BIOPOLYMERS VOL. 10, PP. 175-219 (1971)

Stereochemistry of Nucleic Acids and Their Constituents. X.* Solid-State Base-Stacking Patterns in Nucleic Acid Constituents and Polynucleotides

C. E. BUGG and J. M. THOMAS, *Institute of Dental Research and Laboratory of Molecular Biology, University of Alabama Medical Center, Birmingham, Alabama 35233* and M. SUNDARALINGAM and S. T. RAO, *Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706*

Synopsis

The base-stacking patterns in over 70 published crystal structures of nucleic acid constituents and polynucleotides were examined. Several recurring stacking patterns were found. Base stacking in the solid state apparently is very specific, with particular modes of interaction persisting in various crystalline environments. The vertical stacking of purines and pyrimidines in polynucleotides is similar to that observed in crystals of nucleic acid constituents. Only partial base overlap was found in the majority of the structures examined. Usually, the base overlap is accomplished by positioning polar substituents over the ring system of an adjacent base. The stacking interactions are similar to those found in the crystal structures of other polar aromatic compounds, but are considerably different from the ring–ring interactions in nonpolar aromatic compounds. Apparently, dipole-induced dipole forces are largely responsible for solid-state base stacking. It is found that halogen substituents affect base-stacking patterns. In general, the presence of a halogen substituent results in a stacking pattern which permits intimate contact between the halogen atom and adjacent purine or pyrimidine rings. Considering differences in the stacking patterns found for halogenated and nonhalogenated pyrimidines, a model is proposed to account for the mutagenic effects of halogenated pyrimidines.

INTRODUCTION

During the past several years, a number of investigations have suggested that, in aqueous solutions, parallel stacking of purine and pyrimidine bases is a major stabilizing force in oligo- and polynucleotides,[2–10] complexes of purines and pyrimidines with oligonucleotides and polynucleotides,[11–16] and aggregates of nucleosides, nucleotides, purines, and pyrimidines.[17–22] Stacking interactions are of considerable importance since, in addition to stabilizing polynucleotides, they apparently play an important role in governing the binding of smaller aromatic compounds to nucleic

* For part IX of this series, see Sundaralingam and Arora.[1]

© 1971 by John Wiley & Sons, Inc.

acids. However, little is known about the specific factors which are responsible for base stacking. Since base stacking apparently does not occur in nonaqueous solvents,[23,24] the base–base interactions have been attributed to hydrophobic factors. Theoretical studies[25–27] have stressed the possible importance of dipole–dipole, dipole–induced dipole, London dispersion, and monopole–monopole interactions.

In order to provide additional information about the types of interactions which accompany the parallel stacking of purines and pyrimidines, we have examined the stacking patterns in fibers of polynucleotides and crystals of nucleic acid constituents. These interactions have been compared with those found in the crystal structures of other aromatic compounds.

Results obtained in this investigation suggest that dipole–induced dipole forces are largely responsible for base stacking in the solid state. Only partial overlap of bases was found in the majority of structures examined. Usually, this overlap is accomplished in a highly specific manner by positioning polar substituents of one base over the ring of a neighboring base. The observed interactions are closely analogous to those found in other polar aromatic compounds but are considerably different from the ring–ring interactions in crystals of nonpolar aromatic compounds. The base-stacking patterns are described, and possible relationships to biological systems discussed.

METHODS

Calculations were based on crystallographic parameters obtained from published crystal structure analyses. Least-squares planes through the atoms of the purine and pyrimidine rings were calculated by the method of Blow.[28] Projections perpendicular to these planes were calculated and the positions of the atoms in these projections were plotted on a high speed printer. Interplanar spacings and all distances between the atoms of stacked bases were calculated. Hydrogen atoms, when shown, were plotted assuming trigonal bonding to the heavy atoms of the bases. Fiber structures of polynucleotides, along with the crystal structures of about 65 nucleosides, nucleotides, purines, and pyrimidines, were examined. In addition, we examined the stacking interactions in the crystal structures of a number of other aromatic compounds.

DESCRIPTION OF FIGURES

All figures show the stacking of two bases as viewed perpendicular to the least-squares plane of the base on top, which is represented by the darker lines. In most cases the bases are related by lattice translations or crystallographic inversion centers; the planes of the bases are consequently parallel. The legends for the figures list the interplanar spacings d for these parallel stacked pairs. The dihedral angles θ between base planes are listed for base pairs which are not exactly parallel. The figure legends also list the interbase distances which are shorter than 3.5 Å and the shortest

0(1)′-base distances. The crystal structures are grouped to show common modes of interaction. The labels on the drawings describe the components (other than water) which are present in the crystal structures. The ribose moieties are shown for a number of the nucleosides and nucleotides; in other cases only the glycosidic carbon atom or the carbon atom plus the ribose ring oxygen atom, 0(1)′, are shown. In Figures 2, 3, and 15–19, nitrogen atoms are depicted by solid circles; oxygen atoms by small open circles; and methyl groups by large open circles; the hydrogen atoms are not shown in these figures. The figures depict only nearest neighbor base stacking patterns. Hydrogen bonding and other important stabilizing interactions present in the crystal structures are not shown.

RESULTS

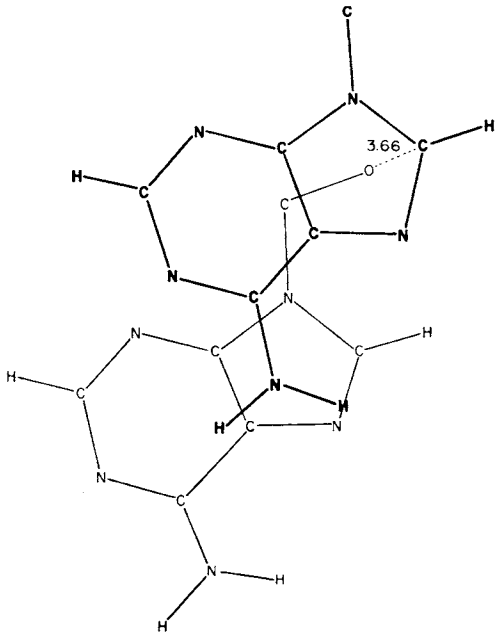
Nucleic Acid Constituents

Base stacking, loosely defined here as partial overlap or close approach of bases which are parallel or approximately parallel, was found in the greatest number of the crystal structures examined. The stacking in these structures is depicted in Figures 1–15. No stacking was found in crystals of 3-methylcytosine hydrobromide,[29] monoclinic cytidylic acid b,[30] 2′-deoxycytidine hydrochloride,[31] cytosine-5-acetic acid,[32] 6-thiopurine riboside,[33] 5-bromouridine,[34] 3-methyluracil hydrobromide,[35] and 3′-*O*-acetyladenosine.[36]

A striking feature that is common to most of the structures depicted is the specific manner in which the bases partially overlap.[37] Extensive overlap of adjacent bases is the exception rather than the rule; usually the bases are positioned so that only a few atoms overlap an adjacent base. In the majority of cases, the bases stack with electronegative heteroatoms forming close contacts with the aromatic ring systems.

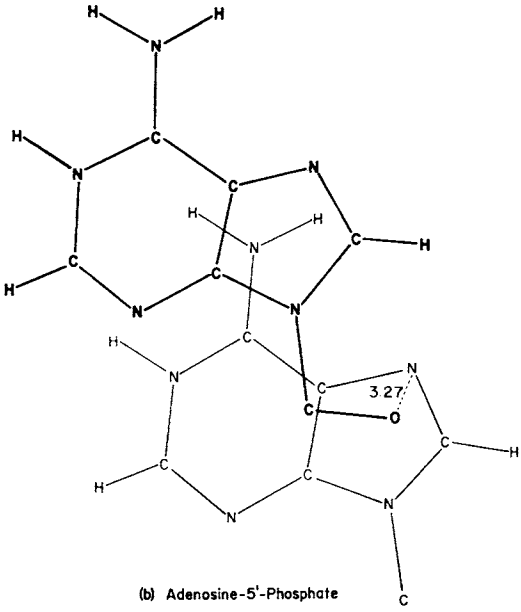
Numerous examples display the recurrence of specific types of stacking interactions. An especially significant example of a particular type of interaction which is found in different crystal structures is shown in Figure 1. Figures 1*a*, 1*b*, and 1*c* show the stacking found for three adenine nucleosides and nucleotides; Figure 1*d* shows the self-association of deoxyguanosine in a co-crystal with 5-bromodeoxycytidine. Despite other large differences in these crystal structures, the stacking interactions are very similar. In all four structures, a polar substituent (either an amino group or a carbonyl oxygen atom) is positioned above the imidazole ring of an adjacent purine. In addition, the ribose oxygen atoms, 0(1)′, form close contacts with the adjacent bases.

Interactions between atom 0(1)′ of the ribose rings and adjacent purine or pyrimidine bases is an important feature in the crystal structures of nucleosides and nucleotides.[42] In many cases, the sugar-to-base contact appears to be the dominant interaction between adjacent molecules, with little or no base overlap. Examples of such crystal structures are shown in Figures 2–4. In some of these structures the distances between 0(1)′



(a) Deoxyadenosine Monohydrate

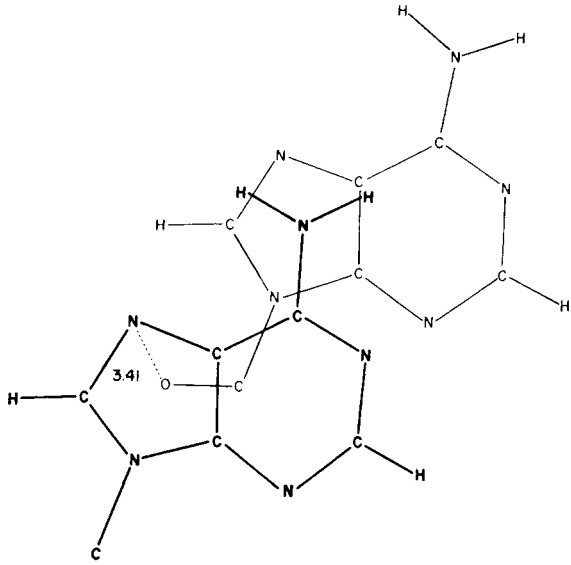
d=3.40



(b) Adenosine-5'-Phosphate

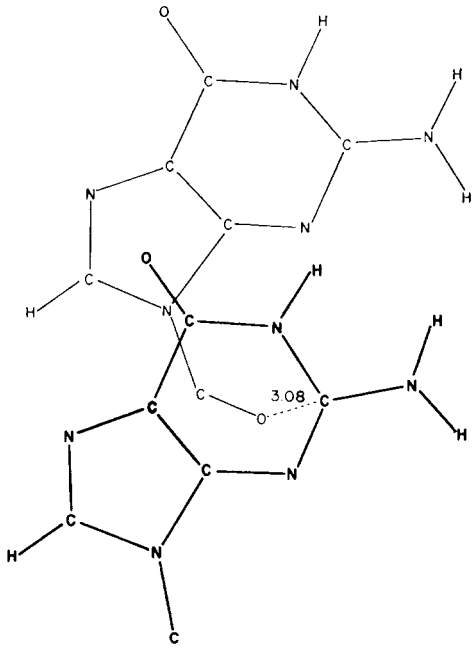
d=3.46

Fig. 1 (*continued*)



(c) Adenosine and 5-Bromouridine

d=3.36



(d) Deoxyguanosine and 5-Bromodeoxycytidine

d=3.40

Fig. 1. Similar modes of stacking for deoxyguanosine and adenine derivatives: (*a*) d = 3.40 Å, C(6)–N(9) = 3.46 Å;[38] (*b*) d = 3.46 Å, O(1)′–N(9) = 3.49 Å, O(1)′–C(8) = 3.29 Å, O(1)′–N(7) = 3.27 Å, O(1)′–C(5) = 3.45 Å, C(4)–N(6) = 3.45 Å;[39] (*c*) d = 3.36 Å, C(4)–O(1)′ = 3.41 Å, C(5)–O(1)′ = 3.41 Å, C(6)–N(9) = 3.40 Å, N(6)–C(5) = 3.47 Å, N(7)–O(1)′ = 3.41 Å, C(8)–O(1)′ = 3.42 Å, N(9)–O(1)′ = 3.45 Å;[40] (*d*) d = 3.40 Å, N(1)–O(1)′ = 3.31 Å, C(2)–O(1)′ = 3.08 Å, N(3)–O(1)′ = 3.13 Å, C(4)–O(1)′ = 3.20 Å, C(5)–O(1)′ = 3.48 Å, C(6)–N(9) = 3.36 Å, C(6)–O(1)′ = 3.48 Å.[41]

and the atoms of an adjacent base are considerably less than the sum of the van der Waals radii of the atoms involved.

In addition to the examples shown in Figure 1, a number of other structures display base–base contacts involving interactions of amino or

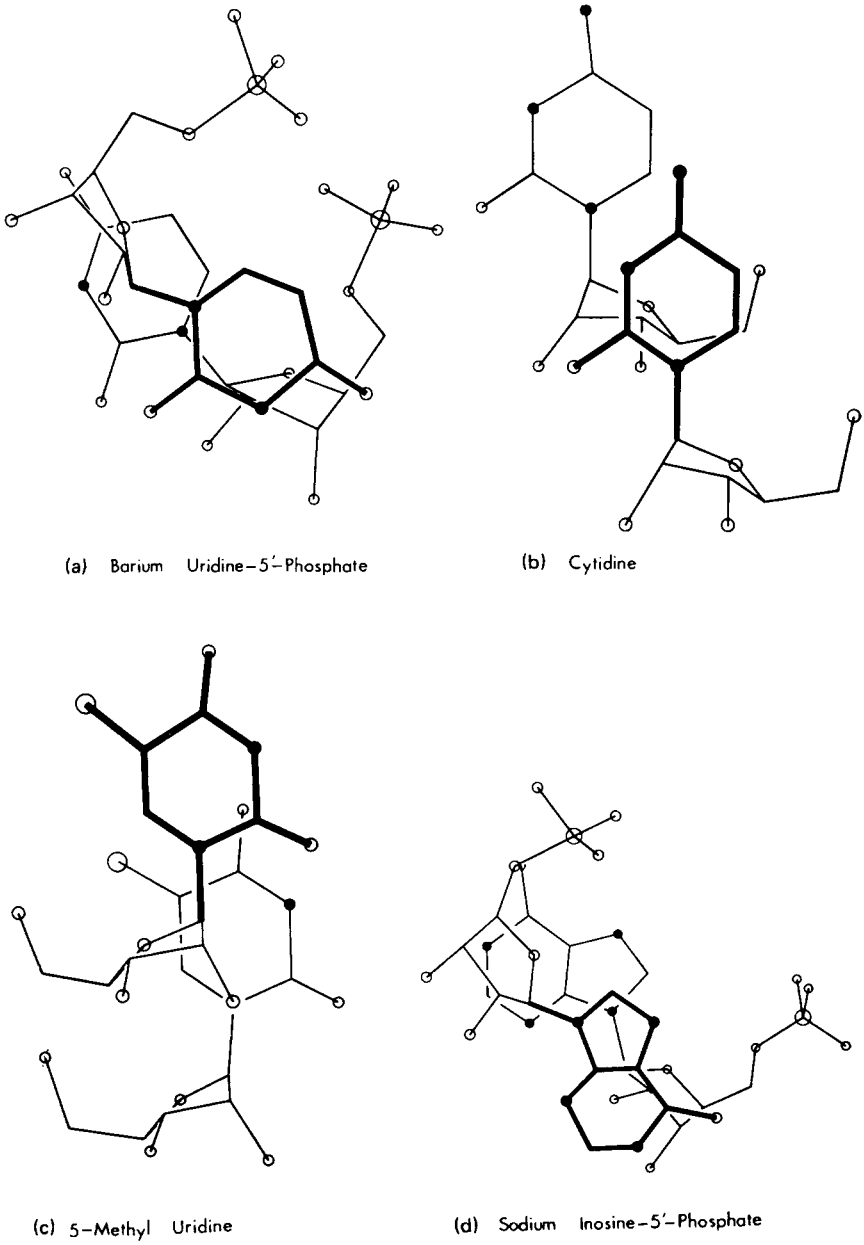
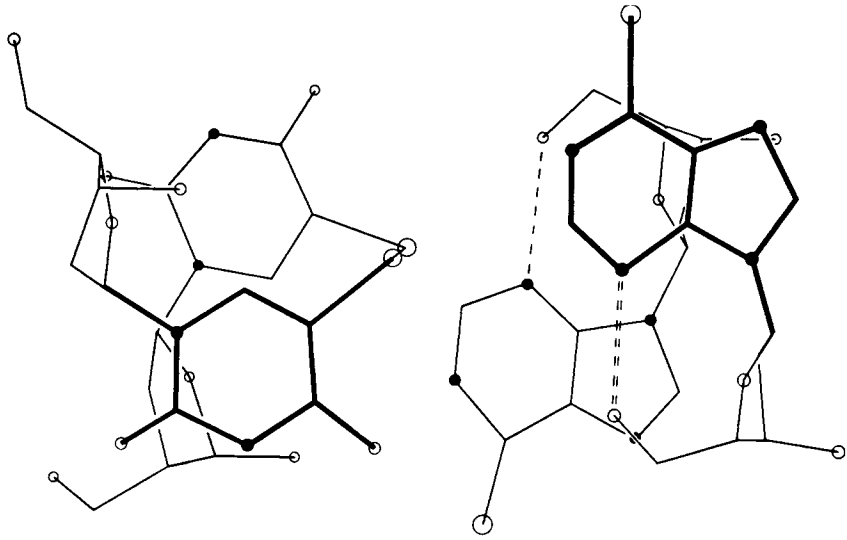


Fig. 2 (*continued*)



(e) 5-[1-(2'-Deoxy-α-D-ribofuranosyl)-uracilyl] disulfide (f) 6-Thiopurine Riboside

Fig. 2. Interactions of ribose rings with purines and pyrimidines: (*a*) $\theta = 3.3°$, O(1)'–C(2) = 3.48 Å, O(1)'–N(3) = 3.30 Å, O(1)'–C(5) = 3.14 Å, O(1)'–C(4) = 2.74 Å, O(2)–O(2) = 3.18 Å, C(2)–O(1)' = 3.48 Å, N(3)–O(1)' = 3.30 Å, C(5)–O(1)' = 3.14 Å, C(4)–O(1)' = 2.74 Å;[43] (*b*) $d = 3.52$ Å, O(1)'–N(1) = 3.28 Å, O(1)'–C(2) = 3.18 Å, O(1)'–N(3) = 3.40 Å, O(1)'–C(6) = 3.47 Å;[44] (*c*) $d = 3.59$ Å, O(1)'–C(5) = 3.25 Å, O(1)'–C(6) = 3.20 Å;[45] (*d*) $\theta = 21.7°$, O(1)'–C(4) = 3.29 Å, O(1)'–C(5) = 3.17 Å, O(1)'–C(6) = 3.11 Å, C(4)–C(6) = 3.45 Å;[46] (*e*) $\theta = 35.0°$, N(1)–O(1)' = 3.19 Å, C(2)–O(1)' = 3.06 Å, C(6)–C(6) = 3.26 Å, O(2)–O(1)' = 3.24 Å, O(1)'–C(2) = 3.07 Å, O(1)'–O(2) = 3.37 Å, O(1)'–N(3) = 3.21 Å;[47] (*f*) $\theta = 3.3°$, N(1)–O(1)' = 3.49 Å, C(2)–O(1)' = 3.43 Å, N(3)–O(1)' = 3.26 Å, C(4)–O(1)' = 3.02 Å, C(5)–O(1)' = 3.08 Å, C(6)–O(1)' = 3.34 Å, O(1)'–C(8) = 3.17 Å.[33]

carbonyl groups with the ring systems of adjacent bases. Interactions of amino groups with purines and pyrimidines are shown in Figure 5. This type of stacking is also found in the crystal structures of other amino-substituted aromatic compounds (e.g., Figure 21*a*).

