Conformational Properties of Dinucleoside Monophosphates in Solution: Dipurines and Dipyrimidines†

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ABSTRACT: In order to obtain information about the conformational features in a polyribonucleotide at the nearest neighbor level, detailed nuclear magnetic resonance studies of the dinucleoside monophosphates ApA, ApG, GpA, UpU, CpC, UpC, and CpU were undertaken. Proton spectra were recorded at 100, 220, 270, or 300 MHz for D_2O solutions, 0.01–0.03 M, pD 7.4 at 20 ± 2 °C. Spectra of ApA, ApG, UpU, and UpC were also recorded in the temperature range of 70–90 °C. Unambiguous signal assignments of *all* proton resonances were made with the aid of selectively deuterated dimers. Complete, accurate sets of nuclear magnetic resonance (NMR) parameters were derived for each nucleotidyl unit by simulation-iteration methods. A complete set of chemical shift and coupling constant data was also obtained for all the constituent monomeric units at a concentration and ionization state comparable to that of the dimers. Conformational properties were evaluated quantitatively for most of the bonds in the dinucleoside monophosphates using procedures developed in earlier studies. All of the dimers have a flexible conformational framework in aqueous solution. While flexibility is allowed and alternate conformations are accessible, these molecules nevertheless attempt to achieve conformational identity by showing preferences—sometimes overwhelming preferences—for certain orientations. Thus the ribose rings exist as equilibrium mixtures of C2′-endo ⇄ C3′-endo conformers with a bias for the C3′-endo pucker in most cases. The C4′–C5′ bonds of both nucleotidyl units show significant preference (70–85%) for a gg conformation. Similarly, the dominant conformer (80–90%) about C5′–O5′ is g′g′. Even though an unambiguous determination of the orientation about C3′–O3′ cannot be made, there is suggestive evidence that the orientation of the 3′ phosphate group is coupled to the ribose conformational equilibrium and it is likely that a $^3Eg^- \rightleftarrows {}^2Eg^+$ equilibrium exists with a bias for the $^3Eg^-$ coupled conformation in which the H3′–C3′–O3′–P dihedral angle is about 34–38°. The individual nucleotidyl units in the dimers differ in several key ways from corresponding monomer conformations. Specifically, the ribose equilibrium C2′-endo ⇄ C3′-endo shifts in favor of C3′-endo upon dimerization, the only exception being UpU. The C4′–C5′ and C5′–O5′ bonding network in the dimer forms a stable conformational unit and no correlation exists in the dimers between the conformational preference of this fragment and ribose conformer population. The temperature data for the dimers and dimerization data clearly indicate that the transition C2′-endo → C3′-endo is directly related to χ_{CN} changes brought about by dimerization and stacking. Such coupling of ribose conformation with stacking enabled a quantitation of the ratio of stacked and unstacked populations for a given dimer from ribose couplings. The percentage populations of the stacked species vary from dimer to dimer with ApA and CpC displaying a maximum of 35–40% stacked population and UpU less than 10%. It is further shown that the stacking process can also be monitored by the shift nonequivalence for H5′, H5″ of the 5′-nucleotidyl unit of the dimer. The data are in accord with the existence of extended conformations in equilibrium with two folded structures, a compact base-stacked right helical structure and a loosely base-stacked loop structure characteristic of a left helical form. It is suggested that the O3′–P–O5′ frame is highly flexible and acts as the swivel enabling the 3′- and 5′-nucleotidyl units to stack–destack depending on the nature of the π-ring system in the bases and solvent and temperature conditions. Stacking will cause a modest decrease in χ_{CN} with an accompanying increase in C3′-endo populations, the latter in turn shifting the 3′ phosphate group from g^+ to g^- domains. In short we envision a coupled series of conformational events at the onset of stacking made feasible by the swivel nature of the O3′–P–O5′ bridge.

The overall three-dimensional geometry of a biologically functional polyribonucleotide is determined by the sequence and by various secondary and tertiary interactions such as complementary base pairing, local and cross-strand hydrogen bonding, base stacking, and dipole–dipole and electrostatic interactions. A number of key structural features (base pairing, loop structure, cross-strand hydrogen bonding, etc.) for $tRNA^{Phe}$ were defined recently by x-ray crystallographic (Kim et al., 1974; Robertus et al., 1974) and nuclear magnetic studies (Shulman et al., 1973; Kearns and Shulman, 1974). In solution, the overall geometry of a tRNA molecule is, to a significant extent, determined by a combination of intra- and intermolecular forces. In order to determine the conformational properties at the nearest neighbor level, there has been intense effort in the past to delineate the stereochemical details of small segments of nucleic acids by theoretical calculations (Pullman et al., 1972; Perahia et al., 1973, 1974a,b; Olson, 1973, 1975a–c; Yathindra and Sundaralingam, 1974a,b; Broyde et al., 1974), optical (Brahms et al., 1967; Catlin and Guschlbauer, 1975; Davies and Tinoco, 1968), magnetic resonance (Chan and Nelson, 1969; Bangerter and Chan, 1969; Barry et al., 1972; Smith et al., 1973; Ts'o et al., 1969; Altona et al., 1974; Hruska and Danyluk, 1968), and x-ray diffraction

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studies (Hingerty et al., 1975; Kim et al., 1973; Rubin et al., 1972; Seeman et al., 1975; Suck et al., 1973; Sussman et al., 1972).

Among all the available analytical tools, nuclear magnetic resonance (NMR)[1] spectroscopy is unique for investigation of small nucleic acid segments in solution because of its sensitivity to minute structural, conformational, and environmental variations. However, complete analyses of their ^1H NMR spectra are difficult because of extensive peak overlap and line-broadening complications. Danyluk and co-workers (Kondo and Danyluk, 1972; Kondo et al., 1973, 1975) circumvented the above problem in dimers and trimers by selectively deuterating one or more residues in a given segment of a nucleic acid (Kondo and Danyluk 1972, 1974). In the meantime Sarma and co-workers (Lee et al., 1975; Evans et al., 1975), using 300-MHz ^1H and 40.5-MHz ^{31}P NMR measurements and extensive computer simulations, reported the *complete* analysis of the NMR spectra of nondeuterated ApA and dApdA. There was complete agreement between the sets of data and conformational details from both laboratories.

In this paper we present a systematic and comprehensive proton NMR study and conformational deductions for all naturally occurring dipurine and dipyrimidine nucleoside monophosphates except GpG. A similar study on all the remaining eight mixed dimers will be presented shortly in a separate communication. Ts'o and co-workers (1969) previously reported a 100-MHz study of 15 dinucleoside monophosphates; but their work dealt with base and H1′ signals only. It may be noted that dipurine and dipyrimidine dinucleoside monophosphates are constitutionally analogous to those segments of nucleic acids that contain either purines or pyrimidines.

Experimental Section

Materials. The 3′ and 5′ mononucleotides and dinucleoside monophosphates were purchased from Sigma Chemical Co., P-L Biochemicals, Collaborative Research, Calbiochem, Miles Laboratories, and Boehringer Mannheim. All the samples were lyophilized three times from 99.8% D_2O and the final solutions were made up in "100%" D_2O to a concentration of 0.01–0.03 M. The pD was measured with Fisher Accument Model 320 and Beckman Research pH meters.

Synthesis of Deuterated Derivatives. Syntheses of selectively deuterated dimers such as *ApA, ApA*, GpA*, and CpC* (the asterisk indicates the deuterated residue) were achieved by both enzymatic and chemical procedures described in detail elsewhere (Kondo and Danyluk, 1972; Kondo et al., 1973). Deuterated nucleotides were isolated from RNA fractions of fully deuterated blue-green algae, *Synchococcus lividus,* and were then condensed with the appropriate protonated nucleotide derivative to yield the dimers. Comparison of spectra for protio and selectively labeled compounds along with spectra of the respective monomers allowed a direct assignment of all the signals.

Measurement of Spectra. Proton spectra were recorded with a variety of spectrometers (Varian 100, 220, and 300, and Bruker 270 in both CW and FT modes). ^{31}P NMR spectra in FT mode were recorded at 40.5 MHz using the Varian HA-100D system. Details of the 100-MHz instrumentation are given elsewhere (Sarma and Mynott, 1973a). The 220-MHz spectra were recorded using the spectrometer system at Argonne National Laboratory, details of which are given in an earlier work (Davies and Danyluk, 1974). The 270-MHz spectra were obtained using a Bruker HX-270 system at Southern New England High Field Facility at New Haven, Connecticut. The 300-MHz spectra were recorded at the Institute of Polymer Science, University of Akron, Akron, Ohio. The Bruker HX-270 system is equipped with a BNC data system and is capable of performing 16K transforms. The Varian 300-MHz system is interfaced to a 620 Varian data system capable of performing 16K transforms. Each of the spectrometer systems cited above is equipped with a variable temperature accessory. The 100-MHz spectra where the phosphorus nuclei were decoupled were recorded with irradiation derived from a Digilab 50-80 PD plug-in amplifier. In the 220-MHz system, ^{31}P decoupling experiments utilized a Schomandl ND 100 M generator set at a decoupling frequency near 89 MHz.

All the spectra were measured at 20 ± 2 °C and several dimers were also recorded at elevated temperatures. Both TSP (3-trimethylsilylpropionate-*2,2,3,3-d*$_4$ sodium salt) and TMA (tetramethylammonium chloride) were used as internal references. The chemical shifts recorded using TMA as an internal standard were converted relative to TSP using a conversion factor of 3.206 ppm.

