Synthesis of Some New Thieno[3,4-*d*]pyrimidines and their *C*-Nucleosides [1]

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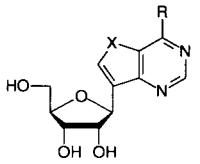
Received November 16, 1992

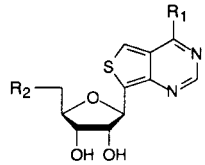
The synthesis of several new thieno[3,4-*d*]pyrimidine *C*-nucleosides **5-8** is described. The known 5-ribosylated methyl 4-(formylamino)thiophene-3-carboxylate key intermediate **20** was obtained as a mixture of anomers in significantly improved yield by condensation of the sugar **15** with methyl 4-(formylamino)thiophene-3-carboxylate **19** in nitromethane at 60° in the presence of stannic chloride. Attempts to prepare the C-7 ribosylated compound **21**β by direct condensation of the bicyclic base **10** with **15** gave instead the N-1 ribosylated nucleoside **16**. The synthesis of the corresponding and previously unknown thieno[3,4-*d*]pyrimidine bases **12** and **13** is described along with stability studies on the 4-methylthio derivative **12**. Preliminary biological studies indicate that adenosine analogue **7** is a potent growth inhibitor of several mammalian tumor cell lines.

J. Heterocyclic Chem., **30**, 509 (1993).

As part of an ongoing program on the synthesis and biological evaluation of purine-like *C*-nucleosides, we have reported the synthesis of the thieno[3,4-*d*]pyrimidine *C*-nucleoside **4** [2]. This compound is an analogue of inosine and of the antibiotic *C*-nucleoside formycin B. It is also closely related in structure to several synthetic 9-deazapurine *C*-nucleosides **1-3** prepared in our laboratory in the past, many of which have shown a wide range of important biological activities. In particular, 9-deazainosine (**2**, R = OH) has demonstrated significant activity against *Pneumocystis carinii* infections in the rat [3a-b], *in vitro* growth inhibitory activity against *Giardia lamblia* [4] and was also found to be a good reversible inhibitor of purine nucleoside phosphorylase (PNP) [5].



1: X = O, R = OH or NH_2
2: X = NH, R = OH or NH_2
3: X = S, R = OH or NH_2



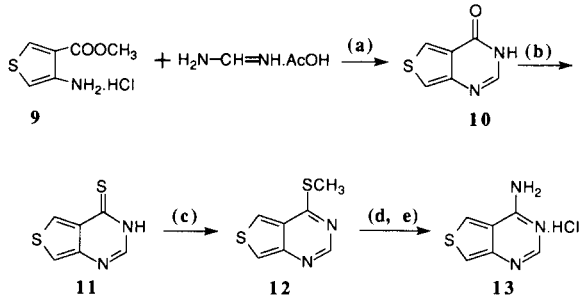
4 : R1 = OH, R_2 = OH
5 : R1 = SH, R_2 = OH
6 : R_1 = SCH_3, R_2 = OH
7 : R_1 = NH_2, R_2 = OH
8 : R_1 = OH, R_2 = I

In preliminary biological studies, **4** has also shown significant activity against *Pneumocystis carinii* in the rat [6]. In view of this observation, the synthesis of the corresponding 4-substituted derivatives **5-7** was deemed important. Derivatives **5** and **6** are of potential biomedical interest in view of the known anticancer activity of the nucleosides of 6-mercaptopurine and its methylthio derivative [7]. Compound **7**, on the other hand, represents an analogue of adenosine and of the antibiotic *C*-nucleoside formycin. We have previously reported the synthesis of 9-deazaadenosine (**2**, R = NH_2) [8], a compound which exhibits pronounced growth inhibitory activity against several murine and human tumor cell lines. The synthesis of the corresponding 5'-iodo compound **8** as a potential inhibitor of purine nucleoside phosphorylase [PNP] was also of relevance in view of our previous observation that 5'-deoxy-5'-iodo-9-deazainosine is a highly potent inhibitor of the enzyme PNP [5].

Since we envisaged the synthesis of *C*-nucleosides **5-8** to involve elaboration of the desired bicyclic moiety onto a common ribosyl precursor (*eg.* **20**β), we first investigated suitable conditions for the synthesis of the parent bicyclic heterocycles which might also be compatible with the presence of the ribosyl moiety at C-7. Thieno[3,4-*d*]pyrimidin-4(3*H*)-one **10** was first prepared in 1953 by Baker *et al* [9] by two different routes; by reaction of methyl 4-(formylamino)thiophene-3-carboxylate **19** with ammonium formate in formamide to give **10** in 50% yield or by cyclization of 4-(formylamino)thiophene-3-carboxamide with sodium methoxide to give **10** in 93% yield. During the course of our investigation, however, we found it more convenient to prepare **10** by cyclization of the known amino ester **9** [9] with formamide acetate in refluxing absolute ethanol in 79% yield (Scheme 1). While thiation of **10** with phosphorus pentasulfide in refluxing pyridine to afford **11** has been reported [10], compounds **12** and **13**, which are the heterocyclic bases of the desired *C*-nucleosides **6** and **7**, had not been reported prior to this work. *S*-Methylation of thione **11** with excess methyl iodide in 1 *N* aqueous sodium hydroxide gave **12** in 83% yield. Ammonolysis of **12** with saturated methanolic ammonia gave the corresponding 4-amino derivative which was isolated in 90% yield as its hydrochloride **13**. In the course of these studies, we found that methylthio derivative **12** exhibited unexpected properties under relatively mild acidic and basic solvolytic conditions at ambient temperature (Scheme 2). Thus, overnight treatment of a solution of **12** in methanol with 0.1 *N* aqueous hydrochloric acid or with

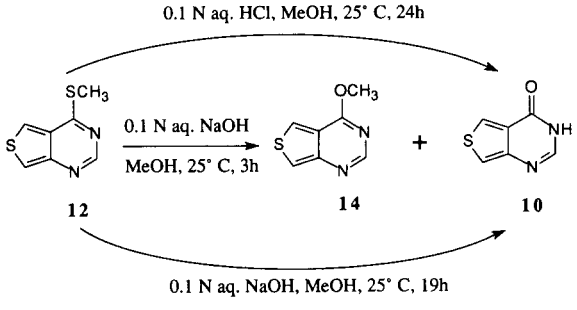
0.1 *N* aqueous sodium hydroxide affords the corresponding 4-oxo compound **10** (isolated in 61% and 54% yield respectively). When the reaction in base was allowed to proceed for only 3 hours, the corresponding 4-methoxy compound **14** could be isolated in 51% yield. Gradual conversion of **14** into **10** was detected by tlc with longer times. The structure of **14** was confirmed by ^1H and ^{13}C nmr spectroscopy with the latter showing the characteristic methoxyl resonance at δ 54.4 ppm.

