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Studies of the Conformation of Modified Dinucleoside Phosphates Containing 1,N^6-Ethenoadenosine and 2′-O-Methylcytidine by 360-MHz ^{1}H Nuclear Magnetic Resonance Spectroscopy. Investigation of the Solution Conformations of Dinucleoside Phosphates†

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ABSTRACT: Seven dinucleoside monophosphates containing ϵA (1,N^6-ethenoadenosine) and 2′-O-methylcytidine were studied by 360-MHz proton magnetic resonance and compared with unmodified dimers and component monomers at 4, 20, 45, and 75 °C. These studies show that the dimers exhibit preference for the gg and g′g′ conformations for the C-4′-C-5′ and C-5′-O-5′ bonds, respectively, and that dimerization induces an increase of the population and inflexibility of the 3′-endo conformation for the ribose ring. Three stacked (or stable) conformations for dimers, I, II, and III, in equilibrium with an unstacked (or open) form in solution, are suggested by dimerization shifts of ribose protons. Conformation I exhibits anti, gg, 3′-endo, ϕ' = 203 to ~211°, ω' = 300°, ω = 290°, g′g′, gg, 3′-endo, and anti conformation from the 5′ end to the 3′ end of the dimer. Conformation II shows anti, gg, 3′-endo, ϕ' = 203–211°, ω' = 30°, ω = 100°, g′g′, gg, 3′-endo, and anti conformation. Conformation III is anti, gg, 2′-endo, ϕ' = 260°, ω' = 50°, ω = 220°, g′g′, gg, 3′-endo, and anti ($\chi \simeq 100°$) conformation. The dimers, PupPu and PupPy, prefer conformations I and II, while PypPu and PypPy prefer conformation II. Introduction of ϵA for the base of -pN induces an increase of conformations II and III, while the ϵA substitution for the Np- residue induces an increase of conformation I. 2′-O-methylation of the Cp- residue of CpC decreases conformation I and increases conformation II. Based on the stable solution conformations of these dimers, a possible conformation of the anticodon loop is proposed, which is an alternative to the one observed in the crystal of tRNA^{Phe}.

The conformations of dinucleoside monophosphates (or dimers) have been investigated in the past decade by a number of different methods, e.g., theoretical calculations (Olson, 1975a,b; Broyde et al., 1974; Perahia et al., 1974; Davis, 1967), optical methods such as UV,[1] ORD, CD, and Raman spectroscopy (Warshaw and Tinoco, 1965, 1966; Davis and Tinoco, 1968; Brahms et al., 1967; Warshaw and Cantor, 1070; Kiser, 1975), and x-ray diffraction (Rubin et al., 1972; Sussman et al., 1972; Kim et al., 1973; Day et al., 1973; Hingerty et al., 1975; Rosenberg et al., 1973; Suck et al., 1973). In addition, nuclear magnetic resonance studies have provided information about molecular conformations and their dynamic nature. The first NMR data on dinucleosides were reported by Hruska and Danyluk (1968). They used the temperature dependency of the coupling constant, $J_{1',2'}$, to deduce that the ribose groups in ApA, GpA, and ApA 2′,3′-cyclic phosphate were in 3′-endo conformations at low temperature and in 2′-endo conformations at high temperature. NMR studies of the base protons and 1′-ribose proton by Ts'o et al. (1969), Chan and Nelson (1969), and Bangerter and Chan (1969) suggested a right-handed base-base stacking conformation for dinucleoside monophosphates. Smith et al. (1973) and Alderfer and Ts'o (1977) attempted to determine the phosphate–ribose conformation of dinucleoside phosphates, using vicinal-coupling constants between ^{13}C and ^{31}P. Altona et al. (1974) first published a complete NMR data set for the dinucleoside N^6-MeApU. A year later, the conformations of dApdA and ApA were analyzed by Evans et al. (1975) and Lee et al. (1975b), based on the NMR data obtained by line-shape simulation. Meanwhile, another group (Kondo and Danyluk, 1976) also worked on ApA using selective dueteration. Finally, the conformations of 15 dinucleoside phosphates (all common dimers except GpG) at 10 to ~30 mM, 20 °C, were studied by NMR using selective deuteration, as well as computer line-shape simulations (Lee et al., 1976; Ezra et al., 1977). Similar

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[1] Abbreviations used are: UV, ultraviolet; ORD, optical rotatory dispersion; CD, circular dichroism; ϵA, 1,N^6-ethenoadenosine; NMR, nuclear magnetic resonance; EDTA, ethylenediaminetetraacetic acid.

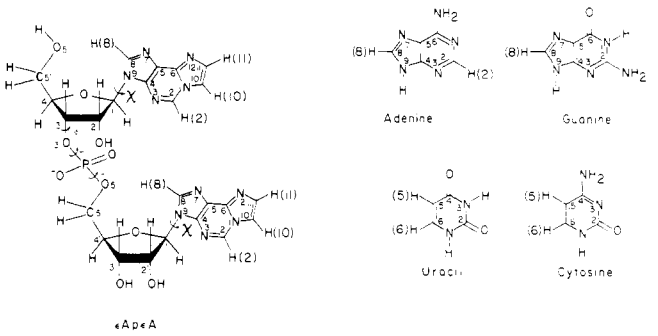


FIGURE 1: The structure and numbering system of ϵApϵA and the unmodified bases.

studies on 2′-*O*-MeApA (Singh et al., 1976) and 2′-*O*-methyl CpC (Cheng and Sarma, 1977) were carried out recently. Detailed conformations of the dimers, such as glycosidic bond (χ angle), ribose ring, and C-4′–C-5′, C-5′–O-5′, and C-3′–O-3′ bonds, were discussed in depth.

ϵA (1,N^6-ethenoadenosine) can be considered as a synthetic analogue of the Y base in the anitcodon loop of yeast $tRNA^{Phe}$. 2′-*O*-Methylcytidine is occasionally found at the 5′ side of the anticodon loop. Such modification of the nucleotide residues in the anticodon loop of tRNA may play an important role in the stabilization of the loop conformation. In this paper, we present conformational analyses of ϵApϵA, ApϵA, ϵApG, Gpϵ A, ϵApU, Upϵ A, and 2′-*O*-MeCpC (CmpC), together with their corresponding unmodified dimers, utilizing 360-MHz ^{1}H NMR spectroscopy. We hope to better understand the function of the nucleotidyl modification of the dimers. The modified dimers also serve as an excellent probe to help determine the conformation of the dinucleoside phosphates. In this work, the bases of the modified and unmodified dimers are assumed to be anti. The ribose conformation is expressed as percent 3′-endo conformation, estimated by $10(10 - J_{1',2'})$. The conformation of the C-4′–C-5′ bond (in percent gg) is computed from $10(13 - (J_{4',5'} + J_{4',5''}))$ (Lee and Sarma, 1976a,b). The G-5′–O-5′ conformation in percent g′g′ is calculated from $100(25 - (J_{5'\text{-P}} + J_{5''\text{-P}}))/20.8$ (Lee and Sarma, 1976a,b). The dihedral angle between H-3′ P is calculated using the modified Karplus equation $^3J_{HP} = 18.1 \cos^2 \theta_{HP} - 4.8 \cos \theta_{HP}$ (Lee and Sarma, 1976a,b). The conformation of the phosphodiester linkage and the orientation of the bases are estimated from space-filling Corey–Pauling–Koltun (CPK) models using maximum base–base stacking, maximum hydrophobic interaction, and minimum steric hindrance as criteria. The existence of the three proposed structures (I, II, and III) is established by specific dimerization shifts of the ribose protons. Based on the sequence-dependent conformational properties discussed for the dinucleoside phosphates a possible conformation for the anticodon loop is proposed.

Materials and Methods

ApA, ApG, GpA, ApU, UpA, CpC, CmpC, and the 3′- or 5′-mononucleotides were purchased from Sigma Chemical Co. The ϵA-containing compounds except for Apϵ A were synthesized from regular nucleotides and chloroacetaldehyde according to the method of Tolman et al. (1974). Apϵ A was synthesized and purified according to a procedure by Follman (1967) and Watts (1977). NMR samples, with 3-trimethylsilyl-d_4 propionate as internal reference and 0.5 mM EDTA to remove any possible paramagnetic ions, were prepared in commercial (Bio-Rad) 100% D_2O at 10 mM, pH 7, for ϵApϵA, ϵApG, Gpϵ A, ϵApU, Upϵ A and 5 mM, pH 7, for Apϵ A, ApA, ApG, GpA, ApU, UpA, CpC, and CmpC. At these concentrations, the effect of intermolecular interaction is considered to be negligible (Ts'o et al., 1969; Chan and Nelson, 1969). The conditions for the mononucleotide samples are the same as those of the dimers except for the pH value. For the monomer samples, the pH is 5, at which point the ionization state of the phosphate group is minus one, or the same as that of the dimers at pH 7. 2′-*O*-Me-3′-CMP was not available. All the ^{1}H NMR spectra were recorded with 16K data points at 4, 20, 45, and 75 °C by a Brucker HXS 360-MHz spectrometer, equipped with a Nicolet 1080 computer unit (Stanford Magnetic Resonance Laboratory, Stanford University).

