Electronic Supplementary Information

Few Constraints Limit the Design of Quinone Methide-Oligonucleotide Self-Adducts for Directing DNA Alkylation

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Materials. Oligonucleotides and their 5'-hexamethyl aminolinker derivatives were obtained from IDT (Coralville, Iowa) and radiolabeled as indicated under standard conditions with [γ-^{32}P]ATP and T4 polynucleotide kinase. Oligonucleotide conjugates were synthesized by coupling N-hydroxysuccimidyl esters of the quinone methide precursor (QMP, 3-(4'-t-butyldimethylsilyloxy-3'-acetoxymethyl-phenyl)propionate) and diphenyl acetate (ϕ_2) alternatively to oligonucleotides containing a 5'-aminolinker as described previously.[1] Each conjugate was purified by C-18 reverse phase HPLC using triethylammonium acetate 50 mM pH 5 and a gradient of 10-55% CH_3CN over 30 min (1 mL/min). Products were desalted using ZipTips and then characterized by either electrospray ionization (1 M ammonium formate:methanol, 1:1) or MALDI-TOF (2',4',6'-trihydroxyacetophenone:citric acid dibasic ammonium salt 1:1 as the matrix) mass spectroscopy (see Tables S1 and S2).

Thermal studies of Ω2-GGG. The hairpin-forming **Ω2-GGG** (1.4 - 5.9 μM) was pre-incubated for 15 min at 40 °C in 100 mM sodium phosphate pH 7, 100 mM NaCl, and 100 mM KF prior to analysis. After this pre-incubation, the temperature was reduced from 40 to 15 °C in steps of 1 °C lasting for 1 min each. Temperature was then increased from 15 °C to 90 °C at the same rate. The ΔA_{260} vs. temperature was monitored for duplicate sets of three samples each with a Varian Cary 100 Bio UV-Vis spectrophotometer, and the melting temperature was calculated using the software of this instrument.

General procedure for deprotection of oligonucleotide quinone methide precursor (QMP) conjugates and subsequent formation of their self-adducts.[1] Reaction was initiated by adding 0.1 - 1 M KF to the oligonucleotide conjugates (2.2 μM) in 25 - 100 mM MES pH 7. Incubation was maintained under ambient conditions and monitored by analytical C-18 reverse phase HPLC using triethylammonium acetate 50 mM pH 5 and a gradient of 10-55% CH_3CN over 30 min (1 mL/min). Samples were isolated from chromatography for mass spectroscopy (Table S1), but crude reaction mixtures were used without purification for subsequent alkylation of target oligonucleotides.

General procedure for target alkylation by quinone methide (QM) oligonucleotide self-adducts.[1] The self-adducts generated as described above were diluted to 1.1 μM by addition of a 5'-diphenylacetylated target strand for HPLC analysis equivalent to that described above. Alternatively, self-adducts were diluted equivalently with a 5'-[^{32}P]-labeled target strand (1.0 μM) in 100 MES pH 7 for analysis by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using A_{260} for HPLC studies and phosphoimagery for gel studies.

References

1. Q. Zhou, S. E. Rokita *Proc. Natl. Acad. Sci. (USA)* **2003**, *100*, 15452-15457.

Table S1. Deprotection of DNA Conjugates and Subsequent Formation of Their Self-Adducts.

quinone methide DNA conjugate	ESI-MS $(M-3H^+)^{3-}$			
	loss of TBDMS		self-adduct of conjugate	
	calc	obs	calc	obs
5'-QMP-d(ACG TCA GGT GGC ACT), **QMP-OD1**	1663[a]	1663[a]	1663.4	1663.1
5'-QMP-d(TCG TCT GGT GGC TCT), **QMP-OD2**	1654.0	1653.8	1634.0	1633.7
5'-QMP-d(ATG TTA GGT GGT ATT), **QMP-OD3**	1683.0	1682.9	1663.0	1662.9
5'-QMP-d(ACT TCA TTT TTC ACT), **QMP-OD4**	1621.3	1620.9	1601.3	1601.1

[a]Q. Zhou, S. E. Rokita *Proc. Natl. Acad. Sci. (USA)* **2003**, *100*, 15452-15457.

