

## Dynamics and Energetics of Single-Step Hole Transport in DNA Hairpins

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**Abstract:** The dynamics of single-step hole transport processes have been investigated in a number of DNA conjugates possessing a stilbenedicarboxamide electron acceptor, a guanine primary donor, and several secondary donors. Rate constants for both forward and return hole transport between the primary and secondary donor are obtained from kinetic modeling of the nanosecond transient absorption decay profiles of the stilbene anion radical. The kinetic model requires that the hole be localized on either the primary or the secondary donor and not delocalized over both the primary and the secondary donor. Rate constants for hole transport are found to be dependent upon the identity of the secondary donor, the intervening bases, and the location of the secondary donor in the same strand as the primary donor or in the complementary strand. Rate constants for hole transport are much slower than those for the superexchange process used to inject the hole on the primary donor. This difference is attributed to the larger solvent reorganization energy for charge transport versus charge separation. The hole transport rate constants obtained in these experiments are consistent with experimental data for single-step hole transport from other transient absorption studies. Their relevance to long-distance hole migration over tens of base pairs remains to be determined. The forward and return hole transport rate constants provide equilibrium constants and free energies for hole transport equilibria. Secondary GG and GGG donors are found to form very shallow hole traps, whereas the nucleobase deazaguanine forms a relatively deep hole trap. This conclusion is in accord with selected strand cleavage data and thus appears to be representative of the behavior of holes in duplex DNA. Our results are discussed in the context of current theoretical models of hole transport in DNA.

### Introduction

The migration of positive charge (holes) can occur over distances of several dozen base pairs in duplex B-DNA.<sup>1–3</sup> Hole migration is of current interest as it relates to both oxidative strand cleavage in DNA<sup>4</sup> and the development of molecular electronic devices.<sup>5</sup> Experimental evidence for long-distance hole migration in DNA is based primarily upon strand cleavage studies in duplexes possessing multiple guanine-containing sites.<sup>1–3</sup> Oxidative cleavage displays base-sequence selectivity, occurring selectively at guanines possessing purine versus pyrimidine neighboring bases, GG sequences being more reactive than GA sequences.<sup>6,7</sup> Increased selectivity is observed for cleavage at GGG sequences.<sup>8–11</sup> This selectivity has been

attributed to the formation of delocalized hole traps at GG and GGG sequences.<sup>12</sup> Even greater selectivity is observed for cleavage at sites containing 7-deazaguanine or 8-oxoguanine, bases which have substantially lower ionization potentials than does G.<sup>13</sup>

The mechanism for charge migration over long distances in DNA is the subject of continuing experimental and theoretical study. The most widely adopted model is a multistep hole hopping mechanism initially proposed by Schuster.<sup>14</sup> Jortner<sup>15</sup> and Giese<sup>16</sup> have suggested that holes migrate via a random walk between sites localized on G, GG, or GGG. An alternative mechanism in which delocalized holes migrate via a polaronlike

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mechanism has been advocated by Schuster<sup>17</sup> and by Conwell.<sup>18</sup> In either mechanism, hole migration is assumed to be more rapid than reactions of the oxidized nucleobases with water. This is the first step in a series of chemical reactions leading to the formation of base-labile sites which, following treatment with hot piperidine, can be located by means of gel sequencing experiments.

Strand cleavage studies have provided information about the relative rates of hole migration versus reaction with water as a function of base sequence. Both Schuster<sup>19</sup> and Barton<sup>3</sup> have found that strand cleavage initiated by photoinduced electron transfer in B-DNA containing multiple GG sites separated by four to eight base pairs displays a shallow dependence upon the distance between the appended oxidant and the GG site. Giese<sup>16</sup> has employed an ingenious method of photochemical cleavage of a nucleotide derivative to irreversibly oxidize a carbohydrate electron donor and study the competition between its reaction with water and single-step and multiple-step charge migration processes. Combining strand cleavage results with kinetic data reported from our laboratory, Giese has calculated rate constants for single-step charge migration as a function of the electron donor, nature of the bridge, and length of the bridge and has also modeled the cleavage patterns in more complex duplexes containing multiple G sites and a single GGG site.<sup>20</sup> Nakatani et al.<sup>11</sup> have reported that the efficiency of hole transport between two GGG sites separated by a TTXTT sequence is highly dependent upon the identity of the base X.

The strand cleavage studies of Giese, Schuster, Barton, Saito, and their co-workers have attracted considerable attention from theoreticians interested in hole transport processes.<sup>21</sup> In fact, the number of theoretical papers on DNA hole transport dynamics has far outpaced the output of experimentalists. In many cases, theoretical predictions have preceded the availability of direct experimental measurements of hole transport dynamics and equilibria and thus need to be revisited in light of recent experimental data.

There are but a handful of reports of direct measurements of the dynamics for hole transport between electron donors (hole acceptors) separated by nucleobases in DNA. Shafirovich et al.<sup>22</sup> have investigated the distance dependence of hole transfer from the cation radical of 2-aminopurine to guanine separated by variable numbers of thymine or adenine bases on the microsecond time scale by means of two-photon ionization followed by transient absorption. Kawai and Majima<sup>23</sup> have measured rate constants on the microsecond time scale for hole transport from a G-rich base sequence to pyrene across a T:A base-paired sequence consisting of two to four base pairs by means of pulse radiolysis with time-resolved transient absorption detection. The short-time resolution of both experiments is limited by the time required for scavenging of the solvated electron. Thus, their studies have been limited to relatively slow hole transport processes.

We have employed femtosecond time-resolved transient absorption spectra in hairpin-forming, bis(oligonucleotide) conjugates possessing a stilbenedicarboxamide linker (**Sa**, Chart 1, conjugates **1a–f**) to investigate the dynamics of charge separation (hole injection) and charge recombination (Figure 1a).<sup>24,25</sup> The singlet state of **Sa** can photooxidize guanine but none of the other natural nucleobases. Increasing the number of nonreactive A:T base pairs separating the **Sa** hairpin linker from a single G:C base pair allowed for the first systematic investigation of the distance dependence of electron-transfer dynamics in a DNA conjugate.<sup>26</sup> Analysis of the sequential decay of the **Sa** singlet and anion radical, **Sa<sup>•-</sup>**, provided the rate constants for both charge separation and charge recombination. It occurred to us that the introduction of a second electron donor might result in hole transport from the primary G to the secondary donor, with a concomitant increase in the **Sa<sup>•-</sup>** decay time (Figure 1b). By combining transient absorption spectroscopy on the femtosecond to microsecond time scale with kinetic modeling, we have proven for the first time that it is possible to obtain rate constants for both forward and return hole transport from G to the secondary donors GG, GGG, and Z as a function of hole transport distance, the nature of the intervening and adjacent bases, and the location of the secondary donor in either the same strand as the primary donor or its complement.<sup>27–29</sup> We report here the detailed results of this investigation, followed by a comparison of our results with experimental data from other laboratories and several current theoretical treatments of hole transport in DNA.

## Results

**Structure and Spectra.** Procedures for the synthesis and characterization of hairpin-forming bis(oligonucleotide) conjugates possessing stilbenedicarboxamide (**Sa**) linkers have been described.<sup>30,31</sup> These procedures were used to prepare the 26 **Sa**-linked hairpins shown in Chart 1. All of these conjugates form stable hairpin structures in aqueous solution containing 0.1 M NaCl and 30 mM sodium phosphate, pH 7.2 (standard buffer). The circular dichroism spectra of several of the hairpins have been obtained and display maxima near 280 and 220 nm and minima near 250 nm characteristic of B-DNA.<sup>32</sup> The thermal dissociation profile of **1a** provides a melting temperature  $T_M = 59$  °C. All of the other conjugates in Chart 1 possess additional G:C or Z:C base pairs and have melting temperatures too high to measure in standard buffer (>75 °C). Minimized structures for conjugates **1c** and **2b** obtained using the MM<sup>+</sup> force field within Hyperchem V5.01a are shown in Figure 2. These structures are similar to that determined by X-ray crystallography for a stilbene diether-linked hairpin.<sup>33</sup>

