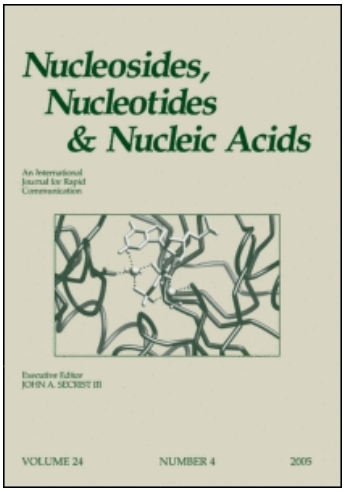


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Imidazole Nucleosides IV Nucleosides of 5 (4)-Formamido-imidazole-4 (5)carboxamide

Hans Guglielmi[a]; Klaus Albert[b]
[a] Physiologisch-Chemisches Institut der Universität Tübingen, Tübingen [b] Institut für Organische Chemie der Universität Tübingen, Tübingen

To cite this Article Guglielmi, Hans and Albert, Klaus(1993) 'Imidazole Nucleosides IV Nucleosides of 5 (4)-Formamido-imidazole-4 (5)carboxamide', Nucleosides, Nucleotides and Nucleic Acids, 12: 2, 215 — 224
To link to this Article: DOI: 10.1080/07328319308021207
URL: http://dx.doi.org/10.1080/07328319308021207

NUCLEOSIDES & NUCLEOTIDES, 12(2), 215-224 (1993)

IMIDAZOLE NUCLEOSIDES IV NUCLEOSIDES OF 5(4)-FORMAMIDO-IMIDAZOLE-4(5)CARBOXAMIDE

Hans Guglielmi*

Physiologisch-Chemisches Institut der Universität Tübingen, Hoppe-Seyler-Straße 4, D-7400 Tübingen

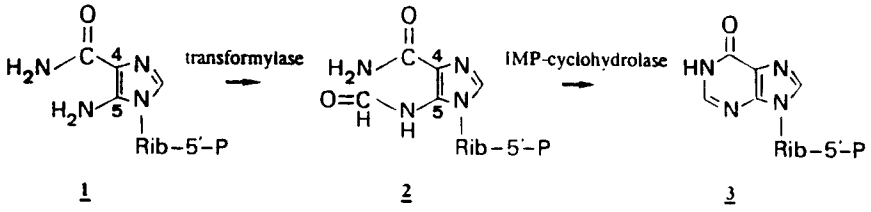
Klaus Albert

Institut für Organische Chemie der Universität Tübingen, Auf der Morgenstelle 18, D-7400 Tübingen

Abstract: Nucleosides of 5(4)-aminoimidazole-4(5)-carboxamide were formylated with sodium formate, formic acid and acetic anhydride to the ß-D-ribo-, α-D-arabino-, α-L-arabino- and ß-D-xylofuranosides of 5-formamidoimidazole-4-carboxamide, and to the ß-D-ribo-, ß-D-arabino-, α-D-arabino- and α-L-arabinopyranosides of 4-formamidoimidazole-5-carboxamide.

In the *de novo* synthesis of purines, a formylation reaction of 5-amino-1-(ß-D-ribofuranosyl)imidazole-4-carboxamide-5'-phosphate 1 to 5-formamido-1-(ß-D-ribofuranosyl)imidazole-4-carboxamide-5'-phosphate 2 occurs by transformylase (E.C.2.1.2.3.). In a following step, inosine-5'-phosphate 3 is formed by IMP-cyclohydrolase (inosinicase, E.C.3.5.4.10.) (FIG.1). Both enzymatic activities are associated or are located on the same protein [1-4].

IMP 3 is the precursor of the purine nucleotides AMP and GMP, which are components of nucleic acids. An inhibition of the enzymes transformylase and IMP-cyclohydrolase by modified imidazole nucleosides might lead to a depression of nucleic acid biosynthesis and therefore result in anti-viral or anti-tumor drugs. We are interested in preparing analogous nucleosides of 5(4)-formamidoimidazole-4(5)-carboxamide.



