We have recently shown that the structurally simplified P[1,4]B cross-linker DSB-120[23] (II) (Figure 8) can form a DNA-DNA interstrand cross-linker between guanines in the 10-mer duplex shown in Scheme II without appreciable distortion of the duplex, as predicted by this combined NMR and molecular modeling study (Wang and Hurley, unpublished results). This compound is considerably more potent that the monoalkylation compound and is not expected to show the cardiotoxicity previously associated with anthramycin and sibiromycin.[13]

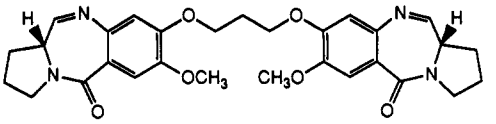

Figure 8. Structure of DSB-120 (II).[23]

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Synthesis and Antifolate Evaluation of 10-Ethyl-5-methyl-5,10-dideazaaminopterin and an Alternative Synthesis of 10-Ethyl-10-deazaaminopterin (Edatrexate)

J. R. Piper,*,† C. A. Johnson,† G. M. Otter,‡ and F. M. Sirotnak‡

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35255, and Memorial Sloan-Kettering Cancer Center, New York, New York, 10021. *Received March 26, 1992*

Previous findings suggesting that 5,10-dialkyl-substituted derivatives of 5,10-dideazaaminopterin warranted study as potential antifolates prompted synthesis of 10-ethyl-5-methyl-5,10-dideazaaminopterin (**12a**). The key step in the synthetic route to **12a** was Wittig condensation of the tributylphosphorane derived from 6-(bromomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine (**7a**) with methyl 4-propionylbenzoate. Reaction conditions for the Wittig condensation were developed using the tributylphosphorane prepared from 6-(bromomethyl)-2,4-pteridinediamine (**7b**) as a model. Each of the respective Wittig products **8a** and **8b** was obtained in 75–80% yield. Hydrogenation of **8a** and **8b** at their 9,10-double bond afforded 4-amino-4-deoxy-10-ethyl-5-methyl-5,10-dideazapteroic acid methyl ester (**9a**) and 4-amino-4-deoxy-10-ethyl-10-deazapteroic acid methyl ester (**9b**). This route to **9b** intersects reported synthetic approaches leading to 10-ethyl-10-deazaaminopterin (10-EDAM, edatrexate), an agent now in advanced clinical trials. Thus the Wittig approach affords an alternative synthetic route to 10-EDAM. Remaining steps were ester hydrolysis of **9a,b** to give carboxylic acids **10a,b** followed by standard peptide coupling with diethyl L-glutamate to produce diethyl esters **11a,b**, which on hydrolysis gave **12a** and 10-EDAM (**12b**), respectively. The relative influx of **12a** was enhanced about 3.2-fold over MTX, but as an inhibitor of dihydrofolate reductase (DHFR) from L1210 cells and in the inhibition of L1210 cell growth in vitro, this compound was approximately 20-fold less effective than MTX (DHFR inhibition, K_i = 4.82 ± 0.60 pM for MTX, 100 pM for **12a**; cell growth, IC_{50} = 3.4 ± 1.0 nM for MTX, 65 ± 18 nM for **12a**).

The classical antifolate methotrexate (MTX), an inhibitor of dihydrofolate reductase (DHFR), remains the only folate analogue in established clinical use for the treatment of cancer. Because of the vital centrality of folate-dependent enzymes in cell proliferation, folate antimetabolites offer high therapeutic promise, especially if greater selectivity of antitumor action can be achieved. Recent investigations have included efforts to identify patterns of structural modifications in those antifolate candidates which have greater antitumor selectivity than MTX.[1,2]

Positions 5 and 10 of classical antifolates are sites where modifications can be made that affect cellular uptake and retention of the candidate drugs without compromising effective binding and inhibition of DHFR.[1] Some analogues modified at these positions are known to accumulate at greater differential levels relative to MTX in tumor than in normal proliferative tissue.[3-6] In a highly responsive tumor, both membrane transport and FPGS work in tandem to provide high levels of the 4-aminofolates.[1,7-10]

These differences between tumor and normal tissues and the enhancement of the accumulation differential through structural modification were exploited in the 10-deazaaminopterin series. This series, particularly the 10-ethyl analogue (10-EDAM), exhibited markedly enhanced

† Southern Research Institute.
‡ Memorial Sloan-Kettering Cancer Center.

