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Nucleoside cyclic 3′,5′-phosphates: chair–twist equilibria of the phosphate rings of methyl phosphate and phenylphosphonate derivatives of cTMP

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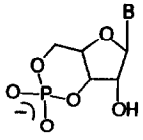
ABSTRACT

The cis and trans forms of thymidine methyl cyclic 3′,5′-phosphate (**9**) and of the corresponding phenylphosphonate derivatives (**10**) were prepared in two steps from the corresponding cyclic amide. ^{1}H NMR spectroscopy showed the phosphorus-containing six-membered rings of *cis*-**9** (MeO and thymin-1-yl *cis*) to be in the chair conformation, but *trans*-**9** to be ~ 40% in the twist conformation in pyridine-d_6 and 33% in acetone-d_6. For *trans*-**9**, there was resistance of conversion of the chair form into the twist form with the MeO group pseudo-axial with a $\Delta G°(C \rightarrow T)$ value of 3.0 kcal/mol. Similarly, for *cis*-**10** (23–39% twist population in CD_3CN, acetone-d_6, and $CDCl_3$), a $\Delta G°(C \rightarrow T)$ value of 0.5–0.6 kcal/mol for placement of the phosphoryl oxygen pseudo-axial was obtained. Both values are taken to be approximations for $\Delta G°(C \rightarrow T)$ for cTMP. For *trans*-**10**, the twist conformation was populated to the extent of 6–13% with a $\Delta G°(C \rightarrow T)$ value of 1.3 kcal/mol.

INTRODUCTION

The importance of adenosine cyclic 3′,5′-phosphate (cAMP, **1**) and guanosine cyclic 3′,5′-phosphate (cGMP, **2**) in the regulation of cell metabolism is well established[1]. Detailed knowledge of the conformational properties of these molecules is essential for a full understanding of their interactions with their principal target enzymes, protein kinases 1 and 2, and the phosphodiesterases[2]



1 B = adenin-1-yl
2 B = guanin-9-yl

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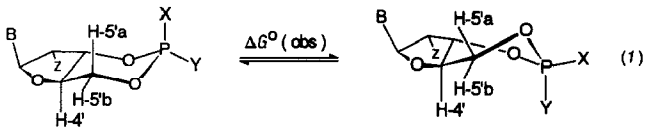


(1)

A B

3 B = various bases, Z = H, OH, X = R_2N, Y = O

4 B = Thy, Z = H, X = O, Y = O

cis-**9** B = Thy, Z = H, X = MeO, Y = O

trans-**9** B = Thy, Z = H, X = O, Y = MeO

cis-**10** B = Thy, Z = H, X = Ph, Y = O

trans-**10** B = Thy, Z = H, X = O, Y = Ph

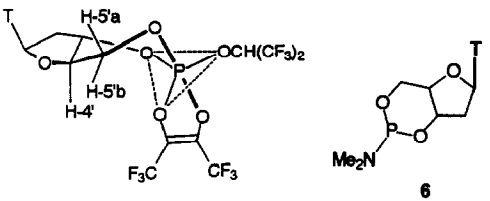
cis-**11** B = Thy, Z = H, X = PhO, Y = O

trans-**11** B = Thy, Z = H, X = O, Y = PhO

cis-**12** B = Thy, Z = H, X = O, Y = Me_2N

responsible for their hydrolysis to the 5′-phosphate. Recent studies have addressed the question of the change in free energy required to convert the lower-energy chair conformation of the phosphate ring of a cyclic nucleotide into the twist form. Thus, we have reported[3] a ^{1}H NMR study of the chair–twist equilibria (**A** ⇌ **B**) for a series of cyclic 3′,5′-phosphoramidate derivatives (**3**) of 3′,5′-cyclic phosphates derived from purine and pyrimidine ribo- and 2′-deoxyribo-nucleosides. An analogous investigation of the *trans* phenyl phosphate derivative (**11**) of thymidine cyclic 3′,5′-phosphate also has been published[4]. From these investigations, it was concluded that the free energy required to convert the chair phosphate form of cAMP into the twist conformation in organic solvents was 1–3 kcal/mol.

On the basis of ^{1}H NMR studies of solutions, it was determined[5] that the five-coordinate molecule **5**, a model for the intermediate or the transition state of the phosphodiesterase-catalyzed hydrolysis of cAMP to its 5′-phosphate, is in the twist form rather than the chair conformation[5]. These results led us to suggest[5]



5

6

the *possibility* that the phosphodiesterase binds cAMP itself in the twist conformation and then delivers the water molecule to form a trigonal bipyramidal, five-coordinate phosphorus intermediate (or transition state) with the phosphorus-contain-

ing ring still in the lower-energy twist form with the attacking water and scissle P–O-3′ bond co-apical (Fig. 1)[5a]. However, no experimental evidence concerning this point yet exists for the enzymic system. Indeed, the recent results of Holmes et al.[6] indicate that intermolecular hydrogen bonding is sufficient to compel such adducts to be in the *chair* conformation, and they suggested that hydrogen bonding and other interactions within the active site could constrain the phosphorus-containing ring to be attached diequatorially to phosphorus.

In earlier work, dialkylamino[3] and phenoxy[4] substituents were employed to drive the equilibrium **A** ⇌ **B** (equation *1*) towards the twist conformation **B**. Dissection of the free energy components of the equilibrium **A** ⇌ **B**, expressed by the conformers of Scheme 2 and equation *3*, assumes that ΔG° measured for the chair–chair equilibrium **C** ⇌ **E** in the monocyclic system is directly transferable to dissection of the equilibrium **A** ⇌ **B** via Scheme 2. However, it has been shown by X-ray crystallography[7] that the phosphate rings of cyclic nucleotides are distorted such that axial H-3′ is closer to the axial substituent on phosphorus than is axial H-5′. Thus, ΔG° values from chair–chair equilibria (**C** ⇌ **D**) of monocycles are only good estimates of the driving force for the reorientation of the *trans*-fused cyclic nucleotide phosphate rings from the chair to the twist conformation (**A** ⇌ **B**). Indeed, the errors involved in this assumption are likely to be responsible in large part for the range (0.5–2.2 kcal/mol) of ΔG°(C → T) values noted[3,4] for cTMP.

