Supplementary Information

The Role of Spatial Arrangement of Aromatic Rings on the Binding of *N*,*N*'-Diheteroaryl Guanidine Ligands to the G2C4/G2C4 motif DNA

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Table S1. Relative potential energy (kcal/mol) of the conformers of DQG, D3iQG, D1iQG, Q3iQG, and DQzG at ωB97XD/6-31G(d, p) level.

	DQG	D3iQG	D1iQG	Q3iQG	DQzG	DQU	DNpG
[a]The number of conformers	21	19	32	36	11	9	32
Rank 1	[b]0.00	[b]0.00	[b]0.00	[b]0.00	[b]0.00	[b]0.00	0.00
2	[b]2.24	[b]2.17	[b]3.41	[b]0.13	[b]0.46	[b]4.33	0.01
3	9.37	7.27	7.25	[b]2.28	[b]1.03	[b]7.02	0.01
4	9.44	7.50	9.21	7.59	[b]1.18	7.04	0.24
5	10.59	8.72	10.35	7.81	[b]1.42	12.34	0.25
6	16.48	13.58	14.37	8.78	[b]2.22	14.72	2.06
7	17.22	14.34	16.03	9.20	[b]2.41	20.08	2.96
8	17.79	14.40	16.35	9.29	16.45		3.33
9	17.82	15.00	16.45	10.74	16.92		
10	17.84		16.75	15.17			
11	18.56		16.88	15.83			
12			17.15	15.96			
13				16.20			
14				16.26			
15				16.90			
16				17.29			

[a]The conformers with the same energy (converged same structures) are not described.

[b]Planar conformers

Table S2. Summary of superimposition of ring A and B and expected H-bonding pattern in DQzG.

	DQG	DQzG				
	conf-1	conf-1	conf-2	conf-3	conf-4	conf-5
ring A[a]	–	0	1	0	0	1
ring B[a]	–	2	2	1	1	1
H-bonding[b]	DD/AA	ADD/DAA	ADD/DAA	ADD/DAA	ADD/DAA	ADD/DAA
ΔT_m (°C)	19.5			20.2		

[a]The number of ring A and B overlapped with that of DQG conf-1. [b]Hydrogen bonding pattern between DQG analogs and cytosine.

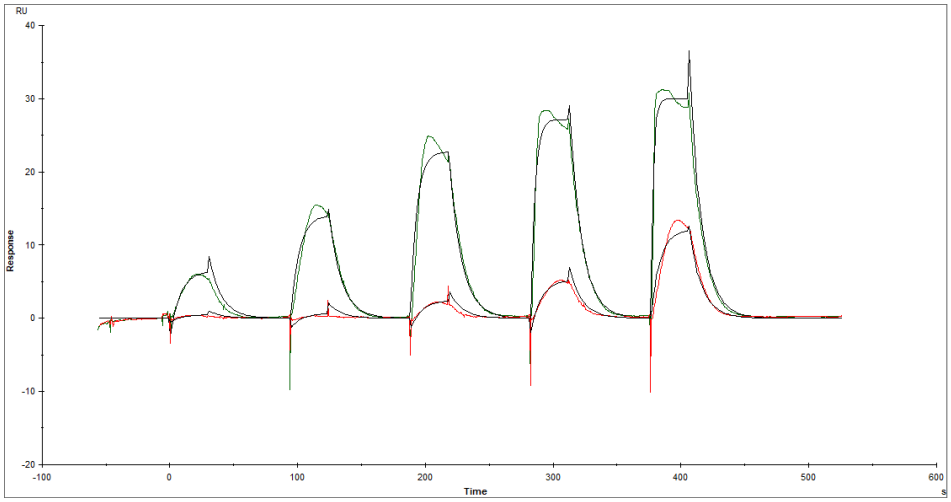
Conf-4 and 5 of DQzG were excluded because they have abundance ratios of 7 and 5%, respectively, in quantum mechanical calculations (Table S1), require a factor contributing 0.5~1 kcal/mol more stabilized energy for G2C4/G2C4 unit in dsDNA than conf-1 and 2, and overlap only one of two ring Bs more important than ring As with conf-1 of DQG upon hydrogen bonding to cytosine (Figure S5 and Table S2).

Table S3. The apparent rate constants (k_{aapp} , k_{dapp}) and dissociation constant (K_{Dapp}) of ligands binding to 5'-Biotinylated G2C4/G2C4 DNA assumed 1:1 binding model by BIAevaluation software.

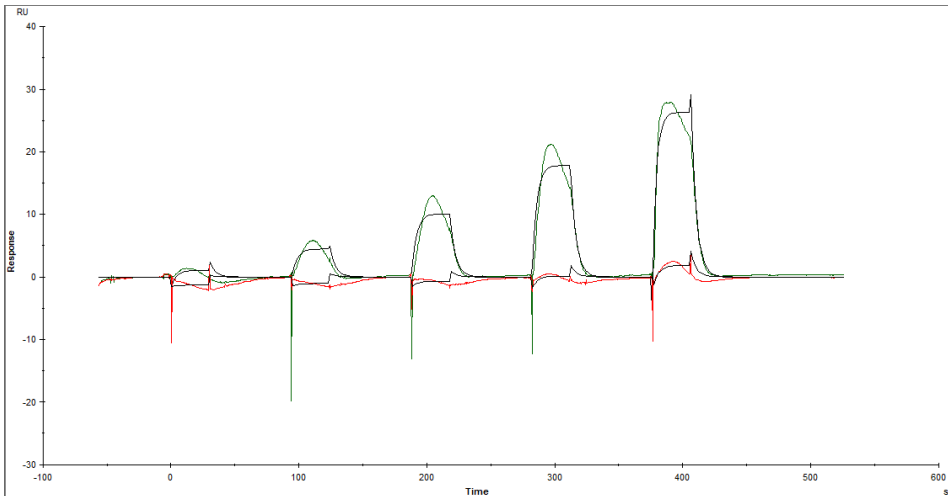
Analyte	k_{aapp} (1/Ms)	SE (k_{aapp})	k_{dapp} (1/s)	SE (k_{aapp})	K_{Dapp} (M)	Chi^2 (RU^2)[a]
DQG	9.94E+04	1.70E+03	0.1031	0.0019	1.04E-06	1.11
D3iQG	2.30E+04	9.70E+02	0.2361	0.0025	1.03E-05	1.27
D1iQG	8.24E+04	2.00E+03	0.2851	0.0035	3.46E-06	0.608
Q3iQG	9.09E+04	1.00E+03	0.1255	0.00072	1.38E-06	1.29
DQzG	5.40E+05	4.80E+03	0.03382	0.00013	6.27E-08	2.91

[a]A measure of the closeness of fit, calculated as the mean squared residual of the difference between the measured data and the fitting curve.

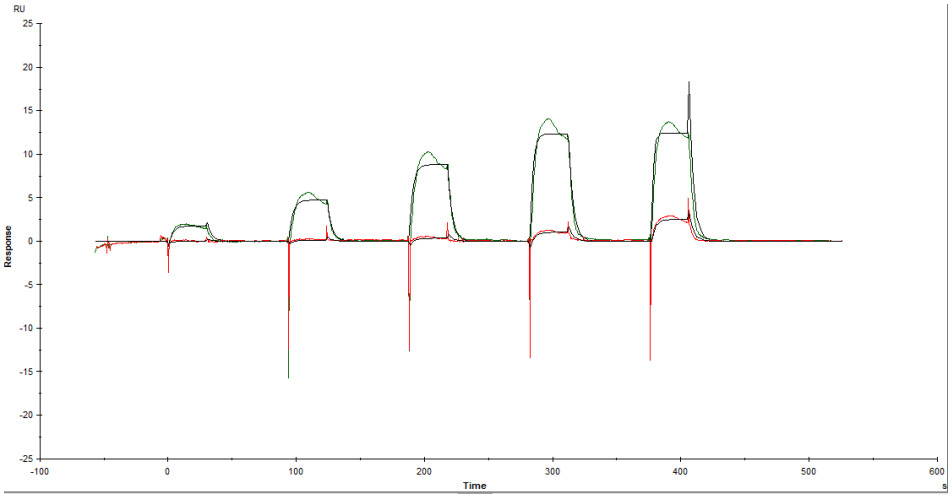
DQG: The fitting curve (black line), the sensorgram at 0.025 - 0.4 μM (red line), and 0.25 - 4.0 μM (green line).



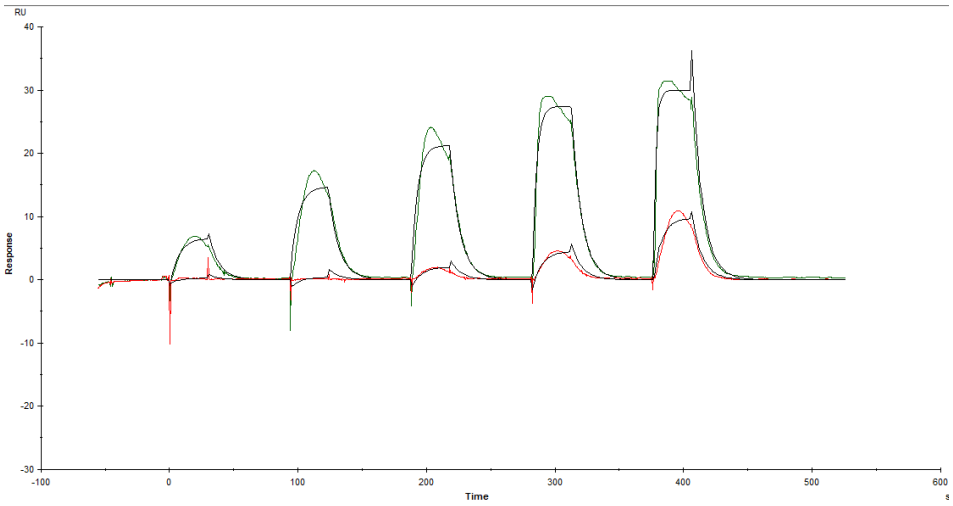
D3iQG: The fitting curve (black line), the sensorgram at 0.025 - 0.4 μM (red line), and 0.25 - 4.0 μM (green line).



