THE SYNTHESIS OF *N*-{2-AMINO-4-SUBSTITUTED [(PYRROLO[2,3-*d*]-PYRIMIDIN-5-YL)ETHYL]BENZOYL}-L-GLUTAMIC ACIDS AS ANTINEOPLASTIC AGENTS[^]

Chuan Shih* and L. S. Gossett

Cancer Research Division, Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, Indiana 46285, U.S.A.

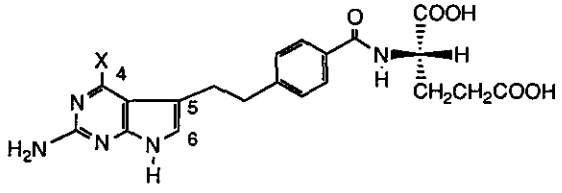
Abstract - A series of *N*-{2-amino-4-substituted[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acids were synthesized. In this current synthesis, compound 2-amino-4-chloro-pyrrolo[2,3-*d*]pyrimidine (**4**) was selected as an important precursor for the preparation of key intermediates such as **5b**, **10b**, **15a** and **15b**. These highly functionalized pyrrolo[2,3-*d*]pyrimidines were then later coupled with either 4-ethynylbenzoylglutamate or 4-iodobenzoylglutamate in a palladium catalyzed Heck reaction and thus provided the basic skeleton of the targeted molecules. The availability of the chlorine atom at the 4-position of the pyrrolopyrimidine nucleus has allowed us to introduce different substituents at this position efficiently. By this approach, we were able to prepare a variety of 4-substituted pyrrolo[2,3-*d*]pyrimidine based folate antagonists (**2a-2g**) which are closely related to the novel thymidylate synthase inhibitor LY231514. In vitro analysis has demonstrated that some of these agents are highly cytotoxic against human leukemic cells (CCRF-CEM) in culture.

Pyrrolo[2,3-*d*]pyrimidine based folate antagonists represent a new class of folate antimetabolites which do not possess the conventional bicyclic 6-6 fused ring system such as pteridine, quinazoline[1] or pyrido[2,3-d]pyrimidine.[2] Several exciting new compounds in this structure class have recently been identified and are currently being developed as novel anticancer agents. This includes the potent thymidylate synthase (TS) inhibitor LY231514[3] (**1**) discovered by the Lilly/Princeton group and

[^]*Dedicated to Professor Edward C. Taylor on the occasion of his 70th birthday*.

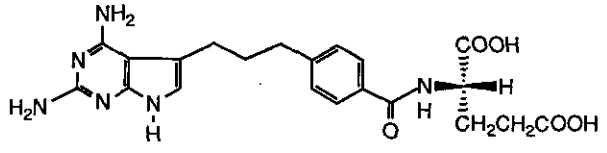
the potent DHFR inhibitor TNP-351[4] which was reported recently by researchers from Takeda.

LY231514 is a novel and non-quinazoline based thymidylate synthase inhibitor. This agent is a superb substrate for the enzyme folylypolyglutamate synthetase and the resulting polyglutamates are potent and tight-binding inhibitors for the enzyme TS. LY231514 has demonstrated excellent antitumor efficacy against a thymidine kinase deficient tumor (L5178Y/TK$^-$/HX$^-$) as well as other human tumor xenografts (VRC5, GC3) in vivo[5] and is currently under phase I clinical evaluation. As part of ongoing structure-activity relationship studies on LY231514, we are interested in examining the effects of various groups at the 4 position of the pyrrolo[2,3-*d*]pyrimidine nucleus. Herein, we report our synthetic efforts in preparing various 4-substituted analogs of LY231514.



1 X=OH (**LY231514**)

2a X=Cl
2b X=SH
2c X=H
2d X=H (5,6-dihydro)
2e X=OMe
2f X=NEt$_2$
2g X=NH$_2$

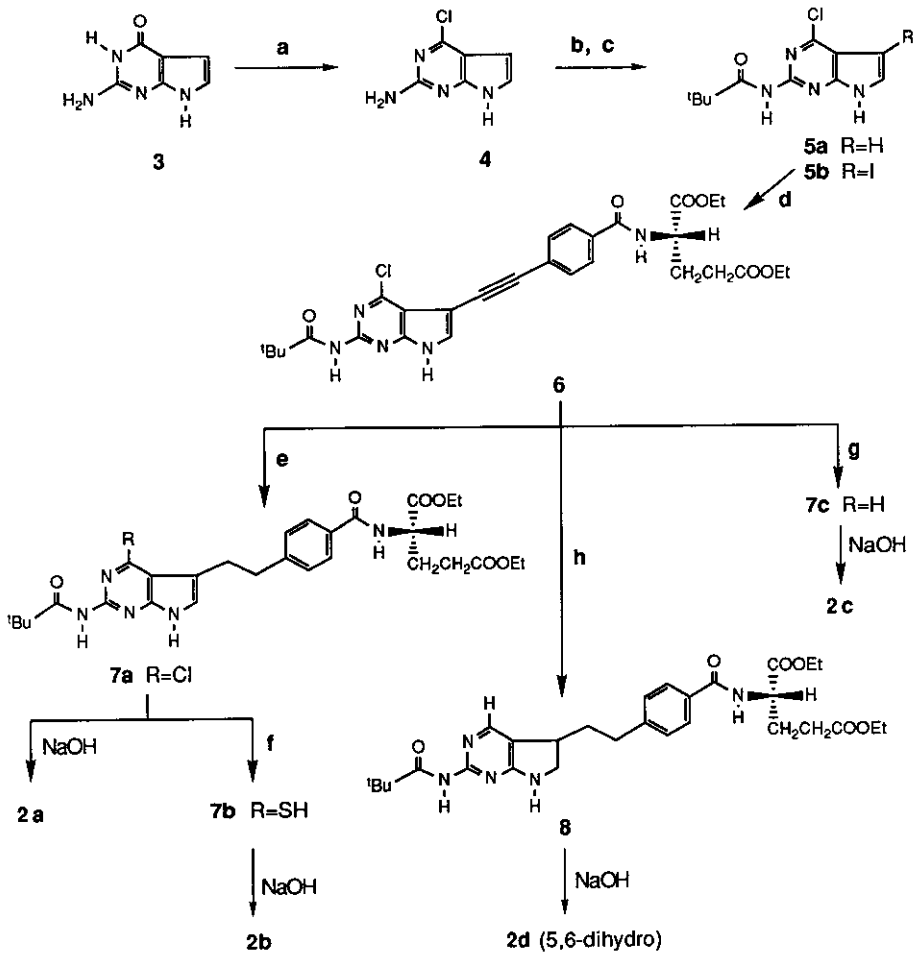


TNP-351

In order to keep the overall synthetic plan versatile and flexible so that different functional groups can be efficiently introduced into the 4 position of the pyrrolopyrimidine ring, we started our synthesis by using the 2-amino-4-chloropyrrolo[2,3-d]pyrimidine (**4**) as the basic building block. Compound (**4**) can be readily converted into other key intermediates such as **5b**, **10b**, **15a** and **15b**. These highly functionalized pyrrolo[2,3-*d*]pyrimidines were then subsequently used in palladium catalyzed Heck coupling reactions for the assembling of the basic skeleton of the targeted molecules. The availability

of the chlorine atom at the 4 position of the pyrrolopyrimidine ring thus allows various functionalities to be introduced into this position either before or after the Heck coupling step (Schemes I-III).

