Pyrrolopyrimidine Nucleosides. VI. Synthesis of 1,3 and 7-β-D-Ribofuranosylpyrrolo[2,3-*d*]pyrimidines *via* Silylated Intermediates (1a)

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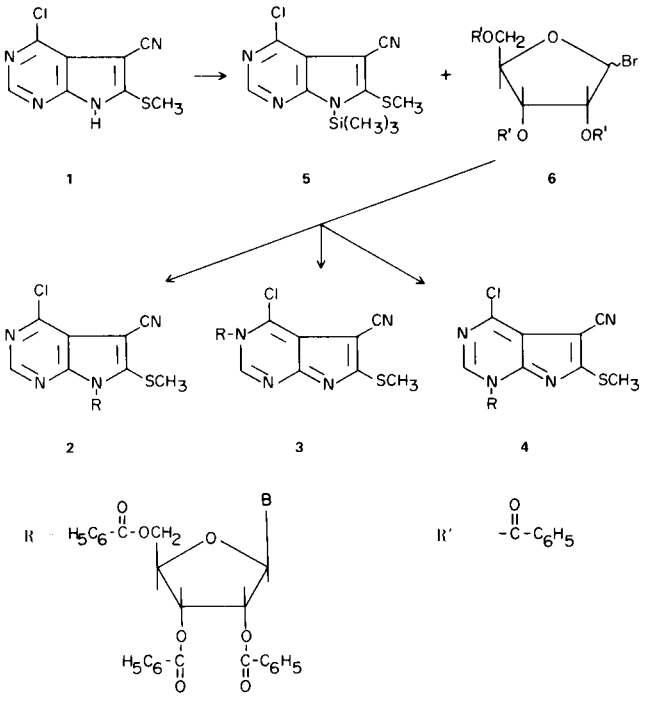
The ribosylation of several silylated pyrrolo[2,3-*d*]pyrimidines by the Wittenberg procedure has produced 1,3 and 7-ribosylpyrrolo[2,3-*d*]pyrimidine derivatives in high yield. Structure assignments have been made on the basis of the ultraviolet spectra of model compounds and further confirmed by chemical conversion to derivatives of established structure. A convenient ribosylation procedure utilizing silver oxide, a halosugar, and a silylated pyrrolo[2,3-*d*]pyrimidine derivative in acetonitrile has been described.

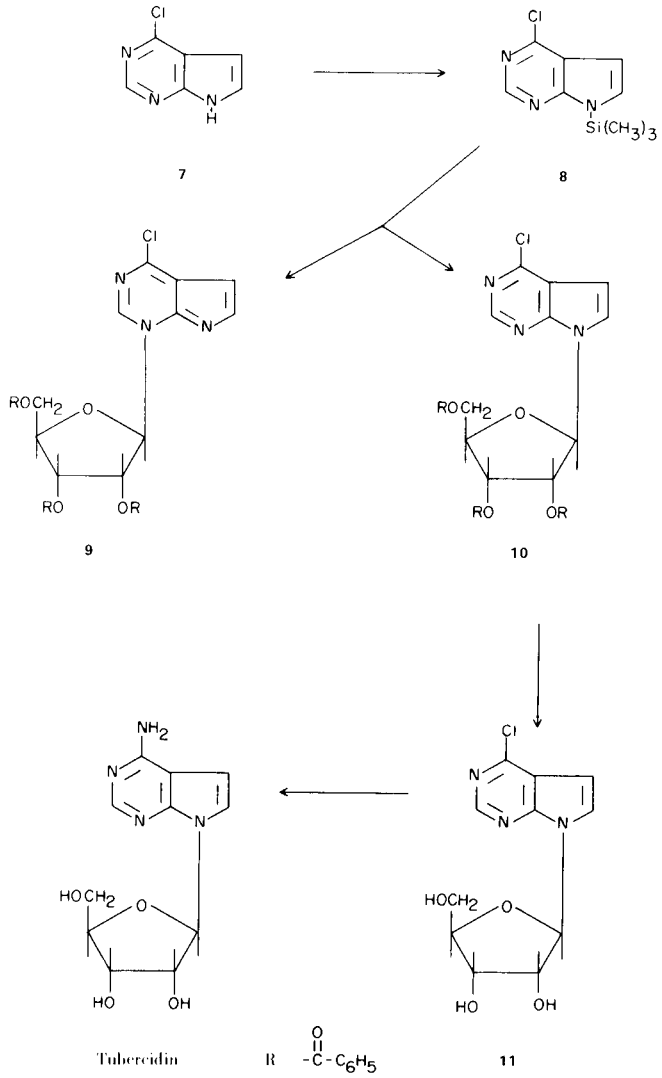
The fusion procedure for ribosylation of pyrrolo[2,3-*d*]pyrimidines has recently been applied to the synthesis of toyocamycin, sangivamycin, and tubercidin (3). However, the fusion procedure for the ribosylation of other mono-, di- and trisubstituted pyrrolo[2,3-*d*]pyrimidines has furnished poor yields of nucleoside material. Extensive decomposition of carbohydrate and aglycon derivatives also made nucleoside isolation very laborious. The search for a more efficient ribosylation procedure prompted our investigation of ribosylation reactions employing trimethylsilyl derivatives. Application of the procedure described by Wittenberg (4) to silylated pyrrolo[2,3-*d*]pyrimidines furnished 1,3 and 7-β-D-ribofuranosylpyrrolo[2,3-*d*]pyrimidines in good yields.