The structures of ϵApϵA and the unmodified bases are shown in Figure 1, along with the numbering system for the molecules. The assignment of the NMR signals is rather complicated. The base protons of the regular dimers were assigned according to Ts'o et al. (1969), Lee et al. (1976), and Ezra et al. (1977). The base protons of CmpC were assigned by comparison of their resonances with those of CpC, 3′-CMP, and 5′-CMP. In addition, the line width of the signals also gives clues in assigning these protons. For 3′-ϵAMP and 5′-ϵAMP, the assignment of the base protons follows Secrist et al. (1972), where the assignment was carried out by selective deuteration, resulting in $\delta_{H\text{-}2} > \delta_{H\text{-}8} > \delta_{H\text{-}10} > \delta_{H\text{-}11}$. For the dimers containing ϵA, the base proton resonances were assigned by comparison between these dimers, a comparison with corresponding unmodified dimers, and a comparison with the component monomers. The assignment of the base protons for ϵApϵA thus obtained enables us to arrive at a reasonable set of dimerization shifts for these protons, even though such an assignment for ϵApϵA is not as clear cut as that for the other ϵA-containing dimers. The assignment of the ribose proton signals was carried out by computer line-shape simulation using the LAOCN III program containing an inserted Lorentzian line-shape plotting routine. The assignment of the geminal 5′ and 5″ proton resonances is according to Remin and Shugar (1972) and Davies and Rabczenko (1975), but in disagreement with Son and Guschlbauer (1975). By this assignment, these geminal protons show a characteristic NMR pattern, i.e. $\delta_{H'\text{-}5} > \delta_{H\text{-}5''}$ and $J_{4',5'} < J_{4',5''}$ with H-5″ gauche to H-4′ and C-3′ in gg conformation (see C-4′–C-5′ conformation in the Discussion). The characteristic NMR data pattern of these geminal protons is always found for nucleosides and mononucleotides with both C-4′–C-5′ and glycosidic bonds rotating freely. In the case of dinucleoside phosphates, a complication arises for the data of these geminal protons because of the shifting effect of the neighboring residue. For the -pN residue of the dimer, H-5′ is shifted further downfield than H-5″ by dimerization (see Discussion, evidence for the existence of conformation I) so that this residue retains the pattern of the geminal protons. According to Remin and Shugar's assignment (1972), $J_{4',5'} < J_{4',5''}$ for the 3′-mononucleotides and this is also true for the Np- residue of the dimer, since dimerization essentially does not perturb the conformation of the C-4′–C-5′ bond in this residue (Table V). Thus, the H-5′ and H-5″ resonances of the Np- residue are assigned unambiguously. H-5′ and H-5″ in the Np- residue of the Npϵ A dimers show a "crossover" of the NMR data pattern, i.e. $\delta_{H\text{-}5'} < \delta_{H\text{-}5''}$ and $J_{4',5'} < J_{4',5''}$. This is due to the large population of conformation III (see Discussion) for these dimers, as well as the stronger ring current shift potential for ϵA than any other base. Figure 2 shows an example of the computer line-shape simulation of the ribose protons of Apϵ A and CmpC. The chemical-shift and coupling constant data of the dimers are shown in Table I which is stored in microfilm and can be requested (see paragraph at end of paper).

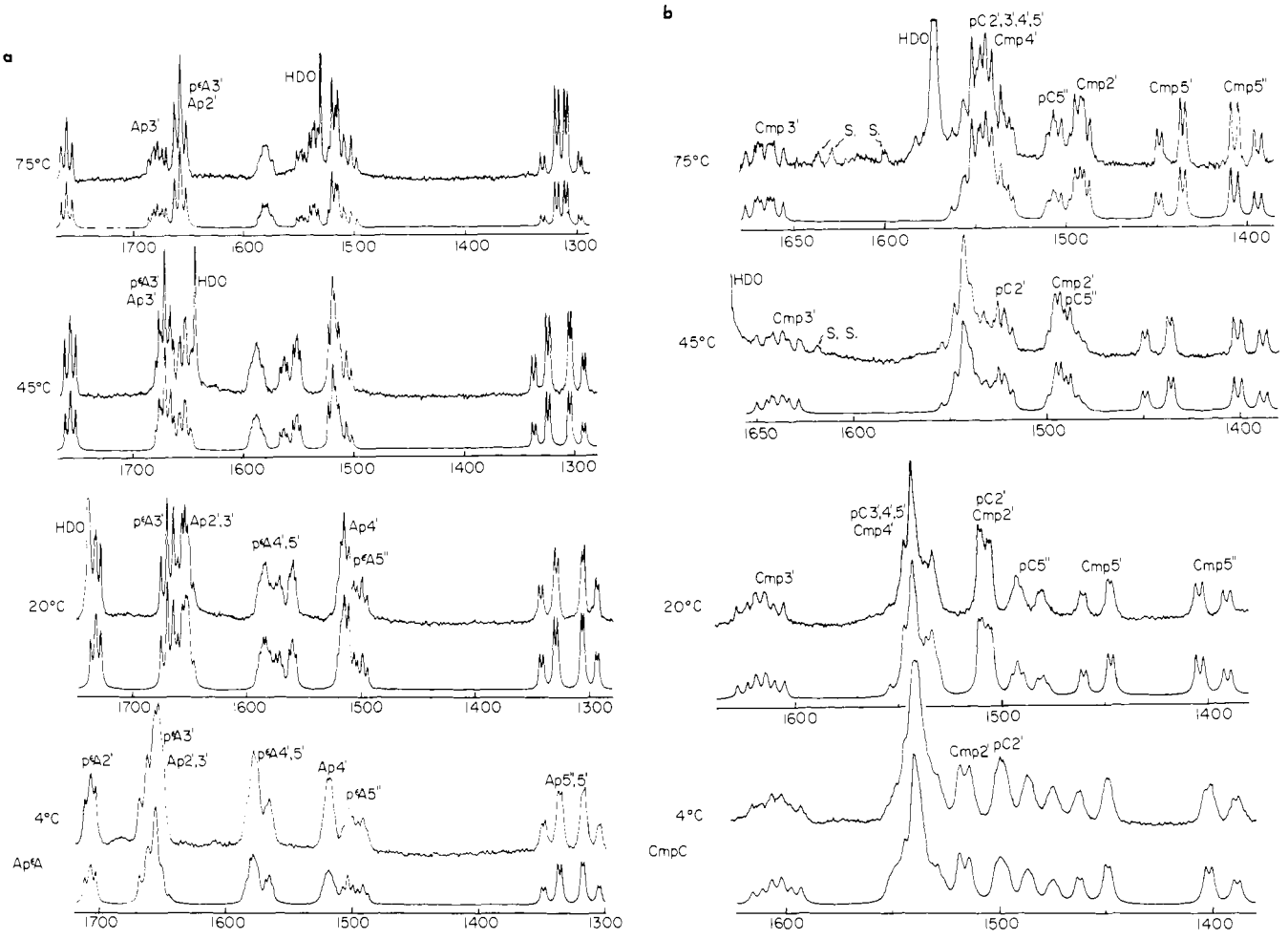


FIGURE 2: ^{1}H NMR (360-MHz) spectra of the ribose protons (not including H-1') of ApεA (a) and CmpC (b) with the simulations (underneath) at 4, 20, 45, and 75 °C. Chemical shifts (Hz) are measured downfield from the internal reference 3-trimethylsilyl-d_4 propionate.

Results

Chemical-Shift Changes. The dimerization shift is defined as the chemical-shift difference between a proton in a dimer and its component monomer, $\Delta\delta = \delta_{monomer} - \delta_{dimer}$. The causes of the dimerization shift are the ring current effect of the neighboring base, the diamagnetic effect (or anisotropy) of the electron-rich groups of the neighboring residue, the change of the local conformation, and perhaps changes in hydration and in ionic effects. Table II (in microfilm, available on request) shows the dimerization shifts of all the protons of the dimers. Parts of the data are plotted in Figures 3–6. There are three general features shown by these data. (a) Assuming the dimers are in the same conformation, the shielding potential of the bases is in the order of $\epsilon A \geq$ purine > pyrimidine. (b) Dimerization selectively shifts the proton signals in a sequence-dependent fashion, except for the H-3′ of Np- and the H-4′ of the -pN residue. (c) The dimerization shift is always reduced when the temperature increases, except for H-5′ and H-5″ of the Np- residue. The base protons and H-1′ show large upfield shifts if the shielding base is not uracil (Figure 7). H-4′, H-5′, and H-5″ of the Np- residue and the two H-2′ protons experience large upfield shifts if the shielding base is not a pyrimidine. H-3′ and H-4′ of the -pN residue exhibit only small chemical-shift changes. In all cases, H-3′ of the Np- residue is shifted upfield, while H-5′ and H-5″ of the -pN are shifted downfield. A similar trend of dimerization shifts had been reported for regular dimers earlier (Lee et al., 1976; Ezra et al., 1977).

