Tetrahedron Vol. 42, No. 18, pp. 5073 to 5080, 1986
Printed in Great Britain.

0040-4020/86 $3.00+.00

THE USE OF ^{15}N-NMR SPECTROSCOPY FOR ASSIGNING THE STRUCTURES OF ISOMERIC N^7- AND N^9-SUBSTITUTED PURINES.

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(*Received in UK* 3 *July* 1986)

Abstract: An ^{15}N-NMR study has shown a considerable difference in electronic structures between the isomeric N^7 and N^9 substituted purines. A comparison of ^{15}N chemical shifts amongst the seven pairs of N^7 and N^9 isomers has revealed that (1) the N^3 resonances are shielded by 18 - 20 ppm in all N^9 isomers; (2) the amino nitrogens at the C-2 or at C-6 positions are always shielded by 3 - 4 ppm in the N^7 isomers; (3) the N^7 chemical shifts in the N^7 isomers are always more shielded by 6 - 7 ppm than the N^9 resonances in the N^9 isomers. It has a also been found by protonation studies that the N^9 of the N^7 isomer is much more basic than the N^7 of the N^9 isomer.

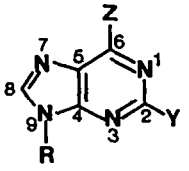
An unequivocal characterization of the N^7 and the N^9 isomers of purines that arise after a condensation reaction of the aglycone with an alkyl or aryl halides or a halosugar, is complex and can yield uncertain results if one of the isomer has not been previously synthesized through an unambiguous route and subsequently spectroscopically characterized[1]. Mostly, it is the N^7 isomer which is synthesized for this purpose by alkylation of guanosine or 2'-deoxyguanosine followed by a deglycosylation step under an acidic condition. Such careful comparison of spectroscopic properties (^{1}H, ^{13}C and UV) between authentic N^7 isomer with that of the N^9 isomer has allowed unambiguous characterization of the latter[2]. ^{1}H- and ^{13}C-NMR studies on pairs of N^7 and N^9 isomers of substituted purines have shown that certain chemical shifts can be used for an unambiguous assignments of the regioisomers[17]. We herein report a study on the ^{15}N-NMR spectroscopy of seven pairs of authentic N^7 and N^9 isomers of substituted purines. This study showed that carefully assigned ^{15}N resonances in these N^7 and N^9 isomers may indeed assist in understanding the differences in electronic properties in a pair of the N^7 and N^9 isomers of purines, which leads to unambiguous assignments of their respective structures.

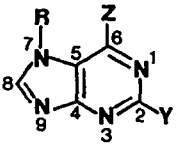
Assignments of the ^{15}N resonances in the N^7 and N^9 isomers

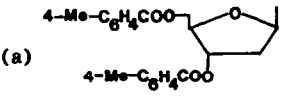
The chemical shifts of different nitrogens and their assignments are shown in Table 1. There are three kinds of nitrogens in amino-purines: the amino nitrogen, the "azine- or pyridine-like"

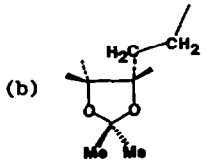
nitrogen and the "pyrrole-like" nitrogen. Each of these types of nitrogens have their own characterestic chemical shifts; for example, amino nitrogens absorb at a higher field than the ring nitrogens and the "N-pyrrole" absorb at a higher field than the "N-azine" type nitrogens[3].

Similarly, these three categories of nitrogens also have different ^{15}N-^{1}H coupling constants. Both the nitrogen atoms of the imidazole part have a scalar coupling with H-8 but the coupling constant between the "N-azine" and H-8 is always larger (10-12 Hz) than that of "N-pyrrole" and H-8 (7-9 Hz)[3,4,5] which makes it convenient to assign the N^7 and N^9 atoms from the proton coupled spectra of the N^7 and N^9 isomers. However, it is difficult to assign the N^1 and N^3 correctly in a C-2 and C-6 disubstituted compounds. In such cases, effects of substituents at C-2 and C-6 on N^1 and N^3 chemical shifts have to be carefully monitored in order to elucidate the substituent increments. In this work, N^1 and N^3 chemical shifts have been monitored with -Cl and $-NH_2$ as substituents with respect to the parent 9--methylpurine (1) and 7-methylpurine (2) in DMSO[6]. The introduction of the -Cl atom at the C^6 position, as in 5, shields the N^1 and N^3 by 3.2 and 0.4 ppm respectively in comparison with 1. Städeli and coworkers[7] have observed a shielding of 3.7 and 5.6 ppm for N^1 and N^3 respectively due to -Cl substitution at the C^4 of pyrimidines. It is possible that the lower shielding of N^1 and N^3 that we observe in the purine system is because of a larger delocalization of the chlorine lone pair in purine ring than in the pyrimidine. The variation of the chemical shifts of the sp^2 hybridized nitrogens has been reported[8,9] to be dependent upon the changes in the medium. We observed that the N^3 and N^9 resonances in 5 were deshielded by 2.5 ppm upon changing the solvent from chloroform to DMSO while the chemical shifts of the N^1 and N^7 did not change. Since the compounds discussed in this paper are neither acidic nor basic in a neutral medium, it is, therefore, assumed that the solvent-induced shift and the concentration effects are minimized. The effect of the $-NH_2$ group at C-2 position on N^1 and N^3 chemical shifts have been elucidated by comparing their resonances in compounds 1 and 7. Such a comparison clarified that the N^1 and N^3 are shielded by 37 and 50 ppm respectively due to the $-NH_2$ group at the C-2 position. A similar shielding of the N^3 was observed in guanosine as compared to inosine[10] and in other 2-amino substituted pyrimidines[11].









