# The Synthesis of a Pyrido[3,4-*d*]pyrimidine Analog of Pteroic Acid (1)



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A multistep synthesis of ethyl 5-amino-2-methylpyridine-4-carboxylate (**5a**) starting from ethyl acetopyruvate and nitroacetamide is described. The condensation of **5a** with benzoylcyanamide gave 2-amino-3-benzoyl-6-methylpyrido[3,4-*d*]pyrimidin-4(3*H*)one (**10**), which could be hydrolyzed in alkali to give 2-amino-4-hydroxy-6-methylpyrido[3,4-*d*]pyrimidine (**9**). Free radical bromination of **10** in bromotrichloromethane gave a mixture of the bromo- and chloromethyl- derivatives (**11**). Fusion of **11** with ethyl *p*-aminobenzoate, followed by alkaline hydrolysis gave the corresponding pteroic acid analog (**12**).

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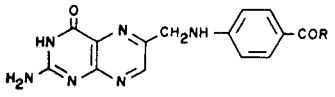
Numerous reports have described attempts at modification or substitution of functionality on the folic acid (**1**) and pteroic acid (**2**) molecules in order to obtain effective folate antagonists. A number of fused pyrimidine

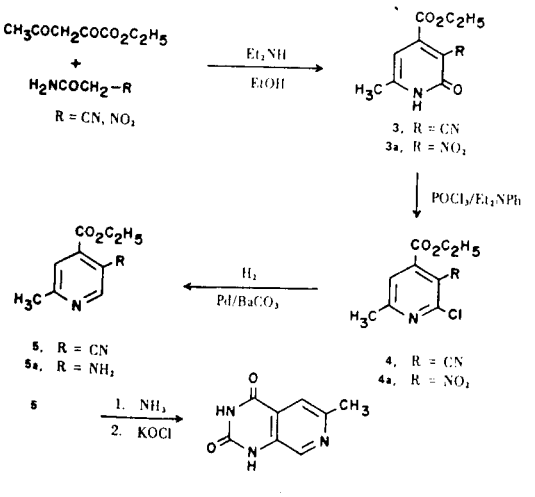


1, R = NHCHCO$_2$H
CH$_2$CH$_2$CO$_2$H
2, R = OH

ring systems have been substituted for the pteridine system in **1** and **2**. Pyrido[2,3-*d*]pyrimidine and pyrido[3,2-*d*]pyrimidine analogs of pteroic acid have been previously described (2,3), however no pyrido[3,4-*d*]pyrimidines have been previously incorporated into folate or pteroate derivatives.

The relative lack of literature concerning pyrido[3,4-*d*]pyrimidines is largely due to the restricted availability of 3-aminopyridine-4-carboxylic acids (4). As a result, several syntheses have been described in which the ring system has been formed without the use of the appropriate pyridine aminoacids (4-7). As an example, the first synthesis of a 6-methylpyrido[3,4-*d*]pyrimidine (**6**) was described by Reider and Elderfield (Scheme I) (5). Substitution of nitroacetamide for cyanoacetamide in Scheme I has allowed the synthesis of ethyl 5-amino-2-methylpyridine-4-carboxylate (**5a**) (8). This compound, **5a**, afforded a more accessable entry into 6-methylpyrido[3,4-*d*]pyrimidine systems, directed towards the syntheses of folate and pteroate analogs.

SCHEME I



Results and Discussion.

The synthesis of **3a** (Scheme I) was achieved with the substitution of nitroacetamide for cyanoacetamide in a previously described Guareshi-Thorpe pyridone synthesis (9). The yields of **3a** were much lower than those obtained for **3** under identical conditions. Variation of solvent, base, temperature, and reaction time gave no substantial increase in yield. The relative non-nucleophilicity of acinitroacetamide *versus* cyanoacetamide anion, and the competing self-condensation of ethyl acetopyruvate (10) are cited as the principal reasons for the low yields.

