Phe-OH, 13734-34-4; BOC-MeTyr($Cl_2$Bzl)-OH, 57817-43-3; H-Gly-Gly-OH, 556-50-3; H-Phe-Leu-Arg(Tos)-MeArg(Tos)-D-Leu-NHEt·TFA, 103613-82-7; BOC-Cys(MBzl)-OH, 61925-77-7; H-Cys(MBzl)-Arg(Tos)-MeArg(Tos)-D-Leu-NHEt·TFA, 122623-94-3; BOC-D-Cys(MBzl)-OH, 61925-78-8; H-Gly-OEt·HCl, 623-33-6; H-D-Cys(MBzl)-Gly-OEt·TFA, 103614-00-2; H-Phe-Cys(MBzl)-Arg(Tos)-MeArg(Tos)-D-Leu-NHEt·TFA, 122623-96-5; H·Tyr-D-Cys-Gly-Phe-Cys-Arg-MeArg-D-Leu-NHEt, 122623-97-6.

# Synthesis and Antifolate Properties of 9-Alkyl-10-deazaminopterins

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Reformatski condensation of benzyl 2-bromopropionate with 4-carbomethoxybenzaldehyde, followed by dehydration afforded benzyl 2-methyl-*p*-carbomethoxycinnamate (**4a**). Hydrogenation over a Pd catalyst gave the hydrocinnamic acid **5a**. Conversion to the chloromethyl (**6a**) and azidomethyl ketone (**7a**) was followed by hydrogenation to the aminomethyl ketone (**8a**). Direct N-alkylation by 2,4-diamino-5-nitro-6-chloropyrimidine followed by reductive ring closure in Zn–HOAc and subsequent saponification of the benzoate ester yielded 4-amino-4-deoxy-9-methyl-10-deazapteroic acid (**11a**). Coupling with diethyl L-glutamate and saponification afforded 9-methyl-10-deazaminopterin (**13a**). The 9-ethyl analogue (**13b**) was similarly prepared from benzyl 2-bromobutyrate. The 9-methyl analogue (**13a**) was 21 times more potent than MTX as an inhibitor of cell growth in L1210 cells. The reason for this enhanced cytotoxicity in L1210 is unclear, since enzyme inhibition and transport parameters were similar to those of MTX. In human Manca leukemia cells growth inhibition was not dramatic and paralleled MTX.

Past communications[1,2] from this laboratory have documented the synthesis and selective antitumor activity of 10-alkyl-10-deazaminopterin compounds. These deazapteridine analogues of methotrexate (MTX) were found to have enhanced transport into tumor tissues and were sparing of sensitive host epithelial and bone marrow cells. Such modification gave analogues with much improved therapeutic indices compared with MTX, and one compound, 10-ethyl-10-deazaminopterin, has undergone extensive clinical trials with favorable results.[3,4] It was also of interest to determine whether the 10-position variants were uniquely responsible for the selective transport into tumor cells. It was noted in early studies with antifolate compounds that 9-methylaminopterin[5,6] showed activity approximating that of MTX in bacterial and mouse tumor model assays. In this paper we report studies with 9-alkyl-10-deazaminopterin analogues.

## Chemistry

The synthesis of 9-methyl-10-deazaminopterin (**13a**) began with 2-bromopropionic acid (**1a**) (Scheme I). This acid was converted to its benzyl ester (**2a**) by treatment with benzyl alcohol catalyzed by *p*-toluenesulfonic acid in benzene.[7] Attempts to convert **2a** to a dimethyl α-phosphono ester gave low yields of product with scrambled alkoxy groups. We thus abandoned plans for an Emmons–Horner condensation with *p*-carbomethoxybenzaldehyde and resorted to Reformatski condensation of **2a** with the aldehyde as promoted by Zn dust in benzene. Instead of directly yielding the expected cinnamate ester (**4a**), the reaction afforded the tertiary alcohol ester **3a**. This carbinol was resistant to direct dehydration, but was converted to **4a** by treatment with $POCl_3$ followed by dehydrohalogenation of the chloro intermediate with diazabicyclononene in 61% yield from **3a**. The benzyl 2-methyl-*p*-carbomethoxy intermediate **4a** was hydrogenated over Pd black at ordinary pressure to saturate the olefinic bond with concomitant hydrogenolysis of the benzyl ester to give the 2-methyl-*p*-carbomethoxyhydrocinnamic acid **5a** in 87% yield.

