Folate Analogues. 31. Synthesis of the Reduced Derivatives of 11-Deazahomofolic Acid, 10-Methyl-11-deazahomofolic Acid, and Their Evaluation as Inhibitors of Glycinamide Ribonucleotide Formyltransferase[1]

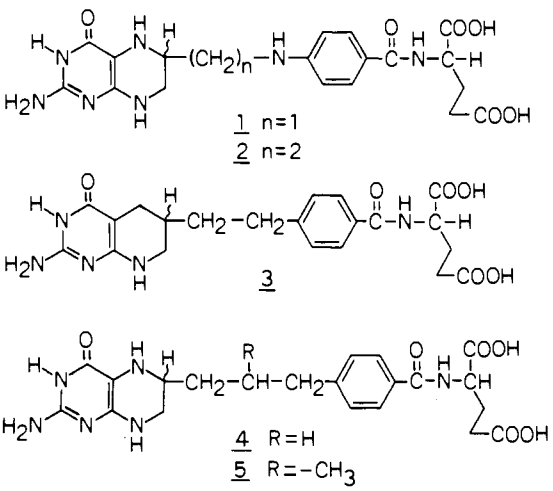
M. G. Nair,*,† B. R. Murthy,† Sharadbala D. Patil,† R. L. Kisliuk,‡ J. Thorndike,‡ Y. Gaumont,‡ R. Ferone,§ D. S. Duch,§ and M. P. Edelstein§

Department of Biochemistry, University of South Alabama, Mobile, Alabama 36688, Department of Biochemistry, Tufts University Health Science Campus, Boston, Massachusetts 02111, and Wellcome Research Laboratory, Burroughs Wellcome Company, Research Triangle Park, North Carolina 27709. *Received July 11, 1988*

The Boon–Leigh procedure, involving condensation of a 6-chloro-5-nitropyrimidine (**22**) with an α-amino ketone (**20** or **21**) followed by reduction of the nitro group, cyclization, and L-glutamylation, led to the formation of 11-deazahomofolate (**29**) and its 10-methyl derivative (**30**). The corresponding (6*R*,*S*)-5,6,7,8-tetrahydro (**4**, **5**) and 7,8-dihydro (**31**, **32**) derivatives were prepared by catalytic hydrogenation. (6*S*)-11-Deazatetrahydrohomofolate was prepared from **29** by enzymatic reduction. Compounds **29** and **30** had little effect ($IC_{50} > 2 \times 10^{-5}$ M) on *Lactobacillus casei* glycinamide ribonucleotide (GAR) formyltransferase but (6*R*,*S*)-11-deazatetrahydrohomofolate (**4**) is a potent inhibitor of this enzyme ($IC_{50} = 5 \times 10^{-8}$ M). It is at least 100 times more inhibitory than **33**, the 6*S* compound, indicating that the 6*R* component of the mixture having the unnatural configuration at C6 (**34**) is responsible for the potent inhibition. Compound **4** is a much weaker inhibitor of murine (L1210) and human (MOLT-4) leukemia cell GAR formyltransferases ($IC_{50} > 1 \times 10^{-5}$ M). (6*R*,*S*)-11-Deaza-10-methyltetrahydrohomofolate (**5**) ($IC_{50} = 1.1 \times 10^{-5}$) is 200 times weaker than **4** against *L. casei* GAR formyltransferase. However, 11-deaza-10-methyldihydrohomofolate (**32**) is more inhibitory ($IC_{50} = 5.5 \times 10^{-7}$ M) than **5** or **30**. None of the compounds showed inhibition of *L. casei* aminoimidazolecarboxamide ribonucleotide (AICAR) formyltransferase, dihydrofolate reductase, or thymidylate synthase. The dihydro derivatives **31** and **32** are 5% as active as dihydrofolate as substrates for *L. casei* dihydrofolate reductase. Compound **4** showed moderate inhibition of the growth of *L. casei*, *Streptococcus faecium*, MOLT-4 cells, and MCF-7 human breast adenocarcinoma cells.

Inhibition of folate-dependent enzymes such as dihydrofolate reductase (DHFR, EC 1.5.1.3) and thymidylate synthase (TS, EC 2.1.1.45) is a useful approach for the chemotherapy of various forms of human cancers. The well-known DHFR inhibitor methotrexate (MTX) and 5-fluorouracil (5FU), which is a precursor of the powerful TS inhibitor 5-fluorodeoxyuridine monophosphate (5FdUMP), are widely used in human medicine.[2] Recently, 10-propargyl-5,8-dideazafolate (PDDF) and its polyglutamates were shown to be potent inhibitors of TS derived from several species.[3-5] Analogues of this lead compound are being developed in several laboratories because the clinical utility of PDDF is limited by its solubility. A few compounds structurally related to tetrahydrofolic acid (**1**) have also exhibited interesting antitumor activity.[6,7] For example, Mead and co-workers demonstrated that tetrahydrohomofolic acid (**2**) was active against a methotrexate-resistant strain of L1210 leukemia.[8] Hakala then showed that homofolate derivatives block purine biosynthesis in cultured cells by virtue of their inhibition of GAR formyltransferase.[9] Inhibition of GAR formyltransferase or AICAR formyltransferase could block purine biosynthesis and lead to cytotoxicity. Recently, the synthesis and antitumor activity of a specific inhibitor of GAR formyltransferase has been reported.[7,10] This compound, 5,10-dideazatetrahydrofolate (DDATHF, **3**) exhibited a wide spectrum of antitumor activity.[10] The excellent therapeutic index obtained with DDATHF provided strong impetus for the development of specific inhibitors of GAR and AICAR formyltransferases.

In this context it was appealing to develop inhibitors of purine biosynthesis that possess an intact pteridine ring system because, unlike DDATHF, they are likely to be substrates for DHFR and be converted to tetrahydro forms potentially capable of inhibiting de novo purine biosynthesis. Such compounds could be useful against those tumors that are resistant to MTX by virtue of elevated



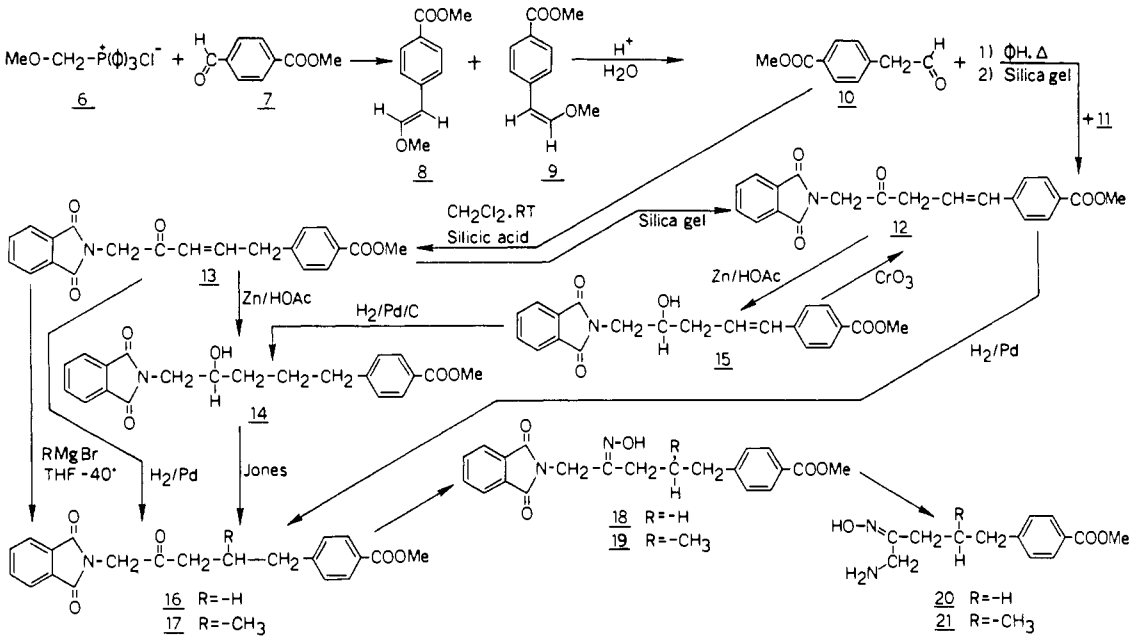
levels of DHFR.[11,12] It was therefore of interest to synthesize and evaluate the biological activity of 11-deaza-

† University of South Alabama.
‡ Tufts University Health Science Campus.
§ Burroughs Wellcome Company.

