Methotrexate Analogues. 12. Synthesis and Biological Properties of Some Aza Homologues

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Methotrexate analogues, in which an additional nitrogen atom is inserted between the phenyl ring and the carbonyl group of the side chain, were prepared by photochemical methods. The compounds were less inhibitory toward dihydrofolate reductase and thymidylate synthetase derived from *Lactobacillus casei* than was methotrexate. They were also less cytotoxic against human lymphoblastic leukemia cells (CCRF-CEM). In vivo against L-1210 leukemia in mice, the aza homologue of methotrexate showed significant antitumor activity (%ILS = 55%) compared to methotrexate (%ILS = 88%).

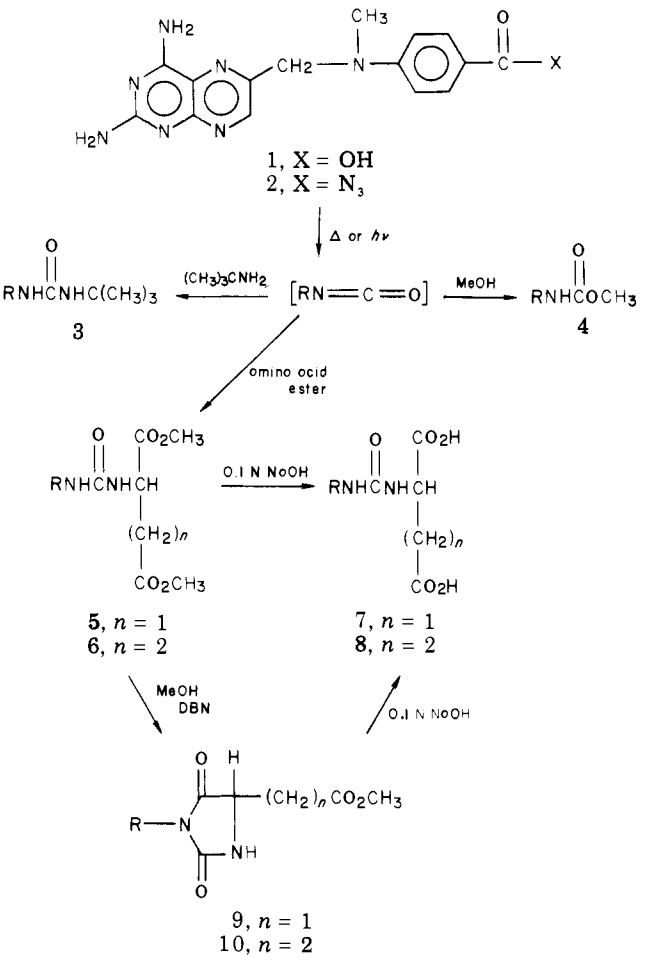
The synthesis and biological properties of homologues of folic acid and its derivatives have been reported by a number of investigators. These homologues include those in which the C^9–N^{10}-bridge region has been lengthened by additional atoms[1-9] and those in which spacer atoms have been inserted between the phenyl ring and the carbonyl group of the side chain.[10,11] A classical example is homofolic acid,[1] which differs structurally from the parent compound in that it contains an additional methylene group between positions 9 and 10. Tetrahydrohomofolic acid was shown to be a specific inhibitor of the enzyme thymidylate synthetase derived from *Escherichia coli*[12] and to have significant antitumor activity against an antifolate-resistant variant of L-1210 leukemia.[13] A stable derivative, 5-methyltetrahydrohomofolic acid,[14] showed improved antileukemic activity,[15] and this compound is currently undergoing further development with an aim toward clinical trial.[16]

One of the most widely used and most effective antitumor agents is the antifolate methotrexate (4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid, amethopterin, MTX).[17] Its mechanism of antitumor action involves strong inhibition of the enzyme dihydrofolate reductase, thus interfering with the biosynthesis of reduced folates which are essential for one-carbon transfer metabolism, including the biosynthesis of nucleotides. For the past several years we have been engaged in the preparation and biological evaluation of MTX analogues designed to improve the transport properties of the drug[18,19] and/or to alter its enzyme-binding properties in the direction of reduced inhibition of dihydrofolate reductase and enhanced inhibition of other folate enzymes such as thymidylate synthetase.[20]

Several years ago, we found[19] that the compound 4-amino-4-deoxy-N^{10}-methylpteroic acid (1, Scheme I) reacted with diphenylphosphoryl azide to form a stable carbonyl azide **2**. The isolation of this compound provided the starting material for the preparation of several MTX analogues in which a nitrogen spacer atom is inserted between the phenyl ring and the carbonyl group of the side chain. We were interested in studying the growth-inhibitory and enzyme-inhibitory properties of these compounds and in their in vitro and in vivo antitumor evaluation.

Chemistry. The pteroic acid analogue **1** (Scheme I) has been previously totally synthesized in this laboratory.[18] However, in this work it was prepared by enzymatic cleavage of MTX[21] with carboxypeptidase G. Treatment of this compound with diphenylphosphoryl azide[22] in Me_2SO precipitated the yellow azide **2** (as the Me_2SO monosolvate) in excellent yield. The azide was stable at 0 °C for a prolonged period of time and was only slightly photosensitive in ordinary light. Upon heating or irradiation with ultraviolet light (Rayonet apparatus, 300-nm lamps), in dimethylacetamide (DMAC), the azide decomposed with loss of nitrogen via a Curtius rearrangement. The intermediate isocyanate could not be isolated, but in the presence of excess *tert*-butylamine or MeOH the urea **3** or the carbamate **4**, respectively, could be isolated in good yields.