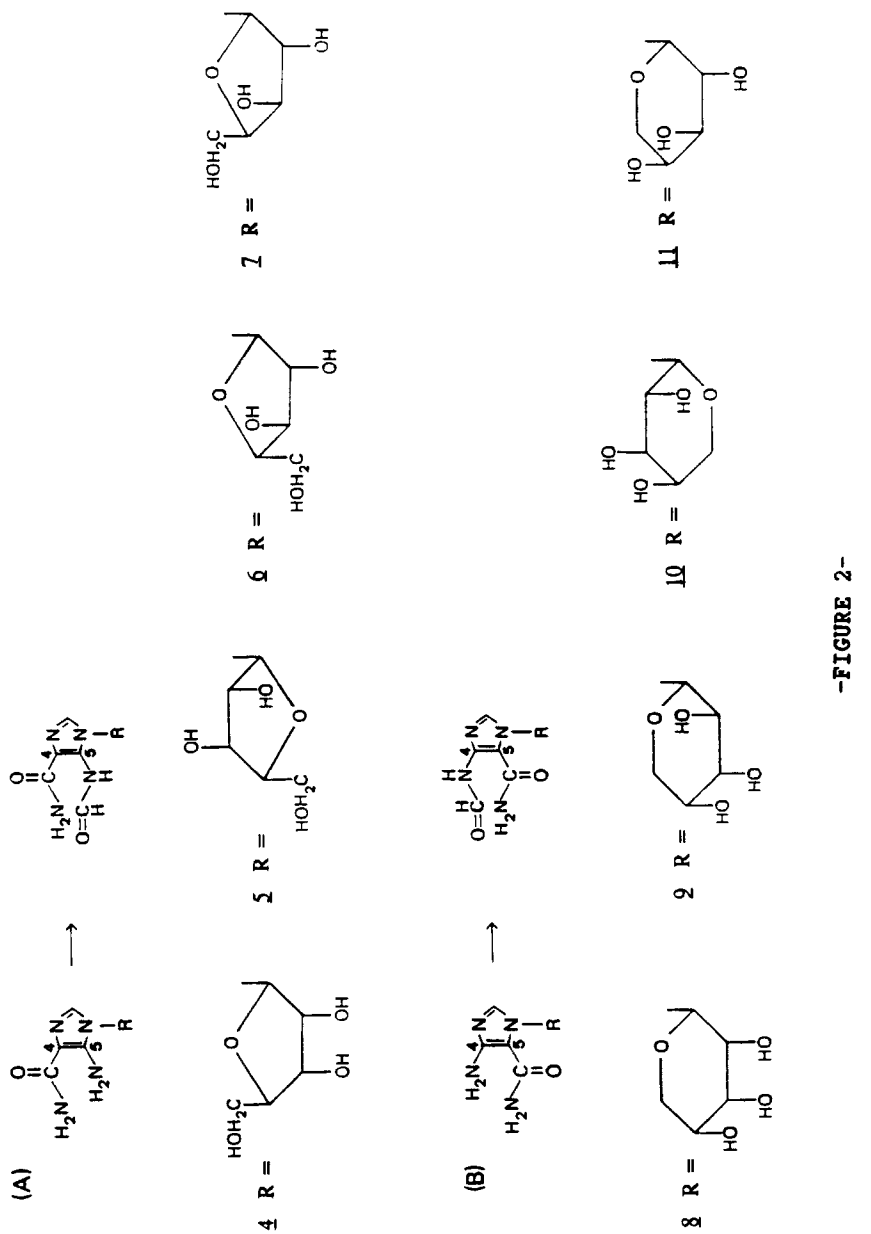
1 2 3

-FIGURE 1-

The formylation reaction of the amino group of 5(4)-aminoimidazole-4(5)-carboxamide with formic acid, acetic anhydride and sodium formate was first reported by Shaw[5] and later used by Flaks[1] to prepare 2. Later investigations revealed that as a side effect of this reaction an opening of the imidazole ring may occur[6] and that in addition to the formylation reaction an acetylation of the amino group takes place[7].

We used the above method to prepare 1-glycosyl-5-formamidoimidazole-4-carboxamides 4-7 (FIG. 2,A) and 1-glycosyl-4-formamidoimidazole-5-carboxamides 8-11 (FIG. 2,B), starting from the corresponding aminoimidazole nucleosides[8].

