A Simple Ligand that Selectively Targets CUG Trinucleotide Repeats and Inhibits MBNL Protein Binding

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PHYSICAL SCIENCES: Chemistry

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Supporting Information

Materials:

Anhydrous solvents and starting materials were purchased by the Aldrich Company and used without further purification unless otherwise specified. Water was purified by a Millipore Direct Q-5 purification system. DNA oligomers were obtained from Integrated DNA Technologies purified by standard desalting. RNA was obtained from Dharmacon, Inc. Chemical reactions were performed under nitrogen. 6-Chloro-2-methoxy-9-phenoxyacridine[i] and 6-chloro-2-methoxy-9-aminopropyl-acridine[ii] were synthesized according to literature procedure.

High and low resolution 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. Circular dichroism was performed on a Jasco J-720 spectropolarimeter. 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. Analytical thin-layer chromatography (TLC) was performed on 0.2 mm silica 60 coated on aluminum plates with F254 indicator. Flash column chromatography was performed on 40-63 μm silica gel (SiO_2) mixed with Fluorescence Indicator green 254 nm in quartz columns to allow monitoring of fractions with a UV lamp. Solvent mixtures used for chromatography are reported as percent volume (v/v) or as volume ratio (v:v). The purity of all ligands was estimated to be >99% by ^{1}H NMR (see S25–S27) and by HPLC (see S28–S30). Instrumentation for UV-Vis absorption, fluorescence, and isothermal titration calorimetery are described below.

Synthesis:

***N*-(4-Amino-butyl)-[1,3,5]triazine-2,4,6-triamine** (**2a**). To 5.7 mL (69 mmol) of 1,3-diaminopropane in an oil bath at 130 °C was added 1.0 g (6.9 mmol) of 2,6-diamino-4-chloro-[1,3,5]triazine slowly over 30 minutes. The reaction was stirred for 1.5 h. and cooled to 80 °C. The excess diamine was removed *in vacuo* to produce a white solid. The solid was suspended in MeOH and conc. NH_4OH was added until the suspension became strongly alkaline. The suspension was then filtered and condensed. The resulting white solid was triturated in hot EtOH to produce 0.96 g (5.2 mmol, 76%) of a white solid: ^{1}H NMR (400 MHz; DMSO-d_6) δ 7.56 (s, 2H), 6.65 (t, J = 6.0, 1H), 6.16 (d, J = 95.3, 4H), 3.22 (q, J = 5.9, 2H), 2.71 (t, J = 6.8, 2H), 1.71 (quintet, J = 6.4 Hz, 2H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 166.8, 166.2, 36.7, 36.2, 28.8; *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 184.1311; found 184.1310.

***N*^{2}-(4-(6-Chloro-2-methoxyacridin-9-ylamino)propyl)-1,3,5-triazine-2,4,6-triamine** (**2**). To a refluxing suspension of 0.16 of **2a** (0.87 mmol) and 6-chloro-2-methoxy-9-phenoxyacridine (0.87 mmol) in 20 mL of freshly distilled MeCN was added 20 drops of TFA. The reaction was stirred overnight, allowing the product to form as a bright yellow precipitate. The suspension was cooled to ambient temperature, filtered, and washed with cold MeCN and Et_2O. Column chromatography with a solvent gradient from 10 to 20% MeOH in CH_2Cl_2 containing 2% NH_4OH provided 0.38 g (0.58 mmol, 71%) of the TFA salt after acidification: ^{1}H NMR (500 MHz; DMSO-d_6) (TFA free) δ 8.34 (d, J = 9.5, 1H), 7.84–7.80 (m, 2H), 7.65 (s, 1H), 7.44 (d, J = 8.1, 1H), 7.31 (d, J = 8.4, 1H), 6.60 (t, J = 6.0, 1H), 6.00 (d, J = 58.8, 4H), 3.91 (s, 3H), 3.81 (t, J = 7.2, 2H), 3.27 (q, J = 6.2, 2H), 1.92 (quin, J = 6.9, 2H); ^{13}C NMR (125 MHz; DMSO-d_6) δ 167.2, 166.4, 155.2, 151.3, 146.4, 143.5, 134.5, 127.10, 126.93, 124.8, 122.8, 122.5, 116.5, 113.7,

101.5, 55.8, 47.1, 37.3, 30.7; *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 425.1605; found 425.1594.

***N*-(8-Amino-octyl)-[1,3,5]triazine-2,4,6-triamine** (**3a**). To a melted suspension of 1.7 g (11.8 mmol) of 1,8-diaminooctane at 80 °C was added diaminochlorotriazine (0.344 g, 2.36 mmol) in one batch and heated to 130 °C overnight. The reaction was cooled to ambient temperature and dissolved in MeOH. Methanolic ammonia was added until basic and the solvent was removed *in vacuo* to produce a yellow orange solid. Column chromatography with a solvent gradient from 10 to 20% MeOH in CH_2Cl_2 containing 2% NH_4OH provided 503 mg (1.99 mmol; 84%) of **3a** as a white amorphous solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 6.43 (t, J = 5.6, 1H), 6.10 (br s, 2H), 5.93 (br s, 2H), 3.18 (q, 2H), 1.50–1.43 (m, 2H), 1.38–1.35 (m, 2H), 1.28 (br s, 8H); ^{13}C NMR (100 MHz, DMSO-d_6) 167.28, 166.93, 166.37, 41.52, 39.84, 32.94, 29.49, 29.09, 29.01, 26.54, 26.46; *m/z* (ESI) 254.2 (M^+); *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 254.2093; found 254.2095.

***N*-[8-(6-Chloro-2-methoxy-acridin-9-ylamino)-octyl]-[1,3,5]triazine-2,4,6-triamine** (**3**). To a refluxing suspension of 0.12 g (0.47 mmol) of **3a** and 0.16 g (0.47 mmol) of 2-methoxy-6-chloro-9-phenoxyacridine in 10 mL of freshly distilled MeCN was added 10 drops of TFA and left overnight. The mixture was cooled to ambient temperature and methanolic ammonia was added until strongly alkaline. The solvent was removed *in vacuo* to produce a yellow oily film. Column chromatography with a solvent gradient from 10 to 20% MeOH in CH_2Cl_2 containing 2% NH_4OH provided 0.17 g (0.335 mmol; 72%) of **3** as an orange oily foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 9.6, 1H), 7.90–7.85 (m, 2H), 7.67 (d, J = 2.4, 1H), 7.47–7.35 (m, 2H), 7.08 (br s, 1H), 6.41 (t, J = 6.0, 1H), 6.10 (br s, 1H), 5.93 (br s, 1H), 3.96 (s, 3H), 3.78 (t, J = 6.4, 2H), 3.14 (q, J = 6.8, 2H), 1.78–1.71 (m, 2H), 1.44–1.15 (m, 10H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.25, 166.91, 166.34, 155.08, 150.87, 147.42, 144.87, 133.84, 129.74, 126.74, 126.15, 124.29, 122.54, 116.84, 114.21, 101.02, 55.69, 49.41, 45.48, 30.46, 29.42, 28.45, 28.77, 26.39, 26.31; *m/z* (FD) m/z 495.3 (M+); *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 495.2388; found 495.2379.

***N*-(12-Amino-dodecyl)-[1,3,5]triazine-2,4,6-triamine** (**4a**). To a melted suspension of 1.21 g (6.03 mmol) of 1,12-diaminododecane at 90 °C was added 0.18 g (1.21 mmol) of diaminochlorotriazine in one batch and heated to 110 °C overnight. The reaction was cooled to ambient temperature and dissolved in MeOH. Methanolic ammonia was added until basic and

the solvent was removed *in vacuo* to produce a yellow orange solid. Column chromatography with a solvent gradient from 10 to 20% MeOH in CH_2Cl_2 containing 2% NH_4OH provided 0.18 g (0.59 mmol; 49%) of **4a** as a white amorphous solid: ^{1}H NMR (500 MHz; DMSO-d_6) δ 6.38 (t, *J* = 5.8, 1H), 5.96 (d, *J* = 87.6, 4H), 3.13 (t, *J* = 6.7, 2H), 1.42 (dt, *J* = 13.0, 6.5, 2H), 1.32 (dt, *J* = 13.1, 6.8, 2H), 1.24 (s, 18H); ^{13}C NMR (125 MHz; DMSO-d_6): δ 167.2, 166.9, 166.3, 41.6, 33.2, 29.5, 29.20, 29.15, 29.03, 26.59, 26.54; *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 310.2719; found 310.2717.

***N*-[12-(6-Chloro-2-methoxy-acridin-9-ylamino)-dodecyl]-[1,3,5]triazine-2,4,6-triamine** (**4**). A suspension containing 0.15 g (0.49 mmol) of **4a** and 0.18 g (0.52 mmol) of 2-methoxy-6-chloro-9-phenoxyacridine in 10 mL of freshly distilled MeCN and 10 drops of TFA was heated to 90 °C for 4 h. The reaction was then cooled to ambient temperature and methanolic ammonia was added until basic. The solvent was removed *in vacuo* to produce a yellow oily film. Column chromatography with a solvent gradient from 10 to 20% MeOH in CH_2Cl_2 containing 2% NH_4OH provided 0.21 g (0.37 mmol; 72%) of **4** as an orange oily foam: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, *J* = 8.0, 1H), 7.91–7.86 (m, 2H), 7.65 (d, *J* = 2.4, 1H), 7.46–7.34 (m, 2H), 6.94 (t, *J* = 5.2, 1H), 6.43 (t, *J* = 6.0, 1H), 6.11 (br s, 2H), 5.93 (br s, 2H), 3.96 (s, 3H) 3.79–3.74 (m, 2H), 3.20–3.15 (m, 2H) 1.76–1.68 (m, 2H), 1.48–1.41 (m, 2H), 1.33–1.17 (m, 18H); ^{13}C NMR (125 MHz; CD_3OD): δ 161.7, 158.26, 158.17, 157.3, 141.8, 141.3, 129.5, 128.7, 125.2, 121.5, 118.6, 115.3, 111.2, 104.1, 57.2, 50.5, 50.04, 50.00, 42.1, 31.0, 30.7, 30.52, 30.44, 30.36, 28.0; *m/z* (FD) m/z 551.3; *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 551.3014; found 551.3026.

***N*^{1}-(Pyridin-2-yl)butane-1,4-diamine** (**5a**). To 5.0 mL (50 mmol) of 1,4-Diaminobutane was heated in an oil bath at 130 °C was added 2-chloropyridine 1.0 mL (11 mmol) slowly over 30 minutes. The mixture was stirred for 24 h and cooled to 80 °C. The excess diamine was removed *in vacuo* to produce a thick yellow oil. Column chromatography with a solvent gradient from 10 to 20% MeOH in CH_2Cl_2 containing 2% NH_4OH provided 1.55 g (9.39 mmol, 89%) of a light yellow oil: ^{1}H NMR (400 MHz; $CDCl_3$) δ 7.93 (ddd, *J* = 5.0, 1.8, 0.7, 1H), 7.25 (ddd, *J* = 8.6, 7.0, 1.8, 1H), 6.40 (ddd, *J* = 7.1, 5.1, 0.8, 1H), 6.23 (d, *J* = 8.4, 1H), 4.94 (s, 1H), 3.13 (t, *J* = 6.8, 2H), 2.58 (t, *J* = 6.9, 2H), 1.65 (s, 2H), 1.54–1.46 (m, 2H), 1.44–1.36 (m, 2H); ^{13}C NMR (100 MHz; $CDCl_3$) δ 159.1, 148.4, 137.6, 112.4, 106.8, 42.09, 41.96, 31.2, 27.0; *m/z* HRMS (ESI) calcd for $[M+H]^+$: 166.1344; found 166.1345.

N^1-(6-Chloro-2-methoxyacridin-9-yl)-N^4-(pyridin-2-yl)butane-1,4-diamine (**5**). To a refluxing suspension of 0.55 g of **5a** (3.3 mmol) and 1.2 g (3.3 mmol) of 6-chloro-2-methoxy-9-phenoxyacridine in 30 mL of freshly distilled MeCN was added 30 drops of TFA. The reaction was stirred overnight, allowing the product to form as a bright yellow precipitate. The suspension was cooled to ambient temperature, filtered, and washed with cold MeCN and Et_2O. Column chromatography with a solvent gradient from 0 to 10% MeOH in CH_2Cl_2 provided 1.36 g (3.3 mmol, quantitative yield) as a bright yellow amorphous solid: ^{1}H NMR (400 MHz; DMSO-d_6) δ 13.97 (s, 2H), 9.69 (t, *J* = 5.0, 1H), 8.93 (s, 1H), 8.50 (d, *J* = 9.8, 1H), 7.91–7.81 (m, 5H), 7.68 (dd, *J* = 9.3, 2.4, 1H), 7.49 (dd, *J* = 9.1, 1.5, 1H), 6.98 (d, *J* = 9.0, 1H), 6.81 (t, *J* = 6.6, 1H), 4.14 (q, *J* = 6.2, 2H), 3.93 (s, 3H), 3.35 (t, *J* = 6.3, 2H), 1.98 (dt, *J* = 14.2, 7.1, 2H), 1.70 (dt, *J* = 14.5, 7.0, 2H); ^{13}C NMR (100 MHz; DMSO-d_6) δ 155.80, 155.69, 152.9, 142.9, 139.9, 138.9, 136.0, 134.3, 128.5, 127.0, 123.0, 120.5, 117.5, 113.8, 112.9, 111.9, 109.8, 103.2, 55.9, 48.2, 41.1, 26.3, 25.1; *m/z* HRMS (ESI) calcd. for $[M+H]^+$: 407.1639; found 407.1638.

Modeling

Minimized MMFFx complexes of ligand **1** binding duplex **A** through the major and minor grooves. Modeling was performed by MOE and high resolution rendering by VMD. While modeling predicts binding through either major or minor grooves, 9-aminoacridine derivatives display a preferred binding orientation in which the 9-amino group lies within the minor groove. Listed below are examples of preferred acridine binding:

(1) Adams, A.; Guss, J. M.; Collyer, C. A.; Denny, W. A.; Prakash, A. S.; Wakelin, L. P. G. *Mol. Pharmacol.* **2000**, *58*, 649–658.

(2) Todd, A. K.; Adams, A.; Thorpe, J. H.; Denny, W. A.; Wakelin, L. P. G.; Cardin, C. J. *J. Med. Chem.*, **1999**, *42*, 536–540.

(3) Adams, A.; Guss J. M.; Collyer, C. A.; Denny, W. A.; Wakelin, L. P. G. *Nucleic Acids Res.* **2000**, *28*, 4244–4253.

(4) Malinina, L.; Soler-Lopez, M.; Aymami, J.; Subirana, J. A. *Biochemistry*, **2002**, *41*, 9341–9348.

(5) Hopcroft, N, H.; Brogden, A. L.; Searcey, M.; Cardin, C. J. *Nucleic Acids Res.* **2006**, *34*, 6663–6672.

(6) Fan, J.; Ohms, S. J.; Boyd, M.; Denny, W. A. *Chem. Res. Toxicol.* **1999**, *12*, 1166–1172.

(7) Rojsitthisak, P.; Romero, R. M.; Haworth, I. S. *Nucleic Acids Res.* **2001**, *29*, 4716–4723.

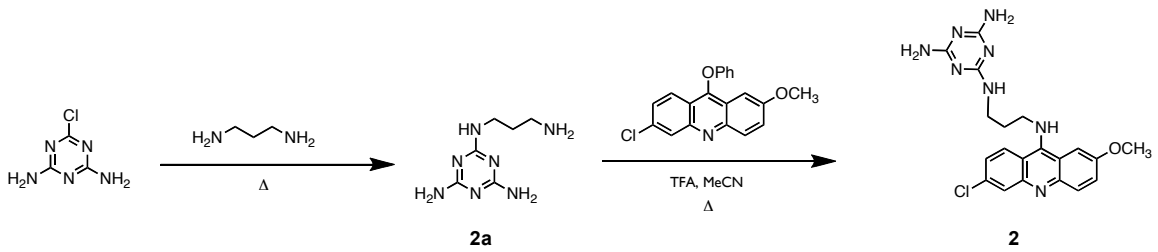


Figure S1. Synthesis of ligand 2.