Stacking interactions involving carbonyl oxygen atoms are represented in Figures 6 and 7. In all of these structures, the carbonyl groups are positioned above adjacent rings; in the largest percentage of cases, the corresponding interplanar spacings are short. It is noteworthy that similar interactions are also found in a number of crystalline molecular complexes.[61–64] As demonstrated by Figure 7*e*, thione derivatives are also capable of forming similar contacts.

Figure 8 shows an interesting mode of stacking which is found in several of the halogenated pyrimidines. In these structures, the halogen atoms are positioned in close contact with neighboring pyrimidines. Similar interactions between halogen substituents and aromatic rings are also

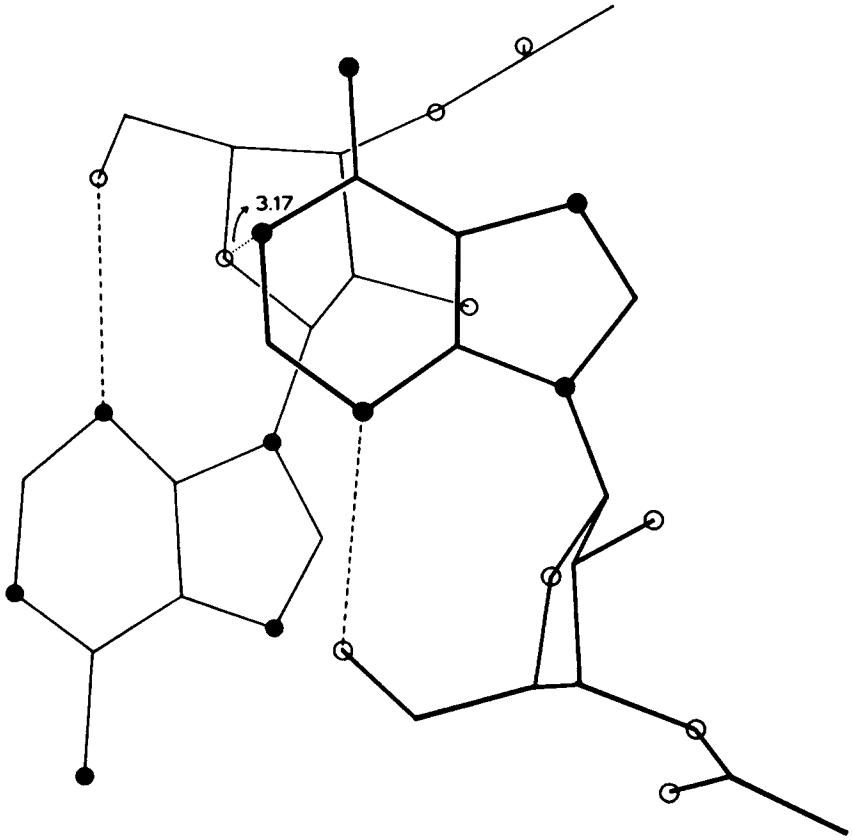


Fig. 3. Ribose-purine interaction in the crystal structure of 3′-*O*-acetyladenosine; θ = 34.3°, N(1)–O(1)′ = 3.17 Å.[36]

found in crystals of the halogenated purines, as shown in Figures 9 and 11*e*.

Another type of recurring interactions is that involving the ring nitrogen atoms of purines and pyrimidines. The roles of these atoms in stacking are depicted in Figures 10 and 11. Again, it is found that the stacking permits the nitrogen atoms to interact with the ring systems of neighboring bases.

Solution studies have suggested that stacking is pH-sensitive; for most dinucleotides, stacking is essentially eliminated at low pH.[7] In agreement with the solution studies, no stacking interactions were found in the crystal structures of protonated pyrimidines. However, most of the protonated purines were found to stack in a characteristic fashion with atom N(3) in close proximity to atom N(7) of a parallel base. The stacking found in these structures is shown in Figure 12. A similar mode of stacking is also found for nonprotonated 9-ethylhypoxanthine, as indicated in Figure 11*e*.

We were somewhat surprised at the absence of extensive superposition of bases in the majority of the structures examined. The crystal structures of

guanosine and inosine, which are isomorphous in spite of the differences in hydrogen bonding,[81,82] suggested that purine superposition might be an important stabilizing force in a number of other crystal structures. The stacking found in the guanosine and inosine structures is shown in Figure 13. Examination of purine stacking in related structures demonstrates that this degree of overlap is unusual. However, several purine crystal structures do show moderate degrees of base overlap which is accompanied by short interplanar separations; these structures are depicted in Figure 14. As in the majority of other structures, the stacking is accomplished so that, in most instances, oxygen or nitrogen atoms are positioned over adjacent bases.

While superposition of purines is usually accompanied by short interplanar spacings, extensive overlap of pyrimidines results in large separations between bases. Three examples of stacking of this type are shown in Figure 15.

Polynucleotides

Figures 16–20 show the intrastrand base stacking interactions found in the helical structures of several polynucleotides. Similar to the crystal structures of the constituents, stacking in the polynucleotides involves only partial overlap of the bases. Again, this partial overlap is accomplished by positioning oxygen or nitrogen atoms in close contact with adjacent bases. Several of the stacking patterns found in the nucleic acid constituents also appear in the polynucleotides. For example, it may be noted that the overlap of bases in polyadenylic acid (Fig. 20) is similar to that found for the adenine derivatives depicted in Figure 1.

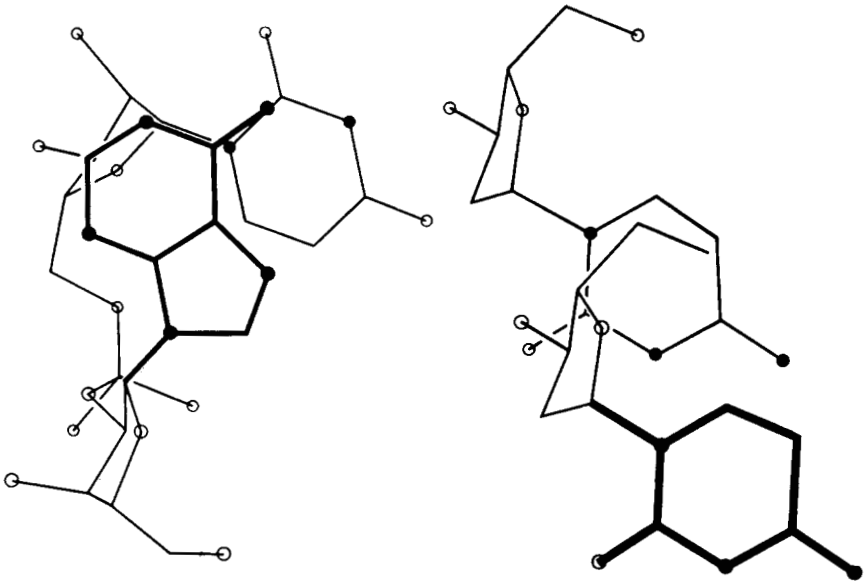
In addition to the intrastrand stacking depicted in Figures 16–20, some of the double helical polynucleotide structures also show interstrand stacking. It is found that interstrand stacking is especially important in the structures of A-DNA and RNA. For some base sequences, interstrand stacking is the predominant type. For example, although there is minimal intrastrand stacking for the pyrimidine–purine sequence in A-DNA (Fig. 16*b*), there is a great deal of interstrand stacking for this sequence. In RNA (Figs. 19*b* and 19*d*), where the uracil residual lacks the methyl group which is found on thymine residues in DNA, interstrand stacking is the predominant type for both the pyrimidine–pyrimidine and the pyrimidine–purine sequence. However, it is noteworthy that only intrastrand stacking is found in the B form of DNA.

Other Aromatic Compounds

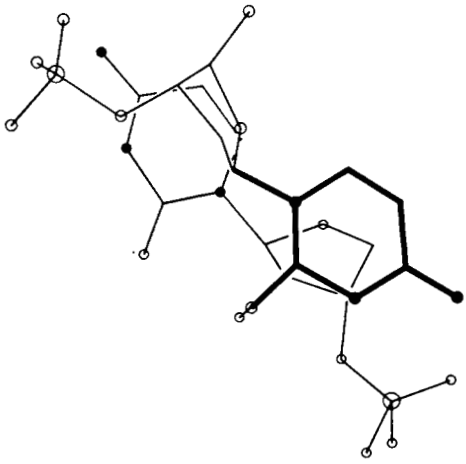
Many of the interbase spacings found for the structures depicted in Figures 1–14 are in the range which is typical of aromatic charge transfer (donor–acceptor) complexes. Since these complexes contain polar compounds and the charge transfer process apparently contributes little to their ground state stability,[93,94] it is likely that the complexes are stabilized by the same forces which are in effect in crystals of nucleic acid constituents.

Therefore the stacking interactions in a number of charge transfer complexes were also examined.

Selected examples of the stacking in these complexes are shown in Figure 21. Figures 21*a*, 21*b*, and 21*c* can be compared with the similar modes of purine and pyrimidine stacking shown in Figures 5–8. Similar to the nucleic acid constituents, these aromatic systems are rarely positioned in such

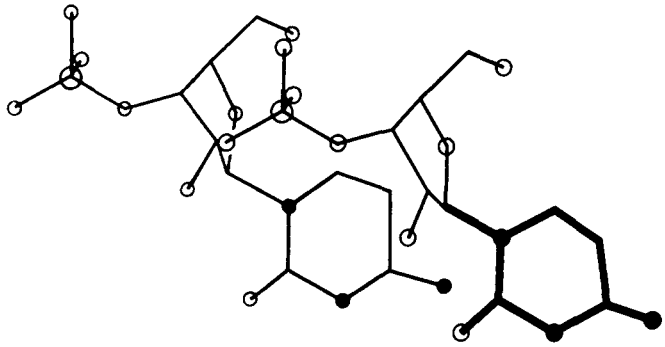


Adenylyl–2′–Phosphate–5′–Uridine (b) Deoxycytidine · HCl

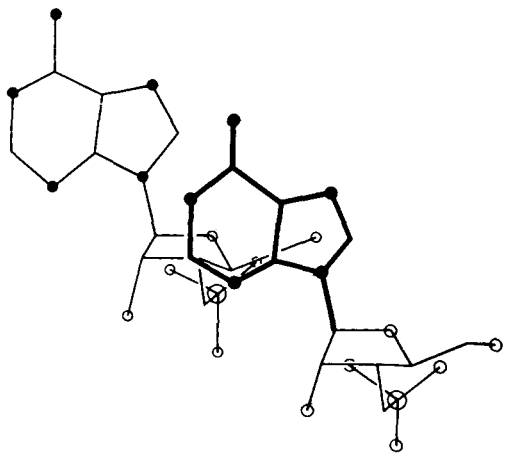


(c) Cytidine–3′–Phosphate (Orthorhombic)

Fig. 4 (*continued*)



(d) Cytidine–3′–Phosphate (Monoclinic)

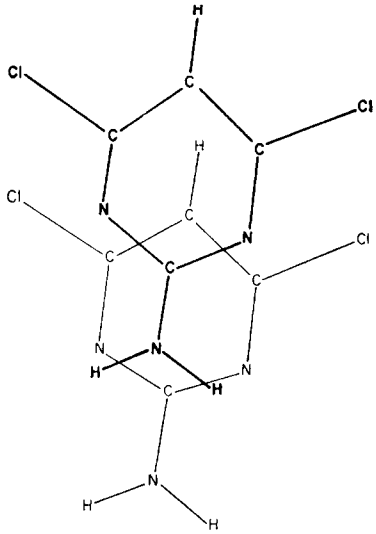


(e) Adenosine–3′–Phosphate

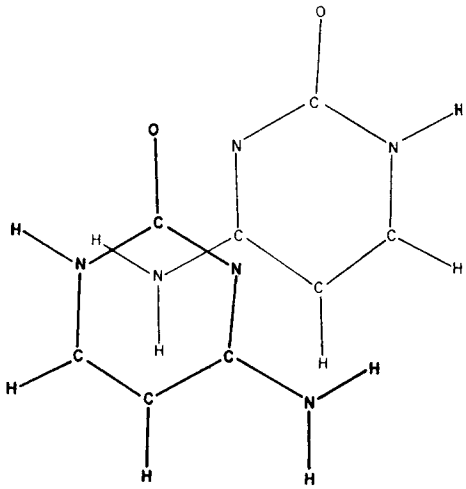
Fig. 4. Additional interactions involving atom O(1)′ of nucleosides and nucleotides: (*a*) θ = 14.8°, N(1)–O(1)′ = 3.05 Å, C(2)–O(1)′ = 2.97 Å, C(6)–O(1)′ = 3.46 Å;[48] (*b*) *d* = 3.26 Å, O(1)′–C(2) = 3.14 Å, O(1)′–N(3) = 3.28 Å;[31] (*c*) θ = 22.3°, N(1)–O(1)′ = 3.02 Å, N(3)–O(1)′ = 3.45 Å, C(6)–O(1)′ = 3.11 Å, C(5)–O(1)′ = 3.41 Å, C(2)–O(1)′ = 3.21 Å;[49] (*d*) *d* = 3.25 Å, O(1)′–C(5) = 3.13 Å, C(2)′–O(6) = 3.07 Å;[30] (*e*) *d* = 3.34 Å, N(1)–O(1)′ = 3.21 Å, C(2)–O(1)′ = 3.14 Å, N(3)–O(1)′ = 3.37 Å, O(9)–O(3)′ = 3.18 Å, N(9)–O(5)′ = 3.31 Å, C(4)–O(5)′ = 3.38 Å, C(8)–O(5)′ = 3.39 Å.[50]

a manner that there is any great amount of ring overlap. Rather, the charge transfer complexes are also usually characterized by the interaction of polar substituents with adjacent aromatic systems. Figure 21*e* shows an exception to this rule; in this unusual case the charge transfer process evidently dominates the interaction.[100]

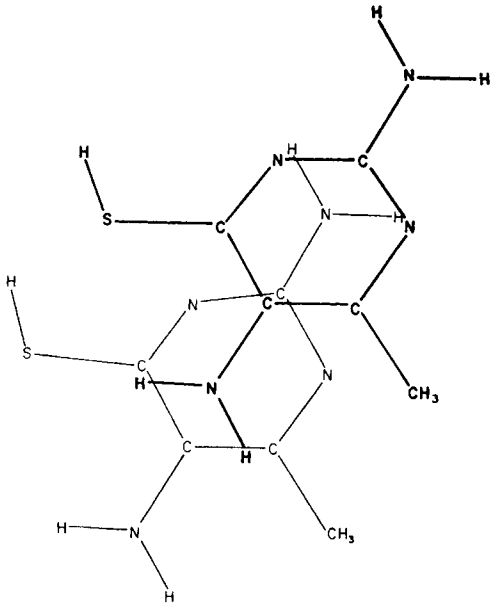
Although not depicted in the enclosed figures, partial overlap of parallel rings was found in crystals of acridine[101] and ethidium bromide.[102] No



