Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supporting Information

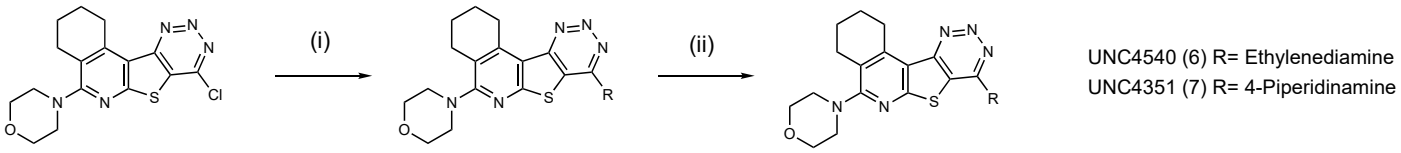
General Information

Analytical LCMS data for all compounds were acquired using an Agilent 6110 series system with the UV detector set to 220 and 254 nm. Samples were injected (<10 µL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 um, C18 column at room temperature. A mobile phase of A (H_2O + 0.1% acetic acid) and B (MeOH + 0.1% acetic acid) was used with a linear gradient from 10% to 100% B in 7.0 min, followed by a flush at 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization source. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Varian Mercury spectrometer at 400 MHz for proton (^{1}HNMR) and 100 MHz for carbon (^{13}CNMR); chemical shifts are reported in ppm (δ). Analytical thin-layer chromatography (TLC) was performed with silica gel 60 F254, 0.25 mm pre-coated TLC plates. TLC plates were visualized using UV 254 nm, I_2 impregnated silica gel, potassium permanganate with charring, and phosphomolybdic acid with charring. Reverse phase or normal phase chromatography was used to purify reaction mixtures using a Teledyne Isco CombiFlash Rf 200 chromatography unit equipped with the UV detector set to 220 nm and 254 nm. Suitable variations in the purification method (flow rate, solvent system) were made as needed to achieve ideal separation for each compound. All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by ^{1}HNMR and LCMS.

Chemistry Abbreviations:

Trifluoroacetic acid (TFA), Dichloromethane (DCM), round bottom flask (rbf), thin layer chromatography (TLC), triethylamine (TEA), dimethylformamide (DMF), N-Methyl-2-pyrrolidone (NMP), tetrahydrofuran (THF)

Scheme 1. Synthesis of compounds 1 – 11.

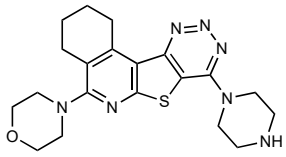


Intermediate 1

UNC4540 (6) R= Ethylenediamine
UNC4351 (7) R= 4-Piperidinamine

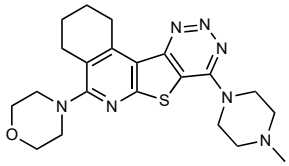
UNC4601 (3) R= (*R*)-2-Methylpiperazine
UNC4684 (4) R= (*S*)-2-Methylpiperazine
UNC4511 (5) R= 1,4-Diazepane

Reagents and conditions: (i) NHR_1R_2, K_2CO_3, CH_3CN, reflux, 43-74% (ii) 50% TFA in DCM, 0 °C, 50-95%.



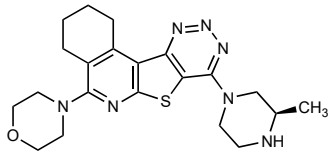
4-(8-(piperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-yl)morpholine, **UNC10201652** (**Inh9, 1**)

Synthesis of **UNC10201652** followed routes and characterization as previously described.[1]

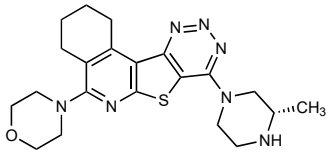


4-(8-(4-methylpiperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-yl)morpholine, **UNC4510** (**2**)
Synthesis of **UNC4510** followed routes and characterization as previously described.[1]

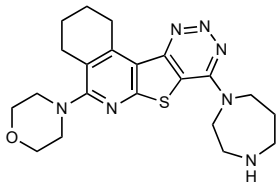
General Procedure A ((i) and (ii))
To a solution of intermediate **1** (0.41 mmol) in CH_3CN (2 mL) was added K_2CO_3 (300 mg) and the respective amine (1.04 mmol), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and filtered. Solvent was removed by rotary evaporation to obtain a crude material. The crude material was adsorbed onto silica gel and purified by normal phase automated Teledyne Isco chromatography generally using a gradient of ethyl acetate in hexanes to afford the desired product. For compounds **3** – **8**, an additional boc deprotection step was performed to achieve the desired product. To a solution of the boc protected intermediate in CH_2Cl_2 (1 mL) at 0°C was added trifluoroacetic acid (1 mL), and the reaction mixture was stirred at room temperature until completion. The solution was washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and vacuum concentrated to afford the desired product. When necessary, the crude material was adsorbed onto silica gel and purified by normal phase automated Teledyne Isco chromatography using a CH_2Cl_2/MeOH/NH_3 solvent system to afford the desired product.



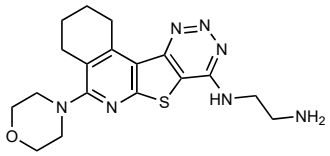
(R)-4-(8-(3-methylpiperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-yl)morpholine, **UNC4601** (**3**)
Compound **3** was synthesized by general procedure A using tert-butyl (*R*)-2-methylpiperazine--carboxylate (208 mg, 1.04 mmol). Following boc deprotection, the crude mixture was purified using a hexane/ethyl acetate gradient system to afford the TFA salt of **UNC4601** (30 mg, 16% over two steps) as a pale-yellow oil.
LC-MS (λ = 254 nm): 99%, t_R = 5.5 min. MS (ESI+): 426 [M+H]+
1H NMR (400 MHz, Chloroform-*d*) δ 4.80 (d, *J* = 12.6 Hz, 2H), 3.87 (t, *J* = 4.6 Hz, 4H), 3.75 (t, *J* = 6.6 Hz, 2H), 3.68 – 3.59 (m, 1H), 3.40 – 3.28 (m, 8H), 3.24 – 3.09 (m, 1H), 2.72 (t, *J* = 5.9 Hz, 2H), 2.08 – 1.94 (m, 2H), 1.87 – 1.76 (m, 2H), 1.37 (d, *J* = 5.8 Hz, 3H)



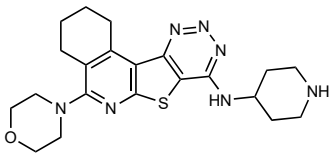
(S)-4-(8-(3-methylpiperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-yl)morpholine, **UNC4684 (4)**
Compound **4** was synthesized by general procedure A using tert-butyl (*S*)-2-methylpiperazine-1-carboxylate (208 mg, 1.04 mmol) to afford the TFA salt of **UNC4684** (27 mg, 15%, over two steps) as an orange solid.
LC-MS (λ = 254 nm): 99%, t_R = 5.4 min. MS (ESI+): 426 [M+H]+
^{1}H NMR (400 MHz, Chloroform-*d*) δ 5.21 (br s, 1H), 4.87 – 4.73 (m, 2H), 3.94 – 3.80 (m, 5H), 3.73 – 3.49 (m, 5H), 3.42 – 3.25 (m, 5H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.02 – 1.91 (m, 2H), 1.79 (m, 2H), 1.50 (d, *J* = 6.0 Hz, 3H).



4-(8-(1,4-diazepan-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-yl)morpholine, **UNC4511** (**5**)
Compound **5** was synthesized by general procedure A using tert-butyl 1,4-diazepane-1-carboxylate (157 mg, 0.78 mmol) to afford the TFA salt of **UNC4511** (40 mg, 71% over two steps) as a yellow solid.
LC-MS (λ = 254 nm): 99%, t_R = 5.8 min. MS (ESI+): 426 [M+H]+
1H NMR (400 MHz, Chloroform-d) δ 9.82 (s, 1H), 4.45 – 4.34 (m, 4H), 4.17 (t, J = 5.9 Hz, 2H), 3.93 – 3.80 (m, 4H), 3.54 (t, J = 5.2 Hz, 2H), 3.42 (t, J = 6.6 Hz, 2H), 3.29 (t, J = 4.5 Hz, 4H), 2.52 (d, J = 7.0 Hz, 4H), 2.00 – 1.89 (m, 2H), 1.78 – 1.68 (m, 2H).

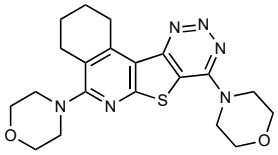


N1-(5-morpholino-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-8-yl)ethane-1,2-diamine, **UNC4540** (**6**)
Compound **6** was synthesized using general procedure A using ethane-1,2-diamine (0.023 mL, 0.41 mmol) to afford **UNC4540** (10 mg, 18%) as a yellow solid.
LC-MS (λ = 254 nm): 99%, t_R = 5.8 min. MS (ESI+): 386 [M+H]+
1H NMR (400 MHz, Methanol-d4) δ 3.90 – 3.86 (m, 4H), 3.82 (t, J = 6.3 Hz, 2H), 3.61 (t, J = 6.8 Hz, 2H), 3.05 (t, J = 6.3 Hz, 2H), 2.78 (t, J = 6.0 Hz, 2H), 2.11 – 1.94 (m, 2H), 1.94 – 1.75 (m, 2H). Four protons masked by solvent peak.



5-morpholino-N-(piperidin-4-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-8-amine, **UNC4351** (**7**)
Compound **7** was synthesized by general procedure A using tert-butyl 4-aminopiperidine-1-carboxylate (208 mg, 1.04 mmol) to afford the TFA salt of **UNC4351** (47 mg, 59% over two steps) as a white solid.
LC-MS (λ = 254 nm): 99%, t_R = 5.4 min. MS (ESI+): 426 [M+H]+

1H NMR (400 MHz, Chloroform-d) δ 6.69-6.61 (m, 1H), 4.69 (br s, 1H), 3.93 – 3.71 (m, 4H), 3.59 (q, J = 8.4, 7.0 Hz, 4H), 3.32 – 3.23 (m, 4H) 3.20 – 3.12 (m, 2H), 2.64 (d, J = 6.0 Hz, 2H), 2.42 (d, J = 12.1 Hz, 2H), 2.27 – 2.05 (m, 2H), 1.94 – 1.68 (m, 4H),

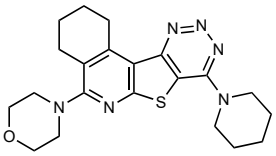


4,4'-(1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinoline-5,8-diyl)dimorpholine, **UNC4365** (**8**)

Compound **8** was synthesized using general procedure A using morpholine (89 mg, 1.03 mmol). The crude mixture was purified using a DCM/MeOH/NH_3 gradient to afford the TFA salt of **UNC4365** (14 mg, 8%) as a white solid.

LC-MS (λ = 254 nm): 99%, t_R = 6.4 min. MS (ESI+): 413 [M+H]+

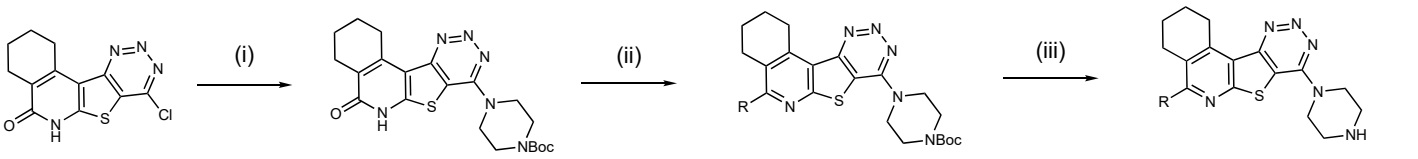
1H NMR (400 MHz, Chloroform-*d*) δ 4.05 (t, *J* = 4.8 Hz, 4H), 3.87 (dt, *J* = 7.1, 4.7 Hz, 8H), 3.75 (t, *J* = 6.6 Hz, 2H), 3.32 (t, *J* = 4.6 Hz, 4H), 2.72 (t, *J* = 5.9 Hz, 2H), 1.97 (m, 2H), 1.85 – 1.72 (m, 2H).



4-(8-(piperidin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-yl)morpholine, **UNC10201651** (**9**)

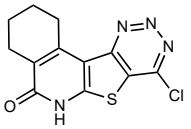
Synthesis of **UNC10201651** followed routes and characterization aligned as previously described.[1]

Scheme 2. Synthesis of UNC10206579 (10) & UNC10206581 (12).



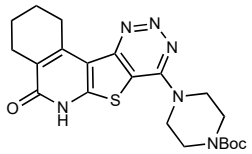
Intermediate 2 | Intermediate 3 | Intermediate 4 (a) R= Piperazine (b) R= $NH(CH_3)$ | UNC10206579 (10) R= Piperazine UNC10206581 (12) R= $NH(CH_3)$

Reagent and Conditions: (i) Boc-piperazine, K_2CO_3, CH_3CN, reflux, 71%; (ii) a. NaH, b. $PhN(Tf)_2$ c. R-NH, 37-58% (iii) 20% TFA in DCM, 0 °C, 80-99%.



8-chloro-2,3,4,6-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5(1H)-one, **Intermediate 2**

Synthesized according to J. C. A. Hunt et al. *Bioorg. Med. Chem. Lett. 17* (2007) 5222-5226).[2]

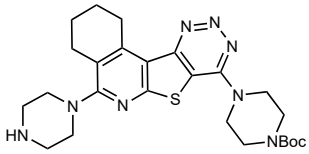


tert-butyl 4-(5-oxo-1,2,3,4,5,6-hexahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-8-yl)piperazine-1-carboxylate, **Intermediate 3**

To a 100 ml rbf equipped with a stir bar was added **intermediate 2** (2.0 g, 6.8 mmol), potassium carbonate (1.888 g, 13.66 mmol), and tert-butyl piperazine-1-carboxylate (1.53 g, 8.2 mmol). The sealed vessel was degassed and purged with nitrogen (x3) following which acetonitrile (20 mL) was added and the sealed vessel was refluxed overnight. The mixture was cooled to rt, water was added dropwise to precipitate the desired product. The mixture was filtered under vacuum and washed with water (30 ml) and purified by reverse phase flash Teledyne Isco chromatography (H2O in ACN + 0.1% TFA) to afford the TFA salt of **intermediate 3** (2.14 g, 71 %) as a light tan solid.

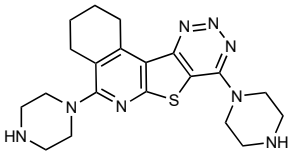
LC-MS (λ = 254 nm): 99%, t_R = 6.5 min. MS (ESI+): 443 $[M+H]^+$

1H NMR (400MHz, DMSO-d6) d = 4.00 - 3.86 (m, 4 H), 3.61 - 3.49 (m, 4 H), 3.44 - 3.39 (m, 2 H), 1.78 (s, 6 H), 1.43 (s, 9 H)



tert-butyl 4-(5-(piperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-8-yl)piperazine-1-carboxylate, **Intermediate 4a**

Intermediate 3 (1.000 g, 2.260 mmol) was added portion wise to a stirred suspension of sodium hydride (65.1 mg, 2.7 mmol) in DMF (15 mL) under nitrogen at 0°C. The reaction mixture was warmed to room temperature to give a brown solution and was stirred for 30 minutes. N-Phenyl-bis(trifluoromethanesulfonimide) (807 mg, 2.26 mmol) was added in one portion and the mixture was stirred further for 20 min, following which tert-butyl piperazine-1-carboxylate (631.3 mg, 3.4 mmol) was added and the reaction mixture stirred overnight at rt. The reaction was slowly quenched with water (5 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford **intermediate 4a**. The crude material was used in the next reaction without further purification (0.427 g, 37% yield).

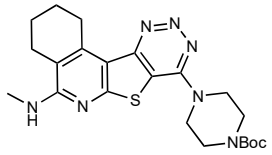


5,8-di(piperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinoline, **UNC10206579 (10)**

Crude **intermediate 4a** was continuously stirred (400 mg, 0.78 mmol) in DCM (8 mL) maintained at 0 °C and TFA (8 mL) was added dropwise. The reaction mixture was constantly monitored while it stirred for 30 min. Upon completion, the volatiles were evaporated under reduced pressure. The crude mixture was purified by reverse phase flash Teledyne Isco chromatography (H2O in ACN + 0.1% TFA) to afford the TFA salt of **UNC10206579** (399 mg, 98.8 %) as a yellow solid.

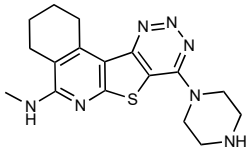
LC-MS (λ = 254 nm): 99%, t_R = 3.5 min. MS (ESI+): 411 $[M+H]^+$

1H NMR (400 MHz, methanol-d4) d = 4.30 (t, J = 5.1 Hz, 4 H), 3.68 (t, J = 6.5 Hz, 2 H), 3.59 (t, J = 4.7 Hz, 4 H), 3.50 (t, J = 5.1 Hz, 4 H), 3.44 (t, J = 4.7 Hz, 4 H), 2.83 (t, J = 5.9 Hz, 2 H), 2.07 - 1.98 (m, 2 H), 1.89 - 1.78 (m, 2 H)



tert-butyl 4-(5-(methylamino)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-8-yl)piperazine-1-carboxylate, **Intermediate 4b**

Intermediate 3 (1.000 g, 2.260 mmol) was added portion wise to a stirred suspension of sodium hydride (65.1 mg, 2.7 mmol) in DMF (15 mL) under nitrogen at 0°C. The reaction mixture was warmed to room temperature to give a brown solution and was stirred for 30 minutes. N-Phenyl-bis(trifluoromethanesulfonimide) (807 mg, 2.26 mmol) was added in one portion and the mixture was stirred further for 20 min, following which tert-Butyl methylcarbamate (445.4 mg, 3.4 mmol) was added and the reaction mixture stirred overnight at rt. The reaction was slowly quenched with water (5 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford **intermediate 4b.** The crude material was used in the next reaction without further purification (596 mg, 58% yield).



