Pteridines. 50. Unequivocal Total Synthesis of Deoxyurothione[1]

Edward C. Taylor* and Lawrence A. Reiter

Department of Chemistry, Princeton University, Princeton, New Jersey 08544

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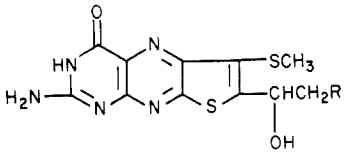
Deoxyurothione (**2**), a model for the unique urinary thienopterin urothione (**1**), has been synthesized in nine steps from 2-amino-3,5-dicyano-6-chloropyrazine (**7**, 16% overall yield) and from 2-amino-3-cyano-5-(oximinomethyl)pyrazine 1-oxide (**3**, 9% overall yield). The synthesis involves initial construction of the condensed thienopyrazine **9b**, introduction of the methylthio substituent to give **12b**, sodium borohydride reduction of the acetyl group to give the 1-hydroxyethyl substituent found in **2**, simultaneous protection of the side-chain hydroxyl group and deprotection of the 3-amino group to give **17**, annulation of the pyrimidine ring by guanidine cyclization to **18**, and final aqueous acidic hydrolysis. The physical and chemical properties of **2** are very similar to those reported for urothione (**1**) itself.

In view of the ubiquitous importance of pterin cofactors in biological alkylation, hydroxylation, dehydrogenation, and electron-transport reactions,[2] it is remarkable that nothing is known about the biogenetic origin or biochemical role of urothione (**1**). This unusual thienopterin was

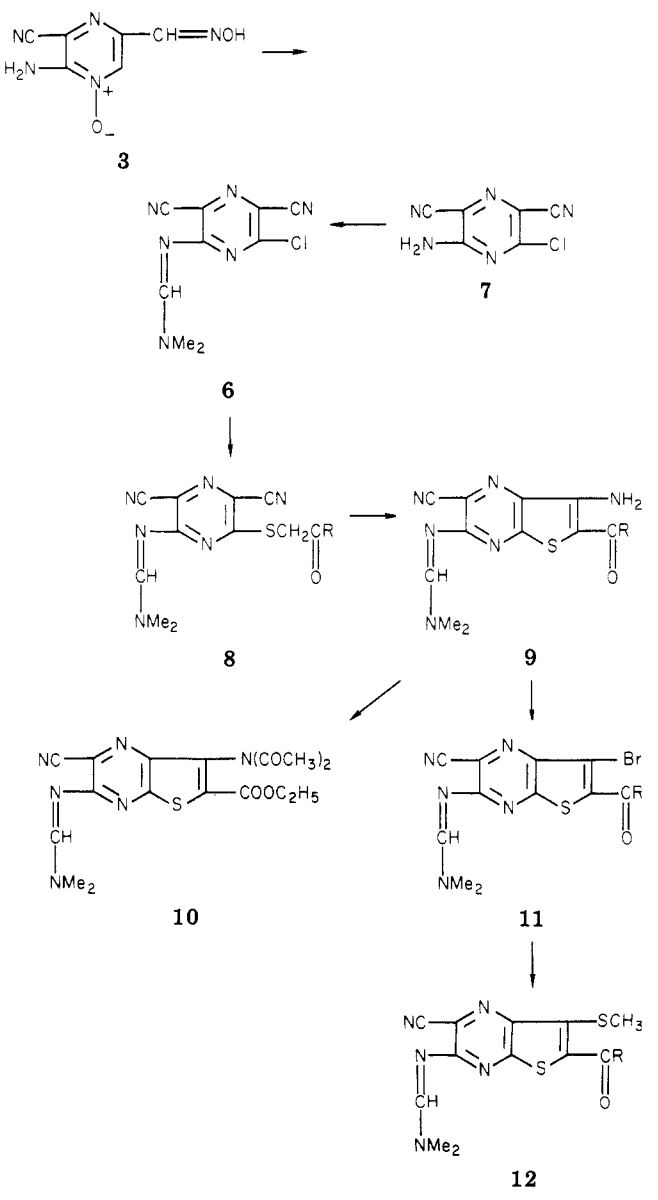


1, R = OH
2, R = H

first isolated from human urine in 1940,[3] but despite intensive efforts by Tschesche and his co-workers in the mid-1950s,[4-6] it was not until 27 years later that a structure for urothione was suggested and a total synthesis described.[7-11] However, apparently as a consequence of the minute amount of urothione available synthetically (0.3% yield from a preformed pteridine intermediate) and the extraordinary difficulties associated with isolation of **1** from natural sources,[6] the physiological activity and significance of urothione remain a mystery. We describe in this paper model studies on a synthetic approach to urothione which have led to an unequivocal total synthesis of deoxyurothione (**2**) and provide additional information bearing on the validity of the assigned structure **1** for urothione itself.

