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The Steric Structure of Nucleosides, Nucleotides, and Their Derivatives

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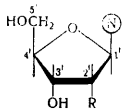
This review deals with the literature data on the conformations and intramolecular interactions of nucleosides, nucleotides, and their derivatives: nucleotide esters, nucleoside cyclic phosphates, oligonucleotides, nucleoside pyrophosphates (including nucleotide coenzymes), ATP, and nucleotidopeptides.
The problem of the degree of bending of the pentofuranose ring and its effect on the orientation of the ring substituents, including the effect on the structure of the *cis*-2',3'-glycol group, which is revealed in a number of specific reactions, is discussed together with the question of the relative positions of the base and sugar in nucleosides. The bibliography includes 268 references.

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I. INTRODUCTION

During the last decade an enormous amount of data on the properties and structure of nucleosides, nucleotides, and their derivatives has accumulated. It has been established unambiguously that nucleotides are as a rule *N*-glycosides of D-ribose or 2-deoxy-D-ribose†. In all nucleosides the carbohydrate residue is in the cyclic furanose form. The glycosidic centre in the nucleosides has the β-configuration (*trans*-position of C_1,-N and $C_{2'}$-$O_{2'}$ bonds or *cis*-position of C_1,-N and $C_{4'}$-$C_{5'}$)‡.



R = OH or H, (N) - purine or pyrimidine base

The studies on the determination of the configuration of nucleosides carried out with the aid of refined chemical methods by Todd's school are recognised as classical and are described in a number of reviews including reviews in the Russian language[1-3]. In recent years much attention has been devoted to physicochemical methods for the determination of the configuration of nucleosides, among which NMR spectroscopy[4-8] and spectropolarimetry[9-20] are undoubtedly promising.

At the present time the chemistry of nucleosides has reached a level such that it is necessary to investigate not only the configurations (i.e. the distribution of atoms about the dissymmetric part of the molecule—for example the asymmetric carbon atom or ring), but also the real geometrical formula of the molecules as a whole—their conformation. This problem is particularly important in connection with the study of various polyfunctional derivatives of nucleosides, in which the specific steric structure of the molecule can lead to the interaction and hence the modification of the properties of groups which are not directly linked.

†In this review we shall not deal with synthetic and natural anomalous nucleosides (the "minor" components of nucleic acids and nucleoside antibiotics), among which may be derivatives of sugars other than D-ribose and 2-deoxy-D-ribose and also *C*-glycosides.

‡The following abbreviations have been adopted in the present review: RNA and DNA—ribonucleic and deoxyribonucleic acid respectively; UMP—uridine 5'-monophosphate; ADP—adenosine 5'-diphosphate; ATP—adenosine 5'-triphosphate; NDPS—nucleoside diphosphate sugar; UDPG—uridine diphosphate glucose; NAD—oxidised nicotinamide-adenine dinucleotide; NADP—oxidised nicotinamide-adenine dinucleotide phosphate; NADH—reduced nicotinamide-adenine dinucleotide; FAD—flavine-adenine dinucleotide; THF—tetra hydrofuran; NMR—nuclear (here as a rule proton) magnetic resonance; ESR—electron resonance.

2. RIBOSE AND 2-DEOXYRIBOSE

The form of the nucleoside molecule is to a large extent determined by the conformation of the five-membered carbohydrate ring. The tetrahydrofuran ring, which constitutes the basis of ribose and 2-deoxyribose, is nonplanar like cyclopentane[21], since all its C–C bonds would then have the eclipsed conformation characterised by maximum energy[22]. Due to the effect of the oxygen atom in the furanose ring, more and less stable conformations appear when the ring is bent. It is readily seen that, when the C_2 or C_3 atom deviates from the plane of the ring, a greater number of bonds passes from the eclipsed to the *gauche*-conformation than when the C_1, C_4, or the oxygen atom deviates from the plane of the ring. Accordingly the

conformations with the C_2 or C_3 (or both) atoms outside the ring are more stable.

The deformation of the ring of D-ribose and its 2-deoxy-derivative probably occurs even more readily than in tetrahydrofuran, since the *gauche*-disposition of the OH groups and the CH_2OH group leads to an even greater energy gain, compared with the eclipsed disposition, than the *gauche*-disposition of the hydrogen atoms in tetrahydrofuran. Furthermore, it has been found that the average length of the C_2–C_3 bond in many ribose derivatives is 1.528 ± 0.008 Å[23] (i.e. is close to the value 1.533 ± 0.003 Å found for the ordinary C–C bond[24]). In this case with the eclipsed disposition of the 2- and 3-hydroxyls the distance between their oxygen atoms must be less than 2.5 Å. Since the van der Waals radius of oxygen is 1.40 Å,[25] evidently a planar conformation of the ribose ring is impossible[26].

By analogy with tetrahydrofuran, the most favourable conformations for ribose are those in which the C_2 or C_3 atoms deviate from the average plane of the ring[27,28]. This is the so called primary or main deformation of the furanose ring. The out-of-plane atom (C_2 or C_3) can be displaced to the side where the C_5 atom is located or to the opposite side. This corresponds to possible *endo*- and *exo*-conformations. The deviation of the out-of-plane carbon atom from the average plane of the ring is very considerable: 0.5–0.6 Å. It is interesting that the *endo*-conformation is most often encountered in nucleosides (see Table 1).

Here, however, one should note that the eclipsed or partially eclipsed conformation of the C_2,–C_3, bond in 2-deoxyribose is characterised by a lower strain compared with ribose and can probably actually occur in solution (because there is a hydrogen atom in the 2-position and not an OH group as in ribose). Indeed a conformation of the carbohydrate ring in which the ring oxygen atom is displaced from the average plane of the ring, and not the C_2, or C_3, atom, has been in fact observed with the aid of NMR spectroscopy for a number of deoxyribose nucleosides (thymidine, deoxyuridine, deoxyadenosine 3′-phosphate) (*O-endo*-conformation, see Table 1)[29,30].

Table 1. Conformation of the pentose ring in nucleosides and nucleotides.

Compound	X Ray diffraction data		NMR spectroscopic data	
	conformation	references	conformation	references
Ribose-5-phosphate	C_2,-*endo*	31		
Uridine	C_3,-*endo*	28		
Cytidine	C_3,-*endo*	28. 32. 33	C_3,-*endo*	48
5-Bromouridine	C_2,-*endo*	34		
Adenosine	C_3,-*endo*	28		
Adenosine-5-bromouridine complex	C_3,-*endo* C_3,-*endo*	35		
Uridine 5′-phosphate	C_2,-*endo*	36		
Uridine 3′,5′-cyclic phosphate	C_3,-*endo*	37		
Cytidine 3′-phosphate	C_2,-*endo*	38—41	C_3,-*endo*	48
Adenosine 5′-phosphate	C_3,-*endo*	42—44	C_2,-*endo*	48
Adenosine 3′-phosphate	C_3,-*endo*	45		
Adenosine 3′,5′-cyclic phosphate	C_4,-*exo*	46		
Adenylyl-(2′–5′)-uridine Adenosine residue Uridine residue	C_2,-*endo* C_3,-*endo*	47		
Thymidine	C_3,-*exo*	49 *	O-*endo*	29. 30
Deoxyuridine			O-*endo*	29. 30
5-Fluorodeoxyuridine	C_2,-*endo*	38		
5-Bromodeoxyuridine	C_2,-*endo*	34. 51		
5-Iododeoxyuridine	C_2,-*endo*	52		
Deoxyadenosine	C_3,-*exo*	53		
Deoxyguanosine-5-bromodeoxy-uridine complex	C_2,-*endo* C_2,-*endo*	54		
Thymidine 5′-phosphate	C_3,-*endo*	55		
Deoxyadenosine 5′-phosphate			O-*endo*	29. 30

*Thymidine is the only nucleoside whose structure was also investigated by ESR spectroscopy[50].

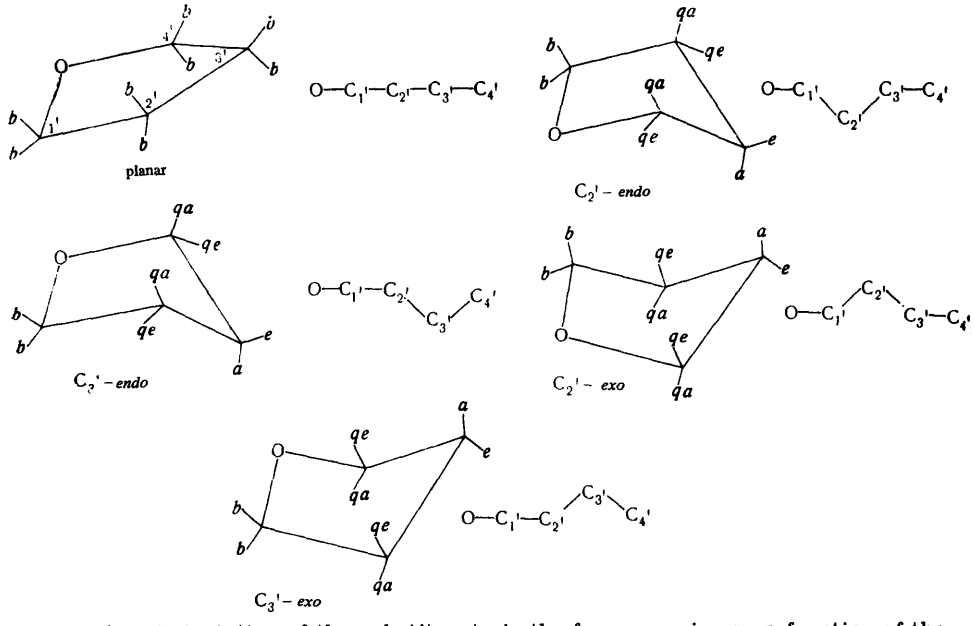


Figure 1. Orientation of the substituents in the furanose ring as a function of the nature of its bending (*a* and *e*—axial and equatorial respectively, *qa* and *qe*—quasi-axial and quasiequatorial, *b*—bisector orientation).

Apart from the primary (main) bending, secondary (minor) bending occurs in the ribose residue of nucleosides: as a rule, the four atoms of the ribose ring, determining its average plane, are also not completely coplanar. However, in this case the deviation is outside the limits of the experimental error, not exceeding 0.05 Å (i.e.8–10% of the primary bending)[38].

The consequences of the bending of the furanose ring in nucleoside are deviations of the C–C and C–O bond lengths from the normal values (up to ±0.11 Å) and of the valence angles from the tetrahedral value (up to ±9°)[21,28-53].

The external angles and bond lengths in the ribose ring also deviate from the normal values. As a result, the substituents at the carbon atoms of ribose are oriented as shown in Fig. 1. Here, however, one should note that, according to NMR spectroscopic data, the orientation of substituents depends to some extent on their nature: in the isomeric 2′- and 3′-*O*-benzylnucleosides the angle of twist between the $C_1,-H_1$, and $C_2,-H_2$, bonds is smaller in the 2′ isomer than in the 3′-isomer[56].

3. CONFORMATION OF THE HETEROCYCLIC BASE

X-Ray diffraction analysis of nucleosides and nucleotides showed that the heterocyclic bases are in many cases almost planar, i.e. the deviations of the ring atoms from the average plane are within the limits of experimental error[32,35,36,45,47,55] (see also Nyburg's review[57]).

However, a number of cases have been described in which the atoms of the base were found to deviate from coplanarity. The bending is particularly pronounced in pyrimidine derivatives. For example, in cytidine[38] and cytidine 3′-phosphate[21,30] the N_1 and C_4 atoms deviate from the average plane of the base to the same side§. The deviations amount to about 0.05 Å, so that the pyrimidine base adopts the form of a shallow boat. In purines the deviations of the heterocyclic ring atoms from the average plane are somewhat smaller.