Scheme 1



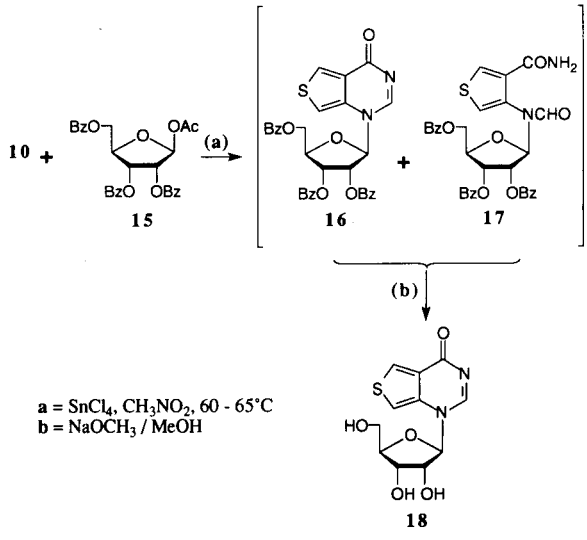
(a) NEt_3, EtOH, Reflux
(b) P_2S_5, Pyridine, Reflux
(c) CH_3I, NaOH;
(d) NH_3 / MeOH
(e) HCl / MeOH

Scheme 2



We have previously reported the synthesis of the nucleoside **21**β by *C*-glycosylation of **19** in refluxing dichloroethane in the presence of stannic chloride to give the key intermediate **20**β. This was then deformylated and annulated to give **21**β (Scheme 4) [2]. Since the direct C-7 glycosylation of the corresponding thienopyrimidine compound **10**, if successful, would represent an even shorter route to the nucleoside **4** and its derivatives, an attempt was made to apply the methodology recently described by Girgis *et al.* [11] to the synthesis of **21**β. These authors achieved the direct *C*-glycosylation of 9-deazaguanine in nitromethane in the presence of stannic chloride. Sugar **15** was therefore heated with the bicyclic base **10** in nitromethane at 60° in the presence of stannic chloride for 30 minutes. However, no *C*-nucleoside could be detected under these conditions. Instead, the formation of the *N*-nucleoside **16** [2] was observed by tlc along with another, very minor product later identified as **17** (Scheme 3). Monitoring the reaction by tlc also indicated that extending reaction times beyond 1.5 hours led to a decreased production of **16**. An attempt to isolate this compound by flash chromatography on silica gel led to its partial conversion to the more stable ring opened compound **17** to give roughly a 1:1 mixture of the two compounds. We have previously observed the susceptibility of **16** to ring opening under aqueous conditions [2]. Debenzoylation of the mixture with sodium methoxide in methanol afforded only pure **18**. This indicated that the formamido carboxamide **17** underwent ring closure during debenzoylation to give **18**. The ring closure of 4-(formylamino)thiophene-3-carboxamide with sodium methoxide is well documented [9]. The cyclization of **17** to **18** was further confirmed by conversion of an authentic sample of debenzoylated **17**, prepared by a different route [2], to **18** by treatment with sodium methoxide.