Scheme I



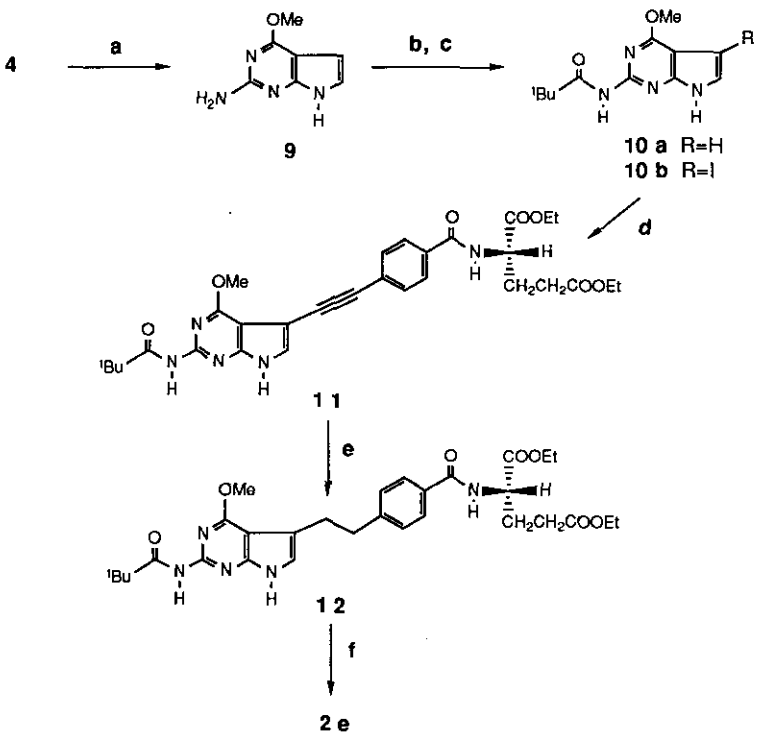
(a) $POCl_3$; (b) $(CH_3)_3CCOCl$, pyridine; (c) NIS, THF; (d)$[(Ph)_3P]_4Pd$, CuI, Et_3N, diethyl 4-ethynylbenzoyl-L-glutamate, DMF; (e) H_2, 10% Pd/C, 4 h; (f) thiourea, 2-methoxyethanol, 100°C; (g) H_2, 10 % Pd/C, NH_4OH (h) H_2, 10 % Pd/C, 18 h

This is first exemplified by the preparation of 4-Cl, 4-SH and 4-H analogs of LY231514 (Scheme I). Compound 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**4**) was prepared according to the literature

procedure.[6] Pivaloylation (73%) followed by regiospecific iodination (*N*-iodosuccinimide, THF, 83%) of **4** gave the 4-chloro-5-iodopyrrolopyrimidine (**5b**). The iodination reaction of **5a** with NIS occurred regiospecifically at the 5 position of pyrrolopyrimidine ring and produced no other regioisomer. Compound (**5b**) was then used readily in a palladium catalyzed coupling reaction with diethyl 4-ethynylbenzoyl-L-glutamate[7] in DMF (10 mol % of tetrakis(triphenylphosphine)palladium(0), copper(I) iodide and triethylamine) and gave the fully assembled and protected compound (**6**) (60 %). The palladium catalyzed carbon-carbon bond formation occurred selectively and exclusively at the 5-iodo position of the pyrrolopyrimidine ring and no coupling has been observed between the 4-ethynylbenzoylglutamate and the 4-chloro position of **5b**. The structure of compound (**6**) was established by ^{1}H nmr, mass spectrum and elemental analysis. The ethynyl bridge in compound (**6**) then can be reduced catalytically (H_2, 10 % Pd/C, 4 h, 95 %) to give intermediate (**7a**), with the chlorine group still remained intact at the 4 position of the pyrrolopyrimidine ring. Treatment of **7a** with thiourea in anhydrous 2-methoxyethanol at 100°C gave the mercapto derivatives **7b** (50 %). Saponification of **7a** and **7b** respectively in aqueous NaOH then gave the 4-chloro- (**2a**) and the 4-mercapto (**2b**) derivatives of LY231514. Hydrogenation of compound (**6**) in the presence of concentrated ammonium hydroxide (10 % Pd/C, 20 h) effectively reduced the ethynyl bridge as well as dehalogenated the chlorine group at the 4 position and led to compound (**7c**) (60 %); introduction of conc. ammonium hydroxide in the reaction mixture can significantly prevent the reduction of the pyrrole ring.[8] Prolonged hydrogenation (10 % Pd/C, 18 h) of **6** in the absence of ammonium hydroxide then led to the fully reduced (both at the bridge and pyrrole portion) and dehalogenated compound (**7d**) (40 %). Again, saponification of **7c** and **7d** in aqueous NaOH respectively gave the 4-deoxy (**2c**) and the corresponding 5,6-dihydro-4-deoxy (**2d**) analogs of LY231514.

Alternatively, we have also investigated the possibility of incorporating substituents into the 4 position of the pyrrolopyrimidine nucleus prior to the Heck coupling step. This is exemplified by two different approaches each illustrated separately in Scheme II and Scheme III. The chlorine group in compound (**4**) can be readily displaced under nucleophilic condition.[9] For example, treatment of **4** with sodium methoxide in refluxing methanol gave 2-amino-4-methoxypyrrolo[2,3-*d*]pyrimidine (**9**) in over 70 % yield. The replacement of the chlorine with a methoxy group at the 4 position of pyrrolopyrimidine did not affect the subsequent pivaloylation (80 %) and regioselective iodination (82%) steps and compound (**10b**) was obtained in good overall yields. Compound (**10b**) was then readily converted via a similar sequence (palladium catalyzed Heck coupling, hydrogenation and saponification) to the 4-methoxy analog (**2e**) of LY231514 (Scheme II).

Scheme II

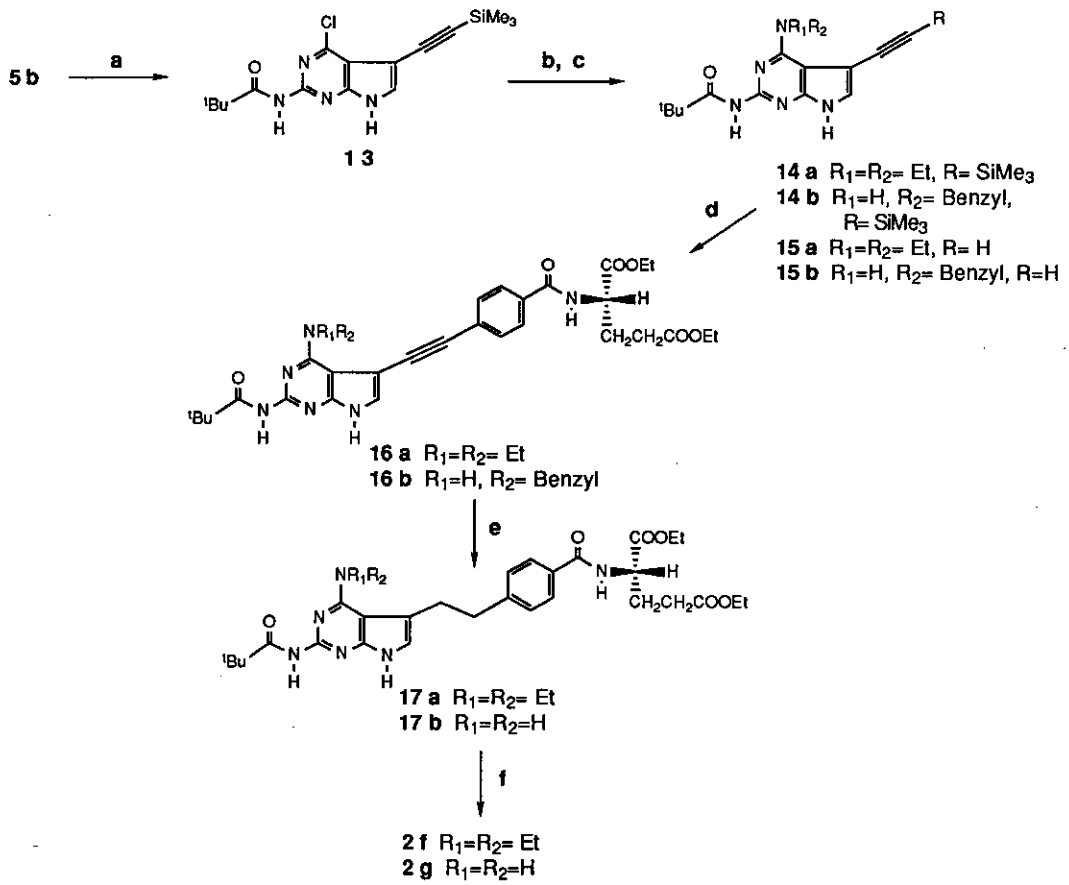


(a) NaOMe, reflux; (b) $(CH_3)_3CCOCl$, pyridine; (c) NIS, THF;
(d) $[(Ph)_3P]_4Pd$, CuI, Et_3N, diethyl 4-ethynylbenzoyl-L-glutamate, DMF; (e) H_2, 10 % Pd/C; (f) 0.5 N NaOH

