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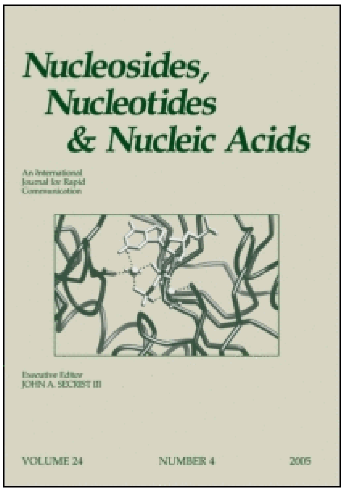

Nucleosides, Nucleotides and Nucleic Acids

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Nucleosides and Nucleotides. 98. Synthesis and Antitumor Activities of 5-Ethynylimidazole-4-carboxamide and -carbonitrile Derivatives

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NUCLEOSIDES & NUCLEOTIDES, 9(8), 1067-1078 (1990)

NUCLEOSIDES AND NUCLEOTIDES. 98.
SYNTHESIS AND ANTITUMOR ACTIVITIES OF 5-ETHYNYLIMIDAZOLE-4-CARBOXAMIDE AND -CARBONITRILE DERIVATIVES[1]

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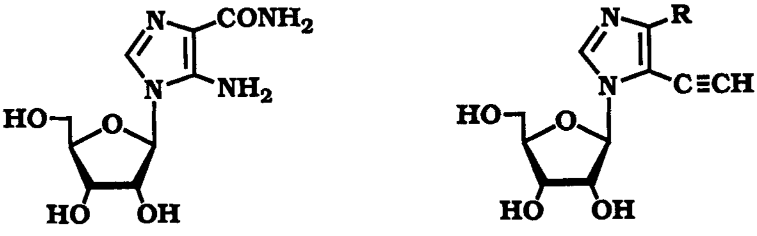
ABSTRACT: 5-Ethynyl-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (**4**) and -carboxamide (**5**) and 5-ethynyl-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (**11**) and -carboxamide (**12**) have been synthesized from the corresponding 5-iodo derivatives **2** and **7** by a palladium-catalyzed cross-coupling reaction with (trimethylsilyl)acetylene. The aglycons, 5-ethynylimidazole derivatives **14** and **15** were synthesized by the hydrolytic cleavage of the corresponding nucleosides. The antileukemic activity of these nucleosides and base analogues are also described.

INTRODUCTION

We have reported the synthesis of 5-alkynyl-1-β-D-ribofuranosylimidazole-4-carboxamides and -carbonitriles using palladium catalyzed cross-coupling reactions of the corresponding 5-iodo imidazole nucleosides with various terminal alkynes.[2,3] Since these nucleosides have structural similarities to 5-amino-1-β-D-ribofuranosylimidazole-4-carboxamide (AICAR), whose 5'-phosphate is an important intermediate of purine nucleotide biosynthesis, and have a chemically reactive functional group at the 5-position, we considered

