The synthesis of bilobalide has been accomplished in 17 steps from 3-furaldehyde. The key transformations are the stereoselective aldol condensation of enolate **5** with aldehyde **4**, the stereoselective photocycloaddition of enone **3**, and the regioselective Baeyer–Villiger oxidation of cyclobutanone **2**. The selective oxidations in the final steps are also worthy of note.

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Supplementary Material Available: A list of ^{1}H and ^{13}C NMR data for compounds **1–17** (2 pages). Ordering information is given on any current masthead page.

Trapping and Isolation of an Alternate DNA Conformation

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DNA can assume many conformations that differ from the B-form double helix.[1] Alternate structures like cruciforms[2] and Z-DNA[3] are of interest as they are thought to serve regulatory functions in vivo. On large molecules like plasmids, atypical geometries are formed as a result of torsional stress, whereas related conformations in oligodeoxyribonucleotides can be induced by changes in temperature or salt concentration.[4] Unusual DNA structures are often recalcitrant to physicochemical characterization because of the narrow range of conditions under which they exist. We have recently developed a general method based on disulfide bond crosslinking that stabilizes the secondary structure of synthetic oligodeoxyribonucleotides without perturbing their native geometries.[5,6] We report the application of this chemistry to trap, isolate, and characterize a "premelting intermediate" of the d(CGCGAATTCGCG)$_2$ dodecamer.

Crystallographic and NMR studies show that d(CGCGAATTCGCG)$_2$ forms a B-DNA duplex.[7,8] UV thermal denaturation experiments confirm these findings: in high salt buffer a monophasic transition is observed which represents melting of the duplex to a random coil.[9] However, biphasic melting profiles are obtained when the buffer contains $[\mathrm{Na}^+] \leq 10$ mM.[10] Breslauer proposed that the first transition in these biphasic curves defines premelting of the duplex to a hairpin, while the second transition represents conversion of the hairpin to a random coil. To examine this premelting intermediate we synthesized **1**, which has the terminal residues of the parent dodecamer replaced with N^3-(mercaptoethyl)thymidine. These substitutions were introduced to stabilize the premelting intermediate with a disulfide crosslink.[5] Control experiments show that the melting profiles of **1** are analogous to those of d(CGCGAATTCGCG)$_2$ ($T_{\mathrm{m}}^{1} = 27.0$ °C and $T_{\mathrm{m}}^{2} = 60.1$ °C; 1 mM NaCl, pH 8, 50 μM in **1**), suggesting that both dodecamers denature along a similar pathway.

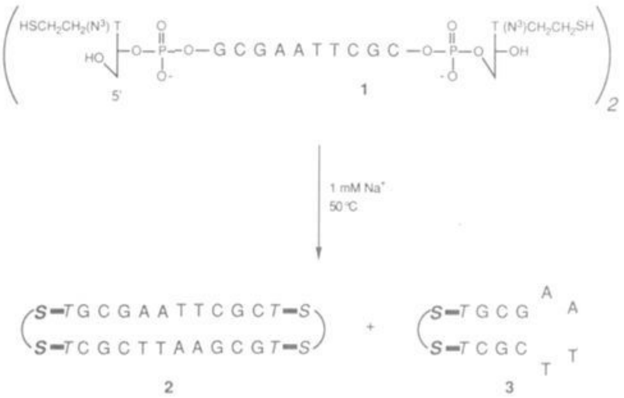

Figure 1. Synthesis of the crosslinked oligomers. The oxidation was performed at 50 °C, which is past the first transition but before significant onset of the second transition. Attempts to crosslink the denatured dodecamer at 75 °C afforded high molecular weight products and trace amounts of **3**.

