recorder, and a circulating constant-temperature bath. Glycinamide ribonucleotide was prepared according to the literature procedure.[8] 5,8-Dideazafolate (1) and N^{10}-acetyl-5,8-dideazafolate (7) were prepared as described previously.[1] Glycinamide ribonucleotide transformylase was purified from L5178Y as previously described.[3]

Enzyme Assays. Glycinamide ribonucleotide transformylase activity was assayed by monitoring the production of 5,8-dideazafolate at 295 nm ($\Delta\epsilon = 18.9 \times 10^3$ mM^{-1}).[2] The assays were performed at 35 °C and pH 7.5 with the solution containing 100 mM potassium phosphate, pH 7.5, 1 mM (α,β) glycinamide ribonucleotide, and enzyme in 0.5 mL. For the inhibition studies, the concentration of N^{10}-formyl-5,8-dideazafolate was varied from 1.25 to 7.5 μM. The inhibitors were maintained at fixed concentrations, which were varied from 0 μM up to 50 μM for the less effective inhibitors. All components, except cofactor, were incubated at 35 °C for 10 min, and the reaction was initiated by the addition of cofactor in a small volume. The rate of product formation was dependent on enzyme concentration and linear with time during the initial part of the reaction. No turnover of cofactor was observed in the absence of either enzyme or substrate.

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Folate Analogues. 30. Synthesis and Biological Evaluation of N^{10}-Propargyl-5,8-dideaza-5,6,7,8-tetrahydrofolic Acid and Related Compounds[1]

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The 5,6,7,8-tetrahydro derivative (1) of the powerful thymidylate synthase inhibitor N^{10}-propargyl-5,8-dideazafolic acid (PDDF) has been synthesized and evaluated for its antifolate activity. A convenient method for the preparation of the key intermediate 2-amino-6-(bromomethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline (18) is described. Two closely related analogues of 1 were also synthesized and evaluated for their antifolate activity and thymidylate synthase inhibition. N^{10}-Propargyl-5,8-dideaza-5,6,7,8-tetrahydrofolate (1) and N^{10}-methyl and N^{10}-hydrogen analogues 2 and 3 were weaker inhibitors of *Lactobacillus casei* thymidylate synthase compared to PDDF. N^{10}-Methyl-5,8-dideaza-5,6,7,8-tetrahydrofolate (2) exhibited the most potent antifolate activity against *L. casei* (IC_{50} = 2.8 nM) and *Streptococcus faecium* (IC_{50} = 0.57 nM). In intact and permeabilized murine leukemia L1210 cells, the replacement of the quinazoline moiety with its tetrahydro derivative resulted in a marked decrease in potency and a loss of the contribution of the propargyl substituent to enzyme inhibition, indicating an altered binding mode to thymidylate synthase.

Coenzyme analogues structurally related to folic acid that are specific inhibitors of thymidylate synthase (TS) are potentially useful antineoplastic agents. Analogues of 5,8-dideazafolic acid that were substituted at N^{10} with various substituents have exhibited varying degrees of inhibition of thymidylate synthase (EC 2.1.1.45).[2,3] The powerful antileukemic agent N^{10}-propargyl-5,8-dideazafolic acid (4) (Chart I) has been shown to be a specific inhibitor of L1210,[4] *Lactobacillus casei*,[5] *Streptococcus faecium*,[5] and human[6] thymidylate synthases. N^{10}-Propargyl-5,8-dideazapteroyl tri-, tetra-, and pentaglutamates were equipotent with 5-fluorodeoxyuridylate as inhibitors of thymidylate synthesis in permeabilized L1210 cells.[7] These polyglutamates of N^{10}-propargyl-5,8-dideazafolic acid (PDDF) isolated as metabolites[8] were shown to be the most potent antifolate inhibitors of *L. casei* and L1210 thymidylate synthases yet described.[7,9,10] In spite of its excellent inhibition ($K_i = 1 \times 10^{-9}$ M) of L1210 TS, the therapeutic utility of PDDF has suffered because of its poor solubility and consequent nephrotoxicity.[11] Several analogues (5–7) of PDDF were subsequently synthesized from this[7] and other laboratories[12] and evaluated for their biological activity. None of these compounds were found to be superior to PDDF as TS inhibitors or as antitumor agents. N^{10}-Propargylfolic acid (8), which is the closest pteridine analogue of PDDF, has been recently synthesized and evaluated for its antifolate activity and TS inhibition.[13] The folate analogue 8 exhibited a considerably lower de-

