# Analogues of 10-Deazaaminopterin and 5-Alkyl-5,10-dideazaaminopterin with the 4-Substituted 1-Naphthoyl Group in the Place of 4-Substituted Benzoyl

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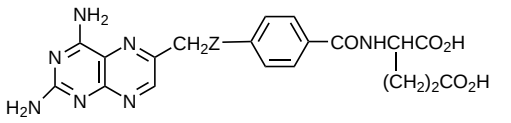
10-Deaza modifications of classical antifolate analogues bearing the 1,4-disubstituted naphthalene ring in place of the 1,4-disubstituted benzene ring were prepared and tested for antitumor activity. Naphthalene analogues (**9a**−**c**, respectively) of 10-deazaaminopterin, 5-methyl-5,10-dideazaaminopterin, and 5-ethyl-5,10-dideazaaminopterin were prepared by a route consisting of *C*-alkylations of the anion derived from 4-carboxy-1-naphthaleneacetic acid dimethyl ester (**2**) by 6-(bromomethyl)-2,4-diaminopteridine (**1a**) and 6-(bromomethyl)-2,4-diamino-5-methyl- and -5-ethyl-5-deazapteridines (**1b** and **1c**, respectively) followed by ester hydrolysis and subsequent decarboxylation to give naphthalene analogues (**7a**−**c**, respectively) of 4-amino-4-deoxy-10-deazapteroic acid and 4-amino-4-deoxy-5-methyl- and -5-ethyl-5,10-dideazapteroic acids. Peptide coupling of **7a**−**c** with L-glutamic acid dialkyl ester followed by mild ester hydrolysis gave target compounds **9a**−**c**. The key advantage of this route is circumvention of a hydrogenation step requiring selectivity as in earlier approaches involving 9,10-olefinic precursors. Steric limitations thwarted plans to prepare the naphthalene analogue of 10-ethyl-10-deazaaminopterin; attempted alkylations of 2-(4-carboxy-1-naphthyl)butyric acid dimethyl ester with **1a** failed as did attempted further alkylation (by EtBr) of the product derived from **1a** and **2**. Growth inhibition tests against three tumor cell lines (L1210, S180, and HL60) showed **9a** to be 4−6-fold more inhibitory than methotrexate but not as inhibitory as 10-ethyl-10-deazaaminopterin; **9b** and **9c** were no more inhibitory than MTX. In tests against the E0771 mammary adenocarcinoma in mice, **9a** was less active than MTX.

Aminopterin (AM) and its $N^{10}$-methyl analogue methotrexate (MTX) act as antitumor agents through potent inhibition of dihydrofolate reductase (DHFR; 5,6,7,8-tetrahydrofolate:$NADP^+$ oxidoreductase, EC 1.5.1.3), the enzyme that promotes metabolism of essential vitamin folic acid. Metabolites of folic acid are involved in the transfer of one-carbon units in biosyntheses that produce thymidylic acid and purine nucleotides required to form nucleic acids.[1−3]

The clinically observed therapeutic superiority of MTX over AM[4] was eventually explained, at least to a major extent, by findings that $N^{10}$-alkyl analogues of AM are accumulated less efficiently than AM into normal proliferative tissue, such as intestinal epithelium, than into tumor tissue. Thus, the higher and more persistent levels of AM in the normal proliferative tissue account, at least partially, for the higher toxic and lower therapeutic effects of AM compared with those of MTX.[5−7]

Studies showing that modification at the 10-position of the classical antifolate structure often produces favorable therapeutic effects with little, if any, reduction in affinity for the target enzyme led to the design of 10-deazaaminopterin (10-DAM) and 10-ethyl-10-deazaaminopterin (10-EDAM).[8−10] Both proved to be more effective than MTX against a group of murine tumors *in vivo* with 10-EDAM proving to be the more effective of the two. Pharmacokinetic studies showed their improved efficacy to be determined to a major extent at the level of membrane transport. Also, differences in the extent of intracellular polyglutamylation were found to contribute to the selective internalization of the agents in tumor cells.[9] The excellent *in vivo* antitumor efficacy of 10-EDAM resulted in it being promoted to clinical trials.[11]

We earlier reported syntheses and antitumor evaluations of analogues of classical antifolates with the naphthalene ring replacing the benzene ring as the only modification of the parent structures.[12] Favorable antitumor activity shown by some of the classical naphthalene analogues prompted us to pursue the corresponding analogues of 10-deaza types.