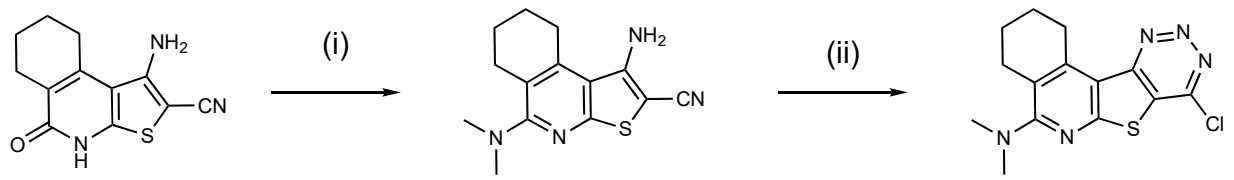
N-methyl-8-(piperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-amine, **UNC10206581 (12)**

Crude **intermediate 4b** was continuously stirred (250 mg, 549 µmol) in DCM (8 mL) maintained at 0 °C and TFA (8 mL) was added dropwise. The reaction mixture was constantly monitored while it stirred for 30 min. Upon completion, the volatiles were evaporated under reduced pressure. The crude mixture was purified by reverse phase flash Teledyne Isco chromatography (H2O in ACN + 0.1% TFA) to afford the TFA salt of **UNC10206581** (190 mg, 97.4 %) as a yellow solid.

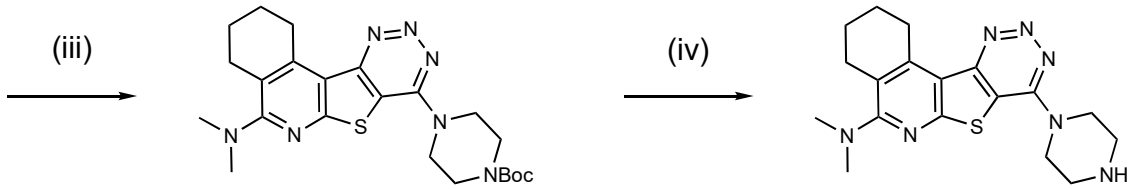
LC-MS (λ = 254 nm): 99%, t_R = 3.6 min. MS (ESI+): 356 $[M+H]^+$

^{1}H NMR (400 MHz, Methanol-d_4) δ 4.37 – 4.32 (m, 4H), 3.64 – 3.59 (m, 2H), 3.53 (m, 4H), 3.23 (s, 3H), 2.59 – 2.51 (m, 2H), 2.04 – 1.91 (m, 4H).

Scheme 3. Synthesis of UNC10206577 (11).

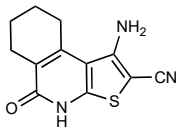


Intermediate 5 Intermediate 6 Intermediate 7

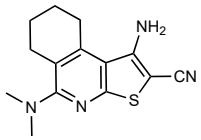


Intermediate 8 UNC10206577 (11)

Reagents and conditions: (i) a. NaH, b. $PhNTf_2$ c. $(CH3)_2NH_2$, 46%; (ii) conc. HCl, $NaNO_2$, H2O, rt, 71% (iii) Boc-piperazine, K_2CO_3, CH_3CN, reflux, 23%; (iv) 20% TFA in DCM, 0 °C, 90%.

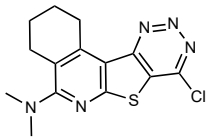


1-amino-5-oxo-4,5,6,7,8,9-hexahydrothieno[2,3-c]isoquinoline-2-carbonitrile, **Intermediate 5**
Intermediate 5 was synthesized according to Hunt et al. *Bioorg. Med. Chem. Lett. 17* (**2007**) 5222-5226)[2].

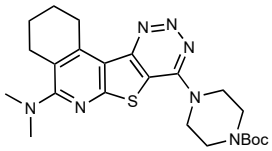


1-amino-5-(dimethylamino)-6,7,8,9-tetrahydrothieno[2,3-c]isoquinoline-2-carbonitrile,
Intermediate 6
Intermediate 5 (500 mg, 2.04 mmol, 1.0 eq.) was added portion wise to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 85 mg, 2.12 mmol, 1.1 eq.) in DMF (10.3 mL) under nitrogen at 0 °C. The reaction mixture was stirred at room temperature for 35-40 min. To the resultant solution was added N-phenyl-trifluoromethanesulfonimide (729 mg, 2.04 mmol, 1.0 eq.) in one portion. After 20 min. dimethylamine (1.06 mL, 2.12 mmol, 1.1 eq.) was added to the reaction mixture and stirred overnight. The reaction was slowly quenched with water (5 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by reverse phase automated Teledyne Isco chromatography using a Methanol/H_2O/0.1% acetic acid solvent system. **Intermediate 6** was obtained as a pale-yellow solid (255 mg, 46%).

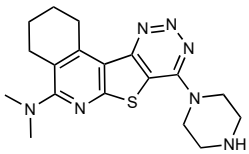
LC-MS (λ = 254 nm): 95%, t_R = 6.1 min. MS (ESI+): 273 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 3.22 (t, *J* = 6.5 Hz, 2H), 2.85 (s, 6H), 2.59 (t, *J* = 5.8 Hz, 2H), 1.84 – 1.72 (m, 2H), 1.60 (m, 2H)



8-chloro-N,N-dimethyl-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-amine, **Intermediate 7**
A solution of sodium nitrite (266 mg, 3.9 mmol, 3.0 eq.) in water (2 mL) was added drop wise, over 30 minutes to a suspension of **intermediate 6** (350 mg, 1.3 mmol, 1.0 eq.) in conc. HCl acid (10 mL) at 0-5°C. The mixture was stirred for an hour at 0-5°C and then allowed to stir at room temperature overnight. Water (100 mL) was added to the precipitated product after which it was filtered, washed with water, and vacuum dried to obtain **intermediate 7** as a pale-yellow solid (291 mg, 71%).
LC-MS (λ = 254 nm): 95%, t_R = 6.8 min. MS (ESI+): 320 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 3.68 (t, *J* = 6.6 Hz, 2H), 3.09 (s, 6H), 2.72 (t, *J* = 5.9 Hz, 2H), 2.04 – 1.93 (m, 2H), 1.81 – 1.72 (m, 2H)

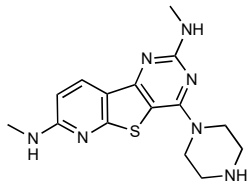


tert-butyl 4-(5-(dimethylamino)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-8-yl)piperazine-1-carboxylate, **Intermediate 8**
To a solution of **intermediate 7** (150 mg, 0.47 mmol, 1.0 eq.) in CH_3CN (4 mL) was added K_2CO_3 (300 mg) and 1-Boc-piperazine (219 mg, 1.17 mmol, 2.5 eq.), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL), and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using a CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 8** was obtained as a white solid (50 mg, 23%).
LC-MS (λ = 254 nm): 99%, t_R = 6.9 min. MS (ESI+): 470 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 4.07 – 3.98 (m, 4H), 3.72 (t, *J* = 6.6 Hz, 2H), 3.62 (dd, *J* = 6.1, 4.3 Hz, 4H), 3.01 (s, 6H), 2.71 (t, *J* = 5.9 Hz, 2H), 2.01 – 1.89 (m, 2H), 1.76 (m, 2H), 1.48 (s, 9H)



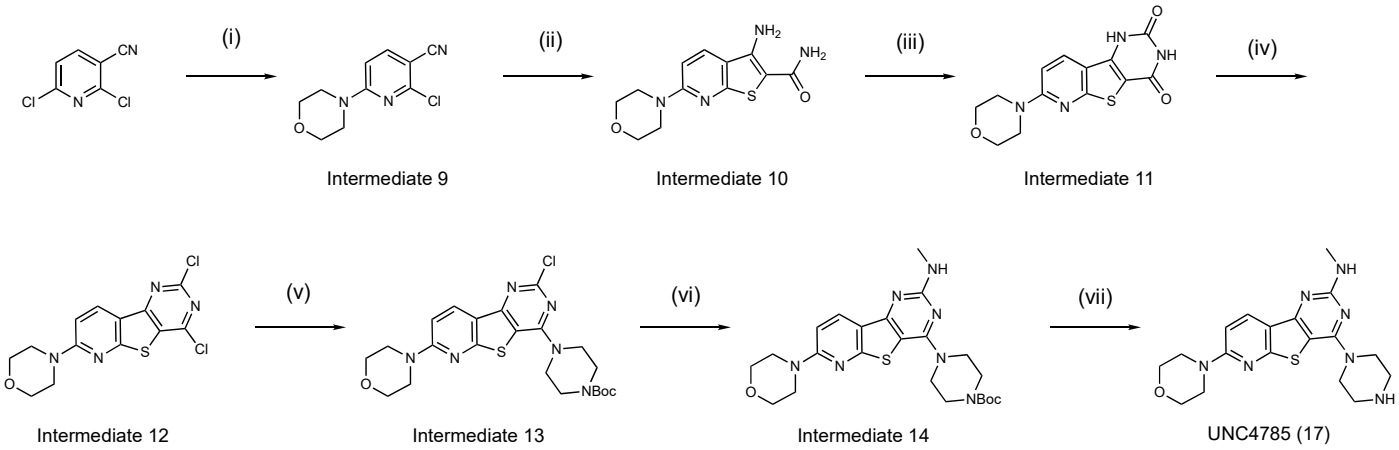
N,N-dimethyl-8-(piperazin-1-yl)-1,2,3,4-tetrahydro-[1,2,3]triazino[4',5':4,5]thieno[2,3-c]isoquinolin-5-amine, **UNC10206577** (**11**)
To a solution of **intermediate 8** (50 mg, 0.106 mmol) in CH_2Cl_2 (1 mL) at 0°C was added trifluoroacetic acid (1 mL), and the reaction mixture was stirred until completion at room

temperature. The solution was vacuum concentrated to obtain the TFA salt of **UNC10206577** (13) as a white solid (35 mg, 90%).
LC-MS (λ = 254 nm): 99%, t_R = 5.6 min. MS (ESI+): 370 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 4.15 – 4.06 (m, 4H), 3.59 (t, *J* = 6.5 Hz, 2H), 3.29 – 3.21 (m, 4H), 3.00 (s, 6H), 2.73 (t, *J* = 5.8 Hz, 2H), 1.98 – 1.87 (m, 2H), 1.77 – 1.64 (m, 2H)

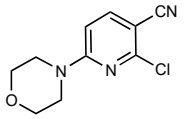


N2,N7-dimethyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidine-2,7-diamine, **UNC4917 (13)**
Synthesis of **UNC4917** followed routes and characterization as previously described.[1]

Scheme 4. Synthesis of UNC4785 (17).

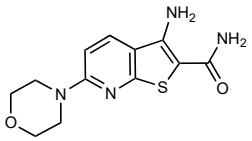


Reagents and Conditions: (i) morpholine, TEA, DMF, rt, 91%; (ii) 2-mercaptoacetamide, K_2CO_3, EtOH, reflux, 68%; (iii) diphosgene, dioxane, reflux, 69%; (iv) $PhPOCl_2$, reflux, 25%; (v) Boc-piperazine, K_2CO_3, ACN, reflux, 93%; (vi) Methanamine, NMP, 100 °C, 79%; (vii) TFA, DCM, 10%.

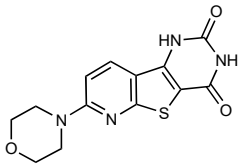


2-chloro-6-morpholinonicotinonitrile, **Intermediate 9**
2,6-Dichloropyridine-3-carbonitrile (300 mg, 1.73 mmol, 1.0 eq.) was dissolved in DMF (2 mL) in a flame dried round bottom flask under nitrogen atmosphere. To the resultant solution was added morpholine (0.15 mL, 1.73 mmol, 1.0 eq.), and triethylamine (0.5 mL, 3.46 mmol, 2.0 eq.). The reaction was stirred overnight at room temperature under nitrogen. The reaction was quenched with sat. aqueous NH_4Cl solution (10 mL). The aqueous phase was separated and extracted with EtOAc (5 x 10 mL). All combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a white crude material. ^{1}H NMR and LC-MS of the crude material showed the presence of both mono- and di-amination

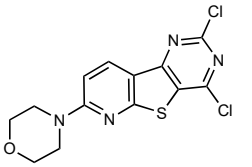
products (6:1, same t_R). The crude material was used in the next reaction without further purification (351 mg, 91 %).



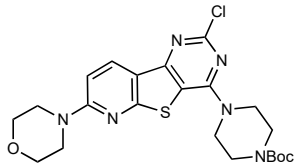
3-amino-6-morpholinothieno[2,3-b]pyridine-2-carboxamide, **Intermediate 10**
Crude **intermediate 9** (350 mg) was dissolved in ethanol (9 mL) in a flame dried round bottom flask under an atmosphere of nitrogen. To the resultant solution was added 2-mercaptoacetamide (100 mg/mL in methanolic ammonia solution, 1.71 mL, 1.88 mmol), and anhydrous K_2CO_3 (521 mg, 3.8 mmol). The reaction was refluxed overnight at 100°C under nitrogen. The reaction was quenched with water (50 mL), which precipitated the desired product. The precipitated product was filtered, washed with water (50 mL), and concentrated under reduced pressure to obtain **intermediate 10** as a pale-yellow solid (299 mg, 68%).
LC-MS (λ = 254 nm): 90%, t_R = 4.6 min. MS (ESI+): 279 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 9.1 Hz, 1H), 7.03 (br s, 2H), 6.93 (d, *J* = 9.1 Hz, 1H), 6.82 (brs, 2H), 3.73 – 3.67 (m, 4H), 3.59 – 3.53 (m, 4H)



7-morpholinopyrido[3',2':4,5]thieno[3,2-d]pyrimidine-2,4(1H,3H)-dione, **Intermediate 11**
Intermediate 10 (150 mg, 1.56 mmol, 1.0 eq.) was dissolved in dioxane (2 mL) in a flame dried round bottom flask under nitrogen atmosphere. To the resultant solution was added trichloromethyl chloroformate (0.07 mL, 0.61 mmol, 1.1 eq.), and the reaction was refluxed for 3 hours at 102°C under nitrogen. The reaction was slowly quenched with water (10 mL). The precipitated product was filtered, washed with water (50 mL), and concentrated under reduced pressure to obtain **intermediate 11** as a yellow solid (118 mg, 69%).
LC-MS (λ = 254 nm): 90%, t_R = 5.0 min. MS (ESI+): 305 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (brs, 1H), 11.29 (brs, 1H), 8.33 (d, *J* = 9.3 Hz, 1H), 7.10 (d, *J* = 9.3 Hz, 1H), 3.70 (m, 4H), 3.68 – 3.60 (m, 4H)



4-(2,4-dichloropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-7-yl)morpholine, **Intermediate 12**
Intermediate 11 (110 mg, 0.36 mmol, 1.0 eq.) was added to phenylphosphonic dichloride (4 mL) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 2 hours at 170°C under nitrogen. The reaction was slowly (possible splattering) quenched with water (10 mL), which precipitated the desired product. The precipitated product was filtered, washed with more water (30 mL), and concentrated under reduced pressure to obtain **intermediate 12** as a brown solid (30 mg, 25%).
LC-MS (λ = 254 nm): 89%, t_R = 5.6 min. MS (ESI+): 342 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.34 (d, *J* = 9.1 Hz, 1H), 6.80 (d, *J* = 9.1 Hz, 1H), 3.85 – 3.75 (m, 8H)

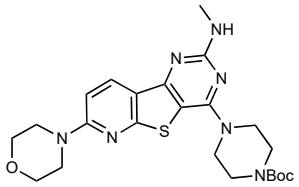


tert-butyl 4-(2-chloro-7-morpholinopyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 13**

To a solution of **intermediate 12** (30 mg, 0.088 mmol, 1.0 eq.) in CH_3CN (2 mL) was added K_2CO_3 (300 mg) and 1-Boc-piperazine (82 mg, 0.43 mmol, 5.0 eq.), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 13** was obtained as a pale-yellow solid (40 mg, 93%).

LC-MS (λ = 254 nm): 98%, t_R = 6.6 min. MS (ESI+): 492 $[M+H]^+$

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.31 (d, *J* = 9.0 Hz, 1H), 6.76 (d, *J* = 9.1 Hz, 1H), 3.97 – 3.90 (m, 4H), 3.85 – 3.77 (m, 4H), 3.73 – 3.65 (m, 4H), 3.58 (m, 4H), 1.47 (s, 9H)

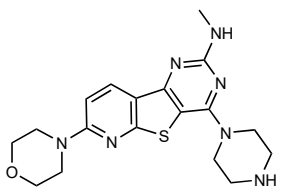


tert-butyl 4-(2-(methylamino)-7-morpholinopyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 14**

Intermediate 13 (40 mg, 0.08 mmol, 1.0 eq.) was added to methylamine (2M in THF, 5 mL) in a flame dried sealed tube under nitrogen atmosphere. The reaction was heated for 5 days at 100°C. The reaction was then quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and purified by reverse phase automated Teledyne Isco chromatography using Methanol/H_2O/0.1% acetic acid solvent system. **Intermediate 14** was obtained as an orange solid (30 mg, 79%).

