recommended by the National Cancer Institute.

Measurement. FT ^{13}C NMR spectra were obtained at 25 MHz with broad-band proton decoupling on a JEOL JNM-FX-100 spectrometer employing the solvent deuterium signal as an internal lock. A total of 20 200–25 800 FID's (8192 points) was averaged to provide the desired signal to noise ratio in the 2.5-kHz frequency spectra. Pulse angles of 45° were employed with no pulse delay. The ambient temperature was room temperature. Tetramethylsilane sealed in a capillary was used as an external reference. All NMR spectra were measured in D_2O solutions. Absorption spectra (AB) were measured in H_2O with a Shimadzu UV 200 recording spectrometer. Circular dichroism (CD) spectra were measured in H_2O with a JASCO J-40 spectropolarimeter. All measurements were performed at room temperature.

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Showdomycin Analogues: Synthesis and Antitumor Evaluation

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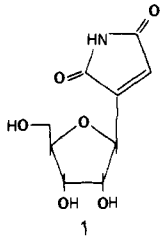
The synthesis of *N*-β-D-ribofuranosyl derivatives of maleimide, 3-methylmaleimide, and 3-chloromaleimide was accomplished in three steps from ribosylamine. The synthetic ribosides can be considered N-nucleoside analogues of showdomycin, which is an antitumor antibiotic of the C-nucleoside type. Although the three analogues were cytotoxic to cultured L1210 cells, no in vivo antitumor activity was found with the murine P388 leukemia test system. Drug transport studies were done in an attempt to trace the biological fate of the analogues.

Showdomycin (1) is an antitumor antibiotic of the C-



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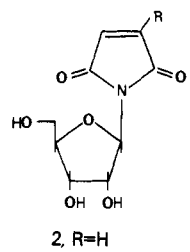
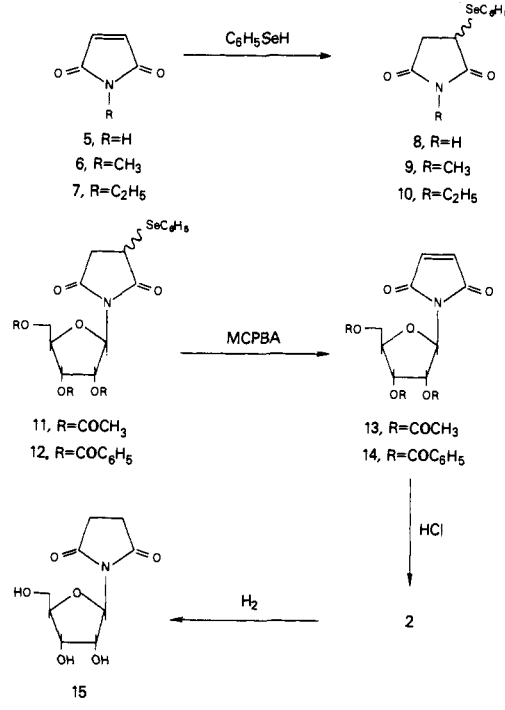


2, R=H
3, R=CH_3
4, R=Cl

nucleoside series that structurally can be considered a ring-contracted analogue of pseudouridine or an analogue of the sulfhydryl reagent *N*-ethylmaleimide (7). Although 1 and 7 inhibit cellular growth in a similar manner, the former is not simply an indiscriminate alkylating agent as is the latter: nucleosides prevent the inhibitory effects of showdomycin but not those of *N*-ethylmaleimide.[3] Apparently, the ribose moiety of 1 contributes to a facilitated entry into cells. Once taken up by the cells, the showdomycin exerts its alkylating capability on intracellular sulfhydryl groups.

Although the therapeutic range of showdomycin is too narrow for use in the treatment of clinical cancer, the specificity which the ribosyl substituent confers on the transport of maleimide across the cell membrane contributes to the continued interest in this antibiotic.[3,4] Also worthy of note is that showdomycin produced a marked radiosensitizing effect on *Escherichia coli*[5] which was enchanced when experiments were done under anoxic conditions.[6] These studies prompted a clinical trial[7] in which

Scheme I



it was shown that showdomycin was effective as a radiosensitizer in the treatment of malignant brain tumors with small dose radiotherapy.

The novel nucleoside structure and biological activity of 1 focused our efforts on the synthesis of analogues of 1 belonging to the naturally occurring N-nucleoside series in which a range of chemical reactivities in the maleimide double bond could be achieved by appropriately selected substituents. In this paper, we report the synthesis and biological evaluation of the *N*-ribosylmaleimides 2–4.

