Synthesis of *N*-{4-[2-(2-Amino-5,6-dihydro-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)-ethyl]benzoyl}-L-glutamic Acid: A Ring-Contracted Analogue of 5,10-Dideaza-5,6,7,8-tetrahydrofolic Acid†

Edward C. Taylor,* Wendy B. Young,‡ and Carsten Spanka§

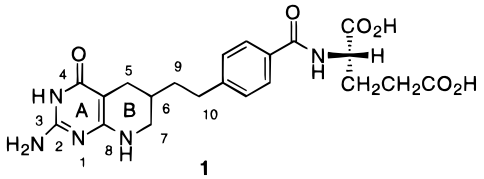
Department of Chemistry, Princeton University, Princeton, New Jersey 08544

Received August 8, 1995⊗

This paper describes the synthesis of *N*-{4-[2-(2-amino-5,6-dihydro-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]-pyrimidin-6-yl)ethyl]benzoyl}-L-glutamic acid (**4**), which can be viewed as a ring-contracted analogue of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, **1**) in which the C-7 methylene group of the latter has been excised and C-6 joined to N-8. This compound exhibits significant activity as an inhibitor of the growth of human (CCRF-CEM) lymphoblastic leukemic cells *in vitro* and apparently acts by blocking *de novo* purine biosynthesis through inhibition of glycinamide ribonucleotide formyltransferase (GAR FTase).

The synthesis of 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF, (6*RS*)-**1**; Lometrexol, (6*R*)-**1**) by Taylor and co-workers[1] represents an important advance in antifolate cancer chemotherapy. Lometrexol has been shown to be an inhibitor of glycinamide ribonucleotide formyltransferase (GAR FTase),[2] the enzyme that catalyzes the first of two formyl transfer reactions in *de novo* purine biosynthesis. Its effectiveness in tumor chemotherapy (Lometrexol is now in Phase II clinical trials) is a result of several factors which include its efficient intracellular conversion to polyglutamates by folylpolyglutamate synthetase (FPGS) and its ability to enter cells by pathways involving both the reduced-folate and the folate-binding membrane protein transport systems. Since Lometrexol is not an inhibitor of dihydrofolate reductase (DHFR), it is fully effective against tumors resistant to methotrexate due to amplification of the DHFR gene.[3,4]

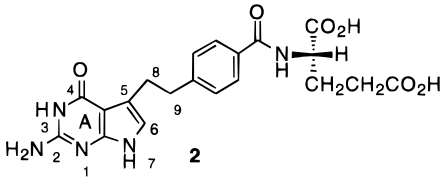
Since the discovery of **1**, many analogues have been prepared in an attempt to uncover the structural requirements for GAR FTase inhibition.[5] We recently prepared **2** (LY231514)[6] as a ring-contracted analogue of **1** in which



1

DDATHF (6RS)
Lometrexol (6R)

C-5 is deleted, C-6 is now joined to the pyrimidine ring, and the B ring is aromatic and therefore planar. A primary motivation for the synthesis of **2** was removal of the chiral center at C-6 of **1**. Interestingly, although **2** (which is also currently in Phase II clinical trials) is extremely active as an inhibitor of tumor growth *in vitro* and *in vivo*, its activity is primarily the consequence of inhibition of thymidylate synthase (TS), not GAR FTase.



2

In a continuation of our program directed toward the synthesis of inhibitors of folate-dependent biochemical processes as potential anticancer agents, we recently prepared **3**[7] as an isomer of **2** in which the ethylbenzoyl glutamate moiety is connected at C-6 rather than C-5. Since compound **3** showed only weak activity as a cell growth inhibitor of CCRF-CEM tumor cells (IC_{50} > 20 μg/mL), we considered the preparation of **4**, which should possess increased flexibility in ring B due to reduction of the 5,6 bond. In addition, compound **4** can be considered a ring-contracted analogue of **1** in which C-7 has been deleted and C-6 joined to N-8.

It is obvious that target molecule **4** differs from **3** only in the state of the reduction of the pyrrole ring. Compound **3** had been synthesized as shown in Scheme 1,[7]

† This paper is dedicated with affection and respect to Professor Yoshifumi Maki on the occasion of his retirement from Gifu Pharmaceutical University, Japan.

‡ Recipient of fellowships from Eli Lilly & Company (administered by the ACS Division of Organic Chemistry), Princeton University (Harold W. Dodds Fellowship), The Association for Women in Science Educational Foundation, and the Josephine De Kármán Foundation.

§ Feodore Lynen Postdoctoral Fellow of the Alexander von Humboldt Stiftung.

⊗ Abstract published in *Advance ACS Abstracts,* December 15, 1995.

(1) (a) Taylor, E. C.; Harrington, P. J.; Fletcher, S. R.; Beardsley, G. P.; Moran, R. G. *J. Med. Chem*. **1985**, *28*, 914. (b) Taylor, E. C.; Wong, G. S. K.; Fletcher, S. R.; Harrington, P. J.; Beardsley, G. P.; Shih, C. In *Chemistry and Biology of Pteridines*; Cooper, B. A., Whitehead, V. M., Eds.; Walter de Gruyter: Berlin, 1986; pp 61−64. (c) Moran, R. G.; Baldwin, S. W.; Taylor, E. C.; Shih, C. *J. Biol. Chem*. **1989**, *264*, 21047.

(2) (a) Moran, R. G.; Baldwin, S. W.; Taylor, E. C.; Grindey, G. B.; Shih, C. *J. Biol. Chem*., **1989**, *264*, 21047. (b) Baldwin, S. W.; Moran, R. G. *Proc. Am. Assoc. Cancer Res*., **1989**, *30*, A1901. (c) Beardsley, G. P.; Moroson, B. A.; Taylor, E. C.; Moran, R. G. *J. Biol. Chem*. **1989**, *264*, 328. (d) Beardsley, G. P.; Taylor, E. C.; Shih, C.; Poore, G. A.; Grindey, G. B.; Moran, R. G. *Proc. Am. Assoc. Cancer Res*. **1986**, *27*, A1027.

(3) Beardsley, G. P.; Taylor, E. C.; Shih, C.; Poore, G. A.; Grindey, G. B.; Moran, R. G. *Proc. Am. Assoc. Cancer Res*. **1986**, *27*, 259.

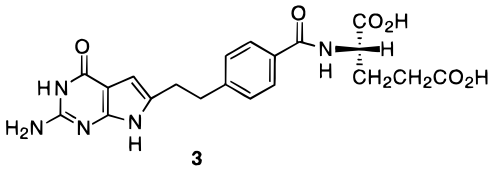
(4) Pizzorno, G.; Moroson, B. A.; Cashmore, A. R.; Taylor, E. C.; Beardsley, G. P. *Proc. Am. Assoc. Cancer Res*. **1988**, *29*, 281.

(5) (a) Taylor, E. C. *J. Heterocycl. Chem*., **1989**, *27*, 1. (b) Taylor, E. C. *Adv. Exper. Med. Biol*. **1993**, *338*, 387.