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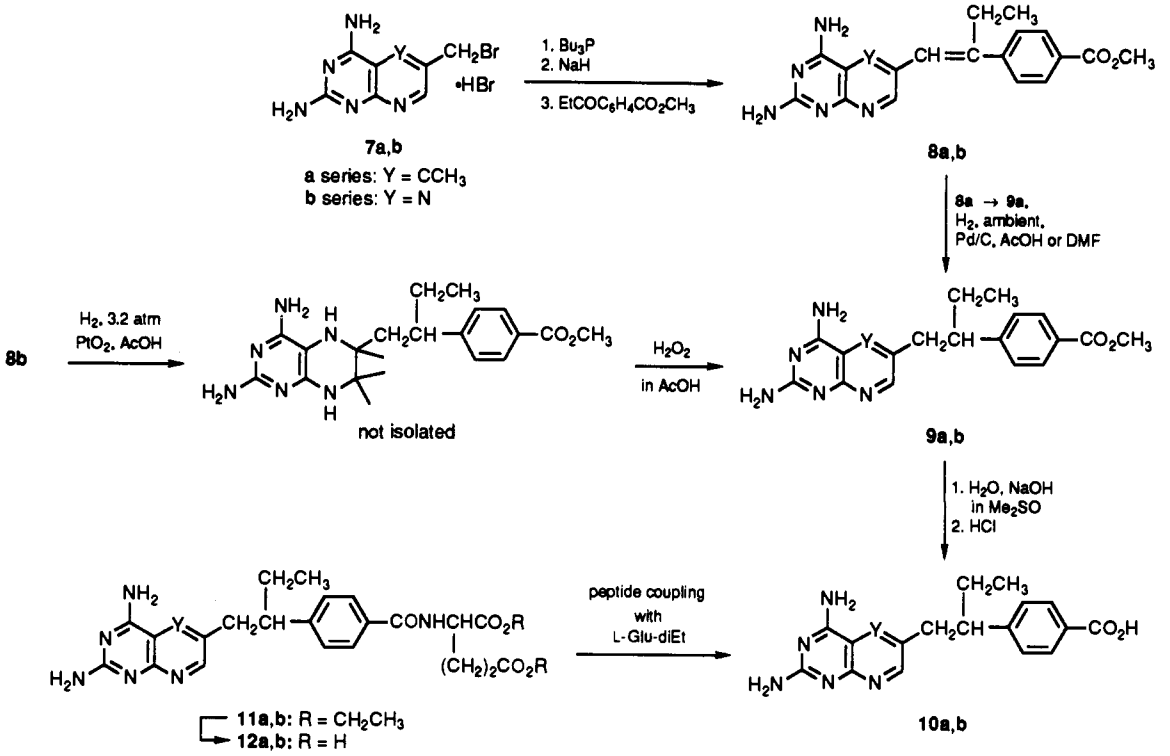
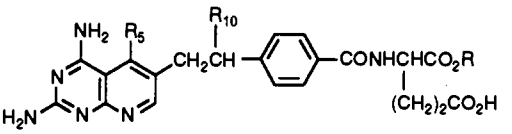
Scheme I



therapeutic selectivity compared to MTX in both animal solid tumor models and human tumor xenografts.[11] This has been attributed to larger differentials in membrane transport inward and intracellular polyglutamylation of 10-EDAM vs MTX in responsive tumors compared to normal proliferative tissues favoring greater accumulation of 10-EDAM as cytotoxic γ-polyglutamates in the tumors.[4,5] 10-EDAM has been shown during clinical trials to have significant therapeutic activity against a variety of solid tumors.[12,13] Results from clinical trials were recently reviewed.[2]

Our studies with the 5-deaza analogues of MTX and aminopterin (AM) have confirmed that position 5 of 4-aminofolates, like position 10, also represents a site where modification can yield analogues with improved therapeutic selectivity, at least in animal models, when compared with MTX.[3,6,14] With 5-alkyl derivatives of 5-deazaaminopterin (5-DAM) and 5-deazamethotrexate (5-DMTX), this may have been achieved, at least in part, by a greater differential in transport inward in tumor cells compared to normal proliferative tissue. Results from studies on the relative antitumor properties of 5-DMTX and 5-DAM analogues compared to MTX in various mammalian cells and in four murine tumor models suggest that these analogues may have significant clinical potential.[3]

