Pyrrolopyrimidine Nucleosides. 18.[1] Synthesis and Chemotherapeutic Activity of 4-Amino-7-(3-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (3′-Deoxysangivamycin) and 4-Amino-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (2′-Deoxysangivamycin)

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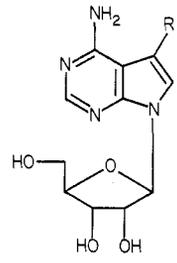
A multistep synthesis, using the nucleoside antibiotic toyocamycin as the starting material, has furnished 6,2′-*S*-cyclosangivamycin (**6**). Desulfurization of 6,2′-*S*-cyclosangivamycin (**6**) with Raney nickel has provided 2′-deoxysangivamycin (**7**). Treatment of sangivamycin (**1c**) with sodium iodide and α-acetoxyisobutyryl chloride has furnished 4-amino-7-[2-*O*-acetyl-3-deoxy-3-iodo-5-*O*-(2,5,5-trimethyl-4-oxo-1,3-dioxolan-2-yl)-β-D-xylofuranosyl]-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**8a**). Dehalogenation of **8a** with 10% palladium on charcoal was followed by a removal of the blocking groups with ammonium hydroxide to give 3′-deoxysangivamycin (**9**) in 49% overall yield. The reaction of sangivamycin (**1c**) with diphenyl disulfide and tributylphosphine gave 5′-(phenylthio)-5′-deoxysangivamycin (**10**). Treatment of **10** with Raney Nickel afforded 5′-deoxysangivamycin (**11**). Antitumor evaluation showed that 3′-deoxysangivamycin had significant activity against the murine leukemia L1210 both in vivo and in vitro, although it was less potent on a molar basis than the parent compound sangivamycin. The 2′- and 5′-deoxysangivamycins did not show significant antitumor activity.

The isolation, characterization, and chemical synthesis[2-4] of the pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics tubercidin (**1a**), toyocamycin (**1b**), and sangivamycin (**1c**), as well as their biological and chemotherapeutic activity,[2-5] created considerable interest in the synthesis of compounds related to this class of adenosine analogues.



1a, R = H (tubercidin)
b, R = CN (toyocamycin)
c, R = $CONH_2$ (sangivamycin)

Although the synthesis of base-modified analogues of tubercidin, toyocamycin, and sangivamycin has been described in some detail,[6] there has been a paucity of research reported on sugar-modified analogues of tubercidin[7] and essentially nothing related to the most clinically promising[8] pyrrolopyrimidine nucleoside antibiotic sangivamycin. The synthesis of a number of analogues of 2′-deoxyadenosine by an enzymatic procedure has been reported recently.[9] However, this approach did not appear to be applicable to the pyrrolo[2,3-*d*]pyrimidine adenosine analogues, since the authors were unsuccessful in their attempt to synthesize 2′-deoxytubercidin. We now report the chemical synthesis and biological activities of the 2′-, 3′-, and 5′-deoxysangivamycins.

Chemistry. Although there have been tremendous advances made in the condensation methods[10] used for the synthesis of nucleosides, the problem of obtaining an anomeric mixture still remains when preparing 2′-deoxyribofuranosides by a condensation procedure. However, 8,2′-*S*-cyclopurine nucleosides, have proven to be useful intermediates for the preparation of anomerically pure 2′-deoxypurine nucleosides.[11] The reactions of 2′,3′-*O*-dibutylstannylene complexes of ribonucleosides with electrophiles have also been reported[12] to yield 8,2′-*O*-cyclopurine nucleosides. We elected to investigate the use of this method for the synthesis of 2′-deoxysangivamycin (**7**) (Scheme I).

6-Bromo-2′,3′-*O*-(dibutylstannylene)toyocamycin (**3a**) was obtained from the reaction of 6-bromotoyocamycin[13]

