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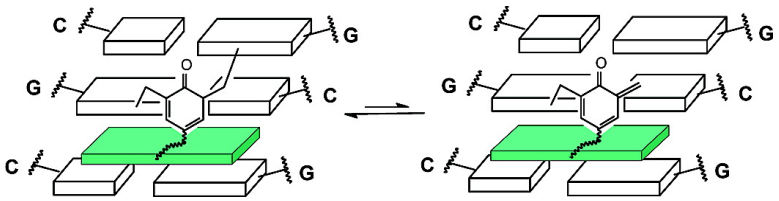

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A Transient Product of DNA Alkylation Can Be Stabilized by Binding Localization

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Abstract: A 9-aminoacridine conjugate of a silyl-protected bis(acetoxymethyl)phenol (bisQMP) was synthesized and evaluated as an inducible cross-linking agent of DNA to test our ability to harness the chemistry of reactive quinone methide intermediates (QM). The acridine component was chosen for its ability to delivery an appendage to the major groove of DNA, and the silyl-protected component was chosen for its ability to generate two quinone methide equivalents in tandem upon addition of fluoride. This design created competition between reaction of (1) the 2-amino group of guanine that reacts irreversibly to form a stable QM adduct and (2) the more nucleophilic N7 group of guanine that reacts more efficiently but reversibly to form a labile QM adduct. This lability was apparently compensated by co-localization of the N7 group and QM in the major groove since the N7 adduct appeared to dominate the profile of products formed by duplex DNA. The controlling influence of acridine was also expressed in the sensitivity of the conjugate to ionic strength. High salt concentration inhibited covalent reaction just as it inhibits intercalation of the cationic acridine. As expected for QM formation, the presence of fluoride was indeed necessary for initiating reaction, and no direct benzylic substitution was observed. The conjugate also cross-linked DNA with high efficiency, forming one cross-link for every four alkylation events. Both alkylation and cross-linking products formed by duplex DNA were labile to hot piperidine treatment which led to ~40% strand scission and ~50% reversion to a material with an electrophoretic mobility equivalent to the parent DNA. All guanines exhibited at least some reactivity including those which were recalcitrant to cross-linking by an oligonucleotide−bisQMP conjugate designed for triplex formation [Zhou, G.; Pande, P.; Johnson, A. E.; Rokita, S. E. *Bioorg. Med. Chem.* **2001**, *9*, 2347−2354].

Alkylating agents that react irreversibly with DNA generate product profiles derived from kinetic competition between accessible nucleophiles. Accordingly, reagents such as dimethyl sulfate and ethyl nitrosourea alkylate the most reactive N and O nucleophiles independent of the relative stability and thermodynamics of the nascent products.[1] In contrast, alkylating agents that react reversibly with DNA may form kinetic intermediates that ultimately give way to an equilibrium distribution of products. Highly functionalized synthetic and biological compounds often bind to regions of DNA that facilitate formation of the thermodynamically favored products and avoid generating covalent intermediates associated with alternative kinetic products.[2]

Notable exceptions illustrate that covalent attachment to DNA need not prevent migration of particular adducts from sites of kinetic reactivity to those of thermodynamic stability established by optimum covalent and noncovalent association. For example, ecteinascidin 743 (Et 743) alkylates the 2-amino group of guanine (GN^2) within 5′-AGT and 5′-AGC sequences with equal efficiency.[3] However, release of Et 743 is faster from the AGT vs AGC sequence and consequently adducts accumulate at AGC sequences for which association is preferred. This reversibility may also provide a mechanism for recycling this promising drug candidate once its DNA adduct is removed by cellular repair processes.[3] The toxicity of malondialdehyde may likewise be enhanced to our detriment by its ability to form adducts with guanine reversibly and return to DNA after excision of its pyrimidopurinone adduct.[4,5] Reversible adduct formation is particularly critical for efficient cross-linking of duplex DNA since initial alkylation anchors an intermediate to a sequence that may not necessarily contain the appropriate nucleotides for subsequent cross-linking. This may in part explain the low levels of cross-linking vs monoalkylation achieved by reagents such as *N*-mustards[6] and diepoxybutane[7] that operate under kinetic control. In contrast, bizelesin containing two cyclopropylpyrroloindole units efficiently cross-links duplex DNA through reaction at the N3 position of two adenosines (AN3) separated by four base pairs.[8] This efficiency is certainly aided by the

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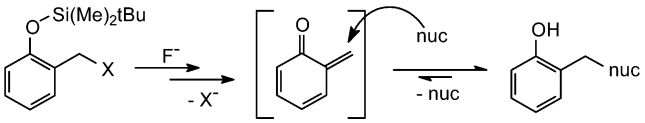
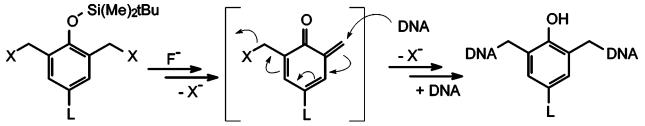
Scheme 1



Scheme 2



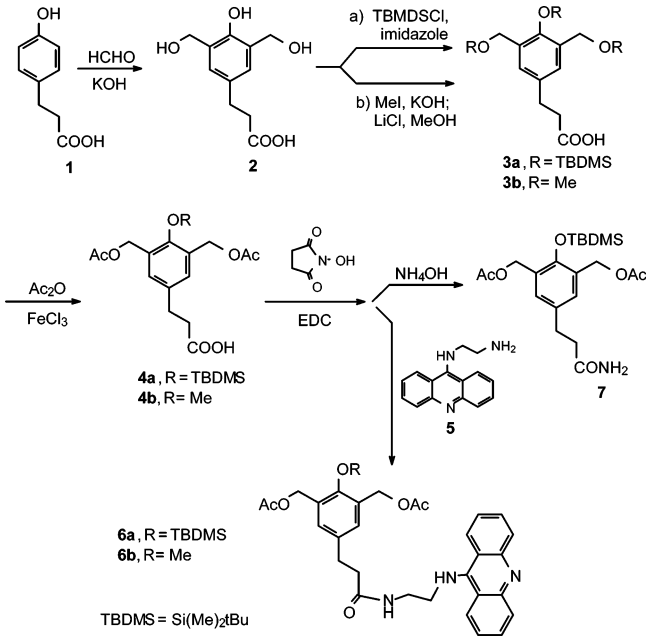
L = H, CH_3, linker+site-directing ligand

ability of bizelesin to migrate from sites supporting only monoalkylation to alternative sites supporting cross-linking.[9]

The highly reactive and transient electrophilic intermediate quinone methide (QM) and related species have been the focus of our laboratory's interest in DNA alkylation and cross-linking for the past decade. Early reports from others describing the reversible[10,11] and transient[12] formation of QM adducts became more instructive when our model studies indicated that the observed specificity of QM for weak nucleophiles was based on the thermodynamics rather than the kinetics of reaction (Scheme 1).[13] Selective preassociation between one of the simplest QM (*o*-benzoquinone methide) and DNA is not easily imagined, and therefore the preferential modification of GN^2 by this QM in duplex DNA can be ascribed to the fundamental thermodynamics of this adduct. Certainly its formation is not kinetically favorable.[13,14] A QM conjugate directed toward this site should then act in synergy with the reaction thermodynamics by increasing the effective concentration of the transient intermediate and providing noncovalent binding stabilization to the final product. The outcome of a complementary strategy of directing QM to a kinetic rather than thermodynamic adduct is not as easily predicted since either target binding or covalent bond formation might dominate the ultimate product profile.

