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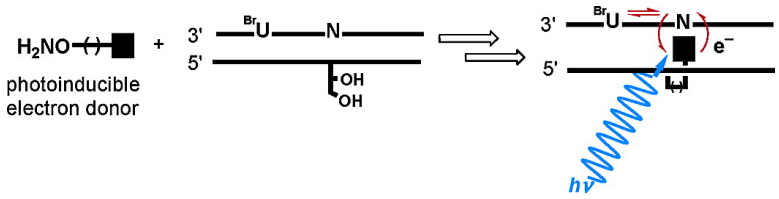

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Reductive Electron Injection into Duplex DNA by Aromatic Amines

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Abstract: An assay based on photoinduced reaction and subsequent cleavage of duplex DNA containing a bromodeoxyuridine (^{Br}U) residue and an abasic site was developed to screen aromatic amines for their ability to initiate charge transfer by reductive electron donation. Two candidates, *N*,*N*,*N*′,*N*′-tetramethyl-1,5-diaminonaphthalene (TMDN) and 1,5-diaminonaphthalene (DAN), expressed the desired activity, and an oligodeoxynucleotide−TMDN conjugate was subsequently prepared to identify additional variables affecting the efficiency of electron injection and transfer into DNA. This system demonstrated only mild sensitivity to molecular oxygen but was strongly inhibited by high concentrations of 2-mercaptoethanol. The nucleobase counter to the attached TMDN strongly modulated charge transfer as evident by a 60-fold decrease in reduction of the distal ^{Br}U when the counterbase A was substituted for C. An inverse relationship between this reduction and quenching of TMDN fluorescence by the counterbase was also discovered and is consistent with a competition between radical recombination and electron migration away from the initial site of its injection into DNA.

The regular array of nucleobases stacking in double helical DNA has long held the attention and imagination of scientists as a possible conductor of charge on the nanometer scale.[1] Advances in this area have since emerged from a range of complementary disciplines. Recently, a short strand of duplex DNA (20 bp) spanning two conductors was shown capable of sustaining a current.[2] Electrochemical devices based on the recognition and conducting properties of DNA have simultaneously been developed for detecting nucleotide sequences, proteins, and small molecules of interest.[3] Furthermore, charge transfer in DNA has been found to influence the susceptibility of certain nucleotide sequences to oxidative damage.[4] One of the most common methods for examining the fundamental nature of charge transfer in DNA is based on photochemical excitation of an electron acceptor or donor.[5−7] In each case, an excited-state chromophore injects charge into DNA by oxidation or reduction of a proximal nucleobase to yield a radical cation or anion that may migrate in processes respectively described as hole transfer (HT) or excess electron transfer (EET).

Structural determinants of HT have recently begun to emerge from the collective efforts of many laboratories exploring numerous complementary systems and employing a wide variety of experimental and theoretical techniques. Both electron tunneling and hopping contribute in a sequence and distance dependent manner to HT.[5,6] Much of the early controversy surrounding this topic arose from initial ambiguities created by contrasting methods used to inject charge and detect its migration. However, this very same diversity ultimately helped to forge a consensus on the essential basis of HT.

By comparison to HT, EET is neither well described nor understood. Most exploration of this alternative mode of charge transfer began only in the past few years, and already seemingly contradictory results have been observed due to different methods of electron injection and EET detection.[8−10] Again, these differences highlight the importance of compiling contrasting systems for defining the kinetics and thermodynamics controlling charge transfer. Initial investigations were based on pulse radiolysis and photoexcitation of nucleotides and heterogeneous samples of DNA.[11−13] Theoretical treatments[14] began

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Table 1. Aromatic Amines as Potential Electron Donors for EET in DNA

donor	λ_{max} (nm)	$E^{1/2}_{ox}$ (V vs SCE)	E_{ox}* (V)[a]	ref
N,*N*-dimethylaniline (DMA)	302	0.83	−3.32	45
N,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD)	332	0.20	−3.04	45
N,*N*,*N*′,*N*′-tetramethylbenzidine (TMB)	345	0.43	−3.17	45
N,*N*,*N*′,*N*′-tetramethyl-1,5-diaminonaphthalene (TMDN)	330	0.58	−2.8	20,46
1,5-diaminonaphthalene (DAN)	334	0.46	−3.06	this work
N,*N*,*N*′,*N*′-tetramethyl-9,10-diaminoanthracene (TMDA)	408	0.84	−1.71	44b, this work

[a] The excited-state oxidation potential E_{ox}* ($D^*/D^{\bullet+}$) was estimated by $E_{ox}^* = E_{ox} - E_{oo}/23.06$ with units of V vs SCE for E_{ox}* and $E^{1/2}_{ox}$ and kcal/mol for E_{oo}.

to appear concurrently with the first experiments based on defined oligodeoxynucleotide sequences containing donors and acceptors at fixed distances.[15,16] The efficiency and dynamics of EET are currently being characterized with steady-state and time-resolved optical spectroscopy.[7,17,18] Simultaneously, EET is being examined through its ability to promote chemistry at a distance from the site of initial electron injection. In one approach, EET is detected by its ability to monomerize a thymine dimer and, in a suitably prepared substrate, cause strand scission.[8,10,16,19] In another approach, EET is detected by reduction and decomposition of a bromodeoxyuridine (^{Br}U) residue that also leads to strand scission.[9,20] Observations based on both strategies very much depend on competition between reversible electron transfer (charge separation and recombination) and irreversible chemistry that ultimately causes strand scission.[8]

A number of donors have been designed for electron injection at the primary acceptors of T and possibly C.[8,12,13,15,16,18,20−22] Each method has the potential to differ in its sensitivity to local environment, orientation with respect to nucleobase stacking, and innate kinetic and thermodynamic properties. Both dihydroflavin[16] and a ketyl radical[8] have been applied in a site specific manner to inject an electron into DNA, and both were successful at driving cleavage of distal thymidine dimers. Our laboratory has developed a complementary method of electron injection based on an aromatic amine as described below. This class of electron donors offers a broad array of variants that are readily available through commercial and synthetic procedures and supports the potential for aerobic study. Aromatic amines are frequently employed in organic photochemistry as electron donors, although they are typically used as a ground-state donor to an excited-state acceptor.[23] This approach has also been extended to nucleotide and DNA studies[24] and is distinct from use of aromatic amines as excited-state electron donors.[20,25]

