Further stereochemical information is clearly needed to identify the factor(s) that determine the elimination specificity of terpene cyclases.

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Sequence-Specific Cross-Linking of Deoxyoligonucleotides via Hybridization-Triggered Alkylation

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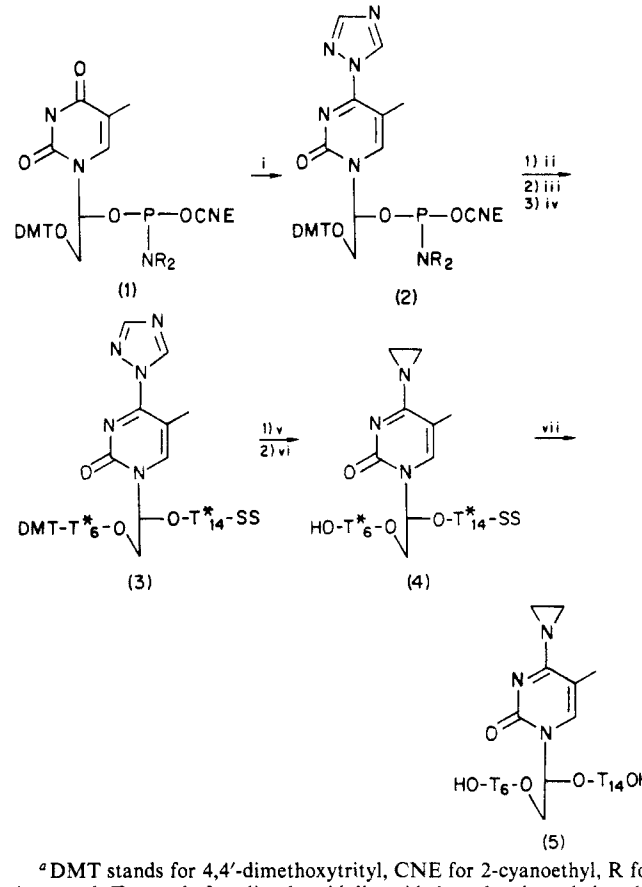
Oligonucleotide analogues that have enhanced binding to a complementary sequence are of potential biological interest. Dimer[1] and oligonucleotide[2] analogues bearing a moiety capable of intercalation show enhanced binding to complementary sequences. Oligonucleotides capable of forming a covalent cross-link with a complementary sequence in a selective manner would be the ultimate in enhanced binding. The incorporation of masked alkylating agents into DNA and RNA has been reported.[3,4] Such systems require the chemical activation of the alkylating moiety and are therefore not compatible with in vivo systems. We have developed an oligodeoxynucleotide analogue that possesses the unique property of being essentially unreactive until it is constrained in a double helix. When so constrained it forms a stable covalent bond with its complementary sequence.

We reasoned that if an analogue of one of the four bases, containing a suitably placed electrophilic center, was constrained in a Watson–Crick bonding scheme, interstrand cross-linking would result due to the proximity of a nucleophilic center on the complementary strand. We chose N^4,N^4-ethanocytosine as the electrophilic base analogue, which ought to be able to form two hydrogen bonds with a guanine base in the target oligomer. We realized that other bases such as adenine or cytosine, which possess nucleophilic amine functions, might also alkylate under these conditions.

The synthesis of deoxyoligonucleotides containing the 5-methyl-N^4,N^4-ethanocytosine (C^e) moiety required some modification of existing synthetic methods. We prepared the amidites **1** and **2** (see Scheme I) and incorporated them in a deoxyoligonucleotide by combining the methods of Huynh-Dinh et al.[5] and Koster et al.[6] (see Scheme I).

Scheme I.[a] Synthesis of the Oligomer Containing 4,4-Ethanocytosine



[a] DMT stands for 4,4′-dimethoxytrityl, CNE for 2-cyanoethyl, R for isopropyl, T_n stands for oligothymidylic acid the subscript n being the number of residues in the oligomer, likewise the * denotes CNE protecting groups on an oligothymidylic acid; SS denotes the solid support (silica gel). (i) Triazole, $POCl_3$ in acetonitrile/triethylamine; (ii) HO–T^*_{14}–SS/4-nitrotetrazole; (iii) I_2/water/lutidine; (iv) six couplings with **1** (addition of T^*_6); (v) 25% dichloroacetic acid in dichloromethane (detritylation); (vi) ethylenimine in water; (vii) concentrated NH_4OH.

