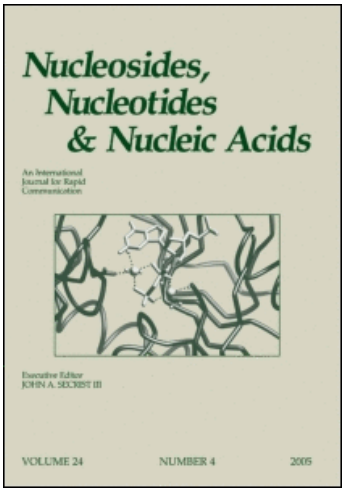

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Nucleosides and Nucleotides. 140. Synthesis and Antileukemic Activity of 5-Carbon-Substituted 1-β-d-Ribofuflranosylimidazole-4-Carboxamides

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NUCLEOSIDES AND NUCLEOTIDES. 140. SYNTHESIS AND ANTILEUKEMIC ACTIVITY OF 5-CARBON-SUBSTITUTED 1-β-D-RIBOFURANOSYLIMIDAZOLE-4-CARBOXAMIDES[#, 1]

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Abstract: Synthesis of 5-carbon-substituted 1-β-D-ribofuranosylimidazole-4-carboxamides are described. Treatment of 5-iodo derivative **8** with methyl acrylate in the presence of palladium catalyst gave (*E*)-5-(2-carbomethoxyvinyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**9**), followed by appropriate manipulations to afford various 5-carbon-substituted imidazole derivatives **1-7**. The antileukemic activities of these imidazole nucleosides are also described.

The nucleosides with a five-membered heterocycle as the base moiety, such as tiazofurin,[2] ribavirin,[3] and bredinin,[4] have a broad spectrum of biological activities, such as antitumor, antiviral, and immunosuppressive activities. Since these nucleosides are structurally similar to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR), the 5'-monophosphate of which is a key intermediate in purine nucleotide biosynthesis, all of these nucleoside 5'-monophosphates are potent inhibitors of inosine

[#]This paper is dedicated to Dr. Yoshihisa Mizuno on the occasion of his 75th birthday.

5'-monophosphate (IMP) dehydrogenase. This enzyme is one of the key rate-controlling enzymes of *de novo* biosynthesis of purine nucleotides, in particular guanine nucleotides and has been considered as a target enzyme for anticancer chemotherapy[5] as well as antiviral[6] and antiparasitic[2] chemotherapy.

In our previous papers,[7] we reported the synthesis and antitumor as well as antiviral activities of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides. Among the alkynyl derivatives, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) proved to be the most active derivative. The antitumor and antiviral activities of EICAR would be related to the inhibition of IMP dehydrogenase and it proved to be one of the most potent inhibitors of the enzyme described thus far.[8] Furthermore, as a preliminary result, it has become apparent that the ethynyl group at the 5-position is important for the activity since the 5-vinyl derivative did not show any antileukemic activity.[7a] These results prompt us to synthesize imidazole nucleosides with other carbon substituents at the 5-position instead of the ethynyl group and evaluate their biological activities. In this paper, we describe the synthesis and antileukemic activity of 5-carbon-substituted 1-β-D-ribofuranosylimidazole-4-carboxamides **1-7** to clarify the structural requirements at the 5-position for the activity (Fig. 1). The synthesis of 5-substituted derivatives involving nitro,[9] triazino,[10] halogeno,[11] and sulfur[12] groups have been reported. For carbon-substituted derivatives, only 5-methyl[12] and 5-cyanomethyl[13] derivatives have been reported to date.

For the synthesis of 5-carbon-substituted 1-β-D-ribofuranosylimidazole-4-carboxamides, we first attempted direct introduction of formyl[14] or cyano[15] group at the 5-position using a reaction of the 5-iodo derivative **8**[7a] under cross-coupling conditions. However, these attempts were unfruitful and resulted in recovery of the starting material or dehalogenation. Therefore, we next examined ozonolysis of (*E*)-5-(2-carbomethoxyvinyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (**9**),[16] followed by appropriate manipulations for the synthesis of target nucleosides (Scheme I). Treatment of 5-iodo derivative **8** with methyl acrylate in the presence of bis(benzonitrile)palladium dichloride gave **9** in 65% yield. The *E*-geometry of **9** was identified by the analysis of its ^{1}H-NMR spectrum, in which the coupling constant of the olefin protons was 16.4 Hz. Ozonolysis of **9** in dichloromethane, followed by reduction with sodium cyanoborohydride under acidic conditions gave hydroxymethyl

