yl)-6,7-dichloro-2,3-dihydrobenzofuran-2-carboxylic acid, 90246-67-6; dichloromethyl methyl ether, 4885-02-3; diethyl bromomalonate, 685-87-0; diethyl 7-chloro-2,3-dihydro-6-methoxybenzofuran-2,2-dicarboxylate, 90246-73-4; 2,3-dihydro-6-methoxy-7-methylbenzofuran-2-carboxylic acid, 90246-75-6; ethyl 2,3-dihydro-5-(*o*-fluorobenzoyl)-6-methoxy-7-methylbenzofuran-2-carboxylate, 90246-80-3; 2-thiophenecarbonyl chloride, 5271-67-0; methyl 7-chloro-2,3-dihydro-6-methoxybenzofuran-2-carboxylate, 90246-85-8; propionyl chloride, 79-03-8; isovaleroyl chloride, 108-12-3; ethyl (*E*)-7-chloro-2,3-dihydro-5-(*o*-fluorobenzoyl)-6-hydroxybenzofuran-2-carboxylate *O*-acetyl oxime, 90246-99-4; 2-(acetoxymethyl)-6,7-dichloro-2,3-dihydrobenzofuran, 90247-20-4; 2-(acetoxymethyl)-6,7-dichloro-2,3-dihydro-5-(*o*-fluorobenzoyl)-benzofuran, 90247-21-5; D-(−)-α-phenylglycinol, 56613-80-0.

Synthesis and Antiviral/Antitumor Activities of Certain Pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone Nucleosides and Related Compounds

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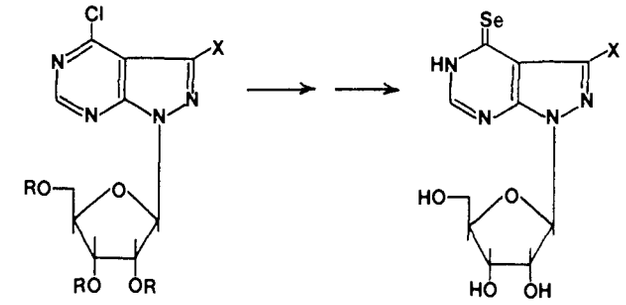
Several pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone ribonucleosides were prepared as potential antiparasitic agents. Treatment of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**5a**) with selenourea and subsequent deacetylation gave 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone (**6a**). A similar treatment of 3-bromo-4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**5b**) with selenourea, followed by debenzoylation, gave the 3-bromo derivative of **6a** (**6b**). Glycosylation of persilylated 4-chloro-6-methylpyrazolo[3,4-*d*]pyrimidine (**7**) with tetra-*O*-acetylribofuranose (**8**) provided the key intermediate 4-chloro-6-methyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**9**). Ammonolysis of **9** gave 4-amino-6-methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**10**), whereas treatment with sodium hydroxide gave 6-methylallopurinol ribonucleoside (**11a**). Reaction of **9** with either thiourea or selenourea, followed by deacetylation, provided 6-methylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione ribonucleoside (**11c**) and the corresponding seleno derivative (**11d**), respectively. The structural assignment of these nucleosides was made on the basis of spectral studies. These compounds were tested in vitro against certain viruses and tumor cells. All the compounds except **11c** exhibited significant activity against HSV-2 in vitro, whereas **11c** exhibited the most potent activity against measles and has a very low toxicity. Compounds **6a**, **6b**, and **11d** were found to be potent inhibitors of growth of L1210 and P388 leukemia in vitro.