At the same time the substituents in the heterocyclic rings of nucleotides and nucleosides (the amino-group, oxygen, the methyl group, the C_1, atom of pentofuranose) are displaced from the average plane of the ring very considerably. The O_2 and C_1, atoms (and also the O_4 and C_7 atoms in thymine) deviate to opposite sides[32,36,52]. The displacement of the C_1, carbon atom of ribose from the average plane of the heterocycl probably constitutes a general rule and reaches a considerable magnitude (up to 0.2–0.3 Å).[18,22,34,36,41,47] As a result the glycoside bond forms an angle with the plane of the base[34], which is 3.5° in adenosine 3′-phosphate for example.

4. RELATIVE POSITIONS OF THE BASE AND SUGAR

The relative positions of the base and sugar play an important role in determining the geometrical form of the nucleoside molecule. A clear albeit an incomplete idea of this is provided by the value of the dihedral angle between the average planes of the base and sugar. It is interesting to note that as early as 1947 Astbury believed, on the basis of the X-ray diffraction data obtained by Hendricks[58], that the planes of the heterocyclic base and the carbohydrate in nucleosides are parallel (quoted after Jordan[59]). However, in 1950 Furberg[28] showed that this conclusion is a result of an incorrect interpretation of the experimental data and that in fact the rings in nucleosides are approximately perpendicular to one another. This proved to be true of all the nucleosides and nucleotides investigated hitherto (see Table 2).

Table 2. Dihedral angles between the planes of the base and the sugar in nucleosides and nucleotides.

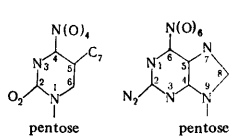
Compound	Angle, °	Refs.
Cytidine	76	21
Thymidine	89	49
	90	50
Deoxyadenosine	69	53
Adenosine-5-bromouridine complex	70 69	35
5-Iododeoxyuridine	81	51
Deoxyguanosine-5-bromodeoxycytidine complex	69 77	54
Adenosine 5′-phosphate	76	43
Adenosine 3′-phosphate	55	45
Thymidine 5′-phosphate	75	55
Uridine 3′,5′-cyclic phosphate	60,62 *	37
Adenosine 3′,5′-cyclic phosphate	138, 137 *	46

*The dihedral angles for the two conformations existing in a single crystal cell (for further details, see Russ. p. 232).

Evidently, for the same dihedral angle between the planes of the base and sugar, there is a possibility of rotation of the base about the glycosidic bond. Therefore, in order to characterise completely the relative positions of the base and sugar, it is necessary to know the magnitude of the angle of twist φ_{CN}. This is the angle formed by the projections of the bonds $C_1,-O_1$, and N_1-C_6 (for pyrimidines) or N_9-C_8 (for purines). If the N_1-C_6 bond of pyrimidine (or the N_9-C_8 bond of purine) and the $C_1,-O_1$, bond of the sugar are antiparallel, then φ_{CN} is believed to be 0°; positive angles are reckoned clockwise and negative angles anticlockwise looking in the direction from C_1, to N.[39,40]

There exist two regions of φ_{CN} angles in which relatively stable conformations may exist: the region with φ_{CN} about −30° (the *anti*-region) and the region with φ_{CN} about +150° (the *syn*-region). Each of these occupies somewhat more than 90°. Examination of molecular models[60] and also calculations based on the results of X-ray diffraction analysis[61] showed that the barrier between the *syn*- and *anti*-regions is determined by steric interactions between the N_3 atom of the base and the oxygen atom of the pentose ring in purine nucleoside and between the oxygen atom of the 2-oxo-group or the hydrogen at the C_6 atom of the base on the one hand and the oxygen atom of the pentose ring or the oxygen at the C_2, atom of the sugar (the hydrogen at the C_2, atom in deoxyribonucleosides) on the other in pyrimidine nucleosides. For most of the

§In this review the system of numbering of the pyrimidine and purine rings adopted in Chemical Abstracts is employed:



nucleosides and nucleotides investigated, the *anti*-orientation of the base is preferred (see Table 3). The *syn*-orientation is as a rule less favourable sterically. Furthermore, for purine nucleoside in which the C_3, atom deviates from the plane of the sugar and for pyrimidine nucleosides with the C_3,-*endo*-conformation of the sugar, the existence of the *syn*-isomers is altogether impossible[61]. However, it is evident that in pyrimidine O_2-5′-cyclonucleosides and in purine N_3,-5′-cyclonucleosides only the *syn*-orientation of the base is possible in consequence of the formation of an additional ring. This was confirmed by X-ray diffraction analysis[62] (see Fig. 2). Nor can one exclude the possibility that the necessary stabilisation of the *syn*-conformation is achieved in certain cases in the polynucleotide chain[61] or in nucleotidopeptides (see Section 12) due to non-covalent interactions.

Table 3. Orientation of the base and sugar in nucleosides and nucleotides.

Compound	φ_{CN}, *	Refs.
Cytidine	−18	32
5-Bromouridine	−56	34
Thymidine	−39	49
5-Bromodeoxyuridine	−43	34
5-Iododeoxyuridine	−67	52
Deoxyadenosine	− 3	51
Adenosine-5-bromouridine complex	−10 −20	35
Deoxyguanosine-5-bromodeoxycytidine complex	+138 −61	54
Uridine 5′-phosphate	−43	36
Uridine 3′,5′-cyclic phosphate	−72,−56 *	37
Thymidine 5′-phosphate	−48	55
Cytidine 3′-phosphate	−42.1	39
Adenosine 5′-phosphate	−18	43
Adenosine 3′,5′-cyclic phosphate	−5.0,+102 *	46
Adenylyl-(2′–5′)-uridine Adenosine residue Uridine residue	−55 −5	47

*See the footnote to Table 2.

There is a hypothesis that the *anti*-orientation of the base in ribonucleosides may be stabilised by the formation of a hydrogen bond between the 2′-hydroxy-group of ribose and the 2-oxo-group of pyrimidine or the N_3 atom of purine. This hypothesis is supported by a number of factors: (1) the ultraviolet absorption spectra of pyrimidine ribonucleosides change appreciably in a highly alkaline medium; such changes were not observed for the corresponding nucleoside 2′-phosphates[63–68]; (2) the basicity of the heterocycle in ribonucleosides is lower than in the corresponding 2′-deoxyribonucleosides[63,64,68–70]; (3) in the infrared spectrum of 3′,5′-di-*O*-acetyladenosine there is an absorption band corresponding to an intramolecular hydrogen bond; a band of this kind is absent from the spectrum of 2′,5′-di-*O*-acetyladenosine[71]; (4) the 2′-hydroxy-group of the ribose residue in nucleosides is more acid than the 3′-hydroxy-group[67,72–75]—this has a significant influence on a number of reactions: in the methylation of ribonucleosides with diazomethane 2′-*O*-methylnucleosides are formed preferentially[76–79] and in the tritylation of uridine[80–83] and tosylation of 5′-*O*-trityluridine[84] the 2′-hydroxy group is attacked appreciably faster than the 3′-hydroxy group; (5) the study of the rate of hydrolysis of a number of ribodinucleotides also led Witzel to the hypothesis of the existence of the hydrogen bond C_2,–O–H...O=C_2 in pyrimidine nucleosides and C_2,–O–H...N_3 in purine nucleosides[85,86]; (6) in the latest papers from Ts'o's laboratory[87] the results of an NMR spectroscopic comparison of adenosine with 2′-deoxyadenosine and 2′-*O*-methyladenosine and of pyrimidine ribonucleoside 5′-phosphates with 2-deoxyribonucleoside 5′-phosphates are regarded as evidence of the existence of a hydrogen bond between the 2′-hydroxy-group of the sugar and the 2-oxo-group of pyrimidine or the N_3 atom of purine.

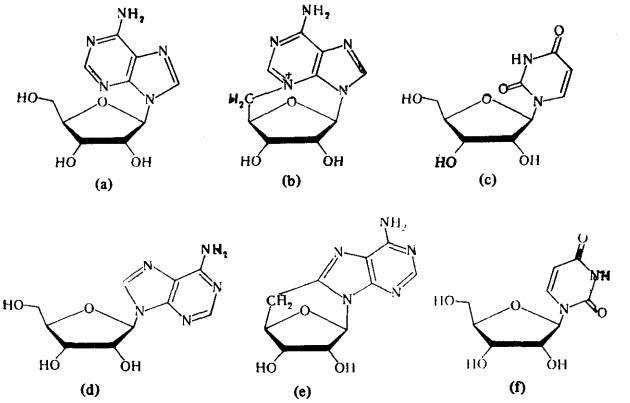
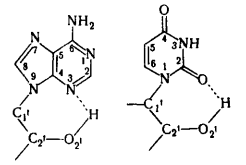


Figure 2. *Syn*- (a, b, and c) and *anti*- (d, e, and f) orientation of the base.

The latter type of hydrogen bond was postulated by Zamecnik[88] in the discussion of the mechanism of the formation of aminoacyl-*t*-RNA from aminoacyladenylates[88].



However, there are a number of data inconsistent with the hypothesis of the existence of such an intramolecular hydrogen bond. Thus in the study of the photochemical addition of water to the 5,6-double bond in pyrimidine nucleosides comparison of the quantum yields for cytidine, 2′-deoxycytidine and cytidine 2′-,3′-, and 5′-phosphates led to the hypothesis that a hydrogen bond forms between the base and the 5′-hydroxy-group of ribose[89,90]. Pitha et al.[91], who observed in the infrared spectra of 2′,3′-di-*O*-substituted nucleosides absorption bands corresponding to a hydroxy-group involved in the formation of a hydrogen bond with the heterocycle, arrived at the same conclusion. Izatt et al. observed a sharp decrease of the acidity of the secondary hydroxyl both in 2′- and in 3′-deoxy-(and methyl-)adenosine and on this basis suggested that a hydrogen bond between 2′- and 3′-hydroxy-groups exists in adenosine[74,75]. It should be noted, however, that this hypothesis does not explain precisely why the 2′-hydroxy-group is the more acid. Finally, on the basis of the determination of the dissociation constants of the 5′-hydroxy-group in the α- and β-isomers of pseudouridine (5-ribofuranosyluracil), Ofengand and Schaefer[92] concluded that the C_5,–O–H...O=C_4 hydrogen bond forms in the natural β-isomer.

5. CONFORMATION OF THE *cis*-GLYCOL GROUP C_2,(OH)–C_3,(OH)

The *cis*-glycol group C_2,(OH)–C_3,(OH) determines a number of important properties of ribonucleosides. Its steric structure can be characterised by the magnitude of the angle of twist φ_{00} between the C_2,–O_2, and C_3,–O_3, bonds. The angle of twist between the true *cis*-bonds is 0°. However, in nucleosides, at least in the solid state, the 2'- and 3'-hydroxy groups deviate from the true *cis*-conformation, which is a consequence (or the cause) of the bending of the ribose ring (see above), and the angle of twist φ_{00} is 43–54°.[23,45] Nevertheless the angle of twist φ_{00} is appreciably less for ribose and other sugar derivatives with the *cis*-glycol group than for sugars with the *trans*-disposition of the 2- and 3-hydroxy-groups, for which the minimum value of φ_{00} is 75° [38] (but not 180° as for the true *trans*-configuration). Thus, as a result of the deformation of the pentose ring, the difference between the values of φ_{00} for the *cis*- and *trans*-isomers is not as great as might have been expected, but it is sufficient to account for the fundamental differences in the reactivities of the compounds. It is known, that in contrast to *trans*-2,3-sugars, ribose derivatives readily react with formation of 2,3-cyclic compounds. A wide variety of 2',3'-alkylidene (isopropylidene, benzylidene, *p*-anisylidene, etc.) derivatives of nucleosides (see, for example, Refs. 1, 2, and 93–101), 2',3'-orthoformates[102] and orthocarbonates[103], 2,3'-carbonates and thiocarbonates[103–112], 2',3'-phenylboronates[113,114], 2',3'-cyclic phosphates (see, for example, Refs. 1, 2, and 114–119), and 2',3'-cyclic phosphites of nucleosides[120] and also of 2',3'-epoxynucleosides[1,121,122] have been described¶. The high rate of oxidation of ribonucleosides with lead tetra-acetate, osmium tetroxide, and periodate, is associated with the ease of formation of intermediate 2',3'-cyclic derivatives[38,123].

