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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.

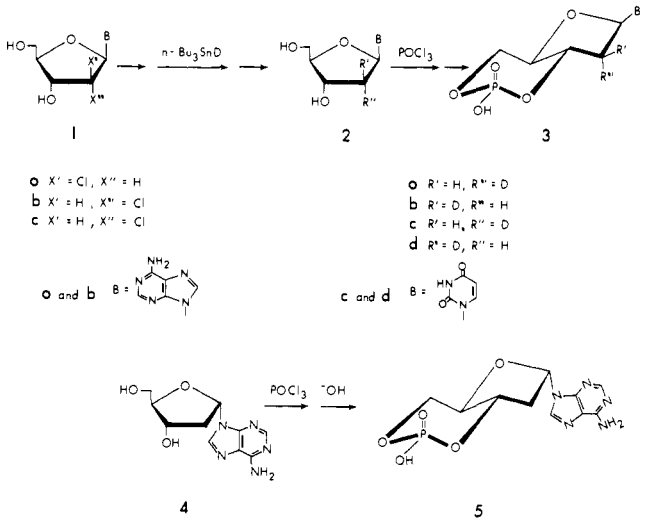
The biologically ubiquitous and potent hormonal "second messenger" cyclic nucleoside 3′,5′-monophosphates (cNMP's)[3] have been investigated extensively from both biomedical and physical-structural viewpoints.[4] Several recent studies have concentrated on their solid state[5] and solution[1,6a-g] geometric 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

of the overall stereochemistry of enzymatic conversion of ribonucleotides to their 2′-deoxy (DNA) counterparts by ribonucleotide reductases was accomplished only after considerable manipulation and syntheses.[8] Application of our new use of cNMP derivatives for H-1′ to H-2′ configuration assignment[1] to cdNMP derivatives makes this problem easilv amenable to experimental investigation.

Treatment of 9-(2-chloro-2-deoxy-β-D-arabinofuranosyl)adenine[9] (**1a**) (Scheme I) with bis(trimethylsilyl)acetamide

Scheme I



(BSA) gave an organic soluble tris(trimethylsilyl) derivative. This product was treated with tri-*n*-butyltin deuteride in the presence of azobisisobutyronitrile (AIBN) in hot benzene.[10] Smooth overall deuterolysis of the chloro function occurred to give 2′-deuterio-2′-deoxyadenosine after deblocking. Careful treatment of this acid-labile purine deoxynucleoside with phosphoryl chloride in cold triethyl phosphate[11] followed by direct cyclization in aqueous base[12] gave 2′-deuterio-cdAMP in 20–30% overall yields.

The NMR spectrum of this product had a narrowly split peak ($J_{1'-2'} \sim 1.5$ Hz) for the anomeric proton of the major ribo isomer (**3a**) which is consistent[1] with a trans orientation of H-1′ and H-2′. Thus, deuterolysis with overall inversion of configuration at C-2′ to give the "natural" ribo-deuterio configuration (**2a**) was predominant (~85%). A peak of minor integrated intensity (~15%) for H-2″ of the minor arabino-deuterio isomer (**2b**) was observed upfield from H-2′ of **2a** in the NMR spectrum of the 2′-deuterio-2′-deoxyadenosines (**2a, 2b**). The anomeric proton of the minor 2′-deuterio-cdAMP isomer (**3b**) is strongly coupled to the cis H-2″ ($J_{1'-2''} \sim 8.5$ Hz) (obtained by peak sharpening upon deuterium heteronuclear decoupling).

An analogous sequence was effected beginning with the less readily accessible[13] 9-(2-chloro-2-deoxy-β-D-ribofuranosyl)-adenine (**1b**). The resulting 2′-deuterio-2′-deoxyadenosines (**2a, 2b**) were formed with, if anything, a higher ratio of ribo (**2a**) to arabino (**2b**) deuterio epimers than with **1a** (H-2″ of the minor isomer **2b** could not be determined by FT-NMR integration of the small sample from **1b**). The predominant product **2a** is seen to be formed from **1b** with overall retention of configuration at C-2′.

Similar treatment of 1-(2-chloro-2-deoxy-β-D-ribofuranosyl)uracil[14] (**1c**) via its benzene-soluble 3′,5′-di-*O*-benzoyl derivative gave the 2′-deuterio-2′-deoxyuridines (**2c, 2d**).[15] The ratio of ribo (~75%) to arabino (~25%) deuterio C-2′ epimers [evaluated by integration of H-2′ and H-2″ (downfield) of the dibenzoyl blocked derivatives] is lower than in the adenine deoxynucleosides, but the ribo product is still markedly predominant in this overall retention of configuration deuterolysis. Similar results were obtained in the radioactivity retention studies of David and Augé using the 2′-bromo-2′-tritio-2′-deoxyuridine derivative and tri-*n*-butyltin hydride followed by an elimination sequence.[16] Phosphorylation[11] of **2c, 2d** at 0–5 °C followed by cyclization[17] using *N,N′*-dicyclohexylcarbodiimide (DCC) gave the 2′-deuterio-cdUMP derivatives (**3c, 3d**). Again, the major trans ribo-deuterio epimer (**3c**) gave rise to a narrowly split anomeric proton peak ($J_{1'-2'} \sim 1.7$ Hz) whereas the cis arabino-deuterio isomer (**3d**) had a strongly coupled H-1′ resonance ($J_{1'-2''} \sim 8.4$ Hz).

The deuterolysis results are easily rationalized since attack of the presumed essentially planar C-2′ radical intermediate on deuterium of the bulky tri-*n*-butyltin deuteride[10a] would be expected to proceed much more readily from the unhindered ribo face trans to the heterocycle at C-1′. However, it is interesting to note that these results are parallel to the biosynthetic transformation of ribonucleotides to 2′-deoxynucleotides in which a free radical mediated enzymatic process has been postulated very recently.[18] The enzymatic conversions proceed with complete stereoselectivity and retention of configuration of attachment of the incoming H-2″ to the ribo face.[8] The present chemical system gives an experimentally convenient biomimetic conversion with greater than 75% stereoselectivity for the "natural" ribo epimer.

Analyses of the furanose backbone conformation of cNMP's both in the solid state[5] and in solution[6] are in agreement with the six-membered phosphodiester "flattened chair" fused trans on the twist-envelope ($^3T_4$–^3E) five-membered ring. However, a systematic comparison of cdNMP conformations has not been reported and variant interpretations regarding cdAMP have been proposed.

