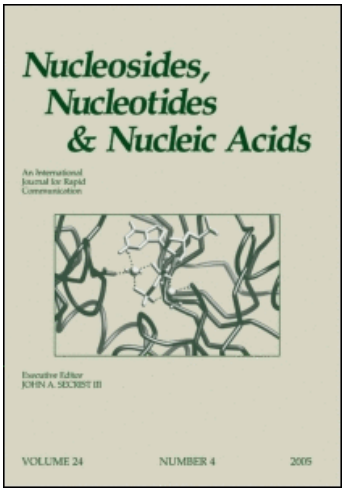


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# An Assessment of Electronic Properties of Pyrimidine and Purine Nucleosides by $^{15}$N-NMR Spectroscopy

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# AN ASSESSMENT OF ELECTRONIC PROPERTIES OF PYRIMIDINE AND PURINE NUCLEOSIDES BY $^{15}$N-NMR SPECTROSCOPY.+

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Abstract: An $^{15}$N-NMR study at natural abundance of $O^4$/$N^3$-substituted pyrimidine and $C^6$-substituted purine ribonucleosides has shown that the exact location of the protecting group (substituent) on either $O^4$ or $N^3$ in pyrimidines has a strong influence on the electronic properties of the resultant pyrimidine system, mainly because of the change of state of hybridization of the $N^3$-nitrogen. The basicity of $N^3$ in some $C^4$-substituted pyrimidines has been studied by following the $^{15}$N chemical shifts of protonated species in the presence of $CF_3COOH$ both in DMSO and in $CH_2Cl_2$ solution. A comparison of the basic character of $N^3$ in $C^4$-substituted pyrimidine and of $N^1$ in $C^6$-substituted purine nucleosides has shown that the magnitude of the $^{15}$N shift of $N^3$ (or $N^1$) upon protonation is governed mainly by the electronic properties of the heteroatom linked to $C^4$ (or $C^6$). It also clearly emerged in this study that there is very litle difference in basicities of $N^3$ of pyrimidine and $N^1$ of purine nucleosides despite the presence of the fused imidazole moiety in the latter.



Recently we have shown that the $N^1$ protection of the $N^2$-protected guanine nucleosides enhanced the basic character of the $N^7$-nitrogen while a $O^6$-aryl protecting group protects the $N^2$-protected guanine residue more satisfactorily against the electrophilic attack of $CH_3I$ than any alkane based $O^6$-protecting group[1,2a]. This report deals with a comparative study delineating the structural similarities between the pyrimidine part of $C^6$-substituted purine nucleosides and the corresponding pyrimidine nucleosides. It was anticipated on the basis of our earlier studies[2-4] that an examination of $\Delta\delta$ $^{15}$N shifts would provide

+ *Dedicated to Professor Wolfgang Pfleiderer on the occasion of his 60th. birthday.*

means to assess the basicities of $N^1$-nitrogen of $C^6$-substituted purine ribosides and $N^3$-nitrogen of $C^4$-substituted pyrimidine nucleosides. We hoped that such a $\Delta\delta$ shift comparison would reveal the influence of the fused imidazole part on the basicity of the $N^1$-nitrogen in $C^6$-substituted purine derivatives. The comparison of magnitudes of $\Delta\delta\,^{15}N$ shifts have been carried out both in $CH_2Cl_2$ and DMSO since we expected that the solvation of proton of $CF_3CO_2H$ in the former medium will be considerably less than in the latter and, therefore, the magnitude of $^{15}NH^+$ shifts will be different in these two acidic media.

Assignment of $^{15}N$ resonances of pyrimidines and purine nucleosides.

