Supplementary Material (ESI) for Chemical Communications

Molecular Recognition of a Thymine Bulge by a High Affinity, Deazaguanine-based Hydrogen Bonding Ligand

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Electronic Supporting Information

Materials:

Anhydrous solvents and starting materials were purchased by the Aldrich Company and used without further purification unless otherwise specified. Water for all experiments was purified by a Millipore Direct Q-5 purification system. DNA oligomers were obtained from Integrated DNA Technologies purified by standard desalting. RNA was provided as page purified oligomers from Dharmacon, Inc. Chemical reactions were performed under nitrogen.

High and low resolution mass spectra were obtained by the Mass Spectrometry Laboratory, School of Chemical Science, University of Illinois. Mass spectra were obtained by field desorption (FD) on a Waters 70-VSE-A and by ESI on a Waters Micromass Q-Tof. Elemental analyses were performed at the University of Illinois, School of Chemical Sciences. Crystallographic analysis was obtained by the X-ray laboratory, School of Chemical Science, University of Illinois. High performance liquid chromatography (HPLC) was performed by a Dynamax SD-200 system with a UV detector set at 254 nm using an Alltech Denali C-18 column (250 × 10 mm) with a dual solvent system of 0.1% TFA/H_2O (Solvent A) and 0.1% TFA/MeCN (Solvent B). Nuclear magnetic resonance (NMR) spectra were recorded on a Varian *Unity* 400, Varian *Unity* 500 or Varian *INOVA* 500NB spectrometer at 21±3 °C unless otherwise mentioned. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (*J*) were reported in Hertz. ^{1}H NMR chemical shifts were referenced to the residual solvent peak at 7.26 ppm in chloroform-*d* ($CDCl_3$) and at 2.50 ppm in DMSO-d_6. ^{13}C NMR chemical shifts were referenced to the center solvent peak at 77.16 ppm for $CDCl_3$ and at 40.45 ppm for DMSO-d_6. The purity of all ligands was estimated to be >99% by ^{1}H NMR (S26–S37) and by HPLC (S38–S40). Instrumentation for UV-Vis absorption, fluorescence, and isothermal titration calorimetery are described below. 2-6-Diheptanoylpyridine was synthesized according to literature procedure.[i]

^{1}H NMR Binding Studies. $CDCl_3$ was distilled from $CaCl_2$, passed through a column of activated (flame-dried) basic alumina, and stored over 4 Å molecular sieves. NMR tubes and volumetric flasks were dried in a 110 °C oven and cooled in a desiccator prior to sample

preparation. Samples (8-12) were prepared by adding aliquots of stock solutions of uracil or thymine and receptor via syringe directly into 1.0 mL volumetric flasks. The samples were transferred (0.700 mL) to NMR tubes via syringe. Spectra were acquired with a 500 MHz spectrometer using temperature control at 20 °C. For titration methods, the concentration of the host was such that no self-association was observed. Data were fit to a 1:1 binding isotherm using standard methods.[ii]

Thermal Denaturation Studies:

Thermal denaturation studies were performed with a temperature controlled Shimadzu 2501PC UV-Vis recording spectrophotometer enabled with an 8-well quartz sample cell (1.0 cm path, 130 μL total volume). Stock solutions of 800 μM DNA oligomers were prepared and annealed in a water bath >90 °C for 5 minutes then allowed to cool to ambient temperature. Five equivalents of the respective ligand was added, followed by 10 μL of 100 mM sodium cacodylate pH 7.0, 10 μL of 1.0 M NaCl, and 10 μL of 10 mM EDTA. The samples were then diluted to 100 μL with water to give final concentrations of 5 μM DNA duplex, 25 μM ligand, 10 mM sodium cacodylate pH 7.0, 100 mM NaCl, and 1.0 mM EDTA. Samples were then placed in the 8-well cell and cooled to 0 °C. The temperature dependent absorbance of each sample was monitored from 0 °C to 90 °C at 260 nm with a ramp rate of 1 °C per minute and monitoring every 0.5 °C. The melting temperature of each curve (inflection point of the sigmoidal transition) was determined by finding the maximum of the first derivative of the curve with Origin 7.0 (MicroCal, Inc. Northampton, MA).

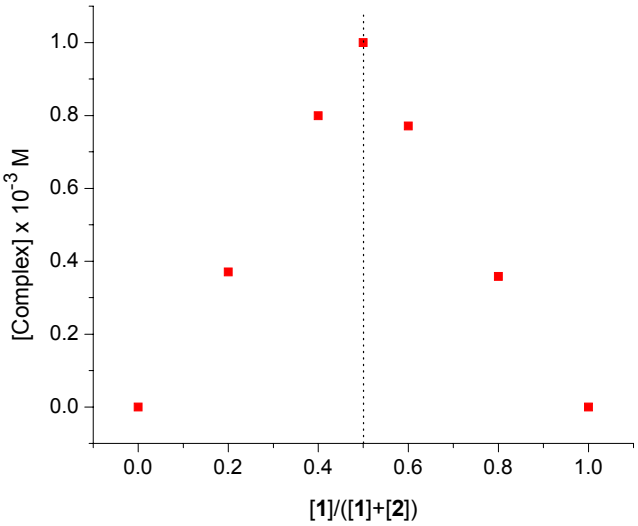
Isothermal Titration Calorimetry:

Isothermal titration calorimetry measurements were performed at 25 °C on a MicroCal VP-ITC (MicroCal, Inc., Northampton, MA). A standard experiment consisted of titrating 10 μL of a 500 μM ligand from a 250 μL syringe (rotating at 300 rpm) into a sample cell containing 1.42 mL of a 5-40 μM DNA/RNA solution. The duration of the injection was set to 24 s, and the delay between injections was 300 s. The initial delay prior to the first injection was 60 s. To derive the heat associated with each injection, the area under each isotherm (microcalories per second versus seconds) was determined by integration by the graphing program Origin 5.0 (MicroCal, Inc. Northampton, MA). The fitting requirements were such that the thermodynamic parameters were derived from curves that produced the lowest amount of deviation. In most cases, fitting to a sequential two-site binding model gave the best-fit data. Analogous low-affinity binding sites have previously been observed in aminoglycoside-16S rRNA interactions.[iii] The ligand stock

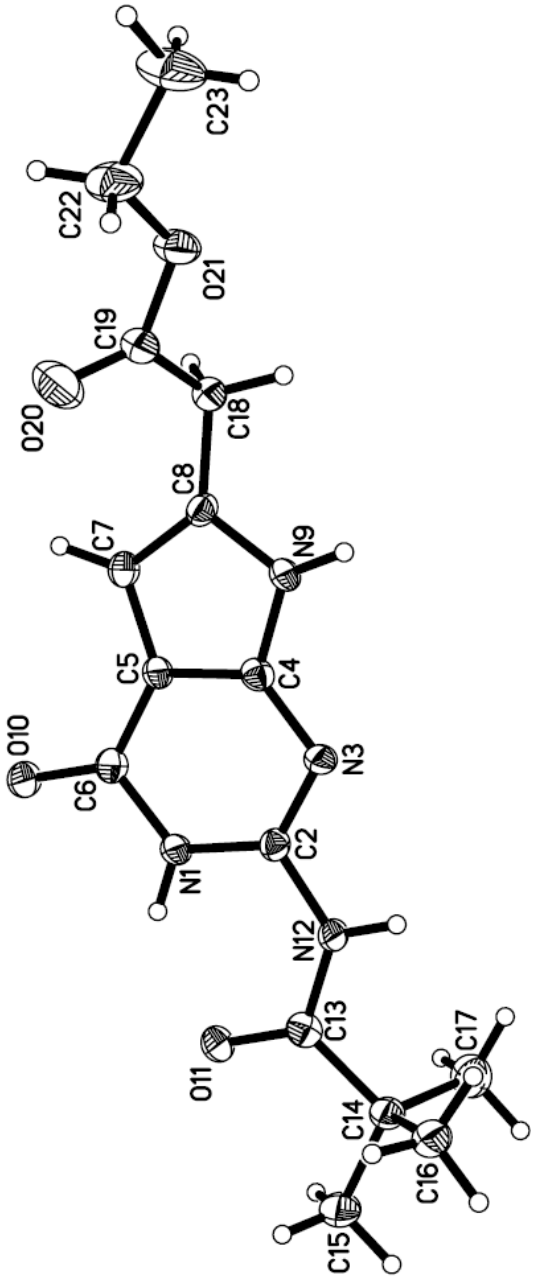
solution was 10 mM in water. The buffer solution for ITC experiments was 10 mM sodium cacodylate pH 7.0, 100 mM NaCl and 1.0 mM EDTA.

Job Plot Method.

The Job plot was performed in $CDCl_3$. Host and guest solutions with concentrations of 2 mM were used. Aliquots were added to each sample to give a total volume of 1 mL and a constant total concentration of host and guest (2 mM).

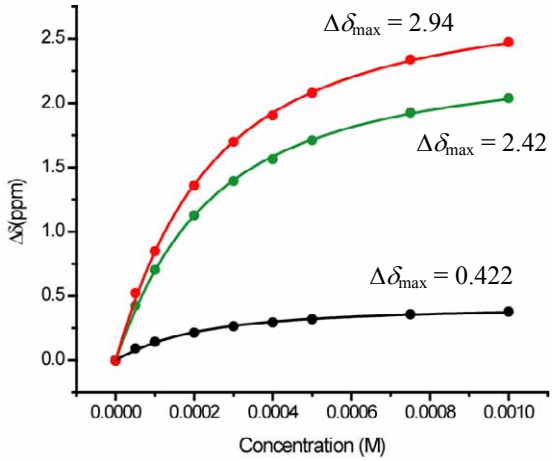


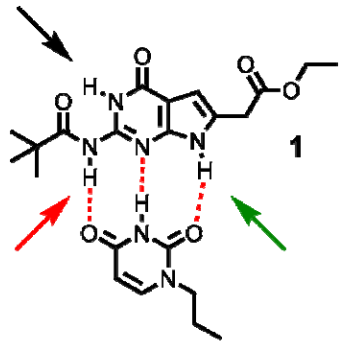
X-ray Crystal Structure of 1 (ORTEP)



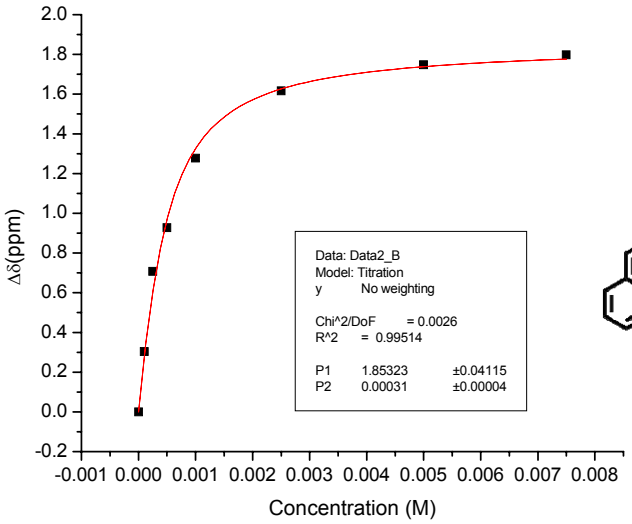
Binding Profiles

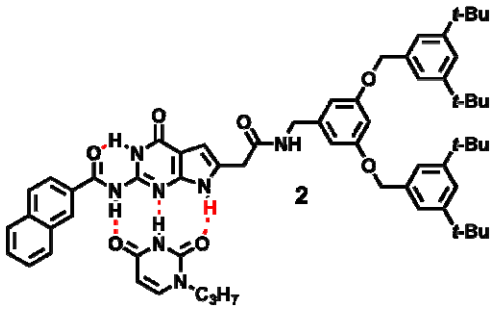
The following are representative binding profiles of selected protons on each receptor. Experiments were done either by titration or dilution method. The reported binding constants in the Results and Discussion are averages of the binding constants of the most visible protons and at least two trials.



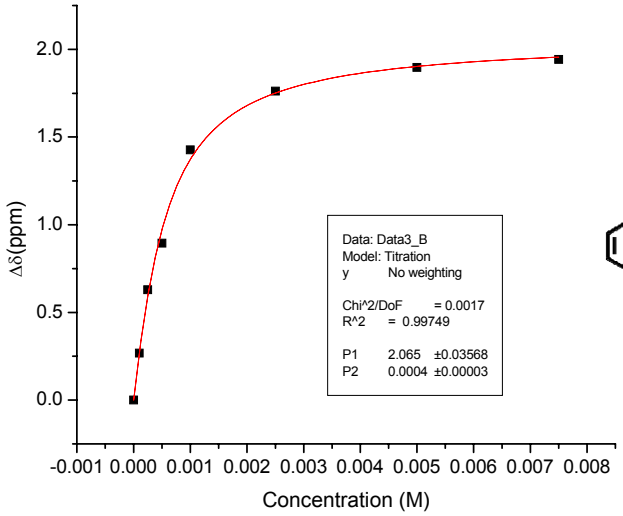


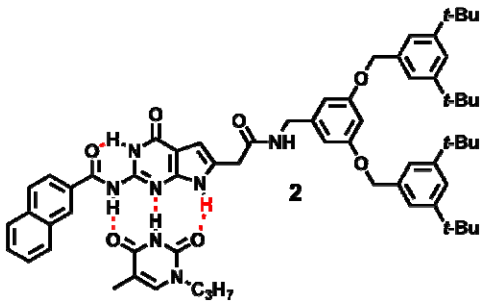
K_{assoc} = 5,800 M^{-1}



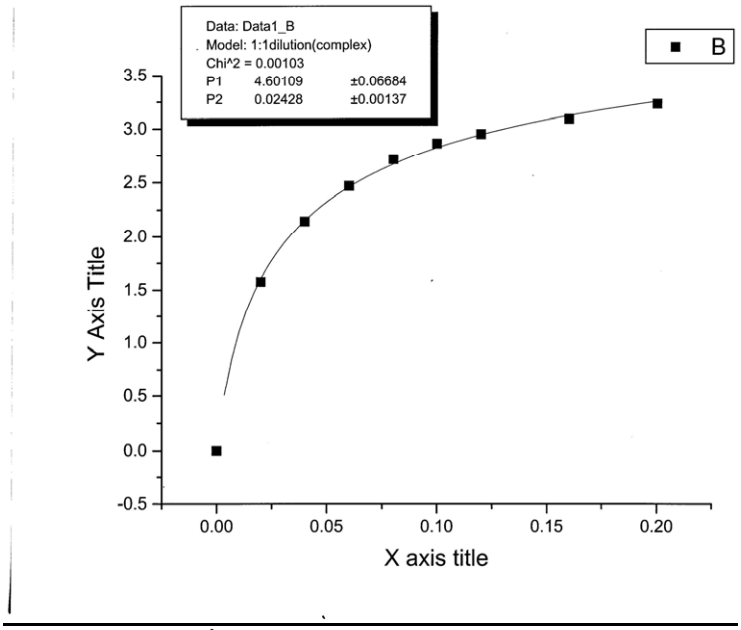


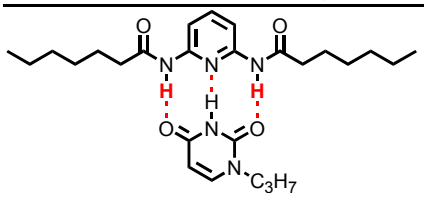
K_{assoc} = 2,860 M^{-1}

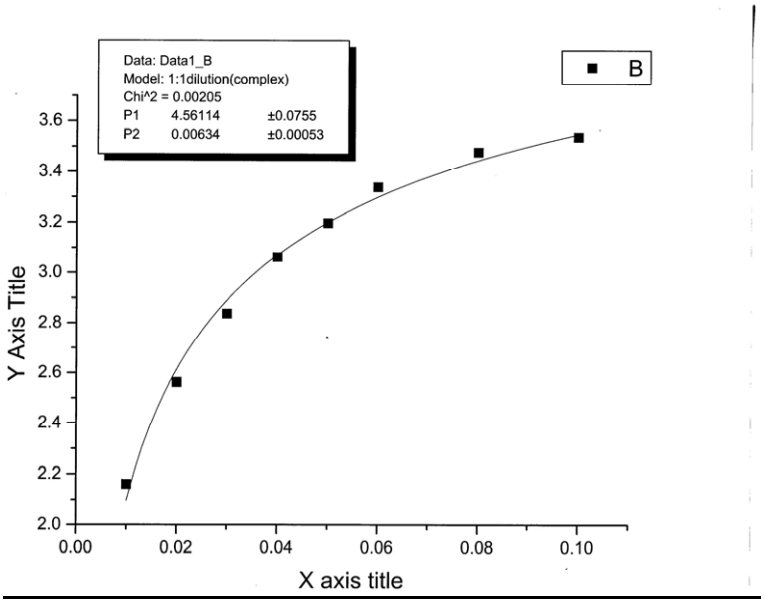




K_{assoc} = 2,560 M^{-1}






Data: Data1_B
Model: 1:1dilution(complex)
Chi^2 = 0.00205
P1 4.56114 ±0.0755
P2 0.00634 ±0.00053
B
Y Axis Title
X axis title
2.0
2.2
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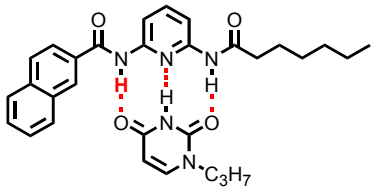
O
N
H
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H
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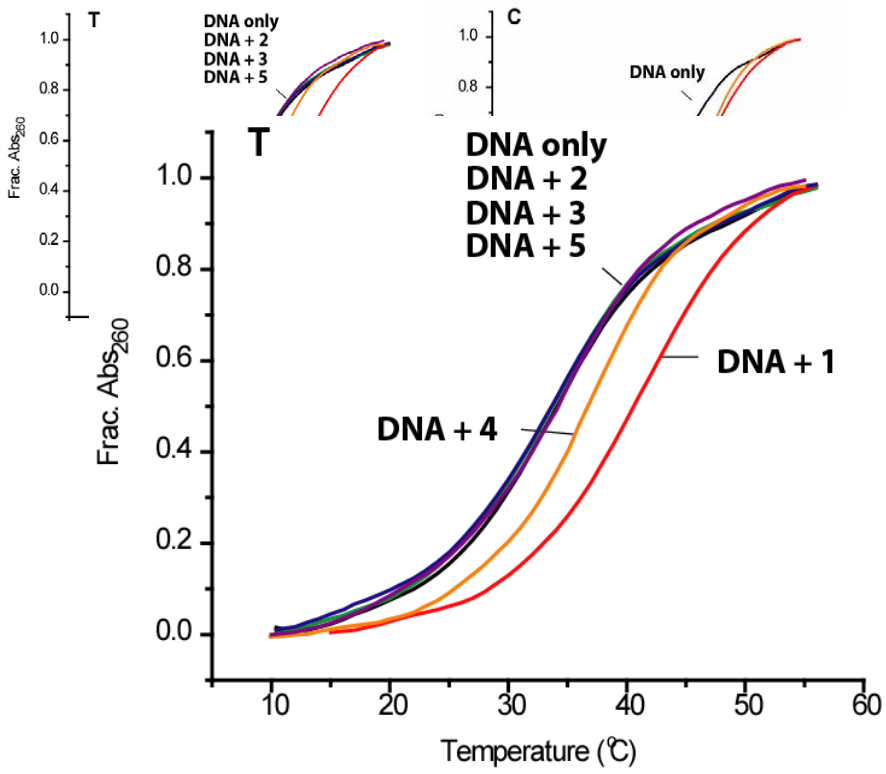
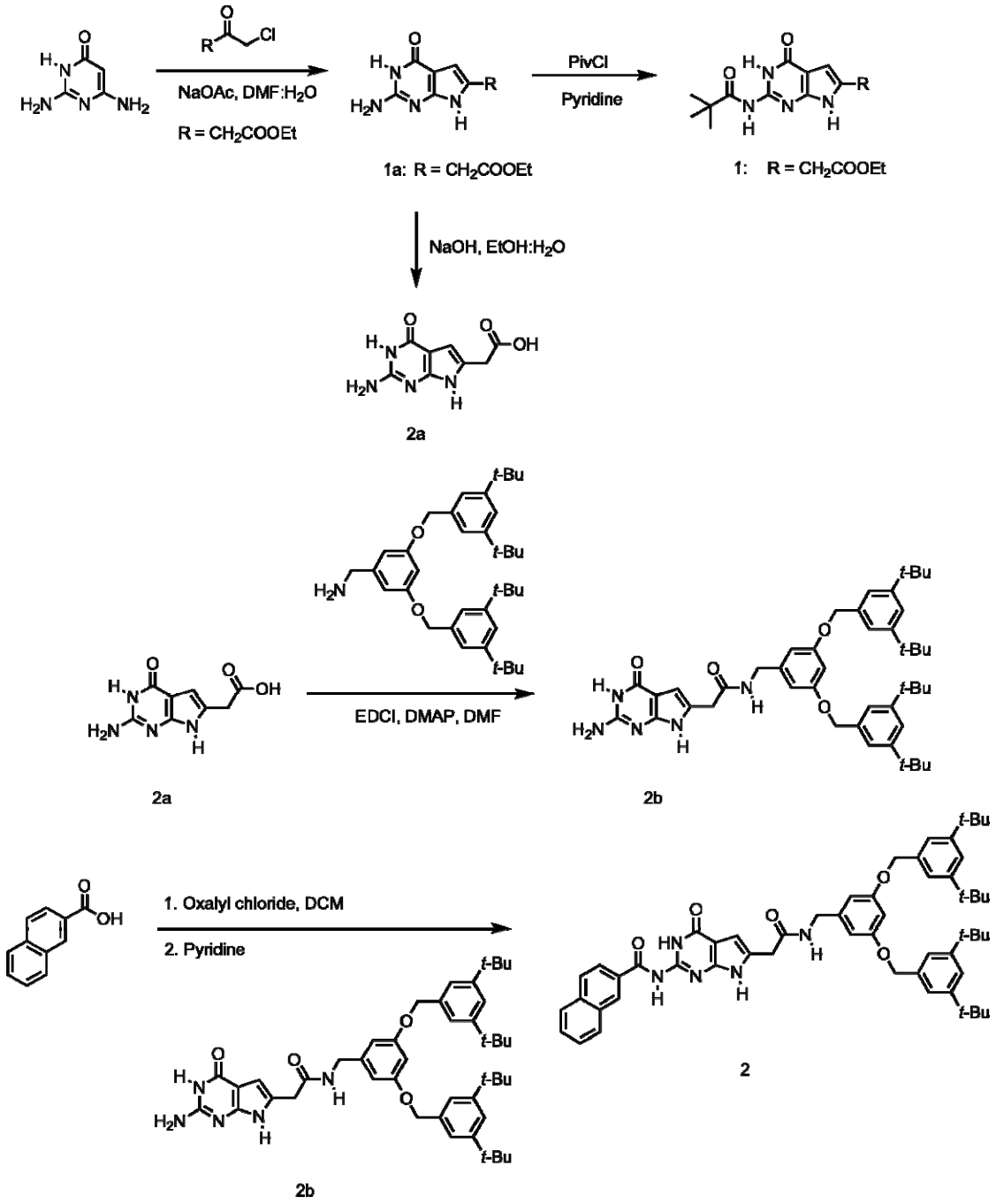
C3H7

Thermal Denaturation Curves



Synthesis



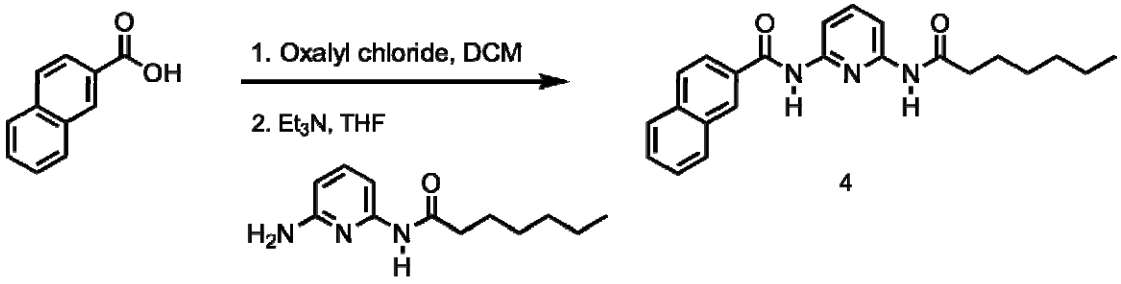
2-Pivaloyl-8-methylene-ethylester-7-deazaguanine (**1**): 7-Deazaguanine-8-methylene-ethylester (300 mg, 1.3 mmol) was dissolved in pyridine (7 mL) and PivCl (184 μL, 1.5 mmol) was added at once. The mixture was stirred at 65 ˚C for 16

h. The solvent was evaporated to dryness and the solid was triturated with acetone. The solid was filtered off and washed with cold acetone to give 301 mg (74%) of a white crystalline solid: ^{1}H-NMR (DMSO-*d*$_6$) δ 11.83 (bs, 1H), 11.46 (bs, 1H), 10.78 (bs, 1H), 6.24 (d, *J* = 2.0, 1H), 4.11 (q, *J* = 7.5, 2H), 3.71 (s, 2H), 1.24 (s, 9H), 1.20 (t, *J* = 7.5, 3H); ^{13}C-NMR (DMSO-*d*$_6$) δ 181.9, 170.9, 157.6, 148.8, 147.4, 127.4, 104.9, 102.4, 61.5, 34.0, 31.7, 27.4, 15.1; MS (FD) 320.2 *m/z*; HRMS (ESI) calcd for $C_{15}H_{20}N_4O_4$, 321.1563; found, 321.1572.

