Inhibitors of Glycinamide Ribonucleotide Formyltransferase as Potential Cytotoxic Agents. Synthesis of 5-Deaza-5,6,7,8-tetrahydrohomofolic Acid, 5-Deaza-5,6,7,8-tetrahydroisohomofolic Acid, and 10-Formyl-5-deaza-5,6,7,8-tetrahydroisohomofolic Acid

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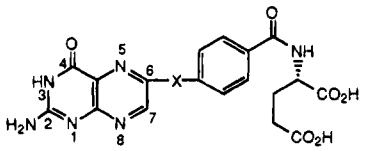
Syntheses of three new analogs of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, Lometrexol)—5-deaza-5,6,7,8-tetrahydrohomofolic acid (**11**), 5-deaza-5,6,7,8-tetrahydroisohomofolic acid (**16a**), and 10-formyl-5-deaza-5,6,7,8-tetrahydroisohomofolic acid (**16b**)—are described.

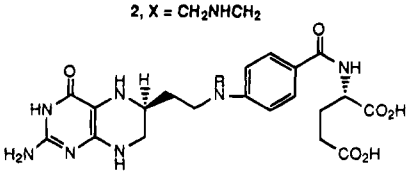
Homofolic acid is the trivial name for the folic acid analog **1** which possesses an additional methylene group between positions C-9 and N-10 of folic acid, while isohomofolic acid is the trivial name given to the folic acid analog **2** with the additional methylene group inserted between N-10 and the benzoylglutamic acid moiety.



1, X = CH_2CH_2NH

9 10 11

2, X = CH_2NHCH_2



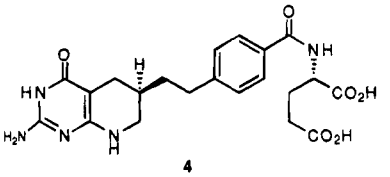
3a, R = H

3b, R = CHO

Some years ago Divekar and Hakala[1] showed that the growth of sarcoma 180 cells was inhibited by **1** after a 24-h preincubation period. It was found by this group that cellular levels of (α-*N*-formyl)glycinamide-1-β-D-ribofuranose 5′-monophosphate (FGAR) were decreased and that 5-aminoimidazole-4-carboxamide (AICA) protected these cells against the cytotoxic effects of **1**. It was therefore postulated that **1** was reduced by sarcoma cells to (6*S*)-5,6,7,8-tetrahydrohomofolic acid (**3a**) which then functioned as an inhibitor of glycinamide ribonucleotide formyltransferase (GAR FTase) in the *de novo* purine biosynthetic pathway.

Slieker and Benkovic[2] later found that (6*S*)-10-formyl-5,6,7,8-tetrahydrohomofolic acid (**3b**) was a potent competitive inhibitor of chicken liver GAR FTase and of the growth of Chinese hamster kidney HKSV28 cells grown *in vitro*.[3] It was proposed by this group that **3b**, after intracellular polyglutamation, might be an inhibitor of both formyltransferases (GAR FTase and 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranose-5′-monophosphate formyltransferase, AICAR FTase) of the *de novo* purine biosynthetic pathway. These observations clearly indicate that elongation of the bridge between the pteridine and (*p*-aminobenzoyl)glutamate moieties of folic acid can lead to compounds exhibiting a significant inhibitory effect on purine biosynthesis.

In 1985 we described the synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, Lometrexol) (**4**).[4] This novel folate analogue is an inhibitor of glycinamide ribonucleotide formyltransferase (GAR FTase), which mediates the conversion of glycinamide ribonucleotide to its *N*-formyl derivative in an early stage of the *de novo* purine biosynthesis pathway; **4** proved to be a potent inhibitor of purine biosynthesis and to possess dramatic cytotoxicity toward a broad variety of solid tumors.[5] Structural features of DDATHF apparently important for its biological activity include, *inter alia*, its stable tetrahydropyridine ring (which contrasts with the oxidatively sensitive tetrahydropyrazine ring present in tetrahydrofolic acid, and in **3a** and **3b**) and replacement of the mechanistically critical 5- and 10-nitrogen atoms of the natural cofactor by methylene groups. Unlike the tetrahydrohomofolic acid analogs **3a** and **3b**, however, DDATHF does not have a homologated bridge region.



