Synthesis and Biological Evaluation of 5-Substituted-4-nitroimidazole Ribonucleosides

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The imidazole nucleosides, 4(5)-bromo-5(4)-nitro-1-β-D-ribofuranosylimidazoles, have been prepared *via* glycosylation of the trimethylsilylated aglycone, 4(5)-bromo-5(4)-nitroimidazole, with tetra-*O*-acetyl-β-D-ribofuranose followed by removal of the acetyl protecting groups. The 5-bromo-4-nitro-1-β-D-ribofuranosylimidazole nucleoside was acetonated to produce 5-bromo-4-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole which was cyclized to provide the corresponding anhydronucleoside 5,5'-anhydro-4-nitro-5-oxo-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole. Sodium hydrosulfide treatment of 5-bromo-4-nitroimidazole nucleoside provided 5-mercapto-4-nitro-1-β-D-ribofuranosylimidazole 5-sodium salt which was alkylated with *E*-1,5-diiodopent-1-ene to yield 5-(*E*-1-iodo-1-penten-5-yl)thio-4-nitro-1-β-D-ribofuranosylimidazole. The corresponding iodine-125-labeled compound was prepared similarly using radiolabeled diiodopentene. The 5-bromo-4-nitroimidazole, 5-mercapto-4-nitroimidazole, and 5-iodopentenylthio-4-nitroimidazole nucleosides were cytotoxic to Molt-3 cells *in vitro* at concentrations higher than 10 μg/mL. The radiolabeled 5-iodopentenylthio-4-nitroimidazole nucleoside showed 2-fold higher uptake in a rapidly growing tumor as compared to uptake in a relatively slower growing tumor in mice.

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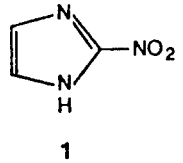
Introduction.

Imidazole nucleosides [1] have been extensively studied as analogues of both antibiotics, such as azomycin (2-nitroimidazole, **1**) [2], bredinin (5-hydroxy-1-β-D-ribofuranosylimidazole-4-carboxamide) [3,4] and pyrazomycin (4-hydroxy-3-β-D-ribofuranoylpyrazole-5-carboxamide) [5], and naturally occurring intermediates [6] of *de novo* purine nucleotide biosynthesis. The imidazole nucleosides exhibit chemotherapeutically important properties [5,7] and serve as useful synthetic intermediates [8-10] for the preparation of related purine nucleosides of biological interest. The possibility that azomycin may exist in nature as a nucleoside analogue suggested the synthesis of 2-nitro-1-β-D-ribofuranosylimidazole (azomycin riboside, **2**) [2]. Subsequent studies demonstrated that certain nitroimidazoles, *i.e.*, azomycin [11] and its analogues [12] metronidazole [13,14] and misonidazole [15], exhibit clinical efficacy as sensitizers of hypoxic tumor cells to radiation in the treatment of human cancer. The radiosensitizing property of these agents appears to be in parallel to their ability to penetrate tumors which contain hypoxic cells and necrotic areas [11,16]. The presence of a nitro group in the imidazole moiety appears to be an essential structural feature for biological activity [17,18]. In addition, *in vitro* studies indicate that introduction of a glycosyl group may further contribute to biological efficacy by both facilitating transport of the nitroimidazole analogue by the nucleoside transport mechanism [19,20] and by increasing the hydrophilicity [21]. For example, azomycin riboside is a substrate for the nucleoside transport mechanism of animal cells, and the riboside is more cytotoxic to hypoxic tumor cells *in vitro* than its aglycone, azomycin [19]. The corresponding 5'-deoxy-5'-iodoazomycin [22] is a more potent cytotoxic agent and also binds selectively and at a faster rate to the hypoxic tumor cells *in vitro* than azomycin or misonidazole. The nitroimidazole nucleosides, which preferentially concentrate in the hypoxic or necrotic areas and bind to hypoxic cells of tumor origin, may potentially be useful as radiolabeled markers for measurement of such regions in solid tumors [22]. The present study describes the synthesis, radiolabeling, and biological evaluation of some 5-substituted-4-nitroimidazole ribonucleosides.



