

Supplementary Material

**Selective Inhibition of MBNL1·CCUG Interaction by Small Molecules Toward Potential Therapeutic Agents for Myotonic Dystrophy Type 2 (DM2)**

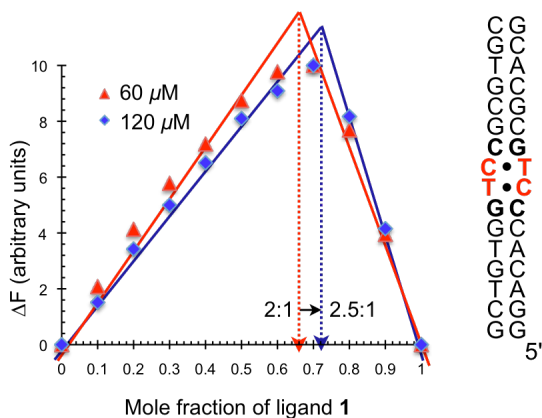
Chun-Ho Wong, Yuan Fu, Sreenivasa Rao Ramisetty, Anne M. Baranger,\*  
Steven C. Zimmerman\*

Department of Chemistry, University of Illinois at Urbana-Champaign, 600 S  
Mathews Avenue, Urbana, Illinois 61801

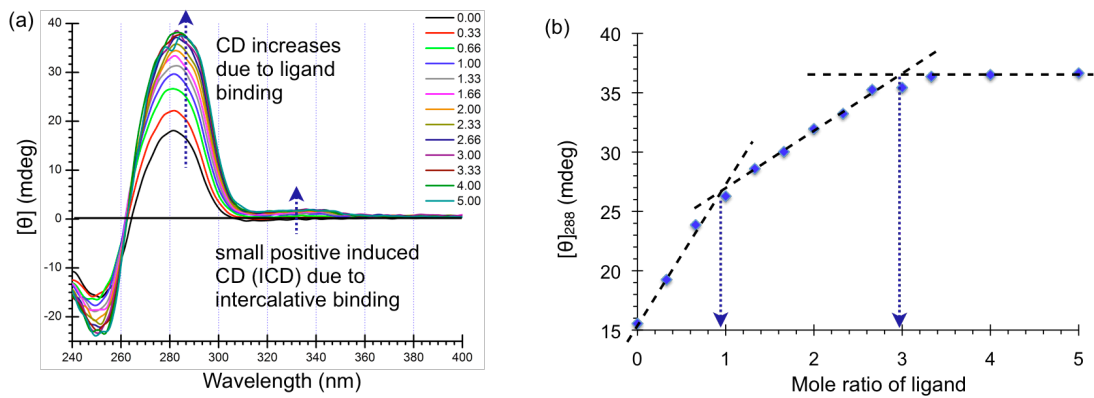
baranger@illinois.edu, sczimmer@illinois.edu

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### Additional biophysical studies for ligand 1:

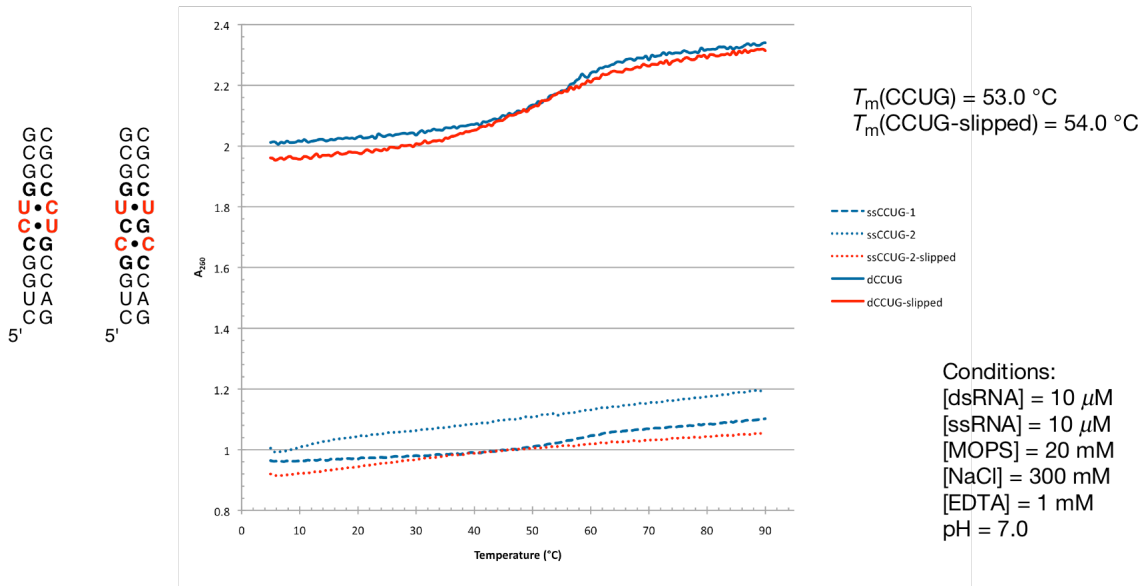


**Figure S1.** Job analysis of the stoichiometry of the DNA complex with ligand 1 with total concentrations of 60  $\mu\text{M}$  ( $\blacktriangle$ ) and 120  $\mu\text{M}$  ( $\blacklozenge$ ).

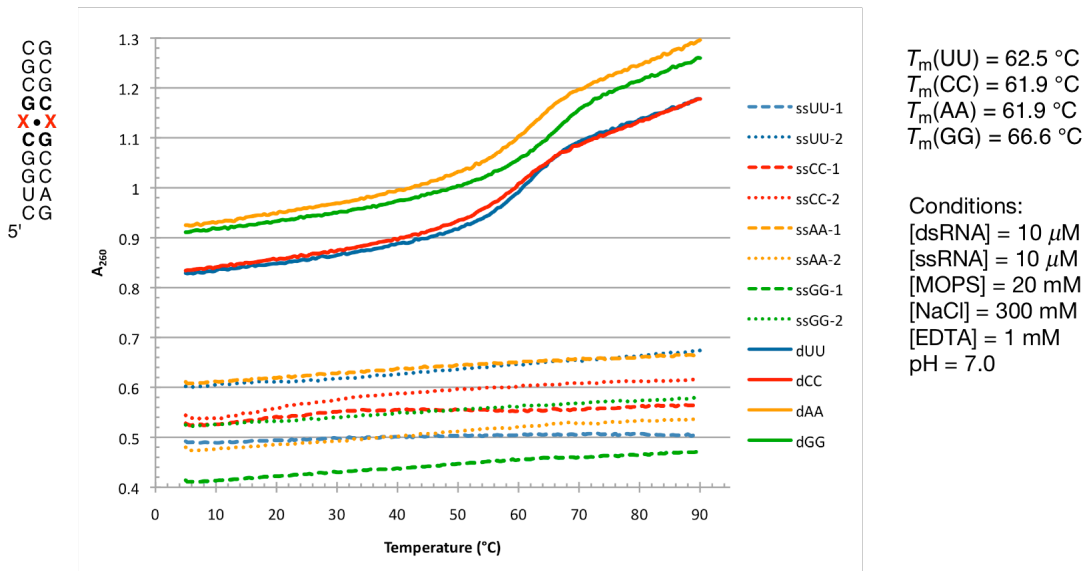


**Figure S2.** (a) CD spectra showing the change in CD signal upon ligand 1 binding. (b) A plot of CD signal at 288 nm versus the mole ratio of ligand to duplex indicating the saturation of binding sites at about 3 equivalents of ligand 1.