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We describe herein the preparation of several novel DDATHF analogs which incorporate the 3-atom bridges found in homofolic acid and in isohomofolic acid. 5-Deaza-5,6,7,8-tetrahydrohomofolic acid (homo-5-DATHF, **11**) parallels the structure of 5,6,7,8-tetrahydrohomofolic acid (**3a**) except for deletion of the 5-nitrogen atom, while 5-deaza-5,6,7,8-tetrahydroisohomofolic acid (**16a**) and its 10-formyl derivative **16b** combine within one system one of the salient structural features of **3a** and **3b** (a homolo-

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(1) Divekar, A. Y.; Hakala, M. T. *Mol. Pharmacol.* **1975**, *11*, 319.

(2) Slieker, L. J.; Benkovic, S. J. *Mol. Pharmacol.* **1984**, *25*, 294.

(3) Benkovic, S. J. *TIBS* **1984**, *9*, 320.

(4) Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1985**, *28*, 914.

(5) For a review of the chemistry and biochemistry of DDATHF, as well as leading references to extensive SAR studies, see: (a) Taylor, E. C. *J. Heterocycl. Chem.* **1990**, *27*, 1–12. (b) Taylor, E. C. In *Chemistry and Biology of Pteridines and Folates*; Ayling, J. E., Nair, M. G., Baugh, C. W., Eds., Plenum Press: New York, 1993; pp 387–408.

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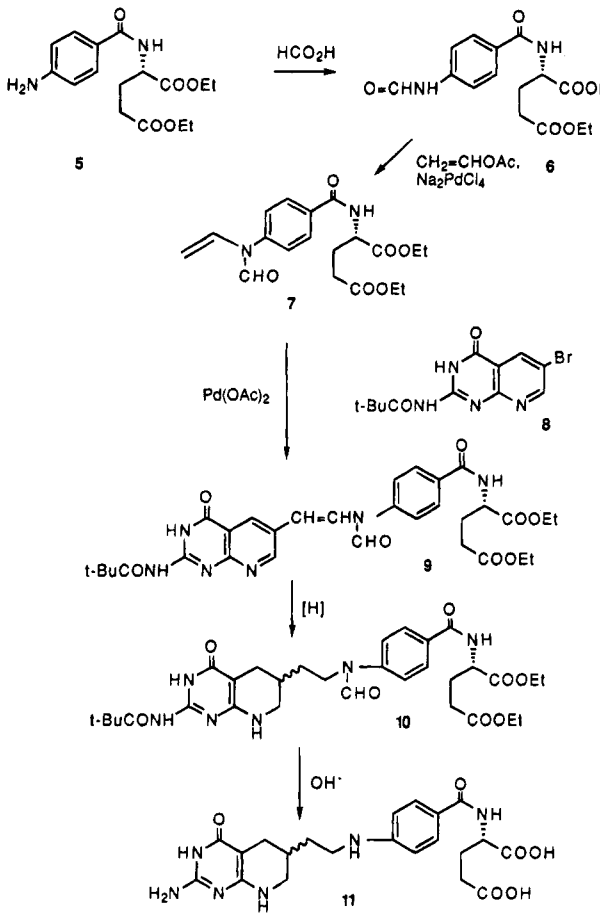
Scheme 1