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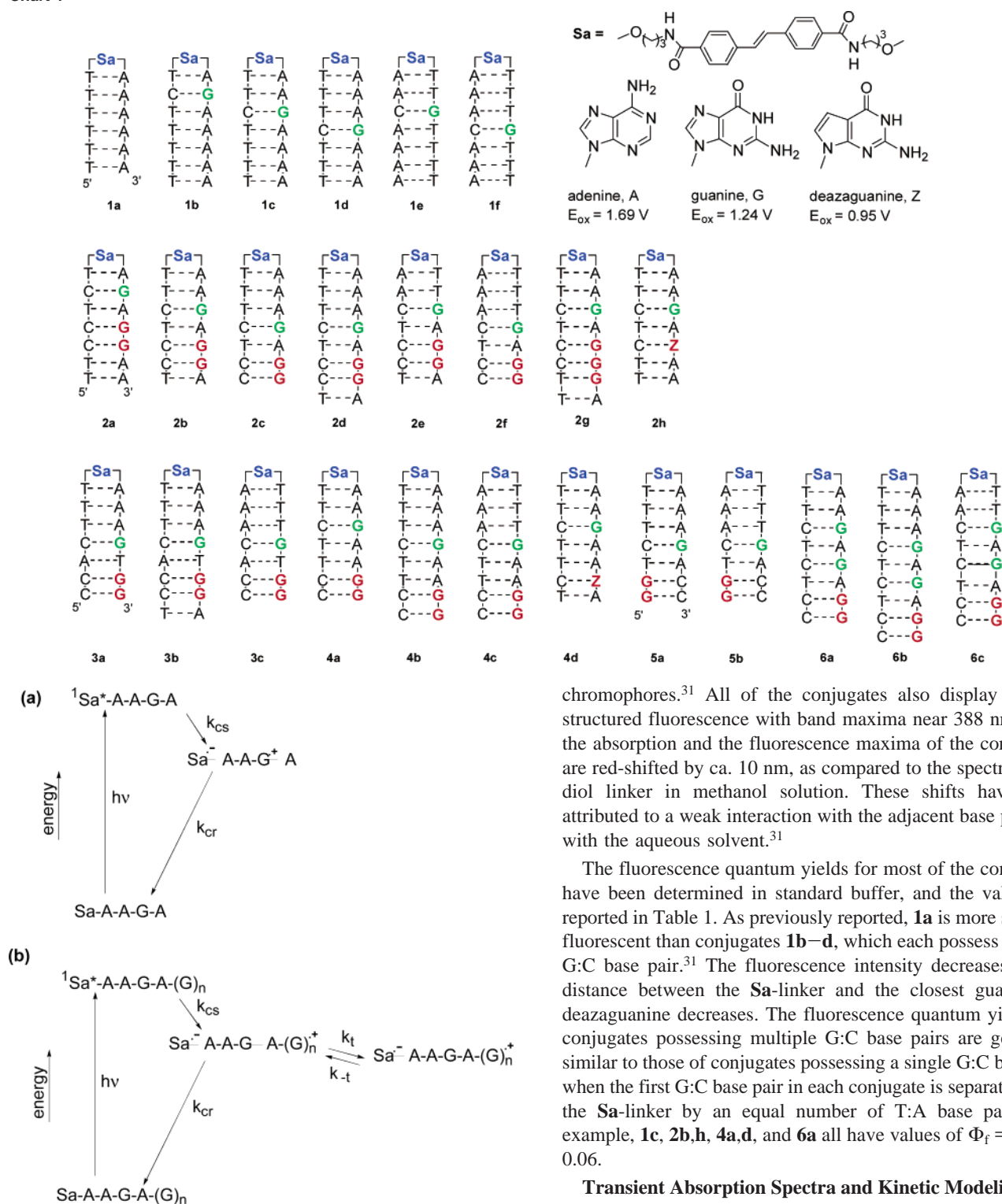
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Chart 1



**Figure 1.** Kinetic scheme for charge separation ( $k_{cs}$ ) and charge recombination ( $k_{cr}$ ) in (a) hairpins containing one or more guanines separated from the stilbenedicarboxamide linker (**Sa**) by two T:A base pairs and (b) hairpins which can also undergo hole transport ( $k_t$  and  $k_{-t}$ ) to a more distal site containing multiple guanines. Only the guanine-containing arm of the hairpin is shown.

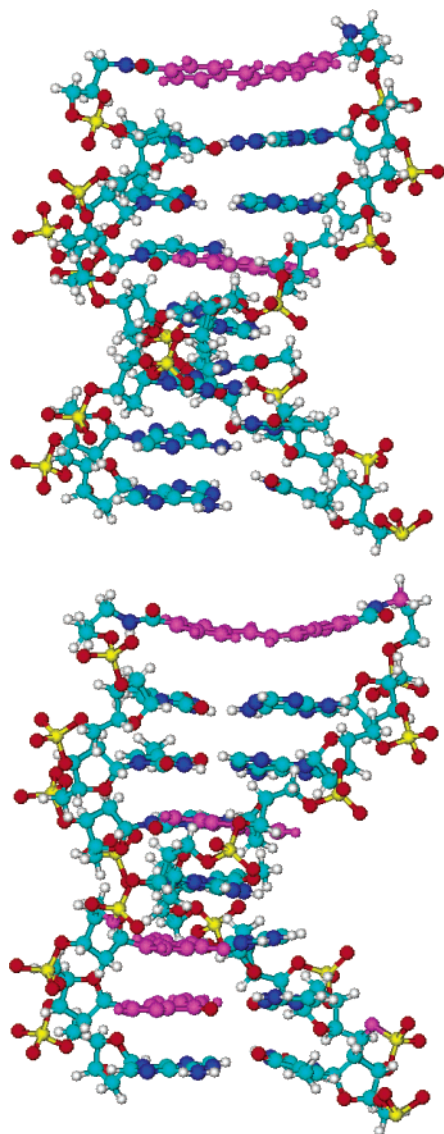
The absorption spectra of all of the conjugates display a long wavelength band with a maximum near 335 nm, attributed to the stilbene linker, and a second maximum near 260, attributed to overlapping absorption of the stilbene and nucleobase

chromophores.<sup>31</sup> All of the conjugates also display weakly structured fluorescence with band maxima near 388 nm. Both the absorption and the fluorescence maxima of the conjugates are red-shifted by ca. 10 nm, as compared to the spectra of the diol linker in methanol solution. These shifts have been attributed to a weak interaction with the adjacent base pair and with the aqueous solvent.<sup>31</sup>

The fluorescence quantum yields for most of the conjugates have been determined in standard buffer, and the values are reported in Table 1. As previously reported, **1a** is more strongly fluorescent than conjugates **1b–d**, which each possess a single G:C base pair.<sup>31</sup> The fluorescence intensity decreases as the distance between the **Sa**-linker and the closest guanine or deazaguanine decreases. The fluorescence quantum yields for conjugates possessing multiple G:C base pairs are generally similar to those of conjugates possessing a single G:C base pair when the first G:C base pair in each conjugate is separated from the **Sa**-linker by an equal number of T:A base pairs. For example, **1c**, **2b,h**, **4a,d**, and **6a** all have values of  $\Phi_f = 0.05–0.06$ .

**Transient Absorption Spectra and Kinetic Modeling.** The time-resolved transient absorption spectra of conjugates **1a–d** have been reported previously.<sup>31</sup> At short times following excitation with the ca. 110 fs, 330 nm laser pulse of a femtosecond amplified Ti-sapphire based laser system, the spectra resemble that of the singlet excited stilbene diamide linker. The transient spectrum of **1a** decays with a decay time of 2.0 ns, with little change in band shape. In contrast, the

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**Figure 2.** Structures for the hairpins **1c** and **2b**. The stilbenedicarboxamide linker (**Sa**, top of structure) and all guanines are darkened. Structures were calculated using the molecular mechanics force field (MM<sup>+</sup>) within Hyperchem V5.01a (Hypercube, Inc., Gainesville, FL). Local minima were optimized assuming normal B-form DNA geometries.

transient spectra of **1b–f** display a time-dependent change in band shape with a decay time  $\tau_s$  attributed to formation of the anion radical of the stilbene diamide linker **Sa**<sup>•−</sup>. The decay of the **Sa**<sup>•−</sup> anion radicals of **1b–f** can be fit to a single exponential with a decay time  $\tau_a$ , attributed to charge recombination with the nucleobase donor cation radical.<sup>31</sup> In the case of **1d** and **1f**, the charge recombination process is too slow to be measured with the femtosecond apparatus ( $\tau_a > 5$  ns), requiring the use of a nanosecond laser system based on a frequency tripled Nd:YAG laser (355-nm excitation, 9-ns total instrument response). The decay times for **1b–f** assigned to the formation and decay of **Sa**<sup>•−</sup>,  $\tau_s$  and  $\tau_a$ , are reported in Table 1.

The formation and decay of **Sa**<sup>•−</sup> in conjugates **1b–f** can be described in terms of a single-step, bridge-mediated electron-transfer mechanism (Figure 1a) in which G or Z serves as an electron donor separated from the **Sa** acceptor by a variable number of T:A base pairs which serve as a bridge, B. The rate constants for charge separation can be calculated from the singlet

decay times,  $\tau_s$ , and the decay time for the hairpin **1a**,  $\tau_o$ , which does not undergo electron-transfer quenching ( $k_{cs} = \tau_s^{-1} - \tau_o^{-1}$ ). Rate constants for charge recombination can be obtained directly from the anion radical decay times ( $k_{cr} = \tau_a^{-1}$ ). Values of  $\tau_s$  and  $\tau_a$  have previously been reported for conjugates **1b–d** and for conjugates related to **1e,f**, with reversed strand polarity.<sup>31</sup> The increase in decay times with increasing bridge length has been analyzed to provide the distance dependence,  $\beta$ , of the charge separation and charge recombination processes.<sup>25</sup>

Conjugates in the series **2–6** (Chart 1) possess multiple G and/or Z bases and were designed to probe the occurrence of hole transport from the primary donor, proximal to the **Sa** acceptor, to a secondary donor (series **2–5**) or tertiary donor (series **6**). A kinetic scheme for hole transport from a primary to a secondary donor is shown in Figure 1b. For all cases in which the picosecond decay of these conjugates has been measured,  $\tau_s$ , the decay component assigned to charge separation, has a value which is similar to that of the corresponding conjugates in series **1**, which possess a single G donor at the same distance from the **Sa**-linker (Table 1). For example, the values of  $\tau_s$  for conjugates **2b,h** and **4d** are all similar to that for **1c** ( $31 \pm 9$  ps).