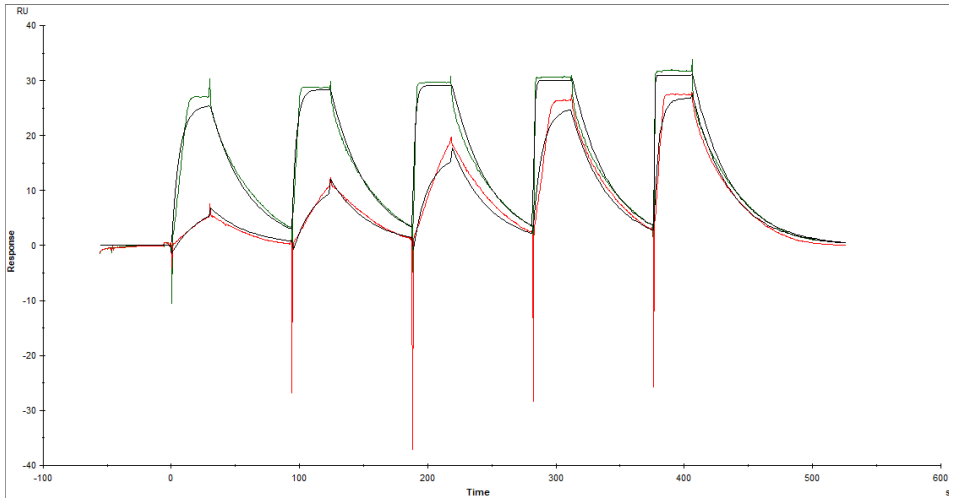
D1iQG: The fitting curve (black line), the sensorgram at 0.025 - 0.4 μM (red line), and 0.25 - 4.0 μM (green line).



Q3iQG: The fitting curve (black line), the sensorgram at 0.025 - 0.4 μM (red line), and 0.25 - 4.0 μM (green line).



DQzG: The fitting curve (black line), the sensorgram at 0.025 - 0.4 μM (red line), and 0.25 - 4.0 μM (green line).



In SPR data analysis of DQzG binding to the hairpin RNA containing G2C4/G2C4, we can estimate the binding stoichiometry by using the equation and values:

$$R_{max} = MW_A \times R_L / MW_L \times n,$$

where in our experiments

n = Stoichiometric ratio (number of binding sites per immobilized RNA)

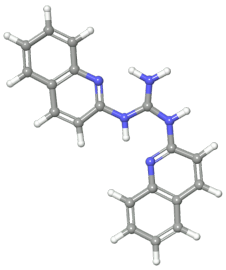
R_{max} (RU) = Maximum binding response, and were about 30 RU

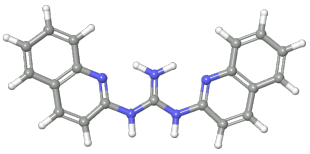
R_L (RU) = immobilization level, and was 515 RU

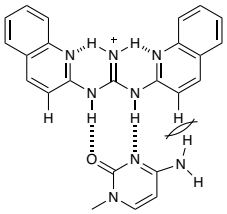
MW_L (Da) = Molecular weight of RNA, as was 10927 Da.

MW_A (Da) = Molecular weight of DQG analogues, and was 315 Da.

(a)

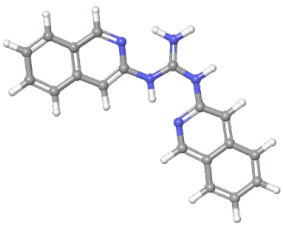


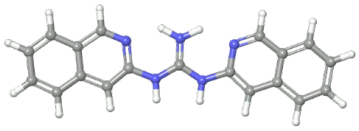




DQG conformer-1 **DQG conformer-2**

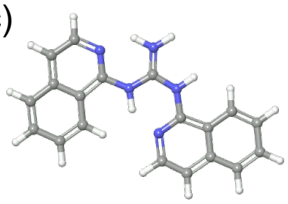
(b)

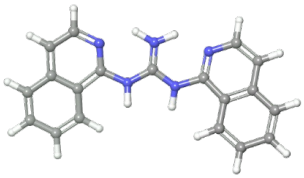


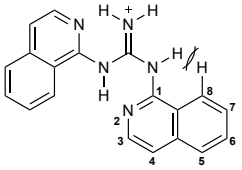


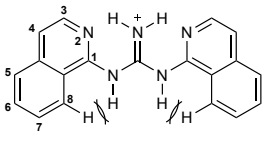
D3iQG conformer-1 **D3iQG conformer-2**

(c)



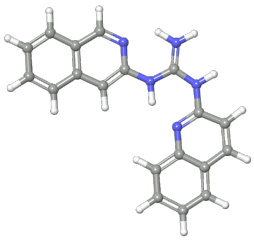


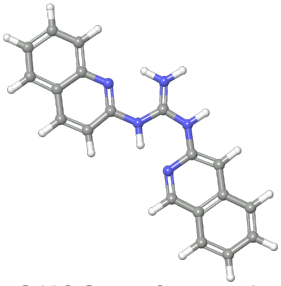


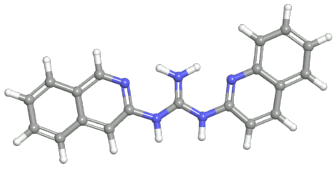


D1iQG conformer-1 **D1iQG conformer-2**

(d)







Q3iQG conformer-1 **Q3iQG conformer-2** **Q3iQG conformer-3**

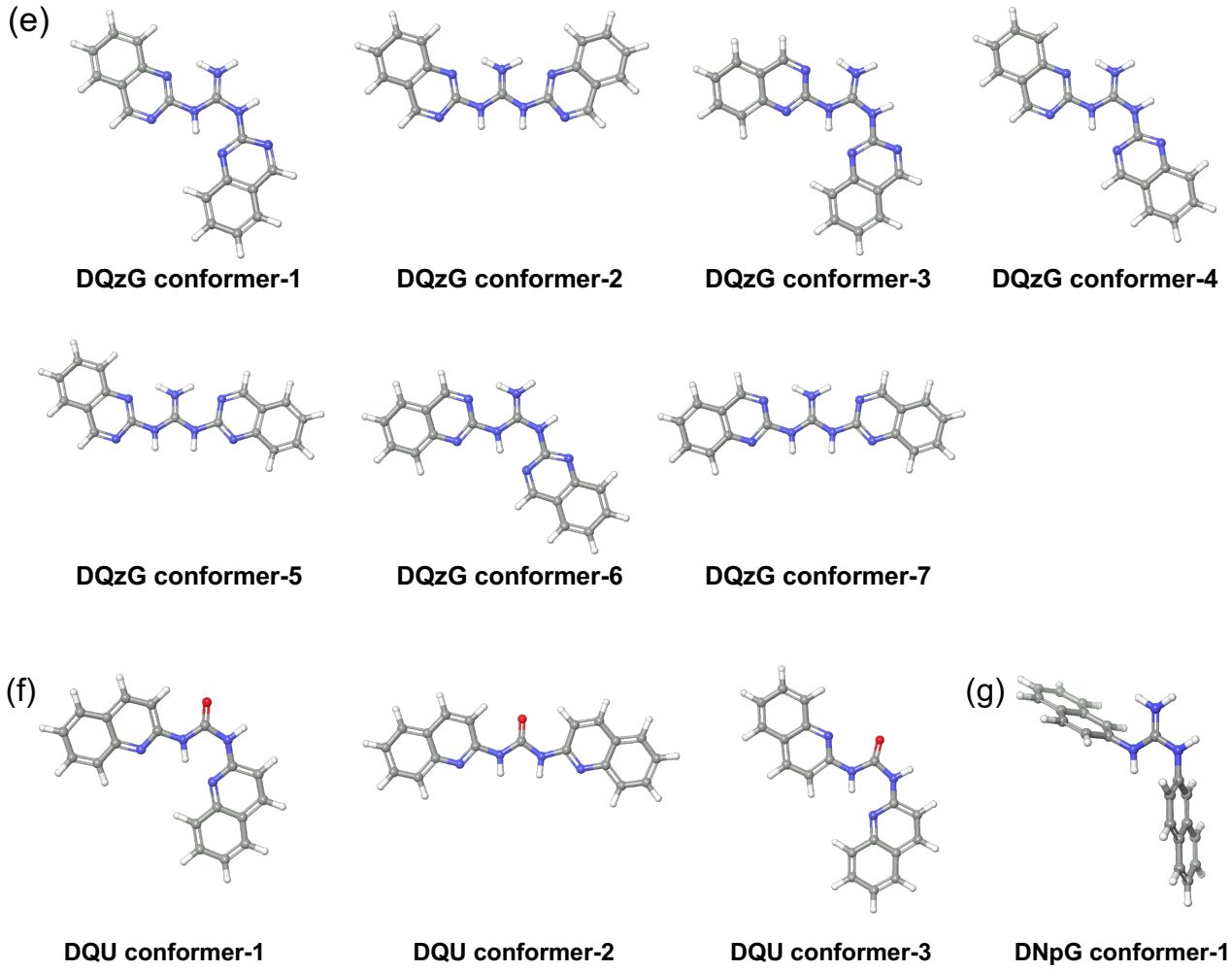


Figure S1. Planar conformers in (a) DQG, (b) D3iQG, (c) D1iQG, (d) Q3iQG, (e) DQzG, and (f) DQU and a representative conformer in (g) DNpG obtained from quantum mechanical calculations.

