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SPECTROSCOPIC, KINETIC AND SEMIEMPIRICAL MOLECULAR ORBITAL STUDIES ON 8-AMINO-, 8-METHYLAMINO- & 8-DIMETHYLAMINO-ADENOSINES

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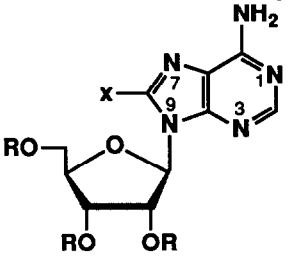
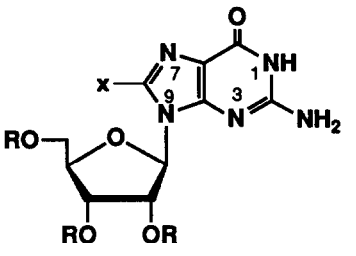
Summary: Kinetic studies on acidic depurination of 8-aminoadenosine, 8-methylaminoadenosine, and 8-dimethylaminoadenosine show their relative rates are respectively 2.7, 2 and 429-fold with respect to adenosine. Structural consequence of 8-amino, 8-methylamino or 8-dimethylamino group in the 8-substituted purine nucleosides have been therefore investigated in order to delineate the influence of these 8-amino substituents on the relative rate of cleavage of glycosyl bond under acidic condition by both ^{15}N- and ^{1}H-NMR spectroscopy in neutral and acidic solutions. ^{15}N-NMR studies showed that the relative amount of protonation at N^1 in 8-amino adenosine and 8-methylaminoadenosine are 66% (44% N^7H^+) and 85% (15% N^7H^+), respectively, while it is 96% (4% N^7H^+) in case of 8-dimethylaminoadenosine. ^{1}H-NMR studies also showed some differences in conformation of sugar moiety of nucleosides in acidic solution in comparison with their conformations in neutral solution. Semiempirical Molecular Orbital calculations have been used to throw light on steric and electronic factors dictated by 8-amino, 8-methylamino or 8-dimethylamino group across C8-N8 bond that control the stabilization of N^7H^+ versus N^1H^+ species. Result from these Semiempirical Molecular Orbital calculations have been subsequently assessed with those obtained by ^{15}N-NMR studies.

Successful rational design of new analogues of nucleosides which should interfere specifically with the biosynthesis of DNA or RNA in cancer cells or virus- or parasite-infected cells requires that the effect of specific structural modification in these analogues on the chemical reactivities, hydrolytic stabilitities, and conformational properties in solution are clearly defined. In this respect, we considered 8-aminoadenosine, 8-methylaminoadenosine, and 8-dimethylaminoadenosine as useful target nucleosides because of the following reasons. Lipophilic derivatives of cytosine and guanosine have been shown to form Watson-Crick type base pairs in nonaqueous solvents over a wide temperature range suggesting that the monomers can be used to understand and possibly to predict structures of polymeric nucleic acids[1], since low dielectric solvents mimic the dielectric environment of the interior of a nucleic acid double helix. Lipophilic derivatives of 8-amino-2′-deoxyadenosine and 8-methylamino-2′-deoxyadenosine are expected to form *both* Watson-Crick and Hoogsteen type base pairs with lipophilic thymidine derivative in non-polar solution while 8-dimethylamino-2′-deoxyadenosine should not. Our initial studies on the base-pairing abilities of lipophilic 8-amino-2′-deoxyadenosine derivative with lipophilic thymidine derivative in $CDCl_3$ have shown that their hydrogen-bonded complexation in 1:1 and 1: 2 molar stoicheiometries at ~20 °C are different[2]. These studies have indicated that oligonucleotide based on 8-amino-2′-deoxyadenosine or 8-methylamino-2′-deoxyadenosine should form intermolecular DNA triplexes which have been shown to be important because of their potential role in chromosome mapping[3] and in antisense gene therapy[4]. Our unpublished work has shown that the glycosidic bond of 8-amino-2′-deoxyadenosine [8-A-2′-dA] and 8-methylamino-2′-deoxyadenosine [8-mA-2′-dA] are ~10 times more acid-labile than that of the parent 2′-deoxyadenosine, which suggests that no acid-labile protecting group can be used in the synthesis of $[8\text{-A-2′-dA}]_8$ & $[8\text{-mA-2′-dA}]_8$. These observations prompted us to study the structural properties of 8-amino- & 8-alkylamino substituted adenosine and guanosine derivatives in

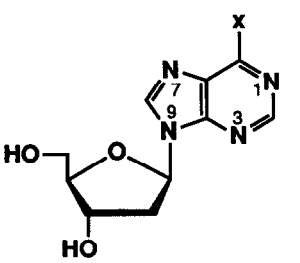
order to understand how does the C^8-amino substituent dictate the acidic depurination in the purine nucleosides! Purine nucleosides with bulky substituent at C-8 adopts a *syn* conformation about the N-glycosidic bond due to



1a : x = H, R = Ac
1b : x = H, R = H
2a : x = Br, R = Ac
2b : x = Br, R = H
3a : x = OH, R = Ac
3b : x = OH, R = H
4a : x = NH_2, R = Ac
4b : x = NH_2, R = H
5a : x = $NHCH_3$, R = Ac
5b : x = $NHCH_3$, R = H
6a : x = $N(CH_3)_2$, R = Ac
6b : x = $N(CH_3)_2$, R = H
15 : x = OMe, R = H



7a : x = H, R = Ac
7b : x = H, R = H
8a : x = NH_2, R = Ac
8b : x = NH_2, R = H
9a : x = $NHCH_3$, R = Ac
9b : x = $NHCH_3$, R = H
10a : x = $N(CH_3)_2$, R = Ac
10b : x = $N(CH_3)_2$, R = H
11 : x = OH, R = Ac



12 : x = H
13 : x = NH_2
14 : x = $N(CH_3)_2$

unfavorable steric and electrostatic interactions between the substituent and the sugar ring[5-9]. Thus, 8-dimethylaminoadenosine (**6b**) and 8-bromoadenosine (**2b**) and corresponding guanosine derivatives have been shown to be predominantly in a *syn* conformation while 8-aminoadenosine (**4b**) and 8-methylaminoadenosine (**5b**) adopt the preferrred *anti* conformation[5-9]. The extreme lability of 8-dimethylaminoadenosine (**6b**) and 8-dimethylaminoguanosine (**10b**) in the acidic medium compared to 8-aminoadenosine (**4b**) and 8-methylaminoadenosine (**5b**) and the corresponding guanosine derivatives (**8b** & **9b**) has been interpreted as being due to their different glycosidic bond conformation and intrinsic relative stabilities[5]. In this paper, We report how different 8-substituents, such as amino, methylamino and dimethylamino influence the electronic properties of the resultant purine ring system **4a** - **6b** & **8a** - **10b**, and how do these alterations of electronic properties affect their chemical reactivities as evident by the relative change of basicities and acidic depurination rates! First, we employ ^{15}N-NMR spectroscopy to determine the protonation sites in the aglycones by studies both in neutral and acidic solutions, secondly, we measure their relative rates of acidic depurination, and show the correlation of relative rates of depurination with the preferential site of protonation in the purine ring as shown by ^{15}N-NMR spectroscopy, third, we deduce the confomations of sugar both in neutral and acidic solutions by ^{1}H-NMR spectroscopy to show if there is any preferential sugar conformation which is responsible for acceleration or decceleration of depurination reaction, and, finally we employ semiempirical molecular orbital calculations in order to understand specific electronic and energetic factors that contribute to the preferential sites of protonations in the aglycones shown by ^{15}N-NMR spectroscopy.

