Restricted Rotation in 6-Methylaminopurine and Analogues. Intramolecular Hydrogen Bonding

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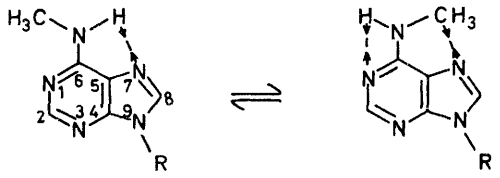
The preferred conformations of 9-alkyl-6-methylaminopurines (m^6A), the corresponding 1-oxides, and 1-alkyl-4-methylaminopyrazolo[3,4-*d*]pyrimidines (4-MeAPP) and the corresponding 5-oxides in chloroform arise from intramolecular hydrogen bonding. m^6A has the *syn*-conformation; m^6A 1-oxide exists mainly as the *anti*-rotamer; 4-MeAPP has similar proportions of *syn*- and *anti*-rotamers, and 4-MeAPP 5-oxide has just the *anti*-conformation. The possible consequences for hydrogen bonding at the nucleic acid level are discussed.

6-METHYLAMINOPURINE (m^6A) present in nucleic acids (RNA and DNA) plays a crucial role in activating segments of information contained in base sequences by locally creating a structural particularity (*e.g.* loop formation) which makes possible recognition by specific proteins (such as restriction enzymes).[1-3] The general understanding of this behaviour is based on the hypothesis that, in nucleic acids, m^6A exists as the *syn*-rotamer [with respect to the position of the exocyclic methyl group near the N(1) atom] whose inability to form the required hydrogen bonding brings about the destabilization of the normal Watson–Crick base arrangement.[4,5]

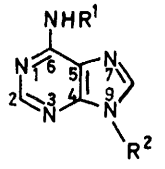
Engel and von Hippel postulated that the preferential *syn*-conformation of m^6A in aprotic media (and tentatively in nucleic acids) arises from interference of the exocyclic methyl group with the adjacent N(7) non-bonding orbital.[5] These authors disregarded intramolecular hydrogen bonding between the hydrogen atom of the exocyclic amino group and N(7) as a possible cause of *syn*-rotamer predominance, though such bonding might be assumed from geometrical considerations. Indeed, crystallographic data for adenine [6] show that the distance and the angle between the amino group and N(7) are not less favourable to hydrogen bond formation



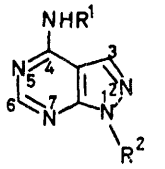
syn *anti*

than the distance and angle between NH–N(1) which give hydrogen bonding in 4-methylaminopyrididine, the pyrimidine part of m^6A.[7]

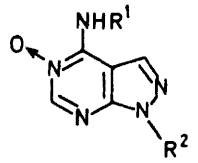
The strength of the N–H vibrator(s) of the exocyclic amino or methylamino groups has proved to be sensitive to intramolecular hydrogen bonding.[8] Valuable information can thus be gained from the analysis of the i.r. spectra in the N–H stretching region (3 600—3 300 cm^{-1}) of dilute solutions in aprotic medium. Accordingly, in chloroform, we undertook the i.r. study of adenines together with *N*-substituted adenine analogues with other potential hydrogen bonding sites. Suitably substituted 9-alkyladenines (1) and (2), 1-alkylaminopyrazolo[3,4-*d*]pyrimidines (3), (4), and (4'), and their corresponding oxides (5)—(8) were selected to permit sufficient solubility in chloroform.



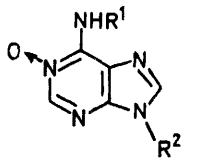
(1) R^1 = H, R^2 = Bu
(2) R^1 = Me, R^2 = Pr^n



(3) R^1 = H, R^2 = Pr^i
(4) R^1 = R^2 = Me
(4') R^1 = Me, R^2 = Pr^i



(5) R^1 = H, R^2 = Pr^i
(6) R^1 = R^2 = Me



(7) R^1 = H, R^2 = Bu
(8) R^1, R^2 = Me

EXPERIMENTAL

Materials.—Adenines. 9-Butyladenine (1) was prepared and purified according to the classical route.[9] 9-Methyl-6-methylaminopurine was obtained by heating an alkaline solution of 1,9-dimethyladenine previously prepared [10] (Dimroth transposition); its formation was monitored by u.v. spectroscopy. 9-Propyl-6-methylaminopurine (2) was obtained from methylation of 2-propyladenine by methyl iodide in dimethylacetamide (DMA) followed by Dimroth transposition in basic aqueous solution.

