Studies on the Molybdenum Cofactor. An Unequivocal Total Synthesis of (±)-Urothione

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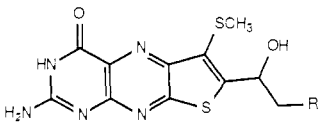
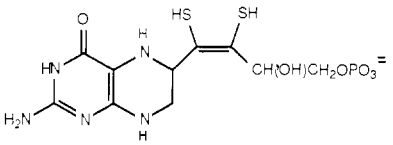
Abstract: Urothione, the urinary metabolite of the molybdenum cofactor, has been synthesized in racemic form in 10 steps from pyrazine **3** in 19% overall yield. Thus, a thiophene ring was fused to pyrazine **3** to give a thieno[2,3-*b*]pyrazine (**14**) which contained latent functionality suitable for the introduction of all substituents in the natural product. The 7-amino group of **14** was converted to a methylthio substituent in two steps, and this transformation was followed by reduction of the ketone of **16** to give **17**, which possesses the requisite 1,2-diol oxidation state on the ethyl side chain. Subsequent adjustment of the protecting groups was followed by pyrimidine ring fusion, giving the 2,4-diaminopteridine **21**. Final acid treatment then gave (±)-urothione (**1**), whose physical, spectral, and chromatographic properties were identical with those of urothione isolated from human urine. This unequivocal synthesis confirms the structural assignment **1** for this unique natural product and makes available for the first time a reasonable source of urothione for further study.

In 1940 Koschara[1] found that a crude mixture of pterins which he had isolated from human urine gave a striking color reaction with warm 80% sulfuric acid which could not be attributed to any previously known pterin. Isolation of a very small amount of this material from large quantities of urine was finally achieved, and a series of degradation and spectroscopic investigations established some but not all of the structural features of this unusual natural product. The urothione structural problem was next attacked by Tschesche in the mid-1950s, who was motivated by the still unknown biological role of urothione.[2,3] He was, however, frustrated by extreme difficulties in obtaining sufficient amounts of material,[4] and many of his structural deductions have subsequently been shown to be in error. In the late 1960s, Goto and co-workers took up the urothione structural problem anew and were finally able, on the basis of more extensive degradation experiments, to propose structure **1**.[5-7] Goto subsequently reported a total synthesis[8,9]



1 R = OH UROTHIONE
2 R = H DEOXYUROTHIONE

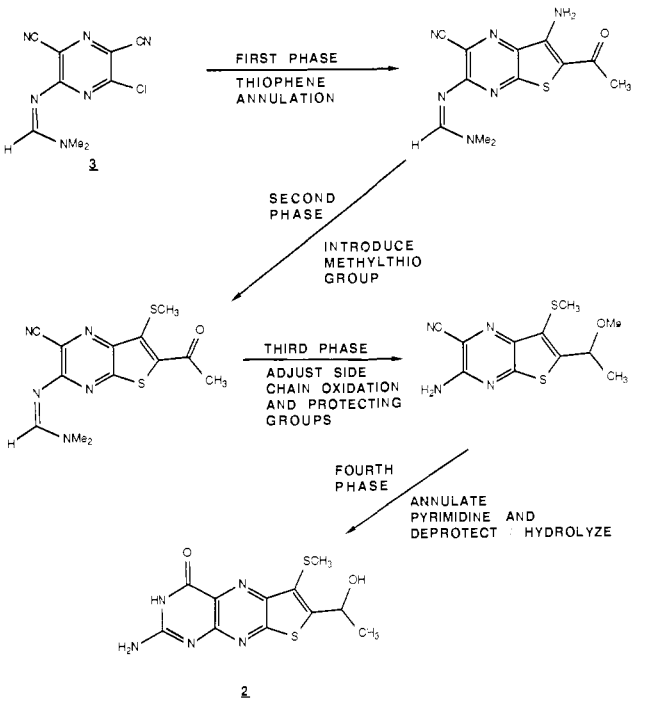


MOLYBDOPTERIN

(in extremely low yield—0.3% from a preformed pterin) of material which appeared to be identical with urothione both spectroscopically and chromatographically. Strong confirmation in support of structure **1** was more recently obtained by an unequivocal total synthesis of deoxyurothione and comparison of its spectral properties with those of **1**.[10]

The biological role of this unique sulfur-containing pterin, however, remained a mystery until 1982. Johnson and Rajagopalan had been investigating a number of young patients suffering from biochemical abnormalities resulting from deficiencies of sulfite oxidase and xanthine dehydrogenase.[11] They were able to show that these patients lacked what has been termed the molybdenum cofactor *and that urine samples from these patients*

Scheme I



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did not contain urothione.[12] A metabolic link between the molybdenum cofactor and urothione was thus suggested, and

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(1) Koschara, W. *Z. Phys. Chem.* **1940**, *263*, 78; **1943**, *277*, 284; **1943**, *279*, 44.
(2) Tschesche, R.; Korte, F.; Heuschkel, G. *Chem. Ber.* **1955**, *88*, 1251.
(3) Tschesche, R.; Heuschkel, G. *Chem. Ber.* **1956**, *89*, 1054.
(4) Tschesche, R. In *Chemistry and Biology of Pteridines*; Wolstenholme, G. E. W., Cameron, M. P., Eds.; Churchill: London, 1954; pp 135-142. (In response to questions by other symposium participants as to whether he had performed one experiment or another, he replied "We have not had enough material to make so many experiments. It is the headache of this investigation. We have had only 30 mg of natural material.")
(5) Goto, M.; Sakurai, A.; Yamakami, H. *Nippon Kagaku Zasshi* **1967**, *88*, 897 (*Chem. Abstr.* **1968**, *69*, 52107*j*).
(6) Goto, M.; Sakurai, A.; Ohta, K.; Yamakami, H. *Tetrahedron Lett.* **1967**, 4507.
(7) Goto, M.; Sakurai, A.; Ohta, K.; Yamakami, H. *J. Biochem.* (*Tokyo*) **1969**, *65*, 611.
(8) Katurai, A.; Goto, M. *Tetrahedron Lett.* **1968**, 2941.
(9) Sakurai, A.; Goto, M. *J. Biochem.* (*Tokyo*) **1969**, *65*, 755.
(10) Taylor, E. C.; Reiter, L. A. *J. Org. Chem.* **1982**, *47*, 528.
(11) Wadman, S. K.; Duran, M.; Beemer, F. A.; Cats, B. P.; Johnson, J. L.; Rajagopalan, K. V.; Saudubray, J. M.; Ogier, H.; Charpentier, C.; Bergber, R.; Smit, G. P. A.; Wilson, J.; Krywawych, S. *J. Inherited Metab. Dis. 6* **1983**, 78, (Suppl 1).

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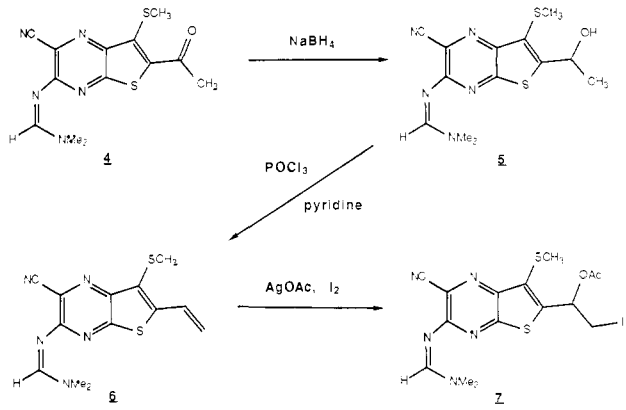
Scheme II