These findings on analogues modified in positions 5 and 10 prompted us to consider variants in the 5,10-dideazaaminopterin series as types worthy of synthesis and evaluation. 5,10-Dideazaaminopterin (1) itself[15,16] and



	R_5	R_{10}
1	H	H
2	CH_3	H
3	H	CH_3
4	H	CH_2CH_3
5	$-(CH_2)_2-$	

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5-methyl (2),[17] 10-methyl (3),[18,19] 10-ethyl (4),[18] and 5,10-ethylene-bridged (5)[20] compounds are known, but no 5,10-dialkyl representative from this series has been reported. We regarded the 5,10-dialkyl type derivative as an important prospective series which warranted study. The representative selected for synthesis was the 5-methyl-10-ethyl derivative **12a** (Scheme I).

Chemistry. Our plan for the synthesis of **12a** was to adapt the route we used earlier to prepare 10-deazaaminopterin (10-DAM).[21] The key steps to 10-DAM were as follows: (a) Wittig condensation of diethyl *N*-(4-formylbenzoyl)-L-glutamate with the triphenylphosphorane derived from 6-(bromomethyl)-2,4-pteridinediamine in *N,N*-dimethylacetamide, (b) catalytic hydrogenation of the resulting olefinic product, and (c) mild oxidation to regenerate the pyrazine moiety, which was unavoidably converted to the 5,6,7,8-tetrahydro form during step b. Use of a similar route leading to 5,10-dideaza compound **12a** first required finding conditions for Wittig condensation of a suitable phosphorane with a derivative of 4-propionylbenzoic acid. This conversion would be different from our experience in the 10-DAM synthesis because the keto precursor to the 10-ethyl target does not react as readily with the required phosphorane as the aldehyde precursor to 10-DAM. We explored conditions required for the Wittig condensation using 6-(bromomethyl)-2,4-pteridinediamine (**7b**, Scheme I) as a model for our eventual work with the 5-methyl-5-deaza analogue **7a**; the full pteridine analogue **7b** was more readily available to us than **7a**. After numerous trial experiments using **7b** we found conditions which proved to be satisfactory. The new conditions (see Scheme I) use the following four main modifications. First, dimethyl sulfoxide proved to be the superior solvent over *N,N*-dimethylacetamide for this conversion. Second, use of the tributylphosphorane alleviated both steric hindrance and solubility problems associated with the triphenylphosphorane. Third, the use of sodium hydride instead of sodium methoxide to generate the phosphorane from the phosphonium bromide caused less unwanted side reactions, and fourth, the use of methyl 4-propionylbenzoate gave a significantly better conversion than the full side-chain precursor diethyl *N*-(4-propionylbenzoyl)-L-glutamate. A procedure based on these conditions consistently afforded the expected olefinic product **8b** in 80% yield. These conditions were then applied with similar results using the (6-bromomethyl)-5-methyl-5-deaza precursor **7a** to give **8a**.

After successful use of **7b** as a model in the identification of effective conditions for the Wittig condensation, we recognized the opportunity to convert **8b** to 10-EDAM (**12b**) and thereby demonstrate an alternative synthesis of this important antifolate drug. Each of the remaining steps outlined in Scheme I leading to 10-EDAM is straightforward. Catalytic hydrogenation of **8b** produced the expected 5,6,7,8-tetrahydro intermediate, which was readily converted to **9b** by treatment with hydrogen peroxide. This synthesis of **9b** is relatively facile compared with reported routes.[22-24] Remaining steps from **9b** to 10-EDAM as outlined in Scheme I have been reported. Thus the preparation of **9b** as described intersects routes previously described and is, therefore, tantamount to a new synthesis of 10-EDAM; however, we completed conversion of **9b** to 10-EDAM using procedures similar to those reported. The remaining steps were ester hydrolysis of **9b** to give carboxylic acid **10b**, followed by coupling with diethyl L-glutamate to give diethyl ester **11b**, which was hydrolyzed to 10-EDAM.

