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Synthesis and Biological Activity of Selected 2-Substituted 6-(β-D-Ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones

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Several 2-substituted 6-(β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones have been prepared by condensation of the appropriate silylated heterocyles with 2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl bromide and the subsequent removal of blocking groups from the carbohydrate moiety with methanolic ammonia. The site of ribosylation was established by comparison of the uv spectra of the nucleosides with that of an appropriate model compound. The anomeric configuration was determined by pmr spectroscopy. These nucleosides have been evaluated for their inhibitory activity against leukemia L1210 and *Escherichia coli* cells *in vitro* and for their effects on leukemia L1210 growth *in vivo*. Among these compounds the 2-methyl, 2-ethyl, and 2-propyl derivatives markedly inhibited the *in vitro* growth of both cell types, the inhibitory concentrations ranging from 5×10^{-7} to 8×10^{-4} *M*. Only the 2-methyl derivative was significantly active against leukemia L1210 *in vivo* with a dose of 200 mg/kg/day × 5, causing a 31% increase in the life span of the tumor-bearing mice.

The characterization[1] of uric acid ribonucleoside isolated[2] from bovine erythrocytes as 3-(D-ribofuranosyl)uric acid has generated interest in the chemical synthesis[3,4] of bicyclic heterocyclic nucleosides with the glycosidic linkage on a nitrogen atom in the pyrimidine ring rather than in the five-membered ring portion of the molecule. 3-(β-D-Ribofuranosyl)adenosine (isoadenosine) was prepared and found[5] to inhibit the growth of various tumor cell lines *in vitro* and *in vivo*, as well as exhibiting some activity against adeno III virus in culture. This interest has been further stimulated by the recent isolation[6] of 7-(D-ribosyl)pyrazolo[3,4-*d*]pyrimidine-4,6-dione (oxoallopurinol ribonucleoside) from the urine of patients treated with allopurinol and the report[7] that oxoallopurinol ribonucleoside, presumably as the corresponding 5′-phosphate derivative, inhibits pyrimidine biosynthesis *de novo*. These findings have prompted us to synthesize a number of 2-substituted 6-(β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones and to study their biological effects.[8,9]

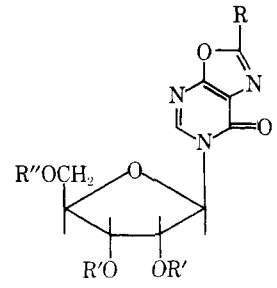
Results and Discussion

Chemistry. The trimethylsilylation of 2-substituted oxazolo[5,4-*d*]pyrimidin-7-ones[10] with hexamethyldisilazane in the presence of a catalytic amount of ammonium sulfate was accomplished in excellent yield. These silyl derivatives (**1a–f**) were subsequently condensed[11] with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide in benzene at reflux temperature in the presence of mercuric oxide–mercuric bromide to furnish good yields of the blocked nucleosides **2a–f** as syrups. Thin-layer chromatography of these syrups revealed the presence of only one nucleoside in each reaction mixture.

Deacetylation of **2a–f** was accomplished with methanolic ammonia to furnish the nucleosides **3a–f** (Table I). That complete deblocking had occurred without ring opening of the labile oxazole ring† was established by uv and pmr spectroscopy. The site of ribosylation was established as N_6 by a comparison of the uv spectral data (Table II) obtained for **3a** and the data reported[12] for 2,6-dimethyloxazolo[5,4-*d*]pyrimidin-7-one. The anomeric configuration of the deblocked nucleosides could not be assigned on the basis of pmr spectral data (Table II) since the peaks assigned to the anomeric proton ($H_{1'}$) of **3b** and **3f** revealed a coupling constant ($J_{1',2'}$) of a magnitude which precluded an unequivocal anomeric assignment.[13] Therefore, the isopropylidene derivatives **4a** and **4b** were prepared from **3b** and **3f** using a standard procedure,[14] and the 5′-*O*-tosyl-2′,3′-*O*-isopropylidene derivative **6** was prepared by tosylation of **4a** (Table I). The pmr spectra of **4a, 4b,** and **6** revealed singlets for their respective anomeric protons, which now allowed an unequivocal assignment of the β configuration to these nucleosides (Table III). This anomeric assignment is further substantiated by utilizing a recently reported[15] criterion for determining anomeric configuration for β-D-ribonucleosides. The difference between the chemical shifts of the two methyl signals for the 2′,3′-*O*-isopropylidene group is between 0.18 and 0.22 ($\Delta\delta$) for β-D-riboncleosides and 0.0 and 0.10 ($\Delta\delta$) for α-D-ribonucelosides. The pmr spectral data in Table III corroborate the β assignment for the nucleosides reported herein.