Z = NH (aminopterin, AM) Z = $CH_2$ (10-DAM)
Z = $NCH_3$ (methotrexate, MTX) Z = $CHCH_2CH_3$ (10-EDAM)

## Chemistry

The syntheses outlined in Scheme 1 used methods based on those reported for syntheses of 10-propargyl-10-deazaaminopterin[13] and other 10-deaza analogues.[14,15] The general route features *C*-alkylation of a malonic ester vinylogue by an appropriate bromomethyl-substituted heteroaromatic precursor followed by ester hydrolysis and decarboxylation to give a 4-amino-4-deoxy-10-deazapteroic acid type intermediate. Standard peptide coupling with L-glutamic dialkyl ester followed by mild hydrolysis then completes the synthesis.

In the current work, bromomethyl compounds were 6-(bromomethyl)-2,4-diaminopteridine (**1a**)[20] and 6-(bromomethyl)-2,4-diamino-5-alkylpyrido[2,3-*d*]pyrimi-

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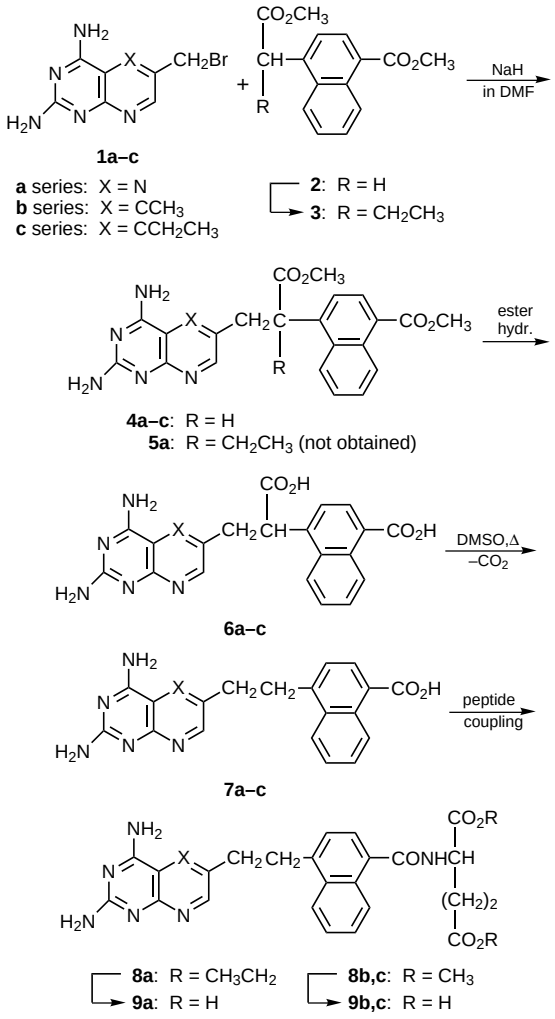
**Scheme 1.** Synthetic Routes to Naphthoyl Analogues of 10-Deazaaminopterin and 5-Alkyl-5,10-dideazaaminopterin (Structures **9a**−**c**)




dines **1b**[12] and **1c**;[21] the malonate vinylogue was 4-carboxy-1-naphthaleneacetic acid dimethyl ester (**2**). The dicarboxylic acid precursor of **2** was prepared in three steps from commercial 1-naphthaleneacetic acid (bromination, displacement of Br by CN, hydrolysis of CN to $CO_2H$) according to reported procedures.[16,17] The Scheme 1 sequence afforded ready access to target compounds **9a**−**c**, naphthalene analogues of 10-DAM, 5-methyl-5,10-dideazaaminopterin, and 5-ethyl-5,10-dideazaaminopterin, respectively. This general route allows syntheses of compounds that would have been difficultly accessible, if at all, by earlier approaches to 10-deaza types. The key advantage in this route is that it does not involve a hydrogenation step as required in routes using Wittig methodology and proceeding through 9,10-olefinic-bridged intermediates.[18] Thus the often formidable task of effecting selective hydrogenation is circumvented. Selective hydrogenation proved to be tedious in earlier syntheses of 5,10-dideazapteridines[18,19] and would be even more difficult in the presence of such easily hydrogenated groups as propargyl.[13] In the present work, if the Wittig approach had been used, hydrogenation in the unsubstituted ring of the naphthalene system would likely have caused difficulties also, judging from our experiences in syntheses of other 1,4-disubstituted naphthalenes.[12]

**Table 1.** Comparisons of 10-Deazanaphthoyl and 5-Alkyl-5,10-dideazanaphthoyl Analogues **9a**−**c** with MTX and 10-EDAM against Growth of L1210, S180, and HL60 *in Vitro*[a]

| | cell growth inhibition, IC50, nM[b] | | |
|---|---|---|---|
| compd | L1210 | S180 | HL60 |
| MTX | 9.8 | 9.9 | 4.6 |
| 10-EDAM | 0.70 | 0.77 | |
| **9a** | 1.5 | 1.7 | 1.1 |
| MTX | 7.3 | 16 | 5.9 |
| **9b** | 8.8 | 29 | 9.4 |
| **9c** | 9.0 | 29 | 9.8 |

[a] Methods reviewed in refs 8 and 9. [b] Average of three determinations.