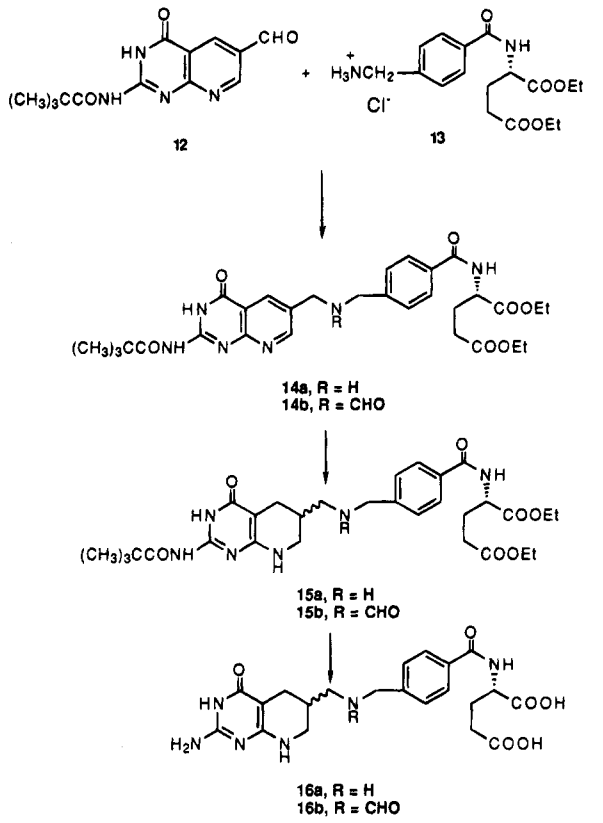


gated bridge region) and of **4** (the tetrahydropyridine ring). Recent papers from our laboratory have described similar composite molecules such as homoDDATHF (which combines the critical structural features of DDATHF (**4**) with homofolic acid (**1**) except for deletion of the bridge nitrogen atom)[6] and 5-DATHF (in which substitution of nitrogen for carbon at position 10 in DDATHF is shown to lead to retention of all of the overall biochemical properties of DDATHF).[7] Both of these latter analogs exhibited very high cytotoxic activity.

Our synthesis of homo-5-DATHF (**11**) is summarized in Scheme 1 and is noteworthy for employing two successive palladium-catalyzed reactions, one to form the $C^{10}-N^{11}$ bond and the other to form the $C^{6}-C^{9}$ bond. Thus, diethyl *N*-(4-aminobenzoyl)glutamate (**5**) was formylated with formic acid at 100 °C to give the *N*-formyl derivative **6** in 91% isolated yield. Treatment of **6** with vinyl acetate in the presence of sodium tetrachloropalladate and cupric chloride under reflux resulted in ethenylation of the formamide nitrogen atom to give diethyl *N*-[4-(*N*-formyl-*N*-ethenylamino)benzoyl]glutamate (**7**) in 51% yield. This material was then coupled with 2-pivaloyl-6-bromo-5-deazapterin[8] (**8**) using palladium acetate, tri-*o*-tolylphosphine, triethylamine, and cuprous iodide in acetonitrile as solvent to give **9** in low (22%) yield. Reduction of both the olefinic double bond and the pyridine ring was achieved with hydrogen in the presence of platinum oxide, in glacial acetic acid as solvent, and the *N*-formyl and *N*-pivaloyl amides as well as the

(6) Taylor, E. C.; Harrington, P. M. *J. Org. Chem.* **1990**, *55*, 3222.
(7) Taylor, E. C.; Hamby, J. M.; Shih, C.; Grindey, G. B.; Rinzel, S. M.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1989**, *32*, 1517.
(8) Taylor, E. C.; Yoon, C.-m. *Synth. Commun.* **1988**, *18*, 1187.

Scheme 2



glutamate ester groupings in the resulting **10** were then simultaneously removed with 1 N NaOH to give homo-5-DATHF (**11**).

5-Deaza-5,6,7,8-tetrahydroisohomofolic acid (**16a**) and its 10-formyl derivative **16b** were prepared as shown in Scheme 2. Diethyl (4-(aminomethyl)benzoyl)glutamate was obtained by a modification of the method of Slavík[9] from 4-(aminomethyl)benzoic acid. Its hydrochloric acid salt **13** was stirred with the aldehyde **12**[8] in acetic acid for 18 h and the resulting Schiff base reduced *in situ* with borane–triethylamine to give **14a** in 52% yield. The N^{10} position was then formylated with a mixture of formic acid and acetic anhydride to afford the formamide **14b** in quantitative yield. Catalytic reduction of **14b** was carried out in glacial acetic acid at 50 psi over platinum oxide for 3 h to give **15b** in 73% yield (formylation at N^{10} is a prerequisite for this catalytic hydrogenation step, since it effectively protects the system against benzylic hydrogenolysis).[7] Selective hydrolysis of the pivaloyl and ester protecting groups, leaving the 10-formyl substituent intact, was accomplished with 0.1 N NaOH at 25 °C for 5 days to give 10-formyl-5-deaza-5,6,7,8-tetrahydroisohomofolic acid (**16b**) in 67% yield as a 50:50 mixture of diastereomers.

Our original strategy was to prepare the parent compound **16a** from **15b** by simultaneous removal of the pivaloyl, ester, and formamide protecting groups utilizing more strenuous basic conditions than employed in the conversion of **15b** to **16b** as described above. Unfortunately, the formamide grouping in **15b** proved to be resistant to basic hydrolysis under the conditions employed. For example, treatment of **15b** with 1.0 N NaOH for 4 days at 25 °C afforded an inseparable mixture of

(9) Slavík, K.; Cihař, R.; Souček, J.; Hermanová, E.; Přistoupilová, K.; Slavíková, V. *Mol. Pharmacol.* **1972**, *8*, 740.