Analysis of Spectra. Initial sets of NMR parameters were obtained directly from the observed spectra. Since the spectra in most cases were not first order, a further refinement was made by simulation of the spectra using a Varian 620i 6-spin NMR simulation program. A final iterated set of parameters was then derived using LAOCOON III or NMREN and NMRIT iterative programs.

It should be noted that both the measurements of spectra and subsequent analyses were conducted independently at two different laboratories (SUNY, Albany and Argonne National Laboratory, Argonne). The results reported in Tables I to IV represent an average of those obtained in each laboratory. Agreement between the independent set of parameters for all of the monomers and dimers was surprisingly good, being ±0.01 ppm for shifts and ±0.2 Hz for couplings.

Results

Assignments. Among the seven dinucleoside monophosphates, ApA, ApG, GpA, UpU, CpC, UpC, and CpU, unequivocal assignments in ApA, GpA, and CpC were made by comparing their 220-MHz spectra with those of the deuterated counterparts, *ApA, ApA*, GpA*, and CpC*. Figure 1 illustrates the spectra of ApA and *ApA, and Figure 2 those for CpC and CpC*. The assignments indicated in the figures are obvious from inspection. Selective deuteration of residues is a particularly important way to assign the H1′ protons of the Xp- and -pX parts. For example in ApG, the Ap- H1′ occurs at a lower field compared with the H1′ resonance of the -pG fragment; in ApA, the situation is reversed (Table I). Under favorable conditions an equally powerful tool to carry out assignments and analyses is the computer simulation of spectra (vide supra). In Figure 3 we have illustrated the experimentally observed and simulated spectra for a homodipyrimidine dinucleoside monophosphate (CpC) and in Figures 4 and 5 a heterodipyrimidine at two different temperatures. In Figure 6 are given the experimental and simulated spectra for a heterodipurine system, ApG. The spectra for a homodipurine have been shown elsewhere (Lee et al., 1975; Kondo and Danyluk, 1974, 1976). The fit between observed and calculated spectra

[1] Abbreviations and symbols used: NMR, nuclear magnetic resonance; CW, continuous wave; FT, Fourier transform; TSP, sodium 3-trimethylsilylpropionate-*2,2,3,3-d*$_4$; TMA, tetramethylammonium chloride; UMP, uridine monophosphate; CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate.

TABLE I: Chemical Shifts[a] of Dinucleoside Monophosphates in D_2O.[b]

Nucleotide	T (°C)	Chemical Shifts (δ, ppm)								
		1′	2′	3′	4′	5′	5″	Δ5′5″[c]	H2(H5)	H8(H6)
ApA Ap-	20	5.835	4.674	4.668	4.359	3.900	3.841	0.059	7.881	8.176
-pA		5.957	4.585	4.512	4.378	4.380	4.186	0.194	8.053	8.245
ApA Ap-	72	5.952	4.707	4.736	4.371	3.849	3.803	0.046	8.146	8.236
-pA		6.067	4.722	4.526	4.390	4.312	4.213	0.099	8.206	8.346
ApG Ap-	20	5.886	4.683	4.663	4.322	3.824	3.788	0.036	8.062	8.224
-pG		5.779	4.636	4.500	4.310	4.306	4.169	0.137		7.909
ApG Ap-	80	5.997	4.774	4.746	4.355	3.850	3.808	0.042	8.183	8.256
-pG		5.873	4.717	4.581	4.375	4.290	4.212	0.078		7.975
GpA Gp-	20	5.673	4.660	4.656	4.310	3.845	3.792	0.053		7.876
-pA		6.056	4.627	4.535	4.388	4.315	4.181	0.134	8.091	8.313
UpU Up-	20	5.892	4.461	4.553	4.338	3.942	3.845	0.097	5.869	7.931
-pU		5.950	4.342	4.346	4.272	4.284	4.140	0.144	5.896	7.936
UpU Up-	89	5.895	4.444	4.595	4.338	3.912	3.824	0.088	5.903	7.823
-pU		5.935	4.372	4.341	4.283	4.233	4.123	0.110	5.927	7.849
CpC Cp-	20	5.782	4.461	4.453	4.327	4.004	3.868	0.136	5.947	7.903
-pC		5.882	4.213	4.300	4.247	4.343	4.121	0.222	5.958	7.943
UpC Up-	20	5.838	4.466	4.542	4.315	3.955	3.850	0.105	5.861	7.902
-pC		5.953	4.284	4.321	4.256	4.334	4.143	0.191	6.033	7.943
UpC Up-	80	5.903	4.463	4.612	4.324	3.929	3.848	0.081	5.916	7.817
-pC		5.979	4.310	4.322	4.266	4.279	4.163	0.116	6.098	7.873
CpU Cp-	20	5.799	4.479	4.464	4.330	4.003	3.871	0.132	5.962	7.918
-pU		5.914	4.299	4.330	4.278	4.333	4.125	0.208	5.817	7.953

[a] Shifts are given relative to internal TSP and are accurate to ±0.005 ppm. [b] pD = 7.4; concentration is 0.02–0.03 M. [c] $\Delta 5'5'' = \delta 5' - \delta 5''$.

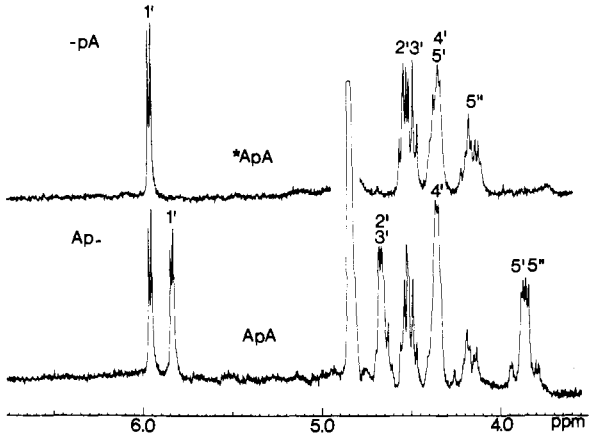

FIGURE 1: The 220-MHz NMR spectra of *ApA (top) and ApA (bottom) at 20 °C, 0.02 M, pH 7.

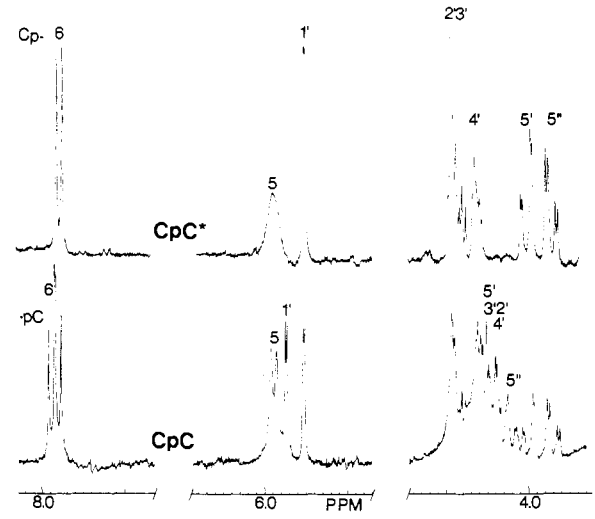

FIGURE 2: The 220-MHz NMR spectrum of CpC* (top) and CpC (bottom) at 20 °C, 0.01 M, pH 7.

is remarkably good in view of the complexity of the systems. It is important to stress that as much as a 30% difference can arise in the magnitude of J values extracted from simple first-order analyses and iteration values; this problem is particularly serious in the H1′ region (Lee et al., 1975), because of virtual coupling effects, i.e., $\delta_{2'} \simeq \delta_{3'}$.

No direct assignments of the 5′ and 5″ hydrogens are possible at present. However, we believe that the low-field hydrogen from the ABX system can be tentatively assigned to H5′ and the high field one to H5″. According to this assignment, which is in agreement with the proposal by Remin and Shugar (1972), the H5′ refers to that geminal hydrogen which is gauche to H4′ and the ether oxygen of the ribose in the traditional gg conformation, and its lower field position compared with H5″ probably reflects the deshielding effect of the contiguous ribose ether oxygen. The present assignments of dipurine base and H1′ signals confirm the earlier results of Ts'o et al. (1969). Selective deuteration also permits an assignment of H5 signals in homo- and heterodipyrimidine dinucleoside monophosphates. This was not attainable in the earlier work (Ts'o et al., 1969). One of the interesting results (Table I) from the pyrimidine assignments is a reversal in shift for H5 in CpU compared with the other dipyrimidines.

Chemical Shifts. The chemical shift data for ApA, ApG, GpA, UpU, CpC, UpC, and CpU at 20 °C and for ApA, ApG, UpU, and UpC at 70–90 °C are summarized in Table I. The data show that, in all cases except ApG, the H1′ of Xp- appears at a higher field than H1′ of -pX. In this series of molecules, it was invariably found that the 2′ and 3′ protons of Xp- appear

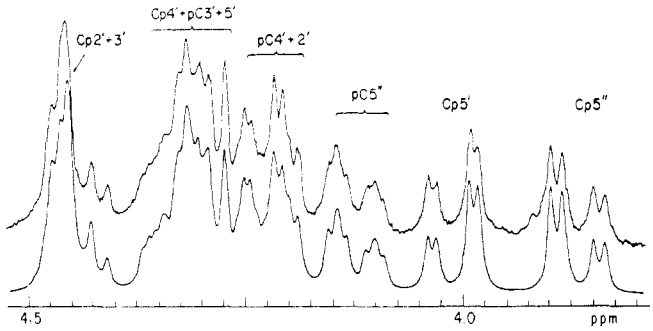

FIGURE 3: The 270-MHz NMR spectrum of CpC (top, 20 °C, 0.03 M, pH 7) and the corresponding line-shape simulation (bottom). The base and 1′ region of the spectra are not shown.