All the assignments of $^{15}N$ chemical shifts were made according to previous studies of purines and pyrimidines[2-5]. For $C^4$-substituted pyrimidines, $N^1$ was easily detected by a large negative NOE because of the adjacent sugar protons. The amide and amine-nitrogens were detected either by NOE spectra or by the magnetization transfer from $^1H$ to $^{15}N$ by INEPT or DEPT pulse sequences. In adenosine and $C^6$-substituted purine ribonucleosides, the cyclic nitrogens were assigned by the INEPT pulse sequence *via* long-range couplings. Thus $N^9$ has a coupling with $H^8$ of ca. 7-9 Hz while $N^7$ has a $^2J_{N^7,H^8}$ of ca. 10-12 Hz. In the pyrimidine part, we have noticed that the $N^1$ usually has a larger coupling with H-2 (ca. 15-17 Hz) than $N^3$ with H-2 (ca. 14-16 Hz).

## RESULT AND DISCUSSION

Distinction between $C^4$ and $N^3$ substituted pyrimidine nucleosides.

Our earlier study[2] has revealed that a distinction between an $O^6$-substituted guanine nucleosides and an $N^1$-substituted guanine nucleosides can be easily done by $^{15}N$ NMR spectroscopy. We expected that the change of the hybridization state of $N^3$ of pyrimidine will also change according to the position of the substitution.

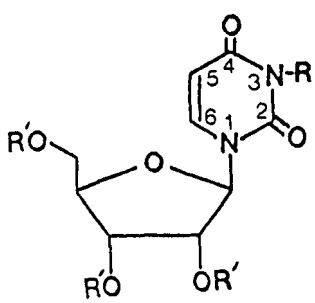
The $^{15}N$ chemical shifts of $N^3$-substituted uridine derivatives (1 - 6) are shown in Table 1. Since the $N^1$-nitrogens in compounds 3 - 6 are standard $sp^3$ hybridized and not in any way different from the parent 2',3',5'-tri-O-acetyl uridine (2), the $N^1$ in these compounds (3 - 6) has a steady chemical shift (-240 to -242 ppm) which is closely compar-

TABLE 1: $^{15}$N chemical shifts[a] of some $N^3$-substituted uridines.

| Compound | $N^1$ | $N^3$ | N-substituent |
|---|---|---|---|
| 1[b] | -237.8 | -223.7 | - |
| 2[b] | -241.9 | -225.6 | - |
| 3[b] | -241.7 | -193.9 | - |
| 4[c] | -237.2 | -222.0 | -13.8 |
| 5[d] | -240.6 | -221.6 | -13.7 |
| 5[e] | -242.2 | -222.3 | -15.5 |
| 6[f] | -240.3 | -216.9 | -10.5 |
| 6[g] | -242.6 | -217.2 | -11.7 |

[a] $CH_3{}^{15}NO_2$ as external reference; [b] from literature (Ref. 4 and 5); [c] 0.35 M in DMSO; [d] 0.55 M in DMSO; [e] 0.55 M in $CH_2Cl_2$; [f] 0.25 M in DMSO; [g] 0.25 M in $CH_2Cl_2$

able to that of 2. The acetyl groups on the sugar moiety in 2 shields the $N^1$ by 3-4 ppm as compared to that of uridine (1). As expected, the $N^3$-nitrogen in compounds 2 - 6 experiences the electron-donating or electron-withdrawing influence of its specific substituents. For the compounds 5 and 6, the $N^3$ chemical shifts are very closely similar to that of uridine (1). On the other hand the electron-withdrawing properties of the $N^3$-benzoyl group in 3 deshields the $N^3$ by ca. 30 ppm as compared to that of the parent compound 2.