Results and Discussion

Our criteria for selecting a series of excited-state electron donors for EET in DNA included easy preparation and conjugation to oligodeoxynucleotides (ODN), an excitation wavelength well resolved from that of DNA, and a potential to operate in the presence of molecular oxygen. In addition, a moderate reduction potential in the ground state was desirable to avoid difficulties during synthetic manipulation, yet a high reduction potential in the excited state was necessary to inject an electron to any of the four nucleotides (T, −2.42; C, −2.59; A, −2.76; G, < −3.00 V vs SCE).[26] Beginning with the simplest aromatic amines, *N*,*N*-dimethylaniline (DMA) could be elaborated synthetically and provide sufficient reducing power (Table 1) as demonstrated by its earlier use in thymine dimer cleavage by electron donation.[25] However, the absorbance maximum of this chromophore is not sufficiently red-shifted to avoid the possibility of direct photoexcitation of DNA. In contrast, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) exhibits the necessary absorbance characteristics but might be easily oxidized in the ground state. Moreover, neither candidate would likely stack within a DNA helix to promote efficient electron transfer. An alternative with a strong possibility for intercalation, *N*,*N*,*N*′*N*′-tetramethyl-9,10-diaminoanthracene (TMDA), was therefore considered. However, this compound did not exhibit a sufficient reduction potential in its excited state to donate an electron to the nucleobases (Table 1). Preliminary experiments also revealed its facile decomposition to 9,10-anthraquinone.[27] This undesirable side reaction is consistent with a report indicating that donation of a second electron from TMDA is more favorable than donation of its first.[28] Finally, three bicylic aryldiamines, *N*,*N*,*N*′,*N*′-tetramethylbenzidine (TMB), *N*,*N*,*N*′,*N*′-tetramethyl-1,5-diaminonaphthalene (TMDN), and 1,5-diaminonaphthalene (DAN), were selected for evaluation as possible electron donors for EET in DNA.

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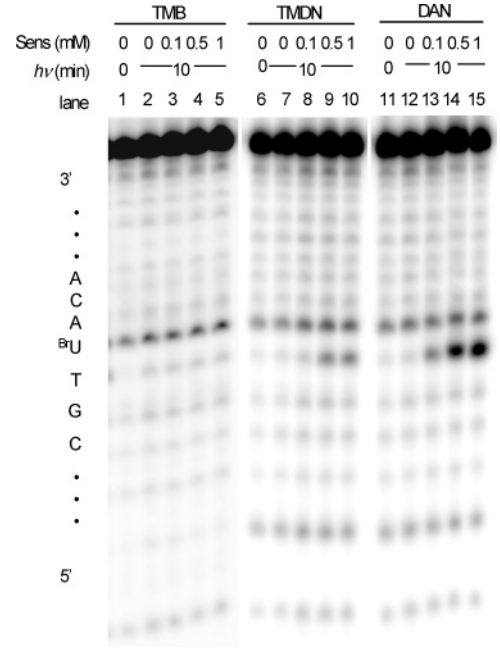


Figure 1. Phosphoimage of 20% denaturing polyacrylamide gels showing strand scission induced by EET from nonconjugated aromatic amines to ODN **2′**/ODN **2**. 5′-[^{32}P]-Labeled ODN **2** (1.0 μM) was annealed with ODN **2′** (1.6 μM) in 10 mM sodium phosphate (pH 7.0) and 90 mM NaCl. The samples were photoirradiated (λ > 335 nm) for the indicated periods in the alternative presence of TMB (lanes 2−5), TMDN (lanes 7−10), and DAN (lanes 12−15) under anaerobic conditions and then reacted with 10% piperidine at 90° C for 30 min.

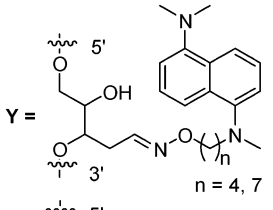
Chart 1. Structure and Sequence of Oligodeoxynucleotides

X = 5-bromo-2'-deoxyuridine

Sequence (5' to 3')

1 GCA GTA CGT TAC ATG ACG
2 GCA GTA C**G**T **X**AC ATG ACG
3 GCA GTA C**C**T **X**AC ATG ACG
4 GCA GTA C**T**T **X**AC ATG ACG
5 GCA GTA C**A**T **X**AC ATG ACG

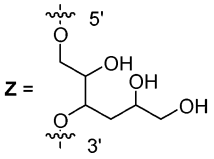
Y =



Sequence (3' to 5')

1' CGT CAT G**Y**A ATG TAC TGC
2' CGT CAT G**Z**A ATG TAC TGC

Z =



Intermolecular Electron Donation. The ability of the selected amines to serve as electron donors was measured in a functional assay prior to designing appropriate oligodeoxynucleotide conjugates. Initial studies demonstrated that duplex DNA containing ^{Br}U was sensitive to irradiation in the presence of TMDN but only if an abasic site within the helix was also present.[20] Reduction of ^{Br}U as detected by strand cleavage was inhibited in a fully complementary helix lacking a proximal binding site for the sensitizer to associate, and presumably stack, with the helix. Accordingly, ODN **2′**/ODN **2** (Chart 1) was irradiated (λ > 335 nm) in the alternative presence of TMB, TMDN, and DAN (Figure 1). A noticeable background cleavage of DNA was evident in all samples due to the innate lability of ^{Br}U under piperidine treatment. This result was independent of sensitizer and time of irradiation as expected. The consequences of EET were evident by strand cleavage at the 5′-neighbor (T) of ^{Br}U.[29] Both TMDN and DAN promoted such cleavage (Figure 1, lanes 9, 10, and 13−15). In contrast, no cleavage was induced by the presence of TMB (Figure 1, lanes 3−5).

Successful cleavage of ODN **2′**/ODN **2** after irradiation and piperidine treatment signifies the dual ability of the sensitizers to associate with the abasic site and donate an electron to the DNA. Neither property alone would adequately support electron injection by the diffusible aromatic amines. The excited state of TMB certainly has the reducing power to donate an electron to DNA (Table 1). Thus, its inability to promote EET is likely a function of its weak binding to DNA or lack of stacking within the abasic site. Previous analysis of the interactions between benzidine and DNA suggested weak binding in the minor groove primarily and only partial intercalation secondarily.[30] Stacking rather than groove binding has already been shown to be critical for efficient HT,[5f,31] and EET is expected to exhibit a similar dependence.

