response time greater than or equal to the mean response time of the control group of 10 mice plus two standard deviations of their mean. Median analgesic doses (ED_{50}) and their 95% confidence limits were determined by the method of Litchfield and Wilcoxon.[10] The mice used in the analgesic assay were closely observed for physical and behavioral changes during the test period and were examined for lethality 24 h after testing. No deaths were noted to occur at any dose levels of the test compounds, although **6b** produced lethality in the smaller mice used during the naloxone-precipitated jumping assay (vp). Naloxone hydrochloride (4 mg/kg) was administered to groups of eight mice, followed by sc ED_{100} doses of **6a-c** and ethoheptazine after a 5-min period. Analgesic activity was then measured 15 min after administration of the test compounds using the tail-flick procedure. Naloxone pretreatment abolished the analgesic activity of **6a** in all animals tested, while analgesic activity was still noted after **6b** and **6c** in one and in two animals, respectively; however, the level of analgesia was deemed insignificant, since the criterion for analgesia was exceeded by an average of only 0.11 s. The procedure of Wiley and Downs was employed in order to obtain data relevant to the physical-dependence capacity of **6b** and **6c**.[11] Swiss-Webster male mice weighing 14-20 g in groups of 10 mice each were given sc injections of **6b** and **6c** 1 h prior to injection of 100 mg/kg naloxone. Saline was administered to a group of mice as a vehicle control, and morphine sulfate was similarly tested as a positive control. All compound doses were calculated as free base and were administered in volumes of 10 mL/kg. Immediately after naloxone injection, mice were individually confined under 4-L beakers on a table top. The proportion of animals jumping at least once and the total number of jumps per treatment group were recorded for 10 min following naloxone injection. Jumping was defined as all four feet simultaneously off the table top. In this assay, a dose of 50 mg/kg of morphine elicited an average of 34.1 jumps with 90% of the naloxone-treated mice jumping. Doses of 25 and 50 mg/kg of **6b** produced 42.4 and 79.5 jumps, respectively, with 100% of the naloxone-treated mice jumping. Doses of 40 and 80 mg/kg of **6c** produced an average of 33.9 and 42.0 jumps in naloxone-treated mice, respectively. These doses of **6c** elicited jumping in 80 and 100% of the test mice. The average number of jumps elicited by morphine, **6b** and **6c** were significantly greater ($p < 0.01$) than those produced by saline injection. Groups of six mice were pretreated with morphine sulfate (5 mg/kg, AD_{100}, sc), followed 10 min later by a sc dose of twice the AD_{50} of **6a-d** and a 200 mg/kg dose of **6e**. The mice were then evaluated for analgesia using the tail-flick procedure 20 min after administration of the test compounds. None of the test compounds produced a significant antagonism of morphine analgesia, except in the case of **6a** which produced a slight shortening of the tail-flick response time in three of the six treated mice.

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Folate Analogues Altered in the C^9-N^{10} Bridge Region. 16. Synthesis and Antifolate Activity of 11-Thiohomoaminopterin[1]

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The synthesis of 11-thiohomoaminopterin (**1**), which is a close analogue of 11-thiohomofolic acid (**2**), has been carried out by modification of the Boon-Leigh procedure. Treatment of 1-chloro-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone (**5**) with sodium azide gave 1-azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone (**6**). After protection of the carbonyl group of **6**, the product **7** was catalytically hydrogenated to 1-amino-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone ketal (**3**). Reaction of **3** with 6-chloro-2,4-diamino-5-nitropyrimidine gave the desired pyrimidine intermediate, which was elaborated to 4-amino-4-deoxy-11-thiohomopteroic acid (**20**) by standard procedures. Alternately, 1-azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone ketal (**7**) was hydrolyzed to the corresponding acid (**8**) and coupled with diethyl L-glutamate to obtain diethyl *N*-[*p*-(1-azido-2-oxo-4-thiobutanoyl)benzoyl]-L-glutamate ketal (**10**), which was used for the large-scale preparation of 11-thiohomoaminopterin (**1**). Although 11-thiohomoaminopterin showed antifolate activity against two folate-requiring microorganisms and inhibited *Lactobacillus casei* dihydrofolate reductase, it did not exhibit any antitumor activity against L-1210 lymphoid leukemia in mice at a maximum dose of 48 mg/kg.