Table I. Cell Growth, Enzyme Inhibition, and Transport Properties for L1210[a]

| compd | L1210 growth inhibn $IC_{50}$, nM | L1210 DHFR inhibn $K_i$, nM | influx[b] $K_i$, μM |
|---|---|---|---|
| **13a** | 0.24 | 0.0052 | 3.0 |
| **13b** | 4.7 | 0.0055 | 8.2 |
| 10-DA | 1.5 | 0.0034 | 1.2 |
| 10-Et-10-DA[c] | 0.65 | 0.0028 | 0.99 |
| MTX | 5.0 | 0.0056 | 3.9 |

[a] See ref 1 for methods. [b] Expressed in terms of competition with [$^3$H]MTX binding, thus a measure of binding to the cellular enzyme responsible for active transport of folates (see ref 11). [c] Data from ref 11.

Acid **5a** was converted to its acid chloride ($SOCl_2$) followed by treatment with $CH_2N_2$ and HCl gas to afford the chloromethyl ketone **6a** in 79% yield. Treatment of **6a** with $NaN_3$ in 80% MeOH gave the azido ketone **7a** (94% yield), which was subsequently hydrogenated (Pd) to the amino ketone **8a**. Past experience[8] has required that the ketone function be blocked as the semicarbazone or ketal before alkylation of 2,4-diamino-5-nitro-6-chloropyrimidine in the Boon–Leigh type[9] of pteridine ring construction.

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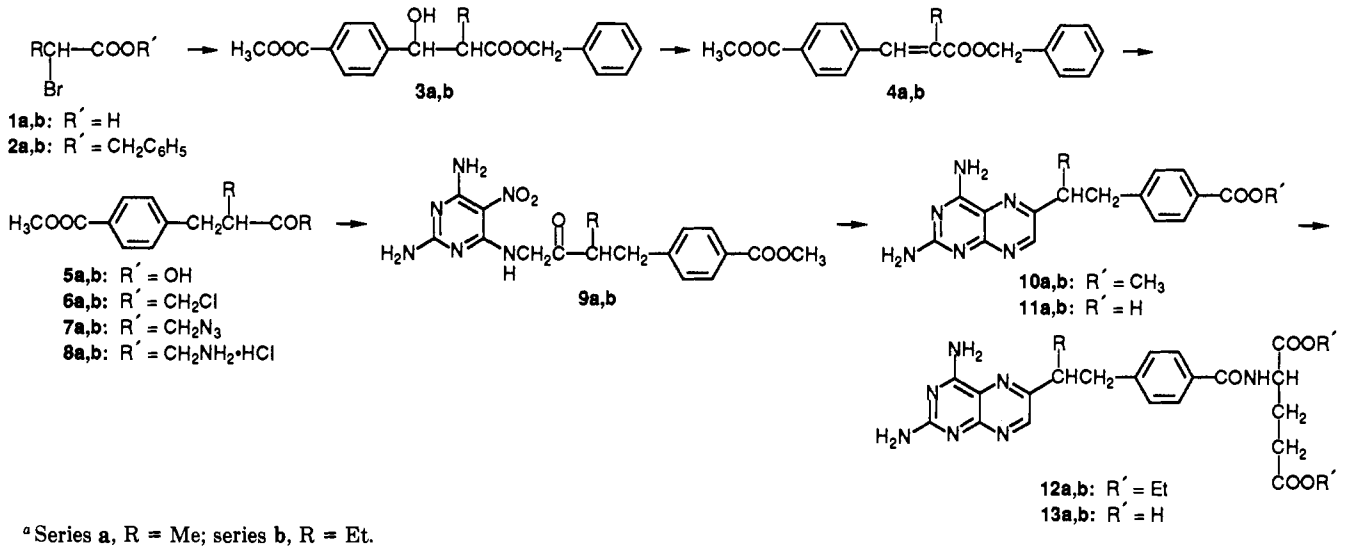
**Scheme I**[a]




[a] Series **a**, R = Me; series **b**, R = Et.

However, attempts to convert **8a** to its semicarbazone gave only unchanged amino ketone even under forcing conditions. The apparent steric hindrance suggested that blocking of the carbonyl was unnecessary. The amino ketone **8a** was then directly reacted with the nitro chloro pyrimidine to afford the 5-nitro-6-pyrimidinylamino ketone **9a** in 83% yield. Reductive ring closure with Zn–HOAc proceeded smoothly to a 7,8-dihydropterin which was treated in situ with dilute $H_2O_2$ to give the pterin, methyl 4-amino-4-deoxy-9-methyl-10-deazapteroate (**10a**) in 39% yield. Following saponification of the benzoate ester with NaOH in 2-methoxyethanol, the 4-amino-4-deoxypteroic acid **11a** was coupled with diethyl L-glutamate via carboxyl activation by isobutyl chloroformate. The diester **12a** was purified by chromatography on silica gel and saponified with 1 N NaOH in 2-methoxyethanol at room temperature to yield 9-methyl-10-deazaminopterin (**13a**). The 10-ethyl analogue **13b** was prepared in a similar manner beginning with 2-bromobutyric acid (**1b**).

