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Acyclic analogues of pyrazofurin: syntheses and antiviral evaluation*

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ABSTRACT

Acyclic analogues of pyrazofurin, including 4-hydroxy-3(5)-{[2-hydroxy-1-(hydroxymethyl)-ethoxy]methyl}-1*H*-pyrazole-5(3)-carboxamide (**36**) and 4-hydroxy-3(5)-[(2-hydroxyethoxy)methyl]-1*H*-pyrazole-5(3)-carboxamide (**27**), that possess the side chains of ganciclovir and acyclovir, respectively, were prepared by heating methyl 4-acetoxy-1-acetyl-3-bromomethyl-1*H*-pyrazole-5-carboxylate (**15**) and sodium acetate in the requisite alcohols or, for **36**, with the sodium alkoxide in dry tetrahydrofuran. These analogues have no antiviral activity, except 4-hydroxy-3(5)-[(3-hydroxypropoxy)methyl]-1*H*-pyrazole-5(3)-carboxamide (**28**), which exhibited slight activity against human cytomegalovirus.

INTRODUCTION

Pyrazofurin (**1**), a naturally occurring *C*-nucleoside antibiotic isolated[1] from *Streptomyces candidus* in 1969, has received considerable attention as a result of its various biological activities. As has been noted[2], in addition to possessing broad-spectrum antiviral activity[3–8], pyrazofurin has been evaluated against several tumor cell lines[9,10], and clinically as an anticancer agent[8,11–13]. More recently, it has been found that **1** has potent activity against influenza viruses A–C[14] and respiratory syncytial virus[15–17]. Although pyrazofurin and its analogues will continue to be pursued for their chemotherapeutic value, the critical hurdle still to be overcome is the inherent toxicity of the drug[5,7].

Several groups have reported syntheses of pyrazofurin[17–22] since its initial preparation[18] in 1972. Derivatives of pyrazofurin have been prepared with the aim of increasing potency or reducing toxicity[2,23–25], but with little success. Considerably reduced toxicity relative to that of the parent compound has been reported for the 5′-phosphate and NAD analogues of pyrazofurin[26]. The synthesis of *N*-nucleoside congeners of pyrazofurin with variations at the carboxamide portion of the nucleoside has been reported, although none of these analogues demonstrated any promise as antitumor or antiviral drugs[21]. Recently, the synthesis of 3(5)-[(2-hydroxyethoxy)methyl]pyrazole-5(3)-carboxamide (**2**), an acyclic analogue of 4-deoxypyrazofurin[25] (**3**) that

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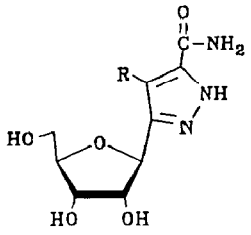
combines the structural features found in the latter and in acyclovir (**4**), was reported[27], although no biological data were given. The synthesis of acyclic nucleosides, structures with the furanose ring replaced by an acyclic carbohydrate moiety, has been a major development in antiviral chemotherapy[27], producing such important drugs as acyclovir[28], ganciclovir[29], and (*S*)-dihydroxypropyladenine[30]. We now report the synthesis of acyclic pyrazofurin analogues, including the analogues of acyclovir (**27**) and ganciclovir (**36**), and their evaluation as potential antiviral drugs.

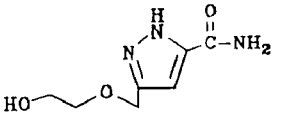
RESULTS AND DISCUSSION

Pyrazofurin (**1**), its α-epimer, 4-deoxypyrazofurin, and carbocyclic pyrazofurin have been synthesized from 1-substituted ribofuranosyl precursors, including **5**[18], **6**[31], **7**[32], **8**[21], and **9**[19]. In each of these starting materials, the ribofuranosyl portion is intact and elaboration of the pyrazole base was required. For the construction of acyclic pyrazofurin analogues, it appeared more appropriate to attach side chains to a suitably blocked pyrazole intermediate possessing functionalized one-carbon units, either a hydroxymethyl or a halomethyl group, at C-3. This approach would allow for the use of both electrophilic and nucleophilic precursors to the side chains. Following an established method[33], methyl pyruvate (**10**) was condensed with ethyl hydrazinoacetate hydrochloride to give the hydrazone **11** in nearly quantitative yield as one isomer. Although the geometry of **11** was not established rigorously, previous work[33] would indicate strongly the *anti*-(*E*) geometry based on the upfield chemical shift (δ 5.98 in $CDCl_3$) for the resonance of the hydrazone NH [*cf.* $\delta \sim 10$ for the *syn*-(*Z*) isomer[33]]. Without crystallization, **11** was cyclized with sodium methoxide in methanol to give the crude pyrazole derivative **12**[33] (90%). In order to minimize the amount of the 3-carboxylic acid derivative **13** formed in the reaction, it was necessary to acidify the chilled reaction mixture prior to further processing. The derivative **12** was acetylated with acetic anhydride–pyridine to afford the blocked pyrazole **14** (85%). Then, by analogy with the method employed[23] to produce acyclic analogues of the *C*-nucleoside formycin A, **14** was treated with *N*-bromosuccinimide and benzoyl peroxide in carbon tetrachloride to give methyl 4-acetoxy-1-acetyl-3-bromomethyl-1*H*-pyrazole-5-carboxylate (**15**, 80–85%). Thus, in four steps in 61% overall yield, an intermediate needed for elaboration of a variety of acyclic side chains was prepared.