Folate antagonists are very widely used in the chemotherapy of neoplastic diseases, and methotrexate continues to be the most important drug belonging to this series.[2-4] The literature on the fate and functions of methotrexate has accumulated to the point that comprehensive review is impractical, if not impossible. Although several close analogues of methotrexate have been synthesized and evaluated, these compounds do not have therapeutic indices high enough to warrant their development as anticancer drugs.[5,6] However, quite recently 10-deazaaminopterin, which is a close analogue of aminopterin in which the 10-amino group was replaced with a methylene group,

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has been synthesized[7,8] and evaluated in several test systems.[9,10] With regard to its inhibition of dihydrofolate reductase and inhibition of growth of *Streptococcus faecium* and *Lactobacillus casei*, 10-deazaaminopterin was classified as one of the most potent antifolates.[8] The in vivo antitumor activity of 10-deazaaminopterin has been shown to be superior to methotrexate following administration in mice against three of five ascites tumors and two of three solid tumors.[8] Although the pharmacokinetics were similar for both methotrexate and 10-deazaaminopterin, the latter had a relatively greater accumulation and improved delivery in tumor.[8] An isomer of folic acid, 10-oxaaminopterin, has been synthesized and evaluated in our laboratory and was found to have significantly different transport properties compared to methotrexate in HeLa cells[11] and showed in vivo antitumor activity against L-1210 lymphoid leukemia in mice comparable to that of methotrexate. Both 10-oxaaminopterin and methotrexate inhibited the uptake of deoxy[2-^{14}C]uridine into DNA and growth of HeLa cells at similar concentrations. These data taken together suggested that some of the analogues of methotrexate and aminopterin which are altered in the C^9–N^{10} bridge region could exhibit superior antitumor properties as a result of altered transport characteristics, affinity for transport receptors, tissue accumulation, distribution, clearance, and/or metabolism. As part of a continuing program aimed at developing folate analogues[1,12-16] which are altered in the C^9–N^{10} bridge region, we describe the synthesis and biological evaluation of 11-thiohomoaminopterin in this paper. The synthesis that follows is different from the one which was previously described by us in a preliminary communication.[16]

Chemistry. The chloromethyl ketone **5**, which was prepared by the Michael addition of *p*-(carbomethoxy)thiophenol and chloromethyl vinyl ketone,[17] was shown to undergo a retro-Michael reaction[18] on exposure to aqueous sodium azide in several solvent systems. Reaction of this chloromethyl ketone with potassium phthalimide resulted in a rearrangement reaction,[18] the product of which was not suitable for the construction of the pteridine ring. Reinvestigation of the reaction between chloromethyl ketone **5** and sodium azide established that the desired azide **6** was formed under strictly anhydrous conditions in aprotic solvents and the yield can be improved by the addition of potassium iodide. Protection of the carbonyl group of **6**, as the ketal in the usual manner, gave 1-azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone ketal (**7**). Catalytic reduction of **7** using 5% Pd on carbon in methanol gave **3**. Alternately, the keto azide **6** could be hydrogenated to the corresponding amino ketone hydrochloride by the addition of a molar equivalent of HCl to the compound prior to hydrogenation. Reaction of **3** with 6-chloro-2,4-diamino-5-nitropyrimidine (**12**) gave the intermediate **13**. Removal of the ketal protective group of this intermediate with aqueous trifluoroacetic acid gave **14**, which on dithionite reduction[19,20] in aqueous DMF gave the corresponding 5-amino compound.

Treatment of this reduction product with a mixture of pyridine and pyridine hydrochloride in ethanol gave methyl 4-amino-4-deoxy-7,8-dihydro-11-thiohomopteroate, which was converted to methyl 4-amino-4-deoxy-11-thiohomopteroate (**19**) by aerobic oxidation[16] in DMF at elevated temperatures. Hydrolysis of this oxidation product (**19**) with 0.33 N NaOH in acetonitrile[21] gave the corresponding pteroic acid **20** in ~33% yield based on the dithionite reduction product. Introduction of the glutamate moiety at this stage either by the solid-phase procedure[14,15] or by coupling in solution by the isobutyl chloroformate method failed to give a good yield of the desired glutamate conjugate (**1**). Since a larger amount of **1** was required for the in vivo antitumor evaluation, incorporation of the glutamate moiety to the side chain prior to the construction of the pteridine ring was considered. This was accomplished by the following procedure.

