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Research Article

NMR spectra of the tautomeric mixture of two forms of 6-azidopurine ribonucleoside labeled with ^{15}N

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Abstract: 6-Azidopurine nucleoside labeled with ^{15}N at N-1 position was synthesized. ^{15}N NMR spectra, ^{15}N-^{1}H and ^{15}N-^{13}C coupling constants were measured. Two well-separated sets of signals for two tautomeric forms were detected. Copyright © 2007 John Wiley & Sons, Ltd.

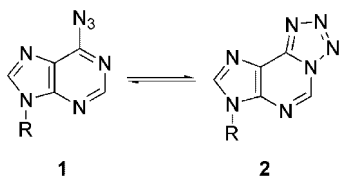
Keywords: ^{15}N NMR; azidopurine; tautomers

Introduction

Azidopurine nucleotides of general formula **1** easily and efficiently give photocrosslinking products and were long ago proposed[1] as photoaffinity labels for studies of protein-DNA/RNA and RNA/RNA interactions.[2–4] They can also potentially posses useful biological activity and for example 9-(β-D-arabinofuranosyl)-6-azidopurine was proposed as a prodrug for antiviral ara-A.[5]

Apart from its photochemical and biological activity, 6-azidopurine (as well as 2-azidopurine) system shows interesting feature of existing in two possible tautomeric forms[6] of azido-azomethine **1** and fused tetrazole **2** structure (Scheme 1).

The two forms exist in equilibrium, and their interconversion is slow enough to enable studying them with spectroscopical methods. Extensive studies[6,7] of this phenomenon were performed, leading to the conclusion that in polar solvents equilibrium is shifted towards tetrazole form, and that electron-withdrawing substituents in the pyrimidine ring favor the azido form.[8] Crystallographic data indicate, that 6-azidopurine exists in solid state as pure tetrazole form.[9]



1 2

Scheme 1

Equilibrium was studied with the use of different methods, ^{1}H NMR and ^{13}C NMR being the methods of choice.[10–12] The ^{15}N NMR spectroscopy, apparently the best natural candidate for this study suffers from low intensity of signals caused by intrinsic properties of nitrogen-15 nucleus as well as from low natural abundance of the isotope. Practically highly concentrated solutions or neat liquids are only suitable for reasonable experiments. For that reason only limited ^{15}N NMR studies of related systems were published.[13] To date no ^{15}N NMR data for the compound were available.

We have synthesized ^{15}N-labeled 6-azidopurine for mechanistic studies of its photochemical transformations,[14] and measured its ^{15}N NMR spectra.

Results and discussion

Synthesis

The synthesis exploited the described route[15] for N-1 labeling of triacetylinosine **3** (Scheme 2). Initial steps of the synthesis were conducted essentially via the published procedure, the only difference being that the intermediate product, N1-nitro-2′3′5′-tri-O-acetylinosine **4** was purified by chromatography. The 1-[^{15}N]-2′,3′,5′-tri-O-acetylinosine **5** was converted into 6-(pyridinium)salt **6** and subjected to substitution with sodium azide,[16] giving the product **7(8)** with 88% yield.

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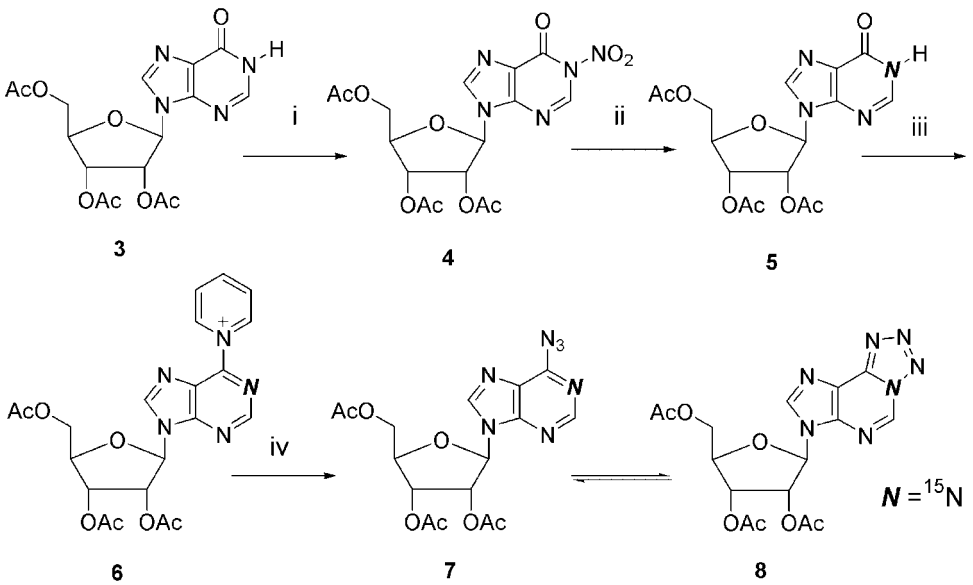