Whilst examining various approaches to certain target structures, it was discovered that warming **15** in 2-propanol in the presence of sodium acetate resulted in displacement of bromide by isopropoxide, and, after treatment of the product with methanolic sodium methoxide, **17** was isolated. Similar displacements occurred when ethylene glycol, 1,3-dihydroxypropane, 2-hydroxyethyl ether, 2-mercaptoethanol, and solketal (1,2-*O*-isopropylideneglycerol) were employed in the presence of sodium acetate, and the acyclic pyrazole esters **18**–**22** were obtained in moderate to good yields. A similar attempt to prepare **23** from **15** by treatment with sodium acetate in 1,3-di-*O*-benzylglycerol was unsuccessful. Only traces of **23** were obtained along with small amounts of the compound produced by displacement of bromide by the acetate ion. An



1 R = OH
3 R = H



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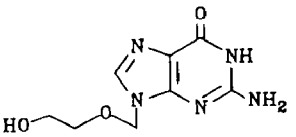

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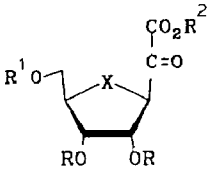
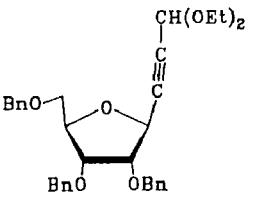


5 R = R^1 = Ac, R^2 = CH_3,
X = O
6 R^2 = CH_3,R,R = $C(CH_3)_2$,
R^1 = $SiMe_2tBu$, X = CH_2



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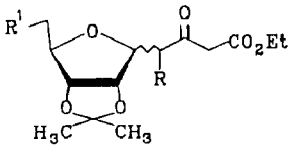
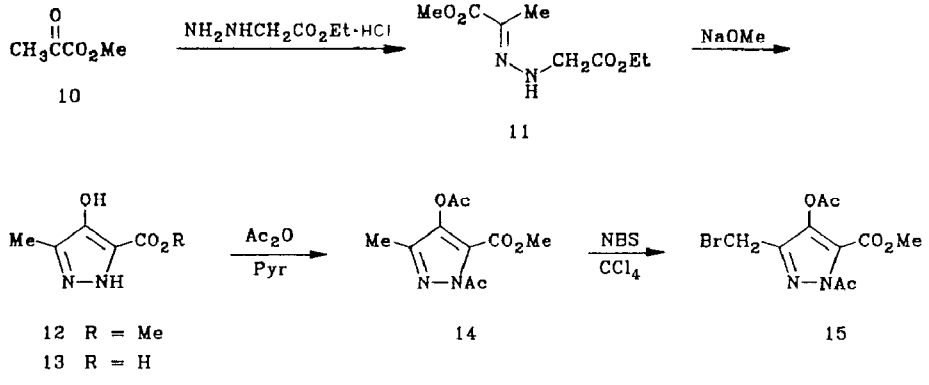


8 R = CO_2Et, R^1 = p-$NO_2C_6H_4CO_2$
9 R = H, R^1 = $OCPh_3$



10

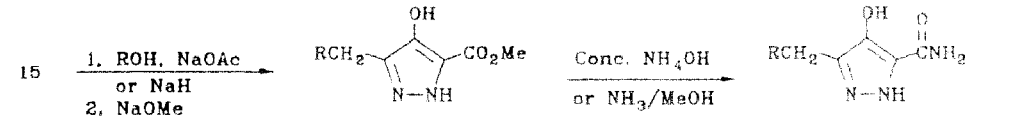
11

12 R = Me
13 R = H

14

15

alternative procedure for the synthesis of **23** is discussed below. The hydroxymethyl derivative **16** was obtained from **15** in two steps. Treatment of **15** with sodium acetate and benzyl alcohol gave **24**, hydrogenolysis (Pd/C) of which in methanol afforded **16** (30% from **15**). Compound **22** was hydrolyzed with aqueous 80% acetic acid to give **25**.



16 R = OH
17 R = $OCHMe_2$
18 R = $O(CH_2)_2OH$
19 R = $O(CH_2)_3OH$
20 R = $(OCH_2CH_2)_2OH$
21 R = $S(CH_2)_2OH$
22 R = OCH_2 (2,2-dimethyl-1,3-dioxolan-4-yl)
23 R = $OCH(CH_2OBn)_2$
24 R = OCH_2Ph
25 R = $OCH_2CHOHCH_2OH$

26 R = H
27 R = $O(CH_2)_2OH$
28 R = $O(CH_2)_3OH$
29 R = $(OCH_2CH_2)_2OH$
30 R = $S(CH_2)_2OH$
31 R = OCH_2 (2,2-dimethyl-1,3-dioxolan-4-yl)
32 R = OCH_2Ph
33 R = OH
34 R = $OCH_2CHOHCH_2OH$
35 R = $OCH(CH_2OBn)_2$
36 R = $OCH(CH_2OH)_2$

In order to obtain the target compounds, it was necessary to convert the methyl esters **18**–**22** and **24** into their carboxamides and then to carry out deblocking reactions. Ammonolysis was attempted first, using the ester **12** as a model compound. When **12** was treated at room temperature with saturated methanolic ammonia, little or no reaction took place. It was necessary to stir **12** in conc. ammonia for 2 days or heat it in a steel bomb at 95° for 5 h[19] in saturated methanolic ammonia in order to produce the carboxamide derivative **26**. In a similar fashion, the esters **18**–**22** and **24** were converted into the corresponding amides **27**–**32** by reaction with either conc. ammonia at room temperature or ammonia in methanol at elevated temperatures. Compounds **22** and **24** were more cleanly and efficiently ammonolyzed, via **31** and **32**, if their blocking groups were retained until after treatment with ammonia. Hydrogenolysis of **32** and hydrolysis of **31** in aqueous 80% acetic acid gave **33** and **34**, respectively.

