Synthesis and in Vitro Biological Evaluation of *N*-[(5-Amino-1-β-D-ribofuranosyl-1*H*-imidazol-4-yl)carbonyl]-3-(hydroxynitrosamino)-L-alanine (L-Alanosine AICO Ribonucleoside)

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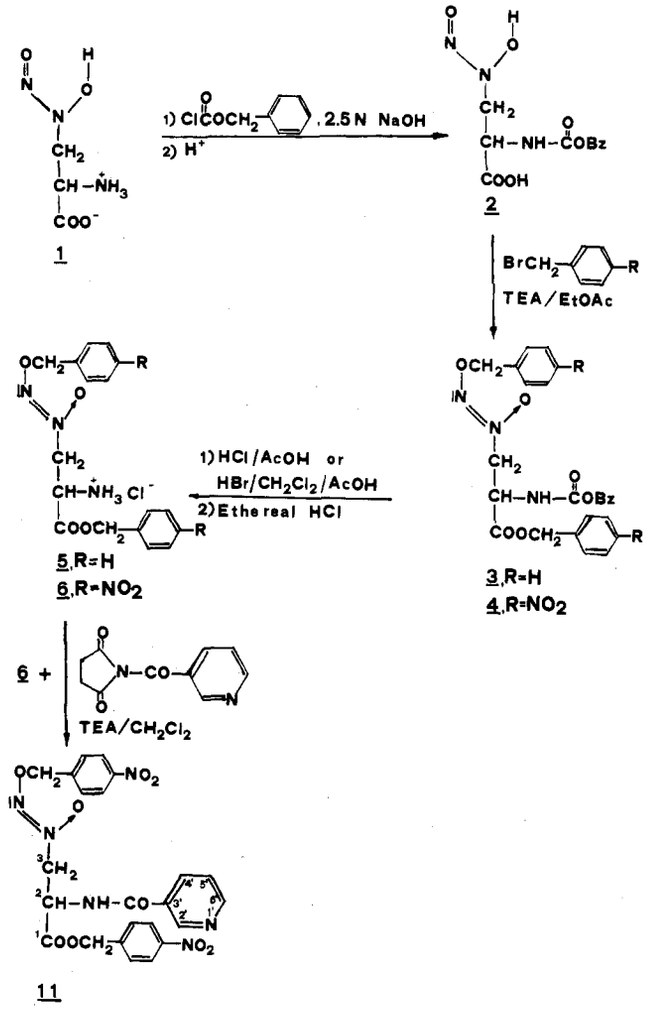
L-Alanosine [3-(hydroxynitrosoamino)-L-alanine] is an antitumor antibiotic that at the present is undergoing phase II clinical trials. Its mode of action as well as its metabolism has been extensively studied, and the metabolite *N*-[(5-amino-1-β-D-ribofuranosyl-1*H*-imidazol-4-yl)carbonyl]-3-(hydroxynitrosoamino)-L-alanine ribonucleotide (L-alanosine AICOR) proved to be an extremely potent inhibitor of de novo purine biosynthesis and is thus primarily responsible for the antitumor activity of the drug. The synthesis of the corresponding ribonucleoside, i.e., *N*-[(5-amino-1-β-D-ribofuranosyl-1*H*-imidazol-4-yl)carbonyl]-3-(hydroxynitrosamino)-L-alanine ribonucleoside (L-alanosine AICO ribonucleoside), was accomplished by condensation of a suitably protected derivative of L-alanosine with *N*-succinimidyl-5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxylate followed by the removal of the protective groups. The biological activity of L-alanosine AICO ribonucleoside was tested in vitro on whole tumor cells and on the isolated enzyme adenylosuccinate synthetase and in vivo on murine experimental leukemia. The compound was found to be inactive in these tests.

L-Alanosine [3-(hydroxynitrosoamino)-L-alanine, 1] is an antitumor antibiotic produced by fermentation of *Streptomyces alanosinicus* n. sp. ATCC 15710,[1,2] which can also be obtained by synthetic methods.[3-5] Extensive tests carried on at the National Cancer Institute (Bethesda, MD) have indicated that this compound has highly antitumor activity in L1210 and P388 murine leukemias.[6] At present, L-alanosine is undergoing phase II clinical trials.

The mode of action of L-alanosine has been extensively studied.[7-13] Due to its structure, L-alanosine is a bioisosteric analogue of L-aspartic acid and can substitute it in several enzymatic reactions.[9] In de novo purine biosynthesis, the metabolite resulting from the condensation of AICOR (5-aminoimidazole-4-carboxylic acid ribonucleotide) with L-alanosine, designated L-alanosine AICOR, is an extremely potent inhibitor of adenylosuccinate synthetase,[10] the enzyme that catalyzes the conversion of IMP into AMP. As a consequence, the availability of adenine nucleotides is drastically reduced and cells cease to grow; this effect is reversed by the administration of adenine.[11]

