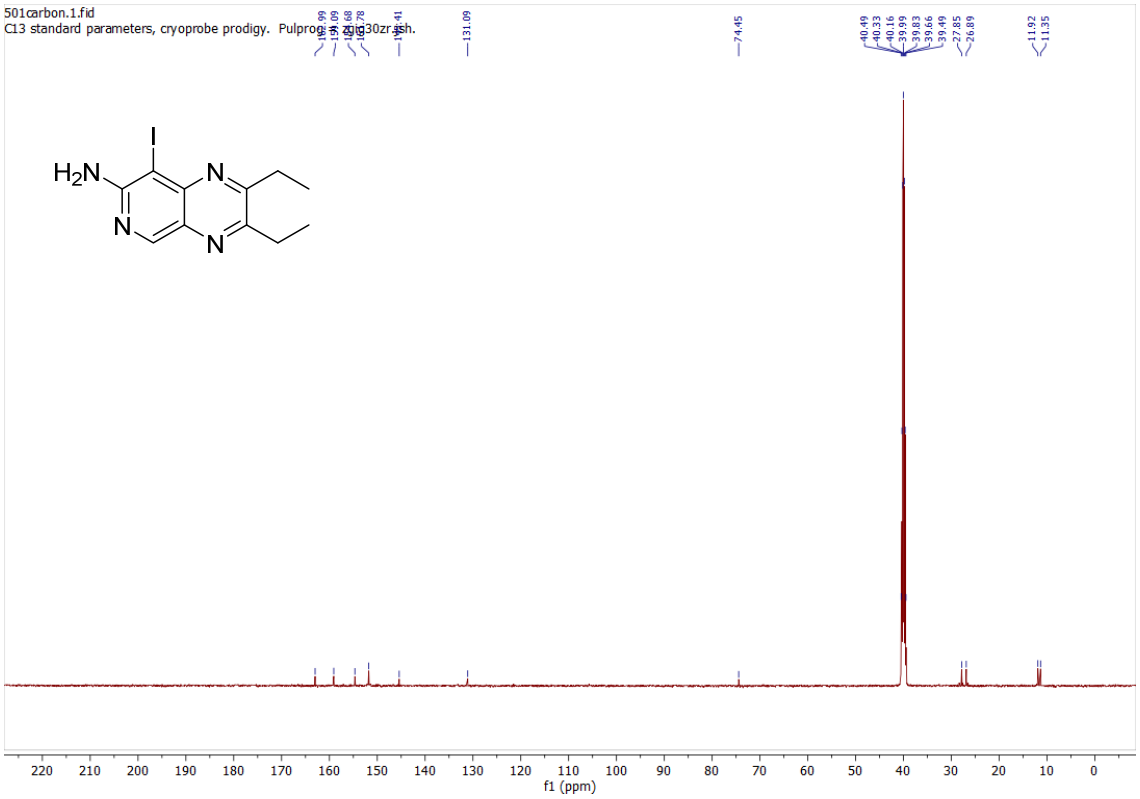
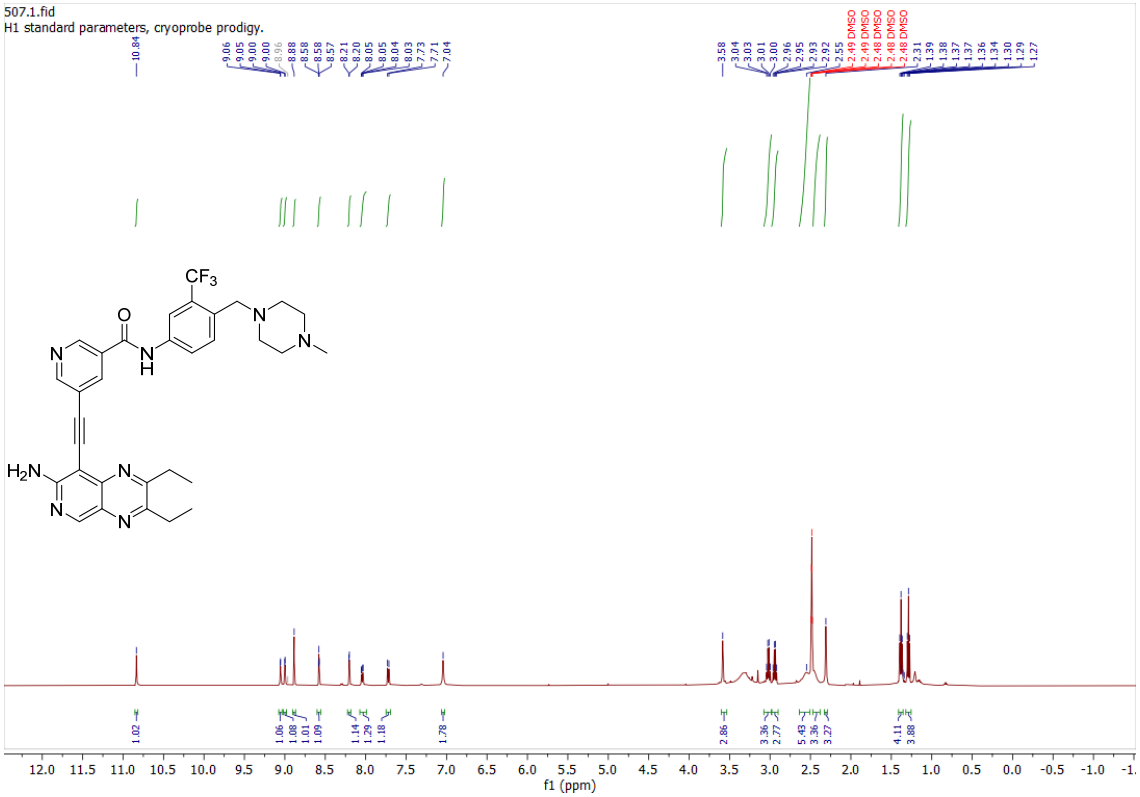


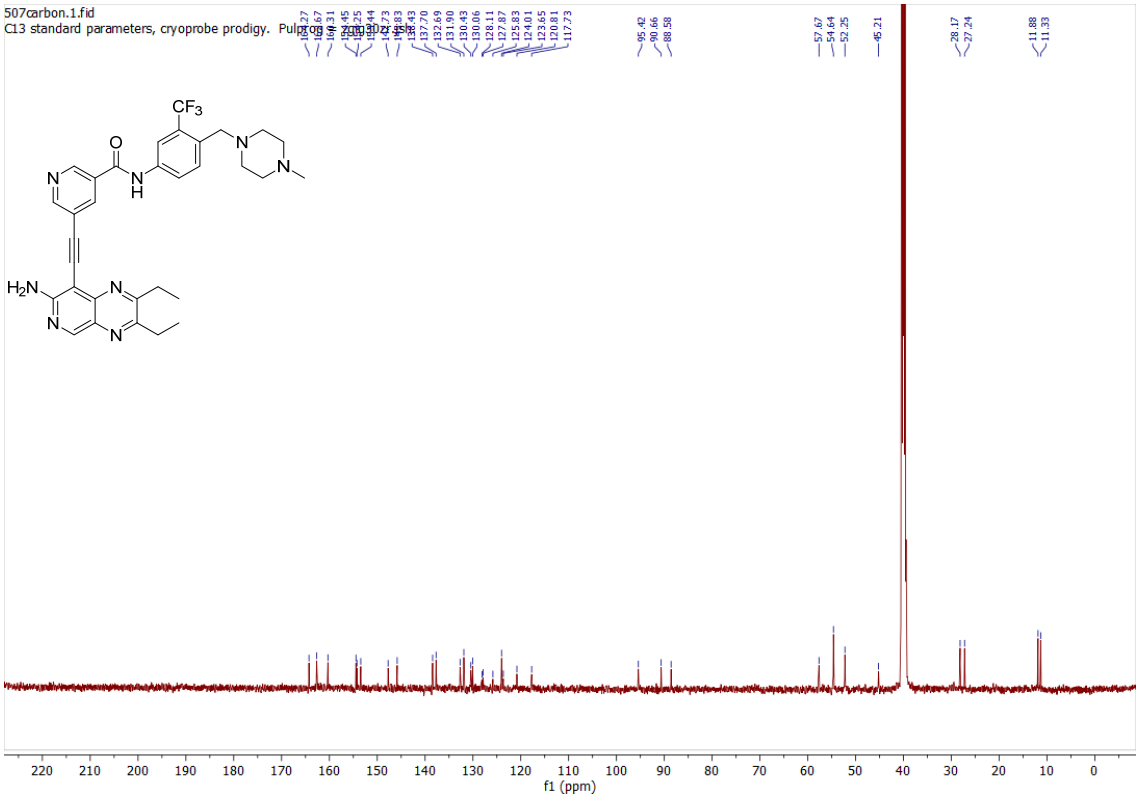
Fig. S1. ^{1}H and ^{13}C Spectras

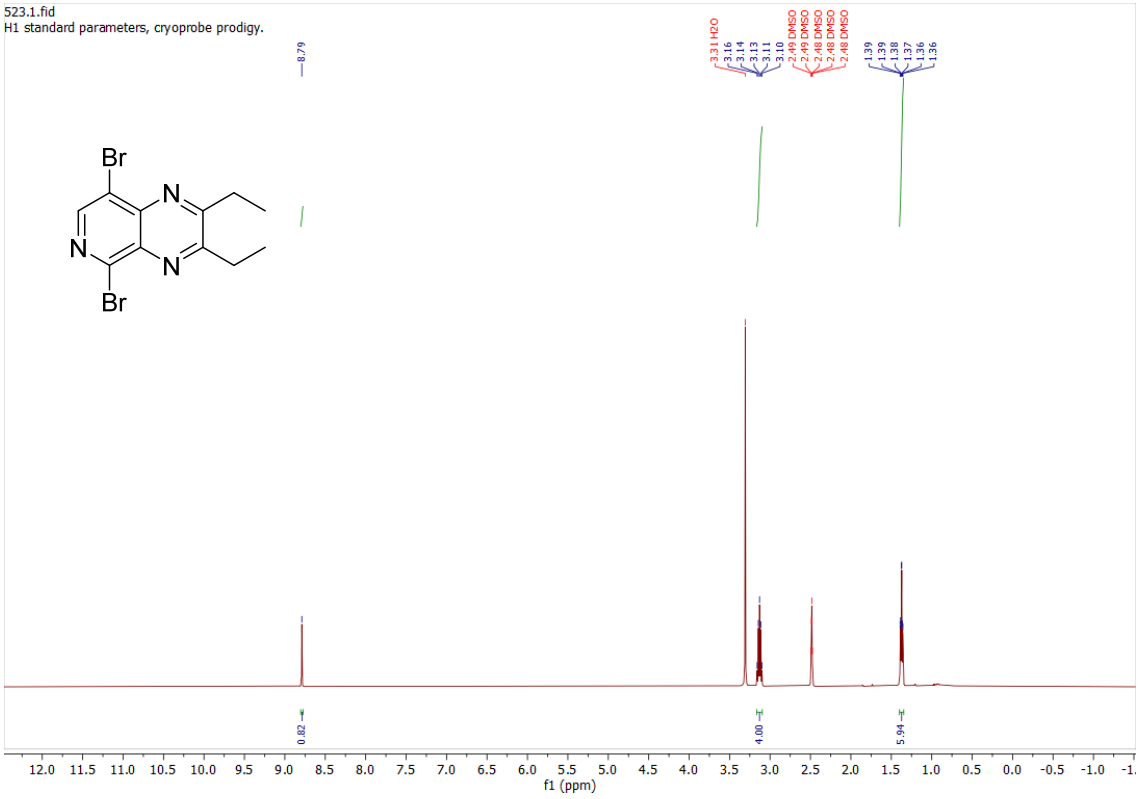


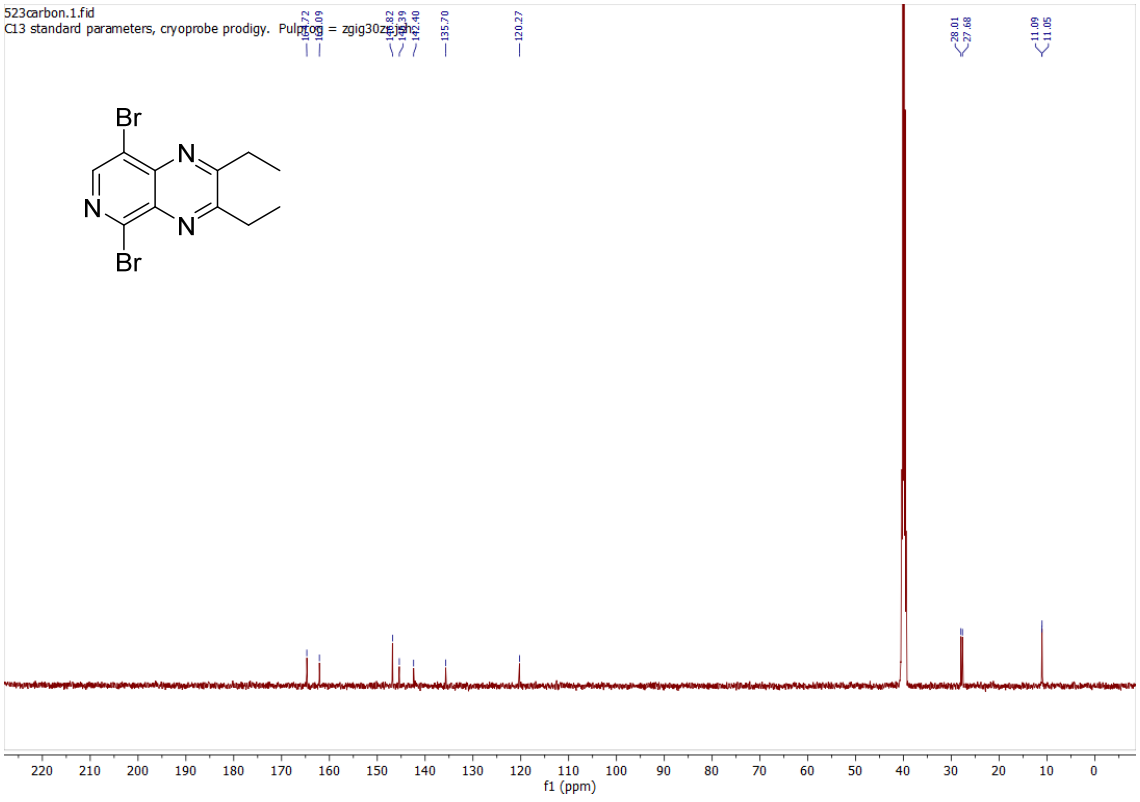

501.1.fid
H1 standard parameters, cryoprobe prodigy.
8.73
6.49
3.31 H2O
3.01
3.00
2.98
2.98
2.97
2.96
2.96
2.94
2.93
2.91
2.49 DMSO
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
1.36
1.35
1.34
1.33
1.29
1.28
1.26
I
H2N
N
N
N
0.87
2.05
2.46
2.10
3.36
3.00
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

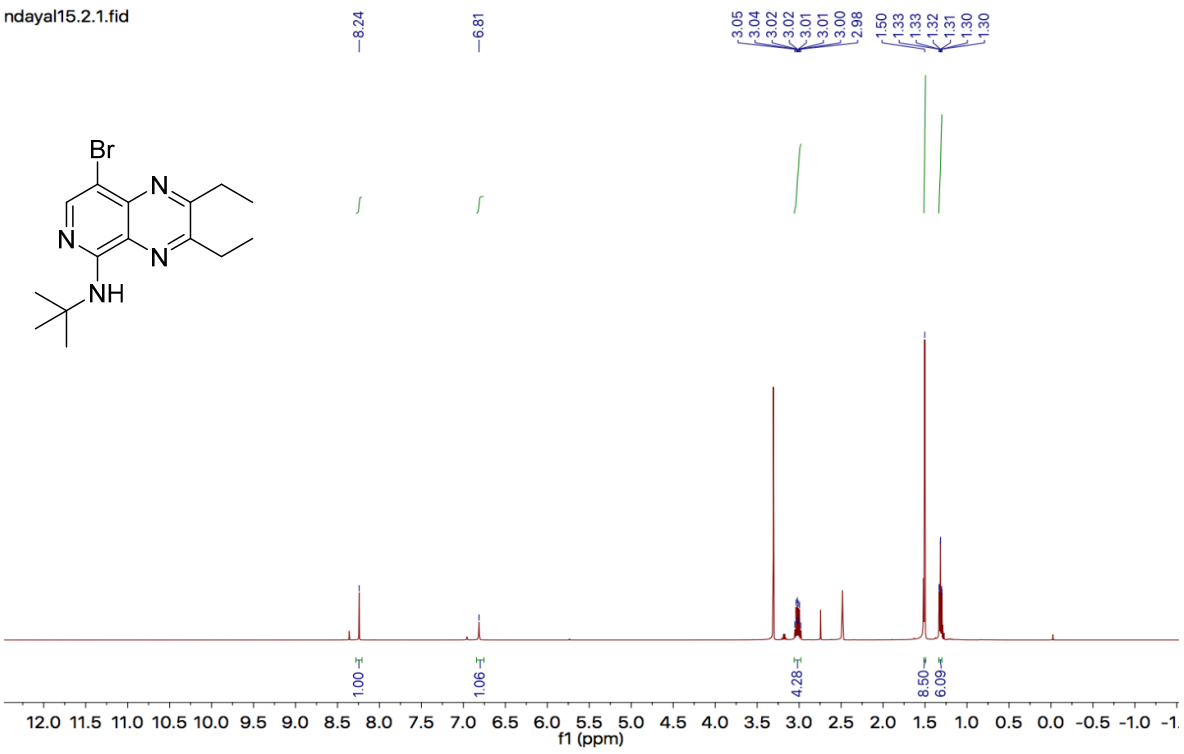

501carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog: zgdg30zn_sh.
162.99
159.09
142.68
140.78
131.41
131.09
74.45
40.49
40.33
40.16
39.99
39.83
39.66
39.49
27.85
26.89
11.92
11.35
I
H2N
N
N
N
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

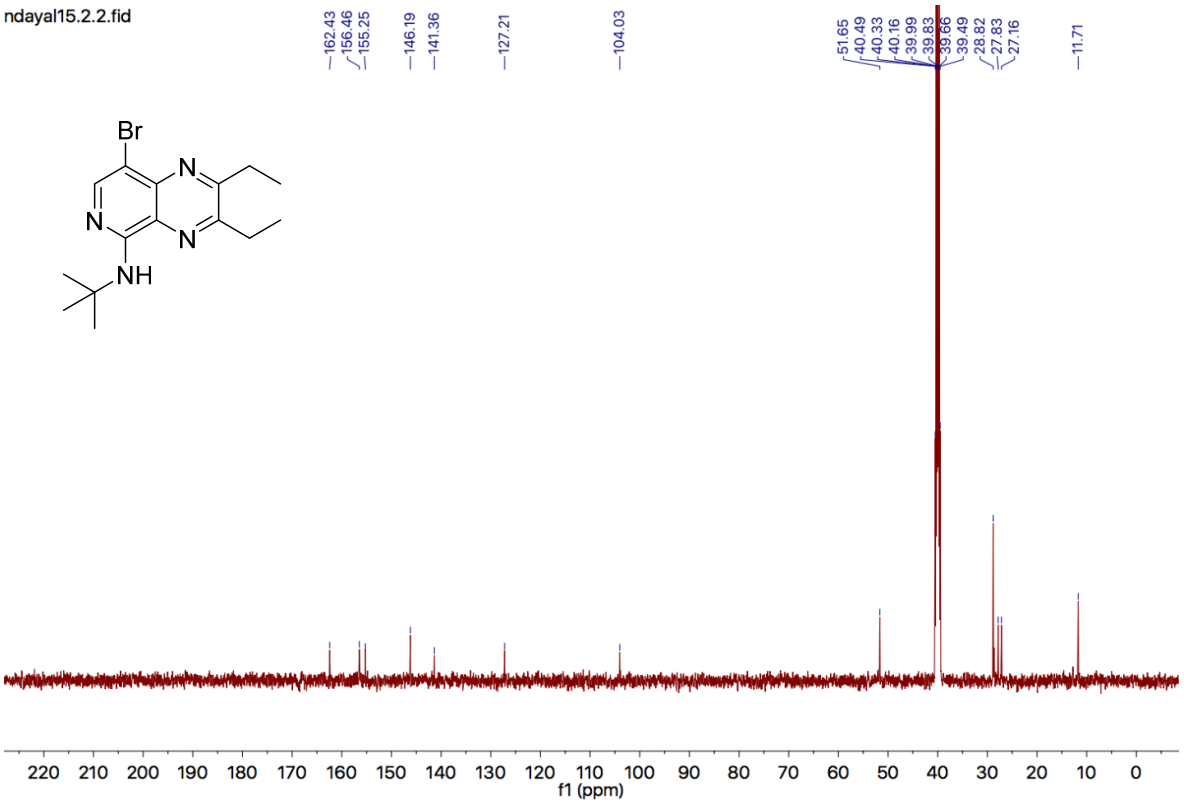

507.1.fid
H1 standard parameters, cryoprobe prodigy.
f1 (ppm)

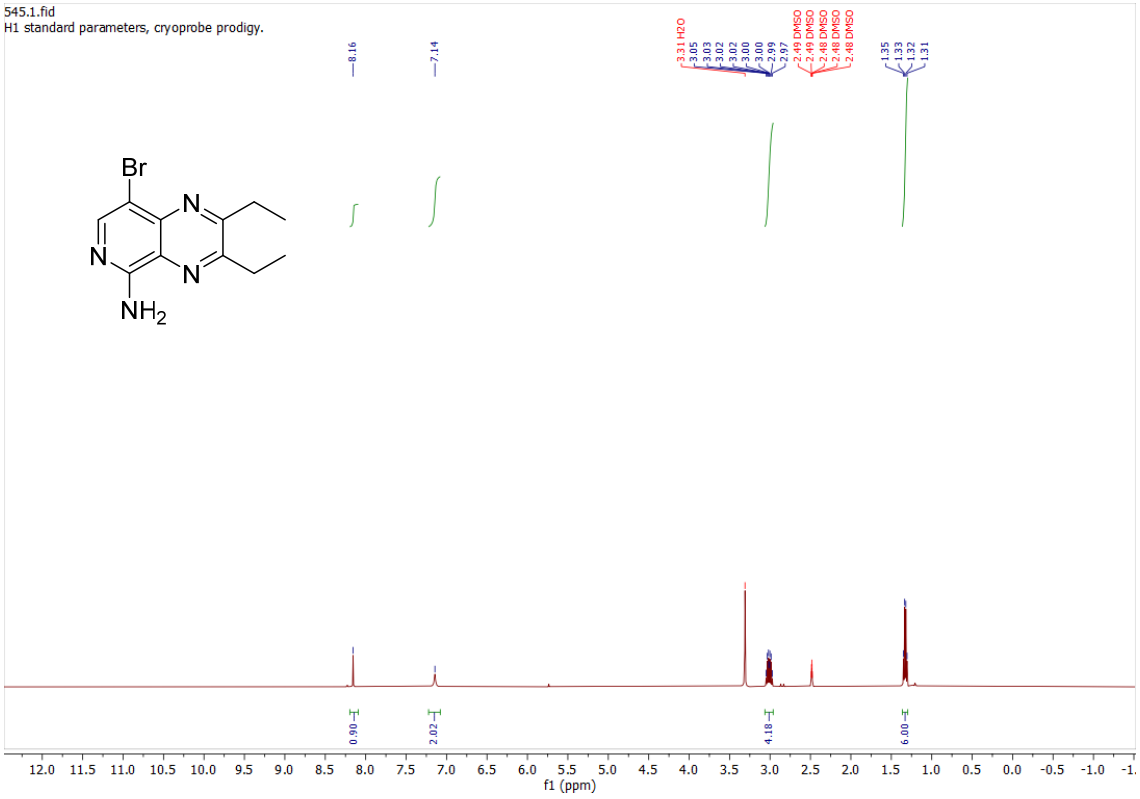

507carbon.1.fid
C13 standard parameters, cryoprobe prodigy.
f1 (ppm)

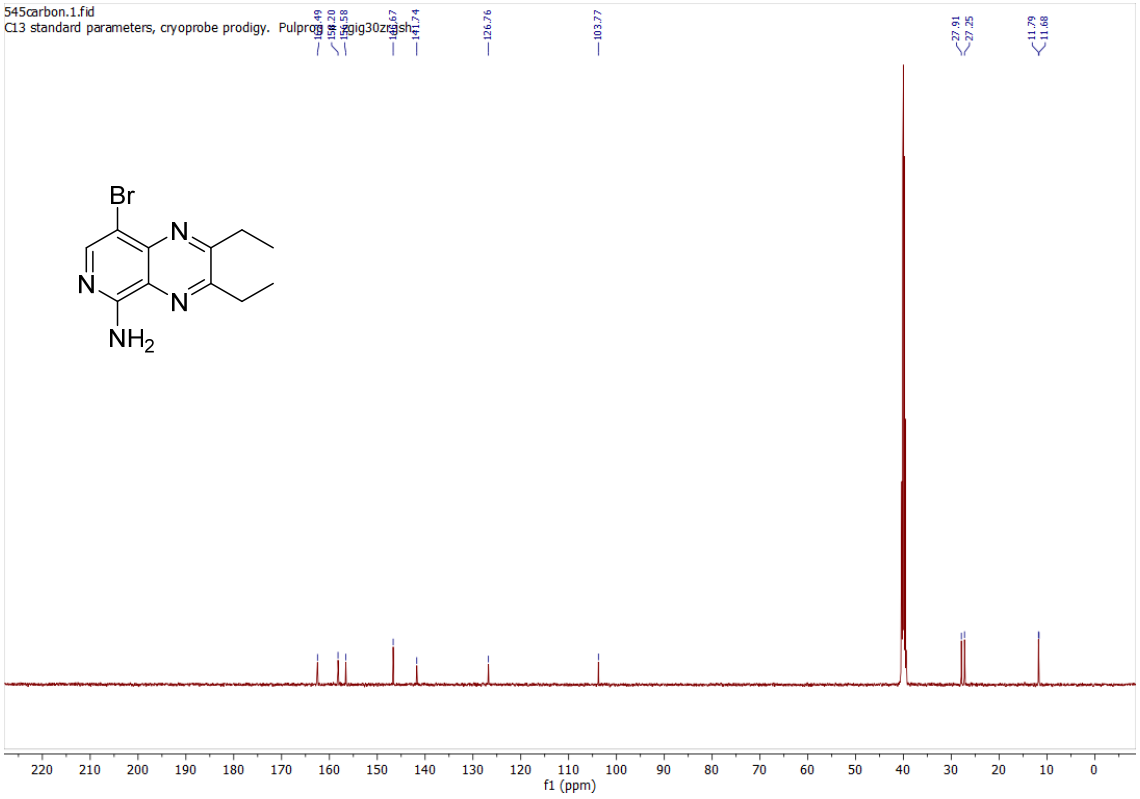

523.1.fid
H1 standard parameters, cryoprobe prodigy.
Br
N
N
N
Br
8.79
3.31 H2O
3.16
3.14
3.13
3.11
3.10
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
1.39
1.39
1.38
1.37
1.36
1.36
0.82
4.00
5.94
f1 (ppm)

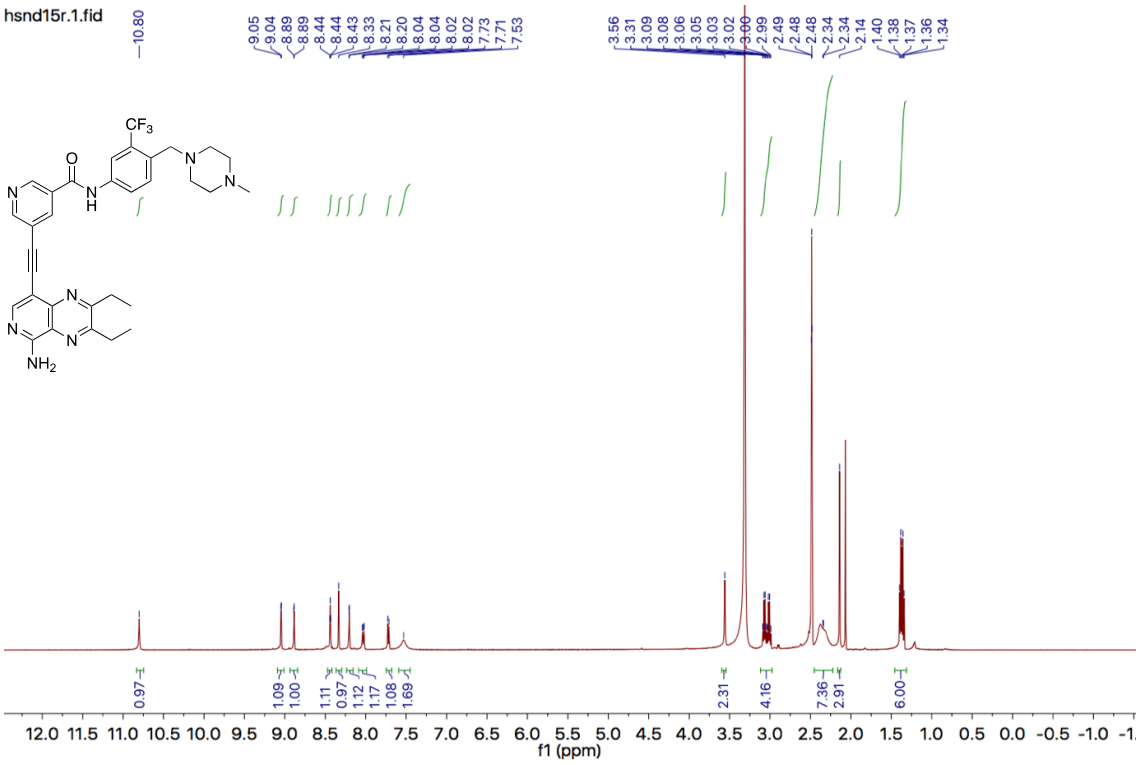

523carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog = zgig30zr.ish
Br
N
N
N
Br
162.72
162.09
143.82
143.39
142.40
135.70
120.27
28.01
27.68
11.09
11.05
f1 (ppm)

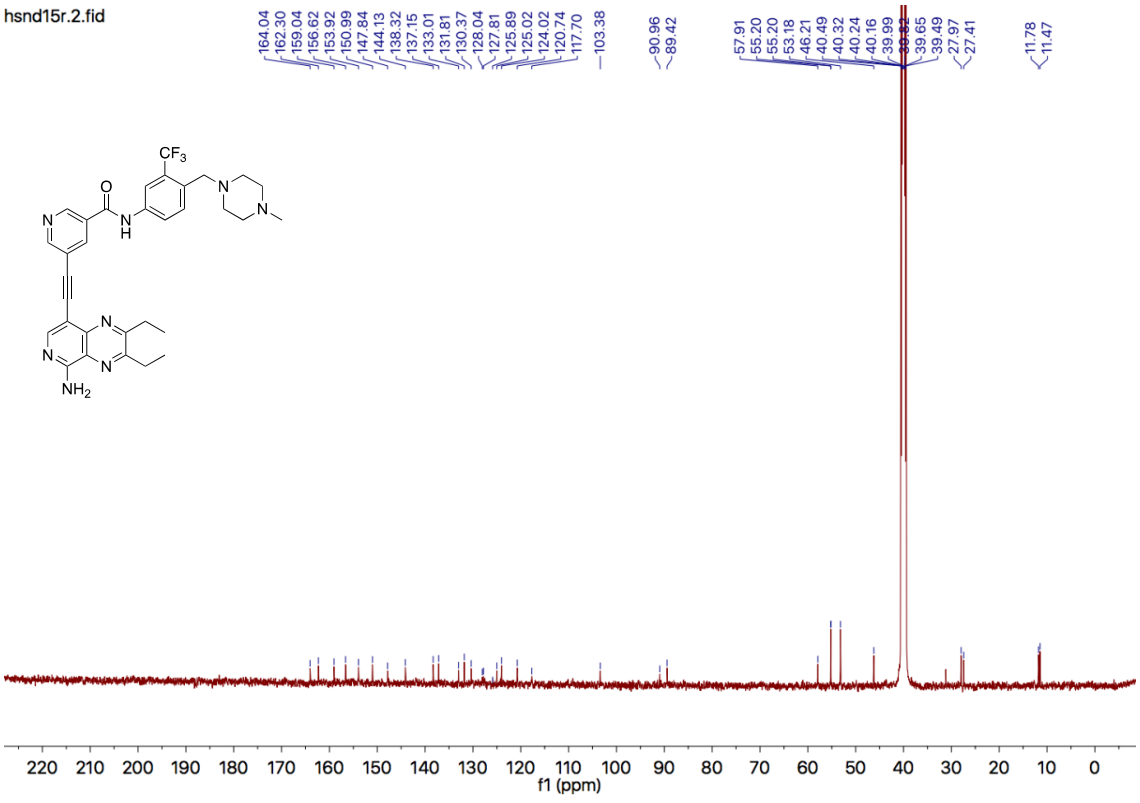

ndayal15.2.1.fid
8.24
6.81
3.05
3.04
3.02
3.02
3.01
3.01
3.00
2.98
1.50
1.33
1.33
1.32
1.31
1.30
1.30
Br
N
N
N
NH
1.00
1.06
4.28
8.50
6.09
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

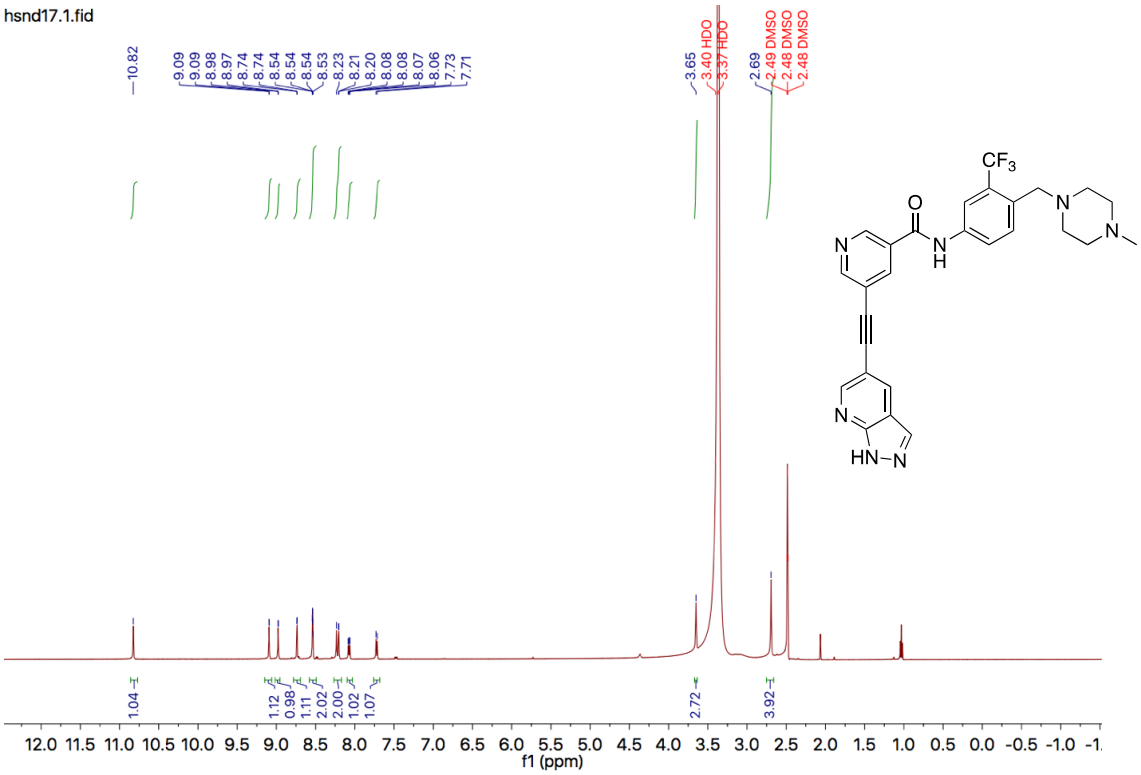

ndayal15.2.2.fid
162.43
156.46
155.25
146.19
141.36
127.21
104.03
51.65
40.49
40.33
40.16
39.99
39.83
39.66
39.49
28.82
27.83
27.16
11.71
Br
N
N
N
NH
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

