Electronic Supplementary Information

Gas-phase isolation of diethyl guanosine 5'-monophosphate and its conformational assignment

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1. Synthesis of diethyl guanosine 5'-monophosphate.

General. Nuclear magnetic resonance (NMR) spectra were measured on a JEOL JNM-ECP500 instrument. The chemical shifts of ^{1}H NMR in D_2O are expressed in parts per million (ppm) relative to HOD (δ 4.65). Chemical shifts of $^{31}P\{^{1}H\}$-NMR spectra are reported downfield in ppm relative to external 85% H_3PO_4 at 0.00 ppm. Signal patterns of ^{1}H NMR as well as $^{31}P\{^{1}H\}$ NMR are indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal. HRMS (FAB^+) measurements were performed on a JEOL JMS-700. HPLC analysis was carried out using a COSMOSIL 5C_{18}-AR-II column [Nacalai Tesque, 4.6 (diameter) mm x 250 (height) mm] on a Waters 2695 Separations Module chromatograph with a Waters 2996 Photodiode Array detector. Preparative HPLC was achieved using a COSMOSIL 5C_{18}-AR-300

column [Nacalai Tesque, 20 (diameter) mm x 250 (height) mm] on an ÄKTA explorer (Amersham Biosciences). Normal-phase medium-pressure liquid chromatography (MPLC) was conducted using a Purif-Pack SI-60 column (size 120) on a Purif-compact (Moritex, Japan).

Materials. Dehydrated DMF (Wako), 2',3'-*O*-isopropylideneguanosine (SIGMA), a 0.97 mol/L solution of *tert*-butylmagnesium chloride in THF (Kanto), diethyl chlorophosphate (Tokyo Kasei), trifluoroacetic acid (Kishida), and a 1 mol/L triethylammonium hydrogen carbonate in water (Nacalai Tesque) were commercially supplied. Powdery molecular sieves 3Å were used after drying the commercially supplied sieves (Nacalai Tesque) at 200 °C for 12 h under reduced pressure (ca. 2 mmHg). Imidazolium triflate[s1] and a 1.0 M *tert*-butyl hydroperoxide/toluene solution[s2] were prepared according to the reported methods.

Preparation of diethyl diisopropylaminophosphoramidite. A 500-mL, three-necked, round-bottomed flask equipped with a magnetic stirring bar, a 100-mL dropping funnel and an argon inlet was charged with dry THF (60 mL) and diethyl chlorophosphate (10 mL, 70 mmol). Under an argon atmosphere, the solution was cooled to 0 °C, and diisopropylamine (31 mL, 220 mmol) in THF (40 mL) was added dropwise over 1 h. After addition was complete, the mixture was allowed to warm to room temperature (rt) and was stirred at this temperature for 48 h. The $^{31}P\{^{1}H\}$ NMR of the reaction mixture indicated that reaction proceeded quantitatively. The resulting slurry was filtered by use of celite and then THF was evaporated under reduced pressure (ca. 2 mmHg) to afford the crude material. Distillation of the crude gave pure diisopropylarainophosphoramidite

(43−44 °C/1.9–2.0 mmHg, 7.55 g, 51%). Spectral and physical data were consistent with reported values.[s3,s4]

Synthesis of diethyl guanosine 5'-monophosphate (3). (a) Phosphorylation of 1 by the phosphoramidite method. A mixture of 2',3'-*O*-isopropylideneguanosine (103 mg, 0.32 mmol), powdery 3Å molecular sieves (404 mg), and $(C_2H_5O)_2P[N(i\text{-}C_3H_7)_2]$ (150 mg, 0.67 mmol) in DMF (5 mL) were stirred for 20 min at rt, and then imidazolium triflate (144 mg, 0.66 mmol) was added and the mixture was stirred for 3 days at rt.[s1] A 1.0 M *tert*-butyl hydroperoxide/toluene solution[s5] (1.3 mL, 1.3 mmol) was added and the mixture was stirred for 1 h, filtered by celite and the solvent was evaporated. To the resulting residue, dichloromethane (20 mL) was added and the organic phase was washed with H_2O (20 mL), dried with Na_2SO_4 and the solvent was evaporated. The crude material was purified by MPLC (size 120, CH_2Cl_2:CH_3OH = 100:0 → 93:7) to afford **2** (113 mg, 77%):[s6] HRMS (FAB^+) calcd for $C_{17}H_{27}N_5O_8P$ ($M + H^+$) *m*/*z* 460.1597, found *m*/*z* 460.1600.

(b) Phosphorylation of 1 by the hydroxy-activated phosphotriester method. To a solution of the nucleoside **1** (480 mg, 1.48 mmol) in DMF (9.0 mL) was added dropwise at rt a 0.97 M solution of *tert*-butylmagnesium chloride (3.2 mL, 3.1 mmol) in THF. After stirring for 10 min, diethyl chlorophosphate (300 μL, 2.1 mmol) was added and stirring was continued for 1.7 h. The mixture was diluted with CH_2Cl_2 (70 mL), quenched with saturated aqueous NH_4Cl solution (70 mL). The organic phase was separated and the aqueous phase was back extracted with CH_2Cl_2 (40 mL x 2). The combined organic

layers were dried with Na_2SO_4 and evaporated to afford an oily material, which was purified by MPLC (size 120, CH_2Cl_2:CH_3OH = 100:0 → 90:10) to give **2** (492 mg, 72%).