7-Deazaguanine-8-methylcarboxylic acid (**2a**): 8-methylene-ethylester-7-deazaguanine (3.0 g, 12.6 mmol) was dissolved in H_2O:EtOH (1:1 v/v) mixture and NaOH(s) (540 mg) was added. The solution was heated to 90 ˚C and stirred for 2 h. The solvent was fully evaporated and the residue was triturated with 2 N HCl (30 mL). The precipitated pink solid (2.6 g, 99%) was filtered and washed with water: mp>300 °C; ^{1}H-NMR (DMSO-*d6*) δ 12.40 (bs, 1H), 10.90 (s, 1H), 10.21 (s, 1H), 6.07 (s, 2H), 6.02 (s, 1H), 3.52 (s, 2H); ^{13}C-NMR (DMSO-*d6*) δ 172.5, 158.3, 152.4, 126.0, 102.2, 100.9, 34.1; MS(FD) 208.1 *m/z*.

6-{[3,5-bis-(3,5-di-*tert*-butyl-benzyloxy)-benzylcarbamoyl]-methyl-7-Deazaguanine- (**2b**) – **2a** (1.2 g, 5.8 mmol) was suspended in DMF (20 mL) and EDCI (1.2 g, 6.3 mmol) and DMAP (0.2 g, 1.6 mmol) were added at once. The suspension was stirred at room temperature for 10 min, after which **2c** (3.6 g, 6.3 mmol) dissolved in DMF (10 mL) was added dropwise. The solution was stirred at room temperature for 18 h. The solvent was fully evaporated, and the solid was suspended in $CHCl_3$ (100 mL) and washed with water (1 × 80 mL) and brine (1 × 80 mL). The organic layer was dried with Na_2SO_4, filtered, and evaporated to dryness to give a solid that was purified by column chromatography using a 10% MeOH:CH_2Cl_2 gradient. The solvent was evaporated to give 2.75 g (65%) of a white solid: mp 162-165 ˚C; ^{1}H-NMR (DMSO-*d*$_6$) δ 10.84 (s, 1H), 10.12 (s, 1H), 8.30 (t, *J* = 6.0, 1H), 7.36 (m, 2H), 7.27 (m, 4H), 6.59 (m, 1H), 6.53 (m, 2H), 5.99 (bs, 2H), 5.00 (s, 4H), 4.72 (d, *J* = 5.5, 2H), 3.42 (s, 2H), 1.29 (s, 36H); ^{13}C-NMR (DMSO-*d*$_6$) δ 170.0, 160.6, 159.4, 153.0, 152.1, 153.1, 142.8, 137.0, 125.9, 123.2, 122.5, 107.3, 100.9, 100.9, 71.1, 43.3, 35.9, 35.5, 32.3; MS (FD) 733.5 *m/z*; Anal. Calcd. for $C_{49}H_{59}N_5O_4$: C, 73.14; H, 8.10; N, 9.54. Found: C, 72.86; H, 8.04; N, 9.40.

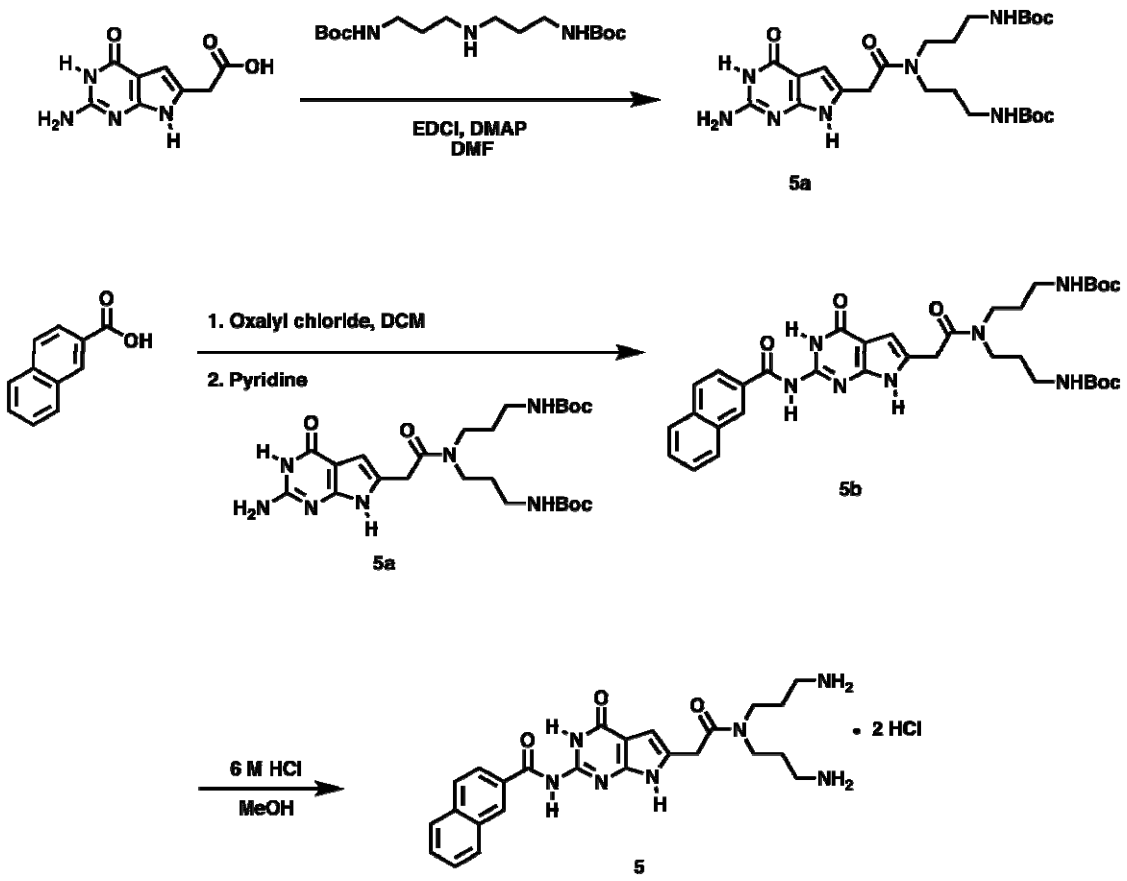
2-Naphthylamide-(6-{[3,5-bis-(3,5-di-*tert*-butyl-benzyloxy)-benzylcarbamoyl]-methyl}-7-deazaguanine (**2**) - 2-Naphthoic acid (400 mg, 2.3 mmol) was suspended in CH_2Cl_2 (12 mL) and oxalyl chloride (10 mL) was added at once. The solution was stirred at room temperature for 18 h, and the solvent was fully evaporated. After co-evaporating with CH_2Cl_2, the dried acid chloride was dissolved in pyridine (4 mL), and added dropwise to a solution of **2b** (1.55 g, 2.1 mmol) in pyridine (30 mL). The solution was stirred at 65 ˚C for 18 h. The solvent was fully evaporated, and the solid was taken up in $CHCl_3$ (30 mL), and washed with water (1 × 20 mL) and brine (1 × 20 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated to dryness to give a yellow solid that was purified by column chromatography using a 10% MeOH:CH_2Cl_2 gradient. The solvent was evaporated to give 1.66 g (89%) of a yellow foam: mp 168-171 ˚C; ^{1}H-NMR ($CDCl_3$) δ 12.13 (s, 1H), 10.62 (bs, 1H), 10.18 (bs, 1H), 8.32 (s, 1H), 7.76 (m, 4H), 7.53 (m, 1H), 7.47 (m, 1H), 7.40 (t, *J* = 2.0, 2H), 7.23 (d, *J* = 2.0, 4H), 6.57 (bt, *J* = 2.0, 1H), 6.41 (s, 1H), 6.35 (s, 2H), 6.19 (bs, 1H), 4.90 (s, 4H), 4.03 (bs, 2H), 3.51 (s, 2H), 1.31 (s, 36H); ^{13}C-NMR (CDCl3) δ 169.9, 169.2, 160.7, 158.1, 151.4, 148.8, 147.4, 140.5, 135.9, 135.5, 132.4, 129.7, 129.6, 129.4, 128.7, 128.5, 127.8, 127.2, 124.1, 122.7, 107.0, 105.3, 102.5, 101.1, 77.6, 71.4 44.0, 36.3, 31.2, 31.8; MS (FD) 887.6 *m/z*; HRMS (ESI) calcd for $C_{56}H_{65}N_5O_5$, 888.5064; found, 888.5076; Anal. Calcd. for $C_{56}H_{65}N_5O_5$: C, 75.73; H, 7.38; N, 7.89. Found: C, 75.75; H, 7.27; N, 7.87.



Heptanoic acid (6-amino-pyridin-2-yl)-amide (4a) – To a 50 mL round-bottom flask was added 2,6-diaminopyridine (2.4 g, 22.0 mmol) dissolved in THF (20 mL) and Et_3N (2.55 mL). Heptanoyl chloride (2.8 mL, 18.3 mmol) dissolved in THF (10

mL) was added dropwise at 0 °C. The reaction was allowed to stir at ambient temperature for 18 h. The solvent was evaporated and the residue was dissolved in 60 mL of H_2O. The product was extracted with $CHCl_3$ (3 x 60 mL) and the combined organic layers were dried with Na_2SO_4, filtered and concentrated in vacuo to give a brown solid/oil that was purified by column chromatography in silica (4 × 10 cm) using 1:4 MeCN:$CHCl_3$ as eluent. The solid was recrystallized with 1:1 Et_2O: Hexanes to give 2.40 g (59%) of white needles: mp range: 67 – 68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (bs, 1H), 7.53 (bs, 1H), 7.44 (t, *J* = 8.4, 1H), 6.24 (dd, *J* = 7.6, 0.8, 1H), 4.27 (bs, 2H), 2.33 (t, *J* = 8.0, 2H), 1.70 (quin., *J* = 8.0, 2H), 1.34 (m, 6H), 0.88 (t, *J* = 6.8, 3H); ^{13}C NMR δ 172.1, 157.4, 150.3, 140.5, 103.6, 38.0, 31.7, 29.1, 25.7, 22.7, 14.3; MS (FD) *m/z* 221.2 (M^+); Anal. Cal'd for: C, 65.13; H, 8.65; N, 18.99. Found: C, 65.23; H, 8.57; N, 18.77.

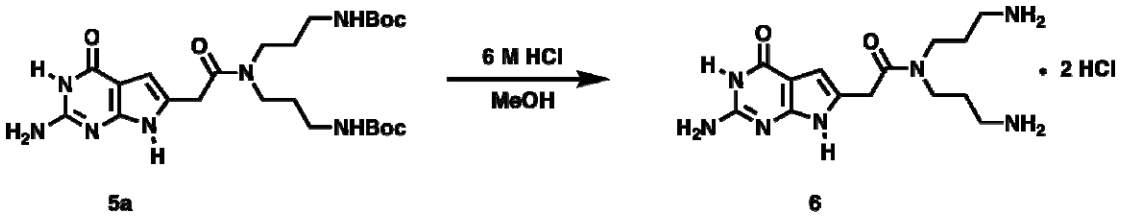
Naphthalene-2-carboxylic acid (6-heptanoylamino-pyridin-2-yl)-amide (**4**) – To a solution of naphthoic acid (75 mg, 0.4 mmol) in CH_2Cl_2 (4 mL) was added oxalyl chloride (1 mL) dropwise. The reaction was stirred at room temperature for 20 h. The solvent was evaporated and co-evaporated with CH_2Cl_2. Once fully dried, the acid chloride was dissolved in THF (1 mL) and added slowly to a solution of **4a** (88 mg, 0.4 mmol), Et_3N (73 μL, 0.5 mmol), and THF (15 mL). The reaction was stirred at room temperature for 20 h. The solvents were evaporated and the residue was dissolved in $CHCl_3$ (30 mL) and washed with water (1 × 30 mL) and brine (1 × 30 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo to give a dull yellow solid that was purified by column chromatography, using 15% MeCN:$CHCl_3$ as eluent. The solvent was evaporated to give 114 mg (76%) of a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.42 (s, 1H), 8.40 (bs, 1H), 8.12 (d, *J* = 8.0, 1H), 7.96 (m, 5H), 7.60 (m, 3H), 2.40 (t, *J* = 8.0, 1H), 1.74 (quin., *J* = 8.0, 2H), 1.39 (m, 2H), 1.32 (m, 4H), 0.90 (t, *J* = 7.0, 3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 171.9, 165.8, 149.88, 149.85, 141.2, 135.3, 132.7, 131.5, 129.3, 129.1, 128.4, 128.11, 128.03, 127.3, 123.6, 109.97, 109.85, 38.0, 31.8, 29.1, 25.5, 22.7; *m/z* MS(FD) 375.2.



7-Deazaguanine-8-methylene-*bis*-3-Boc-propylamine amide (**5a**) – To 1.1 g (5.5 mmol) of 7-deazaguanine-8-methylene acetic acid **2a** and 2.0 g (6.0 mmol) of *tert*-butyl 3,3'-azanediylbis(propane-3,1-diyl)dicarbamate in 50 mL of DMF at 0 °C was added 0.6 g (4.9 mmol) of DMAP followed by 1.6 g (8.3 mmol) of EDCI. The suspension was stirred at room temperature for 20 h. The solvent was evaporated to dryness, and the brown solid was suspended in CH_2Cl_2 and added directly to silica gel. Purification by column chromatography using a MeOH gradient from 0 to 10% in CH_2Cl_2 produced 1.95 g (3.7 mmol, 68%) of a yellow foam: ^{1}H NMR (500 MHz; DMSO-d_6): δ 10.78 (s, 1H), 10.15 (s, 1H), 6.92 (t, J = 5.0, 1H), 6.74 (t, J = 5.0, 1H), 6.00 (s, 2H), 5.89 (s, 1H), 3.53 (s, 2H), 3.24 (dt, J = 31.8, 7.5, 4H), 2.91 (dq, J = 35.7, 6.1, 4H), 1.60 (dquintet, J = 53.0, 7.0, 4H), 1.36 (s, 18H); ^{13}C NMR (125 MHz; DMSO- d_6): δ 168.9, 158.5, 155.69, 155.52, 152.0, 151.1, 129.4, 124.8, 115.2, 99.86, 99.74, 77.61, 77.50, 45.3, 42.9, 37.61, 37.51, 32.3, 28.8, 28.3, 27.8; *m/z* (FD) 521.3; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 522.3040; found 522.3047.

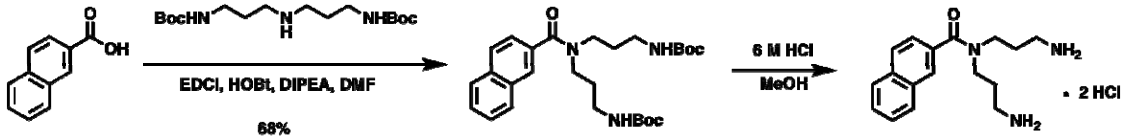
2-Naphthylamide-7-deazaguanine-8-methylene-*bis*-3-Boc-propylamine amide (5b) – To 80 mg (0.47 mmol) of 2-Naphthoic acid suspended in 2 mL of CH_2Cl_2 was added 2 mL of oxalyl chloride stirred at room temperature for 18 h. The solvent was removed under reduced pressure and co-evaporated with CH_2Cl_2. The dried acid chloride was dissolved in 1 mL of pyridine, and added dropwise to a solution of 220 mg (0.42 mmol) of **5a** in 12 mL of pyridine. The solution was stirred at 65 ˚C for 18 h. The solvent was fully evaporated *in vacuo*, and the solid was taken up in $CHCl_3$ (60 mL), and washed with water (1 × 50 mL) and brine (1 × 50 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated to dryness to give a yellow solid that was purified by column chromatography using a MeOH gradient from 0 to 10% in CH_2Cl_2 to produce 243 mg (0.36 mmol, 85%) of a yellow foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.99 (s, 1H), 11.81 (s, 1H), 11.57 (s, 1H), 8.73 (s, 1H), 8.11-8.02 (m, 4H), 7.71-7.63 (m, 2H), 6.96 (t, *J* = 5.4, 1H), 6.77 (t, *J* = 5.4, 1H), 6.20 (d, *J* = 1.8, 1H), 3.70 (s, 2H), 3.32 (d, *J* = 8.1, 2H), 3.25 (t, *J* = 7.2, 2H), 2.98 (q, *J* = 5.7, 2H), 2.90 (q, *J* = 6.3, 2H), 2.55-2.49 (m, 4H), 1.69 (dt, *J* = 13.9, 6.6, 2H), 1.58 (dt, *J* = 13.6, 5.6, 2H), 1.37 (s, 18H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 169.5, 169.3, 157.4, 156.39, 156.21, 148.5, 146.6, 135.5, 132.5, 130.44, 130.27, 130.0, 129.23, 129.19, 128.8, 128.4, 127.7, 125.1, 105.0, 101.5, 78.29, 78.18, 45.9, 43.7, 38.34, 38.22, 33.0, 29.5, 28.9, 28.5; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 676.3459; found 676.3453.

2-Naphthylamide-7-deazaguanine-8-methylene-*bis*-3-propylamine amide hydrochloride (**5**): To 15 mL of 6 N HCl in MeOH at 0 ˚C was added 1.5 g (2.2 mmol) of **5b** and stirred at room temperature 1 h. The solvent was fully removed under reduced pressure to give 1.21 g (2.2 mmol, 99%) of a dull yellow foam: ^{1}H NMR (500 MHz, D_2O) δ 7.98 (s, 1H), 7.59 (t, *J* = 7.9, 2H), 7.51 (d, *J* = 8.5, 1H), 7.32 (t, *J* = 4.0, 1H), 7.10 (t, *J* = 3.9, 2H), 5.98 (s, 1H), 3.67 (s, 2H), 3.45 (q, *J* = 7.9, 4H), 2.98 (dt, *J* = 26.7, 7.5, 4H), 1.95 (ddt, *J* = 25.3, 15.8, 8.5, 4H); ^{13}C NMR (125 MHz; CD_3OD) δ 171.6, 169.3, 158.8, 149.4, 146.2, 135.6, 132.5, 129.6, 129.2, 128.59, 128.54, 128.45, 127.6, 127.0, 123.6, 104.3, 101.3, 65.6, 45.4, 42.6, 37.0, 32.3, 26.6, 25.6; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 476.2410; found 476.2404.



5a 6

7-Deazaguanine-8-methylene-*bis*-3-Boc-propylamine amide (**6**): To 15 mL of 6 N HCl in MeOH at 0 ˚C was added 2.0 g (3.7 mmol) of **5a** and stirred at room temperature 1 h. The solvent was fully evaporated to give 1.44 g (3.6 mmol, 98%) of a dull yellow foam: ^{1}H NMR (500 MHz; D_2O) δ 6.35 (s, 1H), 3.89 (s, 2H), 3.53 (t, *J* = 7.9, 2H), 3.47 (t, *J* = 7.0, 2H), 3.05-3.02 (m, 2H), 2.96 (t, *J* = 7.3, 2H), 2.05-1.99 (m, 2H), 1.93 (quintet, *J* = 7.2, 2H); ^{13}C NMR (125 MHz; CD_3OD) δ 171.6, 158.4, 151.4, 141.3, 126.7, 101.9, 100.4, 45.3, 42.5, 36.9, 31.8, 26.5, 25.5; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 322.1991; found 322.1993.