Pyrazolopyrimidines. 4-Amino-1-isopropylpyrazolo-[3,4-*d*]pyrimidine (1-Pr^i-4APP) (3) was a gift from Ciba-Geigy. 1-Methyl-4-methylaminopyrazolo[3,4-*d*]pyrimidine (1,4-Me_2-APP) (4) was prepared according to the published procedure with *N*-methylformamide replacing formamide in the condensation with 5-amino-4-cyano-1-methylpyrazole,[11] or by Dimroth transposition of the previously obtained 1,5-dimethyl-4-APP.[12] 4-Methylamino-9-isopropylpyrazo[3,4-*d*]pyrimidine (4-Me-9-Pr-APP) (4') was prepared by methylation of (3) followed by Dimroth rearrangement, and purified by g.l.c.

N-Oxides. Compounds (5)—(8) were prepared from

oxidation of the corresponding 4APPs and adenines by H_2O_2 in acetic acid under conditions reported for adenine 1-oxide and 4APP 5-oxide synthesis.[13,14] The progress of the reactions was monitored by t.l.c. on alumina (Merck; 0.1 mm thickness; used without further treatment) with ethanolic NH_3 as eluant. Compounds with an R_F lower than that of the starting bases formed slowly. Even after two weeks the reactions were not complete; the products corresponding to low R_F were then separated by t.l.c. (Merck: alumina; 2 mm thick; used without further treatment; ethanolic NH_3 as eluant). The compounds were extracted from alumina with ethanol; ethanol was then removed under vacuum. Molecular weights (mass spectrometry) were as expected for the various *N*-oxides. No well defined m.p.s were observed since (5)—(8) started to decompose around 270 °C. Attributing the 1-oxide structure of adenine oxides was readily done on the basis of the close similarity of their u.v. spectra with that of adenine 1-oxide.[13] Since no u.v. spectra of authentic 4APP 5-oxides were available, evidence for the 5-oxide structure was tentatively gained from the following observation. Aqueous solutions of 4APP oxides were subjected to strong u.v. irradiation for variable periods; isosbestic spectra which are consistent with increasing proportions of 4-amine-6-hydroxypyrazolo[3,4-*d*]pyrimidines were observed; this behaviour is similar to that of aqueous adenine 1-oxide solutions which, upon irradiation, lead to isoguanine.[15]

I.R. Spectra.—Spectra were recorded with a Perkin-Elmer 225 i.r. spectrometer using Infrasil cells (1 or 2 cm optical length) in conjunction with Fluka (Spectro unstabilized) or Baker (G. C. grade) chloroform, and 500 mπ optical length cells in conjunction with acetonitrile (Prolabo).

N.M.R. Spectra.—The spectra of the deuteriated analogues of compounds (2), (4), and (4′) were recorded on a JEOL C-60 H-L n.m.r. spectrometer fitted with a variable temperature device.

RESULTS AND DISCUSSION

The results of the i.r. study of the amino, deuterioamino, and methylamino derivatives are presented in the Table and in Figure 1. Typical substrate concentrations range from 8×10^{-4} to 5×10^{-3}M; no frequency shifts are observed when concentrations are varied within this range.

I.r. absorption frequencies of NH stretching in chloroform

	ν/cm^{-1}	ν/cm^{-1}	$\Delta\nu$/cm^{-1}
Adenine			
NH_2 (1)	3 413 (3 392 [a])	3 526 (3 494 [a])	113
NHD (1)	3 468	3 468	0
NHMe (2)	3 449 (*syn*) (3 408 [a])		
4-APP			
NH_2 (3)	3 417	3 530	113
NHD (3)	3 463 (*anti*)	3 484 (*syn*)	21
NHMe (4)	3 432 (*anti*)	3 461 (*syn*)	29
4-APP 5-oxide			
NH_2 (5)	3 328	3 508	180
NHD (5)	3 340 (*anti*)	3 488 (*syn*)	148
NHMe (6)	3 290 (*anti*)		
Adenine 1-oxide			
NH_2 (7)	3 350	3 500	150
NHD (7)	3 382 (*anti*)	3 475 (*syn*)	93
NHMe (8)	3 302 (*anti*)	3 420w (*syn*)	118

[a] In CH_3CN.

