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Pyrrolopyrimidine Nucleosides II. The Total Synthesis of 7-β-D-Ribofuranosylpyrrolo[2,3-*d*]pyrimidines Related to Toyocamycin (1, 1a)

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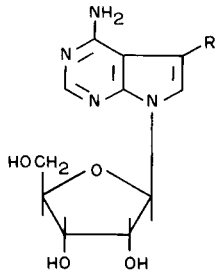
The first synthesis of a 7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidine by direct ribosidation of a preformed pyrrolo[2,3-*d*]pyrimidine has now been accomplished *via* the fusion procedure. Subsequent functional group transformations furnished the 6-methylthio derivative of the nucleoside antibiotic toyocamycin. Preparation of the 1-, 3- and 7-methyl isomers of 4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine was accomplished and has provided an unequivocal assignment for the actual site of ribosidation by a comparison of ultraviolet absorption spectra. Factors utilized for the assignment of anomeric configuration are discussed.

Isolation of the antibiotics tubercidin (3) (I) toyocamycin (4,5) (II) and sangivamycin (6) (III) was subsequently followed by their structural elucidation and it is of interest to note that all three antibiotics



I, R = -H

II, R = -CN

III, R = -C-NH$_2$ (O)

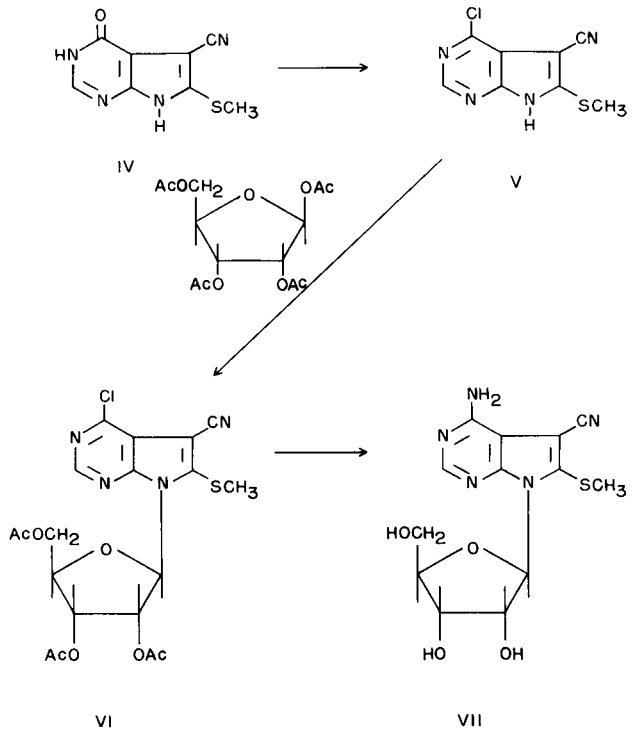
were shown (7-11) to be pyrrolo[2,3-*d*]pyrimidine nucleoside derivatives. It has been recently reported (12) that an antibiotic isolated (13) from *Streptomyces* species No. 1037 and designated only as antibiotic 1037 is identical in all respects to toyocamycin.

Tubercidin has been shown to serve as a substrate for several enzymes as evidenced by the formation of cofactors (14,15) (nicotinamide-tubercidin dinucleotide), incorporation (14,16,17) into RNA and DNA, enzymatic formation of 2'-deoxytubercidin (14,16) and in addition has also demonstrated significant activity against a variety of experimental tumors (16,18-23). 7-β-D-Ribofuranosylpyrrolo[2,3-*d*]-4-pyrimidone, derived from tubercidin, has also exhibited (24,26) some antineoplastic activity. Sangivamycin has demonstrated (6) significant activity against leukemia 1210 in mice, cytoxicity against HeLa cells, and in a phase I toxicity study (27) on humans produced no evidence of toxicity at maximally tolerated doses. The antitumor activity of toyocamycin is significant (16,23, 26) but its clinical use has been precluded by severe toxicity and would suggest that structural modifications might possibly reduce the toxicity within allowable limits. This broad spectrum of chemotherapeutic and biological activity has created considerable interest in the synthetic preparation of these nucleoside antibiotics and their related derivatives.

Of the synthetic routes available for the preparation of imidazole (28), pyrimidine (29,30) and purine (30,31) nucleosides, direct glycosidation of the appropriate preformed aglycone appears to be the method of choice and therefore by analogy should be the preferred procedure for the preparation of pyrrolopyrimidine nucleosides. On this *a priori* premise, the synthesis of tubercidin *via* direct ribosidation of the heavy metal salt of either 4-amino- or 4-chloropyrrolo[2,3-*d*]pyrimidine (32,33) was attempted (34) and found to afford only an intractable resinous mixture from which none of the desired product could be isolated. Ribosidation of the chloromercury salt of 4-amino-5-cyanopyrrolo[2,3-*d*]pyrimidine has produced (35) nucleoside material, however the yield was so low (less than 1%) that complete characterization (anomeric configuration, actual site of glycosidation and complete elemental analysis) of the nucleoside was unattainable. The preceding difficulties strongly suggested that an alternate method for the direct ribosidation of a preformed pyrrolo[2,3-*d*]pyrimidine derivative might be more rewarding.