In 1968 Smith and Jardetzky reported the ^1H NMR spectrum of cdAMP and analyzed the conformation as C-3′ endo, C-4′ exo ($^3T_4$) based on combination splitting peak widths for H-1′-H-2′,H-2″ (since the anomeric proton signal was an apparent triplet).[6b] Chladek and Nagyvary prepared cyclic 5′-thio-2′,5′-dideoxynucleoside 3′,5′-phosphorothioates from thymidine, deoxyadenosine, and deoxycytidine.[6h] An "irregular quartet-like feature" was noted for the H-1′ signal in the NMR spectrum of the thymidine product whereas the anomeric proton peaks of both the adenine and cytosine products "appeared as the usual triplet" with J = 8–9 Hz.[6h] Smith and co-workers examined cdTMP and analyzed the conformation as 4′-exo ($_4$E) with $J_{1'-2'}$ = 2.4 Hz and $J_{1'-2''}$ = 8.9 Hz.[6c] Kainosho and Ajisaka have recently reached the same conformational conclusions regarding cdTMP from both computer simulation and lanthanide shifted measurements.[6e] Lee and Sarma[6f] have very recently published an NMR study of rigid derivatives including cdTMP and cdAMP. Their analysis[6f] essentially conforms to that of the previous studies for cdTMP.[6c,e] However, "an unusual $^3E \rightleftarrows {}^0E$ equilibrium" was proposed for cdAMP to explain "an unusual situation in which $J_{1'-2'} = J_{1'-2''} = 5.2$ Hz and $J_{2'-3'} = J_{2''-3'} = 9.1$ Hz" as obtained from their computer simulated spectra.[6f] They suggested that a lack of rigidity in the furanose backbone of cdAMP allows this molecule to exist in the flexible $^3E \rightleftarrows {}^0E$ equilibrium. However, examination of molecular models reveals no support for or rationalization of such proposed flexibility. As noted by these authors, H-2′ and H-2″ have nearly identical chemical shifts in cdAMP.[6f] A rigid trans-fused ring structure with H-1′ weakly coupled to H-2′ (trans) of the strongly coupled (and equivalently shifted) H-2′–H-2″ geminal system (and strongly coupled H-2′–H-3′ vicinal system) fulfills conditions which can result in "virtual coupling" effects.[19] This could mask actual coupling parameters and result in capricious conformational conclusions. That such "virtual coupling" complications were operative was suggested by the dimethylformamide (DMF) solvent effect on the H-1′ resonance of

Table I. ^{1}H NMR Chemical Shifts of cdNMP Compounds[a]

Compd	H-1′ [b]	H-2′,H-2″ [c]	H-3′ [c]	H-4′ [c]	H-5′,H-5″ [c]	H-2[d](H-5)[e]	H-8[d](H-6)[e]
cdGMP	6.38	2.68–2.88	5.16	4.04	4.49, 4.34		7.96
cdCMP	6.39	2.50–2.74	*f*, *g*	4.05	*f*, 4.38	6.14	7.76
cdAMP	6.58	2.80–2.96	*g*, *h*	4.15	*h*, *h*	8.24	8.32
2′-D-cdAMP	6.57	2.78–2.91	*g*, *i*	4.18	*i*, 4.47	8.24	8.34
α-cdAMP	6.54	2.72–3.28	*j*	*j*	*j*, *j*	8.23	8.46
cdUMP	6.35	2.43–2.84	*g*, *k*	3.97	*k*, 4.33	5.94	7.76
2′-D-cdUMP	6.35	2.50–2.64	*g*, *l*	3.99	*l*, 4.36	5.96	7.76

[a] All spectra were determined in D_2O. Values are δ ppm downfield from external Me_4Si (internal HDO peak set at δ 4.80). [b] See Tables II and III for coupling constants and peak shapes. [c] Multiplets. [d] Singlets, purine H-2 and H-8. [e] Doublets, J_{5-6} = 7.6–8.0 Hz, pyrimidine H-5 and H-6. [f] 4.54–4.93. [g] HDO peak broadens downfield limit. [h] 4.36–5.0. [i] 4.60–5.12. [j] 4.26–4.84. [k] 4.52–4.97. [l] 4.54–4.84.

Table II. "Apparent" and Praseodymium Shift-Deuterium Substitution Determined ^{1}H Coupling Constants of cdNMP Compounds[a]

Compd (Pr^{3+})[b]	$J_{1'-2'}$[c]	$J_{1'-2''}$[c]
cdGMP (80 μL)	4.0 (~2.5)	6.8 (~7.6)
cdCMP (30 μL)	3.0 (2.2)	7.6 (8.0)
cdAMP (70 μL)	5.3 (2.1)	5.3 (7.9)
2′-D-cdAMP	1.4	8.5
α-cdAMP (20 μL)	~6.8 (~7.5)	~7.8 (~7.5)
cdUMP (80 μL)	4.0 (2.4)	7.0 (8.4)
2′-D-cdUMP	1.7	8.4
cdTMP (0.8 M)[d]	(2.4)[d]	(8.9)[d]

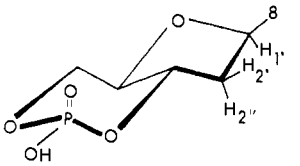
[a] Coupling values in Hz. [b] Volume of 0.8 M $Pr(NO_3)_3$ in 0.1 M DCl/D_2O which gave the coupling values noted. Further additions of lanthanide had negligible effect on these couplings. [c] Values in parentheses are praseodymium-shifted coupling parameters. [d] Taken from ref 5e.

cdAMP noted earlier by Smith and Jardetzky.[6b] This has now been conclusively demonstrated both by deuterium substitution at C-2′ and by differentially shifting the H-2′ and H-2″ NMR frequencies paramagnetically using praseodymium.

The chemical shift values for the cdNMP compounds are listed in Table I and their H-1′ spin coupling parameters in Table II. As seen in Table II, the anomeric proton of both the cyclic ribo-2′-deuterio-2′-deoxyadenosine and uridine nucleotides have $J_{1'-2'} \sim 1.6$ Hz (trans) and the corresponding minor product arabino-2′-deuterio derivatives have $J_{1'-2''} \sim 8.5$ Hz (cis) couplings. These limiting coupling values are approached in all the protio β-cdNMP spectra upon addition of sufficient praseodymium nitrate solution. Mean values for the lanthanide shifted β-cdNMP "apparent coupling" parameters from Table II are $J_{1'-2'} = 2.3 \pm 0.1$ Hz (trans) and $J_{1'-2''} = 8.2 \pm 0.4$ Hz (cis) (which, it should be noted, cannot be considered *actual* J_{AX} and J_{BX} coupling constants since they are obtained from the X resonance of the ABX system[19]).

