(3 mL) to yield 68 mg of crude 4·HCl. The HCl salt was dissolved in water. The aqueous solution was basified with $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ extracts were pooled and concentrated to a small volume, with care being taken not to bring the solution to dryness. The solution was loaded to a silica gel column, which was eluted at 10 psi ($CHCl_3$–acetone–Et_3N, 90:10:3). The eluate fractions containing pure product were pooled. An equal volume of toluene was added, and the solvents were partially evaporated, with care being taken not to bring the solution to dryness. The addition and evaporation of toluene was repeated several times to insure that all the triethylamine had been removed. The solution was acidified with ethereal HCl and evaporated to dryness in vacuo to leave 38 mg (51% yield) of 4·HCl: mp 93 °C dec; $[\alpha]^{25}_D$ −157.2° (*c* 0.5, MeOH); TLC, R_f 0.69 (ether–acetone–Et_3N, 90:10:3); IR (KBr) 2090 (s, N=C=S stretching) cm^{-1}; NMR ($CDCl_3$, free base) δ 6.77–6.52 (2 d, 1 H each, Ar *H*), 4.49 (d, *J* = 7.47 Hz, C-5*H*); EIMS, *m/e* 368 (M^+). Anal. ($C_{21}H_{24}N_2O_2S$·HCl) C, H, N.

***N*-[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6β-yl]iodoacetamide (5).** To the primary amine 14 (50 mg, 0.153 mmol) in 2-propanol (2.5 mL) and THF (5 mL) at −70 °C was added a solution of *N*-(iodoacetoxy)succinimide[18] (48 mg, 0.17 mmol) in THF (2.5 mL). The mixture was stored at −8 °C for 10 h and at 25 °C for an additional 8 h. A solution of methanesulfonic acid in methanol then was added to pH 1.5. Toluene (10 mL) was added, and all of the solvents were evaporated in vacuo to leave an oily residue, which was crystallized from acetone (5 mL) to afford 49 mg (54%) of 5·CH_3SO_3H. The product was recrystallized from toluene and ethanol: mp 272–273 °C dec; $[\alpha]^{25}_D$ −115.8 °C (*c* 0.5, MeOH); TLC, R_f 0.58 (EtOAc–MeOH–NH_4OH, 90:10:3); IR (KBr) 1640 (s, amide C=O) cm^{-1}; NMR ($CDCl_3$, free base) δ 6.82 (d, *J* = 8.24 Hz, amide N*H*), 6.74–6.49 (2 d, 1 H each, Ar *H*), 4.38 (d, *J* = 7.69 Hz, C-5*H*), 3.70 (s, 2 H, CH_2I); EIMS, *m/e* 494 (M^+). Anal. ($C_{22}H_{27}N_2O_3I$·CH_3SO_3H) C, H, N.

(*E*)-4-[[17-(Cyclopropylmethyl)-4,5α-epoxy-3-hydroxymorphinan-6β-yl]amino]-4-oxo-2-butenoic Acid Methyl Ester (6). To the primary amine 14 (54 mg, 0.165 mmol) and K_2CO_3 (0.11 g, 0.825 mmol) in water (0.5 mL) and THF (1.5 mL) at 25 °C was added dropwise a solution of (*E*)-4-chloro-4-oxo-2-butenoic acid methyl ester (24.5 mg, 0.165 mmol) in THF (3 mL) over a 5-min period. After 48 h the solvent was removed in vacuo, and the residue was dissolved in water (25 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 layers were pooled, dried (Na_2SO_4), filtered, and evaporated to leave 50 mg of crude **6**. The residue was dissolved in methanol (1 mL) and triethylamine (0.2 mL) and stirred for 48 h. Removal of solvents followed by silica gel column chromatography (ether–MeOH–NH_4OH, 95:5:2) left 18 mg (25%) of pure **6**: mp 159–161 °C; $[\alpha]^{25}_D$ −149° (*c* 0.5, MeOH); TLC, R_f 0.49 (EtOAc–MeOH–NH_4OH, 90:10:3); IR (KBr, HCl salt) 1744 (s, ester C=O) 1679 (s, amide C=O) cm^{-1}; NMR ($CDCl_3$) δ 7.25–6.64 (2 d, 1 H each, olefinic *H*), 6.96–6.45 (2 d, 1 H each, Ar *H*), 4.48 (d, *J* = 7.69 Hz, C-5*H*); EIMS, *m/e* 438 (M^+). Anal. ($C_{25}H_{30}N_2O_5$·0.5H_2O) C, H, N.

Acknowledgment. This work was supported by the National Institute of Drug Abuse. We thank Victoria Darrow Elliott and Mary Schwartz for capable technical assistance.

Registry No. 3, 107819-63-6; 3·2HCl, 107819-74-9; 4, 107819-64-7; 4·HCl, 107819-77-2; **5**, 107819-65-8; **5**·CH_3SO_3H, 107819-78-3; **6**, 107819-66-9; **7**, 16590-46-8; **8**, 107819-67-0; **9**, 107819-68-1; **9**·HCl, 107846-39-9; **10**, 107819-69-2; **11**, 107819-70-5; **12**, 107819-71-6; **12**·HCl, 107819-75-0; **13**, 107819-72-7; **13**·2HCl, 107819-76-1; **14**, 107819-73-8; MeNH$(CH_2)_2$OH, 109-83-1; (*E*)-ClC(O)CH=CHC(O)OMe, 17081-97-9; *N*-(iodoacetoxy)succinimide, 39028-27-8.

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Synthesis, Structure, and Biological Activity of Certain 2-Deoxy-β-D-*ribo*-hexopyranosyl Nucleosides and Nucleotides[1]

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2-Deoxy-β-D-*ribo*-hexopyranosyl nucleosides with adenine (**2**), hypoxanthine (**17**), guanine (**23**), cytosine (**13**), and uracil (**7**) as the aglycon were synthesized by the Lewis-acid-catalyzed condensation of an appropriate trimethylsilylated heterocyclic base and 2-deoxy-1,3,4,6-tetrakis-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose (**5**) to provide the desired β anomers in good yield. When the synthesis of **7** via an S_N2 displacement was attempted by reaction between silylated uracil and 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl bromide (**8**), the major product, 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**9**), had retained the α configuration at the anomeric carbon. The structures of both anomers of 1-(2-deoxy-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione were assigned by single-crystal X-ray methods. The anomeric configuration and conformation of other nucleosides were determined by proton magnetic resonance analysis of the 4-nitrobenzoylated nucleosides. Nucleoside 6′-monophosphates of **7**, **13**, and **2** and the 4′,6′-cyclic monophosphate of **2** were also prepared. All 2′-deoxy-D-*ribo*-hexopyranosyl nucleosides and 6′-monophosphate derivatives were tested in vitro for antiviral and antitumor activity. The guanosine analogue **23** was moderately active against HSV-2 virus. The UMP analogue, 1-(2-deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**28**), demonstrated moderate activity against HSV-2 and parainfluenza 3 virus and was also active against L1210 (ID_{50} = 39 μM) and P388 (ID_{50} = 33 μM) leukemic cell lines. Two compounds, 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**2**) and 9-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,6-diaminopurine (**24**), were substrates for adenosine deaminase (EC 3.5.4.4) with K_m values of 57 and 90 μM, respectively. 6-Amino-7-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine, **18**, was a competitive inhibitor of ADase (K_i = 0.1 mM).

Certain hexopyranosyl nucleoside analogues have been useful as probes of enzymes involved in nucleic acid metabolism. The nucleoside 1-(2-deoxy-β-D-*erythro*-hexopyranosyl)thymine[3] (**1**) is a potent and specific inhibitor of mammalian uridine phosphorylase (K_i = 12 μM)[4] and has recently been utilized to characterize various mammalian pyrimidine phosphorylase activities.[5,6]

(1) This report is taken, in part, from the Ph.D. Dissertation of L.D.N., Brigham Young University, 1985.

(2) Present address: Nucleic Acid Research Institute, Costa Mesa, CA 92626.

(3) Zorbach, W. W.; Durr, G. J. *J. Org. Chem.* **1962**, *27*, 1474.

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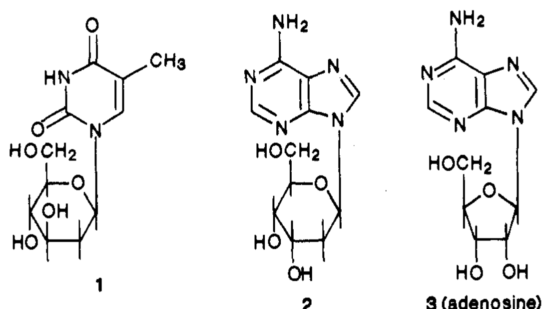
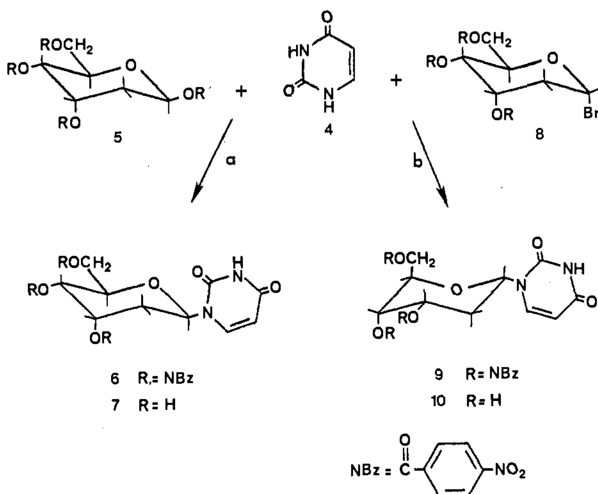
Hexopyranosyl nucleosides containing 2-deoxy-β-D-*ribo*-hexopyranose as the carbohydrate moiety possess obvious structural similarities to ribo- and 2-deoxyribonucleosides. 6-Amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**2**), for example, possesses a *cis*-diol function at C3′–C4′, which could mimic the C2′–C3′ portion of adenosine (**3**). In addition, the configuration at C2′–C3′ of **2** is similar to that found in 2′-deoxyadenosine.



A comparison of Dreiding models of **2** and **3** demonstrates that when the *cis*-diol groups of these molecules are superimposed the position of the heterocyclic base of **2** is slightly altered from that of **3**. The actual stereopositions, however, depend upon the conformation of the pyranose ring, which may exist in either of two chair forms ($^4C_1$ or $^1C_4$)[7] or the less likely boat or skew forms.[8]

Certain nucleoside antibiotics including blasticidin,[9] gougerotin,[10] hikizimycin,[11] mildiomycin,[12] and miharamycin[13] contain hexopyranosyl sugars and demonstrate distinct and varied biological activity. Hikizimycin exhibits anthelmintic activity.[14] Blasticin S, in addition to possessing important antifungal activity,[15] has shown antitumor[16] and antiviral activity.[17,18] Blasticidin and gougerotin act as nonfunctional aminoacyl-tRNA analogues to block protein elongation[19] and have been utilized as probes for the peptidyl transferase activity of both prokaryotic and eukaroyotic ribosomes.[20,21] The recently reported gougeroutin analogues, bagougeramine A and B,[22] also possess insecticidal activity.[23]

Scheme I



An antiviral hexopyranosyl nucleoside antibiotic, SF-2140, inhibits the in vitro replication of certain strains of influenza A and B viruses.[24]

2-Deoxyhexopyranosyl nucleoside antibiotics include pentopyranine A and B,[25] which significantly inhibit RNA synthesis in Ehrlich ascites cells,[26] and the amicetin group of antibiotics, which inhibit protein synthesis.[27]

In the present study, 2-deoxy-β-D-*ribo*-hexopyranosyl analogues of various naturally occurring nucleosides have been synthesized to investigate molecular structures and compare interactions against normal substrates with certain target enzymes. Additionally, the analogue compounds have been tested for antitumor and antiviral activity.