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(7) (a) Anzai, K.; Matsui, M. *Agr. Biol. Chem.* **1973**, *37*, 345. (b) Jain, T. C.; Russell, A. F.; Moffatt, J. G. *J. Org. Chem.* **1973**, *38*, 3179. (c) Robins, M. J.; Fouron, Y.; Muhs, W. H. *Can. J. Chem.* **1977**, *55*, 1260, and references cited therein.
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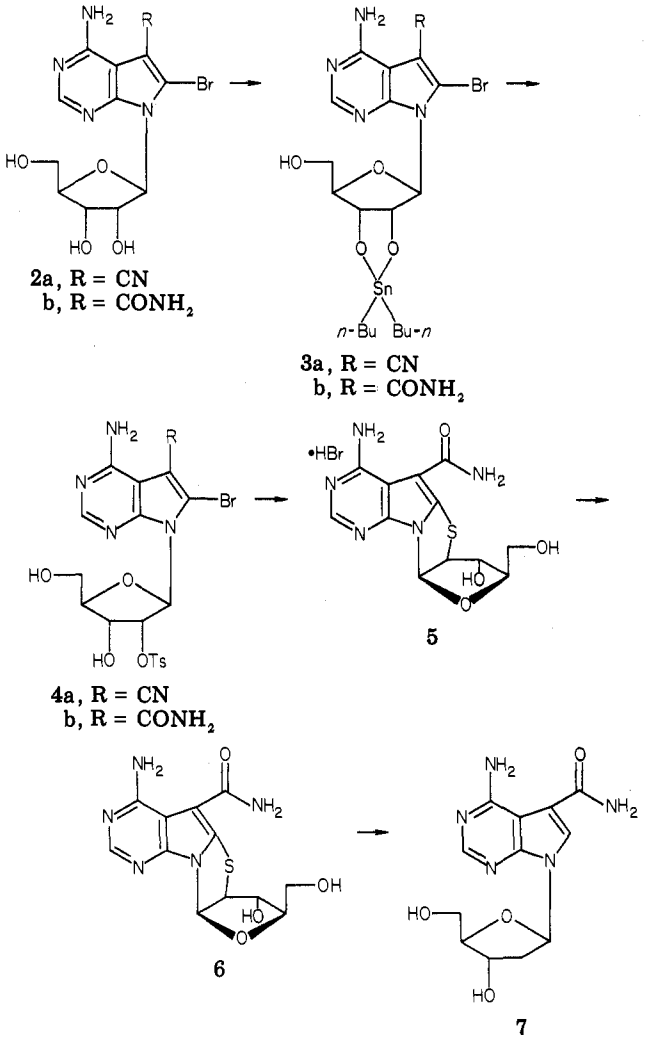
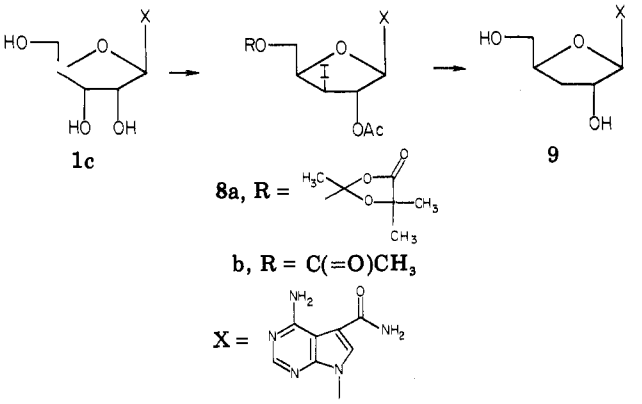
Scheme I

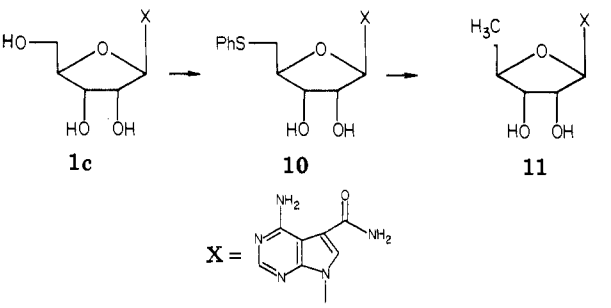


(**2a**) with di-*n*-butyltin oxide. This nucleoside (**3a**) was then treated with tosyl chloride and triethylamine in methanol to give 6-bromo-2′-*O*-tosyltoyocamycin (**4a**). The conversion of **4a** to 6-bromo-2′-tosylsangivamycin (**4b**) was accomplished (66% yield) with hydrogen peroxide and ammonium hydroxide. An alternative and versatile synthesis of **4b** was accomplished by the tosylation of 6-bromo-2′,3′-*O*-(dibutylstannylene)sangivamycin (**3b**). The formation of an 8,2′-*S*-cyclo bond has been achieved by treating 8-bromo-2′-*O*-(arylsulfonyl)adenosine with sodium hydrogen sulfide.[14] However, we found that this procedure was unsuitable for the conversion of **4b** into 6,2′-*S*-cyclo-4-amino-6-mercapto-7-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**6**). It was subsequently found that when thiourea and the nucleoside **4b** were heated at reflux in 1-propanol, a white crystalline compound was isolated and characterized as 6,2′-*S*-cyclosangivamycin hydrogen bromide (**5**). ^{1}H NMR spectroscopy revealed a 0.5-ppm downfield shift for the anomeric proton, and a very small coupling constant was observed between the 2′ proton and the 3′ proton ($J_{2',3'} \simeq 0$), which is a characteristic feature of 8,2′-cyclopurine nucleosides.[15] The nucleoside **6** was obtained by treating **5** with aqueous triethylamine. Treatment of compound **6** with Raney nickel gave a white crystalline product. ^{1}H NMR spectroscopy of this solid showed a 0.5-ppm downfield shift for

