prepared by using an analogous procedure. Purification by column chromatography gave yields of approximately 40%; however, overall yields were higher and purity of the final product was unaffected if this intermediate was not purified before the detritylation.

1-*S*-Hexadecyl-2-*O*-methyl-*rac*-thioglycerol. 1-*S*-Hexadecyl-2-*O*-methyl-3-*O*-trityl-*rac*-thioglycerol (2.0 g, 0.0031 mol) was dissolved in 125 mL of CH_2Cl_2 and cooled to 0 °C under nitrogen. Boron trifluoride–methanol complex (50%, 0.4 mL) was added in one portion. The yellow solution was stirred for 1 h, an additional 0.4-mL portion of BF_3·2MeOH was added, and stirring was continued for 1 h. Water (50 mL) was added and the organic fraction separated and washed with two additional 50-mL portions of water. The CH_2Cl_2 fraction was dried over Na_2SO_4, filtered, and concentrated. The residue was dissolved in 10 mL of petroleum ether, and a small amount of insoluble material (TrOH) was filtered and discarded. Chromatography on silica gel with 9:1 petroleum ether/ether gave pure alcohol (750 mg, 63%) as a waxy solid. NMR ($CDCl_3$): δ 0.87 (t, 3 H, CH_3), 1.2–1.4 (m, 26 H, $(CH_2)_{13}$), 1.58 (p, 2 H, SCH_2CH_2), 2.0 (br s, 1 H, OH), 2.54 (t, 2 H, SCH_2CH_2), 2.62 (d of d, 2 H, $CHCH_2S$), 3.40 (m, 1 H, CH), 3.44 (s, 3 H, OCH_3), 3.75 (d of d, 2 H, CH_2OH). The 1-*S*-hexadecyl-2-*O*-ethyl and the 1-*S*-octadecyl-2-*O*-methyl and -ethyl ethers (1-*S*-alkyl-2-*O*-alkyl-*rac*-thioglycerols) were prepared in a similar manner. Use of crude tritylated starting material gave yields of approximately 50% in two steps from the 1-*S*-alkyl-3-*O*-trityl-*rac*-thioglycerols.

1-*SO*$_2$-Hexadecyl-2-*O*-ethyl-*rac*-sulfonylglycerol. 1-*S*-Hexadecyl-2-*O*-ethyl-*rac*-thioglycerol (0.9 g, 2.5 mmol) was dissolved in methanol (15 mL). Oxone (2.8 g, 9 mmol) in 15 mL of water was added dropwise. The cloudy solution was stirred overnight at room temperature. Water (30 mL) was added and the solution extracted with chloroform (3 × 30 mL). The organic fractions were combined, washed with NaCl solution (20 mL), dried over Na_2SO_4, filtered, and concentrated. The resulting solid (800 mg, 2.0 mmol, 80% yield) was used without further purification. NMR ($CDCl_3$): δ 0.7–2.0 (m, 34 H $(CH_2)_{14}CH_3$ and CH_3), 2.8–3.4 (overlapping m, 4 H, $CH_2SO_2CH_2$), 3.4–4.0 (overlapping multiplets, 3 H, OCH_2 and OCH).

