Notes

Convenient Synthesis of 10-Deazaaminopterin via a Pteridine Ylide

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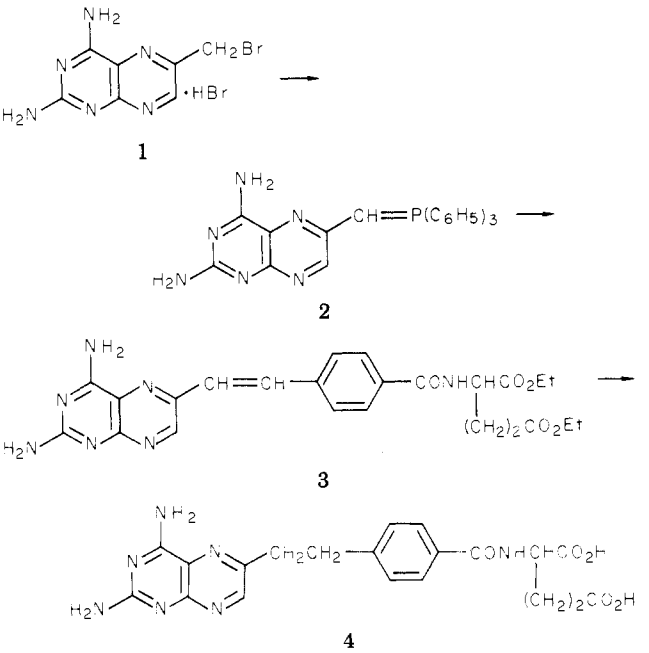
10-Deazaaminopterin, a potential antitumor agent now undergoing clinical trials, has been synthesized by a new approach involving the Wittig reaction. The ylide obtained by reaction of 6-(bromomethyl)-2,4-pteridinediamine with triphenylphosphine in Me_2NAc, followed by treatment with NaOMe, underwent smooth reaction with diethyl *N*-(4-formylbenzoyl)-L-glutamate to give the vinyl precursor of the subject compound. Catalytic hydrogenation (Pt in glacial AcOH) of this product at ambient conditions led to uptake of 3 molar equiv of H_2. Exposure to air during saponification of the ester groupings apparently gave the 7,8-dihydro compound according to UV spectral data, and further oxidation with H_2O_2 led to 10-deazaaminopterin.

The folate analogue methotrexate is active against some experimental neoplasms and in certain forms of human cancer. Acute lymphocytic leukemia and choriocarcinoma are prominent examples of tumors highly susceptible to this agent. Other human cancers for the most part, however, are only marginally responsive or not susceptible at all.[1] A correlation between uptake of methotrexate and responsiveness of a group of murine tumors has been reported,[2] and in 11 clinical cases the susceptibility of the leukemia to the drug was related to the relative ability of the leukemia cells to take up tritiated methotrexate.[3] 10-Deazaaminopterin (**4**) is significantly more active than methotrexate against certain animal neoplasms[4] because of more favorable transport characteristics: there is a greater persistence of exchangeable levels of **4** than methotrexate in tumor tissue but not in the drug-limiting normal proliferative tissue of the small intestine. The carrier transport mechanism of tumor, but not normal, cells has a much greater affinity for **4** than for methotrexate.[5] These data would seem to suggest a broader spectrum of clinical potential for **4** and provide the basis for trials that are underway.[6]

The first synthesis of **4** consisted of a sequence based on the Boone–Leigh method that led to 4-amino-4-deoxy-10-deazapteroic acid and introduction of the glutamic acid grouping by two coupling methods.[7] Recently, the preparation of the pteroic acid by an improved procedure via condensation of 2,4,5,6-tetraaminopyrimidine with 2-bromo-4-(4-carboxyphenyl)butyraldehyde was described.[8]

We report a simple three-step procedure (Scheme I) for the preparation of **4** in which the intact side chain is attached directly to the pteridine moiety via the ylide **2** prepared from 6-(bromomethyl)-2,4-pteridinediamine hydrobromide (**1**), available as an intermediate in large-scale synthesis of methotrexate.[9] The approach is related to that used earlier for the synthesis of 10-deazapteroic acid[10] and the recent synthesis of 5,8,10-deazafolic acid,[11] but this is the first example of the preparation of a pteridine ylide and its use in the Wittig reaction.