Over the past several years we have developed and exploited a new synthetic approach to pteridines which, by an initial unequivocal synthesis of pyrazine intermediates followed by annulation of the pyrimidine ring, avoids the structural ambiguities and technical difficulties associated with the classical synthetic approach to this ring system.[12]

Scheme I[a]



[a] a, R = OC_2H_5; b, R = CH_3.

Application of this strategy to the synthesis of deoxyurothione (**2**) required the construction of a pyrazine intermediate suitably functionalized for eventual annulation

(1) For the previous paper in this series, see: Taylor, E. C.; Dumas, D. J., submitted for publication in *J. Org. Chem.*

(2) Kisliuk, R. L.; Brown, G. M., Eds; "Chemistry and Biology of Pteridines"; Elsevier/North-Holland: New York, 1979; see also extensive references cited therein.

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(4) Tschesche, R.; Korte, F.; Heuschkel, G. *Chem. Ber.* **1955**, *88*, 1251-1258.

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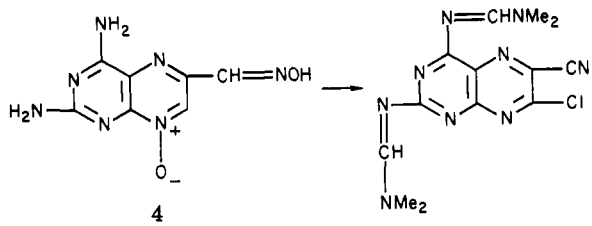
of the requisite thiophene and pyrimidine rings. Pyrazine **3** appeared to be ideal for this purpose, since the *o*-aminonitrile functionality present in **3** (Scheme I) is a well-known precursor to fused pyrimidines,[13] and the previously described conversion of **4** to **5**[14] suggests that