3-β-D-Ribofuranosylpyrazol[4,3-d]pyrimidine-7-(6*H*)-thione[1] (thioformycin B, **2**) has shown significant activity (ED_{50} = 3.6 μM) against *Leishmania tropica* in human monocyte-derived macrophages in vitro[2] and is less toxic than formycin B (**1**). Recently Santi and co-workers[3] have found that 3-β-D-ribofuranosylpyrazolo[4,3-*d*]pyrimidine-7(6*H*)-selone[4] (selenoformycin B, **3**) is more active than thioformycin B but less active than formycin B against *L. tropica* promastigotes in vitro with an ED_{50} of 0.2 μM. Both allopurinol (pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one) and its ribonucleoside (**4**) exhibited significant activity against several species of *Leishmania*[5,6] and *Trypanosoma*.[7] Allopurinol ribonucleoside (**4**) is a structural analogue of formycin B and, as in the case of formycin B, the glycosidic linkage in 4 appears to be remarkably stable toward enzymatic hydrolysis, especially in various parasitic systems.[8,9] In view of these observations, it was decided to synthesize hitherto unreported sulfur and selenium derivatives of certain pyrazolo[3,4-*d*]pyrimidine nucleosides containing the exocyclic thiono and seleno function at position 4.

Scheme I



5a, X = H; R = Ac

b, X = Br; R = Bz

6a, X = H

b, X = Br

Chemistry. The selenopurine nucleosides are generally synthesized from the corresponding halonucleosides with either sodium hydrogen selenide[10–13] or selenourea.[4] Re-

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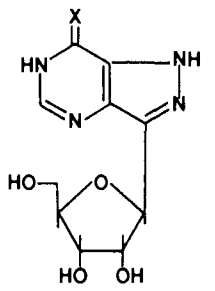
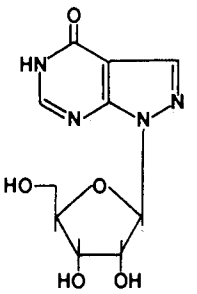


1, X = O

2, X = S

3, X = Se

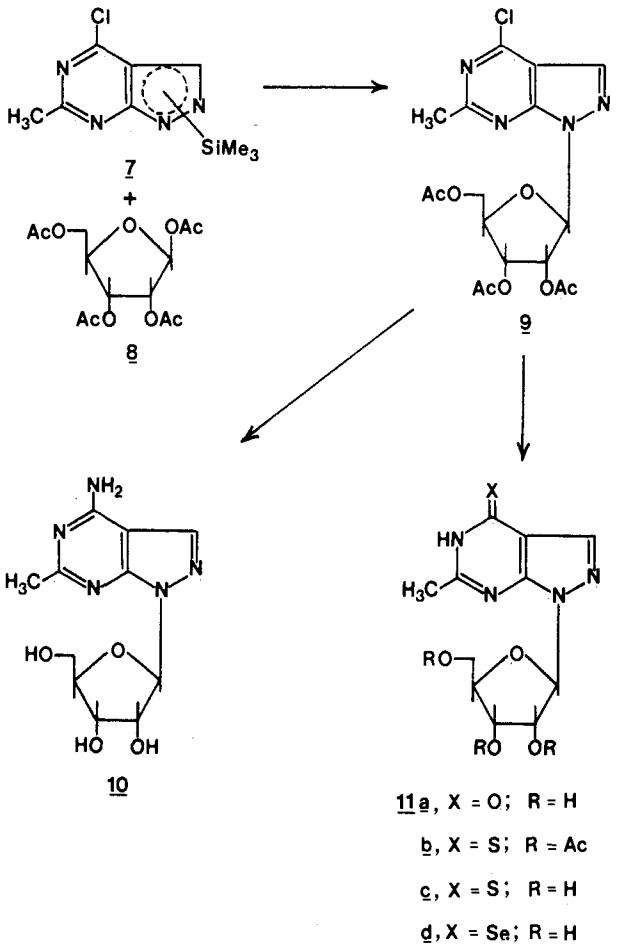


4

cently, the synthesis of several 6-selenopurine nucleosides, nucleotides, and cyclic nucleotides has been reported by Shiue and Chu[14] employing the 6-aminopurine glycosides and H_2Se in aqueous pyridine in a sealed tube. For our purposes, the halonucleoside-selenourea procedure proved to be satisfactory. Treatment of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**5a**)[1] with selenourea in ethanol at 0–5 °C and subsequent deacetylation with methanolic sodium methoxide gave 1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone (**6a**). The UV spectrum of **6a** exhibited a large bathochromic shift, as compared to allopurinol ribonucleoside (see Experimental Section), which is due[10] to the presence of the aromatic NHC=Se. A similar treatment of 3-bromo-4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine (**5b**)[15] with selenourea readily gave 3-bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone (**6b**). As in the case of **6a**, a large bathochromic shift (100 nm in pH 1 and 69 nm in pH 11) in the UV spectrum of **6b** as compared to 3-bromoallopurinol ribonucleoside[15] was observed.

