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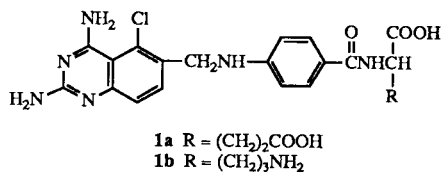
Received July 30, 1990

The target folate analogue 5-trifluoromethyl-5,8-dideazafolic acid has been elaborated in a seven-step reaction sequence beginning with 2-fluoro-6-trifluoromethylbenzotrile. The bridge-reversed isomer 5-trifluoromethyl-5,8-dideazaisofolic acid was prepared in six steps using the common intermediate 2,6-diamino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline. The key intermediate 2-amino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline was prepared using two distinct synthetic routes and the resulting products were found to be identical.

*J. Heterocyclic Chem.*, **27**, 2101 (1990).

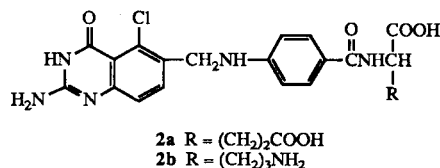
The introduction of small nonpolar substituents at position five of the quinazoline nucleus of folate analogues can have a dramatic influence upon biological activity. Some important examples of this phenomenon have been described using antibacterial [1], antimycobacterial [2], antimalarial [3] and antitumor [4,5] test systems. In each of these studies the highly active compounds were 2,4-diaminoquinazolines and the enzymatic target was assumed to be dihydrofolate reductase. The enzyme folypolyglutamate synthetase (FPGS) is responsible for the intracellular conversion of naturally occurring folates into poly- $\gamma$ -L-glutamyl derivatives, a process which has been deemed to be essential for cell survival [6,7].

Folate analogues such as methotrexate which contain a terminal L-glutamate residue are also substrates for FPGS and the resulting longer chain polyglutamates of methotrexate are selectively retained by tumor cells in the absence of extracellular drug [8-11]. Recently, a wide variety of quinazoline analogues of folic acid (5,8-dideazafolates) were evaluated as substrates for hog liver FPGS [12]. The substrate activities as measured by  $V_{max}/K_m$  varied by greater than 100-fold depending upon structure. However, within each homologous series of compounds the 5-chloro modifications were consistently the most efficient substrates. Careful kinetic studies have revealed that compounds **1a** and **2a** are the best substrates for FPGS reported thus far being approximately 20-fold superior to (6*S*)-tetrahydrofolate [13]. On the other hand, the corresponding five chloro analogues bearing a terminal L-ornithine in place of a L-glutamate moiety, **1b** and **2b**, are



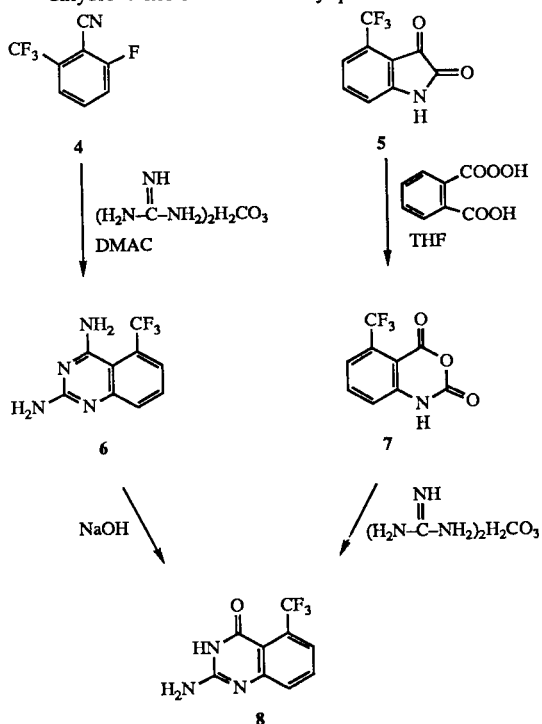
by far the most potent inhibitors of FPGS known [13]. Therefore, it was of interest to synthesize and evaluate

other 5,8-dideaza analogues of folic acid possessing more hydrophobic substituents located at position five. This paper describes the synthesis of 5-trifluoromethyl-5,8-dideazafolic acid, **3a**, and 5-trifluoromethyl-5,8-dideazaisofolic acid, **3b**, (*cf.* Scheme II). The biological testing of these new folate analogues is currently in progress.

