Pyrrolopyrimidine Nucleosides. III. The Total Synthesis of Toyocamycin, Sangivamycin, Tubercidin, and Related Derivatives[1]

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Abstract: The structures of toyocamycin and sangivamycin have been unequivocally established as 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1**) and 4-amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2**), respectively. The total synthesis of all the presently known pyrrolo[2,3-*d*]pyrimidine nucleoside antibiotics (toyocamycin, sangivamycin, and tubercidin) has now been accomplished *via* the acid-catalyzed fusion procedure. The synthesis of several new pyrrolo[2,3-*d*]pyrimidine derivatives required for utilization in the fusion procedure is reported. Factors which are involved in a successful fusion reaction, *e.g.*, the number and magnitude of electron-withdrawing substituents and the juxtaposition of these substituents in relation to the site of glycosidation, are discussed.

The antibiotic toyocamycin was first isolated[3] from *Streptomyces toyocaensis* in 1956 and subsequently[4] from a *Streptomyces* strain No. 1922. The unusual biological properties[3,4] and antitumor activity[5] of this nucleoside antibiotic has stimulated considerable interest in the total synthesis of toyocamycin and related derivatives.[6,7]

Recently, antibiotic 1037[8] has been shown to be identical[9] with toyocamycin. Unamycin B, isolated from a culture of *Streptomyces fungicidicus*,[10] and antibiotic E-212, isolated[11] from *Streptomyces sp.* E-212, have been described as related to toyocamycin. In a previous communication[12] from our laboratory it was unequivocally established that these antibiotics were also identical with toyocamycin, inasmuch as their chromatographic mobilities in four solvent systems and uv and ir spectra were identical with those observed for an authentic sample of toyocamycin. Vengicide was isolated from *Streptomyces vendargensis*[13] and assigned the empirical formulas $C_{24}H_{29}O_9N_{10}$ and $C_{24}H_{30}O_{10}N_{10}$. Since a number of physical properties of this antibiotic were similar to those of toyocamycin, a sample was obtained for structural investigation. Rigorous comparison of physical data with toyocamycin showed conclusively that the two antibiotics were identical. Preliminary degradation studies[4,14] have resulted in a tentative structure assignment for toyocamycin as 4-amino-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine, although it has been recently pointed out[15] that neither the position of glycosidation, the anomeric configuration, nor the sugar moiety have been definitely established. Sangivamycin has been isolated from an unidentified species of *Streptomyces*[16] and was referred to as BA-90912. It was assigned an empirical formula of $C_{12}H_{17}O_6N_5$ and was reported to possess cytotoxicity against HeLa cells grown in cell cultures and to exhibit significant activity against leukemia L1210 in mice. Sangivamycin has produced no evidence of toxicity in humans at significant doses[17] and is presently undergoing human clinical trial against leukemia.[18] A number of biochemical investigations have been accomplished using tubercidin, toyocamycin, and sangivamycin as substitute analogs for adenosine and adenosine monophosphates. It has been reported[19] recently that all three of the above pyrrolopyrimidine nucleoside antibiotics are incorporated at the 3′ terminals of tRNA. The tRNA with tubercidin at the terminal end functioned satisfactorily in the incorporation of amino acids into polypeptides and the coding properties of polymers containing tubercidin were found to be indistinguishable from the same polymers containing adenosine. However, the acceptor ability of tRNA with toyocamycin and sangivamycin was much less than that exhibited by tubercidin. The 5′-diphosphate derivatives of tubercidin and toyocamycin were enzymatically reduced to the 2′-deoxyribonucleotide derivatives with a ribonucleoside reductase from *Escherichia coli*.[20] However, the 5′-diphosphate derivative of sangivamycin was not reduced to the 2′-deoxyribonucleotide and this must

(1) This work supported in part by Research Contract No. PH 43-65-1041, Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service.

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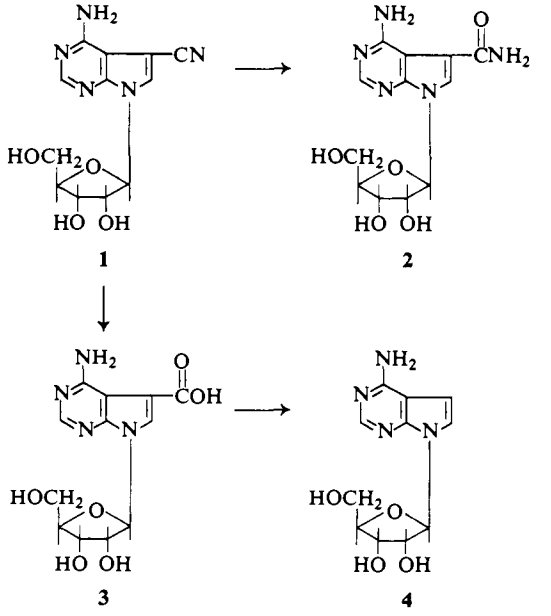
be attributed to the replacement of the cyano group at position 5 by a carboxamido group. This would suggest that the type or size of group at position 5 may be a very critical factor in the ability of a pyrrolopyrimidine nucleoside to function as substrate for certain enzymatic reactions. It has also been reported[21] that toyocamycin is incorporated into RNA and DNA and that toyocamycin was found at internal positions of RNA which suggests that RNA synthesis continued after the incorporation of toyocamycin. The above findings are of considerable interest since these investigations were completed prior to the final structure determination of sangivamycin and toyocamycin.