Scheme 3

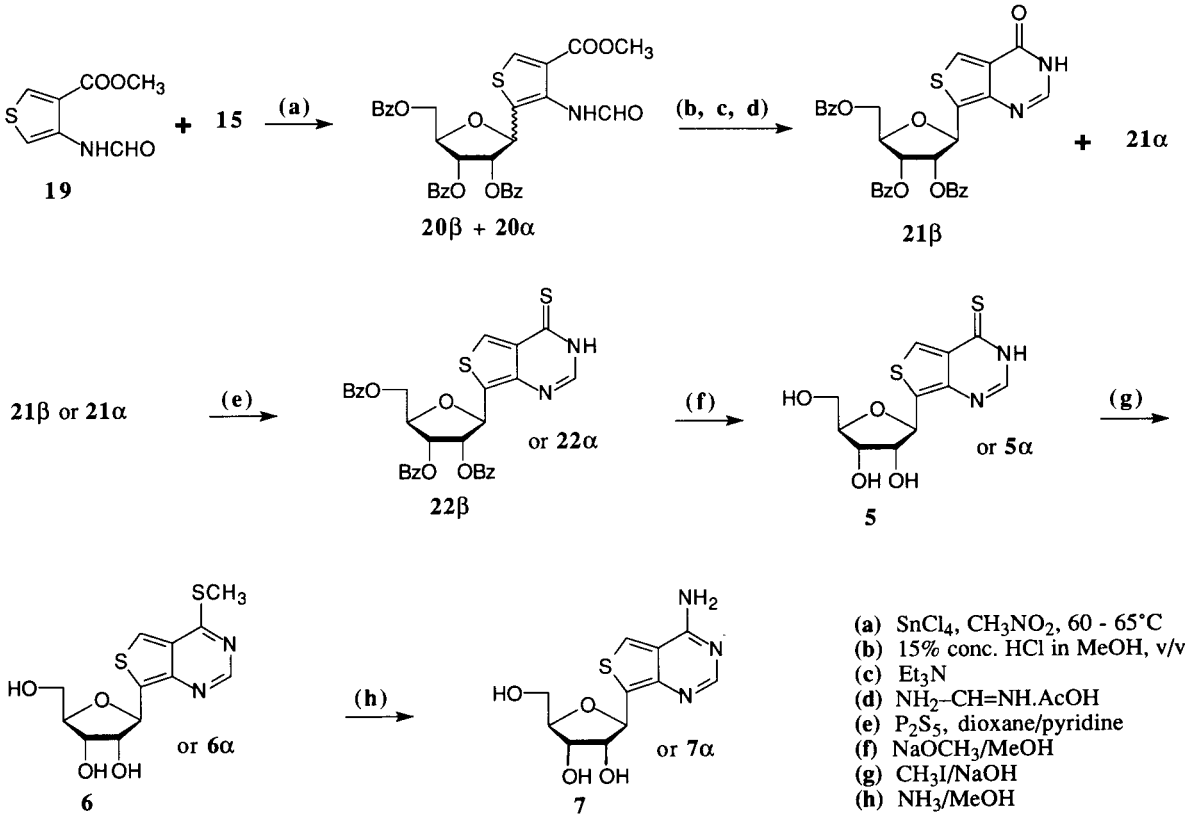


a = $SnCl_4$, CH_3NO_2, 60 - 65°C
b = $NaOCH_3$ / MeOH

When the condensation of **15** with **19** was carried out under the conditions reported by Girgis *et al* [11], the combined yield of **20**β + **20**α increased significantly from 13% [2,12] to over 35% (Scheme 4). No attempt was made to separate the anomers **20**β and **20**α since our previous experience [2] had shown us that both compounds anomerize during the subsequent deformylation step using aqueous methanolic hydrochloric acid. Instead, this anomeric mixture was first deformylated and then cyclized using formamide acetate to give a mixture of the required nucleosides **21**β and **21**α. These anomers were then separated chromatographically and debenzoylated as described previously [2].

Reaction of **21**α with phosphorus pentasulfide in pyridine gave **22**α in 78% yield (Scheme 4). However, under these same conditions, **21**β gave **22**β in only 34% yield. By using a mixture of dioxane and pyridine (9:1), the yield of

Scheme 4

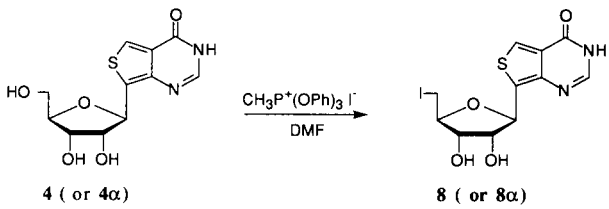


22β was increased to 49% (based on the amount of **21**β consumed). Both **22**β and **22**α were readily debenzoylated with methanolic sodium methoxide to give the thiones **5** and **5**α. Reaction of these anomers with excess methyl iodide in 0.1 *N* aqueous sodium hydroxide gave the corresponding methylthio compounds **6** and **6**α in greater than 60% yield.

The 4-amino compounds **7** and **7**α were prepared in excellent yields by reaction of the corresponding methylthio compounds with methanolic ammonia at 45°.

The synthesis of the 5'-iodo compounds **8** and **8**α was accomplished most conveniently (albeit in modest yields) by reaction of the corresponding 5'-hydroxy compounds **4** and **4**α [2] with methyl triphenoxyphosphonium iodide (Rydon's reagent) in dimethylformamide at ambient temperature. The ^{13}C nmr spectrum of the two compounds showed the characteristic iodomethyl resonances in the 9-12 ppm range.

Scheme 5



The determination of growth inhibitory activities of **5**, **6**, and **7** *in vitro* against a number of tumor cell lines [13] indicated that the adenosine analogue **7** is by far the most cytotoxic with IC_{50} values of 0.0046, 0.015 and 0.0092 μM against L1210-C1, Sarcoma 180 and HL60 cell lines. This is comparable to the activity of lead compound 9-deazaadenosine (**2**, R = NH_2; IC_{50} = 0.0041, 0.0011 and 0.0024 μM in those same lines). Also quite significant are the IC_{50} values for methylthio derivative **6** against those same cell lines (0.412, 0.263 and 0.211 μM respectively). The thiono derivative **5** was much less active with IC_{50} values of 17.6, 29.1 and 8.17 μM respectively. The 5'-iodo derivative **8** was found to be a very good inhibitor of purine nucleoside phosphorylase with a K_i value of 6.3 μM when tested at several concentrations *vs.* 40 μM of inosine [14].

EXPERIMENTAL

General Procedures.

Melting points were determined on an Electrothermal digital melting point apparatus and are uncorrected. The ^1H and ^{13}C nmr spectra were run on a Varian XL-200 spectrometer and the chemical shifts were measured relative to tetramethylsilane (TMS). Where possible, signal assignments were confirmed by selective decoupling experiments. First order values are given for chemical shifts and coupling constants. Microanalyses were performed by M.H.W. laboratories, Phoenix, Az. Thin layer chromatography (tlc) was performed on 250 μm silica gel GHLF plates

(Analtech, Inc.), and the substances were visualized by short-wave (254 nm) uv light and/or by spraying with 10% ethanolic sulfuric acid and charring. Preparative column chromatography was performed by standard flash chromatographic techniques on Merck silica gel 60 (230-400 mesh ASTM). The uv spectra were obtained on a Gilford Response II spectrophotometer. Unless stated otherwise, the molar extinction coefficients were measured in 0.05 *M* phosphate buffer, *p*H 7.00, at 25°.

Thieno[3,4-*d*]pyrimidin-4(3*H*)-one (**10**).

A mixture of **9** (5.23 g, 28.24 mmoles), formamidine acetate (15.0 g, 0.14 mole), triethylamine (2.73 g, 27 mmoles) and absolute ethanol (50 ml) was refluxed for one hour. The mixture was cooled in ice for 30 minutes and filtered. The solid was washed thoroughly first with ethanol then with water. Crystallization from methanol gave colorless needles of **10** (3.24 g, 79%), mp 285-291° (lit [9] mp 270-272°).

4-(Methylthio)thieno[3,4-*d*]pyrimidine (**12**).