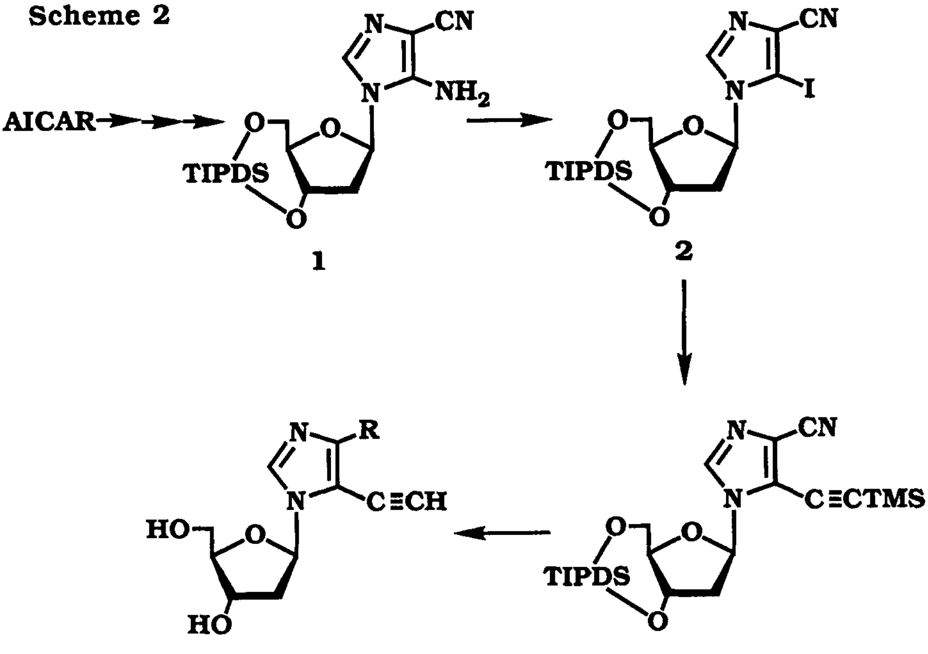
Scheme 1



AICAR

R = $CONH_2$; EICAR
R = CN ; EICNR

Scheme 2



1

2

3

4 ; R = CN
5 ; R = $CONH_2$

that they could act as antimetabolites with a potential of being an irreversible inhibitor of IMP dehydrogenase, which is one of the target enzymes for anticancer chemotherapy[4] as well as antiviral[5] and antiparasitic[6] chemotherapy. The inhibition of IMP dehydrogenase would cause cell death due to a decrease in the guanine nucleotide content in the cell.

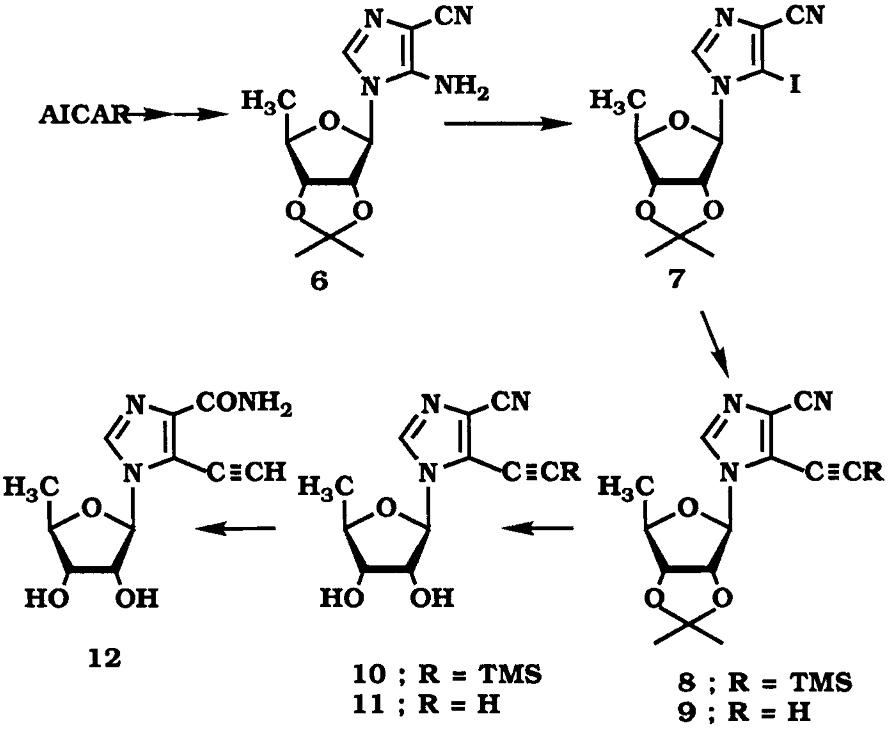
Among the 5-alkynylimidazole nucleosides, 5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide (EICAR) was found to be the most cytotoxic to murine leukemia L1210 cells *in vitro*.[2,3] Also, its 4-cyano derivative (EICNR) showed potent antileukemic activity. Moreover, EICAR has potent antitumor activity against various human tumor cells including leukemia, lymphoma, carcinoma, and adenocarcinoma cells from various tissues in culture, and its spectrum of activity is quite similar to 5-fluorouracil. We also demonstrated that EICAR significantly increased the life span of mice bearing L1210 and P388 leukemias *in vivo*.[3]

To clarify further the structural requirements for the antitumor activity, several nucleosides and base analogues were synthesized. In this paper, we wish to describe the synthesis and antileukemic activity of the sugar derivative of EICAR, 5-ethynyl-1-(2-deoxy- or 5-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide or carbonitrile and the aglycons, 5-ethynylimidazole-4-carboxamide and -carbonitrile.