A partial structure elucidation of sangivamycin[22a] revealed that the empirical formula previously reported[16] included 1 mol of water and the correct empirical formula was actually $C_{12}H_{15}O_5N_5$. This investigation[22] also established the presence of a carboxamido, an aromatic amino group, and a basic pyrrolo[2,3-*d*]pyrimidine structure. It has been reported,[15] without experimental detail, that sangivamycin is structurally similar to toyocamycin and, in fact, that sangivamycin may be obtained by controlled acid hydrolysis of toyocamycin. Although these experimental procedures have recently been published,[22b] the structural assignment of sangivamycin was still based solely on its structural relationship to toyocamycin.

Treatment of toyocamycin (**1**) in our laboratory with 30% hydrogen peroxide in concentrated ammonium hydroxide solution gave a 95% yield of colorless crystals (**2**) whose ir spectrum showed no nitrile absorption in the 2200-cm^{-1} region. A comparison of the ultraviolet absorption and infrared spectra of **2** with the corresponding spectra of an authentic sample of sangivamycin established that the samples were identical. Paper chromatographic mobilities of **2** and sangivamycin in four solvent systems were identical. We wish to report the complete structural assignment of toyocamycin and sangivamycin on the basis of the following evidence.

The 2′,3′-O-isopropylidene derivative of toyocamycin (**1a**) was prepared in 85% yield by the modification of a reported procedure[23] utilizing 2,2-dimethoxypropane and perchloric acid in acetone. Treatment of **1a** with *p*-toluenesulfonyl chloride in pyridine[24] furnished 2′,3′-O-isopropylidene-5′-O-*p*-toluenesulfonyltoyocamycin (**1b**). A pmr spectrum of the 5′-O-tosyl derivative in chloroform-*d* exhibited a doublet at δ 6.05 ($J_{1,2}$ = 2 cps) which was attributed to the anomeric proton. However, a pmr spectrum of the above tosyl derivative in dimethyl-d_6 sulfoxide revealed that intramolecular quaternization to form the N_1-5′ cyclonucleoside occurs very readily in a solvent which is able to promote the nucleophilicity of the electron pair on N-1. This conclusion was supported by the appearance in the pmr spectrum of a sharp singlet at δ 6.65 (1 proton) assigned to the anomeric proton. An ultraviolet absorption spectrum (Table I) of 2′,3′-O-isopropylidene-N_1,5′-toyocamycin cyclonucleoside tosylate showed an absorption maximum at 284 nm. This bathochromic shift of 11 nm in the ultraviolet absorption maxima is characteristic of intramolecular quaternization and confirms cyclonucleoside formation. The steric requirements of a pyrrolo[2,3-*d*]pyrimidine N_1,5′-cyclonucleoside are such that only a β-D-nucleoside has the 5′-methylene in a position which is favorable for bonding with N-1.[24,25] Thus, the anomeric configuration of sangivamycin and toyocamycin must be β.[26] The site of ribosidation and the nature of the sugar moiety remained to be resolved before the characterization of toyocamycin and sangivamycin could be considered complete.

Sangivamycic acid (**3**), the carboxylic acid derivative of sangivamycin has been previously prepared[4] as an intermediate but was never characterized. The acid **3** in our laboratory was an intermediate isolated in low yield from the treatment of toyocamycin with 3 *N* hydrochloric acid at reflux temperature under a nitrogen atmosphere for 12 hr. Decarboxylation of sangivamycic acid was accomplished by immersion of **3** in an oil bath preheated to 238° for approximately 10 sec which gave a dark amber melt. An aqueous ethanol extract of this melt furnished tubercidin [**4**, 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine] in 13% yield. The identity of **4** was confirmed by a mixture melting point which showed no depression, rigorous spectral comparison, and identical chromatographic mobilities in four solvent systems with authentic tubercidin. The structure of tubercidin has been previously established[27] as 4-amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. Thus, this conversion of toyocamycin to tubercidin (**4**) has firmly established the actual site of ribosidation as N-7 for toyocamycin and the structure of the carbohydrate moiety as D-ribofuranosyl. On the basis of the above data, the complete structures for toyocamycin and sangivamycin are 4-amino-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine and 4-amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine, respectively.



