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Nucleic Acid Related Compounds. 26. A "Geometry-Only" Method for Determining the Anomeric Configuration of Nucleosides Based on the H-1′ NMR Signal of Cyclic α and β 3′,5′-Mononucleotides[1]

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Abstract: Conversion of ribonucleosides to their cyclic 3′,5′-monophosphates (3′,5′-cNMP's) provides a derivative with a trans-fused six- to five-membered ring system. The spin-spin coupling of the anomeric proton (H-1′) with H-2′ of these conformationally rigid cyclic mononucleotides is now uniquely controlled by the geometry of the fused molecular system. The anomeric proton of β anomers gives rise to a sharp singlet [$J_{1'-2'}$(trans) $\lesssim$ 0.7 Hz] whereas the corresponding ^1H NMR signal for α anomers appears as a doublet [$J_{1'-2'}$(cis) $\gtrsim$ 3.5 Hz] in over 200 examples with *no observed exception.* Some 15 additional examples of arabinofuranosyl nucleoside and cyclonucleoside 3′,5′-cNMP's also exhibit the expected anomeric proton doublet [$J_{1'-2'}$(cis) = 6–7 Hz]. Limitations of and exceptions to previous approaches to the determination of anomeric configuration of ribofuranosyl compounds are discussed.

Previous approaches to the determination of anomeric configuration of ribonucleosides have employed chemical reactions, chiroptical methods, and NMR spectroscopy. However, all of these methods have involved specific structural requirements in the base moiety and/or conformational effects which have proven to be empirically useful in many cases but which are not fully understood or predictable a priori. Certain of these methods are applicable only if both anomers are available for comparison.

Hudson's rules of isorotation[3] for carbohydrate derivatives

had been applied to nucleosides, but in 1961 were found to fail for certain anomeric pyrimidine deoxynucleosides.[4] In fact, a later ORD/CD "rule" proposed reversed sign trends for the long-wavelength ("B_{2u}") transition of purine [(+)α; (−)β][5a] and pyrimidine [(−)α; (+)β][5b] ribonucleosides. However, ORD/CD spectra of nucleosides are complex[6] and the long wavelength spectral envelope often contains several different electronic transitions. Even the so-called B_{2u} transition is dependent on intrinsic transition dipole vector orientation in the planar heterocyclic base, glycosyl rotamer populations, sugar conformation, substituent transition coupling, etc. Changes in solvent and temperature as well as sugar and base substituents can change the *sign* as well as magnitude of the B_{2u} transition.[6]

Periodate oxidation of the nucleoside 2′,3′-*cis*-glycol unit and borohydride reduction of the resulting dialdehyde gives a triol structure with chirality remaining only at C-1′. Comparison of nucleoside anomers gives an enantiomeric triol pair.[7] However, this method has not been explored extensively and both anomers are required at present unless one of the resulting triols is known. Also, it is not known experimentally if relative sign inversions might occur with different base structures which exhibit such effects in the ORD/CD approach.

Purine[8a] 3-N→C-5′ and pyrimidine[8b] 2-O→C-5′ cyclonucleoside formation provides unequivocal proof of cis orientation of the base and C-5′ (β-D configuration). However, synthetic nucleosides such as 3-deazaadenosine do not possess the requisite functionality (N-3).[9] In addition, certain β anomers react sluggishly[10] which could make a negative result somewhat uncertain if only one of the anomers were available.

^{1}H NMR spin coupling approaches include assigning a trans H-1′ to H-2′ configuration if $J_{1'-2'} \lesssim 1$ Hz,[11] and using 2′,3′-*O*-isopropylidene functionalization[12] to reduce $J_{1'-2'}$. However, no definitive assignments are possible with intermediate or large coupling values. The singlet anomeric proton resonance observed for two 2′,3′-anhydro lyxo nucleosides[1b,13] (H-1′ and H-2′ formally cis) represent technical exceptions to the $J_{1'-2'} \lesssim 1$ Hz trans criterion.

^{1}H NMR chemical shift approaches include: (1) 2′-*O*-acetyl methyl shifts measured before and after hydrogenation of the base;[14] (2) chemical shift limits for the 2′-*O*-acetyl signal;[15] (3) diamagnetic shielding of the anomeric proton by a cis 2′-hydroxyl group;[16] and (4) the chemical shift difference ($\Delta\delta$) between the two methyl singlets of 2′,3′-*O*-isopropylidene ribonucleosides.[17a-e] (We have recently observed an H-4′ multiplicity effect which augments this isopropylidene shift criterion.[17f]) Methods (1) and (2) require base anisotropy [and an easily reducible double bond for (1)] and are rationalized assuming a favorable acetyl methyl to base conformational population. Chemical shifts are solvent dependent and an exception to (2) has been noted recently.[17c] One exception to (3) has been observed with a benzyl ether derivative,[18] and since shift differences can be small,[19] both anomers must be available for comparison. Method (4) has been qualified to exclude anisotropic substituents[17d] and requires an "aromatic" (anisotropic) base at C-1′.[17e] Nuclear Overhauser effect (NOE) measurements[20] are dependent on sugar conformer and glycosyl rotamer populations in these mobile molecules and are inapplicable when the chemical shifts of sugar protons are overlapped. A relaxation time method noted with carbohydrate derivatives has not been explored widely and requires advanced instrumental sophistication.[21] ^{13}C NMR has been investigated in only an exploratory manner with respect to anomeric configuration.[22]

