Structural Chemistry of Cyclic Nucleotides. III. Proton Magnetic Resonance Studies of β-Pyrimidine Nucleotides

David K. Lavallee[1] and Charles L. Coulter*

Contribution from the Department of Anatomy, University of Chicago, Chicago, Illinois 60637. *Received May 8, 1972*

Abstract: A 100-MHz pmr study of tetraethylammonium salts of 2′,3′-CMP, 2′,3′-UMP, and 3′-CMP in CD_3OD, D_2O, and ethylene glycol over a temperature range of −50 to 150° has been done to investigate the solution conformations of these nucleotides. J_0 values for the Karplus equation were derived from the solution coupling constants and the crystal-structure geometry of 2′,3′-CMP. We suggest J_0 = 7.16 Hz for a dihedral angle between vicinal protons of 0 to $\pi/2$ and J_0 = 10.1 Hz for $\pi/2$ to π as suitable values for nucleotide protons. Populations of conformations of the exocyclic CH_2OH group were derived for several nucleotides and nucleosides. The barrier to rotation is small. The near constancy of the H(6) chemical shift for 2′,3′-CMP and the marked change in this shift for 3′-CMP with temperature can be accounted for by assuming a fixed conformation for the base in 2′,3′-CMP and a syn–anti equilibrium for 3′-CMP. The barrier to rotation for 3′-CMP in methanol lies between 3 and 7 kcal/mol. 2′,3′-CMP appears to be locked in the syn conformation. Consideration of published data suggests that 5′-pyrimidine nucleotides are also syn–anti mixtures at room temperature.

Solution properties and conformations of nucleotides and nucleosides have been extensively studied in recent years.[2-8] The conformations of these compounds are of particular interest because they are well defined in the crystalline state[9] and relevant to nucleic acid structure and to the mechanism of action of ribonuclease and deoxyribonuclease. Nucleotides and nucleosides have been studied in a sufficiently wide range of crystal environments to permit generalization to solution conformation in some cases. An example is the previous paper in this series[10] on the crystal structure of β-cytidine 2′,3′-cyclic phosphate, an intermediate in the ribonuclease-catalyzed hydrolysis of ribonucleic acid. This structure was both unusual and especially rigidly defined because of the bicyclic ribose–phosphodiester ring system.

We report here a pmr[11] investigation of the solution conformations of 2′,3′-CMP and related nucleotides. We have found that the bases in 2′,3′-CMP and 2′,3′-UMP are in the syn conformation about the β-glycosidic bond over a wide temperatute range; 3′-CMP in methanol or water is a mixture of syn and anti conformers with the anti conformer the lower energy form. The barrier to base rotation in methanol was estimated to be between 3 and 7 kcal/mol. In addition, estimates of the populations of conformers about the C(4′)–C(5′) bond were made using the Karplus equation.[12] The J_0 values needed to calibrate this equation were derived from the solution coupling constants and the crystal-structure geometry[10] of 2′,3′-CMP.

Experimental Section

Instrumentation. Pmr spectra were recorded with Varian Associates A-60 and HA-100 spectrometers. Line positions on the HA-100 spectra were measured relative to an internal lock signal by counting the sweep oscillator frequency to 0.1 Hz. Field widths for the averaging computer were determined by counting the sweep oscillator frequency at each end of the spectrum to the nearest 0.1 Hz several times and averaging. A Nicolet 1080 computer was used to obtain the spectra for all variable temperature runs and for the calculated spectra. Each spectrum was taken with the same machine parameters except for the phase adjustment, which was very sensitive to temperature changes. For spectra taken in CD_3OD as a solvent, the methyl proton signal from a small amount of CH_3OH added to the solution was used as a line-width standard. Temperature calibration was made from the separation of OH proton signal and the CH_3 proton signals of methanol for temperatures of −60 to 50° and from the separation of the OH and CH_2 proton signals of ethylene glycol from 40 to 150°.[13] In all cases where spectra were taken above room temperature control spectra were taken at room temperature afterwards to ensure that no decomposition had taken place. At temperatures above 120° such decomposition was evident in some cases, and these spectra were not used in the analysis.

Materials. The following compounds were purchased from the indicated companies: Sigma Chemical Co., St. Louis, Mo., 2′,3′-CMP; Schwarz/Mann, 2′,3′-CMP; Calbiochem, 2′,3′-UMP as the cyclohexylguanidinium salt; Stohler Chemical Co., CD_3OD (99.5%) and D_2O (99.8%); Aldrich Chemical Co., tetramethylsilane, an internal reference standard; E. Merck Co., Germany, 3-trimethylsilylpropanesulfonic acid (Na salt), an internal reference standard; J. T. Baker Chemical Co., ethylene glycol. All reagents were of the highest purity, and when possible were used without further purification.

The 2′,3′-CMP, 2′,3′-UMP, and 3′-CMP were converted to tetraethylammonium salts using the $(Et_4N)^+$ form of a Dowex 50W-2X ion exchange resin. Water was removed by flash rotary evaporation at room temperature followed by several hours in a vacuum desiccator. D_2O was exchanged for the remaining H_2O by two dissolution–flash rotary evaporation cycles.

