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SYNTHESIS AND BIOLOGICAL ACTIVITIES OF 4-(D-ALANYLAMINO)-2-AMINO-2,3,4-TRIDEOXY-L-*threo*-PENTOSE (3-DEOXYPRUMYCIN)*

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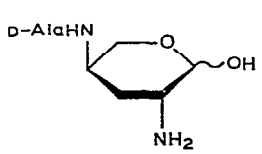
ABSTRACT

Three analogs of prumycin [4-(D-alanylamino)-2-amino-2,4-dideoxy-L-arabinose], *i.e.*, the 3-deoxygenated (**1**), that having the 2-amino and 3-hydroxyl groups interchanged (**2**), and that having C-2 deaminated (**3**), were synthesized. Examination of the antifungal and antileukemic activities showed that, for **1**, the former activity was more potent against some fungi, whereas the latter was slightly weaker, than that of prumycin.

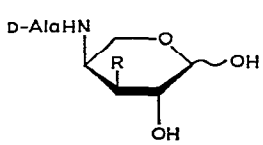
INTRODUCTION

Studies on the antimicrobial activity of all seven synthetic prumycin diastereoisomers, in which its 2,4-diaminopentose moiety had been altered, proved that only the L-*lyxo* isomer showed moderate activity[1], indicating that the activity would be very sensitive to the configuration, namely, the relative, spatial arrangement of such functional groups as the 4-(D-alanylamino) and the 2-amino. In order to elucidate more clearly the role of these functional groups for development of activity, three analogs of prumycin were synthesized. The first was the simplest model-compound, having only these two functional groups, *i.e.*, 4-(D-alanylamino)-2-amino-2,3,4-trideoxy-L-*threo*-pentose (**1**), which is the 3-deoxygenated analog of prumycin. Similar deoxygenation has often been found to be successful in the development of powerful, new aminoglycoside antibiotics, such as[2] 3′,4′-dideoxykanamycin B, against resistant



1

D-Ala = *(R)*-$CH_3CH(NH_2)CO$



2 R = NH_2
3 R = OH

*Amino Sugars, Part XXXIV. For Part XXXIII, see ref. 1.

bacteria, and found to be reasonable by study of the mechanism of acquiring resistibility[3]. The second analog was 4-(D-alanylamino)-3-amino-3,4-dideoxy-L-arabinose (**2**), in which the 2-amino and 3-hydroxy functions were interchanged (one of the positional isomers), and the third was 2-deaminated prumycin, namely, 4-(D-alanylamino)-4-deoxy-L-arabinose (**3**).

RESULTS AND DISCUSSION

Synthesis of **1** started with a mixture (**4**), from ammonolysis of benzyl 2,3-anhydro-4-azido-4-deoxy-β-L-ribopyranoside, that was composed of benzyl 2-amino-4-azido-2,4-dideoxy-β-L-arabinopyranoside and benzyl 3-amino-4-azido-3,4-dideoxy-β-L-xylopyranoside. The former had previously been used for the synthesis of prumycin[4]. As both isomers should be converted into the same 2,3-epimino derivative, which was selected as a suitable intermediate for the synthesis of **1**, the ammonolysis mixture (**4**) was used without separation of the two components. For deoxygenation, the introduction of an iodine atom at C-3, followed by selective reduction, was chosen, because direct, selective reduction of the aziridine ring might be difficult. The selectivity of the ring-opening reaction was then examined by using four 2,3-epimino-β-L-lyxopyranoside derivatives (**9**–**12**) having different substituents on the nitrogen atom in the epimino ring.

Mixture **4** was treated with *p*-anisaldehyde in 1,4-dioxane, to give a mixture of *N*-anisylidene derivatives (**5**), which was then converted into a mixture of the corresponding mesylates (**6**). Then, **6** was *N*-de-anisylidenated with M hydrochloric acid, to give a mixture of benzyl 2-amino-4-azido-2,4-dideoxy-3-*O*-mesyl-β-L-arabinopyranoside (**7a**) and benzyl 3-amino-4-azido-3,4-dideoxy-2-*O*-mesyl-β-L-xylopyranoside (**7b**), which could be separated on silica gel. However, for the next reaction, it was not necessary to separate these isomers, and the mixture (**7**) in 10:1 *N*,*N*-dimethylformamide (DMF)–water, in the presence of sodium acetate, was heated at 70–80°, to afford the 2,3-epimino derivative (**9**), in 62% yield from **4**. The corresponding *N*-acetyl (**10**) and *N*-(benzyloxycarbonyl) (**11**) derivatives of **9** were prepared in the usual way. Furthermore, reduction of **6** with sodium borohydride afforded the corresponding *N*-(*p*-methoxybenzyl) derivative (**8**), which was then cyclized in the same way, to give the *N*-(*p*-methoxybenzyl)-2,3-epimino derivative (**12**) in 43% yield from **4**. The structure of these 2,3-epimino derivatives was readily confirmed by the signals of the protons attached to the epimino ring, which resonate at higher field (δ ~2.6 for **8** and **12**, and δ 2.9–3.0 for **10** and **11**) than those of the usual ring-protons of pyranosides, as shown in the Experimental section. The structure was also ascertained by the small coupling-constants between H-4 and the two protons on C-5 (2.3–2.7 and 1.5 Hz, respectively), indicating that they adopt the $^{O}H_{5}$ conformation almost exclusively.