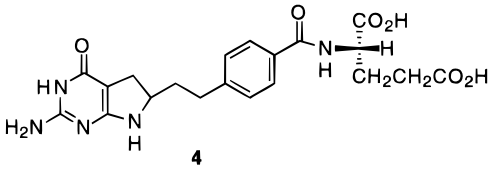
(6) Taylor, E. C.; Kuhnt, D.; Shih, C.; Rinzel, S. M.; Grindey, G. B.; Barredo, J.; Jannatipour, M.; Moran, R. G. *J. Med. Chem*., **1992**, *35*, 4450.

(7) Taylor, E. C.; Young, W. B.; Chaudhari, R.; Patel, H. H. *Heterocycles* **1993**, *36*, 1897.

0022-3263/96/1961-1261$12.00/0



3



4

and it was anticipated that **4** should be available through reduction of the pyrrole nucleus at some intermediate step in the synthesis. However, since it was not clear how or when this reduction would be accomplished, preliminary work focused on reduction of the model compound **5**.

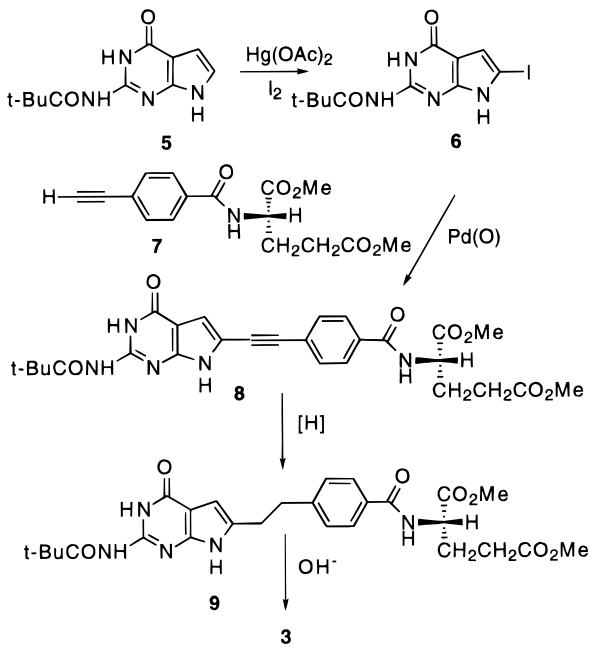
Literature reports on attempts to reduce pyrrolo[2,3-*d*]pyrimidines to 5,6-dihydro systems were not at all encouraging.[8] Attempts in our hands to reduce **5** to **10** by catalytic hydrogenation using several different catalysts (PtO_2, Pd/C, Pt/C) in a variety of solvents (MeOH, CH_2Cl_2, TFA) also failed; only recovered starting material was obtained. Attempted reduction using $NaBH_3CN$ was also unsuccessful. However, since it is well documented that the presence of an electron-withdrawing group on the pyrrole nitrogen greatly facilitates ring reduction,[9] we turned to an examination of appropriate N-7 substituted derivatives of **5**.

Treatment of **5** with 3 equiv of sodium hydride followed by addition of 2.1 equiv of acetyl chloride led, as expected, to the N-7 acetylated derivative **11** (90%, Scheme 2). Unfortunately, reduction of **11** with 50 psi of hydrogen for 12 h in a CH_2Cl_2/HOAc solution, using PtO_2 as catalyst, gave a mixture of deacetylated starting material **5** (47%) together with a low yield of the desired dihydro compound **13** (30%). Extensive modifications of the reaction conditions failed to improve the yield of **13**.

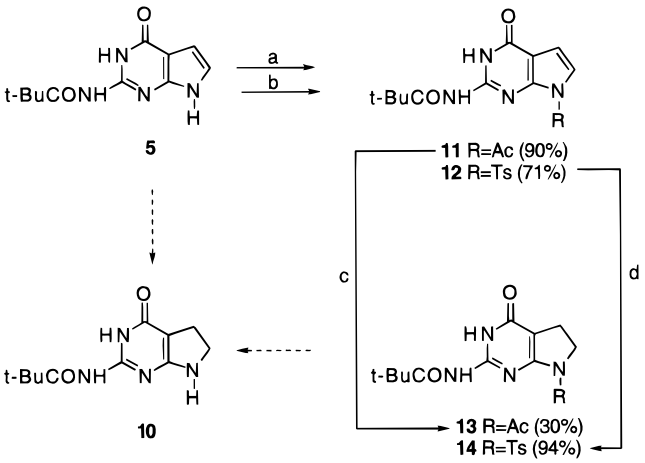
Due to the ease with which **11** underwent deacetylation during hydrogenation, the more durable tosyl protecting (and electron-withdrawing) group was chosen for further studies.[10] Treatment of **5** with 3.3 equiv of NaH and 1.0 equiv of TsCl in DMF produced **12** in 71% yield. After numerous attempts to reduce **12** using catalytic hydrogenation methods with a range of catalysts (PtO_2, 5% Pd/C, 5% Pt/C) it was finally found that with Pearlman's catalyst (10% $Pd(OH)_2$/C) in MeOH, under 1 atm of hydrogen for 5 h, reduction of **12** to **14** could be accomplished in high (94%) yield. Conversion of **14** to the depivaloylated/detosylated pyrrolopyrimidine **15** could then be carried out either with methanolic NH_3/metallic Na at −78 °C[11,12] or by heating at 100 °C for 18 h in concentrated H_2SO_4 (Scheme 3). We then turned our attention to the preparation of our desired target compound **4**.

Schemes 4 and 5 illustrate the reactions utilized in the synthesis of **4**. The 6-iodo compound **6**[7] was tosylated at

Scheme 1

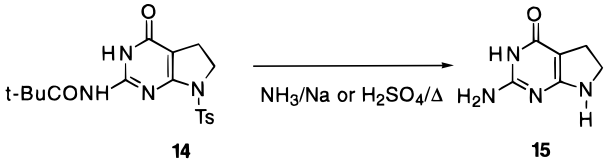


Scheme 2



Reagents: a: NaH, CH_3COCl; b: NaH, TsCl; c: PtO_2, 50 psı H_2; d: $Pd(OH)_2$, 14.4 psi H_2.

