Supporting Information for

Novel Oxidatively Activated Agents Modify DNA and are Enhanced by *Ercc1* Silencing

by

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Figure S1: HPLC of benzethenoguanine adducts after purification

Once purified and placed in NMR solvent, 5 μL was injected into the HPLC using the conditions listed in the text. Both compounds are pure.

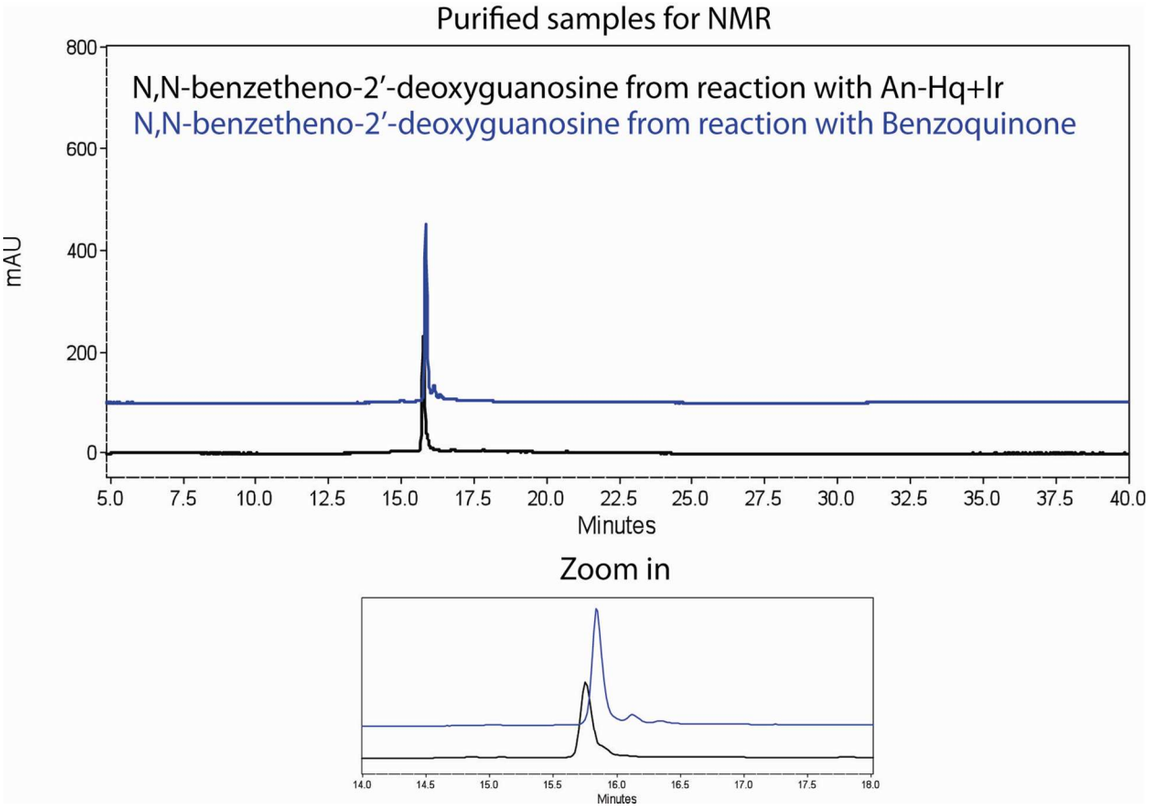
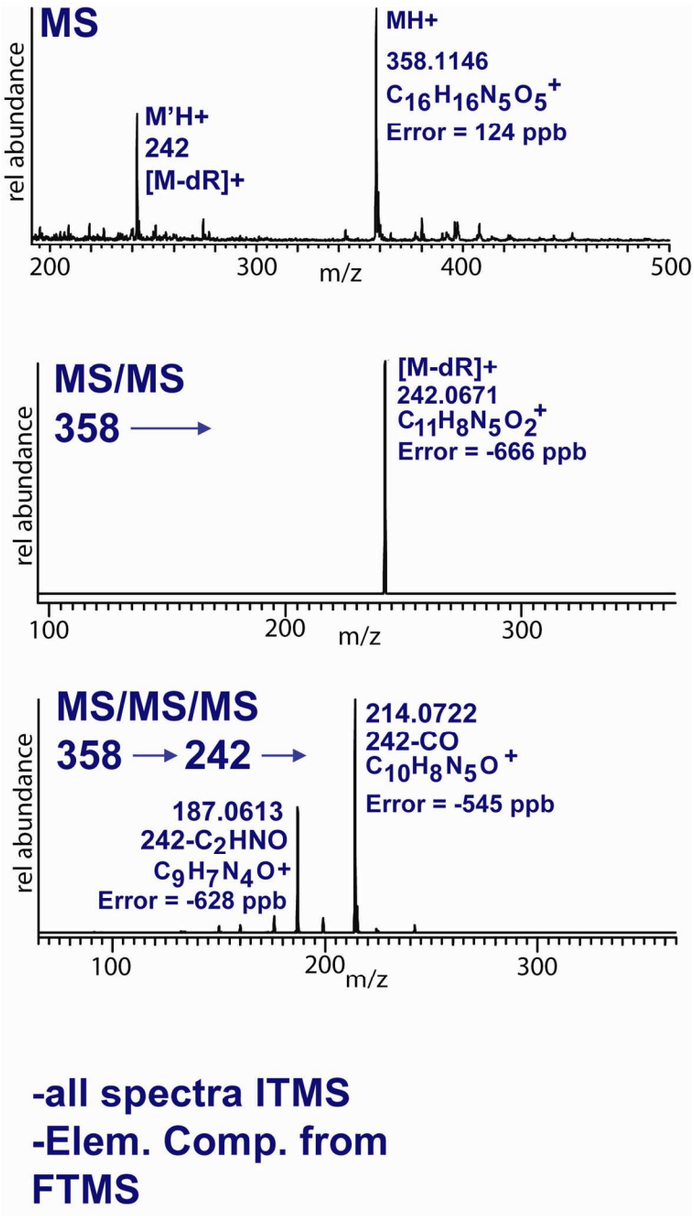


