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Complete List of Authors:	Deeyaa, Blessing; Johns Hopkins University, chemistry Rokita, Steven; Johns Hopkins University, Dept of Chemistry

Title: Migratory Ability of Quinone Methide-Generating Acridine Conjugates in DNA

Authors: Blessing Deeyaa and Steven E. Rokita*

Contact: Department of Chemistry, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218 USA. rokita@jhu.edu

Abstract:

The dynamic nature of nucleic acid alkylation by simple *ortho* quinone methides (QM) and their conjugates has provided numerous opportunities ranging from sequence selective targeting to bipedal walking in duplex DNA. To enhance the diffusion rate of adduct migration, one of two sites for QM generation was deleted from a bisQM conjugate of acridine to remove the covalent anchor to DNA that persists during QM regeneration. This conversion of a bisfunctional cross-linking agent to a monofunctional alkylating agent allowed adduct diffusion to traverse an extrahelical -TT- bulge that previously acted as a barrier for its bisfunctional analog. An electron rich derivative of the monofunctional acridine conjugate was additionally prepared to accelerate the rates of DNA alkylation and QM regeneration. The resulting stabilization of this QM effectively enhanced the rate of its release from adducts attached at guanine N7 in competition with an alternative and detrimental deglycosylation pathway. Intercalation by the acridine component was not sufficient to hold the transient QM intermediates within duplex DNA and consequently these electrophiles diffused into solution and were subject to quenching by solvent and a model nucleophile, β-mercaptoethanol.

Introduction

Application of quinone methide intermediates (QM) continues to expand across a variety of fields including chemistry, biology and materials science as our understanding of their reactivity and methods of preparation gain increasing sophistication. QMs have been integrated into numerous strategies for organic synthesis[1-3] including a variety of stereoselective transformations.[4,5] QM

and their related azaquinone methides additionally provide the basis for numerous self-immolating materials with wide-ranging relevance to optical sensing, drug delivery, polymers and surface modification.[6-9] While QMs are sometimes designed as passive carriers of drugs, QMs may also be generated in situ for their own therapeutic activities.[10-13] In biochemistry, QMs have served in mechanism-based inactivation of enzymes,[14] activity-based screening of molecular libraries,[15] protein conjugation,[16] nucleic acid alkylation and cross-linking[17-19] and many more opportunities are on the horizon.[20] The transient nature of QMs requires their generation in situ from transformation of precursors using methods ranging from photochemistry,[21-23] oxidation,[24-26] reduction,[27,28] hydrolysis,[10,14] and deprotection of silylated derivatives (Scheme 1).[29]

[insert Scheme 1]

The reversibility of QM reaction broadens the utility of these intermediates as illustrated in synthesis of chiral BINOL ligands,[30], dynamic kinetic resolution of 2-sulfonylalkyl phenols,[31] and patterning solid surfaces.[7] The strong nucleophiles of DNA (guanine N7, adenine N1 and cytosine N3, Scheme 1) readily couple with simple *ortho*-QMs and the similarly strong nucleofugacity of these same groups allows facile regeneration of the QMs.[32,33] This reversibility supports sequence specific alkylation and cross-linking of nucleic acid targets through formation of reversible self-adducts that require no external trigger such as fluoride, oxidation or photochemical excitation to initiate reaction.[34-38] Reversible cross-linking of nucleic acids with bisfunctional quinone methides (bisQMs) containing two electrophilic sites extends the effective lifetime of these intermediates by their repetitive regeneration under aqueous conditions for more than 13 days.[36,39] The dynamics of this reaction supports exchange of cross-links between DNA strands and migration of a bisQM within duplex DNA.[36,40]

Slow migration of the bisQM within DNA currently limits its potential use in a cellular environment. Despite the short half-life of a model QM adducts (hours),[33,41] diffusion of

cross-links in DNA formed by an electron rich bisQM-acridine conjugate required 7 days to diffuse across 9 base pairs.[40] Furthermore, no such diffusion was observed for a less electron rich derivative that had previously supported exchange between cross-linked strands of DNA strands. Diffusion within a duplex was possible only after a methylene bridge to the QM was replaced with an electron-donating oxygen to form an ether linkage.[40] This is consistent with the general sensitivity of QMs to substituent effects.[41-43] Initial formation and regeneration of the electron deficient QM is promoted by electron-donating substituents and its lifetime is similarly extended by these substituents. Migration of the conjugate is thought to involve a transient regeneration of a QM from a nucleobase adduct that is followed by a conformational rearrangement to allow subsequent alkylation at an alternative nucleobase. The extent of rearrangement and distance the QM may diffuse is constrained by the remaining covalent attachment of the QM and non-covalent intercalation of the acridine appendage.

As described below, monofunctional QM derivatives have now been prepared and characterized with DNA to test if the overall rate of QM migration within a duplex can be enhanced in the absence of the second QM equivalent that allows for cross-linking. The QM precursor (QMP) conjugated to acridine (QMP-Acr) is analogous to the original methylene linked precursor to the bisQM species and retains the acridine to maintain its general affinity for duplex DNA (Scheme 2). An electron rich conjugate linked by an ether bridge has also been prepared (Scheme 2). The linkage in this case is *para* to the nascent exo-methylene group of the QM to maximize the QM dynamics that are dependent on both the nature and site of substitution.[41] The corresponding conjugate used previously for cross-linking contained an ether bridge at the related *meta* position that is less effective than that in a *para* position to stabilize the QM.[41] Diffusion of the monofunctional QM conjugate is now shown to be greatly enhanced over that of its bisfunctional analog. Loss of a covalent anchor during QM regeneration also allows release of the reagent from DNA duplex despite the presence of a strong intercalator.

