Synthesis of 4-[(1,3-Diaminopyrrolo[3',4':4,5]pyrido[2,3-*d*]-pyrimidin-8-yl)benzoyl]-L-glutamic Acid as a Potential Antifolate

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This paper is dedicated to the memory of Professor Roland K. Robins

The synthesis of 4-[(1,3-diaminopyrrolo[3',4':4,5]pyrido[2,3-*d*]pyrimidin-8-yl)benzoyl]-L-glutamic acid (**18**), a potential antifolate and anticancer agent, has been achieved starting from 1,4-dibromobutan-2-ol with alkyl *p*-aminobenzoic acids. Condensation of these two agents gave 1-(4-alkoxycarbonylphenyl)pyrrolidin-3-ols **7a,b**, which were oxidized to the corresponding pyrrolidin-3-one derivatives **8a,b**. Compounds **8a,b** were converted into 1,3-diamino-8-(4-alkoxycarbonylphenyl)-7,8-dihydro-9*H*-pyrrolo[3',4':4,5]pyrido[2,3-*d*]pyrimidines **12a,b** in 4 steps. Saponification of **12b** the benzoate ester and coupling with di-*tert*-butyl glutamate afforded a mixture of 7,8-dihydro product **16** and its aromatized derivative **17**. Finally hydrolysis of esters **16** or **17** gave only the title compound **18**. The 7,8-dihydro tricyclic derivatives were easily air-oxidized to form their fully aromatized compounds. The title compound **18** was one tenth less active than MTX against HL-60 cells in culture.

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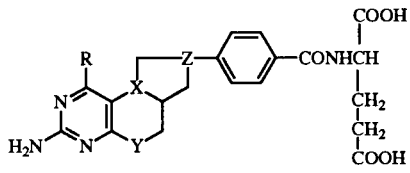
It is well known that mammals require folic acid (FA) as an essential growth factor. FA is first enzymatically reduced in mammalian cells to 7,8-dihydrofolic acid (FAH_2), which is further converted into 5,6,7,8-tetrahydrofolic acid (FAH_4). Once FA has been reduced to the cofactor form FAH_4, myriad enzymes then utilize various derivatives of FAH_4 in one-carbon transfer reactions involving the formation of thymidylic acid, synthesis *de novo* of purine nucleotides, interconversion among several amino acids, and initiation of peptide chain synthesis. For example, one of FAH_4 coenzyme, 5,10-methylenetetrahydrofolic acid **1**, donates the methyl group necessary for the conversion of 2'-deoxyuridylic acid (dUMP) to thymidylic acid (dTMP). Exploitation of this essential biochemical pathway for the rational design of specific thymidylate synthase (TS) inhibitor and hence as an antitumor agent is of interest.

The inhibitory action of analogues of aminopterin and methotrexate with modification at 5-, [1-6] 8-, [7-9] 10-, [10-11] 5,8-, [12-15] or 5,10- [16] position(s) (*i.e.*, deazaminopterin derivatives) on dihydrofolate reductase (DHFR) and TS is well-established. It could be expected that compounds bearing an uncleavable methylene bridge between 5- and 10-positions may be capable of interacting with TS but can not be involved in the one-carbon transfer reaction resulting in inhibition of this enzyme.

One of such compounds, 5,10-methylenetetrahydro-8,10-dideazaminopterin (**2**), has been synthesized by DeGraw *et al* [17]. However, compound **2** was found to be a rather poor inhibitor of TS derived from *L. casei* and DHFR derived from L1210 murine leukemia. It exhibited a weak inhibitory activity against L1210 growth *in vitro*.

In the past years we [4-5] have synthesized 5- and/or 7-substituted 5-deazaminopterin derivatives. We have also established a synthetic method toward the synthesis of 5,10-methanotetrahydro-5-deazaminopterin (**3**) derivatives [18] which bear a novel tricyclic ring, pyrrolo[3',4':4,5]-pyrido[2,3-*d*]pyrimidine (*i.e.*, the 5-deazaminopterin analogue with methylene bridge between C_5 and N^{10}). Unfortunately, the chemistry developed for the synthesis of this tricyclic compound was found not to be applicable to the synthesis of **3**. Recently, a modified method was developed in our laboratory that enabled us to prepare *N*-[(1,3-diaminopyrrolo[3',4':4,5]pyrido[2,3-*d*]pyrimidin-8-yl)benzoyl]-L-glutamic acid (**18**) which would allow us to prepare **3** by catalytic hydrogenation. The synthesis of **18** is described herein.

In our previous publication [18] we described a synthetic method to prepare a novel tricyclic ring, pyrrolo[3',4':4,5]pyrido[2,3-*d*]pyrimidine. The tricyclic compounds were prepared either by adding a pyrrole ring to pyrido[2,3-*d*]-pyrimidine derivative or were constructed by starting with a pyrrolidin-3-one derivative *via* a multistep approach. One of the key intermediate in the latter case, 1-(4-methoxyphenyl)pyrrolidin-3-one, was prepared by starting from