The presence of L-alanosine AICOR in tumors of animals treated with L-alanosine has been demonstrated. In addition, this metabolite was prepared by enzymatic means and its activity confirmed in vitro in a cell-free system.[10] Thus, L-alanosine AICOR appears to be responsible for the antitumor activity of the drug: this effect, however, may be limited by the small amount of the active metabolite produced in vivo, either due to high levels of L-aspartic acid[10] or to the low activity of the enzyme 5-amino-4-imidazole-*N*-succinocarboxamide ribonucleotide (SAICAR) synthetase, which catalyzes the condensation of AICOR with L-alanosine.[8,12] Consequently, L-alanosine AICOR

Scheme I



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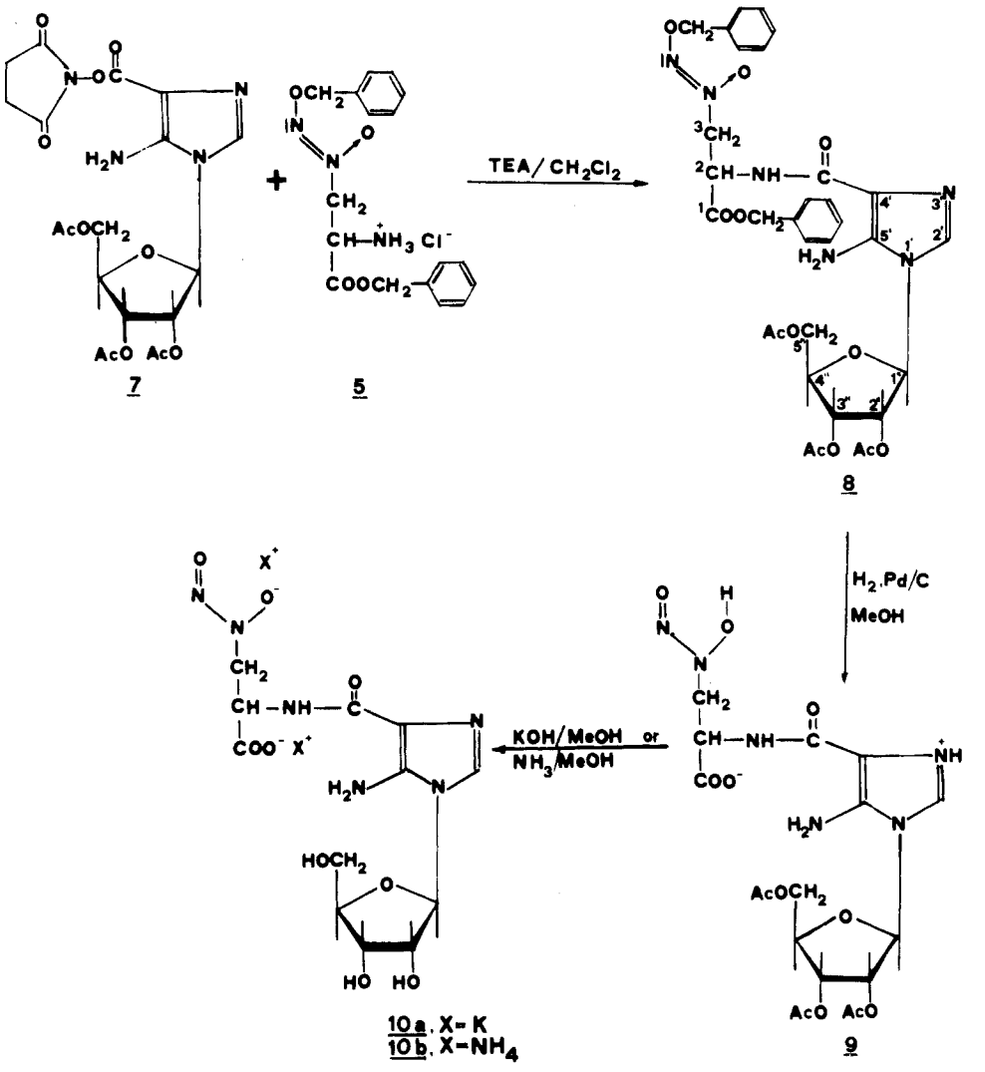
Scheme II



might be a new powerful antineoplastic drug. However, due to the fact that the phosphorylated compound is thought to be unable to enter the cell, the corresponding ribonucleoside was synthesized with the hope that either it was also active as an antimetabolite or after cell penetration it could be phosphorylated to the active metabolite.

