Imidazole Nucleosides, V.

Synthesis of 3′-Fluoro-3′-deoxy-ribofuranosides of 4(5)-Amino-imidazole-5(4)carboxamide

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Fluorinated Imidazole Nucleosides, Structure, UV Data, NMR-Data, Biological Evaluation

The synthesis of the 3′-fluoro-derivatives of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside) and the isomeric 4-amino-1(β-D-ribofuranosyl)imidazole-5-carboxamide (*iso*-AICA-riboside) are described. Structures were confirmed by elemental analysis, UV and ^{1}H NMR spectroscopy. The anti-viral and anti-cancer activities of these imidazole nucleosides were tested.

Introduction

Imidazole nucleosides have been extensively studied as naturally occuring intermediates of the *de novo* purine nucleotide biosynthesis and as enzyme inhibitors of this important anabolic pathway. Therefore, there has been great interest in the synthesis and evaluation of analogues of AICAR (5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide-5′-phosphate) which is one of the intermediates of this process [1]. Furthermore, imidazole nucleosides serve as useful synthetic intermediates for the preparation of related purine nucleosides of biological interest. A recent survey was given by Shaw [2].

The synthesis of imidazole nucleosides can be achieved by condensation of a metal salt of an imidazole derivative with acylated glycosyl halides which leads to the isomeric N-1 and N-3 substituted imidazoles. Furthermore, syntheses have been reported applying the glycosylamine method [2] or alkaline treatment of N-1 substituted purine nucleosides [3]. Imidazole nucleosides with a fluorine atom in the carbohydrate moiety are unknown.

Results

We describe the synthesis of 3′-fluoro derivatives of 5-amino-(1-β-D-ribofuranosyl)imidazole-4-carboxamide (AICA-riboside) and the isomeric 4-amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (*iso*-AICA-riboside). The route is illustrated in Fig. 1. The silver salt of methyl-4(5)-nitroimidazole-5(4)-carboxylate **1** was used as a starting material and prepared by modifying a procedure described earlier [4].

For the synthesis of 3′-fluoro-analogues we utilized 1-O-acetyl-2,5-di-O-benzoyl-3-fluoro-3-deoxy-α,β-D-ribofuranose (**2**) which is available by a twelve step process [5]. It was converted to the unknown 1-chloro derivative **3** which was not further characterized. Condensation of **3** with an excess of **1** in boiling toluene resulted in a mixture of the expected N-1 and N-3 substituted imidazoles **4** and **5**, respectively. They were separated by silica gel column chromatography. Most remarkably, the 5-nitro-4-carboxylic acid derivative (**5**) is the mayor product (23% yield) and not the 4-nitro-5-carboxylic acid derivative (**4**) (13% yield). This was never observed in a series of similar syntheses [6,8]. Compounds **4** and **5** were deblocked and converted to the carboxamides **6** and **7** by treatment with methanolic ammonia. However, 5-nitro-1-(3′-fluoro-3′-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (**7**) did not crystallize and darkened within a few days because of decomposition. A methanolic solution of the foregoing 4-nitroimidazole (**6**) was shaken with a platinium cata-

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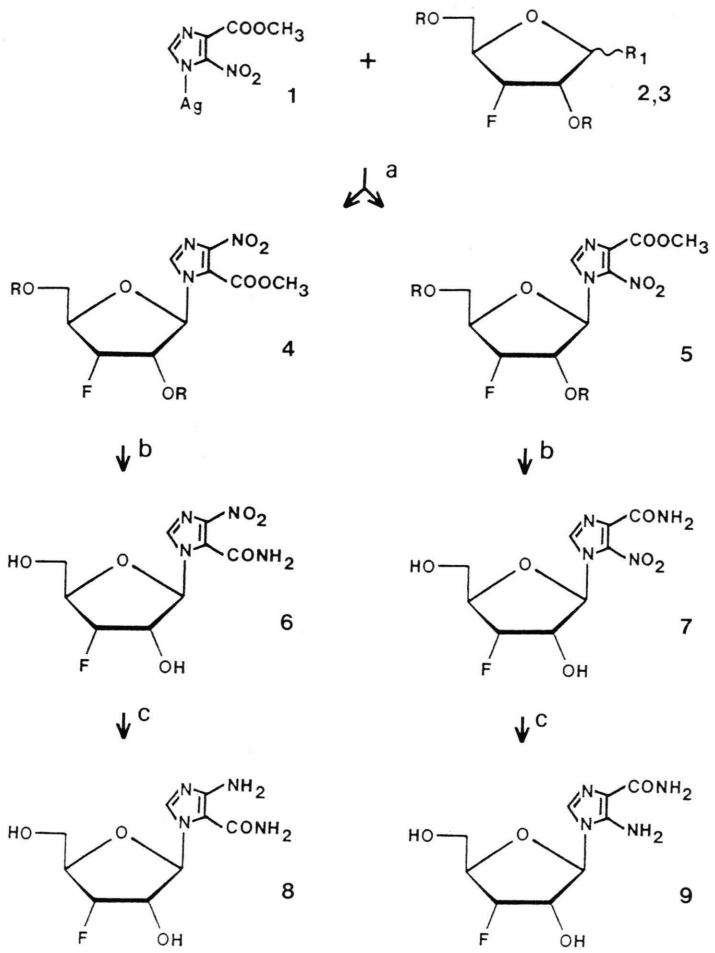