Chlorination of the pyridones (**3** and **3a**) was achieved using a phosphorous oxychloride/diethylaniline combination (11) which afforded increased yields as compared with the phosphorous oxychloride/phosphorous pentachloride system (5). Subsequent hydrogenation of **4a** at atmospheric pressure with a palladium on barium carbonate catalyst (5) resulted in the reduction of the nitro group and hydrogenolysis of the 2-chloro substituent, affording **5a** in good yield. While the initial condensation reaction with nitroacetamide is presently the weak point in this synthesis of **5a**, the potential preparation of 3-nitropyridones through the use of nitroacetamide offers distinct advantages over potential isomeric distributions associated with direct nitration of pyridine intermediates.

Attempts at the preparation of 2-amino-4-hydroxy-6-methylpyrido[3,4-*d*]pyrimidine (**9**) by fusion of **5a** with guanidine or guanidine salts gave multiple, high-melting products which proved difficult to separate and purify (Scheme II). Preparation of 4-hydroxy-2-mercapto-6-methylpyrido[3,4-*d*]pyrimidine (**8**) *via* the benzoylthiourea (**7**) afforded the substrate for potential alternate routes

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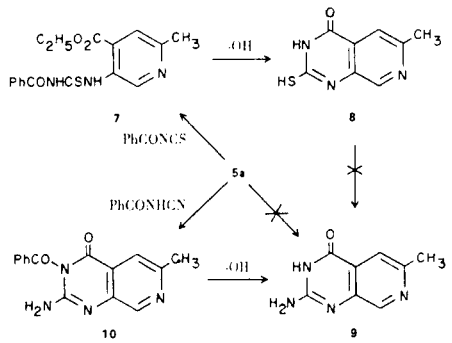
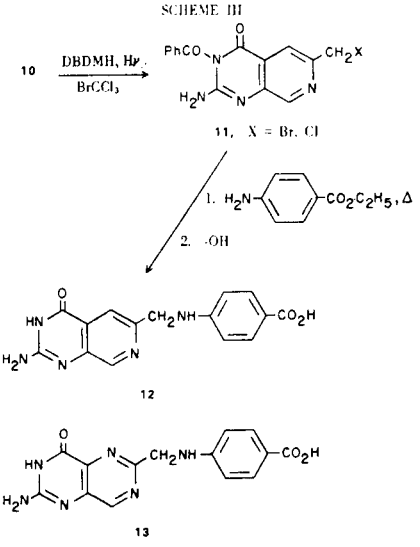
to **9**. The attempted replacement of the 2-mercapto functionality of **8** with ammonia and ammonium chloride in a sealed bomb gave multiple, high-melting products. Previous attempts at this transformation indicated that **9** and 2,4-diamino-6-methylpyrido[3,4-*d*]pyrimidine were present in the final mixture, as judged by high resolution mass spectrometry (8). The synthesis of **9** was achieved by the condensation of **5a** with benzoylcyanamide, affording 2-amino-3-benzoyl-6-methylpyrido[3,4-*d*]pyrimidin-4(3*H*)one (**10**), which was subsequently hydrolyzed to **9**.

The monobromination of 2,4-dihydroxy-6-methylpyrido[3,4-*d*]pyrimidine has been reported by Oakes and Rydon (3). The bromomethyl compound was not isolated; however, it was allowed to react *in situ* with *p*-aminobenzoic acid, and 4-[(2,4-dihydroxypyrido[3,2-*d*]pyrimidin-6-ylmethyl)amino]benzoic acid was recovered as the major product. With either **9** or with 2,4-dihydroxy-6-methylpyrido[3,4-*d*]pyrimidine (**6**) as a bromination substrate, no evidence for the formation of a pteroate derivative was observed after reaction with *p*-aminobenzoic acid.