A solution of **11** (1.00 g, 5.94 mmoles) [10] in 1 *N* aqueous sodium hydroxide (25 ml) was treated with methyl iodide (1.70 g, 11.98 mmoles) and the mixture was stirred at 25°. The methylthio compound **12** starts to crystallize out of solution almost immediately. After 2 hours, it was filtered off, washed with water and dried to give 0.90 g (83%) of **12**. For analytical purposes, a small quantity of the compound was recrystallized from ethanol-water, mp 107-111°; ^{1}H-nmr (dimethyl sulfoxide-d_6): δ 2.69 (s, 3H, CH_3), 8.21 (d, 1H, H-5 or H-7), 8.53 (d, 1H, H-7 or H-5), 8.64 (s, 1H, H-2); ^{13}C-nmr (dimethyl sulfoxide): δ 11.4 (CH_3), 118.6 (C-7 or C-5), 121.8 (C-5 or C-7), 126.3 (C-4a), 147.4 (C-7a), 151.0 (C-2), 167.3 (C-4); uv (*p*H 7.00): λ max (ε), 229.5 (20,560), 310.5 (9,960), 351.5 (6,480).

Anal. Calcd. for $C_7H_6N_2S_2$: C, 46.13; H, 3.32; N, 15.37; S, 35.18. Found: C, 46.29; H, 3.40; N, 15.24; S, 34.92.

4-Aminothieno[3,4-*d*]pyrimidine Hydrochloride (**13**).

A solution of **12** (2.50 g, 13.72 mmoles) in saturated methanolic ammonia (saturated at 0°, 200 ml) was heated in a stainless-steel bomb at 53° for 17 hours. The solvent was removed under reduced pressure and the residual cream colored solid was dissolved in hot methanol (90 ml). The solution was cooled in ice, treated with cold 10% (v/v) methanolic hydrogen chloride (25 ml) and allowed to stand in ice for 30 minutes. The product crystallized out upon addition of warm diethyl ether (140 ml) in portions and refrigeration overnight. The cream colored crystalline solid was collected by filtration, washed with a mixture of methanol-diethyl ether and dried to give **13** (2.30 g, 90%), mp 287-289° dec; ^{1}H-nmr (dimethyl sulfoxide-d_6): δ 7.96 (d, 1H, H-5 or H-7, $J_{5,7}$ = 3.2 Hz), 8.53 (s, 1H, H-2), 9.28 (d, 1H, H-5 or H-7), 9.89 (br s, 1H, NH, exchanged with deuterium oxide), 10.46 (br s, 1H, NH, exchanged with deuterium oxide); ^{13}C-nmr (dimethyl sulfoxide-d_6 + deuterium oxide): δ 112.9, 129.7 (C-5 and C-7), 117.7, 136.9, 158.8 (C-4, C-4a, C-7a), 149.1 (C-2); uv (*p*H ≤3.0): λ max (ε), 219.0 (12,950), 241.5 (7,440), 248.0 (7,660), 283.0 (6,320), 290.5 (6,350), 335.5 (5,170); (*p*H ≥11.0): 225.0 (14,320), 250.0 (6,530), 331.5 (5,480).

Anal. Calcd. for $C_6H_6ClN_3S$: C, 38.41; H, 3.22; N, 22.39; S, 17.08. Found: C, 38.65; H, 3.17; N, 22.43; S, 16.96.

Reaction of 4-(Methylthio)thieno[3,4-*d*]pyrimidine **12** with Dilute Hydrochloric Acid in Aqueous Methanol.

A mixture of **12** (0.15 g, 0.82 mmole), methanol (2.5 ml) and 0.1 *N* aqueous hydrochloric acid (25 ml) was stirred at 25° for 24 hours; tlc (dichloromethane-methanol, 95:5) indicated almost complete disappearance of **12** (R_f = 0.49) and the appearance of a new polar product (R_f = 0.26). The mixture was neutralized with Amberlite IR-45 (OH^-) ion-exchange resin and filtered. The filtrate was evaporated under reduced pressure and the residue was crystallized from methanol to give **10** [15] (0.076 g, 61%), mp 285-286° dec.

Reaction of 4-(Methylthio)thieno[3,4-*d*]pyrimidine **12** with Dilute Sodium Hydroxide in Aqueous Methanol.

A solution of **12** (0.1 g, 0.55 mmole) in methanol (6 ml) was diluted with 0.1 *N* aqueous sodium hydroxide (13 ml) and the mixture was stirred at 25° for 3 hours; tlc (chloroform-methanol, 97:3) indicated almost complete disappearance of **12** (R_f = 0.38) and the appearance of a major product (R_f 0.28) and a more polar minor product (R_f = 0.05). The solution was neutralized with Amberlite IRC-50 (H^+) ion-exchange resin and filtered. The filtrate was evaporated under reduced pressure and the residue purified by preparative tlc (1.5 mm x 3 plates) developing twice with dichloromethane-methanol, 98:2.

The less polar product (R_f = 0.28) was extracted with dichloromethane-methanol, 75:25 and identified as **14** [16] (0.046 g, 51%), mp 101-103°; ^{1}H-nmr (dimethyl sulfoxide-d_6): δ 4.1 (s, 3H, OCH_3), 8.13 (d, 1H, H-5 or H-7, $J_{5,7}$ = 3.2 Hz), 8.45 (s, 1H, H-2), 8.50 (d, 1H, H-7 or H-5); ^{13}C-nmr (deuteriochloroform): δ 54.4 (OCH_3), 117.3 (C-5 or C-7), 121.2 (C-5 or C-7), 121.3 (C-4a), 151.9 (C-7a), 153.8 (C-2), 164.7 (C-4).

Anal. Calcd. for $C_7H_6N_2OS$: C, 50.59; H, 3.64; N, 16.86; S, 19.29. Found: C, 50.42; H, 3.76; N, 16.76; S, 19.12.

The more polar product (R_f = 0.05) was extracted with methanol and identified as **10** (0.015 g, 18%, [15]).

When the above reaction was repeated on the same scale and allowed to proceed for 19 hours, the product obtained was entirely the more polar one **10** (R_f = 0.05). The solution was neutralized with 1 *N* aqueous hydrochloric acid and evaporated to dryness under reduced pressure. The resulting pale yellow colored solid was triturated with water, filtered and dried. Crystallization from methanol gave **10** (0.045 g, 54%) in two crops.

