above for 6. After treatment with p-TosOH·H_2O and usual work-up the crude yield of 41 was 5% which recrystallized (MeCN) to pure 41: mp 164.5–167.5°; ir (KBr) 1700 cm^{-1} (C=O); nmr ($CDCl_3$) δ 7.0–7.7 (q, 4, J = 8.5 Hz, aromatic), 3.78 (s, 3, CH_3O), 2.7 (m, broad, 1, CH_3CH), 2.4–1.8 (overlapping m, 5, $CHCO_2H$ and $CH_2C{=}CCH_2$), 1.2–1.05 [doublet overlapping two singlets, J = 6 Hz, 9, $CHCH_3$ and $C(CH_3)_2$], and 0.87 (t, 3, J = 7.5 Hz, CH_2CH_3); scale expansions and spin decouplings confirmed the assignments as well as establishing the stereochemistry; coupling constant of the C-1 proton (10 Hz) was indicative of trans-diaxial coupling; mass spectrum (70 eV) m/e 302. *Anal.* ($C_{19}H_{26}O_3$) C, H.

3-Ethyl-4-(*p*-methoxyphenyl)-2,6,6-trimethyl-Δ^2-cyclohexenecarboxylic Acid (43). The Et_2O extracts which remained after the base extractions in the above experiment were worked up to leave an oil. Distillation [bp 173–182° (0.05 mm)] gave the Δ^2-ene ester 42 (yield, 42%): nmr ($CDCl_3$) δ 1.70 (d, 3, J = 2 Hz, $CH_3C{=}C$). *Anal.* ($C_{21}H_{30}O_3$) C, H. A solution of the ester 42 (1.10 g, 0.003 mol) in ethylene glycol (18 ml) and H_2O (3.6 ml) containing KOH (2.00 g, 0.036 mol) was heated at reflux for 88 hr. Usual work-up gave the acid 43 (0.67 g, 67%): mp 131–132° (MeCN); ir (KBr) 1697 cm^{-1} (C=O); nmr ($CDCl_3$) δ 1.76 (d, 3, J = 2 Hz, $CH_3C{=}C$); mass spectrum (70 eV) m/e 302. *Anal.* ($C_{19}H_{26}O_3$) C, H.

Acknowledgment. The authors wish to thank Dr. J. E. MacNintch for the hypocholesterolemic data, H. D. Taylor and G. M. Sanhueza for the glpc data, A. L. Vulcano for 100-MHz nmr data, and the analytical and spectroscopic departments of these laboratories for their services.

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Synthesis and Antifolate Activity of Isoaminopterin†

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The synthesis of isoaminopterin has been carried out by two methods. The first parallels the method employed for constructing the isofolate framework recently developed in this laboratory, which involves a novel ring closure of a substituted pyrimidine. The second involves the displacement of the halogen of 2,4-diamino-6-chloropteridine by α-amino-p-toluic acid. Isoaminopterin has been tested for its ability to inhibit the growth of two folate-requiring organisms. Inhibition studies of this compound were carried out on the enzyme dihydrofolate reductase. While isoaminopterin is equally potent in its bacteriostatic activity as aminopterin, it is somewhat less inhibitory for dihydrofolate reductase.

As part of a continuing program aimed at the design, synthesis, and biological evaluation of folate analogs which would be less toxic and more specific in their action than currently available drugs, we have recently reported 9-oxofolic acid[1] and isofolic acid.[2] Although the altered isomeric framework of isofolic acid could contribute specific interference with the metabolic pathways in which 5,10-methylenetetrahydrofolate takes part as a cofactor, its 4-amino analog might also elicit antimetabolic activity by inhibiting the enzyme dihydrofolate reductase.[3-5] Since no 4-amino analogs of isofolic acid have been prepared, the preceding assumption of inhibition of DHFR by 4-aminoisofolate is only speculative and warranted examination. It appeared that although 4-aminoisofolate possesses the 2,4-diamino functions at the pteridine moiety as with the classical aminofols, the reversal of substituents at the C_9–N_{10} positions could alter its binding characteristics for DHFR, which should be reflected in altered inhibitory characteristics. Some evidence to support this type of reasoning is available. For example, it has been noted that dihydroisofolic acid, which differs from dihydrofolic acid only in the exchange of the amino and methylene groups at positions 9 and 10, does not behave as a substrate for DHFR.‡