Silylated 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]-pyrimidine (5) (**1**) was heated in benzene at reflux temperature with mercuric oxide and 2,3,5-tri-*O*-benzoyl-D-ribofuranosyl bromide (**5**) to furnish three isomeric nucleosides which were separated by silica gel column chromatography. The ultraviolet spectra exhibited by the three nucleosides were used to tentatively assign the structures **2**, **3**, and **4**. Previous studies (5) from our laboratory have established that the site of *N*-alkylation of 4-amino-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidines could be assigned on the basis of ultraviolet spectra. Alkylation of *N*-3 or *N*-1 produced 15-20 nm and 25-30 nm bathochromic shifts, respectively, while alkylation at *N*-7 produced a small hypsochromic shift if any shift was observed at all. The first nucleoside (**2**) eluted from the column exhibited λ max (ethanol), 307 nm, which was a 5 nm hypsochromic shift relative to the ultraviolet maximum observed for **1**. On the basis of the aforementioned trend, the nucleoside **2** was assigned the structure 4-chloro-5-cyano-6-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine. The second nucleoside (**3**) eluted from the column showed λ max (ethanol), 319 nm (a 7 nm bathochromic shift relative to the observed uv maximum for **1**) and was therefore 4-chloro-5-cyano-6-methylthio-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine. The third nucleoside eluted from the column exhibited an ultraviolet maximum at λ max (ethanol), 331, which was a 19 nm bathochromic shift relative to **1**, and on this basis was assigned the structure 4-chloro-5-cyano-6-methylthio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**). Examination of the pmr spectra of the 7- and 3-ribosyl isomers

REACTION SCHEME I



REACTION SCHEME II



(**2** and **3**) revealed singlets at δ 6.52 and δ 6.64, respectively, which were attributed to the anomeric protons while the pmr spectrum of the 1-ribosyl isomer (**4**) exhibited a doublet at δ 6.31 ($J_{1',2'}$ = 1.0 Hz). On the basis of these data, **2**, **3** and **4** were assigned the *beta* configuration since it is generally accepted that a definite assignment of anomeric configuration can be made when the coupling constant for neighboring *trans*-hydrogens is less than or equal to 1.0 Hz (5).

Similarly, ribosylation of silylated 4-chloropyrrolo[2,3-*d*]pyrimidine (7) (**8**) by an analogous procedure furnished two nucleosides which were successfully separated by silica gel column chromatography. The structure of the major product (18%) was established as 4-chloro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**10**) on the basis of its ultraviolet spectrum (λ max (ethanol) 274 nm) as compared to the ultraviolet spectrum of the aglycon (**7**) (λ max (ethanol), 273 nm). The isomeric nucleoside (11%) was assigned the structure 4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**9**) since the ultraviolet spectrum exhibited a 25 nm bathochromic shift (λ max (ethanol) 298 nm) relative to the aglycon (**7**).

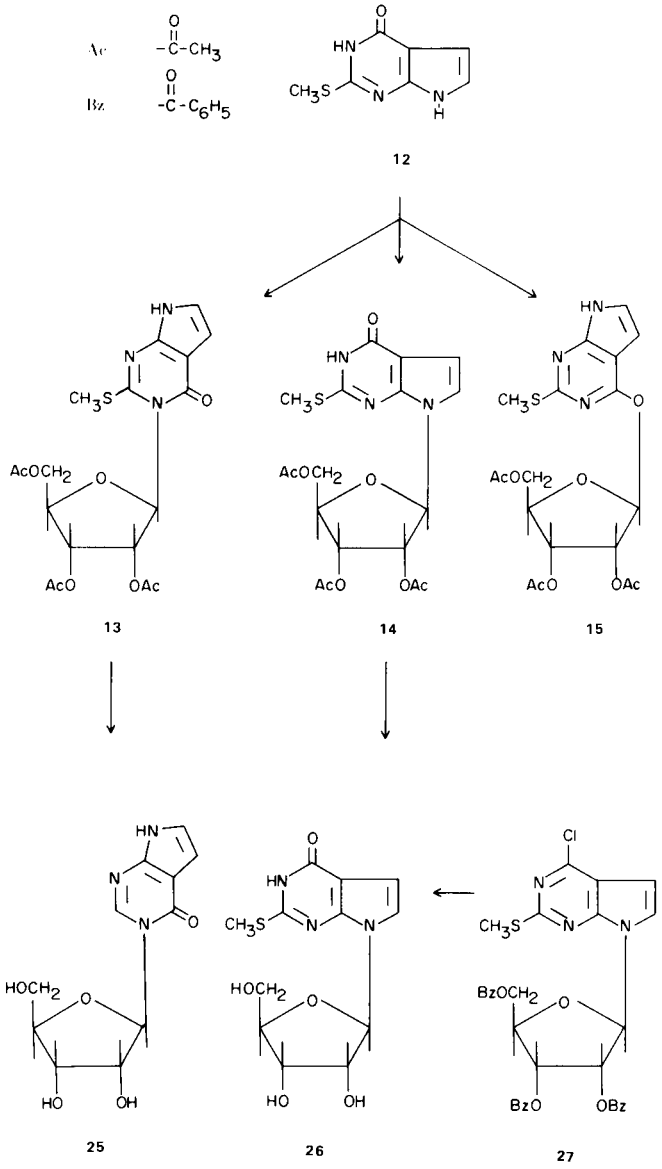
The configuration of the *N*-7 isomer was established as *beta* by treatment of **10** with sodium methoxide in methanol which furnished **11**. A rigorous comparison of **11** with an authentic sample of 4-chloro-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (8), whose anomeric configuration has been definitely established as *beta*, proved them to be identical. The anomeric configuration of the *N*-1 isomer (**9**) was presumed to be *beta* on the basis of the *trans* rule.

Silylated 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**12**) (λ max (ethanol) 271 nm) (9) was ribosylated by the above procedure to furnish three nucleosides which were isolated in yields of 8%, 64% and 7%. These nucleosides were subsequently assigned the structures **13**, **14**, and **15**; however, an initial inspection of the ultraviolet spectra revealed that the three nucleosides possessed very similar absorption maxima (**13**, λ max (ethanol), 275 nm; **14**, λ max (ethanol), 272 nm; **15**, λ max (ethanol), 281 nm).