In the synthesis of the new antifolate candidate 5-methyl-10-ethyl-5,10-dideazaaminopterin (**12a**), hydrogenation of the 9,10-double bond of **8a** to give **9a** was achieved in a manner like that used in the reported synthesis of 5-methyl-5,10-dideazaaminopterin (**4**).[17] In this step, over-reduction involving the pyrido ring of the 5-deaza system is to be avoided because, unlike the pteridine system, an appropriate oxidation method to regenerate the aromatic system is not currently known. Remaining steps as outlined in Scheme I were carried out in similar fashion as for **12b**.

Table I. Comparison of Properties of 10-Ethyl-5-methyl-5,10-dideazaaminopterin (**12a**) with MTX in L1210 in Vitro[a]

compound	DHFR inhibn K_i (pM)	cell growth inhibn IC_{50} (μM)	influx K_m (μM)
MTX	4.82 ± 0.6	0.0034 ± 0.0010	3.51 ± 0.4
12a	100	0.0650 ± 0.018	1.08 ± 0.2

[a] Methods reviewed in ref 3.

Biological Studies. Evaluation of **12a** as an inhibitor of DHFR from L1210 cells showed it to have a K_i of 100 pM, approximately 20-fold less potent than MTX (see Table I). As an inhibitor of L1210 cell growth in vitro, **12a** was also about 20-fold less effective than MTX although the influx of **12a** into L1210 cells was enhanced by about 3.2-fold over that of MTX. The lower growth inhibitory capacity might be due to enhanced efflux coupled with a low rate of polyglutamylation. Steric interference would not appear to account for the lower activity since earlier results from the identically substituted 5-deaza analogue 10-ethyl-5-methyl-5-deazaaminopterin showed it to be more potent than MTX in inhibition of L1210

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DHFR and in vitro cell growth.[6] An overview of the antifolate test results from 12a along with the reported results from 1–5 reveal no compound of the group displays a significant antitumor advantage over MTX. Combination of the features of therapeutically advantageous deaza types modified at positions 5 and 10 did not result in enhanced antitumor effectiveness.

Experimental Section

Examinations by TLC were performed on Analtech precoated (250-μm) silica gel G(F) plates. TLC plates spotted directly with reaction solutions in Me_2SO were kept in vacuo (<1 mm) over P_2O_5 for 45 min before being developed. Unless other conditions are specified, evaporations were performed with a rotary evaporator and 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 by the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. The ^1H NMR spectra data reported were determined with a Nicolet NMC 300 NB spectrometer using Me_4Si as internal reference. Chemical shifts (δ) 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. UV spectra were determined with a Perkin-Elmer Model Lambda 9 spectrometer. Samples were first dissolved in 0.1 N NaOH, and the solutions were then diluted 10-fold with the medium given in the listings. Maxima are expressed in nanometers with the molar absorbance given in parentheses. Molecular weights used in all calculations conform with the compositions listed with the indicated elemental analyses.

Methyl 4-Propionylbenzoate. The reported procedure for conversion of 4′-methylacetophenone to 4-acetylbenzoic acid was adapted to conversion of commercially available 4′-methylpropiophenone to 4-propionylbenzoic acid.[25] The product had mp 157–158 °C after successive recrystallizations from C_6H_6 and H_2O and was homogeneous according to TLC ($CHCl_3$–AcOH, 30:1). Anal. ($C_{10}H_{10}O_3$) C, H. (Other investigators[26] prepared this compound by another method and reported mp 176–178 °C.) We converted the acid to the methyl ester by three methods: (a) direct esterification (CH_3OH, H_2SO_4-promoted), (b) treatment with CH_2N_2, and (c) treatment with CH_3I in DMF containing $(i\text{-Pr})_2NEt$. Each method afforded the pure ester, mp 80–81 °C. An account of method c follows. A solution of $4\text{-EtCOC}_6H_4CO_2H$ (3.56 g, 20.0 mmol), $(i\text{-Pr})_2NEt$ (3.36 g, 26.0 mmol), and MeI (3.98 g, 28.0 mmol) in DMF (25 mL) was kept in a stoppered flask at 20–25 °C for 44 h. Evaporation in vacuo (<1 mm, bath to 30 °C) followed, and the residue was stirred with 3% Na_2CO_3 (30 mL) for 5 min. The methyl ester, mp 80–81 °C (lit.[24] mp 80–81 °C), was collected: yield 60% (2.28 g); homogeneous by TLC (cyclohexane–EtOAc, 3:1). Anal. ($C_{11}H_{12}O_3$) C, H.