Chemistry. The synthetic approach outlined in Scheme II was applied. A suitably protected derivative of L-alanosine (**5**) was condensed with an activated form of 5-amino-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxylic acid (AICO ribonucleoside), i.e., the *N*-succinimidyl-5-amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-imidazole-4-carboxylate (**7**), which was prepared from the commercially (Sigma) available 5-amino-1-β-D-ribofuranosyl-1*H*-imidazole-4-carboxamide (AICA) ribonucleoside by using the procedure previously described.[14]

The preparation of a protected form of **1** was accomplished as described in Scheme I. Preliminary experiments were made to assess whether **1** maintained its optical configuration under the conditions required for condensation and removal of the protecting groups and whether the hydroxynitrosoamino function was stable under the other reaction conditions (hydrogenation, basic hydrolysis). *N*-(Benzyloxycarbonyl)-3-(hydroxynitrosoamino)-L-alanine (**2**) was obtained by treating a solution of **1** with benzyl chloroformate. By reaction of **2** with benzyl bromide or *p*-nitrobenzyl bromide in the presence of TEA, the corresponding N,O-diblocked L-alanosine esters (**3**, **4**) were prepared in fairly good yields. The blocking group on the amino function was displaced by acid treatment, giving the corresponding compounds **5** and **6**. This latter compound (**6**) was used in experiments to optimize the condensation conditions, utilizing the *N*-succinimidyl ester of nicotinic acid as acylating agent. The two compounds were allowed to react for 10 days at room temperature in CH_2Cl_2 in the presence of TEA, obtaining, after silica gel column chromatography, the *N*-(3-pyridinylcarbonyl)-3-[N′-(4-nitrobenzyloxy)azoxy]-L-alanine 4-nitrobenzyl ester (**11**),[15] which showed $[\alpha]^{20}_D$ −36° (*c* 1%, DMF). Condensation of **5** with *N*-succinimidyl-AICO ribonucleoside (**7**) was accomplished in the presence of TEA, giving the fully blocked compound **8**. The removal of the benzyl groups

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(15) The compound melted at 138–140 °C. TLC *Rf* 0.55 ($CHCl_3$/MeOH, 9:1); IR (Nujol) 3350 (ν(NH)), 1735 (ν(C=O), ester), 1650 (ν(C=O), amide I), 1620, 1600 (ν(C=C)), 1525–1500 (δ NH, amide II; $\nu_{asym}NO_2$, ν(N=N)), 1340 ($\nu_{sym}(NO_2)$), 1220, 1190, and 1030 (ν(C–O)), 858, 750, 735, 700 cm^{-1} (γ CH arom); ^1H NMR ($CDCl_3$) δ 4.78 [d, 2, $J_{CH_2(3)-CH(2)}$ = 4 Hz, H(3)], 5.16 [dt, 1, $J_{CH(2)-NH}$ = 6.5 Hz, H(2)], 5.32 (s, 2, CH_2Ph), 5.35, 5.43 (2 d, 2, J_{gem} = 13 Hz, CH_2Ph), 7.30 (d, 1, NHCO), 7.45 [dd, 1, J_{ortho} = 5–8 Hz, H(5′)], 7.50, 7.58 (2 d, 4, J_{ortho} = 8.5 Hz, H arom *m*-NO_2), 8.07 [ddd, 1, J_{ortho} = 8 Hz, J_{meta} = 1.5 Hz, H(4′)], 8.13, 8.27 (2d, 4, H arom *o*-NO_2), 8.82 [dd, 1, J_{ortho} = 5 Hz, J_{meta} = 1.5 Hz, H(6′)], 8.96 [d, 1, H(2′)]. Anal. ($C_{23}H_{20}N_6O_9$) C, H, N.

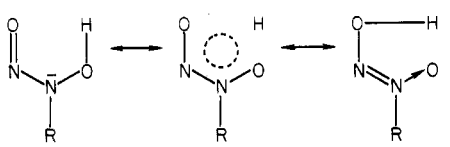
Table I

compd	yield, %	TLC, R_f ($CHCl_3$/MeOH, 9:1, v/v)	mp,[a] °C dec	$[\alpha]^{20}_D$, deg (c, solv)	UV spectral data λ_{max}, nm (ϵ), solv	formula	anal.
2	47.6	0.70	130	−33.0 (1, 0.1 N NaOH)	230 (6770), MeOH 227 (6855), 0.1 N HCl 251 (9430), 0.1 N NaOH	$C_{11}H_{13}N_3O_6$	C, H, N
3	46.2	nd[b]	83	−32.7 (1, DMF)	237 (12515), MeOH	$C_{25}H_{25}N_3O_6$	C, H, N
4	51.5	0.44	139	−31.0 (1, DMF)	260 (29115), MeOH	$C_{25}H_{23}N_5O_{10}$	C, H, N
5	53.5	nd	134	−10.0 (1, DMF)	238 (11265), MeOH	$C_{17}H_{19}N_3O_4$·HCl	C, H, N, Cl
6	79.2	0.35	140	−11.9 (1, DMF)	261 (22200), MeOH	$C_{17}H_{17}N_5O_8$·HCl	C, H, N, Cl
8	31.3	0.80	50	−20.7 (1, MeOH)	240 (18695) and 266 (14230), MeOH	$C_{32}H_{36}N_6O_{12}$	C, H, N
9	95.7	nd	>100	−7.2 (1, MeOH)	235 (11670) and 265 (14045), MeOH	$C_{18}H_{24}N_6O_{12}$	C, H, N
10a	93.4	0.70[c]	>150	−37.8 (1, H_2O)	258 (15630), H_2O	$C_{12}H_{16}N_6O_9K_2$	C, H, N

[a] Melting points were determined by DSC. [b] nd = not determined. [c] NH_4OH/MeOH/$CHCl_3$, 2:2:1 (v/v).

was achieved by catalytic hydrogenolysis. The tri-*O*-acetyl intermediate **9** so obtained was converted into the final *N*-[(5-amino-1-β-D-ribofuranosyl-1*H*-imidazol-4-yl)-carbonyl]-3-(hydroxynitrosoamino)-L-alanine as the dipotassium (**10a**) or diammonium (**10b**) salt (L-alanosine AICO ribonucleoside) by treatment with the methanolic solutions of the corresponding bases. The correct structures of compound **10** and of the intermediates were assigned on the basis of their IR and UV spectra. Some physicochemical data are reported in Table I.