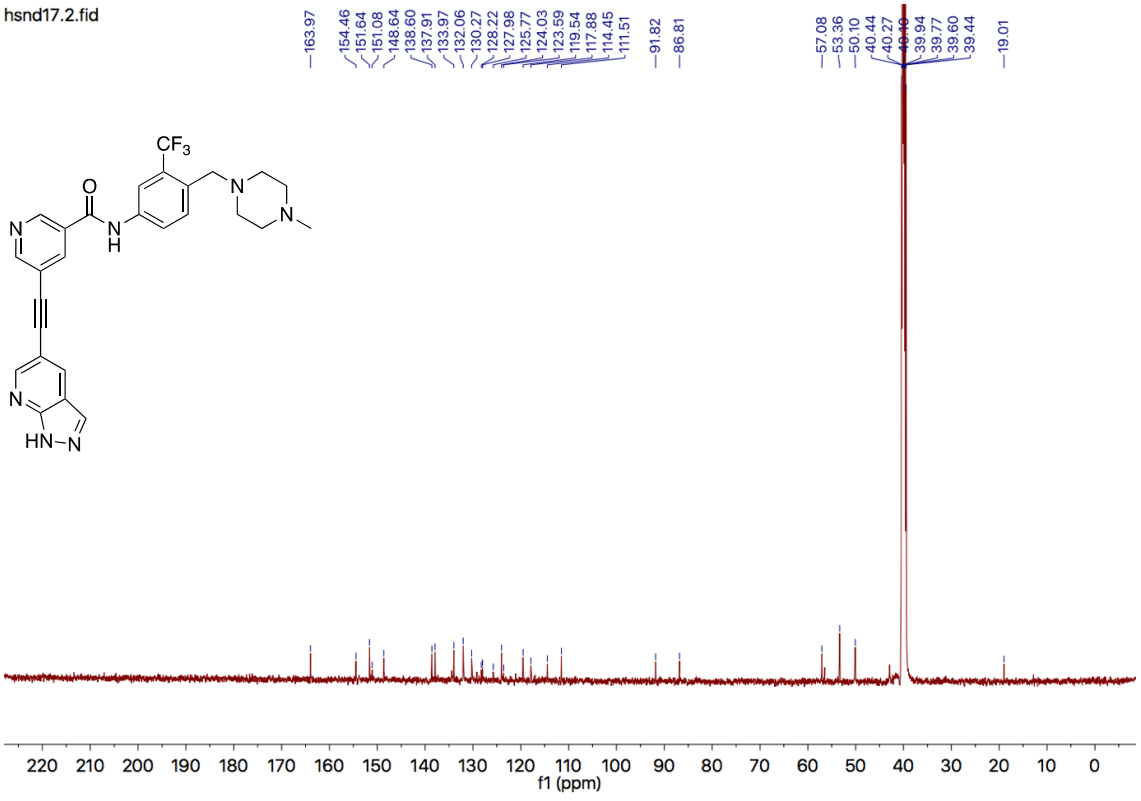

545.1.fid
H1 standard parameters, cryoprobe prodigy.
Br
N
N
N
NH2
8.16
7.14
3.31 H2O
3.05
3.03
3.02
3.02
3.01
3.00
2.99
2.97
2.49 DMSO
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
1.35
1.33
1.32
1.31
0.90
2.02
4.18
6.00
f1 (ppm)

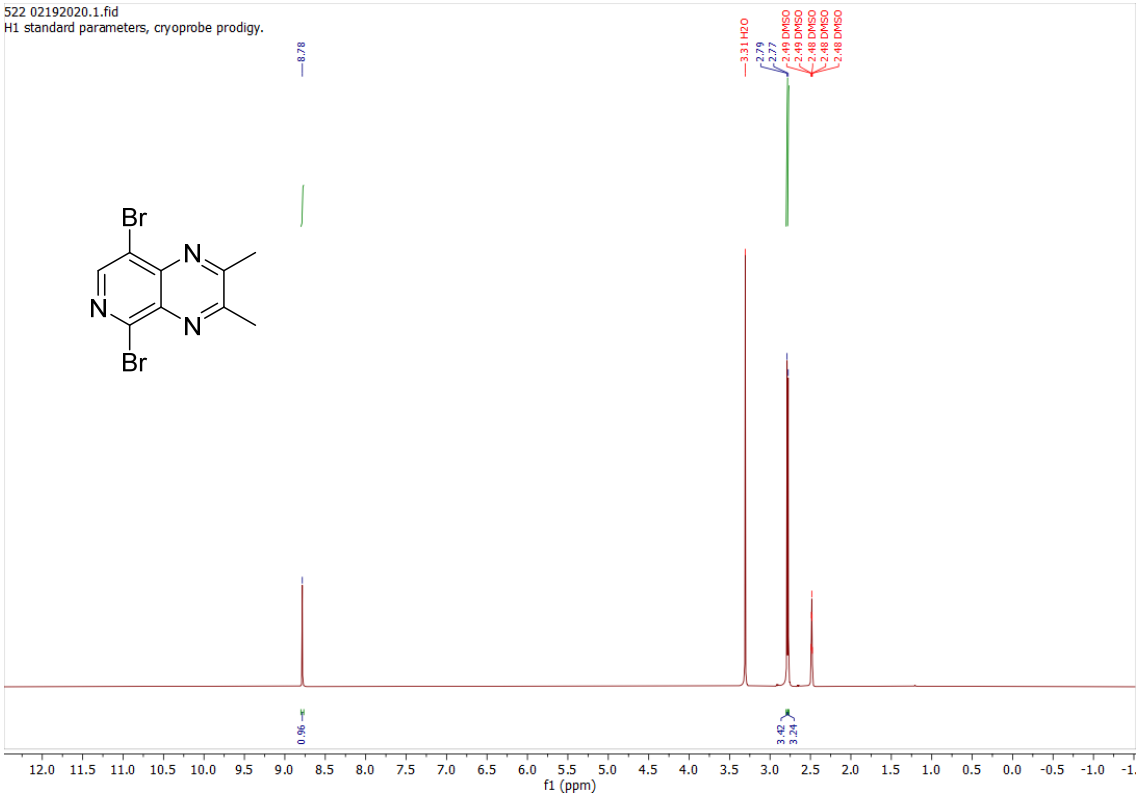

545carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgpg30zgdsh
158.49
154.20
153.58
142.67
141.74
126.76
103.77
27.91
27.25
11.79
11.68
Br
N
N
N
NH2
f1 (ppm)

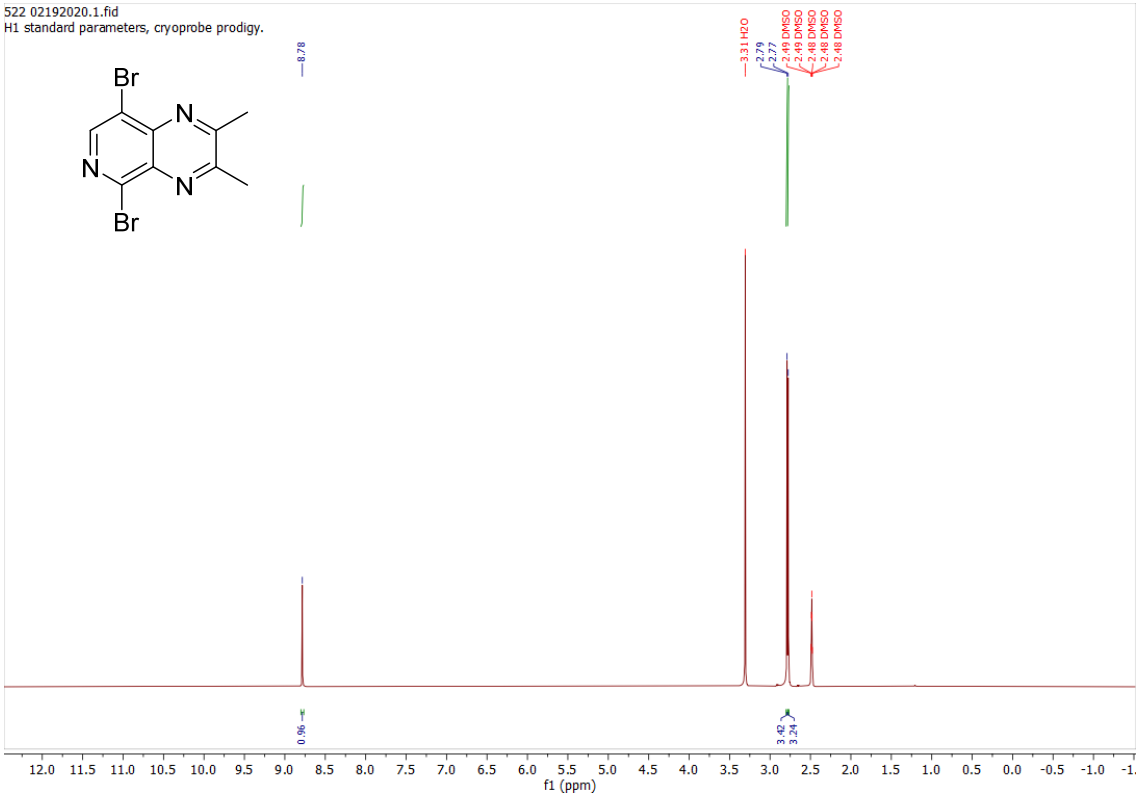

hsnd15r.1.fid
f1 (ppm)

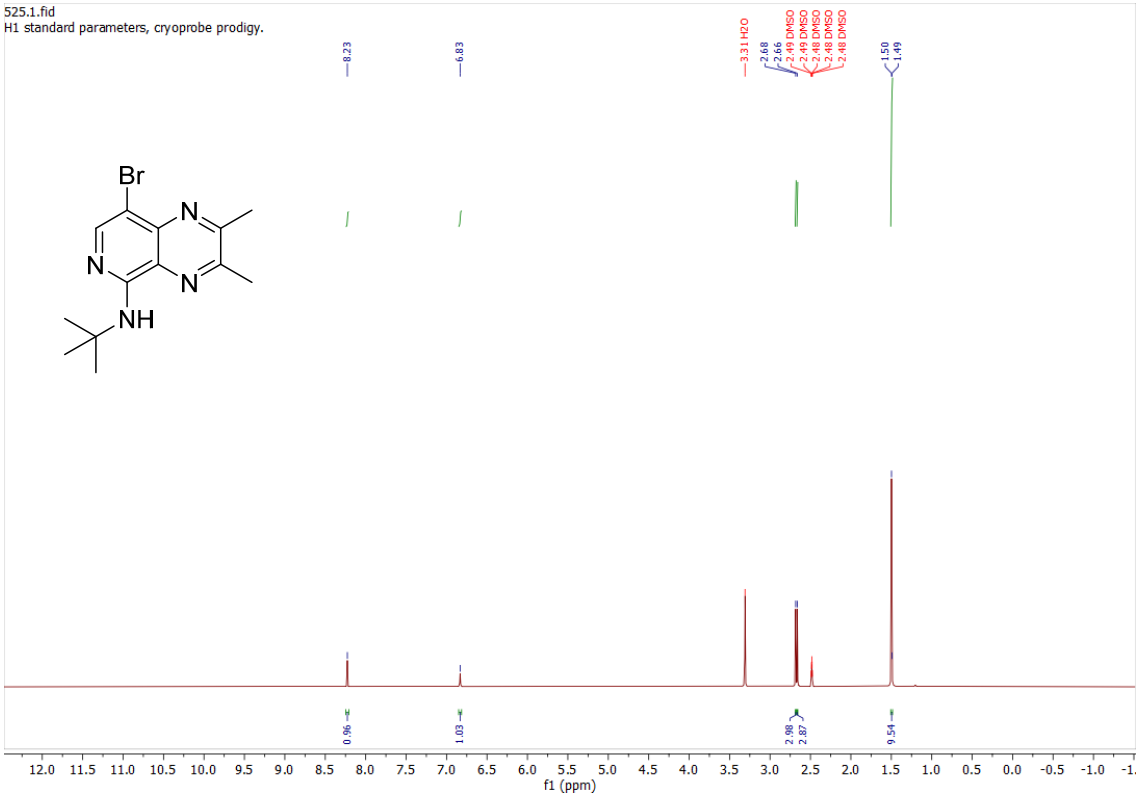

hsnd15r.2.fid
f1 (ppm)

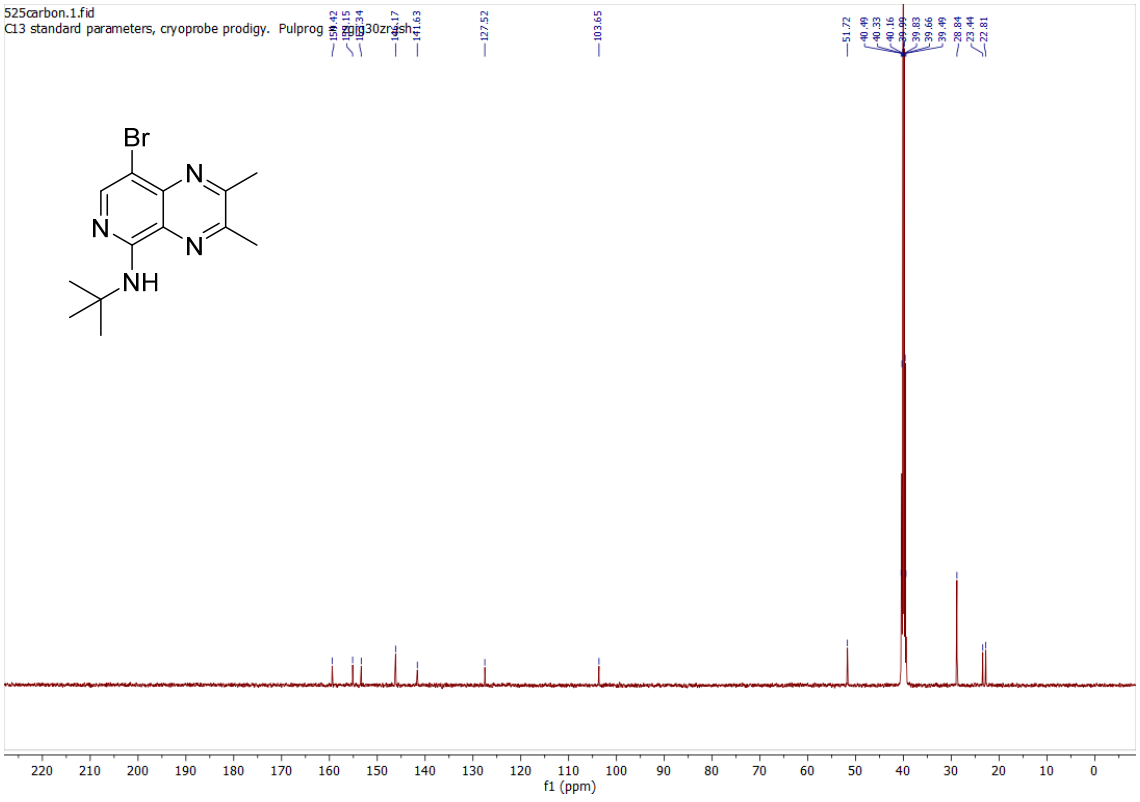

hsnd17.1.fid
10.82
9.09
9.09
8.98
8.97
8.74
8.74
8.54
8.54
8.54
8.53
8.23
8.21
8.20
8.08
8.08
8.07
8.06
7.73
7.71
3.65
3.40 HDO
3.37 HDO
2.69
2.49 DMSO
2.48 DMSO
2.48 DMSO
CF3
O
N
H
N
N
N
HN–N
1.04
1.12
0.98
1.11
2.02
2.00
1.02
1.07
2.72
3.92
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.
f1 (ppm)

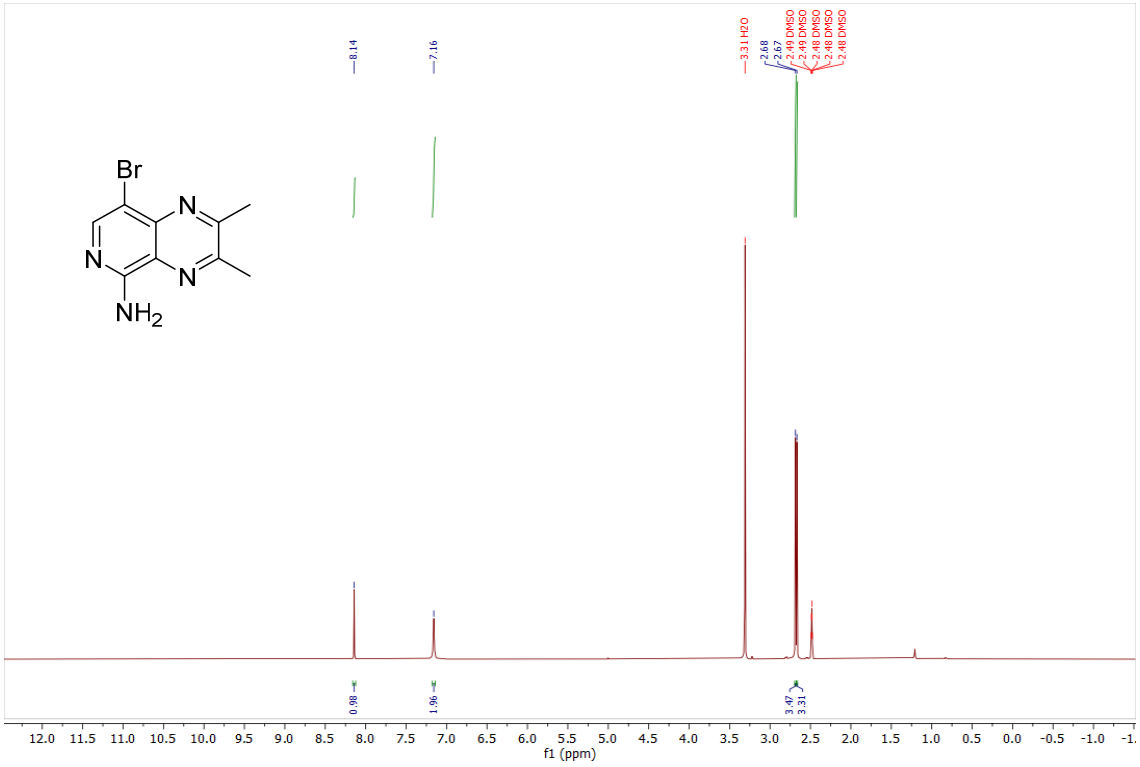

hsnd17.2.fid
163.97
154.46
151.64
151.08
148.64
138.60
137.91
133.97
132.06
130.27
128.22
127.98
125.77
124.03
123.59
119.54
117.88
114.45
111.51
91.82
86.81
57.08
53.36
50.10
40.44
40.27
39.94
39.77
39.60
39.44
19.01
CF3
O
N
H
N
N
N
HN–N
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

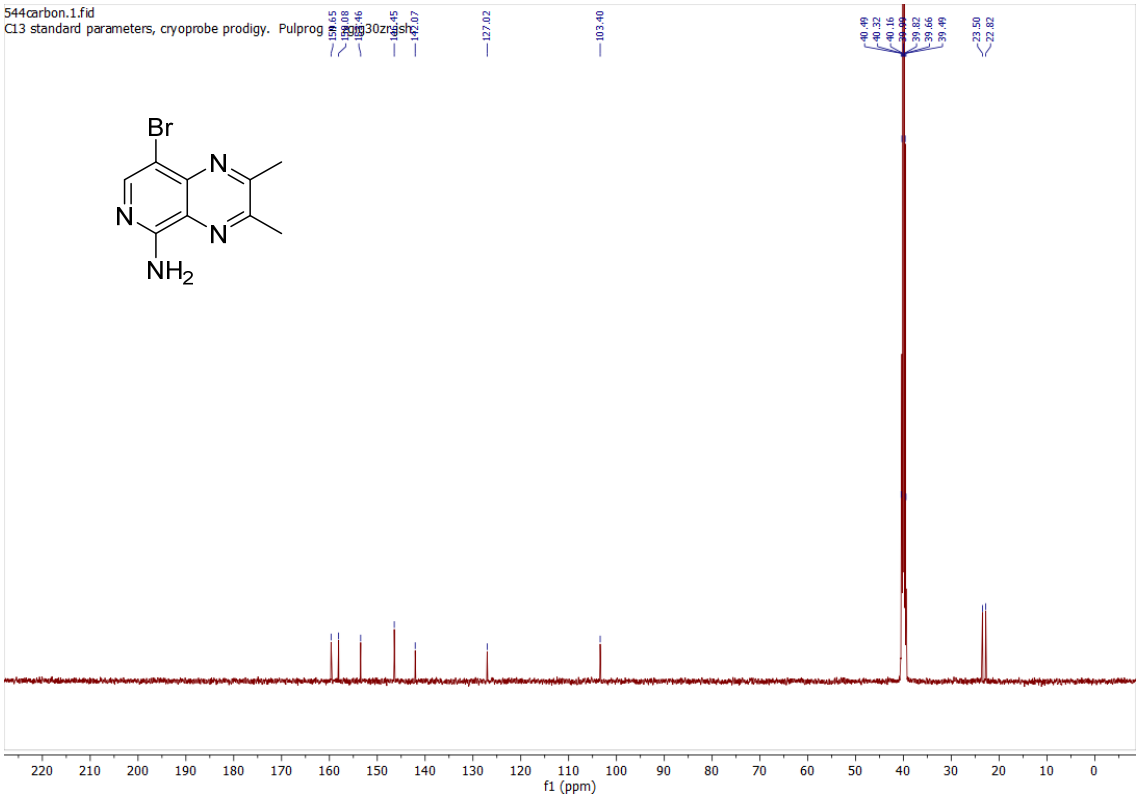

522 02192020.1.fid
H1 standard parameters, cryoprobe prodigy.
8.78
3.31 H2O
2.79
2.77
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
Br
N
N
N
Br
0.96
3.42
3.24
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

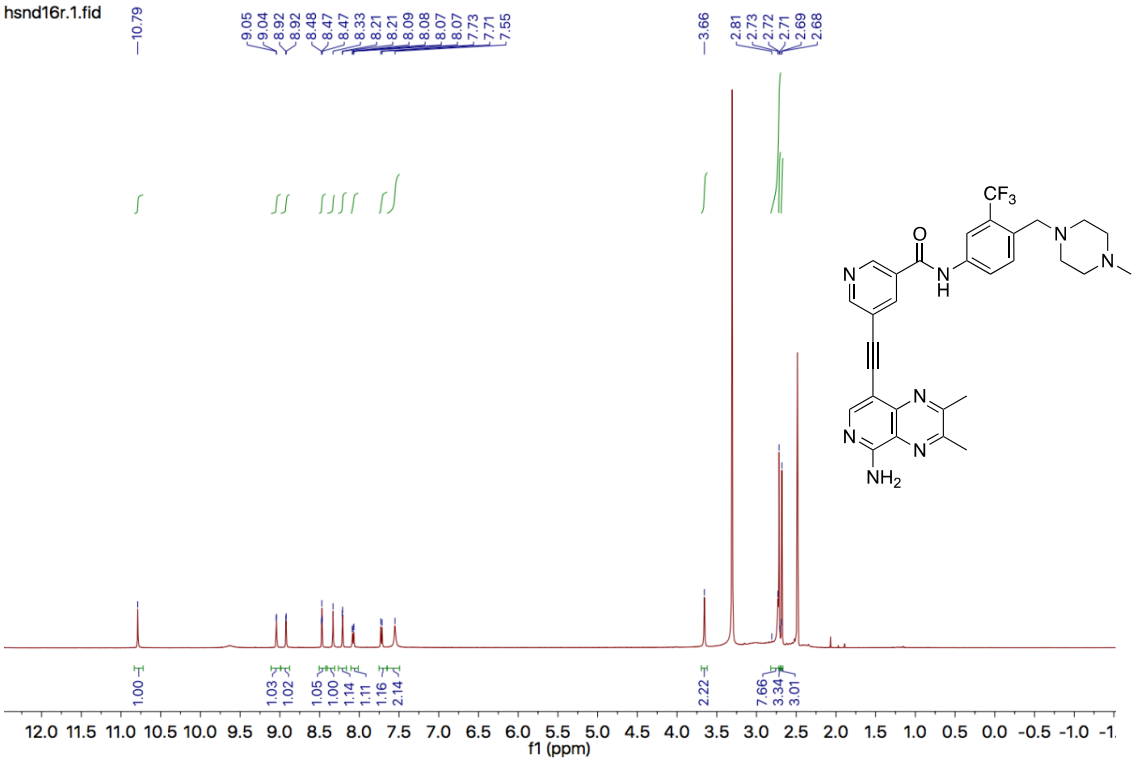

522 02192020.1.fid
H1 standard parameters, cryoprobe prodigy.
8.78
3.31 H2O
2.79
2.77
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
Br
N
N
N
Br
0.96
3.42
3.24
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

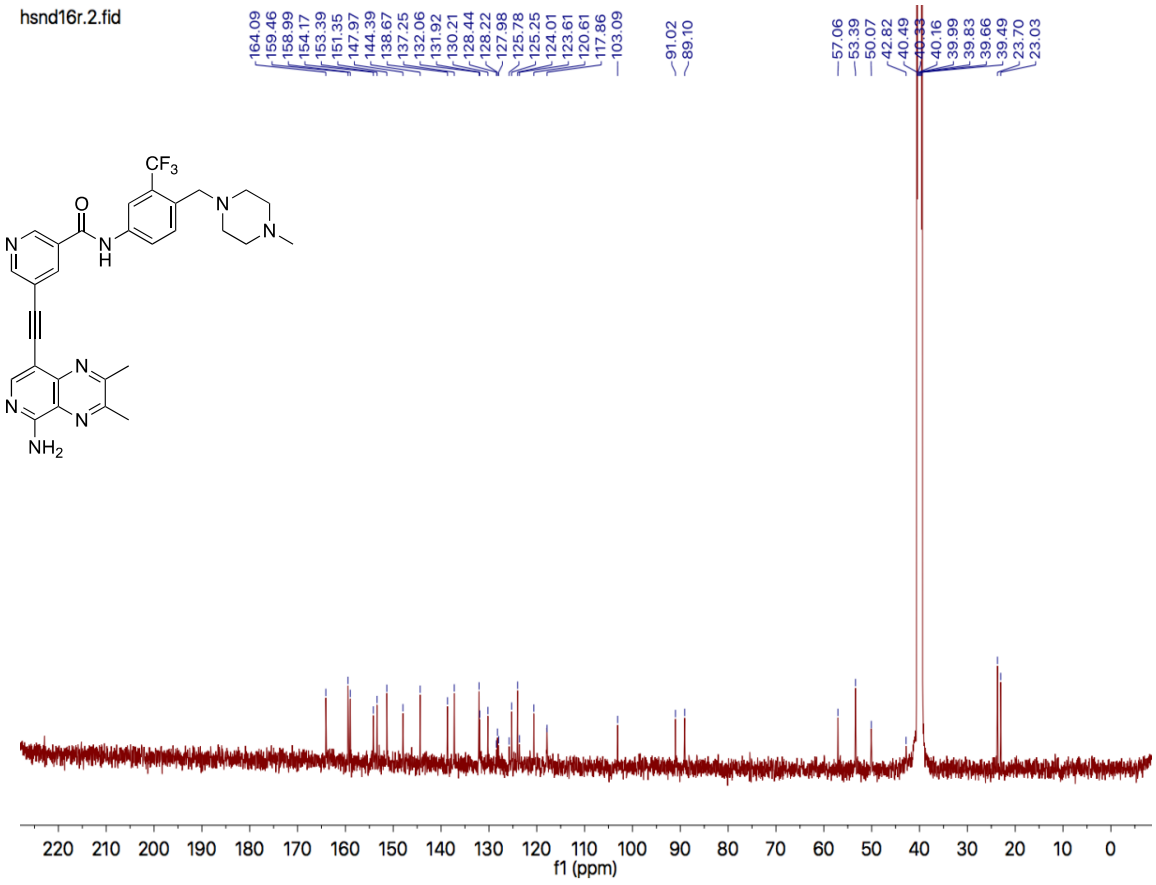

525.1.fid
H1 standard parameters, cryoprobe prodigy.
8.23
6.83
3.31 H2O
2.68
2.66
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
1.50
1.49
Br
N
N
N
NH
0.96
1.03
2.88
2.87
9.54
f1 (ppm)

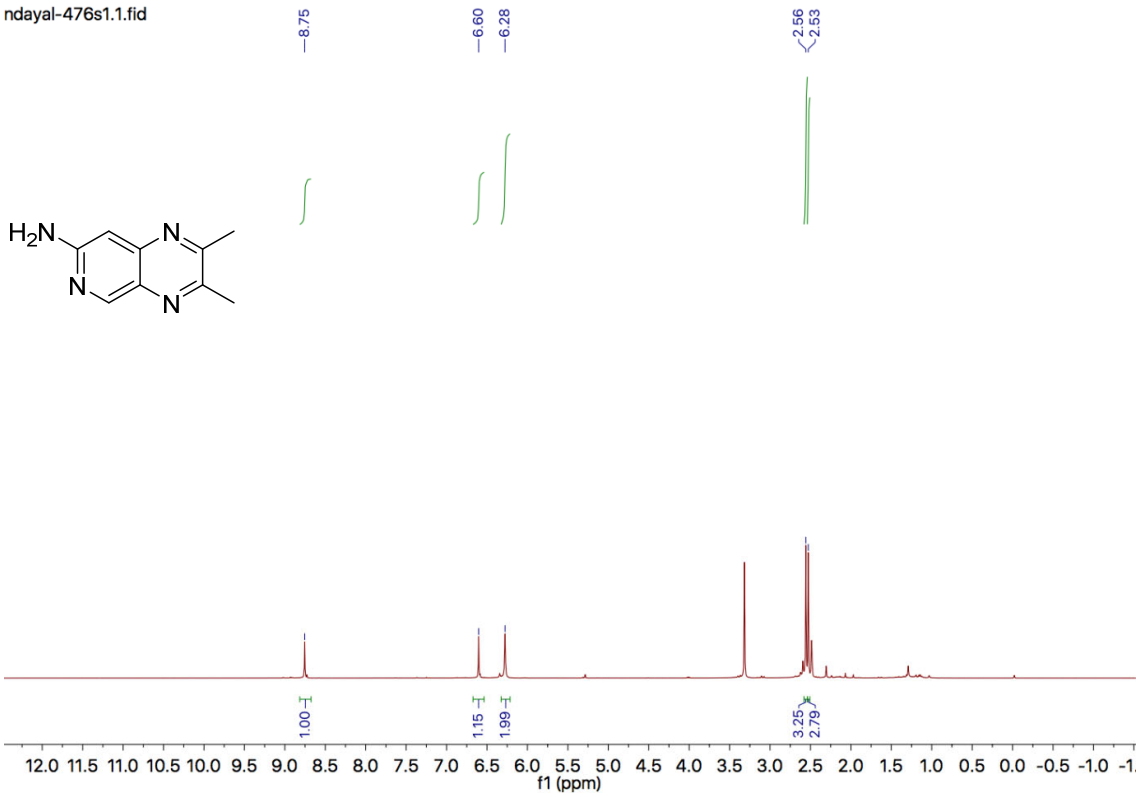

525carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgig30zgdsh
159.42
155.15
150.34
141.17
141.63
127.52
103.65
51.72
40.49
40.33
40.16
39.99
39.83
39.66
39.49
28.84
23.44
22.81
Br
N
N
N
NH
f1 (ppm)

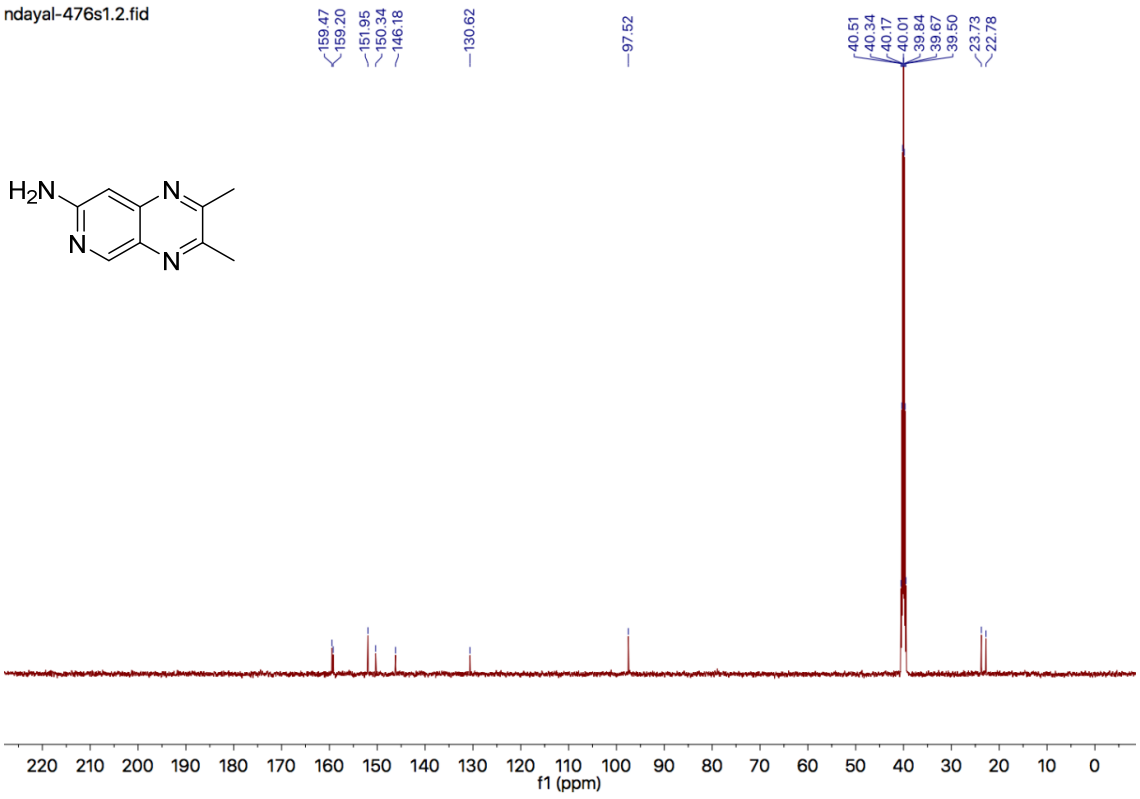

Br
N
N
N
NH2
8.14
7.16
3.31 H2O
2.68
2.67
2.49 DMSO
2.49 DMSO
2.48 DMSO
2.48 DMSO
2.48 DMSO
0.98
1.96
3.47
3.31
f1 (ppm)

