Synthesis of 10-Thia-5-deazafolic Acid and 2-Desamino-2-oxo-10-thia-5-deazafolic Acid

Shyam K. Singh and John B. Hynes*

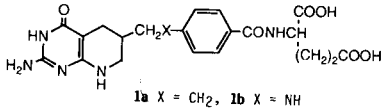
Department of Pharmaceutical Sciences,
Medical University of South Carolina,
Charleston, South Carolina 29425 U.S.A.

Received February 21, 1991

The folate analogue, 10-thia-5-deazafolic acid, was obtained *via* a multistep synthetic sequence beginning with the known intermediate, 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde. Reduction of this aldehyde with sodium borohydride gave 2,4-diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine, which when heated in base gave 2-amino-3,4-dihydro-6-(hydroxymethyl)-4-oxopyrido[2,3-*d*]pyrimidine. Treatment of the latter compound with phosphorus tribromide in tetrahydrofuran afforded 2-amino-6-(bromomethyl)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidine, thus constituting the first successful synthesis of this elusive intermediate. The aforementioned bromomethyl compound reacted smoothly with the sodium salt of ethyl 4-mercaptobenzoate, and the resulting ester was saponified to give 10-thia-5-deazapteroic acid. Conventional peptide bond coupling to di-*tert*-butyl L-glutamate followed by treatment with trifluoroacetic acid afforded the target compound in respectable yield. Attempts to prepare its 5,6,7,8-tetrahydro derivative by catalytic hydrogenation were unsuccessful.

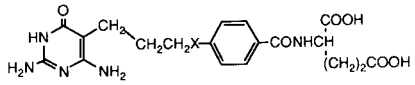
J. Heterocyclic Chem., **28**, 977 (1991).

Recent interest in 5-deaza analogues of folic acid derives largely from observations that several compounds of this type are capable of selectively inhibiting the *de novo* synthesis of purines. For example, 5,10-dideaza-5,6,-7,8-tetrahydrofolic acid (DDATHF), **1a**, owes its broad spectrum of antitumor activity to the inhibition of glycinamide ribonucleotide transformylase (GAR Tfase), the first of two folate requiring enzymes in the biosynthetic pathway leading to inosine monophosphate [1-4]. In addition, 5-deaza-5,6,7,8-tetrahydrofolic acid (5-DATHF), **1b**, is also a potent inhibitor of GAR Tfase as well as the growth of a variety of tumor cell lines in culture [5,6].



1a X = CH_2, **1b** X = NH

Two novel open-chain modifications of **1a** and **1b** have also been described in which the 7 methylene group is deleted. The 5,10-dideaza derivative of this type was designated as 7-DM-DDATHF, **2a**, since it can be considered as the 7-desmethylene analogue of **1a** [7]. It was reported to be somewhat less effective as an inhibitor of the growth of CCRF-CEM cells than **1a** or **1b** [8]. The acyclic counterpart of **1b** has been designated as 5-DACTHF, **2b**. This analogue was also found to be an effective inhibitor of GAR Tfase as well as a weak inhibitor of aminoimidazole ribonucleotide transformylase, the second of the folate-mediated enzymes required for the formation of the purine ring [9]. While 5-DACTHF is approximately 14-fold less potent as an inhibitor of hog live GAR Tfase than DDATHF, it was found to be only 2-fold less effective in inhibiting the growth of MCF-7 cells than DDATHF [10]. The potent cytotoxicity of 5-DACTHF was attributed at least in part to the fact that it is a considerably more efficient substrate than DDATHF for folylpolyglutamate synthetase, the enzyme which converts folates and folate analogues to high molecular weight polyglutamates, which are selectively retained by cells. In addition, it should be noted that polyglutamate metabolites of both DDATHF and 5-DACTHF are considerably more effective as inhibitors of mammalian GAR Tfase than the corresponding monoglutamates [6,9].