Amidite **2** was prepared from **1** and used to prepare the cyanoethyl-protected oligonucleotide **3** by using the standard synthetic cycle.[7,8] The intermediate **3** (attached to silica gel support) was treated with a solution of ethylenimine[9] in water to give the protected oligomer **4**. The intermediate **4** was deprotected at phosphorus and removed from the solid support by brief treatment (~30 min, 25 °C) with concentrated NH_4OH, to yield oligomer **5**. Oligomers **6–10** were prepared by using methoxy-*N*,*N*-diisopropyl amidites[8] by the standard synthetic cycle[7,8] and purified by polyacrylamide gel electrophoresis (20% denaturing gel).

Oligomer **5** (containing the ethanocytidine group) was allowed to hybridize with purified oligomers **6–10**, which had been labeled at the 5′-end with ^{32}P using γ-^{32}P-labeled ATP and polynucleotide kinase (see Figure 1b for conditions). Analysis of these reactions by gel electrophoresis (20% denaturing gel) followed by autoradiography showed the appearance of a higher molecular weight band (Figure 1b). This band **11** was by far most prevalent in the reaction of **5** with **9**, the oligomer-bearing cytosine (Figure 1b, lane 8). A control reaction containing only labeled **5** and buffer did not show any new bands even after 7 days at 24 °C (data not shown). A band of identical mobility to **11** was observed when purified 5′-^{32}P-labeled **5** was allowed to react with unlabeled **9**, demonstrating that this new band was a product of **5** and **9** (data not shown). Product **11** is unaffected by the strongly denaturing

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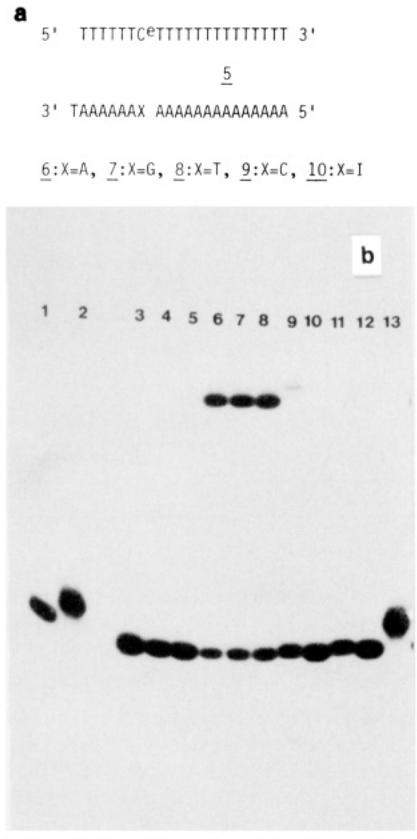

Figure 1. (a) The sequences of the alkylating oligomer **5** and the target oligomers **6**–**10**: A = deoxyadenosine, G = deoxyguanosine, T = thymidine, C = deoxycytidine, I = deoxyinosine. (b) Autoradiogram of 20% denaturing gel. All of these reactions were done in the following buffer: 10 mM NaCl, 10 mM Tris-HCl pH 7.5 2-fold excess of target oligomers (**6**–**10**) at 0 °C for 96 h. An asterisk indicates a ^{32}P label. Lane 1: **9*** [d($A_{14}CA_6T$)] as size standard. Lane 2: **12*** [d(CATCGTCTAGATCTTTTGCCGC)] standard. Lane 3: **5** [d($T_6C^eT_{14}$] which was treated with 80% acetic acid/water for 30 min at 24 °C, then concentrated and allowed to hybridize with **9*** [d($A_{14}CA_6T$)]. Lane 4: **5** [d($T_6C^eT_{14}$) and **9*** [d($A_{14}CA_6T$)] in 50% formamide/buffer. Lane 5: **5** and **9*** in presence of 10-fold excess of unlabeled d(T_{40}). Lane 6: **5** and **9*** in presence of 10-fold excess **12**. Lane 7: The reaction of **5** and **9*** for 96 h (the cross-linked product **11** is the higher band) followed by 100 °C for 5 min. Lane 8: Reaction of **5** [d($T_6C^eT_{14}$)] with **9*** [d($A_{14}CA_6T$)]. Lane 9: Reaction of **5** with **8*** [d($A_{14}TA_6T$)]. Lane 10: Reaction of **5** with **6*** [d($A_{21}T$)]. Lane 11: Reaction of **5** with **7*** [d($A_{14}GA_6T$)]. Lane 12: Reaction of **5** with **10*** [d($A_{14}IA_6T$)]. Lane 13: Reaction of **5** with **12*** [d(CATCGTCTAGATCTTTTGCCGC)].