Chemistry. The initial strategy for synthesizing the *N*-ribosyl analogues called for protection of a requisite maleimide by the addition of benzeneselenol (C_6H_5SeH) across the double bond prior to condensation with a ribose

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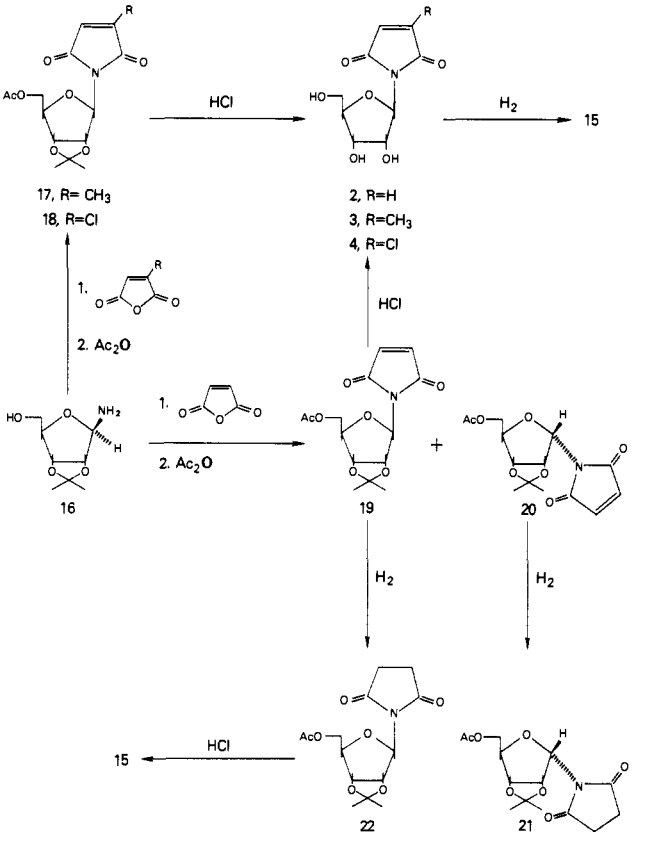
Table I. Chemical and Physical Data

no.	formula	method	% yield	mp, °C	anal.	IR: ν, cm^{-1}	$[\alpha]^{25}_D$, deg
2	$C_9H_{11}NO_6$	A	46	75–85	C, H, N, O	3400 (br), 1775, 1700, 1585	−32 (*c* 0.11, H_2O)
3	$C_{10}H_{13}NO_6$	A	67	oil	C, H, N	3400 (br), 1773, 1705, 1640	−26 (*c* 0.11, CH_3OH)
4	$C_9H_{10}NO_6Cl$	A	89	amorphous	C, H, N	3400 (br), 1850, 1715, 1600	−28 (*c* 0.11, H_2O)
8	$C_{10}H_9NO_2Se$	B	81	107–108	C, H, N, Se	3250 (br), 1780, 1720, 1705 (sh)	
9	$C_{11}H_{11}NO_2Se$	B	69	73.5–74.5	C, H, N, Se	1770, 1695	
10	$C_{12}H_{13}NO_2Se$	B	74	64.5–65.5	C, H, N, Se	1775, 1695	
11	$C_{21}H_{23}NO_9Se$	C	51	oil	C, H, N, Se	1785, 1745, 1725	
12	$C_{36}H_{29}NO_9Se$	C	96	59–63	C, H, N, Se	1785, 1725	
13	$C_{15}H_{17}NO_9$	D	85	oil	C, H, N, O	1735, 1715	−42 (*c* 0.18, DCE)
14	$C_{30}N_{23}NO_9$	D	90	56–60	C, H, N	1780, 1740, 1735, 1720	−41 (*c* 0.12, DCE)
15	$C_9H_{13}NO_6$	E, F	100	113–114	C, H, N	3480, 1787, 1705, 1690	−53 (*c* 0.1, H_2O)
17	$C_{15}H_{19}NO_7$	G	26	75–77	C, H, N	1785, 1740 (sh), 1720, 1645	−22 (*c* 0.14, CH_3OH)
18	$C_{14}H_{16}NO_7Cl$	G	18	oil	C, H, N	1795, 1730 1600	−31 (*c* 0.14, CH_3OH)
19	$C_{14}H_{17}NO_7$	H	36	52–55	C, H, N	1780, 1720, 1600	−25 (*c* 0.11, CH_3OH)
20	$C_{14}H_{17}NO_7$	H	4	oil	C, H, N	1780, 1740, 1720, 1595	+34 (*c* 0.12, CH_3OH)
21	$C_{14}H_{19}NO_7$	E	100	oil	C, H, N	1785, 1740, 1720	+40 (*c* 0.12, CH_3OH)
22	$C_{14}H_{19}NO_7$	E	96	112–114	C, H, N	1785, 1723	−24 (*c* 0.13, CH_3OH)