**Table 2.** Activity of **9a** Compared with MTX against the E0771 Mammary Adenocarcinoma[a]

| compd | dosage[b] (mg/kg) | av wt change (g) | av tumor vol[c] ($mm^3$) | T/C[d] (%) |
|---|---|---|---|---|
| control | | 0.8 | 1204 | 1.00 |
| MTX | 3 | −4.9 | 151 | 0.13 |
| **9a** | 24 | −0.4 | 1150 | 0.96 |
| | 48 | −1.3 | 624 | 0.52 |

[a] Five mice/test, day 10; methods described in refs 8 and 9. [b] Rx daily for 5 days starting day 3 after implantation. [c] Volume ($mm^3$) = $^4/_3\pi r^3$. [d] T/C = treated/control.

We intended to prepare the naphthalene analogue of 10-EDAM, but unlike the analogous homoterephthalate derivative with its aromatic ring of less bulk than naphthalene, the anion derived from **3** could not be *C*-alkylated by **1a** to give the sought intermediate **5a**. The failure of this conversion may be attributed to steric hindrance, and apparently for the same reason, attempted conversion of **4a** (NaH, EtBr in DMF) to **5a** also failed.

## Antitumor Evaluation

In *in vitro* cell growth inhibition tests against the three tumor cell lines as shown in Table 1, the 10-DAM naphthalene analogue **9a** was 4−6-fold more effective in inhibiting cell growth than MTX, but not as effective as 10-EDAM. 5-Alkyl-5,10-dideaza analogues **9b** and **9c** were found to produce essentially the same cell growth inhibition as MTX against the three lines.

The *in vitro* results from **9a** prompted *in vivo* evaluation against E0771 mammary adenocarcinoma in mice. This compound proved to be less effective than MTX as shown by results listed in Table 2. The poor activity of **9a** in mice despite the encouraging results against tumor cells in culture probably reflects pharmacokinetic differences characterizing the evaluation methods. In the *in vitro* tests,[8,9] the candidate drug is in contact with the tumor cells for a protracted period. In the mouse, however, compound **9a** appears to be rapidly cleared from the tumor tissue and also from other murine tissue as indicated by the high tolerance to this agent.

## Experimental Section

Examinations by TLC were performed on Analtech precoated (250 $\mu$m) silica gel G(F) plates. High-performance liquid chromatography (HPLC) assays were made with a Waters Associates ALC-242 liquid chromatograph equipped with an ultraviolet detector (254 nm) and a M-6000 pump using a 30 × 0.29 cm $C_{18}$ $\mu$Bondapak column. Purity assays were done in the reversed-phase isocratic mode with a mobile phase consisting of MeCN (10 or 15% by volume) in 0.1 M NaOAc (pH 3.6). Purifications by preparative TLC were done on Analtech silica gel G(F) plates (2 mm). Column chromatographic purifications were done with silica gel (Merck, 60 A,

