for 2 h or until the arrhythmia returned, and the duration of conversion was noted. The data were analyzed by paired t test, and $p < 0.05$ was considered significant.

Benzamide Antiarrhythmic Plasma Assay. Plasma concentrations of the benzamide antiarrhythmics were quantitated by a modification of the GC procedure of Yamaji and co-workers.[26] The assay was linear from 1 to 10 μg/mL, and concentrations as low as 50 ng/mL in plasma could be detected. Compounds were dissolved in water and administered to Fischer 344 rats via the jugular vein, and blood samples were obtained by cardiac puncture. To 100 μL of plasma were added 500 ng of internal standard and 1 mL of 2 N sodium hydroxide. This mixture was applied to a Chem Elut column (Analytichem Int., Harbor City, CA), and after 15 min the column was eluted with 10 mL of dichloromethane. The eluate was taken to dryness under nitrogen and reconstituted with 50 μL of ethyl acetate, and 2 μL was injected into the GC system. Procainamide was used as the internal standard for the analysis of plasma concentrations of **5** and **8**, and **5** was used as the internal standard for analysis of plasma concentrations of procainamide and *N*-acetylprocainamide. A Hewlett-Packard Model 5890 gas chromatograph, equipped with a nitrogen–phosphorus detector, a Model 3393A reporting integrator, and a Model 7673A autosampler, was used for analysis of plasma concentrations of the benzamides. The capillary column used was an 30-m, 0.32 mm column with a 0.25 μm DB-5 coating (J. W. Scientific, Folsom, CA). The injector and detector temperatures were 300 °C, and the column temperature was 235 °C. Flow rate of carrier helium was 2.7 mL/min. Under these conditions, retention times were as follows: procainamide, 3.45 min; *N*-acetylprocainamide, 7.8 min; dimethylprocainamide, 5.11 min; and *N*-acetyldimethylprocainamide, 8.98 min.

Acknowledgment. We thank Della Nation for preparation of the manuscript and Eric Evenson for technical assistance.

Registry No. 1, 51-06-9; 1 (*N*-acetyl), 32795-44-1; 4, 112740-76-8; 4·2HCl, 112740-75-7; **5**, 112740-74-6; **5**-2HCl, 112740-73-5; **6**, 112740-71-3; **6**·oxalate, 112740-70-2; **7**, 112740-69-9; **7**-oxalate, 112740-72-4; **8**, 112740-77-9; $H_2N(CH_2)_2N(CH_2CH_3)_2$, 100-36-7; 3,5-dimethyl-4-nitrobenzoic acid, 3095-38-3; 3,5-dimethyl-4-nitrobenzoic acid chloride, 3558-73-4; 3-methyl-4-nitrobenzoic acid, 3113-71-1.

(26) Yamaji, A.; Kataoka, K.; Oishi, M.; Kanamori, N.; Hiraoka, E. *J. Chromatogr.* **1987**, *415*, 143.

Synthesis and Antitumor Activity of Quaternary Ellipticine Glycosides, a Series of Novel and Highly Active Antitumor Agents

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A series of ellipticine glycosides [2-*N*-glycosyl quaternary pyridinium salts of three ellipticines: ellipticine (**1**), 9-methoxyellipticine (**2**), and 9-hydroxyellipticine (**4**)] were stereoselectively synthesized in good yields by an improved condensation reaction between ellipticines [**1**, **2**, and 9-acetoxyellipticine (**3**)] and protected (peracylated and perbenzylated) glycosyl halides with cadmium carbonate, followed by deprotection. These glycosides were preliminarily evaluated for their antitumor activity in the L1210 leukemia system. Twenty-six (53%) of the 49 glycosides tested were curative, and five [9-hydroxyellipticine L-arabinopyranoside (**41b**), D-lyxofuranoside (**43a**), L-lyxopyranoside (**44b**), D-xylofuranoside (**49a**), and L-rhamnopyranoside (**56**)] were selected for extended evaluation on the basis of their high levels of activity. The structure–activity relationships are discussed. The selected glycosides showed remarkable activity in six different murine tumor systems with excellent therapeutic ratios; their efficacy surpassed that of doxorubicin against three of these systems. On the basis of these results and ease of formulation, the two glycosides **41b** (SUN4599) and **49a** (SUN5073) were selected for further preclinical evaluation and possible clinical development.

Ellipticine (**1**, 5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole) and 9-methoxyellipticine (**2**) are alkaloids isolated from various plants of the Apocynaceae family.[1-4] These alkaloids and some of their derivatives exhibit antitumor properties in tests with experimental animal tumors.[5-8] Most ellipticine derivatives are insoluble in water because of their hydrophobic structures and this has led to considerable difficulty in developing formulations for clinical use. To date, of these compounds, 9-methoxyellipticine lactate[9] and 9-hydroxy-2-methylellipticinium acetate (celiptium, **5**),[10,11] water-soluble derivatives, have been found to be effective against human myeloblastic leukemia

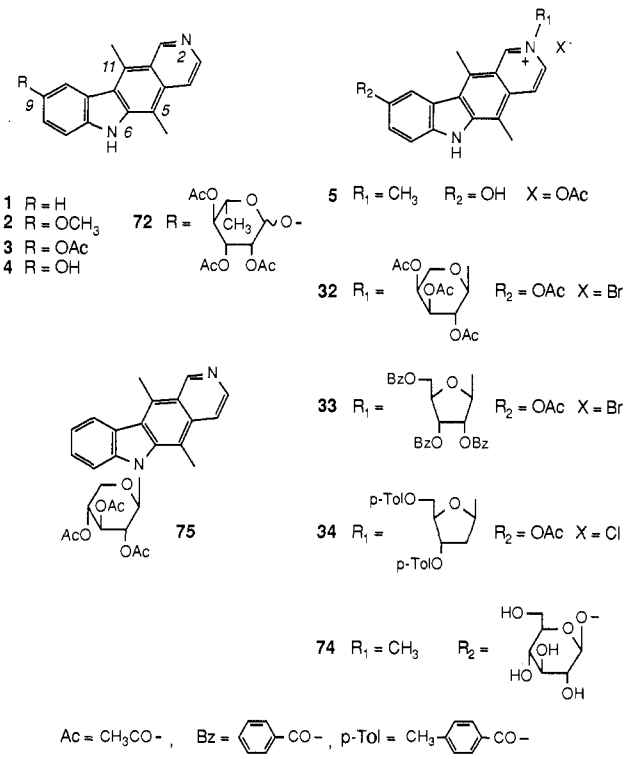
(1) Goodwin, S.; Smith, A. F.; Horning, E. C. *J. Am. Chem. Soc.* **1959**, *81*, 1903.
(2) Loder, J. W. *Aust. J. Chem.* **1966**, *19*, 1947.
(3) Kilminster, K. N.; Sainsbury, M.; Webb, B. *Phytochemistry* **1972**, *11*, 389.
(4) Ahond, A.; Fernandez, H.; Julia-Moore, M.; Poupat, C.; Sânchez, V.; Potier, P.; Kan, S. K.; Sēvenet, T. *J. Nat. Prod.* **1981**, *44*, 193.
(5) Dalton, L. K.; Demerac, S.; Elmes, B. C.; Loder, J. W.; Swan, J. M.; Teitei, T. *Aust. J. Chem.* **1967**, *20*, 2715.
(6) Guthrie, R. W.; Brossi, A.; Mennona, F. A.; Mullin, J. G.; Kierstead, R. W.; Grunberg, E. *J. Med. Chem.* **1975**, *18*, 755.
(7) Werbel, L. M.; Angelo, M.; Fry, D. W.; Worth, D. F. *J. Med. Chem.* **1986**, *29*, 1321.
(8) Archer, S.; Ross, B. S.; Pica-Mattoccia, L.; Cioli, D. *J. Med. Chem.* **1987**, *30*, 1204.
(9) Mathē, G.; Hayat, M.; De Vassal, F.; Schwarzenberg, L.; Schneider, M.; Schlumberger, J. R.; Jasmin, C.; Rosenfeld, C. *Rev. Eur. Etud. Clin. Biol.* **1970**, *15*, 541.
(10) (a) Paoletti, C.; Le Pecq, J. B.; Dat Xuong, N.; Lesca, P.; Lecointe, P. *Curr. Chemother.* **1978**, *2*, 1195. (b) Le Pecq, J. B.; Paoletti, C. U.S. Patent 4 310 667, 1982; Chem. Abstr. **1982**, *96*, 181489*s*.
(11) Juret, P.; Tanguy, A.; Girard, A.; Le Talaer, J. Y.; Abbatucci, J. S.; Dat Xuong, N.; Le Pecq, J. B.; Paoletti, C. *Eur. J. Cancer* **1978**, *14*, 205.

0022-2623/88/1831-1295$01.50/0

and human breast cancer, respectively.

On the basis of the information so far obtained, ellipticine derivatives are generally thought to exert their antitumor activity via their interaction with DNA.[12,13] Some of DNA synthesis inhibitors, e.g., anthracycline antibiotics[14] (intercalation into DNA), bleomycins[15] (cleavage of DNA strand), and epipodophyllotoxin glucosides[16] (inhibition of topoisomerase II) are well known to possess sugar moieties, which seem to strongly influence their bioavailability, permeability, and selective toxicity toward tumor cells. It would be of great interest, therefore, to combine the ellipticine nucleus with a sugar moiety. In particular, sugar moieties are expected to increase the water solubility of such ellipticine derivatives.

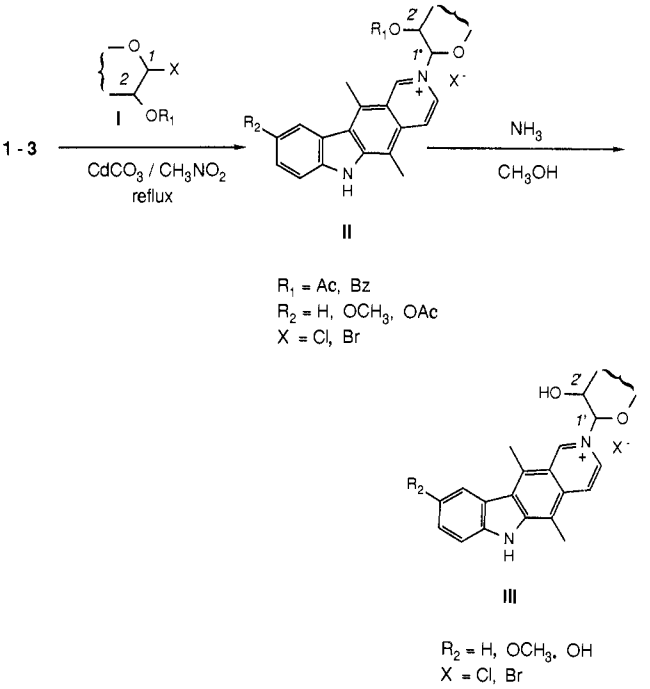
Consequently, we decided to synthesize 2-*N*-glycosyl quaternary pyridinium salts of some ellipticine nuclei (ellipticine glycosides).[17] In this paper, the synthesis and antitumor activity of ellipticine glycosides are reported.