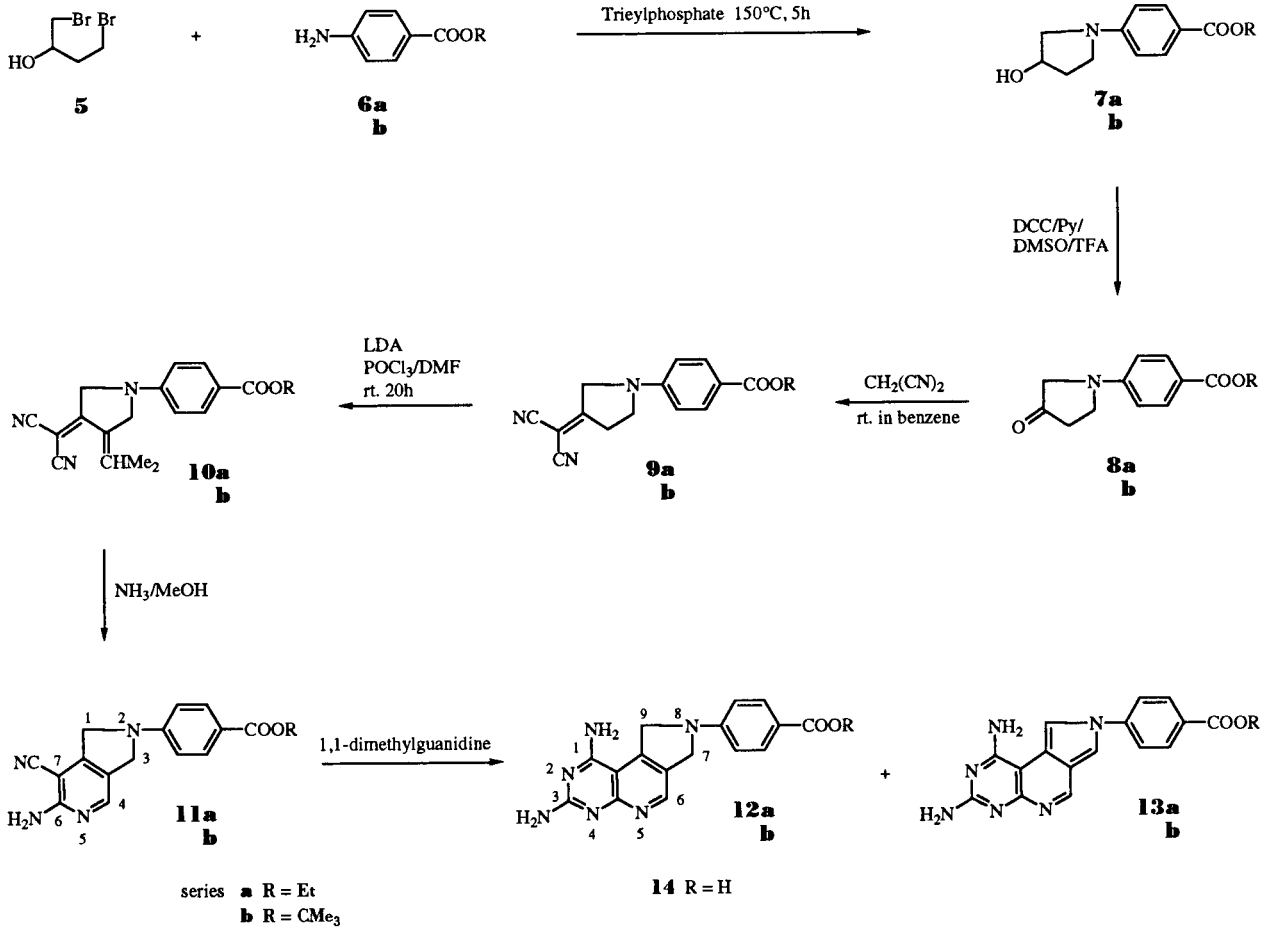
1 R = OH, X = Z = N, Y = NH
2 R = NH_2, X = N, Y = CH_2, Z = CH
3 R = NH_2, X = CH, Y = NH, Z = CH
4 R = OH, X = CH, Y = NH, Z = N

p-anisidine followed by *N,N*-dialkylation with ethyl acrylate and ethyl bromoacetate. The *N,N*-disubstituted *p*-anisidine was converted into the pyrrolidin-3-one *via* intramolecular Aldol condensation and decarboxylation [18]. This methodology, however, is not applicable to prepare 5,10-methano-5-deazaminopterin, since 1-[4-(alkoxycarbonyl)phenyl]pyrrolidin-3-one **8a** or **8b** is required for this synthesis. The very starting material for the synthesis of pyrrolidin-3-ones **8a,b** has to be alkyl *p*-aminobenzoate **6a,b**. *N,N*-Dialkylation of **6a,b** by following the same procedure as described above would not be possible because these compounds contain a less reactive amino function (aniline with an electron-withdrawing function, COOR, at the *para*-position) in comparison with that of *p*-anisidine (aniline with an electron-donating group, OMe, at *para*). The chemistry for the synthesis of 1-[4-(alkoxycarbonyl)-phenyl]-3-pyrrolidin-3-one should, therefore, be used to facilitate the synthesis of the target compounds.

While we were preparing this manuscript, Gangjee *et al.* [19] reported the synthesis of 5,10-methanotetrahydro-5-deazafolic acid (**4**) which was prepared by a Fisher-indole type cyclization of diethyl *N*-[4-[3-[[(2-amino-4-oxopyrimidine-6-yl)hydrazone]methyl]pyrrol-1-yl]benzoyl]-L-glutamate from 2-amino-6-hydrazino-4-oxopyrimidine and diethyl *N*-[4-(3-formyl-1-pyrrolyl)benzoyl]-L-glutamate, followed by the catalytic reduction of the cyclized product and saponification. However, this method is also not suitable for preparing our target molecule, since amination of the 4-hydroxy of 5,10-methanotetrahydro-5-deazafolic acid **4** to form the corresponding 2,4-diamino derivative **3** would be difficult.