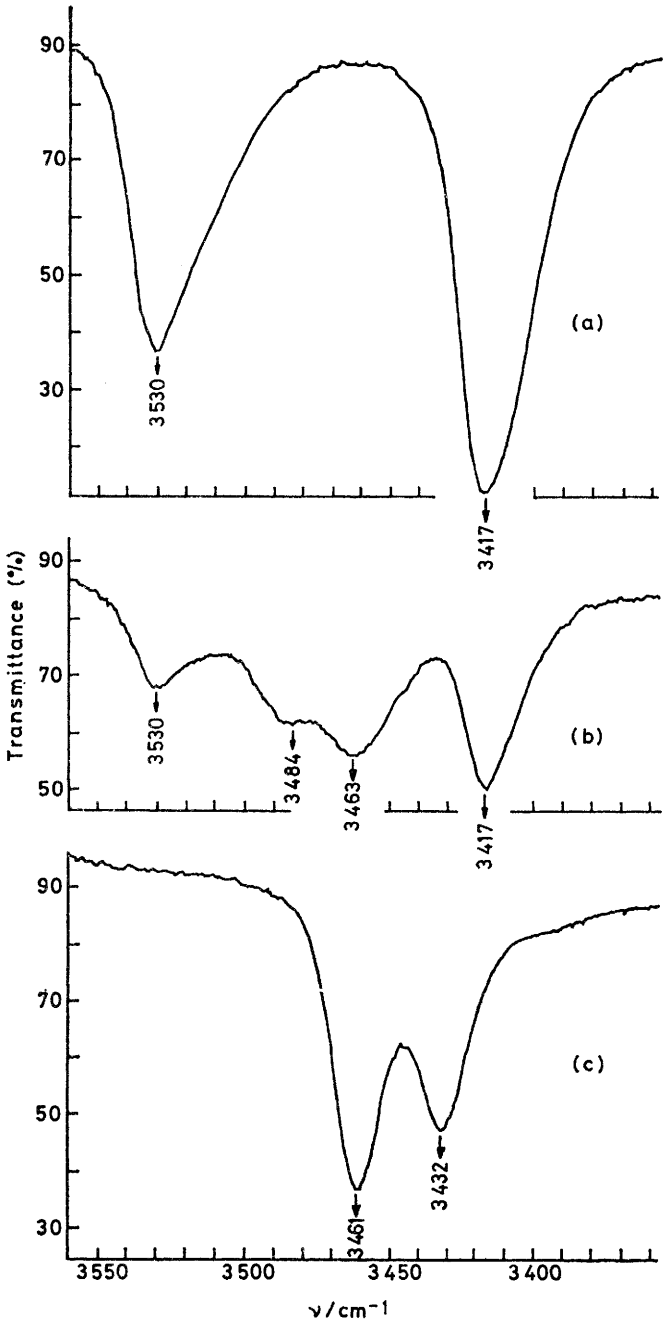


FIGURE 1 I.r. spectra of 4-APPs (3) in chloroform: (a) compound (3); (b) semi-deuteriated (3); (c) compound (4)

The spectra of the semi-deuteriated compounds provide good criteria for asserting that the various compounds exist in chloroform as primary amines.[8,16] Although this is unquestioned in substituted adenines and 4-APP (except for 1-alkyladenine and 5-alkyl-4-APP where imino structures prevail in chloroform),[10,17] the NH_2 group would not be present if the *N*-oxides were to exist as N–OH tautomers. The barycentres of the NHD and NH_2 absorption bands coincide in the oxides (and in the bases), and the difference $\Delta\nu_{NHD}$ is markedly less than $\Delta\nu_{NH_2}$, thus showing that the two vibrations in the non-deuteriated oxides are strongly coupled. They

are consequently assigned to the symmetric and antisymmetric stretchings of an amino group. If the *N*-oxides had the hydroxyimino structure, OH and NH vibrations would be expected to be weakly coupled; the spectrum observed upon semi-deuteriation would not be significantly different from that of the undeuteriated derivative.

It must be emphasized that partial deuteriation which is effected in CH_3OD leads to similar proportions of both rotamers. A slight variation in the ratio [*syn*]/[*anti*] from unity may however occur because the exchange rate of H $\rightleftharpoons$ D decreases when the hydrogen atom of the amino groups forms a hydrogen bond with the neighbouring hydrogen acceptor site. Obviously, no information regarding rotamer populations in NHMe derivatives can thus be gained from the spectra of the semi-deuteriated compounds.