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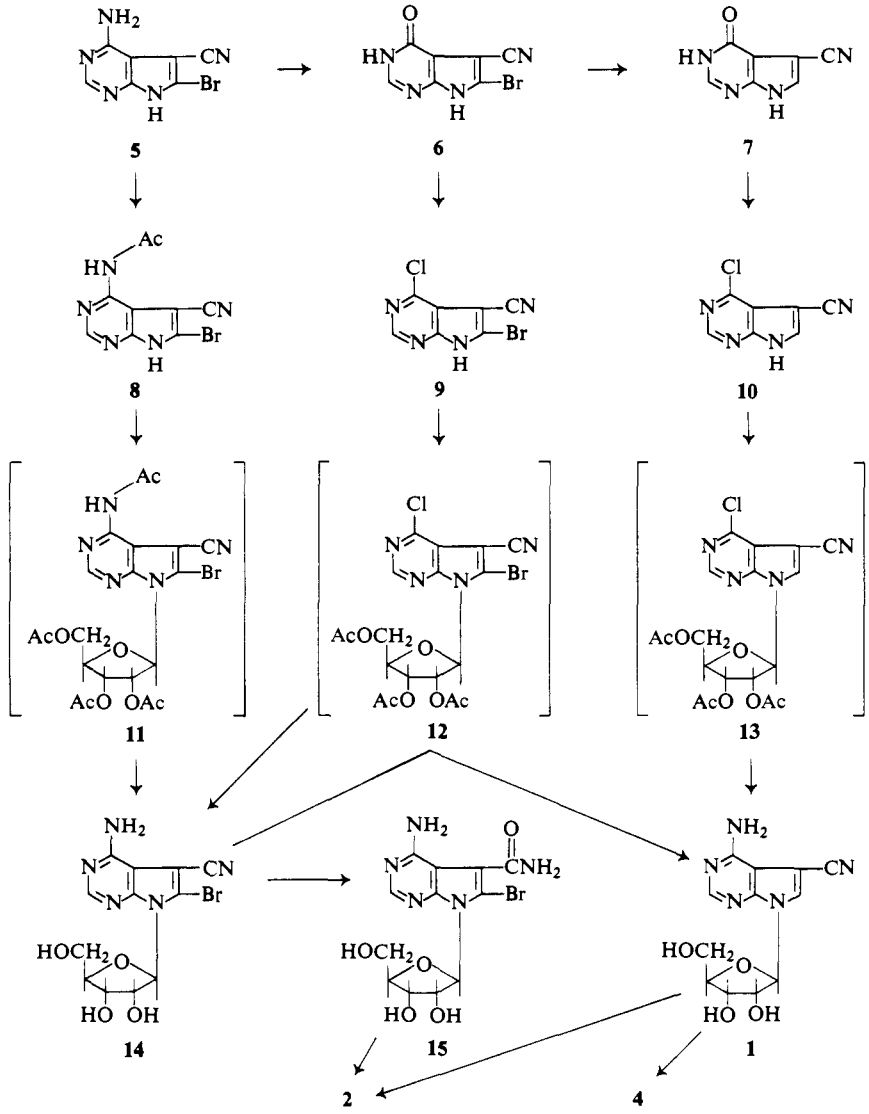

The structural assignments for toyocamycin, sangivamycin, and tubercidin have been further verified by total synthesis. 4-Amino-5-cyano-6-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine[28] has been prepared, but attempts to prepare toyocamycin by desulfurization were unsuccessful. In an effort to circumvent these difficulties, 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) was prepared by ring closure of 2-amino-5-bromo-3,4-dicyanopyrrole[29] with formamidine acetate in 2-ethoxyethanol at reflux temperature. Since electron-withdrawing substituents on a purine moiety have been shown to facilitate the fusion condensation[30] with an acetylated sugar, a route to the synthesis of a 4-acetaminopyrrolo[2,3-*d*]pyrimidine derivative was investigated. A mixture of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**), acetic anhydride, and xylene was heated at reflux temperature for 18 hr to afford a monoacetylated product (**8**), as determined by pmr spectra and elemental analysis, in excellent yield (94%).

The single acetyl group was shown to reside on the 4-amino group by pmr spectra which showed a singlet at δ 2.2 (3 protons) and two distinctly different absorption bands at δ 10.8 (1 proton) and 12.0 (1 proton) attributable to NH protons. The monoacetylated product was thus assigned the structure 4-acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**8**). This is of interest since previous attempts[31] to prepare a monoacetylated pyrrolo[2,3-*d*]pyrimidine with an exocyclic amino group have resulted in the formation of a diacetylated derivative or an intractable mixture.

A mixture of 4-acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**8**) and 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose was heated at 175° in the presence of a catalytic amount of bis(*p*-nitrophenyl) phosphate[32] for 25 min. This reaction mixture was then applied to a column of Woelm neutral alumina and eluted with mixtures of petroleum ether (bp 30–60°)–chloroform. Preparative layer chromatography was then utilized to furnish the tetraacetylated nucleoside **11** as a pale yellow syrup in 9%

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Table I. Ultraviolet Absorption Data of Certain Pyrrolo[2,3-*d*]pyrimidines[a]

