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4-Homopyrazofurin and an acyclic analogue

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ABSTRACT

The synthesis of 4-hydroxymethyl-3(5)-(β-D-ribofuranosyl)pyrazole-5(3)-carboxamide (**2**, 4-homopyrazofurin) is described via a pathway that commences with the dipolar cycloaddition reaction of 2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-diazo-D-allitol (**4**) and methyl 4-benzyloxy-2-butynoate (**5**). Preparation of 3(5)-[(2-hydrox,-ethoxy)methyl]-4-(hydroxymethyl)pyrazole-5(3)-carboxamide (**3**) as a derivative of **2** possessing the truncated acyclic side chain of acyclovir has been accomplished in a similar manner beginning with the reaction of **5** with 1-diazo-2-[(2-benzyloxy)ethoxy]ethane (**13**). Neither compound **2** nor **3** showed any in vitro antiviral activity against human immunodeficiency virus (HIV-1), sandfly fever, Punta Toro, Japanese encephalitis, yellow fever, Venezuelan equine encephalomyelitis, and vaccinia viruses. Both compounds were also nontoxic. These results suggest that direct bonding of the hydroxyl group to the pyrazole ring is important for pyrazofurin based agents to demonstrate biological activity.

INTRODUCTION

Nucleosides of 5-membered heterocycles are playing a prominent role in the design of antiviral agents[1a]. Included in this group is 4-hydroxy-3-β-D-ribofuranosylpyrazole-5-carboxamide (pyrazofurin, **1**), which is a naturally occurring *C*-nucleoside whose usefulness as an antiviral agent is limited by its toxicity[1b]. In that regard, however, De Clercq and Torrence[1c] have suggested that the toxicity of **1** is unlikely to be associated with the structural components that are responsible for its antiviral properties. To evaluate this suggestion for the purposes of producing nontoxic pyrazofurin-derived antiviral agents, we[2] and others[3] have focused on modification of the 4-hydroxyl moiety. To date, there has been no analysis of the relationship between direct bonding of this substituent to the pyrazole ring of **1**

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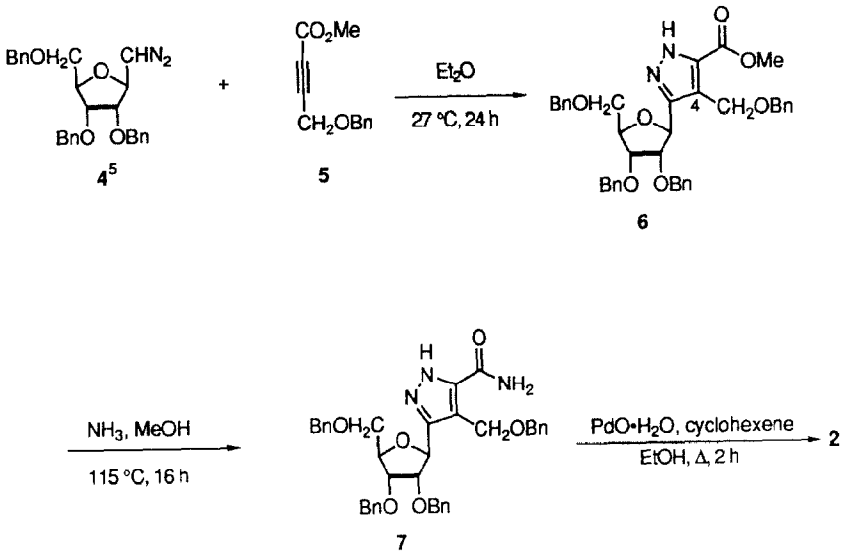
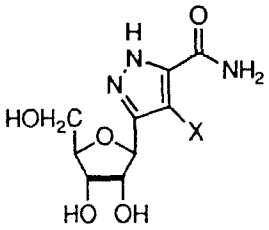
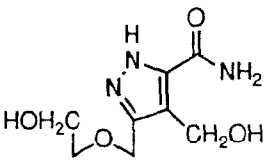


Scheme 1.

and cytotoxicity or antiviral properties. In this latter regard, this paper describes the synthesis of the 4-homologue of **1** (that is, **2**) and, due to the biological properties shown by acyclic nucleosides[4], an acyclic analogue **3**.