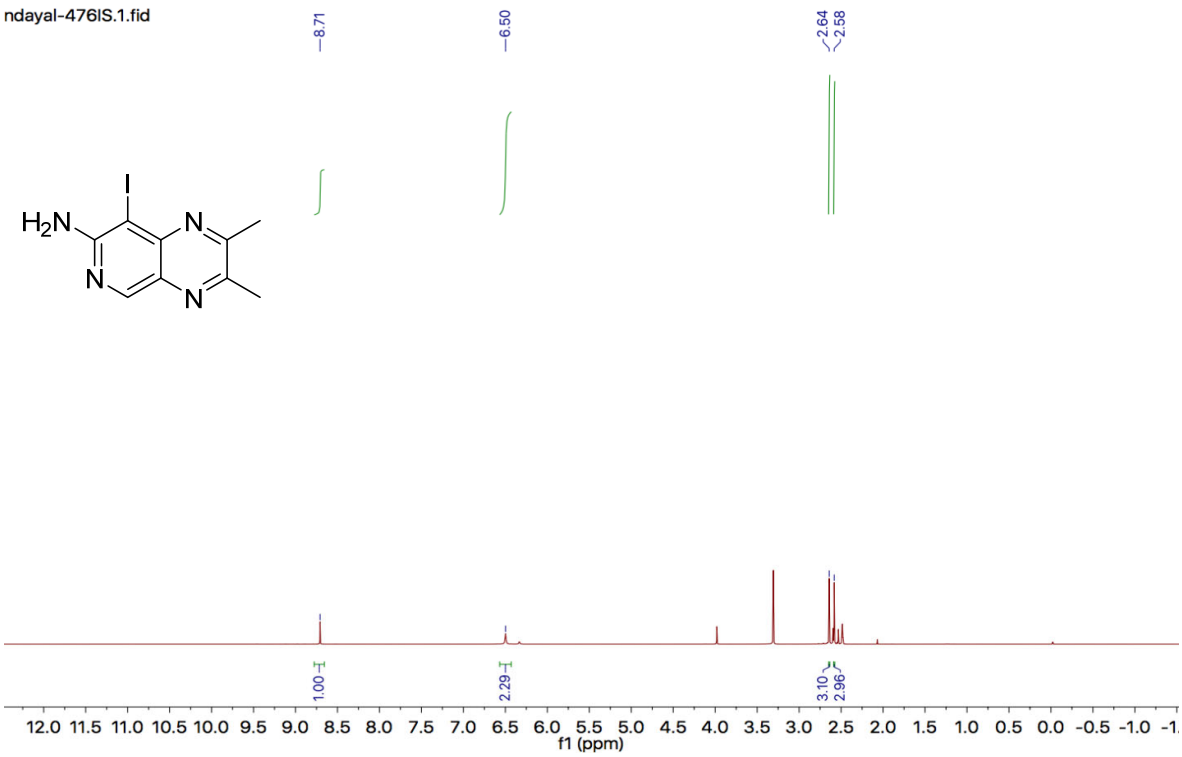

544carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog
Br
N
N
N
NH2
159.65
155.08
150.46
147.45
142.07
127.02
103.40
40.49
40.32
40.16
39.99
39.82
39.66
39.49
23.50
22.82
f1 (ppm)

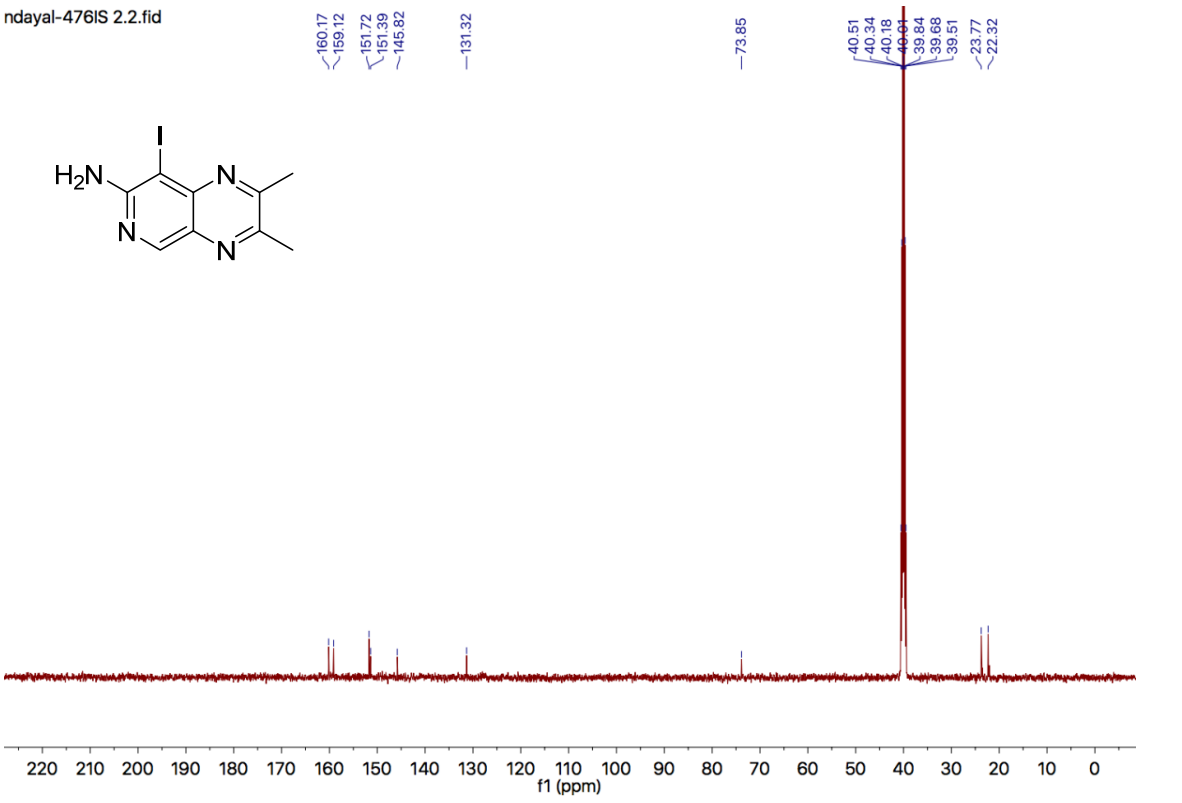

hsnd16r.1.fid
10.79
9.05
9.04
8.92
8.92
8.48
8.47
8.47
8.33
8.21
8.21
8.09
8.08
8.07
8.07
7.73
7.71
7.55
3.66
2.81
2.73
2.72
2.71
2.69
2.68
CF3
O
N
H
N
N
N
N
N
NH2
1.00
1.03
1.02
1.05
1.00
1.14
1.11
1.16
2.14
2.22
7.66
3.34
3.01
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

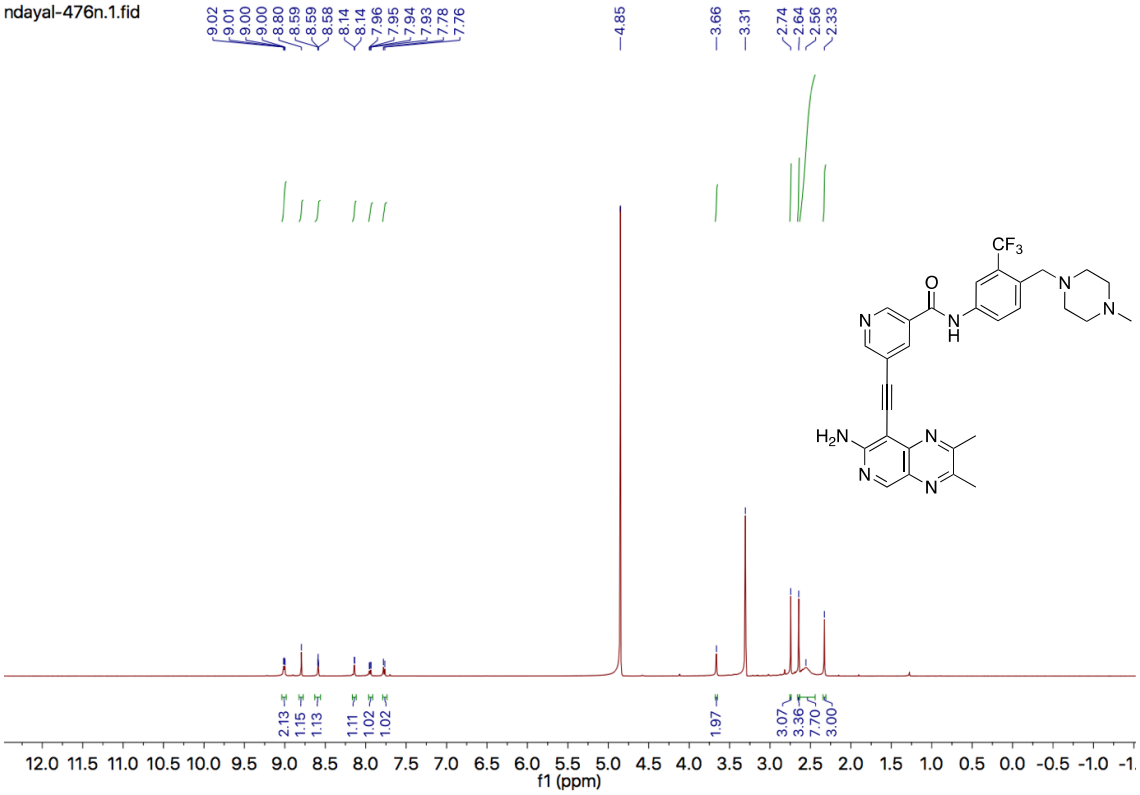

hsnd16r.2.fid
164.09
159.46
158.99
154.17
153.39
151.35
147.97
144.39
138.67
137.25
132.06
131.92
130.21
128.44
128.22
127.98
125.78
125.25
124.01
123.61
120.61
117.86
103.09
91.02
89.10
57.06
53.39
50.07
42.82
40.49
40.33
40.16
39.99
39.83
39.66
39.49
23.70
23.03
CF3
O
N
H
N
N
N
N
N
NH2
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

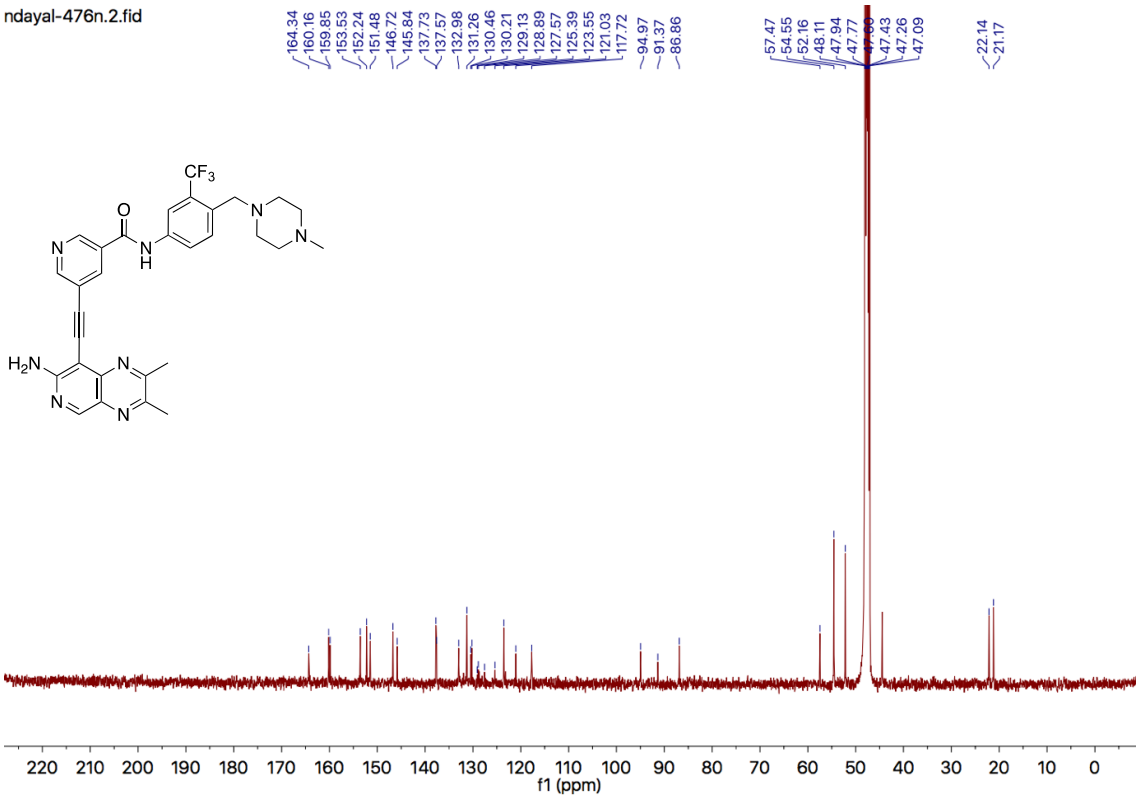

ndayal-476s1.1.fid
8.75
6.60
6.28
2.56
2.53
1.00
1.15
1.99
3.25
2.79
f1 (ppm)


ndayal-476s1.2.fid
159.47
159.20
151.95
150.34
146.18
130.62
97.52
40.51
40.34
40.17
40.01
39.84
39.67
39.50
23.73
22.78
f1 (ppm)

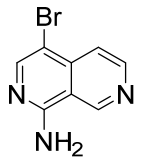

ndayal-476IS.1.fid
8.71
6.50
2.64
2.58
I
H2N
N
N
N
1.00
2.29
3.10
2.96
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


ndayal-476IS 2.2.fid
160.17
159.12
151.72
151.39
145.82
131.32
73.85
40.51
40.34
40.18
40.01
39.84
39.68
39.51
23.77
22.32
I
H2N
N
N
N
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


ndayal-476n.1.fid
9.02
9.01
9.00
9.00
8.80
8.59
8.59
8.58
8.14
8.14
7.96
7.95
7.94
7.93
7.78
7.76
4.85
3.66
3.31
2.74
2.64
2.56
2.33
CF3
O
N
H
N
N
N
H2N
N
N
2.13
1.15
1.13
1.11
1.02
1.02
1.97
3.07
3.36
7.70
3.00
12.0 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 0.0 -0.5 -1.0
f1 (ppm)

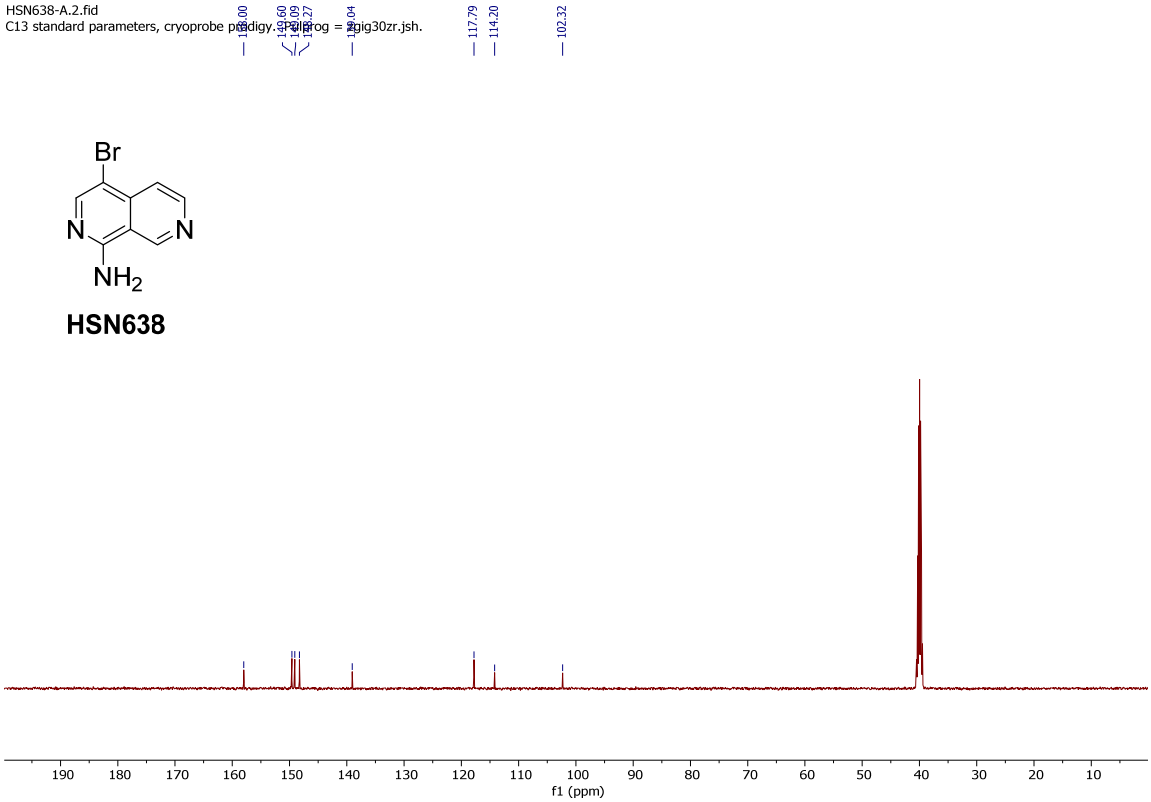

ndayal-476n.2.fid
164.34
160.16
159.85
153.53
152.24
151.48
146.72
145.84
137.73
137.57
132.98
131.26
130.46
130.21
129.13
128.89
127.57
125.39
123.55
121.03
117.72
94.97
91.37
86.86
57.47
54.55
52.16
48.11
47.94
47.77
47.43
47.26
47.09
22.14
21.17
CF3
O
N
H
N
N
N
H2N
N
N
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

HSN638-A.1.fid
H1 standard parameters, cryoprobe prodigy.

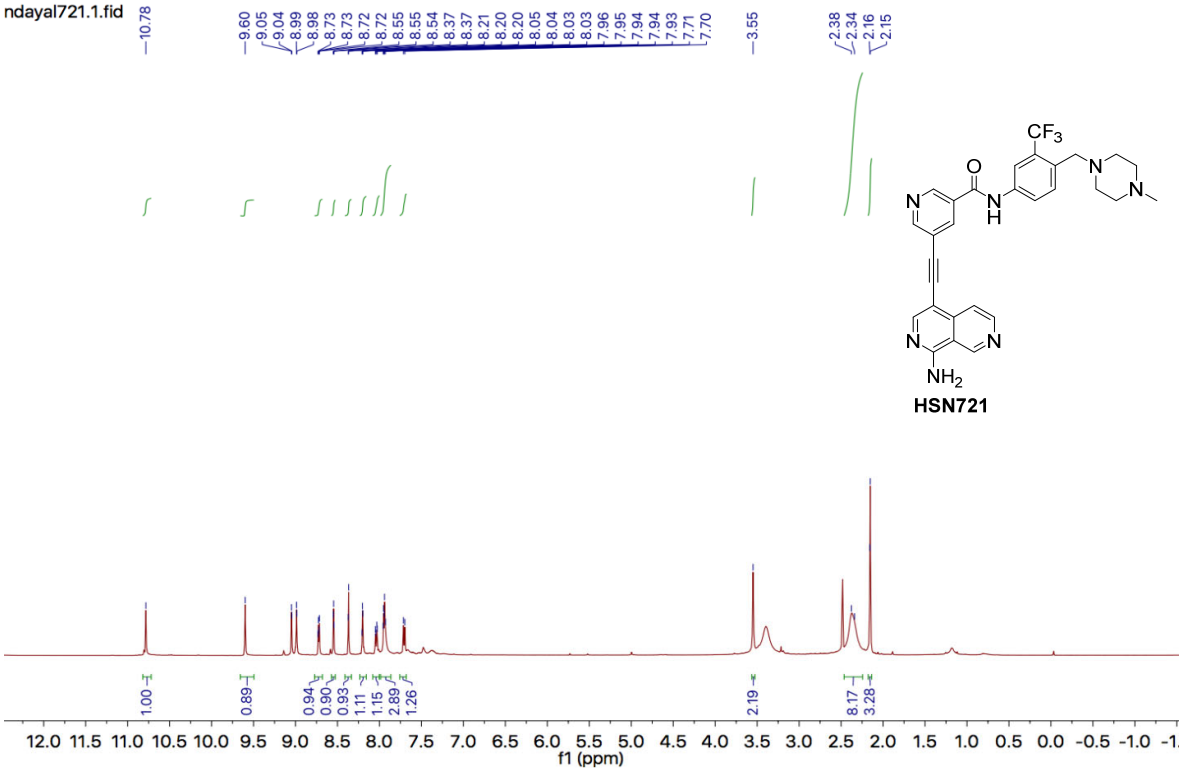


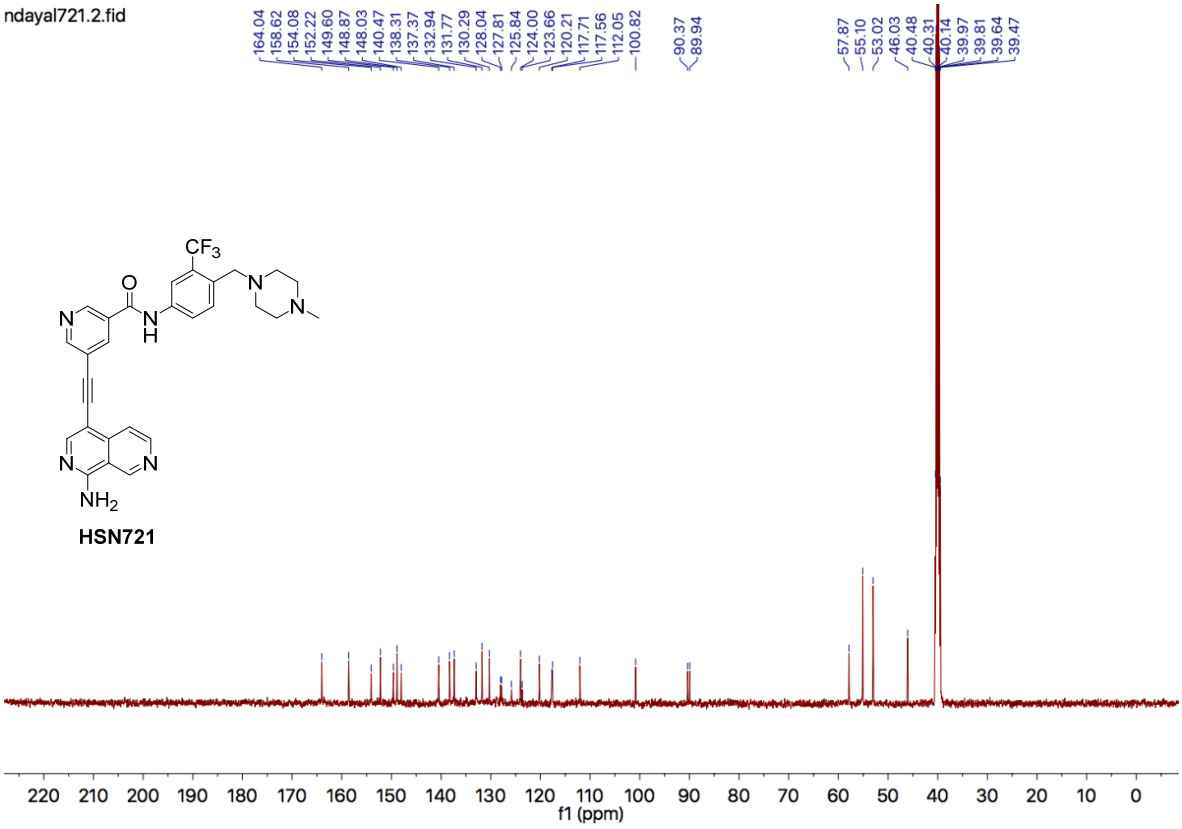
HSN638

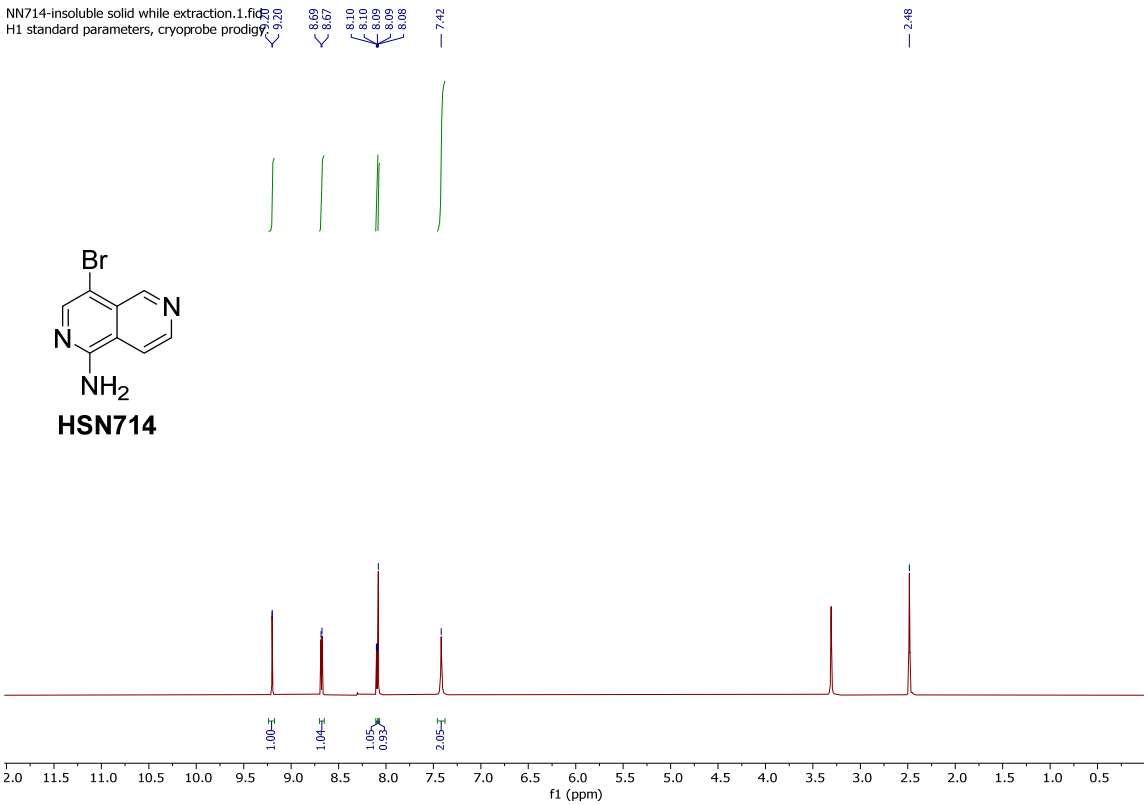
HSN638-A.2.fid
C13 standard parameters, cryoprobe prodigy. prog = zgig30zr.jsh.

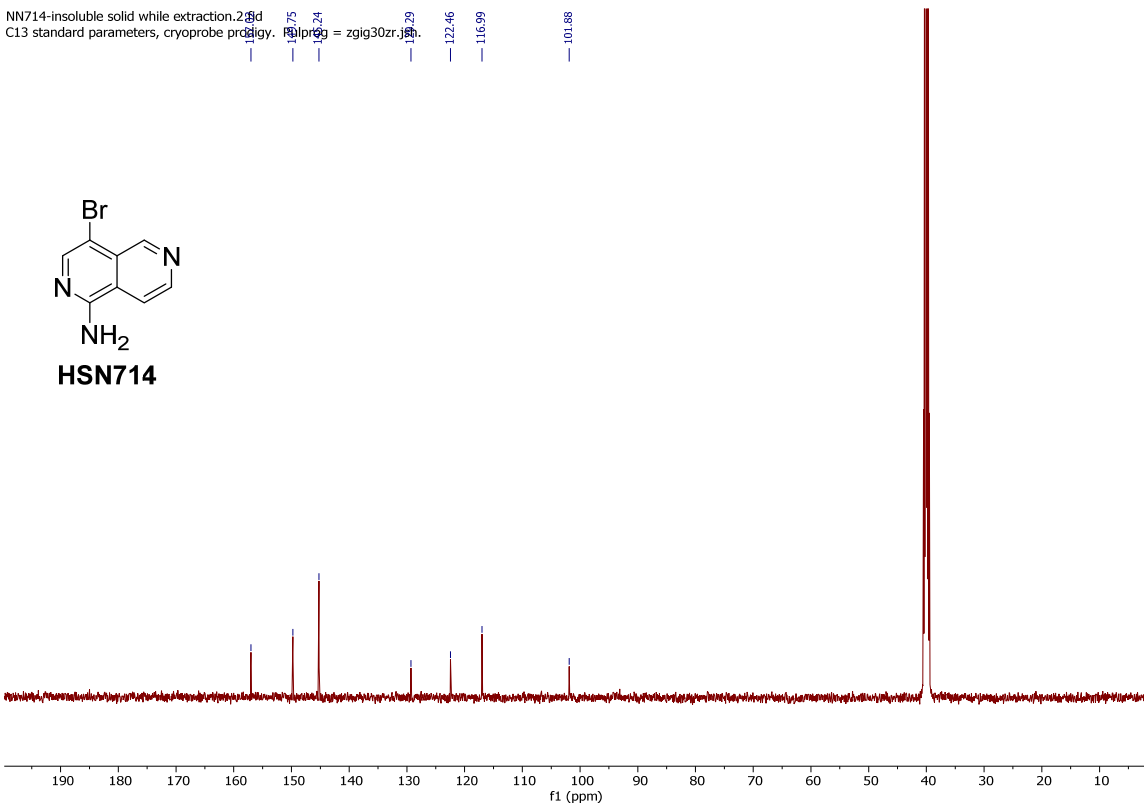


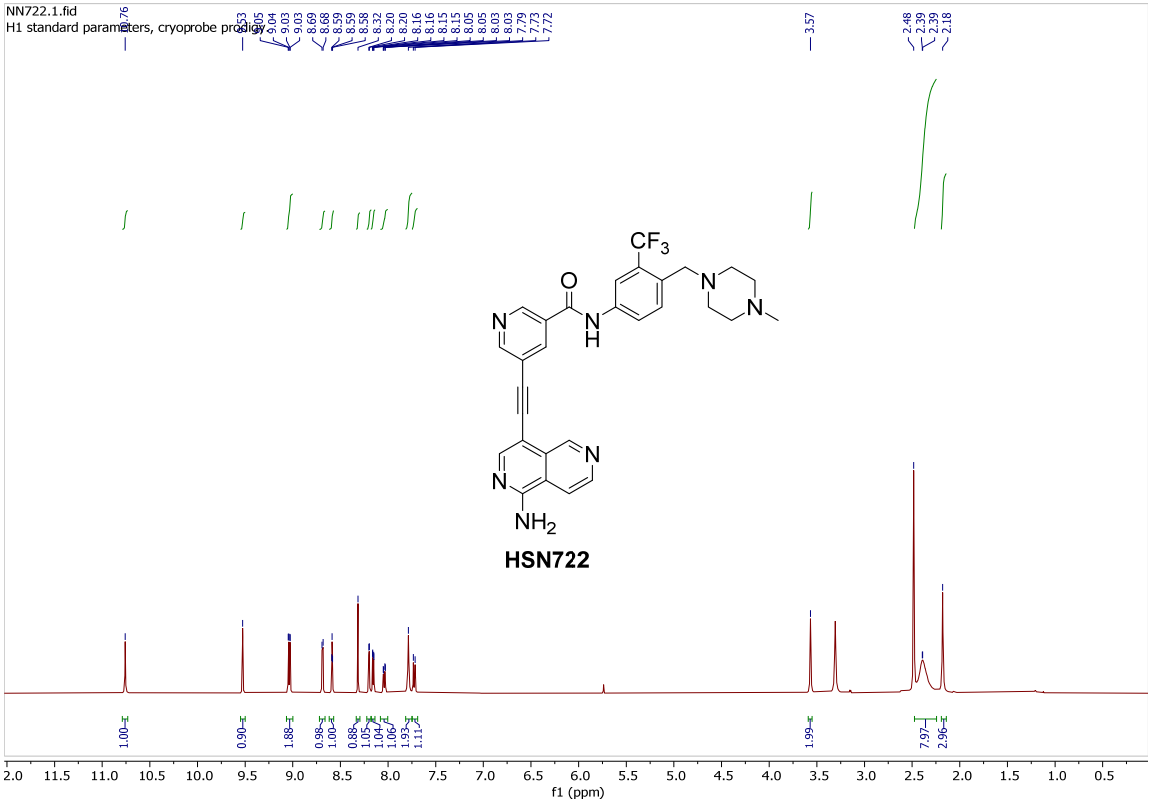
HSN638

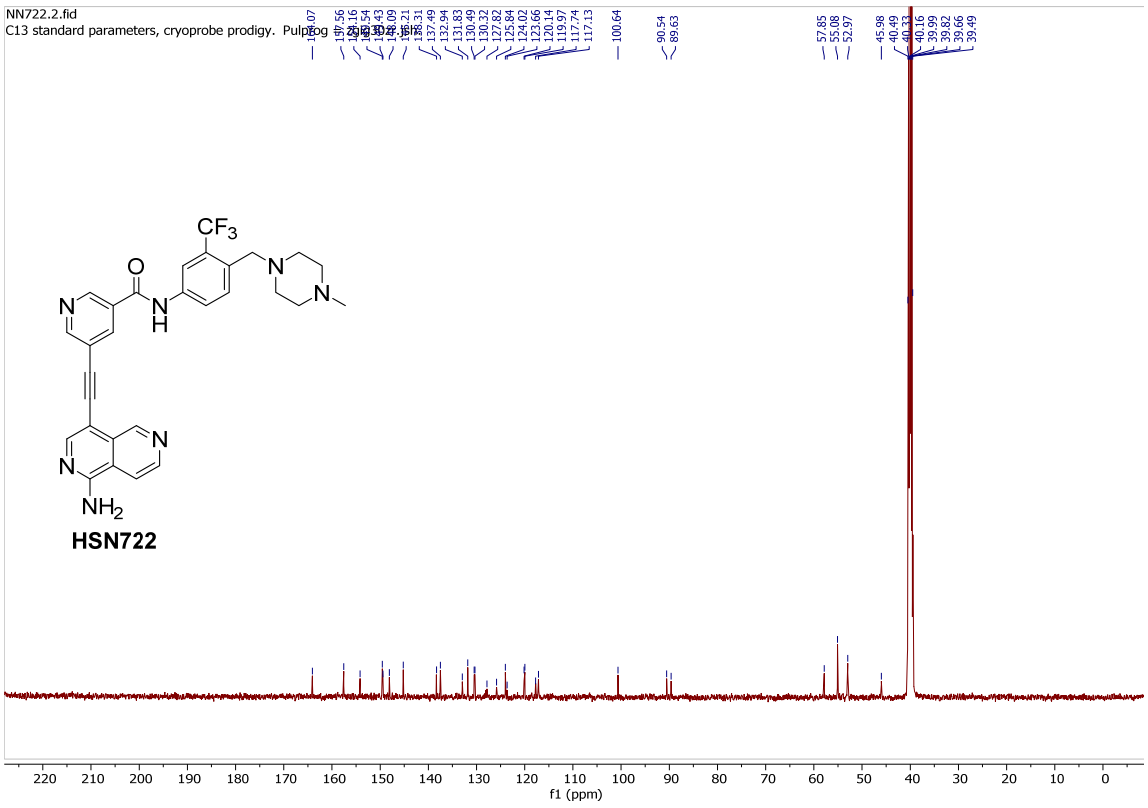

ndayal721.1.fid
10.78
9.60
9.05
9.04
8.99
8.98
8.73
8.73
8.72
8.72
8.55
8.55
8.54
8.37
8.37
8.21
8.20
8.05
8.04
8.03
7.96
7.95
7.94
7.94
7.93
7.71
7.70
3.55
2.38
2.34
2.16
2.15
CF3
O
N
H
N
N
N
N
NH2
HSN721
1.00
0.89
0.94
0.90
0.93
1.11
1.15
2.89
1.26
2.19
8.17
3.28
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 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 0.0 -0.5 -1.0
f1 (ppm)