SCHEME II




SCHEME III




The appreciable solubility of **10** in hot bromotrichloromethane suggested the use of this solvent in potential free radical halogenations. Subsequent bromination attempts using *N,N'*-dibromo-5,5-dimethylhydantoin (DBDMH) (11) with bromotrichloromethane as solvent and benzoyl peroxide as initiator gave a mixture of products (Scheme III). The nmr spectra indicated two resonances at 4.87 (-$CH_2$Br) and 4.95 ppm (-$CH_2$Cl), in addition to small amounts (< 20%) of unreacted **10**. Integration of the -$CH_2$X resonances for various preparations demonstrated that the ratio -$CH_2$Br/-$CH_2$Cl varied from 45:55 to 80:20. The mass spectrum (direct probe) of a sample from a single preparation gave ions at m/e 316 $(M+2)^+$ and 314 $(M^+)$ in the ratio of 0.35/1.00, verifying the 2-amino-3-benzoyl-6-chloromethylpyrido[3,4-*d*]pyrimidin-4(3*H*)one structure (**11**, X = Cl). No molecular ion was observed for the bromomethyl compound (**11**, X = Br). The presence of the chloromethyl compound (**11**, X = Cl) in this reaction is apparently not unusual, based upon the previously reported isolation of variable amounts of both bromo- and chloroalkanes from a Hunsdieker reaction with bromotrichloromethane as the solvent (13).

Fusion of **11** with ethyl *p*-aminobenzoate and subsequent alkaline hydrolysis of the intermediate gave a low yield of a compound identified as 4-[(2-amino-4-hydroxypyrido[3,4-*d*]pyrimidin-6-ylmethyl)amino]benzoic acid (**12**). This compound displayed all the expected properties of a pteroate analog, with the ir and uv spectra being remarkably similar to those reported for 4-[(2-amino-4-hydroxypyrimido[5,4-*d*]pyrimidin-6-ylmethyl)-amino]benzoic acid (**13**) (14). The low yields of **12** were partly attributable to the ethyl *p*-aminobenzoate fusion reaction, suggesting that a 2-amino protecting group might be advantageously used to prevent competing intermolecular reactions of **11** in the molten reaction medium. Due to the low overall yields encountered in this synthesis, no biological data are presently available for the pteroate analog (**12**).

## EXPERIMENTAL

Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Nmr spectra were obtained on a Jeol C60HL spectrometer, infrared spectra were obtained on a Perkin-Elmer Model 257 spectrophotometer, and ultraviolet spectra were obtained on a Unicam SP-800B spectrophotometer. Mass spectra were obtained on a Hitachi Perkin-Elmer RMU-6E spectrometer. Microanalyses were performed by Galbraith Laboratories, Knoxville, Tenn. The DMSO-$d_6$/deuteriochloroform/ solvent for nmr spectra was prepared as a 70:30 (v/v) solution. Bromotrichloromethane (Spectrograde) was obtained from Eastman Organic Chemicals. Thin layer chromatography (tlc) was conducted on microscope slides coated with silica gel GF 254

(Merck), and chromatograms were developed in chloroform/methanol (1:1). Visualization of tlc was accomplished with iodine vapor, and with 254 and 366 nm uv sources.

Ethyl 6-Methyl-3-nitro-2(1*H*)pyridone-4-carboxylate (**3a**).

Ammonium acinitroacetamide (15) (65 g., 0.537 mole) was suspended in 650 ml. of 95% ethanol, and ethyl acetopyruvate (16) (76 ml., 0.540 mole) was added with stirring. The mixture was treated with 3*N* hydrochloric acid to give a *p*H of 7 (wet *p*H paper). Diethylamine was then added with stirring to give a *p*H of 9-10, as the temperature of the reaction mixture gradually rose to 45°. After stirring for 18 hours, the orange solution was stripped of all volatile material and the residue was acidified to *p*H 1 with 6*N* hydrochloric acid. After removal of all volatile material, the semi-crystalline residue was washed with acetone, and the acetone solution was allowed to crystallize. Three crops of crystals could be obtained by further concentration of the acetone solution. Recrystallization of the recovered material from 80% ethanol (Norit) afforded 15.5 g. (13%) of light yellow crystals, m.p. 178.5-180°; ir (potassium bromide): 3200-2500, 1730, 1680, 1660, 1628, 1545, and 1250 $cm^{-1}$; nmr (DMSO-$d_6$/deuteriochloroform): $\delta$ 1.10 (t, 3H, J = 7.5 Hz), 2.15 (s, 3H), 4.13 (q, 2H, J = 7.5 Hz). 6.25 (s, 1H), and 13.15 ppm (br, 1H); ms: m/e (%) 226 (100), 198 (33), 181 (47), 168 (31), 140 (24).