The progress of the reaction can be followed by thin layer chromatography (t.l.c.). Imidazoles were detected by u.v. absorbance and the Pauly reaction. Nucleosides of 5-amino- and 5-formamidoimidazole-4-carboxamide showed a violet colour whilst nucleosides of 4-amino-and 4-formamidoimidazole-5-carboxamide showed a yellow to brown-yellow colour. Despite the variation of reaction time and temperature the reaction was incomplete and byproducts were always formed. For instance, a t.l.c. examination (system A) of the formylation of 5-amino-1-(ß-D-ribofuranosyl)imidazole-4-carboxamide revealed the following substances detected by different colours using the Pauly spray reagent: 5(4)-Formamidoimidazole-4(5)-carboxamide[5] (R_f = 0.36, orange), 5(4)-aminoimidazole-4(5)-carboxamide (R_f = 0.27, blue), nearly half of the starting material (R_f = 0.19, violet), an unknown substance (R_f = 0.15, violet) and the product (R_f = 0.1, violet). The starting material and the product have nearly identical properties, thus 1-glycosyl-5-formamidoimidazole-4-carboxamides 4-7 with a furanose moiety can only be obtai-



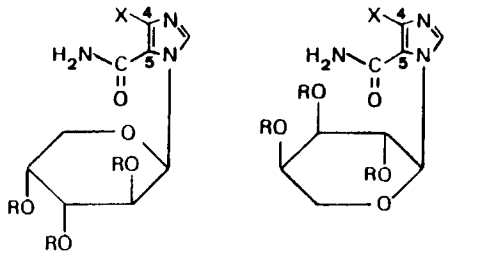
-FIGURE 2-

ned in a pure form by chromatography. Preparative separations of a reaction mixture were performed on preparative columns (180 x 6 cm) packed with cellulose as stationary phase. n-Butanol saturated with water was used as mobile phase. The isolated compounds did not cristallize, and mainly due to the incompleteness of the reaction, the yields were very low, ranging between 19 and 23%.

On the other hand, 1-glycosyl-4-formamidoimidazole-5-carboxamides 8-11 with a pyranose moiety gave much higher overall yields, less byproducts were formed and these compounds crystallized. In this series we used system B for t.l.c. examination. None of the formylated imidazole nucleosides reacted in the Bratton-Marshall assay.

The configuration of the glycosidic bond of all of the described imidazole nucleosides is the same as in the original nucleosides of 4(5)amino- or 4(5)nitroimidazole-5(4)-carboxamide[8,9]. The proton of the aldehyde group can be clearly seen in the ^{1}H-NMR-spectra. It absorbs as a singlet at 8.34 ppm in the ß-D-ribopyranoside 8, and the signal of H-2 of the imidazole ring is observed as a singlet at 8.08 ppm. This holds also true for the ß-D-arabinopyranoside 9, whereas in the ^{1}H-NMR spectrum of α-L-arabinopyranoside 11 two aldehyde signals at 8.69 and 8.19 ppm together with two H-2 signals at 7.95 and 7.81 ppm can be observed. Further splitting of the anomeric proton H-1' at 5.45 ppm and 5.83 ppm (J = 9.3 Hz) is found. This behaviour is due to the existence of two rotation isomers. The H-2 signals coalesce at 325 K and the aldehyde signals at 340 K resulting in an activation energy $\Delta G^{\neq}$ of 16.5 kcal/mol. A similar value is found for D-arabinopyranoside 10. Rotation isomers were also found in the case of imidazole nucleosides with a ß-D-ribopyranose carbohydrate moiety[10] and 5-formamidoimidazole-4-carboxamides with a furanoside carbohydrate moiety 5-7. Ribofuranoside 4 and the corresponding 5'-phosphate[3] 2 do not show rotation isomers.

The synthesis of the precursors of the ß-D-arabinopyranoside 9 with a cis(ß)-configuration has not been described to date. Methyl-4-nitro-1-(2 ,3 ,4 -tri-O-acetyl-ß-D-arabinopyranosyl)imidazole-5-carboxylate 12 (FIG.3) was used as starting material. This compound is one of the products of the condensation of the mercury complex of methyl-4(5)-nitroimidazole-5(4)-carboxylate with 2,3,4-tri-O-acetyl-ß-D-arabinopyranosylbromide. As described previously[9] the main product is



12: R = Ac, X = NO_2 13: R = Ac, X = NO_2
14: R = H, X = NO_2
15: R = H, N = NH_2

-FIGURE 3-

the α-isomer 13. The blocked nucleoside 12 was treated with methanolic ammonia to form 4-nitro-1-(ß-D-arabinopyranosyl)imidazole-5-carboxamide 14. Hydrogenation of 14 with a platinum catalyst[8] yielded 4-amino-1-(ß-D-arabinopyranosyl)imidazole-5-carboxamide 15. The ^{1}H-NMR-spectra of 9 and 15 show the anomeric proton signal as a singlet and indicate the ß-configuration. These assignments agree with those obtained for other imidazole-ß-D-arabinopyranosides[11].