The nanosecond decays for conjugates in series **2–6** are either single or double exponentials. Decay constants and preexponentials are reported in Table 1. The single-exponential decay constants for conjugates **3a** and **4a,b** are similar to those for conjugates in series **1**, which possess a single G donor at the same distance from the **Sa**-linker. Evidently, hole transport does not compete effectively with charge recombination in these conjugates. In all cases for which dual exponential decay is observed, one component is similar to, or shorter than, that of the analogous conjugate in series **1**, and the second component is significantly longer. In a few cases, the **Sa**<sup>•−</sup> transient absorption does not decay completely to the baseline on the time scale of our measurements (ca. 0.1 ms).

Procedures for kinetic modeling of the transient decays using Mathcad 8 Professional<sup>34</sup> have been reported previously.<sup>28</sup> The kinetic modeling protocol requires that two **Sa**<sup>•−</sup> decay components can be resolved and that the shorter-lived component be comparable to or shorter than the value of  $\tau_a$  determined for the corresponding hairpin **1b–f**, with a single donor base. Kinetic modeling of the decay data for conjugates **6a–c** is not feasible due to the larger number of kinetic processes for hairpins capable of two reversible hole transport processes. Typical errors for each of the variable kinetic parameters for fast hole transport (**2f**) and slow hole transport (**4c**) are reported in the footnotes to Table 1.

**Hole Transport Dynamics.** Conjugates **2a–h** possess a primary G donor separated from a secondary GG, GGG, or Z electron donor by a single adenine base, A. In the case of **2a–d,g,h**, the primary donor is separated from the **Sa** acceptor by a (T:A)<sub>n</sub> sequence which is reversed in the case of **2e,f**. For all of these conjugates, the values of  $\Phi_f$  and  $\tau_s$  are similar, within the experimental error ( $\pm 25\%$ ), to those of the analogous conjugates **1b–f** which possess the primary guanine donor in the same location (Table 1). This result indicates that the presence of the secondary donor does not strongly perturb the rate of the primary electron-transfer process. The **Sa**<sup>•−</sup> decay

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**Table 1.** Fluorescence Quantum Yields and Singlet and Radical Ion Decay Times for **Sa**-DNA Conjugates and Rate Constants Obtained from Kinetic Modeling

hairpin <sup>a</sup>	donor sequence <sup>a</sup>	$\Phi_f^b$	$\tau_s, \text{ns}^c$	$\tau_a, \text{ns}^d$	$10^{-7} k_{cr}^e$ $\text{s}^{-1}$	$10^{-7} k_t^e$ $\text{s}^{-1}$	$10^{-7} k_{-t}^e$ $\text{s}^{-1}$
<b>1a</b> <sup>f</sup>		0.38	2.0				
<b>1b</b> <sup>f</sup>	AGAAAAA	0.018	0.0039	0.094	1100		
<b>1c</b> <sup>f</sup>	AAGAAAA	0.050	0.031	1.9	53		
<b>1d</b> <sup>f</sup>	AAAGAAA	0.20	0.46	57	1.8		
<b>1e</b>	TTGTTTT	0.15	0.031	2.6	38		
<b>1f</b>	TTTGTTT	0.31	(0.93)	250	0.40		
<b>2a</b>	AGAGGAA	0.008	0.004	0.10	1000		
<b>2b</b>	AAGAGGA	0.05	0.039	1.5(77), 170(23)	60	5.6	0.75
<b>2c</b>	AAAGAGG	0.14	0.61	29(34), 233(66)	1.5	1.3	0.90
<b>2d</b>	AAAGAGGA			70(30), 470(60), sh (10)			
<b>2e</b>	TTGAGGA	0.13	0.059	2.1(57), 111(43)	37	10.2	1.2
<b>2f</b> <sup>g</sup>	TTTGAGG	0.28	1.1	60(9), 790(91)	0.40	0.84	0.54
<b>2g</b>	AAAGAGGGA		0.022	0.92(56), 295(44)	50	8.7	0.43
<b>2h</b>	AAGAZAA	0.06	0.037	0.82(45), 6000(55)	53	69	0.04
<b>3a</b>	AAAGTGG	0.17		66			
<b>3b</b>	AAAGTGGGA			70(84), 17 000(16)	1.4	0.04	0.006
<b>3c</b>	TTTGTGG	0.33	(1.1)	240(88), 1200(12)	0.41	0.017	0.045
<b>4a</b>	AAGAAGG	0.06		3.7	27		
<b>4b</b>	AAAGAAGG	0.14		55	1.8		
<b>4c</b> <sup>g</sup>	TTTGAAGG	0.33	(1.0)	220(88), 3600(12)	0.38	0.038	0.079
<b>4d</b>	AAGAAZA	0.06	0.026	1.2(86), sh(14)	53	5.0	$\leq 0.01$
<b>5a</b>	AAAGACC	0.17	(0.67)	53(84), 3200(16)	1.6	0.21	0.037
<b>5b</b>	TTTGACC	0.32	(1.1)	223(82), 4800(18)	0.29	0.10	0.033
<b>6a</b>	AAGAGAGG	0.05		3.6(92), 96(8)			<1.0
<b>6b</b>	AAAGAGAGG	0.12		65(62), 430(38)			<0.2
<b>6c</b>	TTGAGAGG	0.17		7.2(83), 112(13), 960(3)			<0.1

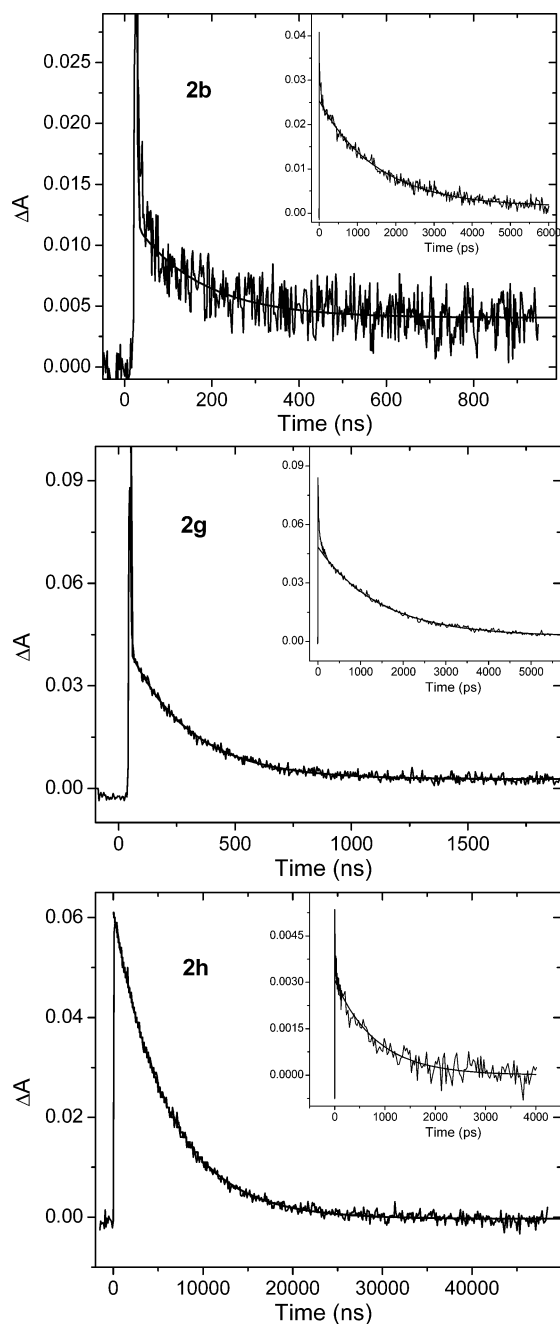
<sup>a</sup> See Chart 1 for conjugate structure and donor strand sequence. <sup>b</sup> Quantum yield for **Sa** fluorescence in aqueous solution. Missing data were not determined. <sup>c</sup> **Sa**: singlet state decay time determined either by picosecond time-resolved transient absorption spectroscopy or by nanosecond fluorescence decay measurements (data in parentheses). Missing data are not determined. <sup>d</sup> **Sa**<sup>•-</sup>: decay time determined by nanosecond time-resolved transient absorption spectroscopy (preexponentials in parentheses). <sup>e</sup> Rate constants determined by nonlinear modeling of the nanosecond decay data. <sup>f</sup> Data from ref 29. <sup>g</sup> Errors obtained from the fitting procedures for **2f**:  $k_{cr} 4.0 \pm 1.8$ ,  $k_t 5.4 \pm 1.5$ ,  $k_{-t} 8.4 \pm 3.0$ , and  $k_t/k_{-t} = 1.6 \pm 1.0$ . For **4c**:  $k_{cr} 3.8 \pm 0.3$ ,  $k_t 4.0 \pm 2.0$ ,  $k_{-t} 8.0 \pm 4.0$ , and  $k_t/k_{-t} = 0.5 \pm 1.0$ .

