Backbone Conformational Properties of Nucleotides in Solution determined by ^{1}H Nuclear Magnetic Resonance Spectroscopy

By **David B. Davies**, Department of Chemistry, Birkbeck College, Malet Street, London WC1E 7HX

The backbone conformational properties of adjacent O(5′)–C(5′) and C(5′)–C(4′) bonds of nucleotides in aqueous solution have been investigated from available n.m.r. results for monomers, dimers, trimers, and polynucleotides. Results for monoribonucleotides show that the backbone conformation depends, primarily, on the glycosidic bond conformation and, to a lesser extent, on the sugar ring conformation of the monomer. On the other hand, the backbone conformational properties of oligoribonucleotides (dimer and trimer) depend, primarily, on the sugar ring conformation (*i.e.* base-stacking on the Altona model) and, to a much lesser extent, on the glycosidic bond conformation. The implications of this nucleotide conformational model for polymer results are discussed.

THE conformational properties of nucleosides and nucleotides at the monomer, dimer, and trimer level have been studied in order to elucidate the principles that govern the behaviour of biologically active nucleic acids. From considerations of many *X*-ray structural studies [1,2] which were supported by semi-empirical potential energy calculations,[3-5] Sundaralingam and his co-workers proposed that the conformations of mononucleotides are essentially 'rigid' and conserved in polynucleotides. Although the analysis of conformations of nucleotides in solution by n.m.r. spectroscopy considers each bond to be flexible, a number of studies have suggested that a highly stable conformational state exists at the monomer [6,7] and dimer [8-10] level which, by extrapolation, is likely to be conserved at the polymer level.[11] The conformational state of nucleotides in solution as observed by n.m.r. spectroscopy is similar to the 'rigid' nucleotide conformation determined by *X*-ray studies making the tacit assumption that the conformational properties about each bond in the nucleotide unit in solution are directly related.

It has recently been suggested that the conformational properties of two nucleotide molecular features, the sugar ring and backbone C(5′)–C(4′) bond, are related and that the conformational behaviour of purine and pyrimidine nucleosides and nucleotides can be rationalised assuming that there is a *ca.* 1 : 1 correspondence between the proportion of the sugar ring *N* conformer and the most stable conformation for the backbone C(5′)–C(4′) bond.[12] This direct correlation between sugar ring and backbone conformational properties is a prerequisite of the existence in solution of the highly stable conformational unit akin to the 'rigid' nucleotide unit observed in the solid state. As properties of the backbone play an important role in determining the overall conformational properties of nucleotides, the backbone conformational properties of nucleotides at the monomer, dimer, and trimer level have been investigated in order to assess further the relevance of the nucleotide conformational unit in solution.

DISCUSSION

(1) *Nomenclature and Notation.*—The backbone conformation of a polynucleotide chain consists of a repeating unit of six single bonds in the sequence shown in the Scheme. Torsion angles for each bond are listed for

—P	O(5′)	C(5′)	C(4′)	C(3′)	C(3′)	P → Chain direction
ω	ϕ	ψ	ψ'	ϕ'	ω′	ref. 13
γ	δ	ε	ζ	α	β	ref. 14
(α	β	γ	δ	ε	ζ)	ref. 14

SCHEME

different systems of nomenclature which are available in the literature, including two possibilities by Rich and his co-workers.[14] In this work the earlier nomenclature of Sundaralingam *et al.*[13] is used until a standardised nomenclature is available. The conformations about four of the six bonds of the sugar phosphate chain of nucleotides in solution, *viz.* ϕ, ψ, ψ', and ϕ', can be determined from vicinal coupling constants observed by ^{1}H, ^{13}C, or ^{31}P n.m.r. spectroscopy.[15] In this work the ϕ and ψ backbone conformational properties of mononucleotides and the —pM fragments of oligonucleotides are investigated.