Scheme II



Scheme III



the anomeric proton (triplet) and a 2.1-ppm upfield shift for the 2′-proton, which is characteristic of 2′-deoxynucleosides.[16] On this basis, the nucleoside was established as being 4-amino-7-(2-deoxy-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (2′-deoxysangivamycin, **7**).

The nucleoside antibiotic cordycepin has been synthesized by the direct condensation method[17] and also by desulfurization of the appropriate 3′-deoxy-3′-sulfur-containing nucleosides.[14] The successful reactions of 2-acetoxyisobutyryl halides[7c] with certain nucleosides prompted us to investigate the use of this reagent for the synthesis of 3′-deoxysangivamycin (**9**) (Scheme II). When sangivamycin (**1c**) was treated with α-acetoxyisobutyryl iodide in acetonitrile, two major compounds were obtained after separation by silica gel column chromatography. The structures of these products were assumed to be 4-amino-7-[3-iodo-3-deoxy-2-*O*-acetyl-5-*O*-(2,5,5-trimethyl-4-oxo-1,3-dioxolan-2-yl)-β-D-xylofuranosyl]pyrrolo[2,3-*d*]-pyrimidine (**8a**) and the 5′-*O*-acetyl derivative **8b** on the basis of ^{1}H NMR and UV spectroscopy. The yields of **8a** and **8b** were calculated as 56 and 20%, respectively. It was found that hydrogenation of either pure compound or a mixture of **8a** and **8b**, with 10% palladium on charcoal at 40 psi of hydrogen, followed by treatment with ammonium hydroxide, gave a good yield of what was assumed to be 4-amino-7-(3-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**9**, 3′-deoxysangivamycin). The ^{1}H NMR spectrum of **9** revealed that the peak assigned to the C-3′ hydrogens of the product appeared at 2.0 ppm, which is similar to the chemical shift observed[2,17] for the C-3′ hydrogens of cordycepin. The structure of **9** was further confirmed by elemental analysis and ultraviolet, mass, and ^{1}H NMR spectra.

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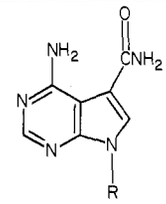
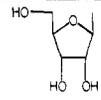
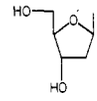
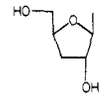
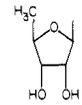
Table I. In Vitro Cytotoxicity and in Vivo Antitumor Testing against Murine Leukemia L1210



			in vivo [b]		
no.	R	in vitro: ID_{50},[a] M	dose per injn,[c] mg/kg	ILS,[d] % of control	host toxicity at higher doses[e]
1c		3.4×10^{-9}	0.25	37	y
7		1.3×10^{-5}	160	4	nt
9		6×10^{-7}	128	48	nt
11		7×10^{-5}	100	5	y

[a] ID_{50} is the concentration required to reduce the growth rate of L1210 cells in culture to half of the control rate. [b] Data are presented for the dose that gave optimal activity or for inactive compounds (ILS < 25%), the highest nontoxic dose tested. Data for compound 1c is the average of seven experiments; others are from single experiments. [c] Administered ip to L1210-bearing mice once daily on days 1–9 after inoculation of the animals with 10^5 tumor cells. [d] ILS is the increase in life span for drug-treated animals as compared to control, untreated tumor-bearing animals, expressed as a percentage of the life span of the untreated tumor-bearing animals. [e] Occurrence of drug-induced shortening of life span resulting from treatment with higher doses: y = yes; nt = no higher dose tested.