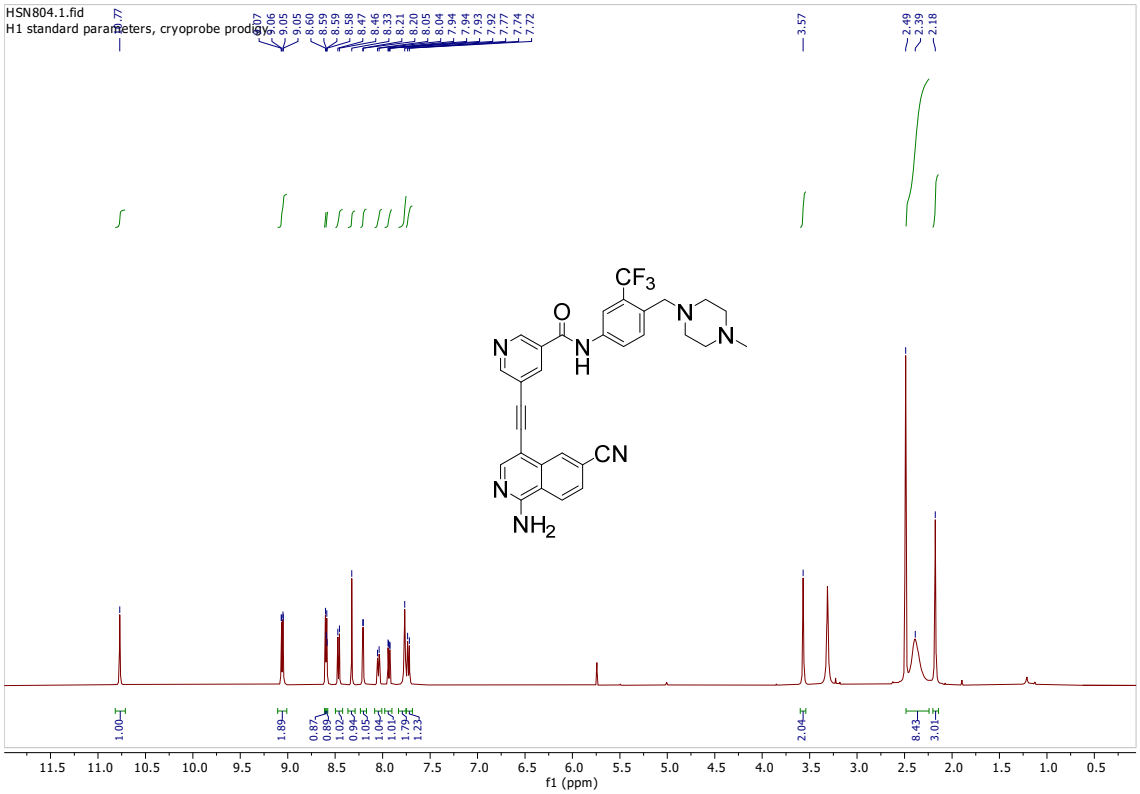

ndayal721.2.fid
164.04
158.62
154.08
152.22
149.60
148.87
148.03
140.47
138.31
137.37
132.94
131.77
130.29
128.04
127.81
125.84
124.00
123.66
120.21
117.71
117.56
112.05
100.82
90.37
89.94
57.87
55.10
53.02
46.03
40.48
40.31
40.14
39.97
39.81
39.64
39.47
CF3
O
N
H
N
N
N
N
NH2
HSN721
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

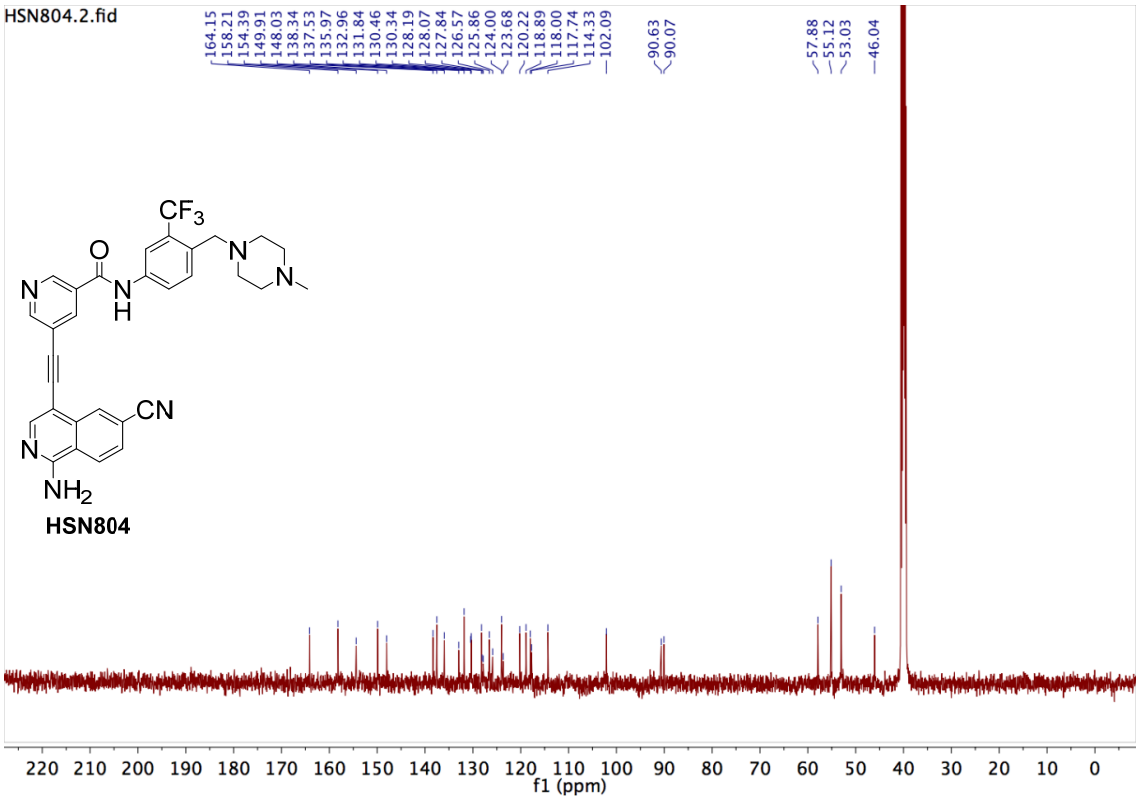

NN714-insoluble solid while extraction.1.fid
H1 standard parameters, cryoprobe prodigy
9.21
9.20
8.69
8.67
8.10
8.10
8.09
8.09
8.08
7.42
2.48
Br
N
N
NH2
HSN714
1.00
1.04
1.05
0.93
2.05
f1 (ppm)


NN714-insoluble solid while extraction.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog = zgig30zr.jsp.
150.29
147.75
139.24
130.29
122.46
116.99
101.88
Br
N
N
NH2
HSN714
f1 (ppm)

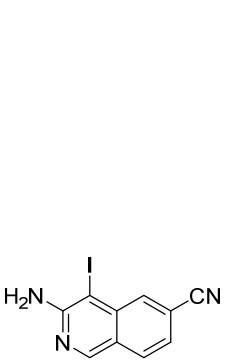

NN722.1.fid
H1 standard parameters, cryoprobe prodigy
HSN722
f1 (ppm)

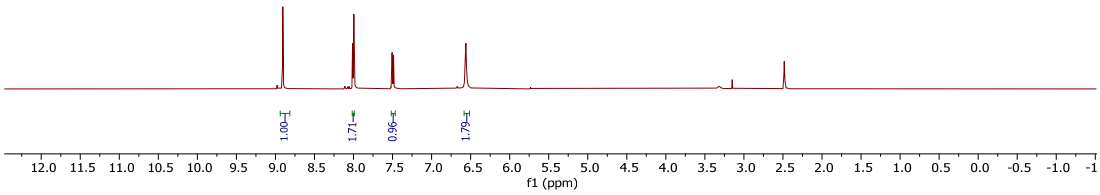

NN722.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgpg30
HSN722
f1 (ppm)


HSN804.1.fid
H1 standard parameters, cryoprobe prodigy
f1 (ppm)

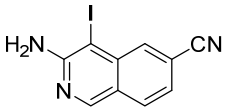

HSN804.2.fid
f1 (ppm)
HSN804

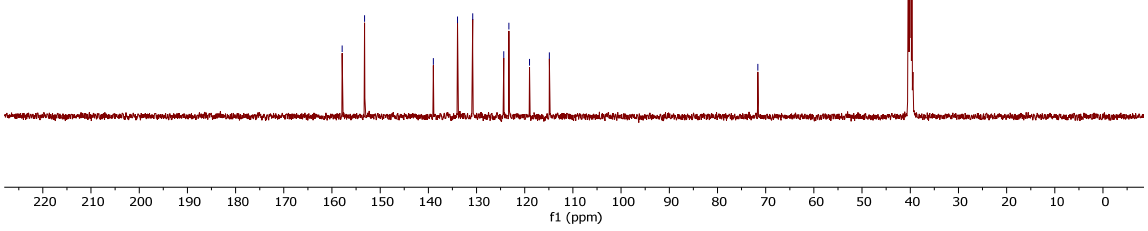
207.1.fid
H1 standard parameters, cryoprobe prodigy.

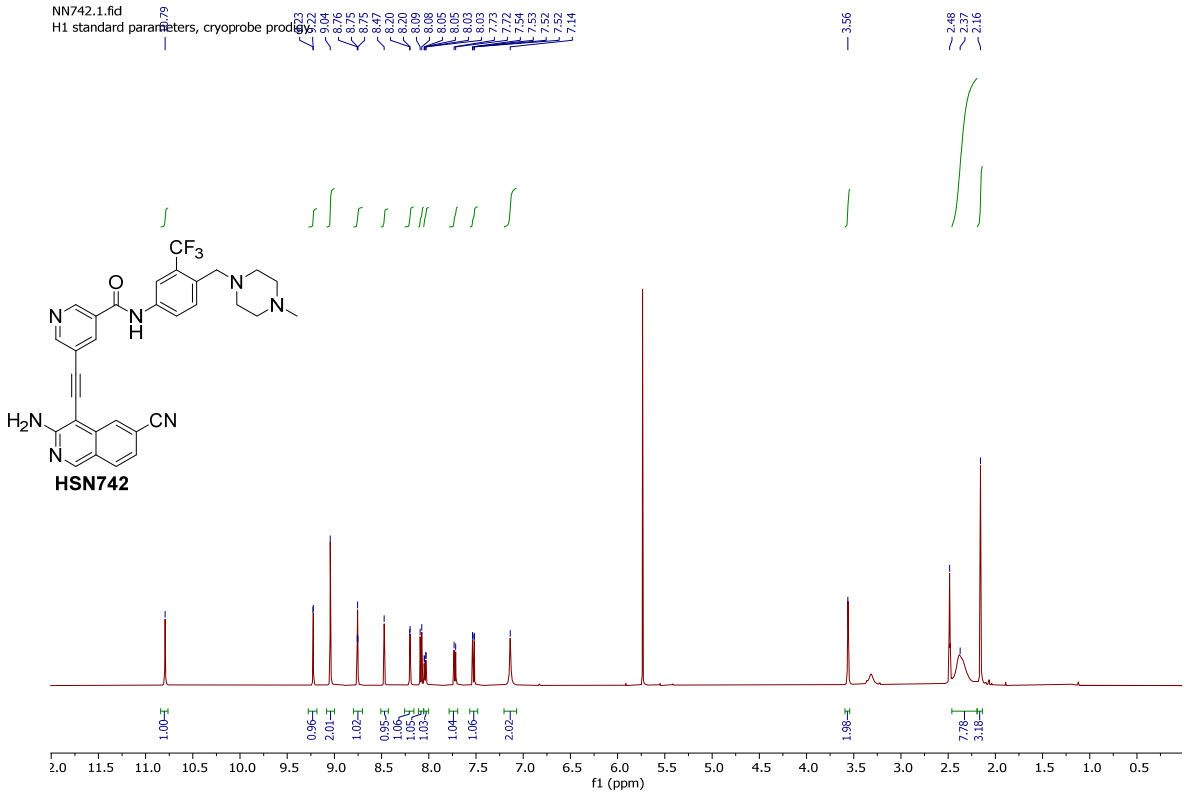


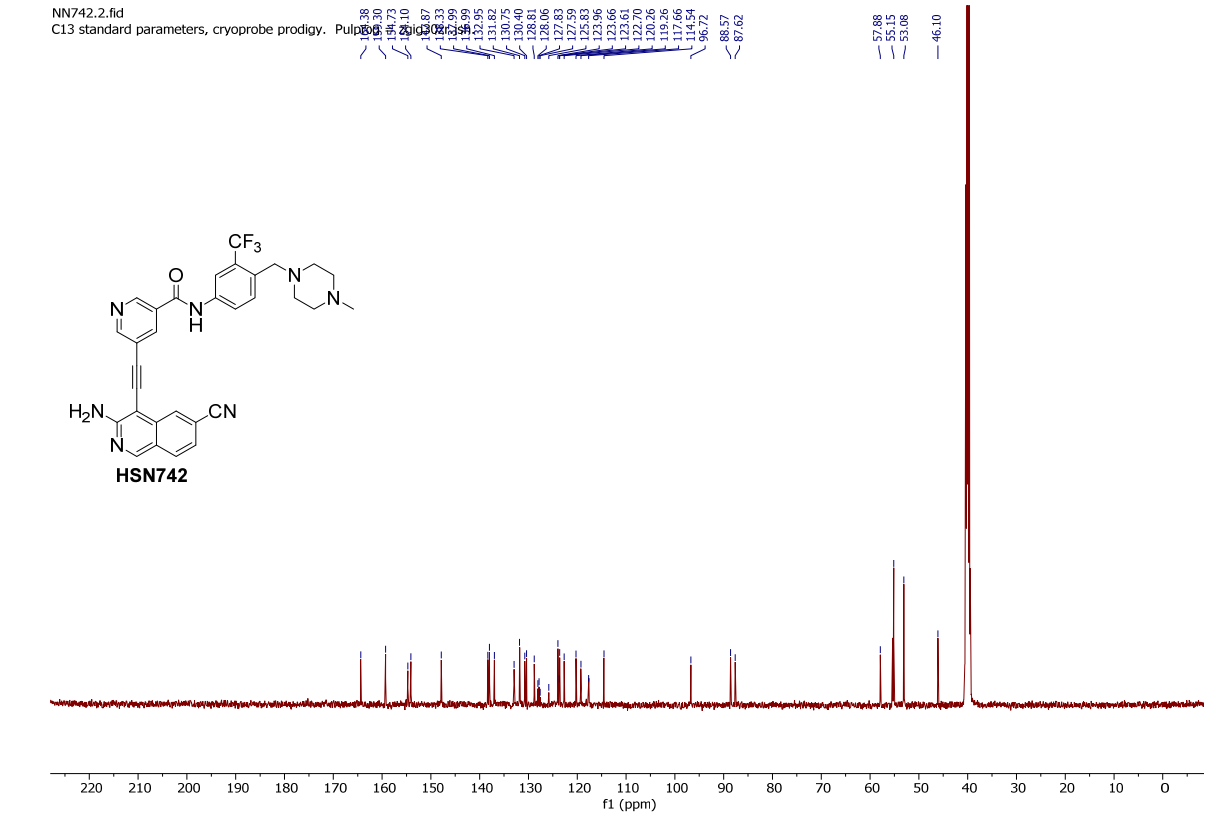


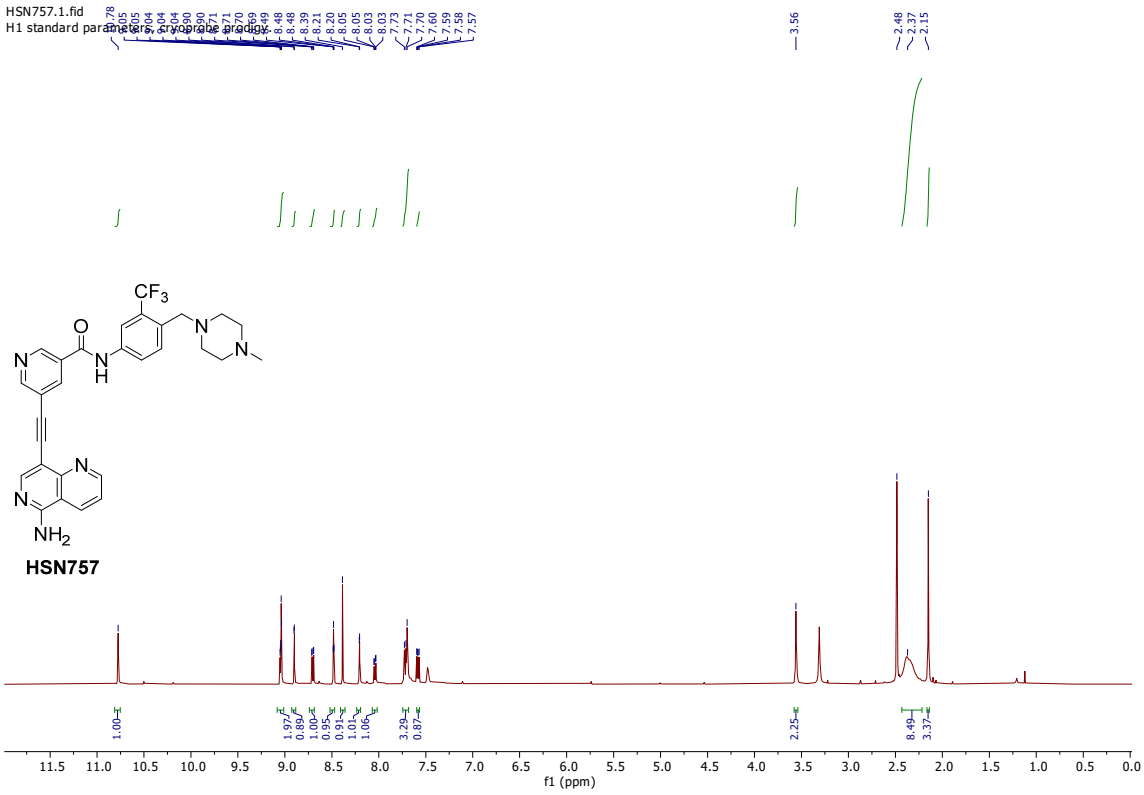
207carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgpg30zr.jsh.

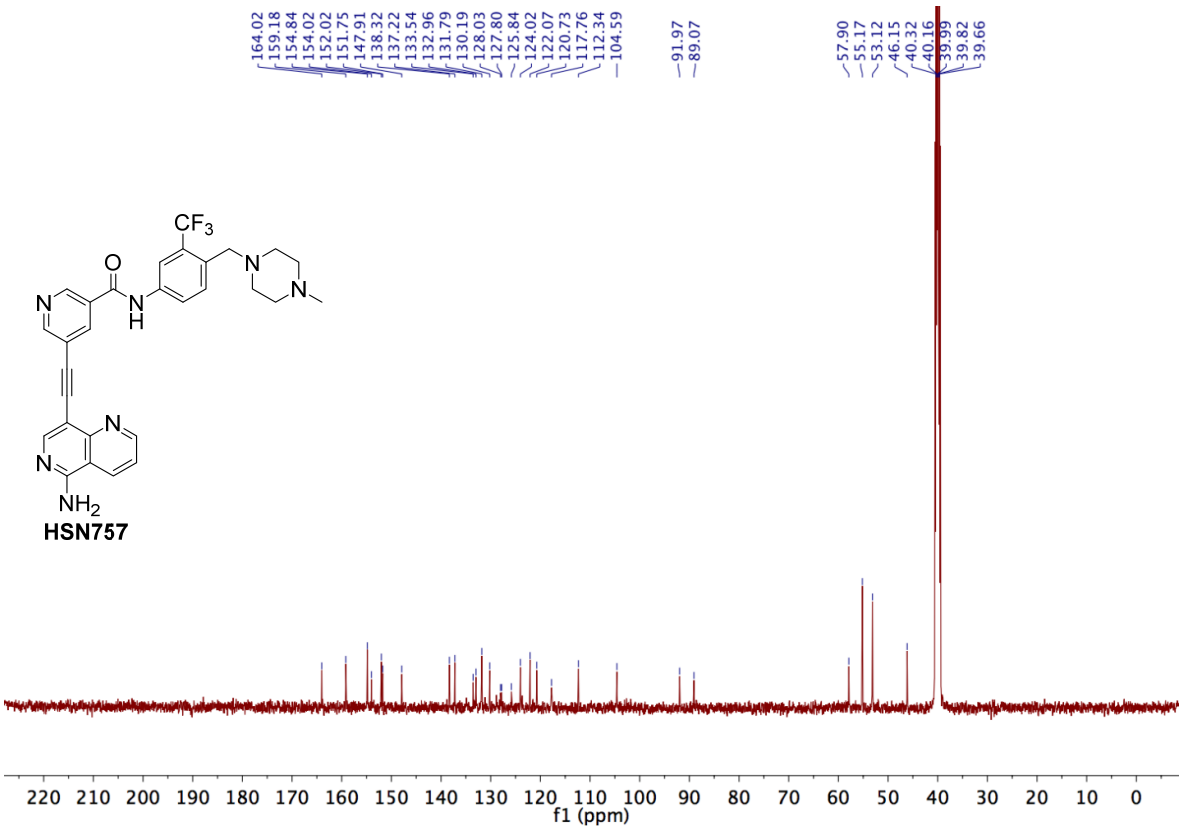




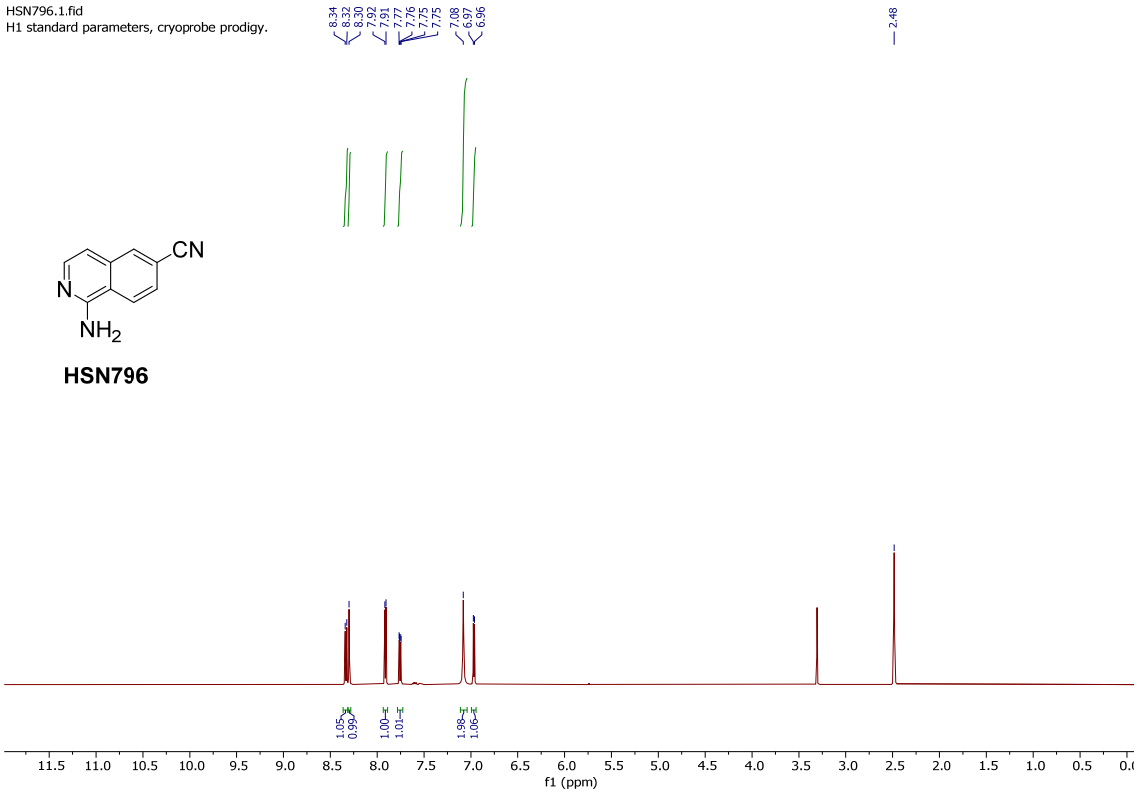

NN742.1.fid
H1 standard parameters, cryoprobe prodigy
HSN742
f1 (ppm)


NN742.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog: zgpg30
HSN742
f1 (ppm)


HSN757.1.fid
H1 standard parameters, cryoprobe prodigy
HSN757
f1 (ppm)

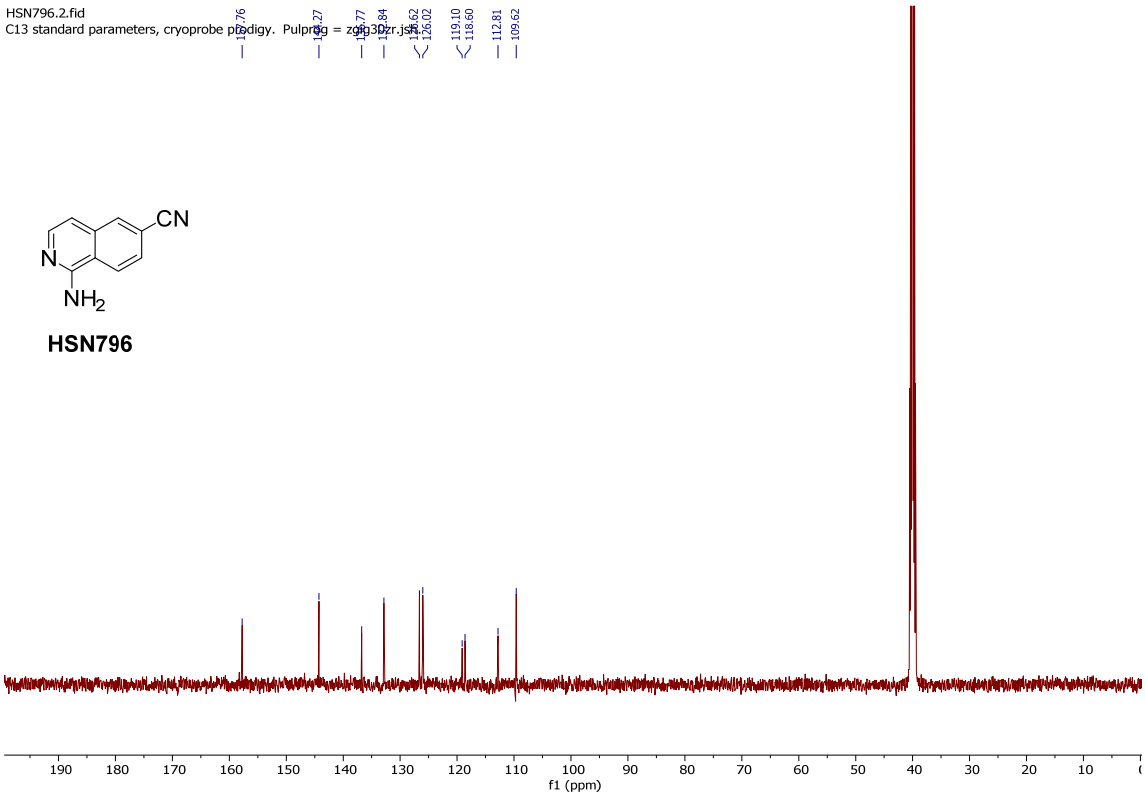

HSN757
f1 (ppm)

HSN796.1.fid
H1 standard parameters, cryoprobe prodigy.

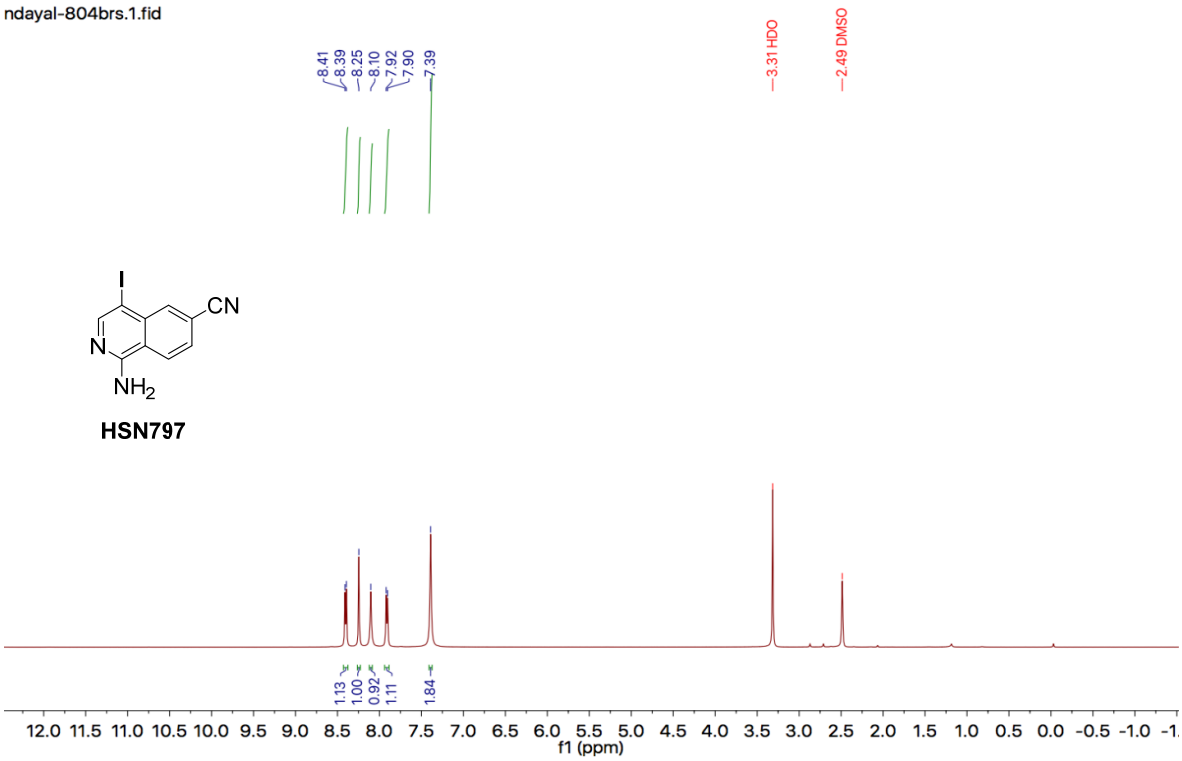


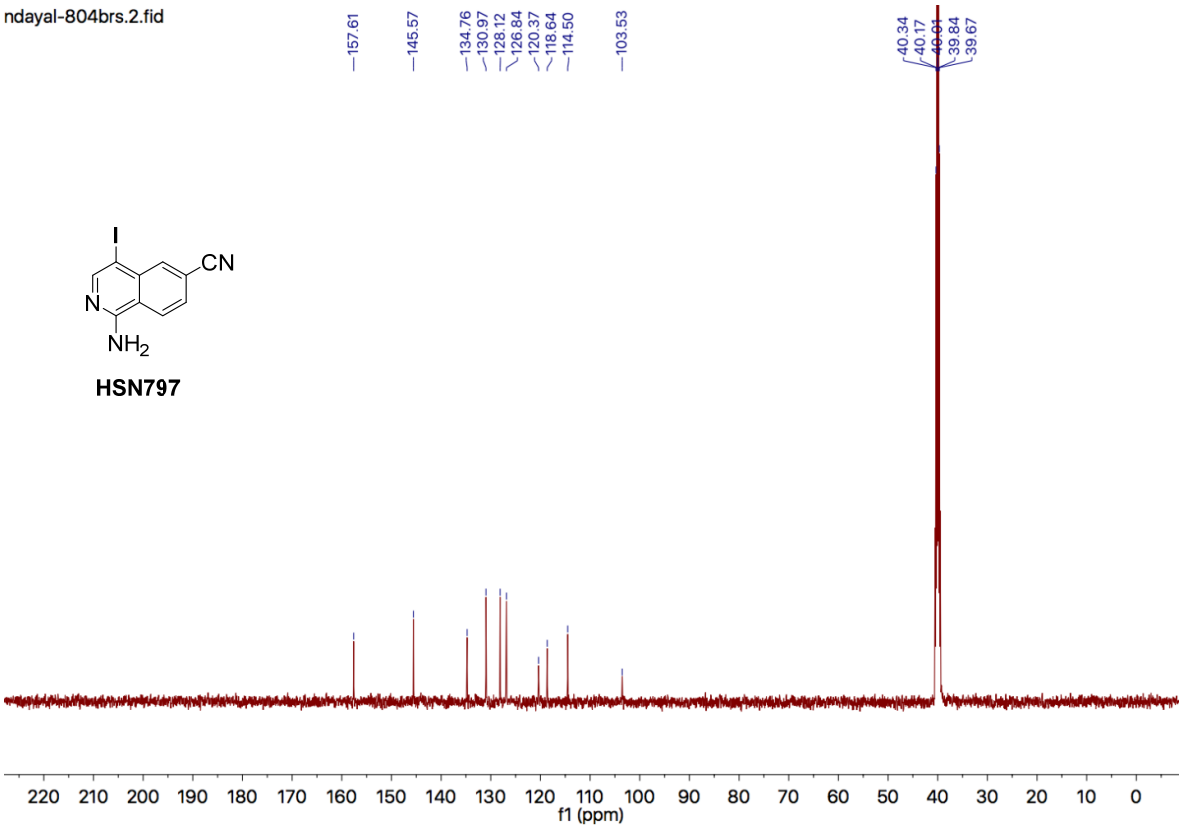
HSN796

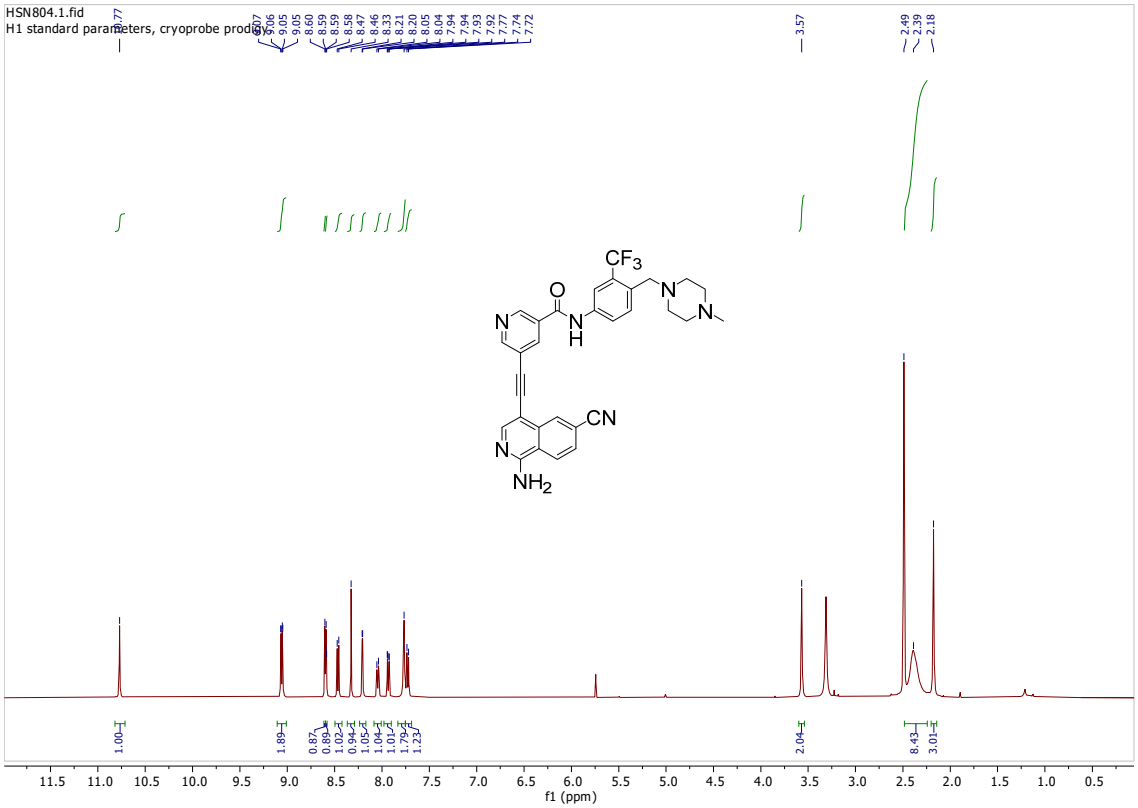
HSN796.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog = zgpg30zr.jst