[a]The entire molecule is distorted with two intramolecular hydrogen bonds.

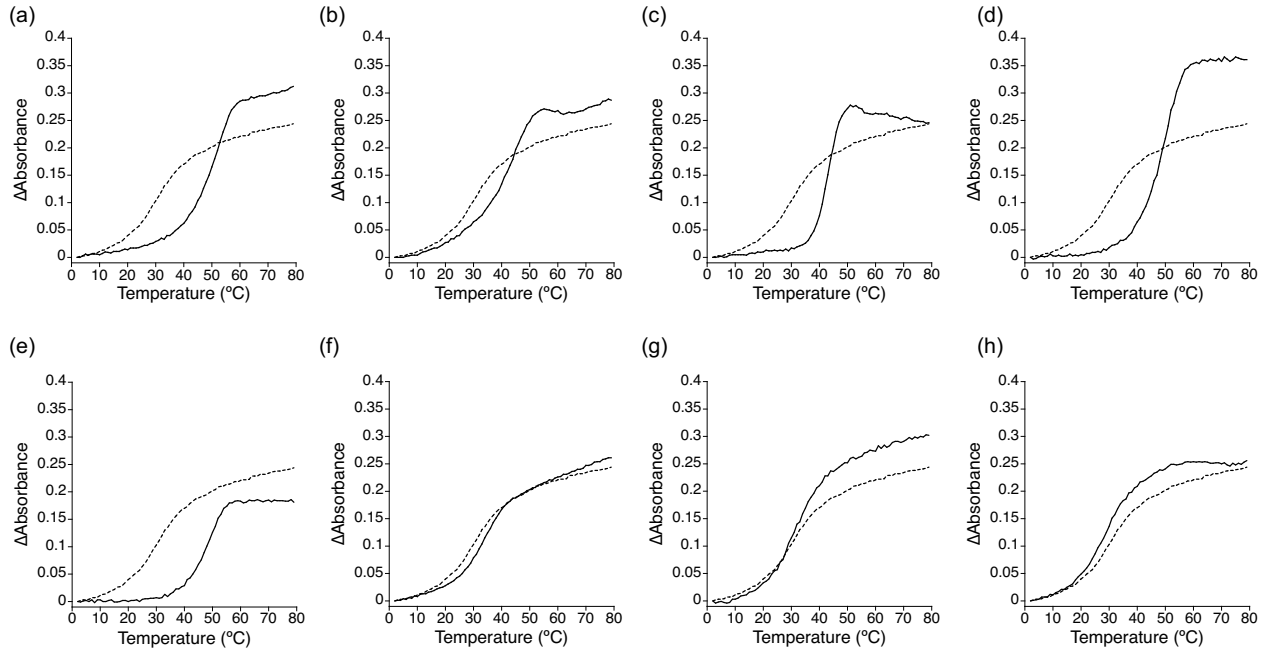


Figure S2. Thermal melting profiles of G2C4/G2C4-dsDNA in the presence (solid line) and absence (dotted line) of ligands, (a) DQG, (b) D3iQG, (c) D1iQG, (d) Q3iQG, (e) DQzG, (f) QG, (g) DNpG, and (h) DQU.

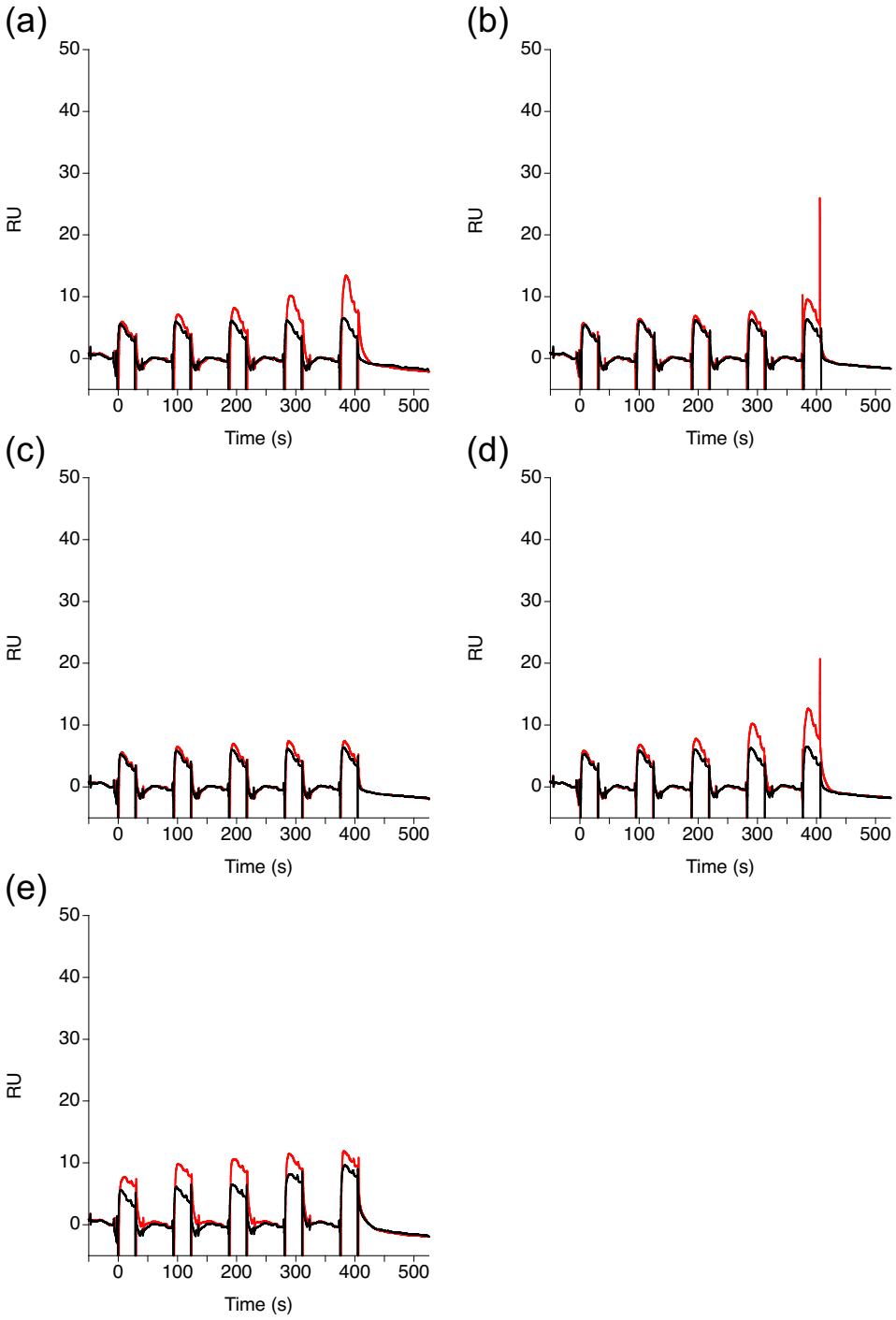


Figure S3. SPR single cycle kinetic analyses of ligand binding to the DNA hairpin 5′-GCATGGCCCCTACGTTTTCGTAGGGGCCATGC-3′. Ligands were applied to the DNA-immobilized surface for 60 seconds, and the sensor surface was subsequently washed with the running buffer for 60 s before the next injection of the ligand. The ligands (a) DQG, (b) D3iQG, (c) D1iQG, (d) Q3iQG, and (e) DQzG were sequentially added at 0.025, 0.050, 0.1, 0.2, 0.4 μM (black line) and 0.25, 0.50, 1.0, 2.0, 4.0 μM (red line).