Chemistry

The trimethylsilyl derivative[28] of uracil (**4**) was glycosylated (Scheme Ia) with 2-deoxy-1,3,4,6-tetrakis-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose[29] (**5**) in the presence of trimethylsilyl trifluoromethanesulfonate[30] (TMS-triflate). The reaction proceeded with retention of configuration about the anomeric carbon to produce the β-anomer of 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**6**), in 75% yield. Deacylation with MeOH/$NaOCH_3$ provided 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**7**) in 76% yield.

Zorbach and co-workers[31] reported the synthesis of 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione and the corresponding cytidine analogue through the common intermediate 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-4-ethoxy-2-pyrimidinone. This intermediate was obtained in 50% yield by the condensation of 2,4-diethoxypyrimidine with 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl bromide. The assignment of anomeric configuration of these nucleosides,

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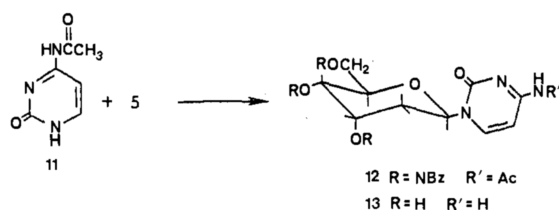
Scheme II



however, was based on optical rotatory dispersion data obtained from furanosyl nucleosides.[31] 1-(2-Deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione, prepared as described by Zorbach and co-workers, was found to be identical with compound 7 of this study by ^{1}H NMR, TLC, and analytical reverse-phase HPLC, thus confirming the reported configuration.

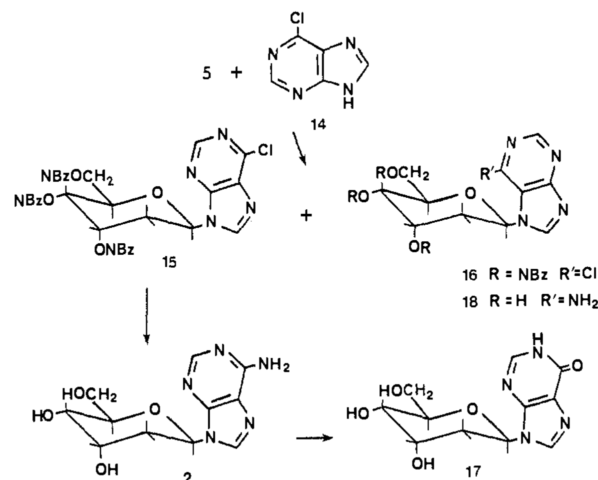
In the present work, we also synthesized the α-anomer of 1-(2-deoxy-D-*ribo*-hexopyranosyl)-2,4-pyrmidinedione to provide a basis for assignment of configuration and conformation of other 2-deoxy-β-D-*ribo*-hexopyranosyl nucleosides. When the halo sugar 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl bromide[29] (8) (Scheme Ib) was stirred with 2,4-bis-*O*-(trimethylsilyl)-uracil in dry CH_3CN, the reaction proceeded with retention of configuration to give the major product 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (9) in 57% yield. In addition, a substantial amount of the glycal 1,5-anhydro-2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hex-1-enitol (structure not shown) was isolated. The identity of the glycal was established by comparison of ^{1}H NMR data with that for similar derivatives of 1,5-anhydro-2-deoxy-D-*ribo*-hex-1-enitol (D-allal) protected with acetyl, benzyl, or benzoyl groups.[32] Treatment of 9 with NH_3/MeOH gave 1-(2-deoxy-α-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (10).

The Lewis-acid-catalyzed glycosylation procedure employing 2′-deoxy-D-*erythro*-pentofuranose has been reported to produce a mixture of anomeric nucleosides.[33] The extent of α-anomer formation in the synthesis of 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (7) by condensation with 5 (Scheme Ia) was determined by performing the glycosylation reaction, as described for 7, without purification. The deblocked anomeric mixture was separated by reverse phase HPLC and the α- and β-anomers amounted to 11% and 89%, respectively.

The method of glycosylation to form the β-uridine analogue 7 was applied with success to other pyrimidine and purine bases. *N*-Acetylcytosine[34] (11) was silylated by treatment with hexamethyldisilazane (HMDS). Reaction between silylated 11 and 5 (Scheme II) in the presence of TMS-triflate provided 4-acetamido-1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2-pyrimidinone (12) in 82% yield. Ammonolysis of 12 provided 4-amino-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2-pyrimidinone (13) in 90% yield. The conformation and anomeric configuration of 12 and similar protected 2-deoxy-β-D-*ribo*-hexopyranosyl purine nucleosides were assigned by comparison of the ^{1}H NMR spectra of these compounds with the spectra of 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α- and -β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (9 and 6, respectively).

The adenosine analogue 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (2) was synthesized by the reaction of 5 with silyl 6-chloropurine (Scheme III). Two products,

Scheme III



15 (62%) and 16 (30%), were isolated and separately treated with MeOH/NH_3 to form 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (2) and the N-7 glycosyl isomer 18. Diazotization of 2 by treatment with $NaNO_2$/acetic acid provided the inosine analogue 17. The synthesis of 2 by condensation of (chloromercuri)-6-benzamidopurine with the bromo sugar 7 was described by Zorbach and Saeki[35] in 1964. Although the isolation of both anomers was mentioned, the yields were low and no anomeric assignments were made. Identification of the products based on the reported physical constants was not possible.

2,6-Dichloropurine (19) was silylated and reacted with 5 (Scheme IV) to form 9-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,6-dichloropurine (20) in 66% yield. Treatment of 20 with aqueous NaOH in dioxane to simultaneously displace the 6-chloro group and deblock gave 22. Subsequent amination of 22 with MeOH/NH_3 produced the guanosine analogue 2-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purin-6-one (23). The intermediate 20 was also treated directly with NH_3/MeOH under varied conditions to form 9-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,6-diaminopurine (24) and the 6-amino-2-chloropurine derivative 25. Catalytic dechlorination of 25 provided an alternate route for the synthesis of 2.

The extent of α-anomer formation in the synthesis of 9-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,6-dichloropurine was determined by ^{1}H NMR. The ^{1}H NMR signals for the C-8 protons of 6-amino-2-chloro-9-(2-deoxy-α- and -β-D-*ribo*-hexopyranosyl)purine are separated by 0.26 ppm ($\alpha C_8\ H$, δ 8.66 ppm and $\beta C_8\ H$, δ 8.40 ppm, see Experimental Section) allowing integration of each peak separately. The ratio of anomers was determined by treating the mixture of blocked anomers in the same manner as described for the synthesis of 6-amino-2-chloro-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (25) and the amount of each anomer was estimated by integration of the peak represented by each anomer. The α- and β-anomers were present to the extent of 18% and 82%, respectively.

Nucleoside 6′-monophosphates of 2, 7, and 13 were synthesized by selective phosphorylation of the primary hydroxyl group[36] at C-6′ by treatment of the unprotected nucleoside with $POCl_3$ in trimethyl phosphate (Scheme V). Phosphate attachment at the $C_{6'}$ hydroxyl group was established by observing the two- and three-bond P–C cou-

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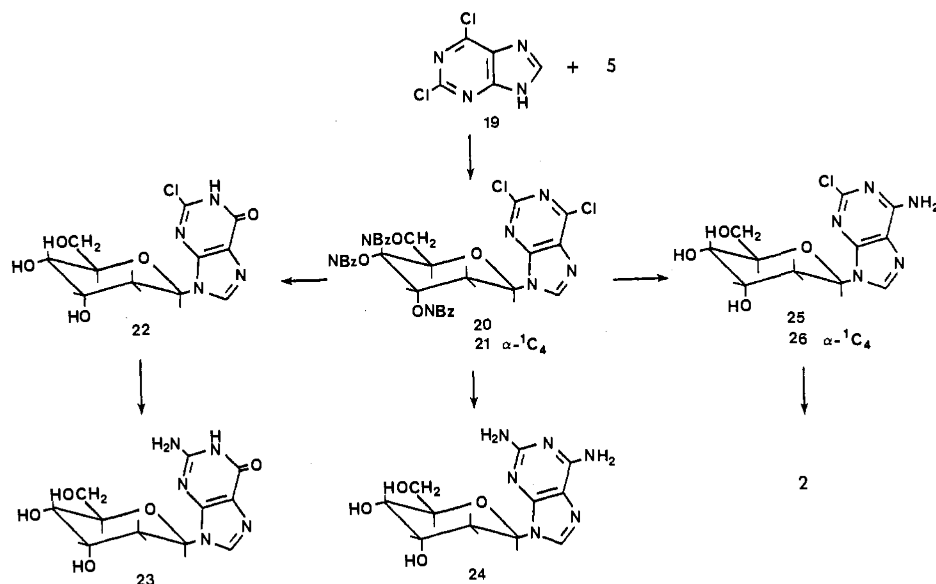
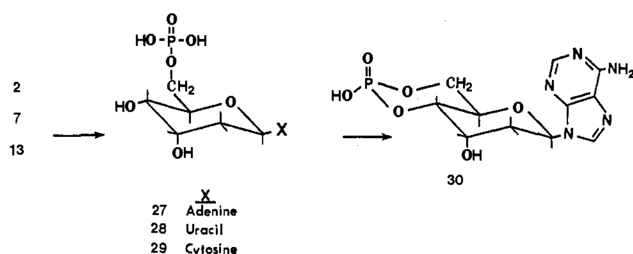
(36) Yoshikawa, M.; Kato, T.; Takenishi, T. *Bull. Chem. Soc. Jpn.* **1969**, *42*, 3505.