Coupling Constants. The coupling constant of pyrimidines, $J_{5,6}$ (~8 Hz), and that of ϵA, $J_{10,11}$ (~2 Hz), are not dependent on the conformations of nucleosides and nucleotides, and are thus omitted. Table I (available in microfilm) contains the coupling constant data of the sugar–phosphate backbones of the dimers. When the temperature is increased, $J_{1',2'}$ increases, $J_{3',4'}$ decreases, and $J_{1',2'} + J_{3',4'}$ (~9.5 Hz) essentially remains unchanged. Such a tendency has been observed before (Lee et al., 1976; Ezra et al., 1977). The value of $J_{2',3'}$ for most of the dimers is independent of temperature, but that for the dimers ApA, ApG, GpεA, εApU, CpC, and CmpC is smaller (4.4–~4.9 Hz) at low temperature and becomes 5.2 Hz only at 75 °C. For monomers, however, $J_{1',2'}$, $J_{2',3'}$, and $J_{3',4'}$ are not changed significantly in the temperature range employed, 4–~75 °C. Generally, comparisons between monomers and dimers show that ($J_{1',2'}$) monomer > ($J_{1',2'}$) dimer, ($J_{2',3'}$) monomer $\geq$ ($J_{2',3'}$) dimer, ($J_{3',4'}$) monomer < ($J_{3',4'}$) dimer. $J_{3'\text{-P}}$ is only slightly sensitive to temperature, and there is little difference in this parameter between monomers and the dimers at high temperature. The four-bond long-range coupling, $J_{2'\text{-P}}$, has a value, for the Np- residue of dimers other than CpC and CmpC, as large as 0.6–~1.2 Hz. No detectable splitting from this four-bond coupling was observed for 3′-mononucleotides. The magnitude of $J_{4',5'}$, $J_{4',5''}$, $J_{5'\text{-P}}$, and $J_{5''\text{-P}}$ of dimers and monomers increases when the temperature is increased. The magnitudes of these constants for dimers are smaller than those for monomers. Another four-bond coupling, $J_{4'\text{-P}}$, is observed for the -pN residue and 5′-mononucleotides, at a magnitude

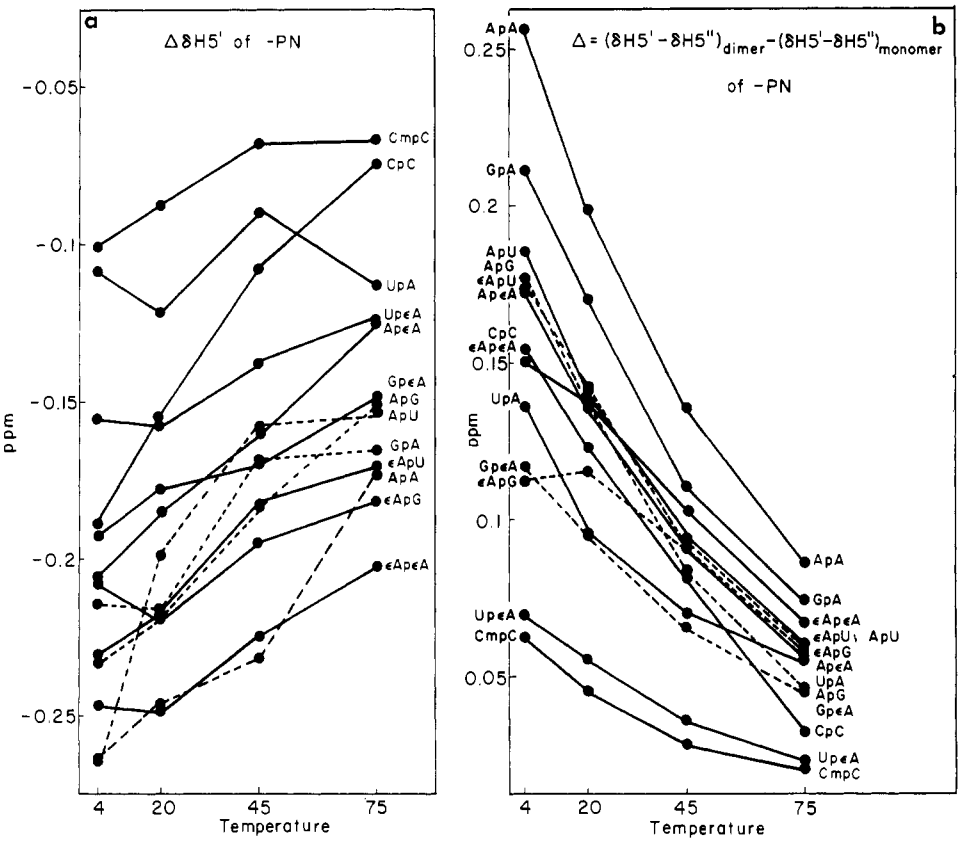


FIGURE 3: Dimerization shift, $\Delta\delta_{H\text{-}i}$ = ($\delta_{H\text{-}i}$)monomer − ($\delta_{H\text{-}i}$)dimer, of H-5′ of the -pN residue (a), and the dimerization induced differentiation of H-5′ and H-5″ of the -pN residue (b), Δ = ($\delta_{H\text{-}5'} - \delta_{H\text{-}5''}$)dimer − ($\delta_{H\text{-}5'} - \delta_{H\text{-}5''}$)monomer.

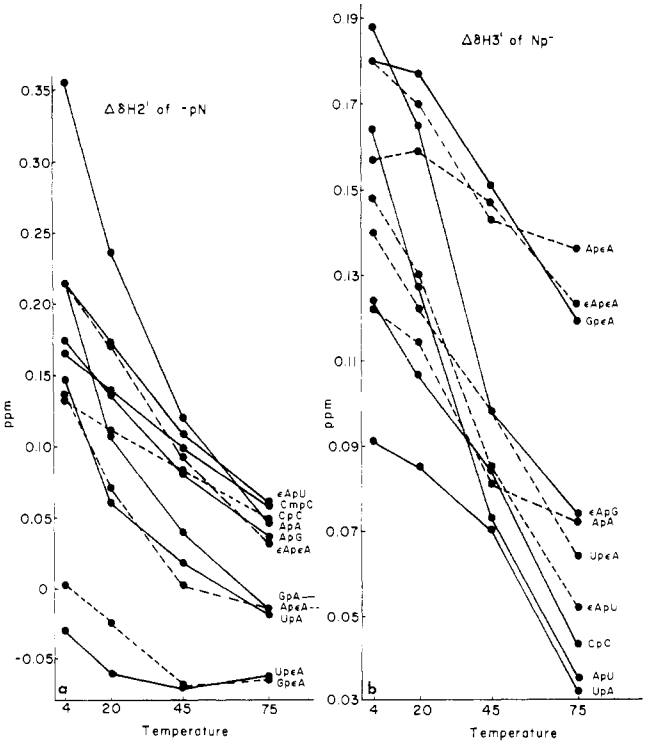
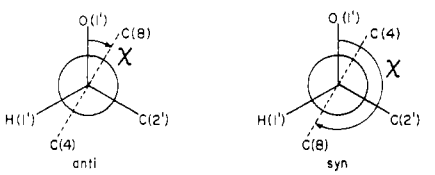


FIGURE 4: Dimerization shift of H-2′ of the -pN residue (a), and H-3′ of the Np- residue (b).

of 1.5–~2.4 Hz, which decreases when the temperature is increased. The coupling constant, $J_{5',5''}$ (−12.8 Hz for Np- and 3′-mononucleotides and −11.8 Hz for -pN and 5′-mononucleotides), is independent of temperature, and consequently is of little value in elucidating conformations in the present study.

Discussion

Conformation of the Glycosidic Bond χ Angle. It is difficult to determine the precise χ angle of the glycosidic bond in solution. However, NMR studies have shown that unmodified nucleoside and nucleotides greatly prefer the anti conformation in solution (Schweizer et al., 1968; Chan and Nelson, 1969; Hruska, 1971; Evans and Sarma, 1974a). Modification or substitution of C–H-8 of purine or C–H-6 of pyrimidine base creates an immense perturbation of the glycosidic conformation, i.e. an increase of the χ angle or a decrease of the anti



conformation (Schweizer et al., 1971; Schweizer and Kreishman, 1973; Wood et al., 1973; Sarma et al., 1974; Lee et al., 1975a). There is no such modification of the base in the dimers under investigation. Thus, it is reasonable to assume that in these dinucleoside monophosphates, the bases are oriented preferentially in the anti conformation. This is consistent with the data reported in single crystals of UpA, GpC, ApU, and $ApA^{+}pA^{+}$ (Rubin et al., 1972; Sussman et al., 1972; Day et al., 1973; Hingerty et al., 1975; Rosenberg et al., 1973; Suck et al., 1973). It has been argued that dimerization induces a decrease of the χ angle in such a way that the magnitude of χ_1 (of the Np- residue) is smaller than that of χ_2 (of the -pN residue) (Lee et al., 1976; Ezra et al., 1977; Bangerter and