The vibration spectra of the NHD compounds which consist of two absorption bands (except for adenine) show the existence of two individual rotamers arising from the restricted rotation about the exocyclic C–N bond,[5] and allow the determination of the N–H vibration frequencies in either *syn*- or *anti*-rotamers. Subsequent comparison of these frequencies with those observed in the methylamino derivative (taking into account frequency decreases upon methylation) provides a convenient means of estimating rotamer populations. Obviously this procedure is valid only if the two N–H vibrators in the deuterioamino and methylamino products have different frequencies so that they can be distinguished. This has been the case in all the examples in this study, except adenine where an estimate of the rotamer population is nevertheless obtained from n.m.r. spectroscopy, which gives conclusive evidence that m^6A exists mainly as the *syn*-rotamer;[5] the absorption band at **3 449** cm^{-1} of (2) in chloroform is thus assigned to this conformation.

4-APPs (3) *and* (4) *and Adenines* (1) *and* (2).—The spectrum of semi-deuteriated 1-Pr^i-4APP (3) shows two sharp bands at **3 463** and **3 484** cm^{-1} ($\Delta\nu_{NHD}$ **21** cm^{-1}; Table). The frequency difference must be understood as arising from hydrogen bonding with the N(5) atom.[7] Assignments of the frequencies to their corresponding rotamers is obtained from study of the 4-APP 5-oxides.

Likewise, the i.r. spectrum of 4-MeAPP (4) presents two absorption bands at **3 432** and **3 461** cm^{-1} ($\Delta\nu_{NHMe}$ **29** cm^{-1}) and is readily interpreted in terms of comparable proportions of *anti*- and *syn*-rotamers. However, a preference for the rotamer showing the high frequency absorption may be postulated from the fact that, in the semi-deuteriated derivative [compound (3)], the absorbance coefficient at **3 481** cm^{-1} is less than at **3 463** cm^{-1}, whereas in compound (4) the absorbance at **3 461** cm^{-1} is stronger than at **3 432** cm^{-1}.

The n.m.r. spectra of deuteriated (4) and (4′) run at different temperatures lead to unequivocally similar observations (Figure 2). Although indirectly, this result casts doubts upon von Hippel's hypothesis concerning the origin of the stabilization of the *syn*-conformation in

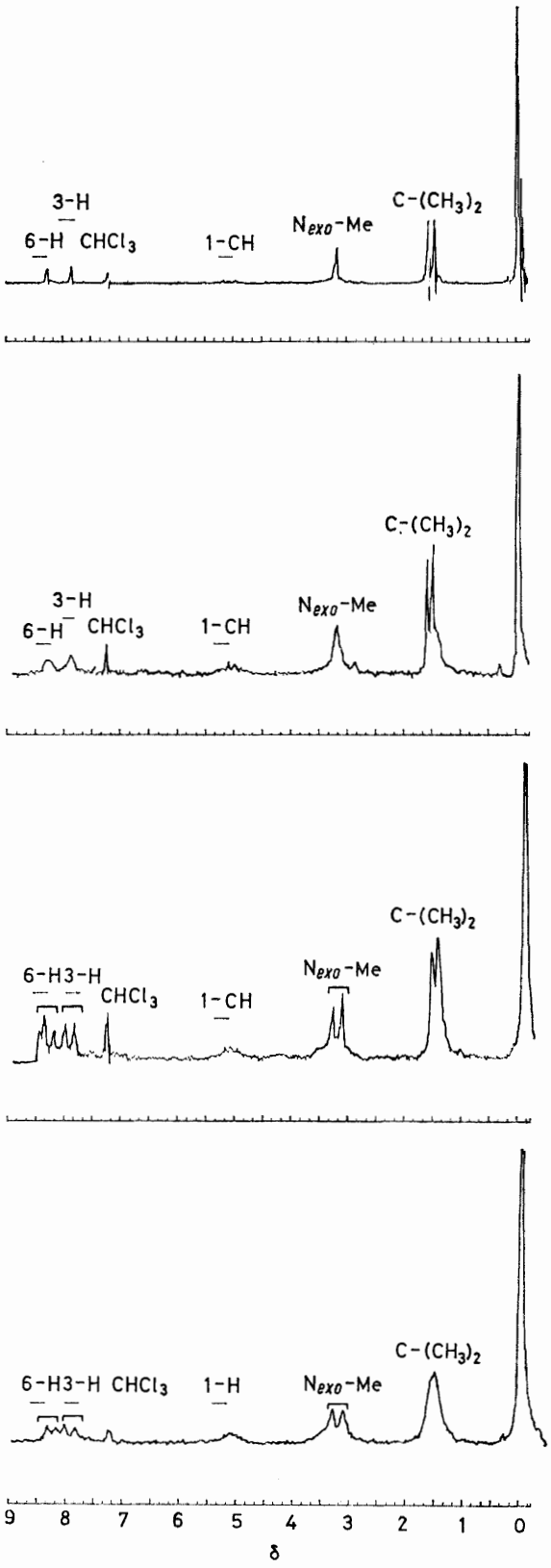
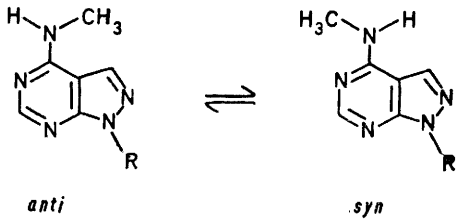