^{15}N NMR spectroscopy. Three different components[10] in the paramagnetic term in nitrogen screening have been used to interpret ^{15}N chemical shifts: (i) the symmetry of the 2p electrons; (ii) the average excitation

energy, especially n, π and π, π* transitions and (iii) the effective nuclear charge in relation with 2p orbital radius. This is in agreement with the relationship observed between ^{15}N chemical shifts and the π electron density of a particular nitrogen atom[11]. An increase of the π charge density on a nitrogen atom causes an upfield shift while an increase of its π bond order leads to a downfield shift[12]. Two types of nitrogens are therefore observed in heteroatomic systems: "pyrrole-like" nitrogen for which the lone pair participates in the conjugation with the π-system, and "pyridine-like" nitrogens for which the lone pair is perpendicular to the π system of the ring. The "pyrrole-like" nitrogen absorbs at higher field than the "pyridine-like" nitrogen due to the difference in their respective π charge densities.On the other hand, due to the availability of the lone pair of the "pyridine-like" nitrogen, it undergoes protonation and experiences an upfield shift[13]. Thus ^{15}N NMR has been proven to be a powerful tool to study the site of protonation in nucleosides and derivatives[14-21].

*Assignment of nitrogen resonances in 8-substituted adenosine and guanosine derivatives **2a - 6b & 7a - 11.***
The chemical shifts of nitrogens in the adenosine derivatives studied are listed in Table 1. The replacement of H at the C-8 position of purine nucleoside by the electron withdrawing Br atom as in **2a** results, as expected, in a downfield shift of the N-7 nitrogen as compared to adenosine. In 8-oxoadenosine **3a**, which is known to exist predominantly in the keto form, the N-7 absorbs at -270 ppm which is a shift expected for a pyrrole-type nitrogen. A $^{1}J_{N7\text{-}H}$ coupling of 90 Hz is also observed indicative of the O=C-NH- tautomeric structure for 8-oxoadenosine. An amino group attached to a heterocyclic ring acts as a mesomeric electron donor and causes an upfield shift for the nitrogen atoms in β-position. The effect of an amino group on the pyridine and pyrimidine system has been investigated[22]. The endocyclic sp^2 hybridized nitrogen in 2-aminopyridine (pK_a = 6.86 at 20 °C) and 2-dimethylaminopyridine (pK_a = 6.99 at 20 °C) move upfield by 50.8 and 49.7 ppm, respectively compared to that of pyridine (pK_a = 5.27 at 20 °C) in DMSO solution[22]. In 2-aminopyrimidine and in 2-dimethylaminopyrimidine[22], the N-1 and N-3 move upfield by 45.1 and 47.2 ppm, respectively, compared to those of unsubstituted pyrimidine in DMSO solution. The effect of an amino group at the C-6 position in purine system as in **12**, **13** or **14** have been investigated[19], and it has been found that substitution of the C-6 proton by an amino or dimethylamino group as in compounds **13** and **14** result in an upfield shift of 43.9 and 44.5 ppm, respectively, for the N-1 nitrogen in DMSO solution (Table 1). The addition of one equivalent of trifluoroacetic acid in the DMSO solutions of **12** (pK_a 1.94), **13** (pK_a 3.67) and **14** (pK_a 3.50) result in upfield shifts of their respective N-1 nitrogen by 20.8, 63.5 and 29.2 ppm respectively due to the formation of N^1H^+ species. It is also evident from ^{15}N-NMR studies that N^6,N^6-dimethyladenosine (**14**) also form the N^3H^+ species upon protonation ($\Delta\delta$ N^3 = 3.2 ppm)[19]. This suggest that N^6,N^6-dimethyladenosine has both N^1 and N^3 as the sites of protonation, clearly both basic sites contribute to its total pK_a of 3.50; $\Delta\delta$ ^{15}N shift of 29.2 ppm for N^1 and 3.2 ppm for N^3 also suggest that the former is more basic than the latter. The chemical reactivities of N^1 and N^3 toward electrophilic methylation reaction in N^6,N^6-dimethyladenine moiety are however quite different: the treatment of 2′,3′,5′-tri-O-benzoyl-N^6,N^6-dimethyladenosine with methyl iodide gives the corresponding N^3-methyl derivative as a sole product[23] showing the enhanced basicity of N^3, while a methylation reaction of 2′,3′,5′-tri-O-benzoyl-adenosine under a similar condition gives only the corresponding N^1-methyl derivative. This contrasting behaviour in protonation versus expression of the chemical reactivity through electrophilic methylation of 2′,3′,5′-tri-O-acetyl-N^6,N^6-dimethyladenosine may be due to the fact that a proton due to its smaller ionic radius may approach the more basic N^1 preferentially, but the dimethylamino group at C^6 blocks the N^1-methylation due to steric reasons, hence N^3-methyl derivative is the thermodynamically most prefered

product. The situation is different in 8-aminoadenosine derivatives **4a**, **5a** and **6a**. The replacement of the proton at C-8 in **1a** by an amino group results in an upfield shift of the N-7 and N-9 nitrogens. The N-1 and

Table 1. ^{15}N NMR chemical shifts of 8-substituted adenosines in the presence and absence of trifluoroacetic acid (TFA) in DMSO, T = 303.2 K c = 0.4 - 0.5 M

Compound	TFA (eq)	δN^1	δN^3	δN^7	δN^9	δN^6	δC^8-N	$\Delta\delta N^1$	$\Delta\delta N^7$
1a	0.0	-144.4	-157.5	-139.1	-215.6	-299.3	-	59.0	-2.1
	1.0	-203.4	-157.3	-137.2	-209.9	-291.6	-		
2a	0.0	-142.3	-158.2	-133.8	-215.6	-297.7	-	38.0	-0.7
	1.0	-180.3	-157.9	-133.1	-211.9	-293.0	-		
3a	0.0	-138.4	-157.8	-271.3	-243.2	-305.8	-	37.0	-1.0
	1.0	-175.4	-158.5	-270.3	-240.4	-302.6	-		
4a	0.0	-141.9	-156.2	-198.0	-241.3	-305.6	-323.9	46.0	23.6
	1.0	-187.9	-157.2	-221.6	-238.7	-298.9	-315.7		
5a	0.0	-141.5	-156.4	-199.2	-242.5	-305.7	-328.7	62.2	10.6
	1.0	-203.7	-157.3	-209.8	-238.3	-298.5	-322.0		
6a	0.0	-141.8	-155.7	-172.1	-233.9	-302.4	-343.0	63.1	2.7
	1.0	-204.9	-155.8	-174.8	-230.0	-295.6	-339.3		

