Supporting information

A prebiotic role for 8-oxoguanosine as a flavin mimic in pyrimidine dimer photorepair

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Material and methods

Oligodeoxynucleotide synthesis and purification. Phosphoramidites for oligodeoxynucleotide synthesis were purchased from Glen Research. Oligodeoxynucleotides were synthesized at the DNA/Peptide Core facility at the University of Utah. The [*cis, syn*] thymine dimer-containing oligodeoxynucleotides were first treated with thiophenol/triethylamine/THF (1/2/2) for 45 min at room temperature to remove the methyl phosphate group [2,3]. The solid support was then washed with THF (10x), methanol (5x), acetonitrile (3x) and dried under argon flow. Oligodeoxynucleotides were cleaved and deprotected in sealed glass vials with concentrated NH_4OH for 16h at 55°C in the dark (in the cases of oligodeoxynucleotides containing **OG**, 0.25M β-mercaptoethanol was added to the deprotection solutions to avoid the oxidation of **OG**). Oligodeoxynucleotides were purified by HPLC on a Dionex DNA Pac PA-100 column with linear gradient of 15% B to 100% B over 30 min (Solvent A: 10% acetonitrile in water; solvent B: 1.5M sodium acetate, 10% acetonitrile in water, pH 7). Oligodeoxynucleotides were then desalted by dialysis against water for 36h at 4°C in the dark. The purity and identity of oligomers were determined by analytical HPLC and mass spectrometry. The [*cis,syn*] thymine dimer-containing oligodeoxynucleotides were quantified by UV-VIS spectroscopy on the Beckman DU 650 spectrometer using extinction coefficient calculated as previously described [4]. Complete sequences are shown in Table S1.

Photorepair of [*cis, syn*] thymine dimer in DNA duplexes. 5μM of thymine dimer-containing DNA was annealed with 1.3 equiv. of the appropriate complementary strand in a buffer solution containing 20mM NaPi, 100mM NaCl, pH 7 by heating at 90°C for 2 minutes and cooling to room temperature over 4h. The DNA duplex was irradiated in polystyrene cuvettes to cut off wavelengths below 300 nm[5] at ambient temperature (22°C) with an FS40 UVB lamp (peak at 313nm, Homephototherapy, OH, USA). The irradiation mixture was then analyzed by HPLC on Hamilton PRP-1 (5μm, 250X4.6mm) column at 70°C with linear gradient of 10% B to 14% B over 25 min (Solvent A: 50mM TEAA in water, pH 7; Solvent B: acetonitrile). Detector was set at 260nm and the flow rate was 0.8mL/min. Under these conditions, the DNA duplex was denatured and single-stranded DNA oligomers eluted in the following order: the thymine dimer T=T strand (18mer), the repaired TT strand (18mer) and the complementary strand (22mer). HPLC peaks corresponding to the thymine dimer strand and the repaired strand were integrated. The peak areas were normalized against extinction coefficients of each strand and used to calculate the thymine dimer repair yield. Thymine dimer repair was plotted as function of irradiation time and fit to exponential curve using OriginPro 8.5 software (Originlab). The repair rate (% min^{-1}) was calculated and this value was used to compare the repair efficiency of different DNA duplexes.[6] The presented data were averaged from three experiments. (See Figure S3.)

Photorepair of [*cis, syn*] uracil dimer. Uracil dimer containing RNA (5'-CACAGCA**U=U**AC AGUACAC-3') was synthesized following a reported procedure[7] except using a higher concentration of acetone photosensitizer (10%), and the oligomer was purified by reversed phase HPLC. The preparations of RNA/DNA and RNA/RNA duplexes and irradiation procedure were the same as described above for the DNA duplex. The irradiation mixture was then analyzed by denaturing HPLC on a Hamilton PRP-1 (5μm, 250x4.6mm) column at 70°C with a linear

gradient of 9% B to 13% B over 25 minutes (Solvent A: 50mM TEAA in water, pH 7; Solvent B: acetonitrile). The detector was set at 260nm and the flow rate was 1.0 mL/min. Under these conditions, the uracil dimer strand and the repaired strand coeluted as a broaden peak at 9 minutes. This peak was isolated and reanalyzed by reversed phase HPLC at room temperature using an Ace C18 column (5µm, 250x4.6mm) with a linear gradient of 4% B to 12% B over 30 minutes (Solvent A: 20mM CH_3COONH_4, pH 7; Solvent B: acetonitrile). Under these conditions, the uracil dimer strand eluted at 10 min and the repaired strand eluted at 12 min. (See figure S4.) These peaks were integrated and used to calculate the repair yield.

