SUPPORTING INFORMATION

Activated ribonucleotides undergo a sugar pucker switch upon binding to a single-stranded RNA template

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Supporting Experimental Procedures

Sample Preparation

Chemically activated monomer ligands (ImpG, ImpdG, MeImpG, MeImpdG) were synthesized and purified as reported previously,[1-3] with minor modifications as follows. For synthesis of ImpG, guanosine 5′-monophosphate free acid (108 mg, 0.31 mmol, purchased from Sigma), was dissolved in 40 ml of anhydrous DMSO. To the stirring solution, imidazole (84 mg, 1.23 mmol), triphenylphosphine (405 mg, 1.54 mmol), triethylamine (0.17 ml, 1.23 mmol) and carbon tetrachloride (0.12 ml, 1.23 mmol) were sequentially added at room temperature. The resulting mixture was stirred at room temperature for 30 min at which time of reaction was monitored by ^{31}P-NMR and found to be completed. The reaction mixture was poured into a beaker containing 3.4 g of sodium perchlorate dissolved in 100 ml of anhydrous acetone. The precipitate was filtered, washed with acetone (3 × 20 ml), and dried in a vacuum desiccator over P_4O_{10}. The product was obtained as a white powder (104 mg, 0.26 mmol, 84% yield). The structure and purity of the final product were verified by ^{1}H and ^{31}P NMR. Another inactivated monomer, dGMP in disodium salt, was purchased from Sigma, and activated with imimdazole or 2-methyl imidazole as described above.

DNA and RNA template of oligonucleotides were purchased from Integrated DNA Technologies. Their sequences and folded structures are shown in Figure 1. NMR samples containing either activated mononucleotides (as monomer) alone or monomer-oligonucleotide (as ss-template) complex were each dissolved in 300 µl D_2O with 100 mM NaCl and 10 mM phosphate buffer (pH 7.8). The oligonucleotide templates alone were heated at 80 °C for several minutes and then slowly annealed

to room temperature before mixing with monomer. NMR samples contained monomer ligand at a concentration of 8 ~ 14 mM, and a template strand at a concentration of 0.5 ~ 0.8 mM.

NMR experiments

NMR experiments were performed on a Varian 400 MHz NMR spectrometer (Oxford AS-400) equipped with a Varian 5 mm broadband PFG (z-gradient) probe. Spectra were collected at 4°C or 25 °C unless otherwise indicated, and data was analyzed using the Varian VnmrJ 2.1B software. Both one-dimensional and two-dimensional ^{1}H spectra were acquired with a spectral width of 4000 Hz. All proton chemical shifts are reported relative to the residual H_2O signal as internal reference (4.96 ppm at 4°C, 4.75 ppm at 25 °C). Proton decoupled one-dimensional ^{31}P spectra were acquired with a spectral width of 10000 Hz. All ^{31}P chemical shifts are reported relative to that of phosphate buffer as internal reference (0 ppm at 4 °C).

Two-dimensional NOESY spectra of monomer ligand alone were collected with a 600 ms mixing time. Two-dimensional transferred NOESY (TrNOESY) spectra of the monomer-template complex were recorded with a 100 ms mixing time. Two pulsed field gradients, G1 (1.0 ms, 14 G/cm) and G2 (1.0 ms, 24 G/cm) were applied at the beginning and end of the mixing time, respectively, to remove undesired magnetization. For both NOESY and TrNOESY experiments, solvent suppression was achieved by a 2.0 s low-power presaturation pulse applied at the water frequency. A total of 16 ~ 32 scans of 2048 complex points were collected for each of the 256 increments in the indirect time domain. The spectral width in F1 and F2 was 4000 Hz, and States-TPPI was used to achieve quadrature in the indirect dimension. The total acquisition time of each two-dimensional experiment was 6 ~ 12 hours. Each direct dimension free induction decay (FID) was apodized using a Gaussian line broadening window function before Fourier transformation, whereas each indirect FID was apodized using a shifted sine bell squared window function. A 512 × 4096 matrix was obtained after zero-filling.