16a and **16b** (more drastic conditions were avoided because of possible racemization of the chiral glutamate). Attempts to remove the acid-sensitive formamide substituent first with 3% sulfuric acid in ethanol for 2 days at 25 °C followed by 1.0 N NaOH also yielded an inseparable mixture of **16a** and **16b**. An alternative route to **16a** was therefore sought.

Catalytic reduction of **14a** to **15a** could be achieved in 37% yield using 5% rhodium-on-charcoal in acetic acid at 50 psi for 48 h. Use of this catalytic system appeared to suppress benzylic hydrogenolysis; by contrast, hydrogenation of **14a** over platinum oxide led predominantly to side products resulting from (double) benzylic cleavage. Hydrolysis of **15a** was accomplished by stirring in 1.0 N NaOH at 25 °C for 3 days and gave 5-deaza-5,6,7,8-tetrahydroisohomofolic acid (**16a**) in 32% yield as a 50:50 mixture of diastereomers. The ^{1}H NMR spectra of compounds **14b**, **15b**, and **16b** all showed evidence of hindered rotation about the formamide C–N bond. Heating these compounds above 70 °C resulted in coalescence of these signals.

Full details on the cytotoxic activity of these new DDATHF analogues **11**, **16a**, and **16b** will be described independently. All three were significantly active as inhibitors of CCRF-CEM cells in vitro [**11**, IC_{50} = 0.0078 μg/mL; **16**, IC_{50} = 0.0104 μg/mL; **16b**, IC_{50} = 0.0680 μg/mL; cf. **4**, IC_{50} = 0.007 μg/mL].

Experimental Section

Diethyl *N*-[4-(*N*-Formylamino)benzoyl]glutamate (6). A solution of 12 g (0.037 mol) of diethyl *N*-(4-aminobenzoyl)-glutamate in 50 mL of formic acid was heated at 100 °C for 1 h and then evaporated under reduced pressure to near dryness. The residue was taken up in 200 mL of methylene chloride, and the solution was washed with 30 mL of saturated sodium bicarbonate solution followed by water (2 × 30 mL) and dried (anhyd $MgSO_4$). The resulting solution was concentrated under reduced pressure, and the solid which separated was recrystallized from ethyl acetate/hexanes to give 11.8 g (91%) of white microcrystals of **6**, mp 102–103 °C. ^{1}H NMR (DMSO-d_6 + 1 drop of D_2O) δ 1.13 (t, 3 H, *J* = 7 Hz), 1.15 (t, 3 H, *J* = 7 Hz), 1.92–2.11 (m, 2 H), 2.40 (t, 2 H, *J* = 7 Hz), 4.01 (q, 2 H, *J* = 7 Hz), 4.07 (q, 2 H, *J* = 7 Hz), 4.39 (m, 1 H), 7.26 and 7.64 (dd, 2 H, *J* = 7.3 Hz, 8.6 Hz), 7.83 and 8.61 (dd, 2 H, *J* = 8.6 Hz, 7.3 Hz), 8.29 and 8.89 (s, s, 1 H). Anal. Calcd for $C_{17}H_{22}N_2O_6$: C, 58.27; H, 6.33; N, 8.00. Found: C, 58.44; H, 6.32; N, 8.00.

Diethyl *N*-[4-(*N*-Formyl-*N*-ethenylamino)benzoyl]-glutamate (7). To a stirred solution of 3.5 g (10 mmol) of diethyl *N*-[4-*N*-formylamino)benzoyl]glutamate (**6**) in 80 mL of vinyl acetate were added 50 mg (0.17 mmol) of sodium tetrachloropalladate and 120 mg of cupric chloride, and the resulting mixture was heated under reflux with exclusion of moisture ($CaCl_2$ tube). After 24 h, the reaction mixture was filtered through Celite and the excess vinyl acetate was removed under reduced pressure. To the residue were added 80 mL of vinyl acetate and 50 mg of sodium tetrachloropalladate, and the above procedure was repeated. The residue obtained after evaporation of excess vinyl acetate was chromatographed on silica gel, using ethyl acetate/hexane (20:1) as eluent. Concentration of the eluate gave 1.92 g (51%) of **7** as a viscous liquid. ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3 H, *J* = 7 Hz), 1.31 (t, 3 H, *J* = 7 Hz), 2.17 (m, 1 H), 2.31 (m, 1 H), 2.48 (m, 2 H), 4.13 (q, 2 H, *J* = 7 Hz), 4.25 (q, 2 H, *J* = 7 Hz), 4.44 (d, 1 H, *J* = 16 Hz), 4.65 (d, 1 H, *J* = 9 Hz), 4.79 (m, 1 H), 7.29 (br d, 1 H, NH), 7.35 (d, 2 H, *J* = 8 Hz), 7.43 (dd, 1 H, *J* = 9 Hz, 16 Hz), 7.96 (d, 2 H, *J* = 8 Hz), 8.23 (s, 1 H). Anal. Calcd for $C_{19}H_{24}N_2O_6$: C, 60.62; H, 6.43; N, 7.44. Found: C, 60.32; H, 6.21; N, 7.32.

