(M + 1)$^+$; ^{1}H NMR (Me_2SO-d_6, XL-100) δ 3.31 (s, H_2O), 3.59 (m, 2 H-5'), 3.94 (m, H-4'), 4.08 (m, H-3'), 4.28 (m, H-2'), 5.15 (dd, $O_{3'}H$), 5.32 (m, H-4' and H-5'), 5.51 (d, H-1', $J_{1',2'}$ = 6 Hz), 6.12 (s, C*H*N_2), 6.58 (s, NH_2), 7.31 (s, H_2); ^{13}C NMR δ 50.29 (CHN_2), 61.02 ($C_{5'}$), 70.24 ($C_{3'}$), 72.69 ($C_{2'}$), 85.43 ($C_{4'}$), 87.40 ($C_{1'}$), 118.06 (C_4), 143.63 (C_5), 179.93 (CO). Anal. Calcd for $C_{10}H_{13}N_5O_5 \cdot 0.7H_2O$: C, 40.57; H, 4.91; N, 23.66. Found: C, 40.65; H, 5.28; N, 23.61.

5-Amino-4-(chloroacetyl)-1-β-D-ribofuranosylimidazole (16). A suspension of 5-amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (**15**; 190 mg, 0.67 mmol) in 15 mL of anhydrous acetonitrile was diluted with 20 mL of ethereal hydrogen chloride (saturated at 20 °C) and the mixture stirred at ambient temperature under anhydrous conditions until the bubbling ceased (about 10 min). The resulting solid was collected under nitrogen atmosphere and dried at ambient temperature and 0.07 nm over P_2O_5 for 1 h: yield 185 mg (95%); mp 155-157 °C dec; TLC ($CHCl_3$/MeOH (3:1), NBP positive).

The analytical sample was obtained from a similar reaction. It was recrystallized from ethanol: mp 154-156 °C dec; UV λ_{max} ($\epsilon \times 10^{-3}$) 300 nm at pH 1 (13.1), 310 nm at pH 7 (12.7), 312 nm at pH 13 (12.7); mass spectrum, *m/e* 291 (M$^+$; ^{1}H NMR (Me_2SO-d_6) δ 1.24 (s, EtOH), 3.32 (s, H_2O), 3.63 (ψt, 2 H-5'), 3.94 (dd, H-4'), 4.05 (dd, H-3'), 4.29 (dd, H-2'), 4.62 (s, $COCH_2Cl$), 5.20 (m, 3'-OH), 5.37 (ψt, 5'-OH), 5.43 (m, 2'-OH), 5.55 (d, H-1', $J_{1',2'}$ = 2 Hz), 7.05 (s, NH_2), 7.38 (s, H_2). Anal. Calcd for $C_{10}H_{14}ClN_3O_5 \cdot 0.5H_2O \cdot 0.3EtOH$: C, 40.48; H, 5.38; N, 13.27. Found: C, 40.39; H, 5.37; N, 13.27.

4-Acetyl-5-amino-1-β-D-ribofuranosylimidazole (18). A solution of 5-amino-4-(diazoacetyl)-1-β-D-ribofuranosylimidazole (**15**; 142 mg, 0.5 mmol) in methanol (10 mL) containing 117 mg of 30% palladium-on-carbon catalyst was hydrogenated at ambient temperature and atmospheric pressure, then filtered, and evaporated to dryness in vacuo. The syrup obtained was purified by preparative thin-layer chromatography on Brinkmann (2-mm) silica gel plates developed in $CHCl_3$/MeOH (3:1). The product was recrystallized from ethanol as a white solid: yield 35 mg (27%); mp 237-238 °C dec; TLC ($CHCl_3$/MeOH (3:1), $(NH_4)_2SO_4$ char); UV λ_{max} ($\epsilon \times 10^{-3}$); 256 nm (sh) at pH 1 (4.30) and 291 nm at pH 1 (13.3), 240 nm at pH 7 (3.07) and 302 nm at pH 7 (13.3), 240 nm (sh) at pH 13 (3.04) and 301 nm at pH 13 (13.4); mass spectrum (EI), *m/e* 257 (M$^+$); ^{1}H NMR (Me_2SO-d_6) δ 2.25 (s, CH_3), 3.60 (ψt, 2 H-5'), 3.93 (dd, H-4'), 4.05 (dd, H-3'), 4.29 (dd, H-2'), 5.19 (d, 3'-OH), 5.35 (ψt, 5'-OH), 5.41 (d, 2'-OH), 5.51 (d, H-1', $J_{1',2'}$ = 6 Hz), 6.79 (s, NH_2), 7.32 (s, H_2). Anal. Calcd for $C_{10}H_{15}N_3O_5$: C, 46.69; H, 5.88; N, 16.33. Found: C, 46.72; H, 6.02; N, 16.05.