Ethylene glycol was vacuum distilled from NaOH, allowed to react with Na under dried N_2, refluxed with Na for 4 hr, and vacuum distilled into a flask containing Linde 4A molecular sieves. The

(1) Postdoctoral trainee under National Institutes of Health Grant GM-94.
(2) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Amer. Chem. Soc.*, **90**, 1042 (1968).
(3) P. O. P. Ts'o and S. I. Chan, *ibid.*, **86**, 4176 (1964).
(4) F. E. Hruska, A. A. Grey, and I. C. P. Smith, *ibid.*, **92**, 4088 (1970).
(5) B. J. Blackburn, A. A. Grey, I. C. P. Smith, and F. E. Hruska, *Can. J. Chem.*, **48**, 2866 (1970).
(6) S. S. Danyluk and F. E. Hruska, *Biochemistry*, 7, 1038 (1968).
(7) (a) S. Fujiwara and M. Uetsuki in "Recent Developments of Magnetic Resonance in Biological Systems," S. Fujiwara and L. H. Piette, Ed., Hirokawa Publishing Co., Tokyo, Japan, 1968, p 1; (b) M. Tsuboi, M. Kainosho, and A. Nakamura, in ref 7a, p 43.
(8) T. Schleich, B. J. Blackburn, R. D. Lapper, and I. C. P. Smith, *Biochemistry*, **11**, 137 (1972).
(9) M. Sundaralingam, *Biopolymers*, 7, 821 (1969).
(10) C. L. Coulter, *J. Amer. Chem. Soc.*, **95**, 570 (1973).
(11) Abbreviations used: pmr = proton magnetic resonance; 2′,3′-CMP = β-cytidine 2′,3′-cyclic phosphate; 2′,3′-UMP = β-uridine 2′,3′-cyclic phosphate; 2′-, 3′-, or 5′-CMP = cytidine 2′-, 3′-, or 5′-phosphate; DSS = 3-trimethylsilylpropanesulfonic acid; TMS = tetramethylsilane; $(Et_4N)^+$ = tetraethylammonium ion.
(12) M. Karplus, *J. Chem. Phys.*, **30**, 11 (1959).
(13) A. L. Van Geet, *Anal. Chem.*, **42**, 679 (1970).

2′,3′-CMP (Et_4N) was dissolved in the ethylene glycol along with DSS in an N_2 drybox. The resulting solution was dried over Linde 4A molecular sieves.

The pD of the D_2O solutions was measured with a Beckman Research pH meter, using a Sargent 30070-10 combination electrode. Using the relation pD = pH (meter reading) + 0.40,[14] the pD values of the solutions for which results are given in this report were: 2′,3′-CMP (Et_4N), 7.25; 3′-CMP (Et_4N), 7.17 and 5.19; 2′,3′-UMP (Et_4N), 8.16; all values ±0.05. pD was adjusted with NaOD. The nucleotide concentrations used in this study were 0.01–0.1 *M*. All experiments were done with solutions of at least two different concentrations, and no concentration dependence of the chemical shifts was observed. Some association of nucleotides in solution cannot be ruled out, but if present, the effect on the pmr spectra is small compared to the effects treated here. Lee, *et al.*,[15] detected no evidence for association in their careful study of cytidine nucleotides.

Results and Discussion

Spectral Assignment. The proton resonances of 2′,3′-CMP (Et_4N) and 2′,3′-UMP (Et_4N) at 32°, 0.05 and 0.01 *M*, respectively, were assigned with the aid of spectra simulated using the Nicolet 1080 NMRCAL program.[16] The observed and calculated spectra for the nucleotide protons of 2′,3′-CMP (Et_4N) at 100 and 60 MHz are shown in Figure 1. Inspection of the agreement between the calculated and observed spectra at 60 MHz can reveal errors in the values of coupling constants and chemical shifts used to simulate the 100-MHz spectrum and is a useful test of an acceptable simulation.[8] Changes in coupling constants of 0.2 Hz were sufficient to cause major intensity changes.

Assignment of the proton signals as shown in Figure 1 can be made unambiguously from the data available for nucleotide and nucleoside pmr spectra.[17] Especially pertinent to the cytidine nucleotides is the determination of proton resonance assignments for uridine by Blackburn, *et al.*,[5] using double-irradiation experiments. The nucleotides investigated also show an unequivocal coupling pattern. The coupling constants and chemical shifts in D_2O, relative to DSS, for 2′,3′-CMP and 2′,3′-UMP are given in Table I. Comparable data were obtained by Smith, *et al.*[18]

An interesting feature of the spectrum of 2′,3′-CMP is the large width of the H(5) signal. Lee, *et al.*,[15] have recently shown that this may be interpreted as the result of a tautomeric equilibrium between the amino and imino forms of the cytosine base. They investigated 5′-CMP, 2′-CMP, 3′-CMP, and several other compounds and concluded that the chemical exchange was of the same nature in all the cytosine-containing compounds studied. This seems the most reasonable explanation for the broad H(5) signals in 2′,3′-CMP. The H(5) signals of 2′,3′-CMP spectra taken in CD_3OD were not broadened like those in D_2O, and the broadening was not evident in 2′,3′-UMP spectra.

Lee, *et al.*,[15] also determined the dependence of the chemical shifts of the H(5) and H(6) protons of cytosine on the pD of the solution. These shifts are very highly pD dependent from pD 3 to 5, the region of the p*K* of cytidine, and pD independent at higher values. Hence, it was important to maintain a pD above 5. The difference between the chemical shifts of the H(6) and H(5) proton signals of 3′-CMP at 32° in this study (1.80 ppm, pD 7.2) agrees well with the value found by Lee, *et al.*,[15] which was 1.82 ppm at pD 7.2.