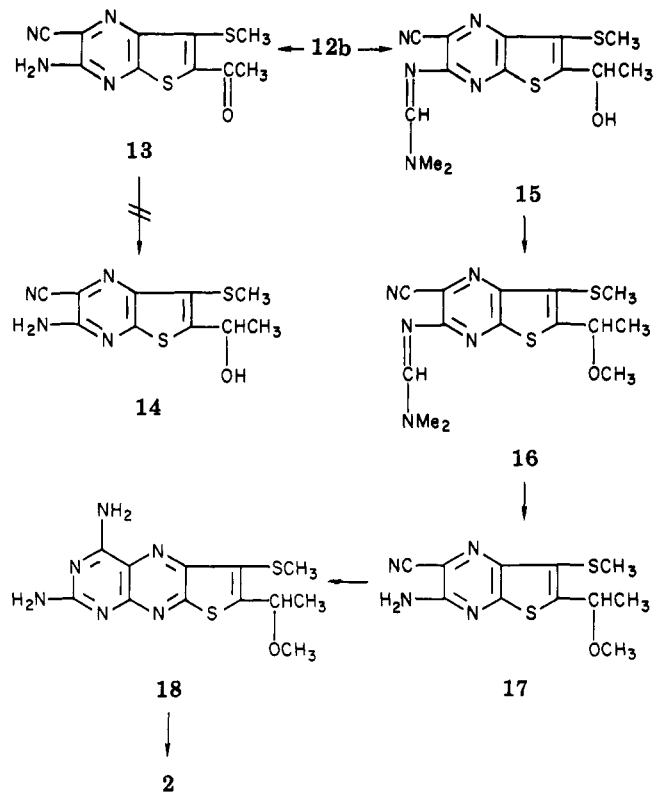


4 **5**

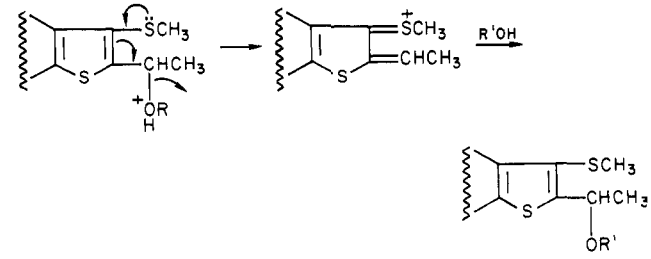
an analogous dehydration/deoxygenation/chlorination reaction of **3** might give an intermediate suitable for direct annulation of a thiophene ring. Treatment of **3** (available as previously described[15] from the condensation of aminomalononitrile tosylate with dioximinoacetone) with phosphorus oxychloride in dimethylformamide gave 6-chloro-3,5-dicyano-2-[[(dimethylamino)methylene]-amino]pyrazine (**6**) in 46% yield. This pyrazine could alternatively be prepared in better yield (85%) from 2-amino-6-chloro-3,5-dicyanopyrazine (**7**)[16] under the same conditions. Condensation of **6** with ethyl α-mercaptoacetate or 1-mercapto-2-propanone then gave the sulfides **8a** and **8b**, respectively, which, by analogy with similarly substituted pyrimidines,[17] pyridines,[18] and pyrazines,[19] underwent intramolecular base-catalyzed cyclization to the thieno[2,3-*b*]pyrazines **9a** and **9b**. The imide **10** was prepared without difficulty by acetylation of **9a** with acetic anhydride, but attempts to displace the imide grouping with methyl mercaptan were unsuccessful. However, treatment of **9a** with sodium nitrite in 48% aqueous hydrobromic acid at 0 °C in the presence of a catalytic amount of copper(I) bromide led to the bromo derivative **11a**, which yielded the desired 3-methylthio derivative **12a** upon reaction with sodium methylmercaptide in warm dimethylformamide.

Since at this point problems were expected with selective manipulation of the ester grouping in **12a** in the presence of the 5-cyano group, we turned our attention to the 2-acetyl-3-aminothieno[2,3-*b*]pyrazine **9b**. This compound was converted in moderate (55%) yield to the 3-bromo derivative **11b** by application of the Sandmeyer procedure described above. Despite numerous attempts to modify the reaction conditions (including the use of Doyle's procedure[20] involving *tert*-butyl nitrite and copper(II) bromide in acetonitrile), yields in this Sandmeyer bromination could not be improved. However, the reaction of **11b** with sodium methylmercaptide in anhydrous THF at room temperature gave the requisite methylthio derivative **12b** in 92% yield.

Scheme II



Acid hydrolysis of **12b** removed the (dimethylamino)-methylene protecting group to give **13** (Scheme II), but the inseparable mixtures which resulted from sodium borohydride reduction of **13** led us to suspect that the anticipated α-hydroxyethyl derivative **14** was unstable. We therefore first reduced **12b** with sodium borohydride in ethanol/tetrahydrofuran to **15** and then examined various methods for protection of the side-chain hydroxyl group. Attempts to prepare the *tert*-butyl ether led to recovery of unchanged starting material, while attempted benzyl ether formation with sodium hydride and benzyl chloride resulted in slow but eventually complete decomposition of **15** without any evidence of ether formation. It was finally found that treatment of **15** with *p*-toluenesulfonic acid in a mixture of trimethyl orthoformate and anhydrous methanol led to rapid formation of the methyl ether **16**, followed more slowly by loss of the (dimethylamino)-methylene protecting group to give **17** in one manipulative step. Cyclization of **17** with guanidine in refluxing methanol then led to the 2,4-diaminopteridine **18**, which was cleanly hydrolyzed to deoxyurothione (**2**) with aqueous acid.[21] Loss of the methoxy group under these acidic conditions (as well as the reverse transformation observed in the conversion of **15** to **16**) is apparently assisted by the ortho-situated methylthio substituent. Deoxyurothione (**2**) is thus available from the pyrazine **6** in 19% overall yield.