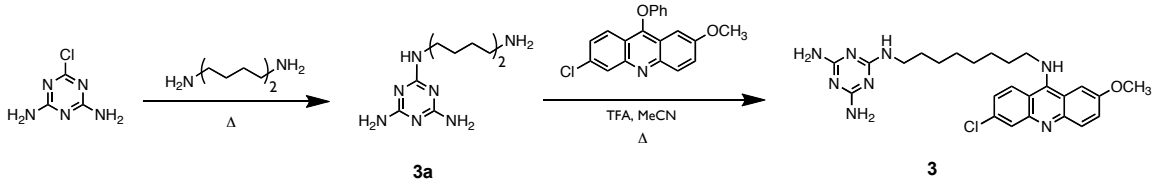


Figure S2. Synthesis of ligand 3.

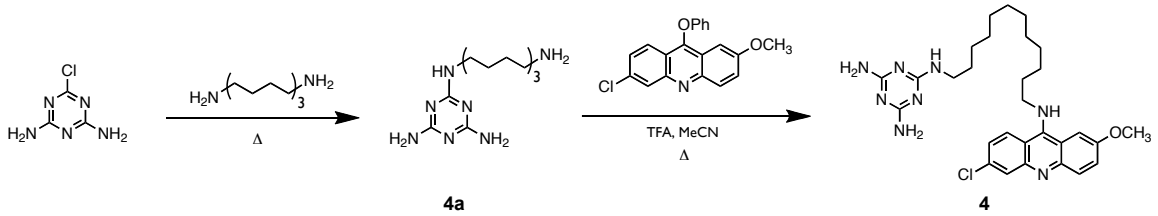


Figure S3. Synthesis of ligand 4.

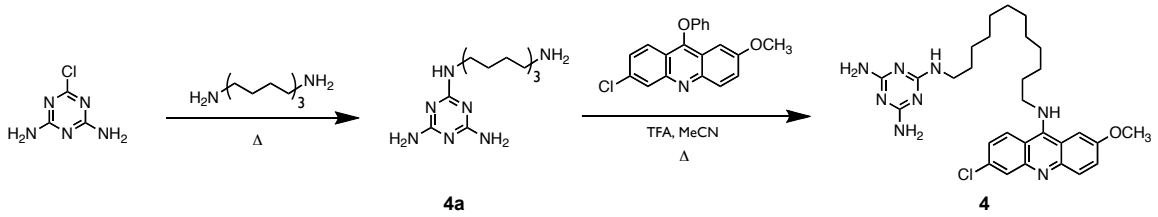


Figure S3. Synthesis of ligand 4.

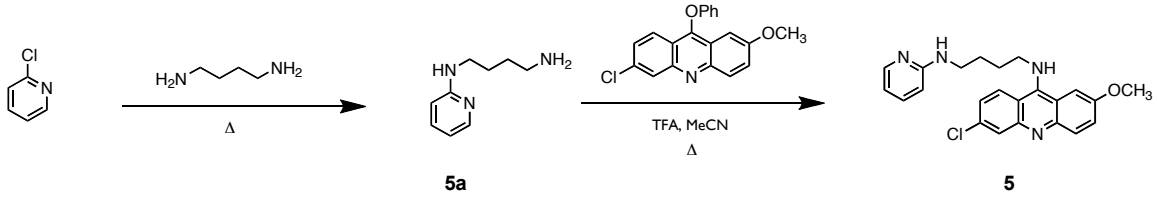


Figure S4. Synthesis of ligand 5.

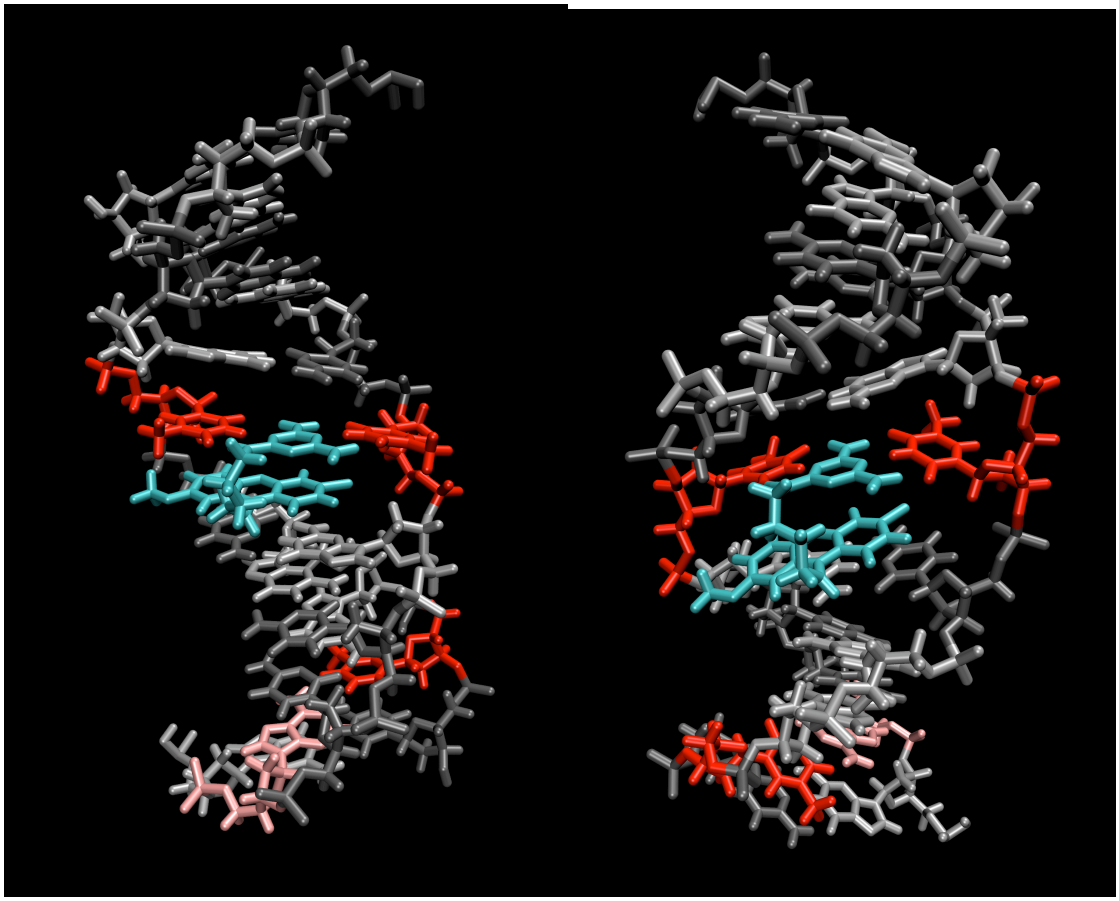
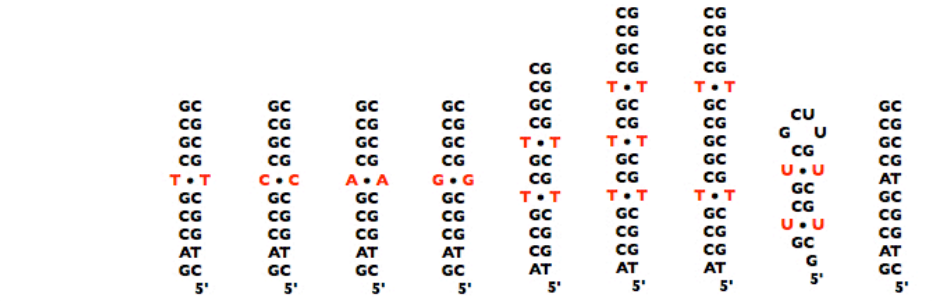


Figure S5. Molecular Modeling of ligand **1** bound to duplex **A** through the major (left) and minor (right) grooves. The modeling was meant only to illustrate the manner in which ligand **1** might fit into the mismatch site as a "stacked intercalator." For example, in the actual complex the acridine nucleus is likely to be protonated.



	A	B	C	D	E	F	I	G	H
Type	DNA	DNA	DNA	DNA	DNA	DNA	DNA	RNA	DNA
T_m (°C)	52	45	NA	54	52	54.5	66.4	57	64
Eq. Lig. **1**	1	1	1	1	2	3	2	2	1
T_m (°C) w/ Ligand	55.1	51	NA	53.6	56.5	61.5	69.5	58	64
ΔT_m (°C)	+3.1	+6.0	-	-0.4	+4.5	+7.0	+3.1	+1.0	0.0

Duplex C did not provide a sigmoidal transition. B-like helix was observed by circular dichroism. Duplex formation was also evident by gel shift assays.

All studies: 12 μM Duplex, 20 mM MOPS pH 7.0, 300 mM NaCl, 1mM EDTA, 1-3 eq. Ligand

Figure S6. Summary table of DNA and RNA melting temperatures in the absence and presence of ligand **1**.

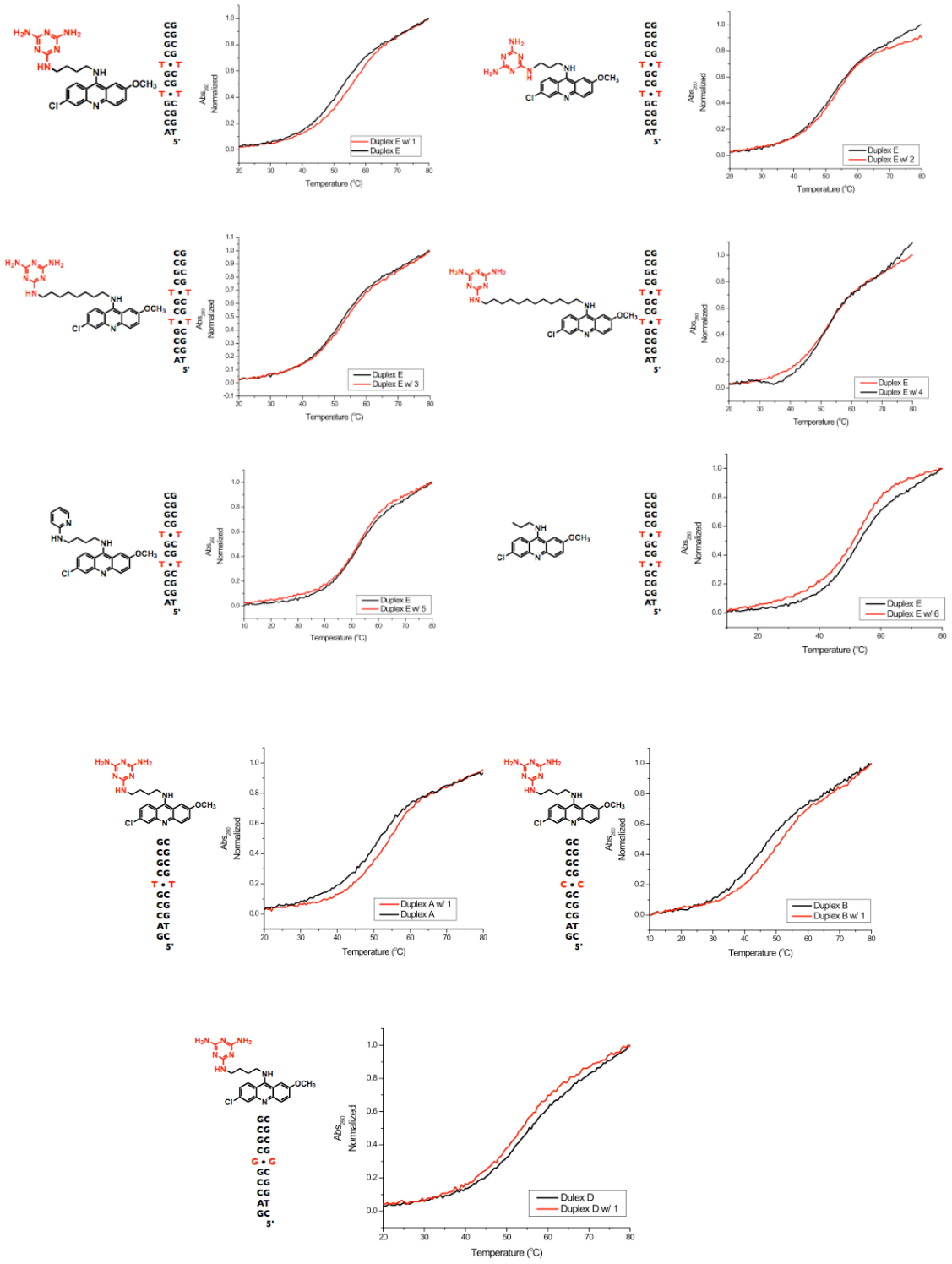


Figure S7. Thermal Denaturation Curves for DNA duplexes containing mismatches in the presence and absence of ligands studied.

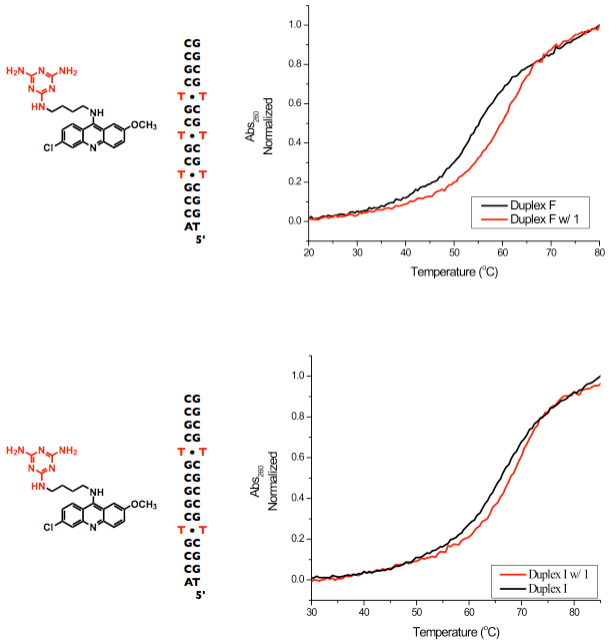


Figure S7 (continued).

