

Short Note

5'-Chloro-5'-deoxy-2',3'-O-isopropylidene-6-fluoro nebularine

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Abstract: In this paper, we report on the synthesis and spectroscopic characterization of the novel nucleoside 5'-chloro-5'-deoxy-2',3'-O-isopropylidene-6-fluoro nebularine, obtained as a side product during the second step of the synthesis of 5'-fluoro-5'-deoxy-5-aminoimidazole-4-carboxamide- β -D-ribose (5'-F-AICAR), a non-phosphorylatable analogue of 5-aminoimidazole-4-carboxamide- β -D-ribose (AICAR).

Keywords: AICAR; acadesine; phosphorylation; fluorination; fluorinated nucleosides; nucleoside analogues; modified nucleosides; chlorinated nucleosides; AMPK

1. Introduction

Nucleoside and nucleotide analogues are synthetic modified compounds that have been developed to mimic their physiological counterparts [1]. Considering that several nucleoside and nucleotide analogues have been approved by the Food and Drug Administration (FDA) for the treatment of viral and cancer diseases and others have entered clinical trials [2,3], many research groups have focused their attention on the preparation of novel compounds to expand the pool of molecules with potential biological activities, with the aim of discovering “leads” that are safer and more effective. For example, the replacement of OH groups of sugar moieties with the isosteric F atom has generated life-saving drugs for the treatment of infectious diseases, such as HIV [4], HBV [5], and HCV [6].

The nucleoside AICAR (1, Figure 1), besides being an intermediate involved in purine biosynthesis, is an activator of the enzyme adenosine monophosphate-activated protein kinase (AMPK) in the 5'-phosphorylated form [7,8]. This activation leads to a cascade of metabolic events, such as the inhibition of basal and insulin-stimulated glucose uptake, lipogenesis, and glucose oxidation [9]. The AMPK pathway is also implicated in the regulation of cell proliferation, and activation by AICAR could result in pro-apoptotic effects [10]. Given the importance of such a molecule, the synthesis of novel AICAR analogues is an appealing goal to better understand its mechanism of action [11–15]. Considering AICAR's low intestinal absorption and poor penetration of the blood–brain barrier, we synthesized a more lipophilic analogue, where the 5'-OH group was replaced by a fluorine atom (2, 5'-F-AICAR) [16].

For the preparation of nucleoside 2, a key step was the 5'-fluorination of compound 4 (Scheme 1) [16]. In this paper, we report on the synthesis and structural characterization of the novel nucleoside 8 (Scheme 2), obtained by a side reaction during the fluorination step. This nucleoside could represent a valuable intermediate for a modular derivatization of the purine base moiety and the sugar residue.

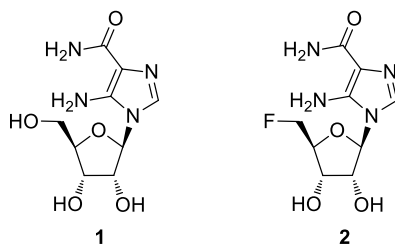
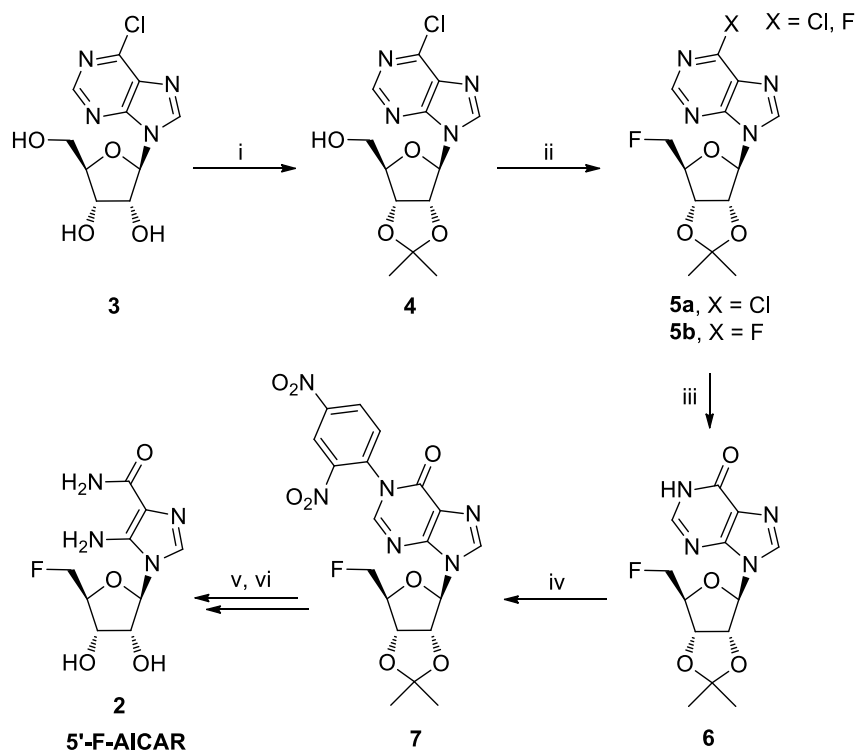


Figure 1. Structures of AICAR (**1**) and 5'-F-AICAR (**2**).