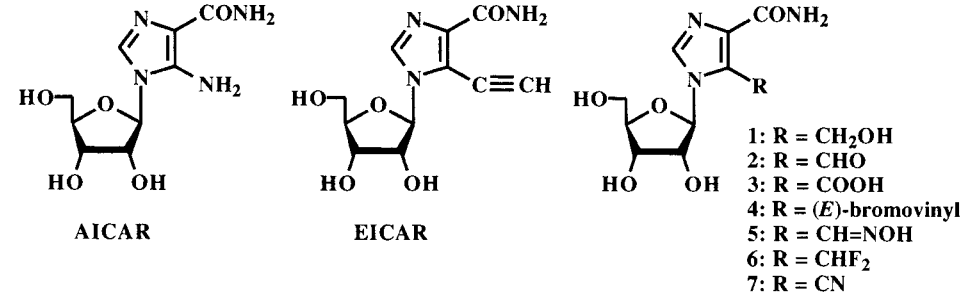
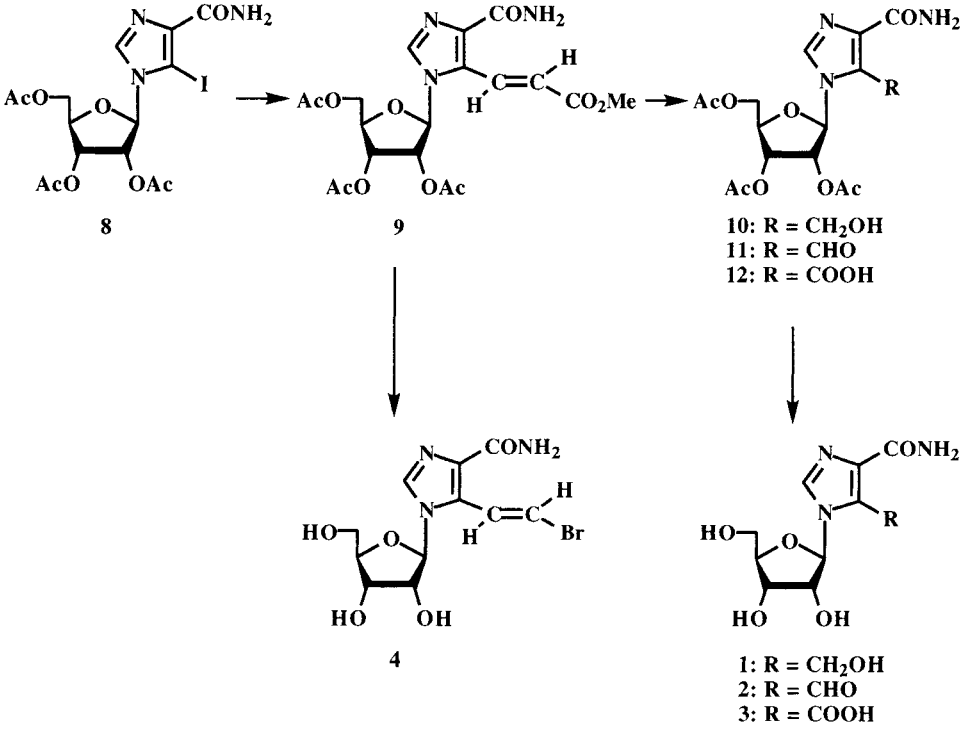


Fig. 1



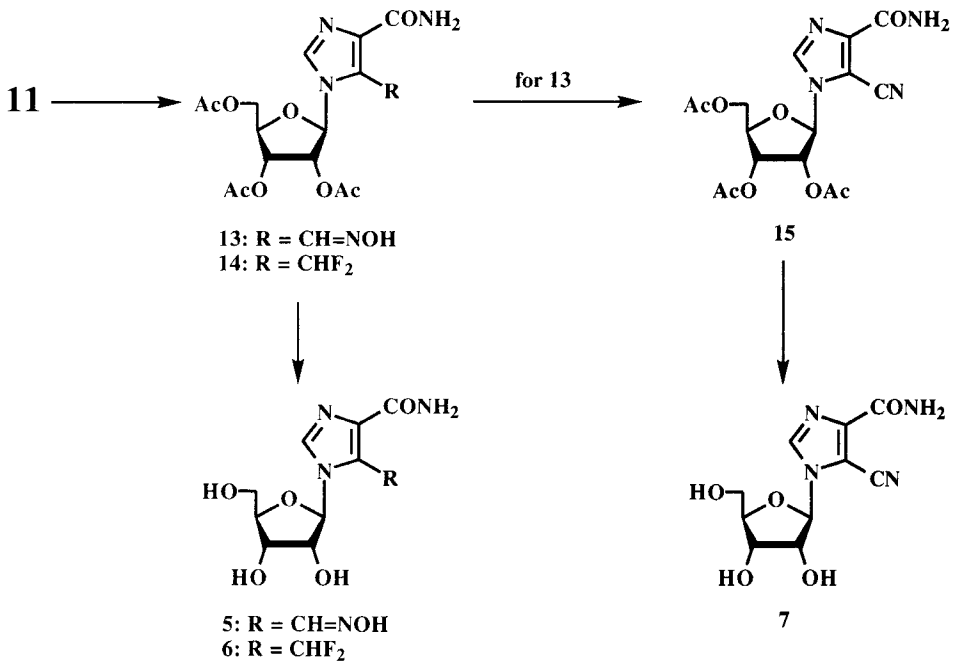
Scheme I

derivative **10** in 65% yield. When **9** was ozonated in dichloromethane, followed by reduction with dimethyl sulfide, formyl derivative **11** along with carboxy derivative **12** were obtained in 54% and 30% yields, respectively. However, from the ozonolysis of **9** in a mixture of dichloromethane and methanol, **11** was only obtained in 85% yield. Deprotection of **10-12** by NH_3/MeOH or NaOMe gave free nucleosides **1-3** in good yields. Compound **9** was further converted to (*E*)-bromovinyl derivative **4** by treatment with *N*-bromosuccinimide after hydrolysis with aqueous alkali.