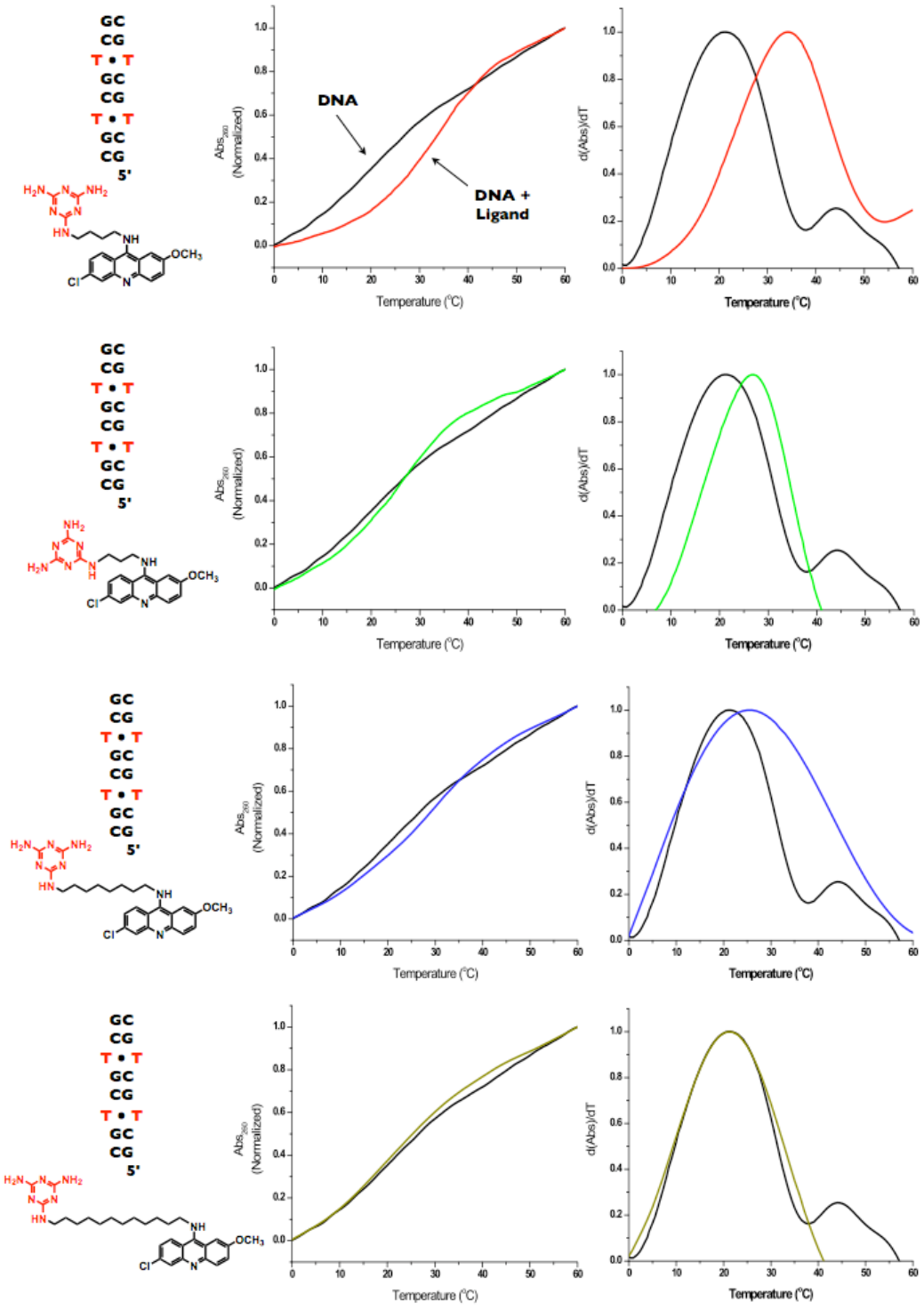


Figure S8. Thermal Denaturation Curves and Corresponding Derivative Curves for $(dGCTGCTGC)_2$ in the presence of ligands **1-5**.

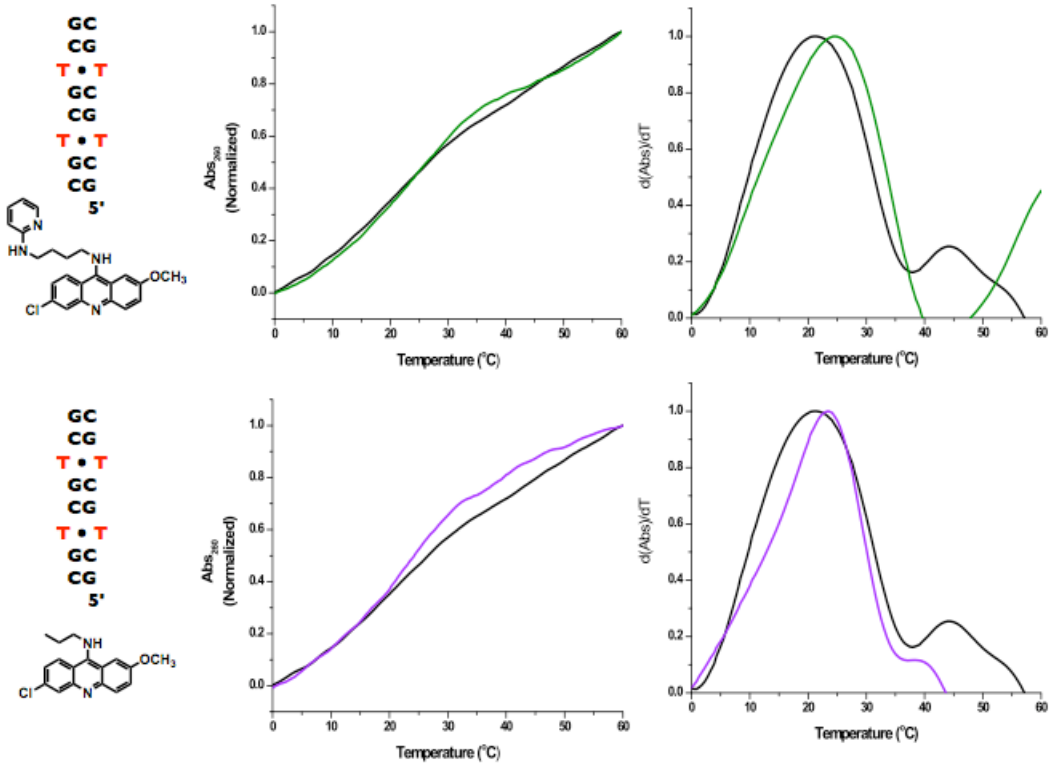


Figure S8 (continued).

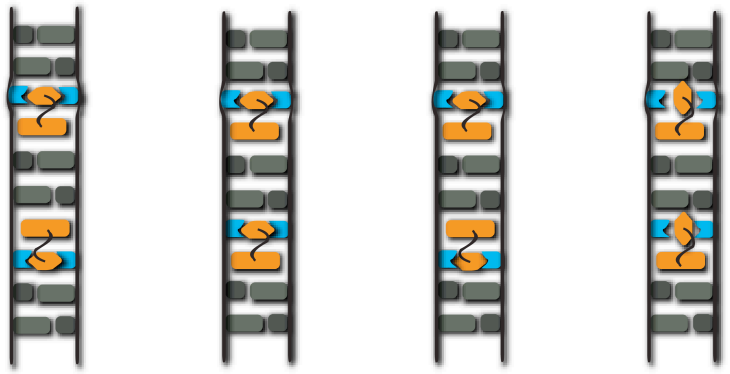


Figure S9. Schematic representation of potential binding modes to CTG and CUG step sequences. (Orthogonal melamine unit indicates partial or no hydrogen bonding in groove)

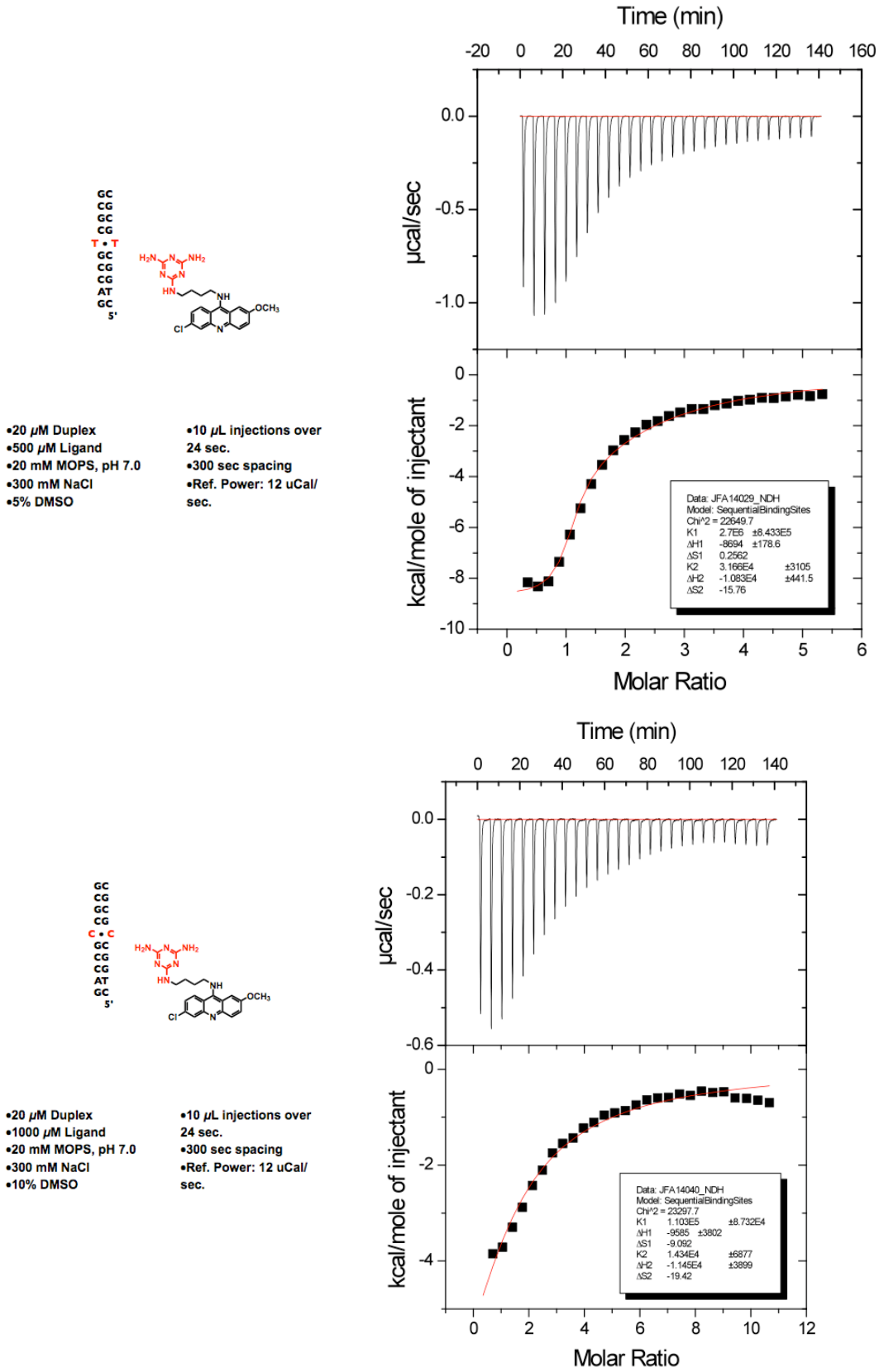
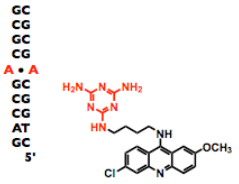
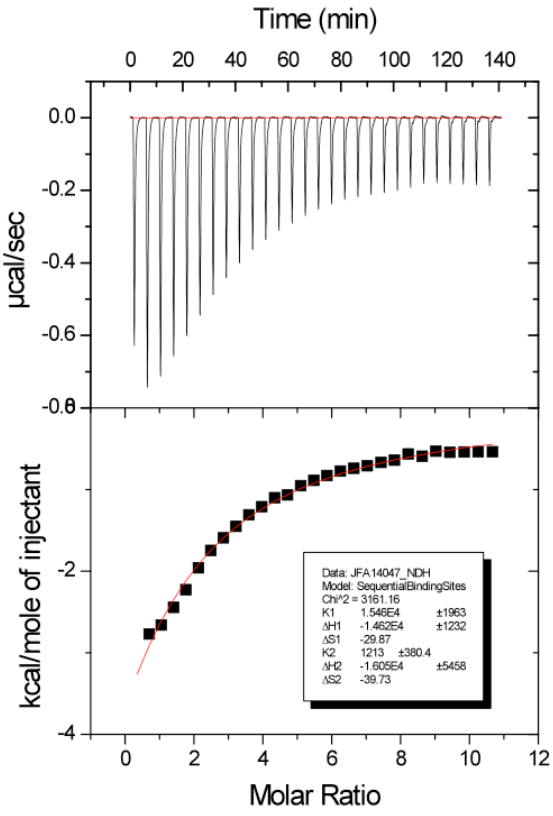


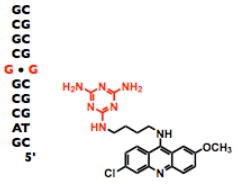
Figure S10. Isothermal Titration Calorimetry Experiments.



•20 μM Duplex
•1000 μM Ligand
•20 mM MOPS, pH 7.0
•300 mM NaCl
•10% DMSO

•10 μL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.





•20 μM Duplex
•1000 μM Ligand
•20 mM MOPS, pH 7.0
•300 mM NaCl
•10% DMSO

•10 μL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 uCal/sec.

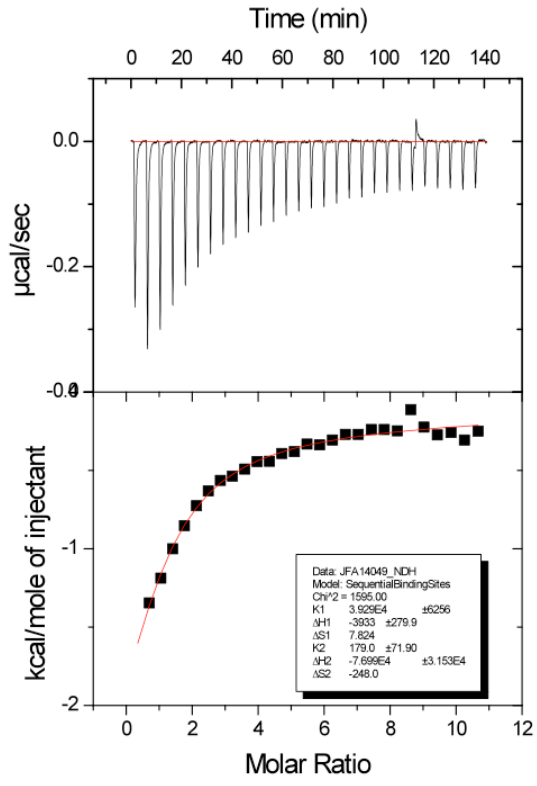


Figure S10. (continued)

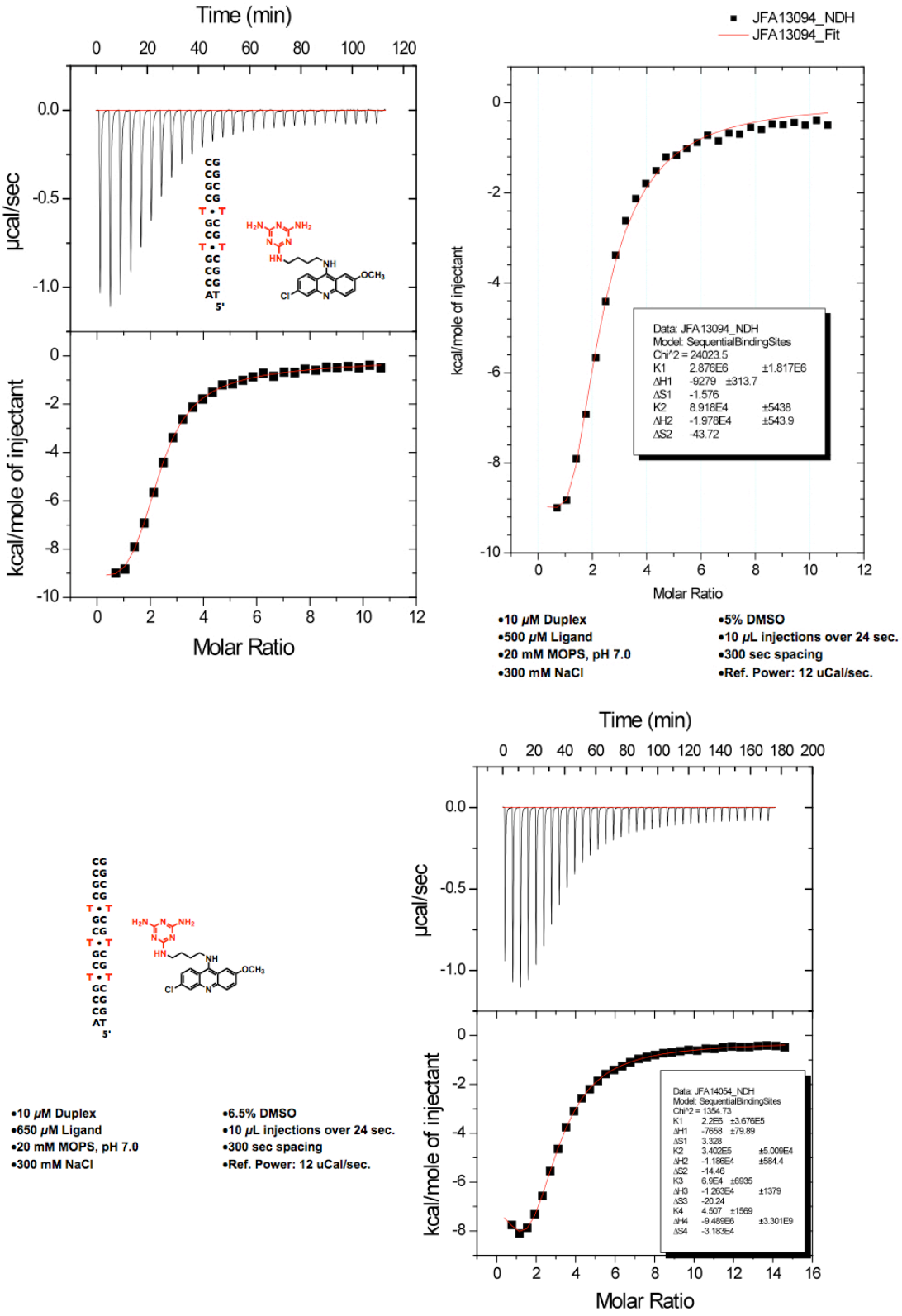


Figure S10. (continued)