for conjugate **2a** is single exponential, with a decay time similar to that for conjugate **1b** (Table 1). This indicates that charge transport from G to GG is too slow to compete with charge recombination in the primary radical ion pair (Figure 1b,  $k_{cr} = 1.1 \times 10^{10} \text{ s}^{-1}$ ). The **Sa**<sup>•-</sup> decays for conjugates **2b–h** are dual exponential. The transient decay curves and fits obtained from kinetic modeling of conjugates **2b,g,h** are shown in Figure 3. Values of  $k_{cr}$ ,  $k_t$ , and  $k_{-t}$  obtained by kinetic modeling are reported in Table 1. Unfortunately, rate constants cannot be extracted from the decay data for **2d** because its short-lived nanosecond decay is longer than that of **1d**. The errors reported for **2e,f** are obtained from the fitting procedure and are typical of the results for hairpins with fast and slow hole transport, respectively. The values of  $k_t$  for **2b** and **2e**, which possess a terminal A:T base pair, are larger than those for **2c** and **2f**, which are lacking the A:T base pair. All of these conjugates have values of  $k_t \leq 1 \times 10^8$ . Assuming a similar value for conjugate **2a**, we found that the yield of hole transport would be ca. 1%, in accord with our failure to detect a long-lived **Sa**<sup>•-</sup> decay component.

Conjugate **2g**, which possesses a GAGGG hole transport sequence, has a value of  $k_t$  slightly larger than that of conjugate **2b** and a value of  $k_{-t}$  which is slightly smaller than that of **2b**. Conjugate **2h**, which possesses a GAZ hole transport sequence, has a significantly larger value of  $k_t$  than for any of the conjugates possessing GAGG hole transport sequences. The value of  $k_{-t}$  for **2h** is smaller than any of the conjugates with GAGG hole transport sequences. Thus, Z appears to be unique in its ability to promote hole transport reactions.

Conjugates **3a–c** possess a primary G donor separated from a secondary GG donor by a single thymine base, T. In the case of **3a**, which possesses a GTGG hole transport sequence, the decay of **Sa**<sup>•-</sup> is single exponential with a decay time similar to that for **1d**. Thus, there is no evidence for the occurrence of hole transport in this hairpin. Evidently, hole transport is too slow to compete with charge recombination ( $k_{cr} = 4 \times 10^6 \text{ s}^{-1}$  for **1d**). In the case of **3b**, which possesses an AAAGTGGGA hole transport sequence, kinetic modeling provides values for forward and return hole transport that are distinctly slower than those for conjugate **2c**, which possesses an AAAGAGGA sequence. This is also the case for **3c**, which possesses a TTTGTGG sequence, when compared to **2f**, which possesses a TTTGAGG sequence. The ratio of forward hole transport rates for **2c/3b** is 33 and for **2f/3c** is 49. The values of  $k_{-t}$  are also smaller for GTGG versus GAGG hole transport sequences.

Conjugates **4a–d** possess a primary G donor separated from a secondary donor by an AA sequence. The secondary donors for **4a–c** and **4d** are GG and Z, respectively. No long-lived **Sa**<sup>•-</sup> decay components are observed for **4a** or **4b**, and their short-lived decay components are similar to those of **1c** and **1d**, respectively. Thus, charge transport does not compete effectively with charge recombination in these conjugates. Low amplitude, long-lived **Sa**<sup>•-</sup> decay components are observed for **4c** and **4d**. Thus, hole transport competes to a small extent with charge recombination in these conjugates. The observation of hole transport for **4c** but not for **4b** (or **4a**) is consistent with the smaller value of  $k_{cr}$  for a TTTG versus AAAG (or AAG) sequence (cf. **1f** vs **1d**). In the case of **4c**, both  $k_t$  and  $k_{-t}$  can be obtained by means of kinetic modeling of the **Sa**<sup>•-</sup> decay.



**Figure 3.** Transient decay of conjugates **2b,g,h** monitored at 575 nm following a 5 ns, 355 nm excitation pulse. Inset shows transient decay following a 0.1 ps, 340 nm exciting pulse.

In the case of **4d**, the long-lived  $\text{Sa}^{\bullet-}$  component does not decay on the microsecond time scale. A value of  $k_{-t} < 10^5 \text{ s}^{-1}$  can be estimated either from the absence of microsecond decay or from the value of  $k_t$  and the equilibrium constant for hole transport between G and Z in conjugate **2h** (vide infra).

Conjugates **5a,b** possess a secondary GG donor in the hairpin strand complementary to the G primary donor. The primary and secondary donors are separated by a single T:A base pair. In both cases, the decay of  $\text{Sa}^{\bullet-}$  is dual exponential. Kinetic modeling provides values of  $k_t$  for **5a** and **5b** which are smaller than the values for intrastrand hole transport in conjugates **2c** and **2f**, respectively. The ratios of rates for intra- versus interstrand hole transport are  $2\text{c}/5\text{a} = 6.2$  and  $2\text{f}/5\text{b} = 8.4$ .

Conjugates **6a–c** possess a GAGAGG hole transport sequence containing three hole donors. Presumably, hole transport from the primary G donor to the tertiary GG donor requires involvement of the secondary G donor, rather than single-step hole transport from the primary G donor to GG. In the cases of **6b** and **6c** (but not **6a**), a  $\text{Sa}^{\bullet-}$  decay component is resolved with a decay time which is longer than that for the corresponding conjugates **2c** and **2e**, which possess a GAGG single-step hole transport sequence. The long decay times are consistent with the arrival of the hole on the tertiary GG donor in **6b,c**. However, the information provided by the experimental decay curves is insufficient to permit kinetic modeling of a process involving two reversible hole transport steps.

## Discussion

The combination of kinetic spectroscopy and kinetic modeling provides the first comprehensive data for the effects of B-DNA base sequence on the dynamics of single-step hole transport from a primary guanine donor to a secondary donor in DNA. These data permit analysis of the effects of the identity of the secondary donor, the identity and number of the intervening bases, and the position of the secondary donor in the same or opposite strands on hole transport. Our kinetic analysis is unique in providing rate constants for both forward and return hole transport, from which hole transport equilibrium data are available. There are, however, several limitations to our method. First, it is limited to relatively fast hole transport processes ( $k_t > 10^6 \text{ s}^{-1}$ ) which can compete with charge recombination of the primary donor and  $\text{Sa}^{\bullet-}$  acceptor radical ions. Second, because the guanine cation radicals cannot be detected via transient absorption spectroscopy, the method is dependent upon analysis of the behavior of the anion radical  $\text{Sa}^{\bullet-}$ . Third, it is unable to resolve the dynamics of processes involving multiple hole transport steps. We have recently detected the formation of the cation radical of a secondary stilbene diether donor formed by hole transport from guanine.<sup>35</sup> This result both establishes the occurrence of hole transport and potentially provides a method for investigating multiple hole hopping processes.

For hole transport to be competitive with charge recombination, the primary guanine donor in our studies must be separated from the  $\text{Sa}$  acceptor by two or three base pairs. Thus, the presence of the stilbene linker should not exert a pronounced influence on hole transport kinetics. The excellent agreement between our data and the other available studies of hole transport dynamics, as discussed below, indicates that our results are representative of the behavior of short segments of duplex B-DNA.

The discussion of our results begins with an analysis of the dynamics of single-step hole transport processes and a comparison to relevant kinetic and strand cleavage data from the literature. Next, the equilibrium data for hole transport between primary and secondary donors are discussed and compared with selected strand cleavage data. Finally, our experimental results are compared with theoretical calculations of hole transport dynamics and equilibria and with current models for the hole transport process.

**Dynamics of Hole Transport from G to GG.** The choice of GG sequences as secondary hole traps was based on the

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conventional wisdom from the strand cleavage literature<sup>4</sup> that G<sub>n</sub> sequences serve as “hole traps” and the use of GG sequences in Schuster’s<sup>19</sup> and Barton’s<sup>3</sup> studies of long-distance hole transport. Conjugates **2a–f** possess a GAGG hole transport sequence and were designed to explore the competition between charge recombination and hole transport in the photogenerated primary radical ion pair (Figure 1b). In all cases except **2a**, the presence of a long-lived decay component provides evidence for the occurrence of hole transport from G to GG. The observation of long-lived Sa<sup>•-</sup> decay for **2b** but not **2a** indicates that hole transport is sufficiently fast to compete with charge recombination in **2b** ( $k_{cr} = 6 \times 10^8 \text{ s}^{-1}$ ) but not in **2a** ( $k_{cr} = 1 \times 10^{10} \text{ s}^{-1}$ ).