Chemistry

Ellipticine 1′,2′-trans-peracylated glycosides (II) can be synthesized by the condensation of ellipticines [ellipticine (**1**),[5] 9-methoxyellipticine (**2**),[5] and 9-acetoxyellipticine (**3**)[6]] with an equimolar amount of peracylated glycosyl halides (I) in aprotic polar solvents (e.g., nitromethane, acetonitrile, etc.).[20] However, this method is not satisfactory due to low yield and poor stereoselectivity. In our previous communication,[21] we reported several examples of the use of $CdCO_3$ with excess (1.5–2.0 equiv) peracylated glycosyl halides (I) to provide predominantly 1′,2′-trans-peracylated glycosides (II) in good yields. Accordingly, we attempted to synthesize various ellipticine 1′,2′-*trans*-glycosides (III) by this improved method followed by deprotection (Scheme I).

Scheme I



Condensation of 9-acetoxyellipticine (**3**) with 2,3,4-tri-*O*-acetyl-β-L-arabinopyranosyl bromide (**6b**)[22] (2 equiv) with $CdCO_3$ in refluxing dry nitromethane afforded 9-acetoxy-2-(2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl)ellipticinium bromide (**32**, containing 3% of the β-anomer[23]) in 97% yield. Deprotection was achieved with methanolic ammonia to give 2-α-L-arabinopyranosyl-9-hydroxyellipticinium bromide (**41b**) containing 3% of the β-anomer[23] in 87% yield. The desired pure α-anomer (**41b**) was obtained by recrystallization from methanol. This procedure was satisfactory for the preparation of other 1′,2′-*trans*-pyranosides, except for the following two cases, xylopyranosides (**50a** and **50b**) and L-rhamnopyranosides (**54–56**), which contained a considerable amount of the corresponding 1′,2′-cis isomer.[24] (See Table I.)

Condensation of **3** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride (**26**)[25] in the presence of $CdCO_3$ failed to give the desired ribofuranoside. However, by use of 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**27a**),[20b] 9-acetoxy-2-

(12) Le Pecq, J. B.; Dat Xuong, N.; Gosse, C.; Paoletti, C. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 5078.
(13) Jain, S. C.; Bhandary, K. K.; Sobell, H. M. *J. Mol. Biol.* **1979**, *135*, 813.
(14) See, for example: Arcamone, F. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J. D., Ed.; Academic: New York, 1980; p 1.
(15) See, for example: Umezawa, H. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J. D., Ed.; Academic: New York, 1980; p 148.
(16) See, for example: Jardine, I. In *Anticancer Agents Based on Natural Product Models*; Cassady, J. M., Douros, J. D., Ed.; Academic: New York, 1980; p 319.
(17) Syntheses of 9-*O*-glycosyl[18] [e.g., 9-β-D-glucopyranosyloxy-2-methylellipticinium (**74**)] and 6-*N*-glycosyl derivatives[19] [e.g., 6-(2,3,4-tri-*O*-acetyl-β-D-xylopyranosyl)ellipticine (**75**)] were reported without their biological properties.
(18) Duguē, B.; Meunier, B.; Paoletti, C. *Eur. J. Med. Chem.* **1983**, *18*, 551.
(19) Bessodes, M.; Antonakis, K.; Dat Xuong, N. *J. Carbohydr. Nucleosides Nucleotides* **1977**, *4*, 215.
(20) See for the preparation of nicotinamide nucleosides: (a) Haynes, L. J.; Todd, A. R. *J. Chem. Soc.* **1950**, 303. (b) Mikhailopulo, I. A.; Pricota, T. I.; Timoshchuk, V. A.; Akhrem, A. A. *Synthesis* 1981, 388 and references cited therein.
(21) Honda, T.; Inoue, M.; Kato, M.; Shima, K.; Shimamoto, T. *Chem. Pharm. Bull.* **1987**, *35*, 3975.
(22) Humoller, F. L. *Methods Carbohydr. Chem.* **1962**, *1*, 83.
(23) Isomer ratio was determined by HPLC analysis.
(24) At present, we cannot suggest a reasonable explanation for these findings.
(25) Davoll, J.; Lythgoe, B.; Todd, A. R. *J. Chem. Soc.* **1948**, 967.

(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)ellipticinium bromide (**33**) was successfully obtained in 77% yield.[24] Deprotection of **33** in methanolic ammonia gave 9-hydroxy-2-β-D-ribofuranosylellipticinium bromide (**47a**) in 80% yield. Other 1′,2′-*trans*-furanosides have also been synthesized stereoselectively by using the corresponding perbenzoylated furanosyl halides. In the case of D-erythro- (**36**) and lyxofuranosides (**43a** and **43b**), a considerable amount of the 1′,2′-cis isomer was detected.[24] (See Table I.)

The 1′,2′-trans relationship of each pyranoside was easily shown by the large trans-diaxial coupling constant ($J_{1',2'}$ = 8.5–9.5 Hz) of the anomeric proton (1′-H) which appears at δ 5.74–6.24 in the ^{1}H NMR spectrum. However, in the case of furanosides, the ^{1}H NMR coupling constant cannot be used to make unambiguous assignment of the anomeric configuration. Therefore, 1′,2′-trans relationships were determined by comparison of the 1′,2′-trans isomers with the corresponding 1′,2′-cis isomers, one of whose structures had been established by an NOE experiment. (The details will be given later.)

The remarkable stereoselectivity of these condensation reaction may be caused by an anchimeric assistance[26,27] which seems to be enhanced by $CdCO_3$.

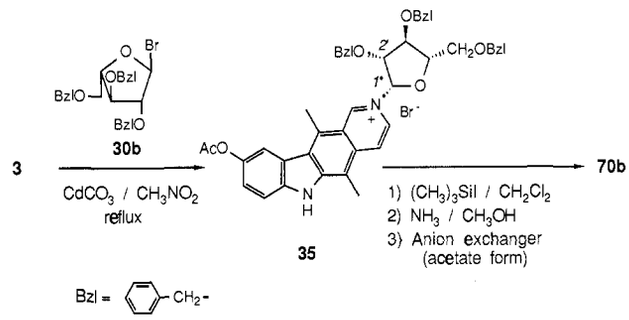
In order to obtain information on the structure–activity relationships of the sugar moieties, peracylated glycoside [2-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-9-hydroxyellipticinium bromide (**58**)], 2′-deoxyglycoside [2-(2-deoxy-β-D-ribofuranosyl)-9-hydroxyellipticinium chloride (**39**)], and 1′,2′-*cis*-glycosides [e.g., 2-β-L-arabinofuranosyl-9-hydroxyellipticinium acetate (**70b**)] were prepared as follows.

Tri-*O*-acetyl-L-rhamnopyranoside (**58**) was obtained only in a low yield (20%) by the condensation of unprotected 9-hydroxyellipticine (**4**)[6] with 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**11**)[28] with $CdCO_3$. It should be noted that 9-(2,3,4-tri-*O*-acetyl-L-rhamnopyranosyloxy)ellipticine (**72**) was not obtained, although Conrow and Bernstein have reported the Königs–Knorr synthesis of aryl glucuronides with $CdCO_3$.[29]

2-Deoxy-β-D-ribofuranoside (**39**) was prepared by the condensation of **3** with crystalline 2-deoxy-3,5-bis-*O*-(*p*-toluoyl)-α-D-ribofuranosyl chloride (**23**)[30] in the presence of $CdCO_3$, followed by the deprotection of the condensation product (**34**) with methanolic ammonia (27% and 76% yields for the condensation and deprotection, respectively, were obtained). The β configuration of **39** was clearly established by ^{1}H NMR; the anomeric proton (1′-H) showed the expected triplet at δ 6.67 (J = 6 Hz) for the β-anomer.[31] Although **23** exhibits no anchimeric assistance effect, it is interesting to note that only the β-anomer (**39**) is obtained. This finding suggests that this condensation must proceed mainly by an S_N2 type reaction.

1′,2′-*cis*-Glycosides have been successfully prepared. It was believed that 1,2-trans-peracylated glycosyl halides would provide predominantly 1′,2′-*trans*-glycosides because of the possibility of anchimeric assistance.[26,27] We further

Scheme II



postulated that 1,2-trans-perbenzylated glycosyl halides in which anchimeric assistance is not possible should afford 1′,2′-*cis*-glycosides by an S_N2 type reaction. Condensation of **3** with 2,3,5-tri-*O*-benzyl-α-L-arabinofuranosyl bromide (**30b**)[32] with $CdCO_3$ gave 9-acetoxy-2-(2,3,5-tri-*O*-benzyl-β-L-arabinofuranosyl)ellipticinium bromide (**35**, containing 4% of the α-anomer[23]) in 49% yield, as was expected. β-L-Arabinofuranoside (**70b**) containing 4% of the α-anomer[23] was obtained in 62% yield by debenzylation (iodotrimethylsilane)[33] and deacetylation (methanolic ammonia), followed by exchange of the counterion from bromide to acetate by an anion exchange resin (acetate form) chromatography (Scheme II). It was recrystallized from methanol to give the desired pure β-anomer (**70b**). Similarly, other 1′,2′-*cis*-glycosides could be prepared by using the corresponding 1,2-trans-perbenzylated glycosyl halides (Table II). The cis relationship between 1′-H and 2′-H of **70b** was confirmed by an NOE experiment; an NOE of 6.6% was observed on 2′-H (δ 4.52) upon irradiation of 1′-H (δ 6.52).

As was expected, all of the ellipticine glycosides thus obtained were found to be easily soluble in water. It must be noted that they decompose thermally before melting [e.g., α-L-arabinopyranoside (**41b**) decomposes into 9-hydroxyellipticine hydrobromide (**73**) (confirmed by ^{1}H NMR), carbon, and a stoichiometric amount of water at 240–245 °C], and therefore they have no distinct melting point. This pyrolysis seems to proceed by a mechanism similar to the demethylation of *N*-methyl quaternary ammonium salts upon heating.[34] The sugar moieties eliminated in this process must have been converted to carbon by dehydration.

