SUPPORTING INFORMATION:

Materials:

Materials/Nucleic Acids. DNA polymers were purchased from Pharmacia Biotech, Inc. (Piscataway, NJ) and were used without further purification. DNA used include poly(dA), lot number: 9017836021; poly(dT), lot numbers: 8107834021 and 9107834021; poly(dA)•poly(dT): lot number: 9067860021; Poly(rA): lot number: 7104110011, Poly(rU): lot number: 9034440021. The concentrations of all the polymer solutions were determined spectrophotometrically using the following extinction coefficients (in units of mol of nucleotide/L)$^{-1}$ cm^{-1}: ε_{257} = 8900 for poly(dA), ε_{265} = 9000 for poly(dT), ε_{260} = 6000 for poly(dA)•poly(dT), ε_{258} = 9800 for poly(rA), ε_{260} = 9350 for poly(rU), ε_{260} = 260,100 for 22dR, ε_{260} = 172,500 for 22dY, ε_{260} = 172,500 for 22dT, ε_{260} = 267,400 for 22A, ε_{260} = 178,800 for 22T.

Chemicals: Neomycin B, 2,4,6-Triisopropylbenzenesulfonyl chloride, Di-tert-butyl dicarbonate, 2-aminoethanethiol hydrochloride and 4-dimethyl-aminopyridine were purchased from Acros (New Jersey) and were used without further purification. 4M HCl/disoxane was purchased from Aldrich and was used without further purification. Methylene chloride, DMF, dioxane were used as received. Pyridine was purified by CaH_2 with distillation before using. BQQ was previously reported. (Escude, C.; Hguyen, C. H.; Kukreti, S.; Janin, Y.; Sun, J.-s.; Bisagni, E.; Garestier, T.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 3591-3596).

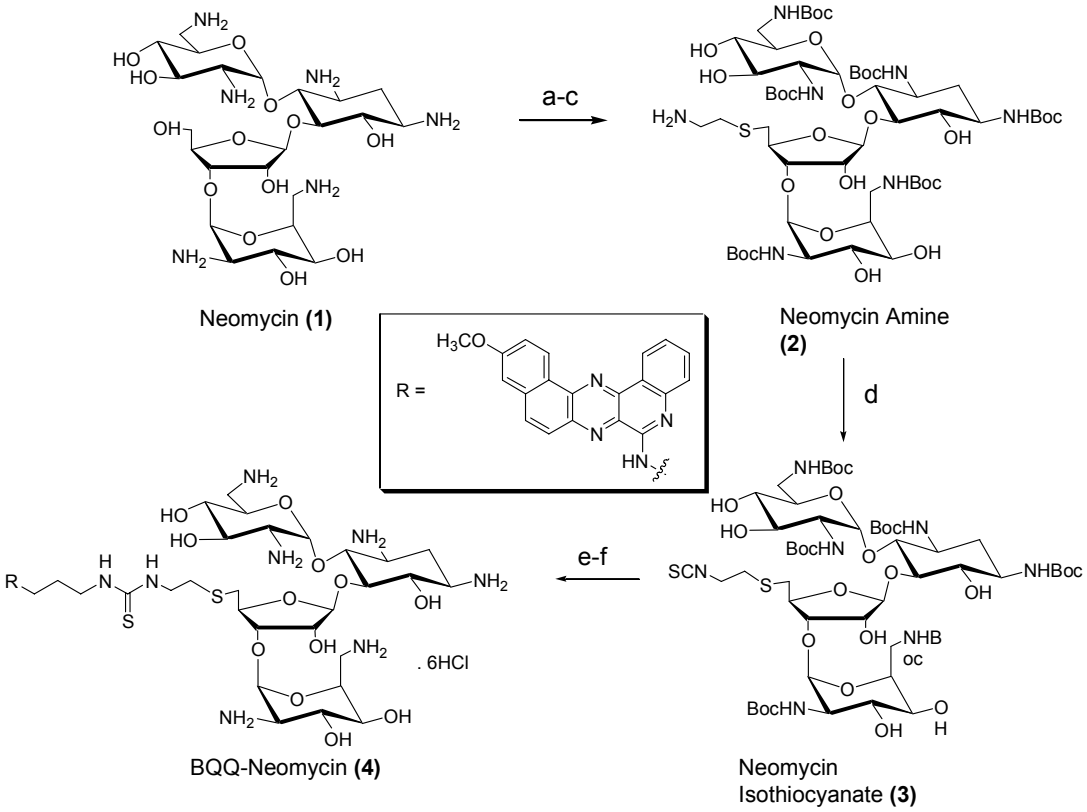


Figure 1. Synthesis of BQQ-Neomycin

BQQ-neomycin Synthesis -Experimental

Compound 1a-1c have previously been reported (Kirk, S. R., Luedtke, N. W., Tor, Y., *J. Am. Chem. Soc.,* 2000, 122, 980-981).