We now report a new synthetic route for the preparation of pyrrolo[2,3-*d*]pyrimidine nucleosides and the first glycosidation of a pyrrolo[2,3-*d*]-pyrimidine *via* the fusion procedure (36). A suc-

cessful fusion has been shown to be influenced by several factors, but presumably the most critical factor is the proper selection of the aglycone utilized. Therefore, we elected to preform a few functional group transformations on a previously synthesized pyrrolo[2,3-*d*]pyrimidine prior to the fusion reaction in an effort to obtain a more likely candidate for fusion. Deamination of 4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (35) with nitrous acid furnished a 63% yield of a pale yellow solid which was identified as 5-cyano-6-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (IV). This reaction was found to proceed to completion more rapidly and a better yield of IV was obtained if the starting material (4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]-pyrimidine) was reprecipitated *in situ* before addition of the sodium nitrite solution. A facile chlorination was observed on treatment of IV with phosphorus oxychloride at reflux temperature and furnished a good yield of 4-chloro-5-cyano-6-methylthiopyrrolo-[2,3-*d*]pyrimidine (V). This provided a pyrrolo-[2,3-*d*]pyrimidine derivative which should function satisfactorily in a fusion reaction. A mixture of 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (V, 0.5 g.) and 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (1.0 g.) was heated at 165° in the absence of a catalyst to afford a 45% yield (based on the recovery of unreacted starting material) of 4-chloro-5-cyano-6-methylthio-7-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VI) as a yellow syrup. Nucleophilic displacement of the 4-chloro group from VI was effected by methanolic ammonia in a sealed vessel at 110° to furnish a derivative of toyocamycin, 4-amino-5-cyano-6-methylthio-7-(β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (VII). The infrared absorption spectra of VII exhibits a band at 2250 cm^{-1} which precludes any transformation of the cyano group.



The *trans* rule (37,38) was utilized initially as the basis for a tentative assignment of beta anomeric configuration for VII.

An inspection of the pmr spectrum for VII revealed an absorption band (1 proton) centered at 6.30 δ as a doublet ($J_{1,2}$ 7.0 cps) which was assigned to the anomeric proton. However, the anomeric configuration of VII could not be assigned on the basis of the coupling constant ($J_{1,2}$) observed for the anomeric proton. It has been determined (39) that in a five membered ring the dihedral angle between neighboring *cis*-hydrogens (α-riboside) and neighboring *trans*-hydrogens (β-riboside) can vary from 0-45° and 75-165° which can produce coupling constants, using the Karplus equation (40), of approximately 3.5-8.0 cps and 0.0-8.0 cps, respectively. Therefore an assignment of anomeric configuration, excluding conformational changes, can only be made for the β-anomer with neighboring *trans*-hydrogens and then only if the coupling constant is less than 3.5 cps, but a smaller coupling constant is desirable. In fact, because of several uncertainties associated with observed coupling constants it is generally accepted (41-43) that this assignment should be applied for neighboring *trans*-hydrogens only when the coupling constant is less than about 1.0 cps which is not the case for VII. However, the pmr spectrum of VI, in deuterated chloroform displayed an absorption band (1 proton) as a doublet ($J_{1,2}$ 2.0 cps) centered at 6.42 δ and was assigned to the anomeric proton. This observed coupling constant ($J_{1,2}$ 2.0 cps) for the anomeric proton of VI is smaller than and closer to 1.0 cps than the coupling constant observed (44) ($J_{1,2}$ 2.4 cps) for the β-anomer of 4-chloro-1-(2',3'-*O*-isopropylidene-D-ribofuranosyl)imidazo[4,5-*c*]pyridine. The anomeric configuration of this compound was definitely established (44,45) as *beta* which supports the *beta* configuration for VI.

An alternate method (46,47) for anomeric assignment utilizing pmr is dependent on the chemical shift of the anomeric proton. It has been observed that the absorption peak for the anomeric proton of C1' - C2' *cis*-nucleosides (α-riboside) appeared at a lower field (approximately 0.5 δ difference) than the corresponding anomeric proton of a C1' - C2', *trans*-nucleoside (β-riboside). This requires a comparison between the α and β anomers for a definite assignment since the chemical shift of a proton may be affected by several diverse factors, *e.g.*, solvent (48,49), electronegativity of substituent groups (50) and hydrogen bonding (51). However, tubercidin (I) and toyocamycin (II) exhibit a doublet centered at 6.18 δ and 6.25 δ for the anomeric proton, respectively and this is in close agreement with the doublet centered at 6.30 δ for VII (run under identical conditions).

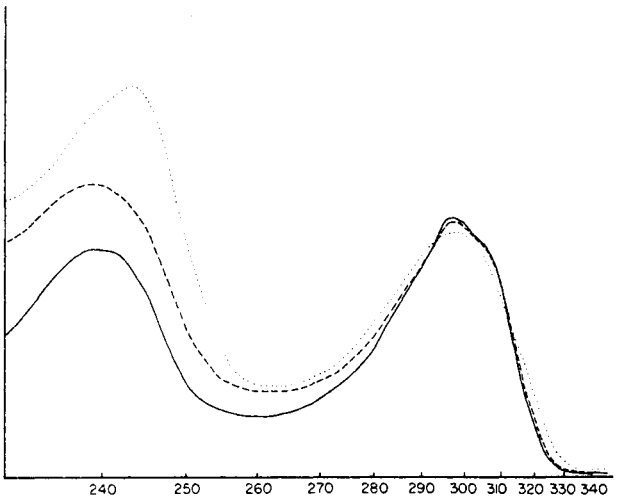


Figure 1. 4-Amino-5-cyano-7-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine. EtOH (——) 5 mg/l; *p*H 1 (·····) 5 mg/l; *p*H 11 (---) 5 mg/l.

