The compound **1b** was converted into its hydrochloride by dissolving it in an equal volume of EtOH and CH_3COCH_3 and treating with 6 *N* HCl. Recrystallization (95% EtOH–CH_3COCH_3) gave crystals of **1b** hydrochloride: mp 205–210° dec; ir (KBr disk) 3500–3100 cm^{-1} (broad); $[\alpha]^{25}D$ −133.8° (*c* 1, H_2O); mass spectrum (70 eV) *m/e* 343 (100%). Anal. ($C_{20}H_{26}ClNO_4 \cdot H_2O$) C, H, N, Cl.

Reduction of Naloxone. Preparation of 2b. A solution of 1.48 g (4 mmol) of naloxone hydrochloride in the minimum volume of H_2O was treated with part of a solution of aqueous NaOH (2.22 g in 130 ml of H_2O) until the mixture turned clear and alkaline. Formamidinesulfinic acid (1.85 g, 10 mmol) was dissolved in the remaining NaOH solution and added to the reaction mixture. The final aqueous volume was made up to 200 ml. Experimental conditions were similar to the previous reaction; however, a 3-hr period was necessary for this reaction to go to completion. On work-up, as in the previous experiment, a white precipitate of **2b** was obtained. This, on drying, weighed 0.52 g (40%): mp 107–110°; TLC R_f 0.70; NMR δ 4.52 (d, 1, J = 6 Hz, 5β-H), 3.68–3.40 (m, 1, 6α-H), 5.94–5.60 (m, 1, vinylic H), 5.26–5.10 (t, 2, gem vinylic H). The compound **2b** was converted to its hydrochloride and recrystallized (95% EtOH–CH_3COCH_3) as in the previous case: mp of **2b** hydrochloride 205–207° dec; $[\alpha]^{25}D$ −158.3° (*c* 0.7, H_2O); mass spectrum (70 eV) *m/e* 329 (100%). Anal. ($C_{19}H_{24}ClNO_4 \cdot H_2O$) C, H, N, Cl.

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Synthesis of N^{10}-Methyl-4-thiofolic Acid and Related Compounds

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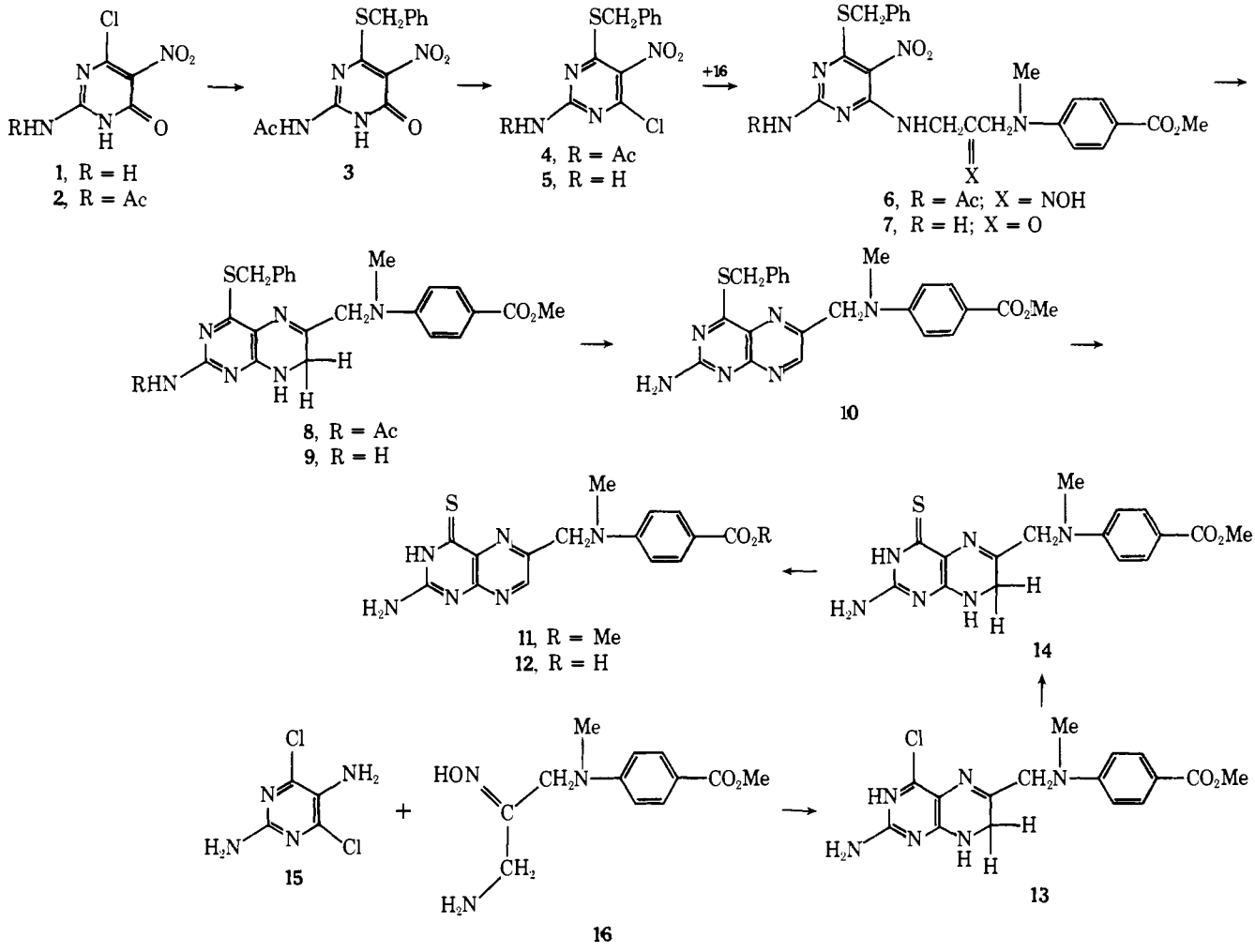
Compound **21** (N^{10}-methyl-4-thiofolic acid) and related compounds were prepared as potential inhibitors of the cofactor forms of tetrahydrofolate. The preparation of 2-acetylamino-4-(benzylthio)-6-chloro-5-nitropyrimidine (**4**) provided an intermediate that was allowed to react with methyl *p*-[(3-aminoacetonyl)methylamino]benzoate oxime (**16**). The oxime function of the resulting 6-substituted aminopyrimidine **6** was hydrolyzed to give the corresponding acetonylaminopyrimidine **7**, which on reductive cyclization gave methyl *p*-[[[2-amino-4-(benzylthio)-7,8-dihydro-6-pteridinyl]methyl]methylamino]benzoate (**9**). This dihydropteridine was oxidized with potassium permanganate, and the product was treated successively with sodium hydrosulfide to replace the benzylthio group and with aqueous sodium hydroxide to hydrolyze the ester function to give *p*-[[(2-amino-3,4-dihydro-4-thioxo-6-pteridinyl)methyl]methylamino]benzoic acid (N^{10}-methyl-4-thiopteroic acid, **12**). Another route to **12** involved the interaction of 2,5-diamino-4,6-dichloropyrimidine (**15**) with **16** to give methyl *p*-[[(2-amino-4-chloro-7,8-dihydro-6-pteridinyl)methyl]methylamino]benzoate (**13**). Displacement of the chloro group of **13** with sodium hydrosulfide followed by the simultaneous air oxidation of the dihydropteridine ring and saponification of the ester group gave **12**. After protection of the 2-amino and 4-thioxo moieties of **12**, the resulting intermediate benzoic acid was coupled with diethyl L-glutamate. The product of this reaction was deblocked to give **21**. Methylation of **21** gave the corresponding 4-(methylthio) derivative **22**, which on reaction with hydrazine gave the 4-hydrazino analog **23** of methotrexate. Reduction of **12** and **21** with sodium hydrosulfite gave the dihydropteridines **24** and **25**, respectively. The title compound was an excellent inhibitor of the growth of *Streptococcus faecium* ATCC 8043. However, this and related compounds were ineffective inhibitors of dihydrofolic reductase and showed no significant activity in either the KB cell culture screen or against L1210 leukemia cells in mice.