Scheme 3

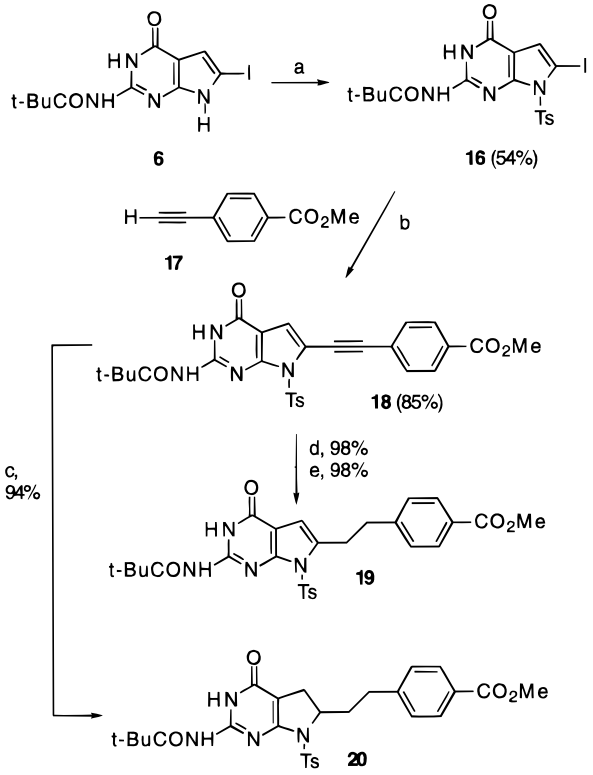


N-7 by treatment with 3.3 equiv of NaH, followed by addition of TsCl and acid workup to generate **16** (54%). Pd-catalyzed coupling of methyl 4-ethynylbenzoate (**17**)[13] with **16** in CH_2Cl_2 gave **18** in 85% yield.

Reduction of **18** under 1 atm of hydrogen using 20% $Pd(OH)_2$/C in 50% MeOH/acetone solution for 12 h gave **20** in 94% yield. With careful manipulation of the reaction conditions, the intermediate reduction product **19** could be isolated. For instance, a 9 mM solution of compound **18** in CH_2Cl_2 using $Pd(OH)_2$ as catalyst under 1 atm of hydrogen for 12 h produced solely compound **19**. Also, the partial reduction of **18** to form **19** could be

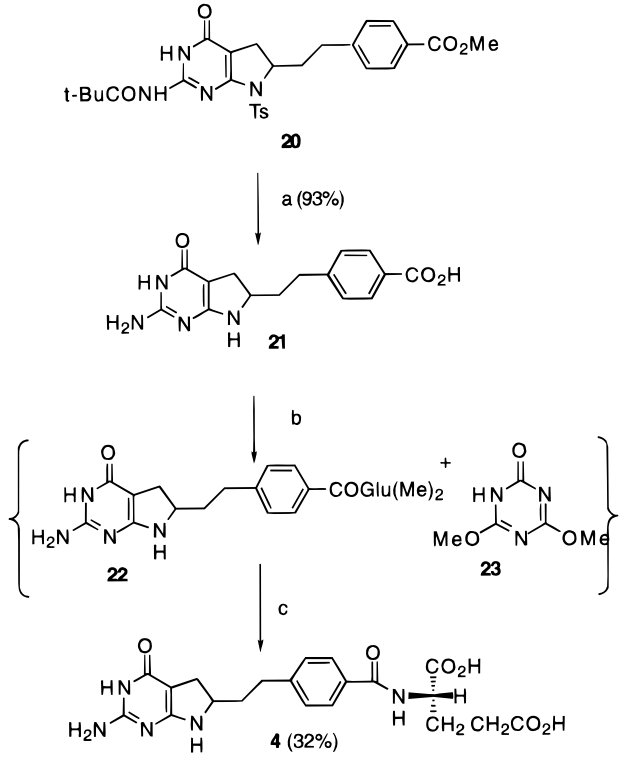
(8) Barnett, C. J.; Wilson, T. M. *Heterocycles* **1993**, *35*, 925.
(9) (a) Signaigo, F. K.; Adkins, H. *J. Am. Chem. Soc.*, **1936**, *58*, 709. (b) Rainey, J. L.; Adkins, H. *J. Am. Chem. Soc.* **1939**, *61*, 1104.
(10) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 2nd Ed.; John Wiley & Sons, NY, 1991.
(11) Roemmele, R. C.; Rapoport, H. *J. Org. Chem.* **1988**, *53*, 2367.
(12) (a) Schultz, A. G.; McCloskey, P. J.; Court, J. J. *J. Am. Chem. Soc.* **1987**, *109*, 6493. (b) Yamazaki, N.; Kibayashi, C. *J. Am. Chem. Soc.* **1989**, *111*, 1396.
(13) Austin, W. B.; Bilow, N.; Kelleghan, W. J.; Lau, K. S. Y. *J. Org. Chem.* **1981**, *46*, 2280.

Scheme 4



Reagents; a: NaH, TsCl; b: $Pd(PPh_3)_4$, CuI, NEt_3; c: 20% $Pd(OH)_2/C$, H_2, 50% MeOH/acetone; d: $Pd(OH)_2$, H_2, CH_2Cl_2; e: PtO_2, H_2, 5% MeOH/CH_2Cl_2

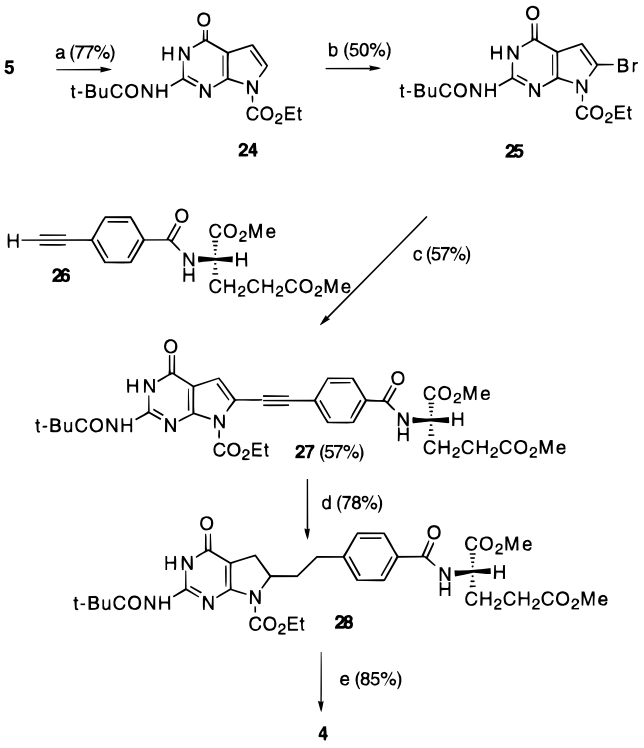
Scheme 5



Reagents: a: H_2SO_4, 100°C; b: dimethyl L-glutamate hydrochloride; 6-chloro-2,4-dimethoxy-1,3,5-triazine, N-methylmorpholine; c: 1M NaOH.

accomplished in 98% yield by exposure of **18** to 50 psi of hydrogen over PtO_2 in 5% MeOH/CH_2Cl_2. Further reduction of **19** to **20** with PtO_2 as the catalyst proved troublesome.