1, X = OH
2, X = CH_2OH



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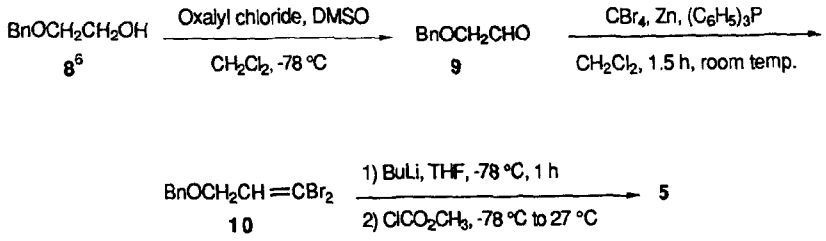
DISCUSSION

Scheme 1 presents the approach used for the preparation of **2** that began with a 1,3-dipolar cycloaddition reaction involving 2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-diazo-D-allitol (**4**) and methyl 4-benzyloxy-2-butynoate (**5**). Since a convenient synthesis of **4** was available[5], it remained to develop a route to the unknown dipolarophile **5**. This is shown in Scheme 2.

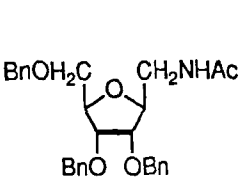
Subjecting 2-benzyloxyethanol (**8**)[6] to a Swern oxidation[7] yielded aldehyde **9**, which was homologated to the *gem*-dibromoalkene **10** by treatment with a reagent prepared from the reaction of carbon tetrabromide with triphenylphosphine[8].



Scheme 2.

Treatment of **10** with butyllithium resulted in a 1,2-elimination to a bromoalkyne intermediate, which underwent metal–halogen exchange to the terminal alkyne anion that was trapped[9] with methyl chloroformate to give **5**.

Reaction of **5** with **4**[5] (Scheme 1), which was generated as needed from 1-acetamido-2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-D-allitol (**11**)[2,5b], proceeded cleanly to yield one detectable regioisomer **6**. The regiochemistry of this cycloaddition was analyzed by comparing the ^{13}C NMR chemical shift for C-4 of **6** (116.51 ppm) with the same carbon of ester **12** (103.47 ppm)[2], which is in agreement with the expected downfield shift (typically, 10–15 ppm) that results when an aromatic proton is replaced by a $-CH_2O-$ group[10].



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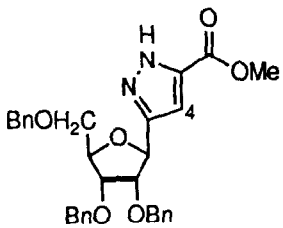

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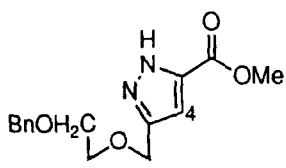

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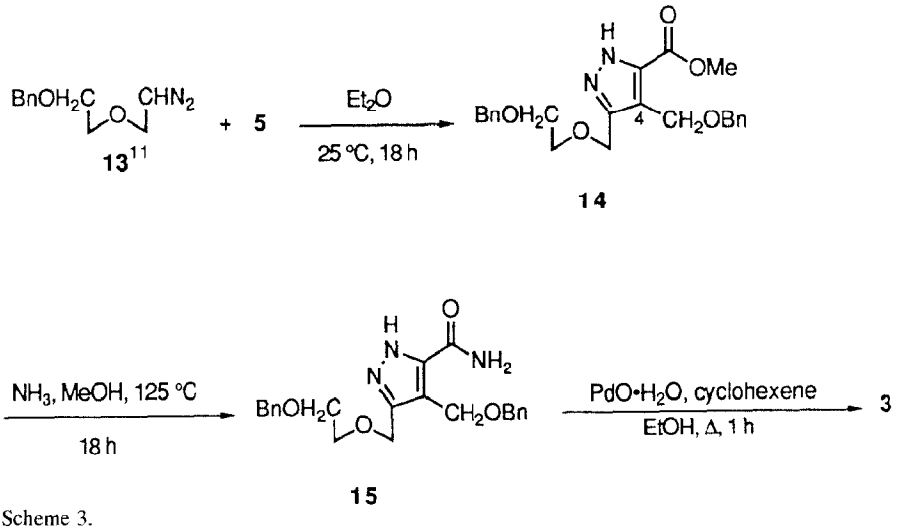
Amidation of ester **6** with ammonia saturated methanol yielded the amide **7**, which was fully deprotected utilizing transfer hydrogenation[11] to provide the desired **2**.