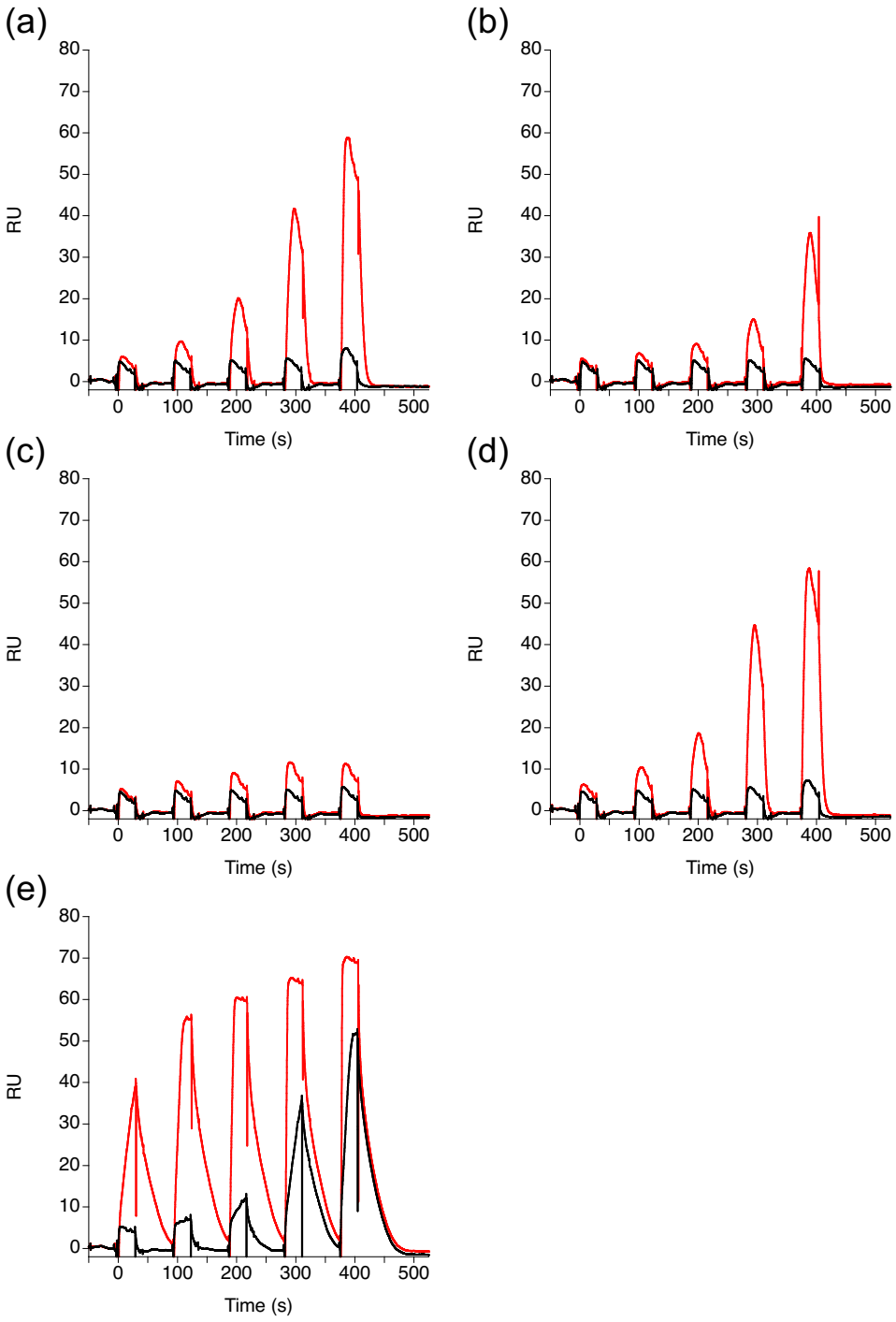


Figure S4. SPR single cycle kinetic analyses of ligand binding to the DNA repeat 5′-(GGCCCC)9-3′. Ligands were applied to the DNA-immobilized surface for 60 seconds, and the sensor surface was subsequently washed with the running buffer for 60 s before the next injection of the ligand. The ligands (a) DQG, (b) D3iQG, (c) D1iQG, (d) Q3iQG, and (e) DQzG were sequentially added at 0.025, 0.050, 0.1, 0.2, 0.4 μM (black line) and 0.25, 0.50, 1.0, 2.0, 4.0 μM (red line).

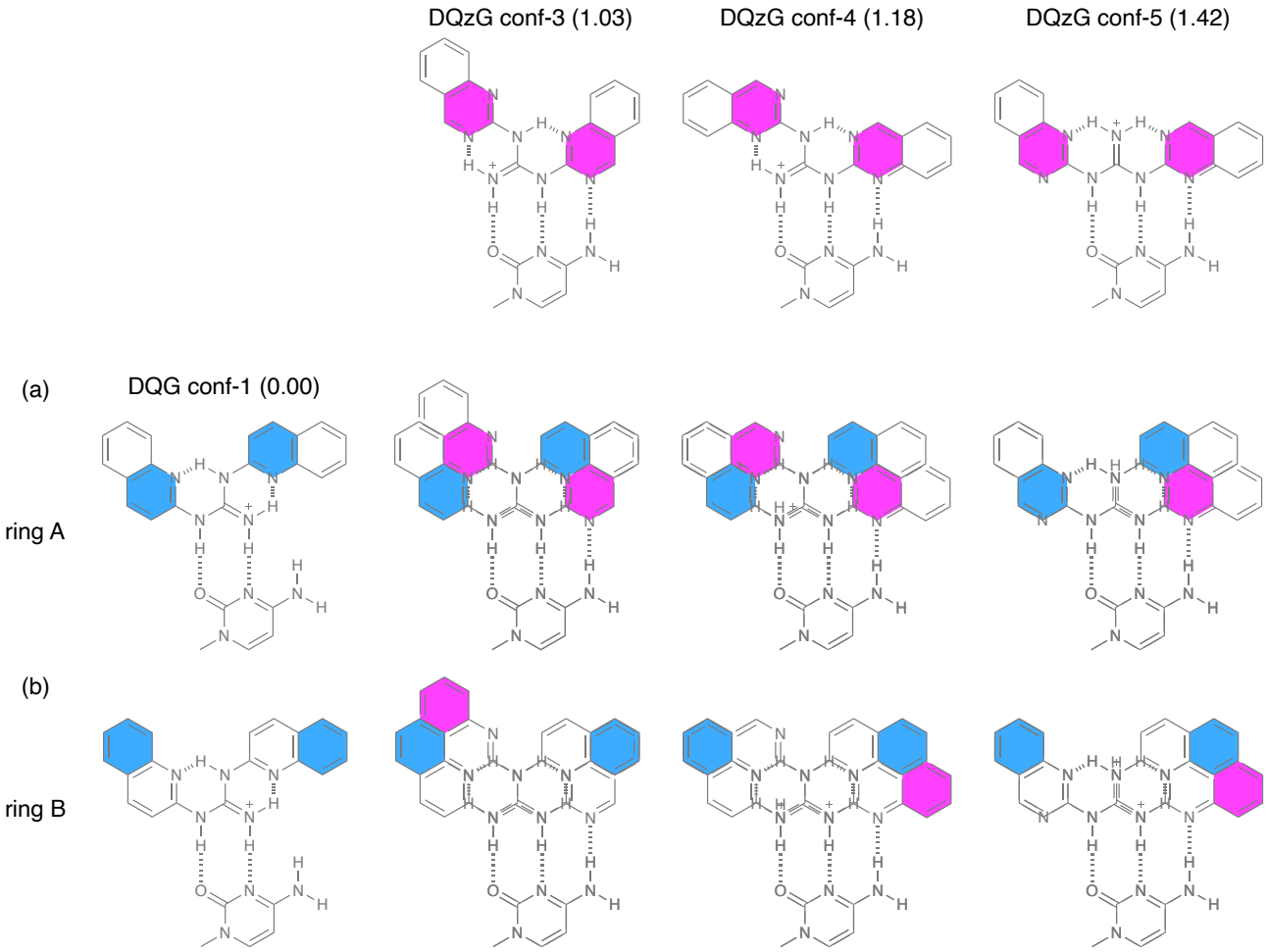
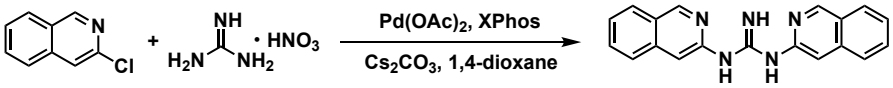


Figure S5. Spatial alignment comparison of (a) ring A and (b) ring B of DQG analogs upon hydrogen bonding to cytosine. DQG conf-1 was superimposed with DQzG conf-3, conf-4, and conf-5 with respect to cytosine. Ring A in (a) or ring B in (b) of DQG conf-1 was colored in cyan, whereas those of others are colored in magenta. In the superimposed figures, the overlapped ring A or B is shown only in cyan; otherwise, both cyan and magenta-colored ring A or B are visible. The numbers in parentheses indicate the energy difference (kcal/mol) from the lowest energy conformation. Plus sings (+) were omitted from the superimposed structures for clarity.

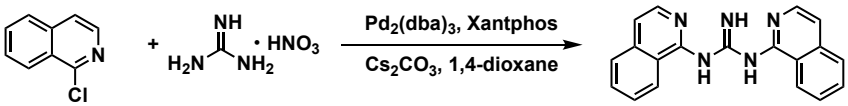
Synthesis of ligands

Synthesis of D3iQG



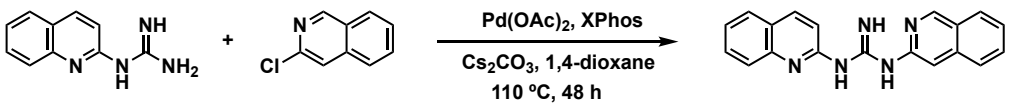
A mixture of guanidine nitrate (373.1 mg, 3.06 mmol), $Pd(OAc)_2$ (0.61 mmol, 137.2 mg), SPhos (1.22 mmol, 501.7 mg) and Cs_2CO_3 (9.17 mmol, 2.99 g) in 1,4-dioxane (25.0 mL) was heated at 100 °C for 1 h under argon atmosphere. And 3-chloroisoquinoline (500.0 mg, 3.06 mmol) in 1,4-dioxane (25.0 mL) was added and heated at 100 ºC for 45 h. The crude product was purified by silica gel chromatography (AcOEt and $CHCl_3$/MeOH = 1:0~10:1). The crude product was removed the solvent, diluted with H_2O and purified by HPLC (eluent: CH_3CN and 0.1% AcOH) to yield pure D3iQG (7.2 mg, 2%). ^{1}H-NMR (CD_3OD, 700 MHz) δ = 9.16 (s, 2H), 8.07 (d, *J* = 8.2 Hz, 2H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.74 (d, *J* = 7.1 Hz, 2H), 7.58-7.55 (m, 4H). ^{13}C-NMR (CD_3OD, 175 MHz) δ = 154.72, 151.48, 149.95, 139.56, 132.83, 129.10, 127.54, 127.29, 127.13, 109.10. HRMS (ESI) Calculated. for $C_{19}H_{15}N_5$: 314.1400 $[C_{19}H_{15}N_5+H]^+$, Found 314.1390.