Such conformational studies have now been extended to cTMP derivatives substituted at phosphorus with axial (*cis*-**10**) and equatorial phenyl (*trans*-**10**), and equatorial methoxy (*trans*-**9**), each of which displaces the equilibrium **A** ⇌ **B** towards the twist conformation. Dissection of these equilibria in terms of equation *3* leads to values for ΔG°(C → T) of 0.6 and 1.3 (Ph) and 3.0 (MeO) kcal/mol. The similarity of these numbers to those derived by the use of R_2N and PhO substituents on phosphorus[3,4] lends further support to the notion that ΔG°(C → T) for the diesters is small.

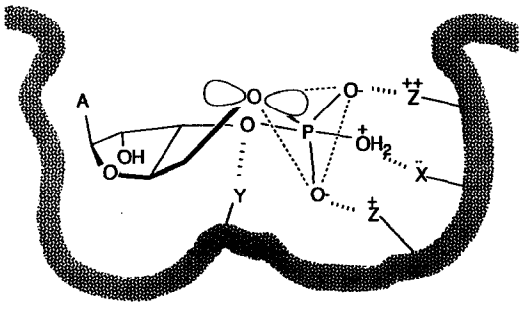


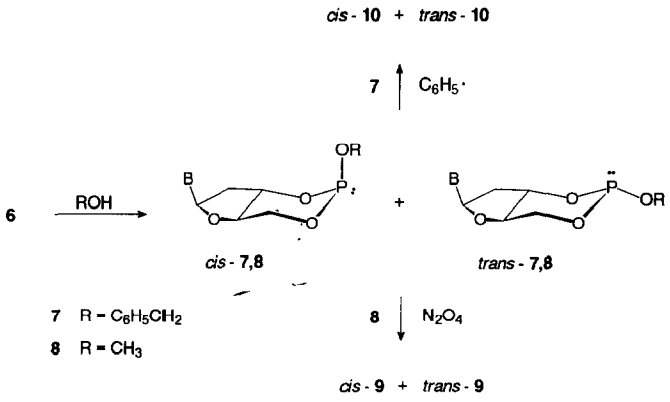
Fig. 1. Speculative representation of enzyme-bound 5-coordinate cAMP–H_2O adduct with twist-form ring (from ref. 5a).

RESULTS

Syntheses.—The cyclic nucleotide derivatives studied were prepared from phosphoramidite **6** according to Scheme 1. Recently, the preparation of **6** in good yields was described[8] by a modification of the procedure of Baschang and Kuvita[9]. The benzyl phosphite **7**, highly enriched in the trans isomer (relation of thymin-1-yl to benzyloxy, trans,cis-ratio 9:1 based on ^{31}P NMR data), was isolated (52%, not optimized) on pyridine hydrochloride-catalyzed reaction of **6** with benzyl alcohol at −78° followed by column chromatography. In an analogous reaction, pure *cis*-**7** was obtained by reaction[10] of the benzyl alcohol with **6** at room temperature then at ~ 100°. The pure methyl phosphite **8**, obtained by the phosphoramidite procedure as a mixture of isomers (cis,trans-ratio ~ 3:2), was oxidized with N_2O_4 to give the methyl phosphate derivative **9**, essentially quantitatively with retention of configuration at phosphorus, and the cis and trans isomers were isolated by chromatography. This preparation was reported previously[10].

Reaction of an 85:15 trans,cis-mixture of the benzyl phosphite **7** with phenyl radicals, obtained by thermal decomposition of phenylazotriphenylmethane, yielded the phenylphosphonate **10** (cis,trans-ratio ~ 85:15, Scheme 1). Chromatography then afforded pure *cis*-**10** (39% yield from **7**). Similarly, pure *cis*-**7** gave *trans*-**10** (32%). This free-radical type of Arbuzov reaction had been used previously to yield acyclic phenylphosphonates[11] and monocyclic 2-phenyl-2-oxo-1,3,2-dioxaphosphorinanes[12].

^1H NMR analysis of the conformations of the 1,3,2-dioxaphosphorinane ring.—$J_{H,H}$ and $J_{H,P}$ values for the cis and trans forms of **9** and **10** are recorded in Tables I and II. Although the spectra for the phosphorus-containing rings were usually close to first order at 300 MHz, the parameters reported are from spectra simulated iteratively by use of the LAOCN3 program. The assumption of a negative value for $J_{4',P}$ generally resulted in a more accurate simulation of the spectral pattern for H-4′. The H-3′ multiplet consistently appeared as an apparent



Scheme 1.

TABLE 1

^{1}H NMR J values for the 1,3,2-dioxaphosphorinane rings of **9–12** at 300 MHz for solutions in acetone-d_6 [a]

Compound	X	Y	J (Hz)								Ref.
			3′,4′	4′,5′a	4′,5′b	5′a,5′b	3′,P	4′,P	5′a,P	5′b,P	
cis-**9**	MeO	O	9.3 [c]	10.7	4.6	−9.4	1.0	−0.6	0.7	21.9	
trans-**9**	O	MeO	9.3 [c]	10.4	5.5	−9.4	1.0	< −0.8 [b]	7.0	14.8	
cis-**10**	Ph	O	9.1 [d]	10.4	5.2	−9.4	1.4	−0.4	6.1	13.3	
trans-**10**	O	Ph	9.2 [d]	10.7	5.0	−9.3	1.7	−0.6	2.2	18.8	
cis-**11**	PhO	O	9.3	10.7	4.5	−9.3	0.9	−0.7	0.7	22.9	4
trans-**11**	O	PhO	9.4	10.4	6.0	−9.6	0.8	−0.6	11.6	9.6	4
cis-**12** [e]	$(Me)_2N$	O	9.1	10.1	6.0	−9.1	~0.2	[f]	14.4	6.0	3

