Synthesis of N-[4-[2-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)-ethylamino]benzoyl]-L-glutamic acid. An Acyclic Analogue of 5,6,7,8-Tetrahydrofolic Acid

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The synthesis of *N*-[4-[2-(2,4-diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)ethylamino]benzoyl]-L-glutamic acid (**2**), a two carbon analogue of 5-DACTHF (**1**) and an acyclic analogue of 5,6,7,8-tetrahydrofolic acid, is reported. The pyrimidinylacetaldehyde diethyl acetal **3**, which was prepared in 2-steps from 2-chloro acetaldehyde diethyl acetal, was converted to **2** in four steps. Compound **2** was less cytotoxic toward Detroit 98 or L cells than 5-DACTHF (**1**).

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Studies on antagonists of folic acid have served as a fertile field of research in the search for chemotherapeutic agents [1-4]. The biologically active cofactor form of folic acid is 5,6,7,8-tetrahydrofolic acid (THF), which functions as a one-carbon transfer agent for many enzymes. We recently reported the synthesis of 5-DACTHF (**1**, 543U76), an acyclic analogue of THF, which inhibits growth of several transformed cell lines *in vitro* [5]. This analogue lacks the tetrahydropyrazine ring of THF, which is replaced with a propylamino bridge to connect the diaminopyrimidinone and benzoylglutamate moieties. The synthesis of pyrimidine analogues of THF that contain an aminoethylamino or aminopropylamino bridge was reported by Tong *et al.* [6,7]. Baker's group studied compounds similar to **1** that contain a 6-methyl or 6-phenyl in place of the 6-amino substituent [8,9]. We have prepared an ethylamino analogue of **1** to investigate the effect of different bridge lengths on activity. The synthesis and activity of **2** and an *N*-methyl analogue **8** are reported herein.

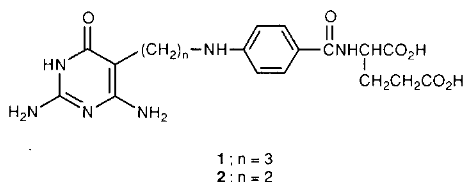


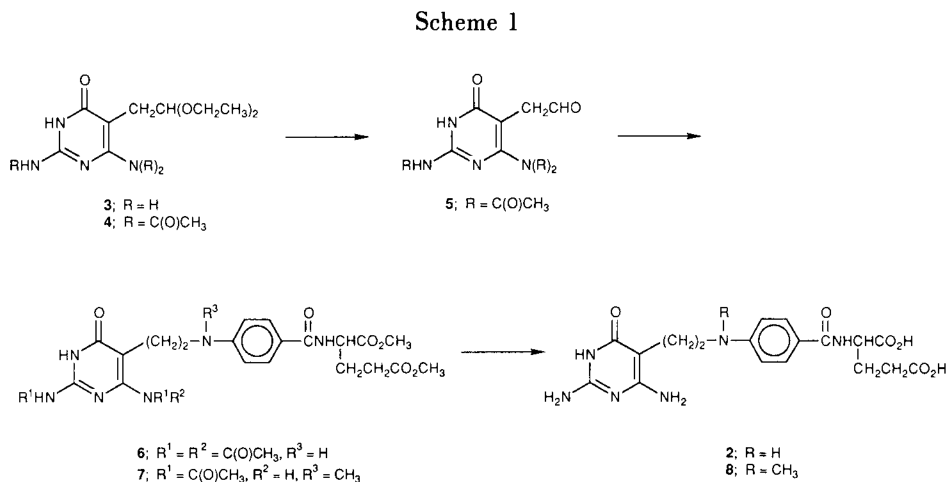
Figure 1

Chemistry.

Compound **2** was prepared in four steps from acetal **3** [10] (Scheme 1). The amino groups in **3** were protected by acetylation with acetic anhydride in pyridine to give the triacetamido pyrimidine **4** in 30% yield. The acetal moiety in **4** was hydrolyzed with silica gel impregnated with 10% aqueous oxalic acid [11] to give triacetylated pyrimidinyl acetaldehyde **5** in fair yield. Alternatively, trans-acetalation to give **5** was accomplished by stirring **4** in acetone in the presence of *p*-toluenesulfonic acid. When the acetal **4** was hydrolyzed with warm water, a water soluble, polar product was obtained, which, in analogy with the reported synthesis of **1**, probably was a diacetylated hemiaminal. Formation of this polar by-product during preparation of **5** from **4** led to the low yields.