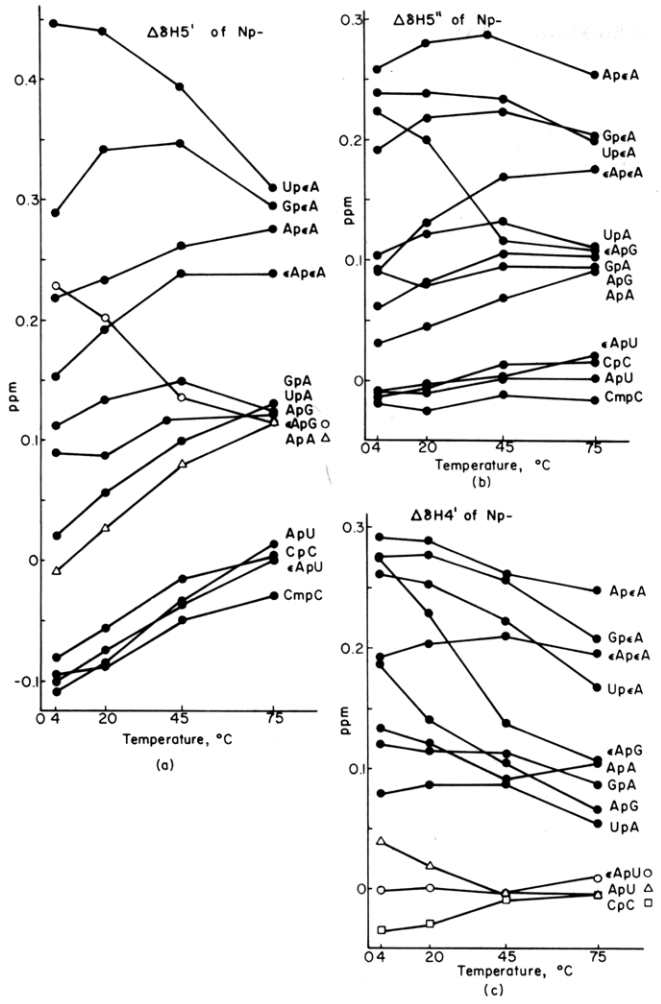


FIGURE 5: Dimerization shift of H-5′ (a), H-5″ (b), and H-4′ (c) of the Np- residue.

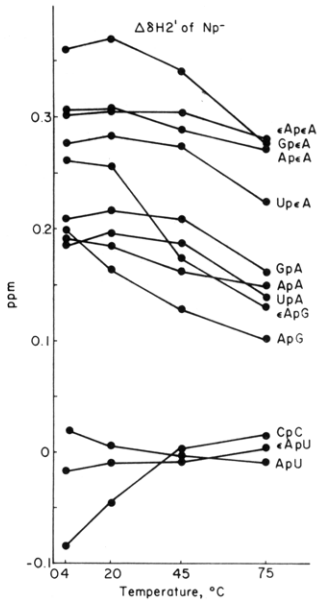


FIGURE 6: Dimerization shift of H-2′ of the Np- residue.

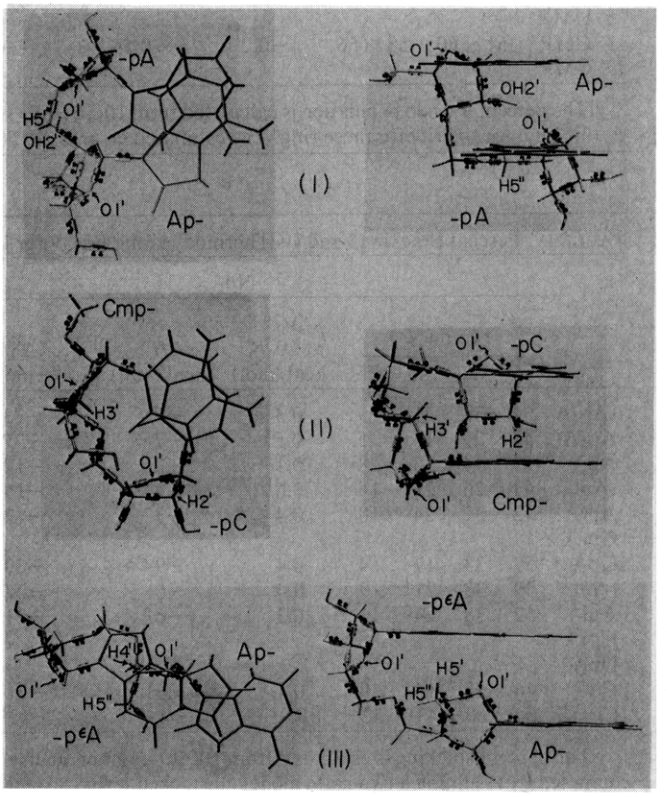
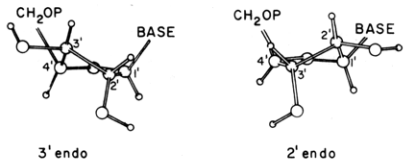


FIGURE 7: Conformations I (ApA), II (CmpC), and III (ApϵA) translated from CPK models. The pictures on the left-hand side are taken perpendicular to the bases, while those on the right-hand side are taken parallel to the bases.

Chan, 1969). This may be true; however, the chemical-shift changes of H-1′ and H-2′ can be mainly attributed to the ring current of the neighboring base, rather than to the change of the χ angle. A clear example is that considerable upfield shifts of H-1′ and H-2′ are observed when the neighboring base is ϵA or a purine, but small shifts of these protons are observed if the neighboring base is uracil.

Ribofuranose Conformation. The conformation of the ribofuranose ring is described by the equilibrium system, 3′-endo $\rightleftharpoons$ 2′-endo (Altona and Sundaralingam, 1972, 1973; Davies



and Danyluk, 1974; Evans and Sarma, 1974b). The population of the 3′-endo conformer, estimated from % 3′-endo = 10(10 − $J_{1',2'}$) (Altona and Sundralingam, 1972, 1973; Davies and Danyluk, 1974; Evans and Sarma, 1974b), is shown in Table III. It can be seen that the 3′-endo population of the dimers decreases drastically as the temperature is increased, while that of the mononucleotides remains essentially unchanged. The thermodynamic properties, computed and shown in Table III, indicate that the 3′-endo conformation is stabilized by dimerization. They also show that the dimers (except for UpA, UpϵA, and GpϵA) tend to approach the properties of the mononucleotides (in terms of 3′-endo conformation) as the temperature increases. Furthermore, x-ray crystallography has shown a 3′-endo conformation for each ribose group in GpC (Day et al., 1973; Hingerty et al., 1975), ApU (Rosenberg et al., 1973), UpA (Rubin et al., 1972; Sussman et al., 1972), and $ApAp^+A^+$ (Suck et al., 1973). Therefore, if we assume that a completely stacked dimer has pure 3′-endo conformation for both ribose rings, the fraction of 3′-endo found in the dimer can be used to estimate the amount of stacking. The fraction of 3′-endo present in each constituent mononucleotide is used as

TABLE III: Percent 3′-Endo Population[a] and Its Thermodynamic Data[b] for Dimers and Monomers.

	Np- or 3′-NMP							-pN or 5′-NMP						
	Temp (°C)				ΔG° at 20 °C	ΔH°	ΔS°	Temp (°C)				ΔG° at 20 °C	ΔH°	ΔS°
	4	20	45	75	(kcal/mol)	(kcal/mol)	(cal/(mol deg))	4	20	45	75	(kcal/mol)	(kcal/mol)	(cal/(mol deg))
ϵApϵA	73	64	55	49	−0.3	−2.8	−8	70	62	59	56	−0.3	−1.5	−4
ApϵA	66	55	47	42	−0.1	−2.6	−8	66	62	58	54	−0.3	−1.3	−4
ApA	70	65	60	56	−0.4	−1.6	−4	69	65	58	53	−0.4	−1.9	−5
ϵApG	67	57	55	52	−0.2	−1.5	−4	69	59	54	50	−0.2	−2.0	−6
ApG	63	57	53	50	−0.2	−1.4	−4	61	56	51	47	−0.1	−1.5	−5
GpϵA	58	53	43	42	−0.1	−1.9	−6	67	62	57	51	−0.3	−1.8	−5
GpA	71	60	52	46	−0.2	−2.8	−8	64	58	53	50	−0.2	−1.5	−4
ϵApU	70	61	55	49	−0.3	−2.3	−7	75	66	61	56	−0.4	−2.2	−6
ApU	69	61	53	46	−0.3	−2.6	−8	74	66	61	56	−0.4	−2.1	−6
UpϵA	50	48	47	46	−0.1	−0.4	−2	64	60	57	52	−0.2	−1.3	−4
UpA	62	58	53	51	−0.2	−1.2	−4	58	54	51	48	−0.1	−1.1	−2
CpC	79	74	66	60	−0.6	−2.5	−6	78	74	67	61	−0.6	−2.2	−6
CmpC	86	84	76	70	−1.0	−2.8	−6	78	74	70	66	−0.6	−1.6	−3
3′-ϵAMP	41	42	43	44	0.2	0.3	0.5							
5′-ϵAMP								44	45	46	47	0.1	0.3	0.7
3′-AMP	36	37	38	39	0.3	0.3	0.1							
5′-AMP								42	43	43	44	0.2	0.2	0.1
3′-GMP	39	40	42	44	0.2	0.6	1							
5′-GMP								40	40	42	44	0.2	1.0	3
3′-UMP	56	55	53	51	−0.1	−0.5	−2							
5′-UMP								52	51	50	49	−0.02	−0.3	−1
3′-CMP	61	60	58	56	−0.2	−0.5	−1							
5′-CMP								66	64	62	60	−0.3	−0.7	−1

[a] The percent 3′-endo population is calculated from $10(10 - J_{1',2'})$. [b] The thermodynamic data are computed by assuming 3′-endo ⇌ 2′-endo equilibrium for the ribofuranose ring. The estimated error is ±0.5 kcal/mol for ΔH° and ±1 cal/(mol deg) for ΔS°.