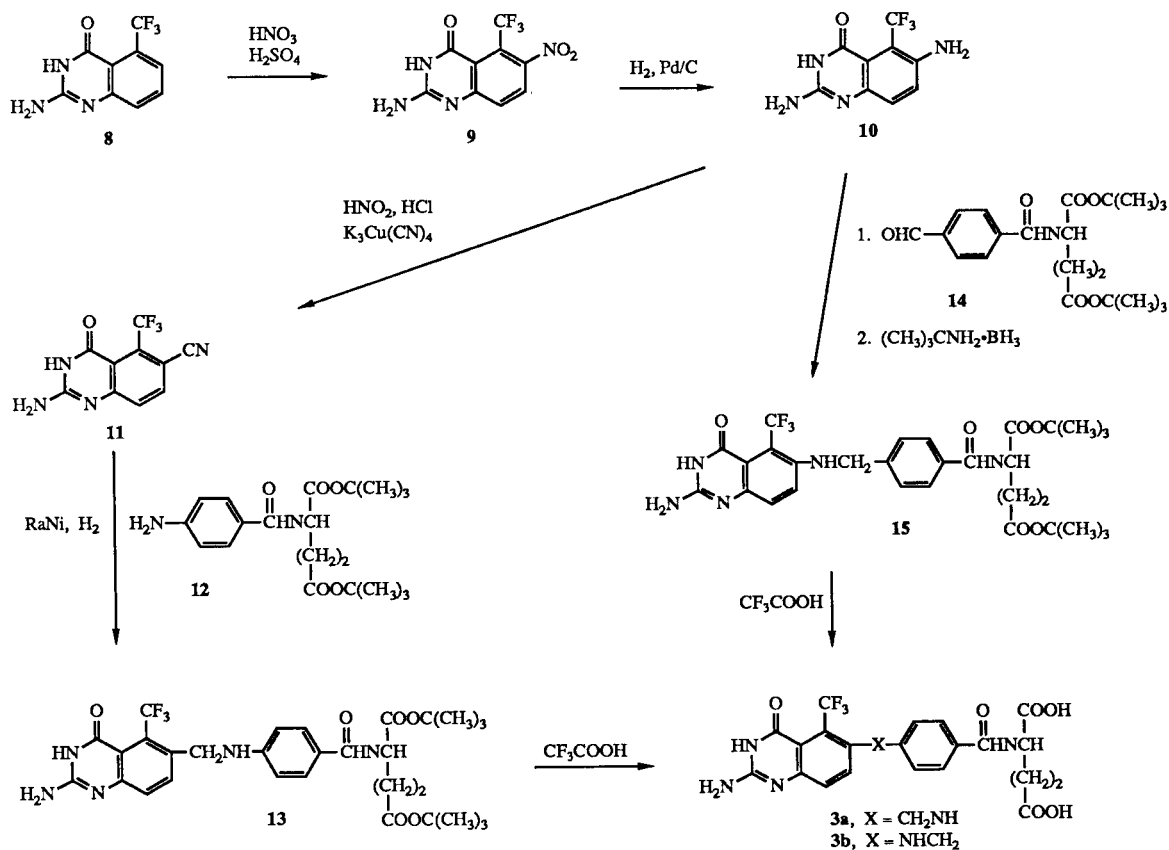


The synthetic routes used to prepare the new 5-trifluoromethyl-5,8-dideaza analogues of folic acid and isofolic acid are depicted in Scheme II. The key intermediate 2-amino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline (**8**) was prepared by two different reaction sequences as shown in Scheme I. The initial approach was to prepare **8** by the condensation of 5-trifluoromethylisatoic anhydride (**7**) with guanidine. Compound **7** in turn was obtained by the oxidation of 5-trifluoromethylisatoic acid (**5**) [14] with monophtalic acid [15] in 48% yield. Reaction of the anhydride **7** with guanidine carbonate in dimethylformamide resulted in a 34% yield of **8**. In view of the poor yield obtained in this reaction and the fact that a large quantity of **8** was required for this synthetic effort, an alternate route for its synthesis was sought. Finally, 2,4-diamino-5-trifluoromethylquinazoline (**6**) was prepared in excellent yield from 2-fluoro-6-trifluoromethylbenzotrile (**4**) utilizing methodology developed recently in this laboratory [16]. Hydrolysis of **6** under basic conditions then afforded the key intermediate **8** in excellent yield, which was shown to be identical to that obtained from the earlier synthetic approach. The nitration of compound **8** gave exclusively the six isomer, 2-amino-3,4-dihydro-4-oxo-6-nitro-5-trifluoromethylquinazoline (**9**). Catalytic hydrogenation of **9** then afforded the 6-amino-modification **10**. Diazotization of **10** under Sandmeyer conditions yielded the required 6-cyano

Scheme I. Alternate Synthetic Routes to 2-Amino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline



Scheme II. Synthetic Routes to 5-Trifluoromethyl-5,8-dideazafolic Acid and 5-Trifluoromethyl-5,8-dideazaisofolic Acid



derivative **11**. Compound **11** was then condensed reductively with di-*t*-butyl *N*-(4-aminobenzoyl)-L-glutamate (**12**) [17] in the presence of Raney nickel to yield the penultimate di-*t*-butyl ester **13**. Removal of the *t*-butyl groups with trifluoroacetic acid then gave 5-trifluoromethyl-5,8-dideazafolic acid (**3a**). The isofolate analogue 5-trifluoromethyl-5,8-dideazaisofolic acid (**3b**) was prepared by reductive