MAGNETIC RESONANCE IN CHEMISTRY, VOL. 36, 442–444 (1998)

Note

^{13}C and ^{15}N NMR Chemical Shift Assignments of *N*-1-(2-Azidoethyl)-4-*R*-pyrimidin-2-ones by ^{1}H,X HMQ(B)C with *z*-Gradient Selection

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Received 30 October 1997; revised 16 January 1998; accepted 27 January 1998

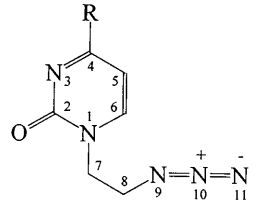
ABSTRACT: ^{13}C and ^{15}N NMR chemical shift assignments based on *z*-gradient selected ^{1}H,X (X = ^{13}C and ^{15}N) HMQC and HMBC experiments are reported for *N*-1-(2-azidoethyl)pyrimidin-2-one (ring system of cytosine), its five 4-R derivatives [where R = NH_2, OCH_3, $N(CH_2)_4$, $NHCH_2CH(CH_3)_2$ and $N(CH_3)_2$] and 2-azidoethyl tosylate. The possibilities of detecting all nitrogens in these molecules containing (i) an azido group at N-1 and (ii) an electronegative substituent at C-4 are limited. First, the terminal nitrogen of the azido group is difficult to observe because the nearest proton (in a CH_2 group) is located four bonds away from it. Second, in contrast to N-1, N-3 in *N*-1-(2-azido-ethyl)-4-pyrimidin-2-ones remained undetected. For that reason, an unsubstituted derivative (R = H) was also prepared, where N-3 was easily observed. © 1998 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; ^{15}N NMR; gradient selection; heterocycles; *N*-1-(2-azidoethyl)-4-R-pyrimidin-2-ones

INTRODUCTION

Although ^{15}N NMR chemical shifts of some azides are known,[1–3] as recently reviewed by Berger *et al.*,[4] there is continuing interest in the chemistry of the azido moiety owing to its unique and partly unknown characteristics.[4–7] First, an erroneous suggestion of a cyclic azide intermediate in the reaction between hydrazine and nitrous acid forming hydrazoic acid has been discarded, based on a ^{15}N NMR study using ^{15}N-labelled hydrazine as a starting material.[5] Second, iminodisulfurylazide containing an azide covalently bound to nitrogen has recently been identified and characterized by ^{14}N and ^{19}F NMR.[6] Third, azido-substituted pyridines, which are known to have a large variety of industrial uses, show very interesting valence tautomeric equilibria determined by multinuclear (^{1}H, ^{13}C and isotope-enriched ^{15}N) magnetic resonance studies.[7]

Substitution of pyrimidin-2-one (a structural fragment in nucleosides and nucleotides) with an azido group can provide very promising structures from the biochemical point of view. The aim of this work was to characterize the novel *N*-1-(2-azidoethyl)-4-R-pyrimidin-2-ones **1**–**6** at natural abundance by proton-detected (inverse) 2 D NMR experiments. As mentioned above, the ^{15}N NMR chemical shifts of these substances are especially useful parameters in estimating the structural and electronic properties which predominantly determine their non-covalent interactions and biological activities.



	R
1	NH_2
2	OCH_3
3	$N(CH_2)_4$
4	$NHCH_2CH(CH_3)_2$
5	$N(CH_3)_2$
6	H

RESULTS AND DISCUSSION

The assignments of ^{1}H, ^{13}C and ^{15}N NMR chemical shifts (Table 1) of **1**–**6** is based on ^{1}H,X HMQC[8] and ^{1}H,X HMBC[9] experiments with *z*-gradient selection and some reference data.[1–5,10,11]

The observed ^{15}N NMR chemical shifts are in agreement with those for a 0.5 M DMSO solution of *N*-1-ribitylcytosine,[10] $\delta(^{15}N\text{-}1) = -229.9$, $\delta(^{15}N\text{-}3) = -172.3$ and $\delta(^{15}NH_2) = -289.0$ ppm; azidobenzene, $\delta(^{15}N\text{-}1\text{-azido}) = -288.5$, $\delta(^{15}N\text{-}2\text{-azido}) = -136.7$ and $\delta(^{15}N\text{-}3\text{-azido}) = -147.4$ ppm; and hydrazoic acid,[5] $\delta(^{15}N\text{-}1\text{-azido}) = -280$ and $\delta(^{15}N\text{-}2\text{-azido}) = -130$ ppm. One of the starting materials, 2-azidoethyl tosylate, gave two cross peaks at -311.5 and -132.1 ppm, confirming the above assignments of the azidoethyl moiety.

The polarization transfer between H-5 and N-3 via ^{3}J(H-5, N-3) is not effective in the ^{1}H,^{15}N HMBC

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Contract/grant sponsor: Academy of Finland; *Contract/grant number*: 8581.