(1) Previous paper: Nair, M. G.; Dhawan, R.; Ghazala, M.; Kalman, T. I.; Ferone, R.; Gaumont, Y.; Kisliuk, R. L. *J. Med. Chem.* **1987**, *30*, 1256.
(2) Bertino, J. R. In *Antineoplastic and Immunosuppressive Agents*; Sartorelli, A. C., Johns, D. G., Eds.; Springer-Verlag: Berlin, 1975; Part II, pp 468–483. Santi, D. V. *J. Med. Chem.* **1980**, *25*, 103. Heidelberger, C.; Danenberg, P. V.; Moran, R. G. *Adv. Enzymol.* **1983**, *54*, 57.
(3) Nair, M. G.; Nanavati, N. T.; Nair, I. G.; Kisliuk, R. L.; Gaumont, Y.; Hsiao, M. C.; Kalman, T. I. *J. Med. Chem.* **1986**, *29*, 1754.
(4) Cheng, Y. C.; Dutschman, G. E.; Starnes, M. C.; Fisher, M. H.; Nanavati, N. T.; Nair, M. G. *Cancer Res.* **1985**, *45*, 598.
(5) Jones, T. R.; Calvert, A. H.; Jackman, A. L.; Brown, S. J.; Jones, M.; Harrap, K. R. *Eur. J. Cancer* **1981**, *17*, 11.
(6) Goodman, L.; DeGraw, J. I.; Kisliuk, R. L.; Friedkin, M.; Pastore, E. J.; Crawford, E. J.; Plante, L. T.; Al-Nahas, A.; Morningstar, J. R.; Kwok, G.; Wilson, L.; Donovan, E. F.; Ratzan, J. *J. Am. Chem. Soc.* **1964**, *86*, 308. DeGraw, J.; Marsh, J. P., Jr.; Acton, E. M.; Crews, O. P.; Mosher, C. W.; Fujiwara, A. N.; Goodman, L. J. *J. Org. Chem.* **1965**, *30*, 304.
(7) Taylor, E. C.; Harrington, S. R.; Fletcher, G. P.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem.* **1985**, *28*, 914.

0022-2623/89/1832-1277$01.50/0

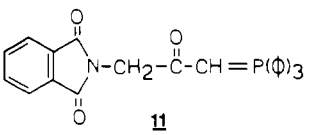
Scheme I



homofolate (**29**), 11-deaza-10-methylhomofolate (**30**), and their reduced derivatives.

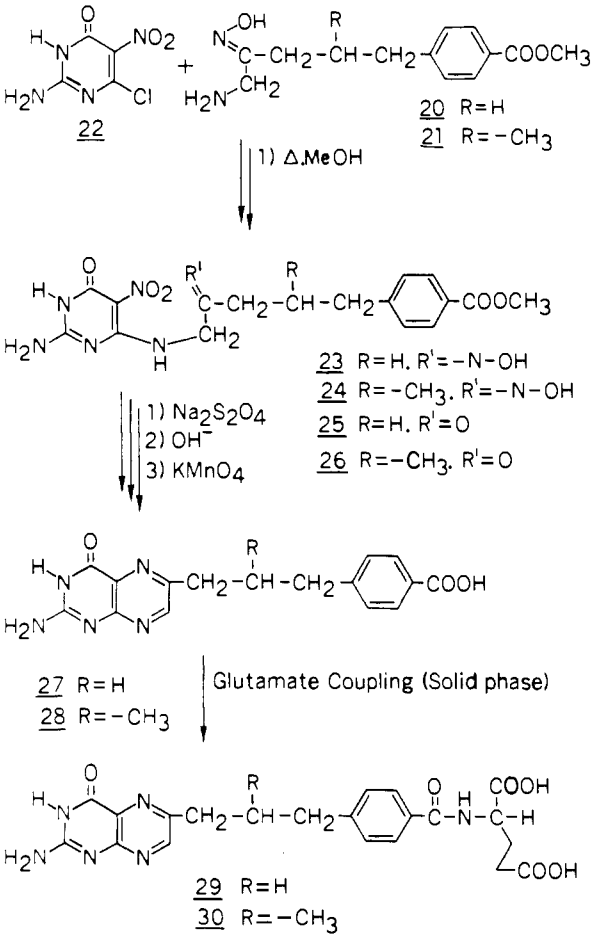
Chemistry

A Boon–Leigh type strategy was considered for the synthesis of both compounds **4** and **5**. The chemistry that has been developed for the preparation of these compounds is outlined in Schemes I–III. The generation of the Wittig reagent derived from (methoxymethyl)triphenylphosphonium chloride (**6**) was attempted by literature procedures[13] using either butyllithium or phenyllithium as a base in diethyl ether or THF. Subsequent reaction of the resulting phosphoranilidine derivative with methyl *p*-formylbenzoate (**7**) gave mixtures of products including the expected enol ethers **8** and **9** in low yields. After a number of trials using different reaction conditions, the procedure was judged to be unsatisfactory for the preparation of **8** or **9** and subsequently abandoned. However, modification of this reaction using methanol as a solvent and NaOMe as a base gave excellent results for the preparation of enol ethers **8** and **9**. The phosphoranilidine derivative from **6** was made in MeOH and it was subsequently reacted with **7** in refluxing benzene. The removal of traces of methanol from the Wittig reagent prior to its reaction with **7** was absolutely essential. Failure to remove methanol from this reaction mixture resulted in the formation of the dimethyl acetal of **7** that was extremely difficult to separate from the mixture of enol ethers **8** and **9**. Conversion of enol ethers **8** and **9** to (*p*-carbomethoxyphenyl)acetaldehyde (**10**) was accomplished by acid hydrolysis in aqueous dioxane at elevated temperature. Reaction of aldehyde **10** with 1-phthalimido-3-(triphenylphosphoranylidene)-2-propanone[14] (**11**) under