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Registry No. **3**, 53459-69-1; **5**, 105400-20-2; **6**, 105400-19-9; **7**, 73240-42-3; **10**, 105400-21-3; **12**, 105400-22-4; **14**, 105400-23-5; **15**, 105400-24-6; **16**, 105400-25-7; **18**, 105400-26-8; *N*-carbethoxyphthalimide, 22509-74-6.

Prostaglandin-H Synthase Inhibition by Malonamides. Ring-Opened Analogues of Phenylbutazone

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Recent reports of serious concern regarding the safe clinical use of phenylbutazone and its hydroxylated metabolite (oxyphenbutazone) as antiinflammatory agents have prompted the further investigation of ring-opened (malonamide) derivatives as potentially preferable therapeutic derivatives. Earlier reports have claimed reduced toxicity among similar derivatives. These studies reveal the relative degree of prostaglandin-H (PGH) synthase inhibitory activity among a series of malonamide derivatives. Contrary to observations in the pyrazolidinedione series, incorporation of a nonpolar butyl side chain in these malonamides was not beneficial but, rather, detrimental to enzyme-inhibitory activity. Although none of the reported nonbutylated malonamides was as potent an inhibitor of this enzyme as phenylbutazone, they all showed some inhibitory activity. PGH synthase inhibitory activity was especially pronounced in the bis(*p*-hydroxy anilide) derivatives, even extending to succinamide and adipamide derivatives. Of some interest is the observation that all of these *p*-hydroxy anilide derivatives were more potent inhibitors of this enzyme than acetaminophen.

The history of phenylbutazone (**1**) as a nonsteroidal antiinflammatory (NSAI) agent began in 1949 when the drug (which had been originally used as a solubilizing agent for aminopyrine) was introduced for the treatment of rheumatoid arthritis, acute gout, and allied disorders.[1] Clinically, phenylbutazone is 6 times more potent than the salicylates, but 5 times less potent than indomethacin. Phenylbutazone is an effective NSAI agent, but serious toxicity (especially aplastic anemia and agranulocytosis) limits its use in long-term therapy.[1,2]

A significant concern has been expressed about the toxicity of phenylbutazone (**1**) and oxyphenbutazone (**2**) in a number of recent reports[3,4] and letters to the editor.[5-8] Phenylbutazone and oxyphenbutazone have, in fact, been removed from the market in Bahrain, Jordan, and Norway.[8] Similar decisions are pending in Australia, the United States, and several other countries.[5,8] Meanwhile, the product labeling of phenylbutazone and oxyphenbutazone in the United States is being revised to reflect concern about the serious toxicity mentioned above.[4] A recent report in the *Drug and Therapeutics Bulletin*[3] acknowledged that while some physicians consider phenylbutazone to have special value in acute gout and an-

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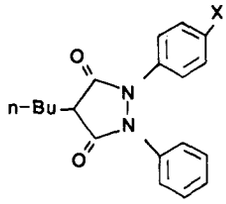
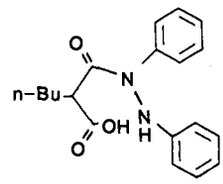
kylosing spondylitis, both phenylbutazone and oxyphenbutazone would have to show some major advantage over the many other nonsteroidal antiinflammatory drugs now available for their risk/benefit ratio to be acceptable.

