Hydroxymethylation and Aminomethylation of 6-Mercaptopurine and Its Derivatives

Kevin G. Siver [1], Kenneth B. Sloan*, Robert P. Waranis [1] and Akram Saab

Department of Medicinal Chemistry, Box J-485, University of Florida, Gainesville, Florida 32610

Received February 8, 1988

Labile aminomethyl and hydroxymethyl derivatives of 6-mercaptopurine (I) (6-MP) and S^6-acyloxymethyl-6-MP have been converted to stable acetyloxymethyl derivatives by their reaction with acetic anhydride. Analysis of the reaction products and comparison of their ^{1}H nmr spectra and hplc chromatograms with those of acetyloxymethyl derivatives of known structures suggested 1) that the aminomethyl derivatives of 6-MP were 7-substituted derivatives, 2) that the aminomethyl derivative of S^6-acetyloxylmethyl-6-MP was a 9-derivative, 3) that the hydroxymethyl derivative of 6-MP was a mixture of 7-substituted and S^6,3-disubstituted derivatives, and 4) that the hydroxymethyl derivative of S^6-pivaloyloxymethyl-6-MP was a 9-substituted derivative. In addition, a previously unreported dialkyl derivative of 6-MP **VI** was isolated from its reaction with aminomethylating agent and characterized. Analyses of the ^{1}H nmr spectra and hplc chromatograms of the reaction of **VI** with acetic anhydride suggested that **VI** was a 1,7-disubstituted derivative.

J. Heterocyclic Chem., **25**, 1077 (1988).

Introduction.

Because of a continuing interest in developing prodrug approaches to enhancing the delivery of polar, heterocyclic drugs through skin [2-5], hydroxymethyl and aminomethyl derivatives of 6-mercaptopurine (6-MP) (I) have been prepared and the aminomethyl derivatives have been evaluated in diffusion cell experiments for their abilities to enhance the delivery of 6-MP through skin [6]. Since the amniomethyl derivatives were found to be very effective in enhancing the delivery of 6-MP through skin, it was important to determine the structure of these types of derivatives of 6-MP and S^6-substituted 6-MP.

Previously, Bryant and Harmon [7] had reported that aminomethylation of 6-MP had taken place on the 9-position of 6-MP, that hydroxymethylation had failed under a variety of conditions and that hydroxymethylation and aminomethylation of S^6-substituted 6-MP had taken place on the 9-position. In the case of the hydroxymethyl derivatives of S^6-substituted 6-MP the labile compounds were converted to stable ones by reaction with isocyanate. However that was not the case for the labile aminomethyl derivatives and the assignment of their structures was made on spectroscopic ground rather than conversion to stable compounds of known structure.

In this paper results from the acylation of the aminomethyl and hydroxymethyl derivatives of 6-MP and S^6-substituted 6-MP to give acyloxymethyl derivatives of 6-MP will be presented which show that the aminomethyl derivatives of 6-MP are 7-derivatives, that the hydroxymethyl derivative of 6-MP is a mixture of mono-(7-) and dialkylated (S^6,3-) species, and that the aminomethyl and hydroxymethyl derivatives of S^6-substituted 6-MP are indeed 9-derivatives.

Results and Discussion.

The syntheses of the aminomethyl derivatives of 6-MP **II-V** were accomplished under quite different conditions than those previously reported where ethanol was used as a solvent and aqueous formaldehyde was used as the source of formaldehyde. In order to obtain more anhydrous conditions, paraformaldehyde was used instead of aqueous formaldehyde. Similarly, since the aminomethyl derivatives that result are unstable in protic solvents [8], a nonprotic solvent such as ether was used. These changes gave more reproducibly uniform products in higher yields. Since excess alkylating agent was used in the preparation of **II-V** and the products were relatively insoluble in ether, the opportunity for the dialkylation of 6-MP, which had not been previously described, was also minimized by the use of ether as a solvent. On the other hand, when dichloromethane, was used as a solvent, alkylation of 6-MP with four equivalents of aminomethylating agent gave dialkylated products in two cases. In those cases the monoalkyl products that were initially formed were relatively soluble in dichloromethane, *i.e.*, **III** and **IV**. Thus, they were in solution and available for a second alkylation while other, less soluble monoalkyl products were not. Even the use of a single equivalent of aminomethylating agent in dichloromethane gave 1:1 mixtures of unreacted 6-MP and dialkyl products. In the case of the reaction of 6-MP with two equivalents of dipropylamine and formaldehyde in dichloromethane an analytically pure product **VI** was isolated. Compound **VI** was stable in dimethyl sulfoxide but not in chloroform in which it decomposed to the monoalkyl derivative **IV**.