Azide **7** was hydrolyzed to the crystalline acid **8**. The carboxyl group of this acid was activated by the isobutyl chloroformate method and was coupled with diethyl L-glutamate to obtain **10**. Hydrogenation of **10** with 5% Pd on carbon gave the corresponding amine **4**, which, on reaction with nitrochloropyrimidines **12** and **11**, gave the respective pyrimidine intermediates **15** and **17**. Removal of the ketal group from **15** and **17** as described for **13** gave **16** and **18**. Dithionite reduction of **16** accompanied by cyclization of the product to the dihydropteridine and its oxidation to the diethyl ester of **1** were carried out using similar procedures which were employed for the conversion of **14** to **20**. Hydrolysis of the diethyl ester of **1**, using the procedure of Kim et al.,[21] gave the final product **1**, which was purified by ion-exchange chromatography. The light yellow compound thus obtained showed UV and NMR spectral characteristics which were in complete agreement with structure **1**. Using a similar procedure, **18** was converted to 11-thiohomofolic acid[18] in good yield (Scheme I).

Biological Evaluation and Discussion. The preliminary evaluation of the antifolate activity of 11-thiohomoaminopterin was done using two folate-requiring microorganisms. Compound **1** showed 50% inhibition of the growth of *Lactobacillus casei* (ATCC7469) and *Streptococcus faecium* (ATCC8043) at 0.1 and 2.0 ng/mL. The homopteroate analogues **19** and **20** were also evaluated for their antifolate activities using the above procedure and they exhibited I_{50} values of 400 and 100 ng/mL for *L. casei* and 16 and 100 ng/mL for *S. faecium*.

The antifolate activity of compound **1** is potent, although it is ~10 times weaker than methotrexate under identical assay conditions. Therefore, 11-thiohomoaminopterin (NSC 313384) was evaluated as a potential anticancer agent at the National Cancer Institute. On a treatment schedule of QD 1D × 09 in mice bearing L-1210 lymphoid

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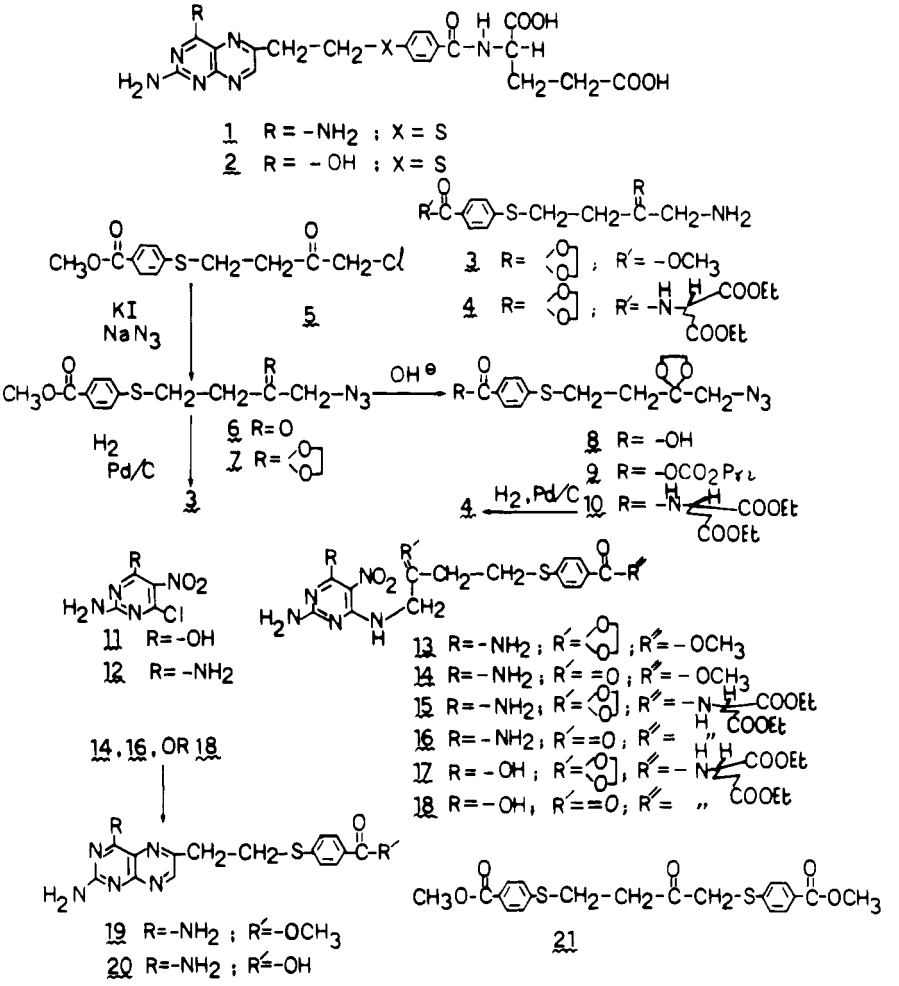
Scheme I