The compounds obtained by nitrosation of N-monosubstituted hydroxylamine exist as an intermediate structure of at least two main forms,[16] i.e., hydroxynitrosoamino (I) or diimide *N*-oxide (II).[17] Compound **1**



I II

was found to exist preferentially as structure I.[2] In fact, the UV spectrum shows an absorption maximum at 228 nm (ε 7350) in 0.1 N HCl shifting to 250 nm (ε 9395) in 0.1 N NaOH attributable[18,19] to the $\pi \rightarrow \pi^*$ transition in form I while it lacks[5] the absorption maximum at 220 nm characteristic of form II.[18] Further evidence is given by the IR spectrum (Nujol) showing bands at 1280, 1250, 1160 (ν(N=O)), 985 (ν(N–N)), and 670 cm^{-1} (N–N=O deformation) and the absence of the band ν(N=N) at ~1500 cm^{-1}.[20,21] Finally, in the mass spectrum (DIS, 160 °C) the peak at M − 16 (corresponding to a loss of oxygen from the –N=N→O system) does not occur, while the presence of a peak at m/z 30 is due to the N=O group. The colorimetric determination[22] of the *N*-nitroso group also gives a positive result. Compounds **2**, **9**, and the final products **10a** and **10b** possess the UV, IR, and colorimetric properties outlined above for **1**, i.e., they are in the hydroxynitrosoamino form (I). The alkyl derivatives **3–6**, **8**, and **11** are in form II, in fact, while they have the IR absorption at 1500 cm^{-1} (ν(N=N)), they lack the other bands characteristic of form I and they give a negative N–NO colorimetric test.

Table II

compd	in vitro activity: inhib of cell proliferation: ED_{50},[a] mM	in vitro activity: inhib of enzymatic activity: ED_{50},[b] mM
L-alanosine (**1**)	0.002	7
L-alanosine AICO ribonucleoside (**10a**)	>0.1	>1
L-alanosine AICOR	nt[c]	0.0003

[a] ED_{50}: concentration that inhibits the proliferation of tumor cells by 50%. [b] ED_{50}: concentration that inhibits the activity of adenylosuccinate synthetase by 50%. Data for L-alanosine and L-alanosine AICOR are from the literature;[9] the data for L-alanosine AICO ribonucleoside (**10a**) are from D. A. Cooney. [c] Not tested.

Biological Activity. L-Alanosine AICO ribonucleoside (**10a**) was tested in vitro on HL60 human promyelocitic tumor cells. Inhibition of cell proliferation was measured as inhibition of [^{3}H]methylthymidine incorporation into the cell DNA (see Experimental Section). Compound **10a** is inactive at concentrations up to 100 times the concentration of L-alanosine that inhibits the proliferation of treated cells by 50% (Table II).

In order to test whether the lack of activity of L-alanosine AICO ribonucleoside (**10a**) might be due to a lack of penetration into cells, it was tested in a cell-free system against adenylosuccinate synthetase, the enzyme that is inhibited by the L-alanosine metabolite (L-alanosine AICOR). As shown in Table II, compound **10a** was inactive (D. A. Cooney, personal communication). L-Alanosine AICO ribonucleoside (**10a**) was also tested in vivo in mice

(16) The matter has been reviewed by Zeeh and Metzger: Zeeh, B.; Metzger, H. In "Methoden der Organishen Chemie (Houben-Weyl)", G. Thieme Verlag: Stuttgart, 1971; Vol. X/1, Chapter 5, p 1273.
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(21) Avram, M.; Mateescu, GH. D. In "Infrared Spectroscopy"; 1st ed.; Wiley-Interscience: New York, 1972; Chapter 5, pp 324–326.
(22) Eisenbrand, G.; Preussmann, R. *Arzneim.-Forsch.* **1970**, *20*, 1513.

bearing P388 lymphocytic leukemia; it failed to increase the survival time of treated mice (data not shown).

In conclusion, it appears that the unphosphorylated metabolite is not active as an inhibitor of the isolated enzyme. The lack of activity on the whole cells might be due either to a lack of phosphorylation or to the fact that the metabolite is not transported through the cell membrane.

