A Convenient Synthesis of Aminopterin and Homologs *via* 6-(Bromomethyl)-2,4-diaminopteridine Hydrobromide (1)

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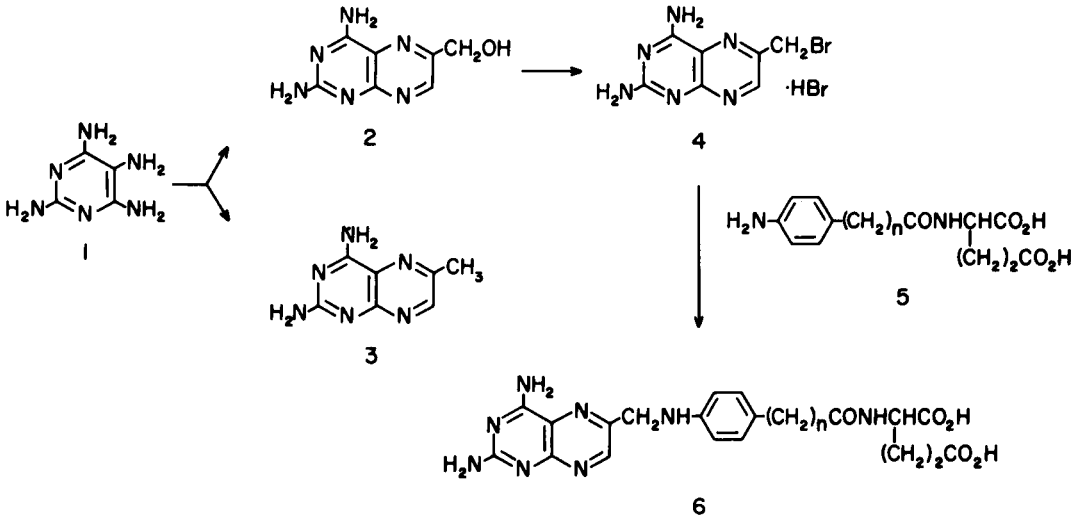
Sir:

We wish to describe a facile preparation of 6-(bromomethyl)-2,4-diaminopteridine hydrobromide (**4**) and its conversion to the anticancer agent aminopterin (**6**, n = 0) and homologs (**6**, n = 1, 2) in good yields and high purity. This simple approach to **6**-types represents a marked improvement over methods used previously that give low yields of products requiring purification by laborious and tedious techniques (2).

The preparation of **4** has not heretofore been reported. There have been several reports on the preparation of folic acid from 2-amino-6-(halogenomethyl)-4-pteridinol (3), but the approach apparently preferred in more recent syntheses of folic acid and its analogs is that *via* 2-acetamido-4-hydroxy-6-pteridinecarboxaldehyde (4). 7-Methylaminopterin and 7-methylamethopterin were recently prepared (5) from the corresponding 6-(bromomethyl)-substituted pteridine by methods similar to those reported earlier for the preparation of 7-methylfolic acid and 7,10-dimethylfolic acid (6). In those reports the 6-(bromomethyl)-7-methyl-substituted pteridines were derived from 6,7-bis(bromomethyl)- precursors, which were prepared by condensation of the appropriate pyrimidines and dibromodiacetyl.

Essential features of the preparation of **4** are as follows. The material obtained directly from the condensation of 2,4,5,6-tetraaminopyrimidine (**1**) and 1,3-dihydroxyacetone according to a reported procedure (7) consisted mainly of 2,4-diamino-6-pteridinemethanol (**2**) and 2,4-diamino-6-methylpteridine (**3**) with **2** in dominance over **3** by a ratio of approximately 5:1. The relative amounts were estimated from pmr spectral data in deuteriotrifluoroacetic acid; compound **2** gave signals at δ 5.3 (6-CH_2O-) and δ 9.1 (7-H), and **3** at δ 2.8 (6-CH_3) and δ 8.8 (7-H). Treatment of a suspension of the crude mixture of **2** and **3** in boiling ethanol with an equimolar amount of 48% hydrobromic acid gave their hydrobromides, and the greater solubility of **3**·HBr in ethanol allowed its nearly complete removal from the desired **2**·HBr. The pmr spectrum of the product (typically obtained in 39% yield) showed only **2**·HBr and **3**·HBr with **2**·HBr in dominance by 16-20:1, depending on the extent of extraction with boiling ethanol. Treatment of the **2**·HBr thus prepared with triphenylphosphine dibromide (8) (four molar equivalents, preformed *in situ* from triphenylphosphine and bromine) in DMAC at 20-25° for 1.5-2 hours led to **4**, but pmr spectral data showed the relative proportion of **4** (δ 4.7, 6-CH_2Br, in deuteriotrifluoroacetic acid) to **3**·HBr in each of three runs to be



