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The NMR study of derivatives of substituted inosine – The precursors of AICAr

Jacek Włostowski, Hanna Krawczyk *

Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland

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ABSTRACT

In this article we describe how the simultaneous presence and interaction of the aromatic ring at position 1 of an inosine derivative with the acetyl group at positions 2, 3, 5 of the furanose ring convert conformation of the nucleoside into the major conformer in solution. During the synthesis of AICAr, we obtained products (***A*** and ***B***) of inosine protected with 2,4-dinitrochlorobenzene, with two sets of NMR signals, each with very similar chemical shifts. Analysis of experimental NMR spectra and theoretical GIAO – DFT calculations were performed to obtain information about the stereochemistry of the products.

1. Introduction

Adenylosuccinate lyase (ASL) catalyzes two reactions in the biosynthesis of purine nucleotides. These are the conversion of SAICA-ribotide into aminoimidasole carboxamide ribotide and the conversion of adenylosuccinate into AMP. A defect in the enzyme (Adenylosuccinate lyase deficiency, ADSL; OMIM: **103,050**) usually leads to accumulation of two detectable compounds: SAICA-riboside (**SAICAr**), derived from SAICA-ribotide and succinyladenosine (**S-Ado**) derived from adenylosuccinate (**AICAr**, Fig. 1 supplementary data). In 2004, Marie et al. [1] identified an excessive amount of 5-amino-1-(β-D-rybofuranosyl)imidazole-4-carboxamide (**AICAr**, Fig. 1 Supplementary data) in the urine of a female infant. This condition was caused by the 5-aminoimidazole 4-carboxamide ribonucleotide formyltransferase/inosine monophosphate cyclohydrolase deficiency (**ATIC**; OMIM: **608,688**). Just as in the case of ADSL deficiency, the succinylpurines SAICAr and S-Ado accumulate in **ATIC** deficiency, but to a lesser extent. Nucleoside analogues that inhibit *de novo* purine biosynthesis have shown anti-cancer and anti-virial features [2]. This is the reason why there is a wide interest in both synthesis and evaluation of analogues of AICAR.

On the other hand, the biochemical systems encountered by a drug molecule are extremely complex. Therefore, it should not be surprising that factors affecting the drug interactions and contributing to its final effect are also manifold [3]. It is, however, known that *inter alia* chemical structure parameters, such as resonance, inductive effect, oxidation potentials, type of bonding and stereochemistry, affect the drug activity. It should be taken into account that the addition of nitrogroups (having a negative inductive effect and electron-withdrawing moiety) to the molecule can cause changes of electrosteric interactions. The resulting new derivatives are likely to exhibit pharmacological properties, e.g. introduction of NO_2 substituent to derivatives of 1,4-benzodiazepin-2-on in position 7 increases the hypnotic and sedative action of drug [3].

During the synthesis of AICAr, we obtained products (***A*** and ***B***) of inosine protected with 2,4-dinitrochlorobenzene (DCNB), each with two sets of NMR signals, with very similar chemical shifts. De Napoli et al. [4] in their investigations obtained the mixture of deoxydiastereomers of 3′,5′-di-*O*-acetyl-2′-deoxy-1-(2,4-dinitrophenyl)-inosine. There was no information about regio and steric interactions between the purine and the phenyl ring (*syn*- or *anti*-form). There are two possible forms of diastereomers, *syn*- or *anti*-conformation. Analysis of experimental NMR spectra and theoretical GIAO – DFT calculations (Gaussian 03W, PCM [5,6]) were performed to obtain information about the stereochemistry of the products.

2. Results and discussion

Performing the calculation for the tautomeric forms of inosine ***AI***, ***BI*** and ***CI*** (Table 1) we could observe that the most energetically stable was the ***AI*** (0 kJ/mol). Inosine in this form was firstly acetylated to give 2′,3′,5′-tri-*O*-acetylinosine and then reacted with 2,4-dinitrochlorobenzene (DCNB) (Fig. 2 Supplementary data). In the NMR spectra of the products we observed two sets of signals with very similar chemical shifts. First, we tried to prove that the products were regioisomers or diastereoisomers. (Steric interaction be-

* Corresponding author.
E-mail address: hkraw@ch.pw.edu.pl (H. Krawczyk).