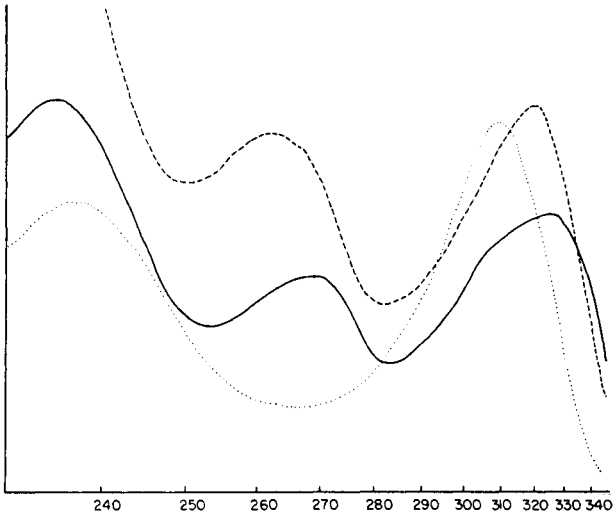


Figure 2. 4-Amino-5-cyano-1-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine. EtOH (——) 5 mg/l; *p*H 1 (·····) 5 mg/l; *p*H 11 (---) 5 mg/l.

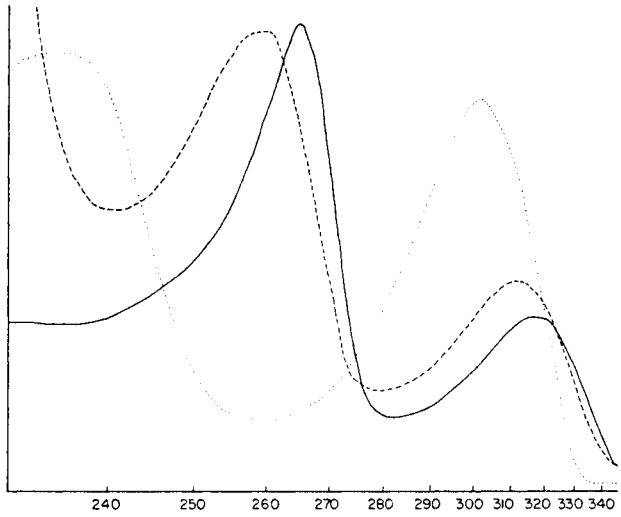


Figure 3. 4-Amino-5-cyano-3-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine. EtOH (——) 2.5 mg/l; *p*H 1 (····) 5 mg/l; *p*H 11 (---) 2.5 mg/l.