The lability of nucleoside 3'-phosphate esters in an alkaline medium is explained by the presence of an adjacent *cis*-hydroxy-group (see, for example, Refs. 1, 2, 93, and 124–126). The elucidation of this effect of the 2'-hydroxy-group assisted in the determination of the structure of RNA (see, for example, Refs. 1, 2, 93, 127 and 128).

There is a hypothesis that the presence of the *cis*-hydroxyl contributes to enhanced activity of the ester group in the *O*-aminoacyl derivatives of adenosine and *t*-RNA in consequence of the formation of a hydrogen bond between the 2'-hydroxy-group and the carbonyl oxygen of the aminoacyl residue[87–128]. In the majority of ribonucleosides and nucleotides in the crystalline state, the C_2,–O_2, and C_3,–O_3, bonds were found to have the *tg*-conformation, i.e. the O–H bond (O–P bond in 3'-phosphate) is oriented *trans* relative to the C_2,–C_3, bond and its orientation is *gauche* relative to the C_2,–C_1, bond or correspondingly the C_3,–C_4, bond (see Fig. 3).

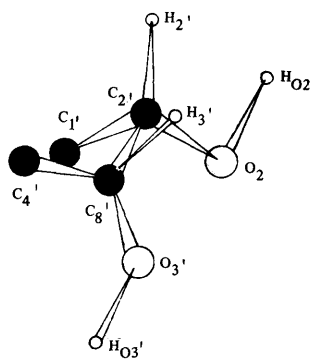


Figure 3. Conformation of the *cis*-glycol group (C_2,-*endo*-bending of the ribose ring).

6. CONFORMATION OF THE C_4,–C_5, BOND

For the exocyclic carbon–carbon C_4,–C_5, bond in the crystalline state, three types of conformation were observed (see Table 4 and Fig. 4): (a) *gg*—*gauche*-orientation of the C_5,–O_5, bond relative to the C_4,–O_1, bond and relative to the C_4,–C_3, bond; (b) *gt*—*gauche*-orientation of the C_5,–O_5, bond relative to the C_4,–O_1, bond and *trans*-orientation relative to the C_4,–C_3, bond; (c) *tg*—*trans*-orientation of the C_5,–O_5, bond relative to the C_4,–O_1, bond and *gauche*-orientation relative to the C_4,–C_3, bond.

Table 4. Conformation of the C_4,–C_5, bond in nucleosides and nucleotides

Compound	conformation	$\varphi_{OO'}$, °	$\varphi_{OC'}$, °	Refs.
Ribose-5-phosphate	*gg*	67	56	31
Cytidine	*gg*			28, 32
Thymidine	*gt*			49
5-Fluorodeoxyuridine	*tg*	173	68	52
Deoxyadenosine	*gt*	68	173	23
Uridine 5'-phosphate	*gg*	54	70	36
Uridine 3',5'-cyclic phosphate	*tg*	176,174*	63,60*	37
Cytidine 3'-phosphate	*gg*	75	44	39,40
Thymidine 5'-phosphate	*gg*	60	57	55
Adenosine 5'-phosphate	*gg*	78	40	42, 43
Adenosine 3'-phosphate	*gt*	56.7	171.7	45
Adenylyl-(2'–5')-uridine				
Adenosine residue	*gg*	73	45	47
Uridine residue	*gg*	62	57	

*See the footnote to Table 2.

As shown in Table 4, the *gg*-conformation is encountered most frequently, at least in the crystalline state. Probably this is because in this conformation the intramolecular interaction between the substituent at the C_5, atom, the base, and the sugar ring is a minimum[21].

7. STRUCTURE OF THE PHOSPHATE RESIDUE IN NUCLEOTIDES

It has been found for the crystals of all the nucleotides investigated that, when the 5'-hydroxy group is esterified by a phosphate residue (nucleoside 5'-phosphate), the O_5,–P bond occupies the *trans*-position relative to the C_4,–C_5, bond, thus ensuring the most extended form of the molecule.

¶Owing to the large number of studies in the field of 2',3'-cyclic derivatives of ribonucleosides, it is not claimed that the selection of literature on this problem presented here is exhaustive.

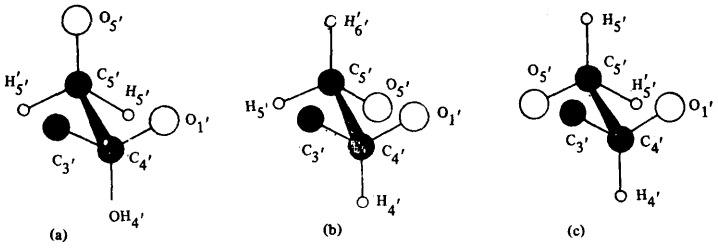


Figure 4. Conformation of the C_4,–C_5, bond: a) *gg*; b) *gt*; c) *tg*.

The phosphate residue in nucleotides in the crystalline form and in neutral solutions is present at the monoanion (this applies to both 3'- and 5'-phosphates). The P–O distance in nucleotides, as in other phosphoric acid esters[129–131], proved to be much shorter (by 0.24–0.09 Å[23,36,38,43–45]) than the calculated value of 1.71 Å,[132] the bond length decreasing in the following sequence: P–OR > P–OH > P–O' > P=O. This confirms the presence in phosphoric acid esters of π-conjugation between the *d*-orbitals of phosphorus and the *p*-electrons of oxygen[133,134]. In other words, the phosphorus–oxygen bond in phosphoric acid esters has partial double bond character[133]. The decrease of the double bond character between the phosphorus and oxygen as a result of the breakdown of p_π–d_π conjugation results in a significant weakening of the phosphate ester bond. This happens, for example, in nucleoside cyclic phosphates.

8. NUCLEOSIDE CYCLIC PHOSPHATES

Nucleotides can form both five-membered 2',3'-cyclic phosphates and six-membered 3',5'-cyclic phosphates. The five-membered phosphate rings are strained[1,135], which is the main reason for their extremely great reactivity compared with non-cyclic-esters[1,93,135,136].

Probably in consequence of the strain of the five-membered phosphate ring, p_π–d_π conjugation between the oxygen and phosphorus breaks down, which is the reason for the lability of the P–O bond[134]. The much greater stability of the six-membered nucleoside 3',5'-cyclic phosphates is evidence of a lower strain of the ring. However, it follows from a comparison of the time required for 50% decomposition in 1 *N* sodium hydroxide at 100°C of thymidine 3',5'-cyclic phosphate, in which the six-membered phosphate ring is formed with participation of the *trans*-hydroxy-groups[137], 1,2-isopropylidenexylofuranose-3,5-cyclic phosphate, in which a phosphate ring of the same size is formed by *cis*-hydroxy-groups[136,138], and propane-1,3-diol cyclophosphate[136] (2,4, and 72 h respectively) that in nucleoside 3',5'-cyclic phosphates the phosphate ring is also strained, probably as a result of the *trans*-orientation of the C_4,–C_5, and C_3,–O_3, bonds relative to the pentose ring. Because of this configuration of the sugar, the only possible form of the six-membered phosphate ring is the chair conformation. For it to exist, the nature of the bending of the furanose ring must be altered and the C_3,-*endo*-C_4,-*exo*-conformation is the most probable[48,139]. Indeed, studies with the aid of X-ray diffraction showed that in the conversion of uridine 5'-phosphate into uridine 3',5'-cyclic phosphate the conformation of ribose changes from C_2,-*endo*-[36] to C_3,-*endo*-[37] and in the conversion of adenosine 5'-phosphate into adenosine 3',5'-cyclic phosphate the change is from C_3,-*endo*-[42,44] to C_4,-*exo*-[46] (Table 1). The change of the conformation of pentose probably entails the occurrence of some strain in the furanose ring. This is believed[136] to be the cause of the lowered stability of the glycoside bond in nucleoside 3',5'-cyclic phosphates compared with the corresponding nucleosides and nucleotides. On treatment with 1 *N* sodium hydroxide at 100°C, adenosine 3',5'-cyclic phosphate yields adenine, while under these conditions adenosine itself is stable[140]; thymidine 3',5'-cyclic phosphate splits off thymine completely on treatment with 0.1 *N* hydrochloric acid at 100°C for 5 min, while thymidine 5'-phosphate remains stable for more than 3 h under these conditions[136].

An interesting feature of the structure of nucleoside 3',5'-cyclic phosphates is the presence in the same crystal cell of two molecules with different conformations of the glycoside bond; in uridine 3',5'-cyclic phosphate both molecules have the *anti*-orientation of the base relative to the sugar[37], but in adenosine 3',5'-cyclic phosphate one molecule has the *anti*-orientation and the other the *syn*-orientation[46] (so far this is only the second, after deoxyguanosine, case of *syn*-orientation, see Table 3).

9. INTRAMOLECULAR INTERACTIONS IN NUCLEOTIDE ESTERS

When nucleotide esters other than cyclic phosphates are examined, the dependence of the properties of the phosphodiester group on the nature of the substituents in the sugar ring is striking. Thus it is known that *p*-nitrophenyl esters of various nucleotides are hydrolysed in different ways: *p*-nitrophenyl esters of thymidine 3'- and 5'-phosphates are hydrolysed with alkali via the intermediate formation of thymidine 3',5'-cyclic phosphate[141,142], while this behaviour was not observed for the *p*-nitrophenyl ester of uridine 5'-phosphate[142]. The different mechanisms of hydrolysis may be associated with the different conformations of the pentose ring, which may favour or hinder the mutual approach of the 3'-hydroxy-group and the 5'-phosphate group. In uridine 5'-phosphate and thymidine 5'-phosphate crystals different conformations of the carbohydrate ring have indeed been observed (see Table 1). Unfortunately the data are so far insufficient to confirm or reject this hypothesis.

The interaction of hydroxy- and phosphate-groups in *trans*-positions occurs also in other nucleotide derivatives. For example the formation of adenosine 3',5'-cyclic phosphate in the hydrolysis of ATP with barium hydroxide[140],

and the conversion of the methyl ester of thymidylyl-(3′–*N*)-phenylalanine into thymidine 3′,5′-cyclic phosphate on standing in dimethylformamide[143] have been described (for a more detailed treatment, see Section 12).

10. DINUCLEOSIDE PHOSPHATES AND OLIGONUCLEOTIDES

Dinucleoside phosphates constitute a special group of nucleotide esters. The study of adenylyl-(2′–5′)-uridine by X-ray diffraction revealed almost parallel disposition of the two bases and their partial overlapping[39] (Fig. 5). The study of solutions of dinucleoside phosphates and di-, tri- and oligo-nucleotides shows that the parallel orientation of the bases is most likely for these compounds also in solution.

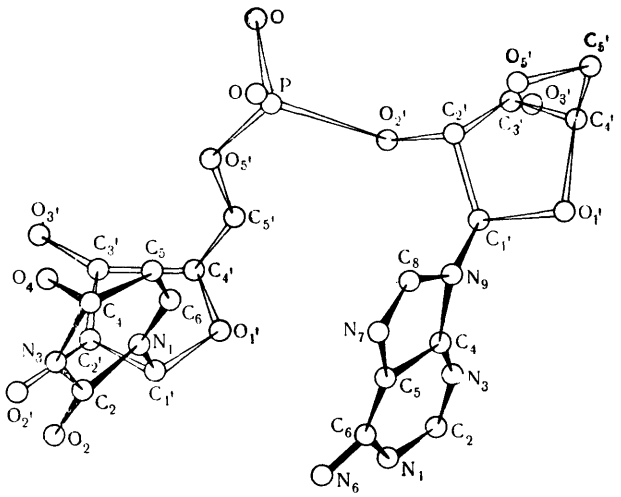


Figure 5. Model of the adenylyl-(2′–5′)-uridine molecule according to X-ray diffraction analysis[47].