The route we envisaged for the preparation of the reamining deoxysangivamycin derivative (5′-deoxysangivamycin) was based on the fact that sulfur-containing compounds are good intermediates for 5′-deoxynucleoside synthesis. Sangivamycin (**1c**) was treated with diphenyl disulfide and tri-*n*-butylphosphine in pyridine[18] to give 5′-(phenylthio)-5′-deoxysangivamycin (**10**) in 80% yield (Scheme III). A downfield shift of the peak asssigned to the 5′ protons and a disappearance of the signal for the 5′-hydroxy group indicated that a selective substitution had occurred at the 5′ carbon. Desulfurization of compound **10** with Raney nickel gave a white solid, which was assigned the structure 4-amino-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**11**, 5′-deoxysangivamycin). Additional confirmation for the proof of structure for **11** was determined by ^1H NMR, UV, and mass spectroscopy, as well as elemental analysis.

All of the monodeoxy derivatives of the nucleoside antibiotic sangivamycin have been synthesized, vide supra, and an evaluation of their anticancer activity is described in the following section.

Antitumor Results and Discussion

Sangivamycin (**1c**) and its three deoxy derivatives, **7**, **9**, and **11**, were evaluated for their activity against murine leukemia L1210 in vitro and in vivo (Table I). The parent compound **1c** was the most effective, and the 3′-deoxy derivative (**9**) also had significant antitumor activity both in vitro and in vivo. Using high-pressure liquid chromatographic methods (to be published elsewhere[19]), we checked the samples of compounds **7**, **9**, and **11** that were used for cytotoxicity testing for possible contamination with sangivamycin (**1c**). Compound **7** was found to contain 0.05% **1c**, but **1c** was not detectable in **9** or **11** (detection limit = 0.01%). Thus, the slight cytotoxic effects of **7** and **11** could have been due to contamination with trace amounts of **1c**, since 0.03% **1c** in **7** and 0.005% **1c** (less than the detection limit) in **11** would have been sufficient to produce the observed growth inhibition. However, the cytotoxic activity of 3′-deoxysangivamycin (**9**) was two orders of magnitude greater than could be accounted for by an undetectable amount of **1c**; i.e., the ID_{50} is two orders of magnitude lower than would result from the presence of 0.01% **1c** in the sample of **9**. Therefore, we have concluded that the activity observed for **9** was due to 3′-deoxysangivamycin, per se. This compound (**9**) also showed in vivo antitumor activity against murine leukemia L1210. The ILS produced by the optimal (highest tested) dose of **9** compared favorably with that obtained at the optimal dose of sangivamycin (**1c**) (Table I). In contrast, compounds **7** and **11** were inactive in the in vivo antitumor testing.

The 2′- and 3′-deoxy derivatives of another pyrrolo[2,3-*d*]pyrimidine antibiotic, tubercidin (**1a**), were recently reported[20] to be virtually ineffective in inhibiting the growth of L1210 cells in vitro. On the other hand, 3′-deoxyadenosine, in the presence of the adenosine deaminase inhibitor 2′-deoxycoformycin, caused a 50% decrease in the growth rate at about 5×10^{-7} M,[20] a value comparable to the ID_{50} reported here for 3′-deoxysangivamycin (**9**), per se (Table I). Since sangivamycin resists deamination, it appears that 3′-deoxysangivamycin may be viewed as a deamination-resistant analogue of 3′-

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(19) Schroeder, W.; Cupps, T. L.; Townsend, L. B., accepted for publication in *J. Chromatog.*
(20) Cass, C. E.; Selner, M.; Tan, T. H.; Muhs, W. H.; Robins, M. J. *Cancer Treat. Rep.* **1982**, *66*, 317.

deoxyadenosine, which has been reported to have in vivo as well as in vitro antitumor activity when administered in combination with 2′-deoxycoformycin.[5] 3′-Deoxytubercidin should also resist deamination, since the parent compound tubercidin is not deaminated.[5] The observation[20] that 3′-deoxytubercidin was, nevertheless, ineffective as an antitumor agent in vitro further suggests that the antitumor activity of 3′-deoxysangivamycin cannot be totally accounted for by its resistance to deamination.