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Koschara originally characterized urothione by two color tests[2] which we have repeated with deoxyurothione (2). Thus, heating urothione (1) with 80% sulfuric acid was reported to result in the appearance of a cherry-red color; we observed a similar color reaction upon treatment of 2 under the same conditions. A solution of urothione in dilute sulfuric acid was reported to be nonfluorescent under UV irradiation, but a mossy green fluorescence was observed after treatment of this solution with permanganate. In analogous fashion, a nonfluorescent solution of 2 in dilute sulfuric acid exhibited a bright green fluorescence upon treatment with permanganate. Furthermore, the published ultraviolet spectra of urothione[7-9] in 0.1 N HCl and in 0.1 N NaOH are virtually superimposable with the spectra exhibited by deoxyurothione (2). Attempts to adapt the above synthetic scheme to the synthesis of urothione (1) (the structure of which appears to be confirmed by our results) are in progress.

Experimental Section

6-Chloro-3,5-dicyano-2-[[(dimethylamino)methylene]amino]pyrazine (6). Method A. Phosphorus oxychloride (21 mL, 230 mmol) was added dropwise to an ice-cold suspension of the oxime of 2-amino-3-cyano-5-formylpyrazine 1-oxide (3;[15] 10.0 g, 55.8 mmol) in DMF (150 mL). After the addition was complete, the reaction mixture was stirred overnight at room temperature, and the resulting dark brown solution was poured into ice–water (800 mL) and stirred for 1 h. The precipitated solid was collected by filtration, washed well with water, and recrystallized from ethanol (Norite) to give 4.6 g (46%) of yellow needles: mp 130–132 °C; NMR ($CDCl_3$) δ 8.73 (s, 1), 3.36 (s, 6); IR (KBr) 2240, 1640 cm^{-1}.

Anal. Calcd for $C_9H_7ClN_6$ (mol wt 234.66): C, 46.07; H, 3.01; N, 35.82. Found: C, 46.05; H, 3.08; N, 35.54.

Method B. Phosphorus oxychloride (5.5 mL, 60 mmol) was added dropwise to a slurry of 2-amino-6-chloro-3,5-dicyanopyrazine[16] (7.18 g, 40 mmol) in DMF (80 mL) at 0 °C. The reaction mixture was worked up as described above to give (after recrystallization from ethanol) 7.98 g (85%) of fine yellow needles (mp 130–132 °C) identical in every respect with the material obtained by method A.

Ethyl [[3,5-Dicyano-2-[[(dimethylamino)methylene]amino]-6-pyrazinyl]thio]acetate (8a). Sodium (1.0 g, 43.5 mmol) was dissolved in dry methanol (150 mL), the solution cooled to −70 °C, and ethyl 2-mercaptoacetate (5.0 g, 41.7 mmol) added followed by the dropwise addition of 6 (5.0 g, 20.3 mmol) in DMF (25 mL) at −70 °C. The reaction mixture was stirred for 1 h and poured into ice–water, and the precipitated solid was collected by filtration, washed with water, and recrystallized from aqueous methanol to give 5.5 g (85%) of fine yellow needles: mp 165–166 °C; NMR ($CDCl_3$) δ 8.77 (s, 1), 4.18 (q, 2), 3.86 (s, 2), 3.33, 3.28 (d, 6), 1.23 (t, 3); IR (KBr) 2240, 2235, 1730, 1640 cm^{-1}.

Anal. Calcd for $C_{13}H_{14}N_6O_2S$ (mol wt 318.37): C, 49.04; H, 4.43; N, 26.40. Found: C, 49.23; H, 4.44; N, 26.37.

7-Amino-6-carbethoxy-2-cyano-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (9a).[22] A suspension of 8a (910 mg, 2.6 mmol) in ethanolic sodium ethoxide (from 50 mL of ethanol and 60 mg, 2.6 mmol, of sodium) was stirred at room temperature for 3 h, acidified with acetic acid, and diluted with water (50 mL), and the precipitated orange solid was collected by filtration and recrystallized from ethanol to give 780 mg (86%) of fine orange needles: mp 258–259 °C; NMR (Me_2SO-d_6) δ 8.75 (s, 1), 6.13 (br s, 2), 4.22 (q, 2), 3.28, 3.27 (d, 6), 1.38 (t, 3); IR (KBr) 3500, 3400, 3370, 2230, 1680, 1625 cm^{-1}.