Kinetic modeling of the nanosecond decay data according to the kinetic scheme in Figure 1b provides rate constants for charge recombination and forward and return hole transport for all of these hairpins except **2a** and **2d**. The values of  $k_{cr}$  are constrained by our model to be similar to those for the analogous hairpins **1b–f** which possess a single G donor. The value of  $k_t$  is slightly higher for the TTGAGGA sequence of **2e** than the AAGAGGA sequence of **2b**; however, the difference in  $k_t$  values is similar to the error inherent in our modeling procedure. Thus, the identity of the neighboring base in a XGAGGA sequence (X = A or T) has, at most, a minor effect on  $k_t$ . The values of  $k_t$  for sequences **2c** and **2f**, which are lacking a terminal A:T base pair, are smaller than those for **2b** or **2e**. This may reflect the ability of the terminal A:T base pair to stabilize a hole on the adjacent GG sequence. The values of  $k_{-t}$  for **2b,c,e,f** are similar.

The values of  $k_t$  for conjugates **2b** and **2e** are smaller than their values of  $k_{cr}$ , in accord with the smaller amplitude of the long-lived versus short-lived Sa<sup>•-</sup> decay components. Similarly, the values of  $k_t$  for conjugates **2c** and **2f** are comparable to their  $k_{cr}$  values, in accord with larger amplitudes for the long-lived decay component (Table 1). Assuming a value of  $k_t$  for conjugate **2a** similar to that for **2b**, we found that the yield of hole transport for **2a** would be <1%, consistent with the absence of a long-lived decay component (Table 1). Because the yield of charge injection in Sa-linked hairpins decreases rapidly when there are four or more A:T base pairs separating the Sa acceptor and G donor, the optimum yields of long-lived Sa<sup>•-</sup> are obtained for conjugates such as **2c,d,f**, which have three intervening A:T base pairs.

Conjugates **3a–c** have GTGG hole transport sequences and thus provide for a direct comparison of interstrand hole transport with GAGG sequence. No evidence for hole transport was observed for **3a**, even though efficient hole transport was observed for **2c**, which has a GAGG donor sequence. In the case of **3b,c**, low amplitude, long-lived Sa<sup>•-</sup> decay components were observed, indicating that hole transport occurs but is slower than charge recombination. Kinetic modeling provides a value of  $k_t = 4 \times 10^5 \text{ s}^{-1}$  for **3b** and a somewhat smaller value for **3c**, which lacks a terminal A:T base pair. Conjugate **3a** also lacks a terminal base pair. Assuming a value of  $k_t$  for **3a** similar to that for **3c**, we found that the efficiency of hole transport would be ca. 1%. Thus, it is not surprising that no long-lived decay component was resolved for this conjugate. Comparison of the values of  $k_t$  for **3b,c** with those for hairpins possessing GAGG sequences indicates that the hole transport via A is 30–40-fold more rapid than hole transport via T. The value of  $k_t$

for **3b** is in excellent agreement with the value of  $k_t = 5 \times 10^5 \text{ s}^{-1}$  reported by Shafirovich et al.<sup>22</sup> for hole transport from the cation radical of 2-aminopurine (AP) to GG in the sequence [AP]TGG.

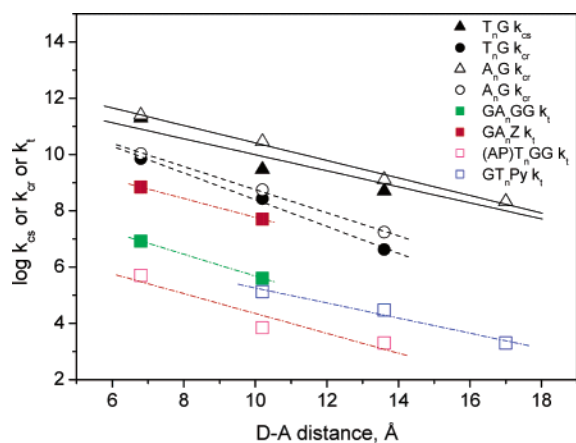
**Dynamics of Hole Transport from G to GGG and Z.** We observe a long-lived Sa<sup>•-</sup> decay component of moderate amplitude for conjugate **2g** which has a GAGGG donor sequence. Kinetic analysis provides a value of  $k_t = 8.7 \times 10^7 \text{ s}^{-1}$  for **2g**, which is only slightly larger than the value obtained for **2b**, which has a GAGG sequence. The value of  $k_{-t}$  for **2g** is slightly smaller than that for **2b**. Thus, GGG is only a slightly better hole trap than is GG. Giese<sup>16</sup> and Saito<sup>8,9</sup> have employed GGG sequences in their strand cleavage studies and assumed that they were deep hole traps. However, Nakatani et al.<sup>11</sup> observed that hole migration could occur between sites in the sequence GGGTTATTGGG and is more rapid than strand cleavage.

The electrochemical oxidation potential of the modified nucleobase 7-deazaguanine (Chart 1, Z) is ca. 0.3 V lower than that of G.<sup>36</sup> We observe a long-lived Sa<sup>•-</sup> decay component with a large amplitude for conjugate **2h**, which has a GAZ hole transport sequence. Kinetic analysis provides a value of  $k_t = 6.9 \times 10^8 \text{ s}^{-1}$ , which is larger than that for either GAGG or GAGGG hole transport sequences. The value of  $k_{-t} = 4 \times 10^5 \text{ s}^{-1}$  for **2h** is smaller than those for the latter sequences. The value of  $k_t$  is ca. 12 times faster than that for the GAGG sequence in **2b**. Recently, Giese and Biland<sup>13</sup> reported that the relative rate constant for hole migration in the sequences R<sup>+</sup>TZ versus R<sup>+</sup>TG, where R<sup>+</sup> is a photochemically generated oxidized deoxyribose, is 17 times faster for Z versus G. The agreement between our kinetic data and Giese’s strand cleavage data is reassuring. Nakatani et al.<sup>11</sup> have found that the presence of Z in the sequence GGGTTZTTGGG totally inhibits hole transport between the two GGG sites and that strand cleavage occurs at Z in preference to the initially oxidized GGG site. Thus, Z appears to form a much deeper hole trap than do GG or GGG.

**Distance Dependence of Hole Transport Dynamics.** Conjugates **4a–c** have GAAGG hole transport sequences and were designed to determine the dynamics of hole transport across an AA bridge. Only in the case of **4c**, which has the slowest charge recombination rate of these three conjugates, were we able to detect a long-lived Sa<sup>•-</sup> decay component. Kinetic analysis provides a value of  $k_t = 3.8 \times 10^5 \text{ s}^{-1}$ , which is 22-fold slower than the value for the GAGG sequence in **2f**. A similar value of  $k_t$  for **4a,b** would result in a hole transport efficiency of <2%. Conjugate **4d** has a GAAZ sequence, and thus hole transport would be expected to be more rapid and exergonic than that for a GAAGG sequence. A long-lived decay component was observed for conjugate **4d**; however, it did not decay appreciably on the microsecond time scale of our measurements. Kinetic modeling provided an estimated value of  $k_t = 5.0 \times 10^7 \text{ s}^{-1}$ , appreciably faster than the value for **4c**, but 14-fold slower than the value for the GAZ sequence in **2h**.

The distance dependence of our hole transport data for GA<sub>n</sub>-GG and GA<sub>n</sub>Z sequences ( $n = 1, 2$ ) is shown in Figure 4, along with our data for photoinduced charge separation and charge recombination in Sa-linked hairpins containing a single G:C base pair. Also shown in Figure 4 are the data of Shafirovich

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**Figure 4.** Distance-dependent kinetics for charge separation (solid lines) and charge recombination (dashed lines) of conjugates possessing a single G donor in a polyA sequence (empty symbols) or polyT sequence (filled symbols); and for hole transport (dot-dash line) from G to GG (red symbols), from G to Z (green symbols), from aminopurine to GG (pink symbols, data from ref 22), and from G to pyrene (blue symbols, data from ref 23).

et al.<sup>22</sup> for hole transport in  $[\text{AP}^+]\text{T}_n\text{GG}$  systems and the data of Kawai et al.<sup>23</sup> for hole transport in  $\text{G}^+\text{T}_n\text{Py}$  systems, where Py is a pyrene donor. There are several notable features of this figure. First, the slopes of the  $k_t$  plots are similar both to each other and to the slope obtained by Meggers et al.<sup>16</sup> from relative strand cleavage yields. Second, these slopes are similar to those for  $k_{cs}$  obtained in our earlier studies of photoinduced charge separation and in related studies from other laboratories.<sup>26,37,38</sup> Third, the values of  $k_t$  are distinctly smaller than the values of  $k_{cs}$  or  $k_{cr}$  at the same donor–acceptor distance. Fourth, the values of  $k_t$  are much smaller for T versus A bridges, whereas the differences are much smaller for  $k_{cs}$  and  $k_{cr}$ .<sup>31</sup> The theoretical basis for these observations will be discussed below.

**Dynamics of Inter- versus Intrastrand Hole Transport from G to GG.** Conjugates **5a,b** have GACC sequences in the hairpin arm containing the primary G donor and a complementary CTGG sequence in the other arm. This presents an interstrand G to GG hole transport sequence in which a single A:T base pair separates the primary and secondary donors. The observation of dual exponential nanosecond decay of  $\text{Sa}^{\bullet-}$  permits the use of kinetic modeling to obtain values of  $k_t$  and  $k_{-t}$  (Table 1). Comparison of the values of  $k_t$  for **5a,b** with those for **2c,f**, which have GAGG hole transport sequences, provides values for the ratio of inter- versus intrastrand hole transport for hairpins which are lacking a terminal A:T base pair. The resulting values of 6.2 and 8.4 indicate that there the kinetic penalty for cross strand hole transport is smaller than that for changes in the transport sequence from GAGG to either GTGG or GAAGG.