## Biological Evaluation

The two 9-alkyl analogues **13a** and **13b** were initially evaluated for their ability to inhibit growth in L1210 cells in culture, their transport influx properties, and their ability to inhibit DHFR derived from L1210 cells. In Table I data are presented showing these observations in direct comparison with MTX and 10-deazaminopterin. Historical data for 10-ethyl-10-deazaminopterin is also included. It was rather surprising that the 9-methyl analogue **13a** was about 21 times more cytotoxic than MTX and about 6 times greater than 10-deazaminopterin. The 9-ethyl compound **13b** was approximately equivalent to MTX. The $K_i$ for inhibition of DHFR derived from L1210 was similar to that of MTX for both compounds. There was evidence for modest enhancement of transport into tumor for **13a** compared with MTX, but **13b** showed a significant increase in transport influx $K_i$. 10-Deazaminopterin showed a considerably enhanced transport profile as previously observed.[1] 10-Ethyl-10-deazaminopterin is more favorably transported into the L1210 cells than all of the compounds shown in Table I, but was not as potent as **13a** for growth inhibition.

Since the potent growth inhibition displayed by **13a** against L1210 was not accounted for by transport enhancement or increased potency for inhibition of DHFR from the L1210 cells, we examined the effect on other enzymes in the folate pathway such as thymidylate synthase, GAR transformylase, and AICAR transformylase as shown in Table II. In addition we also measured the inhibition of DHFR from a human source. Both **13a** and **13b** were found to be ineffective as inhibitors of TS, GAR T-fase, and AICAR T'fase. Although the data were obtained from bacterial enzymes, the conclusions are unlikely to differ with mammalian enzymes. Activity against the DHFR from human Manca leukemia cells showed that the 9-Me compound was similar to MTX, but somewhat more potent than the 9-Et analogue. A growth inhibition assay was also carried out in Manca cells, but **13a** did not show unusual activity in this system. In fact, it was a little less active than MTX, but considerably more effective than the 9-Et analogue.

Table II. Enzyme Inhibition Data ($IC_{50}$, $\mu$M)

| compd | TS[a] *L. casei* | GAR T'fase[b] *L. casei* | AICAR T'fase[c] *L. casei* | DHFR[a] Manca[d,e] |
|---|---|---|---|---|
| **13a** | >100 | >50 | >50 | 0.04 |
| **13b** | >100 | >50 | >50 | 0.07 |
| MTX | – | – | – | 0.03 |

[a] See ref 1 for methods. [b] Assayed by the method of Smith et al.: Smith, G. K.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 4034. [c] Assayed as described by Baggott and Krumdieck: Baggott, J. E.; Krumdieck, C. L. *Biochemistry* **1979**, *18*, 1036. [d] Enzyme supplied by Dr. J. H. Freischeim (Pendergast, N.; Delcamp, T.; Smith, P.; Freischeim, J. H. *Biochemistry* **1988**, *27*, 3664). [e] Growth inhibition in Manca cells ($IC_{50}$, nM): **13a**, 15.0; **13b**, 35.0; MTX, 10.0.

It should be noted that the method used for the synthesis of **13a,b** would produce a diastereomeric mixture that is completely racemic about C-9. This could of course influence biological activity although we observed only small differences between pure diastereomers for related C-10 substituted analogues.[2] We expect to prepare and evaluate pure diastereomers in the C-9 series also.

Further work to elucidate the nature of the potent cytotoxicity observed for **13a** against L1210 in vitro and evaluation of this analogue in murine tumors in vivo is under way.

## Experimental Section

Elemental analyses were obtained from Galbraith Laboratories, Knoxville, TN. The $^1H$ NMR spectra were taken on a Varian 360A or a JEOL FX90Q spectrometer. Mass spectra were run on a LKB 9000 GC–MS spectrometer or a Ribermag R10-10C MS system. Ultraviolet spectra were taken on a Perkin-Elmer 552

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or Perkin-Elmer-Coleman 575. Melting points were determined on a Thomas-Hoover Uni-melt apparatus. HPLC was run on a Waters Novapak $C_{18}$ column with 25% MeOH/75% 0.1 M $NaH_2PO_4$, pH 5.5, as solvent.