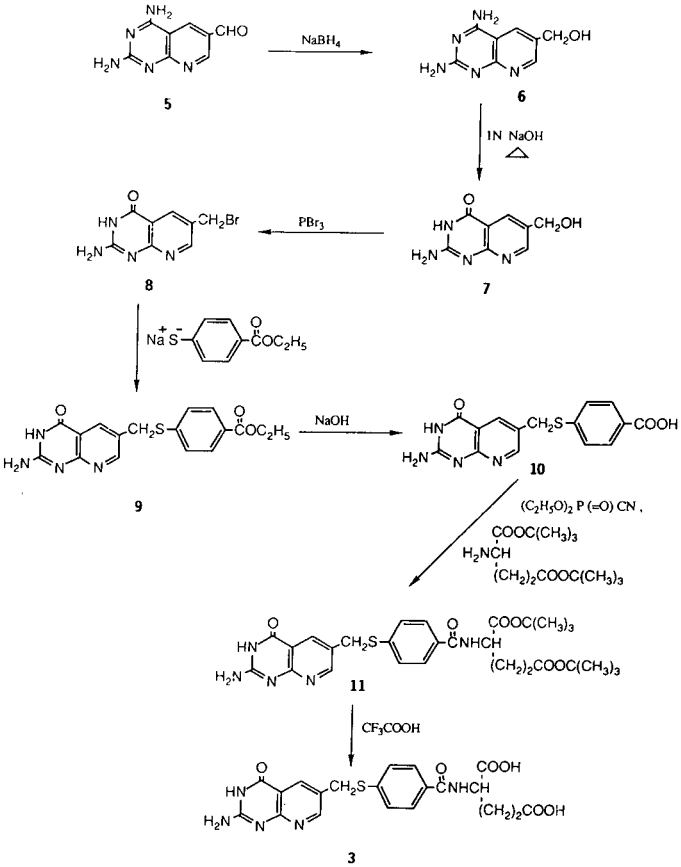
2a X = CH_2, **2b** X = NH

In this paper we describe the synthesis of 10-thia-5-deazafolic acid, **3**, as well as unsuccessful attempts to prepare 10-thia-5-deaza-5,6,7,8-tetrahydrofolic acid, a logical analogue of compounds **1a** and **1b**.

The synthetic route employed for preparing 10-thia-5-deazafolic acid, **3**, is depicted in Scheme I. The key starting material, 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde, **5**, was obtained according to the literature method [3]. It was then reduced using sodium borohydride to afford 2,4-diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine, **6**, in good yield. The nmr spectrum of **6** was in good agreement with that reported earlier for **6** which had been prepared by an entirely different route [11]. Basic hydrolysis of **6** then provided 2-amino-3,4-dihydro-6-(hydroxymethyl)-4-oxopyrido[2,3-*d*]pyrimidine, **7**, in nearly quantitative yield. Several different reagents were evaluated for the conversion of **7** to the corresponding bromomethyl derivative, **8**. Finally, it was found that this elusive intermediate could be obtained readily by treating **7** with phosphorus tribromide in anhydrous tetrahydrofuran.

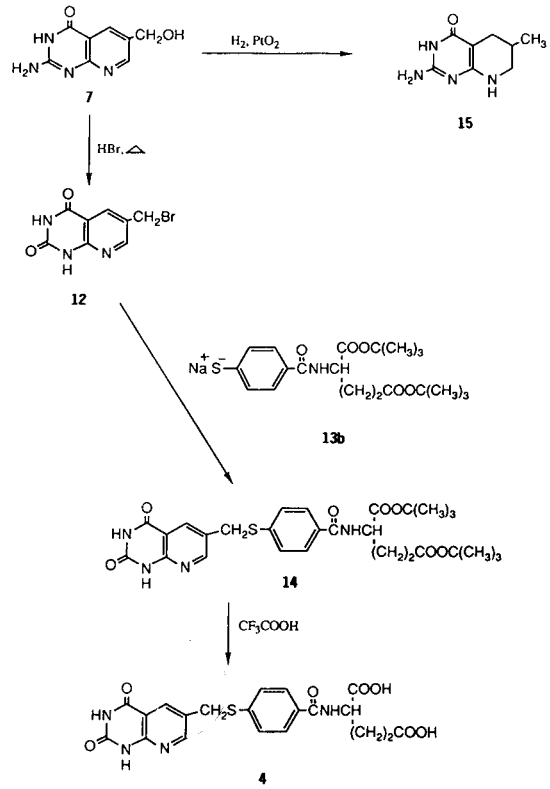
Treatment of crude **8** with the sodium salt of ethyl 4-mercaptobenzoate (generated *in situ*) gave ethyl 10-thia-5-deazapteroate, **9**, which after saponification yielded the key intermediate 10-thia-5-deazapteroic acid, **10**. This was then coupled to di-*tert*-butyl L-glutamate using diethyl phosphorocyanidate as the peptide bond forming reagent to afford di-*tert*-butyl 10-thia-5-deazafolate, **11**. Finally, the treatment of **11** with anhydrous trifluoroacetic acid gave 10-thia-5-deazafolic acid **3**. Attempts to hydrogenate the pyridine ring using platinum oxide or 10 percent palladium on carbon as the catalyst resulted in hydrogenolysis of the carbon-sulfur bond as indicated by the presence of 2-amino-3,4-dihydro-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine, **15**, in the reaction mixture. As shown in Scheme II an authentic sample of **15** was obtained by the catalytic hydrogenation of **7** using platinum oxide and fully characterized.