Synthesis of neomycin isothiocyanate (3)

In a 25ml round-bottom flask, neomycin amine (11mg, 0.00863mmol) was dissolved in 10ml anhydrous methylene chloride under N_2. 1,1'-Thilcarbonyldi-2(1H)-pyridone (3mg, 1.5equiv.) was added into the flask and stirred overnight at room temperature. TLC monitored the reaction. Flash column chromatography (elute: 4% CH_3OH in CH_2Cl_2) provided white solid (10mg, yield: 88%) Rf: 0.55 (10% CH_3OH in CH_2Cl_2); ^{1}HNMR (500MHz, methanol-d_4, 25°C): 5.3 (br, 1H), 5.13 (1H), 4.92 (1H), 4.23 (1H), 4.15(2H), 3.89 (2H), 3.76 (2H), 3.40-3.52 (6H), 3.0-3.3 (9H), 2.85-2.9 (4H), 1.89 (2H), 1.4 (m, 54H).

Synthesis of BQQ-neomycin conjugate (Boc-protected) (4')

In a 25ml round-bottom, BQQ (7mg, 0.0183mmol, 1.1 eqiv.) was dissolved in 5ml anhydrous DMF under N_2. Neomycin isothiocyanate (20.6mg, 0.0157mmol) was added into and stirred under N_2 for 7hrs at room temperature. Solvent was removed by vacuum pump. Flash column chromatography (elute: 5% CH_3OH in CH_2Cl_2) offered yellow solid (23mg, yield: 86%) Rf: 0.38 (10% CH_3OH in CH_2Cl_2); ^{1}HNMR (500MHz, methanol-d_4, 25°C): 8.6 (1H), 8.4 (1H), 7.54-7.58 (4H), 7.2 (1H), 7.0 (1H), 6.8 (1H), 5.40 (br, 1H), 5.37 (1H), 5.11 (1H), 4.9 (1H), 4.23 (1H), 4.09(2H), 3.82 (4H), 3.76 (2H), 3.67 (2H), 3.44-3.56 (6H), 3.0-3.3 (9H), 2.84-2.86 (4H), 2.7 (4H), 2.38 (2H), 2.03 (2H), 1.94 (1H), 1.4 (m, 54H). MS (MALDI-TOFMS): calcd 1699, obsd ($M+H^+$)1700.

Synthesis of BQQ-neomycin conjugate (4)

Compound 1d (16mg) was dissolved in dioxane (0.9ml, treated over alumina). 1, 2-ethanedithiol (0.003 ml) was added followed by 4M HCl/dioxane (0.9ml). The solution

was swirled for 5 min by hand upon which a pale yellow precipitate formed. Further precipitation was induced by adding ether and hexane (0.9ml each). Precipitate was recovered by centrifugation and several washes with ether and hexane. The pale-yellow solid was redissolved in water and lyophilized overnight. 6.8 mg yellow solid was obtained. ^{1}HNMR (500MHz, methanol-d_4, 25°C): 9.3 (1H), 9.0 (1H), 8.1 (1H), 7.9 (1H), 7.6 (1H), 7.4 (4H), 5.40 (br, 1H), 5.37 (1H), 5.11 (1H), 4.9 (1H), 4.23 (1H), 4.09(2H), 3.82 (4H), 3.76 (2H), 3.67 (2H), 3.44-3.56 (6H), 3.0-3.3 (9H), 2.84-2.86 (4H), 2.7 (4H), 2.38 (2H), 2.03 (2H), 1.94 (1H); MS (MALDI-TOFMS) calcd 1098, obsd (M^+): 1098. Yield: 58%.