[insert Scheme 2]

Results and discussion

Design and synthesis

Monofunctional QM-acridine conjugates were designed for comparison with the prior bisQM cross-linking agents (Scheme 2). The QM precursor QMP-Acr lacked only the substitution representing the second latent QM in order to characterize the migration of a derivative capable of DNA alkylation but not cross-linking (Scheme 1). An additional QM precursor eQMP-Acr was also synthesized to test the consequences of a derivative with an enhanced ability to regenerate and stabilize the QM intermediate based on the electron donating properties of an ether, rather than methylene, linkage. Contribution of the substituent was further increased by placing the ether linkage on the *para*, rather than *meta*, position relative to the nascent electrophilic site.[41] Synthesis of both conjugates followed literature procedures and are detailed in the supplementary information. For QMP-Acr, 3-(4-hydroxylphenyl)proprionic acid was first hydroxymethylated and then silylated (Scheme S1).[34,44] The resulting benzyl siloxyl ether was selectively substituted with acetate and the carboxylate was activated for coupling to *N*-(9-acridyinyl)-1,2-diaminethane. For eQMP-Acr, 2,4-dihydroxybenzaldehyde was alkylated with methyl bromoacetate, saponified and silylated (Scheme S2).[38] The aldehyde was next reduced and condensed with acetate. Activation of the carboxylate and coupling with the acridine derivative followed the same procedures as those used for QMP-Acr. Both products were purified by crystallization and confirmed by standard NMR and MS characterization.

[insert Fig. 1] [insert Scheme 3]

DNA alkylation by QM-acridine conjugates

The corresponding bisfunctional QM predominantly alkylates DNA at guanine N7 and reaction with the monofunctional eQMP-Acr and QMP-Acr was consequently monitored at this site as

well.[45] The duplex formed by OD1:OD2 was used as a model of DNA and reaction sites were determined by fragmentation of the radiolabeled strand induced by standard piperidine treatment (Scheme 3). Little time dependence was observed for DNA alkylation after 1 h for eQMP-Acr, whereas alkylation with QMP-Acr continued to accumulate for the first 24 h (Fig. 1). These trends are reproducible and indicate that QMP-Acr generates an almost 4-fold greater yield of alkylation than that with eQMP-Acr (Fig. 2A). However, eQMP-Acr reacts much more quickly and produces its maximum yield in little more than 2 h rather than the 24 h required for QMP-Acr (Fig. 2). The relative rates are consistent with the expected substituent effects for which electron donating groups accelerate formation of the transient QM. The dramatic difference in yield was unexpected since the two corresponding bisQMP derivatives characterized previously generated similar yields of products.[40] The contrasting behavior of the monofunctional derivatives eQMP-Acr and QMP-Acr may reflect an interplay between the rates of QM reaction and their potential to dissociate from DNA after QM regeneration. This is distinct from the bisQMP derivatives that remain anchored by a covalent bond to DNA during QM regeneration. The electron rich nature of eQMP-Acr both promotes reversible formation of the QM and extends the lifetime of this reactive intermediate to enhance the opportunities for partitioning between subsequent addition of DNA and water.[41] Guanine N7 adducts remain reversible but water adducts persist and therefore quench further reaction.[36,39] These contrasting stabilities may also explain the slight decline in yield with eQMP-Acr after 24 h (Fig. 2A). The different geometry of tethering the QMs to acridine could also influence the efficiency of reaction but this effect is expected to be minor due to the length and flexibility of the full linker (Scheme 2).

[insert Fig. 2]

Low yields of reaction at adenines are also evident after treatment with QMP-Acr (Fig. 1) and, when background signals are low, equivalent products are detected after treatment with

eQMP-Acr as well (Fig. S1). Both results reflect in part the relative lability of such adducts. Some of the DNA fragments generated indirectly by QMP-Acr decrease after extended incubation suggesting a loss of product. Analogous adducts formed by eQMP-Acr are even more labile as expected for this activated system and their corresponding DNA fragments are no longer detectable after 1 h (Fig. S1). Adducts of adenine N1 were previously known to dominate the initial product profile but then quickly dissipate when a nucleotide model system was monitored over time.[33] The N1 position of adenine is also expected to participate in migration of interstrand cross-linking since cross-links are capable of traversing a region with G/T on one strand and C/A on the complementary strand.[40] An adduct containing adenine was additionally identified by mass spectrometry during reaction with a conjugate of QM and metal cyclens and this too diminished over time.[46] When the reversibility of QM reaction was quenched by selective oxidation, an N1 adenine adduct was observed after treatment of single- and double-stranded DNA with a simple, unconjugated QM.[47]

Susceptibility of eQMP-Acr and QMP-Acr to quenching by water

The relative rates of QM formation for eQMP-Acr and QMP-Acr were additionally compared by their characteristic quenching with water in solutions lacking DNA (Fig. 3A). Initial deprotection of these precursors first yields their benzyl acetate intermediates that subsequently generate the transient QMs. In the absence of competing nucleophiles, water quenches the QMs to form the benzyl alcohols that are incapable of QM regeneration and consequently no longer effective alkylating agents. The persistence of the benzyl acetates can therefore be monitored by following the diminishing ability of the reaction mixture to alkylate DNA over time. Accordingly, eQMP-Acr and QMP-Acr were preincubated with fluoride to initiate QM formation for 0 - 24 h prior to addition of DNA. The resulting alkylation of guanine N7 by eQMP-Acr was lost very rapidly over a single hour of preincubation (Fig. 3B). This rapid generation and quenching of the

QM formed by eQMP-Acr is consistent with its rapid reaction in the presence of DNA as well (Figs. 1 & 2). The yield of DNA alkylation decreased more slowly during preincubation of QMP-Acr and maintained for 8 h before addition of DNA (Fig. 3B). Neither QM precursor supported DNA alkylation after their preincubation with water for more than 24 h and hence the dynamic reaction observed after such a period as described below can only be attributed to the extended QM lifetime established by reversible reaction with DNA as examined through transfer of QM between DNA strands.