The 4-amino-4-deoxy derivatives of folic acid and its N^{10}-methyl derivative, aminopterin and methotrexate, are among the most active anticancer agents in use today. Both compounds interact with dihydrofolic reductase to give complexes with low dissociation constants (pseudo-irreversible) that inhibit the function of this enzyme. In con-

Scheme I



trast, a 4-thio analog (**21**) of folic acid, lacking a 4-amino group, should be a weaker inhibitor of dihydrofolic reductase. However, this type of compound might serve as a substrate for the enzyme in the same manner as folic acid producing 4-thiotetrahydro derivatives that might block one or more of the functions of the cofactor forms of tetrahydrofolates.[1]

The route used for the preparation of **21** and its methylthio derivative **22** was reported earlier.[2] In this paper we describe the experimental details and some improvements in the original synthesis of **21** as well as the use of **22** as an intermediate for the preparation of the 4-hydrazino analog (**23**) of methotrexate.

Previously, the preparation of the 4-thiopteroic acid **12** in an overall yield of about 14% involved nine steps as shown in Scheme I. This route utilized the interchangeability of 4-(alkylthio) and 4-thio groups to prevent the loss of the alkylthio moiety via hydrolysis during reactions carried out under basic conditions.[3] Key steps in the reaction sequence involved the conversion of **3** to the chloropyrimidine **4** and the reductive cyclization of the ketone **7** to give **9** in 77% yield. The blocked thio group of **7** allowed the reduction of the nitro group without poisoning the catalyst. In previous work the conversion of the oxime corresponding to **6** to a dihydropteridine was successful,[4] but in the present work the reductive cyclization of **6** gave only a 12% yield of **8**.