The potential conflict between adduct stability and site-directed targeting became a concern while investigating sequence-selective cross-linking of DNA by a conjugate constructed of a triplex-forming oligonucleotide and a quinone methide precursor designed for tandem alkylation (Scheme 2).[15] A parent bis-functionalized precursor (bisQMP, L = CH_3) derived from an earlier monofunctionalized precursor (QMP) had been designed to share a five-atom bridge between alkylation sites common to *N*-mustards which cross-link GN7s in the major groove.[6,16] and only one atom longer than the four-atom bridge of mitomycin C that cross-links GN^2's in the minor groove.[17] This parent was competent at cross-linking DNA, but the extent of reaction at GN7 was ambiguous and could have suffered from

Scheme 3



competition between thermodynamic and kinetic products.[18] An oligonucleotide−bisQMP conjugate was then expected to enhance reaction at GN7 due to its selective binding within the major groove adjacent to a sequence 5′-CG that is favored for bisQMP reaction in the major groove.[18] Surprisingly, only monoalkylation and no cross-linking was detected for the conjugate.[15] A bisQMP−acridine conjugate has now been synthesized and characterized to address whether results with the triplex-forming conjugate were unique to this initial trial or more representative of a potential limitation in directing a reversibly reactive group to form adducts lacking intrinsic thermodynamic stability.

Results

Synthesis of bisQMP, Its Acridine Conjugate and Related Derivatives. Attempts to elaborate our original silyl-protected bis(bromomethyl)phenol[18] for coupling to site-directing ligands was stymied by the instability of the benzylic bromides. Consequently, these substituents were replaced with acetates which allowed for facile synthesis of the desired activated ester and its coupling to primary amines. Hydroxyphenylpropionic acid **1** was first hydroxymethylated with aqueous formaldehyde under basic conditions to provide the bis(hydroxymethyl) derivative **2** (Scheme 3).[19] Selective silylation was not required in this scheme since the benzylic acetoxy groups could be introduced efficiently into the trisilylated species **3a** through a method developed by Ganem and Small.[20] Accordingly, **3a** was treated with $FeCl_3$ and Ac_2O to produce the bis(acetoxymethyl) compound **4a** in 94% yield. The carboxylic acid was next activated for coupling by preparation of its *N*-hydroxysuccinimidyl ester intermediate. This was condensed with 9-[*N*-(2-aminoethyl)]aminoacridine[21−23] **5** to generate the desired acridine conjugate **6a** and with ammonia to generate a nonconjugated

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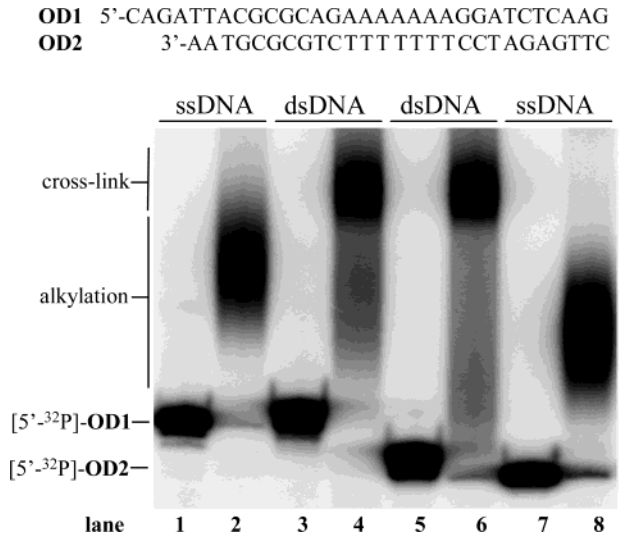

Figure 1. Reaction of single- and double-stranded DNA with the bisQMP conjugate **6a**. 5′-[^{32}P]-**OD1** (30 nCi, 3.0 μM, lane 1) and 5′-[^{32}P]-**OD2** (30 nCi, 3.0 μM, lane 7) were alternatively incubated with **6a** (60 μM) for 4 h (20 °C) in 25% aqueous acetonitrile (6 mM MES, pH 7) and KF (10 mM) (lane 2 and 8, respectively). Duplex DNA was formed by alternately annealing 5′-[^{32}P]-**OD1** (30 nCi, 3.0 μM) with **OD2** (3.3 μM, lane 3) and 5′-[^{32}P]-**OD2** (30 nCi, 3.0 μM) with **OD1** (3.3 μM, lane 5) and incubated with **6a** under the conditions described above (lanes 4 and 6, respectively).

control derivative **7**. The labile silyl group of **6a** was also replaced with a stable methyl group to distinguish between DNA alkylation resulting from direct benzylic substitution and QM formation. This additional derivative **6b** was constructed in parallel to the silyl species **6a** starting from permethylation of **2**,[24] saponification and benzylic substitution.[20]

The bisQMP−Acridine Conjugate 6a Cross-Links Duplex DNA Efficiently. Activities of both conjugated and nonconjugated derivatives of the bisQMP were examined with the same oligonucleotide duplex used previously for investigating delivery of the bisQMP by triplex formation (**OD1**/**OD2**, Figure 1).[15] This permitted direct comparisons between the two bisQMP conjugates and focused attention on competition between kinetic and thermodynamic product formation while simultaneously avoiding potential complications caused by use of different nucleotide sequences. The original design incorporated a polypurine tract for triple strand recognition and oligonucleotides of different lengths for distinguishing between strand alkylation and cross-linking by gel electrophoresis.

Samples of single- and double-stranded DNA (3 μM) were almost completely consumed within 4 h (20° C) in the presence of excess bisQMP conjugate **6a** and fluoride (Figure 1). The mobilities of the parent oligonucleotides and their alkylation products were unique and readily separated by denaturing gel electrophoresis. In contrast, cross-linked DNA containing both strands should express identical gel mobilities regardless of whether **OD1** or **OD2** is labeled. This common mobility was indeed observed after treating duplex DNA with conjugate **6a** (Figure 1, lanes 4 and 6). Interestingly, this conjugate cross-linked the duplex with little accumulation of monoalkylation.

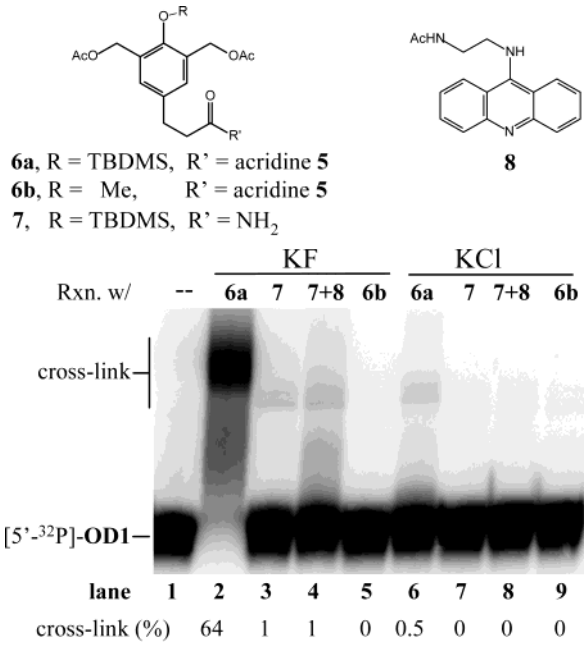

Figure 2. Cross-linking efficiency of bisQMP derivatives in the presence and absence of fluoride. Duplex DNA formed by 5′-[^{32}P]-**OD1** (30 nCi, 3.0 μM) and **OD2** (3.3 μM) (lane 1) was incubated for 12 h (20° C, 6 mM MES, pH 7, 25% aqueous acetonitrile) in the alternate presence of **6a** (60 μM, lanes 2 and 6), **7** (60 μM, lanes 3 and 7), **7** and **8** (60 μM each, lanes 4 and 8), or **6b** (60 μM, lanes 5 and 9). Reactions were initiated by addition of either KF (10 mM KF, lanes 2−5) or KCl (10 mM, lanes 6−9).

Acridine Conjugation and Quinone Methide Formation Are Essential for Efficient Cross-Linking. The high efficiency of cross-linking was not innate to the simple bisQMP component (**7**) but rather depended on the attached acridine derivative to direct and localize the reactive intermediate.