TABLE IV: Percent Stacking[a] and Its Thermodynamic Data[b] for Dimers.

	Np-							-pN						
	Temp (°C)				ΔG° at 20 °C	ΔH°	ΔS°	Temp (°C)				ΔG° at 20 °C	ΔH°	ΔS°
	4	20	45	75	(kcal/mol)	(kcal/mol)	(cal/(mol deg))	4	20	45	75	(kcal/mol)	(kcal/mol)	(cal/(mol deg))
ϵApϵA	54	38	21	9	0.3	−7	−23	46	31	24	17	0.5	−4	−14
ApϵA	47	29	15	5	0.5	−7	−27	39	31	22	13	0.5	−4	−15
ApA	53	44	35	28	0.1	−3	−10	46	39	26	16	0.3	−4	−15
ϵApG	44	26	21	14	0.6	−4	−15	48	32	21	11	0.4	−5	−19
ApG	42	32	24	18	0.4	−3	−12	35	27	16	5	0.6	−9	−32
GpϵA								41	31	20	8	0.5	−6	−20
GpA	52	33	17	4	0.4	−9	−30	40	30	19	11	0.5	−5	−17
ϵApU	49	33	21	9	0.4	−6	−22	46	31	22	14	0.5	−4	−17
ApU	52	38	24	11	0.3	−6	−21	45	31	22	13	0.5	−4	−17
UpϵA								36	27	20	9	0.6	−4	−17
UpA								27	19	14	7	0.8	−4	−17
CpC	46	35	19	9	0.4	−6	−21	35	28	13	3	0.6	−8	−29
CmpC	64[c]	60[c]	43[c]	32[c]	0.2[c]	−4[c]	−12[c]	35	28	21	15	0.6	−3	−12

[a] The percent stacking is estimated from $100[(J_{1',2'}\text{monomer} - (J_{1',2'}\text{dimer}]/(J_{1',2'}\text{monomer}$ (see text). [b] The thermodynamic data are computed by assuming a two-state model, unstacked form ⇌ stacked form for the dimer conformations (see text). The estimated error is ±1 kcal/mol for ΔH° and ±2 cal/(mol deg) for ΔS°. [c] $J_{1',2'}$ of 3′-CMP is substituted for that of 2′-*O*-Me-3′-CMP.

a measure of the stacked form. Thus, the % stacking = $100[(J_{1',2'}\text{monomer} - (J_{1',2'}\text{dimer}]/(J_{1',2'}\text{monomer}$ (Lee et al., 1976; Ezra et al., 1977). This calculation gives an independent estimate of the stacking from each nucleotide unit in the dimer. Table IV gives the percent stacking and the thermodynamics for the equilibrium between the stacked and unstacked forms. For the dimers UpA, UpϵA, and GpϵA, the Np- residue shows a 3′-endo population less than (or equal to) the corresponding 3′-mononucleotides at high temperatures. This suggests the presence of a stacked form, in which the ribose conformation of the Np- residue is 2′-endo. Thus, the percent stacking of these molecules can only be estimated from the -pN residue (see conformation III in the Discussion). In the other dimers, Np- and -pN residues show reasonably consistent data of percent stacking. At 4 °C, 27–64% of the dimers are in the stacked forms. For the dimers containing no pyrimidines, the order of percent stacking is ϵApϵA ≃ ApA ≥ ϵApG > ApϵA ≃ GpϵA > ApG; for those containing only one pyrimidine, ϵApU ≃ ApU > Upϵa > UpA; and for those containing two pyrimidies, CmpC > CpC. The cross-relation of the percent stacking of the first two sets of molecules is ϵApϵA ≃ ϵApU; ApG > UpϵA. The correlation between base-base stacking and dimerization shifts of protons will be discussed in the later sections. The magnitude of ΔH° stacking

TABLE V: Percent gg Population[a] and Its Thermodynamic Data[b] for Dimers and Monomers.

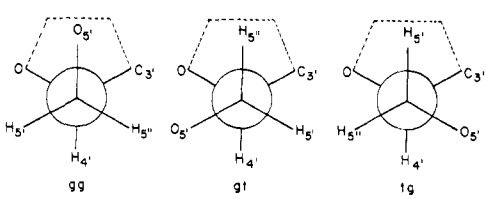
	Np- or 3′-NMP							-pN or 5′-NMP						
	Temp (°C)				$\Delta G°$ at 20 °C	$\Delta H°$	$\Delta S°$	Temp (°C)				$\Delta G°$ at 20 °C	$\Delta H°$	$\Delta S°$
	4	20	45	75	(kcal/mol)	(kcal/mol)	(cal/(mol deg))	4	20	45	75	(kcal/mol)	kcal/mol)	(cal/(mol deg))
ϵApϵA	78	72	66	58	−0.6	−2.5	−6	77	72	59	52	−0.6	−3.2	−9
ApϵA	76	74	70	64	−0.6	−1.6	−3	71	65	59	53	−0.4	−2.1	−6
ApA	69	69	66	63	−0.5	−0.8	−1	86	79	68	58	−0.8	−4.0	−11
ϵApG	72	66	59	58	−0.4	−1.7	−4	74	70	65	57	−0.5	−2.0	−5
ApG	73	70	66	62	−0.5	−1.4	−3	80	77	63	55	−0.7	−3.4	−10
GpϵA	68	66	63	60	−0.4	−0.9	−2	76	72	65	56	−0.6	−2.5	−7
GpA	72	68	63	58	−0.4	−1.7	−4	82	76	67	57	−0.7	−3.3	−9
ϵApU	69	65	61	56	−0.4	−1.5	−4	84	76	67	55	−0.7	−3.8	−11
ApU	75	72	66	60	−0.6	−1.9	−5	84	79	72	64	−0.8	−2.9	−7
UpϵA	78	72	65	59	−0.6	−2.4	−6	72	67	62	55	−0.4	−2.0	−5
UpA	72	68	62	56	−0.4	−1.9	−5	76	70	63	55	−0.5	−2.5	−7
CpC	73	70	63	57	−0.5	−2.0	−5	82	78	72	64	−0.7	−2.5	−6
CmpC	75	68	63	59	−0.4	−1.9	−5	90	84	76	70	−1.0	−3.6	−9
				Av	−0.5	−1.7	−4.2				Av	−0.6	−2.9	−7.8
3′-ϵAMP	70	64	60	54	−0.4	−1.8	−5							
5′-ϵAMP								76	73	68	63	−0.6	−1.7	−4
3′-AMP	72	70	68	65	−0.5	−0.9	−1							
5′-AMP								77	74	70	66	−0.6	−1.5	−3
3′-GMP	70	66	61	55	−0.4	−1.7	−5							
5′-GMP								71	65	58	52	−0.4	−2.2	−6
3′-UMP	68	64	58	52	−0.3	−1.8	−5							
5′-UMP								84	80	73	67	−0.8	−2.7	−6
3′-CMP	69	65	61	58	−0.4	−1.3	−3							
5′-CMP								84	80	74	67	−0.8	−2.6	−6
				Av	−0.4	−1.5	−3.8				Av	−0.6	−2.1	−5

[a] The percent gg population is estimated from $10(13 - (J_{4',5'} + J_{4',5''}))$. [b] The thermodynamic data are computed by assuming (gt + tg) ⇌ gg equilibrium for the conformation of the C-4′-C-5′ bond. The estimated error is ±0.5 kcal/mol for $\Delta H°$ and ±1 cal/(mol deg) for $\Delta S°$.

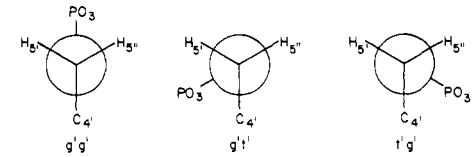
ranges from −3 to −9 kcal/mol and that of $\Delta S°$ stacking ranges from −12 to ∼−29 cal/(mol deg). These data are comparable to those estimated by optical methods (Davis and Tinoco, 1968; Brahms et al., 1967; Watts, 1977).

The small magnitude (4.4 to ∼4.9 Hz vs. 5.2 Hz) of $J_{2',3'}$ shown by ApA, ApG, GpϵA, ϵApU, CpC, and CmpC at low temperature suggests that the ribose in these dimers has larger puckering amplitude ($^{N}\tau_m$ = 36 to ∼42°, $^{S}\tau_m$ = 39 to ∼45°) than the usual puckering amplitude ($^{N}\tau_m$ = 34 to ∼38°, $^{S}\tau_m$ = 37 to ∼40°) shown by a ribose moiety with $J_{2',3'}$ = 5.2 Hz (Davies and Danyluk, 1974). Such perturbation of the ribose conformation is probably caused by the high degree of base-base stacking of these dimers under these conditions.