Name	pH 1 λ_{max}, nm	pH 1 $\varepsilon_{max} \times 10^{-3}$	EtOH λ_{max}, nm	EtOH $\varepsilon_{max} \times 10^{-3}$	pH 11 λ_{max}, nm	pH 11 $\varepsilon_{max} \times 10^{-3}$
4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (**2**, sangivamycin)	273 228	12.8 9.5	278 229	15.1 8.2	277 227	14.4 14.1
4-Amino-5-cyano-7-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1a**)	271 233	15.9 17.9	288[b] 278 230	10.9 16.6 10.3	286[b] 276 227	10.5 15.9 11.3
4-Amino-5-cyano-7-(2′,3′-O-isopropylidene-5′-O-toluenesulfonyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**1b**)	273 213	10.7 23.8	273 221	11.2 24.8	272 210	8.9 24.8
4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (**3**)	274 227	11.8 13.2	279 231	13.5 7.6	277	13.9
4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**)	280 230	15.2 14.5	284 250	13.8 7.8	293 245	11.7 16.4
4-Acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**8**)	297	9.0	312 282 250	4.5 7.0 12.9	315 282 248	5.3 7.0 14.0
4-Amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**14**)	281 231	17.4 16.3	284	18.3	283	17.8
4-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**1**, toyocamycin)	272 232	12.2 16.0	288[b] 278 231	9.8 15.1 9.3	287[b] 277 227	9.3 14.3 10.2
4-Amino-6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-5-carboxamide (**15**)	280	16.0	285	15.5	283	15.2
6-Bromo-5-cyanopyrrolo[2,3-*d*]-4-pyrimidone (**6**)	272	16.1	277 245	12.9 12.9	287 241	13.7 22.1
5-Cyanopyrrolo[2,3-*d*]-4-pyrimidone (**7**)	263 221	12.2 14.9	263	10.7	273 242	12.0 13.3
6-Bromo-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (**9**)	285 225	9.8 44.0	312 273 245	4.1 8.2 25.4	307 272 243	5.1 16.5 21.4
4-Chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (**10**)	272 220	5.9 25.9	273 246 220	6.3 7.3 25.0	315 272 243	3.2 7.2 23.6

[a] Spectra were obtained with a Beckman DK-2 ultraviolet spectrophotometer. [b] Shoulder.

yield (calculated on the basis of unrecovered starting material). Complete deacetylation of **11** was accomplished with methanolic ammonia (previously saturated at 0°) at room temperature to furnish a 92% yield of 4-amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**14**). The position of ribosidation was assigned at N-7 on the basis of the ultraviolet absorption. Recent studies in this laboratory have shown that ribosidation or alkylation[28] of 4-amino-5-cyano-6-substituted pyrrolo[2,3-*d*]pyrimidines in the pyrimidine ring produces a 15–30-nm bathochromic shift while alkylation in the pyrrole ring produces only a small hypsochromic shift if any shift is observed at all. The ultraviolet absorption maxima recorded in ethanol for 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) and the nucleoside derivative (uv, maximum at 284 nm in EtOH) are identical (Table I).

Removal of the 6-bromo group of **14** with 5% palladium on powdered charcoal in a hydrogen atmosphere afforded an 88% yield of 4-amino-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**1**, toyocamycin), mp 243°, $[\alpha]^{27}D$ $-55.6 \pm 1.3°$ (*c* 1.0, 0.1 *N* HCl).[33] A mixture melting point of the above product and toyocamycin showed no depression. The ultraviolet absorption, infrared, and pmr spectra of **1** and toyocamycin were superimposable. The chromatographic mobilities of **1** in four solvent systems were identical with those observed for authentic toyocamycin. This has provided the first total synthesis of toyocamycin. Therefore, the conversion of toyocamycin to tubercidin (*vide supra*) also constitutes the first total synthesis of the pyrrolopyrimidine nucleoside antibiotic tubercidin.

6-Bromosangivamycin was prepared in 65% yield by treatment of 4-amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**14**) with 30% hydrogen peroxide in concentrated ammonium hydroxide solution at room temperature. The structure of this nucleoside was established as 4-amino-6-bromo-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**15**) on the basis of elemental analysis and pmr spectra. Absorption peaks

(33) Optical rotation of an authentic sample of toyocamycin under similar conditions was $[\alpha]^{27}D$ $-55.7 \pm 0.9°$.

at δ 7.7 (2 protons) and 7.9 (2 protons) were assigned to the 4-amino and 5-carboxamido groups, respectively. Removal of the 6-bromo group from 4-amino-6-bromo-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**15**) with 5% palladium on powdered charcoal in a hydrogen atmosphere proceeded smoothly to afford 4-amino-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2**, sangivamycin). Comparison of this product with natural sangivamycin and with **2** prepared from toyocamycin showed them to be identical.

Since the fusion reaction of 4-acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine proceeded in a low yield, efforts were directed toward the preparation of other pyrrolopyrimidine derivatives which might furnish a nucleoside product *via* the fusion reaction in higher yield. Two of the factors which determine the facility of a particular heterocyclic derivative in the fusion[28,34] reaction are the number and magnitude of electron-withdrawing substituents and the steric environment of the nitrogen atom involved in the site of glycosidation. Halogen-substituted purines and pyrrolo[2,3-*d*]pyrimidines have been utilized successfully[28,30,34] in fusion reactions and the resulting acylated halogenonucleosides isolated and characterized. Therefore, the synthesis of certain 4-chloropyrrolo[2,3-*d*]pyrimidine derivatives was investigated. Deamination of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) by the addition of a sodium nitrite solution to a fine suspension of the heterocycle in 50% aqueous acetic acid furnished a good yield of 6-bromo-5-cyanopyrrolo[2,3-*d*]-4-pyrimidone (**6**). It was found that the deamination proceeded faster and with less decomposition if **5** was reprecipitated *in situ* before the deamination procedure was initiated. Treatment of **6** with phosphorus oxychloride at reflux temperature followed by hydrolysis furnished 6-bromo-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (**9**) in 84% yield. To investigate the possibility that the steric requirement of the 6-bromo group might hinder the ribosidation at the adjacent position (N-7), a route for the preparation of 4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine was investigated. Dehalogenation of **6** in 50% ethanolic ammonium hydroxide with 5% palladium on carbon in a hydrogen atmosphere afforded 5-cyanopyrrolo[2,3-*d*]-4-pyrimidone (**7**) in excellent yield. Chlorination of **7** in phosphorus oxychloride at reflux temperature furnished 4-chloro-5-cyanopyrrolo-[2,3-*d*]pyrimidine (**10**) in 60% yield.