In order to further demonstrate the versatility of this synthetic approach, we have also elected to first introduce the ethyl bridge equivalent as well as the 4-substituents into the pyrrolo[2,3-*d*]pyrimidine nucleus before conducting the Heck coupling reaction. This is shown in Scheme III in the preparation of various 4 amino analogs of LY231514. Reaction of compound (**5b**) first with trimethylsilylacetylene in the presence of tetrakis(triphenylphosphine)palladium(0) and copper(I) iodide in DMF gave the 2-trimethylacetylamino-4-chloro-5-trimethylsilylethynylpyrrolo[2,3-*d*]pyrimidine (**13**). Compound (**13**) was then further reacted with various amine nucleophiles such as diethylamine (80%) and benzylamine (79%) in anhydrous 2-methoxyethanol so that various nitrogen atom based functional groups could be introduced into the 4 position of the pyrrolopyrimidine nucleus. The silyl protecting group on compounds (**14a**) and (**14b**) was then removed by fluoride ion in high yields to give the 4-aminosubstituted 5-ethynylpyrrolopyrimidines (**15a**) and (**15b**). Compounds (**15a**) and (**15b**) were then coupled successfully with diethyl 4-iodobenzoyl-L-glutamate under similar palladium catalyzed

reaction condition (tetrakis(triphenylphosphine)palladium(0), copper(I) iodide, DMF) to give compounds such as **16a** and **16b**. Subsequent catalytic hydrogenation and saponification then gave the 4-diethylamino (**2f**) and 4-amino (**2g**) analogs of LY231514.

Scheme III



(a) $[(Ph)_3P]_4Pd$, CuI, Et_3N, trimethylsilylacetylene, DMF; (b) 2-methoxyethanol, diethylamine (or benzylamine for **14 b**); (c) Bu_4NF, THF; (d) $[(Ph)_3P]_4Pd$, CuI, Et_3N, diethyl iodobenzoyl-glutamate, DMF; (e) H_2, 10 % Pd/C; (f) 1.0 N NaOH

In summary, the introduction of the chlorine atom at the 4 position of the pyrrolo[2,3-*d*]pyrimine nucleus creates a versatile group that can be readily replaced or substituted by other functionalities. This combines with the regiospecific iodination of the pyrrolo[2,3-*d*]pyrimidine nucleus and the powerful Heck coupling synthetic strategy has allowed us to prepare various 4-substituted analogs of LY231514 with high versatility and efficiency. Among all the new analogs that have been prepared,

we have found that compounds such as **2b**, **2c**, **2d** and **2g** are highly cytotoxic agents (with IC_{50} ranging from 0.001 - 0.060 μg/ml) against the human T-cell derived lymphoblastic leukemic cells (CCRF-CEM).[10] The detailed biochemical, pharmacological and mechanism of action studies of these new pyrrolopyrimidine based antifolates will be presented elsewhere.

EXPERIMENTAL

Melting points are all uncorrected and were determined in open capillary tubes using a Thomas-Hoover apparatus for temperatures below 250°C and a Meltemp or Electrothermal apparatus for temperatures above 250°C. ^{1}H Nmr data were obtained with a General Electric QE300 MHz instrument using residual solvent as an internal standard and chemical shifts are reported in ppm downfield from TMS. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, qn = quintet, m = multiplet, and br = broad), coupling constant (Hz), integration. Flash chromatography was performed on EM Reagents silica gel 60 (230-400 mesh). Analytical thin layer chromatography (tlc) was performed with Merck 0.25 mm silica gel 60-F plates utilizing uv visualization. Mass spectra and elemental analysis were performed by the physical chemistry department, Eli Lilly and Company, Indianapolis, Indiana. Commercial reagents were utilized without further purification. Anhydrous solvents were distilled before use.

2-Amino-4-chloropyrrolo[2,3-*d*]pyrimidine (**4**)

To a 100 ml round-bottomed flask was charged 2.0 g (13.3 mmol) of 2-aminopyrrolo[2,3-*d*]pyrimidin-4-one suspended in 20 ml of phosphorous oxychloride. The reaction was heated to reflux for 2 h. After cooling to room temperature, the solvent was removed in vacuo, and the residue was treated with 30 ml of ice water in an ice bath. The insoluble material was filtered away and the filtrate was treated with concentrated NH_4OH to adjust the pH to 2. The resulting precipitate was filtered, washed with water and 20 ml of ether, and dried in a vacuum oven to give 1.1 g (49%) of 2-amino-4-chloro-pyrrolo[2,3-*d*]pyrimidine (**4**) as a pale yellow solid: mp 225-226°C (decomp., lit.,[6] 217°C); ir (KBr) 700, 741, 812, 887, 920, 1203, 1272, 1310, 1385, 1407, 1428, 1485, 1512, 1564, 1616, 1637, 2822, 2927, 2965, 3111, 3190, 3309, 3414, 3450 cm^{-1}; ^{1}H nmr (DMSO-d_6) δ 6.22 (d, J=3.3 Hz,1H) 6.46 (s, 2H), 7.06 (d, J= 2.7 Hz, 1H), 11.43 (s, 1H).

2-Pivaloylamino-4-chloropyrrolo[2,3-*d*]pyrimidine (**5a**)

To a 100 ml round-bottomed flask was charged 4.0 g (23.8 mmol) of 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine dissolved in 50 ml of anhydrous pyridine. To this solution was added 10.2 ml (83 mmol) of pivaloyl chloride, and after several minutes a precipitate began forming. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. The volatiles were removed in vacuo, and the residue was dissolved in 1 l of chloroform. The organic layer was then washed twice with 0.1 N HCl, dried over Na_2SO_4 and then concentrated in vacuo. The crude residue was flash

chromatographed on silica gel eluting with a gradient of 100% chloroform to 2% MeOH/$CHCl_3$ to give 4.4 g (73%) of **5a** as an off-white solid: mp 217-220°C (decomp.); ir (KBr) 1371, 1390, 1421, 1459, 1493, 1510, 1573, 1615, 1694, 2871, 2963, 3127, 3163, 3422 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.20 (s, 9H), 6.50 (d, J= 3.3 Hz, 1H), 7.51 (d, J= 3.2 Hz, 1H), 10.02 (s, 1H), 12.31 (s, 1H); ms (m/z) = 378 (FD); Anal. Calcd for $C_{11}H_{13}N_4OCl$: C, 52.28; H, 5.18; N, 2217. Found: C, 52.55; H, 5.18; N, 22.09.

2-Pivaloylamino-4-chloro-5-iodopyrrolo[2,3-*d*]pyrimidine (**5b**)

To a 100 ml round-bottomed flask covered with aluminum foil was charged 1.50 g (5.94 mmol) of 2-pivaloylamino-4-chloropyrrolo[2,3-*d*]pyrimidine dissolved in 30 ml of anhydrous THF, followed by the addition of 1.47 g (6.5 mmol) of *N*-iodosuccinimide. The dark brown solution was stirred at room temperature under a nitrogen atmosphere for 1 h. The solvent was removed in vacuo, and the residue was dissolved in chloroform. The organic layer was washed with water, dried over Na_2SO_4 and concentrated in vacuo. The crude residue was then flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 2% MeOH/$CHCl_3$ to give 1.86 g (83%) of **5b** as a tan solid: mp 242-244°C (decomp.); ir (KBr) 759, 780, 803, 916, 936, 965, 1021, 1161, 1178, 1227, 1259, 1287, 1315, 1368, 1414, 1453, 1500, 1565, 1603, 1708, 2871, 2967, 3213, 3425 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.20 (s, 9H), 7.74 (s, 1H), 10.09 (s, 1H), 12.67 (s, 1H); ms (m/z) = 378 (FD).