Scheme I



The carbonyl azide **2** does not react appreciably with an excess of the amino acid esters dimethyl L-aspartate or dimethyl L-glutamate at room temperature in DMAC. After 6 h, TLC (silica gel; 5–20% MeOH–$CHCl_3$) showed

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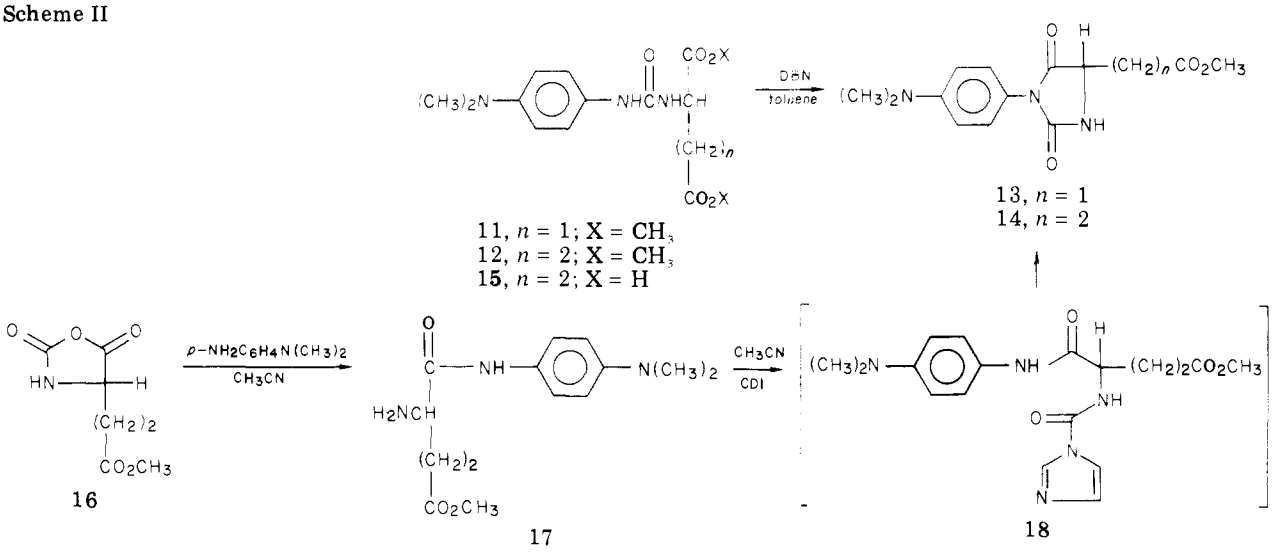
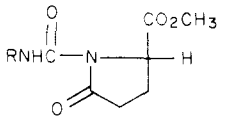
Scheme II



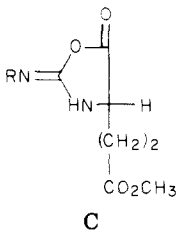
the formation of no new products. Only after 24 h was the formation of a small amount of a new product evident in each case.[23] The sluggish reactivity of the carbonyl azide toward direct displacement by the amino group of the amino acid esters is most likely due to the deactivation of the carbonyl group by resonance electron donation of the *p*-amino moiety (N^{10}). However, under photolytic conditions (see Experimental Section) the amino acid esters reacted with the intermediate isocyanate to give the aza homologues **5** and **6**, respectively, in about 50% yields. These products were found to be homogeneous by TLC and NMR and were optically active.

Hydrolysis of the diesters **5** and **6** with dilute NaOH in MeOH gave the desired diacids **7** and **8** in good yields. High-performance liquid chromatography (LC) on a reverse phase column did not show the presence of any major impurities. No MTX (<0.5%) was found as a contaminant in compound **8** and none of the corresponding aspartate analogue was found as a contaminant of **7**. Polarimetric measurements on the diacids **7** and **8** revealed that they were racemic. The reason for this was not apparent at first, since it is known[24] that optically active MTX diesters are not appreciably racemized under conditions of dilute alkaline hydrolysis. The racemization of the chiral center in **7** and **8** must therefore be caused by the presence of the additional nitrogen atom in these compounds. Subsequent experiments, as described below, provided some insight into this matter.

We found that the glutamate diester **6**, upon prolonged heating in MeOH or $CHCl_3$, was slowly converted into a new racemic compound and that this conversion was greatly accelerated by the addition of the strong organic base 1,5-diazabicyclo[3.4.0]non-5-ene (DBN). Elemental and NMR analysis of the new compound showed the loss of CH_3OH from the starting material. Alkaline hydrolysis gave the diacid **8**. Three possible structures for the compound are the hydantoin **10** (N-acylation via cyclization of the α-carbomethoxy group), the pyrollidinone (structure B; N-acylation via cyclization of the γ-carbomethoxy group), and the 2-iminooxazolidin-5-one (structure C; O-acylation via cyclization of the α-carbomethoxy group). It was subsequently found that the aspartate diester **5** also reacted under the same conditions to give a racemic product whose NMR, IR, and UV spectra were similar to that of the product derived from **6**. This evidence indicated that both new products were structurally similar and tentatively eliminated the pyrollidinone structure B, since **5** cannot form a pyrollidinone. Since it is known[25] that hydantoins with a chiral center at the 5 position and a phenyl substituent at the 3 position racemize easily under basic conditions and since the NMR spectra of both compounds showed no evidence of geometrical isomers (syn and anti, as might be expected for structure C),[26] we favored the hydantoin structures **9** and **10** for these products. Indeed, if **9** and **10** are intermediates in the alkaline hydrolysis of the diesters **5** and **6**, then this accounts for the racemic nature of the diacids **7** and **8**. Because, on the basis of the spectral data, we could not distinguish unequivocally between the hydantoin structures and the oxazolidinone structures C, we initiated work with model compounds in order to further verify the structural nature of the cyclized products.