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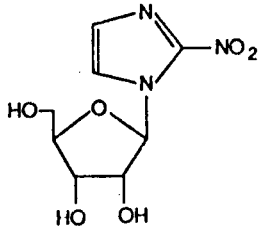

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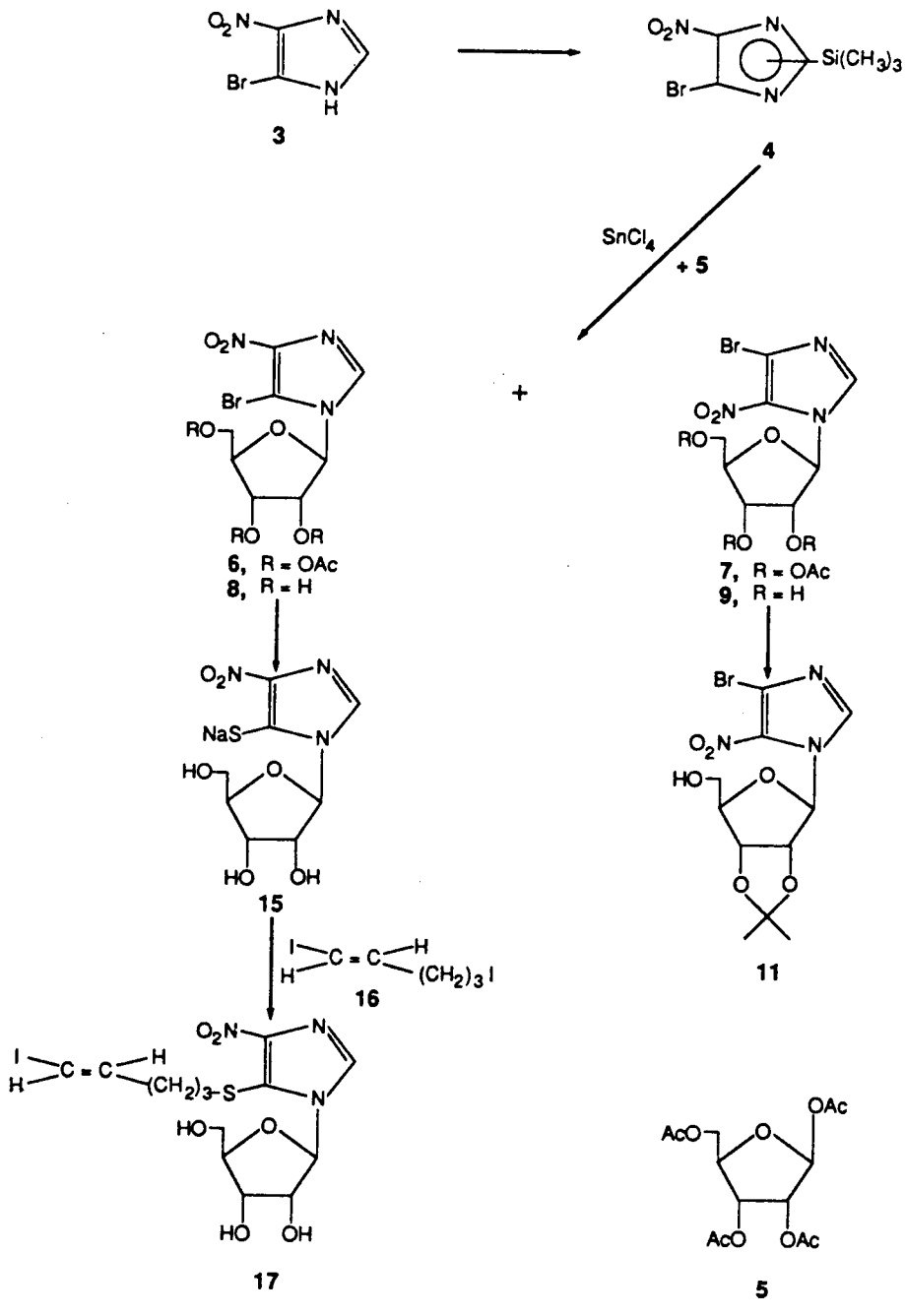
Chemistry.