N-3 nitrogens show a slight downfield shift (ca 2-3 ppm) while the exocyclic N-6 nitrogens move upfield by 3-6 ppm. The magnitude of the upfield shift for the N-7 and N-9 nitrogens depend however on the nature of the substituent at the exocyclic N-8. In 8-aminoadenosine (**4a**) and 8-methylaminoadenosine (**5a**), the N^7 absorbs at -198.0 and -199.2 ppm respectively (-140 ppm in adenosine), while in 8-dimethylaminoadenosine (**6a**), the N^7 absorbs at -172.1 ppm. The N^9 absorbs at -241.0 and -242.5 ppm in **4a** and **5a** (-211.8 ppm in adenosine) while it absorbs at -233.9 ppm in **6a**. The exocyclic N-8 shows an upfield shift of 4.9 ppm and 19.1 ppm in 8-methylaminoadenosine and 8-dimethylaminoadenosine respectively compared to 8-aminoadenosine. It is worthy of note that this situation is encountered in aniline derivatives[24], N-methylation of aniline leads to a shielding of 12 ppm which was attributed to the effect of methyl group. However, introduction of bulky alkyl substituent in the *ortho* position of N,N-dimethylaniline results in a larger shielding of the nitrogen (by 39 ppm to 55 ppm depending on the bulk of the *ortho* substituent) which is attributed to a steric inhibition of delocalization of nitrogen lone-pair. In 8-dimethylaminoadenosine (**6a**), the N^7 has more sp^2 character compared to that in 8-aminoadenosine (**4a**) and 8-methylaminoadenosine (**5a**). Probably steric effects exerted by two methyl groups in 8-dimethylaminoadenosine **6a** enforce a non-planar conformation of the 8-NMe_2 moiety and reduce the delocalization of the lone pair into the imidazole ring (see the semiempirical MO calculation part, vide infra)[25]. On the other hand, 8-aminoadenosine and 8-methylaminoadenosine adopts preferentially an *anti* conformation. This promotes hydrogen bonding between 8-NH_2 or 8-MeNH and 5′-OH and restricts the rotation about N^8-C^8 bond which may favour the delocalization of the N^8 lone pair for the stabilization of the N^7H^+ species. In **4a** and **5a**, the upfield shift observed for the N^7 and N^9 is an indication of a strong conjugation of the NH_2 or NHMe group at the C-8 position with the imidazole ring leading to a guanidine type system.

The assignments of the nitrogen resonances for 8-substituted guanosine derivatives (**7a**, **8a**, **9a** **10a**, & **11**) are listed in Table 2. The effect of an 8-oxo, 8-amino, 8-methylamino and 8-dimethylamino group on the resonances of the N-7 and N-9 nitrogens are very similar to what is observed in the adenosine derivatives. An exocyclic 8-amino group increases the electron density at the N-7 and N-9 nitrogens as can be seen from their upfield shift compared to guanosine.

Table 2. ^{15}N NMR chemical shifts of 8-substituted guanosines in the presence and absence of trifluoroacetic acid (TFA). DMSO, T = 303.2 K c = 0.4 - 0.5 M

Compound	TFA (equiv)	δN^1	δN^3	δN^7	δN^9	δN^2	δC^8-N	$\Delta\delta N^7$
7a	0.0	-233.5	-216.1	-131.5	-215.7	-307.0	....-	26.3
	1.0	-233.1	-217.0	-157.8	-213.8	-304.8		
8a	0.0	-232.4	-211.4	-190.6	-237.7	-308.3	-327.6	67.9
	1.0	-232.9	-214.9	-258.5	-241.4	-303.3	-314.6	
9a	0.0	-231.8	-212.0	-191.3	-239.8	-308.6	-332.6	53.6
	1.0	-232.4	-214.6	-244.9	-242.1	-304.2	-320.1	
10a	0.0	-231.3	-213.9	-162.4	-231.9	-307.7	-347.2	36.1
	1.0	-238.8	-214.2	-198.5	-233.5	-305.6	-339.7	
11	0.0	-233.8	-214.4	-272.5	-240.9	-306.6	-	-
	1.0	-234.0	-214.4	-272.6	-240.9	-306.8		

Protonation study. In all 8-substituted adenosines (**2a** - **6a**), the N-1 nitrogen is the primary site of protonation (Figure 1). In **1a**, **2a** and **3a**, protonation at N^1 results in a slight downfield shift of the N-7 and N-9 resonances (Figure 1). In 8-aminoadenosine derivatives (**4a**, **5a** and **6a**), the N-7 nitrogen moves upfield upon addition of trifluoroacetic acid (TFA). In 8-aminoadenosine (**4a**), the N-1 and N-7 nitrogens move upfield by 46.0 and 23.6 ppm respectively upon addition of 1.0 eq. of TFA. In 8-methylaminoadenosine (**5a**) the N^1 and N^7 resonances are shifted upfield by 62.2 and 10.6 ppm, while in 8-dimethylaminoadenosine (**6a**) they are shifted by 63.1 and 2.7 ppm, respectively. From these data, it seems clear that the extent of N^7 versus N^1 protonation is controlled by the nature of the substituent at the 8-amino group. Thus the relative amount of protonation at N^1 in 8-NH_2 adenosine and 8-NHMe adenosine are 66% (44% N^7H^+) and 85% (15% N^7H^+), respectively, while it is 96% (4% N^7H^+) in case of 8-NMe_2 adenosine (Figure 2). The total $\Delta\delta = \delta[(N^7 - N^7H^+) + (N^1 - N^1H^+)]$ is approximately 70 ppm for 8-aminoadenosine (**4a**) and 8-methylaminoadenosine (**5a**) and they also have similar pK_{a1} values (4.0). A possible explanation for the difference in the ratio of N-1 versus N-7 protonation in 8-aminoadenosine and 8-methylaminoadenosine can be that the bulky 8-methylamino group in the latter do not stabilize the protonation at N-7 as efficiently as the 8-amino group in the former. This trend is particularly noticeable in 8-dimethylaminoadenosine in which only 4% of N^7H^+ is observed. This also can be explained as due to the inability of the lone-pair of 8-dimethylamino group to be coplanar with the π electrons of the imidazole, and therefore can not delocalize the N^7H^+ in the imidazole system. Our subsequent semiempirical molecular orbital calculations have lent support to this (vide infra). In all guanosine derivatives, the N^7 is the first site of protonation (Table 2, Figure 3). Additional effect of 8-amino substituents on the $\Delta\delta N^7$

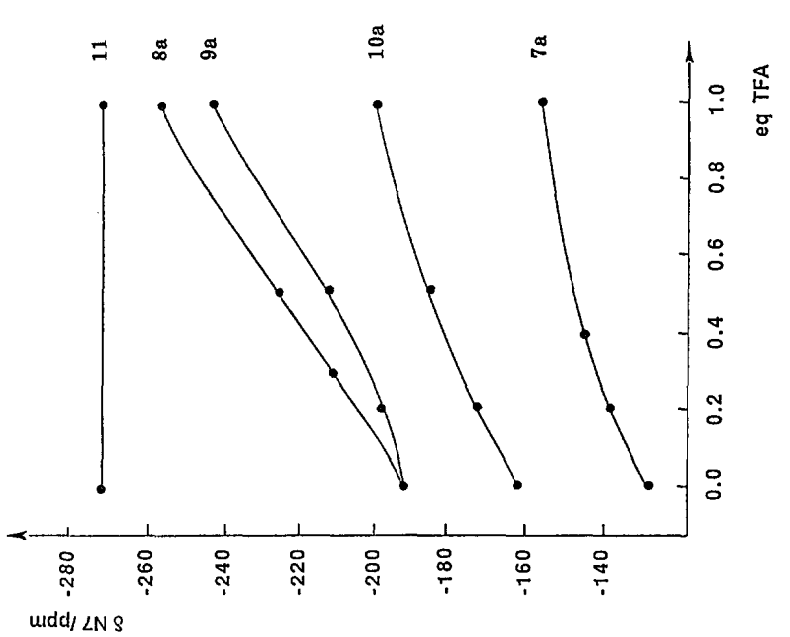


Figure 3: Dependance of ^{15}N chemical shifts of N^7 of guanosine and 8-substituted derivatives as a function of concentration of CF_3COOH.