A similar sequence of reactions (Scheme 3) was employed to prepare the acyclic analogue **3**. In accomplishing that synthesis, the regiochemistry of the cycloaddition reaction that produced **14** was also confirmed using ^{13}C NMR data in which C-4 for **14** showed the same downfield shift trend when compared to that of **16**[11] (that is, 118.26 ppm for **14** versus 107.18 ppm for **16**) as described herein for **6** relative to **12**.

Compounds **2** and **3** were evaluated for their in vitro antiviral activities against (*i*) human immunodeficiency virus (HIV-1), (*ii*) the RNA-containing viruses sandfly fever (bunyavirus), Punta Toro (bunyavirus), Japanese encephalitis (flavivirus),



Scheme 3.

yellow fever (flavivirus), Venezuelan equine encephalomyelitis (alphavirus), and (*iii*) the DNA containing vaccinia virus. Both compounds were nontoxic and inactive against all of the viruses tested (in MT2 cells for HIV-1 and Vero cells for the others) up to 100 μg/mL in the HIV assay and up to 320 μg/mL in the others. These results, together with the data of Buchanan and co-workers[3b] with 4-deoxypyrazofurin, suggest that the presence of a hydroxyl group directly bound to the C-4 of the pyrazole ring of pyrazofurin is necessary for biological activity, and that structural variations seeking less toxic antiviral agents derived from **1** should be conducted at other centers.

EXPERIMENTAL

General.—Melting points were determined on a Mel-Temp capillary melting point apparatus and are uncorrected. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. Infrared spectra were recorded on a Beckman model FT 1100 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol FX90Q spectrometer (operated at 90 and 22.5 MHz, respectively) in $CDCl_3$ or Me_2SO-d_6 referenced to internal tetramethylsilane (Me_4Si) at 0.0 ppm. Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm E. Merck Silica Gel 60-F_{254} precoated silica gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to iodine vapor. Column chromatography was performed on Aldrich silica gel (230–400 mesh, 60 Å) eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H and ^{13}C NMR) homogeneous materials.

Methyl 4-benzyloxy-2-butynoate (**5**).—To an oven-dried flask equipped with a mechanical stirrer, gas inlet, two pressure-equalizing addition funnels, and a gas bubbler was added a mixture of dry, distilled CH_2Cl_2 (150 mL) and oxalyl chloride

(35 mL of a 2.0 M solution in hexane; 70 mmol oxalyl chloride) under Ar. The addition funnels were charged with anhyd Me_2SO (10 mL, 141 mmol) CH_2Cl_2 (30 mL) and 9 g (59 mmol) of 2-benzyloxyethanol (**8**)[6] in dry, distilled CH_2Cl_2 (60 mL), respectively. The reaction flask was cooled to −78°C, and the Me_2SO solution was added dropwise (< 5 min), with stirring, to the oxalyl chloride solution. Upon completion of the addition, the solution of **8** in CH_2Cl_2 was added dropwise to the mixture, and the resultant mixture was allowed to stir at −78°C for 10 min. After this period of time, anhyd Et_3N (42 mL, 581 mmol) was added dropwise to the mixture, and the mixture was then allowed to warm to room temperature. Water (300 mL) was then added to the mixture, the phases separated, and the aqueous phase was extracted with CH_2Cl_2 (300 mL). The combined organic phases were washed with satd aq NaCl (300 mL), dried ($MgSO_4$), filtered, and concentrated by means of a rotary evaporator. The resulting yellow syrup, which gave a positive, 2,4-dinitrophenylhydrazone test indicating conversion to the aldehyde **9**, was used without further purification for the next reaction sequence.