The precursor, 4(5)-bromo-5(4)-nitroimidazole [23] (**3**, Scheme I), was prepared from commercially available 2,4,-5-tribromoimidazole. The glycosylation *via* fusion [10] of a mixture of **3** and tetra-*O*-acetyl-β-D-ribofuranose (**5**) in the presence of an acid catalyst yielded a mixture of four nucleosides, as monitored by tlc, including 5-bromo-4-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (**6**)

Scheme I

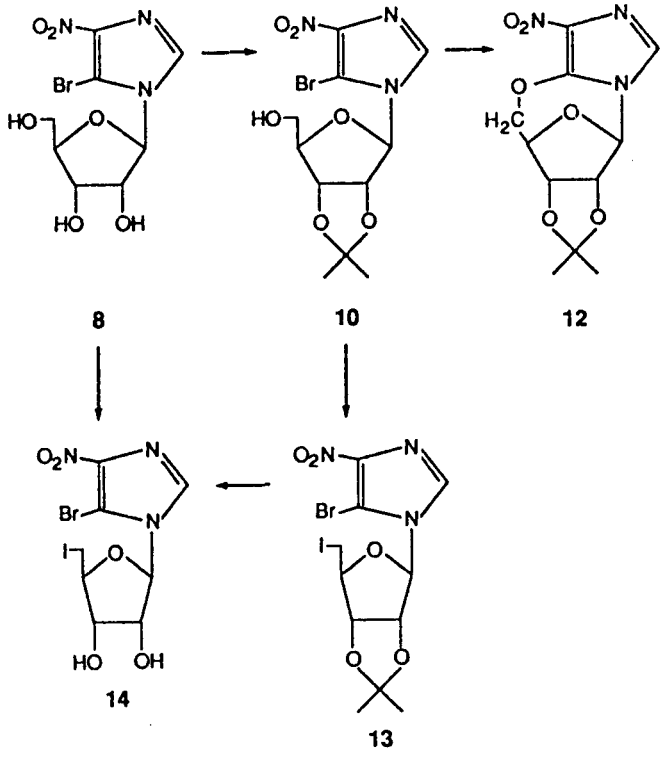


and 4-bromo-5-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (**7**). Consequently, the fusion procedure for the synthesis of these nucleosides was not pursued. Alternatively, **3** was silylated and the silyl intermediate, 5-bromo-4-nitro(1 or 3)-trimethylsilylimidazole (**4**), was condensed with **5** in the presence of stannic chloride catalyst to yield a mixture of **6** and **7** which were separated by silica gel column chromatography. Compounds **6** and **7** were conveniently deacetylated in methanol and ammonium hydroxide to yield 5-bromo-4-nitro-1-β-D-ribofuranosylimidazole (**8**) and 4-bromo-5-nitro-1-β-D-ribofuranosylimidazole (**9**), respectively.

The analytical data [10] (uv and mp) for both 4-nitro-nucleosides **6** and **8** were within acceptable ranges. The mp (67-68°) observed for compound **7**, however, was significantly lower than that reported in the literature [10] (192-193°). Compound **9**, the deacetylated nucleoside analogue of **7**, has not been described in the literature

[10,24]. These results indicated that further derivatization of **8** and **9** was required for structural determinations. Nucleosides **8** and **9** were acetonated (Scheme I and Scheme II) to provide 5-bromo-4-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**10**) and 4-bromo-5-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**11**). In the ^{1}H nmr spectrum of **10** and **11**, the differences in chemical shifts of the methyl groups of the isopropylidene moieties were equal to 0.25 and 0.27, respectively, suggesting [25] the β-configuration for these nucleosides. Also, **10** readily cyclized in dimethylformamide containing sodium hydride to yield 5,5'-anhydro-4-nitro-5-oxo-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**12**). This result confirmed both the β-configuration and the N^1-nitrogen position adjacent to the bromide leaving group as the site of glycosylation in **10**, and consequently in the parent **8**. On the other hand, compound **11** eluded our attempts at cyclization under similar conditions suggesting the nitrogen adjacent to the nitro group as the site of glycosylation in **11**, and therefore in its precursor **9**.

Scheme II



The halogenated nucleosides are useful precursors for exchange-labeling [22] with Na^{125}I. Consequently, compound **10** was treated with methyltriphenoxyphosphonium iodide (Rydon reagent) [26] to provide 5-bromo-4-nitro-1-(5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole (**13**), which after removal of the isopropylidene group yielded 5-bromo-4-nitro-1-(5-deoxy-5-iodo-β-D-ribofuranosyl)imidazole (**14**). Alternatively, direct iodination of **8** with Rydon reagent also resulted in formation of **14**. Treatment of compound **8** with sodium hydrosulphide (NaSH) provided 5-mercapto-4-nitro-1-β-D-ribofuranosylimidazole as a sodium salt (**15**). Isolation of **15** as the sodium salt, even after treatment with acetic acid, indicates stronger acidic character of the thiol (SH) group in **15** as compared to acetic acid. Our attempts to replace bromine with SH group in **7** or **9** were futile. The mercapto compound **15** was alkylated with *E*-1,5-diiodopent-1-ene (**16**) [27] to form 5-(*E*-1-iodopenten-5-yl)thio-4-nitro-1-β-D-ribofuranosylimidazole (**17**). The iodopentene moiety was introduced because this moiety as a radioiodide ligand [27] exhibits minimal *in vivo* deiodination and is therefore suitable for radiolabeling of compounds for biological evaluation.