The route used for the synthesis of the targeted compound **15** is shown in Scheme 1. The key intermediates, 1-

Scheme 1

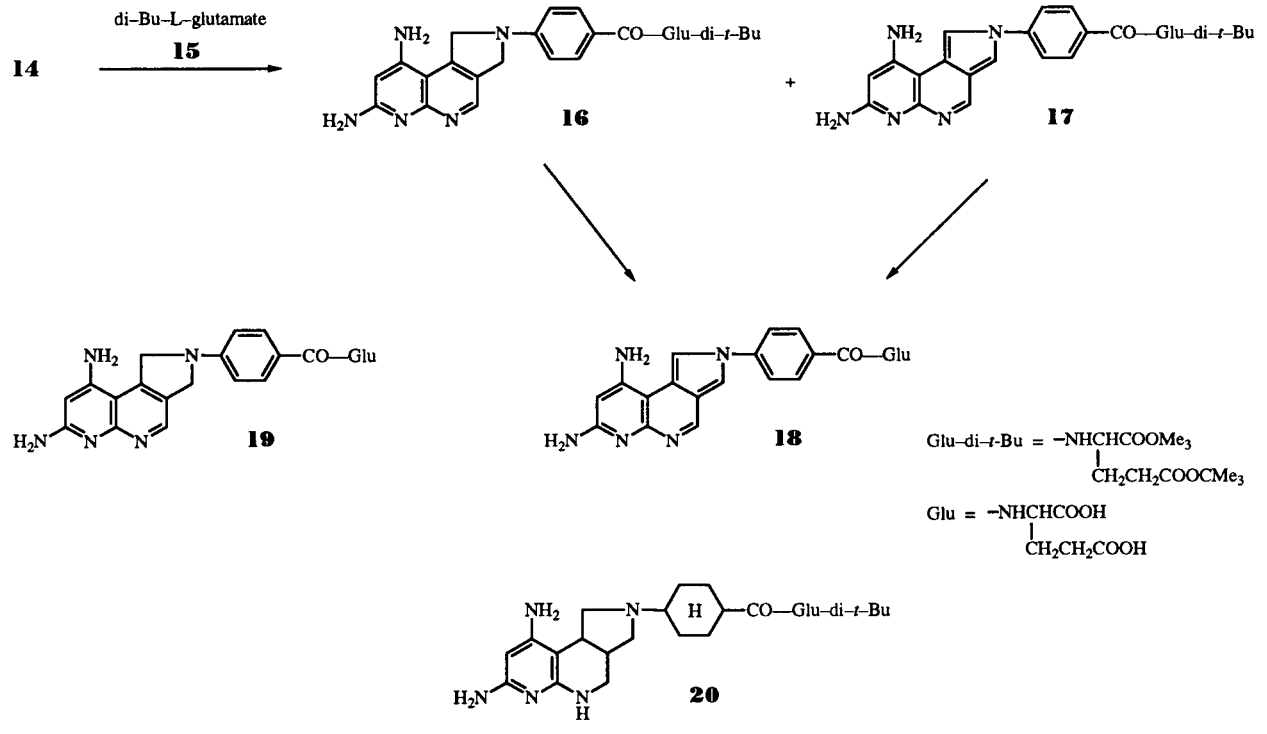


(4-alkyloxyphenyl)pyrrolidin-3-ones **8a,b**, were prepared by the following procedure: Alkyl *p*-aminobenzoate **6a** or **6b** was treated with 1,4-dibromobutan-2-ol (**5**) in triethyl phosphate in the presence of potassium carbonate under reflux for 10 hours to give 1-(alkyloxyphenyl)pyrrolidin-3-ols **7a,b** in *ca.* 50% yield. The pyrrolidin-3-ols **7a,b** were then oxidized by treatment with DCC/DMSO/pyridine/-TFA to form pyrrolidin-3-ones **8a,b** in good yield. The synthesis of compound **8b** was originally reported by Taylor *et al.* [20-21] who condensed pyrrolidin-3-ol with *t*-butyl 4-fluorobenzoate, followed by oxidation of the product, 1-[4-(*t*-butoxycarbonyl)phenyl]pyrrolidin-3-ol [20-22]. Our methodology for the synthesis of **8b** is superior to the reported one, since 1,4-dibromobutan-2-ol is inexpensive in comparison with pyrrolidin-3-ol. Besides, under the facile reaction conditions, various *N*-substituted pyrrolidin-3-ols could be prepared from anilines with electron-withdrawing or electron-donating substituent at the *para*-position.