Synthesis of D1iQG



A mixture of guanidine nitrate (373.1 mg, 3.06 mmol), $Pd(OAc)_2$ (0.61 mmol, 137.2 mg), XPhos (1.22 mmol, 582.8 mg) and Cs_2CO_3 (9.17 mmol, 2.99 g) in 1,4-dioxane (25.0 mL) was heated at 100 °C for 1 h under argon atmosphere. And 1-chloroisoquinoline (500.0 mg, 3.06 mmol) in 1,4-dioxane (25.0 mL) was added and heated at 100 ºC for 21 h. The crude product was purified by silica gel chromatography (AcOEt and $CHCl_3$/MeOH = 1:0~10:1). The crude product was removed the solvent, diluted with H_2O and purified by HPLC (eluent: CH_3CN and 0.1% AcOH) to yield pure D1iQG (3.0 mg, 0.3%). ^{1}H-NMR (CD_3OD, 600 MHz) δ = 8.61 (d, *J* = 8.2 Hz, 2H), 8.21 (d, *J* = 5.5 Hz, 2H), 7.84 (d, *J* = 8.2 Hz, 2H), 7.75 (t, *J* = 7.2 Hz, 2H), 7.68 (d, *J* = 8.2 Hz, 2H), 7.33 (d, *J* = 5.5 Hz, 2H). ^{13}C-NMR (CD_3OD, 175 MHz) δ = 170.33, 156.81, 139.97, 139.10, 131.67, 128.10, 127.93, 125.40, 115.71. HRMS (ESI) Calculated. for $C_{19}H_{15}N_5$: 314.1400 $[C_{19}H_{15}N_5+H]^+$, Found 314.1391.

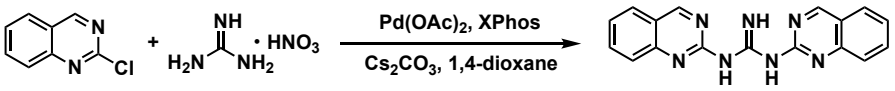
Synthesis of Q3iQG



A mixture of 1-(quinolin-2-yl)guanidine (447.6 mg, 2.40 mmol), $Pd(OAc)_2$ (0.96 mmol, 216.0 mg), XPhos (1.92 mmol, 917.2 mg) and Cs_2CO_3 (7.21 mmol, 2.35 g) in 1,4-dioxane (20.0 mL) was heated at 110 °C for 1 h under argon atmosphere. And 3-chloroisoquinoline (393.2 mg, 2.40 mmol) in 1,4-dioxane (20.0 mL) was added and heated at 100 ºC for 45 h. The crude product was purified by silica gel chromatography (AcOEt/Hex = 1:1 and $CHCl_3$/MeOH = 1:0~10:1). The crude

product was removed the solvent, diluted with H_2O and purified by HPLC (eluent: CH_3CN and 0.1% AcOH) to yield pure Q3iQG (34.3 mg, 6%). ^{1}H-NMR (CD_3OD, 700 MHz) δ = 9.12 (s, 1H), 8.11 (d, *J* = 8.8 Hz, 1H), 8.02 (d, *J* = 8.2 Hz, 1H), 7.82-7.77 (m, 3H), 7.66-7.61 (m, 2H), 7.56 (s, 1H), 7.43 (t, *J* = 7.3 Hz, 1H), 7.35 (t, *J* = 7.3 Hz, 1H), 7.15 (d, *J* = 8.9 Hz, 1H). ^{13}C-NMR (DMSO-D6, 175 MHz) δ = 158.59, 154.25, 150.11, 145.70, 137.87, 137.35, 130.90, 129.53, 127.89, 127.52, 126.30, 125.71, 124.77, 124.36, 123.99, 123.63, 118.646, 107.36, 99.51. HRMS (ESI) Calculated. for $C_{19}H_{15}N_5$: 314.1400 $[C_{19}H_{15}N_5+H]^+$, Found 314.1400.

Synthesis of DQzG

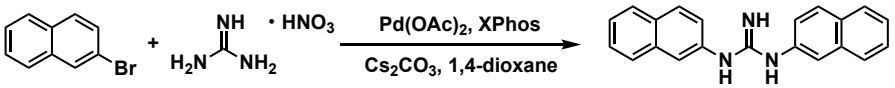


A mixture of guanidine nitrate (148.3 mg, 1.22 mmol), 2-chloroquinazoline (200.0 mg, 1.22 mmol), $Pd(OAc)_2$ (0.24 mmol, 54.6 mg), XPhos (0.49 mmol, 231.7 mg) and Cs_2CO_3 (3.65 mmol, 1.19 g) in 1,4-dioxane (6.0 mL) was heated at 100 °C for 20 h under argon atmosphere. The reaction mixture was added to water and the solvent was evaporated. The resulting mixture was added to chloroform and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was removed the solvent, diluted with H_2O and purified by HPLC (eluent: CH_3CN and 0.1% AcOH) to yield pure DQzG (11.8 mg, 6%). ^{1}H-NMR (CD_3OD, 700 MHz) δ = 9.36 (s, 2H), 7.98 (d, *J* = 7.9 Hz, 2H), 7.91 (dt, *J* = 7.4, 1.3 Hz, 2H), 7.87 (d, *J* = 8.3 Hz, 2H), 7.53 (t, *J* = 7.2 Hz 2H). ^{13}C-NMR (CD_3OD, 175 MHz) δ = 163.88, 160.28, 157.37, 151.29, 136.18, 128.99, 127.04, 126.61, 122.63. HRMS (ESI) Calculated. for $C_{17}H_{13}N_7$: 316.1305 $[C_{17}H_{13}N_7+H]^+$, Found 316.1296.

Synthesis of DQU

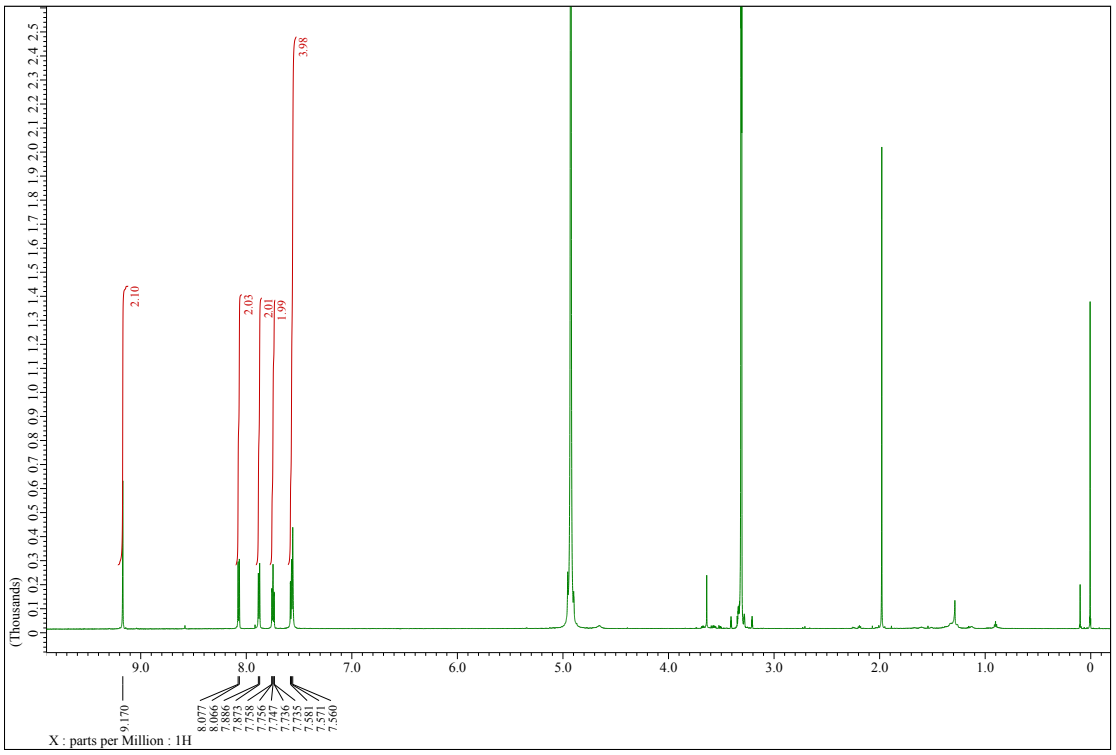
A solution of triphosgene (82.3 mg, 0.28 mmol) in 3.0 mL of CH_2Cl_2 was added to a solution of 2-aminoquinoline (0.20 g, 1.39 mmol) and 4-dimethylaminopyridine (20.3 mg, 1.67 mmol) in 3 mL of CH_2Cl_2. The resulting solution was stirred at room temperature for 9 h. The cold water was added to the reaction solution. The resulting mixture was added to chloroform and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by silica gel chromatography ($CHCl_3$/MeOH = 10:1) to give DQU (11.1 mg, 13%). ^{1}H-NMR (400 MHz, DMSO-d6) δ = 11.52 (br), 8.37 (d, *J* = 9.2 Hz, 1H), 7.94-7.88 (m, 3H), 7.76 (td, *J* = 7.6, 1.4 Hz, 1H), 7.51 (td, *J* = 7.6, 1.4 Hz, 1H). ^{13}C-NMR (151 MHz, DMSO-d6) δ = 152.8, 152.4, 146.2, 139.3, 130.8, 128.4, 127.2, 125.6, 125.3, 114.3. HRMS (ESI) Calculated. for $C_{19}H_{14}O$: 315.1240 $[C_{19}H_{14}O+H]^+$, Found 315.1242.

