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(6R,6S)-5,8-Dideaza-5,6,7,8-tetrahydroaminopterin (**1**) and (6R,6S)-5,8-dideaza-5,6,7,8-tetrahydro-methotrexate (**2**) were synthesized as potential inhibitors of dihydrofolate reductase (DHFR) and as antitumor agents. Cyclohexanone-4-carboxaldehyde dimethyl acetal, a key intermediate [10] was synthesized from cyclohexane-1,4-dione monoethylene ketal, which was converted *via* a Wittig reaction to its exocyclic 4-methylene derivative which in turn, was converted to the 4-aldehyde *via* a hydroboration-oxidation sequence. Selective protection of the 4-aldehyde as the dimethylacetal and cyclization with dicyandiamide afforded the 6-dimethylacetal of 2,4-diamino-5,6,7,8-tetrahydroquinazoline. Protection of the 2,4-diamino moieties and selective deprotection of the 6-aldehyde followed by reductive amination with *p*-aminobenzoyl-L-glutamate afforded 2,4-bisacetamido-5,8-dideaza-5,6,7,8-tetrahydroaminopterin (**11**). Deprotection of **11** afforded **1**. Compound **2** was obtained from **11** *via* *N*<sup>10</sup>-methylation and deprotection. The *N*<sup>10</sup>-methyl analogue **2** was 2-10 fold more potent than **1** as an inhibitor of various DHFRs. In the *in vitro* preclinical screening program of the National Cancer Institute, compound **2** inhibited the growth of eighteen of the twenty nine tumor cell lines in culture at a GI<sub>50</sub> <1.0 × 10<sup>-8</sup> M.

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Dihydrofolate reductase (DHFR, EC 1.5.1.3) catalyzes the NADPH-dependent reduction of 7,8-dihydrofolate, and at a much slower rate, of folate, to 5,6,7,8-tetrahydrofolate (FH<sub>4</sub>). Since tetrahydrofolate serves as a carrier of one-carbon units required to build nucleic acids and certain amino acids, inhibition of DHFR and consequent depletion of FH<sub>4</sub> pools leads to cessation of cell growth [2]. The key structural modification of the 4-hydroxy group of folic acid, the pteridine substrate of DHFR, to a 4-amino group transforms the molecule into a potent inhibitor, aminopterin (AMT) (Figure 1). The *N*-10 methyl analog of AMT, *viz.* methotrexate (MTX), is clinically used in cancer chemotherapy [3]. Deazapteridines, in which the 5-, 8- and/or 10-nitrogens are replaced by carbon, have also been found to be potent inhibitors of various folate-metabolizing enzymes, in particular, of dihydrofolate reductase and thymidylate synthase (TS) [4]. 5,8-Dideaza(quinazoline) analogs of AMT and MTX (Figure 1) are as inhibitory as AMT and MTX against

mammalian DHFR and towards several tumor cell lines [5-8]. Although several 2,4-diamino-5,8-dideazafolate analogs have been synthesized, there is a considerable dearth of information on tetrahydroquinazoline antifolates. This probably stems from the difficulty in the synthesis of such analogs.

Some of the earliest reported tetrahydroquinazoline analogs were the 5,8-dideaza-5,6,7,8-tetrahydrofolic acid [9] and 5,8-dideaza-5,6,7,8-tetrahydroaminopterin **1** [10]. In antibacterial assays, the folate analog (2-amino-4-hydroxy compound) was found to be thirty times less active than AMT. Interestingly, the 2,4-diamino analog **1**, was found to be six to eight times more active than AMT, against *S. faecium* and *P. cerevisiae*.

