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SYNTHESIS AND CROSSLINKING PROPERTIES OF A DEOXYOLIGONUCLEOTIDE CONTAINING N^6,N^6-ETHANODEOXYADENOSINE

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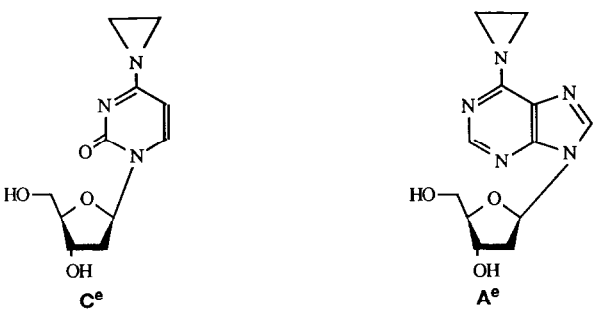
Abstract: We report the synthesis of 5' HO-dT_6(N^6,N^6-ethanodeoxyadenosine)T_{14}. This oligonucleotide specifically crosslinks with 5' HO-$dA_{14}CA_6T$ at pH 7.5 and 20°C with a half-life of approximately one day.

We recently reported the synthesis of deoxyoligonucleotides containing a modified nucleoside N^4,N^4-ethanodeoxycytidine (C^e, Fig. 1)) (1,2). These oligomers have the interesting property of covalently crosslinking to a complementary oligonucleotide upon hybridization. Surprisingly, the crosslinking reaction is most efficient when C^e is opposite a deoxycytidine in the complementary oligomer. Molecular modeling suggests that such a pyrimidine-pyrimidine interaction is not optimum and perhaps a more efficient system would be an adenosine analog opposite deoxycytidine. In this communication we report the synthesis of a deoxyoligonucleotide containing N^6,N^6-ethanodeoxyadenosine (A^e, Fig. 1) and its crosslinking properties with complementary oligomers.



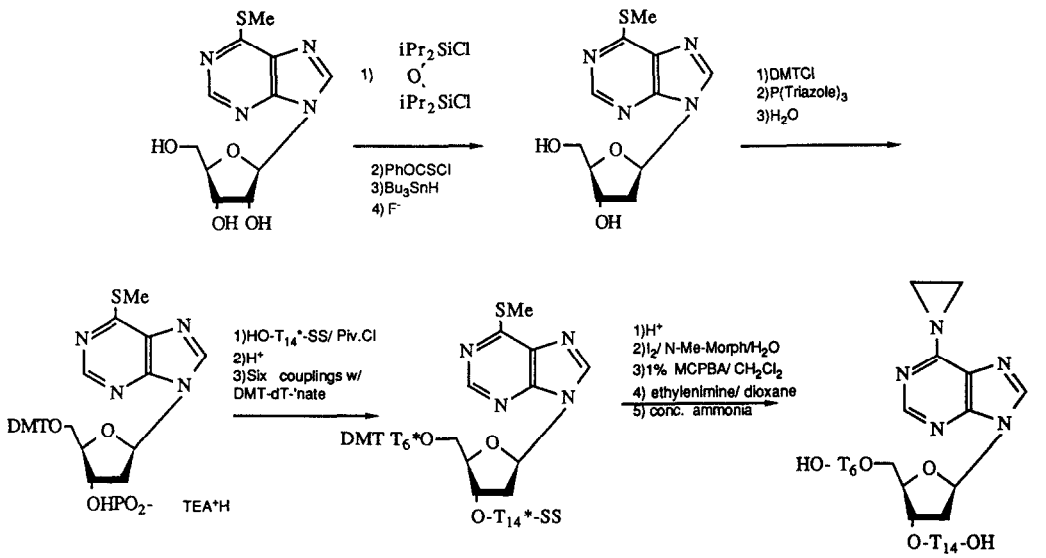
C^e A^e

Fig. 1 Structures of C^e and A^e

The synthesis of oligonucleotide containing Ae is accomplished by first incorporating 6-methylthiopurine deoxyriboside into an oligonucleotide. Wetzel and Eckstein have shown 6-methylthiopurine riboside can be oxidized to the sulfone and N^6-alkyl adenosine derivatives generated by the reaction of amines with the sulfone (3). Our investigation of such a scheme on the commercially available compound, 6-methylthiopurine riboside showed that oxidation of the methylthiopurine proceeded rapidly and quantitatively using MCPBA in CH_2Cl_2 and subsequent displacement with aziridine yielded the N^6,N^6-ethanoadenosine as the major product (isolated yield, 60 percent). This was characterized by H'NMR and FAB Mass Spectra analysis. Interestingly, the ethanoadenosine is significantly more stable to nucleophiles than ethanocytidine. Stability in conc. 7M NH_4OH at 20°C showed half-lives of 4 hrs and greater than 40 hrs for the C and A derivatives, respectively.

The synthesis of deoxyoligonucleotides containing 6-methylthio- purines requires the corresponding deoxynucleoside (Scheme I). This was synthesized in a 4-step straightforward manner from 4 methylthio- purine riboside by the selective deoxygenation procedure of Robbins (yield from riboside was 50 percent) (4). This nucleoside was then protected at the 5' hydroxyl with dimethoxytritylchloride and converted to the 3' triethylammonium H-phosphonate salt via the phosphoryl tris triazolide (yield from deoxynucleoside was 70 percent) (5). This monomer was then suitable for incorporation into deoxyoligonucleotides at distinct positions via the H-phosphonate synthesis method (5).

