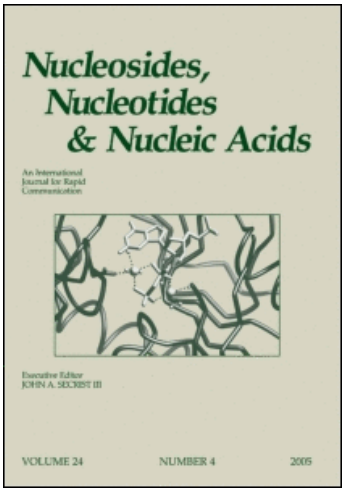

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Oxygen Chiral Phosphate in Ribo- and 2'-Deoxyribodinucleoside Monophosphates by Oxidation of Phosphite Intermediates

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OXYGEN CHIRAL PHOSPHATE IN RIBO- AND 2'-DEOXYRIBODINUCLEOSIDE MONOPHOSPHATES BY OXIDATION OF PHOSPHITE INTERMEDIATES

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ABSTRACT: Oxidation of dinucleoside monophosphite triesters of ribo- and deoxyribonucleosides with iodine-[^{18}O]H_2O furnished diastereoisomeric phosphate triesters having the oxygen labels in the P=O group. Chromatographic separation of the isomers followed by deprotection yielded oxygen chiral dinucleoside monophosphates. The absolute configuration of [^{18}O]UpA has been established.

The elucidation of the stereochemical course of nucleotide and polynucleotide processing enzymatic reactions has been accomplished by the use of phosphorthioates[1]. Advances have been made in the study of these reactions by employing oxygen labelled phosphate esters[2]. The synthesis of a stereospecifically oxygen labelled phosphate group in the internucleotide bond of a dinucleoside monophosphate has been accomplished by the stereospecific replacement of sulfur in a deoxydinucleoside phosphorthioate diastereoisomer. We report a novel facile, universal method for the stereochemically controlled oxygen labeling of ribo- and deoxyribodinucleoside monophosphates[3]: As depicted in the formula the isotopomers of [^{18}O]uridylyl(3',5')adenosine containing an oxygen chiral phosphate were obtained from a dinucleoside monophosphite triester by oxidation with iodine-[^{18}O]H_2O.

The synthesis of the phosphite 1 was achieved by condensation of the 3'-phosphoramidite of 2',5'-bis-tert-butyl-dimethylsilyl-uridine with N^6-benzoyl-2',3'-bis-tert-butyl-dimethylsilyl-adenosine. After oxidation with iodine-[^{18}O]H_2O the diastereoisomers 2 and 3 were separated

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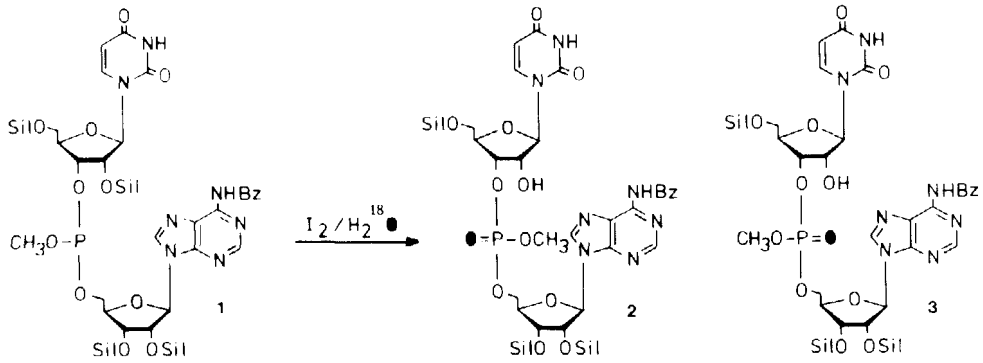
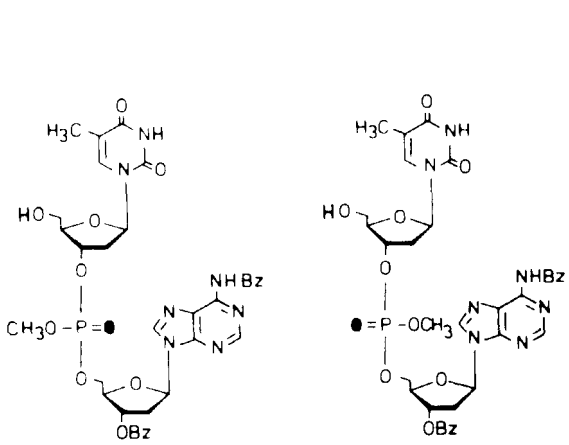


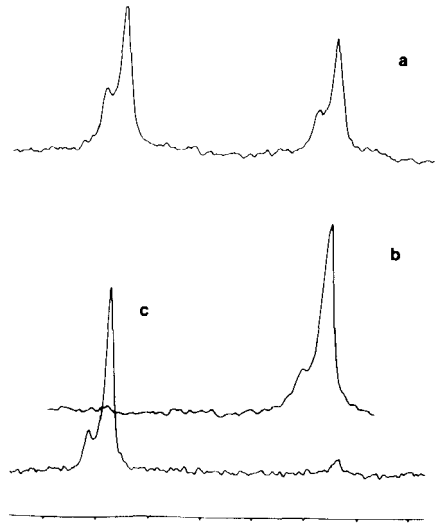
chromatographically. The ratio of the reaction products depends strongly on the method and conditions of condensation. The methyl group was cleaved off by the action of thiophenol-triethylamine, the benzoyl groups were removed with ammonia and desilylation was accomplished with tetrabutylammonium fluoride. The resulting UpA's were purified on a DEAE-Sephadex column. The structure of the isomers was confirmed by ^{1}H and ^{13}C NMR spectroscopy. Cleavage with phosphodiesterase produced uridine and adenosine 5'-monophosphate. The incorporation of ^{18}O isotope was confirmed by ^{31}P NMR spectroscopy. An upfield isotopic shift (2.4 Hz) of the $^{18}O=P$ signal compared to that of unlabelled material was observed in aqueous EDTA.

Hydrolysis of the major isotopomer with nuclease P1 in $[^{17}O]H_2O$ with inversion of configuration yielded $[^{16}O,^{17}O,^{18}O]$AMP. This was converted into the isotopomers of the cyclic 3',5' phosphate with inversion of configuration. Methylation of the latter followed by ^{31}P NMR spectroscopy established the absolute isotopic configuration of $[^{16}O,^{17}O,^{18}O]$AMP as S_P. Therefore the absolute configuration of the major isomer (3) of the phosphite oxidation was S_P. Consequently, the corresponding $[^{17}O, ^{18}O]$ UpA has the R_P configuration.

Similar to the synthesis of chiral ^{18}O-labelled UpA the ^{18}O-labelled protected dinucleoside monophosphates d(CpA), d(TpA), and d(ApA) have been prepared. The corresponding $[^{18}O]$ d(GpA) has been described recently[4]. In contrast to the ribodinucleoside monophosphates the deoxy compounds are difficult to separate. However, separation was achieved after 5'-detritylation employing flash chromatography.



4(R_P,S_P)



The figure exhibits the ^{31}P NMR signals of detritylated compound 4 (R_P, S_P) as a diastereomeric mixture (a) and as separated isomers (b)[faster migrating zone] and (c)[slower migrating zone]. Configurational analysis of the chiral oxygen labelled deoxynucleoside monophosphates is under investigation.

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