(a) 2-Amino-4,6-Dichloropyrimidine
d=3.32

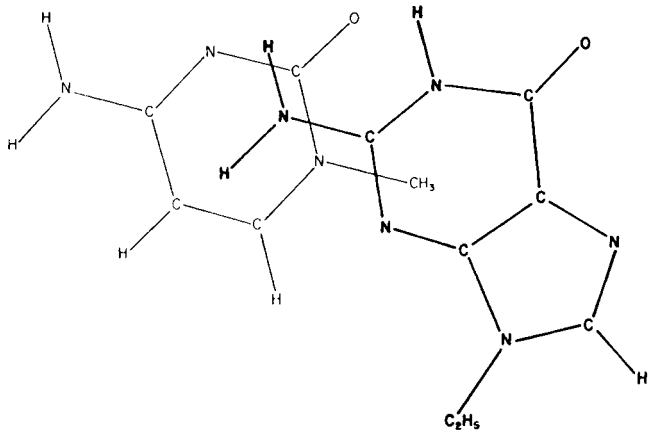


(b) Cytosine Monohydrate
d=3.3

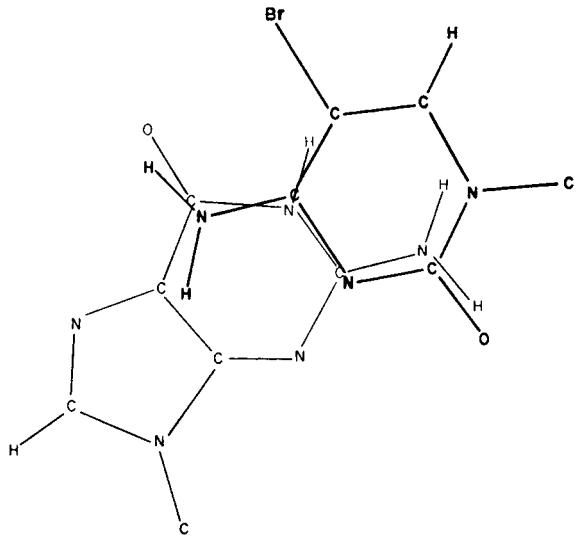


(c) 2,5-Diamino-4-Mercapto-6-Methylpyrimidine
d=3.58

Fig. 5 (*continued*)

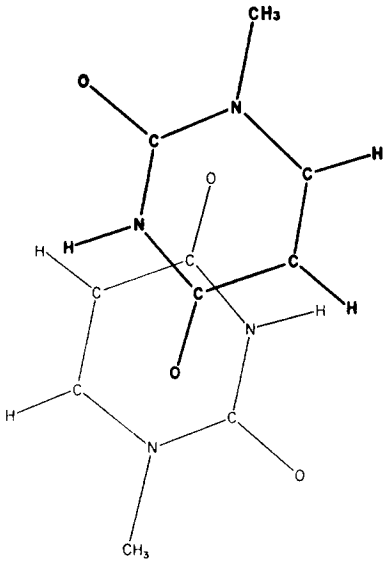


(d) 9-Ethyl Guanine and 1-Methyl Cytosine
d=3.4



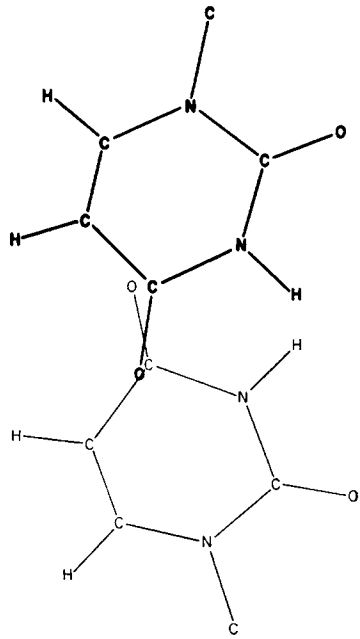
(e) Deoxyguanosine and 5-Bromodeoxycytidine
d= 3.4

Fig. 5. Interactions of amino groups with purines and pyrimidines: (*a*) d = 3.32 Å, N(1)–C(5) = 3.41 Å, N(1)–C(6) = 3.42 Å, C(2)–C(4) = 3.49 Å, C(2)–C(5) = 3.37 Å, N(2)–C(2) = 3.39 Å, N(2)–N(3) = 3.42 Å, N(3)–C(4) = 3.48 Å;[51] (*b*) θ = 2.21°, N(3)–C(4) = 3.44 Å;[52] (*c*) d = 3.58 Å;[53] (*d*) (similar stacking observed in the isomorphous 9-ethylguanine-1-methyl-5-fluorocystosine structure) θ = 6.45°, N(2)–N(1) = 3.48 Å, C(2)–N(1) = 3.41 Å;[54] (*e*) θ = 0.03°, C(2)–N(2) = 3.37 Å, N(3)–C(2) = 3.40 Å, C(4)–N(1) = 3.38 Å, N(4)–C(6) = 3.33 Å.[41]



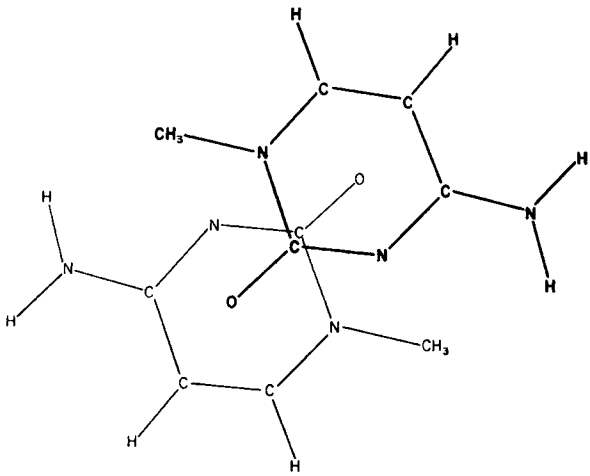
(a) 9-Ethyladenine and 1-Methyluracil

d=3.34



(b) Barium Uridine-5'-Phosphate

d=3.2



(c) 9-Ethylguanine and 1-Methylcytosine

d=3.25

Fig. 6 (*continued*)

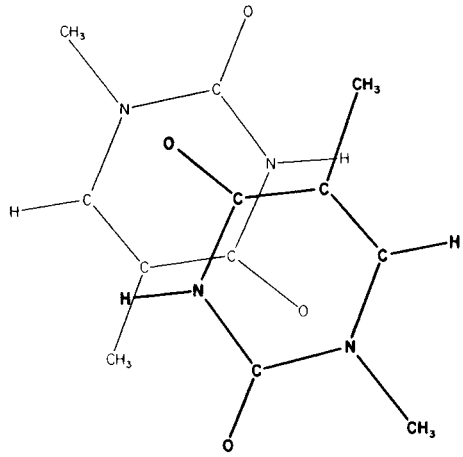


Fig. 6. Pyrimidine stacking interactions involving two carbonyl groups: (*a*) d = 3.34 Å, C(4)–C(4) = 3.40 A;[55] (*b*) θ = 3.29°, N(3)–O(6) = 3.46 Å, C(4)–C(4) = 3.35 Å, C(4)–O(4) = 3.12 Å, C(5)–O(4) = 3.27 Å, O(4)–O(4) = 3.38 Å;[43] (*c*) (similar stacking observed in the isomorphous 9-ethylguanine-1-methyl-5-fluorocytosine structure) d = 3.25 Å, N(1)–N(3) = 3.45 Å, C(2)–C(2) = 3.30 Å, C(2)–N(3) = 3.48 Å, O(2)–C(4) = 3.47 Å;[54] (*d*) = 3.28 Å, N(1)–O(4) = 3.39 Å, C(2)–O(4) = 3.42 Å, N(3)–C(4) = 3.36 Å, N(3)–C(5) = 3.39 Å, C(4)–C(4) = 3.37 Å.[56]

stacking was found in the aromatic hydrocarbons benzene,[103] naphthalene,[104] anthracene,[105] and phenanthrene.[106]

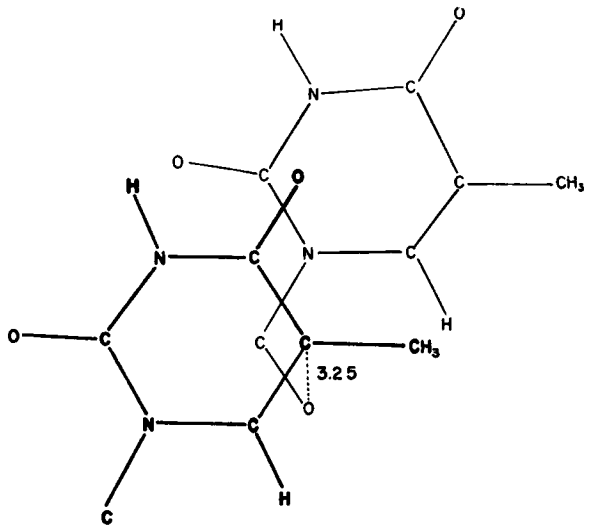
DISCUSSION

Stacking Forces

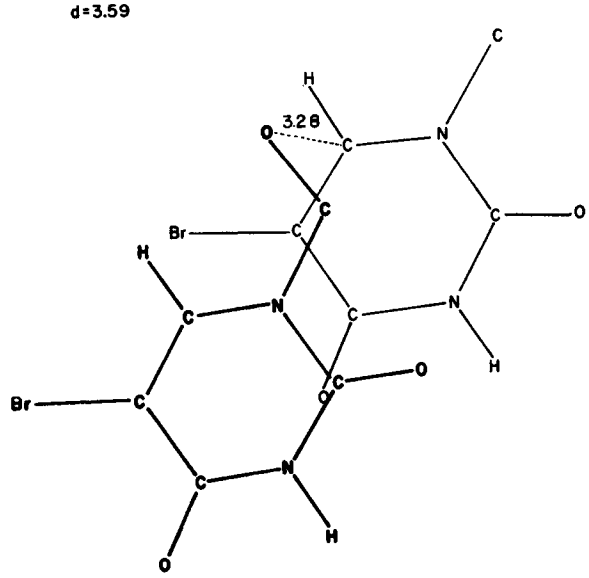
The most striking feature of these structures is the recurring interaction of heteroatoms with adjacent bases. As can be seen by molecular orbital calculations[107] or by valence-bond considerations,[108] the purines and pyrimidines possess considerable charge asymmetry with the nitrogen and oxygen atoms of the bases carrying large partial charges; consequently, bonds involving the heteroatoms have relatively large bond moments. Thus these stacking forces appear to be mainly of the dipole–induced dipole type, involving the electrostatic interaction of a partial bond moment with a polarizable π-electron system.[109,110] Interactions of this type in other crystal structures have been described.[94] Less specific London dispersion forces would be expected to further stabilize the stacked configuration.

The observed solid-state stacking suggests that association involving partial overlap of bases is the most common type of interaction. This type of interaction is probably a primary stabilizing force in the solid state, as evidenced by the close approaches of parallel bases in these crystal struc-

tures (e.g., the interplanar separations found in graphite and crystals of polynuclear aromatic molecules are usually about 3.4–3.5 Å). The recurring modes of stacking in these structures indicate that the stacking interactions are surprisingly specific. In many cases, the base stacking is even more specific than hydrogen bonding. For example, the hydrogen-bonding



(a) 5-Methyl Uridine
d=3.59



(b) 5-Bromouridine and Adenosine
d=3.54

Fig. 7 (*continued*)

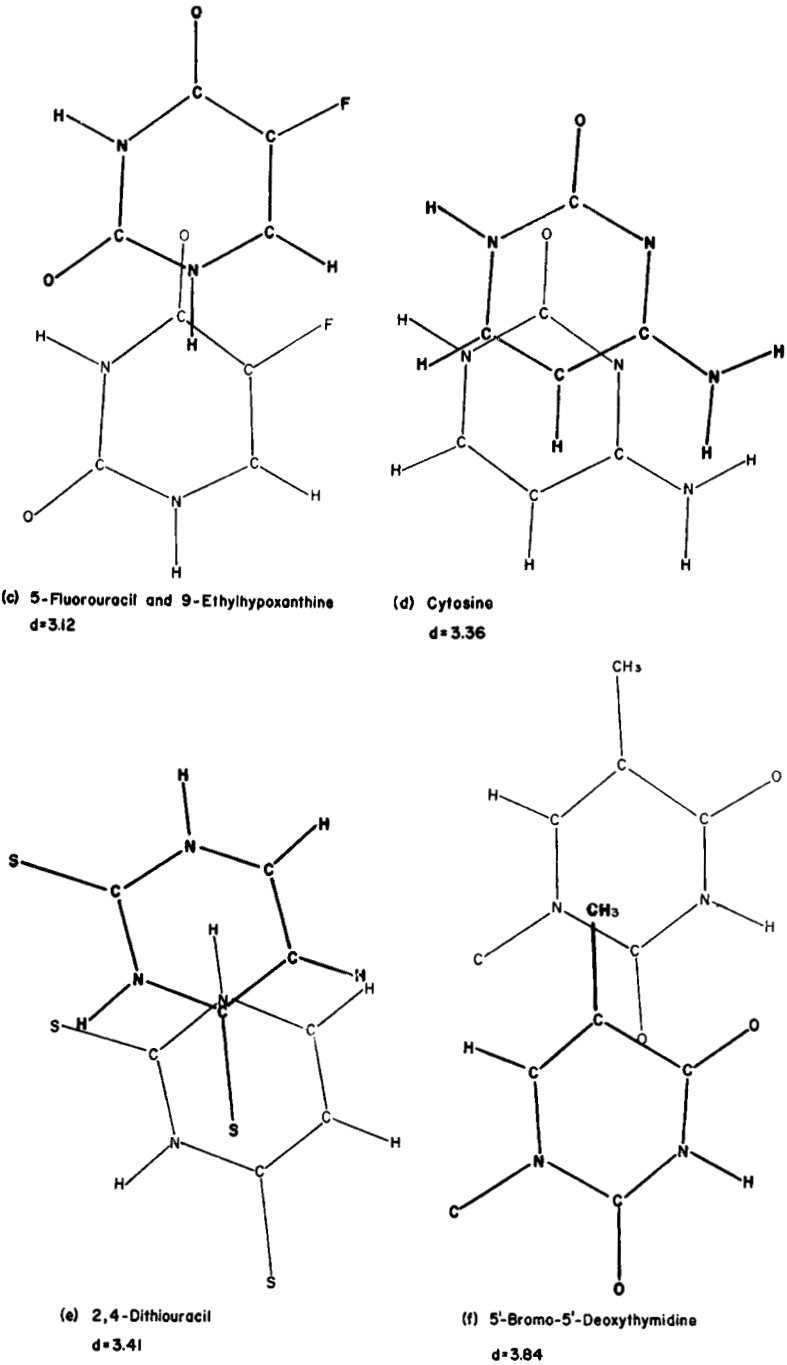


Fig. 7. Overlap involving a single carbonyl oxygen atom or thione sulfur atom: (*a*) d = 3.59 Å, C(5)–O(1)′ = 3.25 Å, C(6)–O(1)′ = 3.20 Å;[31] (*b*) d = 3.54 Å, O(1)′–C(5) = 3.35 Å, O(1)′–C(6) = 3.28 Å;[40] (*c*) d = 3.12 Å, N(1)–C(4) = 3.20 Å, C(2)–C(4) = 3.49Å;[57] (*d*) d = 3.36 Å, N(1)–O(2) = 3.44 Å, C(2)–O(2) = 3.40 Å, C(4)–N(3) = 3.42 Å, C(5)–N(3) = 3.50 Å, C(6)–N(1) = 3.39 Å, C(6)–C(2) = 3.48 Å;[58] (*e*) d = 3.41 Å, C(6)–N(3) = 3.44 Å;[59] (*f*) d = 3.84 Å.[60]

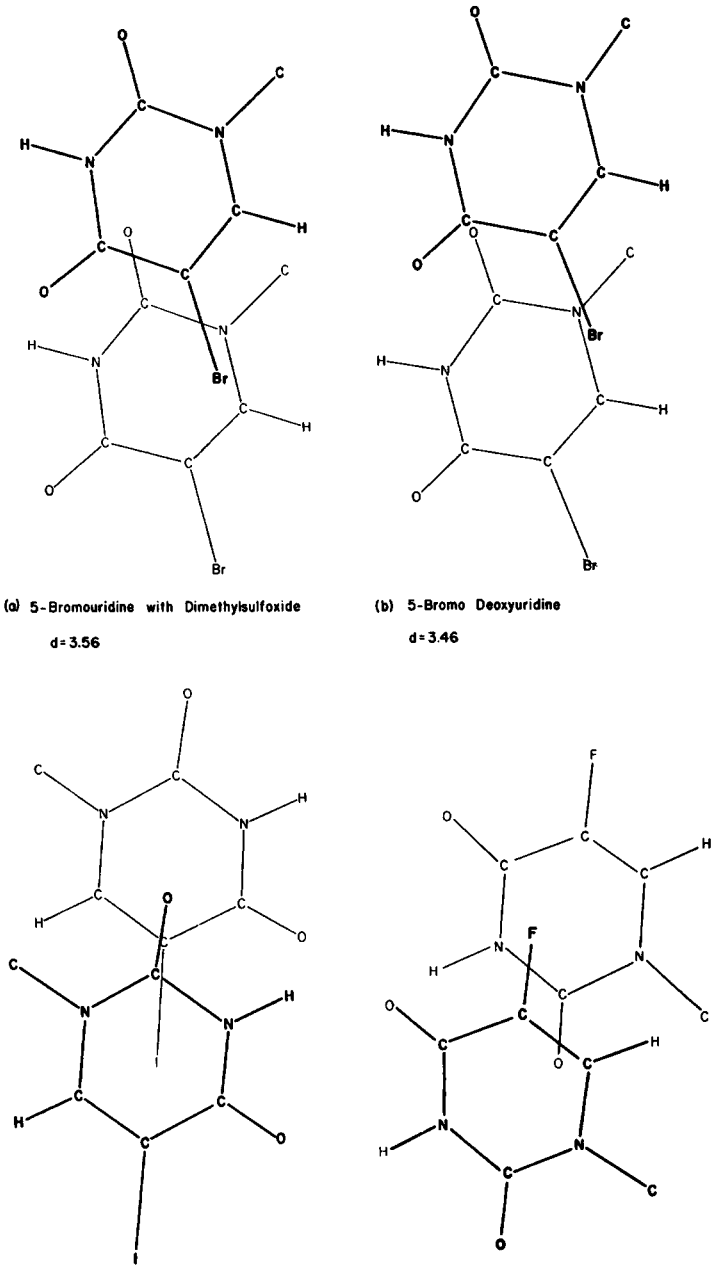


Fig. 8 (*continued*)

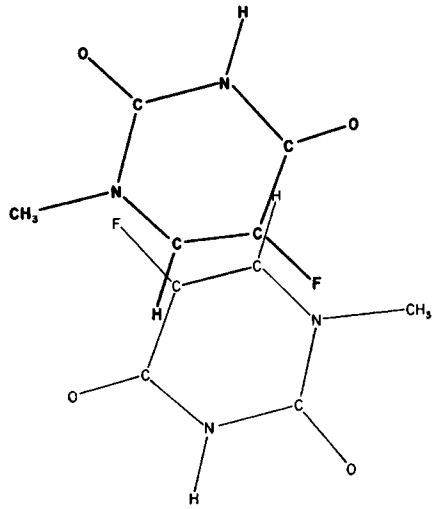


Fig. 8. Pyrimidine stacking interactions involving halogen atoms: (*a*) d = 3.56 Å;[65] (*b*) d = 3.46 Å;[34] (*c*) d = 3.68 Å;[66] (*d*) d = 3.31 Å, C(6)–C(2) = 3.50 Å, C(6)–O(2) = 3.32 Å, C(5)–C(2) = 3.37 Å, C(5)–O(2) = 3.43 Å, F(5)–N(3) = 3.37 Å, F(5) – C (2) = 3.49 Å;[67] (*e*) d = 3.40 Å, N(1)–F(5) – 3.38 Å, C(5)–C(6) = 3.45 Å, C(6)–F(5) = 3.50 Å.[68]

schemes are completely unrelated in the four crystal structures shown in Figure 1; however, the base–base stacking association and the interactions involving 0(1)′ are very similar in the four structures. The stacking patterns in polynucleotides also display a number of these common features.