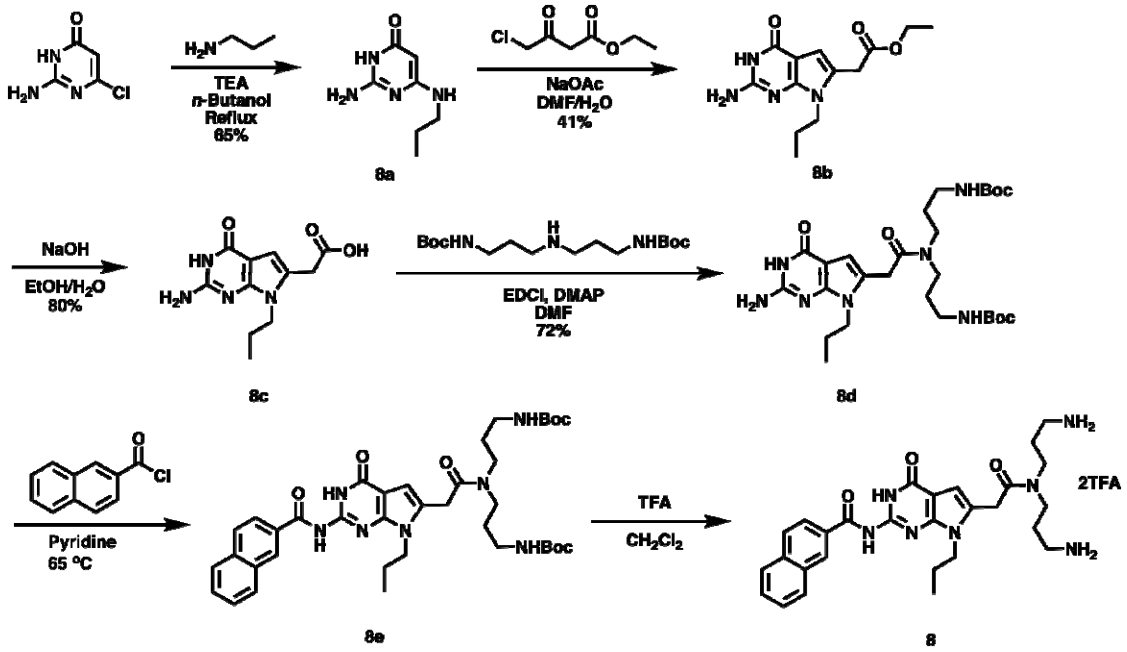


7a 7

2-Naphthoylamide-*bis*-3-Boc-propylamine amide (**7a**): To 1.0 g (5.8 mmol) of 2-naphthoic acid, 1.9 g (5.8 mmol) of *tert*-butyl 3,3'-azanediylbis(propane-3,1-diyl)dicarbamate, and 0.64 g (5.2 mmol) of DMAP in 30 mL of DMF at 0 °C was added 1.7 g (8.7 mmol) of EDCI. The solution was stirred at room temperature for 20 h. The solvent was evaporated to dryness, and the clear oil was suspended in 200 mL of CH_2Cl_2 and washed sequentially with 100 mL of saturated $NaHCO_3$, 100 mL of brine, and 100 mL of water. The organic layer was dried over Na_2SO_4 followed by removal of solvent under reduced pressure to produce 2.2 g of crude clear foam. Purification by column chromatography using a MeOH gradient from 0 to 10% CH_2Cl_2 produced 2.1 g (4.1 mmol, 74%) of a clear brittle foam: ^{1}H NMR (500 MHz; DMSO-d_6) δ 8.00-7.95 (m, 3H), 7.89 (s, 1H), 7.59-7.55 (m, 2H), 7.43 (dd, *J* = 8.4, 1.5, 1H), 6.87 (s, 1H), 6.64 (s, 1H), 3.43 (s, 2H), 3.21 (s, 2H), 3.02 (s, 2H), 2.73 (s, 2H), 1.73 (s, 2H), 1.62 (s, 2H), 1.39 (s, 9H), 1.20 (s, 9H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 172.4, 156.4, 156.1, 134.0, 133.6, 132.9, 128.62, 128.49, 128.0, 127.2, 127.0, 126.1, 79.1, 46.9, 42.1, 37.8, 29.6, 28.7, 28.4, 28.04, 123.88; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 486.2968; found 486.2962.

2-Naphthylamide-*bis*-3-propylamine amide hydrochloride (**7**) – To 15 mL of 6 N HCl in MeOH at 0 ˚C was added 1.0 g (2.1 mmol) of **3a** and stirred at room temperature 1 h. The solvent was fully evaporated to give 0.73 g (2.1 mmol, 99%) of a clear colorless foam: ^{1}H NMR (500 MHz; D_2O) δ 8.03 (d, *J* = 8.5, 1H), 7.97 (sextet, *J* = 4.5, 2H), 7.94 (s, 1H), 7.65-7.60 (m, 2H), 7.47 (dd, *J* = 8.4, 1.6, 1H), 3.66 (t, *J* = 7.0, 2H), 3.41 (t, *J* = 7.7, 2H), 3.11 (t, *J* = 7.5, 2H), 2.68 (dd, *J* = 8.8, 7.2, 2H), 2.09 (quintet, *J* = 7.3, 2H), 1.89 (dt, *J* = 15.6, 7.8, 2H); ^{13}C NMR (125

MHz; CD_3OD) δ 174.7, 135.0, 134.21, 134.04, 129.9, 129.4, 128.9, 128.5, 128.1, 127.0, 124.4, 47.7, 43.1, 38.3, 38.0, 27.6, 26.7; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 286.1919; found 286.1912.



2-amino-6-(propylamino)pyrimidin-4(3*H*)-one (**8a**): To a refluxing suspension of 4.1 g (28 mmol) of 2-amino-6-(propylamino)pyrimidin-4(*3H*)-one and 7.8 mL (56 mmol) of triethylamine in 200 mL of *n*-butanol was added 4.7 mL (56 mmol) of propylamine and left overnight. The clear yellow solution was cooled to ambient temperature and washed with 100 mL (3×), dried over Na_2SO_4 and condensed under reduced pressure. The resulting solid was suspended in a 10 mL of *n*-butanol and triturated in 600 mL of pentane to produce 3.1 g (18.2 mmol) of an off-white amorphous solid: mp 248-250 ˚C; ^{1}H NMR (400 MHz; DMSO-d_6) δ 9.64 (s, 1H), 6.35 (s, 1H), 6.11 (s, 2H), 4.39 (s, 1H), 2.97 (s, 2H), 1.45 (sextet, J = 7.2, 2H), 0.85 (t, J = 7.4, 3H); ^{13}C NMR (100 MHz; DMSO-d_6) δ 164.5, 163.3, 155.1, 75.2, 42.9, 22.4, 11.8; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 169.1089; found 169.1091.

ethyl 2-(2-amino-4-oxo-7-propyl-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)acetate (**8b**): To a suspension of 2.1 g (12.4 mmol) of **8a** and 1.2 g (14.9 mmol) of NaOAc in 65 mL of DMF and 15 mL of water was added 3.4 mL (24.9 mmol) of ethyl-4-chloroacetoacetate and stirred for 4 days. The mixture was diluted to 1.0 L with water, filtered, and extracted with 400 mL of $CHCl_3$ (3×). The combined organic layers were dried over Na_2SO_4 and condensed under reduced pressure to produce a red crude solid. The solid was suspended in a minimal amount of MeCN and precipitated with 1:1 Et_2O/hexanes to produce 1.4 g (5.1 mmol, 41%) of a light brown solid:

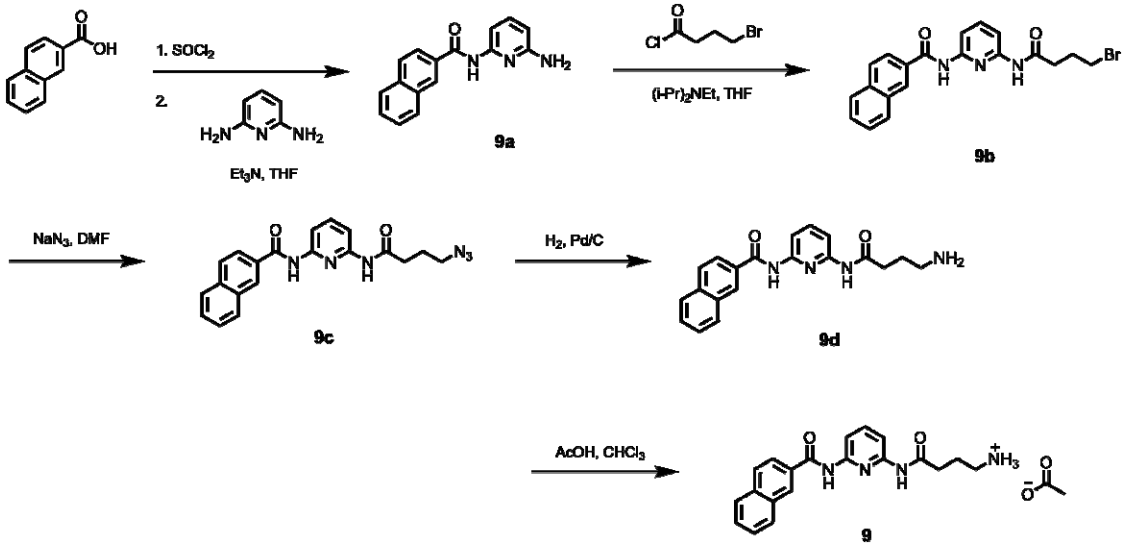
mp 204-205 ˚C; ^{1}H NMR (400 MHz; DMSO-d_6) δ 10.25 (s, 1H), 6.19 (s, 2H), 6.09 (s, 1H), 4.09 (q, J = 7.1, 2H), 3.79 (t, J = 7.6, 2H), 3.71 (s, 2H), 1.59 (dq, J = 15.1, 7.5, 2H), 1.19 (t, J = 7.1, 3H), 0.83-0.79 (m, 3H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 170.3, 158.5, 152.4, 150.9, 124.7, 101.3, 99.4, 60.7, 43.3, 32.4, 23.1, 14.2, 11.2; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 279.1457; found 279.1448; Anal. calcd. for $C_{13}H_{18}N_4O_3$: C, 56.10; H, 6.52; N, 20.13. Found: C, 56.83; H, 6.51; N, 19.97.

2-(2-amino-4-oxo-7-propyl-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)acetic acid (**8c**): To a refluxing solution of 0.63 g (2.3 mmol) of **8b** in 30 mL of 1:1 EtOH:water was added 0.18 g (4.5 mmol) of NaOH and stirred for 4 h. The solution was cooled to ambient temperature, neutralized with conc. HCl and condensed under reduced pressure to produce an off-white crude precipitate. The resulting solid was triturated in cold water to produce 0.45 g (1.8 mmol, 80%) of and off-white solid: mp dec. > 250 ˚C: ^{1}H NMR (500 MHz; DMSO-d_6) δ 12.52 (s, 1H), 10.23 (s, 1H), 6.18 (s, 2H), 6.08 (s, 1H), 3.80 (t, J = 7.7, 2H), 3.61 (s, 2H), 1.61 (sextet, J = 7.5 Hz, 2H), 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 172.0, 158.6, 152.3, 150.9, 125.5, 101.2, 99.3, 43.3, 32.6, 23.1, 11.2; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 251.1144; found 251.1149.

***tert*-butyl 3,3'-(2-(2-amino-4-oxo-7-propyl-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)-acetylazanediyl)bis(propane-3,1-diyl)dicarbamate** (**8d**): To 0.25 g (0.81 mmol) of (**8c**) and 0.43 g (1.3 mmol) of *tert*-butyl 3,3'-azanediylbis(propane-3,1-diyl)dicarbamate in 15 mL of DMF at 0 °C was added 0.11 g (0.89 mmol) of DMAP followed by 0.20 g (1.0 mmol) of EDCI. The suspension was stirred at room temperature for 20 h. The solvent was evaporated to dryness, and the resulting solid was suspended in 200 mL of CH_2Cl_2 and washed sequentially with 100 mL saturated Na_2CO_3, 100 mL of brine, and 100 mL of water. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. Purification by column chromatography using a MeOH gradient from 0 to 10% in CH_2Cl_2 produced 0.33 g (0.58 mmol, 72%) of a light yellow foam: ^{1}H NMR (400 MHz; DMSO-d_6) δ 10.21 (s, 1H), 6.94 (t, J = 5.2, 1H), 6.77 (t, J = 5.6, 1H), 6.16 (s, 2H), 5.97 (s, 1H), 3.77 (t, J = 7.6, 2H), 3.65 (s, 2H), 3.26 (dt, J = 31.7, 7.5, 4H), 2.92 (dq, J = 25.6, 6.2, 4H), 1.70-1.51 (m, 6H), 1.36 (d, J = 3.7, 15H), 0.81 (t, J = 7.4, 3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 170.2, 160.6, 156.6, 152.14, 152.02, 126.8, 101.5, 100.3, 79.7, 79.3, 46.3, 44.3, 43.5, 38.4, 37.8, 32.9, 29.9, 28.80, 28.74, 28.3, 23.8, 11.6; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 564.3510; found 564.3508.

***tert*-butyl3,3'-(2-(2-(2-naphthamido)-4-oxo-7-propyl-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)acetylazanediyl)bis(propane-3,1-diyl)dicarbamate** (**8e**): To 147 mg (0.85 mmol) of 2-Naphthoic acid suspended in 2 mL of CH_2Cl_2 was added 2 mL of oxalyl chloride stirred at room temperature for 18 h. The solvent was removed under reduced pressure and co-evaporated with CH_2Cl_2. The dried acid chloride was dissolved in 2 mL of pyridine, and added dropwise to a solution of 247 mg (0.44 mmol) of **8d** in 12 mL of pyridine. The solution was stirred at 65 ˚C for 18 h. The solvent was fully evaporated *in vacuo*, and the solid was taken up in $CHCl_3$ (60 mL), and washed with water (1 × 50 mL) and brine (1 × 50 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated to dryness to give a yellow solid that was purified by column chromatography using a MeOH gradient from 0 to 10% in CH_2Cl_2 to produce 175 mg (0.25 mmol, 56%) of a yellow foam: ^{1}H NMR (500 MHz; DMSO-d_6) δ 12.06 (s, 1H), 11.86 (s, 1H), 8.75 (s, 1H), 8.07 (td, *J* = 21.4, 8.6, 4H), 7.67 (tdd, *J* = 14.4, 6.4, 1.4, 2H), 6.96 (t, *J* = 5.3, 1H), 6.78 (t, *J* = 5.5, 1H), 6.27 (s, 1H), 3.98 (t, *J* = 7.6, 2H), 3.81 (s, 2H), 3.36 (d, *J* = 7.4, 2H), 3.25 (t, *J* = 7.4, 2H), 2.98 (q, *J* = 6.0, 2H), 2.90 (q, *J* = 6.3, 2H), 1.71 (dq, *J* = 15.0, 7.5, 4H), 1.59 (dt, *J* = 14.0, 7.0, 2H), 1.37 (d, *J* = 1.4, 18H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz; $CDCl_3$) δ 169.5, 168.0, 157.8, 156.48, 156.37, 148.1, 145.6, 135.6, 132.5, 129.52, 129.41, 129.26, 129.05, 128.89, 128.0, 127.4, 123.5, 104.6, 102.3, 79.6, 79.2, 46.2, 44.4, 43.3, 38.2, 37.6, 32.6, 29.9, 28.66, 28.59, 28.1, 23.8, 11.5; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 718.3928; found 718.3937.

***N*-(6-(2-(bis(3-aminopropyl)amino)-2-oxoethyl)-4-oxo-7-propyl-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2-naphthamide** (**8**): To a solution of 130 mg (0.181 mmol) of **8e** in 5 mL of CH_2Cl_2 was added 2 mL of TFA dropwise and stirred for 1 h. The solution was condensed under reduced pressure and the resulting oil was dissolved in 10 mL of water. The solution was filtered and purified by RP-HPLC using a gradient of 4:1 0.1% TFA/H_2O: 0.1% TFA/MeCN to 0.1% TFA/MeCN over 30 min to produce 0.121 mg (0.163 mmol, 90%) of a light yellow clear film as the bis TFA salt: ^{1}H NMR (500 MHz; CD_3OD) δ 8.46 (s, 1H), 7.94-7.81 (m, 4H), 7.54-7.48 (m, 2H), 6.38 (s, 1H), 4.01 (t, *J* = 7.7, 2H), 3.92 (s, 2H), 3.55 (dt, *J* = 26.2, 7.4, 4H), 3.04 (t, *J* = 7.6, 2H), 2.96 (t, *J* = 7.2, 2H), 2.01 (dquintet, *J* = 26.9, 8.1, 4H), 1.75 (sextet, *J* = 7.6, 2H), 0.92 (t, *J* = 7.4, 3H); ^{13}C NMR (125 MHz; CD_3OD) δ 172.3, 170.6, 159.8, 150.2, 147.2, 136.7, 133.6, 131.1, 130.62, 130.45, 130.37, 129.65, 129.51, 128.7, 128.0, 124.9, 104.8, 102.7, 46.6, 45.4, 43.8, 38.22, 38.05, 32.8, 27.8, 26.7, 24.5, 11.5; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 518.2880; found 518.2880.



Naphthylene-2-carboxylic acid (6-amino-pyridin-2-yl)-amide (9a). To 2.0 g (11.6 mmol) of 2-naphthoic acid suspended in $SOCl_2$ (40 mL) and the solution was refluxed (80 °C) for 20 h. The mixture was cooled and the excess $SOCl_2$ was evaporated. The residue was co-evaporated with benzene and placed on high-vacuum to give a tan solid. A solution of 2,6-diaminopyridine (1.5 g, 14.0 mmol) in Et_3N (1.6 mL, 11.6 mmol) and THF (40 mL) was prepared and cooled in an ice-bath. The dried acid chloride dissolved in THF (20 mL) was added dropwise and the reaction was allowed to stir at room temperature for 20 h. The solvents were evaporated and the residue was dissolved in $CHCl_3$ (60 mL) and washed with H_2O (2 x 100 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo to give a brown solid. Purified by column chromatography using 1:4 MeCN:CH_2Cl_2 mixture as eluent. The product **5a** was isolated and the solvent was evaporated to give a white solid that was recrystallized with $CHCl_3$. After two recrystallizations, the crops were combined to give a total of 2.23 g of a white crystalline solid (73%): ^{1}H-NMR (500 MHz; DMSO-d_6) δ 10.27 (s, 1H), 8.66 (s, 1H), 8.07-8.00 (m, 4H), 7.67-7.61 (m, 2H), 7.44 (dt, J = 17.6, 8.4, 2H), 6.28 (dd, J = 7.8, 1H), 5.84 (s, 2H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 165.8, 158.9, 150.8, 139.2, 134.6, 132.4, 132.0, 129.4, 128.7, 128.2, 127.9, 127.0, 124.8, 104.3, 102.4; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 264.1137; found 264.1137.

Naphthylene-2-carboxylic acid (6-4-bromo-butyrylamino) pyridin-2-yl)-amide (9b): 4-bromobutyric acid chloride (176 µL, 1.52 mmol) dissolved in THF (5 mL) was added dropwise to a solution of **5a** (400 mg, 1.50 mmol) dissolved in THF (25 mL) and (*i*-Pr)$_2$NEt (264 µL, 1.52 mmol) and stirred at ambient temperature. The THF was evaporated and co-evaporated with $CHCl_3$. The residue was dissolved in $CHCl_3$ (50 mL) and washed with 5% (*w*/*v*) $NaHCO_3$ (1 x 50

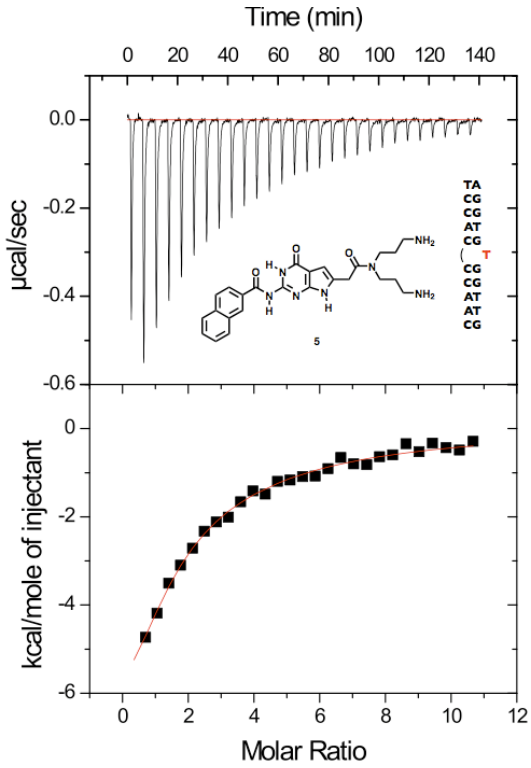
mL) and H_2O (2 x 50 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo to give a brown residue. The product was purified by column chromatography using 1:4 MeCN:CH_2Cl_2 as eluent. The product fractions were collected, and the solvent was evaporated to give 615 mg (98%) of a yellow oil. ^{1}H-NMR (400 MHz; $CDCl_3$): 8.42 (s, 2H), 8.15 (d, J = 8.4, 1H), 7.99-7.91 (m, 5H), 7.80 (t, J = 8.0, 1H), 7.68 (bs, 1H), 7.60 (dquintet, J = 7.2, 2.4, 2H), 3.55 (t, J = 6.4, 2H), 2.63 (t, J = 6.8, 2H), 2.30 (quintet, J = 6.4, 2H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 171.8, 166.7, 151.06, 150.90, 140.8, 135.1, 132.7, 132.1, 129.8, 129.2, 128.7, 128.3, 127.5, 125.1, 111.3, 110.4, 35.1, 28.7; *m/z* FD 412.2; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 412.0661; found 412.0658.

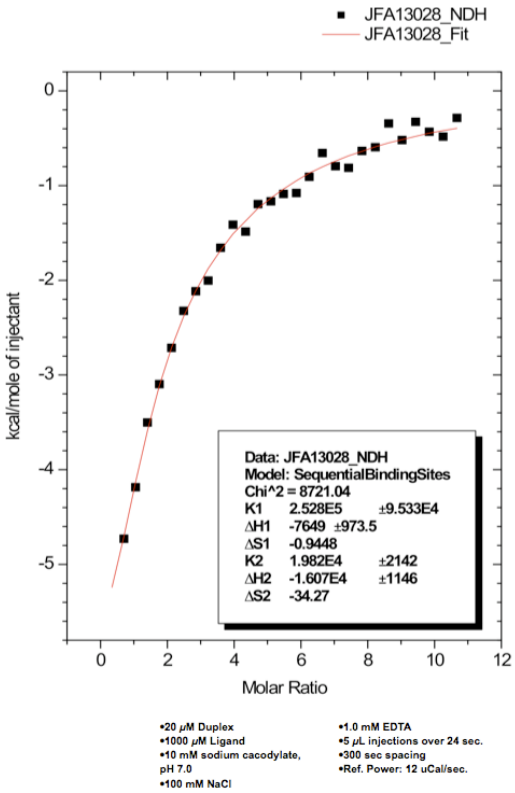
Naphthylene-2-carboxylic acid (6-4-azido-butyrylamino) pyridin-2-yl)-amide (9c): NaN_3 (48 mg, 0.74 mmol) was added to a solution of **5b** (200 mg, 0.49 mmol) in DMF (10 mL) and the reaction was allowed to stir at ambient temperature for 20 h. The DMF was evaporated, and the residue was dissolved $CHCl_3$ (20 mL) and washed with H_2O (1 x 20 mL) and brine (1 x 20 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated in vacuo to give a yellow oil that was purified by column chromatography using 5% MeCN:CH_2Cl_2 as eluent. The product was isolated, and the solvents were evaporated to give 90 mg (50%) of a yellow oil: 1H-NMR (500 MHz; DMSO-d_6): δ 10.51 (s, 1H), 10.29 (s, 1H), 8.63 (s, 1H), 8.07-8.00 (m, 4H), 7.85-7.82 (m, 3H), 7.66-7.60 (m, 2H), 3.38 (t, J = 6.9, 2H), 2.52 (t, J = 7.3, 2H), 1.85 (quintet, J = 7.1, 2H). ^{13}C NMR (125 MHz; DMSO-d_6) δ 171.7, 166.2, 150.9, 150.7, 140.3, 134.8, 132.4, 131.8, 129.4, 128.9, 128.4, 128.0, 127.2, 124.8, 110.9, 110.1, 50.6, 33.4, 24.5; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 375.1569; found 375.1559.