Attempted reductive amination of **5** with dimethyl *N*-(4-aminobenzoyl)-L-glutamate [12] and sodium cyanoborohy-

Scheme 1



dride [13] in methanol failed to give an isolable product. When the reaction was performed in acetic acid, however, reductive amination was rapid and clean to give triacetylated **6** in 45% yield. The *N*-methyl intermediate **7** was prepared by *in situ* reductive alkylation of **6** with aqueous formaldehyde and sodium cyanoborohydride [5,13]. Mono-deacetylation of the intermediate occurred under these conditions; column chromatography gave **7** in low yield.

Removal of the blocking groups on **6** and **7** was accomplished with warm 1 *N* sodium hydroxide to give **2** and **8**, respectively. These compounds were light sensitive; the solids slowly turned yellow when exposed to sunlight. This degradation was minimized by including small amounts of 2-mercaptoethanol in the solvent during hydrolysis and recrystallization.

Biological Results.

Compounds **2** and **8** were tested against Detroit 98 and L cells in cell culture by the previously described method [5]. Under conditions where **1** caused 50% inhibition of Detroit 98 cell growth at 0.11 μM and 50% inhibition of L cell growth at 0.088 μM, **2** had IC_{50}s of 0.7 μM and 0.4 μM, respectively. The *N*-methyl analogue **8** was less toxic with IC_{50}s of 7 μM and 0.75 μM, respectively. Thus, removal of a methylene from the propylamine bridge of **1** resulted in agents with reduced cell culture cytotoxicity.

Compounds **2** and **8** were also tested against human dihydrofolate reductase (DHFR) [5], but exhibited less than 40% inhibition at 300 μM and 40 μM, respectively. This level of activity contrasts with the good DHFR inhibition reported by Baker for the 6-methyl and 6-phenyl congeners of **1**, which had K_is of 2 μM and 0.09 μM, respectively [8,9,14].

EXPERIMENTAL

Melting points were taken in capillary tubes on a Mel-Temp block and are uncorrected. The ^{1}H nmr spectra were recorded on a Varian XL-100-15-FT, a Varian T-60, or a Varian FT-80A spectrometer and referenced to tetramethylsilane as an internal standard. The uv absorption spectra were measured on a Unicam SP 800 or Cary 118 UV-VIS spectrophotometer. The solutions for uv measurements were prepared by dissolution of compound in 10 ml of methanol followed by dilution with 0.1 *N* hydrochloric acid, *p*H 7.0 phosphate buffer or 0.1 *N* sodium hydroxide to a volume of 100 ml. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on tlc on Whatman 200 micron MK6F plates of silica gel with fluorescent indicator. All compounds were analyzed for C,H,N and gave combustion values within 0.4% of theoretical. Pyridine was dried over calcium hydride.

N-[4-[2-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)ethylamino]-benzoyl]-L-glutamic Acid (**2**).

A crude preparation of **6** (prepared on a 3.3 mmole scale but without recrystallization) was dissolved in 1 *N* sodium hydroxide (100 ml) and ethanol (50 ml) containing 2-mercaptoethanol (0.25 ml). The reaction was heated with stirring to 55° for 18 hours and then spin evaporated *in vacuo* to a small volume. The *p*H of the ice-bath cooled solution was adjusted to 4 with hydrochloric acid (12 *N*) to precipitate the product, which was recrystallized from methanol-ether and dried *in vacuo* at 100° to give 0.52 g (35%) of **6**, mp 180-185°; uv (*p*H 7.0 buffer + 10% methanol): λ max 280 nm (ϵ 23000); ^{1}H nmr (DMSO-d_6): δ 1.05 (t, 0.6 H, J = 6 Hz, OCH_2CH_3), 1.98 (br m, 2 H, $CHCH_2$), 2.25 (br m, 4 H, CH_2CO_2 + CH_2CH_2N), 2.95 (br m, 2 H, CH_2N), 3.4 (q, 0.4 H, J = 6 Hz, OCH_2CH_3), 4.45 (br q, 1 H, NCH), 5.75 (br s, 2 H, NH_2), 5.98 (br s, 2 H, NH_2), 6.47 (d, 2 H, J = 9 Hz, Ar), 7.57 (d, 2 H, J = 9 Hz, Ar), 7.95 (br s, 1 H, NH).

Anal. Calcd. for $C_{18}H_{22}N_6O_6 \cdot H_2O \cdot 0.1\ Et_2O$: C, 49.79; H, 5.68; N, 18.93. Found: C, 49.62; H, 5.53; N, 18.87.

2-(2-Acetylamino-4-diacetylamino-1,6-dihydro-6-oxo-5-pyrimidinyl)acetaldehyde Diethyl Acetal (**4**).