Scheme 6



Reagents; a: NaH, $ClCO_2Et$ b: NBS/CH_2Cl_2 c: $Pd(PPh_3)_4$, Et_3N, CuI d: H_2, Pd/C e: 1 N NaOH, then HCl

The protecting groups were removed by heating **20** in concentrated H_2SO_4 at 100 °C for 90 min followed by an alkaline workup to afford **21** (93%). Coupling of **21** with dimethyl L-glutamate was accomplished using the 6-chloro-2,4-dimethoxy-1,3,5-triazine[14] /N-methymorpholine technique, as we have described previously.[7] Since attempted isolation of **22** from the crude reaction mixture by column chromatography on silica gel afforded a 1:1 mixture of the desired product **22** and 2,4-dimethoxy-6(5*H*)-oxo-1,3,5-triazine (**23**), the crude reaction mixture was stirred with 1 N NaOH at room temperature for 12 h and then acidified. In this way compound **4** was isolated in 32% yield (from **21**) as a 1:1 mixture of diastereomers.

A more efficient synthesis of **4** resulted from the use of an ethoxycarbonyl N-7 protecting group rather than an *N*-Ts group (Scheme 6). Thus, reaction of 2-pivaloyl-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**5**) with 3 equiv of NaH, followed by 1 equiv of ethyl chloroformate, smoothly gave the 7-ethoxycarbonyl derivative **24**. In contrast to the behavior of **5** itself toward halogenation reactions, **24** underwent monobromination with *N*-bromosuccinimide at 0 °C in CH_2Cl_2 solution to give exclusively the 6-bromo derivative **25**. Standard Pd-catalyzed coupling of **25** with dimethyl *N*-(4-ethynylbenzoyl)-L-glutamate (**26**)[15] proceeded as expected to provide **27**; catalytic reduction of the acetylenic and pyrrole systems to give **28**, followed by final deprotection with dilute alkali, then gave target compound **4**.

Biological evaluation showed that **4** was an active inhibitor of the growth of human (CCRF-CEM) lympho-

(14) (a) Dudley, J. R.; Thurston, J. T.; Schaefer, F. C.; Holm-Hansen, D.; Hull, C. J.; Adams, P. *J. Am. Chem. Soc.* **1951**, *73*, 2986. (b) Kaminski, Z. J. *Tetrahedron Lett.* **1985**, *26*, 2901.

(15) Taylor, E. C.; Wong, G. S. K. *J. Org. Chem.* **1989**, *54*, 3618.

blastic leukemic cells *in vitro* (IC_{50} = 0.071 μg/mL). Thymidine (5 μM) did not protect the cells from the cell growth inhibitory effects of **4**, while hypoxanthine (100 μM) and aminoimidazolecarboxamide (300 μM) was each able to reverse the cell growth inhibitory effects of **4**. These data indicate that the activity of **4** (presumably following intracellular polyglutamylation) is the result of inhibition of GAR FTase. Thus, **4** is indeed an inhibitor of purine biosynthesis, as initially predicted. However, since both **2** (LY231514) and its dihydro derivative are thymidylate synthase (TS) inhibitors[8] and thus poor inhibitors of the purine biosynthetic pathway enzymes, it can be seen that very minor changes in structure can determine the preferred enzymatic inhibition target.

Experimental Section

2-(Pivaloylamino)-4(3*H*)-oxo-7-acetyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (11). In a 200 mL round-bottom flask, flushed with nitrogen, NaH (80% in mineral oil, 0.16 g, 6.45 mmol) in $DMF_{(anh)}$ (10 mL) was stirred at 0 °C. To this mixture was added 2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-pivaloyl-7-deazaguanine, **5**) (0.50 g, 2.15 mmol) dissolved in $DMF_{(anh)}$ (15 mL) dropwise over 15 min. The mixture was stirred an additional 30 min, and acetyl chloride (0.36 g, 4.60 mmol) was added dropwise over a period of 30 min. The mixture was warmed to rt and stirred overnight, $AcOH_{(gl)}$ was carefully added to neutralize the solution, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography over silica gel with 0.5% MeOH/CH_2Cl_2 as the eluent to yield 0.53 g (90%) of **11** as a gum: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.04 (s, 1 H), 8.06 (s, 1 H), 7.49 (d, 1 H, J = 3.8 Hz), 6.70 (d, 1 H, J = 3.8 Hz), 2.83 (s, 3 H), 1.35 (s, 9 H); MS *m*/*e* (relative intensity) 276 (M^+, 38), 234 (88), 150 (100); HRMS calcd for $C_{13}H_{16}N_4O_3$ 276.1224, found 276.1208.

2-(Pivaloylamino)-4(3*H*)-oxo-5,6-dihydro-7-acetyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (13). A mixture of **11** (0.10 g, 0.36 mmol), PtO_2 (0.05 g), and 5% MeOH/HOAc was shaken under 50 psi of hydrogen for 12 h. The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the product was purified by column chromatography over silica gel with 100% CH_2Cl_2 to 1% MeOH/CH_2Cl_2 as the eluent to yield 0.03 g (30%) of **13** as a white solid and 0.04 g (47%) of **5**. **13**: mp 230−232 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.84 (s, 1 H), 8.12 (s, 1 H), 4.04 (t, 2 H, J = 9.0 Hz), 2.86 (t, 2 H, J = 9.0 Hz), 2.52 (s, 3 H), 1.34 (s, 9 H); MS *m*/*e* (relative intensity) 278 (M^+, 50), 236 (100), 151 (60); HRMS calcd for $C_{13}H_{18}N_4O_3$ 278.1381, found 278.1372. Anal. Calcd for $C_{13}H_{18}N_4O_3$: C, 56.10; H, 6.52; N, 20.13. Found: C, 55.78; H, 6.50; N, 19.89.

2-(Pivaloylamino)-4(3*H*)-oxo-7-tosyl-7*H*-pyrrolo[2,3-*d*]-pyrimidine (12). In a 200 mL round-bottom flask, flushed with nitrogen, NaH (80% in mineral oil, 0.21 g, 7.08 mmol) in $DMF_{(anh)}$ (5 mL) was stirred at 0 °C. To this mixture **5** (0.50 g, 2.14 mmol) dissolved in $DMF_{(anh)}$ (5 mL) was added dropwise over 15 min. The mixture was stirred an additional 30 min and TsCl (0.40 g, 2.14 mmol) in $DMF_{(anh)}$ (5 mL) was added dropwise. The mixture was warmed to rt and stirred overnight, $AcOH_{(gl)}$ was carefully added to neutralize the solution, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography over silica gel with 1% MeOH/CH_2Cl_2 as the eluent to yield 0.59 g (71%) of **12** as a white powder: mp 279−280 °C. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.16 (s, 1 H), 10.80 (s, 1 H), 8.14 (d, 2 H, J = 8.3 Hz), 7.40 (d, 2 H, J = 8.3 Hz), 7.35 (d, 1 H, J = 3.8 Hz), 6.61 (d, 1 H, J = 3.8 Hz), 2.39 (s, 3 H), 1.25 (s, 9 H); IR (KBr) 3224, 3153, 3133, 2857, 1654, 1591, 1541, 1372, 1131, 647, 619 cm^{-1}; MS *m*/*e* (relative intensity) 388 (M^+, 56), 232 (72), 149 (100), 107 (27), 99 (27), 85 (81); HRMS calcd for $C_{18}H_{20}N_4O_4S$ 388.1207, found 388.1208. Anal. Calcd for $C_{18}H_{20}N_4O_4S$: C, 55.65; H, 5.19; N, 14.43. Found: C, 55.57; H, 5.23; N, 14.40.