Treatment of **11** with hydroxylamine hydrochloride in pyridine gave hydroxyiminomethyl derivative **13** in 69% yield, which was converted into cyano derivative **15** in good yield on treatment with phenyl chloroformate, followed by heating at 100 °C. Difluoromethyl derivative **14** was obtained in 70% yield when **11** was treated with diethylaminosulfur trifluoride in dichloromethane. Deprotection of **13-15** by NH_3/MeOH or Et_3N/MeOH gave free nucleosides **5-7** in good yields (Scheme II).

The antileukemic activity of the synthetic nucleosides toward murine L1210 cells in culture is summarized in Table 1. Data for EICAR is also shown for comparison. These nucleosides except **1** and **2** did not show any significant tumor cell growth inhibitory activity up to 100 μg/mL, while **1** and **2** had IC_{50} values of 19 and 18.5 μg/mL, respectively. However, **1** and **2** were almost 100 times less potent than EICAR. In a previous study,[7a] we examined antileukemic activity of 5-alkynyl-4-carboxamide derivatives, in which even the 5-phenylethynyl derivative had an IC_{50} of 20.6 μg/mL. As can be seen from these results, the alkynyl group, especially an ethynyl group at the 5-position, is important for the activity arising from inhibition of IMP dehydrogenase.

We have already suggested that EICAR would act as an alkylating agent of the active site of IMP dehydrogenase and demonstrated that it reacted with NaSMe in MeOH to give (Z)-5-(2-methylthiovinyl)-1-β-D-ribofuranosylimidazole-4-carboxamide, as a preliminary experiment.[7a] Quite recently, Antonino *et al.*[17] proposed that one possible mechanism for IMP dehydrogenase involves nucleophilic attack at the C2-position of IMP by one cysteine residue, Cys-331 of IMP dehydrogenase. Though it is not known whether the cysteine residue is alkylated by the ethynyl group of EICAR, the reactive site of EICAR, that is the terminal position of the ethynyl group, would be expected to be spatially close to the C2-position of IMP. On the other hand, the nucleosides synthesized in this study do not have an alkylating site close to the Cys-331.



Scheme II

Table 1. Inhibitory Effects of 5-Ethynylimidazole Derivatives on the Growth of Murine Leukemia L1210 Cells in Culture[8b]

compds	R	IC_{50}, μg/mL
EICAR	ethynyl	0.18
1	CH_2OH	19
2	CHO	18.5
3	COOH	>100
4	(*E*)-bromovinyl	>100
5	CH=NOH	>100
6	CHF_2	>100
7	CN	>100

In the case of **2** and **7**, the substituents at the 5-position are expected to have alkylating ability, however the reactive site of these nucleosides would be apart from the Cys-331. Furthermore, it is not known whether these nucleosides are metabolized to 5'-monophosphate derivatives by cellular kinase(s), though EICAR is metabolized to its 5'-monophosphate mainly by adenosine kinase.[8b] The less potency of these nucleosides **1**-**7** might be considered in terms of these factors. The detailed structure-activity relationships for substituents at the 5-position, including activation by cellular kinase(s) is a subject for further studies.

Experimental Section

Physical data were measured as follows: Melting points were measured on a Yanagimoto Mp-3 micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on JEOL JNM FX-100 or JEOL GX-270 instruments in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. UV spectra were recorded with a Shimadzu UV-260 spectrophotometer. Mass spectra were recorded on a JEOL JMS DX-303 or JEOL JMS HX-110 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. Silica gel used for column chromatography was YMC gel 60A (70-230 mesh).

(*E*)-5-(2-Carbomethoxyvinyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole-4-carboxamide (9). A mixture of **8** (495 mg, 1.0 mmol), bis(benzonitrile)-palladium dichloride (36 mg, 10 mol%), triethylamine (0.16 mL, 1.2 mmol), and methyl acrylate (0.18 mL, 2.0 mmol) in dry acetonitrile (5 mL) was heated at 100 °C for 16 h in a sealed glass tube. The reaction mixture was filtered through a Celite pad and washed with EtOH. The combined filtrate and washings were concentrated *in vacuo* and the residue was purified on a silica gel column (2.3 x 14 cm), eluted with hexane/AcOEt (1:2-1:3), to give **9** (293 mg, 65%, crystallized from $CHCl_3$-Et_2O): mp 161 °C; EI-MS *m/z* 453 (M^+); ^{1}H-NMR ($CDCl_3$) 8.06 (d, 1 H, vinyl proton, J = 16.4 Hz), 7.83 (s, 1 H, H-2), 7.16 (br s, 1 H, NH), 6.94 (d, 1 H, vinyl proton, J = 16.4 Hz), 5.96 (d, 1 H, H-1', $J_{1',2'}$ = 5.6 Hz), 5.57 (t, 1 H, H-2', $J_{2',1'} = J_{2',3'}$ = 5.6 Hz), 5.49 (br s, 1 H, NH), 5.42 (dd,

1 H, H-3', $J_{3',2'}$ = 5.6, $J_{3',4'}$ = 3.4 Hz), 4.48~4.34 (m, 3 H, H-4', 5'a, b), 3.82 (s, 3 H, methyl), 2.16, 2.15, 2.11 (each s, each 3 H, acetyl). *Anal.* Calcd for $C_{19}H_{23}N_3O_{10}$: C, 50.33; H, 5.11; N, 9.27. Found: C, 50.16; H, 5.05; N, 9.20.