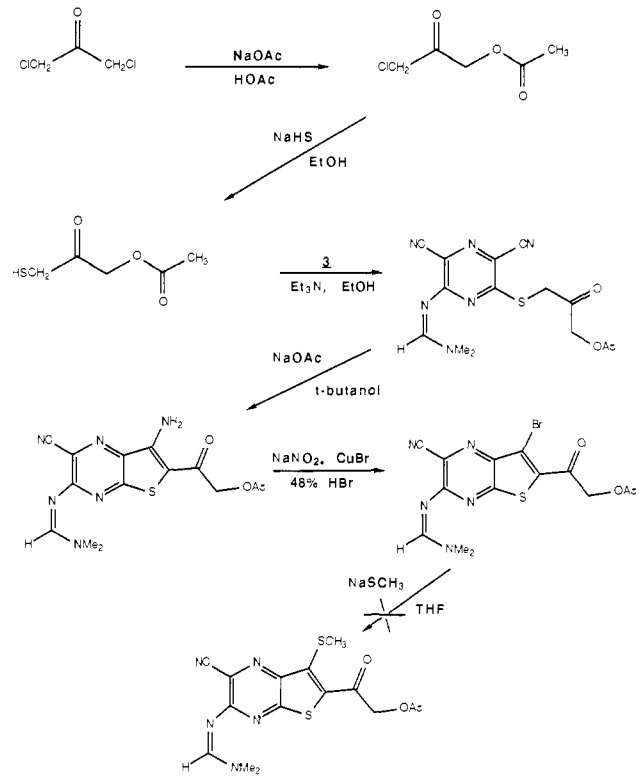
subsequent structural studies on the molybdenum cofactor have established that urothione is, in fact, the urinary metabolite of the pterin portion (termed molybdopterin) of the molybdenum cofactor.[13]

As a part of program currently under way in our laboratory aimed at the total synthesis of the molybdenum cofactor, molybdopterin, and certain oxidative degradation products of molybdopterin (forms A and B),[13] we now report a total synthesis of urothione by an unambiguous sequence of synthetic reactions which firmly establishes structure **1** as correct and provides methodology which should prove to be useful in synthetic approaches to the family of sulfur-containing natural products derived from the molybdenum cofactor.

A few years ago we reported an unambiguous synthesis of a monodeoxy analogue of urothione.[10] This model study took advantage of methods developed by us in the early 1970s[14,15] which largely avoid the classical problems of pteridine synthesis. Thus, the pyrimidine ring of the pteridine system is not annulated until late in the synthetic sequence, at which point the functionalities destined to be at positions 6 and 7 of the pteridine ring have been introduced and suitably protected. This model synthesis began with pyrazine **3** (see Scheme I) and proceeded to monodeoxy urothione **2** through four principal synthetic phases. In the first phase, a thiophene ring was fused across C-5 and C-6 of the pyrazine ring by a sequence of two steps. The first of these involved displacement of the C-6 chloro group of **3** with an α-mercapto ketone, which provided the sulfur atom of the eventual thiophene ring as well as C-7 and the C-7 ethyl group of the final thienopteridine. The second step involved treatment with base, which induced ring closure and provided an amino group at the β-position of the fused thiophene ring which, in the second phase of the synthesis, was manipulated to introduce the requisite β-methylthio substituent. In the third phase, the oxidation state of the ethyl side chain was adjusted, as were the protecting groups on the side-chain alcohol and the pyrazine 2-amino group. Finally, the pyrimidine ring was annulated and the protecting groups removed. We now describe a successful adaptation of this model strategy to the synthesis of racemic urothione.

In order to extend the above model study to a preparation of urothione, we needed at some point in the synthetic sequence to incorporate a second hydroxy group on the 7-ethyl side chain of the thienopteridine nucleus. Two approaches were examined toward this end. This first of these involved further functionalization (i.e. oxidation) of one of the intermediates from the model study. The second approach involved introduction of the requisite hydroxy group, in a latent form, as part of the α-mercapto ketone, with the intent of carrying this latent alcohol through a sequence

Scheme III



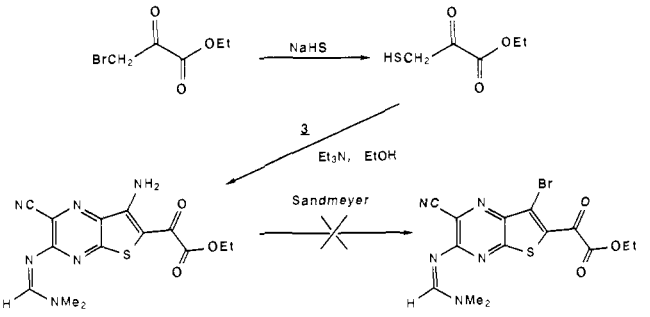
of reactions paralleling all four phases of the model study.

During attempted execution of the first approach, we examined the oxidation of intermediates containing both the 6-methylthio group and the amidine-protected 2-amino functionality in the pyrazine starting material, i.e. compounds **4** and **5** (see Scheme II). It was expected that selective carbon oxidation of these compounds would be difficult, but because of the limited opportunities available for introduction of the methylthio group, it was hoped that manipulation of **4** and **5** might provide an adequate opportunity of diverting compounds from the model series to the natural series. In the event, however, we were unable to oxidize selectively the methyl ketone of intermediate **4**. In addition to the obvious chemical interference by the methylthio group, our efforts were frustrated both by the apparently extremely limited enolization of the methyl ketone under a variety of reaction conditions and by the extremely low solubility of 4, which precluded the use of many possible oxidation procedures. By contrast, the olefin **6**, derived by dehydration of alcohol **5**, could be selectively oxidized under Prevost conditions, although a variety of other oxidants proved to be unselective. Unfortunately, the iodo acetate product of the Prevost reaction (**7**) could not be further selectively transformed into a useful intermediate. Attempts to replace the iodo functionality with an oxygen-containing moiety resulted, among other reactions, in both dehydroiodination and intramolecular cyclizations. These failures to transform either **4** or **5** into useful intermediates for the synthesis of urothione led us to focus our efforts on the second approach.

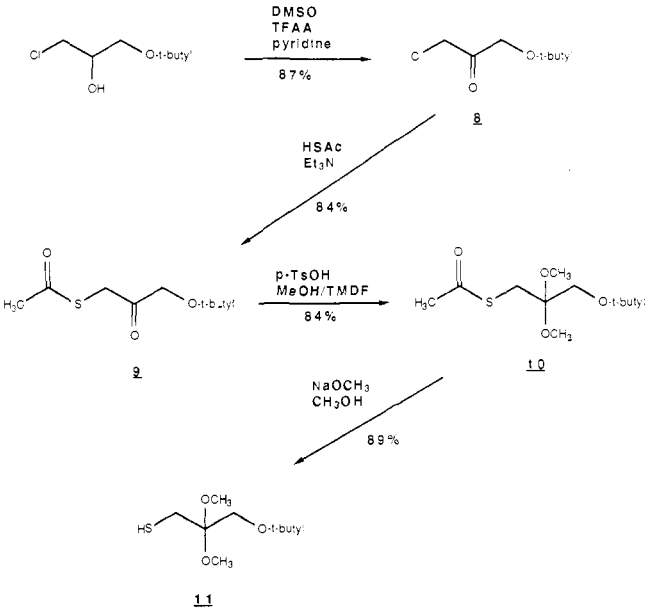
Our first attempt to carry the second hydroxy group of the 7-ethyl side chain through all four phases of the projected synthetic sequence involved the use of an acetoxy group as the latent alcohol (see Scheme III). This protected alcohol funtionality, however, proved to be insufficiently robust. Thus, in the second phase of the sequence, the Sandmeyer reaction proceeded in poor yield, both under the conditions used in the model series and under those described by Doyle,[16] and attempts to introduce the methylthio group led to extensive decomposition. We then sought to carry the hydroxy group through the synthetic sequence in the form of an ester which would eventually be reduced to a hydroxymethyl

(12) Johnson, J. L.; Rajagopalan, K. V. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6856.
(13) Kramer, S. P.; Johnson, J. L.; Ribeiro, A. A.; Millington, D. S.; Rajagopalan, K. V. *J. Biol. Chem.* **1987**, *262*, 16357.
(14) Taylor, E. C.; Perlman, K. L.; Sword, I. P.; Sequin-Frey, M.; Jacobi, P. A. *J. Am. Chem. Soc.* **1973**, *95*, 6407.
(15) Taylor, E. C.; Perlman, K. L.; Kim, Y.-H.; Sword, I. P.; Jacobi, P. A. *J. Am. Chem. Soc.* **1973**, *95*, 6413.
(16) Doyle, M. P.; Siegfried, B.; Dellaria, J. F., Jr. *J. Org. Chem.* **1977**, *42*, 2426.