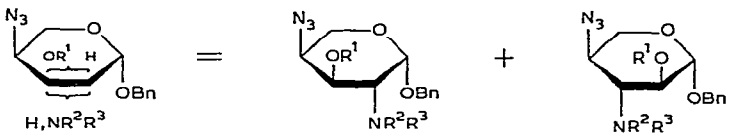
The reaction of **9**, **10**, and **11** with sodium iodide gave two positional isomers (**13** and **17**, **14** and **18**, and **15** and **19**, respectively) in the ratios shown in Table I, whereas that of **12** gave the 3-iodo derivative (**16**) exclusively. Although these 2,3-

TABLE I

RATIOS OF ISOMERS PRODUCED BY THE REACTION OF 4-AZIDO-2,3,4-TRIDEOXY-2,3-EPIMINO-β-L-LYXO-PYRANOSIDE DERIVATIVES

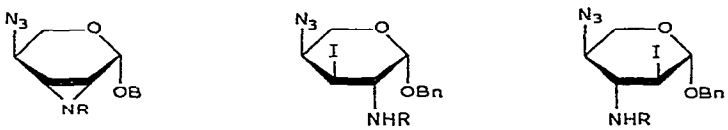
Substrate	*Total yield (%)*	*Products*		
		3-Iodo derivative	*(Ratios in %)*	*2-Iodo derivative*
9	55[a]	**13**	(85:15)[b]	**17**
10	73	**14**	(67:33)[c]	**18**
11	81	**15**	(80:20)[b]	**19**
12	93	**16**	(100:0)[b]	

[a]Isolated as *N*-benzyloxycarbonyl derivatives. [b]Determined by the amounts of the products isolated. [c]Determined by the intensity of n m.r signals, as described in the Experimental section.



β-L-arabino (a) β-L-xylo (b)

4 $R^1 = R^2 = R^3 = H$
5 $R^1 = H, R^2, R^3 = p\text{-}MeOC_6H_4CH$
6 $R^1 = Ms, R^2\ R^3 = p\text{-}MeOC_6H_4CH$
7 $R^1 = Ms\ R^2, R^3 = H$
8 $R^1 = Ms, R^2 = H, R^3 = p\text{-}MeOC_6H_4CH_2$



9 R = H
10 R = Ac
11 R = Z
12 R = $p\text{-}MeOC_6H_4CH_2$

13 R = H
14 R = Ac
15 R = Z
16 R = $p\text{-}MeOC_6H_4CH_2$

17 R = H
18 R = Ac
19 R = Z

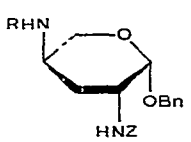
Z = $PhCH_2OCO$

epimino sugars adopt the $^OH_5$ conformation, which is favorable for substitution on C-2 from the electro-stereochemical point of view (Fürst–Plattner rule[5]), the preponderant products were, in all cases, the 3-iodo derivatives, indicating the larger,

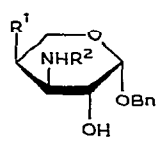
positive, inductive effect of the anomeric carbon atom, as widely proved in the reaction of 2,3-anhydroaldopentopyranosides[6]. The introduction of an electron-withdrawing group onto the nitrogen atom facilitates substitution at C-2. The results may be explained by the acceleration of the ring-opening reaction, causing relative decrease in predominance of the electrostatic over the electro-stereochemical effect for regioselectivity.

In consideration of the yield, and readiness of deprotection, compound **15** was used for the synthesis of **1**. Selective reduction of the azido and iodo groups with hydrogen in the presence of Raney nickel gave the 4-amino-3-deoxy derivative (**20**) in 45% yield. 3-Deoxygenation was confirmed by its n.m.r. spectrum, which showed typical signals due to methylene protons at C-3 (δ 1.6–2.1). This selective reduction proceeded in better yield (~70%) on using nickel chloride and sodium borohydride[7], *i.e.*, "nickel boride" formed *in situ*. Condensation of **20** with *N*-(benzyloxycarbonyl)-D-alanine (*N*-Z-D-Ala) with *N*,*N*-dicyclohexylcarbodiimide (DCC) in acetonitrile gave, in 78% yield, **21**, which was then hydrogenolyzed in the presence of palladium–charcoal in methanol–water containing hydrochloric acid, to give **1** as the dihydrochloride in good yield.

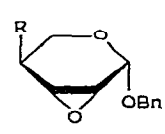
On the other hand, compound **2** was similarly synthesized from a minor ammonolysis product (**22**) of benzyl 2,3-anhydro-4-azido-4-deoxy-β-L-lyxopyranoside[8] (**26**) by *N*-(benzyloxycarbonyl)ation, selective reduction with hydrogen (Raney nickel), condensation with *N*-Z-D-Ala, and hydrogenolysis *via* compounds **23**, **24**, and **25**. 4-(D-Alanylamino)-4-deoxy-β-L-arabinose (**3**) was prepared from **26** by reduction of the azido group, condensation with *N*-Z-D-Ala and simultaneous opening of the oxirane ring, and hydrogenolysis *via* compound **28**. The ready ring-opening, accompanied by condensation of the alanine moiety, may be explained by neighboring-group participation of the amido carbonyl group[9].