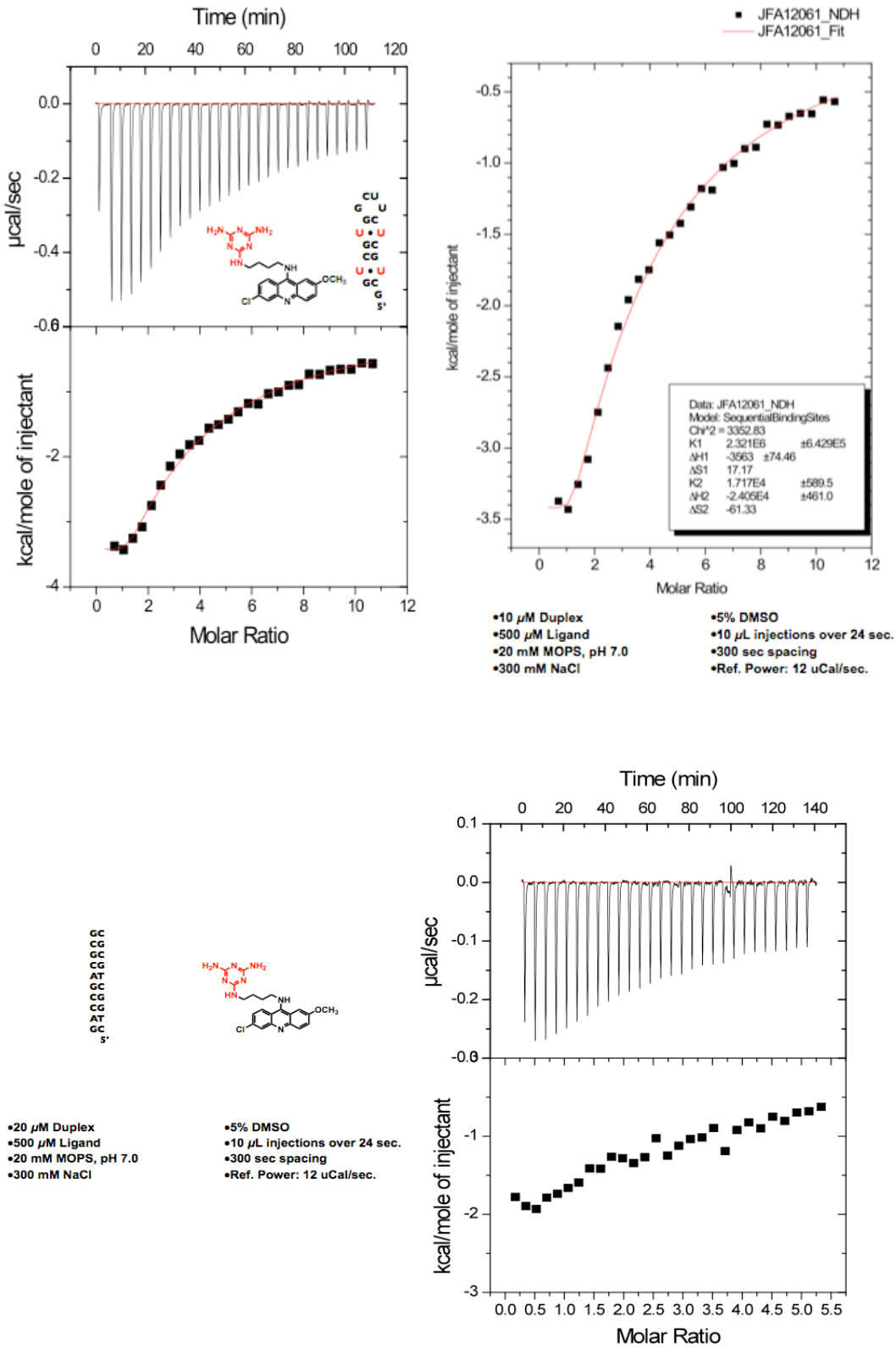
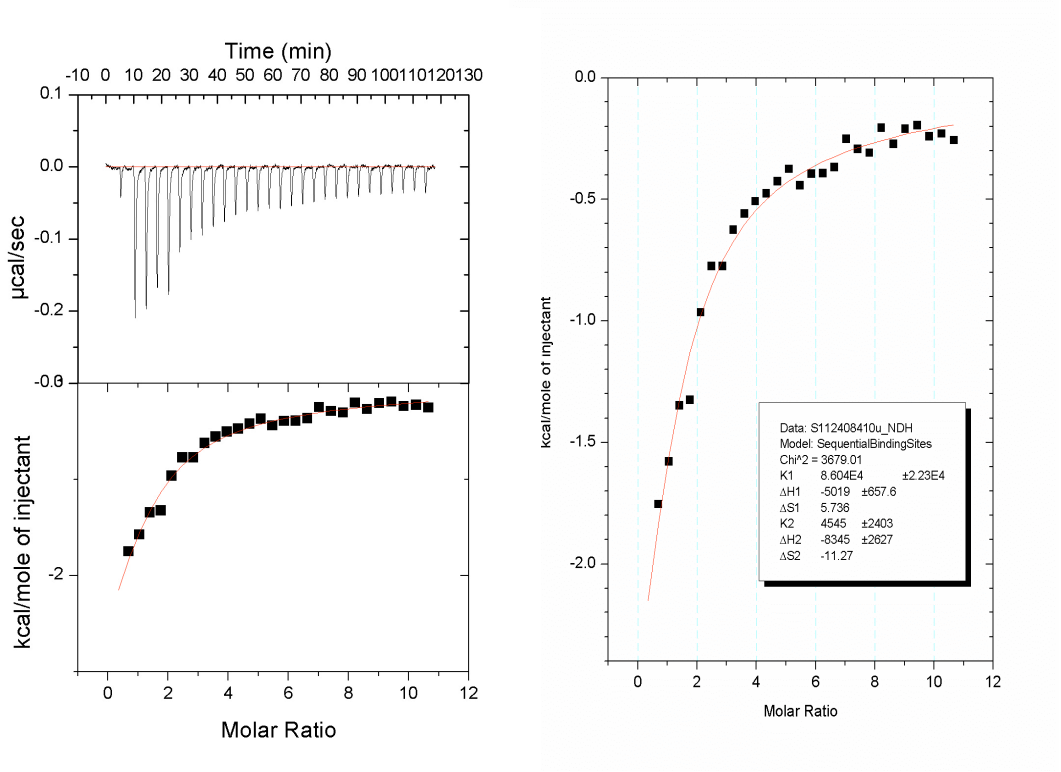
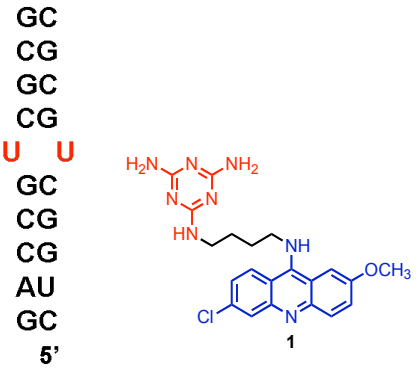


Figure S10. (continued)



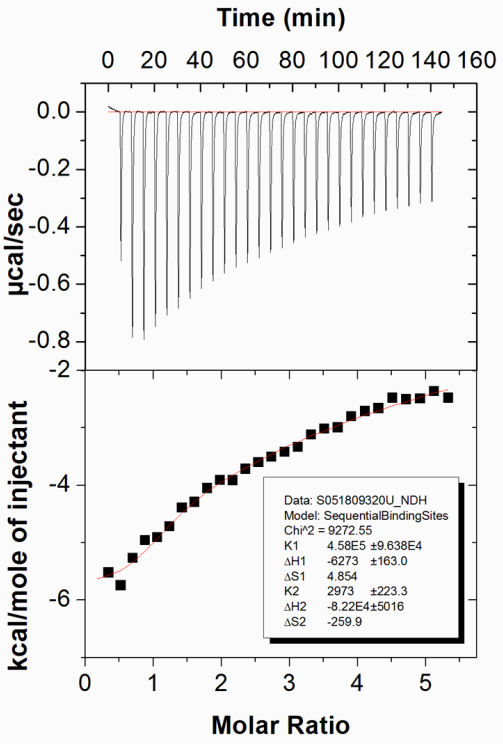
- 10 µM tRNA Duplex
- 500 µM Ligand
- 20 mM MOPS, pH 7.0
- 300 mM NaCl
- 5% DMSO
- 10 µL injections over 24 sec.
- 300 sec spacing
- Ref. Power: 12 µCal/sec.

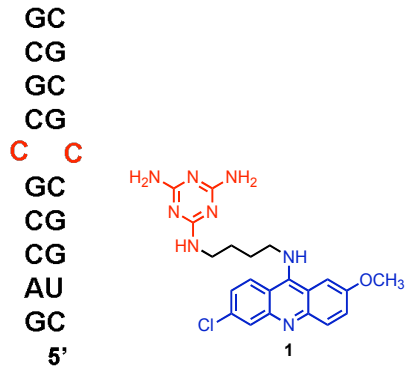
Figure S10. (continued)



•20 μM Duplex
•500 μM Ligand
•20 mM MOPS, pH 7.0
•300 mM NaCl

•5% DMSO
•10 μL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 μCal/sec.





•20 μM Duplex
•500 μM Ligand
•20 mM MOPS, pH 7.0
•300 mM NaCl

•5% DMSO
•10 μL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 μCal/sec.

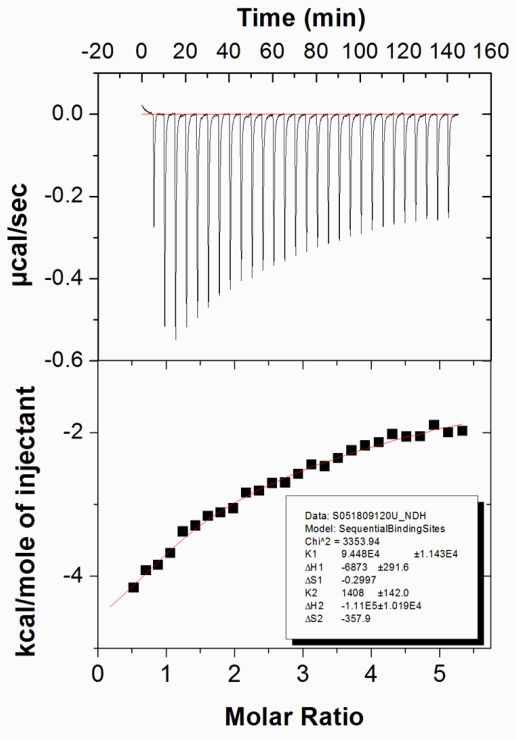
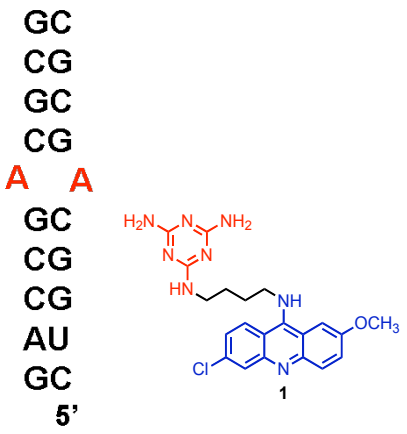
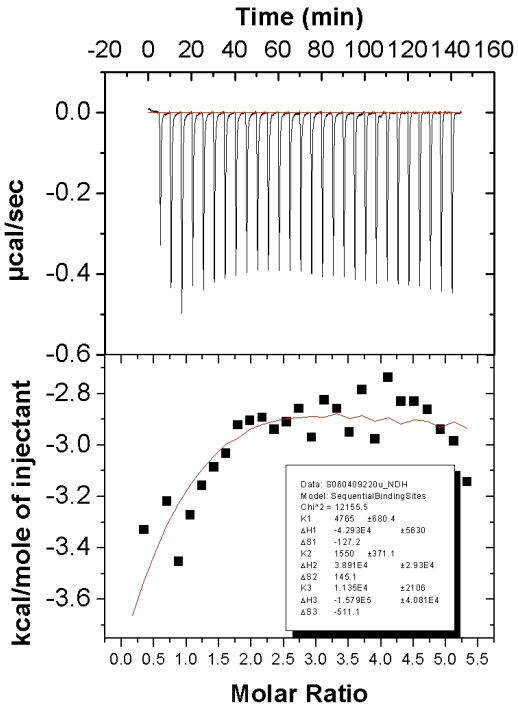


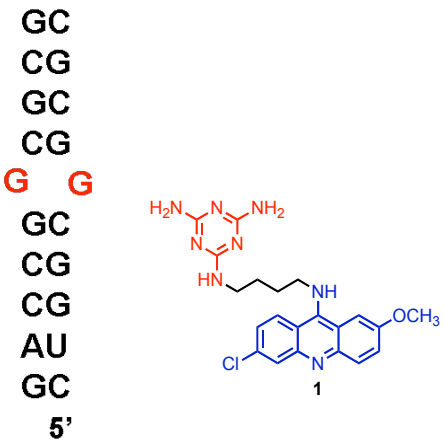
Figure S10. (continued)



•20 μM Duplex
•500 μM Ligand
•20 mM MOPS, pH 7.0
•300 mM NaCl

•5% DMSO
•10 μL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 μCal/sec.





•20 μM Duplex
•500 μM Ligand
•20 mM MOPS, pH 7.0
•300 mM NaCl

•5% DMSO
•10 μL injections over 24 sec.
•300 sec spacing
•Ref. Power: 12 μCal/sec.