Table S2. Characterization of Oligonucleotide Conjugates by Mass Spectroscopy.

	ESI-MS $(M+Na^+-6H^+)^{5-}$	
quinone methide DNA conjugate	calc (m/z)	obs (m/z)
5'-QMP-d(CGCTCTCCTCCTCCACCTCCGAGCGCAG) **QMP-Ω1-CAG**	1783.2	1784.4
quinone methide DNA conjugate	MALDI-TOF MS (m/z)	
5'-QMP-d(ACGCTCTCCTCCTCCACCTCCGAGCGTAAA) **QMP-Ω2-AAA**	9522.2	9521.4
5'-QMP-d(ACGCTCTCCTCCTCCACCTCCGAGCGTCCC) **QMP-Ω2-CCC**	9451.2	9450.5
5'-QMP-d(ACGCTCTCCTCCTCCACCTCCGAGCGT) **QMP-Ω2**	8582.6	8583.3
5'-QMP-d(ACGCTCTCCTCCTCCACCTCC) **QMP-OD5**	6688.4	6687.5

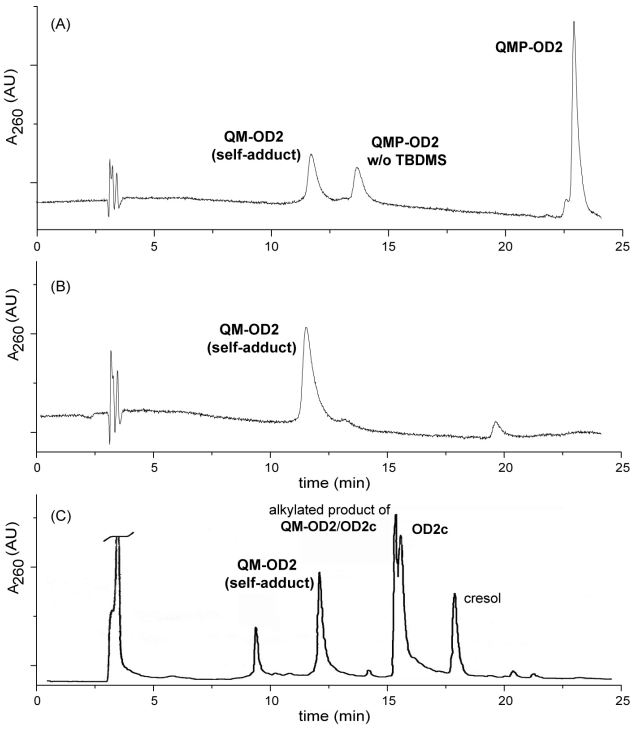


Figure S1. Reverse-phase (C-18) chromatography monitors deprotection of a quinone methide precursor (QMP)-oligonucleotide conjugate, its conversion to a quinone methide (QM) self-adduct and ultimate alkylation of a complementary target. KF (1 M) was added to 5'-QMP-d(**T**CGTC**T**GGT GGC**T**CT) (**QMP-OD2**, 2.2 μM) in 100 mM MES pH 7 to initiate reaction, and the resulting solution was incubated under ambient conditions. Aliquots were removed over time and analyzed by HPLC. Chromatograms after (A) 4 h and (B) 24 h incubations are provided as examples. An equal volume of the complementary target 3'-d(GAATC**A**GCAG **A**CCACCG**A**GA)-ϕ_2 (**OD2c**, 2.0 μM in 100 MES pH 7) modified at its 5'-aminolinker with diphenylacetate to aid chromatographic resolution was then added. The solution was incubated under ambient conditions, and aliquots were removed over time for HPLC analysis and *meta*-cresol was added as a chromatographic standard. (C) The chromatogram after 5 days is provided as an example.