Fusion of the 6-bromo derivative (**9**) with 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose at 135° in the presence of a catalytic amount of dichloroacetic acid under a water aspirator vacuum for 25 min furnished a 31% yield (calculated on the basis of unrecovered starting material) of acetylated nucleoside which was isolated as a pale yellow syrup by dry column chromatography.

Fusion of the debrominated derivative (**10**) under identical conditions furnished a 28% yield of acetylated nucleoside which was purified by dry column chromatography.[35] Variation of the acidic fusion catalyst produced little or no variation in yield. Fusion without an acidic catalyst proceeded in nearly the same yield, presumably due to the formation of acetic acid as a by-product of the sugar decomposition at high temperatures. The success of the fusion reaction is also greatly influenced by the solubility of the heterocyclic base in the hot melt. The more soluble the base in the reaction melt, the greater the ease with which the reaction proceeds. The fusion mixtures prepared from analytically pure samples of the heterocyclic bases **8**, **9**, or **10** were found to undergo the fusion reaction very poorly, while the impure or unrecrystallized heterocyclics melted at a lower temperature and were found to be much more soluble in the hot melt.

Since the 6-bromo derivative (**9**) and the derivative with no substituent at the 6 position (**10**) seem to afford nearly identical yields of acetylated nucleoside, it would seem that the addition of another electron-withdrawing substituent (6-bromo) is nearly compensated for by the removal of possible steric hindrance at position 6.

Removal of the blocking groups with concomitant amination of the acetylated chloro nucleosides (**12** and **13**) proceeded smoothly. Treatment of 6-bromo-4-chloro-5-cyano-7-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**12**) with methanolic ammonia at 110° in a sealed tube furnished 4-amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**14**) in good yield. Debromination followed by a comparison of the product to authentic toyocamycin showed them to be identical in every respect.

Amination of **13** with anhydrous methanolic ammonia in a sealed tube at 110° afforded 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1**, toyocamycin) in 78% yield. Rigorous comparison showed the product to be identical with toyocamycin, which confirms the β configuration and site of ribosidation of the fusion product.

Experimental Section[36]

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (2, Sangivamycin). Method 1. To 1.5 g of 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1**) in 50 ml of concentrated ammonium hydroxide was added 5 ml of 30% H_2O_2 and the mixture stirred for 2 hr at room temperature. After 10 min the starting material had gone into solution and a heavy white solid slowly separated from solution. The mixture was allowed to stand at 5° for 18 hr and the solid was collected by filtration and washed thoroughly with 50% aqueous ethanol. The product (1.52 g, 95%) was recrystallized from water to yield an analytical sample, mp 260°.

Anal. Calcd for $C_{12}H_{15}N_5O_5 \cdot H_2O$: C, 44.04; H, 5.24; N, 21.40. Found: C, 43.92; H, 5.42; N, 21.48.

Method 2. To 4-Amino-6-bromo-5-carboxamido-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**15**, 250 mg) dissolved in 200 ml of hot 50% aqueous ethanol was added 250 mg of 5% palladium on charcoal. The mixture was shaken under 40 psi of hydrogen for 5 hr. The charcoal was removed by filtration and then washed with hot aqueous ethanol (75 ml). The filtrate was concentrated *in vacuo* and then allowed to stand at 5° for 12 hr. The white crystals which separated from solution were collected by filtration to furnish an 83% yield of product (155 mg). Chromatographic mobilities, ultraviolet, infrared, and pmr spectra were identical with those of the product prepared by method 1.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxylic Acid (3). One gram of 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**1**) was dissolved in 3 *N* hydrochloric acid (40 ml) and the solution heated at reflux temperature

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(36) Pmr spectra were obtained on a Varian A-60 instrument using tetramethylsilane as an internal standard. Optical rotations were obtained with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Melting points were observed on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed by Heterocyclic Chemical Corp., Harrisonville, Mo., and M-H-W Laboratories, Garden City, Mich.

for 12 hr under a nitrogen atmosphere. After cooling at 5° for 18 hr, the long colorless needles which had separated from solution were collected by filtration. This solid was absorbed on a preparative tlc plate (20 × 40 cm) of SilicAR 7GF and developed with a 5% aqueous NH_4HCO_3 solution. The ultraviolet absorbing band with the greatest mobility was removed and extracted with hot water to yield after evaporation *in vacuo*, 72 mg of a white solid which contained some inorganic salts. Recrystallization from water gave an analytical sample (35 mg) which melted at 238° with decomposition.