In order to confirm the tentatively assigned nucleoside structures, the synthesis of all the methylated 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidones was initiated. The actual site of ribosylation was then assigned by uv comparison with the model methyl compounds.

Nucleophilic displacement of chloride from 4-chloro-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**16**) by methoxide in methanol furnished 4-methoxy-2-methylthiopyrrolo-[2,3-*d*]pyrimidine (**17**) in 95% yield (λ max (ethanol), 280 nm). A comparison of the ultraviolet spectra of **17** and the nucleoside (**15**) obtained in 7% yield proved them to be very similar. The nucleoside (**15**) was found to give a positive Fehlings Test, characteristic of an *O*-glycoside (10,11). Inspection of the infrared spectrum of 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (8) (7-deazainosine) revealed a carbonyl stretching absorption at 6.02 microns which was attributed to the keto group at position 4. The ir spectrum of 7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone exhibited two separate and distinct carbonyl absorptions at 5.72 and 6.02 microns (the absorption at 5.72 microns was assigned to the acetyl groups on the carbohydrate portion of the molecule). Examination of the infrared spectrum of the nucleoside (**15**) revealed a single carbonyl absorption at 5.72 microns (acetyl groups). The lack of a carbonyl absorption at 6 microns lends further support for the structural assignment of this nucleoside as an *O*-riboside. The anomeric configuration was assigned as *beta* on the

REACTION SCHEME III



basis of a singlet at δ 6.82 (anomeric proton) observed in the pmr spectrum. On the basis of these data, the structure of the nucleoside (7% yield) was assigned as 2-methylthio-4-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyloxy)pyrrolo[2,3-*d*]pyrimidine (**15**). Attempts to rearrange the *O*-riboside to a *N*-riboside with mercuric bromide in acetonitrile (12) at reflux temperature were unsuccessful.

Methylation of 4-chloro-2-methylthiopyrrolo[2,3-*d*]-pyrimidine (**16**) with methyl iodide in the presence of excess aqueous sodium hydroxide afforded 4-chloro-7-methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**18**) in quantitative yield. The site of methylation was assigned as *N*-7 since the ultraviolet spectrum of the methyl derivative **18** was almost identical to the ultraviolet spectrum of the starting material, 4-chloro-2-methylthiopyrrolo[2,3-*d*]-pyrimidine (**16**). Treatment of **18** with sodium hydroxide in a sealed vessel at 100° furnished 7-methyl-2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**19**) in good yield (λ max (ethanol), 272 nm).

Ring closure of 4-amino-1-methyl-2-methylthio-6-pyrimidone (**20**) (13) with 30% chloroacetaldehyde furnished 3-methyl-2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**21**) (λ max (ethanol), 276 nm). The only remaining *N*-methyl isomer of **12** was prepared in two steps from 6-amino-1-methyl-2-thio-4-pyrimidone (**22**) (14). Methylation of **22** with methyl iodide in excess aqueous base furnished 6-amino-1-methyl-2-methylthio-4-pyrimidone (**23**) in excellent yield. Ring closure of **23** was effected with 30% chloroacetaldehyde to produce 1-methyl-2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**24**) (λ max (ethanol), 285 nm). A comparison of the ultraviolet spectra of the three *N*-methyl derivatives of **12** with the spectra of the two as yet uncharacterized nucleosides, eliminated *N*-1 as a site of ribosylation for either of the nucleosides. Therefore, the ribosyl moiety for these two nucleosides must reside at *N*-3 and *N*-7. However, the ultraviolet maxima for the *N*-3 (**21**) and *N*-7 methyl (**19**) derivatives were not sufficiently different so as to permit unequivocal differentiation of the nucleoside derivatives.

This prompted a chemical proof of structure which would allow an absolute structural assignment for these nucleosides. Deacetylation and dethiation of **13** furnished nucleoside material which was shown by thin layer chromatography in four solvent systems to be different from 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (7-deazainosine), prepared by unequivocal synthesis (15). The anomeric configuration of the original nucleoside (8% yield) was unequivocally established as *beta* on the basis of a singlet (δ 6.77) observed in the pmr spectrum which was attributed to the anomeric proton. Thus the structure of the nucleoside (8%) was assigned as 2-methylthio-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**13**). The structure of the corresponding dethiated product was thereby established as 3-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]-4-pyrimidone (**25**, 1-ribosyl-7-deazahypoxanthine).