A number of notations have been used to describe particular conformations, usually staggered, about ϕ and ψ bonds. In general, n.m.r. descriptions based on the terms *gauche* and *trans* (*viz.* ψ bond: *gg*, *gt*, and *tg* and ϕ bonds; *g′g′*, *g′t′*, and *t′g′*) differ from those used in *X*-ray crystallographic and theoretical potential energy calculations (g^+, t, and g^- for each bond). An alternative description of these conformers has been suggested; it is based on the Klyne–Prelog [16] conformational nomenclature and can be used to specify conformational

properties about any bond in an unequivocal and convenient way. The notation is described in terms of the nomenclature suggested by Sundaralingam *et al.*[13] but is consistent with all conformational nomenclature. The symbols ϕ, ψ, *etc.*, represent the O(5′)–C(5′) and C(5′)–C(4′) bonds, *etc.*, and the notations +, a, and — represent the 60° (+sc), 180°(ap), and 300°(—sc) conformations, respectively. The relation between the various conformational notations for ψ and ϕ bonds are shown in Figures 1 and 2 respectively. The terms *gauche* and *trans* may still be used to denote spin-coupling relationships.

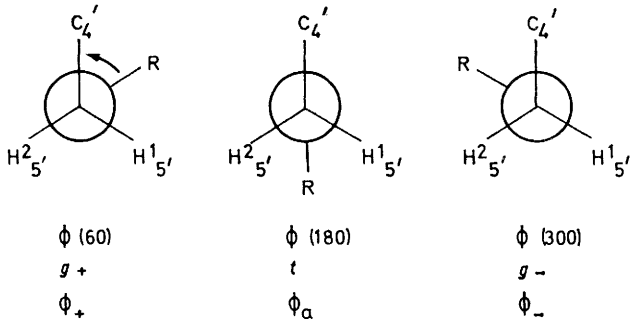

FIGURE 1 Classical staggered rotamers for C(5′)–C(4′) bonds, ψ

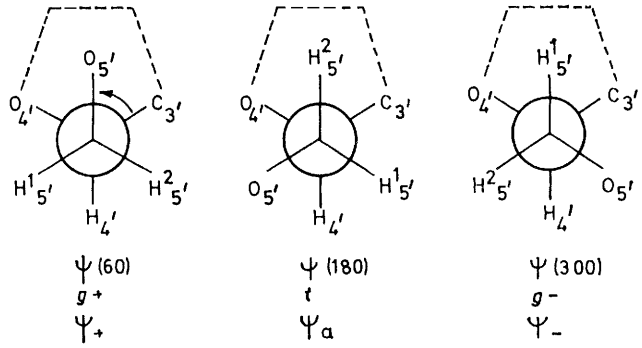

FIGURE 2 Classical staggered rotamers for O(5′)–C(5′) bonds, ϕ

(2) *ψ,φ Backbone Conformations.*—The conformational properties of ψ and ϕ bonds of nucleotides in solution are conveniently determined from appropriate vicinal proton–proton ($J_{4'5'}$ and $J_{4'5''}$ for ψ bonds) and proton–phosphorus [$J_{P5'}$ and $J_{P5''}$ for ϕ bonds] spin-coupling constants.[15] Observed ^3J values are analysed in terms of population distributions of the three staggered conformers shown for both ψ and ϕ bonds in Figures 1 and 2, respectively. Populations of one conformer for each bond can be determined from sums of observed coupling constants according to equations (1) and (2) where Σ and

$$p(gg) = p(\psi_+) = (13 - \Sigma)/10 \quad (1)$$

$$p(g'g') = p(\phi_a) = (25 - \Sigma')/20.8 \quad (2)$$

Σ' have their customary meaning † and $p(gg)$ and $p(g'g')$ represent the populations of the so-called *gauche–gauche* conformers in which either H(4′) or P(5′) atoms exhibit *gauche* relationships to the two C(5′) protons, respectively. The populations of these same conformers are denoted by $p(\psi_+)$ and $p(\phi_a)$, respectively. For the derivation of equations (1) and (2), respectively, *gauche* and *trans* coupling constants suggested by Hruska and Sarma and their co-workers [17–20] have been utilised for the ψ bond fragment (J_g 1.5, J_t 11 Hz) and those recently suggested by Sarma and his co-workers [21] have been used for the ϕ bond fragment (J_g 2.1 and J_t 22.9 Hz).

A plot of Σ' against Σ represents the ϕ,ψ backbone conformational properties of ribonucleotides as a two-dimensional map as shown in Figures 3 and 4 for purine and pyrimidine nucleotides, respectively; in general, Σ and Σ' data have been determined by high-field ^{1}H n.m.r. observations and the magnitudes checked by computer simulation of the spectra. The errors in Σ and Σ' magnitudes ($\pm$0.2 Hz for monomers and dimers and $\pm$0.5 Hz for polymers) are shown in Figures 3 and 4.