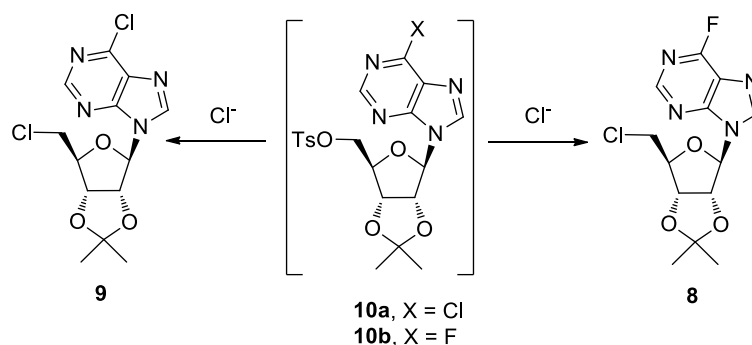
2. Results and Discussion

For the synthesis of 5'-F-AICAR (**2**), we used as the starting material the commercially available 6-chloronebularine (**3**, Scheme 1), which was readily transformed into its 2',3'-*O*-isopropylidene derivative **4** [17]. After 5'-fluorination, the 6-chloropurine was converted into hypoxanthine and the obtained nucleoside **6** transformed into the derivative **7**. The strong electron-withdrawing 2,4-dinitrophenyl group, introduced at the N1 hypoxanthine position, allowed us to obtain the 5'-F-AICAR **2** through a 1,2-diaminoethane-mediated purine ring degradation [18].

In the second step of the synthesis, the 5'-OH group of nucleoside **4** was replaced by a 5'-F in a one-pot tosylation/fluorination sequence, through the treatment with the tosyl fluoride/tetrabutylammonium fluoride (TsF/TBAF) reagent system with tetrahydrofuran (THF) as solvent, according to Shimizu's procedure [19]. As previously observed by Ashton and Scammells [19], we noted (TLC monitoring in *n*-hexane/AcOEt; 1:1) that the process led not only to the formation of the expected fluorinated product **5a** with *R_f* = 0.48 (Scheme 1), but also to the formation of the bis-fluorinated product **5b** with *R_f* = 0.42, as a consequence of the substitution in species **4** of the C6 chlorine atom by a fluoride ion.



Scheme 1. Reagents and conditions: (i) acetone, 2,2-dimethoxypropane (DMP), *p*-toluenesulfonic acid (*p*-TsOH), 2 h, r.t.; (ii) TsF, TBAF, THF, reflux, 18 h; (iii) 0.1 M NaOH, 4 h, r.t.; (iv), K₂CO₃, 1-chloro-2,4-dinitrobenzene, *N,N*-dimethylformamide (DMF), 3 h, 80 °C; (v) 1,2-diaminoethane, DMF, 16 h, 50 °C; (vi) 50% trifluoroacetic acid (TFA) in H₂O, 4 h.



Scheme 2. Proposed mechanisms for the formation of nucleosides **8** and **9**.

The TLC also showed a third spot with $R_f = 0.52$, whose corresponding product was isolated (10% yield) and analyzed by ^1H -NMR. The spectrum evidenced the presence of two compounds in a ratio of 1:8, which were resolved only by means of high-performance liquid chromatography (HPLC). The nucleoside in lower amounts and with $R_t = 21.2$ min was assigned as the novel 5'-chloro-5'-deoxy-2',3'-*O*-isopropylidene-6-fluoro nebularine (**8**, Scheme 2). Its structure was established through 1D-, 2D-NMR, and HRESI-MS experiments (see Supplementary Materials). In detail, in the ^1H -NMR spectrum the 5'- $\text{H}_{a,b}$ protons resonated as two doublets of doublets at 3.81 and 3.70 ppm, whereas in the ^{13}C -NMR spectrum the 5' C carbon resonated at 43.4 ppm. These data were consistent with the presence of a 5' C -Cl bond [20]. In the HMBC spectrum, the 2-H proton at 8.69 ppm correlated with the C6 purine carbon at 159.9 ppm, which appeared as a doublet with $J = 261.8$ Hz, as a consequence of the coupling with the F atom.

A similar compound, with the 2'- and 3'-ribose hydroxyls protected as acetates, was synthesized by Robins et al. [21] and used to obtain fluorescent probes for detecting the cellular uptake of the drug gemcitabine.