Calculation of the corresponding vicinal dihedral angles (and the resulting conformational information) is dependent upon the Karplus approximation[20,21] and "constant" values[22] chosen. Lee and Sarma have noted that "there could be errors as much as ±10% in the computed torsion angles" [5f] based on coupling uncertainties, etc. However, whereas the calculated trans H-1′ to H-2′ angles vary by less than ±5% using three approximation treatments[20-22] of the same data, the small cis H-1′ to H-2″ angle calculations are very sensitive to the approximation constants used, and calculated angles[20-22] differ by more than ±65% using the same large coupling data. Uncertainties involving electronegatively substituted and strained systems in general[20b,23] and 2′-deoxynucleoside derivatives in particular[21] have been discussed. The generally employed Karplus–Abraham et al. treatments[20,22] suggest that the trans H-1′ to H-2′ dihedral angles are of the order of 115–120° and that the cis H-1′ to H-2″ angles are small (0–15°) in these compounds.

These data are compatible with an essentially constant $_4E$–$_4T^3$ conformation range for all of these rigid 2′-deoxy compounds (i). There is no evidence to support any unusual



i

flexibility or abnormal conformational effects[6f] in cdAMP. As noted by Kainosho and Ajisaka,[6e] electrostatic dipolar repulsion of base at C-1′ and the 2′-hydroxyl group could result in orientations approaching antiperiplanar for these substituents in the corresponding cyclic 3′,5′-ribonucleotides. Attendant 3′–4′ torsional adjustment would result in a ^3E conformation range predominance as observed. Removal of this polar effect in the 2′-deoxy compounds would be expected to allow conformational relaxation to the $_4T^3$–$_4E$ range in agreement with our present data.

If the apparent splittings in the unshifted spectrum of α-cdAMP approximate actual spin-coupling parameters (vide infra), then dihedral angles in the range of ~25 and ~150° [20,22] ($J_{1'-2',2''} \sim 6.8$ and 7.8 Hz) are suggested. These angles are compatible with a $^3T_4$–^3E conformation range, which can be rationalized by the "outward" torsional displacement of the base (now exo at C-1′) owing to interactions with the quasi-axial H-4′ and H-2″ on the α face. Again, this interpretation is consistent with minor pseudorotational adjustments on a basically rigid $_4E$–$_4T^3$–$^3T_4$–^3E ribofuranose backbone.

A final aspect of this study involves assessment of the relative chemical shifts of the protons at C-2′. Table III contains a tabulation of the H-2′, H-2″ multiplet peak width limits with the corresponding H-1′ peak shape for the cdNMP compounds in D_2O (as neutral salts), in 0.1 M DCl/D_2O solution, and with increasing concentrations of praseodymium nitrate in the acidic medium (required for solubility). The 2′-protons of cdCMP are chemically shifted (multiplet spread ~0.24 ppm) sufficiently to give an apparent doublet of doublets for H-1′ initially in D_2O. Acidification had little effect but addition of lanthanide effected 2′-methylene broadening until by 30 μL addition ($\Delta\delta \sim 0.57$ ppm) a sharply resolved doublet of doublets was obtained for the H-1′ peak. Similarly, the 2′-methylene bandwidth for cdUMP in D_2O (0.41 ppm) is sufficiently spread to give a doublet of doublets for H-1′. This is consistent with the relative peak widths observed for the 2′-deuterio (0.14 ppm) and protio (0.41 ppm) cdUMP's which indicates a significant H-2′, H-2″ chemical shift difference in the protio product. At 80 μL addition, the 2′ protons have been widely

Table III. Chemical Shift Envelope Limits for H-2′,H-2″ and H-1′ Peak Shapes for cdNMP Compounds with Increasing Additions of Praseodymium Nitrate[a,b]

Compd	D_2O	0.1 M DCl	10 μL	20 μL	*c, d*	*e, f*	*g, h*
cdGMP	2.68–2.88 (0.20) dd	2.76–2.96 (0.20) dd	2.90–3.08 (0.18) t	2.98–3.15 (0.17) t	3.20–3.38[d] (0.18) br t	3.30–3.51[e] (0.21) dd	3.37–3.62[g] (0.25) dd
cdCMP	2.50–2.74 (0.24) dd	2.54–2.78 (0.24) dd		2.83–3.19 (0.36) dd	2.85–3.42[c] (0.57) dd		
cdAMP	2.80–2.96 (0.16) t	2.80–2.98 (0.18) t	2.94–3.13 (0.19) t	3.05–3.25 (0.20) br t	3.15–3.38[c] (0.23) dd	3.34–3.69[f] (0.35) dd	3.48–3.86[h] (0.38) dd
2′-D-cdAMP	2.78–2.91 (0.13) d						
α-cdAMP	2.72–3.28 (0.56) br t	2.88–3.20 (0.32) t		3.20–3.41 (0.21) t	3.40–3.59[d] (0.19) t	3.52–3.72[e] (0.20) t	3.68–3.94[h] (0.26) t
cdUMP	2.43–2.84 (0.41) dd	2.42–2.82 (0.40) dd	2.52–2.96 (0.44) dd	2.56–3.04 (0.48) dd	2.67–3.21[d] (0.54) dd	2.74–3.34[e] (0.60) dd	2.82–3.60[g] (0.78) dd
2′-D-cdUMP	2.50–2.64 (0.14) d						

[a] Spectra were determined in D_2O, then made 0.1 M in DCl, followed by additions of 0.8 M $Pr(NO_3)_3$ in 0.1 M DCl/D_2O. Values of the H-2′,H-2″ envelope limits are δ ppm downfield from external Me_4Si (internal HDO peak set at δ 4.80). [b] The widths of the H-2′,H-2″ envelope limit (Δδ) values are in parentheses and the H-1′ peak shapes are dd = doublet of doublets, t = triplet, and br t = broadened triplet with some splitting of center peak. [c] 30 μL. [d] 40 μL. [e] 60 μL. [f] 70 μL. [g] 80 μL. [h] 100 μL.

spread (~0.78 ppm) and a sharply resolved H-1′ doublet of doublets is observed.