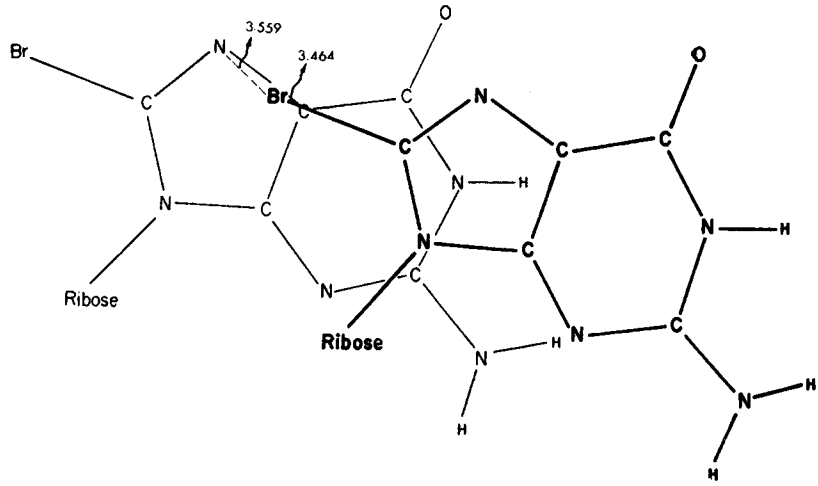


Fig. 9. Stacking in the crystal structure of 8-bromoguanosine; d = 3.48 Å, Br–C(5) = 3.464 Å, Br–N(7) = 3.559 Å.[69]

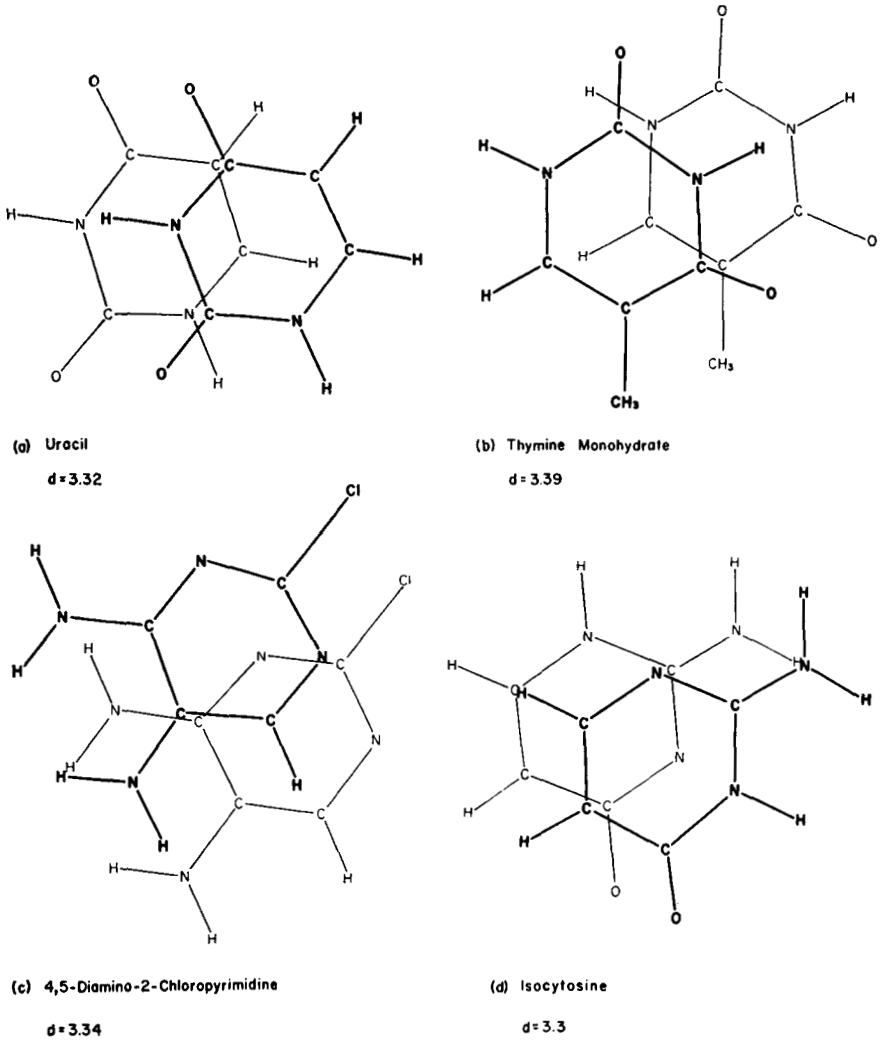


Fig. 10. Pyrimidine overlap involving one ring nitrogen atom: (*a*) d = 3.32 Å, C(2)–N(1) = 3.32 Å, C(4)–C(5) = 3.33 Å, O(2)–N(1) = 3.49 Å;[70] (*b*) d = 3.39 Å, C(4)–C(5) = 3.42 Å, C(2)–N(1) = 3.43 Å;[71] (*c*) d = 3.34 Å, N(1)–C(2) = 3.34 Å, N(1)–N(3) = 3.49 Å, C(5)–C(4) = 3.39 Å;[72] (*d*) θ = 9.80°, N(1)–C(2) = 3.48 Å, C(2)–N(3) = 3.45 Å, N(3)–N(3) = 3.42 Å, C(4)–C(4) = 3.42 Å, C(4)–O(4) 3.23 Å, C(5)–C(4) = 3.25 A, C(5)–O(4) = 3.36 Å, O(4) – O(4) = 3.31 Å.[73]

It thus appears that, in addition to acting as a stabilizing force, stacking interactions probably play a major role in establishing and maintaining the structural specificity of the nucleic acids.

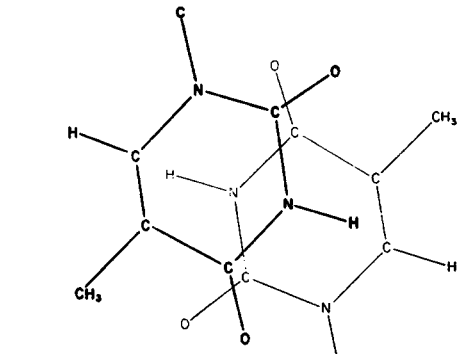
The solid-state stacking apparently cannot be rationalized on the basis of permanent, molecular dipole–dipole interactions. Base stacking occurs regardless of whether the dipole moments of the bases are parallel, antiparallel, or in an intermediate orientation. Among the structures exam-

ined, there are numerous examples in which bases are stacked with their dipole moments approximately parallel and thus in the most unfavorable configuration for dipole-dipole interactions; a particularly good example of this is found in the guanosine structure (Fig. 13), where the dipole moments[25] of adjacent guanine moieties are almost exactly parallel and superimposed.

It is interesting that the strength of association between the bases in aqueous solution decreases in the order purine–purine, purine–pyrimidine, pyrimidine–pyrimidine.[17] The polarizabilities of purines are considerably greater than those of pyrimidines;[25,111] this suggests that induction effects are also important in solution base stacking and may provide an explanation for the observed differences in the stacking properties of purines and pyrimidines. Experimentally, the degree of self-association of nucleosides in aqueous solution correlates with the polarizabilities of the bases.[112] Thus it is possible that dipole–induced dipole forces are also largely responsible for base stacking in aqueous solutions. Since the specific stacking interactions reported here have been found to persist in a number of different crystalline environments, it would not be surprising if these same stacking patterns are also important in solution.

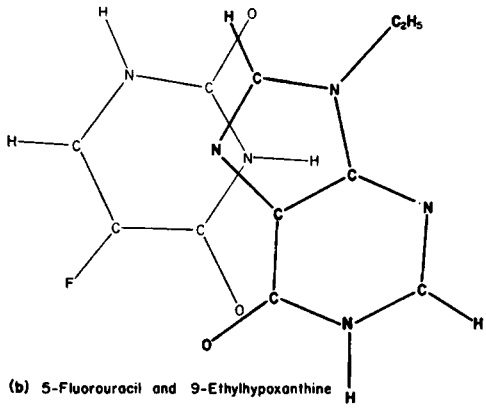
Whereas purines and pyrimidines and aromatic charge transfer complexes usually do stack in the solid state, aromatic hydrocarbons such as benzene,[102,103] naphthalene,[104] anthracene,[105] and phenanthrene[106] crystallize in a characteristic herringbone pattern, with no overlap between parallel molecules. However, when heteroatoms are substituted in the rings or polar substituents are added to aromatic hydrocarbons, stacking interactions become a dominant feature of the crystal structures.[113] For example, there is considerable molecular superposition in the crystal structure of acridine[100] as contrasted to the absence of stacking in anthracene; similarly, in spite of the absence of molecular overlap in the crystal structure of phenanthrene, the crystal structure of ethidium bromide[101] displays stacking interactions between parallel phenanthridinium rings. In brief, nonpolar hydrophobic compounds do not generally stack in the solid state, whereas polar compounds usually do.

The apparent absence of base stacking in nonaqueous solution[10,23] has been interpreted as evidence that stacking interactions are due to hydrophobic factors involving aggregation which is primarily the consequence of solvent effects (for discussion see Felsenfeld and Miles[114]). The manner in which hydrophobic factors cause the aggregation of nonpolar substituents in aqueous solution has been discussed by Kauzmann;[115] for the most part, such aggregations are due primarily to the favorable entropy changes accompanying the removal of nonpolar substituents from the water phase. However, it is doubtful that such solvent effects could be totally responsible for causing the aggregation of relatively polar purines and pyrimidines in aqueous solutions.[112] It is especially unlikely that solvent considerations alone can account for the observation that, in aqueous solution, the self-association of adenosine is greater than that of ribosyl purine,[112] since the



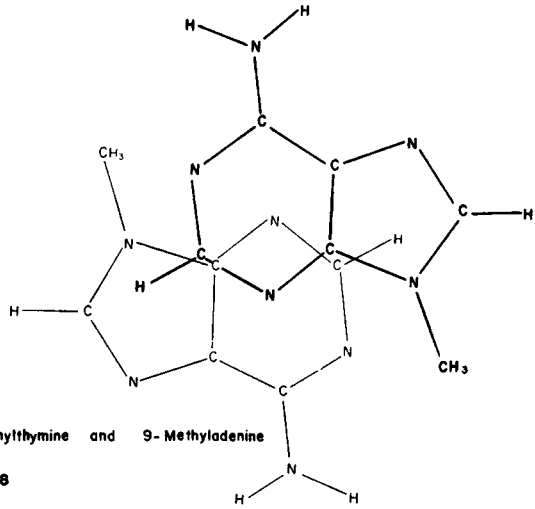
(a) Calcium Thymidine-5'-Phosphate

d=3.3



(b) 5-Fluorouracil and 9-Ethylhypoxanthine

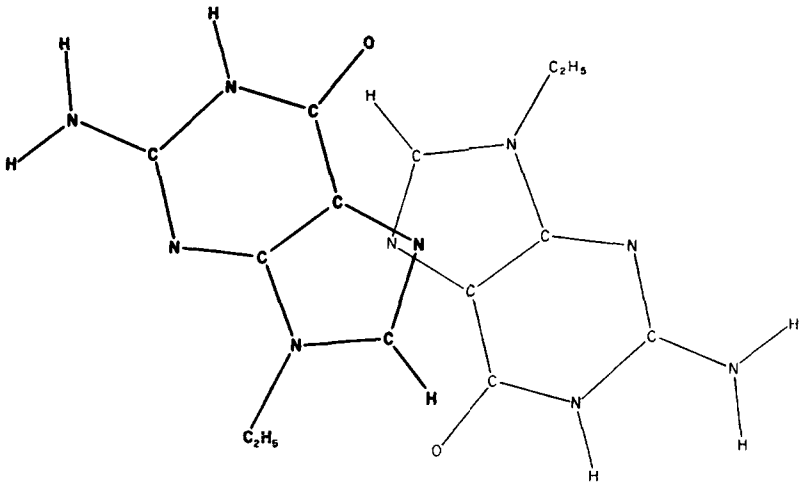
d=3.2



(c) 1-Methylthymine and 9-Methyladenine

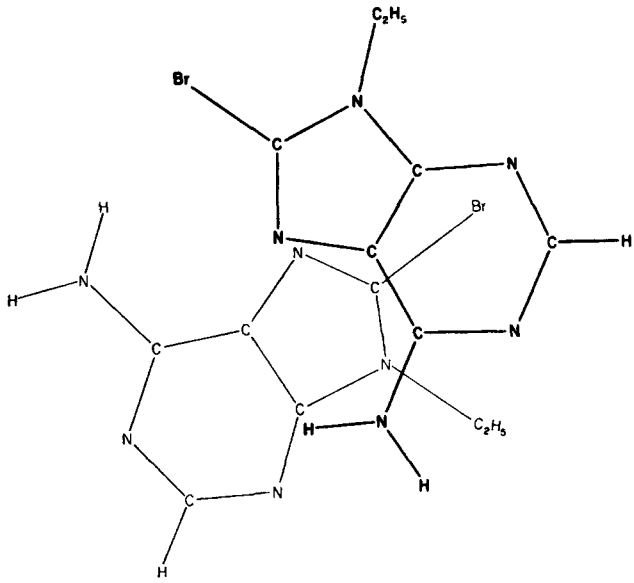
D=3.28

Fig. 11 (*continued*)



(d) 9-Ethylguanine and 5-Methylcytosine

d = 3.27



(e) 8-Bromo-9-Ethyladenine and Phenobarbital

d = 3.3

Fig. 11. Overlap involving two ring nitrogen atoms: (*a*) θ = 167.75°, C(4)–C(2) = 3.20 Å, C(4)–O(2) = 3.36 Å, O(4)–O(2) = 3.37 Å, O(4)–N(1) = 3.40 Å, O(4)–O(2) = 3.35 Å, O(4)–C(2) = 3.29 Å;[74] (*b*) θ = 3.79°, C(5)–N(3) = 3.36 Å, C(5)–C(4) = 3.42 Å, N(7)–N(3) = 3.26 Å, N(7)–C(2) = 3.38 Å, N(7)–C(4) = 3.41 Å, C(8)–N(3) = 3.47 Å, C(8)–C(2) = 3.26 Å, C(8)–O(2) = 3.39 Å;[57] (*c*) *d* = 3.28 Å, C(2)–C(4) = 3.29 Å, C(2)–N(9) = 3.48 Å, N(3)–C(4) = 3.44 Å, C(4)–C(2) = 3.29 Å, N(9)–C(2) = 3.48 Å, C(4)–N(3) = 3.44 Å;[56] (*d*) (similar stacking observed in the isomorphous 9-ethylgaunine-1-methyl-5-fluorocytosine structure) *d* = 3.27 Å, C(5)–N(7) = 3.45 Å, N(7)–N(7) = 3.31 Å, N(7)–C(5) = 3.45 Å;[54] (*e*) θ = 7.20°, C(5)–C(8) = 3.50 Å, C(6)–N(9) = 3.41 Å, N(7)–N(7) = 3.50 Å, N(6)–N(9) = 3.45 Å.[75]