intermediate. At an appropriate stage in the synthesis the double bond could then be regenerated via oxidation, resulting in spontaneous elimination of benzeneselenenic acid (C_6H_5SeOH).[8] Accordingly, maleimide (**5**) was reacted with benzeneselenol to provide a stable addition product in good yield (**8**, Scheme I). Adducts **9** and **10** also were smoothly formed from the selenol with **6** and **7**, respectively. Using the Vorbrüggen procedure,[9] the maleimide adduct (**8**) was trimethylsilylated with bis(trimethylsilyl)trifluoroacetamide (BSTFA) and condensed in the presence of stannic chloride with either ribose tetraacetate or 1-acetylribose tribenzoate to give **11** and **12**, respectively. The maleimide double bond was regenerated by treatment of **11** and **12** with *m*-chloroperoxybenzoic acid (MCPBA) to give **13** and **14**. Tribenzoates **12** and **14** were obtained in high yield as crystalline solids (Table I), but, unfortunately, all hydrolysis attempts failed to remove the benzoyl protecting groups without extensive destruction of the product. However, acid hydrolysis of **13** gave isoshowdomycin (**2**) in 46% yield following simple column chromatography. The synthetic plan shown in Scheme I, although successful in the preparation of **2**, lacked the synthetic generality necessary also to yield analogues **3** and **4**. Attempts to apply the Vorbrüggen ribosylation reaction directly on maleimide derivatives, without protection of the double bond, gave no isolatable products.

A synthesis (Scheme II) based on ribofuranosylamine **16** as a key intermediate had sufficient generality to provide **2**, as well as the methyl (**3**) and chloro (**4**) analogues. Amide **16** as the crystalline tosylate salt[10] was readily

Scheme II



prepared and reacted as anticipated with the appropriate maleic anhydrides in the presence of triethylamine. The resulting maleamic acids were not isolated but cyclized directly to maleimides **17**–**19** with acetic anhydride containing sodium acetate.[11] Except for a small amount of

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Table II. Ultraviolet Maxima for Water Solutions of the Ribosylmaleimides

no.	UV: λ_{max}, nm (ϵ)	
2	215 (13 100)	282 (360)
3	218 (13 600)	275 (250)
4	227 (12 900)	280 (330)

the α-anomer (**20**) which formed along with **19**, only products having the β configuration were isolated. Removal of the protecting groups of **17–19** by acid hydrolysis gave the desired showdomycin analogues **2–4**. Analogue **2** obtained by this route was identical with that given by the synthetic sequence described earlier (Scheme I).

The structure assignments for **2–4** are supported by a number of observations. The UV spectra (Table II) show the characteristically weak maleimide chromophore.[12] The NMR spectra (Table III) of **2** and **4** show the olefinic protons as sharp singlets. As expected, the olefinic proton of **3** is weakly coupled (J = 2 Hz) to the allylic methyl group and appears as a quartet at δ 6.48. The isolation of the α-anomer **20** (4% yield) along with the predominant β-anomer **19** (36%) enroute to the synthesis of **2** was of considerable utility in assigning configurations at $C_{1'}$. Since the chemical shift of the anomeric proton of **19** was observed at higher field (δ 5.78) than the corresponding proton of **20** (δ 6.01), the former can be assigned the β configuration and the latter the α configuration.[13] In an unsuccessful synthesis of **2**, Schwartz and Lerner[14] prepared the tri-*O*-acetate of isoshowdomycin, to which an anomeric configuration could not be unequivocally assigned and which was too unstable to be successfully deacetylated. The optical rotation in dichloroethane was reported to be +121° (*c* 1.93), and the anomeric proton at 60 MHz in $CDCl_3$ solution appeared as a doublet at δ 6.07 (J = 4 Hz). The 60-MHz NMR of triacetate **13**, which was prepared during the course of this study, exhibited the anomeric proton as a doublet at a substantially higher field (δ 5.65, J = 4 Hz). Using the same NMR argument as above,[13] the triacetate reported by Schwartz and Lerner undoubtedly has the α configuration, and triacetate **13** is the corresponding β-anomer. The levorotatory specific rotation observed with **13** (Table I), in contrast to the dextrorotatory triacetate of Schwartz and Lerner, is a further indication of the β configuration for **13**. Indeed, all of the levorotatory compounds in Table I have a β assignment, and the two compounds having the α configuration (**20** and **21**) are dextrorotatory.

Catalytic hydrogenation of the isoshowdomycin (**2**) obtained from the method of Scheme I gave a succinamide derivative (**15**) which was identical with that obtained via the synthetic route (**19** → **22** → **15**) given in Scheme II. Hydrogenation of the chloro analogue **4** also gave **15**, which established the β configuration for **4**. Attempts to hydrolytically remove protection groups from the α-anomer **20** resulted in decomposition. A similar hydrolytic instability was observed earlier[14] for the α-anomer of **13**.