Efforts were then initiated to synthesize 6-methylallopurinol ribonucleoside (**11a**), since the purine analogue 2-methylinosine[16] exhibited very significant antimalarial and antitrypanosomal activity (ED_{50} = 0.21 μM) in vitro.[17] For the synthesis of **11a** and other related compounds, 4-chloro-6-methylpyrazolo[3,4-*d*]pyrimidine[18] was found to be a viable starting material. Glycosylation of the trimethylsilyl derivative (**7**) of 4-chloro-6-methylpyrazolo[3,4-*d*]pyrimidine with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**) in the presence of the catalyst trimethylsilyl trifluoromethanesulfonate (TMS-triflate)[19] gave 4-chloro-6-methyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (**9**) as the only major nucleoside product in 69% yield after purification by silica gel column chromatography. Treatment of **9** with methanolic ammonia at 120 °C for 16 h gave a good yield of 4-amino-6-methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (**10**). When compound **9** was treated with 1 N sodium hydroxide in aqueous dioxane, 6-methylallopurinol ribonucleoside (**11a**) was obtained.

Scheme II



7 + 8 → 9 → 10

11a, X = O; R = H

b, X = S; R = Ac

c, X = S; R = H

d, X = Se; R = H

For the synthesis of 6-methyl-4-thio- or -4-selenopyrazolo[3,4-*d*]pyrimidine ribonucleosides (**11c** and **11d**), compound **9** was found to be a suitable starting material. Reaction of **9** with thiourea in ethanol at room temperature gave 6-methyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (**11b**). Subsequent deacetylation of **11b** with methanolic sodium methoxide afforded 6-methylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione ribonucleoside (**11c**) in good yield. A similar treatment of **9** with selenourea, followed by deacetylation, furnished 6-methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4-(5*H*)-selone (**11d**), which revealed a large bathochromic shift (99 nm in pH 1 and 97 nm in pH 7) in the UV spectrum as compared to that of **11a**.

Determination of the site of glycosylation was accomplished by UV spectroscopy and was based on the preliminary work done by Lichtenthaler and Cuny.[20] It has been observed[20,21] that when the pyrazolo[3,4-*d*]pyrimidine ring has been alkylated or glycosylated at the N-1 site, the resulting UV spectrum resembles very closely the spectrum of the unsubstituted heterocycle. However, when the heterocycle is substituted at N-2, the resulting spectrum shows a distinct bathochromic shift in the wavelength of maximum absorption with respect to the unsubstituted heterocycle. The essentially identical UV absorption

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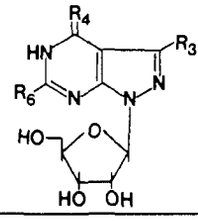
Table I. In Vitro Antiviral Activity of 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone and Certain Related Compounds



compd	R_3	R_4	R_6	ED_{50},[a] M				toxic level, M
				Para 3	measles	VV	HSV-2	
6a	H	Se	H	$>5 \times 10^{-3}$	1.2×10^{-6}	9.6×10^{-6}	5×10^{-6}	1.6×10^{-4}
6b	Br	Se	H	$>5 \times 10^{-3}$	1.3×10^{-6}	5×10^{-6}	9×10^{-6}	5×10^{-5}
10	H	NH	CH_3	$>5 \times 10^{-3}$	1.2×10^{-5}	5×10^{-3}	3.5×10^{-5}	none
11a	H	O	CH_3	$>5 \times 10^{-3}$	1.2×10^{-5}	1.6×10^{-3}	5×10^{-5}	5×10^{-3}
11c	H	S	CH_3	$>5 \times 10^{-3}$	1.4×10^{-9}	1.2×10^{-3}	3.2×10^{-4}	5×10^{-3}
11d	H	Se	CH_3	1.3×10^{-5}	1.3×10^{-6}	1.6×10^{-5}	5×10^{-6}	5×10^{-4}
12	H	S	H	$>5 \times 10^{-3}$	1.2×10^{-5}	2.9×10^{-4}	1.6×10^{-4}	none