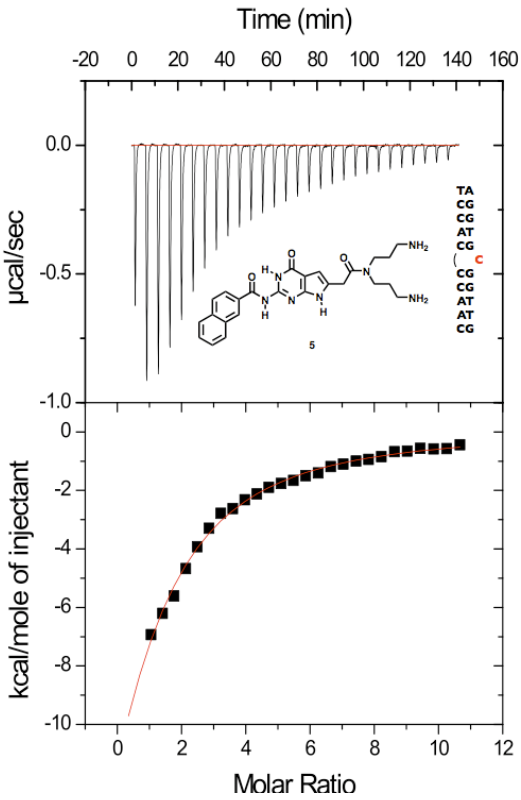
Naphthylene-2-carboxylic acid (6-4-amino-butyrylamino) pyridin-2-yl)-amide (9d): 5c (90 mg, 0.24 mmol) was dissolved in cold MeOH (20 mL), and Pd/C was carefully added (50 mg; 10% on act. ca.). The air in the solution was evacuated and re-pressurized with a H_2 balloon. The reaction was hydrogenated for 2 h using the hydrogenation apparatus at atmospheric pressure. The solution was filtered through Celite and the Celite was washed with 5% NEt_3/MeOH. The solvent was evaporated to give a faint yellow oil 54 mg (65%) that was purified with column chromatography using 1% Et_3N/5% MeOH/CH_2Cl_2. The product was isolated and the solvents evaporated to give 54 mg (65%) of a yellow oil which was carried directly to the next step.

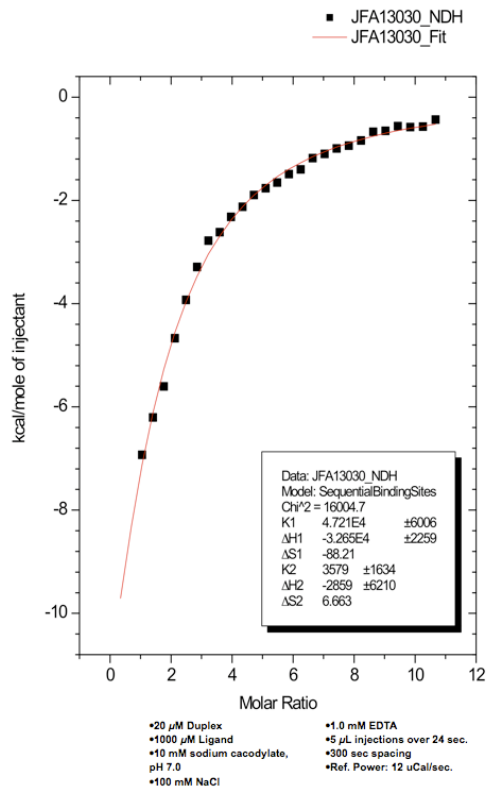
Naphthylene-2-carboxylic acid (6-4-ammonium-butyrylamino) pyridin-2-yl)-amide acetate (9): 5d (300 mg, 0.86 mmol) was suspended in $CHCl_3$ (20 mL) and AcOH (65 mg, 1.1 mmol, glacial) was added dropwise. The reaction was allowed to stir at room temperature for 20 min and the solvents were evaporated. The pink oil was triturated with $CHCl_3$ to give 270 mg (78%) of a white solid **5** that was filtered and washed with more $CHCl_3$: ^{1}H-NMR (400 MHz; D_2O): 8.18 (s, 1H), 7.87 (d, J = 7.6, 1H), 7.80 (d, J = 8.8, 2H), 7.68 (m, 1H), 7.61 (t, J = 8.0, 1H), 7.55-7.51 (m, 3H), 7.22 (d, J = 8.0, 1H), 2.97 (t, J = 7.6, 2H), 2.33 (t, J = 7.6, 2H), 1.88 (quintet, J = 8.4, 2H), 1.87 (s, 3H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 172.0, 166.7, 151.00, 150.88, 140.9, 135.1, 132.7, 132.1, 129.8, 129.2, 128.7, 128.3, 127.6, 125.1, 111.3, 110.4, 39.2, 33.5, 23.5; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 363.1821; found 363.1813.

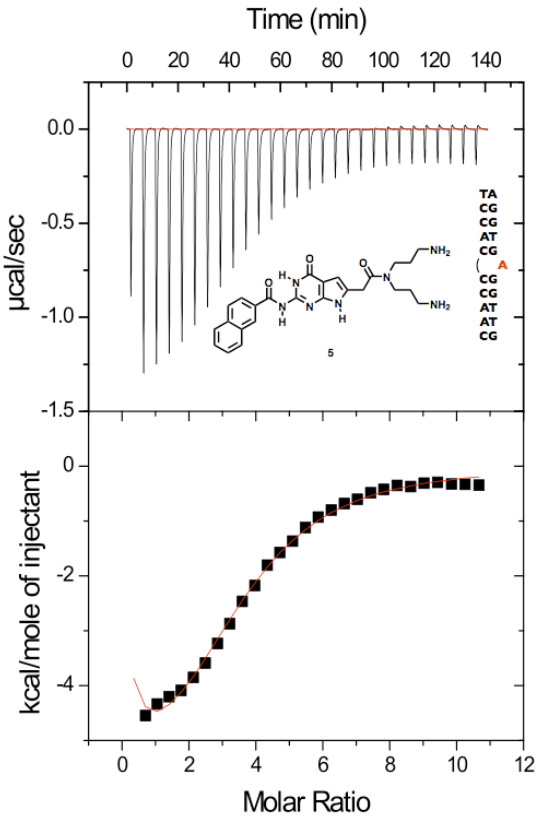
Isothermal Titration Calorimetry:







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µcal/sec
kcal/mole of injectant
Molar Ratio
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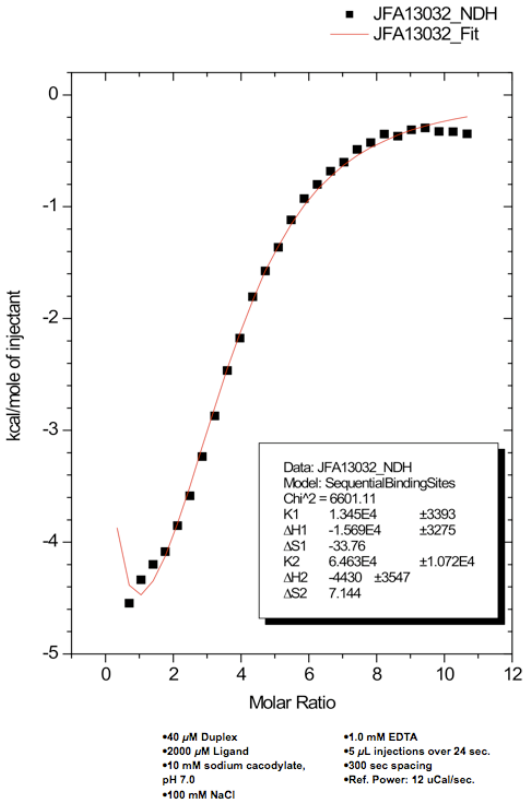
CG
CG
AT
CG
A
CG
CG
AT
AT
CG


JFA13032_NDH
JFA13032_Fit
kcal/mole of injectant
Molar Ratio
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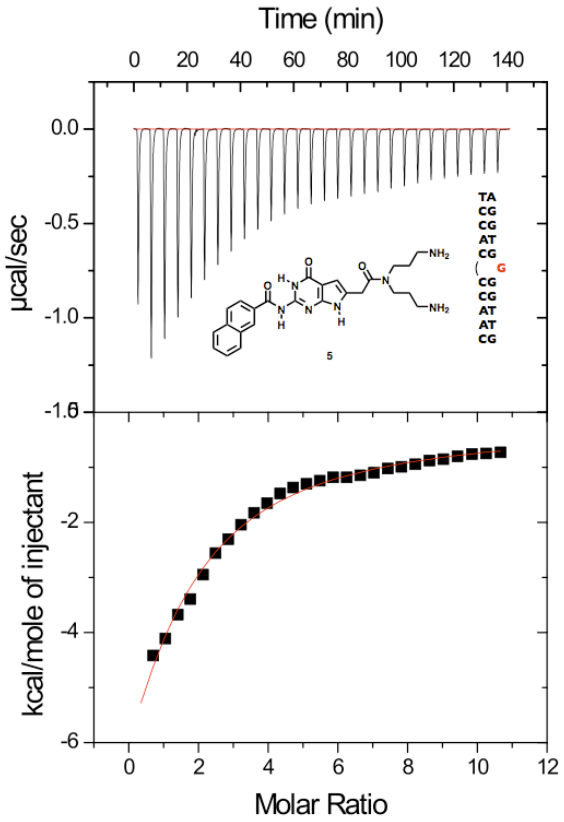
Chi^2 = 6601.11
K1 1.345E4 ±3393
ΔH1 -1.569E4 ±3275
ΔS1 -33.76
K2 6.463E4 ±1.072E4
ΔH2 -4430 ±3547
ΔS2 7.144
•40 µM Duplex
•2000 µM Ligand
•10 mM sodium cacodylate, pH 7.0
•100 mM NaCl
•1.0 mM EDTA
•5 µL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.


Time (min)
µcal/sec
kcal/mole of injectant
Molar Ratio
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CG
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AT
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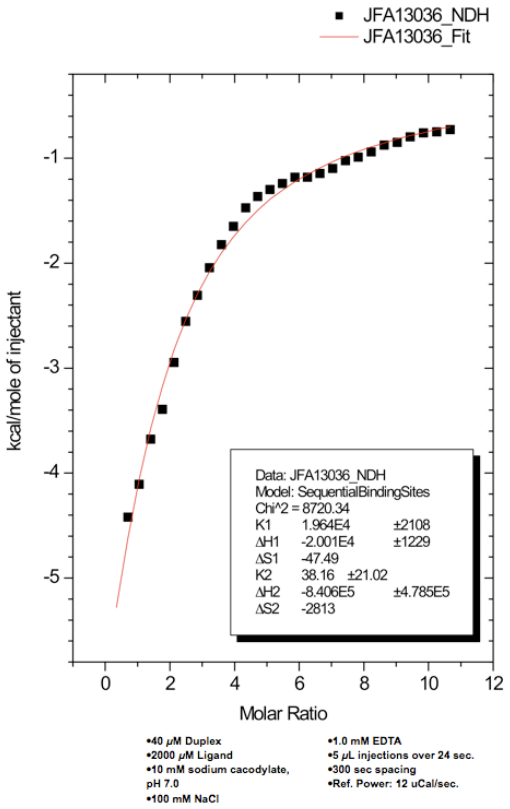
CG
CG
AT
AT
CG


JFA13036_NDH
JFA13036_Fit
kcal/mole of injectant
Molar Ratio
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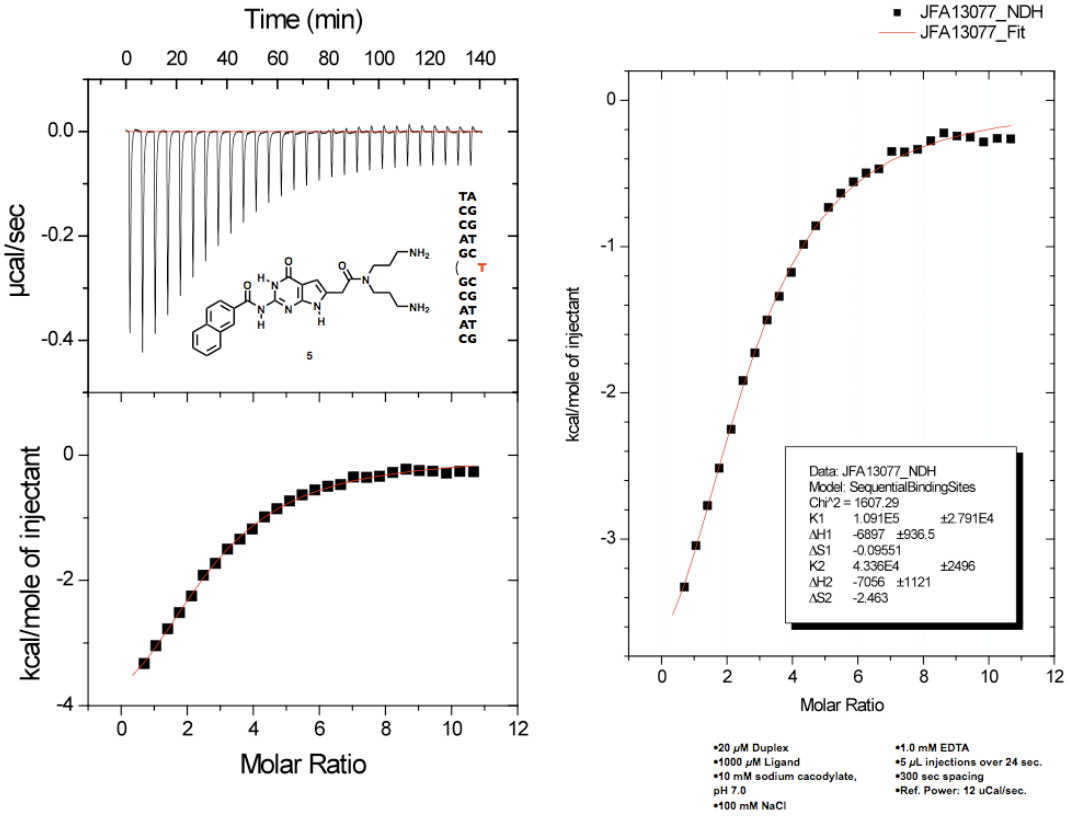
Model: SequentialBindingSites
Chi^2 = 8720.34
K1 1.964E4 ±2108
ΔH1 -2.001E4 ±1229
ΔS1 -47.49
K2 38.16 ±21.02
ΔH2 -8.406E5 ±4.785E5
ΔS2 -2813
•40 µM Duplex
•2000 µM Ligand
•10 mM sodium cacodylate, pH 7.0
•100 mM NaCl
•1.0 mM EDTA
•5 µL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.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-0.4
µcal/sec
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CG
CG
AT
GC
T
GC
CG
AT
AT
CG
5
0
-2
-4
kcal/mole of injectant
0 2 4 6 8 10 12
Molar Ratio
JFA13077_NDH
JFA13077_Fit
0
-1
-2
-3
kcal/mole of injectant
Data: JFA13077_NDH
Model: SequentialBindingSites
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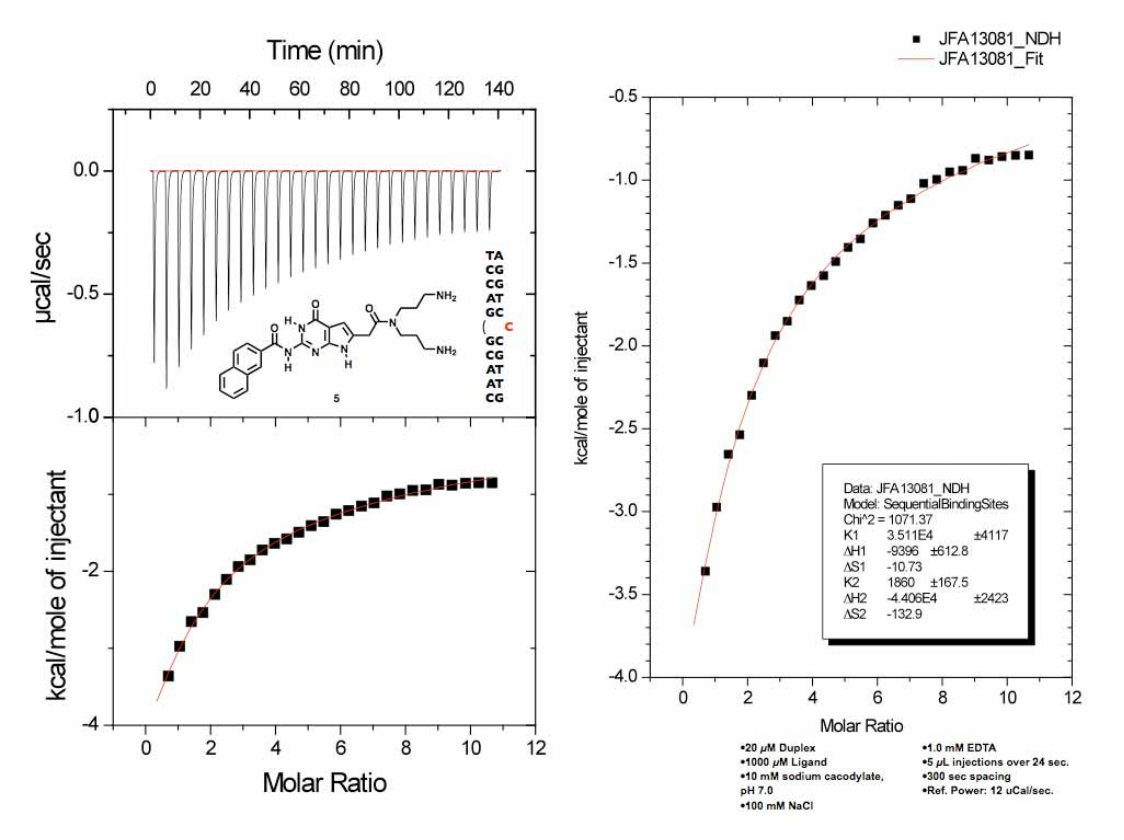
ΔH1 -6897 ±936.5
ΔS1 -0.09551
K2 4.336E4 ±2496
ΔH2 -7056 ±1121
ΔS2 -2.463
0 2 4 6 8 10 12
Molar Ratio
•20 µM Duplex
•1000 µM Ligand
•10 mM sodium cacodylate, pH 7.0
•100 mM NaCl
•1.0 mM EDTA
•5 µL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.


Time (min)
µcal/sec
kcal/mole of injectant
Molar Ratio
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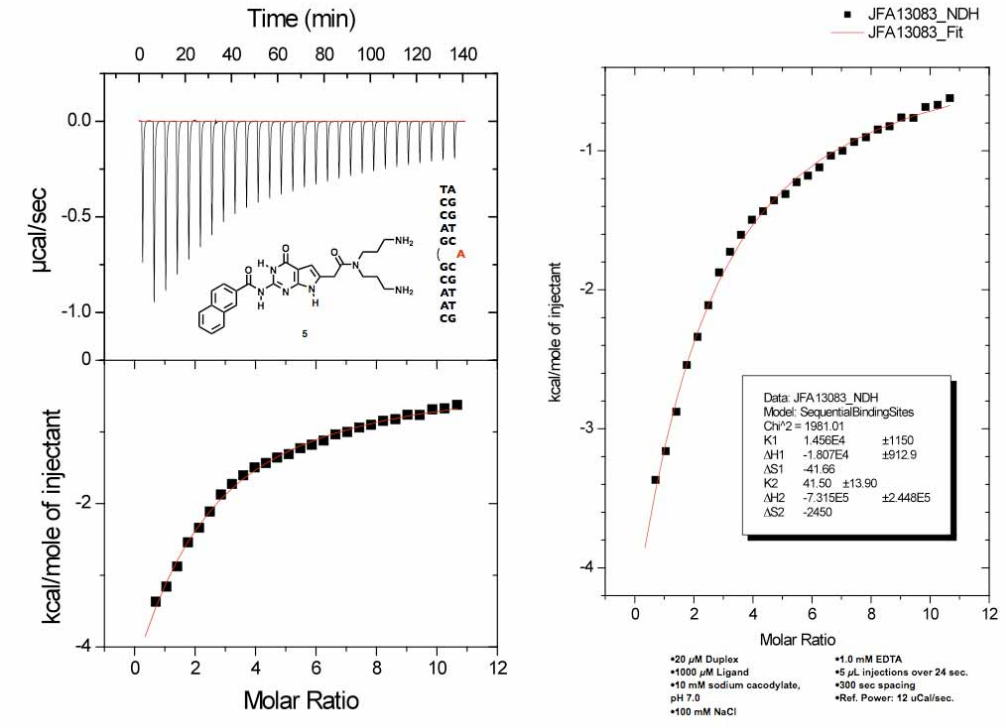
CG
AT
GC
C
GC
CG
AT
AT
CG
JFA13081_NDH
JFA13081_Fit
Data: JFA13081_NDH
Model: SequentialBindingSites
Chi^2 = 1071.37
K1 3.511E4 ±4117
ΔH1 -9396 ±612.8
ΔS1 -10.73
K2 1860 ±167.5
ΔH2 -4.406E4 ±2423
ΔS2 -132.9
•20 µM Duplex
•1000 µM Ligand
•10 mM sodium cacodylate, pH 7.0
•100 mM NaCl
•1.0 mM EDTA
•5 µL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.