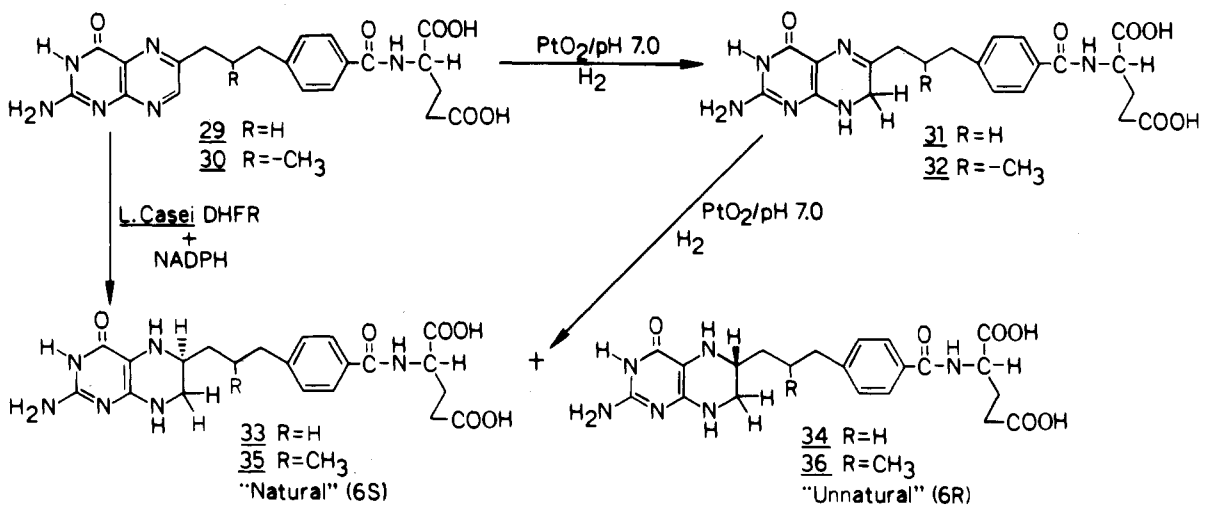
11

Scheme II



(8) Mead, J. A. R.; Goldin, A.; Kisliuk, R. L.; Friedkin, M.; Plante, L.; Crawford, E. J.; Kwok, G. *Cancer Res.* **1966**, *26*, 2374.
(9) Hakala, M. *Cancer Res.* **1971**, *31*, 813.
(10) Beardsley, P. G.; Taylor, E. C.; Grindey, G. B.; Moran, R. G. In *Chemistry and Biology of Pteridines*; Cooper, B. A., Whitehead, V. M., Ed.; Walter de Gruyter: New York, 1986; p 953.
(11) Misra, D. K.; Humphreys, S. R.; Friedkin, M.; Goldin, A.; Crawford, E. J. *Nature (London)* **1961**, *189*, 39.
(12) Nair, M. G.; Colleen, S.; Chen, S. Y.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1980**, *23*, 59.
(13) Levine, S. G. *J. Am. Chem. Soc.* **1958**, *80*, 6150.
(14) Nair, M. G. *J. Org. Chem.* **1985**, *50*, 1879.

Scheme III



neutral conditions gave a major product **12**, which was isolated after column chromatography over silica gel. The mass spectrum of **12** exhibited the expected molecular ion at 363, and its NMR resonances were consistent with the expected structure **13** (vide infra). Treatment of **12** with Zn/HOAc did not give the expected saturated **16**, but surprisingly gave a homoallylic alcohol **15** (M^+ 365), which on oxidation gave the starting ketone **12** in quantitative yield. Examination of the fragmentation pattern of **12** at this stage revealed the presence of fragments at *m/e* 363, 203, and 160 in the mass spectrum of **12**. These chemical, NMR, and mass spectral data of **12** clearly established that the product isolated from the Wittig reaction was not the expected conjugated ketone **13**, but was indeed **12**. Catalytic hydrogenation of **15** to alcohol **14** and subsequent Jones oxidation gave a saturated ketone **16**, which was the expected Zn/HOAc reduction product of **13**. Although **12** was useful for the construction of target compound **4**, after conversion to **16**, the preparation of compound **5** required enone **13** as a crucial intermediate according to the projected synthetic strategy. Therefore, attempts were made at this stage to monitor the presence of the elusive enone **13** during the reaction of **10** and **11**. It was obvious that either the conditions employed during the Wittig reaction or the procedures used for the isolation and purification of the Wittig product might have caused the migration of the double bond of the initially formed enone **13** out of conjugation from the carbonyl group. Examinations of the crude product obtained by the reaction of aldehyde **10** and reagent **11** at room temperature revealed the formation of only a single major product, the R_f value of which on silica gel plate using several solvent systems was very close to but not identical with that of **12**. This result indicated that compound **13** was indeed formed during the reaction as the initial product, but isomerization of the double bond to **12** took place, presumably during column chromatography of the reaction mixture on silica gel. Therefore, the crude reaction product of **10** and **11** was chromatographed on other supports such as acidic, basic, and neutral alumina and Florisil. In each case, the compound that was isolated from the column was the undesired ketone **12**. Finally, the problem was solved by chromatography of the reaction mixture on silicic acid hydrate (Baker) whereupon the elusive isomeric ketone **13** was isolated in good yield. The NMR spectra of **12** and **13** displayed proton resonances as expected. Significant differences in the spectra of **12** and **13** were observed in the chemical shifts of the phthalimidomethyl protons (δ 4.84 and 4.56), the allylic or benzylic protons of the butenone moiety (δ 3.74 and 3.85), and the vinyl protons (δ 6.7, 8.1 and 6.15, 7.7). Conversion of **13** to **16** was accomplished by mild reduction with Zn/HOAc at 25 °C or by catalytic hydrogenation. Reduction of **13** with Zn/HOAc at higher temperatures resulted in the formation of alcohol **14**, which was identical with the catalytic hydrogenation product of **15**.

Having constructed the crucial and common phthalimide intermediate **13**, we next directed our attention toward the preparation of **17**. Reaction of **13** with methylmagnesium bromide in the presence of CuBr at −25 °C gave the conjugate addition product **17** in 41% yield. Compounds **16** and **17** were elaborated to pteroic acid analogues **27** and **28** by employing the following common procedures.[15-17] Reaction of **16** or **17** with hydroxylamine gave the corresponding oximes **18** or **19**, which on hydrazinolysis gave the masked α-amino ketones **20** or **21**. Reaction of **20** or **21** with 6-chloro-2,4-diamino-5-nitropyrimidine (**22**) gave the corresponding pyrimidine intermediates **23** or **24** (Scheme II). Treatment of **23** or **24** with TFA/HCl at elevated temperatures resulted in the removal of the oxime protective groups with the formation of the nitro ketones **25** or **26**, respectively. Dithionite reduction of **25** or **26** was followed by base treatment that resulted in the simultaneous cyclization of the reduced products to their respective dihydropteridines and the hydrolysis of the methoxycarbonyl moieties to the carboxylic acids. These dihydrohomopteroic acid analogues, without isolation, were oxidized directly to the homopteroic acid analogues with $KMnO_4$ in presence of MeOH. Compound **27** has been previously prepared by De Graw and co-workers[18] by a synthetic route different from that reported here, but represents an alternative for the synthesis of 11-deazahomopteroic acid. Finally, homopteroic acid analogues **27** and **28** were converted to compounds **29** and **30** by coupling with diethyl L-glutamate by the solid-phase procedure followed by hydrolysis and purification by ion-exchange chromatography. Catalytic hydrogenation of **29** for 12 h yielded the (6*R*,*S*)-tetrahydro mixture **4** (Scheme III), which was purified by DEAE-cellulose chromatography. Hydrogenation of **30** for 12 h yielded a

(15) Ghazala, M.; Nair, M. G.; Toghiyani, T. R.; Kisliuk, R. L.; Gaumont, Y.; Kalman, T. I. *J. Med. Chem.* **1986**, *29*, 1263.
(16) Nair, M. G.; Otis, E. B.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1983**, *28*, 135.
(17) Nair, M. G.; Chen, S. Y.; Kisliuk, R. L.; Gaumont, Y.; Strumpf, D. *J. Med. Chem.* **1979**, *22*, 850.
(18) DeGraw, J. I.; Tsakotellis, T.; Kisliuk, R. L.; Gaumont, Y. *J. Heterocycl. Chem.* **1971**, *8*, 105.