The chemical shift difference between H-2′ and H-2″ is extremely small in cdAMP. As seen in Table III, the combined peak width for the protium parent (0.16 ppm) is only marginally wider than that of H-2′ of the deuterium derivative (0.13 ppm) in D_2O. An "apparent" smoothly symmetrical triplet is observed for H-1′. Acidification and addition of lanthanide resulted in progressive broadening of the 2′-methylene envelope with an accompanying resolution of the H-1′ "triplet" to a "broadened triplet" at 20 μL (0.20 ppm) and a sharply resolved doublet of doublets at 70 μL (0.35 ppm).

The situation with cdGMP is the most interesting and complex. As seen in Figure 1A and Table III the initial spectrum in D_2O has a 2′-methylene bandwidth of 0.20 ppm centered at δ 2.78 and a doublet of doublets for H-1′ at δ 6.38. Acidification (Figure 1B) resulted in an ~1 ppm downfield shift of H-8 (adjacent to the site of protonation at N-7)[34] and contraction of the peak for H-1′ to a "broadened triplet". Addition of 10–30 μL of lanthanide resulted in *narrowing* of the 2′-methylene envelope to ~0.17 ppm with accompanying collapse of the H-1′ resonance to an "apparent" symmetrical triplet (Figure 1C). Further additions of praseodymium resulted in broadening of the H-2′, H-2″ envelope (chemical shift separation) with concomitant resolution of the H-1′ peak to a "broadened triplet" (Figure 1D) and finally a separated doublet of doublets (Figure 1E). At this point the H-3′ resonance has "crossed over" that of H-1′, and other sugar protons are shifted markedly downfield.

A similar trend was observed with α-cdAMP (**5**). The initial spectrum in D_2O has a 2′-methylene envelope bandwidth of ~0.56 ppm and a "broadened triplet" peak for H-1′. Acidification caused reduction of the H-2′, H-2″ bandwidth to ~0.32 ppm with an accompanying collapse of the H-1′ peak to an apparent triplet. Addition of 20–60 μL of lanthanide solution caused narrowing of the 2′-methylene envelope to ~0.20 ppm. Addition of a total of 100 μL of praseodymium solution increased the 2′-methylene bandwidth to ~0.26 ppm, but no resolution of the apparent triplet for H-1′ occurred. All resonance peaks experience a progressive paramagnetic shifting in an absolute sense, but we are concerned here with the relative H-2′ vs. H-2″ shifting (bandwidth broadening).

If it is assumed that the praseodymium cation associates exclusively (for all practical purposes) with the cyclic phosphate anion,[6d] and in an essentially constant statistically populated region of space closer to the less sterically crowded

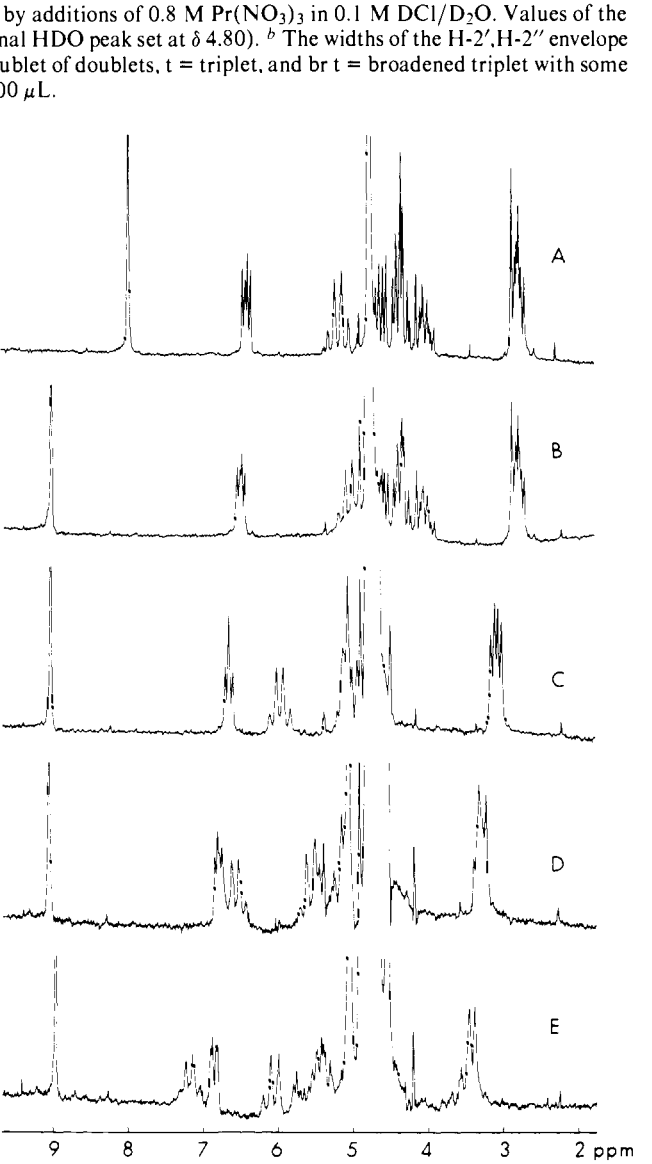


Figure 1. ^1H FT-NMR spectra (100 MHz) of cdGMP in (A) D_2O, (B) 0.1 M DCl, (C) plus 30 μL, (D) plus 50 μL, (E) plus 70 μL (cumulative total) of $Pr(NO_3)_3$ solution (see Experimental Section for details).

equatorial oxygen, then an interesting trend emerges. The exo H-2″ (which is slightly closer to the equatorial phosphate-oxygen in all the pseudorotational conformations as measured on Dreiding molecular models) resonates downfield from the

endo H-2′ in cdCMP, cdUMP, cdTMP,[6c,e,f,24] and cdAMP. This is consistent with the 2′-deuterio cdAMP and cdUMP spectra and with the direct progressive widening of the 2′-methylene envelope of these compounds with increasing concentration of lanthanide (i.e., H-2″ shifted progressively downfield relative to H-2′). In contrast, the H-2′ cis to guanine must resonate at lower field than H-2″ in cdGMP. A deshielding zone in the 2′,3′-endo region of this molecule is also indicated by the resonance shift of H-3′, which is at least 0.15 ppm downfield from the H-3′ peak of the other cdNMP's (see Table I). An initial narrowing of the 2′-methylene envelope occurs with addition of praseodymium followed by successive broadening. This is consistent with the initially higher field resonance of H-2″ "crossing over" that of H-2′ and then moving to progressively lower relative fields with increasing lanthanide concentration. The exo H-2″ (now cis to adenine) in α-cdAMP appears to be deshielded (and H-2′ more strongly deshielded) compared to the β anomer. These relative effects are reduced by protonation of the base as seen by the marked reduction in the 2′-methylene bandwidth (Table III). Addition of lanthanide results in further narrowing followed by progressive broadening of this 2′-envelope, as the H-2″ resonance presumably "crosses over" that of H-2′ to progressively lower relative fields. However, as noted (vide supra and Table III), the ~0.26 ppm bandwidth attained at 100 μL addition did not result in resolution of the apparent triplet for H-1′.

