A Novel Synthetic Approach to Pyrrolo[2,3-*d*]pyrimidine Antifolates[1]

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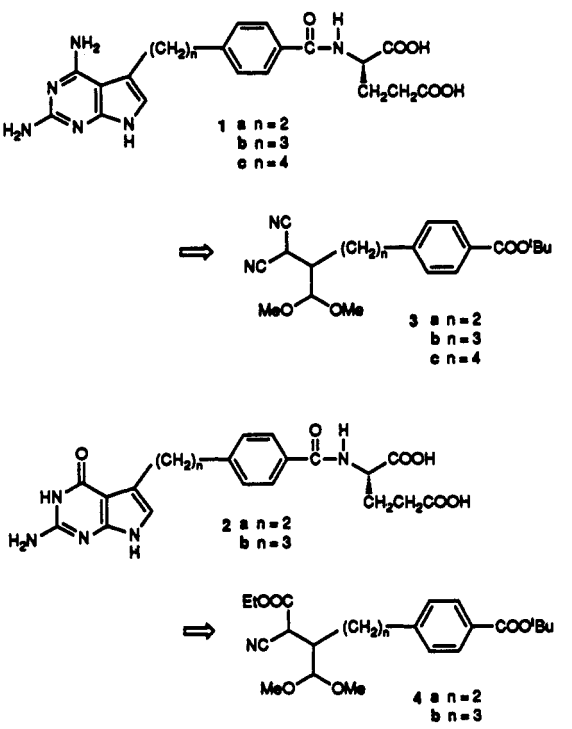
A novel and efficient synthetic method for the synthesis of pyrrolo[2,3-*d*]pyrimidine antifolates is described. The key reaction of this method is the photo-initiated free radical addition of bromomalononitrile or ethyl bromocyanoacetate to an enol ether to afford the backbone skeleton of the targeted antifolate molecule. The key intermediates **3** or **4** are smoothly converted to the pyrrolo[2,3-*d*]pyrimidine antifolates **1** or **2** in three steps and in high overall yield.

Folic acid is an important vitamin related to the biosynthesis of nucleic acids and amino acids. Dihydrofolate reductase (DHFR) is one of the most important enzymes involved in the biosynthesis.[2] Methotrexate (MTX) strongly inhibits DHFR and has been applied clinically as an anticancer agent since 1953. Although MTX is active against acute lymphoblastic leukemia and choriocarcinoma, it has limitations because of toxicity and lack of efficacy as a single agent against most human solid tumors.[3] In order to overcome these limitations, a large number of analogues of MTX have been synthesized and evaluated.[4] However, although several promising candidates have reached experimental trial, none has at this point succeeded in replacing MTX for general clinical use.

Fused heteroaromatic rings of previously known antitumor active antifolates had been mostly limited to pteridines or related 6-6 fused heterocyclic rings. Antifolates with 6-5 fused ring system have attracted little attention since purine-based compounds did not show any antitumor activities.[5] However, our first report[6,7] on the 5-substituted pyrrolo[2,3-*d*]pyrimidine antifolates and their potent growth inhibitory activity against tumor cells showed eminent possibilities for a 6-5 fused ring system as a heterocyclic moiety of antifolates.

In this paper, we report a novel, efficient, and alternative method for the synthesis of pyrrolo[2,3-*d*]pyrimidines, featuring a photo-initiated free radical addition of bromomalononitrile or ethyl bromocyanoacetate to the corresponding enol ethers as a key step. Using this methodology, we synthesized pyrrolo[2,3-*d*]pyrimidine antifolates with two, three, or four carbons in the bridge region and either a 4-amino or 4(3*H*)-oxo substituent in the heteroaryl moiety.

Scheme I



Our method involves initial construction of an acyclic precursor followed by closure of the heterocyclic ring. Retrosynthetic analysis led to the dimethoxymethyl compounds **3** and **4** as possible acyclic intermediates (Scheme I).

In order to prepare these intermediates, we adopted the photo-initiated free radical addition of bromomalononitrile to olefins. Although this reaction was reported in 1967,[8] enol ethers have not been utilized as the olefinic partner. If bromide **6** (EWG = CN or COOEt) added to the enol ether regioselectively,[9] the reaction would directly give

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(1) Presented at the 202nd National Meeting of the American Chemical Society, New York City, NY, August 25–30, 1991.