Biological Evaluation.

Nucleosides **8**, **15**, and **17** were evaluated [29,30] for their ability to inhibit the expression of HIV in cell culture using the Molt-3 cell line at concentrations of 0.01, 0.1 and 10 μg/ml. The compounds were devoid of any biological activity at these concentrations but showed cytotoxicity at concentrations higher than 10 μg/ml. The radiolabeled nucleoside, [^{125}I]**17**, was evaluated for tumor uptake *in vivo* in tumor-implanted nude mice (3 animals/time point) at 1 hour after intravenous administration. Compound **17** (Table 1) showed significant tumor uptake (1.6% injected dose/g) in Balb-C mice bearing Line-1 carcinomas. Line-1 is a spontaneous lung carcinoma representing a rapidly growing tumor with potential development of necrotic and hypoxic areas in the tumor. In contrast, compound **17** showed low (0.8% injected dose/g) tumor uptake in nude mice bearing SW 948 human colorectal carcinoma xenografts, which represent a slower growing tumor model. The data (Table 1) indicate a two-fold higher concentration of compound **17** in the rapidly growing tumor than in the slower growing tumor. The uptake of **17** in blood, kidneys, and small and large intestine was relatively high in both experiments. Although the uptake of **17** in the tumor as compared to other organs (Table 1) was not high enough to have any clinical significance, the study points out differences in the tumor uptake of this compound in the slowly growing versus rapidly growing (potentially hypoxic) tumors. Similar agents, if high tumor uptake relative to normal organs can be demonstrated, may also have potential in clinical diagnosis of the metabolic differences between slow and fast growing tumors.

EXPERIMENTAL

Melting points (mp) were determined in capillary tubes with a Thomas Hoover (Uni-melt) capillary melting point apparatus and

Table I

Uptake of 5-(E-1-[^{125}I]idio-1-penten-5-yl)thio-4-nitro-1-β-D-ribofuranosylimidazole in Tissues of Tumor Bearing Female Mice Following Intravenous Administration [a]

Tumor model	Time after injection	Mean % injected dose/g						
		Tumor	Blood	Kidney	Small Intestine	Large Intestine	Liver	Lung
Spontaneous lung carcinoma Line-1 tumor cells (10^6) [b]	1 hour	1.62	2.97	3.44	5.09	2.58	2.89	2.29
SW-948 colorectal carcinoma [c]	1 hour	0.76	3.19	4.45	6.40	2.75	3.94	1.97

[a] Three animals per group were used. Other tissues analyzed were spleen and bone. [b] ID = 0.11 μCi in 0.1 ml saline. [b] ID = 0.23 μCi in 0.1 ml saline.

are uncorrected. Thin-layer chromatographic analyses (tlc) were performed with 250 μm thick layers of silica gel G PF-254 coated on glass plates (Analtech, Inc.). Spots on the tlc plates were detected by observation under short-wave uv light or exposure to iodine vapor. The thin-layer chromatograms were charred after spraying with a solution of 5% sulfuric acid in methanol. A dark brown to balck color indicated the presence of sugar. Analtech silica gel GF (20 x 20 cm, 1,000 microns thickness) plates were used for preparative chromatography. The low-resolution mass spectra (ms) were recorded at 70 eV with a Kratos MS 25 instrument. The proton nuclear magnetic resonance spectra (^{1}H nmr) were obtained at 60 MHz with a Varian 360-L instrument. Samples (30-40 mg) were dissolved in the solvents indicated, and the resonances are reported downfield (δ) from the internal tetramethylsilane standard. The presence of exchangeable protons was confirmed by treatment with deuterium oxide followed by reintegration of the nmr spectrum. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

Materials.

All chemicals and solvents were analytical grade and were used without further purification. Tribromoimidazole was purchased from Aldrich Chemical Company (Milwaukee, WI). Sodium [^{125}I]iodide was purchased from New England Nuclear, Inc. (North Billierica, MA).

Biological Experiments.