Supporting Text

Comments on the insensitivity of the *J* coupling constants approach for determining the sugar pucker of the template-bound monomer

Both activated and nonactivated monomers bind weakly to ss-template, with K_ds ranging from tens to hundreds of mM (Zhang and Szostak, in preparation). Therefore, the exchange between free and bound monomer is much faster than the NMR time scale. As a result, the signals of free and bound monomer will merge together and the observed resonances and *J* coupling constants would be a fractionally weighted average of those of free and bound monomer. Because it is necessary to use a large excess of monomer over template in order to achieve high template occupancy, the observed $^3J_{H1'-H2'}$ would be dominated by the contribution from free monomer even in the presence of template. The presence of overlapping signals from the template would further complicate this approach. As a result, the *J* coupling constant approach lacks the sensitivity required to determine the conformation of template bound monomers.

Supporting Figures

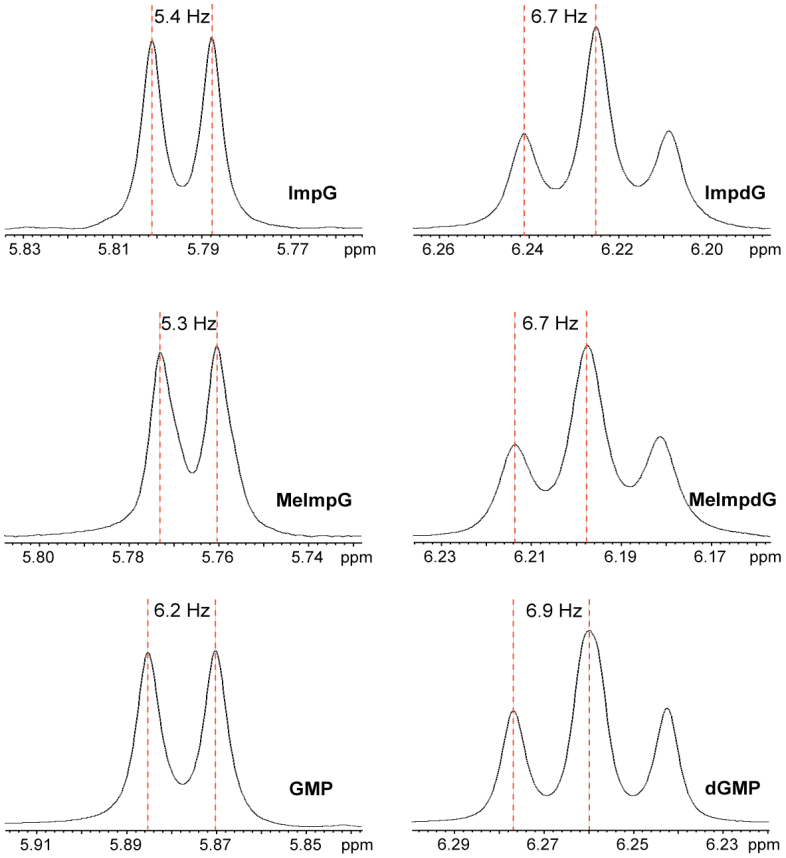

Figure S1. Expanded sugar H1′ region of one-dimensional ^{1}H spectra of free monomer ligands at 4 °C. The $^{3}J_{H1'\text{-}H2'}$ value of each monomer is labeled. NMR samples contained 8 ~ 14 mM free monomer in 300 µl D_2O with 100 mM NaCl and 20 mM phosphate buffer (pH 7.8).