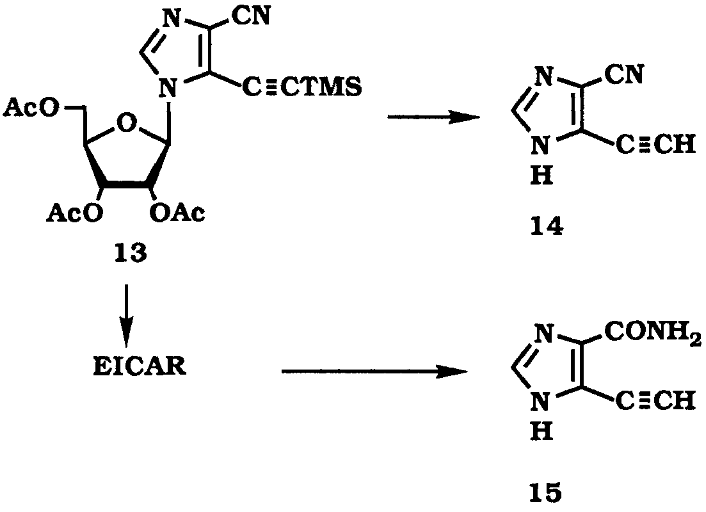
CHEMISTRY

For the synthesis of 2'-deoxy EICAR (**5**), we chose AICAR as a starting material, which was further converted into 5-amino-1-[2-deoxy-3,5-*O*-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-β-D-ribofuranosyl]imidazole-4-carbonitrile (**1**) by the method reported by us.[7] Treatment of **1** with isoamyl nitrite in diiodomethane for 30 min at 100°C afforded the 5-iodo derivative **2** in 64% yield as white crystals. The palladium-catalyzed cross-coupling reaction of **2** with (trimethylsilyl)acetylene was done using 5 mol% of bis(benzonitrile)-palladium dichloride and triethylamine in acetonitrile. The reaction proceeded smoothly to produce 5-[2-(trimethylsilyl)ethynyl] derivative **3** in 59% yield without formation of the undesired dimer.[3] Compound **3** was then deprotected with tetrabutylammonium fluoride

Scheme 3



Scheme 4



(TBAF) in tetrahydrofuran (THF) to give 5-ethynyl-1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carbonitrile (**4**) in 97% yield. Treatment of **4** with hydrogen peroxide in NH_4OH/MeOH caused hydrolysis of the 4-carbonitrile to give 5-ethynyl-1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide (**5**) in 84 yield as crystals.

The synthesis of the 5'-deoxy derivative of EICAR is shown in scheme 3. As a starting material for the reaction sequence of 5-ethynyl-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (**11**) and -carboxamide (**12**), 5-amino-1-(5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (**6**) was prepared by the reported procedure.[8] Compound **6** was converted into the corresponding 5-iodo nucleoside **7** in 66% yield as described above, which was then coupled with (trimethylsilyl)acetylene in the presence of bis(benzonitrile)palladium dichloride, producing the 5-[2-(trimethylsilyl)ethynyl] derivative **8** in 41% yield. Deblocking of **8** with 90% aqueous trifluoroacetic acid (TFA) afforded 5-[2-(trimethylsilyl)ethynyl]-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (**10**), which was then treated with NH_3/MeOH to furnish **11**. Alternatively, **8** was treated with TBAF to give **9**, whose the isopropylidene group was removed by treatment with 90% aqueous TFA to produce **11** in good yield. Hydrolysis of the 4-cyano group in 11 to give 12 was accomplished with hydrogen peroxide in NH_4OH/MeOH.

Hydrolytic cleavage of the glycosidic linkage in **13** was next examined. When **13** was heated under reflux in a mixture of 1 N HCl and MeOH, 4-cyano-5-ethynylimidazole (**14**) was produced in 96% yield as white crystals, without formation of the further hydrolyzed 4-carboxamide derivative **15**. Treatment of **14** with hydrogen peroxide in NH_4OH/MeOH at room temperature, however, did not afford **15**, because of the dissociation of the N^1-H under these conditions. To prepare 4-carboxamide-5-ethynylimidazole (**15**), EICAR was heated for 4 h at 100°C in 1 N HCl to furnish **15** in 38% yield.

ANTILEUKEMIC ACTIVITY

The antileukemic activity of the nucleosides and bases toward murine L1210 cells in culture is summarized in Table I. Data for EICAR and EICNR are also shown for comparison.