[a] Ambient temperature. [b] Coupling could not be defined clearly; maximum value, 0.8 Hz. [c] Probable error, as calculated by the LAOCN3 program, 0.009–0.013 Hz. [d] Probable error, 0.010–0.022 Hz. [e] In toluene-d_8. [f] < 1 Hz.

broad quartet, but its analysis was difficult because it was generally without features to which line assignments could be made. Hence, only the limiting values of the transitions beneath each peak, based on the frequencies of the outer edge and point of maximum intensity, could be made. Because the LAOCN3 program is limited to seven spins, the protons were divided into five-spin (H-3′, H-4′, H-5′a, H-5′b, and P) and six-spin (H-1′, H-2′a, H-2′b, H-3′, H-4′, and P) systems which were simulated independently. Because of the greater detail of the H-4′ multiplet, the value for $J_{3',4'}$, a parameter common to the simulation of the H-5′ and H-2′ resonances, was taken from analysis of the 5-spin set. The assignments of chemical shifts for transitions within peaks did not vary from those calculated by more than 0.2 Hz, the computer resolution of the data set.

As for the three- and four-coordinate cyclic nucleotide derivatives[3,4,13], the assignments of H-5′a,5′b were based on the larger value of $J_{4',5'a}$ compared to that

TABLE II

^{1}H and ^{31}P chemical shift data for the 1,3,2-dioxaphosphorinane rings of **9** and **10** for solutions in acetone-d_6 [a]

Compound	X	Y	δ (ppm)[b]					R.m.s. error[h]
			H-3′	H-4′	H-5′a	H-5′b	P [c]	
cis-**9**	MeO	O	4.94 [f]	3.98	4.45	4.61	−6.3 [d]	0.034
trans-**9**	O	MeO	4.91	3.97	4.36	4.52	−4.6 [d]	[i]
cis-**10**	Ph	O	4.88 [g]	4.22	4.52	4.81	15.2 [e]	0.037
trans-**10**	O	Ph	5.15 [g]	4.17	4.71	4.68	19.6 [e]	0.032

[a] Ambient temperature. [b] Internal Me_4Si. [c] External aq 85% H_3PO_4. [d] Acetone-d_6. [e] $CDCl_3$. [f] Probable error calculated by the LAOCN3 program, 0.007 ppm. [g] Probable error, 0.007–0.011 ppm. [h] Root-mean-square error in the ^{1}H line positions. [i] LAOCN3 analysis not needed; first-order spectrum.

of $J_{4',5'b}$ that arises from the essentially antiperiplanar relation of H-5'a and H-4' in both the chair and twist conformations. The parameters for a four-coordinate 1,3,2-dioxaphosphorinane ring that is expected to be largely in the chair conformation are illustrated by those for *cis*-**9** (Table I). For comparison, those for the phenyl phosphate analog, *cis*-**11**, are also recorded. On the basis of the known[14] axial preferences of such substituents in monocyclic ring systems of this type, the PhO group should favor population of the chair conformation more than the MeO group. The small value (0.7 Hz) for $J_{5'a,P}$ for each cis phosphate is evidence that the substitution of PhO for MeO has not altered the equilibrium and that each is completely in the chair conformation. The large $J_{5'b,P}$ values noted for *cis*-**9** and *cis*-**11** are consistent with the antiperiplanar relationship of the atoms involved and the well-defined Karplus relation[15] for $^3J_{H,P}$.

By contrast, *trans*-**9** exhibits a much increased $J_{5'a,P}$ value (7.0 Hz), whereas $J_{5'b,P}$ is reduced to 14.8 Hz. This finding is parallel to the change in $J_{5'b,P}$ reported earlier[7] for *trans*-**11** (Table I), although, for that compound, the change in $J_{5'b,P}$ values in a given solvent was even larger. In conformation **B** of equation *1*, H-5'a is essentially pseudo-equatorial and will experience a large coupling to phosphorus. The opposite is true for H-5'b, which is pseudo-axial. These resonances are assigned readily on the basis of the large $J_{4',5'a}$ value predicted from the essentially antiperiplanar relation of those protons in **B** (Dreiding models).

A similar analysis of the ^{1}H NMR data shows that *cis*-**10** is destabilized in the chair conformation and that the twist form is also populated. By contrast, *trans*-**10** is primarily in the chair form in equilibrium with only a small percentage of the twist form.

The mole fractions (N) of **A** and **B** populated are estimated readily, as shown[3,4], by use of equation *2*. An analogous equation involving $J_{5'b,P}$ also yields the mole fractions of the conformers of equation *1*. For conformer **A** of *trans*-**9**,

$$N(\mathbf{B}) = \frac{J_{5'a,P}(\text{obsd}) - J_{5'a,P}(\mathbf{A})}{J_{5'a,P}(\mathbf{B}) - J_{5'a,P}(\mathbf{A})} \qquad (2)$$

$J_{H,P}$ values close to those for *cis*-**11** and *cis*-**9** should be reasonable estimates for $J_{5'a,P}$ and $J_{5'b,P}$. Therefore, 1.0 and 22.5 Hz, respectively, were chosen for the assumed values (Table III). As the sum of $J_{H,P}$ observed for *trans*-**9** is less than that for *cis*-**9**, it is evident that the $J_{H,P}$ sum in the twist form **B** is reduced from that of **A**. Almost certainly this stems from the fact that the degree of twisting in the non-chair conformer is not sufficient to bring H-5'a and H-5'b into fully pseudo-equatorial and pseudo-axial positions, respectively. Inspection of Dreiding models shows that the torsion angles H–C–O–P consequently will be greater than the 60° angle of a perfect chair for H-5'a and less than the optimal 180° angle for H-5'b. The Karplus relation[15] for $J_{H,P}$ then predicts reduced values for both $J_{5'a,P}$ and $J_{5'b,P}$. As reasonable estimates for the twist conformation **B** of *trans*-**9**, 19.0 Hz ($J_{5'a,P}$) and 0.5 Hz ($J_{5'b,P}$), used in previous studies of chair–twist equilibria[4], were chosen. The agreement for both *trans*-**9** and *trans*-**11** seen in Table III between