In contrast to the previous report [7], it was not possible to obtain analytically pure aminomethyl derivatives of 6-MP when morpholine or other low p*K*a amines such as *N*-methylpiperazine were used in the aminomethylation reaction. In those cases there were multiple N-CH_2-N type absorptions present in the ^{1}H nmr spectra of the initial products which could not be removed by recrystalliza-

tion. A wide variety of solvents (ethanol, dimethylformamide, acetone, ether and dichloromethane) were tried in the aminoethylation reactions of the morpholine type amines with 6-MP to give a single aminomethyl product but without success.

The uv spectra of the aminomethyl derivatives **II-VI** were all quite similar to that of 6-MP itself in acetonitrile (Table I). Since analysis of ^{13}C nmr spectra of 6-MP suggests that it exists primarily as the 1-H, 7-H tautomer in dimethyl sulfoxide solution [9], the similarity of the uv spectra of **II-VI** to that of 6-MP would suggest that the monoalkyl derivatives are 1- or 7-derivatives and that the dialkyl derivatives are 1,7-derivatives. It was not possible to obtain definitive ^{13}C nmr spectra of the aminomethyl derivatives and the ^1H nmr spectra of the derivatives were not helpful either in defining the structures of **II-VI**. Generally the ^1H nmr spectra of **II-VI** in deuteriodimethyl sulfoxide exhibited two sharp singlets for the 2-*H* and 8-*H* hydrogens in the region δ 8.2-8.5 and a broad multiplet for the N-C*H*$_2$-N hydrogens in the region δ 5.0-6.0 which integrated for two or four hydrogens depending on the derivative. On the other hand, the ^1H nmr spectrum of **VI** in deuteriochloroform clearly showed two different N-C*H*$_2$-N absorptions.

Table I

Ultraviolet Absorbances of Aminomethyl Derivatives of 6-MP in Acetonitrile

Compound	UV λ max nm (log ε)			
6-MP	232 (s, 3.83) [a]	288 (s, 3.50)	330 (4.27)	344 (s, 4.05)
II	233 (s, 3.84)	297 (s, 3.68)	330 (4.30)	344 (s, 4.06)
III	232 (s, 3.85)	291 (s, 3.58)	330 (4.29)	344 (s, 4.07)
IV	232 (s, 3.86)	294 (s, 3.63)	330 (4.29)	345 (s, 4.07)
V		297 (s, 3.50)	330 (4.20)	344 (s, 3.99)
		309 (s, 3.98)		
VI	230 (s, 3.89)	293 (s, 3.62)	330 (4.29)	345 (s, 4.03)

[a] The symbol s signifies a shoulder.

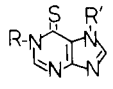
In order to more clearly establish the structures of these aminomethyl derivatives of 6-MP, they were acetylated with acetic anhydride according to a modification of the method of Roth and Brandes [10] to give acetyloxymethyl derivatives of 6-MP. Because the aminomethyl derivatives were mono-derivatives the remaining unalkylated N—H (or S-H) group in 6-MP was also available for acylation. Thus, the use of large excesses of acetic anhydride apparently led to ring acylated species which subsequently rearranged to a large number of products. The use of pyridine as an acid scavenger in the reaction also led to numerous unidentified products. In addition, since **II-V** were relatively insoluble in most solvents except dimethyl sulfoxide, the non-homogeneous reaction conditions that resulted when the former solvents were used also led to excess acetylating reagent being available for reaction with the small amounts of **II-V** that were in solution at any one time. For these reasons the reaction conditions that gave the cleanest results involved dissolving the aminomethyl derivative in deuteriodimethyl sulfoxide and allowing it to react with only two equivalents of acetic anhydride. In this way the progress of the reaction could also be followed by ^1H nmr until all of the N-C*H*$_2$-N absorbance in the region δ 5.0-6.0 had disappeared.

The positions and intensities of the 2-*H*, 8-*H* and N- or S-C*H*$_2$O absorptions in the nmr spectra of the reaction between **II** and acetic anhydride showed that the major component was 7-acetyloxymethyl-6-MP (**VII**, 65%). The remaining components consisted of 6-MP (20%) and small amounts of 9-acetyloxymethyl-6-MP (**VIII**, 5%) [11] and *S*^{6},3-bisacetyloxymethyl-6-MP (**IX**, 10%) [5]. These results were confirmed by hplc analyses. In one instance the ^1H nmr reaction mixture containing two equivalents of acetic anhydride and 200 mg of **III** in 2 ml of deuteriodimethyl sulfoxide was subsequently diluted with 2 ml of water. A small amount of **VII** was isolated from the precipitate which was identical with authentic **VII** (see below) by ^1H nmr, hplc and uv. Thus, the aminomethyl derivatives of 6-MP have been assigned 7-alkyl structures.

