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^{17}O NMR OF DIASTEREOMERIC 3',5'-CYCLIC THYMIDINE METHYL PHOSPHATES, METHYLPHOSPHONATES, AND N,N-DIMETHYL PHOSPHORAMIDATES. PHOSPHORUS CONFIGURATION OF P-CHIRAL [^{17}O, ^{18}O]-NUCLEOSIDE PHOSPHATE DIESTERS

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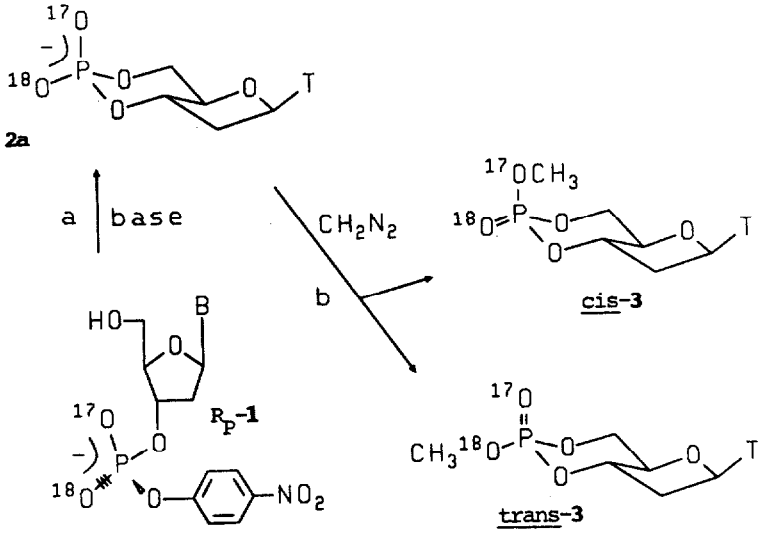
Summary: ^{17}O NMR spectra of the title compounds show well-separated P=O^{17} peaks and configurationally diagnostic $\delta^{17}O$ values and line widths. Use of these features to assign configurational purity to (^{17}O, ^{18}O) P-chiral nucleoside monophosphate diesters is proposed.

^{17}O NMR has become of practical use only in the past few years but is now routinely available through present-day, commercial, high-field instruments. It has been applied to nucleotides to reveal the position on phosphorus of ^{17}O label and the degree of negative charge on oxygen.[1] Furthermore, ^{17}O NMR has had limited use in the study of enzymic phosphoryl transfer reactions, particularly in the characterization of the configurations of ^{17}O-labeled, P-chiral phosphodiesters. Examples are the thymidine cyclic 3',5'-monophosphates, **2a** and **2b**, for which the chemical shifts of the axial and equatorial ^{17}O atoms differ by 1.9 ppm.[1d] Cyclic diester **2a** (^{17}O axial) is formed on the stereospecific (inversion) cyclization (reaction 1a) of the P-chiral 4-nitrophenyl thymidine diester, R_P-**1**.



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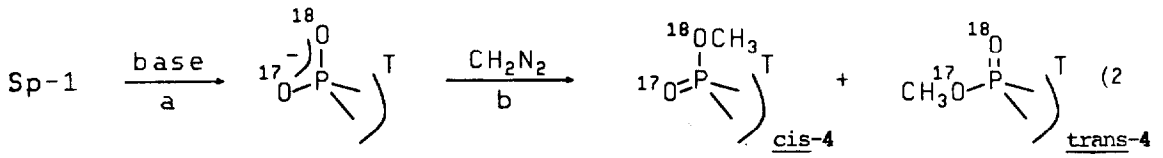
The latter is a useful substrate in the study of the stereochemistry at phosphorus of 3'-phosphodiesterases.[2] Cyclization of S_P-**1** (reaction 2a) yields **2b** (^{17}O equatorial). From the ratio of ^{17}O (axial)/^{17}O(equatorial) peak intensities (**2a**/**2b**), one can <u>in principle</u> very readily determine the optical purity of the chiral phosphorus in **1**.

