The aziridination experiments with olefins (Table I) and enolsilanes (Table II) were carried out with 5.0 and 1.5 equiv of substrates, respectively. For those cases where the olefin might be considered as the valuable reaction component, 1.0 equiv of substrate may be employed with negligible loss in yield if the substrate concentration is increased to 1.0 M (checked for those experiments described in Table I, entry 11; Table II, entries 1, 2).

Ongoing studies are being directed toward extending the scope and developing enantioselective variants of this reaction.[6] It is our intention to develop a new catalytic, asymmetric enolate amination procedure to complement methods previously reported from these laboratories for the asymmetric synthesis of amino acids.[13]

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**Supplementary Material Available:** Experimental procedures and spectral data for all compounds (4 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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## Synthesis of a Conformationally Restricted DNA Hairpin

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*Summary:* A synthetic method based on disulfide bond crosslinking between modified thymidine bases has been developed to stabilize the conformation of DNA hairpin structures.

Physical studies of oligodeoxynucleotides provide a rich source of information regarding DNA structure.[1] Yet, such investigations can be hampered by the dynamic properties of these molecules.[2] This problem is often encountered in studies of hairpin stem-loop structures. At the DNA or salt concentrations required for crystallographic or NMR work, self (or partially self) complementary sequences can dimerize or oligomerize.[3] Indeed, only one X-ray[4] and several NMR[5] structures of DNA hairpins have been determined. Here, we describe a general method to stabilize the molecular architecture of DNA hairpins and apply it to prepare a conformationally restricted stem-loop structure whose sequence comes from the ColE1 cruciform.[6] Unlike many other procedures to crosslink oligodeoxynucleotides, this chemistry does not perturb native DNA structure.[7]

On a B-DNA duplex the pyrimidine N-3 position faces toward the center of the helix so that at the site of a T-T mismatch, the two N-3 atoms converge (to within 4.5 Å).[1] Molecular modeling studies suggest that if this mismatch is located at the terminus of a duplex, a six-atom linker can crosslink these N-3 positions without disrupting the native geometry of the helix. To bridge this distance, we have alkylated the 2'-deoxythymidine N-3 nitrogen with a mercaptoethyl linker so synthesis of an oligodeoxynucleotide with this base at the 3' and 5' termini permits formation of an intramolecular disulfide bridge across the helix.[8] In this scheme, the mercaptoethyl bridge comes

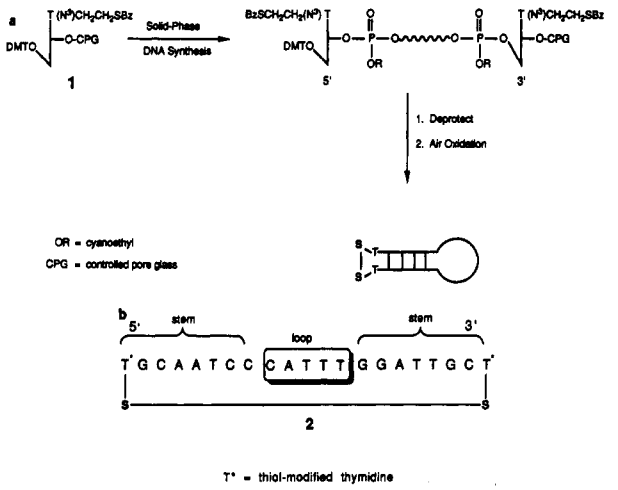


**Figure 1.** (a) General synthetic route to crosslinked hairpins. As depicted schematically, the lowest energy conformation of the crosslink places the disulfide bond and alkyl chains below the base of the stem. In this the geometry there are no eclipsing interactions in the linker and the C-S-S-C dihedral angle is 81°. (b) Sequence of the crosslinked hairpin.

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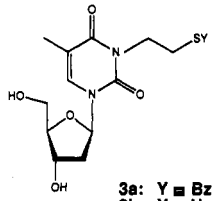
from 2-mercaptoethanol and a benzoyl group is used to protect the thiols (Figure 1a).

In preliminary experiments the stability of the benzoyl moiety was examined by treating samples of **3a** for 24 h with each reagent used during solid-phase DNA synthesis (0.1 M $I_2$ in pyridine/THF/$H_2O$; $Ac_2O$/pyridine/DMAP; 3% $CHCl_2CO_2H$ in $CH_2Cl_2$; 0.5 M tetrazole in $CH_3CN$).[9]




**3a**: Y = Bz
**3b**: Y = H

In each case **3a** was quantitatively recovered and was shown by $^1H$ NMR, FAB-MS, and TLC to be identical with the starting material. Next, the reactivity of the benzoyl group was tested under conditions employed to cleave an oligodeoxynucleotide from CPG. Reaction of **1** with concentrated $NH_4OH$ in the presence of excess DTT (to trap the acrylonitrile formed upon removal of the phosphate cyanoethyl protecting groups) for 1 h at room temperature afforded a 95% recovery of **3b**. Further treatment with $NH_4OH$ (6 h, 55 °C), which is used to remove the benzoyl and isobutryl amino protecting groups on the bases, yielded **3b** and several unidentified products. These side reactions were suppressed by switching to rapid deprotecting amidites[10] which were completely removed by $NH_4OH$ in 7 h at room temperature.

