another 1.5 g of solid, mp 217–220°. The total yield of product was 53%. It crystallized from *i*-PrOAc, mp 220–225° dec.

5-(4-Chloro-3-sulfamoylphenyl)-3-methyl-4*H*-1,2,4-thiadiazine 1,1-Dioxide (8). NH_3 was bubbled through a solution of the chlorosulfonyl compound 7 (7.1 g, 0.02 mol) in acetone (100 ml) for 10 min. After evaporation of the solvent, the residue was dissolved in hot 5% aqueous NaOH solution, filtered, and acidified with 3 *N* HCl to precipitate a cream-colored solid. It was collected by filtration, washed with H_2O, and triturated with Me_2CO to afford 5.8 g (86.5%) of the title compound 8, mp 303–310° dec. The crude product was dissolved in hot MeOH (50 ml) and excess Et_2NH. Addition of *i*-Pr_2O and cooling gave 3.6 g of the diethylammonium salt of 8, mp 300–305° dec. This salt was dissolved in hot 5% aqueous NaOH, filtered, acidified with 3 *N* HCl, and cooled to afford analytically pure 8.

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Synthesis and Antifolate Activity of 10-Deazaminopterin

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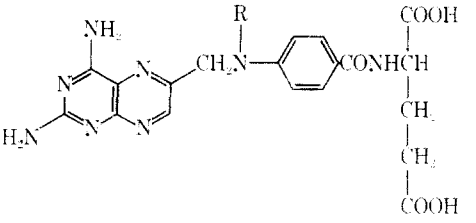
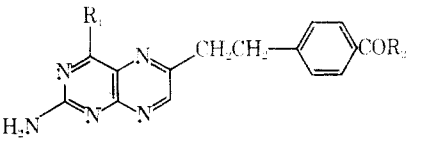
The antimicrobial and antitumor activities of the powerful dihydrofolic reductase inhibitors aminopterin (I) and its N^{10}-methyl derivative, methotrexate (MTX, II), are well known. Numerous analogs have been made to further improve the potency, cell penetration, and toxicity properties of these compounds. As part of a continuing program to investigate structure–activity relationships in folic acid analogs, we were interested in the effects of replacement of the nitrogen atom in the side chain of aminopterin. The synthesis and biological activity of 10-deazaminopterin are reported in this paper.



I. R = H
II. R = CH_3



III. $R_1 = R_2 = OH$
IV. $R_1 = OH$; R_2 = glutamyl
V. $R_1 = NH_2$; $R_2 = OH$
VI. $R_1 = NH_2$; R_2 = glutamyl

In a previous communication[1] we reported the synthesis of 10-deazapteroic acid (III) and noted the potent growth inhibitory action of III and its tetrahydro derivative against *Streptococcus faecium*, a folate dependent bacteria. Struck, *et al.*,[2] confirmed the activity of III and also reported the activity of the glutamate conjugate, 10-deazafolic acid (IV). Encouraged by the activity of the 10-deaza compounds we subsequently prepared[3] 2,4-diamino-6-*p*-carboxyphenethylpteridine (V). The compound possessed one-half the potency of aminopterin and $\frac{1}{14}$ that of amethopterin against *S. faecium*. A sixfold loss of activity was observed after reduction to the tetrahydro form. Only moderate activity was noted against *Lactobacillus casei*.

Coupling of V with glutamic acid to form 10-deazaminopterin (VI) has resulted in an analog with powerful antifolate activity. In Table I it can be seen that VI and its dihydro (VI-H_2) and tetrahydro (VI-H_4) derivatives are strong inhibitors of bacterial growth in *S. faecium* and *L. casei*. In *S. faecium* the minimum inhibitory concentrations (MIC) are similar to those for MTX and aminopterin and their corresponding reduced forms. However, VI-H_2 was the most potent antifolate we have seen for the inhibition of *L. casei*. Half maximal inhibition occurred at 5×10^{-12} *M* at a ratio of antimetabolite to folate of 1:500. It is also of interest that this compound has some activity against MTX resistant strains of *L. casei* and *S. faecium*. The tetrahydro-VI was also very potent and was about nine times more active than the respective tetrahydro MTX and tetrahydroaminopterin against *L. casei*. Strong activity against *Pediococcus cerevisiae* was also observed for VI-H_4.