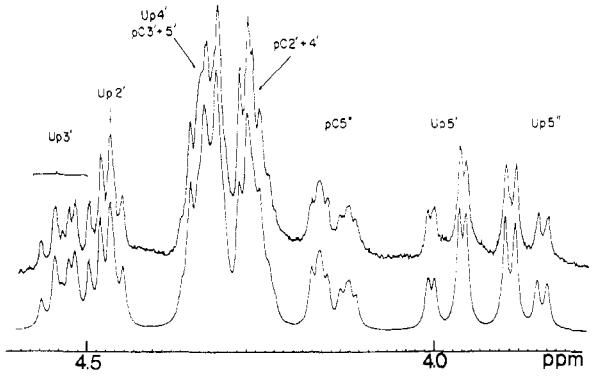

FIGURE 4: The 270-MHz NMR spectrum of UpC at 20 °C (top) along with the corresponding line shape simulations (bottom).

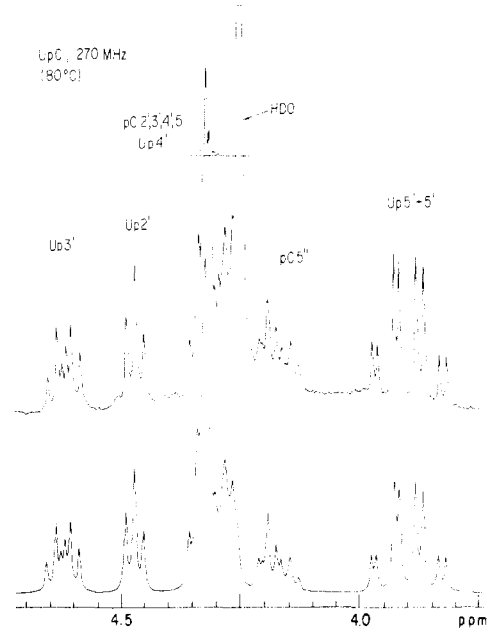

FIGURE 5: The 270-MHz NMR spectrum of UpC at 80 °C (top) along with the simulated spectrum (bottom).

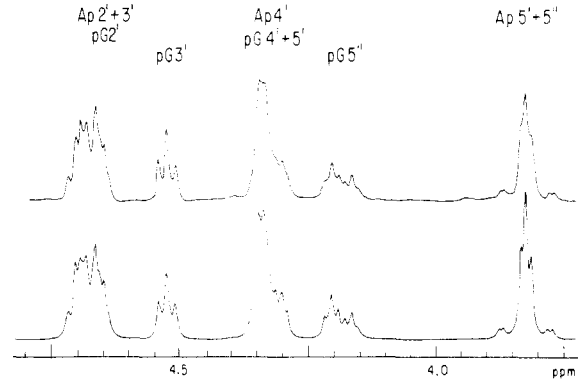

FIGURE 6: The 300-MHz NMR spectrum of ApG at 20 °C (top) along with the corresponding computer simulated spectrum (bottom).

at lower field than the same protons in -pX. In general for the dipurine systems, H2′ occurs at a lower field compared with H3′, the effect being most noticeable for the 5′ nucleotide. In the case of the py–py dimers, no such trends are seen and considerable variability occurs for the 2′ and 3′ proton chemical shifts. The chemical shifts of the 4′ proton of Xp- show a remarkable constancy irrespective of whether one is dealing with a pu–pu or a py–py dimer; the observed maximum spread is 0.05 ppm. Even though H4′ of -pX shows a variation of 0.13 ppm, the variation is extremely small within the dipyrimidine dimers, i.e., 0.03 ppm. In addition in the dipyrimidine series, H4′ of Xp- appears at a lower field compared with H4′ of -pX; this is reversed for dipurines.

In all cases examined, the 5′,5″ protons of Xp- appear at a considerably higher field than the respective protons of -pX. Examination of the chemical shift data for 5′ and 5″ (Table I) unravels some trends. Thus it was found that, within their classes, the chemical shifts of the 5″ proton of -pX have a constant value for purines and pyrimidines. Also, H5″ of Xp- has a constant chemical shift in the pyrimidine systems indicating that this proton is insensitive to pyrimidine sequence effects. On the other hand the 5′ protons of Xp- and -pX show sequence-dependent variations in chemical shifts.

The Ap- H8 and H2 in ApA are located at a higher field than the corresponding protons in ApG. It is also seen that the -pA H8 and H2 in ApA appear at higher field compared with the corresponding protons in GpA. Comparison of the data for ApG and GpA indicates that H8 of the guanine nucleotide appears at a higher field in GpA; also H8 and H2 of Ap- in ApG are located at a higher field compared with the corresponding protons in GpA, i.e., the 5′-nucleotidyl moiety has a greater shielding effect than the 3′-nucleotidyl part for the purines. Comparison of the UpU and UpC pair shows that -pC does not significantly affect H5 and H6 chemical shifts of Up-. However, data on UpU and CpU suggest that Cp- significantly affects the H5 chemical shifts of the -pU moiety. Comparison of CpC and UpC indicates that removal of Cp- and substitution by Up- has a pronounced effect on -pC H5 chemical shifts, i.e., Cp- increases the shielding of the base protons in the -pC part of CpC.

Increasing the temperature from 20 to ≃80 °C considerably perturbed the ApA and ApG chemical shifts. In general it was found that higher temperature caused a shift to lower field of the ribose and the base protons, the effect being maximum for the base protons and the ribose 1′, 2′, and 3′ protons. A similar temperature dependence has been observed for base and H1′ protons by Ts'o et al. (1969) and Hruska and Danyluk (1968). With respect to the chemical shifts of 5′ and 5″ protons ApG and ApA display a different behavior. In ApG, only the 5″ proton of -pG showed a detectable shift to lower field with increasing temperature. In ApA, temperature increase caused an increased shielding of 5′ and 5″ protons of Ap- and H5′ of -pA. In the case of UpC, increasing the temperature caused a deshielding of 1′ and 3′ protons of the Up- with no significant effect on 2′, 4′, and 5″ protons of Up-. The 5′ proton of Up-

TABLE II: Coupling Constants[a] for Dinucleoside Monophosphates in D_2O.[b]

Nucleotide	T (°C)	Coupling Constants (Hz)										
		1′2′	2′3′	3′4′	4′5′	4′5″	3′P	4′P	5′P	5″P	5′5″	5 6
ApA Ap-	20	3.6	5.2	5.5	2.5	3.5	7.6	<0.5			13.0	
-pA		4.0	5.3	5.8	2.8	3.7		2.0	3.0	3.3	−12.7	
ApA Ap-	72	4.8	5.2	4.4	2.6	4.0	8.8				−12.8	
-pA		4.6	5.1	4.6	2.8	4.2		1.8	4.9	4.9	−11.6	
ApG Ap-	20	4.6	4.9	4.7	2.4	3.4	8.2				−12.8	
-pA		4.4	5.2	4.8	2.8	3.6		2.2	4.0	4.0	−11.8	
ApG Ap-	80	5.4	5.2	3.8	2.9	3.8	8.0				−13.1	
-pG		4.9	5.4	4.6	2.6	4.7		1.0	4.8	5.3	−11.8	
GpA Gp-	20	4.0	5.1	5.2	2.7	4.0	8.0				−13.0	
-pA		4.2	5.4	5.3	2.5	3.0		2.0	3.7	3.9	−11.8	
UpU Up-	20	4.2	5.2	5.3	2.4	4.0	8.2				−13.1	8.1
-pU		3.9	5.1	5.0	2.4	3.2		2.0	4.4	4.5	−11.9	8.2
UpU Up-	89	4.8	5.3	4.9	2.5	4.3	8.1				−13.0	8.1
-pU		4.4	5.2	4.9	2.7	3.5			4.9	4.9	−11.8	8.1
CpC Cp-	20	3.0	5.1	7.0	2.5	3.3	8.3				−13.0	7.7
-pC		2.9	5.1	6.6	2.2	2.6		2.0	4.0	3.6	−11.7	7.7
UpC Up-	20	3.9	5.2	5.9	2.7	3.9	8.4				−12.9	8.1
-pC		3.5	5.2	6.0	2.5	2.8		2.0	3.8	4.0	−11.8	7.7
UpC Up-	80	4.8	5.4	5.2	2.8	4.4	8.2				−13.0	
-pC		3.0	5.2	6.2	2.8	4.2		1.8	5.2	5.2	−12.0	
CpU Cp-	20	3.2	5.1	6.8	2.3	3.8	8.6				−13.3	7.8
-pU		3.3	5.2	6.1	2.2	2.8		2.0	3.6	3.6	−11.9	8.2

[a] Coupling constants are accurate to ±0.1–0.2 Hz. [b] Solution conditions are the same as in Table I.

showed a slight shift to higher fields. The -pC residue of UpC displayed a contrast with Up- in its temperature dependence. Thus H3′ of -pC was insensitive to temperature variation, while H5′ showed a larger shift to higher field and H1′ a smaller shift to lower field compared with the corresponding protons of the Up- part. Elevation of temperature causes H5 to shift to lower and H6 to higher fields in UpC. The base protons in UpU also showed similar temperature dependence while only a minimal influence was observed on ribose proton chemical shifts in UpU.