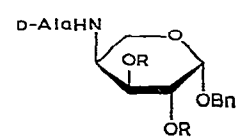
20 R = H
21 R = *N*-Z-D-Ala



22 $R^1 = N_3, R^2 = H$
23 $R^1 = N_3, R^2 = Z$
24 $R^1 = NH_2, R^2 = Z$
25 R = *N*-Z-D-Ala, $R^2 = Z$



26 R = N_3
27 R = NH_2



28 R = H
29 R = Ac

The antifungal activities of these synthetic prumycin analogs (**1**–**3**) against 12 representative phytopathogenic fungi were examined, and the preliminary results were expressed in terms of the inhibition ratio of hypha growth, together with the minimum inhibitory concentration of prumycin reported[10], as shown in Table II. Although the antifungal activity of these analogs could not be compared directly with that of prumycin, in the case of **1**, more-potent activity against some fungi (such as *Diaporthe citri*) was observed. Because the antitumor activity of prumycin

TABLE II

ANTIFUNGAL ACTIVITY OF PRUMYCIN ANALOGS, EXPRESSED AS INHIBITION RATIOS OF HYPHA GROWTH

Fungi	*Inhibition ratios of hypha growth*									*Reported MIC values of prumycin*[b]
	1			**2**			**3**			
	Concentration[a]			*Concentration*[a]			*Concentration*[a]			
	100	*10*	*1*	*100*	*10*	*1*	*100*	*10*	*1*	
Sclerotinia sclerotiorum	100	30.4	13.0	7.2	1.4	0	8.7	7 2	4.3	<25
Botrytis cinera	100	65.8	31.6	10.5	2 6	0	18.4	13 2	7 9	<25
Diaporthe citri	100	12 1	15 2	15 2	9.1	6.1	9 1	6 1	3.0	200–400
Cochliobolus miyabeanus	44.0	10 0	10 0	16.0	8.0	0	22.0	18.0	20.0	400–800
Pyricularia oryzae	18.6	7.1	0	0	0	0	7.1	0	0	800
Rhizoctonia solani	54.8	19.4	0	0	0	0	0	0	0	>800
Pythium debaryanum	16.9	1.1	0	0	0	0	0	0	0	>800
Fusarium oxysporum	15.6	0	0	2 2	0	0	0	0	0	>800
Vabalsa ceratoperum	100	16 2	0	13.5	16 2	16 2	29.7	8.1	0	—[c]
Colletoriclum lagenorium	100	13.0	4 3	17.4	8.7	8.7	13.0	4.3	0	—
Roseltinia necatrix	60.7	0	0	3.6	0	0	0	0	0	—
Alternaria mali	23 9	13.0	13.0	13.0	8.7	4.3	13.0	8.7	4.3	—

[a]Concentration in μg/mL. [b]Minimum inhibitory concentration in μg/mL (see ref 10). [c]Not reported.

TABLE III

ANTILEUKEMIC ACTIVITY OF **1**

Compound	*Dosage (mg/kg)*[a]	*Average survival time, in days (T)*[b]	*T/C*
Control	—	10.6(C)	1.00
1	50	12.2	1.15
1	100	12.8	1 21
1	200	12.9	1.22
Prumycin[c]	100	—	1.35

[a]One and five days after leukemia P-388 inoculation. [b]Six CDF_1 mice/group [c]Reported (see ref 11) using BDF_1 mice by single administration one day after leukemia P 388 inoculation.

has recently been reported[11], that of **1** against leukemia P 388 was also examined; it showed a moderate activity, albeit weaker than that of prumycin, as shown in Table III.

These results indicate the importance of the 4-(D-alanylamino) and 2-amino functions, and that the 3-hydroxyl group also plays an appreciable role in the development of the biological activities.

EXPERIMENTAL

General. — Melting points were determined with a Mel-Temp melting-point apparatus and are not corrected. Optical rotations were measured in chloroform at

c 1.0, unless stated otherwise, using a 0.5-dm tube with a Carl Zeiss LEP-A1 or a JACSO DIP-4 polarimeter. I.r. spectra were recorded with a Hitachi EPI-G2 grating spectrometer. N.m.r. spectra were recorded at 100 MHz with a JEOL JNM PS-100 spectrometer for solutions in chloroform-*d* containing tetramethylsilane as the internal standard, unless stated otherwise. Chemical shifts and coupling constants are given in δ and Hz units, respectively, and i.r. frequencies in cm^{-1}. Column chromatography and preparative t.l.c. were performed on Wakogel C-200 (Wako Pure Chemical Industries, Ltd.) and Kieselgel 60 HF_{254} (Merck), respectively. Evaporations were conducted under diminished pressure at a temperature not exceeding 50°.