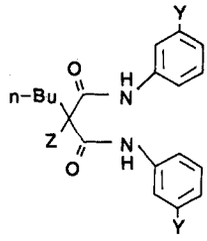


1, X = H

2, X = OH



3



4, Y = NH_2, Z = Et

5, Y = Z = H

As a result of the liabilities of phenylbutazone and oxyphenbutazone resulting from their reported toxicity, potentially less toxic analogues of phenylbutazone and oxyphenbutazone have been sought. Previous observations had indicated that ring-opened analogues of phenylbutazone such as hydrazide **3**[9] and malonamides **4**[10,11] and **5**[12] demonstrated good in vivo antiinflammatory activity in model systems. Hydrazide **3** and malonamide **4** were equipotent and less toxic than phenylbutazone,[10,11] while malonamide **5**[12] was less potent but also less toxic. These reports of in vivo antiinflammatory activity for such nonacidic malonamides (**4** and **5**) contradict the generally accepted "requirement" for acidic properties among useful in vivo antiinflammatory agents.[1] Acidity is not essential for in vitro PGH synthase inhibition.[13]

Accordingly several open-ring (malonamide) derivatives of phenylbutazone were synthesized and evaluated for potential antiinflammatory activity according to their inhibition of prostaglandin-H (PGH) synthase in vitro. Substitution in the aromatic rings of the proposed malonamides with substituents such as *p*-OCH_3, *p*-OH, *p*-CH_3, *p*-Cl, and *p*-NO_2 was accomplished with the intent of increasing potency as observed with several para-substituted derivatives in the phenylbutazone series.[10] The contribution to activity provided by the nonpolar 4-position butyl side chain was also examined in these ring-opened analogues.

Chemistry

The syntheses of the malonamides were accomplished by the high-temperature condensations between the appropriate aniline derivative and diethyl butylmalonate or diethyl malonate. The chemical syntheses of malonamides **5**, **6**, **8**, **10**, **12**, **14**, and **16** have been reported;[10,14-20] however, their effect on PGH synthase has not been previously reported. As in the literature procedures, these malonamides were all formed by heating a neat mixture of aniline derivative and the malonate diester. Use of a base catalyst such as sodium methoxide[21] was not advantageous in attempts to improve this procedure. Each reaction was heated until the distillation of ethanol ceased, indicating completion of the reaction. The resulting malonamides were quite insoluble and often crystallized upon cooling of the reaction mixture. A stoichiometric ratio of the aniline derivative and malonate ester was generally used, but in some cases an increase in the relative amount of malonate ester was necessary to minimize side reactions. In some cases the formation of the half malonamide ester was observed. After poor results were obtained in the synthesis of **15** from the direct condensation reaction between 4-aminophenol and diethyl butylmalonate, **15** was synthesized from anisidine derivative **13** in quantitative yield by boron tribromide O-demethylation.[22]

On the basis of good PGH synthase inhibitory activity (vide infra) shown by the two 4-hydroxyl-substituted malonamides **15** and **16**, several related compounds containing the bis(4-hydroxy anilide) functionality were prepared (Table I). Compounds **17**, **18**, and **20** were synthesized in high-temperature-condensation reactions between the appropriate ethyl esters and aniline derivatives in yields of 3%, 27%, and 22%, respectively. Compound **19** was synthesized from succinyl chloride and 4-aminophenol in 71% yield according to a modified procedure of Nakanishi and Tsuda.[23]

Results

The malonamides, consistent with previous in vivo assay results,[9,10,11] were all less active than phenylbutazone in the inhibition of microsomal PGH synthase (Table I). Nonetheless, several of them showed promising activity. In particular, the two derivatives **16** and **17** with *p*-hydroxyl substituents were the most potent. The unsubstituted malonamide **6** and malonamide **10** with a *p*-chloro substituent were also reasonably active. In contrast to the phenylbutazone series, the butyl side chain in these malonamides generally proved to be detrimental to activity. Except in the ring-hydroxylated series, most of the butyl-substituted malonamides actually showed slight stimulation of the enzyme at lower concentrations (100 μM); insolubility at higher concentrations prevented testing at these levels.