Figure S2: MS/MS of An-Hg-benzetheno-2'-deoxyguanosine

MS analysis performed as described in manuscript.



242.0670=benzethenoguanine; 214= likely loss of C_6=O_6 on guanine (also possible on phenol); 214=loss of N_7-C_5-C_6=O_6 (upper portion of guanine)

Figure S3: NMR of N1,N2- benzetheno-2'-deoxyguanosine+D20

Note: reaction with benzoquinone shown. Both reactions give the same exchangeable protons). Methods as described in Material and Methods section of manuscript.

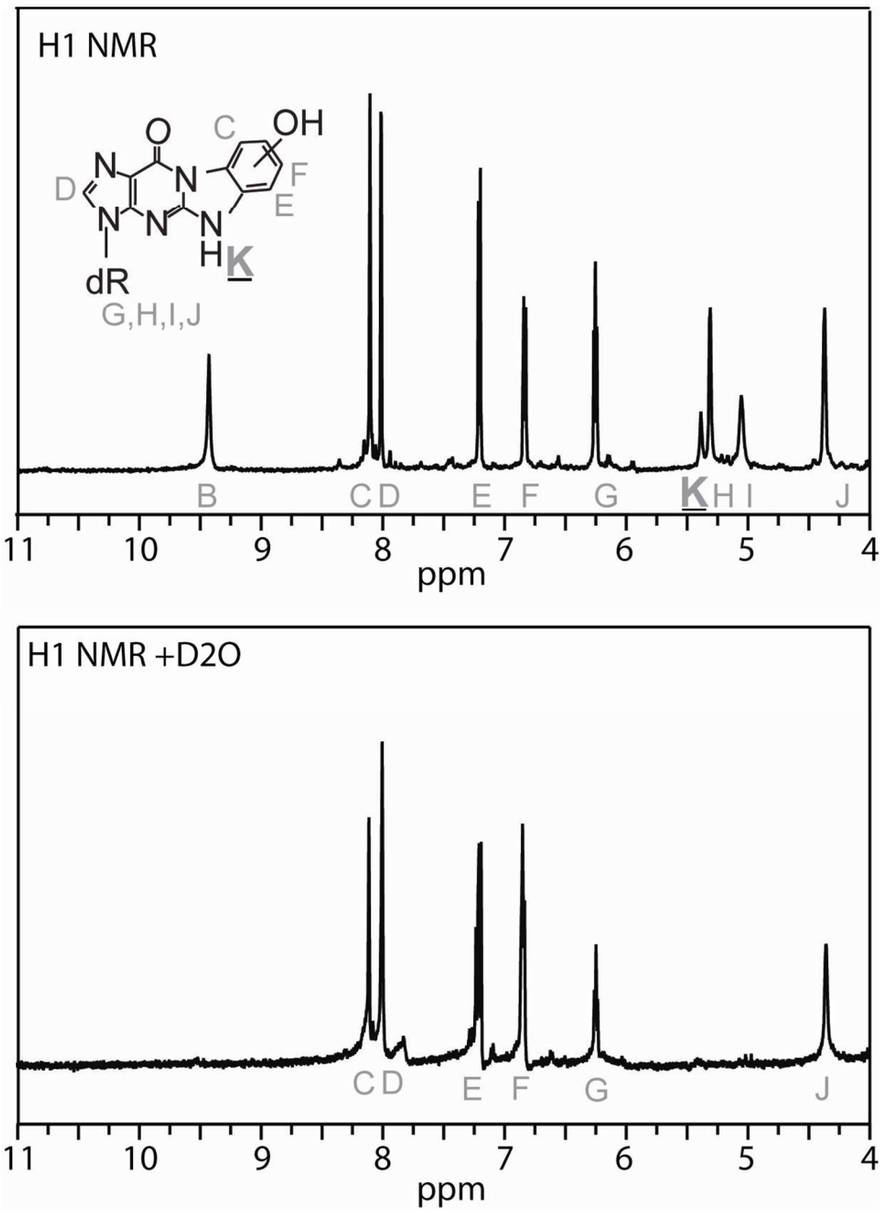


Figure S4: Rapid degradation of hydroxy-N2,N3-benzetheno-2'-deoxyguanosine