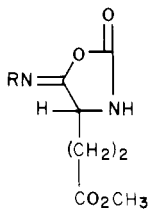
B



C

p-(Dimethylamino)benzoic acid was treated with diphenylphosphoryl azide to give the carbonyl azide. This compound was photolytically rearranged in the presence of dimethyl L-glutamate to give the optically active diester **12** (Scheme II). The aspartate analogue **11** was prepared by direct coupling of dimethyl L-aspartate with 4-(dimethylamino)phenyl isocyanate. Under reaction conditions similar to those for the MTX analogues, these diesters were cyclized with DBN in toluene to give racemic products **13** and **14**, and alkaline hydrolysis of **12** gave racemic diacid **15**. Spectral data on compounds **13** and **14** indicated that they had similar structures, but we could not distinguish between the hydantoin structure and the oxazolidinone structure C.

A synthetic scheme was then devised, which unequivocally proved that compound **14** had the hydantoin structure shown in Scheme II. The *N*-carboxyanhydride of γ-methyl L-glutamate **16** was treated with *p*-(dimethylamino)aniline in CH_3CN to give the anilide **17**. We anticipated that this 2-aminoamide might react with *N,N'*-carbonyldiimidazole[27] (CDI) to give, as an intermediate,[28] the *N*-imidazolylcarbonyl derivative **18**. The latter could be expected to react further by intramolecular ring closure to yield either the hydantoin **14** (via C–N ring closure)[29] or the 5-iminooxazolidin-2-one (structure D, via



D

C–O ring closure). In the actual event, **17** reacted smoothly with the carbonyldiimidazole in CH_3CN, without any added base, to yield an *optically active* product which was identified as enantiomeric **14**. This product was rapidly converted by DBN in MeOH to a racemic product, identical in all respects with racemic **14** obtained by cyclization of the diester **12**. Since the products of both of these reaction pathways are identical and since the hydantoin structure is the only possible product common to both pathways, it follows that compound **14** (and therefore compound **13**, based on similar spectral data) must be the hydantoin. Based on this work with the model compounds, it seems reasonable to conclude that compounds **9** and **10** also have the hydantoin structures shown in Scheme I.

Biological Results. The compounds listed in Table I were evaluated for growth-inhibitory activity against the folate-dependent organism *Lactobacillus casei* (ATCC 7469) and also for inhibition against the enzymes dihydrofolate reductase and thymidylate synthetase, derived from this organism. None of the compounds were comparable to MTX in their inhibition of *L. casei* growth. Against dihydrofolate reductase the diacids **7** and **8** were equipotent inhibitors, although less effective than MTX, and the hydantoin **10** was the least effective inhibitor. The diacids were only weak inhibitors of thymidylate synthetase.

These same compounds (Table II) were evaluated for inhibitory activity, in vitro against the CCRF-CEM human lymphoblastic leukemia cell line in culture and in vivo against L-1210 leukemia in mice. In culture, as well as in vivo, the MTX aza homologue **8** was more effective than the aspartate analogue **7**, and the hydantoin **10** was the least effective inhibitor, showing no antitumor activity in vivo. The significant in vivo antitumor activity shown by compound **8**, compared to the other compounds, may be due in large part to better penetration into the cell, possibly via the same active-transport mechanism as MTX. In this respect, we surmise that the L enantiomer, since it resembles MTX in spatial configuration, would be transported more readily than the D enantiomer and would have the greater antitumor effectiveness.

Experimental Section

IR spectra were taken with a Perkin-Elmer Model 137B or a Beckman Acculab 8 double-beam recording spectrophotometer. Quantitative UV spectra were measured on Cary Model 11 and 15 spectrophotometers. Proton NMR spectra were determined on a Varian T-60 instrument with Me_4Si as internal standard. Optical rotations were measured in a 1-dm quartz cell on a Perkin-Elmer 241MC spectropolarimeter; cell temperature was maintained by a Lauda RC-3B circulator. LC analyses were performed on a Waters Associates, Inc. (Milford, MA), Model ALC202 equipped with a U6K injector, a M-6000 pump, and a Schoeffel Spectral Flow Monitor Model SF770, monitoring at 270 nm. The column was a Waters Associates C_{18} Bondapak micropore reverse phase. Photochemical reactions were performed under N_2 in quartz vessels in a Rayonet RS (RPR-204) preparative reactor containing four 3000-Å lamps.

TLC was routinely performed on Eastman 13181 silica gel with 5–20% MeOH–$CHCl_3$ or on Eastman 13254 cellulose for the carboxylic acids with 5% $NaHCO_3$ or the buffer system 0.5 M NaCl, 0.02 M 2-mercaptoethanol, 0.05 M KH_2PO_4 (pH 6.75).