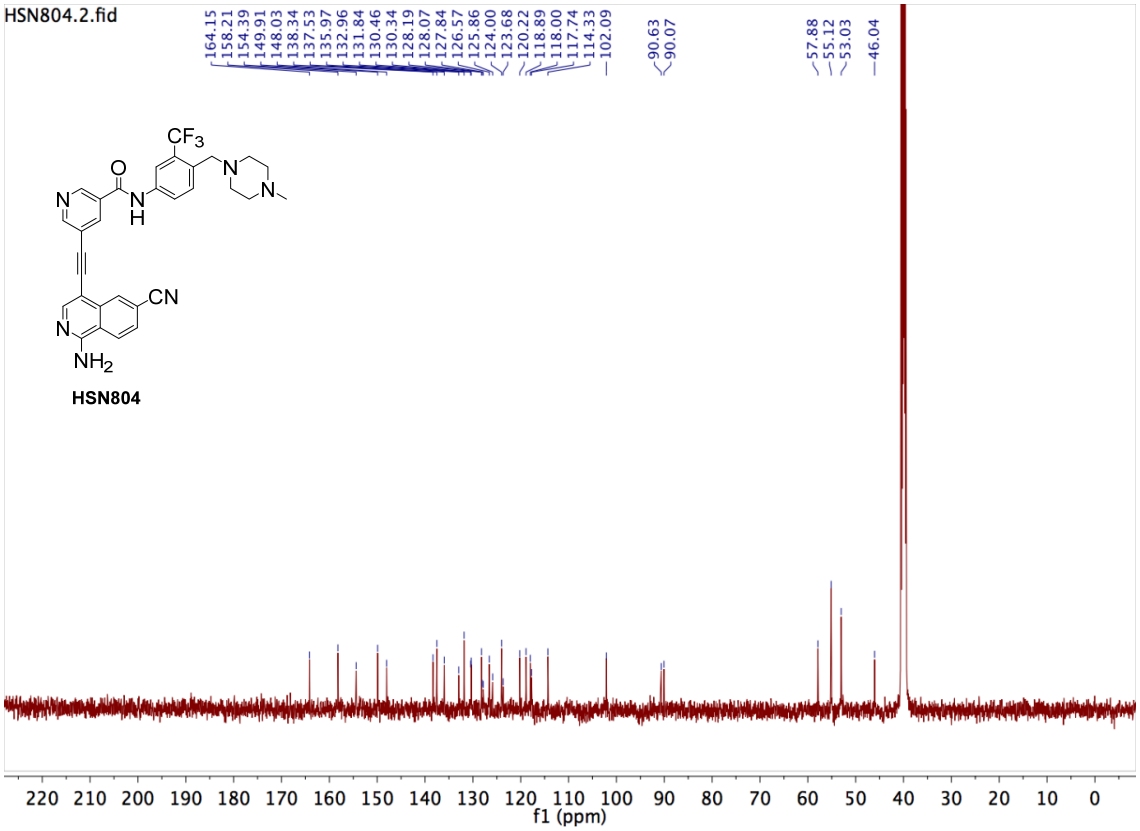


HSN796

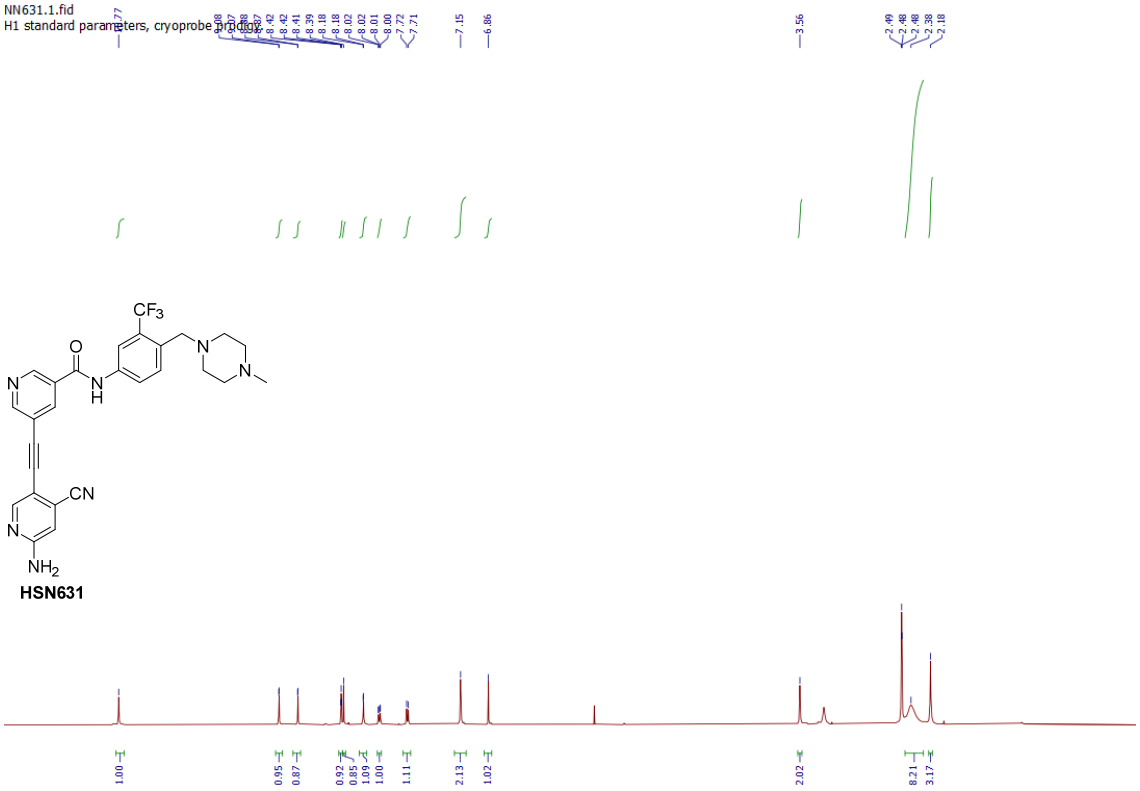

ndayal-804brs.1.fid
8.41
8.39
8.25
8.10
7.92
7.90
7.39
3.31 HDO
2.49 DMSO
I
CN
N
NH2
HSN797
1.13
1.00
0.92
1.11
1.84
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

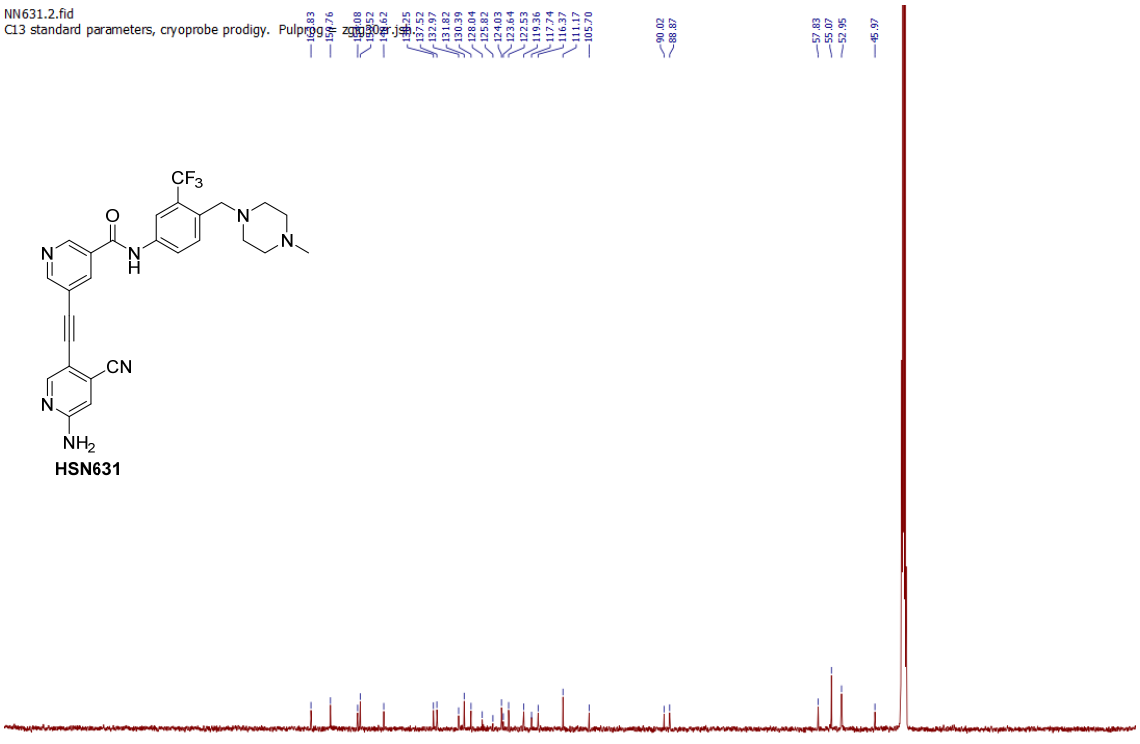

ndayal-804brs.2.fid
157.61
145.57
134.76
130.97
128.12
126.84
120.37
118.64
114.50
103.53
40.34
40.17
40.01
39.84
39.67
I
CN
N
NH2
HSN797
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

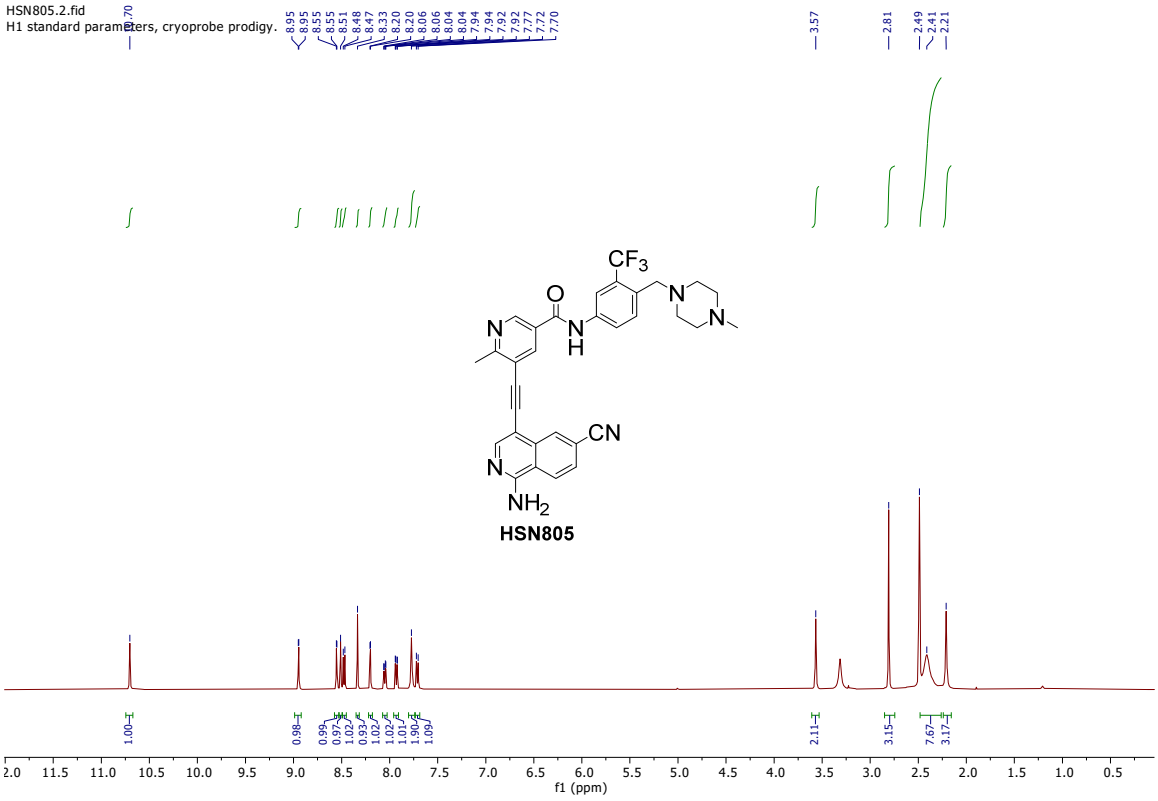

HSN804.1.fid
H1 standard parameters, cryoprobe prodrive
f1 (ppm)

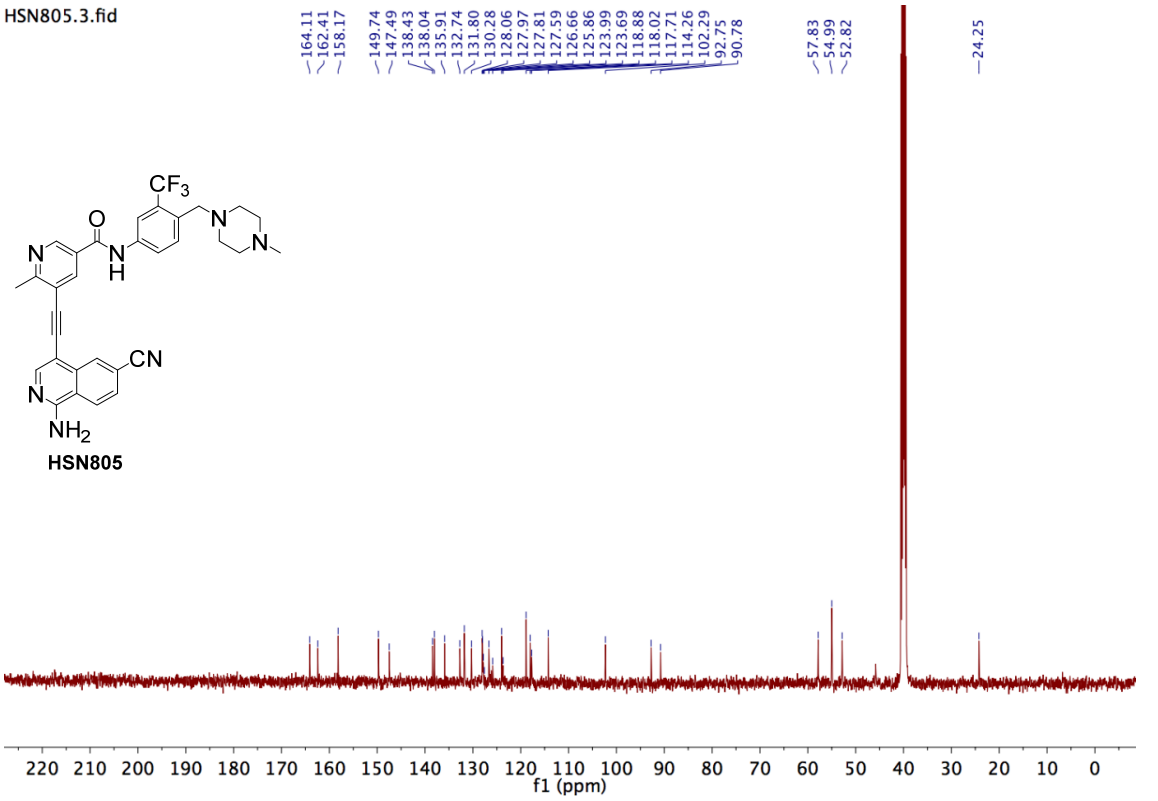

HSN804.2.fid
f1 (ppm)

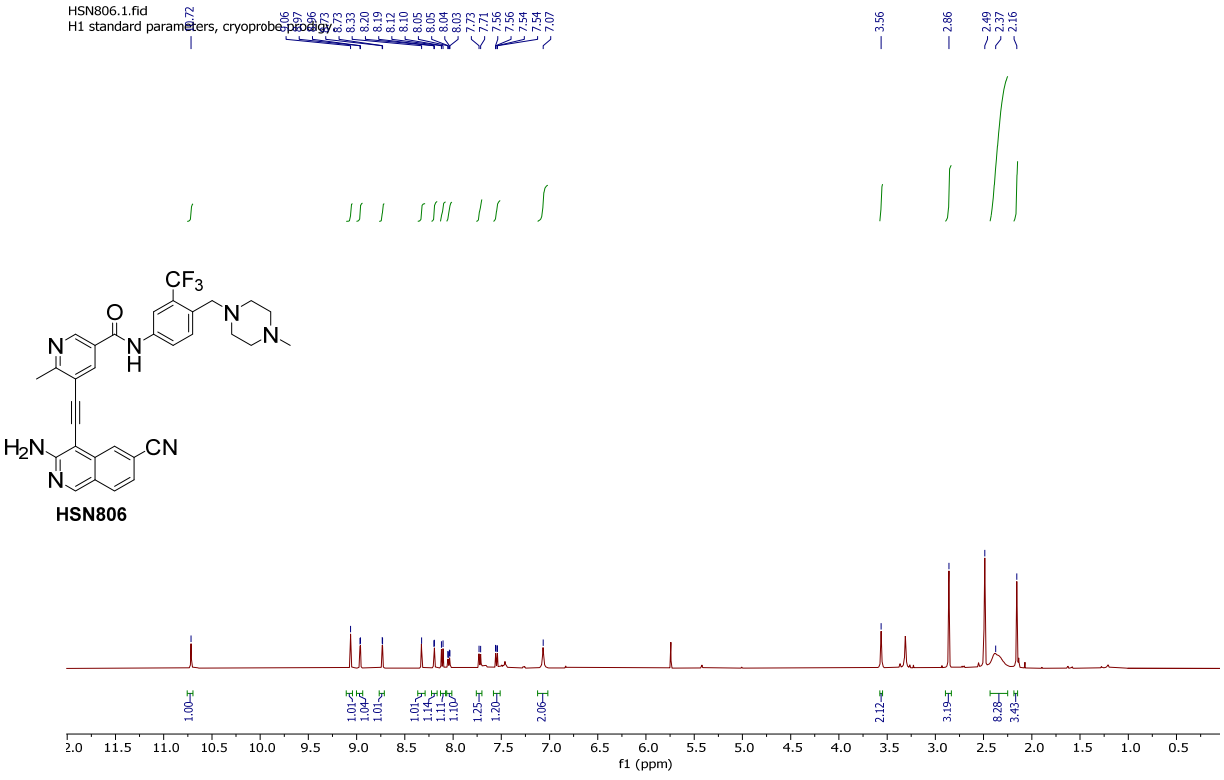
HSN804

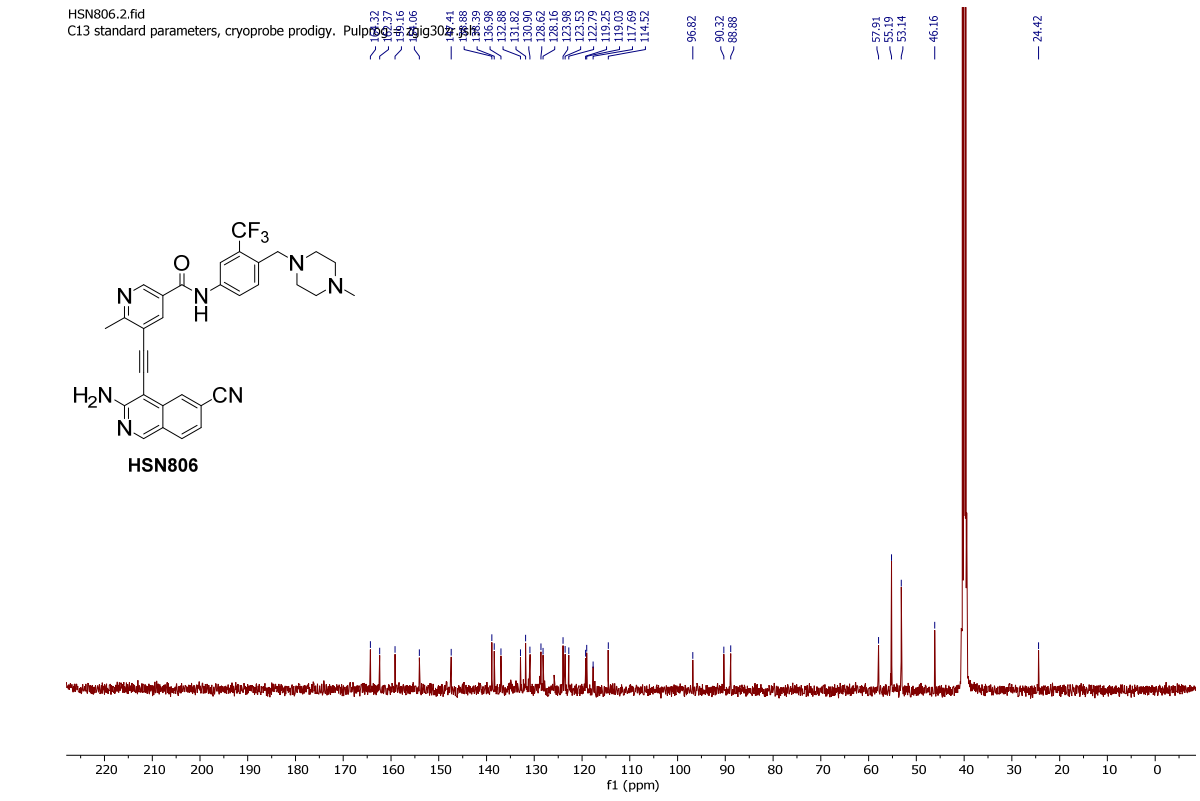

NN631.1.fid
H1 standard parameters, cryoprobe prodigy
HSN631

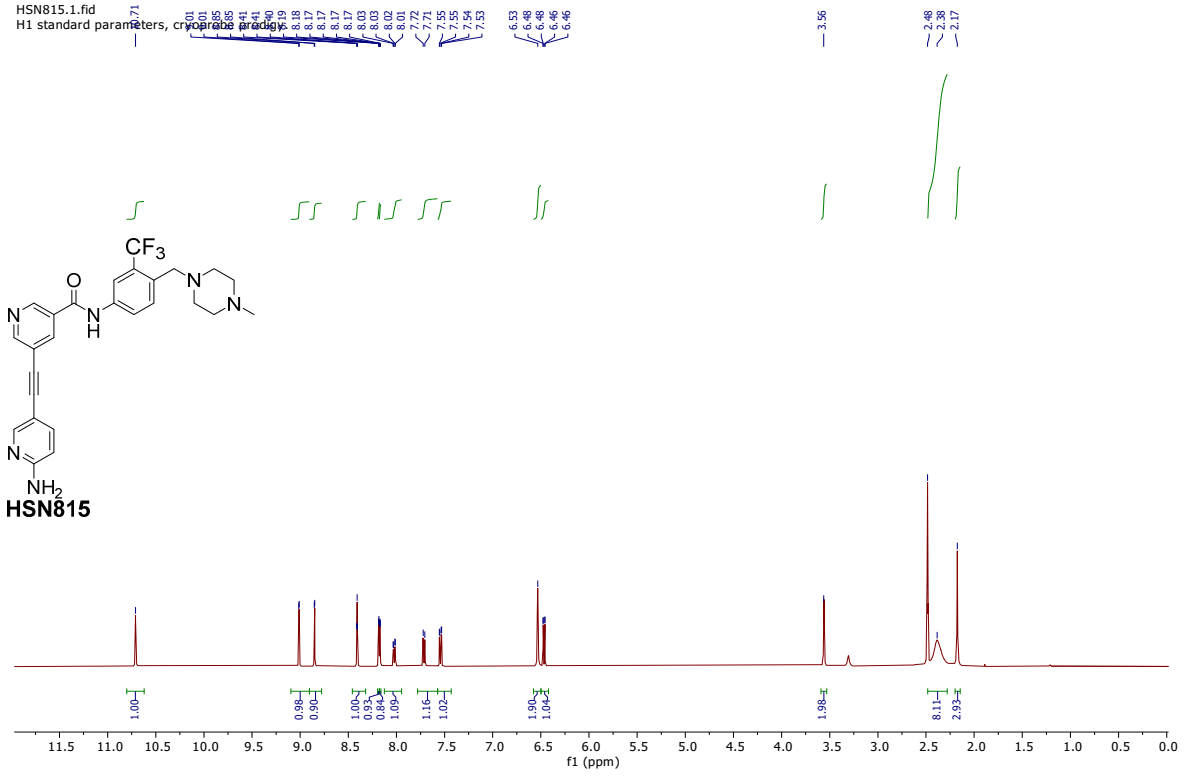

NN631.2.fid
C13 standard parameters, cryoprobe prodigy
HSN631

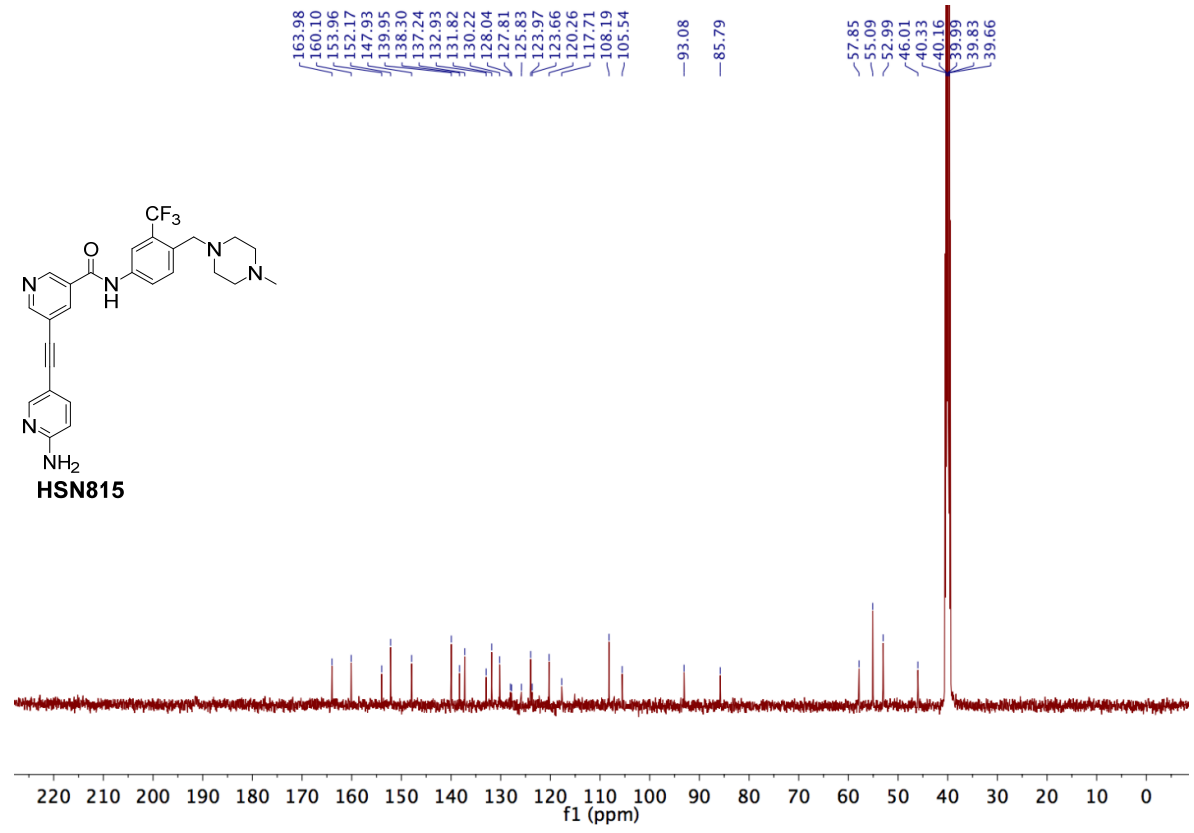

HSN805.2.fid
H1 standard parameters, cryoprobe prodigy.
HSN805
f1 (ppm)

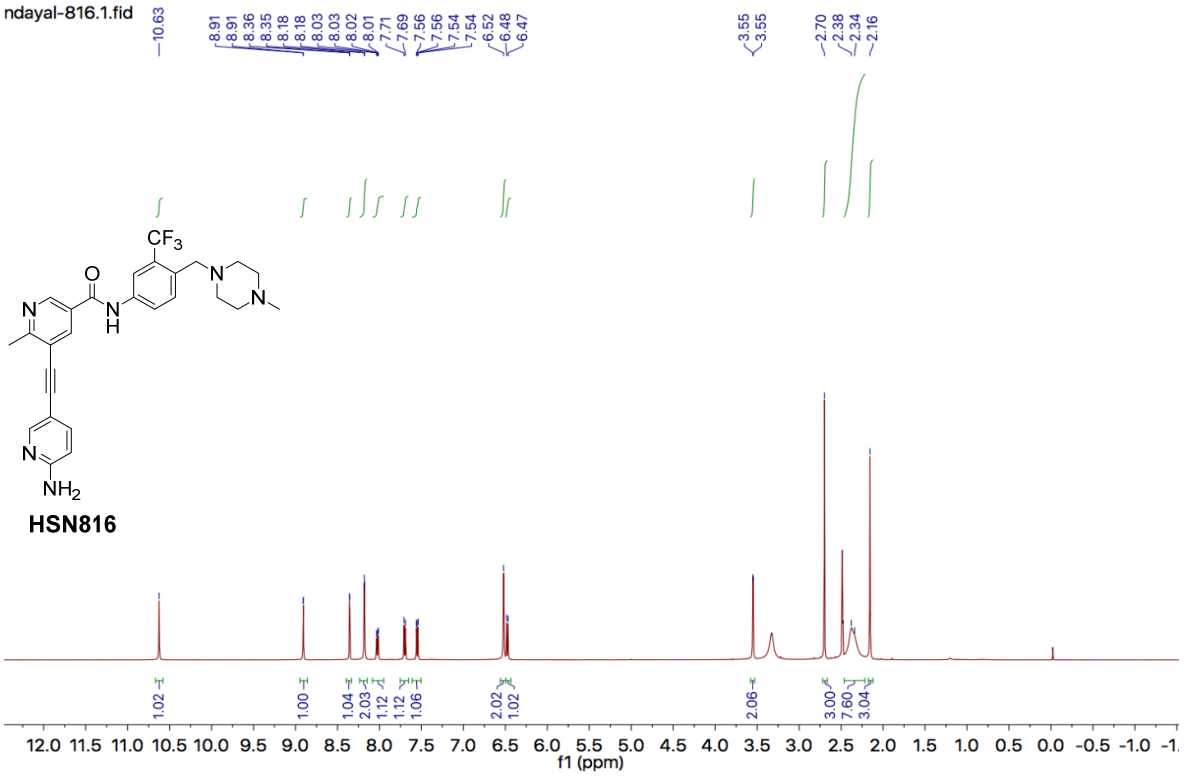

HSN805.3.fid
HSN805
f1 (ppm)

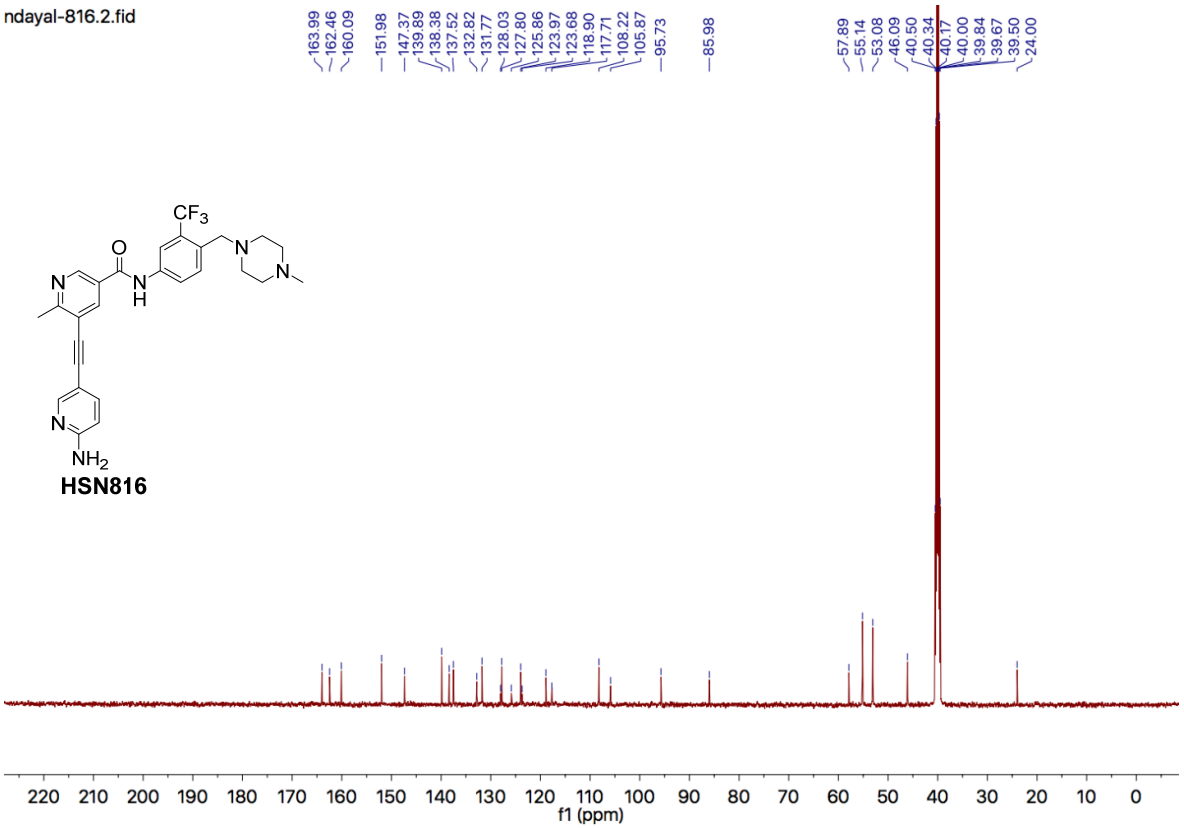

HSN806.1.fid
H1 standard parameters, cryoprobe prodigy
HSN806
f1 (ppm)

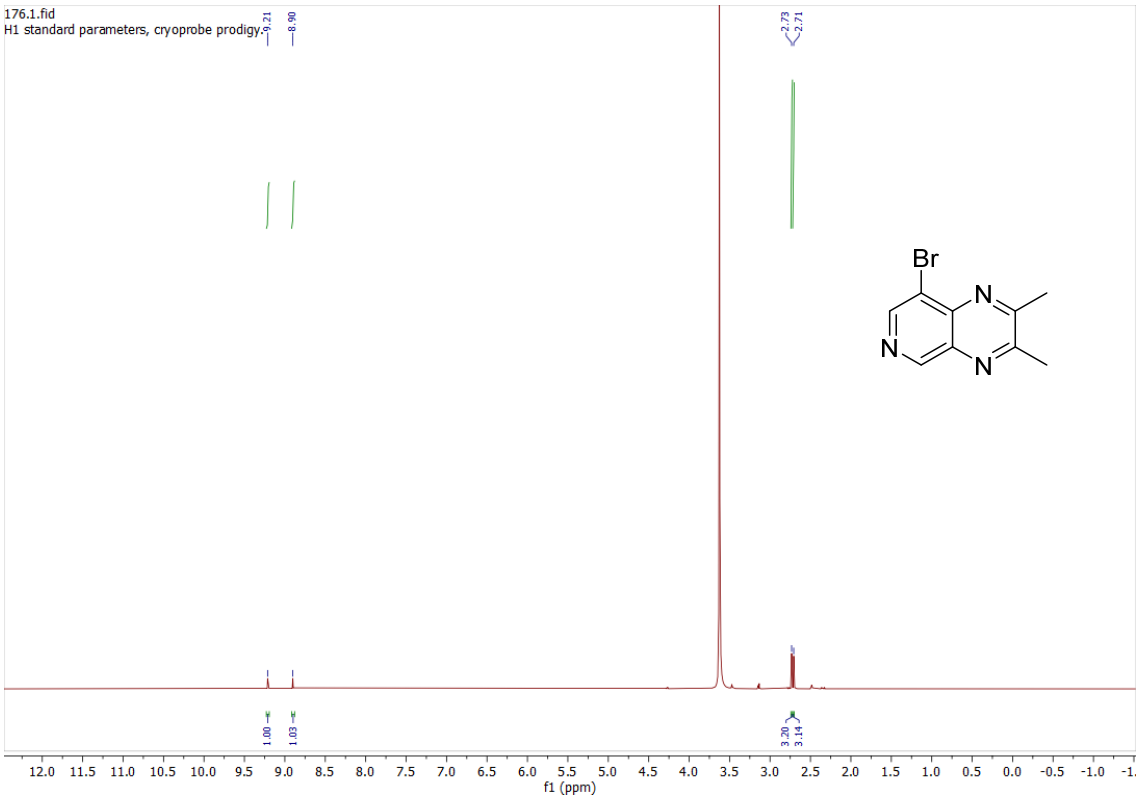

HSN806.2.fid
C13 standard parameters, cryoprobe prodigy
HSN806
f1 (ppm)