**Benzyl 2-Bromopropionate (2a).** Benzyl 2-bromopropionate was prepared in 85% yield by reaction[7] of 2-bromopropionic acid with benzyl alcohol as catalyzed by *p*-toluenesulfonic acid in benzene with $H_2O$ separation: bp 104–107 °C (0.8 mm); NMR ($CDCl_3$) $\delta$ 1.8 (3 H, d, $CH_3$), 4.4 (1 H, q, CH), 5.18 (2 H, s, $CH_2$), 7.4 (5 H, s, Ar).

**Benzyl 2-Bromobutyrate (2b).** A solution of 26.5 g (0.25 mol) of 2-bromobutyric acid in 125 mL of $SOCl_2$ was heated at reflux for 3 h. Excess $SOCl_2$ was removed in vacuo followed by the addition and evaporation of 50 mL of toluene. The residual liquid was dissolved in 50 mL of toluene, chilled to 0–5 °C, and a mixture of 25.8 mL (0.25 mol) of benzyl alcohol and 32.9 mL (0.25 mol) of *s*-collidine was added dropwise over 30 min. The mixture was kept at ambient temperature for 18 h and partitioned between 250 mL of $H_2O$ and 150 mL of $Et_2O$. The ether extract was washed successively with 100 mL of 2 N HCl, 100 mL of $H_2O$, and 75 mL of saturated $NaHCO_3$ followed by drying over $MgSO_4$. Evaporation of solvent in vacuo gave 59.0 g of a yellow oil. Distillation through a short Vigreux head at 0.2 mm afforded 42.0 g (65%), bp 110–115 °C. The infrared spectrum was very similar to that of the propionate ester **2a**.

**Benzyl 2-Methyl-3-hydroxy-*p*-carbomethoxyhydrocinnamate (3a).** A mixture of 12.7 g (0.077 mol) of methyl 4-formylbenzoate, 8.2 g (0.125 g-atom) of Zn dust, and 100 mL of benzene was heated to boiling, and 12 mL of benzene was removed by distillation. The mixture was cooled to room temperature and a solution of 20.0 g (0.082 mol) of the bromo ester **2a** in 37 mL of dry benzene was added slowly followed by a catalytic amount of $I_2$. The mixture was stirred at reflux for 18 h. After cooling the mixture was treated with 150 mL of $H_2O$ and 37 mL of 6 N HCl (dropwise addition). The mixture was stirred for 30 min and separated. The aqueous portion was extracted with 200 mL of $Et_2O$, and the combined organic extracts were washed with 100 mL of $H_2O$, dried over $MgSO_4$, and evaporated to leave 25.6 g (100%) of a pale yellow oil: IR (film) $\lambda$ 2.86 $\mu$ (OH), 5.82 (esters), 7.80 (benzoate C–O); NMR ($CDCl_3$) $\delta$ 1.1 (3 H, q, $CH_3$), 2.85 (2 H, m, OH + C*H*$CH_3$), 3.9 (3 H, s, $COOCH_3$), 4.83 (1 H, m, ArC*H*), 5.13 (2 H, d, benzyl $CH_2$), 7.35 (7 H, d, 3′,5′-ArH + benzyl ArH), 7.95 (2 H, d, 2′,6′-ArH).

The ethyl homologue **3b** was similarly obtained in 100% yield. The IR spectrum was in agreement for the product with key bands similar to those of **3a**.

**Benzyl 2-Methyl-*p*-carbomethoxycinnamate (4a).** To a solution of 25.6 g (0.077 mol) of the hydroxy ester **3a** in 79 mL of pyridine was added dropwise 8.45 mL (0.091 mol) of $POCl_3$. The resulting solution was stirred for 3 h at room temperature and then heated at 100 °C for 30 min. The solvent was removed in vacuo and the residue treated with 300 mL of 1 N HCl. The mixture was thrice extracted with 125-mL portions of $Et_2O$ and the ether extract washed with 100 mL of $H_2O$, dried over $MgSO_4$, and evaporated to leave 18.8 g (70%) of the chloro intermediate; IR (film) *no* OH; NMR ($CDCl_3$) shift of 3-proton to 5.1 (C*H*Cl).