Table I. Inhibition of *L. casei* (ATCC 7469) Growth and Dihydrofolate Reductase and Thymidylate Synthetase Derived from *L. casei* by MTX Analogues[a]

	concn for 50% inhibn		
compd	*L. casei* growth, ng/mL	dihydrofolate reductase, M ($\times 10^{-8}$)	thymidylate synthetase, M ($\times 10^{-6}$)
5	6	60	
6	6	30	
7	150	5	190
8	8	8	250
10	104	400	
MTX	0.01	0.3	100

[a] Microbiological assays[30] and enzyme assays[31] were carried out as described previously. See ref 20 for substrate concentrations in the enzyme assays.

Combustion analyses were performed by Galbraith Laboratories, Knoxville, TN. Where analyses are indicated only by the symbols of the elements, the results were within ±0.4% of the theoretical values. Melting points were measured in vacuum-sealed Pyrex capillary tubes on a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, MA) and are corrected.

Dimethyl L-aspartate hydrochloride and dimethyl L-glutamate hydrochloride were prepared from the L acids with MeOH and HCl gas and were recrystallized from MeOH–THF. The aspartate hydrochloride had $[\alpha]^{25}_D$ +16.36 (*c* 4.438, MeOH) and +12.51 (*c* 6.514, 0.1 N HCl). The glutamate hydrochloride had $[\alpha]^{25}_D$ +25.3 (*c* 2.095, 1 N HCl), lit.[33] $[\alpha]^{21}_D$ +26.0 (*c* 5.0, H_2O).

4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoic Acid (1). NaMTX (equivalent to 12.0 g, 26.4 mmol) was dissolved in 400 mL of 0.1 M Tris buffer. Carboxypeptidase G (~50 units)[34] and $ZnCl_2$ (8 mg) were added and the pH of the solution was adjusted to 7.2 with concentrated HCl. The solution was shaken in an incubator at 38 °C for 2 days, and the mixture (pH 8.4) was then adjusted to pH 3.5 with dilute HCl. The precipitated yellow solid was filtered; washed with H_2O, EtOH, and Et_2O; and dried: yield 9.5 g (97.5% for $C_{15}H_{15}N_7O_2 \cdot 0.5HCl \cdot 1.5H_2O$);[18] UV (0.1 N NaOH) λ_{max} 258 nm (ϵ 24 150), 286 (21 220), 371 (7070).

4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]benzoyl Azide (2).[19] A solution of 1 (10.41 g, 31.17 mmol), diphenylphosphoryl azide (12.87 g, 46.76 mmol, Aldrich Chemical Co.), and Et_3N (8.7 mL, 62.34 mmol) in Me_2SO (150 mL) was stirred under N_2 for 20 h. THF (200 mL) was then added and the precipitate was filtered to give 7.27 g of bright yellow solid. The THF was rotary evaporated and the Me_2SO solution was diluted with H_2O to give a second crop of 2.47 g: total yield 87%; NMR (CF_3CO_2D) δ 3.7 (s, 3 H, N^{10}-CH_3), 5.4 (s, 2 H, C^9-H_2), 8.2 (A_2B_2 center; J = 10 and 30 Hz, 4 H, arom), 9.1 (s, 1 H, C^7-H), a peak at δ 2.95 (6 H) showed the presence of 1 mol of Me_2SO; UV (EtOH) λ_{max} 318 nm (ϵ 42 500).

***N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenyl]-*N'*-*tert*-butylurea (3).** A solution of **2** (249 mg, 0.581 mmol, Me_2SO monosolvate) and *tert*-butylamine (5.0 mL) in dry DMAC (35 mL) was heated to 60 °C for 20 h. Et_2O was added to the cooled solution to precipitate a yellow solid, which was filtered and recrystallized from DMAC–CH_3CN to yield 150 mg (0.394 mmol, 68%) of **3**: mp 222 °C dec; NMR (Me_2SO-d_6, D_2O) δ 1.20 (s, 9 H, *t*-Bu), 3.0 (s, 3 H, N^{10}-CH_3), 4.55 (br s, 2 H, C^9-H_2), 6.6–7.4 (A_2B_2, 4 H, arom), 8.6 (s, 1 H, C^7-H). This compound decomposed rapidly in CF_3CO_2D. Anal. ($C_{19}H_{25}N_9O$) C, H, N. This compound was also prepared by photolysis of a solution of **2** and excess *tert*-butylamine in DMAC, but the yield was only 6%.

Methyl *N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenyl]carbamate (4). After deoxygenation with a N_2 sweep, a solution of **2** (428 mg, 1.0 mmol, Me_2SO monosolvate) in MeOH (25 mL) was photolyzed for 2.75 h. The volume was then reduced to 25 mL and ether was added to precipitate a yellow-orange solid which was collected by centrifugation and dried to yield 275 mg (0.777 mmol, 78%). Chromatography on silica gel with 10% MeOH–$CHCl_3$ gave the analytical sample: mp 262 °C dec; NMR (CF_3CO_2D) δ 3.6 (s, 3 H, N^{10}-CH_3), 3.85 (s, 3

H, O-CH_3), 5.3 (br s, 2 H, C^9-H_2), 7.6 (s, 4 H, arom), 8.85 (s, 1 H, C_7-H). Anal. ($C_{16}H_{18}N_8O_2$) C, H, N. This compound was also prepared in 78% yield by thermolysis of **2** in refluxing MeOH for 3 h.