**Melting curves for RNA used:**

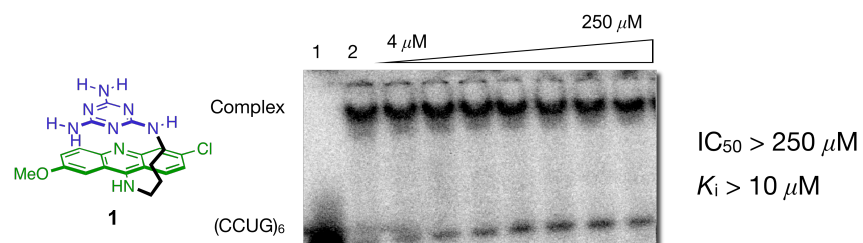


**Figure S4.** Melting curves for RNA duplex containing two mismatches and their corresponding single strands.



**Figure S5.** Melting curves for RNA containing a single mismatch and their corresponding single strands.

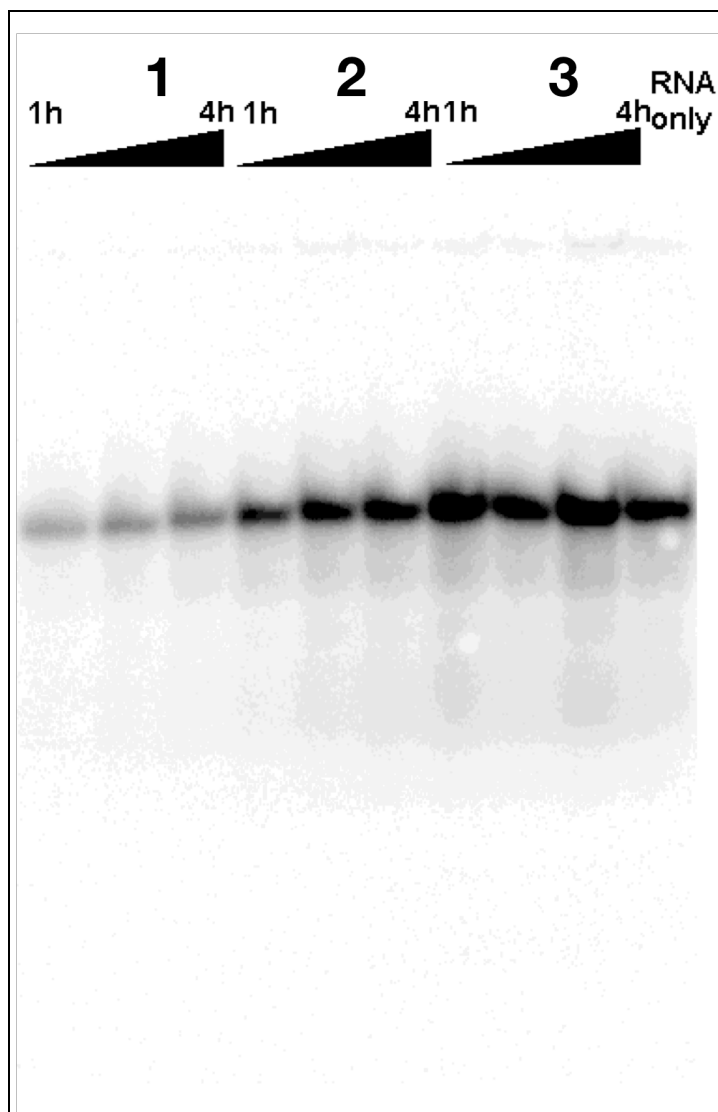
**EMSA:**



**Figure S6.** EMSA of ligand **1** in the presence of 100 nM tRNA. Control lane 1 (C1): RNA only. Control lane 2 (C2): RNA + MBNL1.