Diethyl *N*-[4-{*N*-[2-(2-(Pivaloylamino)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethen-1-yl]-*N*-formylamino}benzoyl]glutamate (9). A mixture of 1.49 g (4.5 mmol) of 2-pivaloyl-6-bromo-5-deazapterin,[8] 1.92 g (5.1 mmol) of diethyl *N*-[4-(*N*-formyl-*N*-ethenylamino)benzoyl]-glutamate (**7**), 50 mg of palladium acetate, 140 mg of tri-*o*-tolylphosphine, 21.4 mg of cuprous iodide, 4.5 mL of triethylamine, and 120 mL of acetonitrile was heated under reflux, under a nitrogen atmosphere, for 48 h. The reaction mixture was then concentrated under reduced pressure and the residue chromatographed on silica gel with 4% methanol in ethyl acetate. Concentration of the eluate, trituration of the residual solid with ethyl acetate, and filtration gave 630 mg (23%) of **9**, mp 103–105 °C. ^{1}H NMR ($CDCl_3$) δ 1.26 (t, 3 H, *J* = 7 Hz), 1.33 (t, 3 H, *J* = 7 Hz), 1.34 (s, 9 H), 2.21 (m, 1 H), 2.53 (m, 2 H), 4.16 (q, 2 H, *J* = 7 Hz), 4.28 (q, 2 H, *J* = 7 Hz), 4.82 (m, 1 H), 5.94 (d, 1 H, *J* = 15 Hz), 7.39 (br d, 1 H, NH, *J* = 7 Hz), 7.44 (d, 2 H, *J* = 8.2 Hz), 8.04 (d, 2 H, *J* = 8.2 Hz), 8.11 (d, 1 H, *J* = 15 Hz), 8.32 (s, 1 H), 8.39 (br s, NH), 8.44 (s, 1 H), 8.77 (s, 1 H), 12.07 (br s, NH). Anal. Calcd for $C_{31}H_{36}N_6O_8$: C, 59.99; H, 5.85; N, 13.54. Found: C, 59.71; H, 5.71; N, 13.33.

Diethyl *N*-[4-{*N*-[2-(2-(Pivaloylamino)-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-*N*-formylamino}benzoyl]glutamate (10). A mixture of 300 mg (0.48 mmol) of diethyl *N*-[4-{*N*-[2-(2-pivaloylamino)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)ethen-1-yl]-*N*-formylamino}benzoyl]glutamate (**9**) and 25 mg of amorphous platinum oxide catalyst in 50 mL of glacial acetic acid was hydrogenated in a Parr apparatus at 50 psi for 8 h and then filtered through Celite. The filtrate was concentrated under reduced pressure and the residue dissolved in 7% methanol in methylene chloride and passed through a short column of silica gel. Concentration of the eluate gave a solid which was triturated with ethyl acetate, collected by filtration, and dried to give 165 mg (55% yield) of **10**, mp 188–190 °C. ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3 H, *J* = 7 Hz), 1.28 (s, 9 H), 1.31 (t, 3 H, *J* = 7 Hz), 1.55–1.65 (m, 3 H), 2.02–2.22 (m, 2 H), 2.31 (m, 1 H), 2.48 (m, 2 H), 2.69 (m, 1 H), 3.01 (m, 1 H), 3.41 (m, 1 H), 3.98 (m, 2 H), 4.14 (q, 2 H, *J* = 7 Hz), 4.25 (q, 2 H, *J* = 7 Hz), 4.65 (br s, 1 H, NH), 4.77 (m, 1 H), 7.24 (d, 2 H, *J* = 8.4 Hz), 7.77 (br s, NH), 7.89 (d, 2 H, *J* = 8.4 Hz), 8.47 (s, 1 H), 11.23 (br s, NH). Anal. Calcd for $C_{31}H_{42}N_6O_8$: C, 59.41; H, 6.76; N, 13.41. Found: C, 59.20; H, 6.73; N, 13.29.