[insert Fig 3]

Dynamics of QM transfer between DNA strands

The dynamic nature of a bisfunctional QM derivative was first demonstrated by its ability to isomerize from an intra- to interstrand cross-link within DNA and further verified by the persistence of its interstrand cross-linking after exchange of complementary strands of DNA.[36,39] An analogous migration of adducts was consequently measured for the monofunctional analogs described here. OD1 was incubated alternatively with eQMP-Acr and QMP-Acr for 24 h to ensure all of the precursor was consumed by alkylation of DNA or quenching by water (Fig. 4A). Only the reversible products formed with OD1 would then sustain subsequent QM regeneration and transfer to other sites in DNA. This was observed by migration of the QM adducts from their initial sites in OD1 to the radiolabeled and complementary strand OD2. As before, transfer to guanine N7 was monitored by its piperidine-induced strand fragmentation. The relative rates for transfer of adducts formed by eQMP-Acr and QMP-Acr are similar and both approach a maximum after ~ 40 h (Figs. 4B and S2). The yield of transfer to OD2 is larger for QMP-Acr than for eQMP-Acr. This may reflect both the greater initial yield of adducts formed by QMP-Acr and the donor strand OD1 and the greater susceptibility of the electron-rich QM intermediate to be quenched by water as evident from the initial studies of alkylating the OD1-OD2 duplex (Figs. 1 & 2). However, the rate of transfer is not enhanced by the faster reaction of eQMP-Acr versus that

of QMP-Acr and may suggest the rate of QM (re)generation does not limit its transfer between strands within duplex DNA.

[insert Fig. 4]

Designing the monofunctional acridine conjugates was motivated by a desire to enhance the diffusion of its dynamic electrophile through duplex DNA relative to the bisfunctional analogs. The latter was guaranteed to remain held within an individual duplex due to its sequential and not concurrent regeneration of the QMs. In contrast, QM regeneration for the monofunctional derivatives removes this covalent constraint and thus the reactive intermediate may diffuse far from its original site within duplex DNA. Additionally, the monofunctional QM may even escape the duplex and diffuse to alternative targets. The extent to which the QM became exposed to solvent was qualitatively compared by its susceptibility to quenching in the added presence of a competitive thiol nucleophile, β-mercaptoethanol (βme). Although water acts as a quenching agent as well, thiols are orders of magnitude more efficient at trapping QMs.[21] Previously, QMs that were covalently anchored within a duplex demonstrated little sensitivity to βme at even mM concentrations.[34,39,40] BisQM equivalents did not dissipate during intra- to interstrand transfer nor during migration within a duplex in the presence of βme. The monofunctional QM conjugates of acridine diverge from this behavior. The transient QM intermediates formed by both eQMP-Acr and QMP-Acr were fully quenched by βme (5 mM) while monitoring their transfer from OD1 to OD2 (Fig. S3). This provided the first indication that QMs generated by monofunctional precursors may become significantly more exposed to solvent than their bisQM counterparts.

Interduplex transfer of monofunctional QM conjugates

The consequences of removing the covalent constraints for QM diffusion are more dramatically illustrated by the ability of the monofunctional QM adducts to migrate past a barrier that previously impeded diffusion of a bisQM counterpart. While diffusion of a bisQM proceeded

stepwise along the entire sequence of a canonical duplex of DNA, an intervening extrahelical -TT- bulge was capable of blocking this process.[40] These results helped to confirm the intraduplex nature of bisQM migration and consequently response to a -TT- bulge was used to test the type of diffusion available to the monofunctional QM as well. In analogy to the prior study with the bisQM-acridine conjugate, OD3 was treated with the monofunctional QMP-Acr conjugate and subsequently annealed with OD4 containing a region complementary to OD3 and a 3'-extension (Fig. 5A). Next, radiolabeled OD5 was added as the ultimate acceptor of QM transfer to displace OD3 and form a full complement with OD4 in addition to a central -TT- bulge. Transfer of the QM from OD4 and its migration along OD5 was again detected at guanine N7 by its lability to hot piperidine treatment. Under these conditions, the distribution of OD5 adducts did not accumulate progressively from the 3' to 5' terminus but instead formed concurrently on both sides of the -TT- bulge as evident from the relatively uniform fragmentation at all guanines (Fig. 5B).

[Insert Fig. 5]

This observation markedly contrasts the behavior of the bisQM conjugate and suggests that the monofunctional QM conjugate freely diffuses from its parent duplex of OD4:OD5 before reacting to form new adducts. Alternatively, these results could have also been generated if the original precursor QMP-Acr had persisted until addition of OD5, but control experiments in which QMP-Acr was pre-incubated in the absence of DNA produced no adducts upon subsequent addition of OD5 (Fig. 5B, lane *b*). Finally, the distribution of adducts on OD5 formed by QM transfer from OD4 were similar to those generated by direct reaction of the duplex OD5:OD4 (Fig. 5B, lanes *c*, *d*). A similar profile of strand fragmentation caused by guanine N7 alkylation was observed after the first day of QM migration and persisted for all subsequent days of analysis. This suggests a relatively rapid equilibration to the thermodynamic array of products. Thus, loss of the covalent but dynamic anchor of the bisQM resulted in a monofunctional QM derivative that

readily diffused without the constraints of intraduplex transfer. The lack of a stepwise migration also indicated that the attached intercalator did not offer sufficient kinetic stability to hold even the most transient QM intermediate within a single duplex DNA. These results also help to rationalize the enhanced sensitivity of the monofunctional QMs versus bisQMs to competing nucleophiles as described above.

Electronics control partitioning of guanine N7 adducts between reversible and irreversible chemistry.