[a] The concentration of compound that resulted in a 50% reduction of plaque formation, as compared with nondrug controls.

spectra of 6-methylallopurinol[18] and **11a** indicated the site of glycosylation in **11a**, and hence **9**, to be N_1. The anomeric configuration was assumed to be β in view of the small coupling constant of the anomeric proton[22] in 6-methylallopurinol ribonucleoside (**11a**).

Biological Evaluations

A. Antiviral Activity. The sulfur and selenium derivatives of pyrazolo[3,4-*d*]pyrimidine nucleosides synthesized during this study were tested against herpes simplex type 2 (HSV-2, 233), vaccinia (VV), parainfluenza type 3 (Para 3), and measles viruses in vitro (Table I). Of all the compounds, **11c** exhibited the most potent activity against measles and has a very low toxicity, whereas compounds **10** and **12** are moderately active with no apparent cytotoxicity at the 50% plaque reduction level. Compounds **6a**, **6b**, and **11d** are also significantly active against measles in vitro, but they are slightly toxic. Although compounds **6a**, **6b**, **10**, **11a**, and **11d** all showed marked activity against HSV-2 in vitro, **10** has no toxicity. 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (**12**), used as the reference compound, showed potent activity against measles and VV, whereas **6a** and **6b** exhibited marked activity against VV in vitro but were accompanied by toxicity. All other compounds were inactive against the viruses used. As a result of these in vitro antiviral studies, compound **11c** appears to be the most potent agent against measles; however, further studies are needed to confirm its antiviral potency in vivo.

B. Antitumor Activity. The pyrazolo[3,4-*d*]pyrimidine nucleosides synthesized during this study were also tested against L1210 and P388 leukemia in vitro (Table II). Compounds **6a**, **6b**, and **11d** were all found to be potent inhibitors of the growth of these cell lines. The reference compound **12** and other compounds under study were found to be inactive in vitro.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded on either a Varian EM-390 or on a JEOL FX-90 Q spectrometer. The chemical-shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard. The presence of H_2O or other solvents as indicated by elemental analyses was verified by NMR. Infrared spectra (IR) were obtained on a Beckman

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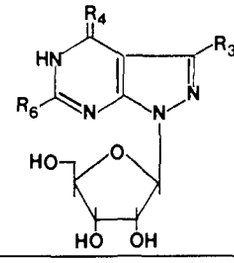
Table II. In Vitro Antitumor Activity of 1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone and Certain Related Compounds



compd	R_3	R_4	R_6	ID_{50},[a] M	
				L1210	P388
6a	H	Se	H	2.5×10^{-6}	2.7×10^{-6}
6b	Br	Se	H	2.7×10^{-6}	2.3×10^{-6}
10	H	NH	CH_3	*b*	*b*
11a	H	O	CH_3	*b*	*b*
11c	H	S	CH_3	*b*	*b*
11d	H	Se	CH_3	3.1×10^{-6}	3.1×10^{-6}
12	H	S	S	*b*	*b*

[a] Inhibitory dose 50 (ID_{50}) is the concentration of the compound in the culture media that produced 50% inhibition of the tumor cell growth as compared to the untreated controls. [b] Inactive at 10^{-4} M.

Acculab 2 spectrophotometer and are expressed in reciprocal centimeters. Ultraviolet spectra (UV; sh = shoulder) were recorded on a Cary Model 15 spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Inc. Knoxville, TN, and Robertson Labs, Florham Park, NJ. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Thin-layer chromatography (TLC) was run on silica gel 60 F-254 (EM Reagents) plates. J. T. Baker silica gel (70–230 mesh) was used for column chromatography. Detection of components on TLC was by UV light and with 10% H_2SO_4 in MeOH spray followed by heating. Evaporations were carried out under reduced pressure with the bath temperature below 30 °C.