†The oxazole ring is, however, susceptible to ring opening. This was established by the frequent pmr spectroscopic monitoring of a reaction mixture of the 2-ethyl nucleoside derivative **3b** dissolved in NaOD; at least two additional compounds can be formed.

Table I. Chemical–Physical Data for Some Oxazolo[5,4-*d*]pyrimidin-7-one 6-Ribonucleosides



Compd no.	R	R′	R″	Mp, °C	% yield (purified)	Formula[a]
3a	$-CH_3$	H	H	181.5	42	$C_{11}H_{13}N_3O_6$
3b	$-CH_2CH_3$	H	H	172	55	$C_{12}H_{15}N_3O_6$
3c	$-CH_2CH_2CH_3$	H	H	191–192	32	$C_{13}H_{17}N_3O_6 \cdot H_2O$
3d	$-CH(CH_3)CH_3$	H	H	197–198	16	$C_{13}H_{17}N_3O_6$
3e	$-CH_2CH_2CH_2CH_3$[b]	H	H	164–165	37	$C_{14}H_{19}N_3O_6$
3f	$-C_6H_5$	H	H	258–259	43	$C_{16}H_{15}N_3O_6$
4a	$-CH_2CH_3$	Isopropylidene	H	165–166	41	$C_{15}H_{19}N_3O_6$
4b	$-C_6H_5$	Isopropylidene	H	248	45	$C_{19}H_{19}N_3O_6$
5	$-CH_2CH_3$	$-C(=O)C_6H_5$	$-C(=O)C_6H_5$	110	73	$C_{33}H_{27}N_3O_9 \cdot 0.5H_2O$
6	$-C_2H_5$	Isopropylidene	Tosyl	75–80	25	$C_{22}H_{25}N_3O_8S$

[a] All compounds were analyzed for C, H, and N and the results were within ±0.4% of theoretical values. [b] The base was prepared by the procedure outlined in ref 10; mp 168–169°.