The main component with $R_t = 24.2$ min was instead identified as the known 5'-chloro-5'-deoxy-2',3'-O-isopropylidene-6-chloro nebularine **9** [20].

A plausible mechanism for the formation of compounds **8** and **9** is described in Scheme 2. The Cl⁻ ions, derived from the partial Cl⁻/F⁻ exchange at the C6 purine position during the 5'-fluorination step, yielded the nucleosides **8** and **9** by S_N2 displacement of the tosylate groups in the intermediates **10b** and **10a**, respectively.

3. Materials and Methods

All the reagents and solvents for the chemical syntheses were obtained from commercial sources and used without further purification. The ^1H -, ^{19}F - and ^{13}C -NMR spectra were acquired on 400/700 MHz instruments (Bruker, Billerica, MA, USA) using CDCl_3 as the solvent. The chemical shifts were reported in parts per million (δ) relative to the residual solvent signal (^1H : CHCl_3 7.27; ^{13}C : CDCl_3 77.0) and assigned by 2D-NMR experiments. All the NMR spectra were processed using the iNMR software package (Nucleomatica, Molfetta, Italy). The HRESI-MS spectra were recorded in positive mode on a Thermo Orbitrap XL mass spectrometer (ThermoFisher, Waltham, MA, USA). The column chromatography was performed by using silica gel 60, 70–230 mesh (Merck, Darmstadt, Germany). The TLC analyses were performed using 0.2 mm thick F254 silica gel plates (Merck, Darmstadt, Germany). The TLC spots were detected under UV light (254 nm). The high-performance liquid chromatography (HPLC) was performed on a UP-2075 Plus pump equipped with a UV-2075 Plus UV detector (Jasco, Cremella, Italy) using a 5 μm , 250-10 Si column (Purosphere® STAR, Merck, Darmstadt, Germany) eluted with *n*-hexane/AcOEt, 6:4 with a flow rate of 2.0 mL/min.

5'-Chloro-5'-deoxy-2',3'-O-isopropylidene-6-fluoro nebularine (8). To a stirred solution of 4 (0.28 g, 0.86 mmol) in dry THF (7.5 mL), TsF (0.30 g, 1.7 mmol) and TBAF (2.6 mL of a 1.0 M solution in dry THF, 2.6 mmol) were added and the mixture refluxed for 16 h (TLC monitoring: *n*-hexane/AcOEt; 1:1).

The solvent was removed under reduced pressure and the crude residue purified by silica gel flash chromatography (*n*-hexane/AcOEt, 6:4) to afford, in addition to the two expected compounds 5a and 5b [19], the inseparable mixture of the two nucleosides 8 and 9 with *R_f* = 0.52 (10% yield). The mixture was purified by HPLC (see above), thus obtaining the pure compound 8. Colorless foam (3.5 mg, 1.2% yield). ¹H-NMR (400 MHz; CDCl₃): δ 8.69 (s, 1H, 2-H), 8.29 (s, 1H, 8-H), 6.24 (d, *J* = 2.6 Hz, 1H, 1'-H), 5.40 (dd, *J* = 6.4, 2.7 Hz, 1H, 2'-H), 5.13 (dd, *J* = 6.4, 3.1 Hz, 1H, 3'-H), 4.58–4.55 (m, 1H, 4'-H), 3.82 (dd, *J* = 11.6, 6.1 Hz, 1H, 5'-H_a), 3.70 (dd, *J* = 11.6, 4.9 Hz, 1H, 5'-H_b), 1.66 (s, 3H, CH₃), 1.42 (s, 3H, CH₃); ¹⁹F-NMR (377 MHz; CDCl₃): δ −68.4 (s); ¹³C-NMR (176 MHz; CDCl₃): δ 159.9 (d, *J* = 261.8, 6-C), 154.5 (d, *J* = 11.1, 4-C), 152.2 (d, *J* = 14.3, 2-C), 144.0 (8-C), 121.15 (d, *J* = 29.2, 5-C), 115.1 (C_q acetonide), 91.3 (1'-C), 86.2 (4'-C), 84.2 (2'-C), 82.3 (3'-C), 43.9 (CH₂Cl), 27.1 (CH₃), 25.3 (CH₃); HRESI-MS *m/z* 329.0809, ([*M* + *H*]⁺ calcd. for C₁₃H₁₅ClFN₄O₃ 329.0817).

Supplementary Materials: The following are available online, Figure S1: copies of ¹H- and ¹⁹F-NMR spectra of compound 8; Figure S2: copy of ¹³C-NMR spectrum of compound 8; Figure S3: copy of HMBC spectrum of compound 8; Figure S4: copy of HRESI-MS spectrum of compound 8.

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