Photorepair of [*cis, syn*] thymine dimer in single-stranded DNA. The irradiation procedure for single-stranded DNA was the same as described above for the DNA duplex. The irradiation mixture was then analyzed by HPLC on Ace C18 column (5µm, 250X4.6mm) with linear gradient of 5% B to 15% B over 25 min (Solvent A: 20mM CH_3COONH_4, pH 7; Solvent B: acetonitrile). Under these conditions, the thymine dimer strand eluted at 10 min and the repaired strand eluted at 12.5 min. (See figure S7.)

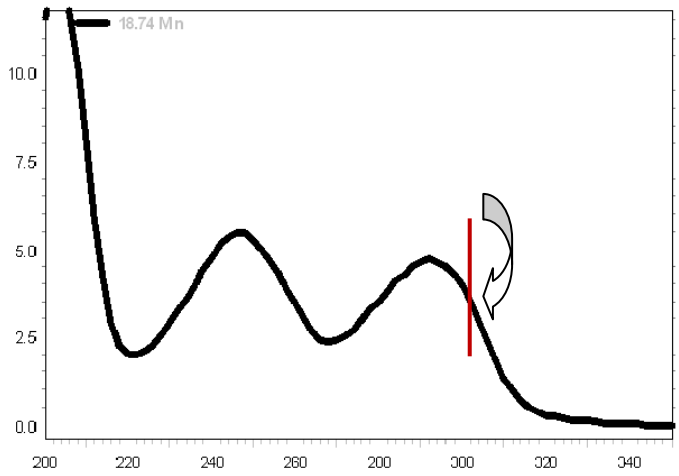


Figure S1. UV spectrum of OG. Irradiation at wavelengths >300 nm (red bar) photoexcites OG, but not normal bases

Table S1. Complete sequences studied. Sequences 1-8 are DNA:DNA duplexes. Sequence 5S is single-stranded DNA

	Sequences
1A	5’-CACAGCA**T=T**ACAGTACAC-3’ 3’-TCTGTGTCG**O**A ATGTCATGTGT-5’
1C	5’-CACAGCC**T=T**ACAGTACAC-3’ 3’-TCTGTGTCG**O**A ATGTCATGTGT-5’
C1	5’-CACAGCA**T=T**ACAGTACAC-3’ 3’-TCTGTGTCG**T**A ATGTCATGTGT-5’
C2	5’-CACAGCC**T=T**ACAGTACAC-3’ 3’-TCTGTGTCG**G**A ATGTCATGTGT-5’
2	5’-CACAGCA**T=T**ACAGTACAC-3’ 3’-TCTGTGTCGTA A**O**GTCATGTGT-5’
3	5’-CACAGCA**T=TO**CAGTACAC-3’ 3’-TCTGTGTCGTA AAGTCATGTGT-5’
4A	5’-CACAGC**OT=T**ACAGTACAC-3’ 3’-TCTGTGTCGAA ATGTCATGTGT-5’
4C	5’-CACAGC**OT=T**ACAGTACAC-3’ 3’-TCTGTGTCGCA ATGTCATGTGT-5’
5	5’-ACAGC**O**A**T=T**ACAGTACAC-3’ 3’-TCTTGTCGATA ATGTCATGTGT-5’
6	5’-ACAGC**O**T**T=T**ACAGTACAC-3’ 3’-TCTTGTCGAAA ATGTCATGTGT-5’
7	5’-CACAGCA**T=T**A**O**CAGTACA-3’ 3’-TCTGTGTCGTA ATAGTCATGTT-5’
8	5’-CACAGCA**T=T**T**O**CAGTACA-3’ 3’-TCTGTGTCGTA AAAGTCATGTT-5’
RNA/DNA	5’-CACAGCA**U=U**ACAGUACAC-3’ 3’-TCTGTGTCG**O**A ATGTCATGTGT-5’
RNA/RNA	5’-CACAGCA**U=U**ACAGUACAC-3’ 3’-UCUGUGUCG**O**A AUGUCAUGUGU-5’
5S	5’-ACAGC**O**A**T=T**ACAGTACAC-3’