***N*-[4-{*N*-[2-(2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]amino}benzoyl]-glutamic Acid (5-Deaza-5,6,7,8-tetrahydrohomofolic Acid) (11).** A homogeneous solution of 100 mg (0.22 mmol) of diethyl *N*-[4-{*N*-[2-(2-(pivaloylamino)-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-*N*-formylamino}-benzoyl]glutamate (**10**) in 10 mL of 1 N NaOH was stirred at rt under nitrogen for 3 days, and the mixture was then acidified to pH 5 with glacial acetic acid. The precipitated solid was collected by filtration, washed with 4 mL of water followed by 4 mL of ethanol, and dried to give 60 mg (82%) of **11**, mp 212 °C dec. ^{1}H NMR (DMSO-d_6 + CF_3COOD) δ 1.49–1.66 (m, 2 H), 1.79–2.14 (m, 3 H), 2.32 (t, 2 H, *J* = 7.4 Hz), 2.42–2.56 (m, 2 H), 2.87 (dd, 1 H, *J* = 12.4, 8.7 Hz), 3.21–3.36 (m, 3 H), 4.37 (dd, 1 H, *J* = 9.7, 4.9 Hz), 7.04–7.06 (m, 2 H), 7.83 (d, 2 H, *J* = 8.3 Hz). Anal. Calcd for $C_{21}H_{26}N_6O_6$: C, 55.02; H, 5.72; N, 18.33. Found: C, 55.27; H, 5.55; N, 18.02.

Diethyl [4-(Aminomethyl)benzoyl]glutamate Hydrochloride (13). Compound **13** was prepared by a modification of the literature procedure.[9] To a solution of *N*-((*o*-nitrophenyl)sulfenyl)-4-(aminomethyl)benzoic acid.[9] (15.4 g, 0.051 mol) and triethylamine (15.48 g, 21.33 mL, 0.153 mol) in methylene chloride (300 mL) was added diethyl L-glutamate (24.45 g, 0.102 mol) followed by 4-(dimethylamino)pyridine (0.62 g, 5.1 mmol). The reaction mixture was cooled to 0 °C and DCC (11.58 g, 0.056 mol) added. After 1 h at 0 °C, the mixture was stirred at ambient temperature for 18 h. The mixture was then stored at 0 °C for 20 h and filtered, and the insoluble material was collected by filtration and washed with a small amount of cold methylene chloride. The methylene chloride filtrate was washed with 0.5 N H_2SO_4 (3 × 150 mL) followed by an aqueous saturated solution of sodium bicarbonate (3 × 150 mL), dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was mixed with absolute ethanol saturated with HCl gas (100 mL), and

anhydrous ether was added (1 L). The precipitate which formed was collected and air-dried and dissolved in ethanol (300 mL) with warming, and ether was added. The white solid which separated was collected by filtration to give 11.07 g (59% yield) of **13** which was used in the next step without further purification.

Diethyl *N*-[*N*-((2-(Pivaloylamino)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl)-4-(aminomethyl)benzoyl]glutamate (14a). A mixture of 2-pivaloyl-6-formyl-5-deazapterin (**12**) (3.0 g, 10.94 mmol), **13** (4.47 g, 12.25 mmol), and sodium acetate (1.0 g, 12.25 mmol) in glacial acetic acid (70 mL) was stirred at 25 °C for 18 h. To this solution was added borane–triethylamine complex (0.42 g, 0.54 mL, 3.65 mmol), and the mixture was stirred at 25 °C for 2 h. The solvent was removed under reduced pressure and the residue dissolved in methylene chloride (200 mL). The mixture was extracted once with water and twice with a saturated aqueous solution of sodium bicarbonate. The aqueous layers were back extracted with methylene chloride (75 mL), and the combined methylene chloride extracts were dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the residue recrystallized from ethyl acetate to give 3.4 g (52% yield) of **14a**: mp 149–151 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.10–1.17 (m, 6 H), 1.22 (s, 9 H), 1.90–2.15 (m, 2 H), 2.40 (t, 2 H, J = 7.40 Hz), 3.75 (s, 2 H), 3.77 (s, 2 H), 3.97–4.10 (m, 4 H), 4.36–4.44 (m, 1 H), 7.41–7.44 (m, 2 H, AA'BB'), 7.80–7.83 (m, 2 H, AA'BB'), 8.35 (d, 1 H, J = 2.09 Hz), 8.65 (d, 1 H, J = 7.38 Hz), 8.76 (d, 1 H, J = 2.09 Hz); mass spectrum, *m/z* (relative intensity) 594 (3, M^+), 538 (31), 537 (100), 521 (22), 392 (24), 334 (41), 274 (21), 259 (29), 202 (44), 174 (27), 134 (55), 131 (22), 118 (25), 105 (29), 84 (22). Anal. Calcd for $C_{30}H_{38}N_6O_7$: C, 60.59; H, 6.44; N, 14.13. Found: C, 60.75; H, 6.68; N, 14.33.