(c) $AcO-(CH_2)_3-CH_2-$

(d) $H_3C-(CH_2)_3-CH_2-$

1: R = Me, Y = Z = H

2: R = Me, Y = Z = H

3: R = Et, Y = H, Z = NH_2

4: R = Et, Y =H, Z = NH_2

5: R = (a), Y = H, Z = Cl

6: R = (a), Y = H, Z = Cl

7: R = (b), Y = NH_2, Z = H

8: R = (b), Y = NH_2, Z = H

9: R = (c), Y = NH_2, Z = Cl

10: R = (c), Y = NH_2, Z = Cl

11: R = (d), Y = NH_2, Z = Cl

12: R = (d), Y = NH_2, Z = Cl

13: R = (b), Y = NH_2, Z = Cl

14: R = (b), Y = NH_2, Z = Cl

The assignments of ^{15}N resonances in authentic compounds of all N^7 and N^9 isomers were achieved through their ^{15}N-^1H coupled spectra using a INEPT pulse sequence[6,12] as shown, for example, for a pair 5 and 6 (Fig. 1). It is relatively simple to assign N^1 vs. N^7 (N^9) in compounds 9 to 14 through their INEPT spectra because the N^7 (N^9) appears as a doublet.
On the other hand, in the INEPT experiment of compounds 5 to 8, both N^1 and N^7 (N^9) appear as doublets , but the $^2J_{N^1,H^6(H^2)}$ has a larger coupling constant[3,6,11] than the $^2J_{N^7(N^9),H^8}$.

The amino group at the C^6 position, as in 3, shields the N^1 and N^3 by 42 and 28 ppm. A similar set of resonances are also found in adenosine and 2'-deoxyadenosine[10]. It thus becomes apparent that the nature of the substituent at the N^7 or N^9 influences the chemical shifts of N^1 and N^3 very slightly (ca. 2 ppm).

Previously, we have shown[13] with a series of C^2-(para-t-butylbenzamido)-C^6-substituted purine-N^9-ribosides that the N^3 chemical shifts do not noticeably change with the change of the C^6 substituent. Thus, for the para-t-butylbenzamido- group at the C-2 position, the N^3 resonates at ca. -160 ppm. The effect of para-t-butylbenzamido- group compared to $-NH_2$ at the C-2 position is a deshielding of the N^3 by 20 ppm[13]. Therefore, the N^3 in 2-aminopurine is expected to have a chemical shift of ca. -180 ppm for the N^9 isomers. This value should be corrected by the shielding contribution of the -Cl atom at the C^6 position. In fact, the N^3 resonance in compounds 9, 11 and 13 has chemical shifts between 179.8 - 182.9 ppm. Similarly, upon calculation of the effect of substituent increments of the -Cl and $-NH_2$ groups at the C-6 and C-2 respectively on the N^1 resonance, one arrives at a theoretical value of ca. -143 - ppm while the N^1 chemical shifts in compounds 9, 11 and 13 are around -146 ppm. This difference in values can be partly explained by the nature of substituents at N^9, solvent and concentration effect. The ^{15}N resonances of the N^7 isomers were similarly assigned.