The dynamic behavior of an electrophilic QM depends on both rates of coupling and release of its nucleophilic partner.[41-43] Adducts of guanine N7 support an additional pathway of deglycosylation in competition to QM regeneration (Scheme 4).[33] This alternative releases the unreactive nucleobase adduct that no longer supports reversible chemistry. Hence, QM migration and transfer can be suppressed by this deglycosylation although its contribution should be subject to manipulation by a predictable structure-activity relationship. Substituents on the nascent QM were not expected to affect the rates of deglycosylation significantly but were already known to affect the rates of QM reformation.[41] The electron donating ether substituent *para* to the nascent electrophile in eQMP-Acr not only promotes QM formation relative to its *meta* substituted methylene analog but also minimizes the frequency of deglycosylation. This was confirmed by comparing DNA strand fragmentation induced by piperidine that represents the yield of both alkylation and deglycosylation to that induced by human apurinic/apyrimidinic endonuclease (APE1) that solely represents the yield of deglycosylation. A majority of adducts generated by QMP-Acr were sensitive to APE1 treatment alone whereas a minority of adducts generated by eQMP-Acr were sensitive to this same treatment (Fig. 6). Thus, quenching the reversibility of QM by deglycosylation of guanine can be minimized by an electron rich and highly reversible QM derivative. This consideration should now be added to the design criteria for reversible

electrophiles to traverse multiple guanine residues in the future.

[insert Scheme 4] [Insert Fig. 6]

Conclusion

The original ether-linked bisQMP-acridine conjugate demonstrated efficient cross-linking that migrated within duplex DNA. This unusual property has the potential to frustrate cellular repair mechanisms and offer greater potency than chemotherapeutic regiments based on nitrogen mustards that react irreversible and generate cross-links in low efficiency. However, applications of bisQMP have been limited in part by its slow rate of transfer along duplex DNA. The cross-linking ability of the QM has now been compromised in this study to test if a monofunctional derivative could promote diffusion of the transient QM intermediate between nucleophilic sites of DNA. An enhanced rate of adduct transfer from one strand to the entire length of an acceptor DNA has now been achieved (Fig. 5). This process likely involves full release of the intermediate from the parent duplex to explain its ability to traverse an extrahelical -TT- that had previously blocked migration of the bisQMP derivative.[40]

An electron rich analog eQMP-Acr was also investigated and designed to enhance the reversibility of QM formation by replacing the methylene linker with an ether linker and changing its position relative to the nascent electrophilic center (Scheme 2). Substitution *para* to this center promotes reversible reaction significantly more than that of an analogous *meta* substitution.[41] Use of the *para* derivative containing an ether linker in a triplex-forming conjugate had already succeeded at sequence specific alkylation while the equivalent *meta* derivative containing a methylene linker had failed.[38,48] In the current study, eQMP-Acr demonstrated the anticipated acceleration in QM formation and subsequent DNA alkylation relative to that of the QMP-Acr standard (Fig. 2). Similarly, alkylation by eQMP-Acr biased partitioning of its guanine N7 adducts towards regenerating the QM rather than terminating reaction by deglycosylation and release of the nucleobase adduct (Fig 6). However, the enhanced

regeneration of the QM by adducts based on the electron rich eQMP-Acr conjugate in combination with its enhanced diffusion from the confines of duplex DNA increased its susceptibility to quenching from competing nucleophiles modeled in this study by solvent and βme (Figs. 2 and S3). These final limitations should be ameliorated in the future by combining the highly reversible nature of electron rich QMs with a high affinity ligand for DNA that is released less readily than acridine.

Experimental section

Materials

Sodium fluoride, 2-(*N*-morpholino)ethanesulfonic acid (MES), boric acid, and Tris base were purchased from Fisher Scientific. Aqueous solutions were prepared using water that was purified to a resistivity of 18 MΩ-cm. Oligonucleotides were synthesized by Integrated DNA Technologies and radiolabeled at the 5-terminus using [γ-^{32}P]-dATP (Perkin Elmer) and T4 polynucleotide kinase (New England Biolabs) following manufacturer's protocols. Human apurinic/apyrimidinic endonuclease (APE1) was purchased from New England Biolabs. Polyacrylamide gels were prepared using 40% acrylamide (19:1 acrylamide:bis-acrylamide) purchased from Bio-Rad. The monofunctional QMP used for coupling to acridine was prepared according to Zhou and Rokita[34] with adaptation by Mark Hutchinson[49] based on a procedure by Nagata et al.[44] for the initial hydroxylmethylation. eQMP-Acr was prepared as described previously.[38] For details, see supplemental information.

General methods

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. DNA samples treated with QMPs as described below were dried, redissolved with loading solution (0.05

% bromophenol blue, 0.05 % xylene cyanol FF, and10 mM EDTA in formamide)) and separated on denaturing 20% acrylamide gels using 1200 V for 4 h. Gels were then transferred onto a Molecular Dynamics phosphoimager screen for 4 hours, and images was scanned with a Typhoon 9410 Variable Mode Imager. DNA fragments were quantified using ImageQuant TL software and reported relative to total signal.

Alkylation of DNA with QM derivatives

The desired ^{32}P radiolabeled oligonucleotide or duplex DNA (3.0 μM, 50 nCi) was incubated under ambient temperature for 24 h with the indicated concentration of the QMP (0 - 240 μM) in presence of NaF (10 mM), MES (10 mM, pH 7) and 20% acetonitrile. Samples were then dried under reduced pressure with a SpeedVac. For subsequent detection of alkylation at guanine N7, the dried samples were resuspended with 10% aq. piperidine and heated to 90 ^{o}C for 30 min. The samples were again dried, resuspended in formamide loading solution, and subjected to 20% denaturing polyacrylamide gel electrophoresis.