Scheme IV



Scheme V



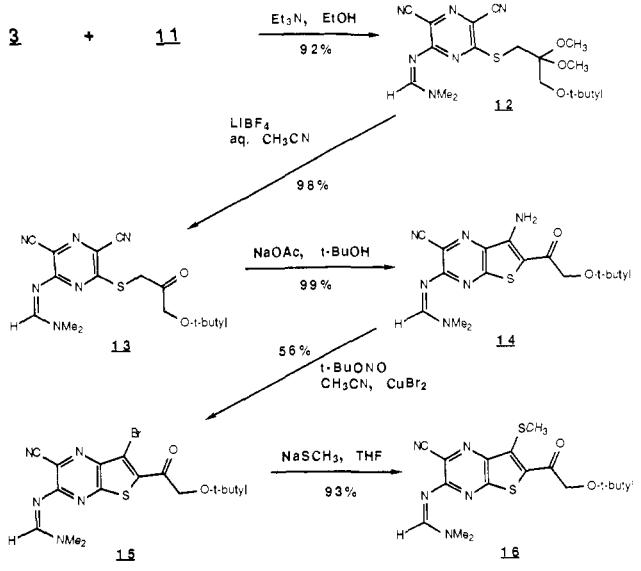
group late in the reaction sequence. This strategy failed because the substantial electron-withdrawing property of the α-ketoester functionality blocked diazotization of the β-situated amino group (see Scheme IV).

We then examined the possible utilization of a *tert*-butyl ether as a latent primary hydroxy group in the hope that such a functionality would be stable through the projected sequence of synthetic reactions, that it would be sufficiently removed from reaction sites so that steric problems would not arise, and that it might be removed simultaneously with the 4-amino group of the projected 2,4-diaminopteridine intermediate **21** when the latter was to be converted to a pterin. The required highly functionalized mercaptan (**11**) was synthesized as outlined in Scheme V. Thus, 1-*tert*-butoxy-3-chloro-2-propanol was converted to the ketone **8** by a Pfitzner–Moffatt oxidation.[17] Treatment of **8** with thiolacetic acid gave the expected thiolester **9**, which was converted to its ketal **10** with trimethyl orthoformate in methanol. The thiolacetate grouping was then efficiently cleaved with sodium methoxide in methanol to give the desired mercaptan **11**. The overall yield for these four steps was 55%. It was necessary to protect the ketone in this intermediate as a ketal, since attempts to prepare the α-mercapto ketone itself from either **8** or **9** failed.

With the requisite mercaptan **11** now in hand, we were ready to examine the first phase of the projected synthetic sequence to urothione—annulation of the thiophene ring onto pyrazine **3**. Thus, reaction of **3** with **11** in ethanol in the presence of triethylamine yielded the desired sulfide **12** in excellent yield (see Scheme VI). In order to activate the methylene group α to sulfur prior to thiophene ring annulation, cleavage of the ketal in **12** was necessary, but this proved to be difficult because of the need to retain the acid-sensitive formamidine protecting group on the

(17) Pero, R. W.; Babiaz-Tracy, P.; Fondy, T. P. *J. Med. Chem.* **1977**, *20*, 644.

Scheme VI



2-amino substituent in the pyrazine ring. Selective ketal cleavage by exchange with acetone in the presence of *p*-toluenesulfonic acid monohydrate was moderately successful, but it was capricious because of its critical dependence on trace concentrations of water in the solvent. This problem of selective ketal cleavage was effectively solved, however, by utilization of lithium tetrafluoroborate in aqueous acetonitrile,[18] which led in quantitative yield to the desired ketone **13**. Completion of the first phase in this synthetic sequence, involving cyclization of **13** to the thienopyrazine **14**, was effected, again in quantitative yield, by treatment of **13** with sodium acetate in *tert*-butyl alcohol.

Introduction of a methylthio group, as the second phase in the reaction sequence, could not be accomplished under the conditions which we had shown to be successful in the model series. Presumably this was because of the sensitivity of the *tert*-butyl ether in **14** to the cold 48% hydrobromic acid employed in the Sandmeyer conversion of the amino group of **14** to a bromo substituent. Fortunately, application of Doyle's procedure[16] to **14** led to a moderate yield of the desired bromo compound **15**, although, as in the model series, we were unable to account for material lost during this step. Although TLC analysis of the crude mixture indicated that a clean conversion of the amine to the bromo compound had occurred, we were unable to obtain **15** consistently in better than 60% yield despite extensive modifications of the reaction and workup procedures. Subsequent treatment of **15** with sodium methylmercaptide resulted in a clean and high-yield conversion to the desired sulfide **16**, thus completing the second phase of the reaction sequence.

The third phase—adjustment of side-chain oxidation state and protecting groups—began smoothly with sodium borohydride reduction of **16** to alcohol **17** (see Scheme VII). At this point in the model series, we had found it necessary to protect the "benzylic" hydroxy group on the ethyl side chain before deprotecting the 2-amino group in the pyrazine ring. This step had been accomplished in the model series by treatment of the alcohol with *p*-toluenesulfonic acid in a 1:1 mixture of trimethyl orthoformate and methanol, which resulted in rapid conversion of the hydroxy group to a methoxy grouping prior to the (slower) cleavage of the formamidine protecting group in the pyrazine ring. On the assumption that similar protection of the "benzylic" hydroxy grouping would be necessary in the natural series, **17** was subjected to the same reaction conditions. However, conversion of the hydroxy group into the corresponding methoxy moiety was not as rapid as in the model series, leading to a mixture of the desired **19** and the fully deprotected derivative **20**, which could not then be further converted to **19**. This problem could be avoided, however, by converting **17** to **19** in two discrete steps, the first

(18) Lipshutz, B. H.; Harvey, D. F. *Synth. Commun.* **1982**, *12*, 267.

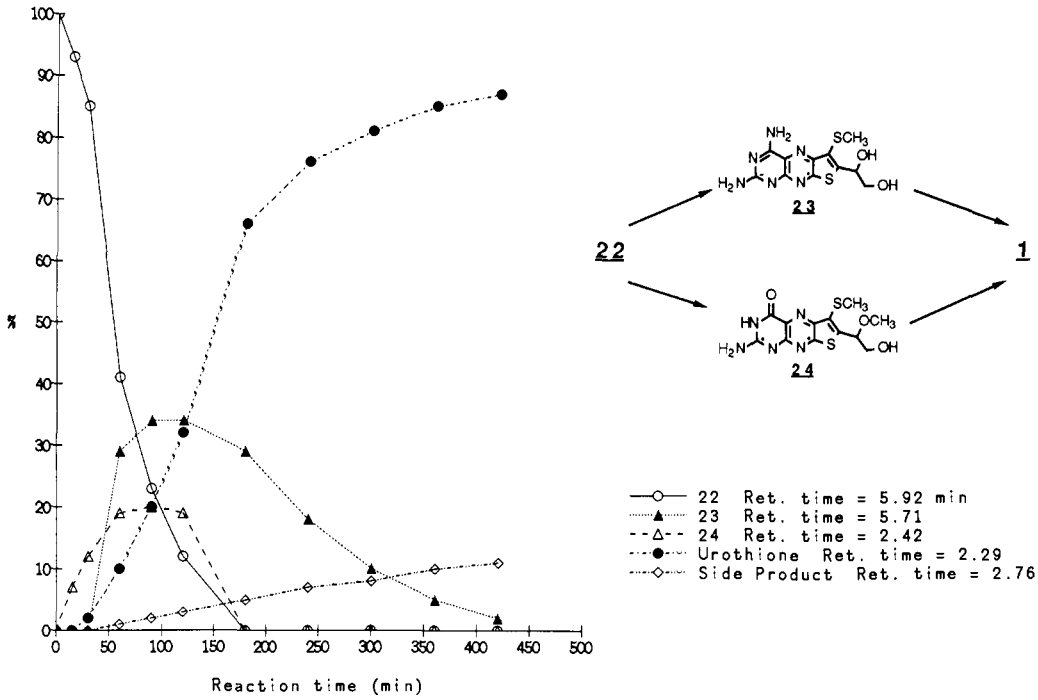


Figure 1. Hydrolysis of **22** in 3 M phosphoric acid. The HPLC analysis was performed on a Rainin HPX system using a Rainin 5 μM C-8 Microsorb column (4.6 × 250 mm) and eluting with 85% pH 2.5 phosphate buffer/15% acetonitrile at a flow rate of 1 mL/min and with UV detection at 254 nm.