Discussion and Results

We have been interested in devising an ^{1}H NMR method for defining anomeric configuration which would be dependent *solely* on the geometry of substitution at C-1′ and C-2′. Examination of molecular models indicated that trans fusion of a six-membered ring to the furanose ring would produce a rigid structure with distinctly unique H-1′ and H-2′ dihedral angle ranges. The biologically ubiquitous β nucleoside 3′,5′-cyclic monophosphates (cNMP's) have been investigated extensively owing to their potent biochemical activity as hormonal "second messengers",[23] These natural products have the desired rigid six- to five-membered trans-fused ring system as determined by single crystal x-ray analysis[24] and NMR spectroscopy.[25] The ICN group[26] and Moffatt[27] have used the anomeric singlet first observed by Jardetzky for cAMP[25a] as a criterion for confirming the presence of an intact cyclic phosphodiester ring of synthetic cNMP's. However, the valuable potential of this system for establishing the anomeric configuration of precursor nucleosides has gone unrecognized.

We have now synthesized certain α- and β-cNMP derivatives from the corresponding ribonucleosides and have examined a large amount of data in the literature.[28-30] As predicted from examination of molecular models, the H-1′ resonance of the β anomeric cyclic nucleotides appears as a sharp singlet, $J_{1'-2'} \lesssim 0.7$ Hz (trans), whereas that of the α anomers is a well-resolved doublet, $J_{1'-2'} \gtrsim 3.5$ Hz (cis).

Most synthetic coupling methods produce predominant or even exclusive formation of β-ribonucleosides,[31] and the naturally occurring nucleosides from RNA's[32] as well as ribonucleoside antibiotics[33] have the β configuration. Therefore, the invariability of any criterion is especially critical in evaluating the β anomeric structure. Examination of Table IA reveals that substitution of bulky and/or electronegative groups at C-2 and/or C-8 (as well as C-6) of the purine ring has no effect on the H-1′ singlet. Thus syn (bulky C-8 substituted), anti (presumably favored by bulky C-2 substitution), and potentially crowded and restricted intermediate (C-2, C-8 disubstituted) rotameric conformations of the base at C-1′ are tolerated without observed exception. Some N-1 substituted cAMP derivatives in Table IB would bear a positive charge on the base at neutral pH in aqueous solution. However, any electrostatic attraction of the base for anionic phosphate has no observed effect on the H-1′ singlet.

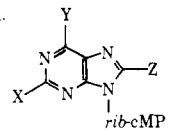
The consistency of the anomeric singlet with heterocyclic base changes at C-1′ is demonstrated by the entries of Table IC. Substitution at O-2′ is also tolerated as seen in Table ID. Especially significant in this regard is the anomeric signal invariance with 2′-*O*-tosyl substitution. The aromatic sulfonate is strongly electronegative at C-2′ and the phenyl ring can overlap[34a] with the aromatic purine base in these 2′ isomers.[34b]

Table IE contains even more demanding examples. Two of the "5′-methyl" cAMP derivatives (the altro isomer, $R_1 = H$, $R_2 = CH_3$; and the dimethyl, $R_1 = R_2 = CH_3$, compound) have axial methyl groups on the phosphodiester backbone "chair". Again however, these compounds as well as the two allo derivatives ($R_1 = CH_3$, $R_2 = H$, Z = H, Br) were reported to have singlet H-1′ resonance peaks.[27] Finally, three examples of β anomers of this basic trans-fused system with sulfur and nitrogen hetero atoms replacing oxygen have been noted. The H-1′ resonance peaks for the two cyclic phosphorothioates (X = O) were reported[29a] to have "*J* ca. 1 Hz", and a singlet was reported[29b] for the cyclic phosphorothioamidate (X = NH) of Table IE.

Thus, with a variety of heterocyclic bases and an extensive array of substitution patterns involving base, sugar, and

Table I.[a] β-D-*ribo*-3′,5′-cNMP Derivatives with Anomeric Proton "Singlets" ($J_{1'-2'} \lesssim 1$ Hz)

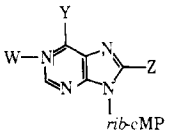
A.