Synthesis of DNpG

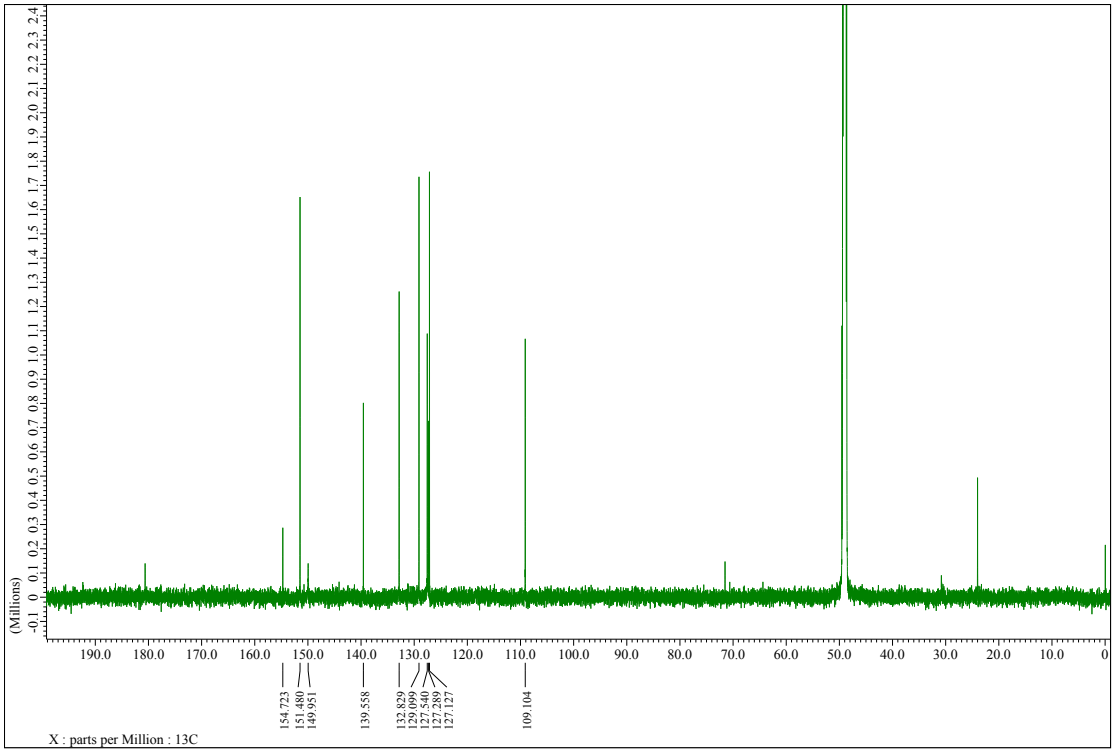


A mixture of guanidine nitrate (118.5 mg, 0.97 mmol), 2-bromonaphthalene (200.0 mg, 0.97 mmol), $Pd(OAc)_2$ (0.19 mmol, 43.6 mg), XPhos (0.39 mmol, 185.0 mg), and Cs_2CO_3 (5.83 mmol, 1.90 g) in 1,4-dioxane (4.0 mL) was heated at 70 °C for 24 h under argon atmosphere. The reaction mixture was added to water and the solvent was evaporated. The resulting mixture was added to chloroform and washed with water and brine. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by amino silica gel chromatography (Hex/AcOEt = 1:1) to give DNpG (19.4 mg, 13%). ^{1}H-NMR (CD_3OD, 600 MHz) δ = 7.82 (dd, *J* = 8.8, 1.9 Hz, 2H), 7.78 (d, *J* = 8.2 Hz, 2H), 7.75 (d, *J* = 8.2 Hz, 2H), 7.71 (s, 2H), 7.39-7.43 (m, 4H), 7.35-7.36 (m, 2H). ^{13}C-NMR (CD_3OD, 151 MHz) δ = 153.14, 142.87, 135.92, 131.67, 129.84, 128.59, 128.10, 127.17, 125.28, 124.07, 119.18. HRMS (ESI) Calculated. for $C_{21}H_{17}N_3$: 312.1495 $[C_{21}H_{17}N_3+H]^+$, Found 312.1491.