leukemia, 11-thiohomoaminopterin failed to exhibit any antitumor activity (% T/C < 125) at doses ranging from 0.38 to 48 mg/kg. In order to gain some insight as to why 11-thiohomoaminopterin failed to show antitumor activity, it was examined in vitro for its ability to inhibit *L. casei* dihydrofolate reductase (DHFR) and thymidylate synthase (TS), and these results were compared with those of MTX. Under identical assay conditions, 1 exhibited I_{50} values of 6×10^{-5} M for TS and 4×10^{-7} M for DHFR (I_{50} values for MTX were 4×10^{-5} and 9.6×10^{-9} M). We have previously observed that the I_{50} values of three closely related analogues, in which their "bridge regions" were altered but not elongated, were very similar to MTX with regard to their inhibition of *L. casei* DHFR. These three compounds were 10-deazaaminopterin,[7] 10-oxaaminopterin,[14] and 10-thioaminopterin.[13,21] The former two compounds exhibited in vivo antitumor activities comparable to or superior to MTX in L-1210 leukemia in mice, and the latter showed strong inhibition of human leukemia cell DHFR ($I_{50} = 7.9 \times 10^{-9}$ M). From these results it would appear that elongation of the bridge region of classical aminofols results in their decreased inhibition of *L. casei* DHFR. It is noteworthy that this result is in direct contrast with the corresponding 7,8-dihydro-4-hydroxy analogues,[1,14,17,20] whose substrate activity to this enzyme increases as a result of bridge elongation.[1] In addition, we have also observed that 1 competes less efficiently ($I_{50} = 25 \times 10^{-6}$ M) compared to 10-thioaminopterin ($I_{50} = 2.7 \times 10^{-6}$ M) or 10-oxaaminopterin ($I_{50} = 0.74 \times 10^{-6}$ M) with MTX transport[11,22] in HeLa cells. Thus, it appears that the inactivity of 1 at the tested dose levels can be explained in terms of its decreased transport and binding to the enzyme DHFR compared to MTX and suggest that classical aminofols whose bridge regions are further elongated than in aminopterin or MTX are high risks for development as potential inhibitors of DHFR having the desirable transport characteristics.

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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 a 90-MHz Perkin-Elmer R-32 spectrometer with Me_4Si as internal lock signal. Field strength of the various proton resonances are expressed in δ (parts per million) and coupling constants in hertz. Peak multiplicity is depicted as usual: s, singlet; d, doublet; t, triplet; q, quartet; br, broadened singlet or unresolved multiplet, the center of which is given. UV spectra were determined on a Beckman Model 25 spectrophotometer. Ion-exchange chromatography was carried out on DEAE-cellulose in the chloride form with 2.5 × 20 cm packing unless otherwise specified. A linear NaCl gradient of 1 L each of 0–0.5 M NaCl in 0.005 M phosphate buffer, pH 7, was used to elute the column. Elemental analyses were either by Galbraith Laboratories, Inc., Knoxville, Tenn., or Integral Microanalytical Lab, Raleigh, N.C. 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.