5-Hydroxymethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (10). A dry CH_2Cl_2 solution (20 mL) of **9** (500 mg, 1.1 mmol) was cooled at -78 °C and ozonized oxygen was bubbled through this solution for 15 min at -78 °C. After the starting material was completely consumed, N_2 gas was bubbled through the solution to remove excess ozonized oxygen. A suspension of sodium cyanoborohydride (274 mg, 4.4 mmol) in acetic acid (4 mL) was added to the solution at -78 °C and then the reaction mixture was allowed to warm to room temperature and was stirred for 30 min. The reaction mixture was diluted with $CHCl_3$ (100 mL), which was washed with saturated aqueous $NaHCO_3$, followed by brine. The separated organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified on a silica gel column (2.7 x 15 cm), eluted with 0-4% EtOH in $CHCl_3$, to give **10** (285 mg, 65%) as a yellow oil: EI-MS *m/z* 399 (M^+); ^{1}H-NMR ($CDCl_3$) 7.68 (s, 1 H, H-2), 7.16 (br s, 1 H, NH), 5.91 (d, 1 H, H-1', $J_{1',2'}$ = 6.0 Hz), 5.81 (br s, 1 H, NH), 5.47 (dd, 1 H, H-2', $J_{2',1'}$ = 6.0, $J_{2',3'}$ = 5.5 Hz), 5.40 (dd, 1 H, H-3', $J_{3',2'}$ = 5.5, $J_{3',4'}$ = 3.8 Hz), 5.27 (br t, 1 H, CH_2OH), 4.88 (m, 2 H, CH_2OH), 4.40 (m, 3 H, H-4', 5'a, b), 2.15 (s, 6 H, acetyl x 2), 2.11 (s, 3 H, acetyl).

5-Hydroxymethyl-1-β-D-ribofuranosylimidazole-4-carboxamide (1). Compound **10** (240 mg, 0.60 mmol) was dissolved in NH_3/MeOH (saturated at 0 °C, 10 mL), and the mixture was kept for 1.5 h at room temperature. The solvent was removed *in vacuo* and the residue was purified on a silica gel column (2.7 x 5 cm), eluted with 5-40% EtOH in $CHCl_3$, to give **1** (137 mg, 83%, crystallized from EtOH): mp 154-155 °C; FAB-MS *m/z* 274 (M^++1), 547 ($2M^+$+1); UV λ_{max}(H_2O) 237 nm (ε 9300); UV λ_{max} (0.5 N NaOH) 240 nm (ε 9900); ^{1}H-NMR (DMSO-d_6) 7.99 (s, 1 H, H-2), 7.41, 7.20 (each br s, each 1 H, NH_2), 5.74 (d, 1 H, H-1', $J_{1',2'}$ = 4.9 Hz), 5.49 (dd, 1 H, CH_2OH, J = 6.1, 5.5 Hz), 5.45 (d, 1 H, 2'-OH, $J_{2'\text{-OH},2'}$ = 6.0 Hz), 5.16 (d, 1 H, 3'-OH, $J_{3'\text{-OH},3'}$ = 5.5 Hz), 5.05 (dd, 1 H, 5'-OH, $J_{5'\text{-OH},5'a}$ = 4.9, $J_{5'\text{-OH},5'b}$ = 5.5 Hz), 4.93 (dd, 1 H, C*H*aOH, J = 5.5, 13.2 Hz), 4.76 (dd, 1 H, C*H*bOH, J = 6.1, 13.2 Hz), 4.16 (ddd, 1 H, H-2', $J_{2',1'}$ = 4.9, $J_{2',2'\text{-OH}}$ = 6.0, $J_{2',3'}$ = 5.5 Hz), 4.05 (dt, 1 H, H-3', $J_{3',2'}$ = $J_{3',3'\text{-OH}}$ = 5.5, $J_{3',4'}$ = 5.0 Hz), 3.90 (ddd, 1 H, H-4', $J_{4',3'}$ = 5.0, $J_{4',5'a}$ = 3.9, $J_{4',5'b}$ = 3.3 Hz), 3.66 (ddd, 1 H, H-5'a,

$J_{5'a, 4'}$ = 3.9, $J_{5'a, 5'b}$ = 12.1, $J_{5'a, 5'-OH}$ = 4.9 Hz), 3.56 (ddd, 1 H, H-5'b, $J_{5'b, 4'}$ = 3.3, $J_{5'b, 5'a}$ = 12.1, $J_{5'b, 5'-OH}$ = 5.5 Hz). *Anal.* Calcd for $C_{10}H_{15}N_3O_6$: C, 43.96; H, 5.53; N, 15.38. Found: C, 43.96; H, 5.66; N, 15.20.

5-Formyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (11) and 4-carbamoyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-5-carboxylic acid (12). A dry CH_2Cl_2 solution (60 mL) of **9** (1.99 g, 4.4 mmol) was cooled at -78 °C and ozonized oxygen was bubbled through this solution for 20 min at -78 °C. After the starting material was completely consumed, N_2 gas was bubbled through the solution to remove excess ozonized oxygen. Dimethyl sulfide (0.55 mL, 7.5 mmol) was added to the solution at -78 °C and then the reaction mixture was allowed to warm to room temperature. The solvent was removed *in vacuo* and the residue was purified on a silica gel column (3.5 x 12 cm), eluted with hexane/AcOEt (1:1-1:3), to give **11** (990 mg, 54%, crystallized from EtOH-hexane) and **12** (268 mg, 30%, crystallized from EtOH).