Biological Results and Discussion

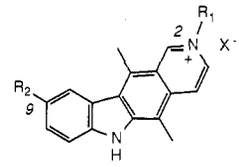
Preliminary Screening Results. Tables I and II show the antitumor effects of ellipticine glycosides against intraperitoneally (ip) implanted L1210 leukemia when administered ip for 5 consecutive days. Forty five (92%) of the 49 glycosides tested showed significant activity (ILS > 25%) and 26 (58%) of the 45 active glycosides were curative (i.e., mice tested survived for 80 days or longer). Five of the 26 highly active glycosides [L-arabinopyranoside (**41b**), D-lyxofuranoside (**43a**), L-lyxopyranoside (**44b**), D-xylofuranoside (**49a**), and L-rhamnopyranoside (**56**)] were selected for extended evaluation in various murine tumor systems on the basis of their high levels of activity against L1210 leukemia.

These preliminary screening results revealed some interesting structure–activity relationships against L1210 leukemia as follows.

(1) 9-Hydroxyellipticine glycosides show much higher activity than the corresponding ellipticine glycosides and

(26) Overend, W. G. In *The Carbohydrates Chemistry and Biochemistry*, 2nd ed.; Pigman, W., Horton, D., Ed.; Academic: New York, 1972; Vol. 1, Part A, p 292 and references cited therein.
(27) Hough, L.; Richardson, A. C. In *Rodd's Chemistry of Carbon Compounds*, 2nd ed.; Coffey, S., Ed.; Elsevier: Amsterdam, 1967; Vol. 1, Part F, p 323 and references cited therein.
(28) Bebault, G. M.; Dutton, G. G. S.; Warfield, C. K. *Carbohydr. Res.* **1974**, *34*, 174.
(29) Conrow, R. B.; Bernstein, S. *J. Org. Chem.* **1971**, *36*, 863.
(30) Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777.
(31) Robins, M. J.; Robins, R. K. *J. Am. Chem. Soc.* **1965**, *87*, 4934.
(32) Barker, R.; Fletcher, H. G., Jr. *J. Org. Chem.* **1961**, *26*, 4605.
(33) Jung, M. E.; Lyster, M. A. *J. Org. Chem.* **1977**, *42*, 3761.
(34) Wilson, N. D. V.; Joule, J. A. *Tetrahedron* **1968**, *24*, 5493.

Table I. Ellipticine 1′,2′-*trans*-Glycosides



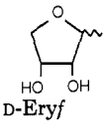
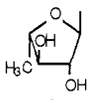
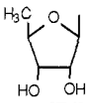
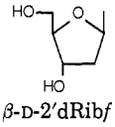
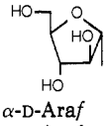
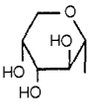
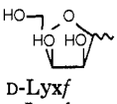
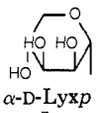
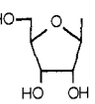
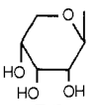
compd[a]	R_1[b]	R_2	X^-[c]	yield,[d] %	formula[e]	$[\alpha]_D$,[f] deg (*c*)	^{1}H NMR:[g] 1′-H, δ ($J_{1',2'}$, Hz)	effect on L1210 leukemia[h] optimal dose, mg/kg	ILS %[i]	cures[j]
36[k]	 D-Ery*f*	OH	Cl	29[k]	$C_{21}H_{21}N_2O_4Cl\cdot 2H_2O$	−240 (0.14)	6.26 (d, 6.5),[l] 6.45 (d, 6.5)[m]	5	122	0/6
37	 α-L-5′dAra*f*	OH	Cl	74	$C_{22}H_{23}N_2O_4Cl\cdot 2H_2O$	−24 (0.19)	6.39 (d, 2.5)	30	>261	1/6
38	 β-D-5′dRib*f*	OH	Cl	28	$C_{22}H_{23}N_2O_4Cl\cdot xH_2O$[n]	−240 (0.19)	6.28 (d, 5.5)	30	>230	1/6
39	 β-D-2′dRib*f*	OH	Cl	21	$C_{22}H_{23}N_2O_4Cl\cdot xH_2O$[n]	−88 (0.20)	6.67 (t, 6.0)	10	>390	2/6
40a	 α-D-Ara*f*	OH	Br	54	$C_{22}H_{23}N_2O_5Br\cdot 1.5H_2O$	+52 (0.22)	6.35 (d, 3.0)	20	91	0/6
40b	α-L-Ara*f*	OH	Br	54	$C_{22}H_{23}N_2O_5Br\cdot 1.5H_2O$	−38 (0.11)	6.35 (d, 3.0)	10	>379	2/6
41a	 α-D-Ara*p*	OH	Br	84	$C_{22}H_{23}N_2O_5Br\cdot 2H_2O$	+230 (0.20)	5.74 (d, 8.5)	20	>606	3/6
41b	α-L-Ara*p*	OH	Br	84	$C_{22}H_{23}N_2O_5Br\cdot 2H_2O$	−240 (0.19)	5.74 (d, 8.5)	30	>860	5/6
42	α-L-Ara*p*	OCH_3	Br	72	$C_{23}H_{25}N_2O_5Br$	−180 (0.41)	5.76 (d, 9.0)	30	56	0/6
43a[o]	 D-Lyx*f*	OH	Cl	74[o]	$C_{22}H_{23}N_2O_5Cl\cdot xH_2O$[n]	+250 (0.42)	6.27 (d, 6.5),[l] 6.43 (d, 6.5)[m]	30	>967	6/6
43b[o]	L-Lyx*f*	OH	Cl	74[o]	$C_{22}H_{23}N_2O_5Cl\cdot xH_2O$[n]	−280 (0.14)	6.27 (d, 6.5),[l] 6.43 (d, 6.5)[m]	10	>552	3/6
44a	 α-D-Lyx*p*	OH	Cl	21	$C_{22}H_{23}N_2O_5Cl\cdot 1.5H_2O$	+400 (0.09)	6.02 (d, 9.0)	10	>651	4/6
44b	α-L-Lyx*p*	OH	Cl	21	$C_{22}H_{23}N_2O_5Cl\cdot 1.5H_2O$	−320 (0.19)	6.02 (d, 9.0)	30	>786	5/6
45	 β-D-Rib*f*	H	Br	24	$C_{22}H_{23}N_2O_4Br$	−150 (0.24)[p]	6.30 (d, 5.0)	20	80	0/6
46	β-D-Rib*f*	OCH_3	Br	45	$C_{23}H_{25}N_2O_5Br$	−180 (0.08)[q]	6.29 (d, 5.0)	30	76	0/6
47a	β-D-Rib*f*	OH	Br	62[r]	$C_{22}H_{23}N_2O_5Br$	−180 (0.16)	6.28 (d, 5.0)	10	138	0/6
47b	β-L-Rib*f*	OH	Br	62[r]	$C_{22}H_{23}N_2O_5Br$	+190 (0.11)	6.28 (d, 5.0)	10	>391	2/6
48a	 β-D-Rib*p*	OH	Br	39	$C_{22}H_{23}N_2O_5Br\cdot 1.5H_2O$	−330 (0.20)	5.98 (d, 9.0)	30	112	0/6
48b	β-L-Rib*p*	OH	Br	39	$C_{22}H_{23}N_2O_5Br\cdot 1.5H_2O$	+310 (0.28)	5.98 (d, 9.0)	30	>944	6/6

Table I (Continued)