Coupling Constants. The coupling constant data are summarized in Table II. The values for $J_{1'2'}$ and $J_{3'4'}$ show a maximum variation of 1.7 and 2.3 Hz, respectively, with the magnitude for $J_{2'3'}$ maintaining values between 4.9 and 5.4 Hz at 20 °C. The observed average for the sum $J_{1'2'} + J_{3'4'}$ was 9.5 Hz. The Xp- and -pX nucleotides of dipurines displayed, in general, the same magnitude for the sum $J_{4'5'} + J_{4'5''}$ (Σ) of about 6.2 Hz. On the other hand Xp- and -pX of the dipyrimidines showed an average difference of 1 Hz in the magnitude of Σ, the 5′-nucleotidyl value being smaller. In all cases examined the magnitude of the four bond coupling $J_{4'P}$ in the -pX part was essentially constant with a value of 2 to 2.2 Hz at 20 °C. The sum $J_{5'P} + J_{5''P}$ (Σ') maintained values between 7 and 8 Hz in all cases except ApA (Σ' = 6.3 Hz) and UpU (8.9 Hz). The value for $J_{H(3')P}$ shows no significant variation among the dimers. In the dipurines, ApA and ApG, and the dipyrimidine UpU, elevation of temperature caused an increase in $J_{1'2'}$, decrease in $J_{3'4'}$, and increases in the magnitudes of Σ and Σ'.

Dimerization Shifts. In Table III are provided the data for the dimerization effects, defined as the difference in NMR parameters between monomer and dimer. The monomer data were obtained at pD 5.5, a pD at which the phosphate ionization state is the same as for the dimer. No corrections for concentration effects were made but all measurements were carried out at 0.01–0.03 M solutions. The data (Table III) show some definite trends and patterns in chemical shift changes as a result of dimerization. Thus the H1′ protons are upfield ($\Delta\delta$ positive) in all cases in the dimers, the effect being pronounced in dipurines and less so in pyrimidines. Equally informative is the observation that the 3′-nucleotidyl unit is more sensitive than the 5′ residue in this respect. In the case of the 2′ protons, the dipurines display the same trend as H1′. On the other hand, dimerization produces less of a change for the 2′ protons in the dipyrimidines and $\Delta\delta$ may be either positive or negative (Table III). With respect to the 3′ protons, in Xp- of the dipurines these shift to higher field, while a minimal effect is seen for the same protons in -pX. The dipyrimidines showed a considerably smaller dimerization effect on H3′ chemical shift. The dimerization effect on H4′ was roughly comparable to that of H3′ for both homodimer sets. The effect of dimerization on H5′ chemical shifts is very dramatic and worth examining in detail. It is seen that (Table III) in the three dipurines, ApA, ApG, and GpA, H5′ of -pX is shifted substantially to *lower* fields whereas H5′ of Xp- shows shifts to *higher* fields. In the case of the dipyrimidines CpC, UpC, and CpU, H5′ of Xp- and -pX fragments shifts to lower fields, the effect being noticeably higher for -pX. Among the homodipyrimidines the H5′ chemical shifts of -pX of UpU show the minimum variation due to dimerization. For the 5″ hydrogen the effect of dimerization is small especially in the dipyrimidines; in dipurines the pattern followed is similar to H5′. The H2 and H8 base protons in the purine dimers shift to higher fields as a result of dimerization. In the case of the dipyrimidines, the dimerization effects for the base protons are small compared with the purine systems, in agreement with earlier observations (Ts'o et al., 1969).

Dimerization Effect on Coupling Constants. The influence of dimerization on J values for the dinucleoside monophosphates is summarized in Table IV. In general, $J_{1'2'}$ decreases,

TABLE III: Dimerization Shifts.

Nucleotide	δ(monomer)-δ(dimer) (ppm) 1′	2′	3′	4′	5′	5″	Δ[a]	H2(H5)	H8(H6)
ApA Ap-	0.260	0.200	0.124	0.140	0.058	0.067	0.009	0.295	0.156
-pA	0.180	0.170	0.00	0.015	−0.245	−0.051	0.194	0.201	0.250
ApG Ap-	0.209	0.191	0.129	0.177	0.134	0.120	−0.014	0.114	0.108
-pG	0.165	0.119	−0.005	−0.040	−0.182	−0.045	0.137		0.203
GpA Gp-	0.276	0.176	0.115	0.108	0.070	0.073	0.003		0.161
-pA	0.081	0.128	−0.024	−0.005	−0.160	−0.046	0.114	0.163	0.182
UpU Up-	0.068	0.002	0.019	−0.028	−0.014	−0.003	0.011	0.046	−0.026
-pU	0.040	0.021	0.032	0.023	−0.116	−0.050	0.066	0.054	−0.055
CpC Cp-	0.170	−0.028	0.099	−0.027	−0.056	−0.008	0.048	0.137	−0.003
-pC	0.117	0.122	0.037	0.039	−0.145	−0.028	0.117	0.166	0.099
UpC Up-	0.122	−0.007	0.030	−0.015	−0.027	−0.002	0.025	0.054	−0.003
-pC	0.046	−0.037	0.016	0.027	−0.136	−0.050	0.086	0.091	−0.099
CpU Cp-	0.153	−0.046	0.088	−0.037	−0.055	−0.011	0.044	0.122	−0.012
-pU	0.076	0.064	0.046	−0.003	−0.173	−0.035	0.138	0.133	0.038

[a] $\Delta = [(\delta_{5'}-\delta_{5''})_{dimer} - (\delta_{5'}-\delta_{5''})_{monomer}]$.

TABLE IV: Dimerization Effects on Coupling Constants.

Nucleotide	J(monomer)-J(dimer) (Hz) 1′2′	2′3′	3′4′	4′5′	4′5″	3′P	4′P	5′P	5″P	5′5″	5 6
ApA Ap-	2.6	0	−2.6	−0.1	−0.2	0.3				0.3	
-pA	1.7	−0.1	−2.1	0.3	−0.6		−0.3	2.0	1.7	0.7	
ApG Ap-	1.6	0.3	−1.8	0	−0.1	−0.3				0.1	
-pG	1.7	0	−1.4	0.6	−0.2		−0.2	1.1	1.1	0	
GpA Gp-	1.8	0.1	−2.1	0.4	−0.4	−0.3				0.3	
-pA	1.5	−0.2	−1.6	0.6	0.4		−0.3	1.3	1.1	−0.2	
UpU Up-	0.7	0	−0.1	0.5	0.2	0				0.3	0.1
-pU	0.6	0	−0.7	−0.1	−0.4		0.1	−0.6	0.1	0.1	−0.1
CpC Cp-	1.2	0.2	−1.4	0.2	0.8	0.2				0.1	0
-pC	1.2	−0.1	−1.5	0.2	1.2		0	0.2	1.5	−0.1	−0.1
UpC Up-	1.0	0	−0.7	0.2	0.3	−0.2				0.1	0.1
-pC	0.6	−0.2	−0.9	−0.1	1.0		0	0.4	1.1	0	−0.1
CpU Cp-	1.0	0.2	−1.2	0.4	0.3	−0.1				0.4	−0.1
-pU	1.6	−0.1	−1.8	0.1	0		0.1	0.7	1.4	0.1	−0.1

$J_{3'4'}$ increases, and $J_{2'3'}$ remains essentially constant upon dimerization, these effects being very small for UpU. No general patterns were observed in the magnitude of $\Delta\Sigma$ and $\Delta\Sigma'$, some showing increases and others decreases (Table IV). Dimerization had no significant effect on the magnitudes of $J_{3'P}$, $J_{4'P}$, $J_{5'5''}$, and J_{5-6}.

Discussion

Conformational Nomenclature. A complete conformational description of a dinucleoside monophosphate structure requires the determination of ribose ring conformations, the relative orientation of base and ribose rings (χ_1 and χ_2 dihedral angles) as well as the specification of dihedral angles about the six bonds of the ribophosphate backbone: C5′-C4′, C3′-O3′, O3′-P of the 3′-nucleotidyl fragment (Xp-) and P-O5′, O5′-C5′, and C5′-C4′ bonds of the -pX moiety. In the notation of Figure 7, these are the ψ_1, ϕ_1', ω_1', ω_1, ϕ_2, and ψ_2 angles, respectively. The conformational nomenclature utilized in the present work is consistent with the Jerusalem proposal recommended to IUPAC and IUB as a standard nomenclature for polynucleotides (Sundaralingam et al., 1973).

Conformation of the Ribose Ring. The ribofuranose ring conformation in a dinucleoside monophosphate may be treated as a simple C2′-endo (^{2}E) ⇄ C3′-endo (^{3}E) equilibrium (Sarma and Mynott, 1972; Evans and Sarma, 1974a) or as an equilibrium of entities with precise phase angles and amplitude of pucker in the pseudorotational itinerary (Altona and Sundaralingam, 1973). Although the ribofuranose system may exist as a continuum of conformational states, the presence of these states cannot be detected by the present NMR technique. A detailed error analysis of the Altona-Sundaralingam ap-

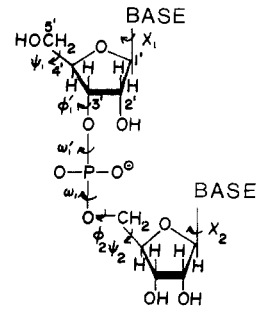

FIGURE 7: Conformational nomenclature for dinucleoside monophosphates (Sundaralingam et al., 1973).