Diethyl *N*-[*N*-((2-(Pivaloylamino)-3,4-dihydro-4-oxopyrido[2,3-*d*]pyrimidin-6-yl)methyl)-10-formyl-4-(aminomethyl)benzoyl]glutamate (14b). To a solution of **14a** (1.0 g, 1.7 mmol) in 97% formic acid (10 mL) was added acetic anhydride (0.35 g, 0.33 mL, 3.5 mmol), and the mixture was stirred at 25 °C for 1 h. The solvent was removed under reduced pressure, and the residue was taken up in methylene chloride. The reaction mixture was extracted twice with a saturated aqueous solution of sodium bicarbonate, the aqueous layers were back extracted with methylene chloride, and the combined methylene chloride extracts were dried over anhydrous magnesium sulfate. Evaporation to dryness gave **14b** in quantitative yield; this compound was used in the next step without further purification. ^{1}H NMR (Me_2SO-d_6) δ 1.11–1.18 (m, 6 H), 1.24 (s, 9 H), 1.90–2.15 (m, 2 H), 2.39–2.44 (m, 2 H), 4.39–4.42 (m, 3 H), 4.53–4.55 (m, 2 H), 7.21–7.44 and 7.34–7.37 (m, 2 H, AA'BB'), 7.76–7.78 and 7.81–7.84 (m, 2 H, AA'BB'), 8.12 and 8.13 (s, 1 H), 8.61–8.78 (m, 2 H).

Diethyl *N*-[*N*-((2-(Pivaloylamino)-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)methyl)-10-formyl-4-(aminomethyl)benzoyl]glutamate (15b). To a solution of **14b** (1.09 g, 0.17 mmol) in glacial acetic acid (50 mL) was added platinum oxide (164 mg), and the suspension was shaken in a Parr hydrogenation apparatus under an atmosphere of hydrogen (50 psi) at 25 °C for 3 h. The reaction mixture was filtered through Celite, and the filtrate was evaporated under reduced pressure. The residue was taken up in methylene chloride (100 mL) and extracted twice with a saturated aqueous solution of sodium bicarbonate. The aqueous layers were back extracted with methylene chloride (75 mL), and the combined methylene chloride extracts were dried over anhydrous magnesium sulfate. Evaporation of the solvent under reduced pressure and radial chromatography of the residue, eluting with 5% methanol in methylene chloride, gave 0.8 g (73%) of **15b**. The analytical sample was recrystallized from acetonitrile: mp 149–151 °C; ^{1}H NMR (Me_2SO-d_6) 1.11–1.16 (m, 6 H), 1.18 (s, 9 H), 1.83–2.15 (m, 4 H), 2.30–2.47 (m, 3 H), 2.79–3.20 (m, 4 H), 3.98–4.11 (m, 4 H), 4.36–4.43 (m, 1 H), 4.53–4.54 (m, 2 H), 6.40–6.51 (m, 1 H), 7.33–7.36 (m, 2 H, AA'BB'), 7.80–7.85 (m, 2 H, AA'BB'), 8.11 and 8.38 (s, 1 H), 8.67–8.73 (m, 1 H); mass spectrum, *m/z* (relative intensity) 626 (6, M^+), 565 (12), 306 (17), 277 (24), 260 (13), 249 (43), 248 (100), 176 (11), 164 (26), 117 (15), 84 (21). Anal. Calcd for $C_{31}H_{42}N_6O_8$: C, 59.41; H, 6.75; N, 13.41. Found: C, 59.14; H, 6.73; N, 13.64.