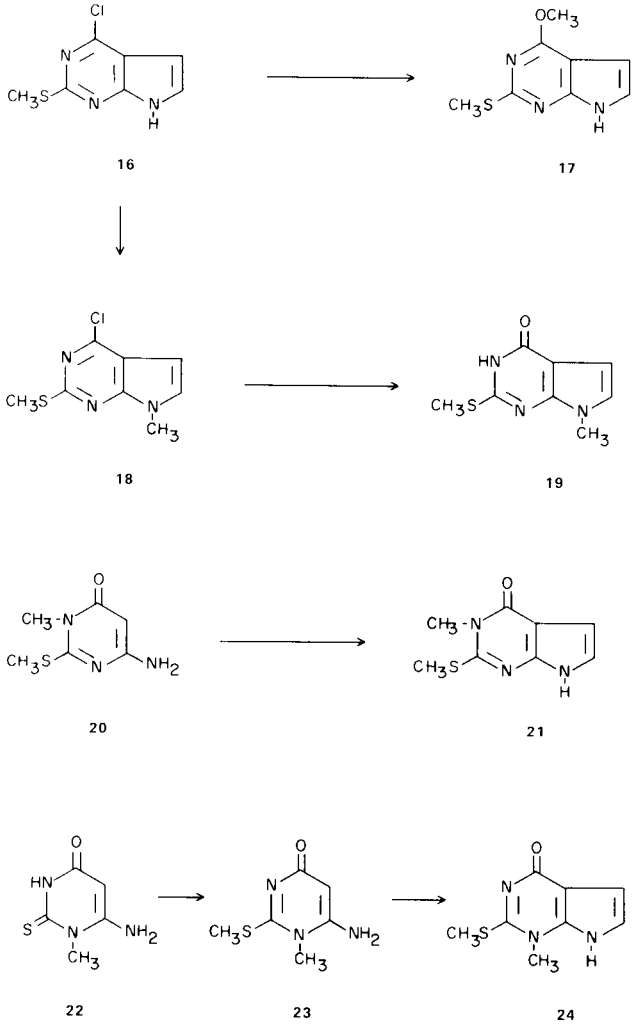
Our attempts to dethiate the remaining nucleoside (64% yield) under a variety of conditions were unsuccessful. Treatment of this nucleoside with sodium methoxide in methanol furnished a *N*-ribosyl derivative of **12**, which at this point was presumed to be the *N*-7 isomer **26**.

4-Chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**27**) was debenzoylated with a large excess of sodium methoxide in a small amount of methanol. Water was then added to the reaction mixture and the mixture heated in a sealed tube at 110° for 18 hours. Elemental analysis and spectral exam-

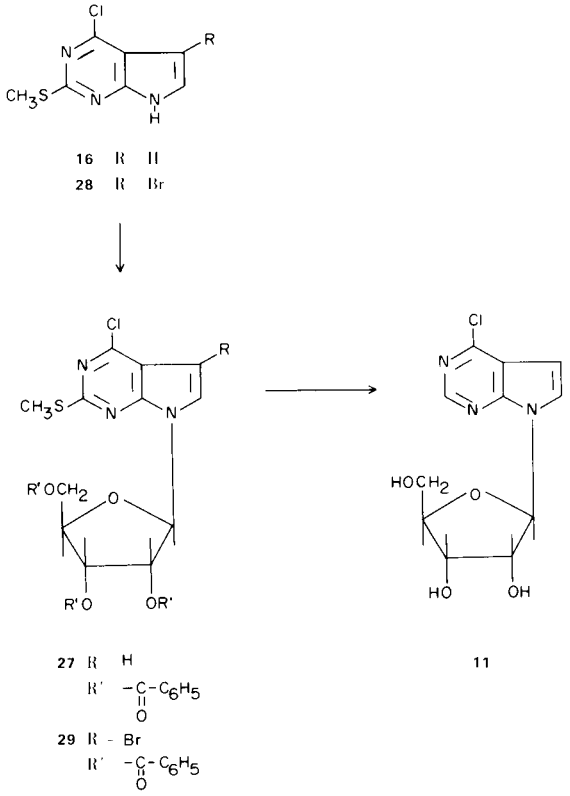
ination of the product which was isolated indicated that nucleophilic displacement of chloride by hydroxide had occurred to furnish 2-methylthio-7-(β-D-ribofuranosyl)-pyrrolo[2,3-*d*]-4-pyrimidone (**26**). A rigorous spectral and chromatographic comparison of the nucleophilic displacement product with the nucleoside prepared by deacetylation of the original nucleoside (64% yield) showed them to be identical. Therefore, the structure of the original nucleoside (64%) was unequivocally assigned as 2-methylthio-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]-4-pyrimidone (**14**).

Thus we have demonstrated that the ultraviolet trend previously described (5) is consistent for the *N*-methyl isomers of 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone, although the bathochromic shifts are not as pronounced as those observed in other pyrrolopyrimidines. Methylation at *N*-7 or **12** produced a 1 nm bathochromic shift from the ultraviolet maximum observed for the starting material, 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**12**), while methylation at *N*-3 and *N*-1 produced 5 nm and 14 nm bathochromic shifts, respectively.

REACTION SCHEME IV



REACTION SCHEME V



The above procedure for ribosylation which requires mercuric oxide in a non-polar solvent, is a modification of the Koenigs-Knorr reaction for the preparation of aliphatic and aromatic glycosides. Since silver salts have been utilized with excellent results in the Koenigs-Knorr synthesis (16), the application of similar conditions was investigated in the anticipation that higher yields of nucleoside material might result. Ribosylation of 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**12**) in acetonitrile in the presence of one equivalent of silver oxide furnished a 95% yield of nucleoside material. Column chromatography of the reaction mixture resolved three nucleosides which were identified by comparison with authentic samples as **13**, **14** and **15** in 11%, 7% and 78% yields, respectively.

Ribosylation of silylated 5-bromo-4-chloro-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**28**) in the presence of one equivalent of silver oxide in acetonitrile furnished a 48% yield of nucleoside material. The nucleoside exhibited a singlet at δ 6.72 in the pmr spectrum which was attributed to the anomeric proton and established the anomeric configuration as *beta*. A comparison of the ultraviolet spectra of the nucleoside and the aglycon (**28**) revealed no

appreciable shift in ultraviolet absorption maximum. This established the site of ribosylation as *N*-7 and the complete structural assignment of the nucleoside as 5-bromo-4-chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranpyrimidine (**29**).