Methyl 4-[2-(2,4-Diamino-6-pteridinyl)-1-ethylethenyl]benzoate (8b). A solution of **7b**·HBr[27] (3.95 g of 85% purity,[28] 10.0 mmol) and Bu_3P (6.07 g, 30.0 mmol) in dry Me_2SO (150 mL) was stirred at 55 °C (bath temperature) for 30 min, and then allowed to cool to 25 °C. (The reaction mixture was stirred under N_2 throughout the reaction period.) Methyl 4-propionylbenzoate (1.92 g, 10.0 mmol) was added followed by NaH (0.80 g of 60% dispersion in oil, 20.0 mmol). Evolution of H_2 occurred while the mixture became red. During several hours, the color gradually changed to orange, and TLC ($CHCl_3$–MeOH, 3:1) revealed the olefinic product as a major spot of $R_f \approx 0.8$. After 45 h at 20–25 °C, TLC examinations indicated no further change. Most of the Me_2SO was then removed by distillation in vacuo (<1 mm, bath to 40 °C). The fluid residue was stirred with Et_2O to give an orange solid which was collected with the aid of Et_2O. The solid was then washed thoroughly with H_2O and dried to give **8b** in 80% yield (2.82 g). Although essentially homogeneous by TLC, **8b** prepared in this manner was a mixture of cis–trans isomers with the cis form in dominance by a molar ratio of approximately 4.5:1 estimated from ^1H NMR data.[29] Spectral data: MS, m/z 351, MH^+ for $C_{16}H_{18}N_6O_2$; ^1H NMR (Me_2SO-d_6) for the cis isomer δ 1.05 (t, CH_3CH_2), 2.60 (q, CH_3CH_2), 3.86 (s, CH_3O), 6.66 (s, CH=C), 7.35 and 7.96 (2 d, C_6H_4), 8.06 (s, C^7-H); for the trans isomer δ 1.10 (t, CH_3CH_2), 3.18 (q, CH_3CH_2), 3.88 (s, CH_3O), 7.00 (s, CH=C), 7.76 and 8.00 (2 d, C_6H_4), 8.80 (s, C^7-H).

Methyl 4-[1-[(2,4-Diamino-6-pteridinyl)methyl]propyl]benzoate (9b). A solution of **8b** (2.82 g, 8.00 mmol) in glacial AcOH (400 mL) containing PtO_2 (0.75 g) was hydrogenated in a Parr shaker at 3.2 atm (46 psi) for 18 h. Mass spectral examination showed disappearance of **8b** with formation of the tetrahydro derivative of **9b** with m/z 357, MH^+ for $C_{16}H_{24}N_6O_2$. The catalyst was removed by filtration, and the residue was treated with 3% H_2O_2 (10 mL). The solution was stirred open to air for 7.5 h before more 3% H_2O_2 (5 mL) was added (total H_2O_2, 0.45 g, 13 mmol). Stirring with exposure to air was continued 16 h longer. TLC ($CHCl_3$–MeOH–concentrated NH_4OH, 4:1:0.05) then showed one spot ($R_f \approx 0.7$). Most of the AcOH was removed by evaporation (bath to 40 °C). The residual solution (about 25 mL) was diluted with H_2O (250 mL), and the resulting solution was treated with concentrated NH_4OH solution to pH 7 to give **9b** as a yellow solid; yield 73% (2.06 g). Spectral data: MS, m/z 353, MH^+ for $C_{18}H_{20}N_6O_2$; ^1H NMR (Me_2SO-d_6) δ 0.76 (t, CH_3CH_2), 1.70 (m, CH_2CH_3), 3.0–3.3 (br m, $C^9H_2C^{10}H$), 3.80 (s, CH_3O), 6.52 (br s, NH_2), 7.36 and 7.84 (2 d, C_6H_4), 7.50 (d, NH_2), 8.35 (s, C^7-H). This spectral data is in agreement with that reported for **9b** prepared by other routes.[22-24]