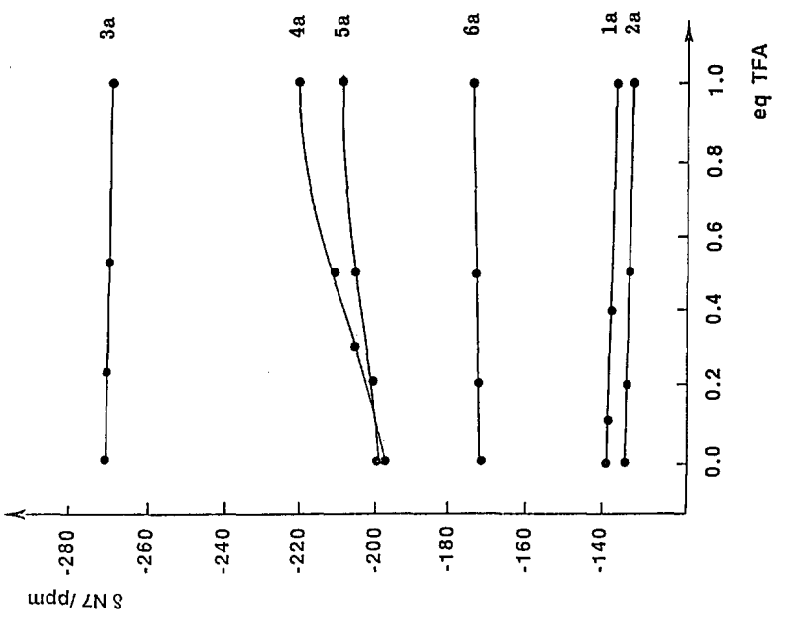


Figure 1: Dependance of ^{15}N chemical shifts of N^7 of adenosine and 8-substituted derivatives as a function of concentration of CF_3COOH.

with respect to guanosine in DMSO - TFA mixture are as follows: 8-NH_2 G > 8-NHMe G > 8-NMe_2 G > G and correlate with the relative basicities of these compounds[5]. In guanosine (**7a**), the electron density at N^9 decreases upon protonation at N^7 and this is seen in the slight downfield shift of N^9 upon addition of TFA.

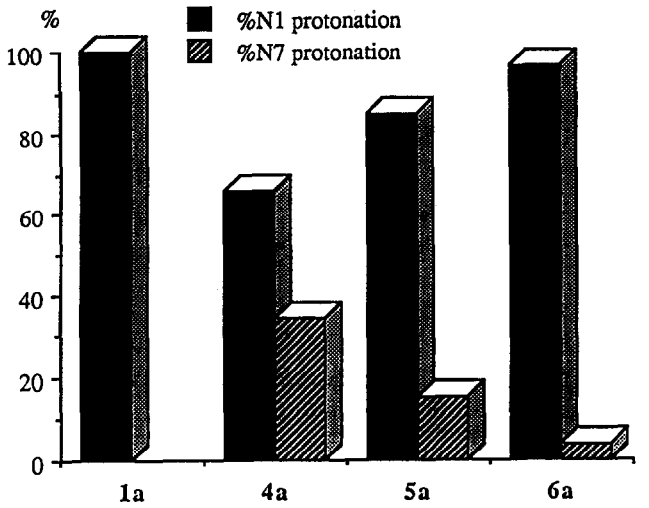


Figure 2: Relative population of N^1 versus N^7 protonation in adenosine **1a**, 8-aminoadenosine **4a** and 8-methylaminoadenosine **5a** and 8-dimethylaminoadenosine **6a**

Kinetics of the acidic hydrolysis of the N-glycosidic bond. Most of the purine nucleosides[26,27] and some of their isosteric analogues, such as benzimidazole nucleosides[28], are hydrolyzed under acidic conditions by a mechanism involving a rapid initial protonation of the base moiety and a subsequent rate-limiting heterolysis to the free base and a resonance stabilized glycosyl carbenium ion (Scheme 1). The bulk of experimental evidence, including pH-rate profiles, structural effects, the secondary deuterium isotope effects and the values of entropy

Table 3: Second order rate constants, acidity constants and relative rates of depurination for adenosine and some of its derivatives at 363.2 K and ionic strength 0.1 M (NaCl).

Compound	k_2 / $10^{-3}dm^3\ mol^{-1}\ s^{-1}$	pK_{a1}	k_{obs} (pH = pK_a)/s^{-1}	k_{rel}(pH = pK_{a1})
1b	4.64 (a)	3.3	$3.50\ 10^{-6}$	1
2b	12.7 (b)	3.7	$2.53\ 10^{-6}$	0.72
4b	62.3	4.0	$9.43\ 10^{-6}$	2.7
5b	50.1	4.0	$6.85\ 10^{-6}$	2.0
6b	8910	3.7	$1.50\ 10^{-3}$	429
15	1.49(b)	3.6	$3.74\ 10^{-7}$	0.11

(a) from ref. 26; (b) from ref 32. Ionic strength unknown, extrapolated to 363.2 K by using Arrhenius equation.

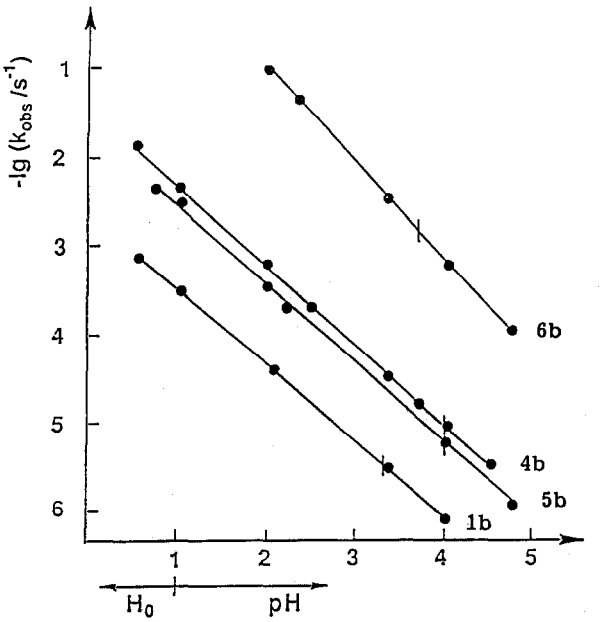
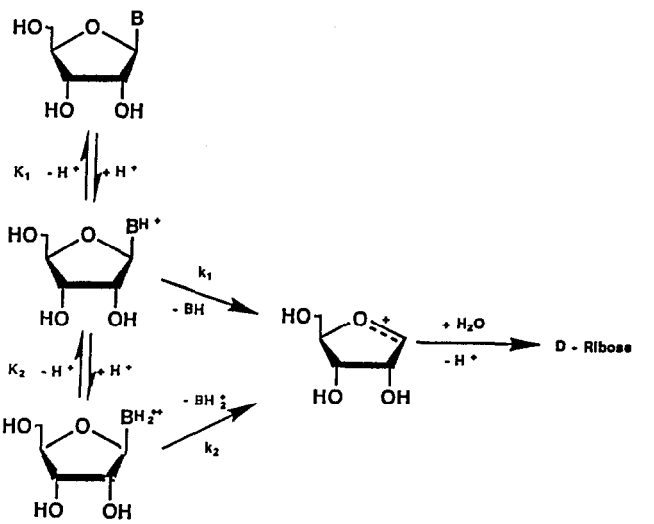