1. R = R' = H
2. R = H, R' = Acetyl
3. R = Benzoyl, R' = Acetyl
4. R = 4-Nitrophenylsulfonylethyl, R' = H
5. R = 4-Nitrophenylsulfonylethyl, R' = Acetyl
6. R = 4-Nitrophenylethyl, R' = Benzoyl

(A) (B) (C) (D)

(A) & (B) are keto-enol tautomers

(C) : $N^3$- substituted pyrimidine nucleosides

(D) : $O^4$- substituted pyrimidine nucleosides

Scheme: 1



It was clearly expected that the electronic consequence of trapping the enol tautomer of the $N^3/O^4$ lactam function of uridine as its $O^4$-substituted derivatives (Scheme 1 and Table 2) would enormously affect the $N^1$ and $N^3$ chemical shifts of the resultant pyrimidine system. The result of this transformation is however more noticeable in $N^3$ than in $N^1$ in compounds 8 - 19. Thus the $N^1$ in these compounds lies always in the range from -219 to -233 ppm while the $N^3$, now in a $sp^2$ hybridized state, is drastically deshielded as compared to those in compounds 1 - 6. In fact, the extent of the deshielding that the $N^3$ experiences in different $C^4$-substituted pyrimidines 7 - 19 is directly related to the nature of the $C^4$ substituent. The $N^3$ chemical shifts for the $O^4$-aryl and $O^4$-alkyl substituted compounds (9 - 14) seem to be very similar as for the $N^4$-acyl-substituted pyrimidines (15, 16 and 19) (ca. -150 to -155 ppm). However, the $N^4$-benzamido group in 16 deshields the $N^3$ more significantly (by ca. 13 ppm) than the corresponding $N^3$ by the $N^4$-acetamido group in 15. The influence of the heteroatom at $C^4$ on the chemical shift of $N^3$ is more noticeable as shown by the deshielding influence of the thioaryl group[6] as a substituent in 11; similarly we observed a considerable shielding of $N^3$ by the amino substituent at $C^4$ in 7 and 8.

It is explicit in the above discussion that the $^{15}N$-NMR spectroscopy offers a good scope to distinguish between a $N^3$ or $O^4$ substituted derivative of lactam function of uridine which may form as a result of trapping either its -NH-CO- or -N=C(OH)- tautomer (Scheme 1) by a suitable electrophile. Recently Claessen et al.[7] have reported that the

Table 2: $^{15}$N chemical shifts[a] of some C$^4$-substituted pyrimidines in neutral and acidic DMSO and/or $CH_2Cl_2$* solutions. T = 303 K.

| Compound[+] | Equiv. of TFA | $N^1$ | $N^3$ | N-substituent |
|---|---|---|---|---|
| 7[b] | 0 | -228.3 ( - ) | -171.4 ( - ) | -287.3 ( - ) |
| | 1 | -227.2 ( - ) | -237.3 ( - ) | -275.2 ( - ) |
| 8[c(f)] | 0 | -232.3 (-233.6) | -171.2 (-177.2) | -285.2 (-284.9) |
| | 1 | -231.7 (-232.6) | -235.2 (-236.9) | -272.5 (-275.3) |
| 9(c) | 0 | - (-225.6) | - (-155.3) | - |
| 10(d) | 0 | - (-220.7) | - (-152.0) | - (-15.8) |
| | 1 | - (-219.8) | - (-154.5) | - (-15.8) |
| 11(d) | 0 | - (-222.2) | - (-119.1) | - |
| | 1 | - (-219.1) | - (-136.7) | - |
| 12(b) | 0 | - (-222.3) | - (-151.5) | - |
| | 1 | - (-220.3) | - (-158.5) | - |
| 13(e) | 0 | - (-225.8) | - (-155.9) | - |
| | 1 | - (-223.8) | - (-162.0) | - |
| 14[f(f)] | 0 | -223.2 (-224.7) | -154.5 (-155.3) | - 10.5 (-12.3) |
| | 1 | -223.3 (-224.2) | -154.6 (-157.2) | - 10.6 (-11.9) |
| 15[b(e)] | 0 | -218.5 (-219.2) | -146.3 (-152.4) | -232.2 (-234.0) |
| | 1 | -218.4 (-216.0) | -148.9 (-206.8) | -232.4 (-235.8) |
| 16[f(f)] | 0 | -218.5 (-219.3) | -135.6 (-139.3) | -239.1 (-239.5) |
| | 1 | -218.6 (-217.8) | -135.6 (-187.0) | -236.5 (-240.8) |
| 17[b] | 0 | -213.1 ( - ) | -154.1 ( - ) | -238.8 ( - ) |
| | 1 | -212.2 ( - ) | -157.8 ( - ) | -238.5 ( - ) |
| 18[d] | 0 | -214.7 ( - ) | -150.1 ( - ) | -256.6 ( - ) |
| | 1 | -213.7 ( - ) | -162.9 ( - ) | -256.8 ( - ) |
| 19(b) | 0 | - (-220.3) | - (-151.0) | - (-258.4) |
| | 1 | - (-218.0) | - (-201.8) | - (-255.6) |