Fig. 1. R = benzoyl, 2: R_1 = acetyl, 3: R_1 = Cl.
a): boiling toluene, b): MeOH/NH_3, c): Pt/H_2.

lyst in an atmosphere of hydrogen for 5 h to produce the 4-aminoimidazole (**8**) as described [6]. In a similar manner, the 5-nitroimidazole (**7**) was reduced to the 5-aminoimidazole (**9**). The orientation of the isomeric nucleosides **8** and **9** was established by UV spectra. They were compared to those of the corresponding nucleosides without fluorine atom [4]. One of the isomers has the same absorption maximum as 5-amino-1-(β-D-ribofuranosyl)-imidazole-4-carboxamide (266 nm) which could be converted to inosine, identical with the naturally occuring product. Therefore, this isomer is 5-amino-1-(3′-fluoro-3′-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (**9**) (268 nm). Likewise, the isomeric nucleoside 8 has the same absorption maximum (275 nm) as 4-amino-1-(β-D-ribofuranosyl)imidazole-5-carboxamide (272 nm), which could be converted to isoinosine. Furthermore, the position of the amino group can easily be determined with the Pauly reaction [7]. As found earlier, all nucleosides of 4-amino-imidazole-5-carboxamide led to a yellow or brown-yellow colour and nucleosides of 5-amino-imidazole-4-carboxamide showed a violet colour [6]. **8** produced a yellow colour, and therefore it is a 4-amino-imidazole. In contrast, we obtained a violet colour with **9**, thus confirming a 5-amino-imidazole nucleoside.

The *structures* of the synthesized compounds are confirmed by ^{1}H NMR data, which are listed in Table I. Fig. 3 shows the ^{1}H NMR spectrum of compound **4** including peak picking and integration. The multiplet at 4.67 ppm arises from the two diastereotopic protons H-5′ and H-5″. Because of their magnetical non-equivalency an ABC-spectrum was found and the chemical shift

Table I. ^{1}H NMR spectral data of compounds **4–6, 8, 9.**

No.	H-2	H-1′	H-2′	H-3′	H-4′	H-5′	H-5′′	others
4	8.37 s	6.52 d	5.91 ddd	5.72 ddd	4.87 dddd	4.66 dd	4.70 dd	3.79 s (OCH_3), 8.06–7.53 m (2 Bz)
5	8.37 s	6.61 d	6.03 ddd	5.71 ddd	4.88 ddt	4.71 d	4.71 d	3.84 s (OCH_3), 8.07–7.51 m (2 Bz)
6	8.23 s	5.65 d	4.65 dddd	5.05 ddd	4.27 dddd	3.33 ddd	3.37 ddd	6.09 d (OH-2′), 5.27 dd (OH-5′), 8.32 s ($CO\text{-}NH_2$)
8	7.75 s	6.00 d	4.45 dddd	4.96 ddd	4.18 dddd	~ 3.56 ddd	~ 3.56 ddd	6.04 d (OH-2′), 5.12 dd (OH-5′), 6.91 s ($CO\text{-}NH_2$), 5.41 s (NH_2)
9	7.32 s	5.54 d	4.50 dddd	4.90 ddd	4.20 dddd	3.55 ddd	3.65 ddd	5.90 d (OH-2′), 5.44 dd (OH-5′), 6.74 s ($CO\text{-}NH_2$), 6.01 s (NH_2)