As mentioned above, the acyclic derivative **23** with a protected ganciclovir-like side chain could not be produced by the general method. A modest yield of **23** was obtained by treating **15** with 5 equiv. of the sodium salt of 1,3-di-*O*-benzylglycerol (from sodium hydride in dry tetrahydrofuran). Crude **23** was treated directly with methanolic ammonia in a steel bomb at 95° for 5 h to give, after chromatography, **35** (12% from **15**). Removal of the benzyl groups from **35** by transfer hydrogenation in cyclohexene/ethanol with 20% palladium hydroxide on carbon produced the target compound **36**.

Compounds **12**, **14**–**18**, **21**, **26**–**30**, **32**–**34**, and **36** were inactive against HIV-1, Punta Toro, yellow fever, pinchinde, Japanese Encephalitis, sandfly fever, vaccinia,

adenovirus 2, vesicular stomatitis, Venezuelan Equine Encephalomyelitis, and Rift Valley Fever viruses. Additionally, **16**, **18**, **19**, **21**, **27**–**30**, and **36** were evaluated for their ability to inhibit human cytomegalovirus (AD169) in MRC5 cells, utilizing a virus-yield-reduction assay. At the greatest non-cytotoxic dose (100μM), **28** exhibited slight activity, reducing virus progeny yield by 1.2 ($\log_{10}$ plaque forming units).

EXPERIMENTAL

Melting points were determined on a Mel-Temp apparatus and are uncorrected. N.m.r. spectra (internal Me_4Si) were recorded with a Nicolet NMC 300NB spectrometer operating at 300.635 MHz for ^1H and at 75.6 MHz for ^{13}C. Mass spectra were recorded with a Varian MAT 311A mass spectrometer in the f.a.b. or e.i. mode. Microanalyses were performed by the Molecular Spectroscopy Section of the Organic Chemistry Research Department at the Southern Research Institute. Chromatography column sizes are given as width × length.

Methyl 2-[(ethoxycarbonylmethyl)hydrazono]propanoate (**11**). — Sodium acetate (6.36 g, 77.6 mmol) and methyl pyruvate (**10**; 7.92 g, 77.6 mmol) were dissolved in methanol (97 mL) and water (32 mL). Ethyl hydrazinoacetate monohydrochloride (12 g, 77.6 mmol) was added and the solution was stirred at room temperature for 48 h. The solvents were evaporated and a solution of the residue in chloroform was washed with water. The pH of the water layer was adjusted to ~7 with M NaOH and the solution was extracted twice with chloroform. The chloroform layers were combined, dried ($MgSO_4$), and filtered, and the solvent was evaporated under reduced pressure to give a yellow oil that, on drying *in vacuo* over P_2O_5, crystallized as a pale-yellow solid. The yield of crude material, which could be used directly in the next step, varied between 80 and 100%. Crystallization from ether–light petroleum (b.p. 30–60°) (5:1) provided **11**, m.p. 65°. E.i.-mass spectrum: *m/z* 202. ^1H-N.m.r. data ($CDCl_3$): δ 1.29 (t, 3 H, $CO_2CH_2CH_3$), 2.03 (s, 3 H, $CH_3C=N$), 3.82 (s, 3 H, CO_2Me), 4.23 (m, 4 H, CH_3CH_2O and $NHCH_2$), 5.98 (bs, 1 H, NHN); [$(CD_3)_2SO$]: δ 1.22 (t, 3 H, $CO_2CH_2CH_3$), 1.87 (s, 3 H, $CH_3C=N$), 3.64 (s, 3 H, CO_2CH_3), 4.05 (d, 2 H, $NHCH_2CO_2$), 4.11 (q, 2 H, $CO_2CH_2CH_3$), 7.67 (t, 1 H, $NNHCH_2$).

Anal. Calc. for $C_8H_{14}N_2O_4$: C, 47.52; H, 6.98; N, 13.85. Found: C, 47.30; H, 6.97; N, 13.90.