The *in vitro* experiments to study the inhibition of HIV-1 expression in MOLT-3 cells and cytotoxicity to these cells were performed by Dr. P. S. Sarin, National Cancer Institute, National Institutes of Health, using a protocol described in the literature [29,30]. For *in vivo* tumor uptake studies, athymic female nude mice bearing SW 948 human colorectal carcinoma xenografts were obtained from Oak Ridge Associated Universities, Oak Ridge, TN, (courtesy of Dr. James E. Crook) and tumor-bearing female Balb-C mice implanted with 10^6 Line-1 spontaneous lung carcinoma cells were obtained from ORNL Biology Division (courtesy of Dr. Stephen J. Kennel). Mice inoculated with Line-1 cells (10^6) develop 1 cm diameter tumors in one week (rapidly growing tumor) and mice inoculated with SW 948 cells (10^6) develop 5-10 mm diameter tumors in three weeks (slowly growing tumor). The nucleoside radiolabeled with ^{125}I was formulated in normal saline. The solution was filtered through a 22-μm Millipore filter and injected *via* a lateral tail vein into the ether-anesthetized animals. At a given time interval after the injection, the animals were reanesthetized with ether and killed by cervical fracture. The tumor and other desired organs were excised, rinsed with saline solution, blotted dry, placed in tared vials, and weighed. The samples were counted for radioactive contents in a Packard Minaxi 5000 sodium iodide auto gamma counter. Results are expressed as % injected dose/g (ID/g) tissue.

5-Bromo-4 nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole (**6**) and 4-Bromo-5-nitro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-imidazole (**7**).

A suspension of 4(5)-bromo-5(4)nitroimidazole (**3**, 3.0 g, 15.6 mmoles) and ammonium sulfate (200 mg) in anhydrous hexamethyldisilazane (HMDS, 50 ml) was refluxed in the presence of chlorotrimethylsilane (1 ml) until a clear solution was obtained (16 hours). The excess HMDS was removed by distillation under reduced pressure and the remaining syrup was dissolved in anhydrous dichloroethane (120 ml) under argon and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**5**, 5.0 g, 15.7 mmoles) was added. The reaction mixture was cooled (0-5°) and stannic chloride (3.0 ml, 1.4 equivalents) was added slowly. The reaction mixture was stirred at room temperature for 18 hours and poured slowly with stirring into a saturated aqueous sodium bicarbonate solution while keeping the solution neutral (*p*H ≥ 7). The microcrystalline tin salts were removed by filtration through a celite pad, and the residue was washed with dichloromethane (120 ml). The filtrate was successively washed with 10% aqueous sodium bicarbonate solution and water. The organic phase was separated and dried (sodium sulfate). Evaporation under reduced pressure provided a crude mixture of 4-nitro- and 5-nitronucleosides (3.8 g, 54% overall yield) which were separated by silica gel column chromatography using increasing gradient of chloroform (25-90%) in hexane. Compound **6**: was obtained in 33% yield (2.3 g), tlc (chloroform) R_f 0.33; mp (crystallization with ethanol) 89-91° (lit [10] mp 92-95°); ^{1}H nmr (deuteriochloroform): δ 7.95 (1H, H-2), 6.0 (1H, d, H-1', $J_{1',2'}$ = 4.0 Hz), 5.68-5.26 (3H, m, H-2', H-3' and H-4'), 4.60-4.38 (2H, m, H-5'), 2.18-2.00 (9H, s, 3 x $COCH_3$); uv (*p*H 1): λ max 312 nm: uv (*p*H 13): λ max 309 nm. Compound **7** was obtained in 14% yield (1.0 g), tlc (chloroform) R_f 0.22, mp (crystallization with chloroform:methanol 67-68°; ^{1}H nmr (deuteriochloroform): δ 7.90 (1H, s, H-2), 5.90 (1H, d, H-1', $J_{1',2'}$ = 3.8 Hz) 5.60-5.30 (2H, m, H-2', H-3'), 4.85-4.65 (1H, m, H-4'), 4.4-4.10 (2H, m, H-5') 2.0 (9H, s, 3 x $COCH_3$); uv (*p*H 1): λ max 313 nm: uv (*p*H 13): λ max 309 nm.

Anal. Calcd. for $C_{14}H_{16}BrN_3O_9$: C, 37.33; H, 3.55; Br, 17.78; N, 9.33. Found: C, 37.29; H, 3.55; Br, 17.57; N, 9.23.

5-Bromo-4-nitro-1-β-D-ribofuranosylimidazole (**8**).