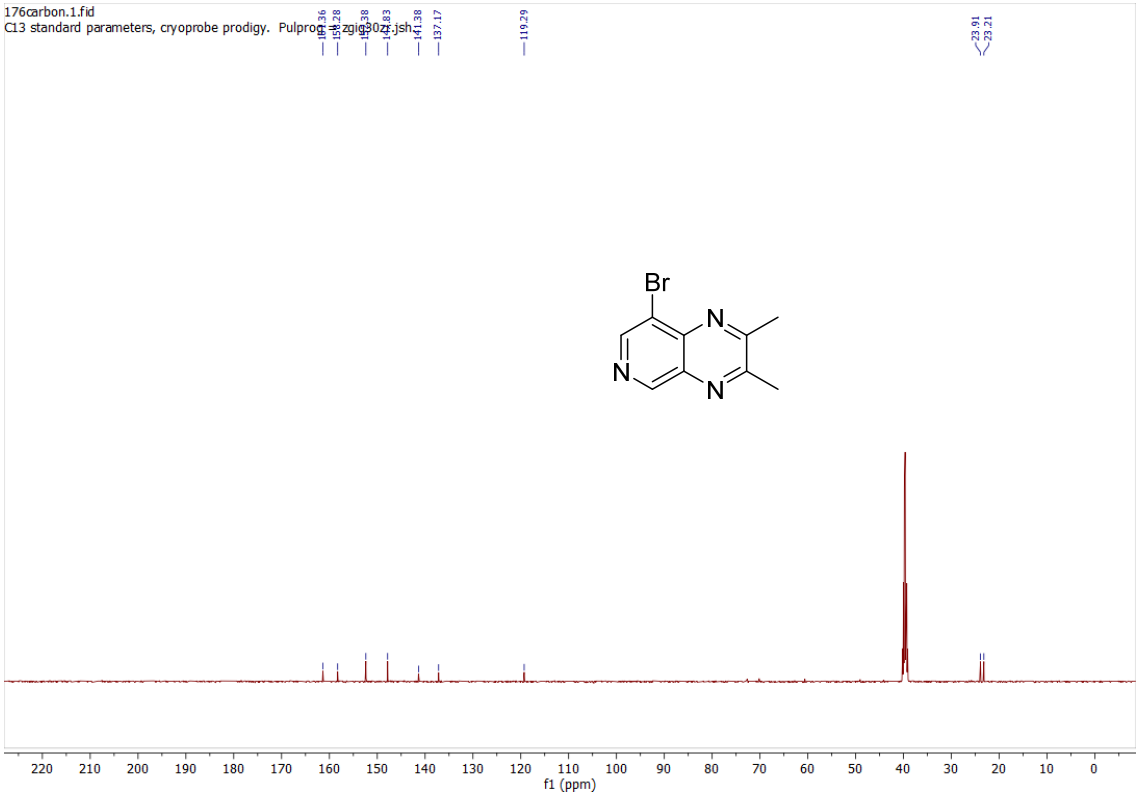

HSN815.1.fid
H1 standard parameters, cryoprobe pradigy
HSN815
f1 (ppm)

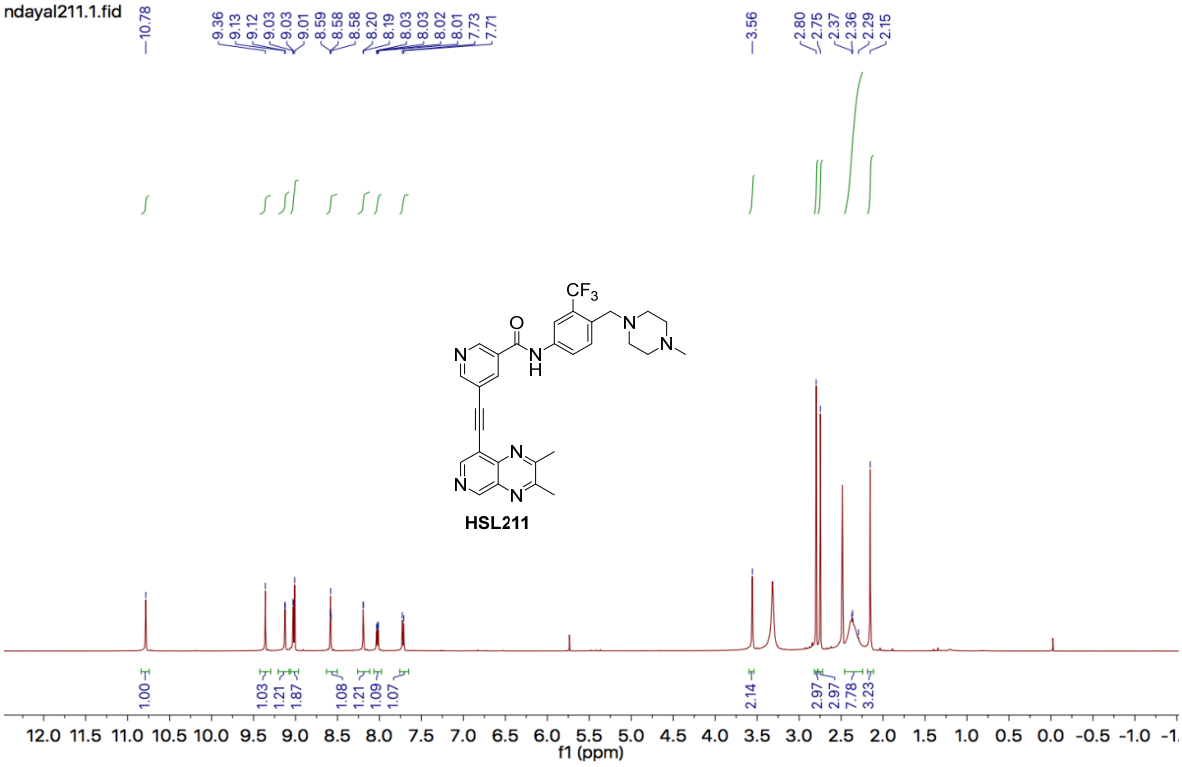

HSN815
f1 (ppm)

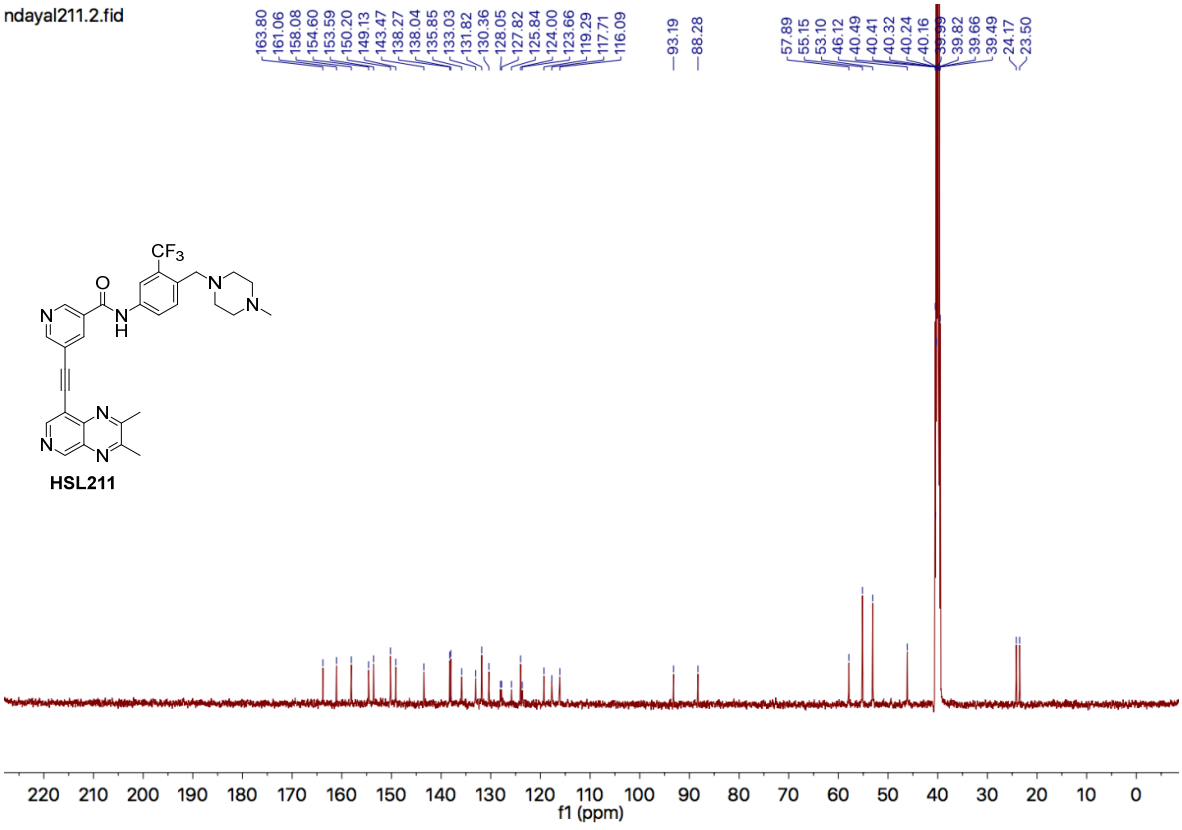

ndayal-816.1.fid
HSN816
f1 (ppm)


ndayal-816.2.fid
HSN816
f1 (ppm)

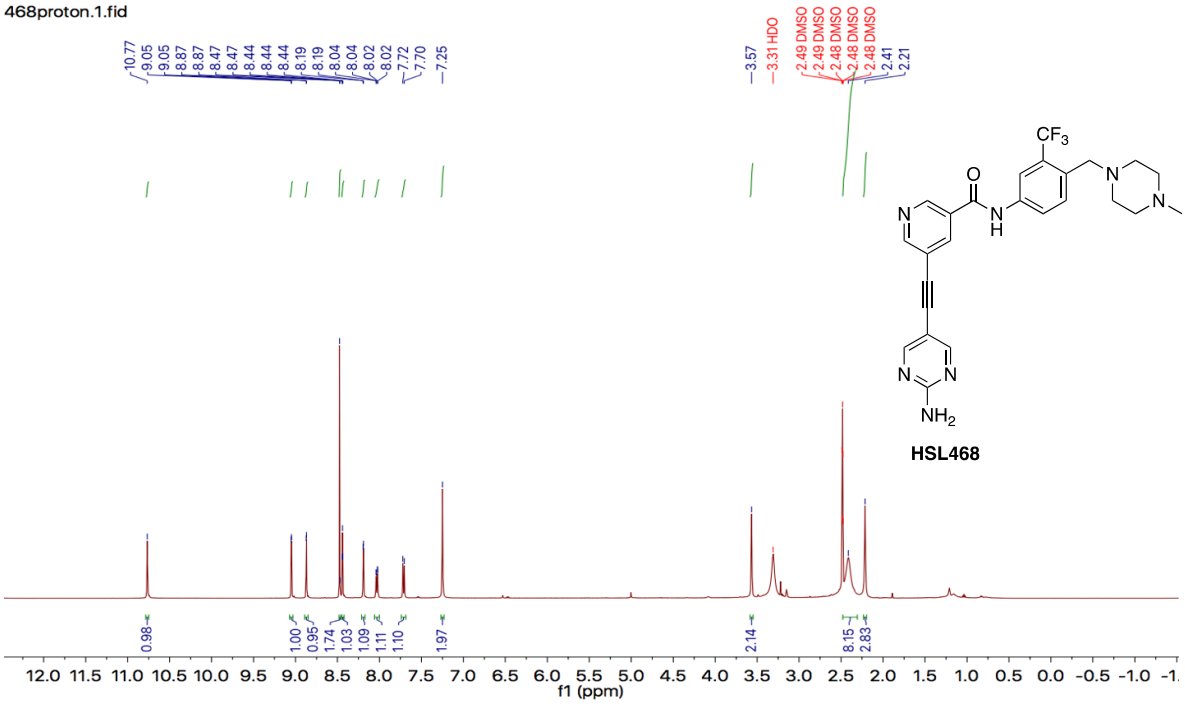

176.1.fid
H1 standard parameters, cryoprobe prodigy
9.21
8.90
2.73
2.71
Br
N
N
N
1.00
1.03
3.20
3.14
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


176carbon.1.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgig30
159.36
154.28
145.38
143.83
141.38
137.17
119.29
23.91
23.21
Br
N
N
N
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)


ndayal211.1.fid
10.78
9.36
9.13
9.12
9.03
9.03
9.01
8.59
8.58
8.58
8.58
8.20
8.19
8.03
8.03
8.02
8.01
7.73
7.71
3.56
2.80
2.75
2.37
2.36
2.29
2.15
CF3
O
N
H
N
N
N
N
HSL211
1.00
1.03
1.21
1.87
1.08
1.21
1.09
1.07
2.14
2.97
2.97
7.78
3.23
12.0 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 0.0 -0.5 -1.0
f1 (ppm)


ndayal211.2.fid
163.80
161.06
158.08
154.60
153.59
150.20
149.13
143.47
138.27
138.04
135.85
133.03
131.82
130.36
128.05
127.82
125.84
124.00
123.66
119.29
117.71
116.09
93.19
88.28
57.89
55.15
53.10
46.12
40.49
40.41
40.32
40.24
40.16
39.99
39.82
39.66
39.49
24.17
23.50
CF3
O
N
H
N
N
N
N
HSL211
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

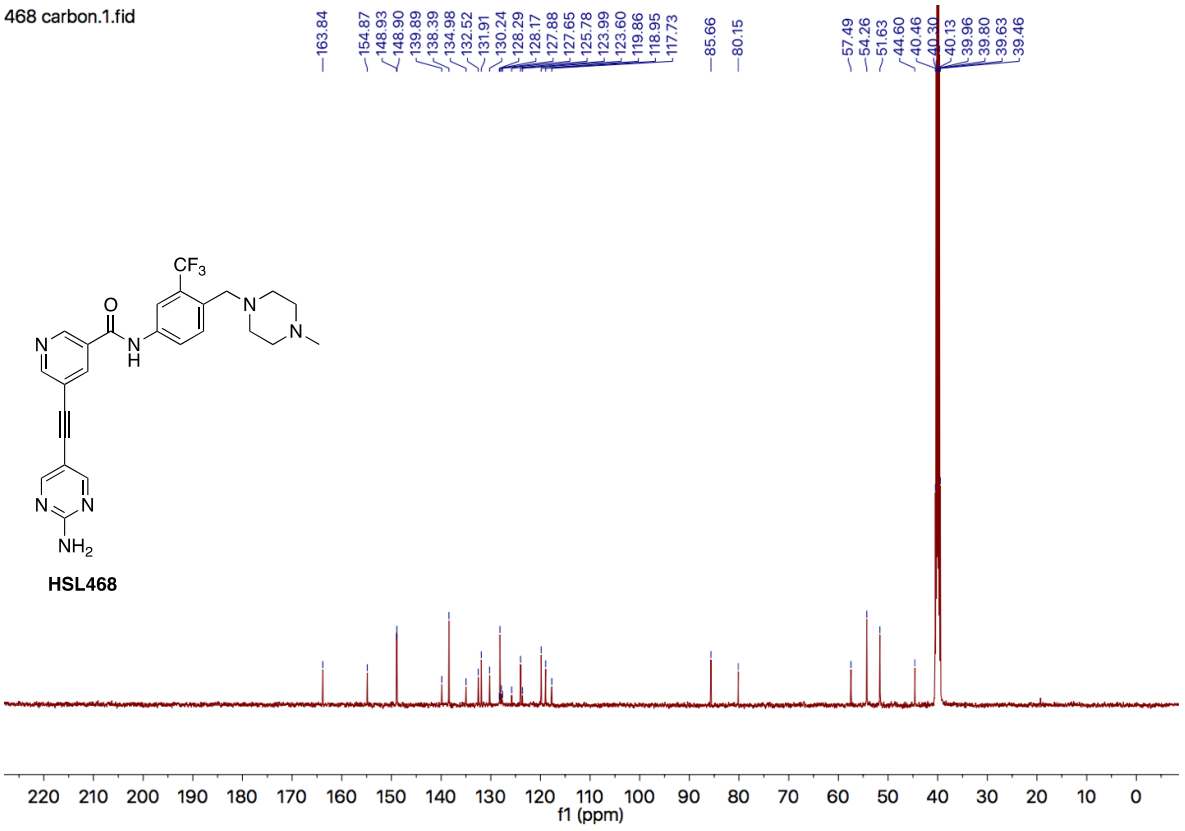
468proton.1.fid



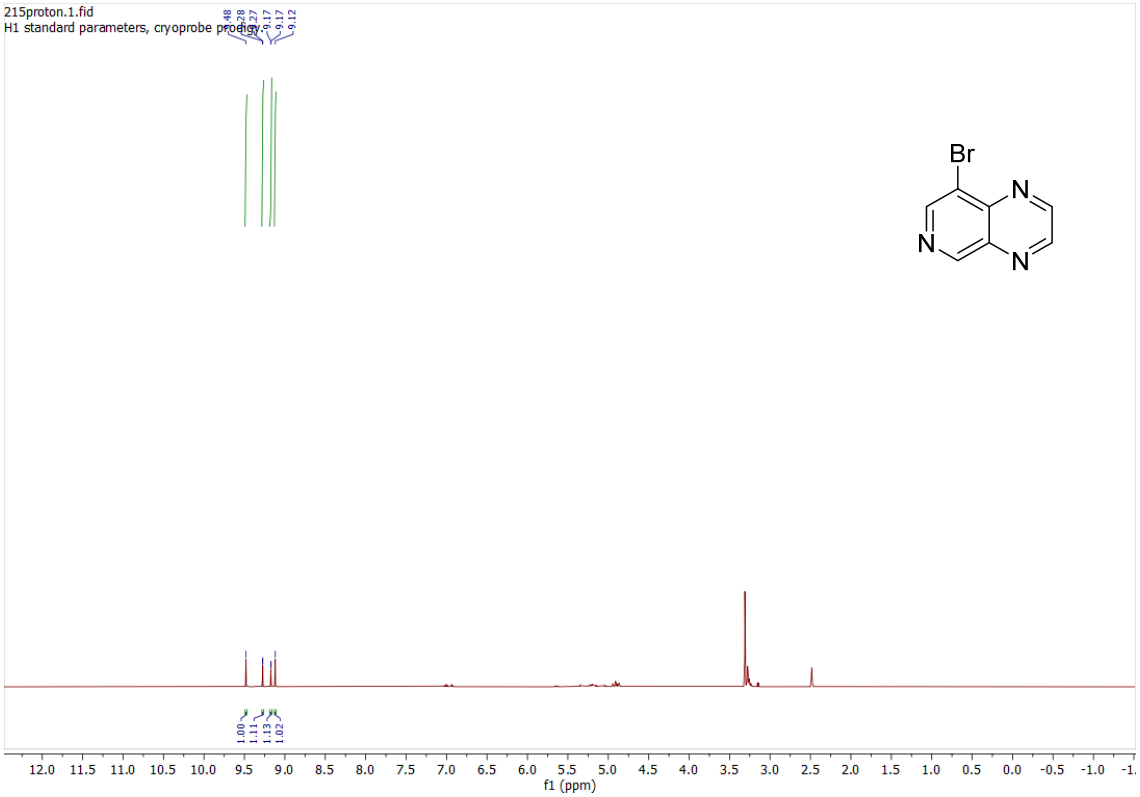
HSL468

\

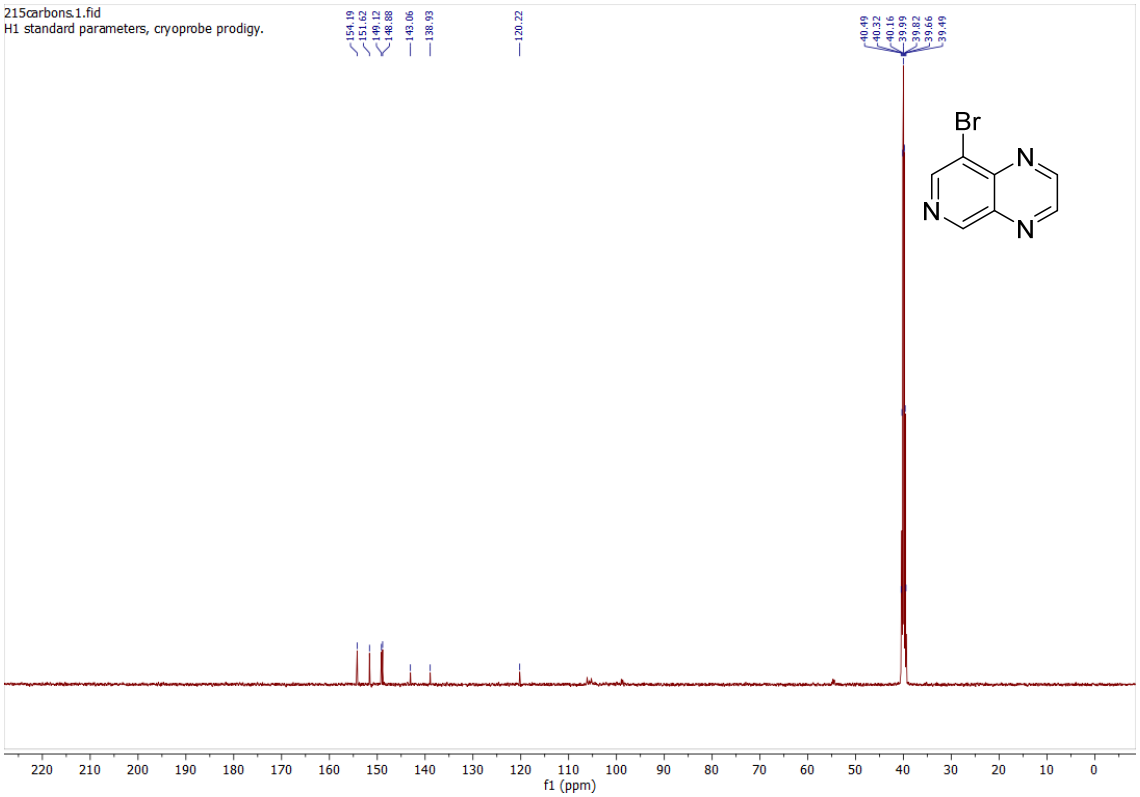
468 carbon.1.fid

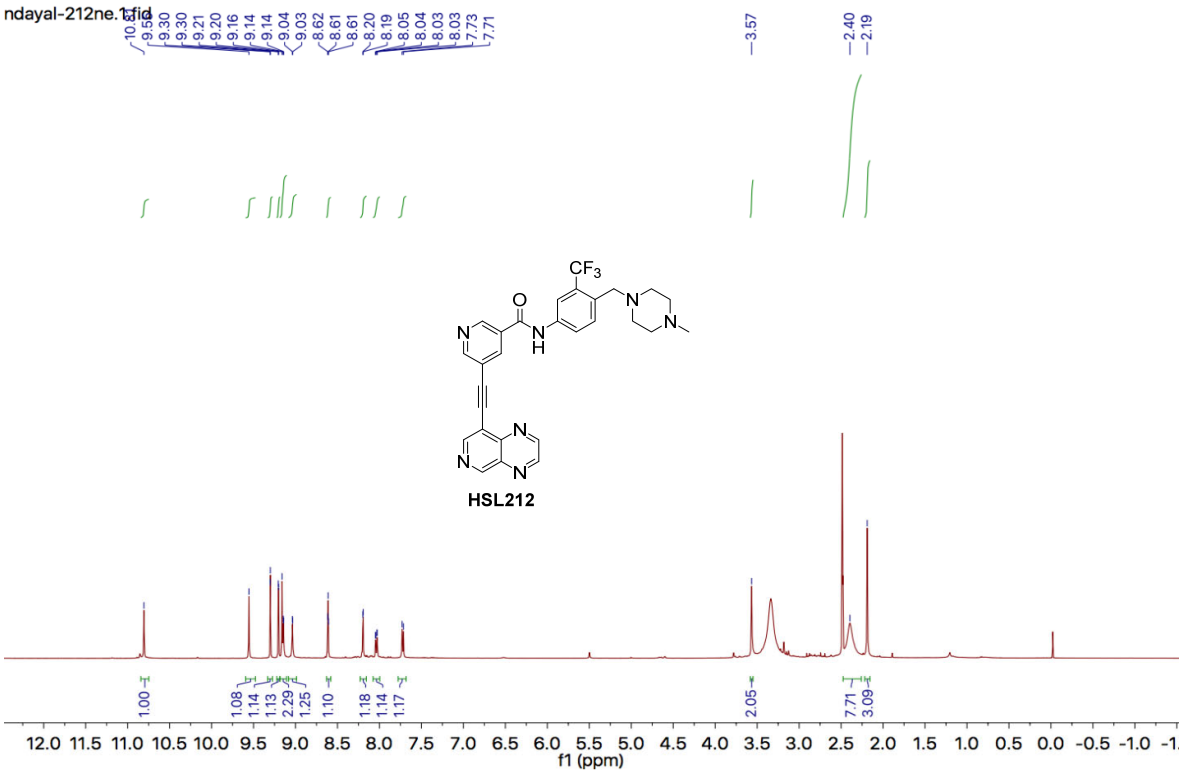


HSL468

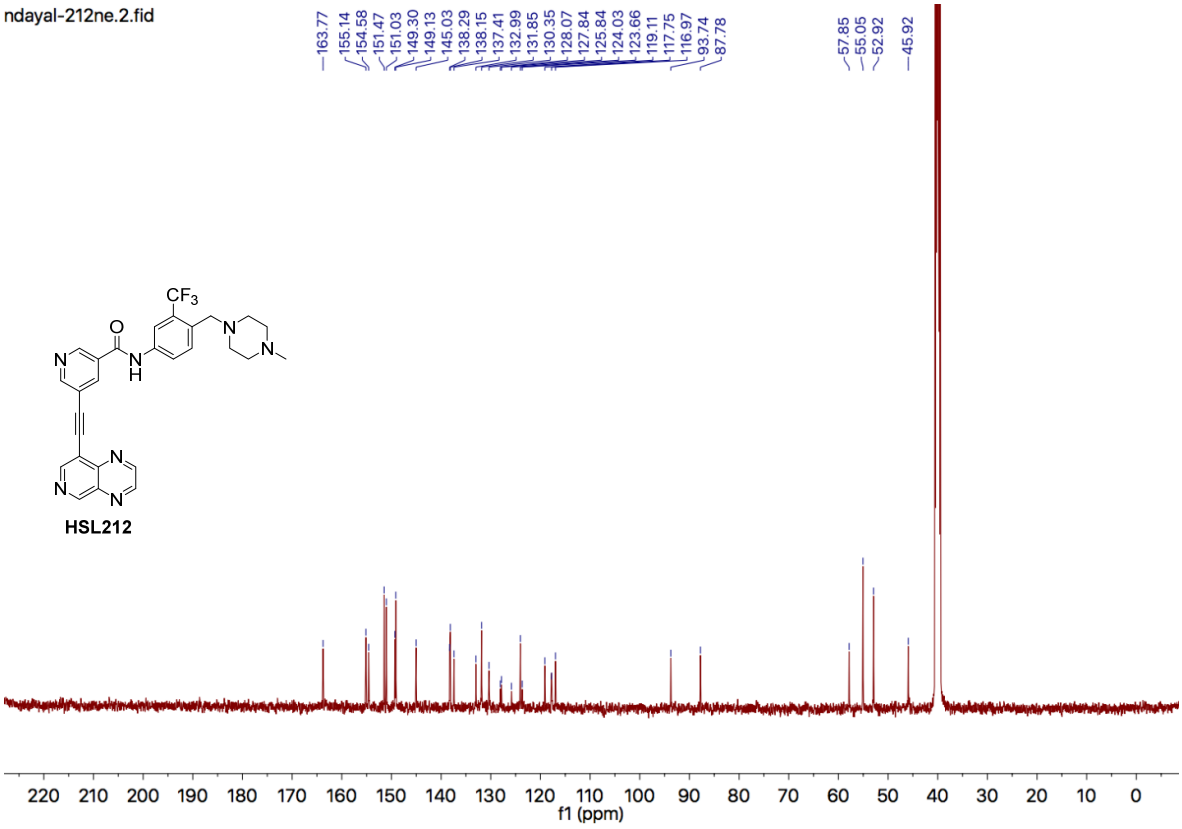

215proton.1.fid
H1 standard parameters, cryoprobe prodigy
9.48
9.28
9.27
9.17
9.17
9.12
Br
N
N
N
1.00
1.11
1.13
1.02
f1 (ppm)

\

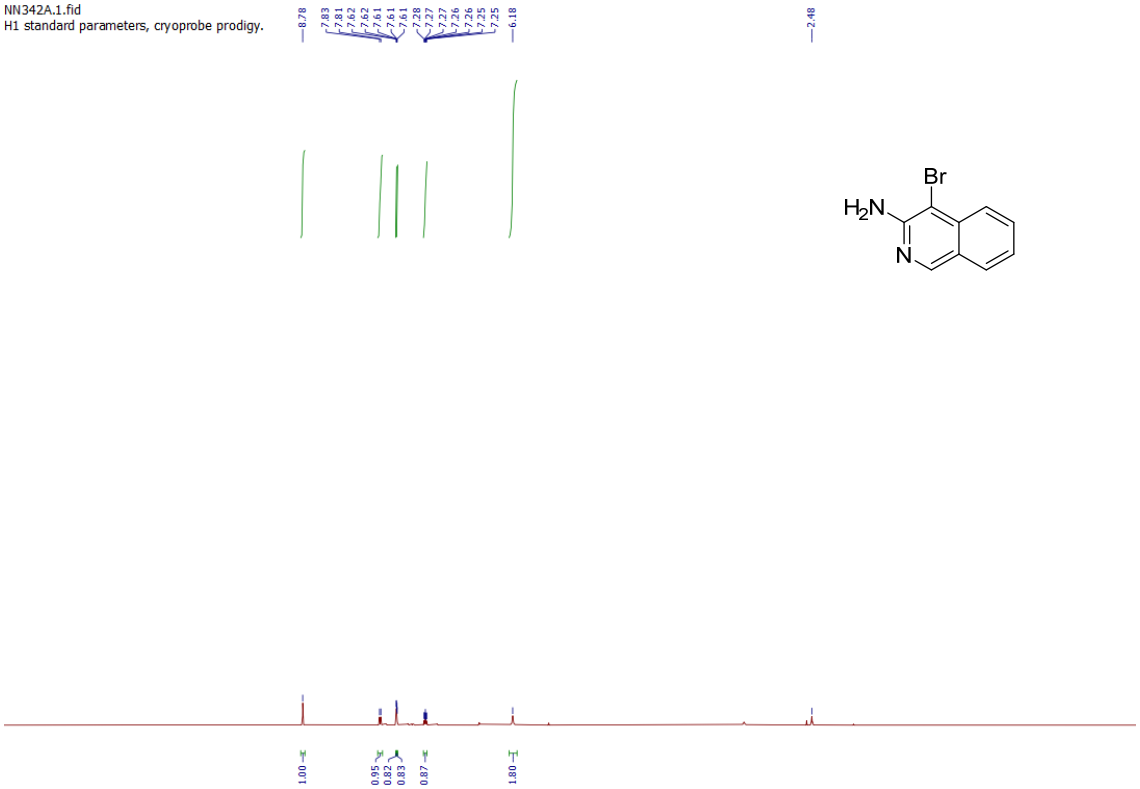

215carbons.1.fid
H1 standard parameters, cryoprobe prodigy.
154.19
151.62
149.12
148.88
143.06
138.93
120.22
40.49
40.32
40.16
39.99
39.82
39.66
39.49
Br
N
N
N
f1 (ppm)


ndayal-212ne.1.fid
HSL212
f1 (ppm)

\

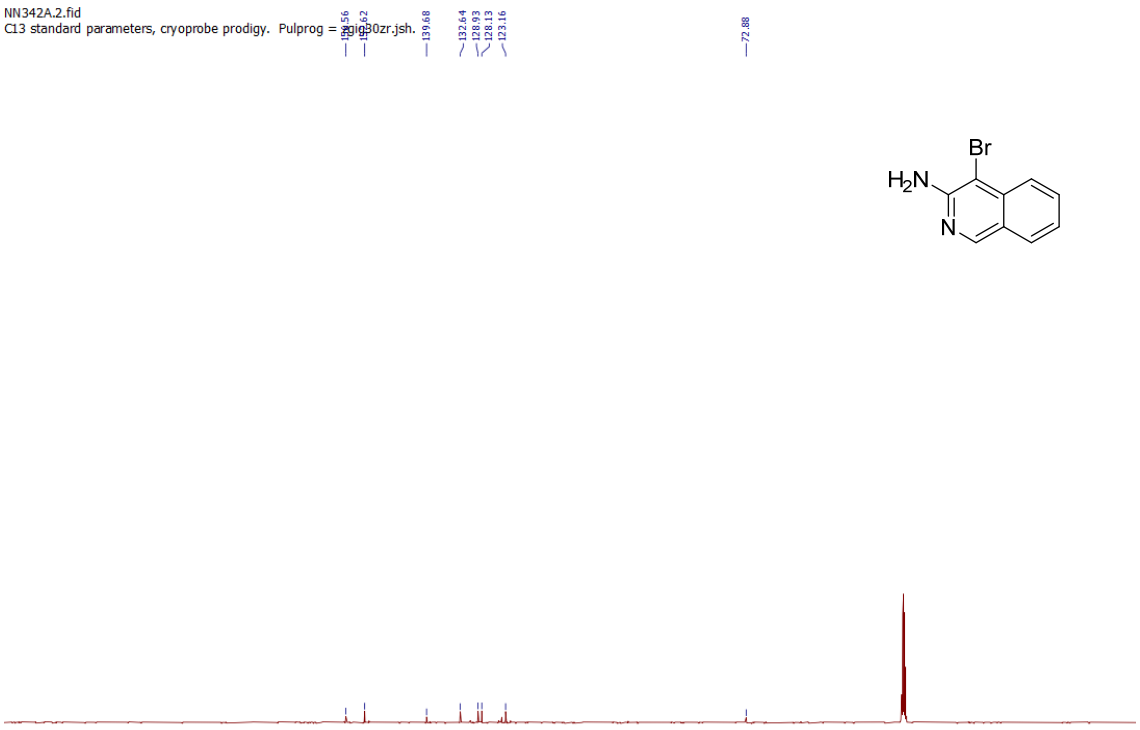

ndayal-212ne.2.fid
HSL212
f1 (ppm)

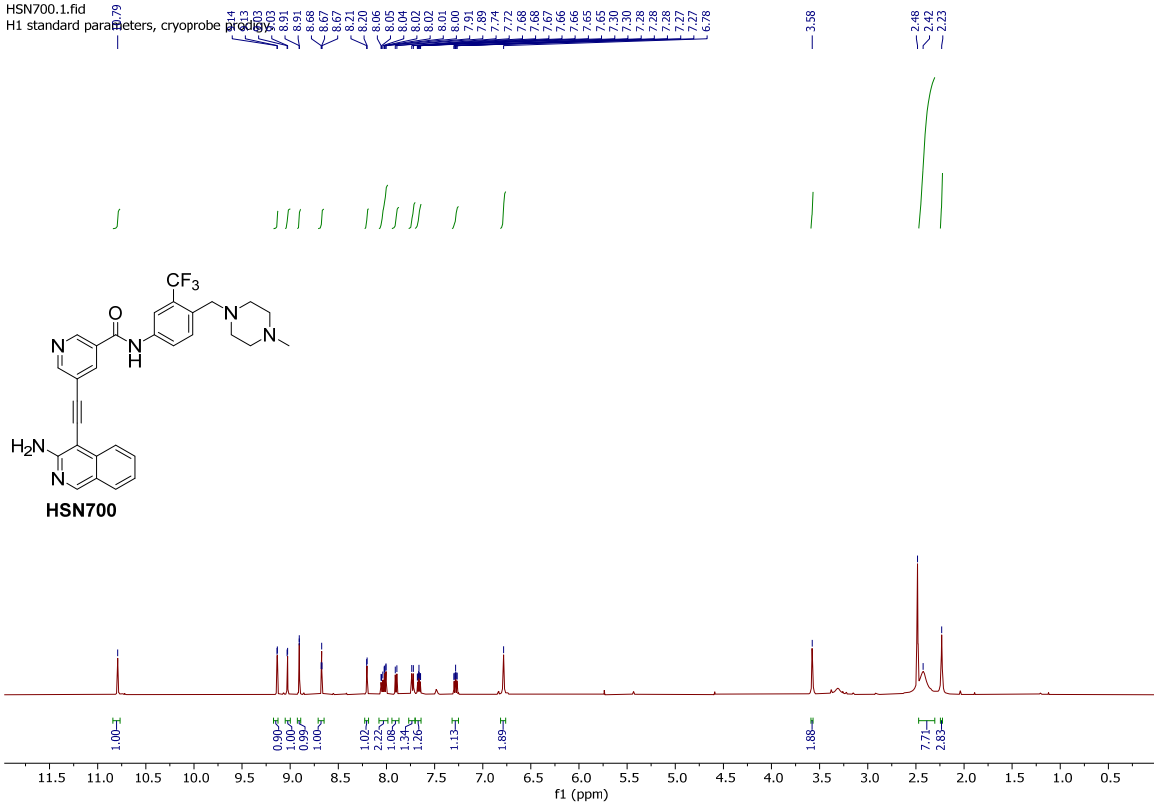
NN342A.1.fid
H1 standard parameters, cryoprobe prodigy.