Ribosylation of 4-chloro-2-methylthiopyrrolo[2,3-*d*]-pyrimidine (**16**) by a similar procedure furnished an 82% yield of a single product. The assignment of *N*-7 as the site of ribosylation was made on the basis of the ultraviolet spectrum which showed no appreciable change from the spectrum of the aglycon (**16**) other than the addition of a peak at short wavelength due to the benzoate groups. The anomeric configuration of the nucleoside was unequivocally established as *beta* and the site of ribosylation was further confirmed as *N*-7 by debenzoylation of the nucleoside with sodium methoxide in methanol and the addition of Raney nickel to the reaction mixture. The dethiated product was isolated in low yield and identified as 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**11**) by a rigorous comparison with an authentic sample (8). This established the structure of the original nucleoside (82% yield) as 4-chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**27**).

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover unimelt and are uncorrected. Infrared, ultraviolet, and nuclear magnetic resonance spectra were recorded on a Beckmann IR-5A spectrometer, Cary 14 ultraviolet spectrometer, and a Varian A-60 high resolution spectrometer, respectively. Tetramethylsilane was utilized as an internal standard in the nuclear magnetic resonance spectrometer.

4-Chloro-5-cyano-6-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**2**), 4-Chloro-5-cyano-6-methylthio-3-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**3**), and 4-Chloro-5-cyano-6-methylthio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**4**).

Silylated (17) 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]-pyrimidine [prepared from 0.5 g. of 4-chloro-5-cyano-6-methylthiopyrrolo[2,3-*d*]pyrimidine (**1**)] was dissolved in 40 ml. of anhydrous benzene and then added to a suspension of mercuric oxide (0.5 g.) in 30 ml. of benzene, which had been dried by the distillation of 10 ml. of benzene from the mixture. Tri-*O*-benzoyl-D-ribofuranosyl bromide (prepared from 1.2 g. of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribofuranose) dissolved in 40 ml. of anhydrous benzene was added to the reaction mixture. The suspension was stirred at reflux temperature and protected from moisture for 18 hours. The reaction mixture was cooled and the mercuric salts were filtered and washed well with chloroform. Methanol (25 ml.) and water (10 ml.) were added to the filtrate and washings and the mixture was warmed on a steam bath for one hour. The solvent was then removed *in vacuo* and the residue was extracted with chloroform until the extracts were colorless. The volume of the combined extracts was reduced to *ca.* 20 ml. and applied to a silica gel column (100 g. of Baker Silica Gel packed in chloroform). Elution with chloroform-methanol (49:1) resolved the nucleoside components. Three nucleoside bands were collected (determined by thin layer chromatography of individual column fractions).

The first nucleoside to be eluted was obtained as a colorless syrup after removal of the eluent. The nucleoside could be obtained in a solid form by adding a concentrated chloroform solution of the nucleoside dropwise to 750 ml. of rapidly stirred *n*-pentane. The resultant white solid was collected by filtration (250 mg., 17%). This nucleoside was established as the *N*-7 isomer on the basis of the uv spectrum (λ max (ethanol), 307 nm).

Anal. Calcd. for $C_{34}H_{25}ClN_4O_7S$: C, 61.03; H, 3.77; N, 8.37. Found: C, 61.00; H, 3.78; N, 8.11.

The second nucleoside to be eluted from the column was isolated as a hard pale yellow foam (31% yield) and was characterized as the *N*-3 isomer (λ max (ethanol) 319 nm).

Anal. Calcd. for $C_{34}H_{25}ClN_4O_7S$: C, 61.03; H, 3.77; N, 8.37. Found: C, 60.78; H, 3.73; N, 8.40.

The third nucleoside eluted from the column was isolated as a bright yellow syrup, which slowly crystallized from cold methanol to furnish yellow crystals (685 mg., 46%), m.p. 198°. The nucleoside was established as the *N*-1 isomer on the basis of the uv spectrum (λ max (ethanol), 331 nm).

Anal. Calcd. for $C_{34}H_{25}ClN_4O_7S$: C, 61.03; H, 3.77; N, 8.37. Found: C, 61.32; H, 3.88; N, 8.15.

4-Chloro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (**10**) and 4-Chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9**).

The preceding procedure was utilized with the following reactants: trimethylsilyl derivative of 0.5 g. of 4-chloropyrrolo[2,3-*d*]-pyrimidine in 40 ml. of benzene, mercuric oxide (750 mg.) in 30 ml. of dried benzene, and tri-*O*-benzoyl-D-ribofuranosyl bromide (from 1.75 g. of the 1-*O*-acetyl derivative) in 40 ml. of benzene. The volume of the combined chloroform extracts was reduced to *ca.* 20 ml. and the concentrated solution applied to a silica gel column (100 g. Baker Silica Gel packed in chloroform). Elution with chloroform-methanol (49:1) furnished three different uv absorbing fractions. Homogeneous fractions, established by TLC to contain the same uv absorbing material, were pooled and the solvents were removed *in vacuo*. The third component eluted from the column was shown by TLC to be starting material, 4-chloropyrrolo[2,3-*d*]pyrimidine (**7**). The first compound eluted from the column was obtained as a colorless foam (λ max (ethanol), 273 nm). Precipitation from *n*-pentane furnished a white amorphous powder (230 mg., 18%) which was assigned the structure 4-chloro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]pyrimidine (**10**) on the basis of the uv spectrum.

Anal. Calcd. for $C_{32}H_{24}ClN_3O_7$: C, 64.27; H, 4.05; N, 7.03. Found: C, 64.30; H, 4.13; N, 6.87.

The second nucleoside was isolated as a pale yellow foam which could be crystallized from warm ethanol (190 mg., 11%). The structure was assigned as 4-chloro-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**9**) on the basis of uv spectrum (λ max (ethanol), 298 nm).

Anal. Calcd. for $C_{32}H_{24}ClN_3O_7$: C, 64.27; H, 4.05; N, 7.03. Found: C, 64.51; H, 4.26; N, 7.20.

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

Method 1.