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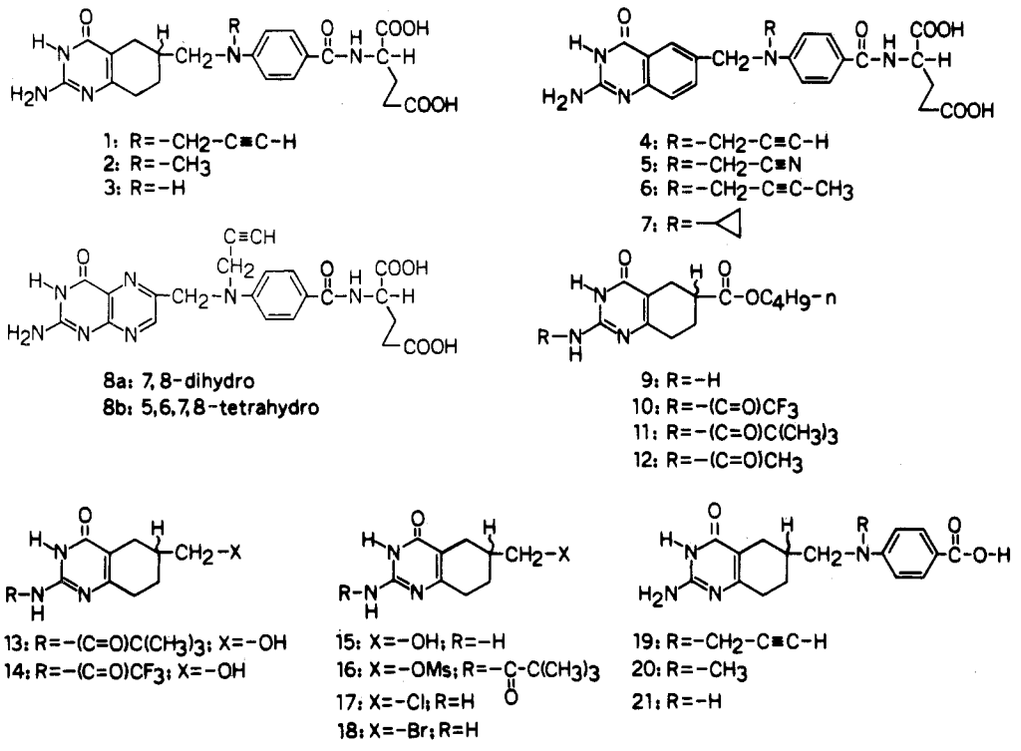
Chart I



gree of TS inhibition compared to the quinazoline analogue 4. 7,8-Dihydro-N^{10}-propargylfolic acid (**8a**) and the 5,6,7,8-tetrahydroderivative **8b** (which was obtained by the enzymatic reduction of **8a**) were also poor inhibitors of TS. From these results, it appeared that the presence of N atoms at the 5- and 8-positions of classical folate analogues is detrimental to TS inhibition irrespective of the nature of substituents at N^{10}. In order to determine whether coplanarity of the quinazoline ring coupled with the presence of the propargyl group at N^{10} in folate analogues is necessary to create favorable binding interactions with TS, we decided to replace the quinazoline ring of PDDF with a 5,6,7,8-tetrahydroquinazoline ring. Since compound **1** does not possess N atoms at either the 5- or 8-position, any significant change in the enzyme inhibitory activity of **1** compared to PDDF must be related to the difference in geometry (and aromaticity) of the quinazoline ring. As part of our attempt to unravel structure–activity relationships[5-7,9] among 5,8-dideazafolate analogues, we report the chemical synthesis and biological evaluation of N^{10}-propargyl-5,8-dideaza-5,6,7,8-tetrahydrofolic acid (**1**) and a few related analogues in this paper.

Chemistry

The most convenient synthetic strategy for the construction of N^{10}-substituted 5,8-dideaza-5,6,7,8-tetrahydrofolic acid derivatives appeared to be the alkylation of [*p*-(monoalkylamino)benzoyl]-L-glutamic acids with a 2-amino-6-(halomethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline.[14] However, Koehler et al. previously reported[14] the reaction between 2-amino-6-(chloromethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline (**17**) and (*p*-aminobenzoyl)-L-glutamic acid resulting in the formation of a product **3**, which had resisted attempted purification. These authors also reported extensive degradation of the reagents under their experimental conditions. Subsequently, DeGraw and co-workers prepared pure **3** by employing an alternate synthetic method involving the reductive alkylation of (*p*-aminobenzoyl)-L-glutamic acid with 2-acetamido-4-hydroxy-6-formyl-5,6,7,8-tetrahydroquinazoline.[15] We felt that the bromomethyl derivative **18** or a suitably protected derivative of **15** such as mesylate **16** could be used for the alkylation of amines such as ethyl *p*-(*N*-propargylamino)benzoate under milder reaction conditions, and the desired target compound can be prepared conveniently by this procedure. Therefore, our initial attempts were directed toward the preparation of mesylate **16** and 2-amino-6-(bromomethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline (**18**).

The known 2-amino-6-carbo-*n*-butoxy-4-hydroxy-5,6,7,8-tetrahydroquinazoline (**9**) was prepared according to the literature procedure.[14] Although direct reduction of **9** with a combination of $NaBH_4$ and $AlCl_3$ in the form of a slurry had been previously reported,[14] a more convenient reduction procedure in homogeneous solution for the preparation **15** was desired. In order to increase the lipid solubility of **9**, it was converted to the 2-trifluoroacetyl and 2-trimethylacetyl derivatives **10** and **11**, respectively. The use of the known 2-acetyl derivative **12** was not considered because of the anticipated difficulty in the selective removal of this protective group at a later stage in presence of the sensitive propargyl group.[13] Both compounds **10** and **11** were soluble in THF and they could be reduced easily to the alcohols **15** and **13** with Superhydride in excellent yield. During the reduction, the trifluoroacetyl group of **10** was easily removed, and the alcohol **15** was the only product isolated. All attempts to selectively reduce the ester function of **10** while preserving the trifluoroacetyl group failed. However, the 2-trifluoroacetyl derivative **14**

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was prepared by the direct trifluoroacetylation of **15** with trifluoroacetic anhydride. Reaction of alcohol **13** with mesyl chloride in pyridine gave the desired mesylate **16** in low yield. The structure of the mesylate was confirmed by NMR and high-resolution mass spectrometry. The mesylate **16** was surprisingly stable and failed to react with either ethyl *p*-aminobenzoate or ethyl *p*-(*N*-propargylamino)benzoate under a variety of reaction conditions, including fusion. Subsequently, the corresponding tosylate was made, which was also unreactive with ethyl *p*-aminobenzoate or the propargylamine. These failures prompted us to prepare the chloromethyl compound **17**. Reaction of **17** with ethyl *p*-(*N*-propargylamino)benzoate as described previously for the preparation of **3**, resulted in extensive degradation of the propargylamine, and the NMR spectrum of the isolated product was inconsistent with the required structure.

