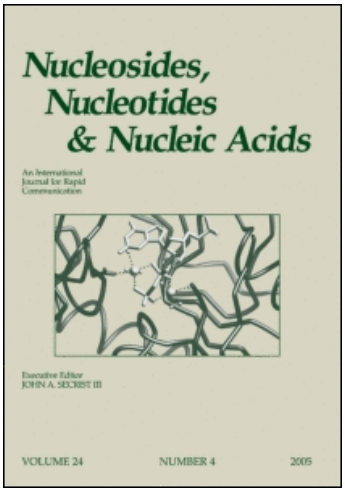

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PHOSPHORAMIDITES OF [^{18}O] CHIRAL (Rp)- AND (Sp)-CONFIGURATED DIMER-BLOCKS AND THEIR USE IN AUTOMATED OLIGONUCLEOTIDE SYNTHESIS

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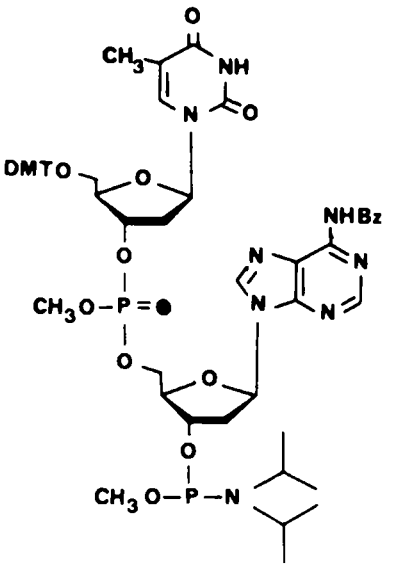
Summary: The N,N-diisopropylphosphoramidites 1 and 2 of appropriately protected chiral diastereoisomers of d(T-[P-^{18}O]-A) (6a and 6b, resp.) have been synthesized. They were employed in solid-phase synthesis to yield the octamers d(GAGT-(Rp)-[P^{18}O]-ACTC) and d(GAGT-(Sp)-[P^{18}O]-ACTC).

Nucleotides with chiral phosphate groups generated by O-labelling ('oxygen chiral isotopes') are useful probes for the elucidation of the stereochemical course of enzymatically catalyzed hydrolytic phosphodiester cleavage. However, the solid-phase method employing monomeric phosphoramidites[1] does not allow the incorporation of [^{18}O] phosphate in a stereochemically controlled fashion.

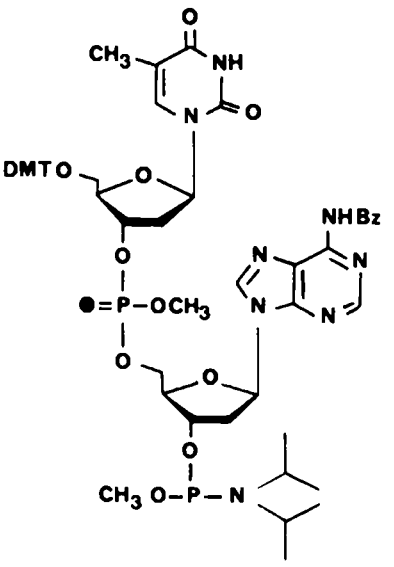
In order to incorporate oxygen chiral phosphodiester moieties into DNA-fragments, appropriately protected and optically pure [^{18}O] chiral (Rp)- and (Sp)-configurated phosphoramidites, such as 1 and 2, have been synthesized[2].

Condensation of the $(MeO)_2$Tr-thymidine phosphoramidite with 3'-silylated N^6-benzoyl-2'-deoxyadenosine in MeCN yielded the diastereomeric phosphite triesters 3. These esters were not isolated, but were oxidized with H_2[^{18}O]/I_2 to yield the diastereomeric phosphate triesters 4a/b. All effords to separate these fully protected diastereoisomers failed.