The pyrrolidin-3-ones **8a,b** were treated with malononitrile under nitrogen at 15° for 1.5 hours to afford 1-[4-(ethoxy or *t*-butoxycarbonyl)phenyl]-3-(dicyanomethylene)-pyrrolidine **9a,b** in moderate yield. The reaction at low temperature is necessary to prevent polymerization of the product. Compounds **9a,b** were lithiated with lithium diisopropylamide mono(tetrahydrofuran) in dry THF in a dry ice/acetone bath followed by treatment with (dimethylamino)methylene chloride to give **10a,b**. Treatment of **10a,b** with methanolic ammonia in a sealed container at 120° for 5 hours gave 6-amino-7-cyano-2,3-dihydro-1*H*-pyrrolo[3′,4′:4,5]pyrimidines **11a,b** in good yield. Compounds **11a,b** were then reacted with *N,N*-dimethylguanidine sulfate in *N,N*-dimethylformamide in the presence of potassium *t*-butoxide at 100° for 20 hours. After removal of the solvent, the residue was triturated with ethanol and the solid residue was collected by filtration to yield a mixture of 1,3-diamino-N^8-[4-(ethoxycarbonyl) or 4-(*t*-butoxycarbonyl)phenyl]-7,8-dihydro-9*H*-pyrrolo[3′,4′:4,5]pyrido-[2,3-*d*]pyrimidines **12a,b** and aromatized compounds **13a,b** in a ratio of *ca.* 8:1 as estimated by the nmr analyses. The nmr (trifluoroacetic acid) spectrum of the mixture revealed the presence of two methylene signals at δ 4.49 and 4.93 (broad singlets), four protons as an AB quartet for the benzene ring at δ 6.32 and 7.52, H-8 as a singlet at δ 8.38 for **12b**. On the other hand, the four benzene protons of compound **13b** shifted to the higher field appearing at δ 7.34 and 7.90 (AB quartet) for the benzene ring. The three singlets appeared at δ 8.36, 8.68 and 9.08 were assigned for H-5, H-7, and H-8. The mixture was inseparable by chromatography. However, the pure **12b** was obtained in 65-75% yield when the mixture was washed successively with *N,N*-dimethylformamide, ethanol, and acetone.

Saponification of the ethyl ester of **12a** to form the corresponding 4-amino-5,10-methano-5-deazapteroic acid (**14**) under basic conditions was rather difficult. However, the acid liable *tert*-butyl ester **13b** could easily be converted

Scheme 2



into **14** by treatment with trifluoroacetic acid in methylene chloride at -10°, with hydrogen chloride in nitromethane or with 96% formic acid at room temperature.

The extraordinary insolubility of **14** was extremely difficult to couple with di-*t*-butyl L-glutamate (**15**). We found that the condensation of **14** and **15** proceeded better when the fine powdered compound **14** was suspended in a mixture of dry dimethyl sulfoxide-dimethylformamide (1:1) in an ultrasonic bath for 1 hour, followed by treatment with **15**. By modifing the known procedure [23], **14** and **15** were condensed to give a mixture of **16** and its aromatized derivative **17** (Scheme 2). The mixture was separated by several chromatographies. However, we were unable to isolate the pure **16** which was always contaminated with traces of **17**. The behavior of compound **17** is similar to that of **12** and aromatized easily due to the air oxidation. Removal of the *t*-butyl groups in **17** was achieved by treatment with hydrogen chloride in nitromethane to yield the targeted compound **18**. It should be noted here that saponification of the mixture of **16** containing traces of **17** afforded only the aromatized product **18** instead of the desired compound **19**.

Attempts to prepare 5,10-methanotetrahydro-5-deazaminopterin (**3**) from **16** or **18** under various catalytic hydrogenation conditions (using platimic oxide as the catalyst in 1*N* hydrochloric acid, acetic acid or trifluoroacetic acid) were unsuccessful. In most cases, the reduction of these compounds led to decomposition or formation of a mixture of unidentified products. Occasionally, we could isolate the fully aromatized compound from the reaction mixture. When **16** was reduced using 1 equivalent of platinic oxide in 1*N* hydrochloric acid (*p*H of the reaction mixture was 3-4) at 50 psi hydrogen pressure for 18 hours, no reduction occurred by monitoring with thin layer chromatography (chloroform-methanol, 4:1). The hydrogenation of **16** was continued for additional 20 hours after additional platinic oxide (1 equivalent) was charged. We found that the main product isolated from the reaction mixture by column chromatography was **20** in which both pyrrolopyrido- and benzene-rings were reduced. Purification of compound **20** was rather difficult and contaminated with some impurities. We did not obtain a clean nmr spectrum of **20**. However, the mass spectrum of this reduced compound revealed a main mass peak (m/z) at (574 M + H [$C_{21}H_{31}N_7O_5$]). Further investigation of **20** was not undertaken since this compound was not our target.

The inhibitory activity against the growth of human leukemic HL-60 cells *in vitro* by compound **18** was compared with that of MTX. Compound **18** was about three times less active than MTX with IC_{50} value of 0.039 μM. The IC_{50} value for MTX was 0.012 μM.

EXPERIMENTAL

General Methods.

Melting points are uncorrected and were determined on a Thomas-Hoover capillary apparatus. Column chromatography was performed on silica gel G60 (70-230 mesh; ASTM, Merk). Elemental analyses were performed by M.H.W. Laboratories, Phoenix, AZ. The pmr spectra were recorded on a JEOL PFT-100 or JEOL FX90Q spectrometer with tetramethylsilane as the internal standard. Chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (multiplet). The ir spectra were recorded on a Perkin-Elmer Infracord Model 137B spectrometer and the uv spectra on Gilford RESPONSE UV-vis spectrophotometer.

1-(4-Ethoxycarbonylphenyl)pyrrolidin-3-ol (**7a**).