The preparation of bromomethyl derivative **18** was next attempted. Reaction of **15** with hydrobromic acid gave the corresponding 6-bromomethyl derivative, either as the hydrochloride or as the free base depending on the nature of the workup of the reaction product. Reaction of *p*-aminobenzoic acid with 2-amino-6-(bromomethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline hydrobromide (**18**·HBr) in DMAc with MgO as an acid scavenger gave the desired product 5,8-dideaza-5,6,7,8-tetrahydropteroic acid in acceptable yield. Encouraged by these results **18**·HBr was reacted under similar conditions with ethyl *p*-(*N*-propargylamino)benzoate, and the resulting product was hydrolyzed to **19**. Similarly, reaction of **18**·HBr with *p*-(methylamino)benzoic acid gave the pteroate analogue **20** in good yield. These analogues **19** and **20** were converted to their respective mixed anhydrides by reaction with isobutyl chloroformate and coupled with L-glutamic acid by the solid-phase procedure. Alternately, diethyl [*p*-(*N*-propargylamino)benzoyl]-L-glutamate or diethyl [*p*-(*N*-methylamino)benzoyl]-L-glutamate was reacted with **18**·HBr and the corresponding product was hydrolyzed and purified by ion-exchange chromatography to target compounds **1** or **2** directly. For the purpose of comparison, compound **3** was also prepared in a similar manner by reaction of the bromomethyl compound **18** with (*p*-aminobenzoyl)-L-glutamic acid in good yield. Compounds **1–3** thus obtained were mixtures of diastereomers due to the asymmetry at C^6 of the tetrahydroquinazoline ring. Reaction of the various amines with the bromomethyl compound **18** obtained as the free base gave very poor yields of the pteroate and folate analogues. The structures of all products were established by a combination of NMR spectroscopy and high-resolution and FAB mass spectrometry.

Biological Evaluation and Discussion

We have recently shown that introduction of heteroatoms such as N at the 5- and 8-positions of the quinazoline ring of PDDF as in N^{10}-propargylfolate[13] resulted in substantial loss of inhibition of thymidylate synthase. Geometric distortions of the planarity of the pyrazine ring of propargylfolate by successive reductions to dihydro and tetrahydro propargylfolates were also detrimental to TS inhibition. The present study was undertaken to determine the effect of altering the coplanarity of the quinazoline ring of PDDF on TS inhibition. This was accomplished by substituting the fused benzene ring of PDDF with a more flexible cyclohexene ring. We observed a striking difference in TS inhibition caused by this change in geometry of the quinazoline ring of PDDF. The parent compound **4** was 1000 times more inhibitory to *L. casei* thymidylate synthase than the target compound **1** (Table I). The closely related N^{10}-methyl analogue **2** was also a weak inhibitor of this enzyme. In fact, none of the N^{10}-substituted 5,8-dideaza-5,6,7,8-tetrahydrofolate or pteroate derivatives exhibited significant inhibition of *L. casei* thymidylate synthase. Since it has been shown previously that the inhibition of TS by tetrahydrohomofolate was species specific,[16] the inhibitory activity of these compounds on thymidylate synthesis in intact and permeabilized L1210 cells was next examined. These determinations were carried out as described previously.[7,13] Compound **1** did not show any significant effect in intact L1210 cells although PDDF exhibited marked inhibition of thymidylate synthesis in this system. From the enzyme-inhibitory data with intact L1210 cells, it would appear that the tetrahydroquinazolines are not effectively transported into these cells. However, in permeabilized L1210 cells, the inhibition of thymidylate synthesis by **1** was also much weaker ($IC_{50} = 1.1 \times 10^{-5}$ M) than PDDF ($IC_{50} = 2.8 \times 10^{-8}$ M). It is interesting that, in L1210 cells, there is a lack of any effect by the propargyl group on the thymidylate synthase inhibitory activity in these cellular enzyme systems—a remarkable contrast with the parent quinazolines, suggesting a different binding mode for the tetrahydroquinazoline derivatives. From these results (Table II) it was concluded that the topography of the binding site of thymidylate synthase has strong geometric restrictions, and coplanarity of the quinazoline ring appears to be essential for favorable binding interactions. Compounds **1** and **2** were further tested as inhibitors of *E. coli* and calf thymus thymidylate synthases. In order to assess the potential of compounds **2**, **15**, and **18** as antibacterial agents they were also tested for their ability to inhibit purified *E. coli* dihydropteroate synthase. None of the compounds showed any significant inhibition of these enzymes. (Table IV). When tested as inhibitors of the growth of MCF-7 cells in culture, compounds **1** and **2** were