Figure S2. Reverse-phase (C-18) chromatography monitors deprotection of a quinone methide precursor (QMP)-oligonucleotide conjugate and its conversion to a quinone methide (QM) self-adduct. KF (1 M) was added to 5'-QMP-d(A**T**GT**T** AGGTGG**T**A**T**T) (**QMP-OD3**, 2.2 μM) in 100 mM MES pH 7 to initiate reaction, and the resulting solution was incubated under ambient conditions. Aliquots were removed over time and analyzed by HPLC. Chromatograms after 4 and 24 h incubations are provided as examples.

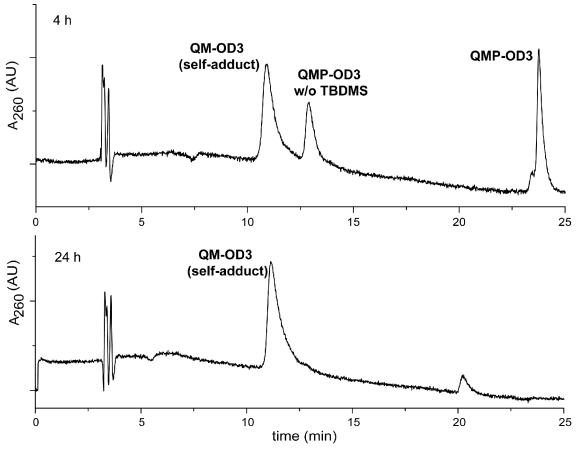


Figure S3. Reverse-phase (C-18) chromatography monitors target alkylation by a complementary quinone methide (QM)-oligonucleotide self-adduct. The self-adduct of **QMP-OD3** (2.2 μM) was generated as described above and combined with an equal volume of its complementary target 3'-d(GAATCT**A**CA**A**TCC ACC**A**T**A**A)-ϕ_2 (**OD3c**, 2.0 μM in 100 MES pH 7) modified at its 5'-aminolinker with diphenylacetate to aid chromatographic resolution. The solution was incubated under ambient conditions, and aliquots were removed over time (0 - 7 days) for HPLC analysis as described above. *meta*-Cresol was added as a chromatographic standard. Chromatograms after 0 and 7 days of **OD3c** alkylation are provided as examples.

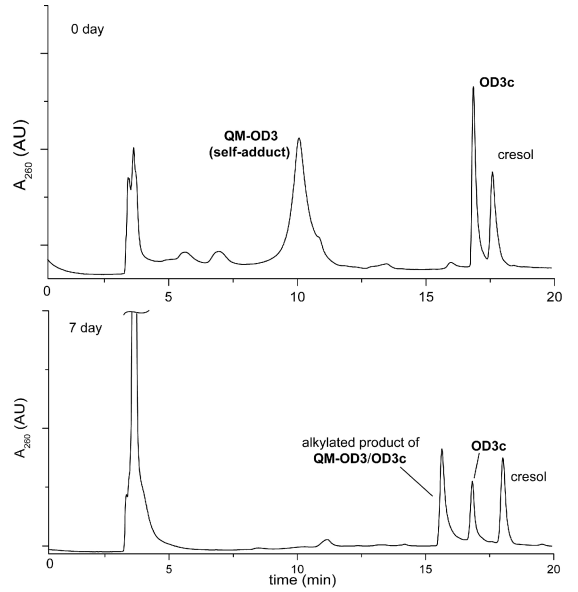
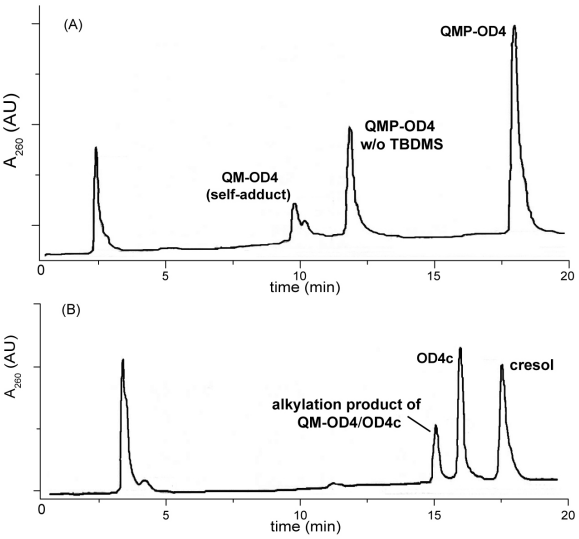