Physical data for **11**: mp 143-144 °C; FAB-MS *m/z* 398 (M^++1); ^{1}H-NMR ($CDCl_3$) 10.60 (s, 1 H, CHO), 8.13 (s, 1 H, H-2), 7.20 (br s, 1 H, NH), 6.52 (d, 1 H H-1', $J_{1', 2'}$ = 2.7 Hz), 5.62 (br s, 1 H, NH), 5.40 (dd, 1 H, H-2', $J_{2', 1'}$ = 2.7, $J_{2', 3'}$ = 5.5 Hz), 5.31 (dd, 1 H, H-3', $J_{3', 2'}$ = 5.5, $J_{3', 4'}$ = 7.7 Hz), 4.43 (m, 3 H, H-4', 5'a, b), 2.19 (s, 6 H, acetyl x 2), 2.08 (s, 3 H, acetyl). *Anal.* Calcd for $C_{16}H_{19}N_3O_9$: C, 48.36; H, 4.82; N, 10.58. Found: C, 48.36; H, 4.82; N, 10.43.

Physical data for **12**: mp 164-165 °C; FAB-MS *m/z* 414 (M^++1); ^{1}H-NMR ($CDCl_3$) 16.16 (s, 1 H, COOH), 8.19 (s, 1 H, H-2), 7.64 (br s, 1 H, NH), 6.76 (d, 1 H H-1', $J_{1', 2'}$ = 1.8 Hz), 6.18 (br s, 1 H, NH), 5.48 (dd, 1 H, H-2', $J_{2', 1'}$ = 1.8, $J_{2', 3'}$ = 5.2 Hz), 5.29 (dd, 1 H, H-3', $J_{3', 2'}$ = 5.2, $J_{3', 4'}$ = 8.2 Hz), 4.49 (m, 1 H, H-4'), 4.45 (m, 2 H, H-5'a, b), 2.19, 2.18, 2.06 (each s, each 3 H, acetyl). *Anal.* Calcd for $C_{16}H_{19}N_3O_{10}$: C, 46.49; H, 4.63; N, 10.17. Found: C, 46.47; H, 4.65; N, 10.09.

In the same manner as described above, ozonolysis of **9** (453 mg, 1.0 mmol) in MeOH (20 mL)-CH_2Cl_2 (10 mL) gave **11** (337 mg, 85%).

5-Formyl-1-β-D-ribofuranosylimidazole-4-carboxamide (2). A MeOH solution of 1 N NaOMe (50 μL) was added to a solution of **11** (200 mg, 0.5 mmol) in MeOH (5 mL). After 30 min, a MeOH solution of 1 N NaOMe (100 μL) was added

and the mixture was stirred for 1 h. The mixture was neutralized with aqueous 1 N HCl and concentrated *in vacuo*. The residue was purified on a silica gel column (1.7 x 15 cm), eluted with 5-30% EtOH in $CHCl_3$, to give **2** (97 mg, 71%, crystallized from EtOH): mp 184-187 °C; FAB-MS *m/z* 272 (M^++1), 543 ($2M^+$+1); UV λ_{max} (H_2O) 276 nm (ε 9800); UV λ_{max} (0.5 N NaOH) 245 nm (ε 8300); ^{1}H-NMR (DMSO-d_6) 10.47 (s, 1 H, CHO), 8.60 (s, 1 H, H-2), 7.83, 7.65 (each br s, each 1 H, NH_2), 6.23 (d, 1 H, H-1', $J_{1',2'}$ = 2.2 Hz), 5.47 (d, 1 H, 2'-OH, $J_{2'\text{-OH},2'}$ = 4.9 Hz), 5.17 (t, 1 H, 5'-OH, $J_{5'\text{-OH},5'a}$ = $J_{5'\text{-OH},5'b}$ = 4.9 Hz), 5.07 (d, 1 H, 3'-OH, $J_{3'\text{-OH},3'}$ = 5.5 Hz), 4.07 (m, 2 H, H-2', 3'), 3.92 (m, 1 H, H-4'), 3.76 (m, 1 H, H-5'a), 3.60 (m, 1 H, H-5'b). *Anal.* Calcd for $C_{10}H_{13}N_3O_6$: C, 44.28; H, 4.83; N, 15.49. Found: C, 43.93; H, 4.94; N, 15.27.