TABLE V: Population Distribution of Conformers in Dinucleoside Monophosphates and Their Components.

			Dimer					Monomer[b]				
			Ribose Ring[a]		Backbone[c]			Ribose Ring[a]		Backbone[c]		
Nucleotide	T (°C)	% Stacked[e]	% ^{3}E	K_{eq}[d]	%gg	%g′g′	θPH[g]	%^{3}E	K_{eq}[d]	%gg	%g′g′	θPH[g]
ApA Ap-	20	38 ± 2	58	1.4	79		±37	31	0.5	82		±36
-pA			61	1.6	74	90		40	0.7	77	72	
ApA Ap-	72	19 ± 4	46	0.9	73		±33					
-pA			48	0.9	64	73						
ApG Ap-	20	25 ± 2	49	1.0	81		±35	31	0.5	82		±36
-pG			51	1.0	75	82		37	0.6	71	71	
ApG Ap	80	16 ± 3	40	0.7	72		±36					
-pG			48	0.9	66	72						
GpA Gp-	20	30 ± 2	55	1.2	72		±36	33	0.5	72		±37
-pA			56	1.3	85	84		40	0.7	77	72	
UpU Up-	20	8 ± 5	56	1.3	75		±35	56	1.3	68		±35
-pU			53	1.1	83	77		46	0.8	89	75	
UpU Up-	89	9 ± 2	52	1.1	71		±35					
-pU			52	1.1	77	73						
CpC Cp-	20	35 ± 1	74	2.9	81		±35	60	1.5	71		±35
-pC			69	2.2	92	84		55	1.2	77	75	
UpC Up-	20	18 ± 2	62	1.6	73		±35	56	1.3	68		±35
-pC			63	1.7	87	83		55	1.2	77	75	
UpC Up-	80	0–4[f]	55	1.2	67		±35					
-pC			65	1.9	69	70						
CpU Cp-	20	33 ± 2	66	1.9	78		±33	60	1.5	71		±35
-pU			64	1.8	90	85		46	0.8	89	75	

[a] Computed by using $J_{1'2'} + J_{3'4'} = 9.5$ Hz for the dimers and $J_{1'2'} + J_{3'4'} = 9.3$ for the monomers. [b] Monomer data for solutions at pD = 5.4. [c] Rotamer equations used: gg = $(13.7 - \Sigma)/9.7$; g′g′ = $(25 - \Sigma')/20.8$. [d] K_{eq}, ^{2}E ⇄ ^{3}E; estimated errors in K_{eq} are ±0.2. [e] % stacked = $(J_{3'4'}(\text{dimer}) - J_{3'4'}(\text{monomer}))/(9.5 - J_{3'4'}(\text{monomer}))$. [f] Computed from the magnitude of $J_{1'2'}$ and $J_{3'4'}$ Up-ribose moiety. [g] In degrees.

proach by Evans and Sarma (1974a) and Davies and Danyluk (1974) showed that for the majority of nucleotide systems the method is not in reality an improvement over the simple qualitative approach of ^{2}E ⇄ ^{3}E equilibrium (Sarma and Mynott, 1972) and therefore for the sake of simplicity the latter approach will be employed in this work.

In order to compute the population of ^{2}E and ^{3}E conformers the value for $J_{1'2'} + J_{3'4'}$ was obtained by averaging the experimentally determined values of 28 ribose moieties (from a collection of dimers in the present and a following paper on mixed dimers). This value was found to be 9.5 ± 0.3 Hz. For the mononucleotides, the experimentally determined average value for $J_{1'2'} + J_{3'4'}$ was found to be 9.3 ± 0.3 Hz. Using these values the percentage of ^{3}E population and K_{eq} for ^{2}E ⇄ ^{3}E were computed for the dimers and monomers; the data are summarized in Table V. Inspection of the data shows some very important trends. Both 5′ and 3′ mononucleotides of adenosine and guanosine, at a pH when the phosphate is a monoanion, show definite preference for ^{2}E pucker, the preference being detectably more for the 3′ monophosphates. Upon dimerization, the ribose moieties of Xp- and -pX of dipurines shift substantially toward ^{3}E conformation, the shift being less for the -pX part. The ribose conformations of the pyrimidine mononucleotides 5′-UMP, 5′-CMP, 3′-UMP, and 3′-CMP show a contrasting picture to their purine counterparts 5′-AMP, 5′-GMP, 3′-AMP, and 3′-GMP, in the sense that the pyrimidine monomers display a substantial ^{3}E population compared with the purines (Table V). Also noticeable within the pyrimidine series is a higher preference for ^{3}E conformer population in 3′ monophosphates than in corresponding 5′ mononucleotides. This is again in contrast to their purine counterparts.

The effect of dimerization on the population distribution of ribose conformers in the pyrimidines *does not* follow the general pattern observed for the purine analogues. Each of the dipyrimidines behaves atypically. Thus in going from 3′-UMP and 5′-UMP to UpU, as well as from 3′-UMP and 5′-CMP to UpC, the ribose conformational distribution does not alter significantly. But the ribose moieties in CpC undergo a decisive change to increased ^{3}E pucker upon dimerization. In CpU, the ribose moiety of the -pU fragment undergoes a substantial shift to ^{3}E form compared with 5′-UMP. The implications of the preceding ribose ring conformational changes are discussed later in terms of base–base interactions and changes in glycosyl torsion angles.

In both purine and pyrimidine series, elevation of temperature causes a depopulation of ^{3}E conformer (Table V), the effect being pronounced for the 3′-nucleotidyl unit. This observation is similar to that reported for ApA (Hruska and Danyluk, 1968; Evans et al., 1975).

In short, the data in Table V indicate that the sugar moiety in both dipurine and dipyrimidine dinucleoside monophosphates at pD 7.4, 0.01–0.03 M concentrations in aqueous solution, exists as an equilibrium mixture of ^{2}E ⇄ ^{3}E conformers with a bias for ^{3}E pucker. Although no crystal data are yet available for dipurine and dipyrimidine dinucleoside monophosphates, the crystal data for ApApA show that the ribose rings of all three residues display ^{3}E pucker (Suck et al., 1973).

Phosphodiester Backbone Conformations. C4′–C5′ (ψ_1, ψ_2) and C5′–O5′ (ϕ_2). The population distribution of conformers about C4′–C5′ and C5′–O5′ bonds can be computed using expressions developed and discussed elsewhere (Hruska et al., 1973; Wood et al., 1973a,b; Sarma et al., 1974; Evans and Sarma, 1974a). These expressions were recently modified by

Lee and Sarma (1976). The presently used J values for pure rotamers are as follows: C4′–C5′, J_g = 2.0 Hz, J_t = 11.7 Hz; for C5′–O5′, J_g = 2.1 Hz, and J_t = 22.9 Hz. The modified expressions as well as the computed populations of gg (about C4′–C5′) and g′g′ (about C5′–O5′) for monomeric components and dimers are compiled in Table V. The data reveal that, in the 3′ and 5′ mononucleotides as well as in all the dimers, the C4′–C5′ bonds show a distinct preference for the gg conformation. In the case of the dipurines, the 3′ residue shows a greater degree of preference for the gg orientation than the 5′ part; in the case of the dipyrimidines the situation is reversed.

A comparison of the gg population vis à vis the ribose conformation indicates that no general correlation of the type postulated by Hruska (1973) exists between the two, i.e., high gg is associated with high ^{3}E populations. Thus no difference in the gg population is observed for the Cp- of CpC and the -pU of UpU even though these two moieties display substantial differences in their ^{3}E populations (74 vs. 53%). Very much related to the lack of correlation between ribose pucker and orientation of the exocyclic linkage is the observation that dimerization has no dramatic effect on the time-average orientation of C4′–C5′ bonds, despite a significant increase in the ^{3}E population. For example in the ApA, ApG, and GpA series, on the average, dimerization causes a 20% increase in ^{3}E populations while there is no effect on the gg population for these compounds. In this respect the pyrimidine dinucleoside monophosphates present a contrast even though no regularity can be seen within them as a class. In the case of CpC, the C4′–C5′ bonds of the Cp- and -pC fragments show an apparent increase in gg from 71–77% in monomers to 81–92% in dimers coupled with an approximately 14% increase in ^{3}E. On the other hand in UpU, dimerization causes a slight (7%) increase in gg population in Up- and a 6% reduction in -pU with no change in ^{3}E populations. In CpU the gg population of -pU is unaffected despite an 18% increase in ^{3}E population as a result of dimerization. In UpC, both fragments make modest gains in gg and ^{3}E population during dimerization.

The population distribution of conformers about C5′–O5′ (Table V) shows high preference for the g′g′ orientation in both monomers and dimers irrespective of the nature of the base. In addition, the population of the g′g′ conformer increases upon dimerization, UpU being the single exception. No correlation between gg and g′g′ populations of the type found for the monomers (Wood et al., 1973b) is found for the dimers. The data indicate that the C4′–C5′, C5′–O5′ bonds in the dimers form a highly stable conformational unit and the implications of this with respect to ribose ring conformation are discussed in a subsequent section.

