values of those phases. Evaluations of the nonlinear optical properties of **2** are in progress and will be reported in due course.[17]

Experimental Section

The synthesis of **2a** has been described elsewhere.[11] X-ray analysis: Siemens P3f, Cu Kα, -165 (2) °C, highly oriented graphite crystal. 2θ range: 4.0–114.0°; scan speed 2.00–12.00°/min; scan range (ω) 0.80°. Background measurement: stationary crystal and stationary counter at beginning and end of scan, each for 16.7% of total scan time. A total of 1897 reflections collected, 1823 independent reflections (R_{int} = 4.02%); 1331 reflections observed ($F > 4.0\sigma(F)$); no absorption correction. Yellow prisms, 0.20 × 0.30 × 0.50 mm, $C_{15}H_{24}N_6O_6 \cdot C_7H_8$, formula weight 476.5; monoclinic, space group *Cc*, a = 16.944 (6) Å, b = 17.189 (8) Å, c = 11.169 (4) Å, β = 129.84 (3)°, V = 2498 (2) Å^3; 25 peaks to determine cell, 2θ range of cell peaks 45.00, 49.00°; Z = 4; density (calcd) 1.267 g/cm^3; absorption coefficient 0.741 mm^{-1}, F(000) 1016. Solution by direct methods,[18] full-matrix least-squares; absolute structure η = 1 (3); extinction correction χ = 0.0015 (4), where $F^* = F(1 + 0.002\chi F^2/\sin(2\theta))$?; hydrogen atoms riding model, isotropic U; weighting scheme $w^{-1} = \sigma^2(F) + 0.0006F^2$; 266 parameters refined; R (obsd data) = 6.96, R_w = 7.06%; Gof = 1.60; largest and mean Δ/σ 0.040, 0.003; largest difference peak 0.32 eÅ^{-3}, largest difference hole -0.29 eÅ^{-3}.

Acknowledgment. We thank the National Institutes of Health for partial financial support under GM.29549 and the Humboldt-Stiftung for a *Feodor Lynen* fellowship to J.J.W.

Registry No. 2a, 132699-66-2; *i*-PrNH$_2$, 75-31-0; $C_6H_5CH_3$, 108-88-3.

Supplementary Material Available: Atomic coordinates for **2a**·$C_6H_5CH_3$ (heavy and hydrogen atoms), bond lengths, bond angles, anisotropic displacement coefficients, and stereoviews of packing diagrams for **2a**$_1$ and **2a**$_2$ (7 pages). Ordering information is given on any current masthead page.

(17) Wolff, J. J.; McMahon, R. J.; Nelsen, S. F. Manuscript in preparation.

(18) Sheldrick, G. M. SHELXTL PLUS, Version 4.2; Siemens Analytical X-ray Instruments, Inc., Madison, WI, 1990.

Conformations of the Phosphorus-Containing Rings of Nucleoside Cyclic 3′,5′-Phosphoramidates. The Question of the Chair to Twist Free Energy Change for cAMP

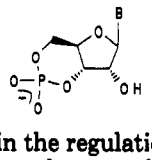
Alan E. Sopchik, Jozsef Beres, Jeno Tomasz, and Wesley G. Bentrude*

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

Received June 4, 1991

A series of nucleoside cyclic 3′,5′-phosphoramidates has been studied by ^{1}H NMR spectroscopy. For *cis*-**9–20** a chair–twist equilibrium has been characterized for the six-membered 1,3,2-dioxaphosphorinane (phosphoramidate) ring. The chair–twist equilibrium constant was estimated on the basis of the proton–phosphorus coupling constants for the 5′a and 5′b protons and found to vary with the nature of the amino group on phosphorus, the solvent, and to a lesser degree, the 2′-substituent (H or OH), and the heterocyclic base (purine or pyrimidine). The replacement of a pyrimidine base (uracil) with a purine base (adenine) shifts the equilibrium toward the chair conformation by only 0.1–0.3 kcal/mol. The presence of a 2′-OH also favors the chair form to a small extent (0.2–0.4 kcal/mol). The observed equilibrium constant for the *N,N*-dimethyl phosphoramidate derived from thymidine is used to estimate an intrinsic resistance of the six-membered ring to chair to twist interconversion ($\Delta G°$(C → T)) in three solvents of only 0.5–0.8 kcal/mol. Correction of this value by 0.5 ± 0.2 kcal/mol (change of base and 2′-substituent) gives an estimated $\Delta G°$(C → T) for cAMP of 0.8–1.5 kcal/mol. Similarly corrected, the value of $\Delta G°$(C → T) for cAMP, based on the previously studied trans phenyl cyclic 3′,5′-phosphate derived from thymidine, becomes 2.5–2.9 kcal/mol. The potential for chair–twist conversion on binding of cAMP to an enzyme is pointed out, although no experimental evidence regarding this question exists. *trans*-**9–20** all are shown to exist in the chair conformation. ^{1}H NMR reveals no evidence for conformational change in the sugar rings of these molecules on chair to twist conversion. The relative destabilization order for an axial amino substituent in the series **9–20** was found to be Me_2N > piperidinyl > $PhCH_2NH$ > PhNH.

Both adenosine cyclic 3′,5′-monophosphate, cAMP (**1**), and guanosine cyclic 3′,5′-monophosphate, cGMP (**2**), play



1 B = Adenine
2 B = Guanine

central roles in the regulation of cell metabolism.[1] cAMP binds to the regulatory subunits of protein kinases I and II to free the catalytic subunit that is responsible for the phosphorylation leading to activation of certain key metabolic enzymes.[2] The enzymatic, hydrolytic ring-opening conversion of cAMP to adenosine 5′-monophosphate (5′-AMP) is accomplished by specific isozymic phosphodiesterases.[3] A detailed understanding of the binding of cAMP to the active site is critical to a full understanding of the interaction of cAMP with protein kinases (PK's) or phosphodiesterase (PDE's).

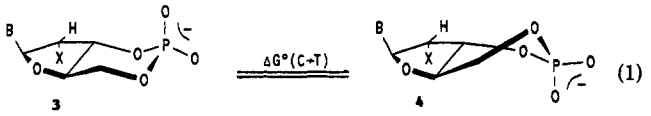
(1) See, for example the review series: *Adv. Cyclic Nucleotide Res.* **1971–1983**, *1–15*. *Adv. Cyclic Nucleotide Res. Protein Phosphorylation Res.* **1983–1987**, *16–20*. *Adv. Second Messenger Phosphoprotein Res.* **1988–1990**, *21–24*.

(2) Taylor, S. S.; Buechler, J. A.; Yonemoto, W. *Ann. Rev. Biochem.* **1990**, *59*, 971.

(3) Beavo, J. A. *Adv. Second Messenger Phosphoprotein Res.* **1988**, *22*, 1.

0022-3263/91/1956-5911$02.50/0

The relative ease with which the phosphate ring of a cyclic nucleoside is converted from the lower energy chair form to the twist conformation, **3** ⇄ **4**, was reported earlier



(1)

from this laboratory. Neutral derivatives, **5** (preliminary study)[4] and **6**,[5] of the cyclic 3′,5′-monophosphate diester of thymidine, cTMP, were used as probes of the conformational properties of this ring. The study of **6** showed that the intrinsic free energy change that resists the observed chair to twist conversion of the phosphate ring of cTMP, $\Delta G°$(C → T), under the stereoelectronic influence of the phenoxy group on phosphorus is only about 2 kcal/mol.