[a] $CH_3{}^{15}NO_2$ as external reference; [(b)] b 0.8 M; [(c)] 0.45 M; [(d)] d 0.5 M; [(e)] e 0.95 M; [(f)] 0.3 M.

*The values in parenthesis denote the chemical shifts in $CH_2Cl_2$.

[+]The superscripts in parenthesis denote the concentration of the substrate in $CH_2Cl_2$.

reaction of 4-nitrophenylsulphonyl ethene with uridine under a base catalized condition gave the $O^4$-(4-nitrophenylsulfonylethyl) derivative. A reexamination of the structure of this adduct by $^{15}N$-NMR spectroscopy has clearly shown that the compound produced in the above reaction is not the $O^4$-substituted derivative but the $N^3$-(4-nitrophenylsulfonylethyl)uridine (4) whose $^{15}N$-NMR data are shown in Table 1. It should be noted that if the $O^4$-substituted product were formed in the above reaction[7], we should have observed the $N^3$- nitrogen chemical shift very closely similar (ca. -150 to -155 ppm) to any other $O^4$-substituted compounds 9, 10, 12, 13 or 14. But this is not the case! The $N^3$ chemical shift of the reaction product[7] resembles more closely to that of a $sp^3$ hybridized nitrogen and, therefore, the structure of the product is $N^3$-(4-nitrophenylsulfonylethyl)uridine (4).

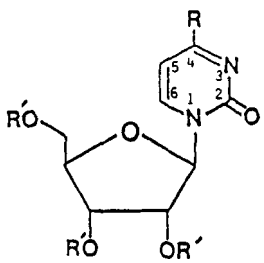


Protonation studies of $C^4$ substituted pyrimidines.

We envisioned that the basic character of the $N^3$ of a $C^4$ substituted pyrimidine nucleoside should be considerable influenced by the electronic properties of the $C^4$ substituent. We therefore decided to probe the basic characters of $N^3$ in the pyrimidines 8 - 19 by its ability to be protonated by a strong acid, trifluoroacetic acid (TFA), both in DMSO and in $CH_2Cl_2$.

As the chemical shifts of $N^3$ of $C^4$-substituted pyrimidines, the protonation of $N^3$ in these compounds is also mainly affected by the nature of the atom directly bonded to $C^4$. This implies that the lone pair of the $C^4$-heteroatom of the $C^4$-substituted pyrimidines is directly involved in the stabilization of the $(N^3H)^+$ (protonated pyrimidine) species. Thus, an addition of one equivalent of TFA to cytidine 7 in DMSO and to its derivative 8 in $CH_2Cl_2$ subjects the $N^3$ to an upfield shift by ca. 60 ppm. Clearly, the stabilization of the $(N^3H)^+$ in compounds 7 and 8 comes from the participation of the lone-pair of the $C^4$-$NH_2$ substituent. However, when the oxygen atom is linked to $C^4$, the $N^3$ is very weakly protonated as seen in compounds 10 - 14. The delocalization of the oxygen lone-pair is understandably less favoured than that of nitrogen lone-pair because of their respective electronegativities which is evident by the comparison of protonation shifts of $N^3$ in compounds 12 and 13 (ca. 6 ppm) with those of compounds 8, 15 and 16 (ca. 45 to 60 ppm) in $CH_2Cl_2$. A noticeable protonation of $N^3$ in compound