Dimethyl *N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenylcarbamoyl]-L-aspartate (5). To a stirred solution of dimethyl L-aspartate hydrochloride (11.86 g, 60 mmol) in dry DMAC (100 mL) was added Et_3N (10 mL, 70 mmol). After filtering the Et_3N·HCl and briefly rotary evaporating to remove excess Et_3N, a solution of **2** (2.57 g, 6.0 mmol, Me_2SO monosolvate) in DMAC (200 mL) was added. The solution was photolyzed for 12 h and then evaporated almost to dryness at 40 °C with a mechanical pump. The residue was added to vigorously stirred CH_3CN (75 mL) to precipitate an orange solid, which was filtered, washed with Et_2O, and dried to yield 2.25 g (4.46 mmol, 78%) of crude **5**. Chromatography on silica gel with 15% MeOH–$CHCl_3$ gave 1.49 g (51%) of desired product: mp 195 °C dec; $[\alpha]^{25}_D$ −7.6 (*c* 1.05, 1 N HCl); NMR (CF_3CO_2D) δ 3.2 (d, *J* = 6 Hz, β-CH_2), 3.65 (br s, 3 H, N^{10}-CH_3), 3.90 (s, 3 H, γ-carbomethoxy), 3.95 (s, 3 H, α-carbomethoxy), 5.0 (t, *J* = 6 Hz, α-CH), 5.35 (br s, 2 H, C^9-H_2), 7.6 (s, 4 H, arom), 8.85 (s, 1 H, C^7-H). Anal. ($C_{21}H_{25}N_9O_5$·0.2H_2O) C, H, N.

Dimethyl *N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenylcarbamoyl]-L-glutamate (6). A solution of **2** (1.67 g, 3.90 mmol, Me_2SO monosolvate) and dimethyl L-glutamate (3.35 g, 19 mmol) in dry DMF (250 mL) was deoxygenated with a N_2 sweep for 30 min. The solution was photolyzed for 7 h with monitoring by TLC, and the volume was then reduced to 20 mL at 40 °C with a mechanical pump. This was added to vigorously stirred Et_2O (250 mL) to precipitate a yellow-orange solid, which was filtered and dried over P_2O_5 under vacuum to yield 1.87 g of crude product. Chromatography on silica gel (150 g) with 10% MeOH–$CHCl_3$ gave 1.0 g (2.01 mmol, 51.3%) of **6**: mp 214 °C dec; $[\alpha]^{25}_D$ −7.0 (*c* 0.215, 1 N HCl); NMR (CF_3CO_2D) δ 2.0–2.8 (m, 4 H, $-CH_2CH_2-$), 3.6 (s, 3 H, N^{10}-CH_3), 3.8 (s, 3 H, γ-carbomethoxy), 3.9 (s, 3 H, α-carbomethoxy), 4.6 (t, *J* = 7 Hz, α-CH), 5.2 (br s, 2 H, C^9-H_2), 7.6 (s, 4 H, arom), 8.8 (s, 1 H, C^7-H). Anal. ($C_{22}H_{27}N_9O_5$) C, H, N.

***N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenylcarbamoyl]-DL-aspartic Acid (7).** A mixture of **5** (932 mg, 1.93 mmol), 0.1 N NaOH (90 mL), and MeOH (60 mL) was stirred under N_2 for 1.5 h. The solution was then acidified to pH 3.5 with dilute HCl, and the orange solid was filtered and dried to yield 698 mg (1.53 mmol, 79%) of **7**: mp 220–235 °C dec; UV (0.1 N HCl) λ_{max} 244 nm (ε 32 500), 337 (10 000), 287 (sh, 6600), 350 (sh, 8700); UV (0.1 N NaOH) λ_{max} 258 nm (ε 30 450), 390 (5740); NMR (CF_3CO_2D) δ 3.2 (d, 2 H, β-CH_2), 3.55 (br s, 3 H, N^{10}-CH_3), 5.0 (t, 1 H, α-CH), 5.2 (br s, 2 H, C^9-H_2), 7.5 (s, 4 H, arom), 8.7 (s, 1 H, C^7-H). Anal. ($C_{19}H_{21}N_9O_5$·0.5 H_2O) C, H, N.

***N*-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenylcarbamoyl]-DL-glutamic Acid (8).** Similarly, diester **6** was hydrolyzed to yield **8** in 93% yield: mp 240 °C dec; UV (0.1 N HCl) λ_{max} 245 nm (ε 34 400), 337 (9900), 285 (6340), 350 (sh, 8840); UV (0.1 N NaOH) λ_{max} 258 nm (ε 30 450), 390 (5740); NMR (CF_3CO_2D) δ 2.0–3.0 (m, 4 H, $-CH_2CH_2-$), 3.65 (br s, 3 H, N^{10}-CH_3), 4.4 (m, 1 H, α-CH), 3.4 (br s, 2 H, C^9-H_2), 7.7 (s, 4 H, arom), 9.0 (s, 1 H, C^7-H). Anal. ($C_{20}H_{23}N_9O_5$·0.5H_2O) C, H, N. This compound had a small optical rotation, $[\alpha]^{25}_D$ +1.3 ± 0.2 (*c* 1.3, 0.1 N NaOH), compared to MTX $[\alpha]^{25}_D$ +18.6 ± 0.2 (*c* 1.5, 0.1 N NaOH).

Both **7** and **8** were subjected to LC analyses using as eluent 0.005 M phosphate buffer containing 12.5% EtOH and 0.02 M in tetraethylammonium chloride at pH 7.65, with a flow rate of 1.5 mL/min. Both compounds showed a major peak with only minor amounts of impurities. Compound **8** showed a minor peak which had the same retention time as MTX, but it was less than 0.5%.