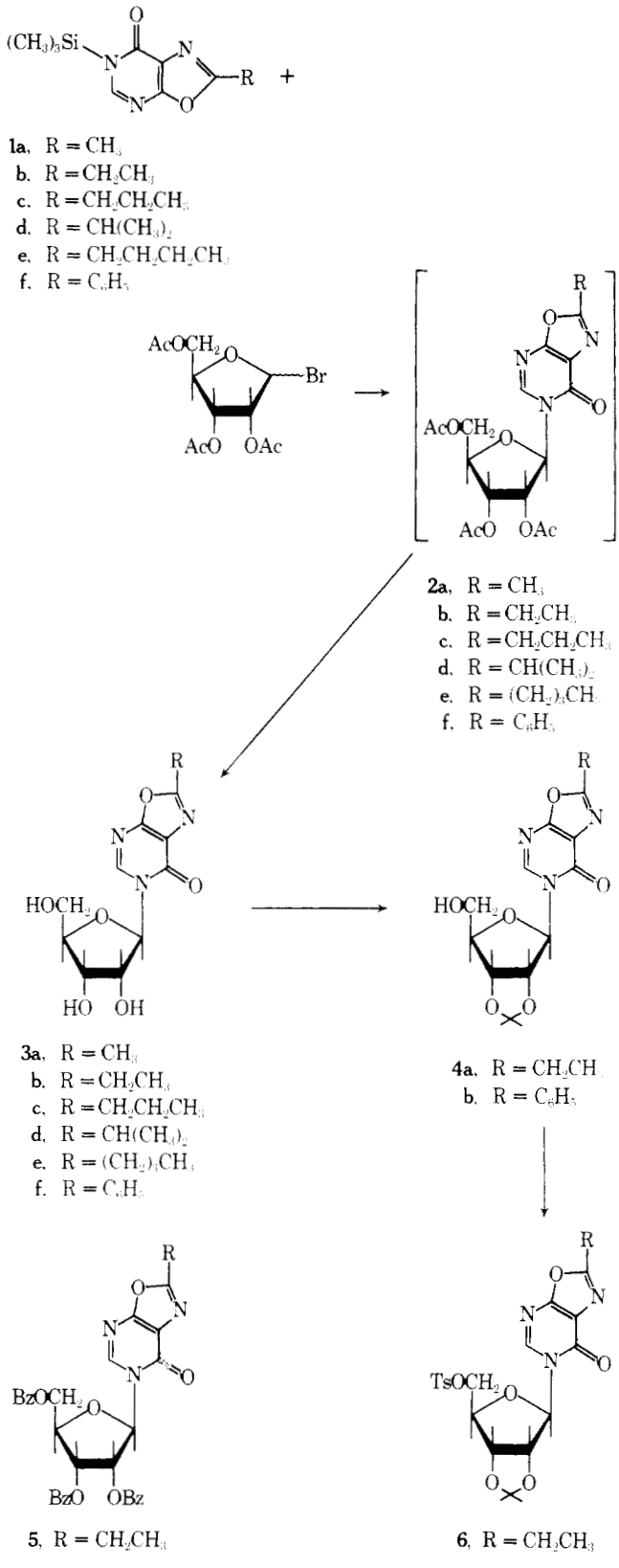
Table II. Spectral Data for Some 2-Substituted 6-(β-D-Ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones[a,b]

Compd	Uv, λ max, nm (ε × 10^3)			Pmr		
	MeOH	pH 1	pH 11	δ $H_{1'}$[c]	$J_{H_{1'},H_{2''}}$, Hz	δ H_6
3a	236 (17.5), 246.5 (sh, 14.3), 274 (8.9)	240 (16.4), 246 (sh, 14.7), 270 (8.7)	240 (15.2), 246 (sh, 14.1), 295 (11.9)	6.18	2.6	8.98
3b	240 (25.6), 246 (sh, 21.1), 275 (12.8)	241 (24.4), 247 (sh, 22.0), 271 (12.2)	241 (21.7), 246 (sh, 20.5), 290 (14.5)	6.16	3.0	8.96
3c	240 (14.9), 247 (sh, 13.4), 275 (8.4)	241 (14.0), 247 (sh, 13.1), 272 (7.5)	241 (14.0), 246 (sh, 13.1), 290 (10.6)	6.22	3.4	8.84
3d	239 (17.8), 247 (sh, 15.1), 274 (9.2)	241 (16.1), 247 (sh, 14.9), 271 (8.7)	241 (16.5), 247 (sh, 15.5), 290 (11.5)	6.18	2.6	9.01
3e	240 (15.8), 246 (sh, 13.9), 274 (8.8)	240 (15.0), 246 (sh, 13.8), 270 (8.6)	241 (15.1), 246 (sh, 14.0), 291 (10.8)	6.20	2.8	9.01
3f	286 (sh, 26.9), 295 (29.6), 303 (sh, 28.4)	286 (sh, 29.4), 293 (32.8), 304 (sh, 30.4)	305 (36.2)	6.10	2.7	8.80
6	275 (10.0)	238 (29.6), 246 (31.8), 280 (20.7)	238 (36.8), 243 (sh, 36.1), 278 (20.1)			

[a] Ultraviolet spectra were obtained on a Beckman DK-2 spectrophotometer. [b] Pmr spectra were recorded in DMSO-d_6 on a Varian A-56/60 spectrometer using TMS as an internal standard. [c] In all cases $H_{1'}$ peak shape was a doublet.

