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The Oxyamino-Aldehyde Coupling Reaction: An Efficient Method for the Derivatization of Oligonucleotides

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Abstract: Oligonucleotides containing an aldehydic function at any preselected position in the sequence have been prepared using post-synthetic oxidation of an alkene as the key reaction. These were efficiently coupled to a reporter molecule tethered to an oxyamino linker. © 1997 Elsevier Science Ltd.

The functionalization of oligodeoxyribonucleotides has found wide applications with the rapid growth of biotechnologies. These include incorporation of fluorescent, lipophilic, intercalating, crosslinking, alkylating or DNA cleaving entities into oligonucleotides.[1] Such derivatizations require site-specific modification of the oligonucleotide and a number of procedures have been described.[1] Most strategies involve preliminary attachment of a nucleophile-bearing tether to a monomeric nucleoside, which is then incorporated into DNA by automated synthesis, and subsequent linkage of the reporter to the tethered nucleophile. The nucleophile most generally used is a primary amine or a thiol.[1]

Electrophilic functions have been rarely introduced into synthetic DNA. Modifications at the 5'-OH of the terminal residue with an aldehyde or carboxylic acid have been reported by Kremsky *et al.*[2] and more recently by Jones and Coll.[3] These methods however are limited to the derivatization at the 5'-end of the oligonucleotide. For functionalization at preselected positions inside the sequence, the aldehydic function of the abasic site has been used by Imbach[4] and more recently by Mirzabekov.[5] However, these methods require a further reduction step after reaction of the aldehyde with amino or hydrazino compounds. Furthermore, destabilization of the double stranded oligonucleotide was induced by the abasic site.

In this paper, we report incorporation of an aldehydic group at any preselected position of the oligodeoxyribonucleotide and a rapid and convenient derivatization by an oxyamino label.

1/ Preparation of the aldehydic oligonucleotide.

The C_8 position of adenine was selected for the introduction of the tether functionalized at the extremity by the aldehyde precursor. The attachment of the linker could be accomplished via alkylation of the thiol **1** which was readily prepared from 8-bromodeoxyadenosine.[6] The aldehyde must be masked during the oligonucleotide

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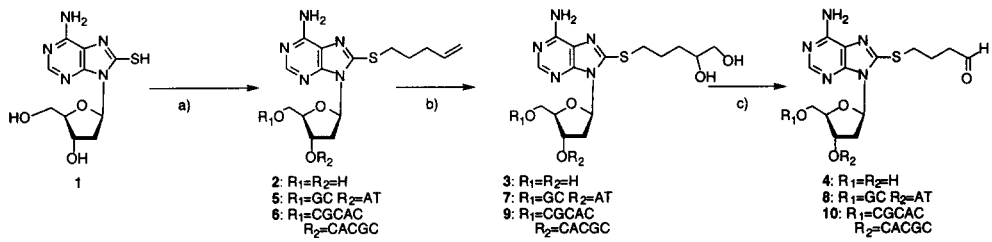
synthesis. However, use of the classical acetal protection was found to be unsuitable as the acidic deprotection step led to depurination. We thus used a post-oxidation strategy. From a retro synthetic point of view, the aldehydic function can be viewed as arising from a 1,2 diol resulting from oxidation of an alkene. Incorporation of a protected diol has been reported by some authors [2, 3]. In our case, we decided to incorporate directly the alkene as it is quite suitable and versatile for oligonucleotide synthesis. Compound **2** was prepared from thiol **1** by alkylation with 1-bromo-4-pentene in basic conditions.

Scheme 1



a) 1-Bromo-4-pentene, K_2CO_3, DMF b) OsO_4, NMMO, H_2O_2, c) $NaIO_4$, H_2O, dark

Oxidation of the alkene into the diol was first studied at the nucleoside level. Oxidation of **2** was performed at room temperature in the dark in a acetone/water solution using a catalytic amount of osmium tetroxyde (0.04 eq.) in the presence of N-methylmorpholine-N-oxyde (NMNO) and hydrogen peroxyde. The reaction was found to be highly selective affording quantitatively diol **3** (89 % yield in isolated product). In these conditions, no oxidation at sulfur could be detected. The aldehydic function was then generated from the vicinal diol using sodium periodate (4 eq.) in water. The HPLC analysis showed exclusive formation of the aldehyde **4** in a very short time ($t_{1/2}$ < 15 min). Compound **4** was purified by semipreparative HPLC using reverse phase and obtained in a 63% yield.[7] The structure of **4** was confirmed by ^{1}H-NMR and MS.

The phosphoramidite derivative of **2** was then prepared for the automated oligonucleotides synthesis. The exocyclic amine on compound **2** was first benzoylated using benzoyl chloride and subsequent basic treatment to cleave the formed ester on the 3' and 5' hydroxyl groups of the sugar. Standard dimethoxytritylation and phosphitylation yielded the phosphoramidite synthon.

