Pyrrolopyrimidine Nucleosides. IV. The Synthesis of Certain 4,5-Disubstituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidines Related to the Pyrrolo[2,3-*d*]pyrimidine Nucleoside Antibiotics (1a)

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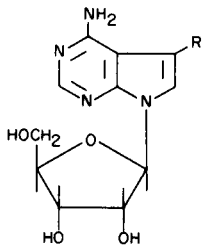
The treatment of 4-chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**) with *N*-bromoacetamide in methylene chloride has furnished the 5-bromo derivative of **4** which on subsequent deacetylation provided a good yield of 5-bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**6**). Assignment of the halogen substituent to position **5** was made on the basis of pmr studies. Treatment of **6** with methanolic ammonia afforded 4-amino-5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**8**, 5-bromotubercidin) and a subsequent study has revealed that the 4-chloro group of **6** was replaced preferentially in a series of nucleophilic displacement reactions. The analogous synthesis of 4,5-dichloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13b**) and 4-chloro-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13a**) from **4** furnished 5-chlorotubercidin (**15**) and 5-iodotubercidin (**14**), respectively, on treatment of **13b** and **13a** with methanolic ammonia. The possible biochemical significance of these tubercidin derivatives is discussed.

Isolation of the antibiotics, tubercidin (**1**) (2), toyocamycin (**2**) (3,4) and sangivamycin (**3**) (5) was subsequently followed by their structural elucidation as pyrrolo[2,3-*d*]pyrimidine nucleosides (6-11). It is of interest that toyocamycin was recently reported (11) to also be identical to several other antibiotics, unamycin B (12), antibiotic E-212 (13) and vengicide (14). These nucleoside antibiotics have all demonstrated significant biological and chemotherapeutic activity (15, 16). The only difference between tubercidin and the other pyrrolopyrimidine nucleoside antibiotics is the absence of a functional group at position five. This investigation is part of a general study involving pyrrolo[2,3-*d*]pyrimidines and pyrrolo[2,3-*d*]pyrimidine nucleosides. The present work (17) describes the synthesis of several new and interesting 4,5-disubstituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidines by the direct introduction of a functional group at position five *via* electrophilic substitution.



1, R = H
2, R = CN
3, R = C-NH2 (O)

Preliminary investigations on the introduction of a functional group at position five *via* electrophilic substitution in tubercidin suggested that a modification of the group at position four and acetylation of the carbohydrate moiety of tubercidin was desirable in order to provide sufficient stability to the molecule for the isolation of a product. Treatment of 4-chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**) (18) with *N*-bromoacetamide in methylene chloride furnished a good yield of the acetylated nucleoside (**5**) as a dark oil which resisted all attempts at crystallization. Removal of the blocking groups from the carbohydrate moiety of **5** with methanolic ammonia was achieved without a concomitant nucleophilic displacement of either the 4-chloro or 5-bromo group, to afford 5-bromo-4-chloro-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**6**). Assignment of the bromo group to position five was accomplished by pmr spectra (19) which revealed absorption peaks at δ 8.8 (1 proton singlet) and δ 8.3 (1 proton singlet) which were assigned