Figure S4. Reverse-phase (C-18) chromatography monitors deprotection of a quinone methide precursor (QMP)-oligonucleotide conjugate, its conversion to a quinone methide (QM) self-adduct and ultimate alkylation of a complementary target. KF (1 M) was added to 5'-QMP-d(AC**T**TCA**T**T**T**T**T**C ACT) (**QMP-OD4**, 2.2 μM) in 100 mM MES pH 7 to initiate reaction, and the resulting solution was incubated under ambient conditions for 24 h. Aliquots were removed over time and analyzed by HPLC. The chromatogram after (A) 6 h incubation is provided as an example. An equal volume of its complementary target 3'-d(GAATCTG**A**AGTAAA**A**AGTGA)-ϕ_2 (**OD4c**, 2.0 μM in 100 MES pH 7) modified at its 5'-aminolinker with diphenylacetate to aid chromatographic resolution was subsequently added. This final solution was incubated under ambient conditions, and aliquots were removed over time for HPLC analysis and *meta*-cresol was added as a chromatographic standard. (B) The chromatogram after 10 days is provided as an example.



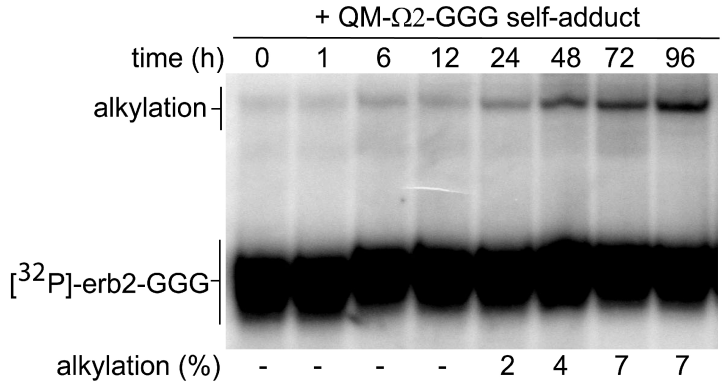


Figure S5. Time dependent alkylation of target DNA with a hairpin-forming self-adduct containing a 3'-GGG extension. The quinone methide (QM) self-adduct of 5'-QMP-d(ACGCTCTCCTCCTCCACCTCCGAGC GTGGG) (**QMP-Ω2-GGG**, 1.1 μM) in 100 mM MES pH 7 was generated *in situ* by addition of 100 mM KF and incubation under ambient conditions for 24 h. The target strand 5'-[^{32}P]-d(AGGAGGAGGTGGAG GAGGAGAGCGTGGG) ([^{32}P]-**erb2-GGG**, 1.0 μM, 90 nCi) was then added at 0 h and incubated under ambient conditions. At the indicated times, samples were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.