The initial hole transport studies of Meggers et al.<sup>16</sup> established that long-distance hole transport can occur via a hole transport sequence in which guanines are located in both strands of the hole transport region. Wagenknecht et al.<sup>39</sup> have also observed long-distance hole transport in duplexes containing

**Table 2.** Equilibrium Constants and Free Energy Changes for Hole Transport

hairpin <sup>a</sup>	donor sequence <sup>a</sup>	$K_{\text{ht}} = k_t/k_{-t}$ <sup>b</sup>	$-\Delta G_{\text{ht}}$ , eV <sup>c</sup>
<b>2b</b>	AAGAGGA	7.5	0.052
<b>2c</b>	AAAGAGG	1.4	0.008
<b>2e</b>	TTGAGGA	8.5	0.055
<b>2f</b>	TTTGAGG	1.6	0.012
<b>2g</b>	AAGAGGGA	20	0.077
<b>2h</b>	AAGAZAA	1700	0.19
<b>3b</b>	AAAGTGG	6.7	0.049
<b>3c</b>	TTTGTGG	> 0.5	> -0.02
<b>4c</b>	TTTGAAGG	> 0.5	> -0.02
<b>4d</b>	AAAGAAZA	> 500	> 0.16
<b>5a</b>	AAAGACC	> 5	> 0.043
<b>5b</b>	TTTGACC	> 3	> 0.028

<sup>a</sup> See Chart 1 for conjugate structure and donor strand sequence.

<sup>b</sup> Equilibrium constant for hole transport calculated using the values of  $k_t$  and  $k_{-t}$  from Table 1. <sup>c</sup> Free energy change for hole transport.  $\Delta G_{\text{ht}} = -RT(\ln K_{\text{ht}})$ .

guanines in both strands but assumed that hole transport occurred in a single strand. This assumption was based on a previous observation that intrastrand fluorescence intensity quenching of singlet aminopurine by G or Z is much more efficient than interstrand quenching.<sup>36</sup> To our knowledge, there are no direct comparisons of the dynamics of inter- versus intrastrand hole transport from other laboratories.

**Multistep Hole Transport.** Strand cleavage studies have amply demonstrated the occurrence of hole transport involving multiple G, GG, and GGG sites.<sup>1,40</sup> Conjugates **6a–c** were designed in the hopes of obtaining direct evidence for the occurrence of two sequential hops by means of transient absorption spectroscopy. Long-lived  $\text{Sa}^{\bullet-}$  decay components are in fact observed for **6b–c**, indicative of the occurrence of hole transport. Unfortunately, our modeling protocol cannot extract the five rate constants expected for two reversible hole transport steps and charge recombination from the transient decays. In each case, there is a long-lived component with a decay time similar to that for the corresponding conjugates with a single GAGG hole transport sequence (**2b,c,e**), but with a smaller amplitude. The smaller amplitude may reflect less efficient hole transport from a primary G donor to a secondary G versus GG donor. Only in the case of **6c** is there an additional  $\text{Sa}^{\bullet-}$  decay component of longer lifetime that might be assigned to charge recombination from GG. The low amplitude of this component indicates either that the efficiency of the two-hop process is very low or that we cannot observe the decay of the charge-separated species on the microsecond time scale of our transient experiments. Hole transport systems designed to provide more efficient multistep hole transport are currently under study in our laboratory.

**Hole Transport Equilibria.** Both forward and return hole transport rate constants are obtained from our kinetic modeling procedure and can be used to calculate the equilibrium constants and free energies for reversible hole transport (Table 2). Conjugates AAGAGGA (**2b**), TTGAGGA (**2e**), and AAAGTGG (**3b**) have values of  $K_{\text{ht}} = 7.5 \pm 1$  ( $\Delta G_{\text{ht}} = -0.052 \pm 0.03$  eV). Thus, the presence of T versus A bases on either side of the primary donor has little effect on the hole transport equilibrium. The small value of  $\Delta G_{\text{ht}}$  for G versus GG cation radicals is consistent with the small difference in rate constants for oxidation of G versus GG sequences in duplex DNA reported

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**Table 3.** Comparison of G, GG, and GGG Sequences: Relative Populations, Cleavage Yields, and Reactivities on a per-Sequence and per-G Basis

property of sequence	G	GG	GGG
population <sup>a</sup>	1	7.5	20
cleavage yield <sup>b</sup>	1	3	5
cleavage rate <sup>c</sup>	1	0.4	0.2
cleavage rate per G <sup>d</sup>	1	0.2	0.1

<sup>a</sup> Relative equilibrium population based on equilibrium data in Table 2.

<sup>b</sup> Consensus value for the relative cleavage yields in sequences containing G, GG, and GGG sites. <sup>c</sup> Cleavage rate = (cleavage yield)/population.

<sup>d</sup> Cleavage rate per G = (cleavage yield)/(population × number of G's per site).

by Sistare et al.<sup>41</sup> A somewhat larger value of  $K_{ht} = 20$  ( $\Delta G_{ht} = -0.077$  eV) is obtained for conjugate **2g**, which has a GAGGGA hole transport sequence. Smaller values,  $\Delta G \approx 0 \pm 0.02$  eV, are observed for conjugates with GAGG (**2c,f**), GTGG (**3c**), and GAAGG (**4c**) sequences, lacking a terminal A. Evidently, the energy of a hole on a terminal AGG site is similar to that of an internal AGA site. Thus, the data for **2c,f** provide an estimated value of  $10^7$  s<sup>-1</sup> for the rate constant for reversible hole transport in a GAG sequence. The increase in the value of the rate constants for hole transport from G to G, GG, and GGG (ca. 1:4.3:6.7) parallels the modest increase in hole stabilization energy.

The small stabilization energies for G versus GG or GGG are consistent with experimental strand cleavage data for short duplexes containing a limited number of G, GG, and GGG sites, which display a relatively low level of strand cleavage selectivity. Hickerson et al.<sup>10</sup> reported a ratio of 1:3.7:5.3 for strand cleavage at G, GG, and GGG sites using a chemical oxidant, and Nakatani et al.<sup>8</sup> reported a ratio of ca. 1:2:3.5 using a photochemical oxidant. Similarly, Muller et al.<sup>7</sup> report a ratio of ca. 1:2.5 for strand cleavage at AG versus GG sites, and Yoshioka et al.<sup>9</sup> report a ratio of ca. 1:2 for strand cleavage at GG versus GGG sites. From these data, a “consensus” ratio of 1:3:5 can be estimated for cleavage at G versus GG versus GGG. Thus, cleavage is less selective than would be expected on the basis of the equilibrium ratio of 1:7.5:20 provided by our equilibrium data. The ratio of relative cleavage yield to relative population provides a measure of the relative reactivity on a per-site basis of 1:0.4:0.2. Giese and Spichty<sup>20</sup> arrive at a similar ratio of 1:0.7:0.3, on the basis of their analysis of strand cleavage ratios for several duplexes. The agreement between these results, which were obtained by totally different methods, is reassuring. Bixon and Jortner<sup>42</sup> obtain a somewhat larger ratio for G versus GGG, 1:0.6, on the basis of their analysis of Giese's data. The relative reactivity on a per-G basis is, of course, even lower. Our data provide a ratio of 1:0.2:0.1 for reactivity at G, GG, and GGG sites on a per-G basis. These relationships are summarized in Table 3.

The small difference in G versus GG versus GGG hole stability and lower reactivity of the more stable sites is, of course, precisely the combination of stability and reactivity that is required for the observation of hole transport over long distances containing multiple G and GG sites. Hole transport between two GGG sites separated by a TTGTT sequence appears to be somewhat slower than cleavage at the initially

oxidized GGG site.<sup>11</sup> To our knowledge, the efficiency of strand cleavage in duplexes possessing multiple GGG sites has not been studied.