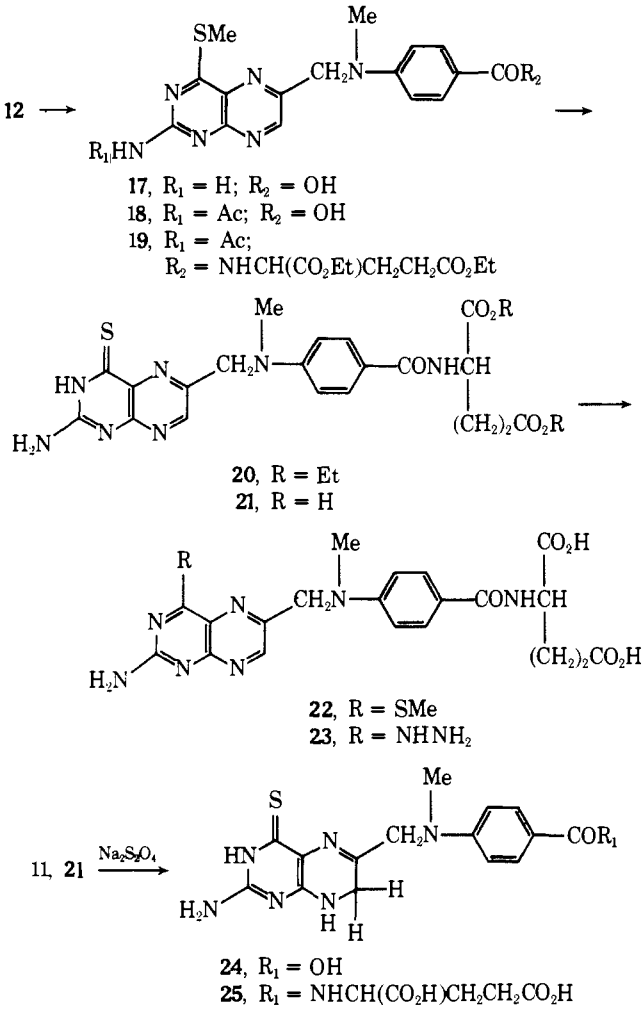
The availability of 2,5-diamino-4,6-dichloropyrimidine (**15**)[5] from other work prompted an investigation of the condensation of the aminoacetone oxime **16** with **15** to give **13**. Although this reaction was slow, these reactants gave a 61% yield of **13** after 336 hr at 64°. At this temperature the aminoacetone oxime **16** appeared to be stable, but the use of a higher temperature resulted in a lower yield of **13**. In contrast to most dihydropteridines, **13** is stable, which is attributed to the electron-withdrawing effect of the 4-chloro group.[6] Treatment of **13** with hydrated NaSH appeared to give a mixture of **11** and **12** (TLC) formed via air oxidation and partial hydrolysis of **14**. Complete oxidation and hydrolysis were effected by treatment of the mixture with base to give **12** in an overall yield of 43%.

Protection of the thioamido and amino moieties of **12** was effected by alkylation with MeI to give **17** followed by treatment of **17** with Ac_2O to give **18**. Reaction of **18** with isobutyl chloroformate gave a solution of the mixed anhydride, which was treated with diethyl L-glutamate to give **19**.[7] Removal of the blocking groups from **19** gave crude **20**, which was saponified to give **21**. Methylation of **21** gave the intermediate 4-(methylthio)pteridine **22**, which was treated with aqueous hydrazine to give **23**. Also, treatment of **11** and **21** with $Na_2S_2O_4$ gave samples of the unstable dihydropteridines **24** and **25**, respectively, which were tested without further purification (Scheme II).

As expected, the 4-thiofolic acid **21** [I_{50}/I_{50}(MTX) > 10^4] was a considerably less effective inhibitor of dihydrofolic reductase from pigeon liver than methotrexate (MTX $I_{50} > 10^{-8}$ *M*).[8] Similarly, the 4-(methylthio) derivative **22** [I_{50}/I_{50}(MTX) = 2.5×10^3] and the 4-hydrazino analog **23** [I_{50}/I_{50}(MTX) = 1.4×10^3] of MTX were poor inhibitors as were the intermediates **9**–**12** and **17**. Furthermore, neither **21** nor **22** was a substrate for this enzyme.

When tested against *Streptococcus faecium* ATCC

Scheme II



8043,[9] compounds **12** (ED_{50} 1.9×10^{-7} *M*), **17** (ED_{50} 5.5×10^{-7} *M*), **21** (ED_{50} 5.2×10^{-9} *M*), and **22** (ED_{50} 2.4×10^{-8} *M*) inhibited the growth of this bacterium (MTX, ED_{50} 7×10^{-10} *M*). However, although **23** (ED_{50} 4.1 μg/ml) exhibited borderline activity in the KB cell culture screen,[10] compounds **12, 21, 22, 24,** and **25** showed no significant cytotoxicity. In addition, **12, 21,** and **22** showed no activity against L1210 leukemia cells implanted intraperitoneally in mice on the single-dose schedule.[10]

Experimental Section

Melting points were determined on a Kofler Heizbank or, when indicated, on a Mel-Temp or Mettler FP1 apparatus. Absence of melting point data indicates an indefinite melting point. The ultraviolet absorption spectra were determined with a Cary Model 17 spectrophotometer. [Each solution contains 90% solvent indicated and 10% dissolving solvent: (a) EtOH; (b) DMSO–MeOH (4:46); (c) 0.01 *N* NaOH; (d) 0.1 *M* $HOCH_2CH_2SH$–0.1 *N* NaOH (1:1).] The infrared spectra were determined in pressed KBr disks with a Perkin-Elmer Model 521 spectrophotometer; only major bands in the 1700–1500-cm^{-1} region are given. The ^1H NMR spectra were determined in 3–7% w/v solutions in DMSO-d_6 with a Varian XL-100-15 or A-60A spectrometer at a probe temperature of about 37° with tetramethylsilane as an internal reference. The relative peak areas are given to the nearest whole number, and chemical shifts quoted in the case of multiples are measured from the approximate center.

2-Acetylamino-4-chloro-5-nitro-6(1*H*)-pyrimidinone (2). Crude 1[11] (3.00 g) was stirred with acetone (400 ml), filtered, and evaporated to dryness. A suspension of the residual aminopyrimidine (2.18 g, 11.5 mmol) in Ac_2O (22 ml) containing a drop of concentrated H_2SO_4 was heated slowly with stirring to 130° over a period of 1 hr. The mixture was cooled and the solid collected, washed with Et_2O, and dried in vacuo (P_2O_5): yield 1.34 g (50%); mp 256–258° dec; λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), 0.1 *N* HCl, 301 (br, 6.7), 325 (sh, 4.8); λ_{max} pH 7, 275 (sh, 4.63), 332 (2.85); λ_{max} 0.1 *N* NaOH, 275 (sh, 4.63), 353 (3.46); ν_{max} 1680, 1610, and 1540 cm^{-1}. Anal. ($C_6H_5ClN_4O_4$) C, H, Cl, N.