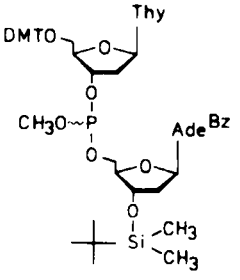
The 4a,b t.-butyl-dimethylsilyl residue was then selectively removed by the action of Bu_4NF to yield compounds 5a/b, which



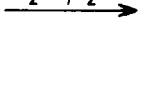
1 (S_P)

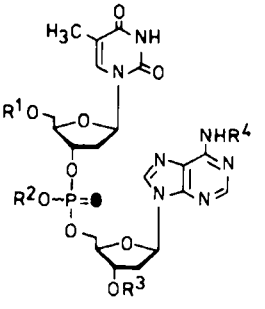


2 (R_P)



3





4-7a/b

	R^1	R^2	R^3	R^4
4a,b	$(MeO)_2Tr$	Me	$(t\text{-}Bu)Me_2Si$	Bz
5a,b	$(MeO)_2Tr$	Me	H	Bz
6a,b	H	H	H	H
7a,b	H	Me	H	H

a,b: opposite configuration at P(V)

showed a good separation on silica gel employing flash-chromatography. The pure diastereoisomers 5a and 5b were isolated in a product ratio of about 1:1.

To establish the absolute configuration at the P-atom of compounds 5a and 5b, they were converted into the fully deprotected compounds 6a and 6b. These compounds were then methylated at the phosphate moiety. A 1:1 mixture of [^{18}O]-labelled and unlabelled d(T-A) was analyzed by ^{31}P NMR spectroscopy.

These methylation products of d(T[P-^{18}O]-A)(7a)/d(T-A) and of d(T[P-^{18}O]-A)(7b)/d(T-A) showed different ^{31}P NMR peak patterns (Fig. 1a,b). Since the methylation experiment was carried out previously with d(T-(Rp)-[P^{18}O]-A)[3] the ^{31}P NMR pattern of this methylation product was essentially identical to that exhibited in Fig. 1b. Hence 6b has (Rp)-configuration. After configurational assignment compounds 5a and 5b were phosphinylated at the 3'-position. To study the utility of the phosphoramidites 1 and 2, solid-phase synthesis of the octamers 8 and 9 was carried out. The synthesis was performed on a DNA-synthesizer using Fractosil-500 bound N-benzoyl-5'-O-dimethoxytrityl-2'-deoxycytidine as polymeric support. The cycles of oligomerization followed a protocoll developed by Beaucage and Caruthers[1]. In the third reaction cycle the phosphoramidites 1 and 2 were employed as coupling blocks instead of the monomeric phosphoramidites[4].

d(G-A-G-T-(Rp)-[P^{18}O]-A-C-T-C) d(G-A-G-T-(Sp)-[P^{18}O]-A-C-T-C)

8 9

The efficient synthesis of the octamers 8 and 9, resp., demonstrates that the dimer blocks 1 and 2 are as applicable as monomeric phosphoramidites.

The nucleoside content of the oligomers was confirmed by enzymatic cleavage with snake venom phosphodiesterase followed by alkaline phosphatase and was analyzed on reverse-phase