*N*-10 Methylation of 2,4-diamino antifolates can increase [4,8,11] or decrease [4,12] DHFR inhibition depending upon the nature of the heterocyclic ring and the source of the DHFR. As part of our continuing interest in classical and nonclassical analogs of folic acid [11], we report here, a convenient synthesis of 5,8-dideaza-5,6,7,8-tetrahydromethotrexate (**2**), from compound **1**. Compound **1**, previously reported by DeGraw *et al.* [10], was also synthesized in this study to re-evaluate its DHFR

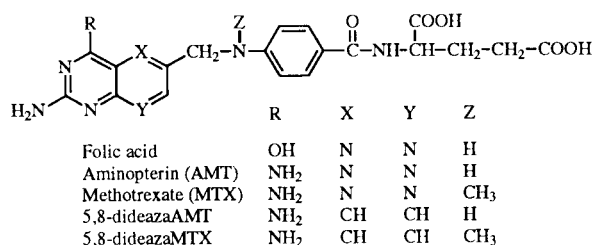
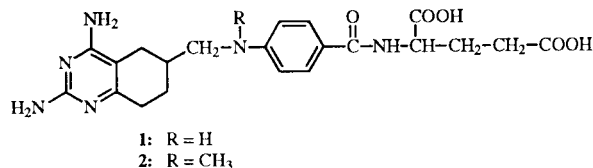