The blocked nucleoside **6** (2.0 g, 4.4 mmoles) was dissolved in methanol (5 ml) and ammonium hydroxide (5 ml) was added. The mixture was stirred for 1.5 hours at room temperature. The solvent was evaporated under reduced pressure at ambient temperature and coevaporated with ethanol. The residue on crystallization with ethanol yielded (0.8 g, 55%) needle-shaped crystals of **8**, mp 165-166° (lit [10] mp 174-175°); ^{1}H nmr (perdeuteriomethanol): δ 8.6 (1H, s, H-2), 5.90 (1H, d, H-1', $J_{1',2'}$ = 3.8 Hz), 4.50-4.48 (3H, m, H-2', H-3' and H-4'), 4.02-3.80 (2H, m, H-5').

4-Bromo-5-nitro-1-β-D-ribofuranosylimidazole (**9**).

Compound **7** (0.5 g, 1.1 mmoles) was dissolved in methanol (3 ml) and ammonium hydroxide (2 ml) was added. The mixture was stirred at room temperature for 1 hour. The solvent was evaporated under reduced pressure. The crude product was purified by silica gel column chromatography using a solution of chloroform:ethyl acetate (1:1 v/v) for elution to yield 170 mg (47%) of **9**, mp 176-177°; ^{1}H nmr (perdeuteriomethanol): δ 8.50 (1H, s, H-2), 5.90 (1H, d, H-1', $J_{1',2'}$ = 3.5 Hz), 4.50-4.10 (2H, m, H-2' and H-3'), 3.95-3.75 (1H, m, H-4'), 3.50-3.25 (2H, m, H-5').

Anal. Calcd. for $C_8H_{10}BrN_3O_6$: C, 29.63; H, 3.08; Br, 24.69; N, 12.96. Found: C, 29.93; H, 3.16; Br, 24.52; N, 12.88.

5-Bromo-4-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**10**).

Nucleoside **8** (100 mg, 0.3 mmole) was added slowly to a cold (0°) stirred solution of perchloric acid (69-72% aqueous, 0.05 ml), anhydrous acetone (1.0 ml), and 2,2-dimethoxypropane (0.3 ml). The stirring was continued for 45 minutes while maintaining the temperature below 0°. The reaction solution was poured slowly into a cold stirred solution of aqueous ammonium hydroxide (20%) while maintaining the solution at *p*H ≥7.0. The solvent was evaporated under reduced pressure and coevaporated with ethanol. The residue was dissolved in chloroform and washed with water. The organic phase was dried (sodium sulfate) and evaporated to afford a crude product, which was crystallized from chloroform to yield (80 mg, 71%) of **10**, mp 147°; ^{1}H nmr (deuteriochloroform): δ 8.48 (1H, s, H-2), 6.10 (1H, d, H-1', $J_{1',2'}$ = 0.5 Hz), 5.05 (2H, m, H-2' and OH), 4.70 (1H, m, H-3'), 4.60 (1H, m, H-4'), 4.10 (2H, m, H-5'), 1.75 and 1.50 (2 x 3H, 2 x s, each 3H, 2 x CH_3).

Anal. Calcd. for $C_{11}H_{14}BrN_3O_6$: C, 36.26; H, 3.86; N, 11.54. Found: C, 36.54; H, 3.80; N, 11.22.

4-Bromo-5-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-imidazole (**11**).

Compound **9** (0.1 g, 0.3 mmole) was added to a cold (0°) solution of perchloric acid (69-72% aqueous, 0.1 ml), anhydrous acetone (1.0 ml) and 2,2-dimethoxypropane (0.3 ml). The stirring was continued for 45 minutes at 0°. The reaction mixture was poured into a cold solution of 30% aqueous ammonium hydroxide. The solvent was evaporated to provide a residue, which was dissolved in chloroform and washed with water. The organic phase was dried (sodium sulfate) and evaporated under reduced pressure to give a residue which was crystallized (chloroform) to yield (95 mg, 85%) of **11**, mp 178-179°; ^{1}H nmr (deuteriochloroform): δ 8.48 (1H, s, H-2), 6.10 (1H, d, H-1', $J_{1',2'}$ = 0.5 Hz), 5.05 (2H, m, H-2' and OH), 4.70 (1H, m, H-3'), 4.60 (1H, m, H-4'), 4.10 (2H, m, H-5'), 1.75 and 1.50 (2 x 3H, 2 x s, 2 x CH_3).