Time (min)
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kcal/mole of injectant
Molar Ratio
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JFA13083_Fit
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Model: SequentialBindingSites
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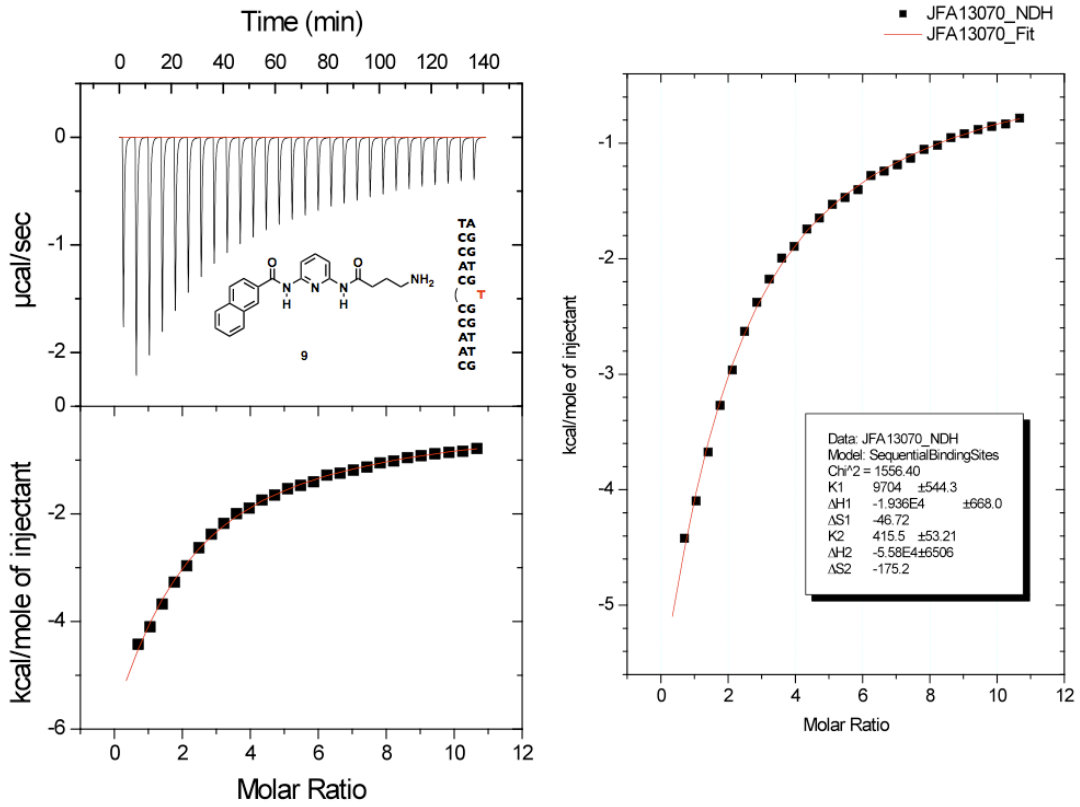
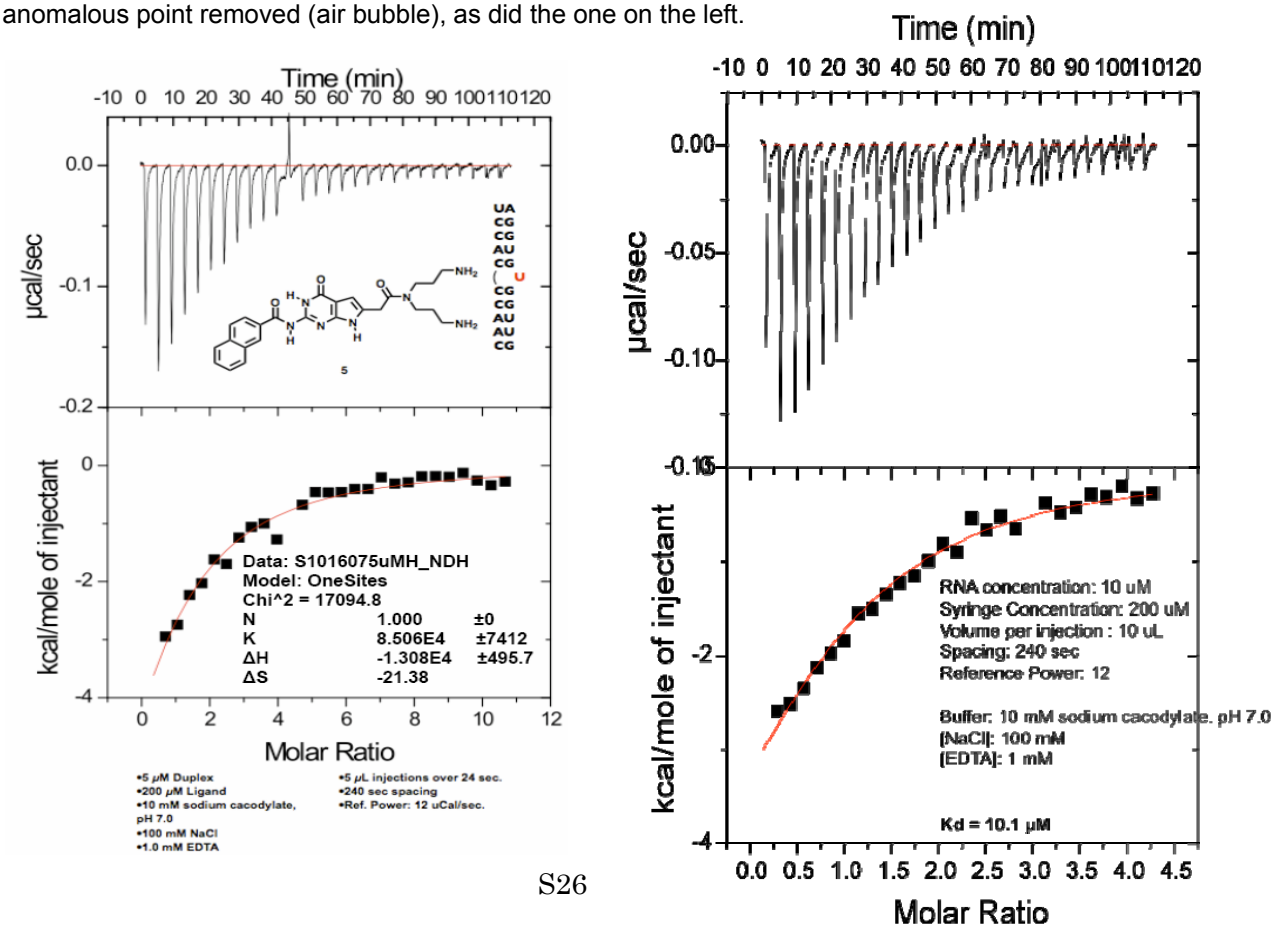
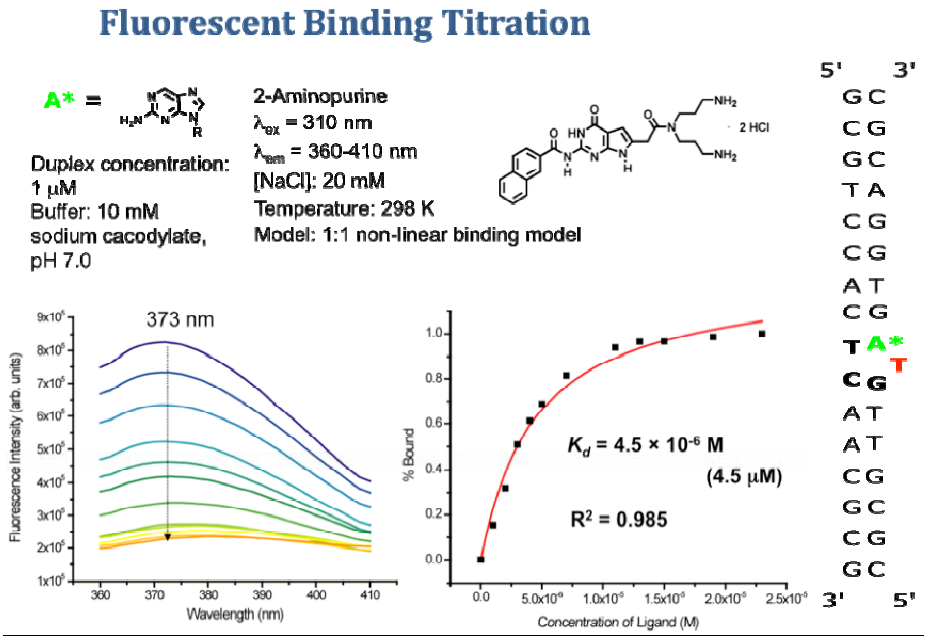
ΔH1 -1.807E4 ±912.9
ΔS1 -41.66
K2 41.50 ±13.90
ΔH2 -7.315E5 ±2.448E5
ΔS2 -2450
•20 µM Duplex
•1000 µM Ligand
•10 mM sodium cacodylate, pH 7.0
•100 mM NaCl
•1.0 mM EDTA
•5 µL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.



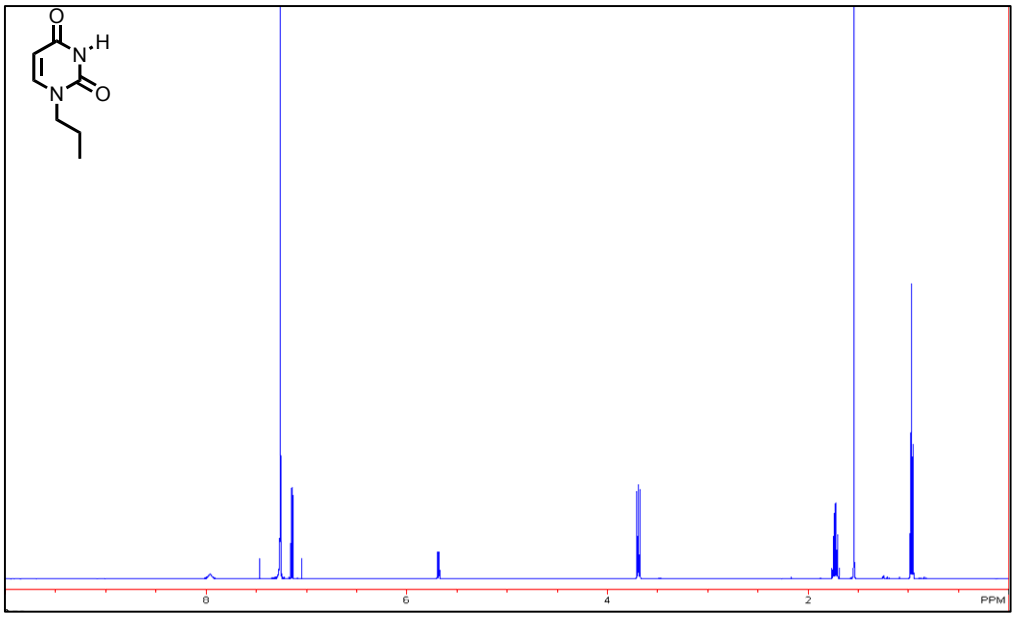
Two representative RNA titrations shown below. The one on left (also shown in text) had one anomalous point removed (air bubble), as did the one on the left.



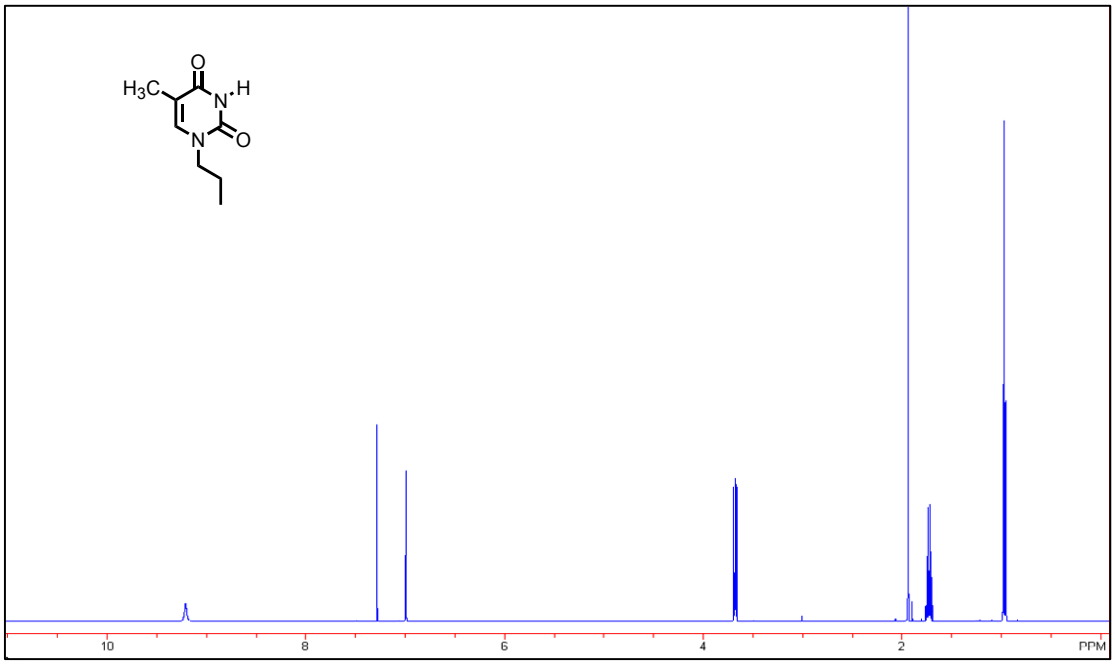


^{1}H NMR of Key Compounds

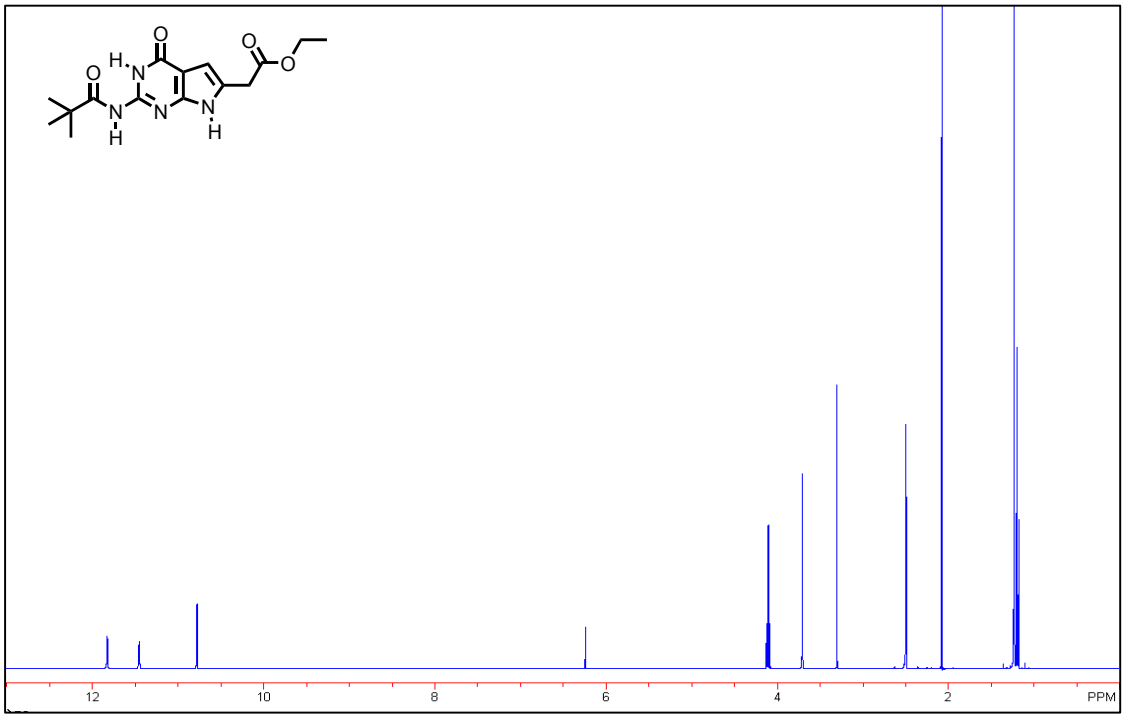
n-propyl uracil ($CDCl_3$)



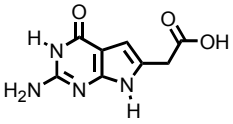
n-propyl thymine ($CDCl_3$)

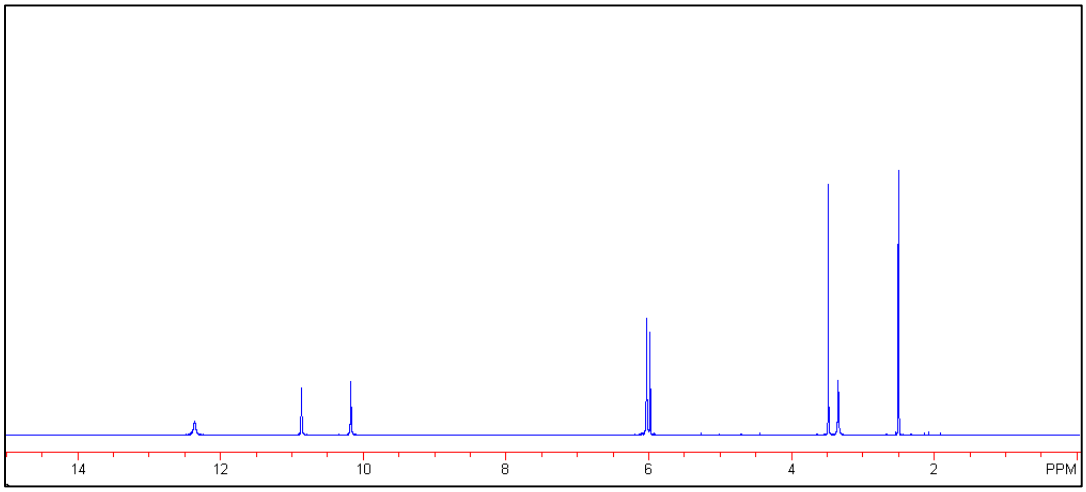


1 (DMSO-d_6)

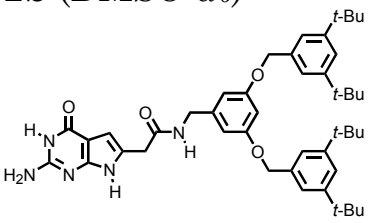


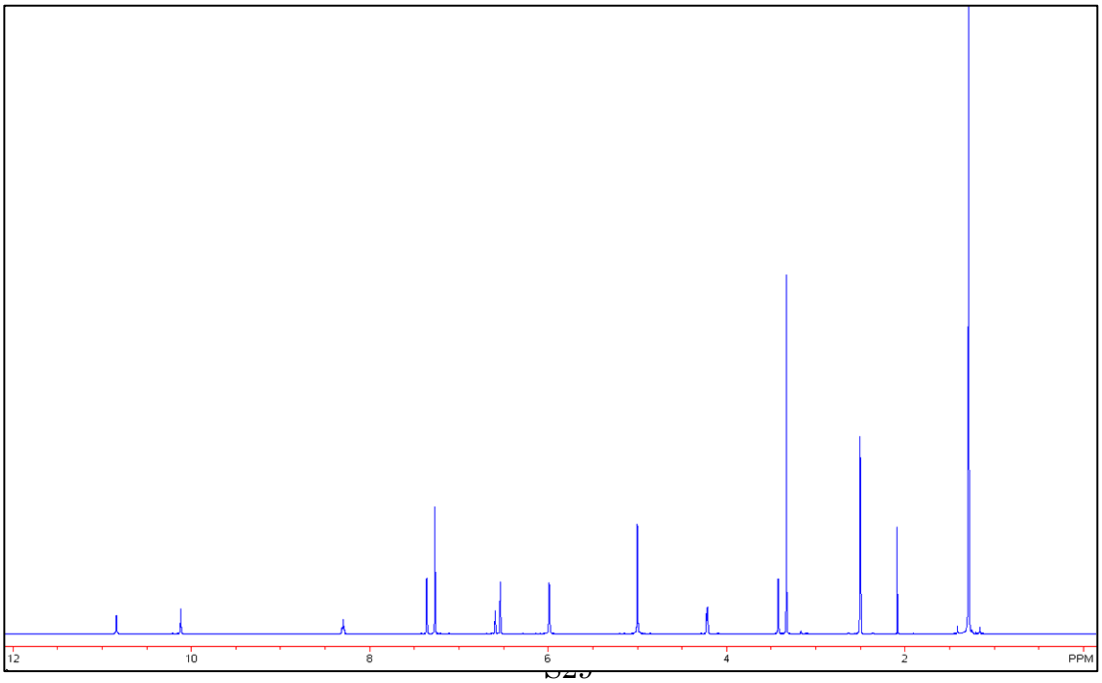
2a (DMSO-d_6)



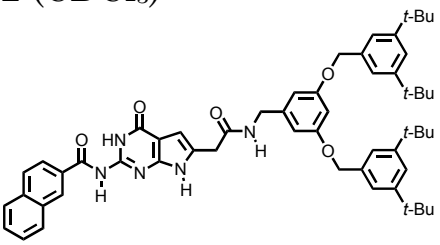


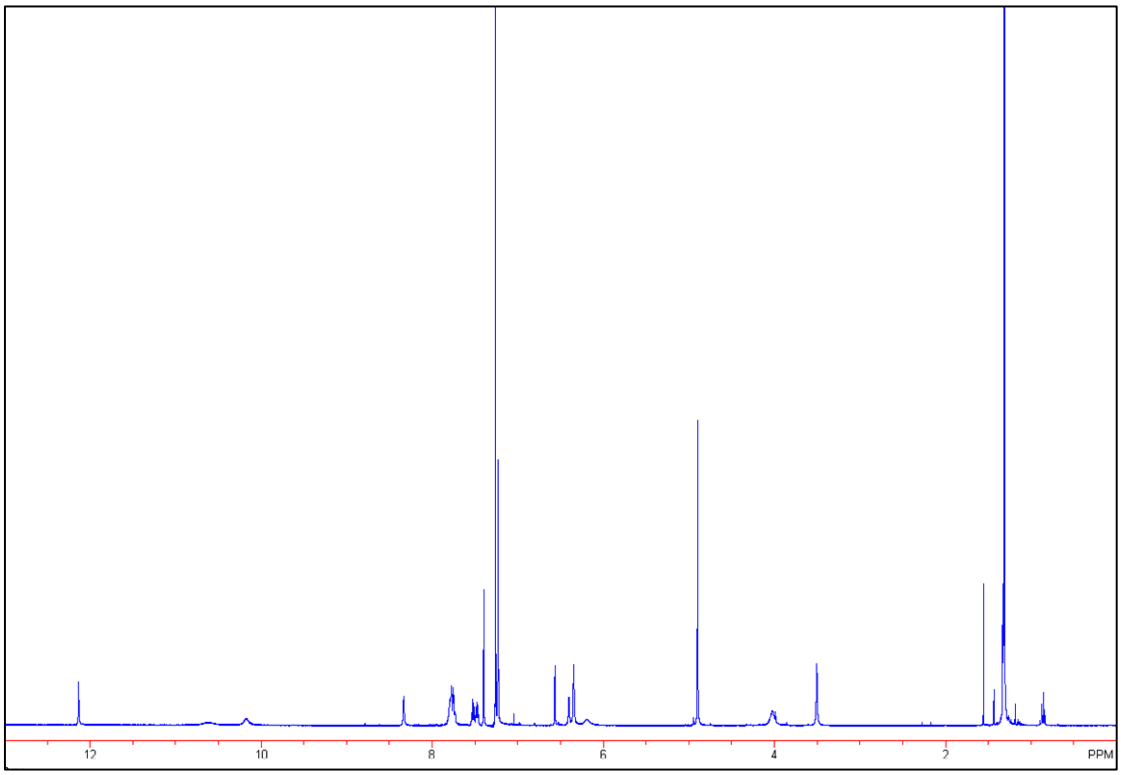
2b (DMSO-d_6)