4-Chloro-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (0.5 g.) (**10**) was dissolved in absolute methanol (25 ml.) and 100 mg. of sodium methoxide was then added. The solution was protected from moisture and heated on a steam bath for 30 minutes. After standing for 24 hours at room temperature,

the methoxide solution was neutralized by the addition of IRC-50 resin. The resin was removed by filtration and the neutral filtrate was evaporated to dryness *in vacuo*. The successive evaporation of three 50 ml. portions of ethanol *in vacuo* caused the light amber syrup to harden. Recrystallization from aqueous methanol furnished 155 mg. of a white solid (68%), m.p. 168°. Rigorous chromatographic and spectral comparison of the product with an authentic sample of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-pyrimidine (8) showed them to be identical. A mixture melting point showed no depression.

Method 2.

4-Chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (0.5 g.) (**27**) was dissolved in absolute methanol (25 ml.) and 100 mg. of sodium methoxide was added. The solution was protected from moisture and heated on a steam bath for 30 minutes. The reaction mixture was then allowed to stand at room temperature for 24 hours before 15% aqueous ammonia (75 ml.) and 2.0 g. of W_4 Raney nickel (wet weight) were added. The mixture was heated at reflux temperature for one hour and then another 2.0 g. of Raney nickel was added. After a total reaction time of three hours, TLC showed one major spot to be present. The nickel catalyst was filtered and washed with hot 50% aqueous ethanol (2 x 100 ml.). Evaporation of the filtrate and washings to dryness *in vacuo*, followed by the successive evaporation of two 75 ml. portions of absolute ethanol, furnished a hard gum. Two recrystallizations from aqueous methanol afforded 58 mg. (21%) of a white solid, m.p. 167°. A rigorous comparison of the product (including mixture melting point) with the product prepared by Method 1 and the authentic sample of 4-chloro-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine, showed them to be identical in every respect.

2-Methylthio-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**13**), 2-Methylthio-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**14**) and 2-Methylthio-4-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyloxy)pyrrolo[2,3-*d*]pyrimidine (**15**).

Method 1.

The same ribosylation procedure was used as outlined above with the following reactants: Trimethylsilyl derivative of 2.0 g. of 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone in 40 ml. of benzene, mercuric oxide (2.5 g.) and mercuric bromide (4.0 g.) in 40 ml. of benzene, and tri-*O*-acetyl-D-ribofuranosyl bromide (from 3.6 g. of tetra-*O*-acetyl-β-D-ribofuranose) in 40 ml. of anhydrous benzene. The extracts were combined and the volume was reduced to 10-15 ml. before being placed on a silica gel column. Resolution of three nucleoside bands was accomplished in an identical manner to that of Method 2.

The *O*-riboside (**15**, 7% yield) was shown by thin layer chromatography (4 systems) to be identical to 2-methylthio-4-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyloxy)pyrrolo[2,3-*d*]pyrimidine prepared by Method 2.

The second nucleoside band was identified as 2-methylthio-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**14**, 64% yield) by spectral and chromatographic comparison with the 7-ribosyl isomer prepared by Method 2.

The third nucleoside (**13**, 8% yield) was shown to be identical to the 3-ribosyl isomer prepared by Method 2 by means of rigorous spectral chromatographic comparisons.

Method 2.

Tri-*O*-acetyl-D-ribofuranosyl bromide [prepared from 2.6 g. of tetra-*O*-acetyl-β-D-ribofuranose] was dissolved in 50 ml. of anhydrous acetonitrile and this solution was added to 1.28 g. of silver oxide and silylated 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone [prepared from 2.0 g. of 2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**12**)]. The flask was stoppered and the solution stirred for 48 hours at room temperature in the dark. The salts were collected by filtration and washed with chloroform. Methanol (25 ml.) and water (10 ml.) were added and the mixture warmed on a steam bath for one hour. Evaporation of the solvent *in vacuo* furnished an amber syrup which was extracted (3 times) with chloroform until the chloroform extracts were colorless. The extracts were combined and the volume was reduced to 10-15 ml. The concentrated solution was then placed on a silica gel column (100 g. Baker Silica Gel packed in chloroform). Elution with chloroform/-methanol (49:1) resolved three nucleoside bands.

The first nucleoside (**15**) eluted from the column was initially isolated as a colorless foam and was subsequently precipitated by addition of a concentrated chloroform solution to a large volume of *n*-pentane (76% yield). A concentrated methanolic solution of the nucleoside crystallized after standing several weeks at 5°. The nucleoside was identified as 2-methylthio-4-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyloxy)pyrrolo[2,3-*d*]pyrimidine (**15**) after examination of pmr, uv, and ir spectra and comparison with model compounds. The nucleoside gave a positive Fehlings test.

Anal. Calcd. for $C_{18}H_{21}N_3O_8S$: C, 49.20; H, 4.82; N, 9.56. Found: C, 49.45; H, 4.96; N, 9.40.

The second nucleoside (**14**) eluted from the column was isolated as a honey colored syrup (7% yield) which could not be crystallized. Uv, ir, and pmr spectral analysis and other chemical proof allowed the structure of the nucleoside to be assigned as 2-methylthio-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**14**).

Anal. Calcd. for $C_{18}H_{21}N_3O_8S$: C, 49.20; H, 4.82; N, 9.56. Found: C, 49.13; H, 4.73; N, 9.75.

The third nucleoside (**13**) eluted from the column was isolated as an amber syrup (11% yield) which was not crystallized. Subsequent investigation showed the nucleoside (**13**) to be the 3-ribosyl isomer, 2-methylthio-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**13**).

Anal. Calcd. for $C_{18}H_{21}N_3O_8S$: C, 49.20; H, 4.82; N, 9.56. Found: C, 48.95; H, 4.54; N, 9.75.

4-Methoxy-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**17**).