QM transfer from alkylated DNA

The indicated oligonucleotide (3.0 μM) was pre-incubated independently with eQMP-Ac and QMP-Acr (240 μM) in the presence of NaF (10 mM), MES (10 mM, pH 7.0) and 20% acetonitrile for 24 h under ambient conditions before addition of the acceptor oligonucleotide (3.3 μM) and further incubation for the specified time under ambient condition. DNA was then treated with 10% aq. piperidine (90 ^{o}C, 30 min), dried with a SpeedVac, resuspended in formamide loading solution and then subjected to 20% denaturing gel electrophoresis.

Conflicts of interest

There are no conflicts to declare about the authors.

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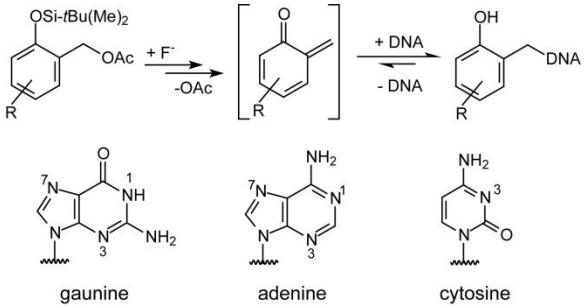
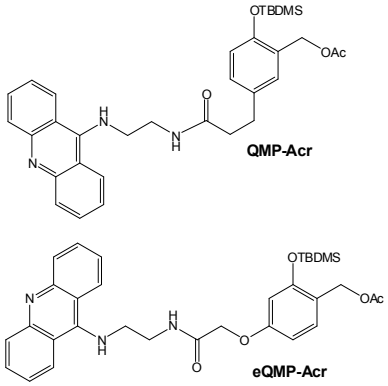
1. W.-J. Bai, J. G. David, Z.-G. Feng, M. G. Weaver, K.-L. Wu and T. R. R. Pettus, 2014, **47**, 3655-3664.
2. M. S. Singh, A. Nagaraju, N. Anand and S. Chowdhury, *RSC Adv.*, 2014, **4**, 55924.
3. S. Mukhopadhyay, C. Gharui and S. C. Pan, *Asian J. Org. Chem.*, 2019, **8**, in press (DOI: 10.1002/ajoc.201900466).
4. K. H. Jensen, T. P. Pathak, Y. Zhang and M. S. Sigman, *J. Am. Chem. Soc.*, 2009, **131**, 17074–17075.
5. J. Rodriguez and D. Bonne, *Chem. Comm.*, 2019, **55**, 11168-11170.
6. L. Weinschenk, D. Schols, J. Balzarini and C. Meier, *J. Med. Chem.*, 2015, **58**, 6114-6130.
7. P. Arumugam and V. V. Popik, *J. Am. Chem. Soc.*, 2012, **134**, 8408-8411.
8. M. E. Roth, O. Green, S. Gnaim and D. Shabat, *Chem. Rev.*, 2016, **116**, 1309−1352.
9. S. Gnaim and D. Shabat, *Acc. Chem. Res.*, 2019, **52**, 2806-2817.
10. T. Dunlap, S. C. Piyankarage, G. T. Wijewickrama, S. Abdul-Hay, M. Vanni, V. Litosh, J. Luo and G. R. J. Thatcher, *Chem. Res. Toxicol.*, 2012, **25**, 2725-2736.
11. J. L. Bolton, *Curr. Org. Chem.*, 2014, **18**, 61-69.
12. Y. Wang, H. Fan, K. Balakrishnan, Z. Lin, S. Cao, W. Chen, Y. Fan, Q. A. Gurthrie, H. Sun, K. A. Teske, V. Gandhi, L. A. Arnold and X. Peng, *Eur. J. Med. Chem.*, 2017, **133**, 197-207.
13. D. Yoo, E. Jung, J. Noh, H. Hyun, S. Seon, S. Hong, D. Kim and D. Lee, *ACS Omega*, 2019, **4**, 10080-10077.
14. J. K. Myers and T. S. Widlanski, *Science*, 1993, **262**, 1451-1453.
15. K. D. Janda, L.-C. Lo, C.-H. L. Lo, M.-M. Sim, R. Wang, C.-H. Wong and R. A. Lerner, *Science*, 1997, **275**, 945-948.
16. J. Liu, S. Li, N. A. Aslam, F. Zheng, B. Yang, R. Cheng, N. Wang, S. Rozovsky, P. G. Wang, Q. Wang and L. Wang, *J. Am. Chem. Soc.*, 2019, **141**, in press (DOI: 10.1021/jacs.1029b01738).
17. C. Percivalle, F. Doria and M. Freccerro, *Curr. Org. Chem.*, 2014, **18**, 19-43.
18. H. Wang, *Curr. Org. Chem.*, 2014, **18**, 44-60.
19. S. Cao and X. Peng, *Curr. Org. Chem.*, 2014, **18**, 70-85.
20. A. Minard, D. Liano, X. Wang and M. Di Antonio, *Bioorg. Med. Chem.*, 2019, **27**, 2298-2305.
21. E. Modica, R. Zanaletti, M. Freccero and M. Mella, *J. Org. Chem.*, 2001, **66**, 41-52.
22. P. Wang, R. Liu, X. Wu, H. Ma, X. Cao, P. Zhou, J. Zhang, X. Weng, X. L. Zhang, X. Zhou and L. Weng, *J. Am. Chem. Soc.*, 2003, **125**, 1116-1117.
23. L. Diao and P. Wan, *Can. J. Chem.*, 2008, **86**, 105-118.
24. J. L. Bolton, L. G. Valerio and J. A. Thompson, *Chem. Res. Toxicol.*, 1992, **5**, 816-822.
25. S. Cao, Y. Wang and X. Peng, *Chem. Eur. J.*, 2012, **18**, 3850-3854.
26. M. F. McLaughlin, E. Massolo, T. A. Cope and J. S. Johnson, *Org. Lett.*, 2019, **21**, 6504-6507.
27. M. Chatterjee and S. E. Rokita, *J. Am. Chem. Soc.*, 1994, **116**, 1690-1697.