Scheme I - Synthetic Route to 10-Thia-5-deazafolic Acid



In an initial attempt to prepare 2-amino-6-(bromomethyl)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidine, **8**, compound **7** was heated under reflux in 48% hydrobromic acid. The crude product thus obtained was then treated with the sodium salt of di-*tert*-butyl 4-mercaptobenzoyl-L-glutamate, **13b**, obtained from the sodium borohydride reduction of tetra-*tert*-butyl 4,4′-dithiobisbenzoyl-L-glutamate, **13a**, as shown in Scheme II. The product, which was purified to homogeneity by column chromatography over silcia gel, had a high resolution nmr spectrum which was consistant with di-*tert*-butyl 2-desamino-2-oxo-10-thia-5-deazafolate, **14**. This structure was confirmed by negative ion fast atom bombardment mass spectroscopy. Treatment of **14** with trifluoroacetic acid then afforded 2-desamino-2-oxo-10-thia-5-deazafolic acid **4**. It is apparent, therefore, that the reaction of **7** with 48% hydrobromic acid results in exocyclic bromination as well as the concomitant hydrolysis of the amino group located at position two to yield **12**. It should be noted that in a recent study, the compound 1,4-dihydro-1,6-dimethyl-4-oxo-2-trimethylacetamidoquinazoline underwent hydrolytic deamination when heated in the presence of hydrobromic acid to yield 1,6-dimethyl-2,4-dioxo-1,2,3,4-tetrahydroquinazoline [12].

Scheme II - Synthetic Route to 2-Desamino-2-oxo-10-thia-5-deazafolic Acid



EXPERIMENTAL

Melting points were determined on a Mel-temp apparatus and are uncorrected. Elemental analyses were performed by Atlantic Microlabs, Inc., Norcross, GA. Solvation due to water was confirmed by the presence of a broad peak centered at *ca* δ 3.4 ppm in the ^{1}H nmr spectrum which was transformed into a sharp singlet (DOH) by addition of deuterium oxide. All intermediates were free of significant impurities on tlc using silica gel media (Kodak

- 13181). Free acids **3** and **4** were assayed on cellulose media (Kodak 13254). Column chromatographic separations were performed on Baker silica gel (60-200 mesh). The ^1H high resolution nmr spectra were acquired on a 400 MHz (Varian VXR-400) instrument unless otherwise stated. The ^1H chemical shifts are presented in parts per million downfield from tetramethyl-silane as the internal standard and the relative peaks are given to the nearest whole number. The electron impact mass spectra were obtained off probe using a Finnigan 4521 mass spectrometer and the fast atom bombardment mass spectra (FAB) were obtained on a Finnigan MAT 212 spectrometer using argon bombardment by Dr. Michael Walla, Chemistry Department, University of South Carolina.

2,4-Diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine (**6**).

Sodium borohydride (1.0 g, 26.4 mmoles) was added in portions during a 10 minute interval to a stirred suspension of 2,4-diaminopyrido[2,3-*d*]pyrimidine-6-carboxaldehyde (**5**) [3] (5.0 g, 26.4 mmoles) in 2-methoxyethanol (1000 ml) at ambient temperature. The reaction mixture was stirred for 2 hours and then sodium borohydride (1.0 g) was again added as before. The suspension was stirred for 48 hours at ambient temperature. After removal of the solvent under reduced pressure, the yellow residue was stirred with water (180 ml) and the *p*H of the suspension was lowered to 8.0 by the addition of 1 *N* hydrochloric acid. This mixture was stirred while being heated at about 70° for 20 minutes. The suspension was cooled in ice bath for 30 minutes, the solid isolated by filtration and dried under vacuum at 100° for 18 hours to afford 4.25 g (84%) of **6**, mp >345° dec; ^1H nmr (DMSO-d_6): δ 4.52 (s, 2, CH_2), 5.28 (br s, 1, OH), 6.52 (br s, 2, NH_2), 7.69 (br s, 2, NH_2), 8.35 (br s, 1, 5-H), 8.61 (br s, 1, 7-H); ms: (FAB) m/e 192 $(M+H)^+$. The nmr was in agreement with that reported earlier for **6**, which was prepared by a different synthetic route [11].