Thus, 3′-deoxysangivamycin (**9**) will be further investigated as an antitumor agent. As compared with sangivamycin (**1c**), its lesser potency on a molar basis may provide a wider therapeutic dosage range without host toxicity. The major clinical toxicities of sangivamycin were hypotension and flushing, which could be minimized if the drug was infused slowly.[21] These side effects indicated that sangivamycin had a vasodilator effect such as has been observed for adenosine.[22] Studies on the structure–activity requirements for coronary vasodilation showed that 3′-deoxyadenosine was only 6% as effective as adenosine, on a molar basis.[23] If a similar difference in vasodilating potency applied to sangivamycin (**1c**) and 3′-deoxysangivamycin (**9**), it would at least partially offset the requirement for higher doses of **9** to achieve a therapeutic effect comparable to **1c** (see Table I). This prediction was supported by the observations that **9** was nontoxic to the host at 128 mg/kg, the highest dose tested, while **1c** caused drug-induced shortening of the host life span when the dose exceeded 0.25 mg/kg.

The biochemical mechanism of action for 3′-deoxysangivamycin is unknown, but the most likely hypothesis would be that its effects would resemble those of sangivamycin and/or cordycepin (3′-deoxyadenosine), which interfere with various aspects of RNA transcription and posttranscriptional modifications.[5,24]

Experimental Section

Proton magnetic resonance (^{1}H NMR) spectra were obtained with JEOL C60H, Varian A56/60, and Varian EM-390 spectrometers (solution in dimethyl-d_6 sulfoxide or chloroform-*d*) with chemical-shift values reported in δ (parts per million) relative to an internal standard (sodium 2,2-dimethyl-2-silapentane-5-sulfonate or tetramethylsilane). Ultraviolet spectra were recorded on a Beckman Acta CIII spectrophotometer. Melting points were determined with a Thomas-Hoover capillary apparatus and are uncorrected. The optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Preparative thin-layer chromatography was performed on glass plates coated with silica gel (1.50 mm, SilicAR 7GF, Mallinckrodt). Compounds of interest were detected by either ultraviolet lamp (Mineralight, 254 nm) or treatment with sulfuric acid, followed by charring. Open-bed column chromatography was carried out on SilicAR CC7 (Mallinckrodt) using gravity flow. The columns were packed as slurries with the elution solvent. All solvent proportions are given by volume. Evaporations were performed in a rotary evaporator under reduced pressure (water aspirator) or in vacuo at 40 °C unless otherwise stated. All compounds were dried in vacuo at 80 °C for 10 to 15 h before submission for elemental analysis. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. The presence of water of crystallization in the elemental analyses was verified by ^{1}H NMR.

4-Amino-6-bromo-5-cyano-7-[2,3-*O*-(dibutylstannylene)-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine [6-Bromo-2′,3′-*O*-(dibutylstannylene)toyocamycin, 3a]. A mixture of 6-bromotoyocamycin (**2a**; 577 mg, 1.56 mmol) and di-*n*-butyltin oxide (390 mg, 1 equiv) in methanol (50 mL) was heated at reflux for 3 h to produce an amorphous solid. This solid was collected by filtration to give 841 mg (92%, 1.43 mmol) of a white solid. A sample was recrystallized from ethanol–acetone to yield colorless prisms of **2a**, mp 206–208 °C. Anal. ($C_{19}H_{28}H_5O_4BrSn$) C, H, N.

4-Amino-6-bromo-7-[2,3-*O*-(dibutylstannylene)-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide [6-Bromo-2′-3′-*O*-(dibutylstannylene)sangivamycin, 3b]. 4-Amino-6-bromo-7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**2b**; 2.60 g, 6.70 mmol) and di-*n*-butyltin oxide (1.733 g, 6.93 mmol) were dissolved in methanol (150 mL), and the mixture was heated at reflux for 2.5 h. The reaction mixture was allowed to stand at 5 °C for 12 h, and the crystalline precipitate was collected by filtration to yield 3.71 g (91%) of **3b**, mp 215–217 °C. Anal. ($C_{19}H_{30}BrN_5O_5Sn$) C, H, N.

4-Amino-6-bromo-5-cyano-7-(2-*O*-*p*-toluenesulfonyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (6-Bromo-2′-*O*-tosyltoyocamycin, 4a). 6-Bromo-2′,3′-*O*-(butylstannylene)toyocamycin (**3a**, 841 mg) was dissolved in 50 mL of methanol. Tosyl chloride (4.1 g, 15 equiv) and triethylamine (3.0 mL, 15 equiv) were then added, and the solution was stirred for 3 h at room temperature. The white crystals that had formed were collected by filtration to yield 480 mg (64%) of **4a**: mp 188–191 °C; IR (KBr) 2240 (CN), 1180 (covalent tosylate) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 8.14 (s, 1 H, H-2), 7.39 (d, 2 H, Ts H_a), 7.10 (d, 2 H, Ts H_b), 6.15 (d, 1 H, H-1′, $J_{1',2'}$ = 6.5 Hz), 2.36 (s, 3 H, Ts CH_3). Anal. ($C_{18}H_{16}N_5O_6SBr$) C, H, N.