Our assay based on noncovalent association with DNA containing an abasic site and ^{Br}U residue allowed rapid identification of two candidates for further study on distance and sequence dependence of EET. The greater activity of DAN vs TMDN is consistent with their relative reducing power in the excited state (Table 1), although contributions from binding and stacking cannot be excluded without further study. To date, our characterization of EET has focused on derivatives of TMDN despite its weaker activity in this simple screen. This choice avoided possible complications due to a reported (but slow) depurination of DNA promoted by certain arylamines including DAN.[32]

Design and Synthesis of an Oligodeoxynucleotide−TMDN Conjugate. While the noncovalent screen represented the most expedient method for identifying electron donors of interest, detailed analysis of EET in DNA requires a covalent oligodeoxynucleotide conjugate. Only a covalent system could ensure fixed distances between an electron donor and acceptor. The intermolecular model above was readily converted for intramolecular studies by oxidation of ODN **2′** with periodate to yield ODN **3′** containing a deoxyribose residue as described previously.[20,33] This residue establishes an equilibrium between its furanose and acyclic forms. The latter presents an aldehyde for selective condensation with a variety of reagents.[34] Use of an abasic site also provided the possibility for an aromatic appendage to stack within the duplex at the site of covalent attachment.[34b,35] This minimizes the diversity of likely orientations between DNA and the chromophore and concurrently stabilizes stacking for efficient electron injection. Finally,

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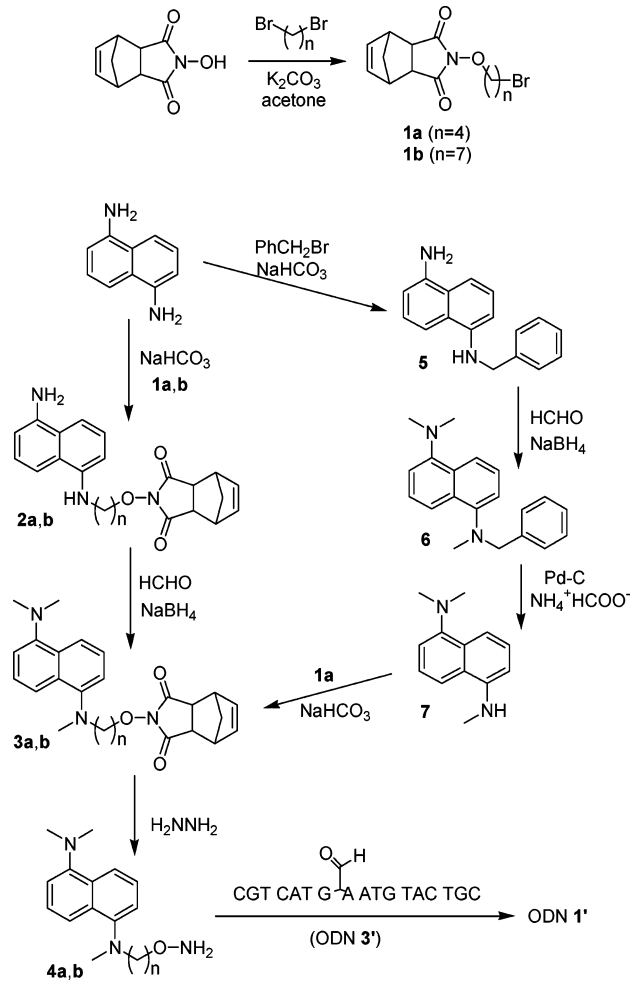
Scheme 1



selecting an internal (rather than terminal) position within duplex DNA for linking the chromophore creates a system that is amenable to study inter- and intrastrand transfer of an electron in 3′-5′ and 5′-3′ directions.[9]

Preliminary attempts to couple an alkylamine derivative of TMDN by reductive amination to the abasic aldehyde-containing **ODN 3′** under standard conditions were unsuccessful.[34b,e]

Subsequently, aminooxy derivatives were designed to enhance the potential for condensation with the aldehyde.[34a,f] Two linker lengths were chosen for initial evaluation, since length can greatly influence the nature and activity of oligodeoxynucleotide conjugates as illustrated during investigation of HT[31] and earlier development of antisense reagents.[36] One derivative containing four methylene units between the aminooxy and naphthylamine groups was used to generate a relatively restricted conjugate, and an alternative containing seven methylene units was used to allow greater conformational freedom.

The most effective strategy for preparing the TMDN−oligodeoxynucleotide conjugates began with alkylation of 1,5-diaminonaphthalene by the appropriately protected bromoalkyl derivatives **1a,b** (Scheme 1). Methylation and deprotection of the resulting products **2a,b** under standard conditions yielded the desired aminooxy intermediates (**4a,b**). Alternative protection of 1,5-diaminonaphthalene (**5**) followed by methylation and deprotection yielded an intermediate **7** that did not react efficiently with the bromoalkyl derivative **1a** (Scheme 1). Condensation of **4** and ODN **3′** likely formed both cis and trans oxime isomers. These did not resolve during reversed-phase HLPC purification of the products ODN **1′**$_4$ (n = 4) and ODN **1′**$_7$ (n = 7), and no additional separations were attempted. Each product was confirmed by ESI-MS and fluorescent properties of the conjugates were comparable to those of the nonconjugated derivative TMDN (emission maximum at 440 nm with excitation at 330 nm) (Figure 2). Only the fluorescence quantum yield decreased approximately 5-fold after coupling the chromophore to ODN **3′** (Supporting Information).