2-(Pivaloylamino)-4(3*H*)-oxo-5,6-dihydro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (14). A mixture of **12** (0.10 g, 0.26 mmol), 10% $Pd(OH)_2$/C (0.20 g), and MeOH (5 mL) was stirred under 1 atm of H_2 for 5 h. The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the product was purified by column chromatography over silica gel with 100% CH_2Cl_2 to 1% MeOH/CH_2Cl_2 as the eluent to yield 0.095 g (94%) of **14** as a white solid: mp > 237 °C dec; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.86 (s, 1 H), 8.80 (s, 1 H), 7.84 (d, 2 H, J = 8.1 Hz), 7.31 (d, 2 H, J = 8.1 Hz), 3.95 (t, 2 H, J = 9.0 Hz), 2.83 (t, 2 H, J = 9.0 Hz), 2.43 (s, 3 H), 1.33 (s, 9 H); IR (KBr) 3542, 3408, 3161, 2964, 2929, 1661, 1563, 1393, 1352, 1168, 1139, 654, 534 cm^{-1}; MS *m*/*e* (relative intensity) 390 (M^+, 43), 306 (10), 151 (18), 91 (26), 78 (100); HRMS calcd for $C_{18}H_{22}N_4O_4S$ 390.1364, found 390.1355. Anal. Calcd for $C_{18}H_{22}N_4O_4S$: C, 55.37; H, 5.68; N, 14.35. Found: C, 55.08; H, 5.63; N, 14.13.

2-Amino-4(3*H*)-oxo-5,6-dihydro-7*H*-pyrrolo[2,3-*d*]pyrimidine (15). Method A. In a 50 mL, two-neck round-bottom flask a solution of **14** (200 mg, 0.51 mmol) in MeOH (5 mL) was added to $NH_{3(liq)}$ (20 mL) at −78 °C. To this mixture was added Na (590 mg, 25.7 mmol) in increments. The reaction was stirred for 30 min at −78 °C, quenched with $NH_4Cl_{(aq)}$ (2 mL), warmed to rt, and acidified to pH 4 with $AcOH_{(gl)}$. The solvent was removed under reduced pressure, H_2O was added and the precipitate was filtered, washed with H_2O (2 × 5 mL), CH_2Cl_2 (2 × 5 mL), Et_2O (2 × 5 mL), and hexanes (2 × 5 mL) and dried overnight in a vacuum oven. The crude product was recrystallized from H_2O (5 mL) to yield 43 mg (55%) of **15** as a white solid, mp > 272 °C dec, (lit.[16] mp 278−280 °C dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 9.81 (s, 1 H), 6.28 (s, 2 H), 3.32 (t, 2 H, J = 8.8 Hz), 2.54 (t, 2 H, J = 8.8 Hz), 1.86 (s, 1 H); MS *m*/*e* (relative intensity) 152 (M^+, 15), 78 (92); HRMS calcd for $C_6H_8N_4O_1$ 152.0700, found 152.0718. Anal. Calcd for $C_6H_8N_4O$: C, 47.36; H, 5.30; N, 36.82. Found: C, 47.62; H, 5.51; N, 36.60.

Method B. A mixture of **14** (0.043 g, 0.11 mmol) and H_2SO_4 (2 mL) was heated to 100 °C for 18 h. The mixture was cooled, ice−water (5 mL) was added, the solution was neutralized to pH 4 with 1 M NaOH, the solvent was removed under reduced pressure, and the remaining solid was dried overnight in a vacuum oven. The product was not further purified. Spectral data were identical with those reported above.

2-(Pivaloylamino)-4(3*H*)-oxo-6-iodo-7-tosyl-7*H*-pyrrolo-[2,3-*d*]pyrimidine (16). To a mixture of NaH (95% in mineral oil, 0.48 g, 19.1 mmol) and $DMF_{(anh)}$ (10 mL) at 0 °C was added 2-(pivaloylamino)-4(3*H*)-oxo-6-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidine (2-pivaloyl-8-iodo-7-deazaguanine, **6**)[5] (2.09 g, 5.8 mmol) in $DMF_{(anh)}$ (15 mL) dropwise over 30 min. The mixture was stirred an additional 30 min, and TsCl (3.3 g, 17.4 mmol) was added dropwise. The mixture was warmed to rt and stirred overnight, $AcOH_{(gl)}$ was carefully added to neutralize the solution, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography over silica with 100% CH_2Cl_2 to 1% MeOH/CH_2Cl_2 as the eluent to yield 1.6 g (54%) of **16** as a white powder: mp 219−221 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.12 (s, 1 H), 9.01 (s, 1 H), 7.95 (d, 2 H, J = 8.1 Hz), 7.32 (d, 2 H, J = 8.1 Hz), 7.02 (s, 1 H), 2.43 (s, 3 H), 1.34 (s, 9 H); IR (KBr) 3203, 2950, 2915, 2865, 2851, 1661, 1590, 1541, 1407, 1379, 1189, 1147, 1084 cm^{-1}; MS *m*/*e* (relative intensity) 514 (M^+, 46), 388 (81), 360 (100), 276 (55), 233 (54), 149 (93); HRMS calcd for $C_{18}H_{19}IN_4O_4S$ 514.0174, found 514.0173.

Methyl 4-[2-(2-(Pivaloylamino)-4(3*H*)-oxo-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)ethynyl]benzoate (18). In a 100 mL, two-neck round-bottom flask equipped with a reflux condensor were combined **16** (0.30 g, 0.58 mmol), methyl 4-ethynylbenzoate (**17**)[13] (0.14 g, 0.87 mmol), tetrakis(triphenylphosphine)palladium(0) (0.07 g, 0.058 mmol), CuI (0.01 g, 0.058 mmol), Et_3N (0.24 g, 2.32 mmol), and CH_2Cl_2 (15 mL), and the mixture was heated to reflux for 3 h. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel with 0.5%

(16) Granik, V. G.; Glushkov, R. G., *Khim.-Farm. Zh.* **1967**, *1*, 16.

MeOH/CH_2Cl_2 as the eluent to give 0.27 g (85%) of **18** as a white solid: mp 228−230 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.15 (s, 1 H), 8.81 (s, 1 H), 8.07 and 7.98 (AA′BB′, 4 H), 7.67 and 7.31 (AA′BB′) 4 H), 7.0 (s, 1 H), 3.95 (s, 3 H), 2.43 (s, 3 H), 1.36 (s, 9 H); MS *m/e* (relative intensity) 546 (M^+, 100), 392 (89), 307 (96), 265 (30), 91 (80); HRMS calcd for $C_{28}H_{26}N_4O_6S$ 546.1575, found 546.1566.