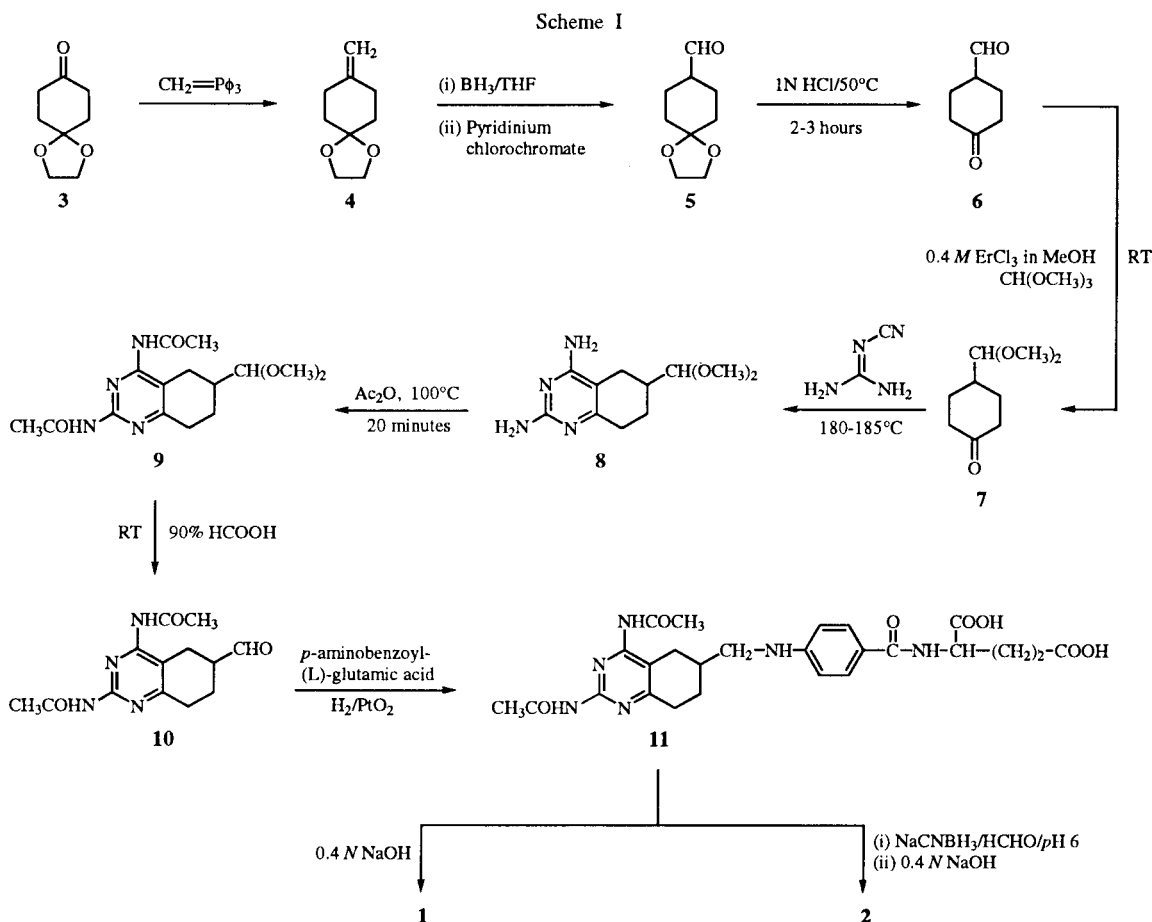
Figure 1



inhibitory effect and also to evaluate the effect of *N*-10 methylation on DHFR inhibition and antitumor activity.

The synthetic strategy was designed to afford a common intermediate, 2,4-bisacetamido-5,6,7,8-tetrahydroquinazoline-6-carboxaldehyde **10** (Scheme I), to which various classical and nonclassical side chains could be conveniently appended. This intermediate **10** appeared to

hydrofuran, followed by pyridinium chlorochromate. Compound **5** was deprotected to the ketoaldehyde **6** by acidic hydrolysis in 1 *N* hydrochloric acid at 50°. Selective protection of an aldehyde in the presence of a ketone has been reported using lanthanoid chlorides as catalysts [17a,b]. Although cerium chloride proved to be unsuccessful in our case, selective protection of the alde-



be the most logical choice, since earlier attempts by DeGraw *et al.* [13] to synthesize a suitable 6-substituted 2,4-diamino-5,6,7,8-tetrahydroquinazoline such as the 6-halomethyl or the mesylate of the 6-hydroxymethyl derivative, for the synthesis of **1**, were unsuccessful. Compound **10** was previously synthesized as a precursor to **1**, by DeGraw *et al.* [14] from cyclohexanone-4-carboxaldehyde dimethyl acetal (**7**). We devised an alternate route to intermediate **7**, starting from commercially available cyclohexane-1,4-dione monoethylene ketal **3** (Scheme I). Compound **3** was converted to its known exocyclic methylene derivative **4**, via a Wittig reaction [15]. This exocyclic methylene was then transformed to the aldehyde **5** by the method of Rosowsky *et al.* [16] using a hydroboration-oxidation sequence with borane in tetra-

hyde functionality of **6** was achieved using 0.4 *M* erbium chloride in methanol in the presence of trimethyl orthoformate, to afford the desired intermediate **7** in 66% yield.

Cyclocondensation of **7** with dicyandiamide afforded the 2,4-diamino-5,6,7,8-tetrahydroquinazoline-6-carboxaldehyde dimethyl acetal (**8**), which was converted to the protected aldehyde **10** [10].

Reductive amination of **10** with *p*-aminobenzoyl-L-glutamic acid, with hydrogen/platinum dioxide in glacial acetic acid, afforded the bisacetamido condensed product **11**, which was directly deprotected with 0.4 *N* sodium hydroxide to afford **1**. The *N*<sup>10</sup>-methyl analog **2** was synthesized by methylation of the protected bisacetamido derivative **11** with 37% aqueous formaldehyde and sodium cyanoborohydride [7,18] at room temperature and

pH 6, followed by deacetylation with 0.4 *N* sodium hydroxide to give **2**.

Compounds **1** and **2** were evaluated as inhibitors of rat liver (RL), *P. carinii*, *T. gondii* [19,20] and *L. casei* [21] DHFR and the results are reported in Table I. The *N*-10

methyl analog **2** was found to be two (*L. casei* DHFR) to ten times (*P. carinii* DHFR) more active than **1**. However, **2** was two to five times less inhibitory than MTX, except against *T. gondii* DHFR. The inhibitory potency of **1** towards rat liver DHFR was twenty times less than MTX. It is apparent that *N*-10 methylation provides for an increase in DHFR inhibitory potency in all of the DHFRs tested.

