Supplementary Information:

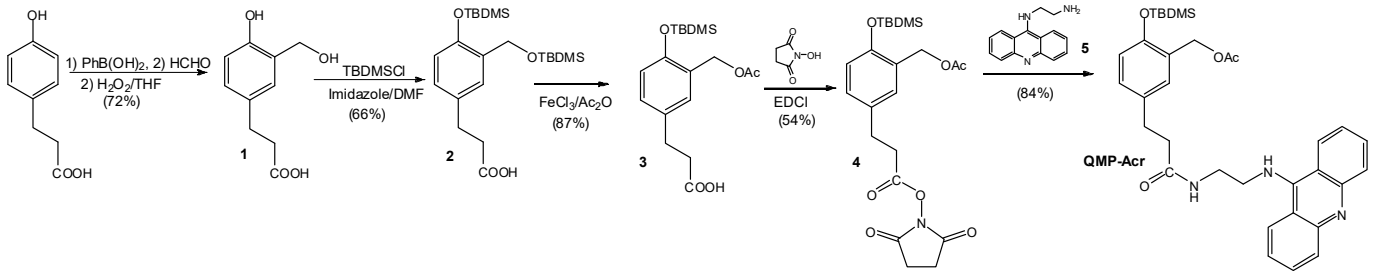
Migratory Ability of Quinone Methide-Generating Acridine Conjugates in DNA

Blessing Deeyaa and Steven E. Rokita*
Department of Chemistry, Johns Hopkins University,
3400 N. Charles Street, Baltimore, Maryland 21218, USA
rokita@jhu.edu

GENERAL METHODS AND MATERIALS

Absorbance spectra were recorded on a Hewlett-Packard 8453 UV/Vis spectrophotometer and DNA concentrations were calculated using ε_{260} values provided by the manufacturer. High resolution mass spectra (HRMS) by Fast Atom Bombardment (FAB) were determined on a VG70S magnetic sector spectrometer. Nuclear magnetic resonance (NMR) was recorded on a Bruker Avance spectrometer (^{1}H: 400 MHz, ^{13}C: 100 MHz). Aqueous solutions were prepared using water that was purified to a resistivity of 18 M Ω-m. Human apurinic/apyrimidinic endonuclease (APE1) was purchased from New England Biolab.

SYNTHETIC PROTOCOLS



Scheme S1 Protocol for synthesis of QMP-Acr as adapted from the literature.[1-3]

3-(3-Hydroxymethyl-4-hydroxyphenyl)propionic acid (1) 3-(4-Hydroxyphenyl)-propionic acid (3.00 g, 18.1 mmol) was added to a solution of phenylboronic acid (2.30 g, 19.0 mmol), trichloroacetic acid (1.47 g, 9.00 mmol), and paraformaldehyde (2.80 g, 90.0 mmol) in benzene (30 mL). The reaction was heated to reflux for 4 h, cooled to room temperature, and solvent was evaporated under reduced pressure. A white solid was collected, dissolved in ether (50 mL), washed with water (50 mL), and saturated aqueous sodium bicarbonate (3 × 50 mL). The organic layer was dried over $MgSO_4$. Solvent was evaporated, and a white solid was collected. This solid was then dissolved in THF (10 mL). Hydrogen peroxide (30 %, 5 mL) was added, and the solution was stirred at 0 °C for 2 h. The reaction was diluted with water (50 mL) and extracted with ether (3 × 50 mL). The organic layers were combined, dried over $MgSO_4$, and concentrated under reduced pressure. A light beige oil was collected, and purified by silica gel chromatography (2:1 hexanes:ethyl acetate and 0.5% acetic acid) to yield **1**, the desired white solid (2.56 g, 72%). ^{1}H NMR (400 MHz, CD_3OD) d ppm 2.54 (t, *J*=7.6 Hz, 2 H), 2.82 (t, *J*=7.6 Hz, 2 H), 4.62 (br s, 2 H), 6.68 (d, *J*=8.2 Hz, 1 H), 6.95 (d, *J*=8.0 Hz, 1 H), 7.12 (br s, 1 H).