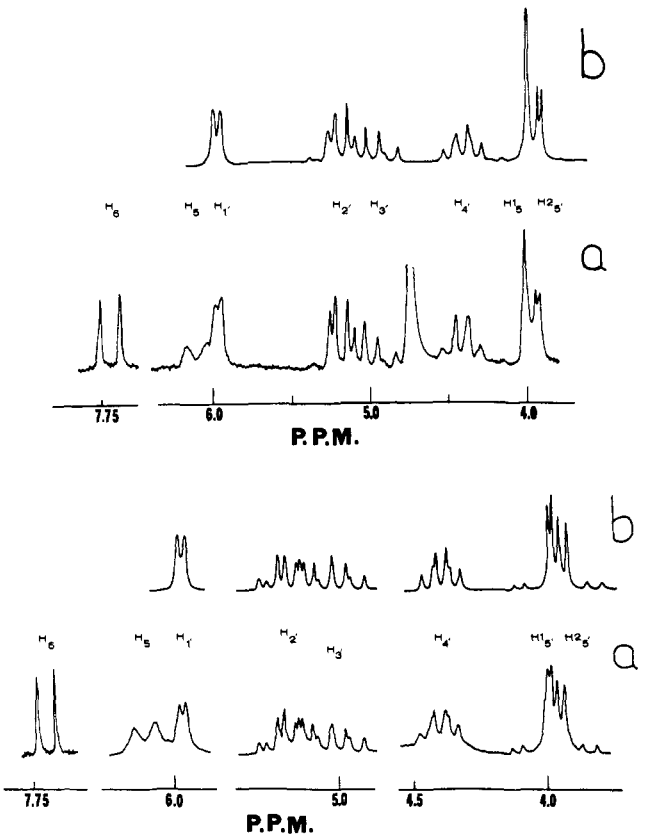


Figure 1. The observed (a) and calculated (b) nmr spectra of 2′,3′-CMP in D_2O. Chemical shifts are relative to internal DSS. H(6) and H(5) were not included in the simulations. Upper spectrum is at 60 MHz and the lower at 100 MHz. The unlabeled peak in the observed 60-MHz spectrum is due to HDO.

Table I. Chemical Shifts and Coupling Constants of Cytidine 2′,3′-Cyclic Phosphate and Uridine 2′,3′-Cyclic Phosphate in D_2O

	Chemical shifts, ppm[a]			Coupling constants, Hz[a]	
	2′,3′-CMP	2′,3′-UMP		2′,3′-CMP	2′,3′-UMP
H(6)	7.695	7.700	J_{56}	7.90	7.95
H(5)	6.108	5.876	$J_{1',2'}$	3.05	3.10
H(1′)	5.964	5.930	$J_{1'-P}$[b]	0.75	0.75
H(2′)	5.229	5.192	$J_{2',3'}$	6.87	7.00
H(3′)	5.050	4.983	$J_{2'-P}$[b]	7.17	9.70
H(4′)	4.408	4.339	$J_{3',4'}$	5.40	5.75
H-1(5′)	4.023	3.950	$J_{3'-P}$[b]	12.20	12.10
H-2(5′)	3.944	3.881	$J_{4',5'-1}$[c]	3.80	3.55
			$J_{4',5'-2}$[c]	5.45	5.60
			$J_{5'-1,5'-2}$	−12.70	−12.50

[a] These values give the best calculated fit to observed spectra. Chemical shifts are relative to DSS as internal standard. Estimated accuracies are 0.01 ppm and 0.10 Hz; sample temperature = 32°; pD = 7.3 for 2′,3′-CMP and 8.2 for 2′,3′-UMP. [b] These are approximate values; accurate P–H coupling constants require P decoupling. D. B. Davies and S. Danyluk (to be published) have done this for 2′,3′-CMP and 2′,3′-UMP. [c] Cannot be distinguished from one another.

Conformation of the Exocyclic CH_2OH Group.

(14) P. K. Glascoe and F. A. Long, *J. Phys. Chem.*, 64, 186 (1960).
(15) G. C. Y. Lee, J. H. Prestegard, and S. I. Chan, *J. Amer. Chem. Soc.*, 94, 951 (1972).
(16) Nicolet Instrument Corporation, Madison, Wis., 1971.
(17) C. D. Jardetzky and O. Jardetzky, *J. Amer. Chem. Soc.*, 82, 222 (1960).
(18) I. C. P. Smith, H. H. Mantsch, R. D. Lapper, R. Deslauriers, and T. Schleich in "Conformations of Biological Molecules and Polymers," E. Bergman and B. Pullman, Ed., Academic Press, New York, N.Y., in press.

Table II. Relative Populations of Classically Staggered Rotational Isomers about C(4′)–C(5′) in Aqueous Solution at 30°

	2′,3′-CMP	2′,3′-UMP	3′-CMP	Uridine	Pseudouridine	3′-UMP
$J_{4',5'-1}$[a]	3.8 Hz	3.6	3.1	3.0[c]	3.2[d]	3.0[e]
$J_{4',5'-2}$[a]	5.5 Hz	5.6	3.6	4.4[c]	4.6[d]	4.0[e]
P_I[b]	0.25 (0.36)	0.26 (0.37)	0.55 (0.67)	0.47 (0.58)	0.43 (0.54)	0.52 (0.64)
P_{II}[b]	0.47 (0.42)	0.49 (0.44)	0.26 (0.20)	0.35 (0.30)	0.37 (0.32)	0.30 (0.24)
P_{III}[b]	0.28 (0.22)	0.25 (0.19)	0.19 (0.13)	0.18 (0.12)	0.20 (0.14)	0.18 (0.12)

[a] H-1(5′) and H-2(5′) cannot be distinguished; interchanging $J_{4',5'-1}$ and $J_{4',5'-2}$ interchanges P_{II} and P_{III}. [b] Using $J_{0°}$ = 7.16 Hz and $J_{180°}$ = 10.1 Hz for the direct entries and $J_{0°}$ = 9.27 Hz and $J_{180°}$ = 10.36 Hz from Blackburn, *et al.*,[5] for the values in brackets. The population accuracy is estimated to be about 10%. [c] Reference 4. [d] Reference 5. [e] Reference 8.