The bisQMP conjugate **6a** generated cross-linked products with a conservatively estimated yield of 64% as suggested by quantifying the material migrating only within the indicated region for cross-linked products relative to the total material distributed throughout the analytical separation (Figure 2, lane 2). In contrast, only 1% of the duplex at most was cross-linked under comparable conditions with the nonconjugated derivative **7** (Figure 2, lane 3). This low yield is consistent with the activity of another nonconjugated derivative (L = CH_3, Scheme 2) that required significantly higher concentrations (450 μM vs 60 μM) to generate a maximum of 7.5% cross-linking.[18] In addition, the activity of **7** was not detectably affected by addition of an untethered acridine derivative (**8**) (Figure 2, lane 4), and thus intercalation by itself neither inhibited nor promoted reaction. Similarly, intercalation and concomitant stabilization of duplex DNA is not likely responsible for the low gel mobility of the material ascribed to be cross-linked DNA since the products formed by the bisQMP containing (**6a**) and lacking (**7**) acridine migrated analogously.

Fluoride- and Ionic Strength-Dependence of DNA Cross-Linking. Cross-linking exhibited a specific requirement for fluoride that could not be satisfied with chloride (Figure 2, lanes 2−4 vs 6−8). This is consistent with an initial loss of the silyl-protecting group from **6a** and subsequent formation of an intermediate QM prior to DNA reaction.[18] If benzylic displacement had alternatively been responsible for cross-linking, then substitution of the silyl group with a methyl group (**6b**) should have allowed for efficient cross-linking that was independent

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of fluoride. On the contrary, no alkylation or cross-linking of DNA was detected after incubation of **OD1**/**OD2** with **6b** (Figure 2, lanes 5 and 9). The low level of cross-linking produced by the silyl derivative **6a** in the absence of fluoride (Figure 2, lane 6) is then best explained by the potential of **6a** to undergo a limited degree of spontaneous desilylation during long periods of incubation. Reaction time of these experiments (12 h) was extended well beyond that necessary for complete reaction of **6a** (4 h, Figures 1 and 3) to offer ample time for possible alternative modes of reaction to generate observable products.

The response of DNA cross-linking to ionic strength provided complementary evidence of acridine's dominant role in directing reaction of its attached bisQMP in **6a**. Intercalation of cationic dyes is very sensitive to ionic strength, and high concentrations of Na^+ and K^+ effectively compete for DNA binding.[25−27] Increasing the concentration of KF used to initiate reaction of **6a** under standard conditions from 10 to 100 mM decreased the yield of cross-linking from 65 to 56%. A further increase of KF concentration to 500 and 1000 mM suppressed the yield even more to 26 and 13%, respectively. Such a decrease is consistent with inhibition of intercalation since neither QM formation or its alkylation of DNA is inhibited by high ionic strength.[18,28] Interestingly, DNA cross-linking by **6a** (20 μM) was not affected by addition of an 8-fold higher concentration of the free acridine derivative **8** (160 μM). Reversible and noncovalent interactions of **8** were consequently not competitive with the reversible and covalent alkylation of **6a**.

Kinetics and Stoichiometry of DNA Alkylation and Cross-Linking. The rate and stoichiometry of DNA reaction were examined to identify the origin of the low-mobility products and detect if a threshold of alkylation was required prior to cross-linking. These experiments also served as a necessary prerequisite for determining the specificity of DNA modification described below. Although cross-linking of duplex DNA was expected to generate products of low mobility in denaturing gel electrophoresis, changes in mobility due to covalent attachment of acridine derivatives were less predictable. Alkylation by the simple, nonconjugated bisQMP had not previously affected DNA mobility,[18] but products of reaction between single-stranded DNA and conjugate **6a** certainly did exhibit retarded mobility (Figure 1).

A small shift in mobility under denaturing conditions was also evident by gel electrophoresis for the labeled strand (**OD1**) in duplex DNA as the duration of its exposure to the bisQMP−acridine conjugate **6a** increased (Figure 3, lanes 1−7). Concurrently, a distinct low-mobility product was observed from the earliest time of analysis, even before the mobility of **OD1** had changed significantly (0.5 h, Figure 3, lane 2). This low-mobility product is hence not likely caused by the gradual accumulation of multiple adducts but rather caused by an interstrand cross-link as previously suggested by reaction of **6a** and duplex DNA containing alternatively labeled strands (Figure 1). Appearance of a cross-link from the outset suggests that this process is quite competitive with monoalkylation. The small changes in mobility

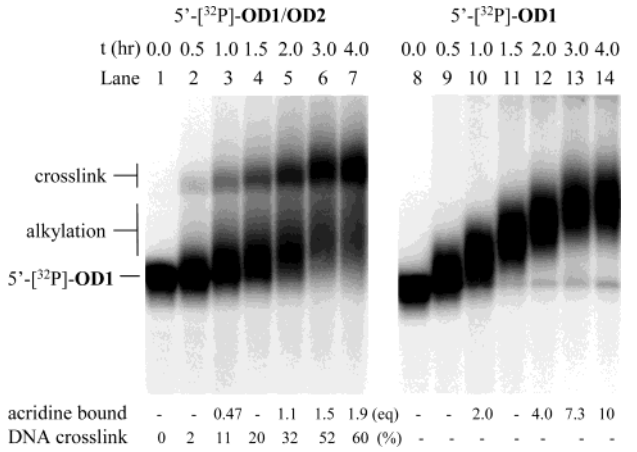

Figure 3. Time dependence of reaction between the bisQMP−acridine conjugate **6a** and double- and single-stranded DNA. Duplex DNA formed by 5′-[^{32}P]-**OD1** (30 nCi, 3.0 μM) and **OD2** (3.3 μM) was incubated (20 °C) with **6a** (60 μM) in 25% aqueous acetonitrile 6 mM MES pH 7 for the indicated time (lanes 1−7). Addition of KF (10 mM) was used to initiate reaction. Identical conditions were used to examine the reaction of single-stranded DNA (5′-[^{32}P]-**OD1**, 30 nCi, 3.0 μM) in the absence of **OD2** (lanes 8−14). The extent of cross-linking and covalent attachment of acridine was measured as described in the Experimental section.

of both **OD1** and its cross-linked products as a function of reaction time were presumably the result of a limited degree of further alkylation. Within 3 h, **OD1** was consumed, and a majority of cross-link had formed in the presence of a 20-fold excess of **6a** (Figure 3, lane 6). Maximum cross-linking was achieved by 4 h (Figure 3, lane 7).

The mobility change of single-stranded DNA caused by reaction with **6a** also neared completion at the end of the same 4 h incubation although consumption of single-stranded parent **OD1** was accomplished in less than 1.5 h (Figure 3, lanes 11 and 14). In contrast to the electrophoretic behavior of the products formed by double-stranded DNA, those from single-stranded DNA presented a continuum of progressively lower mobility (Figure 3, lanes 9−14). This result is indicative of a buildup of alkylation at multiple sites within **OD1**. Alternative formation of intrastrand cross-links is possible, but again these would likely have exhibited a distinct, rather than continuous, change in gel mobility.