Another reason for the synthesis of 4-aminoisofolic acid comes from the work of Kisliuk and coworkers who investigated the ability of the classical aminofols aminopterin,[6] methotrexate,[7] and their dihydro and tetrahydro derivatives to inhibit the enzyme thymidylate synthetase and concluded that the tetrahydro forms are more powerful inhibitors than the parent compounds. Since formaldehyde

†Trivial names in general usage will be used for these compounds: isoaminopterin = *N*-[*p*-[[(2,4-diamino-4-deoxy-6-pteridinyl)amino]methyl]benzoyl]glutamic acid; methotrexate = *N*-[*p*-[[(2,4-diamino-6-pteridinyl]methyl]methylamino]benzoyl]glutamic acid; aminopterin = *N*-[*p*-[[(2,4-diamino-6-pteridinyl)methyl]amino]benzoyl]glutamic acid; folic acid = *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)methyl]amino]benzoyl]glutamic acid; homofolic acid = *N*-[*p*-[[(2-amino-4-hydroxy-6-pteridinyl)ethyl]amino]benzoyl]glutamic acid. Other abbreviations include DHFR, dihydrofolate reductase; DMSO, dimethyl sulfoxide; THF, tetrahydrofuran; DEAE, diethylaminoethyl; DMF, dimethylformamide; Boc, *tert*-butyloxycarbonyl.

‡C. M. Baugh, R. L. Kisliuk, and M. G. Nair, unpublished work, University of South Alabama and Tufts University, 1974.

was present in the assay medium and it is known that reaction of these tetrahydro compounds with formaldehyde forms the corresponding 5,10-methylene derivatives, it was impossible to determine the structure of the material which actually contributed to this inhibition. Although tetrahydroisoaminopterin cannot form the methylene bridge, it would be interesting to see whether this compound is inhibitory to thymidylate synthesis. To clarify some of these problems which would also contribute to a better understanding of the structure-activity relationships in this series, the synthesis of isoaminopterin (4-aminoisofolic acid) was undertaken.