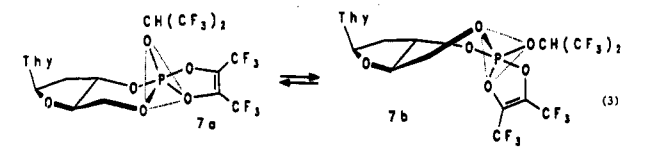
(2)

5a B = Thy, Z = Me_2N, Y = O **5b**

6a B = Thy, Z = O, Y = PhO **6b**

Clearly, so small an amount of energy can readily be overcome by the energy of binding of cAMP to an enzyme active site. Moreover, the *possibility* was suggested that cAMP is in fact enzyme bound in the twist conformation, particularly if some chemical advantage of doing so could be realized. *However, no direct evidence regarding this question exists.*

More recently, it was shown[6] that a thymidine-based model for the pentacovalent phosphorus, P(V), cAMP–H_2O adduct that has been proposed as an intermediate or transition state in PDE-catalyzed hydrolysis of cAMP *features the six-membered ring in a twist conformation*, [**7b**] ≫ [**7a**]. Furthermore, it was noted[6,7] that there may

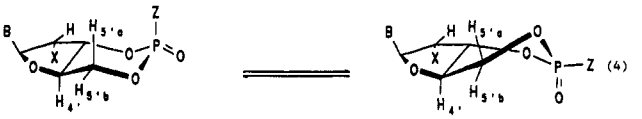


(3)

be a chemical advantage to this conformation in that the lone pair p orbital on O5′ is lined up in the twist form (as shown in **8**), *but not in the chair conformation*, to assist

kinetically both the formation of this adduct and its decay to 5′-AMP. The transition state for a one-step, inline displacement process would be similarly aided. It was suggested that the enzyme may bind cAMP in the twist form leading to the direct formation of the adduct in the twist conformation.[6,7] Again, there is *no experimental evidence* that this occurs.

The chair to twist free energy change, based on study of **6**, was estimated with a cTMP derivative that, unlike cAMP, bears no 2′-OH and contains a pyrimidine base rather than a purine heterocycle. Furthermore, the reorientation of the equatorial PhO was driven by *stereoelectronic* factors. Thus, the purpose of the research reported herein, utilizing **9–20**, is as follows: (1) to assess the affect on the chair–twist equilibrium shown by A ⇄ B, eq 4 (and by inference on **3** ⇄ **4**), of varying the nature



(4)

A B

cis-9	B = Ade, X = OH, Z = Me_2N	
cis-10	B = Ade, X = OH, Z = PhNH	
cis-11	B = Ade, X = OH, Z = $PhCH_2NH$	
cis-12	B = Ade, X = OH, Z = $N(CH_2)_5$	
cis-13	B = Ade, X = H, Z = $N(CH_2)_5$	
cis-14	B = Ade, X = H, Z = $PhCH_2NH$	
cis-15	B = Thy, X = H, Z = Me_2N	
cis-16	B = 5-I-Ura, X = H, Z = $PhCH_2NH$	
cis-17	B = 5-isoPr-Ura, X = H, Z = $PhCH_2NH$	
cis-18	B = 5-CF_3-Ura, X = H, Z = Me_2N	
cis-19	B = Ura, X = OH, Z = $N(CH_2)_5$	
cis-20	B = Ura, X = OH, Z = $PhCH_2NH$	

of the C2′ substituent (H or OH) and the identity of the nucleoside base and (2) to employ amino groups that display preferences for the pseudoequatorial position largely for *steric* reasons. In fact, only small effects (<1 kcal/mol) of either change were found. On replacement of the thyminyl group of **5** with the adeninyl moiety and the functionalization of C2′ with an α-OH (cTMP → cAMP), the two effects work in the same direction, but the total effect is no more than 0.3–0.7 kcal/mol. The intrinsic free energy change, $\Delta G°$(C → T), for the chair to twist conversion of the phosphate ring of **6** (2.2 kcal/mol), therefore, needs to be increased to no more than 3 kcal/mol to be applicable to the ring of cAMP.

Results

Preparations. The preparations of cyclic 3′,5′-phosphoramidates **9–20** were reported earlier. Activation of the cyclic 3,5-monophosphate diester precursor by Ph_3P/CCl_4[8] or triisopropylbenzenesulfonyl[9] chloride, followed by addition of the requisite amine, gave **9–14**, **16**, **17**, **19**, and **20**. The remaining cyclic 3′,5′-phosphoramidates were obtained by the efficient cyclization of the 2′-deoxynucleoside with $(Me_2N)_3P$ followed by *t*-BuOOH oxidation.[10] Individual diastereomers were separated chromatographically. The cis and trans geometries (relation of base to amino group, Z) were assigned by the known[6,11] upfield shifts of the ^{31}P resonances of the cis diastereomers of such compounds relative to those of the trans diastereomers. This is a general finding for 1,3,2-dioxaphosphorinanes and has been confirmed by X-ray crystallography for **15**[12] and **17**.[8]

(4) (a) Bentrude, W. G.; Sopchik, A. E. *Tetrahedron Lett.* **1981**, 307. (b) Sopchik, A. E.; Bajwa, G. S.; Nelson, K. A.; Bentrude, W. G. In *Phosphorus Chemistry*; Quin, L. D., Verkade, J. G., Eds.; ACS Symposium Series No. 171; American Chemical Society: Washington, DC, 1981; pp 217–220.

(5) Nelson, K. A.; Bentrude, W. G.; Setzer, W. N.; Hutchinson, J. P. *J. Am. Chem. Soc.* **1987**, *109*, 4058.

(6) Yu, J. H.; Arif, A. M.; Bentrude, W. G. *J. Am. Chem. Soc.* **1990**, *112*, 7451.

(7) Yu, J. H.; Sopchik, A. E.; Arif, A. M.; Bentrude, W. G. *J. Org. Chem.* **1990**, *55*, 3444.

(8) Beres, J.; Bentrude, W. G.; Parkanji, L.; Kalman, A.; Sopchik, A. E. *J. Org. Chem.* **1985**, *50*, 1271.

(9) Bentrude, W. G.; Tomasz, J. *Synthesis* **1984**, *1*, 27.

(10) Bentrude, W. G.; Khan, M. H.; Saadein, M. R.; Sopchik, A. E. *Nucleosides Nucleotides* **1989**, 1359.

(11) Maryanoff, B. E.; Hutchins, R. O.; Maryanoff, C. A. *Top Stereochem.* **1979**, *11*, 187.

(12) Bentrude, W. G.; Sopchik, A. E.; Bajwa, G. S.; Setzer, W. N.; Bates, R. B.; Ortega, R. B. *Acta Crystallogr., Sect. C* **1986**, *C42*, 584 and unpublished results.

Table I. Coupling Constants and Estimated Percentage of Conformation B for the 1,3,2-Dioxaphosphorinane Rings of *cis*-9–20 at 300 MHz, Ambient Temperature

compd	solvent	3′4′	5′a5′b	4′5′a	4′5′b	3′P	5′aP	5′bP	Σ	% twist (B)
9[a]	DMSO-d_6	10.0	−9.2	10.1	5.6	0.8	9.0	12.1	21.1	42
10	DMSO-d_6	10.0	−9.4	10.2	5.0	1.9	1.7	19.4	21.1	5
11[g]	DMSO-d_6	9.4	−9.1	10.1	4.9	1.3	4.1	17.2	21.3	16
12	DMSO-d_6	9.8	−9.0	9.8	5.4	0.6	8.2	13.1	21.3	37
13[a]	DMSO-d_6	8.7	−9.3	10.5	5.8	1.0[b]	9.9	11.2	21.1	47
14[a]	DMSO-d_6	9.1	−9.5	10.3	5.1	*c*	5.3	16.0	21.3	23
15[a]	toluene-d_8	9.1	−9.1	10.1	6.0	~0.2[b]	14.4	6.0	20.4	72
16	acetone-d_6	9.6	−9.4	10.3	5.4	1.0	7.3	14.0	21.3	33
17[f]	acetone-d_6	9.1	−9.4	10.3	5.3	1.1	6.8	14.4	21.2	31
18	acetone-d_6	9.2	−9.2	10.3	5.7	*c*	12.0	8.5	20.7	59
19[a]	DMSO-d_6	9.4	−9.2	10.5	5.8	*d*	9.3	10.9	20.2	46
20	DMSO-d_6	10.0	−9.3	10.0	5.4	*c*	4.9	16.9	21.8	19
cAMP[e]	D_2O	8.9	−9.7	10.7	4.8	2.0	1.7	21.4	23.1	0[h]
cTMP[e]	D_2O	9.2	−9.5	10.6	4.7	1.7	2.2	20.4	22.6	0[h]

[a] Iteratively refined by use of the LAOCN3 or LAOCN5 program. [b] J_{HP} value required to simulate spectrum. [c] Broadening of $H_{3'}$ resonance indicated the presence of an unresolved coupling, 0.5–1.0 Hz. [d] Nature of overlap of spectra of protons $H_{3'}$ and H_5 prevented band-shape fitting. [e] Reference 13. [f] $H_{1'}$, $H_{2'}$, $H_{3'}$, regions interatively refined by use of LAOCN5 program. [g] $H_{3'}$, $H_{4'}$, $H_{5'}$ regions iteratively refined by use LAOCN5 program. [h] Assumed from J_{HP} values of $H_{5'a}$, $H_{5'b}$.