The inhibition of *L. casei* dihydrofolate reductase by 10-deazaminopterin and its reduced forms is appreciable (Table II) but is not correlated with growth inhibition because the latter is 100, 1000, and 5000 times greater than reductase inhibition with the unreduced, dihydro, and tetrahydro compounds, respectively. Inhibition of thymidylate synthetase by VI and derivatives is not outstanding but is detectable with the reduced forms.

Standard procedures for the coupling of V, *via* its bis(trifluoroacetyl) derivative, and diethyl glutamate were not undertaken due to the possibility of deamination of the 4 position during hydrolysis of blocking groups. Instead, the carboxyl group of unblocked V was activated

Table I. Bacterial Growth Inhibition of 10-Deazaminopterin and Its Reduced Forms

	MIC (ng/ml) for 50% inhibition[a,g]				
	S. faecium[b] (ATCC 8043)	*S. faecium*[b] MTX resistant	*L. casei*[b] (ATCC 7649)	*L. casei* MTX resistant	*P. cerevisiae*[c]
10-Deazaminopterin (VI)	0.2	>2000	0.02	>2,000	86
VI-H_2[e]	0.01	200	0.002[d]	60	2.5
VI-H_4[f]	0.03	>2000	0.007	250	0.18
Methotrexate (MTX)	0.15	>6000	0.01	38,000	60
7,8-Dihydro-MTX[e]	0.01		0.009		
5,6,7,8-Tetrahydro-MTX[f]	0.05		0.06		
Aminopterin	1.0		0.03		210
7,8-Dihydroaminopterin[e]	0.01		0.01		
5,6,7,8-Tetrahydroaminopterin[f]	0.07		0.06		

[a]None of the compounds supported growth in the absence of folate at a level of 2000 ng/ml. [b]Folate, 1 ng/ml, growth inhibition reversed by added folate. *S. faecium:* medium of L. M. Flynn, V. B. Williams, B. L. O'Dell, and A. G. Hogan, *Anal. Chem.*, **23,** 180 (1951); *L. casei:* BBL folic acid assay broth. [c]5-Formyltetrahydrofolate, 1 ng/ml (Difco CF assay medium). [d]5×10^{-12} *M*. [e]From $Na_2S_2O_4$ reduction of parent compound: M. Friedkin, E. J. Crawford, and D. Misra, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **21,** 176 (1962). [f]From H_2–PtO_2 reduction in neutral aqueous solution: R. L. Blakley, *Biochem. J.*, **65,** 331 (1957). [g]All compounds were diluted in potassium ascorbate, 6 mg/ml (pH 6.0), and added to growth media aseptically after autoclaving: H. A. Bakerman, *Anal. Biochem.*, **2,** 558 (1961).

Table II. Enzyme Inhibition of 10-Deazaminopterin and Its Reduced Forms

	Molarity for 50% inhibition[c]	
	Dihydrofolic reductase[a]	Thymidylate synthetase[a]
VI	4.5×10^{-9}	$>1 \times 10^{-4}$
VI-H_2[b]	4.5×10^{-9}	1×10^{-5}
VI-H_4[b]	1.0×10^{-7}	5.7×10^{-6}
MTX	3.3×10^{-9}	$>1 \times 10^{-4}$

[a]Derived from *L. casei* (ATCC 7649). [b]Reduced forms were not active as substrates for either enzyme. [c]Conditions: DHF, 50 μM; NADPH,[f]80 μM; Tris HCl, 0.05 *M*; 2-mercaptoethanol, 0.01 *M*; EDTA, 0.001 *M*; protein, 0.0012 mg; pH 7.4; 30°; reaction initiated with enzyme.

with *i*-BuOCOCl under controlled conditions. *tert*-Butyloxycarbonylglutamic acid α-benzyl ester was esterfied to Merrifield resin through the γ-COOH group.[4] After deprotection the amino group of resin-bound glutamate was then coupled with the mixed anhydride of V. Cleavage of coupled product from the resin with 2 *N* NaOH–dioxane at ambient temperature afforded VI in 70% yield.