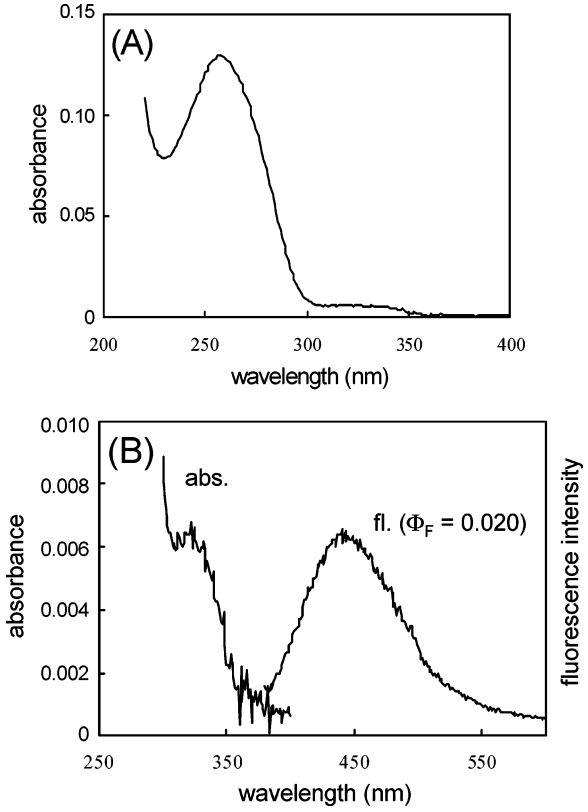


Figure 2. (A) UV absorption and (B) fluorescence spectra of ODN **1′**$_4$ (1.0 μM) in sodium phosphate (10 mM, pH 7.0) and NaCl (90 mM) at ambient temperature. Fluorescence was observed using photoexcitation at 330 nm.

Electron Transfer within Duplex DNA From Covalently Bound TMDN (n = 4, 7) to a ^{Br}U Residue. The TMDN conjugate ODN **1′**$_7$ induced piperidine lability at the residue (T9) immediately preceding ^{Br}U after irradiation (λ > 335 nm) in a duplex with ODN **2** in a manner similar to that observed previously for the duplex of ODN **1′**$_4$ and ODN **2**.[20] The extent of this strand scission was dependent on the duration of irradiation as expected for a photochemically induced electron injection into the helical DNA and EET to ^{Br}U (Figure 3). No equivalent scission was observed in the absence of the sensitizer (Figure 1, lanes 1, 6, and 11) nor was such scission detected previously in the absence of the ^{Br}U residue (ODN **1**/ODN **1′**$_4$).[20] The only other site of piperidine lability was again due to the intrinsic instability of ^{Br}U under the combination of heat and alkaline conditions. An additional product also appeared to form during irradiation as evident from the accumulation of a material migrating more slower than the parent oligodeoxynucleotide

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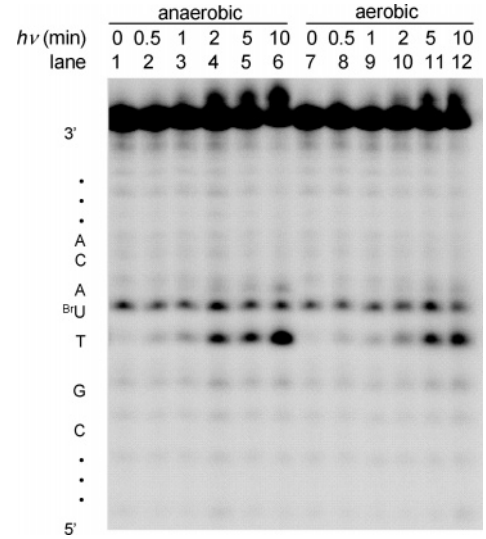

Figure 3. Phosphoimage of a 20% denaturing polyacrylamide gel showing strand scission induced by EET in ODN **1′$_7$**/ODN **2**. 5′-[^{32}P]-Labeled ODN **2** (1.0 μM) was annealed with ODN **1′$_7$** (1.6 μM) in 10 mM sodium phosphate (pH 7.0) and 90 mM NaCl. Samples were photoirradiated (λ > 335 nm) for the indicated periods under anaerobic (lanes 1−6) and aerobic (lanes 7−12) conditions alternatively and then treated with 10% piperidine at 90° C for 30 min.

(Figure 3, lanes 4−6, 11, and 12). The structure of this species is currently under investigation and may result from an addition reaction to the intermediate uridinyl radical that becomes competitive with the more common intrastrand hydrogen atom abstraction leading to strand cleavage.[37]

The TMDN conjugates ODN **1′$_4$** and ODN **1′$_7$** were considerably more efficient than free TMDN at initiating EET to ^{Br}U in ODN **2**. Nearly a 100-fold excess of TMDN could still not rival the activity of a 1.6-fold excess of conjugate ODN **1′$_7$** (Figures 1 vs 3). At least for the conjugated system, EET appears to be mediated by the intervening nucleobases of duplex DNA. Photoinduced reduction of ODN **2** by ODN **1′$_4$** is not affected by the presence of nitrous oxide (saturated) used to quench diffusible, hydrated electrons,[20] and EET is dependent on the nucleotide sequence and polarity (3′-5′ vs 5′-3′) separating the electron donor and ^{Br}U acceptor.[9] Most significantly, ambient concentrations of molecular oxygen did not inhibit the overall process by more than 20% (n = 4) and 31% (n = 7) (Figure 4). Aerobic conditions also did not induce oxidative degradation of the oligodeoxynucleotide system, or at least no new sites of alkaline lability were detected after irradiation in the presence of O_2 (Figure 3). Other systems developed to date for study of EET rely on anaerobic conditions.

While an initial assay based on noncovalent association between potential electron donors and ODN **2′**/ODN **2** provided an expedient screen to identify candidates for electron injection, the ultimate efficiency of these donors depends on the linkage used for their covalent attachment to DNA. For example, the conjugate containing a bridge of four methylene groups (ODN **1′$_4$**) supported a greater extent of EET than the comparable conjugate containing a bridge of seven methylene groups (ODN **1′$_7$**) (Figure 4). This difference in activity cannot easily be ascribed to selective denaturation of DNA structure, since both

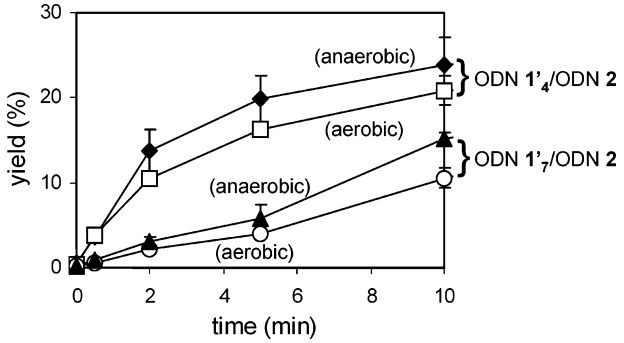

Figure 4. Effect of linker length on excess electron transfer under anaerobic and aerobic irradiation (λ > 335 nm). The indicated duplexes with the TMDN derivative attached through four and seven methylene units were treated under standard conditions (10 mM sodium phosphate, pH 7.0, 90 mM NaCl). Yields were calculated from the individual intensity of strand cleavage at the residue directly 5′ to ^{Br}U and related to the total intensity determined for each sample by gel electrophoresis. Each point represents an average value from no less than two independent experiments, and the indicated uncertainty represents the range of individual determinations.