Methyl 4-hydroxy-3(5)-methyl-1H-pyrazole-5(3)-carboxylate[22] (**12**). — Sodium (3.10 g, 135 mmol) was added to cooled (0–5°) MeOH (200 mL) and, after the sodium had reacted, crude **11** (10.40 g, 51.4 mmol) was added in one portion. The solution was boiled under reflux for 4 h, then chilled in an ice-bath, and conc. HCl (11.2 mL) was added slowly during 10 min (if necessary, more conc. HCl or solid $NaHCO_3$ was added to bring the pH to near neutral as judged by wet pH paper). Most of the MeOH was then evaporated, the residue was dissolved in H_2O, and the pH of the solution was adjusted to ~7. The solution was extracted with EtOAc (7 × 40 mL) and the solvent was evaporated from the combined extracts to give a crude product (90%) that was adequate for use in subsequent reactions. An analytical sample, obtained by column

chromatography on silica gel (230–400 mesh) with chloroform–methanol (9:1), had m.p. 154–155°; lit.[22] m.p. 140–142°. E.i.-mass spectrum: *m/z* 156. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 2.10 (s, 3 H, CH_3), 3.77 (s, 3 H, CO_2CH_3), 8.79 and 8.56 (bs, 1 H, OH) (tautomers), 12.76 (bs, 1 H, NH).

Anal. Calc. for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 45.82; H, 5.36; N, 17.89.

Methyl 4-acetoxy-1-acetyl-3-methyl-1H-pyrazole-5-carboxylate (**14**). — A solution of crude **12** (5.78 g, 37 mmol) in acetic anhydride (50 mL) and pyridine (27 mL) was heated at 90° for 3 h, then cooled. The solvents were evaporated, and a solution of the residue in ether was washed with H_2O (2 × 30 mL), dried (Na_2SO_4), and filtered, and the ether was evaporated. The orange-tinted residue was triturated with cold ether, to give **14** (7.55 g, two crops, 85%), as fine white needles, m.p. 75–77°. E.i.-mass spectrum: *m/z* 240. N.m.r. data [$(CD_3)_2SO$]: ^{1}H, δ 2.33 (s, 3 H, $COCH_3$), 2.4 (s, 3 H, $COCH_3$), 2.68 (s, 3 H, CH_3), 3.84 (s, 3 H, CO_2CH_3); ^{13}C, δ 10.64 (3-CH_3), 20.03, 22.39 ($OCOCH_3$ and $NCOCH_3$), 52.10 (CO_2CH_3), 134.38, 134.56, 136.61 (C-3,4,5), 160.25 (CO_2CH_3), 168.33, 171.38 (NCO and OCO).

Anal. Calc. for $C_{10}H_{12}N_2O_5$: C, 50.02; H, 5.00; N, 11.67. Found: C, 49.73; H, 5.15; N, 11.66.

Methyl 4-acetoxy-1-acetyl-3-bromomethyl-1H-pyrazole-5-carboxylate (**15**). — A mixture of **14** (3.0 g, 12.5 mmol), benzoyl peroxide (250 mg, 1.03 mmol), *N*-bromosuccinimide (4.90 g, 27.5 mmol), K_2CO_3 (1.0 g, 7.23 mmol), and CCl_4 (120 mL) was boiled under reflux for 2 h, then chilled to 5–10°, and filtered, and the solvents were evaporated *in vacuo*. The residue was triturated with 2-propanol to give **7** (3.28 g, 82%), m.p. 94–96°. F.a.b.-mass spectrum: *m/z* 319. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 2.38 (s, 3 H, OAc), 2.73 (s, 3 H, NAc), 3.87 (s, 3 H, CO_2CH_3), 4.86 (s, 2 H, $BrCH_2$).

Anal. Calc. for $C_{10}H_{11}BrN_2O_5$: C, 37.64; H, 3.47; N, 8.78. Found: C, 37.73; H, 3.63; N, 8.64.

General procedure for the preparation of the esters **16**–**22** *and* **25**. — A homogeneous solution or a heterogeneous mixture of **15** (0.16M) in the requisite alcohol with 5 equiv. of NaOAc was heated at 90° for 2 h. Following cooling and filtration of each mixture, the alcohol was evaporated *in vacuo* at 40–85° (depending on the boiling point of the alcohol). A 0.16M solution of each residue in MeOH was stirred in the presence of 2 equiv. of NaOMe for 0.5 h at room temperature, then neutralized with M HCl, and the solvent was evaporated *in vacuo*. Each residue was purified by chromatography on Silica Gel 60 (70–230 mesh) and crystallized from, or triturated with, the indicated solvent to provide analytical samples. Where exceptions occurred or where deblocking was necessary, conditions are given. In this manner, the following compounds were prepared.

Methyl 4-hydroxy-3(5)-hydroxymethyl-1H-pyrazole-5(3)-carboxylate (**16**). — The ester **24** was prepared from **15** (4.0 g, 12.5 mmol) and benzyl alcohol, then chromatographed (4 × 22 cm column) with $CHCl_3$–MeOH (97:3) to provide a clear oil (2.66 g) that was homogeneous by t.l.c. A solution of this oil in MeOH (100 mL) was stirred under H_2 (1 atm) with 10% Pd/C (1.0 g) and a catalytic amount of $CHCl_3$[35] for 16

h, then filtered through Celite, and the solvent was evaporated *in vacuo*. The residue was purified by chromatography on silica gel (EtOAc–MeOH, 98:2). The white solid, obtained after removing the solvent from the product-containing fractions, was triturated with EtOH to provide **16** (650 mg, 30% from **15**), m.p. 135–136.5°. F.a.b.-mass spectrum: *m/z* 173. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.79 (s, 3 H, CO_2CH_3), 4.41 (s, 2 H, CH_2OH), 4.7–5.6 (bs, 1 H, $HOCH_2$), 7.6–9.2 (bs, 1 H, HO-4), 12.7–13.2 (bs, 1 H, NH).

