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Reversible formation of DNA G-quadruplex hairpin dimers from stilbenediether conjugates†

Frederick D. Lewis,* Yansheng Wu and Ligang Zhang
Department of Chemistry, Northwestern University, Evanston, Illinois 60208-3113, USA.
E-mail: lewis@chem.northwestern.edu

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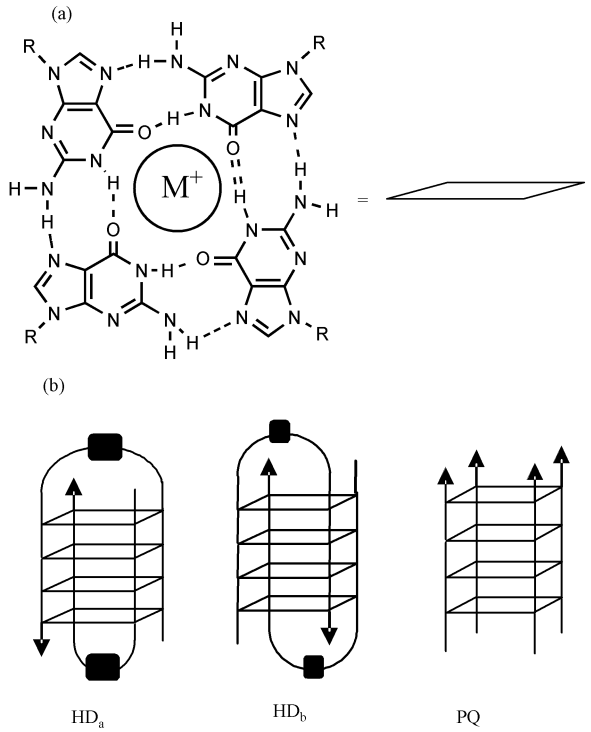
Rapid and reversible G-quadruplex hairpin dimer formation is observed for bis(oligonucleotide) conjugates possessing stilbenediether (Sd) linkers connecting two short poly(G) sequences.

We have previously reported that bis(oligonucleotide) conjugates possessing stilbenediether (Sd) linkers and complementary arms with as few as two or three bases can form stable base-paired hairpin structures.[1,2] Introduction of G:G mismatches results in only a minor decrease in the stability of Sd-linked hairpins.[2] Thus it occurred to us that Sd-linked conjugates containing poly(G) arms might adopt hairpin structures capable of dimerizing to form antiparallel hairpin dimer (HD) G-quadruplex structures. We report here the rapid and reversible formation of HD structures possessing as few as two tetrads.

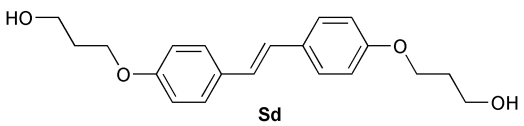
G-rich oligonucleotides can form G-quadruplex structures containing two or more guanine tetrads.[3,4] The tetrad is composed of a cyclic array of four guanines, each engaged in two Hoogsteen base-pair interactions (Scheme 1a). The formation of intramolecular G-quadruplexes in telomere repeat sequences has stimulated considerable interest in the structure and properties of G-quadruplexes.[5] G-quadruplexes can form upon folding of a single strand possessing four polyG sequences, dimerization of two G-hairpins possessing G:G stems, or tetramerization of four separate strands. Two distinct hairpin dimer (HD) structures (Scheme 1b) have been identified. Dimers with diagonal loops (HD_a) generally have four or more bases connecting the two G-domains and are proposed to form *via* folding of an intermolecular antiparallel G:G duplex.[6] Dimers possessing lateral loops (HD_b) generally have shorter connecting base sequences that promote Watson–Crick hairpin formation and are proposed to form *via* hairpin dimerization.[7] Tetramers adopt parallel quadruplex (PQ) structures in which all four strands have the same polarity (Scheme 1b).[8] G-rich oligonucleotides can also assemble into larger aggregates known as G-wires.[9] Both PQ and G-wire structures have high thermodynamic stability and are resistant to thermal dissociation.