The presence of **IX** in the reaction product probably results from rearrangement of the initial products, since **IX** became the major product if a large excess of acetic anhydride or non-homogeneous reaction conditions were used. The presence of **VIII** in the reaction product more likely was the result of the presence of some 9-aminomethyl derivative in the starting material. The broadness of the N-C*H*$_2$-N absorption in the ^1H nmr spectra of the aminomethyl derivatives certainly does not preclude the presence of two mono-isomers.

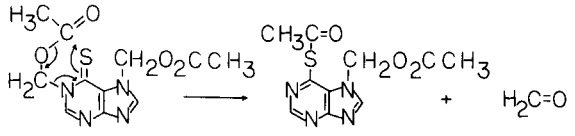
The assignment of the structure of the dialkylated derivative **VI** was also partially based on the reaction of **VI** with acetic anhydride in deuteriodimethyl sulfoxide. Again, 7-acetyloxymethyl-6-MP (**VII**) was the major product (about 60%) and there were about two equivalents of *N,N*-dipropylacetamide formed. However, there were a number of components that could not be identified by ^1H nmr and hplc. The fact that **VII** was the major product identifies one site of aminomethyl alkylation as the 7-position. Compound **VI** can not be a *S*^{6},7-isomer because **VI** exhibits a uv maximum at 330 nm while an *S*^{6},7-isomer would exhibit a uv maximum below 300 nm [3]. This leaves only the 1,7- and 3,7-isomers as possibilities for the structure of **VI**. Usually 1,7-dialkyl isomers exhibit uv maxima that are quite close to that of 6-MP in the same solvent while 3,7-isomers exhibit uv maxima that are shifted 20 nm to longer wavelengths [3]. Since the uv maximum exhibited by **VI** is quite similar to that of 6-MP in acetonitrile, **VI** has been assigned a 1,7-dialkyl structure.

The fact that **VII** was the major product instead of a dialkyl product can be rationalized by assuming that the 1,7-bisacetyloxymethyl product is formed first. An intramolecular attack of the sulfur on the carbonyl of the 1-acetyloxymethyl group through a six-membered transition state yields formaldehyde and the corresponding *S*^{6}-acetyl-7-acetyloxymethyl-6-MP (see Figure 1). Subsequent facile hydrolysis of the *S*-acetyl group would then give rise to **VII**. Similar rearrangements of acyloxymethyl derivatives have been previously reported by Rasmussen and Leonard [12].



R = H I
R = H, R' = $CH_2N(CH_3)_2$ II
R = H, R' = $CH_2N(C_2H_5)_2$ III
R = H, R' = $CH_2N(C_3H_7)_2$ IV
R = H, R' = $CH_2N(CH_2)_5$ V
R = R' = $CH_2N(C_3H_7)_2$ VI

Figure 1



In order to clarify whether aminomethylation of an *S*^{6}-substituted 6-MP derivative resulted in 7- or 9-substitution, *S*^{6}-acetyloxymethyl-6-MP was converted to its piperidylmethyl derivative **X** using essentially the same conditions that were used to prepare **II-V**. A solution of **X** in pyridine was then allowed to react with acetic anhydride for one hour to give a crude product that was 75% *S*^{6},9-bisacetyloxymethyl-6-MP (**XI**) by ^{1}H nmr spectroscopy. Analytically pure **XI** [5] was isolated from the reaction mixture by crystallization. Assignment of a *S*^{6},9-structure to **X** was also based on its uv spectrum which exhibited a maximum at 274 nm and a shoulder at 280 nm. This is characteristic of a *S*^{6},9- as opposed to a *S*^{6},7-structure which exhibits a uv maximum which is about 10 nm longer in wavelength and which also exhibits shoulders on either side of the maximum [3]. All other dialkyl structures absorb at much longer wavelengths. Thus, aminomethylation of *S*^{6}-substituted 6-MP does give 9-substitution [7].