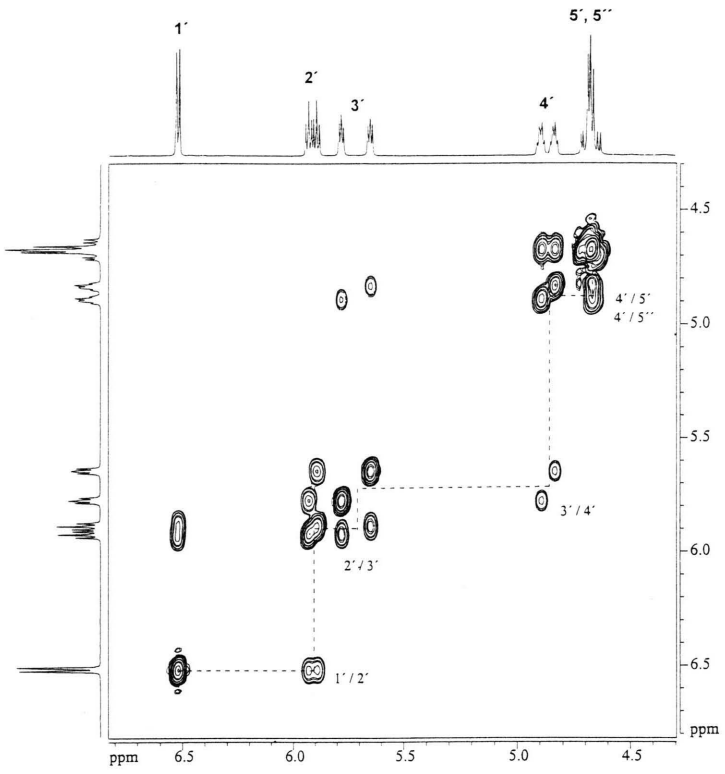

Fig. 2. ^{1}H, ^{1}H COSY spectrum of compound **4**.

values as well as the coupling constants were simulated with the BRUKER WinDaisy program. The results are listed in Table I and II. The COSY spectrum in Fig. 2 depicts all crosspeaks of the aliphatic protons. Proton H-5′ couples with the proton H-5″ with the typical geminal coupling constant of 12.2 Hz and with the vicinal proton H-4′ ($^3J_{\text{H-5′, H-4′}}$ 5.4 Hz). H-4′ has also a crosspeak with proton H-3′. So the doublet at 5.72 ppm belongs to the proton H-3′. The typical coupling constant of 52.4 Hz indicates the coupling to a fluor atom. The coupling constants to the β-H-atoms prove also the existence of the fluor atom ($^3J_{\text{H-2′, F}}$ 14.8 Hz; $^3J_{\text{H-4′, F}}$ 20.7 Hz). The coupling constants J_{HF} are collected in Table III. The COSY spectrum in Fig. 2 shows also clearly the crosspeaks H-3′/H-2′ and H-2′/H-1′. The singulet at 8.37 ppm is caused by the proton H-2. The multiplets between 8.06 ppm and 7.53 ppm arise from the 10 protons of the two benzoyl groups. All other structures can be proved in an analogous way by NMR spectroscopy. Table I to III show the results of all compounds. Of interest are the accidental isochronous protons H-5′ and H-5″ of compound **5**. Therefore, the spectrum exhibits only a doublet of an AB-spectrum at 4.71 ppm.

Table II. Coupling constants J_{HH} of compounds **4–6, 8, 9.**

No.	H-1′, H-2′	H-2′, H-3′	H-3′, H-4′	H-4′, H-5′	H-4′, H-5″	H-5′, H-5″	H-2′, OH-2′	H-5′, OH-5′	H-5″, OH-5″
4	5.4	5.4	3.6	5.4	4.0	12.2	–	–	–
5	4.5	4.9	4.5	4.0	4.0	–	–	–	–
6	7.6	4.0	4.0	4.0	4.0	12.1	6.7	5.4	5.4
8	8.0	4.4	4.4	3.5	3.5	~ 12	~ 6.2	~ 4.8	~ 4.8
9	8.0	4.4	4.4	4.0	4.4	11.9	6.2	4.9	4.9