Conformations of the C-4′-C-5′ and C-5′-O-5′ Bonds. The population of the gg conformer for the C-4′-C-5′ bond, shown in Table V together with the thermodynamic data, is estimated from % gg = $10(13 - (J_{4',5'} + J_{4',5''}))$ (Lee and Sarma, 1976a,b). The gg conformation, which is more populated than gt + tg, decreases as the temperature increases (Table V). We



notice that dimerization does not induce a large change in the conformation in this bond (the change in gg is ±11% or less). The average thermodynamic data suggest that the C-4′-C-5′ bond of the -pN residue ($\Delta H°_{av}$ = −2.9 kcal/mol, $\Delta S°_{av}$ = −7.8 cal/(mol deg)) is only slightly stabilized by dimerization (for 5′ NMP, $\Delta H°_{av}$ = −2.1 kcal/mol, $\Delta S°_{av}$ = −5 cal/(mol deg)), while that of the Np- residue is not affected by dimerization (this is important for the assignment of H-5′ and H-5″ of this residue, discussed in the section of Materials and Methods).



The g′g′ population of the C-5′-O-5′ bond in the -pN residue, computed from % g′g′ = $100(25 - (J_{5'\text{-P}} + J_{5''\text{-P}}))/20.8$ (Lee and Sarma, 1976a,b), and shown in Table VI, reveals that the g′g′ confomer is more stable than g′t′ + t′g′, and decreases with increasing temperature. As in the case of the C-4′-C-5′ bond of the same residue, dimerization only slightly perturbs the C-5′-O-5′ conformation (+ 11% g′g′ or less). The stability of the C-5′-O-5′ conformation ($\Delta H°_{av}$ = −2.0 kcal/mol, $\Delta S°_{av}$ = −3.9 cal/(mol deg), Table VI) is not very different from that of the 5′-NMP ($\Delta H°_{av}$ = −1.6 kcal/mol, $\Delta S°_{av}$ = −3.2 cal/(mol deg)).

The preference for gg and g′g′ conformations along the C-4′-C-5′ and C-5′-O-5′ bonds is also suggested by the large long-range four-bond coupling, $J_{4'\text{-P}}$ (1.5 to ∼2.4 Hz), which is indicative of more than a 50% population of a *trans-coplanar* conformation for the H-4′-C-4′-C-5′-O-5′-P linkage (Sarma et al., 1973).

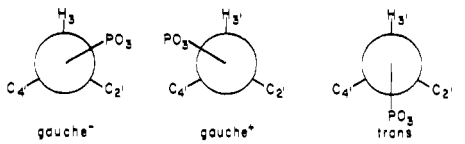
Conformation along the C-3′-O-3′ Bond of the Np- Residue. The conformation of the C-3′-O-3′ bond is described in principle as the time average of three stable conformers—

TABLE VI: Percent g'g' Population[a] and Its Thermodynamic[b] Data for the -pN Residue of the Dimers and the 5'-NMP.

	Temp (°C)				$\Delta G°$ at 20 °C (kcal/mol)	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/(mol deg))
	4	20	45	75			
ϵApϵA	84	80	76	73	−0.8	−1.8	−3
ApϵA	82	79	76	72	−0.8	−1.5	−2
ApA	87	83	79	74	−0.9	−2.3	−4
ϵApG	79	77	75	72	−0.7	−1.0	−1
ApG	86	81	77	72	−0.8	−2.3	−5
GpϵA	80	78	74	71	−0.7	−1.4	−2
GpA	88	84	78	173	−1.0	−2.7	−6
ϵApU	86	83	77	70	−0.9	−2.6	−6
ApU	87	83	77	70	−0.9	−2.8	−6
UpϵA	84	80	76	72	−0.8	−1.9	−4
UpA	84	81	77	72	−0.8	−1.9	−4
CpC	82	80	79	76	−0.8	−1.0	−0.3
CmpC	91	88	82	76	−1.2	−3.2	−7
				Av	−0.8	−2.0	−4
5'-ϵAMP	82	79	74	69	−0.8	−1.9	−4
5'-AMP	82	78	73	67	−0.7	−2.1	−5
5'-GMP	78	74	70	66	−0.6	−1.6	−3
5'-UMP	78	76	73	69	−0.7	−1.3	−2
5'-CMP	80	77	74	71	−0.7	−1.3	−2
				Av	−0.7	−1.6	−3.2

[a] The percent g'g' population is estimated from $100(25 - (J_{5'\text{-P}} + J_{5''\text{-P}}))/20.8$. [b] The thermodynamic data are computed by assuming (g't' + t'g') ⇌ g'g' equilibrium for the conformation of the C-5'-O-5' bond. The estimated error is ±0.5 kcal/mol for $\Delta H°$ and ±1 cal/(mol deg) for $\Delta S°$.

gauche⁻, gauche⁺, and trans. However, considerable steric hindrance (easily seen in the CPK model) is involved in the



trans conformation, in which the phosphate group is underneath the ribose ring. Furthermore, because of the tremendous steric restriction from the nearby O-H-2' and C-H-4' groups (CPK model), one cannot make a trans conformer from a gauche one. It thus seems reasonable to rule out the possibility of the trans conformation in solution. From the coupling constant, $J_{3'\text{-P}}$, the dihedral angle of H-3'-C-3'-O-3'-P was calculated using the Karplus equation $^3J_{HP} = 18.1 \cos^2 \theta_{HP} - 4.8 \cos \theta_{HP}$ (Lee and Sarma, 1976a,b). It falls in the range of ±29 to ∼±37°, corresponding to $\phi' = 203$ to ∼277° in the nomenclature used for polynucleotide conformations. This is somewhat different from the "real" gauche⁺ ($\phi' = 300°$) and gauche⁻ ($\phi' = 180°$) conformations.

The dimers CmpC and CpC exhibit larger magnitude (8.4 to ∼9.7 Hz) of $J_{3'\text{-P}}$ corresponding to the smaller dihedral angle of $\theta_{H\text{-}3'\text{-P}}$ than the other dimers (7.8 to ∼8.6 Hz). This is attributed to the high 3'-endo population (Table III) of the ribose in the Np- residue of CmpC and CpC, since in this conformation a large dihedral angle of $\theta_{H\text{-}3'\text{-P}}$ would be subject to the steric interaction between the phosphate and C-2' and between the phosphate and C-4'. $J_{3'\text{-P}}$ decreases slightly (0 to ∼1 Hz) when the temperature is increased from 4 to 75 °C. This indicates only a small increase of the angle $\theta_{H\text{-}3'\text{-P}}$ with increasing temperature. Using $J_{3'\text{-P}}$ alone, it is not possible to differentiate the gauche⁺ from the gauche⁻ conformers. This can only be carried out by ^{13}C NMR, with accurate measurement of $^3J_{C\text{-}2'\text{-P}}$ and $^3J_{C\text{-}4'\text{-P}}$ and using the correct Karplus dependence (Smith et al., 1973; Blackburn et al., 1973; Lapper et al., 1973; Lapper and Smith, 1973, Schleich et al., 1976; Alderfer and Ts'o, 1977). The magnitude of the long-range four-bond coupling constant, $J_{2'\text{-P}}$, which is consistently observed in the dimer (except for CpC and CmpC), is an indication of the existence of gauche⁺-2'-endo conformation, in which the four bonds of H-2'-C-2'-C-3'-O-3'-P are nearly *trans coplanar* (Sarma et al., 1973). Such four-bond coupling was observed before (Lee et al., 1976; Ezra et al., 1977). The magnitude (0.6 to ∼1.2 Hz) of this four-bond coupling is less than half of 2.7 to ∼3 Hz (Sarma et al., 1973), possibly suggesting that the population of the gauche⁺-2'-endo conformation is less than 50%.