**Table 3.** $^1$H NMR Spectral Data for **2**, **3**, and **9a**−**c**

| compd no. (solvent) | chemical shifts ($\delta$, relative to TMS) |
|---|---|
| **2** ($CDCl_3$) | 3.64 (s, $CH_2CO_2CH_3$), 3.97 (s, $CO_2CH_3$), 4.08 (s, $CH_2CO_2CH_3$), 7.4 (d, $C^2$-H), 7.58 (m, $C^6$-H and $C^7$-H), 8.00 (m, $C^8$-H), 8.09 (d, $C^3$-H), 8.95 (m, $C^5$-H) |
| **3** ($CDCl_3$) | 0.97 (t, $CH_2CH_3$), 1.96, 2.30 (2 m, $CH_2CH_3$, nonequivalent), 3.64 (s, $CHCO_2CH_3$), 3.99 (s, $CO_2CH_3$), 4.35 (t, $CHCO_2CH_3$), 7.54 (d, $C^2$-H), 7.6 (m, $C^6$-H and $C^7$-H), 8.13 (d, $C^3$-H), 8.19 (m, $C^8$-H), 8.96 (m, $C^5$-H) |
| **9a** ($Me_2SO$-$d_6$) | 1.92, 2.09 (2 m, $CHCH_2CH_2$, nonequivalent), 2.4 (m, $CHCH_2CH_2$), 3.22, 3.61 (two t, $C^9H_2$, $C^{10}H_2$), 4.46 (m, $NHCHCH_2$), 6.55 (br s, $NH_2$), 7.44 (d, $C^{2'}$-H), 7.51 (d, $C^{3'}$-H), 7.54−7.71 (m, overlapping, $NH_2$, $C^{6'}$-H, and $C^{7'}$-H), 8.28 (m, overlapping, $C^{5'}$-H and $C^{8'}$-H), 8.61 (s, $C^7$-H), 8.72 (d, CONH) |
| **9b** ($Me_2SO$-$d_6$) | 1.94, 2.08 (2 m, $CHCH_2CH_2$, nonequivalent), 2.39 (t, $CHCH_2CH_2$), 2.69 (s, $CH_3$), 3.04, 4.41 (two t, $C^9H_2$, $C^{10}H_2$), 4.45 (m, $NHCHCH_2$), 6.88 (br s, $NH_2$), 7.36 (d, $C^{2'}$-H), 7.43 (br s, $NH_2$), 7.52 (d, $C^{3'}$-H), 7.54−7.65 (m, overlapping, $C^{6'}$-H, C $^{7'}$-H), 8.21 (d, $C^{8'}$-H), 8.30 (m, overlapping, $C^7$-H, $C^{5'}$-H), 8.64 (d, CONH) |
| **9c** ($Me_2SO$-$d_6$) | 1.18 (t, $CH_2CH_3$), 1.94, 2.08 (2 m, $CHCH_2CH_2$, nonequivalent), 2.4 (t, $CHCH_2CH_2$), 3.08 (m, overlapping, $CH_2CH_3$ and $C^9$-$H_2$ or $C^{10}$-$H_2$), 3.34 (t, $C^9$-$H_2$ or $C^{10}$-$H_2$), 4.45 (m, $NHCHCH_2$), 6.78, 7.3 (two br s, $NH_2$), 7.38 (d, $C^{2'}$-H), 7.5−7.68 (m, overlapping $C^{3'}$-H, $C^{6'}$-H, $C^{7'}$-H), 8.23 (d, $C^{8'}$-H), 8.32 (d, $C^{5'}$-H), 8.4 (s, C $^7$-H), 8.64 (d, CONH) |

230−400 mesh for flash chromatography). When solubility limitations made it necessary, crude products to be purified were dispersed in silica gel for application to the column. Dispersal was achieved by evaporating *in vacuo* a solution of the crude product in DMF containing suspended silica gel (3 g of 60−200 mesh per gram of crude product). Evaporations were performed with a rotary evaporator; higher boiling solvents (DMF, $Me_2NAc$, $Me_2SO$) were removed *in vacuo* (<1 mm, bath to 35 °C), and more volatile solvents, with a $H_2O$ aspirator. Products were dried *in vacuo* (<1 mm) at 22−25 °C over $P_2O_5$ and NaOH pellets. Final products were dried and then allowed to equilibrate with ambient conditions of the laboratory. Analytical results indicated by element symbols were within ±0.4% of the theoretical values. Spectral determinations and elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. $^1$H NMR spectral data on key intermediates and target compounds are listed in Table 3. The $^1$H NMR spectra were determined with a Nicolet NMC 300 NB spectrometer using $Me_4Si$ as internal reference. Chemical shifts ($\delta$) listed for multiplets were measured from the approximate centers, and relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on a Varian MAT 311A mass spectrometer in the fast-atom-bombardment (FAB) mode.

**4-Carboxy-1-naphthaleneacetic Acid Dimethyl Ester (2).** A solution of the parent dicarboxylic acid[16,17] (1.75 g, 7.61 mmol) in MeOH (70 mL) containing concentrated $H_2SO_4$ (1.0 mL) was refluxed 4 h, cooled, and evaporated. The gummy residue was dissolved in $CH_2Cl_2$ (40 mL), and the solution was washed three times with $H_2O$ (10-mL portions), dried ($Na_2SO_4$), and evaporated. The residue was dissolved in cyclohexane−EtOAc (85:15) and applied to a silica gel column, which was eluted using the same solvent. Evaporation of combined homogeneous fractions ($R_f$ 0.5; cyclohexane−EtOAc, 3:1) gave pure **2**, mp 68−70 °C, in 77% yield (1.50 g). Anal. ($C_{15}H_{14}O_4$) C, H. Mass spectrum: $m/z$ 259, $MH^+$. $^1$H NMR data is given in Table 3.