129 examples
(ref 25e, 28a,b, d–f, h, j–n, p, r)

X = H, Cl, R, Ar, NH_2, NHR, NR_2, OH, SH, SR
Y = Cl, NH_2, NHR, NR_2, NHOH, NHOR, $NHCO_2R$, $NHCO_2Ar$, OH, OR, SH, SR
Z = H, Br, Cl, I, R, COR, COAr, $CONH_2$, C(=NNHG)R, N_3, NH_2, NHR, NR_2, OH, OR, SH, SR, SAr

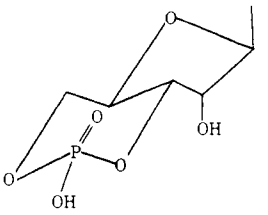
B.



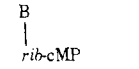
8 examples
(ref 28f, p)

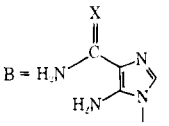
W = Me, O, OH, OR
Y = NH, NH_2, OH
Z = H, Br, SCH_2Ph

rib-cMP =

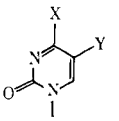


C.

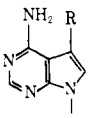




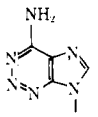
X = NH, NOH, NOR, O, S
7 examples (ref 28f)



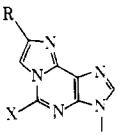
X = NH_2, OH
Y = H, F
4 examples
(ref 25e, 36)



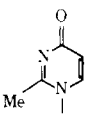
R = H, CN, $CONH_2$
(ref 28g)



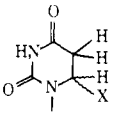
(ref 28j)



R = H, Ph
X = Br, Me, N_3, NH_2, NMe_2, OH, OMe, SH, SR
17 examples (ref 28f, q)

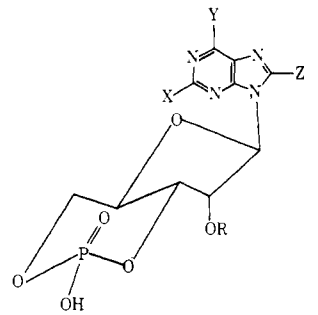


(unpublished data)



X = H, OH
3 examples
(ref this work, 39b)

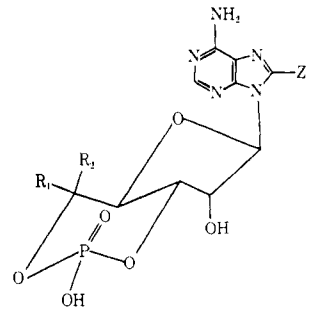
D.



29 examples
(ref 28c, e, i, k, n, o, 30b, d)

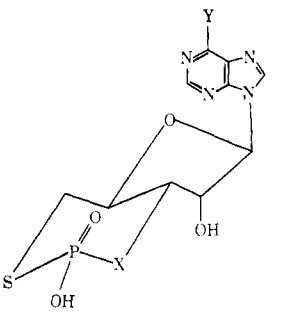
X = H, NH_2
Y = Cl, NH_2, NHCOR, OH, SH
Z = H, Br, Cl, OH, SH, SR, SAr
R = Me, 2,4-$C_6H_3(NO_2)_2$, Ts, COR′, CONHR′, CONHAr

E.



4 examples
(ref 27)

Z = H, Br
R_1 = H, Me
R_2 = H, Me



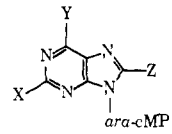
3 examples
(ref 29a, b)

Y = NH_2, OH
X = NH, O

[a] The predominant tautomers of most OH and SH substituted heterocycles are the corresponding keto and thione forms.

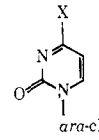
backbone loci in over 200 β-cNMP derivatives, the anomeric proton resonance peak has $J_{1'-2'} \lesssim 1$ Hz and would appear as a singlet at ~1 Hz resolution *without a noted exception.*[35] Long-range coupling of the anomeric proton with fluorine of the 5-fluorouracil[36a] and 5-fluorocytosine[36b] cNMP's gives observed doublets ($^5J_{H\text{-}1'\text{-}F\text{-}5}$ = 1.5–2 Hz) for H-1′. However, these doublets collapse to singlets ($J_{1'-2'} < 0.7$ Hz) upon fluorine heteronuclear decoupling. The observed $J_{1'-2'} \lesssim 1$ Hz

Table II.[a,b] β-D-*arabino*-3′,5′-cNMP Derivatives with Anomeric Proton Doublets ($J_{1'-2'}$ = 6–7 Hz)

A. 
ara-cMP

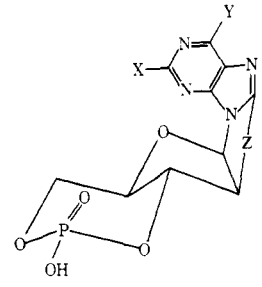
X = H, NH_2
Y = NH_2, OH
Z = H, NH_2, OH, OMe

8 examples
(ref 30b–d)

B. 
ara-cMP

X = NH_2, OH, SH; SMe

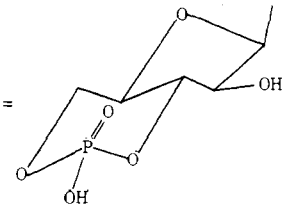
(ref 30a)

C. 

