Supporting Information

Klenow Fragment Discriminates Against the Incorporation of the Hyperoxidized dGTP Lesion Spiroiminodihydantoin into DNA

Ji Huang, Craig J. Yennie, and Sarah Delaney*

From the Department of Chemistry, Brown University, Providence, RI 02912, USA

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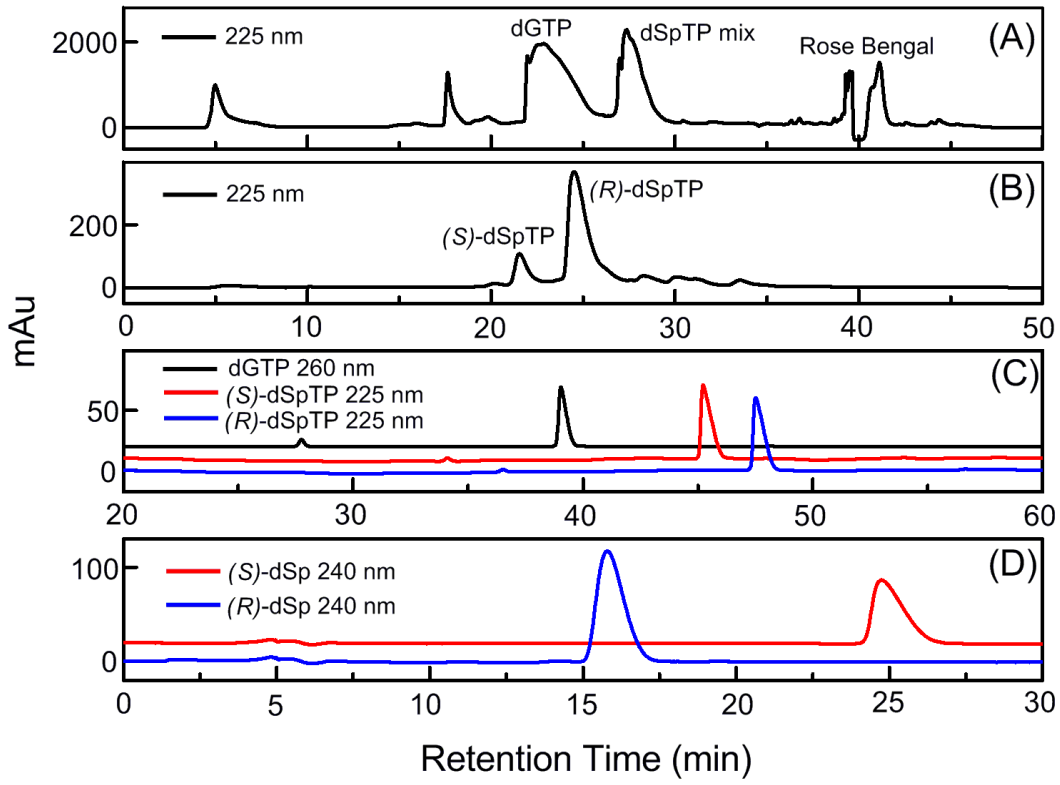

Figure S1. HPLC purification and characterization demonstrating (A) the mixture of dSpTP diastereomers was separated from dGTP by ion pairing C18 HPLC; (B) separation of *(S)*- from *(R)*-dSpTP on an ion pairing C18 HPLC column; (C) anion exchange HPLC of dGTP (monitored at 260 nm), *(S)*- and *(R)*-dSpTP (monitored at 225 nm), respectively (chromatogram at 225 nm was baseline corrected due to the background signal of ammonium bicarbonate); (D) characterization of the dSp nucleoside diastereomers by HyperCarb HPLC column. The minor peak observed in panel C for dGTP, (*S*)-dSpTP, and (*R*)-dSpTP corresponds to the dNDP hydrolysis product which occurs during freeze/thaw cycles of dNTPs. Importantly, the dNDP version is not a substrate for polymerase and would not influence our kinetic results.