The rule proposed by Imbach[15] for distinguishing between α- and β-ribosides based on the difference in chemical shifts of *gem*-dimethyl singlets does not hold for the isopropylidene derivatives in this study, and, thus, they exemplify a structural type which was shown to be another exception[16] to the proposed rule.

Table III. Proton NMR Chemical Shifts at 100 MHz

		^{1}H NMR[a]			
no.	solvent	H-1'	acetyl	*gem*-dimethyl	pyrroline
2	CD_3OD	5.55 (d, J = 5, 1 H)			6.86 (s, 2 H)
13	$CDCl_3$	5.65 (d, J = 4, 1 H)	2.06 (s, 3 H) 2.08 (s, 6 H)		6.78 (s, 2 H)
15	CD_3OD	5.55 (d, J = 5, 1 H)			2.71 (s, 4 H)
19	$CDCl_3$	5.78 (d, J = 2, 1 H)	2.06 (s, 3 H)	1.35 (s, 3 H), 1.55 (s, 3 H)	6.76 (s, 2 H)
22	$CDCl_3$	5.82 (d, J = 2) 1 H)	2.04 (s, 3 H)	1.34 (s, 3 H), 1.54 (s, 3 H)	2.72 (s, 4 H)
20	$CDCl_3$	6.01 (d, J = 5, 1 H)	2.10 (s, 3 H)	1.32 (s, 3 H), 1.49 (s, 3 H)	6.73 (s, 2 H)
21	$CDCl_3$	6.01 (d, J = 5, 1 H)	2.08 (s, 3 H)	1.31 (s, 3 H), 1.49 (s, 3 H)	2.68 (s, 4 H)
17	$CDCl_3$	5.78 (d, J = 2, 1 H)	2.06 (s, 3 H)	1.35 (s, 3 H), 1.55 (s, 3 H)	2.08 (d, J = 2, 3 H) 6.36 (d, J = 2), 1 H)
18	$CDCl_3$	5.79 (d, J = 2, 1 H)	2.05 (s, 3 H)	1.35 (s, 3 H), 1.55 (s, 3 H)	6.70 (s, 1 H)
3	CD_3OD	5.50 (d, J = 5, 1 H)			2.06 (d, J = 2, 3 H), 6.48 (q, J = 2, 1 H)
4	CD_3OD	5.53 (d, J = 5, 1 H)			6.93 (s, 1 H)

[a] Chemical shifts are reported in δ units downfield from tetramethylsilane. J values are recorded in hertz. Abbreviations used are: s, singlet, d, doublet; q, quartet.

Biological Evaluation. Antitumor Evaluation. The P388 murine leukemia test system, which has been described previously,[17,18] was selected for in vivo antitumor testing of compounds **2–4** and **13**. Drugs in 0.9% saline solution (Tween 80 was required to solubilize compound **13**) were injected intraperitoneally daily beginning 24 h after tumor implantation and continuing for 9 days (nine injections). Using this test procedure, showdomycin (**1**) at 4–8 (mg/kg)/day increased the survival of test animals bearing P388 leukemia by approximately 50% over untreated control groups. However, the showdomycin analogues (**2–4** and **13**) did not significantly increase survival times when tested in the same way. The approximate LD_{50} values for **2–4** and **13** observed with the daily adminis-

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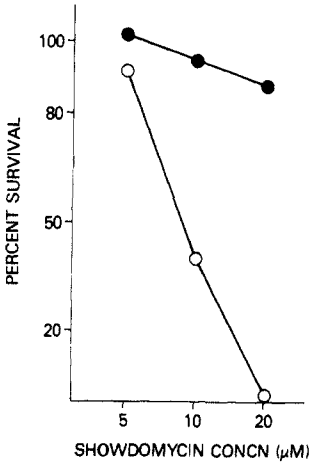

Figure 1. The effect of showdomycin (1, ○) and showdomycin in combination with a nucleoside transport inhibitor (HNBMPR, 7 μM, ●) on the growth of cultured L1210 cells with respect to a control population. Dose–response curves are shown as semilogarithmic plots.