Scheme I



The I_{50} value for **4** against DHFR derived from pigeon liver[12] was 0.014 μM, while that for MTX was 0.026 μM. In cloning suppression tests against H.Ep.-2 cells,[13] **4** gave an ED_{50} of 0.9 nM and MTX gave 2.4 nM.

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Experimental Section

Diethyl *N*-[4-[2-(2,4-Diamino-6-pteridinyl)vinyl]-benzoyl]-L-glutamate (3). A mixture of 1-$(CH_3)_2CHOH$[9] (2.00 g, 5.05 mmol) and triphenylphosphine (1.39 g, 5.30 mmol) in Me_2NAc (75 mL) was stirred at 60–63 °C for 1.5 h. The pale-yellow solution was cooled to 25 °C and diethyl *N*-(4-formylbenzoyl)-L-glutamate[14] (1.78 g, 5.30 mmol) was added, followed immediately by solid NaOMe (0.60 g, 11.1 mmol). The dark-red solution was stirred at 25 °C overnight (17 h) while it became orange colored and developed a green-yellow fluorescence. The solution was treated with Norit, filtered (Celite mat), and evaporated in vacuo (<1 mm, bath to 45 °C). The dark-orange, viscous residue was stirred with C_6H_6 (20 mL) at 40–45 °C until a fluid mixture formed. Treatment of the cooled mixture with Et_2O (30 mL) gave a yellow precipitate, which was collected with the aid of Et_2O, dried in vacuo, and redissolved in Me_2NAc (20 mL). The clarified (Norit, Celite) solution was then treated with H_2O (100 mL) to give **3** as a yellow solid. After cooling for 2 h in a refrigerator, the solid was collected, washed with H_2O followed by Et_2O, and dried in vacuo (25 °C over P_2O_5): yield 2.05 g (78%); UV λ_{max} ($\epsilon \times 10^{-3}$), 0.1 N HCl, 314 nm (30.5), 385 (18.2); pH 7, 318 (28.0), 407 (17.8); 0.1 N NaOH, 319 (30.0), 407 (18.1); ^1H NMR (Me_2SO-d_6) δ 1.2 (m, CH_3CH_2), 2.1 (m, $CHCH_2CH_2CO$), 4.1 (m, CH_3CH_2O), 4.4 (m, $NHCHCH_2$), 6.8 (s, NH_2), 7.5 (d, one vinyl proton, J = 16 Hz, trans), 7.6–8.3 (br complex m, C_6H_4, NH_2, remaining vinyl proton), 8.7 (d, CONH), 8.9 (s, C_7H); MS (field desorption) m/e 493 (M^+). Anal. ($C_{24}H_{27}N_7O_5 \cdot 1.5H_2O$) C, N; H: calcd, 5.81; found, 5.39. Thin-layer chromatography [Analtech SGGF plates, $CHCl_3$–MeOH (3:1)] revealed a major UV-absorbing spot (R_f 0.68) and a much weaker spot (R_f 0.72), possibly indicating the presence of a small amount of cis isomer not detected in the ^1H NMR spectrum.