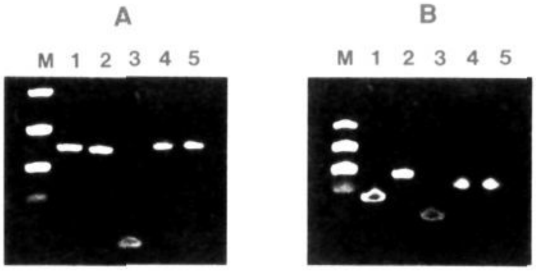

Figure 2. Electrophoretic analysis of the modified oligomers. In both gels lane M contains single-stranded markers 30, 22, 16, and 12 bases long. (A) 20% polyacrylamide nondenaturing gel: lane 1, d(CGCGAATTCGCG)$_2$; lane 2, **2**; lane 3, **3**; lane 4, dodecamer **1** produced by reduction of **2**; lane 5, dodecamer **1** produced by reduction of **3**. (B) 20% polyacrylamide denaturing gel: lane 1, d(CGCGAATTCGCG)$_2$; lane 2, **2**; lane 3, **3**; lane 4, dodecamer **1** produced by reduction of **2**; lane 5, dodecamer **1** produced by reduction of **3**.

Air oxidation of the sulfhydryl groups was achieved by heating **1** with vigorous stirring under the conditions used for the melting studies (Figure 1). After 24 h the solution tested negative for thiol groups with Ellman's reagent. HPLC analysis of the reaction mixture revealed two major products in a 16:1 ratio. On a nondenaturing gel, the minor component (**2**) migrated with d(CGCGAATTCGCG)$_2$, suggesting that this compound is the (bis)crosslinked dodecamer (Figure 2A).[11] However, the major product (**3**) migrated below the 12-mer size marker, indicative of a hairpin structure.[12] Reduction of **2** or **3** with DTT afforded

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(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: Berlin, 1984. Patel, D. J.; Shapiro, L.; Hare, D. *Annu. Rev. Biophys. Biophys. Chem.* **1987**, *16*, 423–454. *Unusual DNA Structures, Proceedings of the First Gulf Shores Symposium*; Wells, R. D., Harvey, S. C., Eds.; Springer-Verlag: Berlin, 1988. Wemmer, D. E. *Curr. Opin. Struct. Mol. Biol.* **1991**, *1*, 452–458.

(2) Müller, U. R.; Fitch, W. M. *Nature* (*London*) **1982**, *298*, 582–585. Horwitz, M. S. Z.; Loeb, L. A. *Science* **1988**, *241*, 703–705. Lilley, D. M. J. *Chem. Soc. Rev.* **1989**, *18*, 53–82. Bianchi, M. E.; Beltrame, M.; Paonessa, G. *Science* **1989**, *243*, 1056–1059.

(3) An, G. *BioEssays* **1987**, *7*, 211–214. Wittig, B.; Dorbic, T.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2259–2263.

(4) Roy, S.; Weinstein, S.; Borah, B.; Nickol, J.; Appella, E.; Sussman, J. L.; Miller, M.; Shindo, H.; Cohen, J. S. *Biochemistry* **1986**, *25*, 7417–7423. Jovin, T. M.; Soumpasis, D. M.; McIntosh, L. P. *Annu. Rev. Phys. Chem.* **1987**, *38*, 521–560. Sheardy, R. D.; Winkle, S. A. *Biochemistry* **1989**, *28*, 720–725. Xodo, L. E.; Manzini, G.; Quadrifoglio, N.; van der Marel, G. A.; van Boom, J. H. *Biochimie* **1989**, *71*, 793–803.

(5) Glick, G. D. *J. Org. Chem.* **1991**, *56*, 6746–6747.

(6) For a related method, see: MacMillan, A. M.; Verdine, G. L. *J. Org. Chem.* **1990**, *55*, 5931–5933. MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603–2616. Ferentz, A. E.; Verdine, G. L. *J. Am. Chem. Soc.* **1991**, *113*, 4000–4002.

(7) Wing, R.; Drew, H.; Takano, T.; Broka, C.; Tanaka, S.; Itakura, K.; Dickerson, R. E. *Nature* (*London*) **1980**, *287*, 755–758.

(8) Patel, D. J.; Kozlowski, S. A.; Marky, L. A.; Broka, C.; Rice, J. A.; Itakura, K.; Breslauer, K. J. *Biochemistry* **1982**, *21*, 428–436. Nerdal, W.; Hare, D. R.; Reid, B. R. *Biochemistry* **1989**, *28*, 10008–10021.

