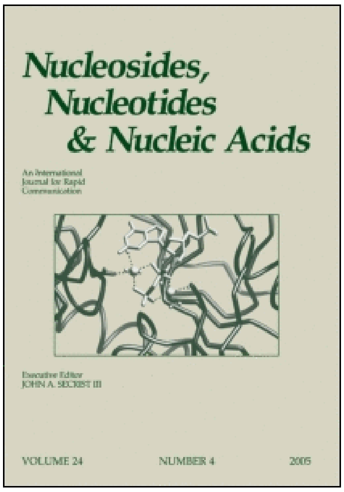

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TRANS-ADENOSINE 3',5'-CYCLIC N,N-DIMETHYLPHOSPHORAMIDATE ETHER SOLVATE, A CYCLIC NUCLEOTIDE WITH AN EQUATORIAL DIMETHYLAMINO GROUP ON PHOSPHORUS

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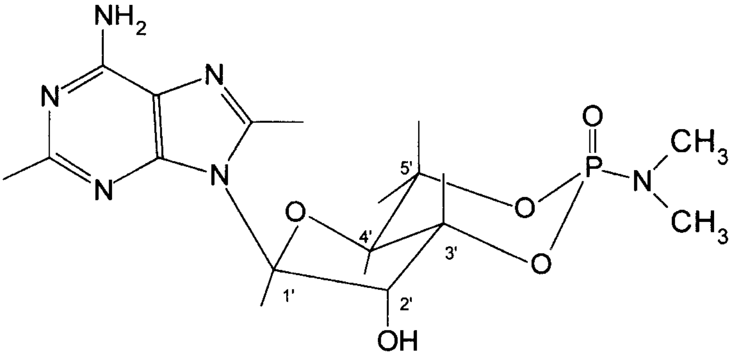
ABSTRACT. *trans-Adenosine 3',5'-cyclic N,N-dimethylphosphoramidate ether solvate crystallizes as monoclinic prisms elongated about the **b**-axis. The dioxaphosphorinane ring adopts a flattened chair conformation with an almost planar dimethylamino substituent equatorial. The purine ring conformation is anti. The ribose ring exists in a $^3T_4$ conformation.*

INTRODUCTION. *Cis* (R_P) and *trans* (S_P) phosphorus diastereoisomers of adenosine 3',5'-cyclic N,N-dimethylphosphoramidate are useful tools for studying the specificity of enzymes involved in the metabolism of adenosine 3',5'-cyclic monophosphate (cyclic AMP), a fundamental intracellular bioregulatory molecule.[1,2,3] In certain respects they can be considered as a nucleotide prodrug with a masked negative charge.[4] Depending on the configuration about the phosphorus, the diastereoisomers may act either as agonists or as antagonists of cyclic AMP action.[5] Dependence of acid hydrolysis rate upon phosphorus configuration has also been observed: the S_P diastereoisomer hydrolysed about one order of magnitude faster than the R_P diastereoisomer did.[6] Configurational assignment for adenosine 3',5'-cyclic N,N-dimethylphosphoramidate diastereoisomers has earlier been done only by ^{31}P NMR spectroscopy.[7] Therefore it is important to make unequivocal assignment about phosphorus by X-ray techniques. The present paper describes the crystal structure of *trans*-adenosine 3',5'-cyclic N,N-dimethylphosphoramidate (**1**) ether solvate. Analogous compounds include the crystal structures of *cis*- and *trans*-thymidine 3',5'-cyclic N,N-dimethylphosphoramidates,[8,9] *cis*-

2'-deoxy-adenosine 3',5'-cyclic N-phenylphosphoramidate[10] and *trans*-5-isopropyl-2'-deoxy-uridine 3',5'-cyclic N-benzyl-phosphoramidate[11].



1

EXPERIMENTAL

The compound was prepared according to Bottka & Tomasz[12] and crystallized by liquid:liquid diffusion of diethyl ether into a methanolic solution at -20°C. The crystals were monoclinic prisms elongated about the **b**-axis. The structure determination was done with a crystal 0.15 x 0.3 x 0.1 mm sealed in a glass capillary with a drop of ether. The crystal data are as follows: $C_{12}H_{17}N_6O_5P.xC_4H_{10}O$. M_r=356.3 (excluding solvent), monoclinic, space group P2$_1$ with unit cell dimensions **a**=9.749(2)Å, **b**=7.720(2)Å, **c**=12.539(3)Å, **ß**=103.64(3)°, **V**=917Å^3, **Z**=2, F(000)=452.