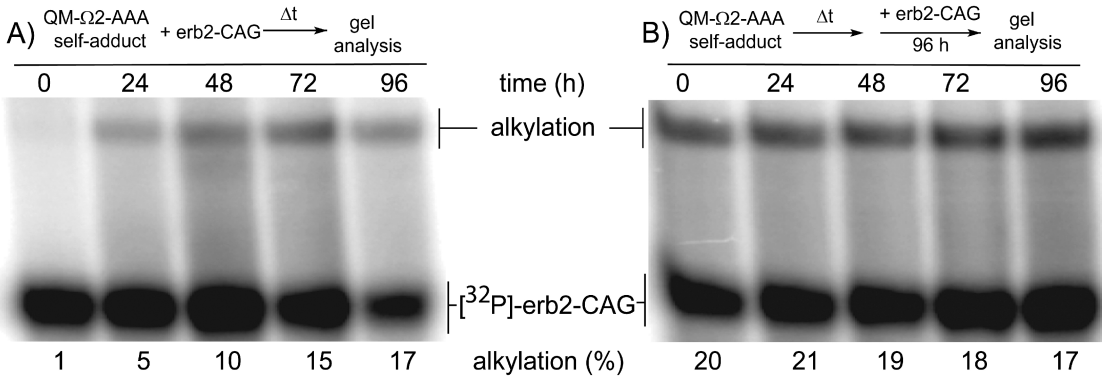


Figure S6. Efficiency and longevity of a hairpin-forming self-adduct containing a 3'-AAA extension for alkylation of target DNA. The quinone methide (QM) self-adduct of 5'-QMP-d(ACGCTCTCCTCCTCC ACCTCCGAGCGTAAA) (**QMP-Ω2-AAA**, 1.1 μM) in 100 mM MES, 100 mM NaCl pH 7 was generated *in situ* by addition of 100 mM KF. (A) To assess the efficiency of target alkylation, incubation was maintained under ambient conditions for 24 h to form the self-adduct. The target strand 5'-[^{32}P]-d(AGGAGGAGGTGG AGGAGGAGAGCGTCAG) ([^{32}P]-**erb2-CAG**, 1.0 μM, 90 nCi) was then added at 0 h and incubated under ambient conditions for the indicated times. (B) To assess the stability of the QM adduct for subsequent alkylation, samples were pre-incubated after KF addition for the indicated times before [^{32}P]-**erb2-CAG** (1.0 μM, 90 nCi) was added. These samples were then incubated for an additional 96 h. All reactions were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.

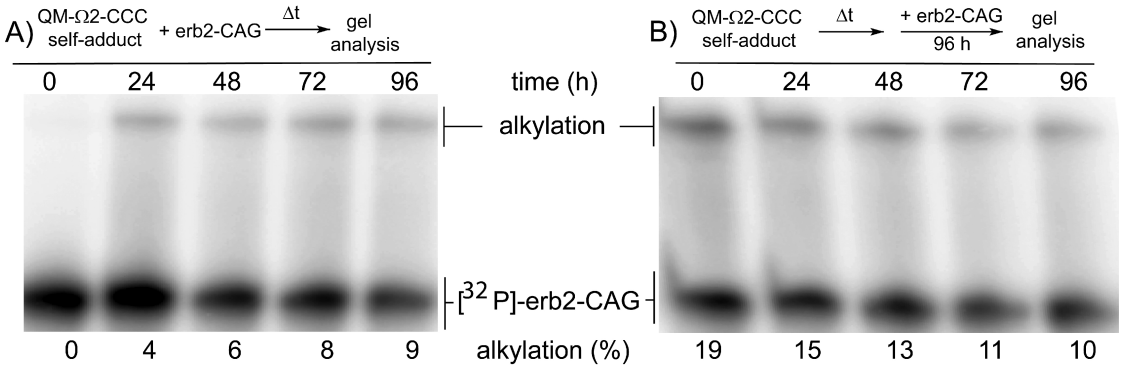


Figure S7. Efficiency and longevity of a hairpin-forming self-adduct containing a 3'-CCC extension for alkylation of target DNA. The quinone methide (QM) self-adduct of 5'-QMP-d(ACGCTCTCCTCCTCC ACCTCCGAGCGTCCC) (**QMP-Ω2-CCC**, 1.1 μM) in 100 mM MES, 100 mM NaCl pH 7 was generated *in situ* by addition of 100 mM KF. (A) To assess the efficiency of target alkylation, incubation was maintained under ambient conditions for 24 h to form the self-adduct. The target strand [^{32}P]-**erb2-CAG** (1.0 μM, 90 nCi) was then added at 0 h and incubation was continued for the indicated times under ambient conditions. (B) To assess the stability of the QM adduct for subsequent alkylation, samples were pre-incubated after KF addition for the indicated times before [^{32}P]-**erb2-CAG** (1.0 μM, 90 nCi) was added. These samples were then incubated for an additional 96 h. All reactions were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.