Experimental

Melting points were determined on a Büchi melting point instrument and are uncorrected. ^{1}H NMR spectra were recorded at 90 MHz on a Bruker WH 90 spectrometer and at 250 MHz on a Bruker AC 250 spectrometer. The Na-salt of deuterated trimethylsilylpropionic acid was used as internal reference. Chemical shifts are reported in parts per million (δ) and signals are expressed as s (singlet), d (doublet) and m (multiplet), measured from the approximate center. Optical rotations were determined in a 5 cm cell with a light electric Zeiss precision polarimeter LEP A2. Ultraviolet absorption spectra were measured on a Shimadzu UV 300 spectrometer using solutions in phosphate buffer (pH7). Thin layer chromatography (t.l.c.) was performed either on precoated aluminium cards (cellulose with fluorescent indicator 254 nm, layer thickness 0.1 mm, 20 x 20 cm, Riedel de Haen), and (A) n-bu-

tanol (saturated with water), (B) n-butanol:conc. ammonia (10:3) as development system, or on precoated silica sheets (layer thickness 0.25 mm, 20x20 cm, ICN Biomedicals) with fluorescent indicator 254/366 nm and (C) CCl_4:EtOAc (1:1) as development solvent system. For preparative t.l.c. we used glass plates, coated with silica gel (40x20 cm, thickness 2 mm, Merck silica gel 7749) and eluent system C. Compounds were detected on t.l.c. plates by u.v. absorbance, the Pauly[12] reaction or by the Bratton-Marshall[13] test. Column chromatography (180x6 cm) was run on cellulose powder (Macherey, Nagel & Co, MN 2100) with eluent system A and detection by u.v. absorbance at 254 nm.

General preparation procedure:

In a typical reaction, 516 mg (2×10^{-3} mol) of a nucleoside of 5(4)-aminoimidazole-4(5)-carboxamide and 680 mg (10^{-2} mol) sodium formate were reacted with 5 ml formic acid and 10 ml acetic anhydride. After heating to 40°C for 2 min, the reaction mixture was kept at room temperature for 30 min and heated to 50°C for further 30 min. After volume reduction by vacuum evaporation, 200 ml of ethyl acetate were added to the mixture resulting in the precipitation of inorganic salts. After cooling, the precipitate was filtered off, the filtrate concentrated by vacuum evaporation and the product precipitated by the additon of ether. The precipitate was separated by centrifugation and washed with ether. The product was either recristallized from methanol (8-11), or the methanolic solutions were separated in two portions on cellulose columns (4-7). The resulting fractions were concentrated by vacuum evaporation at 35°C, dissolved in little methanol and precipitated with ether. The picrates cristallized overnight from a cold saturated methanolic solution of picric acid and a methanolic solution of the nucleosides.

5-Formamido-1-(ß-D-ribofuranosyl)imidazole-4-carboxamide 4:

A colourless, amorphous product, precipitated from methanolic solution by the addition of ether, yield 100 mg (17.5%).

$[\alpha]_{578}^{22}$ -35°, $[\alpha]_{546}^{22}$ -38°, (*c* 1, in H_2O). UV (λ,ε): 205 nm (max) 9 260, 236 nm (max) 6 110. ^{1}H NMR (D_2O): 8.27 (s, 1 H, CHO), 7.86 (s, 1 H, H-2). 5.71 (d, 1 H, H-1', J = 2.4 Hz,4.58 (m, 1 H, H-2'),4.33 (m, 1 H, H-3'),4.22 (m, 1 H, H-4'), 3.83 (m, 2 H, H-5', H-5'').

Picrate: $C_{16}H_{17}O_{13}N_7$ (515.34) Calcd. C 37.29 H 3.32 N 19.02

Found C 37.24 H 3.34 N 18.92. M.p. 190°C (dec.)