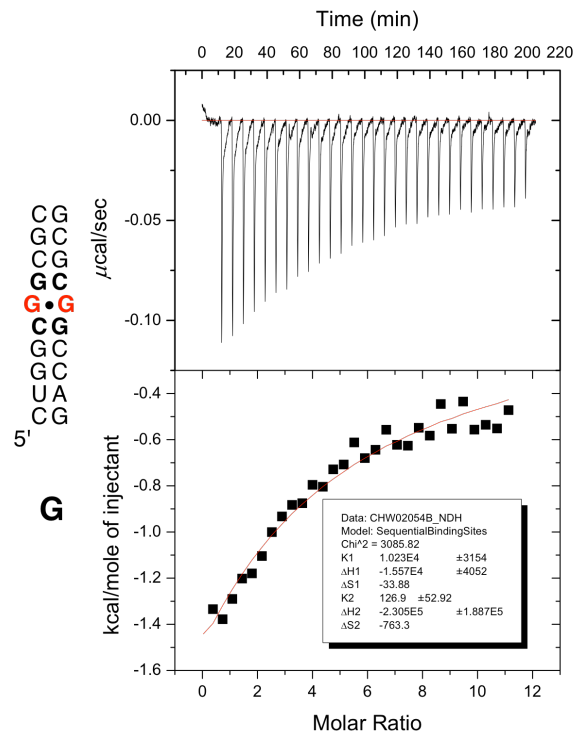
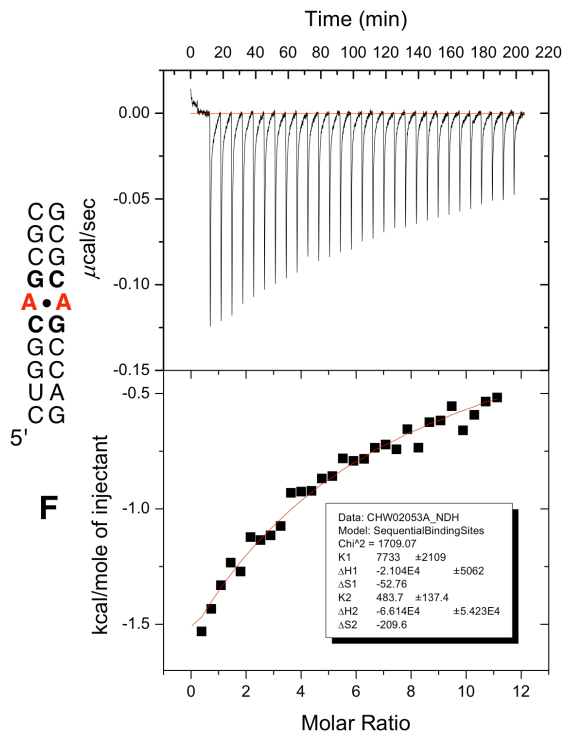
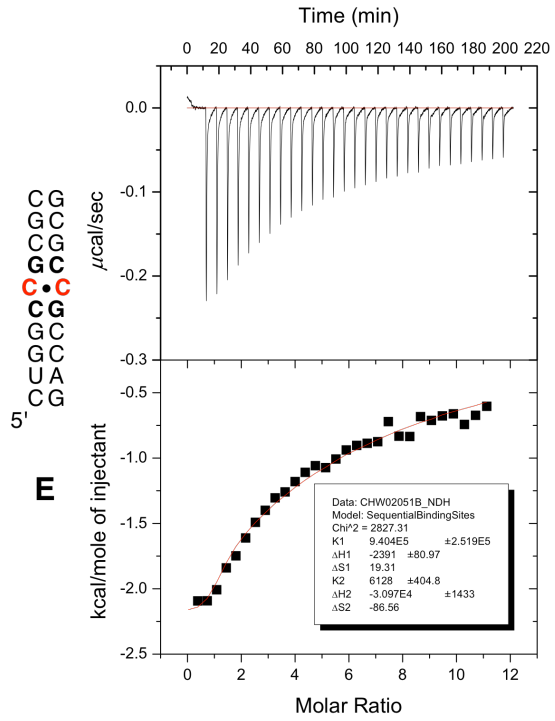
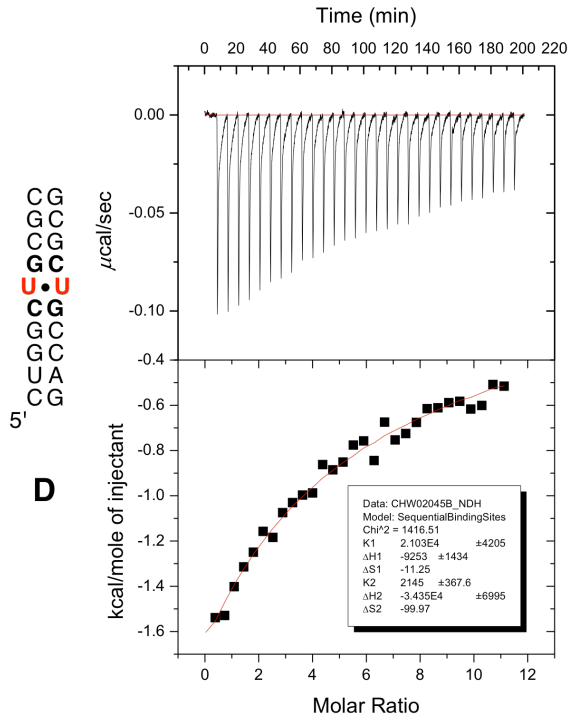
### RNA stability experiment:

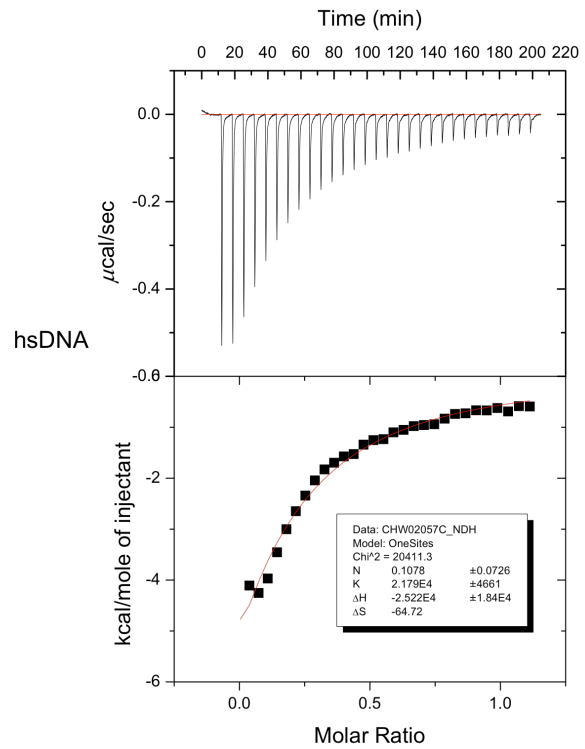
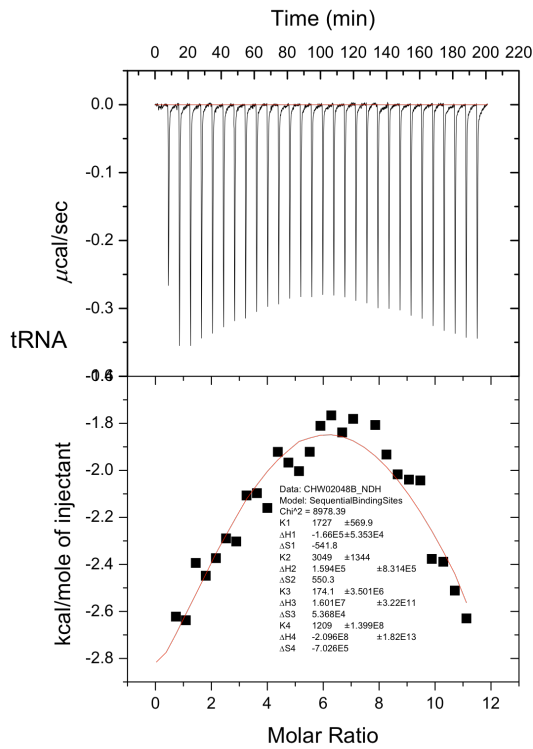
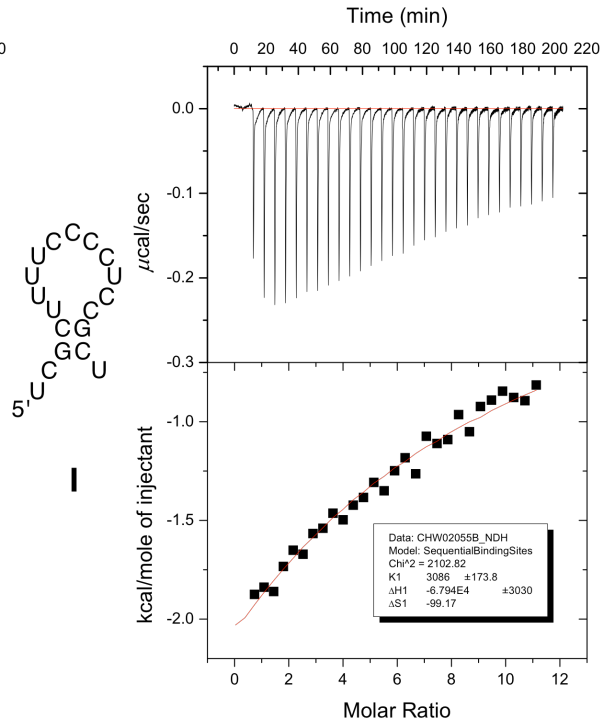
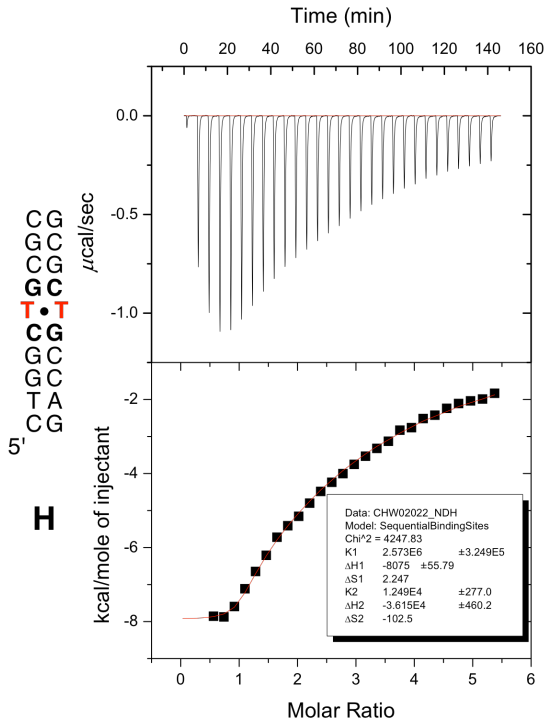
Method: Labeled (CCUG)<sub>6</sub> RNA was annealed by incubation at 95 °C for 1 min and then placed on ice for 20 min in a buffer containing 66 mM NaCl, 6.7 mM MgCl<sub>2</sub> and 27 mM Tris-Cl (pH = 7.5). Ligands dissolved in DMSO were then added to the RNA at a volume ratio of 1:9. The final reaction volume was 20 μl with 1 nM RNA and 200 μM ligands. The reactions were incubated at room temperature for 1–4 h before loading on a 15% polyacrylamide-urea denaturing gel. Electrophoresis was performed at 200 V and the gel was visualized by PhosphorImaging.