The stoichiometry of DNA modification was followed by the gain in UV absorbance at 415 nm due to the attached acridine versus UV absorbance at 260 nm due to both DNA and acridine. The level of incorporating the bisQMP−acridine conjugate **6a** in duplex DNA consistently suggested over the time of incubation that cross-linking occurred at approximately 25% of the rate of monoalkylation (Figure 3). Each cross-linked duplex on average contained no more than 4 acridine equivalents (acridine bound/cross-link). Incorporation of the conjugate **6a** in single-stranded DNA was more rapid and accumulated to a greater extent. Attachment of an average of two acridine derivatives per **OD1** inhibited gel migration noticeably, and a maximum effect was observed after attachment of 8−10 acridine derivatives per strand (Figure 3, lanes 10, 13, and 14). The lack of conformational constraints of single-stranded DNA and its greater accessibility to reagents are presumably responsible for its enhanced reactivity with **6a**.[14,29]

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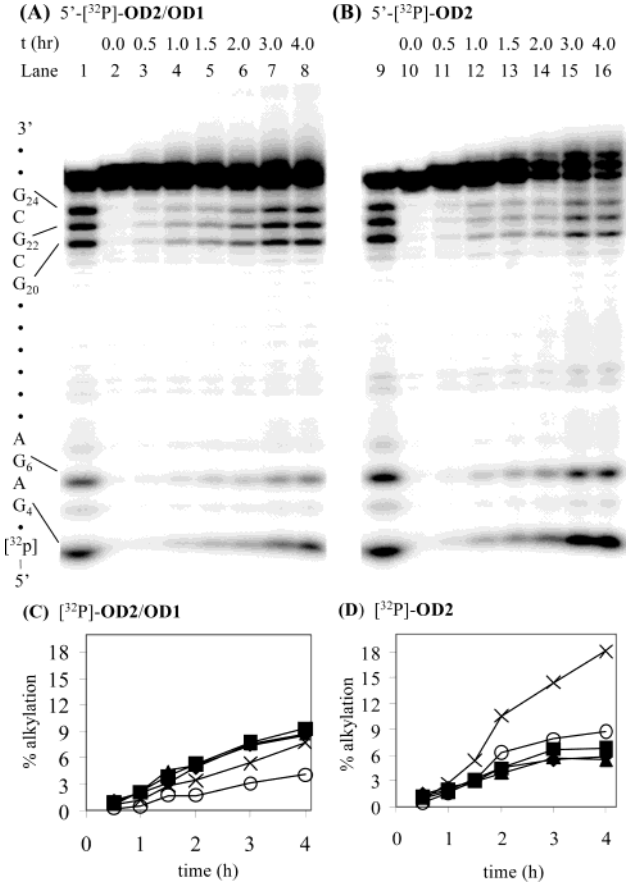

Figure 4. Site specificity of the bisQMP−acridine conjugate **6a** for double- and single-stranded DNA. Identical incubations containing **6a** and either (A) double- or (B) single-stranded DNA as described in Figure 3 were treated with hot piperidine, analyzed by denaturing gel electrophoresis, compared to standard G ladders (lanes 1 and 9) and quantified by phosphoimagery. The resulting strand scission at G_{24} (◆), G_{22} (■), G_{20} (▲), G_6 (○), and G_4 (×) in the (C) double- and (D) single-stranded samples was then calculated relative to the total material of each sample. See Figure 1 for the nucleotide sequences of **OD1** and **OD2.**

Site Specificity of Alkylation and Cross-Linking. Once the efficiency of DNA alkylation and cross-linking by **6a** was found to be substantially controlled by its attached acridine, the site specificity of reaction was also expected to be controlled by this same component. The anticipated selectivity was confirmed by treating product mixtures with hot piperidine, a common method used to induce strand scission at GN7, AN3, and AN7 alkylation.[30] All of the sites of alkylation and cross-linking within duplex DNA were sensitive to this secondary treatment. Of those products present after 4 h under conditions identical to those of Figure 3, approximately 50% reverted to a mobility equivalent to that of the parent strand in denaturing gel electrophoresis (Figure 4A).[31] Another 40% underwent strand scission consistent with alkylation or cross-linking at GN7, and only a trace level of scission at other residues was also observed. The relative levels of each DNA strand fragment indicated that a mild selectivity for the 5′···GCGCG··· sequence of **OD2** remained constant during incubation with **6a** (Figure 4C). Less information can be gleaned from the fraction of oligonucleotide products that reverted to parent-like mobilities, although these products most probably derive from QM adducts of known lability. For example, addition of a model QM (Scheme 1) to AN1 is rapidly reversible.[13] An equivalent CN3 adduct is more stable but ultimately decomposes as well,[12] and finally the GN7 adduct discussed above actually partitions between QM elimination and deglycosylation.[14,32] Only the deglycosylation is easily detected through its sensitivity to piperidine-induced strand scission as illustrated in Figure 4A.

The profile of products generated from single-stranded DNA and **6a** were additionally examined with piperidine treatment, and the results highlight important similarities and differences between reaction of double- and single-stranded DNA. Gel mobility of the single-stranded products again increased after incubation with piperidine, but only a small fraction reverted to a mobility equivalent to that of the parent oligonucleotide (Figure 4B).[31] Approximately 40% of the products present after a 4 h incubation with **6a** and **OD2** still migrated more slowly than unmodified **OD2**. Single-stranded, but not duplex, DNA can then likely form a significant number of stable QM adducts by reacting at GN1, GN^2 and AN^6.[13,14] Another 45% of the alkylated products were subject to scission by piperidine and yielded fragments that also indicated a high selectivity for GN7. However, the relative distribution of alkylation among the G residues did not mimic the mild sequence selectivity detected in the double-stranded reaction (Figures 4C vs 4D). Only G_4 exhibited an apparent high reactivity in single-stranded DNA. However, this observation is also consistent with an accumulation of adducts typically associated with conditions that exceed single hit kinetics. The relative selectivity of G_4 and, to a lesser extent, G_6 increased over the incubation period as expected for multiple alkylations within each target strand. Indeed, an estimated 8−10 equiv of **6a** alkylated single-stranded DNA after a 4 h reaction. Accordingly, few conclusions pertaining to specificity may be drawn from the smallest fragments of this analysis.[33] Note that comparable reaction of OD2 in duplex DNA did not result in an excess of small DNA fragments (Figure 4C) despite evidence for ∼2 adducts per strand (4 per duplex). These seemingly contradictory results can be reconciled if QM release is more facile than deglycosylation for adducts of GN7.

Discussion

A triplex binding oligonucleotide conjugate of bisQMP had previously been constructed in hopes of enhancing the expected efficiency of the major groove reaction of its nonconjugated parent derivative. Although fewer equivalents of this conjugate were necessary for reaction relative to that of the parent, the conjugate was only capable of alkylating individual strands of its target, and no interstrand cross-linking was detected.[15] The constraints of sequence specificity enforced by the attached oligonucleotide were then eliminated in the acridine conjugate **6a** to investigate the potential conflict between thermodynamic and kinetic processes that may limit targeted alkylation of DNA.

An acridine conjugate was chosen as an alternative to the oligonucleotide conjugate for a variety of reasons including the ability of acridine to intercalate with little sequence selectivity,[21,27,34,35] ease of preparation,[22,23] ability to direct reagents to the major groove,[21,22] and its general applicability to coupling

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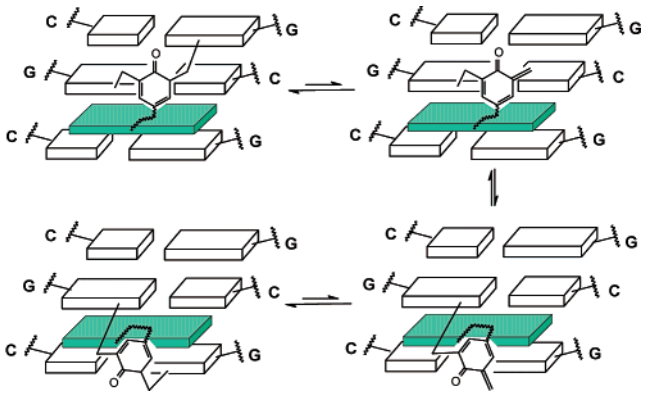
with a wide variety of DNA reagents.[35−39] Most important to our interests were the acridine conjugates of aliphatic and aromatic *N*-mustards.[21,22] The efficacy of DNA alkylation and cytotoxicity of these conjugates increased orders of magnitude over those of their nonconjugated derivatives. Such results were somewhat anticipated since the attached acridine enhances binding of the *N*-mustards to DNA and localizes them in the major groove near their preferred site of reaction, GN7. The extent to which acridine might promote reaction of the bisQMP (Scheme 2) was not as obvious since the energy gained from intercalation would need to offset the inherent instability of a major groove reaction at GN7 and overcome competition from minor groove reaction at GN[2] that yields a thermodynamically favored product.[14] To complicate predictions even further, conjugates appended to the amino group of 9-aminoacridine have also been shown to reside in the minor groove, depending on the linker and conjugate structure.[21,40]