Table II. ^1H Chemical Shifts for 1,3,2-Dioxaphosphorinane Rings of *cis*-9–20 at 300 MHz, Ambient Temperature

compd.	solvent	H3′	H4′	H5′a	H5′b
9[a]	DMSO-d_6	5.11	4.36	4.23	4.60
10	DMSO-d_6	5.23	4.28	4.50	4.66
11[b]	DMSO-d_6	5.05	4.25	4.31	4.54
12	DMSO-d_6	5.19	4.34	4.17	4.58
13[a]	DMSO-d_6	5.33	4.14	4.17	4.54
14[a]	DMSO-d_6	5.24	4.04	4.29	4.50
15[a]	Toluene-d_8	4.60	3.90	3.78	4.10
16	Acetone-d_6	4.90	4.09	4.43	4.57
17[c]	Acetone-d_6	4.89	4.03	4.34	4.55
18	Acetone-d_6	4.90	4.22	4.35	4.60
19[a]	DMSO-d_6	4.57	4.23	4.18	4.58
20	DMSO-d_6	4.46	4.12	4.36	4.54
cTMP[d]	D_2O	4.70	3.91	4.92	4.45
cAMP[d]	D_2O	4.75	4.37	4.39	4.60

[a] Iteratively refined by use of the LAOCN3 or LAOCN5 program. [b] $H_{3'}$, $H_{4'}$, $H_{5'}$ regions iteratively refined; LAOCN5 program. [c] $H_{1'}$, $H_{2'}$, $H_{3'}$ regions iteratively refined; LAOCN5 program. [d] Reference 13.

^1H NMR Results and Conformational Analysis of the 1,3,2-Dioxaphosphorinane Ring. The ^1H NMR spectra of the cis diasteromers of **9–20** were measured at 300 MHz. In Table I are recorded the coupling constants for the 1,3,2-dioxaphosphorinane rings of the compounds in question. The chemical shifts are found in Table II. ^1H NMR parameters for the purine and pyrimidine bases and the amino functionalities were reported in the papers concerning the preparations of **9–20**. Chemical shift differences allowed first-order analyses of the spectra to be made except in a few cases that were refined iteratively by use of spectral simulation programs as noted. For comparison purposes data previously reported for cAMP and cTMP also are recorded in Table I.

The chair–twist equilibrium to which **9–20** are subject is shown by the structures A and B of eq 4. Obviously, the trans ring fusion excludes a chair–chair equilibrium. The key coupling constants are the time-averaged, measured values for the coupling of $H_{5'a}$ and $H_{5'b}$ with phosphorus. The assignments of these protons are based on the large coupling of $H_{5'a}$ with $H_{4'}$ which is necessarily large, since the latter remain antiperiplanar to one another in both the chair and twist structures of equilibrium 4. (Dreiding models; indeed, the $J_{5'a4'}$ values remain nearly constant throughout Table I.) $^3J_{HCOP}$ values obey a Karplus relationship.[11,14] Thus, when the chair form, A, is highly populated, $J_{5'aP}$ is small as seen for **10** (1.7 Hz) and $J_{5'bP}$ is large (19.4 Hz for **10**). (See also J_{HP} for cAMP and cTMP.) By contrast, **13** has more nearly equal values for these couplings, 9.9 ($J_{5'aP}$) and 11.2 Hz ($J_{5'bP}$), evidence for the important contribution of twist conformer B to the equilibrium. For **15** $J_{5'aP}$ (14.4 Hz) is much larger than $J_{5'bP}$ (6.0 Hz), showing that the equilibrium favors twist conformer B.

As shown previously,[5] the mole fractions of A, N(A), and B, N(B), can be estimated from the observed, time-averaged, value of $J_{5'aP}$ by the simply derived eq 5. A completely analogous equation gives estimates of N(A) and N(B) from measured $J_{5'bP}$ values.

$$N(\mathrm{A}) = \frac{J_{5'aP}(\mathrm{obs}) - J_{5'aP}(\mathrm{B})}{J_{5'aP}(\mathrm{A}) - J_{5'aP}(\mathrm{B})} \quad (5)$$

This approach requires resonable, assumed values for the various $^3J_{HP}$ of the individual conformations A and B. For A $J_{5'aP}$ and $J_{5'bP}$ values with a sum of 21.5 Hz were deemed reasonable considering the measured sums for largely chair-form **10** (21.1 Hz), **11** (21.3 Hz), and the cis adenosine derivative with cis NH_2 (unpublished results, 21.7 Hz[15]). Since $J_{5'aP}$ for chair-form rings, A, is generally small (0.9 Hz for *trans*-**15**, and for the cis phenyl phosphate corresponding to **15**,[5] Z = PhO), the value of 1.0 Hz was used for $J_{5'aP}$(A) of eq 5. The number assumed for $J_{5'bP}$(A) was 20.5 Hz (21.5 Hz sum). The sum of $J_{5'aP}$ and $J_{5'bP}$ for a few of the examples of Table I that feature larger populations of twist B is reduced below 21 Hz. Although $H_{5'a}$ and $H_{5'b}$ are essentially interchanged in conformations A and B, the actual values of the coupling constants for pseudoequatorial $H_{5'a}$ and pseudoaxial $H_{5'b}$ in B will depend on the degree of twisting of the six-membered ring. Indeed, Dreiding models show that, on the basis of torsion angle HCOP comparisons, the sum $J_{5'aP}$ + $J_{5'bP}$ for B should be less than that for A unless B is very highly twisted. Assumed values for $J_{5'aP}$(B) (20.0 Hz) and $J_{5'bP}$(B) (0.5 Hz) reflect a reduced torsion angle (HCOP) for $H_{5'a}$ and an increased torsion angle for $H_{5'b}$ compared to those

(13) Blackburn, B. J.; Lapper, R. D.; Smith, I. C. P. *J. Am. Chem. Soc.* **1973**, *95*, 2873.

(14) (a) Bentrude, W. G.; Setzer, W. N. In *^{31}P NMR Spectroscopy in Stereochemical Analysis: Organic Compounds and Metal Complexes*; Verkade, J. G., Quin, L., Eds.; VCH Publishers: Deerfield Beach, FL, 1987; Chapter 11. Lankhorst, P. P.; Haasnoot, C. A. G.; Erkelens, C.; Altona, C. J. *J. Biomol. Struct. Dyns.* **1984**, *1*, 1387. (b) Kung, W.; Marsh, R. E.; Kainosho, M. *J. Am. Chem. Soc.* **1977**, *99*, 5471. (c) Lee, C. H.; Sarma, R. H. *J. Am. Chem. Soc.* **1976**, *98*, 3541.

(15) Tomasz, J.; Bottka, S.; Sopchik, A. E.; Bentrude, W. G. Unpublished results from this laboratory.