Diethyl *N*-{2-pivaloylamino-4-chloro[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (**6**)

To a 100 ml round-bottomed flask covered with aluminum foil were charged 1.5 g (3.96 mmol) of **5b**, 1.38 g (4.16 mmol) of diethyl 4-ethynylbenzoyl-L-glutamate, 0.16 g (0.83 mmol) of copper(I) iodide, and 0.48 g (0.42 mmol) of tetrakis(triphenylphosphine)palladium(0) dissolved in 30 ml of anhydrous DMF, followed by the addition of 1.16 ml (8.3 mmol) of triethylamine. The dark brown solution was stirred at room temperature under a nitrogen atmosphere for 4 h. The volatiles were removed in vacuo and the crude residue was flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 1% MeOH/$CHCl_3$. After collecting the correct fractions, the solvents were removed in vacuo, and the solid was triturated in 40 ml of a 2:1 mixture of hexane/ether to give 1.35 g (58%) of **6** as a tan solid: mp 212-214°C (decomp.); ir (KBr, cm^{-1}) 785, 851, 926, 1020, 1099, 1162, 1300, 1426, 1497, 1568, 1612, 1639, 1737, 2219, 2978, 3214; ^1H nmr (DMSO-d_6) δ 1.00-1.19 (m, 6H), 1.21 (s, 9H), 1.97-2.13 (m, 2H), 2.40-2.47 (m, 2H), 4.43 (m, 1H), 7.60 (d, J= 8.2 Hz, 2H), 7.90 (d, J= 8.2 Hz, 2H), 8.80 (d, J= 7.3 Hz, 1H), 10.14 (s, 1H), 12.80 (s, 1H); ms (m/z) = 582 (FD); Anal. Calcd for $C_{29}H_{32}N_5O_6Cl$: C, 59.84; H, 5.54; N, 12.03. Found: C, 59.54; H, 5.52; N, 11.83.

Diethyl *N*-{2-pivaloylamino-4-chloro-[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (**7a**)

To a 50 ml round-bottomed flask was charged 0.050 g (0.086 mmol) of **6** dissolved in 1 ml of absolute ethanol and 1 ml of dichloromethane, followed by the addition of 0.04 g of 10% Pd/C. The reaction mixture was then stirred under a balloon containing hydrogen for 4 h. The catalyst was filtered, washed thoroughly, and the filtrate was removed in vacuo. The crude residue was then flash chromatographed on silica gel eluting with 2% MeOH/$CHCl_3$ to give 0.45 g (95%) of **7a** as a yellow solid; mp 86-88°C; ir (KBr) 759, 922, 1020, 1098, 1165, 1374, 1427, 1469, 1502, 1540, 1569, 1613,

1648, 1737, 2977, 3244 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.11-1.19 (m, 6H), 1.20 (s, 9H), 1.95-2.09 (m, 2H), 2.41 (t, J= 7.4 Hz, 2H), 2.97-3.15 (m, 4H), 3.98-4.11 (m, 4H), 4.38 (m, 1H), 7.23 (s, 1H), 7.32 (d, J= 8.1 Hz, 2H), 7.78 (d, J= 8.0 Hz, 2H), 8.63 (d, J= 8.0 Hz, 1H), 9.98 (s, 1H), 12.02 (s, 1H); ms (m/z) = 586 (FAB).

N-[2-Amino-4-chloro[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid (**2a**)

To a 15 ml round-bottomed flask was charged 0.015 g (0.026 mmol) of **7a** suspended in 1 ml of 2.0 N NaOH. The mixture was stirred at room temperature for 7 days, and then at 50°C for 4 h. The pale yellow solution was then acidified with 5.0 N HCl, and the white precipitate was filtered, washed with 15 ml of water, and dried in a vacuum oven at 70°C to give 0.0056 g (49%) of **2a** as a tan solid: mp >300°C (decomp.); ir (KBr) 599, 928, 1020, 1225, 1435, 1501, 1555, 1617, 1714, 3337 cm^{-1}; ^1H nmr (300 MHz, DMSO-d_6) δ 1.87-2.09 (m, 2H), 2.32 (t, J= 7.3 Hz, 2H), 2.95 (s, 4H), 4.36 (m, 1H), 6.42 (s, 2H), 6.78 (s, 1H), 7.30 (d, J= 7.7 Hz, 2H), 7.81 (d, J= 7.6 Hz, 2H), 8.49 (d, J= 7.2 Hz, 1H), 11.13 (s, 1H), 12.40 (br s, 2H); ms (m/z) = 446 (FAB).

Diethyl *N*-[2-pivaloylamino-4-mercapto[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamate (**7b**)

To a 15 ml round-bottomed flask was charged 0.22 g (0.37 mmol) of **7a** dissolved in 4 ml of anhydrous 2-methoxyethanol, followed by the addition of 0.20 g (2.6 mmol) of thiourea. The reaction mixture was heated to 100°C under a nitrogen atmosphere for 20 min. After cooling to room temperature, the solvent was removed in vacuo. The residue was then dissolved in 100 ml of chloroform. The organic layer was then washed with water, separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was then flash chromatographed on silica gel with 2% MeOH/$CHCl_3$ to give 0.11g (50%) of **7b** as a yellow solid: mp 185-187°C (decomp.); ir (KBr) 766, 842, 935, 1023, 1163, 1360, 1439, 1564, 1640, 1735, 2975, 3195 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.11-1.24 (m, 15H), 1.90-2.10 (m, 2H), 2.41 (t, J= 7.4 Hz, 2H), 2.98-3.03 (m, 2H), 3.14-3.22 (m, 2H), 3.99-4.11 (m, 4H), 4.38-4.40 (m, 1H), 6.88 (s, 1H), 7.33 (d, J= 8.1 Hz, 2H), 7.77 (d, J= 8.1 Hz, 2H), 8.62, (d, J= 7.4 Hz, 1H), 11.05 (s, 1H), 11.62 (s, 1H), 13.14 (s, 1H); ms (m/z) = 583 (FD); Anal. Calcd for $C_{29}H_{37}N_5O_6S$: C, 59.67; H, 6.39; N, 12.00. Found: C, 59.44; H, 6.33; N, 11.91.

N-[2-Amino-4-mercapto[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid (**2b**)

To a 15 ml round-bottomed flask was charged 0.083 g (0.14 mmol) of **7b** dissolved in 6 ml of 0.5 N NaOH. The reaction mixture was covered with aluminum foil and stirred at room temperature for 5 days. The orange solution was then acidified with 1.0 N HCl. The precipitate was then filtered, washed with water and dried in a vacuum oven at 60°C to give 0.037 g (59%) of **2b** as a tan solid: mp 240-242°C (decomp.); ir (KBr) 767, 817, 968, 1020, 1098, 1191, 1343, 1402, 1446, 1502, 1568, 1637, 1710, 2929, 3333 cm^{-1}; ^1H nmr (DMSO-d_6) δ 2.22-2.35 (m, 4H), 2.94-3.20 (m, 4H), 4.29-4.31 (m, 1H), 6.39 (s, 2H), 6.52 (s, 1H), 7.30 (d, J= 7.9 Hz, 2H), 7.73 (d, J= 7.9 Hz, 2H), 8.48 (d, J= 7.5 Hz, 1H), 10.95 (s, 1H); ms (m/z) = 444 (FAB); Anal. Calcd for $C_{20}H_{21}N_5O_5S$: C, 54.17; H, 4.77; N, 15.79. Found: C, 53.90; H, 4.73; N, 15.52.

Diethyl *N*-{2-pivaloylamino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (**7c**)

To a Parr hydrogenation bottle was charged 0.10 g (0.17 mmol) of **6** dissolved in 8 ml of absolute ethanol and 2 ml of dichloromethane, followed by the addition of 0.05 ml (1.3 mmol) of concentrated ammonium hydroxide. To this solution was then added 0.20 g of 10% Pd/C, and the mixture was hydrogenated at 50 psi for 20 h. The catalyst was filtered and washed thoroughly, and the filtrate was concentrated in vacuo. The residue was then flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 2% MeOH/$CHCl_3$ to give 0.055 g (59%) of **7c** as a pale yellow solid: mp 148-149°C; ir (KBr) 766, 850, 923, 1021, 1097, 1165, 1433, 1477, 1500, 1545, 1582, 1613, 1636, 1701, 1742, 2978, 3225, 3363, 3441 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.11-1.20 (m, 15H), 1.90-2.10 (m, 2H), 2.41 (t, J= 7.4 Hz, 2H), 3.01 (s, 4H), 3.99-4.12 (m, 4H), 4.38-4.41 (m, 1H), 7.14 (s, 1H), 7.32 (d, J= 8.0 Hz, 2H), 7.76 (d, J= 7.9 Hz, 2H), 8.60 (d, J= 7.3 Hz, 1H), 9.69 (s, 1H), 11.57 (s, 1H); ms (m/z) = 551 (FD); Anal. Calcd for $C_{29}H_{37}N_5O_6$: C, 63.14; H, 6.76; N, 12.69. Found: C, 63.02; H, 6.75; N, 12.39.