(2) Blakley, R. L. In *Folates and Pterins*; Blakley, R. L., Benkovic, S. J., Eds.; John Wiley & Sons: New York, 1984; Vol. 1, pp 191–253.

(3) Chabner, B. A.; Myers, C. E. In *Cancer: Principles and Practice of Oncology*, 3rd ed.; DeVita, V. T., Jr., Hellman, S., Rosenberg, S. A., Eds.; Lippincott: Philadelphia, 1989; Vol. 1, pp 350–359.

(4) (a) Montgomery, J. A.; Piper, J. R. In *Folate Antagonists as Therapeutic Agents*; Sirotnak, F. M., Burchall, J. J., Ensminger, W. D., Montgomery, J. A., Eds.; Academic: Orlando, 1984; Vol. 1, pp 219–260. (b) Rahman, L. K. A.; Chharbra, S. R. *Med. Res. Rev.* **1988**, *8*, 95. (c) Rosowsky, A. *Prog. Med. Chem.* **1989**, *26*, 1.

(5) Weinstock, L. T.; Grabowski, B. F.; Cheng, C. C. *J. Med. Chem.* **1970**, *13*, 995.

(6) Miwa, T.; Hitaka, T.; Akimoto, H.; Nomura, H. *J. Med. Chem.* **1991**, *34*, 555.

(7) (a) Akimoto, H.; Hitaka, T.; Miwa, T. European Patent Appl. published on Sept 27, 1989 with publication No. 0 334 636 A2. (Priority: Japan Patent Appl. 63-71149, March 24, 1988 and Japan Patent Appl. 63-245379, Sept 29, 1988); *Chem. Abstr.* **1990**, *112*, 158968n. (b) An American research group reported similar pyrrolo[2,3-*d*]pyrimidine antifolates. Taylor, E. C.; Kuhnt, D. G.; Shih, C.; Grindey, G. B. U.S. Patent 4 996 206 (issued on Feb 26, 1991) and 5 028 608 (issued on July 2, 1991).

(8) Boldt, P.; Schlutz, L.; Etzemüller, J. *Chem. Ber.* **1967**, *100*, 1281.

(9) The Frontier orbital theory (Fleming, I. In *Frontier Orbitals and Organic Chemical Reactions*; John Wiley & Sons: London, 1976; Chapter 5) and some free radical addition reactions with enol ethers (e.g., Tarrant, P.; Stump, E. C. *J. Org. Chem.* **1964**, *29*, 1198) suggest this regioselectivity. Recently, similar regioselective addition of malononitrile radicals to alkenes were reported (Curran, D. P.; Thoma, G. *J. Am. Chem. Soc.* **1992**, *114*, 4436.)

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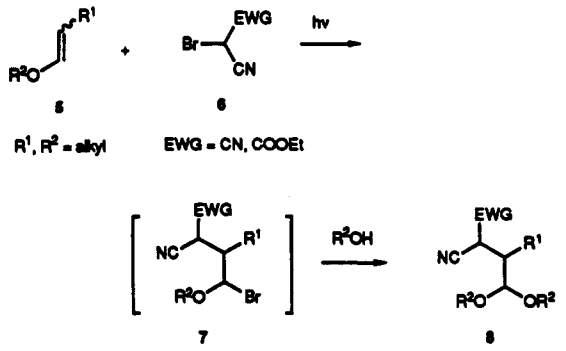
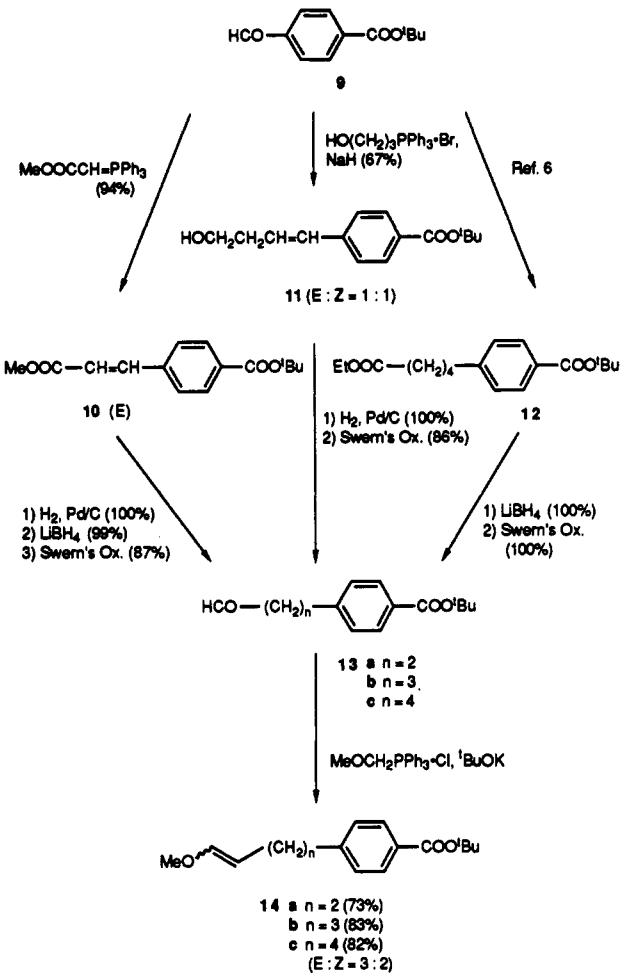
Scheme II