Table I. **Inhibitory Effects of 5-Ethynylimidazole Derivatives on the Growth of Murine Leukemia L1210 Cells In Culture**[a]

compds	IC_{50}, μg/mL[b]
EICAR	**0.18**
EICNR	**1.9**
4	**6.7**
5	> **100**
11	> **100**
12	> **100**
14	**2.9**
15	**4.0**

[a]Cytotoxic activity assay *in vitro* was done by the method of Carmichael *et al.*[11] L1210 cells (1 x 10^4/well) was incubated in the presence or absence of compounds for 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and the OD (570 nm) was measured. Percent inhibition was calculated as follows: % inhibition = [1 - OD (570 nm) of sample well / OD (570 nm) of control well] x 100. [b]IC_{50} (μg/mL) was given as the concentration at 50% inhibition of cell growth.

Among these, EICAR is the most effective inhibitor of the growth of L1210 cells. Compounds EICNR, **5**, **14**, and **15** are potent inhibitors but their potencies are more than 10 times lower than EICAR, and **4**, **11**, and **12** are devoid of antileukemic activity up to 100 μg/mL. From the data on **11** and **12**, phosphorylation at the 5'-position of EICAR by nucleoside kinases is essential for the activity. If 2'-deoxy nucleosides **4** and **5** were activated by nucleoside kinases, they could not be expected to be inhibitors of IMP dehydrogenase. However, **4** had antileukemic activity. Since 5-ethynylimidazoles **14**, **15** have good activity to L1210, they must be activated by certain phosphoribosyltransferases to give the corresponding 5'-nucleotides, like the aglycon of bredinin, 4(5)-carbamyl imidazolium-5(4)-olate, which is known to be transformed to bredinine 5'-monophosphate by adenine phosphoribosyltransferase.[9] This is also a potent inhibitor of IMP dehydrogenase.[10] Since the 5-ethynyl group in **11** and **14** is expected to be the better Michael acceptor than that in **12** and **15**, it is possible that these bases and nucleosides would act as alkylating agents before or after activation to the corresponding nucleotides. Actually, we demonstrated that EICAR reacts with NaSMe in MeOH to form (*Z*)-5-(2-methylthiovinyl)-1-β-D-ribofuranosylimidazole-4-carboxamide.[3] The detailed mechanism of the anti-leukemic action of these compounds is a subject of further studies.

ACKNOWLEDGMENT

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EXPERIMENTAL

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a JEOL JNM-FX 100 (100 MHz) or JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane as 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 confirmed by addition of D_2O. IR spectra were recorded on a JASCO IR report 100 spectrometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. TLC was done on Merck Kieselgel F254 precoated plates. The silica gel used for column chromatography was YMC gel 60A (70-230 mesh).

5-Iodo-1-(2-deoxy-3,5-*O*-TIPDS-β-D-ribofuranosyl)imidazole-4-carbonitrile (2). Isoamyl nitrite (0.6 mL, 4.47 mmol) was added to a solution of **1** (500 mg, 1.07 mmol) in diiodomethane (6 mL) at 100°C. The mixture was stirred for 0.5 h and the cooled mixture was put on a silica gel column (2.3 x 13 cm), eluted with hexane : EtOAc (10 : 1 to 6 : 1). The main UV-absorbing fractions were combined and concentrated to dryness *in vacuo*. The residue was crystallized from hexane to yield **2** (397 mg, 64%); mp 148-149°C. MS *m/z*: 577 (M^+). ^{1}H NMR ($CDCl_3$): 8.06 (s, 1H, H-2), 5.89 (dd, 1H, H-1', $J_{1',2'}$ = 6.8, $J_{1',2''}$ = 2.0 Hz), 4.59 (ddd, 1H, H-3', $J_{3',2'}$ = 7.6, $J_{3',2''}$ = 2.2, $J_{3',4'}$ = 8.1 Hz), 4.08 (m, 2H, H-5'a, b), 3.84 (dt, 1H, H-4', $J_{4',3'}$ = 8.1, $J_{4',5'}$ = 2.9 Hz), 2.48 (m, 2H, H-2'a, b), 1.05 (m, 28H, isoPr). *Anal.* Calcd for

$C_{21}H_{36}IN_3O_4Si_2$: C, 43.67; H, 6.28; N, 7.27. Found: C, 43.27; H, 6.35; N, 7.33.