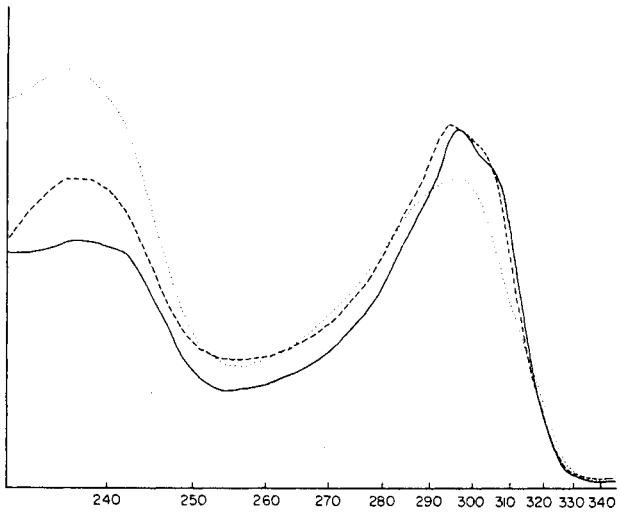
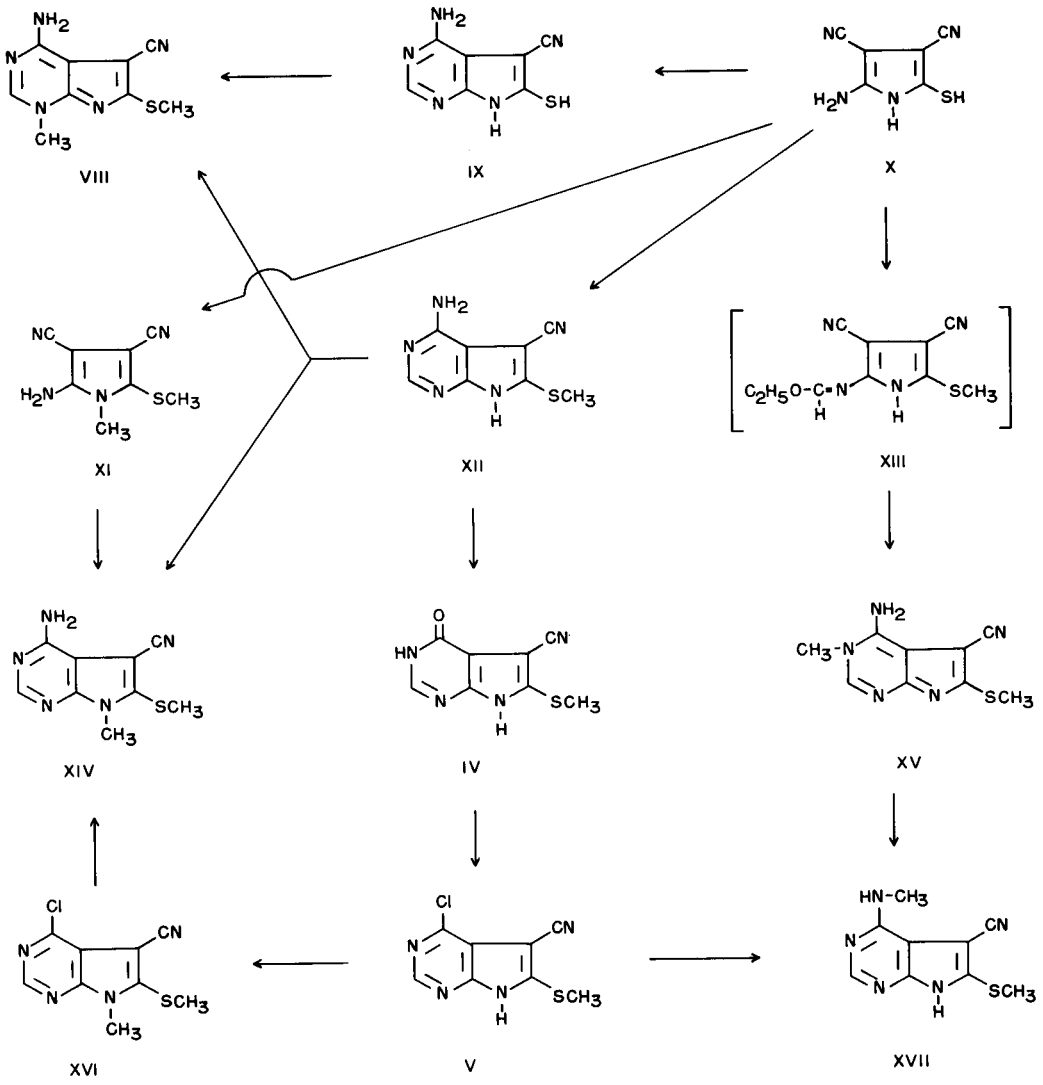


Figure 4. 4-Amino-5-cyano-6-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. EtOH (——) 10 mg/l; *p*H 1 (·····) 10 mg/l; *p*H 11 (---) 10 mg/l.

On the basis of the *trans*-rule, the coupling constant ($J_{1',2'}$) observed for VI and the relative chemical shifts observed for the anomeric proton of tubercidin, toyocamycin and VII, a tentative assignment of *beta* configuration has been made for VI and VII.

Efforts to obtain toyocamycin (II) from VII, by dethiation, using a variety of reaction conditions was unsuccessful. In every instance there was observed a gradual loss of ultraviolet absorbance for the reaction mixture and thin layer chromatography revealed a small amount of unreacted VII accompanied by a large number of minor components. The assignment for actual site of glycosidation, for VII, by a conversion to the known compound toyocamycin was therefore ruled out. An alternate method (52) was to compare the ultraviolet absorption spectra of VII with the ultraviolet absorption spectra of the corresponding *N*-methyl model compound. 4-Amino-5-cyano-"7"-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine has previously been prepared and reported (35) to possess λ max (EtOH), 327 mμ, whereas the nucleoside VII dis-

played λ max (EtOH), 296 mμ. This indicated that we probably had in our possession either the N-1 or N-3 ribosyl compound and prompted the unequivocal preparation of all three possible *N*-methyl isomers of 4-amino-5-cyano-6-methylthiopyrrolo-[2,3-*d*]pyrimidine (XII). It has been previously (35) stated that treatment of 2-amino-3,4-dicyano-5-thiopyrrole (X) with trimethylorthoformate affords 3,4-dicyano-2-ethoxymethyleneamino-5-methylthiopyrrole (XIII). Treatment of XIII with methylamine produced a compound which was initially assumed to be and later definitely established as 4-amino-5-cyano-3-methyl-6-methylthiopyrrolo[2,3-*d*]-pyrimidine (XV). The ultraviolet absorption spectra of XV displayed λ max (EtOH), 318 mμ and appears to eliminate N-3 as the site of glycosidation for VII. Additional corroboration for the structural assignment of XV was obtained when exposure to dilute alkaline conditions resulted in a facile rearrangement (24, 35, 53, 54) with subsequent formation of a new compound which was shown to be 5-cyano-4-methylamino-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XVII). This rearranged product was also found to be identical in all respects to the product isolated from the treatment of 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (V) with methylamine in a sealed vessel at 110° and firmly established the unrearranged compound (XV) as the 3-methyl isomer of XII. Preparation of the "7" methyl isomer in our Laboratory, using the reported (35) procedure for the methylation of 4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XII) unexpectedly produced an isomeric mixture instead of the "7"-methyl isomer. Chromatographic separation of this isomeric mixture furnished two different compounds. One isomer possessed an ultraviolet absorption spectrum of λ max (EtOH), 327 mμ and the other isomer possessed an ultraviolet absorption spectrum