Synthesis of oligomers containing N^6,N^6-ethanoadenine



Scheme I. DMT is dimethoxytrityl, * denotes a 5'-3' H-phosphonate linkage between nucleosides, Piv.Cl is pivaloyl chloride, MCPBA is meta-chloroperbenzoic acid, and SS denotes an ester linkage to a controlled pore glass polymer support.

Previously we reported the synthesis and crosslinking properties of 5' $T_6(C^e)T_{14}$, therefore we chose to synthesize and test $T_6(A^e)T_{14}$ for this study. This oligomer was obtained by first synthesizing T_6(6-methylthiopurine)T_{15} on a polymer support (5) using the monomer described above (Scheme I). After oxidation of the phosphonate backbone to phosphates with .1M I_2 in N-methylmorpholine, H_2O and THF (1/1/18), the polymer was washed with CH_3CN and treated for 5 minutes with 1 percent MCPBA in CH_2Cl_2, washed thoroughly with CH_2Cl_2 containing 1 percent triethylamine, and dried. Further treatment with aziridine/dioxane (1/1) for 30 mins followed by conc. NH_4OH for 30 minutes produced the A^e analog and removed the oligomer from the controlled pore glass support. The presence of the A^e base was confirmed by radiolabeling the 5'OH of the oligomer with P^{32} and subjecting the product oligomer to 1M piperidine 90°C for 15 minutes. Such treatment of the monomer ethanoadenosine had previously shown a clean ring opening of the aziridine yielding a terminal tertiary piperidine. Such a compound would be expected to be protonated at pH 7.5. Gel electrophoresis at pH 7.5 followed by autoradiography of the piperidine treated oligomer showed a band of slower mobility relative to a sample without piperidine treatment (6).

The crosslinking properties of the ethanodeoxyadenosine containing oligomer were tested with 4 complementary radiolabeled oligomers 5'$dA_{14}XdA_6dT$, where X was dA, dG, dC, or dT. Hybridization reactions were performed in 10mM Tris-HCl (pH 7.5) under conditions identical to those previously reported (1). Aliquots were taken at various times and the results after gel electrophoresis and autoradiography are shown in Figure 2. Extremely long reaction times (21 days) showed no further reaction as compared to 70 hours. A crosslinking reaction is observed and as in the case of C^e is very specific for dC in the opposing strand. The ethanoadenosine undergoes a much slower reaction with an opposing dG and dA and no reaction with an opposing dT. This is in contrast with the ethanocytidine case where a slower reaction was observed with dT but not dG and dA (1). The kinetics of the crosslinking reaction of ethanoadenosine with dC have an approximate half-life of 1 day or very similar to those seen previously with ethanocytidine and dC (1).

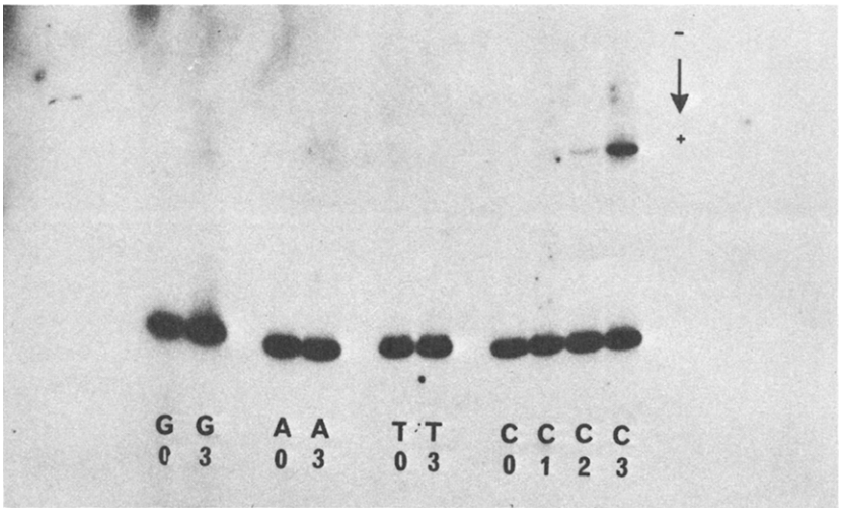


Figure 2. Autoradiogram derived from crosslinking experiment. Reaction consisted of 10 μM HO-$dT_6A^eT_{14}$ and 1 μM radiolabeled p-$dA_{14}XA_6T$ (X=dA, dC, dG, dT) in 10 mM Tris-HCl (pH 7.5). Letters on lanes denote the X of the radiolabeled oligomer and numbers denote reaction time of aliquot: 1; 0.5 hours. 2; 5 hours. 3; 70 hours.

The relative stability of ethanodeoxyadenosine to ammonia should allow for the synthesis of A^e deoxyoligonucleotides containing the four natural heterocycles. The recently introduced exocyclic amino protecting groups (isobutyrl for deoxycytidine and phenoxyacetyl for deoxyadenosine and deoxyguanosine) are completely removed by a four-hour room temperature treatment with concentrated ammonia (7,8). These conditions should result in only trace decomposition of ethanodeoxyadenosine in an oligomer.

The hope in this experiment was that by positioning the ethanoadenosine alkylating group opposite a dC that one would obtain a more "normal" purine-pyrimidine spacing and a faster rate of crosslinking. This was not observed. It is interesting to note that monomer ethanodeoxyadenosine appears to be about 10-fold less reactive to nucleophiles (ammonia) than ethanodeoxycytidine. Given that the two analogs crosslink to dC at about the same rate when constrained in a duplex suggests that a selectivity enhancement is being observed with ethanodeoxyadenosine. The goal of obtaining an oligomer which will crosslink on a biological time scale awaits further analog development.

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