*Anal.* Calcd. for $C_9H_{10}N_2O_5$: C, 47.79; H, 4.46. Found: C, 47.86; H, 4.60.

Ethyl 2-Chloro-6-methyl-3-nitropyridine-4-carboxylate (**4a**).

Previously dried **3a** (5.11 g., 22.6 mmoles), diethylaniline (7.2 ml., 45 mmoles), and 60 ml. of freshly distilled phosphorous oxychloride were refluxed for 24 hours. Excess phosphorous oxychloride was removed under reduced pressure, and the residue was treated with 75 ml. of cold water and 15 ml. of acetone. Upon stirring overnight, a green precipitate was formed, which was filtered off, and washed with water to give 4.77 g. (87%). This material was purified by sublimation at 90° (2 Torr), followed by recrystallization from acetone/water to give white crystals, m.p. 75-76°; ir (potassium bromide): 2975, 1725, 1548, 1295, 1015, and 740 $cm^{-1}$; nmr (deuteriochloroform): $\delta$ 1.38 (t, 3H, J = 7.5 Hz), 2.66 (s, 3H), 4.39 (q, 2H, J = 7.5 Hz), and 7.68 ppm (s, 1H); ms: m/e (%) 244 (44), 216 (30), 199 (46), 184 (31), 170 (30), 134 (100).

*Anal.* Calcd. for $C_9H_9ClN_2O_4$: C, 44.17; H, 3.68; N, 11.45. Foiund: C, 44.34; H, 3.63; N, 11.50.

Ethyl 5-Amino-2-methylpyridine-4-carboxylate (**5a**).

A 2.0 g. sample of **4a** was dissolved in 110 ml. of absolute ethanol, and this solution was hydrogenated at atmospheric pressure, using 2.0 g. of a 5% palladium on barium carbonate catalyst mixed with 2.0 g. of finely ground barium carbonate. After 93% of the theoretical amount of hydrogen had been consumed, the catalyst was filtered off and the filtrate was evaporated to dryness. The yellow solid was washed with a 10% sodium bicarbonate solution, and the solid was recrystallized from 50% ethanol (Norit), affording 1.09 g. (75%) of pale yellow crystals, m.p. 129-130°; ir (potassium bromide): 3425, 3285, 3155, 2970, 1700, 1620, 1589, 1300, and 1205 $cm^{-1}$; nmr (deuteriochloroform): $\delta$ 1.40 (t, 3H, J = 7.5 Hz), 2.43 (s, 3H), 4.35 (q, 2H, J = 7.5 Hz), 5.55 (br, 2H), 7.45 (s, 1H), and 8.13 ppm (s, 1H).

*Anal.* Calcd. for $C_9H_{12}N_2O_2$: C, 59.99; H, 6.72. Found: C, 59.96; H, 6.74.

Ethyl 5-(*N*'-Benzoylthioureido)-2-methylpyridine-4-carboxylate (**7**).