1-β-D-Ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone (6a). A mixture of 4-chloro-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine[1] (**5a**; 2.3 g, 7.37 mmol) and selenourea (1.5 g, 12.0 mmol) in absolute ethanol (25 mL) was stirred at 0–5 °C under anhydrous conditions for 16 h. The reaction mixture was filtered to remove the precipitated metal, which was washed with ethanol (25 mL). Evaporation of the combined filtrate and washings gave an oil, which was dissolved in methanol (50 mL). The pH of the solution was adjusted to 9.0 with $NaOCH_3$ and stirred for 4 h. Some more metal had precipitated, which was removed by filtration, and the filtrate was acidified (pH 4) with Dowex-50 H^+ resin. The resin was removed by filtration and washed with methanol until the washings were colorless. Evaporation of the combined filtrates

gave a yellow solid, which was crystallized from water to yield 1.80 g (66%); mp 231–232 °C; IR (KBr) ν 1590 (C=Se), 3240, 3420 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 347 nm (ϵ 21 200); UV λ_{max} (pH 7) 233 nm (sh) (ϵ 15 200), 341 (16 600); UV λ_{max} (pH 11) 233 nm (sh) (ϵ 15 200), 335 (15 700); NMR (Me_2SO-d_6) δ 6.15 (d, 1, J = 4.8 Hz, $C_{1'}$ H), 8.35 (s, 1, C_6 H), 8.39 (s, 1, C_3 H), 14.30 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{10}H_{12}N_4O_4Se$) C, H, N, Se.

3-Bromo-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone (6b). A mixture of 3-bromo-4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine[15] (**5b**; 5.0 g, 7.38 mmol) and selenourea (1.5 g, 12.0 mmol) in absolute ethanol (75 mL) was stirred at room temperature for 16 h with the exclusion of moisture. The reddish brown solid that separated was collected by filtration and washed with cold ethanol (10 mL). This material was debenzoylated immediately due to its instability. A solution of the above material in methanol (50 mL) was adjusted to pH 9 with $NaOCH_3$, stirred for 15 h at room temperature, and worked up as described for **6a**. Crystallization from aqueous ethanol gave an analytical sample of **6b**, 2.5 g (83.3%); mp 220–221 °C dec; IR (KBr) ν 710 (CBr), 1590 (C=Se), 3340–3520 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 243 nm (sh) (ϵ 2700), 352 (10 200); UV λ_{max} (pH 7) 234 nm (sh) (ϵ 5700), 347 (10 300); UV λ_{max} (pH 11) 232 nm (sh) (ϵ 9000), 345 (13 500); NMR (Me_2SO-d_6) δ 6.05 (d, 1, J = 4.7 Hz, $C_{1'}$ H), 8.29 (s, 1, C_6 H), 14.30 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{10}H_{11}BrN_4O_4Se$) C, H, Br, N, Se.

4-Chloro-6-methyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrazolo[3,4-*d*]pyrimidine (9). Dry 4-chloro-6-methylpyrazolo[3,4-*d*]pyrimidine[18] (5.0 g, 30 mmol) was silylated with hexamethyldisilazane (HMDS, 30 mL) and a catalytic amount of $(NH_4)_2SO_4$. The mixture was refluxed for a total of 4 h to ensure complete silylation. The excess HMDS was removed and the oily residue was placed under high vacuum for 1 h to obtain the trimethylsilyl derivative **7**. To a solution of **7** in dry CH_3CN (200 mL) was added 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**8**; 10.4 g, 33.0 mmol), followed by the TMS-triflate catalyst (7.33 g, 6.0 mL, 33.0 mmol). The clear, amber solution was stirred at ambient temperature with the exclusion of moisture for 16 h. The solvent was evaporated to dryness, and the residual syrup was dissolved in EtOAc (250 mL) and poured into a saturated aqueous $NaHCO_3$ solution (200 mL) with stirring. The organic layer was separated and washed with 5% aqueous $NaHCO_3$ solution (2 × 50 mL), followed by water (3 × 75 mL). The dried (Na_2SO_4) organic phase was evaporated and the residual syrup (16.0 g) was purified by flash chromatography on silica gel with toluene:EtOAc (3:1, v/v) as the solvent. The title compound was isolated as a pale yellow syrup, 8.8 g (69%); IR (neat) ν 790 (CCl), 1760 (C=O of OAc) cm^{-1}; UV λ_{max} (MeOH) 260 nm (ϵ 6200); NMR (Me_2SO-d_6) δ 2.25 (3 s, 9, 3 $COCH_3$), 2.60 (s, 3, C_6 CH_3), 6.28 (d, 1, J = 3.05 Hz, $C_{1'}$ H), 8.18 (s, 1, C_3 H), and other sugar protons. A suitable elemental analysis for **9** could not be obtained due to its shelf life of approximately 48 h.