1-β-D-Ribofuranosylthieno[3,4-*d*]pyrimidin-4(1*H*)-one (**18**).

A mixture of **10** (2.0 g, 13 mmoles), **15** (7.96 g, 16 mmoles) and dry nitromethane (60 ml) was heated at 60° under a nitrogen atmosphere. To this mixture was added freshly distilled stannic chloride (5.14 g, 20 mmoles) and the heating was continued for 1.5 hours; tlc (chloroform-methanol, 97:3) showed the presence of unreacted **10** (R_f = 0.07) and a new blue fluorescent spot with R_f = 0.32. The solution was cooled to ambient temperature and poured into saturated sodium bicarbonate solution (250 ml). The mixture was then stirred for 30 minutes and filtered through celite. The filter cake was washed thoroughly with ethyl acetate and the layers separated. The aqueous layer was extracted once with ethyl acetate and the combined organic layer washed twice with brine and dried over anhydrous sodium sulfate. After filtration and removal of the solvent *in vacuo*, the residue was treated with dichloromethane and the suspension was filtered to remove insoluble **10** (0.36 g, 18%). The filtrate was then subjected to flash chromatography. Unreacted **15** and unidentified sugar by-products were eluted first with dichloromethane-methanol, 99.5:0.5 followed by **16** and **17** which were eluted together (0.83

g) with dichloromethane-methanol, 99:1.

Without further purification, the mixture of **16** and **17** was dissolved in anhydrous methanol (17 ml) and treated with 25 wt% sodium methoxide in methanol (0.29 g, 1.34 mmoles). After stirring the mixture at ambient temperature for 2 hours, tlc (dichloromethane-methanol, 5:1) showed complete disappearance of starting material and the presence of a single new blue fluorescent spot with R_f = 0.16. The solution was neutralized with Amberlite CG-50 (H^+) ion exchange resin and evaporated under reduced pressure. The residue was washed repeatedly with diethyl ether and dried to give **18** (0.33 g, 11% based on amount of **10** reacted, [17]).

Methyl 5-(2,3,5-Tri-*O*-benzoyl-β-(and α-)D-ribofuranosyl)-4-(formylamino)thiophene-3-carboxylate (**20**β and **20**α).

A mixture of **19** (70.0 g, 0.378 mole), **15** (228.8 g, 0.454 mole) and dry nitromethane (1.1 l) was heated to 65° in an oil bath under a nitrogen atmosphere. Freshly distilled stannic chloride (147.7 g, 0.567 mole) was added dropwise over 10 minutes and the heating was continued for 12 hours. The reaction mixture was cooled to ambient temperature, diluted with ethyl acetate (2 l) and stirred vigorously with brine (2.75 l). The mixture was filtered through Celite and the layers were separated. The organic layer was washed with brine (3 l) and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate (1.3 l) and slurried with silica gel (875 g). After removal of the solvent, the material was flash chromatographed over silica gel (875 g) with hexane-ethyl acetate (9%-29% in ethyl acetate) to elute **15** and unidentified sugar side products, benzoic acid and unreacted **19**. Finally, elution with hexane-ethyl acetate, 5:3 gave **20**β and **20**α as a mixture (83.3 g, 35%, based on **19**).

Anal. Calcd. for $C_{33}H_{27}NO_{10}S$: C, 62.95; H, 4.32; N, 2.23. Found: C, 63.08; H, 4.36; N, 2.11.

Unreacted **19** could be recovered in pure form by resubjecting the early fractions containing it to flash chromatography on silica gel using toluene-ethyl acetate (95:5). Final crystallization from hot toluene after evaporation of the appropriate fractions afforded pure **19** (16.93 g, 24% obtained in two crops).

7-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine-4(3*H*)-thione (**22**β).

To a mixture of dry dioxane (800 ml) and dry pyridine (80 ml) (maintained at 115° under a nitrogen atmosphere) were added the nucleoside **21**β (20 g, 33.52 mmoles) [2] and phosphorus pentasulfide (14.88 g, 33.47 mmoles). The mixture was heated with stirring for 1.25 hours. An additional quantity of phosphorus pentasulfide (7.44 g, 16.74 mmoles) was then added and heating continued for an additional hour; tlc (chloroform-methanol, 97:3) indicated the presence of some unreacted **21**β (R_f = 0.28) and the appearance of a new product with R_f = 0.58. The solvents were removed first under reduced pressure and then under high vacuum. The residue was partitioned between water and chloroform and the layers were separated. The organic layer was washed with water and dried over sodium sulfate. Removal of the solvent gave a residue which was purified by flash chromatography using chloroform-methanol, 99.5:0.5 to give unreacted **21**β (7.66 g, 38%) and product **22**β (6.24 g, 49% based on amount of **21**β reacted); ^{1}H-nmr (deuteriochloroform): δ 4.64 (dd, 1H, H-5″, $J_{5'',4'}$ = 3.7 Hz, $J_{5'',5'}$ = 11.9 Hz), 4.75 (m, 1H, H-4′), 4.90 (dd, 1H, H-5′, $J_{5',4'}$ = 2.9 Hz), 5.92-6.03 (m, 3H, H-1′, H-2′, H-3′), 7.20-8.45 (m, 17H, Ph, H-2 and H-5), 10.07 (br s, 1H, CSNH, exchanged with deuterium oxide).

Anal. Calcd. for $C_{32}H_{24}N_2O_7S_2$: C, 62.73; H, 3.95; N, 4.57; S, 10.47. Found: C, 62.57; H, 4.16; N, 4.43; S, 10.56.

7-(2,3,5-Tri-*O*-benzoyl-α-D-ribofuranosyl)thieno[3,4-*d*]pyrimidine-4(3*H*)-thione (**22**α).