**2-(4-Carboxy-1-naphthyl)butyric Acid Dimethyl Ester (3).** A solution of **2** (2.10 g, 8.14 mmol) in DMF (5 mL) was added during 45 min to a cold (0−5 °C), stirred suspension of NaH (325 mg of 60% in oil, 8.14 mmol) in DMF (5 mL). After another 45-min period, the mixture was chilled to −30 °C and then treated during 45 min with a solution of ethyl bromide (890 mg, 8.17 mmol) in DMF (6 mL). The stirred mixture was allowed to warm during 3 h to 10 °C and then left at 20−23 °C for 16 h. Examination by TLC showed complete conversion to **3** ($R_f$ 0.6; cyclohexane−EtOAc, 3:1). The mixture was treated with small pieces of dry ice until a test portion was neutral to test paper. DMF was evaporated, and the residue was stirred with $CH_2Cl_2$−$H_2O$ (50 mL of 4:1). The $H_2O$-washed (3 × 10 mL) $CH_2Cl_2$ layer was dried ($Na_2SO_4$) and evaporated. The residual oil was then purified by silica gel chromatography (as described above for **2**) to give pure **3**, a homogeneous oil, in 50% yield (1.16 g). Anal. ($C_{17}H_{18}O_4$) C, H. Mass spectrum: $m/z$ 287 ($MH^+$); $^1$H NMR data is given in Table 3.

**4-[1-Carboxy-2-(2,4-diamino-6-pteridinyl)ethyl]-1-naphthoic Acid Dimethyl Ester (4a).** The sodium derivative of **2** (from 775 mg, 3.00 mmol) was prepared using NaH (120 mg of 60% in oil dispersion, 3.00 mmol) in DMF as described under the preparation of **3** above. With the temperature of the stirred mixture at −30 °C, a solution of **1a**·HBr[20] (400 mg of 85% purity, 1.00 mmol) in DMF (6 mL) was added during 20 min. The stirred mixture was allowed to warm to 10 °C, kept near 10 °C for 3 h, and then left for 16 h at 20−23 °C. Solid $CO_2$ was then added in small pieces until the mixture was neutral to test paper. DMF was removed *in vacuo*, and the residue was stirred with cold $H_2O$. The collected and dried solid was converted with the aid of DMF to a dried slurry with silica gel. The slurry was applied to a silica gel column, and elution with $CHCl_3$−MeOH (85:15) followed. Homogeneous fractions were combined and evaporated to give **4a** in 60% yield (260 mg). Mass spectrum: $m/z$ 433, ($MH^+$) for $C_{22}H_{20}N_6O_4$. Unchanged **2** was recovered from early fractions.

**4-[1-Carboxy-2-(2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoic acid dimethyl ester (4b)** was prepared by alkylation of the sodium derivative of **2** by 6-(bromomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine[12] (**1b**) in an adaptation of the procedure used to prepare **4a**. The yield of **4b** was 17% (150 mg from 2.0 mmol of **1b**·1.7HBr·0.5$CH_3CO_2H$ and 6.0 mmol of **2**) after purification by elution from a silica gel column by $CHCl_3$−MeOH (9:1). Mass spectrum: $m/z$ 446, $MH^+$ for $C_{24}H_{23}N_5O_4$.

**4-[1-Carboxy-2-(2,4-diamino-5-ethylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoic acid dimethyl ester (4c)** was prepared using 6-(bromomethyl)-2,4-diamino-5-ethylpyrido[2,3-*d*]pyrimidine[21] (**1c**) as described above for the preparation of **4a** from **1a**, yield 25% (116 mg).

**4-[1-Carboxy-2-(2,4-diamino-6-pteridinyl)ethyl]-1-naphthoic Acid (6a).** A solution of **4a** (1.00 g, 2.31 mmol) in 2-methoxyethanol (70 mL) and $H_2O$ (30 mL) was treated with 1 N NaOH (5.5 mL) and then kept at 20−25 °C for 54 h. Solvents were evaporated, and the residue was stirred with $H_2O$ (20 mL) for 16 h. The solution was clarified (Norit, Celite) and treated with 1 N HCl to produce a pH of 3.8 and precipitate **6a**. After the mixture had been chilled in an ice bath, the solid was collected, dried, and used directly in the conversion to **7a** described below.