4-Amino-6-methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine (10). Compound **9** (4.4 g, 10.3 mmol) and methanolic ammonia (saturated at 0 °C, 100 mL) were placed in a steel bomb and heated at 120 °C for 16 h. The contents were evaporated to a small volume (20 mL), whereupon crystallization occurred to yield 2.0 g (69%). A small amount was recrystallized from MeOH for analytical purposes; mp 236–238 °C; IR (KBr) ν 3240–3420 (NH_2, OH) cm^{-1}; UV λ_{max} (pH 1) 217 nm (ϵ 22 100), 256 (8700); UV λ_{max} (pH 7 and 11) 265 nm (ϵ 9300); NMR (Me_2SO-d_6) δ 2.40 (s, 3, C_6 CH_3), 3.19 (solvent CH_3OH), 6.05 (d, 1, J = 4.96 Hz, $C_{1'}$ H), 7.96 (s, 2, NH_2), 8.11 (s, 1, C_2 H), and other sugar protons. Anal. ($C_{11}H_{15}N_5O_4 \cdot CH_3OH$) C, H, N.

6-Methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (6-Methylallopurinol Ribonucleoside, 11a). Compound **9** (4.4 g, 10.3 mmol) was dissolved in dioxane (50 mL) and added dropwise with stirring to an aqueous NaOH solution (1 N, 100 mL) at near boiling temperature. The addition was complete after 1 h and heating was continued for an additional hour. After cooling, the solution was neutralized with Dowex-50 H^+ resin to pH 6. The resulting solution was evaporated to dryness and the residue was crystallized from a small amount of 95% EtOH to yield 1.60 g (55%); mp 182–184 °C; IR (KBr) ν 1690 (C=O), 3250–3500 (OH, NH) cm^{-1}; UV λ_{max} (pH 1 and 7) 248 nm (ϵ 9300); UV λ_{max} (pH 11) 264 nm (ϵ 9000); NMR (Me_2SO-d_6) δ 2.38 (s, 3, C_6 CH_3), 6.03 (d, 1, J = 4.74 Hz, $C_{1'}$ H), 8.01 (s, 1, C_3 H), and other sugar protons. Anal. ($C_{11}H_{14}N_4O_5 \cdot {}^1/_4H_2O$) C, H, N.

6-Methyl-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (11b). A mixture of **9** (2.1 g, 50 mmol) and thiourea (1.0 g, 13.0 mmol) in absolute ethanol (35 mL) was stirred at room temperature for 45 min. The solvent was evaporated and the residue was triturated with ethyl acetate (50 mL). The unreacted thiourea that precipitated was removed by filtration and the filtrate was evaporated to a foam to give 2.0 g (95%) of **11b**; mp 120–122 °C; IR (KBr) ν 1575 (C=S), 1750 (C=O of OAc) cm^{-1}; UV λ_{max} (pH 1) 230 nm (sh) (ϵ 5100), 318 (16 600); UV λ_{max} (pH 7) 230 nm (ϵ 6800), 318 (19 300); UV λ_{max} (pH 11) 230 nm (ϵ 11 900), 315 (15 300); NMR (Me_2SO-d_6) δ 2.08 and 2.10 (2 s, 9, 3 $COCH_3$), 2.50 (s, 3, C_6 CH_3), 6.31 (d, 1, J = 3.05 Hz, $C_{1'}$ H), 8.32 (s, 1, C_3 H), 13.64 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{17}H_{20}N_4O_7S$) C, H, N, S.