amination of **10** to di-*t*-butyl *N*-(4-formylbenzoyl)-L-glutamate (**14**) [18] in the presence of *t*-butylamine-borane complex followed by deprotection of the resulting di-*t*-butyl ester, **15**, with trifluoroacetic 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  $\alpha \delta$  3.4 ppm in the  $^1\text{H}$  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 **3a** and **3b** were assayed on cellulose media (Kodak 13254). Column chromatographic separations were performed on Baker silica gel (60-200 mesh). The  $^1\text{H}$  and  $^{19}\text{F}$  high resolution spectra were acquired on a 400 MHz (Varian VXR-400) instrument. The  $^1\text{H}$  chemical shifts are presented in

parts per million downfield from tetramethylsilane as the internal standard and the relative peak areas are given to the nearest whole number. The  $^{19}\text{F}$  chemical shifts are presented in parts per million relative to fluorotrichloromethane as the internal standard. 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-Fluoro-

6-trifluoromethylbenzonitrile (**4**) was obtained from Accurate Chemical & Scientific Corp., Westbury, NY.

#### 2,4-Diamino-5-trifluoromethylquinazoline (**6**).

A mixture of 25 g (0.132 mole) of 2-fluoro-6-trifluoromethylbenzonitrile (**4**), guanidine carbonate 47.63 g (0.264 mole) in 300 ml of dimethylacetamide was stirred under a nitrogen atmosphere at 128-130° for 5 hours. The course of the reaction was monitored by  $^{19}\text{F}$  nmr. The reaction mixture was allowed to cool to ambient temperature and evaporated to dryness with the addition of ethanol under vacuum to afford a light cream color solid. Next, it was treated with ca 500 ml of ice water and the product was separated by filtration, washed with ice water and dried under vacuum at 75° overnight to yield 29.5 g (98%) of pure cream color product **6**, mp 176-178°;  $^1\text{H}$  nmr (DMSO- $d_6$ ):  $\delta$  6.31 (br s, 2H, 2-NH $_2$ ), 6.59 (br s, 2H, 4-NH $_2$ ), 7.47-7.52 (m, 2H, 6-H and 8-H), 7.59 ppm (t, 1H, 7-H,  $J$  = 15.72 Hz);  $^{19}\text{F}$  nmr (DMSO- $d_6$ ): -54.25 ppm (s, CF $_3$ ); ms: (m/e) 228 (M $^+$ ).

*Anal.* Calcd. for C $_9$ H $_7$ N $_4$ F $_3$ : C, 47.37; H, 3.09; N, 24.55; Found: C, 47.29; H, 3.13; N, 24.50.