of λ max (EtOH), 297 mμ and since neither absorption maximum corresponds to that observed for the 3-methyl isomer, these compounds must be the 1- and 7-methyl isomers of XII. In our Laboratory, the second method reported (35) for the preparation of the "7"-methyl isomer by methylation of 4-amino-5-cyano-6-mercaptopyrrolo[2,3-*d*]pyrimidine (IX) was repeated and only one product was isolated, which possessed an ultraviolet absorption spectrum of λ max (EtOH), 327 mμ. While it was tempting to assign the compound with the ultraviolet absorption value of λ max (EtOH), 327 mμ as the 7-methyl

TABLE I

Ultraviolet Absorption (a) of some Pyrrolo[2,3-*d*]pyrimidines

No.	Compound	λ max (*p*H 1) mμ	ε	λ max (EtOH) mμ	ε	λ max (*p*H 11) mμ	ε
X	2-Amino-3,4-dicyano-5-thiopyrrole	292	7,700	313	11,500	323 229	8,500 8,400
XII	4-Amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine	300 235	15,600 15,800	301 232	15,400 14,600	309 247	13,700 19,100
IV	5-Cyano-6-methylthiopyrrolo-[2,3-*d*]-4-pyrimidone	290	15,900	290	15,500	300 248	14,200 30,900
V	4-Chloro-5-cyano-6-methylthiopyrrolo[2,3-d]pyrimidine	312 238	14,800 20,600	300 312(sh) 258 243	10,100 9,600 20,000 18,900	322 296 253	10,100 9,400 34,800
VI	4-Chloro-5-cyano-6-methylthio-7-(β-D-tri-*O*-acetylribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine	311 229	14,000 29,900	309 229	13,100 28,400	311 231	13,500 26,600
VII	4-Amino-5-cyano-6-methylthio-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine	297 236	15,200 17,900	296 236	16,200 12,200	297 236	17,200 13,200
IX	4-Amino-5-cyano-6-thiopyrrolo[2,3-*d*]pyrimidine	327 242	13,900 18,500	327 241	19,700 22,900	319 239	20,100 25,700
XI	2-Amino-3,4-dicyano-1-methyl-5-methylthiopyrrole	298 229	8,700 15,200	302 257 229	9,000 4,400 15,000	298 229	8,500 13,700
VIII	4-Amino-5-cyano-1-methyl-6-methylthiopyrrolo[2,3-*d*]-pyrimidine	310 238	17,300 12,700	327 269 235	14,300 12,700 15,900	320 264 235	14,700 12,500 14,500
XV	4-Amino-5-cyano-3-methyl-6-methylthiopyrrolo[2,3-*d*]-pyrimidine	302 235	16,700 16,700	318 265	14,000 28,900	312 260	14,700 22,600
XIV	4-Amino-5-cyano-7-methyl-6-methylthiopyrrolo[2,3-*d*]-pyrimidine	299 244	15,100 17,100	297 239	15,000 14,300	298 240	13,600 13,400
XVI	4-Chloro-5-cyano-7-methyl-6-methylthiopyrrolo[2,3-*d*]-pyrimidine	314 232	10,000 17,700	312 231	11,700 19,700	314 232	8,100 13,600
XVII	5-Cyano-4-methylamino-6-methylthiopyrrolo-[2,3-*d*]pyrimidine	304 227	20,600 13,600	304 230	20,800 13,100	310	18,800

(a) All spectra were taken on a Cary Model 15 spectrophotometer.

TABLE II

R_{Ad} (a) for *N*-Methylated-4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidines

No.	Compound	Solvent Systems (b)			
		A	B	C	D
XIV	7-Methyl-	1.09	1.07	1.33	0.69
VIII	1-Methyl-	0.98	1.01	1.21	0.79
XV	3-Methyl-	0.89	0.87	0.92	1.02
	Mixture (c) of 1- and 7-Methyl-	1.07	1.07	1.34	0.69
		0.98	1.01	1.20	0.79

(a) R_f of Compound/R_f of Adenine = R_{Ad}. (b) Solvent A, Methanol:Water [7:3 (v/v)]; Solvent B, Acetone:Water [1:1 (v/v)]; Solvent C, Isopropyl alcohol:Aqueous ammonia 28%:Water [70:5:25 (v/v)]; Solvent D, Isopropyl alcohol:5% Aqueous ammonium sulfate [1:19 (v/v)]. (c) Isomeric mixture obtained from the methylation of XII in our Laboratory. (d) Thin-layer chromatography on glass plates coated with *ca* 250 μ layer of SilicAR 7 GF and developed by the ascending technique.

TABLE III

Melting Points and $\Delta\lambda$ min Values for Certain Pyrrolo[2,3-*d*]pyrimidines

Compound	M.p. °C	λ min (*p*H 1) - λ min (EtOH) = $\Delta\lambda$ min
4-Amino-5-cyano-6-methylthio-pyrrolo[2,3-*d*]pyrimidine		
1-Methyl- (VIII)	315-317°	266-284 = -18 mμ
3-Methyl- (XV)	305-307°	258-283 = -25 mμ
7-Methyl- (XIV)	237-238°	260-255 = + 5 mμ
7-β-D-ribofuranosyl- (VII)	225°	257-254 = + 3 mμ

isomer on the basis of previous (35) data, it is a fact that the actual position of the methyl group in the earlier investigation was not unambiguously established. This prompted the unequivocal preparation of at least one of the two isomers in question to firmly establish the actual site of *N*-methylation.