Of significant interest is the observation that all compounds containing the bis(*p*-hydroxy anilide) function malonamides **15–17**, succinamide **19**, and adipamide **20** showed greater enzyme inhibition than the chosen reference compound acetaminophen. This might be expected in comparison on a molar basis since each of these compounds contains two such moieties per molecule; however, malonamide **12** (*p*-CH_3) showed inhibitory activity similar to that of acetaminophen, and malonamides **8** (*p*-NO_2) and **18** (*m*-HO) showed some inhibitory activity without the

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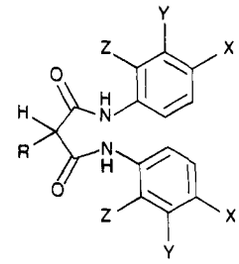
Table I. Malonamides as Inhibitors of PGH Synthase



no.	R	X	Y	Z	% control[a] 100 μM	% control[a] 500 μM	IC_{50},[b] mM
1 (PB)					11 ± 0.5	0	0.01
5	*n*-Bu	H	H	H	105 ± 2	insol	
6	H	H	H	H	65 ± 3	55 ± 4	
7	*n*-Bu	NO_2	H	H	126 ± 3	insol	
8	H	NO_2	H	H		88 ± 5	
9	*n*-Bu	Cl	H	H	96 ± 5	insol	
10	H	Cl	H	H	64 ± 3	48 ± 3	0.42
11	*n*-Bu	CH_3	H	H		97 ± 8	
12	H	CH_3	H	H		66 ± 1	
13	*n*-Bu	OCH_3	H	H	102 ± 6	insol	
14	H	OCH_3	H	H	78 ± 6	insol	
15	*n*-Bu	OH	H	H	76 ± 6	36 ± 1	0.29
16	H	OH	H	H	67 ± 3	25 ± 1	0.19
17	*n*-Bu	OH	H	CH_3	57 ± 4	31 ± 1	0.15
18	H	H	OH	H		86 ± 2	
19	$(HOPhNHCOCH_2)_2$				79 ± 7	27 ± 1	0.24
20	$(HOPhNHCOCH_2CH_2)_2$				67 ± 4	26 ± 1	0.23
acetaminophen	$HOPhNHCOCH_3$					67 ± 1	>0.50

[a] These values are the mean of triplicate determinations (± standard deviation) for the rate of oxygen consumption in the presence of the indicated compound concentration as compared to the uninhibited control. The specific activity of this microsomal PGH synthase preparation was 52 ± 4 nmol of O_2/min^{-1} mg^{-1}. [b] IC_{50} values were determined from the mean curve of percent inhibition against log concentration. The mean curve was obtained from three determinations per concentration.

p-hydroxy anilide substructure. Although acetaminophen itself does not exhibit an in vivo antiinflammatory effect, it is a widely used antipyretic/analgesic that has been claimed to selectively inhibit brain PGH synthase.[24] *lands* has attributed the selectivity of acetaminophen inhibition to its phenolic antioxidant properties that he has demonstrated to be responsible for reversible inhibition of PGH synthase in tissues with lower lipid peroxide concentrations.[13] The phenolic compounds discussed herein may be acting in a similar manner. These results suggest that such bis(*p*-hydroxy anilides) might be further evaluated as analgesics rather than nonsteroidal antiinflammatory agents.

Experimental Section

A. General Procedures. Melting points were determined with a Thomas-Hoover or Mel-Temp capillary apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 281 spectrophotometer. Nuclear magnetic resonance spectra were obtained with a JEOL FX90Q or Nicollet NT 300 spectrometer. Mass spectra were obtained on an AEI MS-30 spectrometer. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Thin-layer chromatography was performed on precoated silica gel plates (HLF, Analtech, Newark, DE). All chemicals and solvents were reagent grade and were distilled or recrystallized according to literature procedures when necessary. Ultracentrifugation was performed on a Beckman L2-65B ultracentrifuge.

B. Chemistry. 2-*n*-Butyl-*N*,*N*′-bis(4-methoxyphenyl)malonamide (13). A mixture of diethyl *n*-butylmalonate (0.025 mol, 5.4 g) and *p*-anisidine (0.05 mol, 6.2 g) was stirred at 160–170 °C for 3 h and the ethanol (1.3 g) formed was allowed to distill from the reaction mixture. The cooled reaction mixture was taken up in ethyl acetate (100 mL) and stirred for 45 min to obtain after filtration **13** (6.9 g, 75%) as an off-white crystalline solid. Malonamide **13** was recrystallized from ethanol to afford white needles: mp 198–199 °C; IR (Nujol) 3300, 3040, 3005, 1675, 1605, 820, 800 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.78–0.90 (t, *J* = 6 Hz, 3 H), 1.12–1.32 (m, 4 H), 1.73–1.98 (m, 2 H), 3.37 (t, *J* = 7 Hz, 1 H), 3.70 (s, 6 H), 6.83 (d, *J* = 9 Hz, 4 H), 7.47 (d, *J* = 9 Hz, 4 H), 9.72 (s, 2 H); MS, *m*/*e* (rel intensity) 370 (M^+, 25), 178 (42), 149 (12), 123 (100), 108 (22). Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