Table I. Growth Inhibition

compound	IC_{50}, μM			
	S. faecium	*L. casei*[a]	MCF-7[b]	MOLT-4[b]
11-deazahomofolate (**29**)	0.12	2.2		
(6*R*,*S*)-11-deazatetrahydrohomofolate (**4**)	0.03	0.54	4.5	3.4
11-deaza-10-methylhomofolate (**30**)	0.25	4.0		
11-deaza-10-methyl-7,8-dihydrohomofolate (**32**)	0.04	1.7		
(6*R*,*S*)-11-deaza-10-methyltetrahydrohomofolate (**5**)	0.128	3.8		
methotrexate	0.9×10^{-3}	0.02×10^{-3}		

[a] Folate concentration 1 ng/mL (for methods, see: DeGraw, J. I.; Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M.; Nair, M. G. *J. Med. Chem.* **1974**, *17*, 552). [b] MCF7 human breast adenocarcinoma cells were maintained as monolayer cultures in RPMI 1640 medium containing 10% dialyzed heat-inactivated fetal calf serum. MOLT-4 human leukemia cells were maintained as suspension cultures in RPMI 1640 medium containing 10% dialyzed fetal calf serum. In both cases 10 nM calcium leucovorin replaced folic acid as the folate source. For determination of the IC_{50} values, MCF-7 cells were seeded at a density of 7.5×10^4 cells/mL. Drug was added 2 h after seeding, and cells were counted at 72 h.

mixture of the dihydroform **32** and the 6*R*,*S* tetrahydroform **5**. Compounds **32** and **5** could be resolved by DEAE-cellulose chromatography, whereas compounds **31** and **4** under the same conditions yielded a mixture containing 60% **31** and 40% **4**. (6*S*)-11-Deazatetrahydrohomofolate (**33**) was prepared from **29** by incubation with *L. casei* DHFR and NADPH (Scheme III).

Biological Results and Discussion

Compounds **29** and **30** and their reduced derivatives were initially evaluated as inhibitors of growth. The results of Table I show that 11-deazahomofolates inhibit growth in bacterial and mammalian systems, but their effects are much weaker than those of MTX. They are less potent against *L. casei* than against *S. faecium*, whereas with MTX the reverse is true. 11-Deazatetrahydrohomofolate (**4**) is 4 times more potent than the unreduced form (**29**) in inhibiting bacterial growth. With the corresponding 10-methyl compounds **5** and **30**, the difference in inhibition is much less pronounced; however, the dihydro compound **32** is more potent than **5** or **30**. (6*S*)-11-Deazatetrahydrohomofolate (**33**) and **4**, the diastereomeric mixture, showed similar inhibitory activity against the growth of *L. casei*. Compound **4** was moderately inhibitory to the growth of MCF-7 and MOLT-4 cells.

(6*R*,*S*)-11-Deazatetrahydrohomofolate (**4**) is a potent inhibitor of *L. casei* GAR formyltransferase ($IC_{50} = 5 \times 10^{-8}$ M), being about 5 times more effective than (6*R*,*S*)-tetrahydrohomofolate (Table II). However, **4** is a poor inhibitor of GAR formyltransferases from L1210 and MOLT-4 cells. Since the 6*S* compound **33** is at least 100 times weaker then the 6*R*,*S* form (**4**) for the *L. casei* enzyme, the inhibitory effect of **4** must be due to the 6*R* diastereomer (**34**) having the unnatural configuration at C6. It is of interest in this connection that the unnatural diastereoisomer at C6 of the substrate 10-formyltetrahydrofolate is a potent inhibitor ($K_i = 7.5 \times 10^{-7}$ M) of GAR formyltransferase from chicken liver.[19] In addition, the unnatural diastereomer at C6 of tetrahydrohomopteroyl-Glu_6 ($IC_{50} = 1 \times 10^{-7}$ M) is 40 times more inhibitory than the corresponding natural diastereomer for *L. casei* GAR formyltransferase.[20] In contrast, GAR formyltransferase from L1210 cells is inhibited almost equally by the natural ($IC_{50} = 3 \times 10^{-7}$ M) and unnatural ($IC_{50} = 7 \times 10^{-7}$) C6 diastereomers of tetrahydrohomopteroyl-Glu_6[21] and the individual C6 diastereomers of the potent GAR formyltransferase inhibitor 5-10-dideazatetrahydrofolate show similar growth inhibition of L1210 cells.[22] Thus the stereospecificity of GAR formyltransferase inhibition varies with enzyme source.

Table II. Inhibition of GAR Formyltransferase

compound	IC_{50}, M		
	L. casei[a]	L1210[a]	MOLT-4[b]
11-deazahomofolate (**29**)	$>2.5 \times 10^{-5}$	$>2.5 \times 10^{-5}$	
(6*R*,*S*)-11-deazatetrahydrohomofolate (**4**)	5×10^{-8}	1.8×10^{-5}	1.4×10^{-5}
(6*S*)-11-deazatetrahydrohomofolate (**33**)	$>5 \times 10^{-6}$		
11-deaza-10-methylhomofolate (**30**)	$>2 \times 10^{-5}$		
11-deaza-10-methyl-7,8-dihydrohomofolate (**32**)	5.5×10^{-7}		
(6*R*,*S*)-11-deaza-10-methyltetrahydrohomofolate (**5**)	1.1×10^{-5}		
(6*R*,*S*)-tetrahydrohomofolate	1×10^{-6}	$>2 \times 10^{-5}$	

[a] Assayed as described (Smith, G. K.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 404) except at 30 °C and the buffer was Tris·HCl, 50 mM, pH 7.5. Substrate concentrations were 40 μM (*L. casei*) and 60 μM (L1210) for (6*R*)-10-formyltetrahydrofolate and 0.24 mM glycinamide ribonucleotide for both extracts. [b] GAR formyltransferase purified and assayed as described (Daubner, S. C.; Young, M.; Sammons, R. D.; Courtney, L. F.; Benkovic, S. F. *Biochemistry* **1986**, *25*, 2951).

Compound **5**, the 10-methyl derivative of **4**, is 200 times less potent than **4** as an inhibitor of *L. casei* GAR formyltransferase. Of the 10-methyl derivatives, the 7,8-dihydro compound **32** shows the most striking inhibition ($IC_{50} = 5.5 \times 10^{-7}$ M). The unreduced compounds **29** and **30** were not inhibitory (Table II).

Compounds **4**, **5**, and **29–32** did not inhibit DHFR, TS, or AICAR formyltransferase activity in extracts of *L. casei* ($IC_{50} > 2 \times 10^{-5}$ M). Compounds **4** and **5** showed no activity as substrates for *L. casei* TS even with 10 times the usual enzyme concentration. This result was expected because the absence of the N11 precludes the possibility of forming the 5,11-methylene bridge necessary for substrate activity. Tetrahydrohomofolate (**2**)[23,24] and 8-deazatetrahydrohomofolate,[25] which contain an N11, both show weak substrate activity for TS.