#### 5-Trifluoromethylisatoic Anhydride (**7**).

To a solution of freshly prepared monoperphthalic acid [15] (29.15 g, 0.16 mole) in dry ether (250 ml) was slowly added 21.5 g (0.1 mole) of 4-trifluoromethylisatin (**5**) [14] in dry tetrahydrofuran (100 ml) at 0°. The mixture was stirred at 0° for 5 hours at which time the tlc indicated complete disappearance of the starting material **5**. The reaction mixture was allowed to warm up to room temperature in 1 hour, diluted with ca 300 ml of ether and carefully washed with saturated sodium bicarbonate solution until the evolution of carbon dioxide gas stopped. The ethereal solution was dried on anhydrous sodium sulfate and evaporated under vacuum to afford 17 g (74%) of the crude product which upon recrystallization from a mixture of ethyl acetate and hexane yielded 11 g (48%) of pure 5-trifluoromethylisatoic anhydride (**7**), mp 232-234° dec;  $^1\text{H}$  nmr (DMSO- $d_6$ ):  $\delta$  7.46 (d, 1H, 6-H or 8-H,  $J$  = 8.36 Hz), 7.66 (d, 1H, 6-H or 8-H,  $J$  = 7.68 Hz), 7.88 (t, 1H, 7-H,  $J$  = 16.16 Hz), 12.00 ppm (br s, NH);  $^{19}\text{F}$  nmr (DMSO- $d_6$ ):  $\delta$  -58.91 ppm (s, CF $_3$ ); ms: (m/e) 231 (M $^+$ ).

*Anal.* Calcd. for C $_9$ H $_4$ N $_2$ O $_3$ F $_3$ : C, 46.76; H, 1.74; N, 6.06. Found: C, 46.48; H, 1.79; N, 6.03.

#### 2-Amino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline (**8**).

##### Method A.

A mixture of 16.8 g (0.073 mole) of 5-trifluoromethylisatoic anhydride (**7**) and guanidine carbonate (19.7 g, 0.11 mole) in 230 ml of dimethylformamide was heated under reflux for 28 hours. The reaction mixture was cooled to ambient temperature, 4 g (0.22 mole) of guanidine carbonate was added and the heating was continued for an additional 7 hours. Next, it was diluted with 1 liter of water and refrigerated for 20 hours to precipitate 4.5 g of a

light gray solid, which was isolated by filtration. A second crop of 3.2 g solid was recovered from the filtrate after evaporation under vacuum. The combined crude product (7.7 g, 44%) was recrystallized twice from dimethyl sulfoxide-water to yield 5.9 g (34%) of pure **8**, mp >250° dec;  $^1\text{H}$  nmr (DMSO- $d_6$ ):  $\delta$  6.56 (br s, 2H, 2-NH $_2$ ), 7.45-7.48 (m, 2H, 6-H and 8-H), 7.66 (t, 1H, 7-H,  $J$  = 15.84 Hz), 11.17 ppm (br s, 1H, 3-NH);  $^{19}\text{F}$  nmr (DMSO- $d_6$ ):  $\delta$  -56.94 ppm (s, CF $_3$ ); ms: (m/e) 229 (M $^+$ ).

*Anal.* Calcd. for C $_9$ H $_6$ N $_3$ OF $_3$ ·0.3H $_2$ O: C, 46.08; H, 2.84; N, 17.91. Found: C, 46.07; H, 2.70; N, 18.15. (Dried under vacuum at 100° for 12 hours).

##### Method B.

A suspension of 23.75 g (0.104 mole) of 2,4-diamino-5-trifluoromethylquinazoline (**6**) in 1.5 liter of 1N sodium hydroxide was stirred and heated under reflux in a nitrogen atmosphere for 5 hours. The solution was cooled to ambient temperature and acidified to pH 3.0 with 1N hydrochloric acid to effect precipitation. The light yellow solid was collected by filtration, washed with ice water (10 ml) and dried under vacuum at 80° overnight to afford 22.48 g (94%) of **8**, identical by mp, tlc and nmr with the sample described in Method A.