5-Formamido-1-(α-D-arabinofuranosyl)imidazole-4-carboxamide 5:
A colourless, amorphous product, precipitated from methanolic solution by the addition of ether, yield 130 mg (22.7%).
$[\alpha]_{578}^{22}$ +53°, $[\alpha]_{546}^{22}$ +58°, (*c* 1, in H_2O). UV (λ,ε): 205 nm (max) 10 300, 236 nm (max) 6 160. ^{1}H NMR (D_2O): 8.38 and 8.22 (2s, 1 H, CHO), 8.13 and 8.02 (2 s, 1 H, H-2),5.76 (d, 1 H, H-1', J = 1.9 Hz),4.62 (m, 1 H, H-2'), 4.28 (m, 2 H, H-3', H-4'), 3.82 (m, 2 H, H-5', H-5''). Picrate: $C_{16}H_{17}O_{13}N_7$ (513.34) Calcd. C 37.29 H 3.32 N 19.02 Found C 37.53 H 3.47 N 18.96. M.p. 170 °C (dec.).

5-Formamido-1-(α-L-arabinofuranosyl)imidazole-4-carboxamide 6:
A colourless, amorphous product, precipitated from methanolic solution by the addition of ether, yield 130 mg (22.7%).
$[\alpha]_{578}^{22}$ -42°, $[\alpha]_{546}^{22}$ -45°, (*c* 1, in H_2O) UV (λ,ε) : 205 nm (max) 8 920, 236 nm (max) 4 290. ^{1}H NMR (D_2O): 8.38 and 8.22 (2 s, 1 H,CHO), 8.12 and 8.04 (2 s, 1 H, H-2),5.68 (d, 1 H, H-1', J = 1.9 Hz),4.49 (m,1 H, H-2'),4.28 (m, 2 H, H-3', H-4'), 3.82 (m, 2 H, H-5', H-5''). Picrate: $C_{16}H_{17}O_{13}N_7$ (513.34) Calcd. C 37.29 H 3.32 N 19.02 Found C 37.30 H 3.41 N 19.08. M.p. 170°C (dec.).

5-Formamido-1-(ß-D-xylofuranosyl)imidazole-4-carboxamide 7:
A colourless, amorphous product, precipitated from methanolic solution by the addition of ether, yield 110 mg (19%).
$[\alpha]_{578}^{22}$ -33°, $[\alpha]_{546}^{22}$ -40°, (*c* 1 in H_2O). UV (λ,ε): 205.5 nm (max) 9 360, 226 nm (min) 690, 236.5 nm (max) 7 200. ^{1}H NMR (D_2O):8.43 and 8.27 (2 s, 1 H, CHO) 8.11 and 8.04 (2 s, 1 H, H-2), 5.68 (d, 1 H, H-1', J = 0.8 Hz), 4.59 (m, 1 H, H-2'), 4.41 (m, 2 H, H-3',H-4'), 4.00 (m, 2 H, H-5', H-5''). Picrate: $C_{16}H_{17}O_{13}N_7$ (513.34) Calcd. C 37.29 H 3.32 N 19.02 Found C 37.16 H 3.31 N 18.93. M.p. 185°C (dec.).

4-Formamido-1-(ß-D-ribopyranosyl)imidazole-5-carboxamide 8:
The reaction mixture was worked up as described, the product was precipitated with ether and recristallized from methanol. It was slightly soluble in water.
M.p. 206°C, yield 350 mg (61%).
$[\alpha]_{578}^{22}$ +85°, $[\alpha]_{546}^{22}$ +100°, (*c* 1, in H_2O). UV (λ,ε): 208 nm (max) 12 560, 228 nm (min) 5 760, 254 nm (max) 7 680.^{1}H NMR (D_2O):8.34

(s,1 H,CHO),8.08 (s,1 H, H-2), 5.84 (d, 1 H, H-1', J = 9.9 Hz). $C_{10}H_{14}O_6N_4$ (286.24) Calcd. C 41.96 H 4.93 N 19.57 Found C 41.89 H 4.86 N 19.06.