Scheme 2 Reaction conditions i– iii.[16] *p*-Cl-(C_6H_4)-OP(O)Cl_2, pyridine; iv NaN_3, DMF, 10 min.

The ^1H and ^{13}C NMR spectra agree with the published data[17–19] for the unlabeled compound.

Having the N-1 labeled compound gave the excellent opportunity to study the equilibrium with ^{15}N NMR spectroscopy. Nitrogen-15 resonance signals were well separated (difference of 2 ppm) and the assignment was straightforward on the basis of their different ^{15}N-^1H coupling constant.[20] Nitrogen nuclei of azido form **7** exhibited grater two-bond coupling constant with neighboring protons than nitrogens of tetrazolo form **8** (Figure 1).[20]

As expected, in relatively non-polar chloroform both forms **7** and **8** could be observed in the ratio of 3:2, respectively. In the ^{13}C NMR spectrum both carbon

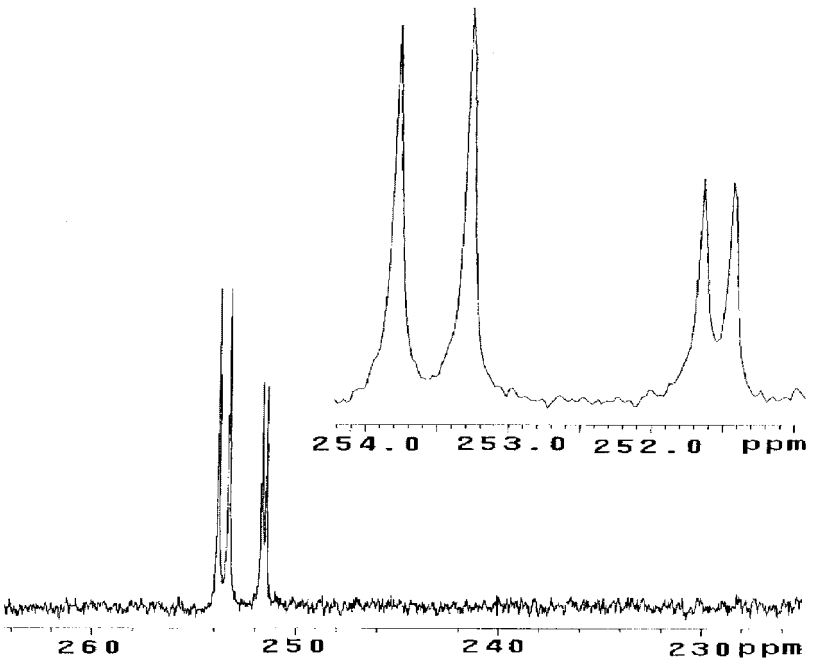

Figure 1 ^{15}N NMR spectrum of the tautomeric mixture of **7** and **8** in $CDCl_3$. N-1 signal at 253.5 ppm ($J^2_{N\text{-}H}$=15.1 Hz) belongs to **7**, the one at 251.5 ppm ($J^2_{N\text{-}H}$=6.3 Hz) belongs to **8**.

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atoms bonded with the labeled nitrogen atom showed coupling (see Table 1). In methanol solution only tetrazolo form was detected.

Except the labeled N-1 resonance signal, we were able to detect and assign two other nitrogen resonances in 2D correlation ^{15}N -^{1}H spectrum. The H-8 signals of both tautomers exhibited cross-peaks with neighboring N-7 and N-9 atoms (Figure 2).

Due to lower intensity unambiguous assignment of the remaining four nitrogen signals (N-3 and azido/tetrazolo atoms) was not possible. All the ^{13}C resonances were assigned for both tautomers (Table 1).