A reagent mixture was prepared at 0°C by the addition of (*i*) 15.7 g (240 mmol) of Zn dust and (*ii*) 79.6 g (240 mmol) of CBr_4 to 62.94 g (240 mmol) of triphenylphosphine in dry, distilled CH_2Cl_2 (300 mL) at 0°C. The mixture was allowed to warm to room temperature where it was stirred for 12 h and then cooled to 0°C. This cooled mixture was mechanically stirred, and to it was added, dropwise, the crude aldehyde (assumed to be 59 mmol) obtained above dissolved in dry, distilled CH_2Cl_2 (30 mL). The resultant mixture was allowed to stir at room temperature for 1.5 h, after which time pentane (1.2 L) was added. The mixture was then filtered by suction, and the residue remaining was extracted again with CH_2Cl_2 (300 mL), followed by re-treatment of the solution with pentane (1.2 L), cooling in an ice–H_2O bath and filtering. This procedure was repeated for three additional cycles. The combined CH_2Cl_2–pentane filtrates were then dried ($MgSO_4$) and concentrated on a rotary evaporator to yield a yellow oil that was purified by column chromatography (9:1 hexane–EtOAc) to yield 6.25 g of **10** (35% from 2-benzyloxyethanol): bp 105 °C at 1 torr; $R_f = 0.5$ (9:1, hexane–AcOEt); IR (neat) 3066, 2925, 2880, 1628, 1430, 1100, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H, ArH), 6.63 (t, 1 H, CH), 4.51 (s, 2 H, $ArCH_2$), and 4.04 (d, 2 H, CH_2).

A solution composed of **10** (6.29 g, 20.56 mmol) dissolved in anhyd THF (100 mL) in an oven-dried flask was cooled to −78°C under Ar. Butyllithium (42 mmol, 26.18 mL of a 1.6 M solution in hexanes) was then added to the mixture dropwise, with stirring, under Ar. The mixture was stirred for 1 h at −78°C after which methyl chloroformate (3.16 mL, 41 mmol) was added in one portion. The mixture was allowed to warm to room temperature and was then poured over satd aq NH_4Cl (100 mL). The organic phase was separated and the aqueous phase was extracted with Et_2O (2 × 200 mL). The combined organic phases were then washed with satd brine (100 mL), dried ($MgSO_4$), filtered, and concentrated on a rotary evaporator to yield a brown syrup. The syrup was purified by column chromatography (9:1 hexane–EtOAc) to yield **5** (3 g, 72%): $R_f = 0.31$ (9:1

hexane–EtOAc); IR (neat) 3033, 2970, 2860, 2233, 1725, 1440, 1270, 1250, 1095, 1065, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.33 (s, 5 H, ArH), 4.60 (s, 2 H, $ArCH_2$), 4.27 (s, 2 H, CH_2), and 3.77 (s, 3 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 153.53, 136.79, 128.50, 128.12, 83.59, 78.01, 72.05, 56.72, and 52.71. Anal. Calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92. Found: C, 70.44; H, 5.69.

Methyl 3(5)-(2,3,5-tri-O-benzyl-β-D-ribofuranosyl)-4-(benzyloxymethyl)pyrazole-5(3)-carboxylate (**6**).—A mixture of **11**[2,5b] (1 g, 2.1 mmol), dissolved in 20 mL of a 1:1 mixture of CCl_4–glacial AcOH containing 1 g of anhyd AcONa was cooled to 3°C in an ice–H_2O bath, treated with 4 mL of liquid N_2O_4, and then stirred for 1.5 h at 3°C. Following this, the solution was poured over 100 mL of ice–H_2O with subsequent vigorous stirring of the resultant mixture for 0.5 h. The organic layer was then separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 25 mL). The combined organic layers were washed with satd aq $NaHCO_3$ solution (25 mL), dried ($MgSO_4$), filtered, and the filtrate was concentrated using a rotary evaporator to yield 2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-(*N*-nitroso)acetamido-D-allitol as a light-green syrup: IR (neat) 1730, 1500 cm^{-1}. This syrup, which showed no IR absorption at 3311 cm^{-1} (NH) or 1653 cm^{-1} (CO) to suggest unreacted **11**, was used immediately in the subsequent reaction.