11 (ca. 17 ppm) can be explained by the larger polarisability of the sulfur atom than the oxygen atom. It is also clear that an amide function in compounds 15 - 19 does not stabilize the protonation of $N^3$ as much as the amino group. This can be understood by further delocalization of the nitrogen lone pair in the amide part. Although, the $N^3$ of $N^4$-amides in 15 and 16 do get easily protonated in $CH_2Cl_2$ (upfield shift of ca. 50 ppm) but the extent of the $N^3$ protonation in these compounds in DMSO solution is relatively small as seen in only a few ppm shift of the $N^3$ nitrogens; on the other hand the compound 8 shows protonation in both solvents (ca. 60 ppm).



7. R = Amino, R' = H
8. R = Amino, R' = Acetyl
9. R = 4-$CH_3C_6H_4O$- , R' = Acetyl
10. R = 4-$NO_2C_6H_4O$- , R' = Acetyl
11. R = Phenylthio, R' = Acetyl
12. R = Phenoxy, R' = Acetyl
13. R = Methoxy, R' = Acetyl
14. R = 4-Nitrophenylethoxy, R' = Acetyl
15. R = Acetamido, R' = Acetyl
16. R = Benzamido, R' = Acetyl
17. R = Benzamido, R' = H
18. R = 9-Fluorenylmethoxycarbonyl, R' = H
19. R = 9-Fluorenylmethoxycarbonyl, R' = Acetyl

Protonation studies of $C^6$ substituted purines.

It was considered natural to extend above studies to the $C^6$ substituted purine nucleosides because of the structural similarities of the -$C^6$=$N^1$- part in the purines in 20 - 27 with the -$C^4$=$N^3$- part in pyrimidines in 8 - 19 as far as the effect of the β substituent was concerned for the stabilization of the protonated $sp^2$ hybridized nitrogens $N^1$ and $N^3$ in purine and pyrimidine system respectively. Thus we have studied the effect of several $C^6$-substituents on the protonation of the respective $N^1$-nitrogen in several purine nucleosides (20 - 27). These studies have been also carried out both in neutral and acidic $CH_2Cl_2$ and in DMSO solution (Table 3). The $N^1$ in adenosine (20 or 21) experiences an upfield shift of ca. 60 ppm in both $CH_2Cl_2$ and in DMSO. We did not, how-

Table 3: $^{15}N$ chemical shifts[a] of some $C^6$-substituted purines in neutral and basic DMSO and/or $CH_2Cl_2$* solutions. T = 303 K.