Alternatively, the mixed anhydride of V was coupled with trimethylsilyl glutamate[5] and the silyl esters were cleaved with 1% Na_2CO_3 at room temperature. After chromatography on DEAE-cellulose the product was found to be identical with VI produced by the resin technique. Since prolonged treatment of V with 1% Na_2CO_3 did not cause deamination, the product obtained by the two different coupling procedures was established as the 2,4-diamino compound VI, as was also verified by elemental analysis.

Experimental Section

10-Deazaminopterin (VI). Method A. Compound V[3] (155 mg, 0.5 mmol) was dissolved in DMSO (15 ml) and 15 ml of dry THF was added. The solution was chilled and *N*-methylmorpholine (0.625 mmol) was added. The solution was kept at 0° for 15 min and *i*-BuOCOCl (0.5 mmol) was added with stirring. After 15 min this product was coupled with the NH_2 deprotected α-benzyl-γ-glutamyl resin ester.[4] The coupled product was cleaved from the resin by shaking for 1 hr at room temperature in a mixture of 20 ml of 2 *N* NaOH and 20 ml of dioxane under N_2.

The solution was adjusted to pH 7.5, diluted to 1 l., and chromatographed on a DEAE Cl column (20 × 3 cm) with elution by 0.15 *M* NaCl. The peak fractions were concentrated to 250 ml *in vacuo*, acidified to pH 4.5 (HOAc), and chilled 15 hr. The pale yellow product was collected by filtration (154 mg, 70%): $\lambda^{pH\ 13}$ 256 nm (32,500), 372 (7475); $\lambda^{pH\ 1}$ 243 (26,800), 340 (8800); chromatography on analytical DEAE column, single uv-absorbing band. *Anal.* ($C_{20}H_{21}N_7O_5 \cdot H_2O$) C, H, N.

Method B. A mixture of glutamic acid (147 mg, 1 mmol), hexamethyldisilazane (10 ml), and 30 mg of H_2SO_4 was stirred at reflux until solution was complete. The solvent was removed *in vacuo* and the residual silyl ester dissolved in 15 ml of DMSO–THF (1:1) and coupled with the mixed anhydride of V. After 15 hr the solution was concentrated to 15 ml *in vacuo* and treated with 100 ml of 1% Na_2CO_3 for 2 hr. The solution was adjusted to pH 7 (1 *N* HCl), diluted to 700 ml, and chromatographed on DEAE as above. Only two peaks were eluted—one corresponded with starting material V and the other was identical with product VI obtained by method A. A control experiment where V was exposed to similar treatment with 1% Na_2CO_3 gave no deamination to 10-deazapteroic acid (III)[1] as shown by analytical DEAE chromatography.

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Synthesis of 1-Deaza-N^{10}-methylfolic Acid and Related Compounds

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Recent tests showed that 3-deazamethotrexate was more effective against leukemia L1210 in mice, more cytotoxic in cell culture, and a better inhibitor of the dihydrofolate reductase enzyme (pigeon liver) than 1-deazamethotrexate. These results indicated that the 1-nitrogen contributed more to activity than the 3-nitrogen in these ring systems.[1] In contrast, a dihydro-1-deazapteridine† precursor of 1-deazamethotrexate showed activity against leukemia L1210 in mice,[2] and the preparation of a number of 1-deazapteridines is currently being investigated. In

†Compound **10a**, ref 2. Preliminary results indicated that this compound gave a 30% (qd 1-9) increase in lifespan against leukemia L1210 in mice at a dose of 25 mg/kg.