28. H. Bauer, K. Fritz-Wolf, A. Winzer, S. Kühner, S. Little, V. Yardley, H. Vezin, B. Palfey, R. H. Schirmer and E. Davioud-Charvet, *J. Am. Chem. Soc.*, 2006, **128**, 10784-10794.
29. S. E. Rokita, in *Quinone Methides*, ed. S. E. Rokita, Wiley, Hoboken, 2009, pp. 297-327.
30. S. Colloredo-Mels, R. T. Dorr, D. Verga and M. Freccero, *J. Org. Chem.*, 2006, **71**, 3889-3895.
31. P. Chen, K. Wang, W. Guo, X. Liu, Y. Liu and C. Li, *Angew. Chem. Int. Ed.*, 2017, **56**, 3689-3693.
32. W. F. Veldhuyzen, A. J. Shallop, R. A. Jones and S. E. Rokita, *J. Am. Chem. Soc.*, 2001, **123**, 11126-11132.
33. E. E. Weinert, K. N. Frankenfield and S. E. Rokita, *Chem. Res. Toxicol.*, 2005, **18**, 1364-1370.
34. Q. Zhou and S. E. Rokita, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 15452-15457.
35. C. S. Rossiter, D. Kumar, E. Modica and S. E. Rokita, *Chem. Commun.*, 2011, **46**, 1476-1478.
36. H. Wang and S. E. Rokita, *Angew. Chem. Int. Ed.*, 2010, **49**, 5957-5960.
37. Y. Liu and S. E. Rokita, *Biochemistry*, 2012, **51**, 1020−1027.
38. C. Huang, Y. Liu and S. E. Rokita, *Signal Transd, Target Ther*, 2016, **1**, 16009; doi:16010.11038/sigtrans.12016.16009.
39. H. Wang, M. S. Wahi and S. E. Rokita, *Angew. Chem. Int. Ed.*, 2008, **47**, 1291-1293.
40. F. Fakhari and S. E. Rokita, *Nature Commun.*, 2014, **5**, 5591 (doi: 5510.1038/ncomms6591).
41. E. E. Weinert, R. Dondi, S. Colloredo-Melz, K. N. Frankenfield, C. H. Mitchell, M. Freccero and S. E. Rokita, *J. Am. Chem. Soc.*, 2006, **128**, 11940-11947.
42. S. Cao, R. Christiansen and X. Peng, *Chem. Eur. J.*, 2013, **19**, 9050-9058.
43. F. Doria, A. Lena, R. Bargiggia and M. Freccerro, *J. Org. Chem.*, 2016, **81**, 3665-3673.
44. W. Nagata, K. Okada and T. Aoki, *Synthesis*, 1979, 365-368.
45. W. F. Veldhuyzen, P. Pande and S. E. Rokita, *J. Am. Chem. Soc.*, 2003, **125**, 14005-14013.
46. T. Lönnberg, M. Hutchinson and S. E. Rokita, *Chem. Eur. J.*, 2015, **21**, 13127-13186.
47. M. P. McCrane, M. Hutchinson, O. Ad and S. E. Rokita, *Chem. Res. Toxicol.*, 2014, **27**, 1282-1293.
48. C. Huang and S. E. Rokita, *Front. Chem. Sci. Eng.*, 2016, **10**, 213-221.
49. M. A. Hutchinson, B. Deeyaa, S. R. Byrne, S. J. Williams and S. E. Rokita, in preparation.

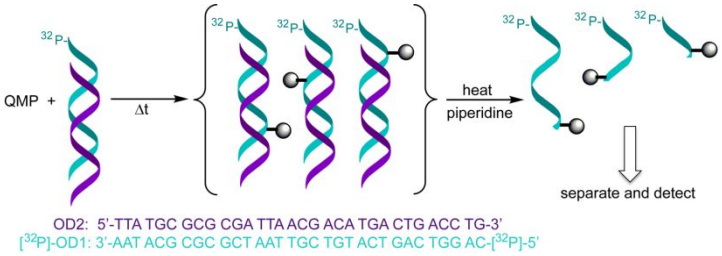
Figures and Legends:



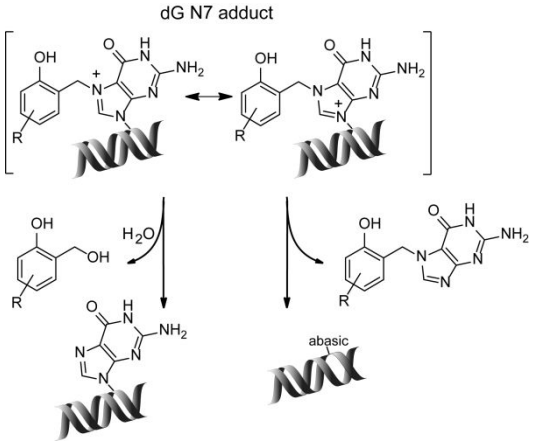
Scheme 1 Reversible alkylation of quinone methides with selected nucleophiles of DNA.



Scheme 2 Quinone methide precursors conjugated to acridine (TBDMS, *tert*-butyldimethylsilyl).



Scheme 3 Alkylation and detection of guanine N7 by a quinone methide precursor (QMP).



Scheme 4 Partitioning between quinone methide regeneration and deglycosylation.