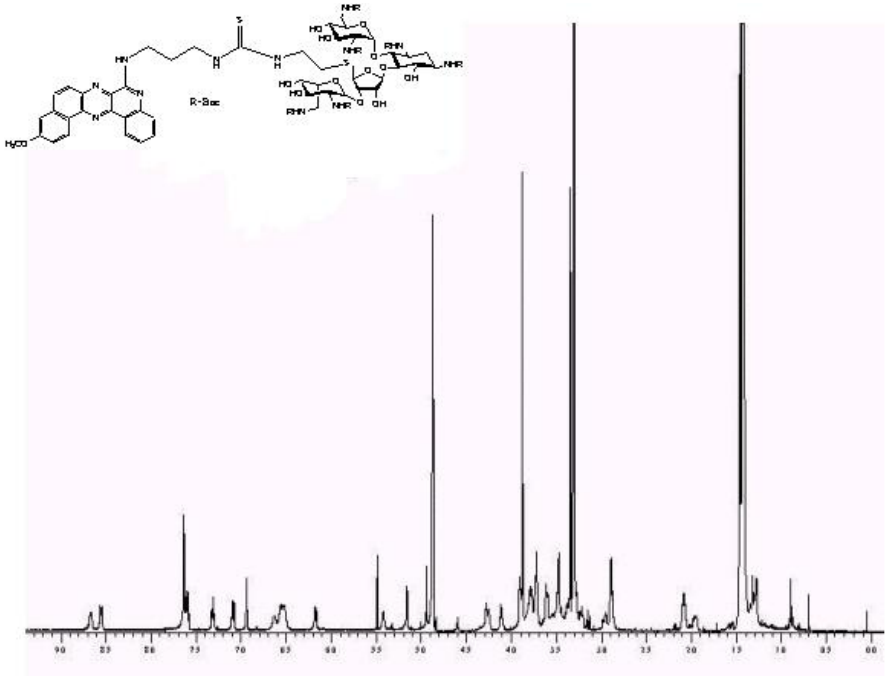


Figure 2. ^{1}H NMR of 4' in CD_3OD

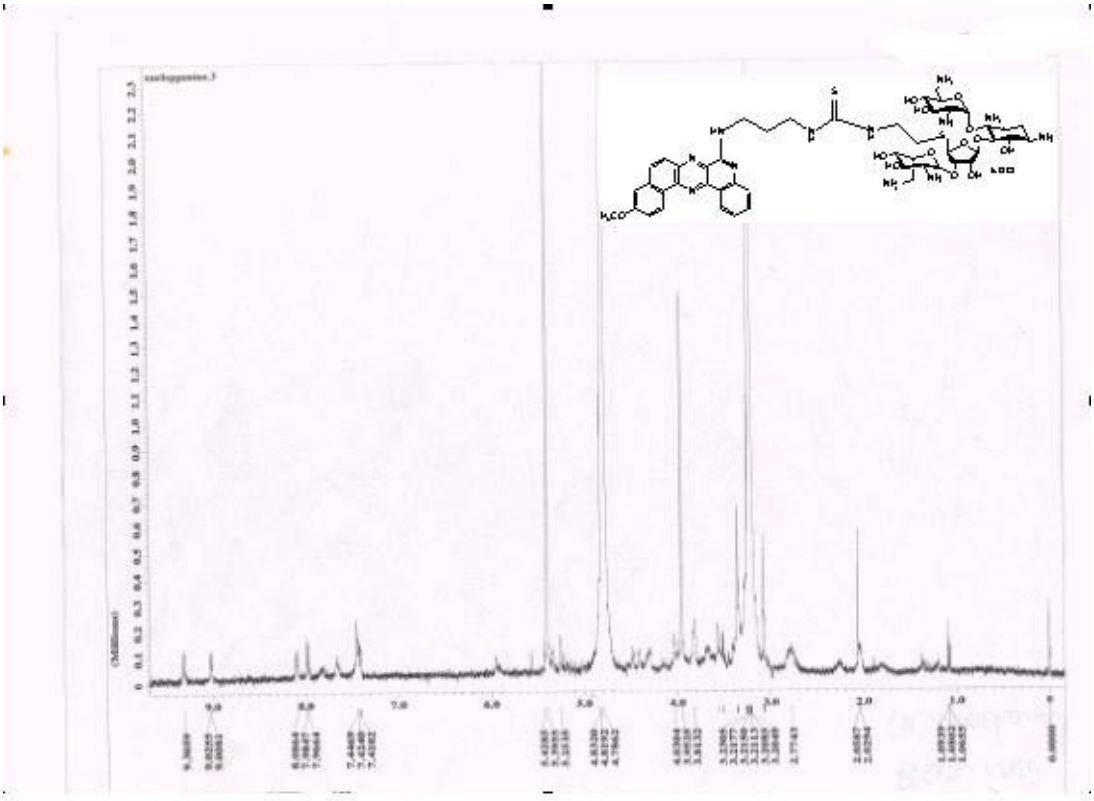


Figure 3. ^{1}H NMR of 4 in CD_3OD

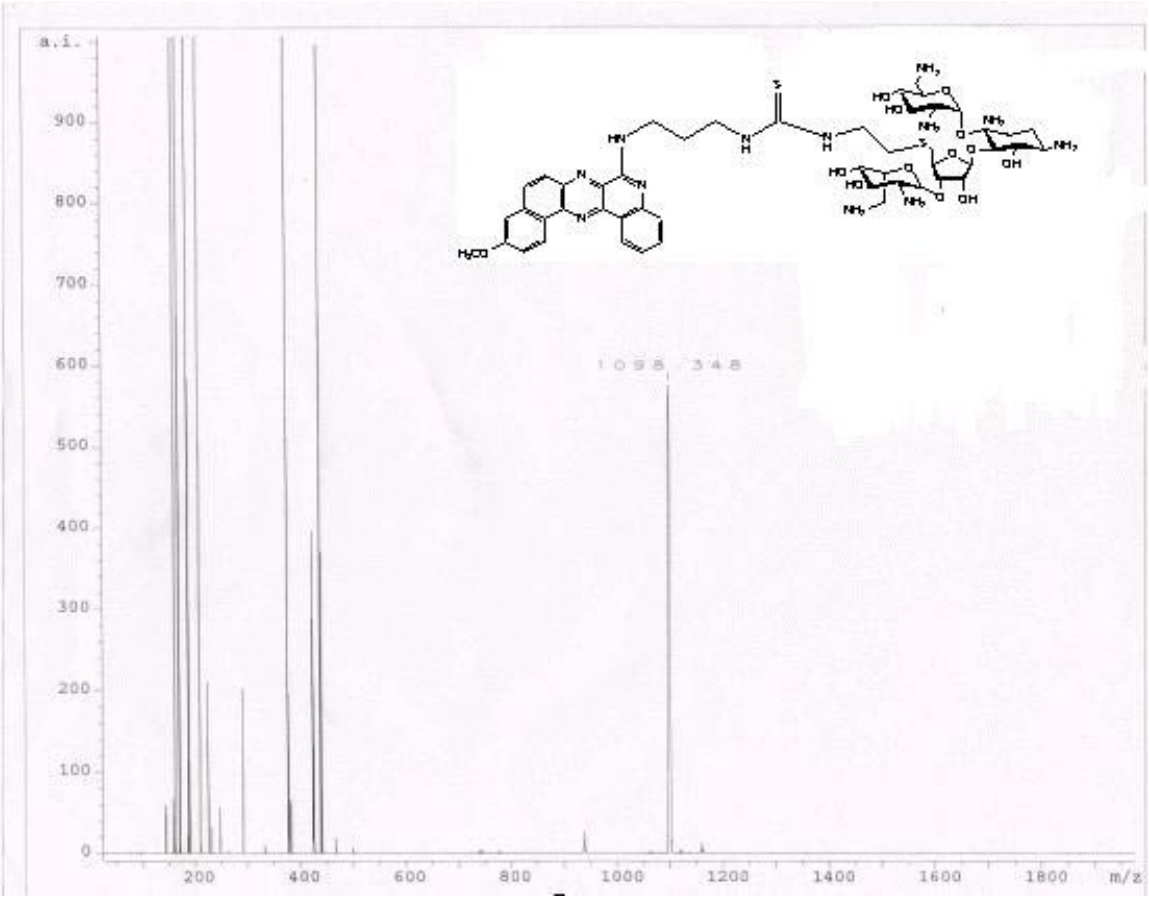


Figure 4a. MALDI-TOFMS of 4