Conformation along the P-O-3' and P-O-5' Bonds. It is not possible, by direct measurement of NMR data, to ascertain the conformation along the phosphodiester linkage O-3'-P-O-5', since there are no coupling constants existing for this purpose. In order to determine the conformation of this linkage, CPK models were employed to construct the possible stacked (or stable) conformations of dimers. The guidelines for constructing these models were: (a) anti, gg and g'g' for the glycosidic, C-4'-C-5' and C-5'-O-5' bonds, (b) gauche⁺ or gauche⁻ for the C-3'-O-3' bond, (c) a 3'-endo or 2'-endo conformation for the ribose ring, and (d) rotation along the P-O-3' and/or P-O-5' bonds in order to achieve a conformation with minimum steric hindrance and maximum interaction between the hydrophobic portions of the molecule, i.e. base-base stacking or base-hydrophobic group overlapping. The model thus constructed should be consistent with the observed dimerization shifts of the various protons. Three stacked (or stable) conformations I, II, and III were found (Figure 7). The dinucleoside phosphates may be represented primarily as a mixture of these conformations plus an open (unstacked) conformation. The amount of each of the four conformations will depend on the molecule and the temperature. In conformation I, ω'(P-O-3' bond) and ω(P-O-5' bond) are 300 and 290°, respectively, with ϕ'(C-3'-O-3' bond) equal to 210° (or gauche⁻), and both riboses in the 3'-endo conformation. The polarities (defined by the vector from C-3'-C-2' to O-1') of the two ribose ring oxygens are in the same directions. This corresponds to the g⁻g⁻ conformation observed in single crystals

of GpC (Day et al., 1973; Hingerty et al., 1975), ApU (Rosenberg et al., 1973) and the ApA^+ part of $ApAp^+A^+$ (Suck et al., 1973). This conformation displays more base-base stacking than a similar one constructed with stick-ball models which show $\omega' = 280°$ and $\omega = 285°$ (Lee et al., 1976). In conformation II, ω', ω, and ϕ' are 30, 100, and 210°, respectively, with both ribose rings in the 3′-endo conformation. The polarities of the two ribose ring oxygens are in opposite directions. Except for the value of ω', this corresponds to the g^+g^+ conformation found in single crystals of UpA (Rubin et al., 1972; Sussman et al., 1972) and the Ap^+A^+ part of $ApAp^+A^+$ (Suck et al., 1973). In these crystals, ω' is 80°; this provides very little intramolecular base-base stacking, presumably because of the strong intermolecular base stacking and other intermolecular interactions (hydrogen bonding) in the crystal. Conformation II exhibits more base-base stacking than a similar conformation proposed (Lee et al., 1976) from stick-ball models which showed very little base-base stacking ($\omega' = 90°$, $\omega = 80°$). In conformation III, ω', ω, and ϕ are 50, 220, and 260° (gauche$^+$), respectively, with the ribose conformation being 2′-endo for the Np- residue and 3′-endo for -pN. The polarities of the two ribose ring oxygens are nearly perpendicular to each other. The base of the -pN residue overlaps H-4′, H-5′, and H-5″ of the Np- moiety, with H-5′ nearer to the base than H-5″. The χ angle of the -pN residue is very large (~100°), so that the 3′-endo conformation of the ribose is favored by the repulsion between the base and C-H-2′ group. A 3′-endo conformation for the ribose of the Np- residue in conformation III is unlikely, since it produces two tilted bases rather than two parallel bases. The 2′-endo conformation of this residue is consistent with the gauche$^+$ conformer of the C-3′-O-3′ bond (Lee et al., 1976; Ezra et al., 1977). The population of conformation III for UpA, UpϵA, and GpϵA, suggested by the large upfield shift of H-4′, H-5′, and H-5″ of the Np- residue (presented in the later section), is probably the main reason the Np- residue of these dimers does not exhibit its monomer property (2′-endo-3′-endo equilibrium) for the ribose at high temperature. Strikingly, these three conformations are similar to ones proposed by Davis (1967) using potential energy calculations without considering the solvent effect.

Correlation between the Proposed Conformations and the Observed Proton Dimerization Shifts. (I) Evidence for the Existence of Conformation I. It is well known that the diamagnetic anisotropy of a nearby electron-rich group tends to deshield the proton signal. Consistently, large downfield shifts of H-5′ of the -pN residue were observed (Figure 3a) upon dimerization. This can only be attributed to the juxtaposition of the OH-2′ group from the neighboring residue in conformation I. However, small downfield shifts are also observed for H-5″ and H-4′ of the same residue (Table II). These are probably due to the change of the local conformations such as χ angle, gg, g′g′, and 3′-endo conformations, etc., induced by dimerization. Such a small downfield contribution is also present for H-5′. More specifically, the measure of conformation I can be expressed by the difference (Δ) between the differentiation of H-5′ and H-5″ before and after dimerization, i.e. $\Delta = (\delta_{H\text{-}5'} - \delta_{H\text{-}5''})_{dimer} - (\delta_{H\text{-}5'} - \delta_{H\text{-}5''})_{monomer}$. Such evidence has been observed previously for the unmodified dimers (Lee et al., 1976; Ezra et al., 1977). Figure 3b shows that Δ is very temperature dependent; thus when the temperature increases, the population of conformation I decreases. It also illustrates that the dimers, excluding PypPy, show more conformation I, when the size of the base is in the order of Np- ≥ -pN. For example, at 4 °C the order of conformation I populations is GpA > GpϵA; ApA > ϵApϵA ≃ ApϵA; ϵApU ≃ ApU > UpA > UpϵA. ϵApG shows less conformation I than ApG at low temperature (4, 20 °C) but more conformation I at high temperature (45, 75 °C). In the case of CpC and CmpC, 2′-O-methylation seems to reduce conformation I, because of steric hindrance by the methyl group.

(II) Evidence for the Existence of Conformation II. The upfield shift of H-2′ of the -pN residue and H-3′ of Np- (Figure 4) is a strong indication for the existence of conformation II. Only in conformation II is the geometry of the molecule such that these protons are sandwiched between the two bases. There they experience a strong shielding from the neighboring base. However, GpϵA and UpϵA show a downfield shift for H-2′ (Figure 4a). This is due to the compensation of the upfield shift from conformation II by the downfield shift exerted by the large χ_2 angle (~100°) (Giessner-Prettre and Pullman, 1977b) because of the large fraction of conformation III (see next section). H-3′ of the -pN residue behaves like H-2′, but to a lesser extent (Table II). The shift contributed from the increased conformational purity (3′-endo) of the ribose and the decrease of χ angle of the glycosidic bond is considered to be negligible except for UpϵA and GpϵA. The other evidence for the existence of conformation II is the large upfield shift of the pyrimidine base protons of the Np- residue (Table II). In conformation II, the pyrimidine base proton region of the Np- residue overlaps the other base, which is not found in any other conformations.

In a previous report (Ezra et al., 1977), the "left-handed stack" conformation, which is similar to, but different than conformation II, was proposed from the upfield shift of H-5′ and H-5″ of the Np- residue. These shifts are not consistent with the structure in CPK models. However, the upfield shift of these two protons and H-4′ of the same residue can be explained by the existence of conformation III, discussed in the next section.

(III) Evidence for the Existence of Conformation III. The large dimerization shifts of H-5′, H-5″, and H-4′ (Figure 5) of the Np- residue, and the fact that $\delta_{H\text{-}5'}$ is more sensitive to dimerization than $\delta_{H\text{-}5''}$ (Figure 5), can only be explained by the existence of conformation III. In this conformation, the base of the neighboring residue, -pN, is next to these protons with H-5′ nearer to the base than H-5″. Thus, H-5′ experiences a larger upfield shift than H-5″ by the ring current of the neighboring base. In addition to the ring current of the neighboring base, the decrease of the χ angle (Giessner-Prettre and Pullman, 1977a,b), the decrease of the gauche$^+$ conformation of the C-3′-O-3′ bond, and the diamagnetic anisotropy of the =O and $-NH_2$ groups on the pyrimidine base will affect the chemical shifts of these protons. For an assumed change of χ angle from 70 to 20° (induced by dimerization, in the anti range) a downfield shift occurs for H-4′, H-5′, and H-5″ in purine nucleosides (Giessner-Prettre and Pullman, 1977b). This effect is negligible in the pyrimidine nucleoside (Giessner-Prettre and Pullman, 1977a). The decrease of the gauche$^+$ conformer of the C-3′-O-3′ bond removes the deshielding phosphate group from H-4′ and H-5″; consequently these two protons are shifted upfield. The downfield shift of these protons in the dimers NpPy (Figure 5) is caused by the deshielding effect of the =O and $-NH_2$ groups on the pyrimidine base. It is thus difficult to estimate the temperature dependence of conformation III from Figure 5, because of so many competing factors which affect the chemical shifts of these protons. From the magnitude of the shift, in general, the sequence dependence of conformation III is in the order of NpϵA > NpPu > NpPy.

(IV) Dimerization Shift of H-2′ of the Np- Residue. The dimerization shift of H-2′ of the Np- residue is composed of

an upfield shift caused by the neighboring base in conformations I and II, an upfield shift caused by the increased 3′-endo conformation, and a downfield shift caused by the phosphate group in the increased 3′-endo-gauche$^-$ conformation. The contribution from the χ change is not appreciable as was discussed in the previous section. Figure 6 shows that in NpϵA and NpPu, the upfield shift is much larger than the downfield shift, while in NpPy, the upfield shift is compensated or overcome by the downfield shift. The overall shift of H-2′ is reduced by increasing temperature, except for the H-2′ of ϵApU and ApU, which is not very sensitive to temperature.