X = H, NH_2
Y = NH_2, OH
Z = NH, NMe, O, S

5 examples
(ref 30b, d)

[a] The predominant tautomers of most OH and SH substituted heterocycles are the corresponding keto and thione forms.

[b] *ara*-cMP = 

is probably an upper limit for the actual H-1′ to H-2′ coupling since long-range ^{31}P (phosphate) to H-1′ coupling ($^5J_{P-H-1'} \sim 0.7$ Hz) has been reported recently and $J_{1'-2'} \sim 0.4$ Hz was estimated by double resonance line width sharpening.[25e]

Table IIA contains data on β-D-arabinofuranosylpurine and Table IIB pyrimidine 3′,5′-cNMP derivatives. These compounds and the related purine 8-Z→2′-arabino cyclonucleoside 3′,5′-cNMP's listed in Table IIC have the common six- to five-membered trans-fused phosphodiester furanose ring system, but now with cis H-1′ to H-2′ geometry. The anomeric proton resonance of these C-2′ arabino epimers appears as a strongly coupled doublet, $J_{1'-2'}$ = 6–7 Hz.[37]

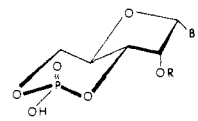
Data on the α-D-ribofuranosylpurine and pyrimidine 3′,5′-cNMP's prepared for this study are listed in Table III. The naturally occurring base derivatives exhibit anomeric proton resonance doublets with $J_{1'-2'}$ = 3.5–3.9 Hz. Since electronegative substituents can reduce spin coupling values on a vicinal ethane system,[38] tosyl and acetyl derivatives were investigated. Again it is significant to note that the electronegative and aromatic (base stacking) *p*-toluenesulfonyl group caused negligible alteration of $J_{1'-2'}$.

We had prepared the 2′,3′-*O*-isopropylidene derivatives of α- and β-uridine and found that Δδ values for the methyl singlets are 0.094 (α) and 0.199 (β) in conformity with Imbach's criterion ($\alpha < 0.15 < \beta$).[17c-e] However, hydrogenation of the 5,6 double bond of uracil in these nucleosides gave dihydro derivatives with Δδ 0.160 (α) and 0.184 (β). Imbach has since reported the same results[17e] and indicated that his criterion should be limited to "aromatic" base substituents at C-1′ since the observation is primarily a result of base anisotropy effects on the endo methyl group of α anomers.

As seen in Tables IC and III, our approach is not limited by such anisotropy considerations. Hydrogenation of β-cUMP gives the dihydro product which has an anomeric proton singlet. Photohydration[39a] of β-cUMP gives presumed diastereomeric 6-hydroxydihydro products,[39b] again with singlet H-1′ peaks. Hydrogenation of α-cUMP gives the dihydro derivative with an anomeric proton doublet, $J_{1'-2'}$ = 5 Hz.

The uniformly singlet H-1′ resonance peak observed with the β-cNMP's (Table I) is compatible with the $^3T_4$–^3E–$^3T_2$ conformation range of the furanose ring[24a] ($\phi_{H-1'-H-2'} \sim$ 105–90°).[40] This range is seen to be relatively unstrained with Dreiding molecular models and reduces 1′–2′ orbital eclipsing. It is also compatible with solid state x-ray data.[24] The $J_{1'-2'}$ = 6–7 Hz coupling observed for the β-arabino products (Table II) corresponds to $\phi_{H-1'-H-2'} \sim$ 28–35°. This is compatible with the $^3T_2$ conformation range. The enhanced puckering (relative to the ribo C-2′ epimers) presumably arises from increased 1′–2′ torsional repulsion of the cis-oriented electronegative 2′-hydroxyl and 1′-base substituents. The α-cNMP's with naturally occurring bases have $J_{1'-2'}$ = 3.5–3.9 Hz couplings (Table III) which correspond to $\phi_{H-1'-H-2'} \sim$ 49°. This would require a "maximally puckered" $_2T^3$–$_2E$ conformation, which would result from the combined steric and dipolar torsional repulsion of the cis 2′-hydroxyl and 1′-base, and base-H-4′