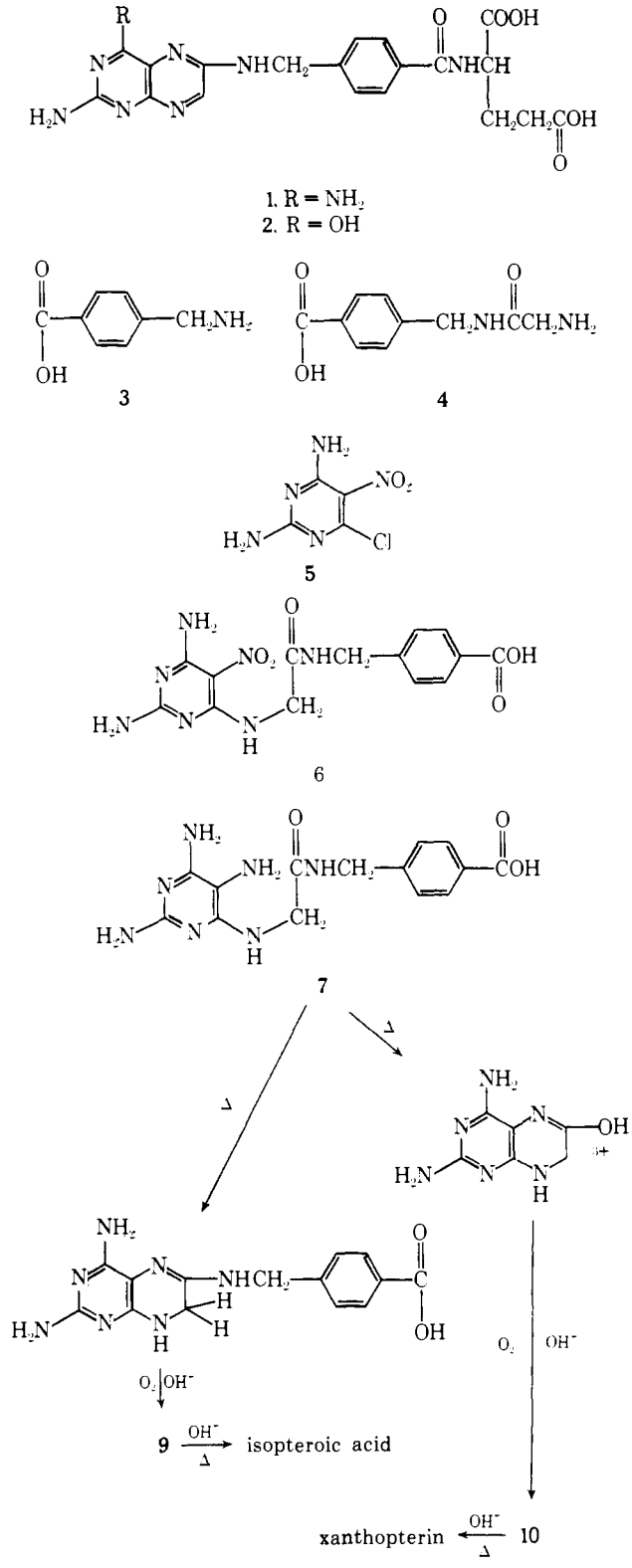
Two approaches to the synthesis of **9** were considered and both worked equally well. The first approach was a modification of the Boon and Leigh method,[8] which also included a novel ring closure reaction previously perfected in this laboratory,[2] for the synthesis of isofolic acid (**2**). The conversion of the commercially available *p*-carboxybenzaldehyde to α-amino-*p*-toluic acid[1] (**3**) and its modification to **4** have been described previously.[2] 2,4-Diamino-6-chloropyrimidine was nitrated according to the procedure of Pfleiderer[9] to the 5-nitro compound **5**. Reaction of 2,4-diamino-6-chloro-5-nitropyrimidine with **4** in refluxing aqueous EtOH in the presence of 1 equiv of triethylamine resulted in the smooth displacement of the halogen at position 6 with formation of intermediate **6**. Reduction of the 5-nitro group of **6** to the corresponding amino compound by catalytic hydrogenation presented problems. However, it was observed that this reaction could be carried out smoothly with sodium hydrosulfite at alkaline pH at a temperature of 50–60°. The progress of the reduction was monitored by the decrease in the uv absorbance at 350 nm. When the reduction was complete, the compound **7** was isolated as a white precipitate after acidifying the cooled clear solution with glacial HOAc. Prolonged exposure to air decomposes **7** to a purple compound unsuitable for further investigations. After drying for 18 hr *in vacuo*, the product **7** was subjected to the ring closure reaction as described previously with molecular sieve in refluxing bis(2-ethoxyethyl) ether.[2] Contrary to expectations, only a small amount of ring closure occurred as evidenced by the uv absorption spectrum of the product. This observation prompted the employment of more vigorous conditions for ring closure (Scheme I).

Intermediate **7** was pyrolyzed[10-12] at its melting point in a nitrogen atmosphere, whereupon the mass softened and resolidified. The dark brown product thus obtained was dissolved in 0.1 *N* NaOH. The solution was diluted and adjusted to pH 8 and MnO_2 oxidation was carried out with stirring for 12 hr under aerobic conditions. Upon DEAE cellulose chromatography of the crude product, two major products were eluted, of which the more polar material was subsequently identified as 4-amino-4-deoxyisopteroic acid (**9**) by spectroscopic and chemical methods. Significant among the data is the nmr spectrum in D_2O containing NaOD, which showed the resonance of the C_7 proton as a singlet at 8.01, the characteristic resonances due to the aromatic protons as a pair of doublets at 7.83 and 7.36, and the C_{10}-methylene protons at 4.36 ppm. The less polar material showed a uv spectrum in base identical with that of xanthopterin.[13] However, in 0.1 *N* HCl, the spectrum differed markedly from that of xanthopterin, suggesting the product to be 4-aminoxanthopterin (**10**). This was subsequently confirmed by the deamination of **10** to xanthopterin by hydrolysis and comparison with an authentic sample by physical and chromatographic methods.