4-Formamido-1-(ß-D-arabinopyranosyl)imidazole-5-carboxamide 9:
The reaction mixture was worked up as described, the product was precipitated with ether and recrystallized from methanol. It is slightly soluble in water.
M.p.183°C, yield 260 mg (45%).
$[\alpha]^{35}_{578}$ +100°, $[\alpha]^{35}_{546}$ +116° (*c* 1, in H_2O). UV (λ,ε):211 nm (max) 13 480, 230 nm (min) 3 920, 256 nm (max) 9 200. ^{1}H NMR (D_2O): 8.35 (s,1 H, CHO), 8.05 (s, 1 H, H-2), 6.13 (s,1-H,H-1'). $C_{10}H_{14}O_6N_4$ (286.24) Calcd. C 41.96 H 4.93 N 19.57 Found C 41.72 H 4.88 N 19.69.

4-Formamido-1-(α-D-arabinopyranosyl)imidazole-5-carboxamide 10:
The reaction mixture was worked up as described, the product was precipitated with ether and recrystallized from methanol. M.p. 223°C (dec.), yield 400 mg (70%).
$[\alpha]^{22}_{578}$ -47°, $[\alpha]^{22}_{546}$ -49°, (*c* 1 in H_2O). UV (λ,ε): 207 nm (max) 12 720, 229 nm (min) 6 160, 258 nm (max) 8 600. ^{1}H NMR (D_2O): 8.67 and 8.17 (2 s,1 H,CHO), 7.92 and 7.75 (2 s, 1 H, H-2), 5.46 and 5.31 (2 d,1 H, H-1', J = 9.3 Hz). $C_{10}H_{14}O_6N_4$ (286.24), Calcd. C 41.96 H 4.93 N 19.57 Found C 42.08 H 4.95 N 19.42.

4-Formamido-1-(α-L-arabinopyranosyl)imidazole-5-carboxamide 11:
The reaction mixture was worked up as described, the product was precipitated with ether and recrystallized from methanol. M.p. 224 °C (dec.), yield 390 mg (68%).
$[\alpha]^{22}_{578}$ +46°, $[\alpha]^{22}_{546}$ + 53°, (*c* 1, H_2O). UV (λ,ε): 208 nm (max) 12 360, 229 nm (min) 6 240, 257 nm (max) 8 400. ^{1}H NMR (D_2O): 8.69 and 8.19 (2 s,1 H,CHO), 7.95 and 7.81(2 s, 1 H, H-2), 5.45 and 5.38 (2 d,1 H, H-1', J = 9.4 Hz). $C_{10}H_{14}O_6N_4$ (286.24), Calcd. C 41.96 H 4.93 N 19.57 Found C 41.85 H 4.84 N 19.74.

Methyl-4-nitro-1-(2 ,3 ,4 -tri-O-acetyl-ß-D-arabinopyranosyl)-imidazole-carboxylate 12:
In an earlier publication[10] we have described the condensation of the mercury complex of methyl-4(5)nitroimidazole-5(4)-carboxylate with

2,3,4-tri-O-acetyl-ß-D-arabinopyranosyl-bromide. The workup and isolation, performed as described yielded methyl-4-nitro-1-(2',3',4'-tri-O-acetyl-α-D-arabinopyranosyl)imidazol-5-carboxylate 13. A new t.l.c. examination (system C) of the mother liquors revealed the presence of the ß-isomer (R_f = 0.72) and further α-isomer (R_f = 0.6). They were separated on preparative t.l.c. plates with system C as the solvent system. The upper band yielded after elution with $CHCl_3$ and cristallization from ethanol methyl-4-nitro-1-(2',3',4'-tri-O-acetyl-ß-D-arabinopyranosyl)imidazole-5-carboxylate 12, the ß-isomer of 13. M.p. 134°C, yield 500 mg (11.6%).
$[\alpha]_{578}^{22}$ +9.5°, $[\alpha]_{546}^{22}$ +9°, (*c* 2, in $CHCl_3$). UV (λ,ε):274 nm (max) 2 920, 256 nm (min) 2 670 in ethanol. ^{1}H NMR ($CDCl_3$): 7.76 (s, 1 H, H-2), 6.33 (d, 1 H, H-1', J = 1.3 Hz), 5,53 (m, 3 H, H-2',H-3',H-4'), 4.15 (m, 2 H,H-5', H-5''), 3.91(s, 3 H,CH_3),2.24 (s, 3 H, Ac), 2.05) (s, 3 H, Ac), 2.01 (s, 3 H, Ac). $C_{16}H_{19}O_{11}N_3$ (429.3), Calcd. C 44.77 H 4.46 N 9.79 Found C 44.77 H 4.50 N 9.74.