Table III. Effect of Changing Base on Chair–Twist Equilibrium A ⇄ B for Various *cis*-Phosphoramidates

compd	solvent	base	2′-X	Z	$J_{5'aP}$	$J_{5'bP}$	% twist	$\Delta\Delta G°$, kcal/mol
11	DMSO-d_6	Ade	OH	$PhCH_2NH$	4.1	17.2	16	−0.12 ± 0.08
20	DMSO-d_6	Ura	OH	$PhCH_2NH$	4.9	16.9	19	
12	DMSO-d_6	Ade	OH	$(CH_2)_5N$	8.2	13.1	37	−0.22 ± 0.05
19	DMSO-d_6	Ura	OH	$(CH_2)_5N$	9.3	10.9	46	
16	acetone-d_6	5-I-Ura	H	$PhCH_2NH$	7.3	14.0	33	0.05 ± 0.05
17	acetone-d_6	5-*i*-Pr-Ura	H	$PhCH_2NH$	6.8	14.4	31	
18	acetone-d_6	5-CF_3-Ura	H	$(CH_3)_2N$	12.0	8.5	59	0.05 ± 0.04
15[a,b]	acetone-d_6	Thy	H	$(CH_3)_2N$	12.3	8.3	60	

[a] Iteratively refined by LAOCN3 program. [b] Data independent of that for 15 in Table I.

Table IV. Effect of Changing 2′-Substituent on Chair-Twist Equilibrium A ⇄ B for *cis*-11–14[a]

compd	base	2′-X	Z	$J_{5'aP}$	$J_{5'bP}$	% twist	$\Delta\Delta G°$, kcal/mol
11	Ade	OH	$PhCH_2NH$	4.1	17.2	16	−0.26 ± 0.08
14	Ade	H	$PhCH_2NH$	5.3	16.0	23	
12	Ade	OH	$(CH_2)_5N$	8.2	13.1	37	−0.24 ± 0.05
13	Ade	H	$(CH_2)_5N$	9.9	11.2	47	

[a] DMSO-d_6 solvent.

in A. (Sum of $J_{5'aP}$ (B) plus $J_{5'bP}$ (B) equals 20.5 Hz.)

Twist- or boat-form six-membered rings normally are very flexible. However, it is very clear from Dreiding models that the phosphorus-containing, nonchair rings of these molecules are strongly biased toward a *single* twist form. This results from the five/six trans ring fusion. Furthermore, were a boat rather than twist conformation becoming populated, the J_{HP} values for compounds such as **15** would be approaching equality. Instead, one sees cases for which $J_{5'aP} > J_{5'bP}$. In fact with Z = Et_2N, even larger $J_{5'aP}$ and smaller $J_{5'bP}$ values than those for **15** are observed.[16]

The estimates of percentage twist conformation listed in Table I are averages of the two calculated from $J_{5'aP}$ and $J_{5'bP}$. The reasonableness of the assumed $^3J_{HP}$ values for conformations A and B is supported by the closeness of the percentages calculated. For the 16 cases of Tables I and III–VII, the difference in calculated percentages (not given) averaged 1.5. Cases were about equally divided between those for which $J_{5'aP}$ or $J_{5'bP}$ gave the larger calculated percentage. The precision of measured J_{HP} values is about 0.2 Hz. Trial calculations readily show that if $J_{5'aP}$ and $J_{5'bP}$ are *both* simultaneously changed by +0.2 or −0.2 Hz, the average of the calculated values of percentage twist is unchanged, although the difference in the two calculated percentages increases. If, however, the $^3J_{HP}$ values move in opposite directions by 0.2 Hz, the calculated average of percentage twist form increases or decreases by 1%. The agreement between percentages obtained from the two $^3J_{HP}$ values is little changed.

We showed in the paper describing the equilibrium for **6**[5] that changes in assumed J_{HP} values of 0.5–1.0 Hz changed the averaged values of percentage twist conformation by at most 2%. Even more important, *differences in conformer population for a given compound from solvent to solvent were unchanged.* Taken together with the premises of the above paragraph, it appears that *for the purposes of comparison* the percentages of twist conformer listed in Table I can be considered to be accurate within ±*1*%. The error limits in $\Delta G°$ given in the tables are calculated using percentages varied by 1% about the values given. For example in Table V, comparison of **9** and **15** were made with percentage twist values for **9** of 41–43% and for **15** of 56–58%.

In the following sections, we look at the effects on chair–twist equilibrium, A ⇄ B, of systematically changing the heterocyclic base, the 2′-substituent, X, and the amino group, Z, on phosphorus. For ease of comparison, data from Table I are grouped in Tables III–V according to structural change. Additionally, the heterocyclic base, C-2′ and Z substituents, and solvent are listed. All direct comparisons are made in the same solvent. Data from Table I are used in the other tables except when a different solvent (designated in the tables) is required for comparison purposes.

Change of Heterocyclic Base. In Table III two pairs of ribonucleoside phosphoramidates are first compared (**11** vs **20** and **12** vs **19**). With Z = $PhCH_2NH$ a barely discernible effect is seen. However, in the second case, Z = piperidinyl, a clearly measurable change occurs. The replacement of the pyrimidine base, uracil, with the purine base, adenine, *decreases the percentage of twist conformation (from 46 to 37%).* The effect on the free energy of equilibrium is small, however, of the order 0.1–0.3 kcal/mol.

The reliability of the measurements is supported by the results in Table III for **15**–**18** in acetone-d_6. Pairs of pyrimidine bases with the same Z display the same population of twist conformer within the error limits of the method. In each case a polar substituent at C-5 of uracil is compared with a nonpolar one, iodine vs isopropyl and CF_3 vs CH_3. The equilibrium is unaffected by change in pyrimidine base.

Nature of the 2′-Substituent. Table IV records the effect of the 2′-OH by comparing cyclic 3′,5′-phosphoramidate derivatives of adenosine and 2′-deoxyadenosine. There can be no doubt that for the pair with Z = $PhCH_2NH$ and that with Z = piperidinyl *the presence of the 2′-OH moves the equilibrium measurably towards the chair conformer.* This is obvious from simple inspection of the change in J_{HP} values and the 7–10% decrease in twist population (from 23 to 16% and 47 to 37%). Nonetheless, *the effect on $\Delta G°$ is relatively small,* of the order 0.2–0.4 kcal/mol.

Simultaneous Change in Both Heterocyclic Base and 2′-Substituent. To see whether the effects of substituent change on the A ⇄ B equilibrium are essentially additive, several cases in which both the heterocyclic base and 2′-substituent are changed were examined. The results in Table V verify the additive nature of the effects. Compounds **15** and **9** differ in both base and 2′-substituent. The largest change in percent twist conformer in DMSO-d_6 noted thus far is seen. Changes in J_{HP} of 2.5–3.5 Hz are noted, and a change from 57 to 42% in the amount of twist conformer populated is observed. Moreover, the relatively large effect is what would be expected from the conclusions drawn from Tables III and IV if *the effects of structural*

(16) Sopchik, A. E.; Bentrude, W. G. Unpublished results from this laboratory.

(17) Galdecki, Z.; Glowka, M. L. *Acta Crystallogr., Sect. B* **1981**, *B37*, 1136. Bentrude, W. G.; Setzer, W. N.; Newton, M. G.; Meehan, E. J., Jr.; Ramli, E.; Khan, M.; Ealick, S. *Phosphorus, Sulfur, Silicon* **1991**, *57*, 25.

(18) Kirby, A. J. *The Anomeric Effect and Related Stereoelectronic Effects at Oxygen*; Springer-Verlag: West Berlin, Heidelberg, New York, 1983.

Table V. Effect of Changing 2′-OH and Base on Chair–Twist Equilibrium A ⇌ B for *cis*-Phosphoramidates

compd	solvent	base	2′-X	Z	$J_{5'aP}$	$J_{5'bP}$	% twist	$\Delta\Delta G°$, kcal/mol
15[a]	DMSO-d_6	Thy	H	Me_2N	11.5	8.8	57	0.36 ± 0.05
9	DMSO-d_6	Ade	OH	Me_2N	9.0	12.1	42	
19	DMSO-d_6	Ura	OH	$(CH_2)_5N$	9.3	10.9	46	0.02 ● 0.05
13	DMSO-d_6	Ade	H	$(CH_2)_5N$	9.9	11.2	47	
20	DMSO-d_6	Ura	OH	$PhCH_2NH$	4.9	16.9	19	0.14 ● 0.07
14	DMSO-d_6	Ade	H	$PhCH_2NH$	5.3	16.0	23	

[a] Data independent of those in Table I.