ϕ,ψ Backbone conformational properties of ribonucleotides plotted in Figures 3 and 4 include data for 5′-mononucleotides,[6,17,22–27] dinucleoside phosphates,[8–10,28–32] a trinucleoside diphosphate,[11] and two polynucleotides.[11,33] A wide variation in backbone conformational behaviour is exhibited by the results for monomers, oligomers, and polymers. It is found that nucleotide backbone conformational properties reflect other conformational properties in monomers (glycosidic bond *syn–anti* equilibrium which may be mediated by the sugar ring conformational equilibrium) and dimers (base-stacking effects as well as, possibly, changes in *syn–anti* equilibria).

(3) syn–anti *Effect for Nucleoside 5′-Monophosphates.*—The approximately linear correlation between Σ' and Σ of the common mononucleotides observed by Wood *et al.*[20] is shown as the dashed line in Figures 3 and 4, linking nucleotides with predominant *syn-* and *anti*-type conformations. Nucleotides with base-rings in predominant *syn*-type conformations (8-bromo- and 8-methylthiopurines,[22] orotidylic acid [20]) are characterised by high values of both Σ' and Σ corresponding to relatively low populations of ψ_+ (*gg*) and ϕ_a (*g′g′*) conformations as a consequence of repulsive interactions between charged phosphate groups and the base-ring. Molecules with base-rings in predominant *anti*-type conformations (all common purine and pyrimidine 5′-nucleotides [6]) exhibit characteristically low values of Σ' and Σ indicating high populations of both ψ_+ and ϕ_a conformers. From the slope of the *syn–anti* line [$\Delta\Sigma'/\Delta\Sigma$ *ca.* 0.54 and $\Delta p(\phi_a)/\Delta p(\psi_+)$ *ca.* 0.25], it is found that the effect of base-ring changing from *anti-* to *syn*-type conformations destabilises the favoured C(5′)–C(4′) bond conformer (ψ_+) about four times as much as the favoured O(5′)–C(5′) bond conformer (ϕ_a). This effect might be expected in view of the proximity of the base-ring to the C(5′)–C(4′) bond.

It is suggested that the base-ring *syn* ⇌ *anti* equilibrium is, primarily, reflected by the C(5′)–C(4′) bond

† $\Sigma = (J_{4'5'} + J_{4'5''})$ and $\Sigma' = (J_{P5'} + J_{P5''})$.