Purified samples were placed in 25 mM acidic acid-25mM sodium acetate (pH 4.7) and heated for 15 min at 50 ^{o}C. HPLC conditions were as follows: a Cosmosil 5C18-PAQ Waters column was used (4.6ID, 150mm in length). The gradient (solvent A=98.5% water, 1.5% acetonitrile and solvent B=5% water, 95% acetonitrile) was linear: 0% B for 2 min, 100% B over 20 min. Absorbance was followed at 330 nm. Black trace are of samples lacking heat and low pH.

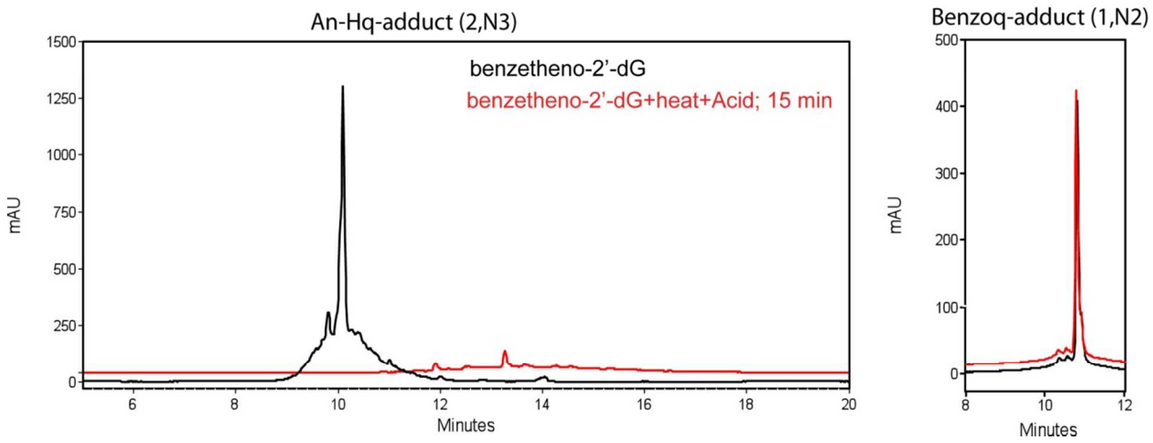
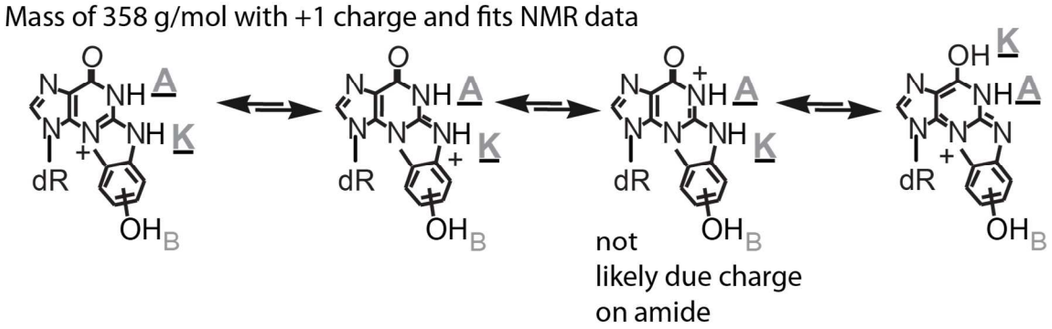