#### 2-Amino-3,4-dihydro-4-oxo-6-nitro-5-trifluoromethylquinazoline (**9**).

Concentrated sulfuric acid, 98%, (250 ml) was added dropwise to fuming nitric acid, 90% (250 ml) stirred at 0° and the mixture was stirred and cooled (ice bath) under nitrogen. To this solution was added portionwise 2-amino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline (**8**) (22.4 g, 0.094 mole) with stirring at 0° over a period of 30 minutes. After stirring the reaction mixture at 0° for 30 minutes it was allowed to warm up to ambient temperature and the stirring was continued for 24 hours. The course of the reaction was followed by  $^{19}\text{F}$  nmr. Next, the reaction mixture was poured onto crushed ice (500 g) and the pH of the light yellow suspension was adjusted to 8.5 by dropwise addition of concentrated ammonium hydroxide, with stirring and cooling below 20°. The yellow solid was isolated by filtration, washed with ice water (15 ml) and dried at 80° under vacuum for 18 hours to afford 21.73 g (79%) of crude product which upon recrystallization from dimethyl sulfoxide-water yielded 16.82 g (61%) of pure bright yellow needles of **9**, mp >300° dec (preliminary softening);  $^1\text{H}$  nmr (DMSO- $d_6$ ):  $\delta$  6.95 (br s, 2H, 2-NH $_2$ ), 7.54 (d, 1H, 8-H,  $J_{8,7}$  = 9.12 Hz), 7.99 (d, 1H, 7-H,  $J_{7,8}$  = 9.16 Hz), 11.50 ppm (s, 1H, 3-NH);  $^{19}\text{F}$  nmr (DMSO- $d_6$ ):  $\delta$  -53.31 ppm (s, CF $_3$ ); ms: (m/e) 274 (M $^+$ ).

*Anal.* Calcd. for C $_9$ H $_5$ N $_4$ O $_3$ F $_3$ ·H $_2$ O: C, 36.99; H, 2.41; N, 19.17. Found: C, 37.24; H, 2.73; N, 18.87.

#### 2,6-Diamino-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline (**10**).

A solution of 16 g (0.055 mole) of the 6-nitro derivative **9** in dimethylformamide (900 ml) and 2-methoxyethanol (100 ml) containing 16 g of 10% palladium-charcoal was hydrogenated at ca 45 psi until the theoretical uptake of hydrogen had occurred (30 minutes). The fluorescent greenish reaction mixture was filtered through Celite and the filtrate evaporated to dryness with the help of added portions of ethanol. The resultant yellowish solid was collected, washed with ether (25 ml) and dried under vacuum at 80° for 18 hours to afford 12.98 g (91%) of crude product which upon recrystallization from dimethyl sulfoxide-water yielded 9.83 g (69%) of pure yellow solid **10**, mp >235° dec;  $^1\text{H}$  nmr

(DMSO- $d_6$ ):  $\delta$  5.73 (br s, 2H, 6-NH<sub>2</sub>), 6.50 (s, 2H, 2-NH<sub>2</sub>), 7.14-7.22 (app. q, 2H, 7-H and 8-H), 11.30 ppm (br s, 1H, 3-NH); <sup>19</sup>F nmr (DMSO- $d_6$ ):  $\delta$  -50.09 ppm (s, CF<sub>3</sub>); ms: (m/e) 244 (M<sup>+</sup>).

*Anal.* Calcd. for C<sub>9</sub>H<sub>7</sub>N<sub>4</sub>OF<sub>3</sub>·0.75H<sub>2</sub>O: C, 41.95; H, 3.32; N, 21.74. Found: C, 41.67; H, 3.01; N, 22.44.

2-Amino-6-cyano-3,4-dihydro-4-oxo-5-trifluoromethylquinazoline (**11**).