(V) Dimerization Shifts of H-1′ and the Base Protons. The data of H-1′ and the base protons (Table II) indicate the degree of overall base-base interaction in a straightforward way. These protons are in the regions directly involved in base-base stacking. However, it is impossible to differentiate the exact contributions from each of the stacked conformations. This can only be estimated qualitatively or semiquantitatively. In conformation I, for the -pN residue, H-5 experiences a strong upfield shift, since it is directly on top of the neighboring base. H-6 and H-8 show lesser upfield shifts, because they are farther from the base, and they are shifted downfield by the nearby $OH_{2'}$ group of the other residue. When the neighboring base is a pyrimidine, its electron-rich groups, such as $-NH_2$ and $=O$, give some deshielding effect on these protons. H-2, H-10, and H-11 show strong, medium, or weak shifts, depending upon whether the base of the Np- residue is ϵA, a purine, or a pyrimidine. For the base protons of the Np- residue, H-5, H-6, and H-8 experience medium shifts, since they are not located directly on top of the neighboring base. H-2, H-10, and H-11 show weak or strong shifts, depending upon whether or not the -pN base is a pyrimidine. H-1′ of the Np- residue shows stronger upfield shifts than H-1′ of the other residue, because it is nearer to the neighboring base. In conformation II, all the base protons are influenced strongly by the base-base stacking, with H-6 and H-8 showing the least extent. The two H-1′ protons are approximately equidistant from the nearby base of the other residue. For the dimer of NpϵA in conformation III, protons H-5, H-6, and H-8 of the Np- residue are shielded by ϵA, while H-2, H-10, and H-11 of -pϵA are shielded by the base of the Np- residue. For NpPu dimers, H-6 and H-8 of the Np- residue and H-2 of the Pu base are shielded. For NpPy dimers, there is little interaction between the bases showing in the base protons shifts. H-1′ of the Np- residue experiences a very strong upfield shift from the base of the -pN residue, while that of the -pN residue is far away from the shielding effect of the Np- base. When the χ angle decreases from 70 to 20° (as estimated from a Kendrew model), H-1′ is shifted upfield by the ring current (Giessner-Prettre and Pullman, 1977a,b) and H-8 of purine and H-6 of pyrimidine base approach the ring oxygen (O-1′) which tends to shift these two protons downfield by its diamagnetic anisotropy.

The complicated H-1′ and base proton shifts alone of the unmodified dimers cannot lead to a conclusive solution of the detailed dynamic conformations of these molecules, as has been attempted before by several researchers (Ts'o et al., 1969; Chan and Nelson, 1969; Bangerter and Chan, 1969). This can better be carried out by the selective dimerization shifts of the ribose protons as was discussed earlier.

Significance of Conformations I, II, and III to Unmodified and Modified Dinucleoside Monophosphates. The quantitative analysis of the populations of each conformation for a dimer is not possible at present. However, semiquantitative aspects of the dimer conformations can be obtained by correlating the degree of base-base stacking in CPK models and the observed proton dimerization shifts (data from this paper and from the previous reports, Lee et al., 1976; Ezra et al., 1977). In all cases, conformation II shows very much base-base overlapping in molecular models, as well as large dimerization shifts of H-2′ of -pN and H-3′ of the Np- residues. The experimental data (Figure 4 of this paper; Table III, Lee et al., 1976; Table III, Ezra et al., 1977) indicate a large population of this conformation for dinucleoside monophosphates. For the dimers of PupPu and PupPy, conformation I also shows very much base-base stacking and a large dimerization shift of H-5′ of the -pN residue. Figure 3a of this paper and Table III of Lee et al. (1976) and Table III of Ezra et al. (1977) suggest a large population of conformation I for these dimers. For PypPu and PypPy, conformation I exhibits small base-base overlapping, together with a small downfield shift for H-5′ of the -pN residue. A small population of this conformation for these dimers is expected. Conformation III shows little base-base interaction, and a small dimerization shift of H-5′ of the Np- residue for unmodified dimers. This suggests a small population of conformation III for these molecules (see Figure 5a of this paper; Table III of Lee et al., 1976; Table III of Ezra et al., 1977 [the assignments for H-5′ and H-5″ in this table should be reversed according to Remin and Shugar, 1972]). Also, the dimers show more base-base stacking when Pu, as opposed to Py, is the base of the Np- residue.

Introduction of ϵA as the Np- residue of the dimers induces an increase of conformations I (Figures 3a and b) and II (Figure 4). Introduction of ϵA as the -pN residue induces an increase of conformations II (Figure 4) and III (Figure 5) by increasing the degree of base-base overlap. 2′-O-Methylation of Cp- in CpC reduces the amount of conformation I (Figures 3a and 3b) but increases conformation II. However, it has been shown that CmpC exhibits more stacking than CpC (Table IV of this paper; Cheng and Sarma, 1977; Warshaw and Cantor, 1970; Drake et al., 1974). This suggests that the decrease of conformation I is smaller than the increase of conformation II. All in all, 2′-O-methylation of CpC helps to stabilize conformation II.

A Possible Conformation of the tRNA Anticodon Loop. The anticodon of tRNA consists of seven nucleotide residues. In spite of its complexity, several research groups (Kan et al., 1974, 1975; Maelicke et al., 1975; Kreishman et al., 1974) have tried to determine its solution conformation by studying either the intact $tRNA^{Phe}$ or fragments of its anticodon loop. Recently, an anticodon loop conformation was reported for a $tRNA^{Phe}$ crystal (Kim et al., 1974; Klug et al., 1974) in which a right-handed stacked (or g^-g^-) conformation was observed for all component dinucleotides, except for U-Gm which is an open conformation (defined as π_3 turn; Kim and Sussman, 1976). tRNAs of known sequences have a common sequence in the anticodon loop (Holmquist et al., 1973), i.e. (5′)-Py-U-N-N-N-Pu*-N*-(3′), where Py = pyrimidine; U = uracil; N = anticodon base; Pu* = purine or modified purine base; N* = arbitrary base. Assuming that the anticodon loop is flexible enough that its residues are allowed to express their stable conformations as the free dimers in solution, we would like to propose a possible conformation for the anticodon loop. This conformation will be based on the information discussed earlier in this paper and the data reported previously (Lee et al., 1976; Ezra et al., 1977). The proposed anticodon loop conformation is: conformation II (or g^+g^+) for Py-U and N-Pu* fragments; open (or unstacked) conformation for U-N fragment; conformation I (or g^-g^-) for all other segments including the loop-stem linkage. As shown previously, dipyrimidine dimers prefer to exist in conformation II, which is enforced by 2′-O-methylation of the Np- residue, occasionally found in anticodon loop. The dimers (such as NpPu*) have

shown promotion of conformation II and suppression of conformation I, when the order of the size of the bases is N < Pu*, which is always found in the anticodon loop. The dimers with the sequence of UpN have been reported to show the least stacking (Lee et al., 1976; Ezra et al., 1977), and, as a result, an open conformation is proposed for this linkage. For the dimers of Pu*pN*, conformation I is favorable when the order of the size of the base is Pu* ≥ N*, always found in the anticodon loop. The loop-stem linkages are assumed to be in conformation I, which is stabilized by the nearby base pair of the anticodon stem. There are three turning points of the molecular structure (or discontinuities of stacking) at the Py-U, U-N, and N-Pu* regions. Besides, N and N* stack to the same side of Pu*, with more stacking for N* than N (ϵApN stacks more than NpϵA, Table IV; YpA stacks more than ApY, Kan et al., 1975). Although the interresidue and/or intraresidue hydrogen bonding is possibly involved in stabilizing the anticodon loop conformation (Quigley and Rich, 1976; Sundaralingam et al., 1976) the proposed conformation, based only on the nature of the base–base stacking in dimers, shows a more compact loop structure than the one found in crystals. The anticodon triplet is in a right-handed stacked conformation, stabilized by the loop structure between the anticodon triplet and the stem. The compact loop structure and the isolated environment of the anticodon triplet from the other residues provide a possible model for two tRNAs coexisting side by side in the A and P sites of the ribosome during protein synthesis.

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Supplementary Material Available

Chemical shifts and coupling constants (Table I) and proton dimerization shifts (Table II) (5 pages). Ordering information is given on any current masthead page.

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Interaction of Oligoribocytidylates with T7 DNA in Neutral and Acid Media†

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ABSTRACT: Oligoribocytidylates of chain length 4 to 12 were found to interact with native T7 DNA at neutral and slightly acid pH. The results suggest that binding occurred at deoxycytosine clusters which may be displaced by the oligomers at neutral pH, while a local triple-stranded structure would be formed at acid pH. Transcription of DNA-$(Cp)_n$ complexes by *Escherichia coli* RNA polymerase showed a decrease in level without affecting the specificity of the transcription, suggesting that oligocytidylate binding did not occur on the promoters.

Many reports concern the binding of short oligonucleotides to single-stranded nucleic acids: for example, to 5S RNA to determine the length and location of loops in the secondary structure (Lewis & Doty, 1970); to charged or uncharged tRNA to reproduce interactions similar to the codon-anticodon associations (Uhlenbeck et al., 1970; Danchin & Grunberg-Manago, 1970; Schimmel et al., 1972); or to denatured DNA to study interactions between the complementary strands (McConaughy & McCarthy, 1967). Niyogi & Thomas (1967) and Rüger & Bautz (1968) have also used RNA fragments varying in length to study the recurrence of specific sequences in natural DNA.

It would be interesting to deal with a double-stranded polymer or DNA—instead of a single-stranded one as previously shown—to mimic replicative or transcriptional processes. Only a few reports have appeared on that topic. Holloman et al. (1975) have observed the binding of deoxyribooligonucleotides to ϕX 174 supercoiled DNA. They suggest that such complexes may simulate early steps in genetic recombination. More recently, it has been shown that RNA can hybridize to double-stranded DNA in the presence of formamide (Thomas et al., 1976). One of the DNA strands is displaced and a stable RNA-DNA structure, called R-loop, is formed.

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