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PREPARATION OF DIASTEREOMERIC THYMIDINE 3',5'-CYCLIC METHYLPHOSPHONATES. ASSIGNMENT OF R_p AND S_p CONFIGURATIONS BY ^{13}C NMR

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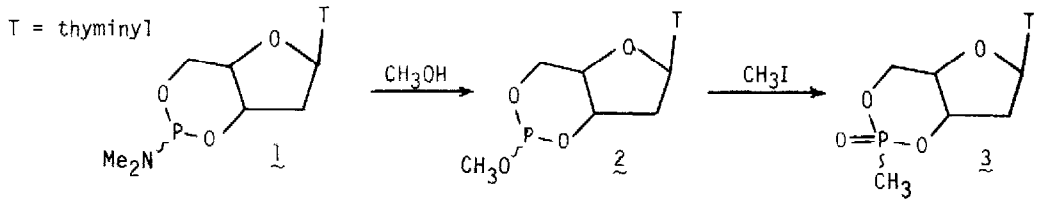
Summary: The diastereomers of thymidine 3',5'-cyclic methylphosphonate have been prepared and separated. A use of ^{13}C NMR for the assignment of their phosphorus configurations is demonstrated which should be generally applicable to P-derivatized cyclic nucleotides.

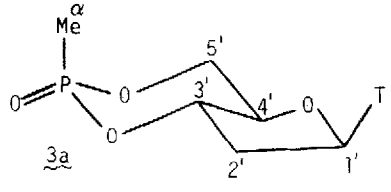
3',5'-Cyclic nucleoside monophosphates, e.g. cAMP and cGMP, play a central regulatory role in cell metabolism. Intense recent interest in analogs of the naturally-occurring cyclic nucleotides,[1] including those derivatized at phosphorus, (e.g., 1-3) stems from their potential as mimics or antagonists,[1] molecular receptor-site probes,[2] or storage forms of the parent cyclic nucleotides.[3] Furthermore, certain 3',5'-cyclic nucleoside N-phenyl phosphoramidates[4] serve as precursors to chiral 5'- and 3',5'-cyclic phosphorothioates and the corresponding ^{18}O-labeled cyclic diesters (formed on subsequent stereospecific reactions), all useful in study of the stereochemistry of enzymatic processes. A wide variety of functionality at phosphorus is necessary for these purposes. Furthermore, ready assignment of phosphorus configurations, R_p or S_p, to the individual diastereomers is imperative. We report here a facile, high-yield preparation of a 3',5'-cyclic nucleoside alkylphosphonate (3), a type of functionality not previously available,[5] and a generally applicable, straightforward, ^{13}C NMR method for assignment of phosphorus configuration.



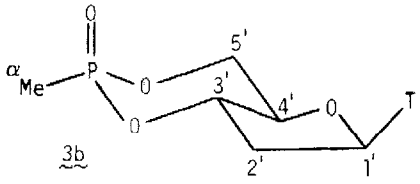
Methanolysis of phosphoramidite 1, as previously reported,[6] gives a 95% isolated yield of methyl phosphite, 2, as a 60/40 mixture of diastereomers. On reaction with MeI as solvent at room temperature, 2 is converted to a 50/50 mixture of diastereomeric methylphosphonates, 3 (^{13}P NMR at 26.2 and 30.2 ppm downfield from external OPA in DMSO-d_6), in 80-90% yields. Medium pressure liquid chromatography (85/15 EtOAc/EtOH on SiO_2) separates diastereomers 3a and 3b quickly and

near-quantitatively giving from 1g of 2 on one mplc pass 200-400 mg amounts of each methylphosphonate.[7]

Assignment of phosphorus configurations to 3a (R_p) and 3b (S_p) were made by comparisons of their ^{13}C NMR data (Table I) with those of two model methylphosphonates: 4, whose structure had been previously determined unequivocally by an X-ray crystallographic study of the cis isomer (t-Bu and Me cis);[8] and, 5, also well studied structurally[9] (NMR data from ref. 10.) ^{13}C



3a



3b

NMR parameters for 4 and 5 appear in Figure 1. The ^{13}C chemical shifts for $C_{4'}$ and $C_{5'}$ of 3a and 3b were assigned by single-frequency proton decoupling techniques. Trans-4 and cis-5 are known from PMR data to populate in solution the chair conformations shown in Figure I. For cis-4 and trans-5, the conformers shown are highly populated, although some conformational averaging occurs.[11]

Table I. ^{13}C NMR Parameters for 3a and 3b

	3a		3b	
Carbon	$\delta^{13}C^a$	J_{CP} (Hz)	$\delta^{13}C$	J_{CP} (Hz)
CH_3P	9.16	137.0	11.58	142.0
1'	83.03	<0.5	83.18	<0.5
2'	34.13	7.8	34.23	7.7
3'	75.93	4.5	73.86	5.2
4'	73.12	8.5	73.61	6.4
5'	68.62	8.5	67.94	8.6

[a]In DMSO-d_6. Chemical shifts in ppm downfield from internal TMS.