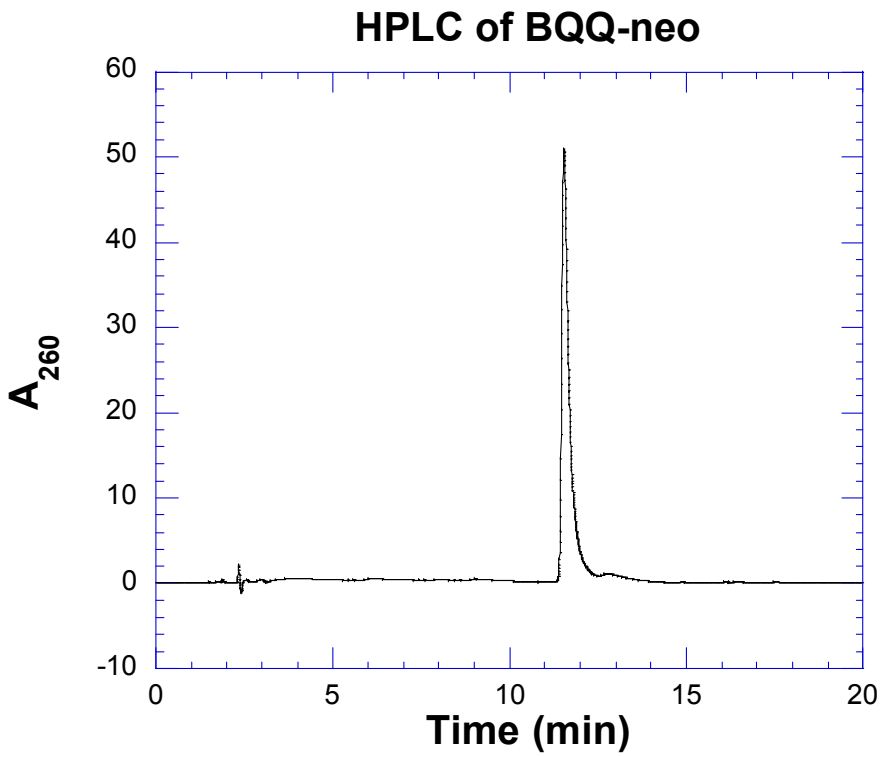


Figure 4b. HPLC of BQQ-neomycin

Condition: Buffer A: 0.1% CF_3COOH/H_2O, Buffer B: 0.08% CF_3COOH/CH_3CN; 0-30% of buffer B over buffer A during 2 min, 30%-100% buffer B over buffer A during 23 min.