Oligonucleotides have a number of specific properties: (1) on degradation of nucleotides, the intensity of ultraviolet absorption increases (hyperchromic effect) (see, for example, Refs. 1, 89, 144, and 145); (2) spectropolarimetry of oligonucleotides reveals a powerful cotton effect, which is not equal to the sum of the effects of the component mononucleotides, and the independence of the specific rotation of oligonucleotides of concentration[16,17,145–152]; (3) the circular dichroism of oligonucleotides does not agree with the sum of the effects associated with the circular dichroism of the component nucleotides[153,154]; (4) the fluorescence spectra of di- and poly-nucleotides and of the component monomers are qualitatively different[155,156]; (5) the NMR spectra of deoxyoligonucleotides reveal a shift of the proton signals from thymine relative to the mononucleotide[157].

The facts enumerated above indicate the occurrence of powerful interaction between the bases in oligonucleotides, which is shown even in dinucleotide phosphates. Several types of such interaction are possible, for example horizontal association via hydrogen bonds or vertical association as a result of π-electron interactions between the heterocyclic systems. Since dinucleoside phosphates which are derivatives of dimethylaminopurine exhibit a greater hyperchromism at all pH values than the adenine derivatives[158], it is unlikely that the interaction of the bases is determined by hydrogen bonds, particularly since it has been shown for diadenylic acid, for example, that the energy of interaction between the bases exceeds the energy of the hydrogen bond[157,159]. Furthermore, the disappearance of hyperchromism in dinucleotides such as adenylyl-(3′–5′)-uridine and adenylyl-(3′–5′)-cytidine 3′-phosphate on saturation of the 5,6-double bond by hydrogenation[160] or photolysis[161] demonstrates the important role of the π-electron systems of the bases in their interaction.

Finally, the study of aqueous solutions of mononucleotides, nucleosides, and purine and pyrimidine bases by NMR spectroscopy[162–165], osmometry[166,167], and ultracentrifugation[169] revealed a high degree of association of their molecules. It was then established that the interaction between the purine derivatives is favoured more than the interaction between purine and pyrimidine derivatives and the latter is in turn favoured more than the interaction between pyrimidine derivatives. On this basis, it was concluded that the mechanism of association consists in the arrangement of the bases in a pile, one on top of the other, as a result of the hydrophobic interactions between the π-electron systems of the bases. It is interesting that guanosine 3′- and 5′-phosphates and isoguanosine [6-amino-2-hydroxy-9-(β-D-ribofuranosyl)-purine] are believed to be arranged in a pile in the form of hydrogen-bonded tetramers with generation of a spiral ordered structure, which leads to the formation of a gel[169–175].

Association complexes of monomers in solution are not broken down by water molecules. This is explained by the presence of strong water–water hydrogen bonds. On the other hand, in dimethylformamide and in dimethyl sulphoxide such hydrogen bonds are not formed because of the absence of proton donors in the molecules of these solvents. However, since they are proton acceptors, they form hydrogen bonds with the molecules of the solute, breaking down its ordered structure[163].

The interaction between bases takes place, and actually to a greater extent than between monomers, in oligo- and poly-nucleotides. In these substances the interaction between the π-electrons of adjacent rings essentially gives rise to a new electronic system, which in fact determines the specific optical properties of the entire molecule[1,176,177].

The study of homogeneous oligo- and poly-nucleotides substituted in the heterocyclic ring (see, for example, Refs. 159 and 178–187) showed that the stability of the secondary structure of polymers increases somewhat on substitution, although at the same time the capacity for the formation of a double spiral with a complementary chain may be reduced. Probably hydrophobic interactions rather than hydrogen bonds play a decisive role in the stabilisation of the secondary structures of DNA and RNA (see, for example, Refs. 187 and 188 and also Tikhonenko's review[189])†.

†This review does not deal with the interactions between bases arising as a result of the formation of hydrogen bonds in accordance with the complementarity principle, since there is an extensive literature, including reviews, on this question, closely related to the study of the structure and functions of nucleic acids, which is outside this scope of the problem discussed here.

The significant role of hydrophobic forces and the stabilisation of the secondary structure of polynucleotides is also indicated by the observed association of purines with dinucleotides[190]: probably purine penetrates the dinucleotide molecule between the bases of the latter and, as a result of π-electron interaction with each of these, it, as it were, draws them together, forming a structure similar to a three-layer sandwich. In this connection there is an interesting hypothesis[191] that the possible cause of the biological activity of the notorious drug thalidomide is its similarity to nucleosides as regards capacity for hydrophobic interactions, as a result of which the thalidomide molecule may penetrate between the bases of DNA, altering its secondary structure.

11. DINUCLEOSIDE PYROPHOSPHATES AND NUCLEOTIDE COENZYMES

Evidently the interaction of the π-electron systems of the bases can also occur in P^1P^2-dinucleoside pyrophosphates. In addition, since the pyrophosphate link is more flexible than the phosphate link, it probably ensures a closer approach and maximum overlapping of the bases, which is reflected in larger hyperchromic effects of these compounds compared with dinucleotides[150,163].

An analogous phenomenon is observed also for other dinucleoside pyrophosphates, in particular the nucleotide coenzymes (flavine-adenine dinucleotide, nicotinamide-adenine dinucleotide). In the hydrolytic or enzymic dissociation of the pyrophosphate bond in FAD and NADH the ultraviolet absorption increases[192-197], which is evidence of interaction between the bases. This is confirmed also by fluoroscopic data. It has been found that nicotinamide forming part of reduced NAD fluoresces not only at 340 nm, as in the free state, but also at 260 nm,[198-201] the fluorescence being quenched by the addition of methanol to the solution of NADH.[202] On the other hand, the riboflavin forming part of FAD fluoresces more weakly than in the free state[203]. Furthermore the fluorescence of riboflavin is quenched on addition to its solution of purines[204-206], phenols[207], and chlorotetracycline[208]. By analogy with oligonucleotides, it was suggested that the interaction of the π-electron systems of adenine and the reduced nicotinamide or riboflavin is accompanied by an energy transfer[195,202,205] (see also Beinert's review[209]). The formation of such a complex is confirmed by spectropolarimetry[210,211] and NMR spectroscopy[212,213], and it has been shown at the same time that the π-complex form of NADH may be in equilibrium with an uncoiled form and that in certain enzymic reactions complete uncoiling of the coenzyme molecule takes place[213] (see Fig. 6).

The hypothesis of the π-electron nature of the interaction between the bases in nucleotide coenzymes is supported also by certain results of studies on their analogues: energy transfer, albeit much less effective than for NADH, has been observed for nicotinamide-uracil dinucleotide[214] and nicotinamide-phenol dinucleotide[215], while for nicotinamide-purine dinucleotide[216], nicotinamide-6-(β-hydroxyethylamino)purine dinucleotide[217], and nicotinamide-α-adenine dinucleotide[218] energy transfer almost as effective as for NADH has been observed. However, on the other hand, the absence of fluorescence in reduced nicotinamide-hypoxanthine dinucleotide[219] and the decrease of the fluorescence on alkylation of riboflavin in FAD at N_3 [220-222] may be regarded as evidence in favour of a considerable role of hydrogen bonds in the intramolecular association of the bases in nucleotide coenzymes.

It is most likely that intramolecular complexes in NADH and FAD are due to the interaction of the π-electron systems of the bases and are stabilised by hydrogen bonds.

An intramolecular complex, in the formation of which hydrogen bonds play the main role, is formed in nucleotide coenzymes of another type—nucleoside diphosphate sugars.

Examination of various natural NDPS [223,224] revealed a definite correspondence between the structure of the heterocyclic base and that of the residue of the nucleoside monosaccharide. Furthermore, there is a relation between the nature of the nucleotide residue in the NDPS and the type of biochemical reactions in which the NDPS is involved[223,224]. Since the NDPS react via the non-nucleoside monosaccharide residue, it was suggested that the specificity of the biochemical properties of NDPS is associated with the conformation of the monosaccharide, which in turn is determined by the structure of the nucleoside. The nucleoside residue may affect the structure of the monosaccharide residue as a result of the formation of intramolecular bonds with the latter, hydrogen bonds being the most likely. The equatorial orientation of the hydroxy-groups at the $C_{2''}$ and $C_{3''}$ atoms of the monosaccharide observed in all NDPS suggested that such bonds may arise between the –NH–CO–group in positions 3 and 4 of the base and the hydroxy-groups at the $C_{2''}$ and $C_{3''}$ atoms of the sugar (see, for example, Fig. 7). For uridine diphosphate sugars, there is also a possibility of a hydrogen bond between the 2-oxo group of the base and the 2′-hydroxy-group of ribose, as in other uracil ribonucleotides (see above).

The study of the activity of various synthetic analogues of uridine diphosphate glucose in enzymic reactions typical

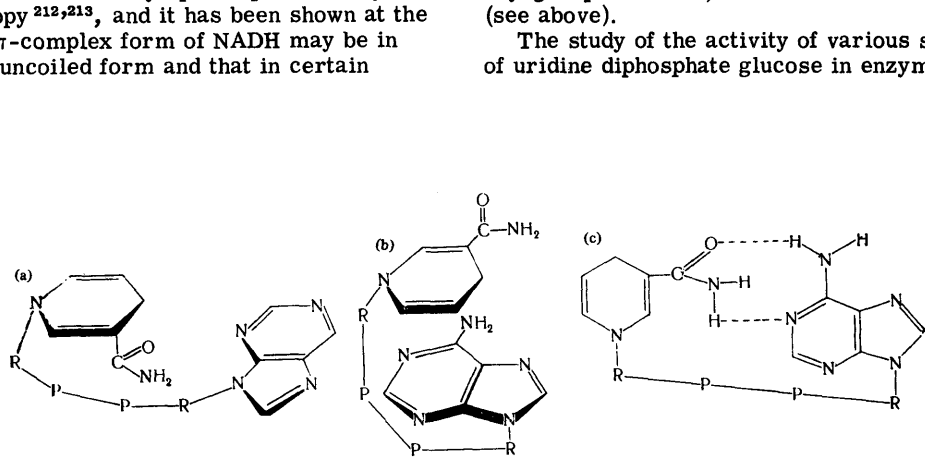


Figure 6. Possible forms of the NADH molecule: a) uncoiled; b) with vertical association of the bases; c) with hydrogen bonds[213].

for it[225-230] confirms the hypothesis that the specificity of biochemical reactions with participation of NDPS is determined by the steric structure of the NDPS molecule, since all the active analogues of UDPG contain the same grouping NH–C=O (or NH–C=S) in positions 3 and 4 of the heterocycle and consequently they can form similar intramolecular complexes.

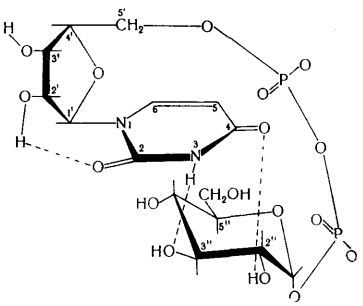


Figure 7. Schematic representation of the steric structure of the UDPG molecule[223].

The hypothesis of an intramolecular bond between the heterocycle and the sugar in NPDS was confirmed chemically by studying the rate of the catalytic hydrogenation (rhodium on alumina) of the carbon–carbon 5,6-double bond in UDPG and UMP-5'.[217] The considerable retardation of the reaction involving UDPG compared with UMP may be regarded as evidence for a significant difference in the steric accessibility of the double bonds in these compounds for the addition of hydrogen or for fixation on the surface of the catalyst, in consequence of their conformational differences. Similar results were obtained for 6-aza-UDPG and 6-aza-UMP, while *N*-methyl-UDPG, CDPG, and 2'-deoxy-UDPG are hydrogenated at the same rate as the corresponding nucleoside monophosphates[231]. The decrease of the differences in the rate of hydrogenation of UDPG and UMP in the presence of 7 M urea may be due to the breakdown of the hydrogen bond, which weakens the specific steric structure of the molecule[231].