Notable correlations amongst the ^{13}C parameters of 3-5 which allow phosphorus configurations to be assigned are the following. The resonance of the axial methyl substituent on phosphorus is upfield-shifted in each pair of isomers. This appears to be a normal γ-gauche effect. A smaller $|^1J_{CP}|$ also is associated with the axial methyl.[12] Unlike the methyl carbons, C_4 and C_6 of 4 and 5 and $C_{3'}$ and $C_{5'}$ of 3 do not display the normal γ-gauche effect but instead are downfield-shifted by the axial methyl on phosphorus. Apparently, phosphoryl oxygen has an important role in determining the chemical shifts of the γ carbons. The latter sort of correlation looks to be general

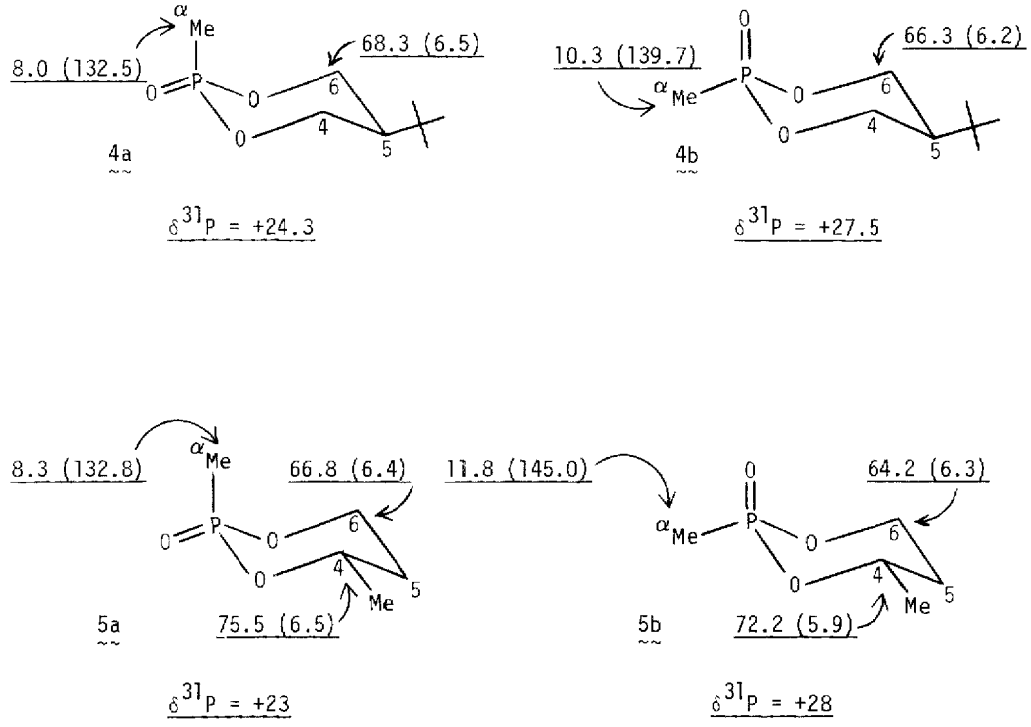


Figure I. ^{13}C and ^{31}P spectral data for model methylphosphonates. ^{13}C chemical shifts in ppm downfield from internal TMS. ^{31}P chemical shifts in ppm downfield from external H_3PO_4. Phosphonate 4 in DMSO-d_6. Solvent not given for 5[10]. J_{CP} values in parentheses, Hz.

for compounds like 4 and 5 with a variety of substitutuents on phosphorus except H.[13,14] It also can be noted for the thymidine 3',5'-cyclic methyl phosphates[15] and N,N-dimethylphosphoramidates.[4,12c] (The 3' carbon shifts are especially affected.) However, this correlation and its usefulness in assigning phosphorus configurations in 1,3,2-dioxaphosphorinanes has not previously been pointed out.

The relative ^{31}P chemical shifts (DMSO-d_6) determined for 3a (δ 26.2) and 3b (δ 30.2) are also supportive of the phosphorus configurations assigned, the axial-methyl diastereomer having the higher-field resonance. This chemical shift order, also seen for 4 and 5 (Figure I), is consistent with what is normally, though not without exception, found for 2-oxo and 2-thio-2-substituted-1,3,2-dioxaphosphorinanes.[14] However, with the ^{13}C NMR correlations noted above, one need not rely on ^{31}P evidence alone to assign phosphorus configurations in such ring systems. This could be especially important in cases in which diastereomers have closely similar ^{31}P chemical shifts.

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