Synthesis of 10-Propargylfolic Acid from 2-Amino-6-(bromomethyl)-4(1*H*)-pteridinone

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6-(Bromomethyl)-2,4-pteridinediamine hydrobromide (**1**) is readily converted to 2-amino-6-(bromomethyl)-4(1*H*)-pteridinone hydrobromide (**2**) by treatment with 48% hydrobromic acid. Compound **2** is of interest for direct attachment of the (2-amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl group to appropriate side-chain precursors of analogues of folic acid, particularly those bearing functional groups incompatible with conditions required for hydrolytic deamination of the corresponding 2,4-diaminopteridine analogues. An example of the use of **2** in this connection is demonstrated through synthesis of 10-propargylfolic acid.

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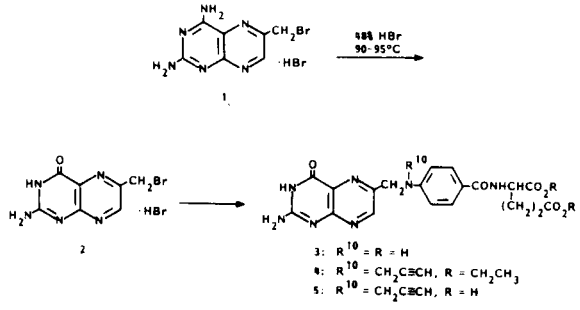
We reported that 6-(bromomethyl)-2,4-pteridinediamine hydrobromide (**1**) could be converted through hydrolytic deamination in 48% hydrobromic acid to 2-amino-6-(bromomethyl)-4(1*H*)-pteridinone hydrobromide (**2**) and that alkylation of *N*-(4-aminobenzoyl)-L-glutamic acid with **2** affords folic acid (**3**) [1]. Details were not published, however, and several investigators have recently requested information on these conversions. In this report we wish to describe a simple conversion of **1** [2] to **2** and the use of **2** to prepare **3** and 10-propargylfolic acid (**5**) (see Scheme I).

Scheme I

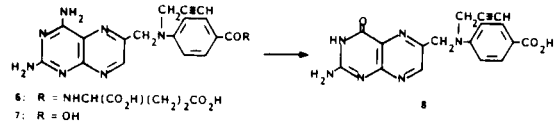


The propargyl compound **5** was selected for synthesis for two reasons. First, it is the pteridine analogue of the quinazoline antifolate 10-propargyl-5,8-dideaazafolic acid [3] now in clinical evaluation [4]; we plan to test **5** for its possible antifolate activity. Second, the lability of the propargyl group precludes use of a standard method for converting available 2,4-diaminopteridines to the corresponding 2-amino-4-oxo analogues; synthesis of **5** from **2** serves to demonstrate the value of **2** in circumventing that obstacle.

One expressed reason for interest in **2** and its conversion to folic acid is the potential for application of this approach to the synthesis of authentic samples of folylpolyglutamates for use as reference samples in studies on folate metabolism [5]. Thus alkylation of an appropriate *N*-(4-aminobenzoyl)oligo-γ-glutamate [6,7] with **2** will afford the corresponding folylpolyglutamate. The 2,4-diamino compound **1** has been used in related fashion in alkylations of *N*-[4-(methylamino)benzoyl]oligo-γ-glutamates to prepare poly-γ-glutamyl analogues of the anticancer agent methotrexate [8].

Although 2,4-diamino compounds accessible from **1** and appropriate side-chain intermediates may often be converted through hydrolysis to the corresponding 2-amino-4-oxo analogues [9-11], the conditions required to effect this conversion are not compatible with some functionalities. The peptide linkages of the folylpolyglutamates mentioned above could be degraded under the hydrolytic conditions required. Similarly, as alluded to above, **5** could not be prepared by hydrolytic deamination of 10-propargylaminopterin (**6**) [12] (see Scheme II).

Scheme II



When the usual conditions using refluxing 1*N* sodium hydroxide [9-11] were applied to **6**, the propargyl group was removed [13] leaving **3** as the only product identifiable by mass spectral examination. Acidic hydrolysis experiments followed. Treatment of **6** with refluxing 1*N* hydrochloric acid produced a mixture of unchanged **6** along with **5** and both 10-propargylpteroic acid (**8**) and its 4-amino-4-deoxy analogue **7**. These results showed the carboxamide function of **6** to be about as labile in refluxing 1*N* hydrochloric acid as its 4-amino group. A brief (five minutes) treatment of **6** with refluxing 4*N* hydrochloric acid effected complete deamination and left the propargyl group in place, but considerable cleavage of the carboxamide group also took place to produce a mixture of **5** and **8**. This finding prompted an experiment on the fate of **7** in refluxing 4*N* hydrochloric acid; as expected, deamination was complete within five minutes without effect on the

propargyl group thereby affording **8**. Results from these hydrolysis experiments are summarized in Table 1.