4-[1-[(2,4-Diamino-6-pteridinyl)methyl]propyl]benzoic Acid (10b). A solution of **9b** (1.30 g, 3.69 mmol) in Me_2SO (70 mL) to which 1 N NaOH (6.8 mL) was added was kept at 20–25 °C for 29 h while **9b** disappeared from thin-layer chromatograms done on specimens of the reaction solution. Me_2SO was removed by short-path distillation in vacuo (<1 mm, bath to 35 °C). The residue was dissolved in H_2O (45 mL), and the solution was clarified (Norit, Celite) and then acidified with 1 N HCl to pH 5.0. A yellow gel formed. The mixture was frozen using a dry ice–acetone bath. Slow thawing in a refrigerator at about 3 °C afforded **10b** as a granular, easily collected solid; yield 77% (1.04 g). Spectral data: UV, λ_{max} 242 (ϵ 29 500), 279 (5680), 340 nm (10 400) at pH 1; 237, plateau (ϵ 22 200), 256 (26 200), 371 nm (7320) at pH 7; 236, plateau (ϵ 22 200), 256 (26 800), 371 nm (7490) at pH 13. The spectral data agrees with reported results.[22-24] Anal. ($C_{17}H_{18}N_6O_2 \cdot 1.5H_2O$) C, H; N: calcd, 23.00; found, 22.53.

Diethyl *N*-[4-[1-[(2,4-Diamino-6-pteridinyl)methyl]propyl]benzoyl]-L-glutamate (11b). A stirred mixture of **10b**·1.5H_2O (1.00 g, 2.74 mmol), Et_3N (1.11 g, 11.0 mmol), and DMF (115 mL) was treated at 20–25 °C with *i*-BuOCOCl (380 mg, 2.78 mmol). After 15 min, the resulting solution was treated with diethyl L-glutamate hydrochloride (657 mg, 2.74 mmol). Three more additions of *i*-BuOCOCl (1.37, 0.69, and 0.69 mmol) were made at 15-min intervals and each was followed 5 min later by an equimolar amount of diethyl L-glutamate hydrochloride. The course of the conversion was followed by TLC, and a chro-

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(28) Material prepared as described in ref 27 was estimated from its integrated ^1H NMR spectrum to be of 85% purity; other matter present was 2-PrOH (12%) and the 6-methyl analogue (3%).

(29) Our previous ^1H NMR studies of cis–trans mixtures of analogues of **8a,b** showed chemical shifts due to the trans isomers occur downfield from corresponding shifts due to the cis forms. This observation was made via coupling constants produced by an analogous cis–trans pair bearing the C^9H=$C^{10}H$ group (see ref 17). Also, ^1H NMR spectral data reported in ref 19 provide additional examples.

matogram observed 1.5 h after the final addition of diethyl L-glutamate hydrochloride revealed one major UV-absorbing spot ($R_f \approx 0.5$; $CHCl_3$–MeOH–concentrated NH_4OH, 7:1:0.04). DMF was then removed in vacuo (<1 mm, 25–30 °C). The residue was dissolved in a minimum volume of $CHCl_3$–MeOH (4:1), and the solution was applied to a column (ca. 325-mL volume) of silica gel (230–400 mesh) poured from $CHCl_3$–MeOH–concentrated NH_4OH (9:1:0.025). Elution by the same solvent combination followed. Fractions of 25–30 mL each were collected, and fractions 6–10 were homogeneous by TLC. The pooled fractions were evaporated, and the yellow solid residue was triturated with Et_2O and collected; yield 67% (961 mg). Spectral data: MS, m/z 524, MH^+. Anal. ($C_{26}H_{33}N_7O_5 \cdot 0.2H_2O$) C, H, N.