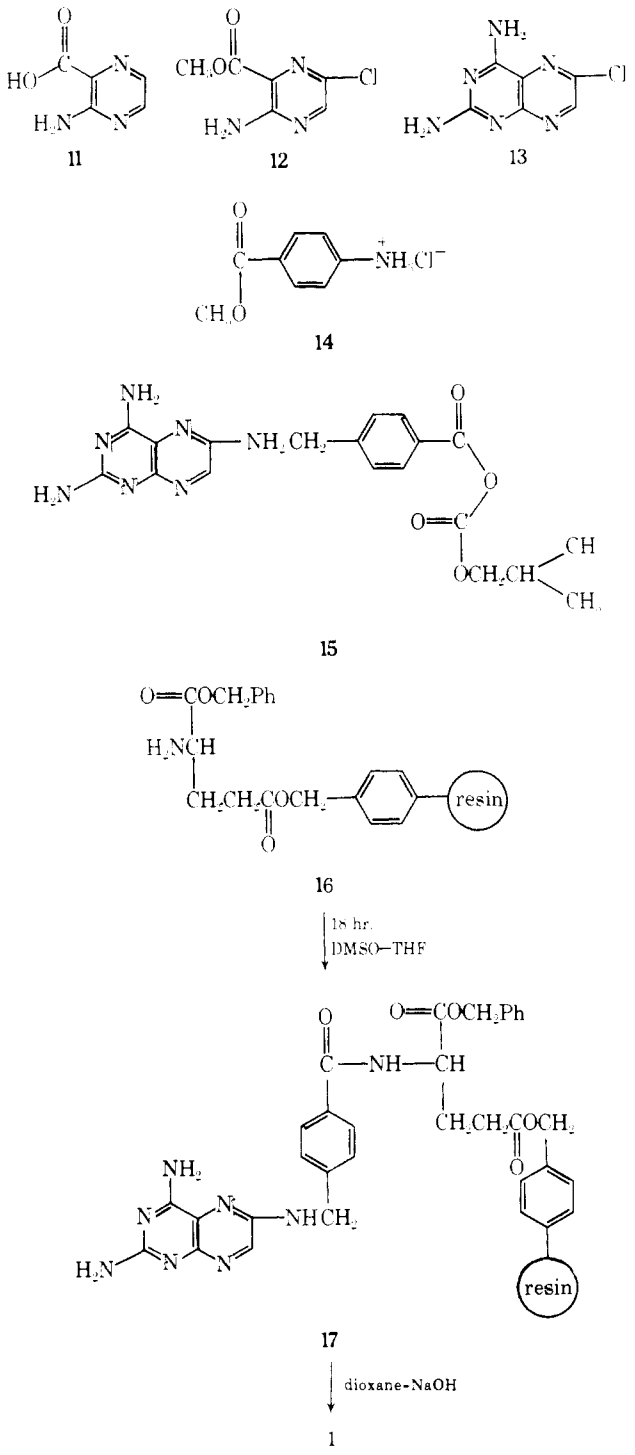
The mechanism of this pyrolytic ring closure reaction has been outlined by us earlier.[2] The conversion of **7** to **9** further illustrates the generality of this ring closure reaction in the construction of pteridines. The cleavage of the side chain, *i.e.*, α-amino-*p*-toluic acid, was expected according to the proposed mechanism, with the concomitant formation of **10**. Reduction of **9** with dithionite at 50° results in the formation of the dihydro derivative, which was found to be stable. The compound, after destruction of dithionite, was reoxidized to **9** without loss of side chain, substantiating that the formation of **10** takes place during the pyrolytic step of the synthesis.

The second approach pursued in the construction of the

Scheme I



Scheme II



isofolate framework was based on a report§ that 6-chloro-2,4-diaminopteridine (13) can result in displacement of the 6-chloro group by reaction with substituted benzylamines at moderately high temperatures.& The synthesis of 2,4-diamino-6-chloropteridine was thus undertaken, starting from commercially available 3-amino-2-pyrazinecarboxylic acid (11). Methyl 3-amino-6-chloro-2-pyrazinecarboxylate (12) was prepared according to the procedure of Bicking, *et al.*[14] Compound 12 was later converted to the desired 6-chloropteridine 13 through a number of intermediary steps according to the procedure of Cragoe and Jones[15] (Scheme II).

§We are grateful to Dr. E. F. Elslager of Merck & Co. for providing us with the experimental details of this synthesis.

& E. F. Elslager, private communication, Merck & Co.