Table III. α-D-*ribo*-3′,5′-cNMP Derivatives

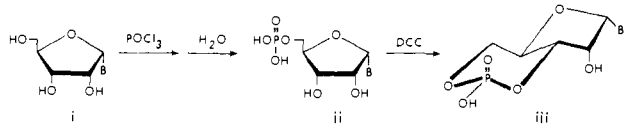


B	R	^{1}H NMR[a] $J_{1'-2'}$[b]	H-1′	H-2(5)[c]	H-8(6)[c]	Solvent	λ_{max}, nm (ε)	Anal.[d]
Adenin-9-yl	H	3.6	6.67	8.23	8.41	D_2O	(H$^+$) 257 (12 700) (OH$^-$) 259 (13 300)	C, H, N
Adenin-9-yl[e]	Ac	3.9	6.89	8.38	8.60	D_2O	(H_2O) 258	
Adenin-9-yl[f]	Ts	3.8	6.60	8.01	8.22	0.1 M NaOD/D_2O		
Cytosin-1-yl	H	3.5	6.57	6.35	8.00	D_2O	(H$^+$) 280 (12 800) (OH$^-$) 274 (8800)	C, H, N
Uracil-1-yl	H	3.5	6.47	6.04	7.89	D_2O	(H$^+$) 263 (7800) (OH$^-$) 263 (6300)	C, H, N
5,6-Dihydro-uracil-1-yl	H	5.0	6.17	2.90[g]	3.92[h]	D_2O		

[a] Chemical shifts in δ ppm downfield from Me_4Si (external). [b] Coupling in Hz. [c] Purine H-2 and H-8, pyrimidine H-5 and H-6. [d] Values within ±0.4% of theoretical. [e] δ 1.82 (s, 3, $COCH_3$), 5.91 ("t", $J_{2'-1'} \sim J_{2'-3'}$ = 3.8 Hz, 1, H-2′). [f] δ 2.35 (s, 3, CH_3–Ar). 5.61 ("t", $J_{2'-1'} \sim J_{2'-3'}$ = 3.7 Hz, 1, H-2′), 7.03–7.39 (m, 4, Ar). [g] (t, J = 6.2 Hz, 2, H-5a, 5b). [h] (m, 2, H-6a, 6b).

interaction. However, these electronegativity and bond angle/distance distorsions could also significantly affect the Karplus dependency.[38] Hydrogenation of α-cUMP resulted in increasing the anomeric coupling to $J_{1'-2'} = 5$ Hz in the dihydro product which corresponds to $\phi_{H\text{-}1'\text{-}H\text{-}2'} \sim 41°$. This slight relaxation to a less puckered $_2T^3$ range can be rationalized by the possible reduction in dipolar repulsion of the 2′-hydroxyl for the saturated base and by the increased conformational mobility of this now flexible six-membered alicyclic substituent at C-1′.

Direct conversion of α-ribonucleosides (i) to their 5′-monophosphates (ii) proceeds cleanly in high yields using phosphoryl chloride with certain technical improvements (see Experimental Section) in the general method of Yoshikawa et al.[41] Cyclizations to the 3′,5′-cNMP's (iii) by the dicyclohexylcarbodiimide (DCC) procedure of Khorana and coworkers[42] proceed in >70% yields. A new method employing base-catalyzed cyclization of 5′-trichloromethyl phosphonates (prepared in one step from the phosphonic acid dichloride) makes these 3′,5′-cNMP derivatives readily accessible.[43]



Summary and Conclusions

Although the assignment of anomeric configuration of naturally occurring[32,33] (and a multitude of synthetic[31b]) ribonucleosides is a critical consideration for structure assignment, no generally applicable method independent of specific structural features or empirically fortuitous conformational effects had been described. We now report such a method which is based on the straightforward conversion of a ribonucleoside to its 3′,5′-cNMP derivative and determination of its H-1′ to H-2′ coupling constant. Examination of data for over 200 examples of variously substituted trans (H-1′ to H-2′, β-D-ribo) compounds and 23 examples of cis (α-D-ribo and β-D-arabino) products reveals that the H-1′ peak appears as a singlet ($J_{1'-2'} \lesssim 1$ Hz) for trans (β) and a moderate to strongly coupled doublet ($J_{1'-2'} \gtrsim 3.5$ Hz) for cis (α) anomers of ribonucleoside 3′,5′-cNMP's *without observed exception.* It may be possible to construct cNMP's in which steric and other substituent effects could cause anomeric proton coupling values to fall outside normal limits or exhibit long-range or virtual coupling effects. However, the latter effects can be identified experimentally.

Therefore, our proposed criterion for defining the anomeric configuration of ribonucleosides is: $J_{1'-2'} \lesssim 1$ Hz for β anomers and $J_{1'-2'} \gtrsim 3$ Hz for α anomers of their 3′,5′-cNMP derivatives.