Table I. Inhibition of Thymidylate Synthase and Dihydrofolate Reductase by H_4-PDDF and Analogues

compd	IC_{50}, M: dihydrofolate reductase (*L. casei*)	IC_{50}, M: thymidylate synthase (*L. casei*)
1	1×10^{-5}	2.3×10^{-5}
2	1.6×10^{-5}	1.2×10^{-4}
3	3×10^{-5}	$>2.8 \times 10^{-4}$
19	1.9×10^{-4}	6.1×10^{-4}
20	$>2.0 \times 10^{-4}$	$>2.0 \times 10^{-4}$
21	$>2.0 \times 10^{-4}$	$>2.0 \times 10^{-4}$
PDDF (**4**)	4.9×10^{-6}	2.1×10^{-8}

Table II. Inhibition of Thymidylate Synthesis in L1210 Cells

compd	IC_{50}, M: intact	IC_{50}, M: permeabilized[a]
H_4-PDDF (**1**)	1.1×10^{-3}	1.1×10^{-5}
PDDF (**4**)	8.3×10^{-6}	2.8×10^{-8}
H_4-DDF (**3**)	1.0×10^{-3}	1.2×10^{-5}
DDF	1.0×10^{-4}	1.0×10^{-5}

[a] L1210 cells were permeabilized by incubation at 4 °C for 20 or 40 min in the presence of 400 μg/mL sodium dextran sulfate (MW 500 000; Pharmacia) followed by washing and resuspension. Enzyme activity was determined at pH 7.4 by measuring the tritium released into water from 1 μM [5-^{3}H]dUMP by 5×10^6 cells during 30 min at 37 °C. The assay mixture contained 0.4 mM *d,l*-L-methylenetetrahydrofolate. DDF: 5,8-dideazafolic acid.

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Table III. Antimicrobial Activities of H_4-PDDF and Analogues

	Streptococcus faecium		*Lactobacillus casei*	
compd	ATCC 8043	MTX-resistant	ATCC 7469	MTX-resistant
1	1.8	350	4.7	>10 000
2	0.57	13	2.8	>20 000
3	140	900	7.5	>20 000
19	26	14 000	1 000	>20 000
20	5.2	8 200	15 800	>20 000
21	400	>20 000	>20 000	>20 000
PDDF (4)	0.13	20	0.4	6 400

Table IV. Inhibition of *E. coli* and Calf Thymus Thymidylate Synthases and *E. coli* Dihydropteroate Synthase by Selected H_4-Quinazoline Analogues

	% inhibn/concn (μM)		
	thymidylate synthase[a]		*E. coli* dihydropteroate synthase[b]
compd	*E. coli*	calf thymus	
1	50/14 ± 1	50/7 ± 0.2	–
2	50/38 ± 5	50/12 ± 0.4	35.1/650
15			1.3/650
18			7/520

[a] Thymidylate synthase assays were performed in the presence of 20 μM [^{3}H]dUMP, 100 μM [6-R,S] H_4folate, 6.5 mM HCHO, 20 mM $MgCl_2$, and 150 mM Tris·HCl at pH 8.0 with purified enzymes. [b] Assayed as described in: Ferone, R.; Webb, S. R. In *Chemistry and Biology of Pteridines*; Pfeiderer, W., Ed.; Walter de Gruyter: New York, 1975; pp 61–71.

inactive up to a concentration of 50 μM.

Recently, a number of 5,10-dideazafolate analogues were reported to exhibit significant antifolate activity, although they were neither inhibitors of dihydrofolate reductase nor thymidylate synthase. 5,10-Dideaza-5,6,7,8-tetrahydrofolic acid, which is a structural analogue of **1**, was shown to be a specific inhibitor of GAR-transformylase having potential clinical utility. In order to evaluate the potential antifolate activity of compounds **1** and **2**, they were tested as inhibitors of the growth of two folate-requiring microorganisms, *L. casei* (ATCC 7469) and *S. faecium* (ATCC 8043). All compounds exhibited some degree of antifolate activity in these systems. PDDF was 10 times more active than **1** in both systems. However, the antibacterial activity of compound **2** was more impressive. PDDF was only 5 times more active than **2** against *S. faecium* and 7 times more active against *L. casei*. Both PDDF and **2** were equally active against the methotrexate resistant strain of *S. faecium* (Table III). It has been shown that these compounds do not inhibit *L. casei* DHFR to any significant extent (Table I). It is conceivable that the moderate antifolate activity exhibited by **1** and **2** may be due to inhibition of other folate-dependent enzymes or metabolism to more active inhibitors of TS. These possibilities are being investigated.

Experimental Section

Melting points were determined on a Fisher Model 355 digital melting point analyzer. NMR spectra were run in $CDCl_3$ or TFA on a 90-MHz Perkin-Elmer Model R-32 spectrometer with Me_4Si as a reference standard. Me_4Si was used as an external standard for all spectral determinations in TFA unless otherwise specified. Field strength of the various proton resonances is expressed in parts per million, and peak multiplicity is depicted as follows: s, singlet; br, broadened 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. High-resolution mass spectra of selected compounds were obtained from the mass spectrometry center at the Research Triangle Institute, and FAB mass spectra were determined by Dr. Susan Weintraub, Department of Pathology, University of Texas. 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 were within ±0.4% of the theoretical values.

6-Carbo-*n*-butoxy-4-hydroxy-2-(trifluoroacetamido)-5,6,7,8-tetrahydroquinazoline (10). To a stirred suspension of **9** (5 g) (prepared according to literature procedure)[14] in a 100-mL round-bottomed flask was added 25 mL of trifluoroacetic anhydride followed by dropwise addition of trifluoroacetic acid (7.3 mL) when a clear solution was obtained. The progress of the trifluoroacetylation was monitored by observing the slow disappearance of the NMR signal due to the presence of the 2-amino group at 7.6 ppm. After 7 h, the mixture was evaporated and the gum thus obtained was triturated with 50 g of ice, filtered, washed with water, and dried: yield 5.74 g; mp 173–174 °C. Anal. ($C_{15}H_{18}F_3N_3O_4·H_2O$) C, H, N.