Intensity data were collected on a Syntex P2$_1$ diffractometer. Cell parameters refined by least-squares method from the setting angles of 15 reflections. Intensities collected by $\Theta/2\Theta$ scan for $3° < 2\Theta < 124°$ with Cu Kα radiation (λ=1.5418Å graphite monochromator). No absorption or extinction correction was considered. Data reduced to Fo and σ(Fo), data with $I < 2\sigma(I)$ considered observed (1443 of 1567 unique data). Structure was solved (IBM 3084) by SHELXS-86[13]. All nonhydrogen atoms of the nucleotide molecule were visible in the map corresponding to the lowest CFOM. Difference maps revealed three 'solvent' atoms in a hydrophobic channel extending through the lattice centered on one diad axis. Application of symmetry generated an infinite chain of atoms separated at distances nominally corresponding to covalently linked atoms. This was most easily interpretable as an incommensurate structure with disordered ether molecules in roughly an extended conformation along the diad axis. These atoms were assigned as oxygen atoms with equal fractional occupancy factors of 0.72 and refined isotropically without hydrogen atoms. Refinement was by full-matrix least squares with an expanded version of SHELXL-76[14], using form factors contained within the program. Hydrogen atoms bonded to carbon atoms were geometrically restrained (C-H, 0.99Å). The positions of hydrogen atoms bonded to oxygen or nitrogen were determined from difference maps and refined. The methyl groups were best modelled as rigid groups pivoting on carbon with six staggered hydrogen atoms in a regular hexagon with occupancy factors of 0.5 and a C-H distance of 0.99Å. Non-hydrogen atoms were refined anisotropically, $\Sigma w|Fo-Fc|^2$ was minimized with unit weights. The final $R=\Sigma|Fo-Fc|/\Sigma Fo$ was 0.054 corresponding to a $Rw=\Sigma w|Fo-Fc|^2/\Sigma wFo^2$ of 0.061. The final difference map contained three peaks with p=0.25 e/ $Å^3$ but was otherwise featureless. The final Δ/σ for non-hydrogen atoms of the nucleotide was 0.1 and for solvent atoms 0.5. Attempts to impose a restrained geometry on the included solvent led to poorer agreement and refinement divergence; range of hkl: h -11,10, k 0,8, l 0,14.

RESULTS AND DISCUSSION

The molecular structure with the atomic numbering is shown in FIG. 1. The final atomic fractional co-ordinates and standard deviations are listed in TABLE 1. The bond lengths and bond angles are given in TABLES 2 and 3. These confirm the earlier *trans* (S_P) assignment for the configuration of the chiral P atom.[7]

The equatorial N,N-dimethylamino substituent on P is almost planar with N 0.15Å from the plane of C(11), C(12) and P(1). As expected, torsion angle C11-N10-P1-O10 is close to 180° (-173.7°). The dioxaphosphorinane ring has a chair conformation with appreciable flattening at the P end such that the angles P-O(3')-C(3') and P-O(5')-C(5') are enlarged to 117.5(8)° and 125.5(8)°, respectively. Distances of P and C4' from the plane of C3'-O3'-C5'-O5' atoms are 0.48Å and -0.75Å, respectively. The planarity of equatorial N,N-dialkylamino substituents and the flattening of the ring at the P, are