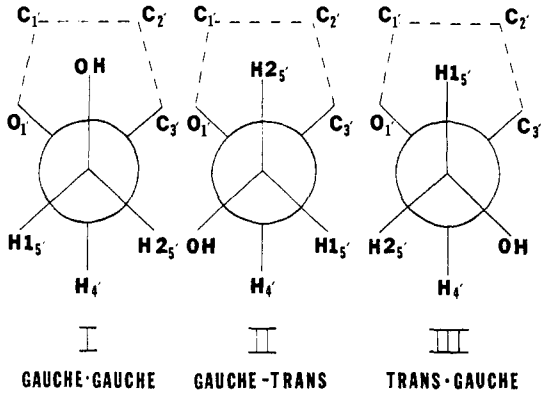


Figure 2. Schematic diagram of the rotational isomers about C(4′)-C(5′).

Changes in the respective proton–proton coupling constants can be used to investigate the dihedral angles between H(4′) and H-1(5′), and H(4′) and H-2(5′). Hruska, *et al.*,[4] and Schleich, *et al.*,[8] estimated populations of conformational states about C(4′)–C(5′) for a variety of uridine nucleosides and nucleotides. The relation between the dihedral angle of protons on vicinal carbon atoms and the proton–proton coupling constant is given by the Karplus equation[12] (1) where

$$J_{HH'} = J_0 \cos^2 \phi - 0.28 \text{ Hz} \quad (1)$$

$J_{HH'}$ is the vicinal coupling constant, ϕ is the dihedral angle between H–C–C′ and C–C′–H′. J_0 has two values, one for $0° \leq \phi \leq 90°$ and one for $90° \leq \phi \leq 180°$, here termed $J_{0°}$ and $J_{180°}$. Values of J_0 from 8 to 16 Hz have been used.[19] The choice of J_0 values has previously been difficult and necessarily arbitrary in the case of nucleosides and nucleotides, since there were no fixed dihedral angles for these molecules in solution. In the careful study of Blackburn, *et al.*,[5] the values suggested by Abraham, *et al.*,[20] from a study of a number of carbohydrate systems were applied to uridine.

In the crystal structure of 2′,3′-CMP,[10] the protons H(2′) and H(3′) were nearly eclipsed in both of the nucleotides in the symmetric unit even though the ribose conformations and the conformations about C(4′)–C(5′) were different. Rotation about C(4′)–C(5′) can give rise to three different conformations corresponding to O(5′) being gauche or trans to O(1′) and gauche or trans to C(3′), as illustrated in Figure 2. The 2′,3′-CMP nucleotides in the crystal were gauche–gauche and gauche–trans;[10] the H(2′)–C(2′)–C(3′)–H(3′) dihedral angles ($\phi_{2',3'}$) were 4 and 1° for these cases. If we assume an average value of $\phi_{2',3'}$ in solution of 2.5°, we can calculate $J_{0°}$ from eq 2, which

$$J_{0°} = (J_{2',3'} + 0.28)/(\cos^2 (2.5)) \quad (2)$$

gives a $J_{0°}$ value of 7.16 Hz. The dihedral angles would be 19° under conditions of maximum additive error, giving a $J_{0°}$ of 8 Hz. Thus the rather low accuracy of these dihedral angles from the crystal analysis (±5°), since they involve hydrogen atoms, should not be a limiting factor. Some torsional flexing in solution such as suggested by Lapper, *et al.*,[21] could also be accommodated. To calculate $J_{180°}$ we used the H(3′)–H(4′) dihedral angle. This angle was 144 and 133° for the nucleotides in the crystal. A calculation of $J_{180°}$ based upon the mean $\phi_{3',4'}$ gives a value of 10.1 Hz. This value is less reliable than $J_{0°}$, since it is sensitive to angle variation.

Proton H(4′) is also coupled to the two C(5′) protons. The chemical shifts and coupling constants will differ for these protons in the different conformations as the environments will differ (Figure 2). If the various rotational isomers interconvert rapidly with respect to the nmr time scale, the coupling constants between H(4′) and H-1(5′) and between H(4′) and H-2(5′) will be average weighted according to the relative populations of the three isomers. The dihedral angles involving these protons in the crystal[10] do not differ significantly from 60 and 180° for gauche and trans. The weighted coupling constant may be expressed as

$$J_{4',5'-1} = P_I J_{60°} + P_{II} J_{60°} + P_{III} J_{180°} \quad (3)$$

$$J_{4',5'-2} = P_I J_{60°} + P_{II} J_{180°} + P_{III} J_{60°} \quad (4)$$

where P_I is the gauche–gauche fraction and P_{II} and P_{III} the gauche–trans and trans–gauche populations. Setting $P_{II} = (1 - P_I)y$, where y is $P_{II}/(P_{II} + P_{III})$, and $P_{III} = (1 - P_I)(1 - y)$ and substituting the Karplus equation (1) for $J_{60°}$ and $J_{180°}$ using the previously derived values of $J_{0°}$ and $J_{180°}$ give two simultaneous equations in two unknowns, P_I and y. Solution of these equations gives the populations shown in Table II. The gauche–gauche conformation is the most frequently observed in the crystals,[8,22,23] but the energy differences among the conformers can be taken up by packing advantages to give gauche–trans or trans–gauche. In the cases of 2′,3′-CMP and 3′-CMP, and as noted by Blackburn, *et al.*,[5] for uridine, there is little change in $J_{4',5'-1}$ and $J_{4',5'-2}$ with temperature, suggesting that the energy differences between various