Although bacterial and protozoan cells lack transport mechanisms for uptake of folate and classical folate inhibitors, and this renders **2** an unlikely therapeutic agent for treatment of *P. carinii* and *T. gondii* infections, it is interesting to note that the classical analog **2** was extremely potent against *P. carinii* and *T. gondii* DHFR. It was eight times more potent than trimetrexate, a nonclassical quinazoline analog, against *P. carinii* DHFR and equipotent with trimetrexate against *T. gondii* DHFR.

On the basis of the DHFR inhibitory results in Table I, compound **2** was selected for evaluation as an inhibitor of the growth of tumor cells in culture, in the *in vitro* preclinical screening program of the National Cancer Institute [22]. The results are reported in Table II. In eighteen of the twenty-nine cell lines tested, **2** exhibited a  $GI_{50} < 1.0 \times 10^{-8}$  M. These encouraging results have prompted us to further evaluate **2** as a potential antitumor agent.

## EXPERIMENTAL

All evaporations were carried out *in vacuo* with a rotary evaporator or by short-path distillation. Analytical samples were dried *in vacuo* (0.2 mm Hg) in an Abderhalden drying apparatus over phosphorus pentoxide and refluxing ethanol or toluene. Thin-layer chromatography was performed on Eastman Kodak silica gel chromatogram plates with fluorescent indicator. Spots were visualized by uv light (254 and 350 nm). Proportions of solvents used are by volume. All analytical samples were homogenous on tlc in at least three different solvent systems. Melting points were determined by the capillary method on a Fisher-Johns melting point apparatus and are uncorrected. Purifications by gravity column and flash chromatography were carried out using Merck silica gel 60 (230-400 mesh). Infrared (ir) spectra were determined neat or in pressed potassium bromide discs on a Perkin-Elmer 1430 ratio recording infrared spectrophotometer and are reported in reciprocal centimeters. The  $^1H$  nmr spectra were recorded on a Varian EM-360 (60 MHz) or Bruker WH-300 (300 MHz) spectrometers. The  $^{13}C$  nmr spectra were obtained on a Bruker WH-300 instrument at 75.46 MHz, 90 pulse and 14  $\mu$ s. The data was accumulated by 16K size with 0.5 second delay time and 90° tip angle. The chemical shift ( $\delta$ ) values are expressed in ppm (parts per million) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, t = triplet, m = multiplet, exch = protons exchangeable by addition of deuterium oxide. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, GA, or Galbraith Laboratories, Knoxville, TN. Elemental compositions are within 0.4% of the calculated values. All solvents and chemicals were purchased from Aldrich Chemical Co. and Fisher Scientific and were used as received.

Table I

Inhibitory Concentrations ( $IC_{50}$ ) in nM Against DHFR from Various Sources [a]

| Compound     | Rat Liver | <i>L. casei</i> | <i>P. carinii</i> | <i>T. gondii</i> |
|--------------|-----------|-----------------|-------------------|------------------|
| <b>1</b>     | 51        | 20.1            | 54                | 48               |
| <b>2</b>     | 12        | 9.7             | 5.2               | 8.4              |
| MTX          | 2.5       | 20              | 1.3               | NA               |
| Trimetrexate | 3         | 40.7            | 42                | 10               |

[a] Values for methotrexate inhibition in rat liver and *P. carinii* DHFR were obtained in a separate study [19]. NA = Not available.