To a solution of 5 g (0.0194 mole) of **10** in 55 ml of 2*N* hydrochloric acid cooled in ice bath was added during a 2 minute period to a cold solution of 1.44 g (0.0209 mole) of sodium nitrite in 10 ml water. The mixture was stirred at 2° in ice bath for 20 minutes and then added to a stirred solution containing 10.68 g (0.1640 mole) of potassium cyanide and 4.33 g (0.4374 mole) of cuprous chloride in 38 ml of water maintained at 2°. After 5 minutes of stirring the reaction mixture was diluted with 300 ml of ice water and stirred at 2° for 30 minutes. Next, it was warmed to 35° for 30 minutes and then stirred at ambient temperature for 1 hour. The pH of the reaction mixture was adjusted to 10 by the addition of concentrated ammonium hydroxide with cooling in ice bath. The reaction mixture was evaporated to dryness under vacuum with the help of added portions of dimethylformamide to produce a dark residue which was extracted with methanol. The crude product obtained after the evaporation of the methanol was purified on a silica gel column prepacked in chloroform. Elution of the column with 100% chloroform to 30% methanol in chloroform in 10% increments afforded (2.93 g, 55%) of product which was recrystallized from dimethyl sulfoxide-water to yield 2.44 g (45%) of pure **11**, mp > 350° dec; <sup>1</sup>H nmr (DMSO- $d_6$ ):  $\delta$  7.08 (br s, 2H, 2-NH<sub>2</sub>), 7.51 (d, 1H, 8-H, J<sub>8,7</sub> = 8.76 Hz), 7.97 (d, 1H, 7-H, J<sub>7,8</sub> = 8.76 Hz), 11.44 ppm (br s, 1H, 3-NH); <sup>19</sup>F nmr (DMSO- $d_6$ ):  $\delta$  -53.71 ppm (s, CF<sub>3</sub>); ms: (m/e) 254 (M<sup>+</sup>).

*Anal.* Calcd. for C<sub>10</sub>H<sub>8</sub>N<sub>4</sub>OF<sub>3</sub>·1.25H<sub>2</sub>O: C, 43.41; H, 2.73; N, 20.24. Found: C, 43.72; H, 2.89; N, 19.87.

Di-*t*-butyl 5-Trifluoromethyl-5,8-dideazaisofolate (**13**).

A mixture of 0.50 g (1.807 mmoles) of **11** and 0.82 g (2.17 mmoles) of di-*t*-butyl *N*-(4-aminobenzoyl)-L-glutamate (**12**) [17] in 62 ml of 70% acetic acid was stirred for 20 minutes at ambient temperature. To the resulting dark yellowish brown solution was then added Raney nickel (ca 0.30 g) and the mixture hydrogenated until hydrogen uptake ceased (20 hours). The process was repeated with an additional ca 0.30 g of Raney nickel. Charcoal was added and the reaction mixture was filtered through Celite and then basified to pH 8.5 with concentrated ammonium hydroxide. After refrigeration, the precipitated solid was collected by filtration, washed with water and then with ether in order to remove unreacted **12**. The cream colored solid was dried under vacuum at 75° overnight to yield 0.58 g (52%) of pure product **13**. Another 0.12 g (11%) of product was recovered from the ether washing by evaporating the solvent and purifying the residue on a silica gel column packed in chloroform. The product was eluted with 2% methanol in chloroform. Appropriate fractions were pooled and evaporated to dryness to afford a light yellow product, mp > 160° dec with preliminary softening; <sup>1</sup>H nmr (DMSO- $d_6$ ):  $\delta$  1.38 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.40 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.82-2.03 (two m, 2H, glu- $\beta$ -CH<sub>2</sub>), 2.29 (t, 2H, glu- $\gamma$ -CH<sub>2</sub>, J = 15.36 Hz), 4.23-4.30 (m, 1H, glu- $\alpha$ -CH), 4.46 (br s, 2H, NHCH<sub>2</sub>), 6.54 (d, 2H, 3'-H and 5'-H, J<sub>0</sub> = 8.92 Hz), 6.80-6.87 (t embedded in a br s, 3H, NH-CH<sub>2</sub> and 2-NH<sub>2</sub>), 7.33 (d, 1H, 8-H, J<sub>8,7</sub> = 8.72 Hz), 7.62 (app. t, 3H, 7-H, 2'-H and 6'-H, J = 17.76 Hz), 8.07 ppm

(d, 1H, CONH, J = 7.68 Hz); <sup>19</sup>F nmr (DMSO- $d_6$ ):  $\delta$  -50.16 ppm (s, CF<sub>3</sub>); ms: (FAB) m/e 620 (M + H)<sup>+</sup>.

*Anal.* Calcd. for C<sub>30</sub>H<sub>36</sub>N<sub>5</sub>O<sub>6</sub>F<sub>3</sub>: C, 58.15; H, 5.85; N, 11.30. Found: C, 57.95; H, 6.03; N, 11.12.