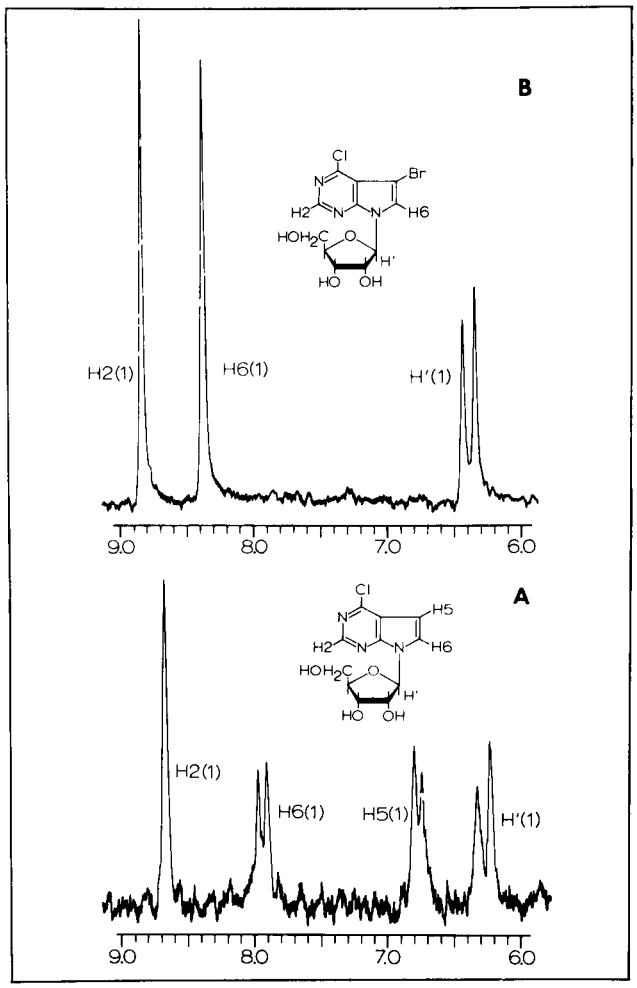


Figure 1. Pyrrolopyrimidine Nucleosides. IV. PMR spectra: A, 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**); B, 5-Bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**6**).

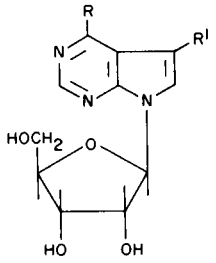
to the aromatic protons at C2 and C6, respectively. This assignment was based on previous work from this laboratory (19) which established the position of the aromatic protons at C2, C5 and C6 in the pyrrolo[2,3-*d*]pyrimidine ring system. The aromatic protons of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (18) occur in the pmr spectrum (Figure 1.A) at δ 8.7 (1 proton singlet, H2), δ 8.02 (1 proton doublet, H6) and δ 6.78 (1 proton doublet, H5). That substitution had indeed occurred in the pyrrole ring at position five was evident by the appearance of two singlets (δ 8.8 and δ 8.3) instead of two doublets and the disappearance of the absorption band at δ 6.78 (H5) (Figure 1.B). The shift to lower field for both absorption peaks (H2 and H6 of **6**) was expected due to the deshielding effect of the bromo group. In fact, there was even observed a shift to lower field for the absorption peak assigned to the anomeric proton. Treatment of **6** with methanolic ammonia in a sealed reaction vessel at 125° effected nucleophilic displacement of only one halogen group as evident by pmr spectra [absorption peak at δ 6.8 (2 protons, broad singlet)].

Elemental analysis established the structure of the nucleoside as 4-amino-5-bromo-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**8**, 5-bromotubercidin). Hydroxylamine in isopropyl alcohol effected a facile nucleophilic displacement of the 4-chloro group of **6** to furnish 5-bromo-4-hydroxylamino-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**9a**). Proof for the site of nucleophilic displacement was furnished by elemental analysis and also by the conversion of **9a** to **8** on treatment with Raney nickel. Treatment of **6** with methylamine and dimethylamine furnished 5-bromo-4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9b**) and 5-bromo-4-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9c**), respectively. When **6** was dissolved in aqueous thiourea in the presence of a catalytic amount of formic acid there was produced a good yield of 5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thione (**7**). The assignment of the thione form to **7** was supported by the appearance of an absorption peak (broad singlet, 1 proton) at δ 13.25 which is attributable to a proton residing on a heterocyclic ring nitrogen (20). Treatment of **7** in sodium methoxide with methyl iodide at room temperature furnished a good yield of 5-bromo-4-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**10**). The site of methylation was assigned on the basis of ultraviolet absorption (hypsochromic shift, Table I) and pmr spectroscopy (based on the expected chemical shift between a methyl group residing on an exocyclic mercapto group and ring nitrogen, value observed being δ 2.6). Treatment of **7** with 30% hydrogen peroxide in concentrated ammonium hydroxide at room temperature effected a facile conversion of sulfur to oxygen to furnish a good yield of 5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-one (**11**). Deamination of **8** produced a nucleoside (**11**) which was shown to be identical in all respects with **11** prepared from **7**. (See Scheme I)

The introduction of a different group at position five *via* electrophilic substitution was then investigated. Treatment of 4-chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**) with iodine monochloride in methylene chloride furnished 4-chloro-5-iodo-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**12a**) as a dark syrup. Deacetylation of **12a** with methanolic ammonia was achieved without nucleophilic displacement of either halogen to provide 4-chloro-5-iodo-7-(β-D-