This technique has in fact been used to assign the configuration and approximate the configurational purity of R_P-**1**. However, as was pointed out,[1d] it could not give a true



assessment of configurational purity at 36.6 MHz (270 MHz ^{1}H instrument), even with ^{31}P decoupling, because of overlap resulting from the small chemical shifts difference and the quadrupolar broadening of the resonance lines. Higher fields can give only partial further resolution of the closely spaced, broad resonances. The corresponding P-chiral diester with the 4-nitrophenyl group attached at the 5' position is similarly used in 5'-phosphodiesterase investigations[3] and presumably could likewise be cyclized to **2** from which its configurational purity potentially would be determined.

Cyclic diesters such as **2** are routinely and quantitatively methylated[1d,4] by CH_2N_2 to obtain the methyl triesters (**3** and **4**, equations 1b and 2b) in the widely used, methylation/cyclization, ^{31}P NMR method[2,4,5] for assignment of phosphorus configuration to (^{16}O, ^{17}O, ^{18}O) P-chiral nucleoside monoesters such as thymidine 3'- and 5'-monophosphate. We report here that for the labeled triesters **trans-3** and **cis-4** the individual axial or equatorial P=O[17] functionalities will have distinctly different ^{17}O chemical shifts. In the Table are recorded data for the analogous (^{17}O, ^{16}O)-triesters, **7** and **8**, prepared by O_2/AIBN[6] or H_2O/I_2[7] oxidation of the ^{16}O- or ^{17}O-phosphite triester,[8] **5** or **6**, equations 3 and 4. The

Table. ^{17}O NMR Parameters for 7-12.

Compound	Solvent	T, °C	$\delta^{17}O$[a]	J_{PO}[b]	Line Width[c]
7	CD_3CN	80	87.7	160	46
8	CD_3CN	80	78.2	156	73
9	CD_3CN	80	29.1[d]		227
10	CD_3CN	80	24.7[d]		310
11	CD_3CN	80	86.2	156	55
12	CD_3CN	80	96.0	160	61
13	$CDCl_3$	60	119	167	157
14	$CDCl_3$	60	116[d]		352

[a] In ppm downfield from external H_2O. Accurate to ± 0.2 ppm. [b] In Hz. Accurate to ± 10 Hz. [c] Line width of $^{17}O\{^{31}P\}$ spectrum. [d] J_{PO} within line width.

resonances for **7** and **8** are well-separated at 54.2 MHz (Figure); and major differences are seen in the P-decoupled peak line widths.

We propose that the cyclization of **1** to **2** shown, followed by methylation (equations 1 and 2) and determination of the ^{17}O NMR spectrum of the resultant set of labeled diastereomeric methyl triesters, trans-**3** and cis-**4**, presents the most efficient way to determine accurately the absolute configuration and configurational purity of isotopically

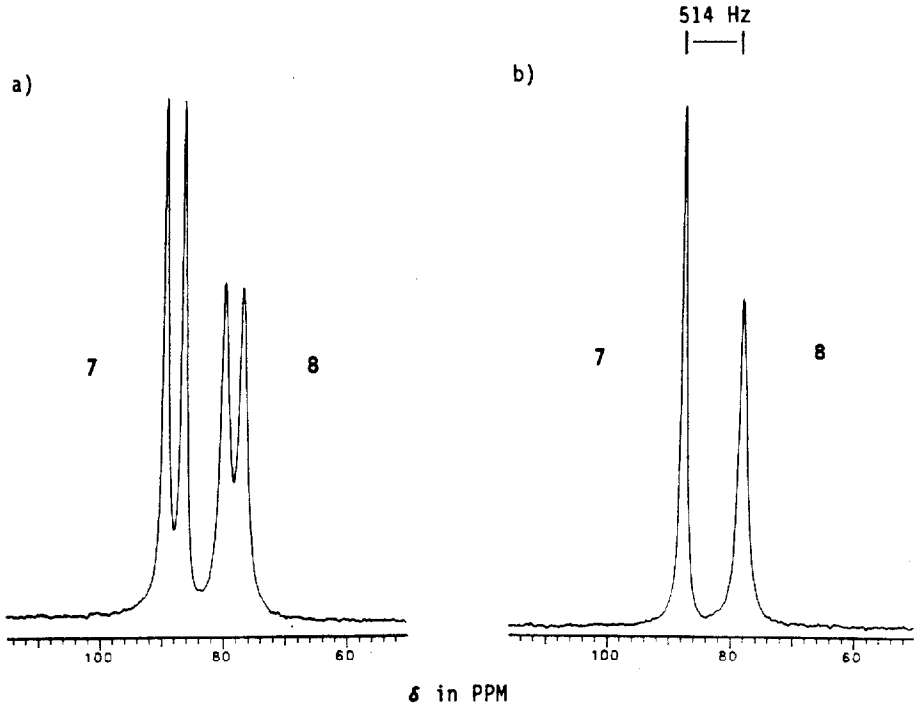
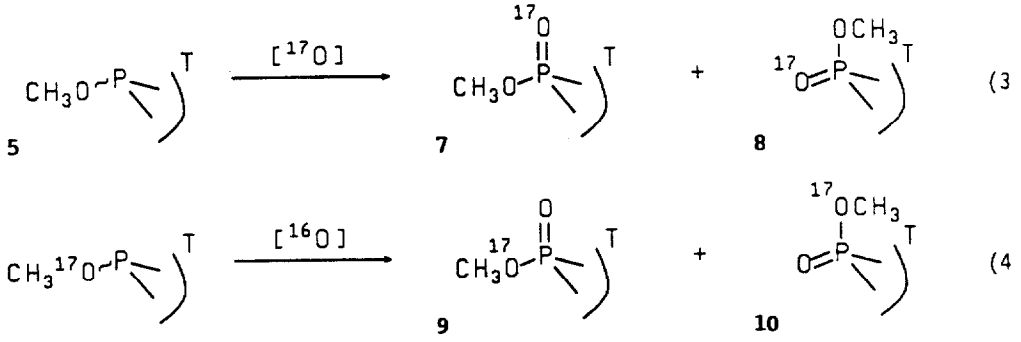