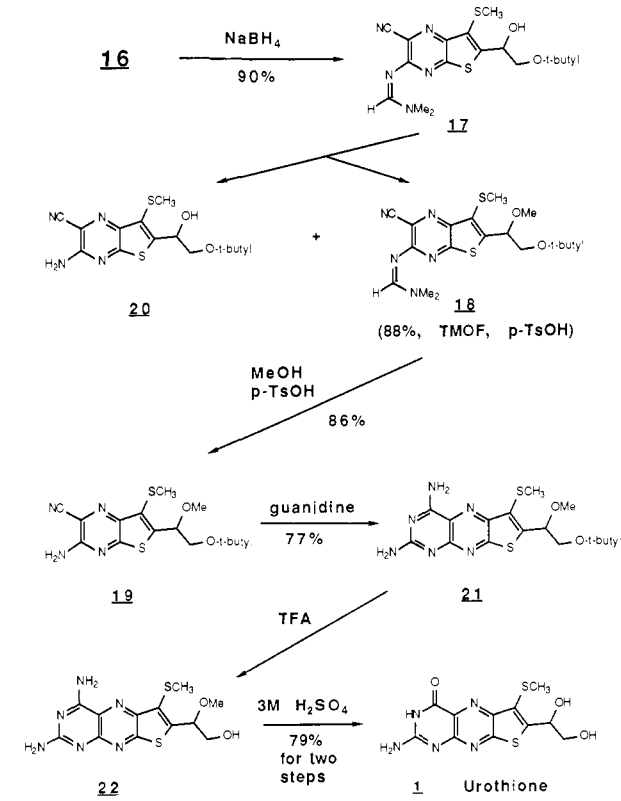
involving introduction of the "benzylic" methoxy group by treatment of **17** with an acid catalyst and trimethyl orthoformate and the second involving cleavage of the amidine functionality in **18** with an acid catalyst in methanol. In this way, **19** could be prepared from **17** in 67% yield.

With compound **19** now in hand, we were ready to investigate the final phase of the projected urothione synthesis—pyrimidine ring annulation and deprotection/hydrolysis. Treatment of **19** with an excess of guanidine in refluxing methanol gave the 2,4-diaminopyrimidine derivative **21** in 77% yield. Removal of the protecting groups and hydrolysis of the 4-amino group in **21** were the only remaining operations. Our model study had demonstrated that the "benzylic" methoxy group on the 7-ethyl side chain as well as the 4-amino group in the pyrimidine ring could be removed with refluxing 1 N HCl over the course of 2 h. On the assumption that cleavage of the *tert*-butoxy group in **21** would also occur under these conditions, we treated **21** with 1 N HCl under reflux. Rapid formation of a dark reaction mixture, accompanied by the precipitation of unidentified decomposition products, indicated that a more careful study of this reaction was mandatory.

Treatment of **21** with trifluoroacetic acid led to rapid cleavage of the *tert*-butyl ether to give the monodeprotected intermediate **22**, which, in contrast to **21**, was soluble in aqueous acid. By subjecting **22** to refluxing 1 N HCl, 1 M H_2SO_4, or 3 M H_3PO_4, we found that hydrolysis of the 4-amino group and cleavage of the "benzylic" methoxy group occurred at similar rates (see Figure 1). As a result, two different intermediates to urothione, **23** and **24**, were observed. Hydrolysis of the 4-amino group in the annulated pyrimidine ring was clearly much slower in the natural series than it had proved to be in the model study; intermediate **23** was not consumed until 6–7 h of refluxing, by which time a significant amount of an unidentified side product was also formed. In contrast, however, hydrolysis of **22** with 3 M H_2SO_4 led to a more rapid reaction and the smooth conversion of **22** to (±)-urothione within 30 min. The product was isolated by precipitation as originally described by Koschara.

The material as isolated was shown by FAB mass spectroscopy to have a molecular ion at *m/e* 326 (protonated urothione). The UV and IR spectra of this material were essentially superimposable on those published,[7] and its ^{1}H NMR spectrum was consistent with that expected for structure **1**. Furthermore, a triacetate prepared from our synthetic (±)-urothione exhibited spectral

Scheme VII



characteristics (NMR, UV, IR) consistent with structure **1** and which matched those that had been previously published.[7] Finally our sample of synthetic (±)-urothione was compared directly with urothione isolated from human urine; the two samples were identical by HPLC analysis in a variety of solvent systems.[19]

(19) We are deeply indebted to Prof. K. V. Rajagopalan of the Department of Biochemistry, Duke University Medical Center, Durham, NC 27710, for isolating urothione from human urine and carrying out the HPLC comparisons of our synthetic (±)-urothione with the natural product.

This synthesis of urothione and its comparison with the natural product rigorously confirms the structural assignment **1** for this molybdenum cofactor urinary metabolite. The only remaining structural question is the absolute configuration of the side-chain hydroxy group. We plan to assign this stereochemistry indirectly by determining the side-chain stereochemistry of the molybdenum cofactor degradation product form A by a chiral synthesis of both enantiomers and comparison (HPLC analysis using chiral columns) with natural form A isolated from the cofactor.

This efficient synthesis of urothione (10% overall yield in 14 steps from previously known intermediates) now makes possible the preparation of sufficient quantities for further study of its chemistry and biological properties.

Experimental Section

2-Cyano-3-[((dimethylamino)methylene)amino]-7-(methylthio)-6-vinylthieno[2,3-*b*]pyrazine (6). The alcohol **5**[10] (1.61 g, 5.00 mmol) was dissolved in dry pyridine (50 mL) and treated with phosphorus oxychloride (1.54 g, 10.0 mmol). After stirring for 30 min at room temperature, the mixture was refluxed for 4 h. The cooled reaction mixture was diluted with ethyl acetate (300 mL) and washed with 1 N HCl (3 × 100 mL), 5% sodium bicarbonate solution (100 mL), and saturated ammonium chloride solution (100 mL). The extract was dried ($MgSO_4$), filtered, and evaporated in vacuo. The residual solid was recrystallized from methanol (Norite) to give 1.07 g (71%) of orange flakes. **6**: mp 176–177 °C (becomes a glass); mass spectrum, *m/e* (relative intensity) M^+ 303 (43.7), 288 (100), 261 (5.6), 236 (13.4); ^1H NMR ($CDCl_3$) δ 2.54 (3 H, s) (SMe), 3.22 (6 H, s) (NMe_2), 5.48 (1 H, d, *J* = 11 Hz) (CH=C*H*, cis), 5.75 (1 H, d, *J* = 18 Hz) (CH=C*H*, trans), 7.40 (1 H, dd, *J* = 11, 18 Hz) (C*H*=CH_2), 8.68 (1 H, s) (CH=N); IR (KBr) 2210 (CN), 1610 (ring C=N), 970, 905 (olefin) cm^{-1}. Anal. Calcd for $C_{13}H_{13}N_5S_2$ (303.40): C, 51.46; H, 4.32; N, 23.09; S, 21.14. Found: C, 51.23; H, 4.55; N, 22.98; S, 20.89.

6-(1-Acetoxy-2-iodoethyl)-2-cyano-3-[((dimethylamino)methylene)-amino]-7-(methylthio)thieno[2,3-*b*]pyrazine (7). The olefin **6** (100 mg, 0.330 mmol) and silver acetate (220 mg, 1.32 mmol) were slurried together in dry ether (20 mL). Iodine (167 mg, 0.660 mmol) was added in one portion, and after 2 h, the excess oxidant was quenched with 5% sodium bisulfite solution (10 mL). The mixture was filtered through Celite, and the silver satls were washed with ether and ethyl acetate. The organic layer in the filtrate was separated, washed with 5% sodium bicarbonate solution (3 × 25 mL) and saturated ammonium chloride solution (25 mL), dried ($MgSO_4$), filtered, and evaporated in vacuo. The residual solid was recrystallized from ethyl acetate/cyclohexane to give 95 mg (59%) of golden crystals. **7**: mp 152–153 °C (gas evolution and resolidification); mass spectrum, *m/e* (relative intensity) M^+ 489 (4.0), 320 (28.9), 306 (19.5), 288 (50.3), 287 (77.2), 259 (25.4), 254 (33.3), 245 (41.7), 232 (56.7), 141 (100), 127 (40.6), 73 (89.3); ^1H NMR ($CDCl_3$) δ 2.19 (3 H, s) ($COCH_3$), 2.63 (3 H, s) (SMe), 3.26 (6 H, s) (NMe_2), 3.61 (2 H, d, *J* = 6.5 Hz) (OCHCH_2I), 6.63 (1 H, t, *J* = 6.5 Hz) (OC*H*CH_2I), 8.70 (1 H, s) (CH=N); IR (KBr) 2220 (CN), 1740 (ester), 1615 (ring C=N) cm^{-1}. Anal. Calcd for $C_{15}H_{16}IN_5O_2S_2$ (489.36): C, 36.81; H, 3.30; N, 14.31; S, 13.10, I, 25.93. Found: C, 37.02; H, 3.52; N, 14.33; S, 12.91; I, 26.07.