TABLE I

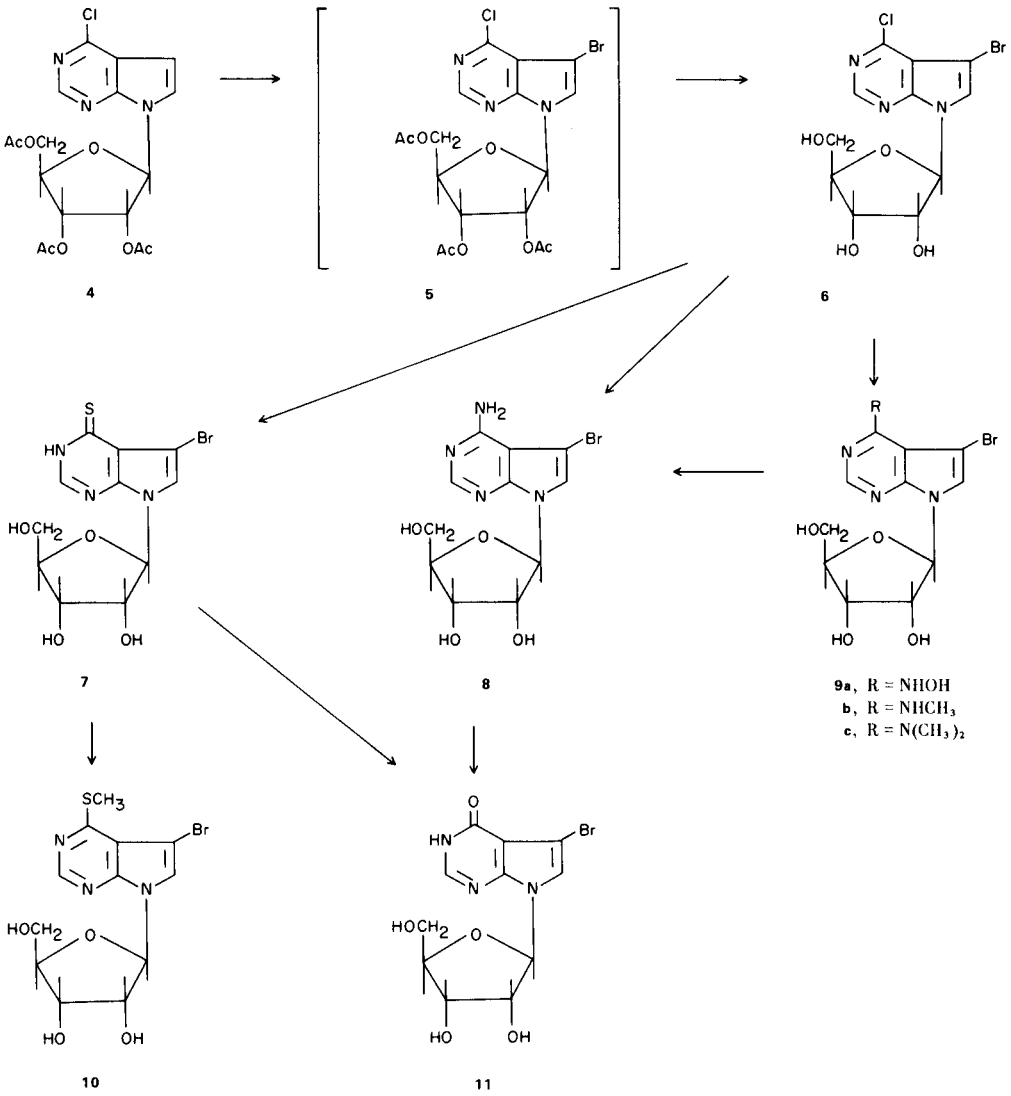
Ultraviolet Absorption Data of Certain 4,5-Disubstituted-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidines (a)



			pH 1		pH 11	
Compound	R	R^1	λ max nm	ε max	λ max nm	ε max
6	Cl	Br	296	8300	296	8300
			270	9100	270	9300
			231	66500	231	63000
9c	$N(CH_3)_2$	Br	291	11600	293	13800
			249	13800		
9b	$NHCH_3$	Br	278	11100	281	11500
			237	18700	231	6400
7	SH	Br	327	18800	317	17550
			277	7860	229	18800
11	OH	Br	265	9000	271	10050
8	NH_2	Br	282	8650	278	9350
			234	21100	228	12500
10	SCH_3	Br	295	9050	305	10150
			269	12000	257	6600
			227	19600	226.5	23700
13a	Cl	I	305	2880	305	2880
			269	2880	269	2880
			234	24300	234	24300
16	NHOH	I	285	8400	283	8400
			244	12650		
14	NH_2	I	285	7900	280	9350
			237	16500		
9a	NHOH	Br	280	10450	272-280	7950
			239	15900		
13b	Cl	Cl	296	3360	296	3360
			270	3360	270	3360
			230	27800	230	26600
15	NH_2	Cl	279	8400	277	9150
			232	21900	224 (b)	10500

(a) Ultraviolet absorption spectra were obtained with a Beckman DK-2 ultraviolet spectrophotometer. (b) Shoulder or inflection.