The bisQMP−Acridine Conjugate 6a Cross-Links DNA in the Presence of Fluoride To Initiate Quinone Methide Formation. Interstrand cross-linking of duplex DNA was the most readily observable product of reaction with **6a** (Figure 1). Conjugation of bisQMP to acridine enhanced its cross-linking efficiency by at least 64-fold (Figure 2, lanes 2 vs 3) in analogy to acridine's effect on the *N*-mustards noted above.[21,22] The response of **6a** to ionic strength also illustrated acridine's controlling influence on the bisQMP. Intercalation by acridine is inhibited by high ionic strength[25−27] and so too is the ability of **6a** to cross-link DNA. In contrast, the nonconjugated parent bisQMP and a monofunctional QMP conjugate of single-stranded DNA were not inhibited by high ionic strength or excessive concentrations of KF (1 M).[18,28]

The bisQMP component again performed as designed for generating the desired quinone methide intermediate. Reaction depended on the presence of fluoride (10 mM) to remove the silyl-protecting group (Figure 2, lanes 2 vs 6). No evidence was detected to support an alternative mechanism based on direct benzylic substitution even though this might have been promoted if an optimal orientation of the nucleophile/electrophile pair had occurred. The lack of such activity is best illustrated by the inability of **6b,** in which the silyl group is substituted by methyl, to react with DNA (Figure 2, lane 5). Although the ratio of cross-linking to simple alkylation was not measured or easily determined for the parent bisQMP,[18] the conjugate **6a** yielded approximately one cross-link out of every four initial alkylations as indicated by the ratio of equivalents of bound acridine vs equivalents of cross-linking (Figure 3). This ratio is quite desirably small when compared to an estimate of 1:20 for a *N*-mustard acridine conjugate.[21] Certainly, the attached acridine promotes initial alkylation for both types of conjugates. Its effect on the subsequent interstrand reaction is less clear, and consequently, differences in cross-linking vs alkylation efficiency may reflect intrinsic differences in the nature of the QM and *N*-mustard. Of course, the reversibility of QM addition[10−14] is one important characteristic that does not seem to be shared by *N*-mustards, or at least no data have yet to suggest this possibility.

Scheme 4



The bisQMP−acridine conjugate, like other acridine conjugates, had the potential to sample all possible cross-linking sites of its duplex target through noncovalent preassociation. In contrast, the previous triplex-forming bisQMP derivative was held to a single region of its target duplex, and this may have restricted interstrand cross-links regardless of the reversibility of QM alkylation.[15] A *N*-mustard held through triplex formation was previously shown to exhibit a similar ability to act only as a mild alkylating agent and not as a cross-linking agent.[41] The possibility that the initial monoalkylated and cross-linked derivative of DNA formed by **6a** could continue to sample alternative covalent structures is currently under investigation. Rather than translocating via monoalkylation and release as described previously for bizelesin,[9] the QM may "walk" from unfavorable to favorable cross-linking sites via transient formation of a monoalkylated intermediate (Scheme 4). This alternative is more reasonable for the bisQMP since its alkylation and cross-linking are coupled in a manner not shared by bizelesin. Whether or not QM migration occurs in this system, the individual adducts of GN7 are still likely to remain labile as expected from model studies.[12−14] The attached acridine may stabilize the overall association of the QM conjugate by intercalation but cannot be expected to alter the dynamics of the QM−GN7 bond.

Acridine Provides Rapid Delivery of bisQMP to GN7 in the Major Groove of Duplex DNA. The acridine conjugate **6a** cross-linked DNA from the earliest time of analysis and did not first require a significant accumulation of monoalkylation (Figure 3, lane 2). The ratio of total acridine attached per duplex vs cross-linking varied little from 4.3 after 1 h to 3.2 upon completion of reaction (4 h). If substantial monoalkylation instead of cross-linking had occurred, then the reaction products would have exhibited a gel mobility intermediate between that of the parent DNA and its cross-linked derivative and similar to the alkylation products of single-stranded DNA (Figures 1 and 3).

Treating the products of duplex reaction with piperidine caused reversal of the cross-link and fragmentation of the phosphodiester backbone in approximately equal proportions (Figure 4A). Both results are consistent with alkylation of GN7

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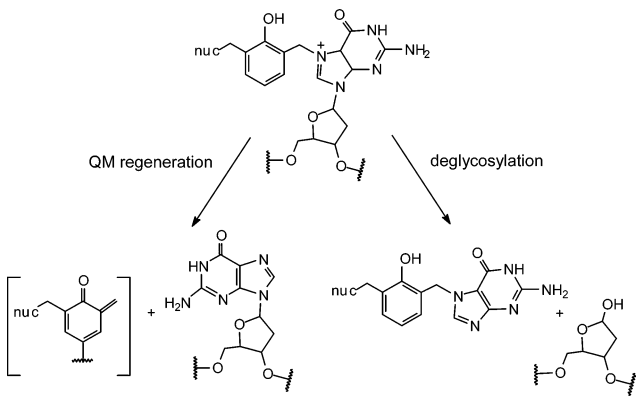
Scheme 5



since its product can undergo elimination of the QM as well as depurination and ultimately strand scission (Scheme 5).[14,32] However, products formed by QM addition to CN3 and AN1 are also expected to eliminate the QM during piperidine treatment. While alkylation at these sites has been observed with *N*-mustards in special cases,[42,43] equivalent reaction with at least the simple *o*-benzoquinone methide is dramatically suppressed in double- vs single-stranded DNA.[14,29] Alkylation of AN7, a weaker nucleophile than GN7 but accessible in the major groove, is not evident after incubation with **6a** since this too would have induced strand scission upon piperidine treatment. In contrast, this site has previously demonstrated competence in nucleophilic reaction since it is a target of alkylation for conjugates containing a long linker between acridine and an aniline-based *N*-mustard.[21]

Since a single piperidine-labile bond in DNA cross-linking (e.g., QM-GN7) is sufficient for the reversion and fragmentation illustrated in Figure 4, little information is available on the second site of attachment. However, cross-linking through two GN7 positions is most satisfying. Participation of nucleophiles in the major groove that can form stable QM adducts (AN,[6] CN[4])[12−14] would only be possible if they acted in concert with GN7. Cross-linking two AN[6] or CN[4] positions would be expected to remain inert to piperidine treatment. Alternative reaction between sites in the major and minor groove would also be unlikely due to the steric constraints of duplex DNA. Finally, cross-linking within the minor groove alone could involve the nucleophiles AN3 and GN[2] but no evidence suggests reaction at either of these sites within duplex DNA. Alkylation at AN3 would have been observed by strand fragmentation after the hot piperidine treatment,[44] and alkylation at GN[2] would have resisted reversal or strand fragmentation after this treatment.[30,45]

Unlike double-stranded DNA, single-stranded DNA was observed to form products of both reversible and irreversible alkylation. Reaction still occurred at GN7 as detected by strand fragmentation after piperidine treatment (Figure 4B). Other products of reaction retained a somewhat diminished gel mobility (vs the parent strand) after equivalent treatment, suggesting the presence of adducts at sites such as GN[2] and AN[6]. Thus, in the absence of the strong directing influence of acridine intercalation, **6a** can act more akin to its parent nonconjugated derivative.