Although Bryant and Harmon [7] reported that a hydroxymethyl derivative of 6-MP could not be isolated, that does not appear to be the case. However, the ^{1}H nmr spectrum of the formaldehyde adduct of 6-Mp (**XII**) suggested that it was a complex mixture, and the composition of the mixture did not change over a wide range of 6-MP:formaldehyde ratios in the reaction. There were eight clearly defined absorptions in the region δ 9-8 where the C_2-*H* and C_8-*H* absorptions normally appear. Two absorptions at δ 8.47 and 8.28 corresponded to the position of the C_2-*H* and C_8-*H* absorptions of 6-MP and accounted for about 33% of the intensity of the absorptions. After deuterium oxide exchange the relative intensities of the absorptions in the δ 9-8 region were not changed. The region δ 7.5-5.5 was also very complex but after deuterium oxide exchange that region was considerably simplified. A doublet that had been centered at δ 5.6 collapsed to a singlet. This doublet appears to be due to the N-CH_2-O absorption of the monohydroxymethyl adduct. Two sets of triplets that had been centered at δ 7.0 and 6.63 disappeared and two broad multiplets centered at about δ 6.1 and 5.97 sharpened considerably. The two broad multiplets centered at δ 6.1 and 5.97 appear to be due to the S- or N-CH_2-O absorptions of the dihydroxymethyl adduct. The absorptions centered at δ 7.0 and 6.63 appeared to be due to OH groups and their multiplicity to be due to coupling with CH_2 groups. Taking into account that $\approx$ 33% of the intensity of the absorptions in the δ 9-8 region was due to 6-MP, and that the remaining absorptions were primarily due to a monohydroxymethyl and a dihydroxymethyl adduct of 6-MP (see below), then the ratio of 6-MP to mono- to dihydroxymethyl-6-MP adducts was about 7:9:5.

In order to identify the structures of the labile adducts, they were converted to stable acetyloxymethyl-6-MP derivatives. Comparison of the ^{1}H nmr spectrum of the crude reaction mixture from the reaction of the formaldehyde adduct of 6-MP with acetic anhydride in pyridine, with those of authentic compounds suggested that the *S*^{6},3-bis- **IX** and 7-acetyloxymethyl-6-MP (**VII**) derivatives had been formed in the ratio of 55:45. Compounds **VII** and **IX** were extracted from the reaction mixture into boiling chloroform from which pure **VII** (15%) crystallized on cooling. The chloroform filtrate contained fairly pure **IX** which was isolated by concentration of the filtrate and crystallization of the residue from acetone (24%). The residue that was left after the chloroform extractions of the crude reaction mixture was composed primarily of 6-MP, 7- and 9-acetyloxymethyl-6-MP (**VIII**), and an unknown component in the ratio of 9:7:5:7 based on the positions and relative intensities of the 2-*H* and 8-*H* absorptions. The identity of **VIII** was based on the comparison of its ^{1}H nmr spectrum with that of an authentic sample (see Experimental) [11]. Thus, on the basis of the conversion of hydroxymethyl-6-MP into acetyloxymethyl derivatives, the reaction product between 6-MP and formaldehyde is primarily a mixture of 7-hydroxymethyl- and *S*^{6},3-bishydroxymethyl-6-MP and 6-MP.

The 7-acetyloxymethyl-6-MP (**VII**) that was isolated from the acetylation of hydroxymethyl-6-MP was identified by its ^{13}C nmr spectrum (see Experimental). The posi-

tion of the carbon absorptions are only consistent with a 7-substituted derivative of 6-MP [9]. In addition, **VII** exhibits a maximum in its uv spectrum at 10 nm longer wavelength than that of 9-acetyloxymethyl-6-MP (**VIII**) [11] which is also consistent with the assigned structure [3].

In order to clarify whether hydroxymethylation of an S^6-substituted 6-MP derivative resulted in 7- or 9-substitution, S^6-pivaloyloxymethyl-6-MP was converted to its hydroxymethyl derivative **XIII** using essentially the same conditions that had been used to prepare **XII**. A solution of **XIII** in pyridine was then allowed to react with pivaloyl chloride for two hours. Analysis of the ^{1}H nmr spectrum of the crude reaction mixture suggested that it contained two components: S^6-pivaloyloxymethyl-6-MP (**XIV**) and S^6,9-bispivaloyloxymethyl-6-MP (**XV**) (4:6). Pure **XIV** and **XV** were isolated from the reaction mixture which were identical with authentic starting material **XIV** and **XV** [3,5].