Experimental Section

Evaporation was done at 40 °C (bath temperature), in vacuo. Melting points were taken from the DSC profiles obtained with a Du Pont Model 990 thermal analyzer. Reactions and final products were monitored by TLC on silica gel Merck 60 F-254 (0.25 mm) plates (spots visualized under UV light at 254 nm). IR spectra (Nujol) were recorded on a Perkin-Elmer Model 580 spectrophotometer. ^{1}H NMR spectra were taken on a Bruker WH-270 cryospectrometer at 270-MHz using Me_4Si (δ 0.00 ppm) as internal standard. UV spectra were obtained with a Perkin-Elmer Model 320 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter in a 0.1-dm cell. Elemental analyses were obtained with a Carlo Erba Model 1106 elemental analyzer. Where analyses are indicated only by symbols of the elements, the analytical results obtained for these elements were within ±0.4% of the theoretical values. Radioactivity was measured with an Intertechnique SL 4000 scintillation counter, with ^{3}H counting efficiency 42%.

***N*-(Benzyloxycarbonyl)-3-(hydroxynitrosoamino)-L-alanine (2).** L-Alanosine (1; 9 g, 60 mmol) was dissolved in 160 mL of 1 N NaOH with stirring at 5 °C, obtaining a solution at pH 13.0 to which 20 mL of 2.5 N NaOH and 14 mL (98 mmol) of benzyl chloroformate were added. Stirring was continued for 6 h at 0–3 °C while the pH was maintained between 12.7 and 13 by adding 2.5 N NaOH dropwise. After standing overnight, the reaction mixture was extracted with 250 mL of a 2:1 mixture of Et_2O–EtOAc. The aqueous phase was cooled at 3 °C, the pH was brought to 2.6 with concentrated HCl and the phase then was extracted three times with 300 mL of EtOAc. The organic phase was washed with water and dried (Na_2SO_4). Removal of the solvent gave a residue, which was triturated with Et_2O (40 mL), filtered, and dried over P_2O_5 in vacuo (4.3 g). By concentration of the mother liquor, an additional 1 g of the compound was obtained. The aqueous phase was brought to pH 13 with 2.5 N NaOH and 6 mL (40 mmol) of benzyl chloroformate was added. By carrying out the reaction as described above, 2.8 g of the compound was obtained (overall yield 8.1 g): IR 3320 (ν(NH)), 3280–2000 (ν(OH)), 1740 (ν(C=O), acid), 1700 (ν(C=O), amide I), 1600 (ν(C=C)), 1545 (δ NH, amide II), 1270, 1250, and 1190 (ν(N=O)), 1265 (ν(C–O)), 935 (ν(N–N)), 735 and 695 (γ CH arom), 670 cm^{-1} (N–N=O); ^{1}H NMR (Me_2SO-d_6) δ 4.37 [d, 2, $J_{CH_2(3)-CH(2)}$ = 5.5 Hz, H(3)], 4.60 [dt, 1, $J_{CH(2)-NH}$ = 8 Hz, H(2)], 5.04 (s, 2, CH_2Ph), 7.35 (s, 5, H arom), 7.80 (d, 1, NHCO), 11.5–12.2 (br, 2, COOH and OH).

***N*-(Benzyloxycarbonyl)-3-[(benzyloxy)-*NNO*-azoxy)]-L-alanine Benzyl Ester (3).** To a solution of 2.8 g (9.8 mmol) of **2** and 2.9 mL (20 mmol) of anhydrous TEA in 180 mL of EtOAc cooled at 10 °C with stirring was added 2.5 mL (21 mmol) of benzyl bromide dissolved in 70 mL of EtOAc. The reaction mixture was allowed to warm to room temperature, stirred for 7 h, and then left for 5 days. The reaction was monitored by TLC. The precipitate was filtered off and the solution was washed with an equal volume of 2% aqueous $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, and concentrated to a small volume. By addition of 100 mL of Et_2O and then *n*-hexane up to cloudiness, a crystalline compound formed that was collected and dried over P_2O_5 in vacuo (2.1 g): IR 3330 (ν(NH)), 1740 (ν(C=O), ester), 1685 (ν(C=O), amide I), 1585 (ν(C=C)), 1520 (δ NH, amide II), 1500 (ν(N=N)), 1225 and 1050 (ν(C–O)), 750, 740, and 700 cm^{-1} (γ CH arom); ^{1}H NMR ($CDCl_3$) δ 4.48–4.61 [2 d, 2, J_{gem} = 12 Hz, $J_{CH_2(3)-CH(2)}$ = 3–2.5 Hz, H(3)], 4.76 [ddd, 1, $J_{CH(2)-NH}$ = 7 Hz, H(2)], 5.08, 5.15, 5.16 (3 s, 6, CH_2Ph), 5.77 (d, 1, NH), 7.35 (s, 15, H arom).

