Tetrahedron Letters No. 51, pp 4457 - 4460, 1977. Pergamon Press. Printed in Great Britain.

THE CRYSTAL STRUCTURE OF THYMIDINE 3',5'-CYCLIC N,N-DIMETHYLPHOSPHORAMIDATE

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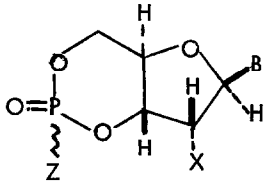
(Received in USA 29 August 1977; received in UK for publication 31 October 1977)

Recently there has been considerable attention given to 3',5'-cyclic nucleotides in which the phosphorus center is derivatized as a triester[1] (1, Z=OR) or phosphoramidate[1f,2] (Z=R_2N). Such derivatives are of biochemical interest as potential storage forms of the 3',5'-cyclic nucleotides themselves (1, Z=X=OH), to be released by hydrolytic P-Z cleavage, and as possible mimics or antagonists of cyclic nucleotide action in protein kinase and phosphodiesterase[3] systems. The ultimate goal of such biochemical work is an understanding of the role that changes in 3',5'-cyclic nucleotide levels (particularly 3',5'-cyclic guanosine and 3',5'-cyclic adenosine phosphates) play in human diseases.[4]



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1a: Z=Me_2N; X=H; B=thymine
1b: Z=EtO; X=HO; B=adenine[1b]

The derivatized 3',5'-cyclic nucleotides (1, Z=RO or Me_2N) can exist in two diastereomeric forms depending on the configuration at phosphorus. Thus, the group Z and the base may be cis or trans to each other. As the biochemical activity of such derivatives will be quite likely stereochemically dependent, it is important to make unequivocal assignments of phosphorus configuration via X-ray techniques. X-ray structures then will provide a firm basis for current applications of ^{13}C and ^{31}P nmr spectroscopy[1a,5] to assign phosphorus stereochemistry in such systems. Furthermore, 3',5'-cyclic nucleotides and model analog systems display unexpectedly high[6], and as yet not understood[6,7] exothermic heats of hydrolysis which may be related to molecular geometry features determinable by X-ray analysis.

We report here an X-ray crystal structure study of thymidine-3',5'-cyclic N, N-dimethylphosphoramidate, 1a, whose synthesis was reported earlier by Baschang and Kvita.[8] This molecule, in which Me_2N and thymine are proved to be trans, does indeed show certain unusual structural features, especially compared to 1b in which EtO and adenine are cis.[1b]

Ia crystallized as needles, space group $P4_1$, with cell dimensions a=b=10.276 (2), c=15.215 (4), and Z=4. The structure was solved by the heavy atom method[9] and refined by full matrix least squares calculations to R=0.085 using the 1489 independent observed reflections measured on an Enraf-Nonius CAD-4 diffractometer with CuK$\bar{\alpha}$ radiation and the ω-2θ scan technique. All hydrogen atomic positions except those on methyl groups were located and included in the refinement. An ORTEP plot[10] of the structure is shown in Figure 1 and bond lengths are given in Table 1.