Through use of standard coupling procedures, **8** could be converted to **5**, but the use of easily prepared **2** offers a more direct choice. Alkylations by **2** in *N,N*-dimethylacetamide of the appropriate side-chain intermediates gave folic acid (**3**) in 80% yield and pure 10-propargylfolic acid diethyl ester (**4**) in 43% yield, after a reprecipitation from a concentrated pyridine solution by addition of water. Hydrolysis of the ester groups of **4** to give **5** was done under mild conditions which did not affect the propargyl group.

EXPERIMENTAL

The proton nmr spectral data reported were determined with a Nicolet NMC 300NB spectrometer for all compounds (except **7** which was done on a Varian XL-100-15) using solutions in hexadeuteriodimethylsulfoxide and tetramethylsilane as internal reference. Mass spectra were recorded in the fast atom bombardment mode on a Varian MAT 311A mass spectrometer equipped with electron impact-field ionization/field desorption and fast atom bombardment ion sources. The uv spectra were determined with a Cary Model 17 spectrometer. Samples bearing carboxyl groups were first dissolved in 0.1*N* sodium hydroxide, and the solutions were then diluted tenfold with the medium given in the listings. Compound **2** was first dissolved in concentrated hydrochloric acid (0.2 ml for 5 mg), and the solution was then diluted with water (to 50 ml) to give the stock solution which was diluted tenfold with the medium given in the uv listings. Maxima are expressed in nanometers with the molar absorbance ($\epsilon \times 10^{-3}$) given in parentheses. Molecular weights used in all calculations conform with the compositions listed with elemental analysis results. The hplc examinations were done with a Waters Associates ALC-242 liquid chromatograph equipped with a uv detector (254 nm) and an M-6000 pump using a 30X0.29 cm C_{18} μBondapak column. The purity assay of **5** was done using reverse-phase in the isocratic mode with a mobile phase consisting of acetonitrile (10 or 15% by volume) in 0.1*M* sodium acetate (*p*H 3.6). Saponification of **4** to give **5** was monitored using a 20-minute linear gradient system with the combination acetonitrile-0.1*M* sodium acetate (*p*H 3.6) changing from 15% acetonitrile to 50%. Except where other conditions are specified, evaporations were performed with a rotary evaporator and a water aspirator. Products were dried *in vacuo* (< 1 mm) at 22-25° over sodium hydroxide pellets and phosphorus pentoxide. Final products were dried and then allowed to equilibrate with ambient conditions of the laboratory. Spectral determinations and some of the elemental analyses were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. Some elemental analyses were also performed at Atlantic Microlab, Inc., Atlanta, GA.

2-Amino-6-(bromomethyl)-4(1*H*)-pteridinone Hydrobromide (**2**).

A solution of **1** (2.00 g of 84% purity [16], 5.06 mmoles) in 48% hydrobromic acid (10 ml) was kept at 90-95° for 30 minutes. The cooled solution became thick with crystalline product, which was collected with the aid of glacial acetic acid, washed thoroughly on the funnel with acetic acid followed by ether, then dried to give **2** as greyish, felt-like matted needles; yield 1.23 g (72%); ms reveals peaks of m/z 256 and 258, $(M + 1)^+$ values for $C_7H_6BrN_5O$; proton nmr: δ 4.95 (2 H, s, CH_2), 8.97 (1 H, s, C-7 H) [17]; uv: λ max, nm ($\epsilon \times 10^{-3}$), 0.1*N* hydrochloric acid, 232 (sh), 252 (11.7), 325 (8.95); *p*H 7, 235 (11.3), 273 (13.6), 346 (6.45); *p*H 13, 253 (22.7), 363 (7.34).

Anal. Calcd. for $C_7H_6BrN_5O \cdot HBr$: C, 24.95; H, 2.09; Br, 47.43; N, 20.78. Found: C, 24.78; H, 2.16; Br, 47.18; N, 20.58.

N-[4-[[(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]amino]benzoyl]-L-glutamic Acid (**3**, Folic Acid).