DL-3-[4-[[(2,4-Diamino-6-pteridinyl)methyl]methylamino]phenyl]-5-(2-carbomethoxyethyl)hydantoin (10) and Compound 9. A solution of **6** (1.87 g, 3.76 mmol) and DBN (0.5 mL, 4.0 mmol) in MeOH (500 mL) was refluxed for 1 h. The solution was then concentrated to 25 mL and cooled in a refrigerator, and the solid was filtered to yield 1.07 g (2.31 mmol, 61%) of **10**. Chromatography on silica gel with 10% MeOH–$CHCl_3$ gave 962 mg (55%) of yellow solid: mp 207 °C dec; NMR (CF_3CO_2D) δ 2.0–2.9 (m, 4 H, $-CH_2CH_2-$), 3.65 (s, 3 H, N^{10}-CH_3), 3.8 (s, 3 H, $-OCH_3$), 4.55 (t, 1 H, α-CH), 5.35 (br s, 2 H, C^9-H_2), 7.8 (A_2B_2, 4 H, arom), 9.0 (s, 1 H, C^7-H). Anal. ($C_{21}H_{23}N_9O_4$) C, H, N. Alkaline hydrolysis of **10** gave the racemic diacid **8** in 75% yield, identical (TLC, IR, and NMR) with that obtained from the diester **6**.

Similarly, diester **5** gave compound **9** in 69% yield: mp 160–170 °C; NMR (CF_3CO_2D) δ 3.15 (d, *J* = 6 Hz, 2 H, $-CH_2CO$), 3.6 (br s, 3 H, N^{10}-CH_3), 3.8 (s, 3 H, $-OCH_3$), 4.6 (t, *J* = 6 Hz, 1 H, α-CH), 5.25 (br s, 2 H, C^9-H_2), 7.65 (A_2B_2, *J* = 9 and 14 Hz, 4 H, arom), 8.75 (s, 1 H, C^7-H). Anal. ($C_{20}H_{21}N_9O_4$) C, H, N.

4-(Dimethylamino)benzoyl Azide. This compound was prepared in 87% yield from 4-(dimethylamino)benzoic acid, diphenylphosphoryl azide, and Et_3N in DMF: mp 96–97 °C dec, lit.[35] mp 97 °C; NMR ($CDCl_3$) δ 3.05 [s, 6 H, $-N(CH_3)_2$], 7.3 (A_2B_2, *J* = 8 and 60 Hz, 4 H, arom).

Dimethyl *N*-[[4-(Dimethylamino)phenyl]carbomoyl]-L-glutamate (12) and L-Aspartate Analogue 11. Dimethyl L-glutamate (26.2 g, 0.15 mol) and 4-(dimethylamino)benzoyl azide (9.51 g, 0.05 mol) were dissolved in CH_2Cl_2 (250 mL), deoxygenated with a N_2 sweep for 15 min, and then photolyzed for 6.5 h. Evaporation left a solid, which was chromatographed on silica gel with 5% MeOH–$CHCl_3$ to give 8.3 g (0.024 mol, 49%) of **12**: mp 161–162 °C; $[\alpha]^{25}_D$ −29.3 (*c* 3.3, 1 N HCl); UV (EtOH) λ_{max} 264 nm (ε 19 530), 305 (sh, 1890); NMR (CF_3CO_2D) δ 2.0–2.8 (m, 4 H, $-CH_2CH_2-$), 3.3 [br s, 6 H, $-N(CH_3)_2$], 3.75 (s, 3 H, γ-carbomethoxy), 3.8 (s, 3 H, α-carbomethoxy), 4.5 (t, *J* = 6 Hz, 1 H, α-CH), 7.35 (s, 4 H, arom). Anal. ($C_{16}H_{23}N_3O_5$) C, H, N.

The aspartate analogue **11** was prepared by direct coupling of equimolar amounts of 4-(dimethylamino)phenyl isocyanate and dimethyl L-aspartate hydrochloride in the presence of excess Et_3N in DMF solution. Addition of H_2O and extraction with CH_2Cl_2 gave the crude product in 50% yield. Recrystallization from CH_2Cl_2–isopropyl ether gave the pure diester: mp 146–147 °C; $[\alpha]^{25}_D$ −10.3 (*c* 0.9, 1 N HCl); NMR (CF_3CO_2D) δ 3.2 (d, *J* = 6 Hz, 2 H, β-CH_2), 3.35 [s, 6 H, $-N(CH_3)_2$], 3.8 (s, 3 H, β-carbomethoxy), 3.9 (s, 3 H, α-carbomethoxy), 5.0 (t, *J* = 6 Hz, 1 H, α-CH), 7.55 (s, 4 H, arom). Anal. ($C_{15}H_{21}N_3O_5$) C, H, N.

DL-3-[4-(Dimethylamino)phenyl]-5-(2-carbomethoxyethyl)hydantoin (14) and Compound 13. A solution of **12** (675 mg, 2.0 mmol) and DBN (0.2 mL) in toluene (25 mL) was refluxed for 30 min. The solution was evaporated to dryness and the residue was recrystallized from isobutyl alcohol to yield 461 mg (1.51 mmol, 75.5%) of colorless crystals: mp 117–118 °C; UV (EtOH) λ_{max} 264 nm (ε 18 680); NMR ($CDCl_3$) δ 2.0–2.7 (m, 4 H, $-CH_2CH_2-$), 3.0 [br s, 6 H, $-N(CH_3)_2$], 3.7 (s, 3 H, $-OCH_3$), 4.2 (t, *J* = 6 Hz, 1 H, α-CH), 7.0 (A_2B_2, *J* = 9 and 27 Hz, 4 H + 1 H under high-field doublet, arom + NH). Anal. ($C_{15}H_{19}N_3O_4$) C, H, N.