TABLE III

Estimated populations of **B** (equation *1*)

Compound	X	Y	Solvent	Observed *J* (Hz)		Assumed *J* (Hz)				**B**, % based on			
				5′a,P	5′b,P	5′a,P(**A**)	5′b,P(**A**)	5′a,P(**B**)	5′b,P(**B**)	5′a,P	5′b,P	Δ	Average
trans-**9**	O	MeO	Pyridine-d_5	8.3	11.7	1.0	22.5	19.0	0.5	41	38	3	40
trans-**9**	O	MeO	Acetone-d_6	7.0	14.8	1.0	22.5	19.0	0.5	33	33	0	33
cis-**10**	Ph	O	$CDCl_3$	7.7	11.7	1.0	22.5	19.0	0.5	37	49	12	43
cis-**10**	Ph	O	$CDCl_3$	7.7	11.7	1.0	20.5	19.0	0.5	40	44	4	42
cis-**10**	Ph	O	$CDCl_3$	7.7	11.7	0.5	19.0	19.0	0.5	39	39	0	39
cis-**10**	Ph	O	Acetone-d_6	6.1	13.3	1.0	20.5	19.0	0.5	28	36	8	32
cis-**10**	Ph	O	Acetone-d_6	6.1	13.3	0.5	19.0	19.0	0.5	30	31	1	31
cis-**10** [a]	Ph	O	CD_3CN	4.7	14.9	0.5	19.0	19.0	0.5	23	22	1	23
trans-**10**	O	Ph	$CDCl_3$	1.6	19.9	0.5	21.0	19.0	0.5	6	6	0	6
trans-**10**	O	Ph	Acetone-d_6	2.2	18.8	0.5	21.0	19.0	0.5	9	11	2	10
trans-**10** [a]	O	Ph	CD_3CN	2.6	18.0	0.5	21.0	19.0	0.5	11	15	4	13
trans-**11** [4]	O	PhO	$CDCl_3$	8.7	13.3	1.0	22.5	19.0	0.5	43	42	1	42
trans-**11** [4]	O	PhO	Acetone-d_6	11.6	9.6	1.0	22.5	19.0	0.5	59	59	0	59

[a] At 500 MHz.

TABLE IV

^{1}H NMR parameters for the 2′-deoxyribose rings of cTMP, **9**, and **10**[a,b]

Compound	J (Hz)							δ (ppm)					R.m.s. error[d]
	1′,2′a	1′,2′b	2′a,2′b	2′a,3′	2′b,3′	3′,4′	3′,P	H-1′	H-2′a	H-2′b	H-3′	H-4′	
cTMP[c,21]	8.9	2.4	−13.3	10.8	8.0	9.2	1.7	6.30	2.59	2.50	4.70	3.91	
cis-**9**	9.4[e]	2.4	−13.3	10.2	8.1	9.3	1.0	6.40[g]	2.66	2.64	4.94	3.98	0.040
trans-**9**	9.2[e]	2.6	−13.3	10.4	8.1	9.3	1.0	6.32[g]	2.52	2.48	4.91	3.97	0.058
cis-**10**	9.2[f]	2.7	−13.4	10.1	8.3	9.1	1.4	6.39[h]	2.69	2.65	4.88	4.22	0.033
trans-**10**	9.3[f]	2.6	−13.4	10.2	8.4	9.2	1.7	6.47[h]	2.70	2.64	5.15	4.17	0.052

[a] Acetone-d_6 at ambient temperature except where noted. [b] Determined by iterative use of the LAOCN3 simulation program. [c] In D_2O [d] Root-mean-square error in the ^{1}H line positions. [e] Probable error calculated by the LAOCN3 program, 0.008–0.019 Hz. [f] Probable error, 0.006–0.013 Hz. [g] Probable error, 0.005–0.0018 ppm. [h] Probable error, 0.004–0.017 ppm.

TABLE V

Effects of solvent and temperature on the chair–twist equilibrium for *trans*-**9**

Solvent	Temp. (°)	Observed J_{HP} (Hz)		**B**, % based on		
		5'a,P	5'b,P	5'a,P	5'b,P	Average
Acetone-d_6	26	7.0	14.8	33	33	33
Methanol-d_4	26	7.1	14.6	34	36	35
Acetonitrile-d_3	26	7.5	14.3	36	37	37
Nitromethane-d_3	26	7.7	14.8	37	35	36
Pyridine-d_5	26	8.3	14.2	41	38	40
Acetonitrile-d_3	72	8.0	13.6	39	40	40
Nitromethane-d_3	96	7.9	13.8	38	40	39

the percentages of twist conformer, **B**, calculated independently by use of experimental $J_{5'a,P}$ or $J_{5'b,P}$ values, lends credence to the assumed coupling constants.

For *cis*-**10**, the data in Table I show that the sum of the $J_{H,P}$ values is reduced to ~ 19.5 Hz in each of the three solvents. Not surprisingly, Table III shows that, for *cis*-**10**, use of the same assumed $J_{H,P}$ values for **A** and **B** that were applied successfully to the data for *trans*-**9** and *trans*-**11**, leads to wide differences in the estimates of the percentage of **B** based on measured $J_{5'a,P}$ and $J_{5'b,P}$ values. Since the $J_{H,P}$ sum for *trans*-**10** (21.0–21.5 Hz, Tables I and II) is smaller than that for **9** and **11**, reduced values for $J_{5'a,P}$ and/or $J_{5'b,P}$ were employed. The best agreement was noted when $J_{H,P}$ values of 0.5 and 19.0 Hz were used interchangeably. However, the *average* percentage of twist calculated (Table III) is not affected much over the range of assumed coupling constants. The low $J_{H,P}$ sum for *cis*-**10** suggests that the chair form may be somewhat distorted (see Discussion), since this sum[16] for chair–chair equilibria in 2-oxo-2-phenyl-1,3,2-dioxaphosphorinanes is 22–24 Hz.

