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THYMIDINE NUCLEOSIDE 3',5'-CYCLIC PHOSPHORAMIDITES AND PHOSPHITES. CONFIGURATION AT PHOSPHORUS IN TRIVALENT AND PENTAVALENT CYCLIC NUCLEOTIDES BY ^{31}P AND ^{13}C NMR.

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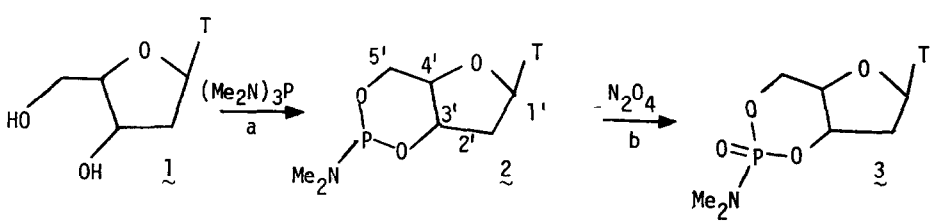
3',5'-cyclic nucleotides play a central role in cell metabolism. Recently, there has been considerable interest in the synthesis and biological activity of cyclic nucleotides derivatized at phosphorus to form pentavalent phosphate triesters and phosphoramidates.[1] These derivatives potentially may act as cyclic nucleotide mimics, as antagonists of cyclic nucleotide action, or as storage forms of the natural diester nucleotides themselves. Certain of them exhibit in vivo anti-cancer activity.[1e] Such systems are cis/trans diastereoisomeric about phosphorus, and it is quite probable that biological activity will be highly dependent on phosphorus configuration.

We report here the isolation of a 3',5'-cyclic deoxynucleotide, 2, which contains trivalent rather than pentavalent ring phosphorus. Application of ^{13}C and ^{31}P nmr methods to 2 and the tri- and pentavalent phosphorus heterocycles, 3 - 5, derived from it leads to the assignment of phosphorus configuration in these derivatives. Furthermore, such trivalent phosphorus compounds may have important biological activity themselves, are also synthetically useful, and to our



(1)

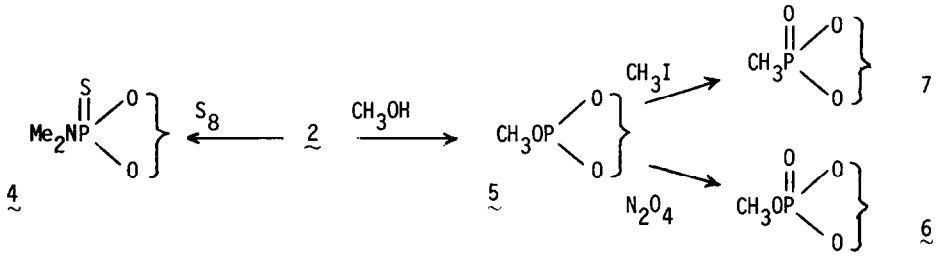
T = Thymine

knowledge have not been previously available in pure form.

The phosphoramidite 2 was easily isolable following reaction 1a[2] by a fast column chromatography (<30 min) of the highly impure product mixture on silica gel using 50:50 EtOAc:Et_2O as eluting solvent. All reaction byproducts remained on the column giving 2 in >95% purity as shown by ^{31}P nmr ($\delta^{31}P$, $CDCl_3$, -144.9, Table I). A second ^{31}P peak (3-5%) at δ -139.6 is very likely the other geometrical isomer of 2. The mono-trimethyl silyl derivative of 2 showed a molecular ion at m/e = 387 amu. N_2O_4 oxidation of 2 in CH_2Cl_2 at -15° gave the known[2a] phosphoramidate, 3, in near

quantitative amounts. (Molecular ion of mono-TMS derivative of 3 at m/e 403 amu, $\delta^{31}P$ = -7.2, DMSO-D_6.)

Methanol at 70 ± 5° converted 2 (dioxane solution) to the triester, 5, a 60/40 mixture of geometrical (cis/trans) isomers > 95% pure by ^{31}P nmr (Table I), and isolated in > 90% yields (based on 2) following rapid chromatography (molecular ion of mono-TMS derivative of 5 at m/e = 374 amu). N_2O_4 treatment of the 60/40 isomeric mixture of pure 5 yielded near-quantitative amounts of phosphate 6 (62/38 isomer ratio as determined from ^{31}P nmr peaks at +6.4 and +4.7 ppm, respectively, acetone-D_6).



In Table I are given the ^{13}C and ^{31}P nmr parameters for the deoxyribose and phosphorus ring portions[3] of 2, 3, and 5, along with ^{13}C parameters from the literature for sodium 3',5'-thymidine cyclic phosphate (3',5'-TMP).[4] The x-ray crystal structure of 3[5] shows the phosphoramidate ring

TABLE I. NMR PARAMTERS FOR 2, 3, 5 AND 3',5'-TMP

$\delta^{13}C^a(J_{CP}{}^b)$

Carbon	2	3	3',5'-TMP[c]	cis-5	trans-5
1'	84.3 (<0.5[d])	85.2 (<1.2[d])	86.5 (<0.2)	83.0 (<1.2[d])	83.5 (<1.2[d])
2'	36.6 (5.6)	35.8 (8.7)	35.7 (8.3)	36.2 (8.9)	36.2 (8.9)
3'	75.4 (9.0)	77.0 (4.1)	76.0 (4.8)	69.8 (<1.2)	71.0 (3.4)
4'	75.7 (12.8)	75.0 (4.0)	77.5 (4.5)	75.7 (7.3)	74.0 (19.8)
5'	67.3 (<0.5)	69.1 (7.5)	68.4 (7.0)	66.8 (4.3)	68.8 (4.5)
Me_2N	35.0 (21.7)	36.3 (4.9)			
CH_3O				50.1 (18.7)	49.7[e] (10)