(9) Marky, L. A.; Blumenfeld, K. S.; Kozlowski, S.; Breslauer, K. J. *Biopolymers* **1983**, *22*, 1247–1257.

(10) In 1 mM NaCl buffer that is 50.6 μM in d(CGCGAATTCGCG)$_2$, $T_{\mathrm{m}}^{1} = 33.2$ °C and $T_{\mathrm{m}}^{2} = 62.4$ °C.[9]

(11) **2** was obtained exclusively when the crosslinking was performed at 25 °C.

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1, which migrated like d(CGCGAATTCGCG)$_2$.

The modified DNAs were also characterized by their susceptibility to enzymatic digestion. The d(CGCGAATTCGCG)$_2$ dodecamer contains an *Eco*RI recognition site (GAATTC), and since this enzyme only cleaves double-stranded DNA, hairpin **3** should not be a substrate. Indeed, [^{32}P] end-labeled **3** was not cleaved by *Eco*RI endonuclease. However, both **1** and **2** were cut as efficiently as d(CGCGAATTCGCG)$_2$, indicating that these two molecules exist in duplex form. Next, we examined the conformational integrity of the modified DNAs. On a denaturing gel, **2** and **3** migrated as a dodecamer and as a hairpin, respectively, whereas reduced duplex **1** migrated like d(CGCGAATTCGCG)$_2$ (Figure 2B). In thermal denaturation experiments, **2** displayed a sharp melting transition at 94 °C in contrast to the biphasic curve obtained with the parent dodecamer.[13] Upon heating from 10 to 99 °C, the absorbance of **3** rose linearly to just over 3% of the initial optical density value.

To summarize, disulfide crosslinking was employed to trap a thermally induced DNA hairpin. These results suggest that the d(CGCGAATTCGCG)$_2$ premelting intermediate is also a hairpin structure and demonstrate the utility of our modification in the synthesis of both ground-state and non-ground-state DNA conformations. The ease with which this crosslink is introduced coupled with the finding that it does not inhibit the action of kinases or endonucleases should make these disulfide-crosslinked DNAs useful in studies of nucleic acid structure and function.

Supplementary Material Available: Procedures for the synthesis and characterization of the cross-linked oligomers (4 pages). Ordering information is given on any current masthead page.

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(12) The ^{1}H NMR spectrum of **3** shows three G/C base pairs which is consistent with the proposed structure.

(13) Bis-crosslinked **2** is similar to DNA dumbbells; see, for example: Erie, D. A.; Jones, R. A.; Olson, W. K.; Sinha, N. K.; Breslauer, K. J. *Biochemistry* **1989**, *28*, 268-273. Ashley, G. W.; Kushlan, D. M. *Biochemistry* **1991**, *30*, 2927-2933.

Intramolecular Coupling of Two Radical Centers through 1,1-Diphenylethylene Chromophores. Isomeric Dinitroxides Vinylogous to Trimethylenemethane, Tetramethyleneethane, and Pentamethylenepropane

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Our motive for the syntheses and analyses of isomeric vinylidenebis(radical X-substituted benzenes) **1** has two facets. As an extension of our efforts to design and construct new organic polymers in high-spin ground states, we became interested in knowing whether polymers **2** would be high-spin and how strong the electron spins in X could couple each other in **2**.[1] For this purpose, studies of dimeric prototypes should be very instructive. On the other hand, *p,p'*-, *m,p'*-, and *m,m'*-**1** (X = CH$_2$·) are related in connectivity to trimethylenemethane (**3**) tetramethyleneethane (**4**), and pentamethylenepropane (**5**), respectively. Since the ground spin states of **4** and **5** are still controversial, in contrast to the well-established triplet of **3**,[2] we thought the delineation of the manner in which the electron spins couple in isomeric **1** might shed light on the discussion of the ground-state spins of **4** and **5**.