6-Carbo-*n*-butoxy-4-hydroxy-2-(trimethylacetamido)-5,6,7,8-tetrahydroquinazoline (11). To a refluxing suspension of 10 g of **9** in 500 mL of dioxane was added portionwise 100 mL of triethylamine mixed with 10 mL of dioxane followed by dropwise addition of 12 mL of trimethylacetyl chloride. After the additions were complete, a yellowish green solution was formed and fine white crystals of triethylamine hydrochloride began to separate. The reflux was continued for another 35 min, and the solution was filtered hot to remove the amine hydrochloride. The filtrate was concentrated to 100 mL to which 100 mL of ethanol was added and chilled overnight at 4 °C. White crystals of **11** thus obtained were filtered, washed with ethanol, and dried: yield 4.88 g; mp 184–185 °C; NMR ($CDCl_3$) δ 4.2 (t, 2 H, OCH_2CH_2), 1.35 (s, 9 H, trimethylacetyl, 1.0 (t, 3 H, CH_2CH_3). Anal. ($C_{18}H_{27}N_3O_4$) C, H, N.

4-Hydroxy-6-(hydroxymethyl)-2-(trimethylacetamido)-5,6,7,8-tetrahydroquinazoline (13). To a solution of 1.75 g (5 mmol) of **11** in 200 mL of dry THF under nitrogen was added dropwise 36 mL of lithium triethylborohydride (Superhydride) (36 mmol) during a period of 3 h. Examination of the reaction mixture by TLC at this stage indicated complete conversion of the starting material. The reaction mixture was poured over 500 g of crushed ice with stirring. After all the ice had melted, the solution was evaporated under reduced pressure at 35 °C to 25 mL and acidified to pH 5.5 with 6 N HCl. The solution was kept for 3 days at 4 °C whereupon large fluffy crystals of **13** were formed, which were filtered, washed with ice-cold water, and dried over P_2O_5 in vacuum: yield 750 mg; mp 204–206 °C; NMR ($CDCl_3$) δ 3.89 (br, 2 H, hydroxymethyl), 1.8–3.0 (c, 7 H, tetrahydroquinazoline), 1.4 (s, 9 H, trimethylacetyl). Anal. ($C_{14}H_{21}N_3O_3$) C, H, N, O.

2-Amino-4-hydroxy-6-(hydroxymethyl)-5,6,7,8-tetrahydroquinazoline (15). To a 500-mL three-neck round-bottomed flask fitted with a dropping funnel was added a solution of 2 g (5.3 mmol) of the trifluoroacetyl derivative **10** in 200 mL of THF. The flask was flushed with dry nitrogen, and a solution of 30 mL (30 mmol) of Superhydride was slowly added to the flask with stirring. The progress of the reaction was monitored by TLC by withdrawing aliquots at intervals. After 2 h, all the reagent was consumed and no starting material remained in the reaction mixture. The slightly greenish yellow solution thus obtained was poured carefully to 400 g of crushed ice and stirred. After all the ice had melted, the solution was evaporated under reduced pressure to 25 mL, and the pH was adjusted to 5.5. On refrigeration overnight compound **15** separated as a white powder, which was filtered, washed with ice-cold water, and dried over P_2O_5 in vacuum: yield 982 mg; mp 300 °C; NMR (TFA) δ 7.3 (br, 2 H, 2-NH_2), 4.22 (s, 2 H, hydroxymethyl), 3.65–2.7 (c, 7 H, tetrahydroquinazoline); UV (0.1 N NaOH) λ_{max} 276 nm (ϵ 5,442). Anal. ($C_9H_{13}N_3O_2·0.25H_2O$) C, H, N, O.

4-Hydroxy-6-(hydroxymethyl)-2-(trifluoroacetamido)-5,6,7,8-tetrahydroquinazoline (14). To a stirred suspension of 1.95 g (10 mmol) of **15** in 30 mL of trifluoroacetic anhydride was added slowly 5 mL of trifluoroacetic acid during a period of 10 min. A few minutes after the addition was complete a clear solution was obtained. The stirring continued for 18 h, and the reaction mixture was evaporated to dryness under reduced pressure. On trituration with 50 g of ice, a white solid was separated, which was filtered, washed with water, and dried: yield

2.9 g; mp 172 °C; NMR (TFA) δ 4.1 (d, 2 H, hydroxymethyl); MS, m/e 291.0833 ($C_{11}H_{12}F_3N_3O_3$ requires m/e 291.0830).