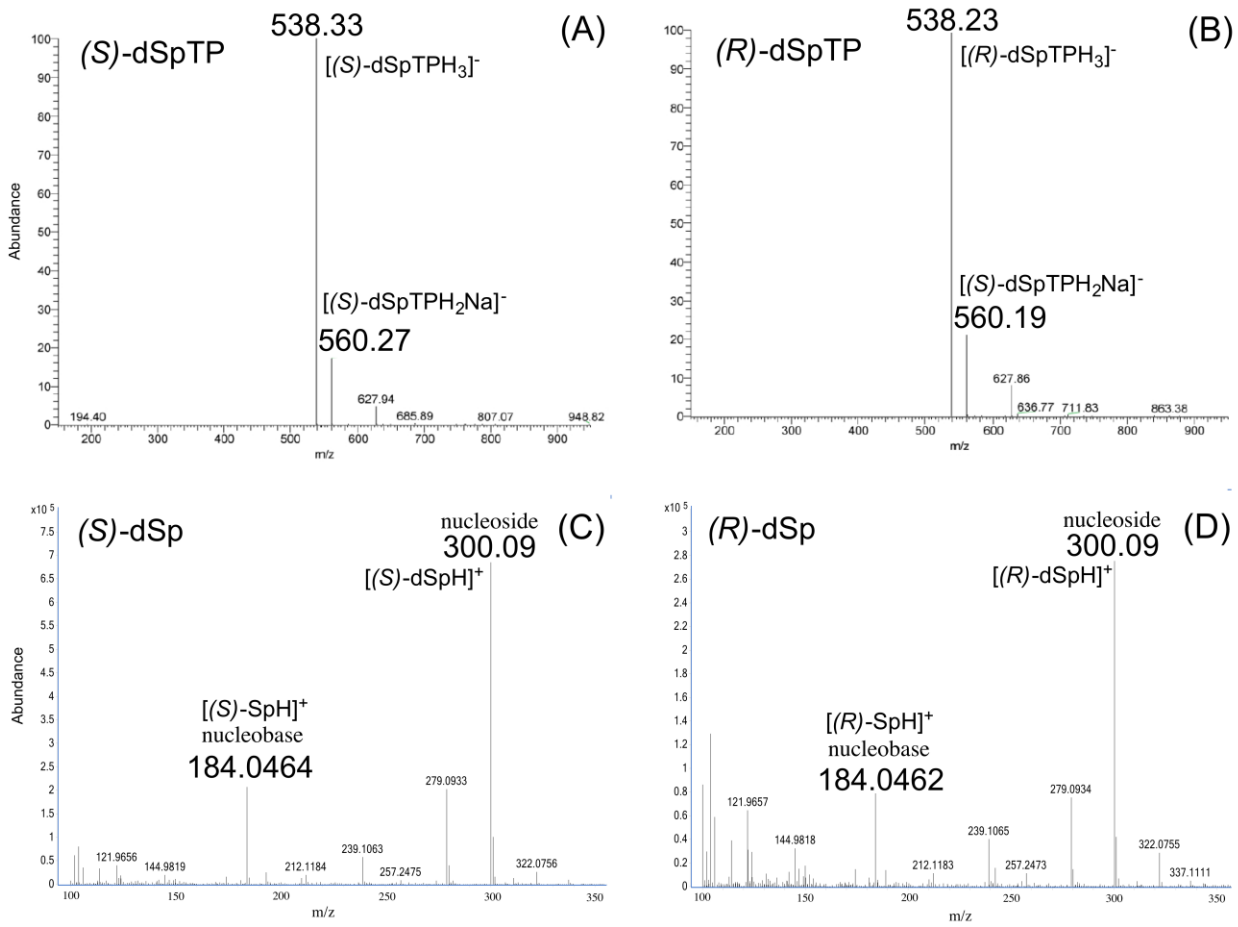

Figure S2. Negative ion mode ESI-MS analysis for nucleotide triphosphates (A) *(S)*-dSpTP and (B) *(R)*-dSpTP. Positive ion mode ESI-MS analysis for nucleosides (C) *(S)*-dSp and (D) *(R)*-dSp. The expected mass for neutral nucleotide, nucleoside and nucleobase are 538.99, 299.09, 183.04 respectively.