The *N*-nitrosoamide prepared above (assumed to be 2.1 mmol) was dissolved in 10 mL of Et_2O and mixed vigorously with an ice-cold solution of 1.2 g of KOH dissolved in 3 mL of H_2O. The mixture was stirred for 45 min at 3°C after which time the IR spectrum of the components in the Et_2O layer showed the formation of a strong band at 2065 cm^{-1} (CHN_2) and no band at 1500 cm^{-1} (NO). The mixture was then diluted with Et_2O (20 mL) and H_2O (25 mL), and the layers were separated. The Et_2O layer was washed with H_2O (10 mL) and dried rapidly, first by swirling over KOH pellets and decantation, followed by anhyd $MgSO_4$. Following filtration, the golden-colored filtrate containing 2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-diazo-D-allitol (**4**) was used immediately in the subsequent reaction; IR (neat) 2065 cm^{-1}.

The aforementioned solution of **4** was added to a solution of **5** (0.64 g, 3.15 mmol) in anhyd Et_2O (10 mL). The mixture was stirred for 24 h at 27°C, after which time TLC analysis (1:1 EtOAc–hexane) indicated that the reaction was complete. (During this time, the solution color changed from golden to light yellow.) The reaction mixture was concentrated using a rotary evaporator, and the residue was purified by column chromatography (1:1 EtOAc–hexane) yielding **6** (0.82 g, 60% from **11**) as a colorless syrup: $R_f = 0.40$ (1:1 EtOAc–hexane); IR (neat) 3250, 3033, 2900, 1725, 1450, 1133, 1080, 750 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.1 (br s, 1 H, pyrazole NH), 7.33 (m, 20 H, ArH, 5.5 (s, 1 H, H-1′), 4.9–3.58 (m, 15 H), 3.9 (s, 3 H, OCH_3); ^{13}C NMR ($CDCl_3$) δ 163.10, 143.58, 141.25, 138.00, 137.90, 137.48, 136.65, 128.67, 128.43, 128.34, 128.30, 128.25, 128.14, 127.87, 127.75, 127.73, 127.71, 127.65, 116.51, 81.13, 79.28, 76.93, 76.11, 73.42, 72.66, 72.51, 71.72, 67.42, 62.45, and 51.75. Anal. Calcd for $C_{39}H_{40}N_2O_7$: C, 72.20; H, 6.22; N, 4.32. Found: C, 72.41; H, 6.20; N, 4.20.

3(5)-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-4-(benzyloxymethyl)pyrazole-5(3)-carboxamide (**7**).—A solution of **6** (830 mg, 1.28 mmol) in freshly distilled MeOH (20 mL) was saturated with NH_3 at 3 °C, and the resulting mixture was heated in a sealed glass tube for 16 h at 115 °C. Upon cooling, TLC analysis (6:4 EtOAc–hexane) indicated that the reaction had proceeded to completion. The solution was then concentrated using a rotary evaporator, and the residue was purified by column chromatography (6:4 EtOAc–hexane) yielding **7** (790 mg, 97%) as a colorless syrup: $R_f = 0.42$ (6:4 EtOAc–hexane); IR (neat) 3300, 3200, 3020, 2910, 2800, 1690, 1590, 1450, 1380, 1200, 1100, 1080, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 12.5 (br s, 1 H, pyrazole NH), 7.25 (m, 20 H, ArH), 6.30 (br d, 2 H, NH_2), 5.47 (s, 1 H, H-1′), and 4.9–3.58 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 164.58, 143.78, 142.75, 137.93, 137.77, 137.44, 136.68, 128.56, 128.23, 128.01, 127.69, 114.90, 80.72, 79.37, 76.00, 73.51, 72.38, 72.27, 71.56, 68.15, and 62.52. *Anal.* Calcd for $C_{38}H_{39}N_3O_6$: C, 72.02; H, 6.20; N, 6.63. Found: C, 72.17; H, 6.10; N, 6.43.