Diethyl *N*-[*N*-((2-(Pivaloylamino)-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)methyl)-4-(aminomethyl)benzoyl]glutamate (15a). To a solution of **14a** (0.37 g, 0.62 mmol) in glacial acetic acid was added 5% rhodium-on-charcoal (0.125 g), and the suspension was shaken in a Parr apparatus under an atmosphere of hydrogen (50 psi) at 25 °C for 48 h. The reaction mixture was filtered through Celite to remove the catalyst, and the filtrate was evaporated under reduced pressure. The residue was dissolved in methylene chloride and extracted twice with a saturated aqueous solution of sodium bicarbonate. The reaction mixture was dried over anhydrous magnesium sulfate and the solvent removed under reduced pressure. The residue was recrystallized from ethyl acetate to give 0.233 g (37% yield) of **15a**: mp 146–147 °C; ^{1}H NMR (Me_2SO-d_6, exchanged D_2O) δ 1.09–1.15 (m, 6 H), 1.15 (s, 9 H), 1.90–2.17 (m, 4 H), 2.40 (t, 2 H, J = 7.3 Hz), 2.50–2.60 (m, 1 H), 2.75–2.85 (m, 1 H), 2.85–2.97 (m, 1 H), 3.96–4.07 (m, 4 H), 4.10–4.11 (m, 2 H), 4.37–4.43 (m, 1 H), 7.55–7.57 (m, 2 H, AA'BB'), 7.85–7.88 (m, 2 H, AA'BB'), 8.76 (d, 1 H, J = 7.37 Hz); mass spectrum, *m/z* (relative intensity) 598 (0.8, M^+), 395 (10), 261 (11), 250 (17), 249 (100), 248 (15), 247 (12), 164 (20), 162 (15), 145 (18), 133 (15), 118 (28), 117 (13), 112 (13), 84 (22), 71 (10). Anal. Calcd for $C_{30}H_{42}N_6O_7$: C, 60.19; H, 7.07; N, 14.04. Found: C, 60.30; H, 6.85; N, 13.96.

***N*-[*N*-((2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)methyl)-10-formyl-4-(aminomethyl)benzoyl]glutamic Acid (10-Formyl-5-deaza-5,6,7,8-tetrahydroisohomofolic Acid) (16b).** A solution of **15b** (0.5 g, 0.8 mmol) in 0.1 N NaOH was stirred at 25 °C for 5 days. The reaction mixture was filtered, and the filtrate was acidified to pH 4 with 0.5 N HCl. The white solid which formed after standing at 0 °C for 1 h was collected by filtration and washed with water. The product was dried *in vacuo* over P_2O_5 to give 0.26 g (67% yield) of **16b**: mp, gradual decomposition over 178 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.75–2.23 (m, 5 H), 2.20–2.42 (m, 3 H), 2.65–2.83 (m, 1 H), 3.00–3.17 (m, 2 H), 4.36–4.40 (m, 1 H), 4.44–4.60 (m, 2 H), 5.95 (s, 2 H), 6.29 (s, 1 H), 7.28–7.36 (m, 2 H, AA'BB'), 7.69–7.92 (m, 2 H, AA'BB'), 8.10 and 8.36 (s, 1 H), 8.57 (t, 1 H, J = 7.78 Hz), 9.80 (br s, 1 H). Anal. Calcd for $C_{32}H_{26}N_6O_7$: C, 54.32 H, 5.39; N, 17.28. Found: C, 54.67; H, 5.46; N, 17.02.

***N*-[*N*-((2-Amino-3,4-dihydro-4-oxo-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)methyl)-4-(aminomethyl)benzoyl]glutamic Acid (5-Deaza-5,6,7,8-tetrahydroisohomofolic Acid) (16a).** A solution of **15a** (0.20 g, 0.34 mmol) in 1 N NaOH (5 mL) was stirred at 25 °C for 3 days. The reaction mixture was filtered and the pH adjusted to 6 by dropwise addition of 0.5 N HCl. After standing at 0 °C for 2 h, the white solid was collected by filtration, washed with cold water, and dried *in vacuo* over P_2O_5 to give 0.049 g (32% yield) of **16a**: mp 263 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.80–2.25 (m, 4 H), 2.27 (t, 2 H, J = 6.95 Hz), 2.73–3.00 (m, 2 H), 3.28–3.31 (m, 1 H), 4.05 (s, 2 H), 4.15 (s, 2 H), 4.21 (s, 2 H), 4.36–4.41 (m, 1 H), 7.41–7.45 (m, 2 H, AA'BB'), 7.80–7.82 (m, 2 H, AA'BB'), 8.61 (s, 1 H). Anal. Calcd for $C_{21}H_{26}N_6O_6 \cdot H_2O$: C, 54.95; H, 5.82; N, 17.97. Found: C, 54.07; H, 5.48; N, 17.76.

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