Large-Scale Synthesis of 6-Bromo-2′-tosylsangivamycin (4b). 6-Bromotoyocamycin (**2a**; 7.77 g, 20 mmol) and di-*n*-butyltin oxide (5.27 g, 1 equiv) were added to 1 L of methanol, and the reaction mixture was heated at reflux for 1.5 h. The reaction mixture was cooled to room temperature, with tosyl chloride (59.2 g, 15 equiv) and triethylamine (43.5 mL) then being added while cooling the solution in an ice bath. The reaction mixture was stirred at room temperature for 3 h, during which time white crystals precipitated from the solution. The moist crystals were then suspended and stirred in a mixture of 30% hydrogen peroxide (34 mL), concentrated ammonia solution (68 mL), and methanol (270 mL) at room temperature for 4 h. Concentration of the solvent yielded 4-amino-6-bromo-7-(2-*O*-*p*-toluenesulfonyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (4b; 6.93 g, 13.1 mmol, 66% from 6-bromotoyocamycin).

4-Amino-6-bromo-7-(2-*O*-*p*-toluenesulfonyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (2′-*O*-Tosylsangivamycin, 4b). Method 1. 2′,3′-*O*-(Dibutylstannylene)-6-bromosangivamycin (**3b**; 3.68 g, 6.06 mmol) was dissolved in methanol, with triethylamine (12.7 mL, 15 equiv) and tosyl chloride (17.3 g, 15 equiv) then being added. The mixture was allowed to stir at room temperature for 1 h, and the solvent was then evaporated in vacuo. Water (120 mL) was added to the residue, and the solution was extracted with ether (50 mL). The aqueous layer was separated and washed with ether (50 mL × 3). The aqueous layer was then concentrated to 100 mL in vacuo and allowed to stand at 5 °C for 18 h to yield **4b** (2.28 g, 71%): mp 191–193.5 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.0 (br, 2, 5-$CONH_2$), 7.96 (s, 1 H, H-2), 7.76 (br, 2 H, 4-NH_2), 7.47 (d, 2 H, Ts H_a), 7.01 (d, 2 H, Ts H_b, 6.15 (m, 2 H, H-1′ and 3′-OH), 5.86 (t, 1 H, H-2′), 5.73 (br, 1 H, 5′-OH), 4.42 (m, 1 H, H3′), 4.08 (m, 1 H, H-4′), 3.63 (m, 2 H, H-5′), 2.26 (s, 3 H, Ts CH_3); IR (KBr) 1180 (covalent tosylate) cm^{-1}. Anal. ($C_{19}H_{21}N_5O_7Br$·0.5 H_2O) C, H, N.

Method 2. 6-Bromo-2′-tosyltoyocamycin (**4a**; 153 mg) was stirred in a mixture of 30% hydrogen peroxide (0.5 mL), concentrated ammonia solution (1 mL), and methanol (4 mL) at room temperature. After 2 h, the solution became a clear, yellow color, and then a solid began to separate from the solution. After 5.5 h, the solvent was removed in vacuo, and water (5 mL) was then added to the residue. The mixture was allowed to stand at 5 °C for 18 h to yield **4b** (104 mg, 0.19 mmol, 66%), identical in all respects with the product obtained by method 1.

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6,2'-Cyclo-4-amino-6-mercapto-7-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyrimidine-5-carboxamide Hydrobromide (6,2'-*S*-Cyclosangivamycin Hydrobromide, 5). 6-Bromo-2'-tosylsangivamycin (**4b**, 6.93 g, 13.1 mmol) was dissolved in 1-propanol (400 mL) and then saturated with argon gas. Thiourea (2.55 g, 3 equiv) was added to the solution, and the solution was heated at reflux for 3 h. More thiourea (2.55 g) was then added, and heating at reflux was continued for an additional 3.5 h. The reaction mixture was allowed to stand for 19 h at room temperature. The solid was removed by filtration and then recrystallized from an ethanol–water mixture to give **5** (2.96 g, 56%): mp 267–269 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 9.24 and 7.44 (2 br s, 4 H, 4-NH_2, 5-$CONH_2$), 8.47 (s, 1 H, H-2), 6.52 (d, 1 H, H-1', $J_{1',2'}$ = 6.5 Hz), 6.0 (br, OH), 4.99 (d, 1 H, H-2'), 4.46 (m, 1 H, H-3'), 4.08 (q, 1 H, H-4'), 3.45 (m, 2 H, H-5'). Anal. ($C_{12}H_{13}N_5O_4S$·HBr) C, H, N.

