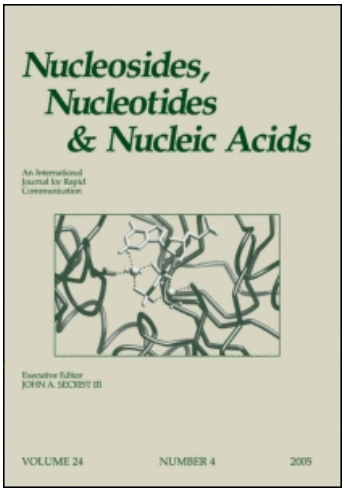

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Aldehyde Functions in Synthetic Oligonucleotides

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ALDEHYDE FUNCTIONS IN SYNTHETIC OLIGONUCLEOTIDES

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Abstract: new nucleoside analogs with a masked aldehyde function have been synthesized and incorporated in oligodeoxyribonucléotides. After unmasking the aldehyde function, the oligomers were easily coupled to biotin derivatives by reductive amination. Reversed phase HPLC proved to be an unvaluable tool to characterize and purify these conjugates.

It is often essential to introduce reactive functions on oligodeoxyribonucleotides. Indeed, cold labeling, immobilisation on a solid support, hybridization-triggered reactions of a synthetic oligonucleotide with a complementary target or affinity labeling of proteins related to DNA metabolism require a site-specific modification of the oligomer. One procedure is to introduce a primary alkylamino function into synthetic DNA. Numerous conjugates were obtained by this route. Electrophilic functions were rarely introduced into synthetic DNA, although they are required for protein affinity labeling.

Lemaitre et al.[1] oxidized a 3'-terminal ribose residue to generate a bis-aldehyde function at the 3'-end of antisense DNA. Kremsky et al.[2] coupled an aldehyde function to the 5'-end of an oligonucleotide. We describe here a method to attach one or several aldehyde functions at any position of a synthetic oligomer. This approach keeps the 5'-OH function free, so that a radioisotope labeling by a kinase remains feasible. It also allows the introduction of a run of aldehyde functions, if multiple conjugation is required.

Our strategy of synthesis is sketched in Figures 1 and 2. On a retrosynthetic point of view, the aldehyde function is made from a glycol that can be suitably protected during oligonucleotide synthesis, and the glycol is

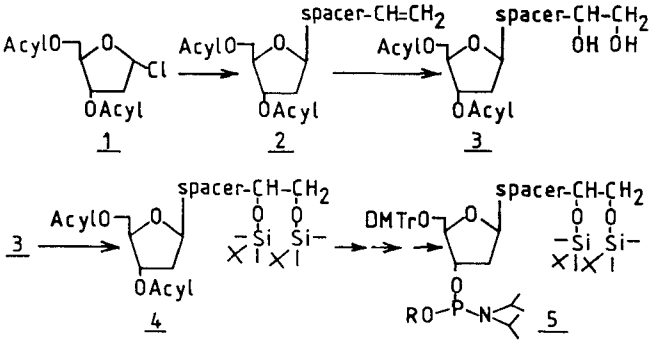

FIGURE 1

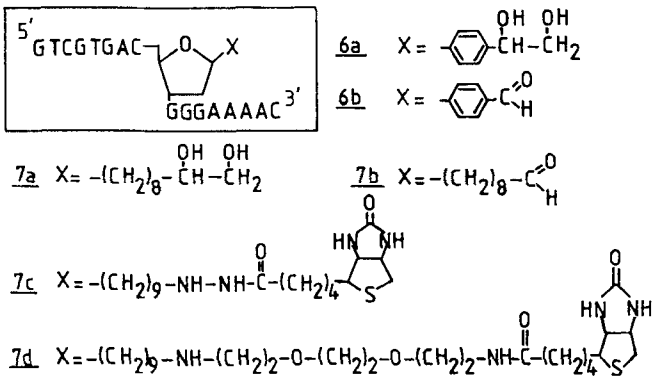

FIGURE 2

generated from an alkene. The required nucleoside analog is easily obtained by coupling a cadmium organometallic derivative of an alkene to O-acylated α-1-chloro-1,2-dideoxy-D-ribofuranose. Typical procedures concerning these synthesis can be found in our previous publications[3].