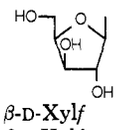
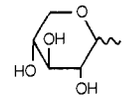
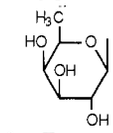
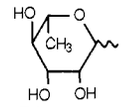
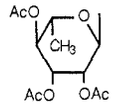
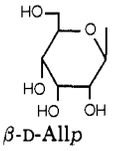
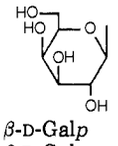
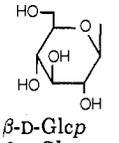
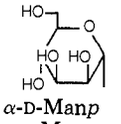
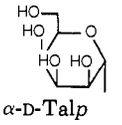
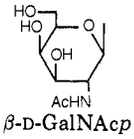
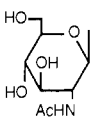
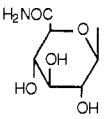
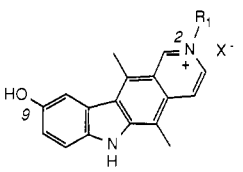
compd[a]	R_1[b]	R_2	X[-c]	yield,[d] %	formula[e]	$[\alpha]_D$,[f] deg (*c*)	^{1}H NMR:[g] 1′-H, δ ($J_{1',2'}$, Hz)	effect on L1210 leukemia[h] optimal dose, mg/kg	ILS %[i]	cures[j]
49a	 β-D-Xyl*f*	OH	Cl	70	$C_{22}H_{23}N_2O_5Cl\cdot H_2O$	+150 (0.17)	6.32 (s)	30	>682	4/6
49b	β-L-Xyl*f*	OH	Cl	70	$C_{22}H_{23}N_2O_5Cl\cdot H_2O$	−170 (0.35)	6.32 (s)	30	>387	2/6
50a[s]	 D-Xyl*p*	OH	Br	57[s]	$C_{22}H_{23}N_2O_5Br\cdot 2H_2O$	−230 (0.12)	5.80 (d, 9.0),[l] 6.34 (s)[m]	30	>857	5/6
50b[s]	L-Xyl*p*	OH	Br	57[s]	$C_{22}H_{23}N_2O_5Br\cdot 2H_2O$	+260 (0.16)	5.80 (d, 9.0),[l] 6.34 (s)[m]	10	>396	2/6
51	 β-D-Fuc*p*	H	Br	32	$C_{23}H_{25}N_2O_4Br$	−170 (0.15)[q]	5.83 (d, 9.0)	30	20	0/6
52	β-D-Fuc*p*	OCH_3	Br	56	$C_{24}H_{27}N_2O_5Br$	+46 (0.19)[p]	5.82 (d, 8.5)	30	20	0/6
53a	β-D-Fuc*p*	OH	Br	80	$C_{23}H_{25}N_2O_5Br$	−210 (0.14)	5.78 (d, 9.0)	30	124	0/6
53b	β-L-Fuc*p*	OH	Br	80	$C_{23}H_{25}N_2O_5Br$	+190 (0.19)	5.78 (d, 9.0)	30	>583	3/6
54[t]	 L-Rha*p*	H	Br	65[t]	$C_{23}H_{25}N_2O_4Br$	−20 (0.11)[u]	6.26 nd, 9.0)[v]	40	40	0/6
55[w]	L-Rha*p*	OCH_3	Br	72[w]	$C_{24}H_{27}N_2O_5Br$	−8 (0.20)[p]	6.24 (d, 9.0)[v]	30	56	0/6
56[x]	L-Rha*p*	OH	Br	88[x]	$C_{23}H_{25}N_2O_5Br\cdot 2H_2O$	−300 (0.12)	6.22 (d, 8.5)[v]	30	>693	4/6
57[x]	L-Rha*p*	OH	OAc	76[x]	$C_{25}H_{28}N_2O_7$	−280 (0.36)	6.24 (d, 8.5)[v]	30	>615	3/6
58		OH	Br	20	$C_{29}H_{31}N_2O_8Br$	+59 (0.11)[u]	6.67 (d, 8.5)	60	89	0/6
59	 β-D-All*p*	OH	Br	28	$C_{23}H_{25}N_2O_6Br\cdot H_2O$	−380 (0.28)	6.05 (d, 9.0)	10	>254	1/6
60	 β-D-Gal*p*	H	Br	53	$C_{23}H_{25}N_2O_5Br$	+53 (0.12)[u]	5.87 (d, 9.0)	40	39	0/6
61	β-D-Gal*p*	OCH_3	Br	62	$C_{24}H_{27}N_2O_6Br$	+45 (0.11)[p]	5.83 (d, 9.0)	10	14	0/6
62a	β-D-Gal*p*	OH	Br	72	$C_{23}H_{25}N_2O_6Br$	−290 (0.16)	5.82 (d, 8.5)	20	>729	4/6
62b	β-L-Gal*p*	OH	Br	72	$C_{23}H_{25}N_2O_6Br$	+320 (0.21)	5.82 (d, 8.5)	2.5	58	0/6
63a	 β-D-Glc*p*	OH	Br	44	$C_{23}H_{25}N_2O_6Br\cdot xH_2O$[n]	−300 (0.12)	5.88 (d, 9.0)	10	52	0/6
63b	β-L-Glc*p*	OH	Br	44	$C_{23}H_{25}N_2O_6Br\cdot xH_2O$[n]	+310 (0.17)	5.88 (d, 9.0)	5	58	0/6
64a	 α-D-Man*p*	OH	Br	31	$C_{23}H_{25}N_2O_6Br\cdot xH_2O$[n]	+300 (0.27)	6.15 (d, 8.5)	10	88	0/6
64b	α-L-Man*p*	OH	Br	31	$C_{23}H_{25}N_2O_6Br\cdot xH_2O$[n]	−300 (0.24)	6.15 (d, 8.5)	5	>362	2/6
65	 α-D-Tal*p*	OH	Br	36	$C_{23}H_{25}N_2O_6Br\cdot 1.5H_2O$	+410 (0.13)	6.09 (d, 9.0)	10	>212	1/6
66	 β-D-GalNAc*p*	OH	Cl	24	$C_{25}H_{28}N_3O_6Cl\cdot 3.5H_2O$	−190 (0.13)	5.82 (d, 9.5)	30	8	0/6

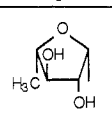
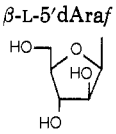
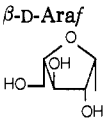
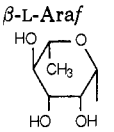
Table I (Continued)

compd[a]	R_1[b]	R_2	X^-[c]	yield,[d] %	formula[e]	$[\alpha]_D$,[f] deg (*c*)	^{1}H NMR:[g] 1′-H, δ ($J_{1',2'}$, Hz)	effect on L1210 leukemia[h] optimal dose, mg/kg	ILS %[i]	cures[j]
67	 β-D-GlcNAc*p*	OH	Cl	24	$C_{25}H_{28}N_3O_6Cl \cdot xH_2O$[n]	−280 (0.10)	5.93 (d, 9.5)	30	39	0/6
68		OH	Br	33	$C_{23}H_{24}N_3O_6Br \cdot H_2O$	−220 (0.13)	5.93 (d, 9.0)	5	49	0/6

[a] The pure 1′,2′-*trans*-glycosides obtained by recrystallization from methanol were used for measurement of physicochemical data, unless otherwise stated. The glycosides without recrystallization were administered to the L1210 leukemia bearing mice. [b] *f*, furanosyl; *p*, pyranosyl. [c] The counterions of the glycosides were determined by means of ion chromatography. [d] Isolated yield before recrystallization based on the corresponding ellipticines (**1**–**4**): containing a small amount (<5%) of the 1′,2′-cis isomer, unless otherwise stated. All the isomer ratios were determined by HPLC analyses. [e] C, H, and N analyses were within ±0.4% of the theoretical values, unless otherwise stated. [f] In water containing 1% CF_3CO_2H at 25 °C, unless otherwise noted. [g] In Me_2SO-d_6. [h] BDF_1 mice were implanted ip with 10^5 cells, dosed ip on days 1–5, and deaths were recorded for 80 days. [i] Increase in mean life span. [j] Number of survivors/total at 80 days. [k] α-:β-anomer = 12:88. [l] 1′-H signal of the 1′,2′-trans isomer. [m] 1′-H signal of the 1′,2′-cis isomer. [n] Consistent and correct results could not be obtained because the glycoside is extremely hygroscopic. [o] α-:β-anomer = 76:24. [p] In methanol at 29–31 °C. [q] In water at 29–31 °C. [r] β-Anomer only. [s] α-:β-anomer = 29:71. [t] α-:β-anomer = 91:9. [u] In ethanol at 29 °C. [v] The 1′-H signal of the β-anomer could not be observed because of overlapping with that of the α-anomer. [w] α-:β-anomer = 90:10. [x] α-:β-anomer = 92:8.

Table II. 9-Hydroxyellipticine 1′,2′-*cis*-Glycosides



compd[a]	R_1[b]	X^-[c]	yield,[d] %	MS (SIMS), *m*/*z*	$[\theta]_\lambda$[e] (λ, nm)	^{1}H NMR:[f] 1′-H, δ ($J_{1',2'}$, Hz)	effect on L1210 leukemia[h] optimal dose, mg/kg	ILS %[i]	cures[j]
69	 β-L-5′dAra*f*	OAc	29	$[C_{22}H_{23}N_2O_4]^+$ 379	+590 (314) −720 (227)	6.59 (d, 5.0)	30	>217	1/6
70a	 β-D-Ara*f*	OAc	30	$[C_{22}H_{23}N_2O_5]^+$ 395	−800 (320) −610 (262) +340 (231)	6.55 (d, 6.0)	30	>262	1/6
70b	 β-L-Ara*f*	OAc	30	$[C_{22}H_{23}N_2O_5]^+$ 395	+690 (320) +560 (256) −470 (229)	6.55 (d, 6.0)	30	>269	1/6
71	 β-L-Rha*p*	Br	16	$[C_{23}H_{25}N_2O_5]^+$ 409	+800 (285)	6.21 (s)[g]	40	78	0/6

[a] The pure 1′,2′-*cis*-glycosides obtained by recrystallization from methanol were used for measurement of physicochemical data. None gave consistent and correct analytical data because of their extreme hygroscopicity. The glycosides without recrystallization were administered to the L1210 leukemia bearing mice. [d] Isolated yield before recrystallization based on 9-acetoxyellipticine (**3**): containing a small amount (<5%) of the 1′,2′-trans isomer as determined by HPLC analysis. [e] Molecular ellipticity [deg/(mol/dL) dm] in water. [f] In Me_2SO-d_6 containing 1% CF_3CO_2H, unless otherwise noted. [g] In Me_2SO-d_6. [b,c,h–j] Same as footnotes in Table I.

9-methoxyellipticine glycosides [compare D-fucopyranoside (**53a**) with **51** and **52**, L-rhamnopyranoside (**56**) with **54** and **55**, and D-galactopyranoside (**62a**) with **60** and **61**]. This finding suggests the importance of a hydroxyl group at 9-position.

(2) Peracylated glycosides are less active than the corresponding deacylated glycosides [compare tri-*O*-acetyl-L-rhamnopyranoside (**58**) with L-rhamnopyranoside (**56**)], suggesting the importance of hydroxyl groups in the sugar moieties (i.e., hydrophilicity).

(3) The level of activity does not depend on the nature of the counterion [compare L-rhamnopyranoside bromide (**56**) with the corresponding acetate (**57**; for the preparation, see the Experimental Section)].

(4) The introduction of an amide linkage into the sugar moieties drastically reduces activity [compare 2-acetamido-2-deoxy-D-galactopyranoside (**66**) with D-galactopyranoside (**62a**)].

Table III. Effects of Selected Ellipticine Glycosides in Syngeneic Tumor Systems[a]

	L1210 leukemia						P388 leukemia						B16 melanoma (ip-ip)		colon 38 carcinoma (sc-ip)	
	ip-ip			ip-iv			ip-ip			ip-iv						
compd	dose, mg/kg	ILS %	cures	dose, mg/kg	ILS %	cures	dose, mg/kg	ILS %	cures	dose, mg/kg	ILS %	cures	dose, mg/kg	ILS %	dose, mg/kg	T/C %
	40	>713	4/6	30	>344	2/6	40	>789	6/6	20	>394	2/6	30	74	50	4
	20	>251	1/6	20	198		20	>549	3/6	10	83		20	60	40	7
41b	10	113		10	82		10	>214	1/6				10	46	30	59
	5	57					5	94					5	44	20	66
	1.25	37					1.25	65					2.5	38		
	40	>281	1/6	32	134		40	>220	2/6	32	176		20	77	40	8
	20	>503	3/6	16	81		20	>510	4/6	16	82		10	44	30	19
43a	10	>377	2/6	8	49		10	>332	2/6				5	39	20	61
	5	102					5	158								
	1.25	46					1.25	68								
	40	>668	4/6				40	>526	4/6				20	87	40	5
	20	>249	1/6				20	>585	4/6				10	61	30	18
44b	10	>238	1/6		NT		10	>369	2/6		NT		5	41	20	37
	5	68					5	>212	1/6							
	1.25	46					1.25	87								
	40	59		16	168		40	112		16	>417	2/6	20	52	30	2
	20	>244	1/6	8	102		20	>628	5/6	8	122		10	64	20	36
49a	10	>272	1/6	4	68		10	>376	2/6				5	42		
	5	73					5	>418	3/6				2.5	38		
	1.25	55					1.25	110								
	80	>1016	6/6	4	38		80	>513	4/6	8	76		30	58	80	2
	40	>712	4/6	2	9		40	>789	6/6	4	63		20	81	60	3
56	20	>248	1/6				20	>677	5/6				10	62	40	19
	10	107					10	>341	2/6				5	50	30	52
	5	67					5	109								
	1.25	56					1.25	85								
ellipticine (1)	120[b]	128			NT		60[b]	139			NT			NT		NT	
9-hydroxyellipticine (4)	60[b]	79			NT		30[b]	91			NT			NT		NT	
celiptium (**5**)	5[b]	48			NT		5[b]	90			NT		2.5[b]	35	5[b]	38
doxorubicin	2.5[b]	90		2.5[b]	30		2.5[b]	>675	5/6	2.5[b]	65		2.5[b]	>257[c]	2.5[b]	31

[a] Details of the evaluation methods are described in the Experimental Section. ILS %: increase in mean life span. Cures: number of survivors/total at 80 days. T/C %: inhibition ratio. NT: not tested. [b] Optimal dose. [c] Mice surviving for 80 days (4/6) were observed.