5-[2-(Trimethylsilyl)ethynyl]-1-(2-deoxy-3,5-O-TIPDS-β-D-ribofuranosyl)imidazole-4-carbonitrile (3). Compound **2** (577 mg, 1 mmol), bis(benzonitrile)palladium dichloride (18 mg, 5 mol%), triethylamine (0.16 mL, 1.2 mmol), and (trimethylsilyl)acetylene (0.17 mL, 1.2 mmol) in acetonitrile (5 mL) was heated at 100°C for 2 h under argon atmosphere in a sealed glass tube. The reaction mixture was filtered through a Celite pad and washed with ethanol. The combined filtrate and washings were concentrated to dryness *in vacuo* and the residue was purified by a silica gel column (2.3 x 19 cm), eluted with hexane to 5% EtOAc in hexane. The main UV-absorbing fractions were combined and concentrated to dryness. The residue was crystallized from hexane to afford **3** (322 mg, 59%); mp 128-129°C. MS *m/z*: 547 (M^+). ^1H NMR ($CDCl_3$): 7.85 (s, 1H, H-2), 6.00 (dd, 1H, H-1', $J_{1',2'}$ = 6.4, $J_{1',2''}$ = 2.4 Hz), 4.56 (ddd, 1H, H-3'), 4.06 (m, 2H, H-5'a, b), 3.84 (dt, 1H, H-4'), 2.50 (m, 2H, H-2'a, b), 1.04 (m, 28H, isoPr), 0.29 (s, 9H, TMS). *Anal.* Calcd for $C_{26}H_{45}N_3O_4Si_3$: C, 56.99; H, 8.28; N, 7.67. Found: C, 56.77; H, 8.45; N, 7.70.

5-Ethynyl-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (4). A THF solution of TBAF (1 M, 0.99 mL, 1 mmol) was added to a solution of **3** (164 mg, 0.3 mmol) in THF (5 mL) at 0°C. The mixture was stirred for 10 min at 0°C and the solvent was removed *in vacuo*. The residue was purified by a silica gel column (1.8 x 6 cm), eluted with 5% to 15% EtOH in $CHCl_3$. The fractions containing **4** were combined and concentrated to dryness giving a glass (73 mg, 97%). ^1H NMR (DMSO-d_6): 8.29 (s, 1H, H-2), 6.08 (dd, 1H, H-1', $J_{1',2'}$ = 6.4, $J_{1',2''}$ = 6.6 Hz), 5.37 (s, 1H, 5-acetylene proton), 5.36 (d, 1H, 3'-OH, $J_{3'OH,\ 3'}$ = 4.4 Hz), 4.97 (t, 1H, 5'-OH, *J* = 5.3 Hz), 4.29 (m, 1H, H-3'), 3.86 (m, 1H, H-4'), 3.53 (m, 2H, H-5'a, b), 3.30 (m, 2H, H-2'a, b). Exact MS *m/z* Calcd. for $C_{11}H_{11}N_3O_3$ (M^+): 233.0800. Found: 233.0782.

5-Ethynyl-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (5). A mixture of **4** (110 mg, 0.47 mmol) in NH_4OH-MeOH (1.5 mL each) containing hydrogen peroxide (30%, 0.1 mL) was stirred for 0.5 h at room temperature. The mixture was concentrated to dryness and the residue was purified by a silica gel column (1.8 x 10 cm), eluted with 5%-15% EtOH in $CHCl_3$. The main UV-absorbing fractions were concentrated to dryness *in vacuo*. The residue was crystallized from EtOH/hexane to afford **5** (100 mg, 84%); mp 174-175°C. MS *m/z*: 251 (M^+). ^1H NMR (DMSO-d_6): 8.09 (s, 1H, H-2), 7.34, 7.24 (each br s, 2H, $CONH_2$), 6.08 (t, 1H, H-1', $J_{1',2'} = J_{1',2''} = 6.6$ Hz), 5.33 (d, 1H, 3'-OH, $J = 4.2$ Hz), 4.96 (t, 1H, 5'-OH, $J = 5.4$ Hz), 4.87 (s, 1H, 5-acetylene proton), 4.33 (m, 1H, H-3'), 3.84 (m, 1H, H-4'), 3.31 (m, 2H, H-5'a, b), 2.40 (m, 2H, H-2'a, b). *Anal.* Calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.72. Found: C, 52.49; H, 5.17; N, 16.59.