Table VI. Selected ^{1}H NMR Parameters for the 1,3,2-Dioxaphosphorinane Rings of the Trans Diastereomers of 9–20 at 300 MHz

		J_{HH} or J_{HP} (Hz)							δ			
trans-	solvent	3′4′	5′a5′b	4′5′a	4′5′b	3′P	5′aP	5′bP	H3′	H4′	H5′a	H5′b
9	*a*	9.6	−9.2	9.4	4.6	1.5	0.6	21.4	5.11	4.19	4.39	4.55
10	*a*	9.8	−9.0	10.0	4.9	1.6	1.4	20.7	5.29	4.38	4.49	4.63
11	*a*	9.6	−9.1	10.4	4.9	1.7	1.5	20.8	5.12	4.18	4.36	4.49
12	*a*	9.1	−9.0	10.6	5.0	1.0	*d*	21.4	5.08	4.16	4.37	4.52
13	*a*	9.3	−9.3	10.4	4.9	*d*	0.8	21.4	5.69	3.95	4.35	4.47
14	*a*	9.8	−9.3	10.2	5.1	*d*	1.7	20.3	5.32	3.95	4.35	4.45
15[c]	*b*	9.2	−9.3	10.7	4.8	1.4	0.9	21.6	4.92	3.93	4.48	4.49
16[c]	*b*	9.3	−9.4	10.6	4.8	1.7	1.6	21.2	4.99	3.95	4.49	4.55
17[c]	*b*	9.3	−9.4	10.5	4.8	1.6	1.5	21.0	4.94	3.91	4.47	4.49
19	*a*	10.0	−9.1	10.2	5.1	*d*	*d*	21.1	4.57	4.03	4.39	4.51
20[c]	*a*	9.9	−9.1	10.3	4.9	1.9	1.4	21.0	4.58	4.06	4.42	4.48

[a] DMSO-d_6. [b] Acetone-d_6. [c] Iteratively refined by use of LAOCN3 program. [d] Peak broadened by J_{HP} (<1.0 Hz) but splitting not resolved.

Table VII. Selected ^{1}H NMR Parameters for the Sugar Rings of Representative Cyclic 3′,5′-Phosphoramidates at 300 MHz, Ambient Temperatures

		J_{HH} (Hz)						δ (ppm)				
compd	solvent	1′2′a	1′2′b	2′a2′b	2′a3′	2′b3′	3′4′	1′	2′a	2′b	3′	4′
cTMP[a]	D_2O	2.4	8.9	−13.3	8.0	10.8	9.2	6.30	2.50	2.59	4.70	3.91
cis-14[b] (23)[d]	DMSO-d_6	2.4	8.6	−13.3	7.6	10.4	9.1	6.48	2.74	2.71	5.24	4.04
cis-13[b] (47)	DMSO-d_6	1.7	8.9	−12.3	7.4	10.6	8.7	6.49	2.79	2.72	5.33	4.14
cis-17[b,e] (31)	acetone-d_6	2.4	9.2	−13.0	8.3	10.4	9.1	6.27	2.58	2.56	4.89	4.03
cis-15[b] (51)	CD_3CN	2.7	9.2	−13.3	8.4	10.4	9.2	6.28	2.42	2.51	4.67	4.07
cis-15[b,f] (69)	$CDCl_3$	2.7	9.2	−13.3	8.8	9.4	9.2	5.97	2.54	2.52	4.60	4.21
trans-13	DMSO-d_6	2.2	8.7	−13.1	8.2	10.6	9.3	6.47	2.79	2.69	5.29	3.95
trans-14	DMSO-d_6	1.6	8.8	−12.8	8.2	10.8	9.8	6.47	2.80	2.65	5.32	3.95
trans-15[b]	acetone-d_6	2.7	9.1	−13.1	8.4	10.3	9.3	6.42	2.60	2.53	4.92	3.93
cAMP[a]	D_2O	0.8			5.2		8.9	6.09	4.71		4.75	4.37
cis-9[b] (42)	DMSO-d_6	~0[c]			5.1		10.0	6.05	4.66		5.11	4.36
cis-10 (5)	DMSO-d_6	~0[c]			5.0		10.0	6.04	4.62		5.23	4.28
cis-11 (6)	DMSO-d_6	~0[c]			4.8		8.8	6.02	4.61		5.05	4.25
cis-12 (37)	DMSO-d_6	~0[c]			5.0		9.8	6.04	4.67		5.19	4.34
cis-19[b] (46)	DMSO-d_6	~0[c]			4.8		9.4	5.65	4.33		4.57	4.23
cis-20 (19)	DMSO-d_6	~0[c]			5.2		10.0	5.69	4.29		4.46	4.12
trans-9	DMSO-d_6	~0[c]			5.2		9.6	6.03	4.68		5.11	4.19
trans-11	DMSO-d_6	~0[c]			5.2		9.6	6.02	4.69		5.12	4.18
trans-19	DMSO-d_6	~0[c]			4.9		10.0	5.70	4.29		4.57	4.03
trans-20[b]	DMSO-d_6	~0[c]			5.3		9.9	5.71	4.38		4.58	4.06

[a] pH 7.2, 23 °C.[13] [b] Iteratively refined by use of LAOCN3 program. [c] Small unresolved J_{HP} present (<1.0 Hz). [d] Percentage twist conformer (B) present. [e] $H_{1'}$, $H_{2'}$, $H_{3'}$ regions interatively refined; LAOCN5 program. [f] At 500 MHz.

change are additive. Both replacement of a pyrimidine base (thymidine, **15**) by a purine (adenine, **9**) and of 2′-H (**15**) by 2′-OH are predicted to decrease the population of twist conformer. That the small effect of base change, noted in Table III, is real is shown by the numbers in Table V for **19** vs **13** and **20** vs **14**. In these pairs, the changes in base and 2′-substituent should work in *opposite* directions. The compensatory effects are seen in the small net changes in twist conformer population seen. In the first pair (**19** vs **13**), the change is within experimental error. In the second pair (**20** vs **14**) the very small but real net effect is in the direction expected if the 2′-substituent plays a slightly dominant role as the effects seen in Tables III and IV predict. The comparison of **13** with **19** may be less reliable in that the change in J_{HP} values relative to one another are in the same direction rather than opposite. Also, the discrepancy between the percent twist calculated by use of $J_{5'aP}$ and $J_{5'bP}$ for **19** is nearly the largest seen for all 16 compounds for which measurements are reported.

Variation in Amino Substituent, Z. The series **9–12** shows a decrease of ease of accomodation of Z axial in the order PhNH > $PhCH_2NH$ > piperidinyl > Me_2N. The same ordering of $PhCH_2NH$ and piperidinyl is seen in **13** vs **14** and in **19** vs **20**. These observed relative conformational energies can be readily understood in terms of orientation about the axial P–N bond[17] and the anomeric effect.[18] These groups will be discussed, along with other amino substituents, in a subsequent paper.

***trans*-9–20.** In Table VI are compiled the pertinent ^{1}H NMR parameters for the 1,3,2-dioxaphosphorinane rings of the corresponding trans diasteromers of **9–20**. The conformations of the 1,3,2-dioxaphosphorinane rings can be inferred, as for *cis*-**9–20**, from the coupling constants $J_{5'aP}$ and $J_{5'bP}$ for the readily assigned protons 5′a and 5′b.

Table VIII. $\Delta G°$(C → T) Based on the A ⇄ B Equilibrium for 5 in Various Solvents

solvent	$\Delta G°$ Me_2N[a]	% twist	$\Delta G°$(Obs)	$\Delta G°$(C → T)
C_6H_6	−1.1	72[b]	−0.6	0.5
pyridine	−1.0	57[c]	−0.2	0.8
CH_3CN	−0.8	51[c]	0.0	0.8

[a] Reference 19. [b] Measured in toluene-d_8. [c] Sopchik, A. E. Unpublished results from this laboratory.