Data for 11-deazadihydrohomofolate (**31**) are not included in Tables I and II because it proved difficult to obtain this compound free from **4** by DEAE-cellulose chromatography. A column fraction containing 60% **31** and 40% **4** showed the same activity as **4** in the tests shown in Tables I and II using *L. casei* systems. Both **31** and **32** were reduced at 5% the rate of dihydrofolate when tested

(19) Smith, G. K.; Benkovic, P. A.; Benkovic, S. J. *Biochemistry* **1981**, *20*, 4034.
(20) Kisliuk, R. L.; Gaumont, Y.; Thorndike, J.; Schmid, F. A.; Sirotnak, F. M.; Piper, J. R.; Murthy, B. R.; Nair, M. G. *Pharmacol. Ther.* In press.
(21) Thorndike, J.; Gaumont, Y.; Kisliuk, R. L.; Sirotnak, F. M.; Murthy, B. R.; Nair, M. G.; Piper, J. R. *Cancer Res.* In press.
(22) Moran, R. G.; Taylor, E. C.; Shih, C.; Beardsley, G. P. *Proc. Am. Assoc. Cancer Res.* **1987**, *28*, 274.
(23) Kisliuk, R. L.; Gaumont, Y. In *Chemistry and Biology of Pteridines*; Iwai, K., Akino, M., Goto, M., Iwanami, Y., Eds.; International Academic Printing Co.: Tokyo, 1970; p 357.
(24) Scanlon, K. J.; Cashmore, A. R.; Moroson, B. A.; Dryer, R. N.; Bertino, J. R. *Mol. Pharmacol.* **1981**, *19*, 481.
(25) DeGraw, J. I.; Colwell, W. T.; Brown, V. H.; Sato, M.; Kisliuk, R. L.; Gaumont, Y.; Thorndike, J.; Sirotnak, F. M. *J. Med. Chem.* **1988**, *31*, 150. Gaumont, Y.; Kisliuk, R. L.; Broom, A. D.; DeGraw, J. I.; Colwell, W. T. FASEB J. 2, A1554, 1988.

in the *L. casei* DHFR assay. Under the same conditions, the rate with dihydrohomofolate as substrate was 20% the rate with dihydrofolate. The presence of **4** along with **31** did not alter the DHFR assay. 11-Deaza-10-methylhomopteroic acid (**28**) exhibited an IC_{50} value of 1.5×10^{-5} M when assayed as an inhibitor of *E. coli* dihydropteroate synthase according to the procedure of Ferone et al.[26] Our data do not establish the mechanism of the growth-inhibitory effects of 11-deazahomofolates. The relatively poor inhibition of *L. casei* growth by **4** in spite of its potent inhibition of GAR formyltransferase may be due to limited membrane transport of the inhibitor.

Experimental Section

Melting points were determined on a Fisher Model 355 digital melting point analyzer. NMR spectra were run in $CDCl_3$ or CF_3COOH on 90-MHz Perkin-Elmer spectrometer with Me_4Si as an internal standard unless otherwise mentioned. Field strength of the various proton resonances is expressed in parts per million, and peak multiplicity is depicted as follows: s, singlet; br, broad singlet; d, doublet; t, triplet; q, quartet; c, unresolved multiplet, the center of which is given. Ultraviolet spectra were recorded on a Bausch and Lomb spectronic 2000 spectrometer interfaced with a Commodore superpet computer or a Gilford Response spectrometer. All HPLC analyses were done on a Waters 600 multisolvent delivery system equipped with a Model 481 UV detector and a Waters 740 data module. All mass spectra were determined by Dr. Susan Weintraub, University of Texas, San Antonio, TX. Elemental analysis were done by Galbraith Laboratories, Inc., Knoxville, TN. 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.

1-Methoxy-2-(4-carbomethoxyphenyl)ethylene (8 and 9). To a stirring solution of 20.57 g (60 mmol) of (methoxymethyl)triphenylphosphonium chloride (**6**) in 150 mL of MeOH was added 13.8 mL of a 25% solution of NaOMe in MeOH. The reaction mixture was kept at room temperature for 30 min and evaporated. The residue was mixed with 100 mL of benzene and reevaporated under reduced pressure. This procedure was repeated twice to ensure complete removal of MeOH from the product. To the crude Wittig reagent thus obtained were added 7.44 g (45.4 mmol) of methyl *p*-formylbenzoate (**7**) and 200 mL of benzene, and the mixture was refluxed for 3 h and then stirred at room temperature for 18 h. The reaction mixture was evaporated to about 50 mL and applied on a large silica gel column prepared in benzene. On elution of the column with benzene, 7.61 g of the desired product was obtained (87%, based on **7**) as a mixture of the cis and trans isomers: NMR ($CDCl_3$) δ 8.05, 7.72 (d, d, 4 H, aromatic), 7.25, 5.85 (d, d, 2 H, trans vinyl, J = 16 Hz), 6.3, 5.3 (d, d, 1 H, cis vinyl, J = 8 Hz), 3.9 (s, 3 H, carbomethoxy), 3.8, 3.71 (s, s, 3 H, OMe). The product mixture was obtained as a semisolid, which could not be crystallized. Anal. ($C_{11}H_{12}O_3$) C, H.

4-Carbomethoxyphenylacetaldehyde (10). To a cooled solution of 7.25 g (37.76 mmol) of the enol ether mixture **8** and **9** in 182 mL of THF and 34 mL of water was added 3.5 mL of concentrated H_2SO_4. The reaction mixture was refluxed for 11 h, during which time all the starting material had been consumed, as evidenced by TLC. The reaction mixture was evaporated under reduced pressure to remove THF and extracted with 500 mL of benzene. The organic layer was washed repeatedly with water until the washings were free of acid. After drying with Na_2SO_4, the benzene extract was evaporated to a viscous liquid, the NMR spectrum of which was consistent with the desired structure. **10**: yield 5.75 g (86%); NMR ($CDCl_3$) δ 9.7 (t, 1 H, aldehyde), 7.95, 7.2 (d, d, 4 H aromatic), 3.85 (s, 3 H, carbomethoxy), 3.7 (d, 2 H, CH_2). This compound was obtained as a gum and was used directly for the next step.

5-(*p*-Carbomethoxyphenyl)-1-phthalimido-4-penten-2-one (12). Compound **11** was prepared according to a previous procedure published from this laboratory.[14] A mixture of 3.0 g (18.53 mmol) of **10** and 8.58 g (18.53 mmol) of **11** in 100 mL of benzene was refluxed for 40 h, evaporated to 30 mL, and applied to a silica gel column (100 g) made in benzene. The column was eluted successively with benzene, CH_2Cl_2, and a mixture of CH_2Cl_2/MeOH (99:1). Minor components that were eluted in the earlier fractions were discarded. The major product was eluted in the CH_2Cl_2/MeOH mixture. **12**: yield 3.5 g (54%); mp 146–8 °C; NMR ($CDCl_3$) δ 7.95, 7.2 (d, d, 4 H, aromatic), 7.7 (C, 4 H, phthalimide), 7.7, 6.15 (d, d, 2 H, trans vinyl, J = 18 Hz), 4.56 (s, 2 H, phthalimidomethyl), 3.8 (s, 3 H, carbomethoxy), 3.6 (d, 2 H, allylic); MS m/e 363. Anal. ($C_{21}H_{17}NO_5$) C, H, N.

5-(*p*-Carbomethoxyphenyl)-1-phthalimido-3-penten-2-one (13). A solution of 7.0 g (39.3 mmol) of **10** and 18.20 g (39.3 mmol) of **11** in 300 mL of CH_2Cl_2 was stirred at room temperature for 40 h. After evaporation to a small volume, the crude reaction mixture was chromatographed on a column made of silicic acid hydrate (Baker). Elution of the column with a mixture of CH_2Cl_2/hexane (3:1) gave pure **13** as a white solid: yield 8.0 g (56%); mp 124–5 °C; NMR ($CDCl_3$) δ 7.95–7.2 (c, 8 H, aromatic), 7.1, 6.15 (d, d, 2 H olefin), 4.63 (s, 2 H, phthalimidomethyl), 3.85 (s, 3 H, carbomethoxy), 3.6 (d, 2 H benzylic); MS m/e 363. Anal. ($C_{21}H_{17}NO_5$) C, H, N.