LC-MS (λ = 254 nm): 99%, t_R = 5.6 min. MS (ESI+): 487 $[M+H]^+$

^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.50 (brs, 1H), 8.61 (d, *J* = 8.4 Hz, 1H), 6.82 (d, *J* = 9.3 Hz, 1H), 4.05 (dd, *J* = 6.1, 4.3 Hz, 4H), 3.83 – 3.78 (m, 4H), 3.73 – 3.69 (m, 4H), 3.65 – 3.60 (m, 4H), 2.99 (d, *J* = 3.5 Hz, 3H), 1.23 (s, 9H)



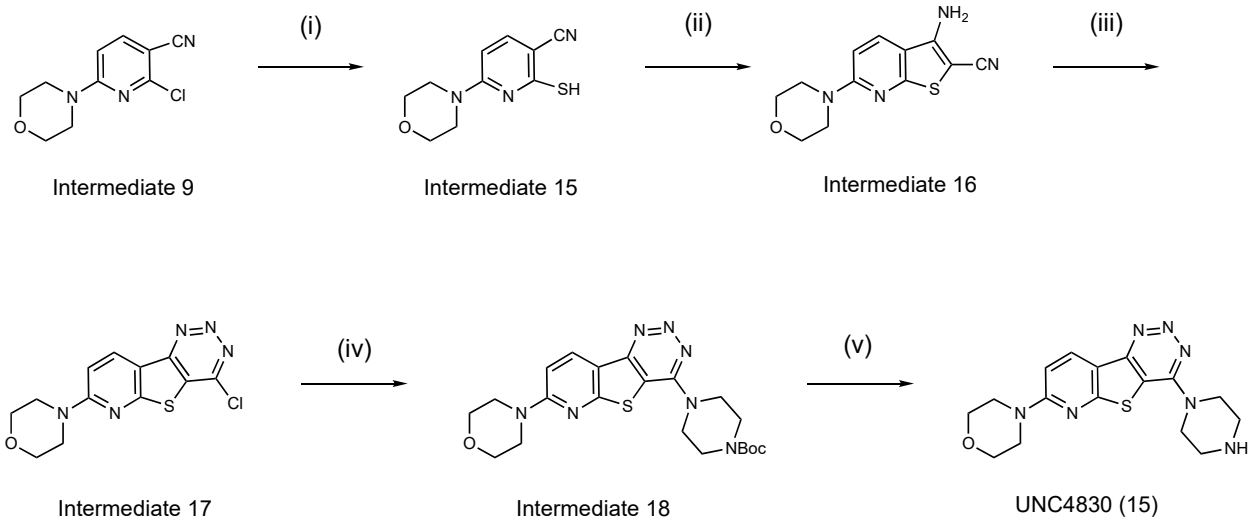
N-methyl-7-morpholino-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-2-amine, **UNC4785** (**17**)

To a solution of **intermediate 14** (30 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated and the crude material was recrystallized (methanol and CH_2Cl_2) to obtain **UNC4785 (17)** as a white solid (10 mg, 10%).

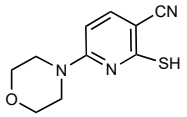
LC-MS (λ = 254 nm): 99%, t_R = 4.2 min. MS (ESI+): 387 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (brs, 1H), 8.23 (d, *J* = 9.1 Hz, 1H), 7.08 (d, *J* = 9.1 Hz, 1H), 4.02 (s, 4H), 3.68 (m, 8H), 3.29 (s, 4H), 2.89 (s, 3H)

Scheme 5. Synthesis of UNC4830 (15).

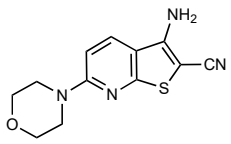


Reagents and conditions: (i) Na_2S, DMF 90 °C, 75%; (ii) $ClCH_2CN$, KOH, DMF, 72% (iii) conc. HCl, $NaNO_2$, H2O, rt, 67% (iv) Boc-piperazine, K_2CO_3, CH_3CN, reflux, 14%; (v) 20% TFA in DCM, 0 °C, 69%.



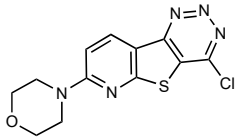
2-mercapto-6-morpholinonicotinonitrile, **Intermediate 15**

Into a flame-dried flask was added **intermediate 9** (see Scheme 4) (400 mg, 1.8 mmol, 1.0 eq.), Na_2S (209 mg, 2.7 mmol, 1.5 eq.) and DMF (9 mL) under argon. The reaction mixture was stirred at room temperature for 2 hours. Then the reaction was heated at 90 °C overnight. The precipitated product was filtered and vacuum dried to obtain **intermediate 15** (300 mg, 75%) as a yellow solid which was used in the next step without further purification.

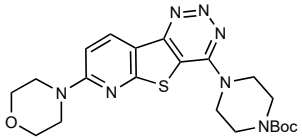


3-amino-6-morpholinothieno[2,3-b]pyridine-2-carbonitrile, **Intermediate 16**

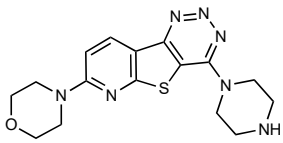
To a solution of **intermediate 15** (400 mg, 1.8 mmol, 1.0 eq.) in 2 mL of DMF was added 2-chloroacetonitrile (0.15 mL, 1.99 mmol, 1.1 eq.), and stirred at rt for an hour. Then the first portion of aq. KOH (10% w/v, 1.0 mL) was added to the reaction mixture and continued stirring at rt overnight. After overnight a second portion of aq. KOH (10% w/v, 1.0 mL) was added to the reaction mixture and stirred for another 4 hours at room temperature. Then water (20 mL) was added to the precipitated solid product, filtered, and vacuum dried to obtain **intermediate 16** as a yellow solid (339 mg, 72%).
LC-MS (λ = 254 nm): 90%, t_R = 5.3 min. MS (ESI+): 261 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.14 (d, *J* = 9.1 Hz, 1H), 6.98 (d, *J* = 9.2 Hz, 1H), 3.69 – 3.65 (m, 4H), 3.59 – 3.55 (m, 4H).



4-(4-chloropyrido[3',2':4,5]thieno[3,2-d][1,2,3]triazin-7-yl)morpholine, **Intermediate 17**
A solution of sodium nitrite (262 mg, 3.8 mmol, 3.0 eq.) in water (2 mL) was added drop wise, over 30 minutes to a suspension of **intermediate 16** (330 mg, 1.3 mmol, 1.0 eq.) in conc. HCl acid (7 mL) at 0-5 °C. The mixture was stirred for an hour at 0-5 °C and then allowed to stir at rt overnight. Water (100 mL) was added to the precipitated product, filtered, washed with water, and vacuum dried to obtain **intermediate 17** as an orange-yellow solid (258 mg; 67%).
LC-MS (λ = 254 nm): 59%, t_R = 6.1 min. MS (ESI+): 308 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.53 (d, *J* = 8.8 Hz, 1H), 6.33 (d, *J* = 8.8 Hz, 1H), 3.89 – 3.80 (m, 4H), 3.73 – 3.66 (m, 4H).



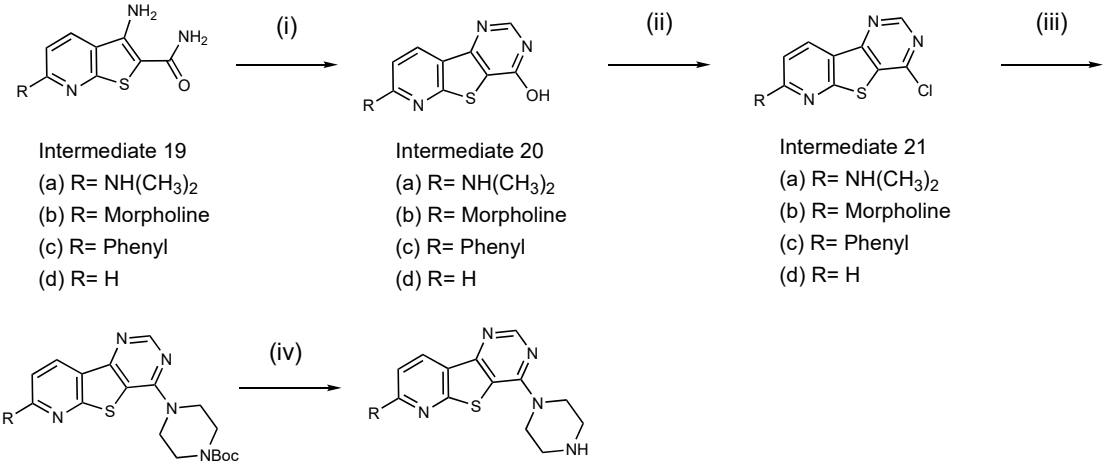
tert-butyl 4-(7-morpholinopyrido[3',2':4,5]thieno[3,2-d][1,2,3]triazin-4-yl)piperazine-1-carboxylate, **Intermediate 18**
To a solution of **intermediate 17** (258 mg, 0.84 mmol) in CH_3CN (7 mL) was added K_2CO_3 (300 mg) and 1-Boc-piperazine (780 mg, 4.19 mmol, 5.0 eq.), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL), organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and rotary evaporated to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 18** was obtained as a reddish brown solid (53 mg, 14%).
LC-MS (λ = 254 nm): 85%, t_R = 6.3 min. MS (ESI+): 459 $[M+H]^+$



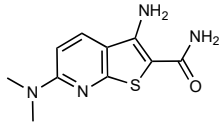
4-(4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d][1,2,3]triazin-7-yl)morpholine, **UNC4830 (15)**
To a solution of **intermediate 18** (53 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at 0 °C was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated to obtain a reddish-brown crude material.

The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system to obtain **UNC4830 (15)** as a red oil (27 mg, 69%).
LC-MS (λ = 254 nm): 99%, t_R = 4.4 min. MS (ESI+): 359 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.50 (d, *J* = 9.1 Hz, 1H), 7.22 (d, *J* = 9.2 Hz, 1H), 4.11 (m, 4H), 3.79 – 3.64 (m, 8H), 3.27 (t, *J* = 5.2 Hz, 4H).

Scheme 6. Synthesis of UNC4764 (14), UNC4746 (16), UNC4600 (18) and UNC4666 (22).

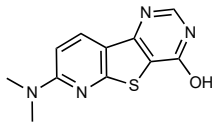


Reagents and conditions: (i) triethyl orthoformate, dioxane, reflux, o/n, 61-99% (ii) phosphoryl chloride, N_2, 106 °C, 90 mins, 55-99% (iii) Boc-piperazine, K_2CO_3, CH_3CN, reflux, 17-81% (iv) 20% TFA in DCM, 0 °C, 89-99%.

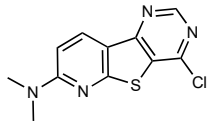


3-amino-6-(dimethylamino)thieno[2,3-b]pyridine-2-carboxamide, **Intermediate 19a**
2,6-Dichloropyridine-3-carbonitrile (300 mg, 1.73 mmol, 1.0 eq.) was dissolved in DMF (2 mL) in a flame dried round bottom flask under nitrogen atmosphere. To the resultant solution was added 2 M dimethyl amine in THF (0.865 mL, 1.73 mmol, 1.0 eq.), and triethylamine (0.5 mL, 3.46 mmol, 2.0 eq.). The reaction was stirred overnight at room temperature under nitrogen. The reaction was quenched with sat. aqueous NH_4Cl solution (10 mL). The aqueous phase was separated and extracted with EtOAc (5 x 10 mL). All combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a white crude material. ^{1}H NMR and LC-MS of the crude material showed the presence of both mono- and di-amination products (2:1, same t_R). The crude material (300 mg, 1.65 mmol) was dissolved in ethanol (9 mL) in a flame dried round bottom flask under an atmosphere of nitrogen. To the resultant solution was added 2-mercaptoacetamide (100 mg/mL in methanolic ammonia solution, 1.8 mL, 1.98 mmol), and anhydrous K_2CO_3 (547.3 mg, 3.96 mmol). The reaction was refluxed overnight at 100°C under

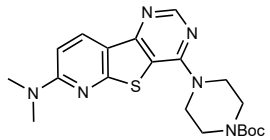
nitrogen. The reaction was quenched with water (50 mL), which precipitated the desired product. The precipitated product was filtered, washed with water (50 mL), and concentrated under reduced pressure to obtain a pale-yellow solid (297 mg, 76% over 2 steps).
LC-MS (λ = 254 nm): 90%, t_R = 4.6 min. MS (ESI+): 279 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.11 (d, *J* = 9.1 Hz, 1H), 7.03 (brs, 2H), 6.93 (d, *J* = 9.1 Hz, 1H), 6.82 (brs, 2H), 3.73 – 3.67 (m, 4H), 3.59 – 3.53 (m, 4H)



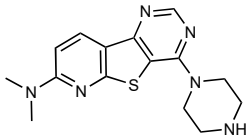
7-(dimethylamino)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-ol, **Intermediate 20a**
Intermediate 19a (250 mg, 1.05 mmol) was added to triethyl orthoformate (7 mL, excess) followed by p-toluenesulfonic acid monohydrate (19.9 mg, 0.105 mmol) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed overnight at 148°C under nitrogen. The reaction was quenched with water (15 mL) which precipitated the desired product. The precipitated product was filtered, washed with water, DCM, methanol and concentrated under reduced pressure to afford **Intermediate 20a** as a yellow solid which was used in the next reaction without further purification (145 mg, 56%).
LC-MS (λ = 254 nm): 90%, t_R = 5.1 min. MS (ESI+): 289 $[M+H]^+$



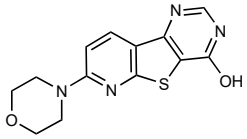
4-chloro-N,N-dimethylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-7-amine, **Intermediate 21a**
Intermediate 20a (145 mg, 0.59 mmol,) was added to phosphoryl chloride (3 mL, excess) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 90 minutes at 106°C. The reaction was concentrated under reduced pressure to obtain a dark brown crude material. This crude material was used in the next reaction without further purification (150 mg, 96%).
LC-MS (λ = 254 nm): 86%, t_R = 6.1 min. MS (ESI+): 308 $[M+H]^+$



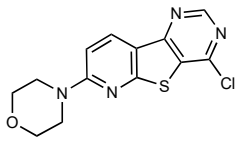
tert-butyl 4-(7-(dimethylamino)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 22a**
To a solution of **intermediate 21a** (155 mg, 0.59 mmol, 1.0 eq.) in CH_3CN (7 mL) was added K_2CO_3 (300 mg) and 1-Boc-piperazine (549.6 mg, 2.4 mmol, 5.0 eq.), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 22a** was obtained as a yellow solid (140 mg, 57%).
LC-MS (λ = 254 nm): 99%, t_R = 6.3 min. MS (ESI+): 457 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 1H), 3.95 – 3.88 (m, 4H), 3.84 – 3.66 (m, 8H), 3.61 – 3.54 (m, 4H), 1.47 (s, 9H)



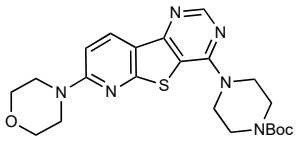
N,N-dimethyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-7-amine, **UNC4764 (14)**
To a solution of **intermediate 22a** (140 mg, 0.34 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated and the desired product was recrystallized to obtain **UNC4764 (14)** as a pale-yellow solid (85 mg, 59%).
LC-MS (λ = 254 nm): 99%, t_R = 4.6 min. MS (ESI+): 315 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.12 (brs, 1H), 8.72 (s, 1H), 8.31 (d, J = 9.1 Hz, 1H), 6.97 (d, *J* = 9.1 Hz, 1H), 4.13 (m, 4H), 3.32 (m, 4H), 3.18 (s, 6H).



7-morpholinopyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-ol, **Intermediate 20b**
Intermediate 16 (see scheme **5**) (150 mg, 0.54 mmol, 1.0 eq.) was added to triethyl orthoformate (5 mL) followed by p-toluenesulfonic acid monohydrate (10.2 mg, 0.05 mmol, 0.1 eq.) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed overnight at 148°C under nitrogen. The reaction was quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure to obtain a yellow crude product, which was used in the next reaction without further purification (155 mg, 99%).
LC-MS (λ = 254 nm): 90%, t_R = 5.1 min. MS (ESI+): 289 $[M+H]^+$

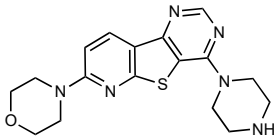


*4-(4-chloropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-7-yl)morpholine***, Intermediate 21b**
Intermediate 20b (150 mg, 0.52 mmol, 1.0 eq.) was added to phosphoryl chloride (3 mL) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 90 minutes at 106°C. The reaction was concentrated under reduced pressure to obtain a dark black crude material. This crude material was used in the next reaction without further purification (158 mg, 99%).
LC-MS (λ = 254 nm): 86%, t_R = 6.1 min. MS (ESI+): 307 $[M+H]^+$

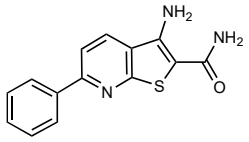


tert-butyl 4-(7-morpholinopyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 22b**
To a solution of **intermediate 21b** (150 mg, 0.5 mmol, 1.0 eq.) in CH_3CN (7 mL) was added K_2CO_3 (300 mg) and 1-Boc-piperazine (455 mg, 2.4 mmol, 5.0 eq.), and the reaction mixture was heated

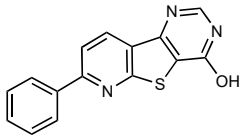
under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 22b** was obtained as a yellow solid (39 mg, 17%)
LC-MS (λ = 254 nm): 99%, t_R = 6.3 min. MS (ESI+): 457 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.61 (s, 1H), 8.32 (d, *J* = 9.0 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 1H), 3.95 – 3.88 (m, 4H), 3.84 – 3.66 (m, 8H), 3.61 – 3.54 (m, 4H), 1.47 (s, 9H)



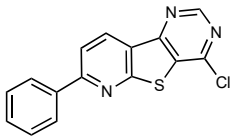
4-(4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-7-yl)morpholine, **UNC4746 (16)**
To a solution of **intermediate 22b** (39 mg, 0.08 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated to obtain the TFA salt of **UNC4746 (16)** as a pale-yellow solid (29 mg, 99%).
LC-MS (λ = 254 nm): 99%, t_R = 4.6 min. MS (ESI+): 357 $[M+H]^+$
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.92 (brs, 1H), 8.67 (s, 1H), 8.34 (d, *J* = 9.1 Hz, 1 H), 7.14 (d, *J* = 9.1 Hz, 1H), 4.11 – 4.05 (m, 4H), 3.75 – 3.67 (m, 8H), 3.30 (s, 4H)



3-amino-6-phenylthieno[2,3-b]pyridine-2-carboxamide, **Intermediate 19c**
Intermediate 19c was synthesized according to Liu et al. *Bioorg. Med. Chem. Lett*. *23* (**2013**) 2349-2352)[3].