strands exhibit very similar melting temperatures with ODN **2** (T_m = 53° C and 54° C for n = 4 and 7, respectively). Interestingly, the fluorescence emission maximum of ODN **1′$_7$**/ODN **2** (454 nm) was slightly red-shifted when compared to ODN **1′$_4$**/ODN **2** (444 nm) and was also subject to less quenching in the presence of ODN **2** (Φ_F = 0.018 vs 0.015 for ODN **1′$_7$** vs ODN **1′$_4$**). The excess methylene groups in ODN **1′$_7$** likely interfere with an optimal orientation of the TMDN derivative for productive electron injection into the helical DNA. The greater activity of ODN **1′$_4$** does not support the possibility that a longer linker would shift the site of intercalation toward the electron acceptor ^{Br}U. If this had occurred, then the longer linker of ODN **1′$_7$** might have enhanced rather than attenuated EET.

Electron Transfer in the Presence of Exogeneous Reductants. Little information is yet available on the irreversible processes that compete with EET and ^{Br}U reduction after electron injection. The weak inhibition of EET observed under aerobic conditions suggests that at least one such process is dependent on O_2 or the general oxidizing conditions of air-saturated buffer. For complementary HT, O_2 has already been proposed to trap transient radical cations formed by A and G,[6,38] and water appears to act analogously as well.[39] Although this latter trapping is unavoidable, anaerobic or reducing conditions may prevent O_2-dependent quenching. A strong reducing agent dithionite has been used to generate the necessary dihydro form of an oligodeoxynucleotide−flavin conjugate in one of the first studies on EET in DNA.[16] These conditions may also help to avoid certain quenching processes that could have affected the aerobic results of Figure 4. Similar reducing conditions were therefore tested with ODN **1′$_4$**/ODN **2**. Strand scission based on electron injection by the TMDN derivative and reduction of ^{Br}U was indeed slightly stimulated by the presence of 50 mM sodium dithionite after brief irradiation (<2 min) but then inhibited upon prolonged irradiation (2−10 min exposure) (Supporting Information).

Another reductant, glutathione, was recently shown to trap an intermediate ketyl radical that would otherwise inject an

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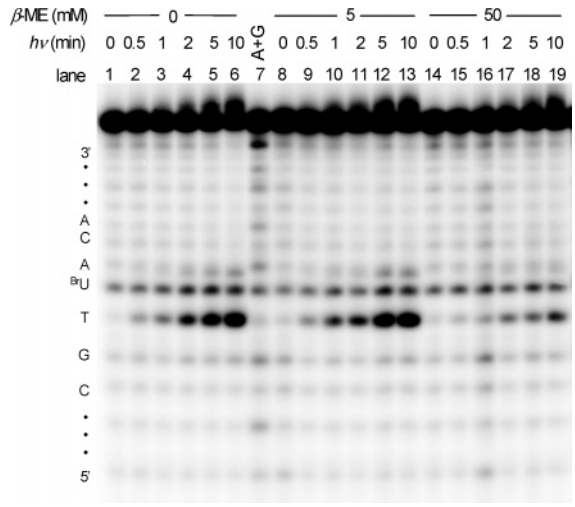

Figure 5. Effect of 2-mercaptoethanol on excess electron transfer. ODN **1′$_4$**/ODN **2** (1.0 μM) in 10 mM sodium phosphate (pH 7.0) and 90 mM NaCl was irradiated in the absence (lanes 1−6) or presence of 5 mM (lanes 8−13) or 50 mM (lanes 14−19) 2-mercaptoethanol (βME). Lane 7, A + G sequencing.

electron into duplex DNA.[8] Although the TMDN derivative is not expected to exhibit an equivalent sensitivity, the uridyl radical formed spontaneously after EET might be trapped by reduction or addition reactions.[40] Addition of 50 mM 2-mercaptoethanol as a substitute for glutathione almost completely suppressed strand scission during irradiation of ODN **1′$_4$**/ODN **2** (Figure 5). In contrast, the presence of a 10-fold lower concentration of this thiol did not affect the yield of strand scission. Collectively, electron injection and EET detection have both demonstrated a sensitivity to reductants, but no evidence yet suggests that EET itself is also sensitive to reductants.

Electron Injection and Fluorescence Quenching as a Function of the Nucleobase Counter to the Attached TMDN Derivative. The nucleobase environment of duplex DNA surrounding the bound TMDN derivative was expected to influence its photochemical properties based on related studies with pyrene[18b] and fluorene[41] conjugates. Fluorescence emission from these conujugates was particularly susceptible to the adjacent nucleobases (intrastrand) and counternucleobases (interstrand). Our efforts to date have focused on perturbations caused by the counternucleobase. This residue is contained within the same DNA strand as ^{Br}U, the probe for EET, and has the possibility of serving as the primary acceptor of the injected electron.

Fluorescence of the TMDN conjugate ODN **1′$_4$** exhibited the same relative susceptibility to quenching by the counterbase (C > T > G > A, Table 2) as that observed for the bimolecular model of TMDN and free nucleosides (Supporting Information). Although the fluorescence quantum yield of TMDN was greatly reduced when linked to a single-stranded oligodeoxynucleotide ODN **3′** (Figure 2 and Supporting Information), little additional quenching was caused by the presence of certain complementary sequences. For example, the fluorescence yield of ODN **1′$_4$** decreased by less than 20% in the presence of ODN **5** containing A counter to the TMDN derivative. The parallel trends in quenching of TMDN and its DNA bound derivative suggest that this chromophore may interact similarly with free nucleotides and the counterbase in duplex DNA. Such an interaction appears to be accommodated within the helical structure, since the various counterbases do not significantly perturb the thermal denaturation of this structure (Table 2). For a much larger chromophore such as phenanthridinium, the counterbases are thought to adopt an extrahelical position and do not affect fluorescence yields.[42]

Table 2. Effect of the Counterbase on Rate of ^{Br}U Reduction and Strand Cleavage (v_i), Thermal Melting (T_m), and Fluorescence Quantum Yield (Φ_F)

DNA	counterbase	v_i (% min^{-1})	T_m (°C)	Φ_F
ODN **1′$_4$**−ODN **5**	A	14.8	52	0.017
ODN **1′$_4$**−ODN **2**	G	9.2	53	0.015
ODN **1′$_4$**−ODN **4**	T	1.3	52	0.009
ODN **1′$_4$**−ODN **3**	C	0.2	54	0.004

Perhaps surprisingly, the extent of fluorescence quenching of ODN **1′$_4$** inversely correlates to the effectiveness of EET in duplexes differing only by their nucleobase counter to TMDN (Table 2). This inverse relationship may at first seem contradictory if both processes rely on electron injection into duplex DNA. However, each activity may simply be controlled by different steps within a multistep process involving electron injection, back electron transfer, charge neutralization, EET to reduce ^{Br}U, and ultimately oxidation of the DNA phosphoribose backbone.