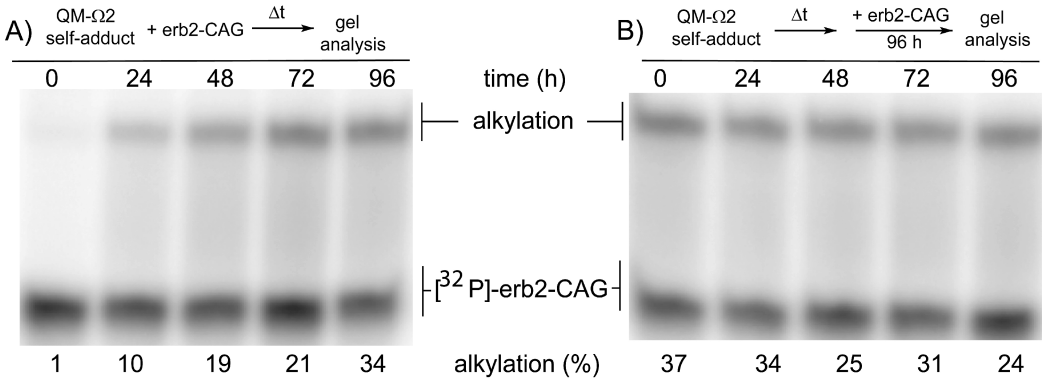


Figure S8. Efficiency and longevity of a hairpin-forming self-adduct lacking a 3'-extension for alkylation of target DNA. The quinone methide (QM) self-adduct of 5'-QMP-d(ACGCTCTCCTCCTCCACCTCCG AGCGT) (**QMP-Ω2**, 1.1 μM) in 100 mM MES, 100 mM NaCl pH 7 was generated *in situ* by addition of 100 mM KF. (A) To assess the efficiency of target alkylation, incubation was maintained under ambient conditions for 24 h to form the self-adduct. The target strand [^{32}P]-**erb2-CAG** (1.0 μM, 90 nCi) was then added at 0 h and incubation was continued for the indicated times under ambient conditions. (B) To assess the stability of the QM adduct for subsequent alkylation, samples were pre-incubated after KF addition for the indicated times before [^{32}P]-**erb2-CAG** (1.0 μM, 90 nCi) was added. These samples were then incubated for an additional 96 h. All reactions were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.