**4-[1-Carboxy-2-(2,4-diamino-5-methylpyrido[2,3-*d*]-pyrimidin-6-yl)ethyl]-1-naphthoic Acid (6b).** The diester **4b** (145 mg, 0.326 mmol) was saponified during 24 h at 20−23 °C in $Me_2SO$ (5 mL) containing 4 N NaOH (0.2 mL). The solvent was removed *in vacuo* (bath at 25 °C), and the residue was dissolved in $H_2O$ (5 mL). Treatment of the solution with AcOH to pH 5.0 caused precipitation of **6b**, yield 83% (136 mg). Mass spectrum: $m/z$ 418, $MH^+$ for $C_{22}H_{19}N_5O_4$. This sample was dried and pulverized for direct conversion to **7b**.

**4-[1-Carboxy-2-(2,4-diamino-5-ethylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoic acid (6c)** was obtained from **4c** (116 mg, 0.253 mmol) as described for the preparation of **6a** from **4a**, yield 95% (104 mg). Mass spectrum: $m/z$ 432, $MH^+$ for $C_{23}H_{21}N_5O_4$.

**4-[2-(2,4-Diamino-6-pteridinyl)ethyl]-1-naphthoic Acid (7a).** The sample of **6a** described above was dissolved in $Me_2SO$, and the solution was heated at 115−120 °C for 5.5 h. The solvent was then removed by distillation (<1 mm, bath to 40 °C). The residue was stirred with $H_2O$ (20 mL), and the suspension was treated with 1 N NaOH (about 2.5 mL) until solution occurred. After clarification (Norit, Celite), the solution was treated dropwise with 1 N HCl to cause pH 3.8 and precipitate **7a**. The mixture was chilled in an ice bath, and the solid was collected and dried, overall yield (from **4a**) 35% (312 mg). Mass spectrum: *m/z* 361 ($MH^+$) for $C_{19}H_{16}N_6O_2$.

**4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoic Acid (7b).** The sample of **6b** (113 mg) described above was suspended in $Me_2SO$ (5 mL), and the stirred mixture was heated at 117−120 °C under $N_2$ for 1 h. Removal of the $Me_2SO$ *in vacuo* followed by treatment of the residue with $H_2O$ gave **7b** in 80% yield (98 mg). Assay by HPLC revealed 88% purity. Mass spectrum: *m/z* 374, $MH^+$ for $C_{21}H_{19}N_5O_2$.

**4-[2-(2,4-Diamino-5-ethylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoic Acid (7c).** Decarboxylation of **6c** (104 mg, 0.241 mmol) as described for the conversion of **6b** to **7b** afforded **7c**; 90% yield (84 mg). Assay by HPLC indicated 84% purity. Mass spectrum: *m/z* 388, $MH^+$ for $C_{22}H_{21}N_5O_2$.

***N*-[4-[2-(2,4-Diamino-6-pteridinyl)ethyl]-1-naphthoyl]-L-glutamic Acid Diethyl Ester (8a).** A stirred mixture of **7a** (300 mg, 0.83 mmol), $Et_3N$ (590 mg, 5.84 (mmol), and DMF (5 mL) was treated at 20−23 °C with *i*-BuOCOCl (230 mg, 1.68 mmol). After 1 h, the resulting solution was treated with diethyl L-glutamate hydrochloride (480 mg, 2.0 mmol), and stirring at 20−23 °C was continued for 2 h. Two more additions of *i*-BuOCOCl (58 mg, 0.42 mmol each time) were made at 2-h intervals, and each was followed 5 min later by diethyl L-glutamate hydrochloride (120 mg, 0.50 mmol each time). The mixture was then stirred 18 h before DMF was evaporated. The residue was stirred with $H_2O$, dried, and then slurred with silica gel for application to a silica gel column. Elution by $CHCl_3$−MeOH (7:1) afforded pure **8a** in 23% yield (102 mg). Mass spectrum: *m/z* 546, $MH^+$ for $C_{28}H_{31}N_7O_5$.

***N*-[4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoyl]-L-glutamic Acid Dimethyl Ester (8b).** Coupling of **7b** (98 mg, 0.26 mmol) with dimethyl L-glutamate hydrochloride mediated by *i*-BuOCOCl was carried out as described for the preparation of **8a**. The filtered solution was evaporated and the residue washed with $H_2O$. The dried crude product was dissolved in the minimum of DMF, and the solution was applied to a preparative thin-layer chromatography plate which was developed with $CHCl_3$−MeOH (5:1). The band due to **8b** was extracted with MeOH; yield 35% (50 mg). Mass spectrum: *m/z* 531, $MH^+$ for $C_{28}H_{30}N_6O_5$. Two other runs gave similar results.