5-(*p*-Carbomethoxyphenyl)-2-hydroxy-1-phthalimidopentane (14). In a typical experiment 3.63 g (10 mmol) of **13** was dissolved in 75 mL of hot glacial HOAc and the solution was cooled to 40 °C. To this solution, with stirring, was added portionwise 15 g of zinc dust during a period of 3 h. During the entire addition the temperature was maintained at 40 °C to prevent precipitation of **13** from the solvent. The reaction mixture was filtered and the residue was washed three times with 50-mL portions of CH_2Cl_2. The combined filtrate was evaporated under reduced pressure, triturated with 100 g of ice, and filtered. The product **14** was recrystallized from MeOH: yield 3.2 g (87.6%); mp 158 °C; NMR ($CDCl_3$) δ 8.05, 7.25 (d, d, 4 H, aromatic), 7.9 (c, 4 H, phthalimide), 4.5 (s, 2 H, phthalimidomethyl), 4.05 (c, 1 H, HCOH), 3.95 (s, 3 H, carbomethoxy), 2.65 (c, 6 H, methylenes); MS m/e 367. Anal. ($C_{21}H_{21}NO_5$) C, H, N.

5-(*p*-Carbomethoxyphenyl)-2-hydroxy-1-phthalimido-4-pentene (15). This experiment was carried out with **12** in a similar manner as described for the preparation of **14**. The product was crystallized from MeOH: yield 3.35 g; mp 175–6 °C; NMR (TFA) δ 7.75, 7.2 (d, d, 4 H, aromatic), 7.45 (c, 4 H, phthalimide), 7.68, 6.3 (d, d, 2 H, vinyl), 4.51 (c, 1 H, HCOH), 4.5 (t, 2 H, phthalimidomethyl), 3.7 (s, 3 H, carbomethoxy), 3.3 (d, 2 H, allylic); MS m/e 365. Anal. ($C_{21}H_{19}NO_5$) C, H, N.

Jones Oxidation of 15. To a stirring solution of 1.825 g (5 mmol) of **15** in 100 mL of acetone was added dropwise 12 mL of freshly prepared Jones reagent (26.72 g of CrO_3 in 23% H_2SO_4 in H_2O) during a period of 20 min. The acetone solution was decanted and evaporated to 15 mL under reduced pressure, and 100 g of ice was added. On trituration, a white crystalline material was formed, which was filtered, washed several times with water, and dried in vacuum. The compound thus isolated in almost quantitative yield was identified to be **12** in all respects.

Hydrogenation of 12 or 13. Preparation of 5-(*p*-Carbomethoxyphenyl)-1-phthalimidopentan-2-one (16). Identical procedures were used for both compounds. A solution of 3.63 g (10 mmol) of either **12** or **13** was made in 200 mL of EtOAc and hydrogenated at 10 psi with 363 mg of 5% palladium on carbon as a catalyst for 18 h and filtered. The filtrate was evaporated under reduced pressure, and the solid residue was recrystallized from MeOH. Compound **16** was obtained in quantitative yield from both **12** and **13**: mp 126–7 °C; MS m/e 365; NMR ($CDCl_3$) δ 8.1, 7.35 (d, d, 4 H, aromatic), 7.9 (c, 4 H, phthalimide), 4.51 (s, 2 H, phthalimidomethyl), 3.95 (s, 3 H, carbomethoxy), 2.65 (c, 6 H, methylenes). Anal. ($C_{21}H_{19}NO_5$) C, H, N.

Jones oxidation of **14**, as described for **15**, gave **16** in quantitative yield, which was identical in all respects with an authentic sample of **16** obtained by hydrogenation of either **12** or **13**.

5-(*p*-Carbomethoxyphenyl)-4-methyl-1-phthalimidopentan-2-one (17). A stirring mixture of 5.4 g (15 mmol) of enone **6** and 900 mg of CuBr in 300 mL of dry THF under N_2 was cooled to −25 °C. To this mixture was added dropwise a solution of 10 mL of 3 M CH_3MgBr in THF, diluted to 100 mL with dry THF during a period of 1 h. After 2 h at −25 °C, the solution was allowed to stir at room temperature for 12 h, and a saturated

(26) Ferone, R.; Webb, S. R. In *Chemistry and Biology of Pteridines*; Pfleiderer, W., Ed.; Walter de Gruyter, New York, 1975; p 61.

solution of 50 mL of NH_4Cl in water was slowly added followed by 100 mL of distilled water. The solvents were evaporated under reduced pressure to ~50 mL, and it was extracted with two 200-mL portions of EtOAc. The EtOAc extract was washed repeatedly with water, dried over Na_2SO_4, and evaporated.

The residue thus obtained was chromatographed on a silica gel column, and the desired product was isolated as a white crystalline material: yield 2.3 g (41%); mp 100–1 °C; NMR ($CDCl_3$) δ 8.0–7.3 (c, 8 H, aromatic, phthalimide), 4.47 (s, 2 H, phthalimidomethyl), 3.95 (s, 3 H, carbomethoxy), 2.65 (d, 2 H, benzylic), 2.45 (c, 3 H, acetonyl, $HCCH_3$), 1.01 (d, 3 H, methyl); MS *m/e* 379. Anal. ($C_{22}H_{21}NO_5$) C, H, N.

5-(*p*-Carbomethoxyphenyl)-1-phthalimidopentan-2-one Oxime (18) and 5-(*p*-Carbomethoxyphenyl)-4-methyl-1-phthalimidopentan-2-one Oxime (19). A solution of 3.65 g (10 mmol) of **16** and 903 mg (13 mmol) of hydroxylamine hydrochloride in 400 mL of a 1:1 mixture of pyridine and MeOH was refluxed for 1 h and evaporated, and the residue was triturated with 100 g of ice. The resulting solid was filtered, washed with water, and dried: yield 3.5 g (92%); mp 164 °C; MS (FAB) *m/z* 381 (MH^+). Anal. ($C_{21}H_{20}N_2O_5$) C, H, N.

Compound **19** was also prepared in a similar manner starting from 4.52 g (12 mmol) of **17** and 1.04 g (15 mmol) of hydroxylamine hydrochloride: yield 4.69 g (99%); mp 131–2 °C; MS (FAB) *m/z* 395 (MH^+). Anal. ($C_{22}H_{22}N_2O_5$) C, H, N.

1-[(2-Amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]-5-(*p*-carbomethoxyphenyl)-2-pentanone Oxime (23) and Its 4-Methyl Analogue (24). A. Preparation of 1-Amino-5-(*p*-carbomethoxyphenyl)-2-pentanone Oxime (20) and Its 4-Methyl Analogue (21). To a solution of 3.80 g (10 mmol) of **18** in 500 mL of MeOH at 45 °C was added under N_2 480 mg (15 mmol) of anhydrous hydrazine, and the mixture was stirred for 48 h. Examination by TLC at this stage indicated that all starting material had been consumed. To this reaction mixture 10 mL of 1 N HCl was added and most of the methanol was evaporated off at reduced pressure. After dilution to 100 mL, the pH of the resulting solution was adjusted to 3.0 by 0.1 N HCl and filtered. The pH of the filtrate was adjusted carefully to 8.5 by concentrated NH_4OH, whereupon an oily residue was formed in the filtrate. This residue was extracted repeatedly with EtOAc (3 × 50 mL). The combined EtOAc extract was washed several times with water, dried over Na_2SO_4, and evaporated to obtain a semisolid: NMR ($CDCl_3$) δ 8.05, 7.32 (d, d, 4 H, aromatic), 3.85 (s, 3 H, carbomethoxy), 3.95 (d, 2 H, aminomethyl), 2.4–2.75 (c, 6 H, methylenes). The aminomethyl protons appeared as a doublet due to the presence of syn and anti isomers. This compound was used for the next step without further characterization; yield 2 g, 80%.