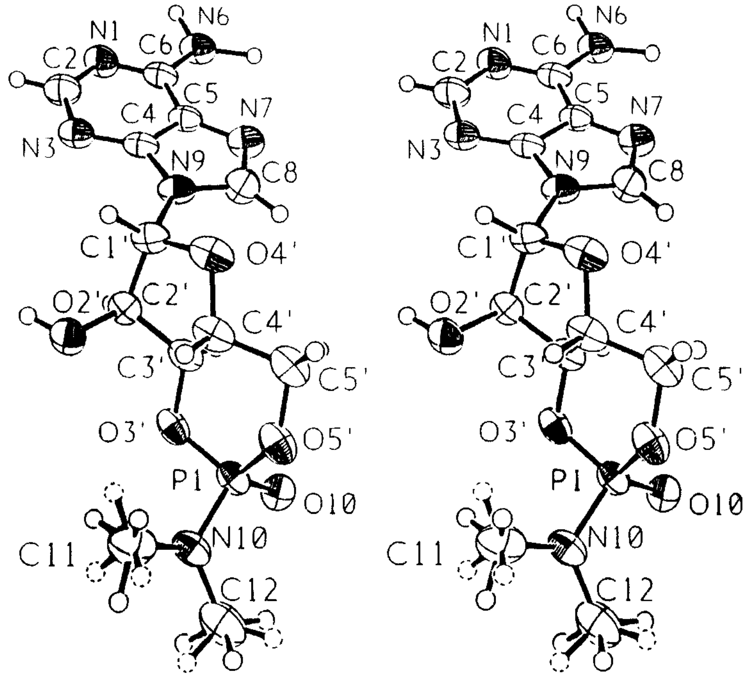


FIG. 1 Stereo ORTEP plot of compound **1**. Thermal ellipsoids are shown at 50% probability level.

general characteristics of 2-oxo-1,3,2,-dioxaphosphorinanes.[9,11,15] The P-O(3'), P-O(5') and P-N(10) bond distances of 1.58(1), 1.60(1) and 1.62(1)Å, respectively are indicative of single bonds with a normal distance of 1.483(7)Å for the P-O(10) bond. The P-N(10) bond length is similar to that observed for 2-oxo-1,3,2,-dioxaphosphorinane having substituted amino groups equatorial at the P on a chair-shaped ring[11,16]. An exception is *trans*-thymidine 3',5'-cyclic N,N-dimethylphosphoramidate that has a P-N bond distance of 1.55(1)Å.[9] This bond shortening used to be regarded as a proof of the increased π bonding between the P and the equatorial N of trigonal planar geometry. Considering the P-N(10) bond length of 1.62(1)Å in **1**, we would preferably interpret the bond shortening observed for the thymidine analogue as a property of an incomplete model (no hydrogen atoms were positioned on the N,N-dimethyl groups in the structure determination).

Compound **1** has an *anti* conformation with a glycosidic torsion angle, χ=C(4)-N(9)-C(1')-O(4') of -155°. In crystal structures of nucleoside 3',5'-cyclic phosphates and

TABLE 1 Atom co-ordinates ($\times 10^4$) and equivalent isotropic and anisotropic temperature factors ($Å^2 \times 10^3$) for **1**