FIGURE 2 N.m.r. spectra showing restricted rotation of (4′) in $CDCl_3$ (5×10^{-2}M) at (a) room temperature; (b) 0 °C; (c) −20 °C; (d) −40 °C

6-methylamino purine (m^{6}A). If steric interaction of the exocyclic methyl group with N(7) were the only cause of the *syn*-preference, then replacing N(7) by a C–H



group (when going from m^{6}A to 4-MeAPP) would cause an increase in the hindrance in 4-MeAPP,[18] and, consequently, would lead to the stabilization of the sole *syn*-rotamer of this compound. The fact that both rotamers of 4-MeAPP (4) are present means that hydrogen bonding of the exocyclic N–H group with N(5) is strong enough to match the steric hindrance of the methyl group by C(3).

Likewise, in m^{6}A, there is hydrogen bonding between the exocyclic NH and N(1) comparable in magnitude with that of the N–H–N(5) bond in 4-MeAPP. Since steric interaction of the methyl group is markedly weaker with N(7) in adenine than with C(3) in 4APP,[18] the N–H–N(1) hydrogen bond would offset the hindrance with N(7) and lead to stabilization of the *anti*-rotamer of m^{6}A. As the opposite is unequivocally observed (*syn*-conformation of m^{6}A is preferred), one must conclude that there is some kind of stabilizing interaction, namely hydrogen bonding between the exocyclic N–H and N(7), whose energy should at least be equal to the difference between the energy of hydrogen bonding at N(1) and that of steric hindrance produced by the methyl group at N(7). The equivalence of exocyclic N–H stretchings in semi-deuteriated adenine (1) (Table) suggests that exocyclic N–H · · · N(1) and exocyclic N–H · · · N(7) bondings have similar energies and, consequently, that the contribution from the steric hindrance to stabilization of the *syn*-rotamer of (2) is weak.

Thus, rotamer stabilization in m^{6}A arises from two competing hydrogen bonds and a steric effect: *syn*-conformation is favoured by N–H–N(7) bonding, whereas the *anti*-form is stabilized by N–H–N(1) bonding, but is destabilized by the steric interaction of the methyl group. This means that, although weak, as shown above, the steric effect plays a determining role in providing a small amount of additional stabilization energy. However, the main contribution to preference for *syn*-conformation arises from hydrogen bonding.

Additional evidence for intramolecular hydrogen bonding in m^{6}A is tentatively gained from the fact that the frequency of NH vibrations in semi-deuteriated (1) (3 468 cm^{-1}) is weaker than that of the band for semi-deuteriated (2) (3 484 cm^{-1}, which, as shall be seen subsequently, is attributed to the ' free ' N–H vibration corresponding to the *anti*-conformation) and comparable with that of the N–H vibration involved in hydrogen-bonding in (2). We also observed that the n.m.r. spectra of compound (2) run in $CDCl_3$ show a resonance line at δ 3.5 assigned to the *anti*-rotamer (Figure 3); this line is not present when the spectrum is taken in CD_3CN. This is understood as arising from formation of intermolecular hydrogen bond between the solvent and the methylamino group (as suggested by the decrease in frequency of the N–H vibrations when going from chloroform to acetonitrile; Table). The resulting destruction of the intramolecular hydrogen bonds [N–H–N(1) and N–H–N(7)] leaves only the steric effect of the methyl group, thus stabilizing the *syn*-conformation even more on going from chloroform to acetonitrile.

It must be stressed that observation of only one absorption in (2) can by no means be considered as evidence for the absence of exocyclic N–H · · · N(7) bonding since, if this were the case, two bands would be observed [as in (4)].