The interaction of the base with the part of the molecule remote from it takes place in NDPS, as in FAD and NADH, because of the presence of the diphosphate unit which is fairly flexible due to the freedom of rotation characteristic of single bonds. A similar unit is present also in other nucleotide coenzymes, including nucleoside di- and triphosphates, among which adenosine 5'-diphosphate (ADP) and adenosine 5'-triphosphate (ATP) undoubtedly merit the greatest attention. Szent-Györgyi put forward the hypothesis[232] (see also later textbooks translated into Russian[233,234]) that the ADP molecule is "folded" in such a way that the terminal phosphate groups are in the immediate vicinity of the N_6 and N_7 atoms of adenine, forming with the latter a tetradentate chelate compound, using Mg^{2+} as a connecting link. A number of indirect infrared[235-237] and ultraviolet[238] spectroscopic and spectropolarimetric[239-240] data have been interpreted as favouring this hypothesis, so that it received wide recognition and the structure proposed by Szent-Györgyi for ATP may be found in many textbooks and reviews (see, for example, Refs. 241 and 242). Nevertheless the attempts to obtain direct proof of the interaction between Mg^{2+} and adenine were unsuccessful[243-252], although there is no doubt that a complex of Mg^{2+} with the phosphate group is really formed[243-255] (see also Bock's review[256]). In this connection it is suggested that in ADP and ATP the phosphate groups (via Mg^{2+}) and adenine form separate bonds with enzymes[244,256,257]. At the same time it should be noted that NMR spectroscopic data for ATP complexes with divalent zinc[245,247] and copper[245,246] ions are not inconsistent with the structure of the type proposed by Szent-Györgyi, i.e. a chelate complex of the metal ion with the nitrogen atoms of adenine and two phosphate groups, and it has been found that for the zinc complex the participation of the β- and γ-phosphate groups is more likely while for the copper complex the α- and β-phosphate groups are more likely to be involved. In addition, it has been established that Mn^{2+} may interact with adenine and with α-, β-, and γ-phosphate residues[245]. Probably Mn^{2+} can form complexes of different types. It is suggested that this capacity of the Mn^{2+} ion may explain why it can replace both Mg^{2+} and Zn^{2+} in enzymic reactions specific with respect to the ions of these metals[245]. The conformation of the possible complexes of ADP and ATP with metal ions is discussed in detail in other reviews[258,259].

12. INTRAMOLECULAR INTERACTIONS IN NUCLEOTIDOPEPTIDES

The amino-acid and peptide derivatives of nucleotides with a covalent bond between the fragments (nucleotidopeptides) play an important role in metabolic processes (see, for example, Refs. 260, 261). Unfortunately, from the chemical standpoint these compounds have been inadequately investigated. An exception are the nucleotidopeptides with a phosphoramidic nucleotidopeptide bond, for which the relation between the structure and chemical properties has been investigated systematically[262-268].

The polyfunctional nature of nucleotido-(P–N)-peptides and the large number of single bonds permitting a wide variety of relative positions of the individual parts of the molecule impart to the molecule a considerable capacity to assume the conformation suitable for intramolecular interactions between groups which are not directly linked. The effect of these interactions on the properties of the nucleotidopeptide bond may be very great because of the specific properties of both the phosphorus atom (capacity for p_π–d_π conjugation with atoms which are not directly linked to it) and the nitrogen atom (tendency to undergo protonation not only by the protons from the medium but also from the proton-donor groups of the same molecule).

The study of nucleotidyl-(5'–N)-amino-acids, in which the amino-acid residue does not contain additional functional groups, showed that the 5'-phosphoramidate bond in these compounds is readily hydrolysed in an acid medium and is stable in neutral and alkaline media[262-266] as in simple amides of phosphoric acid and its esters. However, in a weakly acid medium (pH 2.5–5) the hydrolytic stability of the phosphoramidate bond depends on the nature of the heterocyclic base of the nucleotide[262,263]. Examination of the structure of nucleotidyl-(5'–N)-amino-acids led to the hypothesis that the interaction of the base with the phosphoramino-acid residue takes place via hydrogen bonds, as shown in Fig. 8, for example. Although the *anti*-orientation of the base is typical for nucleotides, as shown on Russ. p. 227, it is possible that in this case (schemes a, b, and d) the energy required to overcome the barrier between the *anti*- and *syn*-regions is compensated by the formation of hydrogen bonds.

Some confirmation of the proposed structures was obtained by infrared and ultraviolet spectroscopy[262], but more detailed information about the intramolecular interactions in nucleotide-(P–N)-peptides was obtained by the study of their hydrolytic stability[262–268]. Fig. 8 shows that in the amino-acid derivative of guanosine 5′-phosphate (b) the phosphoramidate nitrogen is protonated even in a neutral medium, which probably accounts for the higher rate of its hydrolysis compared with the uridine 5′-phosphate derivative. On the other hand, adenylyl-(5′–N)-phenylalanine and uridylyl-(5′–N)-phenylalanine have similar stabilities, as was to be expected on the basis of the structures (a), (c), and (d) in Fig. 8. The decrease of the differences between the rates of hydrolysis of the phosphoramidate bond in phenylalanine derivatives of various nucleotides on raising the temperature[263] and on increasing the acidity of the medium[262,263] may be a consequence of the breakdown of specific hydrogen bonds.

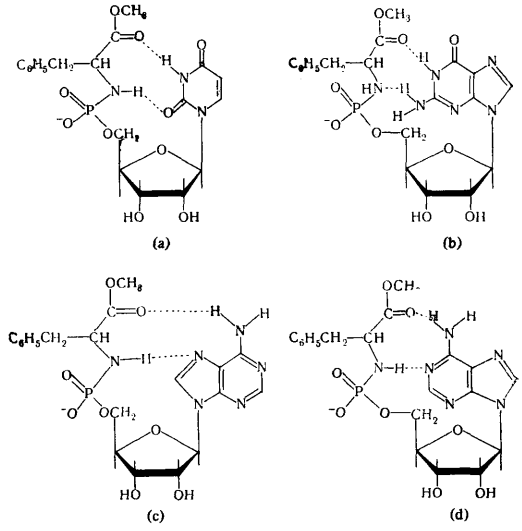
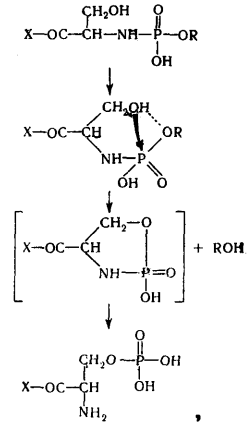


Figure 8. Schematic representation of the possible hydrogen bonds in methyl esters of uridylyl-(5′–N)-phenylalanine (a)[262], guanylyl-(5′–N)-phenylalanine (b)[263], and adenylyl-(5′–N)-phenylalanine (c)[264] or (d).

In the presence of the nucleotidopeptide molecule of other functional groups, the properties of the phosphoramidate bond can change. Thus the introduction into the 3′(2′)-position of the nucleotide fragment of an additional phosphate group leads to some stabilisation of the P–N bond, but only at a pH less than 2.5.[266] Probably under these conditions the 5′-phosphoramidate and the 3′(2′)-phosphate groups are close together, which may be favoured by the *gg*-conformation of the $C_{5'}$–$O_{5'}$ bond and the $C_{2'}$-*endo*-bending of the ribose ring. However, at higher pH this interaction breaks down as a result of the electrostatic repulsion between the dissociated phosphate groups.

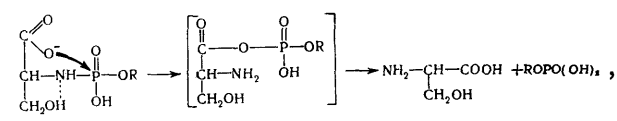
On going from amino-acid to peptide derivatives, the stability of the phosphoramidate bond also increases somewhat. The study of the rate of hydrolysis of a number of derivatives of guanosine 5′-phosphate at a pH less than 2.0 and at different temperatures led to the conclusion that the decisive factor in such increase of stability is the occurrence of steric hindrance in the protonation of the phosphoramidate nitrogen by the protons of the medium[263]. If, however, protonation takes place intramolecularly, for example with participation of the guaninine amino-group (see Fig. 8), an increase in the length of the peptide chain does not lead to any appreciable change in the stability of the phosphoramidate bond[263].

Examination of the hydrolytic stability of nucleotido-(5′–N)-peptides shows that the appreciable differences in the stability of the phosphoramidate bond are nevertheless only quantitative. However, when the peptide unit contains functional groups, there is a possibility of a fundamentally new type of dissociation of the nucleotidopeptide bond. Thus nucleotidyl-(5′–N)-amino-acids with a free carboxy-group[267] and esters of nucleotidyl-(5′–N)-serines with a free serine hydroxy-group[268] are hydrolysed on treatment with acid not only at the P–N bond with formation of the nucleotide and the amino-acid but also at the phosphoester bond with formation of the nucleoside and the phosphoramino-acid. One of the possible mechanisms of hydrolysis at the phosphoester bond includes intramolecular protonation of the phosphoramidate unit and the formation as an intermediate of a cyclic phosphoramino-acid derivative:



where ROH is the nucleoside and X is the peptide or OCH_3.

The intramolecular interaction between the hydroxy-group of serine and the phosphoramidate group is observed also in an alkaline medium, resulting in the dissociation of the phosphoramidate unit (simple phosphoramidates are stable in an alkaline medium)[268]. This dissociation takes place in different ways depending on which groups participate in the catalytic step. If this step involves the simultaneous participation of the hydroxy- and carboxy-groups, then the phosphoramidate bond dissociates (the carboxy-group may be either adjacent or remote, for example the terminal carboxy-group in the tri- and tetra-peptide derivative of the nucleotide where serine is the *N*-terminal amino-acid):

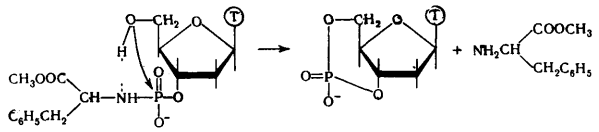


where ROH is the nucleoside.

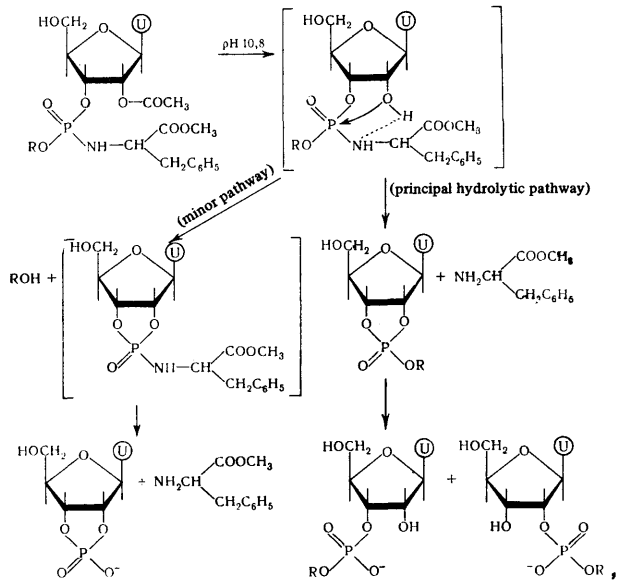
If only the hydroxy-group takes part in the catalytic step, then in alkaline degradation the phosphoester group dissociates by the acid hydrolytic mechanism described above, with formation of an intermediate five-membered cyclic phosphate, which dissociates in an alkaline medium to the *N*-phosphopeptide.

Thus a structure with a characteristic "loop" at the amino-acid end of the molecule is typical for nucleotidyl-(5′–N)-amino-acids with an additional functional group in the amino-acid fragment.