Anal. Calc. for $C_6H_8N_2O_4$: C, 41.86; H, 4.68; N, 16.27. Found: C, 41.68; H, 4.95; N, 16.00.

*Methyl 4-hydroxy-3(5)-[(1-methylethoxy)methyl]-1*H*-pyrazole-5(3)-carboxylate* (**17**). — After following the general procedure with **15** (1.5 g, 4.7 mmol) and 2-propanol, chromatography (2 × 20 cm column) with $CHCl_3$–MeOH (98:2) followed by removal of solvents *in vacuo* and trituration of the residue with light petroleum (b.p. 30–60°)–ether (60:40) gave **17** (500 mg, 50% over 2 steps), m.p. 74–75°. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 1.09 [d, 6 H, $CH(CH_3)_2$], 3.63 (m, 1 H, CHO), 3.79 (s, 3 H, CO_2CH_3), 4.37 (s, 2 H, OCH_2), 8.6 (bs, 1 H, OH), 13.1 (bs, 1 H, NH).

Anal. Calc. for $C_9H_{14}N_2O_4$: C, 50.46; H, 6.59; N, 13.08. Found: C, 50.18; H, 6.70; N, 13.32.

*Methyl 4-hydroxy-3(5)-[(2-hydroxyethoxy)methyl]-1*H*-pyrazole-5(3)-carboxylate* (**18**). — After following the general procedure with **15** (2.0 g, 6.3 mmol) and 1,2-ethanediol, chromatography (2.5 × 15 cm column) with EtOAc followed by removal of solvents *in vacuo* and trituration with EtOAc gave **18** (560 mg, 41% over 2 steps), m.p. 122–123°. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.3–3.6 (m, 4 H, OCH_2CH_2O), 3.79 (s, 3 H, CO_2CH_3), 4.42 (s, 2 H, ring CH_2), 7.5–9.0 (bs, 2 H, 2 OH), 13.2 (bs, 1 H, NH).

Anal. Calc. for $C_8H_{12}N_2O_5$: C, 44.44; H, 5.60; N, 12.96. Found: C, 44.36; H, 5.69; N, 12.96.

*Methyl 4-hydroxy-3(5)-[(3-hydroxypropoxy)methyl]-1*H*-pyrazole-5(3)-carboxylate* (**19**). — After following the general procedure with **15** (3.0 g, 9.4 mmol) and 1,3-propanediol, chromatography (6.5 × 5 cm column, 70–230 mesh silica gel) with EtOAc–MeOH (98:2) followed by removal of solvents and some residual 1,3-propanediol *in vacuo* gave **19** as a clear, homogeneous oil that was used without further purification for the synthesis of amide **28**. Some transesterification occurred during this reaction. F.a.b.-mass spectrum: *m/z* 231.

*Methyl 4-hydroxy-3(5)-{[2-(2-hydroxyethoxy)ethoxy]methyl}-1*H*-pyrazole-5(3)-carboxylate* (**20**). — After following the general procedure with **15** (3.5 g, 11 mmol) and bis(2-hydroxyethyl) ether, chromatography (6.5 × 5 cm column) with chloroform–methanol (5:1) gave **20** as an oil (~2.5 g) that was used without further purification in the preparation of amide **21**. Some transesterification occurred during this reaction. F.a.b.-mass spectrum: *m/z* 261.

*Methyl 4-hydroxy-3(5)-{[2-hydroxyethyl)thio]methyl}-1*H*-pyrazole-5(3)-carboxylate* (**21**). — A mixture of compound **15** (6.50 g, 20.4 mmol), sodium acetate (8.37 g, 94 mmol), and 2-mercaptoethanol (36 mL) was heated at 60° for 1 h. Residual 2-mercaptoethanol was evaporated *in vacuo* with mild heating. The residue was dissolved in dry pyridine (18 mL), acetic anhydride (36 mL) was added, and the suspension

was stirred at room temperature overnight (18 h). After the solvents had been evaporated *in vacuo* with mild heating, the residue was triturated with 2-propanol, the solution was decanted, and the solvent was evaporated. The residue was triturated with light petroleum, then treated with 3 equiv. of sodium methoxide in methanol to effect deacetylation. The residue was extracted with ethyl acetate (Soxhlet) for 2 days. The ethyl acetate was evaporated and the residue was purified by chromatography (4.4 × 39 cm column, 230–400 mesh silica gel) with chloroform–methanol (95:5) to produce **21** (2.9 g, 63%) as a yellow oil that crystallized on standing, and had m.p. 94–95°. F.a.b.-mass spectrum: *m/z* 233. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 2.58 (t, 2 H, $HOCH_2CH_2$), 3.50 (t, 2 H, $HOCH_2CH_2$), 3.68 (s, 2 H, ring SCH_2), 3.78 (s, 3 H, $COOCH_3$), 4.78 (m, 1 H, *H*OCH), 8.31 and 8.85 (HO-4), 13.01 (bs, NH).

Anal. Calc. for $C_8H_{11}N_2O_4S$: C, 41.37; H, 5.21; N, 12.06. Found: C, 41.63; H, 5.44; N, 12.04.