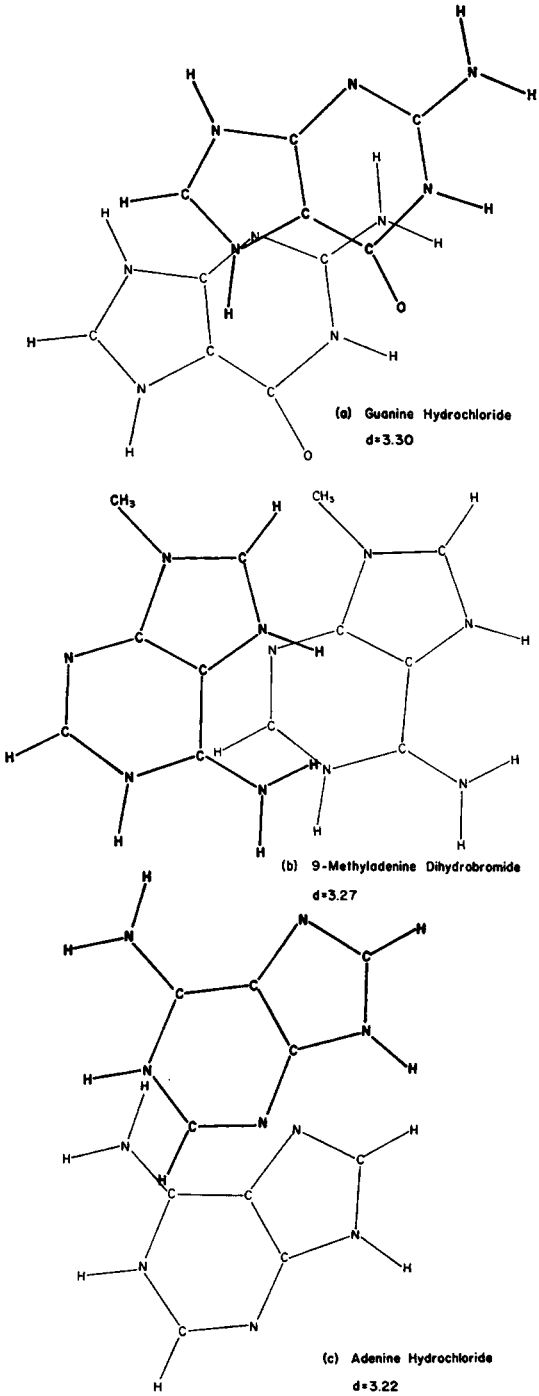
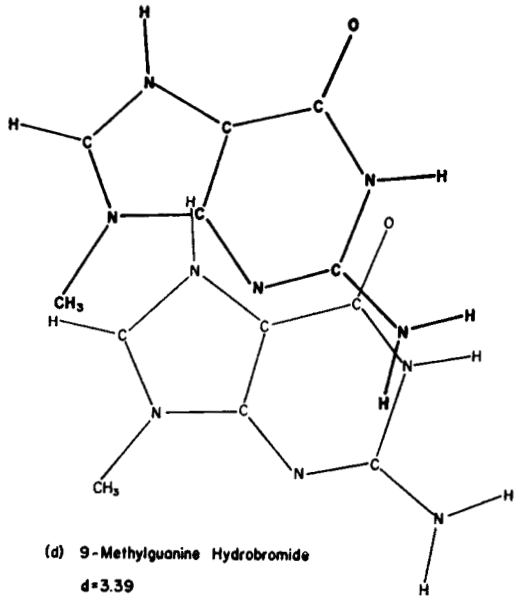
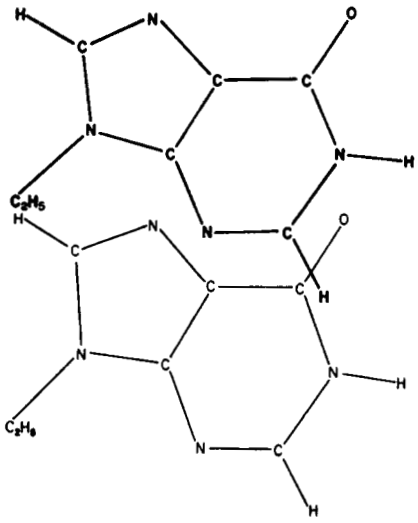


Fig. 12 (*continued*)



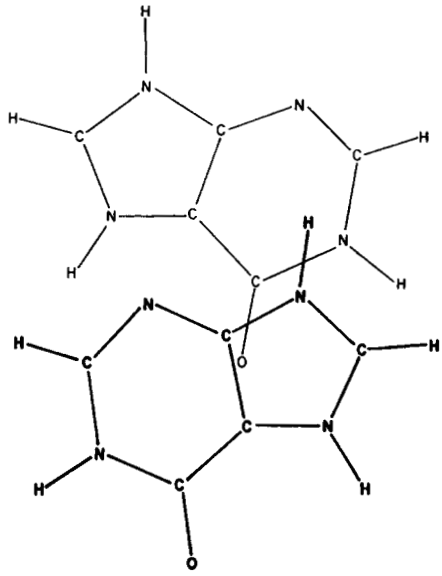
(d) 9-Methylguanine Hydrobromide

d=3.39



(e) 5-Fluorouracil and 9-Ethylhypoxanthine

d=3.34



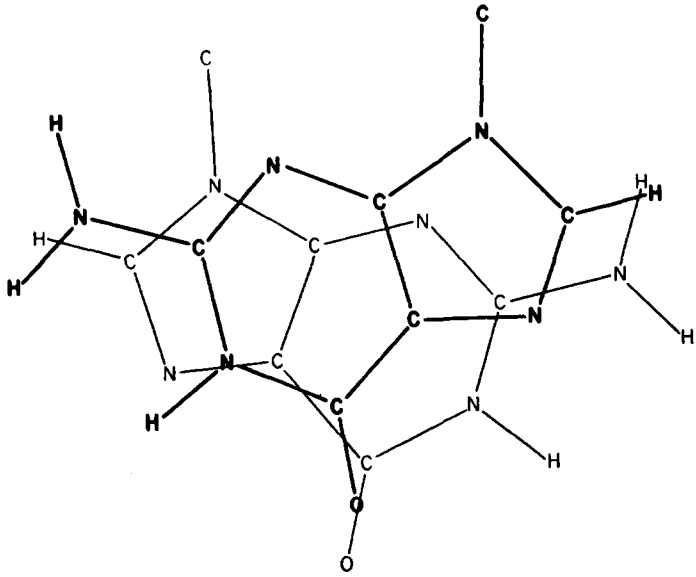
(f) Hypoxanthine Hydrochloride

d=3.20

Fig. 12. Stacking of protonated purines and the similar stacking of nonprotonated 9-ethylhypoxanthine: (*a*) d = 3.30 Å, N(1)–N(2) = 3.36 Å, C(5)–C(2) = 3.42 Å, C(5)–N(3) = 3.40 Å, C(5)–N(2) = 3.46 Å, C(6)–C(2) = 3.43 Å, C(6)–N(2) = 3.28 Å, N(7)–N(3) = 3.31 Å, N(7)–C(4) = 3.40 Å;[76] (*b*) d = 3.27 Å, C(5)–N(3) = 3.43 Å, N(7)–N(3) = 3.40 Å N(6)–N(1) = 3.35 Å, N(6)–C(2) = 3.37 Å;[77] (*c*) d = 3.22 Å, C(2)–C(6) = 3.46 Å; C(2)–N(6) = 3.46 Å, N(3)–C(5) = 3.44 Å, N(3)–N(7) = 3.26 Å, C(4)–N(7) = 3.49 Å;[78] (*d*) d = 3.39 Å, N(1)–O(6) = 3.48 Å, C(2)–C(6) = 3.42 Å, C(2)–O(6) = 3.48 Å, N(3)–C(5) = 3.45 Å, N(2)–N(1) – 3.42 Å, N(2)–C(6) = 3.47 Å;[79] (*e*) d = 3.34 Å, C(2)–C(6) = 3.45 Å, C(2)–O(6) = 3.49 Å, N(3)–C(5) = 3.43 Å, N(3)–N(7) = 3.46 Å;[57] (*f*) d = 3.20 Å, N(3)–N(7) = 3.40 Å, N(9)–O(6) = 3.43 Å, N(9)–N(1) = 3.35 Å, N(9)–C(6) = 3.24 Å, C(4)–O(6) = 3.22 Å, C(4)–C(6) = 3.37 Å, C(5)–O(6) = 3.31 Å.[80]

polar amino group should enhance the solvation properties of the nucleoside. Although solvent effects probably play a role in causing the aggregation of purines and pyrimidines in aqueous solution, it is possible that other factors, presumably the specific types of polar interactions described here, also stabilize the stacked states.

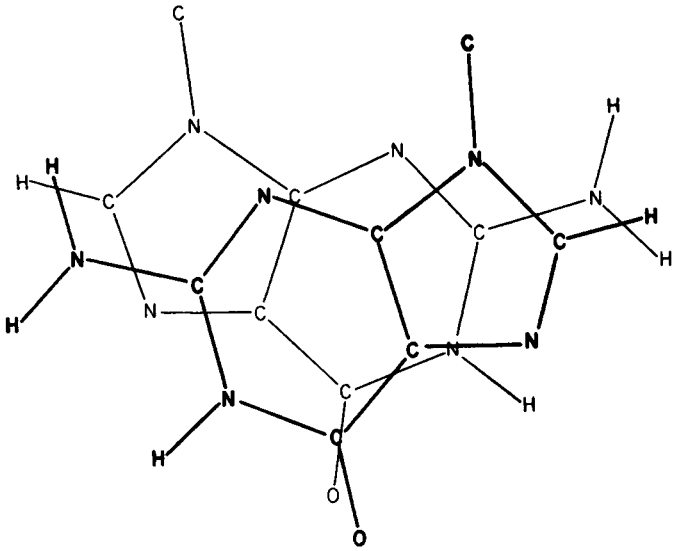
It is also possible that the absence of purine and pyrimidine stacking in nonaqueous solutions is partially due to specific solvent–base interactions which are similar to the interactions depicted here. Many of the nonaqueous solvents utilized for studying base–base interactions consist of molecules with polar substituents which might interact with purines and pyrimidines, thus competing with the forces causing self-association of the bases. This possibility is supported by work which demonstrates a pronounced correlation between the polarizabilities of solvents and their abilities to act as denaturing agents[116] for nucleic acids. Specific interactions between chloroform and aromatic compounds have been demonstrated;[117,118] perhaps similar interactions are partially responsible for the observed absence of base stacking in chloroform solutions.[23] Similarly, the absence of base stacking in dimethyl sulfoxide and dimethylformamide[19,23] may be due to interactions between solvent oxygen atoms and the bases. These interactions might be similar to those depicted in Figures 6 and 7. In this respect, it is also of interest that certain anions will denature nucleic acids and will increase the water solubilities of purines and pyrimidines.[119–121] Robinson



(a) Guanosine

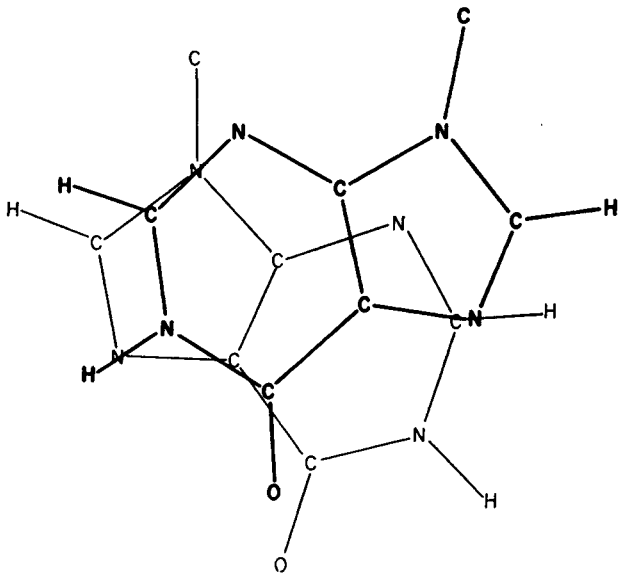
d=3.3

Fig. 13 (*continued*)



(b) Guanosine

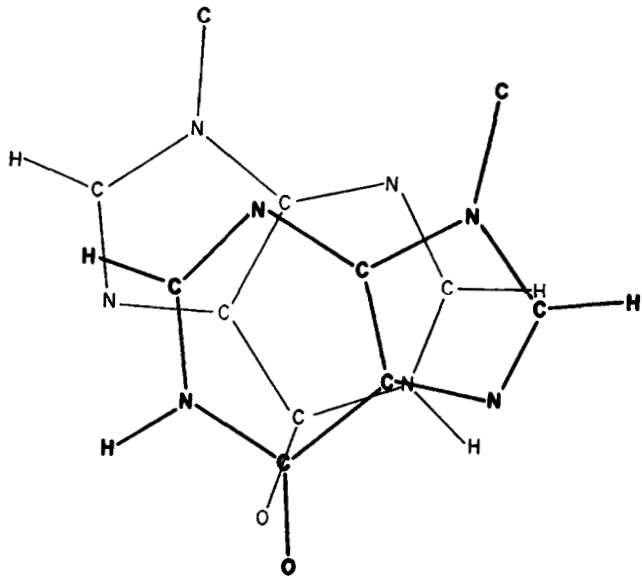
d≈3.3



(c) Inosine

d≈3.3

Fig. 13 (*continued*)

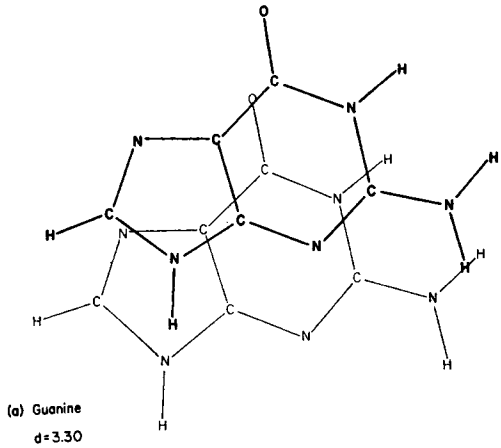


(d) Inosine

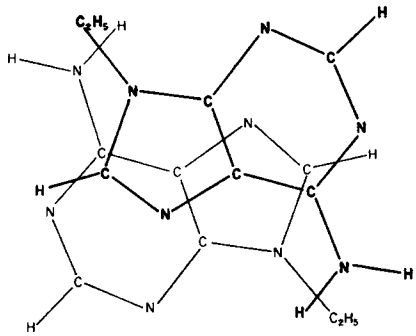
d≈3.3

Fig. 13. Purine stacking in the crystal structures of guanosine and inosine:[81,82] (*a*) θ = 0.61°, C(2)–C(8) = 3.40 Å, C(2)–N(9) = 3.33 Å, N(3)–C(4) = 3.49 Å, N(3)–N(9) = 3.36 Å, C(4)–N(3) = 3.37 Å, C(4)–C(4) = 3.41 Å, C(5)–C(2) = 3.43 Å, C(6)–C(5) = 3.35 Å, C(6)–C(6) = 3.33 Å, N(7)–N(1) = 3.49 Å, N(7)–C(2) = 3.29 Å, N(7)–N(2) = 3.47 Å, C(8)–C(2) = 3.47 Å, C(8)–N(2) = 3.39 Å, N(9)–N(3) = 3.47 Å, N(2)–C(8) = 3.34 Å, O(6)–C(6) = 3.28 Å, O(6)–O(6) = 3.30 Å, N(1)–C(5) = 3.34 Å, N(1)–N(7) = 3.37 Å; (*b*) θ = 0.82°, N(1)–C(5) = 3.49 Å, C(2)–C(5) = 3.40 Å, C(2)–N(7) = 3.34 Å, N(3)–C(4) = 3.30 Å, N(3)–N(9) = 3.46 Å, C(4)–N(3) = 3.45 Å, C(4)–C(4) = 3.46 Å, C(5)–N(1) = 3.33 Å, C(5)–C(6) = 3.41 Å, C(6)–C(6) = 3.35 Å, C(6)–O(6) = 3.36 Å, N(7)–C(1) = 3.43 Å, C(8)–C(2) = 3.46 Å, C(8)–N(2) = 3.33 Å, N(9)–C(2) = 3.41 Å, N(9)–N(3) = 3.43 Å, N(2)–N(7) = 3.45 Å, N(2)–C(8) = 3.38 Å, O(6)–O(6) = 3.37 Å; (*c*) θ = 3.61°, N(1)–C(5) = 3.40 Å, N(1)–N(7) = 3.39 Å, C(2)–N(9) = 3.40 Å, N(3)–N(9) = 3.44 Å, C(4)–N(3) = 3.34 Å, C(4)–C(4) = 3.44 Å, C(5)–C(2) = 3.41 Å, C(5)–N(3) = 3.42 Å, C(5)–C(4) = 3.48 Å, C(6)–C(5) = 3.31 Å, C(6)–C(6) = 3.35 Å, N(7)–N(1) = 3.45 Å, N(7)–C(2) = 3.21 Å, C(8)–C(2) = 3.46 Å, N(9)–N(3) = 3.43 Å, O(6)–C(6) = 3.22 Å, O(6)–O(6) = 3.28 Å; (*d*) θ = 2.76°, N(1)–C(5) = 3.41 Å, N(1)–N(7) = 3.45 Å, C(2)–C(5) = 3.32 Å, C(2)–N(7) = 3.28 Å, C(2)–C(8) = 3.42 Å, N(3)–C(4) 3.21 Å, N(3)–C(5) = 3.46 Å, N(3)–N(9) = 3.36 Å, C(4)–C(2) = 3.48 Å, C(4)–N(3) = 3.40 Å, C(4)–C(4) = 3.42 Å, C(5)–N(1) = 3.35 Å, C(5)–C(6) = 3.44 Å, C(6)–C(6) = 3.34 Å, C(6)–O(6) = 3.40 Å, N(7)–N(1) = 3.48 Å, N(9)–C(2) = 3.43 Å, N(9)–N(3) = 3.43 Å, O(6)–O(6) = 3.39 Å.

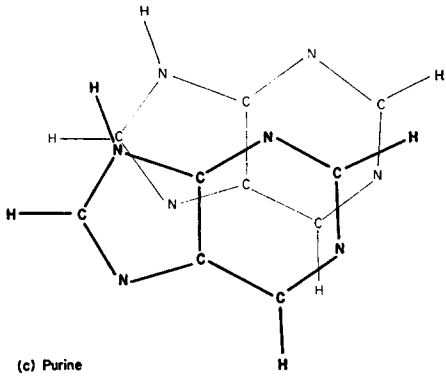
and Grant[121] present evidence that these effects are due to direct interactions of the anions with the bases; possibly these interactions also can be attributed to dipole–induced dipole forces involving the polar anions and the polarizable bases.