Significant advantages of this approach include (1) it is based solely on the geometrical orientation of protons on a uniform rigid ring system and does not rely on specific structures or empirical effects, (2) it defines either anomer uniquely and thus is *applicable when only one anomer is available,* (3) it involves measurement of a single resonance peak in an ordinarily unobstructed region of the ^{1}H NMR spectrum ($\delta \sim 6$), (4) no techniques or instrumentation beyond an ^{1}H NMR spectrometer capable of ~1 Hz resolution are required, and (5) the straightforward synthesis of the cyclic nucleotide (which itself may be of significant interest as a hormonal messenger analogue) can be reversed by chemical and/or enzymatic hydrolysis if recovery of the precursor nucleoside is required.

Experimental Section

General Procedures. NMR spectra were recorded on a Varian HA-100 instrument with Me_4Si as external reference. UV spectra were recorded on Cary 15 or Pye Unicam SP1700 spectrophotometers. IR spectra were recorded on a Perkin-Elmer 421 grating spectrometer. Mass spectra were determined by the Mass Spectroscopy Laboratory staff of this department on AEI MS-2, MS-9, MS-12, or MS-50 instruments using direct probe introduction at 150–230 °C and 70 eV. Melting points were determined on a Reichert Microstage and are uncorrected. Optical rotations were measured on Perkin-Elmer 141 or 241 polarimeters using a 10-cm, 1-mL microcell. Elemental analyses were determined by the microanalytical laboratory of this department or Galbraith Laboratories, Inc. TLC was performed on Eastman Chromatogram Sheet (silica gel 13181 with 6060 indicator or cellulose 13254 with 6065 indicator) or Brinkmann-Merck (silica gel 60F-254) precoated glass plates in the indicated solvents. Developed chromatograms were evaluated under 2537 Å UV light or by spraying with 5% H_2SO_4 in EtOH and charring (glass plates) for non-UV absorbing compounds. Descending paper chromatography (PC) was performed on Whatman No. 1 paper in the solvents indicated. Electrophoresis was effected using a Savant HV-3000A flat-plate apparatus with Whatman No. 1 paper or a Camag Thin-Layer Electrophoresis (after Pastuska) apparatus with the above noted Eastman cellulose sheets. Evaporations using a Buchler rotating evaporator with a dry ice cooled Dewar condenser under water aspirator or mechanical oil pump vacuum were carried out below 40 °C. The evaporator vacuum release was vented through a Drierite ($CaSO_4$) tower so that only dried air was allowed to enter. Pyridine was refluxed over and then distilled from calcium hydride, then from chlorosulfonic acid (*caution*), and finally from KOH pellets and stored over activated (>200 °C) 4A sieves. Dimethyl sulfoxide was distilled from calcium hydride in vacuo and stored over activated 4A sieves. α-Uridine, α-cytidine, α-5′-UMP, and α-5′-CMP were purchased from Terramarine Bioresearch. β-cUMP was purchased from Sigma Chemical Co., and 5% Rh/Al_2O_3 catalyst was purchased from Engelhard Industries.

Standard ^{1}H NMR samples were prepared by dissolving 15 mg of the cNMP in 1.2 mL of 0.1 M triethylammonium bicarbonate (TEAB, pH 7) solution and evaporating to dryness in vacuo. Addition of ~2 mL of D_2O and reevaporation was repeated 3–5 times allowing only dried air to enter the evaporator. The residue was then dissolved in 0.3 mL of D_2O, the solution filtered through cotton wool, and the spectrum recorded using Me_4Si as *external* standard. Final concentrations of cNMP's were 0.05–0.13 M. The spectrum of 2′-*O*-tosyl-α-cAMP was determined in 0.1 M NaOD due to solubility problems and 2′-*O*-acetyl-α-cAMP was determined as the ammonium salt at 0.2 M.

α-Adenosine was prepared by a modification of the procedure of Pichat et al.[44] using four parallel fusion reactions of 6-*N*-octanamidopurine[45] (N^6-octanoyladenine, 4.43 g, 0.017 mol, total) and 1,2,3,5-tetra-*O*-acetylribofuranose (10% molar excess) with *p*-toluenesulfonic acid as catalyst for 10 min at 180 °C in vacuo. The residues were stirred with 50 mL of MeOH–H_2O–Et_3N (2:2:1) for 40 h at room temperature. The pooled solutions were evaporated and the black residue was partitioned between 150 mL of H_2O and 200 mL of Et_2O. The aqueous layer was washed with 200 mL of Et_2O and evaporated. The residue was dissolved in ~20 mL of MeOH–H_2O (4:10) and applied to a column (15 × 43 cm) of Dowex 1-X2(OH^-) resin[46] packed in the same solvent mixture. Elution with 6370 mL of 40% MeOH/H_2O gave two small unidentified peaks. The first 675 mL of 60% MeOH/H_2O was UV transparent and the following 2370 mL contained 15.2% yield of α-adenosine. After 300 mL of UV transparent eluate, the following 4825 mL contained 36.1% yield of β-adenosine. The adenosine anomers had NMR parameters compatible with reported data[44] and were shown to be free of adenine by TLC (cellulose, *t*-BuOH–MeCOEt–H_2O–concentrated NH_4OH, 4:3:2:1). The crude β anomer had $[\alpha]^{24}_D$ −58.6° (*c* 0.6, H_2O) [lit.[7] $[\alpha]_D$ −60.4° (*c* 0.7, H_2O)] and was identical with natural adenosine by all properties compared. The α anomer crystallized slowly from MeOH with diffusion of Et_2O[47] and had mp 220–221 °C; $[\alpha]^{24}_D$ 27.9° (*c* 0.66, H_2O) [lit.[7] mp 201 °C; $[\alpha]_D$ 24° (*c* 0.65, H_2O)]. Mass spectroscopy; α anomer *m/e* 267.0961, calcd for M^+ ($C_{10}H_{13}N_5O_4$) 267.0967; M − 30 (α) ≪ M − 30 (β).[48]