| Compound | Equiv. of TFA | $N^1$ | $N^3$ | $N^7$ | $N^9$ | N-substituent |
|---|---|---|---|---|---|---|
| 20[b] | 0 | -145.3 ( - ) | -158.5 ( - ) | -140.3 ( - ) | -211.8 ( - ) | -299.3 ( - ) |
| | 1 | -205.9 ( - ) | -157.1 ( - ) | -138.0 ( - ) | -205.2 ( - ) | -292.2 ( - ) |
| 21[b] | 0 | -144.4 (-148.5) | -157.5 (-156.7) | -139.1 (-144.8) | -215.6 (-215.1) | -299.3 (-307.1) |
| | 1 | -203.4 (-215.1) | -157.5 (-156.6) | -137.2 (-141.2) | -209.9 (-209.5) | -291.6 (-294.0) |
| 22[c(c)] | 0 | -140.4 (-141.4) | -142.6 (-144.3) | -139.5 (-140.8) | -214.3 (-215.8) | - |
| | 1 | -140.4 (-140.8) | -142.7 (-145.1) | -140.4 ( d ) | -214.2 (-213.9) | - |
| 23[b(b)] | 0 | -112.8 (-113.8) | -140.2 (-142.0) | -139.1 (-139.5) | -214.3 (-216.3) | - |
| | 1 | -112.8 (-123.1) | -140.2 (-142.7) | -139.3 (-153.0) | -214.3 (-213.8) | - |
| 24[b(b)] | 0 | -137.0 (-138.4) | -138.9 (-140.5) | -139.8 (-140.4) | -213.6 (-215.5) | - |
| | 1 | -137.1 (-138.3) | -139.0 (-141.5) | -141.2 ( d ) | -213.6 (-213.6) | - |
| 25[e] | 0 | -120.9 ( - ) | -136.4 ( - ) | -136.4 ( - ) | -211.2 ( - ) | -247.9 ( - ) |
| | 1 | -128.8 ( - ) | -137.3 ( - ) | -153.2 ( - ) | -208.9 ( - ) | -248.0 ( - ) |
| 26[b(b)] | 0 | -120.2 (-129.0) | -134.9 (-139.2) | -136.7 (-140.0) | -215.7 (-215.9) | -249.3 (-252.3) |
| | 1 | -126.4 (-164.9) | -137.3 (-139.4) | -141.6 (-153.8) | -214.5 (-210.8) | -248.3 (-250.9) |
| 27[b(b)] | 0 | -125.7 (-133.2) | -138.9 (-141.8) | -136.9 (-143.7) | -215.1 (-215.4) | -242.9 (-244.1) |
| | 1 | -140.6 (-166.1) | -139.4 (-140.8) | -140.6 (-148.3) | -213.4 (-211.2) | -242.9 (-244.4) |

[a] $CH_3{}^{15}NO_2$ as external reference; [b] 0.5 M; [c] 0.8 M; [d] not found, too broad, [e] 0.6 M

*The values in parenthesis denote the chemical shifts in $CH_2Cl_2$

+The superscripts in parenthesis denote the concentration of the substrate in $CH_2Cl_2$

ever, observe any protonation of $N^1$ when the $C^6$-hetero-substituent was oxygen (22 and 24); on the other hand, a considerable $N^1$ protonation was observed when the $C^6$ hetero-substituent was sulfur as in 23. It was found that due to the possible protonation of $N^7$ in the purine derivatives, the magnitude of the $N^1$ protonation in purine ring system is smaller than the $N^3$ of pyrimidines.

A direct comparison of the $\Delta\delta\,^{15}N$ shifts $N^1$ of purine residues versus $N^3$ of pyrimidine residues either in $C^4/C^6$ substituted pyrimidines/purines or in $N^3/N^1$ substituted pyrimidines/purines is not possible because of the presence of the C-2 carbonyl group in pyrimidines and mainly because of the fused imidazole ring in purines. Fig. 1 illustrates a correlation of $\Delta\delta\,^{15}N$ shifts for each $C^6$- and $C^4$-substituted purine and pyrimidine nucleosides respectively in order to show that the