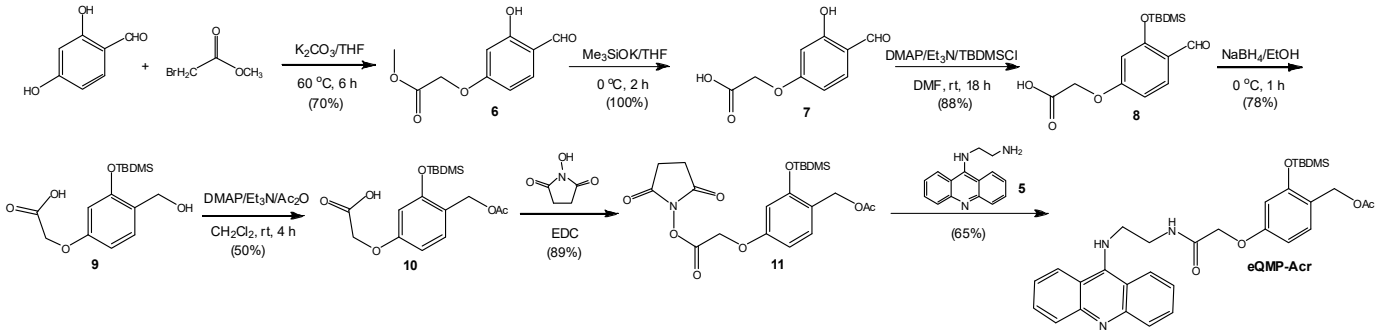
3-(3-*tert*-Butyldimethylsilyoxymethyl-4-*tert*-butyldimethylsilyloxyphenyl)propionic acid (2). Imidazole (3.00 g, 43.8 mmol) and *tert*-butyldimethylsilyl chloride (TBDMSCl, 3.30 g, 21.9 mmol) was added to a solution of **1** (1.00 g, 5.10 mmol) in DMF (6 mL). The reaction was stirred under nitrogen for 18 h, diluted with brine (50 mL), and extracted with ether (3 × 50 mL). The organic layers were combined, washed with saturated aqueous sodium bicarbonate (2 × 50 mL), dried over $MgSO_4$ and solvent was removed under reduced pressure to yield **2** as a colorless oil (3.27 g, 66 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm -0.01 - 0.04 (m, 6 H), 0.26 (s, 6 H), 0.87 (s, 9 H), 0.93 (s, 9 H), 2.61 (t, *J*=7.8 Hz, 1 H), 2.87 (t, *J*=7.8 Hz, 1 H), 4.72 (s, 1 H), 6.65 (d, *J*=8.2 Hz, 1 H), 6.92 (d, *J*=8.0 Hz, 1 H), 7.27 (s, 1 H).

3-(3-Acetoxymethyl-4-*tert*-butyldimethylsilyloxyphenyl)propionic acid (3). Ferric chloride (15 mg, 0.10 mmol) was added to a solution of **2** (1.00 g, 2.36 mmol) in acetic anhydride (2 mL) at 0 °C. The reaction was stirred for 1 h, diluted with ether (50 mL), washed with water (50 mL) and saturated aqueous sodium bicarbonate (50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated under reduced pressure to yield **3** as a colorless oil (0.72 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.27 (s, 5 H), 0.97 (s, 9 H), 2.09 (s, 3 H), 2.74 (d, *J*=8.0 Hz, 2 H), 2.90 (t, *J*=7.7 Hz, 2 H), 5.08 (br s, 2 H), 6.75 (d, *J*=8.0 Hz, 1 H), 6.99 - 7.08 (m, 1 H), 7.14 (br s, 1 H).