Assignment of a S^6,9-structure to **XIII** was also based on its uv spectrum which exhibited a maximum at 274 nm and a shoulder at 280 nm. This is characteristic of a S^6,9- as opposed to a S^6,7-structure which exhibits a uv maximum at about 10 nm longer wavelength and which also exhibts shoulders on either side of the maximum [3]. All other dialkyl structures absorb at much longer wavelengths. Thus hydroxymethylation of S^6-substituted 6-MP does give 9-substitution.

EXPERIMENTAL

The tlc were run on Brinkman Polygram Sil G/UV 254 plates. The mp (corrected) were taken with a Thomas-Hoover capillary apparatus. The ^{1}H nmr spectra were recorded on a Varian EM-390 or T-60 spectrometer and the ^{13}C nmr on a Nicolet NT-300 spectrometer while the uv spectra were recorded on a Cary 210 spectrophotometer. Microanalyses were obtained from Atlantic Microlab Inc., Atlanta, GA. Except for 6-MP, which was obtained from Sigma, the chemical starting materials were obtained from Aldrich. The bulk solvents were obtained from Fisher Scientific. The hplc system consisted of a Beckman model 110A pump with a model 153 uv detector, a Rheodyne model 7125 injector with a 20 μl injector volume, and a Hewlett-Packard model 3392A integrator.

General Procedure for Aminomethylation of 6-MP.

To four equivalents of a secondary amine was added four equivalents of paraformaldehyde. The white suspension was stirred at room temperature for 1-5 minutes as an exothermic reaction ensued. The mixture was then stirred with 10 ml of ether overnight, filtered and allowed to react with 1.70 g (0.01 mole) of 6-MP hydrate for 2-4 hours. The white suspension was diluted with 5 ml of tetrahydrofuran, stirred for an additional 5 minutes and filtered. The residue was washed once with an additional 5 ml of tetrahydrofuran and three times each with 5 ml of ether before being allowed to air dry to give the respective aminomethyl derivatives of 6-MP.

7-(Dimethylamino)methyl-6-mercaptopurine (**II**).

This compound was obtained in 90% yield (1.89 g), mp 234° dec.

Anal. Calcd. for $C_8H_{11}N_5S$: C, 45.92; H, 5.30; N, 33.47. Found: C, 45.78; H, 5.35; N, 33.36.

7-(Diethylamino)methyl-6-mercaptopurine (**III**).

This compound was obtained in 71% yield (1.69 g), mp 178° dec.

Anal. Calcd. for $C_{10}H_{15}N_5S$: C, 50.61; H, 6.37; N, 29.51. Found: C, 50.44; H, 6.39; N, 29.43.

7-(Dipropylamino)methyl-6-mercaptopurine (**IV**).

This compound was obtained in 49% yield (1.31 g), mp 184° dec.

Anal. Calcd. for $C_{12}H_{19}N_5S$: C, 54.31; H, 7.22; N, 26.39. Found: C, 54.11; H, 7.20; N, 26.31.

7-(Piperidyl)methyl-6-mercaptopurine (**V**).

This compound was obtained in 99% yield (2.49 g), mp 218° dec, lit [7] mp 207-208°.

Anal. Calcd. for $C_{11}H_{15}N_5S$: C, 52.99; H, 6.06; N, 28.09. Found: C, 52.82; H, 6.11; N, 27.99.

Preparation of 1,7-Di(dipropylamino)methyl-6-mercaptopurine (**VI**).

To 2.20 g (0.02 mole) of dipropylamine was added 0.60 g (0.02 mole) of paraformaldehyde. The suspension that resulted was stirred for 5 minutes before 15 ml of dichloromethane was added to it. The mixture was stirred at room temperature overnight to give a solution which was dried over sodium sulfate. 6-Mercaptopurine hydrate (1.70 g, 0.01 mole) was added to the dichloromethane solution. The suspension that resulted was stirred at room temperature until it became a clear solution (1 hour). The solution was filtered and concentrated to give an oil (3.6 g) which solidified upon standing in a vacuum desiccator for 2 days to give 3.2 g (mp 65-88°, 84% yield) of **VI** as a dry, waxy, pale yellow solid; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.57 (s, 1, 2-*H*), 8.39 (s, 1, 8-*H*), 5.5-5.0 (broad s, 4, N-C*H*$_2$-N), 2.53 (t, 8, J = 7 Hz, N-C*H*$_2$CH$_2$-); ^{1}H nmr (deuteriochloroform): δ 8.68 (s, 1, 2-*H*), 8.03 (s, 1, 8-*H*), 5.49 (s, 2, N-C*H*$_2$-N), 5.16 (s, 2, N-C*H*$_2$-N), 2.63 (t, 4, J = 7 Hz, N-C*H*$_2$-CH$_2$), 2.54 (t, 4, J = 7 Hz, N-C*H*$_2$-CH$_2$).