Anal. Calcd for $C_{13}H_{14}N_6O_2S$ (mol wt 318.37): C, 49.04; H, 4.43; N, 26.40. Found: C, 49.28; H, 4.60; N, 26.49.

6-Carbethoxy-2-cyano-7-(diacetylamino)-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (10). A suspension of 9a (150 mg, 0.47 mmol) in acetic anhydride (10 mL) was heated under reflux for 5 h, cooled to room temperature, and neutralized with 10% aqueous sodium bicarbonate (200 mL). This mixture was then extracted with chloroform (3 × 25 mL), the extracts were washed with 5% aqueous sodium bicarbonate (25 mL) followed by saturated aqueous ammonium chloride (25 mL), and dried over anhydrous magnesium sulfate. Evaporation under reduced pressure gave a yellow solid which was recrystallized from ethanol to give 130 mg (68%) of yellow needles: mp 235–237 °C; NMR (Me_2SO-d_6) δ 8.78 (s, 1), 4.37 (q, 2), 3.30, 3.27 (d, 6), 2.28 (s, 6), 1.30 (t, 3); IR (KBr) 2220, 1730, 1708, 1700, 1620 cm^{-1}.

Anal. Calcd for $C_{17}H_{18}N_6O_4S$ (mol wt 402.43): C, 50.73; H, 4.51; N, 20.89; S, 7.97. Found: C, 50.47; H, 4.49; N, 20.77; S, 7.90.

7-Bromo-6-carbethoxy-2-cyano-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (11a). To a solution of copper(I) bromide (144 mg, 1.0 mmol) in 48% hydrobromide acid (15 mL) cooled to 0 °C was added the thienopyrazine 9a (320 mg, 1.0 mmol) followed by dropwise addition of 0.55 N sodium nitrite (2 mL). The reaction mixture was stirred at 0 °C for 30 min and then carefully poured into a mixture of sodium bicarbonate (12 g) and ice. The reaction mixture was then diluted with water, and the precipitated solid was collected by filtration, washed with water, and recrystallized from ethyl acetate to give 250 mg (65%) of golden crystals: mp 229–231 °C; IR (KBr) 2220, 1685, 1615 cm^{-1}.

Anal. Calcd for $C_{13}H_{12}BrN_5O_2S$ (mol wt 382.26): C, 40.84; H, 3.16; N, 18.32; S, 8.39. Found: C, 41.08; H, 3.24; N, 18.52; S, 8.27.

6-Carbethoxy-2-cyano-3-[[(dimethylamino)methylene]amino]-7-(methylthio)thieno[2,3-*b*]pyrazine (12a). To a solution of sodium ethoxide (20 mg, 0.42 mmol) in ethanolic DMF (1:1, 10 mL) saturated with methyl mercaptan was added the thienopyrazine 11a (100 mg, 0.26 mmol), and the reaction mixture was heated under reflux for 16 h. Cooling yielded a yellow solid which was collected by filtration and recrystallized from ethanol to give 30 mg (33%) of pure 12a: mp 222–224 °C; IR (KBr) 2220, 1710, 1620 cm^{-1}.

Anal. Calcd for $C_{14}H_{15}N_5O_2S_2$ (mol wt 349.43): C, 48.12; H, 4.33; N, 20.04; S, 18.35. Found: C, 47.88; H, 4.10; N, 19.88; S, 18.09.

1-[[3,5-Dicyano-2-[[(dimethylamino)methylene]amino]-6-pyrazinyl]thio]-2-propanone (8b). To a slurry of 6 (4.69 g, 20 mmol) and 1-mercapto-2-propanone (1.98 g, 22 mmol) in 95% ethanol (200 mL) was added triethylamine (2.12 g, 21 mmol). After 30 min, the reaction mixture was cooled to −20 °C and filtered, and the collected solid was washed with cold ethanol and recrystallized from 2-butanone to give 5.33 g (92%) of a fluffy yellow solid: mp 206–208 °C; NMR (Me_2SO-d_6) δ 8.70 (s, 1), 4.27 (s, 2), 3.28, 3.23 (d, 6), 2.25 (s, 3); IR (KBr) 2225, 2215, 1710, 1615 cm^{-1}.