C3′–O3′(ϕ_1'). Simple steric considerations show three distinct rotamer possibilities about this bond; they are P–O3′ bond trans to C3′–C4′ (g^-, ϕ_1' = 180°), P–O3′ trans to C3′–C2′ (g^+, ϕ_1' = 300°), P–O3′ gauche to both C3′–C4′ and C3′–C2′ (t, ϕ_1' = 60°). Information about the conformational status of the 3′ phosphate group can be obtained from the magnitude of $J_{H3'-P3'}$. We have indicated elsewhere (Lee and Sarma, 1975; Davies and Danyluk, 1975) that one cannot unambiguously distinguish between g^+ and g^- conformers and that for reasons given there, one may indeed exclude any contribution to $J_{H3'-P3'}$ from the t conformer.[2] Computation of the H3′–C3′–O3′–P dihedral angle from the Karplus relation $^3J_{HP} = 18.1\cos^2\theta - 4.8\cos\theta$ using the observed $J_{H3'-P3'}$ values (Table II) yields two ranges ±34–38° and ±122–125°. The range ±122–125° is sterically forbidden and can be excluded. The range ±34–38° could be either in the g^+ ($\phi_1' \sim 306°$) or in the g^- ($\phi_1' \sim 234°$) domains. Below we attempt to distinguish between several possibilities, i.e., pure g^+, pure g^- or an equilibrium of g^+ and g^-.

(1) The 4′ hydrogen of the Xp- fragment shows the least change (maximum variation 0.05 ppm, Table I) in the entire collection of dinucleoside monophosphates examined. One would expect this chemical shift to show sensitivity to the orientation of the 3′ phosphate group because it is near the phosphate in g^+ and away from it in g^- orientations and, to a lesser extent, to the adjacent exocyclic linkage and the base of Xp-. The lack of significant variability in shielding environment about H4′ implies a similar orientation for the C3′–O3′ bond throughout the entire series.

(2) The dimerization shift data for the H4′ hydrogen (Table IV) reveal that, in the case of the dipurines, the 4′ hydrogen undergoes a shielding of 0.11 to 0.17 ppm as a result of dimerization.[3] This is best rationalized on the ground that, in purine 3′ monophosphates, the phosphate group occupies both g^- and g^+ domains and, during dimerization, the equilibrium shifts toward g^- domain. In fact we have presented $J_{H2'-P}$ four-bond coupling evidence that in 3′-AMP the phosphate group can indeed occupy g^+ domains (Lee et al., 1975).

(3) Examination of the dimerization data (Table III) for the pyrimidine series shows that the H4′ chemical shift is relatively insensitive to dimerization. This observation along with the fact that the 4′ hydrogen has the same chemical shift in the purine and pyrimidine series can be explained on the basis that, in the pyrimidine 3′ mononucleotides and dinucleoside monophosphates, the phosphate group favors a g^- domain and only a small change occurs on dimerization.

It is worthwhile to explore the factors which determine the orientation of the 3′-phosphate group. The computed percentage populations of ribose conformers in Table V show that, in the purine mononucleotides 3′-AMP and 3′-GMP, the ribose has a 70% preference for the ^{2}E conformer (i.e., 30% for ^{3}E) whereas, in the purine dimers and pyrimidine monomers and dimers, the percentage ^{3}E population lies between 50 and 75%. These observations viewed in the light of the previous discussion tend to suggest that a g^- orientation for the 3′ phosphate group is preferred in the ^{3}E pucker and that ^{2}E pucker favors g^+. Examination of molecular models shows that in the ^{2}E ribose conformation a g^- orientation may cause repulsion between the 2′OH group and the 3′-phosphate group. Similarly in an ^{3}E pucker there is increased repulsion between the phosphate group and 5′OH in a g^+ orientation.

From the above discussion it is reasonable to state that *the orientation of the 3′-phosphate group is coupled to the ribose pucker* and it is likely that the ribose 3′-phosphate exists in 3′ mononucleotides and in the dimers as an equilibrium system.

$$^3Eg^+ \rightleftharpoons {}^3Eg^- \rightleftharpoons {}^2Eg^+ \rightleftharpoons {}^2Eg^-$$

The above concept of coupling the ribose ring conformation and the orientation of the 3′-phosphate group suggests that the populations of g^- and g^+ are roughly similar to the populations

[2] Recent ^{13}C NMR results indicate the presence of a significant amount of t conformer about the C3′–O3′ bond (Alderfer and Ts'o, 1976); however, as has been previously pointed out, there is a discrepancy between ^{13}C and ^{1}H NMR results (Davies and Danyluk, 1975). We have based our conclusion, i.e., absence of t, on crystal structure data and theoretical calculations.

[3] A contribution to the upfield shift for H4′ could occur from the adjacent purine base in a left-handed loop structure (vide infra). However, examination of molecular models shows that this may not be significant.

of ^3E and ^2E conformers, respectively. It has been reported in recent ^{13}C NMR studies (Alderfer and Ts'o, 1976) that the g^+ rotamer population in 3′-UMP increases from 5% at pH 8.4 to 25% at pH 4.1 at the expense of the g^- population, i.e., g^- decreases from 48 to 25%. Furthermore, we have observed from ^1H NMR studies of 3′-UMP (Ezra et al., 1975) that the ^3E ring pucker decreases from 53 to 48% within the same pH range. These trends are in agreement with the model described above of a coupling between the ring pucker and conformation about the C3′–O3′ bond.

Elevation of temperature in ApG causes a depopulation of ^3E, gg and g′g′ conformers (Table V). The situation is very much similar to that reported for ApA (Evans et al., 1975). However, it should be noted that ApA and ApG conformations do not respond to temperature in identical ways. Thus in ApA the ribose rings of the 3′ and 5′ residues show roughly the same sensitivity to temperature change whereas in ApG the 3′ nucleotide is more sensitive than the 5′. In addition the g′g′ population in ApG suffers a modest loss of 10% as a result of elevation of temperature whereas, in the case of ApA, the reduction is a substantial 17%. These differences are an indication of some of the specific and subtle variations that exist in the intramolecular conformations of the various purine dinucleoside monophosphates.

The high temperature data for UpC and UpU (Table V) indicate that elevation of temperature cases a depopulation of ^3E, gg and g′g′ populations in UpC but no significant change in conformational populations in UpU. The data in Table V further reveal that the dihedral angle θH3′–P is not appreciably perturbed by temperature in accord with the dimerization shift data (vide supra). This observation does not necessarily mean that the time average orientation of the C3′–O3′ is insensitive to temperature effects. It is possible that there may be a shift in the population of conformers from g^- to g^+ domains (or vice versa), but this will not be reflected in the magnitude of $J_{H3'-P3'}$, provided the vicinal angles are the same in g^- and g^+. In UpC the H4′ chemical shift is the same at both 20 and 80 °C indicating that temperature has not affected the orientation of 3′-phosphate group. On the other hand in ApA and ApG, increasing the temperature causes a small but detectable shift of H4′ to lower fields. This can be rationalized if the 3′-phosphate group in ApA and ApG undergoes a small increase in g^+ population with increasing temperature. Such a trend would be in keeping with our earlier thesis that ribose conformation and the orientation of the 3′-phosphate groups are strongly interrelated.

Relative Orientation between Base and Ribose Groups. In NMR studies of the stereochemistry of dinucleoside monophosphates one of the most difficult tasks is the determination of the orientation of the base with respect to the ribose. The information derived from such methods as Mn^{2+} ion binding (Chan and Nelson, 1969) and pH effects (Schweizer et al., 1968; Danyluk and Hruska, 1968) conflicts with that derived from nuclear Overhauser studies with respect to preferential syn, anti orientations (Noggle and Schirmer, 1971; Schirmer et al., 1970, 1972; Hart and Davis, 1971). However, several methods based on zig–zag coupling (Hruska, 1971; Evans and Sarma, 1975), ^{13}C–^1H couplings (Lemieux et al., 1972; Schweizer and Kreishman, 1973), lanthanide-induced pseudocontact shift (Birdsall et al., 1975), perturbation of ribose chemical shifts by the base (Schweizer and Robins, 1973; Sarma et al., 1974; Davies and Danyluk, 1974), and perturbation of the base proton chemical shifts by ribose rings (Evans and Sarma, 1974b) indicate that the qualitative conclusion about the sugar–base torsional preference in nucleic acid components reached by pH effects and Mn^{2+} ion binding studies are essentially true; i.e., in all naturally occurring nucleic acid components in aqueous solution, the base–ribose ring exists as an equilibrium system of syn and anti conformers with preference for the anti orientation. Such a conclusion has also received support from extensive molecular orbital calculations by Berthod and Pullman (1971a,b, 1973a,b), as well as by the semiempirical, potential-energy calculations of Yathindra and Sundaralingam (1973a–c, 1974a,b) and crystal studies of nucleic acid components (Sundaralingam, 1973).

One may obtain a qualitative indication of the trend in glycosidic torsion angle in the present series of molecules from the 1′, 2′, 3′, 5′, and 5″ ribose proton chemical shifts as has been attempted for ApA (Kondo and Danyluk, 1976). For purine–purines the 1′, 2′, and 3′ ribose proton shifts are influenced by a combination of base-stacking effects and χ_{CN} changes, and the individual contributions cannot be sorted out in a simple way. On the other hand, for pyrimidine–pyrimidine dimers where base-stacking effects are manifested to a lesser degree in ribose proton shifts, the dimerization-induced upfield shifts for H1′ (Table III) indicate a decrease of χ_{CN} on dimerization. This trend is further confirmed by temperature measurements which show a deshielding of H1′ and shielding of H6 with increase in temperature (Table I), both of which are consistent with an increase in χ_{CN}. These results are in line with the earlier work of Prestegard and Chan (1969) on CpC.[4] The origin of the H1′ and H6 shift changes arises in each instance from changes in locations of these protons relative to the >C=O and >C=C< bonds and phosphate group, respectively, of the pyrimidine. In the absence of limiting shift data in the pure syn, anti, stacked and unstacked forms, a quantitative evaluation of χ_{CN} change is not possible. Therefore, in the present work no attempt was made to determine χ_{CN} and it was assumed that it prefers the anti domain.