A mixture of **21**α (1.0 g, 1.68 mmoles) [2], phosphorus pentasulfide (0.92 g, 2.06 mmoles) and dry pyridine (10 ml) was heated under a nitrogen atmosphere in an oil bath maintained at 120-125° for 1.75 hours; tlc (chloroform-methanol, 97:3) indicated complete disappearance of **21**α (R_f = 0.24) and the appearance of a new product with R_f = 0.4. The reaction mixture was worked-up and the product was purified exactly as described for **22**β to yield **22**α (0.80 g, 78%); ^{1}H-nmr (deuteriochloroform): δ 4.65 (dd, 1H, H-5″, $J_{5'',4'}$ = 4.6 Hz, $J_{5'',5'}$ = 11.9 Hz), 4.77 (dd partially overlapping multiplet for H-4′, 1H, H-5′, $J_{5',4'}$ = 3.7 Hz), 4.87 (m, 1H, H-4′), 6.02 (dd, 1H, H-3′, J = 7.5 Hz and 4.5 Hz), 6.20 (t, 1H, H-2′), 6.40 (d, 1H, H-1′, J = 3.3 Hz), 7.20-8.72 (m, 17H, Ph, H-2 and H-5), 10.32 (br s, 1H, CSNH, exchanged with deuterium oxide).

Anal. Calcd. for $C_{32}H_{24}N_2O_7S_2$: C, 62.73; H, 3.95; N, 4.57; S, 10.47. Found: C, 62.80; H, 4.19; N, 4.53; S, 10.31.

7-β-(and α-)D-ribofuranosylthieno[3,4-*d*]pyrimidine-4(3*H*)-thione (**5** and **5**α).

A mixture of the appropriate nucleoside (**22**β and **22**α, 1.0 g, 1.63 mmoles) and sodium methoxide (25 wt% solution in methanol, 0.35 g, 1.62 mmoles) in absolute methanol (20 ml) was stirred overnight at 25°. In the case of compound **22**α, an additional quantity of sodium methoxide (25 wt% solution in methanol, 0.035 g, 0.16 mmole) was added at the end of this period and the mixture stirred for an additional hour. The reaction mixture was neutralized with Amberlite IRC-50 (H^+) ion-exchange resin and filtered. The resin was washed repeatedly with methanol followed by washing with water. The filtrate and the methanol-water washings were combined and evaporated to dryness under reduced pressure. Methyl benzoate was removed by triturating the residue with diethyl ether. The resulting solid was collected by filtration, washed with diethyl ether and dried. Crystallization from hot water gave **5** (0.32 g, 65%) as a yellow crystalline solid, mp 209-212° dec; ^{1}H-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 3.82 (app q, 1H, H-4′), 3.93 (t, 1H, H-3′), 4.03 (t, 1H, H-2′), 5.33 (d, 1H, H-1′, $J_{1',2'}$ = 6.3 Hz), 7.84 (s, 1H, H-2), 8.48 (s, 1H, H-5); ^{13}C-nmr (dimethyl sulfoxide-d_6 + 2 drops of deuterium oxide): δ 62.1 (C-5′), 71.5 (C-3′), 77.0 (C-1′), 77.6 (C-2′), 84.9 (C-4′), 128.1 (C-5), 133.1 (C-4a), 137.1 (C-7), 140.2 (C-7a), 141.1 (C-2), 182.5 (C-4); uv (*p*H 7.00): λ max (ε), 234.0 (25,184), 371.0 (13,199).

Anal. Calcd. for $C_{11}H_{12}N_2O_4S_2$: C, 43.99; H, 4.03; N, 9.33; S, 21.35. Found: C, 43.93; H, 4.22; N, 9.09; S, 21.19.

Compound **5**α was similarly crystallized from hot water (0.37 g, 76%), mp 223-226° dec; ^{1}H-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 3.82 (br s, 1H, H-4′), 3.97 (br s, 1H, H-2′), 4.22 (app dd, 1H, H-3′), 5.70 (br s, 1H, H-1′), 7.82 (s, 1H, H-2), 8.49 (s, 1H, H-5).

Anal. Calcd. for $C_{11}H_{12}N_2O_4S_2$: C, 43.99; H, 4.03; N, 9.33; S, 21.35. Found: C, 43.79; H, 4.14; N, 9.13; S, 21.12.

4-(Methylthio)-7-β-D-ribofuranosylthieno[3,4-*d*]pyrimidine (**6**).

A solution of **5** (2.88 g, 9.59 mmoles) in 0.1*N* aqueous sodium hydroxide (100 ml) was treated with methyl iodide (17.5 ml) and the resulting mixture was stirred vigorously at 25° for 1 hour. The excess methyl iodide was removed under reduced pressure and the mixture was cooled in ice for 1 hour. The precipitated yellow colored solid was filtered, washed with cold water and dried (2.15 g). Pure **6** (1.91 g, 64%) was obtained in two crops by crystallization from absolute ethanol, mp 198-201° dec; ^{1}H-nmr (dimethyl sulfoxide-d_6): δ 2.66 (s, 3H, SCH_3), 3.55 (m, 2H, H-5″, H-5′), 3.86 (m, 1H, H-4′), 3.98 (m, 1H, H-3′, changes to t with deuterium oxide), 4.13 (m, 1H, H-2′, changes to t with deuterium oxide), 4.93 (t, 1H, OH-5′, J = 5.6 Hz, partially overlapping δ for OH at δ 5.00, exchanged with deuterium oxide), 5.00 (d, 1H, OH, J = 4.7 Hz, exchanged with deuterium oxide), 5.20 (d, 1H, OH, J = 5.9 Hz, exchanged with deuterium oxide), 5.48 (d, 1H, H-1′, $J_{1',2'}$ = 6.2 Hz), 8.44 (s, 1H, H-5), 8.59 (s, 1H, H-2); ^{13}C-nmr (dimethyl sulfoxide-d_6): δ 11.5 (CH_3), 62.0 (C-5′), 71.5 (C-3′), 77.2 (C-1′), 77.6 (C-2′), 85.0 (C-4′), 120.1 (C-5), 127.1 (C-4a), 136.6 (C-7), 144.2 (C-7a), 150.7 (C-2), 167.8 (C-4); uv (*p*H 7.00): λ max (ε), 229.0 (23,090), 315.0 (9,460), 358.5 (7,306).

Anal. Calcd. for $C_{12}H_{14}N_2O_4S_2$: C, 45.85; H, 4.49; N, 8.91; S, 20.36. Found: C, 46.05; H, 4.45; N, 8.71; S, 20.51.