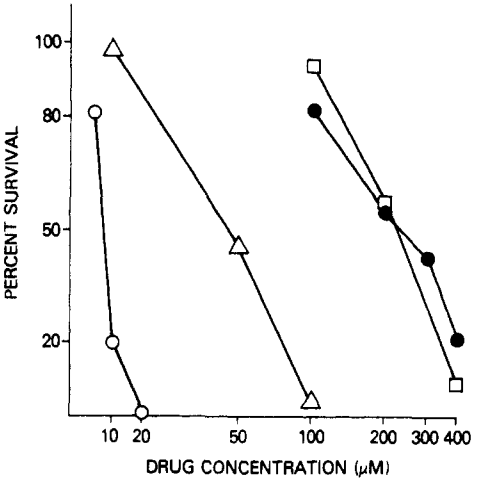

Figure 2. Growth inhibition of cultured L1210 cells by isoshowdomycin triacetate (**13**, ○), chloroisoshowdomycin (**4**, △), methylisoshowdomycin (**3**, □), and isoshowdomycin (**2**, ●). Dose–response curves are shown as semilogarithmic plots.

tration schedule were 35, 100, 18, and 10 (mg/kg)/day, respectively. Triacetate **13** also produced manifestations of numerous neurological toxicities in the mice.

The P388 test system was also used to investigate the potential of the phenylseleno group to latentiate the reactive maleimide double bond. The phenylselenol–maleimide adducts, if metabolically oxidized, would result in the spontaneous elimination of benzeneseleninic acid and the in vivo release of a maleimide derivative. The ejected seleninic acid would probably contribute to a minimal host Accordingly, when adducts **8–10** were tested in the P388 system (day 1, 5, and 9 treatment schedule), no antitumor activity was observed. The acute LD_{50} doses of the adducts **8–10** were approximately 10 times higher than those found for the corresponding maleimides **5–7**. Since the LD_{50} dose levels for the adducts paralleled those of the related maleimides, the toxic entity derived from the adducts is probably the maleimide substructure. Although the selenium compounds, therefore, were apparently latentiated maleimides, toxicity selective for cancer cells was not observed.

Cytotoxicity and Transport Studies. The disappointing antitumor testing results given by analogues **2–4** and **13** prompted us to make some experimental inquiries to determine possible reasons for the failure of these analogues to exhibit antitumor activity.

The potent cytotoxicity shown by showdomycin (**1**) can be attributed to its facilitated passage across the cell membrane via a carrier-mediated nucleoside transport system and to its reactivity as a maleimide sulfhydryl reagent.[20] Figure 1 shows the cytotoxicity[21] of showdomycin (open circles at three concentrations against cultured L1210 cells. The curve defined by the solid circles shows the moderating affect on showdomycin cytotoxicity which is observed in the presence of 6-[(2-hydroxy-5-nitrobenzyl)thio]-9-β-D-ribofuranosylpurine (HNBMPR). Since HNBMPR is a potent specific inhibitor of the nucleoside transport system,[22,23] the cytotoxicity decrease can be attributed to the reduced cellular uptake of **1**.

Figure 2 shows the results for the cytotoxicity evaluations of chloroisoshowdomycin (**4**), methylisoshowdomycin (**3**), isoshowdomycin (**2**), and isoshowdomycin tri-*O*-acetate (**13**). Only triacetate **13** has a level of potency comparable to showdomycin. By contrast, analogues **2** and **3** were only weakly cytotoxic, and the chloro analogue **4** was of an intermediate potency.

When the cytotoxicity experiments were repeated in the presence of 7 μM HNBMPR, no change in the respective curves produced by the analogues (Figure 2) was observed, except in the 200–400 μM region of methylisoshowdomycin (**3**) wherein percent survival values increased from 60 (200 μM) and 9% (400 μM) to 90 and 53%, respectively. The cytotoxicity inhibition of **3** at 200 and 400 μM was further enchanced (95 and 85%, respectively) through the agency of the triterpenoid inhibitor of nucleoside transport, cimicifugoside.[24,25] As expected, cimicifugoside at 7 μM had no effect on the cytotoxicity of chloroisoshowdomycin (**4**) but completely nullified the cytotoxic effect of showdomycin at 5–20 μM.

Discussion

Pseudouridine, 5-(β-D-ribofuranosyl)uracil, in which the linkage of sugar to base occurs via a carbon–carbon bond, is characterized by a relatively poor affinity for the transport protein of the nucleoside uptake system.[26] Since showdomycin (**1**) is also a C-nucleoside and can be considered a ring-contracted analogue of pseudouridine, it seemed likely that **1** might also compete poorly for the nucleoside transport system. Therefore, we initially thought that *N*-ribosylmaleimides might show a greater potency through a more effective utilization of the transport system. Unfortunately, that end was not at all realized with the *N*-ribosyl analogues synthesized during the course of this study. Transport studies with HNBMPR indicated that analogues **2** and **4** did not use the nucleoside transport system. Since both **2** and **4** have

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partition coefficients in the hydrophilic range, passive diffusion across the cell membrane would not be a favorable process, which might explain the weak to moderate cytotoxicity observed for 2 and 4. Isoshowdomycin triacetate (13), although its cytotoxicity was not affected by HNBMPR and, therefore, was not transported by the nucleoside transport system, nevertheless, exhibited a potenty cytotoxicity. Unlike 2, the lipophilic character of 13 perhaps promoted cellular uptake by passive diffusion whereupon the very reactive, unsubstituted maleimide double bond could exert its effect within the cell.