Assignment of H-2′ and H-2″ is critical for the study of ribonucleotide reductase enzymatic stereoselectivity.[8] Fraser-Reid and Radatus synthesized the two 2′-deuterio-2′-deoxycytidines from specifically deuterated carbohydrate precursors and found that the exo H-2″ trans to the base resonated at lower field than H-2′.[8f] Ts'o and co-workers have assigned H-2″ of 2′-deoxyadenosine 5′-monophosphate to higher field (than H-2′) on the basis of diamagnetic shielding by the cis 3′-hydroxyl group.[25] Slessor and Tracey have made the same relative assignments to the 2′-methylene protons of 2′-deoxyadenosine by computer simulation of the 220-MHz spectrum.[26] A computer simulation of the spectrum of 2′-deoxyuridine was consistent with assignment of H-2″ to lower field than H-2′, although the rationalization of "the deshielding effect of a cis hydroxyl group"[27] (at C-3′) is contrary to a body of experimental evidence.[25,28] A systematic analysis of the 220 MHz spectra of the six common 2′-deoxynucleosides by computer simulation[26] is consistent with H-2″ resonating at lower field than H-2′ for the pyrimidine compounds 2′-deoxycytidine, 2′-deoxyuridine, and (2′-deoxy)thymidine. A reversed trend (H-2″ at higher field) was found for the purine compounds 2′-deoxyadenosine, 2′-deoxyguanosine, and 2′-deoxyinosine. The stereochemical outcome of the present deuterolysis studies was readily analyzed by means of the geometrical constraints imposed by closure to the cyclic 3′,5′ nucleotide system.[1] The resulting ~1.6 Hz (trans) and ~8.5 Hz (cis) couplings of H-1′ with H-2′ and H-2″ of the major and minor 2′-deuterio isomers, respectively, are clearly defined since calculation of dihedral angles with the coupling constants reversed ($\phi_{cis} \sim 63°$ and $\phi_{trans} \sim 157°$)[22] corresponds to a geometrically impossible conformation for the trans-fused system. The spectral assignments made previously for the precursor nucleosides 2′-deoxyadenosine[8c,25,26] and 2′-deoxyuridine[26,27] are now confirmed chemically.

Hydrogenation of 2′-deuterio-2′-deoxyuridine (**2a**) (H-2′, δ 2.20) and 2′-deoxyuridine (H-2′, δ 2.20; H-2″, δ ~2.27) gave the corresponding 5,6-dihydro products with H-2′ at δ ~2.18 and H-2″ at δ ~1.96. From these results and the chemical shift tabulation of Slessor and Tracey,[26] certain trends appear consistent in the magnetic effects of the nucleic acid bases on the 2′-methylene protons of 2′-deoxynucleosides. The "aromatic" purine ring appears to exert a deshielding effect on both H-2′ and H-2″. Either this effect is more pronounced on the cis H-2′ and/or the shielding of H-2″ by the 3′-hydroxyl group[25,28] results in the higher relative field resonance of H-2″ in purine β-2′-deoxynucleosides. Assuming that the 5,6-dihydrouracil moiety would contribute only small magnetic effects, our hydrogenation shifts and the tabulated chemical shift data[26] suggest that the unsaturated base of pyrimidine β-2′-deoxynucleosides has negligible influence on H-2′, but exerts a pronounced deshielding effect on the trans H-2″ resulting in a lower relative field resonance of H-2″ in these compounds. Upon saturation of the uracil ring, H-2″ shifts from lower to higher field relative to H-2′. The relative shifts of H-2′ and H-2″ in the dihydro derivative parallel those of the purine compounds (but at higher absolute fields) and are compatible with the expected diamagnetic shielding of H-2″ by the 3′-hydroxyl group.[25,28] Slessor and Tracey have noted that there are no major differences between the conformations of purine and pyrimidine 2′-deoxynucleosides.[26]

Conclusions

As is evident from these studies, chemical shifts of the 2′-methylene protons of 2′-deoxynucleosides and their 3′,5′-cyclic phosphates vary in a presently unpredictable manner. Spin–spin splitting parameters are difficult to obtain from the strongly coupled spectra especially when the chemical shift difference between H-2′ and H-2″ is small. These factors have made spectral analyses and assignments difficult and somewhat tenuous in some instances.

Conversion of the conformationally mobile 2′-deoxynucleosides to the corresponding cyclic 3′,5′-phosphodiesters provides a rigid trans-fused six- to five-membered ring system in which the *actual* H-1′–H-2′,H-2″ coupling parameters appear to be closely consistent. Our use of cyclic 3′,5′-mononucleotides for determination of the relative base to 2′-substituent configuration[1] has now been successfully employed to provide facile assignment of stereochemistry at C-2′ of the biomimetically synthesized 2′-deuterio-2′-deoxy derivatives. The specific 2′-methylene assignments have provided chemical corroboration of previously suggested chemical shift ordering, and a brief analysis of base magnetic effects in the 2′-region has been made.

Deuteration at C-2′ or use of praseodymium induced shifts resolves the virtually coupled H-1′ resonance peak of cdNMP's, in the latter case from an apparent triplet or "broadened triplet" into a measurable ABX doublet of doublets pattern. The narrow range of H-1′ coupling parameters thus observed has allowed formulation of a limiting ${}_4E$–${}^3E$ range of conformations for all the cdNMP's examined. This is consistent with strain effects apparent in Dreiding molecular models and the conformations of the well-studied parent cNMP's and cdTMP. There is no evidence for unusual conformational effects in cdAMP as previously interpreted from virtually coupled spectra.[6f] Such spectra cannot be evaluated successfully on a first-order approximation basis or, apparently, even employing computer simulation of intense field spectra using LAOCOON III.[6f] The presently described methods circumvent such difficulties. This cyclic 3′,5′-monophosphate approach should be readily applicable to the stereochemical analysis of other 2′-substituted ribo or arabino β nucleosides and related carbohydrate systems.