Base–Base Orientation and Overall Intramolecular Conformation. A crucial component of the overall molecular geometry of dinucleoside monophosphates is the relative orientation between the bases; this orientation in turn is closely linked to the magnitude of the angles ω and ω' about the phosphodiester backbone (Figure 8). The importance arises because the nucleotide moieties could maintain their preferred conformation about the sugar–base bond, the ribose ring, and the C3′–O3′, O5′–C5′, C4′–C5′ bonds while the dimer is still capable of existing in a variety of linear and stacked conformations depending upon the magnitudes of ω and ω' (Sundaralingam, 1973; Kim et al., 1973).

No crystal structures have been reported for the seven dinucleoside monophosphates studied in the present work so that one does not have a convenient starting point for discussing the overall conformation in terms of NMR data. However, it is reasonable to state that, in aqueous solution at biological pH and temperature, these molecules exist as equilibrium mixtures of extended (unstacked) and stacked conformers and that elevation of temperature causes an increase in the population of the extended form at the expense of the stacked conformers. In Figure 8 are depicted perspective drawings of two stacked forms and an extended arrangement derived from possible dinucleoside monophosphate structures proposed by Kim et al., 1973. In all three forms the bases are anti; the ribose ring and C4′–C5′ and C3′–O3′ bonds of the 3′-nucleotidyl unit are respectively ^3E, gg, and g^-; the ribose ring, and C4′–C5′ and

[4] For CpC the base and H1′ protons also show contributions from base stacking interactions.

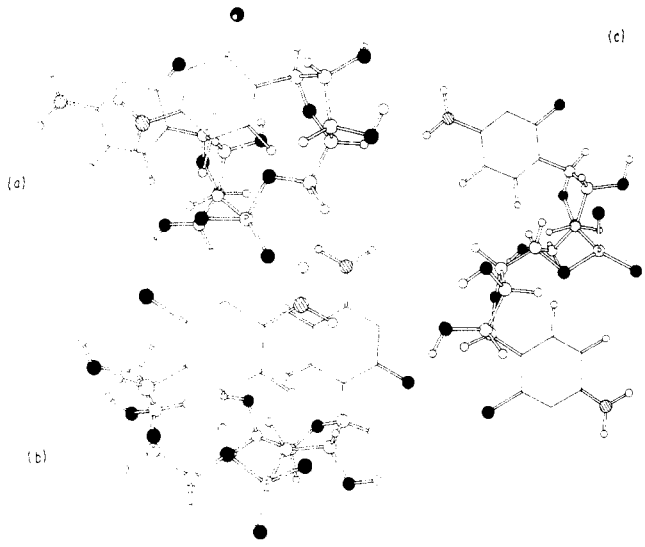

FIGURE 8: Perspective drawings of three possible intramolecular conformations for CpC. In all three projections the 5′-nucleotidyl unit has the following geometry: sugar–base torsion = anti; ribose = ^{3}E; C4′–C5′ = gg; C5′–O5′ = g′g′. The 3′-nucleotidyl unit has the following geometric features: sugar–base torsion = anti; ribose = ^{3}E; C3′–O3′ = g$^-$; C4′–C5′ = gg. The differences among the three are in the magnitude of ω_1 and ω_1' about O5′–P and O3′–P bonds. Thus Figure 8a is a right helical stack, ω_1 = 285° and ω_1' = 280°; Figure 8b is the loop stack, ω_1 = 80° and ω_1' = 90°. Figure 8c is an extended form, ω_1 = 270°, ω_1' ≈ 90°. In all three forms the bases could occupy parallel planes. In the right helical stack (a) the sugar oxygens in both the ribose moieties are oriented in the same direction but in the loop stack (b) they orient in opposite directions. It should be noted that the loop stack cannot form an ordered left helical structure beyond the dimer level.

C5′–O5′ parts of the 5′-nucleotidyl units are ^{3}E, gg, and g′g′, respectively. The *only* differences in the three conformations are in the magnitudes of ω_1 and ω_1' about the O5′–P and P–O3′ bonds. In Figure 8 these have values of 285 and 280° for the regular right helical stack (Figure 8a), 80 and 80° for the loop stack (Figure 8b), and 270 and 90° for the linear form (Figure 8c). These perspectives illustrate dramatically how a simple torsional variation about O5′–P and O3′–P *alone* can cause impressive changes in the shape of polynucleotides.

In earlier work attempts were made to establish base–base orientations and the overall conformation from shift data for base and anomeric protons (Chan and Nelson, 1969; Ts'o et al., 1969). In the absence of additional NMR data, this approach yielded a useful qualitative picture of the overall conformation, but has limitations for quantitative determinations (vide infra).

Without attempting to distinguish between the two stacked forms, one may obtain quantitative information about the population of stacked and nonstacked forms from the ribose coupling constants. This is possible because the ribose coupling constants are highly sensitive to stacking interactions (Hruska and Danyluk, 1968; Chan and Nelson, 1969; Altona et al., 1974; Altona, 1975). It has also been shown recently that for 5′-AMP, over a concentration range of 0.005 to 1.0 M, the value of $J_{1'2'}$ decreases, $J_{3'4'}$ increases, and the sum $J_{1'2'} + J_{3'4'}$ remains constant (Evans and Sarma, 1974a). In a preceding section it was shown that the conformational stability of the C4′–C5′ and C5′–O5′ bonding network is such that it does not influence the ribose-ring conformation; therefore the observed sensitivity of the ribose ring conformational equilibrium to stacking must reflect essentially base–base interactions mediated through χ_{CN} changes.

Assuming a pure ^{3}E conformation for the ribose ring in the fully stacked dimer, a maximum value of 9.5 Hz is expected for $J_{3'4'}$. The value for $J_{3'4'}$ in the monomeric units at low concentration can be taken as that expected for a nonstacked state.[5] Using these limits, the percentage populations of stacked conformers were computed and these are given in Table V. Inspection of the data,[6] shows that, in the case of the purine dinucleoside monophosphates, ApA displays a stacking population of close to 40%, while ApG and GpA have values of 25–30%. The dipyrimidines, CpC and CpU, show a higher stacked population (≃35%) than UpC (≃20%). The remarkable observation is that among all the dinucleoside monophosphates examined UpU shows an overwhelming preference for the extended conformers (i.e., over 90%). The data in Table V also show that, as expected, an elevation of temperature causes a depopulation of the stacked forms.

Another index of the stacking interaction is provided by the chemical shifts of the 5′5″ protons of the 5′-nucleotidyl unit. The Δ5′5″ (δ5′–δ5″) values in Table I clearly show that elevation of temperatures causes significant reductions in the magnitude of Δ5′5″ in ApA, ApG, UpC, and UpU. Thus in ApA at 20 °C the difference in chemical shifts between H5′ and H5″ is 0.194 ppm. This value drops to 0.099 ppm at 72 °C. In ApG 5′5″ decreases from 0.137 ppm to 0.078 ppm over the range 20 to 80 °C. Similarly in UpC, the value of Δ5′5″ undergoes a diminution of 0.075 ppm as the temperature is elevated. These observed changes in Δ5′5″ closely parallel the changes in ^{2}E ⇄ ^{3}E equilibrium and in base proton chemical shifts. Because base ring current effects are also expected to contribute toward the magnitudes of δ5′ and δ5″, the use of these shifts to monitor stacking interactions should be limited to the same class of compounds, i.e., pu–pu, py–py, etc. Thus a comparison of the 5′5″ data (Table I) for ApA, ApG, and GpA reveals that the population of stacked conformers is greater in ApA compared with ApG and GpA, as has been demonstrated earlier from the ribose J data. The Δ5′5″ data for the pyrimidine dimers UpU, CpC, UpC, and CpU unmistakably show that the extent of stacking interaction in UpU is much less compared with the remaining dipyrimidines as has been concluded from the ribose coupling constants. The dimerization Δ data for H5′ and H5″ in Table III also lead to the same conclusions.

From the preceding discussion a generalized model emerges of the stacked and extended dimer forms and the ratios of their populations. It would be desirable to obtain a more quantitative picture of the relative orientation of the bases in the stack. An approach which has been fairly extensively used relies on computed isoshielding lines of purines and pyrimidines to project the geometry of a molecular stack (Ts'o et al., 1969; Sarma and Mynott, 1973b; Evans and Sarma, 1974c; Lee and Sarma, 1975; Kondo and Danyluk, 1976). This approach has obvious limitations (Evans and Sarma, 1974c) and its usefulness for defining specific geometries for dinucleoside monophosphates must be viewed with caution. For example, H3′ of the 3′ residues in the regular stack (Figure 8a) lies with cy-

[5] A similar approach for the analysis of stacking in ApU and its methyl derivatives has been recently reported (Altona, 1975).

[6] The presently reported populations for the stacked forms are in serious conflict with those reported earlier (Bangerter and Chan, 1969; Chan and Nelson, 1969) based on the effect of temperature on chemical shifts. These authors have discussed in extenso the limitations in their calculations. It may further be pointed out that computed populations for the stacked forms vary considerably and depend upon the particular proton selected to carry out the calculations.

lindrical coordinates[7] of $Z = 3.25$ Å and $P = 3.0$ Å from the 5′ base, but the same proton is also buried in an oxygen pool: 3.75 Å from $O(5')_{5'}$, 4 Å from *pro-S* phosphate oxygen, and 2.25 Å from the *pro-R* phosphate oxygen. One therefore cannot neglect the effect of these oxygens on the chemical shift of H3′ in any calculations of stacking effects. A very similar situation exists for the H2′ of the 3′-nucleotidyl units. In view of these factors we feel that a stacked model which may emerge from ring-current considerations alone may be of doubtful validity.