4-Carbamoyl-1-β-D-ribofuranosylimidazole-5-carboxylic acid (3). A MeOH solution of 28% NaOMe (0.6 mL) was added to a suspension of **12** (413 mg, 1.0 mmol) in MeOH (20 mL) and the whole was stirred for 15 min. The mixture was neutralized by addition of Dowex 50 (H^+ form) and the resin was filtered and washed with H_2O and MeOH. The combined filtrate and washings were concentrated to dryness *in vacuo* to give **3** (211 mg, 74%, crystallized from EtOH-H_2O): mp 195 °C (colored); FAB-MS *m/z* 288 (M^++1); UV λ_{max} (H_2O) 250 nm (ε 6800); UV λ_{max} (0.5 N NaOH) 249 nm (ε 7800); ^{1}H-NMR (DMSO-d_6) 17.25 (br s, 1 H, COOH), 8.89, 8.80 (each br s, each 1 H, NH_2), 8.76 (s, 1 H, H-2), 6.55 (d, 1 H, H-1', $J_{1',2'}$ = 1.6 Hz), 5.53, 5.25, 5.05 (each br s, each 1 H, 2', 3', 5'-OH), 4.06 (m, 2 H, H-2', 3'), 3.93 (m, 1 H, H-4'), 3.78 (m, 1 H, H-5'a, $J_{5'a,4'}$ = 2.1, $J_{5'a,b}$ = 12.3 Hz), 3.63 (m, 1 H, H-5'b, $J_{5'b,4'}$ = 2.2, $J_{5'b,a}$ = 12.3 Hz). *Anal.* Calcd for $C_{10}H_{13}N_3O_7$: C, 41.82; H, 4.56; N, 14.63. Found: C, 41.73; H, 4.58; N, 14.49.

(*E*)-5-(2-Bromovinyl)-1-β-D-ribofuranosylimidazole-4-carboxamide (4). Aqueous 0.5 N NaOH (7 mL) was added to a solution of **9** (480 mg, 1.06 mmol) in MeOH (7 mL) and the mixture was stirred for 2 h at room temperature. The mixture was neutralized with aqueous 1 N HCl and concentrated *in vacuo*. The residue was dissolved in H_2O (20 mL) by heating with sodium acetate (174 mg, 2.12 mmol) for 5 min at 100 °C. While it was still hot (about at 60 °C), *N*-bromosuccinimide (189 mg, 1.06 mmol) was added in small portions to the mixture, which was stirred at room temperature. After 3 h, the mixture was heated at 60 °C again and more *N*-bromosuccinimide (189 mg, 1.06 mmol) was added in small portions to the mixture,

which was stirred for 2.5 h more at room temperature. The solvent was removed *in vacuo* and the residue was purified on a silica gel column (2.7 x 10 cm), eluted with 5-40% EtOH in $CHCl_3$, to give **4** (308 mg, 84%) as a white solid. This was further purified by a HPLC (YMC-D-ODS-5, flow 8 mL/min, retention time 20 min) with 30% MeOH-H_2O as eluent: FAB-MS *m/z* 348, 350 (M^++1); UV λ_{max}(H_2O) 274 nm (ε 10200); UV λ_{max}(0.5 N NaOH) 277 nm (ε 10600); ^{1}H-NMR (DMSO-d_6) 8.14 (s, 1 H, H-2), 7.63 (d, 1 H, vinyl proton, J = 14.3 Hz), 7.48 (br s, 1 H, NH), 7.45 (d, 1 H, vinyl proton, J = 14.3 Hz), 7.27 (br s, 1 H, NH), 5.62 (d, 1 H, 2'-OH, $J_{2'\text{-OH}, 2'}$ = 6.2 Hz), 5.60 (d, 1 H, H-1', $J_{1', 2'}$ = 5.5 Hz), 5.28 (d, 1 H, 3'-OH, $J_{3'\text{-OH}, 3'}$ = 5.5 Hz), 5.09 (t, 1 H, 5'-OH, $J_{5'\text{-OH}, 5'a} = J_{5'\text{-OH}, 5'b}$ = 5.3 Hz), 4.29 (ddd, 1 H, H-2', $J_{2', 1'}$ = 5.5, $J_{2', 2'\text{-OH}}$ = 6.2, $J_{2', 3'}$ = 5.1 Hz), 4.06 (ddd, 1 H, H-3', $J_{3', 2'}$ = 5.1, $J_{3', 3'\text{-OH}}$ = 5.5, $J_{3', 4'}$ = 3.7 Hz), 3.97 (m, 1 H, H-4'), 3.68~3.51 (m, 2 H, H-5'a, b). *Anal.* Calcd for $C_{11}H_{14}BrN_3O_5$: C, 37.95; H, 4.05; Br, 22.95; N, 12.07. Found: C, 38.14; H, 3.98; Br, 22.60; N, 11.91.

5-Hydroxyiminomethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (13). Hydroxylamine hydrochloride (168 mg, 2.41 mmol) was added to a solution of **11** (800 mg, 2.01 mmol) in dry pyridine (10 mL) and the mixture was stirred for 30 min at room temperature. The reaction was quenched by addition of acetone and the mixture was concentrated *in vacuo*. The residue was dissolved in $CHCl_3$ and washed with H_2O. The separated organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was coevaporated with toluene and purified on a silica gel column (2.7 x 15 cm), eluted with 0-4% EtOH in $CHCl_3$, to give **13** (576 mg, 69%, crystallized from EtOH-hexane): mp 196-197 °C; EI-MS *m/z* 412 (M^+); ^{1}H-NMR ($CDCl_3$) 9.09 (s, 1 H, C*H*=N), 8.00 (s, 1 H, H-2), 7.79 (br s, 1 H, N-OH), 7.11 (br s, 1 H, NH), 6.44 (d, 1 H, H-1', $J_{1', 2'}$ = 2.8 Hz), 5.77 (dd, 1 H, H-2', $J_{2', 1'}$ = 2.8, $J_{2', 3'}$ = 4.9 Hz), 5.48 (br s, 1 H, NH), 5.33 (dd, 1 H, H-3', $J_{3', 2'}$ = 4.9, $J_{3', 4'}$ = 7.1 Hz), 4.41 (m, 3 H, H-4', 5'a, b), 2.18, 2.15, 2.08 (each s, each 3 H, acetyl). *Anal.* Calcd for $C_{16}H_{20}N_4O_9$: C, 46.60; H, 4.89; N, 13.59. Found: C, 46.51; H, 4.95; N, 13.51.