To a mixture of ethyl *p*-aminobenzoate (**6a**, 16.5 g, 0.10 mole) and anhydrous potassium carbonate (40.0 g, 0.31 mole) in triethyl phosphate (70 ml) was added dropwise 1,4-dibromobutan-2-ol (**5**, 50.0 g, 0.21 mole) with vigorous stirring under nitrogen at room temperature during 30 minutes. The mixture was heated at 150° (oil bath temperature) for 10 hours. After cooling, the mixture was diluted with water (500 ml) and extracted with ether (300 ml x 3). The combined organic extracts were washed with water (200 ml x 2), dried over sodium sulfate, and concentrated *in vacuo* to dryness. The residue was chromatographed on a silica gel column (5 x 40 cm) using chloroform-methanol (100:3 v/v) as the eluent. The major uv-absorbing fraction was concentrated to afford **7a**, 12.2 g (52%), mp 97-98°; ^{1}H nmr (deuteriochloroform): δ 1.36 (t, 3H, $-CH_2CH_3$, J = 7.41 Hz), 2.01-2.22 (m, 2H, 4-CH_2), 2.37 (s, 1H, exchangeable, OH), 3.34-3.62 (m, 2H, 5-CH_2), 3.47 (d, 2H, 2-CH_2, J = 4.66 Hz), 4.30 (q, 2H, $-CH_2CH_3$, J = 7.41 Hz), 4.52-4.66 (m, 1H, 3-CH), 6.46 and 7.87 (2d, each, 2H, ArH, J = 9.01 Hz).

Anal. Calcd. for $C_{13}H_{17}NO_3$: C, 66.38; H, 7.23; N, 5.96. Found: C, 66.21; H, 7.21; N, 5.91.

By following the same procedure, compound **7b**, 1-(4-*t*-butoxycarbonylphenyl)pyrrolidin-3-ol, was prepared from *t*-butyl *p*-aminobenzoate (**6b**, 34.2 g, 0.18 mole) and 1,4-dibromobutan-3-ol (**5**, 50.0 g, 0.21 mole), 23.3 g (50%), mp 164-165°; ^{1}H nmr (deuteriochloroform): δ 1.57 (s, 9H, 3 x CH_3), 2.02-2.24 (m, 2H, 4-CH_2), 2.41 (s, 1H, exchangeable, OH), 3.34-3.63 (m, 2H, 5-CH_2), 3.48 (d, 2H, 2-CH_2, J = 4.39 Hz), 4.59-4.69 (m, 1H, 3-CH), 6.47 and 7.84 (2d, each, 2H, ArH, J = 9.01 Hz).

Anal. Calcd. for $C_{15}H_{21}NO_3$: C, 68.44; H, 7.98; N, 5.32. Found: C, 68.35; H, 7.82; N, 5.34.

1-(4-Ethoxycarbonylphenyl)pyrrolidin-3-one (**8a**).

To a mixture of pyrrolidin-3-ol (**7a**, 17.6 g, 75 mmoles), DCC (46.3 g, 0.225 mole), pyridine (8.35 g, 106 mmoles), dimethyl sulfoxide (214 ml) in dry benzene (400 ml) was added dropwise trifluoroacetic acid (6.32 g, 55.4 mmoles) under nitrogen at 5°. After stirring at room temperature for 20 hours, the mixture was diluted with ethyl acetate (1.2 *l*). The mixture was filtered, and the filtrate was washed with water (300 ml x 3), dried over sodium sulfate, and evaporated *in vacuo* to dryness. The residue was crystallized from hexane to give **8a**, 14.5 g (83%). A small amount of **8a** was purified by column chromatography on silica gel (chloroform) for analysis, mp 134-135°; ^{1}H nmr (deuteriochloroform): δ 1.38 (t, 3H, $-CH_2CH_3$, J = 7.2 Hz), 2.77 (t, 2H, 4-CH_2, J = 7.8 Hz), 3.78 (s, 2H, 2-CH_2), 3.79 (t, 2H, 5-CH_2, J = 7.8 Hz), 4.34 (q,

2H, $-CH_2CH_3$, J = 7.2 Hz), 6.62 and 7.89 (2d, each, 2H, ArH, J = 9.0 Hz).

Anal. Calcd. for $C_{13}H_{15}NO_3$: C, 66.95; H, 6.44; N, 6.01. Found: C, 67.01; H, 6.42; N, 6.01.

In a similar manner, compound **8b**, 1-(4-*t*-butoxycarbonylphenyl)pyrrolidin-3-one (**8b**) was prepared from pyrrolidin-3-ol **7b** (17.1 g, 65 mmoles), 16.1 g (95%), mp 173-174°; ^{1}H nmr (deuteriochloroform): δ 1.58 (s, 9H, 3 x CH_3), 2.76 (t, 2H, 4-CH_2, J = 8.0 Hz), 3.76 (s, 2H, 2-CH_2), 3.77 (t, 2H, 5-CH_2, J = 8.0 Hz), 6.59 and 7.92 (2d, each, 2H, ArH, J = 9.0 Hz).

Anal. Calcd. for $C_{15}H_{19}NO_3$: C, 68.97; H, 7.28; N, 5.36. Found: C, 68.70; H, 7.41; N, 5.69.

3-Dicyanomethylene-1-(4-ethoxycarbonylphenyl)pyrrolidine (**9a**).