Preparation of 1-Azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone (6). The preparation of chloromethyl ketone **5** by the Michael addition of *p*-(carbomethoxy)thiophenol and chloromethyl vinyl ketone has been described by us previously.[18] Treatment of this chloromethyl ketone with sodium azide in aqueous methanol or aqueous acetone gave **21**, which was identified by comparison with an authentic sample.[18] A solution of 1.5 g (5.5 mmol) of chloromethyl ketone **5** in 50 mL of acetone

was stirred with a mixture of 92 mg (0.55 mmol) of potassium iodide and 447 mg (6.88 mmol) of sodium azide, and the progress of the reaction was monitored by TLC. When all the starting material was consumed (4 h), the reaction mixture was evaporated to dryness at reduced pressure and 50 mL of ether was added. The residue was removed by filtration and on evaporation of the ether layer a light yellow syrup was obtained, which was crystallized by the addition of methanol: yield 1.4 g (91%); mp 83–84 °C, NMR ($CDCl_3$) δ 2.83, 3.29 (2 t, 4 H, ethylene), 3.90 (s, 3 H, carbomethoxy), 3.95 (s, methylene), 7.35, 8 (2 d, 4 H, aromatic). Anal. ($C_{12}H_{13}N_3O_3S$) C, H, O.

Conversion of 6 to 1-Azido-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone Ketal (7). In a 500-mL round-bottom flask a suspension of 976 mg (3.5 mmol) of **6** in 5 mL of ethylene glycol was stirred with 80 mg (1.0 mmol) of *p*-toluenesulfonic acid monohydrate at 100 °C for 10 min. Very carefully, 200 mL of dry benzene was added, and the mixture was refluxed under vigorous stirring with the continuous removal of water using a Dean–Stark distilling receiver for 6 h. The benzene was removed under reduced pressure and the reaction mixture was cooled to 30 °C. An excess of (1 g) anhydrous potassium bicarbonate was added to the contents of the flask to neutralize the toluenesulfonic acid. On addition of 300 mL of saturated bicarbonate solution to the flask, a viscous oil was separated, which was extracted in 100 mL of benzene. The benzene layer was washed with water, dried with sodium sulfate, and evaporated to a residue, which crystallized on standing in methanol: yield 969 mg (85%); mp 49 °C; NMR ($CDCl_3$) δ 2.06, 3.03 (2 t, 4 H, ethylene), 3.22 (s, 2 H, methylene), 3.88 (s, 3 H, carbomethoxy), 7.30, 7.92 (2 d, 4 H, aromatic). Anal. ($C_{14}H_{17}N_3O_4S$) C, H, N.

Hydrolysis of 7. Preparation of 1-Azido-4-(*p*-carboxythiophenoxy)-2-butanone Ketal (8). A solution of 969 mg (3 mmol) of **7** in 25 mL of acetonitrile was stirred with 24 mL of 0.5 N NaOH for 6 h. At the end of this period most of the acetonitrile was evaporated off at reduced pressure. The remaining solution was diluted to 100 mL, the pH was lowered to 4.0 by the addition of glacial acetic acid, and the solution chilled for 3 h. The crystals thus formed were removed by filtration, washed with water, and dried: yield 910 mg (~99%); mp 122 °C. Anal. ($C_{13}H_{15}N_3O_4S$) C, H, N.

Catalytic Hydrogenation of 6 and 7. Compound **6** was hydrogenated as a 2% solution in methanol containing an equivalent molar amount of 1 N HCl using 5% palladium on carbon as a catalyst (10% by weight based on the starting material) at 23 psi for 18 h. The catalyst was removed by filtration and the product was characterized as the crystalline picrate **6b**, which was prepared by treating the hydrogenation product with an aqueous solution of picric acid: mp 149–150 °C. Anal. ($C_{18}H_{18}N_4O_{10}S$) C, H, N.