*Benzyl 4-azido-2,3,4-trideoxy-2,3-epimino-β-*L*-lyxopyranoside* (**9**) — A mixture[4] (**4**; 1.75 g, 5.95 mmol) of benzyl 2-amino-4-azido-2,4-dideoxy-β-L-arabinopyranoside and benzyl 3-amino-4-azido-3,4-dideoxy-β-L-xylopyranoside was dissolved in 1,4-dioxane (25 mL), and to this solution was added *p*-anisaldehyde (0.9 mL, 7.4 mmol). After being stirred overnight at room temperature, the solution was evaporated to give a mixture of *N*-anisylidene derivatives (**5**) and the excess of *p*-anisaldehyde as a syrup which was esterified with methanesulfonyl chloride in pyridine. The solution was poured into ice–water, and extracted with benzene. The extract was washed with water, dried (anhydrous magnesium sulfate), and evaporated, to give a mixture of the isomeric *N*-anisylidene-*O*-mesyl derivatives (**6**) and the excess of *p*-anisaldehyde as a syrup; ν_{max}^{NaCl} 2120 (N_3), 1643 (C=N), and 1355 (Ms).

The mixture was treated with M hydrochloric acid (20 mL) under vigorous stirring for 1 h. The solution was washed with benzene to remove the *p*-anisaldehyde liberated, made neutral with sodium hydrogencarbonate, and extracted with chloroform. The extract was dried, and evaporated, to give a syrupy mixture (**7**) of *O*-mesyl derivatives. A portion was separated by preparative t.l.c., to give the *β-*L*-arabino* (**7a**) and *β-*L*-xylo* (**7b**) isomers, which were characterized by i.r. and n.m.r. data; compound **7a**: ν_{max}^{NaCl} 3400 (NH), 2120 (N_3), 1350, and 1177 (Ms) cm^{-1}; n.m.r.: 4.97 (d, H-1, $J_{1,2}$ 3.3), 3.26 (dd, H-2, $J_{2,3}$ 10.4), 4.86 (dd, H-3, $J_{3,4}$ 3.5), 4.12 (m, H-4), 3.70 (dd, H-5*a*, $J_{4,5a}$ 2.3, $J_{5a,5e}$ 10.8), 3.94 (dd, H-5*e*, $J_{4,5e}$ 1.8), 3.17 (s, Ms), 4.48 and 4.62 (ABq, Bn), and 1.62 (s, NH_2); compound **7b**: ν_{max}^{NaCl} 3400 (NH), 2110 (N_3), 1355, and 1178 (Ms) cm^{-1}; n.m.r.: 4.49 (d, H-1, $J_{1,2}$ 7.5), 4.20 (dd, H-2, $J_{2,3}$ 9.6), 2.95 (s, OMs), 4.56, and 4.90 (ABq, Bn).

For the next step, mixture **7** was used without separation. A solution of **7** in DMF (50 mL) and water (5 mL) was heated in the presence of sodium acetate trihydrate (4.5 g) for 10 h at 70–80°, with stirring. To the solution were added chloroform (50 mL) and water (30 mL), the mixture was shaken vigorously, and the chloroform layer was separated, washed with water, dried, and evaporated, to give **9** (0.91 g, 62% from **4**) as a syrup; $[\alpha]_D$ +77.5°, ν_{max}^{NaCl} 3500 (NH) and 2100 (N_3) cm^{-1}; n.m.r.: 5.06 (d, H-1, $J_{1,2}$ 3.0), 2.58 (m, 2 H, H-2,3), 3.47 (m, H-4), 3.99 (dd, H-5, $J_{4,5}$ 2.6, $J_{5,5'}$ 13.0), and 3.53 (dd, H-5′, $J_{4,5'}$ 1.5).

Anal. Calc. for $C_{12}H_{14}N_4O_2$: C, 58.52; H, 5.73; N, 22.75. Found: C, 58.11; H, 5.57; N, 22.59.

Benzyl N-*acetyl-4-azido-2,3,4-trideoxy-2,3-epimino-β*-L-*lyxopyranoside* (**10**). — Compound **9** was acetylated with acetic anhydride–pyridine in the usual way, to give **10** as a syrup in quantitative yield; $[\alpha]_D$ +108 8°; ν_{max}^{NaCl} 2105 (N_3) and 1707 (Ac) cm^{-1}; n.m.r.: 5.11 (d, H-1, $J_{1,2}$ 3.4), 2.96 (dd, H-2, $J_{2,3}$ 5.7), 3.05 (dd, H-3, $J_{3,4}$ 1.5), 3.60 (m, H-4), 4 01 (dd, H-5, $J_{4,5}$ 2.7, $J_{5,5'}$ 12 9), 3.61 (dd, H-5′, $J_{4,5'}$ 1.5), 2.20 (s, Ac), 4.60, and 4.83 (ABq, Bn).

Anal. Calc. for $C_{14}H_{16}N_4O_3$· C, 58.32; H, 5.59; N, 19.44. Found: C, 58.32; H, 5 43; N, 18 98.