Scheme IV



Scheme V



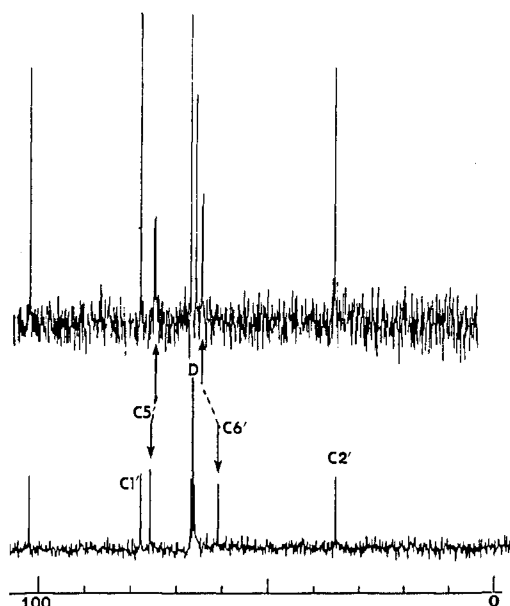


Figure 1. Proton-decoupled ^{13}C NMR (89.6 MHz) spectra of the carbohydrate carbons of 1-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**7**, bottom) and the corresponding 6'-monophosphate derivative **28** (top). Dioxane (D) was used as an internal reference.

pling evident in proton-decoupled ^{13}C NMR spectra (Figure 1). 1-(2-Deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**28**) displayed two doublets (δ 65.19 ppm, C-6'; δ 75.75 ppm, C-5') with the signal for C-6' having the larger shift of 3.22 ppm downfield relative to the signal for the nucleoside. The $C_{6'}$ doublet had $^2J(C_{6'}OP)$ = 5.10 Hz and the $C_{5'}$ doublet had $^3J(C_{5'}C_{6'}OP)$ = 6.38 Hz. Precisely the same pattern of the carbon β to

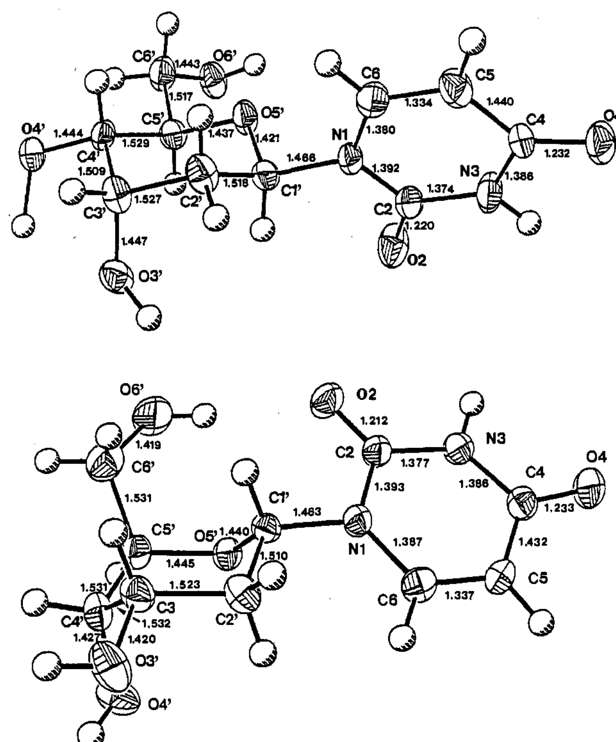


Figure 2. Computer drawings of the anomeric structures of 1-(2-deoxy-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (β-anomer (**7**), top; α-anomer (**10**), bottom).

the phosphate group possessing a larger coupling constant than the carbon α to the phosphate has been observed for uridine 5'-monophosphate.[37,38] The other 6'-monophosphates demonstrated a similar pattern. This represents the first application of the Yoshikawa procedure[36] to hexopyranosyl nucleosides to obtain nucleoside 6'-monophosphates.

The cyclic AMP analogue 6-amino-9-(2-deoxy-4,6-*O*-(hydroxyphosphinyl)-β-D-*ribo*-hexopyranosyl)purine (**30**) was synthesized by treatment of **27** with DCC/pyridine to give **30** in 65% yield.

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Table I. Crystal and Experimental Data for the α- and β-Anomers of 1-(2-Deoxy-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione

	α	β
formula	$C_{10}H_{14}O_6N_2$	$C_{10}H_{14}O_6N_2 \cdot 2.5H_2O$
formula weight	258.2	303.3
$F(000)$	544	644
crystal size, mm	0.3 × 0.3 × 0.3	0.25 × 0.3 × 0.3
space group	$P2_12_12_1$	C_2
a, Å	7.228 (2)	14.677 (9)
b, Å	8.242 (4)	13.282 (4)
c, Å	19.541 (7)	7.452 (4)
β, deg	90	101.62 (4)
V, Å^3	1164.1	1422.8
Z	4	4
D_x, g/cm^3	1.47	1.42
μ, cm^{-1}	1.15	1.16
unique obsd data	999	1371
unobsd data	221	345
R	0.044	0.054
R_w	0.043	0.054
max e/Å^3 values	0.21, −0.22	0.26, −0.24
av Δ/esd value	0.005	0.008

Table II. Positional (×10^4) and Thermal (Å^2 × 10^3) Parameters for Atoms of the α Anomer[a]

atom[b]	1 − x	1 − y	1 − z	U[c]
N1	9209 (4)	8088 (3)	4192 (2)	26 (1)
C2	7783 (5)	9117 (4)	4396 (2)	27 (1)
O2	6159 (4)	8798 (3)	4320 (1)	39 (1)
N3	8377 (4)	10532 (4)	4699 (2)	32 (1)
C4	10187 (5)	11021 (4)	4811 (2)	30 (1)
O4	10463 (4)	12315 (3)	5111 (1)	43 (1)
C5	11560 (5)	9924 (4)	4556 (2)	31 (1)
C6	11051 (5)	8532 (4)	4260 (2)	28 (1)
C1′	8617 (5)	6585 (4)	3860 (2)	23 (1)
C2′	10065 (5)	5267 (4)	3821 (2)	28 (1)
C3′	9214 (5)	3807 (4)	3462 (2)	28 (1)
O3′	10647 (4)	2638 (3)	3395 (1)	41 (1)
HO3′	10182 (60)	1786 (49)	2207 (20)	70 (13)
C4′	8432 (5)	4289 (4)	2763 (2)	31 (1)
O4′	9896 (4)	4742 (3)	2312 (1)	38 (1)
HO4′	10579 (69)	3871 (53)	2253 (25)	78 (17)
C5′	7136 (5)	5753 (4)	2816 (2)	29 (1)
C6′	5248 (5)	5377 (5)	3135 (2)	42 (1)
O6′	4274 (4)	6834 (4)	3275 (2)	50 (1)
HO6′	4939 (83)	7391 (64)	3582 (30)	146 (23)
O5′	8030 (3)	7057 (3)	3184 (1)	26 (1)

[a] Estimated standard deviation values are in parentheses. [b] Hydrogen atoms for which parameters were calculated are omitted. [c] Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor.

Conformation and Anomeric Configuration

A. X-ray Crystallographic Studies. The structures of 1-(2-deoxy-α- and β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**10** and **7**, respectively) in the solid state are shown in Figure 2. Both molecules assumed the conformation in which the aglycon was equatorially oriented. The α-anomer **10** proved to be in the $^1C_4$ conformation and the β-anomer **7** assumed the $^4C_1$ conformation. X-ray crystal data are summarized in Table I. The atomic coordinates are included in Tables II and III. The C_2–N_1–$C_{1'}$–$O_{5'}$ torsion angles and estimated standard deviation (esd) values are −74.6 (3)° and 95.3 (6)° for the α- and β-anomer, respectively. In both compounds, the atoms in the chair conformation alternate 0.22 Å above and below the least-squares plane of the pyranose ring. The heterocycle for each molecule is planar with the largest deviation of any ring atom from the least-squares plane being 0.028 Å for C-2 in the β-anomer and 0.023 Å for N-1 in the α-anomer. One intramolecular hydrogen bond was found in the α-anomer involving the pyranose $C_{6'}OH$ and the pyrimidine $C_2{=}O$. The bond is characterized as follows:

Table III. Positional (×10^4) and Thermal (Å^2 × 10^3) Parameters for Atoms of the β Anomer[a]

atom[b]	1 − x	1 − y	1 − z	U[c]
N1	8869 (2)	1799	7387 (4)	27 (1)
C2	9069 (2)	2824 (3)	7436 (5)	30 (1)
O2	8485 (2)	3479 (2)	7417 (5)	45 (1)
N3	9985 (2)	3052 (3)	7458 (5)	37 (1)
C4	10713 (2)	2381 (3)	7481 (5)	31 (1)
O4	11484 (2)	2706 (3)	7360 (5)	46 (1)
C5	10456 (3)	1340 (4)	7603 (6)	37 (1)
C6	9570 (3)	1092 (3)	7546 (6)	36 (1)
C1′	7887 (2)	1511 (3)	7134 (5)	26 (1)
C2′	7650 (3)	583 (3)	5941 (5)	32 (1)
C3′	6611 (2)	375 (3)	5734 (5)	29 (1)
O3′	6046 (2)	1187 (2)	4809 (4)	33 (1)
HO3′	6317	1520	3933	37 (12)
C4′	6381 (2)	274 (3)	7611 (5)	26 (1)
O4′	5396 (2)	133 (2)	7500 (4)	34 (1)
HO4′	4983 (39)	592 (49)	6177 (70)	92 (19)
C5′	6721 (2)	1182 (3)	8822 (5)	26 (1)
C6′	6608 (3)	1000 (3)	10775 (5)	35 (1)
O6′	6778 (2)	1886 (3)	11913 (4)	37 (1)
HO6′	7339 (30)	2027 (37)	12265 (58)	57 (14)
O5′	7697 (2)	1338 (2)	8904 (3)	30 (1)
OW1	8421 (3)	9035 (4)	2640 (7)	70 (2)
HW1A	8887 (35)	8755 (50)	3670 (65)	98 (23)
HW1B	8529 (47)	8793 (58)	1542 (60)	112 (28)
OW2	9042 (3)	8850 (3)	−672 (6)	72 (2)
HW2A	8880 (36)	8325 (33)	−1502 (61)	66 (17)
OW3	10000	8235 (7)	5000	130 (4)

[a] Estimated standard deviation values are in parentheses. [b] Hydrogen atoms for which parameters were calculated are omitted. [c] Equivalent isotropic U defined as one-third of the trace of the orthogonalized U_{ij} tensor.

bond lengths, $C_{6'}OH$–C_2O, 2.049 (63) Å, $C_{6'}O$–C_2O, 2.939 (4) Å; and bond angle ($C_{6'}O$–$C_{6'}OH$–C_2O) 172.8 (5.2)°.

B. NMR Spectroscopy. ^{1}H NMR methods have been previously utilized to determine anomeric configuration and conformation of protected hexopyranoses[39-41] and 2-deoxypentopyranosyl nucleosides.[42] Vicinal proton–proton interactions, associated dihedral angles, and coupling constants for hexopyranoses have also been described.[43]

The anomers of 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (α, **9**; β, **6**) were obtained at 300 MHz in Me_2SO-d_6. The resulting carbohydrate proton signals are shown in Figure 3. The β-anomer (**6**, upper spectrum) exhibits well-separated signals with a set of doublets centered at 5.52 ppm, for H-4′. In the $^4C_1$ conformation, H-4′ is axially oriented and coupled to H-5′ ($J \simeq 10.5$ Hz), which is also axially oriented. The smaller coupling ($J \simeq 2.8$ Hz) is from H-3′ (equatorial). The H-3′ peak (5.95 ppm) appears as a narrow doublet and is only weakly coupled to neighboring protons. In contrast, the signals for the α-anomer (**9**, lower spectrum) are not well-separated. In the $^1C_4$ conformation, H-3′ is axially oriented and coupled to H-2′a ($J > 10.5$ Hz). The smaller couplings are from H-2′e and H-4′. The H-4′ signal (5.79 ppm) in this conformation appears as an unresolved multiplet.