***N*-[4-[2-(2,4-Diamino-5-ethylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoyl]-L-glutamic Acid Dimethyl Ester (8c).** Coupling of the sample of **7c** (84 mg, 0.217 mmol) with dimethyl L-glutamate hydrochloride mediated by *i*-BuOCOCl as described for the preparation of analogues **8a** and **8b** was followed by workup and purification using preparative TLC as applied to **8b**. The yield of **8c** was 45% (53 mg). Mass spectrum: *m/z* 545, $MH^+$ for $C_{29}H_{32}N_6O_5$.

***N*-[4-[2-(2,4-Diamino-6-pteridinyl)ethyl]-1-naphthoyl]-L-glutamic Acid (9a).** A solution of **8a** (102 mg, 0.187 mmol) in MeOH (70 mL) was treated with $H_2O$ (10 mL) followed by 1 N NaOH (0.42 mL), and the solution was kept at 20−23 °C for 16 h. MeOH was removed by evaporation, and the aqueous solution that remained was diluted with $H_2O$ (10 mL). After another 16 h at 20−23 °C, the solution was filtered and then treated dropwise with 1 N HCl to produce pH 4.0 and give target compound **9a** as a beige solid in yield 80% (79 mg), homogeneous (>99%) by HPLC. Anal. ($C_{24}H_{23}N_7O_5 \cdot 2.5H_2O$) C, H, N. Mass spectrum: *m/z* 490, $MH^+$. $^1H$ NMR, see Table 3.

***N*-[4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoyl]-L-glutamic Acid (9b).** Hydrolysis of ester **8b** (50 mg, 0.094 mmol) as described for the preparation of **9a** afforded pure **9b** in 45% yield (23 mg). Anal. ($C_{26}H_{26}N_6O_5 \cdot 3.7H_2O$) C, H, N. Mass spectrum: *m/z* 503, $MH^+$. $^1H$ NMR, see Table 3.

***N*-[4-[2-(2,4-Diamino-5-ethylpyrido[2,3-*d*]pyrimidin-6-yl)ethyl]-1-naphthoyl]-L-glutamic acid (9c)** was obtained following hydrolysis of diester **8c** (50 mg, 0.085 mmol) as described under analogue **9a**, yield 65% (30 mg). Anal. ($C_{27}H_{28}N_6O_5 \cdot 1.6H_2O$) C, H, N. Mass spectrum: *m/z* 517, $MH^+$. $^1H$ NMR, see Table 3.

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## References

(1) Kisliuk, R. L. The Biochemistry of Folates in *Folate Antagonists as Therapeutic Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. B., Montgomery, J. A., Eds.; Academic Press: Orlando, FL, 1984; Vol. 1, pp 1−68.

(2) *Folates and Pterins. Vol. 1. Chemistry and Biochemistry of Folates*; Blakley, R. L., Benkovic, S. J., Eds.; John Wiley and Sons: New York, NY, 1984.

(3) Moran, R. G. Folate Antimetabolites Inhibitory to *de novo* Purine Synthesis. In *New Drugs, Concepts and Results in Cancer Chemotherapy*; Muggia, F. M., Ed.; Kluwer Academic Publishers: Boston, MA, 1992; pp 65−87.

(4) Hutchinson, D. J.; Schmid, F. A. Experimental Cancer Chemotherapy with Folate Antagonists. In *Folate Antagonists as Therapeutic Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. B., Montgomery, J. A., Eds.; Academic Press: Orlando, FL, 1984; Vol. 2, pp 1−22.

(5) Dembo, M.; Sirotnak, F. M. Membrane Transport of Folate Compounds in Mammalian Cells. In the volume cited in ref 1, pp 173−217.

(6) Rumberger, B. G.; Schmid, F. A.; Otter, G. M.; Sirotnak, F. M. Preferential Selection During Therapy *in vivo* by Edatrexate Compared to Methotrexate of Resistant L1210 Cell Variants with Decreased Folylpolyglutamate Synthetase Activity. *Cancer Commun.* **1990**, *9*, 305−310.

(7) Sirotnak, F. M.; Donsbach, R. C. Further Evidence for a Basis of Selective Activity and Relative Responsiveness during Antifolate Therapy of Murine Tumors. *Cancer Res.* **1975**, *35*, 1737−1744.