6,2'-Cyclo-4-amino-6-mercapto-7-β-D-arabinofuranosylpyrrolo[2,3-*d*]pyrimidine-5-carboxamide (6). 6,2'-*S*-Cyclosangivamycin hydrobromide (**5**; 20 mg, 0.05 mmol) was dissolved with heating in 10 mL of water. The solution was cooled to room temperature, 0.5 mL of triethylamine was added, and the solution was then concentrated to ≃5 mL to yield **6** (0.33 mmol, 66%). The product was recrystallized from an ethanol–water mixture to afford an analytical sample, mp 290 °C. Anal. ($C_{12}H_{13}N_5O_4S$) C, H, N.

4-Amino-7-(2-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (2'-Deoxysangivamycin, 7). 6,2'-*S*-Cyclosangivamycin hydrobromide (**5**; 1.0 g) was dissolved in a mixture of water (70 mL) and dioxane (10 mL) with heating and then neutralized with concentrated ammonia solution. A solid began to separate but was redissolved by the addition of dioxane. The mixture was then heated at reflux with the addition of Raney nickel (4 mL) for 45 min. An addditional 3.3 mL of Raney nickel was then added, and the mixture was heated at reflux for another 1.5 h. The Raney nickel was removed by filtration, and the solvent was evaporated to dryness in vacuo. The resulting solid was crystallized from methanol to yield 340 mg (1.16 mmol, 47%) of **7**: mp 258 °C; mass spectrum, *m/z* 581 (M + 4-Me_3Si); ^{1}H NMR (Me_2SO-d_6) δ 8.12 and 8.15 (2 s, 2 H, H-2 and H-6), 7.8 and 7.4 (2 br s, 4 H, 4-NH_2 and 5-$CONH_2$), 6.52 (t, 1 H, H-1', $J_{1',2'}$ = 7 Hz), 5.30 (d, 1 H, H-3 OH), 4.96 (t, 1 H, H-5' OH), 4.39 (m, 1 H, H-3'), 3.86 (m, 1 H, H-4'), 3.58 (m, 2 H, H-5'), 2.3 (m, 2 H, H-2'). Anal. ($C_{12}H_{15}N_5O_4$·0.5H_2O) C, H, N.

4-Amino-7-[2-*O*-acetyl-3-deoxy-3-iodo-5-*O*-(2,5,5-trimethyl-4-oxo-1,3-dioxolan-2-yl)-β-D-xylofuranosyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8a) and 4-Amino-7-(2,5-di-*O*-acetyl-3-deoxy-3-iodo-β-D-xylofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (8b). To a vigorously stirred solution of dried sodium iodide (12.1 g) in 80 mL of anhydrous acetonitrile was added 4.64 of α-acetoxyisobutyryl chloride, followed immediately by 2.45 g of previously dried sangivamycin (**1c**). The brown reaction mixture was stirred for 1 h at room temperature while being protected from moisture. The mixture was then poured into a stirred solution of 192 mL of saturated aqueous $NaHCO_3$ and 125 mL of saturated $Na_2S_2O_3$ solution. A 100-mL portion of $CHCl_3$ was added, and the layers were separated. The aqueous layer was extracted with $CHCl_3$ (2 × 30 mL), and the combined organic extractions were washed with water and dried over Mg_2SO_4, the Mg_2SO_4 was collected by filtration, and the filtrate was then concentrated to 20 mL. The solution was applied to and isolated from a column of silica gel using $CHCl_3$–EtOH. Compounds **8a** and **8b** were obtained in 56 and 20% yields, respectively, from the evaporation of the appropriate middle fraction (s).