It is also interesting to note that the signals for the anomeric protons are identical in peak shape. This would be expected for the α ($^1C_4$) and β ($^4C_1$) structures as demonstrated in Figure 4. The anomeric proton in each case

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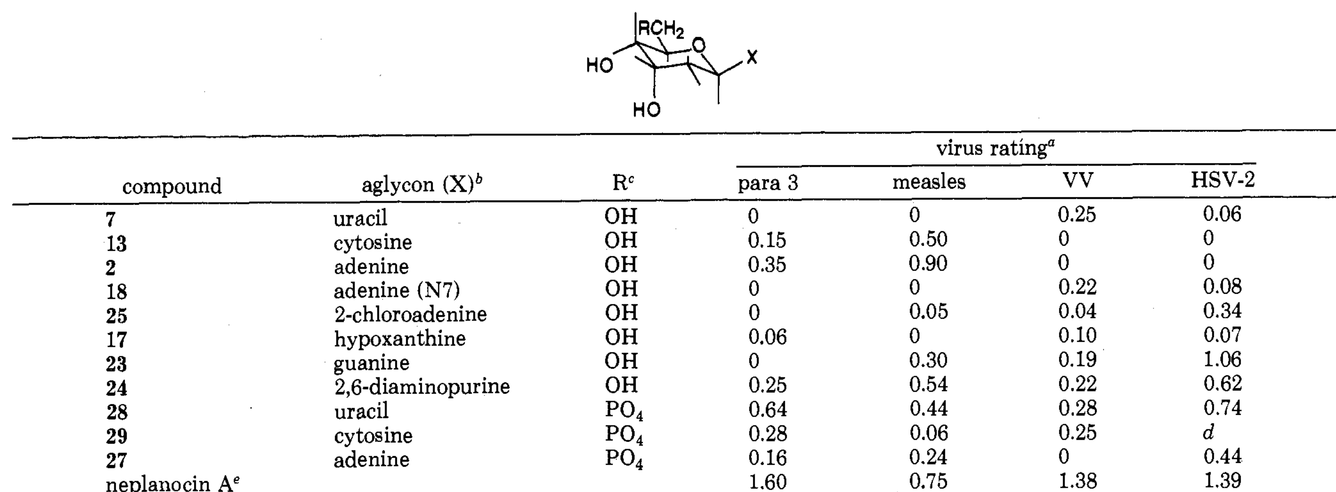
Table IV. Comparative in Vitro Antiviral Activity of 2-Deoxy-β-D-*ribo*-hexopyranosyl Nucleosides and Nucleotides



compound	aglycon (X)[b]	R[c]	virus rating[a] para 3	measles	VV	HSV-2
7	uracil	OH	0	0	0.25	0.06
13	cytosine	OH	0.15	0.50	0	0
2	adenine	OH	0.35	0.90	0	0
18	adenine (N7)	OH	0	0	0.22	0.08
25	2-chloroadenine	OH	0	0.05	0.04	0.34
17	hypoxanthine	OH	0.06	0	0.10	0.07
23	guanine	OH	0	0.30	0.19	1.06
24	2,6-diaminopurine	OH	0.25	0.54	0.22	0.62
28	uracil	PO_4	0.64	0.44	0.28	0.74
29	cytosine	PO_4	0.28	0.06	0.25	*d*
27	adenine	PO_4	0.16	0.24	0	0.44
neplanocin A[e]			1.60	0.75	1.38	1.39

[a] The virus rating (VR) was determined by comparing CPE development in drug-treated cells (T) and virus control cells (C). The CPE value (0–4) assigned to T for each drug level was subtracted from that of C, and the differences (C − T) were totaled. If partial toxicity was evident at any drug level, the C − T of that level was divided by 2. The sum of all C − T values was then divided by 10 times the number of test wells used per drug level. [b] Aglycon attachment is at N1 of pyrimidines or N9 of purines unless otherwise specified. [c] R = PO_4 designates the nucleoside 6′-monophosphate. [d] Not tested. [e] Positive control.

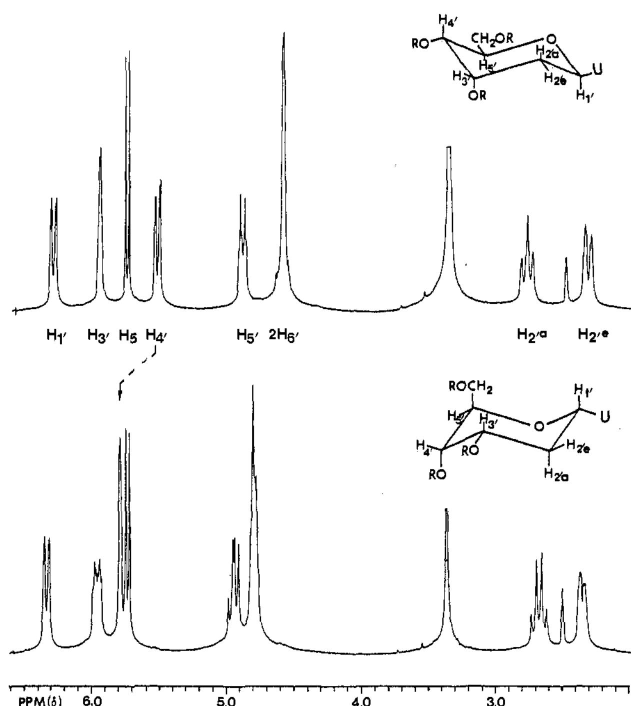


Figure 3. ^{1}H NMR (300 MHz) spectra of the carbohydrate protons of 1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (β-anomer (**6**), top; α-anomer (**9**), bottom).

is axially oriented and is coupled to H-2′a and H-2′e. The resulting signal in each case would be a set of doublets with a large ($J_{1',2'a} \sim 11.0$ Hz) and a small ($J_{1',2'e} \sim 2.0$) coupling component.

Similar ^{1}H NMR chemical shifts and coupling patterns were observed at 90 MHz (figures not shown) for other protected α (**21**) or β (**12**, **15**, **20**) nucleosides and for 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose (**5**), which has not been previously characterized with regard to anomeric configuration.[29]

Biological Evaluations

A. Antiviral Activity. The 2-deoxy-β-D-*ribo*-hexopyranosyl nucleosides and 6′-monophosphate derivatives synthesized during this study were tested (Table IV)

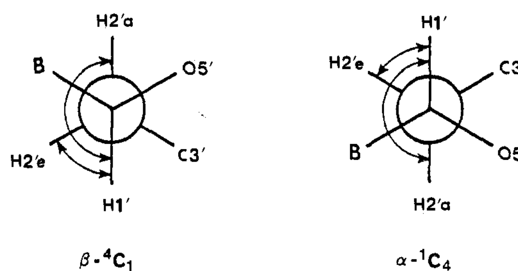


Figure 4. Newman projections viewing from C-1′ to C-2′ of anomeric 2-deoxy-D-*ribo*-hexopyranosyl nucleosides (B = heterocyclic base).

against herpes simplex type 2 (HSV-2), vaccinia (VV), parainfluenza type 3 (para 3), and measles viruses in vitro. The guanosine analogue 2-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purin-6-one (**23**) demonstrated moderate activity against HSV-2. The UMP analogue 1-(2-deoxy-6-*O*-phosophono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione demonstrated moderate activity against HSV-2 and para 3. The other compounds did not exhibit significant in vitro activity.

B. Antitumor Activity. The 2-deoxy-β-D-*ribo*-hexopyranosyl nucleosides and 6′-monophosphate derivatives were also tested against L1210 and P388 cell lines in vitro. Interestingly, the UMP analogue 1-(2-deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**28**) demonstrated activity against both cell lines, with ID_{50} values of 3.9×10^{-5} M (L1210) and 3.3×10^{-5} M (P388). For comparison, tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide)[44] gave values of 1.2×10^{-6} and 1.3×10^{-6}M for L1210 and P388, respectively. All other compounds were inactive.

C. Enzymatic Activity. Adenosine deaminase (ADase EC 3.5.4.4) catalyzes the hydrolysis of C-6 amino group of adenosine or 2-deoxyadenosine to form inosine or 2-deoxyinosine, respectively, and demonstrates broad specificity with regard to carbohydrate-modified substrates.[45-48] However, it has been suggested that ADase

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Table V. Kinetic Parameters of Substrates for Adenosine Deaminase

compound	λ^a	$\Delta\epsilon \times 10^{-3\,b}$	10^5K_m, M	$V_{max}{}^c$
adenosine	265	−8.47	3.1	1.0
2	265	−5.17	5.7	0.06
24	254	2.16	90.0	0.006

[a] Wavelength at which deamination was measured. [b] Maximal change in extinction coefficient (ϵ) at specified wavelength. [c] Relative to adenosine.

will have little bulk tolerance for a carbohydrate larger than the ribofuranose ring.[49] When calf intestine ADase was separately incubated with 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**2**) and 9-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2,6-diaminopurine (**24**), the deaminated products **17** and **23**, respectively, were formed. In comparison with adenosine, **2** was bound by ADase nearly as well, as indicated by the K_m value (Table V), but was deaminated at a much lower rate. The 2,6-diaminopurine compound **24** was deaminated at a drastically reduced rate. 6-Amino-7-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**18**) was also tested for interaction with ADase and found to be a competitive inhibitor (K_i = 0.1 mM). The natural deamination products inosine and 2′-deoxyinosine had K_i values of 0.5 and 0.15 mM, respectively.

The structural requirements that are necessary for efficient activation of cAMP-dependent protein kinase by cyclic nucleotide analogues have been described.[50,51] This kinase requires an unprotected C2′ hydroxyl group on the ribofuranose moiety for efficient activation.[50] The cyclic AMP analogue **30** possesses such a group adjacent to the cyclic phosphate function. The activation of protein kinase (bovine heart) was investigated and the K_a for **30** was found to be 2.6×10^{-4} M. Cyclic AMP tested in parallel had a K_a value of 8×10^{-8} M.

Other enzymes were tested for activity with 2-deoxy-β-D-*ribo*-hexopyranosyl nucleosides or the 6′-monophosphate derivatives. The UMP analogue **28** did not interact with thymidylate synthase (EC 2.1.1.45),[52] an enzyme that is highly specific with regard to the carbohydrate moiety. The inosine (**17**) and guanosine (**23**) analogues were not significant substrates for calf spleen purine nucleoside phosphorylase (PNPase, EC 2.4.2.1).

Experimental Section

General Procedures. Melting points were determined on a Fischer-Johns melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were determined with a JEOL FX-90Q (89.6 MHz) or, where indicated, with an IBM NR300AF (300.1 MHz) spectrometer. The presence of H_2O or NH_3 as indicated by elemental analysis was confirmed by ^{1}H NMR. Proton (^{1}H) and carbon (^{13}C) chemical shifts are in δ (ppm) units downfield from tetramethylsilane (Me_4Si). ^{13}C spectra were obtained with dioxane (67.4 ppm) as reference. Phosphorus (^{31}P) chemical shift values are referenced to 85% H_3PO_4 (external reference). First-order spin coupling constants, J, are expressed in hertz. Infrared spectra (IR) were obtained on a Beckman Acculab 2 spectrophotometer and ultraviolet spectra (UV) were recorded on a Cary Model 118 spectrophotometer. Optical rotations were obtained with a Perkin-Elmer 241 polarimeter (589 nm). Reverse-phase high-pressure liquid chromatography (HPLC) was performed with a Beckman 332 HPLC system on an Ultrasphere-ODS (4.6 × 250 mm) column with ammonium phosphate buffer (50 mM, pH 6.0) containing 3.5% CH_3CN as the mobile phase. Elemental analyses were performed by Robertson Laboratory, Florham Park, NJ, or Galbraith Laboratories, Inc., Knoxville, TN. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 (EM Reagents) plates. Detection of components on TLC was by UV light and with H_2SO_4 (10%) in MeOH spray followed by heating. Silica gel (60–200 mesh) was used for column chromatography. All solvents were reagent grade. Evaporations were carried out under reduced pressure with a rotary evaporator.