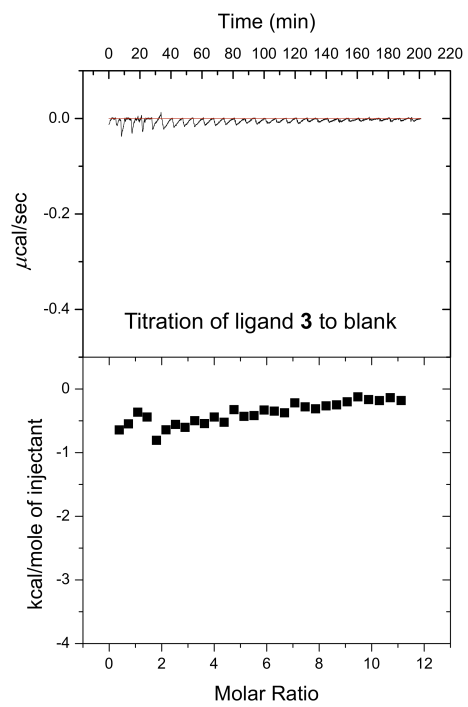
**Figure S7.** These studies showed that ligands 1–3 do not cleave the target RNA.



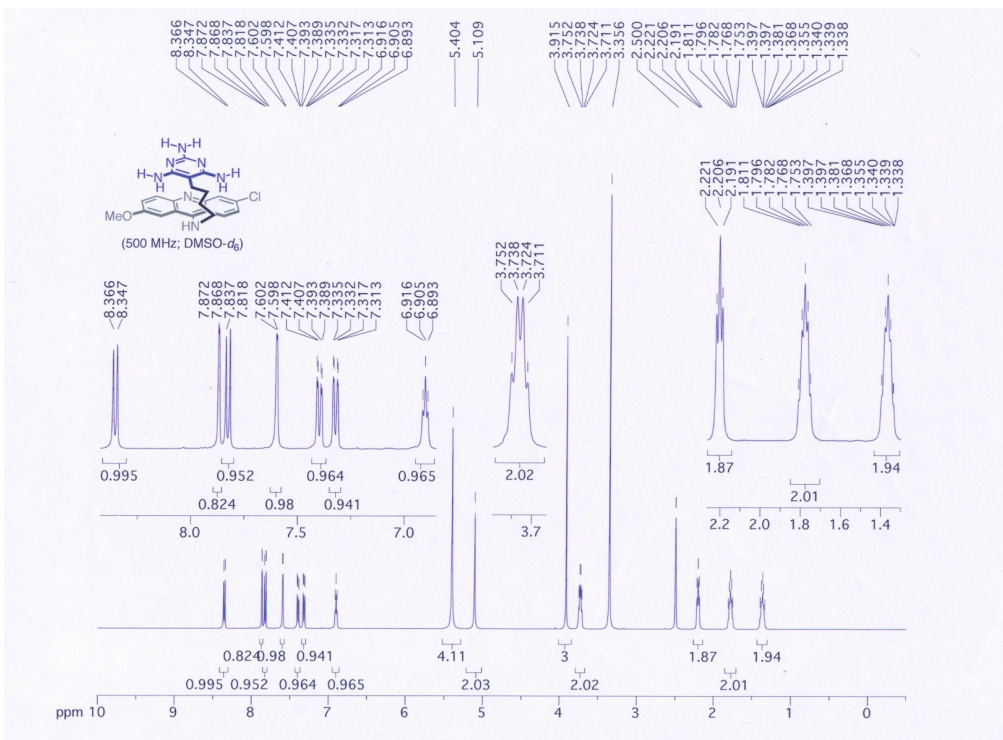




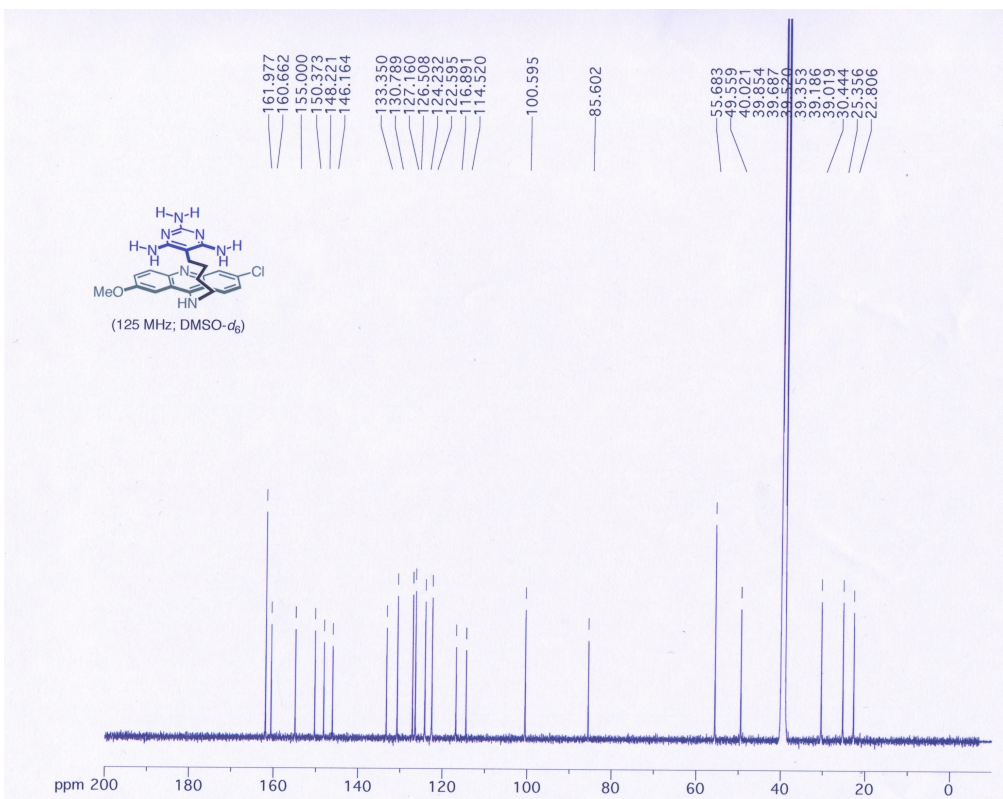