N-{2-Amino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (**2c**)

To a 15 ml round-bottomed flask was charged 0.045 g (0.082 mmol) of **7c** suspended in 2 ml of 1.0 N NaOH. The reaction mixture was stirred at room temperature for 3 days. The yellow solution was acidified with 1.0 N HCl, and the precipitate filtered, washed with water, and dried in a vacuum oven at 70°C to give 0.019 g (57%) of **2c** as a pale yellow solid: mp 260-264°C (decomp.); ir (KBr) 845, 963, 1020, 1093, 1209, 1291, 1333, 1404, 1451, 1500, 1538, 1558, 1614, 1655, 2813, 2857, 2947, 3138, 3353 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.87-2.09 (m, 2H), 2.30-2.34 (m, 2H), 2.90-3.01 (m, 4H), 4.32-4.39 (m, 1H), 6.01 (s, 2H), 6.70 (s, 1H), 7.30 (d, J= 8.1 Hz, 2H), 7.76 (d, J= 8.1 Hz, 2H), 8.41 (s, 1H), 8.50 (d, J= 7.6 Hz, 1H), 10.76 (s, 1H), 12.40 (br s, 1H); ms (m/z) = 412 (FAB).

Diethyl *N*-{2-pivaloylamino[(5,6-dihydropyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (**8**)

To a Parr hydrogenation bottle was charged 0.068 g (0.17 mmol) of **6** dissolved in 6 ml of absolute ethanol and 1 ml of dichloromethane, followed by the addition of 0.2 g of 10% Pd/C. This mixture was then hydrogenated at 50 psi for 18 h. After filtering and washing the catalyst with ethanol, the filtrate was concentrated in vacuo. The crude residue was then flash chromatographed on silica gel eluting with 2% MeOH/$CHCl_3$ to give 0.026 g (40%) of **8** as a white solid: mp 90-92°C; ir (KBr) 853, 925, 1022, 1181, 1398, 1504, 1591, 1623, 1735, 2978, 3308 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.04-1.20 (m, 15H), 1.75-1.85 (m, 1H), 1.93-2.09 (m, 4H), 2.41 (t, J= 7.4 Hz, 2H), 2.69 (t, J= 7.6 Hz, 3H), 3.65-3.69 (m, 1H), 4.00-4.11 (m, 4H), 4.39-4.42 (m, 1H), 7.34 (d, J= 8.0 Hz, 2H), 7.40 (s, 1H), 7.78 (d, J= 8.4 Hz, 2H), 7.81 (s, 1H), 8.62 (d, J= 7.4 Hz, 1H), 9.29 (br s, 1H); ms (m/z) = 554 (FD); Anal. Calcd for $C_{29}H_{39}N_5O_6$: C, 62.91; H, 7.10; N, 12.65. Found: C, 62.61; H, 7.40; N, 12.53.

N-{2-Amino[(5,6-dihydropyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (**2d**)

To a 15 ml round-bottomed flask was charged 0.075 g (0.13 mmol) of **8** suspended in 3 ml of 1.0 N NaOH. The reaction mixture was covered with aluminum foil and stirred at room temperature in 7 days. The yellow solution was acidified with 5.0 N HCl, and the yellow precipitate was filtered,

washed with water and dried in a vacuum oven at 70°C. The filtrate was further concentrated in vacuo and the residue was purified by C-18 reverse phase high performance liquid chromatography eluting with 10% MeCN/89.5% H_2O/0.5% AcOH. The correct fractions were combined, and the solvents were concentrated in vacuo to near dryness and then lyophilized. The precipitated solid and the lyophilized solid were combined to give 0.027 g (48%) of **2d** as a pale yellow solid: mp >300°C (decomp.); ir (KBr) 660, 1180, 1295, 1380, 1500, 1608, 1630, 1640, 2920, 3340 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.89-1.99 (m, 1H), 2.10-2.20 (m, 4H), 2.26 (t, J= 6.8 Hz, 3H), 2.61-2.66 (m, 2H), 4.30-4.40 (m, 1H), 5.76 (br s, 2H), 6.77 (s, 1H), 7.29 (d, J= 7.9 Hz, 2H), 7.51 (br s, 1H), 7.72 (d, J= 7.7 Hz, 2H), 8.10 (br s, 1H); ms (m/z) = 414 (FAB).

2-Amino-4-methoxypyrrolo[2,3-*d*]pyrimidine (**9**)

To a 250 ml round-bottomed flask under a nitrogen atmosphere was charged 2.0 g (11.9 mmol) of 2-amino-4-chloropyrrolo[2,3-*d*]pyrimidine to a stirring solution of 0.92 g (40 mmol) of sodium metal dissolved in 80 ml anhydrous methanol. The yellow solution was heated to reflux for 24 h. After cooling to room temperature, the reaction was neutralized with glacial acetic acid to pH 6.0, and the volatiles were removed in vacuo. The residue was treated with 50 ml of water, and a small amount of an insoluble solid was filtered away. The filtrate was extracted three times with 100 ml of chloroform. The organic layers were combined, dried over Na_2SO_4 and concentrated in vacuo. The crude solid was flash chromatographed on silica gel eluting with 5% MeOH/$CHCl_3$ to give 1.36 g (70%) of 2-amino-4-methoxypyrrolo[2,3-*d*]pyrimidine as an off-white solid: mp 202-204°C (decomp.); ir (KBr) 711, 740, 793, 823, 902, 1056, 1093, 1165, 1194, 1228, 1321, 1334, 1390, 1406, 1440, 1473, 1490, 1579, 1610, 1632, 2828, 3110, 3370, 3484 cm^{-1}; ^1H nmr (DMSO-d_6) δ 3.88 (s, 3H), 5.96 (s, 2H), 6.15 (d, J=2.3 Hz, 1H), 6.78 (d, J= 2.9 Hz, 1H), 10.99 (s, 1H); ms (m/z) = 164 (FD); Anal. Calcd for $C_7H_8N_4O$: C, 51.22; H, 4.91; N, 34.13. Found: C, 51.02; H, 4.95; N, 34.23.

2-Pivaloylamino-4-methoxypyrrolo[2,3-*d*]pyrimidine (**10a**)

To a 50 ml round-bottomed flask was charged 0.90 g (5.48 mmol) of 2-amino-4-methoxypyrrolo[2,3-*d*]pyrimidine dissolved in 12 ml of anhydrous pyridine. To this solution was added 2.4 ml (19.2 mmol) of pivaloyl chloride. The reaction was heated to reflux for 1.5 h under a nitrogen atmoshpere. The volatiles were removed in vacuo, and the residue was dissolved in 9 ml of methanol and cooled in an ice bath. To this stirring solution was added 9 ml of 10% ammonium hydroxide and the resulting precipitate was filtered, washed with water and 10% ammonium hydroxide, and dried to give 1.09 g (80%) of **10a** as a white solid: mp 235-236°C; ir (KBr) 709, 720, 738, 789, 844, 881, 901, 934, 969, 1017, 1049, 1058, 1100, 1167, 1209, 1309, 1358, 1395, 1460, 1516, 1588, 1618, 1693, 2958, 3130, 3185, 3430 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.20 (s, 9H), 6.38 (d, J= 3.2 Hz, 1H), 7.20 (d, J= 3.1 Hz, 1H), 9.49 (s, 1H), 11.77 (s 1H); ms (m/z) = 248 (FD); Anal. Calcd for $C_{11}H_{13}N_4OCl$: C, 58.05; H, 6.50; N, 22.57. Found: C, 57.75; H, 6.48; N, 22.62.