Similarly, diester **11** gave compound **13** in 58% yield: mp 156–158 °C; NMR (CF_3CO_2D) δ 3.2 (d, *J* = 5 Hz, 2 H, β-CH_2), 3.45 [s, 6 H, $-N(CH_3)_2$], 4.75 (t, *J* = 5 Hz, 1 H, α-CH), 7.65 (s, 4 H, arom). Anal. ($C_{14}H_{17}N_3O_4$) C, H, N.

***N*-[[4-(Dimethylamino)phenyl]carbamoyl]-DL-glutamic Acid (15).** A mixture of **12** (2.108 g, 6.25 mmol) and 0.5 N NaOH (30 mL) was stirred at room temperature for 2.5 h. The pH was then lowered to 3 with dilute HCl and the solution evaporated almost to dryness. The precipitated solid was filtered, washed with H_2O (3 × 2 mL), and dried to yield 1.285 g (4.16 mmol, 67%) of colorless product: mp 181 °C dec; $[\alpha]^{25}_D$ −0.4 ± 0.2 (*c* 3.0, 1 N HCl); UV (0.1 N HCl) λ_{max} 244 nm (ε 15 080), 275 (sh, 775); NMR (CF_3CO_2D) δ 2.0–2.8 (m, 4 H, $-CH_2CH_2-$), 3.35 [br s, 6 H, $-N(CH_3)_2$], 4.55 (m, 1 H, α-CH), 7.4 (s, 4 H, arom). Anal. ($C_{14}H_{19}N_3O_5$·0.33H_2O) C, H, N.

L-4-(2-Carbomethoxyethyl)oxazolide-2,5-dione (16). γ-Methyl L-glutamate hydrochloride was prepared from L-glutamic acid and thionyl chloride in MeOH.[36] This compound was converted to the *N*-carboxyanhydride with phosgene gas by the procedure of Coleman[37] in 83% yield: mp 90.5–95 °C; $[\alpha]^{25}_D$ −22.8 (*c* 5.558, *p*-dioxane), lit.[38] $[\alpha]_D$ −20.2 (*c* 1.8, MeCN).

γ-Methyl-L-glutamic Acid 4-(Dimethylamino)anilide (17). To a solution of freshly distilled *N*,*N*-dimethyl-*p*-phenylenediamine (5 mL) in CH_3CN (25 mL), under N_2, was added compound **16** (2.81 g, 15.0 mmol) over a period of 1 h. After stirring the solution for 12 h, the slurry was poured into Et_2O (50 mL) and filtered to yield a first crop of colorless crystals (1.065 g). The

Table II. In Vitro and in Vivo Antitumor Evaluation of MTX Analogues

compd	in vitro (CCRF-CEM)[a] ID_{50}, μg/mL	in vivo (L-1210)[b] opt nontox dose, mg/kg × 3	% ILS[c]
5	3.9		
6	3.5		
7	3.2	400	22
8	0.4	200	55
10	6.7	50-200	0
MTX	0.02	30	88

[a] The assay method has been previously described.[32] [b] Male BDF/1 hybrid mice were inoculated ip with 10^5 L-1210 leukemia cells. Test compounds in 0.1 M phosphate buffer (pH 8)(10 suspended in 10% Tween 80) were injected ip 24 h after tumor implantation (day 1) and on days 4 and 7 (qd 1, 4, 7). [c] % ILS = 100 (T/C − 1), where T and C are median survival times of treated and control animals.

filtrate was concentrated to yield another crop of slightly impure product, which was chromatographed on silica gel with 2% MeOH–$CHCl_3$ to yield an additional 0.46 g of pure product. The total yield was 36.5%: mp 123.5–124.5 °C; $[\alpha]^{25}_D$ +35.04 (*c* 5.416, MeOH); NMR ($CDCl_3$) δ 1.65 (br s, 2 H, $-NH_2$), 1.9–2.6 (m, 4 H, $-CH_2CH_2-$), 2.95 [s, 6 H, $-N(CH_3)_2$], 3.6 (m, 1 H, α-CH), 3.7 (s, 3 H, $-OCH_3$), 6.8–7.6 (q, 4 H, arom). Anal. ($C_{14}H_{21}N_3O_3$) H, N; C: calcd, 60.19; found, 60.63.

Conversion of 17 into 14. To a solution of **17** (464 mg, 1.66 mmol) in CH_3CN (10 mL) was added *N,N'*-carbonyldiimidazole[27] (323 mg, 1.99 mmol). A flocculent precipitate appeared which dissolved within 30 min to produce a much finer precipitate. The mixture was filtered and the filtrate was evaporated to leave a solid, which was recrystallized from EtOAc–isopropyl ether to yield 365 mg (0.978 mmol, 59%) of colorless crystals: mp 116.5–118 °C; $[\alpha]^{25}_D$ −59.80 (*c* 1.438, MeOH). Anal. ($C_{15}H_{19}N_3O_4$) C, H, N. This product was identical (Co-TLC, NMR) with compound **14** obtained by cyclization of diester **12**. The IR spectra (KCl) of both products differed slightly in the fingerprint region, as might be expected for enantiomeric vs. diasteriomeric crystals. The enantiomeric product was rapidly converted into racemic **14** by DBN in MeOH.