Anal. Calcd for $C_{12}H_{12}N_6OS$ (mol wt 288.34): C, 49.98; H, 4.20; N, 29.15. Found: C, 49.95; H, 4.12; N, 29.45.

6-Acetyl-7-amino-2-cyano-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (9b). Sodium (23 mg, 1 mmol) was dissolved in dry ethanol (200 mL), and to this solution was added 8b (2.88 g, 10 mmol). The instantaneous development of a deep orange color indicated that cyclization was immediate. After 1 h of being stirred at room temperature, the reaction mixture was cooled to −20 °C and filtered, and the collected solid was washed with ethanol and dried to give 2.86 g (99%) of an orange solid. The analytical sample (mp 302–303 °C) was prepared by recrystallization from acetone: IR (KBr) 3455, 3405, 3310, 2220, 1620, 1595 cm^{-1}.

Anal. Calcd for $C_{12}H_{12}N_6OS$ (mol wt 288.34): C, 49.98; H, 4.20; N, 29.15. Found: C, 50.12; H, 4.08; N, 29.11.

6-Acetyl-7-bromo-2-cyano-3-[[(dimethylamino)methylene]amino]thieno[2,3-*b*]pyrazine (11b). This compound was prepared from copper(I) bromide (300 mg, 2 mmol), 48% hydrobromic acid (100 mL), sodium nitrite (830 mg, 12 mmol) in water (20 mL), and compound 9b (2.88 g, 10 mmol) as described previously for the preparation of 11a. The crude product was recrystallized from acetonitrile (Norite) to give 1.70–2.34 g (48–66%) of golden crystals: mp 255–256.5 °C; IR (KBr) 2220, 1645, 1610 cm^{-1}.

Anal. Calcd for $C_{12}H_{10}BrN_5OS$ (mol wt 352.23): C, 40.92; H, 2.86; N, 19.88. Found: C, 41.15; H, 2.80; N, 20.01.

6-Acetyl-2-cyano-3-[[(dimethylamino)methylene]amino]-7-(methylthio)thieno[2,3-*b*]pyrazine (12b). An excess of methyl mercaptan (3 mL of liquid) was added to a slurry of sodium hydride (380 mg of a 57% suspension, 9 mmol) in dry THF

(22) We suggest that the simplified method described for the synthesis of 8b be used for future preparations of this material.

(210 mL), and after a few minutes, compound **11b** (2.11 g, 6 mmol) was added. The reaction mixture was stirred at room temperature for 3 h, solvent was removed under reduced pressure, and the residual solids were slurried in water, collected by filtration, washed well with water, and recrystallized from acetonitrile to give 1.77 g (92%) of waxy yellow needles: mp 231–232 °C; NMR (Me_2SO-d_6) δ 8.75 (s, 1), 3.25, 3.23 (d, 6), 2.78 (s, 3), 2.73 (s, 3); IR (KBr) 2220, 1610 cm^{-1}.

Anal. Calcd for $C_{13}H_{13}N_5OS_2$ (mol wt 319.40): C, 48.88; H, 4.10; N, 21.93; S, 20.08. Found: C, 49.16; H, 3.92; N, 21.95; S, 20.27.

6-Acetyl-3-amino-2-cyano-7-(methylthio)thieno[2,3-*b*]pyrazine (13). A slurry of 500 mg (1.56 mmol) of **12b** in 50 mL of 6 N hydrochloric acid was heated under reflux for 30 min, cooled to 0 °C, and filtered, and the collected solid was recrystallized from 2-butanone followed by acetone to give 250 mg (60%) of **13** as bright orange needles: mp 272–273 °C dec; IR (KBr) 3370, 3300, 3160, 2210, 1640 cm^{-1}.

Anal. Calcd for $C_{10}H_8N_4OS_2$ (mol wt 264.32): C, 45.44; H, 3.05; N, 21.20; S, 24.26. Found: C, 45.32; H, 3.30; N, 21.26; S, 23.96.