1-Acetoxy-3-mercapto-2-propanone. A solution of sodium (575 mg, 25.0 mmol) in dry ethanol (50 mL) was saturated with hydrogen sulfide and cooled in an ice bath. 1-Acetoxy-3-chloro-2-propanone[21] (3.01 g, 20.0 mmol) in ethanol (10 mL) was then added dropwise. A precipitate of sodium chloride formed instanteously. After complete addition, the mixture was stirred for 1 h at 4 °C. The sodium chloride was removed by filtration and washed with ethanol. The filtrates were concentrated, and the residual oil was dissolved in ether (50 mL). A small amount of solid was removed by filtration, and the filtrates were concentrated in vacuo. The residual yellow oil crystallized upon trituration with a small portion of ether. The solid was collected and air-dried, yielding 1.23 g (43%) of a slightly odiferous, white powder. An analytical sample was obtained by recrystallization from toluene/cyclohexane: mp 100–102 °C; IR (KBr) 3450 (OH), 1750 (acetate) cm^{-1}. Anal. Calcd for $C_5H_8O_3S$ (148.18): C, 40.53; H, 5.44; S, 21.64. Found: C, 40.81; H, 5.22; S, 22.02.

1-Acetoxy-3-[[3,5-dicyano-2-[((dimethylamino)methylene)amino]-6-pyrazinyl]thio]-2-propanone. Chloropyrazine **3**[10] (502 mg, 2.14 mmol) and the above mercaptan (380 mg, 2.56 mmol) were slurried together in dry ethanol (20 mL). Triethylamine (237 mg, 2.35 mmol) was added in one portion; the reaction ensued immediately. After 45 min, the reaction mixture was cooled to −20 °C for a few hours. The precipitated product was collected, washed with cold ethanol, and recrystallized from 2-butanone to give 650 mg (88%) of a yellow powder. An analytical sample was prepared by recrystallization from acetone: mp 225–226 °C; mass spectrum, *m/e* (relative intensity) M^+ 346 (31.8), 304 (3.6), 273 (100), 246 (47.3), 245 (63.5), 231 (16.3); ^1H NMR (Me_2SO-d_6) δ 2.09 (3 H, s) ($COCH_3$), 3.19, 3.28 (6 H, 2 s) (NMe_2), 4.38 (2 H, s) (CH_2S), 5.01 (2 H, s) (CH_2O), 8.78 (1 H, s) (CH=N); IR (KBr) 2240, 2230 (CN's), 1750 (ester), 1730 (ketone) 1630 (ring C=N) cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_6O_3S$ (346.37): C, 48.54; H, 4.07; N, 24.27; S, 9.26. Found: C, 48.41; H, 4.01; N, 24.02; S, 9.17.

6-(Acetoxyacetyl)-7-amino-2-cyano-3-[((dimethylamino)methylene)-amino]thieno[2,3-*b*]pyrazine. A mixture of the above α-keto sulfide (1.04 g, 3.00 mmol), potassium acetate (300 mg, 3.00 mmol), and some 3-Å molecular sieves was refluxed for 1 h in dry *tert*-butyl alcohol (75 mL). The reaction mixture was cooled to room temperature, diluted with ether (75 mL), and cooled briefly in an ice bath. The orange precipitate was collected, washed with ether, water, and ether again, and recrystallized from acetone with hot filtration to give 990 mg (95%) of bright orange needles: mp 241–242 °C; mass spectrum, *m/e* (relative intensity) M^+ 346 (33.2), 304 (6.2), 273 (100), 259 (4.6), 245 (13.6), 218 (8.5); ^1H NMR (Me_2SO-d_6) δ 2.15 (3 H, s) ($COCH_3$), 3.18, 3.27 (6 H, 2 s) (NMe_2), 4.98 (2 H, s) (CH_2O), 7.88 (2 H, br s) (NH_2), 8.82 (1 H, s) (CH=N); IR (KBr) 3410, 3300 (NH_2), 2225 (CN), 1740 (ester), 1610 (ring C=N, ketone) cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_6O_3S$ (346.37): C, 48.54; H, 4.07; N, 24.27. Found: C, 48.75; H, 4.29; N, 24.05.

6-(Acetoxyacetyl)-7-bromo-2-cyano-3-[((dimethylamino)methylene)-amino]thieno[2,3-*b*]pyrazine. The above amine (350 mg, 1.00 mmol) and cuprous bromide (150 mg, 1.00 mmol) were combined in ice cold 48% hydrobromic acid (35 mL). A solution of sodium nitrite (140 mg, 2.00 mmol) in water (4 mL) was added dropwise with vigorous stirring. Fifteen minutes after complete addition, the mixture was diluted with saturated sodium bromide solution (75 mL) and extracted with chloroform (4 × 25 mL). The chloroform extracts were washed with water (3 × 25 mL) and concentrated in vacuo to give a yellow solid, which was recrystallized from toluene/cyclohexane (Norite) to give 120 mg (29%) of yellow needles: mp 233–234 °C; mass spectrum, *m/e* (relative intensity) M^+ 409, 411 (6.6, 6.6), 336, 338 (100, 99.3), 229 (22.4), 187 (13.9); ^1H NMR (Me_2SO-d_6) δ 2.17 (3 H, s) ($COCH_3$), 3.21, 3.29 (6 H, 2 s) (NMe_2), 5.53 (2 H, s) (CH_2O), 8.87 (1 H, s) (CH=N); IR (KBr) 2220 (CN), 1740 (ester), 1670 (ketone), 1615 (ring C=N) cm^{-1}. Anal. Calcd for $C_{14}H_{12}BrN_5O_3S$ (410.27): C, 40.98; H, 2.95; N, 17.07; S, 7.82. Found: C, 40.99; H, 2.98; N, 17.13; S, 7.58.

Ethyl [7-Amino-3-cyano-2-[((dimethylamino)methylene)amino]-6-thieno[2,3-*b*]pyrazinyl]formate. Sodium (253 mg, 11.0 mmol) was dissolved in dry ethanol (80 mL), and this solution was saturated with hydrogen sulfide and cooled to 4 °C. Ethyl bromopyruvate (1.95 g, 10.0 mmol) in ethanol (10 mL) was added dropwise with cooling, and after complete addition, the mixture was stirred at 4 °C for 1 h. The white precipitate of ethyl mercaptopyruvate was collected, washed with ethanol and ether, and air-dried to give 0.95 g (64%) of a white, odiferous powder. This was slurried in 95% ethanol (55 mL) with chloropyrazine **3**[10] (1.25 g, 5.34 mmol). Triethylamine (593 mg, 5.87 mmol) was added; the mixture immediately became an orange solution. After a few seconds an orange precipitate formed, and after stirring for 1 h at room temperature, the reaction mixture was cooled to −20 °C for a few hours. The precipitate was collected, washed with ethanol, and air-dried to give 1.18 g (64%) of an orange powder. An analytical sample was prepared by recrystallization from a large volume of acetone: mp 285–286 °C; mass spectrum, *m/e* (relative intensity) M^+ 346 (22.3), 273 (100), 245 (6.5); ^1H NMR (Me_2SO-d_6) δ 1.32 (3 H, t, *J* = 7.1 Hz) (ester CH_3), 3.19, 3.29 (6 H, 2 s) (NMe_2), 4.31 (2 H, q, *J* = 7.1 Hz) (ester CH_2), 8.84 (1 H, s) (CH=N); IR (KBr) 3420, 3300 (NH_2), 2220 (CN), 1705 (ester) 1605 (ring C=N, ketone) cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_6O_3S$ (346.37): C, 48.54; H, 4.07; N, 24.27; S, 9.26. Found: C, 48.62; H, 4.02; N, 24.26; S, 9.26.