2-Acetylamino-4-(benzylthio)-5-nitro-6(1*H*)-pyrimidinone (3). A mixture of α-toluenethiol (14.0 g, 113 mmol), K_2CO_3 (14.7 g, 106 mmol), DMAC (320 ml), and **2** (24.5 g, 105 mmol) was stirred for 20 hr, then diluted with H_2O (1 l.), and refrigerated. The crude product was collected by filtration, washed with H_2O, dried in vacuo, triturated with Et_2O (200 ml), and dissolved in a solution of DMAC (600 ml) and 1 *N* HCl (120 ml). The resulting solution was filtered and diluted with H_2O (1 l.) to give a yellow crystalline product which was collected by filtration, washed with DMAC–H_2O (3:10) followed by H_2O, and dried at 65° in vacuo (P_2O_5): yield 30 g (89%); mp 229–231° dec (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), 0.1 *N* HCl, 258 (17.5), 315 (11.3), 348 (10.8); λ_{max} pH 7, 251 (16.6), 351 (10.4); λ_{max} 0.1 *N* NaOH, 353 (sh, 12.2), 356 (12.1); ν_{max} 1685, 1610, 1580, and 1550 cm^{-1}. Anal. ($C_{13}H_{12}N_4O_4S$) C, H, N.

2-Acetylamino-4-(benzylthio)-6-chloro-5-nitropyrimidine (4). A suspension of **3** (15.0 g, 46.9 mmol) in $POCl_3$ (450 ml) was stirred at 80° for 3 hr, poured into an ice–water mixture (7 l.), and stirred until a homogeneous yellow powder formed. A solution of the solid in hot benzene (500 ml) was treated with charcoal, filtered, diluted with cyclohexane (500 ml), and refrigerated. The red and yellow precipitate consisting of a mixture of **4** and **5** was collected, dried in vacuo (P_2O_5), and heated in refluxing Ac_2O (300 ml) for 1 min. Refrigeration of the resulting solution deposited pure **4** which was collected by filtration and dried in vacuo (P_2O_5): yield 10.8 g (68%); mp 180° (Mel-Temp); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), 0.1 *N* HCl, 271 (18.5), 345 (br, 3.39); λ_{max} pH 7, 271 (7.97), 345 (br, 3.18); ν_{max} 1675, 1555, and 1518 cm^{-1}. Anal. ($C_{13}H_{11}ClN_4O_3S$) C, H, Cl, N.

Methyl *p*-[[3-[[2-Acetylamino-6-(benzylthio)-5-nitro-4-pyrimidinyl]amino]acetonyl]methylamino]benzoate Oxime (6). A stirred solution of 4 (2.12 g, 6.24 mmol), **16** (1.57 g, 6.24 mmol),[12] Et_3N (0.871 ml, 6.24 mmol), and EtOH (18 ml) was heated in an oil bath at 59° for 16 hr and cooled to 25°. The yellow product was collected by filtration, washed with EtOH, and dried in vacuo (P_2O_5): yield 1.36 g (39%) of either the syn or anti isomer of **6**; mp ~168° dec (Mettler FP1); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), 0.1 *N* NaOH, 225 (32.3), 263 (18.3), 320 (30.6), 365 (sh, 12.2); ^1H NMR δ 3.07 (s, 3, NCH_3). Anal. ($C_{25}H_{27}N_7O_6S$) C, H, N.

Addition of H_2O to the mother liquor gave 1.87 g (54%) of the other isomer of **6** containing a trace amount (TLC) of the isomer obtained above: mp 100–114°; ^1H NMR δ 2.88 (s, NCH_3). Anal. ($C_{25}H_{27}N_7O_6S$) C, H, N.

Methyl *p*-[[3-[[2-Amino-6-(benzylthio)-5-nitro-4-pyrimidinyl]amino]acetonyl]methylamino]benzoate (7). A solution of a mixture of *syn*- and *anti*-oxime isomers **6** (7.66 g, 13.8 mmol) in dioxane (150 ml) and 1 *N* HCl (150 ml) was stirred at 70° for 1 hr and cooled to 20°. The crystalline product was collected, washed with cold 1:1 dioxane–H_2O and then H_2O, and dried at 100° in vacuo (P_2O_5): yield 5.43 g (77%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent b), EtOH, 264 (18.9), 308 (31.5), 350 (16.4). Anal. ($C_{23}H_{24}N_6O_5S \cdot 0.2HCl \cdot 0.4\ H_2O$) C, H, Cl, N.

Methyl *p*-[[[2-Acetylamino-4-(benzylthio)-7,8-dihydro-6-pteridinyl]methyl]methylamino]benzoate (8). A solution of **6** (400 mg, 0.722 mmol) in EtOH (40 ml) was hydrogenated at 50° for 4 hr in the presence of Raney nickel (870 mg, weighed wet with EtOH). The reaction mixture absorbed 58 ml (2.38 mmol) of H_2 and was evaporated to dryness. The residue was washed with hot EtOH (2 × 25 ml) and extracted with warm DMAC (6 ml). Addition of water to the DMAC extract gave a crystalline product which was collected by filtration, washed with H_2O, and dried in vacuo (P_2O_5): yield 43 mg (12%); ^1H NMR δ 2.18 (s, 3, CH_3CO), 3.05 (s, 3, NCH_3), 3.76 (s, 3, OCH_3), 3.14, 4.20, 4.33 (m, 6, NCH_2, NCH_2, SCH_2), 6.77, 7.75 (m, 4, C_6H_4), 7.16–7.48 (m, C_6H_5), 9.85 (s, 1, NH). Anal. ($C_{25}H_{26}N_6O_3S$) C, H, N.