The chloro intermediate was dissolved in 66 mL of pyridine containing 13.6 mL (0.11 mol) of diazabicyclononene and the mixture heated at 100 °C for 1.25 h. The solvent was removed in vacuo, the residue treated with 350 mL of 1 N HCl, and the mixture thrice extracted with 150-mL portions of $Et_2O$. The extract was washed with 100 mL of $H_2O$, dried over $MgSO_4$, and evaporated to leave 16.2 g of an oil. This material was chromatographed on 450 g of silica gel with elution by $CH_2Cl_2$ to afford 14.5 g (87% or 61% from **3a**) of clear oil: UV (EtOH) $\lambda_{max}$ 280 nm; NMR ($Me_2SO$-$d_6$) $\delta$ 2.1 (3 H, s, $CH_3$), 3.87 (3 H, s, $COOCH_3$), 5.25 (2 H, s, $CH_2$), 7.4 (5 H, s, benzyl Ar), 7.6 (3 H, m, olefin, 3′,5′-ArH), 7.98 (2 H, d, 2′,6′-ArH).

The ethyl homologue **4b** was synthesized in a similar manner from **3b** in a 54% overall yield: UV (EtOH) $\lambda_{max}$ 276 nm.

**2-Methyl-*p*-carbomethoxyhydrocinnamic Acid (5a).** To 0.7 g of 10% Pd/C was added a solution of 6.09 g (19.6 mmol) of **4a** in 110 mL of 2-propanol. The mixture was stirred under an atmosphere of hydrogen at room temperature for 7 h, consuming the theoretical amount of $H_2$. The catalyst was removed by filtration through Celite and the filtrate evaporated in vacuo to dryness. The residual oil was suspended in 100 mL of $H_2O$ and treated dropwise with concentrated $NH_4OH$ with stirring until complete solution was obtained (pH 10–11). The aqueous solution was washed with 50 mL of pentane and adjusted to pH 1–2 with 6 N HCl. The oily precipitate was extracted into $CHCl_3$ (2 × 25 mL) and the solution dried ($MgSO_4$) and evaporated to leave 3.7 g (87%) of a white solid. An analytical sample, mp 65–66 °C, was obtained by recrystallization from pentane/$Et_2O$ (2:1): NMR ($CDCl_3$) $\delta$ 1.2 (3 H, d, $CH_3$), 2.8 (1 H, m, C*H*COOH), 3.05 (2 H, m, $CH_2Ar$), 3.93 (3 H, s, $COOCH_3$), 7.27 (2 H, d, 3′,5′-ArH), 7.97 (2 H, d, 2′,6′-ArH). Anal. Calcd for $C_{12}H_{14}O_4$: C, H.

The ethyl analogue **5b** was similarly obtained in 75% yield: mp 66–67 °C; NMR ($CDCl_3$) $\delta$ 0.95 (3 H, t, $CH_3$), 1.60 (2 H, m, $CH_2CH_3$), 2.65 (1 H, m, C*H*COOH), 2.9 (2 H, m, $CH_2Ar$), 3.90 (3 H, s, $COOCH_3$), 7.35 (2 H, d, 3′,5′-ArH), 8.10 (2 H, d, 2′,6′-ArH). Anal. Calcd for $C_{13}H_{16}O_4$: C, H.

**1-Chloro-3-methyl-4-(*p*-carbomethoxyphenyl)-2-butanone (6a).** A solution of 3.57 g (16 mmol) of acid **5a** in 42 mL of benzene containing 2.1 mL (29 mmol) of $SOCl_2$ was heated at reflux for 2.5 h and evaporated in vacuo. Benzene (10 mL) was added and subsequently evaporated to leave the acid chloride as a syrup. The residue was taken up in 20 mL of $Et_2O$ and added dropwise to a solution of $CH_2N_2$ (from 10.07 g, 98 mmol of *N*-nitrosomethylurea) in 95 mL of $Et_2O$ at 0–5 °C. The solution was kept at 0–5 °C for 1 h and then treated with dry HCl gas and allowed to stand for 15 h. The ether solution was washed with 100 mL of $H_2O$ and 25 mL of cold 0.5 N $Na_2CO_3$, dried over $MgSO_4$, and evaporated to leave 3.20 g (79%) of a pale yellow oil. Chromatography on silica gel with elution by $CH_2Cl_2$ followed by crystallization from $Et_2O$ yielded an analytical sample: mp 53–54 °C; NMR ($CDCl_3$) $\delta$ 1.2 (3 H, d, $CH_3$), 2.72 (1 H, m, C*H*$CH_3$), 3.05 (2 H, m, $CH_2Ar$), 3.92 (3 H, s, $COOCH_3$), 3.97 (2 H, d, $CH_2Cl$), 7.22 (2 H, d, 3′,5′-ArH), 7.97 (2 H, d, 2′,6′-Ar). Anal. Calcd for $C_{13}H_{15}ClO_3$: C, H.

The ethyl analogue **6b** was similarly prepared in 91% yield as a yellow oil used directly in the next step.