Methylation of 2-amino-3,4-dicyano-5-thiopyrrole (X) in dilute sodium hydroxide in the presence of a large excess of methyl iodide furnished 2-amino-3,4-dicyano-1-methyl-5-methylthiopyrrole (XI) which was separated from other components by preparative layer chromatography. The excess methyl iodide is a critical factor (55) since, if the large excess is absent, considerable decomposition with concomitant formation of several other products occurs as the alkyl halide phase is consumed and the product is forced to be in contact with the aqueous alkaline reaction mixture. Treatment of XI with trimethylorthoformate followed by exposure to methanolic ammonia yielded a product which was unequivocally 4-amino-5-cyano-7-methyl-6-methylthio-[2,3-*d*]pyrimidine (XIV) which possessed an ultraviolet absorption spectrum λ max (EtOH), 297 mμ. Additional corroboration for this assignment was obtained by methylation of V which furnished a compound, assumed to be the 7-methyl derivative of V, with an ultraviolet absorption value of λ max (EtOH) 310 mμ which is very similar to the ultraviolet absorption value of λ max (EtOH), 309 mμ displayed by VI. Amination of this compound (XVI) furnished a product identical in all respects with the product (XIV) obtained by ring closure of the pyrrole derivative (XI). On the basis of the methylation studies *vide supra* and from a visual inspection of the ultraviolet absorption curves (Figures 1, 2, 3 and 4) the total structure of VII was assigned as 4-amino-5-cyano-6-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine and VI must therefore possess the structure 4-chloro-5-cyano-6-methylthio-7-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine.

The compound previously reported (35) as the "7"-methyl isomer of XII, with λ max (EtOH), 327 mμ should now be reassigned the structure 4-amino-5-cyano-1-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine (VIII).

In the course of obtaining routine chromatograms for the compounds prepared in this investigation,

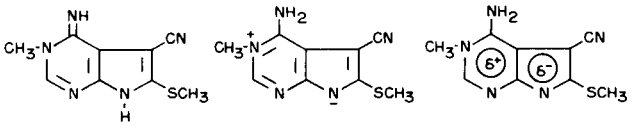
there was observed a correlation between the position of methylation and relative chromatographic mobilities. From the chromatographic data obtained for the 1-, 3- and 7- methyl isomers of XII (Table II), it appears that there exists some similarity between *N*-alkyl pyrrolo[2,3-*d*]pyrimidines and *N*-alkyl purines. The chromatographic behavior of certain purines methylated in the six-membered ring has been shown (52, 56) to be characteristically different from the same purines methylated in the five-membered ring, depending on the choice of solvents. This difference has been attributed (52) to the fact that alkylation of the six-membered ring presumably produces a polar or zwitterionic structure, while alkylation in the five-membered ring produces an uncharged structure. This is manifested in chromatographic mobility by the fact that more polar compounds should be retarded in an aqueous stationary phase, while the uncharged compound should demonstrate less retardation and *per contra*, when the mobile phase is highly ionic the more polar compounds should exhibit a greater chromatographic mobility than the more non-polar compounds. It is evident, from Table II, that the 1- and 3-methylpyrrolo[2,3-*d*]pyrimidines are definitely more retarded than the 7-methyl isomer in solvent systems A, B, and C, and yet, when solvent system D, with a more ionic mobile phase was employed the converse was observed.

Ultraviolet spectral data has also been utilized (52) to distinguish between purines alkylated in the six-membered ring and the five-membered ring. It was shown (52) that the Δ λ min [λ min (*p*H 1) - λ min (alcohol or *p*H 7)] value was positive for certain purines substituted in the five-membered ring and negative for the isomeric purines substituted in the six-membered ring. From an inspection of the Δ λ min values contained in Table III it appears that the difference between substitution in the six-membered ring and the five-membered ring for certain isomeric pyrrolo[2,3-*d*]pyrimidines can be readily ascertained since the 1- and 3-methyl-derivatives possess a large negative Δ λ min while the 7-methyl isomer possesses a positive Δ λ min value. This should prove to be very useful in future investigations on direct glycosidation of a preformed pyrrolo[2,3-*d*]pyrimidine where an assignment of actual site of glycosidation is required.

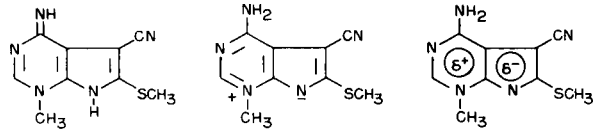
In fact, it has been demonstrated (52) that melting points may even be utilized in a preliminary evaluation of the structure assignment of certain isomeric purines. Melting points of purines substituted in the six-membered ring are considerably higher than the isomeric purines substituted in the five-membered ring. The same trend in melting points is evident for the isomeric methyl derivatives of 4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (Table III) prepared in this investigation since the 1- and 3-methyl derivatives of XII exhibit much higher melting points than does the 7-methyl isomer.