Methyl 3(5)-{[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]methyl}-4-hydroxy-1H-pyrazole-5(3)-carboxylate (**22**). — A mixture of **15** (6.0 g, 18.8 mmol), solketal (110 mL), and sodium acetate (7.71 g, 94 mmol) was heated at 90° for 1.5 h, then filtered, and the solvent was evaporated *in vacuo* at 85°. The resulting burgundy oil was triturated with ethyl acetate, then filtered, and the product in the filtrate was purified by chromatography (70–230 mesh silica gel, 6.8 × 16 cm column) with $CHCl_3$–MeOH (99:1). Two compounds were isolated and identified by f.a.b-mass spectrometry as **22** and the partially deblocked 4-acetoxy derivative. The latter fraction was treated with 1 equiv. of sodium methoxide in methanol at room temperature for 1.5 h. The solution was neutralized with Dowex 50W-X4 (H^+) resin and filtered, the methanol was evaporated *in vacuo*, and the residue was combined with **22** from above to give a pale-yellow oil (4.1 g, 75%). F.a.b.-mass spectrum: *m/z* 247.

Methyl 3(5)-[(2,3-dihydroxypropoxy)methyl]-4-hydroxy-1H-pyrazole-5(3)-carboxylate (**25**). — A solution of **22** in aqueous 80% acetic acid (100 mL) was stirred at 55° for 3 h. The solvent was evaporated *in vacuo*, the residual pale-orange oil was triturated with ether and applied to a column (54 × 270 mm) of silica gel (500 mL, 70–230 mesh), and eluted with $CHCl_3$–MeOH (7:1). Fractions containing **32** were combined, the solvent was evaporated *in vacuo*, and a solution of the resulting yellow oil (2.46 g, 53% from **15**) in water was applied to a column (28 × 130 mm) of BioBeads SM-4 (20–50 mesh, BioRad). The column was washed with water to remove glycerol and then with methanol to elute **25**. The methanol was evaporated *in vacuo* and the residue was triturated with ether to give a pale-yellow oil that crystallized on standing to give **25**, m.p. 80–82°. F.a.b.-mass spectrum: *m/z* 247. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.2–3.5 [m, 4 H, $HOCH_2CH(OH)CH_2O$], 3.56 [m, 1 H, $HOCH_2CH(OH)CH_2O$], 3.78 (s, 3 H, CO_2CH_3), 4.49 (s, 2 H, ring CH_2), 4.47 and 4.65 [bs, 2 H, $HOCH_2CH(OH)CH_2O$], 8.49 and 8.87 (bs, 1 H, ring OH), 13.13 (bs, 1 H, NH).

Anal. Calc. for $C_9H_{14}N_2O_6$: C, 43.90; H, 5.73; N, 11.38. Found: C, 43.95; H, 5.89; N, 11.50.

General procedure for the preparation of the amides **26**–**36**. — A 0.14M solution of the appropriate ester in conc. NH_4OH was stirred at room temperature for 64 h, when

0–10% of the starting material remained. The solvent was evaporated *in vacuo* at 35° (bath), and the residue was adsorbed on a small amount of silica gel by evaporating the solvent from a mixture of a methanolic solution of the residue and Silica Gel 60 (5–10 g, 70–230 mesh). The residue was applied to a column of the same silica gel and eluted with the appropriate solvent. Evaporation of the solvents and trituration of the residue with the indicated solvent gave an analytical sample of the product. In this manner, the following compounds were obtained.

4-Hydroxy-3(5)-methyl-1H-pyrazole-5(3)-carboxamide (**26**). — Ester **12** (1.5 g, 9.6 mmol) was treated with conc. NH_4OH and without chromatography, the product was triturated with water to give crystalline **26** (800 mg, 59%), m.p. 245–247° (dec.). F.a.b.-mass spectrum: *m/z* 142. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 2.12 (s, 3 H, CH_3), 6.7–9.4 (bs, 3 H, OH and NH_2), 12.54 (bs, 1 H, NH).

Anal. Calc. for $C_5H_7N_3O_2$: C, 42.55; H, 5.00; N, 29.77. Found: C, 42.42; H, 5.33; N, 29.68.

4-Hydroxy-3(5)-[(2-hydroxyethoxy)methyl]-1H-pyrazole-5(3)-carboxamide (**27**). — Ester **18** (1.3 g, 6.0 mmol) was converted into **27**, which was eluted (4 × 8 cm column) with EtOAc–MeOH (96:4) and crystallized as the chromatographic solvents were being removed, to give a white solid (627 mg, 50%), m.p. 134–136°. F.a.b.-mass spectrum: *m/z* 202. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.47 (m, 4 H, OCH_2CH_2O), 4.43 (s, 2 H, ring OCH_2), 4.1–9.7 (bs, 4 H, 2 OH and NH_2), 12.9 (bs, 1 H, NH).

Anal. Calc. for $C_7H_{11}N_3O_4$: C, 41.79; H, 5.51; N, 20.89. Found: C, 41.84; H, 5.66; N, 20.76.

4-Hydroxy-3(5)-[(3-hydroxypropoxy)methyl]-1H-pyrazole-5(3)-carboxamide (**28**). — Ester **15** (3.0 g, 9.4 mmol) was converted into **28**, which was eluted with EtOAc–MeOH (98:2). Trituration of the product with EtOAc gave **28** (899 mg, 45%), m.p. 116–122°. F.a.b.-mass spectrum: *m/z* 216. ^{1}H-N.m.r. data [$(CD_3)_2SO$], δ 1.65 (m, 2 H, $CH_2CH_2CH_2$), 3.35–3.55 (m, 4 H, $CH_2CH_2CH_2$), 4.38 (s, 3 H, $HOCH_2$ and ring CH_2O), 6.92, 7.35–7.7, 8.66, 9.4 (bs, 3 H, ring OH and NH_2), 12.8–13.0 (bs, 1 H, NH).