	x/a	y/b	z/c	U_{eq}	U_{11}	U_{22}	U_{33}	U_{23}	U_{13}	U_{12}
N(1)	0180(7)	1243(12)	8597(5)	63	65(4)	77(6)	46(4)	05(4)	27(3)	07(4)
C(2)	0693(9)	0871(14)	7715(6)	65	66(5)	75(7)	55(5)	02(5)	22(4)	14(5)
N(3)	1404(6)	1936(10)	7170(5)	56	51(4)	68(5)	48(4)	05(4)	23(3)	10(4)
C(4)	1519(8)	3519(13)	7612(6)	51	45(4)	68(6)	40(4)	13(4)	15(3)	14(4)
C(5)	1039(9)	4096(14)	8507(7)	51	51(5)	65(6)	38(4)	10(4)	19(3)	10(4)
C(6)	0317(8)	2836(14)	8996(6)	55	48(4)	76(7)	40(4)	13(5)	14(3)	13(4)
N(6)	-195(9)	3274(13)	9894(6)	68	74(5)	85(6)	45(4)	14(4)	34(4)	13(5)
N(7)	1431(8)	5817(12)	8727(6)	65	63(4)	76(6)	56(4)	03(4)	34(4)	06(4)
C(8)	1995(9)	6320(15)	7964(7)	65	69(5)	68(6)	58(5)	-1(5)	34(4)	00(5)
N(9)	2141(6)	4946(12)	7276(4)	54	48(3)	66(4)	47(3)	10(4)	24(3)	16(4)
C(1')	2805(7)	6040(16)	6339(5)	57	54(4)	67(5)	49(4)	16(5)	32(3)	12(5)
C(2')	1810(7)	5455(11)	5224(6)	49	38(4)	61(6)	49(4)	-3(4)	22(3)	-1(4)
O(2')	2260(7)	4629(9)	4369(5)	66	72(4)	68(5)	57(3)	-7(3)	37(3)	-2(3)
C(3')	2036(7)	7372(13)	5154(6)	47	31(3)	70(5)	41(4)	03(4)	13(3)	01(4)
O(3')	1682(5)	8102(9)	4078(4)	53	40(3)	76(4)	44(3)	10(3)	02(2)	-14(3)
C(4')	3627(7)	7502(14)	5639(6)	54	29(3)	86(6)	45(4)	12(4)	16(3)	04(4)
O(4')	3825(5)	6460(10)	6595(4)	60	35(2)	94(5)	50(3)	18(3)	14(2)	07(3)
C(5')	4058(8)	9366(13)	5857(6)	56	36(4)	85(7)	51(4)	01(4)	08(3)	-9(4)
O(5')	3717(5)	9871(11)	4793(4)	61	44(3)	84(4)	54(3)	08(4)	03(2)	-16(4)
C(11)	3327(10)	8505(16)	2395(8)	79	62(5)	102(9)	73(6)	-15(6)	22(5)	11(6)
C(12)	2433(10)	11507(18)	2055(7)	82	71(6)	114(9)	62(6)	26(7)	21(5)	-3(7)
P(1)	2221(2)	10000	3932(1)	50	40(1)	66(1)	44(1)	03(1)	13(1)	-8(1)
N(10)	2521(6)	9933(13)	2717(5)	60	52(3)	83(5)	45(3)	02(5)	18(3)	-4(5)
O(10)	1263(6)	11425(10)	4078(5)	64	54(3)	63(4)	74(4)	-5(4)	30(3)	-7(3)
O(E1)	4563(41)	0731(82)	-519(48)	255	190(29)	255(49)	319(52)	-31(36)	53(28)	00(31)
O(E2)	5110(37)	2626(118)	0022(35)	280	200(27)	397(57)	242(28)	02(36)	49(22)	55(42)
O(E3)	4760(47)	4612(103)	0679(53)	339	241(36)	354(69)	423(53)	165(56)	144(36)	23(39)

The anisotropic temperature factor exponent takes the form:
$-2\pi^2(U_{11} h^2 a^{*2} + \ldots + 2U_{12} h k a^* b^* + \ldots)$

TABLE 2 Bond lengths (Å) for **1**

C(2)-N(1)	1.349(12)	C(6)-N(1)	1.322(14)
N(3)-C(2)	1.358(13)	C(4)-N(3)	1.335(13)
C(5)-C(4)	1.387(13)	N(9)-C(4)	1.371(13)
C(6)-C(5)	1.422(15)	N(7)-C(5)	1.375(13)
N(6)-C(6)	1.378(13)	C(8)-N(7)	1.327(13)
N(9)-C(8)	1.395(14)	C(1')-N(9)	1.471(9)
C(2')-C(1')	1.537(12)	O(4')-C(1')	1.464(13)
O(2')-C(2')	1.403(11)	C(3')-C(2')	1.502(14)
O(3')-C(3')	1.427(11)	C(4')-C(3')	1.530(11)
P(1)-O(3')	1.582(9)	O(4')-C(4')	1.419(11)
C(5')-C(4')	1.506(15)	O(5')-C(5')	1.425(11)
P(1)-O(5')	1.600(7)	N(10)-C(11)	1.465(14)
N(10)-C(12)	1.463(16)	N(10)-P(1)	1.618(8)
O(10)-P(1)	1.483(9)	O(E2)-O(E1)	1.65(6)
O(E3)-O(E2)	1.81(6)	O(E1)-(OE3)d	1.13(7)