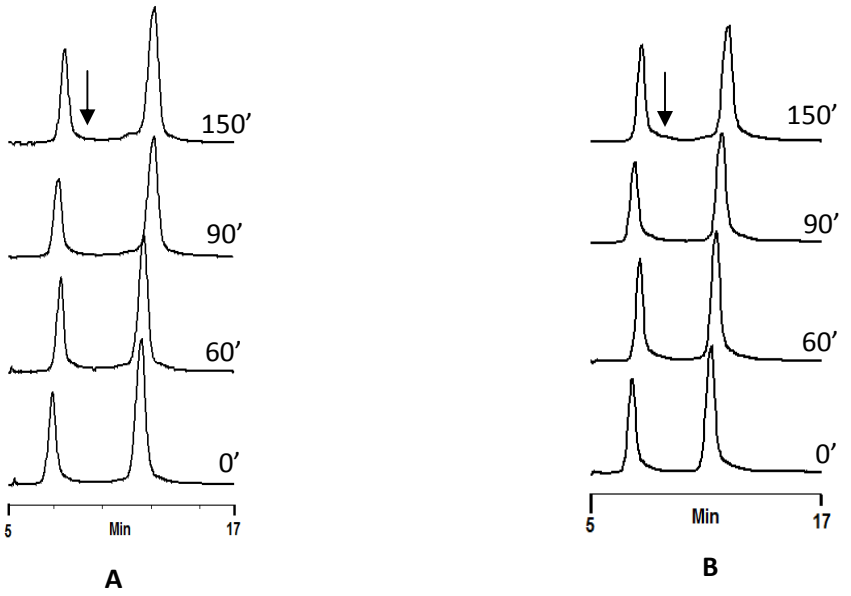
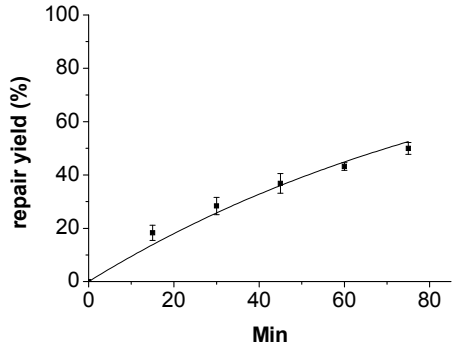
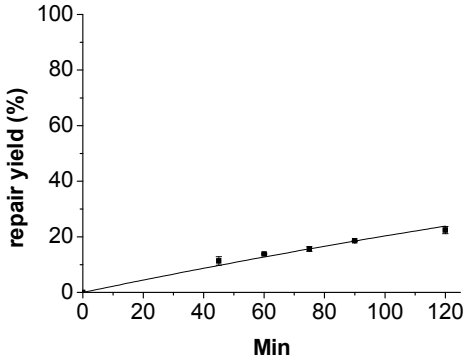


Figure S2. Denaturing HPLC analysis of control duplexes as a function of irradiation time. (**A**) Duplex C1. (**B**) Duplex C2. The black arrow indicates the expected position for the repaired strand. No repair was detectable in both duplexes after 150 minutes of irradiation. The limit of detection is estimated to be ~5%.

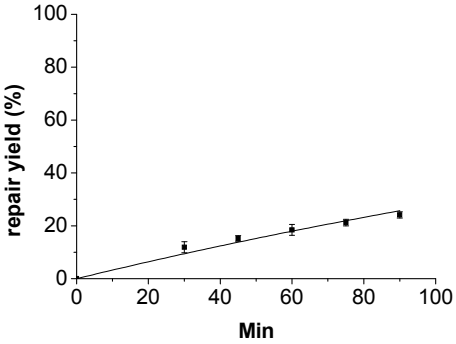
Figure S3. Plot of thymine dimer repair yield (percentage of total DNA) as the function of irradiation time in different DNA duplexes



1A



1C



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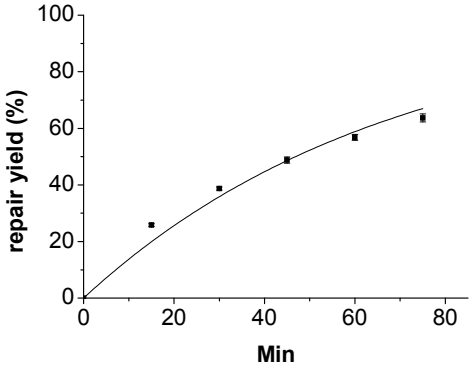