The $J_{H,P}$ values for solutions of *trans*-**10** in acetone and CD_3CN suggest 6–13% depopulation of chair conformer **A** (Table III). Additional evidence for the presence of conformation **B** is the small, progressive change in $J_{5'a,P}$ and $J_{5'b,P}$ in the three solvents (see Discussion).

Conformations of the 2'-deoxyribose rings.—The ^1H NMR data for the ribose rings of the cis and trans forms of **9** and **10** are second order as a result of the small separation of the resonances of H-2'a and H-2'b. Therefore, they were refined iteratively, and the results are recorded in Table IV together with those for cTMP. Little change in the J values, and by inference the ring conformation, results from substitution or changing configuration at phosphorus. Thus, the conformations of the 2'-deoxyribose rings are unperturbed by the chair-to-twist conversion. This result parallels that found[4] for **11** and the large series of nucleoside-based cyclic 3',5'-phosphoramidates[3] for which the percentage of twist form varied greatly.

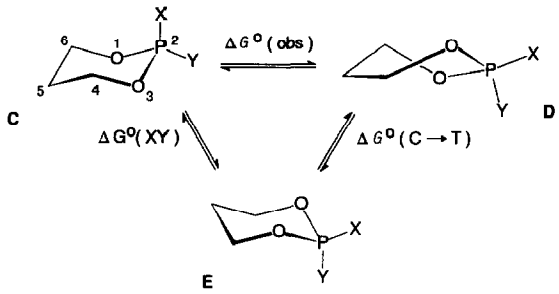
Effects of solvent and temperature on the conformational equilibrium for trans-**9**. —Table V records the variation in J values for the derivatized phosphate rings of

trans-**9** in a series of solvents and in CD_3NO_2 and CD_3CN at two temperatures. The percentage of twist conformations increased slightly at the higher temperatures and decreased slightly in solvents with a dielectric constant higher than that of pyridine-d_5.

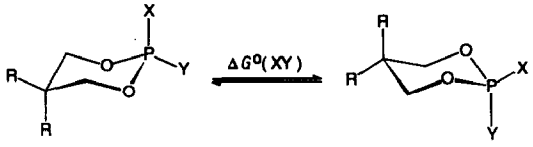
DISCUSSION

The above data show that an equatorial MeO on phosphorus destabilizes the chair conformation **A** and results in the population of the twist conformation **B**. The pseudo-axial preference of the MeO group can be understood in terms of stabilizing anomeric interactions of the lone pair of electrons on O-3′ and O-5′ and the P–OMe σ^* orbital available in the twist form but not in the chair form in which the MeO group is equatorial. The degree of population of the twist form by *trans*-**9** in a given solvent is less than that reported[4] for *trans*-**11**. Thus, the electron-withdrawing phenyl group enhances the n/σ^* stabilization by lowering the energy of the P–O σ^* orbital. The preference of the phenyl group of a 2-oxo-1,3,2-dioxaphosphorinane ring is balanced closely between the equatorial and axial positions and is dependent on the solvent as reflected by the equilibrium constants for the chair–chair equilibria of such rings[16]. That the chair form (**A**) of *cis*-**10** is partially depopulated to form the twist conformation **B** attests to the relative thermodynamic ease of the chair to twist interconversion of these rings.

Estimation of $\Delta G°(C \rightarrow T)$ *for cTMP.*—The chair–twist interconversion for the cyclic nucleotide derivatives can be represented by the isomerization **C** → **D** of Scheme 2. In turn, **C** → **D** is equivalent thermodynamically to the two-component, two-step process **C** → **E** → **D**. $\Delta G°(XY)$ corresponds to the change in free energy for the reorientation of the phenyl group in the chair conformation **A** of *cis*-**10** from axial to equatorial or the methoxy group of *trans*-**9** from equatorial to axial along with the concomitant change in free energy associated with the simultaneous reorientation of the phosphoryl oxygen. These energies have been measured experimentally by determination of $\Delta G°(XY)$ for the chair–chair equilibrium **13** ⇌ **14** in various solvents[14].



Scheme 2.



13 14

Moreover, the resistance of the ring system to the particular chair-to-twist conversion that puts H-4′(C-4′) and the MeO group of *trans*-**9** in the opposite bowsprit positions of **B** (equation *1*) is the free energy of converting **E** into **D** or $\Delta G°(C \rightarrow T)$. The same term for the phenylphosphonate *cis*-**10** (X = Ph, Y = O) also measures the change in free energy for **E** → **D**, in this instance the resistance to the chair-to-twist isomerization for *cis*-**10** that places H-4′(C-4′) opposite a phosphoryl oxygen in the twist form **B**. Equation *3* expresses this dissection of the conversion of **A** into **B**[3,4]. Table VI contains the necessary, measured energy change, $\Delta G°(XY)$, based on the equilibrium **13** ⇌ **14**, from which $\Delta G°(C \rightarrow T)$ is obtained from $\Delta G°$(obsd) for *trans*-**9** and *cis*-**10**, together with the same information for the derivatives *cis*-**12** (Z = Me_2N, Y = O)[3] and *trans*-**11** (Y = O, Z = PhO)[4] previously studied.

$$\Delta G°(C \rightarrow T) = \Delta G°(\text{obsd}) - \Delta G°(XY) \qquad (3)$$