Scheme III



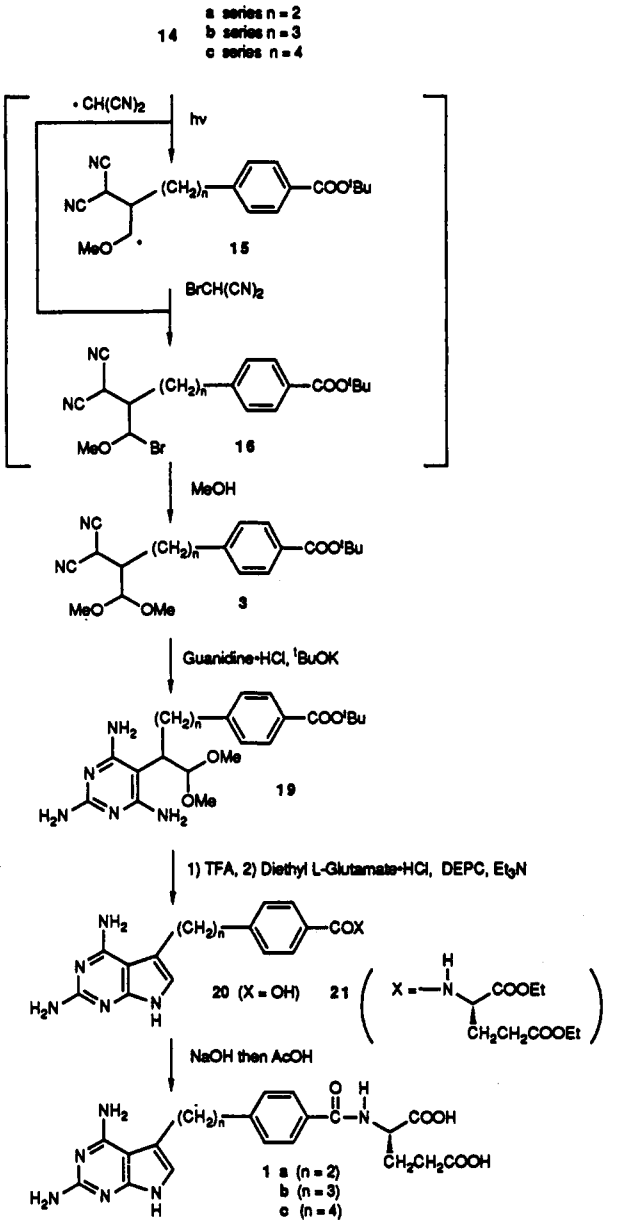
the needed functional moieities of the key intermediate **3** or **4** after alcoholysis (Scheme II).

The enol ether starting materials (**14a–c**) were prepared by the Wittig reactions of aldehydes (**13a–c**) with methyl (triphenylphosphoranylidene)methyl ether. The aldehydes **13a–c** were easily synthesized from *tert*-butyl 4-formylbenzoate[10] (Scheme III).

The following is a representative procedure for the synthesis of **1b** from the enol ether **14b** (Scheme IV). Reaction of the enol ether **14b** with bromomalononitrile under ultraviolet irradiation at 0–5 °C gave the single adduct **16b** which was checked and identified by NMR analysis of the reaction mixture. Having found radical addition of bromomalononitrile to be regiospecific, we

(10) Sunagawa, J.; Matsumura, H.; Inoue, T.; Enomoto, M. Japan Patent Kokai 58-116487, 1983.