Synthesis of the crosslinked hairpin proceeded in a straightforward fashion using β-cyanoethyl phosphoramidite chemistry[11,12] to give the sequence depicted in Figure 1b (5′-*O*-DMT-**3b** was converted to the corresponding phosphoramidite to couple the thermal thymidine). After synthesis, the oligomer was released from the support and deprotected as described above. The crude product was purified by reversed-phase HPLC and detritylated with 5% acetic acid. The DNA (ca. 1.5 mg) was dissolved in phosphate buffer (2 mL, 0.1 mM in DNA, pH 8) and stirred vigorously while exposed to air for 12 h to effect oxidation of the thiol groups.[13,14] Nondenaturing electrophoresis of the reaction mixture on a 20% polyacrylamide gel showed the monomeric hairpin as the major product and trace amounts of a dimeric material. Hairpin **2** was isolated from this mixture (1.2 mg, 22% yield) by reversed-phase HPLC in >97% purity.[15]

The effects of crosslinking were examined by optical melting studies. As seen in Figure 2, the disulfide crosslink in **2** increases $T_m$ by 21 °C relative to the wild-type sequence. In either 0.1 or 1 M NaCl buffer, **2** melts in excess of 96 °C so that values of $T_m$ could not be measured.[17] Both the wild-type and crosslinked hairpins showed a single cooperative melting transition. The transition in **2** is nearly 40% less hyperchromic than the wild-type sequence, indicating that the crosslinked hairpin may not be fully denatured. After reduction with DTT, the melting temperature of **2** (64.2 °C) is nearly identical to the wild-type sequence, demonstrating that the linker does not destabilize the stem helix. Furthermore, when **3** is incorporated at either the 5′ *or* 3′ terminus, the $T_m$ values of resulting hairpins are also similar to the wild-type sequence.[18] These results provide further evidence that the alkyl mercaptan linker does not disrupt the geometry of the stem duplex.

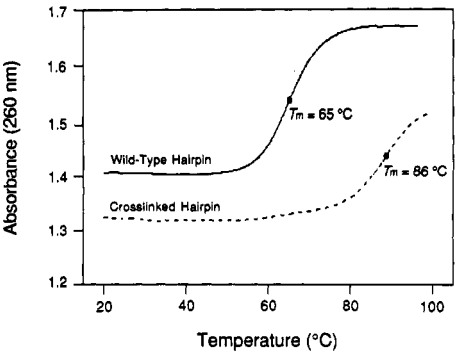



**Figure 2.** Optical melting profiles of the wild-type hairpin (3′ dT, 5′ dA) and **2**. Samples were dissolved in 10 mM NaCl containing 1 mM EDTA, pH 6.6 (8 μM in single strands), and heated at 0.5 °C/min from 20 to 99 °C. The renaturation curves for both hairpins were identical to the melting plots indicating that the $T_m$'s are thermodynamic values.[16] Duplicate experiments resulted in $T_m$ values within 0.2 °C of each other.

To summarize, we have developed chemistry for site-specific incorporation of a crosslink in synthetic DNA hairpins. Our method preserves native DNA conformation, is independent of sequence or steric constraints, and in principle, is not limited just to stem–loop secondary structures. Through proper control of solution conditions, our crosslinking reaction can be used to "trap" various conformations of the same sequence. The chemistry described here is also applicable to the synthesis of RNA hairpins. This avenue of research is particularly interesting given the biological importance[19] of RNA hairpins and the paucity of high-resolution data regarding their three-dimensional geometries.[20] These possibilities along with NMR and crystallographic analysis of the molecules described here are currently being explored.

**Acknowledgment.** This work was supported by grants from the NIH (BRSG RR07050-25 and P60 AR20557-13) and the American Cancer Society (IN-40-32).

**Supplementary Material Available:** Procedures for the synthesis and characterization ($^1H$ NMR, IR, MS, TLC) of **3** and detailed protocols for the preparation and enzymatic degradation of **2** (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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(11) Solid-phase DNA synthesis was performed on a MilliGen-Biosearch-Waters Cyclone Plus instrument and followed the manufacturers protocols.
(12) Synthesized with the 5′ DMT on 1 μmol scale.
(13) The wild-type sequence adopts a hairpin structure under these conditions: Glick, G. D.; Blatt, N. B.; Cain, R. Manuscript in preparation.
(14) After 12 h the reaction gave a negative Ellman's test for thiols.
(15) Enzymatic degradation of the hairpin gave the expected ratios of constituent monomers and the symmetric disulfide of **3b**.
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