The suitability of the term $\Delta G°(C \rightarrow T)$, derived from the equilibria for *trans*-**11** and *cis*-**12**, as an approximation of $\Delta G°(C \rightarrow T)$ for the cyclic nucleotide diester anion, has been discussed in detail[3]. Since the phosphoryl oxygen more nearly resembles the P–O of a diester than does the PhO group of *trans*-**11**, *cis*-**12** (Y = Me_2N, Z = O, equilibrium **A** ⇌ **B**) was deemed the better model. By analogy,

TABLE VI

Estimated $\Delta G°(C \rightarrow T)$ for the equilibrium **A** ⇌ **B** (300 MHz, ambient temperature)

Compound	Twist (%)	$\Delta G°$(obs) [a]	Solvent	$\Delta G°(XY)$ [a]	$\Delta G°(C \rightarrow T)$ [a]	Ref.
trans-**9**	40	0.3	Pyridine-d_5	−2.7 [d]	3.0	
trans-**9**	33	0.4	Acetone-d_6			
cis-**10**	39	0.3	$CDCl_3$	−0.3 ± 0.4	0.6 ± 0.4	
cis-**10** [c]	23	0.7	CD_3CN	0.1 ± 0.2	0.6 ± 0.2	
cis-**10**	31	0.5	Acetone-d_6	0.0 ± 0.2 [b]	0.5 ± 0.2	
trans-**10**	6	1.6	$CDCl_3$	0.3 ± 0.4	1.3 ± 0.4	
trans-**10**	10	1.3	Acetone-d_6	0.0 ± 0.2 [b]	1.3 ± 0.2	
trans-**10** [c]	13	1.1	CD_3CN	−0.1 ± 0.2	1.2 ± 0.2	
trans-**11**	42	0.2	$CDCl_3$	−2.0 [e]	2.2	4
trans-**11**	59	−0.2	Acetone-d_6			4
cis-**12**	51	0.0	CD_3CN	−0.8 [f]	0.8	3

[a] In kcal/mol. $\Delta G°(XY)$, except as noted, measured for **13** ⇌ **14**, R = Me, Et, Ph, ref. 16a,c. Value recorded is the average range determined for the series R = Me, Et, Ph. For example, for *cis*-**10**, the average of the range −0.1 to +0.30 is +0.1. The difference between 0.1 and the extremes is ±0.2. [b] From plots of $\Delta G°(XY)$ versus $1/\epsilon$ (ref. 16c). [c] At 500 MHz. [d] Measured for R = Me only, ref. 17. [e] For R = Me only, ref. 16c. [f] For R = Me only, ref. 16a.

cis-**10** is a better model than is *trans*-**9**. For each model, the value for $\Delta G°(C \rightarrow T)$ is an approximation because the diester will have a partial negative charge on each exocyclic oxygen. Also, as noted earlier[3], because of the lack of symmetry in the 1,3,2-dioxaphosphorinanes of the cyclic nucleotide derivatives, the relief of 1,3-syn-axial repulsions between H-3′ and H-5′ in the conversion **A** → **B** is only approximated by the term $\Delta G°(XY)$ or $\Delta G°(C \rightarrow E)$ measured for monocycles.

The evaluation of $\Delta G°(XY)$ for Scheme 2 (**13** ⇌ **14**) is crucial. This parameter is sensitive to both the nature of the substituent R on the ring and the polarity of the solvent. Where X = Ph and Y = O, several measurements of the equilibrium have been reported, based on IR data and, more commonly, ^1H NMR techniques, usually with good agreement[16]. When R = Me, Et, or Ph, the $\Delta G°$ values ranged from −0.10 to 0.30 kcal/mol in MeCN[16c] and from −0.70 to 0.19 kcal/mol in $CHCl_3$[16c]. The equilibrium was shifted towards **13** (Ph axial) in the more polar solvent MeCN, consistent with the obviously greater dipole moment of **13**. In each solvent, the population of **13** decreased in the order R = Et > Me > Ph. The solvent-polarity effect is also completely consistent with the observed greater population of **A** in CD_3CN compared to that in $CDCl_3$ (Table VI). Since it is difficult to choose between the $\Delta G°(XY)$ values as a function of R, the average between the extremes is recorded in Table VI, together with the difference between the average and the extremes (see footnote *a*). Both solvents yield the same value for $\Delta G°(C \rightarrow T)$, namely, 0.6 kcal/mol.

For acetone, $\Delta G°(XY)$ was not measured[16c], but for each of the equilibria **13** ⇌ **14** (R = Me, Et, Ph), linear plots of $\Delta G°$ versus $1/\epsilon$ were obtained[16c] for the solvents MeCN, pyridine, $CDCl_3$, and CS_2. By use of the dielectric constant (ϵ) for acetone, the range of $\Delta G°(XY)$ recorded in Table VI was obtained from the plots. The value (0.5 kcal/mol, Table VI) for $\Delta G°(C \rightarrow T)$ in acetone is consistent with those obtained for $CDCl_3$ and CD_3CN, for which $\Delta G°(XY)$ was directly measured[16c].

For *trans*-**9**, the dissection can be done only in one solvent, as the equilibrium between **13** and **14** (X = O, Y = MeO) has been measured only in pyridine[17] and only for R = Me. Most likely, a dispersion of values of $\Delta G°(XY)$ would arise for this equilibrium as well for a series of R. Furthermore, it is difficult to measure the equilibrium constant precisely for this equilibrium since, in polar solvents, **14** is nearly completely populated. For the closely related structure with X = EtO and Y = O, two values of $\Delta G°(XY)$ were reported for a solution in pyridine (2.4[16a] and 3.0[16c] kcal/mol) along with a value of 1.50 kcal/mol in CS_2[16a,c]. Surprisingly, the equilibrium **A** ⇌ **B** for *trans*-**9** was found not to respond greatly to changes in the polarity of the solvent (Table V), unlike that for *cis*-**9**. This situation makes the value (3.0 kcal/mol) of $\Delta G°(C \rightarrow T)$ from dissection of equation *3* less reliable for *trans*-**9**. Nonetheless, it is not in great disagreement with that (2.2 kcal/mol) determined[4] for *trans*-**11**.