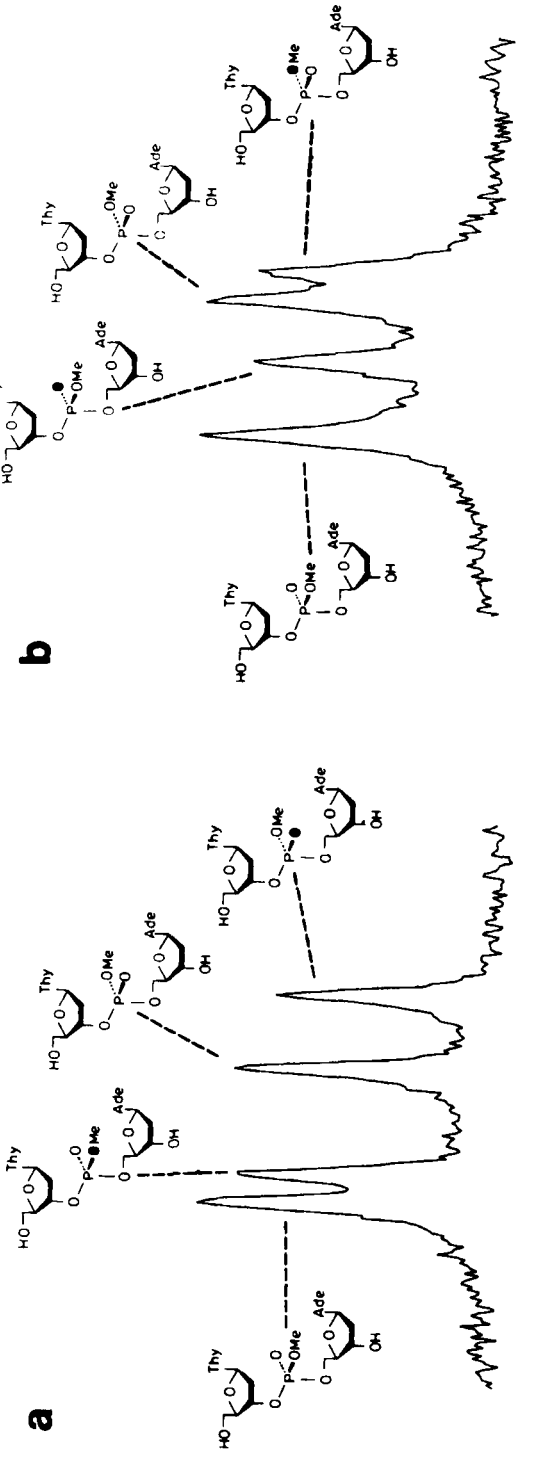


Fig. 1. ^{31}P NMR spectra of 1:1 mixtures of d(T-A) methyl-esters and d(T[pI^{18}O]-A) methyl esters in (D6)Me_2SO.

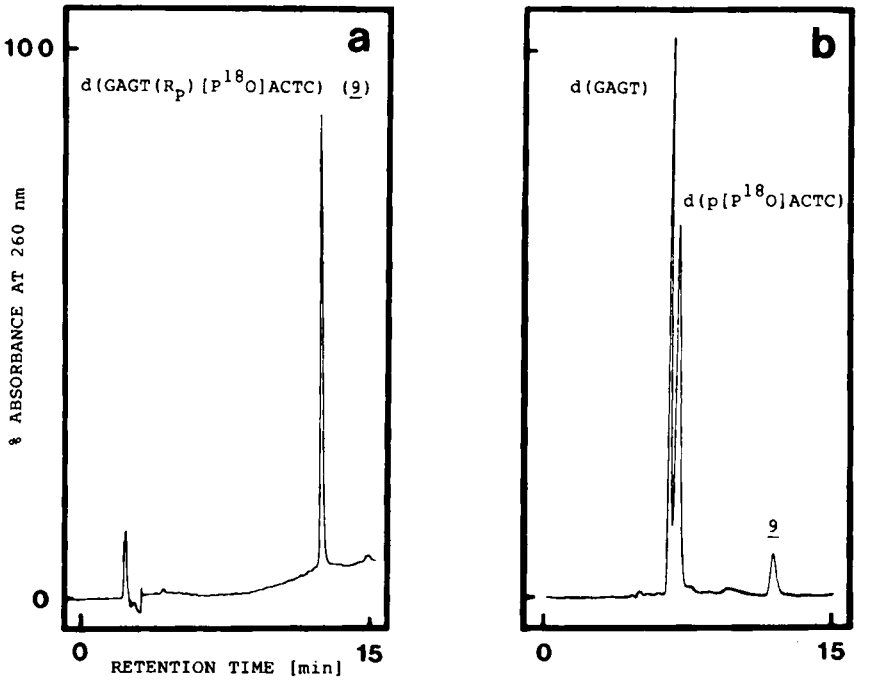


Fig. 2. HPLC-elution profiles of a) the purified oligomer 9 and b) of 9 digested by the endodeoxyribonuclease Rsa I (20^{0}C; 10 mM TRIS-HCl, pH 7.9, 6 mM $MgCl_2$; 5 h).

HPLC. The oligomers 8 and 9 are self-complementary and formed duplexes under appropriate salt conditions. Both exhibited sigmoidal melting profiles with T_M's of 31^{0}C. Due to the sequence d(GTAC), which represents the recognition site of the endodeoxyribonuclease Rsa I, the oligomers should be cleaved enzymatically between dT and dA. As demonstrated in Fig. 2, specific cleavage was observed yielding d(GAGT) and d(p-[P^{18}O]-ACTC).

The same experiments are now carried out in H_2[^{17}O]resulting in chiral d(p-[P^{16}O, ^{17}O, ^{18}O]ACTC). Configurational analysis of[P^{16}O, ^{17}O, ^{18}O]d(AMP), which allows the determination of the stereochemical course of the enzyme, is under investigation.

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