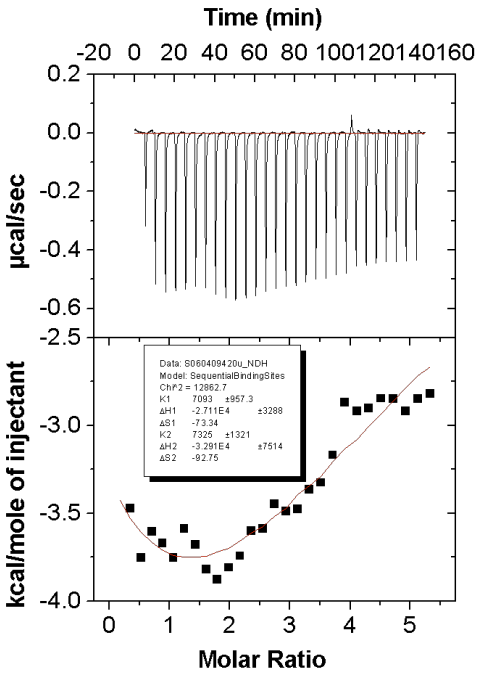


Figure S10. (continued)

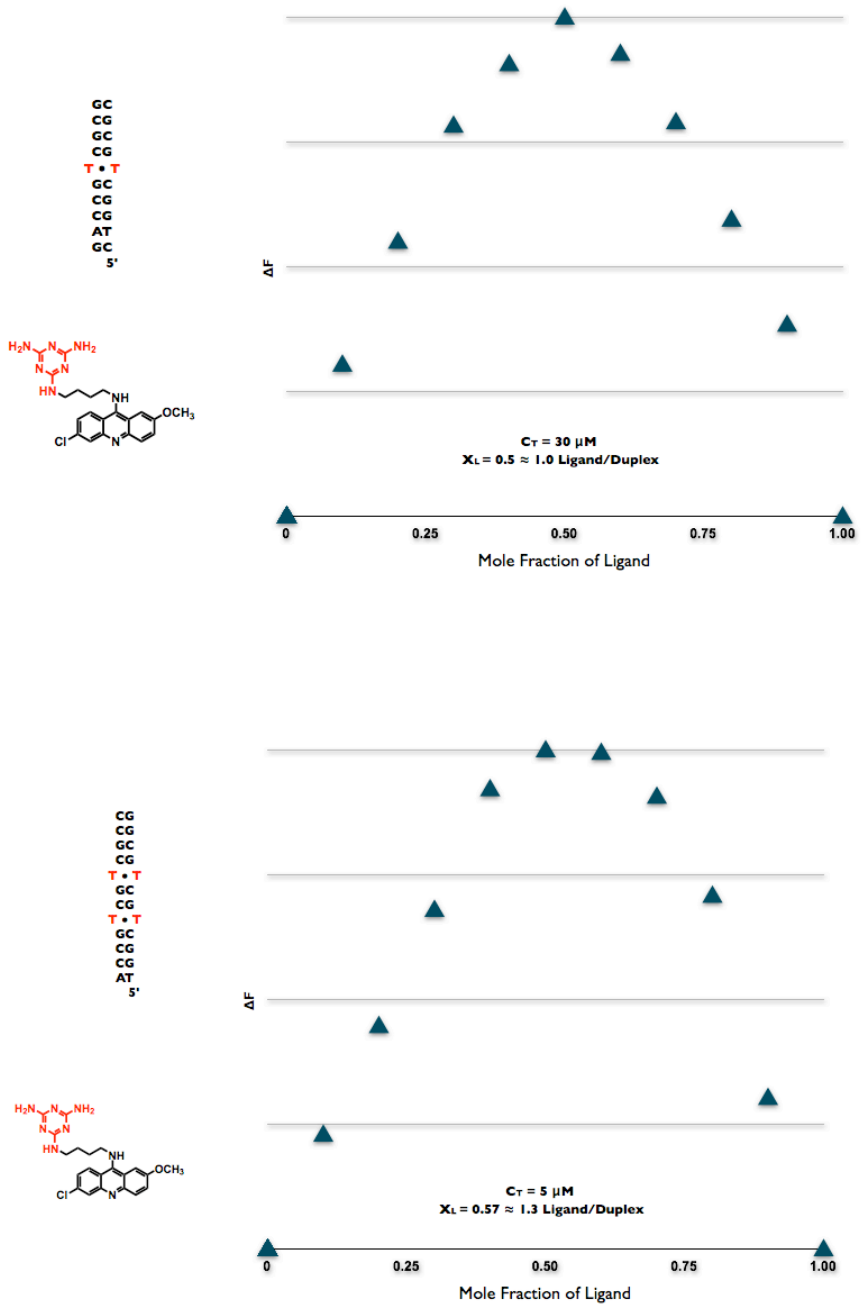


Figure S11. Job plot analysis of ligand **1** with various nucleic acid duplexes.

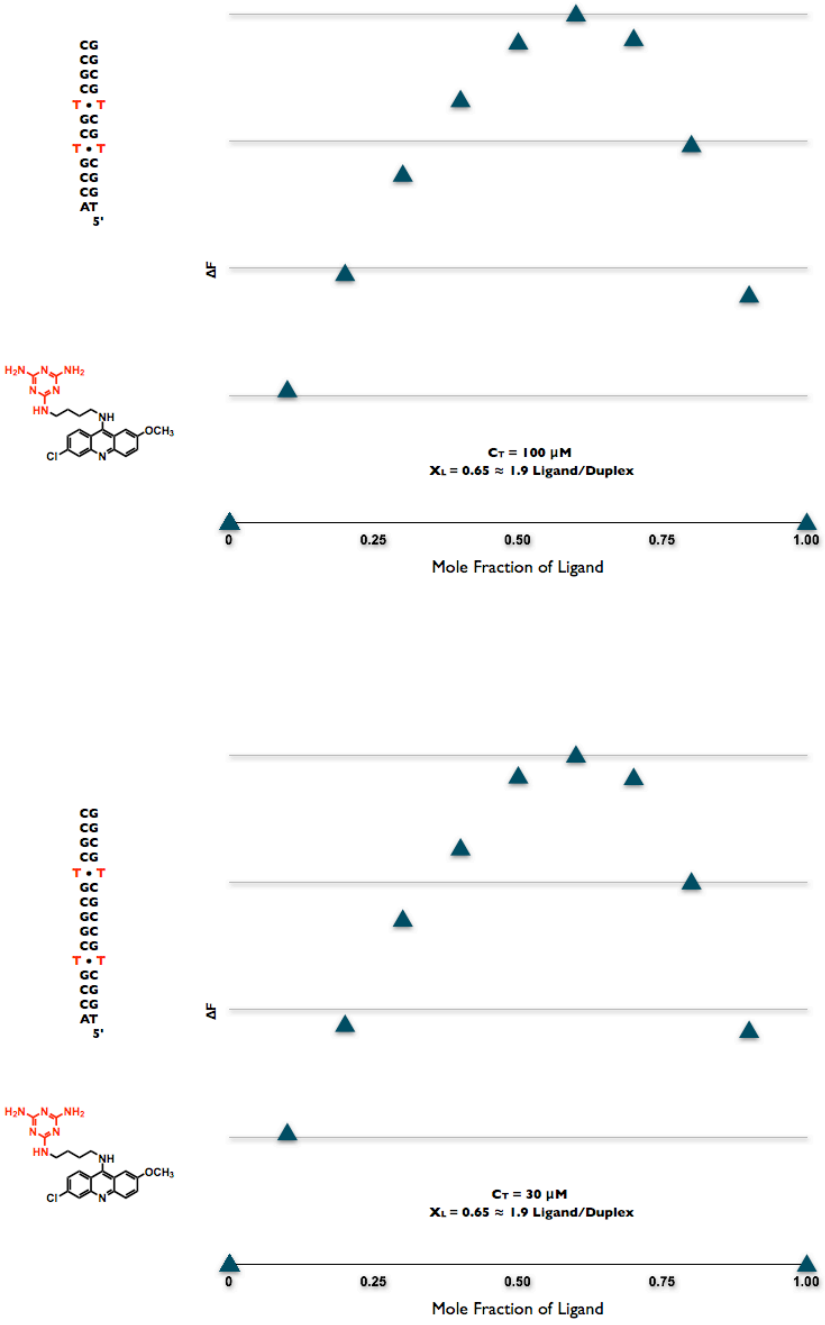
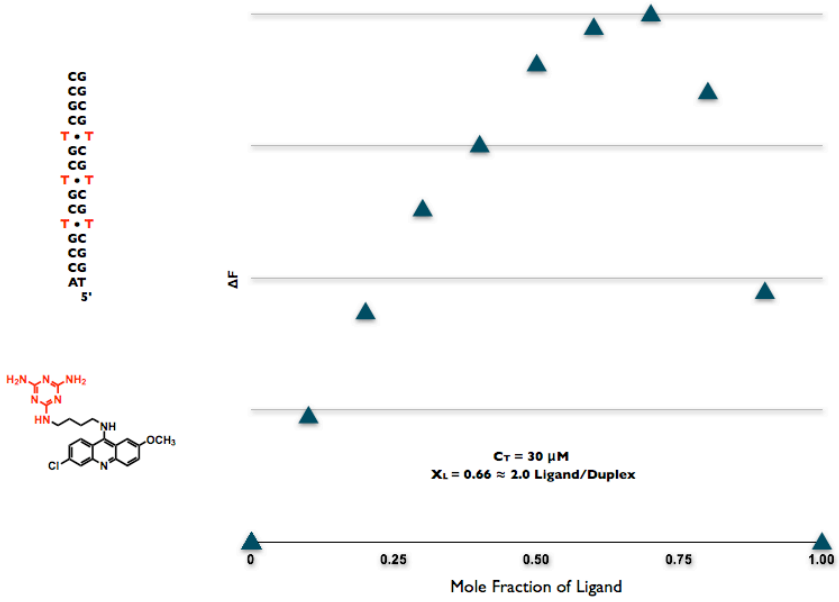


Figure S11. (continued)



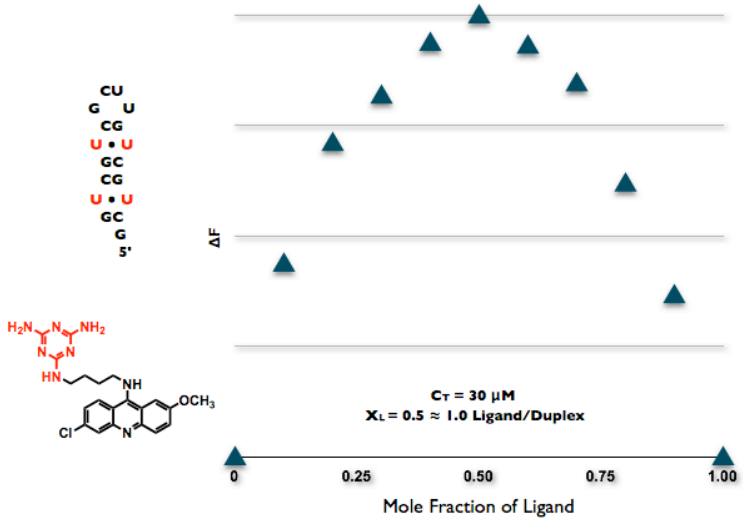


Figure S11. (continued)

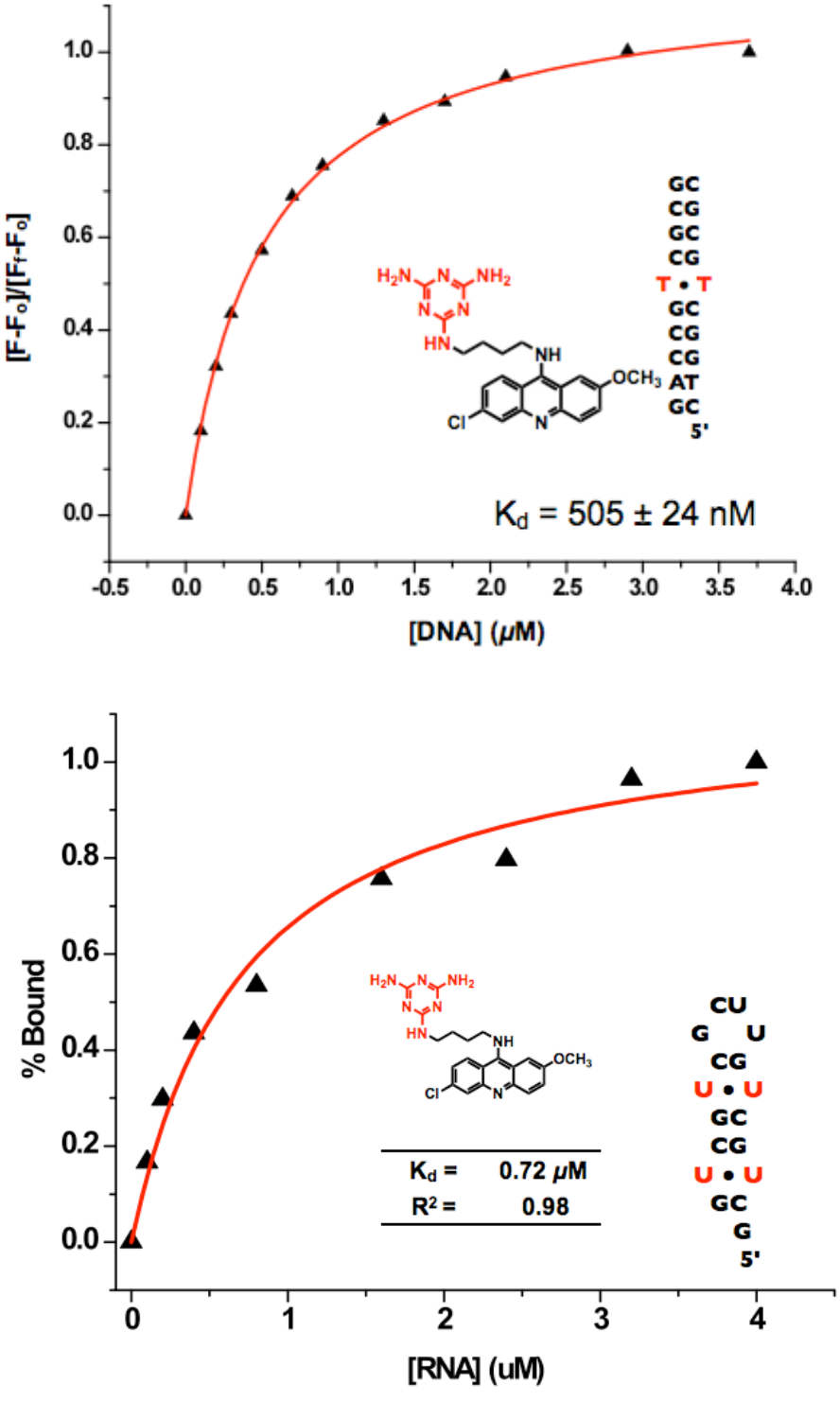


Figure S12. Reverse fluorescence binding titrations.

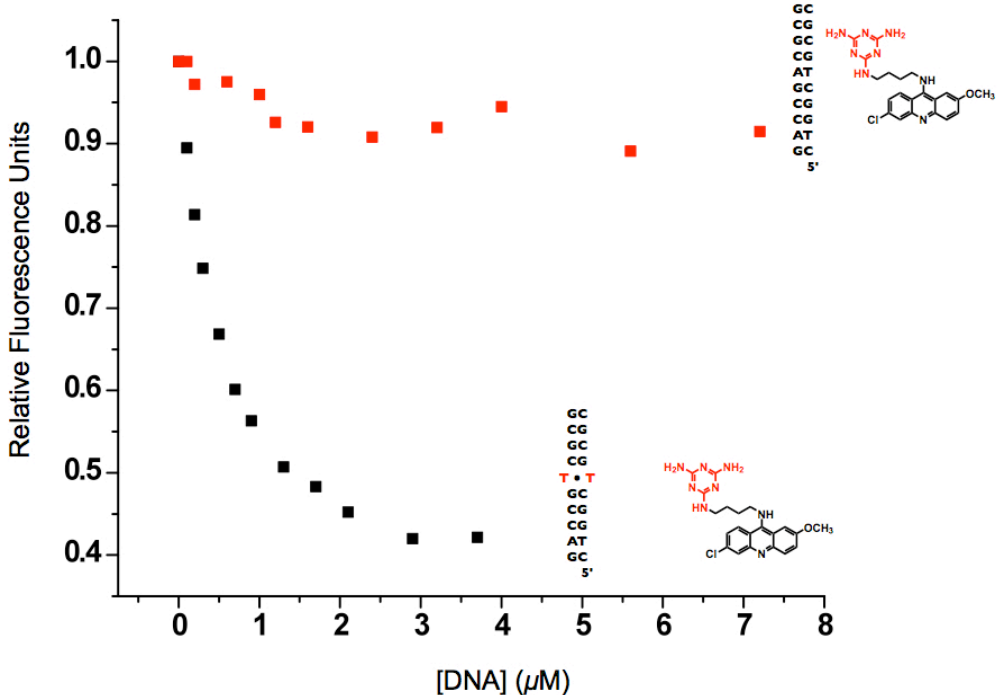


Figure S13. Relative fluorescence change of ligand **1** in the presence of increasing concentration of duplex A (black) and G (red).