TABLE 3 Bond angles (°) for **1**

C(6)-N(1)-C(2)	119.0(9)	N(3)-C(2)-N(1)	128.0(10)
C(4)-N(3)-C(2)	110.4(8)	C(5)-C(4)-N(3)	128.2(9)
N(9)-C(4)-N(3)	127.5(8)	N(9)-C(4)-C(5)	104.4(9)
C(6)-C(5)-C(4)	115.1(10)	N(7)-C(5)-C(4)	112.1(10)
N(7)-C(5)-C(6)	132.8(10)	C(5)-C(6)-N(1)	119.3(8)
N(6)-C(6)-N(1)	120.9(9)	N(6)-C(6)-C(5)	119.7(10)
C(8)-N(7)-C(5)	104.7(9)	N(9)-C(8)-N(7)	111.1(10)
C(8)-N(9)-C(4)	107.7(7)	C(1')-N(9)-C(4)	126.5(9)
C(1')-N(9)-C(8)	125.8(9)	C(2')-C(1')-N(9)	115.8(6)
O(4')-C(1')-N(9)	105.5(8)	O(4')-C(1')-C(2')	107.1(8)
O(2')-C(2')-C(1')	110.9(7)	C(3')-C(2')-C(1')	100.8(8)
C(3')-C(2')-O(2')	109.1(7)	O(3')-C(3')-C(2')	116.0(8)
C(4')-C(3')-C(2')	101.0(8)	C(4')-C(3')-O(3')	110.3(7)
P(1)-O(3')-C(3')	117.5(6)	O(4')-C(4')-C(3')	102.8(7)
C(5')-C(4')-C(3')	110.3(8)	C(5')-C(4')-O(4')	114.3(8)
C(4')-O(4')-C(1')	106.8(6)	O(5')-C(5')-C(4')	103.4(8)
P(1)-O(5')-C(5')	125.5(6)	O(5')-P(1)-O(3')	104.8(4)
N(10)-P(1)-O(3')	102.6(5)	N(10)-P(1)-O(5')	107.4(4)
O(10)-P(1)-O(3')	115.9(4)	O(10)-P(1)-O(5')	111.8(5)
O(10)-P(1)-N(10)	113.5(5)	C(12)-N(10)-C(11)	115.7(8)
P(1)-N(10)-C(11)	120.7(8)	P(1)-N(10)-C(12)	120.5(8)

their ester and amide derivatives both *syn* and *anti* conformations were found.[17] The furanose ring of **1** has a twisted $^3T_4$ conformation, as evidenced by the relatively small torsional angle, C4'-O4'-C1'-C2', ν_o, of -9.0° and the large O4'-C1'-C2'-C3' torsional angle, ν_1, of -20.0°. The pseudorotation parameters are: τ_m=-46.2° and P=29.3.[18] Here the carbon-oxygen bond distances to O(4') are 1.464(13)Å for the C(1')-O(4') bond and 1.419(11)Å for the C(4')-O(4') bond. The shortening of C(4')-O(4') bond and the lengthening of C(1')-O(4') bond relative to nucleotides is a general characteristics of nucleoside 3',5'-cyclic phosphates and derivatives.[8,11,19,20,21,22] and may be regarded as a manifestation of the torsional strain in the furanose ring caused by the cyclization.[19]

In the crystal lattice the purine rings are linked by pairs of hydrogen bonds between N(6)-H and N(7) of 2_1 symmetry-related molecules. Purine rings stack neither upon each other nor upon sugar rings but instead over the phosphoramidate group leading to two short intermolecular contacts - N(10)...C(6), and N(10)...C(5) of 3.48 and 3.50Å. There are three close intermolecular contacts between O(3') and C(2), N(3) and C(4) of the same purine ring (3.31, 3.18 and 3.35Å, respectively.) There is a close contact of 3.66Å by the sugar ring oxygen atom O(4') and N(10) on the opposite side of the phosphoramidate group. Finally, the molecules are linked into continuous chains along the **b**-axis by hydrogen bonds between O(2')-H...O(10)-P. Three intermolecular hydrogen bonds facilitate crystal growth along the **b**-axis: N(7)...H(61)-N(6a), O(3')-H(O3)...N(3b) and O(10)...H(O2'c)-O(2'c) with donor...acceptor distances of 2.96Å 3.18Å and 2.65Å, respectively.

The ether solvent molecules form a chain of atoms contained in a square shaped hydrophobic channel centered on the 2_1 screw axis at x=1/2, z=0 and with a van der Waals edge of 4.8Å. The channel is bounded by the purine rings and by the rotationally disordered N,N-dimethyl groups.

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