Purine Nucleosides. XXI. The Synthesis of 6-Amino-2-methylthio-7-(β-D-ribofuranosyl)purine, an Anomer of the Nucleoside from Factor F, by Ring Closure of an Imidazole Nucleoside[1]

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The synthesis of 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)purine-2,6-dithione (**4**) has been accomplished by ring closure of an imidazole nucleoside with carbon disulfide and pyridine. The preparation of a 7-glycosylpurine with an exocyclic group at position 6 susceptible to nucleophilic displacement was achieved for the first time when deacetylation and methylation of **4** furnished 2,6-bismethylthio-7-(β-D-ribofuranosyl)purine (**3**) in 80% yield. Nucleophilic displacement of the 6-methylthio group was achieved to afford 6-amino-2-methylthio-7-(β-D-ribofuranosyl)purine (**5**). A comparison of certain physicochemical data between **5** and the nucleoside isolated from the naturally occurring vitamin B_{12} analog (factor F) has been made and the actual site of ribosyl attachment for the nucleoside from factor F has now been definitely established as N-7. Treatment of **5** with Raney nickel has provided a new route for the preparation of 6-amino-7-(β-D-ribofuranosyl)purine.

A vitamin B_{12} analog (factor F) was isolated[2] and subsequently characterized[2,3] as (α-methylthioaden-7-yl)cobamide cyanide. This was the first time that a sulfur-containing purine had ever been isolated from a naturally occurring source. Five other purines or purine nucleosides isolated from other naturally occurring analogs of vitamin B_{12} were described[3] as either 7-ribosylpurines or purines obtained from the acid hydrolysis of presumed 7-ribosylpurines. The synthesis of several 7-β-D-ribofuranosylpurines has been recently described[4-6] *via* a route which utilizes imidazole nucleosides[7] as precursors. This included the β anomer of all but one[8] of the purine nucleosides or presumed nucleosides which had been isolated from naturally occurring vitamin B_{12} analogs. However, these studies[4-6] provided 7-β-D-ribofuranosylpurines with a single exocyclic group at C-6 or 2,6-disubstituted 7-β-D-ribofuranosylpurines with the 6 substituent generally restricted to a hydroxyl group. This precluded the synthesis of 6-amino-2-methylthio-7-(β-D-ribofuranosyl)-purine utilizing the routes described above. This prompted us to investigate the possibility of using an imidazole nucleoside with a different type of ring-closure procedure to obtain a 2,6-disubstituted 7-(β-D-ribofuranosyl)purine with an exocyclic substituent more amenable toward nucleophilic displacement. We now wish to report the synthesis of 6-amino-2-methylthio-7 (β-D-ribofuranosyl)purine from an imidazole nucleoside prepared *via* the fusion method.[9]

A mixture of 4-amino-5-cyano-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)imidazole[6] (**1**), pyridine and carbon disulfide was heated at reflux temperature for 2 hr to furnish a yellow solid in 76% yield. The structure of this nucleoside was subsequently established as 7-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)pyrine-2,6-dithione (**4**). This ring closure presumably occurs *via* the *m*-thiazine derivative (**2**) analogous to the reported[10,11] ring closures of certain cyclic nonaromatic, aromatic, and heterocyclic *O*-aminonitriles. To our knowledge this is the first use of this ring closure for the synthesis of a nucleoside. Apparently the formation of **2** is followed by a facile ring-opening and ring-closing rearrangement initiated by pyridine to furnish **4** (Scheme I). The assignment of structure **4** to this nucleoside rather than structure **2** was supported by a comparison of the ultraviolet absorption spectra of **4** and 7-methylpurine-2,6-dithione[12] (Table I). The infrared spectrum revealed a strong absorption band at 1580 cm^{-1} which was assigned[13,14] as C=S stretching and part of a —NC=S system. The thiol form was excluded by the absence of any absorption bands at 2550–2600 cm^{-1} usually attributed to —SH[13-15] stretching. The rearrangement of *m*-thiazines to pyrimidine dithiones with cold dilute sodium hydroxide is well documented.[11] However, treatment of the crystalline nucleoside obtained above with warm 10% sodium hydroxide for a prolonged period of time effected no change in the ultraviolet absorption spectra (long-wavelength maxima) which substantiates the above structural assignment. Treatment of **4** with 10% sodium hydroxide has presumably effected a removal of the blocking groups from the carbohydrate moiety of **4**. Isolation of this deacetylated product was not attempted since the addition of methyl iodide to the reaction mixture furnished a white solid (80% over-all yield) which was characterized as 2,6-bismethylthio-7-(β-D-ribofuranosyl)purine (**3**). This structure assignment was established by the disappearance of the absorption bands in the infrared spectrum attributable to —C=S stretching and the appearance of two peaks (singlets) at δ 2.65 (three protons) and δ 2.80 (three protons) in the pmr spectrum (DMSO-d_6) which indicated the presence of two methyl

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(8) This nucleoside is 6-amino-2-methylthio-7-(β-D-ribofuranosyl)purine.

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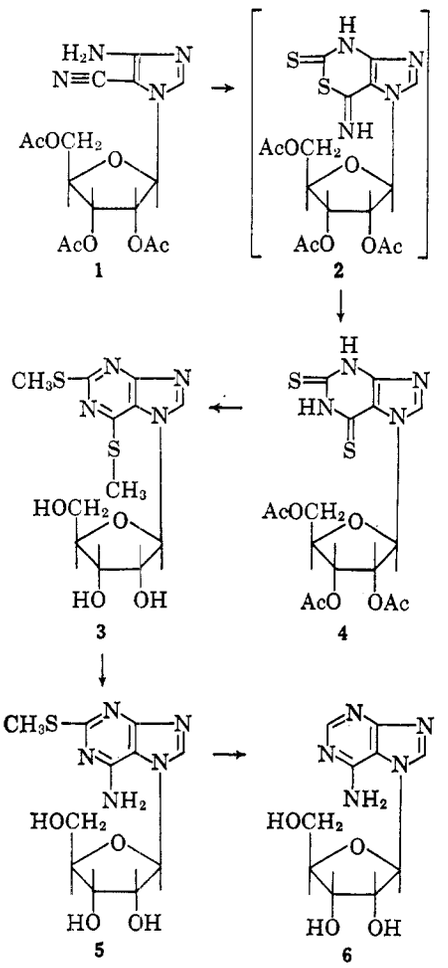
TABLE I
ULTRAVIOLET ABSORPTION SPECTRAL DATA OF CERTAIN 7-SUBSTITUTED PURINES[a]

	pH 1				MeOH				pH 11			
	λ_{max}, nm	ε	λ_{min}, nm	ε	λ_{max}, nm	ε	λ_{min}, nm	ε	λ_{max}, nm	ε	λ_{min}, nm	ε
7-Methylpurine-2,6-dithione[b]	258	13,700	236	4,150	258	17,400	240	12,200	243	15,400	224	14,700
	298	18,400	270	8,400	298	22,600	270	12,100	280	16,700	259.5	10,300
	349	13,500	327	10,300	351	13,100	334	11,900	335	8,100	313	6,400
7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribo-	257.5	16,800	236	9,700	255	13,700	236	9,700	247.5	18,600	223	12,800
furanosyl)purine-2,6-dithione	296	17,700	270	11,100	293	16,400	268	10,600	283	14,600	264	9,700
(**4**)	350	11,100	330	9,700	350	10,000	336	7,500	340	8,000	316	9,700
2,6-Bismethylthio-7-methyl-	261.5	17,000	240.5	4,750	238.5	15,700			240	15,700	223	6,550
purine[b]	316	9,500	299	7,450	257.5	17,400	246	13,800	257.5	15,700	247.5	13,300
					316	8,350	287	3,280	315	9,250	288	4,050
2,6-Bismethylthio-7-(β-D-ribo-	261.5	18,100	245	12,300	240	19,900	248	18,400	240	20,300	223	9,050
furanosyl)purine (**3**)	319	9,400	300	7,600	257	19,900	288	3,300	257	18,100	250	17,300
					317	8,300			315	8,700	290	4,300
6-Amino-2-methylthio-7-(β-D-	247	22,600	272	8,450	240	21,000	274	5,500	238	22,200	274	6,250
ribofuranosyl)purine (**5**)	288	11,600			289	6,600			286.5	6,900		
		0.1 *N* HCl							pH 6–12			
Nucleoside from 2-methylthio-	247	21,100							238	21,400		
adeninecobalamine analog[c]	289.5	10,300							287.5	6,100		

[a] Determined on a Beckman DK-2 spectrophotometer. [b] See ref 12. [c] These data obtained from ref 2.

SCHEME I



groups. A comparison of the ultraviolet absorption data of **3** with the ultraviolet absorption data of 2,6-bis-methylthio-7-methylpurine[12] (Table I) lends further support for these structural assignments (*vide supra*).[16] The preparation of 6-amino-2-methylthio-7-(β-D-ribofuranosyl)purine (**5**) was accomplished in an excellent yield from the treatment of **3** with liquid ammonia in a sealed vessel at 100° for 14 hr.[17] The ultraviolet absorption spectral data observed for **5** compares favorably with the ultraviolet absorption spectral data described[2] for the nucleoside isolated from factor F (Table I). It was of considerable interest that the initial assignment[2] of N-7 for the position of ribosidation for the naturally occurring nucleoside was based on the observation that 7-substituted purines generally exhibit, in comparison with 9-substituted purines, a definite bathochromic shift in the long-wavelength maxima. This comparison was made entirely on the basis of a 9-substituted purine since the 7-substituted, 1-substituted, and 3-substituted model compounds were not available for comparison. However, it has been recently reported[18] that several 7-substituted purines (including nucleosides) were in actuality 3-substituted purines and that the actual site of ribosidation could not be made solely on the basis of a bathochromic shift in the long wavelength maxima relative to that observed for a 9-substituted purine. In fact, it has been found that 1-substituted[19] and 3-substituted purines[20] usually exhibit a bathochromic shift for the long wavelength maxima in comparison with 9-substituted purines. This prompted us to investigate the conversion of **5** into a nucleoside of known structure. Removal of the 2-methylthio group from **5** with Raney nickel furnished 6-amino-7-(β-D-ribofuranosyl)purine (**6**). The structure of **6** has been previously established unequivocally by an alternate route[4] and by utilization of the $\Delta\lambda_{min}$ and $\Delta\delta$ rule.[18,21] Therefore, on the basis of these data (*vide infra*) the actual site of ribosyl attachment for the nucleoside from factor F has now been definitely established as N-7. In addition, the actual site of ribosyl attachment and the anomeric configura-

(16) Methylation of a ring nitrogen atom was excluded by the subsequent conversion of **3** into **6**.

(17) That nucleophilic displacement had occurred at position 6 instead of position 2 was subsequently established by the conversion of **5** into **6**.

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tion of all nucleosides prepared in this investigation were predetermined since the imidazole nucleoside used in the initial ring closure possessed a known anomeric configuration and site of ribosyl attachment. The anomeric configuration of the naturally occurring nucleoside could not be established in the present investigation as a comparison with **5** was inconclusive owing to the lack of physicochemical data reported[2] for the nucleoside isolated from factor F. However, the data presented herein have definitely established that the naturally occurring nucleoside isolated from factor F is 6-amino-2-methylthio-7-(D-ribofuranosyl)purine and corroborates the original site[2] of glycosidation assignment.

The preparation of **3** has provided for the first time a 7-glycosylpurine with an exocyclic group (methylthio) at position 6 which is susceptible toward nucleophilic displacement. Therefore, **3** can obviously be utilized for the preparation of numerous 6-substituted and 2,6-disubstituted 7-(β-D-ribofuranosyl)purines dependent only on the nucleophile employed. The preparation of additional 7-glycosylpurines *via* the route described herein utilizing imidazole nucleosides as precursors and the determination of actual anomeric configuration for the nucleoside isolated from factor F are under active investigation in this laboratory.

Experimental Section[22]

7-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)purine-2,6-dithione (4)—4-Amino-5-cyano-1-(2′,3′,5′-tri-*O*-acteyl-β-D-ribofuranosyl)-imidazole[6] (**1**, 8.5 g) was added to a mixture of pyridine (160 ml) and carbon disulfide (80 ml) and the reaction solution heated for 2 hr at reflux temperature. The yellow solution was evaporated to a foam *in vacuo* on a water bath and the residual foam dissolved in toluene (50 ml) and again evaporated to dryness *in vacuo*. The above procedure was repeated two more times to yield a yellow crystalline solid. Recrystallization of this solid from ethanol (2 l.) afforded a yellow powder (5.5 g) which was collected by filtration. Concentration of the filtrate to 500 ml furnished an additional 2.0 g of product. There was obtained a total yield of 7.5 g (76%) of **4**: mp 178–180°; $[\alpha]^{27}D$ +151.4 (*c* 1.0, pyridine).

Anal. Calcd for $C_{16}H_{18}N_4O_7S_2$: C, 43.44; H, 4.12; N, 12.67. Found: C, 43.08; H, 4.00; N, 12.57.

2,6-Bismethylthio-7-(β-D-ribofuranosyl)purine (3).—7-(2′,3′,-5′-Tri-*O*-acetyl-β-D-ribofuranosyl)purine-2,6-dithione (**4**, 3.0 g) was dissolved in 30 ml of 10% sodium hydroxide and stirred for 16 hr at room temperature. To this clear solution was added 30 ml of water and 6 ml of methyl iodide. The reaction mixture was then stirred for 1 additional hr in a loosely stoppered flask. The resulting white solid (2.4 g, 80.5%) was collected by filtration and washed with cold water (50 ml). A small sample was recrystallized from water for analysis to afford long, colorless, transparent needles: mp 195–197°; $[\alpha]^{27}D$ +18.80 (*c* 1.03, pyridine).

Anal. Calcd for $C_{12}H_{16}N_4O_4S_2 \cdot H_2O$: C, 39.78; H, 5.03; N, 15.47. Found: C, 39.49; H, 4.92; N, 15.40.

6-Amino-2-methylthio-7-(β-D-ribofuranosyl)purine (5).—2,6-Bismethylthio-7-(β-D-ribofuranosyl)pyrine (**3**, 1.0 g) and liquid ammonia (50 ml) were placed in a stainless steel reaction vessel and then heated at 100° for 14 hr. The residue remaining after evaporation of the liquid ammonia was dissolved in hot methanol and the solution then evaporated to dryness *in vacuo* on a steam bath to yield 850 mg of a light tan powder. The tan powder was recrystallized from a small amount of methanol (anhydrous) for analysis to give heavy colorless crystals: mp 206–208° with bubbling; $[\alpha]^{26}D$ −106 (*c* 0.785, pyridine).

Anal. Calcd for $C_{11}H_{15}N_5O_4S$: C, 42.18; H, 4.83; N, 22.36. Found: C, 42.27; H, 4.52; N, 22.22.

Treatment of 5 with Raney Nickel.—6-Amino-2-methylthio-7-(β-D-ribofuranosyl)purine (**5**, 50 mg) was heated at reflux temperature for 3 hr in 2 ml of water containing 350 mg of Raney nickel. An ultraviolet absorption spectrum of the reaction mixture indicated that the reaction was not complete and an additional 500 mg of Raney nickel was then added. After the reaction mixture had been heated at reflux for an additional 3 hr, the Raney nickel was removed by filtration and washed with boiling water (10 ml). The combined filtrate and washing exhibited the following ultraviolet absorption spectral data: pH 1, 272 nm; pH 11, 268 nm; and methanol, 268 nm. Paper chromatography of the reaction mixture with solvent system A (butanol saturated with water) and solvent system B [*n*-propanol, ammonium hydroxide, water, (6:3:1 v:v)] using the descending technique and Whatman No 1 chromatography paper gave the following results (Table II): a major spot[23] with an R_f value identical with that observed for 7-(β-D-ribofuranosyl)adenine, a minor spot with the identical mobility as starting material (**5**), and a trace spot which possessed a smaller R_f value than either of the previously mentioned compounds (**5** and **6**). When the ultraviolet absorbing spots with mobilities identical with 7-β-D-ribofuranosyladenine were excised and eluted with boiling water, there was obtained an ultraviolet absorption spectrum identical with that of 7-β-D-ribofuranosyladenine[4,6] (**6**).

TABLE II

CHROMATOGRAPHIC MOBILITIES ARE RECORDED AS R_f VALUES

	Filtrate	7-β-D-Ribofuranosyladenine (6)	6-Amino-2-methylthio-7-(β-D-ribofuranosyl)-purine (5)
Solvent system A	0.37 minor		0.37
	0.18 major	0.18	
	0.01 trace		
Solvent system B	0.75 minor		0.75
	0.59 major	0.59	
	0.13 trace		

Registry No.—**3**, 16797-71-0; **4**, 16797-72-1; **5**, 16797-73-2; **6**, 485-08-5.

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(22) Melting points were determined with a Thomas–Hoover melting point apparatus and are uncorrected. The nuclear magnetic resonance spectra were obtained on a Varian A-60 high resolution spectrometer utilizing tetramethylsilane as an internal standard and the chemical shifts are expressed as δ, parts per million, from tetramethylsilane. The infrared spectra were recorded with a Beckman IR-5A spectrometer. The optical rotations were obtained with a Perkin-Elmer Model 141 automatic digital readout polarimeter. Elemental analyses were performed by M-H-W Laboratories, Garden City, Mich.

(23) The ultraviolet absorbing spots were detected using short-range (254 nm) ultraviolet light.