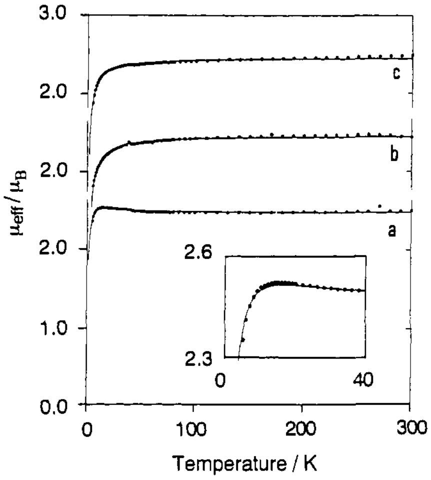
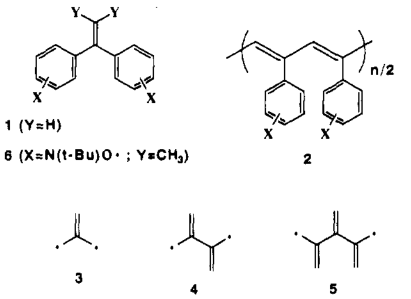


Figure 1. Plots of μ_{eff}/μ_B vs temperature for (a) *p,p'*-, (b) *m,p'*-, and (c) *m,m'*-**6**.



1 (Y=H)
6 (X=N(t-Bu)O· ; Y=CH$_3$)
2
3
4
5

The experimental work has been carried out on the isomeric vinylidene derivatives **6** with additional steric protection.[3] The corresponding dibromo compounds were lithiated with 4 equiv of *tert*-butyllithium and allowed to react with 2 equiv of 2-nitroso-2-methylpropane to give the hydroxyamines, which were then treated with Ag$_2$O to give **6**. Purification was accomplished by chromatography on silica gel to give analytically pure samples.[4]

EPR spectra of **6** in toluene at room temperature consisted of five unperturbed lines ($|J| > |a_N| = 11.2$ G). The dipolar interaction between the nitroxides was unexpectedly small as revealed by their EPR spectra in frozen matrices: spectral widths as a measure of $2D$ were less than 90 G, but $\Delta m_s = 2$ transitions for triplet species were clearly observed at $g = 4$. The signals were too small to study the temperature dependence of their intensities.

The magnetic susceptibility was measured for powder samples of **6** on a Quantum Design SQUID susceptometer in the temperature range 5–300 K. The results are expressed in terms of μ_{eff}/μ_B vs temperature plots in Figure 1. The μ_{eff} values approaching 2.45 μ_B at room temperature are indicative of the near degeneracy of the singlet and triplet states for the three isomers. The plots were analyzed in terms of a Bleaney–Bowers equation

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(1) Iwamura, H. *Adv. Phys. Org. Chem.* **1990**, *26*, 179. Fujii, A.; Ishida, T.; Koga, N.; Iwamura, H. *Macromolecules* **1991**, *24*, 1077.

(2) (a) Dowd, P. *J. Am. Chem. Soc.* **1970**, *92*, 1066. (b) Roth, W. R.; Erker, W. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 503. (c) Borden, W. T.; Davidson, E. R. *J. Am. Chem. Soc.* **1977**, *99*, 4587. (d) Borden, W. T., Ed. *Diradicals*: Wiley: New York, 1982. (e) Du, P.; Hrovat, D. A.; Borden, W. T.; Lahti, P. M.; Rossi, A. R.; Berson, J. A. *J. Am. Chem. Soc.* **1986**, *108*, 5072. (f) Greenberg, M. M.; Blackstock, S. C.; Berson, J. A.; Merrill, R. A.; Duchamp, J. C.; Zilm, K. W. *J. Am. Chem. Soc.* **1991**, *113*, 2318.

(3) A preliminary study shows that *p,p'*-dinitroxide **1** (X = *N*(*t*-Bu)O·) readily undergoes polymerization to form an insoluble solid as soon as the solvent is removed: Ishida, T. Ph.D. Dissertation, University of Tokyo, Tokyo, Japan, 1991.

(4) The empirical factors, *F*, introduced to correct slight reductions in the effective magnetic moment of the samples made for SQUID measurements due to the presence of nondiradical impurities were 0.97, 0.88, and 0.94 for *p,p'*-, *m,p'*-, and *m,m'*-**6**, respectively.

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