α-Amino-*p*-toluic acid[1] was converted to its methyl ester hydrochloride 14 by a standard procedure. Treatment of this material with the chloropteridine 13 in the presence of excess diazabicyclooctane, using dimethyl sulfone as a solvent, at various temperatures and for various lengths of time resulted either in the recovery of the starting pteridine or polymerization to tarry materials. It therefore appeared that under conditions required to displace the halogen at the 6 position, the amino ester was undergoing polymerization. To avoid this possibility, α-amino-*p*-toluic acid (3) was used directly instead of 14. After a series of experiments, a set of satisfactory conditions was developed which provided a yield of 10–15% of 9. The material obtained by either of the two procedures described was indistinguishable in all respects.

To complete the synthesis of isoaminopterin, the problem of making the glutamate conjugate of 9 remained. This was accomplished according to the chemical procedures developed earlier[16,17] for making poly-γ-glutamyl conjugates of 4-amino-4-deoxy-10-methylpteroic acid. Briefly, *tert*-butyloxycarbonylglutamic acid α-benzyl ester was esterified to the chloromethylated Merrifield resin by standard procedure. After deprotection of the Boc-protective group, the resin-bound glutamic acid derivative 16 was allowed to react with the mixed anhydride of 9 and isobutyl chloroformate (15). Cleavage of the product 17 from the resin and hydrolysis of the α-benzyl ester was carried out as reported.[16,18] The product was purified by column chromatography to obtain 1 in good yield. These procedures resulted in the deamination of the 4-amino group to the extent of 2–3% to form isofolic acid 2, which was less polar on a DEAE column and therefore easily separated.

Additional proof of the structure of 9 was obtained by its deamination to isopteroic acid and that of 1 by examination of its nmr spectrum in 0.1 *N* NaOH in D_2O which showed the following resonances: 8.18 (s, one proton, H_7), 7.8 (d, J = 8 Hz, two protons, $H_{2',6'}$), 7.45 (d, J = 8 Hz, two protons, $H_{3',5'}$), 4.45 (c, three protons, C_{10}-methylene and α-proton of glutamic acid), and between 2 and 3.1 ppm (c, four protons, glutamic acid). These observations are in complete agreement with the structure.

Biological Evaluation. Antimetabolic Activity. Isoaminopterin was tested for its ability to inhibit the growth of two folic acid requiring strains of bacteria, *Lactobacillus casei* (ATCC no. 7469) and *Streptococcus faecium* (ATCC no. 8043). These studies were carried out in duplicate tubes containing the appropriate Difco folic acid assay media for these organisms. The results are summarized in Table I.

A number of folic acid related metabolites were used in attempts to reverse the inhibition brought about with isoaminopterin in *L. casei*. Data presented in Table II show that only folic acid and thymidine were able to reverse the inhibition.

Enzyme Inhibition. The ability of isoaminopterin to inhibit the enzyme dihydrofolate reductase was compared to that of methotrexate. A generous gift, donated by Dr. R. L. Kisliuk, of the partially purified enzyme derived from *L. casei* (ATCC no. 7469) was utilized. The progress of the enzymatic reaction was monitored by recording the decrease in absorbance at 340 nm according to the procedure of Kisliuk and coworkers.[6,7] The results show that while isofolic acid is only weakly inhibitory‡ to the enzyme as compared to methotrexate, isoaminopterin is a potent in-

Table I

Compound	Molar concn for 50% inhibition of growth		Molar concn required for 50% inhibition of DHFR[c]
	L. casei[a]	*S. faecium*[b]	
Isoaminopterin	0.90×10^{-13}	45×10^{-13}	14.5×10^{-9}
Methotrexate	1.32×10^{-13}	6×10^{-13}	6.8×10^{-9}

[a]ATCC No. 7469; 4×10^{-10} g of folic acid present in each assay tube. [b]ATCC No. 8043; 4×10^{-9} g of folic acid present in each assay tube. [c]The reaction was started by the addition of 0.33 mg of TPNH to the assay medium. A total of 2.6 μmol of 7,8-dihydrofolate and 0.1 mg of enzyme was present in a total volume of 2.5 ml of solution used for each assay.

hibitor, and the degree of the difference in the inhibition of methotrexate and isoaminopterin is significant. These results are summarized in Table I.