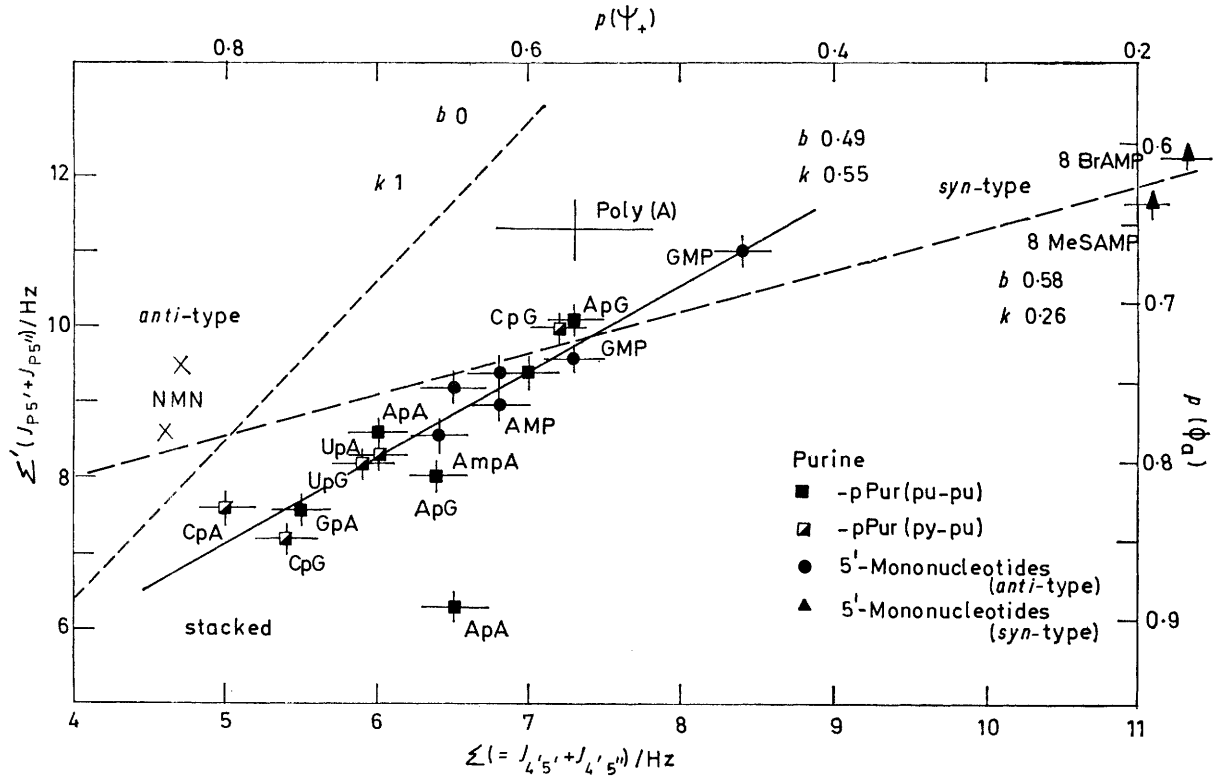

FIGURE 3 Ribonucleotide backbone conformational map, ϕ,ψ. Plot of Σ' *versus* Σ for purine 5'-nucleotides, dinucleoside phosphates, and oligo- and poly-nucleotides