1-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (9) and 1,5-Anhydro-2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hex-1-enitol. 2,4-Bis(trimethylsilyl)uracil[28] (1.68 g, 6.36 mmol) was added to 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl bromide[29] (**8**; 2.86 g, 4.2 mmol) in dry acetonitrile. After 2 h, the solvent was evaporated and the residual syrup was dissolved in 1,2-dichloroethane (150 mL) and washed with $NaHCO_3$ solution (5%, 50 mL). The organic phase was washed with water (2 × 50 mL) and then dried ($CaCl_2$). Two products were separated by column chromatography (silica gel, elution with CH_2Cl_2). Appropriate fractions were evaporated to dryness. Crystallization of the residue from $CHCl_3$ and collection by filtration gave 0.67 g (27%) of 1,5-anhydro-2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hex-1-enitol: mp 125–126 °C; $[\alpha]^{24}_D$ +439.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.9–8.6 (m, 12, 3 OpN*Bz*), 6.75 (d, 1, $J_{1,2}$ = 5.87 Hz, C_1H), 5.94 (dd, C_3H), 5.72 (dd, C_4H), 5.20 (dd, 1, $J_{1,2}$ = 5.87 Hz, $J_{2,3}$ = 5.9 Hz, C_2H), and other sugar protons. Anal. ($C_{27}H_{19}N_3O_{13}$) C, H, N.

The protected nucleoside **9** was eluted with CH_2Cl_2–acetone (9:1, v/v) and was collected as a white powder after evaporation of solvent to give 1.70 g (57%). An analytical sample was crystallized from CH_2Cl_2–ether: mp 148–155 °C; $[\alpha]^{24}_D$ −63.4° (*c* 1.0, CH_2Cl_2); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 11.48 (s, 1, N_3H), 7.97–8.4 (m, 12, 3 OpN*Bz*), 7.92 (d, 1, C_6H, J = 8.10 Hz), 6.34 (dd, 1, $C_{1'}H$), 5.96 (m, 1, $C_{3'}H$), 5.80 (s, 1, $C_{4'}H$), 5.47 (d, 1, C_5H, J = 8.10 Hz), 4.95 (br s, 2, $C_{6'}H$), 2.68 (m, 1, $C_{2'a}H$), 2.35 (m, 1, $C_{2'e}H$). Anal. ($C_{31}H_{23}N_5O_{15}$) C, H, N.

1-(2-Deoxy-α-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (10). 1-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**9**; 1.7 g, 2.41 mmol) was deblocked by stirring for 12 h in methanolic $NaOCH_3$ (300 mL, 0.1 N). The solution was neutralized with Dowex 50-X8 (H^+ form), filtered, and evaporated. The nucleoside was dissolved in water and filtered, and methyl 4-nitrobenzoate was extracted with $CHCl_3$. Compound **10** was adsorbed onto silica gel and loaded on a silica gel column (2.5 × 25 cm, packed in EtOAc). Elution with EtOH–EtOAc (1:1), evaporation of pure fractions, and crystallization from EtOH gave 485 mg of **10** (78%): mp 188–190 °C; $[\alpha]^{24}_D$ 25.8° (*c* 0.5, water); IR (KBr) ν 1670 (>C=O) cm^{-1}; UV λ_{max} (pH 1) 259 nm (ϵ 10 500); UV λ_{max} (pH 7) 259 nm (ϵ 10 500); UV λ_{max} (pH 11) 258.5 nm (ϵ 8200); ^{1}H NMR (Me_2SO-d_6) δ 11.30 (s, 1 N_3H, exchanged with D_2O), 7.76 (d, 1, J = 8.12 Hz, C_6H), 5.81 (m, 1, $C_{1'}H$), 5.65 (d, 1, J = 8.12 Hz, C_5H), 4.6–5.0 (m, 3, 3 O*H*, exchanged with D_2O), 1.55–2.18 (m, 2, $C_{2'a,e}H$), and other sugar protons. Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

1-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (6). To a suspension of 2-deoxy-1,3,4,6-tetrakis-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose[29] (**5**; 8.6 g, 11.3 mmol) in dry acetonitrile (350 mL) was added 2,4-bis-*O*-(trimethylsilyl)uracil (3.0 mL, 13.56 mmol) with protection from moisture. At reflux temperature, trimethylsilyl trifluoromethanesulfonate[30] (TMS-triflate, 0.55 mL, 3.0 mmol) was added and the reaction mixture stirred for 2 h. The reaction mixture was cooled to room temperature and poured into cold sodium bicarbonate solution (5%, 200 mL) and stirred for 5 min, and the aqueous solution was then diluted with water to 1000 mL. The precipitate was collected by filtration and washed with water (2 × 100 mL). Anomerically pure **6** (5.9 g, 75%) was obtained by crystallization of the crude product from nitromethane: mp 326 °C (lit.[31] mp 329 °C); $[\alpha]^{24}_D$ +79.3° (*c* 0.30, CH_3NO_2); ^{1}H NMR (300 MHz, Me_2SO-d_6) δ 11.48 (s, 1, N_3H), 7.97–8.4 (m, 12, 3 OpN*Bz*), 7.92 (d, 2, C_6H, J = 8.10 Hz), 6.29 (dd, 1, $C_{1'}H$), 4.89

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(52) Maley, F.; Nord, L. D.; Robins, R. K., unpublished results.

(m, 1, $C_{5'}H$), 4.59 (br s, 2, $C_{6'}H$), 2.79 (t, 1, $C_{2'e}H$), 2.34 (d, 1, $C_{2'e}H$). Anal. ($C_{31}H_{23}N_5O_{15}$) C, H, N.

1-(2-Deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (7). Treatment of 6 (10.2 g, 14.5 mmol) with freshly prepared methanolic $NaOCH_3$ solution (300 mL, 0.2 N) for 2 h provided 7. The solution was neutralized with Dowex 50-X8 (H^+ form), filtered, and evaporated to dryness. The residue was dissolved in water (300 mL) and methyl 4-nitrobenzoate was extracted with $CHCl_3$. The crude material was adsorbed onto silica gel (20 g, 60–200 mesh). Column chromatography on silica gel (eluted with EtOAc–EtOH, 9:1) provided a white powder, which was crystallized from EtOH–EtOAc (9:1) to give 7 (2.85 g, 76%) as white crystals: mp 145 °C (lit.[31] mp 105–110 °C); $[\alpha]^{24}_D$ +30.0° (*c* 0.50, water); IR (KBr) ν 1660–1720 (>C=O) cm^{-1}; UV λ_{max} (pH 1) 259 nm (ϵ 10600); UV λ_{max} (pH 7) 259 nm (ϵ 10600); UV λ_{max} (pH 11) 258.5 nm (ϵ 8400); ^1H NMR (Me_2SO-d_6) δ 11.34 (s, 1, N_3H, exchanged with D_2O), 7.72 (d, 1, C_6H, J = 8.12 Hz), 5.91 (m, 1, $C_{1'}H$), 5.62 (d, 1, C_5H, J = 8.12 Hz), 4.91 (d, 1, $C_{3'}OH$, J = 2.93, exchanged with D_2O), 4.74 (d, 1, $C_{4'}OH$, exchanged with D_2O), 4.50 (t, 1, $C_{6'}OH$, exchanged with D_2O), 5.54 (m, 1, $C_{4'}H$), and other sugar protons; ^{13}C NMR (D_2O) δ 166.79 (C_4), 152.07 (C_2), 142.95 (C_6), 103.12 (C_5), 78.83 ($C_{1'}$), 76.74 ($C_{5'}$), 67.85 ($C_{4'}$ or $C_{3'}$), 67.00 ($C_{3'}$ or $C_{4'}$), 61.97 ($C_{6'}$), 36.27 ($C_{2'}$). Anal. ($C_{10}H_{14}N_2O_6$) C, H, N.

Crystals suitable for X-ray analysis were grown from 95% EtOH–EtOAc (9:1); mp 108–112 °C. Anal. ($C_{10}H_{14}N_2O_6 \cdot 2H_2O$) C, H, N.

4-Acetamido-1-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2-pyrimidinone (12). *N*-Acetylcytosine[34] (11; 3.3 g, 21.6 mmol) was treated with hexamethyldisilazane (HMDS, 15 mL) and ammonium sulfate (10 mg) for 8 h at 135 °C with exclusion of moisture. Excess HMDS was removed by distillation to provide bis-*O*-(trimethylsilyl)-*N*-acetylcytosine as a white solid, which was dissolved in dry acetonitrile (25 mL) and added to a suspension of 2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose (5; 8.22 g, 10.8 mmol) in acetonitrile (300 mL). TMS-triflate (2.88 mL) was then added. After stirring at 25 °C for 10 days, the reaction mixture was poured into cold aqueous sodium bicarbonate solution (100 mL, 5%) and stirred for 5 min before diluting to 1000 mL with cold distilled water. The white precipitate was collected by filtration and washed with cold water (2 × 200 mL). After thorough drying the crude blocked nucleoside was loaded on a silica gel column (3.0 × 30 cm) and the desired compound was eluted with EtOAc–1,2-dichloroethane (1:1, v/v). Compound 12 was collected after evaporation of the appropriate fractions (6.6 g, 82%) and used directly in the subsequent step. An analytical sample was crystallized from CH_3NO_2–ether: mp 273–274 °C; $[\alpha]^{24}_D$ +69.8° (*c* 0.5, CH_3NO_2); ^1H NMR (Me_2SO-d_6) δ 10.95 (s, 1, N_3H), 8.1–8.5 (m, 12, 3 OpN*Bz*), 8.01 (d, 1, J = 7.46 Hz, C_6H), 7.27 (d, 1, J = 7.46 Hz, C_5H), 6.40 (m, 1, $C_{1'}H$), 2.08 (s, 3 O*Ac*), and other sugar protons. Anal. ($C_{33}H_{26}N_6O_{15}$) C, H, N.

4-Amino-1-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2-pyrimidinone (13). The protected nucleoside 12 (7.6 g, 10 mmol) was stirred in saturated methanolic ammonia (250 mL) at 25 °C for 8 h. After evaporation of the solvent, the residue was dissolved in water and 4-nitrobenzamide was extracted with ethyl ether. The resulting aqueous phase was evaporated to dryness and 13 was obtained by silica gel chromatography (2.5 × 16 cm, eluted with EtOAc–EtOH, 1:1, v/v). After crystallization from 90% aqueous EtOH/ethyl ether, white needles of 13 were collected by filtration (2.4 g, 90%): mp 239–240 °C (lit.[31] mp 239–240 °C); $[\alpha]^{24}_D$ +40.2° (*c* 1.0, water); IR (KBr) ν 1640 (>C=O) cm^{-1}; UV λ_{max} (pH 1) 276 nm (ϵ 13600); UV λ_{max} (pH 7) 266 nm (ϵ 8500); UV λ_{max} (pH 11) 266 nm (ϵ 8000); ^1H NMR (Me_2SO-d_6) δ 7.55 (d, 1, J = 7.21 Hz, C_6H), 7.14 (s, 2, NH_2, exchanged with D_2O), 5.99 (m, 1, $C_{1'}H$), 5.71 (d, 1, J = 7.21 Hz, C_5H), 4.86 (d, 1, J = 2.94 Hz, $C_{3'}OH$, exchanged with D_2O), 4.69 (d, 1, J = 6.54 Hz, $C_{4'}OH$, exchanged with D_2O), 4.43 (t, 1, $C_{6'}OH$, exchanged with D_2O), 3.97 (m, 1, $C_{3'}H$), 1.80 (m, 2, $C_{2'a,e}H$), and other sugar protons. Anal. ($C_{10}H_{15}N_3O_5 \cdot 0.5H_2O$) C, H, N.