(a) Guanine
d=3.30



(b) 9-Ethyladenine and 1-Methyl-5-Fluorouracil
d=3.31



(c) Purine
d=3.38

Fig. 14 (*continued*)

Halogenated Purines and Pyrimidines

The results of several studies suggest that halogen substituents affect base-stacking interactions. For example, it has been demonstrated that the thermal stabilities of double helical polynucleotides are enhanced when halogenated pyrimidines are substituted for the naturally occurring bases,[122–125] and it has been concluded that these effects may be due to altered base-stacking interactions.[122,123] In addition, it has been shown that association of nucleosides occurs to a greater extent in aqueous solutions of 5-bromodeoxyuridine than in solutions of thymidine or uridine.[18,112]

Comparison of the base-stacking patterns in crystal structures of halogenated and nonhalogenated bases suggests that halogen substituents have

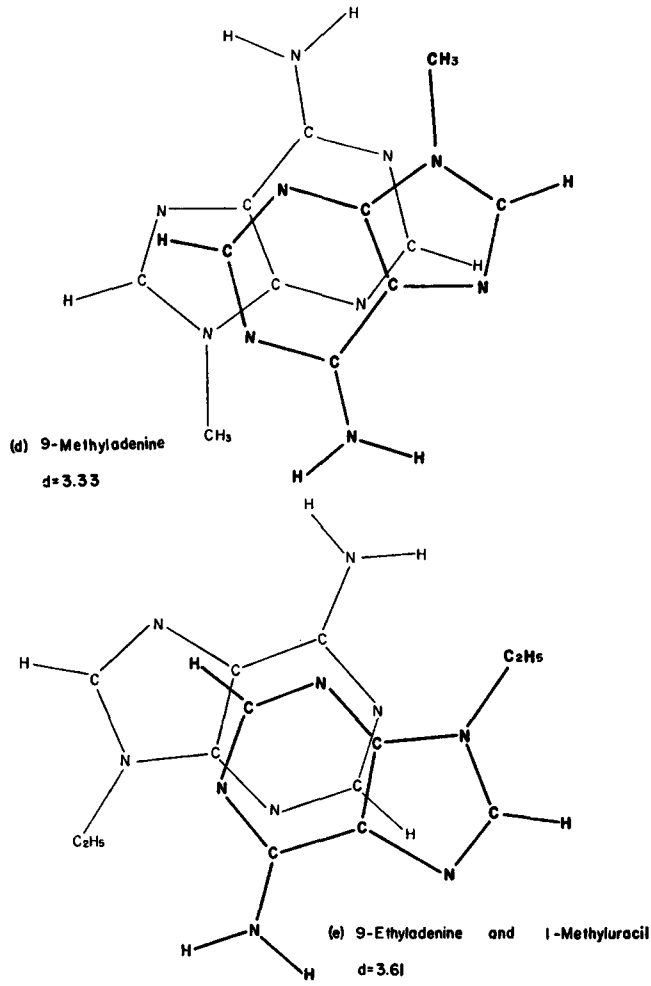


Fig. 14 (*continued*)

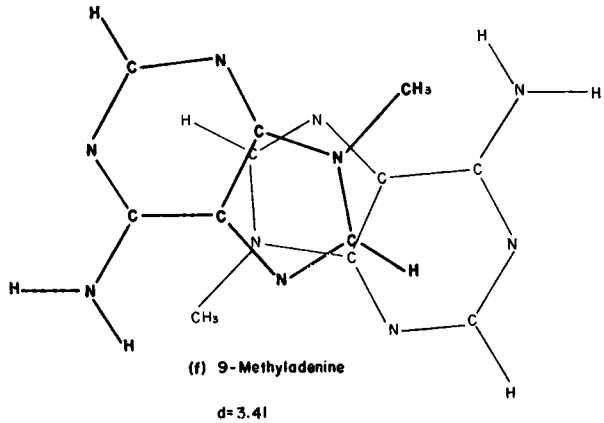


Fig. 14. Purine structures with moderate amounts of base overlap: (*a*) d = 3.30 Å, C(2)–N(1) = 3.35 Å, N(3)–N(1) = 3.40 Å, N(3)–C(2) = 3.40 Å, C(4)–C(5) = 3.36 Å, C(4)–C(6) = 3.42 Å, C(5)–C(6) = 3.43 Å, C(5)–O(6) = 3.44 Å, C(6)–O(6) = 3.35 Å, C(8)–N(7) = 3.30 Å, N(9)–C(5) = 3.39 Å, N(9)–N(7) = 3.46;[81,83] (*b*) d = 3.31 Å, N(1)–C(8) = 3.43 Å, N(3)–N(1) = 3.43 Å, C(4)–N(7) = 3.45 Å, C(5)–(C5) = 3.49 Å, C(6)–C(8) = 3.27 Å, C(6)–N(9) = 3.46 Å, N(9)–N(6) = 3.49 Å, C(9)–N(6) = 3.38 Å;[68] (*c*) d = 3.38 Å, N(1)–C(6) = 3.44 Å, C(2)–N(1) = 3.49 Å, N(3)–C(4) = 3.44 Å, C(4)–C(5) = 3.46 Å, C(4)–N(7) = 3.45 Å, N(9)–C(8) = 3.37 Å;[84] (*d*) d = 3.33 Å, N(1)–C(4) = 3.43 Å, N(1)–N(9) = 3.38 Å, C(2)–C(4) = 3.46 Å, C(2)–C(5) = 3.40 Å, N(3)–C(5) = 3.38 Å, N(3)–C(6) = 3.45 Å;[85] (*e*) d = 3.61 Å;[55] (*f*) d = 3.41 Å, C(4)–C(8) = 3.42 Å, C(5)–N(9) = 3.48 Å.[85]

a pronounced effect on solid-state base stacking. From Figures 8 and 9, it is apparent that interactions of halogen atoms with the ring systems of adjacent purines and pyrimidines is a common feature in the stacking patterns of several crystal structures. In general, the presence of a halogen substituent results in a stacking pattern which permits intimate contact between the halogen atoms and an adjacent base. The effects of halogen substituents on base stacking patterns are especially apparent when the base stacking pattern in the crystal structure of 8-bromoguanosine[69] (Fig. 9) is compared with that in the crystal structure of guanosine[81,82] (Figs. 13*a* and 13*b*). In the guanosine structure, the bases are essentially superimposed with a large amount of purine overlap. When a bromine atom is substituted in the 8-position of the guanine moiety, the stacking is altered so that the bromine atom forms close contacts with a neighboring base; it is noteworthy that the bromine contact with atom C(5) is considerably shorter than the sum of the van der Waals radii of the atoms involved, suggesting a rather strong interaction.

As seen in Figure 7, interaction of halogen atoms with uracil rings is a common feature in several crystal structures of halogenated uracil derivatives. It might be expected that similar interactions would be important in nucleic acids containing halogenated pyrimidines. Incorporation of 5-halogenated uracil derivatives in nucleic acids leads to mutagenesis which ap-

pears to be due to mispairing between the uracil derivatives and guanine.[126] Considering the base-stacking patterns found in crystals of halogenated uracil derivatives, a model which might account for mispairing in nucleic acids is depicted in Figures 22–24.

Figure 22*a* shows the stacking of adjacent pyrimidines in the biologically active B form of DNA, along with the normal Watson-Crick comple-

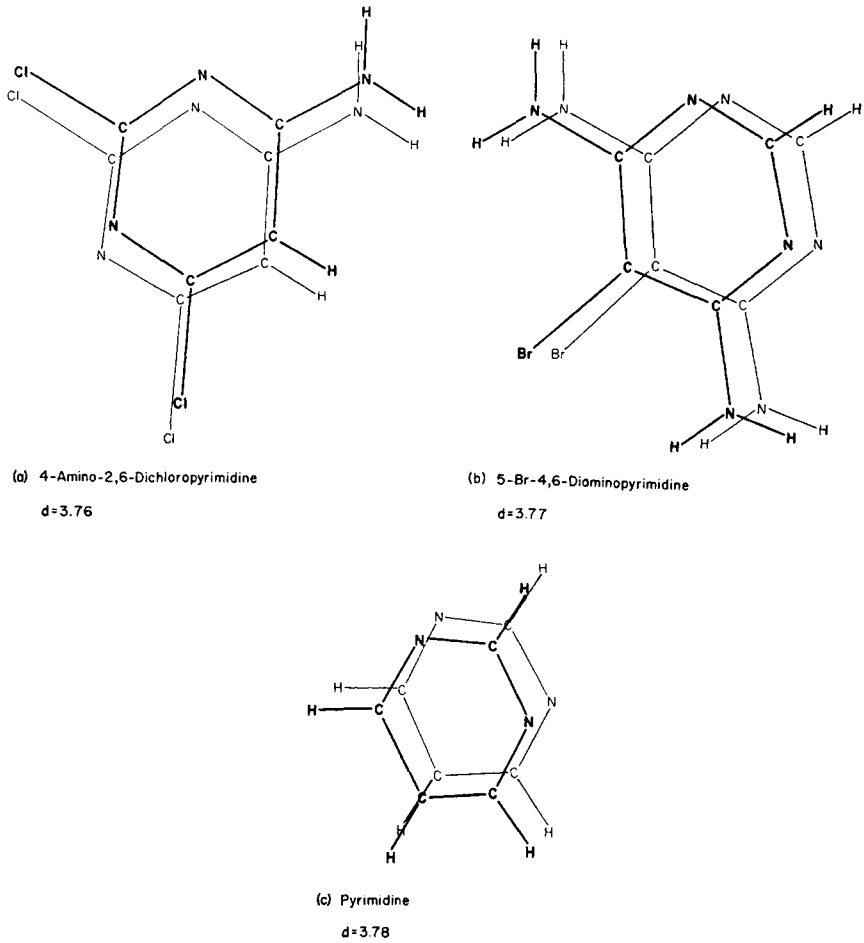


Fig. 15. Pyrimidine stacking involving extensive overlap accompanied by large interplanar spacings: (*a*) d = 3.76 Å;[86] (*b*) d = 3.77 Å;[86] (*c*) d = 3.78 Å.[87]

mentary hydrogen bonding between adenine and thymine. It is obvious that this stacking pattern does not permit the substituent at the 5-position of the pyrimidine to interact with the adjacent pyrimidine ring. Stacking similar to that shown in Figure 22*a* is found in the crystal structures of nonhalogenated uracil derivatives (Fig. 6).

Figure 22*b* shows a modified stacking pattern which results from a slight rotation and translation of the lower uracil residue shown in Figure 22*a*. This modified pattern allows the substituent at the 5-position of the pyrimidine to interact with the neighboring base; as seen in Figure 8, this stacking pattern is similar to that found in the crystal structures of halogenated uracil derivatives. As a consequence of this modified stacking pattern, the uracil derivative assumes an orientation which could result in hydrogen bonding to guanine, as shown in Figure 22*b*. If the normal conformation of

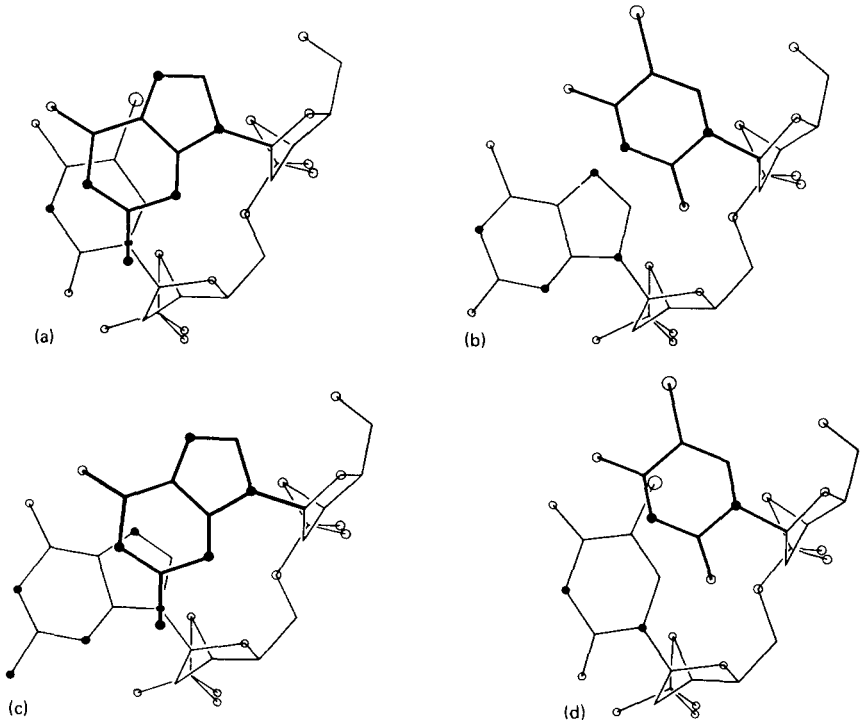


Fig. 16. Intrastrand stacking in the A form of DNA, $\theta = 11.8°$:[88] (*a*) O(6)–O(6) = 3.04 Å; (*b*) N(1) – N(7) = 3.42 Å; (*c*) N(1)–C(5) = 3.48 Å; (*d*) N(1)–C(5) = 3.45 Å.

the complementary strand were maintained, the rotated uracil derivative could only pair with guanine. As shown by Figure 23*a*, adenine could no longer hydrogen-bond to the uracil residue, and incorporation of an adenine residue in the complementary strand would result in an unreasonably short, nonbonded contact. Figure 23*b* shows that a pyrimidine incorporated in the complementary strand would be too far removed from the rotated uracil residue to result in hydrogen bonding.

Interactions between halogen substituents and purines could also lead to mispairing when a halogenated uracil derivative is incorporated adjacent to a purine in DNA. As shown in Figure 24*a*, the normal purine–pyrimidine

intrastrand stacking would not permit a halogen substituent at the 5-position of the pyrimidine to associate with the adjacent purine ring. However, as in the pyrimidine–pyrimidine stacking depicted in Figure 22, interaction of the halogen atom with the purine ring could be accomplished by a slight translation and counterclockwise rotation of the pyrimidine. Figure 23*b* shows that this modified purine–pyrimidine stacking pattern could result in hydrogen bonding between the uracil derivative and a guanine resi-

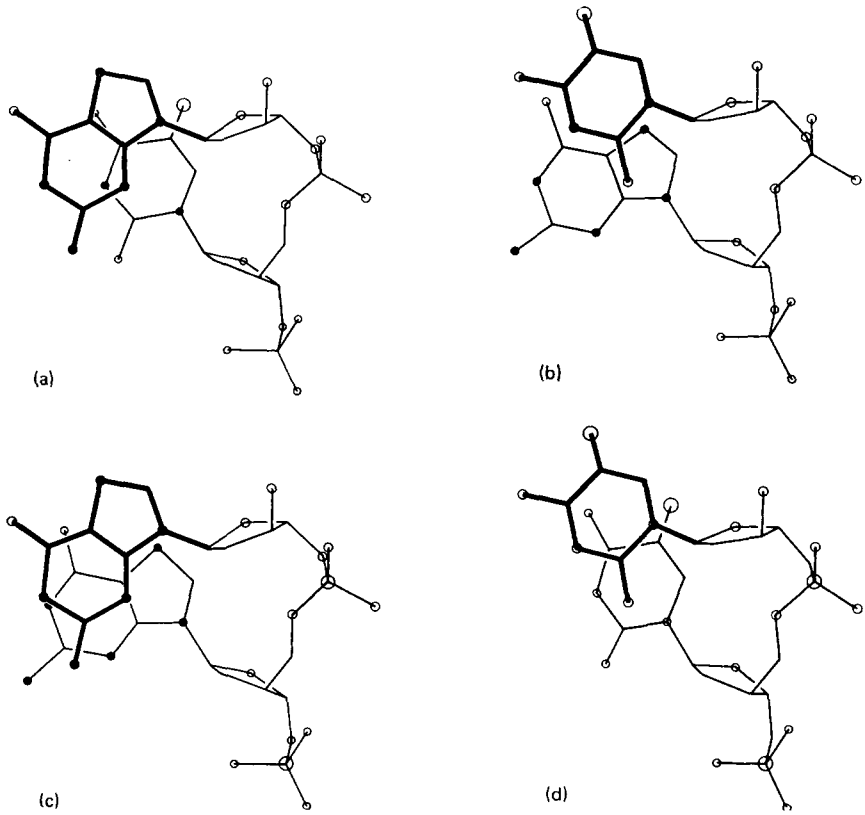


Fig. 17. Intrastrand stacking on the B form of DNA, $\theta = 4.8°$:[89] (*a*), (*b*) no contacts shorter than 3.5 Å; (*c*) N(1) – N(1) = 3.38 Å; (*d*) N(1) – C(6) = 3.48 Å, O(2)–C(5) = 3.47 Å.

due in the complementary strand. Again, it is found that, in this modified orientation, the uracil derivative could not hydrogen bond with adenine or with a pyrimidine.