Drug instability in aqueous solution cannot be implicated in the poor cytotoxicity observed for 2–4. Using a simple NMR method, the half-life of isoshowdomycin (2) in water was found to be approximately 80 h at 27 °C. Water solutions of the chloro analogue 4 were more stable than solutions of 2, whereas methylisoshowdomycin (3) was the most resistant to hydrolysis.

Of the analogues appraised in Figure 2, only the methylisoshowdomycin (3) cytotoxicity was decreased in the presence of transport inhibitors, indicating that only 3 was able to utilize the nucleoside transport system. Although 3 is apparently taken up by active transport, once inside the cell it perhaps is not sufficiently reactive toward sulfhydryl groups to elicit an appreciable cytotoxic response. It has been reported[11] that *N*-ethylmaleimide (a model compound for 2) is 500 times more reactive toward glutathione addition than *N*-ethyl-2-methylmaleimide (a model for 3). In the same study, *N*-ethylmaleimide was about 10 times more reactive than showdomycin (1).

An extensive study[26] of the specificity of the nucleoside transport sysem revealed that considerable structural variation in the nucleoside base is permissible, although the sugar moiety is ostensibly limited to ribose and 2′-deoxyribose. Both purine and pyrimidine nucleosides utilize the identical carrier-mediated transport, suggesting a carrier protein with a flexible active site. In view of those observations it was surprising to find an apparent rigid transport specificity with respect to analogues 2–4. Thus, addition of a methyl group to isoshowdomycin (2) produced an analogue transported by the nucleoside transport carrier system. Replacement of the methyl with a chloro substituent, which has similar space-filling attributes, gave an analogue which again was unable to use the transport system. However, these results can be rationalized by assuming covalent bond formation between the carrier and the maleimide analogue. If 2 and 4 combine with transport protein irreversibly, a condition would ensue whereby 2 and 4 would effectively exclude themselves from the cell interior through carrier inactivation, and potent cytotoxicity would, therefore, not be observed. Since 3 is chemically much less reactive than 2 and 4, it does not inactivate the carrier; therefore, carrier-mediated transport was observed for 3. Studies are in progress to test the validity of this assumption.

Experimental Section

A Cary Model 15 spectrophotometer was used to obtain UV spectra, and a Perkin-Elmer Model 621 was used to record infrared spectra. Proton NMR spectra were recorded with a Varian HA-100D spectrometer. Where appropriate, decoupling experiments were carried out to verify chemical-shift assignments. Specific rotations were measured in a 1-dm cell with a Perkin-Elmer Model 141 polarimeter at the sodium D line. Mass spectra were routinely determined for all new compounds by direct probe insertion with a DuPont 21-492 spectrometer operated with a 75-eV ionizing voltage. When necessary, samples were pertrimethylsilylated with a BSTFA–CH_3CN (1:2) reagent. Although not reported here, mass spectra were in accord with assigned structures. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. Preparative LC was carried out with a Waters Associates, Inc., Model PrepLC/System 500A liquid chromatograph equipped with a PrepPAK-500 silica cartridge. Open column chromatography was accomplished with silica gel 60 (extra pure, 70–230 mesh) obtained from E. Merck, Darmstadt, West Germany. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN. Analytical results for elements indicated by their symbol were within ±0.4% of theoretical values.

1-β-D-Ribofuranosyl-1*H*-pyrrole-2,5-dione (2). Method A. A solution of 19 (4.15 , 14.4 mmol) in methanol (140 mL) containing 6 N aqueous HCl (14 mL) was stirred for 6 h at 40 °C. The reaction solution was evaporated in vacuo and then dried under high vacuum overnight to give a yellow oil (3.67 g) which was purified with preparative LC (ethyl acetate elution) to give 1.33 g of 2 as a colorless oil. Analytically pure 2 was obtained by evaporation of a methanol solution of the oil in the presence of a small amount of silica gel, loading the silica with adsorbed materials onto a silica column, and eluting the product (810 mg, 25%) with ethyl acetate–methanol (95:5).

Preparation of 2 by acid hydrolysis of 13 did not require preliminary purification by preparative LC but was directly obtained in a pure state (46% yield) by silica gel chromatography (EtOAc–CH_3OH, 95:5) of the crude product. Storage of the resulting colorless syrup at 4 °C for several days in a stoppered flask induced crystallization.