***N*-[4-[1-[(2,4-Diamino-6-pteridinyl)methyl]propyl]benzoyl]-L-glutamic Acid (10-EDAM, 12b).** A solution of **11b**·$0.2H_2O$ (930 mg, 1.76 mmol) in MeOH (100 mL) treated with 1 N NaOH (3.9 mL) was kept at 20–25 °C for 40 h. MeOH was removed by evaporation (bath 20–25 °C), and the residue was dissolved in H_2O (50 mL) to which 1 N NaOH (1.9 mL) was added. The solution was kept at 20–25 °C for 6 days, then treated with 1 N HCl to pH 3.8 to precipitate **12b** as a nearly white solid; yield 90% (803 mg). Spectral data: MS, m/z 468, MH^+; UV, λ_{max} 243 (ϵ 29 300), 339 nm (10 300) at pH 1; 255 (ϵ 30 100), 370 nm (7130) at pH 7; 255 (ϵ 32 500), 372 nm (7670) at pH 13; ^1H NMR (Me_2SO-d_6) δ 0.75 (t, CH_3CH_2), 1.70 (m, CH_2CH_3), 1.92 and 2.06 (2 m, $CHCH_2CH_2$, nonequivalent), 2.35 (t, CH_2CO), 3.0–3.4 (br m, $C^9H_2C^{10}H$), 4.36 (q, NHCHCO), 6.56 (br s, NH_2), 7.32 and 7.74 (2 d, C_6H_4), 7.56 (d, NH_2), 8.35 (d, narrowly split, C^7-H), 8.50 (d, CONHCH). The spectral data is supportive of the assigned structure and in agreement with reported results.[22-24] Anal. ($C_{22}H_{25}N_7O_5 \cdot 2H_2O$) C, H, N.

6-(Bromomethyl)-2,4-diamino-5-methylpyrido[2,3-*d*]pyrimidine (7a) Hydrobromide. A sample of **7a**·2HBr[14] (1.00 g) was stirred with refluxing 2-PrOH (200 mL), and the boiling mixture was filtered from insoluble matter (0.07 g). Crystalline **7a**·HBr, solvated by 2-PrOH, separated from the filtrate over a 3-day period; yield 0.49 g. Spectral data: MS, m/z 268 and 270, MH^+ for $C_9H_{10}BrN_5$; ^1H NMR (Me_2SO-d_6) δ 2.78 (s, 5-CH_3), 4.94 (s, CH_2), 8.76 (s, C^7-H); signals due to 2-PrOH δ 1.06 (d, CH_3CH), 3.8 (m, CH_3CH); molar ratio of **7a**:2-PrOH, 1.0:0.9. Anal. ($C_9H_{10}BrN_5 \cdot HBr \cdot 0.9C_3H_7OH$), C, H; N: calcd, 17.37; found, 17.90. A larger sample of **7a**·2HBr (6.40 g) treated with 2-PrOH (2 L) as described above afforded 3.19 g of recrystallized sample used in the preparation of **8a**. Samples of **7a**·HBr thus prepared are stable when protected from light and stored in a refrigerator.

Methyl 4-[2-(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)-1-ethylethenyl]benzoate (8a). Treatment of **7a**·$HBr \cdot 0.9C_3H_7OH$ (3.13 g, 7.76 mmol) with Bu_3P (5.00 g, 24.7 mmol) in Me_2SO (200 mL) with later addition of methyl 4-propionylbenzoate (1.58 g, 8.22 mmol) and NaH (0.66 g of 60% dispersion in oil, 16.5 mmol) was carried out as described for the preparation of **8b**. The course of the conversion was monitored by TLC, and no further change was evident after 44 h; the product appeared as a UV-quenching spot of $R_f \approx 0.6$ ($CHCl_3$–MeOH, 4:1). Isolation as described for **8b** afforded **8a** in 82% yield (2.31 g). The product appeared by TLC to be essentially homogeneous with one UV-absorbing spot, but spectral data which follows showed it to be the expected cis–trans mixture.[29] Spectral data: MS, m/z 364, MH^+ for $C_{20}H_{21}N_5O_2$; ^1H NMR (Me_2SO-d_6) for the cis isomer (in dominance by 2:1) δ 1.05 (t, CH_3CH_2), 2.7 (m, CH_3CH_2) under 2.62 (s, 5-CH_3), 3.81 (s, OCH_3), 6.60 (s, CH=C), 7.22 and 7.82 (2 d, C_6H_4), 7.74 (s, C^7-H); for the trans isomer δ 1.05 (t, CH_3CH_2), 2.7 (m, CH_3CH_2) under 2.65 (s, 5-CH_3), 3.87 (s, OCH_3), 6.82 (s, CH=C), 7.72 and 8.00 (2 d, C_6H_4), 8.38 (s, C^7-H).