Electrophilic-nucleophilic intramolecular catalysis similar to that described above for serine derivatives of nucleoside 5′-phosphates is also observed for the thymidylyl-(3′–N)-phenylalanine ester, which on standing or brief heating in dimethylformamide is converted into thymidine 3′,5′-cyclic phosphate with simultaneous elimination of the amino-acid[143]:



In this case nucleophilic substitution at the phosphorus atom may be favoured by the conformation of the molecule in which the amide nitrogen is close to the 5′-hydroxy-group of deoxyribose (*gg*-conformation of the $C_{5'}$–$O_{5'}$ bond and $C_{3'}$-*endo*-bending of the pentose ring). An analogous intramolecular effect of the hydroxy-group favourably oriented in relation to the phosphoramidate group is most fully shown in ribonucleoside 3′-phosphoramino-acids, where the position of the hydroxyl near the P–N bond is fixed by the sugar ring. Indeed, while the amino-acid derivatives of thymidine 3′-phosphate and 5′-phosphate behave almost identically in aqueous solution regardless of the position of the phosphoramino-acid residue, being stable at pH > 3,[143] the amino-acid derivatives of ribonucleoside 3′-phosphates can exist under these conditions only if the 2′-hydroxy-group is blocked[262]. The role of the 2′-hydroxy-group in this phenomenon is confirmed by the formation of nucleoside 2′,3′-cyclic phosphate on elimination of the protective 2′-acetyl or 2′-tetrahydropyranyl groups under conditions where the isolated P–N bond is stable (pH 10.8 and 4.5 respectively)[262]. An analogous phenomenon was observed in the amino-acid derivatives of dinucleoside phosphates at the internucleotide phosphorus: while thymidylyl-(3′–5′)-thymidino-(P–N)-phenylalanine is quite stable in neutral aqueous solution, its ribo-analogue—2′-*O*-acetyluridylyl-(3′–5′)-uridino-(P–N)-phenylalanine—hydrolyses immediately after liberation of the 2′-hydroxyl:



where R = H for uridylyl-(3′–N)-phenylalanine and R is the nucleoside residue for uridylyl-(3′–5′)-uridino-(P–N)-phenylalanine.

The information obtained in the study of synthetic nucleotidopeptides suggests that, by virtue of numerous conformational possibilities, the individual fragments of the nucleotidopeptide can interact with one another. A consequence of this is extensive variability of the reactivity of nucleotidopeptides. This permits at least two conclusions. Firstly, in the determination of the types of covalent bonds in natural nucleotidopeptides one must use with great caution data obtained in the study of relatively simple structures. This is clearly shown for the phosphoramidate bond as an example, which, while stable in neutral and alkaline media in the case of simple compounds, becomes labile under the same conditions if the conformation of the molecule is such that proton-donor groups are adjacent to the phosphoramidate group. On the other hand, in the presence of functional groups capable of intramolecular catalysis of hydrolysis and sterically similar to the phosphoramidate group, anomalous dissociation of the phosphoramidate unit may occur also in an acid medium: instead of the dissociation of the phosphoramidate bond (as in simple nucleotide amides), the phosphate residue migrates from the nucleoside to the peptide. Thus, on the basis of the structure of the hydrolysis products of nucleotidopeptides, unambiguous conclusions concerning the nature of the bond in the natural compound cannot be arrived at. In the first place, the available information about the intramolecular interactions in nucleotido-(P–N)-peptides shows that even in a fairly simple molecule consisting of mononucleotide and peptide fragments there is considerable scope for self-regulation: the mutual approach of the phosphoramidic group and a proton donor or acceptor leads to an enhancement or correspondingly a decrease of its reactivity. This suggests the possibility of the existence of analogous mechanisms of the activation of the phosphate residue of mononucleotides in biosynthetic processes such as the formation of internucleotide bonds in the presence of specific enzymes. The enzyme–substrate complex of the phosphoramidate type with the appropriate conformation can effect the transfer of the mononucleotide residue to the 3′-hydroxy-group of the terminal nucleotide of the polynucleotide chain being formed. The ease of the regulation of such a process by the transfer of a proton to the phosphoramidic nitrogen or its removal makes this hypothesis very probable.

It may be expected that the study of the intramolecular interactions in more complex nucleotidopeptides, in particular in oligonucleotidopolypeptides with a specified structure (with a specified sequence of mononucleotides and amino-acids), can throw light on the most important problem of modern molecular biology—the problem of mutual "recognition" of specific nucleic acids and enzyme proteins.

REFERENCES

1. A. M. Michelson, "The Chemistry of Nucleosides and Nucleotides" (Translated into Russian), Izd. Mir., Moscow, 1966.
2. N. K. Kochetkov, I. V. Torgov, and M. M. Botvinnik, "Khimiya Prirodnykh Soedinenii" (The Chemistry of Natural Compounds), Izd. Akad. Nauk SSSR, Moscow, 1961.

3. Z. A. Shabarova, Uspekhi Khim., 28, 369 (1959).
4. R. U. Lemieux and M. Hoffer, Canad. J. Chem., 39, 110 (1961).
5. R. U. Lemieux, Canad. J. Chem., 39, 116 (1961).
6. M. J. Robins and R. K. Robins, J. Amer. Chem. Soc., 87, 4934 (1965).
7. R. J. Cushley, K. A. Watanabe, and J. J. Fox, Chem. Comm., 598 (1966).
8. R. J. Cushley, K. A. Watanabe, and J. J. Fox, J. Amer. Chem. Soc., 89, 394 (1967).
9. E. R. Walwick, W. K. Roberts, and C. A. Dekker, Proc. Chem. Soc., 84 (1959).
10. C. Y. Lin, D. W. Urry, and H. Eyring, Biochem. Biophys. Res. Comm., 17, 642 (1964).
11. T. R. Emerson and T. L. V. Ulbricht, Chem. and Ind., 2129 (1964).
12. T. L. V. Ulbricht, J. P. Jennings, P. M. Scopes, and W. Klyne, Tetrahedron Letters, 695 (1964).
13. T. L. V. Ulbricht, T. R. Emerson, and R. J. Swan, Biochem. Biophys. Res. Comm., 19, 643 (1965).
14. G. D. Fasman, C. Lindblow, and L. Grossman, Biochemistry, 3, 1015 (1964).
15. M. R. Lamborg, P. C. Zamecnik, T.-K. Li, J. H. Kägi, and B. L. Vallee, Biochemistry, 4, 63 (1965).
16. L. G. Koreneva, G. A. Dvorkin, and V. V. Smolyaninov, Dokl. Akad. Nauk SSSR, 162, 451 (1965).
17. M. M. Warshaw and I. Tinoco, J. Mol. Biol., 13, 54 (1965).
18. T. Nishimura, B. Shimizu, and I. Iwai, Biochim. Biophys. acta, 157, 221 (1968).
19. I. Frič, J. Smejkal, and J. Farkaš, Tetrahedron Letters, 75 (1966).
20. T. R. Emerson, R. J. Swan, and T. L. V. Ulbricht, Biochem. Biophys. Res. Comm., 22, 505 (1966).
21. K. C. Murdock and R. B. Angier, J. Amer. Chem. Soc., 84, 3758 (1962).
22. J. E. Kilpatrick, K. S. Pitzer, and R. Spitzer, J. Amer. Chem. Soc., 69, 2483 (1947).
23. M. Sundaralingam and L. H. Jensen, J. Mol. Biol., 13, 930 (1965).
24. L. S. Bartell, J. Amer. Chem. Soc., 81, 3497 (1959).
25. E. Müller, "Neueve Anschauungen dev organischen Chemie" (Springer-Verlag, 1956), (Translated into Russian), Inostr. Lit., 1960, p. 639.
26. C. D. Jardetzky, J. Amer. Chem. Soc., 82, 229 (1960).
27. M. Spencer, Acta crystallogr., 12, 59 (1959).
28. S. Furberg, Acta chem. scand., 4, 751 (1950).
29. C. D. Jardetzky, J. Amer. Chem. Soc., 83, 2919 (1961).
30. C. D. Jardetzky, "Trudy V Mezhdunarodnogo Biokhimicheskogo Kongressa. Referaty Sektsionnykh Zasedanii" (Proceedings of the Fifth International Biochemical Congress. Abstracts of Sectional Sessions), Izd. Akad. Nauk SSSR, Moscow, 1961, Vol. 1, p. 163.
31. S. Furberg and A. Mostad, Acta chem. Scand., 16, 1627 (1962).
32. S. Furberg, C. S. Peterson, and C. Romming, Acta crystallogr., 18, 313 (1965).
33. S. Furberg, Acta crystallogr., 3, 325 (1950).
34. J. Iball, C. H. Morgan, and H. R. Wilson, Nature, 209, 1230 (1966).
35. A. E. V. Haschemeyer and H. M. Sobell, Acta crystallogr., 18, 525 (1965).
36. E. Shefter and K. N. Trueblood, Acta crystallogr., 18, 1067 (1965).
37. C. L. Coulter, Science, 159, 888 (1968).
38. M. Sundaralingam, J. Amer. Chem. Soc., 87, 599 (1965).
39. M. Sundaralingam and L. H. Jensen, J. Mol. Biol., 13, 914 (1965).
40. E. Alver and S. Furberg, Acta chem. Scand., 13, 910 (1959).
41. E. Alver and S. Furberg, Acta chem. Scand., 11, 188 (1957).
42. J. Kraut and L. H. Jensen, Nature, 186, 798 (1960).
43. J. Kraut and L. H. Jensen, Acta crystallogr., 16, 79 (1963).
44. D. M. Brown, G. D. Fasman, D. I. Magrath, A. R. Todd, W. Cochran, and M. M. Woolfson, Nature, 172, 1184 (1953).
45. M. Sundaralingam, Acta crystallogr., 21, 495 (1966).
46. K. Watenpaugh, J. Dow, and L. H. Jensen, Science, 159, 206 (1968).
47. E. Shefter, M. Barlow, R. Sparks, and K. Trueblood, J. Amer. Chem. Soc., 86, 1872 (1964).
48. C. D. Jardetzky, J. Amer. Chem. Soc., 84, 62 (1962).
49. P. Tollin, H. R. Wilson, and D. W. Young, Nature, 217, 1148 (1968).
50. B. Pruden, W. Snipes, and W. Cordy, Proc. Nat. Acad. Sci. U. S. A., 53, 917 (1965).
51. N. Camerman and J. Trotter, Acta crystallogr., 18, 203 (1965).
52. N. Camerman and J. Trotter, Science, 144, 1348 (1964).
53. D. G. Watson, D. J. Sutor, and P. Tollin, Acta crystallogr., 19, 111 (1965).
54. A. E. V. Haschemeyer and H. M. Sobell, Acta crystallogr., 19, 125 (1965).
55. K. N. Trueblood, P. Horn, and V. Luzzati, Acta crystallogr., 14, 965 (1961).
56. H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston, and D. R. Trentham, Tetrahedron, 22, 705 (1966).
57. S. C. Nyburg, "X-Ray Analysis of Organic Structures", Acad. Press, New York-London, 1961, p. 251.
58. S. B. Hendricks, J. Phys. Chem., 45, 65 (1941).
59. D. O. Jordan, in "The Nucleic Acids" (Edited by E. Chargaff and J. N. Davidson), Akad. Press, New York, 1955, Vol. 1, p. 453.
60. J. Donohue and K. N. Trueblood, J. Mol. Biol., 2, 363 (1960).
61. A. E. V. Haschemeyer and A. Rich, J. Mol. Biol., 27, 369 (1967).
62. J. Zussman, Acta crystallogr., 6, 504 (1953).
63. J. J. Fox, and D. Shugar, Biochim. biophys. acta, 9, 369 (1952).
64. J. J. Fox, L. F. Cavalieri, and N. Chang, J. Amer. Chem. Soc., 75, 4315 (1953).
65. J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan, and J. O. Lampen, J. Amer. Chem. Soc., 80, 5155 (1958).
66. J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, J. Amer. Chem. Soc., 81, 178 (1959).
67. R. Kuhn and H. Sobotka, Ztschr. phys. Chem., 109, 65 (1924).
68. S. Lewin and D. A. Humphreys, J. Chem. Soc., (B), 210 (1966).
69. L. Grossman, S. S. Levine, and W. S. Allison, J. Mol. Biol., 3, 47 (1961).
70. P. O. P. Ts'o, S. A. Rapaport, and F. J. Bollum, Biochemistry, 5, 4153 (1966).
71. A. M. Michelson, Ann. Review Biochem., 30, 133 (1961).
72. P. A. Levene, L. W. Bass, and H. S. Simms, J. Biol. Chem., 70, 229 (1926).