Biological. The effect of some of the newly prepared nucleosides on the *in vitro* growth of leukemia L1210 and *Escherichia coli* K_{12} cells is shown in Table IV. Substitution at the 2 position with a methyl, ethyl, or propyl group gives rise to a growth inhibitory activity in both the L1210 and *E. coli* systems, whereas an isopropyl or phenyl group at the 2 position results in compounds which are inactive against L1210 cells but, in the case of the phenyl derivative, shows some activity against *E. coli*. The corresponding 2-substituted heterocycles are inactive in these test systems, except for the isopropyl derivative which inhibits the growth of *E. coli* by 50% at 3×10^{-5} *M*.

Preliminary evaluation of the activity of these compounds against leukemia L1210 *in vivo* showed that, at 200 mg/kg/day × 5, the 2-methyl-substituted nucleoside **3a** increased the life span of the tumor-bearing mice by



31%. At this dose, none of the other analogs were significantly active.

Since these nucleosides can be viewed either as derivatives of a pyrimidine nucleus substituted with a cyclic moiety at positions 5 and 6, or as purine analogs with the carbohydrate moiety attached to the pyrimidine moiety, it was of interest to determine whether, in the cells, these compounds act as purine or pyrimidine antagonists. Consequently, an inhibition analysis[16] was carried out, which showed that the inhibition of growth exerted, for example, by the 2-ethyl nucleoside **3b**, is prevented by pyrimidines but not by purines. Thus, the inhibition of the growth of L1210 cells by 1×10^{-4} *M* **3b** was completely prevented by uridine or cytidine at 1×10^{-4} *M*. Smaller concentrations of these metabolites decreased the inhibition to a proportionately smaller extent. 2′-Deoxyuridine or 2′-deoxycytidine was approximately ten times less effective in preventing the inhibition than were the corresponding ribonucleosides and thymidine (at 1×10^{-5} *M*) which appeared to act like a product of the inhibited path, reversing the inhibition to the same extent (80%) at all concentrations of inhibitor. The reversal pattern obtained in *E. coli* was quite similar to that seen in the leukemic cells, thymidine again providing a product effect, whereas the extent of reversal provided by the other pyrimidine nucleosides was approximately proportional to their concentration. Thus, it appears that the compounds act as pyrimidine antagonists, exerting their inhibitory activity along the metabolic path followed by the pyrimidines, rather than as purine analogs which could mimic the feedback effect exerted by purine nucleosides and nucleotides in enzymes concerned with *de novo* purine synthesis.[17]

Table III. Pmr Spectral Data of 2′,3′-*O*-Isopropylidenes of Some Oxazolo[5,4-*d*]pyrimidin-7-one 6-Ribonucleosides

Compd	δ CH_3	Δδ[a]	δ $H_{1'}$	$J_{H_{1'}}$, Hz
4a	1.36, 1.54	0.18	6.18	<1
4b	1.37, 1.58	0.21	6.30	s[b]
6	1.30, 1.50	0.20	6.12	s

[a] Δδ is the difference between the chemical shifts observed for the methyl protons of the isopropylidene group. [b] s = singlet.

Experimental Section

General Procedure for the Preparation of 2-Substituted 6-(β-D-Ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones (3a-f). The silylated heterocycle **1a-f** (0.04 mol) [prepared by heating the heterocycle[10] in hexamethyldisilizane in the presence of a catalytic amount of $(NH_4)_2SO_4$ at reflux temperature] was dissolved in 50 ml of anhydrous benzene and then added to a suspension of $HgBr_2$ (7.5 g) and HgO (7.5 g) in 250 ml of azeotropically dried benzene. 2,3,5-Tri-*O*-acetyl-D-ribofuranosyl bromide (prepared from 10.5 g of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose) dissolved in 50 ml of anhydrous benzene was added to the reaction mixture. The suspension was heated at reflux temperature for 20 hr, protected from moisture. The reaction mixture was cooled, the mercury salts were removed by filtration through a Celite pad, and the pad was washed with chloroform (3 × 50 ml). The filtrate and washings were combined and evaporated *in vacuo* to afford a syrup. This syrup was dissolved in chloroform (350 ml); the solution was washed successively with a 30% aqueous solution of KI (3 × 50 ml), a cold saturated sodium carbonate solution (4 × 50 ml), and distilled water (4 × 50 ml) and was then dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the chloroform mixture evaporated under reduced pressure to afford a syrup. The syrup was treated with methanol, which had been previously saturated with ammonia at 0° (400 ml) and the solution was allowed to stand at room temperature for 20 hr with occasional shaking. The solution was filtered and the filtrate evaporated *in vacuo* to yield a solid material which was collected by filtration. Recrystallization from methanol yielded pure 2-substituted 6-(β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones.