Figure 4: pH-rate profiles for the hydrolysis of adenosine **1b** and 8-substituted derivatives (**4b** - **6b**) at 363.2K. The ionic strength was adjusted to 0.1 mol.dm^{-3} when [H$^+$] < 0.1 mol.dm^{-3}.



Scheme 1

of activation support this mechanism. The pH-rate profiles for the acid catalyzed hydrolysis of compounds **1b**, **4b**, **5b** and **6b** are shown in Fig. 4. The profiles are linear in the whole pH-range studied even passing through an inflection point pH = pK_{a1} (the vertical lines in Fig. 4). This strongly suggests that the reaction is of first order with respect to oxonium ion. The linear dependence of hydrolysis rates means that the relative rates of the hydrolysis via mono- and diprotonated substrates are of the same order of magnitude ($k_1/K_1 \approx k_2/K_2$, see Scheme 1). The mechanism for the acidic hydrolysis of 8-amino- and 8-alkylamino adenosines is most probably the same as above (A-1). The possible anomerization during hydrolysis, as has been observed for thymidine[29] and some deaza analogues of purine nucleosides[30] can be eliminated in the present case, because no anomerization could be observed when the hydrolysis was followed by HPLC and ^{1}H-NMR techniques. According to this A-1 mechanism, the polar nature of a substituent at C^6 has only a moderate effect on the rate of the hydrolysis, the electropositive and electronegative groups being both rate-retarding. Electronegative groups, for example, markedly retard the pre-equilibrium protonation. However, the purine moiety becomes simultaneously a better leaving group. The influences on the initial protonation and rate-limiting heterolysis are opposite and largely cancel each other[26]. A substituent at C^8 is close to the reaction center and therefore it has a larger effect to the rate of hydrolysis than a substituent at C^6. For example, a bulky substituent at C^8 interferes sterically with the reactive centre. It is well documented that steric crowding of atoms decreases in the activated complex in an A-1 type reaction[31]. Thus in this type of mechanism an increase of the rate of hydrolysis is expected in the presence of a big substituent near the reactive centre.

The extreme lability of 8-dimethylaminoadenosine (**6b**) and 8-dimethylaminoguanosine (**10b**) under acidic conditions has been interpreted as due to the 8-dimethylaminonucleosides being locked in the *syn* conformation about the N-glycosidic bond, and therefore the hydrolysis is sterically accelerated[5]. However the role of *syn* $\rightleftarrows$ *anti* equilibrium in the enhancement of acidic depurination rates should not be overestimated. While it is true that 8-dimethylaminoadenosine (**6b**) and -guanosine (**10b**) are predominantly in the *syn* conformation, the dimethylamino substituent is not sufficiently bulky to drive the *syn* $\rightleftarrows$ *anti* equilibrium to 100% *syn* form. Furthermore, 8-bromoadenosine (**2a**) and 8-methoxyadenosine (**15**), which are mainly in *syn* conformation are much more stable in acidic conditions than 8-dimethylaminoadenosine (**6b**)[32]. The 8-methoxyadenosine (**15**) is even more stable than adenosine itself[32]. As shown in the case of 2-substituted benzimidazole nucleosides[28], the real difference in the rates of hydrolysis between the *syn* and *anti* conformers is most probably less than one order of magnitude. Therefore the lability of 8-dimethylaminoadenosine and -guanosine has to result mainly from other electronic factors. We have earlier shown through our studies on the acidic depurination of 2′-deoxyadenosines that the shift of protonation from N^1 to N^7 was responsible for the enahanced rate of hydrolysis of N^6-benzoyl-2′-deoxyadenosine[19]. N^7-protonation makes purine a better leaving group and contributes in the rate-acceleration in the depurination reaction under mildly acidic conditions, when the hydrolysis mainly occurs via monoprotonated substrate. *In the present case, the protonation at N^7 presumably accelerates the hydrolysis of 8-aminoadenosine (**4b**) and 8-methylaminoadenosine (**5b**) only to minor extent.* While the exocyclic amino group increases the electron density at N^7 and makes it more susceptible for protonation, concurrently it enhances the electron-density at N^9 which stabilizes the C1′-N9 bond. These two effects seem to cancel each other almost completely, because the pH-rate profiles are linear. Furthermore, intramolecular hydrogen bonding involving an 8-amino hydrogen as the donor and the oxygen of 5′-OH as the acceptor in 8-amino- and 8-methylamino-adenosine stabilizes the *anti* conformation about the N-glycosidic

bond, which partially decreases the steric acceleration of the acidic hydrolysis. Clearly such a situation in case of 8-dimethylaminoadenosine (**6b**) does not exist in solution. ^{15}N-NMR results show that there is very little N^7H^+ species (~4%) in acidic solution of 8-dimethylaminoadenosine suggesting there must be other electronic reasons for labilities of the glycosidic bond in these nucleosides.