Anal. Calcd. for $C_{11}H_{14}BrN_3O_6$: C, 36.26; H, 3.86; N, 11.54. Found: C, 36.52; H, 3.88; N, 11.14.

5,5'-Anhydro-5-oxo-4-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**12**).

Sodium hydride (60% oil dispersion 27.2 mg, 0.70 mmole) was added to a solution of **10** (170 mg, 0.46 mmole) in anhydrous DMF (1 ml). The mixture was stirred for 18 hours at room temperature. The solvent was evaporated under reduced pressure and coevaporated with water. The residue was dissolved in chloroform and washed with water. The organic phase was dried (sodium sulfate), evaporated under reduced pressure and crystallized (chloroform) to yield (60 mg, 46%) of **12**; mp 167-168°; ^{1}H nmr (deuteriochloroform): δ 7.47 (1H, s, H-2), 5.9 (1H, s, H-1'), 5.35-4.85 (3H, m, H-2', H-3' and H-4'), 3.10-2.90 (2H, d, H-5', $J_{4',5'}$ = 5.0 Hz), 1.60 and 1.40 (2 x 3H, 2 x s, 2 x CH_3); uv (*p*H 1): λ max 315 nm; uv (*p*H 13): λ max 316 nm.

Anal. Calcd. for $C_{11}H_{13}N_3O_6$: C, 46.64; H, 4.59; N, 14.84. Found: C, 46.75; H, 4.71; N, 14.48.

5-Bromo-4-nitro-1-(5-deoxy-5-iodo-β-D-ribofuranosyl)imidazole (**14**).

Method A.

Rydon reagent (600 mg, 1.3 mmoles) was added under argon atmosphere to a solution of 5-bromo-4-nitro-1-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**10**, 300 mg, 0.89 mmole) in anhydrous DMF (2 ml). The mixture was stirred at room temperature for 2.5 hours. Methanol (2 ml) was added and the solvent evaporated under reduced pressure. The residue was dissolved in ethyl acetate (20 ml) and washed with sodium thiosulfate solution followed by water. The organic phase was dried (sodium sulfate) and evaporated under reduced pressure to provide a syrup which was passed through a column packed with silica gel slurry in benzene. Elution with benzene followed by 50% benzene in chloroform provided 5-bromo-4-nitro-1-(5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-ribofuranosyl)imidazole (**13**) with a trace of impurity (diphenylmethylphosphonate); ^{1}H nmr (deuteriochloroform): δ 8.18 (1H, s, H-2), 6.0 (1H, d, H-1', $J_{1',2'}$ = 2.5 Hz), 5.10-4.65 (2H, m, H-2' and H-3'), 4.40-4.15 (1H, m, H-4'), 3.60-3.40 (2H, d, H-5', $J_{4',5'}$ = 4.5 Hz), 1.60 and 1.35 (2 x 3H, 2 x s, 2 x CH_3). The crude product (200 mg, 0.6 mmole) was used without further purification for deblocking by dissolving in 80% formic acid (3 ml). The solution was stirred for 40 hours at 60°. The solvent was evaporated and coevaporated with water (5 x 2 ml) under reduced pressure. Purfication using preparative tlc (50% ethyl acetate in chloroform) yielded (50 mg, 28%) of **14** which was identical (tlc, mp, nmr) to **14** prepared by method B.

Method B.

Rydon reagent (279 mg, 0.62 mmole) was added under argon to a solution of **8** (200 mg, 0.62 mmole) in anhydrous DMF (2.0 ml). The solution was stirred at room temperature for 2 hours. Methanol (2.0 ml) was added and the solvent was evaporated under reduced pressure. The residue was applied on a preparative tlc plate which was developed in 50% (v/v) ethyl acetate in chloroform. The band containing **14** was scraped and eluted with 50% ethyl acetate in methanol to yield (120 mg, 65%) of **14** which was crystallized from methanol, mp 77-78°; ^{1}H nmr (perdeuteriomethanol): δ 8.30 (1H, s, H-2), 5.90 (1H, d, H-1', $J_{1',2'}$ = 4.8 Hz), 4.65-4.30 (1H, m, H-2'), 4.25-3.90 (2H, m, H-3' and H-4'), 3.0-2.8

(2H, d, H-5', $J_{4',5'}$ = 7.0); uv (*p*H 1): λ max 308 nm: uv (*p*H 13): λ max 299 nm.