(5) In a series of pyranosides, it is observed that pentosides and deoxyhexosides (having three hydroxyl groups) are more active than hexosides (having four hydroxyl groups) [compare D-arabinoside (**41a**) and L-fucoside (i.e., 5′-methyl-D-arabinoside) (**53b**) with L-galactoside (i.e., 5′-(hydroxymethyl)-D-arabinoside) (**62b**), also L-lyxoside (**44b**) and L-rhamnoside (i.e., 5′-methyl-L-lyxoside) (**56**) with L-mannoside (i.e., 5′-(hydroxymethyl)-L-lyxoside) (**64b**)]. This tendency suggests the importance of a balance between hydrophilicity and hydrophobicity.

(6) The relationships between each pair of enantiomers are not defined.

(7) The relationships between pyranosides and the corresponding furanosides are not defined.

(8) The relationships between 1′,2′-*trans*-glycosides and the corresponding 1′,2′-*cis*-glycosides are not defined.

Results of Extended Evaluation. Table III shows the effects of the five selected glycosides (**41b**, **43a**, **44b**, **49a**, and **56**) in four different syngeneic tumor systems.

These glycosides were evaluated against both ip implanted leukemias, L1210 and P388, by ip administration for 5 consecutive days at dose levels between 1.25 and 80 mg/kg for each glycoside in order to calculate their therapeutic ratios (optimal dose/dose for ILS 30%). All of the tested glycosides were curative against P388 leukemia as well as L1210 leukemia. Moreover, their effects against P388 leukemia seem to be better than those against L1210 leukemia. The therapeutic ratio (>16 to >64) of each glycoside in both tumor systems was found to be much higher than those of conventional anticancer drugs.

Against L1210 leukemia when administered intravenously (iv) for 5 consecutive days, the four glycosides tested showed good activity and one of them, L-arabinopyranoside (**41b**), was curative. Against P388 leukemia when administered iv, the four glycosides tested also showed marked activity and two of them, **41b** and D-xylofuranoside (**49a**), were curative.

The five selected glycosides were subsequently evaluated against ip implanted B16 melanoma when administered ip for 9 consecutive days. They displayed good activity (ILS > 50%). However their activity against B16 melanoma seemed to be inferior to that observed against the other tumors.

Against subcutaneously (sc) implanted colon 38 carcinoma, when administered ip on days 1, 3, 5, 7, and 9, each of the five glycosides tested remarkably inhibited tumor growth [inhibition ratios (T/C %) < 2–8%].

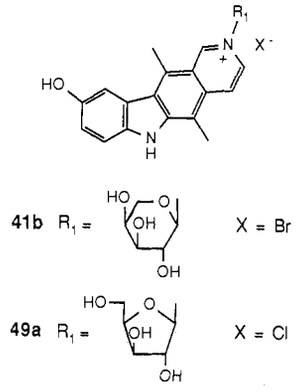
Table IV shows the effects of the two glycosides (**41b** and **49a**) against two different ip implanted allogeneic tumors, Ehrlich ascites carcinoma (EAC) and sarcoma 180, when administered ip for 5 consecutive days. Both glycosides showed curative effects against both tumors; moreover they were particularly effective against EAC.

On the basis of these results, the five selected glycosides were established to have high levels of antitumor activity against various murine tumor models with excellent therapeutic ratios.

The five selected glycosides were much more active than ellipticines (**1** and **4**) and celiptium (**5**) against the murine tumors tested. These findings suggested that the sugar moieties should increase bioavailability, permeability, and/or selective toxicity toward tumors. In comparison with doxorubicin, these glycosides had superior activity in the L1210 (ip-ip, ip-iv), P388 (ip-iv), and colon 38 systems, comparable activity in the P388 (ip-ip), EAC, and sarcoma 180 systems, and inferior activity in the B16 system.

Consequently, although it may be concluded that all of

Table IV. Effects of Selected Ellipticine Glycosides (**41b** and **49a**) in Allogeneic Tumor Systems[a]



compd	EAC (ip-ip) dose, mg/kg	EAC (ip-ip) ILS %	EAC (ip-ip) cures	sarcoma 180 (ip-ip) dose, mg/kg	sarcoma 180 (ip-ip) ILS %	sarcoma 180 (ip-ip) cures
	80	>492	5/6	80	>353	2/6
	40	>518	5/6	40	>339	2/6
41b (SUN4599)	20	>338	1/6	20	282	
	10	>297	1/6	10	117	
	5	95				
	40	>501	5/6	40	>335	2/6
49a (SUN5073)	20	>380	3/6	20	>318	2/6
	10	>318	2/6	10	69	
	5	125				
doxorubicin	2.5[b]	>360	3/6	2.5[b]	>288	1/6

[a,b] Same as footnotes in Table III.

the five selected glycosides are promising anticancer drugs, L-arabinopyranoside (**41b**, code number for development: SUN4599) and D-xylofuranoside (**49a**, SUN5073) have been selected for further preclinical evaluation and possible clinical development because of their higher activity in the two leukemia systems, L1210 (ip-iv) and P388 (ip-iv), and easier formulation in comparison with the other glycosides. Preclinical studies of both glycosides are in progress.[35]

Experimental Section

Specific rotations ($[\alpha]_D$) were determined on a Perkin-Elmer 241 polarimeter or a JASCO DIP-181 polarimeter. Molecular ellipticity ($[\theta]_\lambda$) was measured on a JASCO J-20C spectropolarimeter. Infrared (IR) spectra were measured on a Hitachi 260-10 spectrometer or a Nicolet 5DX spectrometer. Ultraviolet absorption (UV) spectra were measured on a Shimadzu UV-250 spectrometer or a Beckman DU-8 spectrometer. Secondary ion mass (SIMS) spectra were obtained on a Hitachi M-80 mass spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra at 360 MHz with Me_2SO-d_6 as the sample solvent were recorded on a Nicolet NT-360 spectrometer. NOE experiments were carried out on a JEOL FX-100 spectrometer. Chemical shifts (δ) were expressed in ppm (CD_2HSOCD_3 was used as an internal standard) and coupling constants (J) in hertz. Elemental analyses were performed on a Perkin-Elmer 240B elemental analyzer and, where only the symbols for the elements are recorded, were within ±0.4% of the theoretical values. Kieselgel 60 (E. Merck), Sephadex LH-20 (Pharmacia), or AG1-X8 anion exchange resin (acetate form) (Bio-Rad) was used for column chromatography. HPLC analyses were performed on a JASCO Tri Rotar SR2 liquid chromatograph equipped with a JASCO UVIDEC-100-IV UV spectrophotometer [column, Shimadzu Shim-pack CLC-ODS (0.15 m × 6 mm); mobile phase, for the analyses of protected glycosides, CH_3CN/0.02 M sodium phosphate buffer (pH 3) (4:5) and for the analyses of the glycosides, the same solvent system (1:3 or 1:4); flow rate, 1.0 mL/min; wavelength for the detection, 318 nm]. Counterion analyses were performed on a Dionex Ion Chromatograph 10 (column, Dionex HPIC AG3-AS3; mobile phase, aqueous solution containing Na_2CO_3 (2.4 mmol) and $NaHCO_3$ (3.0 mmol) per 1 L; flow rate, 1.5 mL/min). Atomic absorption analyses on cadmium were performed on a Nippon Jarrell Ash AA-8500 spectrometer.

Ellipticine (1),[5] 9-methoxyellipticine (**2**),[5] 9-acetoxyellipticine (**3**),[6] and 9-hydroxyellipticine (**4**)[6] were synthesized according to the reported methods.

Preparation of Peracetylated Pyranosyl Halides. 2,3,4-Tri-*O*-acetyl-β-D-(and L-)arabinopyranosyl bromide (**6a, 6b**),[22] 2,3,4-tri-*O*-acetyl-α-D-(and L-)lyxopyranosyl chloride (**7a, 7b**), 2,3,4-tri-*O*-acetyl-β-D-(and L-)ribopyranosyl bromide (**8a, 8b**),[36] 2,3,4-tri-*O*-acetyl-α-D-(and L-)xylopyranosyl bromide (**9a, 9b**),[37] 2,3,4-tri-*O*-acetyl-α-D-(and L-)fucopyranosyl bromide (**10a, 10b**), 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (**11**),[28] 2,3,4,6-tetra-*O*-acetyl-α-D-allopyranosyl bromide (**12**), 2,3,4,6-tetra-*O*-acetyl-α-D-(and L-)galactopyranosyl bromide (**13a, 13b**),[38] 2,3,4,6-tetra-*O*-acetyl-α-D-(and L-)glucopyranosyl bromide (**14a, 14b**),[39] 2,3,4,6-tetra-*O*-acetyl-α-D-(and L-)mannopyranosyl bromide (**15a, 15b**), 2,3,4,6-tetra-*O*-acetyl-ξ-D-talopyranosyl bromide (**16**), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-galactopyranosyl chloride (**17**), 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride (**18**),[40] and methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (**19**)[41] were prepared according to the reported methods.