1-*S*-Hexadecyl-2-*O*-methyl-*rac*-thioglycero-3-phosphocholine (1). 1-*S*-Hexadecyl-2-*O*-methyl-*rac*-thioglycerol (3.4 g, 0.0094 mol, dried under high vacuum over P_2O_5) and triethylamine (1.21 g, 0.012 mol, freshly distilled) were dissolved in ethanol-free $CHCl_3$ (100 mL) and added dropwise to $POCl_3$ (1.1 mL, 0.012 mol) under nitrogen. The solution was stirred for 30 min at 60 °C. After cooling, pyridine (4.9 mL, freshly distilled over KOH) was added in one portion followed by solid choline tosylate (4.54 g, 0.0165 mol, dried under high vacuum over P_2O_5). The reaction mixture was stirred at room temperature overnight. Water (3 mL) was added and stirring continued for 30 min. The solution was then extracted with solutions of 3% Na_2CO_3 (3 × 70 mL), 5% HCl (2 × 60 mL), and water (2 × 60 mL) with the addition of methanol to break the emulsions that formed. After drying over Na_2SO_4 and filtration, the chloroform was removed on a rotary evaporator. The resulting semisolid was dissolved in hot chloroform (15 mL) and cooled to room temperature. Acetone (30 mL) was added and the solution cooled to −15 °C. The precipitate that formed was filtered and purified by column chromatography on silica gel with use of $CHCl_3$/MeOH/HOAc/H_2O (50:25:8:4) as eluant. Pure fractions were evaporated to an oil, which required a reprecipitation from $CHCl_3$/acetone to give a solid product (3.4 g, 67%) melting with decomposition at 248–251 °C. The 1-*S*-hexadecyl-2-*O*-ethyl-*rac*-thioglycero-3-phosphocholine (2) (semisolid, no mp, lit.[12] mp 238–243 °C), 1-*S*-octadecyl-2-*O*-methyl-*rac*-thioglycero-3-phosphocholine (3) (mp 246–249 °C, lit.[12] mp 251–252 °C), 1-*S*-octadecyl-2-*O*-ethyl-*rac*-thioglycero-3-phosphocholine (4) (mp 242–245 °C), and the 1-*SO*$_2$-hexadecyl-2-*O*-ethyl-*rac*-sulfonylglycero-3-phosphocholine (5) (mp 247–250 °C) were prepared by the same procedure in 40, 69, 58, and 40% yields, respectively.

NMR spectral data for each new phosphocholine (1–5) are given in Table II.

Acknowledgment. We thank Dr. J. T. O'Flaherty for performing the neutrophil degranulation studies. This work was supported in part by NCI CA 12197, by a grant from the Forsyth Cancer Service, and by NIH Grant HL 28491.

Registry No. 1, 103304-63-8; 2, 103304-64-9; 3, 103304-65-0; 4, 103321-05-7; 5, 103304-73-0; (±)-Me$(CH_2)_{15}SCH_2CH(OH)CH_2OH$, 25666-00-6; (±)-Me$(CH_2)_{17}SCH_2CH(OH)CH_2OH$, 25666-01-7; (±)-Me$(CH_2)_{15}SCH_2CH(OH)CH_2OTr$, 103321-06-8; (±)-Me$(CH_2)_{17}SCH_2CH(OH)CH_2OTr$, 91274-06-5; (±)-Me$(CH_2)_{15}SCH_2CH(OMe)CH_2OTr$, 103304-66-1; (±)-Me$(CH_2)_{15}SCH_2CH(OEt)CH_2OTr$, 103304-67-2; (±)-Me$(CH_2)_{17}SCH_2CH(OMe)CH_2OTr$, 103321-07-9; (±)-Me$(CH_2)_{17}SCH_2CH(OEt)CH_2OTr$, 103321-08-0; (±)-Me$(CH_2)_{15}SCH_2CH(OMe)CH_2OH$, 103304-68-3; (±)-Me$(CH_2)_{15}SCH_2CH(OEt)CH_2OH$, 103304-69-4; (±)-Me$(CH_2)_{17}SCH_2CH(OMe)CH_2OH$, 103304-70-7; (±)-Me$(CH_2)_{17}SCH_2CH(OEt)CH_2OH$, 103304-71-8; (±)-Me$(CH_2)_{15}SO_2CH_2CH(OEt)CH_2OH$, 103304-72-9; choline tosylate, 55357-38-5.