Table II

Cytotoxicities of 5,8-Dideaza-5,6,7,8-tetrahydromethotrexate **2**

| panel/cell line            | $GI_{50}(M)$ | panel/cell line | $GI_{50}(M)$ |
|----------------------------|--------------|-----------------|--------------|
| Leukemia                   |              |                 |              |
| CCRF-CEM                   | <1.00E-08    | HL-60(TB)       | <1.00E-08    |
| K-562                      | <1.00E-08    | MOLT-4          | <1.00E-08    |
| RPMI-8226                  | 6.92E-08     |                 |              |
| Non-Small Cell Lung Cancer |              |                 |              |
| A549/ATCC                  | 1.97E-08     | HOP-92          | 4.85E-07     |
| NCI-H23                    | 1.44E-07     | NCI-H460        | <1.00E-08    |
| NCI-H522                   | 7.41E-08     |                 |              |
| Colon Cancer               |              |                 |              |
| COLO 205                   | 2.96E-07     | HCC-2998        | 4.61E-07     |
| HCT-116                    | <1.00E-08    | HCT-15          | <1.00E-08    |
| HT-29                      | <1.00E-08    | KM12            | 8.63E-08     |
| SW-620                     | <1.00E-08    |                 |              |
| CNS Cancer                 |              |                 |              |
| SF-268                     | 9.12E-08     | SF-295          | <1.00E-08    |
| SF-539                     | 1.39E-08     | U251            | 1.45E-07     |
| Melanoma                   |              |                 |              |
| LOX-IMVI                   | <1.00E-08    | M14             | 3.84E-08     |
| SK-MEL-5                   | 7.56E-08     | UACC-257        | 2.60E-07     |
| UACC 62                    | <1.00E-08    |                 |              |
| Ovarian Cancer             |              |                 |              |
| IGROVI                     | <1.00E-08    | OVCAR-3         | 3.38E-07     |
| OVCAR4                     | <1.00E-08    |                 |              |
| Renal Cancer               |              |                 |              |
| 786-0                      | <1.00E-08    | ACHN            | 3.42E-08     |
| SN12C                      | 5.90E-08     | UO-31           | 4.99E-08     |
| Prostate Cancer            |              |                 |              |
| PC-3                       | <1.00E-08    | DU-145          | 2.45E-07     |
| Breast Cancer              |              |                 |              |
| MCF7                       | 3.71E-08     | MCF7/ADR-RES    | 3.03E-07     |
| MDA-MB-435                 | <1.00E-08    | MDA-N           | <1.00E-08    |

(±)-Cyclohexanone-4-carboxaldehyde (**6**).

A solution of 1 g (6.3 mmoles) of 4-formylcyclohexanone ethylene ketal **5** [16] in tetrahydrofuran (10 ml) and 1 *N* hydrochloric acid (10 ml) was heated at 50°, under nitrogen for 3 hours, cooled and poured into a mixture of ethyl acetate (100 ml) and water (100 ml) in a separatory funnel. The organic layer was washed with 5% aqueous sodium bicarbonate (2 x 50 ml) and brine (2 x 50 ml), dried (anhydrous magnesium sulfate) and evaporated to afford a light green liquid (0.46 g, 62%), tlc: silica gel, ethyl acetate-petroleum ether (1:1), *R<sub>f</sub>* 0.42; ir (neat): 2720 (-C-H alde str), 1720-1735 cm<sup>-1</sup> (C=O); <sup>1</sup>H nmr (60 MHz, deuteriochloroform): 2.0 (broad m, 4 H, CH<sub>2</sub>), 2.35 (br m, 5 H, CH, CH<sub>2</sub>), 9.78 (s, 1 H, CHO). This liquid was used without further purification.

(±)-Cyclohexanone-4-carboxaldehyde Dimethyl Acetal (**7**).