Important differences in the ^{15}N chemical shifts in the N^7 and N^9 isomers

A perusal of Table 1 clearly shows that a distinction between an N^7 and an N^9 isomer is possible on the basis of the following general observations which is based on the study of seven pairs of isomers in DMSO solution:

(1) the N^3 resonance is shielded by 18 - 20 ppm in the N^9 isomer;
(2) the amino nitrogen at the C-2 or at C-6 is always shielded by 3 - 4 ppm in the N^7 isomer;
(3) the N^7 chemical shift in the N^7 isomer is always more shielded by 6 - 7 ppm than the N^9 resonance in the N^9 isomer.

Since above observations are independent of the N^7 and N^9 substituents, we attribute these differences of nitrogen resonances between the N^7 and N^9 isomers to a different "building" of the purine ring.

It thus clearly appears that it is possible to arrive at the structures unambiguously for any unknown pair of the N^7 and N^9 isomers of purines which are substituted either by $-NH_2$ and/ or -Cl groups.

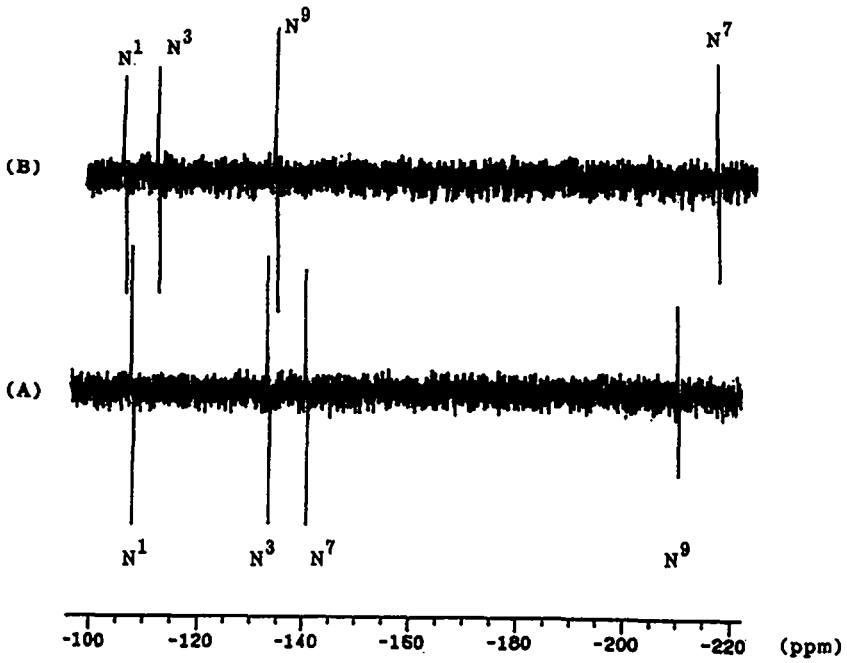


Figure 1. INEPT spectra of compound 5 (A) and 6 (B) in chloroform.

Protonation study

It is clear from the literature[4,6,10,13,14] that the N^7 vs. N^1 activation in the N^9 substituted purines is controlled by the nature of the substituent at the C-2 and C-6 positions. An enhanced N^7 reactivity of guanosine, with respect to 9-substituted-2-aminopurine, is clearly due to the non-aromatic nature of the pyrimidine ring in the former which aids in a direct conjugation of the C-2 amino function to the N^7. Recently, we have shown[13] by ^{15}N-NMR study that the activated nature of the N^7 of guanosine is, indeed, trimmed by (i) derivatizing the $-NH_2$ to an amide and also (ii) by locking the lactam of the guanine moiety to the aromatic O^6-alkyl/aryl derivative. These conclusions were based on the estimation of the difference of the N^7 chemical shift in the neutral and in the protonated medium. The change of ^{15}N chemical shifts of compounds 13 and 14, upon gradual protonation, are shown in Tables 2 and 3 respectively while the figures 2 and 3 show the evolution of each purine-nitrogen as a function of the number of equivalents of trifluoroacetic acid (TFA) added. The N^7 of the N^9 isomer 13, upon addition of one equivalent of TFA, moves upfield by 10 ppm due to protonation while the N^3 and N^9 remain at the same position but the N^1 shifts downfield by 0.4 ppm (Fig. 2).