Methyl 4-[2-(2-(Pivaloylamino)-4(3*H*)-oxo-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)ethyl]benzoate (19). A mixture of **18** (0.38 g, 0.70 mmol), PtO_2 (0.38 g), and 5% MeOH/CH_2Cl_2 (30 mL) was shaken under 58 psi of H_2 for 24 h. The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the product was purified by column chromatography over silica gel with 2% MeOH/CH_2Cl_2 as the eluent to yield 0.38 g of **19** (98%) as an off-white solid: mp 254 °C dec; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.00 (s, 1 H), 8.16 (s, 1 H), 7.97 and 7.85 (AA′BB′, 4 H), 7.30 and 7.29 (AA′BB′, 4H), 6.41 (s, 1 H), 3.91 (s, 3 H), 3.25 (t, 2 H, J = 8.3 Hz), 3.09 (t, 2 H, J = 8.3 Hz), 2.41 (s, 3 H), 1.34 (s, 9 H); MS *m/e* (relative intensity) 550 (M^+, 9), 402 (38), 401 (100), 247 (40); HRMS calcd for $C_{28}H_{30}N_4O_6S$ 550.1888, found 550.1893.

A mixture of **18** (0.10 g, 0.18 mmol), 20% $Pd(OH)_2$/C (0.030 g), and CH_2Cl_2 (20 mL) was stirred under 1 atm of H_2 for 12 h. The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the product was purified by column chromatography over silica gel with 2% MeOH/CH_2Cl_2 as the eluent to yield 0.096 g (98%) of **19** as an off-white solid. The spectral data were identical with those obtained for the product of the first reduction reported above.

Methyl 4-[2-(2-(Pivaloylamino)-4(3*H*)-oxo-5,6-dihydro-7-tosyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)ethyl]benzoate (20). A mixture of **18** (0.09 g, 0.16 mmol), 20% $Pd(OH)_2$/C (45 mg), and 50% MeOH/acetone (10 mL) was stirred under 1 atm of H_2 for 12 h. The mixture was filtered, the filtrate was evaporated to dryness under reduced pressure, and the product was purified by column chromatography over silica gel with 1% MeOH/CH_2Cl_2 as the eluent to yield 0.085 g (94%) of **20** as a white powder: mp 120−122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.85 (s, 1 H), 8.83 (s, 1 H), 7.96 and 7.71 (AA′BB′, 4 H), 7.26 and 7.24 (AA′BB′, 4 H), 4.37−4.34 (m, 1 H), 3.91 (s, 3 H), 2.89 (dd, 1 H, J = 10.1, 15.8 Hz), 2.77−2.69 (m, 2 H), 2.59 (dd, 1 H, J = 3.8, 15.8 Hz), 2.41 (s, 3 H), 2.25−2.20 (m, 1 H), 2.12−2.05 (m, 1 H), 1.32 (s, 9 H); IR (KBr) 3556, 3189, 2964, 2943, 2915, 1710, 1661, 1605, 1563, 1386, 1351, 1274, 1154 cm^{-1}; MS *m/e* (relative intensity) 552 (M^+, 42), 389 (20), 247 (28), 235 (100), 151 (31); HRMS calcd for $C_{28}H_{32}N_4O_6S$ 552.2045, found 552.2026.

4-[2-(2-Amino-4(3*H*)-oxo-5,6-dihydro-7*H*-pyrrolo[2,3-*d*]-pyrimidin-6-yl)ethyl]benzoic Acid (21). A solution of **20** (0.31 g, 0.56 mmol) and $H_2SO_{4(conc)}$ (4 mL) was heated at 100 °C for 90 min and cooled, ice−water (3 mL) was added, and the precipitate was removed by filtration. The collected solid was placed in H_2O (1 mL), 1 N NaOH was added until the solution went clear and the mixture was filtered. To the filtrate was added $AcOH_{(gl)}$ (1 mL), and the precipitate was collected by filtration, washed with H_2O (2 × 5 mL), Et_2O (2 × 10 mL), and hexanes (2 × 10 mL) and dried in a vacuum oven overnight to yield 0.16 g (93%) of **21** as a white solid: mp > 257 °C slow dec; ^{1}H NMR (DMSO-d_6, 270 MHz) δ 13.0 (s, 1 H), 9.65 (s, 1 H), 7.79 and 7.28 (AA′BB′, 4 H), 6.59 (s, 1 H), 6.21 (s, 2 H), 3.67−3.61 (m, 1 H), 2.71 (dd, 1 H, J = 14.2, 10.0 Hz), 2.64−2.53 (m, 2 H), 2.25 (dd, 1 H, J = 14.2, 6.9 Hz), 1.87−1.67 (m, 2H); IR (KBr) 3408, 3338, 3140, 2922, 1640, 1598, 1570, 1252, 1168 cm^{-1}; MS *m/e* (relative intensity) 300 (M^+, 34), 163 (45), 151 (100); HRMS calcd for $C_{15}H_{16}N_4O_3$ 300.1224, found 300.1240.

2-(Pivaloylamino)-4(3*H*)-oxo-7-(ethoxycarbonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (24). A solution of 2-(pivaloylamino)-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidine (**5** , 4.68 g, 20 mmol) in $DMF_{(anh)}$ (200 mL) was placed into an ice bath and treated with NaH (420 mg, 80% suspension in mineral oil, 22 mmol, 1.1 equiv). The mixture was stirred vigorously until the evolution of H_2 ceased completely. To make certain that deprotonation was complete, the purple-colored suspension was stirred for an additional 1 h. Ethyl chloroformate (2.1 mL, 2.39 g, 22 mmol) was added in one portion, and the reaction mixture was allowed to come to rt overnight with stirring and then poured into ice cold water (400 mL). The cloudy solution was extracted as rapidly as possible with EtOAc (3 × 150 mL). The combined organic extracts were washed with brine, dried ($MgSO_4$), and evaporated to dryness to leave a purple solid. Purification by column chromatography over SiO_2 with 50% EtOAc/hexanes as the eluent gave 4.7 g (77%) of **24** as a colorless white powder: mp 186−188 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.14 (br s, 1 H,), 8.60 (br s, 1 H), 7.23 (d, 1 H, J = 4.1), 6.64 (d, 1 H, J = 4.2), 4.42 (q, 2 H), 1.40 (t, 3 H, J = 7.0), 1.28 (s, 9 H); ^{13}C-NMR ($CDCl_3$, 60 MHz) δ 14.1, 26.9, 40.2, 63.9, 105.7, 108.3, 120.8, 147.7, 148.9, 157., 180.0; IR (KBr) 3564, 3463, 3187, 3162, 2982, 2958, 2926 & 2854, 1768, 1753, 1706, 1674, 1609, 1559, 1426, 1374, 1336, 1326, 1289, 1268, 1249, 1166, 1152, 1013 (w), 784, 730 cm^{-1}; MS *m/e* (relative intensity) 306 (100%, M^+), 222 (22%, M^+ − 84, − ^{t}BuCO), 150 (85%, $C_6H_6N_4O$), 133 (21%), 125 (36%), 111 (60%), 97 (90%), 83 (74%), 71 (90%); HRMS calcd for $C_{14}H_{18}N_4O_4$ 306.1328, found 306.1330. Anal. Calcd for $C_{14}H_{18}N_4O_4$: C: 54.89; H, 5.92; N, 18.29. Found: C, 54.67; H, 5.85; N, 18.43.