**1-Azido-3-methyl-4-(*p*-carbomethoxyphenyl)-2-butanone (7a).** To a solution of 2.80 g (11 mmol) of the chloromethyl ketone **6a** in 80 mL of MeOH was added a solution of 5.48 g (84 mmol) of $NaN_3$ in 20 mL of $H_2O$. The resulting solution was kept at ambient temperature for 18 h and MeOH was evaporated in vacuo. The aqueous residue was diluted to 50 mL with $H_2O$ and extracted with 40 mL of $CHCl_3$. The aqueous portion was extracted with another 10 mL of $CHCl_3$ and the $CHCl_3$ extract dried over $MgSO_4$ and evaporated to leave 2.71 g (94%) of a yellow syrup: IR (film) $\lambda$ 4.75 $\mu$ ($N_3$), 5.85 (ketone, ester C=O); NMR ($CDCl_3$) $\delta$ 1.2 (3 H, d, $CH_3$), 2.6–3.2 (3 H, m, $CH_2CH$), 3.8 (2 H, d, $CH_2N_3$), 3.95 (3 H, s, $COOCH_3$), 7.25 (2 H, d, 3′,5′-ArH), 8.0 (2 H, d, 2′,6′-ArH).

The ethyl compound **7b** was similarly obtained as an oil from **6b** in 80% yield; IR (film) $\lambda$ 4.75 $\mu$ ($N_3$).

**1-Amino-3-methyl-4-(*p*-carbomethoxyphenyl)-2-butanone Hydrochloride (8a).** A mixture of 2.32 g (8.9 mmol) of azido ketone **7a**, 400 mg of Pd black, 1.51 mL (18 mmol) of concentrated HCl, and 40 mL of EtOH was stirred under an atmosphere of $H_2$ for 6 h. The catalyst was removed by filtration and the filtrate evaporated in vacuo to leave 0.90 g of solid. The Celite pad was extracted by stirring for 20 min with 20 mL of warm 2-methoxyethanol, followed by filtration and evaporation to afford another 1.53 g of product. The crude solids were washed with $Et_2O$ and acetone and dried to leave 2.0 g (83%) of a white solid, mp 191–193 °C. Recrystallization from EtOH gave an analytical sample: mp 193–195 °C; IR (Nujol) $\lambda$ 5.85 $\mu$ (ketone, ester C=O), no $N_3$ present. Anal. Calcd for $C_{13}H_{17}NO_3$·HCl: C, H, N.

Hydrogenation of azide **7b** afforded the ethyl homologue in 44% yield, characterized as the picrate salt, mp 186–189 °C (from MeOH). Anal. Calcd for $C_{20}H_{22}N_4O_{10}$: C, H, N.

**1-[(2′,4′-Diamino-5′-nitro-6′-pyrimidinyl)amino]-3-methyl-4-(*p*-carbomethoxyphenyl)-2-butanone (9a).** To 12 mL of MeOH containing NaOMe (from 85 mg, 3.68 mg-atom of Na) was added 1.00 g (3.68 mmol) of the amino ketone hydrochloride **8a**. The mixture was stirred for 5 min and treated successively with 34 mL of DMF, 695 mg (3.68 mmol) of 2,4-diamino-5-nitro-6-chloropyrimidine,[10] and 0.49 mL (3.68 mmol)

(10) Brien, D. E.; Cheng, C. C. *J. Med. Chem.* **1966**, *9*, 573.

of *s*-collidine. The mixture was stirred at 90 °C for 30 min and evaporated in vacuo. The residue was stirred with 90 mL of ice water and the yellow solid was collected, washed with $H_2O$, and dried to leave 1.19 g (83%). An analytical sample, mp 189–191 °C, was obtained by extraction into hot MeOH, followed by evaporation and recrystallization from EtOH: UV λ (EtOH) max 235 nm, 340. Anal. Calcd for $C_{17}H_{20}N_6O_5 \cdot 0.25H_2O$: C, H, N.

The ethyl homologue **9b** was obtained in a 67% yield in a similar manner from **8b** as its hydrochloride salt. Crystallization from 2-propanol gave an analytical sample, mp 171–173 °C. Anal. Calcd for $C_{18}H_{22}N_6O_5$: C, H, N.