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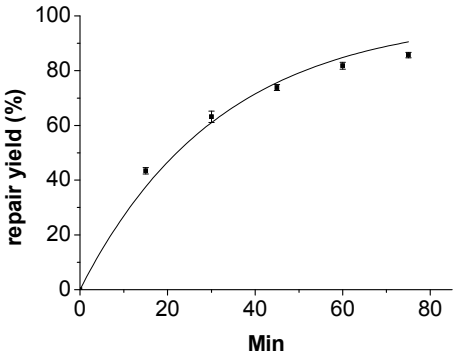
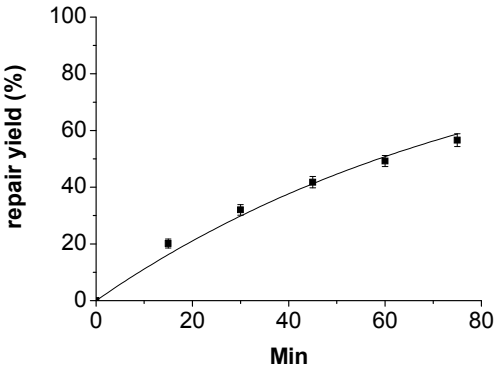
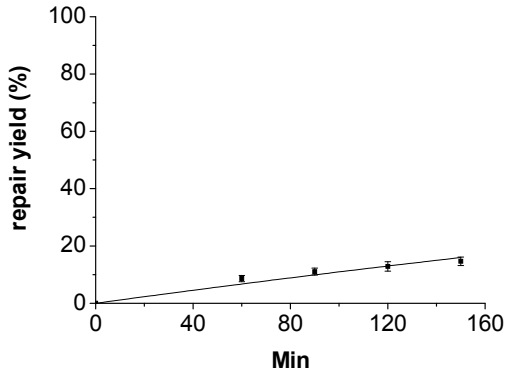


4A



4C



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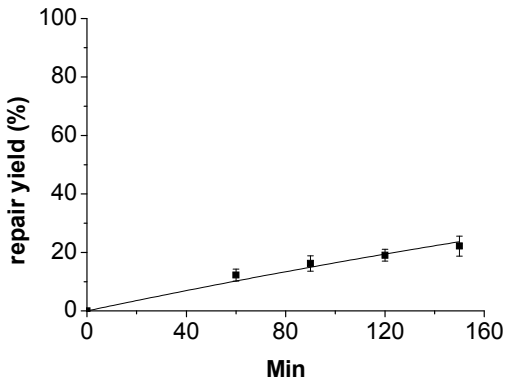

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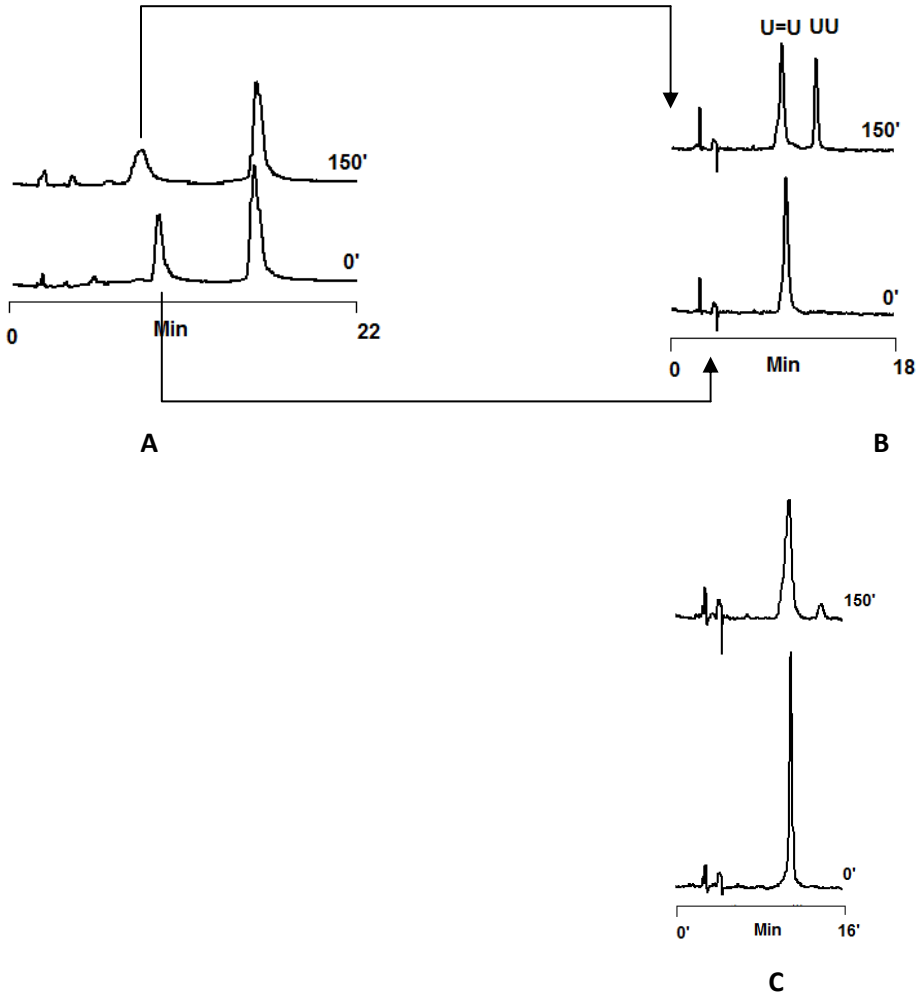