2-*n*-Butyl-*N*,*N*′-bis(4-hydroxyphenyl)malonamide (15). To a suspension of diether **13** (0.01 mol, 3.7 g) in methylene chloride (125 mL) at −78 °C was added boron tribromide (0.04 mol, 10.0 g) in methylene chloride (25 mL) according to the method of McOmie et al.[22] The reaction mixture, which immediately became clear, was allowed to warm to room temperature and was stirred for 42 h. Water (250 mL) was added to quench the reaction. The off-white solid that formed was removed by filtration to afford diphenol **15** (3.4 g, 100%). Malonamide **15** was recrystallized from acetonitrile to give off-white needles: mp 220–222 °C; IR (Nujol) 3290, 1660, 1640, 1605, 1550, 1515, 805 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 0.86 (t, *J* = 6 Hz, 3 H), 1.14–1.34 (m, 4 H), 1.72–1.96 (m, 2 H), 3.35 (t, *J* = 7 Hz, 1 H), 4.76 (br s, 2 H), 6.66 (d, *J* = 9 Hz, 4 H), 7.34 (d, *J* = 9 Hz, 4 H), 9.18 (s, 2 H), 9.60 (s, 2 H); MS, *m*/*e* (rel intensity) 342 (M^+, 6), 164 (20), 135 (18), 109 (100), 80 (19), 55 (15), 28 (21). Anal. ($C_{19}H_{22}N_2O_4$) C, H, N.

In general, malonamide formation was performed by condensation of the requisite aniline derivative with either the butyl-substituted or unsubstituted malonate diethyl ester as described in detail above for malonamide **13**. The pertinent details for the syntheses of the other malonamides discussed in this paper are given in Table II.

C. Enzyme Preparation and Assay. 1. PGH Synthetase Microsomal Preparation. The microsomal enzyme was isolated from sheep seminal vesicles (SSV) obtained from Pel-Freez Biologicals (Rogers, AR) by a modified procedure of Takeguchi et al.[25] Approximately 120 g of SSV was allowed to thaw before

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Table II. Reaction Conditions and Characterization of Malonamide Derivatives

no.	reaction conditions: time, h	reaction conditions: temp, °C	postreaction diluent	% yield	recrystn solvent	mp, °C	(lit. mp or C, H, N det)
5	5.0	160	Et_2O	35	EtOAc	191-192	(lit.[20] 193)
6	5.0	163	EtOH	65		228-229	(lit.[17,18] 223; 225)
7	2.5	185	Et_2O	25	EtOH/acetone (5:1)	211-212	($C_{19}H_{20}N_4O_6$)
8	4.0	185	6 N HCl	31	nitrobenzene	239-242	(lit.[16] 243-245)
9	3.0	165-180	EtOAc	53	CH_3CN/MeOH/acetone (3:1:1)	228-230	($C_{19}H_{20}Cl_2N_2O_2$)
10	4.0	160-175	EtOH	38		256-257	(lit.[15] 261)[a]
11	6.5	168-170	EtOH wash	11		191-193	($C_{21}H_{26}N_2O_2$)
12	3.5	155-160	EtOH wash	60		255-257	(lit.[18,19] 250; 248)[a]
14	2.0	190	hot EtOH	34		232-234	(lit.[14] 234)
16	3.5	185	none	19	hot 0.15 M Na_2CO_3	247-248	(lit.[14] 235)[a]
17	3.0	160	acetone	(3)	CH_3CN (yield after recrystn)	193-195	($C_{21}H_{26}N_2O_4$)
18	3.0	160	hot 1.5 M Na_2CO_3	27		206-208	($C_{15}h_{14}N_2O_4 \cdot {}^1/_2H_2O$)
19[b]	6.0	49	cold 3 N HCl	71	hot EtOH wash	280-282	(lit.[23] >250)
20	6.0	210	2 N HCl	22		265-268	(lit.[23] >250)