6-Chloro-9-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)purine (15) and 6-Chloro-7-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)purine (16). (Trimethylsilyl)-6-chloropurine was prepared by heating a solution of chloropurine (14; 2.07 g, 13.4 mmol) and hexamethyldisilazane (15 mL) at reflux with ammonium sulfate (10 mg) as catalyst. Excess HMDS was removed by distillation in vacuo. Crystalline (trimethylsilyl)-6-chloropurine was dissolved in dry acetonitrile (20 mL) and added to a suspension of 5 (8.6 g, 11.3 mmol) and acetonitrile (300 mL). The reaction mixture was protected from moisture, and the reaction was initiated by the addition of TMS-triflate (3.0 mL). After the mixture was stirred for 12 h at 25 °C, the solvent was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 (300 mL). After washing of the organic phase with aqueous $NaHCO_3$ solution (5%, 150 mL) and water (2 × 100 mL), it was dried ($CaCl_2$) and silica gel (45 g) was added before evaporating to dryness. The compound adsorbed on silica gel was layered on a column (2.7 × 40 cm) of silica gel and eluted with 1,2-dichloroethane–acetone (19:1). Appropriate fractions were collected and evaporated to yield a white powder. Crystallization from CH_2Cl_2–$CHCl_3$ and collection by filtration gave 5.2 g (62%) of 15 as white crystals; mp 263 °C. Recrystallization from $CHCl_2$–ether yielded 4.87 g of pure N-9 isomer 15 (58%): mp 271–272 °C; $[\alpha]^{24}_D$ +58.4° (*c* 0.45, CH_2Cl_2); ^1H NMR (Me_2SO-d_6) δ 9.16 (s, 1, C_8H), 8.87 (s, 1, C_2H), 8.0–8.5 (m, 12, 3 OpN*Bz*), 6.69 (m, 1, $C_{1'}H$), 6.06 (m, 1, $C_{3'}H$), 5.55 (dd, 1, $C_{4'}H$), 4.97 (m, 1, $C_{5'}H$), 4.65 (s, 2, 2$C_{6'}H$), and other sugar protons. Anal. ($C_{32}H_{22}N_7O_{13}Cl$) C, H, N, Cl.

The N-7 isomer 16 was isolated from the same column as above by eluting with 1,2-dichloroethane–acetone (8:2). The residue obtained by evaporation of the collected fractions was crystallized from $CHCl_3$–CH_3NO_2 (1:1, v/v) to yield 2.52 g (30%) of 16: mp 235 °C; $[\alpha]^{24}_D$ +122° (*c* 0.5, CH_3NO_2); ^1H NMR (Me_2SO-d_6) δ 9.04 (s, 1, C_8H), 8.87 (s, 1, C_2H), 8.0–8.44 (m, 12, 3 OpN*Bz*), 6.59 (m, 1, $C_{1'}H$), 6.06 (m, 1, $C_{3'}H$), 5.59 (dd, 1, $C_{4'}H$), 5.01 (m, 1, $C_{5'}H$), 4.64 (s, 2, 2$C_{6'}H$), and other sugar protons. Anal. ($C_{32}H_{22}N_7O_{13}Cl$) C, H, N, Cl.

6-Amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (2). Method 1. 6-Chloro-9-(2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)purine (15; 6.42 g, 8.58 mmol) was cooled (−40 °C) in a stainless steel high-pressure vessel. Liquid ammonia (50 mL) was added, and the vessel was sealed and heated for 12 h at 70 °C. After the bomb was cooled in liquid N_2, the top was removed, cold MeOH (−40 °C, 40 mL) was added, and the vessel and contents were allowed to warm to room temperature. The methanol solution was transferred to a round-bottom flask and evaporated to dryness. The residue was dissolved in water, and the aqueous solution was filtered and washed with ethyl ether (8 × 200 mL) to remove 4-nitrobenzamide. The water was then evaporated under reduced pressure, and the residue was crystallized from water and filtered to yield 2.41 g (87%) of 2 as white crystals: mp 232 °C; $[\alpha]^{24}_D$ +7.2° (*c* 0.50, water), +22.3° (*c* 0.30, MeOH); IR (KBr) ν 3200–3420 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 256 nm (ϵ 14500); UV λ_{max} (pH 7) 259 nm (ϵ 14500); UV λ_{max} (pH 11) 259 nm (ϵ 15100); ^1H NMR (Me_2SO-d_6) δ 8.38 (s, 1, C_8H), 8.17 (s, 1, C_2H), 7.28 (s, 2, NH_2, exchanged with D_2O), 6.06 (m, 1, $C_{1'}H$), 5.02 (d, 1, J = 2.93 Hz, $C_{3'}OH$, exchanged with D_2O), 4.81 (d, 1, J = 6.54 Hz, $C_{4'}OH$, exchanged with D_2O), 4.52 (t, 1, $C_{6'}OH$, exchanged with D_2O), 4.09 (m, 1, $C_{3'}H$), and other sugar protons. Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

Method 2. 6-Amino-2-chloro-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (25; 0.5 g, 1.58 mmol) and Pd/C (40 mg, 10%) were suspended in a KOH solution (0.1 N, 150 mL) in a hydrogenation bottle. The reaction mixture was shaken under H_2 (40 psi) for 8 h. The reaction mixture was filtered through a pad of Celite, the filtrate was neutralized with HCl, and the volume was reduced to 10 mL by evaporation in vacuo. The concentrated solution was applied to a silica gel column (2.5 × 25 cm) and 2 was eluted with EtOAc–EtOH (1:1, v/v). Appropriate fractions were collected and evaporated to dryness. Crystallization from water and collection by filtration provided 0.24 g (55%) of 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine, mp 232 °C. This compound was identical in all respects with the compound obtained by the previous method.

6-Amino-7-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (18). The blocked compound 16 from above (2.5 g) was treated in the same manner as described for the N-9 isomer. After crystallization from water, 0.70 g (90%) of 18 was collected as the hemihydrate: mp 257–258 °C; $[\alpha]^{24}_D$ +7.0° (*c* 0.10, water); IR (KBr) ν 1640 cm^{-1}; UV λ_{max} (pH 1) 218, 272 nm (ϵ 11900, 13200); UV λ_{max} (pH 7) 211, 270 nm (ϵ 18300, 9100); UV λ_{max} (pH 11) 268 nm (ϵ 10500); ^1H NMR (Me_2SO-d_6) δ 8.50 (s, 1, C_8H), 8.23 (s, 1, C_2H), 7.00 (s,

2, NH_2, exchanged with D_2O), 6.06 (m, 1, $C_{1'}H$), 5.13 (d, 1, J = 3.39 Hz, $C_{3'}OH$, exchanged with D_2O), 4.99 (t, 1, J = 4.96 Hz, $C_{6'}OH$, exchanged with D_2O), 4.94 (d, 1, J = 6.54, $C_{4'}OH$, exchanged with D_2O), and other sugar protons. Anal. ($C_{11}H_{15}N_5O_4 \cdot 0.50H_2O$) C, H, N.

9-(2-Deoxy-β-D-*ribo*-hexopyranosyl)purin-6-one (17). A suspension of 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**2**; 0.10 g, 0.36 mmol) in acetic acid (1.5 mL, 2.0 N) was stirred at 0 °C. Sodium nitrite ($NaNO_2$, 0.17 g) was added in portions, and the loosely stoppered flask and contents were allowed to rise to room temperature. After stirring for 48 h, the reaction mixture was twice diluted with water (15 mL) and evaporated (2 × 15 mL) to dryness in vacuo. After crystallization from water, 70 mg (68%) of white needles (**17**) was collected by filtration: mp 184–185 °C; $[\alpha]^{24}_D$ +12.7° (*c* 0.30, water); IR (KBr) ν 1650–1700 (>C=O) cm^{-1}; UV λ_{max} (pH 1) 248.5 nm (ε 11 900); UV λ_{max} (pH 7) 248 nm (ε 11 600); UV λ_{max} (pH 11) 252 nm (ε 12 900); ^1H NMR (Me_2SO-d_6) δ 8.32 (s, 1, C_8H), 8.06 (s, 1, C_2H), 6.02 (m, 1, $C_{1'}H$), 4.10 (m, 1, $C_{3'}H$), and other sugar protons. Anal. ($C_{11}H_{14}N_4O_5 \cdot 0.5H_2O$) C, H, N.

9-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,6-dichloropurine (20) and 9-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-α-D-*ribo*-hexopyranosyl)-2,6-dichloropurine (21). Trimethylsilyl-2,6-dichloropurine was prepared by heating a suspension of 2,6-dichloropurine (**19**; 2.52 g, 13.3 mmol) and ammonium sulfate (10 mg) in hexamethyldisilazane (HMDS, 15 mL). After 2 h at 135 °C, excess HMDS was removed by distillation in vacuo. The crystalline silylated heterocycle was dissolved in acetonitrile (25 mL) and transferred to a flask containing a suspension of 2-deoxy-1,3,4,6-tetrakis-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranose (**5**; 8.6 g, 11.3 mmol) in acetonitrile (300 mL). The reaction flask was protected from moisture, and TMS-triflate (3.0 mL, 16.5 mmol) was added with stirring. After 16 h the solvent was removed under reduced pressure. The resulting syrup was dissolved in CH_2Cl_2 (300 mL) and this solution was washed with aqueous $NaHCO_3$ solution (5%, 100 mL) and water (2 × 100 mL) and dried ($CaCl_2$). After adsorption onto silica gel, both anomers were eluted from a silica gel column (4.0 × 45 cm, packed in 1,2-dichloroethane; eluted with 1,2-dichloroethane–acetone, 19:1). Appropriate fractions were collected and evaporated to dryness. Compound **20** was purified by two cycles of crystallization from CH_2Cl_2–ether to yield 5.68 g (66%): mp 248–249 °C; $[\alpha]^{24}_D$ +36.2° (*c* 0.50, CH_2Cl_2); ^1H NMR (Me_2SO-d_6) δ 9.05 (s, 1, C_8H), 8.45–7.95 (m, 12, 3 Op*NBz*), 6.51 (m, 1, $C_{1'}H$), 6.04 (m, 1, $C_{3'}H$), 5.54 (dd, 1, $C_{4'}H$), 5.00 (d, 1, $C_{5'}H$), 4.62 (s, 2, 2 $C_{6'}H$), and other sugar protons. Anal. ($C_{32}H_{21}N_7O_{13}Cl_2$) C, H, N, Cl.

The filtrates from the above crystallizations were combined and evaporated to dryness. Crystallization twice from CH_3CN provided the pure α-anomer **21** (70 mg, 8%): mp 215–216 °C; $[\alpha^{24}_D$ −25.1° (*c* 1.0, CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 8.42 (s, 1, C_8H), 8.35–7.70 (m, 12, 3 Op*NBz*), 6.42 (m, 1, $C_{1'}H$), 5.57–6.00 (m, 2, $C_{3'}H$,$C_{4'}H$), 4.55–5.15 (m, 3, $C_{5'}H$,2$C_{6'}H$), 4.04 (m, 1, $C_{2'a}H$), and 2.67 (m, 1, $C_{2'e}$). Anal. ($C_{32}H_{21}N_7O_{13}Cl_2$), C, H, N, Cl.