(c) Removal of isopropylidene protector of 2 and purification of 3. **2** (84.0 mg, 0.18 mmol) was dissolved in dichloromethane (2.0 mL) and then, 80% aqueous trifluoroacetic acid (350 μL) was added. The mixture was stirred for 3h at rt and to this, 80% aqueous trifluoroacetic acid (350 μL) was added again. After stirring for another 3 h at rt, the mixture was neutralized with a 1 mol/L triethylammonium hydrogen carbonate in water (7 mL). The solvent was evaporated, and the resulting oily material was diluted with water (6.5 mL) to give the aqueous solution of the crude product. This solution (ca. 2 mL) was subjected to preparative HPLC using COSMOSIL 5C_{18}-AR-300 column [20 (diameter) mm x 250 (height) mm] on an ÄKTA explorer. Elution was carried out under these conditions: [A = H_2O, B = a 20:80 mixture of H_2O and CH_3CN; gradient: 0–10 min A 100%, 10–59 min with a linear gradient A 100% to A 50%/B 50%, 59–78 min B 100%; detection 254 nm and 280 nm; flow rate: 8.0 mL/min; temperature, rt]. This purification procedure was conducted another three times, and the appropriate fractions were evaporated under reduced pressure (ca. 2 mmHg) to afford **3** (56.7 mg, 74%): ^{1}H NMR (500 MHz, D_2O) δ 1.08 (t, *J* = 7.5 Hz, 3H), 1.09 (t, *J* = 7.5 Hz, 3H), 3.82–3.95 (m, 4H), 4.18 (d, *J* = 4.9 Hz, 3H), 4.44 (m, 1H), 4.71 (t, *J* = 4.9 Hz, 1H), 5.78 (d, *J* = 4.0 Hz, 1H), 7.79 (s, 1H); ^{31}P NMR (202 MHz, D_2O) δ –0.08; HRMS (FAB^+) calcd for $C_{14}H_{23}N_5O_8P$ (M + H^+) *m*/*z* 420.1284, found *m*/*z* 420.1284. The purity of **3** was routinely checked by HPLC analysis under the following conditions: column, COSMOSIL 5C_{18}-AR-II [4.6 (diameter) mm x 250 (height) mm]; flow rate, 1.0 mL/min;

detection, 254 nm; eluent and gradient, [A = H_2O, B = a 20:80 mixture of H_2O and CH_3CN, 0–10 min A 100%, 10–30 min with a linear gradient from A 100% to A 50%/B 50%]; temperature, 40 °C; retention time of **3**, 22.0 min.

References

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2. Calculated IR spectra for the *syn* and *anti* conformers of diEtGMP.

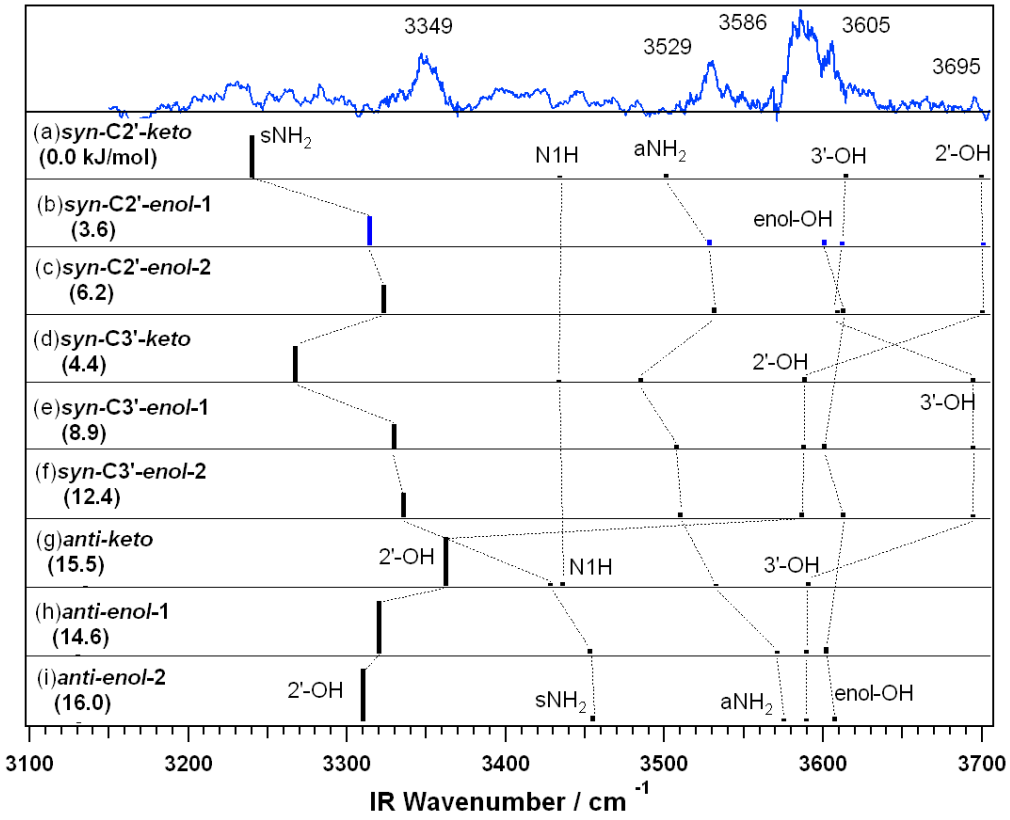

Fig. S1. Calculated IR spectra (scaled by 0.957) for the low-energy conformers of diEtGMP shown in Fig. 3 of the text. The observed IR spectrum shown in the top panel is consistent with the calculated spectrum of the *syn*-C2'-*endo*-*enol*-1 conformer (blue).