***N*-[4-[2-(2,4-Diamino-6-pteridinyl)ethyl]benzoyl]-L-glutamic Acid (10-Deazaaminopterin, 4).** Hydrogenation of **3** (1.70 g, 3.27 mmol) at ambient conditions in AcOH (250 mL) containing Pt (from prior reduction of 200 mg of PtO_2 of 84% purity) resulted in uptake of 200 mL of H_2 during 18 h. The apparently spent catalyst was removed by filtration, and the filtrate was combined with a suspension of fresh catalyst (from reduction of 150 mg of 84% PtO_2) in AcOH (10 mL). Hydrogenation at ambient conditions was resumed, and an additional 50 mL of H_2 was taken up during the next 3 days. The solution filtered from the catalyst was evaporated under reduced pressure (bath to 35 °C). Solutions of the residue in EtOH were then repeatedly evaporated as above to aid in the removal of AcOH. The viscous oil that remained was dissolved in EtOH (40 mL), and the solution was treated with 1 N NaOH (9 mL) to give a solution of pH 9. More 1 N NaOH (8 mL total) was added in four equal portions during the next 5 h. The basic solution was stirred exposed to air at 25 °C for 20 h and was then evaporated (H_2O aspirator, bath to 25 °C) until the EtOH had been removed. Dilution with H_2O (50 mL) followed, and treatment with Norit followed by filtration (Celite and powdered cellulose mat) gave a pale-yellow solution which was treated with AcOH to pH 7.0.[15] This solution was chilled to 5 °C, treated with H_2O_2 (4.5 mL of 30%), kept for 1 h at 20–25 °C, and treated with AcOH to pH 4.3. The yellow solid that separated immediately was collected after the mixture had been kept in a refrigerator overnight. Examination of this solid by reversed-phase high performance LC indicated that saponification was incomplete; a peak due to a more strongly retained component was present along with that due to **4**.[18] The crude material was then suspended in H_2O (60 mL), and sufficient 1 N NaOH was added dropwise to produce a solution of pH 12. After this solution had been kept at 55 °C for 30 min, analysis by high-performance LC as described above revealed disappearance of the more strongly retained component. The cooled solution was clarified (Norit, Celite) and acidified to pH 4.2. After refrigeration, the solid was collected, washed with H_2O, and dried in vacuo (25 °C, P_2O_5) to give 0.91 g of pale-yellow solid. The weight increased to 0.94 g (62% yield) on exposure to ambient conditions of the laboratory: UV λ_{max} ($\epsilon \times 10^{-3}$), 0.1 N HCl, 242 nm (30.0), 337 (10.2), 352 (sh) (9.97); pH 7, 254 (30.5), 372 (7.17); 0.1 N NaOH, 256 (31.1), 372 (7.31); ^1H NMR (Me_2SO-d_6) δ 2.1 (m, $CHCH_2CH_2$), 2.3 (m, CH_2CO_2H), 3.16 (s, $CH_2CH_2C_6H_4$), 4.4 (m, $NHCHCH_2$), 6.80 (s, NH_2), 7.35 and 7.80 (2 d, C_6H_4), 7.74 (s, NH_2), 8.5 (d, CONH), 8.58 (s, C_7 H); MS (field desorption) m/e 440 $(M + 1)^+$. Anal. ($C_{20}H_{21}N_7O_5 \cdot 1.5H_2O$) C, N; H: calcd, 5.19; found, 4.76. Analysis by high-performance LC indicated a purity level of at least 97% with respect to UV-absorbing material. Another reprecipitation from basic solution carried out essentially as before preceded a reprecipitation from Me_2SO solution at 70–75 °C by addition of H_2O of the same temperature. These two operations gave **4** as granular particles in 80% recovery, but, according to high-performance LC and UV spectral data, the purity of this sample was the same as that for which analytical results are given above. Anal. ($C_{20}H_{21}N_7O_5 \cdot 1.25H_2O$) C, H, N.

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(15) The 5,6,7,8-tetrahydro derivative that formed during the reduction step was expected to be converted by air oxidation to the 7,8-dihydro derivative and, possibly to some extent, the fully aromatic system.[16] Indeed, the UV spectra of samples of the solution at this point adjusted to pH 1 and 13 revealed maxima at 290 nm, which suggests that the dihydro form was in dominance since the reported λ_{max} for dihydroaminopterin is 289 nm at pH 0.3, 7, and 13.[17] The subsequent treatment with H_2O_2 caused the UV maxima to change to those listed for **4**.

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(18) Analysis by high-performance LC using a μBondapak C_{18} column and a 20-min linear gradient system with the combination acetate buffer (pH 3.6)–MeCN changing in volume proportions from 85:15 to 1:1 affords an excellent method for monitoring the hydrolysis of alkyl esters of **4** and related compounds.