***N*-Succinimidyl-3-(3-acetoxymethyl-4-*tert*-butyldimethylsilyloxyphenyl)propionate (4)**. *N*-Hydroxysuccinimide (680 mg, 6.4 mmol) was added to a solution of **3** (580 mg, 1.65 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCl, 450 mg, 3.91 mmol) in DMF (5 mL). The reaction was cooled to 0 °C and stirred under N_2 for 22 h. The mixture was diluted with brine (30 mL) and extracted with ether (3 × 30 mL). The organic layers were combined, washed with saturated ammonium chloride, and dried over $MgSO_4$. The solvent was evaporated under reduced pressure to yield **4** as a viscous colorless oil (400 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.23 (s, 6 H), 0.97 (s, 10 H), 2.09 (s, 3 H), 2.77 - 2.91 (m, 8 H), 2.93 - 3.03 (m, 2 H), 5.09 (s, 2 H), 6.76 (d, *J*=8.4 Hz, 2 H), 7.06 (dd, *J*=8.2, 2.2 Hz, 2 H), 7.16 (d, *J*=2.2 Hz, 2 H).

5-(3-((2-(Acridin-9-ylamino)ethyl)amino)-3-oxopropyl)-2-((*tert*-butyldimethylsilyl)-oxy)benzyl acetate (QMP-Acr). Trimethylamine (350 μL, 2.5 mmol) was added to a solution of *N*'-(acridin-9-yl)ethane-1,2-diamine hydrochloride (**5**, 50 mg, 0.21 mmol)[4] in methanol (10 mL) and stirred at room temperature. Compound **4** (400 mg, 0.890 mmol) was dissolved in acetonitrile (8 mL) and added dropwise to the methanol solution. This mixture was stirred for 30 min, and then acetic acid (35 μL, 0.56 mmol) was added dropwise over 5 min. The solvent was removed under

reduced pressure, and the solid residue was dissolved in CH_2Cl_2 (30 mL), washed with water (1 × 30 mL) and brine (2 × 30 mL). The organic layer was dried over Na_2SO_4, and concentrated under reduced pressure. A yellow paste was collected and recrystallized in CH_2Cl_2:ether (1:2) to yield **QMP-Acr**, a yellow crystal (101 mg, 84%). ε_{410} 5500 M^{-1} cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.21 (s, 6 H), 0.97 (s, 10 H), 2.06 (s, 3 H), 2.77 (t, *J*=7.7 Hz, 2 H), 3.02 (t, *J*=7.7 Hz, 2 H), 3.89 (br. s., 2 H), 4.29 (br. s., 2 H), 5.04 (s, 2 H), 6.69 (d, *J*=8.0 Hz, 1 H), 7.10 (d, *J*=8.2 Hz, 1 H), 7.20 (br s, 3 H), 7.51 (t, *J*=6.7 Hz, 2 H), 8.18 (d, *J*=8.2 Hz, 2 H), 8.27 (d, *J*=7.0 Hz, 2 H), 8.59 (br s, 1 H), 9.48 (br s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ -4.05, 18.37, 21.28, 25.84, 31.11, 38.14, 39.72, 52.57, 62.48, 118.74, 119.69, 126.53, 129.56, 130.53, 133.28, 134.50, 152.63, 171.17, 178.00. HRMS (FAB, mNBA) m/z 572.2938. Calcd for $C_{33}H_{42}N_3O_4Si$ ($M + H^+$) 572.2945.



Scheme S2 Protocol for synthesis of eQMP-Acr following a literature procedure.[5]

Methyl (2-(4-formyl-3-hydroxyphenoxy) acetate) (6). Methyl bromoacetate (2.00 mL, 20.0 mmol) was added dropwise over 3 minutes to a solution of 2,4-dihydroxybenzaldehyde (3.00 g, 21.7 mmol) and potassium carbonate (3.00 g, 21.7 mmol) in THF (50 mL) and stirred on ice. The mixture was heated to reflux for 16 h, cooled to room temperature, and potassium carbonate was removed by vacuum filtration. The filtrate was collected and dried under reduced pressure to yield a yellow oil. The product was purified via silica gel chromatography using CH_2Cl_2:ethyl acetate (20:1) to provide a white solid (3.19 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 3.83 (s, 3 H), 4.70 (s, 2 H), 6.39 (d, *J*=2.3 Hz, 1 H), 6.60 (dd, *J*=8.7, 2.4 Hz, 1 H), 7.48 (d, *J*=8.7 Hz, 1 H), 9.75 (s, 1 H), 11.45 (s, 1 H).

