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Supporting Information

General Methods. All air sensitive reactions were carried out under N_2. ^1H NMR were recorded on a GE QE-300 (300 MHz) spectrometer. ^{13}C NMR were recorded on a GE QE-300 (75 MHz) or GN-500 (125 MHz) spectrometer. ^{31}P NMR were recorded on a GE GN-500 (202 MHz, H_3PO_4 external standard) spectrometer. Mass spectra and high-resolution mass spectra were recorded on a VG-ZAB-2FHF mass spectrometer using FAB ionization at the Southern California Regional Mass Spectrometry Facility (University of California, Riverside). Chemicals were purchased from either Sigma, Aldrich or Fluka Chemical Companies. Solvents were purchased from Fisher Scientific. T4 polynucleotide kinase was purchased from Boehringer Mannheim. 5'-(γ-^{32}P)ATP (~3000 Ci/ mmol) was purchased from Amersham. Pyridine and CH_2Cl_2 were distilled over CaH_2 and stored over molecular sieves. THF was freshly distilled from sodium/benzophenone and stored over molecular sieves.

2'-d-Iso-guanosine: To a solution of **3** (75.5 mg, 0.15 mmol) in CH_2Cl_2 (0.660 mL) was added triethylamine (0.064 mL, 0.46 mmol) and triethylamine-trihydrofluoride (0.075 mL, 0.46 mmol) and stirred at room temperature for 5 hours and then concentrated. Flash chromatography (SiO_2, MeOH (25%) / CH_2Cl_2) afforded the product: 27.7 mg (0.1 mmol), 68% yield. ^1H NMR: (DMSO-*d*6) δ 2.14 (m, 1 H), 2.54 (m, 1 H), 3.17 (d, 1 H, J = 4.55 Hz), 3.52-3.58 (m, 3 H), 3.85 (d, 1 H, J = 2.05 Hz), 4.34 (s, 1 H), 5.27 (d, 1 H, J = 3.51 Hz), 6.11 (m, 1 H), 7.71 (br s, 2 H), 7.96 (s, 1 H), 10.73 (br s, 1 H).

Iso-cytosine: Isocytidine (80 mg, 0.33 mmol) was placed in a hydrolysis tube and 98% formic acid (2.5 mL) was added. The tube was sealed and placed in a 175° C oil bath for 30 mins. The black mixture was filtered through cotton and concentrated. Flash chromatography (MeOH (25-75%) / CH_2Cl_2) afforded the product: 21.6 mg (0.19 mmol), 58% yield. ^1H NMR (DMSO-*d*6) δ 5.49 (d, 1 H, J = 6.52 Hz), 5.76 (s, 1 H), 6.53 (br s, 1 H), 7.51 (d, 1 H, J = 6.52 Hz); 13C NMR (DMSO-*d*6) δ 102.56, 155.42, 156.43, 163.05; MS (FAB^+) m/z 111; HRMS (FAB^+) calcd. for $C_4H_5N_3O_1$ (MH^+) 111.0433 found 111.0434.

DNA and RNA Synthesis. Oligodeoxynucleotides were synthesized trityl-off using a controlled pore glass solid support via the phosphite-triester method with an Applied Biosystems 391EP DNA synthesizer (1 μmol scale). Cleavage from the solid-support and deprotection was accomplished by treatment with concentrated NH4OH for 15 hours at 55° C. The solution was then lyophilized to dryness. The residue was taken into 1 mL 100mM triethylammonium acetate (TEAA), pH 7, and purified by reversed-phase HPLC (Hamilton PRP-1, 300 mm x 7 mm, Eppendorf CH-30 column heater, 60° C). RNA was synthesized trityl-off. Deprotection was accomplished using 3:1, EtOH/conc. NH4OH at 55°C for 2 hours. 2'-O-TBDMS groups were removed from RNA employing 1 M tetra-butylammonium fluoride in THF for 24 hrs and then desalted using a NAP column (Pharmacia). A two-stage purification was used for RNA involving intially ion-exchange chromatography (HEMA IEC ion exchange column), followed by reversed-phase chromatograpy as above for DNA (Hamilton PRP-1).

Nucleoside composition of oligomers was confirmed by digestion with snake venom phosphodiesterase and bacterial alkaline phosphatase followed by HPLC comparison with authenic samples purchased from Sigma Chemical Company or authentic samples of iso-cytidine and iso-guanosine prepared as described herein (Alltech, HS, C-18; 20 mM K_2HPO_4 , pH 5.5 (A), MeOH (B), 100% A to 40% B, 20 min.).[72]

General procedure for kinasing and gel electrophoresis. H_2O (5 μL), ATP (1 μL, 660 mM), spermidine (1 μL, 10 mM), 10X kinase buffer (1 μL, 700 mM Tris-HCl pH 7.6, 100 mM $MgCl_2$, 1 mM KCl, 50 mM dithiothreitol), $\gamma\,^{32}P$ ATP (1 μL, 1 μCi) and T4 polynucleotide kinase (1 μL, 30 units) were added to the lyophilized DNA sample (200 pmol) and the mixture incubated at 37°C for 30 minutes. Stop solution (10 μL, 7 M urea, 1X TBE, 0.1% bromophenol blue, 0.1% xylene cyanol) was added and the sample was run through a 20% polyacrylamide, 7 M urea denaturing gel with Tris-Borate-EDTA (TBE, pH 8.6) as the buffer. Autoradiograms were developed in 1-3 hours with an intensifying screen.

Melting experiments. UV absorbance versus temperature profiles were measured on an HP 8452A diode-array UV spectrophotometer in a temperature controlled cell holder with an HP 89090A peltier temperature controller. The temperature of the cell holder was

decreased from 90°C to 0°C in 1°C decrements at a cooling rate of 1 deg./min. The temperature of the solution was monitored by a thermocouple placed in the cell solution. N_2 gas (ice-cold) was passed over the cell at low temperatures to avoid the condensation of moisture. Free energy values and melting temperatures were obtained by non-linear regression using a two-state model. An excellent fit of the experimental data was seen in all cases. All melts were performed with 5 μM total strands in 1 M NaCl, 10 mM sodium phosphate, 0.1 mM EDTA at pH 7.