2-Ethyl-6-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-one (5). The same ribosylation procedure was followed as outlined above, with the following reactants being used. The trimethylsilyl derivative formed from 3.3 g of 2-ethyloxazolo[5,4-*d*]pyrimidin-7-one was dissolved in 40 ml of benzene and added to a suspension of mercuric oxide (5 g) and mercuric

Table IV. Effect of Some 2-Substituted 6-(β-D-Ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-ones on the *in Vitro* Growth of Leukemia L1210 and *E. coli* Cells

Compd	2-Substituent	Concn (*M*) for 50% growth inhibition of Leukemia L1210 cells	Concn (*M*) for 50% growth inhibition of *E. coli* K_{12} cells
3a	$-CH_3$	5×10^{-5}	8×10^{-4}
3b	$-CH_2CH_3$	7×10^{-6}	6×10^{-6}
3c	$-CH_2CH_2CH_3$	3×10^{-5}	5×10^{-7}
3d	$-CH(CH_3)_2$	$>10^{-4}$	$>10^{-3}$
3f	$-C_6H_5$	$>10^{-4}$	4×10^{-4}

bromide (5 g) in 250 ml of benzene and this was then added to 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (prepared from 10 g of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose) dissolved in 40 ml of anhydrous benzene. After work-up of the reaction mixture the chloroform was evaporated to a hard foam residue which was recrystallized from benzene to yield **5** (9 g, 72.8%).

2-Ethyl-6-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-one (4a). To 450 ml of dry acetone was added 1.6 ml of 2,2-dimethoxypropane and 1.5 ml of 70% perchloric acid. The mixture, protected from moisture, was stirred at room temperature for 5 min and then 1.3 g of **3b** was added in one portion. Pyridine (1.6 ml) was added after the mixture had been stirred for 45 min. The volume was reduced to 50 ml *in vacuo* and 23 ml of 10% aqueous sodium carbonate was added before the remaining acetone was removed. Cold water (40 ml) was then added and the solution was allowed to stand at 5° for 12 hr. The white crystalline solid which separated from the solution was collected by filtration and was washed with a small amount of cold water. Recrystallization from methanol afforded 0.60 g (40.7%) of **4a**.

2-Phenyl-6-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-one (4b). Compound **4b** was prepared in the same manner as **4a** by treatment of **3f** (1.4 g) with 1.6 ml of 2,2-dimethoxypropane and 1.5 ml of 70% perchloric acid, followed by 1.6 ml of pyridine. This procedure furnished **4b** (0.7 g, 44.8%) as white needles.

2-Ethyl-6-(2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-one (6). 2-Ethyl-6-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)oxazolo[5,4-*d*]pyrimidin-7-one (**4b**, 0.405 g) was dissolved in pyridine (8 ml) and *p*-toluenesulfonyl chloride (0.286 g) was added to this solution. The reaction mixture was stored in the dark at 5° for 36 hr and was then poured onto ice water (100 ml). The aqueous mixture was extracted with chloroform (2 × 5 ml); the organic layer was washed with 1 *M* H_2SO_4 (2 × 25 ml) and then with water until the aqueous layer was neutral. The chloroform solution was dried over sodium sulfate and its volume was reduced to 10 ml. Methanol (20 ml) was added and the solution was then evaporated to dryness and the residue dried in a vacuum desiccator to afford 0.150 g (25%) of **6**.

Biological. The procedures used for the evaluation of the biological effects of the nucleosides have been described previously.[18]

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