$J_{5'aP}$ values are small and cover a narrow range from slightly less than 1 to 1.7 Hz. By contrast, $J_{5'bP}$ values, though also showing only a small variation, are large, 20.3–21.6 Hz. The sum of the J_{HP} values for $H_{5'a}$ and $H_{5'b}$ ranges only 22.0–22.8 Hz for the cases for which $J_{5'aP}$ was precisely determined. There is no doubt that *trans-9–20 exist essentially entirely in the chair conformation* equivalent to structure A of the equilibrium A ⇄ B but with the Z and P=O moieties interchanged. This result is not surprising for Z equal to Me_2N or $(CH_2)_5N$ in light of the approximately 1 kcal/mol preference of the Me_2N substituent for the equatorial position in monocyclic 2-oxo-1,3,2-dioxaphosphorinanes.[19]

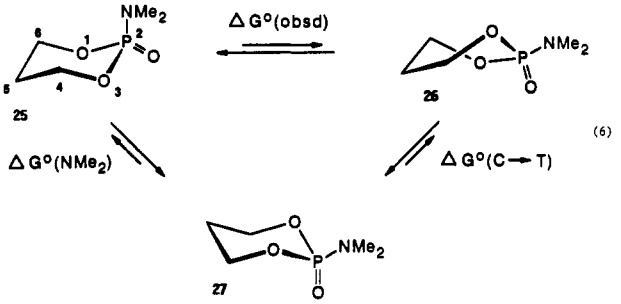
Conformations of the Sugar Rings. Table VII contains ^1H NMR parameters for the ribose and 2′-deoxyribose rings of selected examples from the series of *cis*- and *trans*-9–20. Particularly excluded were any instances in which second-order effects might make the J_{HH} values less reliable, except for those cases for which spectral simulation was done. For the cis diastereomers, both purine- and pyrimidine-based cyclic nucleotides were included along with ribose and 2′-deoxyribose examples and cases with large and small populations of twist conformer. For comparison, the same parameters for cAMP and cTMP are tabulated. As reported earlier for the cis and the trans cyclic 3′,5′-phenyl phosphites and phosphates based on thymidine, e.g., **6a**, as well as 5-coordinate P(V) analogues, **7**, the population of the twist conformation has little effect on the proton–proton coupling constants of the ribose and 2′-deoxyribose rings.[4-7] Changes in sugar ring conformations thus are not coupled to variations in the conformational equilibria of the six-membered rings to which they are trans fused. That the phosphorus containing ring is able to undergo conformational change without perturbing the relatively rigid trans-fused five-membered ring seems consistent with the similarity, shown below, of the free energy change for chair to twist conversion in *cis*-**9–20** to that for simple, monocyclic 2-oxo-1,3,2-dioxaphosphorinane rings.

Discussion

The clear result of this study is that exchange of a pyrimidine for a purine base has at most a very small effect (0.1–0.3 kcal/mol) on the equilibrium **6a** ⇄ **6b** in favor of the chair form. The presence of a 2′-OH biases the equilibrium toward the chair by 0.2–0.4 kcal/mol. Taken together these effects amount to about 0.3–0.7 kcal/mol (average, 0.5 kcal/mol). *This means that estimates of the intrinsic free energy change for chair to twist ring conversion for cAMP based on thymidine systems are not beset by very large errors.*

In the earlier paper[5] for the cis phenyl triester of thymidine cyclic 3′,5′-monophosphate, **6**, a thermodynamic dissection of the equilibrium **6a** ⇄ **6b** allowed an estimate of the intrinsic resistance to the conversion of this ring from the chair (**6a**) to the twist (**6b**), $\Delta G°$(C → T), of 2.2 kcal/mol to be made. The present work would raise that estimate to about 3 kcal/mol for cAMP itself (2.2 + 0.5 = 2.7 kcal/mol).

The same approach to obtain $\Delta G°$(C → T) is illustrated below for **5**(**15**) by the equilibrium involving **25–27**, eq 6.



(6)

The measured equilibrium free energy, $\Delta G°$(obsd) (**25** ⇄ **26**, equivalent to **5a** ⇄ **5b**), is related by eq 7 to the propensity of the Me_2N to be equatorial ($\Delta G°(Me_2N)$), **25** → **27**, and the intrinsic resistance of the ring to chair–twist conversion ($\Delta G°$(C → T)), **27** → **26**. Rearranged, eq 7

$$\Delta G°(\text{obsd}) = \Delta G°(\text{C} \rightarrow \text{T}) + \Delta G°(NMe_2) \quad (7)$$

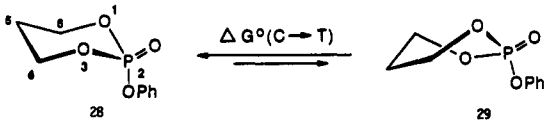
yields $\Delta G°$(C → T), eq 8. The value of $\Delta G°(NMe_2)$ of −1.1

$$\Delta G°(\text{C} \rightarrow \text{T}) = \Delta G°(\text{obsd}) - \Delta G°(NMe_2) \quad (8)$$

kcal/mol in C_6H_6, measured previously in another laboratory,[19] along with $\Delta G°$(obsd) in toluene (−0.6 kcal/mol) yields a $\Delta G°$(C → T) value of 0.5 kcal/mol (−0.6 − (−1.1)). Including the correction of about 0.5 kcal/mol for replacement of the thyminyl by adeninyl and hydrogen by OH at C-2′, $\Delta G°$(C → T) becomes 1.0 kcal/mol. This number is even lower than that determined similarly from the equilibrium (**28** ⇄ **29**) involving **6** of 2.7 kcal/mol.[5]

In Table VIII are recorded measured $\Delta G°$(C → T) values, based on equilibria for **5**(**15**) in benzene, CH_3CN, and pyridine. $\Delta G°$(C → T) determined in these solvents is a bit under 1 kcal/mol. Corrected to cAMP (0.5 kcal/mol) the numbers derived, 1.0–1.3 kcal/mol, may be compared to the 2.7 kcal/mol figure determined from study of **6**.

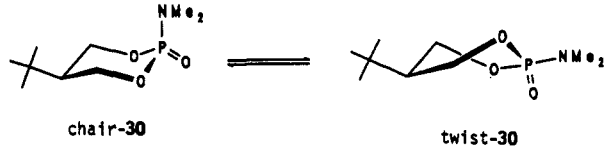
The degree to which the $\Delta G°$(C → T) values determined from the dissection of the equilibria **5a** ⇄ **5b** and **6a** ⇄ **6b** approximate the actual $\Delta G°$(C → T) for the equilibrium **3** ⇄ **4** depends on two factors. The *first* is the accuracy of the estimated driving force for the interconversion **25** → **27** (and its counterpart for **6a** ⇄ **6b**). The *second* is the degree to which steps **27** → **26** and **28** → **29** are good models for the interconversion **3** → **4**.