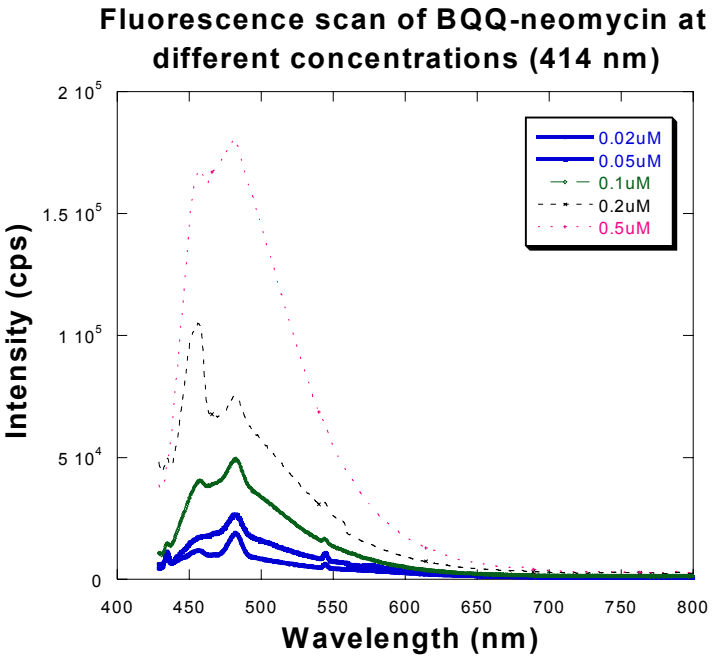


Figure 5. Fluorescence spectrum of BQQ-neomycin

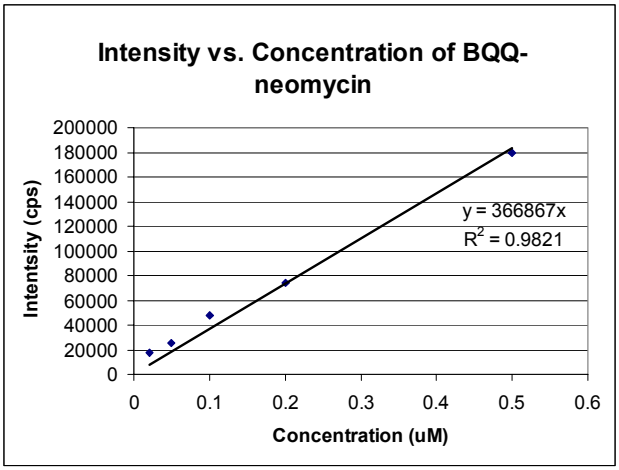
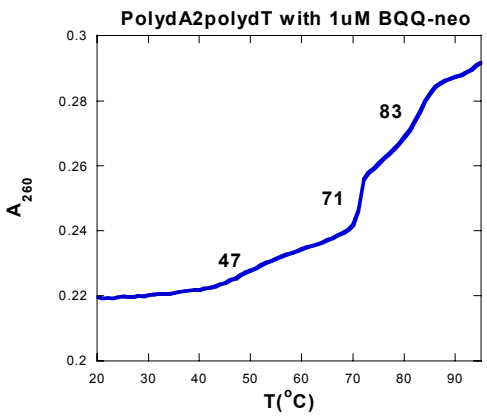
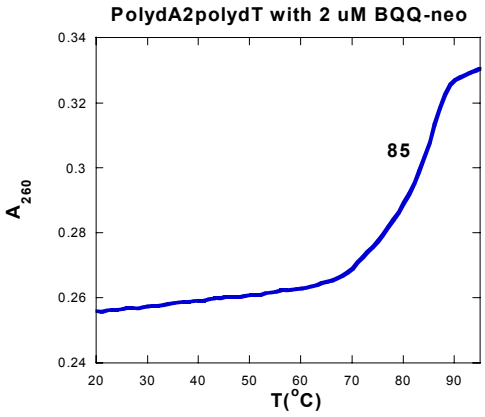


Figure 6: UV melting profiles of the poly(dA)•2poly(dT) in the presence of 150mM KCl at the indicated ligand concentrations. [DNA]=15μM base triplet. Solution conditions: 10 mM sodium cacodylate buffer, 0.5 mM EDTA, pH 7.2. Samples were heated from 20 to 90°C at 5 deg/min, the annealing(90-5°C) and the melting (5-90°C) were conducted at 0.2 deg/min, and the samples were brought back to 20°C at a rate of 5 deg/min.