2-Cyano-3-[[(dimethylamino)methylene]amino]-6-(1-hydroxyethyl)-7-(methylthio)thieno[2,3-*b*]pyrazine (15). To a solution of sodium borohydride (340 mg, 8.75 mmol) in dry ethanol (130 mL) was added compound **12b** (2.54 g, 7.95 mmol) followed by dry THF (130 mL). The reaction mixture became homogeneous after about 1 h of stirring at room temperature, and after 4 h the reaction was judged complete by TLC. Solvents were removed under reduced pressure, and the solid residue was slurried in water, collected by filtration, and washed with water. The filtrates were then acidified with acetic acid and extracted with ethyl acetate (3 × 50 mL). The combined extracts were evaporated to dryness, and the yellow oily residue was combined with the solid collected above and recrystallized from methanol to give 1.88 g (74%) of fine yellow needles: mp 211–213 °C; NMR (Me_2SO-d_6/$CDCl_3$) δ 8.82 (s, 1), 5.52 (q, 1), 3.30, 3.27 (d, 6), 2.52 (s, 3), 1.53 (d, 3); IR (KBr) 3280 (br), 2220, 1620 cm^{-1}.

Anal. Calcd for $C_{13}H_{15}N_5OS_2$ (mol wt 321.42): C, 48.58; H, 4.70; N, 21.79. Found: C, 48.25; H, 4.69; N, 21.42.

2-Cyano-3-[[(dimethylamino)methylene]amino]-6-(1-methoxyethyl)-7-(methylthio)thieno[2,3-*b*]pyrazine (16). A solution of 320 mg (1 mmol) of **15** in a mixture of 16 mL of trimethyl orthoformate and 16 mL of anhydrous methanol containing 190 mg (1 mmol) of *p*-toluenesulfonic acid was stirred at room temperature for 2.5 h and transferred to a separatory funnel with 75 mL of ethyl acetate, and the mixture was washed with 5% aqueous sodium bicarbonate (3 × 25 mL). The organic layer was then separated, dried ($MgSO_4$), and evaporated under reduced pressure to give a yellow oil which crystallized on standing. Recrystallization from anhydrous methanol then gave 250 mg (75%) of **16** as small yellow needles: mp 156–157 °C; NMR (Me_2SO-d_6) δ 8.68 (s, 1), 5.03 (q, 1), 3.19, 3.16 (d, 6), 3.05 (s, 3), 2.41 (s, 3), 1.36 (d, 3); IR (KBr) 2215, 1615 cm^{-1}.

Anal. Calcd for $C_{14}H_{17}N_5OS_2$ (mol wt 335.45): C, 50.12; H, 5.11; N, 20.88; S, 19.12. Found: C, 49.90; H, 4.99; N, 20.71; S, 19.31.

3-Amino-2-cyano-6-(1-methoxyethyl)-7-(methylthio)-thieno[2,3-*b*]pyrazine (17). A mixture of **15** (1.28 g, 4 mmol) in a 1:1 mixture of trimethyl orthoformate and methanol (130 mL) containing *p*-toluenesulfonic acid (760 mg, 4 mmol) was heated under reflux for 16 h. It was then cooled to room temperature, diluted with ethyl acetate (200 mL), washed with 5% aqueous sodium bicarbonate (3 × 100 mL), and saturated aqueous ammonium chloride (100 mL), and the organic layer was separated, dried over anhydrous magnesium sulfate, and evaporated under reduced pressure to give a yellow solid which was recrystallized from methanol: yield 850 mg (76%) of yellow needles: mp 176–177 °C; IR (KBr) 3380, 3305, 3170, 2220, 1650 cm^{-1}.

Anal. Calcd for $C_{11}H_{12}N_4OS_2$ (mol wt 280.37): C, 47.12; H, 4.32; N, 19.98; S, 22.87. Found: C, 47.15; H, 4.26; N, 19.81; S, 22.55.

2,4-Diamino-7-(1-methoxyethyl)-6-(methylthio)thieno-[3,2-*g*]pteridine (18). Sodium (263 mg, 11.4 mmol) was dissolved in dry methanol, and to this solution was added guanidine hydrochloride (1.09 g, 11.4 mmol) followed by the thienopyrazine **17** (1.60 g, 5.71 mmol). The reaction mixture was heated under reflux for 6 h and cooled to −20 °C, and the precipitated solid was collected by filtration, washed well with cold methanol, water, and again with cold methanol, and recrystallized from a 1:1 mixture of DMF and ethanol to give 680 mg (37%) of golden crystals. Dilution of the recrystallization filtrates with water yielded an additional 840 mg of product: total yield 82%; mp >300 °C; NMR (Me_2SO-d_6) δ 7.8 (br s, 2), 6.8 (br s, 2), 5.16 (q, 1), 3.29 (s, 3), 2.62 (s, 3), 1.49 (d, 3); IR (KBr) 3450, 3340, 3120 cm^{-1}.