A solution of **5a** (1.80 g., 10 mmoles) in 10 ml. of dry DMF was heated and stirred at 60° while benzoylisothiocyanate (17) (1.43 ml., 10.5 mmoles) was added dropwise over a period of 2 minutes. The solution was heated for 20 minutes, then cooled and poured into 50 ml. of cold water. The resulting yellow solid was filtered off and washed with cold water to give 3.30 g. (97%). Recrystallization from 95% ethanol gave 2.70 g. of a fine light yellow powder. m.p. 149-150°; ir (potassium bromide): 3270, 3140, 2980, 1715, 1680, 1605, 1560, and 1500 $cm^{-1}$; nmr (deuteriochloroform): $\delta$ 1.40 (t, 3H, J = 7.5 Hz), 2.58 (s, 3H), 4.41 (q, 2H, J = 7.5 Hz), 7.32-7.78 (m, 3H), 7.71 (s, 1H), 7.88-8.15 (m, 2H), 9.47 (s, 1H), 9.51 (br, 1H), and 13.25 ppm (br, 1H).

*Anal.* Calcd. for $C_{17}H_{17}N_3O_3S$: C, 59.48; H, 4.96. Found: C, 59.38; H, 5.06.

4-Hydroxy-2-mercapto-6-methylpyrido[3,4-*d*]pyrimidine (**8**).

One gram (2.9 mmoles) of **7** was dissolved in 15 ml. of water containing 0.8 g. of sodium hydroxide. This yellow solution was refluxed for 10 minutes, then allowed to cool. Carbon dioxide was bubbled through the cold solution, and a white precipitate was formed. Filtration of this solid, and washing with water gave 0.52 g. (92%) of a white powder. Purification was achieved by dissolution in dilute aqueous sodium hydroxide, followed by precipitation with hydrochloric acid. The solid was filtered off, washed with water, and dried *in vacuo* to give a fine, white powder, m.p. greater than 300°; uv max (95% EtOH): 207 ($\epsilon$ 17,000), 227 ($\epsilon$ 15,300), and 296 nm ($\epsilon$ 5,600); ir (potassium bromide): 3100-2700, 1710, 1563, 1475, 1280, and 1159 $cm^{-1}$; nmr (deuteriotrifluoroacetic acid): $\delta$ 2.97 (s, 3H), 8.65 (s, 1H), and 9.01 ppm (s, 1H); ms: m/e (%) 193 (100), 135 (40), 134 (43), 107 (42), 105 (24).

*Anal.* Calcd. for $C_8H_7N_3OS$: C, 49.74; H, 3.63; N, 21.76. Found: C, 49.74; H, 3.63; N, 21.62.

2-Amino-3-benzoyl-6-methylpyrido[3,4-*d*]pyrimidin-4(3*H*)one (**10**).

A solution of **5a** (360 mg., 2 mmoles) in 10 ml. of dry DMF was stirred at 90-95°, after the addition of benzoylcyanamide (18) (146 mg., 1 mmole). An additional portion (146 mg.) of benzoylcyanamide was added after 5 hours, then the solution was heated and stirred for an additional 15 hours. The solution was concentrated under reduced pressure to a volume of 3-4 ml., and was poured into 5 ml. of 95% ethanol. The ethanolic solution was then poured into 35 ml. of ice water, and the resulting suspension was stirred overnight. The solid was filtered off, washed with water, then extracted with diethyl ether to give 196 mg. (35%) of a tan residual solid. The ether extracts of the solid were evaporated to give 66 mg. of unreacted **5a**, raising the overall yield of **10** to 43%. Recrystallization of the tan solid from 70% ethanol (Norit) gave white plates, m.p. 269-270.5° (12).

2-Amino-4-hydroxy-6-methylpyrido[3,4-*d*]pyrimidine (**9**).