However, one may use the dimerization shift data in Table III to arrive at qualitative conclusions with respect to the preference of the dimers for one of the two possible stacked forms (Figures 8a and 8b). The data obviously indicate that significant interaction takes place between the 3′- and 5′-nucleotidyl residues in dipurines and several of the dipyrimidines. A particularly pertinent point is the impact of dimerization on the chemical shift of the 5′ proton of 5′-nucleotidyl units. In all the molecules examined this proton undergoes a *downfield* shift of 0.116 ppm to 0.245 ppm and this is clearly the reflection of rotamer rearrangement about P–O3′ and P–O5′ bonds upon dimerization. Examination of the perspective structures (Figures 8a and 8b) as well as molecular models reveals that in both stacked forms H5′ of the 5′-nucleotidyl unit has a very similar oxygen environment with respect to its relation to O3′ of the 3′-nucleotidyl unit as well as the two *pro-R* and *pro-S* oxygens of the phosphate group (Kondo and Danyluk, 1976). *However, a clear difference exists in the position of H5′ of the 5′ nucleotide in the two stacked forms with respect to its relation to O2′ of the 3′ nucleotide.* The measured distance between O2′ of Xp- and H5′ of -pX is 2.5 Å in the regular stack (Figure 8a) and 6 Å in the loop stack (Figure 8b). Obviously one would expect H5′ of the 5′ unit to be considerably deshielded by O2′, should the molecules show preference for the regular stack. The dimerization data in Table III indicate a pronounced deshielding of this H5′, suggesting that a regular right helical stack is preferred over a loop stack in dipurines and dipyrimidines. This does not rule out a sizeable contribution from a loop stack conformation, and in fact a detailed analysis of base and ribose proton shift behavior for ApA strongly supports the coexistence of right helical, loop, and extended forms for this dimer in solution (Kondo and Danyluk, 1976). A similar equilibrium is likely for other pu–pu dimers.

It is informative to explore the possible reasons for the existence of UpU in an extended conformation and the tendency of the remaining dimers to populate to a greater extent in stacked conformations. Insofar as the O5′–P and O3′–P bonds are flexible, the dinucleotides can easily adopt a stacked mode, provided intramolecular interaction is favored between the bases. The origin of the interaction presumably resides in the nature of the π-ring systems in the bases and the extent of overlap of the π system. Thus for dipurines and dipyrimidine cytosine bases, overlap of the π-ring system will lead to aggregation of the bases in aqueous solution with accompanying changes in the overall conformational properties of these molecules. It is therefore possible to propose the following model for the *coupled* conformational changes due to stacking: a modest decrease in χ with an accompanying increase in ^3E populations, the latter in turn shifting the 3′ phosphate group from g^+ to g^- domains. In short, dimerization of mononucleotides by 3′–5′ phosphodiester formation causes a series of conformational events in a majority of cases and the initiator of these changes is the stacking interaction.

[7] Z and P are the vertical and in-plane cylindrical coordinates, respectively.

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Properties of tRNA Species Modified in the 3′-Terminal Ribose Moiety in an Eukaryotic Ribosomal System†

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ABSTRACT: Phe-tRNA^{Phe} species modified on the 3′-terminal ribose residue were investigated for their ability to participate in individual steps of the elongation cycle using eukaryotic ribosomes from reticulocytes. None of the Phe-tRNAs used, namely Phe-tRNA^{Phe}-C-C-3′dA, Phe-tRNA^{Phe}-C-C-3′-NH_2A, and Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$, can function in the overall process. All modified Phe-tRNA^{Phe} species can be bound nonenzymatically to ribosomes. Phe-tRNA^{Phe}-C-C-3′NH_2A exhibits exceptionally high binding at low Mg^{2+} concentration compared with Phe-tRNA^{Phe}-C-C-A binding. Ac-Phe-tRNA^{Phe} species prepared from the three modified tRNAs, when bound to the donor site, were devoid of donor activity. The enzymatic binding of both Phe-tRNA^{Phe}-C-C-3′dA and Phe-tRNA^{Phe}-C-C-3′NH_2A is less efficient than that of Phe-tRNA^{Phe}-C-C-A but these Phe-tRNA^{Phe} species have acceptor activity. Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$ is not a substrate for the EF-I promoted binding reaction and has no acceptor activity. The nonaminoacylated species, tRNA^{Phe}-C-C-A, tRNA^{Phe}-C-C-3′dA, and tRNA^{Phe}-C-C-3′NH_2A, bind to the ribosome to a larger extent than the corresponding aminoacylated tRNAs, both in the presence and in the absence of poly(U). Peptidyl-tRNA^{Phe}-C-C-3′dA bound to the donor site cannot activate the acceptor site for EF-I promoted binding of Phe-tRNA^{Phe} as does peptidyl-tRNA^{Phe}-C-C-A. Further, it was observed that a correct codon–anticodon interaction influences the recognition of the 3′ terminus of tRNA. Specificity of eukaryotic ribosomes for the 2′- and/or 3′-aminoacylated tRNA species is discussed and compared with the properties of *Escherichia coli* system.

The binding of tRNA[1] to the ribosome is a multiple-site interaction in which at least three partial sequences of the tRNA seem to be involved: the anticodon, the GpTpΨpC sequence, and the 3′-terminal -C-C-A sequence. It is generally accepted that ribosomes active in protein synthesis bind two molecules of tRNA (Leder, 1973). The 3′-terminal nucleotides of both tRNAs interact with the peptidyl transferase on the large ribosomal subunit. This interaction has been studied in detail, mostly with ribosomes from prokaryotes and it was found that the specificity of the acceptor site (A-site) interaction is different from that of the donor site (P-site) interaction in several respects. A complete -C-C-A terminus bearing the peptidyl residue is required for the binding at the P-site (Monro et al., 1968), but a terminal adenosine seems to be sufficient to replace the aminoacyl-tRNA on the A-site.

tRNA with a modified 3′-terminal ribose moiety can be prepared either by a direct chemical treatment with periodate which reacts in a specific way with the 3′-terminal ribose of the tRNA (Cramer et al., 1968), or by incorporating a modified AMP into tRNA-C-C with tRNA nucleotidyl transferase (Sprinzl et al., 1973b). The modified tRNAs used in this work were tRNA^{Phe}-C-C-3′dA, tRNA^{Phe}-C-C-3′NH_2A, tRNA^{Phe}-C-C-$A_{oxi-red}$, tRNA^{Phe}-C-C-2′dA, and tRNA^{Phe}-C-C (Figure 1). The first three of these tRNAs can be aminoacylated (Sprinzl and Cramer, 1973; Fraser and Rich, 1973) yielding Phe-tRNA^{Phe} species on which the migration of the amino acid residue between the 2′ and the 3′ position of the terminal adenosine (Griffin et al., 1966) cannot take place. These nonisomerizable aminoacyl-tRNAs are therefore suitable for the investigation of the specificity of the tRNA–ribosome interaction with respect to the position of the aminoacyl residue bound to tRNA. The activities of these Phe-tRNA^{Phe} species in a ribosomal system derived from *Escherichia coli* have been studied in several laboratories (Ofengand and Chen, 1972; Chinali et al., 1974; Sprinzl et al., 1975; Hecht et al., 1974; Fraser and Rich, 1973). Some disagreement exists, however, concerning the acceptor activities of the above mentioned, nonisomerizable tRNA species. Chinali et al. (1974) reported that Phe-tRNA^{Phe}-C-C-3′dA and Phe-tRNA^{Phe}-C-C-$A_{oxi-red}$, having the aminoacyl residue on the 2′ position, possess some acceptor activity, while Hecht et al. (1974) could not detect acceptor activity for Phe-tRNA^{Phe}-C-C-3′dA and Hussain and Ofengand (1973) could not observe acceptor activity for enzymatically aminoacylated adenosine$_{oxi-red}$. On the other hand an agreement exists that none

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[1] Abbreviations used are: EF-I, EF-II, elongation factors; tRNA^{Phe}-C-C-A, native phenylalanine transfer ribonucleic acid; Phe-tRNA^{Phe}, phenylalanyl-tRNA^{Phe}; Ac-Phe-tRNA^{Phe}, *N*-acetylphenylalanyl-tRNA^{Phe}; tRNA^{Phe}-C-C, tRNA^{Phe} lacking the 3′-terminal AMP; tRNA^{Phe}-C-C-2′dA or tRNA^{Phe}-C-C-3′dA, tRNA^{Phe} lacking the 2′- or 3′-hydroxyl group of the terminal ribose, prepared by enzymatic incorporation of 2′-deoxyadenosine or 3′-deoxyadenosine 5′-phosphate into tRNA^{Phe}-C-C; tRNA^{Phe}-C-C-3′NH_2A, tRNA^{Phe} with the terminal adenosine replaced by 3′-deoxy-3′-aminoadenosine; tRNA^{Phe}-C-C-$A_{oxi-red}$, tRNA^{Phe} lacking the C(2′)–C(3′) bond of the terminal ribose, prepared by periodate oxidation and subsequent borohydride reduction of tRNA^{Phe}-C-C-A; poly(U), poly(uridylic acid); Tris, tris(hydroxymethyl)aminomethane.