Compound **6** (6.37 g, 51 mmoles) and trimethyl orthoformate (40 ml) were dissolved in 0.4 *M* erbium chloride in methanol (130 ml). The solution was stirred at room temperature, under nitrogen, for 2.5 hours, poured into 5% aqueous sodium bicarbonate (500 ml) and extracted with ether. The organic layer was washed with water and brine and dried (anhydrous magnesium sulfate). The solvent was removed *in vacuo* to yield a light green liquid (5.68 g, 65%), tlc: silica gel, ethyl acetate:petroleum ether (1:1), *R<sub>f</sub>* 0.39; ir (neat): 1715 (C=O), 1130, 1105, 1075, 1050 cm<sup>-1</sup>; <sup>1</sup>H nmr (60 MHz, deuteriochloroform): 1.6-2.55 (m, 9 H, (CH<sub>2</sub>)<sub>4</sub>, CH), 3.38 (s, 6 H, OCH<sub>3</sub>), 3.92 (d, 1 H, CH(OCH<sub>3</sub>)<sub>2</sub>).

(±)-2,4-Diamino-5,6,7,8-tetrahydroquinazoline-6-carboxaldehyde Dimethyl Acetal (**8**).

In a three-necked flask fitted with a Dean Stark trap and an immersion thermometer, a mixture of **7** (34.70 g, 0.202 mole) and dicyandiamide (20.35 g, 0.242 mole) was fused, at an internal temperature of 180-185° for 3 hours. The reaction mixture was then cooled to 160° and extracted with 3 x 100 ml boiling water. The aqueous extract was decanted from the residue, and re-extracted with warm chloroform (5 x 100 ml). The residue was treated with hot chloroform (2 x 75 ml) and the combined chloroform extracts were dried (anhydrous magnesium sulfate) and evaporated to yield **8** as a yellow solid (13.65 g, 29%), tlc: silica gel, ethyl acetate:methanol (2:1), *R<sub>f</sub>* 0.28, mp 190-193° (lit [10] mp 195-200°); ir (potassium bromide): 3460, 3360, 3165 (N-H), 1640, 1625, 1585, 1560, 1440, 1130, 1095, 1080 (acetal C-O-C), 1040 cm<sup>-1</sup>; <sup>1</sup>H nmr (300 MHz, DMSO-d<sub>6</sub>): 1.34 (m, 1 H, quinazoline H), 1.8-2.55 (m, 6 H, quinazoline CH<sub>2</sub>), 3.31 (s, 6 H, (OCH<sub>3</sub>)<sub>2</sub>), 4.19 (d, 1 H, -CH(OCH<sub>3</sub>)<sub>2</sub>), 5.52 (s, 2 H, NH<sub>2</sub>, exch), 6.00 (s, 2 H, NH<sub>2</sub>, exch).

(±)-2,4-Diacetamido-5,6,7,8-tetrahydroquinazoline-6-carboxaldehyde Dimethyl Acetal (**9**).

A mixture of 11.53 g (48.4 mmoles) of **8** and 58 ml of acetic anhydride was heated, with stirring, at 100° for 20 minutes. A complete solution resulted within 10 minutes. The solution was evaporated to dryness *in vacuo* and the residue dissolved in hot benzene (25 ml). The solution was then diluted with ether (60 ml), and refrigerated. The precipitated cream solid was collected and washed with ether. A second crop was obtained by evaporating the mother liquor to dryness and recrystallizing the residue with benzene:ether. A total yield of 8.84 g (66%) of **9** was obtained, tlc: (a) silica gel, ethyl acetate:methanol (2:1), *R<sub>f</sub>* 0.64 (b) silica gel, chloroform:methanol (1:1) *R<sub>f</sub>* 0.6, mp 152-154°

(lit [10] mp 156.5-157°); ir (potassium bromide): 3200, 3115 (N-H), 1705 (C=O), 1652, 1585, 1555, 1360, 1305, 1240, 1125, 1085, 1050 (acetal C-O-C) cm<sup>-1</sup>; <sup>1</sup>H nmr (300 MHz, DMSO-d<sub>6</sub>): 1.45 (m, 1 H, quinazoline H), 1.91 (m, 2 H, CH<sub>2</sub>), 2.19 and 2.20 (2s, 6 H, NHCOCH<sub>3</sub>), 2.32 (m, 2 H, CH<sub>2</sub>), 2.76 (m, 2 H, CH<sub>2</sub>), 3.31 (s, 6 H, (OCH<sub>3</sub>)<sub>2</sub>), 4.21 (d, 1 H, CH(OCH<sub>3</sub>)<sub>2</sub>), 9.98 (s, 1 H, NH, exch), 10.27 (s, 1 H, NH, exch).