As with *cis*-**12**, the values of $\Delta G°(C \rightarrow T)$ determined for *cis*-**10** (~ 0.6 kcal/mol) are relatively small. In part, this is because the phosphoryl oxygen is pseudo-axial

in the twist conformation. As noted earlier, this arrangement better approximates the twist form for cTMP than the twist conformation with an alkoxy or phenoxy group pseudo-axial. However, as for *cis*-**12**, it must be re-emphasized that the value of $\Delta G°(XY)$ employed for the pair of substituents X = Ph and Y = O in the dissection of the equilibrium may be less favorable than that for *cis*-**10**. Indeed, as noted in the Introduction, the above-mentioned lack of symmetry of the phosphate ring may lead to abnormally large steric repulsions between the axial phenyl and the axial hydrogen at C-3′ and a more favorable $\Delta G°(XY)$. The fact that smaller $J_{H,P}$ values needed to be used in order to obtain closely similar values for the percentage of the twist conformer from the experimental values of $J_{5'a,P}$ and $J_{5'b,P}$ may mean that the chair form (**A**) for *cis*-**10** is distorted to help relieve the steric repulsions in question (see comment on the assumed $J_{H,P}$ for *cis*-**10** in the Results section). Nonetheless, *the numbers obtained in the present study for* $\Delta G°(C \rightarrow T)$ *lie close to those determined previously for the series of cis phosphoramidates*[3] (see comparisons in Table VI). That the free energy required to convert the phosphorus-containing ring in thymidine cyclic 3′,5′-phosphates from the chair into the twist is relatively small is indisputable. Finally, it should be noted that from an investigation of the effects of changing the nucleobase and HO-2′ on the equilibria for the various cis phosphoramidates[3] it was concluded[3] that *a correction of* 0.5 ± 0.2 *kcal/mol needs to be added to estimates of such values for cTMP in order to make them applicable to cAMP.*

The values of $J_{H,P}$ noted for *trans*-**10** and their variation in $CDCl_3$, CD_3CN, and acetone-d_6 show that only 6–13% of the twist form is populated (Table III). The variation in the **B** population is as expected from the dielectric constants of the solvents[16a,c]. Use of Scheme 2, with X = O and Y = Ph and $\Delta G°(XY)$ for the equilibrium **13** ⇌ **14** (**C** ⇌ **E**), gives a value of 1.3 kcal/mol for $\Delta G°(C \rightarrow T)$. This value is for the chair-to-twist conversion **E** → **D** of Scheme 2 that places the Ph group pseudo-axial in conformation **D** rather than the sterically smaller phosphoryl oxygen that becomes pseudo-axial in the twist form **D** involved in the dissection of the equilibrium for *cis*-**10**. Nonetheless, even 1.3 kcal/mol is a relatively small value for a chair-to-twist interconversion in a six-membered ring.

Effects of solvent and temperature.—The small, but real increase in the population of the twist conformer at higher temperatures for *trans*-**9** (Table V) is a strong indication that its enthalpy is higher than that of the chair conformer. The lack of a large effect of the polarity of the solvent on the chair–twist equilibrium for *trans*-**9** is surprising since that equilibrium for *trans*-**11** was shifted markedly[4] in favor of the twist form on change from $CDCl_3$ to the more polar acetone-d_6. This effect is understood readily in terms of the greater dipole moment of the chair conformer of *trans*-**11** which has been discussed in detail[3,4]. A response of the equilibrium to the polarity of the solvent *opposite* to that of *trans*-**11**[3] was shown by *cis*-**12**, indicative of the reversed relative polarities of its chair and twist conformations relative to those of *trans*-**11**. Indeed, the chair conformation (**A**) for *cis*-**10** also will have a dipole moment smaller than that of the twist form. The

greater population of **A** by *cis*-**10** in CD_3CN and acetone-d_6 compared to $CDCl_3$ (Table VI) accords with the finding[3] for *cis*-**12** and the effect of solvent[16] on the chair–chair equilibrium for **13** ⇌ **14** when X = Ph and Y = O.

Clearly, the energy required to convert the phosphate ring of cAMP into the twist form is low, especially relative to those for cyclohexane (5 kcal/mol)[18] and 1,3-dioxane (8 kcal/mol)[19]. The possibility that this change occurs on binding cAMP to an enzyme, particularly a phosphodiesterase, deserves serious consideration[5], *although no evidence that this occurs is available.*

EXPERIMENTAL

$(MeN_2)_3P$ (Aldrich) was distilled before use. Medium-pressure liquid chromatography (MPLC) was performed on an Altex column (15 × 1000 mm) on Silica Gel 60 (Merck 230–400 mesh) at 35 p.s.i. Elemental analyses were carried out by Galbraith Laboratories, Knoxville, TN. Methylene chloride was distilled from P_2O_5 and then from CaH_2. Melting points are uncorrected. Phenylazotriphenylmethane was prepared as described[20].