***N*-(Benzyloxycarbonyl)-3-[[(4-nitrobenzyl)oxy]-*NNO*-azoxy]-L-alanine 4-Nitrobenzyl Ester (4).** Anhydrous TEA (5.7 mL, 40 mmol) and Na_2SO_4 (20 g) were added with stirring and cooling at 5–10 °C to a solution of 5.7 g (20 mmol) of **2** in 400 mL of EtOAc. To this suspension was added dropwise a solution of 9 g (41 mmol) of 4-nitrobenzyl bromide in 200 mL of EtOAc over 90 min while the temperature was maintained below 10 °C. The reaction mixture was allowed to warm to room temperature and left for 4 days with intermittent stirring. After removal of the insoluble material by filtration, the solution was evaporated to dryness. The oily residue (16 g) was dissolved in 80 mL of CH_2Cl_2 and applied to a column of 800 g of silica gel (Merck, 0.2–0.05 mm) slurried in CH_2Cl_2. Fractions of 200 mL each were collected, eluting with CH_2Cl_2 (1 L), CH_2Cl_2–MeOH (99:1, 3 L), and finally CH_2Cl_2–MeOH (98:2, 2 L). Fractions 17–22 containing the product were pooled and evaporated to dryness. The residue was triturated with 50 mL of EtOAc, 400 mL of Et_2O was added, and the suspension was stirred for 30 min. The solid was filtered, washed with Et_2O, and dried in vacuo (5.7 g). An analytical sample was crystallized from CH_2Cl_2–EtOAc: IR 3305 (ν(NH)), 1750 (ν(C=O), ester), 1690 (ν(C=O), amide I), 1605 (ν(C=C)), 1515 (δ NH, amide II), 1540 and 1350 ($\nu(NO_2)$), 1500 (sh, ν(N=N)), 1230 and 1040 (ν(C–O)), 860, 750, and 700 cm^{-1} (γ CH arom); ^{1}H NMR ($CDCl_3$) δ 4.58, 4.64 [2 dd, 2, J_{gem} = 12 Hz, $J_{CH_2(3)-CH(2)}$ = 3–2.5 Hz, H(3)], 4.78 [ddd, 1, $J_{CH(2)-NH}$ = 6 Hz, H(2)], 5.10, 5.30 (2 s, 6, CH_2Ph), 5.72 (d, 1, NH), 7.36 (s, 5, H arom), 7.50, 7.53 (2 d, 4, J_{ortho} = 8.5 Hz, H arom *m*-NO_2), 8.22, 8.24 (2 d, 4, H arom *o*-NO_2).

3-[(Benzyloxy)-*NNO*-azoxy]-L-alanine Benzyl Ester Hydrochloride (5). Anhydrous HCl was bubbled into a suspension of 4.5 g (9.7 mmol) of **3** in 100 mL of AcOH until all the solid was dissolved (ca. 3.5 h). Bubbling was continued for 1 h and then the solution was poured into 900 mL of Et_2O. After remaining overnight, the supernatant was discarded and the solid was washed with a little Et_2O and then dissolved in 200 mL of water. The pH of the solution was brought to 9 with Na_2CO_3 and then the solution was extracted with EtOAc (3 × 200 mL). The organic extracts were combined, dried (Na_2SO_4), and concentrated to 50 mL. By addition of ethereal HCl, a precipitate formed, which was filtered off, washed with Et_2O, and then dried over P_2O_5 at 40 °C in vacuo. After recrystallization from CH_2Cl_2–MeOH, 1.9 g of **5** was obtained: IR 3300–1900 ($\nu(NH_3^+)$), 1745 (ν(C=O), ester), 1590 (δ NH_3^+), 1600 (ν(C=C)), 1500 (ν(N=N)), 1250 (ν(C–O)), 750 and 700 cm^{-1} (γ CH arom); ^{1}H NMR (Me_2SO-d_6) δ 4.77 [s, 3, H(2) + H(3)], 5.14, 5.22 (2d, 2, J_{gem} = 15 Hz, CH_2Ph), 5.26 (s, 2, CH_2Ph), 7.44 (s, 10, H arom), 9.15 (br, 3, NH_3^+).

3-[[(4-Nitrobenzyl)oxy]-*NNO*-azoxy]-L-alanine 4-Nitrobenzyl Ester Hydrochloride (6). To a solution of 4 g (7.2 mmol) of **4** in 100 mL of CH_2Cl_2 was added 100 mL of AcOH, and then anhydrous HBr was bubbled for 2 h while the solution was stirred. The reaction mixture was evaporated in vacuo. By addition of Et_2O, an oily product separated, which was washed with Et_2O and dissolved in water. The solution was brought to pH 8 with $NaHCO_3$ and extracted with EtOAc. The combined extracts were dried (Na_2SO_4) and concentrated to 100 mL. By addition of ethereal HCl, a precipitate formed, which was filtered off, washed with Et_2O, and dissolved in a mixture of CH_2Cl_2–absolute EtOH. By concentration a crystalline product separated, which was collected and dried over P_2O_5 in vacuo (2.6 g): IR 3300–1900 ($\nu(NH_3^+)$), 1765 (ν(C=O), ester), 1580 (δ NH_3^+), 1610 (ν(C=C)), 1520 and 1350 ($\nu(NO_2)$), 1500 (ν(N=N)), 1225 (ν(C–O)), 815 and 805 cm^{-1} (γ CH arom); ^{1}H NMR (Me_2SO-d_6) δ 4.84 [m, 3, H(2) + H(3)], 5.33, 5.42 (2 d, 2, J_{gem} = 15 Hz, CH_2Ph), 5.47 (s, 2, CH_2Ph), 7.60, 7.68 (2 d, 4, J_{ortho} = 8.5 Hz, H arom *m*-NO_2), 8.25, 8.26 (2 d, 4, H arom *o*-NO_2), 9.11 (br, 3, NH_3^+).