2-Chloro-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purin-6-one (22). 9-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,6-dichloropurine (**20**; 5.0 g, 6.39 mmol) was dissolved in boiling dioxane (30 mL) and added dropwise with stirring to a boiling solution of dioxane in 1 N NaOH (70 mL, 1:1, v/v). After 30 min the reaction was allowed to cool to room temperature, water (250 mL) was added, and the pH was adjusted to pH 4.0 with Dowex 50-X8 (H^+ form). The resin and precipitate were removed by filtration and washed with water (50 mL). The pH of the filtrate was again adjusted to 4.0 with resin and filtered. The filtrate was then washed with ether (7 × 200 mL), and the volume was reduced in vacuo to 30 mL. To this concentrated solution was added MeOH (100 mL) and silica gel (10 g), and the solvent was removed in vacuo. Chromatography on a silica gel column (3.2 × 32 cm) packed in EtOAc and eluted with EtOAc–EtOH (1:1) provided 1.34 g (67%) of **22** as a tan powder; mp >200 °C, gradually decomposing. An analytical sample crystallized from 95% isopropyl alcohol, which analyzed for 0.5 mol of water and 1 mol of isopropyl alcohol; mp >175 °C dec. Anal. ($C_{11}H_{13}N_4O_5Cl \cdot 0.5H_2O \cdot C_3H_9O$) C, H, N, Cl.

9-(2-Deoxy-β-D-*ribo*-hexopyranosyl)-2,6-diaminopurine (24). 9-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,6-dichloropurine (**20**; 3.0 g, 3.8 mmol) was treated with $MeOH/NH_3$ (50 mL) at 145 °C in a stainless steel bomb for 72 h. The resulting solution was evaporated to dryness. The remaining solid was resuspended in water and washed with ether (8 × 200 mL) and the aqueous solution was evaporated to dryness. Compound **24** was purified by separation on a silica gel column (2.5 × 30 cm) with EtOAc–EtOH–H_2O (12:6:1) as the eluting solvent. The pure fractions were collected and evaporated to dryness. Crystallization (H_2O) provided **24** (0.33 g, 27%) as tan crystals: mp >222 °C dec; $[\alpha]^{24}_D$ −9.0° (*c* 0.30, water); IR (KBr) ν 3200–3340 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 251, 290 nm (ε 10 600, 8900); UV λ_{max} (pH 7) 254, 278 nm (ε 9400, 9900); UV λ_{max} (pH 11) 254, 278 nm (ε 9400, 9900); ^1H NMR (Me_2SO-d_6) δ 7.90 (s, 1, C_8H), 6.64 (s, 2, NH_2, exchanged with D_2O), 5.92 (m, 1, $C_{1'}H$), 5.84 (s, 2, NH_2, exchanged with D_2O), and other sugar protons. Anal. ($C_{11}H_{16}N_6O_4 \cdot 1.5H_2O$) C, H, N.

2-Amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purin-6-one (23). Crude 2-Chloro-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purin-6-one (**22**; 1.6 g, 5 mmol) was treated with $MeOH/NH_3$ (50 mL) at 145 °C for 72 h in a stainless steel bomb. The resulting solution was evaporated to dryness, dissolved in water (10 mL), and applied to a charcoal column (2.0 × 4.0 cm). The column was washed with 10% aqueous acetonitrile and **23** was eluted with 30% aqueous acetonitrile. Pure fractions were dried, and the residue was crystallized (H_2O) to provide white crystals (0.56 g, 37%): mp 290 °C, darkens; $[\alpha]^{24}_D$ −14.5° (*c* 0.20, water); IR (KBr) ν 3300–3400 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 255 nm (ε 11 900); UV λ_{max} (pH 7) 252 nm (ε 12 600); UV λ_{max} (pH 11) 255 nm (ε 11 700); ^1H NMR (Me_2SO-d_6) δ 7.91 (s, 1, C_8H), 6.64 (s, 2, NH_2, exchanged with D_2O), 5.86 (m, 1, $C_{1'}H$), 4.92 (d, 1, J = 2.6 Hz, $C_{3'}OH$, exchanged with D_2O), 4.78 (d, 1, J = 6.3 Hz, $C_{4'}OH$, exchanged with D_2O), 4.52 (t, 1, J = 5.50 Hz, $C_{6'}OH$, exchanged with D_2O), 4.03 (m, 1, $C_{3'}H$), and other sugar protons. Anal. ($C_{11}H_{15}N_5O_5 \cdot 0.5H_2O$) C, H, N.

6-Amino-2-chloro-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (25). 9-(2-Deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-β-D-*ribo*-hexopyranosyl)-2,6-dichloropurine (**20**; 5.86 g, 7.48 mmol) was treated with $MeOH/NH_3$ (60 mL) in a stainless steel pressure vessel at 70 °C for 4 h. The resulting solution was separated from crystals of **25** by decantation. The solution was evaporated to dryness and the residue was dissolved in water (50 mL). The collected crystals were also dissolved in the aqueous solution, and the solution was washed with ethyl ether (8 × 200 mL) and evaporated to dryness. The residue was crystallized from boiling water to yield 1.89 g (85%) of **25**: mp >300 °C; $[\alpha]^{24}_D$ −5.3° (*c* 0.30, water); IR (KBr) ν 3200–3460 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 261 nm (ε 11 100); UV λ_{max} (pH 7) 260 nm (ε 12 200); UV λ_{max} (pH 11) 260 nm (ε 12 200); ^1H NMR (Me_2SO-d_6) δ 8.40 (s, 1 C_8H), 7.80 (s, 2, NH_2, exchanged with D_2O), 5.95 (m, 1, $C_{1'}H$), 5.02 (d, 1, J = 2.6 Hz, $C_{3'}OH$, exchanged with D_2O), 4.78 (d, 1, J = 6.6 Hz, $C_{4'}OH$, exchanged with D_2O), 4.50 (t, 1, J = 5.5 Hz, $C_{4'}OH$, exchanged with D_2O), 4.05 (m, 1, $C_{6'}H$), and other sugar protons. Anal. ($C_{11}H_{14}N_5O_4Cl$) C, H, N, Cl.

6-Amino-2-chloro-9-(2-deoxy-α-D-*ribo*-hexopyranosyl)purine (26). For ^1H NMR comparison, **26** was prepared by the same method as described for **25**, except that the α-blocked nucleoside **21** was used as the starting material. After extracting residual 4-nitrobenzamide with ether, the aqueous solution was evaporated to dryness. A sample obtained by crystallization from EtOH was used for NMR analysis: ^1H NMR (Me_2SO-d_6) δ 8.66 (s, 1, C_8H), 7.84 (s, 2, NH_2, exchanged with D_2O), 6.06 (m, 1, $C_{1'}H$), 4.6–5.3 (m, 3, $C_{3',4',6'}OH$, exchanged with D2O), 4.06 (m, 1, $C_{3'}H$), and other sugar protons.

6-Amino-9-(2-deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)purine, Ammonium Form (27). A suspension of 6-amino-9-(2-deoxy-β-D-*ribo*-hexopyranosyl)purine (**2**, 2 mmol) in trimethyl phosphate (5 mL) was maintained at −4 °C. Phosphorus oxychloride (0.4 mL, 4.4 mmol) was added and after 1 h the reaction was terminated by pouring the reaction mixture into cold water (<5 °C, 50 mL). The aqueous solution was allowed to stand at room temperature for several hours. The solution was then washed with $CHCl_3$ (3 × 20 mL) and neutralized with ammonium bicarbonate. The solution was evaporated to dryness in vacuo, redissolved in water (10 mL), and applied to a column of DEAE-Sephadex (HCO_3^- form, 3.2 × 35 cm). After elution of the unreacted nucleoside with distilled water (1000 mL), the

nucleoside 6′-monophosphate **27** was eluted with a linear gradient of ammonium bicarbonate (0–0.6 M). Both the mixing vessel and the reservoir contained 1000 mL. A side product from the reaction that eluted prior to the 6′-monophosphate was detected by analyzing the fractions by thin-layer chromatography (TLC) with CH_3CN–0.1 M NH_4Cl (7:3, v/v) as the developing solvent. Fractions containing **27** were collected and evaporated to dryness in vacuo with the bath temperature <30 °C. Residual ammonium bicarbonate was removed by repeated (4×) evaporations in vacuo and finally by lyophilization to provide 0.63 g (80%) of **27**: ^{1}NMR (Me_2SO-d_6) δ 8.38 (s, 1, C_8H), 8.15 (s, 1, C_2H), 7.31 (s, 2, NH_2, exchanged with D_2O), 6.02 (m, 1, $C_{1'}H$), 2.61 (t, 1, $C_{2'a}H$), 1.98 (d, 1, $C_{2'e}H$), and other sugar protons; ^{31}P NMR (D_2O, pH 11.0) δ 6.56 (s). Anal. ($C_{11}H_{16}N_5O_7P{\cdot}H_2O{\cdot}NH_3$) C, H, N, P.

4-Amino-1-(2-deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2-pyrimidinone, Ammonium Form (29). 4-Amino-1-(2-deoxy-β-D-*ribo*-hexopyranosyl)-2-pyrimidinone (**13**) was treated in the same manner as described for the synthesis the AMP analogue **27**, but the reaction was terminated after 2.5 h and the overall yield of **29** was 83%: ^{1}H NMR (Me_2SO-d_6) δ 7.58 (d, 1, C_6H, J = 7.18 Hz), 7.26 (s, 2, NH_2, exchanged with D_2O), 5.99 (m, 1, $C_{1'}H$), 5.84 (d, 1, C_5H, J = 7.18 Hz), and other sugar protons; ^{31}P NMR (D_2O, pH 11.0) δ 6.73 (s). Anal. ($C_{10}H_{16}N_3O_8P{\cdot}0.5H_2O{\cdot}NH_3$) C, H, N, P.

1-(2-Deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione, Ammonium Form (28). 1-(2-Deoxy-β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**7**) was treated in the same manner as described for the synthesis of the AMP analogue **27**, but the reaction was terminated after 2.5 h. The total yield amounted to 85%: ^{1}H NMR (Me_2SO-d_6) δ 7.67 (d, 1, C_6H, J = 8.10 Hz), 5.90 (m, 1, $C_{1'}H$), 5.58 (d, 1, C_5H, J = 8.10 Hz), and other sugar protons; ^{13}C NMR (H_2O) δ 166.79 (C4), 152.07 (C_2), 142.85 (C_6), 103.15 (C_5), 78.83 ($C_{1'}$), 75.75 ($C_{5'}$, $^3J(C_{6'}C_{5'}OP)$ = 6.4 Hz), 67.85 ($C_{4'}$ or $C_{3'}$), 66.55 ($C_{3'}$ or $C_{4'}$), 65.19 ($C_{6'}$, $^2J(C_{6'}OP)$ = 5.10 Hz), 36.27 ($C_{2'}$); ^{31}P NMR (D_2O, pH 11.0) δ 6.66 (s). Anal. ($C_{10}H_{15}N_2O_9P{\cdot}0.5H_2O{\cdot}NH_3$) C, H, N, P.