1-*tert*-Butoxy-3-chloro-2-propanone (8). 1-*tert*-Butoxy-3-chloro-2-propanol[22] (33.3 g, 0.200 mol), dicyclohexylcarbodiimide (82.5 g, 0.400 mol), pyridine (3 mL), and Me_2SO (20 mL) were dissolved in dry ether (400 mL). This mixture was cooled to 4 °C, trifluoroacetic acid (3 mL) was added, and the reaction was removed from the ice bath. After about 30 min, the reaction spontaneously warmed to a gentle reflux which soon subsided. After cooling back to room temperature, the reaction mixture was placed in an ice bath, and oxalic acid (25 g) in methanol (50 mL)

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was added to destroy the excess DCC. The precipitated urea was collected and washed with ether (3 × 100 mL). The filtrates were washed with water (250 mL), 5% sodium bicarbonate solution (3 × 250 mL), and saturated sodium chloride solution (250 mL), dried (Na_2SO_4), filtered, and evaporated in vacuo. The residual oil was distilled to give 28.5 g (87%) of a colorless liquid. **8**: bp 47 °C (1.0 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.22 (9 H, s) (*t*-Bu), 4.09 (2 H, s) (CH_2O), 4.33 (2 H, s) (CH_2Cl); IR (neat) 1740 (ketone) cm^{-1}. Anal. Calcd for $C_7H_{13}ClO_2$ (164.63): C, 51.07; H, 7.96. Found: C, 50.80, H, 7.96.

1-(Acetylthio)-3-*tert*-butoxy-2-propanone (9). The chloro ketone **8** (4.94 g, 30.0 mmol) and thiolacetic acid (3.43 g, 45.0 mmol) were dissolved in dry ether (75 mL), and triethylamine (3.54 g, 35.0 mmol) in ether (15 mL) was added dropwise with ice cooling. After complete addition, the reaction mixture was stirred for 1 h at room temperature. It was then diluted with ether (50 mL) and washed with water (3 × 50 mL), 5% sodium bicarbonate solution (50 mL), and saturated sodium chloride solution (50 mL). The ether was then dried (Na_2SO_4), filtered, and evaporated in vacuo. The residual oil was distilled to give 5.12 g (84%) of a pale yellow liquid. **9**: bp 64 °C (0.01 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.23 (9 H, s) (*t*-Bu), 2.37 (3 H, s) (CH_3), 3.92 (2 H, s) (CH_2S), 4.10 (2 H, s) (CH_2O); IR (neat) 1730 (ketone), 1690 (thiolester) cm^{-1}. Anal. Calcd for $C_9H_{16}O_3S$ (204.39): C, 52.91; H, 7.90; S, 15.70. Found: C, 52.76; H, 7.63; S, 15.90.

1-(Acetylthio)-3-*tert*-butoxy-2,2-dimethoxypropane (10). The ketone thiolester **9** (6.92 g, 33.9 mmol) was dissolved in a mixture of methanol (35 mL) and trimethyl orthoformate (35 mL) and treated with *p*-toluenesulfonic acid monohydrate (650 mg, 3.4 mmol). Within a short time the reaction turned deep purple. After 18 h, this mixture was diluted with ether (200 mL) and washed with 5% sodium bicarbonate solution (3 × 50 mL) and saturated sodium chloride solution (100 mL). The organic layer was then dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was distilled to give 7.10 g (84%) of a light yellow liquid. **10**: bp 73 °C (0.03 mmHg); ^{1}H NMR (benzene-d_6) δ 1.08 (9 H, s) (*t*-Bu), 2.02 (3 H, s) (CH_3), 3.13 (6 H, s) (OCH_3), 3.38 (2 H, s) (CH_2S), 3.50 (2 H, s) (CH_2O); IR (neat) 1690 (thiolester) cm^{-1}. Anal. Calcd for $C_{11}H_{22}O_4S$ (250.36): C, 52.77; H, 8.86. Found: C, 52.86; H, 8.83.

1-*tert*-Butoxy-2,2-dimethoxy-3-mercaptopropane (11). Sodium (690 mg, 30.0 mmol) was dissolved in dry methanol (150 mL), the ketal thiolester **10** (7.51 g, 30.0 mmol) was added, and the mixture was refluxed for 1 h. After cooling to room temperature, the reaction mixture was diluted with ether (450 mL) and washed with 50% saturated ammonium chloride solution (3 × 150 mL), water (150 mL), and saturated sodium chloride solution (150 mL). The organic layer was then dried (Na_2SO_4), filtered, and evaporated in vacuo. The residue was distilled to give 5.55 g (89%) of a colorless liquid. **11**: bp 45 °C (0.5 mmHg); ^{1}H NMR ($CDCl_3$) δ 1.27 (9 H, s) (*t*-Bu), 1.32 (1 H, t, J = 8 Hz) (SH), 2.80 (2H, d, J = 8 Hz) (CH_2S), 3.23 (6 H, s) (OCH_3), 3.50 (2 H, s) (CH_2O); IR (neat) 2560 (w) (SH) cm^{-1}. Anal. Calcd for $C_9H_{20}O_3S$ (208.32): C, 51.89; H, 9.68; S, 15.39. Found: C, 52.04; H, 9.65; S, 15.35.

1-*tert*-Butoxy-3-[[3,5-dicyano-2-[((dimethylamino)methylene)amino]-6-pyrazinyl]thio]-2,2-dimethoxypropane (12). Chloropyrazine **3**[10] (4.69 g, 20.0 mmol) and mercaptan **11** (5.00 g, 24.0 mmol) were slurried together in dry ethanol (200 mL). Triethylamine (2.23 g, 22.0 mmol) was added in one portion, and the reaction mixture was stirred at room temperature for 4 h. The mixture was cooled to −20 °C for a few hours, and the precipitate was collected, washed with cold ethanol, and recrystallized from dry ethanol to give 7.44 g (92%) of a light yellow powder. Recrystallization from ethyl acetate/cyclohexane gave an analytical sample. **12**: mp 126–127 °C; mass spectrum, *m/e* (relative intensity) M$^+$ 406 (1.2), 319 (69.0), 161 (100), 73 (10.2), 57 (34.5); ^{1}H NMR ($CDCl_3$) δ 1.15 (9 H, s) (*t*-Bu), 3.27 (12 H, s) (NMe_2, OMe), 3.47 (2 H, s) (CH_2O), 3.62 (2 H, s) (CH_2S), 8.68 (1 H, s) (CH=N); IR (KBr) 2220 split (CN's), 1625 (ring C=N) cm^{-1}. Anal. Calcd for $C_{18}H_{26}N_6O_3S$ (406.51): C, 53.18; H, 6.45; N, 20.68. Found: C, 53.32; H, 6.49; N, 20.38.

1-*tert*-Butoxy-3-[[3,5-dicyano-2-[((dimethylamino)methylene)amino]-6-pyrazinyl]thio]-2-propanone (13). The ketal **12** (4.06 g, 10.0 mmol) was dissolved in wet CH_3CN (10 mL, 2% water by volume) and treated with 1 M $LiBF_4$ in wet CH_3CN (10 mL). After stirring at room temperature for 5 h, the mixture was diluted with water (100 mL). The resulting precipitate was collected and washed with water. Drying the wet product under vacuum yielded 3.52 g (98%) of a yellow powder; mp 164–165 °C. Recrystallization from methanol gave an analytical sample. **13**: mp 164–165 °C; mass spectrum, *m/e* (relative intensity) M$^+$ 360 (23.6), 287 (27.7), 273 (86.1), 246 (56.5), 232 (98.6), 83 (20.5), 57 (100); ^{1}H NMR (acetone-d_6) δ 1.26 (9 H, s) (*t*-Bu), 3.30 (3 H, s) (NCH_3), 3.43 (3 H, s) (NCH_3), 4.28 (2 H, s) (CH_2S), 4.46 (2 H, s) (CH_2O), 8.84 (1 H, s) (CH=N); IR (KBr) 2200, 2210 (CN's), 1730 (ketone), 1615 (ring C=N) cm^{-1}. Anal. Calcd for $C_{16}H_{20}N_6O_2S$ (360.44): C, 53.31; H, 5.59; N, 23.32. Found: C, 53.17; H, 5.46; N, 23.57.