4-Hydroxy-6-[(mesyloxy)methyl]-2-(trimethylacetamido)-5,6,7,8-tetrahydroquinazoline (16). A solution of 558 mg (2 mmol) of **13** in 250 mL of dry CH_2Cl_2 was treated with 0.5 mL of triethylamine followed by 458 mg (4 mmol) of methanesulfonyl chloride at 0 °C for 2 h. Examination of the reaction by TLC indicated incomplete conversion of the starting alcohol to the product. The mixture was stirred at room temperature for 18 h more, evaporated at 25 °C under reduced pressure, and triturated with 25 g of ice. The product was extracted in 50 mL of EtOAc, washed with water, dried over Na_2SO_4, evaporated, and chromatographed on a silica gel column. Elution of the column with CH_2Cl_2 gave pure **16**, the NMR spectrum of which exhibited typical resonances of the (mesyloxy)methyl group: yield 133 mg (18.6%); mp 68–70 °C; NMR ($CDCl_3$) δ 4.25 (d, 2 H, (mesyloxy)methyl), 3.8 (s, 3 H, mesyloxy), 1.3 (s, 9 H, trimethylacetamido); MS, m/e 357.1356 ($C_{15}H_{23}N_3O_5S$ requires m/e 357.1358).

The tosylate of alcohol **13** was also made in a similar manner, substituting toluenesulfonyl chloride for methanesulfonyl chloride in the previous reaction. Column chromatography of the crude product on silica gel gave 275 mg of the pure tosylate: mp 167–172 °C; NMR ($CDCl_3$) δ 7.8, 7.3 (d, d, 4 H, aromatic), 4.05 (d, 2 H (tosyloxy)methyl), 2.5 (s, 3 H, tosyl), 1.3 (s, 9 H, trimethylacetamido); MS, m/e 433.1672 ($C_{21}H_{27}N_3O_5S$ requires m/e 433.1671).

2-Amino-6-(chloromethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline (17). This compound was prepared from **15** according to the procedure of ref 13: MS, m/e 213.0669 ($C_9H_{12}ClN_3O$ requires m/e 213.0669).

2-Amino-6-(bromomethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline (18). A suspension of 1.95 g (10 mmol) of **15** in 50 mL of 49% hydrobromic acid was refluxed for 4 h and let stand overnight at 25 °C. The reaction mixture containing a few crystals was evaporated to dryness under reduced pressure. The reaction product was triturated with 50 mL of toluene and reevaporated. This process was repeated three times, whereupon a slightly yellow solid was formed. After the solid was triturated with 100 mL of ether, it was filtered, washed successively with ether, and dried over P_2O_5 in vacuum: yield 3.5 g; mp 223–226 °C; UV (0.1 N NaOH) λ_{max} 275 nm (ε 9482); (H_2O) λ_{max} 263 nm (ε 6760); NMR (TFA) δ 3.30 (br, 2 H, bromomethyl).

The free base **18** was obtained by adding the required amount of $NaHCO_3$ to a solution of 2-amino-6-(bromomethyl)-4-hydroxy-5,6,7,8-tetrahydroquinazoline hydrobromide in water. The white precipitate of **18** thus obtained was filtered, washed with water, and dried: mp 268–272 °C. This material showed a single spot on silica gel TLC plates (CH_2Cl_2/MeOH, 9:1). MS, m/e 257.0166 ($C_9H_{12}BrN_3O$ requires 257.0164). Anal. ($C_9H_{12}BrN_3O$) C, H, N.

General Method for the Synthesis of N^{10}-Substituted 5,8-Dideaza-5,6,7,8-tetrahydropteroic Acids 19–21. In a typical experiment a mixture of 676 mg (2 mmol) of **18**·HBr, 2 mmol of the appropriate *p*-aminobenzoic acid, and 160 mg (4 mmol) of MgO was stirred with 5 mL of DMAc at 120–135 °C for 18 h. The reaction mixture was then poured over 150 g of crushed ice, triturated, and filtered. The crude pteroic acids were purified by ion-exchange chromatography on DEAE-cellulose columns. The column was eluted with a linear gradient of ammonium bicarbonate from 0 to 0.75 M. In each case, the effluent corresponding to the desired product was evaporated to dryness under vacuum and the residue dissolved in a small volume of water. The aqueous solution of the ammonium salt of the product thus obtained was acidified to pH 4.5 with glacial HOAc and refrigerated overnight. The precipitated compounds **19–21** were separated by filtration, washed with water, and dried.

Compound **19**: NMR (TFA) δ 8.2 (d, 2 H, aromatic), 7.65 (d, 2 H, aromatic), 7.5 (br, 2 H, 2-amino), 4.35 (br, 2 H, propargyl), 3.75 (br, 2 H, bridge methylene), 2.5–1.85 (c, 8 H, tetrahydroquinazoline and propargyl); yield 36%; UV (0.1 NaOH) λ_{max} 291 nm (ε 25 020); MS (FAB), m/z 353 $[MH]^+$. Anal. ($C_{19}H_{20}N_4O_3$) C, H, N.

Compound **20**: NMR (TFA) δ 8.2 (d, 2 H, aromatic), 7.55 (d, 2 H, aromatic), 7.45 (br, 2 H, 2-amino), 3.6 (br, 2 H, bridge methylene), 3.2 (s, 3 H, *N*-methyl), 2.5–1.6 (c, 7 H, tetrahydroquinazoline); yield 38%; mp 300 °C; MS (FAB), m/z 329 $[MH]^+$. Anal. ($C_{17}H_{20}N_4O_3$) C, H, N.

Compound **21**: NMR (TFA) 8.0 (d, 2 H, aromatic), 7.35 (C, 4 H, aromatic, 2-amino), 3.35 (br, 2 H, bridge methylene), 2.6–1.5 (c, 7 H, tetrahydroquinazoline); yield 40%; mp 300 °C; UV (0.1 N NaOH) λ_{max} 280 nm (ε 18 281); MS (FAB), m/z 315 $[MH]^+$. Anal. ($C_{16}H_{18}N_4O_3$) C, H, N.