2-Amino-3,4-dihydro-6-(hydroxymethyl)-4-oxopyrido[2,3-*d*]pyrimidine (**7**).

A suspension of 3.0 g (15.7 mmoles) of 2,4-diamino-6-(hydroxymethyl)pyrido[2,3-*d*]pyrimidine (**6**) in 300 ml of 1*N* sodium hydroxide was stirred and heated under reflux in a nitrogen atmosphere for six hours. The suspension was then cooled to ambient temperature and acidified to *p*H 6.0 with 2*N* hydrochloric acid to effect precipitation. The yellow colored solid was collected by filtration, washed with iced water followed by a small amount of acetone and dried under vacuum at 80° overnight to afford 3.0 g (100%) of **7**, mp >310° dec; ^1H nmr (DMSO-d_6): δ 4.52 (s, 2, CH_2), 5.29 (br s, 1, OH), 6.86 (br s, 2, NH_2), 8.15 (d, 1, 5-H, J_m = 1.28 Hz), 8.55 (d, 1, 7-H, J_m = 1.04 Hz); ms: (FAB) m/e 193 $(M+H)^+$.

Anal. Calcd. for $C_8H_8N_4O_2 \cdot 4H_2O$: C, 36.36; H, 6.10; N, 21.20. Found: C, 35.98; H, 5.75; N, 20.97.

2-Amino-6-(bromomethyl)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidine (**8**).

A suspension of 1.92 g (10 mmoles) of predried **7** in 30 ml of anhydrous tetrahydrofuran was stirred under nitrogen for 18 hours with 2 ml of phosphorus tribromide. The precipitated solid was isolated by filtration, washed with a small amount of cold tetrahydrofuran and dried. Its nmr in deuteriotrifluoroacetic acid showed formation of a major product, however a small amount of the starting material was still present. Then the solid was retreated with 2 ml phosphorus tribromide in 30 ml anhydrous tetrahydrofuran for 18 hours as above. The product (2.45 g, 96%), thus obtained was free from any starting material and significant impurities. This compound could not be purified and fully characterized because of the lability of the bromo group, however, it was suitable for use in the preparation of **9**; ^1H nmr (deuteriotrifluoroacetic acid): 90 MHz δ 4.65 (s, 2, CH_2), 8.92 (s, 1, 5-H), 9.13 (s, 1, 7-H); ms: (FAB) m/e 255 and 257.

Ethyl 10-Thia-5-deazapteroate (**9**).

A solution of 4.0 g (11.03 mmoles) of diethyl 4,4'-dithiobisbenzoate [13,14] in 220 ml of ethanol was reduced with 0.834 g (22.06 mmoles) of sodium borohydride at ambient temperature. After 10 minutes of stirring the solvent was removed under reduced pressure at 35° and the resultant oily material was dissolved in 30 ml of anhydrous dimethylacetamide. This was added dropwise to a suspension of the bromomethyl compound **8** (prepared from 3.5 g, 18.23 mmoles of **7** and phosphorus tribromide as described earlier) in 160 ml of anhydrous dimethylacetamide and the resulting mixture was stirred at ambient temperature under N_2 for 74 hours. The solvent was removed under reduced pressure at 47°, the oily residue treated with 300 ml of cold water and *p*H of the suspension brought to 8.5 with 2*N* sodium hydroxide. The suspension was stirred, cooled in ice bath for 30 minutes and the solid collected by filtration. The crude product was washed with water, copious amount of acetone and ether and dried at 80° under vacuum overnight to yield 5.10 g (78%) of **9** pure by tlc (silica gel, chloroform-methanol: 80:20), mp >250° dec; ^1H nmr (DMSO-d_6): δ 1.30 (t, 3, CH_3, J = 6.92 Hz), 4.25-4.35 (q, 2, OCH_2, J = 6.90 Hz), 4.43 (s, 2, CH_2S), 6.60-6.94 (br s, 2, NH_2), 7.47 (d, 2, 3', 5', J_o = 8.28 Hz), 7.84 (d, 2, 2', 6', J_o = 8.28 Hz), 8.23 (s, 1, 5-H), 8.64 (s, 1, 7-H), 11.20 (br s, 1, 3-NH); ms: (FAB) m/e 357 $(M+H)^+$.