**Methyl 4-Amino-4-deoxy-9-methyl-10-deazapteroate (10a).** A mixture of 1.00 g (2.6 mmol) of the nitro ketone **9a**, 2.0 g (30 mg-atom) of Zn dust, and 50 mL of HOAc was stirred at 100 °C for 4 h. The mixture was cooled and filtered and the filtrate treated with 20 mL of $H_2O$ followed by 1.5 mL of 30% $H_2O_2$. After 15 h, the solvent was removed in vacuo followed by addition of 30 mL of $H_2O$ and adjustment of the pH to 8 with 1.5 N $NH_4OH$. After 1 h the yellow solid was collected, washed with $H_2O$, and dried to leave 0.87 g. The crude product was taken up in $CHCl_3$ and chromatographed on 30 g of silica gel with elution by $CHCl_3$/MeOH (97.5:2.5) to afford 0.34 g (39%) of a yellow solid: UV (pH 13) max 242 nm, 370; MS *m/e* 338; NMR ($CDCl_3$/$CD_3OD$) δ 1.3 (3 H, d, $CH_3$), 3.07 (1 H, m, C-9H), 3.27 (2 H, m, 10-$CH_2$), 3.8 (3 H, s, $COOCH_3$), 7.05 (2 H, d, 3′,5′-ArH), 7.77 (2 H, d, 2′,6′-ArH), 8.33 (1 H, s, C-7H). Anal. Calcd for $C_{17}H_{18}N_6O_2 \cdot 0.4H_2O$: C, H, N.

The 9-ethyl compound **10b** was similarly prepared from **9b** in 58% yield: UV (pH 13) max 245 nm, 370; MS *m/e* 352; NMR ($CDCl_3$) δ 0.87 (3 H, t, $CH_3$), 1.80 (2 H, m, $CH_2CH_3$), 2.1 (4 H, m, C-9H, HOAc solvate), 3.10 (2 H, br s, 10-$CH_2$), 3.90 (3 H, s, $COOCH_3$), 7.10 (2 H, d, 3′,5′-ArH), 7.90 (2 H, d, 2′,6′-ArH), 8.43 (1 H, s, C-7H). Anal. Calcd for $C_{18}H_{20}N_6O_2 \cdot CH_3COOH$: C, H, N.

**4-Amino-4-deoxy-9-methyl-10-deazapteroic Acid (11a).** A suspension of 330 mg (0.98 mmol) of ester **10a** in 4 mL of 2-methoxyethanol was treated with 0.7 mL (1.75 mmol) of 10% NaOH. The mixture was stirred at room temperature for 44 h, giving a complete solution after 2 h. The solvent was removed in vacuo and the residue dissolved in 10 mL of $H_2O$ and acidified to pH 5 with HOAc. The pale yellow precipitate was collected by filtration, washed with $H_2O$, and dried to leave 296 mg (94%): UV (pH 13) max 255 nm, 370; MS *m/e* 324; HPLC 98% pure; NMR ($Me_2SO$-$d_6$) δ 1.3 (3 H, d, $CH_3$), 2.8–3.5 (3 H, m, 9-CH, 10-$CH_2$), 7.23 (2 H, d, 3′,5′-ArH), 7.8 (2 H, d, 2′,6′-ArH), 8.48 (1 H, s, C-7H). Anal. Calcd for $C_{16}H_{16}N_6O_2 \cdot 1.5H_2O$, C, H (0.6), N.

The 9-ethyl analogue **11b** was prepared in 54% yield from **10b**; MS *m/e* 338, major fragment 203 (2,4-diamino-6-propylpteridine ion). A satisfactory elemental analysis could not be obtained, and the material was used directly in the next step.

**9-Methyl-10-deazaminopterin Diethyl Ester (12a).** To a solution of 225 mg (0.69 mmol) of the aminopteroic acid **11a** in 5 mL of dry $Me_2SO$ was added 0.19 mL (1.38 mmol) of $Et_3N$ and 0.18 mL (1.38 mmol) of isobutyl chloroformate. The mixture was stirred for 1 h, and then 0.19 mL (1.38 mmol) of $Et_3N$ and 331 mg (1.38 mmol) of diethyl L-glutamate hydrochloride were added. The mixture was stirred under argon for 4 h and the sequence repeated as above at one-half the respective quantities of reagents. After being stirred for 18 h at room temperature, the reaction mixture was treated with 40 mL of ice $H_2O$ and thrice extracted with 20-mL portions of $CHCl_3$. The $CHCl_3$ extract was washed with 20 mL of saturated $NaHCO_3$, dried over $MgSO_4$, and evaporated in vacuo. The residual gum was chromatographed on 40 g of silica gel with elution by $CHCl_3$/MeOH (97.5:2.5) to afford 267 mg (76%) of a yellow semisolid. Crystallization from EtOAc gave an analytical sample: mp 173–174 °C; MS *m/e* 509; NMR ($CDCl_3$) δ 1.25 (9 H, m, $CH_3$), 2.4 (4 H, m, $CH_2CH_2$), 3.02 (3 H, m, 9,10-$CHCH_2$), 4.15 (4 H, q, $OCH_2$), 4.8 (1 H, d, NHC*H*), 7.1 (2 H, d, 3′,5′-ArH), 7.7 (2 H, d, 2′,6′-ArH), 8.47 (1 H, s, 7-H). Anal. Calcd for $C_{25}H_{31}N_7O_5 \cdot 0.5H_2O$: C, H, N.