Methyl *p*-[[[2-Amino-4-(benzylthio)-7,8-dihydro-6-pteridinyl]methyl]methylamino]benzoate (9). A suspension of **7** (5.43 g, 10.6 mmol) in EtOH (540 ml) containing Raney nickel (10 g, weighed wet with EtOH) was hydrogenated at 50° for 24 hr. The reaction mixture, which had absorbed 777 ml (32.0 mmol) of H_2, was evaporated to dryness in vacuo. An extract of the residue in DMAC (100 ml) was filtered and the catalyst was washed with additional DMAC (100 ml). The combined filtrate and wash was diluted with H_2O (300 ml) and refrigerated to give crystalline **9** which was collected by filtration, washed with cold DMAC–H_2O (2:3) and then H_2O, and dried at 78° in vacuo (P_2O_5): yield 3.65 g

(77%); mp ~148°; ^{1}H NMR δ 3.03 (s, 3, NCH_3), 375 (s, 3, OCH_3), 4.00, 4.14, 4.25 (m, 6, NCH_2, NCH_2, SCH_2), 6.16 (s, 2, NH_2), 6.76, 7.74 (m, C_6H_4), 6.84 (s, NH), 7.30 (m, 5, C_6H_5). Anal. ($C_{23}H_{24}N_6O_2S$) C, H, N, S.

Methyl p-[[[2-Amino-4-(benzylthio)-6-pteridinyl]methyl]methylamino]benzoate (10). A solution of **9** (2.05 g, 4.58 mmol) in acetone (1 l.) was stirred, exposed to the air for 16 hr, treated with $MgSO_4$ (551 mg, 4.58 mmol), cooled to ~12°, and treated dropwise with a 0.27% solution of $KMnO_4$ in acetone (179 ml, 3.05 mmol) over a period of 10 min. The resulting mixture was filtered through Celite to remove MnO_2 and the filtrate was evaporated to dryness in vacuo (P_2O_5): yield 1.93 g (94%); mp ~226°; λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent b), EtOH, 276 (22.1), 306 (31.5), 393 (10.4); ^{1}H NMR δ 3.21 (s, 3, NCH_3), 3.75 (s, 3, OCH_3), 4.49, 4.84 (d, 4, SCH_2, NCH_2), 6.83, 7.73 (m, 4, C_6H_4), 7.2–7.6 (m, C_6H_5), 7.43 (s, NH_2), 8.77 (s, 1, 7-CH). Anal. ($C_{23}H_{22}N_6O_2S \cdot 0.4\ H_2O$) C, H, N.

Methyl p-[[(2-Amino-3,4-dihydro-4-thioxo-6-pteridinyl)methyl]methylamino]benzoate (11). A solution of **10** (1.91 g, 4.28 mmol) and hydrated NaSH (1.91 g) in DMAC (100 ml) and H_2O (100 ml) was stirred at 76° for 18 hr and evaporated to dryness in vacuo at 25°. A mixture of the residue with H_2O (80 ml) was acidified to pH 1.5 with 1 *N* HCl and stirred for 1 hr. The resulting precipitate was collected by filtration, washed successively with H_2O (at pH 1), Et_2O, and C_6H_5, and dried in vacuo (P_2O_5) at 65°: yield 1.53 g (96%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), 0.1 *N* HCl, 308 (28.7), 378 (8.62); λ_{max} pH 7, 310 (27.7), 410 (8.17). Anal. ($C_{16}H_{16}N_6O_2S \cdot H_2O$) C, H, N.

Compound **11** (135 mg, 80% yield) was also obtained when **10** (200 mg, 0.448 mmol) was treated with NaSH (200 mg) in refluxing EtOH (20 ml) for 30 min.

p-[[(2-Amino-3,4-dihydro-4-thioxo-6-pteridinyl)methyl]methylamino]benzoic Acid (12). A. A solution of **11** (293 mg, 0.784 mmol) in DMSO (20 ml) was treated dropwise with 1 *N* NaOH (2.35 ml, 2.35 mmol) under N_2, stirred in a stoppered flask at 25° for 19 hr, and evaporated to dryness at <25° on a lyophilization apparatus. A solution of the residue in H_2O (15 ml) was acidified to pH 1.5 with 1 *N* HCl. The red precipitate was collected, washed with H_2O at pH 1.5, dried in vacuo (P_2O_5) at 100°, triturated with MeOH (2 × 4 ml), and redried at 100°: yield 256 mg (91%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent c), 0.1 *N* HCl, 307 (23.0), 380 (9.80); λ_{max} pH 7, 289 (26.8), 412 (9.75); λ_{max} 0.1 *N* NaOH, 287 (26.5), 410 (9.49); ν_{max}, 1660, 1596, 1573, and 1520 cm^{-1}; ^{1}H NMR δ 3.24 (s, 3, NCH_3), 4.83 (s, 2, NCH_2), 6.82, 7.73 (m, 4, C_6H_4), 7.14 (s, 2, NH_2), 8.59 (s, 1, 7-CH). Anal. ($C_{15}H_{14}N_6O_2S \cdot H_2O$) C, H, N, S.