Synthesis of 10-Acetyl-5,8-dideazafolic Acid: A Potent Inhibitor of Glycinamide Ribonucleotide Transformylase[1]

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10-Acetyl-5,8-dideazafolic acid has been synthesized in good yield from the parent compound, 5,8-dideazafolic acid. This quinazoline folate analogue showed no activity as a substrate for the folate-requiring de novo purine biosynthetic enzyme glycinamide ribonucleotide transformylase isolated from the murine lymphoma cell line L5178Y, but proved to be a potent competitive inhibitor, K_i = 1.3 μM, of the purified enzyme.

Recently, it has been amply demonstrated that quinazoline (5,8-dideaza) analogues of reduced folate cofactors can serve as substrate or inhibitors for many of the enzymes that require folate cofactors. This has led to an increased interest in these compounds as potential chemotherapeutic agents. The chemical stability of the quinazolines, relative to the oxidatively labile reduced folate,[2] presents an additional advantage.

In addition to the numerous examples of the interaction of these analogues with dihydrofolate reductase (DHFR)[3] and thymidylate synthase (TS)[4] isolated from a variety of

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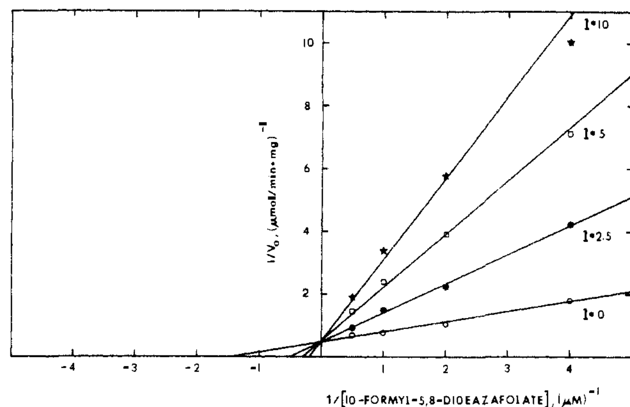
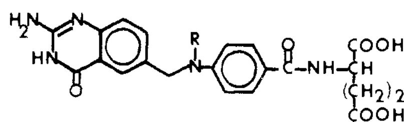


Figure 1. Lineweaver–Burk plot of initial velocity data for inhibition of GAR TFase by 10-acetyl-5,8-dideazafolic acid (3) as a function of 10-formyl-5,8-dideazafolic acid (2) concentration. The concentration of 3 was 0 μM (○), 2.5 μM (●), 5.0 μM (□), and 10.0 μM (★).

sources, recent work from this and other laboratories suggests that the folate-dependent transformylases of de novo purine biosynthesis, glycinamide ribonucleotide transformylase (GAR TFase) (EC 2.1.2.2) and 5-amino-imidazole-4-carboxamide ribonucleotide transformylase (AICAR TFase) (EC 2.1.2.3), interact with these analogues as well. It has been demonstrated that 10-formyl-5,8-dideazafolic acid (2) serves as an efficient substrate for GAR TFase isolated either from the murine lymphoma cell line L5178Y[5] or from chicken liver,[6] but is only a poor substrate for AICAR TFase from chicken liver.[6]

In connection with our continuing studies on mammalian GAR TFase, we sought a competitive inhibitor of the enzyme that would be a useful probe for mechanistic studies and a tool for purification of the enzyme.[5] The 10-acetyl analogue seemed a logical choice as an extension of previous work[5,6] and as a potential inhibitor of GAR TFase.

Chemistry. This paper describes the synthesis of 10-acetyl-5,8-dideazafolic acid (3), a new quinazoline analogue of 10-formyltetrahydrofolic acid, the folic acid cofactor required by the two formyl transferases of de novo purine biosynthesis.[7,8] This analogue (3) was prepared in 80% yield by acetylation of 5,8-dideazafolic acid (1)[9] with acetyl chloride in dimethylacetamide using a modification of the



1, R = H
2, R = CHO
3, R = CH_3CO

procedure employed for the synthesis of 10-acetylfolic acid.[10] The structure of 3 was confirmed by high-resolution NMR, which showed the presence of the acetyl methyl group at δ 1.87 and loss of the triplet at δ 6.84 due to the hydrogen on N-10 in the free base (1).