General Method for the Synthesis of N^{10}-Propargyl-5,8-dideaza-5,6,7,8-tetrahydrofolic Acid (1) and Analogues 2 and 3. These reactions were carried out by heating a mixture of 676 mg (2 mmol) of **18**·HBr, 2 mmol of the substituted (*p*-aminobenzoyl)glutamic acid derivative, and 160 mg (4 mmol) of MgO in 5 mL of DMAc as described previously.

For the preparation of compound **1**, the amino compound used was diethyl [4-(*N*-propargylamino)benzoyl]-L-glutamate. After reaction, the crude diethyl ester of **1** was hydrolyzed with a mixture of 100 mL of 0.1 N NaOH and 30 mL of acetonitrile for 18 h at room temperature. The acetonitrile was removed under vacuum, and the clear solution was adjusted to pH 7.5, diluted to 250 mL, and applied on a DEAE–cellulose column. The column was eluted with a linear NaCl gradient from 0 to 1.0 M at pH 7.0. The fractions corresponding to product **1** were pooled and evaporated to a small volume, and the pH was adjusted to 4.5 with 1 N HCl. The precipitated product after overnight refrigeration was filtered, washed with ice-cold water, and dried over P_2O_5: yield 25%; mp 185–190 °C; NMR (TFA) δ 8.05 (d, 2 H aromatic), 7.8 (d, 2 H, aromatic), 4.95 (t, α-proton of glutamate), 4.45 (br, 2 H, propargyl), 3.9 (br, 2 H, bridge methylene), 2.8–1.6 (c, 8 H, tetrahydroquinazoline, propargyl); UV (0.1 N NaOH) λ_{max} 300 nm (ε 20 971); MS (FAB), m/z 482 $[MH]^+$. Anal. ($C_{24}H_{27}N_5O_6$) C, H, N, O.

For preparation of compound **2** diethyl [*p*-(methylamino)-benzoyl]-L-gutamate was required. This substituted amine was prepared by the direct alkylation of diethyl (*p*-aminobenzoyl)-L-glutamate with iodomethane. The desired monoalkylation product was separated from the dialkylation product by column chromatography on silica gel. After reaction of this amine with **18**·HBr, the crude product was hydrolyzed and purified by ion-exchange chromatography as described above.

Alternately compounds **1** and **2** were prepared from **19** and **20** by the solid-phase procedure. The carboxyl group of each pteroic acid was activated as the mixed anhydride with isobutyl chloroformate[17] and coupled with α-(benzyloxy)-L-glutamate-γ-resin ester. The product was cleaved from the resin and simultaneously deprotected by treating the reacted resin with a mixture of 1:1 2 N NaOH and *p*-dioxane for 1 h[18] and purifying the liberated product by ion-exchange chromatography over DEAE-cellulose. Compound **1** or **2** prepared by either method were identical in all respects.

Compound **2**: NMR (TFA) δ 7.9 (d, 2 H, aromatic), 7.6 (d, 2 H, aromatic), 7.56 (br, 2 H, 2-amino), 5.01 (t, 1 H, α-proton of glutamic acid), 3.55 (br, 2 H, bridge methylene), 3.18 (s, 3 H, *N*-methyl), 2.8–1.6 (c, 7 H, tetrahydroquinazoline); UV (0.1 N NaOH) λ_{max} 312 (ε 22 167); yield 18%; mp 235–238 °C dec; MS (FAB), m/z 458 $[MH]^+$. Anal. ($C_{22}H_{27}N_5O_6$) C, H, N, O.

5,8-Dideaza-5,6,7,8-tetrahydrofolic acid (**3**), which was required to provide comparative biological data, was prepared by the direct alkylation of **18**·HBr with (*p*-aminobenzoyl)-L-glutamic acid in the presence of MgO in DMAc at 125–135 °C. The UV spectrum and melting point of this compound was identical with those reported by DeGraw and co-workers.[15] **3**: NMR (TFA) δ 7.65 (d, 2 H, aromatic), 7.31 (d, 2 H, aromatic), 4.6 (t, 1 H, α-proton of glutamic acid), 3.3 (br, 2 H, bridge methylene), 2.6–1.5 (c, 7 H, tetrahydroquinazoline); MS (FAB), m/z 444 $[C_{21}H_{25}N_5O_6H]^+$.

Biological Evaluation. Permeabilization of the L1210 cells with dextran sulfate was carried out according to the procedure described by Kucera and Paulus.[19] The enzyme-inhibitory activity of analogues in intact cells was determined as described previously.[20] Thymidylate synthase assays with the *L. casei* enzyme were performed according to the procedure of Wahba and Friedkin.[21] Microbiological assays using various strans of *L. casei*

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and *S. faecium* were carried out as described previously.[22]

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Registry No. **1**, 107174-40-3; **2**, 107174-41-4; **3**, 94961-89-4; **4**, 76849-19-9; **9**, 5446-29-7; **10**, 107174-42-5; **11**, 107174-43-6; **13**, 107174-44-7; **13** (*O*-tosylate), 107174-52-7; **14**, 107174-45-8; **15**, 5452-19-7; **16**, 107174-46-9; **17**, 107174-47-0; **18**, 107174-48-1; **18**·HBr, 107174-53-8; **19**, 107174-49-2; **20**, 107174-50-5; **21**, 107174-51-6; $(CF_3CO)_2O$, 407-25-0; Me_3CCOCl, 3282-30-2; MsCl, 124-63-0; TsCl, 98-59-9; $HC{\equiv}CCH_2NHC_6H_4$-*p*-COOH, 107174-54-9; $MeNHC_6H_4$-*p*-COOH, 10541-83-0; $H_2NC_6H_4$-*p*-COOH, 150-13-0; $HC{\equiv}CCH_2NHC_6H_4$-*p*-CO-Glu(OEt)-OEt, 76858-72-5; $MeNHC_6H_4$-*p*-CO-Glu(OEt)-OEt, 2378-95-2; $H_2NC_6H_4$-*p*-CO-Glu-OH, 4271-30-1; thymidylate synthase, 9031-61-2; dihydrofolate reductase, 9002-03-3.