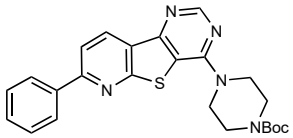


7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-ol, **Intermediate 20c**
Intermediate 19c (80 mg, 0.3 mmol, 1.0 eq.) was added to triethyl orthoformate (2 mL, excess) followed by p-toluenesulfonic acid monohydrate (5.6 mg, 0.03 mmol, 0.1 eq.) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed overnight at 148°C under nitrogen. The reaction was quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a yellow crude product, which was used in the next reaction without further purification (50 mg, 61%).
LC-MS (λ = 254 nm): 70%, t_R = 5.8 min. MS (ESI+): 280 $[M+H]^+$



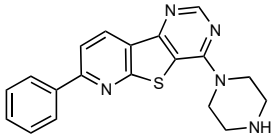
4-chloro-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidine, **Intermediate 21c**

Intermediate 20c (50 mg, 014 mmol, 1.0 eq) was added to phosphoryl chloride (1 mL) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 90 minutes at 106°C. The reaction was concentrated under reduced pressure to obtain a dark black crude material. The crude material was dissolved in CH_2Cl_2 (15 mL) and the organic layer was washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a brown crude product. The crude material isolated as a brown solid was used in the next reaction without further purification (51 mg, 99%).
LC-MS (λ = 254 nm): 80%, t_R = 6.8 min. MS (ESI+): 298 $[M+H]^+$



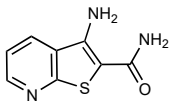
tert-butyl 4-(7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 22c**

To a solution of **intermediate 21c** (50 mg, 0.16 mmol, 1.0 eq.) in ethanol (3 mL) was added 1-Boc-piperazine (157 mg, 0.84 mmol, 5.0 eq.), and the reaction mixture was heated under reflux for 6 hrs. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using EtOAc/hexane solvent system. **Intermediate 22c** was obtained as a yellow solid (50 mg, 70%).
LC-MS (λ = 254 nm): 99%, t_R = 6.8 min. MS (ESI+): 448 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.74 (s, 1H), 8.70 (d, *J* = 8.3 Hz, 1H), 8.17 – 8.13 (m, 2H), 7.96 (d, *J* = 8.4 Hz, 1H), 7.54 – 7.49 (m, 3H), 4.02 (t, *J* = 5.2 Hz, 4H), 3.64 (dd, *J* = 6.5, 4.1 Hz, 4H), 1.51 (s, 9H).



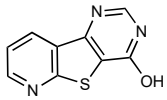
7-phenyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidine, **UNC4600 (18)**

To a solution of **intermediate 22c** (50 mg, 0.08 mmol) in CH_2Cl_2 (1 mL) at 0°C was added trifluoroacetic acid (1 mL), and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated to obtain **UNC4600 (18)** as a pale-yellow solid (45 mg, 89%).
LC-MS (λ = 254 nm): 99%, t_R = 5.4 min. MS (ESI+): 348 $[M+H]^+$
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.76 (s, 1H), 8.74 (d, *J* = 8.4 Hz, 1H), 8.20 – 8.16 (m, 2H), 8.13 (d, *J* = 8.4 Hz, 1H), 7.57 – 7.49 (m, 3H), 4.31 – 4.25 (m, 4H), 3.47 – 3.43 (m, 4H).



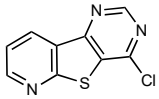
3-aminothieno[2,3-b]pyridine-2-carboxamide, **Intermediate 19d**

Intermediate 19d was synthesized according to B. E. Sleebs et al. *Med. Chem. Comm. 2* (**2011**) 977-981)[4].



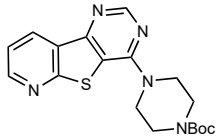
pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-ol, **Intermediate 20d**

Intermediate 19d (200 mg, 1.04 mmol, 1.0 eq.) was added to triethyl orthoformate (5 mL, excess) followed by p-toluenesulfonic acid monohydrate (19.7 mg, 0.01 mmol, 0.1 eq.) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed overnight at 148°C under nitrogen. The reaction was quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with saturated $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure to obtain a pale-yellow product, which was used in the next reaction without further purification (169 mg, 80%).
LC-MS (λ = 254 nm): 90%, t_R = 4.3 min. MS (ESI+): 204 $[M+H]^+$



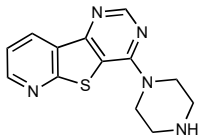
4-chloropyrido[3',2':4,5]thieno[3,2-d]pyrimidine, **Intermediate 21d**

Intermediate 20d (100 mg, 0.33 mmol, 1.0 eq.) was added to phosphoryl chloride (2 mL, excess) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 90 minutes. The reaction was concentrated under reduced pressure to obtain a dark black crude material. The crude material was used in the next reaction without further purification (60 mg, 55%).
LC-MS (λ = 254 nm): 86%, t_R = 5.6 min. MS (ESI+): 222 $[M+H]^+$



tert-butyl 4-(pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 22d**

To a solution of **intermediate 21d (**60 mg, 0.26 mmol, 1.0 eq.) in CH_3CN (7 mL) was added K_2CO_3 (300 mg) and 1-Boc-piperazine (251 mg, 1.3 mmol, 5.0 eq.), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using EtOAc/hexane solvent system. **Intermediate 22d** was obtained as a white solid (80 mg, 81%).
LC-MS (λ = 254 nm): 99%, t_R = 6.0 min. MS (ESI+): 372 $[M+H]^+$
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.87 (brs, 1H), 8.77 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.71 (s, 1H), 8.65 (dd, *J* = 7.9, 1.7 Hz, 1H), 7.47 (dd, *J* = 8.0, 4.7 Hz, 1H), 4.04 – 3.93 (m, 4H), 3.65 – 3.55 (m, 4H), 1.48 (s, 9H).



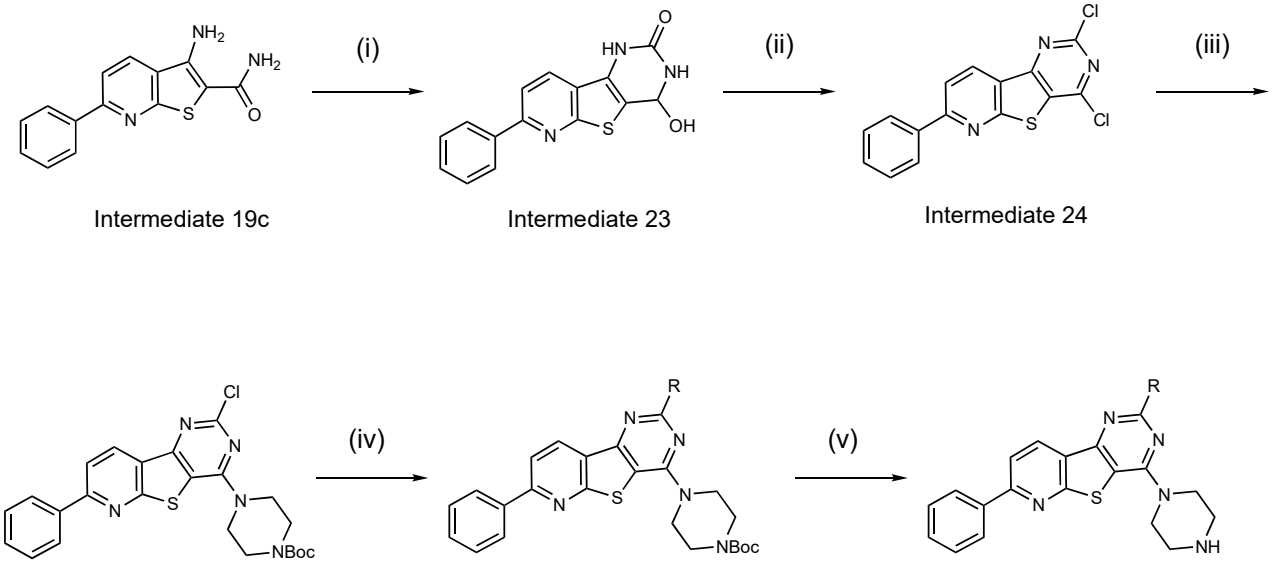
4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidine, **UNC4666 (22)**

To a solution of **intermediate 22d** (80 mg, 0.21 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL), and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated to obtain **UNC4666 (22)** as a pale-yellow solid (80 mg, 97%).

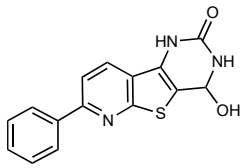
LC-MS (λ = 254 nm): 99%, t_R = 4.3 min. MS (ESI+): 272 $[M+H]^+$

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (br s, 1H), 8.88 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.79 (s, 1H), 8.71 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.69 (dd, *J* = 8.0, 4.6 Hz, 1H), 4.18 – 4.11 (m, 4H), 3.33 (m, 4H).

Scheme 7. Synthesis of UNC4847 (21), UNC4707 (20) and UNC4708 (19).

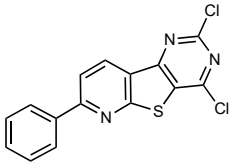


Reagents and conditions: (i) trichloromethyl carbonochloridate, dioxane, reflux, o/n, 61%; (ii) phenylphosphonic dichloride, dioxane, 100 °C, 3 hr, 65%; (iii) boc-piperazine, K_2CO_3, CH_3CN, reflux, 47%; (iv) X-NH_2, THF, 100 °C, 5 d, 38-78%; (v) HCl, dioxane, rt, 51-96%.

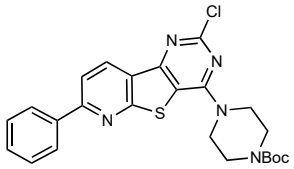


4-hydroxy-7-phenyl-3,4-dihydropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-2(1H)-one, **Intermediate 23**

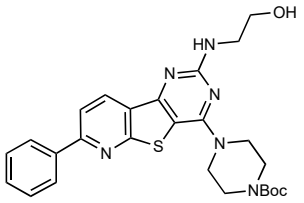
Intermediate 19c (scheme **6**) (917 mg, 3.71 mmol, 1.0 eq.) was dissolved in dioxane (4 mL) in a flame dried round bottom flask under nitrogen atmosphere. To the resultant solution was added trichloromethyl chloroformate (0.5 mL, 4.08 mmol, 1.1 eq.), and the reaction was refluxed for 3 hours at 102°C under nitrogen. The reaction was slowly quenched with water (10 mL). The precipitated product was filtered, washed with water (50 mL), and dried under reduced pressure to obtain **intermediate 23** as a brown solid (674 mg, 61%).
LC-MS (λ = 254 nm): 82%, t_R = 6.3 min. MS (ESI+): 296 [M+H]+
^{1}H NMR (400 MHz, Methanol-d_4) δ 8.56 (d, *J* = 8.5 Hz, 1H), 8.22 – 8.10 (m, 2H), 8.04 (d, *J* = 8.7 Hz, 1H), 7.51 (m, 3H).



2,4-dichloro-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidine, **Intermediate 24**
Intermediate 23 (357 mg, 1.64 mmol, 1.0 eq.) was added to phenylphosphonic dichloride (6 mL, 42.5 mmol) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 2 hours at 170°C under nitrogen. The reaction was slowly (possible splattering) quenched with water (10 mL) at 0 C, which precipitated the desired product. The precipitated product was filtered, washed with water (30 mL), and concentrated under reduced pressure to obtain a dark brown solid. The crude material was used in the next step without further purification (272 mg, 65%).
LC-MS (λ = 254 nm): 60%, t_R = 7.5 min. MS (ESI+): 333 [M+H]+



tert-butyl 4-(2-chloro-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 25**
To a solution of **intermediate 24** (270 mg, 0.81 mmol) in CH_3CN (7 mL) was added K_2CO_3 (561 mg, 4.1 mmol) and 1-Boc-piperazine (757 mg, 4.1 mmol), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 20 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×20 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 25** was obtained as a yellow solid (129 mg, 47%).
LC-MS (λ = 254 nm): 99%, t_R = 7.4 min. MS (ESI+): 482 [M+H]+
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.68 (d, *J* = 8.3 Hz, 1H), 8.14 – 8.09 (m, 2H), 7.93 (d, *J* = 8.4 Hz, 1H), 7.53 – 7.48 (m, 3H), 4.06 – 3.96 (m, 4H), 3.66 – 3.59 (m, 4H), 1.49 (s, 9H).

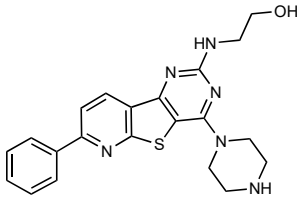


tert-butyl 4-(2-((2-hydroxyethyl)amino)-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 26a**

In a flame dried sealed tube under an atmosphere of nitrogen **intermediate 25** (80 mg, 0.17 mmol, 1.0 eq.) was added to a solution of ethanolamine (51 mg, 0.83 mmol, 5.0 eq.) in NMP (4 mL). The reaction was heated for 5 hr at 90°C. The reaction was then quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a brown crude material. The crude material was adsorbed onto silica gel, and purified by reverse phase automated Teledyne Isco chromatography using Methanol/H_2O/0.1% acetic acid solvent system. **Intermediate 26a** was obtained as a yellow solid (32 mg, 38%).

LC-MS (λ = 254 nm): 99%, t_R = 6.3 min. MS (ESI+): 507 [M+H]+

^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.49 (d, *J* = 8.4 Hz, 1H), 8.13 – 8.08 (m, 2H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.53 – 7.41 (m, 3H), 3.89 (m, 6H), 3.65 (m, 2H), 3.61 – 3.55 (m, 4H), 1.23 (s, 9H). One proton hidden under solvent peak.

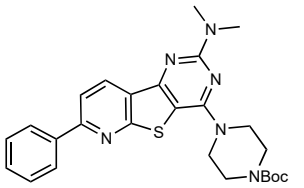


2-((7-phenyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-2-yl)amino)ethan-1-ol, **UNC4847 (21)**

To a solution of **intermediate 26a** (32 mg, 0.06 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL) and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated to obtain **UNC4847 (21)** as a TFA salt and pale-yellow solid (38 mg, 96%).

LC-MS (λ = 254 nm): 99%, t_R = 5.1 min. MS (ESI+): 407 [M+H]+

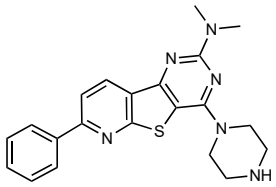
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.93 (brs, 1H), 8.57 (d, *J* = 8.4 Hz, 1H), 8.26 – 8.17 (m, 3H), 7.62 – 7.50 (m, 3H), 4.60 (t, *J* = 5.3 Hz, 1H), 4.06 (m, 4H), 3.77 (m, 2H), 3.60 (t, *J* = 6.1 Hz, 1H), 3.32 (m, 4H).



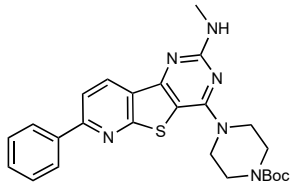
tert-butyl 4-(2-(dimethylamino)-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 26b**

In a flame dried sealed tube under an atmosphere of nitrogen, **intermediate 25** (80 mg, 0.17 mmol, 1.0 eq.) was added to a suspension of 2 M solution dimethylamine in THF (0.17 mL, 0.33 mmol, 2.0 eq.) and NMP (4 mL). The reaction was then quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat.

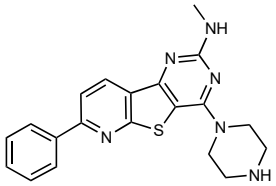
$NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a brown crude material. The crude material was adsorbed onto silica gel, and purified by automated Teledyne Isco chromatography using a gradient of ethyl acetate in hexanes. **Intermediate 26b** was obtained as a yellow solid (54 mg, 67%).
LC-MS (λ = 254 nm): 99%, t_R = 6.3 min. MS (ESI+): 507 [M+H]+



N,N-dimethyl-7-phenyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-2-amine, **UNC4707 (20)**
To a solution of **intermediate 26b** (54 mg, 0.11 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL) and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated, the crude material was adsorbed onto silica gel, and purified by automated Teledyne Isco chromatography using CH_2Cl_2/Methanol/NH_3 solvent system. **UNC4707 (20)** was obtained as a yellow solid (40 mg, 93%).
LC-MS (λ = 254 nm): 99%, t_R = 5.8 min. MS (ESI+): 391 [M+H]+
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.58 (d, *J* = 8.4 Hz, 1H), 8.23 – 8.15 (m, 3H), 7.60 – 7.49 (m, 3H), 4.00 (m, 4H), 3.23 (m, 4H), 3.21 (s, 6H).