The generality of the above observations concerning chromatographic mobilities, Δ λ min values and melting points will require further investigation before being firmly established.

The above results suggest that the overall structure of VIII and XV probably include contributions from the following forms.



XVa XVb XVc



VIIIa VIIIb VIIIc

However, structures VIIIa and XVa (the "imino" form) can be excluded since there was observed an absorption peak (2 protons) at 7.13 δ for VIII and an absorption peak (2 protons) at 7.43 δ for XV in the pmr spectra which can be attributed only to an exocyclic amino group. This established that the predominant tautomeric form (57, 58) of VIII and XV is the "amino" form, rather than the "imino" form, at least in DMSO-d_6. On the basis of the above evidence and of similar investigations on the chemical reactivity in relationship to structure of polar or zwitterionic purines (58, 61), the structure which contributes most to the over-all structure is probably VIIIc and XVc with delocalization of the partial positive charge in the pyrimidine ring and delocalization of the partial negative charge in the pyrrole ring.

Further studies on the relationship of chemical reactivity and site of substitution (alkylation or glycosidation) as well as the extension of this synthetic procedure for the preparation of additional pyrrolopyrimidine nucleosides is presently under active investigation in our Laboratory.

EXPERIMENTAL (62)

5-Cyano-6-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (IV).

Twenty-five grams of 4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]-pyrimidine (35) (XII) was suspended in 500 ml. of distilled water and solid sodium hydroxide (5.0 g.) was added. When solution was effected, glacial acetic acid (500 ml.) was added to achieve a fine suspension and the mixture was heated to 65-70°. Sodium nitrite (25.2 g.) in 120 ml. of distilled water was added dropwise to the above solution over a 45 minute period at which time all the solid had dissolved. The solution was stirred an additional hour, treated with charcoal and filtered hot. A pale yellow solid crystallized out on cooling at

5° for 20 hours. The product was collected by filtration to yield 15.8 g. (63% yield), m.p. 343° (dec.). A small sample was recrystallized from ethanol for analysis. I.R. 2225 cm^{-1} (-C≡N).

Anal. Calcd. for $C_8H_6N_4OS$: C, 46.60; H, 2.91; N, 27.18. Found: C, 46.42; H, 3.08; N, 26.93.

4-Chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (V).

Ten grams of 5-cyano-6-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (IV) was suspended in 150 ml. of phosphorus oxychloride and heated to reflux for 45 minutes (the solid dissolved). Excess phosphorus oxychloride was removed immediately *in vacuo* and the remaining syrup poured cautiously onto ice (keeping an excess present at all times). The tan solid was filtered (7.4 g. crude wt.) and the filtrate extracted with chloroform (4 x 100 ml.) to yield an additional 0.7 g. The combined crude solid was recrystallized from a mixture of methanol-chloroform to yield 6.8 g. of pale yellow crystals (62%), m.p. 246° (dec.). I.R. 2225 cm^{-1} (-C≡N).

Anal. Calcd. for $C_8H_5ClN_4S$: C, 42.79; H, 2.23; N, 24.91. Found: C, 42.81; H, 2.27; N 24.84.

4-Chloro-5-cyano-6-methylthio-7-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VI).

A mixture of one gram of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose and 500 mg. of 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (V) was thoroughly pulverized and placed in an oil bath for 10 minutes at 165°. A water aspirator vacuum was then applied for 30 minutes with continued heating. The dark melt was cooled and extracted with ethyl acetate (200 ml.) and the insoluble residue removed by filtration. The ethyl acetate solution was extracted with 5% aqueous sodium hydroxide at 0° until the aqueous layer was colorless. The organic layer was then washed with saturated sodium chloride (150 ml.) and dried over anhydrous sodium sulfate. The aqueous fractions were adjusted to *p*H 7 with acetic acid and the solid collected by filtration. The total weight of recovered base was 230 mg. (46%). The ethyl acetate was removed *in vacuo* and the dark amber syrup placed on a column (3.5 cm I.D. x 20 cm) of Woelm neutral alumina (activity 1) and eluted with equal volumes (250 ml.) of the following mixtures: chloroform/ligroine--1:4, 2:3, 3:2 and 4:1. The very strongly ultraviolet absorbing fractions were pooled and the solvent removed *in vacuo* to yield 260 mg. of nucleoside material (45% yield) as a bright yellow glass.

4-Amino-5-cyano-6-methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (VII).

Two hundred milligrams of 4-chloro-5-cyano-6-methylthio-7-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (VI) was dissolved in 50 ml. of methanolic ammonia and placed in a sealed vessel and heated at 110° for 8 hours. The cooled orange-colored solution was evaporated to dryness *in vacuo* and the amber syrup triturated for 20 hours with chloroform (150 ml.) at 0°. The solid was collected by filtration and recrystallized from a mixture of water-methanol to furnish 123 mg. (85%) yield of product as fine colorless needles melting at 225°. I.R. 2250 cm^{-1} (-C≡N).

Anal. Calcd. for $C_{13}H_{15}N_5O_4S \cdot {}^1/_2H_2O$: C, 45.20; H, 4.63; N, 20.20. Found: C, 45.34; H, 5.05; N, 20.19.