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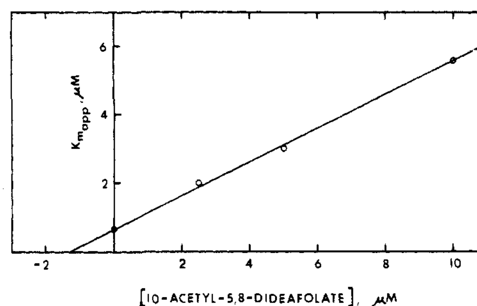

Figure 2. Intercept replot. Plot of $K_{m,app}$ for 10-formyl-5,8-dideazafolic acid (2) as a function of 10-acetyl-5,8-dideazafolic acid (3) concentration.

During the course of this work we have introduced modifications to the published procedures for the syntheses of the precursor free base (1)[9] and the 10-formyl derivative 2[11] which result in preparations of higher purity than that obtained previously.

Biological Evaluation. Compound 3 was tested as a substrate for GAR TFase, which requires 10-formyltetrahydrofolic acid as cofactor,[7,8] but which will use 10-formyl-5,8-dideazafolic acid (2) with comparable efficiency.[5,6] There was no turnover of 3 even with elevated levels of enzyme. When 3 was tested as an inhibitor of the transformylation reaction, it proved to be a potent competitive inhibitor against 2, as illustrated by the Lineweaver–Burk plot (Figure 1), with K_i = 1.3 μM, determined from the intercept replot (Figure 2). The inhibition of GAR TFase from L5178Y by the 10-acetyl analogue 3 compares favorably with that obtained for the chicken liver enzyme with 10-formyl-8-deazafolic acid, K_i = 4 μM.[6]

The interactions of GAR TFase with compound 1 and 10-formylfolic acid were also examined. Compound 1 proved to be competitive against 2 as indicated by the Lineweaver–Burk plot of the initial velocity data (not shown), with a K_i = 2 μM. 10-Formylfolic acid was accepted, albeit poorly, as a substrate by GAR TFase with a $K_{m,app}$ = 60 ± 2 μM. The V_{max} with 10-formylfolic acid, 0.08 μmol/(min·mg), is only 4% of that with 2.

Discussion

10-Acetyl-5,8-dideazafolic acid, as well as the parent free base, proved to be a potent competitive inhibitor of GAR TFase isolated from the murine lymphoma cell line L5178Y, and the 10-acetyl derivative was quite effective as a ligand for affinity chromatography in our recently developed purification scheme for this enzyme.[5] We expect that this new folate analogue will aid in our continuing mechanistic studies on mammalian GAR TFase. We also intend to examine the potential interactions of this new analogue with other folate-requiring enzymes and with L5178Y cells in culture.

Experimental Section

Melting points were determined with a Thomas-Hoover Uni-melt and are uncorrected. Elemental analysis was performed by Mic Anal, Tucson, AZ. DEAE-cellulose (DE-52) was obtained from Whatman, Clifton, NJ. HPLC analyses were performed on a Du Pont 8800 liquid chromatograph equipped with a μBondapak C-18 column (4.6 × 25 cm) with detection at 254 nm. A 15-min linear gradient (0–20% v/v) of MeCN in H_2O adjusted to pH 4 with 10 mM HOAc was used at a flow rate of 1 mL/min. Samples were dissolved in Me_2SO immediately prior to injection. TLC analyses utilized either (A) silica gel (Eastman 13181) with