Anal. Calcd. for $C_{17}H_{16}N_4O_3S \cdot 2H_2O$: C, 52.03; H, 5.14; N, 14.28. Found: C, 51.74; H, 4.87; N, 14.65.

10-Thia-5-deazapteroic Acid (**10**).

A suspension of 3.0 g (7.64 mmoles) of **9** in 65 ml of ethanol, 65 ml of water and 5 ml of 1*N* sodium hydroxide was stirred at ambient temperature for 60 hours. Traces of insoluble material were removed by filtration, and the *p*H of the filtrate was adjusted to 3.5 with 1*N* hydrochloric acid. The precipitated solid was collected by filtration, washed with water and acetone and dried at 80° under vacuum for 18 hours to afford 2.14 g (81%) of **10**, mp >280° dec; ^1H nmr (DMSO-d_6): δ 4.41 (s, 2, CH_2S), 6.82 (br s, 2, NH_2), 7.43 (d, 2, 3', 5', J_o = 8.56 Hz), 7.82 (d, 2, 2', 6', J_o = 8.48 Hz), 8.22 (d, 1, 5-H, J_m = 2.6 Hz), 8.62 (d, 1, 7-H, J_m = 2.4 Hz); ms: (FAB) m/e 329 $(M+H)^+$.

Anal. Calcd. for $C_{15}H_{12}N_4O_3S \cdot H_2O$: C, 52.01; H, 4.07; N, 16.18. Found: C, 51.83; H, 3.98; N, 16.45.

Di-*tert*-butyl 10-Thia-5-deazafolate (**11**).

To a suspension of **10** (2.1 g, 6.09 mmoles) in 150 ml of anhydrous dimethylformamide were added 0.616 g (6.09 mmoles) of triethylamine and 0.99 g (6.09 mmoles) of diethyl phosphorocyanidate in 20 ml of dimethylformamide and the resulting mixture was stirred at ambient temperature under nitrogen for 1.5 hours. Then 1.8 g (6.09 mmoles) of di-*tert*-butyl L-glutamate hydrochloride was added to the reaction mixture and stirring was continued for 18 hours. One more batch of 6.09 mmoles of triethylamine and 6.09 mmoles of diethyl phosphorocyanidate was added to the reaction mixture and it was heated to 65° for 2 hours after

another 18 hours of stirring at ambient temperature under nitrogen. The reaction mixture was filtered hot to remove a small amount of insoluble material and the filtrate was evaporated to dryness. The crude residue was purified on a silica gel column (37 x 14.4 cm) eluting with chloroform-methanol gradients of 98:02 and 95:05. Appropriate fractions were pooled and evaporated to dryness to yield 1.60 g (46%) of pure **11**, mp >220° dec; ^{1}H nmr (DMSO-d_6): δ 1.37 (s, 9, $C(CH_3)_3$), 1.40 (s, 9, $C(CH_3)_3$), 1.84-2.08 (m, 2, glu β-CH_2), 2.31 (br s, 2, glu γ-CH_2), 4.25-4.35 (m, 1, glu, α-CH), 4.41 (s, 2, CH_2S), 6.70 (br s, 2, NH_2), 7.44 (d, 2, 3′, 5′, J_o = 7.44 Hz), 7.78 (d, 2, 2′, 6′, J_o = 7.36 Hz), 8.23 (br s, 1, 5-H), 8.54 (d, 1, CONH, J = 7.20 Hz), 8.63 (br s, 1, 7-H); ms: (FAB) m/e 570 $(M+H)^+$.

Anal. Calcd. for $C_{28}H_{35}N_5O_6S$: C, 59.03; H, 6.19; N, 12.29. Found; C, 58.69; H, 5.85; N, 12.57.

10-Thia-5-deazafolic Acid (**3**).