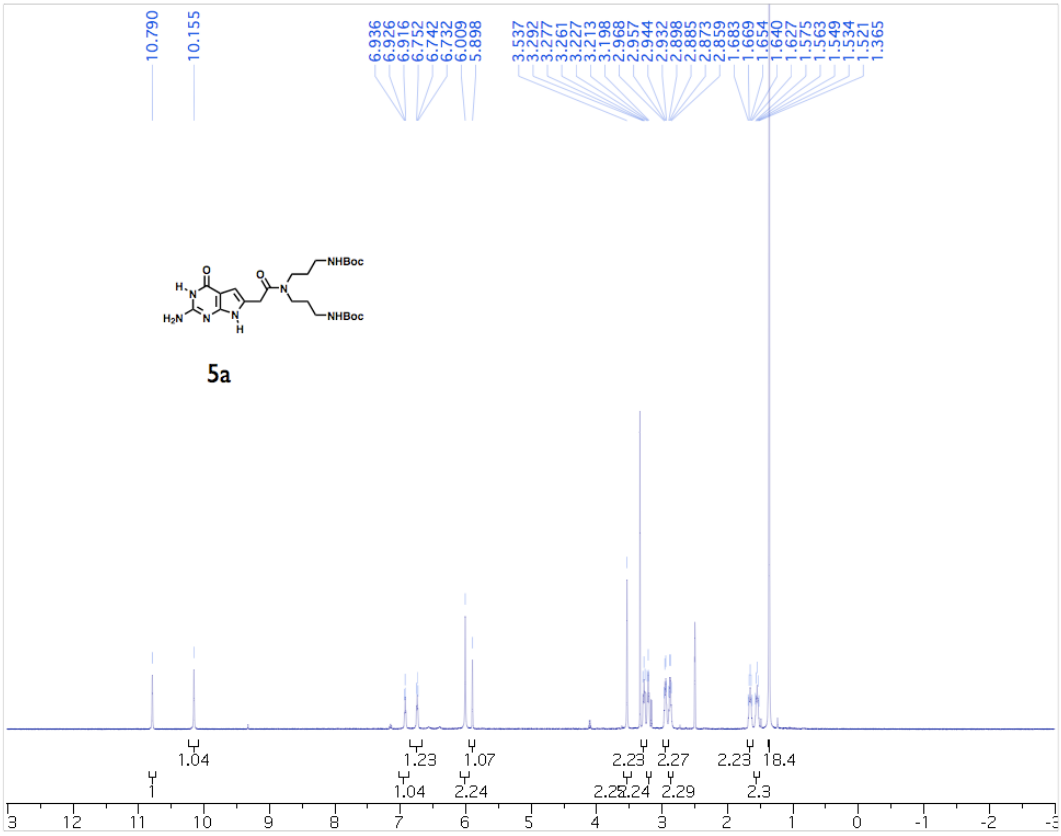




2 ($CDCl_3$)



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1.365
NHBoc
NHBoc
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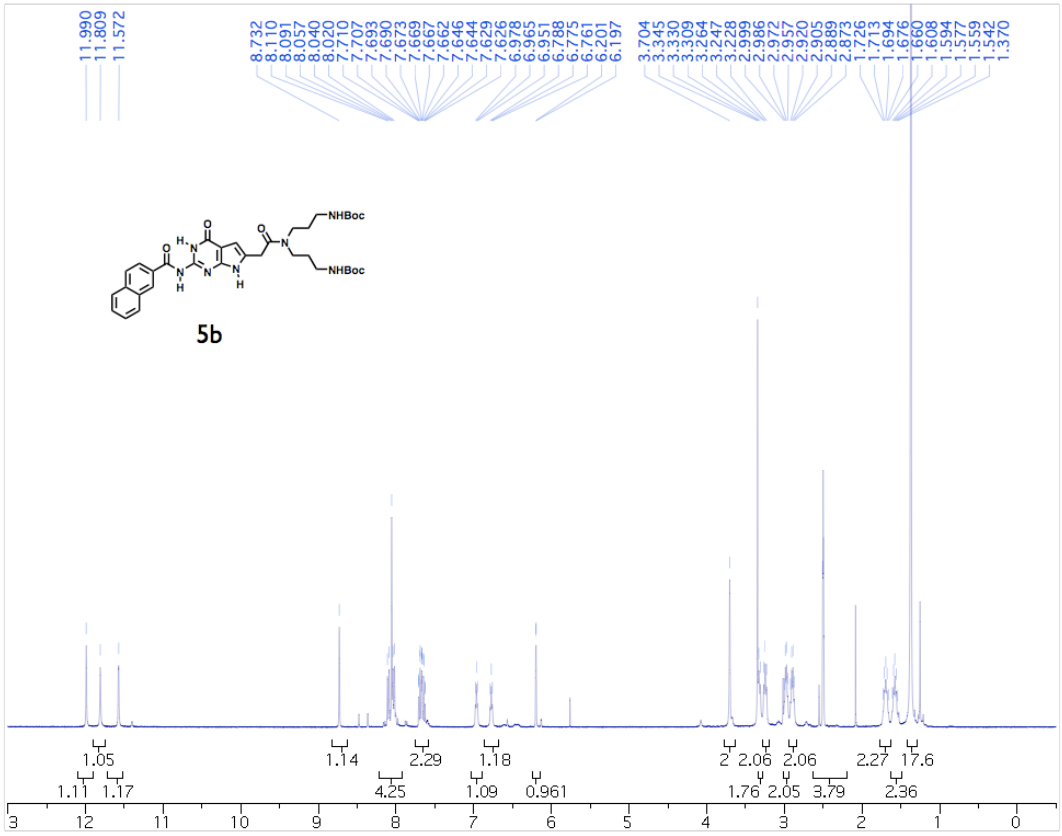
2.3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1.370
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NHBoc
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1.11
1.17
1.14
2.29
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1.18
1.09
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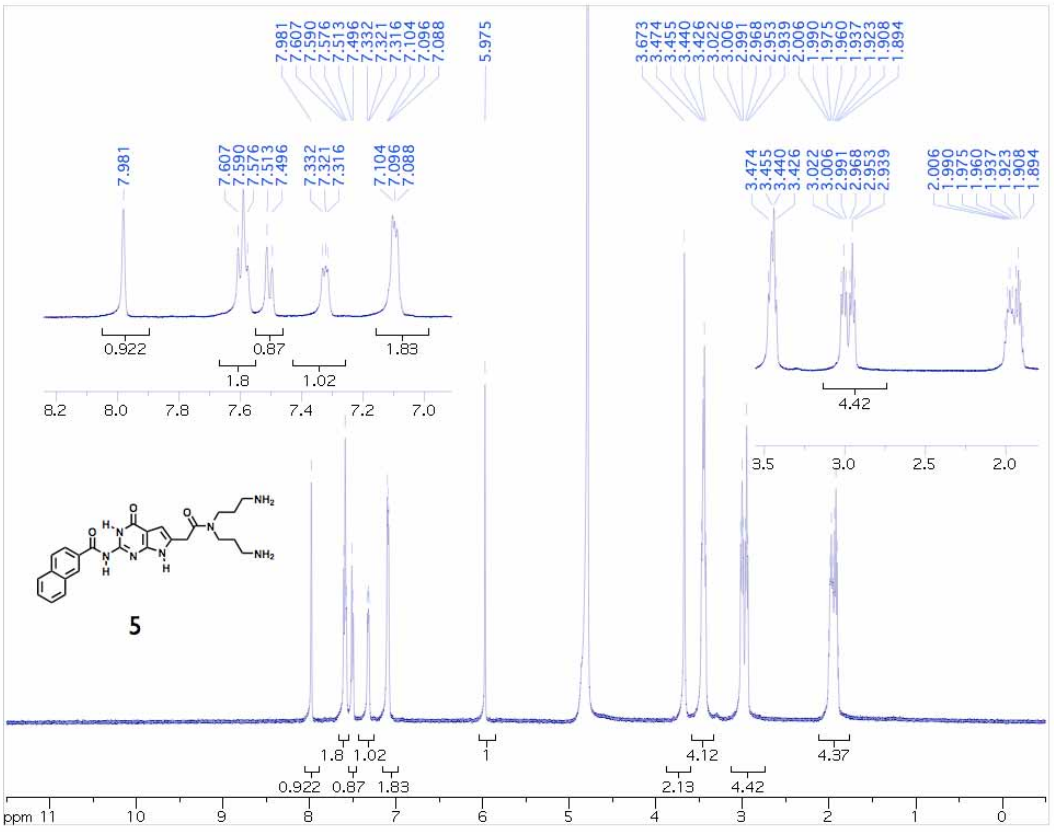
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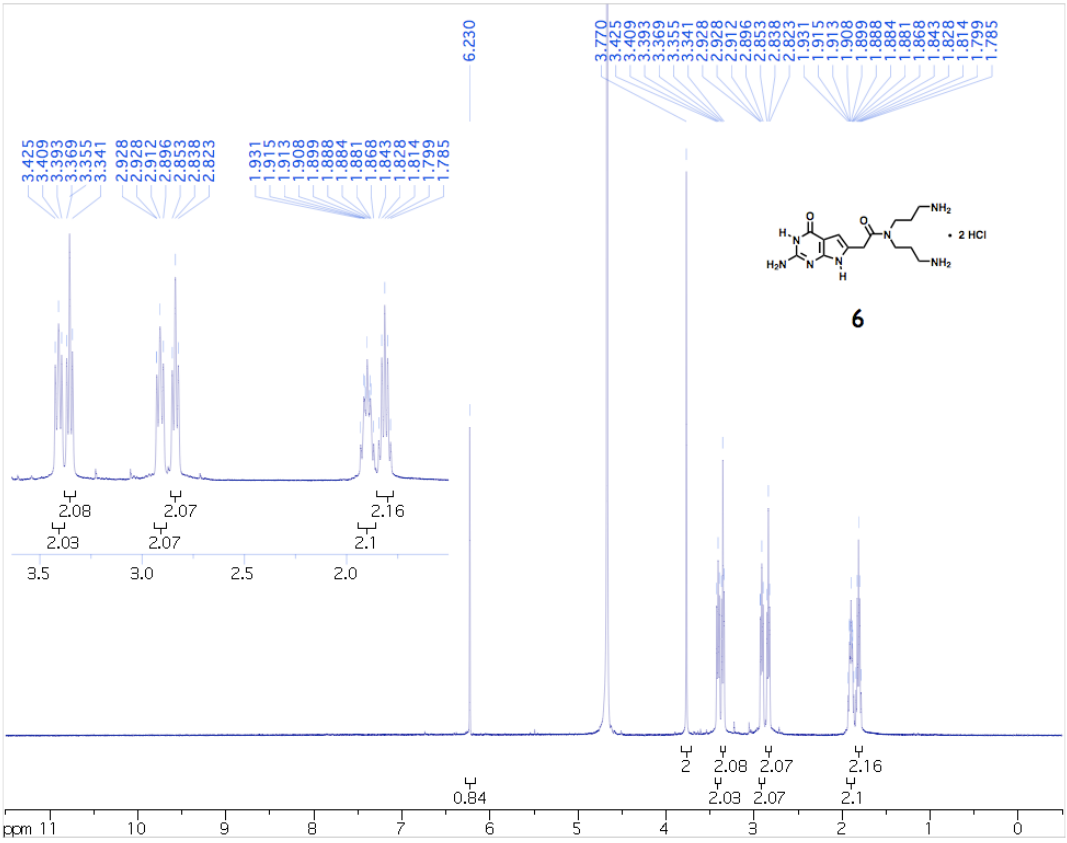
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2.16
2.03
2.07
2.1
3.5
3.0
2.5
2.0
2
2.08
2.07
2.16
0.84
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2.07
2.1
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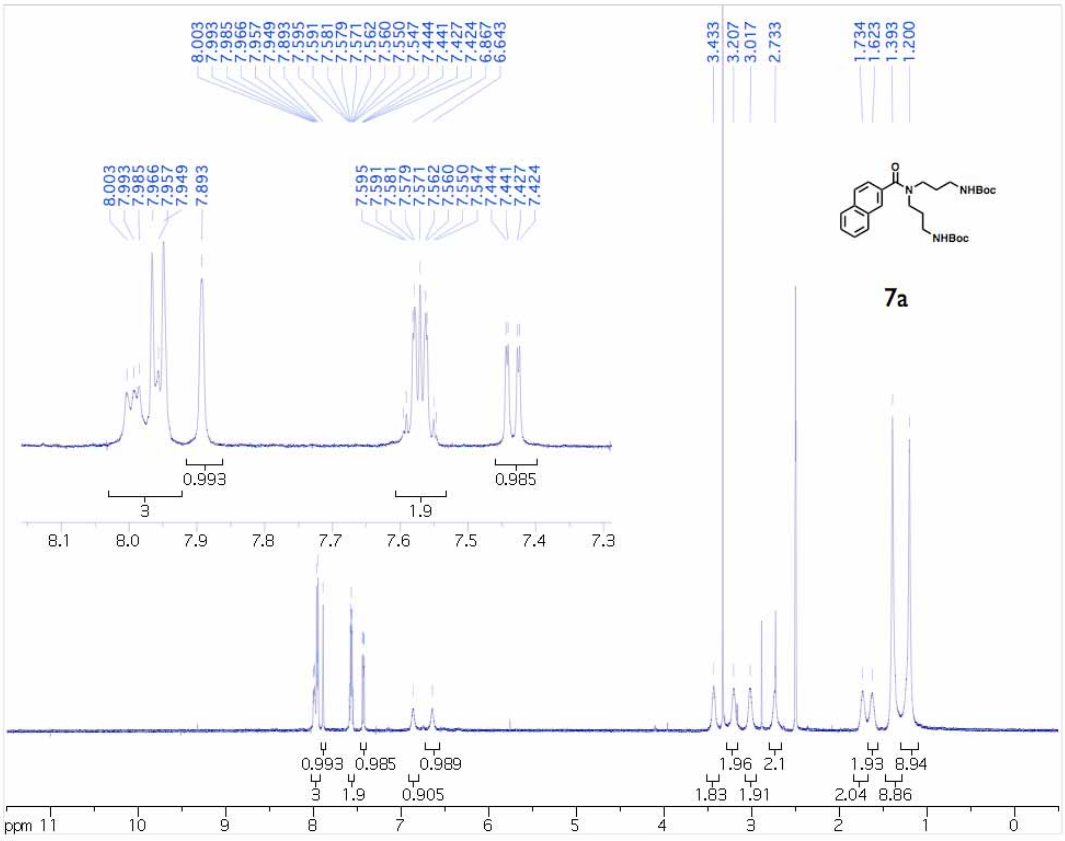
6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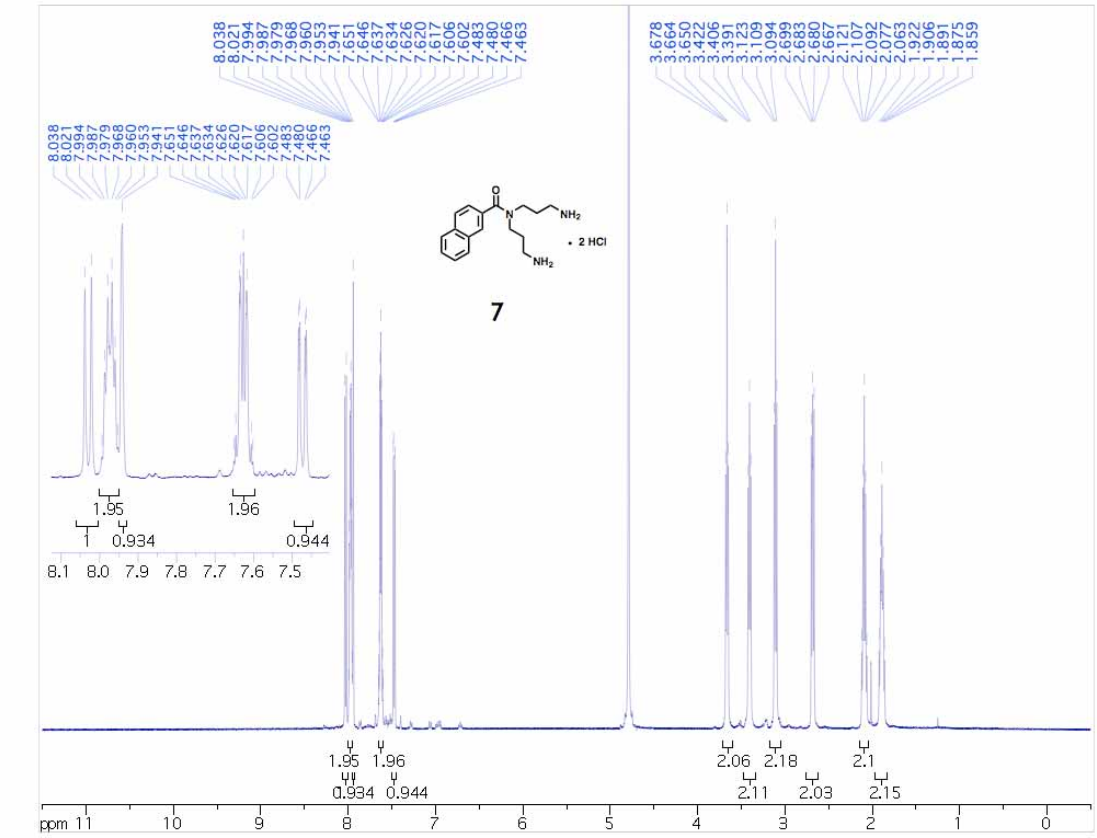
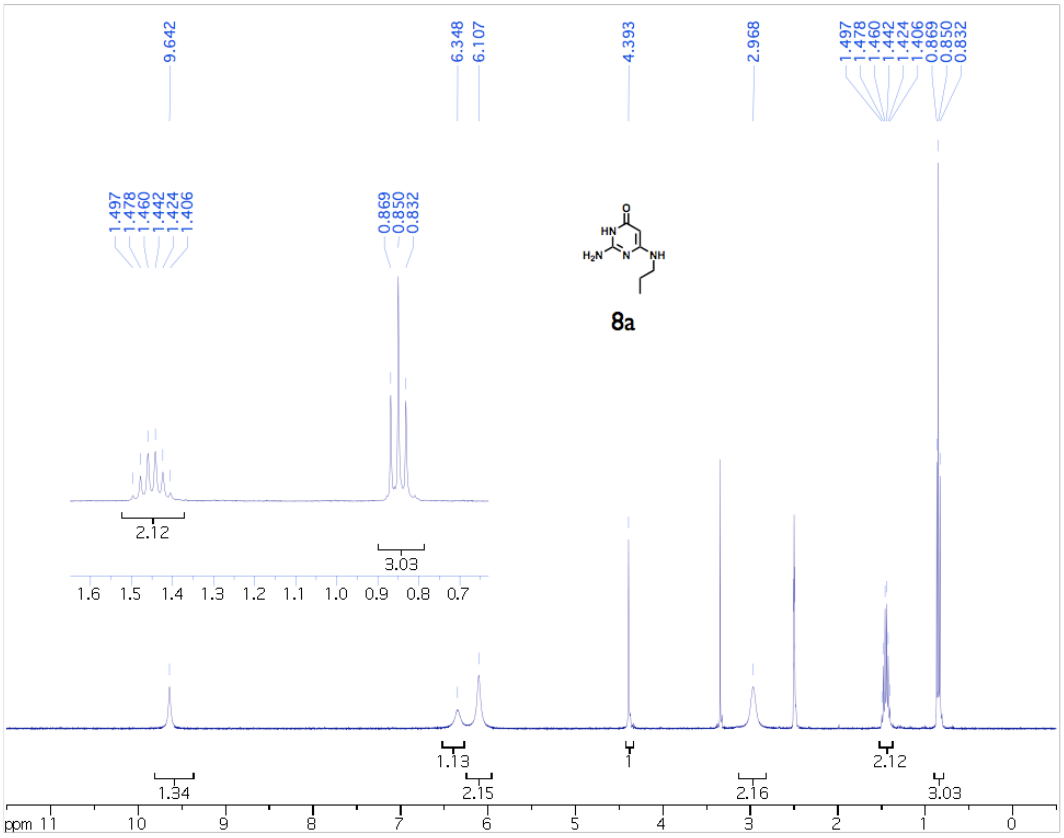
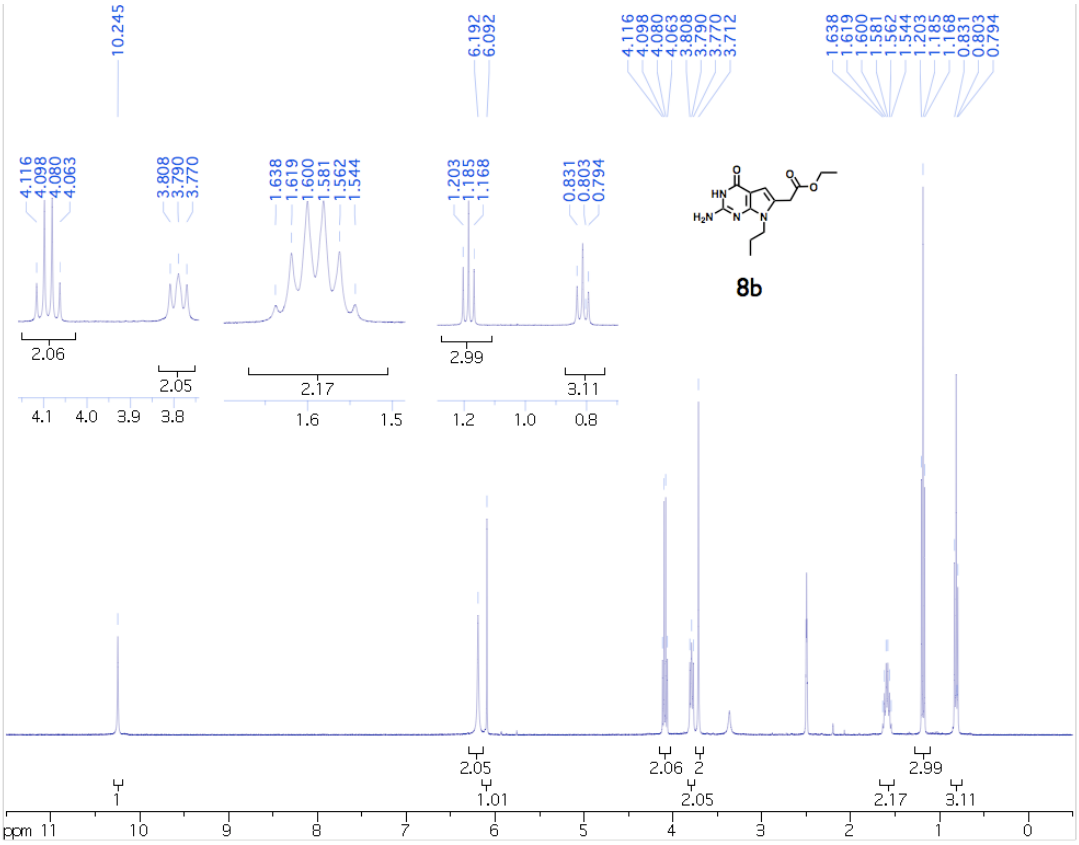
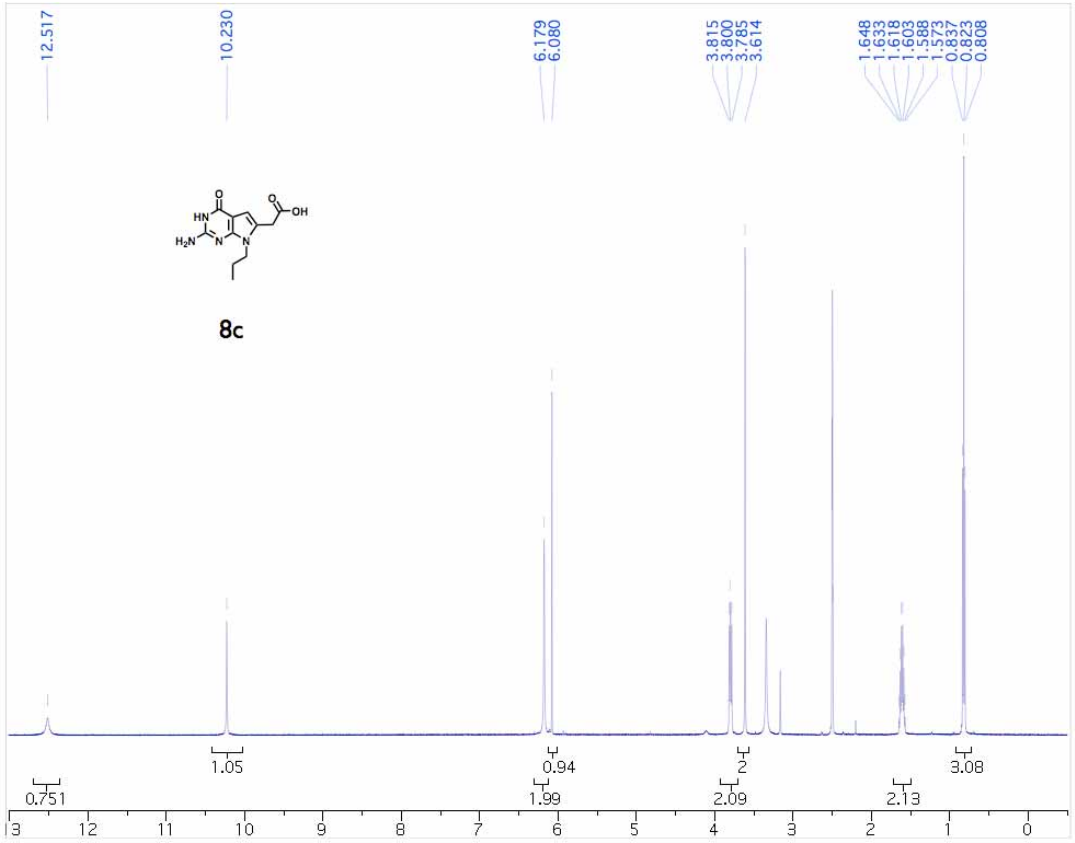
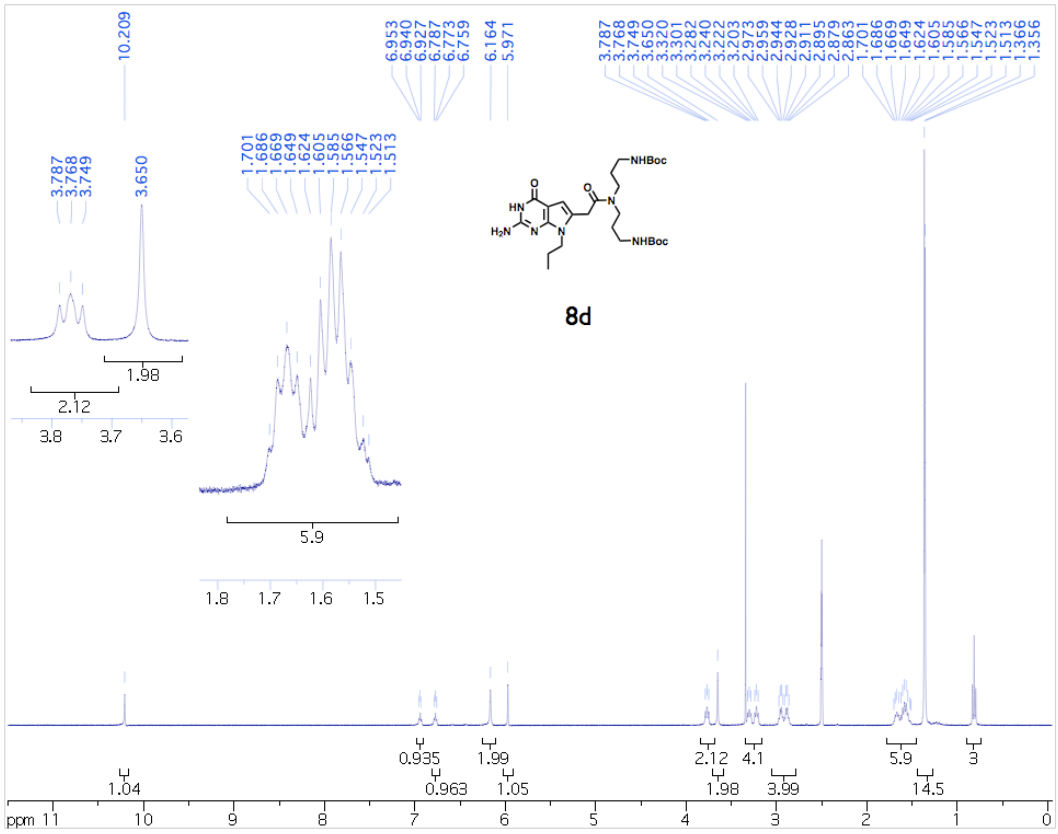
1.393
1.200
NHBoc
NHBoc
7a
0.993
0.985
3
1.9
0.993
0.985
0.989
3
1.9
0.905
1.96
2.1
1.83
1.91
1.93
8.94
2.04
8.86
ppm