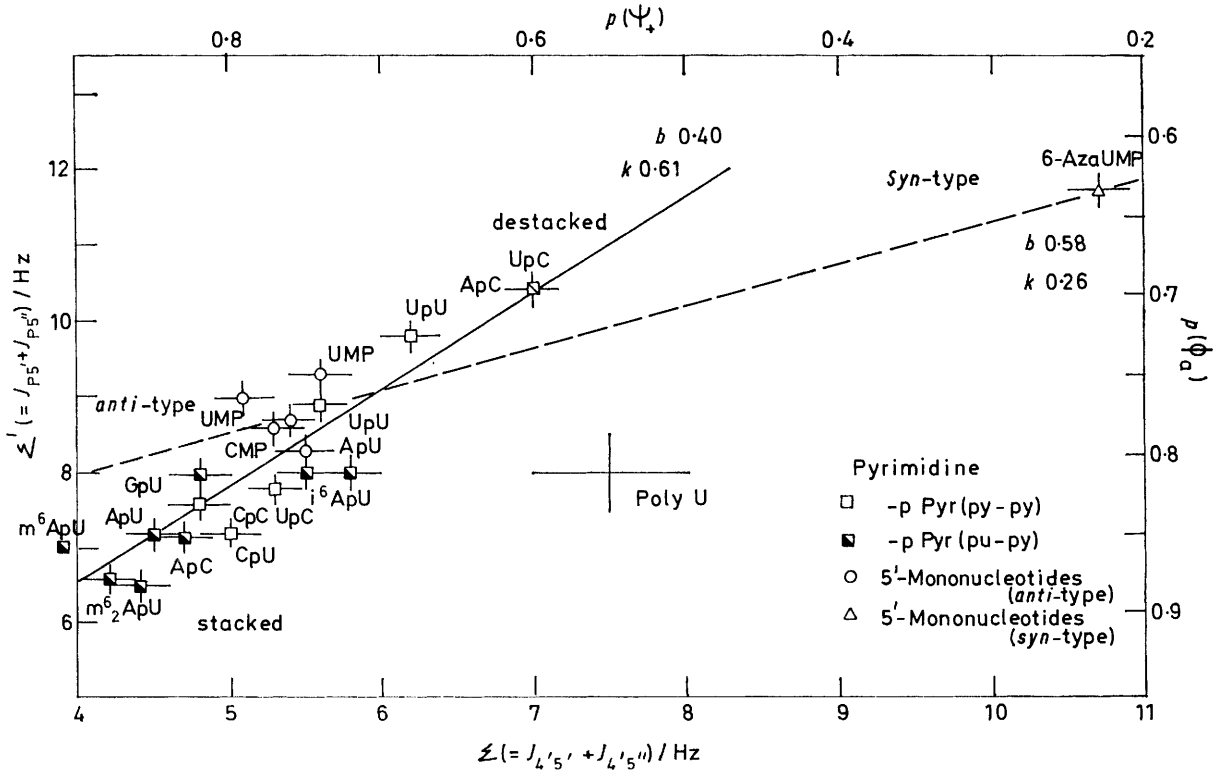

FIGURE 4 Ribonucleotide backbone conformational map, ϕ,ψ. Plot of Σ' *versus* Σ for pyrimidine 5'-nucleotides, dinucleoside phosphates, and polyU

conformational properties of 5′-ribonucleotides, *i.e.* the population of the stable ψ_+ conformer (the so-called *gg* conformer) is proportional to the glycosidic bond *anti*-conformation. Although there is no direct confirmation of this suggestion at present, a number of lines of argument support the proposal.

(i) The correlation indicates that pyrimidine 5′-ribonucleotides [Σ 5—5.5 Hz; $p(\psi_+)$ 75—80%] have stable *anti* conformations in line with results from theoretical potential energy calculations,[3] *X*-ray crystal structures,[2] and many n.m.r. methods [δ methods,[34,35] ^5J(HH) in 5′-UMP,[36] $^3J(^{13}C, H1')$ measurements [37] and lanthanide ion probe methods [38,39]]. Indeed, semi-empirical potential energy calculations showed a striking correlation between glycosyl (χ) and backbone C(5′)–C(4′) bond (ψ) conformations with attractive interactions between 5′-phosphate group and the base-ring stabilising the *anti*-ψ_+ conformations in 5′-ribopyrimidine nucleotides.[3]

(ii) For purine 5′-ribonucleotides nuclear Overhauser enhancements [40] show that 5′-AMP (*ca.* 70%) has greater contributions of the *anti*-conformer than 5′-GMP (*ca.* 50%); at the same time the backbone C(5′)–C(4′) bond conformations of 5′-AMP [Σ *ca.* 6.5 Hz; $p(\psi_+)$ *ca.* 65%] are more stable than 5′-GMP [Σ *ca.* 7.5 Hz; $p(\psi_+)$ *ca.* 55%]. The difference in glycosidic bond *syn* ⇌ *anti* equilibrium between 5′-AMP and 5′-GMP has been confirmed by $^3J(^{13}C,H1')$ measurements [41] and semi-empirical theoretical potential energy calculations.[4,5]

(iii) Extrapolation of the *syn–anti* Σ′ *versus* Σ linear correlation indicates that the *anti*-conformation of purine or pyrimidine 5′-ribonucleotides might be further stabilised up to 100% contribution of the ψ_+ conformer (Σ *ca.* 3 Hz). The results for β-nicotinamide 5′-mononucleotide (NMN; Σ′ *ca.* 8.6 and Σ *ca.* 4.6 Hz, Figure 3) [20,26,27] are in line with this hypothesis because stabilisation of the ψ_+ conformer is caused by interaction between the negatively charged phosphate group and the base-ring with a formal positive charge. In a similar manner extrapolation shows that the proportion of the *syn*-type conformer may increase until complete destabilisation of the ψ_+ conformer (Σ *ca.* 13 Hz). The results for 5′-orotodylic acid (Σ 13 ± 2, Σ′ = 16 ± 2 Hz) [20,*] bear out this hypothesis assuming that the base-ring of the 5′-nucleotide adopts the *syn*-conformation as observed for orotidine by chemical shift,[42] ^5J(HH),[43] and $^3J(^{13}C,H1')$ measurements.[41]