2-Amino-3,4-dicyano-1-methyl-5-methylthiopyrrole (XI).

A mixture of 2-amino-3,4-dicyano-5-thiopyrrole (63) (X, 3.0 g.), 100 ml. of 5% aqueous sodium hydroxide and 45 ml. of methyl iodide was rapidly stirred and heated at reflux for 12 hours. The excess methyl iodide was removed *in vacuo* and the tan solid was collected by filtration. The crude product was recrystallized from a mixture of 2-propanol-methanol to yield 1.4 grams of white needles. Thin layer chromatography (SilicAR 7GF, ethyl acetate/water/1-propanol [4:2:1] (upper phase)) showed two ultraviolet absorbing species to be present. Preparative layer chromatography utilizing the above system was successful in resolving the mixture and the faster moving ultraviolet absorbing band furnished 580 mg. of the desired product. Recrystallization from ethanol (anhydrous) furnished long opaque white needles melting at 241-243°.

Anal. Calcd. for $C_8H_8N_4S$: C, 49.98; H, 4.19; N, 29.15. Found: C, 49.85; H, 4.40; N, 29.03.

4-Amino-5-cyano-7-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XIV).

Method 1.

Five hundred mg. of 2-amino-3,4-dicyano-1-methyl-5-methylthiopyrrole (XI) was dissolved in 25 ml. of trimethylorthoformate and refluxed for 8 hours. The solvent was removed *in vacuo* below 40° and the white solid was covered with 25 ml. of methanolic ammonia (saturated at 0°) and allowed to stand at room temperature for 2 days. The mixture was evaporated to dryness *in vacuo* and the remaining white solid (330 mg.) washed with water and then recrystallized from a mixture of methanol-2-propanol, m.p. 237-238° (dec.). I.R. 2225 cm^{-1} (-C≡N).

Anal. Calcd. for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94. Found: C, 49.44; H, 4.29; N, 31.84.

Method 2.

One hundred milligrams of 4-chloro-5-cyano-7-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XVI) was covered with 50 ml. of methanolic ammonia (saturated at 0°) and placed in a sealed vessel and heated at 100° for 12 hours. The cooled solution yielded long white needles which were collected by filtration to yield 72 mg. (79%) of the desired product. The ultraviolet absorption spectra and R_f values showed the white needles to be identical to those described for the product obtained by Method 1 (see Tables II and III and no depression on mixed melting point was observed).

4-Chloro-5-cyano-7-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XVI).

A mixture of 1.0 g. of 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]-pyrimidine (V), 200 ml. of 1 *N* aqueous sodium hydroxide and 45 ml. of methyl iodide was stirred rapidly and heated at reflux for 30 hours. The excess methyl iodide was removed *in vacuo* and the yellow-orange solid was removed by filtration. Recrystallization from ethanol furnished 470 mg. (44% yield) of yellow needles which melted at 137-138°. I.R. 2240 cm^{-1} (-C≡N).

Anal. Calcd. for $C_7H_7ClN_4S$: C, 45.16; H, 2.96; N, 23.47. Found: C, 45.33; H, 3.08; N, 23.60.

4-Amino-5-cyano-3-methyl-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XV).

Five grams of 2-amino-3,4-dicyano-5-mercaptopyrrole (X) was heated at reflux temperature for 5 hours in trimethyl orthoformate (125 ml.) and the solvent then removed *in vacuo*. The resulting tan solid was covered with 75 ml. of ethanol (anhydrous) which had been previously saturated with anhydrous methylamine and the solution was allowed to stand at room temperature for 48 hours. The product crystallized from solution as tan needles and was removed by filtration. The crude product was recrystallized from a mixture of 2-propanol-methanol to yield 4.6 g. of white needles (69% yield), m.p. 305-307°. I.R. 2200 cm^{-1} (-C≡N).

Anal. Calcd. for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94. Found: C, 49.29; H, 4.36; N, 31.92.

5-Cyano-4-methylamino-6-methylthiopyrrolo[2,3-*d*]pyrimidine (XVII).

Method 1.

One gram of 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (V) was dissolved in 100 ml. of methanol which had been previously saturated with gaseous methylamine and placed in a sealed vessel at 110°. After 8 hours the solution was cooled and the solvent removed *in vacuo*. The remaining tan solid was recrystallized from a mixture of 2-propanol-acetone to yield 780 mg. (85%) of pale yellow needles which melted at 328°. I.R. 2225 cm^{-1} (-C≡N).

Anal. Calcd. for $C_9H_9N_5S$: C, 49.30; H, 4.14; N, 31.94. Found: C, 49.09; H, 4.19; N, 32.21.

Method 2.

One gram of 4-amino-5-cyano-3-methyl-6-methylthiopyrrolo[2,3-*d*]-pyrimidine (XV) was dissolved in 100 ml. of 5% aqueous ammonium hydroxide and the solution heated at reflux temperature for two hours. The solution was neutralized to *p*H 7 with glacial acetic acid while still hot, the solution was allowed to stand at 5° for 4 hours, and the tan solid which had separated was collected by filtration. The tan solid was recrystallized from a mixture of 2-propanol-acetone to yield 700 mg. of crystals which were shown to be identical to those prepared by Method 1 by a comparison of ultraviolet absorption spectra, melting points, and R_f values in three different solvent systems.

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