Experimental Section

General procedures are described in the previous paper.[1] ^{1}H NMR spectra were recorded on a Varian HA-100 spectrometer (CW mode) and on a Varian HA-100 (15-in. magnet, equipped with a Digilab FTS-NMR accessory) for FT mode experiments at 100 MHz. Decoupling and integration experiments in the FT mode were effected using a Brüker WP-60 spectrometer. All FT spectra determined in D_2O were recorded with the HDO peak set at δ 4.80 (downfield from Me_4Si external standard). Samples of cdAMP and cdGMP were

purchased from Sigma Chemical Co., St. Louis, Mo., and 2′-deoxyuridine and 2′-deoxycytidine 5′-monophosphate were purchased from Calbiochem, La Jolla, Calif.

Praseodymium Shifted NMR Spectra. Samples of the cdNMP's (as the sodium salt, except for the uracil derivatives which were ammonium salts) were successively dissolved in 2 mL of D_2O and evaporated in vacuo three times, allowing only dried air to enter the system. The residue was then dissolved in D_2O and the spectrum was recorded. A 20% DCl/D_2O solution (Terochem Laboratories, Edmonton, Alberta) was added to give a final solution of 0.054 M in cdNMP and 0.1 M in DCl and the spectrum was again recorded. The indicated cumulative volumes of a solution of 0.8 M 99.9% $Pr(NO_3)_3$ (Research Organic/Inorganic Chemical Corp., Sun Valley, Calif.) in 0.1 M DCl/D_2O were added and spectra recorded until apparent virtual coupling effects on H-1′ had been minimized by shift separation of H-2′ and H-2″.

Tri-*n*-Butyltin Deuteride. Treatment of a solution of 173 mg (4.1 mmol) of $LiAlD_4$ in 15 mL of Et_2O under a N_2 atmosphere at 0 °C with 3 mL of $(n\text{-Bu})_3SnCl$ according to the procedure of Kuivila and Beumel[29] was followed up to drying the ether phase over $MgSO_4$. Drying agent was removed by filtration and 12.5 mL of dried benzene was added to the filtrate. The Et_2O was evaporated (bath temperature 60–70 °C) and the resulting ~20% stock solution[15] of $(n\text{-Bu})_3SnD$/PhH was used directly in the deuterolysis experiments.

2′-Deuterio-2′-deoxyadenosines (2a, 2b). (A) From 9-(2-Chloro-2-deoxy-β-D-arabinofuranosyl)adenine (1a). A stirred suspension of 285 mg (1 mmol) of **1a**[9b] in 15 mL of dried pyridine was treated with 1 mL of *N,O*-bis(trimethylsilyl)acetamide (BSA) and stirring was continued at room temperature. Additional 1-mL portions of BSA were added after 1 and 2 h. After 3.5 h the resulting solution was evaporated in vacuo and four portions of dried benzene were successively added and evaporated. The residue was dissolved in 30 mL of dried benzene and treated at reflux with 8 mL of the ~20% $(n\text{-Bu})_3SnD$/PhH stock solution and 15 mg of azobisisobutyronitrile (AIBN) initiator for 2.5 h. TLC (silica, upper phase of EtOAc–*n*-PrOH–H_2O, 4:1:2) showed complete conversion to a product with the identical mobility of 2′-deoxyadenosine (hydrolysis of trimethylsilyl groups occurs during chromatography). The solution was evaporated to dryness and MeOH was added and evaporated three times to give a yellow-brown syrup. MeOH (15 mL) and H_2O (15 mL) were added and the milky suspension was refluxed for 15 min, cooled, diluted to 75 mL with H_2O, and extracted with 2 × 50 mL of *n*-pentane. The aqueous layer was evaporated to dryness, and the residue was dissolved in 8 mL of H_2O and applied to a column (1.7 × 25 cm) of Dowex 1-X2 (OH^-) resin packed in H_2O. The column was washed with H_2O which eluted unidentified impurities and the product was eluted with 30% MeOH/H_2O. The appropriate fractions (UV detector) were combined and evaporated to give 219 mg (87%) of crystalline **2a, 2b**: NMR (Me_2SO-d_6) δ 2.26 (m, ~0.15, H-2″ of **2b**), 2.71 (d of d, $J_{2'-1'}$ = 7.6 Hz, $J_{2'-3'}$ = 5.5 Hz, ~0.85, H-2′ of **2a**), 3.58 ("t", J ~ 4 Hz, 2, H-5′, H-5″), 3.89 (m, $J_{4'-5',5''}$ ~ 4 Hz, $J_{4'-3'}$ ~ 2.5 Hz, 1, H-4′), 4.42 (m, 1, H-3′; after D_2O exchange d of d, $J_{3'-2'}$ = 5.6 Hz, $J_{3'-4'}$ = 2.8 Hz), 5.28 (t, 1, 3′-OH), 6.34 (d, $J_{1'-2'}$ = 7.7 Hz, 1, H-1′), 7.29 (s, 2, 6-NH_2), 8.14 (s, 1, H-2), 8.33 (s, 1, H-8). Mass spectrum (MS-50 with computer processing) calcd for M^+ ($C_{10}H_{12}DN_5O_3$) 252.1082, found *m/e* (% rel int, ion) 252.1078 (7.5%, M^+), 222 (21%, $M^+ - OH_2C$-5′), 164 (48%, BHCHO) 163 (100%, BHCH=CHD), 136 (99%, B + 2H), 135 (99%, BH), 118 (13%, sugar ion).

(B) From 9-(2-Chloro-2-deoxy-β-D-ribofuranosyl)adenine (1b). A 5-mg (0.018 mmol) sample of **1b**[13] was trimethylsilylated and treated with 0.3 mL of the ~20% $(n\text{-Bu})_3SnD$/PhH stock solution in 1 mL of dried benzene followed by work-up as described above in method A. The product **2a, 2b** (4 mg, 94%) was identified by comparison with that of the above method A by TLC, NMR, and mass spectra. The mass spectrum had *m/e* 252.1087 (M^+); the NMR peak for H-2″ of the minor isomer **2b** could not be detected by integration in the FT mode.