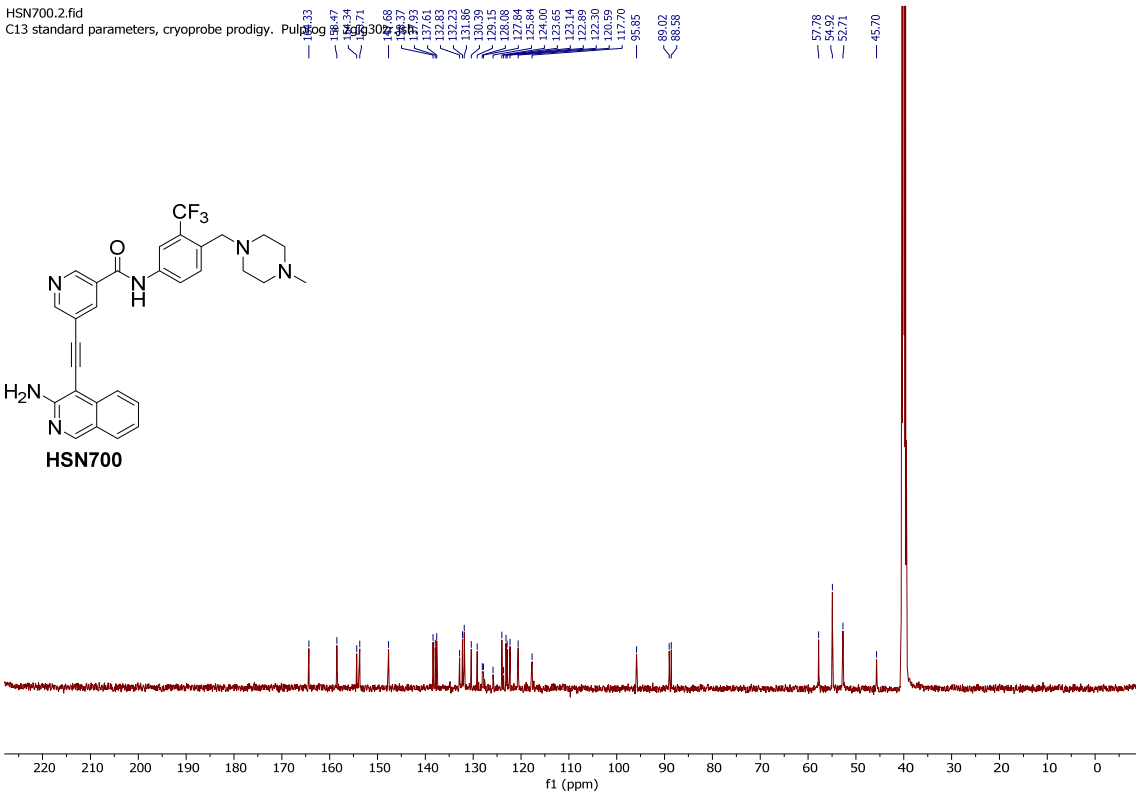
\

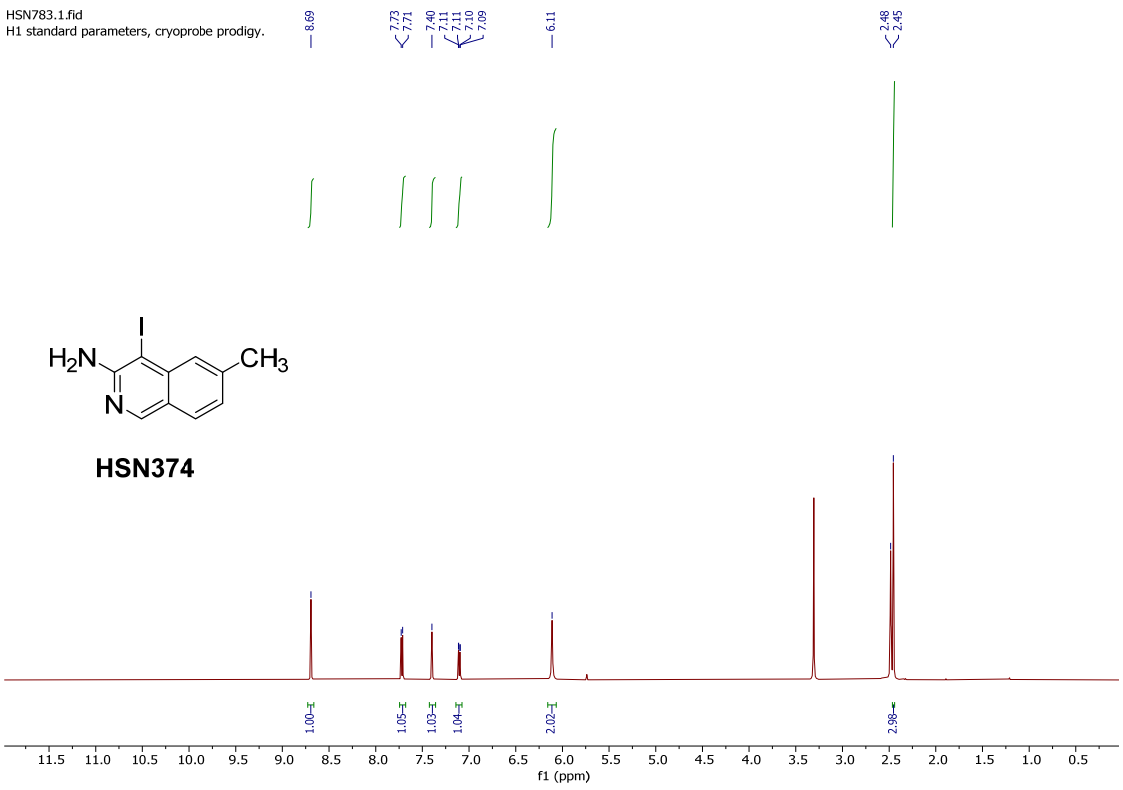
NN342A.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog = zg30zr.jsh.



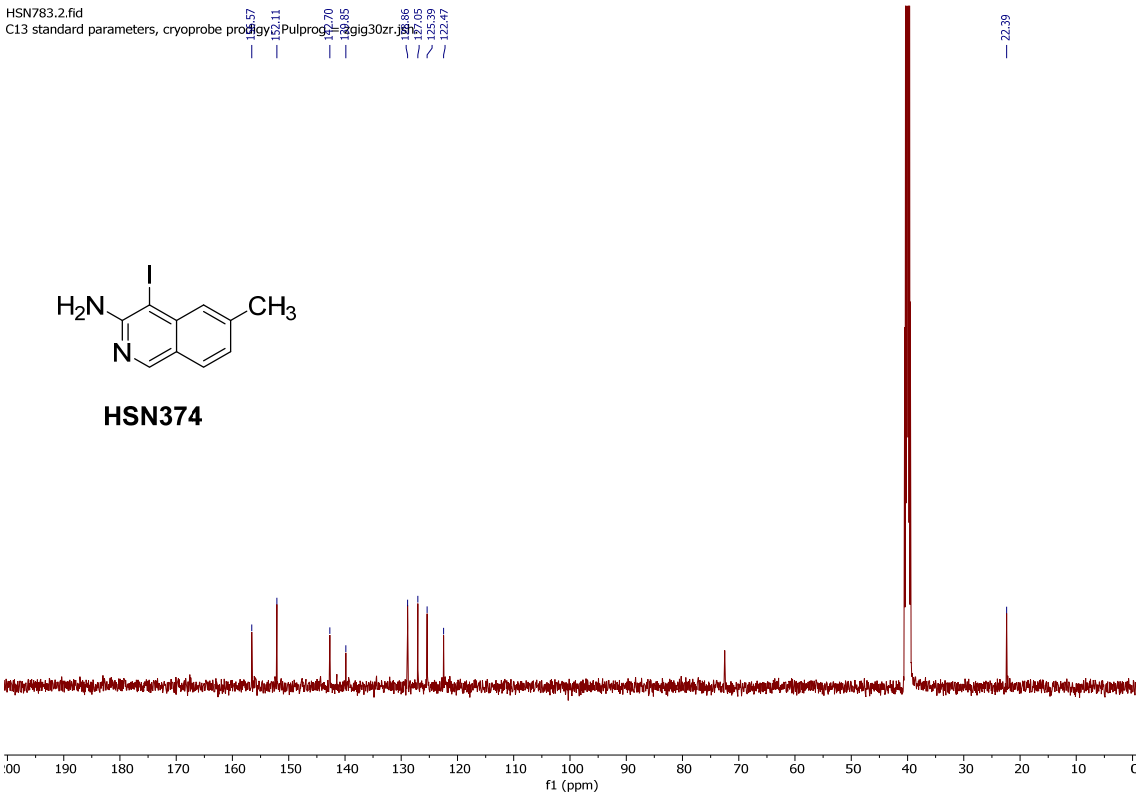

HSN700.1.fid
H1 standard parameters, cryoprobe prodigy
HSN700
f1 (ppm)

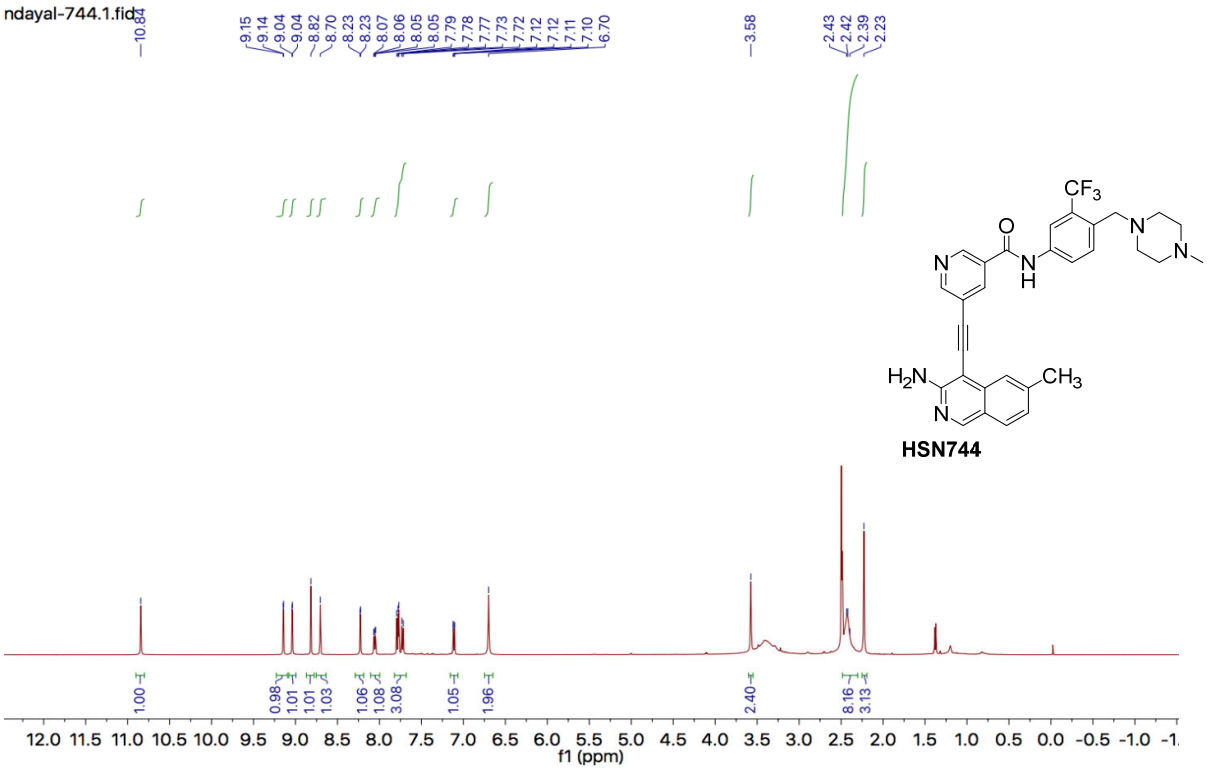
\


HSN700.2.fid
C13 standard parameters, cryoprobe prodigy. Pulprog zgdg30
HSN700
f1 (ppm)

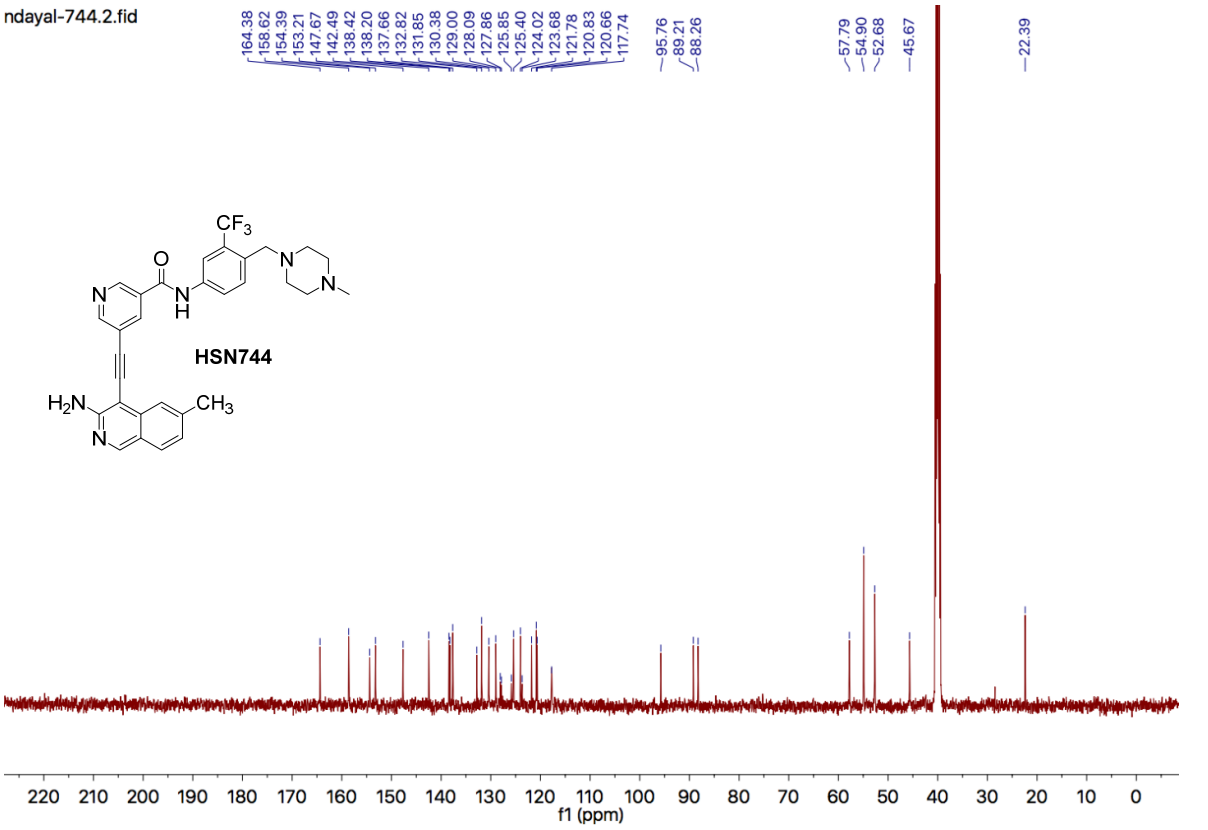

HSN783.1.fid
H1 standard parameters, cryoprobe prodigy.
8.69
7.73
7.71
7.40
7.11
7.11
7.10
7.09
6.11
2.48
2.45
I
H2N
CH3
N
HSN374
1.00
1.05
1.03
1.04
2.02
2.98
11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

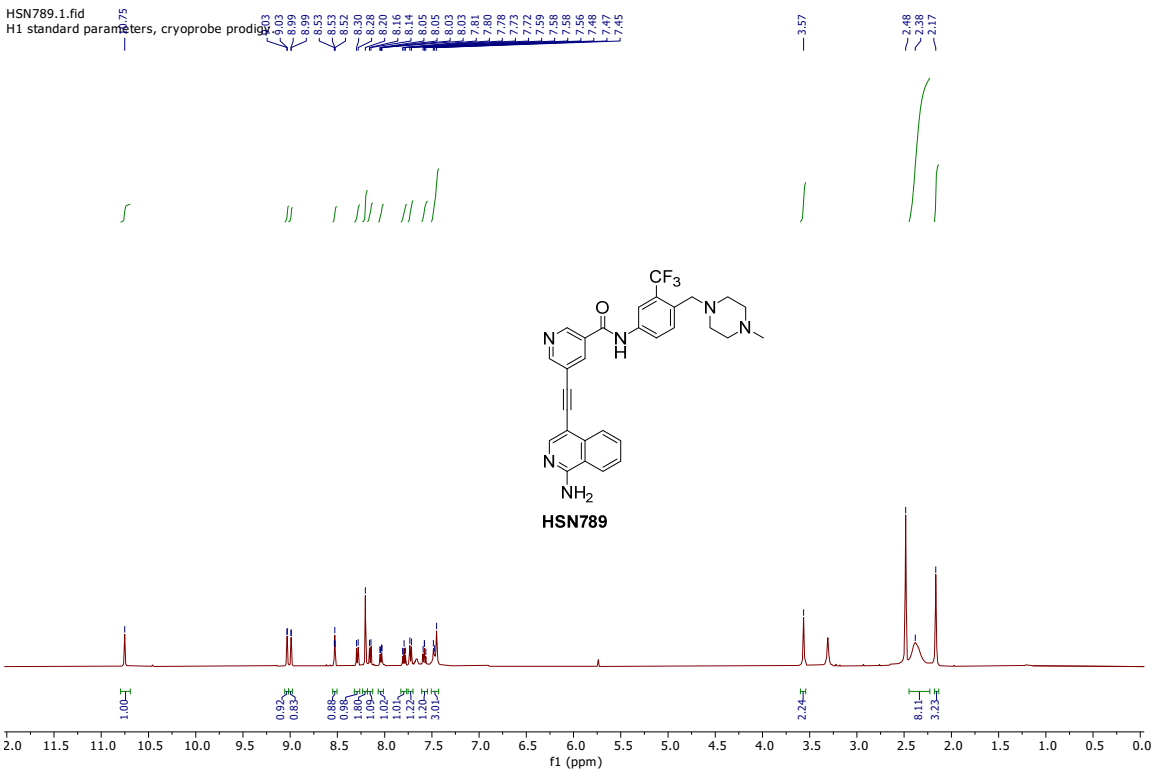
\\\

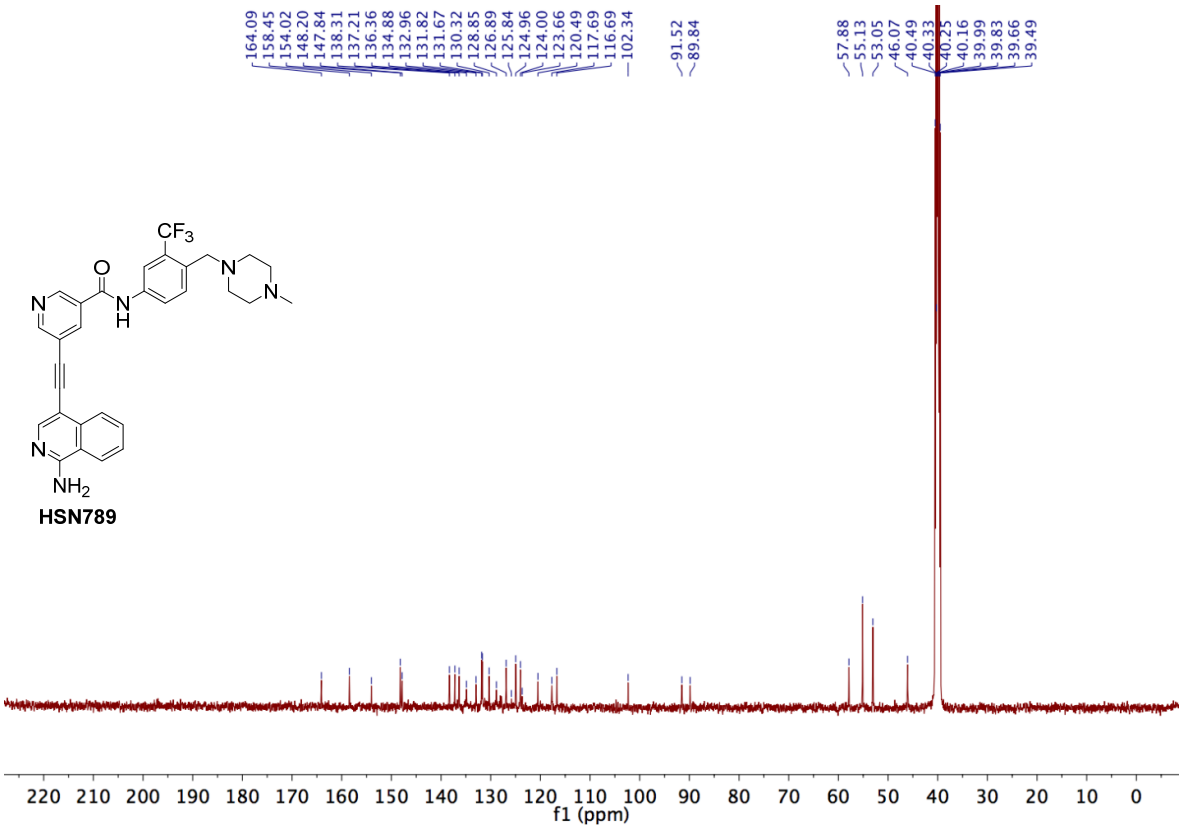

HSN783.2.fid
C13 standard parameters, cryoprobe prodigy, Pulprog: zgig30zr.jsh
156.57
152.11
137.70
130.85
130.86
127.05
125.39
122.47
22.39
I
H2N
CH3
N
HSN374
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)


ndayal-744.1.fid
10.84
9.15
9.14
9.04
9.04
8.82
8.70
8.23
8.23
8.07
8.06
8.05
8.05
7.79
7.78
7.77
7.73
7.72
7.12
7.12
7.11
7.10
6.70
3.58
2.43
2.42
2.39
2.23
HSN744
1.00
0.98
1.01
1.01
1.03
1.06
1.08
3.08
1.05
1.96
2.40
8.16
3.13
f1 (ppm)

\\


ndayal-744.2.fid
164.38
158.62
154.39
153.21
147.67
142.49
138.42
138.20
137.66
132.82
131.85
130.38
129.00
128.09
127.86
125.85
125.40
124.02
123.68
121.78
120.83
120.66
117.74
95.76
89.21
88.26
57.79
54.90
52.68
45.67
22.39
HSN744
f1 (ppm)


HSN789.1.fid
H1 standard parameters, cryoprobe prodigy
HSN789
f1 (ppm)


HSN789
f1 (ppm)

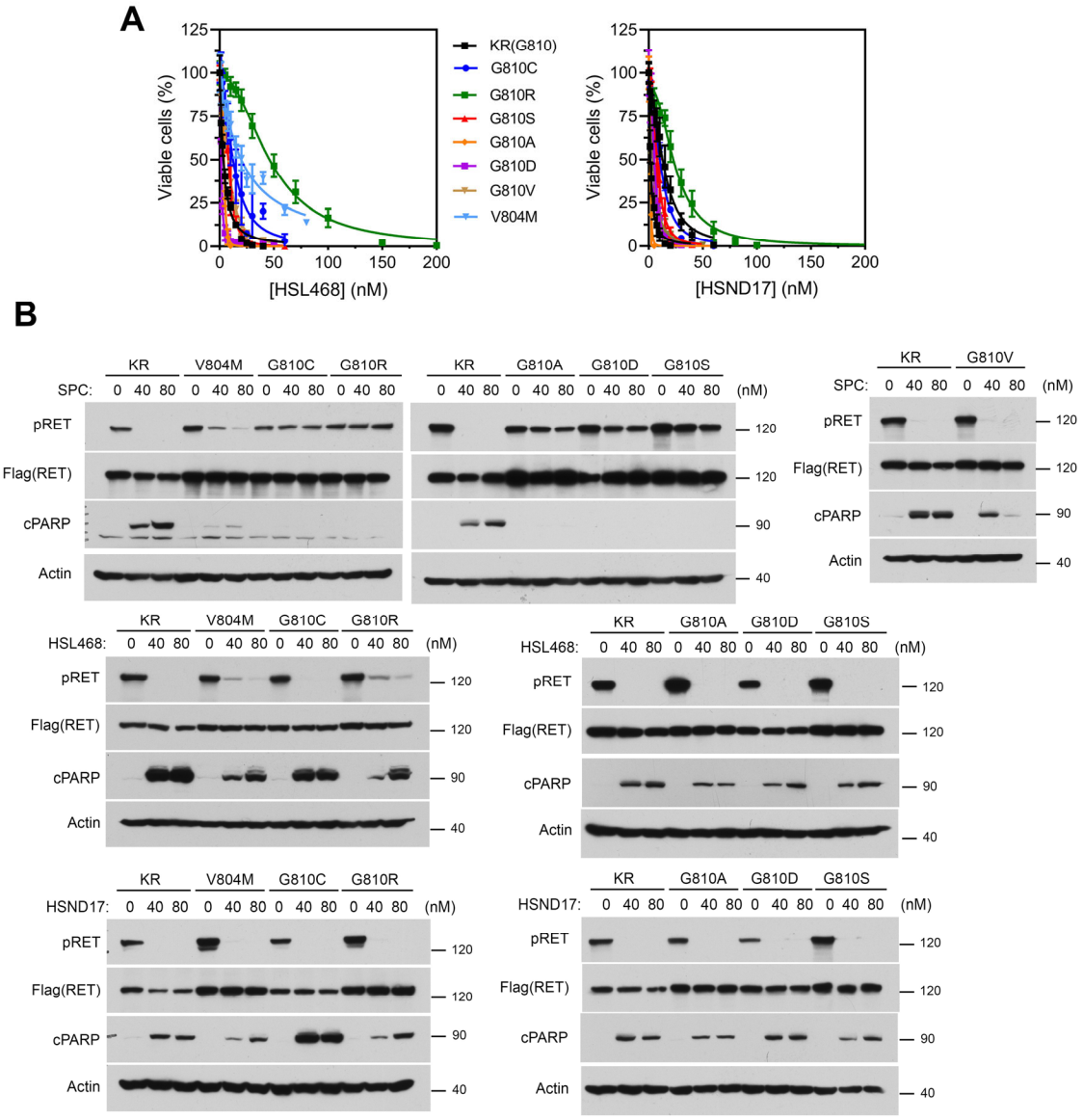


Fig. S2. Sensitivity of different G810 mutants to LOXO292, HSL468 and HSND17. **A,** IC_{50} curves of B/KR and G810 mutant cells to HSL468 and HSND17. **B,** Immunoblotting analyses of RET TKIs on inhibition of KR and its G810 mutants. For analysis of pRET, cells were treated with the indicated concentrations of compounds for 4 h, whereas for cPARP analysis, cells were treated for 16 h. SPC, Selpercatinib

Table S2. Eurofins KINOMEscan™ testing with 10 nM compound concentration.

Kinome panel selectivity: The kinome selectivities of three compounds and ponatinib, which is an FDA approved drug with alkynyl aryl motif, were evaluated at the Contract Research Organization, Eurofins, against ~450 kinases, using their gold standard KINOME*scan*™ screening platform. The KINOME*scan*™ screening platform uses a proprietary active site-directed competition binding assay to quantitatively measure interactions between test compounds and more than 450 human kinases and disease relevant mutant variants. Active site or allosteric binders prevent kinase binding to the immobilized ligand, and therefore the amount of the kinase captured on the solid support, as a percentage of control (no added compound) correlates to binding affinity. Compounds with %Ctr (i.e. control) = 100% denote no binding whereas %Ctr (i.e. control) → 0% denote very strong binders. The compounds were tested at 10 nM and 100 nM.