Figure S4. Photorepair of uracil dimer (**A**) Denaturing HPLC analysis of $RNA^{U=U}$-DNA^{OG} duplex before and after irradiation. (**B**)Reversed phase HPLC analysis of $RNA^{U=U}$ peak isolated from denaturing HPLC. (**C**) Reversed phase HPLC analysis of $RNA^{U=U}$ peak collected from denaturing HPLC analysis of $RNA^{U=U}$-RNA^{OG} duplex before and after irradiation.

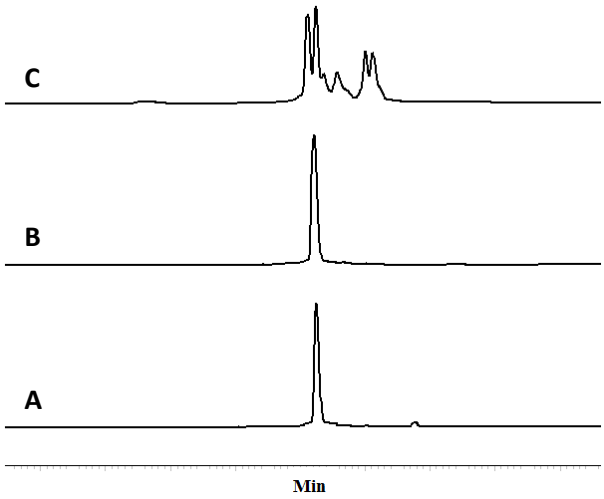


Figure S5. The OG-containing strand was unchanged after the photorepair process. HPLC traces of (**A**) isolated **OG** strand after 75 minutes irradiation of duplex **1A** (50% thymine dimer was repaired) (**B**) initial **OG** strand (**C**) oxidation products of **OG** strand generated by Ir(IV) at pH 7 [1]. Samples were analyzed on Dionex DNA Pac PA-100 column using gradient of 15% B to 100% B over 30 minutes (solvent A: 10% acetonitrile in water, solvent B: 1.5M sodium acetate in 10% acetonitrile in water at pH 7).

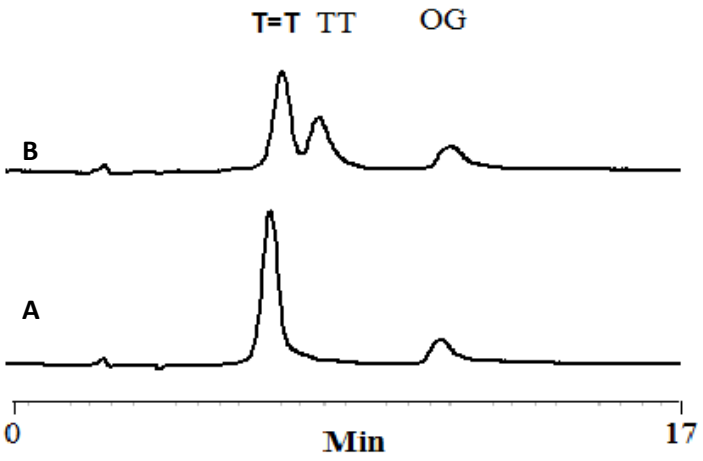


Figure S6. The OG-containing strand demonstrated turnover catalysis. HPLC traces of (**A**) initial duplex **1A** with 5.4-fold excess of thymine dimer strand than the OG strand; (**B**) mixture after 5 cycles of irradiation. 40% of thymine dimer was repaired, which is 2 times that of stoichiometric repair, or ~200% based on the OG-containing strand.