The 9-ethyl-10-deazaminopterin diethyl ester **12b** was similarly obtained from **11b** in 51% yield as a yellow gum: MS *m/e* 523; NMR ($CDCl_3$) δ 0.86 (3 H, t, 9-$CH_2CH_3$), 1.3 (6 H, m, $COOCH_2CH_3$), 1.88 (2 H, m, $CH_2CH_3$), 2.4 (m, $CH_2CH_2$), 3.12 (3 H, m, 9,10-$CHCH_2$), 4.3 (4 H, q, $OCH_2$), 4.8 (1 H, m, C*H*NH), 7.1 (2 H, d, 3′,5′-ArH), 7.7 (2 H, d, 2′,6′-ArH), 8.43 (1 H, s, 7-H).

**9-Methyl-10-deazaminopterin (13a).** A solution of 223 mg (0.44 mmol) of the diester **12a** in 3 mL of 2-methoxyethanol was treated with 3 mL (3 mmol) of 1 N NaOH and the resulting solution kept at room temperature for 4 h. Following dilution with 3 mL of $H_2O$, the pH was adjusted to 4–5 with HOAc. The mixture was evaporated to dryness in vacuo (0.5 mm) without application of heat. The residue was treated with 6 mL of $H_2O$ and the product collected to afford 144 mg (73%): UV (pH 13) max 225 nm (ε 30 391), 370 (6854); MS *m/e* 453; HPLC indicated 99% purity; NMR ($Me_2SO$-$d_6$) δ 1.33 (3 H, d, $CH_3$), 2.0 (2 H, m, $CH_2$-glutamate), 2.27 (2 H, m, $CH_2COOH$), 3.2 (3 H, m, 9,10-$CHCH_2$), 4.35 (1 H, m, C*H*NH), 7.23 (2 H, d, 3′,5′-ArH), 7.75 (2 H, d, 2′6′-ArH), 8.5 (1 H, s, 7-H). Anal. Calcd for $C_{21}H_{23}N_7O_5 \cdot 1.75H_2O$: C, H, N.

9-Ethyl-10-deazaminopterin (**13b**) was similarly prepared in 42% yield by saponification of **12b**: UV (pH 13) max 225 nm (ε 28 550), 370 (6655); MS *m/e* 467; NMR ($Me_2SO$-$d_6$) δ 0.77 (3 H, t, $CH_3$), 1.75 (2 H, m, $CH_2CH_3$), 1.95 (2 H, m, $CH_2$-glutamate), 2.28 (2 H, m, $CH_2COOH$), 3.12 (3 H, m, 9,10-$CHCH_2$), 4.32 (1 H, m, C*H*NH), 7.17 (2 H, d, 3′,5′-ArH), 7.65 (2 H, d, 2′,6′-ArH), 8.4 (1 H, s, 7-H). Anal. Calcd for $C_{22}H_{25}N_7O_5 \cdot 0.75H_2O$: C, H, N.

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**Registry No.** **1a**, 598-72-1; **1b**, 80-58-0; **2a**, 3017-53-6; **2b**, 42115-51-5; **3a**, 122594-12-1; **3b**, 122594-13-2; **4a**, 122594-15-4; **4b**, 122594-16-5; **5a**, 122594-17-6; **5a** (acid chloride), 122594-19-8; **5b**, 122594-18-7; **6a**, 122594-20-1; **6b**, 122594-21-2; **7a**, 122594-22-3; **7b**, 122594-23-4; **8a**·HCl, 122594-24-5; **8b** (picrate salt), 122594-26-7; **9a**, 122594-27-8; **9b**, 122594-28-9; **10a**, 122594-29-0; **10b**, 122594-30-3; **11a**, 122594-31-4; **11b**, 122594-32-5; **12a**, 122594-33-6; **12b**, 122594-34-7; **13a**, 122594-35-8; **13b**, 122622-74-6; 4-(OHC)$C_6H_4$COOMe, 1571-08-0; 4-(MeOCO)$C_6H_4$CH(OH)C(Me)(Cl)$COOCH_2Ph$, 122594-14-3; H-Glu(OEt)-OEt·HCl, 1118-89-4; 2,4-diamino-5-nitro-6-chloropyrimidine, 6036-64-2.

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