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$CHCl_3$/MeOH (4:1 v/v) as eluant, (B) cellulose (Eastman 13254) with 0.1 M potassium phosphate, pH 7.5, as eluant, or (C) cellulose (Eastman 13254) with 0.1 M K_2HPO_4 as eluant. UV spectra were recorded with a Varian 2200 spectrophotometer. The ^{1}H NMR spectra were obtained with a Nicolet QE-300 spectrometer operating at 300 MHz. Chemical shifts are reported in parts per million relative to the lock solvent (Me_2SO-d_6) and, in the case of multiplets, refer to the center of the peak. Relative peak areas are reported to the nearest whole number. Enzyme assays were performed with a Gilford 260 spectrophotometer equipped with an automatic cell programmer and a recorder. Glycinamide ribonucleotide was prepared according to the literature procedure.[11] GAR TFase was purified from L5178Y as described previously.[5]

Diethyl 5,8-Dideazafolate. This was prepared by a modification of the published procedure.[9] A solution of 1 g (3.2 mol) of 2-amino-6-(bromomethyl)-4-hydroxyquinazoline hydrobromide[12] and 1.5 g (4.5 mmol) of diethyl *p*-(aminobenzoyl)-glutamate in dimethylacetamide (22 mL) was stirred for 36 h at ambient temperature. The solution was diluted with H_2O (88 mL), and the pH of the resultant suspension was adjusted to pH 4. After several hours at 4 °C, the precipitate was collected by filtration, washed with H_2O and Et_2O, and dried in vacuo over P_2O_5 to yield 1.1 g (2.2 mmol, 69%) of diester as a yellow powder: TLC (A), R_f = 0.43; NMR (Me_2SO-d_6) consistent with the diethyl ester. A sample was recrystallized from 95% ethanol to yield a pale yellow powder: mp 209–211 °C (lit[3] mp 205–210 °C).

5,8-Dideazafolic Acid (1). This was prepared by a slight modification of the literature procedure[9] and purified by anion exchange chromatography. The compound (0.77 g, 1.7 mmol) was dissolved in 0.2 M NH_4HCO_3, pH 8.0, and applied to a column of DEAE-cellulose (3.3 × 30 cm) that had been equilibrated with this buffer. Stepwise elution with increasing concentrations of NH_4HCO_3, pH 8.0, resulted in elution of the product with 0.7 M NH_4HCO_3, pH 8.0. Product-containing fractions were combined and treated with 2 M equiv of Et_3N over buffer, and the solvent was evaporated under reduced pressure at <50 °C. The residue was dissolved in H_2O (15 mL), and this solution was adjusted to pH 4, which resulted in precipitation of product. After several hours at 4 °C, the precipitate was collected by filtration, washed with H_2O and Et_2O, and dried in vacuo over P_2O_5 to yield 0.57 g (74%) of a white powder: mp 231–233 °C dec (lit[9] mp 232 °C dec); TLC (B), R_f = 0.44; HPLC.

10-Formyl-5,8-dideazafolic Acid (2). A modification of the published procedure[3] was used. Acetic anhydride (0.35 g, 3.4 mmol) was added to a solution of 0.22 g (0.5 mmol) of 1 in 88% HCOOH (1 mL), and the solution was stirred overnight at ambient temperature. The solution was added to H_2O (7 mL), and the pH of the resulting solution was adjusted to pH 4. After the suspension had chilled for several hours, the precipitate was collected by filtration, washed with H_2O and Et_2O, and dried in vacuo over P_2O_5 to yield 0.19 g (0.4 mmol, 80%) of a white powder: mp 221 °C (lit[3] mp >210 °C); TLC (B), R_f = 0.72.