Additions and Corrections

1986, Volume 29

Hans Rollema, Dora Mastebroek, Håkan Wikström,* Kjell Svensson, Arvid Carlsson, and Staffan Sundell: Enantiomers of 3-(3,4-Dihydroxyphenyl)- and 3-(3-Hydroxyphenyl)-*N*-*n*-propylpiperidine: Central Pre- and Postsynaptic Dopaminergic Effects and Pharmacokinetics.

Page 1892. The atomic fractional coordinates in Table VIII were inadvertently given for the *R* enantiomer instead of the *S* enantiomer. A corrected table follows.

Table VIII. Atomic Fractional Coordinates and Equivalent Isotropic Temperature Factors ($\times 10^2$) for the Non-Hydrogen Atoms[a]

atom	*x*	*y*	*z*	U_{eq}
		Conformation A		
Br(1)	0.0319 (1)	0.7096 (–)	0.8073 (1)	8.3 (0.0)
N(1)	0.1917 (4)	0.4924 (5)	0.9668 (4)	7.0 (0.2)
O(1)	0.6991 (3)	0.7155 (4)	0.8120 (3)	7.8 (0.2)
O(2)	0.8745 (4)	0.5618 (5)	0.9370 (3)	8.7 (0.2)
C(1)	0.5235 (5)	0.5924 (5)	0.9764 (4)	6.3 (0.3)
C(2)	0.5495 (5)	0.6602 (5)	0.8994 (4)	6.3 (0.3)
C(3)	0.6671 (5)	0.6512 (5)	0.8863 (4)	6.2 (0.3)
C(4)	0.7579 (5)	0.5739 (6)	0.9474 (4)	6.8 (0.3)
C(5)	0.7325 (5)	0.5062 (6)	1.0252 (5)	7.6 (0.3)
C(6)	0.6160 (6)	0.5154 (6)	1.0390 (5)	8.0 (0.3)
C(7)	0.3942 (5)	0.6060 (6)	0.9895 (4)	6.8 (0.3)
C(8)	0.3209 (5)	0.4842 (6)	0.9580 (5)	7.5 (0.3)
C(9)	0.1980 (6)	0.5315 (8)	1.0752 (5)	9.0 (0.4)
C(10)	0.2674 (7)	0.6511 (8)	1.1053 (5)	9.5 (0.4)
C(11)	0.3990 (6)	0.6441 (8)	1.0975 (5)	9.2 (0.4)
C(12)	0.1172 (6)	0.3753 (8)	0.9361 (5)	8.6 (0.4)
C(13)	0.0914 (10)	0.3452 (9)	0.8266 (8)	12.2 (0.6)
C(14)	–0.0015 (10)	0.2417 (8)	0.7899 (10)	13.6 (0.6)
		Conformation B		
Br(1)	1.4643 (1)	–0.1131 (1)	0.6735 (0)	7.8 (0.0)
N(1)	1.2813 (5)	0.0244 (7)	0.4576 (4)	9.6 (0.4)
O(1)	0.8158 (4)	–0.0819 (4)	0.7034 (3)	7.8 (0.2)
O(2)	0.6351 (4)	0.0631 (5)	0.5752 (4)	10.4 (0.3)
C(1)	0.9951 (5)	0.0641 (6)	0.5519 (4)	6.9 (0.3)
C(2)	0.9692 (5)	–0.0097 (5)	0.6256 (4)	6.6 (0.3)
C(3)	0.8487 (5)	–0.0146 (5)	0.6324 (4)	6.4 (0.3)
C(4)	0.7525 (5)	0.0578 (6)	0.5644 (5)	7.5 (0.3)
C(5)	0.7767 (6)	0.1276 (8)	0.4891 (6)	9.5 (0.4)
C(6)	0.8987 (6)	0.1292 (7)	0.4819 (5)	8.8 (0.4)
C(7)	1.1316 (5)	0.0777 (6)	0.5535 (5)	7.2 (0.3)
C(8)	1.1466 (6)	0.0096 (6)	0.4590 (5)	7.9 (0.3)
C(9)	1.3182 (7)	0.1510 (11)	0.4587 (8)	12.0 (0.6)
C(10)	1.3064 (7)	0.2220 (8)	0.5514 (8)	11.0 (0.5)
C(11)	1.1721 (7)	0.2090 (8)	0.5557 (7)	10.2 (0.4)
C(12)	1.2972 (9)	–0.0510 (12)	0.3678 (6)	13.7 (0.6)
C(13)	1.4211 (10)	–0.0584 (16)	0.3639 (7)	20.0 (1.1)
C(14)	1.4367 (11)	–0.1110 (13)	0.2817 (7)	15.6 (0.8)

[a] $U_{eq} = {}^1/_3(U_{11} + U_{22} + U_{33} + 2U_{13} \cos \beta)$.