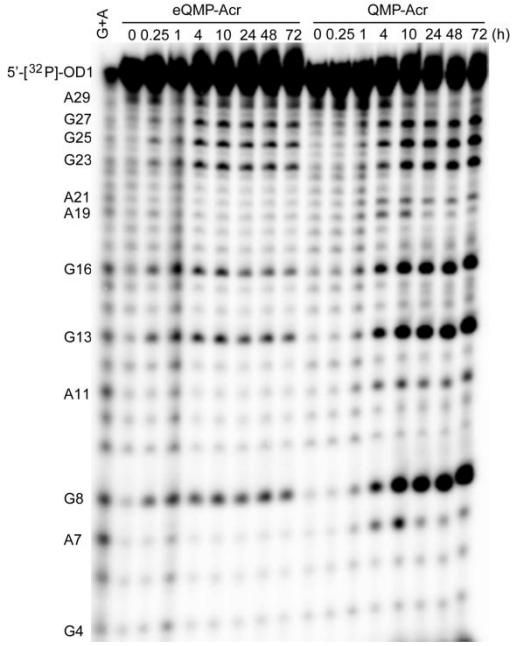


Fig. 1 Time-dependence of duplex DNA alkylation by **eQMP-Acr** and **QMP-Acr**. 5'-[^{32}P]-OD1:OD2 (3.0 μM) was incubated with the specified QMP (120 μM) in the presence of NaF (10 mM) and MES (10 mM pH 7.0) for 0 - 72 h followed by hot piperidine treatment and analyzed by denaturing PAGE (20%).

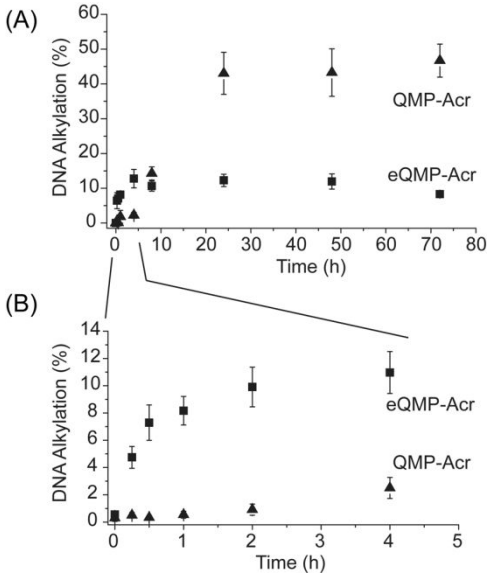


Fig 2 Rate of duplex DNA alkylation by **eQMP-Acr** and **QMP-Acr**. 5'-[^{32}P]-OD1:OD2 (3.0 μM) was treated as described in Fig 1 and total piperidine-induced fragments of DNA were quantified to reflect reaction at guanine N7 (sum of fragments as a percent of the total signal). Data represent the average of three independent determinations from analyses similar to that illustrated in Fig. 1 and the error represents their standard deviation for incubations (A) from 0 - 72 h and (B) from 0 - 4 h. See Fig S1 in the supplementary information for an example of data gathered from 0 - 4 hr.

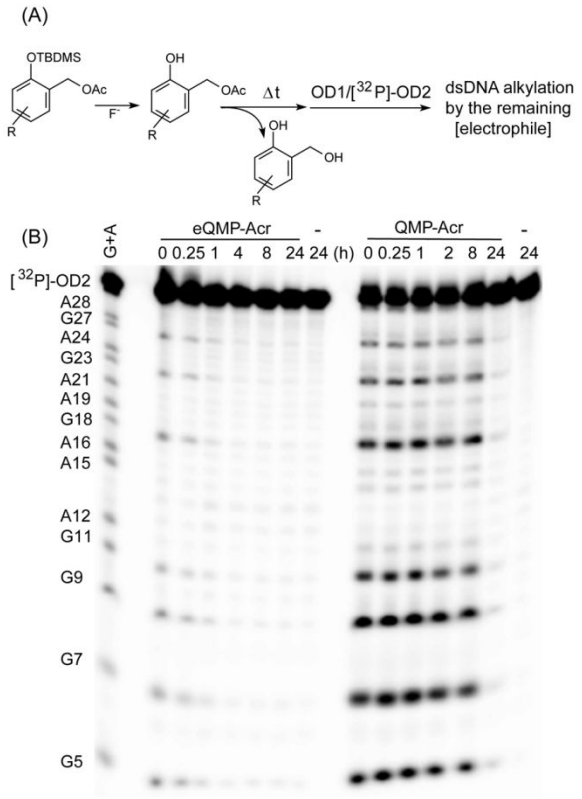


Fig 3 Quenching the quinone methide by hydrolysis. (A) Consumption of the quinone methide intermediate and its precursor was detected by its diminishing ability over time to alkylate duplex DNA. (B) The indicated QMP (240 μM) was incubated in the presence of NaF (10 mM) and MES (10 mM, pH 7.0) from 0 - 24 h followed by addition of 5'-[^{32}P]-OD2:OD1 (3.0 μM) and further incubated for an additional 24 h. DNA was then treated with 10 % hot piperidine and fragmentation products were separated by 20% denaturing gel electrophoresis. Control incubations for 24 h lacking eQMP-Acr and QMP-Acr respectively are marked with "-".