At the oligonucleotide level, key steps are the oxidation of the glycol and the reductive amination. The aldehyde function was generated from the vicinal diol according to the protocol of Bayard et al.[4]. When 2.8 nmoles of our sequences were oxidized by $NaIO_4$ (2.5 ml of a 1.6 mM solution in 0.02 M sodium acetate buffer, pH 4.75) at 0°c for 5h in the dark, and then reduced in situ by $NaBT_4$ (+ 2.5 ml of a 10 mM solution in 0.01 M sodium borate buffer, pH 9) at 4°c for 5 h, the tritium was incorporated into the oligomers. The yield

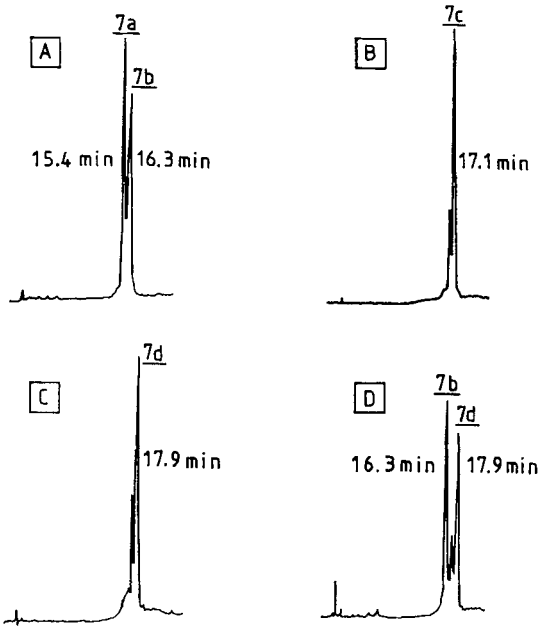

FIGURE 3

Reversed phase HPLC traces. **A**, a mixture of **7a** and **7b**; **B**, crude **7c**; **C**, crude **7d**; **D**, a mixture of **7b** and crude **7d**. Detection at 260 nm. BIORAD Bio-Sil C18 HL 150x4.6 mm column. Linear gradient of eluent II (triethylammonium acetate 0.1M in CH_3CN:H2O, 60/40) in eluent I (triethylammonium acetate 0.1M in CH_3CN:H2O, 5/95), from 0 to 30% in 25 min. Flow, 1ml/min.

of incorporation was determined by passing the sample through two successive NAP-10 cartridges, isolating the high molecular weight fraction and determining both the A_{260} (2.5 nmoles of DNA recovered) and the immobilized tritium content (2.37 nmoles, by scintillation counting). The tritium incorporation yield was 95%. The oxidation of the diol **7a** to the aldehyde **7b** was also conveniently monitored by reversed phase HPLC (Figure 3). Trace A corresponds to a mixture of **7a** and **7b**.

Next, **7b** was coupled by reductive amination ($NaBH_3CN$) with amino-derivatives of biotin (biotin hydrazide from Sigma, to give **7c**, and Biotin-DADOO from Boehringer Mannheim, to give **7d**). This coupling was also monitored by reversed phase HPLC. Trace B corresponds to crude **7c**, and trace C to crude **7d**. Trace D corresponds to a mixture of crude **7c** and the starting aldehyde **7b**.

As a proof of structure, the major peak of trace C was isolated. Aliquots of this oligonucleotide were treated by increasing amounts of avidine, and ultrafiltrated (ULTRAFREE-MC from Millipore, membrane UFC3-LTK, cutoff 30.000). The amount of oligonucleotide in the filtrate was determined by HPLC. This titration by avidine demonstrated that one molecule of biotin was immobilized per oligomer.

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