(19) S. Sternhall, *Quart. Rev., Chem. Soc.*, **23**, 86 (1969).
(20) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLaughlin, *J. Chem. Soc.*, 3699 (1962).
(21) R. D. Lapper, H. H. Mantsch, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **94**, 6243 (1972).
(22) E. Shefter and K. N. Trueblood, *Acta Crystallogr.*, **18**, 1067 (1965).
(23) M. Sundaralingam, *J. Amer. Chem. Soc.*, **87**, 599 (1965).

conformations are not large.[24] The energetic preference for the P_{II} state over the P_{III} state noted by Schleich, *et al.*,[8] is of interest, and seems not to be very sensitive to the J_0 values (Table II) or to salt or high temperatures.[8]

Conformation about the Glycosidic Bond. Donohue and Trueblood[25] discovered that there were essentially two preferred orientations of purine or pyrimidine rings about the glycosidic bond in nucleotides and nucleosides, and the conformations were termed syn and anti. The two minima are broad, but well defined;[9,26] free rotation of the rings and intermediate states would involve close contacts with H(2′) and O(1′) of the ribose. Pyrimidine bases are nearly always in the anti conformation[9] in the solid state, with H(6) over the ribose ring, and optical studies[27-32] have been interpreted to suggest retention of the anti conformation in solution. Recent studies on the pH dependence[6] and on the effect of the 5′-phosphate[2] on proton chemical shifts support the predominance of the anti conformer in purine and pyrimidine 5′-nucleotides. A qualitative method for estimating the amounts of anti and syn forms has been proposed.[33] Lack of variation of coupling constants with temperature in uridine[4] and β-pseudouridine[5] has been cited to show no change in the torsion angle for the temperature ranges studied (5–70° and 20–78°).

Schirmer, *et al.*,[34] have used the nuclear Overhauser effect to show that 2′,3′-isopropylidineuridine exists primarily in the syn conformation in dimethyl sulfoxide solutions. No comparable studies for nucleotides have been reported and no experimental estimates of the syn–anti rotational barrier have been made. The other reported investigations of variable temperature nmr spectra[4,5,8] covered a narrow temperature range. It was felt that a study over a large temperature range might reveal a change in the chemical shift of H(6), the proton likely to be the most sensitive to a syn–anti conformation change. The tetraethylammonium salts were used to allow solutions of reasonable concentration to be prepared using methanol and ethylene glycol. These solvents are useful over a very wide temperature range.

As was noted in part II,[10] the cytosine bases in 2′,3′-CMP crystals seemed likely to be locked in the syn conformation. For 2′- and 3′-pyrimidine nucleotides there is a steric barrier to rotation,[26] but this largely involves simultaneous flexing of the sugar with rotation, and the barrier has been presumed to be low. Figure 3 summarizes the chemical-shift data for H(6) of 2′,3′-CMP (Et_4N) and 3′-CMP (Et_4N) at various temperatures. The data for 3′-CMP (Et_4N) may be treated by assuming an equilibrium exists between two conformers. As the temperature is decreased, the lower energy conformation state becomes more populated and the chemical shift approaches that of the low energy conformer. At high temperatures, the barrier to rotation and the energy difference between the conformers become smaller compared to the available energy so that equal population of the two states is approached. The chemical shift at high temperature would then be intermediate between that of the two conformers.

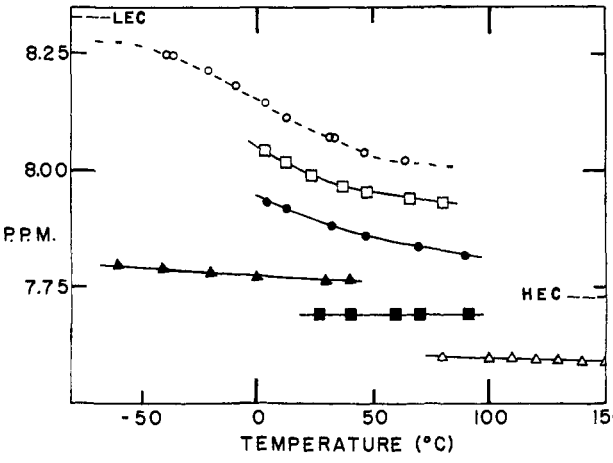


Figure 3. Variation in the chemical shift of H(6) with temperature for 3′-CMP and 2′,3′-CMP: ○, 3′-CMP in CD_3OD; □, 3′-CMP in D_2O at pD 5.2; ●, 3′-CMP in D_2O at pD 7.2; ▲, 2′,3′-CMP in CD_3OD; ■, 2′,3′-CMP in D_2O at pD 7.3; △, 2′,3′-CMP in ethylene glycol. Chemical shifts in CD_3OD and ethylene glycol are relative to internal TMS and those in D_2O to internal DSS. δ_{LEC} and δ_{HEC} are the estimated chemical shifts of the low- and high-energy conformers of 3′-CMP in CD_3OD.

Low-energy and high-energy shifts can each be estimated from an extrapolation of the plot of the observed chemical shift *vs.* temperature. The population at each temperature can then be calculated using the relation

$$\delta_{obsd} = \delta_A P_A + \delta_S P_S \quad (5)$$

where δ_A is the chemical shift of H(6) in the anti conformation, P_A is the population of the anti conformer, δ_S is the chemical shift of H(6) in the syn conformation, and P_S is the population of the syn conformer.