B. A stirred mixture of **13** (615 mg, 1.64 mmol), hydrated NaSH (615 mg), DMAC (10 ml), and H_2O (1 ml) in a stoppered flask was heated in an oil bath at 66° for 18 hr. The filtered reaction mixture was evaporated to dryness at 55° under high vacuum. A suspension of the residue in H_2O (40 ml) was stirred, exposed to air for 15 min, and filtered to remove unreacted **13** (134 mg). The filtrate was acidified with 6 *N* HCl to pH 4 and the orange precipitate of crude **11** containing some **12** [TLC, $CHCl_3$–MeOH (9:1)] was collected in a refrigerated centrifuge, washed with H_2O at pH 4, and dried in vacuo (P_2O_5). A suspension of this sample (400 mg) in DMSO (25 ml) containing 1 *N* NaOH (3.21 ml, 3.21 mmol) was stirred for 19 hr and evaporated to dryness at 25° under high vacuum. A solution of the residue in H_2O (20 ml) was acidified with 1 *N* HCl to pH 1.5. The brown precipitate of **12** was collected in a refrigerated centrifuge, washed with H_2O at pH 1.5, air-dried, triturated with CH_3OH (2 × 5 ml), and dried in vacuo (P_2O_5): yield 328 mg (71%, based on unrecovered **13**). This product was identical with that obtained in procedure A.

Methyl p-[[(2-Amino-4-chloro-7,8-dihydro-6-pteridinyl)methyl]methylamino]benzoate (13). A mixture of **15** (840 mg, 4.69 mmol),[5] **16** (1.18 g, 4.69 mmol),[12] and Et_3N (0.654 ml, 4.69 mmol) in EtOH (34 ml) was stirred at 64° in a stoppered flask for 4 days. The solution was stirred at 25° for 30 min, and the tan gelatinous precipitate of **13** was collected by filtration under N_2, washed with EtOH, and dried in vacuo (P_2O_5): yield 331 mg; melting point darkens above 180°. The filtrate was refrigerated overnight, filtered to remove a brown impurity, and evaporated to dryness in vacuo. A solution of the residue in EtOH (27 ml) containing additional **16** (955 mg) and Et_3N (0.654 ml) was stirred at 64° for 10 days. The product was collected by filtration from the reaction mixture (at 25°) every 48 hr to give an additional 730 mg of **13** (total yield 61%): λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), pH 7, 311 (32.0); ^{1}H NMR δ 3.06 (s, 3, NCH_3), 3.76 (s, 3, OCH_3), 4.05 (s, 2, CH_2), 4.20 (s, 2, CH_2), 6.42 (s, NH_2), 6.79, 7.76 (m, 4, C_6H_4), 7.31 (s, 1, NH). Anal. ($C_{16}H_{17}ClN_6O_2 \cdot 0.7\ H_2O$) C, H, N.

p-[[[2-Amino-4-(methylthio)-6-pteridinyl]methyl]methylamino]benzoic Acid (17). A solution of **12** (1.13 g, 3.14 mmol) in 1 *N* NaOH (6.27 ml, 6.27 mmol) and H_2O (25 ml) at 10° was treated with CH_3I (0.215 ml, 3.45 mmol) and stirred in a stoppered flask at 10° for 15 min and at 25° for 18 hr. The gelatinous solution was acidified to pH 1.5 with 1 *N* HCl, and the brown precipitate was collected, washed with H_2O at pH 1.5, and dried in vacuo (P_2O_5) at 100°: yield 1.13 g (94%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent b), 0.1 *N* HCl, 331 (29.2), 361 (12.2), 377 (sh, 10.8); λ_{max} pH 7, 274 (26.1), 295 (sh, 23.1), 389 (9.35); ν_{max} 1665, 1626, 1600, 1570, 1550, 1520 cm^{-1}; ^{1}H NMR 2.56 (s, SCH_3), 3.24 (s, 3, NCH_3), 4.88 (s, 2, NCH_2), 6.84, 7.74 (m, 4, C_6H_4), 8.81 (s, 1, 7-CH). Anal. ($C_{16}H_{16}N_6O_2S \cdot H_2O \cdot 0.2$-HCl) C, H, Cl, N.

p-[[[2-Acetylamino-4-(methylthio)-6-pteridinyl]methyl]methylamino]benzoic Acid (18). A solution of **17** (757 mg, 1.98 mmol) in Ac_2O (15 ml) was heated at 115° for 5 hr and evaporated to dryness in vacuo. Trituration of the residue with H_2O (10 ml) gave a homogeneous powder, which was dissolved by addition of a minimum amount of concentrated NH_4OH. The solution was rapidly filtered, acidified to pH 1 with 4 *N* HCl, and stirred for 1 hr. The product was collected by filtration, washed with H_2O at pH 1, and dried (P_2O_5) at 100° in vacuo: yield 721 mg (90%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent a), 0.1 *N* HCl, 225 (28.7), 275 (sh, 16.9), 310 (28.7), 361 (10.8), 380 (sh, 8.95); λ_{max} pH 7, 275 (29.2), 295 (sh, 24.2), 365 (8.90). Anal. ($C_{18}H_{18}N_6O_3S \cdot 0.34\ H_2O \cdot 0.07$HCl) C, H, Cl, N.