Benzyl 4-azido-N-(*benzyloxycarbonyl*)-*2,3,4-trideoxy-2,3-epimino-β*-L-*lyxopyranoside* (**11**). — To a solution of **9** (773 mg, 2.8 mmol) in 1,4-dioxane (15 mL) and water (15 mL) were added sodium hydrogencarbonate (340 mg) and benzyl chloroformate (0.62 mL), with stirring. After the solution had been stirred for 3 h at room temperature, ether was added, and the mixture was shaken. The ether layer was washed twice with water, dried, and evaporated, to give a syrup which was purified on a column of silica gel with 15:1 benzene–acetone as the eluant, to afford pure **11** in quantitative yield; $[\alpha]_D$ +81.3°; ν_{max}^{NaCl} 2110 (N_3) and 1732 (Z) cm^{-1}; n.m.r.: 5.07 (d, H-1, $J_{1,2}$ 3.6), 2.94 (dd, H-2, $J_{2,3}$ 6.0), 2 84 (dd, H-3, $J_{3,4}$ 1.5), 3.48 (m, H-4), 3.88 (d, H-5, $J_{4,5}$ 2.3, $J_{5,5'}$ 12.6), 3.45 (dd, H-5′, $J_{4,5'}$ 1.5), 5.01 (s, Z), 4 46. and 4.70 (ABq, Bn).

Anal. Calc. for $C_{20}H_{20}N_4O_4$: C, 63.15, H, 5.30, N, 14.73. Found: C, 63.16; H, 5.43; N, 14.38.

Benzyl 4-azido-2,3,4-trideoxy-2,3-epimino-N-(p-*methoxybenzyl*)-*β*-L-*lyxopyranoside* (**12**). — Mixture **6** was prepared from **4** (1.75 g, 5.95 mmol) as described for **9**. To a solution of **6** in methanol (20 mL) was added sodium borohydride (290 mg, 7.6 mmol), portionwise, with stirring at room temperature. Stirring was continued for 3 h, benzene and water were added, and the benzene layer was separated, washed with water, dried, and evaporated, to give a syrup which was freed of contaminating *p*-methoxybenzyl alcohol on a column of silica gel eluted with 50:1 benzene–acetone, to afford a mixture (**8**) of *N*-(*p*-methoxybenzyl)-*O*-mesyl derivatives. The conversion of **8** into **12** was performed by heating in DMF–water as described for **9**. The yield from **4** was 62%; $[\alpha]_D$ +77.5°; ν_{max}^{NaCl} 3300 (NH) and 2100 (N_3) cm^{-1}; n.m.r.: 5.06 (d, H-1, $J_{1,2}$ 3.0), 2.58 (m, H-2,3), 3.47 (m, H-4), 3.99 (dd, H-5, $J_{4,5}$ 2.6, $J_{5,5'}$ 13.0), and 3.53 (dd, H-5′, $J_{4,5'}$ 1.5).

Anal. Calc. for $C_{20}H_{22}N_4O_3$: C, 65.55; H, 6.05; N, 15.29. Found. C, 65.65; H, 6.04; N, 14.88.

Ring-opening reaction of benzyl 4-azido-2,3,4-trideoxy-2,3-epimino-β-L-*lyxopyranoside derivatives* (**9–12**) *with sodium iodide.* — To a solution of the 2,3-epimino sugar (1.0 mmol) in acetone (10 mL) were added sodium iodide (825 mg, 5.5 mmol), sodium acetate (82 mg, 1.0 mmol), and acetic acid (1 mL), with stirring. The mixture was boiled under reflux for 5 h, and cooled; chloroform and water were added, and the chloroform layer was separated, washed with water, dried, and evaporated, to give a syrup which was purified, or fractionated, by preparative t l c., using 50:1:1 benzene–acetone–ethyl acetate as the eluant. The yields are given in Table I. In the

case of **9**, the reaction products (**13** and **17**) were isolated, and characterized as their *N*-(benzyloxycarbonyl) derivatives **15** and **19**. The ratio of the two isomers was estimated from the amount isolated, except for **10**. For **10**, the ratio was determined from the intensity of the n.m.r. signals of the mixture.

*Benzyl 2-acetamido-4-azido-2,3,4-trideoxy-3-iodo-β-*L*-arabinopyranoside* (**14**) *and benzyl 3-acetamido-4-azido-2,3,4-trideoxy-2-iodo-β-*L*-xylopyranoside* (**18**). — The reaction of **10** with sodium iodide, as just described, gave a mixture of **14** and **18** as a syrup which was characterized by n.m.r. and elemental analysis; n.m.r.: 3.28 (dd, H-5*a* of **18**, 0.4 H), 5.75 (d, NH of **14**, 0.65 H), 6.69 (d, NH of **18**, 0.35 H), 1.98 (s, Ac of **14**, 2 H), and 2.08 (s, Ac of **18**, 1 H).

Anal. Calc. for $C_{14}H_{17}IN_4O_3$: C, 40.37; H, 4.08; N, 13.31. Found. C, 40.63; H, 4.14; N, 12.94.

*Benzyl 4-azido-2-(benzyloxycarbonylamino)-2,3,4-trideoxy-3-iodo-β-*L*-arabinopyranose* (**15**) *and benzyl 4-azido-3-(benzyloxycarbonyl)amino-2,3,4-trideoxy-2-iodo-β-*L*-xylopyranoside* (**19**). — The reaction of **9** with sodium iodide, as already described, yielded **15** and **19**.