The 4-methyl analogue **21** was likewise prepared from **19**. The NMR spectrum of **21** was similar to that of **20** except for the methyl signal at 1.1 and appropriate changes in the methylene envelope centered around 2.4–2.75 ppm; yield, 83%.

B. **Reaction of 20 and 21 with 2-Amino-6-chloro-4-hydroxy-5-nitropyrimidine.** The following procedure was used for the preparation of either **23** or **24**. In a typical experiment 10 mmol of either **20** or **21** was dissolved in 100 mL of MeOH. Compound **22** obtained by nitration of 2-amino-6-chloro-4-hydroxypyrimidine (10 mmol) was also dissolved in 200 mL of hot MeOH. These two solutions were mixed in a 500-mL round-bottomed flask and refluxed under stirring for 1 h, and 1.31 mL (10 mmol) of 4-methylmorpholine was added. The reflux was continued for another 3 h and the solution was evaporated to ~50 mL, whereupon a yellow precipitate was formed. After cooling overnight at 4 °C, the precipitate was collected by filtration, washed with water, followed by a small amount of MeOH, and dried to obtain either **23** or **24** in 75% yield. Compound **23**: mp 182–5 °C; UV λ_{max} (0.1 N NaOH) 338 nm; MS (FAB) *m/z* 405 (MH^+); NMR (TFA) δ 8.1, 7.35 (d, d, 4 H, aromatic), 3.85 (s, 3 H, carbomethoxy), 3.78 (br, 2 H, aminomethyl), 2.4–2.8 (c, 6 H, methylenes). Anal. ($C_{17}H_{20}N_6O_6$) C, H, N. Compound **24**: mp 172–3 °C; UV λ_{max} (0.1 N NaOH) 237 nm; MS (FAB) *m/z* 419 (MH^+); NMR (TFA) δ 8.05, 7.28 (d, d, 4 H, aromatic), 3.82 (s, 3 H, carbomethoxy), 3.72 (br, 2 H, aminomethyl), 2.4–2.8 (c, 5 H, methylenes), 1.05 (d, 3 H, methyl). Anal. ($C_{18}H_{22}N_6O_6$) C, H, N.

1-[(2-Amino-4-hydroxy-5-nitropyrimidin-6-yl)amino]-5-(*p*-carbomethoxyphenyl)pentan-2-one (25) and Its 4-Methyl Analogue (26). To a stirring solution of 2.025 g (5 mmol) of **23** or 2.09 g (5 mmol) of **24** in 50 mL of TFA maintained at 60 °C was slowly added 50 mL of 1 N HCl during a period of 30 min. After the first 15 min of HCl addition, a creamy white precipitate was formed in both cases. The solution was evaporated at 60 °C under reduced pressure to 25–30 mL, chilled, and triturated with 250 g of crushed ice. The off-white precipitate thus obtained was collected by filtration, washed several times with water and finally with a small amount of MeOH, and dried. Compounds **25** and **26** were obtained in almost quantitative yield.

Compound **25**: mp 210–1 °C; MS (FAB) 390 (MH^+); UV λ_{max} (0.1 N NaOH) 232 nm. The NMR spectra of **25** and **26** in TFA were very similar to those of their precursors. Anal. ($C_{17}H_{19}N_5O_6$) C, H, N. Compound **26**: mp 224–5 °C; MS (FAB) 404 (MH^+); UV λ_{max} (0.1 N NaOH) 333 nm. Anal. ($C_{18}H_{21}N_5O_6$) C, H, N.

Conversion of 25 and 26 to 11-Deazahomopteroic Acid (27) and 11-Deaza-10-methylhomopteroic Acid (28). As described previously from this laboratory,[14-17] these multistep procedures involving the dithionite reduction of **25** and **26** followed by the cyclization, hydrolysis, and oxidation of the reduction products to **27** and **28** were carried out in a single sequence without isolation of any of the intermediates. Development of such a sequence has become necessary due to the instability of the reduction products and dihydropteridines with the accompanying difficulties in their characterization. To a stirring solution of 1.95 g (5 mmol) of **25** in 40 mL of DMF maintained at 60–5 °C was added 14 g of sodium dithionite. Distilled water (40 mL) was added gradually portionwise to this solution during a period of 30 min, when a clear solution was obtained, and the color lightened. Examination of an aliquot of this solution by UV spectroscopy indicated complete reduction of the nitro group as evidenced by the disappearance of the absorption peak around 333 nm. The solution was cooled and triturated with 400 g of crushed ice. The white precipitate thus obtained was filtered, washed with water, and stirred under N_2 with a solution of 30 mL of acetonitrile and 100 mL of 0.1 N NaOH for 8 h. The solution was then exposed to air, and oxidation was carried out by adding 15 mL of MeOH followed by dropwise addition of 10 mL of 5% $KMnO_4$ during a period of 20 min. The solution was stirred for an additional 20 min. An aliquot was withdrawn and filtered. The UV spectrum of this solution exhibited a well-defined peak at 360–365 nm, which was indicative of the formation of a pteridine ring system. The main reaction mixture was filtered, the pH was adjusted to 7.5 with 1 N HCl, and it was evaporated to ~50 mL. The clear yellow solution was chilled and acidified to pH 3.5 with glacial HOAc, whereupon a thick yellow precipitate was formed, which was filtered, washed several times with water, and dried. Final purification of the product was carried out by DEAE-cellulose chromatography using a 0–0.5 M NaCl gradient in 0.005 M phosphate buffer at pH 7.0: yield 650 mg (40%); mp >300 °C; UV λ_{max} (0.1 N NaOH) 247 nm (ϵ = 29590), 363 (6480); NMR (TFA) δ 8.78 (s, 1 H, H_7), 8.05, 7.28 (d, d, 4 H, aromatic), 3.05–2.8 (c, 6 H, bridge methylenes); MS (FAB) 326 (MH^+). Anal. ($C_{16}H_{15}N_5O_3$) C, H, N.

The 10-methyl analogue **28** was also prepared according to the procedure described for **27**: yield (from **26**) 47%; mp >300 °C; UV λ_{max} (0.1 N, NaOH) 247 nm (ϵ = 30180), 364 (6512); NMR (TFA) δ 8.8 (s, 1 H, H_7), 8.1, 7.3 (d, d, 4 H, aromatic); 3.04 (d, 2 H, H_9), 2.6–2.8 (c, 3 H, H_{10}, H_{11}), 1.1 (d, 3 H, 10-methyl); MS (FAB) 340 (MH^+). Anal. ($C_{17}H_{17}N_5O_3$) C, H, N.