5-Iodo-1-(5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (7). Isoamyl nitrite (2 mL, mmol) was added to a solution of **6** (1.06 g, 4 mmol) in diiodomethane (20 mL) at 100°C. The mixture was stirred for 20 min and the cooled solution was put on a silica gel column (2.7 x 27 cm), eluted with hexane : EtOAc (5 : 1 to 2 : 1). The main UV-absorbing fractions were concentrated to dryness to give **7** (982 mg, 66%) as a foam. MS *m/z*: 375 (M^+). ^1H NMR ($CDCl_3$): 7.79 (s, 1H, H-2), 5.75 (d, 1H, H-1', $J_{1',2'} = 2.9$ Hz), 4.83 (dd, 1H, H-2', $J_{2',1'} = 2.9$, $J_{2',3'} = 6.3$ Hz), 4.54 (dd, 1H, H-3', $J_{3',2'} = 6.3$, $J_{3',4'} = 3.9$ Hz), 4.35 (dd, 1H, H-4', $J_{4',3'} = 3.9$, $J_{4',5'} = 6.0$ Hz), 1.62, 1.37 (each s, 6H, isoPr), 1.43 (d, 3H, H-5', $J_{5',4'} = 6.6$ Hz).

5-[2-(Trimethylsilyl)ethynyl]-1-(5-deoxy-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole-4-carbonitrile (8). A mixture of **7** (739 mg, 1.97 mmol), bis(benzonitrile)palladium dichloride (36 mg, 5 mol%), triethylamine (0.32 mL, 2.4 mmol), and (trimethylsilyl)acetylene (0.34 mL, 2.4 mmol) in acetonitrile (7 mL) was heated at 100°C for 5.5 h under an argon atmosphere 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 to dryness *in vacuo* and the residue was purified by a silica gel column (2.7 x 20 cm), eluted with hexane : EtOAc (5 : 1 to 2 : 1). The main UV-absorbing fractions were concentrated to dryness. The residue was crystallized from hexane to afford **8** (278 mg, 41%); mp 70-72°C. MS *m/z*: 345 (M^+). ^1H NMR ($CDCl_3$): 7.61 (s, 1H, H-2), 5.80 (d, 1H, H-1', $J_{1',2'} = 2.5$ Hz), 4.99 (dd, 1H, H-2', $J_{2',1'} = 2.5$, $J_{2',3'} = 6.1$ Hz), 4.42 (m, 2H, H-3', 4'), 1.58, 1.35 (each s, 6H, isoPr), 1.40 (d, 3H, H-5'), 0.29 (s, 9H, TMS). *Anal.* Calcd for $C_{17}H_{23}N_3O_3Si$: C, 59.10; H, 6.71; N, 12.16. Found: C, 58.94; H, 6.79; N, 12.14.

5-Ethynyl-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (11). (a) An aqueous TFA solution (90%, 3 mL) containing **8** (50 mg, 0.14 mmol) was stirred for 10 min at room temperature. The solvent was removed *in vacuo* and coevaporated several times with EtOH. The residue was purified on a silica gel column (1.8 x 7 cm), eluted with 0-4% EtOH in $CHCl_3$. The main UV-absorbing fractions were concentrated to dryness giving 5-[2-(trimethylsilyl)ethynyl]-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (**10**, 43 mg, 97%, as a glass); MS *m/z*: 305 (M^+); ^1H NMR (DMSO-d_6 + D_2O): 8.26 (s, 1H, H-2), 5.61 (d, 1H, H-1', $J_{1',2'}$ = 4.9 Hz), 4.39 (dd, 1H, H-2', $J_{2',1'}$ = 4.9, $J_{2',3'}$ = 5.1 Hz), 3.90 (m, 2H, H-3', 4'), 1.30 (d, 3H, H-5', $J_{5',4'}$ = 7.4 Hz), 0.28 (s, 9H, TMS). A solution of **10** (130 mg, 0.43 mmol) in NH_3/MeOH (saturated at 0°C, 3 mL) was kept overnight at room temperature. The solvent was concentrated *in vacuo* and the residue was purified on a silica gel column (1.5 x 5 cm), eluted with 0-6% EtOH in $CHCl_3$. The eluates were concentrated to dryness to afford **11** (81 mg, 76%, as a glass); ^1H NMR (DMSO-d_6): 8.26 (s, 1H, H-2), 5.60 (d, 1H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.58 (d, 1H, 2'-OH, $J_{2'OH,2'}$ = 5.9 Hz), 5.35 (s, 1H, 5-acetylene proton), 5.24 (d, 1H, 3'-OH), $J_{3'OH,3'}$ = 5.1 Hz), 4.38 (m, 1H, H-2'), 3.87 (m, 2H, H-3', 4'), 1.27 (d, 3H, H-5', $J_{5',4'}$ = 6.4 Hz). Exact MS *m/z* Calcd. for $C_{11}H_{11}N_3O_3$ (M^+): 233.0800. Found: 233.0779. (b) Compound **8** (278 mg, 0.81 mmol) in THF (5 mL) was treated with TBAF (1 M in THF, 1.2 mL) for 10 min at room temperature. After removal of the solvent *in vacuo*, the residue was purified on a silica gel column to give **9** (198 mg, 90%), followed by treatment with 90% aq. TFA (10 mL) for 10 min at room temperature. The solvent was concentrated and coevaporated several times with EtOH *in vacuo*. The residue was purified by a silica gel column to afford **11** (164 mg, 97%).