Experimental

The NMR spectra were recorded at 298 K on a Bruker Advance DRX 600 spectrometer operating at frequencies 600.186 MHz (^{1}H) and 60.816 MHz (^{15}N). Proton detected 2D spectra were carried out using 5 mm TBI probe head {$^{1}H/^{31}P$/BB} with a self-shielded z-gradient coil (90° ^{1}H pulse width 10.6 μs and ^{15}N pulse width 19 μs). ^{13}C NMR spectra were measured on a Varian Mercury 300 MHz spectrometer operating at 300.071 MHz (^{1}H) and 75.46 MHz (^{13}C). Two-dimensional ^{1}H-^{15}N gradient selected HMBC experiments were performed using standard pulse sequences from the Bruker pulse library. The delays for evaluation of multiple bond couplings were set to 62.5–250 ms. The chemical shift reproducibility was better than ± 0.05 ppm.

All spectra were measured in anhydrous $CDCl_3$ or CD_3OD and the sample concentrations were 5 mg per 600 μl of solvent. Internal TMS was used as a reference (^{1}H and ^{13}C), ^{15}N NMR spectra were referred to external neat NH_3 and recalculated to nitromethane. The procedure recommended by IUPAC for indirect

Table 1 Chemical shift[a] of NMR resonance signals (^{1}H, ^{13}C and ^{15}N) assigned for **7** and **8**

Compound	H2	H8	H1′	H2′	H3′	H4′	H5′,5″			
7	8.60 (15.1)	8.07	6.14	5.87	5.59	4.40	4.38; 4.32			
8	9.48 (6.3)	8.30	6.27	5.87	5.54	4.48	4.40; 4.35			
	C2	**C4**	**C5**	**C6**	**C8**	**C1′**	**C2′**	**C3′**	**C4′**	**C5′**
7	152.50 (2.5)	151.60	124.52	153.35 (5.1)	142.02	85.68	69.52	72.11	79.41	61.93
8	134.07 (11.2)	141.30	121.99	145.45 (9.7)	141.86	86.35	69.39	72.43	79.65	61.84
	N1	**N7**	**N9**							
7	253.5	168.0	241.0							
8	251.5	172.0	246.5							

[a] $CDCl_3$, ^{1}H and ^{13}C in ppm relative to TMS, ^{15}N in ppm relative to nitromethane; ( )—coupling constant with ^{15}N, in Hz.

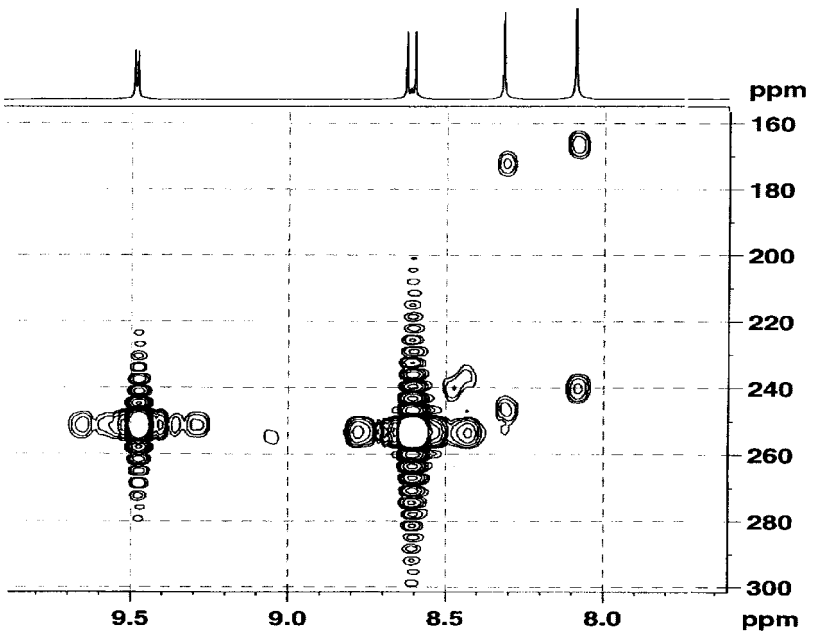

Figure 2 ^{15}N-^{1}H HMBC spectrum of **7**(**8**). Cross-peaks for N-7 appear at 168 (**7**) and 172 (**8**); for N-9 at 241 (**7**) and 246.5 (**8**).

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referencing (Markley *et al.*, 1998) was used with the relative frequency factor (Ξ) of 10.13291 MHz.

Syntheses of compounds **5**[16] and **7(8)**[17] were repeated according to reported procedures.

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