3-(Phenylseleno)-2,5-pyrrolidinedione (8). Method B. A stirred solution of maleimide (5; 4.85 g, 50 mmol) in dry ether (75 mL) was cooled with an ice bath and treated with benzeneselenol[27] (8.64 g, 55 mmol) under a nitrogen atmosphere. After the solution stirred for 2 h at room temperature, TLC analysis indicated the absence of maleimide. Petroleum ether (75 mL) was added to the reaction solution, and the resulting precipitate was stirred with the supernatant for 1–2 h at room temperature and then separated by filtration. After the precipitate was dried in a desiccator ($CaCl_2$), 10.34 g of powder was obtained, which was crystallized from CH_2Cl_2–petroleum ether.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-(phenylseleno)-2,5-pyrrolidinedione (11). Method C. A solution of 8 (5.08 g, 20 mmol) in anhydrous acetonitrile was treated with BSTFA (8 mL, 32 mmol) at room temperature under a nitrogen atmosphere and stirred for 3 h at room temperature. Solvent and volatile materials were removed from the reaction solution under vacuum, and the residual yellow oil was taken up in anhydrous acetonitrile (30 mL). While working in a dry box under a dry nitrogen atmosphere, the above acetonitrile solution was combined at room temperature with tetra-*O*-acetyl-β-D-ribofuranose (7.64 g, 24 mmol) and freshly distilled stannic chloride (8 mL) in anhydrous acetonitrile (30 mL). The mixture, which was protected with a calcium chloride drying tube, was stirred for 24 h at room temperature. While cooling with an ice bath, ice–water (60 mL) was added to the mixture, followed by vigorous stirring for 10 min. The mixture was extracted (3 times) with chloroform, and the combined extracts were shaken successively with brine, saturated sodium bicarbonate solution, and brine. The dried (magnesium sulfate) chloroform extracts were evaporated in vacuo to give 12.7 g of a yellow oil, which was chromatographed on silica gel. Elution with dichloromethane–ethyl acetate (9:1) gave 5.23 g (51%) of 11 as an oil. Continued elution gave 1.53 g (30%) of recovered 8.

1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-1*H*-pyrrole-2,5-dione (13). Method D. *m*-Chloroperoxybenzoic acid (85% purity, 1.12 g, 5.5 mmol) was added to a solution of 11 (2.56 g, 5 mmol) in chloroform (100 mL), which was stirred and cooled with an ice bath. The solution was stirred for 30 min with the ice bath in place, then treated with an additional 0.56 g (2.75 mmol) of peracid, and stirred for ca. 1 h at room temperature, at which time 11 was no longer evident by TLC analysis. The chloroform solution was shaken with saturated sodium bicarbonate solution and then with brine, and dried over magnesium sulfate. Solvent removal under reduced pressure gave a yellow oil (1.92 g), which was chromatographed on a silica gel column. Elution of the

(27) Foster, D. G. "Organic Synthesis"; Horning, E. C., Ed.; Wiley: New York, 1955; Collect. Vol. III, p 771.

column with dichloromethane–ethyl acetate (9:1) gave 1.51 g of 13 as an analytically pure oil.

1-β-D-**Ribofuranosyl-2,5-pyrrolidinedione (15). Method E. From Compound 2.** To a solution of 2 (220 mg, 0.96 mmol) in methanol (10 mL) was added 10% palladium on activated carbon catalyst (44 mg) under a nitrogen atmosphere. The nitrogen was exchanged with hydrogen and the mixture was stirred at room temperature under a hydrogen atmosphere for 40 min. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo to give colorless crystals of 15, which melted at 113–114 °C after recrystallization from methanol–petroleum ether.

Method F. From Compound 22. To a solution of 22 (550 mg, 1.76 mmol) in methanol (17 mL) was added 6 N aqueous HCl (1.7 mL), and the solution was stirred for 6 h at 40 °C. The reaction solution was evaporated in vacuo and then dried under high vacuum to give a viscous brown oil (540 mg). Purification of the oil with preparative TLC (silica; ethyl acetate–methanol, 20:1) provided colorless crystals of 15. The product was identical with 15 obtained from method E in terms of melting point, mixture melting point, and TLC analysis. The NMR and IR spectra of 15 from the two methods were superimposable.