A mixture of pyrrolidin-3-one **8a** (3.50 g, 15 mmoles) and malononitrile (9.08 g, 137 mmoles) in dry benzene (250 ml) was stirred under nitrogen at 15°. The reaction was monitored by thin layer chromatography (toluene/ethyl acetate, 4:1 v/v). After the starting material **8a** was consumed (*ca.* 1.5 hours), the reaction mixture was poured onto a silica gel column (5 x 30 cm). The main fraction was eluted with benzene/ethyl acetate (98:2 v/v) to afford **9a**, 1.53 g (39%), mp 157-158° (ethanol); ir (potassium bromide): ν CN 2215 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.38 (t, 3H, $-CH_2CH_3$, J = 7.2 Hz), 3.30 (t, 2H, 4-CH_2, J = 6.6 Hz), 3.75 (t, 2H, 5-CH_2, J = 6.6 Hz), 4.33 (q, 2H, $-CH_2CH_3$, J = 7.2 Hz), 4.45 (s, 2H, 2-CH_2), 6.61 and 7.97 (2d, each, 2H, ArH, J = 9.1 Hz).

Anal. Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.33; H, 5.34; N, 14.95. Found: C, 68.21; H, 5.37; N, 14.75.

By following the same procedure, compound **9b**, 1-(4-*t*-butoxycarbonylphenyl)-3-dicyanomethylenepyrrolidine, was prepared from pyrrolidin-3-one **8b** (3.30 g, 12.6 mmoles), 2.32 g (60%), mp 163-165° (ethanol); ir (potassium bromide): ν CN 2210 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.58 (s, 9H, 3 x CH_3), 3.31 (t, 2H, 4-CH_2, J = 6.3 Hz), 3.74 (t, 2H, 5-CH_2, J = 6.3 Hz), 4.47 (s, 2H, 2-CH_2), 6.61 and 7.92 (2d, each, 2H, ArH, J = 9.1 Hz).

Anal. Calcd. for $C_{18}H_{19}N_3O_2$: C, 69.90; H, 6.15; N, 13.59. Found: C, 69.98; H, 6.36; N, 13.60.

3-Dicyanomethylene-2-(*N*,*N*-dimethylamino)methylene-1-(4-ethoxycarbonylphenyl)pyrrolidine (**10a**).

Lithium diisopropylamide mono(tetrahydrofuran) (10 ml, 15 mmoles, 1.5 moles in cyclohexane) was added dropwise to a suspension of compound **9a** (2.82 g, 10.0 mmoles) in THF (180 ml, freshly distillated over calcium hydride) at -78°. After the mixture was stirred for 1 hour, (dimethylamino)methylene chloride (freshly prepared from 1.55 ml of DMF, 1.86 ml of phosphorus oxychloride in 20 ml of dry tetrahydrofuran) was added, and the stirring was continued overnight at -65°. The mixture was allowed to warm to room temperature, and the solid product was collected by filtration. The product **10a** was purified by chromatography on silica gel (chloroform-methanol, 500:1 v/v) to yield 1.43 g (43%), mp 195-197°; ir (potassium bromide): ν CN 2210 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.37 (t, 3H, $-CH_2CH_3$, J = 7.2 Hz), 3.32 (s, 6H, $N(CH_3)_2$), 4.32 (q, 2H, $-CH_2CH_3$, J = 7.2 Hz), 4.40 (s, 2H, 5-CH_2), 4.55 (s, 2H, 2-CH_2), 6.51 and 7.93 (2d, each, 2H, ArH, J = 9.0 Hz), 8.31 (s, 1H, s, =CH-).

Anal. Calcd. for $C_{19}H_{20}N_4O_2$: C, 67.86; H, 5.95; N, 16.67. Found: C, 67.96; H, 6.09; N, 16.71.

In a similar manner, compound **10b** was synthesized, 1-(*t*-butoxycarbonylphenyl-3-dicyanomethylene-2-(*N*,*N*-dimethylaminomethylene)pyrrolidine (**10b**) was prepared from **9b** (4.62 g, 15 mmoles), 3.55 g (65%), mp 163-166°; ir (potassium bromide): ν CN 2210 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.58 (s, 9H, 3 x CH_3), 3.32 (s, 6H, $N(CH_3)_2$), 4.43 (s, 2H, 5-CH_2), 4.57 (s, 2H, 2-CH_2), 6.52 and 7.90 (2d, each, 2H, ArH, J = 9.0 Hz), 8.31 (s, 1H, =CH-).

Anal. Calcd. for $C_{21}H_{24}N_4O_2$: C, 69.23; H, 6.59; N, 15.38. Found: C, 68.96; H, 6.49; N, 15.28.

6-Amino-7-cyano-2-(ethoxycarbonylphenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (**11a**).

A mixture of compound **10a** (0.89 g, 2.65 mmoles) in saturated methanolic ammonia (30 ml) was heated in a sealed steel vessel at 120° for 5 hours. After cooling, the white needles were collected by filtration, washed with methanol and recrystallized from methanol to give **11a**, 0.78 g (96%), mp 263-264°; ^{1}H nmr (DMSO-d_6): δ 1.29 (t, 3H, $-CH_2CH_3$, J = 7.0 Hz), 4.23 (q, 2H, $-CH_2CH_3$, J = 7.0 Hz), 4.57 and 4.72 (2 brs, each, 2H, 1-CH_2 and 3-CH_2), 6.70 and 7.83 (2d, each, 2H, ArH, J = 9.05 Hz), 6.96 (brs, 2H, exchangeable, NH_2), 8.24 (s, 1H, 4-H).