Compound **11** (0.25 g, 0.439 mmole) was dissolved in trifluoroacetic acid (25 ml) and stirred at ambient temperature for 3 hours. The solvent was removed under reduced pressure at 45° with the help of added protions of ethanol and ether and the resultant oily residue was treated with 20 ml of water. The suspension was basified to *p*H 11 with 1*N* sodium hydroxide to give a solution which was clarified by filtration, and the filtrate was brought to *p*H 3.5 with 1*N* hyrochloric acid. The precipitated solid was isolated by filtration, washed with water and acetone and dried at 80° under vacuum for 18 hours to obtain 0.16 g (77%) of 10-thia-5-deazafolic acid (**3**), mp >225°; ^{1}H nmr (DMSO-d_6): δ 1.88-2.12 (m, 2, glu β-CH_2), 2.34 (t, 2, glu γ-CH_2, J = 7.44 Hz), 4.33-4.39 (m, 1, glu α-CH), 4.41 (s, 2, CH_2S), 6.73 (br s, NH_2), 7.44 (d, 2, 3′, 5′, J_o = 8.48 Hz), 7.79 (d, 2, 2′, 6′, J_o = 8.32 Hz), 8.23 (d, 1, 5-H, J_m = 2.36 Hz), 8.55 (d, 1, CONH, J = 8.16 Hz), 8.62 (br s, 1, 7-H); ms: (FAB) m/e 458 $(M+H)^+$ and 456 $(M-H)^-$.

Anal. Calcd. for $C_{20}H_{19}N_5O_6S\cdot H_2O$: C, 50.52; H, 4.45; N, 14.73. Found: C, 50.16; H, 4.68; N, 15.09.

Tetra-*tert*-butyl 4,4′-Dithiobis(benzoyl-L-glutamate) (**13a**).

A solution of 1.03 g (3.37 mmoles) of 4,4′-dithiobisbenzoic acid [13,14] and 0.68 g (6.73 mmoles) of 4-methylmorpholine in 20 ml of anhydrous dimethylformamide was cooled to −15°, and the mixed anhydride was formed by the addition of 0.918 g (6.73 mmoles) of isobutyl chloroformate. After 2 minutes, a precooled (−15°) suspension of 1.99 g (6.73 mmoles) of di-*tert*-butyl glutamate hydrochloride, 0.68 g (6.73 mmoles) of triethylamine and 0.68 g (6.73 mmoles) of 4-methylmorpholine in 15 ml of anhydrous dimethylformamide was added at one time. The reaction mixture was placed under nitrogen, stirred at −15° for 45 minutes and then allowed to warm to room temperature and stirred for 18 hours. The reaction mixture was filtered to remove a small amount of water soluble white precipitate and the filtrate evaporated to dryness. The residue was dissolved in 15 ml of chloroform and the solution was washed with 15 ml of water, 15 ml of 10% potassium bicarbonate, 15 ml of water, 2 x 15 ml of 5% citric acid, 15 ml of water and finally with 15 ml of saturated sodium chloride. After drying over magnesium sulfate, the solvent was removed under vacuum and the resulting oily material was purified by applying it to a silica gel column (37 x 14.4 cm) and eluting with chloroform. Appropriate fractions were pooled and evaporated to dryness under reduced pressure to afford 1.65 g (63%) of pure oily material which was triturated with 50 ml of hexane and cooled at 4° for 18 hours to afford a light yellowish solid. The product was collected by filtration and dried under vacuum at ambient temperature for 48 hours to give a yellowish white product, **13a**, mp, 50-54°; ^{1}H nmr (deuteriochloroform): δ 1.38 (s, 9, $C(CH_3)_3$), 1.45 (s, 9, $C(CH_3)_3$), 1.96-2.44 (m, 4, $(CH_2)_2$), 4.57-4.65 (m, 1, α-CH), 6.97 (d, 1, CONH, J = 7.65 Hz), 7.49 (d, 2, 3′, 5′, J_o = 8.40 Hz), 7.74 (d, 2, 2′, 6′, J_o = 8.51 Hz); ms: (FAB) m/e 790 $(M+H)^+$.

Anal. Calcd. for $C_{40}H_{56}N_2O_{10}S_2$: C, 60.90; H, 7.15; N, 3.55. Found: C, 61.25; H, 7.39; N, 3.69.

Di-*tert*-butyl 2-Desamino-2-oxo-10-thia-5-deazafolate (**14**).