Anal. Calcd for $C_{12}H_{14}N_6OS_2$ (mol wt 322.41): C, 44.70; H, 4.38; N, 26.07; S, 19.89. Found: C, 44.92; H, 4.44; N, 26.17; S, 19.63.

2-Amino-7-(1-hydroxyethyl)-6-(methylthio)thieno[3,2-*g*]-pteridin-4(3*H*)-one (Deoxyurothione, 2). A slurry of compound **18** (340 mg, 1.05 mmol) in 1 N hydrochloric acid (50 mL) under nitrogen was heated under reflux for 2 h, cooled to room temperature, and filtered through a fritted-glass funnel to remove a small amount of suspended solid. The filter was rinsed with 1 N hydrochloric acid (50 mL), and the combined filtrates were diluted with 3 N sodium hydroxide (100 mL). The resulting alkaline solution was diluted with water (600 mL), warmed to 70 °C, and acidified with acetic acid (20 mL). The resulting solution, which contained a yellow solid, was allowed to cool slowly to room temperature, stored at 4 °C for 24 h, and then concentrated by centrifugation. The collected solid[23] was washed with water followed by ethanol and dried at 55 °C in vacuo to give 280 mg (86%) of **2** as a yellow microcrystalline powder: mp >300 °C; NMR (NaOD–D_2O) δ 5.4 (q, 1), 2.27 (s, 3), 1.43 (d, 3); UV (0.1 N NaOH) λ_{max} (log ϵ) 268 (4.43), 391 (4.04); UV (0.1 N HCl) 231 (4.33), 277 (4.34), 350 (4.01).

Anal. Calcd for $C_{11}H_{11}N_5O_2S_2$ (mol wt 309.37): C, 42.70; H, 3.58; N, 22.64; S, 20.73. Found: C, 42.60; H, 3.59; N, 22.87; S, 20.55.

This compound was further characterized as its diacetate which was prepared from **2** (100 mg) and acetic anhydride (10 mL). After 30 min of heating under reflux, the reaction mixture was evaporated under reduced pressure, and the residual solid recrystallized from ethanol/ethyl acetate (1:1) to give 70 mg (55%) of **2-acetamido-7-(1-acetoxyethyl)-6-(methylthio)thieno[3,2-*g*]-pteridin-4(3*H*)-one** as a yellow powder: mp >360 °C (with darkening above 240 °C); NMR (Me_2SO-d_6) δ 5.2 (q, 1), 2.65 (s, 3), 2.28 (s, 3), 2.15 (s, 3), 1.65 (d, 3); IR (KBr) 1740, 1715, 1680 cm^{-1}.

Anal. Calcd for $C_{15}H_{15}N_5O_4S_2$ (mol wt 393.44): C, 45.79; H, 3.84; N, 17.80; S, 16.30. Found: C, 45.82; H, 3.81; N, 17.56; S, 16.07.

Registry No. 2, 70741-07-0; **3**, 49699-53-8; **6**, 79917-06-9; **7**, 40559-88-4; **8a**, 79917-07-0; **8b**, 79917-08-1; **9a**, 79917-09-2; **9b**, 79917-10-5; **10**, 79917-11-6; **11a**, 79917-12-7; **11b**, 79917-13-8; **12a**, 79917-14-9; **12b**, 79917-15-0; **13**, 79917-16-1; **15**, 79917-17-2; **16**, 79917-18-3; **17**, 79917-19-4; **18**, 79917-20-7; ethyl 2-mercaptoacetate, 623-51-8; 1-mercapto-2-propanone, 24653-75-6; guanidine hydrochloride, 50-01-1; 2-acetamido-7-(1-acetoxyethyl)-6-(methylthio)-thieno[3,2-*g*]pteridin-4(3*H*)-one, 79917-21-8.

(23) This complex series of dilutions and pH adjustments is adapted from Koschara's original work[3] and appears to be necessary for the formation of a microcrystalline product.