Preparation of 5′-mononucleotides followed the general procedure of Yoshikawa et al.[41] with technical improvements noted in the following example.

α-Adenosine 5′-Phosphate. A solution of 0.6 mL (6.6 mmol) of freshly distilled $POCl_3$ and 6.3 mL of triethyl phosphate was stirred for ~20 min in an ice bath and 0.5 g (1.9 mmol) of α-adenosine was

added. After an additional 4 h (6 h for α-uridine) of stirring at 0 °C, the reaction was complete as judged by hydrolysis of a 1-drop aliquot in ~0.2 mL of H_2O and then TLC (silica, MeCN–1 M NH_4OH, 7:3). The reaction mixture was added dropwise to 125 mL of stirred dry Et_2O (Et_2O–*n*-pentane, 1:1, for α-uridine) and ~5 mL of fresh triethyl phosphate was used to rinse the reaction flask. The resulting white precipitate was filtered, washed well with ~100 mL of dry Et_2O, dissolved in 100 mL of cold H_2O, and allowed to stand at 0 °C for 17 h. The solution was adjusted to pH 9.0 (pH meter) using Et_3N and was then evaporated to dryness. A stiff white solid foam was obtained upon addition and evaporation of Me_2CO followed by EtOH/$CHCl_3$. This glassy product was dissolved in 10 mL of H_2O and applied to a column (3 × 45 cm) of Dowex 1-X2(HCO_2^-). The column was washed with 750 mL of H_2O (small amounts of unidentified materials) and elution was effected using a linear gradient of H_2O (3 L) to 1 M HCO_2H/H_2O (3 L). The appropriate fractions (UV monitor) were pooled and evaporated to give a white powder after several additions and reevaporations of H_2O. This was dissolved in 100 mL of H_2O and adjusted to pH 7.3 using 0.5 M LiOH (saturated $Ba(OH)_2$ for α-cytidylate). After concentration of the solution to a small volume, the product was crystallized using H_2O/MeOH/Me_2CO to give 0.55 g (82%) of the salt of α-5′-AMP in two crops (α-5′-UMP 75%, α-5′-CMP 87%). Anal. Calcd for $C_{10}H_{12}N_5O_7PLi_2 \cdot 1.5\ H_2O$: C, 31.11; H, 3.92; N, 18.14; P, 8.02. Found: C, 31.31; H, 3.81; N, 17.91; P, 8.06. $[\alpha]^{25}_D$ 20.6° (*c* 1, H_2O); UV (H_2O) max 259 nm (ε 15 900) min 227 nm (ε 2600). α-5′-UMP and α-5′-CMP were characterized by comparison with commercial samples. All mononucleotides were homogeneous by PC and electrophoresis.

Cyclic α-3′,5′-nucleoside monophosphates were prepared by the general procedure of Khorana and co-workers using the 5′-mononucleotide morpholine carboxamidine salts.[42] The pyridine-insoluble cytidine derivative salt was treated by the dimethyl sulfoxide modification of Symons.[49] The physical properties of α-cAMP (87%) (recrystallized from EtOH–H_2O,[42] 81%), α-cUMP (90%) (precipitated from MeOH/*i*-PrOH/Et_2O in 80% yield as the triethylammonium salt after unsuccessful attempts to crystallize the free acid and other salts), and α-cCMP (70%) (recrystallized[42] quantitatively) are tabulated in Table III. These compounds were homogeneous by PC and electrophoresis and migrated with known β-cNMP's.