(±)-2,4-Diacetamido-5,6,7,8-tetrahydroquinazoline-6-carboxaldehyde (**10**).

A solution of 7.23 g (22.4 mmoles) of **9** in 90% formic acid (23 ml) was stirred at room temperature for 5 hours, after which no starting material could be detected by tlc. The solution was evaporated to dryness *in vacuo* at room temperature and <1 mm Hg. The residue was dissolved in 25 ml of ethyl acetate and diluted with excess ether. The supernatant was decanted from the gummy residue and cooled to afford white crystals (1.58 g). The gummy residue was dissolved in 6 ml of hot acetic anhydride, diluted with ether and stirred overnight to afford an additional 3.0 g of a cream solid. Total yield of **10** was 4.58 g (74% yield), tlc: silica gel, ethyl acetate:methanol (2:1), *R<sub>f</sub>* 0.59, mp 182-183° (lit [10] mp 185-186.5°); ir (potassium bromide): 3300 (N-H), 2720 (aldehyde C-H str), 1725 (C=O), 1690, 1670, 1590, 1370, 1300 cm<sup>-1</sup>; <sup>1</sup>H nmr (300 MHz, DMSO-d<sub>6</sub>): 1.80 (m, 1 H, quinazoline H), 2.14 and 2.20 (two s, 7 H, NHCOCH<sub>3</sub> and quinazoline H), 2.6-2.95 (m, 5 H, quinazoline CH<sub>2</sub>), 9.70 (s, 1 H, CHO), 10.08 (s, 1 H, NH, exch), 10.31 (s, 1 H, NH, exch).

(6*R*, 6*S*)-5,8-Dideaza-5,6,7,8-tetrahydroaminopterin (**1**).

A mixture of **10** (2.20 g, 7.97 mmoles), *p*-aminobenzoyl-L-glutamic acid (2.12 g, 7.97 mmoles) and platinum oxide (0.44 g) in 22 ml of glacial acetic acid was hydrogenated under atmospheric pressure and room temperature till the theoretical amount of hydrogen was absorbed. The reaction was then filtered through Celite and the filtrate was evaporated to dryness at <50° and 1 mm Hg. The residue was extracted with 2 x 100 ml of boiling water. The aqueous extracts were pooled and refrigerated overnight to deposit a yellow solid. The clear supernatant was decanted and the solid dried at room temperature under vacuum to yield crude 2,4-bisacetamido-5,8-dideaza-5,6,7,8-tetrahydroaminopterin (**11**); ir (potassium bromide): 3340, 3200 (NH), 1720-1700, 1660, 1650 (C=O of amide and carboxyl), 1600, 1500, 1380, 830 (*p*-substituted phenyl), 760 cm<sup>-1</sup>.