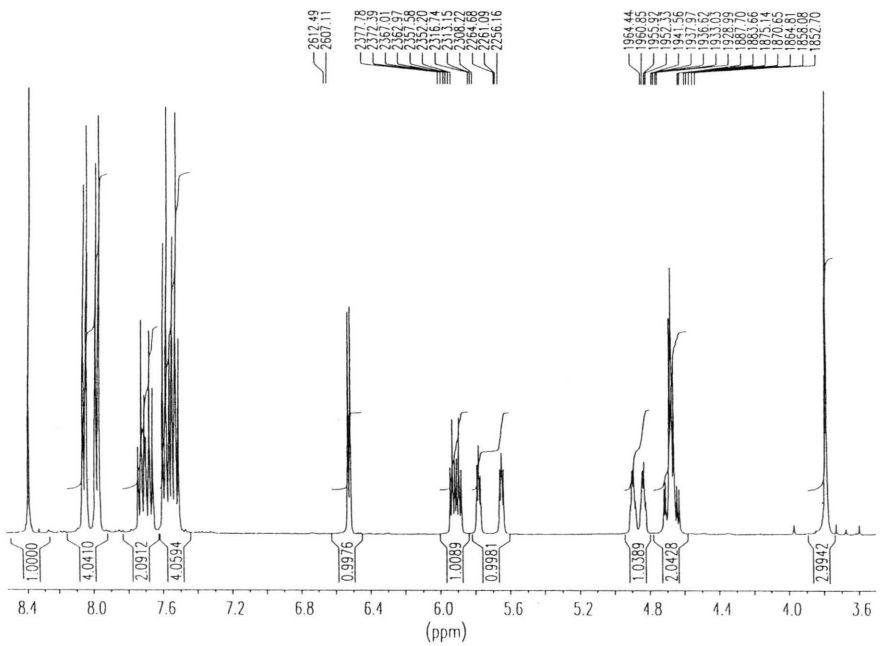

Fig. 3. ^{1}H NMR spectrum of compound **4**.

Table III. Coupling constants J_{HF} of compounds **4–6, 8, 9.**

No.	H-2′, F	H-3′, F	H-4′, F
4	14.8	52.1	22.9
5	12.1	51.6	20.6
6	24.7	54.3	26.5
8	23.4	54.8	27.4
9	26.1	54.8	28.8

The β-configuration of the carbohydrate moiety was also assigned by NMR spectroscopy. The values of the coupling constant $^3J_{H\text{-}1',\,H\text{-}2'}$ in Table II indicate no evidence for the formation of α-nucleosides. Due to the electron-withdrawing effect of the benzoyl group in position 2′, the coupling constants of compound **4** and **5** decrease.

Biological evaluation. Nucleosides 6, 8 and 9 have been evaluated for *in vitro* anti-HIV activity [9]. None of the compounds showed significant inhibitory effects. The anti-cancer activity of these compounds was tested with an *in vitro* model of human tumor cell lines [10]. As primary anti-cancer screen a three-cell line panel was used, consisting of MCF 7 (Breast), NCI-H 460 (Lung) and SF-268 (CNS). All three substances were inactive.

Experimental

^{1}H NMR experiments were recorded with a BRUKER ARX 400 spectrometer. All compounds were dissolved in DMSO-d_6. The temperature was set to 300 K. 32 transients with a time domain of 32 k per FID were coadded using a pulse length of 7.7 μs and a sweep width of 7352.9

Hz. The COSY spectra were recorded with a time domain of 2 k per FID and 256 experiments in F1 dimension. Data processing was performed with XWINNMR and 1D WINNMR software (BRUKER). Zero-filling to 32 k data points and exponential multiplication with a line broadening of 0.3 Hz was applied to the FID prior to Fourier transformation in the F2 dimension.

Melting points were determined on a Büchi melting point instrument and are uncorrected. Optical rotations were determined in a 5 cm cell with a light electric Zeiss precision polarimeter LEP A2 at 22 °C. Ultraviolet absorption spectra were measured on a Contron Uvicon 930 spectrometer using solutions in phosphate buffer (pH 7) unless otherwise stated. Thin-layer chromatography was performed on precoated silica sheets (layer thickness 0,25 mm, ICN Biomedicals) with fluorescent indicator 254/366 nm. Compounds were detected on t.l.c. plates by UV absorbance and the Pauly reaction [7]. Column chromatography (45 x 3 cm) was performed on silica gel 60 Merck 7734. The following solvent systems were used (v/v): Light petroleum (50–70 °C) – ethyl acetate 2:1 (A) and n-butanol saturated with water (B).

Elemental analyses were performed by Mikroanalytisches Labor Pascher, D-53424 Remagen-Bandorf (Germany).

Methyl-4-nitro-1-(2'-5'-di-O-benzoyl-3'-fluoro-3'-deoxy-β-D-ribofuranosyl)imidazole-5-carboxylate (**4**) *and methyl-5-nitro-1-(2'-5'-di-O-benzoyl-3'-fluoro-3'-deoxy-β-D-ribofuranosyl)imidazole-4-carboxylate* (**5**)