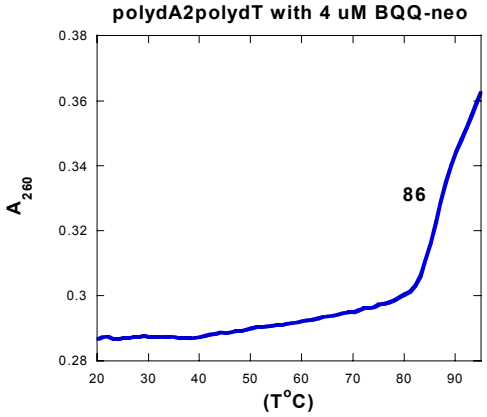


Figure 7: UV melting profiles and annealing profiles of the 2:1 stand mixture **I** in the presence of 150mM KCl at the indicated ligand concentrations. dA_{22} = 1μM/strand; dT_{22} = 2μM/strand [BQQ-neomycin] = 4μM. Solution conditions: 10 mM sodium cacodylate buffer, 0.1 mM EDTA, pH 6.8. Samples were heated from 20 to 90°C at 5 deg/min, the annealing (90-5°C) and the melting (5-90°C) were conducted at 0.2 deg/min, and the samples were brought back to 20°C at a rate of 5 deg/min.

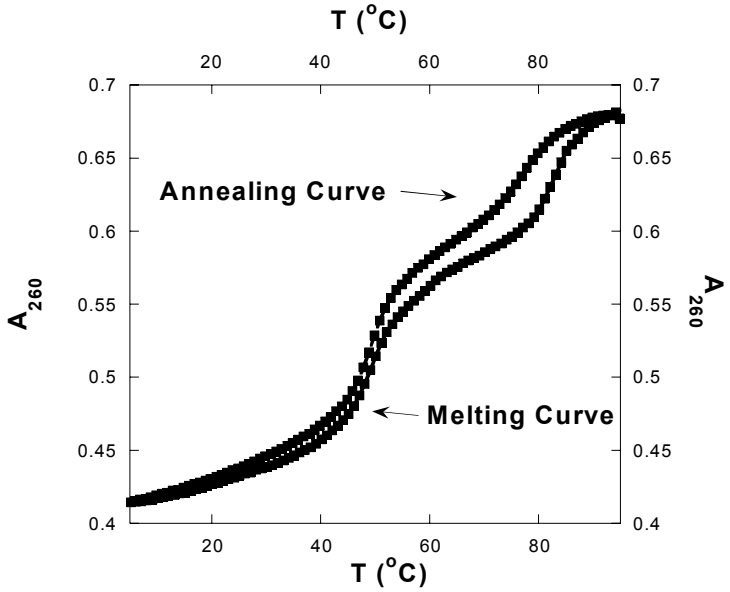
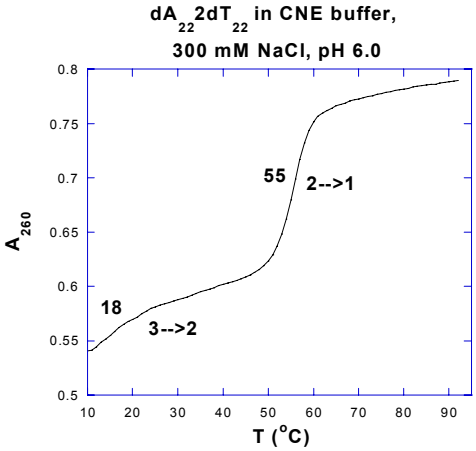
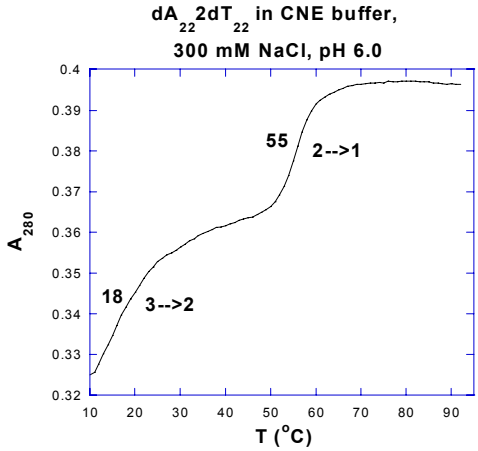


Figure 8. UV melting profiles (260 nm, 280 nm, and 287 nm) of the 1 μM strand $dA_{22}•2dT_{22}$. Solution conditions: 10 mM Sodium cacodylate, 0.5 mM EDTA, 300mM NaCl, pH 6.0. Samples were heated from 10 to 95°C at 0.2 deg/min.