4-Hydroxymethyl-3(5)-β-D-ribofuranosylpyrazole-5(3)-carboxamide(homopyrazofurin) (**2**).—A solution of **7** (748 mg, 1.18 mmol) in 3:1 EtOH–cyclohexene (20 mL) was treated with 100 mg of palladium(II) oxide hydrate. The resultant mixture was refluxed for 2 h after which TLC analysis [4:1:2 EtOAc–PrOH–H_2O (upper phase)] showed complete loss of starting material. The mixture was then cooled and filtered through a pad of Celite that had been washed with hot EtOH. The Celite pad was then washed with hot EtOH, and the combined filtrates were concentrated. The resulting colorless glass was purified by column chromatography (6:2:1 EtOAc–EtOH–H_2O) to yield **2** (226 mg, 70%) as a colorless glass: $R_f = 0.25$ [4:1:2 EtOAc–PrOH–H_2O (upper phase)]; IR (neat) 3500–3100, 2900, 1680, 1600 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.59 (br d, 2 H, NH_2), 5.31 (br s, D_2O exch., 2 H, 2 × OH), 5.29 (br s, D_2O exch., 1 H, OH), 4.78 (d, *J* 5.8 Hz, 1 H, H-1′), 4.52 (s, 2 H, CH_2 at C-4 of pyrazole), and 3.94–3.32 (m, 6 H, H-2′, H-3′, H-4′, H-5′, and 1 exch. H, OH); ^{13}C NMR (Me_2SO-d_6) δ 164.81, 143.08, 140.70, 119.52, 84.95, 75.85, 75.53, 70.70, 61.44 and 52.88. Anal. Calcd for $C_{10}H_{15}N_3O_6 \cdot 0.5MeOH$: C, 43.60; H, 5.92; N, 14.53. Found: C, 43.73; H, 5.76; N, 14.52. (For the purposes of obtaining the microanalytical data for **2**, MeOH was used to transfer the sample to a small vial for delivery to M-H-W Laboratories, and it was not possible to free the sample of all of the MeOH to obtain an analysis free of this solvent.)

Methyl 3(5)-[(2-benzyloxy)ethoxymethyl]-4-(benzyloxymethyl)pyrazole-5(3)-carboxylate (**14**).—A mixture of 1-acetamido-2-[(2-benzyloxy)ethoxy]ethane[11] (1.16 g, 4.9 mmol) dissolved in 1:1 CCl_4–glacial AcOH (30 mL) and 2.32 g of anhyd AcONa was cooled to 3°C in an ice–H_2O bath, treated with liquid N_2O_4 (2 mL), and stirred for 1.5 h at 3°C. Following this, the solution was poured over ice–H_2O (200 mL with subsequent vigorous stirring of the resultant mixture for 0.5 h. The organic layer was then separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL). The combined organic layers were washed with satd aq $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered, and the filtrate was concentrated using a rotary evaporator to yield 1-(*N*-nitroso)acetamido-2-[(2-benzyloxy)ethoxy]ethane[11]

as a light-green syrup. This syrup showed no IR absorption at 3296 cm^{-1} (NH) or 1653 cm^{-1} (CO) to suggest unreacted 1-acetamido-2-[(2-benzyloxy)ethoxy]ethane. The *N*-nitrosoamide prepared in this manner was used immediately for the next reaction.

A solution composed of 1-(*N*-nitroso)acetamido-2[(2-benzyloxy)ethoxy]ethane prepared above (assumed to be 4.9 mmol) in Et_2O (12 mL) was mixed vigorously with an ice-cold solution of 3.3 g of KOH dissolved in H_2O (6 mL). The mixture was stirred for 45 min at 3°C after which time the IR spectrum of the Et_2O layer showed the formation of a strong band at 2067 cm^{-1} (CHN_2) and no band at 1505 cm^{-1} (NO). The mixture was then diluted with Et_2O (50 mL) and H_2O (50 mL), and the layers were separated. The Et_2O layer was washed with H_2O (50 mL) and dried rapidly, first by swirling over KOH pellets and decantation, followed by treatment with anhyd $MgSO_4$, and filtration. The golden-colored filtrate containing **13**[11] was used immediately.