Anal. Calcd for $C_{12}H_{14}N_4O_6$: C, 46.40; H, 4.55; N, 18.06. Found: C, 46.20; H, 5.01; N, 18.05.

4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (4, Tubercidin). 4-Amino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxylic acid (**3**, 200 mg) was placed in several capillary tubes and inserted individually in a Thomas–Hoover melting point apparatus which had been preheated to 238°. The tube was withdrawn from the apparatus when the melt had become dark amber (~10 sec) and then pulverized in a mortar. The residue was extracted with aqueous ethanol (50%) and the solvent then was removed *in vacuo*. The residue was absorbed on a preparative tlc plate (20 × 40 cm) SilicAR 7GF and developed with a 5% aqueous NH_4HCO_3 solution to furnish a 13% yield of nucleoside material which showed no depression in a mixture melting point with tubercidin. Chromatographic mobilities, ultraviolet, infrared, and pmr spectra also showed the product to be identical with an authentic sample of tubercidin.

4-Amino-5-cyano-7-(2′,3′-O-isopropylidene-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (1a). To 450 ml of acetone was added 1.60 ml of 2,2-dimethoxypropane and 2.0 ml of 70% perchloric acid. The mixture was protected from moisture and stirred at room temperature for 5 min then 1.32 g of toyocamycin was added in one portion. Pyridine (1.60 ml) was added after the mixture had been stirred for 45 min. The volume was reduced to *ca.* 50 ml *in vacuo* and then 23 ml of 10% sodium carbonate was added before the remaining acetone was removed. Cold water (100 ml) was added to the aqueous solution and the solution then allowed to stand at 5° for 12 hr. The white solid which had separated from solution was collected by filtration and washed with cold water. Recrystallization from ethanol–water afforded 1.28 g (85%) of white solid, mp 136–137°.

Anal. Calcd for $C_{15}H_{17}N_5O_4$: C, 54.33; H, 5.17; N, 21.13. Found: C, 54.38; H, 5.15; N, 21.07.

4-Amino-5-cyano-7-(2′,3′-O-isopropylidene-5′-O-*p*-toluenesulfonyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (1b). 2′,3′-O-Isopropylidenetoyocamycin (750 mg) was dissolved in pyridine (6 ml) and 480 mg of *p*-toluenesulfonyl chloride was added to this solution. The solution was stored in the dark at 5° for 36 hr and then poured into ice water (250 ml). The aqueous mixture was extracted with chloroform (two 50-ml portions), and the organic layer was then washed with 1 *M* H_2SO_4 (two 50-ml portions) and with water until the aqueous layer was neutral. The chloroform solution was dried over sodium sulfate and the volume then reduced *in vacuo* to 10 ml. Methanol (20 ml) was added and the remaining chloroform then removed *in vacuo*. The solution was allowed to stand at −10° and the white crystals which had separated from solution were collected by filtration to yield 820 mg (75%) of product.

Anal. Calcd for $C_{22}H_{23}N_5O_6S$: C, 54.43; H, 4.77; N, 14.42. Found: C, 54.07; H, 4.79; N, 14.27.

4-Amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (5). Formamidine acetate (15 g) and 2-amino-5-bromo-3,4-dicyanopyrrole[29] (15 g) were dissolved in 275 ml of 2-ethoxyethanol. The mixture was heated at reflux temperature for 36 hr, the dark solution treated with charcoal, and the charcoal removed from the hot solution by filtration. The solvent was removed *in vacuo*, and the resultant brown solid was triturated with water and collected by filtration. Recrystallization of the crude product and treatment with charcoal from a DMF–methanol mixture afforded 8.7 g of pale yellow product (51%). A small sample was recrystallized from methanol–water, mp > 300°.

Anal. Calcd for $C_7H_4BrN_5 \cdot 0.5H_2O$: C, 34.03; H, 2.04; N, 28.35. Found: C, 33.75; H, 1.98; N, 28.13.

4-Acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (8). One gram of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) was suspended in a mixture of acetic anhydride (5 ml) and xylene (30 ml) and the mixture stirred at reflux temperature for 18 hr. The solvent was removed *in vacuo* to yield a cream-colored solid which was then triturated with water. The solid was collected by filtration and recrystallized from a mixture of DMF–MeOH. The pale yellow crystals (94%) melted with decomposition at 265°.

Anal. Calcd for $C_9H_6BrN_5O$: C, 38.59; H, 2.16; N, 25.00. Found: C, 38.47; H, 2.23; N, 24.97.

4-Amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (14). Method 1. 4-Acetamino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (0.75 g) and 1.25 g of 1,2,3,5-tetra-O-acetyl-β-D-ribofuranose which had been mixed in a mortar was fused in an oil bath at 175°. Bis(*p*-nitrophenyl)phosphate (10 mg) was added and a water aspirator vacuum was then applied for 25 min. The hot melt was extracted with ethyl acetate (40 ml), and the unreacted aglycon (610 mg) was removed by filtration. The organic extract was extracted at 0° with three 100-ml portions of 5% aqueous sodium hydroxide and then with water until the aqueous wash was neutral. The organic phase was dried over sodium sulfate and the solvent then removed *in vacuo*. The resultant amber syrup was absorbed on a column of Woelm neutral alumina (2.5 × 30 cm) and eluted with petroleum ether–chloroform mixtures (300-ml portions of 4:1, 3:2, 2:3, 1:4; v/v). The ultraviolet absorbing fractions were collected, the solvent removed, and the syrup separated into four ultraviolet-absorbing bands on preparative tlc plates [SilicAR 7GF; acetone–chloroform–isopropyl ether (1:2:4, v/v, 20 × 40 cm)]. The major band was removed and extracted with chloroform and evaporated to dryness *in vacuo*. The resultant pale yellow syrup (55 mg) was identified as the desired product by pmr spectral analysis (9% yield calculated from unrecovered starting material).