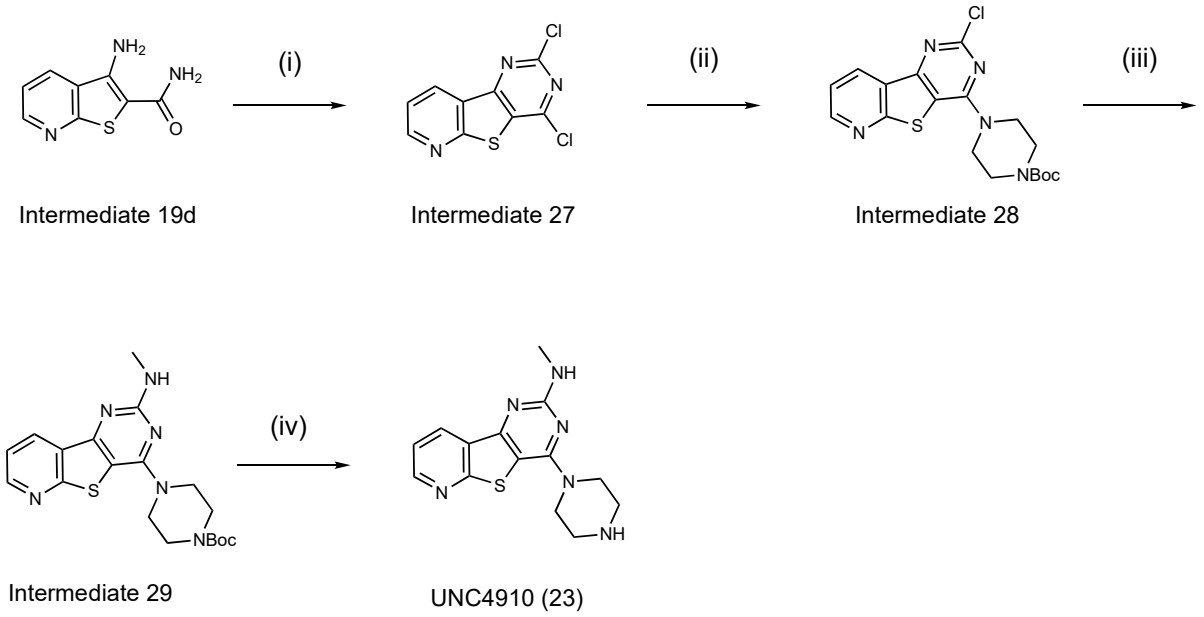
tert-butyl 4-(2-(methylamino)-7-phenylpyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate, **Intermediate 26c**
In a flame dried sealed tube under an atmosphere of nitrogen **intermediate 25** (80 mg, 0.17 mmol, 1.0 eq.) was added to a suspension of 2M solution methanamine in THF (0.17 mL, 0.33 mmol, 2.0 eq.) and NMP (4 mL). The reaction was heated for 5 hrs. at 90°C. The reaction was then quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a brown crude material. The crude material was adsorbed onto silica gel, and purified by automated Teledyne Isco chromatography using a gradient of EtOAc in hexanes. **Intermediate 26c** was obtained as a yellow solid (62 mg, 78%).
LC-MS (λ = 254 nm): 99%, t_R = 6.3 min. MS (ESI+): 507 [M+H]+
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.49 (d, *J* = 8.4 Hz, 1H), 8.13 – 8.08 (m, 2H), 7.82 (d, *J* = 8.4 Hz, 1H), 7.53 – 7.41 (m, 3H), 3.89 (m, 6H), 3.65 (m, 2H), 3.61 – 3.55 (m, 4H), 1.23 (s, 9H).



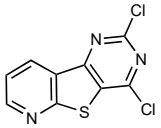
N-methyl-7-phenyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-2-amine, **UNC4708 (19)**

To a solution of **intermediate 26c** (62 mg, 0.13 mmol) in CH_2Cl_2 (2 mL) at 0°C was added trifluoroacetic acid (2 mL) and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated, the crude material was adsorbed onto silica gel, and purified by reverse phase automated Teledyne Isco chromatography using Methanol/H_2O/0.1% acetic acid solvent system. **UNC4708 (19)** was obtained as a yellow solid (20 mg, 51%).
LC-MS (λ = 254 nm): 99%, t_R = 5.4 min. MS (ESI+): 377 [M+H]+
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.00 (brs, 1H), 8.57 (d, *J* = 8.4 Hz, 1H), 8.23 – 8.19 (m, 2H), 8.17 (d, *J* = 8.4 Hz, 1H), 7.61 – 7.48 (m, 3H), 4.05 (t, *J* = 5.2 Hz, 4H), 3.32 (m, 4H), 2.91 (s, 3H).

Scheme 8- Synthesis of UNC4910 (23).

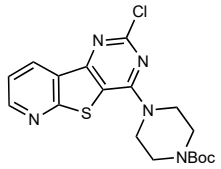


Reagents and conditions: (i) trichloromethyl chloroformate, dioxane, reflux, o/n, 71%; phenylphosphonic dichloride, dioxane, 100 °C, 3 hr, 85%; (ii) boc-piperazine, K_2CO_3, CH_3CN, reflux, 56%; (iii) methanamine, THF, 100 °C, 5 d, 98%; (iv) HCl, dioxane, rt, 63%.

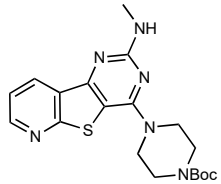


2,4-dichloropyrido[3',2':4,5]thieno[3,2-d]pyrimidine, **Intermediate 27**
Intermediate 19d (**Scheme 6**, 1.2 g, 6.6 mmol, 1.0 eq.) was dissolved in dioxane (5 mL) in a flame dried round bottom flask under nitrogen atmosphere. To the resultant solution was added trichloromethyl chloroformate (0.9 mL, 7.24 mmol, 1.1 eq.), and the reaction was refluxed for 3 hours at 102°C under nitrogen. The reaction was slowly quenched with water (10 mL). The precipitated product was filtered, washed with water (50 mL), and concentrated under reduced pressure to obtain a brown solid (1.2 g). The brown crude product (1.2 g, 6.8 mmol, 1.0 eq.) was added to phenylphosphonic dichloride (10 mL) in a flame dried round bottom flask under nitrogen atmosphere. The reaction was refluxed for 2 hours at 170°C under nitrogen. The reaction was slowly (possible splattering) quenched with water (20 mL), which precipitated the desired product.

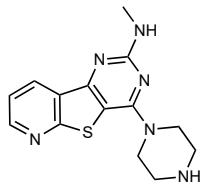
The precipitated product was filtered, washed with water (30 mL), and concentrated under reduced pressure to obtain a pale brown solid (1.2 g, 85%). The product was carried forward to the next step without purification.
LC-MS (λ = 254 nm): 70%, t_R = 6.3 min. MS (ESI+): 256 [M]+



tert-butyl 4-(2-chloropyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate,
Intermediate 28
To a solution of **intermediate 27** (1.2 g) in CH_3CN (20 mL) was added K_2CO_3 (1 g) and 1-Boc-piperazine (2 g, 10.7 mmol), and the reaction mixture was heated under reflux overnight. Upon completion, the reaction was quenched by addition of 50 mL of sat. $NaHCO_3$ solution. The aqueous phase was extracted with CH_2Cl_2 (3×50 mL) and the organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a dark brown crude material. The crude material was adsorbed onto silica gel, and purified by normal phase automated Teledyne Isco chromatography using CH_2Cl_2/MeOH/NH_3 solvent system. **Intermediate 28** was obtained as a yellow solid (1.07 g, 56%).
LC-MS (λ = 254 nm): 99%, t_R = 6.5 min. MS (ESI+): 407 [M+H]+
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.79 (dd, *J* = 4.7, 1.7 Hz, 1H), 8.67 (dd, *J* = 8.0, 1.7 Hz, 1H), 7.48 (dd, *J* = 8.0, 4.7 Hz, 1H), 4.06 – 3.98 (m, 4H), 3.68 – 3.58 (m, 4H), 1.48 (s, 9H).



tert-butyl 4-(2-(methylamino)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-4-yl)piperazine-1-carboxylate,
Intermediate 29
In a flame dried sealed tube under an atmosphere of nitrogen **intermediate 28** (100 mg, 0.25 mmol, 1.0 eq.) and K_2CO_3 (300 mg, 2.14 mmol) was added to a solution of methylamine (2M in THF, 0.62 mL, 1.25 mmol, 5.0 eq.) in NMP (2 mL). The reaction was heated overnight at 90°C. The reaction was then quenched with water (15 mL) and extracted with ethyl acetate (3×20 mL). The combined organic fractions were washed with sat. $NaHCO_3$ solution, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to obtain a brown crude material. The crude material was adsorbed onto silica gel, and purified by reverse phase automated Teledyne Isco chromatography using Methanol/H_2O/0.1% acetic acid solvent system. **Intermediate 29** was obtained as a yellow solid (98 mg, 98%).
LC-MS (λ = 254 nm): 99%, t_R = 6.0 min. MS (ESI+): 401 [M+H]+
^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.24 (d, *J* = 8.8 Hz, 1H), 6.51 (d, *J* = 8.8 Hz, 1H), 5.04 (dd, *J* = 9.9, 4.7 Hz, 1H), 3.94 (t, *J* = 5.1 Hz, 4H), 3.58 (dd, *J* = 6.5, 3.9 Hz, 4H), 3.04 (d, *J* = 5.1 Hz, 3H), 1.47 (s, 9H).



N-methyl-4-(piperazin-1-yl)pyrido[3',2':4,5]thieno[3,2-d]pyrimidin-2-amine, **UNC4910 (23)**

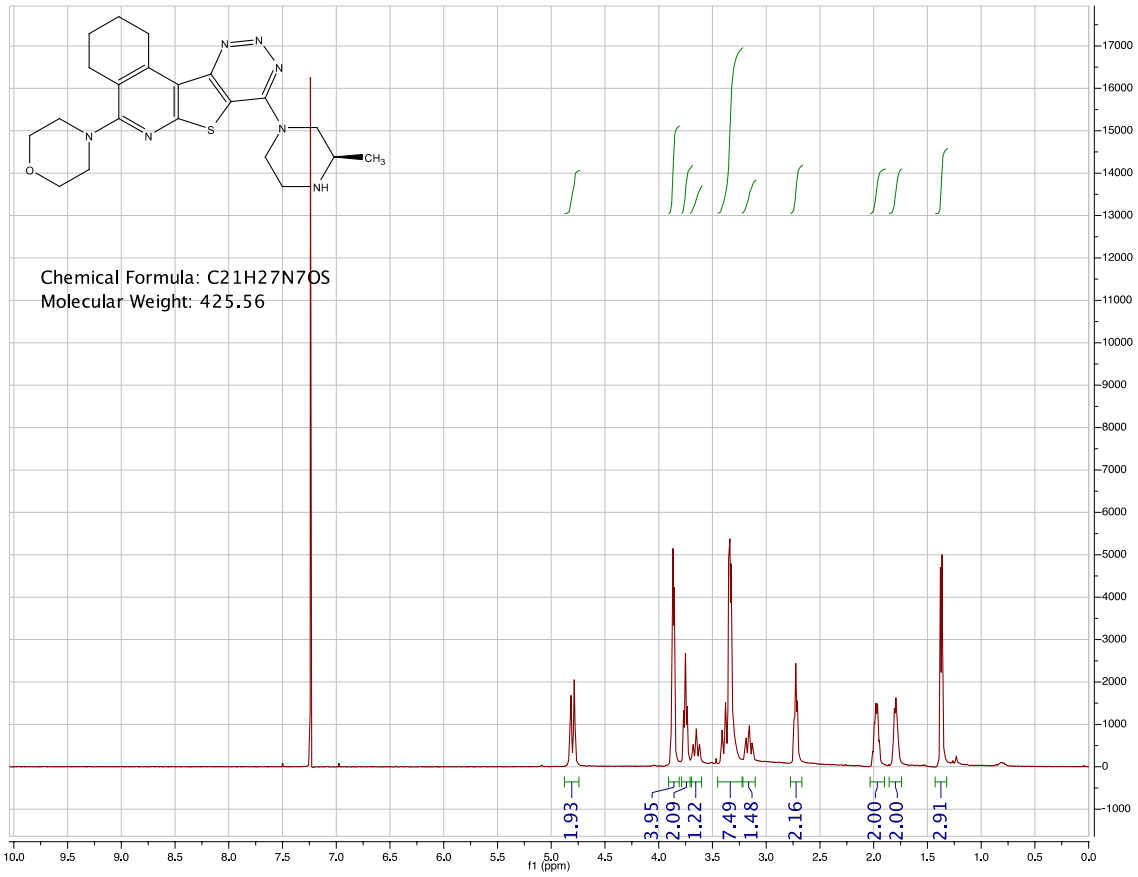
To **Intermediate 29 (**98 mg, 0.24 mmol)) was added HCl/4M in dioxane (2 mL) at 0°C and the reaction mixture was stirred until completion at room temperature. The solution was vacuum concentrated to obtain a pale-yellow product, and the product was recrystallized using CH_2Cl_2 and methanol (53 mg, 63%).

LC-MS (λ = 254 nm): 99%, t_R = 3.7 min. MS (ESI+): 301 [M+H]+

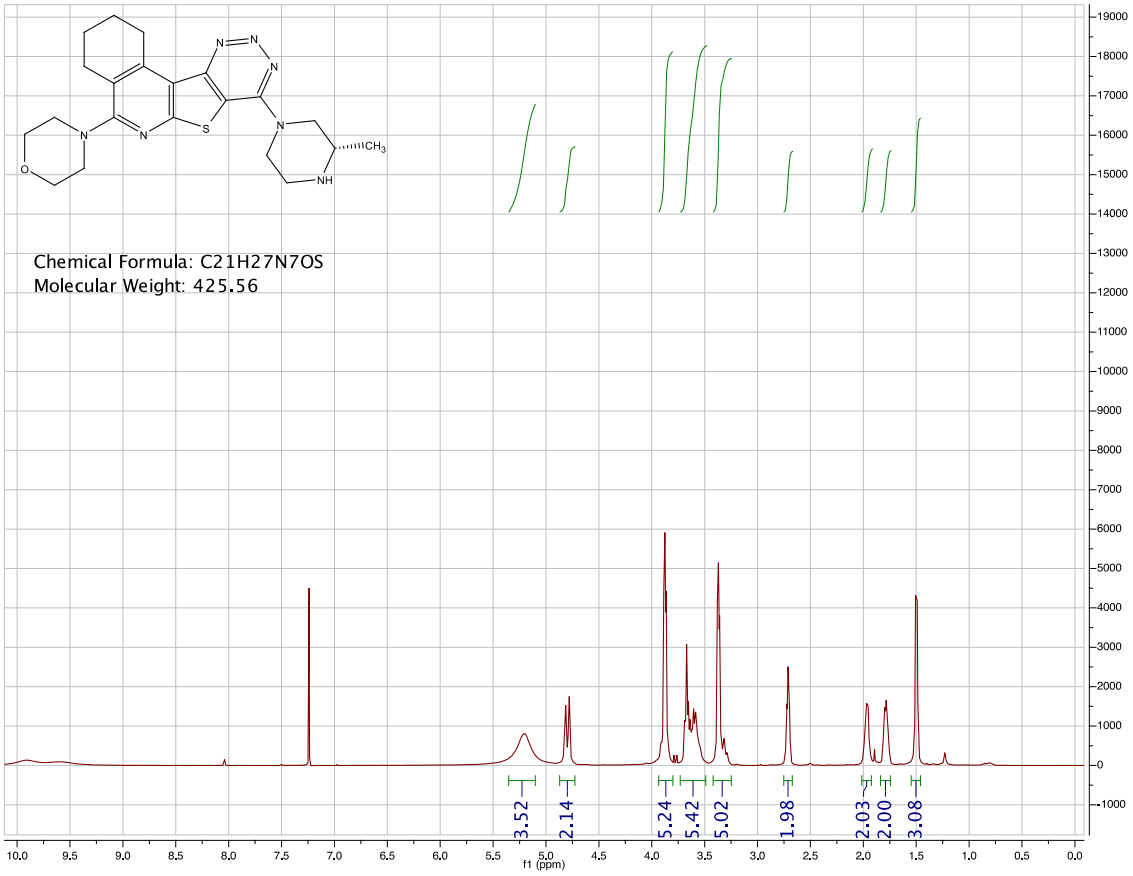
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.76 (d, *J* = 5.5 Hz, 1H), 8.60 (d, *J* = 8.2 Hz, 1H), 7.61 (dd, *J* = 8.2, 4.7 Hz, 1H), 4.16 (t, *J* = 5.1 Hz, 4H), 3.29 (t, *J* = 5.2 Hz, 4H), 2.91 (s, 3H).