The marked difference noted for isoaminopterin and isofolic acid suggests that the presence of the 4-amino group in the isofolate framework is necessary for the inhibition of DHFR. However, the reversal of the substituents at C_9–N_{10} positions dramatically alters the binding characteristics of this molecule as evidenced by the inability of dihydroisofolate to behave as a substrate for this enzyme.‡ The fact that dihydrohomofolate is a substrate for DHFR[19] implies that the bridge amino group must be separated from the pteridine moiety by at least one carbon unit for appropriate enzyme–substrate interaction in compounds possessing the normal 2-amino-4-hydroxypteridine ring.

Studies are now in progress with the di- and tetrahydro derivatives of isoaminopterin with regard to their antimetabolic activities and ability to inhibit the enzymes DHFR and thymidylate synthetase. The antitumor activity of isoaminopterin is also being investigated.

Experimental Section

Melting points are uncorrected and were determined on a Fisher-Johns apparatus. Nmr spectra were run in 0.1 *N* NaOD in D_2O on a 60-MHz, HA-60 Varian spectrometer with [3-(trimethylsilyl)tetradeuterio]sodium propionate as an internal standard. Field strengths of the various proton resonances are expressed in parts per million and coupling constants as cycles per second. Peak multiplicity is depicted as usual: s for singlet, d for doublet, t for triplet, q for quartet, and c for complex. Ultraviolet spectra were determined on a Beckman Model 25 spectrophotometer. All chromatography was carried out on DEAE cellulose (Mannex DEAE, from Schwarz/Mann, Orangeburg, N.Y.) in the chloride form with 1.2 × 22 cm packing unless otherwise specified. A linear NaCl gradient, 0.005 *M* phosphate buffer, pH 7.0, from 0 to 0.5 *M* with respect to NaCl was used to elute the column in a total volume of 2 l. Elemental analyses were by Galbraith Laboratories, Inc., Knoxville, Tenn.** Yields represent the actual amount of pure compound isolated, assuming 100% reaction.

Reaction of 4 with 6-Chloro-2,4-diamino-5-nitropyrimidine (5). Preparation of 6. Compound 4 was synthesized according to the procedure of Nair and Baugh.[2] Nitration of 6-chloro-2,4-diaminopyrimidine was carried out according to a procedure described earlier.[9] In a typical experiment, 10 mmol of 4 was dissolved in 200 ml of water at 100° in round-bottomed flask with stirring. The chloropyrimidine 5, 10 mmol, was dissolved in 500 ml of 95% EtOH and the hot solution slowly added to the boiling aqueous solution through a dropping funnel. After the addition was complete, the solution was allowed to reflux for an additional 3.5 hr. On cooling, cream-colored crystals of 6 appeared. These were removed by filtration and recrystallized from a DMSO–benzene mixture: mp 305–306°. *Anal.* ($C_{14}H_{15}N_7O_5$) C, H, N, O.

Reduction of 6 with Sodium Dithionite. Preparation of 7. 6 (100 mg) was suspended in 15 ml of water and 1 *N* NaOH added to the solution so that the pH did not exceed 10. When all the material had gone into solution, the pH was lowered by the addition of 1 *N* HCl to 7.5 and the mixture was heated to ~50°. Solid dithionite, 500 mg, was added slowly in small portions while adding 1 *N* NaOH dropwise to maintain a pH of 8. Then an additional 100 mg of dithionite was added and the solution chilled to 0°. At this stage the uv absorption peak at 343 nm due to the nitro group had disappeared completely. The solution was then slowly acidified with glacial HOAc to pH 5. The precipitate of 7 was collected by filtration and washed several times with ice-cold distilled water and finally with absolute EtOH. The precipitate was then immediately transferred to a vacuum oven and dried at room temperature for 18 hr over P_2O_5.

Table II. Reversal of Isoaminopterin Inhibition of *Lactobacillus casei* ATCC No. 7469 Growth by Folic Acid[a] and Thymidine

Isoaminopterin/tube	Methionine	Adenine	Thymidine	Turbidity
4×10^{-10} g				0.35
4×10^{-10} g	1×10^{-3} g			0.35
4×10^{-10} g		1×10^{-3} g		0.34
4×10^{-10} g			1×10^{-5} g	0.70
				0.70

[a]All tubes contained 4×10^{-10} g of folate per tube.