Anal. Calc. for $C_8H_{13}N_3O_4$: C, 44.65; H, 6.09; N, 19.53. Found: C, 44.66; H, 6.34: N, 19.57.

4-Hydroxy-3(5)-{[2-(2-hydroxyethoxy)ethoxy]methyl}-1H-pyrazole-5(3)-carboxamide (**29**). — Ester **20** (600 mg, 2.4 mmol) was converted into **29**, which was eluted with EtOAc–MeOH (98:2) and triturated with EtOAc to give **29** (197 mg, 34% over 2 steps), m.p. 130–132°. F.a.b.-mass spectrum: *m/z* 246. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.3–3.6 (m, 8 H, $HOCH_2CH_2OCH_2CH_2$), 4.42 (s, 2 H, ring OCH_2), 4.59 (bs, 1 H, $HOCH_2$), 6.8–9.6 (bs, 3 H, ring OH and NH_2), 12.89, 12.95 (bs, 1 H, NH).

Anal. Calc. for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.13. Found: C, 43.82; H, 6.27; N, 17.31.

4-Hydroxy-3(5)-{[(2-hydroxyethyl)thio]methyl}-1H-pyrazole-5(3)-carboxamide (**30**). — Compound **21** (1.56 g, 6.7 mmol) was treated with conc. NH_4OH (25 mL), as described above, for 3 days. The product was purified by chromatography (4.4 × 12 cm column, 230–400 mesh silica gel), using chloroform–methanol (7:1). The resulting yellow oil crystallized upon standing and was coevaporated with isopropyl

alcohol and ethanol to give **30** (539 mg, 37%), m.p. 121–122°. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 2.70 (t, 2 H, $HOCH_2CH_2$), 3.53 (t, 2 H, $HOCH_2CH_2$), 3.61 (s, 2 H, ring SCH_2), 4.6–5.0 (bs, 1 H, $HOCH_2$), 6.5–9.7 (bs, 3 H, NH_2 and ring OH), 12.8 (bs, 1 H, NH).

Anal. Calc. for $C_5H_{11}N_3O_3S$: C, 38.70; H, 5.10; N, 19.34. Found: C, 38.86; H, 5.54; N, 19.11.

3(5)-{[(2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy]methyl}-4-hydroxy-1H-pyrazole-5(3)-carboxamide (**31**). — A solution of crude **22** (4.6 g, 14 mmol) in saturated methanolic ammonia (100 mL) was heated at 90° for 4 h. The solvent was evaporated *in vacuo*, and the resulting oil was purified by chromatography on Silica Gel 60 (6.5 × 5 cm column, 70–230 mesh) with $CHCl_3$–MeOH (95:5). The product-containing fractions were combined and the solvents were evaporated *in vacuo* to give a colorless oil (1.0 g, 75% based on 3.0 g of recovered starting material) that was used directly to prepare **34**. F.a.b.-mass spectrum: *m/z* 272.

3(5)-Benzyloxymethyl-4-hydroxy-1H-pyrazole-5(3)-carboxamide (**32**). — Compound **24** was prepared from **15** (4.0 g, 12.5 mmol), as described in the general procedure, and without purification was treated with NH_4OH as described above. Chromatography (4 × 15 cm column) of the product with EtOAc gave **32**, which crystallized from the column fractions (1.89 g, 61%) with m.p. 166–167°. F.a.b.-mass spectrum: *m/z* 248. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 4.51 (s, 4 H, CH_2OCH_2), 7.2–7.4 (m, 5 H, aromatic H's), 6.9–7.7, 8.7, 9.5 (bs, 3 H, NH_2 and OH), 12.93 and 13.03 (bs, 1 H, NH).

Anal. Calc. for $C_{12}H_{13}N_3O_3$: C, 58.29; H, 5.30; N, 16.99. Found: C, 58.32; H, 5.50; N, 17.04.

4-Hydroxy-3(5)-hydroxymethyl-1H-pyrazole-5(3)-carboxamide (**33**). — A solution of **32** (1.06 g, 4.3 mmol) in EtOH (50 mL) was stirred under H_2 (1 atm) with 10% Pd/C (250 mg) and a catalytic amount of $CHCl_3$[35] for 18 h, then filtered through Celite, and the solvent was evaporated *in vacuo*. Trituration of the residue with EtOH–EtOAc (1:1) gave **33** (680 mg, 100%), m.p. >250° (dec.). F.a.b.-mass spectrum: *m/z* 158. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 4.42 (s, 2 H, $HOCH_2$), 4.78, 5.14 (bs, 1 H, $HOCH_2$), 6.5–9.5 (bs, 3 H, ring OH and NH_2), 12.74, 12.81 (bs, 1 H, NH).

Anal. Calc. for $C_5H_7N_3O_3$: C, 38.22; H, 4.49; N, 26.74. Found: C, 38.07; H, 4.61; N, 26.34.