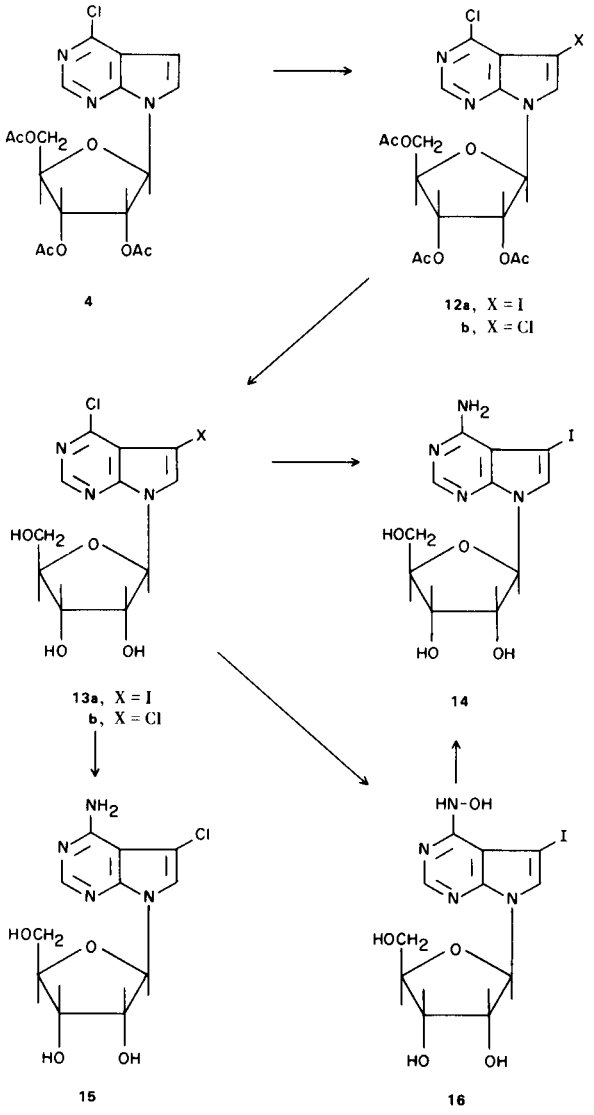
SCHEME I



ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13a**). Pmr spectroscopy established that iodination had occurred at position five [δ 8.7 (H2) and δ 8.3 (H6)]. Treatment of **13a** with hydroxylamine in isopropyl alcohol effected a preferential nucleophilic displacement of the 4-chloro group to afford 4-hydroxylamino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**16**). Reduction of the 4-hydroxylamino group with Raney nickel proceeded smoothly to furnish 4-amino-5-iodo-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**14**, 5-iodotubercidin). Treatment of **13a** with methanolic ammonia in a sealed reaction vessel at 125° provided a more convenient route for the preparation of **14**.

The introduction of a chloro group in the five position of **4** was accomplished with *N*-chlorosuccinimide in methylene chloride at room temperature to afford 4,5-dichloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**12b**) as a syrup. Removal of the blocking groups was accomplished with ethanolic ammonia to furnish 4,5-dichloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13b**). Treatment of **13b** with methanolic ammonia in a sealed reaction vessel at an elevated temperature afforded 4-amino-5-chloro-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**15**, 5-chlorotubercidin). (See Scheme II).

SCHEME II



The above nucleosides are of considerable biochemical and chemotherapeutic interest as tubercidin derivatives. The 5-halotubercidin analogs are of especial interest as potential biochemical tools for a study on the size of group (Cl, Br, I) which can be accommodated in position five of tubercidin without affecting the ability of tubercidin to be effectively bound to the active site of various purine nucleoside enzymes, *e.g.* adenosine kinase. The substitution of halogen for the cyano group of toyocamycin is another way to view the nucleoside derivatives **8**, **14** and **15**. The specificity exhibited by these nucleosides has been *strikingly* demonstrated by 5-bromotubercidin (**8**) which stimulates certain viral growth while simultaneously inhibiting the host cells (16). Further biochemical studies of these nucleosides will be published elsewhere.