Kinetic modeling provides a significantly larger value of  $\Delta G_{ht} = -0.19$  eV for the hole transport sequence GAZAA in conjugate **2h** (Table 2). A similar value of  $\Delta G_{ht}$  can be estimated for the GAAZA sequence in **4d**. Thus, Z functions as a much deeper hole trap than does GG or GGG. The larger value of  $\Delta G_{ht}$  for Z versus GG is also consistent with the faster rate constants for both superexchange charge separation<sup>29,37</sup> and relative rates for hole transport from an oxidized sugar cation to Z versus GG.<sup>43</sup> Nakatani et al.<sup>11</sup> have reported that hole injection into the 5'-end of a 5'-GGGTTZTTGGG sequence results predominantly in cleavage at Z, with a lesser amount at the 5'-GGG and very little at the 3'-GGG. On the basis of our values of  $\Delta G_{ht}$  for hole transport from G to Z and GGG, the relative equilibrium population in a ZTTGGG sequence should be ca. 70:1. Assuming similar rates for the chemical reactions leading to strand cleavage, we found that cleavage at Z should predominate for this sequence. Our estimated value of  $\Delta G_{ht}$  is somewhat smaller than the measured difference in irreversible oxidation potentials of dZ versus dG (ca. 0.3 eV) or calculated ionization potentials of Z versus G (0.39 eV).<sup>11</sup>

#### Comparison of Hole Transport Equilibria with Theory.

The difference in stabilities of cation radicals on G, GG, and GGG sequences was initially addressed by Sugiyama and Saito,<sup>12</sup> who employed ab initio methods to calculate the gas-phase ionization potentials of nucleobases stacked in B-DNA geometries. Their results indicated large differences in potential for holes on G versus GG (0.47 eV) and GGG (0.68 eV) sequences. A similar G versus GG difference was calculated by Prat et al.<sup>44</sup> These values suggest that GG and GGG are, in fact, deep hole traps, and they have been widely cited as evidence to that effect.<sup>45,46</sup>

Voityuk et al.<sup>47</sup> employed semiempirical calculations to determine the effects of neighboring bases on the relative energies of holes localized on a single nucleobase. They find a difference in energy of 0.13 eV between AG<sup>+</sup>G and AG<sup>+</sup>A triples, substantially smaller than the earlier estimates. Recently, Kurnikov et al.<sup>48</sup> have reported that the inclusion of solvation energies has a “leveling effect” on the calculated (ab initio) gas-phase ionization potentials of G, GG, and GGG sequences imbedded in a poly-A sequence. Solvation stabilization is largest for a hole localized on a single G and decreases with increasing delocalization. The net effect is a modest stabilization energy (<0.1 eV) for GG versus G and an even smaller stabilization energy for GGG. These authors point out that both their calculated stabilization energies and our experimental results for the trap depths for G and GG are within  $\sim 2k_B T$  (where  $k_B$  is Boltzmann's constant). It is interesting to note that our experimental values for the relative energies of G, GG, and GGG (0, 0.052, and 0.077 eV) are in excellent agreement with a

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Hückel analysis, according to which the stabilization energy for GGG should be larger than that for GG by  $\sqrt{2}$ .<sup>49</sup>

Quantum calculations for the GAGG sequence by Barnett et al.<sup>50</sup> indicate that the solvated cation is strongly delocalized with similar charge densities on all three guanines. Conwell and Basko<sup>51</sup> have analyzed our results in terms of a delocalized polaron model and find the hole in a AAGAGGAA sequence is delocalized over about six bases, but it is largely confined by the polaron effect to GG. They obtained excellent agreement with our experimental equilibrium data using a value of 0.2 eV for the difference in ionization potentials of adjacent G and A. Thus, either a localized hole hopping mechanism, in which the energy of a localized hole is influenced by neighboring bases, or a polaron mechanism can account for our equilibrium data.

Our experimental results for conjugate **2h**, which possesses a GAZA hole transport sequence, provide a value of  $\Delta G_{\text{ht}} = -0.19$  eV. This value is substantially larger than the values for GAGGA and GAGGGA sequences, indicating that Z is a much deeper hole trap than are GG or GGG. As previously noted, this result is in accord with the results of strand cleavage studies.<sup>11,13</sup> The experimental value is smaller than the values of  $\Delta G_{\text{ht}}$  estimated from the difference in oxidation potentials of dG versus dZ (0.3 V)<sup>36</sup> or their calculated gas-phase ionization potentials (0.39 eV).<sup>11</sup> This apparent “leveling effect” is also observed when comparing the difference in oxidation potentials of dG versus dA (0.45 V) with the energy difference between holes localized on G versus A in B-DNA estimated by Bixon and Jortner<sup>52</sup> from their analysis of multistep hole hopping in DNA or by Conwell and Basko<sup>51</sup> in their polaron model (0.20 eV). In contrast, our studies of the driving force dependence of superexchange electron transfer (hole injection) from the nucleobase to singlet acceptors including **Sa** require a larger difference (ca. 0.45 eV) in G versus A oxidation potentials.<sup>53</sup> This difference can be accounted for by the involvement of the neutral nucleobase in the superexchange process and the oxidized nucleobase in the hole transport process. The relative energies of the oxidized nucleobases should be much more dependent upon interactions with neighboring bases and solvation than is the case for the neutral nucleobases, because solvent and structural relaxation is expected to be more rapid than hole transport (vide infra). Differences in solvation between the neutral and oxidized nucleobases can also account for the faster rates of hole injection versus hole transport, as discussed below.

**Comparison of Hole Transport Dynamics with Theory for G(A)<sub>n</sub>G Sequences.** Prior to our report of the hole transport rates for the GAGG sequence, Bixon et al.<sup>54</sup> estimated a value of  $k_t \approx 10^9$  s<sup>-1</sup> for hole hopping from G to G via two intervening AT base pairs. Incoherent hole transport in DNA has been estimated to occur on the picosecond time scale.<sup>55</sup> On the basis of the behavior of polarons in a vacuum, Conwell and

co-workers also predicted a picosecond time scale for hole motion; however, they recently pointed out that solvation would lead to a decrease in rate.<sup>56</sup> These estimated rates are comparable to our measured value for superexchange charge separation in the **SaAAG** sequence ( $k_{\text{cs}} = 3 \times 10^{10}$  s<sup>-1</sup>), but much faster than the value of  $k_t = 5.6 \times 10^7$  s<sup>-1</sup> for hole transport in GAGGA sequences or the value of  $k_t = 1 \times 10^7$  s<sup>-1</sup> for GAGG sequences lacking the terminal A. The latter number is our best estimate for isoenergetic GAG hole transport.

According to the Marcus theory, the rate constant for an electron-transfer process is described by eq 1

$$k_{\text{ET}} = \frac{2\pi V_{\text{DA}}^2 \exp(-(\Delta G + \lambda_s)/4\lambda_s k_{\text{B}}T)}{\hbar \sqrt{4\pi\lambda_s k_{\text{B}}T}} \quad (1)$$

where  $\Delta G^\circ$  is the free energy of reaction, and  $\lambda_s$  is the solvent reorganization energy.<sup>57</sup> A comparison of our kinetic data for charge separation, charge recombination, and hole transport is shown in Figure 4. For a fixed distance between hole donor and acceptor,  $k_{\text{cs}} > k_{\text{cr}} \gg k_t$ . The larger value of  $k_{\text{cs}}$  relative to that of  $k_{\text{cr}}$  is a consequence of the charge separation and recombination reactions occurring in the Marcus normal and inverted regions, respectively.<sup>53</sup> However, the much slower rate constants for hole transport require further consideration. The observation of much faster hole transport rates for GAZ than for GAGG sequences indicates that the rates of charge recombination and hole transport are dependent upon the driving force for the hole transport processes. The values of  $-\Delta G_{\text{ht}}$  for hole transport from G to GG and G to Z are 0.05 and 0.19 eV, respectively, considerably smaller than the value of  $\lambda_s = 0.41$  eV obtained in our study of the driving-force dependence of DNA bridge-mediated charge separation.<sup>53</sup> Thus, the hole transport processes are in the normal region, and the rates are expected to increase with increasing driving force. In contrast, charge recombination of the charge-separated **Sa<sup>-</sup>AAG<sup>+</sup>** is highly exergonic ( $-\Delta G^\circ \gg \lambda_s$ ), placing it deep in the inverted region. Using the same dielectric continuum model of solvation to describe the dependence of both  $\Delta G^\circ$ <sup>58</sup> and  $\lambda_s$ <sup>57</sup> on the dielectric constant of the medium, we have shown that the free energy at which the maximum rate of electron transfer occurs does not depend to a first approximation on the dielectric constant of the solvent.<sup>59</sup> Only the width of the rate versus free energy profile increases as the static solvent dielectric constant increases. On the other hand, for a charge shift reaction such as hole transport, the free energy at which the maximum rate of reaction occurs depends strongly on the solvent dielectric constant.<sup>60</sup> It is possible that the local dielectric environment that is created following formation of the initial radical ion pair produces an overall solvation environment, that is, a change in  $\Delta G^\circ + \lambda_s$ , that results in a decrease in the rate of subsequent hole transport.

There has been considerable recent interest among theoreticians in the distance dependence of the dynamics of hole

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transport. For  $G(A)_nG$  sequences, Jortner and co-workers<sup>52,61</sup> have proposed that hole transport from G to G occurs via a single-step superexchange process when  $n \leq 3$  or 4. Similar results have been obtained by Berlin et al.<sup>62</sup> For longer  $A_n$  sequences, these workers propose a change in mechanism to thermally induced hopping in which the  $A_n$  bridge is oxidized. Basko and Conwell<sup>63</sup> also predict a change in mechanism from tunneling to on-bridge motion for longer  $A_n$  bridges. Because our results pertain only to short distance hole transport ( $n = 1$  or 2), the latter mechanism is not relevant to our studies.