5-Ethynyl-1-(5-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (12). A solution of **11** (84 mg, 0.28 mmol) in NH_4OH/MeOH (1.5 mL each) containing hydrogen peroxide (30%, 0.1 mL) was stirred for 0.5 h at room temperature. The solvent was removed by evaporation and the residue was purified by a silica gel column (1.8 x 8 cm), eluted with 5-15% EtOH in $CHCl_3$. The eluates were concentrated to dryness and the residue was crystallized from EtOH/hexane to give **12** (47 mg, 68%); mp 171-172°C. ^1H NMR (DMSO-d_6): 8.05 (s, 1H, H-2), 7.35, 7.25 (each br s, 2H, $CONH_2$), 5.62 (d, 1H, H-1', $J_{1',2'}$ = 5.1 Hz), 5.52 (d, 1H, 2'-OH), 5.21 (d, 1H, 3'-OH), 4.86 (s, 1H, 5-acetylene proton), 4.39 (m, 1H, H-2'), 3.87 (m, 2H, H-3', 4'), 1.29 (d, 3H, H-5', $J_{5',4'}$ = 6.1 Hz). Exact MS *m/z* Calcd for $C_{11}H_{13}N_3O_4$ (M^+): 251.0906. Found: 251.0923.

4-Cyano-5-ethynylimidazole (14). A solution of compound **13** (447 mg, 1 mmol) in a mixture of 1 N HCl (10 mL) and MeOH (5 mL) was heated under reflux for 4 h. The cooled mixture was neutralized with 1 N NaOH and concentrated to dryness. The residue was purified on a silica gel column (2.7 x 13 cm), eluted with 0-8% EtOH in $CHCl_3$. The main UV-absorbing fractions were concentrated to dryness and the resulting solid was crystallized from aqueous MeOH to give **14** (112 mg, 96%); mp 169°C (colored from 140°C). MS *m/z*: 117 (M^+). ^1H NMR (DMSO-d_6): 13.70 (br s, 1H, NH), 7.99 (s, 1H, H-2), 5.02 (s, 1H, 5-acetylene proton). *Anal.* Calcd for $C_6H_3N_3$: C, 61.54; H, 2.58; N, 35.88. Found: C, 61.50; H, 2.52; N, 35.78.

5-Ethynyl-1(3)*H*-imidazole-4-carboxamide (15). A solution of EICAR (200 mg, 0.75 mmol) in 1 N HCl (5 mL) was heated under reflux for 4 h. The cooled mixture was neutralized with 1 N NaOH and the solvent was concentrated to dryness. The residue was purified by silica gel column (2.7 x 11 cm), eluted with 10-16% EtOH in $CHCl_3$. The main UV-absorbing fractions were concentrated to dryness and the residue was crystallized from EtOH/hexane to afford **15** (38 mg, 38%); mp 208-209°C (colored from 175°C). MS *m/z*: 135 (M^+). ^1H NMR (DMSO-d_6): 13.0 (br s, 1H, NH), 7.71 (s, 1H, H-2), 7.25, 7.22 (each br s, 2H, $CONH_2$), 4.52 (s, 1H, 5-acetylene proton). *Anal.* Calcd for $C_6H_5N_3O$: C, 53.33; H, 3.73; N, 31.10. Found: C, 53.18; H, 3.66; N, 31.15.

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