In a similar manner, compound **7** was hydrogenated without the addition of HCl. Evaporation of the solvent gave the product whose infrared spectrum established that complete reduction of the azide to the amine has taken place. Without further characterization, this material (**3**) was successfully used for the preparation of **13**. The hydrogenation product of **6**, on treatment with the nitrochloropyrimidine **12**, failed to give **14** but gave tarry reaction products which were unsuitable for further work.

Preparation of 1-[*N*-(2,4-Diamino-5-nitropyrimidin-6-yl)amino]-4-[*p*-(carbomethoxy)thiophenoxy]-2-butanone Ketal (13). This reaction was carried out by refluxing a solution of 594 mg (2 mmol) of the hydrogenation product **3** in 100 mL of methanol with 380 mg (2 mmol) of 6-chloro-2,4-diamino-5-nitropyrimidine for 1 h. After this period, 0.23 mL (2 mmol) of *N*-methylmorpholine was added to the reaction mixture and refluxing continued for an additional 3 h. On evaporation of the solvent under reduced pressure, light yellow crystals were formed, which were collected by filtration, washed successively with water and methanol, and dried: yield 700 mg (78%); mp 142–144 °C. Anal. ($C_{18}H_{22}N_6O_6S$) C, H, N, S.

Deprotection of 13. Preparation of 14. A solution of 1.8 g (4 mmol) of **13** in 50 mL of TFA was slowly heated to 50 °C under stirring. To this solution, during a period of 20 min, 50 mL of water was added and the reaction mixture was evaporated to dryness at reduced pressure. On addition of 200 g of ice and trituration, compound **14** separated as a yellow powder, which was collected by filtration, washed with water, and dried to obtain the analytical sample: yield 1.52 g (94%); mp 215 °C; UV (0.1 N NaOH) λ_{max} 316 nm. Anal. ($C_{16}H_{18}N_6O_5S$) C, H, N, S.

Preparation of 4-Amino-4-deoxy-11-thiohomopteroic Acid (20). (A) Dithionite Reduction of 14. This reaction was carried out by a slight modification of the previously reported procedure. A solution of 1.22 g (3 mmol) of **14** in 60 mL of *p*-dioxane at ~55 °C was treated with 10 g of sodium dithionite and stirred vigorously while 55 mL of distilled water was added portionwise to this mixture during a period of 15 min. At this point, a homogeneous solution was obtained. The solvents were removed under vacuum and the residue was triturated with 250 g of crushed ice. The yellow solid thus obtained was filtered, washed with water, and dried under vacuum: yield 900 mg (80%); UV (0.1 N NaOH) λ_{max} 320 (s) nm, 281.

(B) Oxidation and Hydrolysis of the Dithionite Reduction Product. In a three-neck round-bottom flask containing 100 mL of a 1:1 mixture of methanol and pyridine whose pH was previously adjusted to 5.0 by the addition of concentrated HCl, 900 mg (2.39 mmol) of the dithionite reduction product was added and refluxed for 1 h in an atmosphere of N_2. The solution was evaporated to dryness and on addition of 300 g of crushed ice a yellow solid was formed, which was filtered, washed, and dried: yield 750 mg. This product was suspended in 750 mL of dry dimethylformamide and slowly heated to 120 °C under stirring and maintained at this temperature. Every 30 min an aliquot was withdrawn and its UV spectrum was examined in 0.1 N NaOH. The well-defined λ_{max} at 320 and 280 nm of the starting material gradually shifted to 375 and 260 nm in a 1:4 ratio, and this spectral change remained stable after 2 h, indicating that the oxidation was complete. The solution was cooled and DMF was removed under vacuum. The NMR spectrum of this compound in TFA exhibited resonances expected of the pteridine structure **19**. Hydrolysis of **19** using 0.33 N NaOH in acetonitrile for 4 h and subsequent chromatography of the crude hydrolysate on DEAE-cellulose gave pure 4-amino-4-deoxy-11-thiohomopteroic acid as a yellow powder: yield 240 mg (24% overall from **14**); the UV spectrum of this compound showed λ_{max} (0.1 N HCl) at 338, 282, and 243 nm and was almost identical with that of 4-amino-4-deoxy-10-thiopteroic acid (λ_{max} at 340, 287, and 247 nm); NMR (TFA) δ 3.6 (br t, 4 H, ethylene bridge), 7.4, 8.05 (d, d, 4 H, aromatic), 8.78 (s, 1, H_7, pteridine ring). Anal. ($C_{15}H_{14}N_6O_2S$) C, H, N, S.