2-(Pivaloylamino)-4(3*H*)-oxo-6-bromo-7-(ethoxycarbonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (25). To a stirred solution of **24** (4.70 g, 15.3 mmol) in dry CH_2Cl_2 (150 mL) was added over a period of 1.5 h a solution of NBS (2.73 g, 15.3 mmol, freshly recrystallized from hot H_2O) in dry CH_2Cl_2 (200 mL) at 0 °C. To prevent deterioration of the product the solution was protected from light by covering the flask with aluminum foil. Stirring was continued for an additional 3 h at 0 °C. During this time the color changed from bright yellow to dark yellow with a green tinge. The reaction was quenched by pouring the contents of the flask onto ice-cold 5% aqueous sodium thiosulfate (350 mL). The organic layer was washed (3 × H_2O, 1 x brine), dried ($MgSO_4$), and evaporated. The remaining deep purple solid was purified by flash chromatography (SiO_2 , EtOAc/hexanes 2:1) to give 2.94 g (50%) of **25** as a fluffy colorless solid: mp 211−213 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.11 (br s, 1 H), 8.50 (br s, 1 H), 6.87 (s, 1 H), 4.52 (q, 2 H), 1.47 (t, 3 H, J = 7.2), 1.31 (s, 9 H); ^{13}C-NMR ($CDCl_3$, 68 MHz) δ 14.0, 26.9, 40.3, 64.9, 103.1, 107.6, 110.5, 147.6, 148.5, 149.4, 155.9, 180.2. IR (KBr) 3174 (NH), 2979 & 2919 (CH), 1771 (C═O), 1732 (w), 1663, 1609, 1557 (w), 1413 (w), 1355, 1296, 1257, 1146, 769, 760 cm^{-1}; MS *m/e* (relative intensity) 386, 384 (48%, 45%, M^+); 314, 312 (53%, 51%, M^+ − 72, − $EtCO_2$); 258, 256 (39%, 38%, M^+ − 128, − H_2C═CMe_2 , − $EtCO_2$); 230, 228 (89%, 89%, M^+ − 156, $C_6H_5BrN_4O$); 188, 186 (28%, 32%); 187, 185 (25%, 26%); 150 (68%, $C_6H_6N_4O$); 105 (23%); 85 (100%); HRMS calcd for $C_{14}H_{17}BrN_4O_4$ 384.0432, found 384.0449. Anal. Calcd for $C_{14}H_{17}BrN_4O_4$: C, 43.65; H, 4.45; N, 14.54; Br, 20.74. Found: C, 43.84; H, 4.42; N, 14.45; Br, 20.57.

Dimethyl *N*-{4-[2-(2-(Pivaloylamino)-4(3*H*)-oxo-7-(ethoxycarbonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)ethynyl]-benzoyl}-l-glutamate (27). To a suspension of **25** (385 mg, 1 mmol) in dry MeCN (15 mL)[17] were added freshly prepared $Pd(PPh_3)_4$ (58 mg, 50 mmol, 5 mol %), triethylamine (1 mL), and CuI (5 mg, 25 mmol, 2.5 mol %). The resulting mixture was stirred for 15 min. at 50 °C, and then a solution of dimethyl *N*-(4-ethynylbenzoyl)-L-glutamate (**26**)[15] (455 mg, 1.5 mmol, 1.5 equiv) in MeCN was introduced in one portion via syringe. After a short time all the solid material went into solution, and the color of the mixture turned olive. The reaction mixture was heated under reflux until **25** had been completely consumed (TLC, *ca*. 6 h). To remove solid impurities the almost black mixture was filtered through a fritted glass funnel. The filtrate was evaporated to dryness to afford a black gum which was dissolved in EtOAc (50 mL), washed (3 × H_2O, 1 × brine), dried ($MgSO_4$), and evaporated again to afford a dark brown gum. Column chromatography over SiO_2 with 1.5% MeOH in CH_2Cl_2 as the eluent gave 350 mg (57%) of **27** as a pale yellow microcrystalline solid: mp. 91−93 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 12.17 (br s, 1 H), 8.91 (br s, 1 H), 7.80 (d, 2 H, J = 8.2), 7.50 (d, 2 H, J = 8.3), 7.36 (d, 1 H, J = 7.5), 6.99 (s, 1 H), 4.79 (ddd, 1 H, J = 4.9, 7.7, 12.5), 4.48

(17) Acetonitrile was purchased from Aldrich (sure seal grade). This coupling step failed with standard-grade J. T. Baker acetonitrile.

(q, 2 H, $J = 7.2$), 3.75 (s, 3 H), 3.63 (s, 3 H), 2.48 (m, 2 H), 2.30 (m, 1 H), 2.16 (m, 1 H),1.40 (t, 3 H, $J = 7.2$), 1.28 (s, 9 H). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.8, 27.5, 27.6, 30.9, 41.0, 52.6, 53.0, 53.3, 65.0, 83.9, 94.3, 107.9, 115.0, 116.2, 128.0, 131.9, 132.6, 133.9, 149.3, 149.6, 150.8, 156.9, 167.0, 173.0, 174.3, 181.1; IR (KBr) 3440 (NH), 2954, 2920, 1737, 1676, 1605, 1556, 1540, 1484 (w), 1447 (w), 1420 (w), 1372 (w), 1303, 1245, 1150, 1106 (w), 775 (w) cm^{-1}; MS *m/e* (relative intensity) 607 (17%, M^+), 591 (33%, $M^+ - 16$), 577 (23%, $M^+ - 30$), 563 (100%), 549 (14%), 535 (15%), 479 (18%), 461 (24%), 389 (17%), 305 (14%), 304 (17%), 303 (13%), 277 (27%), 276 (27%), 249 (10%). Anal. Calcd for $C_{30}H_{33}N_5O_9$: C, 59.30; H, 5.47; N, 11.53; O, 23.70. Found: C, 59.59; H, 5.51; N, 11.64.

