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### SYNTHESES OF DNA DUPLEXES CONTAINING A C-C INTERSTRAND CROSS-LINK

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## SYNTHESES OF DNA DUPLEXES CONTAINING A C–C INTERSTRAND CROSS-LINK

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### ABSTRACT

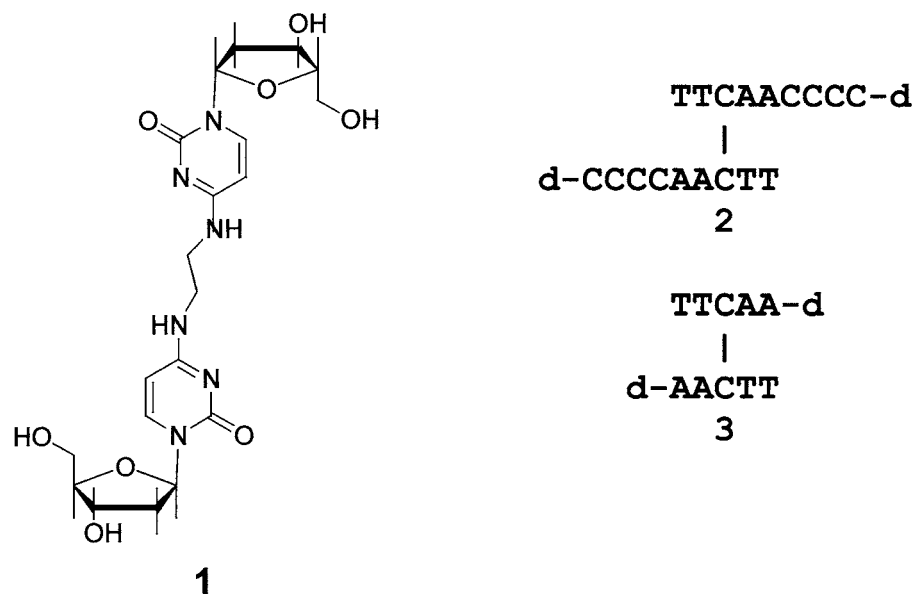
Short DNA duplexes that contain a N<sup>4</sup>C-ethyl-N<sup>4</sup>C interstrand cross-link were prepared on controlled pore glass supports using a DNA synthesizer. The C–C cross-link was introduced *via* a convertible nucleoside on the support or by using a protected C–C cross-link phosphoramidite. An orthogonal protection scheme allowed selective chain growth in either a 3' → 5' or 5' → 3' direction. The cross-linked duplexes were purified by HPLC and characterized by MALDI-TOF mass spectrometry and/or by enzymatic digestion.

Bifunctional alkylating agents can react with DNA to form a variety of products among which are interstrand cross-links (1–3). These latter adducts are believed to be primarily responsible for the antitumor activity of therapeutic bifunctional alkylators. Because interstrand cross-links can be repaired (4–8), a better understanding of the repair process could lead to the development of more effective therapeutic agents. Such studies would be aided significantly by the availability of cross-linked DNA of defined structure.

In this paper we describe methods to prepare a model DNA duplex that contains a N<sup>4</sup>C-ethyl-N<sup>4</sup>C interstrand cross-link. This cross-link, **1**, whose structure is shown in Figure 1, is similar to an N<sup>3</sup>C–N<sup>3</sup>C cross-link that has been identified

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**Figure 1.** Structure of the N<sup>4</sup>C-ethyl-N<sup>4</sup>C cross-link and sequences of the C-C cross-linked duplexes.

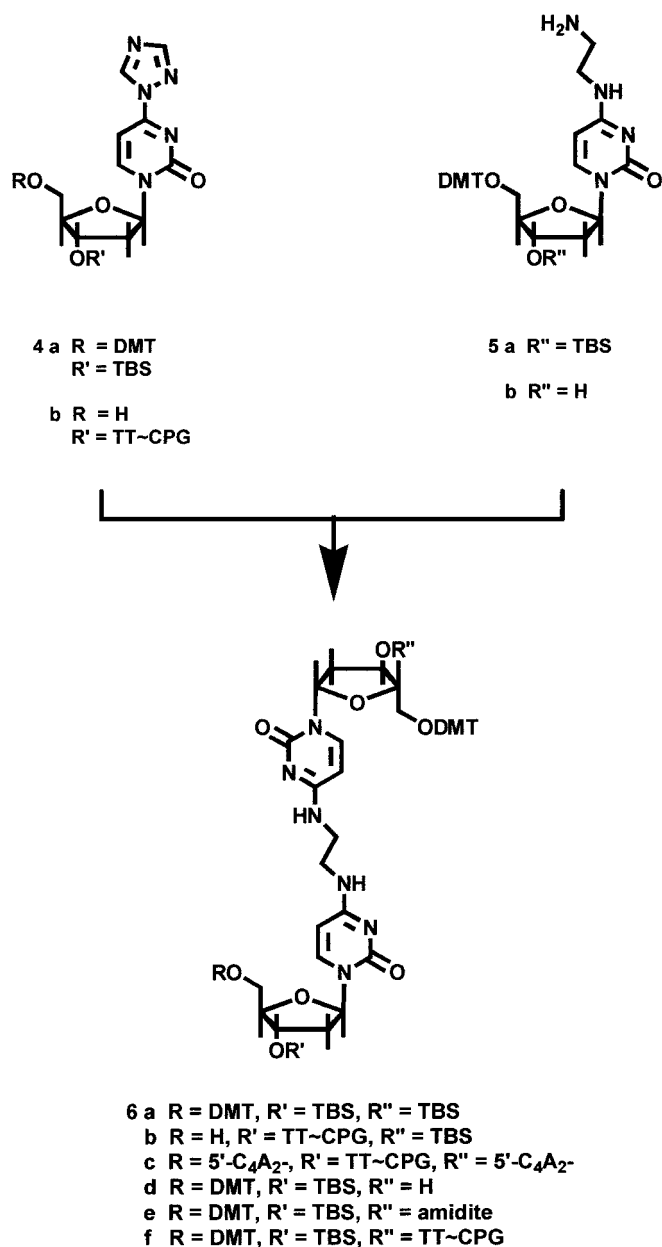
when DNA that contains a C/C mismatch is reacted with mechlorethamine (9). The cross-link was placed in two short DNA duplexes: duplex **2**, which contains two A/T base pairs on either side of the cross-link and four non-paired C residues at the 5'-end of each strand, and duplex **3**, which lacks the 5'-overhanging bases.

Cross-link **1** was synthesized using a convertible nucleoside approach (10) as shown in Figure 2. Protected O<sup>4</sup>-triazolyl-2'-deoxyuridine nucleoside, **4a**, was converted to the N<sup>4</sup>-(2-aminoethyl)-deoxycytidine nucleoside, **5a**, by reaction with ethylenediamine. Displacement of the triazole group of **4a** by the 2-amino group of **5a** yielded protected C-C cross-link **6a**. Removal of the dimethoxytrityl (DMT) and *t*-butyldimethylsilyl (TBS) protecting groups by treatment of **6a** with 0.1 N hydrochloric acid produced C-C cross-link **1**, which was characterized by <sup>1</sup>H NMR spectroscopy.

The convertible nucleoside approach was used to prepare cross-linked duplex **3**. Triazole-derivatized trinucleotide **4b** was first prepared on a controlled glass support (CPG) and reacted with aminoethyl nucleoside **5a** to give support-bound oligomer **6b**. The dimethoxytrityl group was removed selectively from **6b** and chain extensions from the 5'-hydroxyls of each strand were carried out simultaneously using the appropriate protected nucleoside 3'-phosphoramidites to give partial duplex **6c**.

The 5'-hydroxyls of **6c** were acetylated and the *t*-butyldimethylsilyl group was removed by treating the support with tetra-*n*-butylammonium fluoride (TBAF). Initial experiments showed that the TBAF treatment resulted in partial cleavage of the oligomer backbone. This cleavage could be suppressed by treating the oligomer





**Figure 2.** Synthetic scheme for preparing C-C cross-linked duplexes.

with anhydrous triethylamine, which removes the cyanoethyl phosphate protecting groups (11), prior to treatment with TBAF. Although brief, 10 min, exposure to TBAF was sufficient to remove the *t*-butyldimethylsilyl group, it did not result in significant cleavage of the oligomer from the support, a result consistent with the observations of Braich and Damha (12)

Chain extension from the 3'-hydroxyl of TBAF-treated **6c** was carried out in the 5'-direction by coupling with 3'-dimethoxytritylthymidine-5'-phosphoramidite. The fully extended oligomer was then deprotected by treatment with concentrated ammonium hydroxide after removal of the 3'-terminal DMT group. Oligomer **2** was purified by C-18 reversed phase HPLC followed by strong anion exchange (SAX) HPLC. The oligomer migrated as a single band on a polyacrylamide gel after enzymatic phosphorylation with  $\gamma$ -[ $^{32}\text{P}$ ]-ATP and polynucleotide nucleotide kinase. Digestion of the oligomer with a combination of snake venom phosphodiesterase and calf intestinal phosphatase gave dC, dT, dA and the C-C cross-link in the expected ratios, and analysis of the oligomer by MALD-TOF mass spectrometry gave a molecular weight consistent with the structure of the duplex.

An alternative method, which is similar to that used by Hopkins and coworkers to prepare a DNA duplex with an interstrand nitrous acid cross-link (13), was used to prepare C-C cross-linked duplex **3**. Cross-link **6d** was prepared by reaction between triazole-derivatized nucleoside **4a** and aminoethyl-derivatized nucleoside **5b**. This protected cross-link was then converted to its  $\beta$ -cyanoethyl-*N,N'*-(diisopropyl)phosphoramidite derivative **6e**. Phosphoramidite **6e** was coupled to d-TT~CPG to give support bound oligomer **6f**. Extension of the upper and lower strands and deprotection were then carried out to produce duplex **3** which was purified by SAX HPLC. Digestion of **3** with snake venom phosphodiesterase and bacterial alkaline phosphatase gave dT, dA and the C-C cross-link in the expected ratios.

Our studies show that both the convertible nucleoside approach or the phosphoramidite approach in combination with orthogonal protecting groups can be used to prepare C-C cross-linked duplexes that have symmetrical sequences. In the examples shown here the cross-linked bases are opposed, but both methods should be applicable to the preparation of duplexes with a staggered cross-link. The convertible nucleoside approach can also be used to prepare C-C cross-links of different chain lengths. The availability of C-C cross-linked duplexes should provide useful substrates for a variety of biophysical and biochemical studies.

### ACKNOWLEDGMENT

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