A mixture of **2** (180 mg, 0.53 mmole), *N*-(4-aminobenzoyl)-L-glutamic-acid (400 mg, 1.5 mmoles), and *N,N*-dimethylacetamide (4 ml) was stirred in a stoppered flask at 22-25° for 96 hours. Warming with the aid of a 50-60° water bath for 30 minutes then caused complete solution. After another 24-hour period at 22-25°, the orange-colored solution was combined with water (30 ml) to cause precipitation of **3**. Centrifugation followed, and the somewhat gelatinous precipitate that remained following decantation of the supernatant was twice washed with water (30-ml portions) while two repetitions of centrifugation-decantation were done. The precipitate was then stirred with acetone, and another centrifugation followed. After decantation of the acetone, the solid that remained was stirred with ether and was then easily collected by filtration. The ether-washed solid was dried to give **3**, yield 185 mg (80%), homogeneous according to thin-layer chromatography on DEAE-cellulose [14] and identical with a standard sample; uv spectra: λ max in 0.1*N* sodium hydroxide, *p*H 7 standard buffer, and 0.1*N* hydrochloric acid are in agreement with data reported (including molar absorbance values) for authentic **3** monohydrate [15].

Diethyl *N*-[4-[[(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]prop-2-ynylamino]benzoyl]-L-glutamate (**4**).

A stirred solution of diethyl *N*-[4-(prop-2-ynylamino)benzoyl]-L-glutamate (720 mg, 2.00 mmoles) [3,12] in *N,N*-dimethylacetamide (50 ml) was treated with **2** (600 mg, 1.78 mmoles) in one portion. Solution occurred within one minute, but finely divided white solid separated within another minute. The mixture was left to stir in a stoppered flask wrapped in aluminum foil for 66 hours at 23-25° before it was heated in a water bath at 60-65° for one hour, then the bath temperature was raised to 86° where solution occurred. After 5 minutes at 85-90°, the bath was removed, and the solution was allowed to cool to approximately 50°. This solution was combined with water (350 ml) of about the same temperature. The solution that resulted soon began depositing yellow solid. The mixture was allowed to cool to 25° before it was chilled in an ice-water bath for 2 hours. The solid was then collected, washed thoroughly with water, and dried. This crude product (520 mg) was dissolved with the aid of rapid stirring in boiling pyridine (550 ml). The nearly clear solution was clarified (with Norit treatment and filtration through a Celite mat), then evaporated until most of the pyridine had been removed leaving a viscous orange solution. This concentrated solution was mixed with water (300 ml) with rapid stirring. The initial gel-like residue formed yellow solid as it was dispersed. After about one hour of stirring, the mixture was treated with sufficient 20% acetic acid in water to produce *p*H 5-6. Overnight storage in a refrigerator followed. The solid was then collected, washed with water, and dried to give **4**·0.5H_2O in 43% yield (420 mg); ms: m/z 536, $(M + 1)^+$; proton nmr: δ 1.1-1.3 (6 H, two t overlapping, CH_3), 2.1 (2 H, m, $CHCH_2CH_2$), 2.4 (2 H, t, CH_2CO), 3.25 (1 H, t, $HC{\equiv}CCH_2$, J = 2.20 Hz), 4.0-4.2 (4 H, two q overlapping, OCH_2CH_3), 4.36-4.46 (3 H, m overlapping d, NHCHCO and $CH_2C{\equiv}CH$), 4.81 (2 H, s, $CH_2NCH_2C{\equiv}CH$), 6.90 (4 H, d, NH_2 and 3,5-protons of 1,4-phenylene), 7.75 (2 H, d, 2,6-protons of 1,4-phenylene), 8.38 (1 H, d, CONHCH), 8.58 (1 H, s, C-7 H), 11.46 (1 H, s, N-3 H); uv: λ max, nm ($\epsilon \times 10^{-3}$), 0.1*N* hydrochloric acid, 298 (26.3); *p*H 7, 278 (27.5), 290-297 plateau (26.7), 348 (7.97); 0.1*N* sodium hydroxide, 256 (27.3), 293 (26.0), 365 (9.69); ir (potassium bromide): 2108 cm^{-1} (mono-substituted acetylene).

Anal. Calcd. for $C_{25}H_{29}N_7O_6 \cdot 0.5H_2O$: C, 57.35; H, 5.55; N, 18.00. Found: C, 57.32; H, 5.49; N, 18.17.

N-[4-[[(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]prop-2-ynylamino]-benzoyl-L-glutamic Acid (**5**, 10-Propargylfolic Acid).