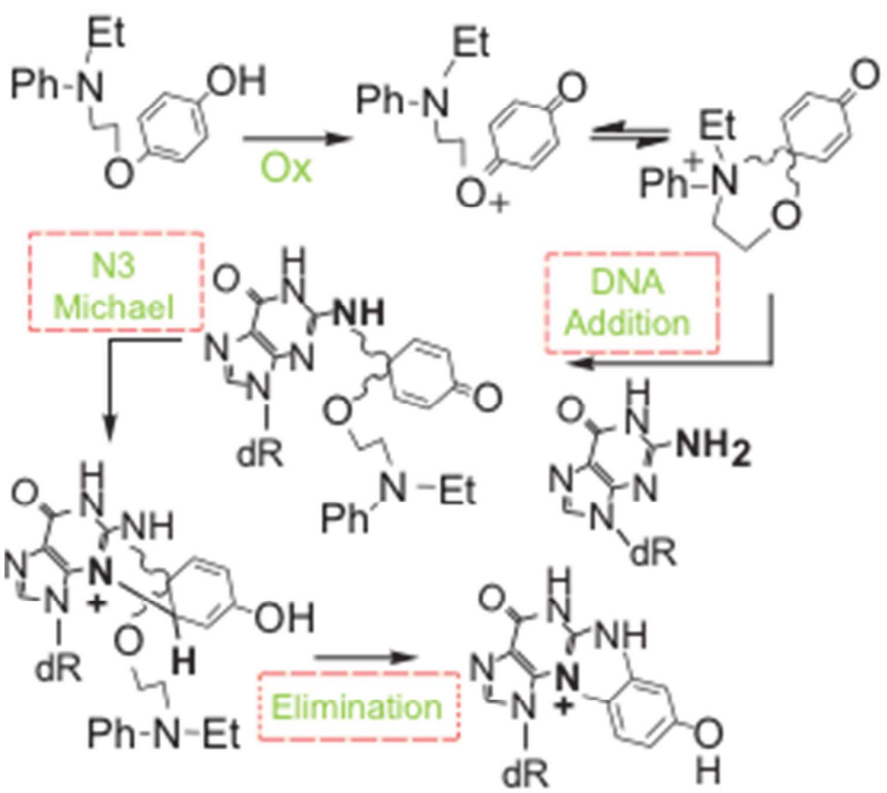
Figure S5: Tautomers and mechanism of N2,N3-benzetheno-2'-deoxyguanosine

Mass of 358 g/mol with +1 charge and fits NMR data



Schiff base not likely due to NMR integrations.

Mechanism



Relative position between phenol and N2,N3 assumed

Figure S6: LC/MS of An-Hq_2-Oligonucleotide Adduct

Observed and Theoretical m/z for $C122H145N46O71P11^{3-}$ proves An-Hq_2 adds to an oligonucleotide *in vitro* in the same manner as seen in the nucleoside reactivity studies. Ms/Ms data of m/z 1244, the triply negatively charged benzetheno-modified oligonucleotide, is provided below.