3′,5′-Di-*O*-benzoyl-2′-deuterio-2′-deoxyuridines. A solution of 710 mg (1.5 mmol) of 3′,5′-di-*O*-benzoyl-2′-chloro-2′-deoxyuridine [prepared from $O^2 \rightarrow$2′-anhydro-1-(3,5-di-*O*-benzoyl-β-D-arabinofuranosyl)uracil[30] by the method described by Holý[15] for the L-enantiomer, with identical mp 166–167 °C] *in* 20 mL of benzene was treated with 1.5 mL of the ~20% $(n\text{-Bu})_3SnD$/PhH stock solution and 20 mg of AIBN. This mixture was then heated at reflux for 1 h at which time TLC (silica, Me_2CO–cyclohexane 1:1) showed complete reaction. The solution was evaporated, the residue was stirred with *n*-hexane, and the insoluble product was filtered and washed well on the filter with *n*-hexane. This white product (605 mg, 92%) had mp 225–227 °C (lit.[31] mp 225–226 °C for the protio compound) and was homogeneous by the TLC system indicated: NMR ($CDCl_3$, Me_4Si internal) δ 2.30 (br t, 0.76, H-2′), 2.72 (m, 0.24, H-2″), 4.57 (m, 1, H-4′), 4.72 (m, 2, H-5′, H-5″), 5.62 (m, 2, H-3′, H-5), 6.40 (d, $J_{1'-2'}$ = 8 Hz, 1, H-1′), 7.51 (m, 7, H-6, *m*- and *p*-benzoyl protons), 8.05 (m, 4, *o*-benzoyl protons), 8.79 (br s, 1, NH-3); mass spectrum calcd for M^+ ($C_{23}H_{19}DN_2O_7$) 437.1333, found *m/e* 437.1337.

2′-Deuterio-2′-deoxyuridines (2c, 2d). A 590-mg (1.34 mmol) sample of the above dibenzoyl product was deblocked by stirring with 50 mL of 0.2 M NaOMe/MeOH for 3 h at room temperature. TLC (silica, 10% MeOH/$CHCl_3$) showed complete reaction. The solution was neutralized with HOAc and evaporated, and the residue was triturated thoroughly with 3 × 10 mL of *n*-pentane. The dried residue was dissolved in ~30 mL of H_2O, 20 mL of saturated NaCl/H_2O was added, and the resulting solution was continuously extracted with MeOAc for 11 h. The organic phase was evaporated and the solid product was dissolved in H_2O and purified from residual salts using a column (2 × 40 cm) of Dowex 1-X2 (OH^-) resin. The column was washed with 600 mL of H_2O and then **2c, 2d** were eluted using ~5000 mL of 0.015 M aqueous triethylammonium bicarbonate (TEAB) solution. Appropriate fractions (UV detector) were combined and evaporated and the residue was treated with H_2O and EtOH and reevaporated to remove TEAB. The solid was recrystallized from EtOH to give 197 mg (68%) of **2c, 2d** (in 2 crops), mp 167–168 °C (lit.[32] mp 167 °C, for the protio compound): NMR (MeOH-d_4, Me_4Si) δ 2.20 (br t, 1, H-2′, H-2″), 3.75 (m, 2, H-5′, H-5″), 3.95 ("q", J ~ 3.2 Hz, 1, H-4′), 4.38 ("q", J ~ 3.2 Hz, 1, H-3′), 5.67 (d, J_{5-6} = 8 Hz, 1, H-5), 6.25 (d, $J_{1'-2'}$ = 7 Hz, 1, H-1′), 7.96 (d, J_{6-5} = 8 Hz, 1, H-6); mass spectrum calcd for M^+ ($C_9H_{11}DN_2O_5$) 229.0809, found *m/e* 229.0811.

Cyclic 2′-Deuterio-2′-deoxyadenosine 3′,5′-Monophosphates (3a, 3b). A 45-mg (0.18 mmol) sample of **2a, 2b** was dissolved in 0.7 mL of $(EtO)_3PO$ by warming and vigorous shaking and the solution was cooled to −5 °C. Freshly distilled $POCl_3$ (35 μL) was added, and the reaction was stirred for 2 h at −5 to 0 °C and then added dropwise to 25 mL of ice-cold 0.1 M NaOH/H_2O. This solution was neutralized *immediately* with 4 M HCl/H_2O to pH 4.0 (pH meter). Activated carbon[9b] (AU-4, 10 g) was added and the slurry was stirred for 1 h at room temperature and then added to a column (2 × 7 cm, 15 g) of the same carbon packed in H_2O. The column was washed with 600 mL of H_2O and the UV transparent wash was discarded. Elution with 700 mL of 2% NH_4OH in 50% EtOH/H_2O and evaporation of appropriate fractions (UV detector) gave an oil which was dissolved in 15 mL of H_2O and applied to a column (2.2 × 27 cm) of Whatman DE-52 (HCO_3^-) DEAE microgranular cellulose packed in H_2O. Elution was begun with H_2O, and at fraction 46 a linear gradient of H_2O (2 L) to 0.18 M TEAB/H_2O (pH 7.0, 2 L) was begun. Fractions (15–20 mL) 84–99 contained 23% (by UV) of the desired **3a, 3b**, 117–150 contained 24% of 2′-deuterio-2′-deoxyadenosine 5′-monophosphate, and 201–231 contained 18% of presumed 2′-deuterio-2′-deoxyadenosine 3′,5′-diphosphate. The two monophosphate derivatives were identified by comparison with known protio samples using TLC (cellulose, *i*-PrOH–concentrated NH_4OH–H_2O 7:1:2) and thin layer electrophoresis (TLE) (cellulose, 0.05 M TEAB/H_2O, pH 7.0) and NMR spectra for **3a, 3b** (see Tables I–III).

Cyclic α-2′-Deoxyadenosine 3′,5′-Monophosphate (5). A 100-mg (0.4 mmol) sample of **4** was dissolved in 1.5 mL of $(EtO)_3PO$ by vigorous shaking for 16 h. This solution was cooled to −5 °C, treated with 90 μL of freshly distilled $POCl_3$, and worked up and purified as described above for the conversion of **2a, 2b** → **3a, 3b**. An 18.5% yield of **5** was obtained plus 17% of α-2′-deoxyadenosine 5′-monophosphate and 39.5% of presumed α-2′-deoxyadenosine 3′,5′-diphosphate as identified by comparison with the β anomers using TLC and TLE.