4-(Methylthio)-7-α-D-ribofuranosylthieno[3,4-*d*]pyrimidine (**6a**).

This compound was obtained in 62% yield from **5**α under conditions similar to those described above for **6**, mp 215-217° dec; ^{1}H-nmr (dimethyl sulfoxide-d_6): δ 2.66 (s, 3H, SCH_3), 3.56 (m, 2H, H-5″ and H-5′), 3.87 (br s, 1H, H-4′), 4.01 (app q, 1H, H-2′, changes to t with deuterium oxide), 4.22 (m, 1H, H-3′, changes to dd with deuterium oxide), 4.75 (t, 1H, OH-5′, J = 5.5 Hz exchanged with deuterium oxide), 4.99 (d, 1H, OH, J = 7.5 Hz, exchanged with deuterium oxide), 5.25 (d, 1H, OH, J = 4.4 Hz, exchanged with deuterium oxide), 5.91 (d, 1H, H-1′, $J_{1',2'}$ = 2.4 Hz), 8.44 (s, 1H, H-5), 8.57 (s, 1H, H-2), mp 215-217° dec.

Anal. Calcd. for $C_{12}H_{14}N_2O_4S_2$: C, 45.85; H, 4.49; N, 8.91; S, 20.36. Found: C, 46.04; H, 4.32; N, 8.69; S, 20.12.

4-Amino-7-β-D-ribofuranosylthieno[3,4-*d*]pyrimidine (**7**).

A mixture of **6** (1.11 g, 3.53 mmoles) and saturated methanolic ammonia (saturated at 0°, 80 ml) was heated in a stainless-steel bomb at 45° for 22 hours with occasional stirring; tlc (chloroform-methanol, 8:2) indicated complete disappearance of **6** (R_f = 0.74) and the appearance of a new polar product with R_f = 0.12. The solvent was removed under reduced pressure and the residual traces of ammonia were removed by entrainment with methanol. The residual solid was crystallized from hot water to give **7** (0.81 g, 81%) in two crops, mp 244-247° dec; ^{1}H-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 3.47 (two dd partly overlapped by HOD signal, 2H, H-5′ and H-5″, $J_{5'',5'}$ = 11.9 Hz, $J_{5'',4'}$ = 4.3 Hz), 3.86 (app q, 1H, H-4′), 3.96 (dd, 1H, H-3′, $J_{3',4'}$ = 3.5 Hz), 4.16 (dd, 1H, H-2′, $J_{2',1'}$ = 6.8 Hz, $J_{2',3'}$ = 5.1 Hz), 5.26 (d, 1H, H-1′), 8.00 (s, 1H, H-5), 8.35 (s, 1H, H-2); ^{13}C-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 62.3 (C-5′), 71.9 (C-3′), 77.0 (C-1′), 77.6 (C-2′), 85.3 (C-4′), 119.6 (C-5), 120.9 (C-4a), 132.1 (C-7), 146.5 (C-7a), 154.1 (C-2), 158.4 (C-4); uv (*p*H 7.00): λ max (ε), 225.0 (29,184), 337.5 (8,125); (*p*H ≤ 3.0): 220.0 (20,840), 244.0 (11,400), 251.5 (11,040), 285.5 (6,670), 294.5 (6,870), 341.5 (6,560).

Anal. Calcd. for $C_{11}H_{13}N_3O_4S$: C, 46.63; H, 4.63; N, 14.83; S, 11.32. Found: C, 46.86; H, 4.42; N, 14.58; S, 11.12.

4-Amino-7-α-D-ribofuranosylthieno[3,4-*d*]pyrimidine (**7**α).

A mixture of **6**α (0.07 g, 0.22 mmole) and saturated methanolic ammonia (saturated at 0°, 10 ml) was heated in a stainless-steel bomb at 45° for 22 hours with occasional stirring; tlc (chloroform-methanol, 8:2) showed complete disappearance of **6**α (R_f = 0.58) and the appearance of a new polar product with R_f = 0.05. The solvent was removed under reduced pressure and the residual traces of ammonia were removed by entrainment once with methanol. The residual solid was subjected to three cycles of trituration-decantation with diethyl ether to give **7**α (0.06 g, ~100% yield); ^{1}H-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 3.83 (br s, 1H, H-4′), 3.97 (br s, 1H, H-2′), 4.16 (m, 1H, H-3′), 5.77 (d, 1H, H-1′, $J_{1',2'}$ = 1.2 Hz), 7.99 (s, 1H, H-2 or H-5), 8.36 (s, 1H, H-5 or H-2).

Anal. Calcd. for $C_{11}H_{13}N_3O_4S \cdot 1/2H_2O$: C, 45.20; H, 4.83; N, 14.38; S, 10.97. Found: C, 45.25; H, 5.00; N, 14.35; S, 11.06.

7-(5′-Deoxy-5′-iodo-α-(and β-)D-ribofuranosyl)thieno[3,4-*d*]pyrimidin-4(3*H*)-one (**8** and **8**α).

A mixture of the appropriate nucleoside **4** and **4**α (1.0 g, 3.52 mmoles), methyltriphenoxyphosphonium iodide (3.18 g, 7.03 mmoles) and dry dimethylformamide (6 ml) was stirred at ambient temperature under a nitrogen atmosphere for 1 hour; tlc (dichloromethane-methanol, 9:1) indicated complete disappearance of the starting material (R_f = 0.14 and 0.05 for **4** and **4**α, respectively) and the appearance of a major new less polar product (R_f = 0.40 and 0.30 for **8** and **8**α, respectively). The reaction was quenched by the addition of methanol (5 ml) and the solvents were removed *in vacuo*. The residue was partitioned between chloroform and water, after which the organic layer was separated and washed once with water. The combined aqueous extracts were washed with chloroform (x2). Removal of the water under reduced pressure gave a yellow colored viscous oil which was dissolved in methanol and slurried with silica gel. After removal of the solvent, the material was flash chromatographed over silica gel using dichloromethane-methanol, 95:5 (for **8**) or 9:1 (for **8**α). The product of iodo-de-hydroxylation of **4** thus obtained was freed from residual traces of organophosphorus by-products by trituration-decantation first with diethyl ether (1x) then with small quantities of methanol (3x) to give **8** (0.17 g, 12%). An analytical sample was obtained by neutralizing a solution of the compound in methanol with Amberlite IR-45 (OH^-) ion exchange resin, filtering off the resin and evaporating the filtrate; ^{1}H-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 3.44 (two dd partly overlapped by the HOD signal, 2H, H-5′ and H-5″, $J_{5'',5'}$ = 10.9 Hz, $J_{5'',4}$ = 5.3 Hz), 3.8 (m, 2H, H-3′ and H-4′), 4.10 (t, 1H, H-2′), 5.39 (d, 1H, H-1′, $J_{1',2'}$ = 6.0 Hz), 7.77 (s, 1H, H-5), 8.42 (s, 1H, H-2); ^{13}C-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 9.7 (C-5′), 74.5, 77.2, 77.6, 82.3 (C-1′, C-2′, C-3′, C-4′), 126.4 (C-5 and C-4a), 135.0 (C-7), 143.6 (C-2), 145.5 (C-7a), 157.7 (C-4).

Anal. Calcd. for $C_{11}H_{11}IN_2O_4S$: C, 33.52; H, 2.81; I, 32.19; N, 7.11; S, 8.13. Found: C, 33.76; H, 3.06; I, 31.90; N, 6.89; S, 7.92.

Compound **8**α obtained after column chromatography was treated with methanol and filtered to remove any insoluble material. The filtrate was concentrated and purified by preparative tlc developing twice with dichloromethane-methanol, 9:1. Compound **8**α was extracted with methanol (0.22 g, 16%). An analytical sample of **8**α was obtained as described above for **8**; ^{1}H-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 3.37 (dd,

1H, H-5″, $J_{5'',4'}$ = 5.2 Hz, $J_{5'',5'}$ = 10.7 Hz), 3.56 (dd, 1H, H-5′, $J_{5',4'}$ = 3.0 Hz), 3.74 (br s, H-4′ partially overlapping HOD peak), 4.02 (br s, 2H, H-2′ and H-3′), 5.80 (d, 1H, H-1′, $J_{1',2'}$ = 2.2 Hz), 7.76 (s, 1H, H-5), 8.40 (s, 1H, H-2); ^{13}C-nmr (dimethyl sulfoxide-d_6 + 2 drops deuterium oxide): δ 11.9 (C-5′), 72.7, 75.2, 77.2, 79.1 (C-1′, C-2′, C-3′, C-4′), 125.1 (C-4a), 127.7 (C-5), 131.8 (C-7), 143.3 (C-2), 145.3 (C-7a), 157.8 (C-4).

Anal. Calcd. for $C_{11}H_{11}IN_2O_4S$: C, 33.52; H, 2.81; I, 32.19; N, 7.11; S, 8.13. Found: C, 33.33; H, 2.79; I, 32.01; N, 6.94; S, 8.33.

REFERENCES AND NOTES

[1] This investigation was supported by grant CH-305 from the American Cancer Society and grants CA-24634 and CA-13330 (Cancer Center NMR Core Facility) from the National Cancer Institute.

[2] S. A. Patil, B. A. Otter and R. S. Klein, *Nucleosides Nucleotides*, **9**, 937 (1990).

[3a] J. W. Smith, M. S. Bartlett, S. F. Queener, M. M. Durkin, M. A. Jay, M. T. Hull, R. S. Klein and J. J. Marr, *Diagn. Microbiol. Infect. Dis.*, **7**, 113 (1987); [b] M. S. Bartlett, J. J. Marr, S. F. Queener, R. S. Klein and J. W. Smith, *Antimicrob. Agents Chemother.*, **30**, 181 (1986).

[4] R. L. Berens and J. J. Marr, *Biochem. Pharmacol.*, **35**, 4191 (1986).

[5] J. D. Stoeckler, J. B. Ryder, R. E. Parks, Jr., M.-Y. Chu, M.-I. Lim, W.-Y. Ren and R. S. Klein, *Cancer Res.*, **46**, 1774 (1986).

[6] Personal communication from Dr. Peter Waltzer, University of Cincinnati, OH.

[7] J. A. Montgomery in Antineoplastic and Immunosuppressive Agents, Part 1, A. C. Sartorelli and D. G. Johns, eds, Springer-Verlag, New York, Heidelberg, Berlin, 1974.

[8a] M.-I. Lim and R. S. Klein, *Tetrahedron Letters*, **22**, 25 (1981); [b] M.-Y. Chu, L. B. Zuckerman, S. Sato, G. W. Crabtree, A. E. Bogden, M.-I. Lim and R. S. Klein, *Biochem. Pharmacol.*, **33**, 1229 (1984).

[9] B. R. Baker, J. P. Joseph, R. E. Schaub, F. J. McEvoy and J. H. Williams, *J. Org. Chem.*, **18**, 138 (1953).

[10] M. Robba and N. Boutamine, *Bull. Soc. Chim. France*, 1629 (1974).

[11] N. S. Girgis, M. A. Michael, D. F. Smee, H. A. Alaghamandan, R. K. Robins and H. B. Cottam, *J. Med. Chem.*, **33**, 2750 (1990).

[12] In our earlier work [2], as much as 78% of **19** was recovered unchanged, whereas in the present study only 24% of **19** could be recovered. Utilization of nitromethane as the solvent in this reaction represents, therefore, a more efficient method for obtaining **20**β and **20**α.

[13] These studies were carried by Glenys Otter at Memorial Sloan-Kettering Cancer Center, NY, NY.

[14] These studies were carried out in Dr. Johanna Stoeckler's laboratory at Brown University, Providence, RI.

[15] This compound was found to be identical with an authentic sample of **10** by nmr spectroscopy. Elemental analyses and melting points were also the same for both.

[16] This compound, while stable for long periods at 0°, is apparently unstable *in vacuo* at higher temperatures.

[17] This compound was found to be identical with an authentic sample of **18** [2] by melting point, tlc and ^{1}H-nmr spectroscopy.