5-Hydroxyiminomethyl-1-β-D-ribofuranosylimidazole-4-carboxamide (5). In the same manner as described for **1**, **13** (196 mg, 0.48 mmol) was treated with NH_3/MeOH (10 mL) gaving **5** (123 mg, 90%, crystallized from isopropanol-hexane): mp 175-178 °C; FAB-MS *m/z* 287 (M^++1); UV λ_{max}(H_2O) 277 nm (ε 12500); UV λ_{max}(0.5 N HCl) 260 nm (ε 10200); UV λ_{max}(0.5 N NaOH) 297 nm (ε 11100); ^{1}H-NMR (DMSO-d_6)

11.51 (s, 1 H, N-OH), 8.82 (s, 1 H, C*H*=N), 8.28 (s, 1 H, H-2), 7.51, 7.31 (each br s, each 1 H, NH_2), 6.27 (d, 1 H, H-1', $J_{1',2'}$ = 3.9 Hz), 5.27 (d, 1 H, 2'-OH, $J_{2'\text{-OH},2'}$ = 5.0 Hz), 5.08 (m, 2 H, 3', 5'-OH), 4.17 (ddd, 1 H, H-2', $J_{2',1'}$ = 3.9, $J_{2',2'\text{-OH}}$ = 5.0, $J_{2',3'}$ = 4.4 Hz), 4.06 (m, 1 H, H-3'), 3.86 (m, 1 H, H-4'), 3.67 (m, 1 H, H-5'a), 3.54 (m, 1 H, H-5'b). *Anal.* Calcd for $C_{10}H_{14}N_4O_6$: C, 41.96; H, 4.93; N, 19.57. Found: C, 41.67; H, 4.93; N, 19.36.

5-Difluoromethyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (14). A dry CH_2Cl_2 solution (12 mL) of **11** (330 mg, 0.83 mmol) was added slowly to a solution of diethylaminosulfur trifluoride (0.56 mL, 4.15 mmol) in dry CH_2Cl_2 (3 mL) and stirred for 25 h at room temperature. The reaction was quenched by addition of 1 M $NaHCO_3$ (10 mL) with being stirred for 30 min. The mixture was diluted with $CHCl_3$ (30 mL), which was washed with H_2O, followed by brine. The separated organic layer was dried (Na_2SO_4) and concentrated *in vacuo.* The residue was purified on a silica gel column (2.4 x 11 cm), eluted with hexane/AcOEt (1:1-1:3), to give **14** (244 mg, 70%) as a white foam: EI-MS *m/z* 419 (M^+); ^{1}H-NMR ($CDCl_3$) 7.93 (s, 1 H, H-2), 7.82 (t, 1 H, CHF_2, $J_{H,F}$ = 52.8 Hz), 7.10 (br s, 1 H, NH), 6.17 (d, 1 H, H-1', $J_{1',2'}$ = 4.7 Hz), 5.59 (br s, 1 H, NH), 5.49 (dd, 1 H, H-2', $J_{2',1'}$ = 4.7, $J_{2',3'}$ = 5.2 Hz), 5.41 (dd, 1 H, H-3', $J_{3',2'}$ = 5.2, $J_{3',4'}$ = 4.9 Hz), 4.45 (m, 1 H, H-4'), 4.41 (m, 2 H, H-5'a, b), 2.20, 2.13, 2.10 (each s, each 3 H, acetyl).

5-Difluoromethyl-1-β-D-ribofuranosylimidazole-4-carboxamide (6). In the same manner as described for **1**, **14** (244 mg, 0.58 mmol) was treated with NH_3/MeOH (10 mL) gaving **6** (110 mg, 65%, crystallized from EtOH-ether): mp 155-156 °C; FAB-MS *m/z* 294 (M^++1); UV λ_{max} (H_2O) 220 nm (ε 8900); ^{1}H-NMR (DMSO-d_6) 8.39 (s, 1 H, H-2), 7.83 (t, 1 H, CHF_2, $J_{H,F}$ = 53.0 Hz), 7.74, 7.57 (each br s, each 1 H, NH_2), 5.78 (d, 1 H, H-1', $J_{1',2'}$ = 4.3 Hz), 5.56 (d, 1 H, 2'-OH, $J_{2'\text{-OH},2'}$ = 5.8 Hz), 5.21 (d, 1 H, 3'-OH, $J_{3'\text{-OH},3'}$ = 5.3 Hz), 5.17 (t, 1 H, 5'-OH, $J_{5'\text{-OH},5'}$ = 5.1 Hz), 4.26 (ddd, 1 H, H-2', $J_{2',1'}$ = 4.3, $J_{2',2'\text{-OH}}$ = 5.8, $J_{2',3'}$ = 4.5 Hz), 4.13 (ddd, 1 H, H-3', $J_{3',2'}$ = 4.5, $J_{3',3'\text{-OH}}$ = 5.3, $J_{3',4'}$ = 4.8 Hz), 3.94 (ddd, 1 H, H-4', $J_{4',3'}$ = 4.8, $J_{4',5'a}$ = 3.3, $J_{4',5'b}$ = 3.2 Hz), 3.71 (ddd, 1 H, H-5'a, $J_{5'a,4'}$ = 3.3, $J_{5'a,b}$ = 12.2, $J_{5'a,5'\text{-OH}}$ = 5.1 Hz), 3.60 (ddd, 1 H, H-5'b, $J_{5'b,4'}$ = 3.2, $J_{5'b,a}$ = 12.2, $J_{5'b,5'\text{-OH}}$ = 5.1 Hz). *Anal.* Calcd for $C_{10}H_{13}F_2N_3O_5$: C, 40.96; H, 4.47; N, 14.33. Found: C, 40.65; H, 4.57; N, 14.21.