Scheme 1 (a) The guanine quadruplex with its metal-binding site and (b) hairpin dimer structures with diagonal loops (HD_a) and lateral loops (HD_b), and parallel quadruplex (PQ).

Conjugates **1–6** (Scheme 2) were prepared, purified, and characterized using methods previously described for the preparation of other conjugates possessing the Sd linker.[2] Their UV absorption spectra consist of a long-wavelength band (λ_{max} ~ 327 nm), assigned to the stilbene π,π* transition, and a short-wavelength band (λ_{max} ~ 260 nm), dominated by the absorption of the nucleobases. They are fluorescent with emission maxima near 382 nm, in accord with the inability of guanine to quench Sd fluorescence.[2] Both the absorption and emission spectra are broadened and their maxima red-shifted by *ca.* 10 nm, when compared to the diol Sd in methanol.

Circular dichroism (CD) spectra of conjugate **1** in aqueous 0.1 M NaCl (in 10 mM phosphate buffer, pH 7.2, heating rate 0.1 °C min^{-1}) are shown in Fig. 1.‡ The CD band at wavelengths longer than 320 nm is attributed to the stilbene linkers (*vide infra*). The low temperature spectra of **1** in the 200–320 nm spectral region (strong positive band and weaker negative band at 295 and 265 nm, respectively) are identical to those reported for G_4-T_4-G_4 and related sequences that form antiparallel hairpin dimers.[10] Heating results in disappearance of the 295 and 265 nm bands and the appearance of bands similar to those reported for monomeric dG_4 (weaker positive band near 255 nm and negative band near 235 nm). These changes are fully reversible upon cooling. Plots of the 295 nm CD band intensities *vs.* temperature provide a CD half-melting temperature (T_M) of 15 °C. UV thermal dissociation profiles (260 nm) provide values of T_M of 15 and 32 °C in the presence of 0.1 M NaCl and KCl, respectively. The Sd linker fluorescence intensity is independent of temperature. The CD spectra of **2** and **3** are more complex than that of **1**.† Their room temperature spectra display strong positive bands near 265 nm and negative bands near 235 nm, with band shapes similar to those reported for parallel quadruplex structures. PAGE gels for **1–3** in the absence of added NaCl are consistent with aggregate formation



1 GG-Sd-GG
2 GGG-Sd-GGG
3 GGGG-Sd-GGGG
4 TGG-Sd-GGT
5 TGGG-Sd-GGGT
6 TGGGG-Sd-GGGGT

Scheme 2 Structures of the Sd linker and bis(oligonucleotide) conjugates **1–6**.

† Electronic supplementary information (ESI) available: CD spectra of **2–4**, UV melting curves for **6**, and PAGE gels for **1–3**, **5** and **6**. See http://www.rsc.org/suppdata/cc/b3/b315265k/

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from **3**, but not from **1** or **2**, even at the high concentrations employed in gel studies.† In the presence of salt, aggregate formation is observed for all three conjugates.

The T-terminated conjugates **4–6** were prepared in an effort to destabilize parallel quadruplex structures and thus favor hairpin dimer formation.[4] The CD spectra of **5** and **6** are shown in Fig. 1. The temperature-dependent CD spectra of **6** are similar to those for **1**, displaying clean conversion from a hairpin dimer structure at low temperature to a single strand structure at high temperatures. Both CD and UV melting curves are reversible, providing values of T_M = 56 °C. The CD spectra of **5** display maxima near 255 and 290 nm at both 5 and 25 °C, consistent with the presence of both single strand and hairpin dimer structures.[11] The temperature dependence of the ratio of these peaks is indicative of a T_M value near room temperature. The spectrum of **4** indicates the presence of only single strands even at 5 °C.† PAGE studies for **5** and **6** reveal bands attributed to both monomer and HD structures in the absence of salt, but only bands attributed to HD structures in the presence of 0.1 M NaCl.† Evidently the presence of terminal thymines inhibits aggregate formation even at the high concentrations employed in gel studies.