5-Trifluoromethyl-5,8-dideazafolic Acid (**3a**).

A solution of **13** (0.30 g, 0.48 mmole) in trifluoroacetic acid (30 ml) was stirred at ambient temperature for 3 hours. The solution was clarified by filtration. The trifluoroacetic acid was evaporated under reduced pressure with the help of added ethanol and ether. The residue was dissolved in 0.1*N* sodium hydroxide (20 ml), filtered and the filtrate was acidified with 2*N* hydrochloric acid to pH 3.5 to precipitate a cream color solid. After overnight refrigeration of the suspension, the product was isolated by filtration, washed with copious amount of water and ether and then dried under *vacuo* at 60° overnight to yield 0.198 g (72%) of **3a** pure by tlc (cellulose, 5% ammonium bicarbonate), mp > 250° dec with preliminary softening; <sup>1</sup>H nmr (DMSO- $d_6$ ):  $\delta$  1.85-2.09 (two m, 2H, glu- $\beta$ -CH<sub>2</sub>), 2.31 (t, 2H, glu- $\gamma$ -CH<sub>2</sub>, J = 14.96 Hz), 4.30-4.37 (m, 1H, glu- $\alpha$ -CH), 4.47 (app. d, 2H, NHCH<sub>2</sub>), 6.53 (d, 2H, 3'-H and 5'-H, J<sub>0</sub> = 8.92 Hz), 6.42-6.66 (br hump, 2H, 2-NH<sub>2</sub>), 6.89 (t, 1H, NHCH<sub>2</sub>, J = 11.76 Hz), 7.36 (d, 1H, 8-H, J<sub>8,7</sub> = 8.76 Hz), 7.63 (d, 2H, 2'-H and 6'-H, J<sub>0</sub> = 8.76 Hz), 7.64 (d, 1H, 7-H, J<sub>7,8</sub> = 8.80 Hz), 8.08 (d, 1H, 6NH, J = 7.68 Hz); <sup>19</sup>F nmr (DMSO- $d_6$ ):  $\delta$  -50.23 ppm (s, CF<sub>3</sub>); ms: (FAB) m/e 506 (M-H)<sup>-</sup> and 508 (M + H)<sup>+</sup>.

*Anal.* Calcd. for C<sub>22</sub>H<sub>20</sub>N<sub>5</sub>O<sub>6</sub>F<sub>3</sub>·3.5H<sub>2</sub>O: C, 46.31; H, 4.77; N, 12.27. Found: C, 46.06; H, 4.58; N, 12.10.

Di-*t*-butyl 5-trifluoromethyl-5,8-dideazaisofolate (**15**).

To a suspension of **10** (0.224 g, 0.87 mmole) in glacial acetic acid (100 ml) and dimethylformamide (25 ml) was added di-*t*-butyl *N*-(4-formylbenzoyl)-L-glutamate (**14**) [18] (0.30 g, 0.767 mmole) in 25 ml of glacial acetic acid containing 3 Å molecular sieves. The reaction mixture was stirred under nitrogen at ambient temperature for 6 hours and then at 70° for 20 hours. Next, the reaction mixture was cooled to room temperature and borane-*t*-butylamine complex (0.025 g, 0.286 mmole) was added and stirred at ambient temperature with the exclusion of moisture for 2 hours and at 70° for 18 hours. The reaction mixture was filtered and the filtrate basified to pH 8.5 with concentrated ammonium hydroxide to precipitate a sticky solid. The product was isolated by filtration, washed with water (25 ml) and hexane (25 ml) and dried at 75° for 18 hours to afford a yellow product which was purified on a silica gel column packed in chloroform. The product was eluted with 1% methanol in chloroform. Appropriate fractions were pooled and evaporated to dryness to yield 0.175 g (32%) of pure **15**, mp > 200° dec with preliminary softening and darkening; <sup>1</sup>H nmr (DMSO- $d_6$ ):  $\delta$  1.38 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.40 (s, 9H, C(CH<sub>3</sub>)<sub>3</sub>), 1.85-2.08 (two m, 2H, glu- $\beta$ -CH<sub>2</sub>), 2.32 (t, 2H, glu- $\gamma$ -CH<sub>2</sub>, J = 15.44 Hz), 4.28-4.36 (m, 1H, glu- $\alpha$ -CH), 4.54 (d, 2H, NHCH<sub>2</sub>, J = 6.04 Hz), 6.50 (br s, 2H, 2-NH<sub>2</sub>), 6.51 (d, 1H, 7-H or 8-H, J<sub>7,8</sub> = 8.28 Hz), 6.59 (t, 1H, NHCH<sub>2</sub>, J = 11.70 Hz), 7.23 (d, 1H, 7-H or 8-H, J<sub>7,8</sub> = 8.32 Hz), 7.44 (d, 2H, 3'-H and 5'-H, J<sub>0</sub> = 8.44 Hz), 7.84 (d, 2H, 2'-H and 6'-H, J<sub>0</sub> = 8.44 Hz), 8.54 ppm (d, 1H, CONH, J = 7.64 Hz); <sup>19</sup>F nmr (DMSO- $d_6$ ):  $\delta$  -54.77 ppm (s, CF<sub>3</sub>); ms: (FAB) m/e 620 (M + H)<sup>+</sup>.