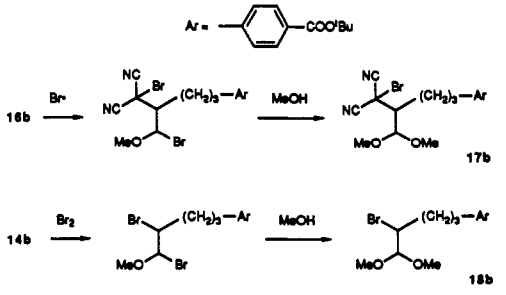
Scheme IV



introduced methanol to the reaction mixture to obtain the desired compound **3b** in almost quantitative yield (98%).[11]

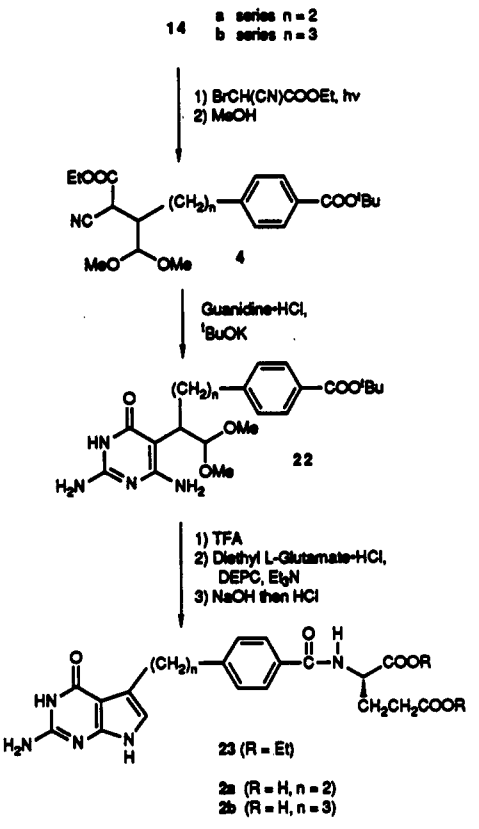
Refluxing **3b** with guanidine in *t*-BuOH gave the triaminopyrimidine **19b** in 97% yield. Acidic deprotection

(11) It should be noted that when the reaction was carried out at 25–30 °C, two byproducts **17b** (5%) and **18b** (8%) were obtained in addition to **3b** (80%).



The bromide **17b** was formed via a 1,3-dibromo compound. A similar 1,3-dibromo compound was reported in the reaction of bromomalononitrile with 2-methylpropene.[8] The bromide **18b** should arise by addition of emerging Br_2 to **3b** and subsequent alcoholysis.

Scheme V



of the dimethyl acetal and *tert*-butyl moiety of **19b** employing CF_3COOH(TFA)–H_2O under an argon atmosphere afforded 4-[3-(2,4-diaminopyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoic acid (**20b**). Without isolation, the acid was coupled with diethyl L-glutamate in 84% yield using diethyl phosphorocyanidate (DEPC)[12] as a coupling reagent in anhydrous DMF. Basic hydrolysis of **21b** with NaOH in THF–H_2O at room temperature for 1 h followed by acid treatment gave *N*-[4-[3-(2,4-diaminopyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic acid (**1b**) in 91% yield. The final product **1b** was identical with that synthesized by the reported procedure.[6] In the same manner, other pyrrolo[2,3-*d*]pyrimidine antifolates with different bridge length, **1a** and **1c**, were synthesized in good yields starting from the enol ethers **14a** and **14c**, respectively.

In order to synthesize 2-amino-4(3*H*)-oxopyrrolo[2,3-*d*]pyrimidine antifolates, ethyl bromocyanoacetate was allowed to undergo the photo-initiated reaction with the enol ethers. Thus, ultraviolet irradiation of ethyl bromocyanoacetate and the enol ether **14b** in CH_2Cl_2 at 0–5 °C afforded the adduct **4b** in 60% yield. A similar procedure as that described for **1b** converted **4b** into **2b** with an overall yield of 58% (Scheme V). In a similar manner, **2a** was synthesized from **14a**.