9-Acetoxy-2-(2,3,4-tri-*O*-acetyl-α-L-arabinopyranosyl)-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Bromide (32). A stirred mixture of **3** (304 mg, 1 mmol), **6b** (678 mg, 2 mmol), and $CdCO_3$ (258 mg, 1.5 mmol) was heated under reflux in dry nitromethane (30 mL) for 10 min. After the precipitate was removed by filtration, nitromethane was evaporated in vacuo to give the residue, which was purified by column chromatography on silica gel [CH_2Cl_2/methanol (95:5)] to provide the crude product containing **32** as the major compound. It was subjected to column chromatography on Sephadex LH-20 for the removal of cadmium salts to afford **32** containing 3% of the β-anomer[23] (596 mg, 97%)

(35) The preliminary studies on metabolism of **41b** in rats revealed that the quaternary glycoside was excreted unchanged in the feces and in the urine under our experimental conditions.

(36) Levene, P. A.; Tipson, R. S. *J. Biol. Chem.* **1931**, *92*, 109.
(37) Weygand, F. *Methods Carbohydr. Chem.* **1962**, *1*, 182.
(38) Hansen, R. G.; Rutter, W. J.; Krichevsky, P. *Biochem. Prep.* **1955**, *4*, 1.
(39) Lemieux, R. U. *Methods Carbohydr. Chem.* **1963**, *2*, 221.
(40) Leaback, D. H.; Walker, P. G. *J. Chem. Soc.* **1957**, 4754.
(41) Bollenback, G. N.; Long, J. W.; Benjamin, D. G.; Lindquist, J. A. *J. Am. Chem. Soc.* **1955**, *77*, 3310.

as an orange solid. Its content of cadmium was determined to be less than 2×10^{-4} w/w % by means of atomic absorption analysis. It was used for the next reaction without further purification: $[\alpha]^{26}_D$ +39° (*c* 0.20, methanol); IR (KBr) 1750, 1640, 1600, 1480, 1420, 1370, 1220, 1060, 940, and 810 cm^{-1}; UV (ethanol) 253 nm (ε 22000), 286 (19000), and 317 (57000); MS (SIMS) *m/z* 563 $[C_{30}H_{31}N_2O_9]^+$; ^1H NMR δ 1.80, 2.00, 2.27 (each 3 H, s, $OCOCH_3 \times 3$), 2.37 (3 H, s, 9-$OCOCH_3$), 2.89 (3 H, s, 5-CH_3), 3.35 (3 H, s, 11-CH_3), 5.51 (2 H, m, AB part of ABX, 2'-H and 3'-H), 6.35 (1 H, t, X part of ABX, 1'-H), 7.48 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.74 (1 H, d, *J* = 9 Hz, 7-H), 8.29 (1 H, d, *J* = 2 Hz, 10-H), 8.57 (2 H, br s, 3-H and 4-H), 10.16 (1 H, s, 1-H), and 12.48 (1 H, br s, 6-H). Consistent and correct analytical datum could not be obtained because of the compound's extreme hygroscopicity.

2-α-L-Arabinopyranosyl-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Bromide (41b). A solution of **32** (596 mg, 0.97 mmol) in methanolic ammonia (81 mL) was stirred for 24 h at 0–5 °C. The solution was concentrated in vacuo to give the residue, which was crystallized from methanol/ethyl acetate to afford **41b** containing 3% of the β-anomer[23] (401 mg, 87%) as an orange solid. It was recrystallized from methanol to give the pure α-anomer (**41b**) as an orange crystalline compound: $[\alpha]_D$ (Table I); IR (KBr) 3250, 1640, 1600, 1480, 1420, 1220, 1200, 1150, 1090, and 1060 cm^{-1}; UV (ethanol) 227 nm (ε 14000), 268 (24000), 281 (21000), and 325 (47000); MS (SIMS) *m/z* 395 $[C_{22}H_{23}N_2O_5]^+$; ^1H NMR δ 2.85 (3 H, s, 5-CH_3), 5.74 (1 H, d, *J* = 8.5 Hz, 1'-H), 7.18 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.55 (1 H, d, *J* = 9 Hz, 7-H), 7.86 (1 H, d, *J* = 2 Hz, 10-H), 8.51 (2 H, br s, 3-H and 4-H), 9.45 (1 H, br s, 9-OH), 10.03 (1 H, s, 10-H), and 12.08 (1 H, br s, 6-H). Anal. (Table I).

Condensation of Ellipticines (1–3) with Peracetylated Pyranosyl Halides. The condensation reaction procedure was analogous to that described for the preparation of **32**.

Preparation of Ellipticine 1',2'-*trans*-Pyranosides. The deprotection reaction was worked up with methanolic ammonia as described for the preparation of **41b** (see Table I).

Preparation of Perbenzoylated Furanosyl Halides. 2,3-Di-*O*-benzoyl-D-erythrofuranosyl chloride (α-:β-anomer = 1:1)[42] (**20**), 2,3-di-*O*-benzoyl-5-deoxy-α-L-arabinofuranosyl chloride (**21**),[43] 2,3-di-*O*-benzoyl-5-deoxy-D-ribofuranosyl chloride (α-:β-anomer = 1.5:1)[42] (**22**),[44] 2-deoxy-3,5-bis-*O*-(*p*-toluoyl)-α-D-ribofuranosyl chloride (**23**),[30] 2,3,5-tri-*O*-benzoyl-α-D-(and L-)arabinofuranosyl bromide (**24a, 24b**),[45] 2,3,5-tri-*O*-benzoyl-D-(and L-)lyxofuranosyl chloride (α-:β-anomer = 2.5:1)[42] (**25a, 25b**), 2,3,5-tri-*O*-benzoyl-D-(and L-)ribofuranosyl bromide (α-:β-anomer = 1:2)[42] (**27a, 27b**),[20b] and 2,3,5-tri-*O*-benzoyl-α-D-(and L-)xylofuranosyl chloride (**28a, 28b**) were prepared according to the reported methods.

9-Acetoxy-2-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Bromide (33). A stirred mixture of **3** (304 mg, 1 mmol), **27a** (1050 mg, 2 mmol), and $CdCO_3$ (258 mg, 1.5 mmol) was heated under reflux in dry nitromethane (30 mL) for 10 min. After the precipitate was removed by filtration, the filtrate was concentrated in vacuo to give the residue, which was purified by chromatography on silica gel [CH_2Cl_2/methanol (95:5)] followed by chromatography on Sephadex LH-20 (methanol) to afford **33** (638 mg, 77%) as an orange solid (the content of Cd $< 2 \times 10^{-4}$ w/w %): $[\alpha]^{26}_D$ −200° (*c* 0.22, methanol); IR (KBr) 1720, 1640, 1590, 1440, 1260, 1100, and 700 cm^{-1}; UV (ethanol) 231 nm (ε 50000), 276 (23000), 284 (23000), and 316 (67000); MS (SIMS) *m/z* 749 $[C_{45}H_{37}N_2O_9]^+$; ^1H NMR δ 2.36 (3 H, s, 9-$OCOCH_3$), 2.90 (3 H, s, 5-CH_3), 3.26 (3 H, s, 11-CH_3), 7.04 (1 H, d, *J* = 5 Hz, 1'-H), 8.23 (1 H, d, *J* = 2 Hz, 10-H), 8.54 (1 H, d, *J* = 7.5 Hz, 4-H), 8.76 (1 H, d, *J* = 7.5 Hz, 3-H), 10.25 (1 H, s, 1-H), and 12.43 (1 H, br s, 6-H). Consistent and correct analytical datum could not be obtained because of the compound's extreme hygroscopicity.

9-Hydroxy-5,11-dimethyl-2-β-D-ribofuranosyl-6*H*-pyrido-[4,3-*b*]carbazolium Bromide (47a). A solution of **33** (638 mg, 0.77 mmol) in methanolic ammonia (67 mL) was stirred for 24 h at room temperature. The solution was concentrated in vacuo to give the residue, which was crystallized from methanol/ethyl acetate to afford **47a** (293 mg, 80%) as an orange crystalline compound: $[\alpha]_D$ (Table I); IR (KBr) 3220, 1650, 1600, 1480, 1430, 1390, 1290, 1220, and 1110 cm^{-1}; UV (ethanol) 226 nm (ε 13000), 268 (23000), 281 (20000), and 323 (46000); MS (SIMS) *m/z* 395 $[C_{22}H_{23}N_2O_5]^+$; ^1H NMR δ 2.84 (3 H, s, 5-CH_3), 3.35 (3 H, s, 11-CH_3), 6.28 (1 H, d, *J* = 5 Hz, 1'-H), 7.18 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.54 (1 H, d, *J* = 9 Hz, 7-H), 7.85 (1 H, d, *J* = 2 Hz, 10-H), 8.52 (1 H, d, *J* = 7.5 Hz, 4-H), 8.63 (1 H, d, *J* = 7.5 Hz, 3-H), 9.45 (1 H, s, 9-OH), 10.26 (1 H, s, 1-H), and 12.04 (1 H, br s, 6-H). Anal. (Table I).

Condensation of Ellipticines (1–3) with Perbenzoylated Furanosyl Halides. The condensation reaction procedure was analogous to that described for the preparation of **33**.

Preparation of Ellipticine 1',2'-*trans*-Furanosides. The deprotection reaction was worked up with methanolic ammonia as described for the preparation of **47a** (see Table I).

2-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Bromide (58). A stirred mixture of **4** (262 mg, 1 mmol), **11** (706 mg, 2 mmol), and $CdCO_3$ (256 mg, 1.5 mmol) was heated under reflux in dry nitromethane (26 mL) for 15 min. After the precipitate was removed by filtration, nitromethane was evaporated in vacuo to give the residue, which was purified by chromatography on silica gel [CH_2Cl_2/methanol (90:10)] followed by chromatography on Sephadex LH-20 (methanol) to afford **58** (123 mg, 20%) as an orange solid (the content of Cd $< 2 \times 10^{-4}$ w/w %): $[\alpha]_D$ (Table I); IR (KBr) 3350, 3150, 1750, 1640, 1590, 1475, 1425, 1380, 1370, and 1220 cm^{-1}; UV (ethanol) 226 nm (ε 15000), 251 (22000), 269 (23000), and 326 (45000); MS (SIMS) *m/z* 535 $[C_{29}H_{31}N_2O_8]^+$; ^1H NMR δ 1.55 (3 H, d, *J* = 7 Hz, 5'-CH_3), 1.85, 2.20, 2.25 (each 3 H, s, $OCOCH_3 \times 3$), 2.86 (3 H, s, 5-CH_3), 3.35 (3 H, s, 11-CH_3), 6.67 (1 H, d, *J* = 8.5 Hz, 1'-H), 7.20 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.56 (1 H, d, *J* = 9 Hz, 7-H), 7.87 (1 H, d, *J* = 2 Hz, 10-H), 8.48 (1 H, d, *J* = 7.5 Hz, 4-H), 8.60 (1 H, d, *J* = 7.5 Hz, 3-H), 9.50 (1 H, s, 9-OH), 10.12 (1 H, s, 1-H), and 12.23 (1 H, br s, 6-H). Anal. (Table I).