4-Amino-7-(3-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (3'-Deoxysangivamycin, 9). Compound **8a** (2.6 g) and 280 mg of sodium acetate were dissolved in a mixture of 50 mL of EtOH and 8 mL of water. This solution was hydrogenated for 18 h at 40 psi of hydrogen in the presence of 0.4 g of 10% Pd/C catalyst. The mixture was partitioned between $CHCl_3$ and water; the organic phase was separated and then washed again with water, dried over Mg_2SO_4, and filtered, and the filtrate was evaporated to dryness in vacuo. The resulting solid was dissolved in 15 mL of methanol, and this solution was then saturated with NH_3 at 0 °C and allowed to stand at room temperature for 20 h. The solution was evaporated to dryness in vacuo, and the resulting solid was crystallized from an EtOH–H_2O mixture to yield 196 mg of compound **9**.

The water layer was treated with 10 mL of 60% ammonium hydroxide at 4 °C for 16 h to yield an additional 430 mg of **9**: total yield 626 mg (2.17 mmol, 49%); mp 280–281 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.19 and 8.13 (2 s, 2 H and H_6), 8.00 and 7.43 (2 br s, 4 H, 5-$CONH_2$ and 4-NH_2), 6.13 (d, 1 H, $J_{1',2'}$ = 1 Hz), 5.69 (d, 1 H, H-2' OH), 5.00 (br, 1 H, H-5' OH), 4.46 (m, 2 H, H-2', H-4'), 3.67 (m, 2 H, H-5'), 2.10 (m 2 H, H-3'). Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

4-Amino-7-[5-(phenylthio)-5-deoxy-β-D-ribofuranosyl]pyrrolo[2,3-*d*]pyrimidine-5-carboxamide [5'-Deoxy-5'-(phenylthio)sangivamycin, 10]. Sangivamycin (1c; 0.87, g, 2.82 mmol) and diphenyl disulfide (1.41 g, 6.27 mmol) were dissolved in 2.8 mL of pyridine, followed by the addition of tributylphosphine (2.25 mL, 9.02 mmol). The mixture was stirred at room temperature for 20 h and then poured into a mixture of water (50 mL) and ether (50 mL). The resulting solid was dissolved in a small amount of ethanol and allowed to stand at 5 °C for 24 h. The solid was recrystallized from a water-ether mixture to give a white solid (770 mg, 68%): mp 106–109 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.02 and 7.99 (2 s, 2 H, H-2, H-6), 7.4–8.3 (br, 4 H, 4-NH_2 and 5-$CONH_2$), 7.1–7.4 (m, 5 H, phenyl), 6.01 (d, 1 H, H-1', $J_{1',2'}$ = 5 Hz), 5.41 and 5.24 (2 d, 2 H, H-2' OH and H-3' OH), 4.31 (q, 1 H, H-2'), 3.8–4.2 (m, 2 H, H-3' and H-4'), 3.30 (m, 2 H, H-5'). Anal. ($C_{18}H_{20}N_5O_4S$) C, H, N.

4-Amino-7-(5-deoxy-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (5'-Deoxysangivamycin, 11). 5'-Deoxy-5'-(phenylthio)sangivamycin (**10**; 0.4 g, 1 mmol) was added to a mixture of 30 mL of dioxane and 20 mL of water. The mixture was heated at reflux with Raney nickel (3 mL) for 45 min. An additional 3 mL of Raney nickel was added, and heating at reflux was continued for 45 min. Raney nickel was removed by filtration, the filtrate was evaporated, and the resulting solid was crystallized from methanol to yield 118 mg (0.40 mmol, 41%) of **11**: mp 262–264 °C; ^{1}H NMR (Me_2SO-d_6) δ 8.12 (s, 2 H, H-2 and H-6), 7.9 and 7.3 (2 br s, 4 H, 5-$CONH_2$ and 4-NH_2), 6.08 (d, 1 H, H-1', $J_{1',2'}$ = 4 Hz), 5.43 and 5.13 (2 d, 2 H, H-2' OH and H-3' OH), 4.24 (q, 1 H, H-2'), 4.1–3.7 (m, 2 H, H-3' and H-4'), 1.35 (d, 3 H, H-5', $J_{4',5'}$ = 6 Hz). Anal. ($C_{12}H_{15}N_5O_4$) C, H, N.

Antitumor Studies. The in vitro cytotoxicity against L1210 was evaluated as described previously.[25] L1210 cells were grown in static suspension culture using Fischer's medium for leukemic cells of mice, and the growth rate over a 3-day period was determined in the presence of various concentrations of the test compound. The ID_{50} was defined as the concentration required to reduce the growth rate to 50% of the control.

The in vivo antitumor data were furnished by the Division of Cancer Treatment using standard National Cancer Institute protocols for evaluation of compounds against the murine leukemia L1210.[26]

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