2-(4-Formyl-3-hydroxyphenoxy)acetic acid (7). Potassium trimethylsilanolate (5.50 g, 43.0 mmol) was added to **6** (3.19 g, 15.2 mmol) in THF (40 mL) at 0 °C. The mixture was stirred on ice for 4 h until the starting material was consumed (monitored by TLC). The solution was adjusted to pH 2.0 using aqueous citric acid (0.1 M) and extracted with ether (3 × 50 ml). The organic layer was dried over sodium sulfate and concentrated under reduced pressure. The crude solid was collected and recrystallized in hexanes:ethanol (10:1) to yield a white solid (3.00 g, quantitative yield). ^{1}H NMR (300 MHz, CD_3CN) δ ppm 4.72 (s, 2 H), 6.42 (d, *J*=2.4 Hz, 1 H), 6.52 - 6.61 (m, 1 H), 7.59 (d, *J*=8.7 Hz, 1 H), 9.74 (d, *J*=0.5 Hz, 1 H), 11.36 (s, 1 H).

2-(3-*tert*-Butyldimethylsilyloxy-4-formylphenoxy)acetic acid (8). Triethylamine (4.0 mL, 28 mmol) and 4-dimethylaminopyridine (DMAP, 0.39 g, 32 mmol) were added to a solution of **7** (3.00 g, 15. 2 mmol) in DMF (30 mL). TBDMSCl (6.72 g, 45.7 mmol) was then added to this solution and allowed to stir under N_2 for 20 h. The reaction was quenched with water (50 mL) and extracted with ether (3 × 50 mL). The organic phases were combined, washed with saturated ammonium chloride (2 × 50 mL) and brine (50 mL) and then dried over sodium sulfate. The mixture was filtered and the solvent was removed under reduced pressure. The crude product was purified by

silica gel chromatography with hexanes:ethyl acetate (2:1) in 1% acetic acid to yield a white solid (4.21 g, 88%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.29 (s, 6 H), 1.03 (s, 10 H), 4.73 (s, 2 H), 6.40 (d, *J*=2.3 Hz, 1 H), 6.57 - 6.61 (m, 1 H), 7.82 (d, *J*=8.7 Hz, 1 H), 10.31 (s, 1 H).

2-(3-*tert*-Butyldimethylsiloxy-4-(hydroxymethyl)phenoxy)acetic acid (9). Compound **8** (2.00 g, 6.36 mmol) was dissolved in ethanol (20 mL) and cooled to 0 °C. Sodium borohydride (240 mg, 6.46 mmol) was then added, and the mixture was stirred for 2 h until the starting material was consumed (monitored by TLC). The reaction was quenched with water (20 mL) and ethanol was removed under reduced pressure. The aqueous layer was acidified with citric acid (1 M) and then extracted with ether (3 x 30 mL). The organic layers were combined and dried over sodium sulfate. Solvent was removed under reduced pressure to yield a white solid (1.54 g, 78 %). ^{1}H NMR (300 MHz, CD_3CN) δ ppm 0.23 (s, 6 H), 0.94 (s, 9 H), 4.56 (s, 2 H), 4.61 (s, 2H), 6.36 (d, *J*=2.5 Hz, 1 H), 6.52 (dd, *J*=8.5, 2.5 Hz, 1 H), 7.25 (d, *J*=8.5 Hz, 1 H).

2-(3-*tert*-Butyldimethylsiloxy-4-(acetoxymethyl)phenoxy)acetic acid (10). Triethylamine (400 μL, 2.64 mmol) and DMAP (32 mg, 0.28 mmol) were added to a solution of **9** (250 mg, 0.79 mmol) in CH_2Cl_2 (10 mL). Excess acetic anhydride (1 mL, 10 mmol) was added to the mixture that was then stirred at room temperature for 2 h, quenched with water (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The organic phases were combined, dried over sodium sulfate, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using hexanes:ethyl acetate (1:1) to yield a white solid (140 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) d ppm 0.24 (s, 6 H), 1.00 (s, 9 H), 2.05 (s, 3 H), 2.27 (s, 2 H), 4.70 (s, 2 H), 5.03 (s, 2 H), 6.42 - 6.50 (m, 2 H), 7.23 (d, *J*=8.3 Hz, 3 H).