(8) Sirotnak, F. M.; DeGraw J. I.; Moccio, D. M.; Samuels, L. L.; Goutas, L. J. New Folate Analogs of the 10-Deaza-aminopterin Series: Basis for Structural Design and Biochemical and Pharmacologic Properties. *Cancer Chemother. Pharmacol.* **1984**, *12*, 18−25.

(9) Sirotnak, F. M.; DeGraw J. I.; Schmid, F. A.; Goutas, L. J.; Moccio, D. M. New Folate Analogs of the 10-Deaza-aminopterin series. Further Evidence for Markedly Increased Antitumor Efficacy Compared with Methotrexate in Ascitic and Solid Murine Tumor Models. *Cancer Chemother. Pharmacol.* **12**, 26−30.

(10) Sirotnak, F. M.; DeGraw, J. I. Selective Antitumor Action of Folate Analogs. In volume cited in Reference 4, pp 43−95.

(11) Reviewed by Berman, E. M.; Werbel, L. M. The Renewed Potential for Folate Antagonists in Comtemporary Cancer Chemotherapy. *J. Med. Chem.* **1991**, *34*, 479−485.

(12) Piper, J. R.; Johnson, C. A.; Maddry, J. A.; Malik, N. D.; McGuire, J. J.; Otter, G. M.; Sirotnak, F. M. Studies on Analogues of Classical Antifolates Bearing the Naphthoyl Group in Place of Benzoyl in the Side Chain. *J. Med. Chem.* **1993**, *36*, 4161−4171.

(13) DeGraw, J. I.; Colwell, W. T.; Piper, J. R.; Sirotnak, F. M. Synthesis and Antitumor Activity of 10-Propargyl-10-deazaaminopterin. *J. Med. Chem.* **1993**, 36, 2228−2231.

(14) DeGraw, J. I.; Colwell, W. T.; Crase, J.; Smith, R. L.; Piper, J. R.; Waud, W. R.; Sirotnak, F. M. Analogs of Methotrexate in Rheumatoid Arthritis. 1. 10-Deazaaminopterins Containing Heteroaroyl Replacements for the Benzoate Moiety. Submitted for publication in *J. Med. Chem.*

(15) Piper, J. R.; DeGraw, J. I.; Colwell, W. T.; Johnson, C. A.; Smith, R. L.; Waud, W. R.; Sirotnak, F. M. Analogues of Methotrexate in Rheumatoid Arthritis. 2. Effects of 5-Deazaaminopterin and 5,10-Dideazaaminopterin and Analogues on Type II Collagen-Induced Arthritis in Mice. Submitted for publication in *J. Med. Chem.*

(16) Ogata, Y.; Okano, M.; Kitamura, Y. The Chemistry of 1-Naphthaleneacetic Acid Derivatives. *J. Org. Chem.* **1951**, *16*, *1588*−1592.

(17) Kiprianov, A. I.; Shulezhko, A. A. Cyanine Dyestuffs with a Naphthalene Nucleus in the Polymethine Chain. *Zh. Org. Khim.* (Engl. Transl.) **1966**, *2*, 1820−1823; *Chem. Abstr.* **1967**, *66*, 47297e.

(18) Piper, J. R.; Johnson, C. A.; Otter, G. M.; Sirotnak, F. M. Synthesis and Antifolate Evaluation of 10-Ethyl-5-methyl-5,10-dideazaaminopterin and an Alternative Synthesis of 10-Ethyl-10-deazaaminopterin (Edatrexate). *J. Med. Chem.* **1992**, *35*, 3002−3006.
(19) Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. Synthesis of the Antileukemic Agents 5,10-Dideazaaminopterin and 5,10-Dideaza-5,6,7,8-tetrahydroaminopterin. *J. Med. Chem.* **1985**, *28*, 914−921.
(20) Piper, J. R.; Montgomery, J. A. Preparation of 6-(Bromomethyl)-2,4-pteridinediamine Hydrobromide and its Use in Improved Syntheses of Methotrexate and Related Compounds. *J. Org. Chem.* **1977**, *42*, 208−211.
(21) Piper, J. R.; Ramamurthy, B.; Johnson, C. A.; Maddry, J. A.; Otter, G. M.; Sirotnak, F. M. Further Evaluation of 5-Alkyl-5-deaza Antifolates. 5-Propyl- and 5-Butyl-5-deaza Analogues of Aminopterin and Methotrexate. *J. Heterocycl. Chem.* **1995**, *32*, 1205−1212.

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