***N*-[[5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-imidazol-4-yl]carbonyl]-3-[(benzyloxy)-*NNO*-azoxy)]-L-alanine Benzyl Ester (8).** To a solution of 3.2 g (8.7 mmol) of **5** in 35 mL of CH_2Cl_2 was added 1.26 mL (9 mmol) of TEA followed by 4.5 g (9.3 mmol) of **7**. The reaction mixture was stirred at room temparature for 2 days, and then an additional 0.15 g (0.3 mmol) of **7** was added and stirring was continued for 3 days. EtOAc (150 mL) was added followed by 50 mL of 5% AcOH. After the mixture was stirred for 20 min, Et_2O (50 mL) was added and the organic layer was separated, washed with water (100 mL), 5% aqueous $NaHCO_3$ (100 mL), then water (100 mL), and treated with charcoal for 10 min. The mixture was filtered and the filtrate was dried (Na_2SO_4) and evaporated to dryness. The oily residue was dissolved in 40 mL of EtOAc, the insoluble material was filtered off, and the solution was poured into 400 mL of Et_2O with

stirring. After filtering on Celite BDH 545, the solution was evaporated to dryness, obtaining an oily residue. Trituration with a mixture of Et_2O-*n*-hexane and filtration gave a gummy solid (1.9 g): IR 3330 ($\nu(NH_2)$), 3410 ($\nu(NH)$), 1745 ($\nu(C{=}O)$, ester), 1640 ($\nu(C{=}O)$, amide I), 1625 (δ NH_2), 1560 ($\nu(C{=}C)$ and $\nu(C{=}N)$), 1510 (δ NH, amide II), 1500 ($\nu(N{=}N)$), 1300–1150 and 1105–1000 ($\nu(C{-}O)$), 780, 750, and 700 cm^{-1} (γ CH arom); ^1H NMR ($CDCl_3$) δ 2.22, 2.25 (2 s, 9, $COCH_3$), 4.53 [m, 3, H(5″) + H(4″)], 4.66, 4.77 [2 dd, 2, J_{gem} = 13 Hz, $J_{CH_2(3)-CH(2)}$ = 4–5 Hz, H(3)], 5.26 [m, 4, H(2) + H(3″) + CH_2Ph], 5.46 (m, 4, CH_2Ph + NH_2), 5.62 [dd, 1, $J_{CH(2'')-CH(1'')}$ = 5.5 Hz, $J_{CH(2'')-CH(3'')}$ = 5.5 Hz, H(2″)], 5.80 [d, 1, H(1″)], 7.28 [s, 1, H(2′)], 7.46 (m, 10, H arom), 7.58 [d, 1, $J_{CH(2)-NH}$ = 7 Hz, NHCO)].

***N*-[[5-Amino-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-imidazol-4-yl]carbonyl]-3-(hydroxynitrosoamino)-L-alanine (9).** A solution of 0.665 g (0.95 mmol) of **8** in 80 mL of anhydrous MeOH was hydrogenated at 4 atm and room temperature in the presence of 450 mg of 5% Pd on C. After addition of Celite analytical filter aid (1 g), the reaction mixture was filtered. The catalyst–Celite pad was washed with 100 mL of MeOH. The clear filtrate was diluted with absolute EtOH (100 mL) and concentrated to a small volume (ca. 40 mL). A crystalline product separated, which was filtered off, washed with Et_2O, and dried in vacuo (0.47 g): IR 3340 ($\nu(NH_2)$), 3400 ($\nu(NH)$), 2100–1800 ($\nu(OH)$), 2560–2200 ($\nu(NH^+)$), 1750 ($\nu(C{=}O)$, ester), 1630 ($\nu(COO^-)$), 1565 ($\nu(C{=}C)$ and $\nu(C{=}N)$), 1510 (δ NH, amide II), 1300–1150 and 1110–1000 ($\nu(C{-}O)$), 980 ($\nu(N{-}N)$), 780 (γ CH arom), 670 cm^{-1} (N–N=O); ^1H NMR (Me_2SO-d_6) δ 2.07, 2.13 (2 s, 9, 3 $COCH_3$), 4.32 [s, 3, H(5″) + H(4″)], 4.50, 4.62 [2 dd, 2, J_{gem} = 14 Hz, $J_{CH_2(3)-CH(2)}$ = 3–5.5 Hz, H(3)], 4.98 [ddd, 1, $J_{CH(2)-NH}$ = 7 Hz, H(2)], 5.36 [m, 1, H(3″)], 5.61 [dd, 1, $J_{CH(2'')-CH(1'')}$ = 5.54, $J_{CH(2'')-CH(3'')}$ = 5.5 Hz, H(2″)], 5.94 [d, 1, H(1″)], 5.94, 6.06 (2 s, 2, NH_2); 7.54 [s, 1, H(2′)], 7.82 (d, 1, NHCO). Evaporation of the mother liquor to dryness gave an additional 0.21 g of an oily product, which was shown by TLC and IR to be the desired compound.