*Anal.* Calcd. for C<sub>30</sub>H<sub>36</sub>N<sub>5</sub>O<sub>6</sub>F<sub>3</sub>·0.25H<sub>2</sub>O: C, 57.73; H, 5.89; N, 11.22. Found: C, 57.47; H, 5.48; N, 10.98.

5-Trifluoromethyl-5,8-dideazaisofolic Acid (**3b**).

A solution of the di-*t*-butyl ester **15** (0.15 g, 0.24 mmole) in trifluoroacetic acid (10 ml) was stirred at ambient temperature for 2 hours. The trifluoroacetic acid was evaporated under reduced pressure with the help of added portions of ether. The yellowish residue was dissolved in 0.1*N* sodium hydroxide, filtered and the pH of the filtrate adjusted to 3.5 with 1*N* hydrochloric acid to precipitate a yellow solid. The product was collected by filtration, washed with water, acetone and ether and dried under vacuum at 75° for 18 hours to yield 0.11 g (90%) of **3b** pure by tlc (cellulose, 5% ammonium bicarbonate), mp >300° dec with preliminary darkening; <sup>1</sup>H nmr (DMSO-*d*<sub>6</sub>): δ 1.88-2.13 (two m, 2H, glu-β-CH<sub>2</sub>), 2.34 (t, 2H, glu-γ-CH<sub>2</sub>, J = 14.92 Hz), 4.35-4.41 (m, 1H, glu-α-CH), 4.52 (d, 2H, NHCH<sub>2</sub>, J = 5.36 Hz), 6.11 (br s, 2H, 2-NH<sub>2</sub>), 6.52 (br s, 1H, NHCH<sub>2</sub>), 6.97 (d, 1H, 7-H or 8-H, J<sub>7,8</sub> = 9.4 Hz), 7.13 (d, 1H, 7-H or 8-H, J<sub>7,8</sub> = 9.24 Hz), 7.41 (d, 2H, 3'-H and 5'-H, J<sub>0</sub> = 8.12 Hz), 7.82 (d, 2H, 2'-H and 6'-H, J<sub>0</sub> = 8.36 Hz), 8.51 ppm (s, CF<sub>3</sub>); ms: (FAB) m/e 506 (M-H)<sup>-</sup> and 508 (M+H)<sup>+</sup>.

*Anal.* Calcd. for C<sub>22</sub>H<sub>20</sub>N<sub>5</sub>O<sub>6</sub>F<sub>3</sub>: C, 52.07; H, 4.01; N, 13.66. Found: C, 51.79; H, 4.01; N, 13.66.

#### Acknowledgement.

This investigation was supported by a grant from the Burroughs Wellcome Company (J.B.H.). The technical assistance of Alpana Pathak was invaluable to the project. A generous gift of Raney 30 was received from Davison Chemical Division, W. R. Grace and Co. and proved to be highly beneficial to this study. One of us (M. G.) was the recipient of a Summer Research Fellowship for College Faculty sponsored by the College of Graduate Studies, Medical University of South Carolina (1985).

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