This intermediate **11** was not purified further but directly deprotected by dissolving it in 40 ml of 0.4 *N* sodium hydroxide and heating on a steam bath for 30 minutes. The solution was acidified while still at 60°, with 6 *N* hydrochloric acid until a white solid precipitated, which was collected by filtration, washed with water and acetone and dried to afford 0.24 g of **1**. Further acidification of the filtrate yielded an additional 0.07 g of a second crop (total yield 8.6%). An analytical sample of **1** was obtained by treating a hot DMSO solution of **1** with charcoal followed by filtration through Celite and reprecipitation with excess water to afford pure **1**, mp 238-250° dec, (lit [10] mp 240-260°); ir (potassium bromide): 3360-3320, 3200 (NH), 2920, 1660-1640, 1600, 1500, 1390, 1180, 830 (*p*-substituted phenyl), 760 cm<sup>-1</sup>; <sup>1</sup>H nmr (300 MHz, DMSO-d<sub>6</sub>): 1.44 (m, 1 H, quinazoline H), 1.97-2.51 (m, 10 H, quinazoline CH<sub>2</sub> and glutamate CH<sub>2</sub>), 3.07 (broad s, 2 H, 9-CH<sub>2</sub>), 4.27 (fused d, 1 H, glutamate -CH), 6.24 (broad s, 1 H, N<sup>10</sup>-H, exch), 6.58 (d, 2 H, 2',6' CH), 7.32 (broad s, 2 H, NH<sub>2</sub>, exch), 7.64 (m, 4 H, 3',5'-CH, NH<sub>2</sub>), 7.87 (m, 1 H, amide NH, exch); <sup>13</sup>C nmr (75

MHz, DMSO- $d_6$ ): 47.7 (C-9), 53.0 ( $\alpha$ -C glutamate), 110.8 (3',5' phenyl CH), 128.8 (2',6' CH), 175.4 (amide C=O).

*Anal.* Calcd. for  $C_{21}H_{26}N_6O_5 \cdot 3H_2O$ : C, 50.80; H, 6.50; N, 16.93. Found: C, 50.87; H, 6.18; N, 17.30.

(6R,6S)-5,8-Dideaza-5,6,7,8-tetrahydromethotrexate (**2**).

Bisacetamido-5,8-dideazatetrahydroaminopterin **11** (0.29 g, 0.55 mmole) was dissolved in a minimum amount of cold 0.4 N sodium hydroxide and the solution was quickly neutralized to pH 6.3 with 1 N hydrochloric acid. To this solution, under nitrogen, was added 37% aqueous formaldehyde solution (0.4 ml, 5 mmoles) and sodium cyanoborohydride (0.11 g, 1.7 mmoles) in one portion. The suspension was allowed to stir at room temperature for 24 hours, maintaining the pH at 6.3 for the first 8 hours, by the periodic addition of 1 N hydrochloric acid. The reaction was then discontinued and basified with 0.4 N sodium hydroxide, added in 1 ml portions with stirring, to pH 13. The resulting solution was warmed on a steam bath for 30 minutes to deacetylate the 2- and 4-amino groups. The solution was then cooled to 50° and neutralized with 6 N hydrochloric acid until a precipitate was obtained. The suspension was refrigerated and the solid collected by filtration, washed with water and acetone, and dried under vacuum to yield 0.13 g (53% from **11**) of a light cream solid. An analytical sample was obtained by treating a hot DMSO solution of **2** with charcoal, filtering through Celite and reprecipitating with excess water. The pure product was collected by filtration, washed with water, ethanol and acetone, and dried under vacuum, mp 258-260° dec; ir (potassium bromide): 3340, 3200, 2920, 1655, 1600, 1500, 1380, 1195, 825, 760  $cm^{-1}$ ;  $^1H$  nmr (300 MHz, DMSO- $d_6$ ): 1.22 (m, 1 H, quinazoline CH), 1.35-2.48 (m, 10 H, quinazoline  $CH_2$  and glutamate  $CH_2$ ), 3.00 (broad s, 3 H, N- $CH_3$ ), 3.37 (m, 2 H, 9- $CH_2$ ), 4.25 (broad s, 1 H, glutamate  $\alpha$ -C), 6.67-7.93 (m, 9 H, phenyl,  $NH_2$ , amide NH), 9.98 (broad hump, COOH); ms: (DCI)  $m/z$  745 ( $M+TMS_4+H$ ).

*Anal.* Calcd. for  $C_{22}H_{28}N_6O_5 \cdot 1.2H_2O \cdot 0.6CH_3CH_2OH$  C, 55.10; H, 6.78; N, 16.62. Found: C, 55.44; H, 6.48; N, 16.25.

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