2-Pivaloylamino-4-methoxy-5-iodopyrrolo[2,3-*d*]pyrimidine (**10b**)

To a 15 ml round-bottomed flask covered with aluminum foil was charged 0.266 g (1.07 mmol) of 2-pivaloylamino-4-methoxypyrrolo[2,3-*d*]pyrimidine and 0.27 g (1.17 mmol) *N*-iodosuccimide dissolved in 6 ml of anhydrous THF. The reaction was stirred under a nitrogen atmosphere for 1.5 h. The solvent was removed in vacuo, and the residue was dissolved in 100 ml of chloroform. The organic layer was washed with water, dried over Na_2SO_4 and concentrated in vacuo. The crude residue was then flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 1% MeOH/$CHCl_3$ to give 0.33 g (82%) of 2-pivaloyl-4-methoxy-5-iodopyrrolo[2,3-*d*]pyrimidine **10b** as an off-white solid: mp 243-244°C (decomp.); ir (KBr) 652, 723, 785, 806, 843, 965, 1010, 1096, 1175, 1212, 1253, 1279, 1328, 1390, 1426, 1461, 1532, 1579, 1620, 1705, 2955, 3200, 3435 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.20 (s, 9H), 4.01 (s, 3H), 7.40 (d, J= 2.0 Hz, 1H), 9.55 (s, 1H), 12.11 (s, 1H); ms (m/z) = 374 (FD).

Diethyl *N*-{2-pivaloylamino-4-methoxy[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (**11**)

To a 25 ml round-bottomed flask covered with aluminum foil were charged 0.20 g (0.53 mmol) of 2-pivaloyl-4-methoxy-5-iodopyrrolo[2,3-*d*]pyrimidine, 0.19 g (0.58 mmol) of diethyl 4-ethynylbenzoyl-L-glutamate, 0.024 g (0.13 mmol) of copper(I) iodide, and 0.074 g (0.06 mmol) of tetrakis(triphenylphosphine)palladium(0) suspended in 5 ml of anhydrous DMF, followed by the addition of 0.18 ml (1.28 mmol) of triethylamine. The dark brown mixture was stirred and heated under a nitrogen atmosphere at 50°C for 0.5 h. The volatiles were removed in vacuo, and the crude residue was flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 1% MeOH/$CHCl_3$ to give 0.085 g (28%) of **11** as a tan solid: mp 215-216°C (decomp.); ir (KBr) 792, 852, 1097, 1165, 1346, 1430, 1527, 1607, 1737, 2216, 2978, 3210 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.06-1.21 (m, 15H), 1.97-2.11 (m, 2H), 2.41-2.51 (m, 2H), 3.99-4.13 (m, 7H), 4.38-4.45 (m, 1H), 7.56 (d, J= 8.2 Hz, 2H), 7.61 (s, 1H), 7.90 (d, J= 8.1 Hz, 2H), 8.79 (d, J= 7.3 Hz, 1H), 9.61 (s, 1H), 12.28 (s, 1H); ms (m/z) = 577 (FD).

Diethyl *N*-{2-pivaloylamino-4-methoxy[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (**12**)

To a 50 ml round-bottomed flask was charged 0.075 g (0.13 mmol) of **11** dissolved in 8 ml of absolute ethanol and 2 ml of dichloromethane, followed by the addition of 0.05 g of 10% Pd/C. The reaction mixture was then stirred under a balloon containing hydrogen for 6 h. The catalyst was filtered, washed thoroughly, and the filtrate was removed in vacuo. The crude residue was then flash chromatographed on silica gel eluting with 3% MeOH/$CHCl_3$ to give 0.062 g (82%) of **12** as a yellow solid: mp 71-74°C (decomp.); ir (KBr) 793, 844, 970, 1020, 1095, 1168, 1338, 1587, 1615, 1648, 1737, 2979, 3259 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.06-1.20 (m, 15H), 1.95-2.09 (m, 2H), 2.41-2.51 (m, 4H), 2.95 (s, 2H), 3.98-4.11 (m, 7H), 4.36-4.40 (m,1H), 6.89 (s, 1H), 7.28 (d, J= 8.1 Hz, 2H), 7.76 (d, J= 8.0 Hz, 2H), 8.62 (d, J= 7.4 Hz, 1H), 9.47 (s, 1H), 11.43 (s, 1H); ms (m/z) = 582 (FAB).

N-[2-Amino-4-methoxy[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl]-L-glutamic acid (**2e**)

To a 15 ml round-bottomed flask was charged 0.07 g (0.12 mmol) of **12** suspended in 4 ml of 0.5 N NaOH. The mixture was stirred at room temperature for 3 days. The pale yellow solution was then acidified with 1.0 N HCl and the tan precipitate was filtered, washed with water and dried in a vacuum oven at 70°C to give 0.044 g (83%) of **2e** as a tan solid: mp 230°C (decomp.); ir (KBr) 777, 958, 1020, 1090, 1153, 1197, 1250, 1394, 1441, 1483, 1538, 1614, 1660, 2941, 3336 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.90-2.07 (m, 2H), 2.32 (t, J= 7.3 Hz, 2H), 2.84-2.95 (m, 4H), 3.96 (s, 3H), 4.33-4.40 (m, 1H), 6.56 (s, 1H), 7.26 (d, J= 8.0 Hz, 2H), 7.78 (d, J= 8.1 Hz, 2H), 8.50 (d, J= 7.6 Hz, 1H), 11.04 (s, 1H), 12.40 (br s, 2H); ms (m/z) = 442 (FAB).

2-Pivaloylamino-4-chloro-5-trimethylsilylethynylpyrrolo[2,3-*d*]pyrimidine (**13**)

To a 100 ml round-bottomed flask covered with aluminum foil were charged 1.0 g (2.6 mmol) of 2-pivaloylamino-4-chloro-5-iodopyrrolo[2,3-d]pyrimidine, 0.2 g(1.04 mmol) of copper(I) iodide, 1.8 ml (13 mmol) of trimethylsilylacetylene, and 0.30 g (0.26 mmol) of tetrakis(triphenylphosphine)palladium(0) dissolved in 15 ml of anhydrous DMF, followed by the addition of 0.72 ml (5.1 mmol) of triethylamine. The dark brown solution was stirred at room temperature under a nitrogen atmosphere for 18 h. The volatiles were removed in vacuo, and the residue was dissolved in 1 l of chloroform. The organic layer was washed with water, dried over Na_2SO_4 and concentrated in vacuo. The crude residue was then flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 1% MeOH/$CHCl_3$ to give 0.49 g (54%) of **13** as a tan solid: mp >300°C; ir (KBr) 626, 758, 785, 860, 926, 1021, 1166, 1232, 1260, 1291, 1432, 1456, 1498, 1568, 1610, 1698, 2161, 2961, 3154, 3424 cm^{-1}; ^1H nmr (DMSO-d_6) δ 0.21 (s, 9H), 1.20 (s, 9H), 7.89 (s, 1H), 10.12 (s, 1H), 12.67 (s, 1H): ms (m/z) = 348 (FD).

2-Pivaloylamino-4-diethylamino-5-trimethylsilylethynylpyrrolo[2,3-*d*]pyrimidine (**14a**)

To a 100 ml round-bottomed flask under a nitrogen atmosphere was charged 0.49 g (1.4 mmol) of **13** suspended in 15 ml of anhydrous 2-methoxyethanol, followed by the addition of 0.87 ml (8.4 mmol) of diethylamine. The mixture was heated to 100°C for 1 h. The volatiles were removed in vacuo, and the residue was flash chromatographed with 3% MeOH/$CHCl_3$ to give 0.43 g (80%) of **14a** as a tan solid; mp 191-194°C (decomp.); ir (KBr) 695, 711, 759, 786, 838, 862, 1040, 1064, 1095, 1176, 1209, 1250, 1304, 1357, 1427, 1532, 1573, 1690, 2144, 2962, 3108, 3428 cm^{-1}; ^1H nmr (DMSO-d_6) δ 0.19 (s, 9H), 1.13-1.18 (m, 15H), 3.86 (q, J= 6.8 Hz, 4H), 7.52 (s, 1H), 9.08 (s, 1H), 11.88 (s, 1H); ms (m/z) = 385 (FD).