73. P. A. Levene, H. S. Simms, and L. W. Bass, J. Biol. Chem., 70, 243 (1926).
74. R. M. Izatt, L. D. Hansen, J. H. Rytting, and J. J. Christensen, J. Amer. Chem. Soc., 87, 2760 (1965).
75. R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, J. Amer. Chem. Soc., 88, 2641 (1966).
76. A. D. Broom and R. K. Robins, J. Amer. Chem. Soc., 87, 1145 (1965).
77. T. A. Khwaja and R. K. Robins, J. Amer. Chem. Soc., 88, 3640 (1966).
78. D. M. G. Martin, C. B. Reese, and G. F. Stephenson, Biochemistry, 7, 1406 (1968).
79. J. B. Gin and C. A. Dekker, Biochemistry, 7, 1413 (1968).
80. J. J. Fox and I. Wempen, Adv. Carbohydrate Chem., 14, 283 (1959).
81. N. C. Yung and J. J. Fox, J. Amer. Chem. Soc., 83, 3060 (1961).
82. J. Žemlička, Coll. Czech. Chem. Comm., 29, 1734 (1964).
83. J. F. Codington and J. J. Fox, Carbohydrate Res., 3, 124 (1966).
84. D. M. Brown, D. B. Parihar, A. Todd, and S. Varadarajan, J. Chem. Soc., 3028 (1958).
85. H. Witzel, Lieb. Ann., 635, 182 (1960).
86. H. Witzel, in "Progress in Nucleic Acids Research" (Edited by J. N. Davidson and W. E. Cohn), Acad. Press, New York–London, 1963, Vol. 2, p. 221.
87. M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, J. Amer. Chem. Soc., 90, 1042 (1968).
88. P. C. Zamecnik, Biochem. J., 85, 257 (1962).
89. D. Shugar, in "The Nucleic Acids" (Edited by E. Chargaff and J. N. Davidson), (Translated into Russian), Inostr. Lit., Moscow, 1962, p. 34.
90. K. L. Wierzchowsky and D. Shugar, Biochim. biophys. acta, 25, 355 (1957).
91. J. Pitha, S. Chládek, and J. Smrt, Coll. Czech. Chem. Comm., 28, 1622 (1963).
92. J. Ofengand and H. Schaefer, Biochemistry, 4, 2832, (1965).
93. H. G. Khorana, "Some Recent Developments in the Chemistry of Phosphate Esters of Biological Interest" (Translated into Russian), Izd. Mir., Moscow, 1964.
94. P. A. Levene and R. S. Tipson, J. Biol. Chem., 106, 113 (1934).
95. P. A. Levene and R. S. Tipson, J. Biol. Chem., 121, 131 (1937).
96. S. Chládek and J. Smrt, Coll. Czech. Chem. Comm., 28, 1301 (1963).
97. J. Žemlička and S. Chládek, Tetrahedron Letters, 3057 (1965).
98. J. Žemlička, S. Chládek, A. Holý, and J. Smrt, Coll. Czech. Chem. Comm., 31, 3198 (1966).
99. C. B. Reese and J. E. Sulston, Proc. Chem. Soc., 214 (1964).
100. A. Hampton, J. C. Fratantoni, P. M. Carroll, and S.-C. Wang, J. Amer. Chem. Soc., 87, 5481 (1965).
101. N. Bagett, A. B. Foster, J. M. Webber, D. Lipkin, and B. E. Phillips, Chem. and Ind., 136 (1965).
102. M. Jarman and C. B. Reese, Chem. and Ind., 1493 (1964).
103. W. V. Ruyle, T. Y. Shen, and A. A. Potchett, J. Org. Chem., 30, 4353 (1965).
104. G. M. Tener and H. G. Khorana, J. Amer. Chem. Soc., 79, 437 (1957).
105. G. M. Tener and H. G. Khorana, J. Amer. Chem. Soc., 80, 1999 (1958).
106. G. M. Tener and H. G. Khorana, Chem. and Ind., 562 (1957).
107. G. M. Tener, R. S. Wright, and H. G. Khorana, J. Amer. Chem. Soc., 78, 506 (1956).
108. G. M. Tener, R. S. Wright, and H. G. Khorana, J. Amer. Chem. Soc., 79, 441 (1957).
109. R. S. Wright, G. M. Tener, and H. G. Khorana, J. Amer. Chem. Soc., 80, 2004 (1958).
110. R. S. Wright, G. M. Tener, and H. G. Khorana, Chem. and Ind., 954 (1957).
111. A. Hampton and A. W. Nichol, Biochemistry, 5, 2076 (1966).
112. A. Hampton and A. W. Nichol, J. Org. Chem., 32, 1688 (1967).
113. A. M. Yurkevich, I. I. Kolodkina, and N. A. Preobrazhenskii, Dokl. Akad. Nauk SSSR, 164, 828 (1965).
114. A. M. Yurkevich, L. S. Varshavskaya, I. I. Kolodkina, and N. A. Preobrazhenskii, Zhur. Obshch. Khim., 37, 2002 (1967).
115. K. H. Scheit and F. Cramer, Tetrahedron Letters, 2765 (1964).
116. R. L. C. Brimacombe, B. E. Griffin, J. A. Haines, W. J. Haslam, and C. B. Reese, Biochemistry, 4, 2452 (1965).
117. M. van Montagy and J. Stockx, Bull. soc. chim. Belges, 74, 341 (1965).
118. M. Ikehara and J. Tazawa, J. Org. Chem., 31, 819 (1966).
119. K. Keck and U. Hagen, Naturwiss, 53, 304 (1966).
120. A. Holý and F. Šorm, Coll. Czech. Chem. Comm., 31, 1562 (1966).
121. N. C. Yung and J. J. Fox, J. Amer. Chem. Soc., 83, 3060 (1961).
122. I. L. Doerr, J. F. Codington, and J. J. Fox, J. Org. Chem., 30, 467 (1965).
123. K. Burton, Biochem. J., 104, 686 (1967).
124. G. A. R. Johnston, Tetrahedron Letters, 2679 (1967).
125. C. B. Reese and D. R. Trentham, Tetrahedron Letters, 2467 (1965).
126. B. E. Griffin, M. Jarman, C. B. Reese, J. E. Sulston, and D. R. Trentham, Biochemistry, 5, 3638 (1966).
127. Sir Alexander Todd (Editor), "Perspectives in Organic Chemistry" (Translated into Russian), Inostr. Lit., Moscow, 1959, p. 174.
128. D. M. Brown and A. Todd, in "The Nucleic Acids" (Edited by E. Chargaff and J. N. Davidson), Acad. Press, New York, 1955, Vol. 1, p. 409.
129. G. H. McCallum, J. M. Robertson, and G. A. Sim, Nature, 184, 1863 (1959).
130. J. Kraut, Acta crystallogr., 14, 1146 (1961).
131. Y. Kyogoku and Y. Iitaka, Acta crystallogr., 21, 49 (1966).
132. V. Schomaker and D. R. Stevenson, J. Amer. Chem. Soc., 63, 37 (1941).
133. D. W. J. Cruickshank, J. Chem. Soc., 5486 (1961).
134. R. L. Collin, J. Amer. Chem. Soc., 88, 3281 (1966).
135. E. Kosower, "Molecular Biochemistry" (Translated into Russian), Izd. Mir., Moscow, 1964.
136. H. G. Khorana, G. M. Tener, K. S. Wright, and J. G. Moffatt, J. Amer. Chem. Soc., 79, 430 (1957).
137. G. M. Tener, H. G. Khorana, R. Markham, and E. H. Pol, J. Amer. Chem. Soc., 80, 6223 (1958).
138. J. G. Moffatt and H. G. Khorana, J. Amer. Chem. Soc., 79, 1194 (1957).
139. M. Smith, G. I. Drummond, and H. G. Khorana, J. Amer. Chem. Soc., 83, 698 (1961).