4-Acetamino-6-bromo-5-cyano-7-(2′,3′,5′-tri-O-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**11**, 200 mg) was covered with 100 ml of methanolic ammonia (saturated at 0°) in a pressure bottle at room temperature. After 24 hr the solvent was removed *in vacuo*. Methanol (75 ml) was added to the residue and the solvent then removed *in vacuo*. This evaporation procedure was repeated two more times to furnish an amber syrup. The amber syrup was dissolved in 20 ml of methanol and 30 ml of water then added. After 48 hr at 5°, tan crystals had separated and were collected by filtration. Recrystallization from water gave an analytical sample (92% yield): mp 245–250° dec; ir 2225 cm^{-1} (C≡N).

Anal. Calcd for $C_{12}H_{12}BrN_5O_4$: C, 38.94; H, 3.27; N, 18.91; Br, 21.58. Found: C, 39.10; H, 3.43; N, 19.18; Br, 21.24.

Method 2. 1,2,3,5-Tetra-O-acetyl-β-D-ribofuranose (750 mg) and 6-bromo-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (9, 257 mg) were thoroughly mixed in a mortar and then placed in an oil bath at 135°. One drop of dichloroacetic acid was added as soon as the initial melt was achieved and water aspirator vacuum was then applied for the duration of the fusion reaction (50 min). The hot melt was triturated with ethyl acetate (25 ml) and the unreacted aglycon (120 mg) removed by filtration. Subsequent extraction of the reaction mixture with 5% aqueous sodium hydroxide at 0° (three 50-ml portions) and neutralization of the combined basic extracts afforded another 25 mg of the heterocyclic aglycon. The organic phase was washed with water until neutral and then dried over sodium sulfate. Dry column chromatography (2.5 × 40 cm column) utilizing SilicAR 7GF and chloroform–acetone (8:2, v/v) as the developing solvent resolved the nucleoside, carbohydrate, and decomposition products. The nucleoside band was removed and extracted with chloroform to afford 68 mg of the nucleoside as a straw-colored syrup (32%, calculated on the basis of unrecovered starting material). The syrup (200 mg) was covered with 100 ml of methanolic ammonia (saturated at 0°) and the solution heated in a sealed tube for 8 hr at 110°. The cooled reaction mixture was evaporated to dryness *in vacuo* and methanol was added to the residue and then removed *in vacuo* until the odor of ammonia was no longer evident. Trituration of the residue with chloroform (three 200-ml portions) at room temperature caused the amber syrup to solidify. Recrystallization of this solid from a methanol–water mixture afforded the product in 62% yield (93 mg).

Uv, ir, and pmr spectra as well as chromatographic mobilities were identical with those of the product prepared by method 1. A mixture melting point showed no depression.

4-Amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (1, Toyocamycin). Method 1. 4-Amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**14**, 500 mg) was dissolved in 200 ml of hot 50% aqueous ethanol. One equivalent of base (0.54 ml of 1 *N* sodium hydroxide) and 500 mg of 5% palladium on charcoal was then added to this solution. The mixture was agitated under 50 psi of hydrogen for 5 hr. The charcoal was then removed by filtration from the solution (pH 7) and washed with 200 ml of hot aqueous ethanol (50%). The volume was reduced to *ca.* 40 ml *in vacuo* and colorless crystals (345 mg, 88%)

separated after standing overnight. An analytical sample was recrystallized from water, mp 243°.

Anal. Calcd for $C_{12}H_{13}N_5O_4$: C, 49.48; H, 4.50; N, 24.04. Found: C, 49.15; H, 4.24; N, 24.06.

Method 2. 1,2,3,5-Tetra-O-acetyl-β-D-ribofuranose (750 mg) and 4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (**10**, 179 mg) were thoroughly mixed in a mortar and then placed in an oil bath at 135°. One drop of dichloroacetic acid was added as soon as the initial melt had been achieved and water aspirator vacuum was applied for the duration of the fusion reaction (50 min). The hot melt was extracted with ethyl acetate (25 ml) and the unreacted aglycon (94 mg) was removed by filtration. Subsequent extraction of the reaction mixture with 5% aqueous sodium hydroxide at 0° (three 50-ml portions) and neutralization of the combined basic extracts afforded another 15 mg of the aglycon. The organic layer was washed with water until the wash was neutral and then dried over sodium sulfate. Dry column chromatography (2.5 × 40 cm column) utilizing SilicAR 7GF and chloroform–acetone (8:2, v/v) as the developing solvent resolved the nucleoside and carbohydrate material. Extraction of the nucleoside band with chloroform and subsequent removal of the solvent afforded 46 mg of nucleoside as a pale yellow syrup (29% calculated on the basis of unrecovered starting material).

