

## Synthesis of oligonucleotides containing an O<sup>6</sup>-G-alkyl-O<sup>6</sup>-G interstrand cross-link

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**Abstract**—A methodology to synthesize oligonucleotides containing an alkyl interstrand cross-link between the two O6 atoms of deoxyguanosine has been developed. This cross-link is designed to serve as a stable structural mimic of the lesion formed in duplex DNA with the bifunctional alkylating agent hepsulfam. The O6-alkyl coupling is performed via a Mitsunobu reaction between a nucleoside and mono-protected 1,7-heptanediol. Solid-phase oligonucleotide synthesis using a nucleoside bis-phosphoramidite allows for the assembly of the cross-linked duplex. Sufficient quantities of this cross-linked duplex were obtained for various structural and biological investigations.

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Bifunctional alkylating agents encompass a class of chemotherapeutic agents whose antitumor activity has been attributed to their ability to cross-link the strands of duplex DNA.<sup>1</sup> These cross-links can inhibit DNA replication and transcription, leading to cell cycle arrest, apoptosis, and inhibition of tumor growth. A problem presented in cancer treatment is the development of resistance to the effects of antitumor agents, which is caused by enhanced repair of interstrand cross-links.<sup>2</sup> A better understanding of the molecular mechanisms of such repair could lead to more effective chemotherapeutic agents and treatment regimens. In order to conduct the various biological and structural investigations associated with probing DNA repair, methods to produce interstrand cross-linked DNA of well-defined structures in sufficient quantities are required.

One example of a bifunctional alkylating agent used clinically in the treatment of chronic myelogenous leukemia is hepsulfam (1,7-heptanediol disulfamate) (Fig. 1). Mass spectrometry and UV spectroscopy studies by Colvin and co-workers has shown that hepsulfam can form a number of N7 alkylated products with guanosine including 1,7-bis(guanyl)heptane. In addition, studies with model oligonucleotides revealed that hepsulfam

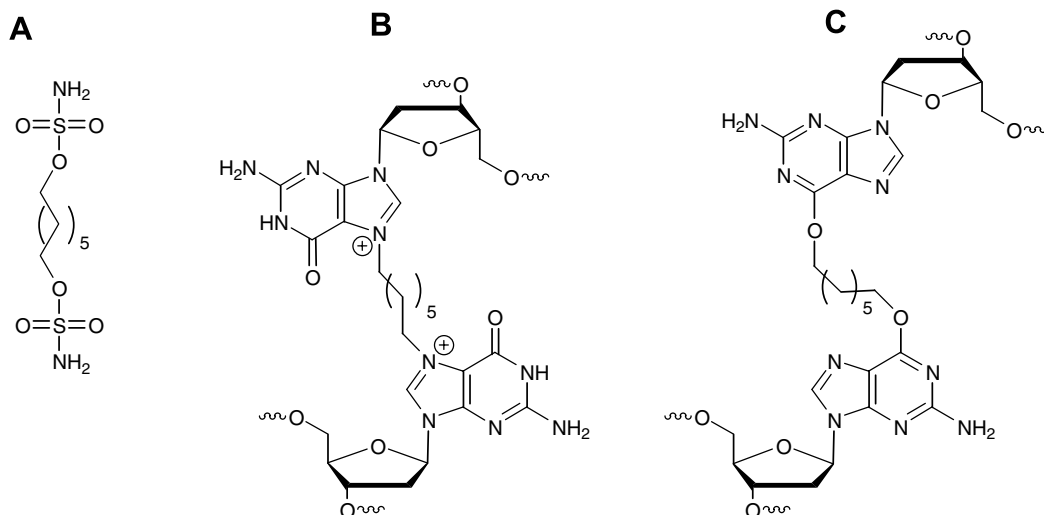
forms interstrand cross-links at 5'-GXC-3' sites.<sup>3</sup> However, the N-7-alkylated bis adducts are unstable with only partial stability imparted to the cross-linked duplex by conversion to the formamidopyrimidyl (FAPY) derivative, which is unstable at room temperature and limits the use of these cross-linked duplexes for structural and biochemical studies to probe DNA repair mechanisms.

This letter describes a methodology to produce interstrand cross-linked DNA, where the alkyl linkage between the two adjacent strands occurs between the O6-positions of deoxyguanosines. The O6 position of deoxyguanosine is a site of alkylation by a number of different agents.<sup>4</sup> It was thought that this may be a structural mimic for the clinically relevant lesion induced in duplex DNA by hepsulfam. In addition, this cross-link should exhibit sufficient chemical stability that would allow for various structural and biochemical investigations related to DNA repair studies. It should be noted that although this O6 cross-linked DNA may serve as a structural mimic of the hepsulfam induced DNA cross-link that occurs at the N7 position of deoxyguanosine, the efficiency and mechanism of DNA repair may be different.