Figure. ^{17}O NMR spectra for **7** and **8** at 54.2 MHz. a) P-coupled. b) P-decoupled. Solvent - CD_3CN at 80 °C.

labeled P-chiral 4-nitrophenyl diesters of nucleoside 3'- and 5'-monophosphates. The individual $P{=}O^{17}$ resonances can be separately integrated. The ratio trans-**3**/cis-**4**, normalized for the relative amounts of cis and trans diastereomers formed, corresponds to R_P-**1**/S_P-**1**.



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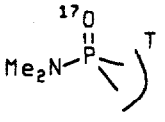
[The cis/trans ratio is easily obtained from the MeO or H_1' peaks in the ^1H spectrum or from the ^{31}P spectrum (^{16}O, ^{18}O and ^{18}O, ^{18}O triesters present in ratio equal to that of ^{17}O-containing triesters).] This approach is more direct and at least as quantitatively accurate as the present best alternative[1d]--hydrogenolysis of **1** to the P-chiral monophosphate, cyclization, methylation and R_P-**1**/S_P-**1** determination by the ^{18}O-shift, ^{31}P NMR method.[2,4,5]

The Table also records the $P{=}O^{17}$ NMR spectral parameters for the thymidine-based cyclic methylphosphonates, **11** and **12**,[10] and the analogous N,N-dimethylphosphoramidates, **13** and **14**.[11] The assignment of configuration at phosphorus based on the observed chemical shifts and line

width effects seen is readily done.[12] Notably, the correlation of diastereomer identity with $\delta^{17}O$ for **13** and **14** is opposite to that seen for **7**, **8**, **11** and **12**. It follows that care must be taken in the use of relative ^{17}O chemical shifts as they may be extremely substituent sensitive. Further investigations of these effects are underway. We have not successfully obtained natural abundance ^{17}O spectra on any of the cyclic nucleotide derivatives. However, the ^{17}O labeled **9-14** are readily available synthetically from reaction of the MeO^{17} phosphite **6** with MeI or Me_2NCl[11].



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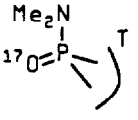

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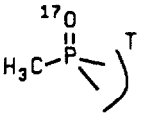

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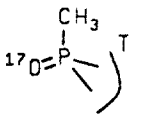

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The ability of ^{17}O NMR spectroscopy to characterize simple six-membered ring phosphate triesters has been reported recently.[13] These materials, however, are readily prepared in reasonably large quantities, isolated, purified, and characterized independently by ^{1}H, ^{13}C and ^{31}P NMR methods. The use of ^{17}O NMR outlined above with enzymatic systems is of particular value in that it is direct, and applicable, following HPLC product isolation, to the relatively small amounts of nucleotide diesters used in enzymatic reactions.

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