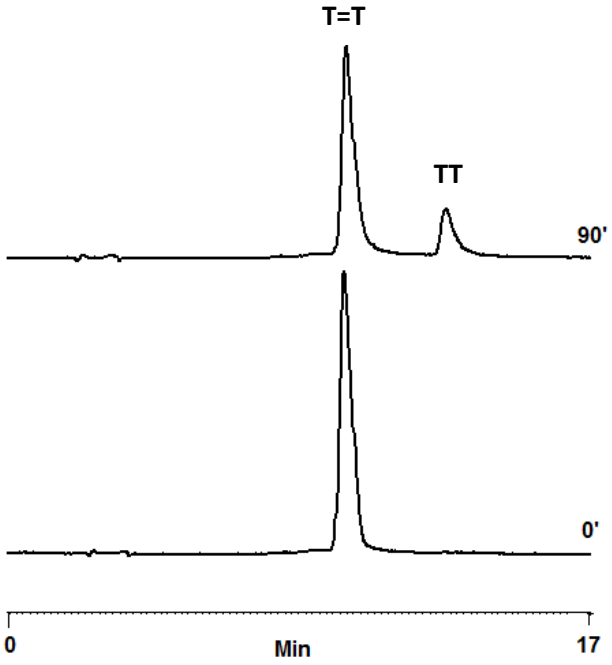
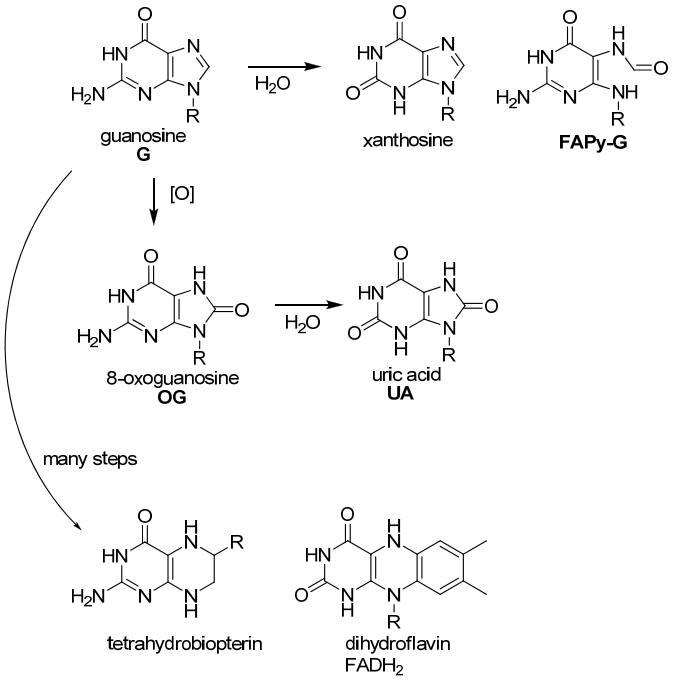


Figure S7. Reversed phase HPLC analysis of single-stranded DNA containing OG and thymine dimer 5S before and after irradiation.



Purine	E_7 (V. vs. NHE)[ref]
Guanosine	**1.3 [8]**
Fapy-dG	1.1 [9]
Xanthosine	1.1 [10]
8-Oxoguanosine	**0.7 [11]**
Urate	0.6 [12]
PterinH_4	0.1 [13]
$FADH_2$	**-0.1 [14]**

Figure S8 and Table S2. Simple chemical transformations of guanosine that yield redox-active ribonucleotides. Guanine can undergo hydrolysis to xanthosine or FAPy-G which both have somewhat lower redox potentials than G. Oxidation to 8-oxo-7,8-dihydroguanosine (OG) leads to a dramatic lowering of the redox potential and further deamination to uric acid ribonucleoside further lowers the potential. For present-day coenzymes (pterins and flavins), several enzyme-catalyzed biosynthetic steps are required that presumably were optimized over millions of years.

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