(4) *Correlation of Effects for Monomers and Oligomers.*—The results for dinucleoside phosphates, a dinucleotide, a trinucleotide diphosphate, polyA, and polyU, do not conform to the *syn–anti* correlation observed for monomers; some points lie above and some below the dashed line as shown in Figures 3 and 4. In particular, the effect of temperature on dinucleoside phosphates (ApA, ApG, UpU, UpC, ApC, and CpG) [9,10] shows variations of Σ′ against Σ magnitudes with slopes (*ca.* 2—3) which indicate that both O(5′)–C(5′) and C(5′)–C(4′) bond conformers (ϕ_a and ψ_+) are destabilised by about the same amounts. This general trend is described by a proportional relationship between ψ_+ and ϕ_a conformational properties as shown in equation (3) where *k* and *b* are constants. Substitution of equations (1) and (2)

$$p(\phi_a) = kp(\psi_+) + b \qquad (3)$$

into (3) generates the linear relationship between Σ′ and Σ according to equation (4); this relation predicts the positive slope (for $k > 0$) found for both purine (Figure 3) and pyrimidine (Figure 4) nucleotide derivatives.

$$\Sigma' = 2.1k\ \Sigma + (25 - 27k - 21b) \qquad (4)$$

A number of solutions of equation (4) are compatible with the observed broad band of results though the wide variation can be explained, in part, by molecules being observed with different solution conditions (temperature, concentration, and pD).

Regression analysis of the Σ′–Σ results for aqueous solutions of purine 5′-nucleotide molecular fragments at the monomer and dimer level shown in Figure 3 indicates an approximately linear relation between $p(\psi_+)$ and $p(\phi_a)$ (correlation coefficient 0.95) with *k* 0.55 and *b* 0.49 excluding the result for polyA which was measured in 1 : 1 ^2H_2O–$(C^2H_3)_2SO$ and excluding one measurement of ApA which was considerably different from others for the same compound. Within the limitations of the measurements it can be seen that similar behaviour is observed for purine mononucleotides and the —pPur molecular fragment in homo- and hetero-dimers. The same conclusion can be made for the Σ′–Σ results of pyrimidine nucleotides in Figure 4 (correlation coefficient 0.91, *k* 0.61, *b* 0.40) except that the linear correlation is slightly different from that for purine nucleotides. Again, the results for polyU do not conform to those for monomers and dimers.

A preliminary analysis of this Σ′–Σ behaviour assumed [15] that there was a *ca.* 1 : 1 relationship between the populations of the most stable conformers for the φ and ψ bonds [*i.e.* $k = 1$ in equation (4)] and that variations in parameter *b* reflected differences in the glycosidic bond conformation, *i.e.* *b*(*syn*-type) *ca.* $0.4—0.5 > b$ (*anti*-type) 0—0.16. The present analysis shows that the linear correlations do not conform to a straightforward 1 : 1 dependence of $p(\phi_a)$ with $p(\psi_+)$ but that *k* is <1 and the magnitude of *k* differs for purine (0.55) and for pyrimidine (0.61) molecular fragments. The linear dependence of $p(\psi_+)$ and $p(\phi_a)$ indicates that the backbone conformational properties are inter-related which is a prerequisite of the existence in solution of a highly stable conformational unit for both purine and pyrimidine 5′-nucleotides and both —pPur and —pPyr molecular fragments of dimers and trimers.

Differences in the slope of $p(\phi_a)$ with $p(\psi_+)$ will reflect features other than backbone conformational properties; it is likely that these are the sugar ring and glycosidic bond equilibrium. We have seen that the glycosidic bond *syn* ⇌ *anti* equilibrium has a marked effect on the

* These results were not included in the *syn–anti* correlation of Figure 4 as they do not conform to the accuracy in *J* (±0.1 to ±0.2 Hz) reported for the other nucleotides.

Σ'–Σ behaviour (k *ca.* 0.25) and that the temperature dependence of dimers has k approaching unity. In the latter case the large changes in sugar ring conformational equilibrium have been used as a measure of base-stacking in dimers [15] according to the model of Altona.[8] The present interpretation of Σ'–Σ behaviour assumes, to a first approximation, that observed magnitudes of k reflect both the sugar ring conformational equilibrium ($k \longrightarrow 1$) and glycosidic bond equilibrium ($k \leqslant 0.25$); the results for purine (0.55) and pyrimidine (0.61) molecular fragments are consistent with the differences found in their glycosidic bond *syn* $\rightleftharpoons$ *anti* equilibrium as discussed in the previous section.