7-Amino-6-(*tert*-butoxyacetyl)-2-cyano-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (14). The ketone **13** (3.32 g, 9.21 mmol) and sodium acetate (755 mg, 9.21 mmol) were combined in *tert*-butyl alcohol (92 mL) and heated to reflux. After 30 min, the reaction was allowed to cool to room temperature and was diluted with ethanol. The precipitated product was collected, washed with ethanol, water, and ethanol, and then suction dried, yielding 3.28 g (99%) of an orange fluffy solid. Recrystallization from 2-butanone gave an analytical sample. **14**: mp 285 °C dec without melting; mass spectrum, *m/e* (relative intensity) M$^+$ 360 (12.5), 273 (100), 248 (13.0), 166 (11.8), 105 (26.3), 57 (16.7); ^{1}H NMR (Me_2SO-d_6) δ 1.27 (9 H, S) (*t*-Bu), 3.19, 3.28 (6 H, 2 s) (NMe_2), 4.19 (2 H, s) (CH_2O), 8.07 (2 H, br s) (NH_2), 8.83 (1 H, s) (CH=N); IR (KBr) 3480, 3355 (NH_2), 2220 (CN), 1635 (ketone), 1615 (ring C=N) cm^{-1}. Anal. Calcd for $C_{16}H_{20}N_6O_2S$ (360.44): C, 53.31; H, 5.59; N, 23.32. Found: C, 53.23; H, 5.38; N, 23.15.

7-Bromo-6-(*tert*-butoxyacetyl)-2-cyano-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (15). The amine **14** (1.80 g, 5.00 mmol) and cupric bromide (2.23 g, 10.0 mmol) were combined in acetonitrile (100 mL). *tert*-Butyl nitrite (1.03 g, 10.0 mmol) was added and the mixture warmed (oil bath ≈ 80 °C). After 30 min, N_2 evolution ceased. Warming was stopped, and the cooled mixture was concentrated to dryness. The residual solid was slurried in 1 M NH_4OH and then collected. After washing with additional 1 M NH_4OH and water, the product was recrystallized from 2-butanone (400 mL) with hot filtration. This gave 1.02 g (48%) of orange-yellow solid; mp 213–214 °C dec. Concentration of the filtrate and recrystallization of the residue gave an additional 164 mg (8%) of product. **15**: mp 216–217 °C dec; mass spectrum, *m/e* (relative intensity) 336, 338 (M$^+$ − CH_2O-*t*-Bu) (4.8, 5.2), 309, 311 (0.6, 0.7), 258 (0.7), 57 (100); ^{1}H NMR (Me_2SO-d_6) δ 1.26 (9 H, s) (*t*-Bu), 3.21, 3.30 (6 H, 2 s) (NMe_2), 4.80 (2 H, s) (CH_2O), 8.87 (1 H, s) (CH=N); IR (KBr) 2220 (CN), 1690 (ketone), 1620 (ring C=N) cm^{-1}. Anal. Calcd for $C_{16}H_{18}BrN_5O_2S$ (424.33): C, 45.29; H, 4.27; N, 16.51. Found: C, 45.68; H, 4.17; N, 16.70.

6-(*tert*-Butoxyacetyl)-2-cyano-3-[[(dimethylamino)methylene]amino]-7-(methylthio)thieno[2,3-*b*]pyrazine (16). Sodium hydride (284 mg, 60% in oil, 7.11 mmol) was slurried in dry THF (300 mL), which was then saturated with methylmercaptan. The bromide **15** (2.51 g, 5.93 mmol) was added in one portion, and the reaction mixture was stirred at room temperature for 1 h. The reaction was quenched with acetic acid (512 mg, 8.5 mmol) and purged with N_2. The solvent was then removed in vacuo; the residue was slurried in water and the solid was collected, washed with water, and recrystallized from 2-butanone (250 mL) to give 1.55 g (67%) of fine golden-yellow needles; mp 228–229 °C dec. Concentration of the filtrate and recrystallization of the residue gave 418 mg (18%) of additional product. Concentration of the filtrate and flash chromatography[20] (60:40 ethyl acetate/hexane) of the residue gave 182 mg (8%) of further product. **16**: mass spectrum, *m/e* (relative intensity) M$^+$ 391 (1.7), 335 (7.4), 318 (1.5), 304 (100), 57 (25.9); ^{1}H NMR (Me_2SO-d_6) δ 1.22 (9 H, s) (*t*-Bu), 2.81 (3 H, s) (SCH_3), 3.18, 3.26 (6 H, 2 s) (NMe_2), 4.64 (2 H, s) (OCH_2), 8.76 (1 H, s) (CH=N); IR (KBr) 2220 (CN), 1670 (ketone), 1620 (ring C=N) cm^{-1}. Anal. Calcd for $C_{17}H_{21}N_5O_2S_2$ (391.51): C, 52.15; H, 5.41; N, 17.89; S, 16.38. Found: C, 52.45; H, 5.42; N, 18.06; S, 16.16.

6-(2-*tert*-Butoxy-1-hydroxyethyl)-2-cyano-3-[[(dimethylamino)methylene]amino]-7-(methylthio)thieno[2,3-*b*]pyrazine (17). The ketone **16** (635 mg, 1.62 mmol) was slurried in a mixture of ethanol (16 mL) and THF (16 mL) and treated with sodium borohydride (67 mg, 1.8 mmol). After 30 min, the reaction was quenched with acetone and concentrated to dryness. The residue was slurried in water and the solid was collected, washed with water, and recrystallized from ethanol (100 mL) to give 575 mg (90%) of fine yellow needles. **17**: mp 199–200 °C dec; mass spectrum, *m/e* (relative intensity) M$^+$ 393 (4.4), 306 (100), 292 (15.6), 272 (6.8), 221 (4.6), 57 (14.1); ^{1}H NMR (Me_2SO-d_6) δ 1.08 (9 H, s) (*t*-Bu), 2.48 (3 H, s) (SCH_3), 3.14, 3.23 (6 H, 2 s) (NMe_2), 3.44 (1 H, dd, J = 5.6, 9.4 Hz) (CHO-*t*-Bu), 3.52 (1 H, dd, J = 6.1, 9.4 Hz) (CHO-*t*-Bu), 5.30 (1 H, br q), (C*H*OH), 6.18 (1 H, d, J = 3.7 Hz) (OH), 8.74 (1 H, s) (CH=N); IR (KBr) 3311 (OH), 2222 (CN), 1627 (ring C=N) cm^{-1}. Anal. Calcd for $C_{17}H_{23}N_5O_2S_2$ (393.52): C, 51.88; H, 5.89; N, 17.80. Found: C, 51.46; H, 5.94; N, 17.73.

6-(2-*tert*-Butoxy-1-methoxyethyl)-2-cyano-3-[[(dimethylamino)methylene]amino]-7-(methylthio)thieno[2,3-*b*]pyrazine (18). The alcohol **17** (555 mg, 1.41 mmol) and *p*-toluenesulfonic acid monohydrate (268 mg, 1.41 mmol) were combined in trimethyl orthoformate (14 mL) and stirred at room temperature. After 1 h, the mixture was diluted with ethyl acetate (50 mL), washed with 50% saturated sodium bicarbonate solution (20 mL) and saturated sodium bicarbonate solution (10 mL),

and dried ($MgSO_4$). The extract was filtered and concentrated to a yellow solid which was recrystallized from methanol (20 mL) to give 412 mg (72%) of yellow fluffy solid: mp 148–150 °C. The filtrate was concentrated, and the residue was recrystallized from methanol to give 92 mg (16%) of additional product. **18**: mass spectrum, *m/e* (relative intensity) M^+ 407 (4.4), 320 (100), 306 (11.6), 288 (10.6), 209 (14.1), 132 (17.9), 57 (27.9); ^1H NMR ($CDCl_3$) δ 1.12 (9 H, s) (*t*-Bu), 2.53 (3 H, s) (SCH_3), 3.19, 3.22 (6 H, 2 s) (NMe_2), 3.38 (3 H, s) (OCH_3), 3.60 (2 H, m) (CH_2O), 5.13 (1 H, *t*, *J* = 5 Hz) (C*H*OMe), 8.62 (1 H, s) (CH=N); IR (KBr) 2223 (CN), 1625 (ring C=N) cm^{-1}. Anal. Calcd for $C_{18}H_{25}N_5O_2S_2$ (407.56): C, 53.04; H, 6.18; N, 17.19. Found: C, 52.66; H, 6.09; N, 16.99.