Preparation of 4-Amino-4-deoxyisopteroic Acid (9). A. Cyclization with Molecular Sieves 3A in Bis(diethoxyethyl) Ether and Diazabicyclooctane. The reduction product 7, 100 mg, was added to 35 ml of bis(diethoxyethyl) ether containing 200 mg of diazabicyclooctane and 2 g of molecular sieves previously saturated with nitrogen. The mixture was heated to reflux in a nitrogen atmosphere and refluxed for 2 hr. The reaction was cooled and 50 ml of diethyl ether added, followed by filtration. The filtrate was discarded. The product, on the filter, was then washed with small amounts of 0.1 *N* NaOH so that the total volume did not exceed 25 ml. The washings were combined and adjusted to pH 8. MnO_2 (500 mg) was added and the mixture stirred in an open beaker for 18 hr. The intense green fluorescent solution was removed from MnO_2 by filtration. The filtrate was adjusted to pH 7.2 with 1 *N* HCl, diluted to 500 ml, chromatographed on a DEAE Cl^- column, and eluted with a NaCl gradient as described. 2,4-Diamino-6-hydroxypteridine was eluted from the column at 0.05 *M* NaCl and 4-amino-4-deoxyisopteroic acid eluted at 0.12 *M* NaCl. These peaks were pooled, evaporated to a small volume, and acidified with glacial HOAc to pH 4 to get 9 and 10. The yield of 10 was estimated to be ~3%.

B. Pyrolysis of 7. In a wide-mouth test tube, 100 mg of 7 was suspended and a slow stream of nitrogen allowed to pass through. The tube was then heated by immersing in a silicone oil bath, while stirring the compound with a glass rod. When the temperature rose to ~250° the mass softened. The temperature was allowed to rise to 270° and held there for 5 min whereupon the soft mass hardened. The tube was taken out of the oil bath, allowed to cool in nitrogen, and triturated with 15 ml of 0.1 *N* NaOH, whereupon complete solution occurred. It was diluted to 200 ml and adjusted to pH 8 and then stirred in an open beaker with 400 mg of MnO_2 for 18 hr. The reaction was worked up as outlined above. A 12% yield of 10 was obtained: nmr (0.1 *N* NaOD in D_2O) 8.01 (s, one proton, H_7), 7.83 (d, J = 8 Hz, two protons, $H_{2',6'}$), 7.36 (d, J = 8 Hz, two protons, $H_{3',5'}$), 4.36 ppm (c, $W_{1/2}$ = 4 Hz, C_{10}-methylene protons); uv λ_{max} max (1 *N* NaOH) 410 nm (ε 6927), 265 (22,815). *Anal.* ($C_{14}H_{13}N_7O_2$-1.5H_2O) C, H, O.

Deamination of 4-Amino-4-deoxyisopteroic Acid to Isopteroic Acid. This conversion was accomplished by refluxing 10 mg of 9 with 10 ml of 1 *N* NaOH in a nitrogen atmosphere for 7 hr. The hydrolysate was adjusted to pH 7 and chromatographed on a DEAE column. A 75% yield of isopteroic acid was obtained which was indistinguishable in all respects from an authentic sample.

Similarly 10 was deaminated according to the same procedure and the formation of xanthopterin was verified by comparison with an authentic sample.

Preparation of 4-Amino-4-deoxyisofolic Acid (1). A. Coupling. *tert*-Butyloxycarbonylglutamic acid α-benzyl ester was esterified to the chloromethylated Merrifield resin as described pre-

**Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements or functions were within ±0.4% of the theoretical values.

viously.[18] The Boc group was removed by treating with 20% trifluoroacetic acid in methylene chloride.

The mixed anhydride **15** was prepared according to the following procedure. **9** (0.169 g, 0.0005 mol) was dissolved in 20 ml of dry DMSO with warming. To the cooled solution was added 20 ml of dry tetrahydrofuran, followed by 0.063 g (0.000625 mol) of *N*-methylmorpholine. The mixture was chilled in an ice bath for 15 min, 0.068 g (0.0005 mol) of freshly distilled isobutylchloroformate was added, and the formation of the mixed anhydride allowed to proceed for an additional 15 min in the ice bath. The solution was then added to the resin-bound glutamic acid α-benzyl ester. After 18 hr at room temperature, the reaction mixture was filtered and the resin washed two times with DMSO and three times with 30-ml portions of *p*-dioxane.