Dimethyl *N*-{4-[2-(2-(Pivaloylamino)-4(3*H*)-oxo-5,6-dihydro-7-(ethoxycarbonyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl}-L-glutamate (28). A solution of the coupling product **27** (150 mg, 0.25 mmol) and 10% Pd/C (150 mg) in methanol (50 mL) was hydrogenated in a Parr shaker at 50 psi of H_2 and at rt for 5 h. The catalyst was removed by means of a fritted glass filter, and the collected filtrate was evaporated to dryness. The residual greenish gum was purified by chromatography over SiO_2 with 5% MeOH in CH_2Cl_2 as the eluent to give 120 mg (78%) of **28** as a colorless glassy syrup, mp 105−108 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 11.95 (br s, 1 H), 9.40 (br s, 1 H), 7.73 (d, 2 H, $J = 7.8$), 7.22 (d, 2 H, $J = 7.7$), 7.07 (d, 1 H, $J = 7.5$), 4.80 (dd, 1 H, $J = 7.2$, 12.3), 4.23 (m, 2 H), 4.06 (m, 1 H), 3.75 (s, 3 H), 3.62 (s, 3 H), 3.07 (dd, 1 H, J=10.2, 15.9), 2.72 (m, 3 H), 2.56 (m, 2 H), 2.32 (m, 1 H),1.50−2.25 (m, 3 H), 1.28 (s, 9 H), 1.27 (t, 3 H, $J = 7.1$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.0, 27.3, 27.1, 27.6, 28.4, 30.9, 36.2, 40.7, 52.5, 52.8, 53.2, 59.2, 91.3, 100.7, 128.0, 129.0, 132.2 , 145.6, 152.1, 160.5, 167.7, 173.2, 174.2, 181.3; IR (KBr) 3420, 3240, 2981, 1744, 1673, 1608, 1556, 1373, 1305, 1246 (w), 1214 (w), 1153, 1108 (w), 777 (w) cm^{-1}; MS *m/e* (relative intensity) 613 (15%, M^+), 584 (100%, $M^+ - 29$, − OMe), 570 (55%, $M^+ - 43$), 556 (78%, $M^+ - 57$, − ^{t}Bu), 409 (26%), 395 (27%), 381 (47%); HRMS calcd for $C_{30}H_{39}N_5O_9$ 613.2787, found 613.2767. Anal. Calcd for $C_{30}H_{39}N_5O_9$: C, 58.72; H, 6.41; N, 11.42. Found: C, 58.47; H, 6.36; N, 11.35.

***N*-{4-[2-(2-Amino-4(3*H*)-oxo-5,6-dihydro-7*H*-pyrrolo-[2,3-*d*]pyrimidin-6-yl)ethyl]benzoyl}-L-glutamic Acid (4). Method A.** To a solution of **21** (130 mg, 0.43 mmol) in DMF (8 mL) was added 6-chloro-2,4-dimethoxy-1,3,5-triazine (0.08 g, 0.45 mmol) and *N*-methylmorpholine (0.052 g, 0.52 mmol) at 0 °C. After the mixture was stirred at 0 °C for 2 h, *N*-methylmorpholine (0.052 g, 0.52 mmol) and dimethyl L-glutamate hydrochloride (0.095 g, 0.45 mmol) were added all at once. The mixture was stirred at 0 °C for 2 h, at rt for 12 h, the solvent was removed under reduced pressure, and the crude product was purified by column chromatography on silica gel with 5% MeOH/CH_2Cl_2 as the eluent to yield 92 mg of a 1:1 mixture of **22** [^{1}H NMR (DMSO-d_6, 270 MHz) δ 9.56 (s, 1 H), 8.64 (d, 1 H, $J = 7.3$ Hz), 7.76 (d, 2 H, $J = 8.3$ Hz), 7.28 (d, 2 H, $J = 8.3$ Hz), 6.60 (s, 1 H), 6.17 (s, 2 H), 4.42−4.39 (m, 1 H), 3.63 (m, 1 H), 3.60 (s, 3 H), 3.54 (s, 3 H), 2.71 (dd, 1 H, $J = 14.2$, 9.9 Hz), 2.66−2.60 (m, 2 H), 2.42−2.38 (m, 2 H), 2.25 (dd, 1 H, $J = 14.2$, 6.6 Hz), 2.08−1.94 (m, 2 H), 1.75−1.67 (m, 2 H); FABMS calcd MH^+ for $C_{22}H_{28}N_5O_6$ 458.2040, found 458.2073] and 2,4-dimethoxy-6(5*H*)-oxo-1,3,5-triazine (**23**) (37%).

To the 1:1 mixture of **22** and **23** (50 mg), 1 N NaOH (10 mL) was added, and the mixture was stirred at rt for 12 h, $HCl_{(concd)}$ (1 drop) was added, the precipitate was filtered, and the crude product was washed with H_2O (2 × 2 mL), Et_2O (2 × 10 mL), and hexanes (2 × 10 mL) and dried in a vacuum oven overnight to yield 32 mg (32% from **21**) of **4** as a white solid: mp 241−243 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 12.30 (br s, 2 H), 9.65 (br s, 1 H), 8.47 (d, 1 H, $J = 7.6$), 7.77 (d, 2 H, $J = 7.9$), 7.29 (d, 2 H, $J = 7.9$), 6.66 (br s, 1 H), 6.24 (br s, 2 H), 4.35 (dd, 1 H, $J = 8.8$, 12.8), 3.65 (m, 1 H), 2.73 (dd, 1 H, $J = 10.1$, 14.1), 2.63 (m, 2 H), 2.31 (m, 3 H), 2.03 (m, 1 H), 1.91 (m, 1 H), 1.70 (m, 2 H); ^{13}C-NMR (DMSO-d_6, 75 MHz) δ 27.1, 31.2, 31.5, 32.2, 39.8 , 53.0, 56.4, 85.4, 128.6, 129.2, 132.5, 146.7, 157.8, 159.8, 167.4, 170.2, 174.6, 175. IR (KBr) 3368, 2980, 2851, 1696 (sh), 1640, 1541, 1504 (w), 1445, 1319, 1259, 1189, 1155, 762, 654 cm^{-1}; FABMS, calcd for MH^+ $C_{20}H_{24}N_5O_6$ 430.1728, found 430.1727. Anal. Calcd for $C_{20}H_{23}N_5O_6$: C, 55.94; H, 5.40; N, 16.31. Found: C, 55.66; H, 5.38; N, 16.00.

Method B. A solution of **28** (92 mg, 0.15 mmol) in 1 N NaOH (3 mL) was stirred for 48 h at rt. The material went almost immediately into solution. At the end of the reaction time a clear pale yellow liquid was obtained. The product was precipitated by careful addition of 1 N HCl. The gel thus obtained was stirred for a short time to facilitate crystallization. The resulting powder was collected by filtration, washed with ice−water (3 × 2 mL), and dried at rt (high vacuum) to afford 55 mg (85%) of **4** as a off-white microcrystalline powder, mp. 241−243 °C. Its spectral properties were identical with those observed for the product obtained by Method A above.

Acknowledgment. We are deeply indebted to the late Dr. G. B. Grindey of Eli Lilly & Co. for the biological evaluations reported herein. Gratitude is expressed to Drs. Chuan Shih and Charles J. Barnett of Lilly for many stimulating discussions, and to Lilly for financial support of this work.

JO951471K