only slightly improved over that of **2**·HBr to **3**·HBr in the starting material. The work-up procedure was as follows. The reaction mixture was treated with ethanol, left in a refrigerator overnight, and evaporated *in vacuo* (bath up to 45°). The residue, a dark oil, gave a solid when stirred with warm benzene. The liquid phase was then removed by decantation, and the benzene-insoluble solid was dissolved in glacial acetic acid at 80°. The cooled solution deposited an off-white crystalline solid, which was ultimately freed of acetic acid by drying *in vacuo* (phosphorus pentoxide) at 110° to give yellow, crystalline product in 60-65% yield (three runs). A sample of **4** ($C_7H_7BrN_6$·HBr) that gave a satisfactory elemental analysis (C, H, Br, and N) (9), although it still contained detectable **3**·HBr and was estimated to be of 95% purity, gave the following uv spectral data: λ max, nm ($\epsilon \times 10^{-3}$), 0.1 *N* hydrochloric acid, 249 (17.1), 339 (10.6), 353 (sh) (9.4); 0.1 *N* sodium hydroxide, 258 (22.1), 372 (7.2). The **4** obtained in this manner proved to be suitable for the preparation of **6**-types.

Treatment of *N*-(*p*-aminobenzoyl)glutamic acid (**5**, n = 0) and its homologs (**5**, n = 1, 2) (three molar equivalents) with **4** in DMAC (20 hours at 20-25°) gave **6** (n = 0, 1, 2) in respective yields of 68, 73, and 39%. Addition of water to the reaction mixtures caused precipitation of the products. Two of the **6**-types (n = 0, 1) required no purification other than thorough washing with water, and **6** (n = 2) was obtained pure following reprecipitation from Norit-treated 0.1 *N* sodium hydroxide solution by addition of an equivalent amount of hydrochloric acid. Each of these products gave satisfactory elemental analyses (C, H, N) (9) and migrated as single uv-absorbing spots on thin-layer chromatograms. The spots from **6** (n = 1, 2) had a thin, faintly fluorescent cap, and that from **6** (n = 0) showed no fluorescence (10). Their pmr spectra were as expected with no indication of the continued presence of **3**. The uv spectrum of **6** (n = 0) agrees with that previously reported (**2a, e**).

Acknowledgment.

N-(*p*-Aminophenylacetyl)glutamic acid (**5**, n = 1) was prepared by Mr. Jerry L. Frye by reduction of *N*-(*p*-nitrophenylacetyl)glutamic acid. *N*-(*p*-Aminohydrocinnamoyl)-glutamic acid (**5**, n = 2) was prepared by Mr. Jerry D. Rose by the following sequence: *p*-nitrocinnamic acid → acyl chloride → *N*-acylated diethyl glutamate → *N*-acylated glutamic acid → **5** (n = 2). Reduction steps in both sequences were by catalytic hydrogenation (5% Pd on charcoal) in water.

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(9) Satisfactory elemental analyses (± 0.4%) were obtained on designated compounds for the elements given in parentheses. Results for **6** (n = 0) correspond to $C_{19}H_{20}N_8O_5 \cdot 1.75H_2O$, those for **6** (n = 1) to $C_{20}H_{22}N_8O_5 \cdot H_2O$, and for **6** (n = 2) to $C_{21}H_{24}N_8O_5 \cdot 2H_2O$.

(10) Thin-layer chromatograms were run on Bakerflex DEAE-cellulose sheets using 0.5 *M* sodium chloride, 0.2 *M* in mercaptoethanol, in 0.005 *M* potassium phosphate buffer at *p*H 7.0. The chromatograms exhibited characteristics like those of related compounds as described by R. B. Angier and W. V. Curran [*J. Am. Chem. Soc.*, 81, 2814 (1959)].