Compound **15**: m.p. 159–160°, $[\alpha]_D$ +199.5°; ν_{max}^{KBr} 3320 (NH), 2100 (N_3), 1688, and 1530 (Z) cm^{-1}; n m r.: 4.87 (d, H-1, $J_{1,2}$ 3.0), 4.28 (ddd, H-2, $J_{2,3}$ 11.7), 4.48 (dd, H-3, $J_{3,4}$ 2.4), 3.96 (m, H-4), 3.72 (dd, H-5*a*, $J_{4,5a}$ 2.3, $J_{5a,5e}$ 12.8), 3.98 (dd, H-5*e*, $J_{4,5e}$ 1.5). 5.05 (d, NH, $J_{2,NH}$ 11.7), 5.11 (s, Z), 4.44, and 4.69 (ABq, Bn).

Anal. Calc. for $C_{20}H_{21}IN_4O_4$: C, 47.31; H, 4.14; I, 25.01; N, 11.04 Found: C, 47.74; H, 4.21; I, 24.82; N, 10.95.

Compound **19**: m.p. 190–193°, $[\alpha]_D$ −101.4°; ν_{max}^{KBr} 3280 (NH), 2105 (N_3), 1690, and 1540 (Z) cm^{-1}; n.m.r.: 4.58 (d, H-1, $J_{1,2}$ 7.5), 3.26 (dd, H-5*a*, $J_{4,5a}$ 9.8, $J_{5a,5e}$ 10.8), 5.15 (s, Z), 4.60, and 4.87 (ABq, Bn).

Anal. Calc. for $C_{20}H_{21}IN_4O_4$: C, 47.31; H, 4.14; I, 25.01; N, 11.04. Found: C, 47.41; H, 4.63; I, 25.53; N, 11.00.

Benzyl 4-azido-2,3,4-trideoxy-3-iodo-2-(p-*methoxybenzyl)amino-β-*L*-arabinopyranoside* (**16**). — The reaction of **12** with sodium iodide as already described gave **16** exclusively, as a syrup, characterized by the following spectral data: ν_{max}^{NaCl} 3400 NH and 2110 (N_3) cm^{-1}; n.m.r.: 4.78 (d, H-1, $J_{1,2}$ 3.2), 3.03 (dd, H-2, $J_{2,3}$ 11.7), 4.54 (dd, H-3, $J_{3,4}$ 3 3), 3.78 (s, OMe), 3.66 (s, *N*-Bn), 4.44 and 4.70 (ABq, *O*-Bn), and 1.92 (s, NH).

This compound has a tendency to recyclize readily at room temperature, and an analytically pure sample could not be obtained.

*Benzyl 4-amino-2-(benzyloxycarbonyl)amino-2,3,4-trideoxy-β-*L*-*threo*-pentopyranoside* (**20**). — *Method A*. To a solution of **17** (1.27 g, 2.5 mmol) in ethanol (50 mL) was added Raney nickel (~1 g), and hydrogen was bubbled in until all of the **17** had disappeared. The catalyst was filtered off, and evaporation of the filtrate gave a syrup which was purified by preparative t.l.c. with 5:1:1 benzene–methanol–ethyl acetate as eluant, to yield **20** in 45% yield; ν_{max}^{KBr} 3370 (NH) and 1724 (Z) cm^{-1}; n.m.r.: 4.82 (d, H-1, $J_{1,2}$ 3.3), 4.12 (m, H-2), 1.64–2.10 (m, H-3*a*,3*e*), 3.16 (m, H-4), 3.42

(dd, H-5*a*, $J_{4,5a}$ 0.5, $J_{5a,5e}$ 12.0), 3.89 (dd, H-5*e*, $J_{4,5e}$ 1.5), 5.06 (s, Z), 4.44, and 4.73 (ABq, Bn).

Method B. To a solution of **17** (1.0 g, 1.9 mmol) in ethanol (40 mL) and 1,4-dioxane (10 mL) were successively added nickel chloride hexahydrate (0.9 g, 3.8 mmol) and sodium borohydride (0.87 g, 22.8 mmol) in methanol (10 mL), portionwise, at room temperature, with stirring. Processing similar to that described in *A* gave **20** in almost 70% yield.

*Benzyl 4-(*N*-benzyloxycarbonyl-*D*-alanyl)amino-2-(benzyloxycarbonyl)amino-2,3,4-trideoxy-β-*L*-threo-pentopyranoside* (**21**). — To a solution of **20** (491 mg, 1.4 mmol) in acetonitrile (15 mL) were added *N*-(benzyloxycarbonyl)-D-alanine (443 mg, 2.2 mmol) and DCC (433 mg, 2.1 mmol) portionwise, with stirring at room temperature. Stirring was continued for 2 days, and the 1,3-dicyclohexylurea that had separated out was filtered off. Chloroform was added to the filtrate, and the solution was washed successively with aqueous sodium hydrogencarbonate and water, dried, and evaporated, to give a syrup which was purified on a column of silica gel with 10:1 benzene–acetone as eluant, to afford **21** in 78% yield; m.p. 139–140°, $[\alpha]_D$ +104.5°; ν_{max}^{KBr} 3325 (NH), 1690 (Z), and 1640 (amide) cm^{-1}; n.m.r.: 4.81 (d, H-1, $J_{1,2}$ 3.3), 1.75–2.05 (m, H-3*a*,3*e*), 3.44 (dd, H-5*a*, $J_{4,5a}$ 1.0, $J_{5a,5e}$ 12.0), 3.86 (dd, H-5*e*, $J_{4,5e}$ 1.5), 5.02 and 5.11 (each s, Z), 4 42 and 4.70 (ABq, Bn), 1.36 (d, C–Me, *J* 7.2), 2.72, 5.10, and 5.86 (NH).