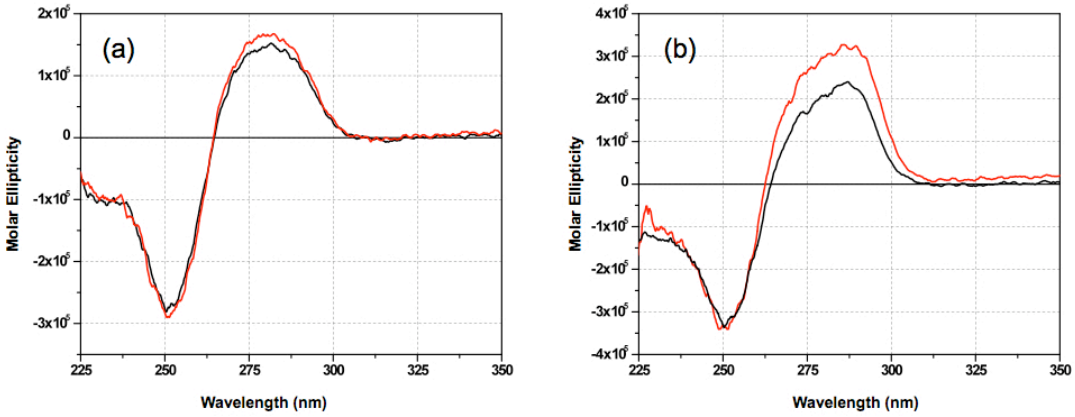


Figure S14. Circular dichroism of ligand-DNA complexes. (a) CD Spectra of d(GCGCTGCCAG)/d(CTGGCAGCGC) (black) and duplex **A** (red) in the presence of 1eq. of ligand **1**. Conditions: 50 μM duplex, 50 μM ligand **1**, 20 mM MOPS, 300 mM NaCl. (b) (a) CD Spectra of d(CCGCTGCTGCTGCCA)/ d(TGGCAGCAGCGAGCGG) (black) and duplex **F** (red) in the presence of 3eq. of ligand **1**. Conditions: 50 μM duplex, 150 μM ligand **1**, 20 mM MOPS, 300 mM NaCl.

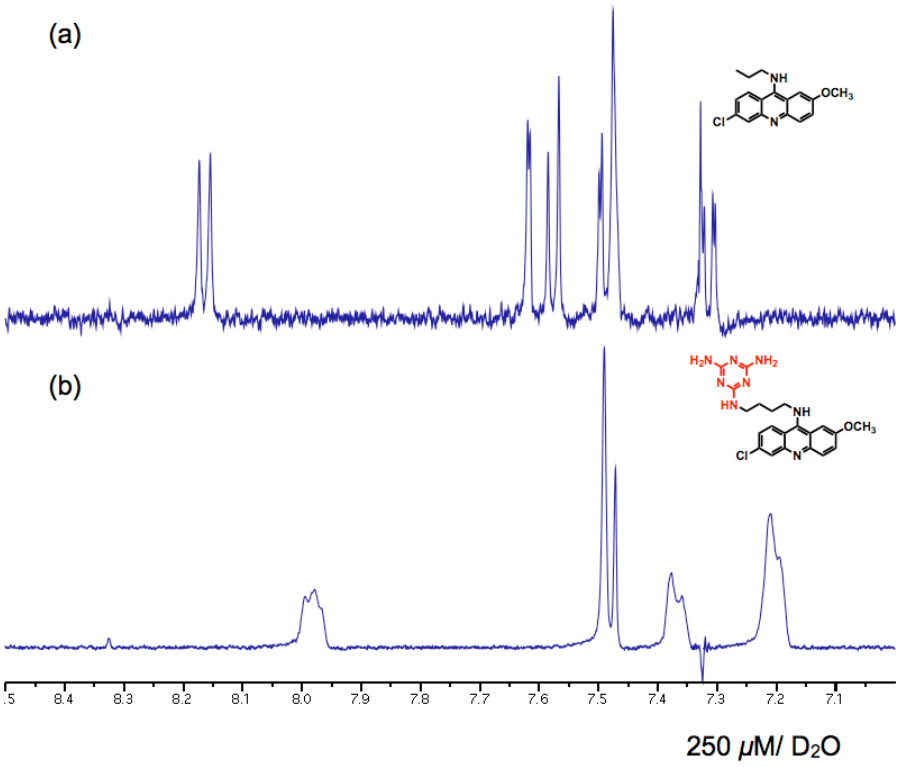


Figure S15. ^{1}H NMR Study confirming stacked conjugate orientation. (a) Proton NMR of the aromatic region of 6-chloro-2-methoxy-9-aminopropyl-acridine at 250 μM concentration in D_2O. (b) Proton NMR of the aromatic region of ligand **1** at 250 μM concentration in D_2O.

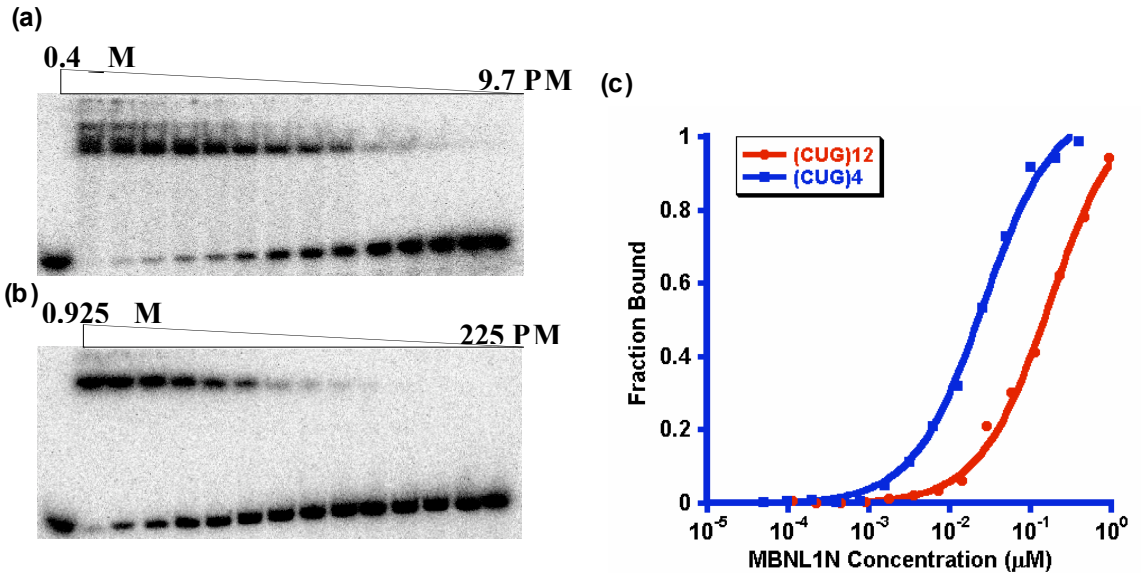


Figure S16. (a) MBNL1N binding to $(CUG)_4$ with protein concentration ranges from 0.4 μM to 9.7 pM (b) MBNL1N binding to $(CUG)_{12}$ with protein concentration ranges from 0.925 μM to 225 pM. (c) Plots illustrating the fraction of $(CUG)_4$ and $(CUG)_{12}$ RNAs bound as a function of MBNL1N concentration in absence of tRNA.

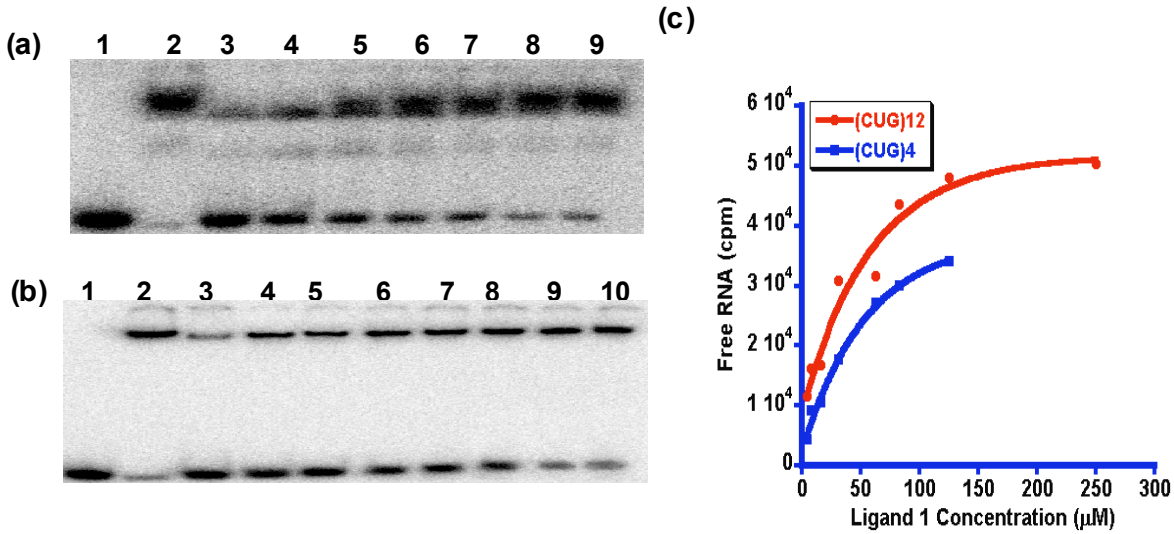


Figure S17. (a) Inhibition of the MBNL1N-$(CUG)_4$ complex with decreasing concentrations of ligand 1 from lanes 3-9: 125 μM, 83.3 μM, 62.5 μM, 31.3 μM, 15.6 μM, 7.8 μM and 3.9 μM, respectively. (b) Inhibition of the MBNL1N-$(CUG)_{12}$ complex with decreasing concentrations of ligand 1 from lanes 3-10: 250 μM, 125 μM, 83.3 μM, 62.5 μM, 31.3 μM, 15.6 μM, 7.8 μM and 3.9 μM, respectively. Lane 1 is a control with only $(CUG)_n$ and lane 2 contains $(CUG)_n$ + MBNL1N complex. (c) Plots illustrating inhibition of $(CUG)_n$-MBNL1N complex with increasing concentrations of ligand **1**.

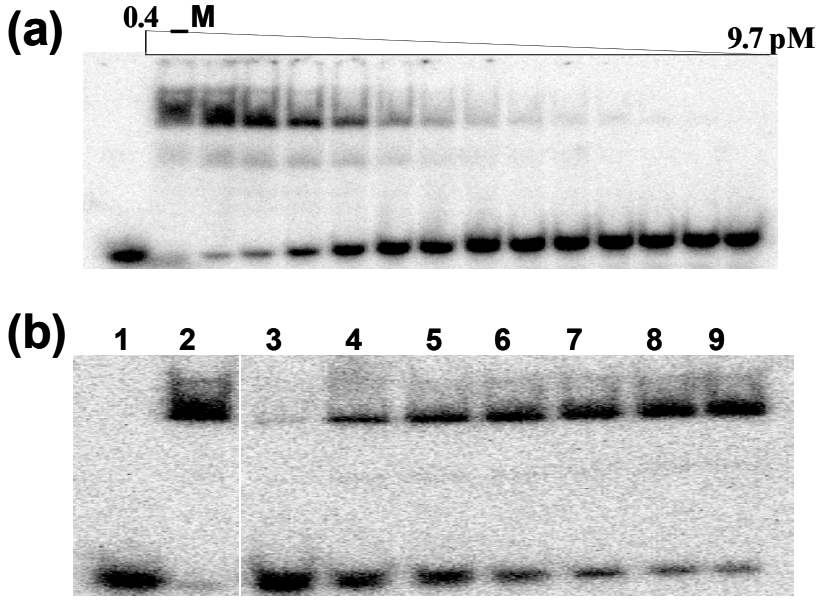


Figure S18. (a) MBNL1N binding to $(CUG)_4$ in the presence of tRNA (0.2 μM) with protein concentration ranges from 0.4 μM to 9.7 pM. (b) Inhibition of the MBNL1N-$(CUG)_4$ complex in the presence of tRNA (0.2 μM) with decreasing concentrations of ligand **1** in lanes 3-9: 125 μM, 83.3 μM, 62.5 μM, 31.3 μM, 15.6 μM, 7.8 μM and 3.9 μM, respectively. Lane 1 is a control with only $(CUG)_4$ + tRNA and lane 2 contains $(CUG)_4$ + MBNL1N complex in the presence of tRNA

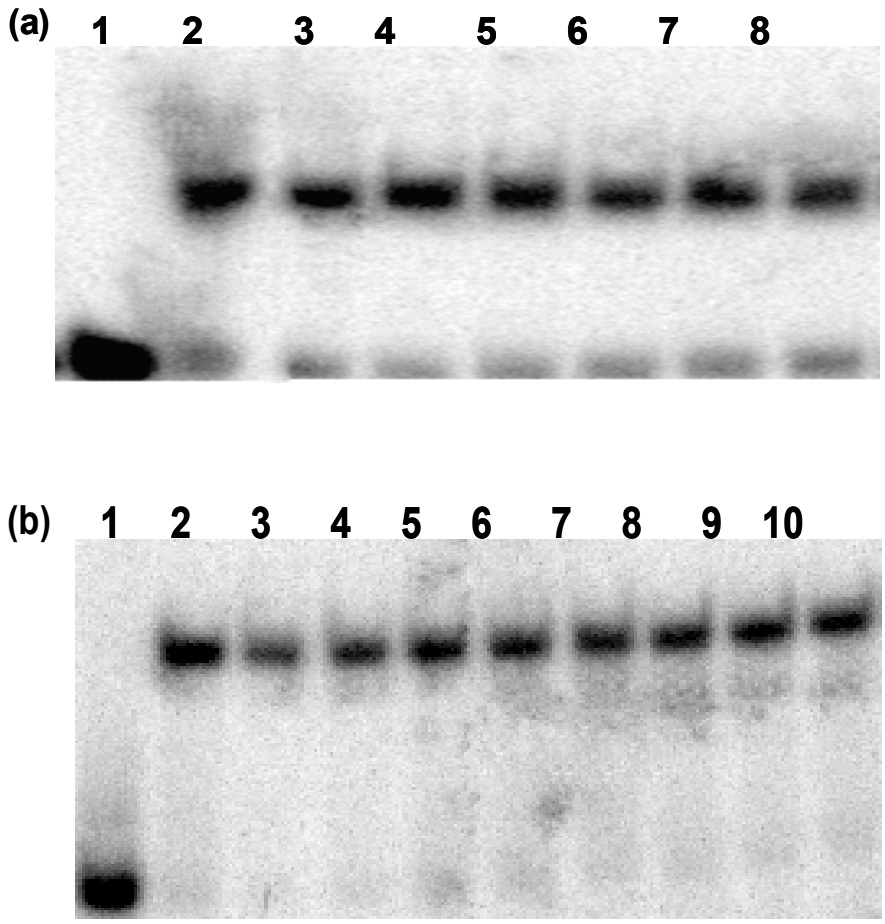


Figure S19. (a). Inhibition of the Sex lethal-tra RNA complex in the presence of tRNA (8 μM) with decreasing concentrations of ligand **1** in lanes 3-8: 125 μM, 83.3 μM, 62.5 μM, 31.3 μM, 15.6 μM, 7.8 μM and 3.9 μM, respectively. Lane 1 is a control with only tra RNA + tRNA and lane 2 contains Sex lethal-tra RNA complex in the presence of tRNA. (b) Inhibition of the U1A-SL2RNA complex in with decreasing concentrations of ligand **1** in lanes 3-10: 500 μM, 250 μM**,** 125 μM, 83.3 μM, 62.5 μM, 31.3 μM, 15.6 μM, 7.8 μM and 3.9 μM, respectively. Lane 1 is a control with only SL2 RNA and lane 2 contains U1A-SL2RNA complex.