2′-Deuterio-2′-deoxyuridine 5′-Monophosphates. A 93-mg (0.41 mmol) sample of **2c, 2d** was dissolved in 1 mL of $(EtO)_3PO$ with vigorous shaking, 10 μL of H_2O was added, and the solution was cooled to 0 °C. Freshly distilled $POCl_3$ (0.2 mL) was added and the reaction was stirred for 3 h at 0 °C. TLC (silica, MeCN–1 M NH_4OH 7:3) showed reaction to be nearly complete. The solution was added dropwise to 25 mL of ice-cold 0.05 M TEAB/H_2O (pH 7.0) and the flask was rinsed with ~2 mL of $(EtO)_3PO$. The combined solution was washed with 3 × 50 mL of Et_2O and the combined organic wash was extracted with 25 mL of H_2O. The combined aqueous phase was evaporated in vacuo and the resulting white residue was dissolved in

a minimum volume of H_2O and applied to a column (2.4 × 20.5 cm) of Bio Rad AG 1-X2 (HCO_2^-) resin packed in H_2O. The column was washed with ~600 mL of H_2O and this wash containing a small UV absorbing peak was discarded. Elution with a linear gradient of H_2O (1.5 L) to 6 M HCO_2H/H_2O (1.5 L) and evaporation of the appropriately combined fractions (UV detector) gave 76% (by UV) of the title compounds which were identical with commercial 2′-deoxyuridine 5′-monophosphate by TLC and TLE.

Cyclic 2′-Deuterio-2′-deoxyuridine 3′,5′-Monophosphates (3c, 3d). A 0.31-mmol (by UV) sample of the above 2′-deuterio-2′-deoxyuridine 5′-monophosphate was converted to the soluble carboxamidinium salt and cyclized using DCC as described for the protium compound.[17] The protium and deuterium products were identical by TLC, TLE, and NMR (except for deuterium substitution, see Tables I–III).

Cyclic 2′-Deoxyuridine 3′,5′-Monophosphate. The protium product was prepared by phosphorylation of 2′-deoxyuridine as described above for the 2′-deuterio derivative followed by cyclization of a 0.29-mmol sample to the title compound[17] in 42% yield (as the ammonium salt).

Cyclic 2′-Deoxycytidine 3′,5′-Monophosphate. The title compound[17] was prepared in 66% yield (UV), 49% as a white precipitated powder, from 94 mg (0.29 mmol) of 2′-deoxycytidine 5′-monophosphate monohydrate using the Symons modification (Me_2SO–pyridine for solubility of unprotected cytidines)[33] of the Khorana–Drummond procedure[17] and the DE-52 (HCO_3^-)–TEAB column purification as described above in the conversion of **2a**, **2b** → **3a**, **3b**.

5,6-Dihydro-2′-deoxyuridine. A 21-mg (0.09 mmol) sample of 2′-deoxyuridine was dissolved in 3 mL of 95% EtOH by vigorous shaking and 17 mg of 5% rhodium on powdered carbon catalyst was added. Hydrogen gas was bubbled through the suspension for 3.5 h at room temperature, the mixture was filtered using a Celite pad, and the catalyst-Celite cake was washed well with MeOH. The filtrate had only end absorption in the UV and TLC (silica, glass plate, upper phase of EtOAc-*n*-PrOH-H_2O 4:1:2, H_2SO_4 charring) showed a homogeneous product with slightly slower migration than 2′-deoxyuridine. Evaporation gave a solid with NMR (MeOH-d_4, Me_4Si) δ ~1.96 (m, 1, H-2″), ~2.18 (m, 1, H-2′), 2.62 (t, $J \sim 6.5$ Hz, 2, H-6a, H-6b), ~3.46 (d of d overlapped with solvent and 5′-H's, $J \sim 7$ Hz, 2, H-5a, H-5b), ~3.66 (m, 2, H-5′, H-5″), ~3.77 (m, 1, H-4′), 4.28 (m, 1, H-3′), 6.26 ("t", $J_{1'-2',2''} = 7.2$ Hz, 1, H-1′); mass spectrum calcd for M + 1 ($C_9H_{15}N_2O_5$) 231.0981, found *m/e* 231.0974, calcd for B ($C_4H_5N_2O_2$) 113.0351, found 113.0349, calcd for sugar ion ($C_5H_9O_3$) 117.0551, found 117.0551.

2′-Deuterio-5,6-dihydro-2′-deoxyuridine. This compound was prepared in an identical manner to that of the protium compound just described and had identical TLC mobility; NMR (MeOH-d_4, Me_4Si) δ 1.96 (m, 0.26, H-2″), 2.17 (m, 0.74, H-2′), 2.61 (t, $J \sim 6.5$ HZ= 2= H-6a, H-6b), ~3.5 (d of d overlapped with solvent and 5′-H's, 2, H-5a, H-5b), ~3.65 (m, 2, H-5′, H-5″), ~3.77 (m, 1, H-4′), 4.27 (m, 1, H-3′), 6.25 (d, $J_{1'-2'} \sim 8$ Hz, 1, H-1′); mass spectrum calcd for M + 1 ($C_9H_{14}DN_2O_5$) 232.1044, found *m/e* 232.1032, calcd for B ($C_4H_5N_2O_2$) 113.0351, found 113.0349, calcd for sugar ion ($C_5H_8DO_3$) 118.0614, found 118.0616.

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References and Notes

(1) For the previous paper in this series see: M. J. Robins and M. MacCoss, *J. Am. Chem. Soc.*, preceding paper in this issue.
(2) Postdoctoral Fellow 1972–1974; Research Associate 1974–1976.
(3) Abbreviations used are: cNMP = cyclic ribonucleoside 3′,5′-monophosphate; cdNMP = cyclic 2′-deoxynucleoside 3′,5′-monophosphate; cdAMP, α-cdAMP, cdGMP, cdCMP, cdUMP, and cdTMP are the corresponding cyclic 3′,5′-monophosphate derivatives of 2′-deoxyadenosine, 9-(2-deoxy-α-D-*erythro*-pentofuranosyl)adenine, 2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyuridine, and (2′-deoxy)thymidine, respectively.
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