Diethyl N-[p-[[[2-Acetylamino-4-(methylthio)-6-pteridinyl]methyl]methylamino]benzoyl]-L-glutamate (19). A solution of **18** (320 mg, 0.786 mmol) and Et_3N (104 mg, 1.02 mmol) in DMAC (2 ml) was cooled in an ice bath and treated with isobutyl chloroformate (139 mg, 1.02 mmol). After stirring at 0° for 1.5 hr, the solution was treated with a mixture of diethyl L-glutamate (245 mg, 1.02 mmol) and Et_3N (1.04 mg) in DMAC (2 ml) and stirred at 25° for an additional 18 hr. The resulting mixture was filtered to remove $Et_3N \cdot HCl$, the filtrate was evaporated to dryness under high vacuum, and the residue was extracted with EtOAc (2 × 10 ml), leaving a brown insoluble residue. A solution of this residue in 2% NH_4OH (10 ml) was filtered and acidified to pH 1 with 6 *N* HCl to give a precipitate of recovered **18** (141 mg). The EtOAc extract was washed successively with 0.3 *N* HCl (2 × 5 ml), H_2O (5 ml), and saturated $NaHCO_3$ (3 × 5 ml). The $NaHCO_3$ extract was acidified to pH 1 to give an additional amount of recovered **18** (68 mg). The EtOAc solution was washed with H_2O (5 ml), dried ($MgSO_4$), and evaporated to dryness in vacuo. The residue was triturated with Et_2O to give an orange solid which was collected by filtration, washed with Et_2O, and dried in vacuo (P_2O_5): yield 115 mg (22%) (65% based on recovered **18**). Anal. ($C_{27}H_{33}N_7O_6S \cdot 0.8$ EtOAc) C, H, N.

N-[p-[[(2-Amino-3,4-dihydro-4-thioxo-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic Acid (21). A stirred mixture of **19** (500 mg, 0.765 mmol), powdered NaSH (500 mg), and EtOH (26 ml) was refluxed under N_2 for 30 min, diluted with EtOH (26 ml), filtered hot, and evaporated to dryness in vacuo. A solution of the residue in H_2O (13 ml) was acidified with 1 *N* HCl to pH 2. The brown precipitate of crude **20** was collected by filtration, washed with H_2O at pH 2, and dried in vacuo (P_2O_5). This residue (378 mg) was dissolved in 1 *N* NaOH (5.30 ml, 5.30 mmol), and the resulting solution was stirred at 25° for 18 hr, diluted with H_2O (5 ml), filtered, washed with $CHCl_3$ (2 × 3 ml), and acidified to pH 2.5. The brown precipitate of crude **21** was collected by filtration, washed with H_2O at pH 2.5, and dried in vacuo (P_2O_5): yield 346 mg. A portion of the crude product (290 mg) was suspended in 0.1 *M* 2-mercaptoethanol (290 ml) and treated with 1 *N* NaOH until a clear solution was obtained. The solution (pH 7.0) was applied to a column (2.8 × 36 cm) of DEAE-cellulose which had been prewashed successively with 0.5 *M* potassium phosphate buffer (pH 7), H_2O, and 0.1 *M* mercaptoethanol. The column was then washed with 0.1 *M* mercaptoethanol (400 ml) and eluted with a linear NaCl gradient (1 l. of 0.7 *M* NaCl in the reservoir and 1 l. of 0.0 *M* NaCl in the mixing bottle; both solutions were 0.005 *M* in pH 7 phosphate buffer and 0.1 *M* in mercaptoethanol). The elution was monitored by uv absorption, and the desired fraction (0.43–0.65 *M*) was lyophilized to dryness. A solution of the residue in a minimum of water (pH 8) was acidified with 1 *N* HCl to pH 2.5. A solution of the resulting precipitate in H_2O (25 ml) containing NH_4OH (3 drops) was acidified to pH 2.5. The orange precipitate was collected in a refrigerated centrifuge, washed with H_2O (pH 2.5), and dried in vacuo (P_2O_5): yield 188 mg (58%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent c), 0.1 *N* HCl, 300 (21.1), 378 (10.5); λ_{max} pH 7, 299 (28.6), 412 (10.7); λ_{max} 0.1 *N* NaOH, 300 (27.6), 410 (10.4); ν_{max} 1710, 1652, 1615, 1590, and 1568 cm^{-1}; ^{1}H NMR δ 1.8–2.4 (m, CH_2CH_2), 3.23 (s, 3, NCH_3), 4.37 (m, NCH), 4.82 (s, 2, NH_2), 6.81,

7.73 (m, 4, C_6H_4), 7.12 (s, 2, NH_2), 8.17 (d, 1, NH), 8.58 (s, 1, 7-CH). Anal. ($C_{20}H_{21}N_7O_5S \cdot 0.9$ HCl $\cdot 0.2H_2O$) C, H, Cl, N, S.

N-[*p*-[[[**2-Amino-4-(methylthio)-6-pteridinyl]methyl]methylamino]benzoyl]-L-glutamic Acid (22).** Methyl iodide (0.014 ml, 0.217 mmol) was added (Hamilton micro syringe) to a stirred solution of **21** (100 mg, 0.197 mmol) in 1 *N* NaOH (0.767 ml) and H_2O (2.7 ml) at 0°. The mixture was stirred for 1 hr at 0° and 18 hr at 25°. Acidification of the resulting solution with 1 *N* HCl to pH 2.5 gave an orange precipitate which was collected by filtration, washed with H_2O at pH 2.5, and dried in vacuo at 65° (P_2O_5): yield 90 mg (90%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent c), 0.1 *N* HCl, 308 (22.9), 362 (9.90), 377 (sh, 8.63); λ_{max} pH 7, 275 (22.4), 303 (25.2), 385 (8.36); λ_{max} 0.1 *N* NaOH, 275 (21.6), 303 (25.3), 384 (8.99); ν_{max} 1710, 1600, 1550, and 1505 cm^{-1}; ^1H NMR δ 1.8–2.4 (m, CH_2CH_2), 2.56 (s, SCH_3), 3.24 (s, 3, NCH_3), 4.36 (m, NCH), 4.86 (s, 2, NCH_2), 6.82, 7.74 (m, 4, C_6H_4), 7.48 (s, NH_2), 8.18 (d, 1, NH), 8.80 (s, 1, 7-CH). Anal. ($C_{21}H_{23}N_7O_5S \cdot 1.3H_2O$) C, H, N, S.