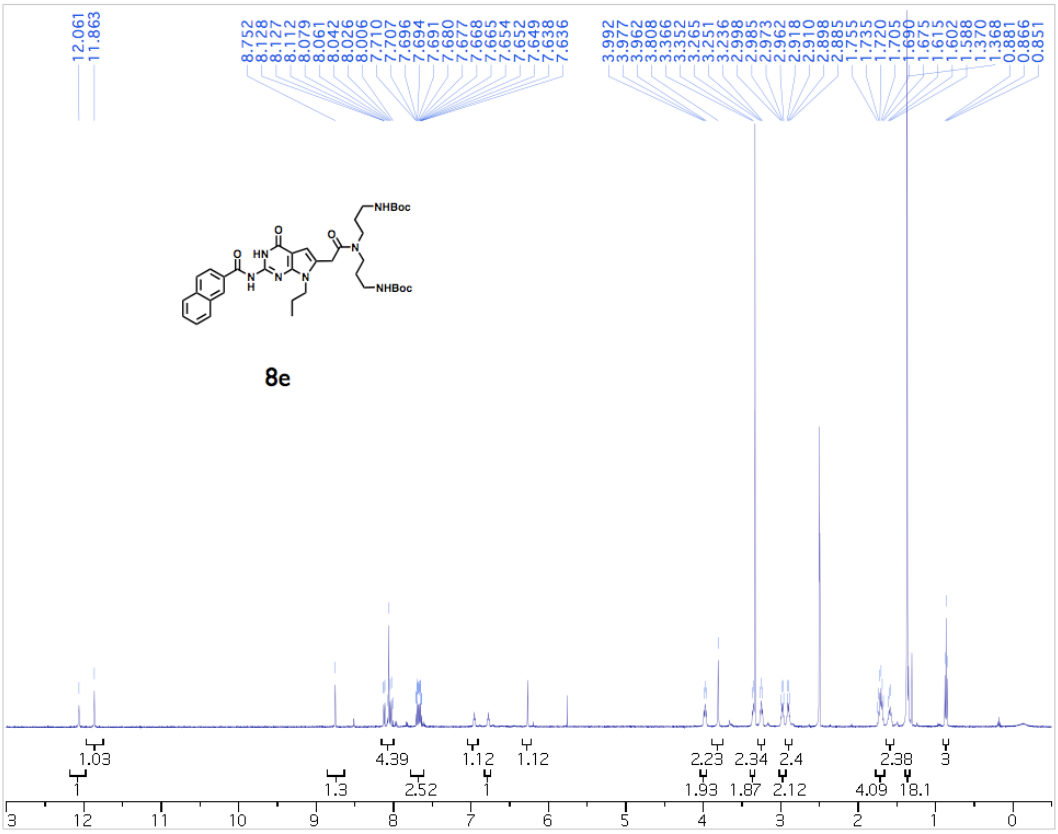

8.038
8.021
7.994
7.987
7.979
7.968
7.960
7.953
7.941
7.651
7.646
7.637
7.634
7.626
7.620
7.617
7.606
7.602
7.483
7.480
7.466
7.463
3.678
3.664
3.650
3.422
3.406
3.391
3.123
3.109
3.094
2.699
2.683
2.680
2.667
2.121
2.107
2.092
2.077
2.053
1.922
1.906
1.891
1.875
1.859
NH2
• 2 HCl
NH2
7
1.95
1.96
1
0.934
0.944
8.1 8.0 7.9 7.8 7.7 7.6 7.5
1.95
1.96
0.934
0.944
2.06
2.18
2.1
2.11
2.03
2.15
ppm 11 10 9 8 7 6 5 4 3 2 1 0

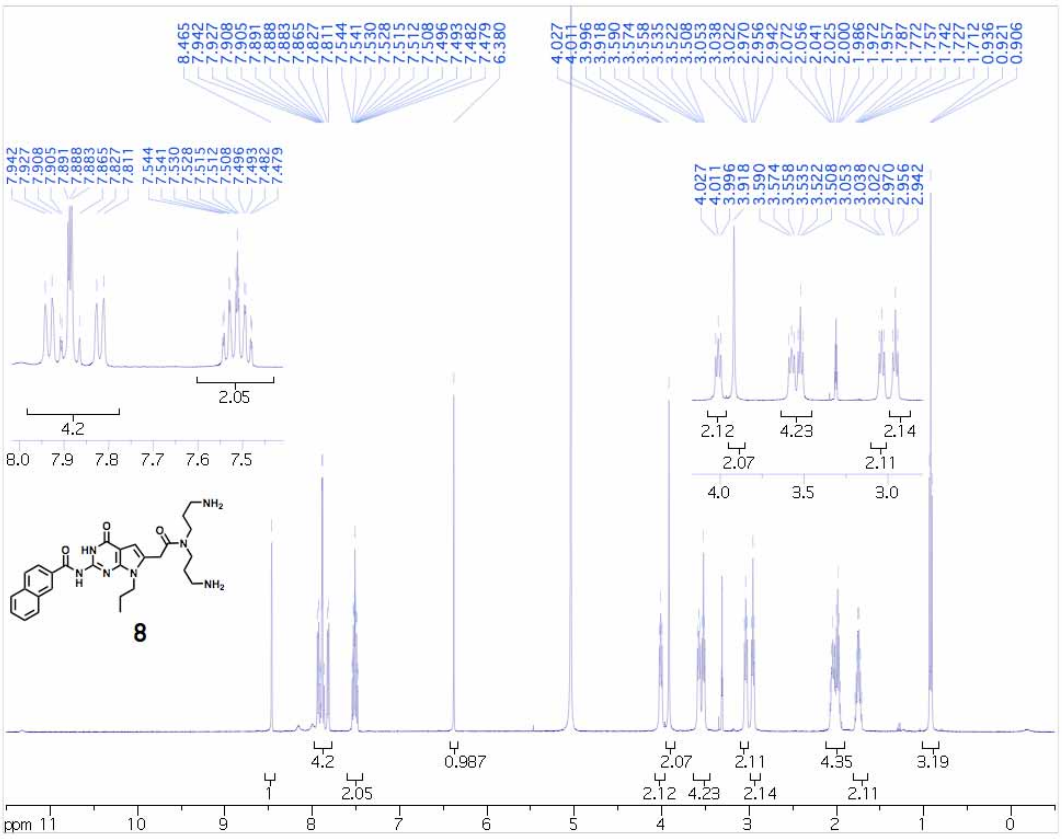

9.642
6.348
6.107
4.393
2.968
1.497
1.478
1.460
1.442
1.424
1.406
0.869
0.850
0.832
8a
2.12
3.03
1.34
1.13
2.15
1
2.16
2.12
3.03
ppm

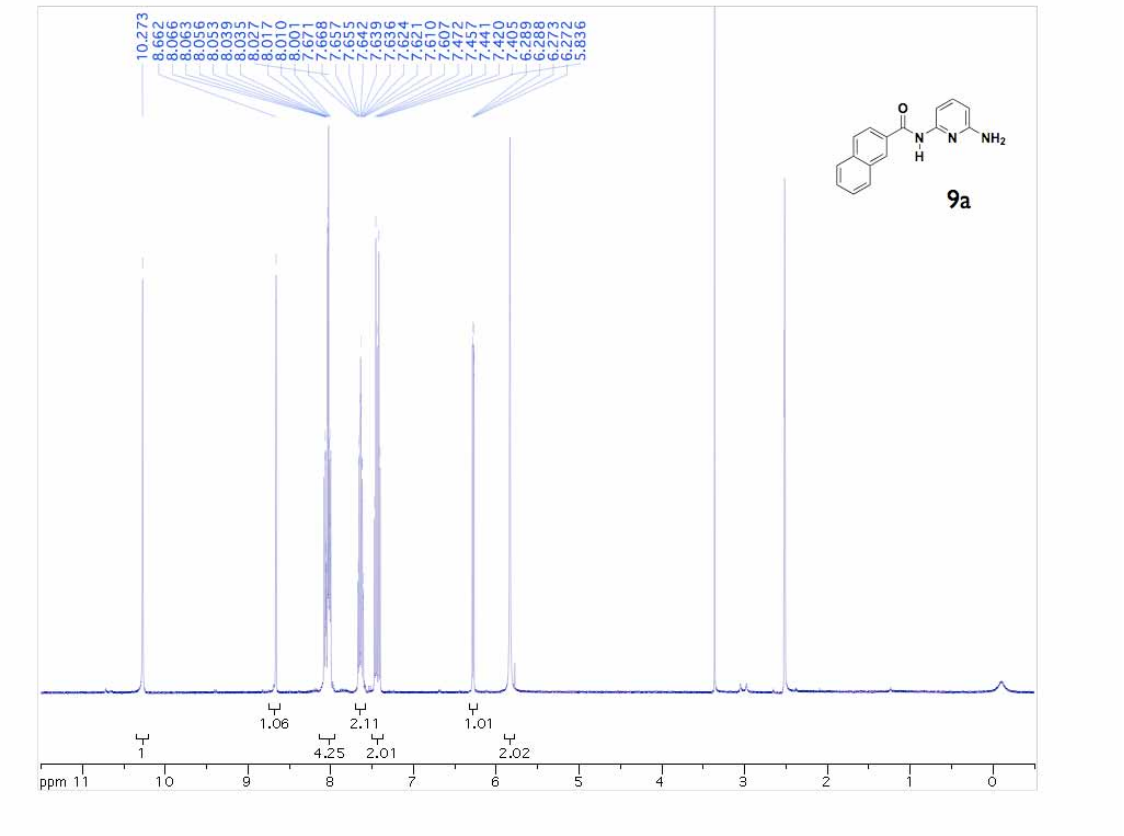

10.245
6.192
6.092
4.116
4.098
4.080
4.063
3.808
3.790
3.770
3.712
1.638
1.619
1.600
1.581
1.562
1.544
1.203
1.185
1.168
0.831
0.803
0.794
8b
2.06
2.05
2.17
2.99
3.11
2.05
2.06
2
1.01
2.05
2.99
2.17
3.11
ppm

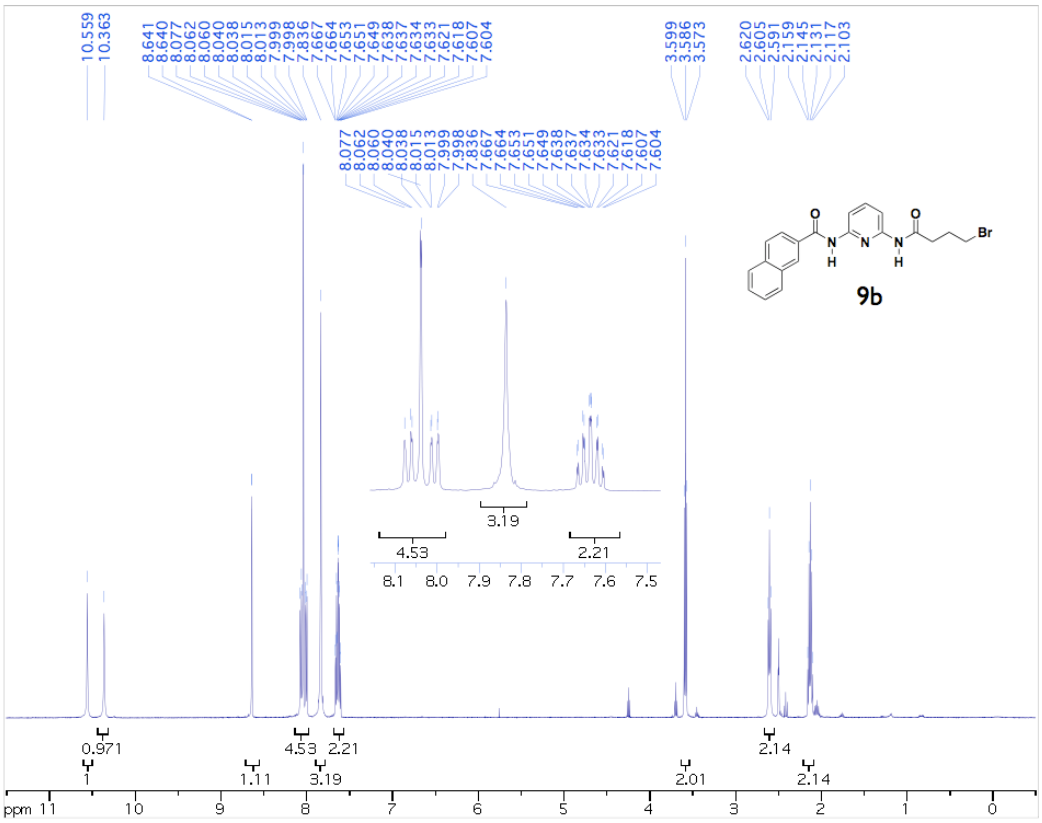

12.517
10.230
6.179
6.080
3.815
3.800
3.785
3.614
1.648
1.633
1.618
1.603
1.588
1.573
0.837
0.823
0.808
HN
O
OH
H_2N
N
N
8c
1.05
0.94
2
3.08
0.751
1.99
2.09
2.13
13
12
11
10
9
8
7
6
5
4
3
2
1
0

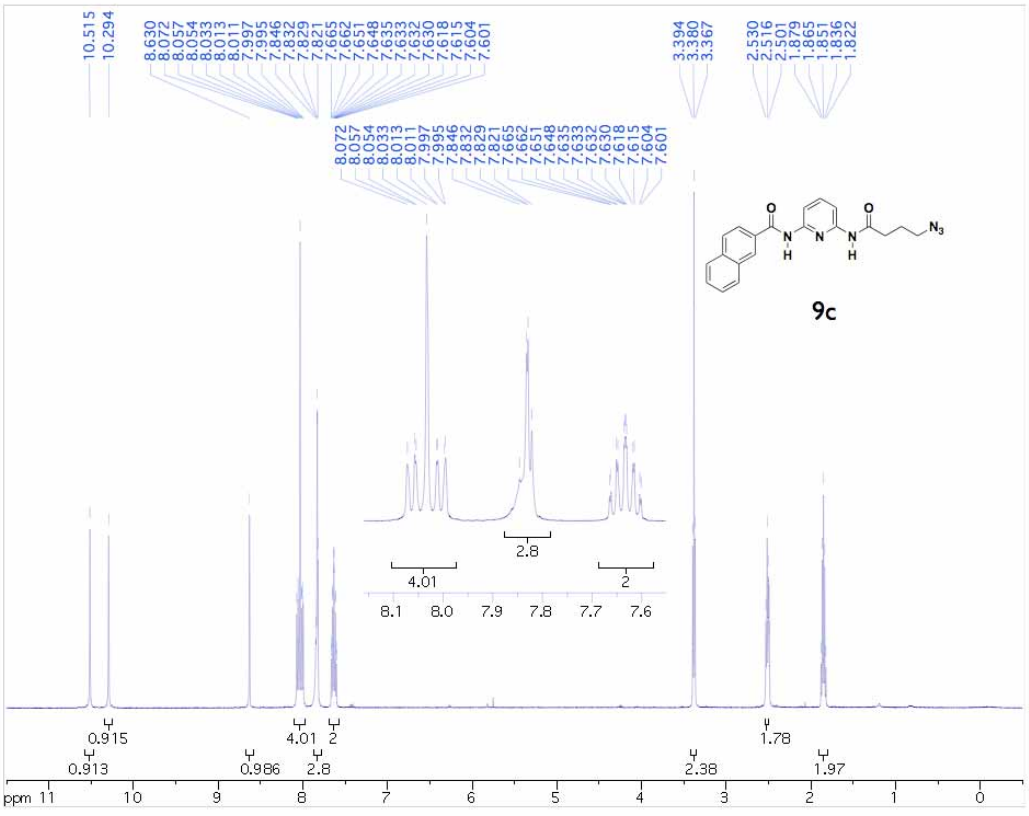

10.209
6.953
6.940
6.927
6.787
6.773
6.759
6.164
5.971
3.787
3.768
3.749
3.650
3.320
3.301
3.282
3.242
3.222
3.203
3.073
2.959
2.944
2.928
2.911
2.895
2.879
2.863
1.701
1.686
1.669
1.649
1.624
1.605
1.585
1.566
1.547
1.523
1.513
1.366
1.356
NHBoc
NHBoc
8d
1.98
2.12
5.9
0.935
1.99
2.12
4.1
5.9
3
1.04
0.963
1.05
1.98
3.99
14.5
ppm