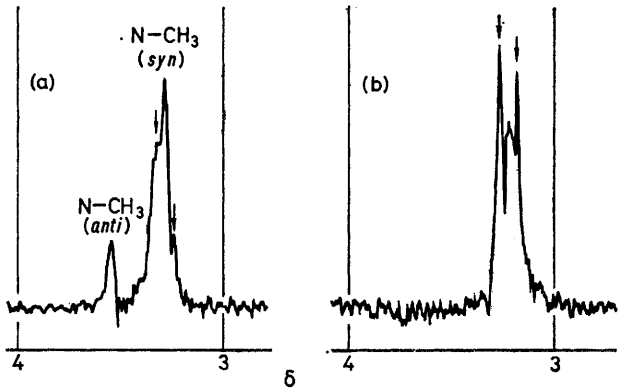


FIGURE 3 N.m.r. spectrum of deuteriated (2) at room temperature (a) in $CDCl_3$; (b) in CD_3CN; arrows indicate resonances of the undeuteriated compound

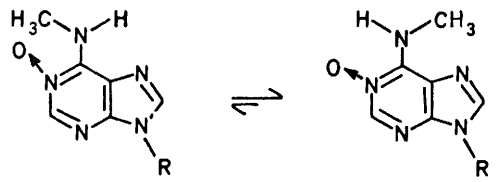
The occurrence of intramolecular hydrogen bonding has been observed subsequently in the 1-oxides of adeninines and in the 5-oxides of 4-APPs where the presence of an oxygen atom is expected to interfere with the hydrogen atom of the exocyclic amino group.

4-APP 5-Oxides (5) *and* (6).—The i.r. spectrum of semi-deuteriated 1-Pr^i-4-APP 5-oxide (5) consists of two bands appearing at 3 340 and 3 488 cm^{-1} (Table). The frequency of the latter absorption is similar to that of the high energy band of semi-deuteriated 4-APP (3), whereas it decreases dramatically in comparison to the weak energy band of (2). This is simply explained by the fact that the absorption at ν_{NH} 3 340 cm^{-1} corresponds to the N–H vibrator where hydrogen is bound to the neighbouring oxygen; the molecule then has the *anti*-conformation. The spectrum of the methylamino derivative (6) shows a single, low-energy absorption band at 3 290 cm^{-1}, thus leading to the conclusion that this compound exists in chloroform mainly as the *anti*-rotamer. The similar frequencies of the high energy bands in semi-deuteriated (5) and (3) strongly suggest that in (3) the absorption at ν_{NH} 3 484 cm^{-1} should be assigned to the *syn*-conformation. This conclusion

opposes previous tentative attributions of exocyclic N–H stretchings in 4-methylaminopyrimidine, the pyrimidine part of (4).[7]

Adenine 1-*Oxides* (7) *and* (8).—The i.r. spectrum of semi-deuteriated (7) is attributed to one 'weakly bound' N–H vibration (3 475 cm^{-1}) and one strongly hydrogen bound N–H stretching (3 382 cm^{-1}) corresponding respectively to the *syn*- and *anti*-rotamers (Table). It is noteworthy that the frequencies of the high energy absorptions are very similar in semi-deuteriated (3) and (5) (3 484 and 3 488 cm^{-1}) and in semi-deuteriated (1) and (7) (3 468 and 3 475 cm^{-1}); however, those of the latter group of compounds are slightly weaker because of hydrogen bonding with the N(7) atom.

The spectrum of the 1-oxide (8) consists of one broad absorption at 3 302 cm^{-1} and a weak band at 3 420 cm^{-1}. Thus, it must be concluded that m^6A 1-oxide (8) has a preferred *anti*-conformation arising from stabilization through strong O • • • H bonding; the *syn*-rotamer is nevertheless present since it is stabilized by the markedly weaker N(7) • • • H bond. The difference in hydrogen bond strengths arises from the fact that the exocyclic N–H • • • O angle is more open than the exocyclical N–H • • • N(7) angle and that the net negative charge at the oxygen is higher than at N(7).



syn *anti*

This study has reasonably established that intramolecular hydrogen bonding is the cause of preferential stabilization of a given rotamer [*syn* or *anti*, depending on the position of the hydrogen acceptor site(s)] in adenines and adenine analogues. The position for chloroform may well be considered to prevail in nucleic acids also. In keeping with the current theories concerning enzyme recognition sites, replacing m^6A by 4-MeAPP (advanced as actually incorporated in DNA [19]) or by *N*-oxides capable of favouring the *anti*-conformation (whose occurrence is this far unreported) would modify the tertiary structure of these sites and thereby disturb the subsequent behaviour of the nucleic acids.

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