A superexchange mechanism is expected to display an exponential dependence of  $k_t$  on the distance separating the donor and acceptor R, as in eq 2

$$k_t = A \exp(-\beta R) \quad (2)$$

where  $\beta$  is determined by the donor–bridge–acceptor energetics.<sup>64</sup> Troisi and Orlandi<sup>46</sup> have calculated values of  $\beta \approx 0.7 \text{ \AA}$  for hole transport in  $G(A)_nG$  systems using the electronic couplings between adjacent bases obtained using an ab initio procedure. A significantly higher value of  $\beta \approx 1.5 \text{ \AA}$  was obtained by Olofsson and Larsson<sup>65</sup> using electronic couplings obtained from a superexchange model. The lower calculated value of  $\beta$  is in good agreement with our data for superexchange charge separation in conjugates **1b–d** and related conjugates that have  $Sa-(A)_nG$  sequences (Figure 4).<sup>31</sup> Our limited data for the distance dependence of  $k_t$  for  $G(A)_nGG$  and  $G(A)_nZ$  sequences<sup>66</sup> are also consistent with a value of  $\beta \approx 0.7 \text{ \AA}$ .

Theoreticians have also considered the difference in the distance dependence of hole transport in  $G(T)_nG$  sequences. Voityuk et al.<sup>67</sup> predict more rapid hole transport for  $T_n$  versus  $A_n$  bridges and an ca. 10-fold decrease in  $k_t$  for each additional bridging base. Olofsson and Larsson<sup>65</sup> also predict more rapid hole transport via  $T_n$  versus  $A_n$  bridges. However, Troisi and Orlandi<sup>46</sup> and Rak et al.<sup>68</sup> have recently reached the opposite conclusion, going so far as to predict that hole transport in  $G(T)_nG$  sequences would occur via the polyA sequence in the complementary strand. We find that hole transport is significantly slower in GTGG versus GAGG sequences (Table 1). Thus, we did not attempt to measure hole transport dynamics in GTGG sequences. However, Shafirovich et al.<sup>22</sup> have measured the dynamics of hole transport in aminopurine( $T$ ) $_nGG$  systems. Their data for  $n = 1–3$  are shown in Figure 4 and are in excellent agreement with our data for  $n = 1$ . They also report much more rapid hole transport for  $A_n$  versus  $T_n$  bridges when  $n = 4$ . Also shown in Figure 4 are the complementary data of Kawai et al.<sup>23</sup> for hole transport from  $G^{+\bullet}$  to pyrene via a  $T_n$  bridge. If hole transport does in fact occur via the complementary  $A_n$  bridge in aminopurine( $T$ ) $_nGG$  systems, then the value of  $\beta$  should be similar to that for systems with  $A_n$  bridges. This appears to be the case for Shafirovich's and Kawai's data.<sup>22,23</sup>

The dynamics of inter- versus intrastrand hole transport have also been the subject of several theoretical investigations. Bixon and Jortner<sup>69</sup> initially estimated a penalty factor of ca.  $1/30$  for inter- versus intrastrand G to G hole transport via a single intervening A:T base pair, on the basis of the matrix elements computed by Voityuk et al.<sup>47</sup> A more recent analysis by Jortner et al.<sup>61</sup> of strand cleavage results reported by Barton et al.<sup>39</sup> led to the proposal that the penalty factor is dependent upon strand polarity, a factor of  $1/3$  being obtained for a 5'-GAC(G) sequence and  $1/40$  for a 3'-GAC(G) sequence (interstrand hole acceptor in parentheses). The origin of this penalty is the reduced electronic coupling between bases in complementary strands. Our experimental data for conjugates **5a,b** (Table 2) provide a penalty of  $1/7$  for inter- versus intrastrand 5'-GACC(GG) hole transport. In view of the difference between our “mini-hairpins”, which contain a single hole transport step, and Barton's duplex, which contains multiple hopping steps, the agreement between our numbers and those reported by Jortner et al. is excellent.

The small penalty for inter- versus intrastrand hole transport lends credence to the proposals of Rak et al.<sup>68</sup> and Troisi and Orlandi<sup>46</sup> that hole transport in  $G(T)_n$  sequences may occur via the complementary  $A_n$  sequence. We, in fact, observe a much larger kinetic penalty for GTGG versus GAGG hole transport than for interstrand GACC(GG) hole transport. If two strand crossings are involved in this process, the average kinetic penalty for each crossing would be ca.  $1/7$ , in good agreement with our value for a single strand crossing. A small penalty for interstrand hole transport might also explain the small difference in the values of  $k_{cs}$  and  $k_{ct}$  observed for photoinduced electron transfer in **Sa**-linked hairpins in which the guanine donor is located in a polyA (**1a–d**) versus polyT (**1e,f**) sequence.<sup>31</sup> Because the **Sa**-linker overlaps with both polyT and polyA sequences, the only kinetic penalty for superexchange electron transfer in **1e,f** would be in coupling of the guanine donor to the complementary polyA sequence. In contrast, interstrand charge separation using an acceptor that overlaps with only one sequence would require two strand crossings, leading to a larger kinetic penalty, as observed by Barton and co-workers for the aminopurine acceptor.<sup>36,70</sup>

## Concluding Remarks

The results of this investigation serve to elucidate several important features of hole transport between two hole traps separated by a small number of base pairs. First, hole transport is slow as compared to the superexchange electron-transfer process employed to generate the holes. For example, for conjugate **1b** which has a **SaAG** charge separation sequence,  $k_{cs} = 2.5 \times 10^{11} \text{ s}^{-1}$ , whereas for conjugate **2b** which has a GAGG hole transport sequence,  $k_t = 5.6 \times 10^7$ . Faster hole transport is observed for the more exergonic GAZ sequence, for which  $k_t = 6.9 \times 10^8$ . The much smaller values of  $k_t$  versus  $k_{cs}$  are indicative of inherent differences between these processes.<sup>59</sup> It is likely that the hole transport process is subject to gating by solvent and nucleobase motion.<sup>71</sup>

Second, the values of  $k_t$  decrease by a factor of 10–20 for each additional base in the  $G(A)_nGG$  hole transport sequence, consistent with a tunneling process. Similar changes in  $k_t$  with

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hole transport distance have been observed by Shafirovich et al.<sup>22</sup> and by Kawai et al.<sup>23</sup> Thus, the changeover from distance-dependent superexchange to distance-independent thermally induced hole hopping, which has been predicted theoretically<sup>52,62,72</sup> and observed in strand cleavage studies,<sup>73</sup> remains to be directly observed by time-resolved spectroscopic methods.

Third, charge transport is ca. 40-fold more rapid for GAGG than for GTGG sequences, but only ca. 7-fold more rapid for intrastrand hole transport versus interstrand hole transport in a GACC sequence. Taken together, these observations are suggestive of a GTGG hole transport process involving two strand crossings. The preferential occurrence of hole transport via all-purine pathways is consistent with recent theoretical predictions and the recent experimental results of Schuster et al.<sup>74</sup>

Fourth, our kinetic analysis provides equilibrium constants and free energy changes for hole transport (Table 2). The relative energies of holes on G, GG, and GGG are 0,  $-0.052$ , and  $-0.077$  eV, indicating that GG and GGG form very shallow hole traps, in magnitude similar to  $2k_B T$ . These differences are consistent with either hole localization or delocalized holes with very small delocalization energies. Comparison of our results with selected strand cleavage data indicates that the rate constants for the chemical reactions leading to strand cleavage decrease with increasing hole stabilization (Table 3). On a per-guanine basis, a single G is 10-fold more reactive than GGG. Equilibrium constants are relatively insensitive to differences between T and A neighboring bases.

Our results provide both the first and the most comprehensive data for hole transport dynamics and equilibria in DNA. However, our method is, at present, limited to the measurement of a single hole transport event in which only one or two base pairs separate the primary and secondary donor. We specifically caution against extrapolation of our results to longer distances,

to multiple hopping processes, or to other hole transport sequences. There is accumulating evidence that hole transport dynamics and equilibria may be highly dependent upon the specific base sequence and sensitive to rapid fluctuations in duplex geometry, solvation, and ion association.

## Experimental Section

**General Methods.** The methods employed for steady-state absorption and fluorescence spectroscopy, the measurement of fluorescence quantum yields and decay times, and femtosecond and nanosecond transient absorption spectroscopy of hairpin-forming bis(oligonucleotide) conjugates have been previously described.<sup>31</sup> Recent enhancements to the femtosecond system have also been reported.<sup>75</sup>

**Materials.** Stilbene-4,4'-dicarboxylic acid was converted to its bis-(3-hydroxypropyl)-amide, as previously described by Letsinger and Wu.<sup>30</sup> Reaction of excess diol with 4,4'-dimethoxytrityl chloride yielded the mono-DMT protected diol, which was reacted with 2-cyanoethyl diisopropylchlorophosphoramidite to yield the monoprotected, mono-activated diol. Bis(oligonucleotide) conjugates (Chart 1) were prepared by means of conventional phosphoramidite chemistry using a Millipore Expedite oligonucleotide synthesizer following the procedure developed by Letsinger and Wu.<sup>30</sup> The conjugates were first isolated as trityl-on derivatives by RP HPLC, and then detritylated in 80% acetic acid for 30 min and then repurified by RP HPLC. A single peak was detected by both RP and IE HPLC. The presence of the stilbene linker was confirmed by absorption and fluorescence spectroscopy. Molecular weights of selected conjugates were determined by means of electrospray ionization mass spectroscopy with a Micromass Quattro II atmospheric pressure ionization (API) spectrometer.

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