The bisQMP−acridine conjugate **6a** demonstrates that efficient cross-linking based on quinone methide formation need not rely on the presence of a single-stranded region extending from duplex DNA as previously observed with a bisQMP lacking the acridine.[18] Likewise, cross-linking is not prohibited from sites within the major groove as implied by data on a bisQMP−oligonucleotide conjugate.[15] The acridine component of **6a** appears to deliver and hold the bisQMP in the major groove in a highly productive manner for cross-linking GN7, a strong nucleophile that has the potential to add to QMs reversibly. Intercalation of the acridine may (1) provide sufficient binding stabilization to overcome the intrinsic instability of QM alkylation at GN7 vs QM alkylation at GN[2] and AN[6] that forms irreversible adducts and/or (2) create a kinetic barrier that prevents migration of the bisQMP to GN[2] and AN[6]. In either event, noncovalent interactions can overcome potential conflicts between thermodynamics and kinetics of bond formation. For the bisQMP−acridine conjugate, reversible QM formation seems to be anchored in the major groove (Scheme 4). In a complementary manner, minor groove binding has previously been shown to anchor reversible reaction of bizelesin.[9] Related mechanisms may also account for the apparent specificity of other synthetic and natural products that react with DNA in a reversible manner.

Experimental Section

General Materials. Organic reagents and starting materials were purchased from Aldrich, Sigma, and Lancaster Chemical Co. and used without further purification. Solvents, buffers, and salts were purchased from Fisher and Sigma. HPLC grade acetonitrile was distilled over calcium hydride under a nitrogen atmosphere prior to use. Silica gel (230−400 mesh) for flash column chromatography was purchased from EM sciences. Oligonucleotides were synthesized by Invitrogen Life Technologies (Rockville, MD), purified by gel electrophoresis under standard conditions,[46] and labeled at their 5′ terminus with 5′-[γ-^{32}P]-ATP as directed by the enzyme supplier (New England Biolabs, Beverly, MA). 4-Morpholineethanesulfonic acid (MES) was purchased from Sigma and Calbiochem. All aqueous solutions were made with water that had been purified by a standard purification system to yield a resistivity of between 17.8 and 18 MΩ.

General Methods. Melting points were measured with a Thomas-Hoover Unimelt apparatus and have been corrected. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on AM 400 and DRX 400 (^{1}H: 400.13 MHz, ^{13}C: 100.61 MHz) spectrometers, and coupling constants are reported in Hz. Low-resolution mass spectra by electron impact (EI) were determined with Hewlett-Packard 5980A and VG 7070E mass spectrometers. High-resolution mass spectra by EI and fast atom bombardment (FAB) were also determined with the VG 7070E mass spectrometer.

3-[4′-Hydroxy-3′,5′-bis(hydroxymethyl)phenyl]propionic Acid (2). Cold aqueous 5 M NaOH was added to 3-(4′-hydroxyphenyl)propionic acid (2.0 g, 12 mmol) to adjust pH to 11, and the resulting solution was combined with formaldehyde (37%, 6 mL).[19] The reaction was stirred at 55° C for 17 h, cooled (5° C), and combined with acetone (100 mL). A resulting orange oil was collected, mixed with methanol (15 mL) and again poured into acetone (150 mL) to form a white precipitate. The solid was collected and washed with acetone to yield

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2 as its sodium salt (2.2 g, 72%). To obtain a melting point, an aliquot of the salt (~15 mg) was dissolved in water, and the pH of the solution was adjusted to pH 2 with 6 M HCl. The resulting suspension was extracted with EtOAc (2 mL), and the organic phase was concentrated under reduced pressure to yield a solid: mp 128−129° C (lit.[19] 130−133° C). ^{1}H NMR (CD_3OD) δ 2.39 (t, J = 7.8, 2H), 2.80 (t, J = 7.8, 2H), 4.66 (s, 4H), 7.00 (s, 2H). ^{13}C NMR (CD_3OD) δ 33.7, 42.4, 64.8, 119.3, 128.4, 129.7, 163.2, 171.1.

3-[4′-*tert*-Butyldimethylsilyloxy-3′,5′-bis(*tert*-butyldimethylsilyloxymethyl)]propionic Acid (3a). Imidazole (3.30 g, 48.5 mmol) was added to a solution of *tert*-butyldimethylsilyl chloride (TBDMSCl, 3.30 g, 21.9 mmol) and the sodium salt of **2** (1.00 g, 4.03 mmol) in 15 mL of DMF. The reaction mixture was stirred at room temperature for 17 h, diluted with brine (100 mL), and extracted with ether (3 × 100 mL). The organic phases were combined, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was redissolved in methanol (50 mL) and potassium carbonate (2.00 g) was added in one portion. The solution was stirred for 1 h and neutralized with 2 M HCl. The mixture was then diluted with water (100 mL) and extracted with ether (3 × 100 mL). The organic phases were combined, washed with brine (3 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel flash chromatography (hexanes:ethyl acetate, 7:3) to yield **3a** as a colorless solid: mp 83−84 °C (1.51 g, 66%). ^{1}H NMR ($CDCl_3$) δ 0.08 (s, 12H), 0.13 (s, 6H), 0.91 (s, 18H), 0.99 (s, 9H), 2.64 (t, J = 8.0, 2H), 2.91 (t, J = 8.0, 2H), 4.66 (s, 4H), 7.17 (s, 2H). ^{13}C NMR ($CDCl_3$) δ −5.3, −3.4, 18.4, 18.7, 25.9, 26.0, 30.3, 35.8, 60.6, 125.6, 131.6, 133.2, 146.5, 179.6. MS (EI) *m*/*z* (rel intens): 570 (M^+ + 1, 1.7), 596 (M^+, 5.0), 511 (43), 495 (6.4), 395 (14), 379 (100), 323 (19), 265 (27), 205 (34), 146 (67). HRMS (FAB, glycerol) *m*/*z* 569.3488. Calcd for $C_{29}H_{57}O_5Si_3$ (M + H^+) 569.3513.

3-[4′-*tert*-Butyldimethylsilyloxy-3′,5′-bis(acetoxymethyl)phenyl]-propionic Acid (4a). Solid ferric chloride (0.10 mg, 0.62 mmol) was added to a solution of **3a** (1.00 g, 1.76 mmol) in acetic anhydride (20 mL) at 0 °C under nitrogen.[20] The reaction mixture was stirred for 30 min and then extracted with ether. The combined organic phases were washed with water and saturated $NaHCO_3$, dried with $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to silica gel flash chromatography (hexane:ethyl acetate, 7:3) and yielded **4a** as a colorless liquid (0.39 g, 94%): ^{1}H NMR ($CDCl_3$) δ 0.17 (s, 6H), 1.00 (s, 9H), 2.07 (s, 6H), 2.74 (m, 2H), 2.91 (m, 2H), 5.06 (s, 4H), 7.13 (s, 2H). ^{13}C NMR ($CDCl_3$) δ −3.8, 18.6, 20.9, 25.8, 29.3, 36.7, 61.6, 127.1, 129.8, 132.9, 149.9, 168.3, 170.7. MS (EI) *m*/*z* (rel intens) 424 (M^+, 0.06), 421 (0.14), 250 (4.5), 215 (5.2), 186 (26), 142 (100). HRMS (FAB, glycerol) *m*/*z* 425.2006. Calcd for $C_{21}H_{33}O_7Si$ (M + H^+) 425.1996.

***N*-Succinimidyl-3-(4′-*tert*-butyldimethylsiloxy-3′,5′-bis(acetoxymethyl)phenyl)propionate.** *N*-Hydroxysuccinimide (0.035 g, 0.30 mmol) was added to a DMF solution (2.0 mL) of **4a** (0.087 g, 0.20 mmol). This mixture was cooled to 4 °C and combined with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 0.046 g, 0.24 mmol). The mixture was then stirred for 20 h at 4 °C, diluted with brine, and extracted with ether (30 mL). The organic phase was washed with brine (4 × 20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The resulting residue was subjected to silica gel flash chromatography (hexane:ethyl acetate, 7:3) to yield the desired activated ester of **4a** (0.071 g, 66%) as a viscous colorless liquid. ^{1}H NMR ($CDCl_3$) δ 0.17 (s, 6H), 1.00 (s, 9H), 2.08 (s, 6H), 2.80 (s, 4H), 2.91 (m, 2H), 2.95 (m, 2H), 5.05 (s, 4H), 7.14 (s, 2H). ^{13}C NMR ($CDCl_3$) δ −3.8, 18.5, 20.8, 25.5, 25.8, 29.5, 32.5, 61.6, 127.1, 129.8, 132.4, 149.9, 167.7, 168.9, 170.7. MS (FAB, glycerol) *m*/*z* (rel. Intensity) 522 (M + H^+, 0.44), 462 (5.0), 404 (3.8), 362 (41), 288 (13), 249 (18), 219 (18), 207 (379), 149 (25), 131 (12), 117 (100). HRMS (FAB, glycerol) *m*/*z* 522.2185. Calcd for $C_{25}H_{36}O_9NSi$ (M + H^+) 522.2159.