6-Methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-thione (11c). A solution of **11b** (2.15 g, 5 mmol) in methanol (75 mL) was adjusted to pH 9 with $NaOCH_3$ and stirred at room temperature for 16 h. The mixture was neutralized with Dowex-50 H^+ resin and the resin was removed by filtration. Evaporation of the filtrate gave a solid which was crystallized from water to yield 1.4 g (93%); mp 240–241 °C; IR (KBr) ν 1570 (C=S), 3100–3380 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 232 nm (sh) (ϵ 7600), 318 (23 000); UV λ_{max} (pH 7) 222 nm (sh) (ϵ 8200), 318 (22 700); UV λ_{max} (pH 11) 235 nm (ϵ 14 500), 314 (19 200); NMR (Me_2SO-d_6) δ 2.49 (s, 3, C_6 CH_3), 6.01 (d, 1, J = 4.5 Hz, $C_{1'}$ H), 8.23 (s, 1, C_3 H), 13.52 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{11}H_{14}N_4O_4S$) C, H, N, S.

6-Methyl-1-β-D-ribofuranosylpyrazolo[3,4-*d*]pyrimidine-4(5*H*)-selone (11d). A solution of **9** (4.27 g, 10.0 mmol) and selenourea (2.0 g, 16.0 mmol) in absolute ethanol (100 mL) was stirred at room temperature for 16 h. The precipitated metal was filtered off and washed with ethanol (15 mL). Evaporation of the combined filtrates gave an oil, which was stirred with methanolic sodium methoxide (50 mL, pH 9) for 4 h. The mixture was neutralized with Dowex-50 H^+ resin and filtered and the filtrate evaporated to dryness. The residual yellow solid was crystallized from water to yield 2.4 g (70%); mp 247 °C; IR (KBr) ν 1590 (C=Se), 2950–3370 (NH, OH) cm^{-1}; UV λ_{max} (pH 1) 240 nm (sh) (ϵ 4800), 347 (19 200); UV λ_{max} (pH 7) 240 nm (sh) (ϵ 6200), 347 (17 300); UV λ_{max} (pH 11) 237 nm (ϵ 14 100), 332 (15 400); NMR (Me_2SO-d_6) δ 2.56 (s, 3, C_6 CH_3), 6.03 (d, 1, J = 4.75 Hz, $C_{1'}$ H), 8.16 (s, 1, C_3 H), 14.06 (br s, 1, N_5 H), and other sugar protons. Anal. ($C_{11}H_{14}N_4O_4Se$) C, H, N, Se.

Antiviral Evaluation. The 50% effective dose (ED_{50}) values were determined by the following plaque reduction method. In this method African green monkey kidney (Vero) or human epithelioid cervical carcinoma (HeLa) cells were seeded into 24-well culture plates (Corning) at a concentration of 2 × 10^5 cells per mL per well and grown for 24 h at 37 °C in 5% CO_2. The plates were infected with 100–150 PFU of virus [herpes simplex type 2 (HSV-2, 233), vaccinia (VV), parainfluenza type 3 (Para 3), and measles] in 0.2 mL and incubated for 30 min at 37 °C in 5% CO_2 to allow adsorption of virus. The test compounds were then added to duplicate wells in seven 0.5-log dilutions (0.1 mL/well), and the cells were overlaid with 1% methylcellulose in EMEM with 2% newborn calf serum (1.0 mL/well). The plates were incubated for 2–3 days to allow for plaque formation. Cells were then fixed with 10% Formalin and stained with 1% crystal violet in water. Plaques were counted, and the ED_{50} was extrapolated graphically from the plotted values. The concentration of compound that resulted in a 50% reduction of plaque formation, as compared with nondrug controls, was designated the ED_{50}.