N-[*p*-[[**(2-Amino-4-hydrazino-6-pteridinyl)methyl]methylamino]benzoyl]-L-glutamic Acid (23).** A solution of **22** (25.0 mg, 0.049 mmol) and 85% hydrazine hydrate (57.9 mg, 0.984 mmol) in H_2O (0.5 ml) was stirred for 5.5 hr and acidified to pH 4 with 1 *N* HCl. The yellow precipitate was collected by filtration, washed with H_2O at pH 4, and dried in vacuo (P_2O_5): yield 19.7 mg (78%); λ_{max}, nm ($\epsilon \times 10^{-3}$) (solvent c), 0.1 *N* HCl, 306 (23.3); λ_{max} pH 7, 221 (24.9), 302 (25.2), 372 (7.10). Anal. ($C_{20}H_{23}N_9O_5 \cdot 2.3H_2O$) C, H, N.

p-[[**(2-Amino-3,4,7,8-tetrahydro-4-thioxo-6-pteridinyl)-methyl]methylamino]benzoic Acid (24).** A solution of 11 (50.0 mg, 0.134 mmol) in 1 *M* mercaptoethanol (4 ml) and 1 *N* NaOH (6 ml) was treated with $Na_2S_2O_4$ (333 mg) and stirred in a stoppered flask under N_2 for 60 hr. The almost colorless solution was filtered under N_2 and acidified with 6 *N* HCl to pH 4. The yellow product was collected in a refrigerated centrifuge, washed with 1 *M* mercaptoethanol (4 ml) and then 0.01 *M* mercaptoethanol (4 ml), and dried in vacuo (P_2O_5): yield 47 mg; λ_{max}, nm (solvent d), 0.1 *N* HCl, 312, 370; λ_{max} pH 7, 251, 293, 328; λ_{max} pH 13, 283, 305.

N-[*p*-[[**(2-Amino-3,4,7,8-tetrahydro-4-thioxo-6-pteridinyl)-methyl]methylamino]benzoyl]-L-glutamic Acid (25).** The reduction of **21** (12.0 mg) to give **25** (3.2 mg) was carried out as described above for **24** except that the time of reaction was 18 hr: λ_{max}, nm (solvent d), 0.1 *N* HCl, 310, 379 (sh); λ_{max} pH 7, 218, 307 (br); λ_{max} 0.1 *N* NaOH, 307 (br).

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Analgesic Activity of the Epimeric Tropane Analogs of Meperidine. A Physical and Pharmacological Study

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Condensation of *cis*-*N*-benzyl-2,5-bis(chloromethyl)pyrrolidine (**6**) and phenylacetonitrile afforded a mixture of epimers **7** and **8**. Compound **8** was readily converted to the meperidine analog **1** prepared earlier by Bell and Archer. Compound **7** was converted to a new tropane analog of meperidine, compound **3**. The ED_{50} of **1** and **3** in the D'Amour–Smith "tail flick" test for narcotic type analgesia, which differs by a factor of only 3 or 4 in potency, supports the accumulated data that suggest that the analgesic activity of the meperidine type is not very sensitive to the conformation of the phenyl group in 4-phenylpiperidines. A proton and ^{13}C magnetic resonance spectral comparison of **1** and **3**, as well as a reevaluation of the conformational requirements of **17–19**, leads to the conclusion that the differences in conformation of **1**, **3**, **17**, and **18** are due to the varying degrees of flattening of the piperidine ring. The ^1H NMR and ^{13}C NMR data are not consistent with the boat conformation suggested earlier for compound **17**.

Almost 15 years after the synthesis of the tropane analog **1** of meperidine (**2**) by Bell and Archer,[1] we have succeeded in preparing the epimeric tropane ester **3**. We hoped that with both epimeric tropane esters **1** and **3** in hand we could contribute to the accumulated information[2-4] relating to the conformational requirement of the phenyl group in the 4-phenylpiperidine analgesics, a topic that has been dealt with in several recent reviews.[5,6]

Chemistry. In 1961, Cignarella et al.[7] reported that *cis*-*N*-tosyl-2,5-bis(chloromethyl)pyrrolidine (**4**) and phenylacetonitrile in the presence of $NaNH_2$ and toluene afforded a single compound, **5**, in 28% yield. This in turn was converted in good yield to **1** (β-ester). We found that *cis*-*N*-benzyl-2,5-bis(chloromethyl)pyrrolidine (**6**)[8] and phenylacetonitrile in the presence of NaH and DMF yielded a 3:1 mixture (42%) of **7** and **8**. The preponderance of isomer **7** over isomer **8** is in complete concordance with the steric control suggested by Dreiding models in the ring closure of the intermediate shown in Scheme I. Fractional crystallization of their HCl salts separated **7** and **8**. The minor component **8** (β-nitrile), when treated with 80% H_2SO_4 at 150° for 1.5 hr followed by reflux with EtOH, afforded the β-