^{1}H-NMR studies on 8-aminoadenosine, 8-methylaminoadenosine and 8-dimethylaminoadenosine. We have next examined if the sugar conformation undergoes any conformational changes in neutral to acidic medium in order to explore its influence on the depurination reaction. Following observations have been made: (1) *g,g* conformation is the prefered C4′-C5′ orientation (γ^+) when the 5′-OH is involved in hydrogen bonding with the N^3 as in the *syn* 8-bromoadenosine, or with 8-amino- in *anti* 8-aminoadenosine, or with 8-methylamino- in *anti* 8-methylaminoadenosine[5-9]. A comparison of γ^+ in neutral and protonated 8-aminoadenosine, 8-methylaminoadenosine and 8-dimethylaminoadenosine shows that the extent of γ^+ population decreases by 3%, 7% and 27%, respectively, in the protonated form suggesting that the hydrogen bonding with 8-amino substituent becomes increasingly less favoured in the following order: 8-dimethylaminoadenosine << 8-methylaminoadenosine < 8-aminoadenosine (Tables 4 & 5). (2) ^{1}H-NMR studies show that the 8-amino substituent in adenosine (amino, methylamino or dimethylamino) prefers the sugar conformation in the 2′-*endo* state (South) in the neutral aqueous solution (Table 5). In the acidic solution (~ pH 1), it is also clearly noticeable that the increase of population of the N conformer in the pseudorotamer equilibrium follows the order: 8-aminoadenosine < 8-methylaminoadenosine < 8-dimethylaminoadenosine. Thus the %N increase in the acidic solution with respect to the neutral solution for 8-aminoadenosine, 8-methylaminoadenosine, and 8-dimethylaminoadenosine are +7%, +9% and +19%, respectively, while their relative rates of acidic depurination are repectively 2.7, 2 and 429-fold with respect to adenosine. This increased relative rates of acidic hydrolysis almost parallels to the relative increase of %N and relative decrease of γ^+ population in 8-aminoadenosine, 8-methylaminoadenosine, and 8-dimethylaminoadenosine. This may suggest that the transition state for the stabilization of the intermediary oxocarbenium ion is presumably favoured by the 3′-*endo* (North) sugar and γ^t conformations. As has been stated above that the 8-amino- group in 8-aminoadenosine can take up amost planar conformation with the imidazole moiety, and hence can partly delocalize the N^7H^+ species (44% N^7H^+ & 66% N^1H^+). The ability to form the N^7H^+ species in 8-methylaminoadenosine is clearly much reduced (15% N^7H^+ & 85% N^1H^+) because of the slightly reduced ability of 8-methylamino group to conjugate with the N^7 as well as possible steric hindrance by the methyl group. On the other hand, the conjugation of the lone-pair of the 8-dimethylamino group with the imidazole moiety is reduced in neutral[25] or in protonated forms due to the energetic reasons with the net result that it can not as efficiently delocalize the N^7H^+ species (4% N^7H^+ & 96% N^1H^+) (see the Semiempirical MO calculations part, vide infra). The 8-dimethylamino lone-pair in protonated 8-dimethylaminoadinosine thus available may presumably interact through the donation of its N^8-lone pair to the neighbouring oxocarbenium ion at O4′-C1′ by a charge-transfer mechanism, and can cause its stabilization, which may furthermore contribute to the kinetic acceleration of the cleavage of the glycosidic bond in contrast to protonated 8-aminoadenosine. On the other hand, the increased relative rates of depurination of 8-aminoadenosine and 8-methylaminoadenosine compared to that of adenosine are clearly due to the fact that in the former the N^7H^+ species is stabilized while in the latter it is only N^1H^+ species that is formed. It may be notied that the relative rates of acidic depurination of 8-aminoadenosine and 8-methylaminoadenosine are almost comparable despite the fact that the relative population of N^7H^+ species in the former is 44% , while in the latter

it is only 15%. *Therefore the acceleration in the acidic depurination of 8-methylaminoadenosine is not entirely due to the participation of N^7H^+ species.* Both in 8-methylaminoadenosine and 8-dimethylaminoadenosine a substantial contribution presumably comes from the delocalization of the oxocarbenium ion by the 8-dimethylamino lone-pair (anchimeric assistance), or from the release of steric strain in activated state due to the bulky 8-amino substituent in the protonated 8-methylaminoadenosine and 8-dimethylaminoadenosine.

Semiempirical molecular orbital calculations (AM1 and PM3). The energetically favoured glycosyl torsion χ(CN)° starting from two preferred staggered C4′ - C5′ conformations (*i.e* $\gamma^+ = 60°$ and $\gamma^t = 180°$) have been determined for neutral, N^7H^+ and N^1H^+ species in both typical 2′-*endo* (S) and 3′-*endo* (N) conformations using both AM1[33a] and PM3[33b] calculations. Essential data from AM1 studies summarizing the conformation of 8-amino and 8-alkylamino group with respect to neutral, N^7H^+ and N^1H^+ species are documented in Table 6. During these computational studies we have monitored the fate of *unconstrained* starting sp^3 geometries of N^8 [torsions: $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a] = -120°$ & $\phi[R^a\text{-}N^8\text{-}C^8\text{-}N^7] = 60°$, ($R^a = R^b$ = H/Me,), while the corresponding bond angles $R^a\text{-}N^8\text{-}C^8$ & $R^b\text{-}N^8\text{-}C^8$ are 109°] in 8-aminoadenosine, 8-methylaminoadenosine and 8-dimethylaminoadenosine. It has been found in AM1 calculations that the sp^3 hybridized N^8 changes to become more or less sp^2 hybridized [torsions: $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a] = 180°$, $\phi[R^a\text{-}N^8\text{-}C^8\text{-}N^7] = 0°$, $R^a = R^b$ = H/Me), while the corresponding bond angles $R^a\text{-}N^8\text{-}C^8$ & $R^b\text{-}N^8\text{-}C^8$ are 120°] depending upon its substituents: H or Me, or the N^7 or N^1protonated form of aglycone. Energetically unfavourable N^7 protonation and consequent delocalization of the N^7H^+ species by specific substituents at N^8 exhibited higher heat of formation. Following conclusions are drawn on the ability of 8-amino-, 8-methylamino- or 8-dimethylamino- group at the C-8 of adenosine to delocalize the N^1H^+ or N^7H^+ species: (1) Most stable N^7H^+ species (ΔH_f = 51.1796 Kcal/mol) of 8-aminoadenosine (S, $\gamma = 154.73°$, χ (CN) = 210°) showed torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a] = -161.44°$ which is 18.56° away from planar sp^2 hybridized nitrogen with the N8-C8 bond order of 1.2958 while the most stable N^1H^+ species (ΔH_f = 50.5794 Kcal/mol) of 8-aminoadenosine (S, $\gamma = 157.47°$, χ (CN) = 210°) showed a torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a] = -144.86°$ which is 35.14° away from planar sp^2 hybridized nitrogen with the N8-C8 bond order of 1.1899. Here, the N^1H^+ species is slightly more stable by 0.6 Kcal than that of the N^7H^+

Table 4: Coupling constants (Hz) for **4b**, **5b**, **6b**, in neutral D_2O at 30 and 80 °C & at pH ~ 1 at 30 °C

Compound	T (°C)	$J_{1'2'}$	$J_{2'3'}$	$J_{3'4'}$	$J_{4'5'}$; $J_{4'5''}$
8-NH_2-A (**4b**)	30°	7.2	5.5	2.2	Σ 4.8
	80°	7.2	6.0	2.9	2.8; 3.1
8-NH_2-A(H^+)	30°	6.7	5.6	3.1	Σ 5.2
8-NHMe-A (**5b**)	30°	7.3	5.7	2.4	2.3; 2.3
	80°	6.8	5.9	3.1	2.6;2.8
8-NHMe-A(H^+)	30°	6.7	5.6	2.9	2.6; 2.6
8-NMe_2-A (**6b**)	30°	7.6	5.5	2.0	2.7; 3.2
	80°	7.2	5.7	2.6	2.8; 3.5
NMe_2-A(H^+)	30°	6.2	5.8	4.0	4.2; 4.2

Table 5: Relative ratios of γ+ and North (3′-*endo*) conformations in 8-amino-, 8-methylamino- and 8-dimethylaminoadenosine at 30 and 80 °C in neutral D_2O and in pH ~ 1