The inter-relation between ψ,ϕ backbone conformational properties of monomers and dimers discussed in this work applies to ribonucleotides. Preliminary analysis of data available for 5′-deoxyribonucleotides d(A, G, U, C, and T), a dinucleoside phosphate [d(TpT)],[44] a dinucleotide [d(TpTp)],[45] and a trinucleoside diphosphate [d(TpTpA)].[46] indicates that a similar correlation is observed for monomer and dimer results (k *ca.* 0.60, b *ca.* 0.42) and that little difference in behaviour is observed between purine and pyrimidine molecular fragments though this result might reflect the limited set of data available and the relatively poor correlation coefficient (0.88). At present there is not sufficient data available on deoxyribonucleotides to make any firm predictions about their backbone conformational behaviour.

The results for polymers do not conform to the trends found for their corresponding monomers and dimers; results for polyA lie above the line for purine molecular fragments (Figure 3) and the results for polyU lie below those for pyrimidine molecular fragments (Figure 4). The results for polyA may be explained in terms of a different conformational equilibrium being stabilised for 1 : 1 ^2H_2O–$(C^2H_3)_2SO$ compared with aqueous solutions because results for 5′-AMP are similar to those of the polymer in the same solvent [11] but different to those for 5′-AMP in aqueous solutions.[6,22,24,31] The results for polyU cannot be explained by a solvent effect as measurements were made in ^2H_2O solutions.[33] The present interpretation of Σ'–Σ behaviour in terms of magnitudes of k depending on the glycosidic bond and sugar ring conformational equilibrium would indicate that a significant population of the *syn*-conformer (or different χ_a region) is found for polymer compared to monomer and dimer.* The magnitudes of Σ and Σ' of polyU in the random-coil form have been calculated taking into account the probability distribution of the six backbone torsion angles using results of energy calculations based on various theoretical methods.[47] It was concluded that the backbone is fairly rigid with mean values of ϕ (*ca.* 180°) and ψ (*ca.* 60°) close to those for ϕ_a and ψ_+ conformations, respectively.[47] However no account was taken, in these calculations, of possible effects on backbone conformations of the base-ring *syn–anti* equilibrium and base-stacking interactions which are shown to be important from the distribution of results in Figure 4 and from calculations on purine ribonucleotides.[48]

The present interpretation of Σ and Σ' magnitudes indicates that the population of the stable nucleotide ϕ,ψ conformational unit depends on the position of the glycosidic bond *syn–anti* equilibrium, the furanose ring equilibrium, and/or base-stacking interactions not only in mononucleotides but also in oligo- and poly-nucleotides in solution. It was previously concluded that 5′-nucleotides [6] and dinucleotide phosphates [9,10] in solution exhibit a highly stable conformational unit akin to the ' rigid ' nucleotide conformation suggested from X-ray studies [1,2] and theoretical calculations [2,4] whereas, in this work, it is shown that the sugar–phosphate ψ and ϕ bonds are flexible with a direct correlation between the preferred conformations (ψ_+ and ϕ_a) of both bonds. The co-operative nature of these preferred conformers is necessary if the stable ψ_+,ϕ_a conformational state exists for monomers and oligomers in solution. Results for dinucleoside phosphates show that as the temperature increases there is a concomitant destabilisation of both ψ_a and ϕ_+ conformers; hence the base-stacking process is accompanied by further stabilisation of the backbone ψ_a,ϕ_+ conformational state compared to the unstacked form. The backbone conformational properties of, say, dinucleoside phosphates are the result of changes in base-stacking properties (Σ' displacements large compared to Σ, slope *ca.* 2—3) together with changes in *syn* $\rightleftharpoons$ *anti* equilibria (Σ' displacements small compared to Σ, slope *ca.* 0.5).

[8/565 *Received*, 29*th March*, 1978]

* It should be noted that the O(5′)–C(5′) bond conformational properties of polyU determined from ^{1}H n.m.r. [0.078M; Σ' 7.5 Hz;[33] $p(\phi_a)$ 82%] and ^{13}C n.m.r. [0.2M; $J(PC_{4'})$ 7 Hz,[25] $p(\phi_a)$ *ca.* 60%] do not agree though these differences may result from different conformational properties of polyU at the two different solution conditions. It may be fortuitous that results for polyU determined by ^{13}C n.m.r. [$p(\phi_a)$ *ca.* 60%, hence Σ' *ca.* 12.4 Hz] lie slightly above the linear correlation found for —pPyr molecular fragments.

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