Freshly distilled acetic anhydride (250 ml) was added to a solution of **3** [10] (50.0 g, 0.206 mole) in dry pyridine. The solution was protected from moisture and heated on a steam bath with magnetic stirring for 5 days. The reaction was spin evaporated *in vacuo* with the addition of 2-methoxyethanol under aspirator and mechanical pump vacuum. The residue was recrystallized from ether to yield 23.0 g (30%) of **4**, mp 128-130°. The analytical sample was prepared by dissolving the sample in chloroform and filtering the solution through a pad of Superfiltrol #19 (Filtrol Corporation). The solution was spin evaporated *in vacuo*, and the residue was recrystallized from ether-cyclohexanes to give analytically pure **4**, mp 129-130°; uv (water): λ max 291 nm (ϵ 11000), 240 (δ 13100); ^{1}H nmr (DMSO-d_6): δ 1.07 (t, 6 H, J = 7 Hz, 2 CH_2CH_3), 2.13 (s, 3 H, CH_3CON), 2.30 (s, 6 H, $(CH_3CO)_2N$), 2.41 (d, 2 H, J = 5 Hz, CH_2CH), 3.46 (br m, 4 H, 2 OCH_2), 4.77 (t, 1 H, J = 5 Hz, CH), 11.75 (br s, 1 H, NH), 11.83 (br s, 1 H, NH).

Anal. Calcd. for $C_{16}H_{24}N_4O_6$: C, 52.17; H, 6.57; N, 15.21. Found: C, 51.82; H, 6.50; N, 15.25.

2-(2-Acetylamino-4-diacetylamino-1,6-dihydro-6-oxo-5-pyrimidinyl)acetaldehyde (**5**).

Method 1.

A slurry of Silica Gel 60 (6.0 g) and aqueous oxalic acid (10%) (0.6 ml) in dichloromethane (12 ml) was stirred for 15 minutes. Compound **4** (1.00 g, 2.7 mmoles) was added, and the mixture was stirred for 18 hours. Sodium bicarbonate (0.20 g, 2.4 mmoles) was added, and after 10 minutes the slurry was filtered and washed with ethyl acetate. The filtrate and wash were combined and spin evaporated *in vacuo* to give 0.37 g (46%) of **5**. The analytical sample was prepared by recrystallization from acetone to give 0.21 g (26%), mp 169-170°; uv (0.1 *N* hydrochloric acid + 10% ethanol): λ max 292 nm (ϵ 9700), 239 (ϵ 12900); (*p*H 7.0 buffer + 10% ethanol): λ max 284 nm (ϵ 7600), 235 (ϵ 12200), (0.1 *N* sodium hydroxide + 10% ethanol): λ max 278 nm (ϵ 10600); ^{1}H nmr (DMSO-d_6): δ 2.28 (s, 3 H, CH_3CON), 2.34 (s, 3 H, CH_3CONAc), 2.36 (s, 3 H, CH_3CONAc), 3.42 (d, 2 H, J = 1.1 Hz, CH_2), 8.2 (br s, 1 H, NH), 9.66 (t, 1 H, J = 1.1, CHO).

Anal. Calcd. for $C_{12}H_{14}N_4O_5$: C, 48.98; H, 4.80; N, 19.08. Found: C, 48.90; H, 4.83; N, 19.26.

Method 2.

A solution of **4** (2.00 g, 5.4 mmoles) and *p*-toluenesulfonic acid monohydrate (0.050 g, 0.26 mmole) in acetone (100 ml) was stirred for 2 days. The solid was collected, washed with acetone,

and dried to give 0.73 g (46%) of **5**, mp 168-170°, which was identical with that prepared by Method 1.

Dimethyl *N*-[4-[2-(2-Acetylamino-4-diacetylamino-1,6-dihydro-6-oxo-5-pyrimidinyl)ethylamino]benzoyl]-L-glutamate Hydrate (**6**).

A solution of **5** (1.74 g, 5.91 mmoles) and dimethyl *N*-(4-aminobenzoyl)-L-glutamate [12] (1.74 g, 5.91 mmoles) in acetic acid (90 ml) was stirred for 10 minutes. Sodium cyanoborohydride (0.84 g, 13.4 mmoles) was added in small portions over 1 hour. After an additional 30 minutes, the reaction was spin evaporated *in vacuo* to a thick oil. The oil was dissolved in ethyl acetate (500 ml) and washed with 5% aqueous sodium bicarbonate until neutral, with water (50 ml), with sodium chloride saturated water (50 ml), and then dried (magnesium sulfate) and spin evaporated *in vacuo*. Recrystallization from ethyl acetate gave 1.50 g (43%) of **6** mp 168-178°. Recrystallization from ethyl acetate (Norite) gave 0.83 g (23%) of the analytical sample, mp 176-179°; uv (0.1 *N* hydrochloric acid): λ max 294 nm (ε 15300), 231 (ε 20400); (*p*H 7.0 buffer + 10% methanol): λ max 300 nm (ε 25700); (0.1 *N* sodium hydroxide + 10% methanol): λ max 291 nm (ε 23300); $[\alpha]_D^{20}$ −0.7° (c 1.0, DMF); ^{1}H nmr (DMSO-d_6): δ 2.15 (s, 3 H, CH_3CON), 2.20 (m, 4 H, $CH_2CH_2CO_2$), 2.28 (s, 6 H, $(CH_3CO)_2N$), 3.20 (br m, 4 H, CH_2CH_2N), 3.58 (s, 3 H, CH_3O), 3.62 (s, 3 H, CH_3O), 4.41 (q, 1 H, J = 7.0 Hz, CH), 6.49 (br s, 1 H, CH_3CONH), 6.54 (d, 1 H, J = 7.0 Hz, ArCON*H*), 11.88 (br s, 1 H, ring NH).