EXPERIMENTAL (21)

5-Bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**6**).

4-Chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**4**, 7.6 g.) (18) was dissolved in methylene chloride (76 ml.) and to this solution was added 2.52 g. of *N*-bromoacetamide dissolved in 76 ml. of methylene chloride. This solution was allowed to stand at room temperature for 24 hours and then washed with 10% aqueous sodium dithionite (2 x 100 ml.), saturated aqueous sodium bicarbonate solution (100 ml.) and cold water (150 ml.). The methylene chloride phase was dried over anhydrous magnesium sulfate and evaporated to dryness *in vacuo* to yield a dark oil. Since this oil resisted all attempts at crystallization, it was dissolved in 100 ml. of methanolic ammonia which had been saturated previously at -10° and allowed to stand at 5° for 2 days. The methanol was removed by evaporation *in vacuo* and the resulting dark syrup was dissolved in 100 ml. of boiling water. The solid which had separated from solution after cooling at 5° for 18 hours was collected by filtration to yield 4 g. of pale brown crystals, m.p. 178-181°. A small sample was recrystallized once more from water for analysis, m.p. 179-180°.

Anal. Calcd. for $C_{11}H_{11}BrClN_3O_4$: C, 36.30; H, 3.04; N, 11.51. Found: C, 36.42; H, 3.20; N, 11.34.

4-Amino-5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**8**).

Method 1.

5-Bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**6**, 5.0 g.) was dissolved in 100 ml. of methanolic ammonia (saturated at -10°) and this solution was heated in a sealed reaction vessel for 5 hours at 125°. The alcohol was removed *in vacuo* and the remaining solid was dissolved in 40 ml. of boiling water. This solution was allowed to stand at 5° for 18 hours and the nucleoside material (3.9 g.) which had crystallized was collected by filtration. This solid was recrystallized from water to yield 2.8 g. of pure **8**, m.p. 231-232° dec. A small sample was dried *in vacuo* at 100° over phosphorus pentoxide for analysis, $[\alpha]_D^{27}$ -52.90° (C=1.0, 50% aqueous acetic acid).

Anal. Calcd. for $C_{11}H_{13}BrN_4O_4$: C, 38.30; H, 3.80; N, 16.22. Found: C, 38.15; H, 3.88; N, 16.37.

Method 2.

5-Bromo-4-hydroxylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**9a**, 0.5 g.) was dissolved in an ethanol-water mixture (50 ml., 2:1, v:v). Two grams of Raney nickel was then added and the mixture was stirred and heated at reflux temperature for one hour. The catalyst was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The resulting solid was recrystallized from water to yield 340 mg. of pure **8**, m.p. 232-235°. The sample prepared by Method 2 exhibited the same chromatographic mobility in 5% ammonium bicarbonate on SilicAR 7GF as that prepared by Method 1. The ultraviolet and pmr spectral data were identical with that obtained for the sample prepared by Method 1.

5-Bromo-4-hydroxylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**9a**).

Two grams of 5-bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**6**) and 4 g. of hydroxylamine (22) were added to 80 ml. of isopropyl alcohol and the reaction mixture was heated at reflux temperature for 1.5 hours. The product separated from the hot solution as it was formed. The reaction mixture was

allowed to cool for 12 hours at 5° and the solid (2.0 g.) was removed by filtration. The solid was recrystallized from a minimum amount of aqueous ethanol to yield 1.5 g. of product, m.p. 196-202° dec.

Anal. Calcd. for $C_{11}H_{13}BrN_4O_5$: C, 36.60; H, 3.64; N, 15.50. Found: C, 36.80; H, 3.80; N, 15.73.

5-Bromo-4-methylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**9b**).

To 50 ml. of anhydrous ethanol containing 1 ml. of anhydrous methylamine was added 1 g. of 5-bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**6**). This solution was heated at reflux temperature for one hour and then evaporated to dryness *in vacuo* to yield 1.3 g. of crude nucleoside material. This solid was recrystallized from water to yield 0.9 g. of **9b**. For analysis, a small sample was dried for one hour over phosphorus pentoxide at approximately 1 mm., m.p. 222°.