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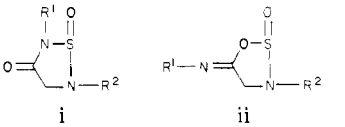

i ii

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Notes

Synthesis of Aza Homologues of Folic Acid

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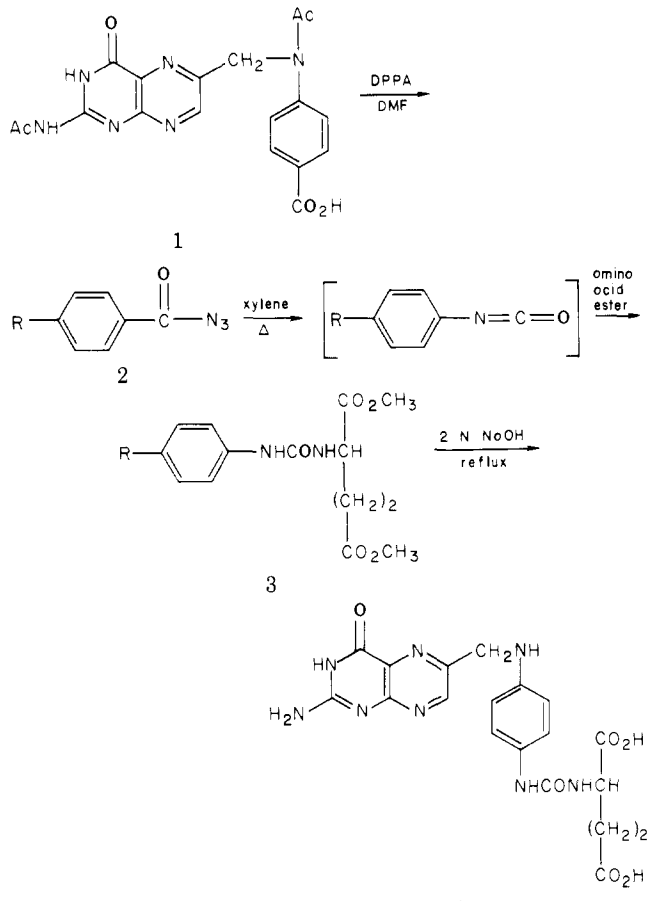
Folic acid analogues containing an additional nitrogen atom between the phenyl ring and the carbonyl group of the side chain were synthesized. None of the compounds showed significant inhibitory activity against human lymphoblastic leukemia cells (CCRF-CEM) in culture or against *Lactobacillus casei* (ATCC 7469) growth. Against L1210 leukemia in mice, the aza homologue of folic acid, **4**, and the aspartic acid analogue, **14**, showed no increase in life span over control animals. These compounds were more toxic in vivo than the corresponding methotrexate analogues. Compound **4** supported the growth of *Streptococcus faecium* (ATCC 8043), and its tetrahydro derivative supported the growth of *Pediococcus cerevisiae* (ATCC 8081). These results strongly suggest that **4** can substitute for folate derivatives as cofactors for serine transhydroxymethylase, thymidylate synthetase, and dihydrofolate reductase.

The development of methotrexate (MTX) analogues with potentially superior clinical properties and whose sole mode of action is inhibition of dihydrofolate reductase (DHFR) is generally held to have little promise.[1,2] However, the design of folate analogues which act as substrates for DHFR,[3] producing "spurious" coenzymes which inhibit other enzymes in the folate cycle, remains an attractive rational strategy[4,5] since its initial proposal.[6] The potent inhibition of thymidylate synthetase and the antitumor activity of several reduced folate, MTX, and quinazoline analogues, in particular homofolic acid derivatives, support this strategy. Accordingly, we have synthesized new aza homologues of folic acid in which an additional nitrogen atom separates the phenyl ring and the carbonyl group.

Chemistry. Folic acid was hydrolyzed with the enzyme carboxypeptidase G to pteroic acid in quantitative yield.[7] Attempts to convert pteroic acid to its pure carbonyl azide with diphenylphosphoryl azide (DPPA) were unsuccessful due to the extremely low solubility of pteroic acid and the carbonyl azide in organic solvents. To improve its solubility, pteroic acid was diacetylated[8] to **1**, which reacted with DPPA in DMF to give **2** in high yield (Scheme I). Photolytic Curtius rearrangement of **2** in the presence of excess dimethyl L-glutamate to yield **3** was impractical because the product was greatly contaminated with the dimethyl folate derivative formed by direct peptide coupling. A similar result precluded the *N*-formylamino protection during the Curtius rearrangement of *p*-aminobenzoyl azide (see below). It is evident that N^{10}-acylation retards resonance deactivation of the carbonyl azide toward nucleophilic attack.[9] Thermolysis of a suspension of **2** in refluxing xylene gave the crude isocyanate which reacted with dimethyl L-glutamate in DMF to give pure **3** in 40% yield. Complete hydrolysis of **3** to the deacylated diacid required refluxing aqueous alkaline conditions and provided racemic **4** in only 10% yield after DEAE-cellulose chromatography. The low overall yield prompted a different approach.

Scheme I



p-Aminophenyl isocyanate, formed by photolysis of *p*-aminobenzoyl azide,[10] reacted in situ with dimethyl

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