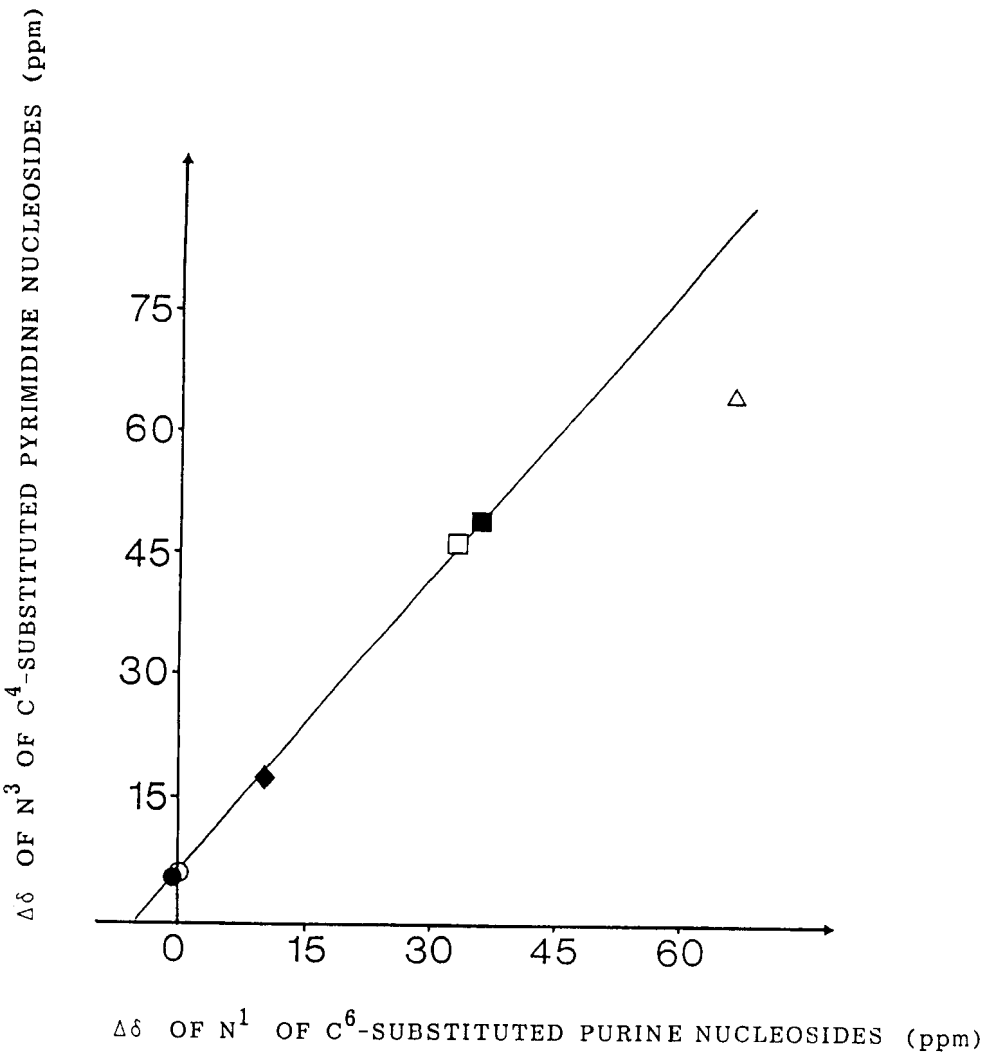



Fig. 1 : A plot of Δδ of $N^3$ of $C^4$-substituted pyrimidine nucleosides as a function of Δδ of $N^1$ of $C^6$-substituted purine nucleosides with common substituents in $CH_2Cl_2$ at 303 K.

( ● ) = phenoxy; ( ○ ) = methoxy; ( ◆ ) = phenylthio
( □ ) = acetamido; ( ■ ) = benzamido; ( △ ) = amino

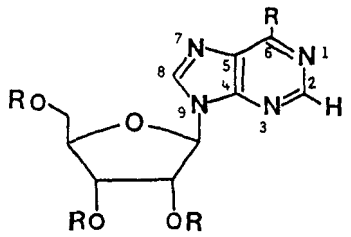
Δδ represents the magnitude of $^{15}$N chemical shift ( δ ) upon protonation.