conditions of 100 °C for 5 min in 50% formamide (Figure 1b, lane 7), strongly suggesting a cross-linked **5** + **9** adduct. The labeled oligomer **5** was stable in dilute buffer solutions at 24 °C for at least 14 days, as evidenced by the fact that the reaction goes to near completion, giving only **11** after this time (data not shown). Only trace amounts of products of similar mobility were present in the reactions of **5** with the other target oligomers, most notably **8** (Figure 1b, lanes 9–12). The reaction of **5** with **9** has a half-life of ~30 h at 24 °C.

Oligomer **5** did not form a cross-linked product with labeled 22-mer d(CATCGTCTAGATCTTTTGCCGC) **12** under conditions identical with the reaction of **5** and **9** (Figure 1b, lane 13), despite the fact that **12** contains seven potentially reactive cytosines. This result is expected since this sequence cannot form a stable duplex with **5**. The observed formation of **11** is inhibited by the presence of either excess T_{40} (Figure 1b, lane 4) or 50% formamide (Figure 1b, lane 5), while excess 22-mer **12** has no effect on the formation of **11** (Figure 1b, lane 6), further demonstrating the requirement for duplex formation. The formation of **11** is dependent on the ethylenimine moiety in oligomer **5**, as demonstrated by the fact that acid-catalyzed ring opening abolishes the cross-linking reaction (Figure 1b, lane 3). Maxam–Gilbert sequencing of **11** gave a pattern consistent with a cross-link to cytosine on the target strand.[10] Since triple-helix formation was likely in this model system, we investigated conditions (excess **6**–**10**) that should give duplex predominantly.[11] In these experiments the results were qualitatively the same as those observed under conditions that favored triplex formation (data not shown).

In summary, we have prepared a synthetic oligodeoxynucleotide **5** which becomes reactive when constrained in a Watson–Crick double helix with **9**. The resulting duplex forms a product (**11**) containing a stable cross-link. The observed selectivity is for cytosine, but so far we have no information on the exact nature of the new bond formed (i.e., N3 vs. N4 linkage). The oligomer **5** is relatively unreactive with amines in solution (e.g., it is not significantly affected by 15 M aqueous ammonia at 22 °C for 30 min) but reactive with a cytosine at the appropriate position of the complementary hybridized oligomer **9**. We hypothesize that this is an example of a significant rate enhancement due to noncovalently bonded (i.e., hydrogen bonded) neighboring group participation.[12] The analogy can be drawn between the model system (**5** and **9**) and the reaction of an enzyme with suitable substrate on an exzyme active site, thus the observed selectivity of **5** for **9** could be explained by sensitive orientation effects or, alternatively, simply by the greater nucleophilicity of cytosine as compared to the other bases. Future experiments will be directed toward answering these questions and to the extension of this system to oligomers containing all four naturally occurring bases.

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Does Ground State Fe^+ React with H_2?

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While numerous investigations of the gas-phase reactions of iron ions have been conducted in recent years,[1-3] none of these has suggested that the reactivity of the ground state may be over an order of magnitude less than that of the excited states. Previous work in our labs on the reactions of H_2 with several states of V^+[4] and Mn^+[5] led us to believe that the ^6D ($(4s)(3d)^6$) ground state and the ^4F ($(3d)^7$) first excited state of Fe^+ should exhibit very different reactivity with H_2 primarily because they differ in their electronic configuration. In the present study, we find that $Fe^+(^4F)$ reacts efficiently with H_2 but $Fe^+(^6D)$ is about 80 times less reactive. This result obtains despite the fact that these states are separted by only 0.25 eV.[6] This work is the first to assess the individual reactivities of such closely spaced electronic levels of a transition-metal ion.[7]

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