^{1}H NMR Spectra for Final Compounds

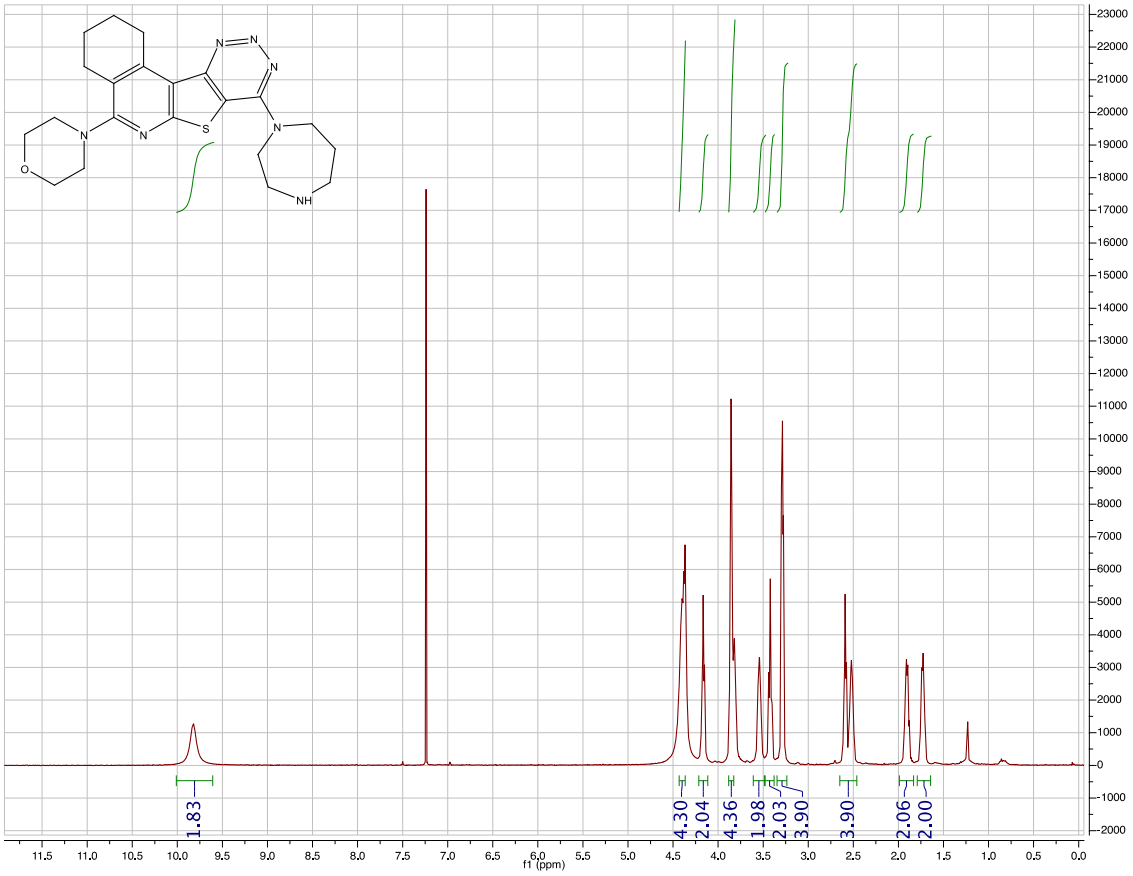
UNC4601 (3), 1H NMR (400 MHz, Chloroform-*d*)



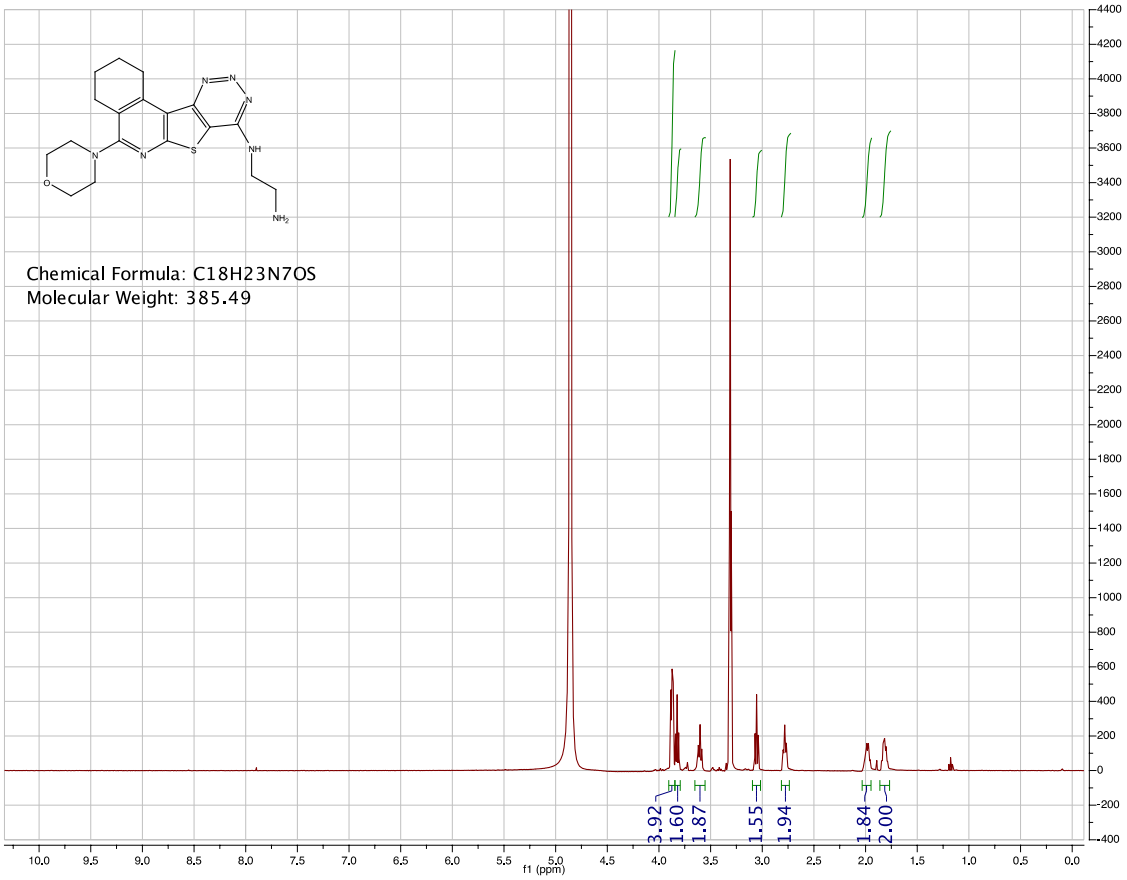
UNC4684 (4), ^{1}H NMR (400 MHz, Chloroform-*d*)



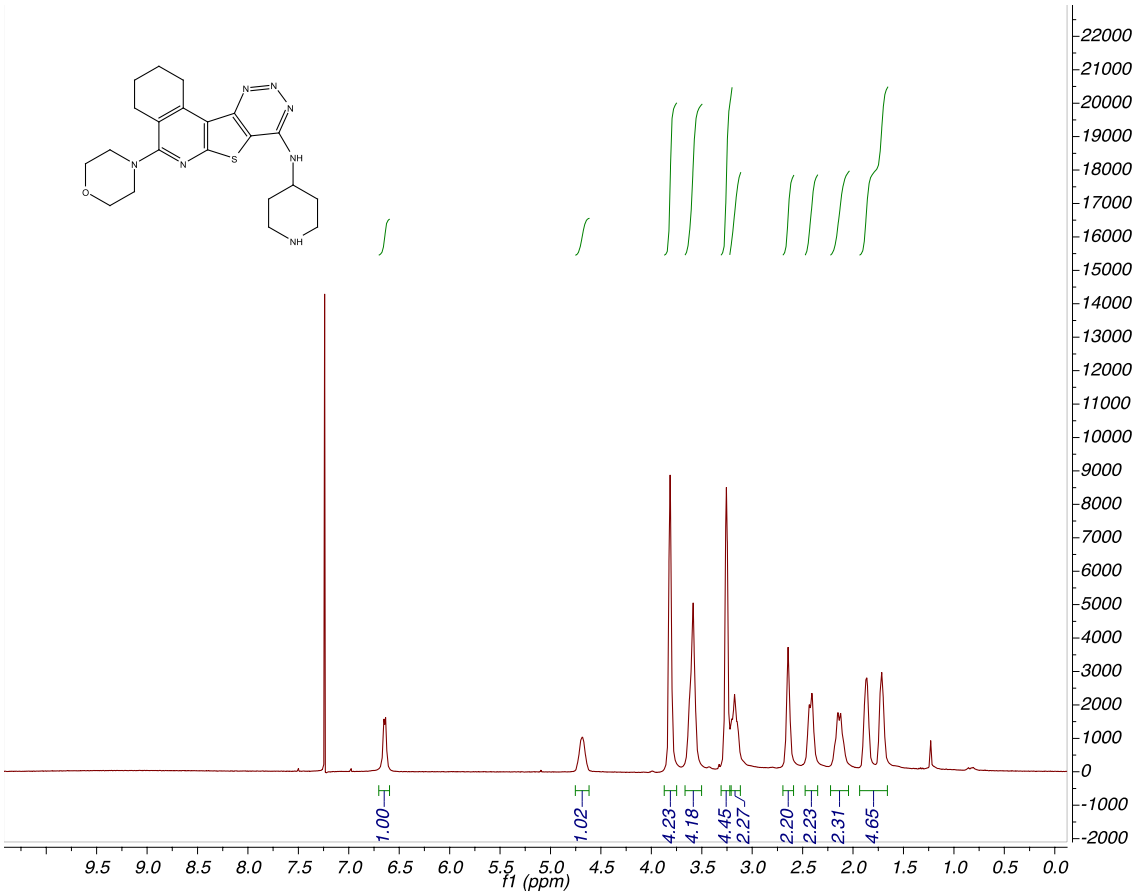
UNC4511 (5), 1H NMR (400 MHz, Chloroform-d)



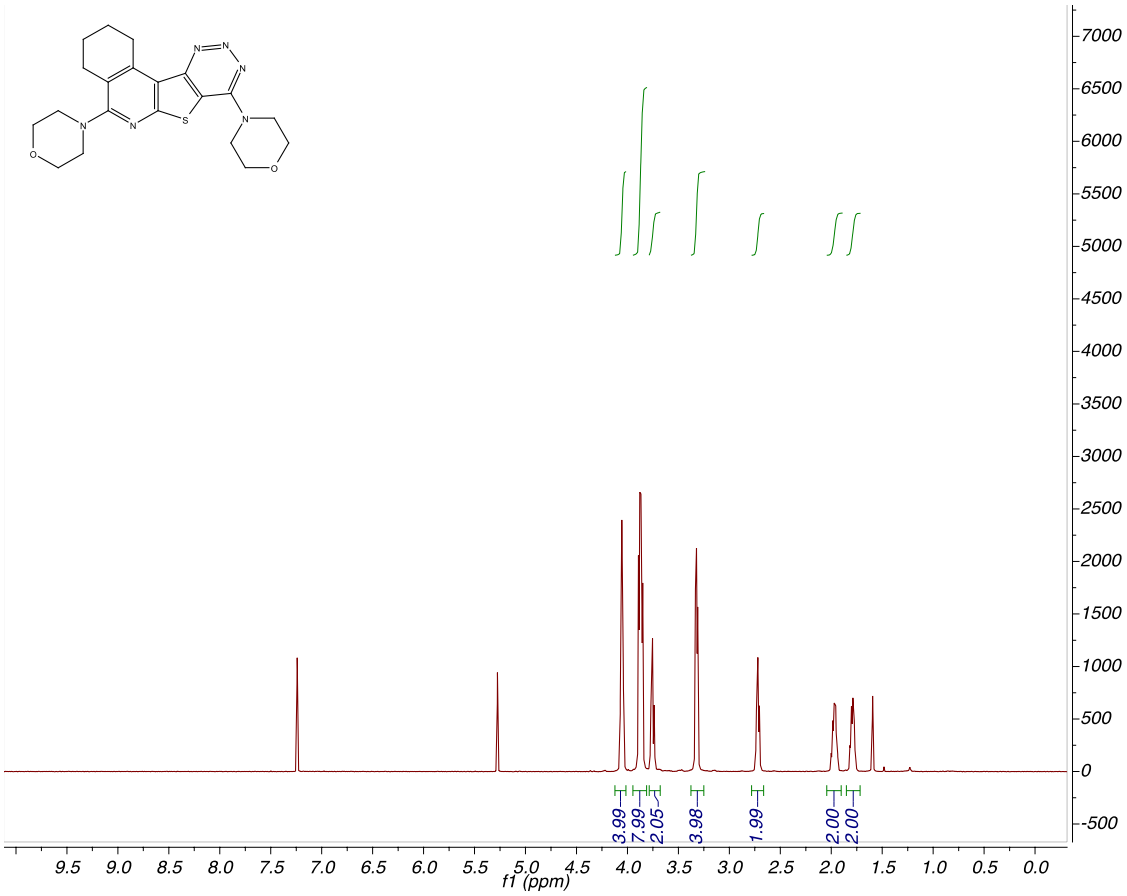
UNC4540 (6), 1H NMR (400 MHz, Methanol-d4)



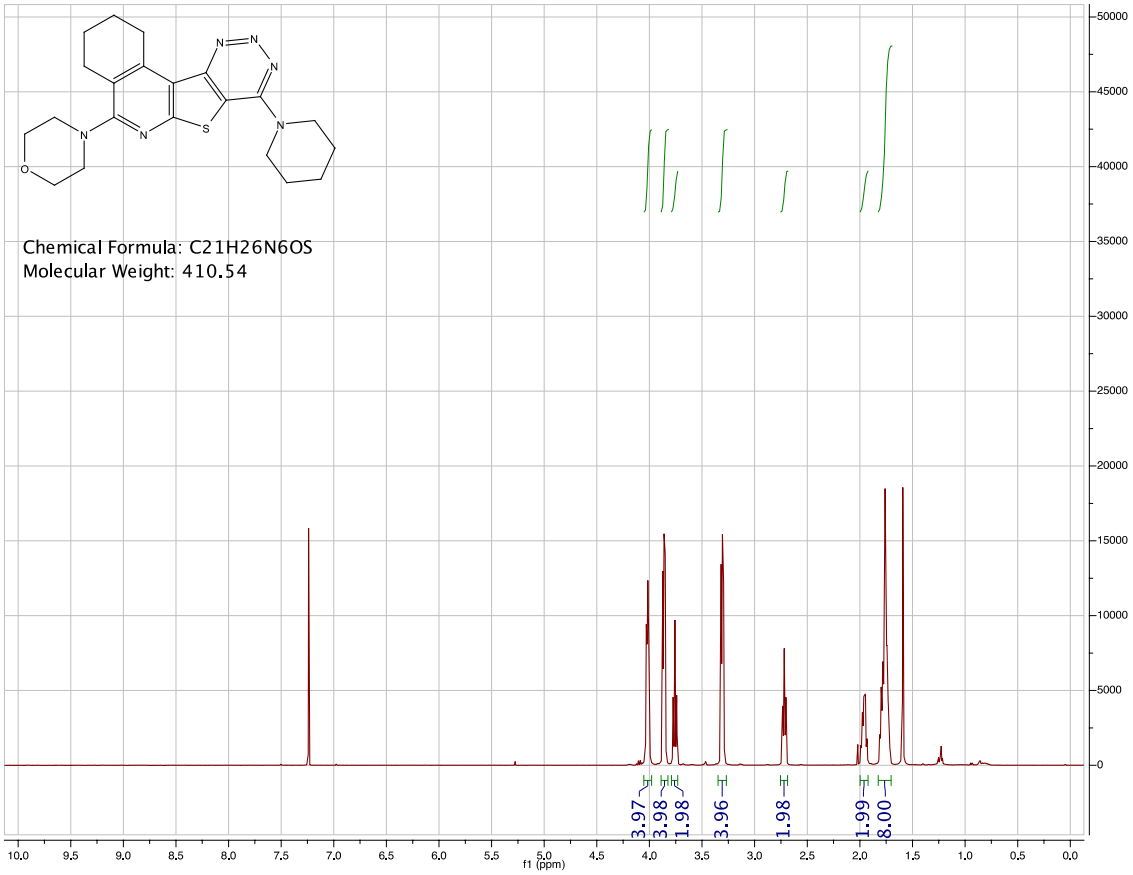
UNC4351 (7), 1H NMR (400 MHz, Chloroform-d)



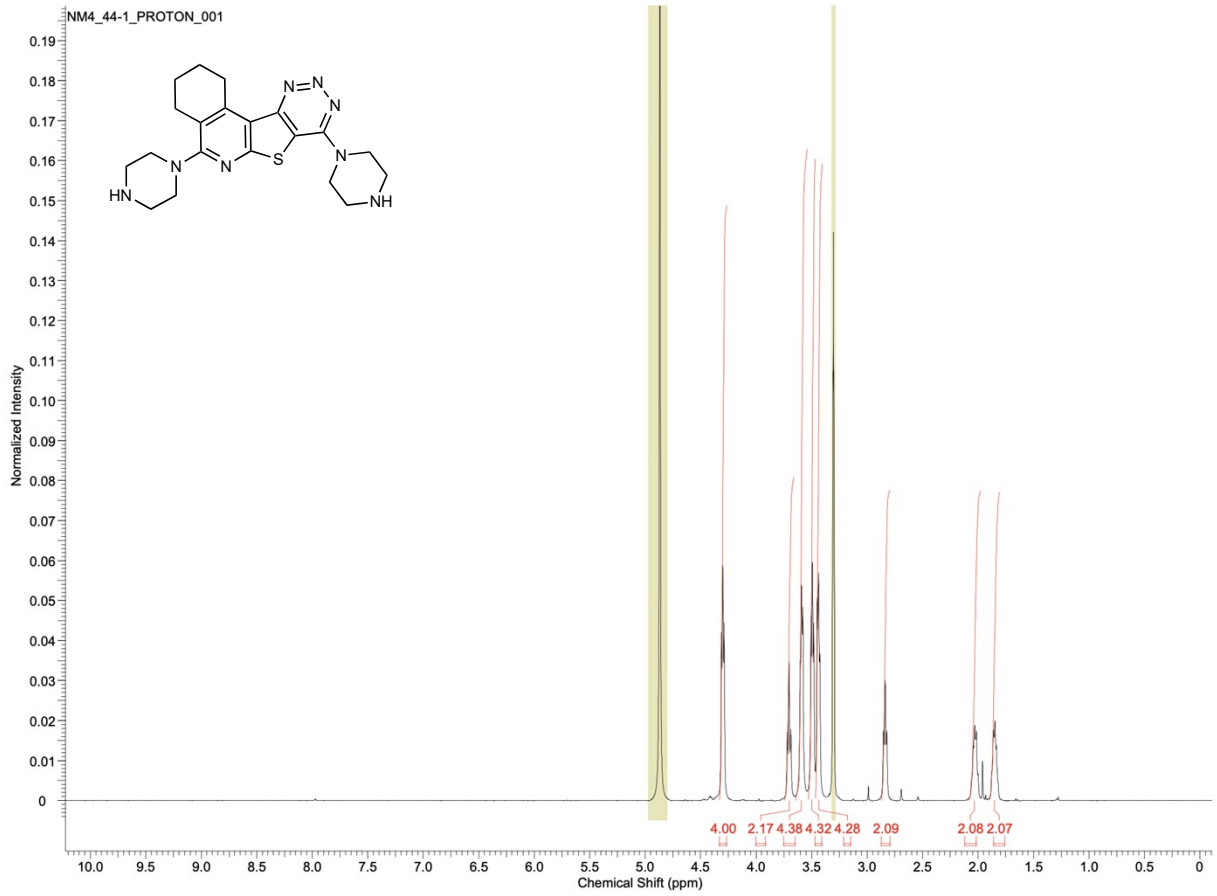
UNC4365 (8), 1H NMR (400 MHz, Chloroform-*d*)