Indeed, some of the disparity in the values for $\Delta G°$(C → T) may arise from use in the thermodynamic analysis (equations 6–8) of $\Delta G°$(Z) values derived from chair–chair equilibria for monocyclic compounds. For example, a component of $\Delta G°$(Z), the magnitude of repulsive synaxial for axial Z (larger for Me_2N than for PhO) in the five/six fused ring system, may be different from that of the monocyclic case. Thus, the X-ray structure of *cis*-**5**(**15**), found to be in chair conformation **5a**, revealed a distortion of the 1,3,2-dioxaphosphorinane ring at the C5′–C4′–C3′ end leading to unequal internuclear distances between the amino nitrogen and the 3′- and 5′-axial hydrogens (2.66 and 3.00 Å, respectively).[12] The shorter distance is slightly less than the sum of the van der Waals distances for H and N (2.75 Å). Interestingly, in much earlier work from this

(19) Majoral, J.-P.; Gergounhou, C.; Navech, J. *Bull. Chim. Soc. Fr.* **1973**, 3146.

laboratory,[20] the ratio of chair–twist conformer populated by **30** in $CDCl_3$ was found to be 67:33, which is very close



chair-30 twist-30

to the 69:31 ratio for **5**(**15**) recorded in Table VI. Perhaps a slightly *increased* (more favorable) value of $\Delta G°(Me_2N)$ for **5** compensates for the inherently higher $\Delta G°(C \rightarrow T)$ for the fused ring systems, based on entropy considerations, and results in the same value of $\Delta G°$(obsd) for **5** and **30**. (For the monocyclic ring systems, two isoenergetic twist structures can be formed with equal probability, which lends to that interconversion a favorable entropy of mixing term of $-RT$ ln 2 or 0.4 kcal/mol.) For **6a** → **6b** $\Delta G°$(PhO) from the monocyclic system is likely to be an excellent approximation since 1,3-synaxial repulsions for PhO are no doubt smaller in both cases than for Me_2N and therefore nearly equal.

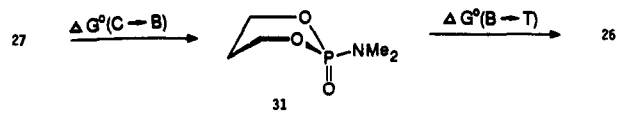
The effects of 1,3-synaxial repulsions between axial groups, Z, on phosphorus and the 3′- and 5′-axial hydrogens on the X-ray parameters of a large number of neutral phosphoramidate, phosphate, and phosphonate derivatives of 3′,5′-cyclic nucleoside diesters have recently been correlated. Both bond length and bond angle effects stemming from steric and stereoelectronic interactions were noted.[21]

With regard to the *second* point raised above, the conversions of **27** → **26** and **28** → **29** can be considered to be reasonably good models for the chair to twist conversion of cyclic phosphate diesters, **3** → **4**. Thus, in the above dissection of the equilibrium **25** ⇄ **26** (equivalent to **5a** ⇄ **5b**), the component $\Delta G°(C \rightarrow T)$, corresponding to **27** → **26** is obtained. In **27** → **26** an axial P=O (**27**) in a chair ring becomes pseudoaxial in a twist-form ring (**26**). That is, the carbon end (C-5) of the chair-form ring moves into position in the twist conformation opposite the pseudoaxial phosphorus–oxygen (P=O) bond. This is quite similar to the conversion **3** → **4** in which an oxygen attached to phosphorus remains equatorial (or pseudoequatorial) and another is transformed from being axial on a chair-form ring to pseudoaxial on a twist-form ring. (It is immaterial to the thermodynamics of the system that it is not the same oxygens undergoing these changes in **3** → **4**.) Similarly, the chair to twist conversion **28** → **29** brings C-5 into position opposite a pseudoaxial phosphorus–oxygen bond, this time P—OPh rather than P=O).

A closer look at the conversion **27** → **26** and **28** → **29** as models for **3** → **4** reveals that there are in fact *two steps and two free energy change components* to such chair to twist conversions, as shown in eq 9. The *first* is the

$$\Delta G°(C \rightarrow T) = \Delta G°(C \rightarrow B) + \Delta G°(B \rightarrow T) \quad (9)$$

increase in energy, $\Delta G°(C \rightarrow B)$ of eq 9, primarily stemming from cross-ring torsional repulsions that result as the chair conformation is converted to the *boat* form precursor to twist form **26** or **29**. (This is shown for **5**(**15**) by the sequence **27** → **31** → **26**.) It seems that as models for **3** → **4**, the conversions **27** → **26** and **28** → **29** should have closely similar increases in the cross-ring steric repulsion components that give rise to an unfavorable $\Delta G°(C \rightarrow B)$. That for the P—OPh pseudoaxial case might be slightly higher since the bond angles on the side of the phosphorus



27 $\xrightarrow{\Delta G°(C \rightarrow B)}$ **31** $\xrightarrow{\Delta G°(B \rightarrow T)}$ **26**

atom opposite the P=O are generally smaller than those on the other side (See, e.g., refs 5, 8, 12, 16, 23, 24), and the P—O bond distance for P—OPh will be greater than that for P=O.[5] The energy increase ($\Delta G°(C \rightarrow B)$) will be offset by a *decrease* in torsional energy included in the term $\Delta G°(B \rightarrow T)$, eq 9, corresponding to the *second step or component* of the chair to twist conversions (**27** → **26** and **28** → **29**). (This is shown for **27** → **26** by **31** → **26**.) Relaxation of the boat conformation to the *twist* form (**26** or **29**) will be accompanied by partial alleviation of repulsive torsional interactions in both **26** and **29** and bring $\Delta G°(C \rightarrow T)$ values closer together for systems **5** and **6** and close to that for **3** → **4**.

Another contributor to the term $\Delta G°(B \rightarrow T)$ of eq 9 stems from the fact that in chair forms **27** and **28** and in the *boat* conformers formed from them (e.g., **31**), a p orbital on each of the 3′ and 5′ oxygens is oriented reasonably well for stabilizing overlap with the adjacent *pseudoaxial* P=O or P—OPh antibonding σ orbital (n/σ^* overlap). Relaxation to the *twist* conformation and accompanying P—O(3′) bond rotation lead to enhanced n/σ^* overlap involving more parallel orientation of the p orbital lone pair on O3′ and the pseudoaxial σ^* orbital on phosphorus (Dreiding models show this clearly). The size of the term $\Delta G°(B \rightarrow T)$ will be thereby become more negative, thus reducing $\Delta G°(C \rightarrow T)$. This n/$\sigma^*$ component of the boat to twist conversion of **3** → **4** in the ionized form is likely somewhat less than that for **27** → **26** (n/$\sigma^*_{P=O}$) and **28** → **29** (n/σ^*_{P-OPh}). In any case, this component of $\Delta G°(B \rightarrow T)$ should be less than 1 kcal/mol.

The above factors, which influence both the accuracy of the dissection of the equilibria **5a** ⇄ **5b** and **6a** ⇄ **6b** and suitability of the derived $\Delta G°(C \rightarrow T)$ values as estimates of $\Delta G°$ for the equilibrium **3** ⇄ **4** both raise and lower the value of $\Delta G°(C \rightarrow T)$ obtained. It is probable, therefore, that the true value for cAMP (**3** and **4**, X = OH, B = adenin-1-yl) lies somewhere within the range of estimates of $\Delta G°(C \rightarrow T)$, 1.0–2.7 kcal/mol. *These values are well below those for cyclohexane, 5 kcal/mol*[5,22] *and 1,3-dioxane, 8 kcal/mol.*[23] It is likely that the reduction in $\Delta G°(C \rightarrow T)$ for the 1,3,2-dioxaphosphorinane ring stems from the long endocyclic P—O bonds and consequent flattening of the ring about phosphorus as well as, to a lesser degree, from improved n/σ^* stabilization in the twist conformation (vide supra). The ring flattening about phosphorus has been demonstrated by X-ray crystallography for chair-form 1,3,2-dioxaphosphorinanes,[24] including **15**[12] and **17**,[8,12] and for twist structures of the related 1,3,2-oxazaphosphorinanes.[25] Chair to twist conversions for cyclohexanes and 1,3-dioxanes obviously lack the benefits of improved n/σ^* orbital overlap available to 1,3,2-dioxaphosphorinanes.