3(5)-[(2,3-Dihydroxypropoxy)methyl]-4-hydroxy-1H-pyrazole-5(3)-carboxamide (**34**). — A solution of **31** (1 g, 3 mmol) in aqueous 80% acetic acid (20 mL) was stirred at room temperature overnight. The acetic acid was evaporated *in vacuo* and the resulting colorless oil was azeotroped with cyclohexane to remove residual acetic acid. The oil crystallized from ethanol to give **34** (509 mg, 14% over 4 steps), m.p. 136–137°. F.a.b.-mass spectrum: *m/z* 232. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.2–3.64 [m, 5 H, $CH_2(OH)CH(OH)CH_2O$], 4.41 (s, 2 H, ring CH_2), 4.49 and 4.65 (bs, 2 H, 2 aliphatic OH), 6.91 and 7.46 (bs, 2 H, $CONH_2$), 8.4–9.6 (bs, 1 H, ring OH), 12.97 (bs, 1 H, NH).

Anal. Calc. for $C_8H_{13}N_3O_6 \cdot 0.25EtOH$: C, 42.06; H, 6.02; N, 17.31. Found: C, 41.73; H, 6.03; N, 17.25.

Methyl 3(5)-{[2-benzyloxy-1-(benzyloxymethyl)ethoxy]methyl}-4-hydroxy-1H-pyrazole-5(3)-carboxylate (**23**) *and 3(5)-{[2-benzyloxy-1-(benzyloxymethyl)-ethoxy]methyl}-4-hydroxy-1H-pyrazole-5(3)-carboxamide* (**35**). — A solution of 1,3-di-*O*-benzylglycerol (Aldrich; 17.07 g, 62.66 mmol) in dry tetrahydrofuran (20 mL) was added dropwise during 0.5 h at room temperature to a stirred suspension of 60% NaH (2.51 g, 62.64 mmol) in dry tetrahydrofuran (60 mL). After an additional hour, a solution of **15** (4.5 g, 14.1 mmol) in dry tetrahydrofuran (20 mL) was added in one portion at room temperature under N_2. The mixture was stirred for 15 min at room temperature, the temperature was raised to 60° for 0.5 h, the mixture was cooled in an ice bath, and HOAc (3.7 g, 62.66 mmol) was added dropwise with deep reddening of the solution. The solvents were evaporated and the residue was partitioned between H_2O and EtOAc. The resulting emulsion was treated with a small amount of HOAc and extracted with EtOAc (4 × 50 mL). The solvent was evaporated from the combined extracts to give a semi-solid residue that was chromatographed on silica gel (8.5 × 4.5 cm column, 70–230 mesh), first with $CHCl_3$ to elute most of the excess of 1,3-di-*O*-benzylglycerol and then with EtOAc to elute **23** as part of a complex mixture (transesterification and some deacylation occurs in these reactions). The solvent was evaporated from the combined product-containing fractions and a solution of the residue (4.5 g) in cold saturated methanolic ammonia (~25 mL) was transferred to a steel bomb with minimal rinsing. The mixture was heated at 95° for 5 h, then cooled, and the solvent was evaporated. A solution of the residue in a small amount of $CHCl_3$ was filtered and chromatographed on silica gel (3 × 25 cm column, 230–400 mesh). More (1.4 g) 1,3-di-*O*-benzylglycerol was eluted with $CHCl_3$–MeOH (98:2) followed by a small amount of **23**. Elution with $CHCl_3$–MeOH (95:5) then gave a clear oil that solidified on standing to give **35** (740 mg, 35% per step over 2 steps). F.a.b.-mass spectrum: *m/z* 412. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.52 [m, 4 H, $(CH_2)_2CH$], 3.80 (bs, 1 H, $CHOCH_2$), 4.47 (s, 4 H, 2 CH_2Ph), 4.56 (s, 2 H, $CHOCH_2$), 7.31 (m, 10 H, 2 Ph), 7.33–7.7 (m, 2 H, $CONH_2$), 8.69 and 9.38 (bs, 1 H, HO-4), 12.89 and 12.96 (bs, 1 H, NH) (the double peaks for NH and HO-4 reflect the presence of tautomers).

4-Hydroxy-3(5)-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}-1H-pyrazole-5(3)-carboxamide (**36**). — A mixture of **35** (520 mg, 1.26 mmol), 20% palladium hydroxide on carbon (100 mg), and 1:1 EtOH–cyclohexene was boiled under reflux for 16 h. Because the reaction was incomplete, more catalyst (20 mg) was added and boiling under reflux was continued for 8 h. The mixture was cooled and filtered through Celite, the solvent was evaporated, and acetone was distilled several times from the residue to leave **36** (239 mg, 82% in 2 crops), m.p. 134–136°. F.a.b.-mass spectrum: *m/z* 232. ^{1}H-N.m.r. data [$(CD_3)_2SO$]: δ 3.2–3.7 [m, 5 H, $(CH_2)_2CH$], 4.56 (s, 2 H, ring OCH_2), 6.86 [bs, 1 H, H of $CONH_2$], 7.44 [bs, 2 H, HO-4 and H of $CONH_2$], 12.84 (bs, 1 H, NH).

Anal. Calc. for $C_8H_{13}N_3O_5$: C, 41.56; H, 5.67; N, 18.17. Found: C, 41.10; H, 5.83; N, 18.29.

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