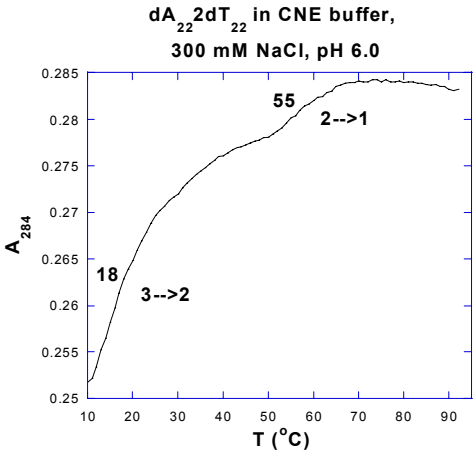
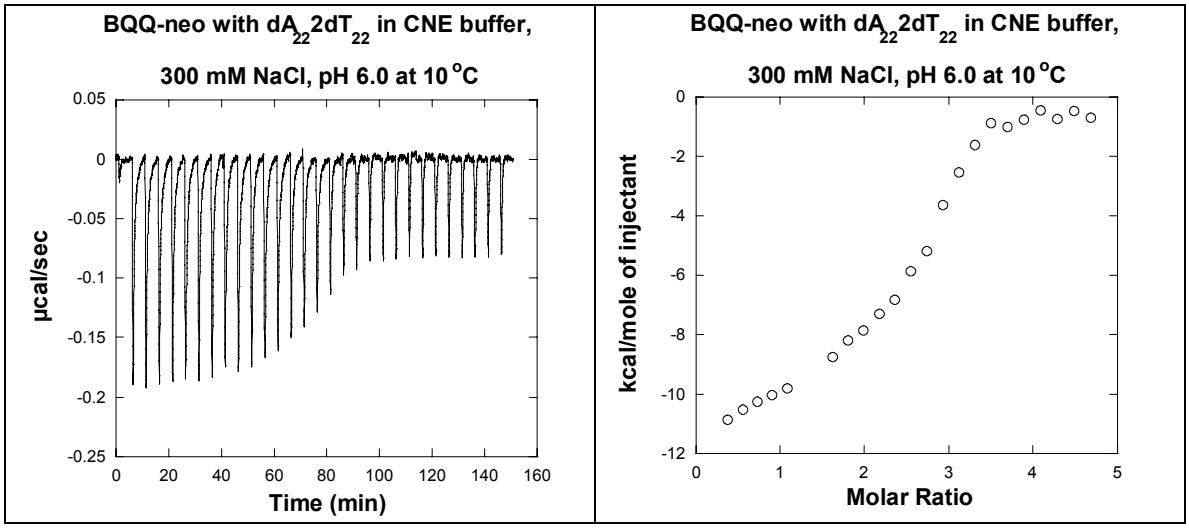
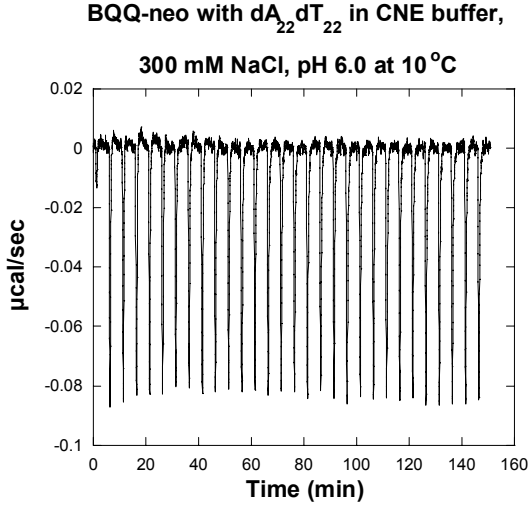


Figure 9. $dA_{22}•2dT_{22}$ triplex (4 μM in strand) titrated by BQQ-neomycin (100 μM) in 10 mM cacodylate, 0.5 mM EDTA, 300 mM NaCl, pH 6.0 at 10°C. 10 μl/inj; 300 sec/inj; 300 rpm stirring; 20 sec inj duration; 2 sec filter; 1.426 ml cell volume.



T (K)	K_1 ($\times 10^6 M^{-1}$)	ΔH_1 (kcal•mol^{-1})	$T\Delta S_1$ (kcal•mol^{-1})	ΔG_1 (kcal•mol^{-1})	N_1
283	4.55±0.82	-7.88±0.06	0.75	-8.6±0.09	1.64±0.2
T (K)	K_2 ($\times 10^7 M^{-1}$)	ΔH_2 (kcal•mol^{-1})	$T\Delta S_2$ (kcal•mol^{-1})	ΔG_2 (kcal•mol^{-1})	N_2
283	3.58±0	-11.6±0.045	-1.82	-9.78	1.19±0.23

Figure 10. dA_{22}•dT_{22} duplex(4 μM in strand) titrated by BQQ-neomycin (100 μM) in 10 mM cacodylate, 0.5 mM EDTA, 300 mM NaCl, pH 6.0 at 10°C. 10 μl/inj; 300 sec/inj; 300 rpm stirring; 20 sec inj duration; 2 sec filter; 1.426 ml cell volume.