2-Pivaloylamino-4-diethylamino-5-ethynylpyrrolo[2,3-*d*]pyrimidine (**15a**)

To a 15 ml round-bottomed flask were charged 0.10 g (0.26 mmol) of **14a** and 0.39 ml (0.39 mmol) of tetrabutylammonium fluoride dissolved in 2 ml of anhydrous THF. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction was then quenched by pouring it into 50 ml of $CHCl_3$ and washing with water. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was then flash chromatographed eluting with 2%

MeOH/$CHCl_3$ to give 0.079 g (97%) of **15a** as a tan solid: mp 71-74°C (decomp.); ir (KBr) 721, 789, 806, 834, 946, 1033, 1091, 1162, 1206, 1279, 1300, 1358, 1370, 1428, 1536, 1571, 1707, 2102, 2969, 3195, 3454 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.11-1.22 (m, 15H), 3.80 (q, J= 6.8 Hz, 4H), 4.01 (s, 1H), 7.47 (s, 1H), 9.02 (s, 1H), 11.81 (s, 1H); ms (m/z) = 314 (FAB).

Diethyl *N*-{2-pivaloylamino-4-diethylamino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (16a) To a 25 ml round-bottomed flask covered with aluminum foil were charged 0.21 g (0.67 mmol) of **15a**, 0.32 g (0.74 mmol) of diethyl 4-iodobenzoyl-L-glutamate, 0.051 g (0.83 mmol) of copper(I) iodide, and 0.086 g (0.074 mmol) of tetrakis(triphenylphosphine)palladium(0) dissolved in 4 ml of anhydrous DMF, followed by the addition of 0.21 ml (1.48 mmol) of triethylamine. The dark brown solution was stirred at room temperature under a nitrogen atmosphere for 1.5 h. The volatiles were removed in vacuo and the residue was flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 2% MeOH/$CHCl_3$. After collecting the correct fractions, the solvents were removed in vacuo, and the solid was triturated in 15 ml of ether to give 0.18 g (45%) of **16a** as an off-white solid: mp 171-174°C (decomp.); ir (KBr) 1092, 1174, 1213, 1261, 1303, 1392, 1426, 1494, 1534, 1571, 1604, 1650, 1669, 1738, 2202, 2933, 2977 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.12-1.21 (m, 21H), 1.96-2.10 (m, 2H), 2.40-2.43 (m, 2H), 3.90 (q, J= 6.4 Hz, 4H), 3.96-4.12 (m, 4H), 4.40-4.45 (m, 1H), 7.54 (d, J= 8.0 Hz, 2H), 7.66 (s, 1H), 7.78 (d, J= 8.1 Hz, 2H), 8.78 (d, J= 7.3 Hz, 1H), 9.12 (s, 1H), 12.01 (s, 1H); ms (m/z) = 619 (FAB).

Diethyl *N*-{2-pivaloylamino-4-diethylamino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (17a) To a Parr hydrogenation bottle was charged 0.17 g (0.27 mmol) of **16a** dissolved in 10 ml of absolute ethanol and 2 ml of dichloromethane, followed by the addition of 0.25 g of 10% Pd/C. This mixture was then hydrogenated at 50 psi for 18 h. After filtering and washing the catalyst thoroughly with ethanol, the filtrate was concentrated in vacuo. The crude residue was then flash chromatographed on silica gel eluting with 2% MeOH/$CHCl_3$ to give 0.13 g (76%) of **17a** as an off-white solid: mp 68-71°C; ir (KBr) 763, 792, 1020, 1083, 1167, 1191, 1260, 1299, 1377, 1421, 1468, 1506, 1537, 1574, 1612, 1648, 1704, 1737, 2872, 2935, 2975, 3292, 3455 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.07-1.19 (m, 21H), 1.90-2.10 (m, 2H), 2.40 (t, J= 7.5 Hz, 2H), 3.30 (s, 4H), 3.52 (q, J= 6.8 Hz, 4H), 3.98-4.11 (m, 4H), 6.91 (s, 1H), 7.26 (d, J= 8.2 Hz, 2H), 7.74 (d, J= 8.1 Hz, 2H), 8.60 (d, J= 7.4 Hz, 1H), 9.15 (s, 1H), 11.26 (s, 1H); ms (m/z) = 623 (FAB); Anal. Calcd for $C_{33}H_{46}N_6O_6$: C, 63.65; H, 7.44; N, 13.49. Found: C, 63.45; H, 7.52; N, 13.46.

N-{2-Amino-4-diethylamino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (2f) To a 15 ml round-bottomed flask was charged 0.065 g (0.10 mmol) of **17a** suspended in 3 ml of 1.0 N NaOH. The reaction mixture was stirred at room temperature for 7 days. The yellow solution was then acidified with 1.0 N HCl, and the precipitate was cooled in an ice bath, filtered, washed with water, and dried in a vacuum oven at 70°C to give 0.021 g (42%) of **2f** as a pale yellow solid: mp 160-163°C (decomp.); ir (KBr) 724, 766, 851, 930, 990, 1020, 1079, 1098, 1206, 1298, 1354, 1401, 1436, 1503, 1533, 1567, 1612, 1656, 2873, 2935, 2975, 3209, 3331 cm^{-1}; ^1H nmr (DMSO-d_6) δ 0.94-1.12

(m, 6H), 1.90-2.07 (m, 2H), 2.30-2.34 (m, 2H), 3.16-3.49 (m, 8H), 4.35-4.36 (m, 1H), 5.59 (s, 2H), 6.56 (s, 1H), 7.24 (d, J= 7.8 Hz, 2H), 7.76 (d, J= 7.8 Hz, 2H), 8.48 (d, J= 7.6 Hz, 1H), 10.58 (s, 1H), 12.15 (br s, 1H); ms (m/z) = 483 (FAB).

2-Pivaloylamino-4-benzylamino-5-trimethylsilylethynylpyrrolo[2,3-*d*]pyrimidine (**14b**)

To a 100 ml round-bottomed flask under a nitrogen atmosphere was charged 0.45 g (1.29 mmol) of **13** suspended in 15 ml of anhydrous 2-methoxyethanol, followed by the addition of 0.84 ml (7.7 mmol) of benzylamine. The mixture was heated to 100°C for 1.5 h. The volatiles were removed in vacuo, and the residue was taken up in chloroform and washed with water. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was then flash chromatographed eluting with 3% MeOH/$CHCl_3$ to give 0.43 g (79%) of **14b** as a tan solid: mp> 300°C (decomp.); ir (KBr) 696, 758, 788, 844, 1082, 1123, 1162, 1176, 1252, 1278, 1352, 1430, 1452, 1491, 1518, 1548, 1616, 1692, 2149, 2959, 3110, 3402, 3432 cm^{-1}; ^1H nmr (DMSO-d_6) δ 0.02 (s, 9H), 1.19 (s, 9H), 4.73 (d, J= 5.3 Hz, 2H), 6.20 (t, J= 5.3 Hz, 1H), 7.19-7.36 (m, 5H), 7.40 (d, J= 7.8 Hz, 1H), 9.24 (s, 1H), 11.87 (s, 1H); ms (m/z) = 419 (FD).

2-Pivaloylamino-4-benzylamino-5-ethynylpyrrolo[2,3-*d*]pyrimidine (**15b**)

To a 15 ml round-bottomed flask were charged 0.30 g (0.71 mmol) of **14b** and 1.08 ml (0.1.08 mmol) of tetrabutylammonium fluoride dissolved in 10 ml of anhydrous THF. The reaction mixture was stirred at room temperature under a nitrogen atmosphere for 30 min. The reaction mixture was then quenched by pouring it into chloroform and washing with water. The organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was then flash chromatographed eluting with a gradient of 100% chloroform to 2% MeOH/$CHCl_3$ to give 0.23 g (93%) of **15b** as a tan solid: mp 218-220°C (decomp.); ir (KBr) 497, 547, 583, 616, 651, 696, 718, 753, 788, 802, 848, 1027, 1070, 1118, 1176, 1214, 1262, 1281, 1351, 1371, 1427, 1459, 1487, 1515, 1543, 1616, 1684, 2106, 2966, 3106, 3314, 3414 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.18 (s, 9H), 4.16 (s, 1H), 6.53 (m, 1H), 7.22-7.33 (m, 5H), 7.38 (d, J= 9.0 Hz, 1H), 9.17 (s, 1H), 11.82 (s, 1H); ms (m/z) = 347 (FD).