equilibrium constants of $N^3H^+$ in pyrimidines and $N^1H^+$ in purines are comparable and, therefore, the electronic influence of the fused imidazole moiety acts as a constant factor. The derivatives containing amino groups, as in compounds 8 and 21, are only exceptions despite the fact that the $pK_a$s of adenosine (ca. 3.5) and cytidine (ca. 4.15) are closely comparable. It is likely that the discrepancies for $C^4$- and $C^6$-amino substituents in compounds 8 and 21 are due to partial protonation of the $-NH_2$ groups, as reflected in their $\Delta\delta\ ^{15}N$ shifts of -9.6 and -13.1 ppm in $CH_2Cl_2$ + TFA, besides the formation of $N^1H^+$ and $N^3H^+$ species, respectively. This explanation also receives support that the amino group in pyrimidines can be indeed protonated[8]. Morever, it is known that the acetylation of cytidine on $N^4$ is relatively easier than on $N^6$ of adenosine, under a mild acetylating condition (acetic anhydride in pyridine at 20 °C), revealing a weaker reactivity of the latter to electrophiles.

Thus, these data and all other previous $^{15}N$-NMR studies[2a-5] clearly suggest that the basicity and reactivity of $N^3$ of $C^4$-substituted pyrimidine nucleoside is quite comparable to that of the $N^1$ of the $C^6$-substituted purines nucleosides, despite the presence of the fused electron-rich imidazole part in the latter. This observation is particularly remarkable in view of the fact that any change of substituent (nature or position) or a change in the aromaticity of the pyrimidine ring, on the other hand, quite considerably influence the reactivity of $N^7$ of the imidazole part[2a-4].

## CONCLUSION

$^{15}N$ NMR spectroscopy has proved to be a very powerfull tool to understand the electronic distribution in nucleobases in purine or pyrimidine nucleosides. Any changes in the aromatic character of the pyrimidine ring in both pyrimidine and purine nucleosides leads to drastic changes in the $^{15}N$ chemical shifts of constituent nitrogens, and, therefore, an unambigous distinction between $C^4/N^3$ and $C^6/N^1$ substitution can be easily achieved. A comparison of the magnitude of the $^{15}N$ shift of $N^3$ in $C^4$-substituted pyrimidine or of $N^1$ in $C^6$ substituted purine nucleosides, upon protonation with $CF_3COOH$ in DMSO and $CH_2Cl_2$ solutions, has shown that the basic character of $N^3$ (or of $N^1$) depends upon the nature and



20. R = Amino, R' = H
21. R = Amino, R' = Acetyl
22. R = Methoxy, R' = Acetyl
23. R = Phenylthio, R' = Acetyl
24. R = Phenoxy, R' = Acetyl
25. R = Benzamido, R' = H
26. R = Benzamido, R' = Acetyl
27. R = Acetamido, R' = Acetyl



the electronic properties of the atom linked to $C^4$ (or $C^6$). Thus the effect of a protecting group on the reactivity of each nitrogen of a nucleobase in a nucleoside can be easily estimated by a $^{15}$N NMR spectroscopy.

## EXPERIMENTAL

$^{15}$N chemical shift determinations were made on a Jeol GX 270 spectrometer at 27.4 MHz. All $^{15}$N-NMR spectra were performed relative to $CH_3{}^{15}NO_2$ in $CD_3NO_2$ in a capillary. The probe temperature was around 30°C. The assignments of $^{15}$N resonances were done by fully proton decoupled condition (NOE) or under an inverse gated proton-noise decoupled mode (without NOE), or using the polarization-transfer pulse sequences INEPT or DEPT. Routinely 16 K data points were used for the acquisition, zero filled to 32 K and Fourier transformed with a broadening factor of 2-3 Hz. The samples were dissolved in distilled $CH_2Cl_2$ or in distilled DMSO. A negative value for the chemical shift denotes an upfield shift.

It nas been observed that the compound 16 has a poor solubility in DMSO and especially in $CH_2Cl_2$; some drops of methanol were therefore added to make a clear $CH_2Cl_2$ solution. Furthermore 16 is not stable in acidic media where a migration of the benzoyl group from $O^4$ to $N^3$ took place[9]. Consequently the accuracy of $^{15}N$ chemical shift for 16 is not as good as for the other compounds.

The nucleosides analogues were prepared according to reported procedures[10-16].

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