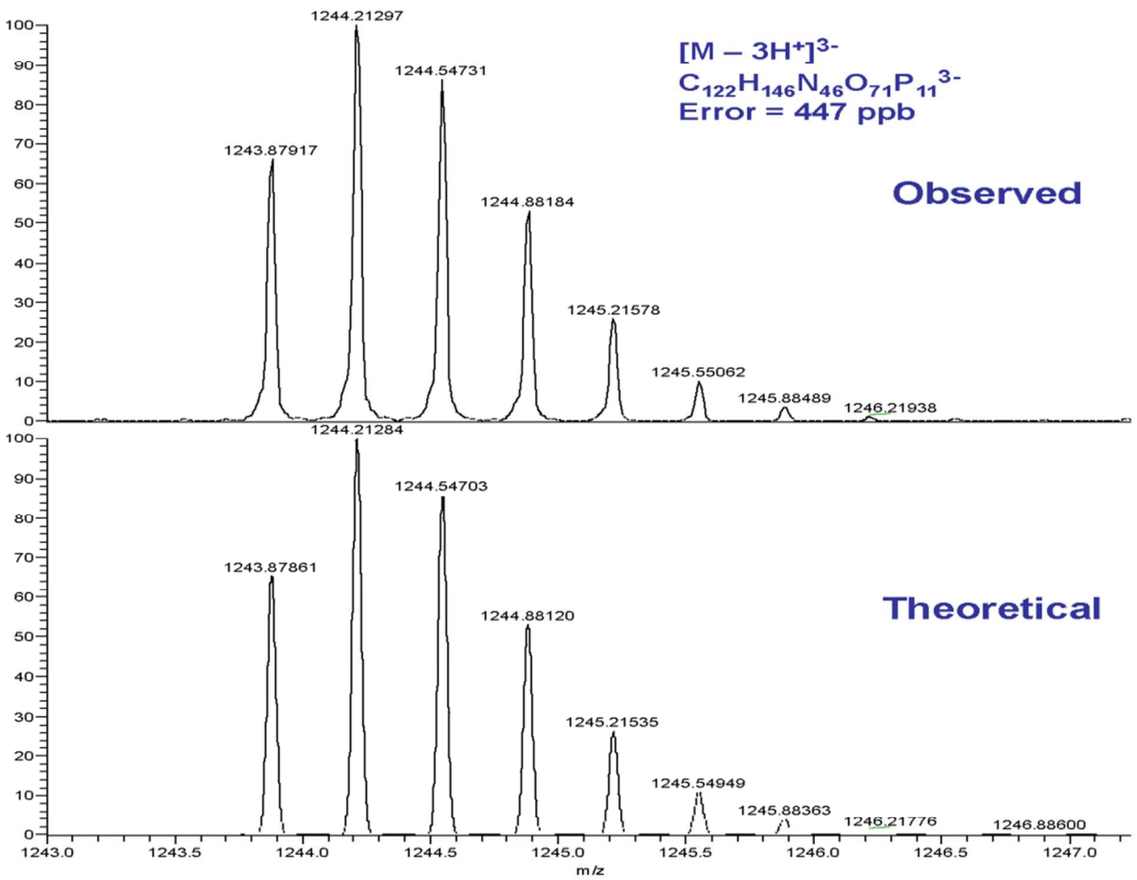


Figure S7: RNAi mediated silencing of *Ercc1* expression.

Knockdown efficiency was assessed by RT-PCR. (Lane 1). Quick-Load 100bp DNA ladder (New England Biolabs, Ipswich, MA). (Lane 2) Analysis of the control line, *da*-Gal4 crossed to the host strain to generate F1 progeny (*da*-GAL4/+) with wild-type *Ercc1* expression. (Lane 3) A lack of *Ercc1* expression was observed when the *da-GAL4* driver line was crossed with the UAS-*Ercc1*^{RNAi} transgenic line to generate F1 progeny (*da*-GAL4/*UAS-Ercc1*^{RNAi}). For both genotypes, no difference was observed in β-actin (control) expression levels. (Lane 4) Negative control.