3-(4′-Methoxy-3′,5′-bis(methoxymethyl)phenyl)propionic Acid (3b). After mixing KOH (flakes, 20.6 g, 364 mmol) in DMSO (2 mL) for 5 min, **2** (5.15 g, 22.8 mmol) and methyl iodide (25 mL, 18 mmol) were added sequentially. This mixture was stirred at room temperature for 1 h, poured into water (100 mL), and extracted with ether (2 × 100 mL). The combined organic solutions were washed with water (2 × 100 mL) and brine (2 × 100 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was then dissolved in methanol (14 mL) and combined with an aqueous solution of LiOH (0.13 g, 3 mmol, 7 mL). This mixture was stirred at 4 °C for 15 h, acidified with HCl to pH 2.0, and extracted with ether (3 × 30 mL). The combined organic phases were washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to silica gel flash chromatography (hexane:ethyl acetate, 7:3) to yield **3b** (0.27 g, 15%) as light yellow-colored viscous liquid. ^{1}H NMR ($CDCl_3$) δ 2.64 (t, J = 7.9, 2H), 2.91 (t, J = 8.0, 2H), 3.40 (s, 6H), 3.76 (s, 3H), 4.48 (s, 4H), 7.20 (s, 2H), 11.2 (bs, 1H). ^{13}C NMR ($CDCl_3$) δ 29.8, 35.2, 57.8, 62.1, 69.1, 129.1, 130.8, 135.81, 154.7, 177.4. MS (FAB) *m*/*z* (rel intens) 269 (M + H^+, 6.0), 237 (93), 191 (42), 147 (65), 91 (49), 55 (100). HRMS (FAB, glycerol) *m*/*z* 269.1385. Calcd for $C_{14}H_{21}O_5$ (M + H^+) 269.1388.

3-(4′-Methoxy-3′,5′-bis(acetoxymethyl)phenyl)propionic Acid (4b). Solid ferric chloride (0.030 g, 0.17 mmol) was added to a solution of **3b** (0.20 g, 0.75 mmol) in acetic anhydride (10 mL) at 20 °C under nitrogen. The mixture was maintained at 80 °C for 24 h, cooled, and extracted with ether (3 × 10 mL). The combined ether extracts were washed with water (2 × 30 mL) and saturated $NaHCO_3$ (4 × 30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to silica gel flash chromatography (hexane:ethyl acetate, 7:3) to yield **4b** (0.22 g, 82%) as a light yellow-colored liquid. ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 6H), 2.77 (m, 2H), 2.93 (m, 2H), 3.82 (s, 3H), 5.15 (s, 4H), 7.23 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 20.7, 29.4, 36.5, 61.1, 62.7, 129.5, 130.5, 135.6, 155.7, 164.8, 170.6. MS (FAB) *m*/*z* (rel intens) 325 (M + H^+, 7.3), 265 (100), 193 (63), 147 (44), 91 (16). HRMS (FAB, glycerol) *m*/*z* 325.1284. Calcd for $C_{16}H_{21}O_7$ (M + H^+) 325.1287.

***N*-Succinimidyl-3-(4′-methoxy-3′,5′-bis(acetoxymethyl)phenyl)-propionate.** *N*-Hydroxysuccinimide (0.11 g, 0.90 mmol) was added to a solution of **4b** (0.20 g, 0.60 mmol) in DMF (2.0 mL). This mixture was cooled to 4 °C and combined with EDC (0.13 g, 0.70 mmol). The mixture was stirred for 20 h at 4 °C, diluted with brine, and extracted with ether (50 mL). The organic phase was washed with brine (4 × 30 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to silica gel flash chromatography (hexane:ethyl acetate, 7:3) to yield the desired activated ester of **4b** (0.18 g, 72%) as a viscous liquid of light yellow color. ^{1}H NMR ($CDCl_3$) δ 2.03 (s, 6H), 2.74 (s, 4H), 2.83 (t, J = 7.6, 2H), 2.92 (t, J = 7.6, 2H), 3.73 (s, 3H), 5.06 (s, 4H), 7.17 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 20.7, 25.3, 29.4, 32.2, 60.9, 62.5, 129.4, 130.2, 135.0, 155.6, 167.5, 168.9, 170.4. MS (FAB) *m*/*z* (rel intens) 422 (M + H^+, 8.99), 362 (100), 290 (62), 175 (66), 147 (82), 91 (60). HRMS (FAB, glycerol) *m*/*z* 422.1447. Calcd for $C_{20}H_{24}O_9N$ (M + H^+) 422.1451.

***N*-(*N*′-Acridinyl-2′-aminoethyl)-3-(4″-*tert*-butyldimethylsilyoxy-3″,5″-bis(acetoxy-methyl)phenyl)propionamide (6a).** Triethylamine (0.16 g, 1.6 mmol) was added to a suspension of **5**[22,23] (0.060 g, 0.20 mmol) in methanol (10 mL). Once this was homogeneous, the activated ester of **4a** (0.080 g, 0.20 mmol) in acetonitrile (10 mL) was added dropwise over 1 min, and finally, acetic acid (0.050 g, 0.80 mmol) was added over 5 min at room temperature. Solvent was removed under reduced pressure, and the residue was dissolved in CH_2Cl_2 (50 mL). This solution was washed with water (1 × 30 mL) and brine (2 × 30 mL), dried ($MgSO_4$), and concentrated under reduced pressure. The solid residue was recrystallized using methylene chloride and diethyl ether to yield **6a** (0.07 g, 65%) as a yellow solid: mp 187−188 °C. ^{1}H NMR ($CDCl_3$) δ 0.17 (s, 6H), 1.01 (s, 9H), 2.08 (s, 6H), 2.78 (t, J = 7.4, 2H), 3.04 (t, J = 7.4, 2H), 3.91 (m, 2H), 4.32 (m, 2H), 5.05 (s, 4H), 7.12 (bs, 1H), 7.20 (s, 2H), 7.46 (t, J = 7.8, 2H), 8.11 (d, J = 8.5, 2H), 8.20 (d, J = 8.5, 2H), 8.48 (t, J = 7.8, 2H), 9.28 (bs, 1H).

^{13}C NMR ($CDCl_3$) δ −3.7, 18.7, 21.0, 25.9, 30.9, 38.1, 39.4, 51.8, 61.9, 112.2, 119.4, 123.5, 124.7, 124.8, 127.1, 130.1, 133.8, 134.1, 139.6, 156.5, 170.9, 175.6. MS (FAB) *m*/*z* (rel intens) 644 ($M + H^+$, 44), 600 (3.9) 524 (3.8), 470 (3.1), 264 (6.1), 221 (26), 209 (16), 195 (100), 180 (14), 117 (49). HRMS (FAB, glycerol) *m*/*z* 644.3187. Calcd for $C_{36}H_{46}O_6N_3Si$ ($M + H^+$) 644.3156.