B. **Deprotection, Cleavage, and Purification.** A mixture of 15 ml of 2 *N* NaOH and 15 ml of purified dioxane was deaerated by bubbling N_2 for 10 min. The resin-bound product was shaken vigorously with this mixture for 1 hr at room temperature in a closed reaction vessel. The vessel was placed in a water bath at 50° for 20 min. After being cooled to room temperature, the mixture was filtered and the filtrate diluted to ~400 ml with H_2O and then adjusted to pH 7. The solution was further diluted to 1 l. and applied to a 5 × 10 cm DEAE column. The column was washed with distilled water and the products were obtained by discharging the resin with 0.5 *M* NH_4OH. The ammonia was driven off *in vacuo* and the ammonium salt of **1** repurified by chromatography on a standard DEAE Cl^- column with the linear sodium chloride gradient described earlier. A small amount of isofolic acid (**2**) was obtained and identified by comparison with an authentic sample. Pure **1** thus obtained showed the following uv absorptions: λ max (0.1 *N* NaOH) 413 nm (ε 5849), 269 (23,729), 248 (23,376); λ max (0.1 *N* HCl) 402 nm (ε 6466) and 260 (28,736). *Anal.* ($C_{19}H_{20}N_8O_5 \cdot 0.5H_2O$) C, H, N, O.

Reduction of 1 with Dithionite. **1** (5 mg) was dissolved in 25 ml of H_2O by the addition of 0.1 *N* NaOH so that the pH reached 10. This solution was slowly heated to 50° and 50 mg of sodium dithionite was added in small portions. The solution became colorless after 5 min. The uv spectrum of an aliquot of this solution showed the disappearance of the absorption at 413 nm. The pH of the solution was adjusted to 3 and after 10 min readjusted to 8. The solution was then filtered, 500 mg of MnO_2 was added, and the reaction mixture was stirred for 16 hr in an open beaker. After removal of MnO_2 by filtration, the compound was chromatographed on a 1.4 × 31 cm DEAE Cl^- column. Two peaks were eluted from the column and the more polar one was identified as **1**. The less polar material which eluted at a NaCl concentration of 0.185 *M* was the dihydro compound as evidenced by its uv spectrum in 0.1 *N* NaOH [λ max 320 (sh) and 290 nm] and also by reoxidation to **1**. No other uv-absorbing material was eluted from the column.

Acknowledgments. This work was supported by Grant No. CI-86N of the American Cancer Society and NIH Grant 1-RO1-CA-16048. The technical assistance of Miss Pay Taylor and Miss Eleanor Braverman is gratefully acknowledged.

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Pyrimido[4,5-*c*]isoquinolines. 2. Synthesis and Biological Evaluation of Some 6-Alkyl-, 6-Aralkyl-, and 6-Aryl-1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinolines as Potential Folate Antagonists[1,†]

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1,3-Diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinolines with lipophilic alkyl, aralkyl, or aryl substituents at position 6 were synthesized *via* a method involving (1) condensation of 2-acyl- or 2-aroylcyclohexanones with cyanoacetamide in the presence of a secondary amine, (2) chlorination of the resultant 4-cyano-3-hydroxy-5,6,7,8-tetrahydroisoquinolines with phenylphosphonic dichloride, and (3) reaction of the chloronitriles with guanidine carbonate in refluxing *N,N*-dimethylformamide. An alternative approach was also discovered when 3-amino-4-cyano-5,6,7,8-tetrahydroisoquinoline proved to undergo ring closure directly on treatment with guanidine carbonate in boiling 1-octanol. The products were potent inhibitors of folate-dependent *Streptococcus faecium* ATCC 8043 and purified dihydrofolate reductase from *Lactobacillus casei* ATCC 7469 when bulky lipophilic groups were present at position 6.

As part of our research program on tricyclic 2,4-diaminopyrimidines as folate antagonists and potential cancer chemotherapeutic agents,[2] it was of interest to prepare some 1,3-diamino-7,8,9,10-tetrahydropyrimido[4,5-*c*]isoquinolines of general structure **1** and to evaluate the biological activity of these compounds with reference to other angular condensed 2,4-diaminopyrimidine types studied previously in our laboratory, such as the bridged pyrimethamine analogs **2**[3-7] and the thieno[2,3-*d*]pyrimidines

†This investigation was supported in part by Research Grant C6516 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.