Since this is an equilibrium process, it can be treated using the relations

$$\Delta G = -RT \ln K = -RT \ln (P_A/P_S) \quad (6)$$

$$\ln (P_A/P_S) = -\Delta H/RT + \Delta S/R \quad (7)$$

The slope of a graph of $\ln (P_A/P_S)$ *vs.* $1/T$ gives ΔH and the intercept yields ΔS. Using the values $\delta_A = 8.325$ ppm and $\delta_S = 7.625$ ppm, we find $\Delta H = 2.8$ kcal/mol and $\Delta S = -5.2$ eu in methanol. The calculated curve for these values is shown as the dashed line in Figure 3. The experiments done in D_2O as a solvent over a more limited temperature range yielded curves with slopes similar to those found for the methanol data, suggesting about the same ΔH in water. Schleich, *et al.*,[8] reported a similar change in the chemical shift (0.064 ppm) for H(6) of 3′-UMP between 23 and 88° in D_2O.

(24) J. A. Pople, W. G. Schneider, and H. J. Bernstein in "High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, N. Y., 1959, p 381.
(25) J. Donohue and K. N. Trueblood, *J. Mol. Biol.*, 2, 363 (1960).
(26) A. E. V. Haschmeyer and A. Rich, *J. Mol. Biol.*, 27, 369 (1967).
(27) R. J. Cushley, K. S. Watanabe, and J. J. Fox, *J. Amer. Chem. Soc.*, 89, 394 (1967).
(28) T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, 6, 843 (1967).
(29) D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U. S.*, 57, 1138 (1967).
(30) T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, *Biochem. Biophys. Res. Commun.*, 19, 643 (1965).
(31) T. L. V. Ulbricht, J. P. Jennings, P. W. Scopes, and W. Klyne, *Tetrahedron Lett.*, 13, 659 (1964).
(32) T. R. Emerson and T. L. V. Ulbricht, *Chem. Ind. (London)*, 2129 (1964).
(33) M. P. Schweizer, J. T. Witkowski, and R. K. Robins, *J. Amer. Chem. Soc.*, 93, 277 (1971).
(34) R. E. Schirmer, J. P. Davis, J. H. Noggle, and P. A. Hart, *J. Amer. Chem. Soc.*, 94, 2561 (1972).

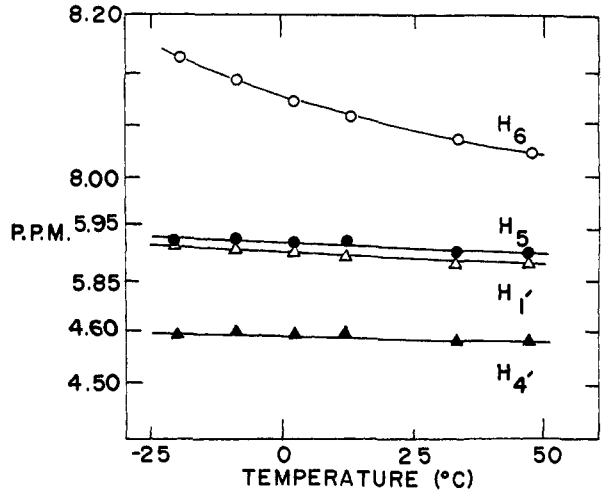


Figure 4. Variation in the chemical shifts of 3′-CMP in CD_3OD with temperature.

The chemical shift of H(6) for the high-energy conformer of 3′-CMP (δ_{HEC} on Figure 3) is close to the chemical shift of H(6) for 2′,3′-CMP. The similarity of 2′,3′-CMP and 3′-CMP would suggest similar values of the chemical shift of H(6) for the same conformations. At high temperatures, the extrapolated value of the shift for 3′-CMP is the mean between δ_{HEC} and δ_{LEC} (Figure 3). The closeness of δ_{HEC} to the chemical shift of 2′,3′-CMP in the same solvent, CD_3OD, implies that the high-energy conformation of 3′-CMP is the same as the conformation of 2′,3′-CMP.

The chemical shift of H(6) of 2′,3′-CMP varies much less with temperature than that of H(6) of 3′-CMP. Solvent effects can cause small chemical-shift changes with temperature as can changes in the ribose conformation. These effects should be similar in the two nucleotides. For a two-state system, a small chemical-shift change with temperature is consistent with either a fixed glycosidic conformation or a very rapid interconversion between the syn and anti conformations. A rapid interconversion which did not cause significant chemical-shift change or broadening over such a large temperature range (−60 to 150°) would require a low energy barrier and nearly equal energies of the two conformations. In this case, the chemical shift would be nearly the average of the syn and anti chemical shifts, since the populations would be nearly equal. If the pyrimidine ring is locked, however, the chemical shift would be expected to be that of the conformation found in the crystalline state, in this case the syn conformation.[10] The agreement between δ_{HEC} and the chemical shift of 2′,3′-CMP is strong evidence for a syn conformation for 2′,3′-CMP in these solutions; it also provides independent evidence for the value of δ_{HEC} used to derive the calculated curve in Figure 3. The anti conformation would then be the low energy, preferred conformation of 3′-CMP, with an H(6) chemical shift of δ_{LEC}. The similarity in the chemical shift of H(6) in 2′,3′-CMP and 2′,3′-UMP (Table I) indicates that 2′,3′-UMP is also in the syn conformation in this case.