2-(2-Deoxy-β-D-ribofuranosyl)-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Chloride (39). A stirred mixture of **3** (304 mg, 1 mmol), **23** (776 mg, 2 mmol), and $CdCO_3$ (258 mg, 1.5 mmol) was heated under reflux in dry nitromethane (30 mL) for 15 min. After the precipitate was removed by filtration, the mixture was concentrated in vacuo to give the residue, which was purified by chromatography on silica gel [CH_2Cl_2/methanol (95:5)] followed by chromatography on Sephadex LH-20 (methanol) to provide 9-acetoxy-2-[2-deoxy-3,5-bis-*O*-(*p*-toluoyl)-β-D-ribofuranosyl]-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium chloride (**34**) (187 mg, 27%) as an orange solid (the content of Cd $< 2 \times 10^{-4}$ w/w %): $[\alpha]^{26}_D$ −240° (*c* 0.12, methanol); IR (KBr) 1720, 1610, 1470, 1270, 1180, 1100, and 750 cm^{-1}; UV (ethanol) 243 nm (ε 46000), 284 (20000), and 314 (59000); MS (SIMS) *m/z* 657 $[C_{40}H_{37}N_2O_7]^+$; ^1H NMR δ 2.19, 2.44 (each 3 H, s, aryl-$CH_3 \times 2$), 2.36 (3 H, s, 9-$OCOCH_3$), 2.86 (3 H, s, 5-CH_3), 3.26 (3 H, s, 11-CH_3), 6.89 (1 H, t, *J* = 6.5 Hz, 1'-H), 7.47 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.73 (1 H, d, *J* = 9 Hz, 7-H), 8.25 (1 H, d, *J* = 2 Hz, 10-H), 8.46 (1 H, d, *J* = 7.5 Hz, 4-H), 8.59 (1 H, d, *J* = 7.5 Hz, 3-H), 10.08 (1 H, s, 1-H), and 12.39 (1 H, br s, 6-H). Consistent and correct analytical datum could not be obtained because of the compound's extreme hygroscopicity. A solution of **34** (187 mg, 0.27 mmol) in methanolic ammonia (20 mL) was stirred for 24 h at room temperature. The solution was concentrated in vacuo to give the residue, which was crystallized from methanol/ethyl acetate to afford **39** (85 mg, 76%) as an orange crystalline compound: $[\alpha]_D$ (Table I); IR (KBr) 3230, 1600, 1470, 1430, 1220, 1100, 1060, and 810 cm^{-1}; UV (ethanol) 207 nm (ε 18000), 227 (13000), 249 (20000), 267 (21000), 280 (19000), and 321 (42000); MS (SIMS) *m/z* 379 $[C_{22}H_{23}N_2O_4]^+$; ^1H NMR δ 2.84 (3 H, s, 5-CH_3), 3.27 (3 H, s, 11-CH_3), 6.67 (1 H, t, *J* = 6 Hz, 1'-H), 7.16 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.53 (1 H, d, *J* = 9 Hz, 7-H), 7.83 (1 H, d, *J* = 2 Hz, 10-H), 8.47 (1 H, d, *J* = 7.5 Hz, 4-H), 8.68 (1 H, d, *J* = 7.5 Hz, 3-H), 9.47 (1 H, br s, 9-OH), 10.27 (1 H, s, 1-H), and 12.08 (1 H, br s, 6-H). Anal. (Table I).

Preparation of Perbenzylated Glycosyl Halides. 2,3-Di-*O*-benzyl-5-deoxy-α-L-arabinofuranosyl chloride (**29**), 2,3,5-tri-*O*-

(42) Isomer ratio was determined by ^1H NMR spectroscopy.
(43) See for the preparation of 5-deoxy-L-arabinose: Taylor, E. C.; Jacobi, P. A. *J. Am. Chem. Soc.* **1976**, *98*, 2301.
(44) See for the preparation of 5-deoxy-D-ribose: Oka, J.; Ueda, K.; Hayaishi, O.; Komura, H.; Nakanishi, K. *J. Biol. Chem.* **1984**, *259*, 986.
(45) Fletcher, H. G., Jr. *Methods Carbohydr. Chem.* **1963**, *2*, 228.

benzyl-α-D-(and L-)arabinofuranosyl bromide (**30a**, **30b**),[32] and 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl bromide (**31**) were prepared according to the reported method.

9-Acetoxy-2-(2,3,5-tri-*O*-benzyl-β-L-arabinofuranosyl)-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Bromide (35). A stirred mixture of **3** (304 mg, 1 mmol), **30b** (966 mg, 2 mmol), and $CdCO_3$ (258 mg, 1.5 mmol) was heated under reflux in dry nitromethane (30 mL) for 10 min. After the precipitate was removed by filtration, the filtrate was concentrated in vacuo to give the residue, which was purified by chromatography on silica gel [CH_2Cl_2/methanol (95:5)] followed by chromatography on Sephadex LH-20 (methanol) to afford **35** containing 4% of the α-anomer[23] (386 mg, 49%) as an orange solid (the content of Cd $< 2 \times 10^{-4}$ w/w %). It was used for the next reaction without further purification: $[\alpha]^{26}_D$ −40° (*c* 0.24, methanol); IR (KBr) 1760, 1600, 1470, 1410, 1360, 1200, 1190, 910, 730, and 690 cm^{-1}; UV (ethanol) 252 nm (ε 25000) and 315 (71000); MS (SIMS) *m/z* 707 $[C_{45}H_{43}N_2O_6]^+$; ^1H NMR δ 2.37 (3 H, s, 9-$OCOCH_3$), 2.85 (3 H, s, 5-CH_3), 3.21 (3 H, s, 11-CH_3), 7.47 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.73 (1 H, d, *J* = 9 Hz, 7-H), 8.23 (1 H, d, *J* = 2 Hz, 10-H), 8.25 (1 H, d, *J* = 7.5 Hz, 4-H), 8.61 (1 H, d, *J* = 7.5 Hz, 3-H), 10.06 (1 H, s, 1-H), and 12.36 (1 H, br s, 6-H). Anal. ($C_{45}H_{43}N_2O_6Br \cdot {}^1/_2H_2O$) C, H, N.

2-β-L-Arabinofuranosyl-9-hydroxy-5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazolium Acetate (70b). A mixture of **35** (289 mg, 0.37 mmol) and iodotrimethylsilane (740 mg, 3.7 mmol) in dry CH_2Cl_2 (19 mL)[33] was stirred at 0–5 °C for 15 h. The red precipitate was filtered and then was dissolved in methanolic ammonia (40 mL). The solution was stirred at 0–5 °C for 4 h. The solution was concentrated in vacuo to give the residue, which was passed through an AG1-X8 (acetate form) resin column to afford **70b** containing 4% of the α-anomer[23] (104 mg, 62%) as a dark-red solid. It was recrystallized from methanol to give pure β-anomer **70b**: $[\theta]_\lambda$ (Table II); IR (KBr) 3180, 1600, 1480, 1400, 1290, 1220, 1120, 1070, and 810 cm^{-1}; UV (ethanol) 210 nm (ε 18000), 227 (14000), 266 (23000), 280 (21000), and 322 (46000); MS (SIMS) (Table II); ^1H NMR (Me_2SO-d_6 containing 1% CF_3CO_2H) δ 1.74 (3 H, s, $CH_3CO_2^-$), 2.85 (3 H, s, 5-CH_3), 3.30 (3 H, s, 11-CH_3), 6.55 (1 H, d, *J* = 6 Hz, 1′-H), 7.17 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.54 (1 H, d, *J* = 9 Hz, 7-H), 7.84 (1 H, d, *J* = 2 Hz, 10-H), 8.46 (1 H, d, *J* = 7.5 Hz, 4-H), 8.54 (1 H, d, *J* = 7.5 Hz, 3-H), 10.20 (1 H, s, 1-H), and 11.98 (1 H, s, 6-H). An NOE of 6.6% was observed on the 2′-H upon irradiation of the 1′-H by 100-MHz ^1H NMR.[46] Consistent and correct analytical datum could not be obtained because of the compound's extreme hygroscopicity.

Condensation of 9-Acetoxyellipticine (3) with Perbenzylated Glycosyl Halides. The condensation reaction procedure was analogous to that described for the preparation of **35**.

Preparation of 9-Hydroxyellipticine 1′,2′-*cis*-Glycosides. Deprotection was achieved with iodotrimethylsilane and methanolic ammonia, and exchange of the counterion was performed by passing through an AG1-X8 (acetate form) resin column as described for the preparation of **70b**.

9-Hydroxy-5,11-dimethyl-2-L-rhamnopyranosyl-6*H*-pyrido[4,3-*b*]carbazolium Acetate (57). A solution of 9-hydroxy-5,11-dimethyl-2-L-rhamnopyranosyl-6*H*-pyrido[4,3-*b*]-carbazolium bromide (**56**, α-:β-anomer = 92:8)[23] (489 mg, 1 mmol) in water (103 mL) was passed through an AG1-X8 (acetate form) resin column. The solution eluted was concentrated in vacuo to give the residue, which was crystallized from methanol/ethyl acetate to afford **57** (α-:β-anomer = 92:8)[23] (401 mg, 86%) as a red solid: $[\alpha]_D$ (Table I); IR (KBr) 3240, 1640, 1600, 1570, 1480, 1410, 1220, and 1060 cm^{-1}; UV (ethanol) 210 nm (ε 19000), 226 (13000), 267 (23000), 281 (20000), and 324 (47000); MS (SIMS) *m/z* 409 $[C_{23}H_{25}N_2O_5]^+$; ^1H NMR δ 1.53 (3 H, d, *J* = 7 Hz, 5′-CH_3), 1.68 (3 H, s, $CH_3CO_2^-$), 2.78 (3 H, s, 5-CH_3), 3.23 (3 H, s, 11-CH_3), 6.24 (1 H, d, *J* = 8.5 Hz, 1′-H), 7.14 (1 H, dd, *J* = 2 and 9 Hz, 8-H), 7.51 (1 H, d, *J* = 9 Hz, 7-H), 7.80 (1 H, d, *J* = 2 Hz, 10-H), 8.36 (1 H, d, *J* = 7.5 Hz, 4-H), 8.51 (1 H, d, *J* = 7.5 Hz, 3-H), and 10.03 (1 H, s, 1-H). All signals of the β-anomer could not be observed because of overlapping with those of the α-anomer. Anal. (Table I).