Anal. Calcd. for $C_{17}H_{16}N_4O_2$: C, 66.23; H, 5.20; N, 18.18. Found: C, 65.96; H, 5.37; N, 17.92.

By following the same procedure, 6-amino-7-cyano-2-(*t*-butoxycarbonylphenyl)-2,3-dihydro-1*H*-pyrrolo[3,4-*c*]pyridine (**11b**) was prepared from **10b** (2.18 g, 6.0 mmoles), 1.98 g (98%), mp 274-276°; ^{1}H nmr (DMSO-d_6): δ 1.52 (s, 9H, 3 x CH_3), 4.57 and 4.71 (2 brs, each, 2H, 1-CH_2 and 3-CH_2), 6.69 and 7.78 (2d, each, 2H, ArH, J = 8.87 Hz), 6.95 (brs, 2H, exchangeable, NH_2), 8.24 (s, 1H, 4-H).

Anal. Calcd. for $C_{19}H_{20}N_4O_2$: C, 67.84; H, 5.99; N, 16.66. Found: C, 67.61; H, 6.06; N, 16.90.

1,3-Diamino-8-(ethoxycarbonylphenyl)-7,8-dihydro-9*H*-pyrrolo-[3',4':4,5]pyrido[2,3-*d*]pyrimidine (**12a**).

1,1-Dimethylguanidine sulfate (2.6 g, 9.5 mmoles) was added to a suspension of potassium *t*-butoxide (2.2 g, 19 mmoles) in dry DMF (8 ml). After stirring at room temperature for 1 hour, **11a** (1.6 g, 4.76 mmoles) was added to the mixture and then heated at 100° (bath temperature) under nitrogen for 18 hours. The crude solid contained two major products, **12a** and **13a**, in a 9:1 ratio by thin-layer chromatography (chloroform-methanol, 4:1 v/v) and nmr analyses. The mixture was not separated, but the filter cake was successively washed with DMF, methanol, and acetone to remove the minor product **13a**. The solid which contained only **12a** was dried *in vacuo*, 250 mg (71%), mp >350°; ^{1}H nmr (trifluoroacetic acid): δ 0.96 (t, 3H, $-CH_2CH_3$, J = 7.4 Hz), 3.97 (q, 2H, $-CH_2CH_3$, J = 7.4 Hz), 4.58 and 5.03 (2 brs, each, 2H, 7-CH_2 and 9-CH_2), 6.45 and 7.61 (2d, each, 2H, ArH, J = 9.05 Hz), 8.46 (s, 1H, 6-H).

Anal. Calcd. for $C_{18}H_{18}N_6O_2$: C, 61.70; H, 5.18; N, 23.99. Found: C, 61.52; H, 5.37; N, 23.71.

In a similar manner, compound **12b**, 1,3-diamino-8-(*t*-butoxycarbonylphenyl-7,8-dihydro-9*H*-pyrrolo[3',4':4,5]pyrido[2,3-*d*]-pyrimidine, was prepared from **11b** (3.36 g, 10 mmoles). After the reaction as described above, the mixture was cooled to room temperature and the yellow solid was collected by filtration. The crude solid contained two major spots with Rf value of 0.41 (**13b**, minor) and 0.38 (**12b**, major with long tail) by monitoring with thin-layer chromatography (chloroform-methanol, 4:1 v/v). The ratio of the two products, **12b**:**13b** = 8:1, was observed in nmr

spectrum which revealed that a pair of signals for the minor product (**13b**, Rf = 0.41) appeared at δ 1.10 (s, 9H, 3 x CH_3), 7.34 and 7.90 (2s, each, 2H, ArH), 8.36, 8.68, and 9.08 (3s, each, 1H, 5-, 7-, and 8-H). The filter cake was washed successively with DMF, methanol and acetone, and dried. Compound **12b** was obtained as a light brown powder, 3.03 g (81%), mp > 350°; ^{1}H nmr (trifluoroacetic acid): δ 1.11 (s, 9H, 3 x CH_3), 4.49 and 4.93 (2 brs, each, 2H, 7-CH_2 and 9-CH_2), 6.32 and 7.54 (2d, each, 2H, ArH, J = 8.87 Hz), 8.39 (s, 1H, 6-H).

Anal. Calcd. for $C_{19}H_{20}N_4O_2$: C, 67.86; H, 5.95; N, 16.67. Found: C, 67.61; H, 6.06; N, 16.90.

1,3-Diamino-8-(hydroxycarbonylphenyl)-7,8-dihydro-9*H*-pyrrolo-[3',4':4,5]pyrido[2,3-*d*]pyrimidine (**14**).

A mixture of compound **12b** (1.89 g, 5.0 mmoles) and trifluoroacetic acid (20 ml) in methylene chloride (150 ml) was strirred at -10° for 1.5 hours. The solvent was removed *in vacuo* at < 20° to dryness. The residue was triturated with chloroform (30 ml) and the solid precipitates were collected by filtration. The solid was suspended in 10% aqueous solution of sodium bicarbonate and neutralized with 1*N* hydrochloric acid. The yellow precipitates were collected by filtration, washed successively with acetone and ether, dried *in vacuo* to give **14**, 1.58 g (98%), mp > 350°; ^{1}H nmr (DMSO-d_6): δ 4.79 and 5.17 (2 brs, each, 2H, 7- and 9-CH_2), 6.89 and 7.86 (2d, each, 2H, ArH, J = 8.9 Hz), 8.81 (s, 1H, 6-H).