Thus, as a consequence of altered base stacking interactions, halogenated uracil derivatives might specifically pair with guanine. This would result in the substitution of an erroneous amino acid into proteins which are constructed under the direction of the altered nucleic acids; therefore, the proposed model could account for the observed mutagenic effects of halo-

genated pyrimidines. Evidently, pairing errors involving halogenated uracil derivatives occur only at a few specific genetic sites, suggesting that the mispairing is somewhat dependent upon the local base composition at the site of substitution in nucleic acids.[126] Since base stacking necessarily involves the adjacent bases, an attractive feature of the model depicted in Figures 22–24 is the possibility that such stacking effects might explain this site-specificity of the mutations produced by halogenated uracil derivatives. It has been demonstrated that stacking forces vary for different combina-

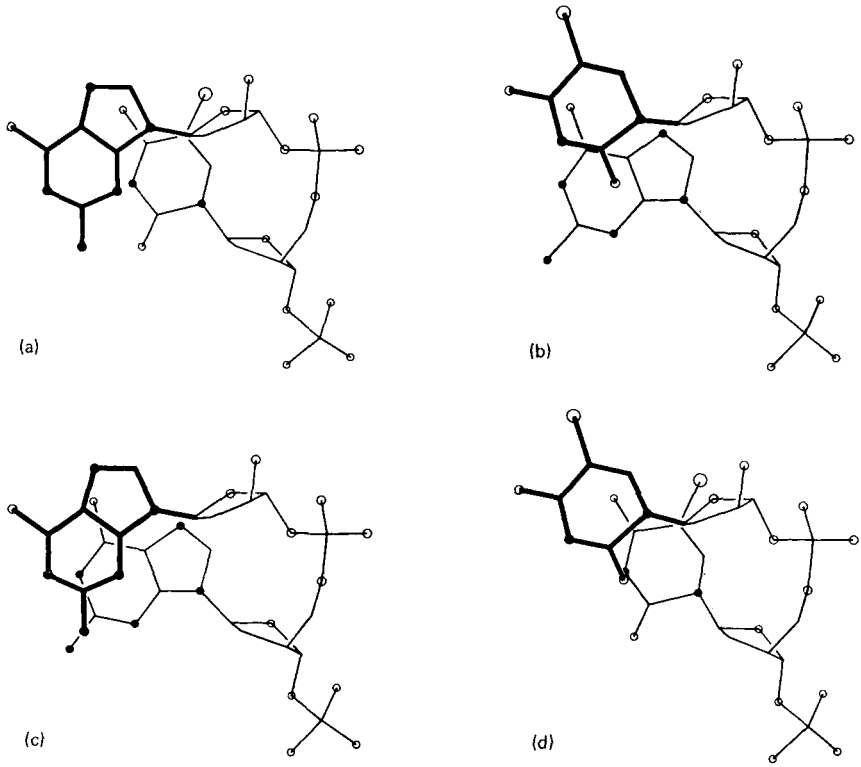


Fig. 18. Intrastrand stacking in the C form of DNA, θ = 11.0°:[90] (*a*), (*b*), (*d*) no contacts shorter than 3.50 Å; (*c*) N(1)–N(1) = 3.36 Å, C(2)–N(1) = 3.36 Å.

tions of bases and are considerably dependent upon base sequence in oligonucleotides.[8,9,112] Consequently, it might be expected that the effects which halogenated pyrimidines have on base stacking would be dependent upon the local sequence of bases at the site of substitution in nucleic acids. Although an altered base-stacking pattern (and the concomitant mispairing with guanine) could result from incorporation of a halogenated uracil residue adjacent to either a purine or a pyrimidine base, it is reasonable to assume that the importance of such stacking effects would be somewhat dependent upon the actual base sequence at the site of substitution.

Role of Atom 0(1)′

The interactions between ribose ring oxygen atoms, 0(1)′, and adjacent purines and pyrimidines is probably also due to dipole–induced dipole forces. The bond between atoms 0(1)′ and C(1)′ in nucleosides and nucleotides usually displays some double-bond character,[42] suggesting that atom 0(1)′ may possess a partial positive charge. This possibility is supported by the fact that atom 0(1)′ never has been found to act as a hydrogen bond

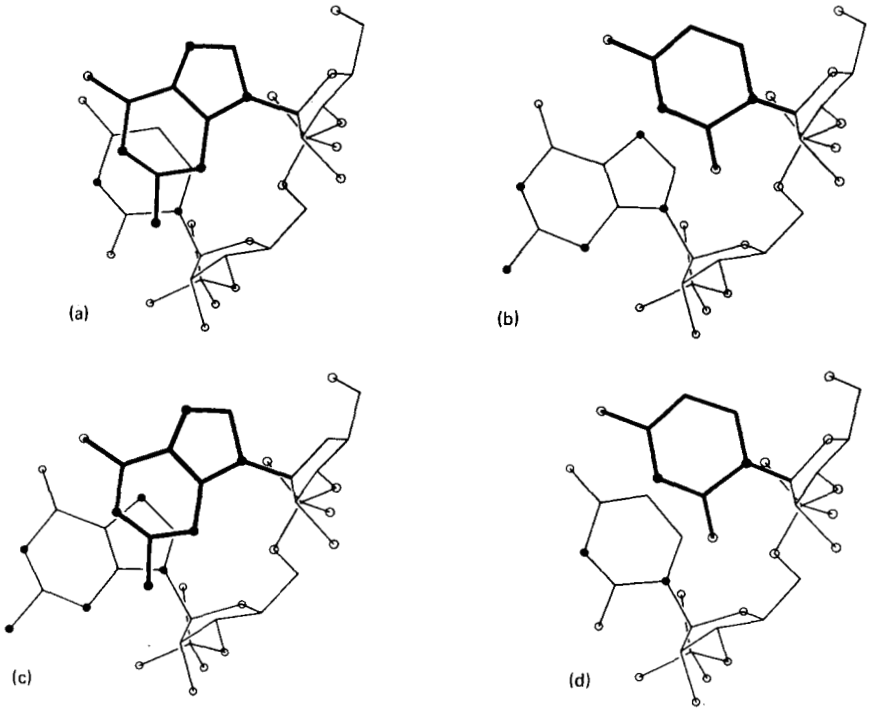


Fig. 19. Intrastrand stacking in model 11 of RNA, $\theta = 5.6°$:[91] (*a*) N(1)–C(4) = 3.16 Å, C(6)–C(4) = 3.08 Å, O(6)–O(4) = 2.91 Å; (*b*) N(3)–N(7) = 3.21 Å, O(2)–C(8) = 3.42 Å; (*c*) N(1)–C(5) = 3.21 Å, N(3)–C(8) = 3.34 Å; (*d*) N(1)–C(5) = 3.17 Å, O(2)–C(4) = 3.36 Å.

acceptor (or, at most, forms only weak hydrogen bonds[127]) in crystal structures of nucleic acid constituents. It is possible that the close contacts between 0(1)′ and the bases may be due to the interaction of the polar oxygen atom with the polarizable bases. Interactions of this type may be important in the binding of aromatic compounds to nucleic acids, since the ribose

oxygen atom is readly available on the external surface of the double-stranded helix.

CONCLUSION

Solid-state base stacking involves a great deal of specificity, with a limited number of stacking patterns recurring in a wide range of different crystal structures. Extensive overlap of bases is unusual; as a rule, purine and pyrimidine stacking occurs with minimal ring overlap and involves inter-

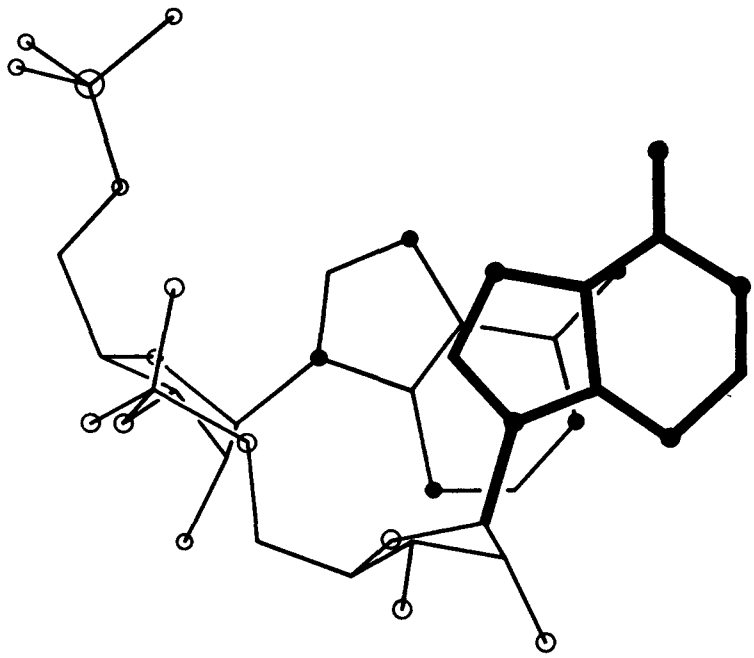
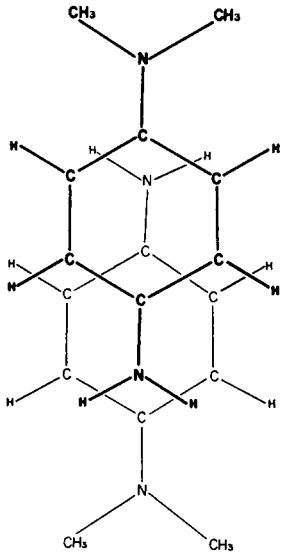


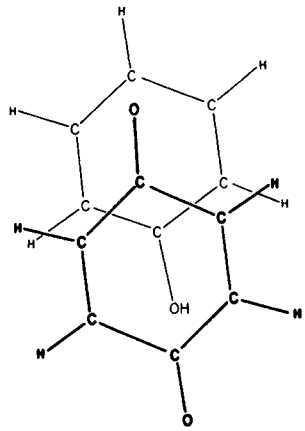
Fig. 20. Intrastrand stacking in polyadenylic acid; $\theta = 5°$, N(9)–C(2) = 3.46 Å.[92]

action between a polar region of one base and the polarizable ring system of another. It is likely that similar specific interactions are also important in aqueous solution and in biological systems. Thus base stacking may make major contributions to the structural specificity, as well as the thermodynamic stability, of nucleic acids.



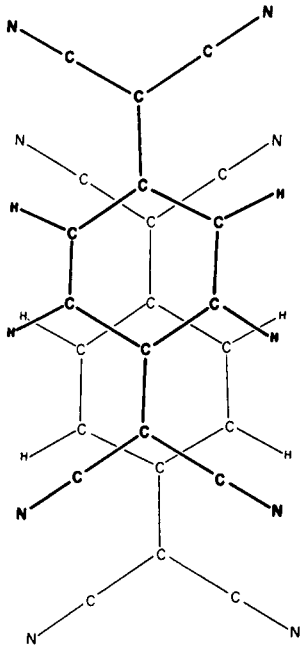
(a) Dimethyl-p-phenylenediamine Bromide

d=3.10



(b) Phenoquinone

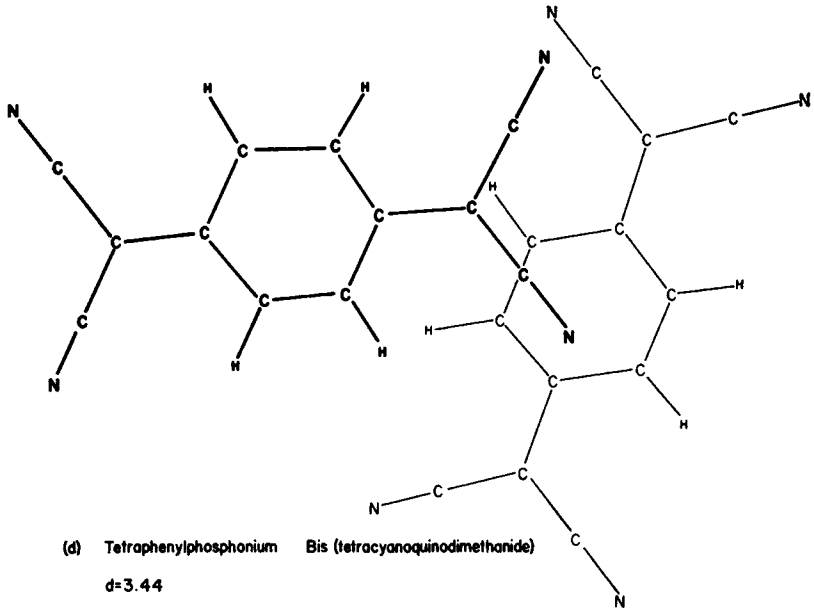
d=3.3



(c) N-Methylphenazinium Tetracyanoquinodimethanide

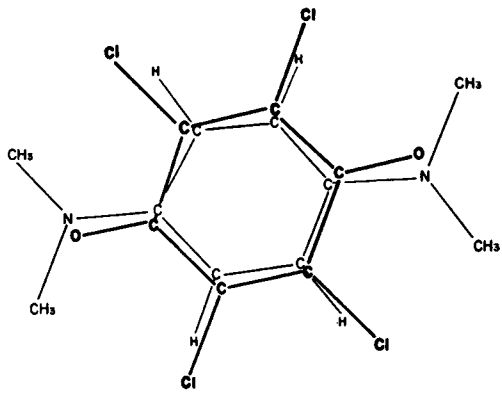
d=3.26

Fig. 21 (*continued*)



(d) Tetraphenylphosphonium Bis (tetracyanoquinodimethanide)

d=3.44



(e) Chloranil with Tetramethyl-p-Phenylenediamine

d=3.28

Fig. 21. Stacking interactions in some charge-transfer complexes: (*a*)d = 3.10 Å;[95] (*b*) d = 3.30 Å (approximately);[96] (*c*) d = 3.20 Å;[97] (*d*) d = 3.44 Å;[98] (*e*) d = 3.28 Å.[99]

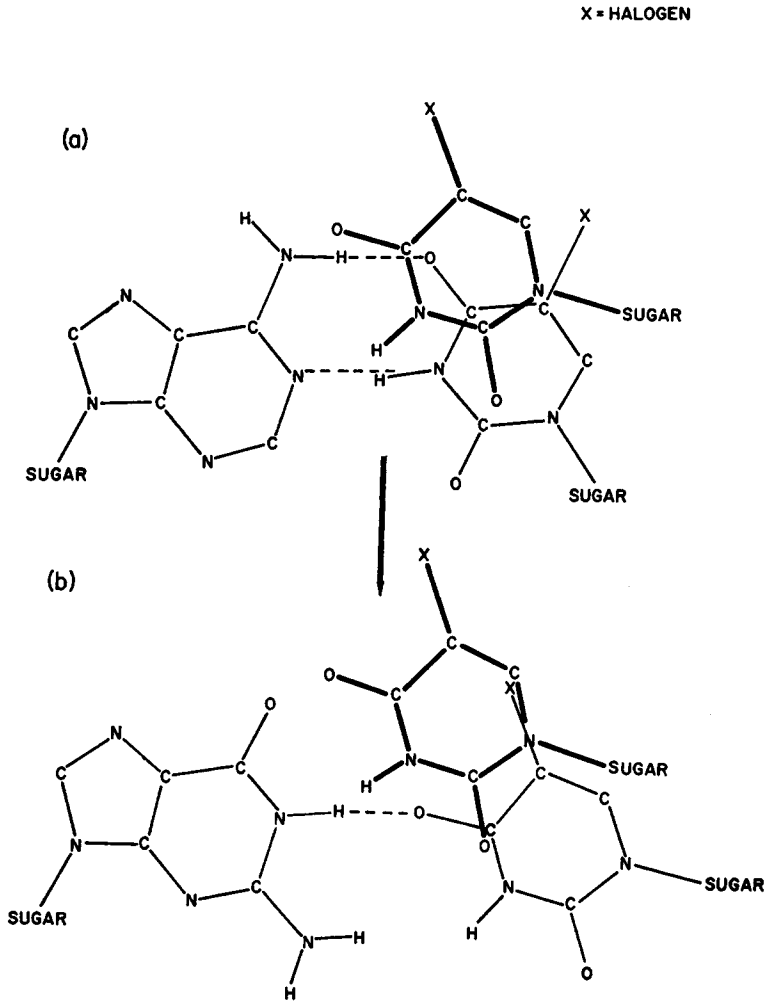


Fig. 22. Model for the mispairing of a halogenated uracil derivative when incorporated adjacent to a second uracil derivative in the B form of DNA: (*a*) normal intrastrand stacking and complementary base pairing with adenine; (*b*) modified intrastrand stacking and resultant mispairing with guanine.

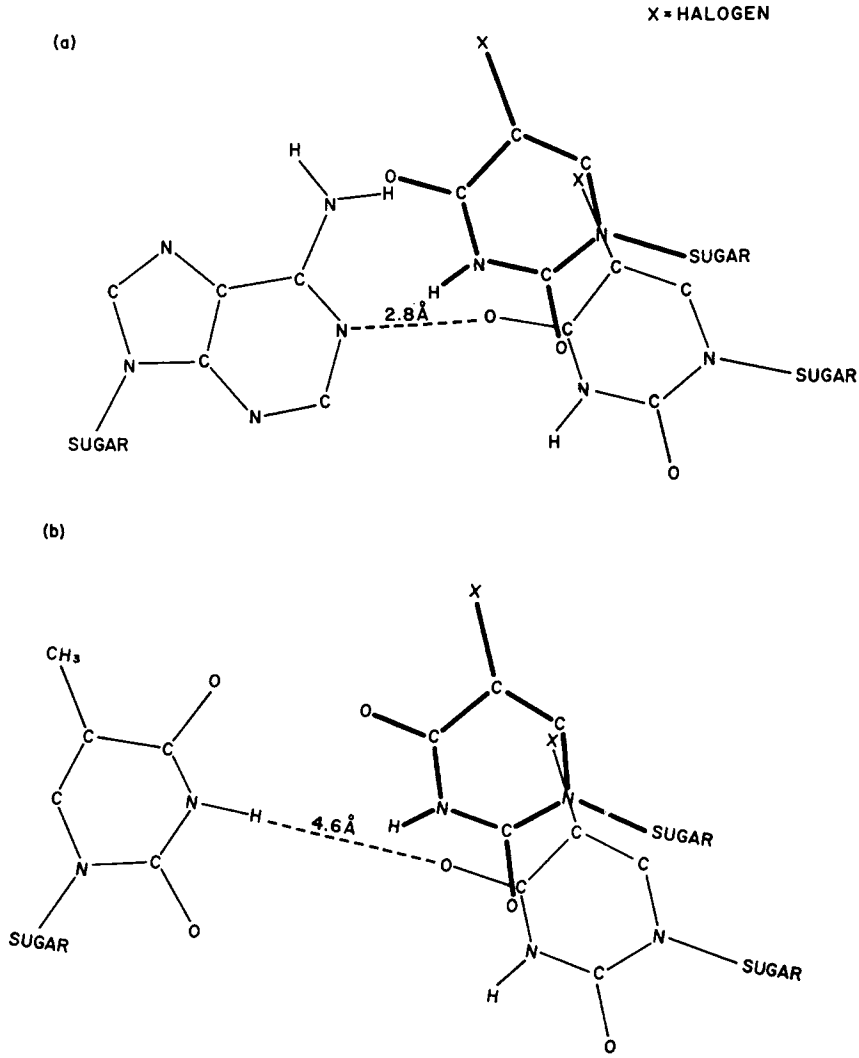


Fig. 23. Inability of a rotated halogenated uracil derivative to hydrogen-bond with bases other than guanine: (*a*) pair with adenine showing a close nonbonded contact; (*b*) pair with a pyrimidine showing that the interbase separation is too great to permit hydrogen bonding.