Anal. Calcd. for $C_{12}H_{15}BrN_4O_4$: C, 40.10; H, 4.18; N, 15.60. Found: C, 39.80; H, 4.26; N, 15.25.

5-Bromo-4-dimethylamino-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**9c**).

Five grams of 5-bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**6**) was dissolved in 250 ml. of ethanol containing 5 ml. of anhydrous dimethylamine. The resulting clear solution was heated at reflux temperature for one hour and then evaporated to dryness *in vacuo*. The residual material was triturated for 15 minutes with 30 ml. of an isopropyl alcohol-water mixture (1:1, v:v) and then allowed to stand at 5° for one hour. The solid (5 g.) was removed by filtration and recrystallized from water to yield 4.1 g. of chromatographically pure nucleoside, m.p. 98-99°.

Anal. Calcd. for $C_{13}H_{17}BrN_4O_4 \cdot H_2O$: C, 40.00; H, 4.86. N, 14.30. Found: C, 40.36; H, 5.02; N, 14.23.

5-Bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thione (**7**).

One gram of 5-bromo-4-chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**6**) and 0.44 g. of thiourea were dissolved in 75 ml. of water. One drop of 25% formic acid was added and the solution was heated at 80° for 2 hours while maintaining the *p*H of the solution at 5 with 25% ammonium hydroxide. The solution was allowed to stand at 5° for 18 hours and the solid which had separated from solution was collected by filtration to furnish 0.88 g. of crude product. A small sample was recrystallized from water for analysis, m.p. 188° dec.

Anal. Calcd. for $C_{11}H_{12}BrN_3O_4S$: C, 35.50; H, 3.33; N, 11.60. Found: C, 35.29; H, 3.56; N, 11.44.

5-Bromo-4-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**10**).

To 30 ml. of methanol containing 16.5 mg. of sodium methoxide was added 1 g. of 5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine-4-thione (**7**). To this solution was added 420 mg. of methyl iodide and the reaction mixture was stirred at 25° for 8 hours. The solid which had separated from solution was collected by filtration and the filtrate was evaporated to dryness *in vacuo*. The combined solids were recrystallized from methanol to yield 500 mg. of chromatographically pure product. A small sample was dried over phosphorus pentoxide *in vacuo* at 100° for 1 hour for analysis, m.p. 210-211°.

Anal. Calcd. for $C_{12}H_{14}BrN_3O_4S$: C, 38.20; H, 3.75; N, 11.14. Found: C, 38.15; H, 3.72; N, 10.99.

5-Bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-one (**11**).

Method 1.

To 100 ml. of concentrated ammonium hydroxide was added 4.4 g. of 5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine-4-thione (**7**). To this solution was added, as fast as possible, 15 ml. of 30% hydrogen peroxide with rapid stirring. The solution was stirred at room temperature for 2 hours and then evaporated to dryness *in vacuo*. The resulting solid was recrystallized from water to yield 2.5 g. of crystalline product, m.p. 254.5-255°.

Anal. Calcd. for $C_{11}H_{12}BrN_3O_5$: C, 38.20; H, 3.49; N, 12.12. Found: C, 38.24; H, 3.59; N, 12.12.

Method 2.

To 2.5 ml. of glacial acetic acid and 40 ml. of water was added 4-amino-5-bromo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*] pyrimidine (**8**, 500 mg.) and then 800 mg. of sodium nitrite. This solution was heated at 70° for one hour and then allowed to stand at 5° for 18 hours. The crude product (300 mg.) which had separated from solution was collected by filtration and recrystallized from water to yield 200 mg. (40%) of chromatographically pure **11**, identical in all respects to **11** prepared by Method 1.

Anal. Calcd. for $C_{11}H_{12}BrN_3O_5$: C, 38.20; H, 3.49; N, 12.12. Found: C, 38.24; H, 3.60; N, 12.30.

4-Chloro-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13a**).