***N*-Hydroxylsuccinimide-2-(3-*tert*-butyldimethylsiloxy-4-(acetoxymethyl)phenoxy)-acetate (11).** Synthesis of **11** was carried out using the same procedure as that described above for **4**. Coupling of **10** (140 mg, 0.395 mmol) with *N*-hydroxysuccinimide (68 mg, 60 mmol) using EDCl (116 mg, 60 mmol) yielded the desired product (160 mg, 89%) as a viscous clear oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm 1.00 (s, 9 H), 2.05 (s, 3 H), 2.82 - 2.87 (m, 4 H), 4.93 (s, 2 H), 5.04 (s, 2 H), 6.40 - 6.48 (m, 1 H), 6.52 (dd, *J*=8.4, 2.5 Hz, 1 H), 7.27 (s, 1 H).

4-(2-((2-(Acridin-9-ylamino)ethyl)amino)-2-oxoethoxy)-2-((*tert*-butyldimentylsilyl)-oxy)benzylacetate (eQMP-Acr). *N*'-(Acridin-9-yl)ethane-1,2-diamine hydrochloride **5** (28 mg, 0.12 mmol)[4] in methanol (10 mL), and trimethylamine (64 mg, 0.64 mmol) were mixed until homogenous. Compound **11** (160 mg, 0.41 mmol) was dissolved in acetonitrile (10 mL) and added dropwise to the methanol solution. The resulting mixture was stirred for 1 h and then acetic acid (0.025 g, 0.41 mmol) was added dropwise over 5 min. Solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (20 mL), washed with water (1 × 20 mL), and brine (2 × 20 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting yellow paste was recrystallized in CH_2Cl_2:ether (1:2) to yield **eQMP-Acr**, a yellow crystal (30 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.21 (s, 6 H), 0.95 (s, 9 H), 1.99 (s, 3 H), 4.07 (dd, *J*=5.0 Hz, 2 H), 4.40 (dd, *J*=4.5 Hz, 2 H), 4.59 (s, 2 H), 4.97 (s, 2 H), 6.53 - 6.61 (m, 2 H), 7.12 (t, *J*=7.7 Hz, 2 H), 7.17 (d, *J*=8.3 Hz, 1 H), 7.39 (t, *J*=7.6 Hz, 2 H), 8.08 (d, *J*=8.4 Hz, 2 H), 8.27 (d, *J*=8.7 Hz, 2 H), 8.61 (br t, *J*=1.0, 1.0 Hz, 1 H), 9.33 (br t, *J*=1.0, 1.0 Hz, 1 H) ^{13}C NMR (100 MHz, $CDCl_3$) δ 4.25, 18.15, 21.01, 25.57, 39.15, 50.80, 61.87, 67.05, 106.05, 119.38, 120.52, 123.44, 131.70, 133.98, 155.43, 158.16, 170.99, 171.10. HRMS (FAB, mNBA) m/z: 574.2738. Calcd for $C_{33}H_{42}N_3O_4Si$ ($M + H^+$): 574.2737.