	8-NH_2-A (**4a**)		8-NHMe-A (**5a**)		8-NMe_2-A (**6a**)	
T (°C)	x(γ+)	x(N)	x(γ+)	x(N)	x(γ+)	x(N)
30° (neutral)	0.87	0.10	0.90	0.08	0.77	0.05
80° (neutral)	0.74	0.10	0.81	0.15	0.72	0.10
30° (acidic)	0.84	0.17	0.83	0.17	0.50	0.24

species suggesting that the N^1H^+ species has a higher population than the N^7H^+ species. Experimental ^{15}N-NMR studies actually show 66% N^1H^+ species and 34% N^7H^+ species. (2) Most stable N^7H^+ species (ΔH_f = 52.4640 Kcal/mol) of 8-methylaminoadenosine (S, γ = 154.70°, χ (CN) = 210°) showed a torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a]$= -163.31° which is 16.69° away from planar sp^2 hybridized nitrogen with the N8-C8 bond order of 1.2839 while its most stable N^1H^+ species (ΔH_f = 51.0170 Kcal/mol, S, γ = 38.66°, χ (CN) = 240°) showed a torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a]$ = -143.56° which is 36.44° away from planar sp^2 hybridized nitrogen with the N8-C8 bond order of 1.1567. Here the energetic requirement of 8-MeNH group to delocalize the N^7H^+ species makes it considerably less stable than the N^1H^+ species by 1.447 Kcal. Experimental ^{15}N-NMR studies actually show 85% N^1H^+ species and 15% N^7H^+ species. (3) Most stable N^7H^+ species (ΔH_f = 66.4171 Kcal/mol) of 8-dimethylaminoadenosine (S, γ = 24.13°, χ (CN) = 30°) showed a torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a]$ = -162.71° which is 17.29° away from planar sp^2 hybridized nitrogen with the N8-C8 bond order of 1.2382 while its most stable N^1H^+ species (ΔH_f = 64.2089 Kcal/mol, S, γ = 66.23°, χ (CN) = 90°) showed a torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a]$ = -134.53° which is 45.47° away from planar sp^2 hybridized nitrogen with the N8-C8 bond order of 1.0441. Here the energy required to delocalize the N^7H^+ species by of 8-Me_2N group is even higher (2.208 Kcal) than 8-MeNH group which makes it certainly much less stable than the N^1H^+ species which is also evident by our ^{15}N-NMR studies (*ie.* ~ 4% N^7H^+ species).

Relative heat of formation [$\Delta(\Delta H_f)$] of N^7H^+ species of 8-dimethylaminoadenosine is much higher than the corresponding N^1H^+ species (the energy of N^7H^+ species is higher by 2.8 Kcal/mol in γ^t and S conformations, 2.2 Kcal/mol higher in γ^+ and S conformations, 2.0 Kcal/mol higher in γ^+ and N conformations, and 2.03 Kcal /mol higher in γ^t and N conformations than the corresponding N^1H^+ species). Sugar conformations therefore do not play significant role in the relative stabilities of N^7H^+ versus N^1H^+ species of 8-dimethylaminoadenosine as would be expected from the *syn* orientation of the 8-dimethylaminoadenine moiety. Relative large difference in the stabilities would seem to disfavour the formation of N^7H^+ species. ^{15}N-NMR data 8-dimethylaminoadenosine shows ~96% N^1H^+ species and ~4% N^7H^+ species. The N^1H^+ species of 8-methylaminoadenosine is more stabilized by 2.44 Kcal than the corresponding N^7H^+ species in S, γ^+ conformations, while in S, γ^t conformations, both N^1H^+ and N^7H^+ species have almost comparable stabilities (ΔE = 0.38 Kcal). ^{15}N-NMR studies however show that it is ~85% protonated at N^1 and ~15% protonated at N^7. The relative stability of N^7H^+ versus N^1H^+ species of 8-aminoadenosine and 8-methylaminoadenosine are dependent upon the sugar conformations because they have preferential *anti* glycosyl torsion. The N^1H^+

Table 6: Semiempirical AM1 calculations on neutral 8-aminoadenosine (**4a**), 8-methylaminoadenosine (**5a**) and 8-dimethylaminoadenosine (**6a**) and on their N^1 and N^7 protonated species (see experimental section for (#).

C8-substituted Adenosines	Sugar pucker# (N or S)	χ (CN)°	H^+ Site	γ^+ / γ^t [°]	Heat of formation (ΔH_f)	Bond order: N^8-C^8	Bond order: N^9-$C^{1'}$	Bond order: N^6-C^6	Bond angle: R^a-N^8-C^8	Bond angle: R^b-N^8-C^8	Torsion $\phi(R^b$-N^8-C^8-$R^a)$
4a	N	180	Neutral	50.98	-87.8354	1.1084	0.8869	1.1793	112.96	115.11	-132.52
		180		-178.94	-87.7613	1.0839	0.8835	1.1994	114.08	112.60	-131.09
	N	180	N^1	48.24	52.1838	1.2190	0.8453	1.2994	116.51	118.76	-148.63
		120		-167.55	55.7496	1.1778	0.8521	1.3077	116.27	117.61	-146.21
	N	180	N^7	50.37	50.7301	1.3096	0.8254	1.2309	119.47	119.25	-158.20
		210		135.41	52.2749	1.3105	0.8350	1.2312	119.33	119.74	-160.00
	S	240	Neutral	47.91	-84.8962	1.0910	0.9278	1.1767	113.79	112.99	-131.22
		240		149.56	-86.8080	1.0762	0.9252	1.1812	112.24	111.94	-127.24
	S	240	N^1	42.79	51.8315	1.2050	0.8866	1.2944	118.76	117.47	-153.59
		210		157.47	50.5794	1.1899	0.8783	1.2957	117.85	115.93	-144.86
	S	210	N^7	55.29	52.0835	1.3071	0.8652	1.2134	119.80	120.69	-176.62
		210		154.73	51.1796	1.2958	0.8637	1.2171	119.46	119.72	-161.44
5a	N	210	Neutral	22.63	-84.3792	1.0778	0.8989	1.1853	113.74	117.53	-136.59
		180		-179.18	-84.6578	1.0627	0.8839	1.1953	112.79	116.62	-133.85
	N	180	N^1	48.31	55.0991	1.2021	0.8468	1.2949	117.33	119.69	151.77
		180		144.74	54.9792	1.2207	0.8489	1.2943	119.36	120.56	-163.27
	N	180	N^7	51.14	52.5791	1.3040	0.8288	1.2190	118.54	122.03	170.15
		180		141.59	52.2784	1.3124	0.8306	1.2222	119.27	122.17	-175.21
	S	240	Neutral	47.33	-81.6351	1.0688	0.9284	1.1757	112.61	116.91	-134.37
		240		171.93	-82.7611	1.0414	0.9232	1.1884	110.47	116.05	-129.79
	S	240	N^1	38.66	51.0170	1.1567	0.8785	1.2963	115.17	118.75	-143.56
		0		-175.86	52.8502	1.1537	0.8462	1.2968	116.96	119.20	-149.14
	S	210	N^7	55.31	53.4668	1.2981	0.8682	1.2081	118.43	122.25	-176.05
		210		154.70	52.4640	1.2839	0.8667	1.2111	118.06	121.80	-163.31
6a	N	90	Neutral	63.21	-77.8443	0.9966	0.9028	1.2005	113.16	112.74	-130.40
		90		-178.55	-78.6904	0.9975	0.9009	1.2051	113.23	112.76	-130.49
	N	90	N^1	12.57	64.9115	1.0358	0.8550	1.3141	114.09	114.53	-133.41
		90		-171.97	65.5703	1.0413	0.8544	1.3186	114.26	114.78	-133.92
	N	90	N^7	38.63	66.8975	1.2157	0.8444	1.2121	118.45	122.54	-162.14
		90		-171.47	67.6092	1.2121	0.8424	1.2273	118.54	122.31	-161.48
	S	60	Neutral	23.43	-76.0535	0.9959	0.9158	1.2035	113.48	112.35	-130.81
		60		179.79	-76.9170	0.9957	0.9179	1.2042	113.44	112.30	-130.68
	S	90	N^1	66.23	64.2089	1.0441	0.8792	1.3102	114.48	114.81	-134.53
		60		148.89	64.6338	1.0311	0.8789	1.3087	114.33	113.86	-133.60
	S	30	N^7	24.13	66.4171	1.2382	0.8541	1.2201	122.07	119.06	-162.70
		90		177.05	67.4359	1.1911	0.8642	1.2178	118.49	121.23	-157.80