NMR spectra.—^1H NMR spectra were recorded in the Fourier-transform mode with a Varian SC-300 or VXR-500 Spectrometer. Computer simulations were done at 100-Hz expansions with a 31-K data base, an acquisition time of 5.459 s, and an accuracy of ±0.2 Hz. Proton-decoupled ^{31}P NMR (32.2 MHz) spectra were obtained with a Varian FT-80A spectrometer; shifts downfield from the standard (external aq 85% H_3PO_4) are positive. Details of the spectral analysis of these types of cyclic nucleotides are given elsewhere[4].

trans-*Thymidine cyclic 3',5'-(benzyl phosphite)* (trans-**7**).—The procedure was analogous to that reported[10] for the preparation of *trans*-**8**. To a stirred solution of the phosphoramidite **6** (178 mg, 0.590 mmol) and pyridine hydrochloride (132 mg, 1.14 mmol) in CH_2Cl_2 (4 mL) at −78 °C was added benzyl alcohol (61 μL, 0.59 mmol) through a septum by a syringe. The mixture was then concentrated under reduced pressure to half volume, EtOAc (1 vol.) was added to precipitate pyridine hydrochloride, the mixture was concentrated under reduced pressure, and more EtOAc was added. After a third replacement of CH_2Cl_2 with EtOAc and reduction of the solution to half volume, the slurry was eluted from a short column of silica gel (Baker, 60–200 mesh) with 1:1 EtOAc–ether. The first portion of the eluate (50–60 mL) was concentrated under reduced pressure, then under high vacuum in the dark to remove benzyl alcohol and leave the product **7** (116 mg, 52%), as a cream-colored foam with trans,cis-ratio of 9:1. As judged from the ^1H and ^{31}P NMR spectra, the purity of **7** was ~95%: NMR data ($CDCl_3$): ^{31}P, δ 129.3 (cis, 122.0); ^1H (90 MHz), δ 9.8 (bs, 1 H, NH-1), 7.2–7.4 (bs, 5 H, Ph), 7.0 (bs, 1 H, H-4), 6.1 (dd, 1 H, H-1'), 4.8 (d, 2 H, $J_{H,P}$ 9 Hz, PhCH_2), 3.8–4.5 (m, 4 H, H-3',4',5'a,5'b), 2.2–2.4 (m, 2 H, H-2'a,2'b), 1.9 (bs, 3 H, Me-5).

cis-*Thymidine cyclic 3',5'-(benzyl phosphite)* (cis-**7**).—To a solution of **6** (232 mg, 0.770 mmol) and pyridine hydrochloride (193 mg, 1.67 mmol) in dry $CDCl_3$ (3 mL),

flushed with N_2, was added benzyl alcohol (80.0 μL, 0.770 mmol) dropwise with shaking. The capped tube was heated in a steam bath to maintain a gentle reflux. When complete isomerization to the cis diastereomer was attained (~ 20 min., ^{31}P NMR data), the solution was worked-up as described for *trans*-7 to give *cis*-7 (98 mg, 34%) as a cream-colored foam, the purity of which was ~ 95% (^1H and ^{31}P NMR data). NMR data ($CDCl_3$): ^{31}P, δ 122.0; ^1H (90 MHz), δ 9.5 (bs, 1 H, NH-1), 7.2–7.4 (m, 5 H, Ph), 6.9 (bs, 1 H, H-4), 6.1 (dd, 1 H, H-1′), 4.9 (d, 2 H, $J_{H,P}$ 10 Hz, PhCH_2), 4.0–4.5 (m, 3 H, H-3′,5′a,5′b), 3.4–3.7 (m, 1 H, H-4′), 2.1–2.5 (m, 2 H, H-2′a,2′b), 1.9 (bs, 3 H, Me-5).

cis-*Thymidine cyclic 3′,5′-phenylphosphonate* (cis-**10**).—A solution of *trans*-**7** (210 mg, 0.554 mmol, trans,cis-ratio 85:15) and phenylazotriphenylmethane (224 mg, 0.665 mmol) in dry benzene (5 mL) was flushed with dry N_2 for 20 min, then heated at 60° for 4 h. Removal of the solvent under reduced pressure afforded a brittle, pale-yellow foam consisting of an ~ 85:15 (cis,trans) mixture. MPLC (9 × 1000 mm column, 20:1 EtOAc–EtOH) gave *cis*-**10** (79 mg, 39%) as a brittle white foam, mp 128–130° (dec). NMR data ($CDCl_3$): ^{31}P δ 15.2 (trans, 19.6); ^1H (300 MHz), δ 7.50–7.88 (m, 5 H, Ph), 6.96 (bs, 1 H, H-4), 6.04 (dd, 1 H, H-1′), 4.82 (ddd, 1 H, H-5′b), 4.67 (apparent q, 1 H, H-3′), 4.37 (ddd, 1 H, H-5′a), 4.24 (ddd, 1 H, H-4′), 2.59–2.72 (m, 2 H, H-2′a,2′b), 1.90 (bs, 3 H, Me-5).

trans-*Thymidine cyclic 3′,5′-phenylphosphonate* (trans-**10**).—Reaction of *cis*-**7** (104 mg, 0.274 mmol) and phenylazotriphenylmethane (123 mg, 0.366 mmol), as described for *cis*-**10**, gave *trans*-**10** (32 mg, 32%) after MPLC (9 × 1000 mm column, 20:1 EtOAc–EtOH); mp 105–108° (dec). NMR data ($CDCl_3$): ^{31}P, δ 19.6; ^1H (300 MHz), δ 7.49–7.93 (m, 5 H, Ph), 7.12 (bs, 1 H, H-4), 6.43 (dd, 1 H, H-1′), 5.07 (apparent q, 1 H, H-3′), 4.76–4.83 (m, 1 H, H-5′a), 4.65 (ddd, 1 H, H-5′b), 3.97 (ddd, 1 H, H-4′), 2.62 (ddd, 1 H, H-2′a), 2.48 (ddd, 1 H, H-2′b), 1.98 (bs, 3 H, Me-5).

Anal. Calcd for $C_{16}H_{17}N_2O_6P$ (*cis,trans*-**10**): C, 52.75; H, 4.70; P, 8.50. Found: C, 53.06; H, 4.80; P, 8.38.

Thymidine cyclic 3′,5′-(methyl phosphates) (cis- *and* trans-**9**).—The preparation of this phosphate was described earlier[10]. To a solution of the phosphite **8** (as a mixture of diastereomers) in CH_2Cl_2 at 0° was added a satd solution of N_2O_4 dropwise until a green tint persisted. The solvent was removed, and MPLC (97:3 EtOAc–EtOH) of the residue separated the diastereomers.

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