[a] Due to variance from reported melting point, confirmatory elemental analyses (C, H, N) were obtained for these compounds. [b] Succinyl chloride in pyridine rather than diester condensation was employed (see ref 23).

removing the associated fat, muscle, and connective tissues. The glands were then cut up into small pieces and added to 120 mL of potassium phosphate buffer (100 mM, pH 8.0) containing 5 mM diethyl dithiocarbamate and 5 mM disodium EDTA. This mixture was then homogenized in a Waring blender for 2 min and centrifuged for 10 min at 12000*g*. The supernatant was filtered through several layers of cheesecloth to remove the fat and recentrifuged for 1.25 h at 100000*g*. The resulting supernatant was decanted, and the pelleted microsomes were cooled to −80 °C and lyophilized. The microsomes were obtained in yields of between 1.0 and 1.5 g, starting from 120 g of crude SSV. The microsomes were dissolved in EDTA buffer[26] (100 mM, pH 8.0) containing 1.0 mM phenol, 2.0 mM glutathione, and 1.5% v/v Tween-20 at a 1.0 mg/mL concentration for enzyme-inhibition studies. The specific activity of these SSV microsomes was 52 ± 4 nmol of O_2 min^{-1} (mg of microsomes)$^{-1}$.

2. Enzyme Assays. Enzyme activity was determined by measuring oxygen consumption in solution with a Yellow Springs Instrument Co. polarographic electrode (Clark oxygen electrode, YSI-5331) in conjunction with a Gilson oxygraph (Model K-1C) as previously described.[27] The YSI reaction chamber was modified to permit smaller sample volumes by uniformly rounding the bottom. The temperature of the reaction chamber was maintained at 37 ± 1 °C with a Haake FG water circulator. The enzyme reaction was initiated by the addition of sodium arachidonate solution (5 mg/mL) to provide a 100 μM final concentration (K_m = 5.9 μM)[28] in the 2-mL reaction chamber. All inhibitors were added as 100 mM solutions in Me_2SO for routine enzyme assays. The levels of Me_2SO used in the enzyme-inhibitor experiments had no effect on enzyme activity itself. Initial enzyme velocities (dO_2/dt) were obtained by measuring the slopes of the resulting oxygen concentration vs. time curves, and reported as a percent of uninhibited control. Values presented in Table I are the result of triplicate determinations expressed as the mean ± standard deviation.

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Synthesis and Biological Properties of 9-(*trans*-4-Hydroxy-2-buten-1-yl)adenine and Guanine: Open-Chain Analogues of Neplanocin A

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Alkylation of adenine (**5a**) or 2-amino-6-chloropurine (**5b**) with excess *trans*-1,4-dichloro-2-butene (4), effected by K_2CO_3 in dimethyl sulfoxide or tetra-*n*-butylammonium fluoride in tetrahydrofuran, led in 90-95% regioselectivity to 9-alkylpurines **6a** and **6b**. The title compounds **2a** and **2b** were obtained by refluxing intermediates **6a** and **6b** in 0.1 M NaOH or HCl. Adenine derivative **2a** is a substrate for adenosine deaminase whereas both **2a** and **2b** exhibit 50% inhibition of the growth of murine leukemia L 1210 cell culture at 1 mM concentration.

Open-chain nucleoside analogues lacking the 2′- or both 2′- and 3′-carbon fragments of ribofuranose moiety are the subject of considerable current interest. Thus, antiviral agent[1] acyclovir (**1a**) is derived from guanosine by removing the 2′,3′-carbon fragment. The corresponding adenine analogue **1b** has a much lower antiviral activity,[1] but it is a substrate for adenosine deaminase.[2] Biologically active open-chain analogues can also be derived from other nucleosides, e.g., 5-benzyluridine[3] (**1c**), as well as from some structurally related antibiotics. For example, N^9-(4-hydroxybutyl)adenine (**1d**), a weak inhibitor of adenosine deaminase,[4] can be regarded as an open-chain analogue of aristeromycin,[5] a naturally occurring carbocyclic analogue

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