140. D. Lipkin, W. H. Cook, and R. Markham, J. Amer. Chem. Soc., 81, 6193 (1959).
141. M. Smith, J. Amer. Chem. Soc., 86, 3586 (1964).
142. A. F. Turner and H. G. Khorana, J. Amer. Chem. Soc., 81, 4651 (1959).
143. V. A. Bakanova, Z. A. Shabarova, and M. A. Trokof'ev, Khimiya Prirodnykh Soedinenii, 35 (1966).
144. H. Simpkins and E. G. Richards, Biochemistry, 6, 2513 (1957).
145. G. H. Beaven, E. R. Holiday, and E. A. Johnson, see Ref. 128, p. 493.
146. J. T. Yang and T. Samejima, J. Amer. Chem. Soc., 85, 4039 (1963).
147. D. N. Holcomb and I. Tinoco, Biopolymers, 3, 121 (1965).
148. C. R. Cantor and I. Tinoco, J. Mol. Biol., 13, 65 (1965).
149. M. M. Warshaw, C. A. Bush, and I. Tinoco, Biochem. Biophys. Res. Comm., 18, 633 (1965).
150. J. N. Vournakis, H. A. Scheraga, G. W. Rushizky, and H. A. Sober, Biopolymers, 4, 33 (1966).
151. Y. Inoue, S. Aoyagi, and K. Nakanishi, Tetrahedron Letters, 3575 (1967).
152. Y. Inoue, S. Aoyagi, and K. Nakanishi, J. Amer. Chem. Soc., 89, 5701 (1967).
153. G. B. Zavil'gel'skii, T. V. Venkstern, and A. A. Baev, Dokl. Akad. Nauk SSSR, 166, 978 (1966).
154. G. B. Zavil'gel'skii and L. Li, Molek. Biologiya, 1, 323 (1967).
155. J. Eisinger, M. Guéron, R. G. Shulman, and T. Yamane, Proc. Nat. Acad. Sci. U. S. A., 55, 1015 (1966).
156. M. Guéron, R. G. Shulman, and J. Eisinger, Proc. Nat. Acad. Sci. U. S. A., 56, 814 (1966).
157. K. H. Scheit, F. Cramer, and A. Franke, Biochim. biophys. acta, 145, 21 (1967).
158. A. M. Michelson, Biochim. biophys. acta, 55, 841 (1962).
159. K. E. van Holde, J. Brahms, and A. M. Michelson, J. Mol. Biol., 12, 726 (1965).
160. C. Janion and D. Shugar, Acta biochim. Polon., 7, 309 (1960).
161. K. L. Wierzchowsky and D. Shugar, Acta biochim. Polon., 7, 377 (1960).
162. M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, J. Amer. Chem. Soc., 87, 5241 (1965).
163. S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, J. Amer. Chem. Soc., 86, 4182 (1964).
164. O. Jardetzky, Biopolymers, Symp. No. 1, 501 (1964).
165. G. K. Helmkamp and N. S. Kondo, Biochim. biophys. acta, 145, 27 (1967).
166. P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, J. Amer. Chem. Soc., 85, 1289 (1963).
167. P. O. P. Ts'o and S. I. Chan, J. Amer. Chem. Soc., 86, 4176 (1964).
168. K. E. van Holde and G. P. Rossetti, Biochemistry, 6, 2189 (1967).
169. M. Gellert, M. N. Lipsett, and D. R. Davies, Proc. Nat. Acad. Sci. U. S. A., 48, 2013 (1962).
170. W. L. Peticolas, J. Chem. Phys., 40, 1463 (1964).
171. H. T. Miles and J. Frazier, Biochim. biophys. acta, 79, 216 (1964).
172. F. B. Howard and H. T. Miles, J. Biol. Chem., 240, 801 (1965).
173. R. V. Ravindranathan and H. T. Miles, Biochim. biophys. acta, 94, 603 (1965).
174. P. K. Sarkar and J. T. Yang, Biochem. Biophys. Res. Comm., 20, 346 (1965).
175. R. B. Homer and S. F. Mason, Chem. Comm., 332 (1966).
176. J. Ladik, Acta Phys. Acad. Sci. Hung., 11, 239 (1960).
177. J. Drobnik and V. Kleinwachter, Biochem. Biophys. Res. Comm., 4, 366 (1965).
178. R. B. Inman and R. L. Baldwin, J. Mol. Biol., 5, 172 (1962).
179. M. Chamberlin and R. L. Baldwin, J. Mol. Biol., 7, 334 (1963).
180. R. B. Inman and R. L. Baldwin, J. Mol. Biol., 8, 452 (1964).
181. J. H. Chen and F. F. Davis, Biochem. Biophys. Res. Comm., 20, 124 (1965).
182. W. Szer, Biochem. Biophys. Res. Comm., 20, 182 (1965).
183. C. W. Abell, L. A. Rosini, and M. R. Ramseur, Proc. Nat. Acad. Sci. U. S. A., 54, 608 (1965).
184. J. Massoulié, A. M. Michelson, and F. Pochon, Biochim. biophys. acta, 114, 16 (1966).
185. F. Pochon and A. M. Michelson, Biochim. biophys. acta, 145, 321 (1967).
186. A. M. Michelson and C. Monny, Biochim. biophys. acta, 149, 88 (1967).
187. G. D. Fasman, C. Lindblow, and E. Seaman, J. Mol. Biol., 12, 630 (1965).
188. V. I. Poltev and B. I. Sukhorukov, Biofizika, 12, 763 (1967).
189. T. I. Tikhonenko, Symposium, "Biosintez Belka i Nukleinovykh Kislot" (Biosynthesis of Proteins and Nucleic Acids) (Edited by A. S. Spirin), Izd. Nauka, Moscow, 1965, p. 260.
190. S. I. Chan, R. W. Bangerter, and H. H. Peter, Proc. Nat. Acad. Sci. U. S. A., 55, 720 (1966).
191. S. Furberg, Acta chem. Scand., 19, 266 (1965).
192. O. Warburg and W. Christian, Biochem. Ztschr., 296, 122 (1938).
193. O. Warburg and W. Christian, Biochem. Ztschr., 298, 150 (1938).
194. A. Kornberg and W. E. Pricer, J. Biol. Chem., 182, 763 (1950).
195. L. G. Whitby, Biochem. J., 54, 437 (1953).
196. J. M. Siegel, G. A. Montgomery, and R. M. Bock, Arch. Biochem. Biophys., 82, 288 (1959).
197. P. M. Bayley, "Abstracts of the 5th International Symposium on the Chemistry of Natural Products", London, July 1968, E23.
198. G. Weber, Nature, 180, 1409 (1957).
199. G. Weber, J. Chim. Phys., 55, 878 (1958).
200. S. F. Velick, J. Biol. Chem., 233, 1455 (1958).
201. I. I. Gol'dshtein, D. I. Roshchupkin, and V. P. Skulachev, Biokhimiya, 31, 927 (1966).
202. E. A. Neifakh, L. A. Tumerman, and S. Frid, Biofizika, 12, 799 (1967).
203. O. A. Bessey, A. H. Lowry, and R. H. Love, J. Biol. Chem., 180, 755 (1949).
204. H. Weil-Malherbe, Biochem. J., 40, 363 (1946).
205. G. Weber, Biochem. J., 47, 114 (1950).
206. K. Burton, Biochem. J., 48, 458 (1951).
207. K. Yagi, J. Okuda, T. Ozawa, and K. Okada, Biochem. Ztschr., 328, 492 (1957).
208. K. Yagi, J. Okuda, T. Ozawa, and K. Okada, Science, 124, 273 (1956).
209. H. Beinert, in "The Enzymes" (Edited by P. D. Boyer, H. Lardy, and K. Myrbäck), Acad. Press, New York–London, 1960, Vol. II, p. 339.
210. I. Listowsky, S. Englard, J. J. Betheil, and S. Seifter, Biochemistry, 5, 2548 (1966).

211. R. T. Simpson and B. L. Vallee, Biochem. Biophys. Res. Comm., 22, 712 (1966).
212. W. L. Meyer, H. R. Hahler, and R. H. Baker, Biochim. biophys. acta, 64, 353 (1962).
213. O. Jardetzky and N. G. Wade-Jardetzky, J. Biol. Chem., 241, 85 (1966).
214. J. E. Wilson, Biochemistry, 5, 1351 (1966).
215. C. Woenckhaus and M. H. Volz, Ber., 99, 1712 (1966).
216. G. Pfleiderer, C. Woenckhaus, K. Scholz, and H. Feller, Lieb. Ann., 675, 205 (1965).
217. H. G. Windmueller and N. O. Kaplan, J. Biol. Chem., 236, 2716 (1961).
218. G. Pfleiderer, C. Woenckhaus, and M. Nelböck-Hochstetter, Lieb. Ann., 690, 170 (1965).
219. S. Shifrin and N. O. Kaplan, Nature, 183, 1529 (1959).
220. B. M. Chassy and D. B. McCormick, Biochemistry, 4, 2612 (1965).
221. N. O. Kaplan, "Trudy V Mezhdunarodnogo Biokhimicheskogo Kongressa, Simp. IV" (Proceedings of the Fifth International Biochemical Congress, Symp. IV), Izd. Akad. Nauk SSSR, Moscow, 1961, p. 3.
222. J. C. M. Tsibris, D. B. McCormick, and L. D. Wright, Biochemistry, 4, 504 (1965).
223. N. K. Kochetkov, E. I. Budovskii, and V. N. Shibaev, Biokhimiya, 28, 741 (1963).
224. N. K. Kochetkov, E. I. Budovskii, and V. N. Shibaev, Uspekhi Biol. Khim., 6, 108 (1964).
225. N. D. Gabrielyan, M. A. Novikova, and G. L. Zhdanov, Dokl. Akad. Nauk SSSR, 151, 1453 (1963).
226. N. D. Gabrielyan and A. V. Venkina, Dokl. Akad. Nauk SSSR, 156, 1379 (1964).
227. N. D. Gabrielyan and A. V. Venkina, Dokl. Akad. Nauk SSSR, 165, 439 (1965).
228. S. H. Goldemberg, Biochim. biophys. acta, 56, 357 (1962).
229. M. Rabinowitz and I. Goldberg, J. Biol. Chem., 238, 1801 (1963).
230. H. Ankel, E. Ankel, and D. S. Feingold, Biochemistry, 5, 1864 (1966).
231. N. K. Kochetkov, E. I. Budovskii, V. N. Shibaev, and G. I. Eliseeva, Dokl. Akad. Nauk SSSR, 159, 605 (1964).
232. A. Szent-Györgyi, in "Enzymes, Units of Biological Structure and Function" (Edited by O. H. Gaebler), Acad. Press, New York, 1956, p. 393.
233. A. Szent-Györgyi, "Bioenergetics" (Translated into Russian), Fizmatgiz, Moscow, 1960, p. 87.
234. A. Szent-Györgyi, "Introduction to a Submolecular Biology" (Translated into Russian), Izd. Mir, Moscow, 1964, p. 108.
235. A. Epp, T. Ramasarma, and L. R. Wetter, J. Amer. Chem. Soc., 80, 724 (1958).
236. F. L. Khalil and T. L. Brown, J. Amer. Chem. Soc., 86, 5113 (1964).
237. I. Feldman and E. Keil, J. Amer. Chem. Soc., 87, 3281 (1965).
238. K. Hotta, J. Brahms, and M. Morales, J. Amer. Chem. Soc., 83, 997 (1961).
239. B. H. Levedahl and T. W. James, Biochim. biophys. acta, 21, 298 (1956).
240. W. G. McCormick and B. H. Levedahl, Biochim. biophys. acta, 34, 303 (1959).
241. B. Pullman and A. Pullman, "Quantum Biochemistry" (Translated into Russian), Izd. Mir, Moscow, 1965, p. 302.
242. M. R. Atkinson and R. K. Morton, in "Comparative Biochemistry" (Edited by M. Florkin and H. S. Mason), Acad. Press, New York, 1960, Vol. 2, p. 1.
243. A. E. Martell and G. Schwarzenbach, Helv. chim. acta, 39, 653 (1956).
244. G. G. Hammes, G. E. Maciel, and J. S. Waugh, J. Amer. Chem. Soc., 83, 2394 (1961).
245. M. Cohn and T. R. Hughes, J. Biol. Chem., 237, 176 (1962).
246. P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, Helv. chim. acta, 47, 992 (1964).
247. J. A. Happe and M. Morales, J. Amer. Chem. Soc., 87, 2077 (1966).
248. G. L. Eichhorn, P. Clark, and E. D. Becker, Biochemistry, 5, 345 (1966).
249. E. Walaas, Acta chem. Scand., 12, 528 (1958).
250. H. Brintzinger, Biochim. biophys. acta, 77, 343 (1963).
251. P. George, R. C. Phillips, and R. J. Rutman, Feder. Proc., 23, 265 (1964).
252. R. C. Phillips, P. George, and R. J. Rutman, J. Amer. Chem. Soc., 88, 2631 (1966).
253. C. H. Oestreich and M. M. Jones, Biochemistry, 5, 2226 (1966).
254. C. H. Oestreich and M. M. Jones, Biochemistry, 5, 3151 (1966).
255. C. H. Oestreich and M. M. Jones, Biochemistry, 6, 1515 (1967).
256. R. M. Bock, in "The Enzymes" (Edited by P. D. Boyer, H. Lardy, and K. Myrbäck), Acad. Press, New York–London, 1963, Vol. VII, p. 1.
257. M. Ikehara, E. Ohtsuka, S. Kitagawa, and Y. Tonomura, Biochim. biophys. acta, 82, 74 (1964).
258. R. J. P. Williams, in "The Enzymes" (Edited by P. D. Boyer, H. Lardy, and K. Kyrbäck), Acad. Press, New York, 1959, Vol. 1, p. 391.
259. R. Phillips, Chem. Rev., 501 (1966).
260. A. A. Bogdanov, Uspekhi Sovrem. Biol., 55, 321 (1963).
261. A. A. Bogdanov and R. S. Shakulov, Symposium, "Nukleinovye Kisloty" (Nucleic Acids), Izd. Mir, Moscow, 1965, p. 245.
262. O. E. Vorob'ev, Z. A. Shabarova, and M. A. Prokof'ev, Vestnik Moskov. Gos. Univ., Ser. Khim., No. 6, 66 (1964).
263. N. I. Sokolova, Z. A. Stumbravichute, P. P. Purygin, Z. A. Shabarova, and M. A. Prokof'ev, Dokl. Akad. Nauk SSSR, 174, 722 (1967).
264. T. S. Ryabova and Z. A. Shabarova, Vestnik Moskov. Gos. Univ., Ser. Khim., No. 5, 98 (1965).
265. E. P. Savel'ev, T. S. Ryabova, I. P. Beletskaya, and Z. A. Shabarova, Dokl. Akad. Nauk SSSR, 155, 1457 (1964).
266. A. B. Solov'eva, N. N. Preobrazhenskaya, N. I. Sokolova, and Z. A. Shabarova, Zhur. Obshch. Khim., 37, 431 (1967).
267. E. P. Savel'ev, N. M. Preobrazhenskaya, Z. A. Shabarova, and M. K. Prokof'ev, Khimiya Prirodnykh Soedinenii, 121 (1967).
268. E. P. Savel'ev, B. A. Yuodka, Z. A. Shabarova, and M. A. Prokof'ev, Vestnik Moskov. Gos. Univ., Ser. Khim., No. 5, 128 (1967).

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