A solution of 1.0 g (5.2 mmoles) of 2-amino-3,4-dihydro-6-(hydroxymethyl)-4-oxopyrido[2,3-*d*]pyrimidine (**7**) in 48% hydrobromic acid (50 ml) was heated under reflux for six hours. The reaction mixture was evaporated to dryness under reduced pressure and dried to afford 1.1 g of crude, dark brown product 6-(bromomethyl)-1,2,3,4-tetrahydro-2,4-dioxopyrido[2,3-*d*]pyrimidine (**12**), which was used without furhter purification; ms: (FAB) m/e 255 and 257 $(M-H)^-$. A solution of 1.0 g (1.268 mmoles) of tetra-*tert*-butyl 4,4′-dithiobisbenzoyl-L-glutamate (**13a**) in 12 ml of ethanol was reduced with 0.145 g (3.8 mmoles) of sodium borohydride at ambient temperature to give **13b**. This was added portionwise to a solution of 0.80 g (2.39 mmoles) of **12** in 30 ml of dimethylformamide, and the resulting mixture was stirred at ambient temperature for 18 hours. The reaction mixture was filtered to remove a small amount of insoluble dark solid and the filtrate was evaporated to dryness under reduced pressure to afford a light yellowish oil which upon trituration with water (15 ml) gave a solid. The product was collected by filtration, washed with cold water and dried at 75° under vacuum to afford 0.95 g of crude product which was purified by applying it to a silica gel column (30 x 8 cm) and eluting with successive gradients of chloroform-methanol:99:01, 98:02 and 97:03. Appropriate fractions were pooled and evaporated to dryness to afford 0.58 g (43%) of pure **14**, mp 166-169°; ^{1}H nmr (DMSO-d_6): δ 1.36 (s, 9, $C(CH_3)_3$), 1.39 (s, 9, $C(CH_3)_3$), 1.84-2.05 (m, 2, glu β-CH_2), 2.31 (t, 2, glu γ-CH_2, J = 7.86 Hz), 4.26-4.32 (m, 1, glu α-CH), 4.42 (s, 2, CH_2S), 7.44 (d, 2, 3′, 5′, J_o = 8.37 Hz), 7.78 (d, 2, 2′, 6′, J_o = 8.55 Hz), 8.28 (d, 1, 5-H, J_m = 2.38 Hz), 8.54 (d, 1, CONH, J = 7.69 Hz), 8.59 (d, 1, 7-H, J_m = 2.39 Hz), 11.43 (br s, 1, 1 or 3 NH), 11.63 (br s, 1, 1 or 3 NH); ms: (FAB) m/e 569 $(M-H)^-$.

Anal. Calcd. for $C_{28}H_{34}N_4O_7S\cdot H_2O$: C, 57.10; H, 6.16; N, 9.52. Found: C, 56.83; H, 5.93; N, 9.22.

2-Desamino-2-oxo-10-thia-5-deazafolic Acid (**4**).

Compound **14** (0.25 g, 0.42 mmole) was dissolved in trifluoroacetic acid (7 ml). After the reaction mixture was stirred at ambient temperature for 2 hours, the solution was evaporated under reduced pressure with the help of added ethanol. The residue was triturated with 3 x 20 ml of ether to afford a yellowish solid which was dissolved in 1*N* sodium hydroxide (20 ml) and stirred with cellulose (0.1 g) and decolorizing charcoal (0.05 g) for 1 hour and filtered. The filtrate was acidified with 2*N* hydrochloric acid to *p*H 3.5 to precipitate a greyish-white solid, which was collected by filtration, washed with water and ether and dried under vacuum at 75° for 6 hours to obtain 0.14 g (70%) of **4** pure by tlc (cellulose, 5% ammonium bicarbonate), mp >225° dec with preliminary darkening; ^{1}H nmr (DMSO-d_6): δ 1.88-2.12 (m, 2, glu

β-CH_2), 2.34 (t, 2, glu γ-CH_2, J = 7.32 Hz), 4.34-4.40 (m, 1, glu α-CH), 4.42 (s, 2, CH_2S), 7.44 (d, 2, 3', 5', J_o = 8.56 Hz), 7.80 (d, 2, 2', 6', J_o = 8.40 Hz), 8.29 (d, 1, 5-H, J_m = 2.36 Hz), 8.56 (d, 1, CONH, J = 7.72 Hz), 8.59 (d, 1, 7-H, J_m = 2.36 Hz), 11.43 (br s, 1, 1 or 3 NH), 11.64 (br s, 1, 1 or 3 NH), 12.20-12.64 (br s, COOH); ms: (FAB) m/e 457 $(M-H)^-$.

Anal. Calcd. for $C_{20}H_{18}N_4O_7S \cdot H_2O$: C, 50.41; H, 4.23; N, 11.75. Found: C, 50.60; H, 4.05; N, 11.67.

2-Amino-3,4-dihydro-6-methyl-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidine (**15**).