Anal. Calcd. for $C_{19}H_{34}N_6S$: C, 60.28; H, 9.05; N, 22.20. Found: C, 60.18; H, 9.08; N, 22.15.

The Reaction of **II** with Acetic Anhydride in Deuteriodimethyl Sulfoxide.

To 7-(dimethylamino)methyl-6-MP (100 mg, 0.00048 mole) was added 2 ml of deuteriodimethyl sulfoxide. The clear yellow solution was then allowed to react with 98 mg (0.00096 mole) of acetic anhydride with a trace of sodium acetate. The reaction progress was followed by ^{1}H nmr spectroscopy and hplc. After 20 hours the reaction was completed as determined by the loss of the N-C*H*$_2$-N absorption in the nmr spectrum. The hplc analysis was obtained under the following conditions; Adsorbosphere C-8 reverse-phase column (4.6 mm x 25 cm), (1:3) methanol/-acetate buffer (0.01 *M*, *p*H 5.0) mobile phase, flow rate 0.9 ml/minute. The retention times for various potential components of the reaction mixture under these conditions were: 6-MP (3.3 minutes), 9-acetyloxymethyl-6-MP (4.0 minutes), 7-acetyloxymethyl-6-MP (5.1 minutes), S^6,3-bisacetyloxymethyl-6-MP (7.5 minutes), S^6-acetyloxymethyl-6-MP (10.1 minutes), S^6,9-bisacetyloxymethyl-6-MP (23.1 minutes). The ^{1}H nmr spectra of the reaction mixture in deuteriodimethyl sulfoxide were analyzed using the position and intensities of the 2-*H*, 8-*H* and S-C*H*$_2$-O and/or N-C*H*$_2$-O absorptions; 6-MP: δ 8.47 (s, 1, 2-*H*), 8.28 (s, 1, 8-*H*), 7-acetyloxymethyl-6-MP, S^6,9-bisacetyloxymethyl-6-MP, and S^6,3-bisacetyloxymethyl-6-MP are given later in the experimental, S^6-acetyloxymethyl-6-MP [11]: δ 8.83 (s, 1, 2-*H*), 8.57 (s, 1, 8-*H*) and 6.07 (s, 2, S-C*H*$_2$-O), 9-acetyloxymethyl-6-MP [10]: δ 8.43 (s, 1, 2-*H*), 8.33 (s, 1, 8-*H*) and 6.18 (s, 2, N-C*H*$_2$-O).

The Reaction of **VI** with Acetic Anhydride in Deuteriodimethyl sulfoxide.

To 1,7-di(dipropylamino)methyl-6-MP (250 mg, 0.00066 mole) was added 2 ml of deuteriodimethyl sulfoxide followed by 142 mg (0.0014 mole) of acetic anhydride and a trace of sodium acetate. The progress of the reaction was followed by ^{1}H nmr and hplc as in the previous experiment.

The Reaction of S^6-Acetyloxymethyl-6-MP with Formaldehyde and Piperidine.

A sample (2 ml) of an ether solution (10 ml) of the reaction between

0.63 g (0.0074 mole) of piperidine and 0.22 g (0.0074 mole) of paraformaldehyde was allowed to react with 165 mg (0.00067 mole) of S^6-acetyloxymethyl-6-MP at room temperature overnight with vigorous stirring. The suspension was filtered and the residue dried to give 142 mg (60% yield) of light yellow solid which was identified as the piperidylmethyl derivative of S^6-acetyloxymethyl-6-MP (**X**) by ^{1}H nmr spectroscopy; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.89 (s, 1, 2-*H*), 8.6 (s, 1, 8-*H*), 6.06 (s, 2, S-CH_2-O), 5.2 (s, 2, N-CH_2-N), 2.06 (s, 3, CH_3), 2.6-2.2 (m, 4, N-CH_2CH$_2$) and 1.7-1.0 (m, 6, -CH_2-); and its uv spectrum; uv (acetonitrile): λ max 274 nm (log ε = 4.13) and 280 nm (shoulder, log ε = 4.07).

The Reaction of S^6-Acetyloxymethyl-9-piperidylmethyl-6-MP (**X**) with Acetic Anhydride.