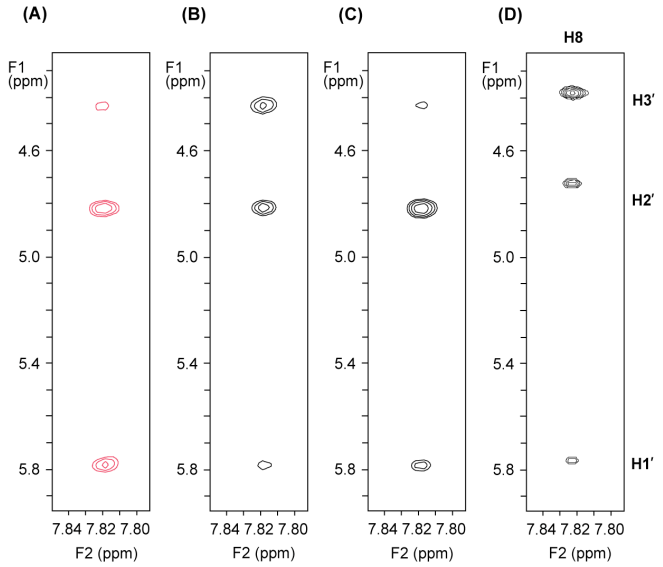

Figure S2. The expanded spectral region between base H8 proton and sugar H1′, H2′, H3′ protons of monomer MeImpG (A to C) and ImpG (D) in D_2O with 100 mM NaCl and 10 mM phosphate buffer (pH 7.8) at 4 °C. (A): NOESY spectrum (mixing time, 600 ms) of 9 mM MeImpG in the absence of ss-template; (B): TrNOESY spectrum (mixing time, 100ms) of 11 mM MeImpG in the presence of RNA ss-template, 0.7 mM strand concentration; (C): TrNOESY spectrum (mixing time, 100ms) of 10 mM MeImpG in the presence of DNA ss-template, 0.5 mM strand concentration; (D): TrNOESY spectrum (mixing time, 100ms) of 8 mM ImpG in the presence of RNA ss-template, 0.5 mM strand concentration. The red cross-peaks in (A) correspond to positive NOEs which have the opposite sign as diagonal peaks; the black cross-peaks in (B), (C) and (D) correspond to negative NOEs which have the same sign as diagonal peaks.

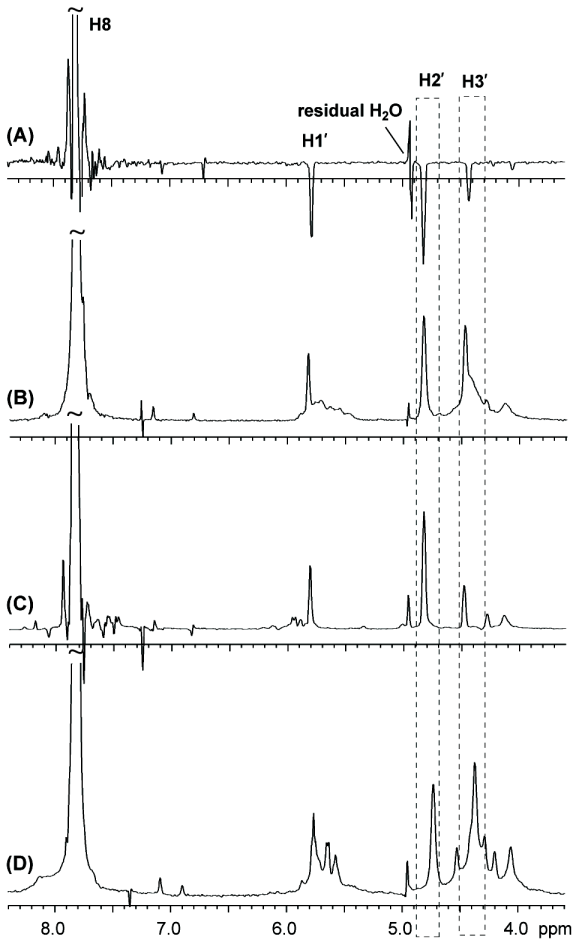

Figure S3. Corresponding 1D slices through the base H8 proton resonances at 7.8 ppm of activated monomer MeImpG (A to C) and ImpG (D) from the 2D NOESY and TrNOESY spectra (shown in Figure S2) of monomer-template complexes. Data were collected in D_2O with 100 mM NaCl and 10 mM phosphate buffer (pH 7.8) at 4 °C. (A): 1D slice of NOESY spectrum (mixing time, 600 ms) of 9 mM MeImpG in the absence of ss-template; (B): 1D slice of TrNOESY spectrum (mixing time, 100ms) of 11 mM MeImpG in the presence of RNA ss-template, 0.7 mM strand concentration; (C): 1D slice of TrNOESY spectrum (mixing time, 100ms) of 10 mM MeImpG in the presence of DNA ss-template, 0.5 mM strand concentration; (D): 1D slice of TrNOESY spectrum (mixing time, 100ms) of 8 mM ImpG in the presence of RNA ss-template, 0.5 mM strand concentration. The downward peaks in (A) correspond to positive NOEs which have the opposite sign as diagonal peaks; the upward peaks in (B), (C) and (D) correspond to negative NOEs which have the same sign as diagonal peaks.

Supporting References

1) Lohrmann, R.; Orgel, L.E. *Nature* **1976**, *261*, 342.

2) Joyce, G. F.; Inoue, T.; Orgel, L. E. *J. Mol. Biol.* **1992**, *114*, 317.

3) Kim, E. K.; Switzer, C. *Current Protocols in Nucleic Acid Chemistry*. John Wiley & Sons, Inc.; New York, **2009**.