Cyclic 5,6-Dihydro-β-uridine 3′,5′-Monophosphate. A solution of 20 mg (0.06 mmol) of cUMP ($Na^+ \cdot H_2O$) in 0.5 mL of H_2O and 3.5 mL of EtOH was hydrogenated in the presence of 29 mg of 5% rhodium on alumina by bubbling H_2 through the suspension in a centrifuge tube for 6 h. The mixture was centrifuged and the 260 nm transparent solution plus 2 × 4 mL of H_2O used to wash the catalyst were applied to a column (1 × 14 cm) of Bio-Rad "Chelex 100" resin (prewashed with dilute EDTA solution and then H_2O) which removed magnetic NMR line-broadening ions. Elution of the column with 100 mL of H_2O under 3 psi pressure and evaporation of the eluate gave a TLC pure [silica, glass plates-charring, MeCN–1 M NH_4OH, 7.5:2.5, R_f (dihydro/5′-UMP) = 3.6, R_f (dihydro/cUMP) = 1.0] residue which was dissolved in D_2O, lyophilized, and examined directly by NMR spectroscopy (see Table III).

Cyclic 5,6-Dihydro-α-uridine 3′,5′-Monophosphate. Treatment of 0.032 mmol of α-cUMP identically with the above procedure for the corresponding β anomer gave the title compound with identical TLC behavior. This product was evaluated by NMR spectroscopy in the same manner (see Table III).

Cyclic 2′-*O*-Tosyl-α-adenosine 3′,5′-Monophosphate. Tosylation of a 33-mg (0.1 mmol) sample of α-cAMP by the procedure of Mian et al.[30b] gave 30 mg (60%) of solid product with IR (KBr disk) 1172 cm^{-1} (OSO_2Ar) and other physical properties listed in Table III.

Cyclic 2′-*O*-Acetyl-α-adenosine 3′,5′-Monophosphate. Acetylation of a small sample of α-cAMP by the procedure of Falbriard et al.[50] (for the β anomer) was monitored by TLC. The product was examined spectroscopically as indicated in Table III.

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Nucleic Acid Related Compounds. 27. "Virtual Coupling" of the Anomeric Proton of Cyclic 2′-Deoxynucleoside 3′,5′-Monophosphates. Reassessment of Conformation Using Praseodymium Shifts and Assignment of H-2′,2″ Signals by Biomimetic Deuteration at C-2′ [1]

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Abstract: Treatment of solubilized trimethylsilyl derivatives of 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)adenine (**1a**) or its 2′-epimer, 9-(2-chloro-2-deoxy-β-D-ribofuranosyl)adenine (**1b**), with tri-*n*-butyltin deuteride under free radical initiating conditions gave the 2′-deuterio-2′-deoxyadenosines (**2a, 2b**) with ~85% selectivity for the natural ribo (down) isomer (**2a**). Analogous treatment of 2′-chloro-2′-deoxy-3′,5′-di-*O*-benzoyluridine gave the 2′-deuterio-2′-deoxyuridines (**2c, 2d**) with ~75% ribo (**2c**) selectivity. Small $J_{1'-2'}$(trans) and large $J_{1'-2''}$(cis) vicinal proton coupling constants were observed for the corresponding major (ribo) and minor (arabino) cyclic 2′-deuterio-2′-deoxynucleoside 3′,5′-monophosphates, respectively. Praseodymium induced shifts resolved the "virtually coupled" anomeric proton triplet of cyclic 2′-deoxyadenosine 3′,5′-nucleotide into an ABX doublet of doublets with splittings approaching those of the respective 2′-deuterio derivatives. The rigid trans-fused six- to five-membered ring system of these cyclic nucleotides allows geometric proton coupling assignments of the protons at C-2′, and praseodymium shifts provide relative chemical shift evaluations. An essentially consistent conformation range for the cyclic 2′-deoxy 3′,5′-nucleotide derivatives of the five common nucleic acid bases is indicated, in contrast to previous interpretations based on "virtually coupled" spectra. Base anisotropy effects in the 2′ region of 2′-deoxynucleosides are discussed.

The biologically ubiquitous and potent hormonal "second messenger" cyclic nucleoside 3′,5′-monophosphates (cNMP's)[3] have been investigated extensively from both biomedical and physical-structural viewpoints.[4] Several recent studies have concentrated on their solid state[5] and solution[1,6a-g] geometric and conformational properties. In contrast, the corresponding cyclic 2′-deoxynucleoside 3′,5′-monophosphates (cdNMP's)[3] have received little attention. Duplicative ^{1}H NMR conformational evaluations of cdTMP,[6c,e,f] a ^{13}C NMR determination of the same compound,[6g] two evaluations of cdAMP,[6b,f] and spectral data for three cyclic 2′-deoxynucleoside 3′,5′-phosphorothioates[6h] have been reported. The isolation of cdAMP from a bacterial culture fluid has been noted very recently.[7]

We have been interested in the valuable potential of these cyclic nucleotides to reveal C-1′-C-2′ configuration patterns. Examination of the usually well-separated H-1′ NMR signal is definitive for assignment of the anomeric cNMP's owing to the geometrical constraints of the trans-fused six- to five-membered phosphodiester furanose backbone.[1] Determination