1-[5-*O*-**Acetyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranosyl]-3-methyl-1*H*-pyrrole-2,5-dione (17). Method G.** At room temperature, a stirring slurry of the tosylate salt of 16[28] (3.61 g, 10 mmol) in chloroform (50 mL) was reacted with citraconic anhydride (1.12 g, 10 mmol) and triethylamine (1.11 g, 11 mmol). The reaction mixture was stirred for 1 h, and then the volatile materials were removed under vacuum to provide a yellow oil. The oil was dissolved in acetic anhydride (20 mL), anhydrous sodium acetate (2 g) was added, and the mixture was stirred at 100 °C for 1 h. In the preparation of compound 18, the sodium acetate was omitted. After the reaction solution cooled to room temperature, ice–water (60 mL) was added and the mixture was stirred for 1 h while cooling with an ice bath. The reaction mixture was dichloromethane extracted (30 mL × 5). The combined extracts were shaken with saturated sodium carbonate solution, followed with brine, and dried ($MgSO_4$). Solvent evaporation gave a brown oil (1.91 g), which was chromatographed on a silica gel column. Elution with dichloromethane–ethyl acetate (9:1) gave 17 (850 mg) as an oil, which gave colorless crystals after standing for several days at room temperature.

1-[5-*O*-**Acetyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranosyl]-1*H*-pyrrole-2,5-dione (19). Method H.** The procedure of Montero et al.[28] was used to prepare 2,3-(1-methylethylidene)ribofuranosylamine (16) as an oil in 70–90% yield from the corresponding tosylate salt.[10] A solution of 16 (1.26 g, 6.7 mmol) in ether (20 mL) was combined with maleic anhydride (0.67 g, 6.7 mmol) with stirring and cooling in an ice bath. The stirring and cooling was maintained for 1 h, during which time a white precipitate separated from solution. Evaporation of the reaction solution at reduced pressure yielded a foam, which was dissolved in acetic anhydride (20 mL) and treated with anhydrous sodium acetate (2 g). The mixture was stirred for 1 h at 100 °C, then cooled to room temperature, and ice–water (60 mL) was added with stirring and cooling with an ice bath. After 30 min, the mixture was extracted with CH_2Cl_2 (20 mL × 5). The combined extracts were washed with saturated sodium carbonate solution and brine. The dried ($MgSO_4$) dichloromethane solution was evaporated in vacuo to afford a brown oil (1.48 g), which was purified with preparative LC (elution with dichloromethane–ethyl acetate, 19:1) to give 19 (765 mg) as a colorless oil, which crystallized after standing at room temperature for several days. Continued elution of the LC column gave a second colorless oil (94 mg) which was the α-anomer (20).

Reaction of a slurry of 16 as the tosylate salt in chloroform with maleic anhydride in the presence of triethylamine gave, after workup as described above, 39% of 19 and 2% of 20.

Solution Decomposition of 2–4. A solution of 2 (70 mg/mL) in sterile water (pH 6.3) containing succinimide (35 mg/mL) as an internal standard was stored in a standard NMR tube at room temperature. At regular intervals (t = 0, 24, 48, 72, and 96 h) NMR spectra were recorded, and the decrease in isoshowdomycin (2) concentration was determined by comparing the integrated areas of the singlet due to the succinimide methylene groups (δ 2.8) with the singlet (δ 6.9) due to the olefinic protons of 2. The solution half-life of 2 was determined graphically from a plot of concentration vs. time. The solution decompositions of 3 and 4 were measured in a similar manner.

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Potential Antitumor Agents. 35. Quantitative Relationships between Antitumor (L1210) Potency and DNA Binding for 4′-(9-Acridinylamino)methanesulfon-*m*-anisidide Analogues

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Factors influencing dose potency of 4′-(9-acridinylamino)methanesulfon-*m*-anisidide (*m*-AMSA) analogues in L1210 assays have been investigated by multiple regression analysis. The dependent variable was D_{40}, the dose to provide 40% life extension in L1210 tests. Independent variables examined were chromatographic R_m values, as a measure of agent lipophilic–hydrophilic balance; R_m^2; log K, where K is the agent–DNA association constant for poly[d(A-T)]; log $T_{1/2}$, the half-life for congener thiolytic cleavage; and agent pK_a values. A regression equation containing terms in R_m^2 and log K was derived with the latter term accepting the greater proportion of the biological variance. DNA binding, of acridine substituted *m*-AMSA variants, is the most important factor influencing dose potency. Modeling of log K for 3-substituted derivatives provided an equation in substituent $\mathcal{R}$ constants and molar refractivities (MR).

During the historical development of the field of quantitative molecular structure–biological activity relationships (QSAR), the almost inevitable lack of knowledge of the strength of interaction between the drug congeners and site of action has required that such interactions be approximated by indirect methods. Within series of substituted drug congeners, changes in drug–site interactions can sometimes be adequately modeled by employing extrathermodynamic substituent parameters. However, such treatments neglect a major contribution that might be made by QSAR methodology. Normally, the site of drug action is inferred from mode of action studies which implicate a critical enzyme, or other cellular macromolecule, as a possible target. The often formidable logistics asso-

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