Fluorescence quenching depends on the efficiency of initial charge separation and should be independent of back transfer and further charge diffusion. In contrast, our detection of EET is the consequence of all productive and competing processes identified above. The duplexes compiled in Table 2 share a common sequence surrounding the ^{Br}U residue, and thus the chemistry of this nucleobase and its transient uridinyl radical likely remain constant. However, differences in the counterbase are expected to influence both forward and back electron transfer. The C and T counterbases of ODN **3** and ODN **4** may offer highly favorable electron acceptors to quench fluorescence as well as efficient channels for back electron transfer to suppress EET. In contrast, the G and A residues of ODN **2** and ODN **5** offer less favorable acceptors but possibly more favorable partitioning between forward and back transfer. Alternatively, electron injection may avoid the counterbases G and A and directly reduce the T residue that is adjacent to both the counterbase and ^{Br}U. Back electron transfer and charge separation in both cases is expected to respond independently as they have in a number of other systems representing both EET and HT in DNA.[29d,43] Back electron transfer can even dominate charge transfer in DNA as illustrated recently with thionine. This sensitizer did not promote irreversible oxidation of guanine, since charge recombination involving the transient guanine radical cation was more efficient than alternative trapping by water or molecular oxygen.[43b]

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Conclusion

Study of EET in DNA requires strategies for both electron injection and transfer-related detection. Discovery of TMDN as a useful electron donor emerged from a narrow set of criteria used to select potential aromatic amines that might stack within duplex DNA, absorb light above 330 nm, and act as an excited-state electron donor. A DNA duplex containing ^{Br}U for detection of EET and an abasic site for interaction with potential electron donors provided a convenient system for identifying candidates for photoinduced electron injection. Covalent conjugation of TMDN to an equivalent duplex was used in turn to illustrate additional variables affecting electron injection. The length of the linker used to attach TMDN to DNA and the nucleobase counter to the bound TMDN both modulate the efficiency of EET as detected by ^{Br}U reduction and subsequent strand scission. Additional characterization of a series of oligodeoxynucleotide−TMDN conjugates has begun to reveal important information on the distance, directionality, and sequence dependence of EET in DNA.[9,20] Discovery of related electron donors such as DAN will allow for future investigations on the relationship between electron injection, EET, and driving force potential.

Experimental Section

General Methods. NMR spectra were recorded on a Bruker AM400 spectrometer (^{1}H, 400 MHz; ^{13}C, 100 MHz) and referenced to residual protons in the solvents. Chemical shifts (δ) are reported in parts per million (ppm), and coupling constants (J) are reported in hertz (Hz). Melting temperatures (T_m) were measured with a Varian Cary 100 UV−vis spectrophotometer, and profiles were measured in 0.5 °C increments with constant monitoring at 260 nm. T_m values were determined for each duplex by calculating the first derivative of the A_{260} profile.

Emission spectra were obtained with a Amico-Bowman Series 2 luminescence spectrometer. Fluorescence quantum yields (Φ_F) were determined from absorbance at 330 nm and integrated intensity of the background corrected fluorescence in comparison to a reference of 10 mM quinine sulfate in 0.5 M H_2SO_4 ($\Phi_F = 0.546$) with a solvent refractive index correction. The optical density of all solutions was about 0.01 at the wavelength of excitation. Since the voltage of the detector was kept constant during those measurements, no further corrections were performed. An error of <10% is estimated for these fluorescence quantum yields. Photoirradiation was performed using a high-pressure Xe-arc (LH151, Spectral Energy Co.) equipped with a LPS255HR power supply (1 kW) and a cutoff glass filter (WG335, Schott). Unless indicated, all samples were purged with N_2 gas before irradiation.

General Materials. Oligodeoxynucleotides were obtained commercially (TriLink BioTechnologies and Midland Certified Reagent), and the TMDN−oligodeoxynucleotide ODN **1′$_4$** was prepared following procedures described in a preliminary communication.[20] TMDN and TMDA were synthesized according to literature procedures.[44] Unless indicated, all other compounds were obtained from commercial sources and used without further purification. Solvents were distilled prior to use, and all aqueous solutions were prepared with water purified to a resistivity of 17.8−18.0 MΩ.

***N*-(7′-Bromoheptyl)-5-norbornene-2,3-dicarboximide (1b).** *N*-Hydroxy-5-norbornene-2,3-dicarboximide (Acros, 0.445 g, 2.5 mmol) in 15 mL of acetone was added dropwise under N_2 to a solution of 1,7-dibromoheptane (2.53 g, 9.8 mmol, Aldrich) and K_2CO_3 (15 g, 8.4 mmol) in dry acetone (5 mL). The resulting mixture was refluxed under N_2 for 19.5 h and cooled. Solid material was then removed by filtration, and the filtrate was concentrated under reduced pressure. The remaining residue was separated by silica gel flash chromatography (hexanes−ethyl acetate 1:0 to 1:1). The desired product was obtained in 86% yield (766 mg). ^{1}H NMR ($CDCl_3$) δ 6.12 (m, 2H), 3.90 (t, 2H, J = 6.52 Hz), 3.39 (m, 2H), 3.37 (t, 2H, J = 6.86 Hz), 3.15−3.14 (m, 2H), 1.82 (m, 2H), 1.73 (m 1H), 1.63 (m, 2H), 1.47 (m, 1H), 1.43−1.28 (m, 6H); ^{13}C NMR δ 172.5, 134.7, 77,5, 51.6, 44.9, 42.8, 34.1, 32.8, 28.5, 28.1, 28.1, 25.5. HRMS (FAB) *m*/*z* calcd for $C_{16}H_{22}BrNO_3$ 355.0783, found 356.0855 ($M + H^+$).