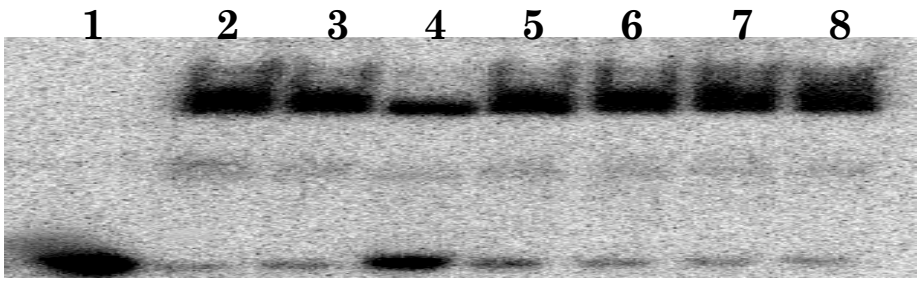
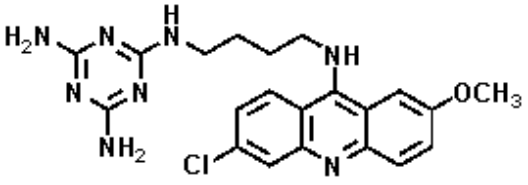
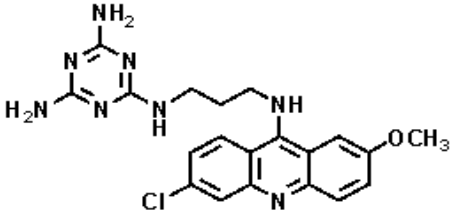


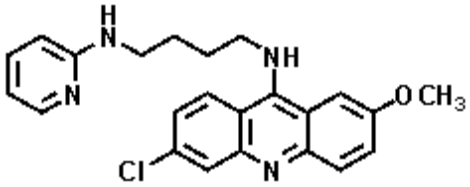
Figure S20. Comparison of inhibition of the MBNL1N-$(CUG)_4$ complex by small molecules. Lane 1 is a control with only $(CUG)_4$, lane 2 is $(CUG)_4$ + MBNL1N (0.2 μM) complex, lane 3 is $(CUG)_4$ + MBNL1N complex + 10 % DMSO and from lane 4-8 is inhibition of complex by 83.3 μM of ligand **1**, **2**, **5**, **6** and Actinomycin D, respectively.



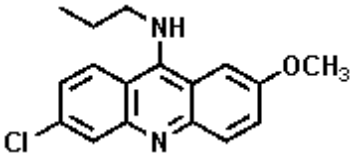
Compound **1**



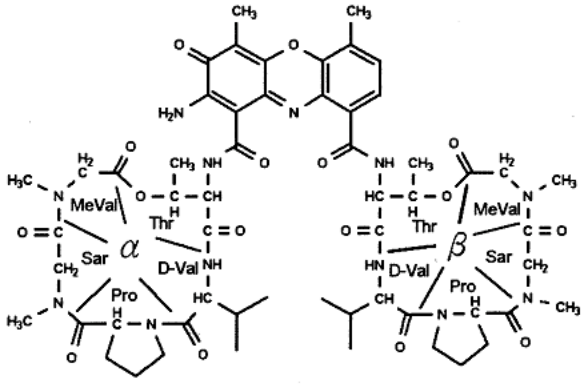
Compound **2**



Compound **5**



Compound **6**



Actinomycin D

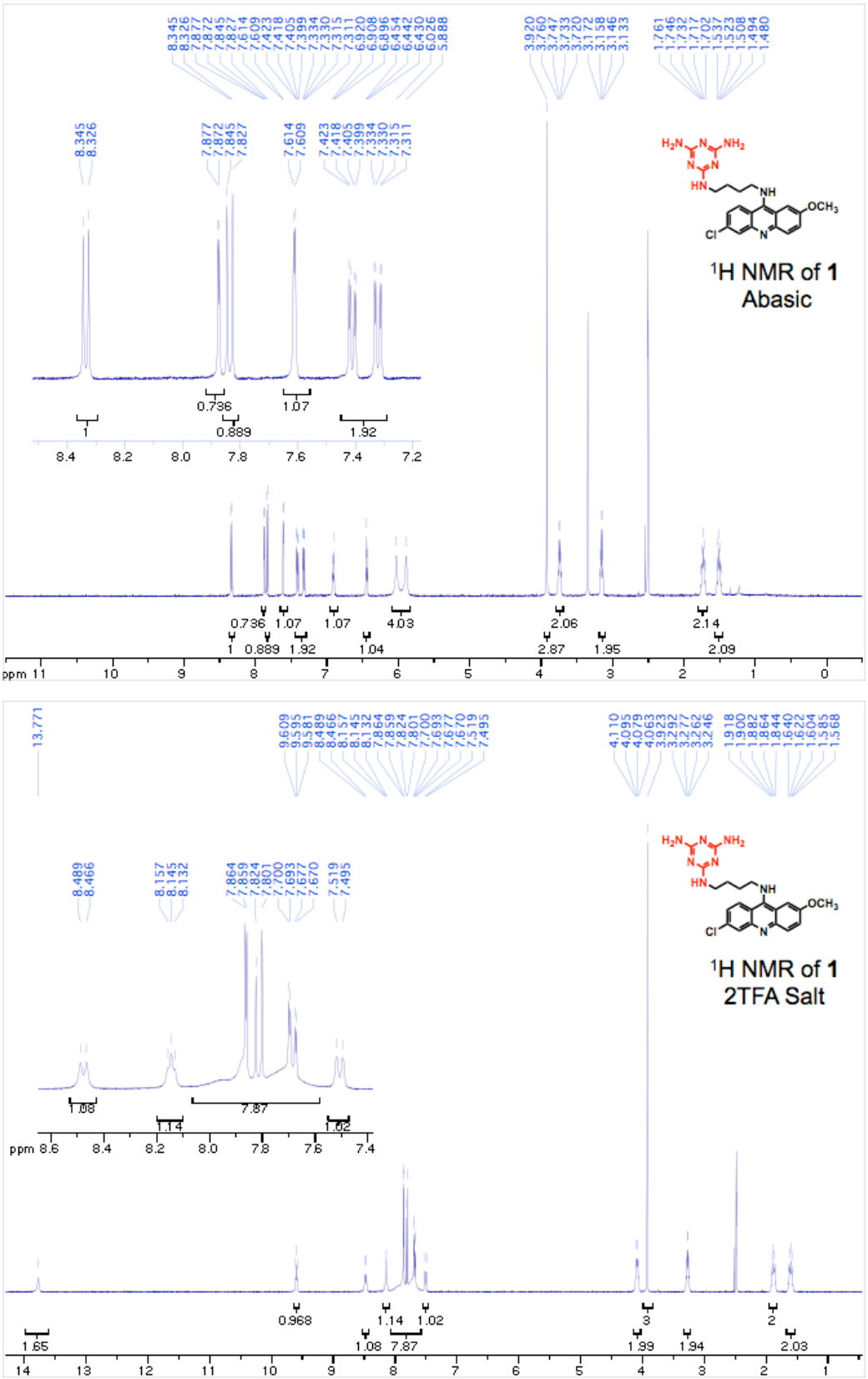
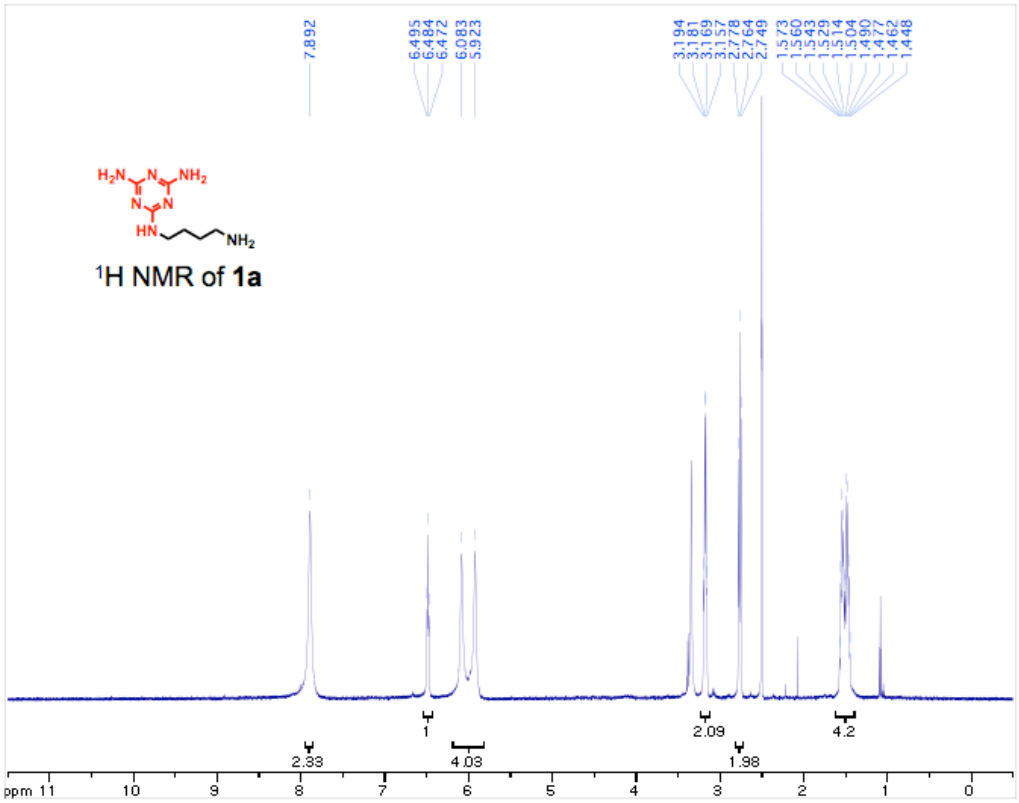


Figure S21. ^{1}H NMR Spectra of Compounds:



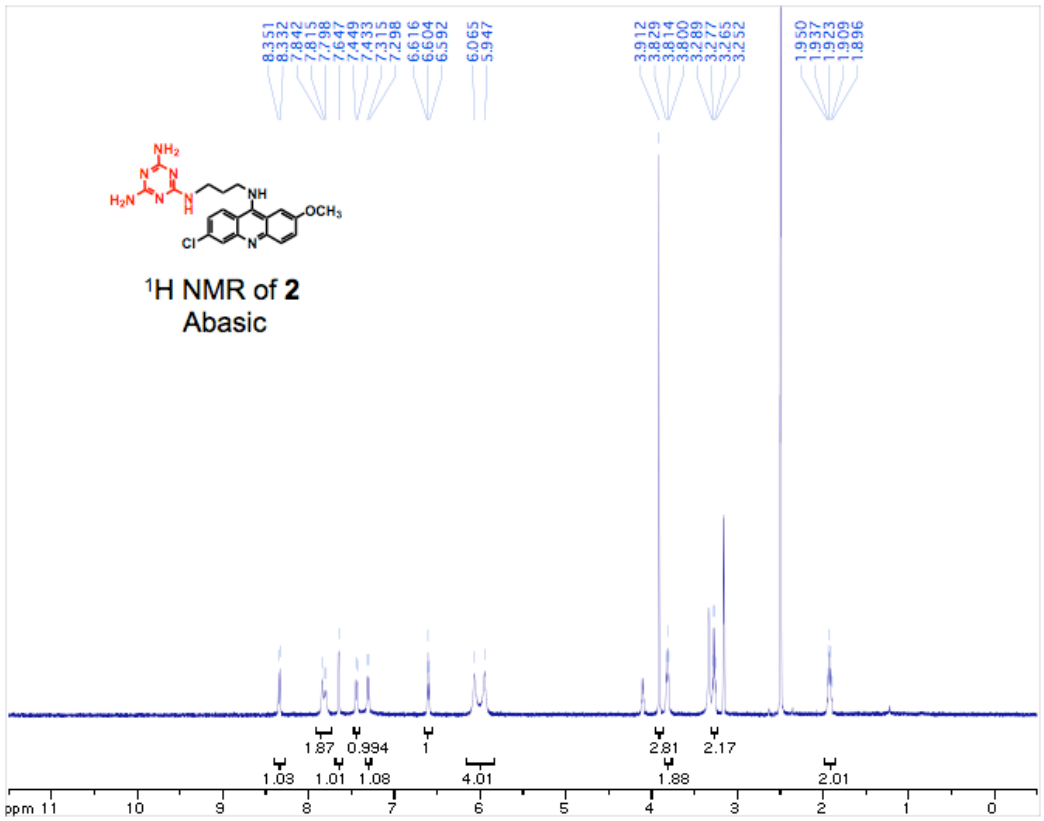


Figure S21. (continued)