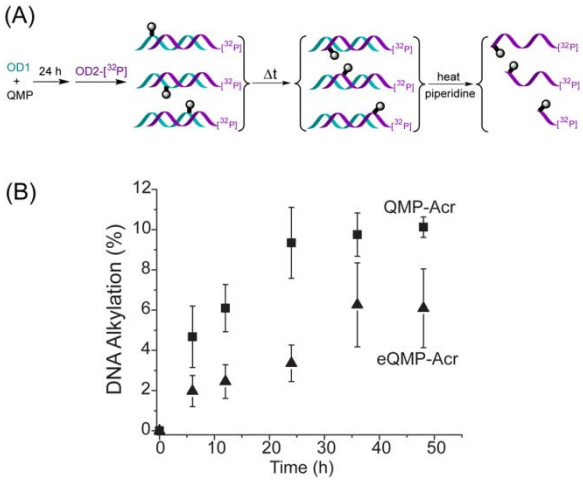


Fig 4 Alkylation transfer between DNA strands based on the reversibility of QM reaction. (A) Reversible regeneration of QM and its alkylation of DNA was evaluated by the QM transfer from a donor OD1 to acceptor OD2 strand of DNA. (B) OD1 (3.0 μM) was incubated alternatively with **eQMP-Acr** and **QMP-Acr** (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 10 % hot piperidine, separated by 20% denaturing gel electrophoresis and quantified by ImageQuant. DNA alkylation of 5'-[^{32}P]-OD2 above background (fragments at t = 0 h) was quantified as the sum of fragments as a percent of the total signal (%). Yields represent an average of three independent trials and error is the standard deviation. A sample fragmentation profile is provided in the supplementary information (Fig S2).

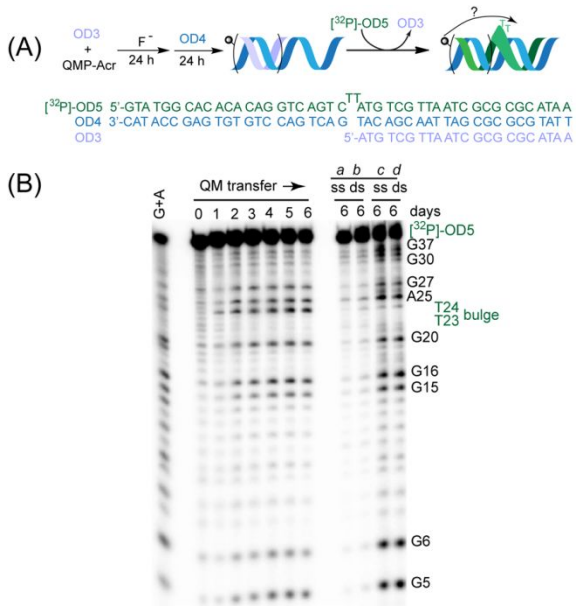


Fig 5 Migration of quinone methide along duplex DNA containing a -TT- bulge. (A) The ability of a QM to migrate past a dinucleotide bulge within duplex DNA was examined by QM transfer to OD5 from OD4 that was alkylated on only one side of the nascent bulge by prior QM transfer from OD3. (B) OD3 (3.0 μM) was treated with **QMP-Acr** (240 μM), NaF (10 mM), MES (10 mM pH 7.0) for 24 h before addition of OD4 (3.0 μM) and further incubation for 24 h. 5'-[^{32}P]-OD5 (3.3 μM) was then added and incubated for 0 - 6 days before treatment with hot piperidine. As controls, (*a*) single (ss, OD5) and (*b*) double (ds, OD4:OD5) strand DNA were incubated for 6 days after **QMP-Acr** (240 μM) had been pre-incubated in MES (10 mM pH 7.0) and NaF (10 mM) for 48 h. Finally, (*c*) single (ss, OD5) and (*d*) double (ds, OD4:OD5) strand DNA was incubated for 6 days with **QMP-Acr** (240 μM) under identical conditions without pre-incubation.

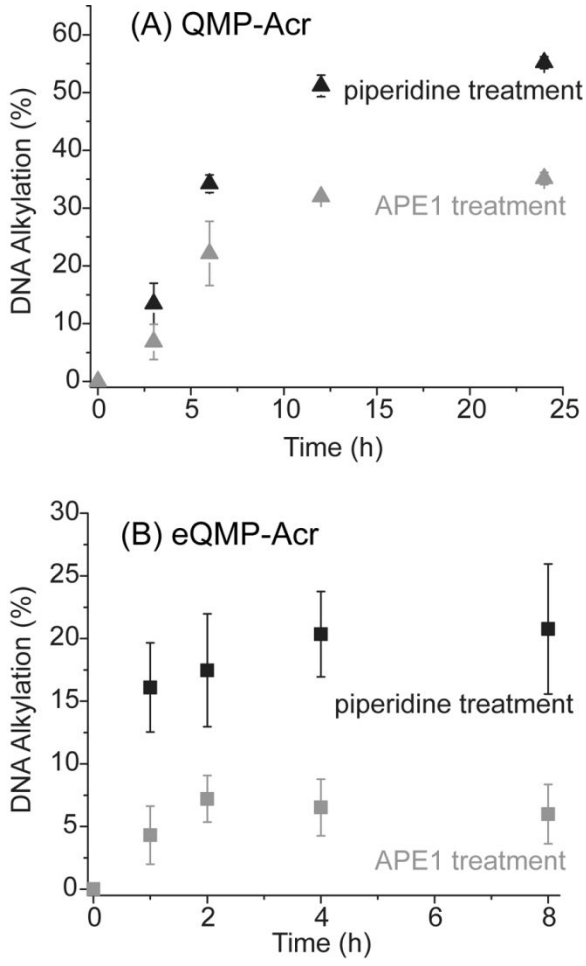


Fig 6 Rate of deglycosylation after alkylation of guanine N7. 5'-[^{32}P]-OD2:OD1 (3.0 μM) was incubated alternatively with (A) **QMP-Acr** and (B) **eQMP-Acr** (240 μM) for 0 - 24 h in the presence of NaF (10 mM) and MES (10 mM pH 7.0) before treating each sample with either 10% hot piperidine or APE1. The sum of fragments (%) was determined according to Fig 4 (three independent repetitions). Sample data are illustrated in supplementary information (Fig S4).

TOC:

Conversion of a bisquinone methide-acridine conjugate to its monofunctional analogue releases the constraints that limit migration of its reversible adducts within DNA.