***N*-(*N*′-Acridinyl-2′-aminoethyl)-3-(4″-methoxy-3″,5″-bis(acetoxymethyl)phenyl)propionamide (6b).** The synthesis of **6b** essentially followed the same procedure as that described above for coupling an activated ester and used *N*-succinimidyl of **4b** (0.03 g, 0.07 mmol) with **5**[22,23] (0.02 g, 0.07 mmol). Recrystallization using methylene chloride and ether yielded **6b** (4.0 mg, 10%) as a yellow solid: mp 137−138 °C. ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 6H), 2.77 (t, $J = 7.3$, 2H), 3.05 (t, $J = 7.3$, 2H), 3.78 (s, 3H), 3.90 (m, 2H), 4.31 (m, 2H), 5.11 (s, 4H), 7.14 (bs, 1H), 7.26 (s, 2H), 7.46 (t, $J = 7.4$, 2H), 8.10 (d, $J = 8.5$, 2H), 8.21 (d, $J = 8.5$, 2H), 8.49 (t, $J = 7.5$, 2H), 9.28 (bs, 1H). ^{13}C NMR ($CDCl_3$) δ 21.1, 31.0, 37.8, 39.4, 51.8, 61.3, 62.9, 112.1, 119.3, 123.5, 124.8, 129.5, 130.8, 134.1, 136.7, 139.5, 155.7, 156.6, 170.8, 175.5. MS (FAB) *m*/*z* (rel intens) 544 ($M + H^+$, 100), 500 (5.2), 442 (3.9), 264 (3.1), 207 (42), 195 (87), 91 (7.0). HRMS (FAB, glycerol) *m*/*z* 544.2428. Calcd for $C_{31}H_{34}O_6N_3$ ($M + H^+$) 544.2447.

3-(4′-*tert*-Butyldimethylsiloxy-3′,5′-bis(acetoxymethyl)phenyl)propionamide (7). The activated ester of **4a** (0.11 g, 0.19 mmol) was dissolved in acetonitrile (12 mL) and water (12 mL), and pH was adjusted to 12 by addition of NH_4OH (29% aqueous solution). The mixture was stirred at room temperature for 5 min, acidified with HCl to pH 2.0, and extracted with ether (2 × 50 mL). The combined ether extracts were washed with water (2 × 50 mL) and brine (2 × 50 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The residue was subjected to silica get flash chromatography (hexane:ethyl acetate 1:1) to yield **7** (19 mg, 21%) as a colorless and viscous liquid. ^{1}H NMR (400.13 MHz, $CDCl_3$) δ 0.18 (s, 6H), 1.01 (s, 9H), 2.09 (s, 6H), 2.50 (t, $J = 8.2$, 2H), 2.91(t, $J = 8.1$, 2H), 5.07 (s, 4H), 5.46 (bs, 1H), 5.58 (bs, 1H), 7.14 (s, 2H). ^{13}C NMR (100.61 MHz, $CDCl_3$) δ −3.7, 18.6, 21.0, 25.9, 30.5, 37.4, 61.8, 127.1, 129.9, 134.1, 149.8, 170.8, 174.3. MS (FAB) *m*/*z* (rel intens) 424 ($M + H^+$, 14), 364 (100), 306 (22), 264 (50), 207 (43), 190 (42), 117 (97). HRMS (FAB, glycerol) *m*/*z* 424.2173. Calcd for $C_{21}H_{34}O_6NSi$ ($M + H^+$) 424.2155.

9-[*N*-(2-Acetylaminoethyl)]aminoacridine (8). Diethylamine (0.50 mL, 6.8 mmol) was added to a solution of **5**[22,23] (25 mg, 0.091 mmol) in acetonitrile (2.5 mL). The solution was stirred at room temperature for 1 min, and acetic anhydride (20 μL, 0.19 mmol) was then added in one portion. The resulting solution was stirred for 2 h at room temperature, and the reaction was neutralized with 0.1 M HCl. The mixture was extracted with chloroform (3 × 30 mL), and the organic phase was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel chromatography (4:1 $CHCl_3$:MeOH). The resulting yellow solid was dissolved in water (5 mL), and the pH was adjusted to 12 with 1 M NaOH. This solution was then lyophilized, and the residue was once again dissolved in water (5 mL), neutralized with 0.1 M HCl, and extracted with $CHCl_3$ (3 × 5 mL). The combined organic phases were dried over $MgSO_4$ and concentrated under reduced pressure to afford **8** as a yellow solid (11 mg, 41%): ^{1}H NMR (400 MHz, DMF-d_7) δ 1.97 (s, 3H), 3.78 (q, $J = 5.6$, 2H), 4.37 (q, $J = 5.6$, 2H), 7.55 (t, $J = 8.0$, 2H), 7.97 (t, $J = 8.0$, 2H), 8.30 (d, $J = 8.0$, 2H), 8.83 (m, 2H), 9.02 (bs, 1H), 10.53 (bs, 1H); ^{13}C NMR (101 MHz, DMF-d_7) δ 22.6, 39.9, 50.8, 113.2, 119.5, 124.0, 126.4, 135.2, 140.4, 158.4, 172.5. HRMS (FAB) *m*/*z* 280.1456 ($M + H^+$); Calcd for $C_{17}H_{17}ON_3$ ($M + H^+$): 280.1450

Oligonucleotide Studies. Duplex DNA was annealed by heating (90° C) 1.0 equiv of the radiolabeled strand and 1.1 equiv of its complementary unlabeled strand in 10 mM MES, pH 7, and then slowly cooling to room temperature (3−4 h). The resulting samples and alternatively the individual oligonucleotide components were then diluted by 30% with addition of the bisQMP conjugate **6a** or its related derivatives dissolved in acetonitrile. Alkylation and cross-linking (20° C) was initiated by diluting the mixtures by another 20% with addition of KF to a final concentration of 10 mM. At the indicated times, reaction was stopped by addition of β-mercaptoethanol (0.3 μL/μL of reaction), and the samples were frozen, lyophilized, resuspended in 10 μL of formamide loading solution (0.05% bromophenol blue and 0.05% xylene cyanol FF in formamide), and finally analyzed by 20% polyacrylamide denaturing gel electrophoresis. Alternatively, samples were resuspended in 10% aqueous piperidine, heated (90° C, 30 min), frozen, and lyophilized. The resulting residue was then dissolved in water (100 μL) and again frozen and lyophilized to remove traces of piperidine. These samples were then also mixed with the loading solution and analyzed under equivalent conditions. Radiolabeled DNA was detected using a Molecular Dynamics PhosphorImager and quantified with ImageQuant software for determining reaction yields (% product relative to total material).

Stoichiometry of Acridine Coupling to Double- and Single-Stranded DNA. Reaction conditions used for gel analysis were maintained for these studies and only the total volume was increased to 800 μL. At the indicated times, β-mercaptoethanol (100 μL) was added to stop reaction, and samples were dialyzed (10000 MW cutoff) against water for 48 h, lyophilized, dissolved in water (1000 μL), and analyzed by UV to determine the acridine:DNA ratio. Absorbance (415 nm) of the $t = 0$ h sample was used as a blank for subsequent A_{415} determinations of acridine. Since the extinction coefficients of acridine at 260 and 415 nm are affected by single- and double-stranded DNA, their corrected values ($\epsilon_{260} = 29.0 \times 10^{-3}$ μM^{-1} cm^{-1} and 25.5×10^{-3} μM^{-1} cm^{-1}, respectively and $\epsilon_{415} = 5.53 \times 10^{-3}$ μM^{-1} cm^{-1} and 4.59×10^{-3} μM^{-1} cm^{-1}, respectively) were determined by titrating acridine with **OD2** and calf thymus DNA. The concentration of DNA recovered from reaction with **6a** was determined from the extinction coefficients provided by the manufacturer (**OD1**: $\epsilon_{260} = 2.61$ μM^{-1} cm^{-1}; **OD2**: $\epsilon_{260} = 3.64$ μM^{-1} cm^{-1}) and the ΔA_{260} remaining after subtracting the contribution of bound acridine.

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Supporting Information Available: Polyacrylamide gel analysis of alkylation and cross-linking of **OD1** after piperidine treatment and details on acridine equivalents bound to DNA (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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