Cellular Cytotoxicity. HeLa and Vero cells were used for normal cytotoxicity studies. These cells were grown overnight on Linbro 96-well plates until near confluency. The medium was then replaced with growth medium containing one of the test compounds at a 100 or 1000 μg/mL concentration. Quadruplet wells were used for each drug concentration, along with an equal number of control (nondrug) wells. Cells were exposed to the drug from 24 to 72 h, after which the cells were harvested by trypsinization, triturated, and serially diluted, and 1/10 dilutions were plated onto Costar 12-well plates. After 3–5 days of incubation in drug-free growth medium, the cells were washed with phos-

phate-buffered saline, fixed with 10% Formalin, and stained with crystal violet. Cell colonies, as representatives of single viable original cells, were counted under a dissecting microscope. Relative plating efficiency, as percent survival, was calculated as the average number of colonies grown from drug-treated cells divided by the average number of control (untreated) cells grown under similar growth conditions.

Antitumor Evaluation. L1210 leukemia and P388 lymphoid neoplasm were maintained in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum. For determination of cell growth inhibition, L1210 and P388 cells were seeded in 13 × 100 tubes at 5 × 10^4 cells/mL (2 mL/tubes). Cells were grown in the presence of the compound of interest, at 4–5 log doses, for 48 h at 37 °C. Cell growth was assessed by cell count, using a Coulter cell counter. Cell growth at each dose level was expressed as a percentage of growth in control tubes and dose resulting in 50% inhibition of growth was determined.

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Resolved 3-(3-Hydroxyphenyl)-*N*-*n*-propylpiperidine and Its Analogues: Central Dopamine Receptor Activity

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Seven enantiomeric pairs of *N*-alkyl analogues of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP, **12**) have been synthesized and evaluated pharmacologically (biochemistry and behavior) in order to examine their ability to interact with central dopamine (DA) receptors, particularly DA autoreceptors. In the *R* series it seems as if all compounds behave as classical DA receptor agonists with affinity and intrinsic activity for both pre- and postsynaptic receptors. The same bifunctional profile seems to be valid for the *S* enantiomers with N-substituents larger or bulkier than *n*-propyl. Likewise, the *S* enantiomers with ethyl or *n*-propyl N-substituents seem to have affinity for both pre- and postsynaptic receptors but intrinsic activity at presynaptic receptors only, thus appearing as antagonists at postsynaptic receptors. In the total series, (*S*)-(−)-3-PPP [(*S*)-**12**] seems to be the most interesting compound both from the theoretical and the therapeutical point of view, possibly attenuating DA function in two different ways by stimulating the presynaptic receptors and blocking the postsynaptic receptors. This compound has been selected for extended pharmacological studies as a potential antipsychotic drug.

The introduction of the modern antipsychotic agents in the 1950's has revolutionized the treatment of schizophrenia and other psychotic conditions. However, these agents are far from ideal. They are not always effective, and, moreover, they may cause serious side effects. Tardive dyskinesia, which can develop during long-term treatment with such drugs, may persist after cessation of treatment, suggesting an irreversible brain lesion within the extrapyramidal system. It is thus obvious that there is a need for more efficacious and less toxic agents in the treatment of schizophrenia. The antipsychotic drugs used today are all centrally acting dopamine (DA) postsynaptic receptor-blocking agents (for a review, see ref 1). In recent years an alternative way of inhibiting central dopaminergic function has attracted considerable interest, i.e., selective stimulation of presynaptic dopaminergic receptors, the so-called DA autoreceptors.[2] Interestingly, low doses of the DA-receptor agonist apomorphine has been found to preferentially activate presynaptic DA receptors,[3] an effect associated with gross behavioral sedation.[2,4] Some clinical studies have reported an antipsychotic action of low doses of apomorphine[5,6] with a concomitant reduction of serum homovanillic acid (HVA) concentrations,[7] which likewise may be explained in terms of preferential activation of dopaminergic autoreceptors. It would appear that the development of selective dopaminergic autoreceptor agonists might offer some promise in the treatment of psychotic conditions.

In two recent papers,[8,9] we have reported on the discovery and properties of the central DA autoreceptor agonist 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (3-PPP, **12**). A structure–activity (SAR) study of analogues of this compound showed that the 3-(3-hydroxyphenyl)piperidine moiety was necessary for high and selective DA-autoreceptor stimulating activity.[9] The optimal N-substituent has been less well defined, although we found that *N*-

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