The results from the protonation study with the N^7 isomer 14 is rather restricted because it started decomposing after the addition of 0.4 equivalent of TFA. However, our limited study in

Table 3 shows that the N^9 is predominantly protonated, with an upfield shift of 26.1 ppm upon addition of 0.4 equiv. of TFA. The protonation of the N^3 is also evident by 8.2 ppm shielding upon addition of 0.4 equiv. of TFA. It seem to be clear that the N^7 and N^3 of the N^7 substituted purines have an enhanced basicity compared to the corresponding N^9 isomer. It appears that the N^9 in the N^7 isomer 14 has a comparable tendency to protonation as the N^7 of guanosine or N^1 of adenosine. A perusal of the literature clarifies[4,13,14] that an acid titration curve for a strongly protonated nitrogen reaches a platau after one equivalent of the acid (TFA) added. We, therefore, extrapolate (Fig. 3) our protonation data with 14 and arrive at a conclusion that the N^9 of 14 should move upfield by 70 - 80 ppm and N^3 by 20 ppm upon addition of one equiv. of the TFA (Fig. 3). This basicity of the N^3 and N^9 atoms of the N^7 isomer 14 can be explained by an absence of appreciable delocalization of the N^3 and N^9 lone pairs. It is, therefore, clear that the delocalization of the π electron-deficient

Table 1 ^{15}N-NMR shifts of some N^7 and N^9 substituted purine derivatives

Compound	N^1	N^3	N^7	N^9	NH_2
1[a]	-115.5	-138.9	-151.2	-250.3	-
2[a]	-114.7	-122.9	-235.6	-149.2	
1[b]	-103.4	-130.0	-140.8	-230.8	
2[b]	-102.3	-109.4	-237.6	-137.0	
3[c]	-147.0	-156.8	-142.2	-216.2	-301.1
4[c]	-144.8	-138.5	-222.8	-137.6	-303.2
5[d]	-107.3	-132.8	-140.1	-210.0	-
5[e]	-106.6	-130.4	-139.5	-207.2	-
6[d]	-105.7	-112.2	-217.4	-134.2	-
7[f]	-139.8	-179.8	-140.2	-225.0	-300.6
8[f]	-141.1	-160.6	-230.0	-138.4	-303.4
9[g]	-146.4	-182.6	-141.4	-221.1	-298.4
10[h]	-143.3	-163.8	-227.8	-141.0	-301.3
11[i]	-146.5	-182.5	-141.6	-220.6	-298.5
12[j]	-143.1	-163.7	-227.3	-141.2	-302.2
13[i]	-146.9	-182.9	-141.6	-222.3	-298.4
14[i]	-143.8	-164.3	-229.0	-141.5	-301.4

[a]in water (ref. 4); [b]in DMSO (ref. 6); [c]in DMSO (ref. 14); [d]in $CHCl_3$ (0.2 M); [e]in DMSO (0.2 M); [f]in DMSO (0.6 M); [g]in DMSO (0.7 M); [h]in DMSO (0.4 M); [i]in DMSO (0.8 M); [j]in DMSO (0.1 M);

pyrimidine and the π electron-rich imidazole system is much constrained in the N^7 isomer than in the corresponding N^9 isomer, however the magnitude of this difference is controlled by the nature of the C-2 and C-6 substituent(s).

Table 2. Study of the ^{15}N chemical shifts of compound 13 upon protonation with trifluoroacetic acid (TFA) at 303 K.

Acid (equiv.)	N^1	N^3	N^7	N^9	NH_2
0	-146.9	-182.7	-141.6	-222.3	-298.4
0.2	-146.3	-182.6	-143.6	-222.0	-298.3
0.6	-145.9	-182.7	-147.4	-221.8	-298.0
1.5	-145.1	-182.9	-155.4	-221.2	-297.1
2.2	-144.4	-183.2	-162.2	-220.8	-297.1

Table 3. Study of the ^{15}N chemical shifts of compound 14 upon protonation with trifluoroacetic acid (TFA) at 303 K.

Acid (equiv.)	N^1	N^3	N^7	N^9	NH_2
0	-143.8	-164.3	-229.0	-141.5	-301.4
0.1	-143.2	-166.1	-228.1	-146.0	-300.7
0.2	-142.7	-168.8	-227.2	-151.2	-300.1
0.3	-142.2	-171.4	-226.0	-158.6	-
0.4	-141.6	-172.5	-225.1	-167.6	-300.2