A stirred mixture of **4**·0.5H_2O (350 mg, 0.642 mmole) in ethanol (25 ml) and water (10 ml) was treated with 1*N* sodium hydroxide (2.4 ml) whereupon solution occurred almost immediately. The yellow solution was kept in a stoppered flask in the dark at 23-25° for 48 hours and was then treated with glacial acetic acid to produce *p*H 4.0. Yellow solid separated readily, and the mixture was left in a refrigerator overnight. The precipitate was too finely divided to collect by filtration. Centrifugation proved satisfactory through two cycles of centrifugation including

wash treatment with water. On the third treatment, however, the precipitate became too finely divided to be centrifuged. The mixture was left overnight in a freezer. After the frozen mixture had been allowed to warm to room temperature, the solid had coagulated sufficiently to allow centrifugation. Three more cycles of centrifugation with wash treatment with cold water (35 ml each treatment) followed. After the final centrifugation, the wet, finely divided solid was transferred to a round-bottom flask with the aid of ethanol. Evaporation of the ethanol followed, then the residual solid was stirred with ether, collected with the aid of ether, and dried, yield 54% (170 mg). Assay by hplc indicated a purity of at least 98%; ms: m/z 480 (M + 1)$^+$; proton nmr: δ 1.90, 2.05 (2 H, two m, $CHCH_2CH_2$, nonequivalent), 2.30 (2 H, t, $CH_2CH_2CO_2H$), 3.22 (1 H, t, $HC\equiv CCH_2$, J = 2.20 Hz), 4.28-4.44 (3 H, m overlapping d, $NHCHCO$ and $CH_2C\equiv CH$), 4.79 (2 H, s, $CH_2NCH_2C\equiv CH$), 6.88 (4 H, d, NH_2 and 3,5 protons of 1,4-phenylene), 7.75 (2 H, d, 2,6-protons of 1,4-phenylene), 8.23 (1 H, d, $CONHCH$), 8.56 (1 H, s, C-7 H), 11.45 (1 H, s, N-3 H), 12.30 (2 H, s, CO_2H); uv: λ max, nm (ε x 10^{-3}), 0.1*N* HCl, 298 (25.0); *p*H 7, 281 (27.9), 347 (7.78); 0.1*N* NaOH, 255 (26.7), 292 (25.7), 366 (9.55); ir (potassium bromide): 2105 cm^{-1} (mono-substituted acetylene).

Anal. Calcd. for $C_{22}H_{21}N_7O_6\cdot 0.7H_2O$: C, 53.70; H, 4.59; N, 19.93. Found: C, 53.66; H, 4.68; N, 20.19.

4-[[(2,4-Diamino-6-pteridinyl)methyl]prop-2-ynylamino]benzoic Acid (**7**, 4-Amino-4-deoxy-10-propargylpteroic Acid).

A mixture of 6.3 mmoles each of **1** (2.5 g of 84% [16]) and 4-(prop-2-ynylamino)benzoic acid (1.1 g) [18] in *N,N*-dimethylacetamide (35 ml) was stirred at 23-25° for 0.5 hour then at 65-75° (bath temperature) for 2 hours while solution occurred. The cooled solution was combined with cold water (200 ml), and the resulting clear solution (*p*H 2.3) was carefully treated with 1*N* sodium hydroxide to raise the *p*H to 5.8 and cause precipitation of **7**. The collected solid was washed with water followed by acetonitrile, then dried; yield 1.50 g. This material was stirred with water (50 ml) while the mixture was treated with 1*N* sodium hydroxide solution (3.5 ml) . The resulting solution was clarified (Norit, Celite) and then treated with 1*N* hydrochloric acid to *p*H 6.1 to reprecipitate **7**, yield 52% (1.27 g); ms: m/z 350, (M + 1)$^+$; proton nmr: δ 3.20 (1 H, t, $HC\equiv CCH_2$), 4.50 (2 H, d, $CH_2C\equiv CH$), 4.84 (2 H, s, CH_2N), 6.65 (2 H, s, NH_2), 6.95 (2 H, d, 3,5-protons of 1,4-phenylene), 7.6 (2 H, br s, NH_2), 7.80 (2 H, d, 2,6-protons of 1,4-phenylene), 8.70 (1 H, s, C-7 H); uv: λ max nm (ε x 10^{-3}), 0.1*N* hydrochloric acid, 243 (16.3), 300 (23.7), 338 (sh); *p*H 7, 260 (26.4), 285 (sh), 372 (7.2); 0.1*N* sodium hydroxide, 259 (27.2), 285 (sh), 372 (7.7).