Evaluation Methods. Animals. Female DBA/2, C57BL/6 mice for the maintenance of tumors and female BDF_1 (C57BL/6 × DBA/2) for the experiments were purchased from Japan Charles River Ltd., Kanagawa. Female ICR mice were purchased from CLEA Japan Inc., Tokyo. The mice were supplied with a pellet diet (CRF-1, Japan Charles River Ltd.) and water ad libitum.

Tumors. L1210 and P388 leukemias were maintained in the intraperitoneal cavity of DBA/2 by weekly transfer. B16 melanoma and colon 38 carcinoma were maintained as sc growing tumors in C57BL/6 mice. Ehrlich ascites carcinoma (EAC) and sarcoma 180 were passaged by weekly transplantation into the intraperitoneal cavity of ICR mice.

Drugs. An appropriate quantity of the drug used for the experiments was dissolved in 0.9% NaCl solution (saline solution) and diluted to the desired concentration with saline just before use. Ellipticine glycosides without recrystallization procedure were used for the experiments. Ellipticines (**1**[5] and **4**[6]) and celiptium (**5**)[10b] were synthesized as described previously. Doxorubicin was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo.

Antitumor Testing. One-tenth milliliter of each diluted ascitic fluid containing tumor cells (10^5 L1210 leukemia cells, 10^6 P388 leukemia cells, 10^7 EAC cells, and 10^7 sarcoma 180 cells) was transplanted into the abdominal cavities of BDF_1 (L1210 and P388 leukemias) and ICR (EAC and sarcoma 180) mice. Each group for each dose level consisted of six mice. The mice were treated with various dose levels of the test drug ip or iv for 5 consecutive days, starting 24 h after tumor cell transplantation. Antitumor activity of the drug was calculated from the ratio of the mean survival time (days) of treated mice to that of control mice (ILS %). The observation period was 80 days.

For B16 melanoma, BDF_1 mice were implanted ip with 0.2 mL of tumor homogenate (25 mg) in saline solution. Various dose levels of the drug were administered ip for 9 consecutive days. Antitumor activity was evaluated by ILS %.

Colon 38 carcinoma experiments were carried out by implanting tumor homogenate (70 mg) into the axillary regions of BDF_1 mice. Various dose levels of the drug were administered ip on days 1, 3, 5, 7, and 9. Evaluation of antitumor activity was assessed by inhibition of tumor growth (treated tumor weight vs control tumor weight, T/C %) on the 20th day after tumor cell inoculation. Tumor weight was estimated by caliper measurement length (*L*) and width (*W*) of the tumor using the following formula:

$$\text{tumor weight (mg)} = LW^2\ (\text{mm}^3)/2$$

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(46) An NOE between 1′-H and 2′-H could not be observed at 360-MHz ^1H NMR.

46, 103481-93-2; **47a**, 103461-17-2; **17b**, 103461-16-1; **48a**, 103461-15-0; **48b**, 103461-14-9; **49a**, 103461-07-0; **49b**, 103461-10-5; **50a**, 114028-11-4; **50b**, 114028-12-5; **51**, 103481-82-9; **52**, 103481-97-6; **53a**, 103461-05-8; **53b**, 103461-06-9; **54**, 114028-13-6; **55**, 114028-14-7; **56**, 114028-15-8; **57**, 114028-17-0; **58**, 103449-53-2; **59**, 103460-98-6; **60**, 103481-74-9; **61**, 103481-91-0; **62a**, 103460-96-4; **62b**, 103460-97-5; **63a**, 113925-86-3; **63b**, 103461-00-3; **64a**, 103449-66-7; **64b**, 103449-67-8; **65**, 103449-68-9; **66**, 103461-29-6; **67**, 103461-28-5; **68**, 103461-30-9; **69**, 103461-42-3; **70a**, 103461-38-7; **70b**, 103461-40-1; **71**, 113925-87-4.

Dinucleotide Analogues as Inhibitors of Thymidine Kinase, Thymidylate Kinase, and Ribonucleotide Reductase

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P^1-(Adenosine-5′)-P^n-(thymidine-5′) tri-, tetra-, penta-, and hexaphosphates (Ap_nT) plus the analogues with a methylene group α,β to the thymidine residue (Ap_ncpT) were synthesized by coupling the appropriate two nucleotides, having activated one by morpholine. These were tested as potential dinucleotide inhibitors of thymidine kinase, thymidylate kinase, and ribonucleotide reductase. All three enzymes bind ATP and thymidine or its nucleotides and therefore might be inhibited by dinucleotides containing adenosine and thymidine. Ap_5T and Ap_6T strongly inhibited all three enzymes (IC_{50} = 2.4–20 μM). Ap_4cpT and Ap_5cpT also strongly inhibited the two kinases (IC_{50} = 4–20 μM) but were much weaker inhibitors of the reductase (IC_{50} = 130 and 230 μM).

Thymidine kinase (EC 2.7.1.21), thymidylate kinase (EC 2.7.4.9), and ribonucleotide reductase (EC 1.17.4.1) are all elevated in malignancy and are essential to DNA synthesis. All three enzymes bind ATP and thymidine or its nucleotides and therefore might be inhibited by dinucleotides containing adenosine and thymidine. Bisubstrate and transition-state analogues have proved effective inhibitors of some enzymes (e.g. Ap_5A inhibits adenylate kinase[1] and PALA inhibits aspartate transcarbamylase[2]); also Hampton et al.[3] have found Ap_3T to be a moderate inhibitor of thymidine kinase. We have synthesized the series Ap_3T–Ap_6T and the corresponding analogues with a methylene group α,β to the thymidine residue and have tested them against the three enzymes of interest. It has been previously shown that insertion of the α,β-methylene residue can confer increased enzyme inhibitor potency;[4] this would also be expected to confer increased chemical and enzymatic stability.

Chemistry

We explored three ways of coupling the nucleoside phosphates: (a) use of 1,1′-carbonyldiimidazole,[5] (b) use of diphenyl chlorophosphate,[6] (c) condensation by the morpholidate method.[7] We found (c) to be the best, particularly for longer chain lengths. Yields of 20% or greater were obtained except in the case of Ap_6T and Ap_5cpT. For Ap_5T our yield (22%) was substantially greater than that of Bone et al.[8] (7.5%). We found that the carbonyldiimidazole route gave a good yield (29%) only in the synthesis of Ap_2cpT (from AMP and α,β-methylene-TDP). In the other reactions that we tried, namely TMP plus either ADP or ATP and Ap_4 plus α,β-methylene-TDP, we were unable to isolate pure product. The diphenyl chlorophosphate route gave good yields of coupling, but owing to disproportionation reactions the major product contained one less phosphorus atom than expected. For example, ADP + TDP gave 6% Ap_4T and 20% Ap_3T and ATP + TDP gave 6% AP_5T and 9% Ap_4T. All compounds were characterized by ^1H and ^{31}P NMR, and their purity was assessed by HPLC. Compounds Ap_3T–Ap_5T, and Ap_2cpT–Ap_4cpT gave satisfactory elemental analyses (CHNP). After material was reserved for biological testing, there was insufficient material for the elementary analysis of either Ap_6T or Ap_5cpT.

Table I

Inhibition of Thymidine Kinase: IC_{50},[a] μM			
Ap_3T	>500	Ap_2cpT	>500
Ap_4T	15.0 (±2.5)	Ap_3cpT	17.0 (±2.2)
Ap_5T	2.5 (±0.3)	Ap_4cpT	9.2 (±0.3)
Ap_6T	6.0 (±0.6)	Ap_5cpT	19.7 (±2.7)
Ap_5A	>500		
Inhibition of Thymidylate Kinase: IC_{50}, μM			
Ap_3T	1230 (±180)	Ap_2cpT	730 (±70)
Ap_4T	290 (±40)	Ap_3cpT	340 (±60)
Ap_5T	8 (±2)	Ap_4cpT	6 (±1)
Ap_6T	6 (±1)	Ap_5cpT	4 (±1)
Ap_5A	>1000		
Inhibition of Ribonucleotide Reductase: IC_{50}, μM			
Ap_3T	>500	Ap_2cpT	460
Ap_4T	77 (±4)	Ap_3cpT	300 (±10)
Ap_5T	14 (±3)	Ap_4cpT	130 (±10)
Ap_6T	20 (±6)	Ap_5cpT	230 (±10)
Ap_5A	>500		

[a] IC_{50} is the concentration of inhibitor required in an assay to reduce the control enzyme rate by 50%. Figures in parentheses are the standard errors.

Biological Results

Thymidine Kinase. By analogy with adenylate kinase, Ap_4T would be expected to be the best inhibitor. We found that Ap_4T was a better inhibitor than Ap_3T, but that unexpectedly the compounds with longer chain lengths were even more potent, with Ap_5T being the best inhibitor of the series. These results are broadly in agreement with those of Bone et al.,[8] but we found a greater difference in potency between the compounds. This may be due to the

(1) Feldhaus, P.; Frohlich, T.; Goody, R. S.; Isakov, M.; Schirmer, R. H. *Eur. J. Biochem.* **1975**, *57*, 197.
(2) Collins, K. D.; Stark, G. R. *J. Biol. Chem.* **1971**, *246*, 6599.
(3) Hampton, A.; Hai, T. T.; Kappler, F.; Chawla, R. R. *J. Med. Chem.* **1982**, *25*, 801.
(4) Barrie, S. E.; Paine, R. M.; Stock, J. A.; Vincent, R. B.; Harrap, K. R. *Purine Metabolism in Man–IV*, Part B; Plenum: New York, 1984; p 371.
(5) Hoard, D. E.; Ott, D. G. *J. Am. Chem. Soc.* **1965**, *87*, 1785.
(6) Michelson, A. M. *Biochem. Biophys. Acta* **1964**, *91*, 1.
(7) Wehrli, W. E.; Verheyden, D. L. M.; Moffatt, J. G. *J. Am. Chem. Soc.* **1965**, *87*, 2265.
(8) Bone, R.; Cheng, Y-C.; Wolfenden, R. *J. Biol. Chem.* **1986**, *261*, 5731.

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