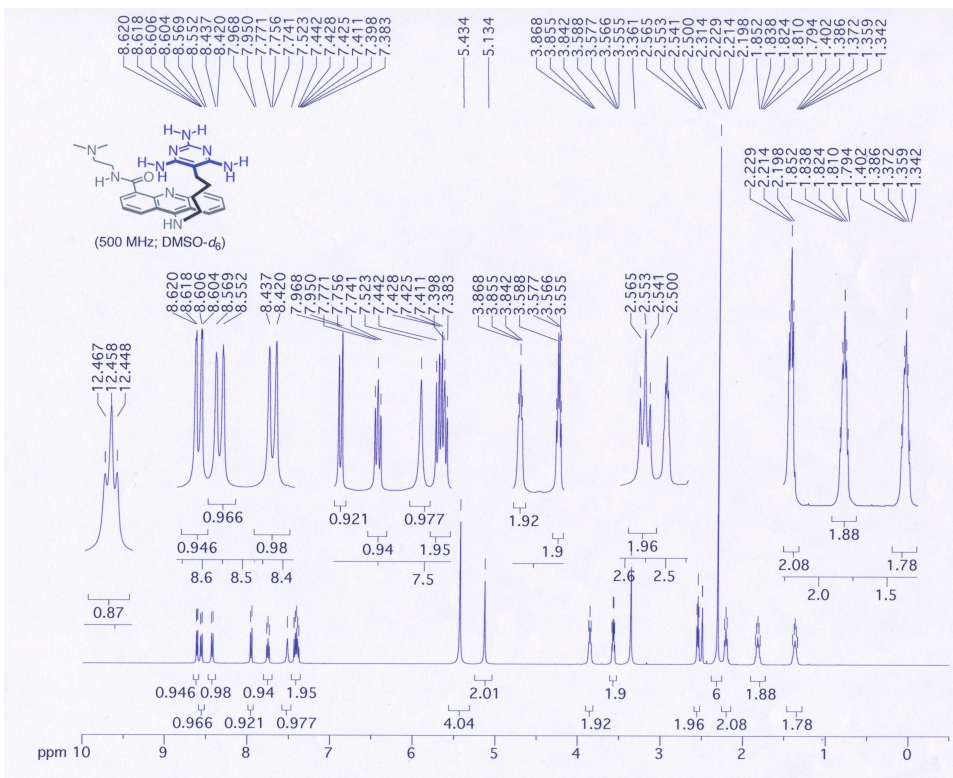
### <sup>1</sup>H NMR of Ligand 2:



### <sup>13</sup>C NMR of Ligand 2:



### <sup>1</sup>H NMR of Ligand 3:



### <sup>13</sup>C NMR of Ligand 3:

