Synthesis and PMR Studies of some Methylated 6-Thiopurine Nucleosides (1)

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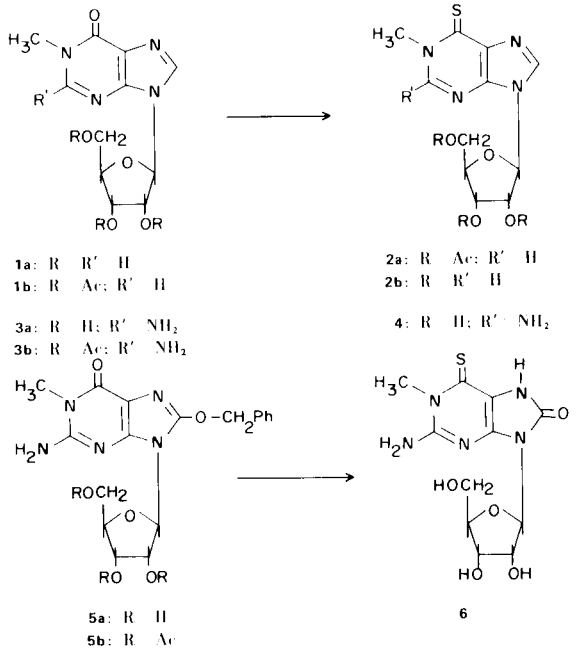
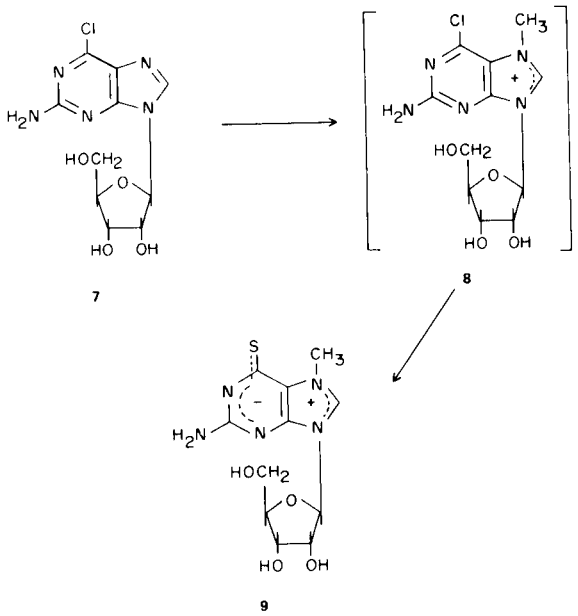
A number of methyl derivatives of purine nucleosides are known to occur in the transfer RNA's (tRNA) of various organisms, including man. Among these are 1-methylinosine (2), 1-methylguanosine (3) and 7-methylguanosine (4). Aberrant methylation of tRNA leading to overproduction or abnormal production of methylated nucleosides has been implicated as a possible step in virus-induced oncogenesis (5) and various alkylating carcinogens have been shown to alkylate guanosine (6) or the guanosine residues in RNA (7) at N-7. Several 6-thio derivatives of naturally occurring purine nucleosides, on the other hand, have shown pronounced antitumor activity; for example, 6-thioinosine and 6-thioguanosine have shown promise as useful antitumor agents (8). In earlier studies dealing with purine nucleosides (9-12) we have made extensive use of proton magnetic resonance (pmr) spectroscopy as a tool both for structure elucidation and for increasing our knowledge concerning the effects of functional group substitutions on the intra and intermolecular interactions which are responsible for the biological properties of those molecules. This report is concerned with the synthesis of some new





N-methyl-6-thiopurine ribonucleosides and with pmr studies of these nucleosides which support certain mechanisms proposed earlier to account for the effects on chemical shift arising from methylation of naturally occurring purine ribonucleosides (9-12).

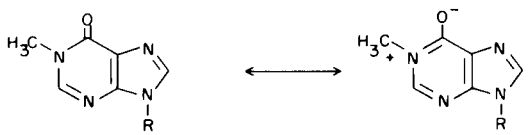
None of the desired compounds could be prepared by direct alkylation of the preformed 6-thionucleosides, since methylation is known to occur on sulfur to give 6-methylthio nucleosides (13). The approach taken, therefore, involved thiation of the appropriate methylated purine ribonucleosides.

Montgomery has reported (14) that tri-*O*-acetyl-1-benzylinosine could not be converted to the 6-thio derivative with phosphorus pentasulfide in refluxing pyridine, the usual conditions of converting purine oxo functions to thiones. Attempts to convert 2′,3′,5′-tri-*O*-acetyl-1-methylinosine (**1b**) to the 6-thio derivative (**2a**) using these conditions also failed. It was therefore decided to use a higher boiling basic solvent for a shorter period of time. The solvent chosen was β-picoline (b.p. 155°). Treatment of **1b** with phosphorus pentasulfide in refluxing β-picoline for two hours followed by deacylation of the crude product

and recrystallization gave 1-methyl-6-thioinosine (**2b**) in 46% yield. Application of similar reaction conditions to the tri-*O*-acetyl derivatives of 1-methylguanosine (**3a**) and 1-methyl-8-benzyloxyguanosine (**5a**) afforded, after deacylation, 1-methyl-6-thioguanosine (**4**) and 1-methyl-8-oxo-6-thioguanosine (**6**). That debenzylation occurred in the conversion of **5b** to **6** is not surprising since phosphorus pentasulfide has been shown to cause even *N*-debenzylation (14). It is noteworthy, however, that no evidence was found for thiation at position 8. This was established through the use of uv spectra and elemental analysis. The uv spectrum of **6** at *p*H 1 is totally unlike that of the known guanosine-8-thione (15) or 2-amino-9-(β-D-ribofuranosyl)-purine-6,8-dithione (16). Elemental analysis revealed that **6** contained only one sulfur atom per molecule; these findings make assignment of thiation at position 6 straightforward.

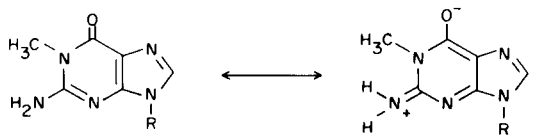
The preparation of 7-methyl-6-thioguanosine (**9**) from 2-amino-6-chloro-9-(β-D-ribofuranosyl)purine (**7**) *via* methylation to intermediate **8** followed by treatment *in situ* with thiourea was described in an earlier communication (1a). Subsequent study has shown that an electron releasing group such as the 2-amino group is required for this methylation reaction, since the 9-ribonucleoside of 6-chloropurine cannot be methylated at all under these conditions.

Examination of the pmr data of these nucleosides reveals some interesting points. In earlier work (9) it was found that 1-methylinosine (**1a**) represented the first example of a 6-substituted purine nucleoside in which the signal for H-2 appeared at lower field than that for H-8. The explanation suggested invoked a contribution to the resonance hybrid of the resonance form (**10**) in which a positive charge



1a **10**

is localized on N-1 (adjacent to H-2). A similar explanation was presented (10) to account for the downfield shift of the 2-amino group resonance of guanosine upon replacement of the proton at N-1 with a methyl or an amino group (cf. structure **11**). Although these explanations seem reasonable, corroborative evidence for them has been lacking.



3a **11**

Such evidence is now available from the pmr data collected in Table 1.

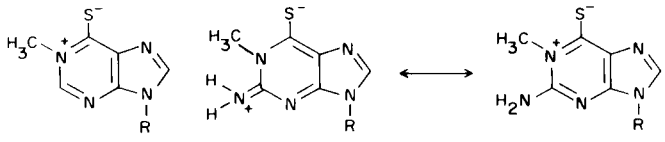
The increased ability of divalent sulfur relative to divalent oxygen in heteroaromatic systems to accommodate a negative charge is readily demonstrated by the greater acidity (17) of pyridine-2-thione (p*K*a 9.97) relative to 2-pyridone (p*K*a 11.6) or of 6-mercaptopurine (p*K*a 7.77) relative to hypoxanthine (p*K*a 8.94). It would therefore be predicted that the resonance hybrids for 1-methyl-6-thioinosine (**2b**) and 1-methyl-6-thioguanosine (**4**) would possess more of the character of charge separated structures **13**, **14**, and **14a** than would the oxo derivatives **1a** and **3a**. On this basis one would expect to observe downfield shifts for both the methyl group at position 1 and the substituent (H or NH_2) at C-2 for the thio nucleosides relative to the

TABLE 1

PMR Data for some Thio and Oxopurine Ribonucleosides (a)

Ribonucleoside	Chemical Shift (ppm)				
	H_2	H_8	H1′	NH_2	CH_3
Guanosine		7.87	5.67	6.42	
6-Thioguanosine		8.00	5.63	6.73	
1-Methylguanosine		7.87	5.68	6.98	3.42
1-Methyl-6-thioguanosine		8.02	5.67	7.32	3.89
Inosine	8.00	8.27	5.85		
6-Thioinosine	8.05	8.37	5.80		
1-Methylinosine	8.33	8.27	5.83		3.53
1-Methyl-6-thioinosine	8.63	8.38	5.83		3.87
1-Methyl-8-oxoguanosine			5.57	6.97	3.37
1-Methyl-6-thio-8-oxoguanosine			5.58	7.37	3.77
7-Methyl-6-thioguanosine		9.70	5.87	7.73	4.33
7-Methyl-6-thioguanosine + NaOH (b)		9.23	5.87	6.23	4.37

(a) Run in DMSO-d_6 with DSS as internal reference. (b) About 30 γ of 1*N* aqueous sodium hydroxide added to the solution to assure complete conversion to the zwitterion.



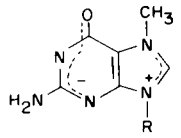
13 14 14a

oxo nucleosides. The data reveal (Table 1) that for each pair of 1-substituted nucleosides large downfield shifts of the appropriate protons are observed. The "thiation shifts" ($\Delta\delta$) for the 1-methyl protons range from 0.34 to 0.47 ppm. Similar shifts are observed for the amino proton of the guanosine derivatives shown in Table 1, suggesting some contribution of **14** to the resonance hybrid. Also, the signal for C-2H in 1-methyl-6-thioinosine (**2b**) is shifted downfield from that of 1-methylinosine (**1a**) by 0.30 ppm, confirming the prediction that resonance structure **13** must make a greater contribution to **2b** than **10** makes to **1a**.

The consistency in magnitude and direction of the "thiation shifts" discussed above is emphasized by the narrow range of chemical shifts of the anomeric sugar proton (C-1'H) within each group studied. Furthermore, the spectra for all nucleosides were run at approximately equal molar concentrations; it has been shown that small changes in concentration produce no change in chemical shift of purine and pyrimidine nucleosides in DMSO (18). It is thus clearly demonstrated that the shift differences obtained are intrinsic in the compounds studied and not artifacts created by experimental techniques.

It is clear that simple resonance arguments for the prediction of proton chemical shifts have no theoretical significance in evaluating anisotropy, ring current, electron localization and other parameters responsible for actual proton chemical shifts. Nonetheless, these arguments are of value in that they enable facile prediction of the direction and, to some extent, the magnitude of chemical shifts in heteroaromatic systems and may provide an experimental basis upon which sound theoretical predictions may be developed.

The pmr spectrum of 7-methyl-6-thioguanosine (**9**) is similar to that previously reported for 7-methylguanosine (**19**). The signal for the proton at C-8 is shifted far downfield from that of 6-thioguanosine (Table 1) and undergoes rapid exchange in the presence of deuterium oxide. In view



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of the betaine structure (**16**) established for such 7-methylribonucleosides (20), it was somewhat surprising to find that the signal for the 2-amino group was also well downfield from that of 6-thioguanosine. Addition of about 0.5 molar equivalents of sodium hydroxide to the DMSO-d_6 solution produced a dramatic upfield shift of the $-NH_2$ signal (from 7.73 to 6.23 δ) and a somewhat smaller (9.70 to 9.23 δ) shift in the H-8 signal. This finding, which is in keeping with our earlier work in this area (20), emphasizes the dependence of chemical shift upon the state of protonation of these quaternized purine derivatives. These data are consistent with the conversion of a partially protonated form of **9** to the betaine in that the 2-amino signal is upfield and C-8H and C-1'H signals are downfield from the corresponding resonances in 6-thioguanosine (Table 1). The data are not consistent with the proposal recently advanced (6) that the formally neutral species of 7-methylguanosine exists as a cation associated with a hydroxide anion.

The availability of the 1-methyl derivatives of 6-thioguanosine and 6-thioinosine permits a definite determination of the tautomeric structure of the latter. The model compounds requisite to such a determination are the 1-methyl derivatives **2b** and **4** and the *S*-methyl derivatives 6-methylthio-9-(β-D-ribofuranosyl)purine (21) and its 2-amino derivative (13). Comparison of the uv spectra of the neutral forms of the nucleosides (Table 2) clearly shows that both 6-thioinosine and 6-thioguanosine must exist predominantly in the thione form, as previously suggested (22,23).

TABLE 2

Uv Data Illustrating Tautomeric Structure of 6-Thioinosine and 6-Thioguanosine

Ribonucleoside	Uv Data for Neutral Molecule (a)	
	λ max	ε max
6-Thioinosine	322 nm	27,900
1-Methyl-6-thioinosine	318	28,600
6-Methylthio-9-(β-D-ribofuranosyl)purine	292	18,600
6-Thioguanosine	342	24,800
	257	8,820
1-Methyl-6-thioguanosine	346	21,200
	261	6,600
2-Amino-6-methylthio-9-(β-D-ribofuranosyl)purine	310	11,000
	245	14,400

(a) Spectral data were obtained from methanol solution.

EXPERIMENTAL

Materials and Methods.

Pmr spectra were obtained using a Jeol C60H spectrometer. Uv spectra were recorded on a Perkin-Elmer 202 or a Beckman DK2 spectrophotometer. Microanalyses were performed by MHW Labo-

ratories. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. Tlc was carried out using Silicar 7GF; solvent system EtOAc/nPrOH/H_2O(4:1:2 v/v) upper phase.

1-Methyl-6-thioinosine (**2b**).

1-Methylinosine (**1a**) (8.0 g., 28 mmoles) was dissolved in a solution of DMF (10 ml.), pyridine (8 ml.) and acetic anhydride (4 ml.). The solution was stirred at room temperature overnight, then poured into water (100 ml.). The solution was extracted with 3 x 50 ml. of dichloromethane; the organic layer was dried over magnesium sulfate. The mixture was filtered, the solvent removed *in vacuo*, the residue was treated twice with toluene and twice with ethanol; the solvent was evaporated *in vacuo* after each treatment.

The resulting glass was dissolved in anhydrous β-picoline (250 ml.), phosphorus pentasulfide (30 g.) was added, and the mixture was refluxed for 2 hours. After removal of most of the solvent *in vacuo*, the black residue was heated with water (400 ml.) on a steam bath for 0.5 hour. The cooled aqueous solution was extracted with 3 x 50 ml. of chloroform. The chloroform solution was filtered through a layer of charcoal on a layer of celite. The chloroform was removed *in vacuo* and the residue was dissolved in methanolic ammonia (saturated at 0°, 150 ml.). The solution was allowed to stand at room temperature overnight. After removal of the solvent *in vacuo*, acetone (150 ml.) was added. The solid was filtered to give 3.9 g. (46%), m.p. 208-210°. One recrystallization from methanol-water gave an analytical smaple; m.p. 214-216°; uv max (*p*H 1): 318 nm (ε 25.7 x 10^3), 231 (14.5 x 10^3); (*p*H 11) 323 (29.8 x 10^3) 234 (14.7 x 10^3); (methanol) 318 (28.6 x 10^3), 232.5 (12.7 x 10^3).