10-Acetyl-5,8-dideazafolic Acid (3). This procedure is modified from that described for the synthesis of 10-acetylfolic acid.[10] A solution of 0.22 g (0.5 mmol) of 1 and 0.12 g (1.5 mmol) of acetyl chloride in dimethylacetamide (3.5 mL) was stirred at ambient temperature for 3 h. The solution was added to H_2O (17.5 mL), treated with charcoal, and filtered. The product was precipitated by adjusting the filtrate to pH 4 with 1 N HCl. This suspension was kept at 4 °C for several hours, and the precipitate was collected by filtration, washed with H_2O and Et_2O, and dried in vacuo over P_2O_5 to yield 0.19 g (0.4 mmol, 80%) of a white powder: mp 216 °C dec; TLC (B), R_f = 0.79; HPLC; UV (10 mM potassium phosphate, pH 7.5) λ_{max} 229 nm (ϵ 46.5 × 10^3), λ_{sh} 252 nm (ϵ 18.2 × 10^3); ^{1}H NMR (Me_2SO-d_6). δ 12.4 (br s, 2, COO*H*), 11.0 (br s, 1, pyrimidinone amide N*H*), 8.6 (d, 1, $J_{NH\text{-}H\alpha}$ = 7.5 Hz, glutamic acid amide N*H*), 7.83 (d, 2, J_{AB} = 8.4 Hz, *p*-phenylene), 7.65 (d, 1, $J_{5,7}$ = 1.2 Hz, H-5), 7.35 (dd, 1, $J_{7,8}$ = 8.3 Hz, $J_{5,7}$ = 1.2 Hz, H-7), 7.27 (d, 2, J_{AB} = 8.4 Hz, *p*-phenylene), 7.09 (d, 1, $J_{7,8}$ = 8.3 Hz, H-8), 6.36 (br s, 2, pyrimidinone exocyclic NH_2), 4.91 (s, 2, H-9), 4.36 (m, 1, glutamic acid C_α-H), 2.33 (t, 2, $J_{\beta,\gamma}$ = 7.4 Hz, glutamic acid C_γ-H), 1.98 (doublet of multiplets, 2, glutamic acid C_β-H), 1.87 (s, 3, NCOCH_3). Anal. ($C_{23}H_{23}N_5O_7$·0.5HCl) C, H, N.

10-Formylfolic Acid. Commercial folic acid was purified as described[6] and formylated by the procedure used for the synthesis of **2**. The UV–vis spectrum of the cream-colored product (74%) was in accord with that reported,[2] and the product was pure by TLC (C), R_f = 0.89.

Enzyme Assays. GAR TFase was assayed by monitoring the production of 5,8-dideazafolic acid at 295 nm ($\Delta\epsilon$ = 18.9 × 10^3).[6] The assays were performed at 35 °C and pH 6.8 with the solution containing 100 mM potassium phosphate, pH 6.8, 1 mM (α, β) glycinamide ribonucleotide, and enzyme in 1 mL. For assays of 10-acetyl-5,8-dideazafolic acid as substrate, the solution contained enzyme at 5 times the usual assay concentration and 0.1 mM 10-acetyl-5,8-dideazafolic acid. All components except cofactor analogue were incubated for 5 min at 35 °C, and then analogue 3 was added in a small volume. For the inhibition studies, the concentration of 10-formyl-5,8-dideazafolic acid was varied from 0.25 to 2.0 μM, while 10-acetyl-5,8-dideazafolic acid was maintained at a fixed concentration, which was varied from 0 to 10 μM. All components, except cofactor, were incubated at 35 °C for 5 min, and the reaction was initiated by the addition of cofactor in a small volume. The inhibition studies with 5,8-dideazafolic acid were conducted as described for those with 10-acetyl-5,8-dideazafolic acid, using fixed concentrations of this analogue from 0 to 25 μM. The production of 5,8-dideazafolic acid 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 glycinamide ribonucleotide.

GAR TFase activity with 10–100 μM 10-formylfolic acid was assayed by monitoring the production of folic acid at 300 nm ($\Delta\epsilon$ = 17 × 10^3).[2] The assay conditions were essentially identical to those with 10-formyl-5,8-dideazafolic acid as substrate (vida infra).

Acknowledgment. This investigation was supported by PHS Grant CA28312, awarded by the National Cancer Institute, DHHS, and, in part, by BRSG Grant RR07062, awarded by the Biomedical Research Support Grant Program, Division of Research Resources, NIH. We wish to thank Dr. John B. Hynes, Medical University of South Carolina, for the HPLC analyses.

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