5-Cyano-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole-4-carboxamide (15). Hydroxylamine hydrochloride (111 mg, 1.60 mmol) was added to a solution of **11**

(503 mg, 1.33 mmol) in dry pyridine (10 mL) and the mixture was stirred at room temperature. After the starting material was completely consumed, the reaction mixture was cooled at 0 °C and phenyl chloroformate (0.32 mL, 2.66 mmol) was added to the mixture. After 30 min, more phenyl chloroformate (0.20 mL, 1.66 mmol) was added and stirred for 1 h, then the reaction mixture was heated for 20 min at 100 °C. The solvent was removed *in vacuo* and the residue was dissolved in $CHCl_3$, which was washed with H_2O, followed by brine. The separated organic layer was dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified on a silica gel column (2.7 x 11 cm), eluted with 0-4% EtOH in $CHCl_3$, to give **15** (430 mg, 86%) as a pale yellow foam: EI-MS *m/z* 394 (M^+); ^{1}H-NMR ($CDCl_3$) 7.87 (s, 1 H, H-2), 6.93 (br s, 1 H, NH), 6.00 (d, 1 H, H-1', $J_{1',2'}$ = 5.5 Hz), 5.77 (br s, 1 H, NH), 5.43 (m, 2 H, H-2', 3'), 4.50 (m, 1 H, H-4'), 4.41 (m, 2 H, H-5'a, b), 2.17, 2.16, 2.13 (each s, each 3 H, acetyl).

5-Cyano-1-β-D-ribofuranosylimidazole-4-carboxamide (7). A solution of **15** (683 mg, 1.73 mmol) in a mixture of MeOH (10 mL) and triethylamine (2 mL) was stirred for 10 h at room temperature. The solvent was removed *in vacuo* and the residue was purified on a silica gel column (3.2 x 6 cm), eluted with 5-30% EtOH in $CHCl_3$, to give **7** (370 mg, 80%, crystallized from EtOH-hexane): mp 169-171 °C; EI-MS *m/z* 268 (M^+); UV λ_{max} (H_2O) 250 nm (ε 9400); UV λ_{max}(0.5 N HCl) 250 nm (ε 9400); UV λ_{max}(0.5 N NaOH) 252 nm (ε 10000); IR 2280 cm^{-1}; ^{1}H-NMR (DMSO-d_6) 8.40 (s, 1 H, H-2), 7.77, 7.64 (each br s, each 1 H, NH_2), 5.68 (d, 1 H, 2'-OH, $J_{2'\text{-OH},2'}$ = 5.9 Hz), 5.66 (d, 1 H, H-1', $J_{1',2'}$ = 5.5 Hz), 5.30 (d, 1 H, 3'-OH, $J_{3'\text{-OH},3'}$ = 4.8 Hz), 5.05 (dd, 1 H, 5'-OH, $J_{5'\text{-OH},5'a}$ = 5.1, $J_{5'\text{-OH},5'b}$ = 5.5 Hz), 4.35 (ddd, 1 H, H-2', $J_{2',1'}$ = 5.5, $J_{2',2'\text{-OH}}$ = 5.9, $J_{2',3'}$ = 4.4 Hz), 4.08 (ddd, 1 H, H-3', $J_{3',2'}$ = 4.4, $J_{3',3'\text{-OH}}$ = 4.8, $J_{3',4'}$ = 4.0 Hz), 3.97 (ddd, 1 H, H-4', $J_{4',3'}$ = 4.0, $J_{4',5'a}$ = 3.7, $J_{4',5'b}$ = 3.9 Hz), 3.62 (ddd, 1 H, H-5'a, $J_{5'a,4'}$ = 3.7, $J_{5'a,5'b}$ = 12,1, $J_{5'a,5'\text{-OH}}$ = 5.1 Hz), 3.58 (ddd, 1 H, H-5'b, $J_{5'b,4'}$ = 3.9, $J_{5'b,5'a}$ = 12.1, $J_{5'b,5'\text{-OH}}$ = 3.9 Hz). *Anal.* Calcd for $C_{10}H_{12}N_4O_5 \cdot 1/4H_2O$: C, 44.08; H, 4.62; N, 20.54. Found: C, 44.30; H, 4.52; N, 20.44.

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