Anal. Calcd. for $C_{11}H_{14}N_4O_4S$: C, 44.3; H, 4.70; N, 18.8. Found: C, 44.2; H, 4.83; N, 18.8.

1-Methyl-6-thioguanosine (**4**).

1-Methylguanosine (**3a**) (24) (4.0 g., 12 mmoles) was dissolved in a solution of acetic anhydride (20 ml.) and pyridine (40 ml.) and stirred overnight at room temperature. Ethanol (50 ml.) and xylene (50 ml.) were added and the solvents were evaporated *in vacuo*. Two additions of ethanol followed by evaporation *in vacuo* gave **3b** as a white foam.

This foam was dissolved in β-picoline (150 ml.). Phosphorus pentasulfide (18.0 g.) was added and the mixture was refluxed for three hours. The reaction was worked up as described above to give **4** (1.60 g., 36%). Recrystallization from water gave the analytical sample, m.p. 174-176°; uv max (*p*H 1): 351 nm (16.6 x 10^3), 264 (8.1 x 10^3); (*p*H 11) 343 (20.8 x 10^3), 259, (7.4 x 10^3); (methanol) 346 (21.2 x 10^3), 261 (6.6 x 10^3).

Anal. Calcd. for $C_{11}H_{15}N_5O_4S{\cdot}H_2O$: C, 39.6; H, 5.19; N, 21.2. Found: C, 39.9; H, 5.14; N, 21.2.

1-Methyl-8-oxo-6-thioguanosine (**6**).

1-Methyl-8-benzyloxyguanosine (**5a**) (10) (8.0 g., 20 mmoles) was acylated and thiated as described for the preparation of **4** to give **6** (2.45 g., 30%), m.p. 205-206° after one recrystallization from water. Another recrystallization from water gave the analytical sample, m.p. 205-206°; uv max (*p*H 1): 353 nm (ε 26.0 x 10^3), 231 (17.1 x 10^3); (*p*H 11) 359 (20.7 x 10^3), 279 (7.9 x 10^3) 235 (16.5 x 10^3); (*p*H 7), 353 (21.6 x 10^3), 230 (16.7 x 10^3).

Anal. Calcd. for $C_{11}H_{15}N_5O_5S$: C, 40.0; H, 4.58; N, 21.3. Found: C, 39.8; H, 4.89; N, 21.0.

7-Methyl-6-thioguanosine (**9**).

Compound **7** (16) (2.0 g., 6.6 mmoles) was dissolved in DMF (10 ml.). Methyl iodide (2.0 ml.) was added and the solution was stirred at room temperature for 15 hours. Excess methyl iodide was removed *in vacuo* and the solution was treated with thiourea (2.0 g.). After 0.5 hour the solution was carefully neutralized with methanolic ammonia (disappearance of the brown color caused by iodine is a good end point for this neutralization). The solution was poured slowly into well-stirred acetone (500 ml.). The residue was filtered, washed with acetone (50 ml.), and dried *in vacuo* (rotary evaporator) to give **9** (1.6 g., 71%), chromatographically homogeneous. Recrystallization from aqueous methanol gave the analytical sample; uv max (*p*H 1): 348 nm (ε 17.3 x 10^3), 254 (6.7 x 10^3); (*p*H 11), 300 (16.7 x 10^3) 240s (15.8 x 10^3); methanol, 329 (14.6 x 10^3).

Anal. Calcd. for $C_{11}H_{15}N_5O_4S{\cdot}3/2H_2O$: C, 38.7; H, 5.28; N, 20.5; S, 9.38. Found: C, 38.7; N, 20.2; H, 5.22; S, 9.12.

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