3-Amino-6-(2-*tert*-butoxy-1-methoxyethyl)-2-cyano-7-(methylthio)-thieno[2,3-*b*]pyrazine (19). The amidine **18** (1.65 g, 4.05 mmol) and *p*-toluenesulfonic acid monohydrate (771 mg, 4.05 mmol) were combined in methanol (40 mL) and refluxed. After 90 min, the mixture was cooled, diluted with ethyl acetate (200 mL), and washed with 50% saturated sodium bicarbonate solution (100 mL) and saturated sodium bicarbonate solution (50 mL), and dried ($MgSO_4$). After filtration and concentration, the resulting oil was taken up in methanol (15 mL). Upon standing overnight, a yellow solid formed which was collected and recrystallized from methanol (15 mL) to give 687 mg (48%) of chunky yellow crystals; mp 153–155 °C. The filtrates from both crystallizations were combined and concentrated, and the residue flash chromatographed (25:75 ethyl acetate/hexane) to give 537 mg (38%) of yellow powder. **19**: mass spectrum, *m/e* (relative intensity) M^+ 352 (1.5), 265 (100), 251 (10.6), 233 (19.4), 189 (28.9), 57 (38.1); ^1H NMR ($CDCl_3$) δ 1.14 (9 H, s) (*t*-Bu), 2.52 (3 H, s) (SCH_3), 3.40 (3 H, s) (OCH_3), 3.64 (2 H, m) (CH_2O), 5.15 (1 H, t, *J* = 5.3 Hz) (C*H*OMe), 5.30 (2 H, br s) (NH_2); IR (KBr) 3358, 3327, 3180 (NH_2), 2217 (CN), 1652 (ring C=N) cm^{-1}. Anal. Calcd for $C_{15}H_{20}N_4O_2S_2$ (352.47): C, 51.11; H, 5.72; N, 15.90. Found: C, 50.80; H, 5.70; N, 15.97.

A lower R_f, yellow side product obtained from the chromatography, 53 mg (4%), was **3-amino-2-cyano-6-(2-hydroxy-1-methoxyethyl)-7-(methylthio)thieno[2,3-*b*]pyrazine**: mass spectrum, *m/e* (relative intensity) M^+ 296 (9.8), 265 (100), 235 (11.0), 233 (22.8), 217 (14.8), 189 (62.4); ^1H NMR ($CDCl_3$) δ 2.26 (1 H, t, *J* = 6.7 Hz) (OH), 2.54 (3 H, s) (SCH_3), 3.44 (3 H, s) (OCH_3), 3.8 (2 H, m) (CH_2O), 5.15 (1H, dd, *J* = 4.5, 6.1 Hz) (C*H*OMe), 5.32 (2 H, br s) (NH_2); IR (KBr) 3395, 3315, 3176 (NH_2), 2224 (CN), 1651 (ring C=N) cm^{-1}.

7-(2-*tert*-Butoxy-1-methoxyethyl)-2,4-diamino-6-(methylthio)thieno-[3,2-*g*]pteridine (21). Guanidine hydrochloride (478 mg, 5.00 mmol) was added to a solution of sodium methoxide (from Na, 115 mg, 5.00 mmol) in dry methanol (20 mL). After about 10 min, *o*-amino nitrile **19** (352 mg, 1.00 mmole) was added, and the mixture was refluxed for 90 min. The cooled mixture was concentrated to a yellow solid. This was triturated with 50% saturated ammonium chloride solution, collected, and washed with water. Flash chromatography (5:95 methanol/chloroform) gave 305 mg (77%) of light yellow solid. **21**: mp 245–246 °C (2-propanol); mass spectrum *m/e* (relative intensity) M^+ 394 (5.3), 307 (42.7), 293 (33.0), 275 (11.1), 231 (16.1), 57 (100); ^1H NMR ($CDCl_3$) δ 1.14 (9 H, s) (*t*-Bu), 1.71 (2 H, br s) (NH_2), 2.56 (3 H, s) (SCH_3), 3.44 (3 H, s) (OCH_3), 3.63 (1 H, dd, *J* = 5.6, 9.7 Hz) (C*H*O-*t*-Bu), 3.73 (1 H, dd, *J* = 5.6, 9.7 Hz) (C*H*O-*t*-Bu), 5.23 (1 H, t, *J* = 5.6 Hz) (C*H*OMe), 5.40 (2 H, br s) (NH_2); IR (KBr) 3461, 3346, 3133 (NH_2), 1661, 1621 (ring C=N) cm^{-1}. Anal. Calcd for $C_{16}H_{22}N_6O_2S_2$ (394.52): C, 48.71; H, 5.62; N, 21.30. Found: C, 48.74; H, 5.56; N, 21.22.

(±)-2-Amino-7-(1,2-dihydroxyethyl)-6-(methylthio)thieno[3,2-*g*]pteridin-4(3*H*)-one ((±)-Urothione) (1). The 2,4-diaminopteridine **21** (197 mg, 0.50 mmol) was dissolved in trifluoroacetic acid (25 mL) and stirred at room temperature for 30 min. The solvent was removed in vacuo, giving **7-(2-hydroxy-1-methoxyethyl)-2,4-diamino-6-(methylthio)thieno-[3,2-*g*]pteridine (22)**: mass spectrum, *m/e* (relative intensity) M^+ 338 (6.2), 307 (52.3), 275 (10.1), 259 (8.1), 231 (15.4), 84 (94.6), 66 (100); ^1H NMR (Me_2SO-d_6) δ 2.61 (3 H, s) (SCH_3), 3.34 (3 H, s) (OCH_3), 3.6 (2 H, m) ($CHCH_2$), 5.2 (1 H, m) (C*H*CH_2). This material was taken up in 3 M H_2SO_4 (25 mL) and refluxed for 30 min. The cooled solution was treated with a small portion of decolorizing carbon and then filtered through Celite. The Celite was rinsed with 1 M H_2SO_4 (25 mL) and then a few milliliters of water. The filtrate was basified with NaOH (12.0 g) in water (350 mL). This solution was warmed to 70 °C and acidified with acetic acid (10.0 mL). As the warm solution slowly cooled, a fine precipitate formed. The precipitate was collected by centrifugation and filtration. The product was rinsed with water and ethanol and was dried, giving 129 mg (79%) of a yellow powder. **1**: mass spectrum (FAB), *m/e* $M^+ + 1$ 326; ^1H NMR (D_2O/NaOD) δ 2.34 (3 H, s) (SCH_3), 3.60 (1 H, dd, *J* = 7.5, 10.9 Hz) ($CHCH_2$), 3.74 (1 H, dd, *J* = 5.7, 10.9 Hz) ($CHCH_2$), 5.41 (1 H, m) (C*H*CH_2); UV (0.1 N HCl) λ_{max} (log ε) 233 (4.15), 277 (4.17), 352 (3.90); (0.1 N NaOH) 270 (4.43), 399 (4.06); IR (KBr) 3257, 3142, 1681, 1608, 1526 cm^{-1}. Anal. Calcd for $C_{11}H_{11}N_5O_3S_2$ (325.37): C, 40.60; H, 3.41; N, 21.53. Found: C, 40.12; H, 3.49; N, 21.26.

2-Acetamido-7-(1,2-diacetoxyethyl)-6-(methylthio)thieno[3,2-*g*]pteridin-4-(3*H*)-one. (±)-Urothione (33 mg, 0.10 mmol) was refluxed for 90 min in acetic anhydride (10 mL). After cooling of the mixture, the solvent was removed in vacuo and the solid residue chromatographed (Chromatotron, 5:95 methanol/chloroform) to give 43 mg (95%) of yellow solid. An analytical sample was obtained by recrystallization from methanol: mp 219–221 °C; mass spectrum, *m/e* (relative intensity) M^+ 451 (4.7), 363 (13), 349 (100), 337 (11), 336 (61), 334 (16), 294 (98), 244 (15), 223 (54), 217 (46), 144 (21), 131 (13), 119 (39), 83 (22); ^1H NMR ($CDCl_3$) δ 2.09 (3 H, s), 2.19 (3 H, s), 2.43 (3 H, s), 2.70 (3 H, s), 4.49 (2 H, d, *J* = 5.1 Hz), 6.80 (1 H, t, *J* = 5.1 Hz), 10.63 (1 H, br s), 12.54 (1 H, br s). Anal. Calcd for $C_{17}H_{17}N_5O_6S_2$ (451.48): C, 45.22; H, 3.80; N, 15.51. Found: C, 44.93; H, 3.66; N, 15.53; exact mass calcd 451.0620, found 451.0632.

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