These results establish the ability of appropriately-designed Sd-linked conjugates to rapidly and reversibly form HD structures. To our knowledge, the dimerization of **1** provides the first example of an HD structure possessing only two tetrads. Molecular modeling indicates that the Sd linkers are too short to form diagonal loops, and thus **1** presumably forms an HD_b structure *via* the dimerization of two hairpins. HD formation presumably also occurs for **2** and **3**, however the HD_b structures may be less stable than PQ structures which possess three or four adjacent tetrads. It is interesting to note that HD_a structures are observed for sequences such as $d(G_3T_4G_3)$ and $d(G_4T_4G_4)$.[6] The formation of a parallel dimer, which is the precursor of both the HD_a and PQ structures, may require a minimum of three G–G base pairs. In the case of $d(G_3T_4G_3)$ and $d(G_4T_4G_4)$ the parallel dimer is able to fold into an HD_a structure, however in the case of **2** or **3**, the Sd linker is too short to permit HD_a formation. Hydrophobic attraction of the Sd linkers may also favor PQ formation in the case of **2** and **3**.

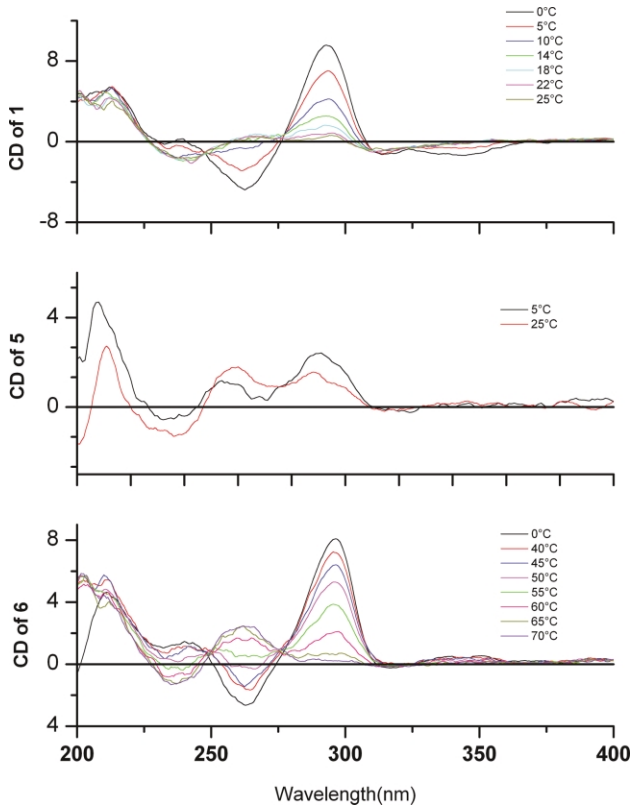

Fig. 1 CD spectra of conjugates **1**, **5**, and **6** in 0.1 M NaCl (10 mM phosphate buffer, pH 7.2). Rates of heating are 0.1 °C min^{-1} for **1** and **6**.

Introduction of 5′- and 3′-thymines destabilizes PQ structures, which are not observed for **4–6**, and to a lesser extent, also destabilizes HD structures. HD formation is not observed for **4**, and the HD structure observed for **5** has a low T_M, similar to that of **1**. Only in the case of **6** is an HD structure with a T_M above room temperature obtained. Additional structural information for the HD dimers formed by **2**, **5**, and **6** is provided by comparison of their 350 nm stilbene CD bands. These bands are attributed to exciton coupling between the two stilbene chromophores, the sign and intensity of which are determined by the distance and dihedral angle ω between their electronic transition dipole moments.[12] The change in sign of the 350 nm band from negative for **1** to positive for **6** is consistent with a helical G-quadruplex structure in which $\omega \sim 180^{\circ}$ in the case of **5**, which displays zero 350 nm CD band intensity. The reversible formation of HD structures from conjugates **1**, **5**, and **6** makes them suitable for investigations of the kinetics and thermodynamics of HD formation and melting as well as for studies of the photoisomerization of the Sd linker.

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Notes and references

‡ The concentration of conjugates (*ca.* 5 μM) was adjusted to provide an absorbance of *ca.* 0.4 at 260 nm. CD spectra were obtained from solutions of conjugates that were deprotected, purified by HPLC, concentrated, and lyophilized following synthesis. Aliquots were thawed and added to solutions of buffer and salt at either 2 °C or room temperature.

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