Alkylation of 1,5-Diaminonaphthalene To Form Its Protected Aminooxy Derivative (2b). The bromide (**1b**, 569 mg, 1.6 mmol) in ethanol (20 mL) was added dropwise to a mixture of 1,5-diaminonaphthalene (792 mg, 0.50 mmol, Aldrich) and $NaHCO_3$ (505 mg, 6.0 mmol) in ethanol−water (2:1, 25 mL) at reflux under N_2 atmosphere. The solution was stirred at 75 °C under N_2 for 19.5 h. The solvent was then removed by evaporation, and the residue was resuspended in water (15 mL). The resulting mixture was acidified to ∼pH 6 and extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The remaining solid was separated by silica gel flash chromatography (hexane−ethyl acetate 4:1 to 1:1). The desired product was obtained in 48% yield (333 mg, 0.77 mmol). ^{1}H NMR ($CDCl_3$) δ 7.33−7.15 (m, 4H), 6.76−6.74 (m, 1H), 6.60 (d, 1H, J = 7.52 Hz), 6.14 (m, 2H), 4.25 (br, 2H), 3.92 (t, 2H, J = 6.62 Hz), 3.40 (m, 2H), 3.23 (t, 2H, J = 7.11 Hz), 3.15−3.14 (m, 2H), 1.78−1.38 (m, 12H); ^{13}C NMR δ 172.5, 144.1, 142.9, 134.8, 125.9, 125.3, 124.4, 124.4, 110.8, 110.1, 109.9, 104.8, 77.4, 51.6, 44.9, 44.5, 42.8, 29.4, 29.2, 28.2, 27.3, 25.7. HRMS (FAB) *m*/*z* calcd for $C_{26}H_{31}N_3O_3$ 433.2381, found 434.2433 ($M + H^+$).

Methylation of 2b To Form 3b. A mixture of the amine (**2b**, 333 mg, 0.77 mmol) and $NaBH_4$ (291 mg) in dry THF (10 mL) was slowly added dropwise to a solution of 3 M H_2SO_4 (0.5 mL) and 37% formaldehyde (0.64 mL, Fisher) at 4 °C. The pH of the mixture was carefully maintained below pH 4 during addition with 3 M H_2SO_4. The mixture was then immediately diluted with water, adjusted to pH ∼6 with 1 N $NaHCO_3$, and extracted with ether. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The remaining solid was separated by silica gel flash chromatography (hexanes−ethyl acetate 4:1 to 6:4). The desired product was obtained in 89% yield (325 mg, 0.68 mmol). ^{1}H NMR ($CDCl_3$) δ 7.91 (d, 2H, J = 8.28 Hz), 7.38−7.34 (m, 2H), 7.066−7.023 (m, 2H), 6.13 (m, 2H), 3.89 (t, 2H, J = 6.75 Hz), 3.40 (m, 2H), 3.15−3.14 (m, 2H), 3.03−2.99 (m, 2H), 2.86 (s, 6H), 2.80 (s, 3H), 1.74−1.29 (m, 12H); ^{13}C NMR δ 172.7, 151.6, 151.4, 1345.0, 131.5, 130.7, 125.3, 119.5, 119.3, 115.8, 114.2, 77.6, 57.6, 51.8, 45.8, 45.1, 45.0, 42.9, 31.4, 29.6, 28.4, 27.9, 27.4, 25.9. HRMS (FAB) *m*/*z* calcd for $C_{29}H_{37}N_3O_3$ 475.2835, found 476.2886 ($M + H^+$).

Deprotection of 3b to *N*-(7-Aminooxyheptyl)-*N*,*N*′,*N*′-trimethyl-1,5-diaminonaphthalene (4b). A solution of **3b** (8.3 mg, 0.017 mmol) and anhydrous hydrazine (1.2 mg, 0.038 mmol) in ethanol (1.5 mL) was refluxed under N_2 for 2 h. The solvent was removed under reduced pressure, and the residue was separated by silica gel flash chromatography (1% methanol in CH_2Cl_2). The desired product was isolated as a viscous liquid in 46% yield (2.6 mg). ^{1}H NMR ($CDCl_3$) δ 7.93 (d, 1H, J = 3.99 Hz), 7.90 (d, 1H, J = 4.01 Hz), 7.37 (dd, 1H, J = 3.75, 7.44 Hz), 7.35 (dd, 1H, J = 3.72, 7.49 Hz), 7.06 (d, 1H, J = 7.48 Hz), 7.04 (d, 1H, J = 7.48 Hz), 5.30 (s, 2H), 3.61 (t, 2H J = 6.66 Hz), 3.02 (t, 2H, J = 7.53 Hz), 2.87 (s, 6H), 2.81 (s, 3H), 1.60−1.51 (m, 4H), 1.30−1.29 (m, 6H); ^{13}C NMR δ 151.3, 151.2, 131.3, 130.5, 125.1, 119.3, 119.1, 115.6, 114.0, 76.4, 57.5, 45.6, 42.7, 29.6, 28.6, 27.7, 27.3, 26.2. HRMS (FAB) *m*/*z* calcd for $C_{20}H_{31}N_3O$ 329.2467, found 329.2477.

***N*-Benzyl-1,5-diaminonaphthalene (5).** 1.5-Diaminonaphthalene (1.58 g, 10 mmol) in ethanol−water (1:1, 200 mL) was briefly refluxed

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and then maintained at 50 °C over 2.5 h under N_2 while a solution of $NaHCO_3$ (2.53 g) and benzyl bromide (858 mg, 5.0 mmol) in ethanol−water (1:1, 150 mL) was added dropwise. The resulting solution was refluxed under N_2 for 16 h, cooled to room temperature, concentrated to ∼100 mL under reduced pressure, acidified to pH 4 with 6 N HCl, and finally extracted with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and then evaporated under reduced pressure. The residue was separated by silica gel flash chromatography (hexanes−ethyl acetate 5:1 to 2:1), and the desired product was isolated as a brown solid in 40% yield (509 mg, 2.0 mmol). ^{1}H NMR ($CDCl_3$) δ 7.50−7.23 (m, 9H), 6.82 (dd, 1H, J = 6.80 and 1.54 Hz), 6.66 (d, 1H, J = 7.50 Hz), 4.53 (s, 2H); ^{13}C NMR δ 144.1, 143.2, 140.0, 129.1, 128.1, 127.8, 126.0, 125.6, 124.6, 124.6, 110.1, 110.5, 110.3, 105.3, 49.0. HRMS (FAB) *m*/*z* calcd for $C_{17}H_{16}N_2$ 248.1313, found 248.1308.