Schweizer, *et al.*,[2] observed H(6) chemical shifts from external TMS of 8.424 for 5′-CMP and 8.403 ppm for 5′-UMP in D_2O, pD 5.9; the values for cytidine and uridine were 8.292 and 8.275 ppm. They attributed these differences to the deshielding effect of the phosphate and concluded that these 5′ nucleotides were in the anti conformation. If we assume that our chemical-shift curve for D_2O (Figure 3) would remain parallel to the curve for CD_3OD at low temperatures, the extrapolated value for the chemical shift of the anti conformer in D_2O, pD 7.2, would be 8.13 ppm or, correcting from internal DSS to external TMS, 8.53 ppm.[35] For 5′-CMP, the phosphate deshielding effect should increase this figure for 100% anti conformer. The most reasonable explanation for the observations summarized in Figure 3 and for those of Schweizer, *et al.*,[2] is that 5′-CMP exists as a syn–anti mixture, with anti the lower energy conformation.

The enthalpy difference between the syn and anti forms of 3′-CMP gives the minimum possible barrier to rotation in methanol. Inspection of the H(6) resonance signals reveals a slight broadening (~0.5 Hz) at low temperatures. This allows a very rough estimate of the maximum barrier to rotation to be made. The relationship between the broadening and the lifetimes of two species in equilibrium is given by[36]

$$\Delta\nu_{obsd} = \Delta\nu_0 + 4\pi P_A^2 P_S^2(\tau_A + \tau_S)\Delta_{AS}^2 \quad (8)$$

where $\Delta\nu_{obsd}$ is the observed line width, $\Delta\nu_0$ is the line width in the absence of exchange, P_A is the population of the anti conformer, P_S is the population of the syn conformer, τ_A and τ_S are the preexchange lifetimes, and $\Delta_{AS} = \delta_A - \delta_S$. The CH_3 signal from a trace of internal CH_3OH was used as a line-width standard. From the value which results, an activation energy may be calculated using eq 9.[37] An examination of the

$$\log 1/\tau(\omega_A - \omega_S) = \log 2k_0/(\omega_A - \omega_S) - E_a/2.3RT \quad (9)$$

broadening at various temperatures and a plot of the left side of eq 9 *vs.* $1/T$ give E_a from the slope and k_0, the frequency factor, from the intercept. $\omega_A/2\pi$ and $\omega_S/2\pi$ are the frequencies of the chemical shift for the anti and syn conformers. This suggests an approximate maximum barrier of 7 kcal/mol. Other effects such as dipole broadening could also account for changes in line width such as observed here, and this would reduce the estimated maximum barrier height.

Other proton resonance signals are also expected to change with the relative populations of the syn and anti conformations,[33,38] but to a smaller extent than H(6). This is shown for the easily resolved signals in Figure 4. Detailed comparisons of chemical shifts of the ribose protons between 2′,3′-CMP and 3′-CMP are difficult because of the unknown effects of the different phosphate orientations. The direction of the shifts with temperature of H(4′) and H(1′) is as predicted[33] for an increase in the syn fraction with increasing temperature.

The structural chemistry of 2′,3′-CMP observed in the crystal thus seems to carry over directly into solution. In addition to allowing characterization of populations of rotational states and barrier heights for pyrimidine nucleotides, the system may allow a more quantitative treatment of the optical rotary dispersion

(35) Based upon a comparison of our value for $\delta_{H(6)}$ of 3′-CMP with that of Schweizer, *et al.*[2]
(36) Reference 22, pp 218–225.
(37) Reference 24, p 367.
(38) H. Dugas, B. J. Blackburn, R. K. Robins, R. Deslauriers, and I. C. P. Smith, *J. Amer. Chem. Soc.*, **93**, 3468 (1971).

data on nucleotides. These studies and enzymatic studies are in progress.

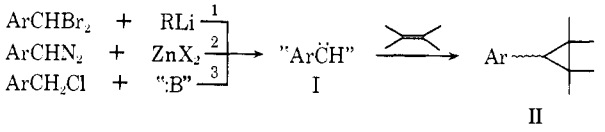
Acknowledgment. For support, we wish to thank the National Science Foundation (GB-25174) and the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago. We also thank the Department of Chemistry for allowing us to use their nuclear magnetic resonance spectrometers, Drs. S. Danyluk and K. Kopple for helpful comments and Dr. Ian Smith for providing copies of his papers before publication.

Communications to the Editor

A Useful Synthesis of Arylcyclopropanes

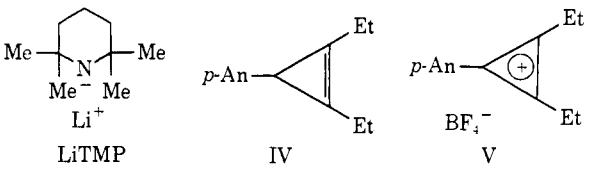
Sir:

In the best published general methods[1,2] for the preparation of arylcyclopropanes (II) by routes involving the reaction of phenyl carbenes or carbenoids (I) with alkenes, the intermediate I is generated: (1) by treatment of the costly and often unstable benzal bromides (but not $ArCHCl_2$[3]) with organolithium reagents,[1] or (2) by zinc halide induced decomposition of the unstable and detonation prone aryldiazomethanes.[2,4] A