4-[1-[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]propyl]benzoic Acid (10a) via the Methyl Ester 9a. Hydrogenation of **8a** to give **9a** and the ester hydrolysis which followed were carried out as described earlier for the preparation of the 10-unsubstituted analogue.[17] In the hydrogenation step, catalyst-poisoning substances were removed when the solution containing **8a** was stirred with 30% Pd/C for 0.5 h and then filtered and treated with 5% Pd/C before hydrogenation. The use of 5% Pd/C allowed greater selectivity in avoiding over-reduction involving the pyrido moiety. Ester **9a** was eluted from a silica gel column using $CHCl_3$–MeOH (4:1).

Following the NaOH-promoted ester hydrolysis in Me_2SO, the acid **10a** was isolated as described above for analogous **10b** except that **10a** did not form a gel when the basic solution was acidified; it precipitated readily at pH 5.0 as a white solid. Spectral data: MS, m/z 352, MH^+; UV λ_{max} 234 (ϵ 39 100), 322 nm (7690) at pH 1; 234 (ϵ 37 600), 335 nm (6020) at pH 7; 236 (ϵ 37 400), 346 nm (6690) at pH 13. Anal. ($C_{19}H_{21}N_5O_2 \cdot 1.9H_2O$) C, H; N: calcd, 18.16; found, 17.63.

***N*-[4-[1-[(2,4-Diamino-5-methylpyrido[2,3-*d*]pyrimidin-6-yl)methyl]propyl]benzoyl]-L-glutamic Acid (5-Me-10-Et-5,10-DDAM; 12a) via the Diethyl Ester 11a.** A solution of **10a**·$1.9H_2O$ (155 mg, 0.402 mmol), *N*-methylmorpholine (125 mg, 1.24 mmol), and $(EtO)_2POCN$ (210 mg of 95%, 1.22 mmol) in Me_2SO (25 mL) was stirred at 20–25 °C for 2 h. More *N*-methylmorpholine (125 mg, 1.24 mmol) was added, followed immediately by diethyl L-glutamate hydrochloride (96 mg, 0.40 mmol). The solution was stirred at 20–25 °C for 62 h before Me_2SO was removed by short-path distillation in vacuo (<1 mm, bath to 35 °C). The residue was stirred with H_2O, and the suspension was treated with 10% $NaHCO_3$ to produce pH 9. The collected and dried crude **11a** (192 mg) was purified by chromatography. Elution by $CHCl_3$–MeOH (4:1) from a column of 70–230-mesh silica gel (10 × 4 cm) to which the crude product had been applied in a dispersion with silica gel gave fractions which were nearly pure in **11a**. The residue (160 mg) from evaporation of the combined fractions was dissolved in MeOH, and the solution was streaked on a 20 × 20 cm preparative TLC plate (Analtech 2-mm silica gel GF) which was developed using $CHCl_3$–MeOH (4:1). The product band, which emitted blue under UV from a 254-nm lamp, was removed and extracted with MeOH. Evaporation of the filtered solution left **11a** (100 mg, 47% yield), homogeneous by TLC, as a pale-yellow glass. Spectral data: MS, m/z 537, MH^+ for $C_{28}H_{36}N_6O_5$.

For ester hydrolysis, the glassy residue (100 mg, 0.186 mmol) was dissolved in MeOH (15 mL), and the solution was treated with 1 N NaOH (0.50 mL). Subsequent treatment and product isolation as described above for the conversion of **11b** to **12b** led to **12a**·$2H_2O$ in 76% yield (73 mg). Spectral data: MS, m/z 481, MH^+; UV λ_{max} 233 (ϵ 41 600), 321 nm (7890) at pH 1; 235 (ϵ 40 600), 335 nm (6100) at pH 7; 237 (ϵ 40 600), 346 nm (6820) at pH 13; ^1H NMR (Me_2SO-d_6) δ 0.75 (t, CH_3CH_2), 1.73 (q, CH_3CH_2), 1.95 and 2.03 (2 m, $CHCH_2CH_2$, nonequivalent), 2.33 (t, CH_2CO), 2.62 (s, CH_3), 2.75 (m, $C^9H_2C^{10}H$), 2.87 and 3.08 (2 m, $C^9H_2C^{10}H$, nonequivalent), 4.34 (q, NHCHCO), 6.84 (br s, NH_2), 7.22 and 7.73 (2 d, C_6H_4), 7.35 (br s, NH_2), 8.05 (d, narrowly split, C^7-H), 8.40 (d, CONHCH). Anal. ($C_{24}H_{28}N_6O_5 \cdot 2H_2O$) C, H, N.

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