To a partial solution of **7** (0.5 g, 2.6 mmoles) in trifluoroacetic acid (40 ml) was added platinum oxide (0.3 g), and the mixture was hydrogenated (45-55 psi) in a Parr apparatus for 3 hours at 25°. The solution was filtered through Celite with the aid of 0.1 g of decolorizing charcoal. The clear almost colorless filtrate was evaporated to dryness under reduced pressure with the help of added portions of ethanol. The white residue was treated with 30 ml of 10% ammonium hydroxide and 15 ml of dimethylformamide to give a light yellow solution which was stirred for 5 minutes and clarified by filtration. The filtrate was evaporated to dryness under reduced pressure to afford a cream colored residue, which was treated with water (15 ml) and collected by filtration. The product was dried under vacuum at 60° overnight to obtain 0.3 g (59%) of white **15**, mp 230-234°; ^{1}H nmr (DMSO-d_6): δ 0.94 (d, 3, CH_3, J = 5.44 Hz), 1.72-1.84 (m, 2), 2.38-2.48 (m, 1), 2.73-2.81 (m, 1), 3.18-3.26 (br d, 1), 6.85-7.25 (two br s, 3, NH_2 and 8-NH), 10.60-11.40 (br s, 1, 3-NH); ms: m/e 180.

Anal. Calcd. for $C_8H_{12}N_4O \cdot 2H_2O$: C, 44.43; H, 7.46; N, 25.91. Found: C, 44.16; H, 7.29; N, 25.95.

Acknowledgement.

This investigation was supported by a grant from the Burroughs Wellcome Company (J.B.H.).

REFERENCES AND NOTES

[1] G. P. Beardsley, B. A. Moroson, E. C. Taylor and R. G. Moran, *J. Biol. Chem.*, **264**, 328 (1989).

[2] G. P. Beardsley, E. C. Taylor, G. B. Grindey and R. G. Moran in Chemistry and Biology of Pteridines, B. A. Cooper and V. M. Whitehead eds, Walter de Gruyter, Berlin, 1986, pp 953-957.

[3] J. R. Piper, G. S. McCaleb, J. A. Montgomery, R. L. Kisliuk, Y. Gaumont, J. Thorndike and F. M. Sirotnak, *J. Med. Chem.*, **31**, 2164 (1988).

[4] R. G. Moran, S. W. Baldwin, E. C. Taylor and C. Shih, *J. Biol. Chem.*, **264**, 21047 (1989).

[5] E. C. Taylor, J. M. Hamby, C. Shih, G. B. Grindey, S. M. Rinzel, G. P. Beardsley and R. G. Moran, *J. Med. Chem.*, **32**, 1517 (1989).

[6] S. W. Baldwin, A. Tse, G. B. Grindey, E. C. Taylor, A. Rosowsky, C. Shih and R. G. Moran, *Proc. Am. Assoc. Cancer Res.*, **31**, 341 (1990).

[7] E. C. Taylor, P. M. Harrington and C. Shih, *Heterocycles*, **28**, 1169 (1989).

[8] O. Russello, A. R. Cashmore, B. A. Moroson, D. E. Wildman, E. C. Taylor and G. P. Beardsley, *Proc. Am. Assoc. Cancer Res.*, **31**, 343 (1990).

[9] J. L. Kelly, E. W. McLean, N. K. Cohn, M. P. Edelstein, D. S. Duch, G. K. Smith, M. H. Hanlon and R. Ferone, *J. Med. Chem.*, **33**, 561 (1990).

[10] S. K. Singh, I. K. Dev, D. S. Duch, R. Ferone, G. K. Smith, J. H. Freisheim and J. B. Hynes, *J. Med. Chem.*, **34**, 606 (1991).

[11a] T.-L. Su, J.-T. Huang, J. H. Burchenal, K. A. Watanabe and J. J. Fox, *J. Med. Chem.*, **29**, 709 (1986); [b] J. I. de Graw, H. Tagawa, P. H. Christie, J. A. Lawson, E. G. Brown, R. L. Kisliuk and Y. Gaumont, *J. Heterocyclic Chem.*, **23**, 1 (1986).

[12] T. R. Jones, R. F. Betteridge, D. R. Newell and A. L. Jackman, *J. Heterocyclic Chem.*, **26**, 1501 (1989).

[13] E. Campaigne and W. W. Meyer, *J. Org. Chem.*, **27**, 2835 (1962).

[14] Y. H. Kim, Y. Gaumont, R. L. Kisliuk and H. G. Mautner, *J. Med. Chem.*, **18**, 776 (1975).