Anal. Calcd. for $C_{16}H_{14}N_6O_2 \cdot 2HCOOH \cdot H_2O$: C, 50.00; H, 4.63; N, 19.44. Found: C, 49.95; H, 4.43; N, 19.65.

Compound **14** was also prepared in 85-95% from **12b** either by treatment with hydrogen chloride in nitromethane at room temperature for 4 hours, with trifluoroacetic acid (5 ml) in methylene-chloride (20 ml) at -10° for 1.5 hours, or with 96% formic acid at room temperature for 20 hours.

Di-*t*-butyl *N*-[4-(1,3-Diamino-7,8-dihydro-9*H*-pyrrolo[3',4':4,5]-pyrido[2,3-*d*]pyrimidin-8-yl)benzoyl]-L-glutamate (**16**).

A dried and fine powdered **14** (97 mg, 0.30 mmole) and 4-methylenemorpholine (30 mg, 0.3 mmole) in dry dimethyl sulfoxide/dimethylformamide (1:1, 2 ml) was ultrasonicated for 1 hour. Isobutyl chloroformate (0.05 ml, 0.3 mmole) was then added into the mixture with stirring at -20°. The mixture was then allowed to stir at room temperature for 0.5 hour and a suspension of di-*t*-butyl L-glutamate hydrochloride (**15**, 89 mg, 0.3 mmole) in dry DMF (1.5 ml) containing 4-methylmorpholine (30 mg, 0.3 mmole) was added. Additional **15** (89 mg) and 4-methylmorpholine (30 mg) were added into the mixture after the mixture was stirred for another 1 hour. The mixture was then stirred for 5 days at room temperature. The reaction was monitored by thin-layer chromatography (chloroform-methanol, 1:1 v/v) which showed that two major products with Rf values of 0.40 and 0.37 were formed. The solvent was removed *in vacuo* to dryness and the residue was dissolved in methanol (20 ml) containing silica gel (5 g), evaporated to dryness, and poured on a silica gel column (2 x 30 cm) using chloroform-methanol (9:1 v/v) as the eluent. Compound **17** was eluted first from the column followed by compound **16**. Compound **16** was obtained in 18% yield (31 mg), mp > 320°; ^{1}H nmr (DMSO-d_6): δ 1.39 and 1.42 (2s, each, 9H, 2 x CMe_3), 1.93-2.03 (m, 2H, CHCH_2), 2.32-2.36 (m, 2H, CH_2COO), 4.35 (m, 1H, C*H*NH), 4.71 and 5.09 (2 brs, each, 2H, 7- and 9-CH_2), 6.86 and 7.85 (2d, each, 2H, ArH, J = 9.0 Hz), 8.68 (s, 1H, 6-H).

Anal. Calcd. for $C_{29}H_{37}N_7O_5$: C, 61.79; H, 6.62; N, 17.40. Found: C, 61.85; H, 6.42; N, 17.21.

Compound **17** was obtained in 28% yield (47 mg), mp > 320°; ^{1}H nmr (DMSO-d_6): δ 1.40 and 1.43 (2s, each, 9H, 2 x CMe_3), 2.05 (m, 2H, CHCH_2), 2.39 (m, 2H, CH_2COO), 4.36 (m, 1H, C*H*NH), 7.89 and 8.16 (2d, each, 2H, ArH, J = 8.8 Hz), 8.08 and 8.44 (2s, each, 1H, 7- and 9-H), 9.18 (s, 1H, 6-H).

Anal. Calcd. for $C_{29}H_{35}N_7O_5$: C, 62.02; H, 6.28; N, 17.46. Found: C, 61.85; H, 6.12; N, 17.15.

N-[4-(1,3-Diamino-pyrrolo[3',4':4,5]pyrido[2,3-*d*]pyrimidin-8-yl)-benzoyl]-L-glutamic Acid (**18**).

Into a suspension of **17** (40 mg, 0.07 mmole) in nitromethane (5 ml) was bubbled dry hydrogen chloride for 0.5 minute in an ice-bath. The mixture was stirred at room temperature for 1 hour and then evaporated to dryness *in vacuo*. The residue was triturated with ether (5 ml x 3) and dried *in vacuo* to give **18**, 18 mg (56%), mp 230-233°; ^{1}H nmr (DMSO-d_6): δ 1.97-2.17 (m, 2H, CHCH_2), 2.38-2.40 (m, 2H, CH_2COO), 4.45 (m, 1H, C*H*NH), 8.06 and 8.15 (2d, each, 2H, ArH, J = 8.6 Hz), 8.71 and 8.75 (2s, each, 1H, 7- and 9-H), 9.36 (s, 1H, 6-H); ms: m/z 449 (molecular ion).

Anal. Calcd. for $C_{21}H_{19}N_7O_5 \cdot H_2O$: C, 53.96; H, 4.53; N, 20.98. Found: C, 54.12; H, 4.39; N, 20.78.

Biological Studies on 5,10-Methano-5-deazaminopterin (**18**).

The cell growth inhibition assay was determined by the XTT assay [24]. The HL-60 cells (1.5 x 10^5/ml) were grown in RPMI 1640 medium containing 10% fetal calf serum, 100 μg/ml streptomycin, and 100 units/ml penicillin, in humidified 5% carbon dioxide at 37°. Five concentrations of compound **18** and MTX were added for up to 72 hour exposure for IC_{50} determination.

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