Anal. Calc. for $C_{33}H_{37}N_3O_7$: C, 66.76; H, 6.48; N, 7.30. Found: C, 66 53; H, 6.40; N, 7.46.

*4-(*D*-Alanylamino)-2-amino-2,3,4-trideoxy-*L*-threo-pentopyranose* (**1**) *dihydrochloride.* — Compound **21** was hydrogenolyzed in methanol–water containing hydrochloric acid, in the presence of palladium–charcoal, as previously reported[5], to give **1** as the dihydrochloride in 94.5% yield; m.p. 145–152° (dec.), $[\alpha]_D$ −14.0° (*c* 0.69, H_2O); ^{13}C-n.m.r.: 192.4 (CO), 95.2 (C-1 of β anomer), 90.1 (C-1 of α anomer), 31.8 (C-3), and 17.9 p.p.m. (CH_3); ratio of α to β anomer, ~1.0:1.0.

Anal. Calc. for $C_8H_{19}Cl_2N_3O_3 \cdot H_2O$: C, 32.66; H, 7.20; N, 14.28. Found: C, 33.09; H, 7.33; N, 13.88.

*Benzyl 4-azido-3-(benzyloxycarbonyl)amino-3,4-dideoxy-β-*L*-arabinopyranoside* (**23**). — Compound **18** (ref. 12) was *N*-(benzyloxycarbonyl)ated as described for **11**, to give **23** in 92% yield; m.p. 104–106°, $[\alpha]_D$ +187.6°; ν_{max}^{KBr} 3430 and 3320 (OH and NH), 2130 (N_3), 1682, and 1535 (Z) cm^{-1}; n.m.r. (exchanged with D_2O): 4.92 (d, H-1, $J_{1,2}$ 3.3), 3.58 (dd, H-2, $J_{2,3}$ 10.5), 4.02 (dd, H-3, $J_{3,4}$ 3.3), 3.66 (dd, H-5*a*, $J_{4,5a}$ 1.8, $J_{5a,5e}$ 12.6), 5.09 (s, Z), 4.51, and 4.75 (ABq, Bn).

Anal. Calc. for $C_{20}H_{22}N_4O_5$: C, 60.29; H, 5.57; N, 14.06. Found: C, 60.06; H, 5.51; N, 13.37.

*Benzyl 4-amino-3-(benzyloxycarbonyl)amino-β-*L*-arabinopyranoside* (**24**). — Compound **23** was hydrogenolyzed in the presence of Raney nickel as described for **20** (Method *A*), to give **24** in 85.1% yield; m.p. 89–94°, $[\alpha]_D$ +15.1°; ν_{max}^{KBr} 3500 and 3350 (OH and NH), 1684, and 1540 (Z) cm^{-1}; n.m.r. (exchanged with D_2O): 4.92 (d, H-1, $J_{1,2}$ 3.8), 3.54 (dd, H-2, $J_{2,3}$ 10.8), 3.93 (dd, H-3, $J_{3,4}$ 4.5), 3.03 (m, H-4), 3.36

(dd, H-5*a*, $J_{4,5a}$ 1.5, $J_{5a,5e}$ 12.0), 3.95 (dd, H-5*e*, $J_{4,5e}$ 1.0), 5.08 (s, Z), 4.48, and 4.75 (ABq, Bn).

Anal. Calc. for $C_{20}H_{24}N_4O_5$: C, 64.50, H, 6.50; N, 7.52. Found: C, 64.31; H, 6.61; N, 7.07.

*Benzyl 4-(N-benzyloxycarbonyl-*D*-alanyl)amino-3-(benzyloxycarbonyl)amino-3,4-dideoxy-β-*L*-arabinopyranoside* (**25**). — Condensation of **24** and *N*-Z-D-Ala was performed as described for **21**, to give **25** in 92% yield; m.p. 162–164°, $[\alpha]_D$ +100.1°; ν_{max}^{KBr} 3340 (OH and NH), 1680, and 1550 (Z and amide) cm^{-1}; n.m r.: 4.90 (d, H-1, $J_{1,2}$ 3.8). 3.63 (dd, H-2, $J_{2,3}$ 10.7), 5.02 (s, Z), 4.44 and 4.68 (ABq, Bn), 1.31 (d, CH_3), 6.00, 5.60, and 2 74 (NH).

Anal. Calc. for $C_{31}H_{35}N_3O_7$: C, 66.29; H, 6.28; N, 7.48. Found: C, 65.91; H, 6.48; N, 7.97.