Kinase	% of control at 10 nM			
	Ponatinib	HSL468	HSL476	HSN608
AAK1	100	100	90	90
ABL1(E255K)-phosphorylated	19	11	20	14
ABL1(F317I)-nonphosphorylated	16	0	4.8	4.4
ABL1(F317I)-phosphorylated	25	23	14	16
ABL1(F317L)-nonphosphorylated	13	0	3.2	0.4
ABL1(F317L)-phosphorylated	5.3	7.5	6.8	1.3
ABL1(H396P)-nonphosphorylated	1.3	0	0.4	0.1
ABL1(H396P)-phosphorylated	17	6.7	11	7.9
ABL1(M351T)-phosphorylated	11	7.9	10	2.8
ABL1(Q252H)-nonphosphorylated	7.6	0	2.6	3.3
ABL1(Q252H)-phosphorylated	18	6.9	16	14
ABL1(T315I)-nonphosphorylated	11	0	5.5	5.5
ABL1(T315I)-phosphorylated	17	9.8	12	3.8
ABL1(Y253F)-phosphorylated	14	3.7	6.5	4.6
ABL1-nonphosphorylated	7.7	0.6	2	1.4
ABL1-phosphorylated	16	5.5	6.7	5.5
ABL2	37	23	32	15
ACVR1	100	100	100	100
ACVR1B	95	100	84	100
ACVR2A	99	100	100	100
ACVR2B	94	100	96	100
ACVRL1	100	100	100	100
ADCK3	100	100	100	100
ADCK4	100	95	95	100
AKT1	88	100	100	100
AKT2	99	77	100	92
AKT3	100	86	78	98
ALK	96	100	100	100
ALK(C1156Y)	87	70	100	100
ALK(L1196M)	91	64	98	91
AMPK-alpha1	100	82	95	94
AMPK-alpha2	100	50	100	100
ANKK1	100	100	69	70
ARK5	94	92	100	79

ASK1	100	97	72	100
ASK2	96	97	96	97
AURKA	86	95	93	100
AURKB	100	100	93	47
AURKC	100	61	85	74
AXL	90	92	36	49
BIKE	100	94	87	84
BLK	8.4	5.1	6.5	12
BMPR1A	87	100	94	95
BMPR1B	100	98	75	82
BMPR2	94	100	100	100
BMX	100	100	100	100
BRAF	55	51	48	25
BRAF(V600E)	28	30	28	11
BRK	98	85	97	100
BRSK1	97	96	100	100
BRSK2	93	100	72	84
BTK	85	84	100	89
BUB1	89	100	100	100
CAMK1	100	100	74	83
CAMK1B	91	92	99	91
CAMK1D	100	100	92	95
CAMK1G	100	95	92	95
CAMK2A	100	100	100	100
CAMK2B	100	100	100	100
CAMK2D	100	87	100	100
CAMK2G	100	100	83	100
CAMK4	100	100	100	100
CAMKK1	100	89	70	95
CAMKK2	90	93	83	98
CASK	83	90	88	93
CDC2L1	93	94	86	81
CDC2L2	92	100	97	93
CDC2L5	44	49	49	86
CDK11	56	97	72	69
CDK2	100	100	82	93
CDK3	100	83	77	100
CDK4	85	81	83	71
CDK4-cyclinD1	99	100	93	93
CDK4-cyclinD3	100	94	97	91
CDK5	100	100	95	100
CDK7	82	11	10	47
CDK8	28	48	56	40
CDK9	97	70	97	95
CDKL1	84	79	85	74
CDKL2	24	41	10	77

CDKL3	100	54	68	94
CDKL5	100	100	88	82
CHEK1	94	100	100	99
CHEK2	91	93	88	100
CIT	97	100	100	100
CLK1	100	78	59	66
CLK2	91	100	100	100
CLK3	100	100	81	79
CLK4	77	57	59	38
CSF1R	24	15	23	5.5
CSF1R-autoinhibited	90	96	100	93
CSK	88	100	76	74
CSNK1A1	90	59	89	100
CSNK1A1L	91	100	89	100
CSNK1D	99	100	100	100
CSNK1E	100	100	100	82
CSNK1G1	100	97	100	100
CSNK1G2	96	100	100	100
CSNK1G3	100	100	79	82
CSNK2A1	100	100	94	100
CSNK2A2	87	95	86	100
CTK	38	100	100	95
DAPK1	97	73	59	89
DAPK2	100	100	100	100
DAPK3	100	100	100	100
DCAMKL1	100	100	85	92
DCAMKL2	91	100	95	88
DCAMKL3	100	100	100	100
DDR1	2.2	0.3	3.3	0
DDR2	6.2	2.2	6.3	2.1
DLK	80	74	77	82
DMPK	96	84	89	93
DMPK2	89	80	100	85
DRAK1	100	94	100	78
DRAK2	100	100	100	100
DYRK1A	100	89	83	87
DYRK1B	93	100	100	88
DYRK2	71	100	88	74
EGFR	100	77	85	98
EGFR(E746-A750del)	88	100	92	100
EGFR(G719C)	92	100	81	94
EGFR(G719S)	91	100	92	95
EGFR(L747-E749del, A750P)	83	80	100	78
EGFR(L747-S752del, P753S)	77	88	91	100
EGFR(L747-T751del,Sins)	83	89	67	100
EGFR(L858R)	100	82	99	100

EGFR(L858R,T790M)	91	100	24	7.6
EGFR(L861Q)	90	100	72	87
EGFR(S752-I759del)	85	99	72	74
EGFR(T790M)	72	98	24	84
EIF2AK1	85	91	100	86
EPHA1	100	84	100	92
EPHA2	80	100	100	100
EPHA3	16	89	100	84
EPHA4	93	92	84	100
EPHA5	100	93	100	100
EPHA6	98	96	89	100
EPHA7	100	100	100	97
EPHA8	14	60	48	60
EPHB1	100	100	100	91
EPHB2	96	100	95	100
EPHB3	100	100	100	100
EPHB4	99	100	94	100
EPHB6	96	100	100	100
ERBB2	75	85	71	68
ERBB3	86	100	100	77
ERBB4	100	100	85	94
ERK1	100	100	100	100
ERK2	100	98	100	100
ERK3	91	91	100	91
ERK4	82	79	98	92
ERK5	80	92	89	100
ERK8	100	100	69	79
ERN1	85	100	100	95
FAK	100	100	100	100
FER	100	100	92	88
FES	100	100	87	100
FGFR1	50	37	58	49
FGFR2	100	70	92	96
FGFR3	100	100	99	100
FGFR3(G697C)	100	92	98	97
FGFR4	100	77	90	85
FGR	74	62	32	81
FLT1	100	93	81	69
FLT3	23	9.1	10	2.7
FLT3(D835H)	54	42	56	36
FLT3(D835V)	82	30	14	11
FLT3(D835Y)	83	21	59	28
FLT3(ITD)	66	24	66	20
FLT3(ITD,D835V)	96	100	55	58
FLT3(ITD,F691L)	62	65	54	29
FLT3(K663Q)	10	15	22	5.9

FLT3(N841I)	7.4	0.3	14	4.6
FLT3(R834Q)	9.6	4.8	9.2	4
FLT3-autoinhibited	99	75	75	47
FLT4	93	70	88	81
FRK	93	68	78	71
FYN	100	77	63	88
GAK	100	98	100	100
GCN2(Kin.Dom.2,S808G)	100	84	100	83
GRK1	97	88	81	84
GRK2	93	100	76	100
GRK3	89	93	100	85
GRK4	100	64	89	82
GRK7	90	100	74	66
GSK3A	100	86	91	94
GSK3B	98	100	100	87
HASPIN	67	93	97	85
HCK	20	64	52	83
HIPK1	93	72	16	79
HIPK2	100	100	20	92
HIPK3	98	100	11	68
HIPK4	100	78	70	60
HPK1	82	82	48	50
HUNK	38	90	100	90
ICK	94	100	69	84
IGF1R	100	93	95	99
IKK-alpha	68	83	15	42
IKK-beta	76	100	22	34
IKK-epsilon	75	100	90	91
INSR	83	86	34	87
INSRR	92	89	91	81
IRAK1	92	100	9.5	57
IRAK3	93	94	100	100
IRAK4	100	100	57	65
ITK	100	100	38	89
JAK1(JH1domain-catalytic)	100	88	100	51
JAK1(JH2domain-pseudokinase)	86	100	61	55
JAK2(JH1domain-catalytic)	93	71	76	66
JAK3(JH1domain-catalytic)	44	35	36	32
JNK1	91	81	100	91
JNK2	77	65	77	72
JNK3	91	88	88	93
KIT	33	17	38	13
KIT(A829P)	4.8	13	13	11
KIT(D816H)	83	76	55	72
KIT(D816V)	100	100	100	100
KIT(L576P)	18	20	26	7

KIT(V559D)	22	12	25	5.7
KIT(V559D,T670I)	20	20	27	18
KIT(V559D,V654A)	94	87	97	77
KIT-autoinhibited	87	86	86	84
LATS1	92	83	100	100
LATS2	100	100	98	100
LCK	12	45	8.3	11
LIMK1	100	100	83	100
LIMK2	100	84	100	92
LKB1	100	98	100	100
LOK	2.2	0.3	1.6	1.7
LRRK2	100	100	100	88
LRRK2(G2019S)	92	91	100	69
LTK	11	77	97	96
LYN	38	32	38	56
LZK	79	95	82	83
MAK	52	74	87	100
MAP3K1	94	100	98	87
MAP3K15	100	100	87	81
MAP3K2	86	99	67	80
MAP3K3	74	67	33	79
MAP3K4	100	100	96	100
MAP4K2	36	89	20	88
MAP4K3	100	93	76	100
MAP4K4	76	96	68	100
MAP4K5	91	100	88	99
MAPKAPK2	100	79	98	77
MAPKAPK5	100	100	80	79
MARK1	87	85	61	60
MARK2	79	92	100	100
MARK3	89	90	100	100
MARK4	100	100	67	81
MAST1	74	56	92	99
MEK1	93	100	93	100
MEK2	96	100	100	100
MEK3	100	100	86	100
MEK4	100	100	95	100
MEK5	58	36	52	72
MEK6	100	83	72	92
MELK	90	83	100	89
MERTK	100	56	48	88
MET	100	93	100	100
MET(M1250T)	100	97	90	91
MET(Y1235D)	100	100	100	86
MINK	81	100	60	71
MKK7	100	100	100	100

MKNK1	93	54	82	56
MKNK2	66	4.1	32	16
MLCK	100	100	100	94
MLK1	100	100	100	100
MLK2	92	97	70	70
MLK3	96	100	97	90
MRCKA	100	91	98	100
MRCKB	99	100	100	100
MST1	100	80	77	100
MST1R	67	96	100	100
MST2	68	92	100	100
MST3	98	87	63	100
MST4	84	100	14	71
MTOR	100	100	100	100
MUSK	69	35	72	36
MYLK	100	36	100	90
MYLK2	86	100	100	83
MYLK4	96	89	90	87
MYO3A	100	70	100	100
MYO3B	80	72	100	100
NDR1	89	100	88	66
NDR2	100	91	100	100
NEK1	92	79	85	89
NEK10	91	100	88	100
NEK11	92	100	69	94
NEK2	100	91	100	100
NEK3	100	94	76	90
NEK4	94	100	91	72
NEK5	69	77	100	91
NEK6	100	99	100	100
NEK7	94	100	61	100
NEK9	97	96	54	76
NIK	96	98	91	89
NIM1	82	98	92	76
NLK	82	54	100	100
OSR1	98	100	76	87
p38-alpha	90	87	75	65
p38-beta	100	100	100	100
p38-delta	64	84	57	100
p38-gamma	100	93	6.3	85
PAK1	100	100	88	96
PAK2	100	86	90	82
PAK3	78	100	51	49
PAK4	98	100	83	90
PAK6	100	100	80	71
PAK7	90	78	96	86

PCTK1	100	77	73	81
PCTK2	76	100	73	80
PCTK3	86	100	89	93
PDGFRA	33	12	9.5	4.9
PDGFRB	18	10	24	5.9
PDPK1	86	100	100	100
PFCDPK1(P.falciparum)	74	100	100	97
PFPK5(P.falciparum)	68	97	97	84
PFTAIRE2	98	89	62	87
PFTK1	91	88	68	97
PHKG1	99	100	100	100
PHKG2	100	100	100	100
PIK3C2B	100	100	80	79
PIK3C2G	95	100	90	100
PIK3CA	85	85	91	95
PIK3CA(C420R)	100	94	89	84
PIK3CA(E542K)	100	74	87	85
PIK3CA(E545A)	100	83	100	100
PIK3CA(E545K)	94	74	89	75
PIK3CA(H1047L)	100	70	100	100
PIK3CA(H1047Y)	83	80	100	69
PIK3CA(I800L)	95	56	88	54
PIK3CA(M1043I)	83	100	89	100
PIK3CA(Q546K)	82	71	73	72
PIK3CB	100	100	100	100
PIK3CD	88	100	67	87
PIK3CG	100	93	70	72
PIK4CB	97	100	79	82
PIKFYVE	97	99	84	95
PIM1	98	98	91	89
PIM2	86	100	100	93
PIM3	98	100	79	84
PIP5K1A	100	78	100	100
PIP5K1C	100	97	68	82
PIP5K2B	100	100	100	100
PIP5K2C	100	100	100	99
PKAC-alpha	92	100	100	87
PKAC-beta	100	99	100	100
PKMYT1	100	86	100	93
PKN1	91	96	88	100
PKN2	93	84	89	100
PKNB(M.tuberculosis)	87	97	79	96
PLK1	80	100	74	70
PLK2	100	100	90	87
PLK3	99	100	84	91
PLK4	88	75	100	100

PRKCD	99	95	88	80
PRKCE	70	85	100	84
PRKCH	78	100	75	64
PRKCI	94	88	92	71
PRKCQ	100	100	100	100
PRKD1	93	100	100	100
PRKD2	100	84	98	92
PRKD3	100	92	74	100
PRKG1	100	70	100	100
PRKG2	89	92	93	74
PRKR	91	66	77	78
PRKX	100	82	84	99
PRP4	79	100	100	88
PYK2	89	100	36	100
QSK	100	75	100	100
RAF1	93	100	88	55
RET	34	8.8	2.6	1.8
RET(M918T)	42	2.5	4.1	2.8
RET(V804L)	91	42	18	34
RET(V804M)	90	54	12	16
RIOK1	100	95	68	77
RIOK2	100	100	85	83
RIOK3	98	89	62	71
RIPK1	82	88	100	86
RIPK2	92	100	96	98
RIPK4	87	85	55	90
RIPK5	100	100	54	93
ROCK1	100	94	77	86
ROCK2	100	100	80	94
ROS1	96	100	75	87
RPS6KA4(Kin.Dom.1-N-terminal)	94	81	100	100
RPS6KA4(Kin.Dom.2-C-terminal)	100	100	89	88
RPS6KA5(Kin.Dom.1-N-terminal)	100	100	76	70
RPS6KA5(Kin.Dom.2-C-terminal)	100	75	100	92
RSK1(Kin.Dom.1-N-terminal)	96	97	82	81
RSK1(Kin.Dom.2-C-terminal)	91	84	60	100
RSK2(Kin.Dom.1-N-terminal)	97	96	73	73
RSK2(Kin.Dom.2-C-terminal)	82	100	100	100
RSK3(Kin.Dom.1-N-terminal)	100	100	83	100
RSK3(Kin.Dom.2-C-terminal)	100	92	64	73
RSK4(Kin.Dom.1-N-terminal)	100	88	73	74
RSK4(Kin.Dom.2-C-terminal)	96	83	66	100
S6K1	81	72	37	55
SBK1	56	100	75	93
SGK	90	100	100	88
SgK110	87	87	100	100

SGK2	100	100	100	100
SGK3	89	100	84	90
SIK	89	90	100	91
SIK2	100	100	76	100
SLK	70	100	59	83
SNARK	92	100	82	75
SNRK	96	91	100	75
SRC	76	86	100	80
SRMS	74	84	90	99
SRPK1	100	100	83	82
SRPK2	85	77	100	94
SRPK3	100	97	96	86
STK16	100	100	88	91
STK33	95	100	98	78
STK35	100	100	96	100
STK36	96	100	100	100
STK39	76	63	93	100
SYK	76	67	63	65
TAK1	15	4.7	4.4	27
TAOK1	67	77	98	94
TAOK2	77	82	98	95
TAOK3	72	57	96	84
TBK1	97	75	84	87
TEC	100	100	100	93
TESK1	100	100	94	100
TGFBR1	100	88	98	73
TGFBR2	100	100	100	100
TIE1	12	23	19	19
TIE2	41	4.8	14	5.1
TLK1	84	81	100	100
TLK2	91	74	72	67
TNIK	65	82	70	97
TNK1	79	54	51	84
TNK2	100	100	100	100
TNNI3K	95	100	93	100
TRKA	28	16	0.3	2.5
TRKB	23	2.6	1.2	4.3
TRKC	63	17	5.6	19
TRPM6	100	100	100	89
TSSK1B	100	98	82	96
TSSK3	89	100	100	75
TTK	99	100	100	100
TXK	92	100	88	83
TYK2(JH1domain-catalytic)	94	77	90	89
TYK2(JH2domain-pseudokinase)	90	100	84	99
TYRO3	100	100	89	100

ULK1	100	95	82	100
ULK2	100	100	100	98
ULK3	93	97	50	91
VEGFR2	45	35	37	33
VPS34	75	82	100	98
VRK2	96	86	100	85
WEE1	98	100	96	100
WEE2	95	95	86	100
WNK1	97	100	100	86
WNK2	88	93	79	92
WNK3	97	100	100	100
WNK4	89	100	92	100
YANK1	89	100	100	80
YANK2	75	96	68	60
YANK3	100	98	100	88
YES	100	69	91	100
YSK1	92	67	85	96
YSK4	92	81	22	55
ZAK	26	8.9	74	12
ZAP70	90	70	95	78

Table S3. Eurofins KINOMEscan™ testing with 100 nM compound concentration

Kinase	% of control at 100 nM			
	Ponatinib	HSL468	HSL476	HSN608
AAK1	100	100	63	91
ABL1(E255K)-phosphorylated	2.6	0.6	2	1.1
ABL1(F317I)-nonphosphorylated	0	0	1.2	0.2
ABL1(F317I)-phosphorylated	9.1	2.8	5.5	5.7
ABL1(F317L)-nonphosphorylated	0	0	0	0
ABL1(F317L)-phosphorylated	1.9	1.2	2	2.2
ABL1(H396P)-nonphosphorylated	0	0.1	0	0
ABL1(H396P)-phosphorylated	3.1	0.5	1.2	0.7
ABL1(M351T)-phosphorylated	1.2	3.3	2.5	2.6
ABL1(Q252H)-nonphosphorylated	0	0.2	0.1	0
ABL1(Q252H)-phosphorylated	3.1	1	0.8	1
ABL1(T315I)-nonphosphorylated	0	0	0	0.1
ABL1(T315I)-phosphorylated	2.6	1	1.6	2.1
ABL1(Y253F)-phosphorylated	2.4	0.4	0.6	0.5
ABL1-nonphosphorylated	1.2	0	0.2	0.1
ABL1-phosphorylated	3.2	0.5	0.5	0.4
ABL2	4.5	5.4	5.5	2.5
ACVR1	100	90	100	100
ACVR1B	100	67	100	100
ACVR2A	100	100	100	87
ACVR2B	100	92	100	83
ACVRL1	100	96	100	100
ADCK3	100	100	100	100
ADCK4	100	95	98	100
AKT1	100	96	100	100
AKT2	100	97	88	92
AKT3	100	83	82	100
ALK	97	92	100	100
ALK(C1156Y)	92	100	77	100
ALK(L1196M)	98	100	100	100
AMPK-alpha1	100	47	91	71
AMPK-alpha2	100	5.3	80	69
ANKK1	88	71	30	100
ARK5	79	81	94	100
ASK1	100	96	100	100
ASK2	87	100	100	94
AURKA	100	76	84	80
AURKB	100	39	47	18
AURKC	92	2.1	45	20
AXL	59	47	4.7	13
BIKE	100	94	97	71
BLK	0.6	0.3	0.4	0.3

BMPR1A	100	68	100	100
BMPR1B	100	100	85	83
BMPR2	100	100	100	100
BMX	33	79	78	100
BRAF	8.3	8.1	9.3	2.9
BRAF(V600E)	2.1	3.7	2.9	0.7
BRK	100	100	100	100
BRSK1	89	100	86	100
BRSK2	100	75	95	100
BTK	87	100	90	100
BUB1	100	100	100	100
CAMK1	100	99	94	87
CAMK1B	91	100	97	86
CAMK1D	100	100	100	100
CAMK1G	100	100	56	88
CAMK2A	100	88	99	100
CAMK2B	100	91	100	96
CAMK2D	100	100	85	100
CAMK2G	100	100	70	100
CAMK4	100	96	91	100
CAMKK1	100	83	21	95
CAMKK2	74	81	35	100
CASK	82	100	100	92
CDC2L1	85	81	93	100
CDC2L2	93	100	87	100
CDC2L5	5.6	13	6.5	33
CDK11	0	0.6	4.6	4.2
CDK2	100	70	21	98
CDK3	100	60	24	89
CDK4	77	100	81	70
CDK4-cyclinD1	100	98	88	100
CDK4-cyclinD3	100	100	84	96
CDK5	100	62	22	100
CDK7	51	1.1	1.3	6.6
CDK8	0	10	4.7	2
CDK9	93	67	96	100
CDKL1	99	53	43	91
CDKL2	0	0	0.7	21
CDKL3	15	1.2	3.7	57
CDKL5	100	100	36	97
CHEK1	100	91	100	99
CHEK2	100	57	100	98
CIT	47	98	100	100
CLK1	59	25	56	38
CLK2	87	40	40	75
CLK3	100	100	96	100

CLK4	43	4.4	25	19
CSF1R	1.3	0.7	0.9	0.6
CSF1R-autoinhibited	100	82	87	83
CSK	27	75	69	66
CSNK1A1	100	69	100	100
CSNK1A1L	100	100	100	100
CSNK1D	95	96	100	100
CSNK1E	100	100	100	100
CSNK1G1	100	91	100	100
CSNK1G2	97	87	100	100
CSNK1G3	100	100	100	100
CSNK2A1	99	100	100	100
CSNK2A2	100	94	100	100
CTK	58	100	82	95
DAPK1	100	92	72	95
DAPK2	92	92	100	100
DAPK3	100	100	90	100
DCAMKL1	100	96	91	100
DCAMKL2	100	89	99	100
DCAMKL3	100	100	97	100
DDR1	0	0.3	0	0
DDR2	0	0.4	0.7	0
DLK	57	93	30	73
DMPK	100	88	90	100
DMPK2	100	100	100	95
DRAK1	100	93	87	100
DRAK2	100	100	100	100
DYRK1A	100	100	96	92
DYRK1B	69	82	68	49
DYRK2	100	54	59	46
EGFR	74	55	60	50
EGFR(E746-A750del)	29	90	65	59
EGFR(G719C)	56	80	82	57
EGFR(G719S)	63	76	84	53
EGFR(L747-E749del, A750P)	51	38	22	19
EGFR(L747-S752del, P753S)	22	69	78	52
EGFR(L747-T751del,Sins)	20	56	45	62
EGFR(L858R)	100	79	81	82
EGFR(L858R,T790M)	84	96	0	9.2
EGFR(L861Q)	46	72	59	64
EGFR(S752-I759del)	40	96	53	24
EGFR(T790M)	72	84	8.9	67
EIF2AK1	85	78	100	100
EPHA1	53	93	100	82
EPHA2	6.7	28	56	64
EPHA3	0.2	54	52	70

EPHA4	16	91	66	60
EPHA5	55	80	100	100
EPHA6	38	54	87	74
EPHA7	94	100	100	100
EPHA8	2.1	8.1	2.7	5
EPHB1	52	100	100	100
EPHB2	21	73	75	94
EPHB3	100	94	100	100
EPHB4	100	96	100	100
EPHB6	70	100	100	89
ERBB2	21	58	23	34
ERBB3	100	100	86	100
ERBB4	45	81	52	84
ERK1	100	94	100	100
ERK2	100	89	100	100
ERK3	100	100	100	100
ERK4	91	81	100	100
ERK5	95	81	100	100
ERK8	96	95	58	99
ERN1	100	100	100	100
FAK	100	95	81	100
FER	100	78	55	100
FES	100	100	79	100
FGFR1	5.2	1	5.8	3.8
FGFR2	21	6.8	31	20
FGFR3	45	26	62	54
FGFR3(G697C)	63	28	75	50
FGFR4	38	12	61	12
FGR	11	13	6.4	14
FLT1	25	30	43	13
FLT3	3.8	3.5	1.8	0.8
FLT3(D835H)	8.6	5.6	7.1	4.3
FLT3(D835V)	22	13	1.8	1.4
FLT3(D835Y)	26	3.6	18	18
FLT3(ITD)	5.6	1.7	9	4.2
FLT3(ITD,D835V)	92	100	57	56
FLT3(ITD,F691L)	17	29	10	5.3
FLT3(K663Q)	69	1.6	5.5	9.4
FLT3(N841I)	0	0	2.2	4.3
FLT3(R834Q)	0	1.9	1.7	1.5
FLT3-autoinhibited	66	66	64	47
FLT4	18	11	11	5.4
FRK	13	15	13	14
FYN	21	26	15	33
GAK	99	92	100	90
GCN2(Kin.Dom.2,S808G)	62	33	86	59

GRK1	100	73	100	100
GRK2	100	100	93	77
GRK3	87	100	60	92
GRK4	100	85	94	84
GRK7	100	100	81	83
GSK3A	92	92	95	100
GSK3B	100	100	100	100
HASPIN	65	100	100	94
HCK	3.1	6.2	6	13
HIPK1	100	33	0.5	46
HIPK2	100	45	0.3	89
HIPK3	100	28	0.5	45
HIPK4	70	31	21	26
HPK1	5	74	3.4	84
HUNK	59	100	100	98
ICK	80	100	42	67
IGF1R	100	87	72	100
IKK-alpha	18	18	1.8	6
IKK-beta	27	25	3.2	5.7
IKK-epsilon	100	100	100	98
INSR	96	75	5.3	100
INSRR	100	85	56	100
IRAK1	46	34	0.3	9.4
IRAK3	100	81	100	100
IRAK4	100	55	16	26
ITK	100	44	4	39
JAK1(JH1domain-catalytic)	76	52	61	57
JAK1(JH2domain-pseudokinase)	88	100	84	87
JAK2(JH1domain-catalytic)	90	37	38	84
JAK3(JH1domain-catalytic)	7.8	4.7	4.6	5.4
JNK1	96	88	100	96
JNK2	54	28	12	25
JNK3	99	92	79	67
KIT	2.2	1.1	2.2	0.5
KIT(A829P)	0.2	11	7.9	6.6
KIT(D816H)	26	23	19	28
KIT(D816V)	63	66	89	45
KIT(L576P)	0	0.8	0	0
KIT(V559D)	0.8	1.2	1	0.5
KIT(V559D,T670I)	2.5	1.6	1.8	1
KIT(V559D,V654A)	30	22	21	8.7
KIT-autoinhibited	88	100	100	100
LATS1	100	73	100	100
LATS2	100	100	85	100
LCK	1	0.8	0.9	1.1
LIMK1	100	72	100	100

LIMK2	100	100	100	74
LKB1	100	100	100	100
LOK	0.2	0.1	0.1	0
LRRK2	90	58	100	75
LRRK2(G2019S)	79	96	100	100
LTK	9.1	75	80	100
LYN	4.7	9.7	17	17
LZK	79	90	52	81
MAK	100	49	62	100
MAP3K1	100	92	93	100
MAP3K15	100	45	56	58
MAP3K2	49	44	13	89
MAP3K3	28	8.2	1.6	22
MAP3K4	100	100	99	100
MAP4K2	2.4	71	0.6	70
MAP4K3	98	93	34	100
MAP4K4	20	65	22	68
MAP4K5	56	100	76	75
MAPKAPK2	100	100	91	100
MAPKAPK5	100	100	91	90
MARK1	100	100	58	76
MARK2	89	89	75	100
MARK3	100	91	100	100
MARK4	100	72	82	99
MAST1	74	74	100	87
MEK1	98	100	100	100
MEK2	100	100	100	100
MEK3	96	100	100	97
MEK4	100	100	100	100
MEK5	3.5	2.3	7.9	13
MEK6	100	100	87	95
MELK	100	34	70	28
MERTK	73	16	9.8	26
MET	91	97	100	100
MET(M1250T)	98	92	88	100
MET(Y1235D)	100	100	100	100
MINK	53	75	10	100
MKK7	100	91	100	95
MKNK1	87	3.6	34	5.8
MKNK2	21	0.4	3.3	1.6
MLCK	100	100	97	100
MLK1	100	94	87	100
MLK2	93	36	42	66
MLK3	100	87	76	100
MRCKA	100	69	100	100
MRCKB	100	100	100	100

MST1	100	84	19	100
MST1R	100	87	100	100
MST2	89	100	60	100
MST3	100	83	49	100
MST4	65	100	25	18
MTOR	100	100	100	99
MUSK	6.7	2.3	12	3
MYLK	100	100	100	100
MYLK2	100	45	87	65
MYLK4	100	74	100	95
MYO3A	93	59	39	100
MYO3B	94	52	100	100
NDR1	100	96	78	100
NDR2	100	86	93	95
NEK1	100	100	95	100
NEK10	98	95	57	100
NEK11	95	92	38	100
NEK2	100	92	100	100
NEK3	72	100	92	78
NEK4	78	98	61	58
NEK5	98	70	100	100
NEK6	100	91	100	100
NEK7	100	100	100	100
NEK9	100	59	41	33
NIK	100	100	85	100
NIM1	86	100	95	99
NLK	42	55	100	100
OSR1	100	90	81	100
p38-alpha	40	27	11	30
p38-beta	43	56	79	74
p38-delta	100	65	3.9	100
p38-gamma	71	75	1.4	74
PAK1	100	77	90	100
PAK2	100	71	94	94
PAK3	5.6	100	40	39
PAK4	100	90	97	96
PAK6	100	87	100	76
PAK7	100	94	91	96
PCTK1	90	29	21	87
PCTK2	52	35	26	100
PCTK3	71	61	35	100
PDGFRA	4.6	2.9	0.8	1.1
PDGFRB	0.7	0.6	0.6	0.2
PDPK1	94	80	75	87
PFCDPK1(P.falciparum)	15	74	7.9	100
PFPK5(P.falciparum)	66	100	88	90

PFTAIRE2	79	30	22	60
PFTK1	46	9.7	6.7	88
PHKG1	100	100	93	100
PHKG2	100	84	100	100
PIK3C2B	97	100	83	88
PIK3C2G	100	100	92	90
PIK3CA	91	90	95	100
PIK3CA(C420R)	100	98	91	100
PIK3CA(E542K)	96	93	95	76
PIK3CA(E545A)	100	88	100	100
PIK3CA(E545K)	93	99	100	81
PIK3CA(H1047L)	100	100	100	100
PIK3CA(H1047Y)	85	100	86	86
PIK3CA(I800L)	89	89	100	81
PIK3CA(M1043I)	95	100	86	100
PIK3CA(Q546K)	70	90	84	87
PIK3CB	100	100	84	100
PIK3CD	97	88	100	88
PIK3CG	96	100	75	81
PIK4CB	82	100	73	69
PIKFYVE	100	100	100	94
PIM1	100	90	96	100
PIM2	100	100	100	100
PIM3	100	92	87	97
PIP5K1A	100	73	100	88
PIP5K1C	79	100	92	97
PIP5K2B	100	86	100	100
PIP5K2C	95	100	100	100
PKAC-alpha	74	100	92	89
PKAC-beta	35	100	97	100
PKMYT1	100	97	100	94
PKN1	100	91	94	100
PKN2	100	75	72	100
PKNB(M.tuberculosis)	95	83	100	100
PLK1	80	94	81	81
PLK2	100	100	100	100
PLK3	100	100	92	100
PLK4	100	83	80	100
PRKCD	76	66	71	82
PRKCE	59	87	90	100
PRKCH	86	76	85	98
PRKCI	100	71	91	85
PRKCQ	100	89	100	98
PRKD1	100	100	100	100
PRKD2	100	28	92	100
PRKD3	100	80	92	97

PRKG1	100	66	100	100
PRKG2	100	94	91	86
PRKR	100	78	85	86
PRKX	100	99	76	92
PRP4	88	99	100	100
PYK2	55	85	4.1	70
QSK	83	95	100	100
RAF1	23	64	57	10
RET	0.4	0.1	0.1	0
RET(M918T)	0.8	0	0.1	0.1
RET(V804L)	8	2.3	0.9	1
RET(V804M)	4.4	3	0.3	0.5
RIOK1	98	100	86	73
RIOK2	96	73	100	30
RIOK3	56	100	73	54
RIPK1	60	76	81	52
RIPK2	32	73	44	56
RIPK4	77	82	9.2	74
RIPK5	100	89	8.6	95
ROCK1	100	98	85	100
ROCK2	99	98	25	72
ROS1	100	83	35	100
RPS6KA4(Kin.Dom.1-N-terminal)	88	74	100	100
RPS6KA4(Kin.Dom.2-C-terminal)	100	100	94	91
RPS6KA5(Kin.Dom.1-N-terminal)	100	53	81	83
RPS6KA5(Kin.Dom.2-C-terminal)	100	100	100	100
RSK1(Kin.Dom.1-N-terminal)	100	76	76	79
RSK1(Kin.Dom.2-C-terminal)	100	94	74	90
RSK2(Kin.Dom.1-N-terminal)	97	43	62	56
RSK2(Kin.Dom.2-C-terminal)	74	100	100	100
RSK3(Kin.Dom.1-N-terminal)	100	53	75	71
RSK3(Kin.Dom.2-C-terminal)	100	72	57	98
RSK4(Kin.Dom.1-N-terminal)	100	69	87	89
RSK4(Kin.Dom.2-C-terminal)	100	72	83	100
S6K1	63	43	10	42
SBK1	100	90	100	100
SGK	99	100	100	68
SgK110	100	69	100	100
SGK2	100	100	100	100
SGK3	100	94	70	67
SIK	28	85	83	22
SIK2	81	47	81	23
SLK	9.9	23	18	42
SNARK	100	100	82	100
SNRK	95	82	94	88
SRC	9.1	19	32	33

SRMS	28	73	28	80
SRPK1	91	100	81	100
SRPK2	100	100	89	100
SRPK3	97	100	95	86
STK16	100	89	90	100
STK33	92	72	58	43
STK35	100	100	91	100
STK36	100	100	100	100
STK39	84	58	79	78
SYK	93	55	47	66
TAK1	2.1	0.3	1.1	5.4
TAOK1	50	49	80	65
TAOK2	54	69	80	72
TAOK3	14	13	56	34
TBK1	100	100	89	94
TEC	100	100	94	90
TESK1	100	87	87	100
TGFBR1	100	94	100	100
TGFBR2	63	85	90	100
TIE1	7	11	9.3	15
TIE2	1.5	0.8	1.8	0.7
TLK1	100	62	88	100
TLK2	100	100	72	66
TNIK	15	44	34	91
TNK1	10	9.2	16	20
TNK2	100	86	100	100
TNNI3K	34	72	37	60
TRKA	2.4	0.3	0	0
TRKB	1.5	0.1	0.1	0.2
TRKC	13	0.7	0.1	0.5
TRPM6	100	100	94	100
TSSK1B	100	100	55	71
TSSK3	100	100	100	91
TTK	100	65	64	100
TXK	20	82	73	79
TYK2(JH1domain-catalytic)	83	63	90	100
TYK2(JH2domain-pseudokinase)	100	100	99	100
TYRO3	100	63	100	100
ULK1	100	94	100	100
ULK2	100	100	100	100
ULK3	64	45	7.3	83
VEGFR2	6.9	7.2	5.8	7.9
VPS34	98	87	100	100
VRK2	98	90	100	100
WEE1	100	100	100	84
WEE2	100	95	100	71

WNK1	100	100	82	100
WNK2	98	83	100	100
WNK3	96	100	100	100
WNK4	100	100	100	100
YANK1	92	87	100	100
YANK2	92	57	86	99
YANK3	100	100	80	75
YES	29	13	28	26
YSK1	100	100	97	100
YSK4	74	26	1.9	28
ZAK	1.9	3.2	22	2.2
ZAP70	98	84	78	90

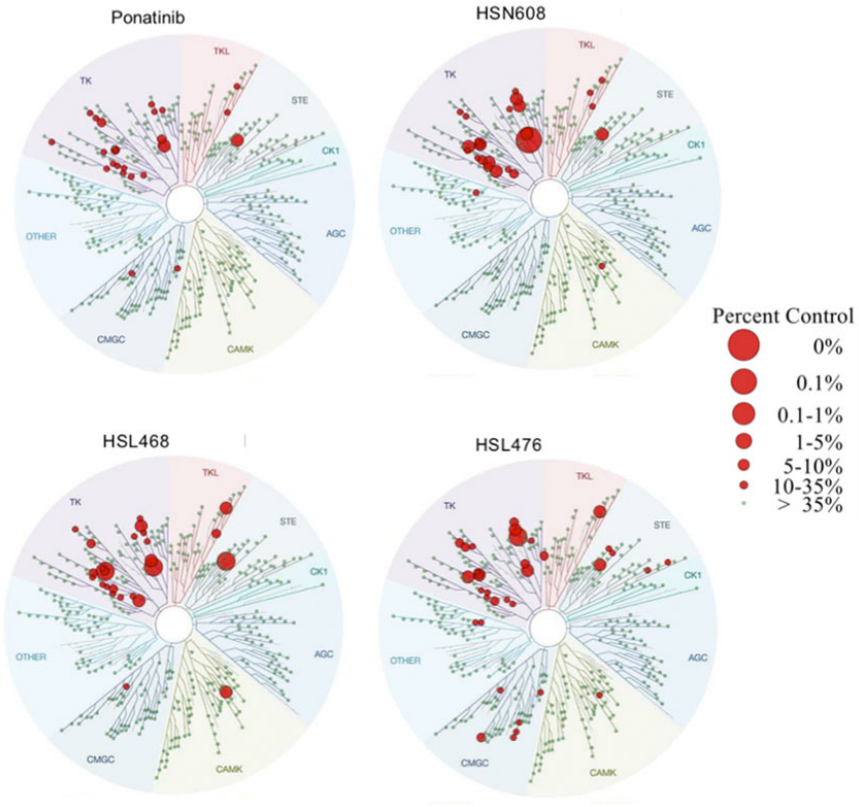


Fig. S3. TREEspot^{TM} interaction map for compounds at 10 nM

Table S4. Metabolic stability in liver microsomes of alkynyl nicotinamides

Compound	Species	$T_{1/2}$ (h)	CL_{int} (µL/min/mg)	Scaled CL_{int} (mL/min/kg)
HSN608	Rat	2.15	5.36	25.09
	Dog	1.38	8.34	48.44
	Human	3.18	3.63	8.99
HSL476	Rat	1.91	12.08	29.47
	Dog	0.37	62.97	110.83
	Human	0.86	26.84	27.6
HSL468	Rat	1.05	22.11	53.95
	Dog	0.56	41.31	72.71
	Human	0.55	41.95	43.13

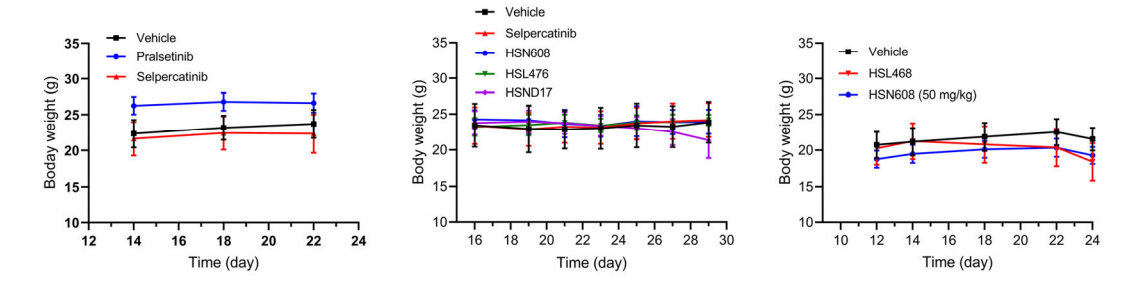


Fig. S4. Body weight monitoring of mice during tumor treatment with RET TKIs. Animal body weights were measured with a scale on the indicated days (see **Fig. 4A** for information on the start and end days of drug treatment). The average body weights from each group of mice are shown.