Preparation of Diethyl *N*-[*p*-(1-Azido-2-oxo-4-thiobutanoyl)benzoyl]-L-glutamate Ketal (4). A solution of 927 mg (3 mmol) of **8** was mixed with 0.42 mL (3.75 mmol) of *N*-methylmorpholine. To this solution 0.4 mL (3 mmol) of freshly distilled isobutyl chloroformate was added and stirred for 20 min. During this period, a solution of 1.44 g (6 mmol) of diethyl L-glutamate hydrochloride in 50 mL of DMF was treated with 0.68 mL (6 mmol) of *N*-methylmorpholine, and the clear solution thus obtained was added to the flask containing the mixed anhydride **9**. After 18 h, the solvent was removed under vacuum at 60 °C, and the product was extracted in ethyl acetate, washed with water, and reextracted with three 50-mL portions of saturated sodium bicarbonate to remove unreacted **8**. The ethyl acetate layer was washed again with water and evaporated to obtain the product, which showed a single spot on TLC and showed NMR signals in accordance with structure **4**: yield 1.1 g (76%).

Reaction of 4 with 6-Chloro-2,4-diamino-5-nitropyrimidine. Preparation of 15 and 16. This reaction was carried out by using the procedure which was described for the preparation of **13**. A solution of 2.97 g (6.2 mmol) of **4** in 200 mL of methanol was refluxed with 1.17 g (6.2 mmol) of **12** and 0.7 mL (6.2 mmol) of *N*-methylmorpholine for 4 h, and the solvent was removed under vacuum. Addition of 500 g of crushed ice to the flask containing the residue and trituration gave a product which did not crystallize in several solvent systems but exhibited λ_{max} (0.1 N NaOH) at 336 and 285 nm. Examination of this product (**15**) by TLC revealed that it was not contaminated with **4**. Compound **15** was dissolved in 50 mL of TFA and slowly heated to 50 °C during a period of 30 min with the portionwise addition of 50 mL of distilled water. The solution was evaporated to dryness under vacuum and on addition of ice a yellow solid was separated, which was filtered, washed, and dried to obtain 2.6 g (72%) of **16**: the UV spectrum of this compound in 0.1 N NaOH showed a single λ_{max} at 315 nm; mp 155 °C. Anal. ($C_{24}H_{31}N_7O_8S$) C, H, N, S.

Reaction of 4 with 2-Amino-6-chloro-4-hydroxy-5-nitropyrimidine (11). Preparation of 17 and 18. The preparation of **17**, which is the 4-hydroxy analogue of **16**, was carried out as described above for **15**. The ketal derivative **17** was not crystalline. Upon removal of the protective group from **17** with TFA and water, **18** was obtained as a light yellow powder in 80% yield: mp 142–143 °C; UV (0.1 N NaOH) λ_{max} 310 nm. Anal. ($C_{24}H_{30}N_6O_9S$) C, H, N, O.

Conversion of 16 to 11-Thiohomoaminopterin (1). The dithionite reduction of **16** and the cyclization and oxidation of the reduction product to diethyl-11-thiohomoaminopterin were carried out exactly as described for the preparation of methyl-4-amino-4-deoxy-11-thiohomopteroic acid (**19**). The noncrystalline diethyl ester thus obtained was hydrolyzed to **1** by using 0.33 N NaOH in acetonitrile for 4 h. The pH of the hydrolysate was adjusted to 7.3 and chromatographed over DEAE-cellulose using the NaCl gradient. The product was eluted from the column at a concentration of ~0.3 M NaCl, which was preceded by the elution of less polar minor impurities. The column effluent corresponding to the desired product was pooled and concentrated to a small volume. Acidification of this concentrate with glacial acetic acid gave a yellow precipitate, which was collected by filtration, washed several times with distilled water, and dried to obtain **1** in 50% yield based on **16**. This product was identical with the glutamate conjugate obtained by the coupling of glutamate with **20** by the isobutyl chloroformate method (vide infra).