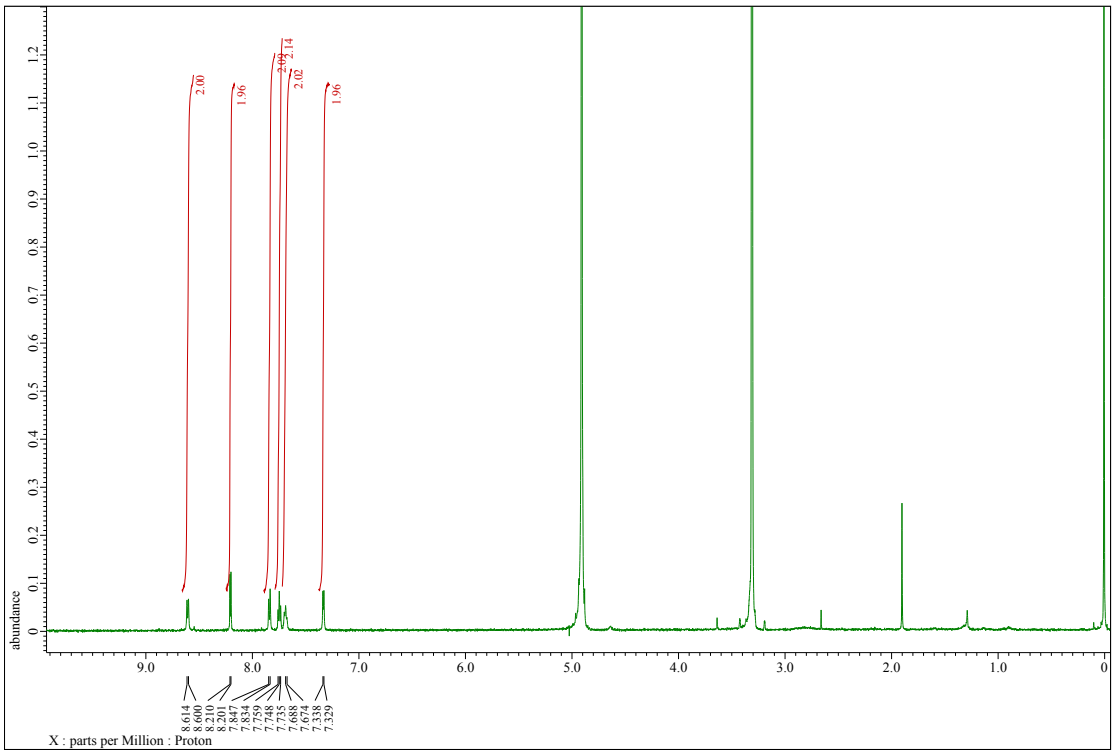
^{1}H NMR of D3iQG



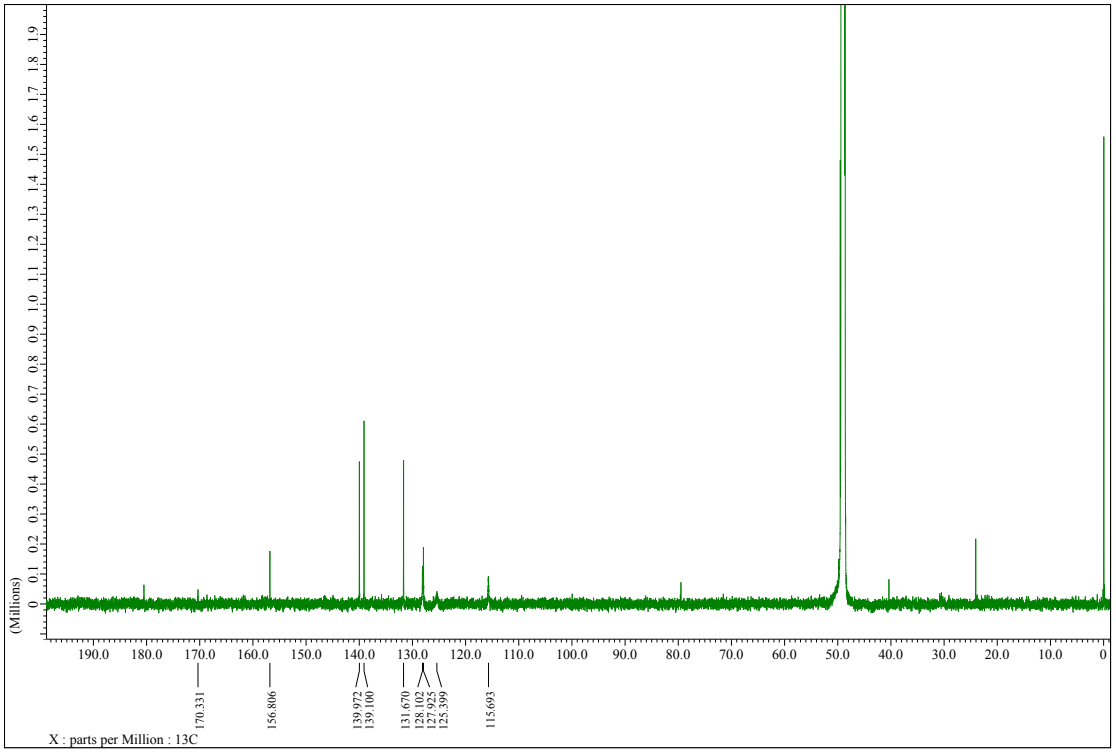
^{13}C NMR of D3iQG



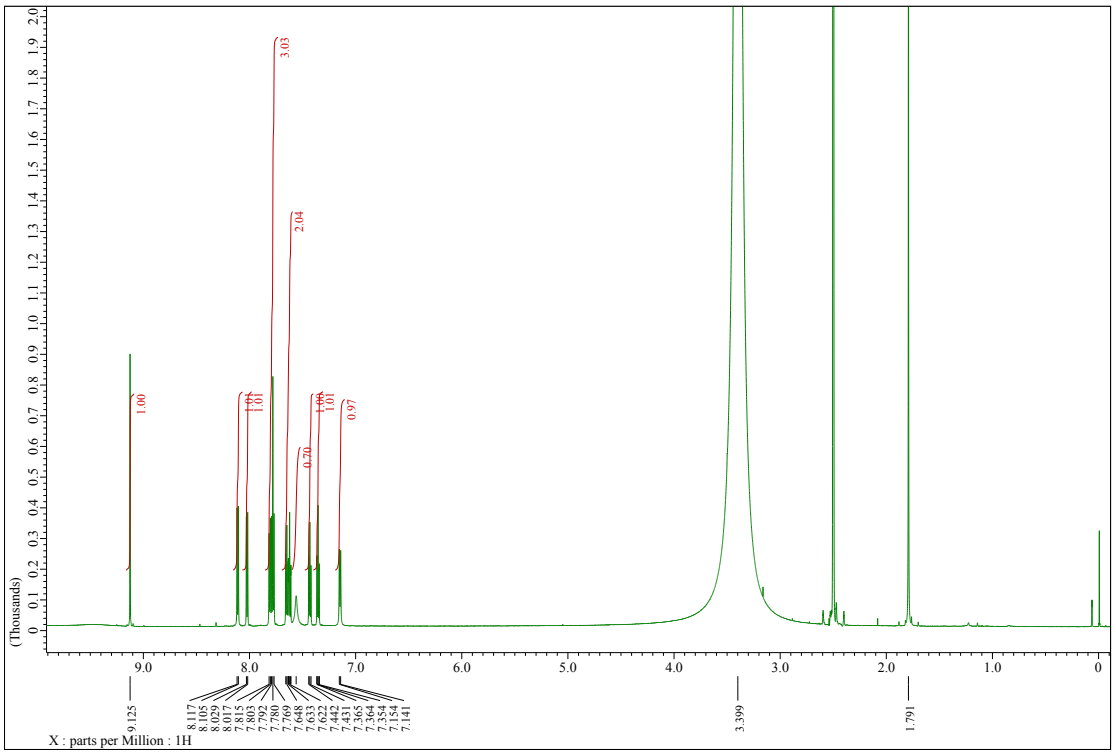
^{1}H NMR of D1iQG



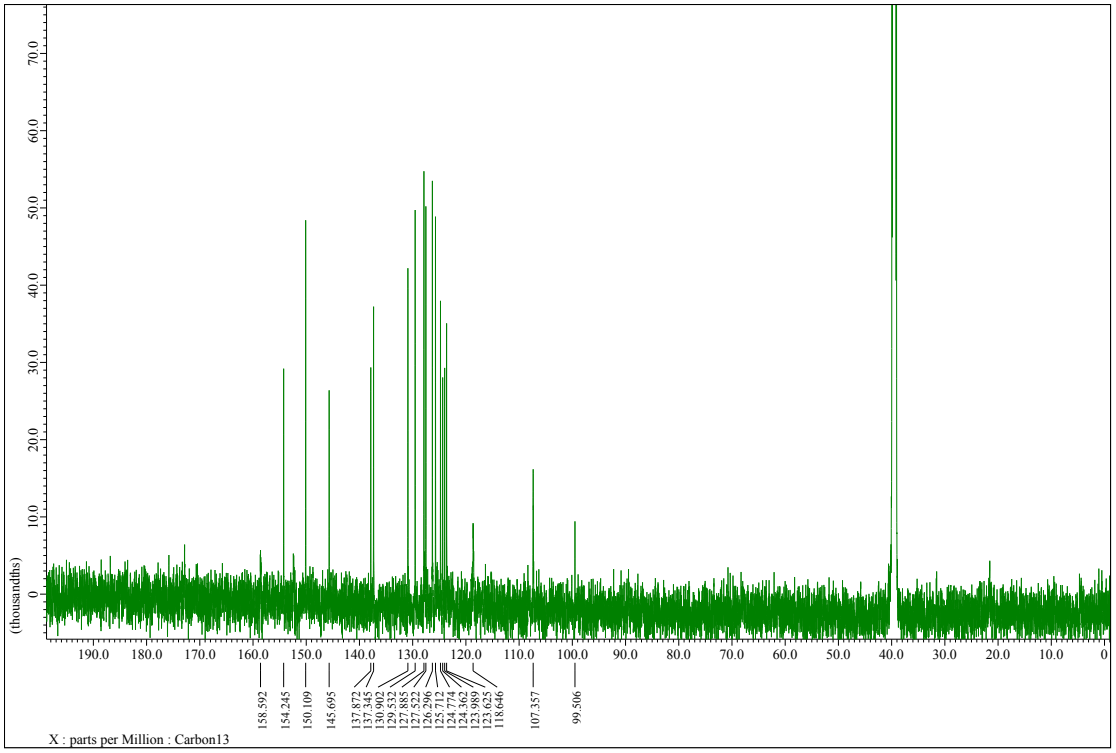
^{13}C NMR of D1iQG



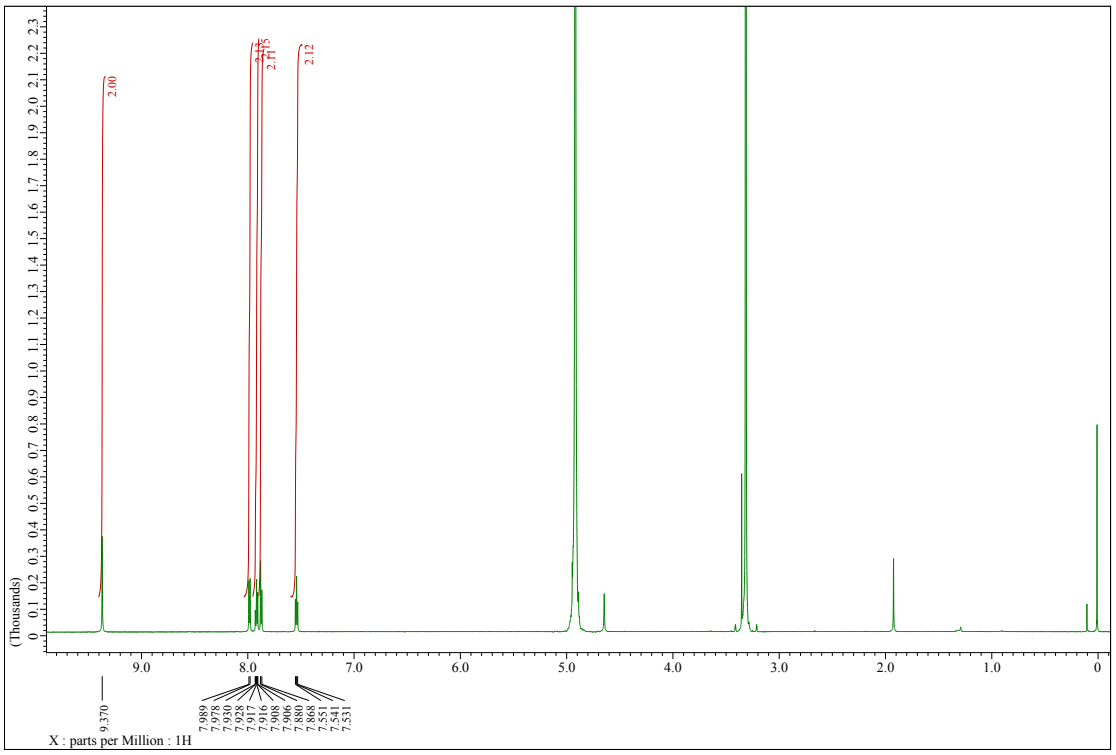
^{1}H NMR of Q3iQG