The aforedescribed solution of **13** (assumed to be 4.9 mmol) was added to a solution of **5** (0.5 g, 2.45 mmol) in Et_2O (10 mL), and the mixture was stirred for 18 h at 25°C. The Et_2O solution was then dried ($MgSO_4$), filtered, and concentrated using a rotary evaporator to afford a yellow syrup. This syrup was purified by column chromatography (3:1 EtOAc–hexane) to yield 0.5 g (50%) of **14** as a colorless syrup: R_f = 0.38 (3:1 EtOAc–hexane); IR (neat) 3220, 3033, 2880, 1725, 1460, 1375, 1150, 1100, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.30 (s, 10 H, ArH), 4.78 (s, 2 H, CH_2), 4.70 (s, 2 H, CH_2), 4.57 (s, 2 H, CH_2), 4.54 (s, 2 H, CH_2), 3.89 (s, 3 H, CH_3), and 3.64 (m, 4 H, CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 162.31, 143.62, 138.74, 138.25, 137.71, 128.39, 127.80, 127.64, 118.26, 73.30, 72.59, 70.05, 69.24, 64.20, 62.08, and 51.90. Anal. Calcd for $C_{23}H_{26}N_2O_5$: C, 67.30; H, 6.39; N, 6.83. Found: C, 67.35; H, 6.47; N, 6.72.

3(5)-[(2-Benzyloxy)ethoxymethyl]-4-(benzyloxymethyl)pyrazole-5(3)-carboxamide (**15**).—A solution composed of **14** (0.5 g, 1.22 mmol) in anhyd MeOH (20 mL) was cooled to 3°C and saturated with anhyd NH_3. The mixture was then heated in a sealed glass tube for 18 h at 125°C. After this period of time, the mixture was cooled and concentrated using a rotary evaporator to yield **15** as a colorless glass (0.475 g, 99%). This glass was crystallized from benzene–hexane to yield crystalline **15**: mp 84–85°C (benzene–hexane); IR (KBr) 3300, 3200, 3100, 2880, 1680, 1600, 1100, 750, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.27 (m, 12 H, ArH, NH_2), 4.80 (s, 2 H, CH_2), 4.63 (s, 2 H, CH_2), 4.50 (s, 4 H, 2 × CH_2), and 3.59 (m, 4 H, CH_2CH_2); ^{13}C NMR ($CDCl_3$) δ 163.61, 144.75, 140.20, 137.87, 137.66, 128.39, 127.91, 127.74, 116.15, 73.19, 72.10, 69.56, 69.29, 64.25, and 62.08. Anal. Calcd for $C_{22}H_{25}N_3O_4$: C, 66.82; H, 6.37; N, 10.63. Found: C, 67.00; H, 6.41; N, 10.66.

3(5)-2-Hydroxyethoxymethyl-4(hydroxymethyl)pyrazole-5(3)-carboxamide (**3**).—Amide **15** (0.55 g, 1.39 mmol) was dissolved in abs EtOH (15 mL) and the mixture was treated with cyclohexene (5 mL) and 50 mg of palladium(II) oxide hydrate. The resultant mixture was refluxed for 1 h, cooled to room temperature and filtered through a pad of Celite. The Celite pad was washed with hot EtOH, and

the combined filtrates were concentrated by means of a rotary evaporator to yield a colorless glass. The colorless glass was crystallized from benzene–EtOH–hexane to yield **3** (250 mg, 84%): mp 158–159°C (benzene–EtOH–hexane); $R_f = 0.55$ (7:3 $CHCl_3$–MeOH); ^1H NMR (Me_2SO-d_6) δ 7.62 (br d, 2 H, NH_2), 4.54 (s, 4 H, $2 \times CH_2$), 4.0 (br s, D_2O exch., 2 H), 3.47 (m, 4 H, CH_2CH_2); ^{13}C NMR (Me_2SO-d_6) δ 164.42, 142.05, 140.31, 120.05, 71.40, 61.92, 60.02, 53.09. Anal. Calcd for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53. Found: C, 44.65; H, 6.12; N, 19.44.

Antiviral assays.—The procedures used to determine the antiviral potential of **2** and **3** were the same as those described by Upadhya and colleagues[12] using MT2 cells for the HIV assays and Vero cells in the assays for sandfly fever, Punta Toro, Japanese encephalitis, yellow fever, Venezuelan equine encephalomyelitis, and vaccinia viruses.

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