Two alkenyl containing oligonucleotides were then synthetized according to standard β-cyanoethyl phosphoramidite chemistry: the 5-mer d(GCXAT) **5** containing the four natural bases and the 11-mer d(CGCACXCACGC) **6**. After deprotection with conc. ammonia for 2 days at r.t., the oligomers **5** and **6** were purified by reverse phase HPLC. Figure 1(A) shows the HPLC profile of the purified oligonucleotide **5**. The bishydroxylation of the double bond was first performed on the oligomer **5**. The reaction was carried out in water in the presence of OsO_4 (0.4 eq) and N-Methylmorpholine N-oxyde at room temperature overnight. Figure 1(B) shows the crude mixture. The reaction was found to be selective affording the oligonucleotide diol **7** which was purified by HPLC. The structure of **7** was established by ^{1}H NMR.[9] In particular, we observed the disappearance of the signals corresponding to the ethylenic protons at 5.06 and 5.86 ppm. The diol was then cleaved oxidatively with 100 eq. of $NaIO_4$ to give aldehyde **8**. As indicated in figure 1(C), the reaction was very clean leading selectively to the oligo-aldehyde **8**. It was noted that even with this large excess of $NaIO_4$ no

oxidation at sulfur was observed. The same procedure was used to prepare the 11-mer **10** (scheme 1). In this case, the structure of the intermediate diol **9** was confirmed by ES-MS.

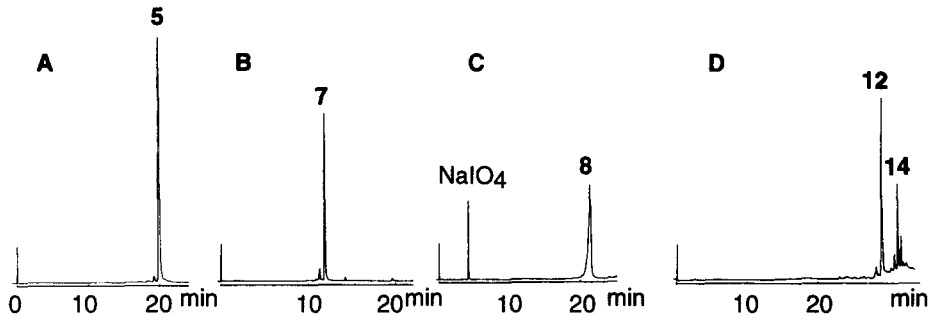


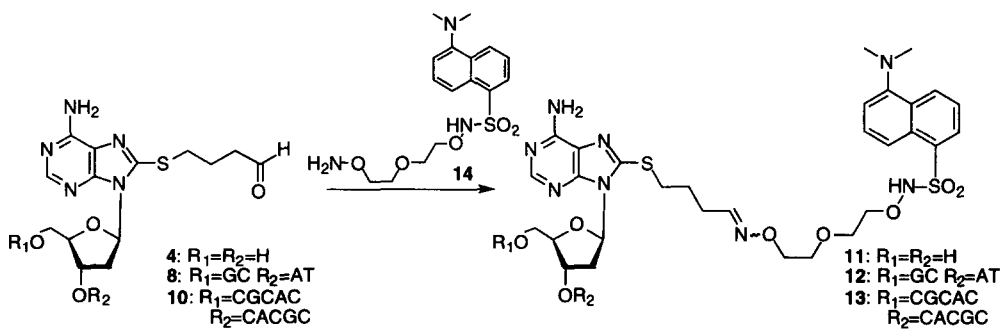
Figure 1: HPLC profiles of A) purified 5-mer **5**, B) crude mixture of the bishydroxylation reaction, C) crude mixture of **8**, D) crude mixture of **12**.

Due to slow decomposition of the aldehyde containing oligonucleotides **8** and **10**, the coupling reaction with the oxyamino label was performed immediately after their obtention. Thus, the structure of the aldehydic oligonucleotides was confirmed by formation of the corresponding oxime ether derivative.

2/ Coupling reaction with the oxyamino probe **14**:

We have recently reported synthesis of the fluorescent label **14** and its reactivity with aldehydic carbonyl groups.[10] Reaction conditions for coupling this oxyamino label were first studied at the nucleoside level. Reaction of the oxyamino probe **14** with **4** was performed in DMF in the dark at room temperature using a slight excess of the oxyamine probe and afforded selectively the coupling product **11**. The structure of **11** was determined by ^{1}H-NMR using COSY techniques and showed that a diastereoisomeric mixture of Z/E compounds in a 50/50 ratio was obtained. The structure of **11** was also confirmed by HR-MS. In regards of this result, the reaction with the oxyamino probe **14** was then performed with the oligonucleotides **8** and **10**. Reactions were carried out in water with a slight excess of oxyamino compound and, as shown in figure 1(D), afforded selectively the oxime ether product. In the case of the 5-mer the structure was established by ^{1}H-NMR[9] and again indicated that a diastereoisomeric mixture of Z/E compounds was obtained (scheme 2).

Scheme 2



In conclusion, reaction between an aldehyde containing oligonucleotide with oxyamino derivatives was found to be highly selective and rapid ($t_{1/2}$ < 30 min). Such efficient derivatization will be of a great interest as it is of a simple use and allows introduction of numerous kinds of reporter molecules at any preselected position.

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