***N*-Benzyl-*N*,*N*′,*N*′-trimethyl-1,5-diaminonaphthalene (6).** A mixture of the amine **5** (498 mg, 2.0 mmol) and $NaBH_4$ (831 mg) in dry THF (2.0 mL) was added dropwise to a solution of 3 M H_2SO_4 (1.5 mL) and 37% formaldehyde (1.8 mL) at 4 °C. The pH of the reaction mixture was carefully maintained below pH 3 by addition of 3 M H_2SO_4. Reaction was then immediately quenched by addition of water (25 mL) and sufficient NaOH (solid) to raise the pH to ∼8. The resulting solution was extracted with ethyl acetate, and the organic layer was washed with $NaHCO_3$, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The dark residue was separated by silica gel flash chromatography (hexanes−ethyl acetate 1:9 to 4:1), and the desired produced was isolated in a 95% yield (523 mg, 1.9 mmol). ^{1}H NMR ($CDCl_3$) δ 7.93 (d, 1H, J = 8.55 Hz), 7.82 (d, 1H, J = 8.57 Hz), 7.29−7.18 (m, 7H), 6.91−6.96 (m, 2H), 4.11 (s, 2H), 2.74 (s, 6H), 2.61 (s, 3H); ^{13}C NMR δ 151.5, 150.8, 139.2, 130.9, 130.5, 128.6, 128.5, 127.2, 125.4, 125.2, 119.6, 118.9, 115.7, 114.2, 61.8, 45.6, 42.0. HRMS (FAB) *m*/*z* calcd for $C_{20}H_{22}N_2$ 290.1783, found 290.1769.

***N*,*N*,*N*′-Trimethyl-1,5-diaminonaphthalene (7).** Ammonium formate (543 mg, 5 equiv) and 10% Pd−C (503 mg) were added to a solution of the amine **6** (500 mg, 1.7 mmol) in dry methanol (10 mL) under N_2 at reflux. The mixture was maintained at reflux for 5 min, diluted with methanol (20 mL), and filtered through a Celite column. The pale pink filtrate was evaporated and extracted with CH_2Cl_2 and H_2O. The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The residue was separated by silica gel flash chromatography (hexanes−ethyl acetate 1:0 to 5:1), and the desired product was isolated in 65% yield (224 mg, 1.1 mmol). ^{1}H NMR ($CDCl_3$) δ 7.64 (d, 1H, J = 8.54 Hz), 7.47 (d, 1H, J = 8.48 Hz), 7.40−7.32 (m, 2H), 7.07 (d, 1H, J = 7.45 Hz), 6.60 (d, 1H, J = 7.30 Hz), 4.39 (br, 1H), 3.00 (s, 3H), 2.88 (s, 6H); ^{13}C NMR δ 151.3, 144.9, 129.5, 125.8, 124.7, 124,6, 114.5, 114.0, 113.4, 103.8, 45.2, 31.1. HRMS (FAB), *m*/*z* calcd for $C_{13}H_{16}N_2$ 200.1313, found 200.1304.

Alkylation of 7 To Form 3a. A solution of **7** (20 mg, 0.10 mmol), *N*-(4-bromobutyl)-5-norbornene-2,3-dicarboximide[20] (31 mg, 0.10 mmol), and $NaHCO_3$ (26 mg) in ethanol−water (2:1, 7.5 mL) was stirred at 50 °C under N_2 overnight. Solvent was then evaporated, and the residue was resuspended in water. This aqueous mixture was extracted with CH_2Cl_2. The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The final residue was separated by silica gel flash chromatography (hexanes−ethyl acetate 1:0 to 1:1) to yield a mixture of the desired product and starting bromide (∼1:5) as indicated by ^{1}H NMR in comparison to the spectra of authentic samples.

Conjugation of 4b and ODN 3′. Periodate oxidation of ODN **2′** and condensation with **4b** to generate ODN **1′$_7$** followed the conditions used to form its analogue, ODN **1′$_4$**, described previously.[20] The conjugate was similarly purified by reversed-phase (C-18) HPLC and isolated in a 37% yield based on absorbance at 260 nm. ESI-MS *m*/*z* for ($M - 3H^+$)/3: calcd 1896.7; found 1896.3.

Photolysis and PAGE Analysis of DNA Conjugates. ODNs **2**−**5** containing ^{Br}U (Midland Certified Reagent Co.) were alternatively radiolabeled at their 5′-termini by use of [γ-^{32}P]ATP (Amersham Bioscience) and T4 polynucleotide kinase (New England Biolabs) following standard procedures. Duplex DNA (0.1−1.0 μM) containing the radiolabeled strand and 1.6 equiv of the TMDN-containing strand was annealed in N_2-saturated buffer (10 mM sodium phosphate, pH 7.0, 90 mM NaCl) by heating to 90 °C followed by slow cooling to room temperature. Samples (10 μL each) were irradiated in microcentrifuge tubes under anaerobic conditions in a N_2 bag at ∼10 °C. DNA was precipitated with 3 M sodium acetate (pH 5.5) and ethanol, treated with 10% piperidine at 90 °C for 30 min, and then dried under reduced pressure. Samples were resuspended in loading buffer (8 M urea, 40% sucrose, 0.025% bromphenol blue, 0.025% xylene cyanol FF) and applied to a 20% denaturing polyacrylamide gel (acrylamide−bisacrylamide 19:1, 7 M urea). Following electrophoresis, strand scission was quantified relative to the total material in each sample by phosphorimagery and its analysis using ImageQuant software (Molecular Dynamics, Sunnyvale, CA).

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Supporting Information Available: Absorption and fluorescence spectra of TMDN, quenching of TMDN fluorescence by nucleotides, effect of added reductant on the yields of EET, fluorescence spectra of ODN **1′$_4$** in the alternative presence of ODNs **2**−**5**, rate of ^{Br}U reduction, and strand cleavage in duplexes formed by ODN **1′$_4$** and ODNs **2**−**5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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