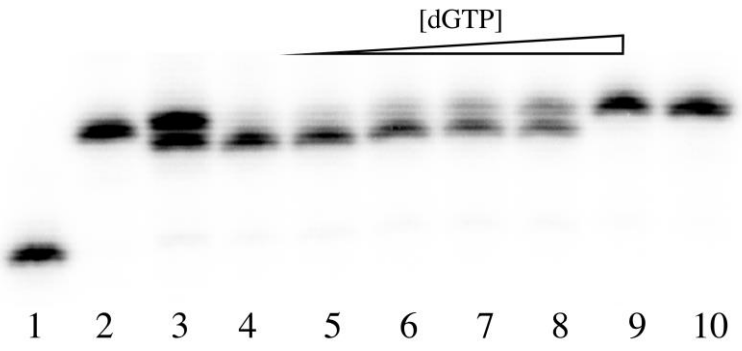

Figure S3. Denaturing PAGE (22.5% acrylamide) showing the migration difference of single nucleotide incorporation product of adding either dGTP or *(S)*-dSpTP. Lane 1: 20 mer primer; lane 2: Single nucleotide incorporation product of adding dGTP; Lane 3: Mixture of single nucleotide incorporation products after incorporating either dGTP or *(S)*-dSpTP; Lane 4: Single nucleotide incorporation product of adding *(S)*-dSpTP; Lane 5-9: Single nucleotide incorporation product of *(S)*-dSpTP in the presence of increasing amount of dGTP (the concentration of dGTP supplemented to the *(S)*-dSpTP were 1.25 nM, 2.5 nM, 5 nM, 10 nM and 20 nM, and the nucleotides were added as the mixture to the pre-incubated DNA/KF^{-} complex); Lane 10: Same as lane 2 (Single nucleotide incorporation product of adding dGTP). The reaction conditions are the same as described in the Experimental Procedures including using 20 nM dGTP and 100 μM dSpTP as the nucleotide concentrations.

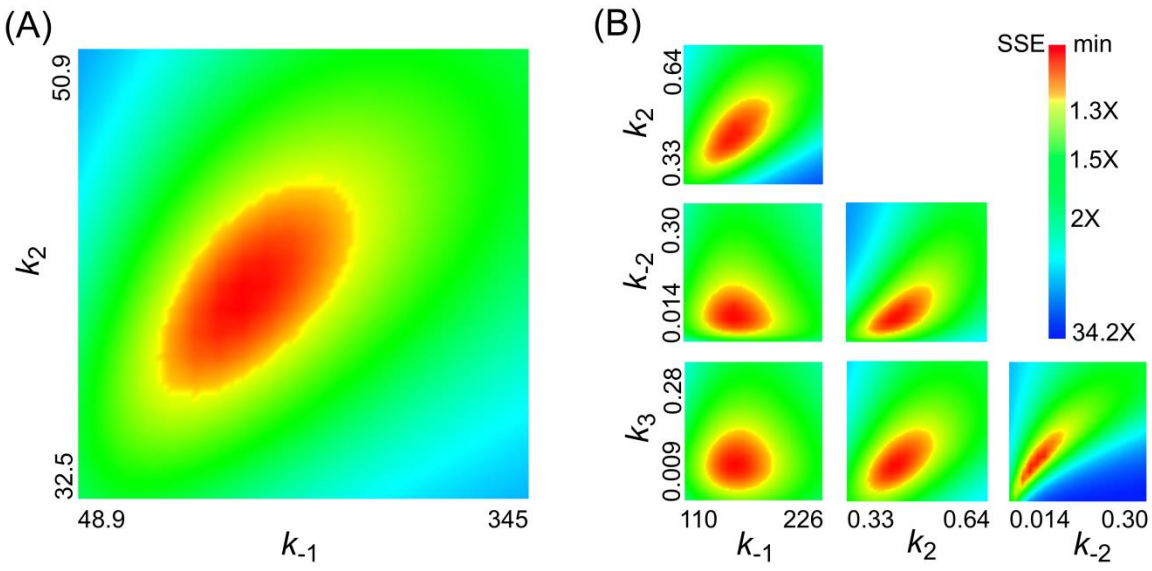

Figure S4. 3-D confidence contours for the rate constants determined by KinTek Global Explorer for (A) dGTP and (B) 8-oxodGTP. For each case the search was carried out up to a sum of squares error (SSE) that is 2-fold higher than the minimum SSE. The upper and lower limits of each parameter were determined using an SSE threshold of 1.2. A red oval shape indicates the pairs of parameters are well constrained. These well-constrained parameters can be compared to those for the diastereomers of dSpTP shown in Figure 4D, E.