Anal. Calcd. for $C_{17}H_{15}N_7O_2\cdot 2.3H_2O$: C, 52.25; H, 5.06; N, 25.09. Found: C, 51.84; H, 5.12; N, 25.43.

Hydrolytic Deamination Experiments on the Propargyl Compounds.

1. Treatment of **6** with 1*N* Sodium Hydroxide.

A solution of **6** (50 mg) [12] in 1*N* sodium hydroxide (1.5 ml) was refluxed under nitrogen for 4 hours. When the cooled solution was acidified with glacial acetic acid a gel-like precipitate formed, but when the mixture was stirred briefly in a hot water bath the precipitate coagulated to a yellow-orange solid which was easily collected by filtration, yield 40 mg; ms gave peaks of m/z 442, (M + 1)$^+$ for **3**; 295, fragment due to pteroyl group, no **5** was detectable.

2. Treatment of **6** with 1*N* Hydrochloric Acid.

A solution of **6** (40 mg) [12] in 1*N* hydrochloric acid (4 ml) was refluxed 30 minutes. The cooled solution was carefully treated with 1*N* sodium hydroxide solution until the *p*H of the mixture reached 4.0. Solid began separating when the *p*H was above 2.5. The mixture was kept overnight in a refrigerator before the tan solid was collected and dried; wt 30 mg; ms: m/z 350, (M + 1)$^+$ for **7**; 351, (M + 1)$^+$ for **8**; 479, (M + 1)$^+$ for unchanged **6**; 480, (M + 1)$^+$ for **5**.

3. Treatment of **6** with 4*N* Hydrochloric Acid.

A stirred suspension of **6** (100 mg) [12] with 4*N* hydrochloric acid (10 ml) was refluxed 5 minutes while solution occurred. Almost immediately after the heat source was removed, solid began precipitating. The solid filtered from the cooled mixture amounted to 20 mg; ms: m/z 480, (M + 1)$^+$ for **5**, and 351, (M + 1)$^+$ for **8**.

4. Treatment of **7** with 4*N* Hydrochloric Acid.

4-[[(2-Amino-3,4-dihydro-4-oxo-6-pteridinyl)methyl]prop-2-ynylamino]-benzoic Acid (**8**, 10-Propargylpteroic Acid).

A stirred suspension of **7** (100 mg, 0.26 mmole) in 4*N* hydrochloric acid (10 ml) was heated to boiling where solution occurred momentarily, but, during the reflux period of 5 minutes, a yellow solid separated. The mixture was allowed to cool, and the solid was collected with the aid of water, yield 60 mg; ms: m/z 351, (M + 1) for **8**; proton nmr: δ 3.27 (1 H, t, $CH_2C\equiv CH$), 4.42 (2 H, d, $CH_2C\equiv CH$), 4.81 (2 H, s, CH_2N), 6.88 (2 H, d, 3,5-protons of 1,4-phenylene), 7.77 (2 H, d, 2,6-protons of 1,4-phenylene), 8.58 (1 H, s, C-7 H).

Table 1

Summary of the Hydrolytic Deamination Experiments on the Propargyl Compounds

Compound No.	Refluxing Medium	Time	Product(s) [a]
6	1*N* NaOH	4 hours	**3**
6	1*N* HCl	30 minutes	**5, 7, 8** [b]
6	4*N* HCl	5 minutes	**5, 8**
7	4*N* HCl	5 minutes	**8**

[a] Detected by mass spectral examination. [b] Unchanged **6** was also present.

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[14] Thin-layer chromatographic examination of **3** was done on DEAE-cellulose sheets as described in [2]. The R_f value was 0.22.

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[16] Prepared as described in [2]. The remainder of the sample of 84% purity consisted mostly of 2-propanol with a small amount of the 6-methyl analogue of **1** present as described in [2].

[17] Any 6-methyl analogue of **1** originally present will be converted to the 6-methyl analogue of **2**. If enough is presented to be detected, it will produce singlets at δ 2.62 (CH_3) and 8.75 (C-7 H). After **2** has been in the hexadeuteriodimethylsulfoxide solution for about 15 minutes, it will have undergone sufficient conversion to the 6-hydroxymethyl analogue that singlets begin to appear at δ 4.72 and δ 8.87; these signals increase with time while those due to **2** decrease.

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