Thus, this sequence of reactions provided a simple (four-reaction sequence from enol ethers) and efficient (62–73% overall yield for 2,4-diamino compounds and 35–37% overall yield for 2-amino-4(3*H*)-oxo compounds based on enol ethers) synthesis of pyrrolo[2,3-*d*]pyrimidine antifolates.

All synthesized compounds (**1** and **2**) inhibited the growth of human tumor cell lines in culture.[13]

(12) Shioiri, T.; Ninomiya, K.; Yamada, S. *J. Am. Chem. Soc.* **1972**, *94*, 6203.

In conclusion, a novel and efficient method for the synthesis of pyrrolo[2,3-*d*]pyrimidine antifolates has been developed. Similar methodology should provide a general route to other biologically important 5-substituted pyrrolo-[2,3-*d*]pyrimidines such as Queuine[14] and Tubercidin analogues.[15]

Experimental Section

Melting points were determined on a Yanaco micro-melting apparatus and are uncorrected. ^1H (200 MHz) and ^{13}C (50.3 MHz) NMR spectra were recorded on a Varian Gemini-200 with tetramethylsilane as internal standard. IR spectra were obtained on a Hitachi 215 infrared spectrometer. Chemical ionization mass spectra (CIMS) were obtained on a Hitachi M-80A spectrometer. Elemental analyses were carried out by Takeda Analytical Research Laboratories, Ltd. Where analyses are indicated only by symbols of the elements, analytical results obtained were within 0.4% of the theoretical values. Flash chromatography was performed with Merck silica gel 60 (230–400 mesh).

Methyl 3-[4-(*tert*-Butoxycarbonyl)phenyl]propenoate (10). A solution of *tert*-butyl 4-formylbenzoate (**9**, 14.2 g, 68.8 mmol) and methyl (triphenylphosphoranylidene)acetate (23.0 g, 68.8 mmol) in 200 mL of toluene was heated to reflux for 1 h. After being cooled to room temperature, the mixture was diluted with 200 mL of MeOH to obtain crystals. Collection of the crystals and purification of the filtrate by flash chromatography afforded 17.0 g (94%) of colorless crystals: IR (KBr) 2985, 1720, 1710, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57 (9 H, s), 3.79 (3 H, s), 6.48 (1 H, d, J = 15.5 Hz), 7.55 (2 H, d, J = 8 Hz), 7.68 (1 H, d, J = 15.5 Hz), 7.97 (2 H, d, J = 8 Hz). Anal. ($C_{15}H_{18}O_4$) C, H.

Methyl 3-[4-(*tert*-Butoxycarbonyl)phenyl]propanoate. A suspension of **10** (22.5 g, 85.8 mmol) and 10% Pd/C (4.0 g) in AcOEt–MeOH (300 mL, 2:1) was stirred vigorously under a hydrogen atmosphere for 4 h. Filtration of the mixture through Celite and evaporation of the solvent in vacuo gave 22.7 g (100%) of colorless oil: IR (neat) 2980, 1745, 1717, 1610 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57 (9 H, s), 2.55 (1 H, dd, J = 6.5, 1.5 Hz), 2.65 (1 H, d, J = 6.5 Hz), 2.94 (1 H, d, J = 6.5 Hz), 3.02 (1 H, dd, J = 6.5, 1.5 Hz), 3.64 (3 H, s), 7.23 (2 H, d, J = 8 Hz), 7.90, (2 H, d, J = 8 Hz). Anal. ($C_{15}H_{20}O_4$) C, H.

***tert*-Butyl 4-(3-Hydroxypropyl)benzoate.** To a solution of methyl 3-[4-(*tert*-butoxycarbonyl)phenyl]propanoate (22.7 g, 85.9 mmol) in 150 mL of Et_2O was added $LiBH_4$ (2.81 g, 129 mmol) at 0 °C, and the mixture was stirred at room temperature for 15 h. The reaction was quenched by adding 1 N $KHSO_4$ solution to bring the pH to 6.5, and the product was extracted into Et_2O (3 × 150 mL). The combined extracts were washed with brine, dried (Na_2SO_4), and concentrated to afford 20.1 g (99%) of colorless oil: IR (neat) 3400, 2978, 2935, 1712, 1607 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.48 (1 H, s), 1.60 (9 H, s), 1.70–2.01 (2 H, m), 2.73 (2 H, t, J = 6.5 Hz), 3.63 (2 H, t, J = 6.5 Hz), 7.22 (2 H, d, J = 8 Hz), 7.89 (2 H, d, J = 8 Hz). Anal. ($C_{14}H_{20}O_3$) C, H.