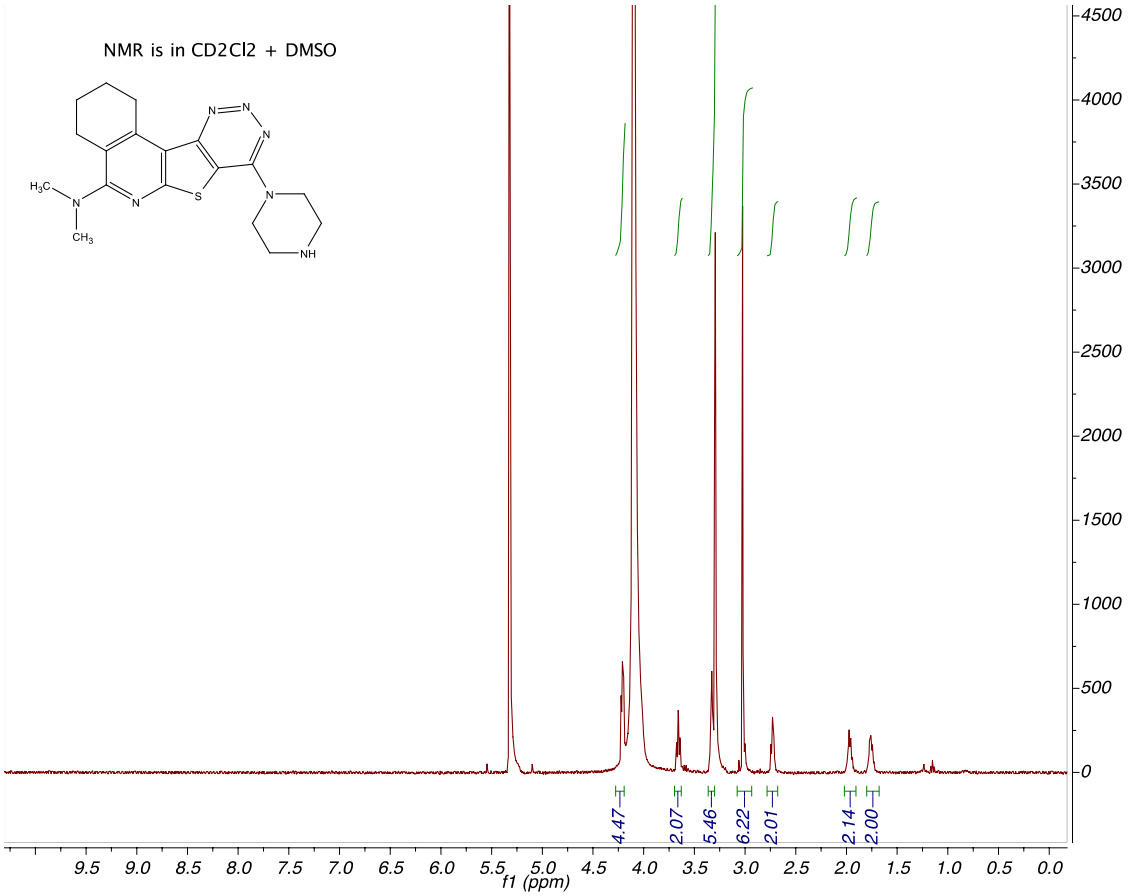
UNC10201651 (9), 1H NMR (400 MHz, Chloroform-*d*)



UNC10206579 (10), 1H NMR (400 MHz, Methanol-d4)



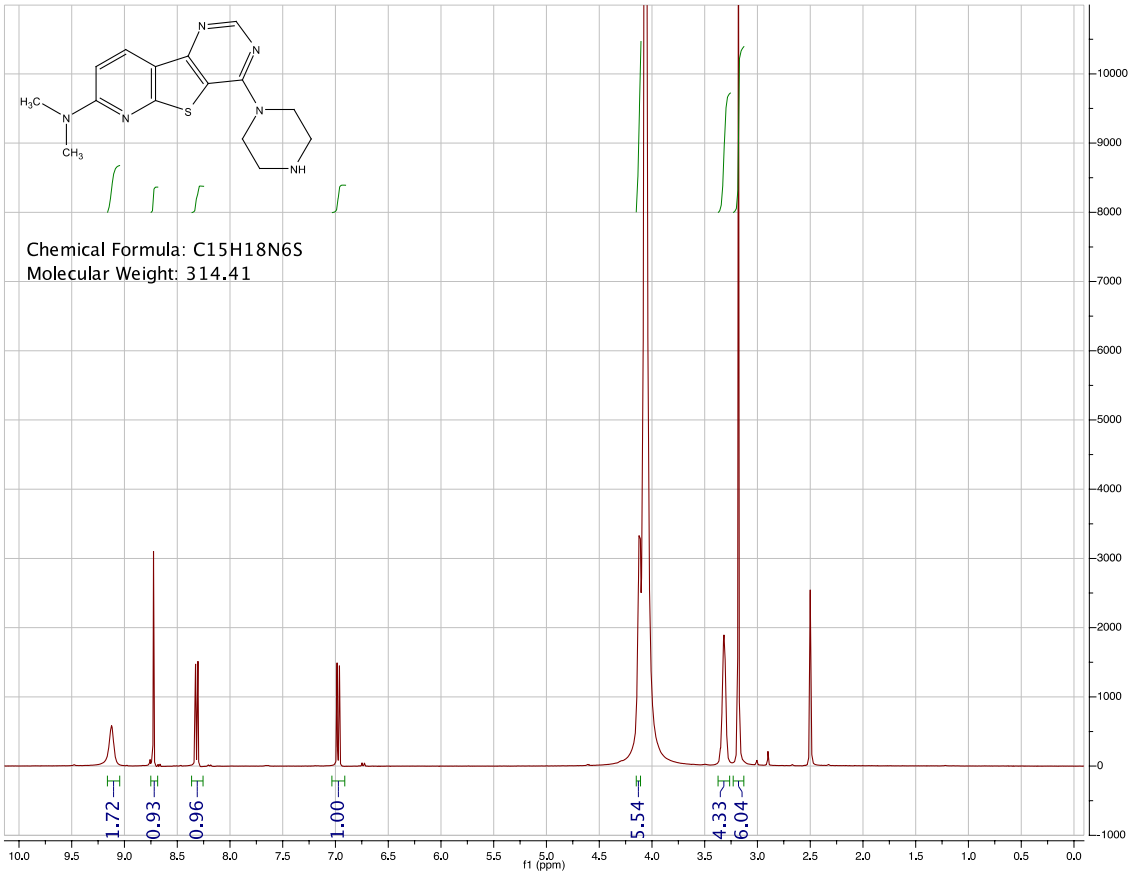
UNC10206577 (11),^{1}H NMR (400 MHz, DMSO-d_6)



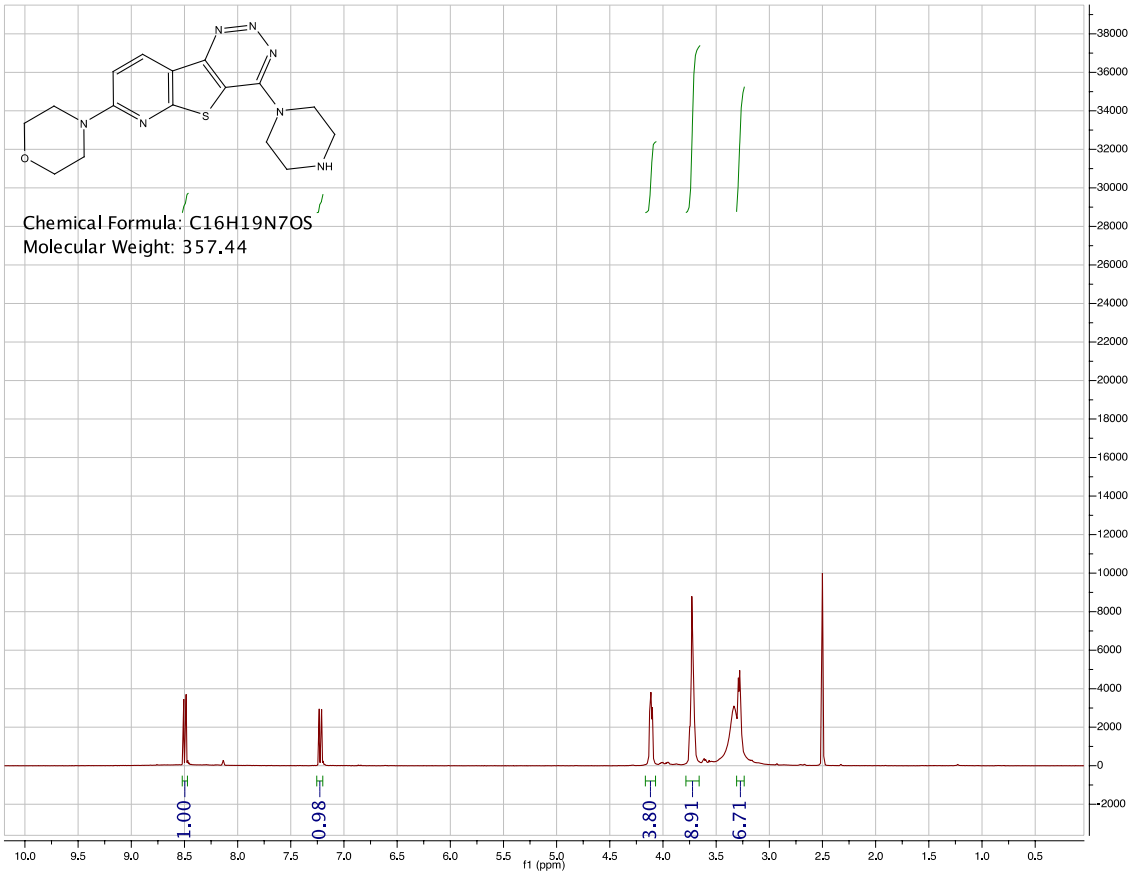
UNC10206581 (12), ^{1}H NMR (400 MHz, Methanol-d_4)

UNC4764 (14), 1H NMR (400 MHz, DMSO-d6)

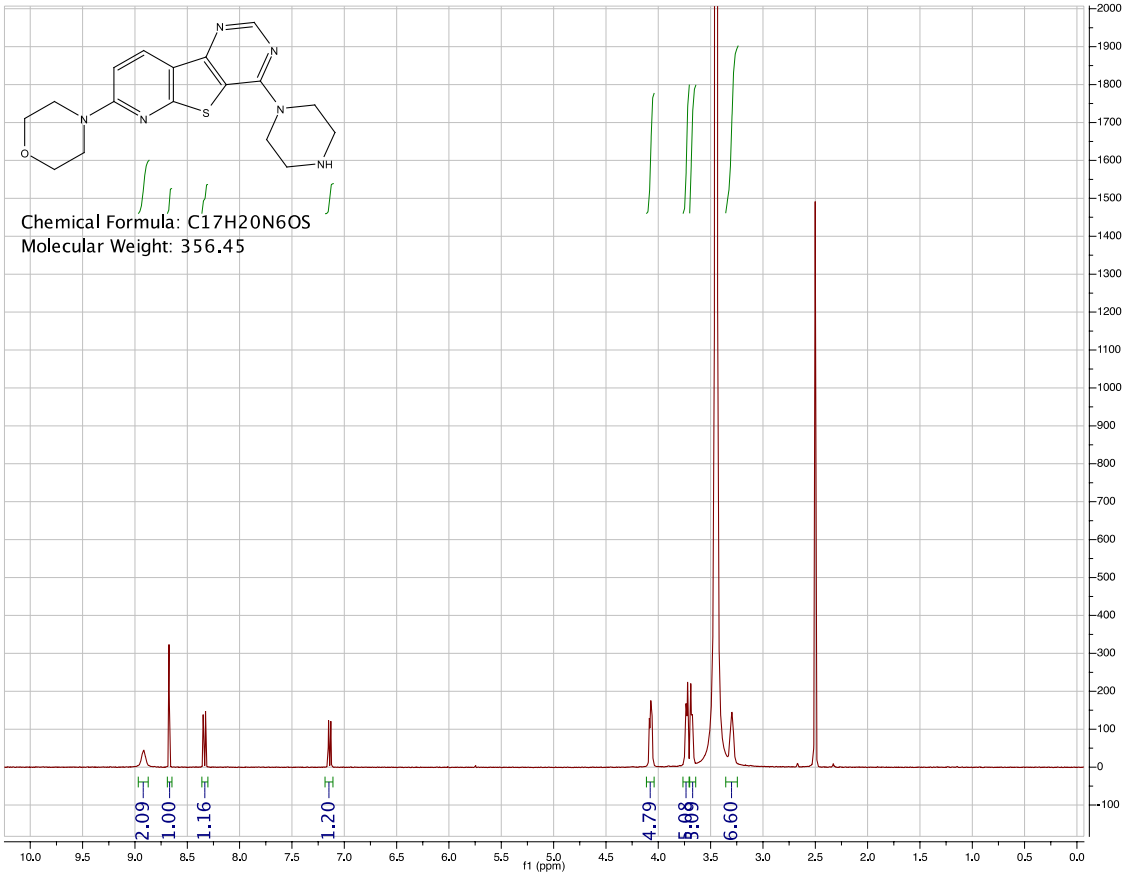
UNC4764 (14), ^{1}H NMR (400 MHz, DMSO-d_6)



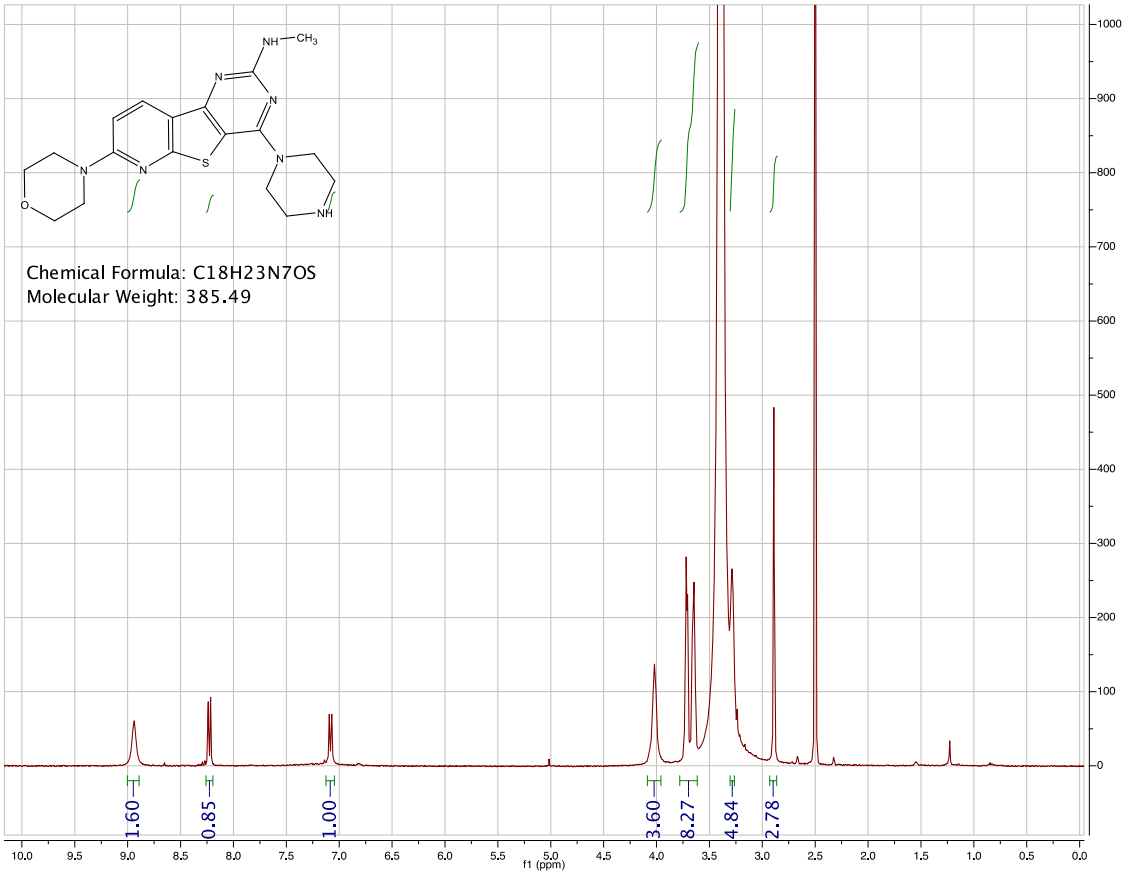
UNC4830 (15), ^{1}H NMR (400 MHz, DMSO-d_6)



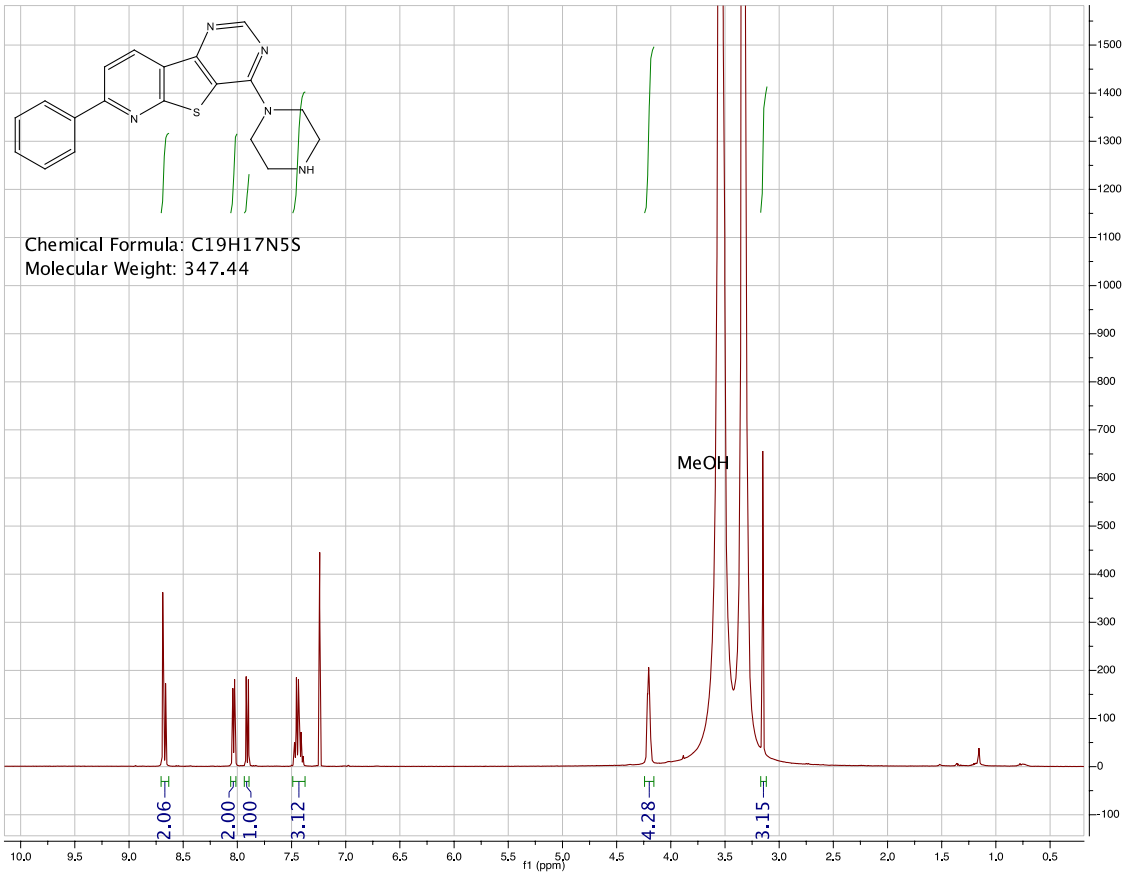
UNC4746 (16), 1H NMR (400 MHz, DMSO-d6)