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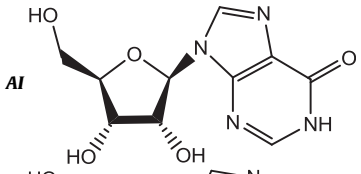
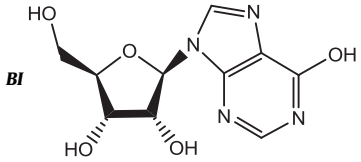
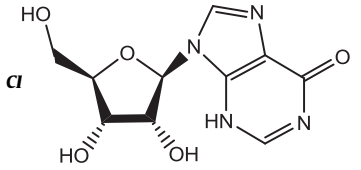
Table 1
Theoretical energy (a.u.) and relative theoretical energy (kJ/mol) calculated using DFT [B3LYP/6-311++G(2d,p)PCM] method and the Gaussian 03W [5,6] program for tautomers ***AI***, ***BI*** and ***CI*** of inosine.

Tautomers	In H_2O	
	Energy (a.u.)	Energy (kJ/mol)
AI 	−983.752157	0
BI 	−983.736444	42
CI 	−983.735529	45

tween the *ortho*-substituent in the aromatic ring and the carbonyl carbon in the purine ring is minimized in an orthogonal geometry, but at the expense of π-electron overlap which decreases with the cosine of the torsion angle and as a result there are two diastereomeric compounds *syn* or *anti* with a torsion angle different from 90°.) Next, we measured the spectra in two different solvents (Fig. 3 Supplementary data) and then in different temperatures. The values of ^{1}H chemical shifts and ^{1}H–^{1}H coupling constants for protons 1,2,3,4,5-CH_2 of the ribose ring and 3, 5, 6 of the phenyl ring in $CDCl_3$ and DMSO-d_6 were obtained by interactive fitting of line positions in the 1D ^{1}H NMR spectra using the program SYMUL written by Gryff-Keller [7] (Table 2). From this analysis we obtained intense lines for the investigated compounds in 1:1 proportion. The ratio is independent of the polarity of the solvent, since the same results have been observed in $CDCl_3$ and DMSO-d_6. Subsequently, we measured ^{1}H NMR spectra of the products at five different temperatures (25–120 °C). Interconversion of stereoisomers possessing diastereotropic nuclei can be monitored by variable-temperature NMR spectroscopy (dynamic NMR) when the reaction is slow on the NMR time scale. A particularly simple situation arises when two nuclei provide sharp signals with equal intensity and undergo chemical exchange resulting in line broadening and coalescence upon heating. In such a case, one can obtain well-resolved signals at low temperatures, where the two nuclei do not show measurable exchange. As the temperature is increased, the exchange rate becomes relatively fast according to the NMR time scale and only one averaged species is observed due to the signal coalescence. In the absence of any coupling, the first-order interconversion rate constant can be calculated as [8]:

$$k_{Tc} = \frac{\Delta v}{2^{1/2}} \tag{1}$$

where k_{Tc} is the rate constant at the coalescence temperature Tc (in Kelvin) and Δv is the difference in the chemical shifts of two signals without exchange (at low temperatures, $T \ll Tc$). In our case the methyl protons are singlets, so the above equation can be applied and we can calculate the free energy activation ($\Delta G^{\#}$) from the equation [8]:

$$\Delta G^{\#} = 19.14 \times 10^{-3} Tc(9.97 + \lg Tc - \lg|\nu_{\boldsymbol{A}} - \nu_{\boldsymbol{B}}|) \tag{2}$$