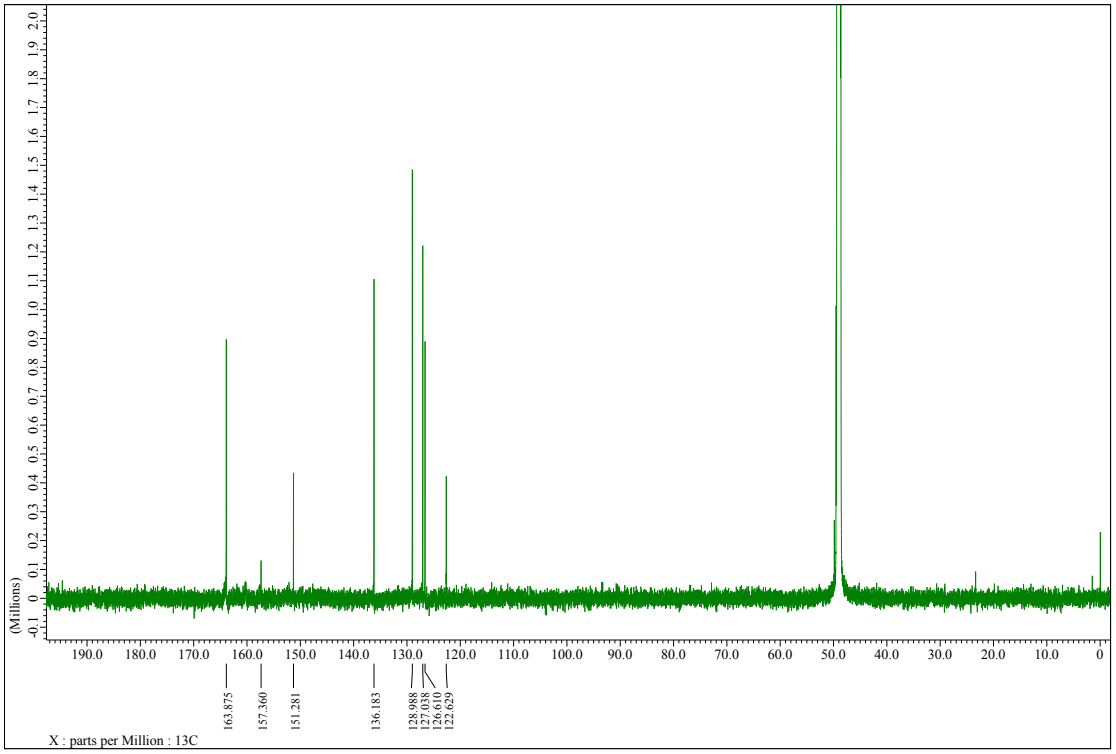
^{13}C NMR of Q3iQG



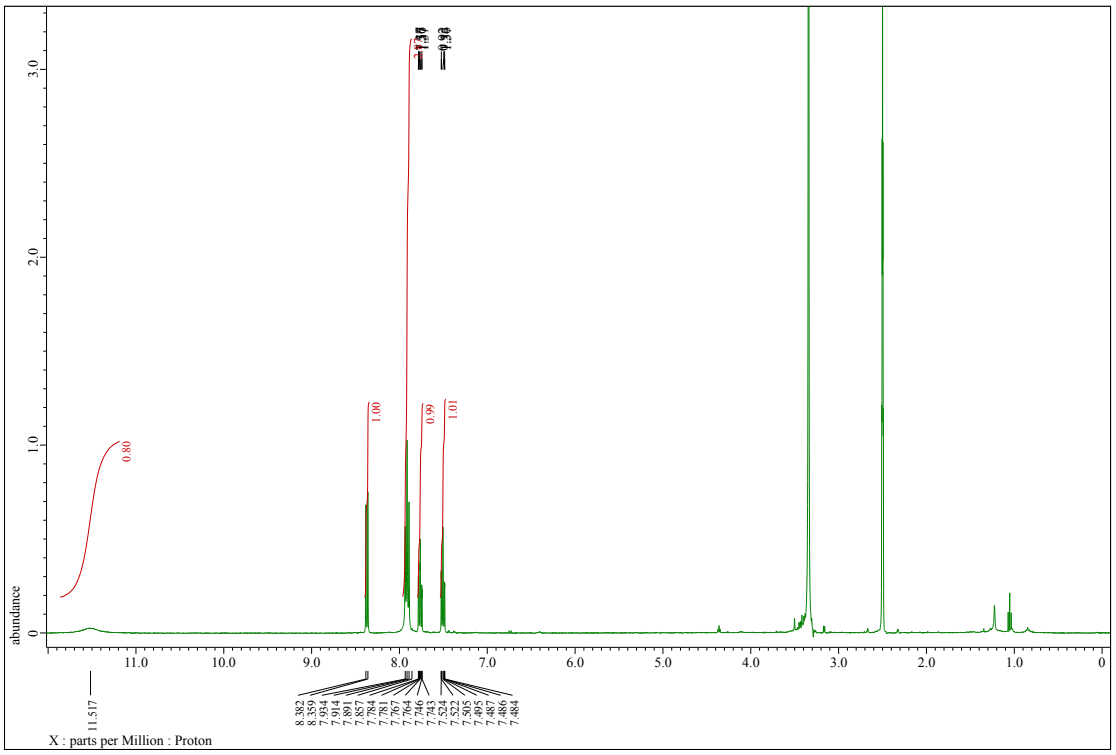
^{1}H NMR of DQzG



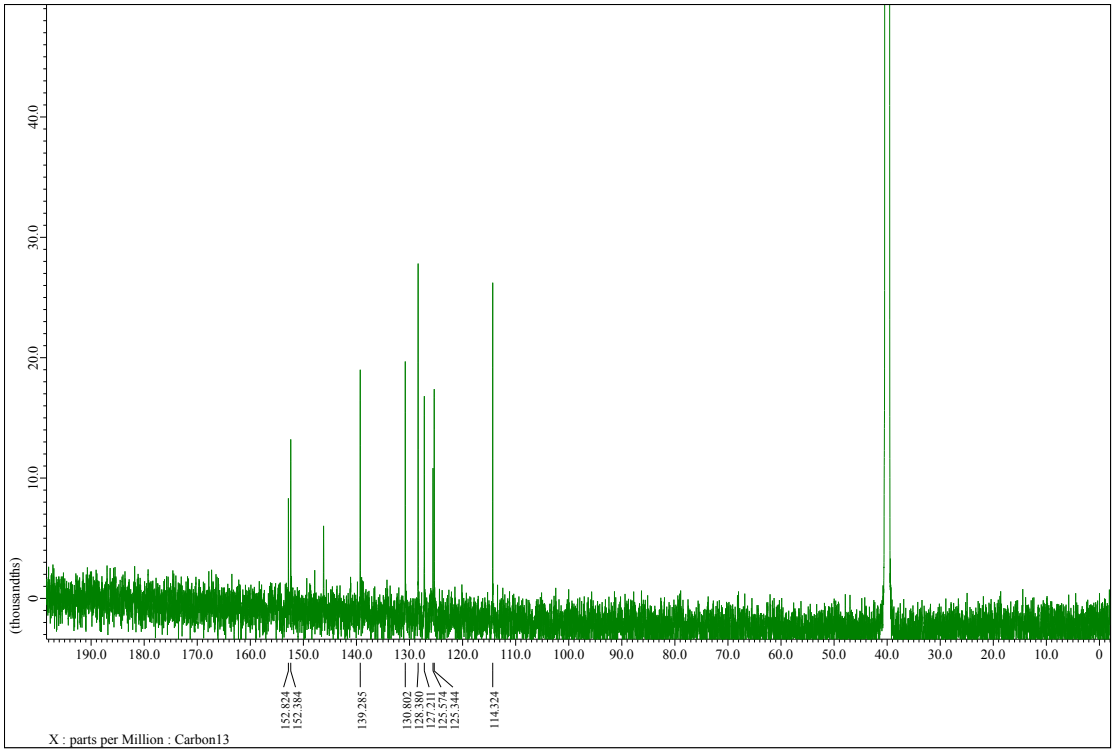
^{13}C NMR of DQzG



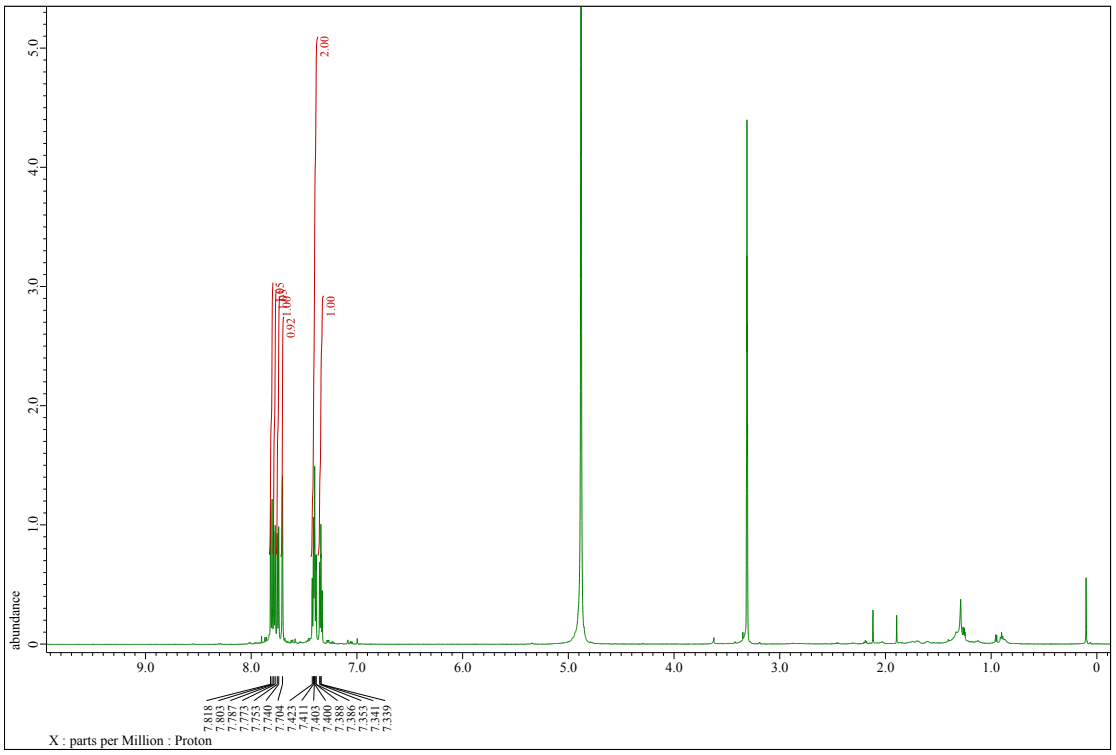
^{1}H NMR of DQU



^{13}C NMR of DQU



^{1}H NMR of DNpG



^{13}C NMR of DNpG