The synthesis of bis-phosphoramidite **6** was accomplished in five steps starting from *N*<sup>2</sup>-phenoxyacetyl-5'-*O*-dimethoxytrityl-3'-*O*-[(allyloxy)carbonyl]-2'-deoxyguanosine **1** (Scheme 1). Although the isobutyryl protecting

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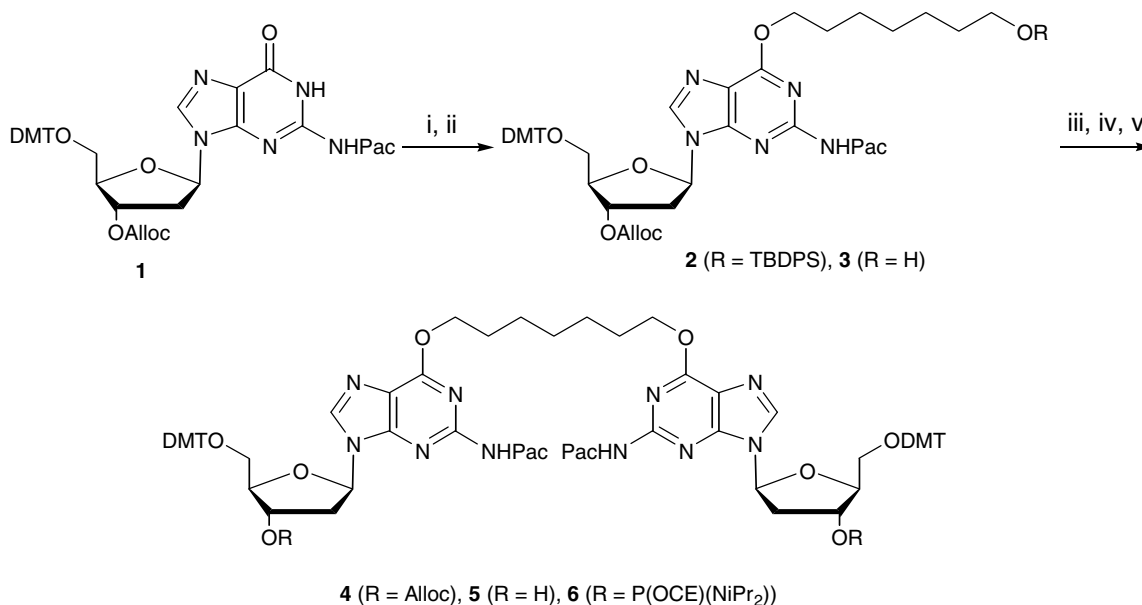


**Figure 1.** Structure of (A) hepsulfam, (B) the interstrand DNA cross-link induced by hepsulfam and (C) the proposed O6-alkyl-O6 deoxyguanosine interstrand cross-link.

group is one of the more popular protecting groups at the N2 position of deoxyguanosine for solid-phase oligonucleotide synthesis, the phenoxyacetyl group would require milder deprotection conditions thus avoiding any potential side reactions that occur in the synthesis of oligonucleotides containing 6-*O*-alkylguanine bases.<sup>5</sup> Compound **1** and 7-(*tert*-butyldiphenylsiloxy)heptanol were converted to **2** via the Mitsunobu reaction with a 74% yield.<sup>6</sup> The *tert*-butyldiphenylsilyl group was then removed using TBAF (1 M in THF) which gave compound **3** in a quantitative yield. Dimer **4** was produced by a second Mitsunobu reaction between **1** and **3** with a yield of 58%. Both 3'-*O*-(alloxy)carbonyl protecting groups were removed from **4** using a catalytic amount of tetrakis(triphenylphosphine)palladium(0) to give compound **5** with a 72% yield,<sup>7</sup> the two 3'-OH function-

alities were then phosphitylated with 3.5 equiv of 2-cyanoethyl *N,N,N',N'*-tetraisopropyl phosphane to give the bis-phosphoramidite **6** in a 70% yield.<sup>8</sup>

Bis-phosphoramidite **6** was used in the assembly of the cross-linked duplex **9**. Bis-phosphoramidites have been used in the synthesis of a number of novel nucleic acid structures including branched nucleic acids and nucleic acid dendrimers.<sup>9</sup> The assembly of the cross-linked oligonucleotide duplex was carried out on a 1  $\mu\text{mol}$  scale using commercially available 3'-*O*-deoxyphosphoramidites (at a concentration of 0.1 M and a coupling time of 120 s). The phenoxyacetyl protecting group at the N2 position of compound **6** makes it necessary to use phenoxyacetic anhydride rather than acetic anhydride as the capping reagent to prevent an undesired



**Scheme 1.** Synthesis of bis-phosphoramidite **6**, where: (i) 7-(*tert*-butyldiphenylsiloxy) heptanol (1.1 equiv), DIAD (1.1 equiv), triphenylphosphine (1.1 equiv), (ii) TBAF (1 M in THF) (1.1 equiv), (iii) **1**, DIAD, triphenylphosphine, (iv) tetrakis(triphenylphosphine)palladium(0) (0.2 equiv), butylamine (5 equiv), formic acid (5 equiv), (v) 2-cyanoethyl *N,N,N',N'*-tetraisopropyl phosphane (3.5 equiv), diisopropylammonium tetrazolidine (3.4 equiv).

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