Chemical Formula: C17H20N6OS
Molecular Weight: 356.45
2.09
1.00
1.16
1.20
4.79
5.08
6.60
f1 (ppm)

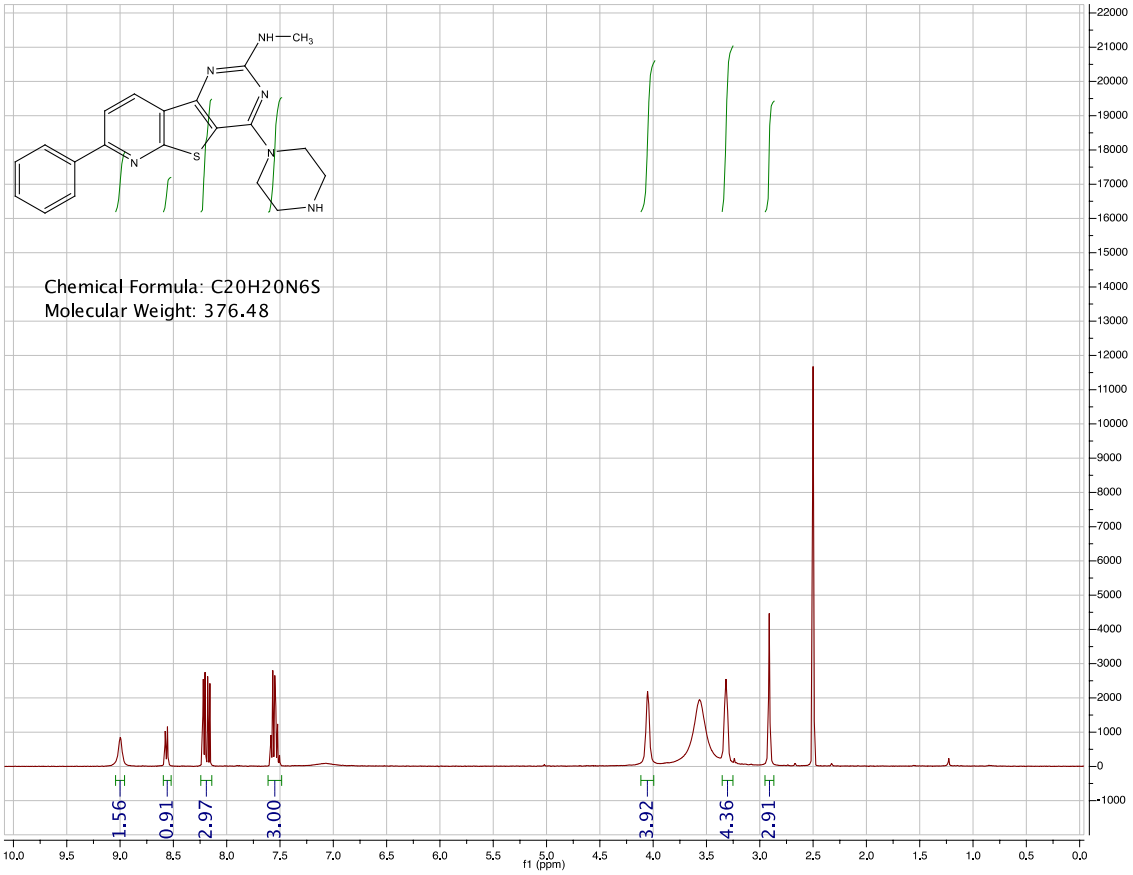
UNC4785 (17), ^{1}H NMR (400 MHz, DMSO-d_6)



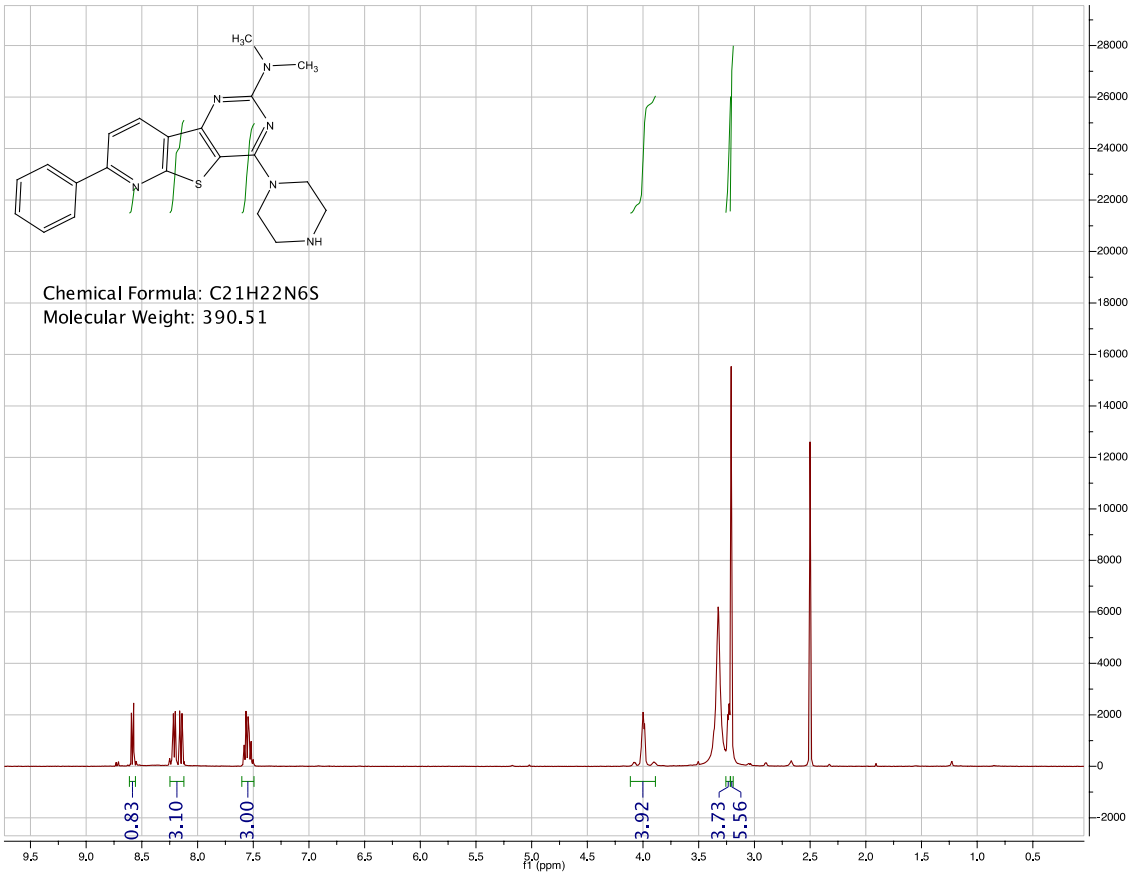
UNC4600 (18), 1H NMR (400 MHz, DMSO-d6)



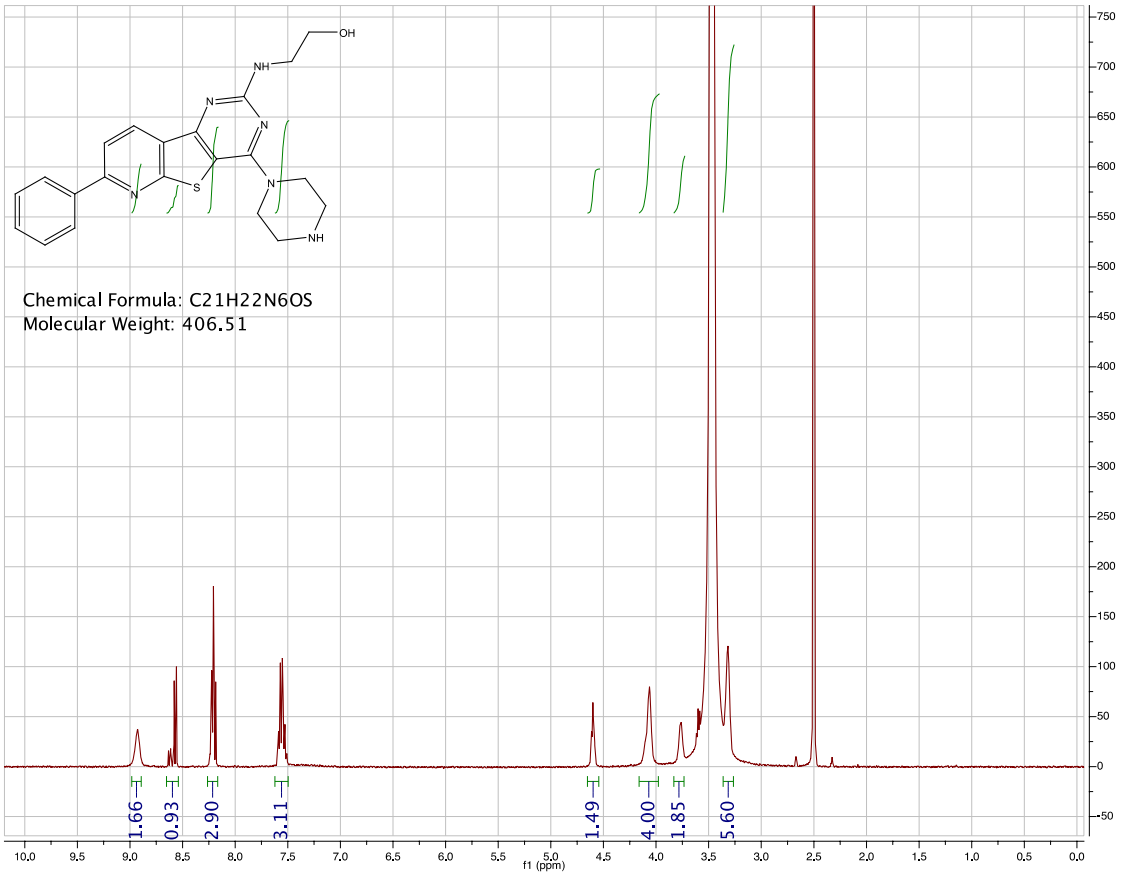
UNC4708 (19), 1H NMR (400 MHz, DMSO-d6)



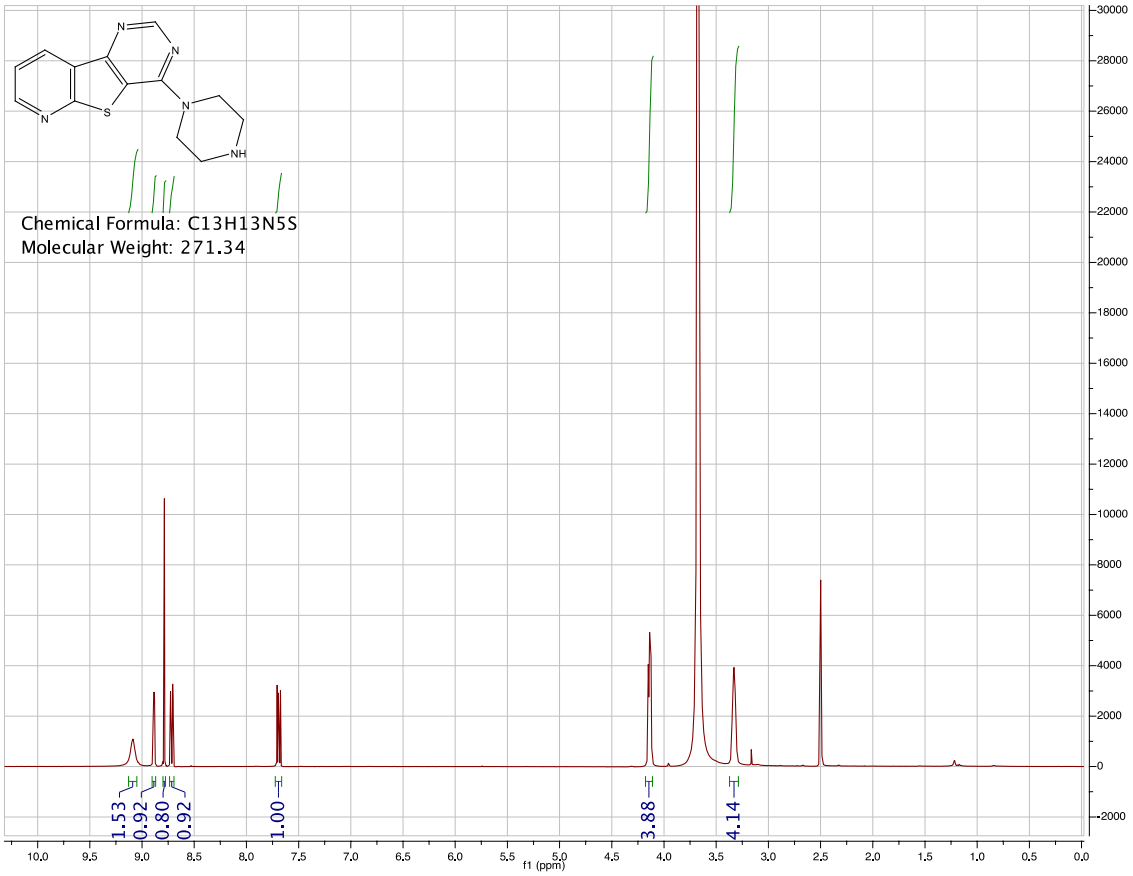
UNC4707 (20), 1H NMR (400 MHz, DMSO-d6)



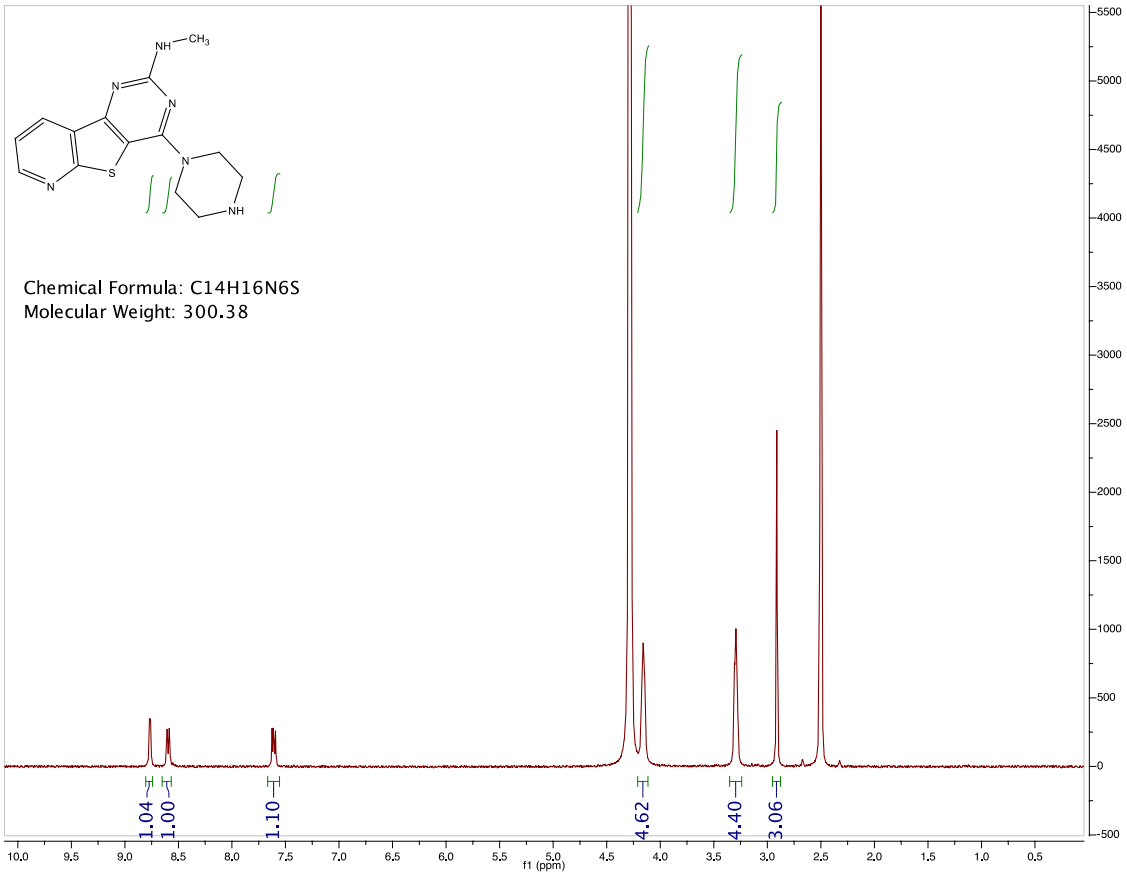
UNC4847 (21), 1H NMR (400 MHz, DMSO-d6)



UNC4666 (22), 1H NMR (400 MHz, DMSO-d6)



UNC4910 (23), 1H NMR (400 MHz, DMSO-d6)



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