Diethyl *N*-{2-pivaloylamino-4-benzylamino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethynyl]benzoyl}-L-glutamate (**16b**) To a 25 ml round-bottomed flask covered with aluminum foil were charged 0.28 g (0.81 mmol) of **15b**, 0.38 g (0.89 mmol) of diethyl 4-iodobenzoyl-L-glutamate, 0.061 g (0.32 mmol) of copper(I) iodide, and 0.094 g (0.081 mmol) of tetrakis(triphenylphosphine)palladium(0) dissolved in 6 ml of anhydrous DMF, followed by the addition of 0.23 ml (1.62 mmol) of triethylamine. The dark brown solution was stirred at room temperature under a nitrogen atmosphere for 1.5 h. The volatiles were removed in vacuo and the crude residue was flash chromatographed on silica gel eluting with a gradient of 100% chloroform to 2% MeOH/$CHCl_3$. After collecting the correct fractions, the solvents were removed in vacuo, and the solid was triturated in 15 ml of a 1:1 ether/hexane mixture to give 0.32 g (61%) of **16b** as a tan solid: mp 222-224°C (decomp.); ir (KBr) 460, 616, 696, 717, 756, 787, 850, 1027, 1116, 1163, 1262, 1303, 1349, 1429, 1484, 1531, 1593, 1645, 1682, 1737, 2199, 2979, 3419 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.04-1.17 (m, 15H), 1.96-2.08 (m, 2H), 2.37-2.43 (m, 2H), 3.97-4.10

(m, 4H),4.37-4.40 (m, 1H), 6.44-6.66 (m, 1H), 7.23-7.30 (m, 5H), 7.31 (d, J= 7.9 Hz, 2H), 7.35 (s, 1H), 7.77 (d, J= 8.2 Hz, 2H), 8.74 (d, J= 7.4 Hz, 1H), 9.22 (s, 1H), 11.97 (s, 1H); ms (m/z) = 652 (FD); Anal. Calcd for $C_{36}H_{40}N_6O_6$: C, 66.24; H, 6.18; N, 12.87. Found: C, 66.29; H, 6.09; N, 12.60.

Diethyl *N*-{2-pivaloylamino-4-amino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamate (**17b**)

To a Parr hydrogenation bottle was charged 0.20 g (0.31 mmol) of **16b** dissolved in 10 ml of absolute ethanol and 2 ml of dichloromethane, followed by the addition of 0.60 g of 10% Pd/C. This mixture was then hydrogenated at 50 psi for 48 h. After filtering and washing the catalyst thoroughly with ethanol, the filtrate was removed in vacuo. The crude residue was then flash chromatographed on silica gel eluting with a gradient of 2% MeOH/$CHCl_3$ to 4% MeOH/$CHCl_3$ to give 0.053 g (31%) of **17b** as a white solid: mp 88-91°C; ir (KBr) 795, 1023, 1185, 1415, 1505, 1632, 1736, 2030, 3351 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.04-1.18 (m, 15H), 1.60-1.70 (m, 2H), 1.80-2.03 (m, 4H), 2.38 (t, J= 7.4 Hz, 2H), 2.58 (t, J= 8.1 Hz, 2H), 3.49-3.53 (m, 1H), 3.95-4.08 (m, 4H), 5.90 (br s, 2H), 6.42 (br s, 1H), 7.26 (d, J= 8.1 Hz, 2H), 7.74 (d, J= 8.0 Hz, 2H), 8.58 (d, J= 7.4 Hz, 1H), 8.80 (br s, 1H); ms (m/z) = 569 (FD).

N-{2,4-diamino[(pyrrolo[2,3-*d*]pyrimidin-5-yl)ethyl]benzoyl}-L-glutamic acid (**2g**)

To a 15 ml round-bottomed flask was charged 0.040 g (0.10 mmol) of **17b** suspended in 2 ml of 1.0 N NaOH. The reaction mixture was stirred at room temperature for 3.5 days. The pale yellow solution was acidified with 5.0 N HCl, and the precipitate was filtered. The solid collected was then washed with water and dried in vacuum at 70°C to give 0.011 g (37%) of **2g** as a white solid: mp 241-243°C (decomp.); ir (KBr) 622, 767, 1095, 1304, 1398, 1456, 1502, 1534, 1639, 2927, 3344 cm^{-1}; ^1H nmr (DMSO-d_6) δ 1.59-1.63 (m, 2H), 1.83-1.99 (m, 4H), 2.26 (t, J= 7.1 Hz, 2H), 2.56 (t, J= 7.8 Hz, 2H), 3.44-3.51 (m, 1H), 4.30 (br s, 1H), 6.06 (br s, 2H), 6.15-6.20 (m, 2H), 6.35 (br s, 1H), 7.24 (d, J= 8.0 Hz, 2H), 7.70 (d, J= 7.7 Hz, 2H), 8.28 (br s, 1H); ms (m/z) = 429 (FAB).

REFERENCES

1. T. R. Jones, A. H. Calvert, A. L. Jackman, S. J. Brown, M. Jones, and K. R. Harrap, Eur. J. Cancer, 1981,17, 11.
2. (a) E. C. Taylor, P. J. Harrington, S. R. Fletcher, G. P. Beardsley, and R. G. Moran, J. Med. Chem., 1985, 28, 914. (b) G. P. Beardsley, E. C. Taylor, G. B. Grindey, and R. G. Moran, "Chemistry and Biology of Pteridines", ed. by B. A. Cooper and V. M. Whitehead, Walter de Gruyter, Berlin, 1986, 953. (c) G. P. Beardsley, B. A. Moroson, E. C. Taylor, and R. G. Moran, J. Biol. Chem., 1989, 264, 328. (d) S. W. Baldwin, A. Tse, G. B. Grindey, E. C. Taylor, A. Rosowsky, C. Shih, and R. G. Moran, Proceedings of American Association of Cancer Research, 1990, 31, 341. (e) S. W. Baldwin, A. Tse, L. S. Gossett, E. C. Taylor, A. Rosowsky, C. Shih, and R. G. Moran, Biochemistry, 1991, 1997.
3. (a) C. Shih, G. B. Grindey, J. A. Engelhardt, S. M. Rinzel, D. Kuhnt,and E. C. Taylor, "7th EORTC-NCI Symposium", Amsterdem, Netherlands, March 17-20, 1992. (b) G. B. Grindey, C.

Shih, C. J. Barnett, H. L. Pearce, J. A. Engelhardt, G. C. Todd, S. M. Rinzel, J. Bewley, L. S. Gossett, T. P. Everson, T. E. Wilson, M. E. Kobierski, M. A. Winter, D. Kuhnt, E. C. Taylor, and R. G. Moran, Proceedings of American Association of Cancer Research, 1992, 33, 411.

4. (a) H. Akimoto, T. Hitika, T. Miwa, K. Yukishige, T. Kusanagi, and K. Ootus, Proceedings of American Association of Cancer Research, 1991, 32, 327. (b) T. Miwa, T. Hitaka, and H. Akimoto, J. Med. Chem., 1991, 34, 555.
5. E. C. Taylor, D. Kuhnt, C. Shih, S. M. Rinzel, G. B. Grindey, J. Barredo, M. Jannatipour, and R. G. Moran, J. Med. Chem., 1992, 35, 4450.
6. F. Seela, H. Steker, H. Driller, and U. Bindig, Liebig Ann. Chem., 1987, 15.
7. E. C. Taylor and G. S. W. Wong, J. Org. Chem., 1989, 54, 3618.
8. F. Seela and H-P. Muth, Liebig Ann. Chem., 1990, 227.
9. F. Seela, B. Westermann, and U. Bindig, J. Chem. Soc., Perkin Trans. I, 1988, 697.
10. The in vitro cytotoxicity data of these analogs were generated by Dr. G. B. Grindey's group at Cancer Research Division, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN.

Received, 27th November, 1992