Six hundred milligrams of 4-chloro-2-methylthiopyrrolo[2,3-*d*]-pyrimidine (9) (**16**) was suspended in 100 ml. of absolute methanol containing 375 mg. (2.3 equiv.) of sodium methoxide. The mixture was heated at reflux for 90 hours protected from moisture, and the pale yellow solution was then neutralized to *p*H 7 by the addition of Dowex 50W-X2. The resin was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. The off-white solid was recrystallized from aqueous methanol to afford a 95% yield (2.8 g.) of fine colorless crystals, m.p. 195-196°.

Anal. Calcd. for $C_8H_9N_3OS$: C, 49.22; H, 4.65; N, 21.52. Found: C, 49.00; H, 4.61; N, 21.22.

4-Chloro-7-methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**18**).

One gram of 4-chloro-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**16**) was suspended in 50 ml. of 1 *N* sodium hydroxide and 10 ml. of methyl iodide was added. The mixture was heated at reflux on a steam bath for 24 hours and the remaining methyl iodide was then removed by distillation. The residue in the flask was extracted with chloroform (2 x 50 ml.) and the aqueous layer was discarded. The chloroform layer was washed with water until neutral and

dried over sodium sulfate. The solvent was removed *in vacuo* and the solid was recrystallized from methanol to afford a quantitative yield of colorless crystals, m.p. 132°.

Anal. Calcd. for $C_8H_8ClN_3S$: C, 44.97; H, 3.77; N, 19.67. Found: C, 45.08; H, 4.08; N, 19.49.

7-Methyl-2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**19**).

Five hundred milligrams of 4-chloro-7-methyl-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**18**) was covered with 100 ml. of 1 *N* sodium hydroxide and then heated in a sealed tube for 14 hours at 100°. The resultant orange solution was filtered and the filtrate neutralized to *p*H 6 with glacial acetic acid. The resultant tan precipitate was filtered as soon as the mixture had cooled. Recrystallization from methanol afforded 340 mg. (74%) of a white solid which was shown to be homogeneous by thin layer chromatography, m.p. 265-267°.

Anal. Calcd. for $C_8H_9N_3OS$: C, 49.22; H, 4.65; N, 21.52. Found: C, 48.97; H, 4.89; N, 21.35.

3-Methyl-2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**21**).

Two grams of 4-amino-1-methyl-2-methylthio-6-pyrimidone (**20**) was suspended in 100 ml. of water containing 30 g. of sodium acetate. The slurry was heated to 80° before 3.6 g. of 30% chloroacetaldehyde was added dropwise over a period of one hour. After an additional 10 minutes of heating, the solid was filtered and washed with 100 ml. of hot water. Extraction of the resultant solid with boiling water (100 ml.) and filtration afforded 1.25 g. of an off-white solid which was shown to be homogeneous by thin layer chromatography. The analytical sample was recrystallized from ethanol and melted at 275°.

Anal. Calcd. for $C_8H_9N_3OS$: C, 49.22; H, 4.65; N, 21.52. Found: C, 49.36; H, 4.90; N, 21.43.

6-Amino-1-methyl-2-methylthio-4-pyrimidone (**23**).

6-Amino-1-methyl-2-thio-4-pyrimidone (**22**, 7.5 g.) was added to 100 ml. of water containing 4.0 g. of sodium hydroxide. As soon as solution was achieved, 15 ml. of methyl iodide was added and the mixture was heated at reflux on a steam bath for three hours. The remaining methyl iodide was removed by distillation and the solution allowed to stand at 5° for 18 hours. The colorless needles (7.2 g.) which had separated from solution were collected by filtration and washed with acetone (88% yield). Recrystallization from water containing a small amount of methanol furnished the analytical sample, m.p. 286°.

Anal. Calcd. for $C_6H_9N_3OS$: C, 42.09; H, 5.30; N, 24.54. Found: C, 42.41; H, 5.34; N, 24.57.

1-Methyl-2-methylthiopyrrolo[2,3-*d*]-4-pyrimidone (**24**).

One gram of 6-amino-1-methyl-2-methylthio-4-pyrimidone (**23**) and 1.5 g. of sodium acetate were dissolved in 50 ml. of water. The temperature of the solution was maintained at 45° in an oil bath before 1.7 g. of 30% chloroacetaldehyde was added. After 20 hours another 1.7 g. of 30% chloroacetaldehyde was added and the temperature was maintained at 45° for another 70 hours. The solvent was evaporated to dryness *in vacuo* and the residue was extracted with absolute methanol (50 ml.). The inorganic salts were filtered, washed with methanol (10 ml.), and the salts were discarded. The volume of the methanol extract was reduced until a solid began to separate (10-15 ml.) and the concentrated solution was then placed on a silica gel column (50 g. packed in chloroform). Elution with methanol-acetone (1:3) resolved the remaining starting material and the product. The fractions containing ring-closed product (determined by uv spectrum) were pooled and evaporated to dryness *in vacuo*. Recrystallization from aqueous acetone furnished an off-white solid (240 mg., 21% yield), m.p. 192-196° dec.

Anal. Calcd. for $C_8H_9N_3OS$: C, 49.22; H, 4.65; N, 21.52. Found: C, 49.32; H, 4.71; N, 21.30.

3-(β-D-Ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**25**).

To 100 mg. of sodium methoxide in 10 ml. of absolute methanol was added a solution of 250 mg. of 2-methylthio-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**13**) in 10 ml. of absolute methanol. After warming for two hours on a steam bath, 60 ml. of water, 20 ml. of concentrated ammonium hydroxide, and 2 g. of wet commercial W_4 Raney nickel were added. The mixture was stirred at reflux temperature for 2 hours before the nickel catalyst was filtered and washed with 100 ml. of hot 5% aqueous ethanol. The filtrate volume was reduced to 50 ml. *in vacuo* and the remaining base was neutralized with excess Dowex 50W-X2. The resin was removed by filtration and washed with methanol. The filtrate and washings were combined and evaporated to dryness *in vacuo* and the resultant translucent syrup was recrystallized from water to furnish a white solid, m.p. 208-210° dec., (63%). The nonidentity of the product to 7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone was established by means of tlc in four solvent systems.