*4-(*D*-Alanylamino)-3-amino-3,4-dideoxy-*L*-arabinopyranose* (**2**) *dihydrochloride.* — Hydrogenolysis of **25** was performed as described for the preparation of **1**,to give **2** as the dihydrochloride in 93% yield; m p. 168–170° (dec.), $[\alpha]_D$ +124.8° (*c* 1.05, H_2O); ^{13}C-n.m.r.: 173.5 (CO), 98.5 (C-1 of β anomer), 93.1 (C-1 of α anomer), and 18.0 p.p.m. (CH_3); ratio of α to β anomer, ~1.3:1.0.

Anal. Calc. for $C_8H_{19}Cl_2N_3O_4 \cdot 0.5\ H_2O$: C, 31.90; H, 6.36; N, 13.95. Found: C, 31.78; H, 6.51. N, 13.58.

*Benzyl 4-(N-benzyloxycarbonyl-*D*-alanyl)amino-4-deoxy-β-*L*-arabinopyranoside* (**28**). — Into a suspension of[12] **26** (1.73 g, 7.0 mmol) and platinum oxide (58 mg) in methanol (40 mL) was bubbled hydrogen with stirring at room temperature, until all of the **26** had disappeared. The catalyst was filtered off, and the filtrate was evaporated, to give, in ~95% yield, crude **27**, which could be identified only by n.m r. spectroscopy, because of its lability; n.m.r. [**27**, 28 mg + $Pr(fod)_3$ 10 mg]: 4.57 (d, H-1, $J_{1,2}$ 3.0), 2.94 (t, H-2, $J_{2,3}$ 3.0), 2.35 (dd, H-3, $J_{3,4}$ 1.5), 1.90 (m, H-4), 2.25 (dd, H-5, $J_{4,5}$ 1.5, $J_{5,5'}$ 11.6), 3.34 (dd, H-5', $J_{4,5'}$ 2.3), and 4.37 and 4.58 (dd, Bn).

Then, condensation of **27** with *N*-Z-D-Ala was performed with DCC in pyridine as reported previously[5], to give **28** in 68% yield from **26**; $[\alpha]_D$ +112.4°; ν_{max}^{NaCl} 3300 (OH and NH), 1700, 1655, and 1524 (Z and amide) cm^{-1}; n.m.r.: 4.82 (d, H-1, $J_{1,2}$ 3.3), 3.66 (dd, H-2, $J_{2,3}$ 9.3), 5.03 (s, Z), 4.46 and 4.71 (ABq, Bn), and 1.31 (d, CH_3).

Anal. Calc. for $C_{23}H_{28}N_2O_7$: C, 62.15; H, 6.35; N, 6.31. Found: C, 61.91; H, 6.39; N, 6.42.

The usual acetylation of **26** gave the corresponding 2,3-diacetate (**29**) in quantitative yield; $[\alpha]_D$ +139.9°; ν_{max}^{NaCl} 3330 (NH), 1740 (ester), 1673, and 1520 (amide) cm^{-1}; n.m.r.: 5.14 (d, H-1, $J_{1,2}$ 3.3), 4.96 (dd, H-2, $J_{2,3}$ 10.5), 5.33 (dd, H-3, $J_{3,4}$ 4.5), 3.49 (dd, H-5*a*, $J_{4,5a}$ 1.5, $J_{5a,5e}$ 12.0), 4.09 (dd, H-5*e*, $J_{4,5e}$ 2.3), 4.26 (q, CH in Ala), 5.11 (s, Z), 4.48 and 4.73 (ABq, Bn), 1.37 (d, CH_3), 5.67 (d, NH), and 1.94 and 2.00 (each s, 2 Ac).

Anal. Calc. for $C_{27}H_{32}N_2O_9$: C, 61.35; H, 6.10; N, 5.30. Found: C, 61.39; H, 6.28; N, 5.24.

*4-(*D*-Alanylamino)-4-deoxy-*L*-arabinopyranose* (**3**) *dihydrochloride.* — Hydrogenolysis of **26** was performed as described for the preparation of **1**, to give **3** as the

dihydrochloride in 96% yield; m.p. 118–122° (dec.), $[\alpha]_D$ +61.6° (*c* 1.12, H_2O); ^{13}C-n.m.r.: 172.5 (CO), 97.9 (C-1 of β anomer), 93.5 (C-1 of α anomer), and 18.0 p.p.m. (CH_3); ratio of α to β anomer, ~2:1.

Anal. Calc. for $C_8H_{17}ClN_2O_5 \cdot 0.5\ H_2O$: C, 36.16; H, 6.82; N, 10.54. Found: C, 35.80; H, 6.48; N, 10.82.

Antifungal activity of **1**, **2**, *and* **3**. — Fungi were grown on potato–sucrose–agar, and inoculated on the same agar containing **1**, **2**, or **3** in three different concentrations. The activity was evaluated after 2 days of incubation at the optimum temperature (21 or 27°, according to the kind of fungus).

Antileukemic activity of **1**. — CDF_1 mice were inoculated intraperitoneally with 1×10^6 cells of P 388 (6 mice/group). A solution of compound **1** in physiological saline solution was administered intraperitoneally into mice, one and five days after leukemia inoculation.

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