Synthesis of N-[p-[[(2-Amino-3,4-dihydro-4-thioxo-6-pteridinyl)methyl]-methylamino]benzoyl]-L-glutamic Acid (N^{10}-Methyl-4-thiofolic Acid) and Related Compounds (1)

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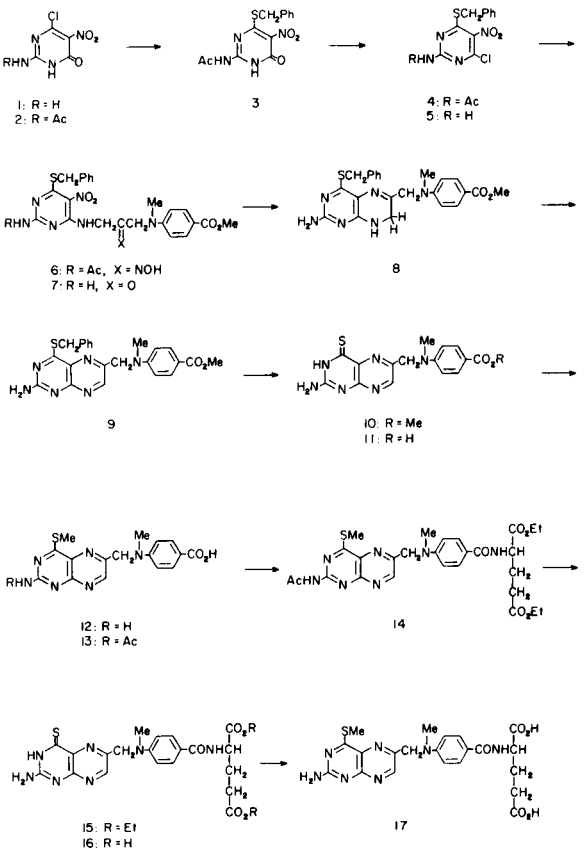
Sir:

Folic acid and its derivatives play a key role in metabolism, and the unnatural 4-amino analogs, aminopterin and methotrexate, are among the most active anticancer agents available today. Similarly, 6-mercaptopurine and 6-thioguanine, the unnatural thio analogs of hypoxanthine and guanine, respectively, are highly active anticancer agents. Many investigators have reported the preparation of thiopteridines, and several have sought a method for the preparation of the thio analog of folic acid (2-6). Direct thiation of folic acid was eliminated from consideration because difficulties have been encountered in the thiation of 2-amino-4(3*H*)oxopteridines (3). Similarly, although the 4-amino group of 2,4-diaminopteridines was readily displaced by hydroxide (7), we found that displacement with hydrosulfide under a variety of conditions was unsuccessful. In this communication, we report the syntheses of both N^{10}-methyl-4-thiopteroic acid and N^{10}-methyl-4-thiofolic acid from a pyrimidine precursor by Boon and Leigh's procedure (8,9). A 4-(alkylthio)-pyrimidine was chosen as a starting material to circumvent the poisoning effect of the thio group on hydrogenation catalysts and to prevent reactions involving the thioamide moiety. However, to avoid hydrolysis of the alkylthio group during reactions carried out under basic conditions, it was necessary to replace the 4-alkylthio group with the 4-thio group. The interchangeability of 4-thio- and 4-alkylthio groups in pteridines has been demonstrated (3), and this interconversion was used throughout the reaction sequence described below.



Acetylation of the known 2-aminopyrimidine **1** (10) with acetic anhydride gave **2**, which was treated with α-toluenethiol in DMAC containing potassium carbonate to give **3** in 89% yield. Chlorodehydroxylation of **3** with phosphorus oxychloride gave a mixture of **4** and **5** that was treated briefly with hot acetic anhydride to give pure **4** (68%). The interaction of **4** with methyl p-[(3-aminoacetonyl)methylamino]benzoate oxime (8) gave the oxime **6** (93%). Hydrolysis of **6** with 1 N hydrochloric acid at 70° gave the α-aminoketone **7** (77%), and hydrogenation of the nitro group of **7** in the presence of Raney nickel at 50° followed by spontaneous ring closure gave the dihydropteridine **8** (77%). Oxidation of **8** with potassium permanganate in acetone gave **9** (94%). Before saponification of the ester group of **9**, the benzylthio group was displaced with sodium hydrosulfide. The ester function of the resulting thiopteridine **10** (96% yield) was hydrolyzed with sodium hydroxide to give N^{10}-methyl-4-thiopteroic acid **11** (91%). The 4-thio group of **11** was blocked by alkylation with methyl iodide to give **12** (94%) followed by treatment of **12** with hot acetic anhydride to give **13** (90%). The reaction of **13** with isobutyl chloroformate in DMAC containing triethylamine gave a solution

of the corresponding mixed anhydride, which was treated with diethyl L-glutamate to give **14** (65%). Replacement of the methylthio group and removal of the acetyl blocking group of **14** was effected with sodium hydrosulfide in refluxing ethanol to give crude **15**, which was not purified but saponified with aqueous sodium hydroxide at room temperature to give N^{10}-methyl-4-thiofolic acid (**16**) (58%). Methylation of **16** in aqueous sodium hydroxide with methyl iodide gave the corresponding methylthio derivative **17** (90%) (11).

These results indicate that the 4-thio group of pteridines is stable to aqueous sodium hydroxide under mild conditions and demonstrate that the alkylthio group protects the sulfur moiety against acylation and displacement during peptide bond formation by the mixed anhydride procedure.

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(11) Satisfactory elemental analyses (± 0.4%) were obtained for all compounds except for **15**, a crude sample of which was converted to **16**.