Conversion of 20 to 11-Thiohomoaminopterin (1). Initial attempts to convert **20** to **1** by the solid-phase procedure which was successfully used for the preparation of 10-thioaminopterin was found to be unsatisfactory due to the low yield of the final product. This was due to the instability of both the reactant (**20**) and product (**1**) under the cleavage conditions which were employed for the release of the resin-bound product. Therefore, the coupling reaction was carried out in solution using the isobutyl chloroformate method.[19,20] A solution of 342 mg (1 mmol) of **20** in a 1:1 mixture of Me_2SO and THF was made by dissolving the compound first in 15 mL of dry Me_2SO at 100 °C, cooling to 30 °C, and then adding 15 mL of dry THF. After cooling this solution in an ice bath, 0.14 mL (1.25 mmol) of *N*-methylmorpholine was added, which was followed by the addition of 0.13 mL (1 mmol) of freshly distilled isobutyl chloroformate. The solution was slowly allowed to warm up to 25 °C during a period of 20 min. A solution of 480 mg (2 mmol) of diethyl L-glutamate hydrochloride in 20 mL of dry Me_2SO was made in a separate flask, neutralized with the addition of 0.23 mL (2 mmol) of *N*-methylmorpholine, and was added to the flask containing the mixed anhydride. After stirring for 18 h at 25 °C, most of the THF was removed by rotary evaporation under vacuum. The bright yellow solution thus obtained was diluted to 150 mL by the addition of 100 mL of distilled water and 20 mL of 1.0 N NaOH and stirred at 25 °C for 6 h to hydrolyze the ester moieties. The pH of the hydrolysate was adjusted to 7.2 and chromatographed over a DEAE-cellulose column using a linear NaCl gradient at pH 7.0 to elute the column. Two major products were eluted from the column, of which the less polar one was identified as **20** and the major one, which was obtained in 60% yield, was identified as **1**. The UV spectrum of this compound was identical with that of 11-thiohomofolic acid in 0.1 NaOH but differed considerably in 0.1 N HCl: UV (0.1 N NaOH) λ_{max} 258 nm (ϵ 32029), 372 (7139); UV (0.1 HCl) λ_{max} 248 nm (ϵ 26944), 337 (9828); NMR (TFA) δ 1.5–2.9 (c, 4 H, glutamate), 3.55 (t, 4 H, ethylene bridge), 4.05 (t, 1 H, glutamate), 7.45, 7.8 (2 d, 4 H, aromatic), 8.78 (s, 1 H, pteridine ring). Anal. ($C_{20}H_{21}N_7O_5S \cdot 1.25H_2O$) C, H, O, S.

Methods Used for Biological Testing. The antitumor data on compound **1** (NSC 313384) were collected at Arthur D. Little, Inc., Boston, MA, under the auspices of the National Cancer Institute using the standard protocol for evaluating (Instruction 14) methotrexate analogues in the L-1210 leukemia test system. Dihydrofolate reductase,[23] thymidylate synthase,[24] and microbiological assays[25] were carried out as described previously.[1]

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Prostaglandins and Congeners. 22.[1] Synthesis of 11-Substituted Derivatives of 11-Deoxyprostaglandins E_1 and E_2. Potential Bronchodilators

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The interesting bronchodilator activity of *l*-11-deoxy-11α-[(2-hydroxyethyl)thio]prostaglandin E_2 methyl ester (**3a**) is described. The preparation of **3a** and its analogues by Michael-type additions to various members of the PGA series or by total synthesis using the lithiocuprate conjugate addition process is also described. Structure–activity relationships in this series are discussed.

Prostaglandins of the E series are potent bronchodilators.[3] However, it also is apparent that the natural prostaglandins will not find clinical application, since they induce cough and irritation of the upper respiratory tract.

(1) For the previous paper in this series, see M. B. Floyd and M. J. Weiss, *J. Org. Chem.*, 44, 71 (1979).

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