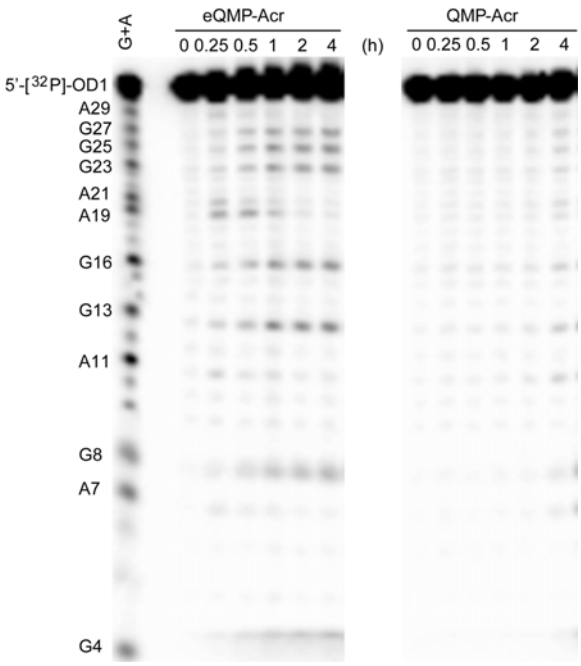


Fig. S1 Time-dependence of duplex DNA alkylation by **eQMP-Acr** and **QMP-Acr**. 5'-[^{32}P]-OD1:OD2 (3.0 μM) was incubated with the specified QMP (120 μM) in the presence of NaF (10 mM) and MES (10 mM, pH 7.0) for 0 - 4 h followed by treatment with hot aqueous piperidine (10%). The resulting DNA fragments were separated by denaturing polyacryl- amide (20%) gel electrophoresis and detected by phosphoimagery.

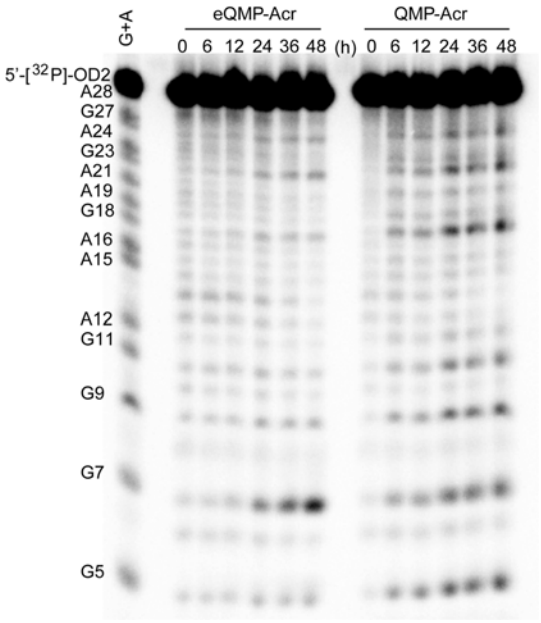


Fig. S2 Alkylation transfer between DNA strands based on the reversibility of QM reaction. OD1 (3.0 μM) was incubated alternatively with **eQMP-Acr** and **QMP-Acr** (240 μM) in the presence of NaF (10 mM) and MES (10 mM, pH 7.0) for 24 h followed by addition of 5'-[^{32}P]-OD2 (3.3 μM) for 0 - 48 h. Samples were subsequently treated with hot aqueous piperidine (10 %). The resulting DNA fragments were separated by denaturing polyacrylamide (20%) gel electrophoresis and detected by phosphoimagery.