species is only 0.25 Kcal /mol (γ^+ and S) or 0.6 Kcal /mol (γ^t and S) more stable than the N^7H^+ species of 8-aminoadenosine. Relatively low difference of relative stabilities between N^7H^+ and N^1H^+ species of 8-aminoadenosine suggest that they have most probably equal likelihood to be protonated. In fact, our ^{15}N-NMR studies show that it is 66% protonated at N^1 and 34% protonated at N^7. Since the ^1H-NMR data show the preferential conformation of the sugar conformation in both 8-aminoadenosine and 8-methylaminoadenosine is South , therefore suggestion by AM1 that N^7H^+ species is more stable than the N^1H^+ species in the North conformations do not have any practical significance.

The comparison of heat of formation (ΔH_f), which is parameterized on the data obtained on model compounds[33a] in gaseous phase, with that of the conformational data by ^1H-NMR spectroscopy in solution phase is partly incompatible. For example, we have observed the free energy-minimum conformation across the C4´- C5´ bond is in γ^+ orientation by ^1H-NMR spectroscopy, but AM1 somtimes prefers the γ^t orientation erroneously. We have furthermore observed that AM1 geometry optimizations on completely unconstrained pentose sugar moiety of nucleosides produce severely flattened pentose sugar. The use of AM1 in conformational studies with nucleosides therefore has to be taken with caution. It is worthy of note that at least in the present case if one tackles the conformations that are predominantly present in solution in NMR time scale (*i.e* S & γ^+), and studies the energetic consequence of formation of N^7H^+ versus N^1H^+ species using AM1 calculations, it correctly predicts the preferred site of protonation for 8-aminoadenosine and 8-methylaminoadenosine and 8-dimethylaminoadenosine as to be N^1H^+ species. The energy difference however for the competing protonation sites is correctly predicted for 8-aminoadenosine and 8-dimethylaminoadenosine, but not for 8-methylaminoadenosine. We have therefore performed AM1 calculations with N^9-methyl-8-amino substituted adenines. Clearly in all three N^9-methyl derivatives (*i.e.* 8-amino-, 8-methylamino and 8-dimethylamino), the N^1H^+ species was more stable by 3.8, 2.6 and 3.5 Kcal/mol, respectively, than the corresponding N^7H^+ species. On the other hand, PM3 calculations[33b] showed the N^7H^+ species as energetically more favourable than the N^1H^+ species for all three 8-amino substituted adenosines or 9-methyladenines.

Work is in progress in this lab to synthesize $[8\text{-A-2´-dA}]_8$ & $[8\text{-mA-2´-dA}]_8$ in order to clearly show the structural features of their triple helix formation with the complementary dT_8 in aqueous solution by high-field ^1H-NMR spectroscopy.

Experimental

Materials All compounds studied were synthetized following normal litterature procedures: 8-bromoadenosine[34], 8-aminoadenosine[35], 8-methylaminoadenosine[36], 8-dimethylaminoadenosine (as methylamino counterpart but by using aqueous dimethylamine), 8-oxoadenosine[37], 8-aminoguanosine (same as above), 8-methylaminoguanosine[38], 8-dimethylaminoguanosine[39], 8-oxoguanosine[40]. Acetylated derivatives were prepared using the procedure of Matsuda *et al*[41]

Kinetic measurements. The progress of acidic hydrolyses was followed by the HPLC technique as described previously[42]. The hydronium ion concentrations of the buffer solutions employed were calculated from the pK_a values of the buffer acids under experimental conditions[43]. The H_0 values of concentrated aqueous acids were taken form literature[44]. The progress of the hydrolysis of 8-dimethylaminoadenosine was followed by recording

the ^1H NMR spectra when it was allowed to hydrolyze in acidic deuterium oxide from 0 to 2 half lives.Only anomeric signal observed was the H1´ doublet of the starting material.

Acidity constants. The pK_a values for the monocations of nucleosides were determined spectrophotometrically (Varian-Cary 2200 spectrophotometer) at 298.2 K as described previously[45]. The results were extrapolated to 363.2 K assuming that the pK_a value depends on temperature as with adenosine[26].

^{15}N NMR measurements. ^{15}N chemical shift determinations were made on a Jeol GX 270 spectrometer at 27.4 MHz. All ^{15}N-NMR spectra were perfomed relative to $CH_3{}^{15}NO_2$ in CD_3NO_2 (reference and lock signal respectivly) in a capillary. The probe temperature was 30 °C. The assignments of ^{15}N resonances were done under proton decoupled mode (without NOE) or by using INEPT pulse sequences. Routinely, 16K data points were used for acquisition, zero filled to 32 K and Fourier transformed with a broadening factor of 2-4 Hz.The samples (c ≈ 0.4 M) were dissolved in distilled DMSO. Because of poor solubility and extreme lability under acidic media, all ^{15}N NMR measurements were done with 2´,3´,5´-triacetylated nucleosides. A negative value for the chemical shift denotes a upfield shift.

Semiempirical AM1 calculations. AMPAC or MOPAC package from QCPE[33a,33b] was used on Silicon Graphics Personal Iris 4D 25G system. Torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a] = 180°$ for idealized sp^2 nitrogen while the corresponding bond angle is 120°. Starting geometries for AM1 and PM3 MO calculations were idealized sp^3 nitrogen, and calculations were run without any constraint on the following starting geometries of N^8: torsion $\phi[R^b\text{-}N^8\text{-}C^8\text{-}R^a] = 120°$ while the corresponding bond angle is 109°. (#) Idealized constraints, based on X-ray crystal structures[46,47], for North (N) and South (S) sugar puckers have been used for all calculations since both AM1 and PM3 tends to planarize the puckered ring which have been observed in NMR spectroscopy. Following sugar torsions O4*-C1*-C2*-C3* (-27.3036°) & C1*--C2*-C3*-C4* (35.7630°) for N-type sugar[46] and O4*-C1*-C2*-C3* (36.8102°) & C1*--C2*-C3*-C4* (-38.4263°) for S-type sugar[47] were fully constrained during the calculations. The energies and relative stability for these starting N or S sugar geometries are unknown, therefore, the AM1 or PM3 calculated ΔH_f can be compared only within S or N conformations.

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