The ease of forming twist conformations for 2-oxo-1,3,2-dioxaphosphorinane rings evidently is not a special property of the trans-fused five/six ring systems dealt with

(20) Bentrude, W. G.; Tan, H.-W. *J. Am. Chem. Soc.* **1973**, *95*, 4666.
(21) Bentrude, W. G.; Setzer, W. N. *J. Org. Chem.*, submitted for publication.
(22) Squillacote, M.; Sheridan, R. S.; Chapman, O. L.; Anet, F. A. L. *J. Am. Chem. Soc.* **1975**, *97*, 3244.
(23) Clay, R. M.; Kellie, G. M.; Riddell, F. G. *J. Am. Chem. Soc.* **1973**, *95*, 4632.
(24) See: Van Nuffel, P.; Van Alsenoy, C.; Lenstra, A. T. H.; Geise, H. J. *J. Mol. Struct.* **1984**, *125*, 1. Warrent, R. W.; Caughlan, C. N.; Hargis, J. H.; Yee, K. C.; Bentrude, W. G. *J. Org. Chem.* **1978**, *43*, 4266 and references cited therein.
(25) Bentrude, W. G.; Setzer, W. N.; Chandrasekaran, S.; Ashby, M. T. *J. Am. Chem. Soc.* **1988**, *110*, 7119 and references cited therein.

here. Considerable previous evidence for the ready formation of twist forms has been presented both in monocyclic rings[26] related to **30** and in trans-fused six/six ring systems.[27] $\Delta G°$(C → T) values of the order 1 kcal/mol or less have been estimated for the monocyclic systems.[20,26]

Of course, a resistance of 1–3 kcal/mol to chair to twist interconversion in cAMP could be easily supplied by binding energies within the active site of a protein kinase or phosphodiesterase if it were chemically advantageous for the phosphate ring to be bound in the twist conformation. As noted earlier, twist structure **8**[6] illustrates a potential stereoelectronic advantage to formation and/or cleavage of a P(V) transition state or intermediate for phosphodiesterase-catalyzed hydrolysis of cAMP to the 5-monophosphate.

A diagrammatic view of cAMP bound in an enzyme active site in the twist form was given in a previous paper.[5] It must be strongly emphasized that the possibility of the formation of the twist conformation of the phosphate ring of cAMP on binding to an enzyme is *completely speculative*. To our knowledge no experimental evidence exists concerning this point.

Finally, it should be stated that other trans-fused five/six 2-oxo-1,3,2-dioxaphosphorinanes, *not derived from nucleosides*, have been observed to exist as equilibrium mixtures of chair and twist forms.[28] In addition certain phosphoramidates derived from cAMP substituted with a methyl group at C-5′ have been examined by ^{1}H NMR.[29] However, none of these systems have been subjected to the thermodynamic dissection utilizing eqs 6 and 7. They, nonetheless, further illustrate the ease with which such chair to twist interconversions are accomplished.

Conclusions

The chair–twist equilibria of *cis*-**9**–**20** (neutral phosphoramidate derivatives of the corresponding cyclic 3′,5′-monophosphates) in a given solvent are affected only a little by changes in the heterocyclic base from pyrimidine to purine (0.1–0.3 kcal/mol) and from removal of the 2′-OH (−0.2 to −0.4 kcal/mol). Discussion of the equilibrium **5a** ⇄ **5b** yields a value for $\Delta G°$(C → T) for **27** → **26** (eq 6) of 0.5–0.8 kcal/mol that when corrected for change in base and 2′-substituent gives a value of $\Delta G°$(C → T) for the corresponding phosphoramidate of cAMP of 1.0–1.3 kcal/mol. A similar correction to the previously determined $\Delta G°$(C → T) value for the trans phenyl ester of cTMP yields an estimate for the cAMP phenyl ester of 2.7 kcal/mol. It is argued that the corrected values of $\Delta G°$(C → T) for the equilibria **27** ⇄ **26** and **28** ⇄ **29** provide a reasonable range of estimates of $\Delta G°$(C → T) for the equilibrium **3** ⇄ **4** for cAMP, i.e, 1.0–2.7 kcal/mol. The relatively low chair to twist free energy changes associated with 1,3,2-dioxaphosphorinane ring systems appear to arise from steric and stereoelectronic effects, i.e., relatively low cross-ring torsional repulsions and improved n/σ^* orbital overlap in the twist form. The energetic ease of conversion of cAMP to the twist form suggests that this conversion *could* accompany its binding to an enzyme. *However, no evidence exists as to whether this possibility in fact occurs.*

Experimental Section

Methods and Materials. ^{1}H NMR spectra were recorded at 300 MHz on a Varian SC-300 or XL-300 spectrometer or at 500 MHz on a Varian VXR-500 instrument (probe temperatures, 24 ± 2 °C). Digital resolution of the proton spectra was 0.2 Hz or less in all cases, and chemical shifts reported are accurate to 0.001 ppm. Computer simulations of second-order proton spectra were performed on an IBM PS/2 Model 50 computer using the NMR simulation program LAOCN5 from the Quantum Chemistry Program Exchange (QCMP049) or on a VAX 11/750 computer with a mainframe version of LAOCN3. Deuterated solvents from Cambridge Isotope Laboratory or Stohler Isotope Chemicals were used as received.

Preparations. The syntheses of **9**–**20** were reported previously.[8-10]

Acknowledgment. Support of this research by Grant 11045 from the National Cancer Institute of the Public Health Service is gratefully acknowledged.

Registry No. *cis*-**9**, 77881-40-4; *trans*-**9**, 77881-41-5; *cis*-**10**, 71960-54-8; *trans*-**10**, 71960-53-7; *cis*-**11**, 94903-45-4; *trans*-**11**, 94903-46-5; *cis*-**12**, 94903-47-6; *trans*-**12**, 94903-48-7; *cis*-**13**, 94844-14-1; *trans*-**13**, 94844-15-2; *cis*-**14**, 94844-12-9; *trans*-**14**, 94844-13-0; *cis*-**15**, 74867-68-8; *trans*-**15**, 74867-69-9; *cis*-**16**, 94844-23-2; *trans*-**16**, 94844-24-3; *cis*-**17**, 94844-27-6; *trans*-**17**, 94844-28-7; *cis*-**18**, 125985-25-3; *trans*-**18**, 125968-35-6; *cis*-**19**, 94844-19-6; *trans*-**19**, 94844-20-9; *cis*-**20**, 94844-17-4; *trans*-**20**, 94844-18-5.

(26) (a) Day, R. O.; Bentrude, W. G.; Yee, K. C.; Setzer, W. N.; Deiters, J. A.; Holmes, R. R. *J. Am. Chem. Soc.* **1984**, *106*, 103. (b) Mosbo, J. A. *Magn. Reson.* **1978**, *11*, 281. (c) Gerlt, J. A.; Gutterson, N. I.; Drews, R. E.; Sokolow, J. A. *J. Am. Chem. Soc.* **1980**, *102*, 1665. (d) Bentrude, W. G.; Yee, K. C. *Chem. Commun.* **1972**, 169. (e) Bentrude, W. G.; Hargis, J. H. *J. Chem. Soc., Chem. Commun.* **1969**, 1114.

(27) Taira, K.; Lai, K.; Gorenstein, D. *Tetrahedron Lett.* **1986**, *42*, 229. Rowell, R.; Gorenstein, D. G. *J. Am. Chem. Soc.* **1981**, *103*, 5894. Gorenstein, D. G.; Rowell, R.; Findlay, J. *Ibid.* **1980**, *102*, 5077. Gorenstein, D. G.; Rowell, R. *Ibid.* **1979**, *101*, 4925. Bouchu, D. *Phosphorus Sulfur Relat. Elem.* **1983**, *15*, 33. Bouchu, D.; Dreux, J. *Tetrahedron Lett.* **1980**, *21*, 2513; Yang, J.-C.; Shach, D. O.; Rao, N. U. M.; Freeman, W. A.; Sosnovsky, G.; Gorenstein, D. G. *Tetrahedron* **1988**, *44*, 6305.

(28) Hermans, R. J. M.; Buck, H. M. *J. Org. Chem.* **1987**, *52*, 5150. Hermans, R. J. M.; Buck, H. M. *Recl. Trav. Chim. Pay-Bas* **1988**, *107*, 82.

(29) Bottka, S.; Radics, L.; Tomasz, J. *Nucleosides Nucleotides* **1989**, *8*, 1217.