4-Nitro-1-(ß-D-arabinopyranosyl)imidazole-5-carboxamide 14:
2.15 g 12 (5 mmol) were treated with methanolic ammonia (150 ml) at 0°C for 50 hours. The solution was evaporated to a gum, which did not cristallize. A solution of the gum in 10 ml methanol was triturated with ether to give a light yellow, amorphous product, yield 1.2 g (83%).
$[\alpha]_{578}^{22}$ -24°, $[\alpha]_{546}^{22}$ -28 °, (*c* 2 in H_2O). UV (λ,ε): 296 nm (max) 3 800, 258.5 nm (min) 2 250. ^{1}H NMR (D_2O): 7.93 and 7.76 (2 s, 1 H, H-2), 5.77 and 5.70 (2 s, 1 H, H-1'), 4.03 (m, 3 H, H-2', H-3', H-4'), 3.74 (m, 2 H, H-5', H-5''). $C_9H_{12}O_7N_4$ (288.2), Calcd. C 37.51 H 4.19 N 19.55 Found C 37.61 H 4.35 N 19.23.

4-Amino-1-(ß-D-arabinopyranosyl)imidazole-5-carboxamide 15:
1.44 g 14 (5 mmol) were dissolved in 100 ml freshly destilled methanol and hydrogenated with a platinium catalyst as described[8]. The catalyst was removed by filtration and the filtrate evaporated to a gum, which did not cristallize. Addition of 100 ml absolute ether caused precipitation of the amorphous product, yield 1.1 g (86%).
$[\alpha]_{578}^{22}$ +42°, $[\alpha]_{546}^{22}$ +51 °, (*c* 1 in H_2O). UV (λ,ε): 275 nm (max) 2 680, 240 nm (min) 1 070. ^{1}H NMR (D_2O): 7.76 (s, 1 H,H-2), 5.98 (s, 1 H,

H-1'), 3.96 (m, 3 H,H-2',H-3',H-4'), 3.77 (m, 2 H,H-5', H-5''). Picrate: $C_{15}H_{17}O_{12}N_7$ (487.34) Calcd. C 36.97 H 3.51 N 20.11. Found C 36.96 H 3.61 N 19.96. M.p. 190°C (dec.)

The authors express sincere thanks to Mrs. Ulrike Wöll for expert assistance.

REFERENCES

1. Flaks, J.G., Erwin, M.J., and Buchanan, J.M., *J. Biol. Chem.* **1957**, *229*, 603-612
2. Geiger, R., and Guglielmi, H., *Hoppe-Seyler's Z. Physiol. Chem.* **1975**, *356*, 819-825
3. Bencovic, S.J., *Trends Biochem. Sci.* **1984**, *9*, 320-322
4. Flannigan, K.A., Hennigan, S.H., Vogelbacker, H.H.,Gots, J.S., and Smith, J.M, *J. Mol. Microbiol.* **1990**, *4*, 381-392
5. Shaw, E., *J. Biol. Chem.* **1950**, *185*, 439-449
6. Baggott, J.E., and Krumdieck, C.L., *Biochemistry* **1979**, *18*, 3501-3506
7. Mueller, W.Th., and Benkovic, S.J., *Biochemistry* **1981**, *20*, 337-344
8. Guglielmi, H., and Jung, A., *Hoppe-Seyler's Z. Physiol. Chem.* **1977**, *358*, 1463-1468
9. Guglielmi, H., *Liebigs Ann. Chem.* **1973**,1286-1293
10. Ferris, J.P., Devadas, B., Huang, C.-H., and Ren, W.-Y., *J. Org. Chem.* 1985, *50*, 747-754
11. Mackenzie, G., Shaw, G., and Robinson, D.H., *J. C. S. Perkin I* 1977, 1094-1103
12. Pauly, H., *Hoppe-Seyler's Z. Physiol. Chem.* **1904**, *42*, 508
13. Bratton, A.C., and Marshall, E.K., *J. Biol. Chem.* **1939**, *128*, 537-588

Received 3/16/92
Accepted 11/23/92