A solution of **X** (95 mg, 0.0003 mole) in 1 ml of pyridine was allowed to react with 0.10 g (≈ 0.001 mole) of acetic anhydride with a trace of sodium acetate at room temperature for 1 hour. The solution was concentrated and an ^{1}H nmr spectrum of the residue showed that it was at least 75% S^6,9-bisacetyloxymethyl-6-MP. The residue was crystallized from acetone to give 15 mg (17% yield, mp 120-122° lit [5] mp 120.5-122.5°) of the S^6,9-derivative; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.93 (s, 1, 2-*H*), 8.68 (s, 1, 8-*H*), 6.26 (s, 2, N-CH_2-O), 6.05 (s, 2, S-CH_2-O) and 2.07 (s, 6, CH_3), and tlc (silica gel, ether) R_f 0.26 were identical with an authentic sample [5].

The Reaction of 6-MP with Aqueous Formaldehyde.

A suspension of 6-MP hydrate (2.5 g, 0.015 mole) in 160 ml of water was treated with 0.2 g of potassium carbonate and 18 ml of 37% aqueous formaldehyde. The initial yellow suspension was stirred at room temperature overnight to give a white suspension. The suspension was filtered and dried to give 2.1 g of solid which was characterized by ^{1}H nmr in deuteriodimethyl sulfoxide (see text).

The Reaction of the Formaldehyde Adduct of 6-MP with Acetic Anhydride and Pyridine.

Acetic anhydride (3 ml, 3.2 g, 0.03 mole) was added to a well-stirred suspension of the formaldehyde adduct of 6-MP (3.6 g, 0.02 mole) in 15 ml of pyridine. The suspension was stirred at room temperature for 3 hours then diluted with 100 ml of ether. The suspension was stirred for an additional 30 minutes then filtered. The residue was washed with ether, dried and suspended in chloroform with stirring overnight. The chloroform suspension was heated to boiling and filtered. The residue was washed three timies with 100 ml of boiling chloroform to give 1.05 g of chloroform insoluble solid which was not processed any further. The combined chloroform solutions were concentrated to 150 ml, cooled and stirred overnight. The chloroform suspension was filtered. That residue was dried to give 0.65 g (15% yield, mp 212-22°) of 7-acetyloxymethyl-6-MP that was 95% pure based on analysis of its ^{1}H nmr spectrum. The 7-acetyloxymethyl-6-MP was recrystallized from methanol to give 0.45 g (mp 229-231°) of analytically pure 7-acetyloxymethyl-6-MP; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.81 (s, 1, 2-*H*), 8.47 (s, 1, 8-*H*), 6.43 (s, 2, N-CH_2-O), 2.09 (s, 3, CH_3); ^{13}C nmr (deuteriodimethyl sulfoxide): δ 171.5 (C-6), 149.8 (C-4), 148.6 (C-8), 146.3 (C-2) and 127.4 (C-5); uv (methanol): λ max 332 nm, 235 nm; uv (methanol, H$^+$): λ max 326 nm, 225 nm; uv (methanol, OH$^-$): λ max 315 nm, 232 nm.

Anal. Calcd. for $C_8H_8N_4O_2S$: C, 42.85; H, 3.60; N, 24.98. Found: C, 42.77; H, 3.65; N, 24.89.

The chloroform filtrate was concentrated to give 1.4 g (24% yield, mp 189-192°) of S^6,3-bisacetyloxymethyl-6-MP that was about 95% pure based on analysis of its ^{1}H nmr spectrum. The bis-compound was recrystallized from acetone to give 0.87 g (mp 198-200°) of analytically pure S^6,3-bisacetyloxymethyl-6-MP, lit [5] mp 200-204°; ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.91 (s, 1, 2-*H*), 8.41 (s, 1, 8-*H*), 6.44 (s, 2, N-CH_2-O), 6.16 (s, 2, S-CH_2-O), 2.08 (s, 6, CH_3); uv (methanol): λ max 322 nm; uv (methanol, H$^+$): λ max 323 nm.

Anal. Calcd. for $C_{11}H_{12}N_4O_4S$: C, 44.59; H, 4.08; N, 18.91. Found: C, 44.66; H, 4.12; N, 18.88.

The Reaction of S^6-Pivaloyloxymethyl-6-MP with Formaldehyde.