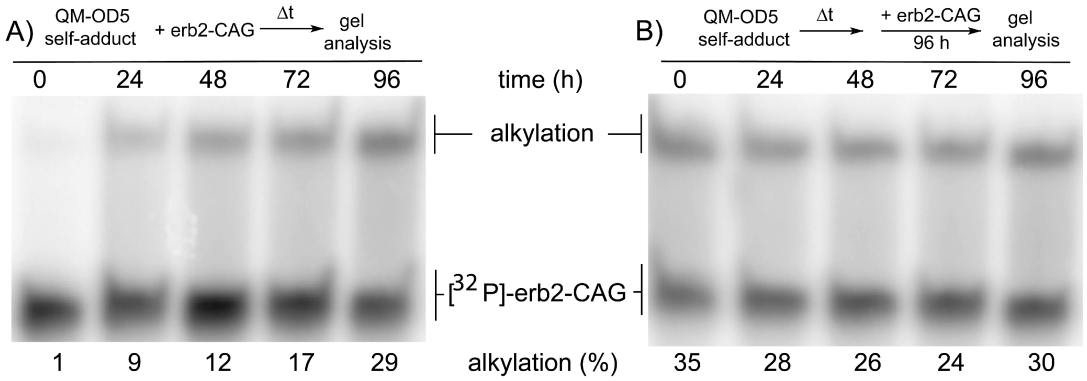


Figure S9. Efficiency and longevity of a self-adduct lacking a hairpin structure prior to alkylation of target DNA. The quinone methide (QM) self-adduct of 5'-QMP-d(ACGCTCTCCTCCTCCACCTCC) (**QMP-OD5**, 1.1 µM) in 100 mM MES, 100 mM NaCl pH 7 was generated *in situ* by addition of 100 mM KF. (A) To assess the efficiency of target alkylation, incubation was maintained under ambient conditions for 24 h to form the self-adduct. The target strand [^{32}P]-**erb2-CAG** (1.0 µM, 90 nCi) was then added at 0 h and incubation was continued for the indicated times under ambient conditions. (B) To assess the stability of the QM adduct for subsequent alkylation, samples were pre-incubated after KF addition for the indicated times before [^{32}P]-**erb2-CAG** (1.0 µM, 90 nCi) was added. These samples were then incubated for an additional 96 h. All reactions were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.

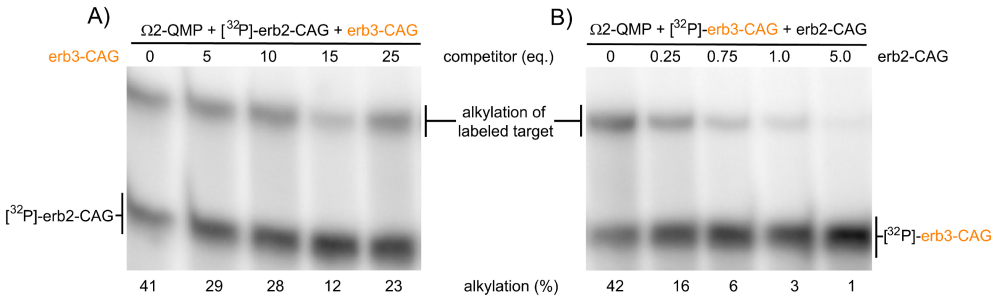


Figure S10. Competition between matched and mismatched targets for alkylation by the hairpin forming conjugate containing a quinone methide precursor. (A) Alkylation of a fully complementary target [^{32}P]-**erb2-CAG** (1.0 µM) by the conjugate **QMP-Ω2** (1.1 µM) in 100 mM MES, 100 mM NaCl pH 7 was initiated by addition of 100 mM KF in the presence of the indicated equivalents of an alternative target containing 3 mispaired nucleotides in the center of the duplex, 5'-d(AGGAGGAGGTGGCAAAGGAGAGCGTCAG) (**erb3-CAG**). (B) Alkylation of the mismatched target [^{32}P]-**erb3-CAG** (1.0 µM) by the conjugate **QMP-Ω2** (1.1 µM) in 100 mM MES, 100 mM NaCl pH 7 was initiated by addition of 100 mM KF in the presence of the indicated equivalents of the fully complementary alternative target **erb2-CAG**. All samples were incubated under ambient conditions for 96 h and then analyzed by denaturing 20% (7 M urea) polyacrylamide gel electrophoresis as described above.