To 660 ml. of methylene chloride was added 22 g. of 4-chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**) (18) and 17.6 g. of iodine monochloride. The solution was allowed to stand for 4 days at room temperature. The solution was washed with 10% sodium dithionite (2 x 300 ml.) and then with 300 ml. portions of water until the wash was neutral. The methylene chloride solution was dried over magnesium sulfate and then evaporated to dryness *in vacuo* to furnish a dark syrup (**12a**). The dark syrup was dissolved in 500 ml. of methanolic ammonia (previously saturated at -10°) and the solution was allowed to stand at 5° for 18 hours. The methanol was removed to yield a brown syrup which was dissolved in 200 ml. of boiling water and a small amount of insoluble solid was removed by filtration. The solid which separated from solution after standing at 5° for 12 hours was collected by filtration. The solid was recrystallized from water with a minimum amount of ethanol to yield 6.5 g. of chromatographically pure product, m.p. 194-196° dec. A small sample was dried over phosphorus pentoxide at approximately 1 mm. for one hour for analysis.

Anal. Calcd. for $C_{11}H_{11}ClIN_3O_4$: C, 32.00; H, 2.68; N, 10.20. Found: C, 32.30; H, 2.63; N, 10.14.

4-Hydroxylamino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**16**).

To a 2-propanolic hydroxylamine solution (23) was added 500 mg. of 4-chloro-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**13a**). The solution was heated at reflux temperature for one hour and then allowed to stand at 5° for 12 hours. The solid which had separated was collected by filtration and recrystallized from a mixture of ethanol-water to furnish 250 mg. of product, m.p. 155-157° dec.

Anal. Calcd. for $C_{11}H_{13}IN_4O_5 \cdot H_2O$: C, 31.00; H, 3.53; N, 13.10. Found: C, 31.30; H, 3.20; N, 12.96.

4-Amino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**14**).

Method 1.

To 100 ml. of methanolic ammonia (previously saturated at -10°) was added one g. of 4-chloro-5-iodo-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**13a**). This solution was heated in a sealed reaction vessel for four hours at 125° and then evaporated to dryness *in vacuo*. The resulting solid was recrystallized from a mixture of ethanol-water to yield 550 mg. of **14**, m.p. 217° dec. A small sample was dried at 0.1 mm. for three hours over phosphorus pentoxide at 110° for analysis.

Anal. Calcd. for $C_{11}H_{13}IN_4O_4$: C, 33.70; H, 3.34; N, 14.30. Found: C, 33.75; H, 3.49; N, 14.35.

Method 2.

4-Hydroxylamino-5-iodo-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**16**, 100 mg.) was dissolved in 10 ml. of ethanol containing 2 drops of water and to this solution was added 400 mg. of Raney nickel which had been thoroughly washed with ethanol. The mixture was heated at reflux temperature for 4 hours and the Raney nickel was removed by filtration. The filtrate was evaporated to dryness and the residue was recrystallized from water to yield 20 mg. of **14**, m.p. 216-217° dec. The sample prepared by Method 2 exhibited the same chromatographic mobility and identical ultraviolet spectra as the sample prepared by Method 1.

4,5-Dichloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13b**).

To 450 ml. of methylene chloride was added 4-chloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**, 10 g.) and 10 g. of *N*-chlorosuccinimide. The solution was allowed to stand for 14 days at room temperature until an ultraviolet spectrum of the reaction mixture exhibited no trace of an absorption peak at 266 nm. The methylene chloride solution was then washed with tap water (3 x 500 ml.), dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo* to afford 4,5-dichloro-7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**12b**) as a syrup. This syrup was dissolved in 250 ml. of ethanolic ammonia (saturated at -10°) and allowed to stand at -25° for 48 hours after which time the syrup was completely in solution. The ethanolic ammonia was removed under water aspirator vacuum using a hot water bath, and the residue was crystallized from a minimum amount of water to afford 3.2 g. (41%) of **13b**, m.p. 98-99°.

Anal. Calcd. for $C_{11}H_{11}Cl_2N_3O_4$: C, 41.30; H, 3.45; N, 13.12. Found: C, 41.38; H, 3.72; N, 12.91.

4-Amino-5-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**15**, 5-chlorotubercidin).

To 100 ml. of methanolic ammonia (saturated at -10°) was added 4,5-dichloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**13b**, 3.0 g.) and the solution was heated for 4 hours at 125° in a sealed reaction vessel. The methanolic ammonia was removed *in vacuo* and the residue was crystallized from water to yield 1.9 g. of **15**. The solid was recrystallized from water to furnish 1.6 g. (57%) of chromatographically pure product, m.p. 226-228°.

Anal. Calcd. for $C_{11}H_{13}ClN_4O_4$: C, 44.10; H, 4.36; N, 18.65. Found: C, 44.10; H, 4.72; N, 18.98.

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