1 g (6 mmol) methyl-4(5)-nitroimidazole-5(4)-carboxylate was dissolved in 150 ml of hot methanol and mixed with the solution of 1 g (6 mmol) silver nitrate in 150 ml of hot methanol. The suspension was treated with 10 ml pyridine, cooled and centrifugated. The precipitate was stirred with methanol, centrifugated again, the precipitate suspended in 400 ml of toluene and dried azeotropically by distillation of 150 ml of the solvent. 1,2 g (3 mmol) 1-O-acetyl-2,5-di-O-benzoyl-3-fluoro-3-deoxy-α,β-D-ribofuranose (**2**) were dissolved in 100 ml of ether and saturated with dry hydrogen chloride gas at 0 °C. After eight days the clear solution was evaporated. The residual sirupy 1-chloro-2,5-di-O-benzoyl-3-fluoro-3-deoxy-α,β-D-ribofuranose (**3**) was dissolved in toluene and re-evaporated with toluene (2x50 ml). The resulting sirup was dissolved in 50 ml anhydrous toluene and added to the suspension of the silver salt. The reaction mixture was stirred and heated under reflux for 1 h with the exclusion of moisture. The cooled mixture was filtered and evaporated to dryness. The residual sirup was fractionated by silica gel column chromatography (A).

Compound **4** was obtained in 13% yield (200 mg).

M.p. 122 °C. R_f = 0,63 (A), $[\alpha]_{578}$ −26°, $[\alpha]_{546}$ −28°, c 1 in $CHCl_3$. UV (λ,ε): 275 nm (max) 10608, 258 nm (min) 4795 in ethanol.

$C_{24}H_{20}N_3O_9F$ (513,45)

Calcd	C 56.14	H 3.90	N 8.19	F 3.69%,
Found	C 56.24	H 3.96	N 8.26	F 3.58%.

Compound **5** was obtained in 23% yield (350 mg).

M.p. 125 °C. R_f = 0,38 (A), $[\alpha]_{578}$ −25°, $[\alpha]_{546}$ −23°, c 1 in $CHCl_3$, UV (λ,ε): 275 nm (max) 8538, 258 nm (min) 6597 in ethanol.

$C_{24}H_{20}N_3O_9F$ (513,45)

Calcd	C 56.14	H 3.90	N 8.19	F 3.69%,
Found	C 56.38	H 4.14	N 8.21	F 3.56%.

4-Nitro-1-(3'-fluoro-3'-deoxy-β-D-ribofuranosyl)-imidazole-5-carboxamide (**6**)

450 mg (0,88 mmol) **4** were treated with methanolic ammonia (100 ml) at 0 °C for 50 h. The solution was evaporated, the residue dissolved in 30 ml of water and extracted two times with 30 ml of ether. The aqueous solution was evaporated. The residue cristallized from ethanol, yield 220 mg (87%). M.p. 225 °C.

UV (λ,ε): 294 nm (max) 5777, 257 nm (min) 2854. R_f = 0,55 (B).

$C_9H_{11}N_4O_6F$ (290,21)

Calcd	C 37.24	H 3.82	N 19.30	F 6.54%
Found	C 37.56	H 3.99	N 19.39	F 6.29%.

5-Nitro-1-(3'-fluoro-3'-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide (**7**)

510 mg (1 mmol) **5** were treated with methanolic ammonia (100 ml) at 0 °C for 50 h. The reaction mixture was processed as described for **6**. The aqueous solution was evaporated to give a gum which did not crystallize and darkened within a few days. Yield 250 mg (86%).

UV (λ,ε): 298 nm (max) 3373, 265 nm (min) 2752. R_f = 0,51 (B).

$C_9H_{11}N_4O_6F$ (290,21)

4-Amino-1-(3'-fluoro-3'-deoxy-β-D-ribofuranosyl)imidazole-5-carboxamide (**8**)

250 mg (0,86 mol) **6** were dissolved in 60 ml of freshly distilled methanol and hydrogenated with a platinium catalyst. The catalyst was removed by filtration and the filtrate evaporated to a sirup which crystallized from ethanol. Recrystallization from water. Yield 210 mg (94%), M.p. 197 °C, R_f = 0,40 (B).

UV (λ,ε): 275 nm (max) 8117, 241 nm (min) 2860.

$C_9H_{13}N_4O_4F$ (260,11)

Calcd	C 41.55	H 5.03	N 21.54	F 7.30%,
Found	C 41.50	H 5.06	N 21.80	F 7.32%.

5-Amino-1-(3'-fluoro- 3'-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide (**9**)

200 mg (0,7 mol) **7** were hydrogenated as described for **8**. Crystallization from ethanol. Recrystallisation from water. Yield 165 mg (91%). M.p. 260 °C (dec.), R_f = 0,50 (B).

UV (λ, ε): 268 nm (max) 9860, 217 nm (min) 3113.

$C_9H_{13}N_4O_4F$ (260,11)

Calcd	C 41.55	H 5.03	N 21.54	F 7.30%,
Found	C 41.50	H 5.04	N 21.60	F 7.45%.

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