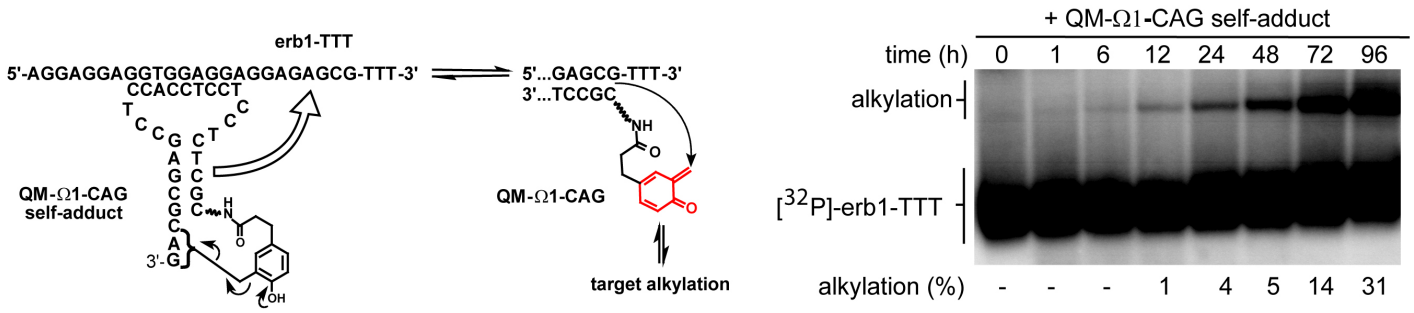


Figure S11. Time dependent alkylation of target DNA containing a 3'-TTT extension. The quinone methide (QM) self-adduct of 5'-QMP-d(CGCTCTCCTCCTCCACCTCCGAGCGTCAG) (**QMP-Ω1-CAG**, 1.1 μM) in 100 mM MES pH 7 was generated *in situ* by addition of 100 mM KF and incubation under ambient conditions for 24 h. The target strand 5'-[^{32}P]-d(AGGAGGAGGTGGAGGAGGAGAGCGTTT) ([^{32}P]-**erb1-TTT**, 1.0 μM, 90 nCi) was then added at 0 h and incubated under ambient conditions. At the indicated times, samples were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.

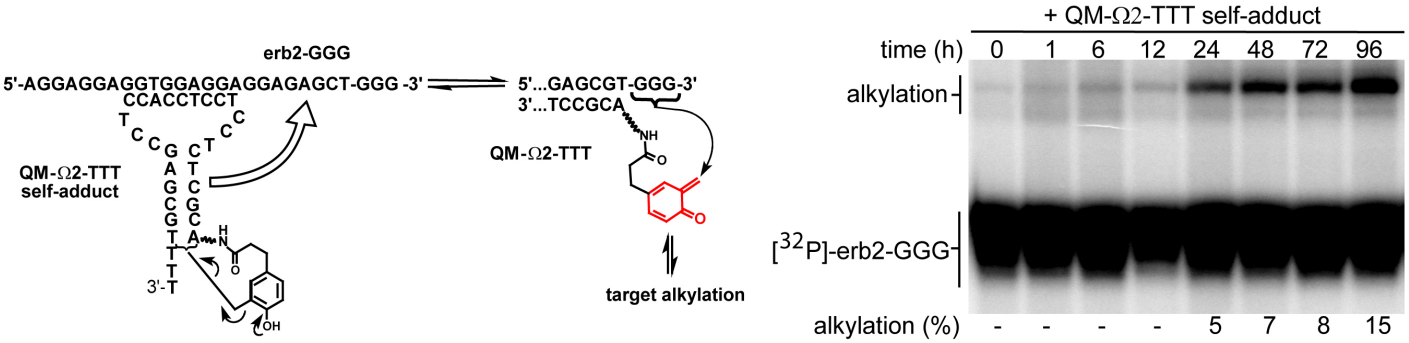


Figure S12. Time dependent alkylation of target DNA with a hairpin-forming self-adduct containing a 3'-TTT extension. The quinone methide (QM) self-adduct of 5'-QMP-d(ACGCTCTCCTCCTCCACCTCC GAGCGTTTT) (**QMP-Ω2-TTT**, 1.1 μM) in 100 mM MES pH 7 was generated *in situ* by addition of 100 mM KF and incubation under ambient conditions for 24 h. The target strand 5'-[^{32}P]-d(AGGAGGAGGTGG AGGAGGAGAGCGTGGG) ([^{32}P]-**erb2-GGG**, 1.0 μM, 90 nCi) was then added at 0 h and incubated under ambient conditions. At the indicated times, samples were analyzed by denaturing 20 % (7 M urea) polyacrylamide gel electrophoresis. Product yields were estimated by the conversion (%) of target strand to its alkylated derivative using phosphoimagery and ImageQuant software.