***N*-[(5-Amino-1-β-D-ribofuranosyl-1*H*-imidazol-4-yl)carbonyl]-3-(hydroxynitrosoamino)-L-alanine Dipotassium Salt (10a).** To a solution of 0.206 g (0.39 mmol) of **9** in 40 mL of MeOH was added dropwise at room temperature 3.6 mL of a solution of 3.2% methanolic potassium hydroxide (2.05 mmol). After the mixture was stirred for 30 min, 40 mL of absolute EtOH was added and the reaction mixture was evaporated to ca. 10 mL. The crystalline precipitate was filtered off, washed with a small amount of absolute EtOH and Et_2O, and dried to obtain 0.17 g of the compound: IR 3700–2000 ($\nu(NH_2)$, $\nu(NH)$, and $\nu(OH)$), 1610 ($\nu(COO^-)$), 1630 ($\nu(C{=}O)$, amide I), 1570 ($\nu(C{=}C)$ and $\nu(C{=}N)$), 1515 (δ NH, amide II), 1255 ($\nu(N{=}O)$), 1130, 1080, and 1060 ($\nu(C{-}O)$), 975 ($\nu(N{-}N)$), 850 (γ CH arom), 680 cm^{-1} (N–N=O); ^1H NMR (D_2O) δ 3.80, 3.87 [2 dd, 2, J_{gem} = 12.5 Hz, $J_{CH_2(5'')-CH(4'')}$ = 3.5–3 Hz, H(5″)], 4.21 [ddd, 1, $J_{CH(4'')-CH(3'')}$ = 3.5 Hz, H(4″)], 4.35 [dd, 1, $J_{CH(3'')-CH(2'')}$ = 5 Hz, H(3″)], 4.37, 4.53 (2 dd, 2, J_{gem} = 9 Hz, $J_{CH_2(3)-CH(2)}$ = 3.5–4.5 Hz, H(3)], 4.61 (dd, 1, $J_{CH(2'')-CH(1'')}$ = 5.5 Hz, H(2″)], 4.84 [m, 1, H(2)], 5.68 (d, 1, H(1″)], 7.52 [s, 1, H(2′)].

The diammonium salt **10b** was prepared by allowing a solution of 180 mg (0.35 mmol) of **9** in 50 mL of 6.5 M methanolic ammonia to stand at room temperature with stirring for 3 h. After evaporation of the solvent, a crystalline product separated. EtOH (50 mL) was added and the solution was concentrated to 20 mL. The solid formed was filtered off, washed with Et_2O, and dried in vacuo over P_2O_5 (0.140 g). This compound showed a TLC behavior identical with that of **10a** and the IR spectrum was compatible with the proposed structure of **10b**: ^1H NMR (Me_2SO-d_6) δ 3.60 [m, 2, H(5″)], 3.91 [m, 1, H(4″)], 4.06 [dd, 1, $J_{CH(3'')-CH(2'')}$ = 5.5 Hz, $J_{CH(3'')-CH(4'')}$ = 5 Hz, H(3″)], 4.33 [m, 4, H(3) + H(2) + H(2″)], 5.48 [d, 1, H(1″)], 5.0–7.4 (br, mobile H), 7.36 [s, 1, H(2′)], 7.45 (d, 1, NHCO).

Determination of in Vitro Cytotoxic Activity. HL60 human promyelocytic cells were cultured in suspension at 37 °C in an atmosphere of 5% CO_2–95% air. Growth medium was RPMI 1640 supplemented with heat-inactivated fetal calf serum (20%), glutamine (0.3 mg/mL), and gentamycin (50 μg/mL). Exponentially growing cells were ensured by seeding them every 24–36 h in fresh growth medium at the concentration of 2×10^5 cells/mL. Cell number was determined in a hemocytometer by a dye-exclusion technique. Cytotoxic activity was determined as inhibition of [^3H]methylthymidine incorporation. Exponentially growing HL60 cells were suspended in fresh growth medium and distributed into microplate wells containing different concentrations of the drugs. Each microwell contained 5×10^4 cells in 0.2 mL. Plates were incubated at 37 °C for 20 h; then cells were exposed to [^3H]methylthymidine (5 μCi/mL) for 2 h. After the 2-h pulse, 0.1 mL was withdrawn from each well and pipetted onto Whatman glass fiber filters that were dipped in cold aqueous solution of 5% TCA and 1% sodium pyrophosphate; the filters were then washed with 5% TCA and dried in vacuo. The radioactivity incorporated into TCA-insoluble material was determined in a liquid scintillation counter. Cytotoxic activity is expressed as the drug concentration that causes 50% inhibition of [^3H]methylthymidine incorporation. The results reported are the average of at least three determinations; the SD ranged from 6.5% to 7.8%.

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