Anal. Calcd. for $C_{11}H_{13}N_3O_5$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.18; H, 4.80; N, 15.41.

2-Methylthio-7-(β-D-ribofuranosyl)pyrrolo[2,3-*d*]-4-pyrimidone (**26**).

Method 1.

2-Methylthio-7-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)pyrrolo-[2,3-*d*]-4-pyrimidone (**14**, 250 mg.) was dissolved in 20 ml. of absolute methanol and 50 mg. of sodium methoxide was added. The mixture was protected from moisture and warmed on a steam bath for one hour and then allowed to stand at room temperature for 24 hours. The solution was adjusted to *p*H 7 with Dowex 50W-X2 and the resin was filtered and washed with methanol. The solvent was evaporated *in vacuo* to furnish an amber syrup. Successive evaporation of three 30 ml. portions of ethanol caused the syrup to harden. Recrystallization from aqueous ethanol furnished an off-white solid in 65% yield.

Anal. Calcd. for $C_{12}H_{15}N_3O_5S$: C, 46.00; H, 4.83; N, 13.41. Found: C, 45.79; H, 4.84; N, 13.55.

Method 2.

Four hundred milligrams of 4-chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**27**) was dissolved in 20 ml. of absolute methanol and 1.0 g. of sodium methoxide was added. The mixture was heated with the exclusion of moisture on a steam bath for one hour. After standing another 12 hours at room temperature the solvent was removed *in vacuo* and the residue was covered with 100 ml. of water and heated in a sealed vessel at 110° for 18 hours. The cooled yellow solution was adjusted to *p*H 7 with Dowex 50W-X2 and the resin was filtered and washed with methanol. The filtrate was evaporated to dryness *in vacuo*. Successive evaporation of four 50 ml. portions of 2-propanol furnished a hard gum which was recrystallized twice from aqueous ethanol to afford a pure product in 28% yield. Rigorous spectral and chromatographic comparison with the product by Method 1 established that the samples were identical.

5-Bromo-4-chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**29**).

Silylated 5-bromo-4-chloro-2-methylthiopyrrolo[2,3-*d*]pyrimidine [prepared from 560 mg. of 5-bromo-4-chloro-2-methylthio-

pyrrolo[2,3-*d*]pyrimidine (6) (**28**)] was dissolved in 40 ml. of anhydrous acetonitrile and 460 mg. of silver oxide was added. Tri-*O*-benzoyl-D-ribofuranosyl bromide (prepared from 1.0 g. of 2,3,5-tri-*O*-benzoyl-1-*O*-acetyl-D-ribofuranose) which had been dissolved in 40 ml. of anhydrous acetonitrile was added to the reaction mixture. The flask was stoppered and the solution stirred at 25° in the dark for three days. Filtration and thorough washings of the silver salts with chloroform furnished an amber solution. Methanol (25 ml.) and water (10 ml.) were added and the resultant solution was warmed on a steam bath for one hour before the solvent was removed *in vacuo*. The residue was extracted with chloroform until the extracts were colorless. The volume of the combined extracts was reduced to *ca.* 20 ml. and the concentrated solution was added to a silica gel column (100 ml. Baker Silica Gel packed in chloroform). Elution with chloroform-methanol (49:1) resolved the nucleoside band. The uv absorbing fractions were collected and the fractions containing uv absorbing species with the same Rf were pooled. One nucleoside band was followed closely by a second band which was identified as starting material by thin layer chromatography. The pooled nucleoside fractions were evaporated to dryness *in vacuo* and the syrup precipitated by the dropwise addition of a chloroform solution to 750 ml. of rapidly stirred *n*-pentane. The pale yellow solid was collected by filtration (700 mg., 48%). The site of ribosylation was assigned as *N*-7 on the basis of the ultraviolet spectrum (λ max (ethanol), 259 nm).

Anal. Calcd. for $C_{33}H_{25}BrClN_3O_7S$: C, 54.77; H, 3.46; N, 5.81. Found: C, 55.01; H, 3.60; N, 5.59.

4-Chloro-2-methylthio-7-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-pyrrolo[2,3-*d*]pyrimidine (**27**).

The same procedure as for **29** was used with the following reagents: trimethylsilyl derivative prepared from 0.4 g. of 4-chloro-2-methylthiopyrrolo[2,3-*d*]pyrimidine (**16**) in 40 ml. of acetonitrile, silver oxide (400 mg.), and tri-*O*-benzoyl-D-ribofuranosyl bromide (prepared from 1.1 g. of 1-*O*-acetyl-tri-*O*-benzoyl-D-ribofuranose) in 40 ml. of acetonitrile. Elution of the nucleoside material was effected with chloroform-methanol (49:1). The nucleoside band was followed closely by a small amount of starting material. The nucleoside fractions were pooled and evaporated to dryness *in vacuo* to furnish a pale yellow foam. The foam was dissolved in a minimum amount of chloroform and then added dropwise to 740 ml. of *n*-pentane, which was rapidly stirred during the addition. The off-white solid was collected by filtration (1.06 g., 82%). The site of ribosylation was assigned as *N*-7 on the basis of the ultraviolet spectrum exhibited by the nucleoside (λ max (ethanol), 250 nm).

Anal. Calcd. for $C_{33}H_{26}ClN_3O_7S$: C, 61.49; H, 4.04; N, 6.52. Found: C, 61.30; H, 4.31; N, 6.25.

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