11-Deazahomofolic Acid (29) and 11-Deaza-10-methylhomofolic Acid (30). These two compounds were prepared from homopteroic acid analogues **27** and **28** by a solid-phase glutamate coupling procedure. To a solution of 0.5 mmol of the desired homopteroic acid analogue in 25 mL of a mixture of DMF/$(Me)_2SO$ (5:3) at 0 °C was added 0.65 mmol (0.07 mL) of *N*-methylmorpholine. After 5 min, 0.5 mmol (0.065 mL) of freshly distilled isobutyl chloroformate was added, and the solution was removed from the ice bath. After 30 min at room temperature, the solution was shaken with 2 g of (1 mmol) of deprotected Merrifield peptide resin that was esterified with *t*-Boc-L-glutamic acid α-benzyl ester. After 18 h, the resin was filtered and shaken vigorously with 20 mL of a mixture of 2 N NaOH and *p*-dioxane (1:1) under N_2 for 1.25 h and filtered. The filtrate was diluted to 450 mL with water, adjusted to pH 7.0 with 1 N HCl, and applied to a DEAE-cellulose column. The column was eluted with a linear NaCl gradient ranging from 0 to 0.5 M in 0.005 M

phosphate buffer at pH 7.0. All fractions corresponding to the product were pooled, evaporated to ~40 mL, and acidified with 1 N HCl to pH 3.5, whereupon a yellow precipitate was formed. The precipitate was filtered, washed with water, and dried; yield 40–50% based on the homopteroic acid used. Compound **29**: mp >300 °C; UV (0.1 N NaOH) λ_{max} 248 nm (ϵ = 30 175), 365 (6758); NMR (TFA) δ 8.91 (s, 1 H, H_7), 7.85, 7.2 (d, d, 4 H, aromatic), 4.35 (t, 1 H, glutamate), 3.5–2.2 (c, 10 H, bridge methylene, glutamate); MS (FAB) 455 (MH^+). Anal. ($C_{21}H_{22}N_6O_6$) C, H, N. Compound **30**: mp >300 °C; UV (0.1 N NaOH) λ_{max} 249 nm (ϵ = 30 370), 365 (6822); NMR (TFA) δ 8.95 (s, 1 H, H_7), 7.80, 7.15 (d, d, 4 H, aromatic), 4.32 (t, 1 H, glutamate α-H), 3.55–2.2 (c, 9 H, bridge protons, glutamate), 1.03 (s, 3 H, methyl); MS (FAB) 469 (MH^+). Anal. ($C_{22}H_{24}N_6O_6$) C, H, N.

Biological Evaluation. (6*R*,*S*)-11-Deazatetrahydrohomofolate (4), (6*R*,*S*)-11-Deaza-10-methyltetrahydrohomofolate (5), 11-Deaza-7,8-dihydrohomofolate (31), 11-Deaza-10-methyldihydrohomofolate (32), and (6*S*)-11-Deazatetrahydrohomofolate (33). A solution of **29** (1 mg/mL) in 0.1 M potassium phosphate, pH 7.0, was hydrogenated 14 h at atmospheric pressure with PtO_2 catalyst (1 mg/mL). The absorbance maximum of the tetrahydro derivative **4** was 303 nm at pH 7.4 with a molar absorbance coefficient of 7000 based on the weight of **29** added. Since dithionite reduction[27] of **29** to completion resulted in **4** rather than the expected **31**, synthesis of the latter was achieved by catalytic hydrogenation as above for 2.5 h. The resulting mixture of **4** and **31** still showed an absorbance maximum at 303 nm but also showed a high absorbance at 340 nm similar to dihydrofolate. Chromatography on DEAE-cellulose[28] (modified by having a 0–1.0 M NaCl gradient rather than 0.2–1.0 M as in ref 22) yielded a single peak with a shoulder. The peak fraction showed a typical tetrahydrofolate spectrum with a single peak at 303 nm (303/340 = 13.0) whereas the shoulder had a higher absorbance at 340 nm (303/340 = 4.2) typical of dihydrofolate. The content of **31** in the mixture (60%) was estimated by assuming the same absorbance coefficient at 340 nm for **31** as was found for **32**.

Hydrogenation of **30** for 14 h as above yielded a roughly equimolar mixture of **5** and **31**, which was resolved into two separate peaks by DEAE-cellulose chromatography.[28] As with **4** and **30**, both **5** and **31** had the same absorbance maximum (308 nm at pH 7.4 for **5** and **31**) with the 308/340 absorbance ratio being 13.3 for **5** and 2.3 for **31**. A molar absorbance coefficient at 308 nm of 7700, based on the weight of the starting material, **30**, was used for **5** and **31**.

Compound **33** was prepared by enzyme catalyzed reduction[29] of **29** at pH 5.5 using *L. casei* DHFR and an enzymatic NADPH regenerating system. The incubation mixture consisted of 0.05 M sodium acetate buffer, pH 5.5, 0.1 M 2-mercaptoethanol, 0.01 M glucose 6-phosphate, 20 μM NADP, 0.17 mM **29**, 0.24 unit/mL glucose-6-phosphate dehydrogenase (Sigma), 384 units/mL DHFR (1 unit of enzyme catalyzes the conversion of 1 μmol of substrate to product/min at 37 °C). The reaction was followed at 340 nm to completion (3 h). Additional 2-mercaptoethanol was added to 0.2 M and the mixture chromatographed on a 1.8 × 10 cm DEAE-cellulose column using a linear gradient generated from 500 mL of 0.005 M Tris·HCl, 0.2 M 2-mercaptoethanol, pH 7.4, and 500 mL of the same solution containing, in addition, 0.5 M NaCl. The product (**33**) had the same absorbance spectrum as **4** prepared by catalytic reduction as above.

Antimicrobial activity of all analogues were determined according to previously published procedures.[30,31] Thymidylate synthase assays were performed according to the procedure of Wahba and Friedkin[32] and DHFR assays by the method of Pastore et al.[33]

Acknowledgment. This investigation was supported by Grants CA 27101, CA 32687 (M.G.N.), and CA 10914 (R.L.K.) from the National Cancer Institute, USPHS/Department of Health and Human Services.

Registry No. (6*R*)-**4**, 119770-36-4; **5**, 119787-36-9; **6**, 4009-98-7; **7**, 1571-08-0; **8**, 119770-37-5; **9**, 119770-38-6; **10**, 106918-32-5; **11**, 96056-26-7; **12**, 119770-39-7; **13**, 119770-40-0; **14**, 119770-41-1; **15**, 119770-42-2; **16**, 119770-43-3; **17**, 119770-44-4; **18**, 119770-45-5; **19**, 119770-46-6; **20**, 119770-47-7; **21**, 119770-48-8; **22**, 1007-99-4; **23**, 119770-49-9; **24**, 119770-50-2; **25**, 119770-51-3; **26**, 119770-52-4; **27**, 31419-64-4; **28**, 119770-53-5; **29**, 119770-54-6; **30**, 119770-55-7; **31**, 119770-56-8; **32**, 119770-57-9; **33**, 119770-58-0; DHFR, 9002-03-3; GAR formyltransferase, 9032-02-4; 2-amino-6-chloro-4-pyrimidinone, 1194-21-4.

(27) Friedkin, M.; Crawford, E. J.; Misra, D. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1961**, *21*, 176.
(28) Kisliuk, R. L.; Gaumont, Y.; Baugh, C. M. *J. Biol. Chem.* **1974**, *249*, 4100.
(29) Fontecilla-Camps, J. C.; Bugg, C. E.; Temple, C., Jr.; Rose, J. D.; Montgomery, J. A.; Kisliuk, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 6114.
(30) Kisliuk, R. L.; Strumpf, D.; Gaumont, Y.; Leary, R. P.; Plante, L. T. *J. Med. Chem.* **1977**, *20*, 1531.
(31) Chaykovsky, M.; Rosowsky, A.; Papathanosopoulos, N.; Chen, K. N.; Modest, E. J.; Kisliuk, R. L.; Gaumont, Y. *J. Med. Chem.* **1974**, *17*, 1212.
(32) Wahba, A. J.; Friedkin, M. *J. Biol. Chem.* **1962**, *237*, 3794.
(33) Pastore, E. J.; Plante, L. Y.; Kisliuk, R. L. *Methods Enzymol.* **1974**, *34*, 281.