Isothermal Titration Calorimetry (ITC):

Isothermal calorimetric measurements were performed at 10 °C on a MicroCal VP-ITC (MicroCal, Inc.; Northampton, MA). In a typical experiment, 10 µL aliquots of 100 µ M BQQ-neomycin were injected from a 250 µL rotating syringe (300 rpm) into an isothermal sample chamber containing 1.42 mL of a DNA triplex or duplex solution that was 4 µM/strand. Each experiment of this type was accompanied by the corresponding control experiment in which 10 µL aliquots of 100 µM drug were injected into a solution of buffer alone. The duration of each injection was 20s, and the delay between injections was 300 s. The initial delay prior to the first injection was 60 s. Each injection generated a heat burst curve (microcalories per second vs seconds). The area under each curve was determined by integration using the Origin (version 5.0) software to obtain a measure of the heat associated with that injection. The heat associated with each drug-buffer injection was subtracted from the corresponding heat associated with each drug-DNA injection to yield the heat of drug binding for that injection.

Competition Dialysis Assay:

Slide-A-lyzer® MINI Dialyzer units (Pierce Chemical Company; www.percenet.com) were first used in the experiment. The commercial products were found to have a high leaking ratio and could not meet the requirement of our experiment. Later, dialyzer units were remade in the lab by using the clusters of Slide-A-lyzer® MINI Dialyzer units. A new membrane (Snake Skin™ pleated dialysis tubing, Pierce Chemical Company) with 7000 MWCO was used to replace the original membrane. The quality of each remade mini dialysis unit was evaluated before experiments by dialysis against water to ensure against leaks. 200 µL water was added into the dialysis units and dialyzed against 400 ml water for 24 hours at room temperature. The volume of water in each unit was then measured. Units with excess volumes of water that exceed the original 200 µl should be discarded. Dialysis units were only used once. By replacing a fresh membrane, a new dialysis unit was generated.

Nucleic acids.

All the nucleic acids were dissolved in BPES buffer (6 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM Na_2EDTA, 185 mM NaCl, pH 7.0). Concentrations of nucleic acids were determined by UV using wavelengths and extinction coefficients. Stock solutions were diluted to 75 µM using the monomeric unit of each nucleic acid as the concentration standard. For single strand, duplex, and triplex, nucleotides (nt), base pairs, and triplets were used, respectively.

Competition dialysis protocol

For each competition dialysis assay, 180 µl of different nucleic acids in the remade mini dialysis units were placed in a MINI dialysis flotation device (Pierce Chemical Company; www.percenet.com) and then dialyzed with 400 ml of 1 µM BQQ-neomycin BPES buffer solution within 72 hrs at room temperature (20-22°C). At the end of the experiment, 150 µl nucleic acids samples were carefully removed to microfuge tubes, and were taken to a final concentration of 1% (w/v) sodium dodecyl sulfate (SDS). Each mixture was allowed to equilibrate for 2 hours. Each nucleic acid solution was then further diluted to overall volume of 4ml by BPES buffer. The concentration of BQQ-neomycin after dialysis was determined by fluorescence (Fluoromax-3, Jobin Yvon, Inc.) An appropriate correction was made due to volume changes. The amount of bound drug was determined by difference, $C_b=C_t-C_f$. Data were plotted as a bar graph using Kaleidagraph Software (Version 3.5, Synergy Software).

Computer Modeling

Step I: DNA (TAT) triplex conformation

The original structure of $dT_{10}dA_{10}dT_{10}$ triplex was obtained from the NMR structure reported by Feigon (*Biochemistry* **1998**). 27 sodium ions were added to neutralize the structure. The conformation was then optimized by AMBER* force field, in water, to within a gradient of 0.5 kJ/molÅ. The movements of the atoms of the external bases were restricted during minimization.

Step II: Neomycin conformation

Neomycin structure was built and optimized with MacroModel and SYBYL programs using AMBER* force field. The all atom AMBER* force field has been used since it contains parameters for both neomycin and the DNA triplex. In accordance with NMR experiments, five of the six-neomycin amines were protonated. Conformational searching was done with Monte Carlo routine from MacroModel, using all atom AMBER* force field, the force field charges, and the continuum GB/SA model of water as implemented in MacroModel. All the flexible bonds were selected.

Step III BQQ-neomycin conformation

BQQ-neomycin conformation was generated based on neomycin conformation. The generated conformation was then optimized by AMBER* force field and water as a solvent.

Step IV Docking

After optimizing the BQQ-neomycin conformation, the structure was docked into Waston-Hoogsteen groove of TAT triplex in different orientations. Restricting the movement of the triplex, the structure of the complex was optimized using AMBER* force field and water as a solvent.

(1) Haq, I.; Ladbury, J. E.; Chowdhry, B. Z.; Jenkins, T. C.; Chaires, J. B. *J. Mol. Biol.* **1997**, *271*, 244-257.