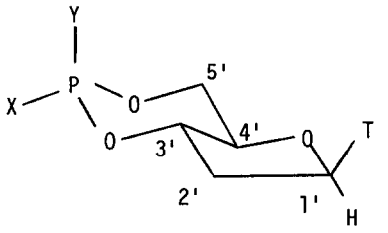
$\delta^{31}P$[f]

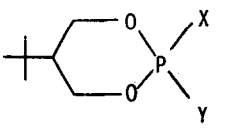
2	3	cis-5	trans-5
-144.9[g]	-7.16[h]	-123.2[i]	-129.5[i]

[a]In ppm downfield from internal TMS, acetone-D_6 solvent except for 3',5'-TMP values determined in D_2O with external TMS. [b]Absolute values in Hz. [c]As sodium salt in D_2O. [d]Spectrum of 2 at 1500 MHz sweepwidth, 1.8 sec. acquisition time, those of 3 and 5 at 5000 MHz, 0.8 sec. acquisition times [e]Uncertainty in δ and J because of weakness of signal intensity. [f]In ppm downfield from external 85% H_3PO_4. [g]$CDCl_3$. [h]DMSO-D_6. [i]Acetone-D_6.

to be a distorted chair with Me_2N equatorial, 8. The ^{31}P shift for 3, -7.16, agrees well with that for trans-2-dimethylamino-5-tert-butyl-2-oxo-1,3,2-dioxaphosphorinane ($\delta^{31}P$ = -6.96, $CDCl_3$) which has both the 5-t- butyl and 2-Me_2N equatorial[6] (12, X = Me_2N, Y = O). The agreement of the other parameters for 3 with those of 3',5'-TMP itself[4] is corraborative of the structure 8 assigned to 3.

Since 3 results almost quantitatively from the stereochemically retentive[7] N_2O_4 oxidation of 2, the trans relationship of the Me_2N and thymine base may be inferred for 2 and likewise the equatorial position of the Me_2N as in 9. (The Me_2N is known to be equatorial at thermodynamic equilibrium in simple cis/trans isomeric carbon-substituted 2-Me_2N-1,3,2-dioxaphosphorinanes[6,7c,8] such as 12[6] (X = Me_2N, Y = lone pair). The structure 9 for derivative 2 is confirmed by the large





12

8	X = Me_2N, Y = O	10	X = CH_3O, Y = e pair
9	X = Me_2N, Y = e pair	11	X = e pair, Y = CH_3O

value (12.8 Hz) of J_{CP} of C-4' which is generally characteristic of a 1,3,2-dioxaphosphorinane[8a,9,10] with an axial electron lone pair on phosphorus. The ^{31}P chemical shift of 2 (-144.9) is very near that reported previously[6] for trans-2-Me_2N-5-t-butyl-1,3,2-dioxaphosphorinane, -142.4 in C_6D_6 (12, X = Me_2N; Y = lone pair).

The power of ^{31}P and ^{13}C nmr to assign cis/trans geometries to these new cyclic nucleotide analogs is even more clearly illustrated by the isomeric phosphites, 5 (Table I). Consistent with assignment of structure 10 and 11 to trans- and cis-5, respectively, are the orderings of their ^{31}P chemical shifts with the upfield value assigned, as established for simple 1,3,2-dioxaphosphorinanes,[6,7c,9a,b] to the axial CH_3O ($\delta^{31}P$ at -124.6 and -131.4 in 12, X = CH_3O, Y = lone pair).[10b] Moreover, trans-5 exhibits the larger J_{CP} for C-4' (19.8 vs. 7.3 Hz) and smaller J_{CP} for the CH_3O. Both couplings are typical of such phosphite systems.[8a,9,10] A small but real apparent γ effect, also observed in previous 1,3,2-dioxaphosphorinanes,[8,9,11] results in upfield shifts of the C-3' and C-5' resonances in cis-5 (11), again indicative of an axial MeO. Certain unusual conformational aspects of the nucleotide-like trivalent phosphorus containing ring systems, 2 and 5, may be signified by the greatly different J_{CP} values for C-3' and C-5' of 2. Also, the percentage of trans isomer of 2 (>95%) and 5 (40%), if these ratios reflect true equilibria, is somewhat higher than expected.[6,7c,9c,12]

The synthetic utility of 2 and 5 is illustrated by the fact that sulfide 4

could not be isolated from S_8 treatment of the crude product mixture containing 2 but resulted quantitatively (based on 2) from the reaction of pure 2 in benzene, mp 218-219° (Anal calcd for $C_{12}H_{18}N_3O_5PS$: C, 41.50%; H, 5.19%; P, 8.93%. Found: C, 41.80%; H, 5.40%; P, 8.74%; mono-TMS derivative molecular ion at m/e 419 amu.) Further, the Arbuzov reaction of 5 with MeI appears quantitative to give stereoisomeric forms of 7. Alkylphosphonate derivatives of 3',5'-cyclic nucleotides are normally difficult to realize and then only in low yields.[1a] Other highly stereoselective reactions of 3 with reagents such as $C_6H_5/NiBr_2$, H_2O, Br_2, etc., can be envisaged as well.

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3. The ^{13}C chemical shifts for the thymine base were routinely comparable to those in 3',5'-thymidine cyclic phosphate itself (reference 4).

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10. a) This relation is also found for the series given by 12, the 2-X-5-tert-butyl-1,3,2-dioxaphosphorinanes (X = Me_2N MeNH, Alkyl, Ph, CH_3O; Y = lone pair). b) Unpublished work from this laboratory.

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