The pale yellow syrup (200 ml) was covered with 100 ml of anhydrous methanolic ammonia (saturated at 0°) and the mixture heated in a sealed tube for 8 hr at 110°. The cooled reaction mixture was evaporated to dryness *in vacuo* until the ammonia odor was no longer present. Trituration of the syrup with chloroform (two 200-ml portions) at room temperature furnished a solid crystalline material. Recrystallization from water afforded the product in 78% yield (110 mg). Chromatographic mobilities, ultraviolet absorption, infrared and proton magnetic resonance spectra were identical with those of the product prepared by method 1 and an authentic sample of toyocamycin; a mixture melting point showed no depression.

4-Amino-6-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-5-carboxamide (15). To 1.64 g of 4-amino-6-bromo-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**14**) in 50 ml of concentrated ammonium hydroxide was added 5 ml of 30% hydrogen peroxide and the mixture stirred for 2 hr at room temperature. The solution was allowed to stand overnight at 5° and the white solid which had separated from solution was collected by filtration and washed well with water (65% yield). A small sample was recrystallized from ethanol–water to furnish colorless needles, mp 221°.

Anal. Calcd for $C_{12}H_{14}BrN_5O_5$: C, 37.13; H, 3.63; N, 18.04. Found: C, 37.05; H, 3.66; N, 18.17.

6-Bromo-5-cyanopyrrolo[2,3-*d*]-4-pyrimidone (6). One gram of 4-amino-6-bromo-5-cyanopyrrolo[2,3-*d*]pyrimidine (**5**) was suspended in 100 ml of water and sodium hydroxide (200 mg) was added to effect a clear solution. To the solution was added 100 ml of glacial acetic acid and the resultant slurry was heated to 80° before sodium nitrite (1.0 g) in 25 ml of water was added dropwise. After the solid had completely dissolved (2–3 hr), the temperature was maintained at 80° for another half hour before the reaction mixture was treated with charcoal and filtered through Celite. The charcoal was washed with 150 ml of hot 50% aqueous acetic acid. The combined filtrate and washings furnished a pale yellow solid (770 mg, 77%) after being allowed to stand for 18 hr at 5°, mp >300°.

Anal. Calcd for $C_7H_3BrN_4O \cdot 0.5H_2O$: C, 33.88; H, 1.63; N, 22.59. Found: C, 33.75; H, 1.64; N, 22.49.

6-Bromo-4-chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (9). 6-Bromo-5-cyanopyrrolo[2,3-*d*]-4-pyrimidone (**6**, 1.0 g) was suspended in 25 ml of phosphorus oxychloride and the mixture stirred on a steam bath (80°) until solution (pale orange) had been achieved (*ca.* 5 hr). Excess phosphorus oxychloride was removed by vacuum distillation and the resultant syrup was poured onto excess ice. The off-white precipitate (0.90 g, 84%) was collected by filtration and air dried. Recrystallization from a methanol–chloroform mixture afforded an analytical sample, mp 250°.

Anal. Calcd for $C_7H_2BrClN_4$: C, 32.65; H, 0.78; N, 21.76. Found: C, 32.86; H, 0.74; N, 21.91.

5-Cyanopyrrolo[2,3-*d*]-4-pyrimidone (7). 6-Bromo-5-cyanopyrrolo[2,3-*d*]-4-pyrimidone (**6**, 200 mg) was dissolved in 50 ml of concentrated ammonium hydroxide, and this solution was added to 50 ml of ethanol containing 200 mg of 5% palladium on charcoal. The mixture was shaken under 40 psi of hydrogen in a Paar hydrogenator for 6 hr. The mixture was then filtered through celite and the catalyst washed with hot ethanol–ammonium hydroxide (1:1, v/v). The volume of the filtrate was reduced *in vacuo* until the ammonia odor was gone and a white solid had precipitated (130 mg, 97%). A small sample was recrystallized from ethanol–water for analysis, mp >330°.

Anal. Calcd for $C_7H_4N_4O$: C, 52.50; H, 2.52; N, 34.99. Found: C, 52.23; H, 2.80; N, 35.07.

4-Chloro-5-cyanopyrrolo[2,3-*d*]pyrimidine (10). 5-Cyanopyrrolo-[2,3-*d*]-4-pyrimidone (**7**, 350 mg) was suspended in 25 ml of phosphorus oxychloride and the mixture stirred for 18 hr on a steam bath. The mixture was heated at 130° for 2 hr until the remaining solid had dissolved and the mixture then poured onto excess ice. A small amount of dark solid was removed from the solution by filtration and discarded. The solution was extracted with chloroform (three 100-ml portions); the extracts were washed with water and then dried over sodium sulfate. The solvent was removed *in vacuo* and the residue recrystallized from a mixture of ethyl acetate–ethanol to yield pale yellow needles (235 mg, 60%), mp >325°.

Anal. Calcd for $C_7H_3ClN_4$: C, 47.08; H, 1.69; N, 31.38. Found: C, 47.23; H, 1.83; N, 31.18.