Anal. Calcd. for $C_8H_9BrIN_3O_5 \cdot 1.5CH_3OH$: C, 23.65; H, 3.11; I, 26.35; N, 8.7. Found: C, 23.87; H, 2.98; I, 26.37; N, 8.9.

5-Mercapto-4-nitro-1-β-D-ribofuranosylimidazole 5-Sodium Salt (**15**).

Compound **6** (9.0 g, 8.9 mmoles) was dissolved in ethanol (15 ml) and sodium hydrosulfide dihydrate (2.45 g, 26.7 mmoles) was added. The solution was refluxed with stirring for 4 hours and evaporated to dryness. The residue was dissolved in a minimum amount of water (≈8.0 ml), neutralized with acetic acid, and the solvent evaporated under reduced pressure. The product after purification by silica gel column chromatography (20 to 35% methanol in ethyl acetate) yielded (1.7 g, 69%) of **15**. A portion (800 mg) of **15** was dissolved in ethanol (slight warming, 80 ml) and filtered. The filtrate was concentrated and the resulting powder was recrystallized with hot water and ethanol to yield 408 mg of **15** as orange-colored crystals, mp 200-210° dec; ms: m/z 277; ^{1}H nmr (DMSO-d_6): δ 7.75 (1H, s, H-2), 6.15 (1H, d, H-1', $J_{1',2'}$ = 2.0 Hz), 4.45-3.95 (2H, m, H-2' and H-3'), 3.65-3.45 (1H, m, H-4'), 3.20-3.0 (2H, m, H-5'); uv (*p*H 1) λ max 280 nm: uv (*p*H 13): λ max 284 nm.

Anal. Calcd. for $C_6H_{10}N_3O_6SNa$: C, 32.10; H, 3.34; N, 14.05; S, 10.70. Found: C, 32.12; H, 3.30; N, 14.07, S, 10.67.

5-(*E*-1-Iodo-1-penten-5-yl)thio-4-nitro-1-β-D-ribofuranosylimidazole (**17**).

Nucleoside **15** (150 mg, 0.54 mmole) was added to a solution of sodium methoxide (29 mg, 0.54 mmole) in anhydrous methanol (2 ml), and the mixture was stirred for 15 minutes at room temperature. Freshly prepared *E*-1,5-diiodopentene [23] (174 mg, 0.54 mmole) was added and the mixture was refluxed for 16 hours and the solvent evaporated under reduced pressure. The residue was dissolved in a mixture of chloroform and water, neutralized with acetic acid and washed with aqueous sodium bicarbonate followed by water. The organic phase was dried (sodium sulfate) and evaporated. The residue (syrup) was purified by silica gel column chromatography (ethyl acetate) to yield **17** (110 mg, 43%). The analytical sample was prepared by preparative tlc (ethyl acetate). The desired band was scraped and eluted with 25% methanol in ethyl acetate; ^{1}H nmr (perdeuteriomethanol): δ 8.4 (1H, s, H-2), 6.57-6.20 (2H, m, CH=CHI), 6.10 (1H, d, H-1', $J_{1',2'}$ = 4.5 Hz), 4.5-4.05 (2H, m, H-2' and H-3'), 3.4-2.85 (3H, m, H-4' and H-5'), 2.3-1.5 (6H, m, $(CH_2)_3$).

Anal. Calcd. for $C_{13}H_{18}IN_3O_6S$: C, 33.15; H, 3.82; S, 6.76. Found: C, 33.15; H, 4.07; S, 6.53.

5-*E*-1-[^{125}I]iodo-1-penten-5-yl)thio-4-nitro-1-β-D-ribofuranosylimidazole ([^{125}I]**17**).

E-1-[^{125}I]-5-Diiodopentene ([^{125}I]**16**) (300 μCi, specific activity 290 mCi/mmole) was prepared in the usual manner [27], dissolved in methanol (0.1 ml) and added to a solution of **15** (3 mg, 10.8 μmoles) in methanol (0.9 ml) containing 10.8 μmoles equivalent of sodium methoxide. The solution was heated (bath temperature 70 to 73°) with stirring under argon atmosphere for 4 hours. The solution was reduced to 0.1 ml by evaporation under argon and applied on a preparative tlc plate. The tlc plate was developed in 5% methanol in ethyl acetate (v/v). The band moving close to the solvent front corresponded to unreacted [^{125}I]**16** (80%). The band corresponding to **17** was scraped and eluted (15% metahnol in ethyl acetate) to yield 31 μCi (10%, or 90% on the basis of [^{125}I]**16** recovered) of [^{125}I]**17**.

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