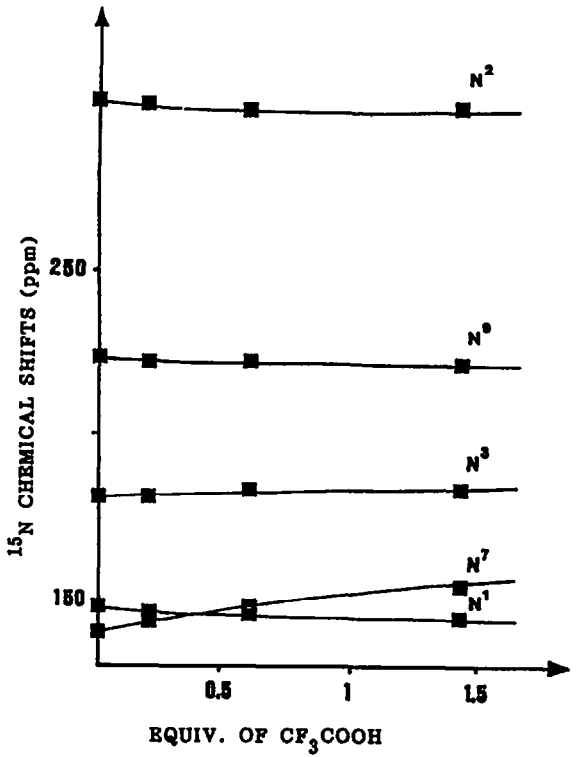


Figure 2. Dependence of ^{15}N chemical shifts (absolute values) with number of equiv. of trifluoroacetic acid at 303 K for the N^9 isomer 13.

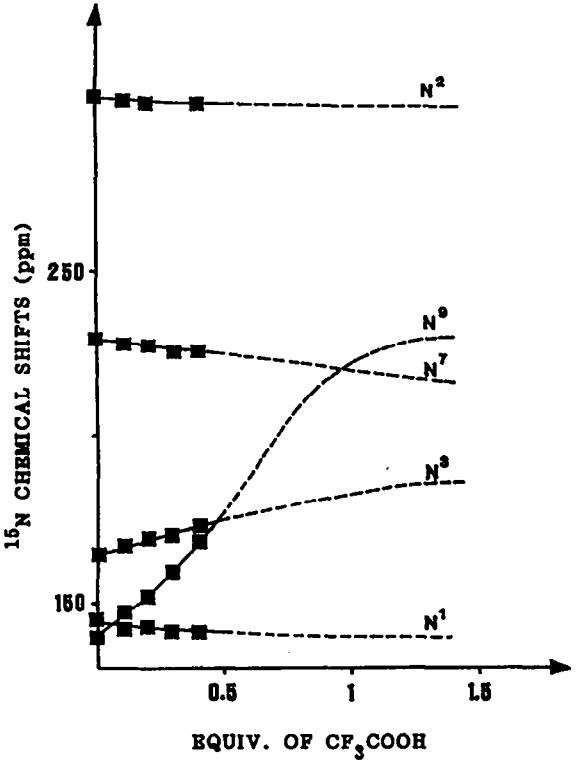


Figure 3. Dependence of ^{15}N chemical shifts (absolute values) with number of equiv. of trifluoroacetic acid at 303 K for the N^7 isomer 14.

——— observed data

- - - extrapolated data from the expected behaviour of a strongly protonated nitrogen (ref. 4, 13 & 14).

Experimental section

All ^{15}N NMR spectra were recorded on Jeol-GX-270 spectrometer at 27.4 MHz with a probe head for 10 mm o.d. sample tubes. The ^{15}N chemical shifts were determined from proton decoupled spectra while INEPT experiments served to assign unambigously each resonance (see text). The chemical shifts (± 0.1 ppm) were measured relative to an external solution of $CH_3{}^{15}NO_2$ (5 x 10^{-3} ml) in CD_3NO_2 (0.35 ml) to provide the reference and the frequency lock respectively. The decoupled spectra with NOE suppressed were recorded with 13 µs of pulse width (45° pluse angle), 1 s acquistition time for 16 K data points, zero filled to 32 K and Fourier transformed with a broadening factor of 2-3 Hz. Useful spectra are obtained with accumulation time of 2-15 h depending on the concentration (Table 1). For the INEPT pulse sequence, ^{1}H -90° = 59 µs, ^{15}N -90° = 26 µs, with $\tau = 1/4\ J_{N,H}$ set at 23 ms and a pulse delay of 2 s a useful spectrum could be obtained in 20 min to 4 h. The spectral range was 9400 Hz involving a digital resolution of 0.6 Hz (0.02 ppm). The probe temperature was around 30° C for all experiments. A negative value for the chemical shift denotes an upfield shift.

The compounds used in this study were prepared according to reported procedures: 6[15], 7 - 14[16].

Acknowledgements

Authors gratefully acknowledge the funding by Wallenbergstiftelsen for the purchase of a Jeol 270 MHz FT NMR spectrometer. Financial supports from Swedish Board for Technical Development and Swedish Natural Science Research Council are also acknowledged. Authors also thank Ingegärd Schiller for the secretarial assistance.

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