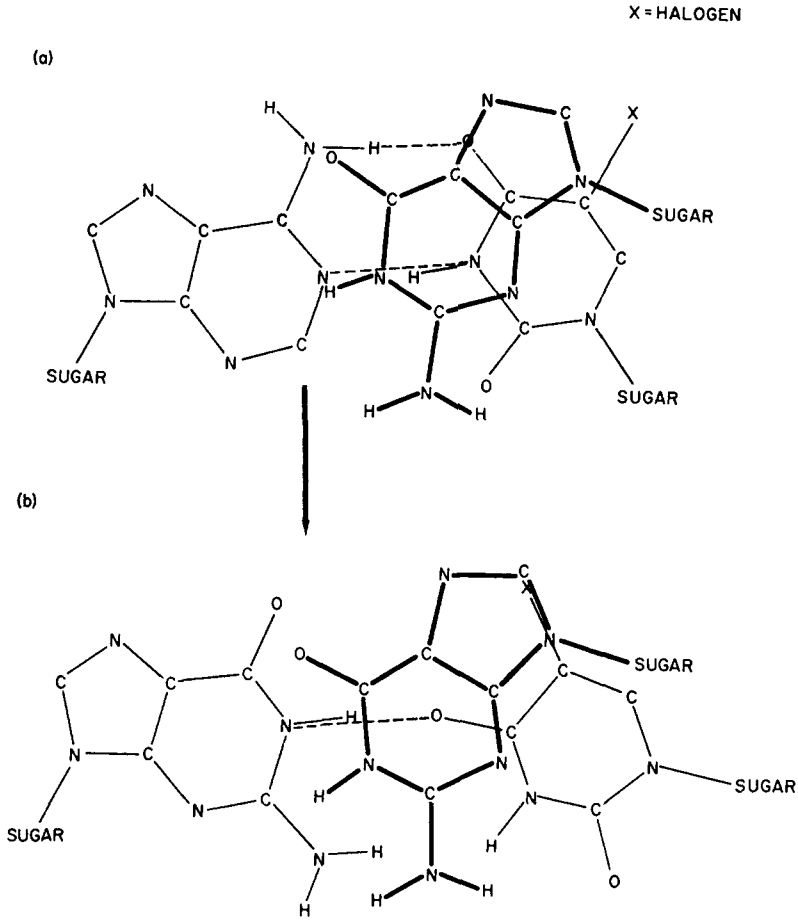


Fig. 24. Model for the mispairing of a halogenated uracil derivative when incorporated adjacent to a purine in the B form of DNA: (*a*) normal intrastrand stacking and complementary base pairing with adenine; (*b*) modified intrastrand stacking and resultant mispairing with guanine.

Work at the University of Alabama was supported by U.S.P.H.S. Research Grant DE-02670.

Work at the University of Wisconsin was supported by U.S.P.H.S. Research Grant GM-17378.

The authors wish to thank Dr. Richard E. Marsh for his many helpful comments and suggestions relating to this work.

References

1. M. Sundaralingam and S. K. Arora, *Proc. Nat. Acad. Sci. U.S.*, **64**, 1021 (1969).
2. M. M. Warshaw and I. Tinoco, *J. Mol. Biol.*, **13**, 54 (1965).
3. C. R. Cantor and I. Tinoco, *J. Mol. Biol.*, **13**, 65 (1965).
4. K. E. Van Holde, J. Brahms, and A. M. Michelson, *J. Mol. Biol.*, **12**, 726 (1965).
5. J. Brahms, A. M. Michelson, and K. E. Van Holde, *J. Mol. Biol.*, **15**, 467 (1966).

6. A. M. Michelson, T. C. V. Ulbricht, R. T. Emerson, and R. J. Swan, *Nature*, **209**, 873 (1966).
7. M. M. Warshaw and I. Tinoco, *J. Mol. Biol.*, **20**, 29 (1966).
8. J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 465 (1967).
9. J. Brahms, J. C. Maurizot, and A. M. Michelson, *J. Mol. Biol.*, **25**, 481 (1967).
10. F. E. Hruska and S. S. Danyluk, *Biochim. Biophys. Acta*, **157**, 238 (1968).
11. S. I. Chan, B. W. Bangerter, and H. H. Peter, *Proc. Nat. Acad. Sci. U.S.*, **55**, 720 (1966).
12. W. M. Huang and P. O. P. Ts'o, *J. Mol. Biol.*, **16**, 523 (1966).
13. F. B. Howard, J. Frazier, M. F. Singer, and H. T. Miles, *J. Mol. Biol.*, **16**, 415 (1966).
14. B. W. Bangerter and S. I. Chan, *Proc. Nat. Acad. Sci. U.S.*, **60**, 1144 (1968).
15. P. O. P. Ts'o and W. M. Huang, *Biochemistry*, **7**, 2945 (1968).
16. P. M. Pitha, W. M. Huang, and P. O. P. Ts'o, *Proc. Nat. Acad. Sci. U.S.*, **61**, 332 (1968).
17. P. O. P. Ts'o, I. S. Melvin, and A. C. Olson, *J. Amer. Chem. Soc.*, **85**, 1289 (1963).
18. P. O. P. Ts'o and S. I. Chan, *J. Amer. Chem. Soc.*, **86**, 4176 (1964).
19. S. I. Chan, M. P. Schweizer, P. O. P. Ts'o, and G. K. Helmkamp, *J. Amer. Chem. Soc.*, **86**, 4182 (1964).
20. M. P. Schweizer, S. I. Chan, and P. O. P. Ts'o, *J. Amer. Chem. Soc.*, **87**, 5241 (1965).
21. G. K. Helmkamp and N. S. Kondo, *Biochim. Biophys. Acta*, **157**, 242 (1968).
22. T. N. Solie and J. A. Schellman, *J. Mol. Biol.*, **33**, 61 (1968).
23. L. Katz and S. Penman, *J. Mol. Biol.*, **15**, 220 (1966).
24. F. E. Hruska, C. L. Bell, T. A. Victor, and S. S. Danyluk, *Biochemistry*, **7**, 3721 (1968).
25. H. DeVoe and I. Tinoco, Jr., *J. Mol. Biol.*, **4**, 500 (1962).
26. S. Lifson and B. H. Zimm, *Biopolymers*, **1**, 15 (1963).
27. P. Claverie, B. Pullman, and J. Caillet, *J. Theoret. Biol.*, **12**, 419 (1966).
28. D. M. Blow, *Acta Cryst.*, **13**, 168 (1960).
29. R. F. Bryan and K. Tomita, *Acta Cryst.*, **15**, 1174 (1962).
30. C. E. Bugg and R. E. Marsh, *J. Mol. Biol.*, **25**, 67 (1967).
31. D. E. Hunt and M. Subramanian, private communication, 1968.
32. R. E. Marsh, R. Bierstedt, and E. L. Eichhorn, *Acta Cryst.*, **15**, 310 (1962).
33. E. Shefter, *J. Pharm. Sci.*, **57**, 1157 (1968).
34. J. Iball, C. H. Morgan, and H. R. Wilson, *Proc. Roy. Soc.* (London), **A295**, 320 (1966).
35. H. M. Sobell and K. Tomita, *Acta Cryst.*, **17**, 122 (1964).
36. S. T. Rao, M. Sundaralingam, and S. K. Arora, *J. Amer. Chem. Soc.*, **92**, 4963 (1970).
37. M. Sundaralingam, S. T. Rao, C. E. Bugg, and J. Thomas, paper presented, at American Crystallographic Association Meeting, March 1969, paper I-5.
38. D. G. Watson, D. J. Sutor, and P. Tollin, *Acta Cryst.*, **19**, 111 (1965).
39. J. Kraut and L. H. Jensen, *Acta Cryst.*, **16**, 79 (1963).
40. A. E. V. Haschemeyer and H. M. Sobell, *Acta Cryst.*, **18**, 525 (1965).
41. A. E. V. Haschemeyer and H. M. Sobell, *Acta Cryst.*, **18**, 125 (1965).
42. M. Sundaralingam, *Biopolymers*, **6**, 189 (1968).
43. E. Shefter and K. N. Trueblood, *Acta Cryst.*, **18**, 1067 (1965).
44. S. Furberg, C. S. Peterson, and C. Romming, *Acta Cryst.*, **18**, 313 (1965).
45. D. E. Hunt and M. Subramanian, *Acta Cryst.*, **B25**, 2144 (1969).
46. S. T. Rao and M. Sundaralingam, *J. Amer. Chem. Soc.*, **91**, 1210 (1969).
47. E. Shefter, M. P. Kotick, and T. J. Bardos, *J. Pharm. Sci.*, **56**, 1293 (1967).
48. E. Shefter, M. Barlow, R. Sparks, and K. N. Trueblood, *Acta Cryst.*, **B25**, 1895 (1969).
49. M. Sundaralingam and L. H. Jensen, *J. Mol. Biol.*, **13**, 914 (1965).

50. M. Sundaralingam, *Acta Cryst.*, **21**, 495 (1966).
51. C. J. B. Clews and W. Cochran, *Acta Cryst.*, **1**, 4 (1948).
52. G. A. Jeffrey and Y. Kinoshita, *Acta Cryst.*, **16**, 20 (1963).
53. E. N. Maslen, D. E. Jukes, and C. J. B. Clews, *Acta Cryst.*, **11**, 115 (1958).
54. E. J. O'Brien, *Acta Cryst.*, **23**, 92 (1967).
55. F. S. Mathews and A. Rich, *J. Mol. Biol.*, **8**, 89 (1964).
56. K. Hoogsteen, *Acta Cryst.*, **16**, 907 (1963).
57. S. H. Kim and A. Rich, *Science*, **158**, 1046 (1968).
58. D. Barker and R. E. Marsh, *Acta Cryst.*, **17**, 1581 (1964).
59. E. Shefter and H. G. Mautner, *J. Amer. Chem. Soc.*, **89**, 1249 (1967).
60. P. M. Huber, *Acta Cryst.*, **10**, 129 (1957).
61. T. Sakurai, *Acta Cryst.*, **19**, 320 (1965).
62. A. W. Hanson, *Acta Cryst.*, **16**, 1147 (1963).
63. B. Kamenar, C. K. Prout, and J. D. Wright, *J. Chem. Soc.*, **1965**, 4851.
64. C. K. Prout and S. C. Wallwork, *Acta Cryst.*, **21**, 449 (1966).
65. J. Iball, C. H. Morgan, and H. R. Wilson, *Proc. Roy. Soc.* (London), **A302**, 225 (1968).
66. N. Camerman and J. Trotter, *Acta Cryst.*, **18**, 203 (1965).
67. D. R. Harris and W. M. MacIntyre, *Biophys. J.*, **4**, 203 (1964).
68. K. Tomita, L. Katz, and A. Rich, *J. Mol. Biol.*, **30**, 545 (1967).
69. C. E. Bugg and U. Thewalt, *Biochem. Biophys. Res. Commun.*, **37**, 623 (1969).
70. R. F. Stewart and L. H. Jensen, *Acta Cryst.*, **23**, 1102 (1967).
71. R. Gerdil, *Acta Cryst.*, **14**, 333 (1961).
72. N. E. White and C. J. B. Clews, *Acta Cryst.*, **9**, 586 (1956).
73. B. D. Sharma and J. F. McConnell, *Acta Cryst.*, **19**, 797 (1965).
74. K. N. Trueblood, P. Horn, and V. Luzzati, *Acta Cryst.*, **14**, 965 (1961).
75. S. H. Kim and A. Rich, *Proc. Nat. Acad. Sci. U.S.*, **60**, 402 (1968).
76. J. M. Broomhead, *Acta Cryst.*, **4**, 92 (1951).
77. R. F. Bryan and K. Tomita, *Acta Cryst.*, **15**, 1179 (1962).
78. W. Cochran, *Acta Cryst.*, **4**, 81 (1951).
79. H. M. Sobell and K. Tomita, *Acta Cryst.*, **17**, 126 (1964).
80. J. Sletten and L. H. Jensen, *Acta Cryst.*, **B25**, 1608 (1969).
81. C. E. Bugg, U. Thewalt, and R. E. Marsh, *Biochem. Biophys. Res. Commun.*, **33**, 436 (1968).
82. U. Thewalt, C. E. Bugg, and R. E. Marsh, *Acta Cryst.*, **B26**, 1089 (1970).
83. U. Thewalt, C. E. Bugg, and R. E. Marsh, *Acta Cryst.*, to be published.
84. D. G. Watson, R. M. Sweet, and R. E. Marsh, *Acta Cryst.*, **19**, 573 (1965).
85. R. F. Stewart and L. H. Jensen, *J. Chem. Phys.*, **40**, 2071 (1964).
86. C. J. B. Clews and W. Cochran, *Acta Cryst.*, **2**, 46 (1949).
87. P. J. Wheatley, *Acta Cryst.*, **13**, 80 (1960).
88. W. Fuller, M. H. F. Wilkins, H. R. Wilson, and L. D. Hamilton, *J. Mol. Biol.*, **12**, 60 (1965).
89. R. Langridge, D. A. Marvin, W. E. Seeds, H. R. Wilson, C. W. Hooper, M. H. Wilkins, and L. D. Hamilton, *J. Mol. Biol.*, **2**, 38 (1960).
90. D. A. Marvin, M. Spencer, M. H. F. Wilkins, and L. D. Hamilton, *J. Mol. Biol.*, **3**, 547 (1961).
91. S. Arnott, M. H. F. Wilkins, W. Fuller, and R. Langridge, *J. Mol. Biol.*, **27**, 535 (1967).
92. A. Rich, D. R. Davies, F. H. C. Crick, and J. D. Watson, *J. Mol. Biol.*, **3**, 71 (1961).
93. R. J. W. LeFévre, D. V. Radford, C. L. D. Ritchie, and P. J. Stiles, *Chem. Commun.*, **1967**, 1221.
94. R. J. W. LeFévre, D. V. Radford, and P. J. Stiles, *J. Chem. Soc. B*, **1968**, 1297.
95. J. Tanaka and N. Sakabe, *Acta Cryst.*, **B24**, 1345 (1968).
96. T. T. Harding and S. C. Wallwork, *Acta Cryst.*, **6**, 791 (1953).

97. C. J. Fritchie, Jr., *Acta Cryst.*, **20**, 892 (1966).
98. P. Goldstein, K. Seff, and K. N. Trueblood, *Acta Cryst.*, **B24**, 778 (1968).
99. J. L. DeBoer and A. Vos, *Acta Cryst*, **B24**, 720 (1968).
100. S. C. Wallwork, *J. Chem. Soc.*, **1961**, 494.
101. D. C. Phillips, *Acta Cryst.*, **9**, 237 (1956).
102. E. Subramanian, J. Trotter, and C. E. Bugg, *J. Cryst. and Mol. Struct.*, in press.
103. E. G. Cox and J. A. C. S. Smith, *Nature*, **173**, 75 (1954).
104. D. W. J. Cruickshank, *Acta Cryst.*, **10**, 504 (1957).
105. D. W. J. Cruickshank, *Acta Cryst.*, **9**, 915 (1956).
106. J. Trotter, *Acta Cryst.*, **16**, 605 (1963).
107. B. Pullman and A. Pullman, in *Quantum Biochemistry*, Interscience, New York, 1963.
108. R. E. Marsh, in *Structural Chemistry and Molecular Biology*, A. Rich and N. Davidson, Eds., Freeman, San Francisco, 1968, p. 484.
109. G. Briegleb, *Z. Physik. Chem.*, **B16**, 249 (1932).
110. G. Briegleb, *Zwischenmolekulare Krafte*, G. Braun, Karlsruhe, Germany, 1949.
111. S. Takashima, *Biopolymers*, **6**, 1437 (1968).
112. P. O. P. Ts'o, in *Molecular Associations in Biology*, B. Pullman, Ed., Academic Press, New York, 1968, p. 39.
113. P. J. Gaultier, C. Hauw, and M. Breton-Lacombe, *Acta Cryst.*, **B25**, 231 (1969).
114. G. Felsenfeld and H. T. Miles, *Ann. Rev. Biochem.*, **36**, 407 (1967).
115. W. Kauzmann, in *Advances in Protein Chemistry*, C. B. Anfinson, M. L. Anson, K. Bailey, and J. T. Edsall, Eds., Vol. 14, Academic Press, New York, 1959.
116. S. Hanlon, *Biochem. Biophys. Res. Commun.*, **23**, 861 (1966).
117. L. W. Reeves and W. G. Schneider, *Can. J. Chem.*, **35**, 251 (1957).
118. W. G. Schneider, *J. Phys. Chem.*, **66**, 2653 (1962).
119. C. F. Emanuel, *Biochim. Biophys. Acta*, **42**, 91 (1960).
120. K. Hamaguchi and E. P. Geiduschek, *J. Amer. Chem. Soc.*, **84**, 1329 (1962).
121. D. R. Robinson and M. E. Grant, *J. Biol. Chem.*, **241**, 4030 (1966).
122. F. B. Howard, J. Frazier, and H. T. Miles, *J. Biol. Chem.*, **244**, 1291 (1969).
123. J. Massoulie, A. M. Michelson, and F. Pochon, *Biochim. Biophys. Acta*, **114**, 16 (1966).
124. A. M. Michelson and C. Monny, *Biochim. Biophys. Acta*, **149**, 88 (1967).
125. R. B. Inman and R. L. Baldwin, *J. Mol. Biol.*, **8**, 452 (1964).
126. R. W. Brockman and E. P. Anderson, in *Metabolic Inhibitors*, Vol. I, J. H. Quastel and R. M. Hochster, Eds., Academic Press, New York, 1963, pp. 239–285.
127. D. C. Rohrer and M. Sundaralingam, *J. Amer. Chem. Soc.*, **92**, 4956 (1970).

Received November 6, 1969
Revised May 10, 1970