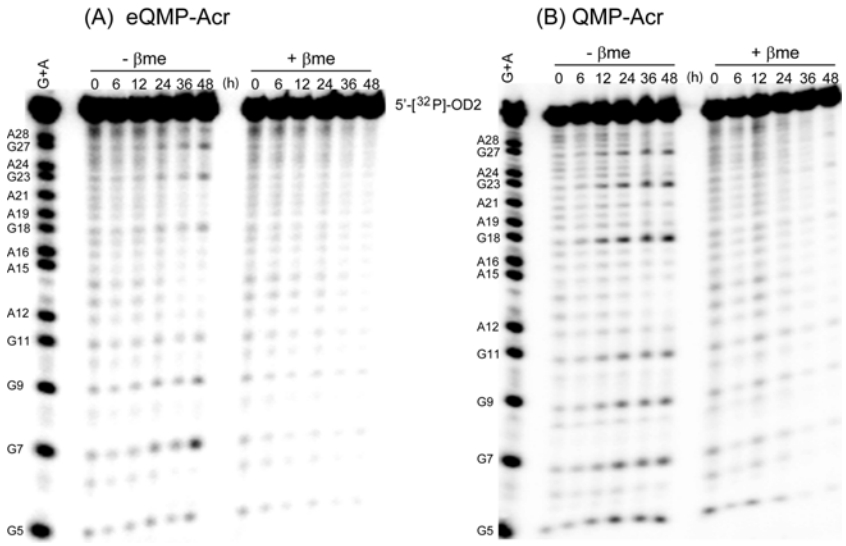


Fig. S3 Transfer of a quinone methide between DNA strands with competition from the highly nucleophilic and diffusible β-mercaptoethanol (βme). OD1 (3.0 μM) was incubated alternatively with (A) **eQMP-Acr** or (B) **QMP-Acr** (240 μM) in the presence of NaF (10 mM) and MES (10 mM, pH 7.0) for 24 h followed by addition of 5'-[^{32}P]-OD2 (3.3 μM) in the indicated absence and presence of βme (5.0 mM) for 0 - 48 h. Samples were subsequently treated with hot aqeous piperidine (10 %) and the resulting DNA fragments were separated by denaturing polyacrylamide (20%) gel electrophoresis and detected by phosphoimagery.

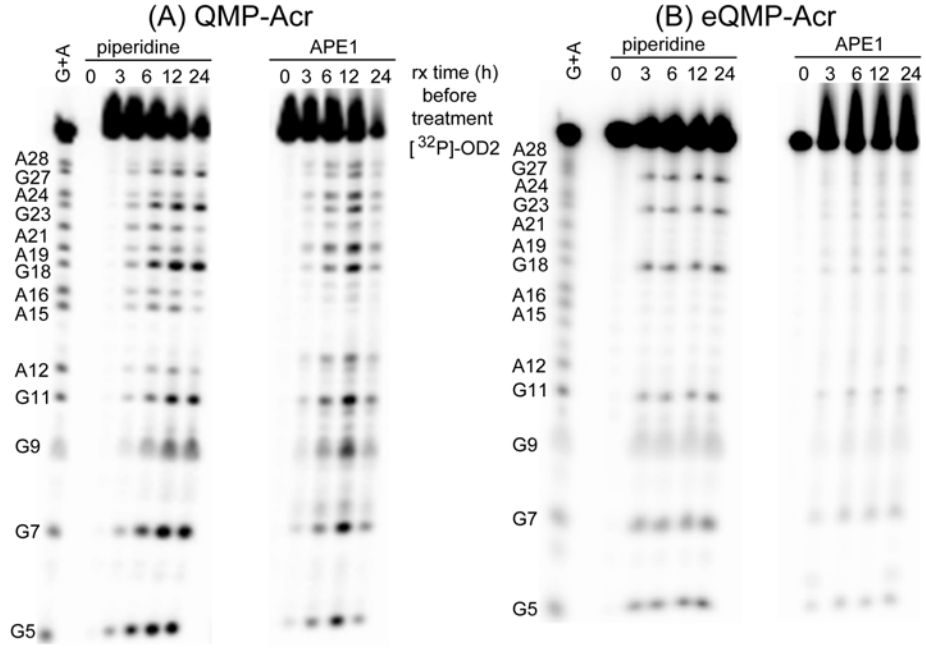


Fig. S4 Relative efficiency of deglycosylation after alkylation of guanine N7 by **eQMP-Acr** versus **QMP-Acr**. 5'-[^{32}P]-OD2:OD1 (3.0 μM) and either (A) **QMP-Acr** or (B) **eQMP-Acr** (240 μM) were incubated for 0 - 24 h in the presence of NaF (10 mM) and MES (10 mM pH 7.0) before treatment with either hot aqeous piperidine (10 %) or APE1 (3U, 37 ºC, 1 h following manufacturer's protocol). The resulting DNA fragments were separated by denaturing polyacrylamide (20%) gel electrophoresis and detected by phosphoimagery.

References

1. Q. Zhou and S. E. Rokita, *Proc. Natl. Acad. Sci. USA*, 2003, 100, 15452-15457.
2. W. Nagata, K. Okada and T. Aoki, *Synthesis*, 1979, 365-368.
3. M. A. Hutchinson, B. Deeyaa, S. R. Byrne, S. J. Williams and S. E. Rokita, in preparation.
4. W. F. Veldhuyzen, P. Pande and S. E. Rokita, *J. Am. Chem. Soc*., 2003, 125, 14005-14013.
5. C. Huang and S. E. Rokita, *Front. Chem. Sci. Eng.*, 2016, 10, 213-221.