9-(2-Deoxy-4,6-*O*-(hydroxyphosphinyl)-β-D-*ribo*-hexopyranosyl)-6-aminopurine, Ammonium Form (30). 6-Amino-9-(2-deoxy-6-*O*-phosphono-β-D-*ribo*-hexopyranosyl)purine (**27**, ammonium form, monohydrate; 200 mg, 0.5 mmol) and 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidine (150 mg, 0.5 mmol) were combined in a reaction vessel. Pyridine was added and evaporated several times to leave a white solid. This solid was dissolved in pyridine (35 mL) and added dropwise into a refluxing solution of dicyclohexylcarbodiimide (500 mg, 2.5 mmol) in pyridine (100 mL). After 2 h the solution was allowed to cool to room temperature and water (7 mL) was slowly added. After the mixture was allowed to stand for 12 h, the solvent was evaporated to dryness in vacuo and the residue was dissolved in water (30 mL). The aqueous solution was filtered and washed with ether (2 × 50 mL). The solution was again evaporated to dryness and the residue was dissolved in water (30 mL) and purified on a column (3.2 × 30 cm) of DEAE-Sephadex as described for the synthesis of **27** to yield 120 mg (65%) of **30**: ^{1}H NMR (Me_2SO-d_6) δ 8.41 (s, 1, C_8H), 8.17 (s, 1, C_2H), 7.47 (s, 2, NH_2, exchanged with D_2O), 6.10 (d, 1, $C_{1'}H$), 2.77 (t, 1, $C_{2'a}H$), 2.04 (d, 1, $C_{2'e}H$), and other sugar protons; ^{31}P NMR (D_2O, pH 11.0) δ 0.71 (s). Anal. ($C_{11}H_{14}N_5O_6P{\cdot}NH_3{\cdot}0.5H_2O$) C, H, N, P.

Single-Crystal X-ray Analysis. Suitable crystals of the anomer pair 1-(2-deoxy-α- and -β-D-*ribo*-hexopyranosyl)-2,4-pyrimidinedione (**10** and **7**, respectively) were mounted on a Nicolet R3 automated diffractometer which utilized graphite monochromated Mo K_α radiation (0.71073 Å). The lattice parameters and orientation matrices for the two crystals were obtained by using a least-squares procedure involving 25 centered reflections for each sample. Structures were solved by using direct methods and the structures were refined by using a blocked-cascading least-squares procedure. All non-hydrogen atoms were refined anisotropically. Hydroxyl protons, with the exception of the β-anomer $C_{3'}OH$, were located in difference maps and their positional and isotropic thermal parameters were refined. The $C_{3'}OH$ of the β-anomer was located in a difference map and then allowed to ride on $C_{3'}O$, otherwise it would refine to an unreasonable location. The O–H bond lengths for water molecules were refined to a value of approximately 0.96 Å. Positions of hydrogen atoms bonded to carbon and nitrogen atoms were calculated on the basis of stereochemical factors and these hydrogen atoms were allowed to ride on their neighboring heavy atoms with C–H and N–H bond lengths fixed at 0.96 Å during refinement. Isotropic thermal parameters of these atoms were set at a value about 20% greater then the U_{eq} of the neighboring atoms and were not refined. All data for which $F \geq 3\sigma(F)$ were considered observed. Weights were based on counting statistics. An empirical extinction correction was applied to both sets of data. Scattering factors for the atoms were obtained from ref 53. All programs used in solution, refining and drawing molecular models of these structures are included in the SHELXTL program package.[54]

Antiviral Evaluation. The compounds were evaluated for their ability to inhibit virus induced cytopathic effect (CPE) produced by four viruses in African Green monkey kidney cells (American Type Culture Collection, Rockville, MD). Viruses used in this evaluation included parainfluenza virus type 3 (para 3) strain C243, measles strain Edmonston, vaccinia virus strain Elstree, and herpes simplex virus type 2 (HSV-2) strain 333. Vero cells were maintained in antibiotic free Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY).

For antiviral experiments, cells were inoculated into 96-well tissue culture plates (Corning Glassworks, Corning, NY) at a concentration of 4×10^4 cells/0.2 mL per well and cultures brought to confluency over 24 h at 37 °C in 5% CO_2 atmosphere. Following incubation, growth medium was removed, and monolayers were inoculated (0.1 mL/well) with a predetermined amount of virus that produces complete destruction of the cell monolayer in 72 h. After 30 min of adsorption of the virus at 37 °C, test compounds were added (0.1 mL/well) in seven 0.5 log concentrations ranging from 10^{-5} to 10^{-2} M, resulting in final well concentrations of 5×10^{-6} to 5×10^{-3} M. At each concentration, duplicate wells were used for evaluation of antiviral activity and single uninfected wells for cytotoxicity estimation.

The degree of virus-induced CPE and compound cytotoxicity was observed microscopically after 72 h of incubation at 37 °C in humidified 5% CO_2 atmosphere. CPE was scored numerically from 0 (normal, uninfected control cells) to 4 (100% cell destruction as in virus infected controls), and a virus rating (VR) was calculated as previously reported.[55] The higher the VR, the better the antiviral activity of the compound, with values greater than 1.0 being considered indicative of significant activity.

Antitumor Evaluation. Compounds were evaluated for their ability to inhibit growth of murine L1210 and P388 leukemia (American Type Culture Collection, Rockville, MD) maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY) and 20 mM HEPES buffer. For growth experiments, cells were adjusted to 10^5 cells/mL and distributed into 24-well tissue culture plates (0.5 mL/well). Test compounds were dissolved in growth medium, sterilized by passage through a 0.22-μm membrane filter, serially diluted, and added to wells (0.5 mL/well). Compounds were tested in duplicate at log concentrations ranging from 10^{-7} to 10^{-4} M. Following 48 h of incubation at 37 °C in CO_2 atmosphere, cell numbers were determined with a Coulter Model ZF cell counter. Cell growth in the presence of test compound was expressed as a percentage of growth in untreated control tubes and the concentration of compound producing 50% inhibition of cell growth was determined (ID_{50}).

Enzyme Studies. Kinetic parameters for calf intestine adenosine deaminase (Sigma Chemical Co.) substrates and inhibitors were determined spectrophotometrically as previously described.[56] Cyclic AMP dependent protein kinase (bovine heart, Sigma) assays were performed as described by Roskoski.[57]

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Substrates activity with purine nucleoside phosphorylase (calf spleen, Sigma) was tested as described by Stoeckler and coworkers[58] using analytical HPLC.

Registry No. **2**, 107796-00-9; **5**, 107796-01-0; **6**, 6129-68-6; **7**, 6067-31-8; **8**, 88293-57-6; **9**, 107712-09-4; **10**, 107796-02-1; **11**, 14631-20-0; **12**, 107712-10-7; **13**, 6027-65-2; **14**, 87-42-3; **15**, 107712-11-8; **16**, 107712-12-9; **17**, 107712-13-0; **18**, 107712-14-1; **19**, 5451-40-1; **20**, 107712-15-2; **21**, 107712-16-3; **22**, 107712-17-4; **23**, 107712-18-5; **24**, 107712-19-6; **25**, 107712-20-9; **26**, 107712-21-0; **27**, 107712-22-1; **28**, 107712-23-2; **29**, 107712-24-3; **30**, 107712-25-4; 2,4-bis(trimethylsilyl)uracil, 10457-14-4; 1,5-anhydro-2-deoxy-3,4,6-tris-*O*-(4-nitrobenzoyl)-D-*ribo*-hex-l-enitol, 107796-03-2; bis-*O*-(trimethylsilyl)-*N*-acetylcytosine, 107712-26-5; adenosine deaminase, 9026-93-1.

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Synthesis and Oral Antifungal Activity of Novel Azolylpropanolones and Related Compounds

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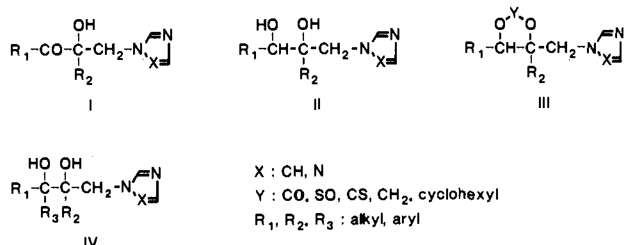
To find orally active antifungal agents, novel imidazolyl- and 1,2,4-triazolylpropanolones I and related compounds II–IV were synthesized. Compounds I were derived from ketones V (method A), α-diketone IX (method B), α-hydroxy ketones X (method C), α-chloro ketone XII (method D), and enones VI (method E). Diols II, synthesized from I with $NaBH_4$, were cyclized to five-membered cyclic compounds III by using *N,N'*-carbonyldiimidazole, thionyl chloride, *N,N'*-(thiocarbonyl)diimidazole, bromochloromethane, 2,2-dimethoxypropane, and cyclohexanone dimethyl ketal. Diols IV were synthesized from I by Grignard reaction (method F), hydroxymethylation of X (method G), and reaction of ketones XXI with 1-[(trimethylsilyl)methyl]-1,2,4-triazole (method H). Compounds I–IV were examined for their antifungal activities in vitro by evaluation of broth dilution MIC values against three species of fungi and the inhibitory effect on pseudomycelium of *Candida albicans*, and they were examined for oral efficacy in vivo against subacute systemic candidiasis in mice and superficial dermatophytosis in guinea pigs. Compounds **2**, **12**, **38**, **39**, and **92** exhibited strong oral antifungal activity. An asymmetric synthesis and the structure–activity relationships of the compounds examined are discussed.

With the advent of ketoconazole,[1] the synthesis of orally active and broad-spectrum antimycotic azoles has been explored actively in recent years.[2] Nevertheless, there is still a need for more potent and better antimycotic drugs. In recent years, (*R,S*)-1-(2,4-dichlorophenyl)-1-(4-fluorophenyl)-2-(1,2,4-triazol-1-yl)ethanol, ICI 153066,[3] was reported to show oral antifungal activity. Therefore our interest was directed to the synthesis of azolylpropanolones and related compounds with the partial structure of ICI 153066. Here we report the synthesis and antifungal properties of new orally active antifungal agents I–IV.[4,5]



Chemistry

The synthetic routes (A–E) to the target compounds I are illustrated in Scheme I (Table I). The starting ketones V reacted with *N,N,N',N'*-tetramethyldiaminomethane in acetic anhydride to give the conjugated ketones VI,[6] which were oxidized to the epoxy ketones VII with hydrogen peroxide in aqueous NaOH. Compounds VII were then treated with 1,2,4-triazole in the presence of NaH in DMF to obtain a mixture of the desired compounds I and the isomeric 1,2,4-triazol-4-yl derivatives VIII as minor byproducts (method A). The triazole isomers I and VIII were separable by chromatography. The structure assignment was made by NMR chemical shift of the triazole ring protons.

Reaction of the α-diketones IX with diazomethane gave the oxiranes VII,[7] which were then treated with 1,2,4-triazole in the presence of NaH in DMF to give a mixture of I and VIII (method B).

Hydroxymethylation of the α-hydroxy ketones X with paraformaldehyde in the presence of $KHCO_3$ afforded the primary alcohols, which were treated with *p*-TsCl to give the tosylate XI. Compound XI was transformed with triethylamine to obtain I and VIII (method C).

Treatment of V with SO_2Cl_2 gave the chloro compounds XII, which were then treated with paraformaldehyde and

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