high-yield synthesis of II from I formed (3) by reaction of a base with a benzyl chloride (usually commercially available, inexpensive) is thus attractive both economically and from a safety standpoint. Numerous past attempts to develop such a method have been unsuccessful.[5-8] With organometallic bases, the products obtained include substances which may be visualized as derived from metal–halogen exchange reactions and from processes involving deprotonation of the alkene trap to an allyl anion. Generally, however, the base is just benzylated. Even when initial α-proton abstraction occurs, the $ArCHCl$ generated does not fragment but is instead alkylated by more $ArCH_2Cl$. The only published exception is the reaction of $PhCH_2Cl$ with *n*-BuLi in cyclohexene from which 7-phenylnorcarane (III) was isolated in a disappointing 14% yield.[6] We have confirmed this result (13%) and have found that MeLi (<9%), PhLi (<5%), and *t*-BuLi (0%) are even less satisfactory for the production of III. In other new tests, no III was isolated with KO-*t*-Bu or with the Schlenk–Hauser base, sodium triphenylmethide,[9] and only traces of III were obtained with lithium bistrimethylsilylamide[10] or with another Hauser base, bromomagnesium diisopropylamide.[11] Calculated yields in these eight experiments using a radioisotope dilution assay to ensure the stability of III in the reaction media and to correct for loss during the sometimes complex isolation schemes required still were only 13, 9, 8, 2, 1–2,[12] 2, 3, and 7%, respectively. The last four reagents above have previously been recommended specifically for their excellence as very strong proton-specific bases.[8-11]

We now report here the successful use of lithium 2,2,-6,6-tetramethylpiperidide (LiTMP) as the base[13] in a practical synthesis of II from $ArCH_2Cl$. In the optimum experimental procedure, a rapidly stirred, concentrated solution (under N_2) of 1 equiv of $ArCH_2Cl$ and the alkene (large excess) in ether is slowly titrated with 1 equiv of a *ca.* 1 *M* solution of LiTMP in ether. The reaction temperature and base addition rate are



(1) G. L. Closs and R. A. Moss, *J. Amer. Chem., Soc.*, 86, 4042 (1964).
(2) S. H. Goh, L. E. Closs, and G. L. Closs, *J. Org. Chem.*, 34, 25 (1969).
(3) Which reacts to give $ArCCl$: R. A. Moss, *ibid.*, 27, 2683 (1962).
(4) Simple photochemical or thermal decomposition of $ArCHN_2$ is a much less satisfactory source of I.[1,2] Also: C. D. Gutsche, G. L. Bachman, and R. S. Coffey, *Tetrahedron*, 18, 617 (1962).
(5) Bases studied include KOH, LiOMe, NaOEt, KO-*t*-Bu, HCONHNa in $HCONH_2$, $LiNH_2$ or $NaNH_2$ in NH_3, ether, THF, hydrocarbon, or $(Me_2N)_3PO$, MeLi, EtLi, *n*-BuLi, Na in NH_3, NaH in THF or $(Me_2N)_3PO$, and "active NaH" in THF. For review of material prior to 1960 see ref 6. For more recent results see ref 7 and S. Bank and M. C. Prislopski, *Chem. Commun.*, 1624 (1970); P. Caubére, *Bull. Soc. Chim. Fr.*, 1293 (1966); P. Caubére and J. Moreau, *ibid.*, 1986 (1970); *Tetrahedron*, 25, 2469 (1969); J. F. Bunnett and J. D. Reinheimer, *J. Amer. Chem. Soc.*, 84, 3284 (1962); P. E. Verkade, K. S. deVries, and B. M. Wepster, *Recl. Trav. Chim. Pays-Bas*, 82, 637 (1963). For new data see ref 8.
(6) G. L. Closs and L. E. Closs, *Tetrahedron Lett.*, 26 (1970).[7]
(7) But note also that the reaction of $PhCH_2Cl$ with *n*-BuLi by a different pathway (similar conditions minus cyclohexene) is used as a quantitative assay for these RLi reagents: H. Gilman and A. H. Haubein, *J. Amer. Chem. Soc.*, 66, 1515 (1944); D. H. Hoeg and D. I. Lusk, *ibid.*, 86, 928 (1964); R. West and W. H. Glaze, *J. Chem. Phys.*, 34, 685 (1961).
(8) R. A. Olofson and C. M. Dougherty, *J. Amer. Chem. Soc.*, 95, 582 (1973).
(9) B. E. Hudson and C. R. Hauser, *ibid.*, 63, 3156, 3163 (1941); D. F. Thompson, P. L. Bayless, and C. R. Hauser, *J. Org. Chem.*, 19, 1490 (1954); W. Schlenk, H. Hillemann, and I. Rodloff, *Justus Liebigs Ann. Chem.*, 487, 135 (1931).
(10) C. R. Krüger and E. G. Rochow, *J. Organometal. Chem.*, 1, 476 (1964), and references therein; D. H. R. Barton, R. H. Hesse, G. Tarzia, and M. M. Pechet, *Chem. Commun.*, 1497 (1969); M. Tanabe and D. F. Crowe, *ibid.*, 1498 (1969); M. W. Rathke, *J. Amer. Chem. Soc.*, 92, 3222 (1970).
(11) F. C. Frostick and C. R. Hauser, *ibid.*, 71, 1350 (1949); C. R. Hauser and H. G. Walker, *ibid.*, 69, 295 (1947).
(12) Depending on reaction solvent: *t*-BuOH, ether, or cyclohexene.
(13) Since 2,2,6,6-tetramethylpiperidine (HTMP) is readily prepared by reduction of 2,2,6,6-tetramethylpiperidone-4 (≡ triacetonamine (i); from acetone, ammonia, and $CaCl_2$), it is potentially inexpensive enough for most synthetic purposes. Because of the value of the derived nitroxides as spin labeled diagnostic reagents in spectroscopy, both HTMP and i are already available from several sources. The unfortunate impurities (*ca.* 10%) in commercial samples are easily removed by distillation. Treatment of HTMP with a commercial RLi (R = Me, *n*-Bu) solution provides a convenient small scale source of LiTMP. The reaction is best performed in the addition funnel just before use.