A sample of **10** (105 mg.) was added to 5 ml. of 0.5*N* sodium hydroxide and the suspension was heated on a steam bath for 20 minutes. Complete dissolution required approximately 5 minutes. The yellow solution was filtered (Norit) and acidified to *p*H 5.5 with acetic acid. The light yellow solid was filtered off and purified by dissolution in dilute base, with subsequent precipitation by addition of acetic acid. Washing of the solid with water and diethyl ether, followed by drying *in vacuo* afforded 53 mg. (80%) of a light yellow solid, m.p. greater than 300°; uv max (0.1*N* sodium hydroxide): 234 ($\epsilon$ 14,200), 269 ($\epsilon$ 10,600), and 346 nm ($\epsilon$ 3,700); ir (potassium bromide): 3450, 3290, 3110, 1690, 1650, 1610, and 1465 $cm^{-1}$; nmr (deuteriotrifluoroacetic acid): $\delta$ 3.06 (s, 3H), 8.71 (s, 1H), and 9.33 ppm (s, 1H).

*Anal.* Calcd. for $C_8H_8N_4O$: C, 54.54; H, 4.58; N, 31.80. Found: C, 53.84; H, 4.64; N, 31.10.

Halogenation of **10**.

A suspension of **10** (706 mg., 2.52 mmoles), DBDMH (360 mg., 1.26 mmoles), and 85 mg. of benzoyl peroxide in 65 ml. of bromotrichloromethane was refluxed and irradiated by suspending the flask one centimeter above a 275 watt sunlamp. Dissolution of **10** was complete upon initiation of reflux. After 1.5 hours of reflux and irradiation, the solvent was removed under reduced pressure, and the tan residue was washed with diethyl ether. Extraction of the solid with hot toluene gave 226 mg. of an off-white powder recovered from the extracts, m.p. greater than 300°. The nmr spectrum (DMSO-$d_6$/deuteriochloroform) of this material indicated resonance values of 4.87 (s, 2H) and 4.95 ppm (s, 2H) for the 6-bromomethyl and 6-chloromethyl compounds respectively, with a ratio of integrations of 74:26. Both halomethyl compounds gave the same resonance values for H-5 (8.27 ppm) and H-8 (9.10 ppm), in addition to the normal aromatic splitting patterns associated with the 3-benzoyl functionality. Small amounts of **10** could be observed due to the presence of the 6-methyl absorption at 2.60 ppm; ms: m/e (%) 316 (4.5), 314 (13), 279 (5), 105 (100).

4-[(2-Amino-4-hydroxypyrido[3,4-*d*]pyrimidin-6-ylmethyl)amino]-benzoic Acid (**12**).

A mixture of **11**, X = Br, Cl (106 mg.) and ethyl *p*-aminobenzoate (530 mg.) was finely pulverized, and placed in a small erlenmeyer flask. Heating of the flask and contents at 100-105° for 20 hours afforded a dark brown melt. Repeated washings of the cooled melt with diethyl ether gave a dark brown residue which was dissolved in 50% ethanol and filtered (Norit). Evaporation of the solvent afforded 20 mg. of a brown powder, which was added to 1 ml. of 0.5*N* sodium hydroxide. The resulting suspension was heated on a steam bath for 20 minutes, filtered (Norit), and the filtrate was acidified with acetic acid. This procedure gave 7 mg. of a tan powder, which was purified by the following procedure. The solid was dissolved in 2 ml. of 0.1*N* sodium hydroxide, the solution was filtered (Norit), and the filtrate was acidified with acetic acid. The yellow-brown precipitate and solution were digested on a steam bath for 10 minutes, the suspension was then cooled, and the solid was filtered off. Repetition of this procedure gave the analytical sample (6 mg.), which was dried *in vacuo* for 2 hours at 100°. Tlc indicated only one zone, $R_f$ 0.60. This material had a melting point in excess of 300°; uv max (0.1*N* sodium hydroxide): 226 ($\epsilon$ 20,900), 277 ($\epsilon$ 25,200), and 343 nm ($\epsilon$ 5,700); ir (potassium bromide): 3310, 3150, 1685-1640, 1600, 1270, and 1180 $cm^{-1}$.

*Anal.* Calcd. for $C_{15}H_{13}N_5O_3$: C, 57.88; H, 4.21; N, 22.50. Found: C, 57.72; H, 4.21; N, 22.39.

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