A suspension of S^6-pivaloyloxymethyl-6-MP (0.50 g, 0.0019 mole) in 6 ml of 37% aqueous formaldehyde was heated on a hot plate until all the solid went into solution, the solution was not allowed to come to a boil. The solution was then allowed to cool overnight to give white crystals. The crystals were filtered, washed three times with 2 ml of water and dried to give 0.51 g (92% yield, mp 140-142°) of S^6-pivaloyloxymethyl-9-hydroxymethyl-6-MP (see below); ^{1}H nmr (deuteriodimethyl sulfoxide): δ 8.88 (s, 1, 2-*H*), 8.63 (s, 1, 8-*H*), 7.0 (t, 1, J = 7 Hz, CH$_2$-O*H*), 6.08 (s, 2, S-CH_2O), 5.67 (d, 2, J = 7 Hz, CH_2-OH), 1.12 (s, 9, (CH_3)$_3$C); uv (acetonitrile): λ max 274 nm (log ε = 4.24) and 280 nm (shoulder, log ε = 4.15).

Anal. Calcd. for $C_{12}H_{16}N_4O_3S$: C, 48.63; H, 5.44; N, 18.91. Found: C, 48.72; H, 5.46; N, 18.81.

The Reaction of the Formaldehyde Adduct of S^6-Pivaloyloxymethyl-6-MP with Pivaloyl Chloride in Pyridine.

The formaldehyde adduct of S^6-pivaloyloxymethyl-6-MP (0.38 g, 0.0012 mole) was dissolved in 4 ml of pyridine and allowed to react with 0.17 g (0.0014 mole) of pivaloyl chloride for 2 hours. The reaction mixture was concentrated to give a solid which was wet with pyridine. The solid was extracted twice with 40 ml of boiling petroleum ether. The residue that remained weighed 130 mg (39% yield, mp 191-192°, lit [11] mp 189-190°) and was identical with authentic S^6-pivaloyloxymethyl-6-MP based on its ^{1}H nmr spectrum [(deuteriochloroform): δ 8.91 (s, 1, 2-*H*), 8.33 (s, 1, 8-*H*), 6.13 (s, 2, S-CH_2-O), 1.2 (s, 9, (CH_3)$_3$C)] and its tlc [(silica gel, ether) R_f 0.13]. The petroleum ether extract was concentrated to 2 ml and allowed to cool. The crystals that formed were filtered to give 85 mg (17% yield, mp 86-89°), lit [3] mp 87-89°) of white solid that was identical with authentic S^6,9-bispivaloyloxymethyl-6-MP based on its ^{1}H nmr spectrum [(deuteriochloroform): δ 8.88 (s, 1, 2-*H*), 8.30 (s, 1, 8-*H*), 6.23 (s, 2, N-CH_2-O), 6.10 (s, 2, S-CH_2-O), 1.19 (s, 18, (CH_3)$_3$C)] and its tlc [(silica gel, ether) R_f = 0.50]. An additional 35 mg (mp 81-87°, 7% yield) of the S^6,9-derivative (one spot on tlc analysis) was obtained upon concentration of the mother liquor.

Acknowledgement.

This work was supported by NIH Grant R01-AM33957.

REFERENCES AND NOTES

[1] Present address: Wyeth-Ayerst Laboratories, Rouses Point, NY, 12979.

[2] K. B. Sloan and N. Bodor, *Int. J. Pharm.*, **12**, 299 (1982).

[3] K. B. Sloan, M. Hashida, J. Alexander, N. Bodor and T. Higuchi, *J. Pharm. Sci.*, **72**, 372 (1983).

[4] K. B. Sloan, S. A. M. Koch and K. G. Siver, *Int. J. Pharm.*, **21**, 251 (1984).

[5] R. P. Waranis and K. B. Sloan, *J. Pharm. Sci.*, **76**, 587 (1987).

[6] K. G. Siver, "Prodrug Approaches to Increase the Topical Delivery of 6-Mercaptopurine", Ph.D. Dissertation, University of Florida, 1987.

[7] C. P. Bryant and R. E. Harmon, *J. Med. Chem.*, **10**, 104 (1967).

[8] H. Bundgaard and M. Johansen, *Int. J. Pharm.*, **9**, 7 (1981).

[9] M.-T. Chenon, R. J. Pugmire, D. M. Grant, R. P. Panzica and L. B. Townsend, *J. Am. Chem. Soc.*, **97**, 4627 (1975).

[10] H. J. Roth and R. Brandes, *Arch. Pharm.*, **298**, 765 (1965).

[11] R. P. Waranis and K. B. Sloan, *J. Pharm. Sci.*, **77**, 210 (1988).

[12] M. Rasmussen and N. J. Leonard, *J. Am. Chem. Soc.*, **89**, 5439 (1967).