CCC 0749-1581/98/060442–03 $17.50

Table 1. $\delta(^{15}N)$ (ppm from external CH_3NO_2), $\delta(^{1}H)$ (ppm from internal TMS) and $\delta(^{13}C)$ (ppm from internal TMS) of *N*-1-(2-azidoethyl)-4-R-pyrimidin-2-ones **1–6** measured in saturated DMSO-d_6 solutions at 30 °C

		$\delta(^{15}N)$ (ppm)					
Compound	4-R	N-1	N-3	N-9	N-10	N-11	*N*-Amino
1	NH_2	−238.3	—[a]	−310.9	−131.5	—[b]	−286.9
2	OCH_3	−229.5	—[a]	−311.2	−131.9	—[b]	—
3	$N(CH_2)_4$	−238.1	—[a]	−311.4	−131.7	—[b]	−270.2
4	$NHCH_2CH(CH_3)_2$	−240.5	—[a]	−310.9	−131.7	—[b]	−278.6
5	$N(CH_3)_2$	−238.3	—[a]	−311.1	−131.8	—[b]	−297.4
6	H	−217.5	−96.0	−311.4	−132.2	—[b]	—
		$\delta(^{1}H)$ (ppm)					
		H-4	H-5	H-6	H-7	H-8	R
1	NH_2	—	5.67	7.55	3.81	3.56	3.56 (NH_2)
2	OCH_3	—	6.01	7.96	3.96	3.64	3.82 (CH_3)
3	$N(CH_2)_4$	—	5.81	7.65	3.84	3.58	—[c]
4	$NHCH_2CH(CH_3)_2$	—	5.72	7.48	3.79	3.55	3.30 (NH)[d]
5	$N(CH_3)_2$	—	7.00	7.66	3.84	3.58	3.02 (CH_3)
6	H	8.57	6.45	8.18	4.04	3.70	—
		$\delta(^{13}C)$ (ppm)					
		C-2	C-4	C-5	C-6	C-7	C-8	R
1	NH_2	155.6	166.1	93.4	146.2	48.2	49.2	—
2	OCH_3	155.2	171.3	94.1	149.3	48.5	48.7	53.6
3	$N(CH_2)_4$	154.9	161.4	92.0	146.0	48.0	49.2	—[e]
4	$NHCH_2CH(CH_3)_2$	155.6	164.1	94.0	144.8	48.1	49.2	—[f]
5	$N(CH_3)_2$	154.7	163.6	90.7	146.2	48.0	49.1	—[g]
6	H	155.4	166.4	103.7	150.1	48.3	49.9	—

[a] ^3J(H-5,N-3) is too small to transfer polarization in $^1H,^{15}N$ HMBC.
[b] ^4J(H-8,N-11) is too small to transfer polarization in $^1H,^{15}N$ HMBC.
[c] δ(H-9) = 3.42, δ(H-10) = 1.93, δ(H-11) = 1.85 and δ(H-12) = 3.37 ppm.
[d] δ(H-9) = 3.07, δ(H-10) = 1.80 and δ(H-11/12) = 0.88 ppm.
[e] δ(C-9) = 46.2, δ(C-10) = 25.0, δ(C-11) = 24.1 and δ(C-11) = 46.3 ppm.
[f] δ(C-9) = 47.2, δ(C-10) = 27.4 and δ(C-11/12) = 20.0 ppm.
[g] δ(C-9) = 36.4 ppm.

experiment because a vicinal coupling constant in unsaturated systems containing an electronegative substituent on the coupling route is only −1 to −2 Hz.[11] Although the delay for the polarization transfer was increased to 200 ms, no correlation peak in the HMBC map was detected. In order to measure $\delta(^{15}N\text{-}3)$, an unsubstituted derivative, *N*-1-(2-azidoethyl)pyrimidin-2-one (**6**), was prepared. It gave a clear cross peak at −96.0 ppm. This value differs considerably, however, from that of *N*-1-ribityl cytosine, δ(N-3) = −172.3 ppm.[10]

The above finding is in agreement with the known substituent effects of amino and alkoxy groups on this type of nitrogen.[11]

In conclusion, by varying substitution at C-4 one can affect the electronic properties of the nitrogens in the pyrimidin-2-one ring. This finding may be significant regarding the non-covalent interactions which are responsible for biochemical activity such as enzymatic catalysis in living organisms.

EXPERIMENTAL

Compounds

Compounds **1–6** were synthesized by reaction of pyrimidin-2-one with 2-azidoethyl tosylate. Details concerning the synthetic procedures will be published separately.[12] The structures of the **1–6** were verified by fast atom bombardment (FAB) mass spectrometry and elemental analyses.[12]

Spectra

All ^1H and ^{13}C NMR spectra and $^1H,^{13}C$ HMQ(B)C and $^1H,^{15}N$ HMQ(B)C contour maps were measured at natural abundance for saturated DMSO-d_6 solutions at 30 °C with a Bruker Avance DPX250 spectrometer

equipped with a 5 mm inverse broadband probehead using *z*-gradient selection. The pulse sequences inv4gs for HMQC[8] and inv4gslplrnd for HMBC[9] in XWIN-NMR software (release 1.3) were applied, using a 50 ms delay in $^{1}H,^{13}C$ HMBC and 100 or 200 ms delays in $^{1}H,^{15}N$ HMBC to transfer polarization between protons and heteronuclei. The gradient program used three sine form gradients in the ratio 5:2:4 for C-13 and 7:3:5 for N-15.

Elemental analyses were performed on a Perkin-Elmer 240-C automatic analyzer. FAB mass spectra were measured using a ZAB-EQ mass spectrometer (VG Analytical, Manchester, UK).

Acknowledgements

Mr Reijo Kauppinen is acknowledged for his help in measuring the NMR spectra. E.K. is grateful to the Academy of Finland for financial support in the form of grant No. 8581.

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