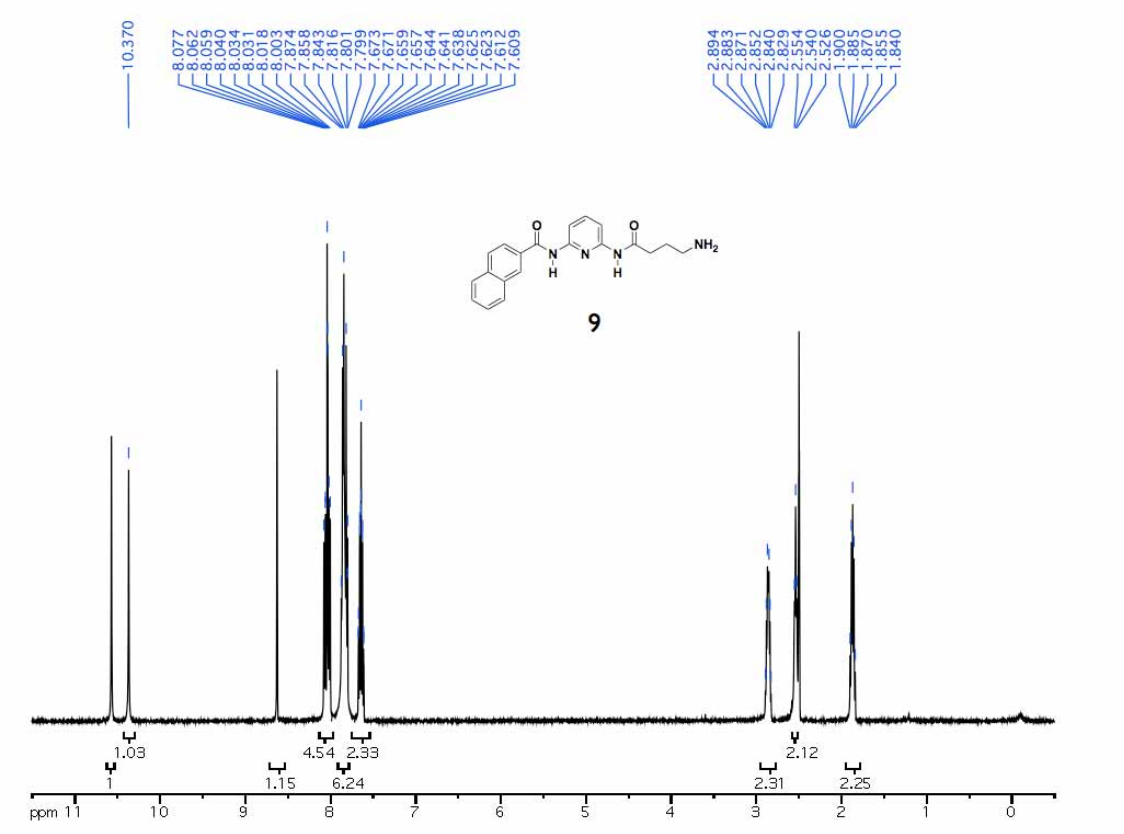

12.061
11.863
8.752
8.128
8.127
8.112
8.079
8.061
8.042
8.026
8.006
7.710
7.707
7.696
7.694
7.691
7.680
7.677
7.668
7.665
7.654
7.652
7.649
7.638
7.636
3.992
3.977
3.962
3.808
3.366
3.352
3.265
3.251
3.236
2.998
2.985
2.973
2.962
2.918
2.910
2.898
2.885
1.755
1.735
1.720
1.705
1.690
1.675
1.615
1.602
1.588
1.370
1.368
0.881
0.866
0.851
NHBoc
NHBoc
8e
1.03
1
1.3
4.39
2.52
1.12
1
1.12
2.23
1.93
2.34
1.87
2.4
2.12
2.38
4.09
3
18.1
3
12
11
10
9
8
7
6
5
4
3
2
1
0


8.465
7.942
7.927
7.908
7.905
7.891
7.888
7.883
7.865
7.827
7.811
7.544
7.541
7.530
7.528
7.515
7.512
7.508
7.496
7.493
7.482
7.479
6.380
4.027
4.011
3.996
3.918
3.590
3.574
3.558
3.535
3.522
3.508
3.053
3.038
3.022
2.970
2.956
2.942
2.072
2.056
2.041
2.025
2.000
1.986
1.972
1.957
1.787
1.772
1.757
1.742
1.727
1.712
0.936
0.921
0.906
2.05
4.2
8.0 7.9 7.8 7.7 7.6 7.5
2.12
4.23
2.14
2.07
2.11
4.0 3.5 3.0
8
4.2
0.987
2.07
2.11
4.35
3.19
1
2.05
2.12 4.23 2.14
2.11
ppm 11 10 9 8 7 6 5 4 3 2 1 0

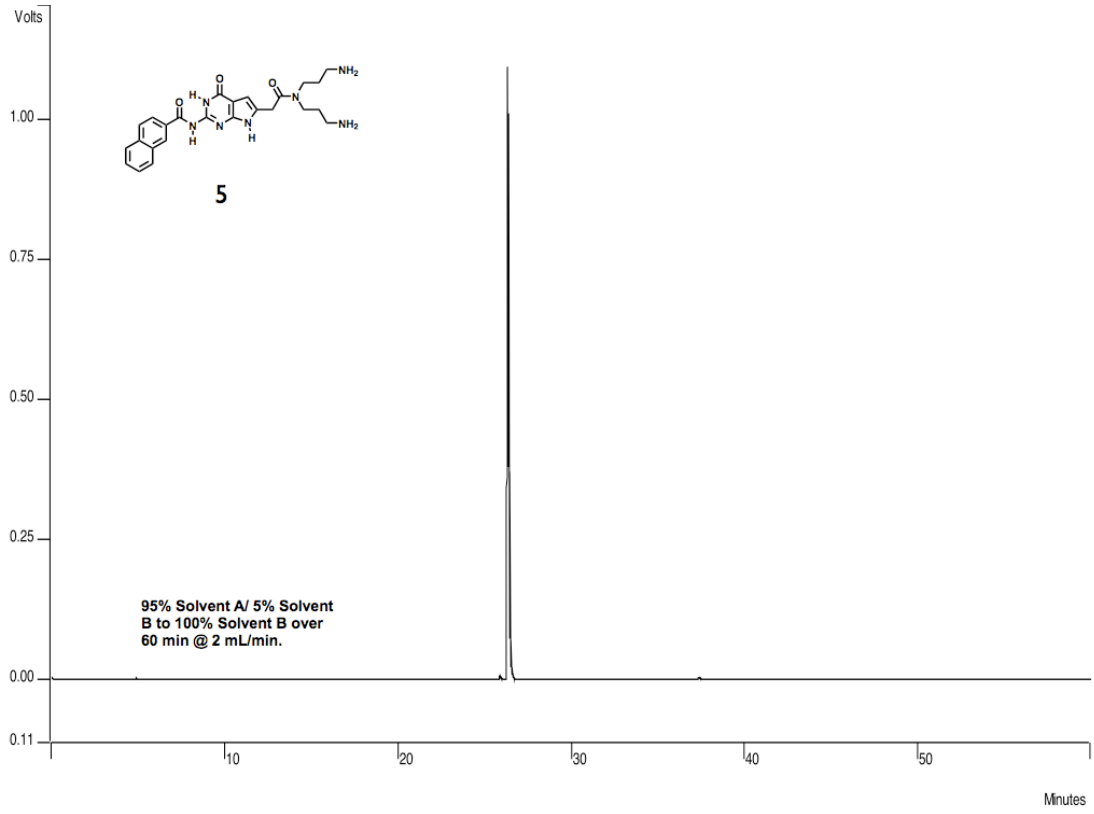

10.273
8.662
8.066
8.063
8.056
8.053
8.039
8.035
8.027
8.017
8.010
8.001
7.671
7.668
7.657
7.655
7.642
7.639
7.636
7.624
7.621
7.610
7.607
7.472
7.457
7.441
7.420
7.405
6.289
6.288
6.273
6.272
5.836
1.06
2.11
1.01
1
4.25
2.01
2.02
ppm 11
10
9
8
7
6
5
4
3
2
1
0
O
N
H
N
NH2
9a


10.559
10.363
8.641
8.640
8.077
8.062
8.060
8.040
8.038
8.015
8.013
7.999
7.998
7.836
7.667
7.664
7.653
7.651
7.649
7.638
7.637
7.634
7.633
7.621
7.618
7.607
7.604
3.599
3.586
3.573
2.620
2.605
2.591
2.159
2.145
2.131
2.117
2.103
8.077
8.062
8.060
8.040
8.038
8.015
8.013
7.999
7.998
7.836
7.667
7.664
7.653
7.651
7.649
7.638
7.637
7.634
7.633
7.621
7.618
7.607
7.604
O
N
H
N
N
H
O
Br
9b
3.19
4.53
2.21
8.1 8.0 7.9 7.8 7.7 7.6 7.5
0.971
1
1.11
4.53
2.21
3.19
2.01
2.14
2.14
ppm 11 10 9 8 7 6 5 4 3 2 1 0

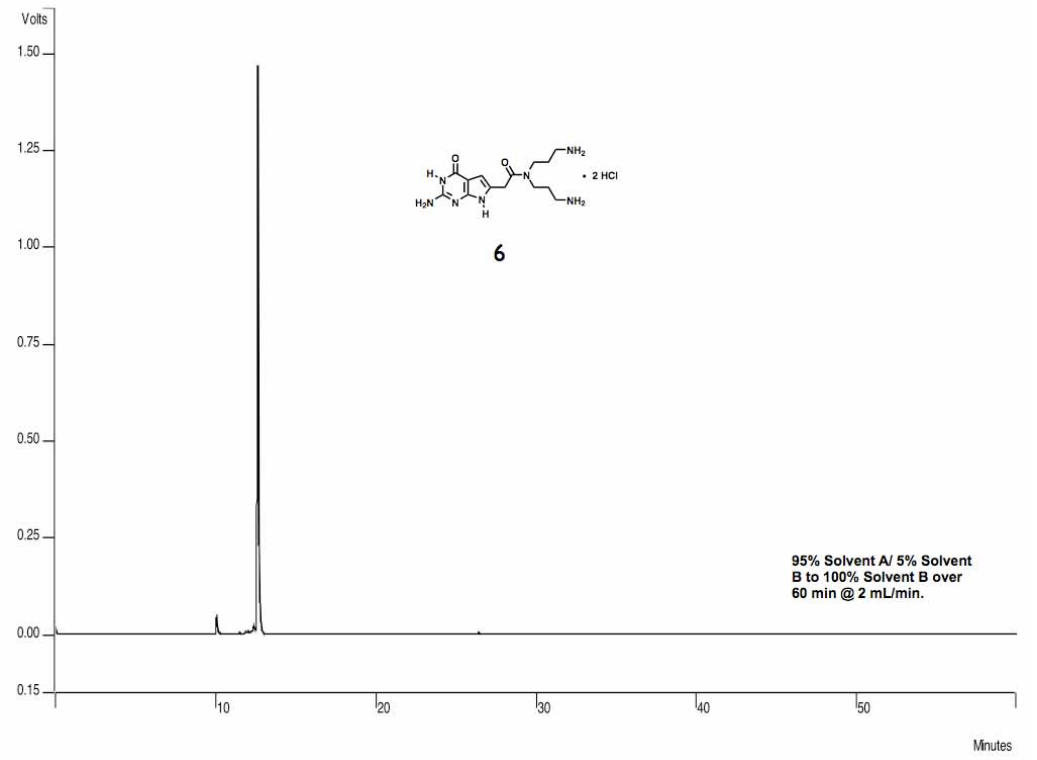

9c
2.8
4.01
2
0.915
0.913
4.01
2
0.986
2.8
2.38
1.78
1.97
ppm



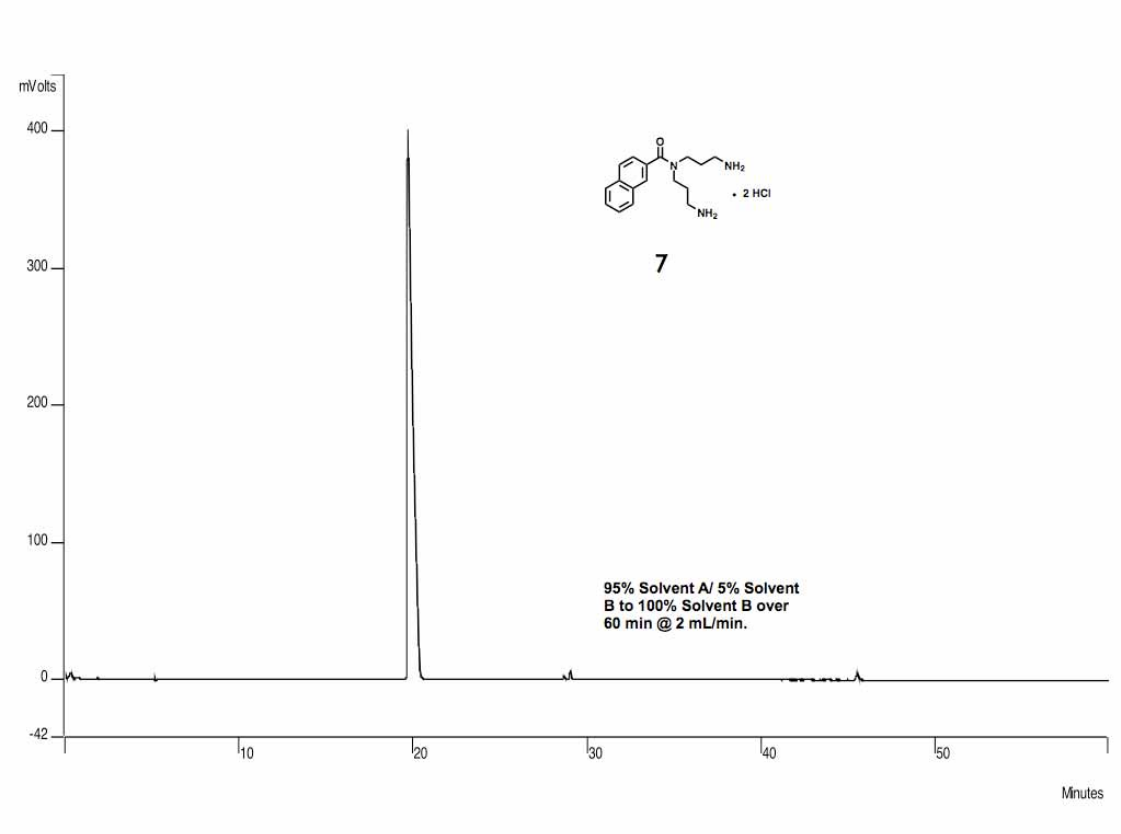
HPLC traces for final compounds:

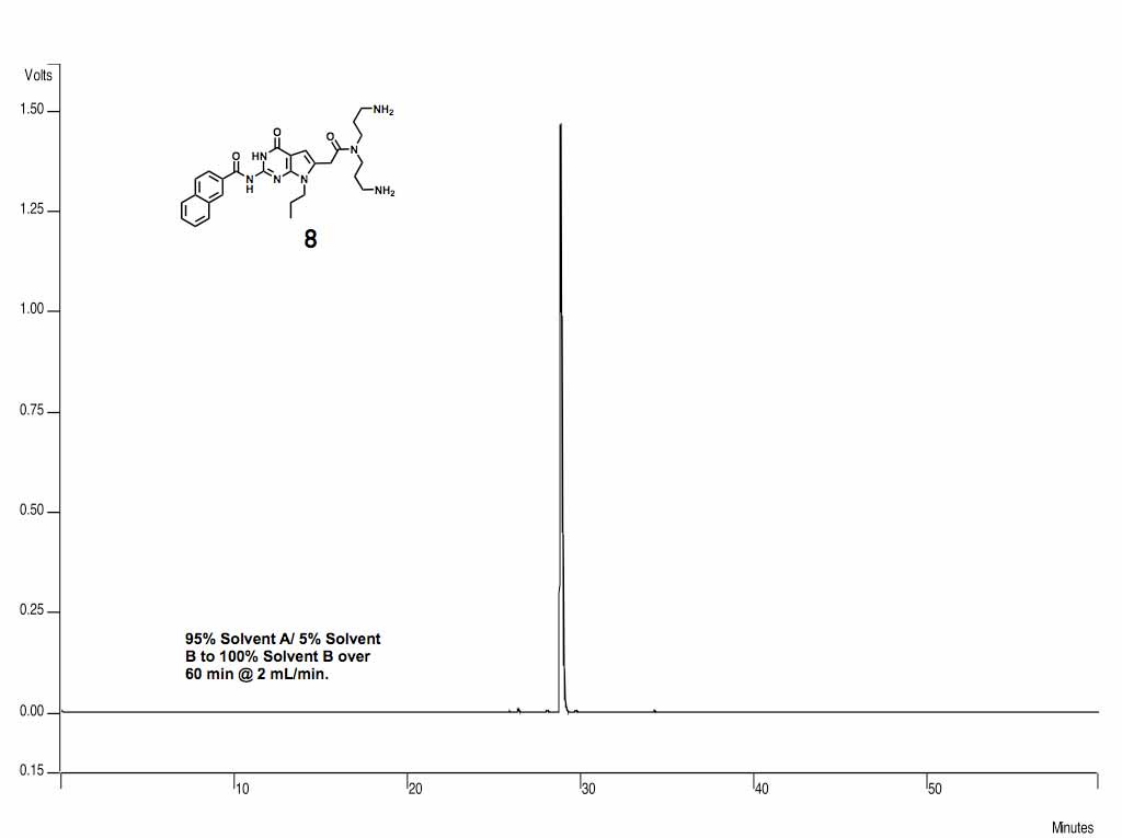

Volts
1.00
0.75
0.50
0.25
0.00
0.11
10
20
30
40
50
Minutes
95% Solvent A/ 5% Solvent
B to 100% Solvent B over
60 min @ 2 mL/min.

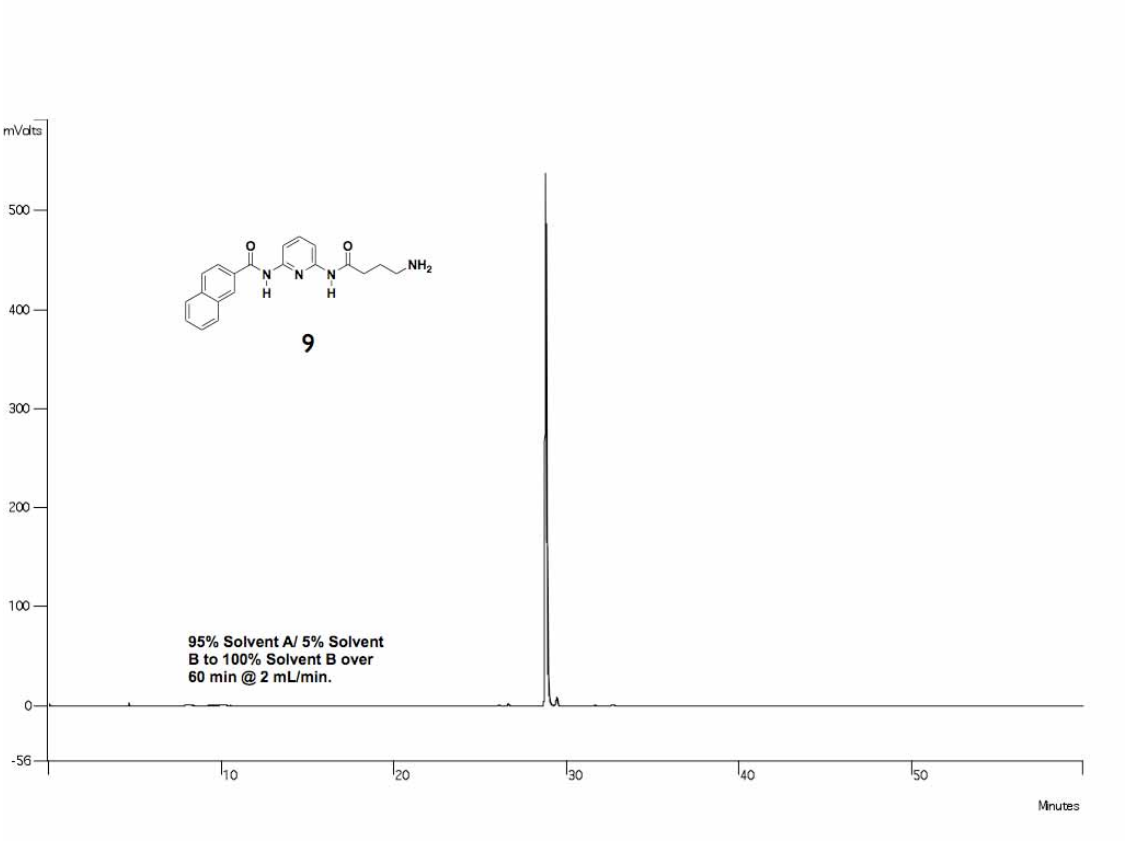
5


Volts
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.15
10
20
30
40
50
Minutes
95% Solvent A/ 5% Solvent
B to 100% Solvent B over
60 min @ 2 mL/min.

6


mVolts
400
300
200
100
0
-42
10
20
30
40
50
Minutes
NH2
• 2 HCl
NH2
7
95% Solvent A/ 5% Solvent
B to 100% Solvent B over
60 min @ 2 mL/min.


Volts
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.15
8
95% Solvent A/ 5% Solvent
B to 100% Solvent B over
60 min @ 2 mL/min.
10
20
30
40
50
Minutes



[i] Kato, T.; Kondo, G.; Kihara, H. *Chem. Lett.* **1997**, 1143–1144.

[ii] Connors, K. A. *Binding Constants: The Measurement of Molecular Complex Stability*; Wiley: New York, 1987.

[iii] Kaul, M; Pilch, D. S. *Biochemistry* **2002**, *41*, 7695–7706.