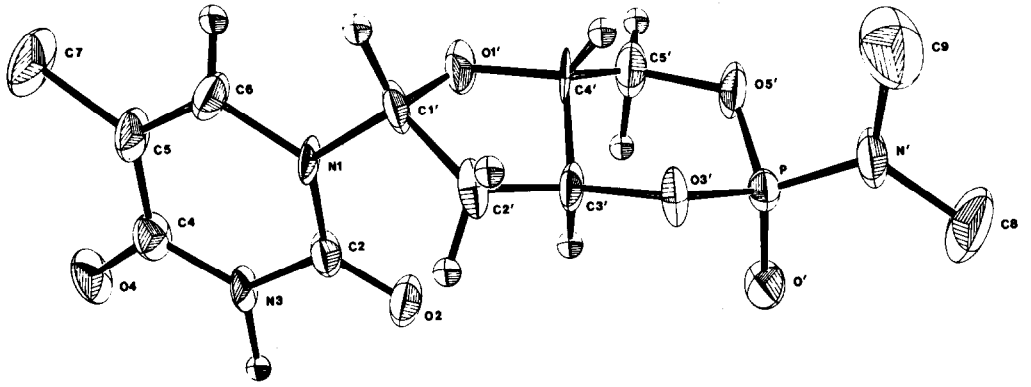

Figure 1. An ORTEP Plot of Structure Ia

The planar Me_2N of Ia is equatorially oriented and lies in the approximate symmetry plane of the distorted chair-form phosphoramidate ring. The largest deviation from the best plane through O'-P-N'-C_8-C_9 is 0.007 Å. The P-N' bond length (1.55 Å) is shortened by >0.05 Å compared to closely related six-membered ring compounds.[11] These two features may reflect increased P-N π-bonding in Ia. The angles P-O_5'-C_5' (124.3(6)°) and P-O_3'-C_3'(115.5(5)°) are similar to those found for Ib[1b] and in the 3',5'-cyclic nucleotides themselves. These distortions from normal P-O-C six-membered ring angles (116-121°)[12] may be related to the exothermic hydrolysis heats of the cyclic nucleotides.[6,7]

Perhaps the most striking aspect of the structure of Ia is seen by comparison of the geometry of its six-membered ring (Me_2N equatorial) to that of phosphate Ib (EtO axial).[1b] In Ia the angle between the O_3'-P-O_5' and O_3'-C_3'-C_5'-O_5' planes is 51.8°, whereas in Ib this angle is 34.9°. Especially notable is the extreme puckering of the Ia ring about C_4', the angle between C_3'-C_4'-C_5' and O_3'-C_3'-C_5'-O_5'- planes being 73.7° in Ia but only 56.9° in Ib. An increased puckering of the phosphorus end of 1,3,2-dioxaphosphorinane rings when the substituent is equatorial rather than axial has been recently noted.[13] However, an interplane angle greater than 59° from puckering about C_4'has not been found in either 1,3,2-dioxaphosphorinanes or 3',5'-cyclic nucleotides.[14]

The ribose ring of Ia contains an almost planar O_1'-C_1'-C_2'-C_3' group (C_1'-C_2' dihedral angle 5.5(9)°) with C_4' lying 0.65 Å out of the best plane through the four atoms and is in a $_4T^3$ conformation.[15] The orientation of the base group is given by the O_1'-C_1'-N_1-C_2 dihedral angle of 74(1)°.

A final important feature of the 1a structure is the strong crystal intermolecular hydrogen bonding between N_3-H and O'[N_3-H, 1.18(1)Å; O'...H, 1.60(1)Å]. This feature is not found[1b] in the crystal structure of 1b and may be related to the cis rather than trans relationship of the P=O and base rings in 1a. The influence of hydrogen bonding on the molecular distortions noted for 1a cannot be assessed at present but may be very important.

Table 1. Distances Between Bonded Atoms

Atoms	Distance, Å (esd.)[a]	Atoms	Distance, Å (esd.)[a]
P-O'	1.461 (7)	N_1-C_1'	1.44 (1)
P-O_3'	1.621 (6)	N_1-C_2	1.36 (1)
P-O_5'	1.583 (7)	N_1-C_6	1.42 (1)
P-N'	1.552 (8)	N_3-C_2	1.39 (1)
O_1'-C_1'	1.44 (1)	N_3-C_4	1.38 (1)
O_1'-C_4'	1.44 (1)	C_1'-C_2'	1.57 (1)
O_3'-C_3'	1.47 (1)	C_2'-C_3'	1.50 (1)
O_5'-C_5'	1.49 (1)	C_3'-C_4'	1.48 (1)
O_2-C_2	1.23 (1)	C_4'-C_5'	1.51 (1)
O_4-C_4	1.26 (1)	C_4-C_5	1.43 (1)
N'-C_8	1.48 (1)	C_5-C_6	1.34 (1)
N'-C_9	1.46 (2)	C_5-C_7	1.49 (2)

[a] The estimated standard deviations given in parentheses do not contain cell constant errors and bond lengths have not been corrected for thermal motion.

Acknowledgment

W.G.B. acknowledges support of this work by the National Cancer Institute of the Public Health Service (grant Ca 11045) and a fellowship from the Alexander von Humboldt Foundation held during the preparation of this manuscript.

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