Anal. Calcd. for $C_{26}H_{32}N_6O_9 \cdot H_2O$: C, 52.88; H, 5.80; N, 14.23. Found: C, 52.99; H, 5.51; N, 14.39.

Dimethyl *N*-[4-[2-(2,4-Bis(acetylamino)-1,6-dihydro-6-oxo-5-pyrimidinyl)ethyl-*N*-methylamino]benzoyl]-L-glutamate (**7**).

A solution of **5** (2.93 g, 10 mmoles) and dimethyl *N*-(4-aminobenzoyl)-L-glutamate [12] (2.93 g, 10 mmoles) in acetic acid (200 ml) was stirred at ambient temperature for 15 minutes. Sodium cyanoborohydride (0.35 g, 5.6 mmoles) was added, and after an additional 15 minutes aqueous formaldehyde (37%) (4.2 ml) was added. After 10 minutes additional sodium cyanoborohydride (1.50 g, 23.9 mmoles) was added, and the reaction was stirred for 30 minutes. The solvent was removed by spin evaporation *in vacuo* at 50°. The viscous oil was dissolved in chloroform-methanol (1:10, 500 ml) and washed with water (50 ml), and 5% aqueous sodium bicarbonate (10 ml) and then dried (magnesium sulfate). After spin evaporation *in vacuo*, the residue was recrystallized from ether to give 3.14 g of a pale yellow powder. The product was purified by open column chromatography on a Silica Gel 60 (63-200 μM, E. Merck No. 7734) column (3 cm x 25 cm) wetted with dichloromethane and eluted with 4% methanol in dichloromethane. The appropriate fractions were combined and spin evaporated *in vacuo*, and the residue was recrystallized from ethyl acetate to yield 1.30 g (23%) of **7**, mp 168-175°; ^{1}H nmr (DMSO-d_6): δ 2.02 (s, 3 H, CH_3CON), 2.15 (s, 3 H, CH_3CON), 1.8-2.3 (m, 4 H, $CH_2CH_2CO_2$), 2.95 (s, 3 H, CH_3N), 3.1-3.7 (br m, 4 H, CH_2CH_2), 3.58 (s, 3 H, CH_3O), 3.63 (s, 3 H, CH_3O), 4.41 (br q, 1 H, J = 7.0 Hz, NCHCO), 6.78 (d, 2 H, J = 9 Hz, Ar), 7.75 (d, 2 H, J = 9 Hz, Ar), 8.3 (br d, 1 H, J = 7.0 Hz, NH), 9.76 (br s, 1 H, ring NH), 11.68 (br s, 2 H, CH_3CONH).

Anal. Calcd. for $C_{25}H_{32}N_6O_8 \cdot 0.5\ H_2O$: C, 54.24; H, 6.01; N, 15.18. Found: C, 53.88; H, 5.75; N, 15.28.

N-[4-[2-(2,4-Diamino-1,6-dihydro-6-oxo-5-pyrimidinyl)ethyl-*N*-methylamino]benzoyl]-L-glutamic Acid (**8**).

A solution of **7** (0.25 g, 0.45 mmole), 2-mercaptoethanol (0.25 ml), 1 *N* sodium hydroxide (20 ml), and ethanol (10 ml) was stirred under nitrogen for 18 hours at 60°. The reaction was cooled in an ice bath, and the *p*H of the solution was adjusted to 4 with hydrochloric acid (12 *N*). The resultant precipitate was collected and recrystallized from ethanol containing 2-mercaptoethanol (0.25 ml) to yield 0.077 g (38%) of **8**, mp 180-188°. This material was a white powder, which became tan upon extended exposure to light; uv (*p*H 7.0 buffer + 10% methanol): λ max 315 nm (ε 20800), 382 (ε 22500); ^{1}H nmr (DMSO-d_6): δ 3.00 (s, 3 H, CH_3N).

Anal. Calcd. for $C_{19}H_{24}N_6O_6 \cdot H_2O$: C, 50.66; H, 5.82; N, 18.66. Found: C, 50.49; H, 5.77; N, 18.52.

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