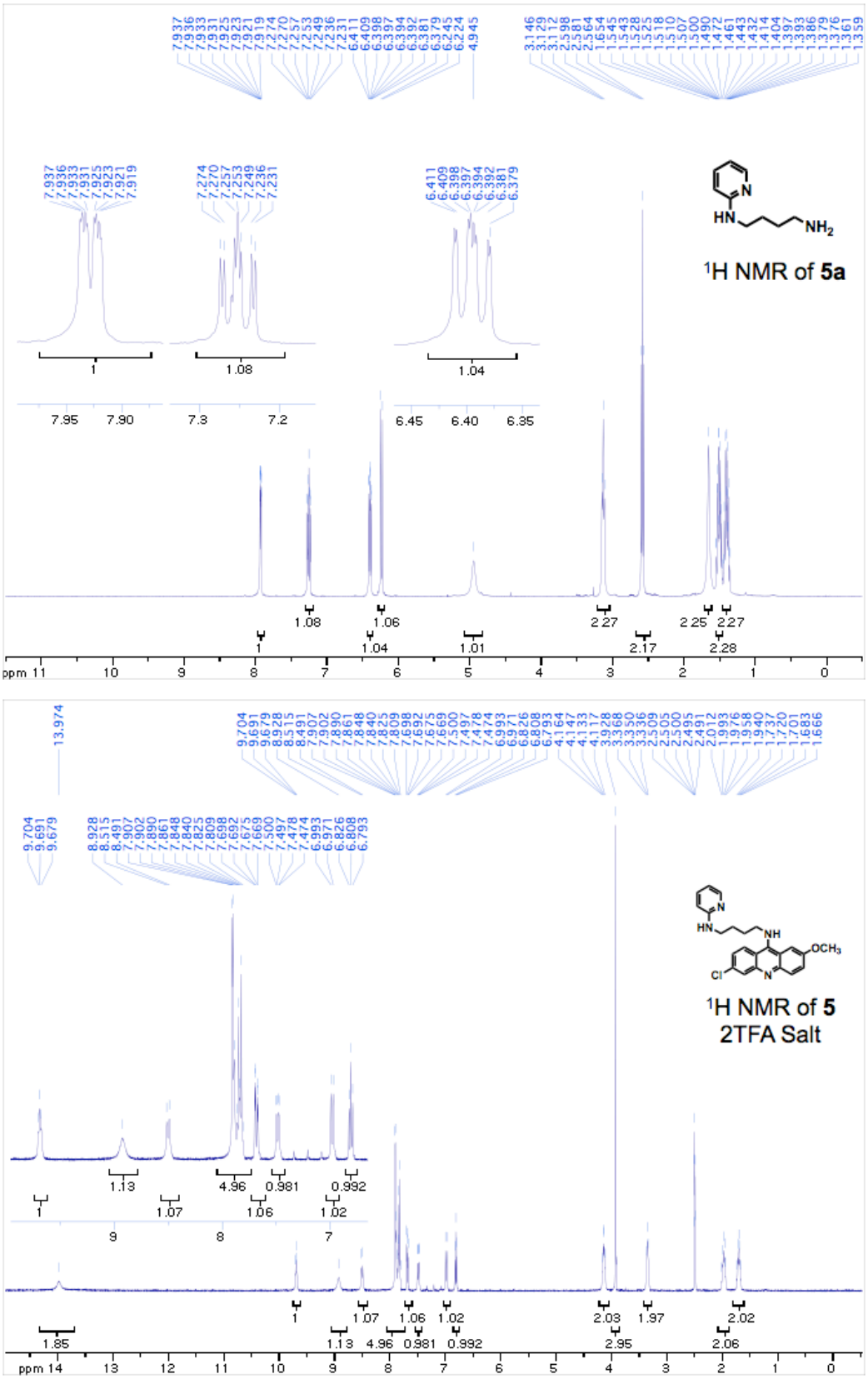
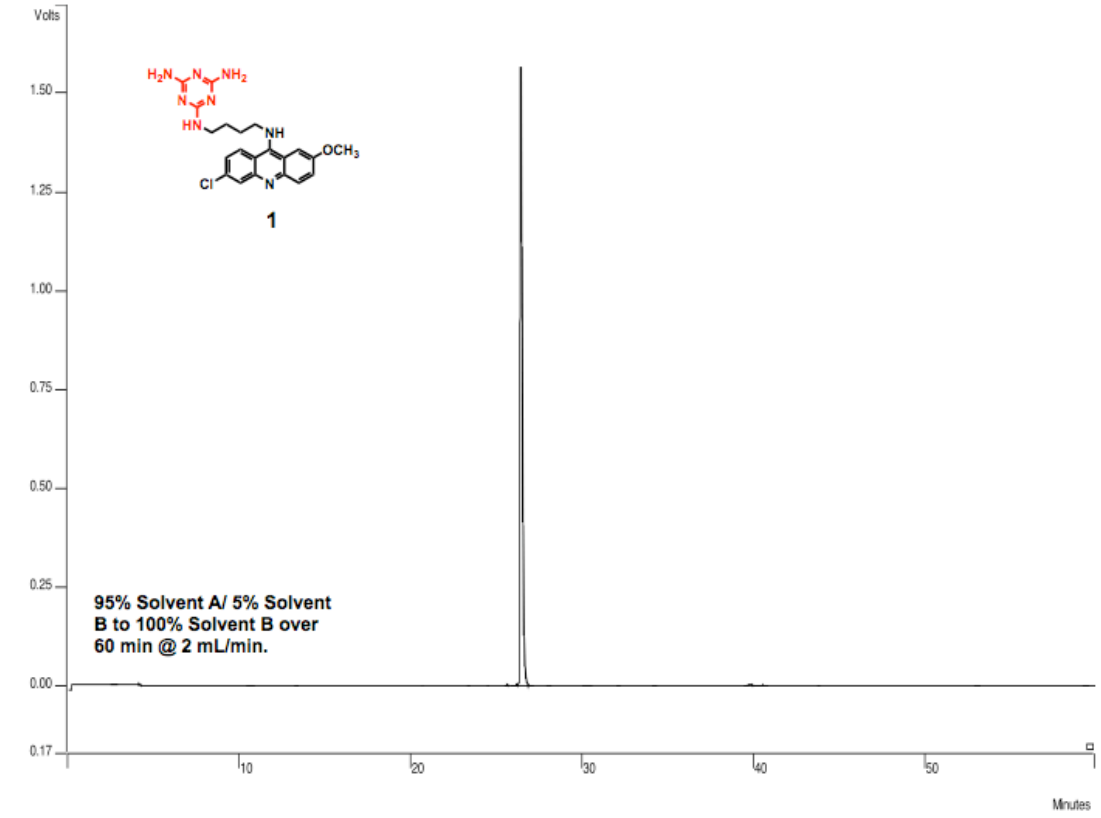


Figure S21. (continued)



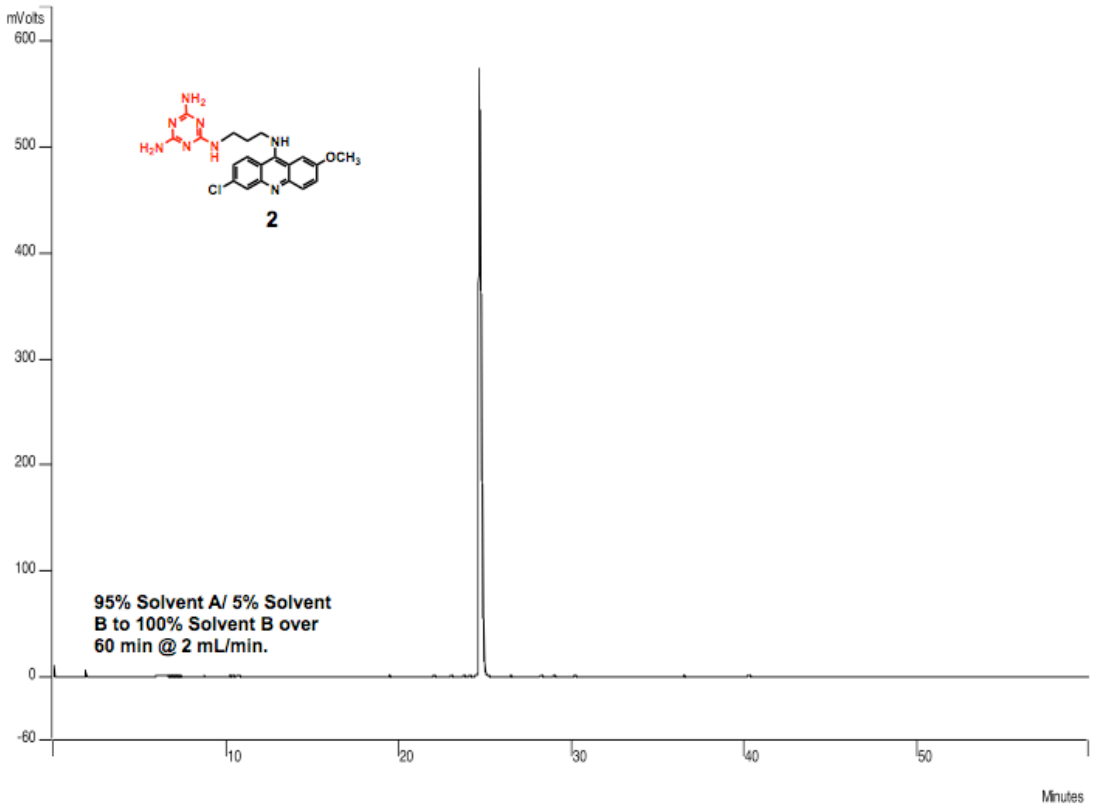
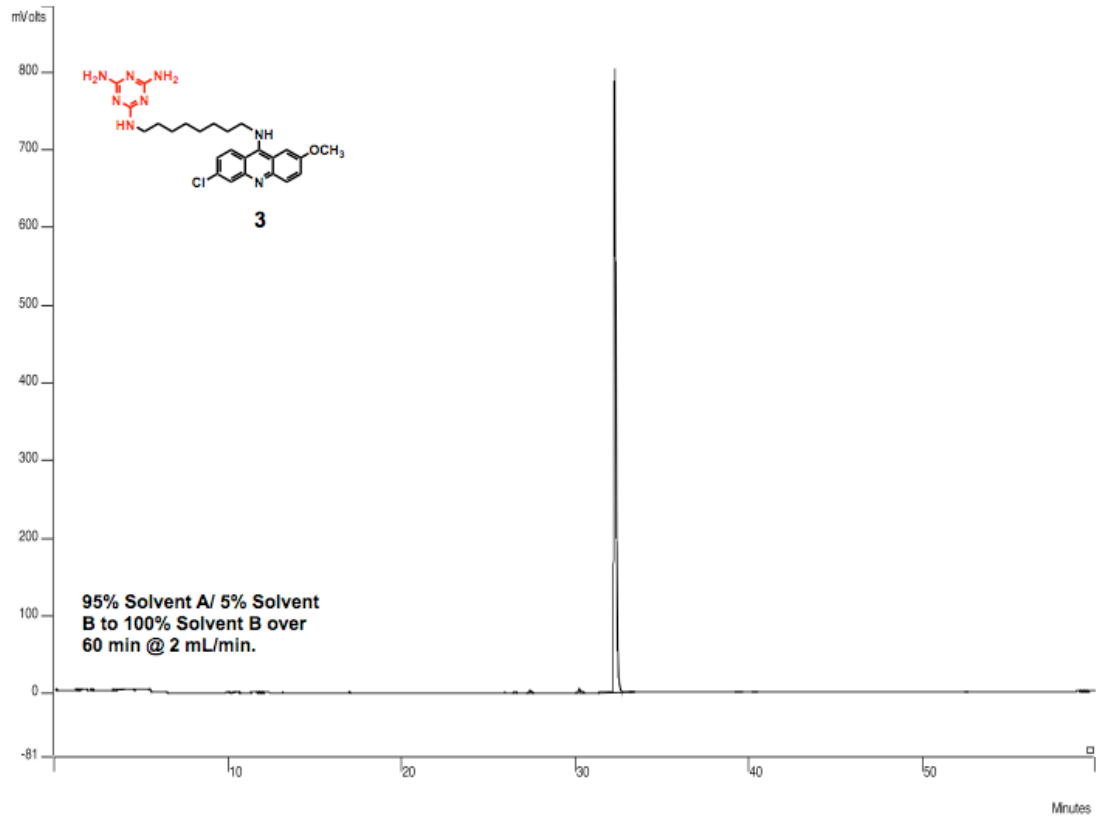


Figure S22. RP-HPLC Traces of Title Compounds:



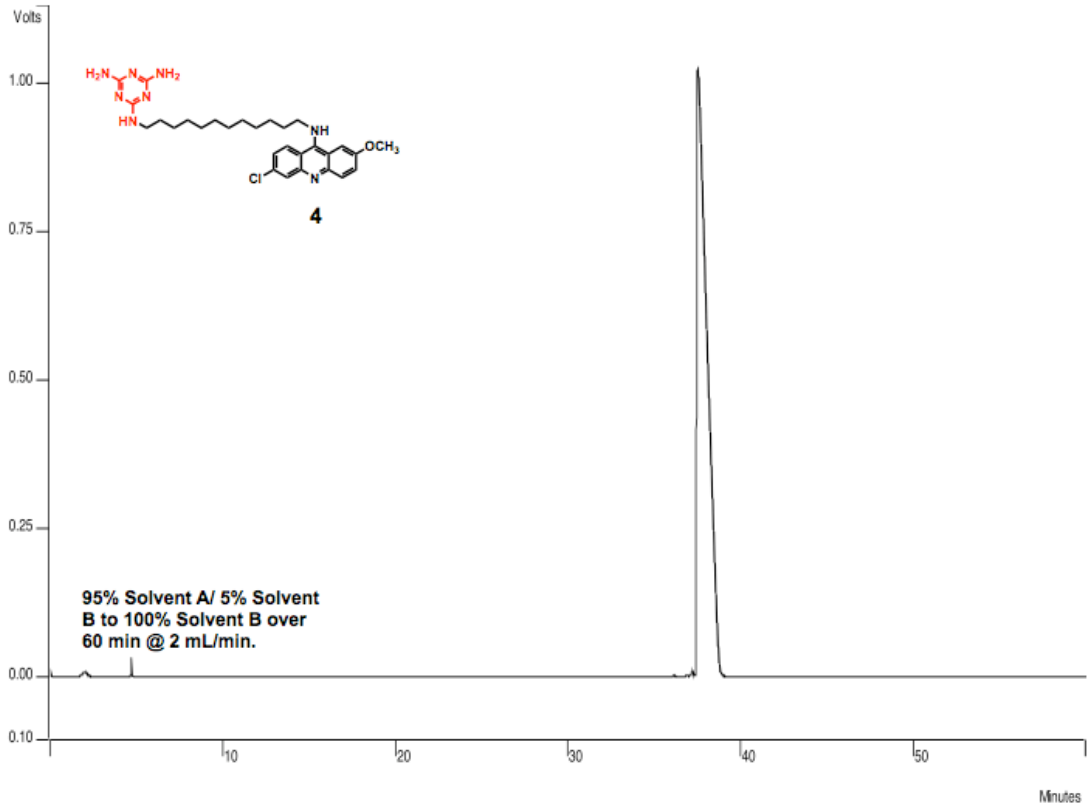


Figure S22. (continued)

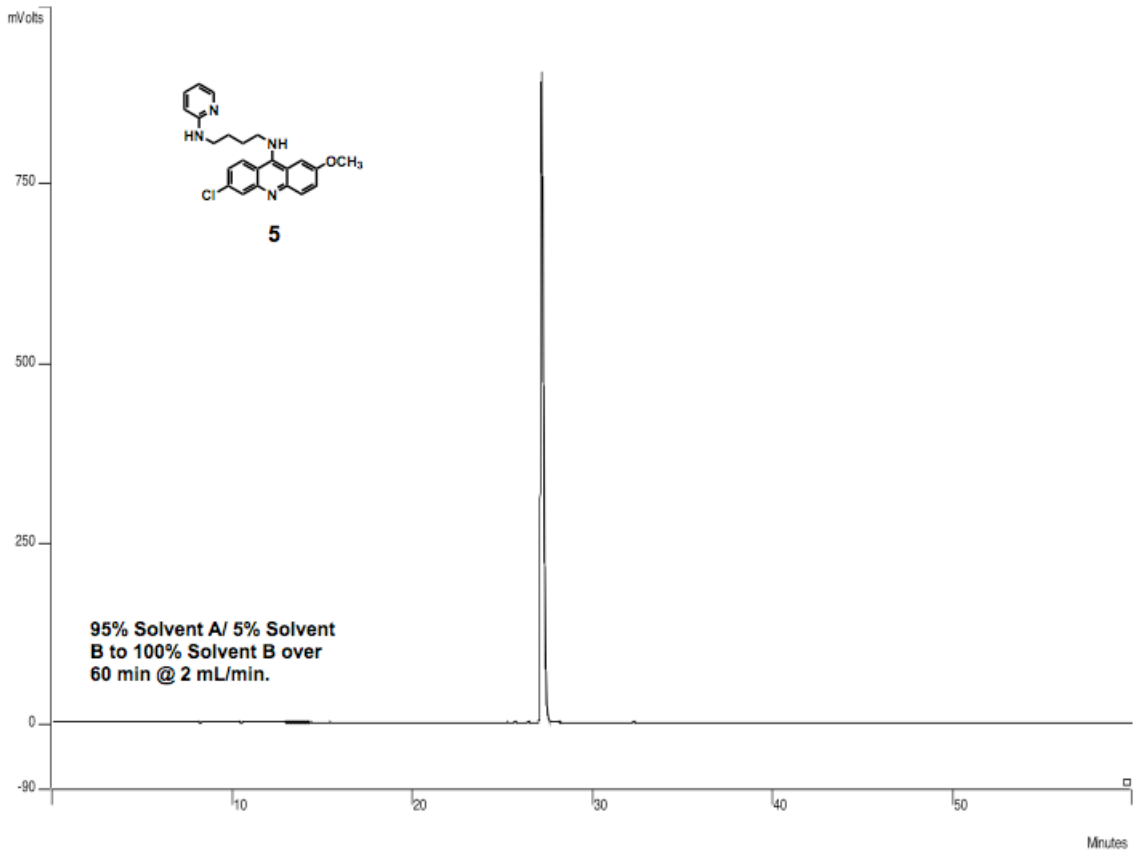


Figure S22. (continued)

[i] Ferlin, M. G.; Marzano, C.; Chiarelotto, G.; Baccichetti, F.; Bordin, F. *Eur. J. Med. Chem.* **2000**, *35*, 827–837.

[ii] Bolte, J.; Demuynck, C.; Lhomme, M. F.; Lhomme, J.; Barbet, J.; Roques, B. P. *J. Am. Chem. Soc.* **1982**, *104*, 760–765.