where $\Delta G^{\#}$ is the free energy of activation in kJ/mol, Tc is the coalescence temperature (in Kelvin) and $\nu_{\boldsymbol{A}}$ and $\nu_{\boldsymbol{B}}$ are the chemical shifts of the nuclei ***A*** and ***B*** in Hz. While raising the temperature of a DMSO solution of the products ***A*** and ***B***, the signals of protons broaden and coalesce, eventually yielding a single line, owing to the rapid interconversion of the two conformers at high temperatures (Figs. 4 and 5 Supplementary data). In the case of aromatic protons one can observe a temperature-dependent variation of chemical shifts. This is related to an interaction of a polar solvent with systems including heteroatoms and polar substituents. This experiment confirms the presence of an energy barrier created by the interactions of the 2-nitro group with the 6-O and 2-H atoms of the purine ring, hindering the complete rotation of the 2,4-dinitrophenyl ring. The chemical shifts of methyl protons (**CH_3**(**4C**)) of ***A*** and ***B*** for the determined rate constants for this process were obtained from the spectrum at 25 °C. The free energy of activation was calculated from the Eq. (2) and was equal to 82.8 kJ/mol (coalescence temperature 112 °C). The H-6 (from the phenyl ring) and H-2, H-8 protons (from the purine ring) play a key role in determining which of the *syn*- or *anti*-conformations (purine and aryl rings) of diastereomers occurs in solutions. First, the optimum structure of the product using the DFT B3LYP/6-311++G(2d,p) method (Table 3) was calculated. The influence of the solvent was described using the polarizable continuum model (PCM) [5]. The calculated energy of the most energetically stable form ***A***-*syn* (a deacetylated form) is −1624.00088391 a.u. This suggests that the ***A***-*syn* forms should prevail in solution. Taking these results into account, we performed the ^{1}H–^{13}C gs-HSQC, gs-NOESY, gs-HMBC 2-D spectra, DPFGSE-NOE (the selective pulse was adjusted on the signal of H-2 proton in the purine ring or H-6 proton in the aryl ring) and HOE (selective irradiation of H-6 proton) experiments. The assignment of signals to atoms in all the molecules was made from these experiments. The phase-sensitive NOESY experiment exhibited negative cross-peaks between the H-8 proton of the purine moiety and the H-1 proton of the ribose cycle for two diastereomers. We did not observe dipolar interaction between H-2 (purine ring) and H-6 (aromatic ring), even in the more sensitive DPFGSE-NOE, but in the HOE experiment we did notice weak dipolar interaction between the carbonyl carbon C-6 (purine ring) and the H-6 proton (NOE effect about 15%, Table 4). These observations tend to indicate that the torsion angle of the purine ring around the glycosidic bond lies in a range corresponding to the *syn* form (we know that from the calculation regardless of the N-type (C2′ exo) or S-type (C2′ endo) furanose ring). It is known [9] that $^3J_{1,2}$ is small (∼1 Hz) for N-type furanose rings but large for S-type rings (∼10 Hz). In our experiment we unfortunately could not determine whether the furanose ring was of the N- or S-type, because of the vicinal coupling constant value ranging from 4.89 to 5.31 Hz (for $CDCl_3$ and DMSO-d_6). The optimum ground-state geometries were calculated for the deacetylated form of ***A*** (form ***C***) (Table 5). It can be observed that for the calculated compound ***C***, the purine ring and the aromatic group are in *syn* form, contrary to experiments. To determine the impact on the substituted aromatic ring, to set the rest of the purine, we calculated the optimum ground-state geometries for compounds ***D***–***G*** (Table 5). Compound ***D*** is a derivative of pyrimidine-4(3H)-one and compounds ***E***–***G*** are derivatives of hypoxanthine. In the phenyl ring molecules ***D*** and ***G*** are placed in positions *ortho* and *para* of the nitro groups, in compounds ***E*** and ***F*** two hydroxy groups and chlorides, respectively. In all cases the rings set the *syn* form (Fig. 2 Supplementary data). The dihedral angles between C(O)6–N1–C1–C2 for ***C***–***G*** range from 61.2° to 88.4° and the distance between C(O)6–H6 is 3.28–3.65 (Å), respectively (Table 6). It can be concluded that the nature of substituents (acceptor or donor substituents) in the aromatic ring as well as the size of the second ring do not affect the conformation. The phenomenon of γ-gauche effects probably plays an important role in interaction between atoms of