***tert*-Butyl 4-(3-Oxopropyl)benzoate (13a).** To a solution of oxalyl chloride (5.91 g, 46.5 mmol) in 100 mL of CH_2Cl_2 under an argon atmosphere was added a solution of DMSO (7.94 g, 102 mmol) in 20 mL of CH_2Cl_2 at –60 °C. After 5 min, a solution of *tert*-butyl 4-(3-hydroxypropyl)benzoate (10.0 g, 42.3 mmol) in 50 mL of CH_2Cl_2 was added in 5 min. The reaction mixture was stirred at –60 °C for 15 min, and then to the mixture was added triethylamine (21.4 g, 212 mmol). The mixture was stirred at –60 °C, for 5 min, allowed to warm to 0 °C in 30 min, and poured into 150 mL of water. Extraction with CH_2Cl_2 (3 × 150 mL) gave a combined organic phase which was dried (Na_2SO_4) and concentrated in vacuo. Purification by flash chromatography

(13) (a) Akimoto, H.; Hitaka, T.; Miwa, T.; Yukishige, K.; Kusanagi, T.; Ootsu, K. *Proc. Am. Assoc. Cancer Res.*, **1991**, *32*, 327 (#1938). (b) Akimoto, H. *Proc. Jpn. Cancer Assoc.* **1991**, *2* (Symposium S-3).

(14) Akimoto, H.; Nomura, H.; Yoshida, M.; Shindo-Okada, N.; Hoshi, A.; Nishimura, S. *J. Med. Chem.* **1986**, *29*, 1749.

(15) Suhadolnik, R. J. *Nucleoside Antibiotics*; John Wiley & Sons, Inc.: New York, 1970; p 298.

(AcOEt/hexane 1:10) gave 8.60 g (87%) of colorless oil: IR (neat) 2980, 1725, 1710, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (9 H, s), 2.80 (2 H, t, J = 7 Hz), 3.00 (2 H, t, J = 7 Hz), 7.25 (2 H, d, J = 8 Hz), 7.92 (2 H, d, J = 8 Hz), 9.83 (1 H, br s). Anal. ($C_{14}H_{18}O_3$) C, H.

***tert*-Butyl 4-(4-Methoxy-3-butenyl)benzoate (14a).** A solution of *t*-BuOK in THF (1 M, 11.0 mL) was added to a suspension of (methoxymethyl)triphenylphosphonium chloride (3.77 g, 11.0 mmol) in 12 mL of toluene at 0 °C, and the mixture was stirred for 10 min. To the suspension was added a solution of **13a** (2.34 g, 10.0 mmol) in 10 mL of toluene. After being stirred for 20 min at 0 °C, 40 mL of Et_2O was added to the mixture. The organic phase was washed with water and brine, dried (Na_2SO_4), and concentrated under reduced pressure. To the residue was added 30 mL of hexane to give crystalline Ph_3PO which was filtered off. Concentration of the filtrate and purification of the residue gave 1.92 g (73%) of colorless oil: IR (neat) 2980, 2945, 1715, 1655, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (9 H, s), 2.24 (1.2 H, td, J = 8, 7 Hz), 2.39 (0.8 H, td, J = 8, 7 Hz), 3.48 (1.8 H, s), 3.56 (1.2 H, s), 4.33 (0.4 H, td, J = 7, 6 Hz), 4.71 (0.6 H, dt, J = 13, 7 Hz), 5.88 (0.4 H, d, J = 6 Hz), 6.28 (0.6 H, d, J = 13 Hz), 7.21 (2 H, d, J = 8 Hz), 7.91 (2 H, d, J = 8 Hz). Anal. ($C_{16}H_{22}O_3$) C, H.

***tert*-Butyl 4-(4-Hydroxy-1-butenyl)benzoate (11).** To a suspension of sodium hydride (4.08 g, 170 mmol) in 400 mL of THF under an argon atmosphere was added (3-hydroxypropyl)-triphenylphosphonium bromide (68.2 g, 170 mmol). After refluxing 4 h, *tert*-butyl 4-formylbenzoate (**9**, 35.1 g, 170 mmol) in 100 mL of THF was added to the mixture which was refluxed for further 1.5 h. The solvent was evaporated under reduced pressure, and to the resulting residue was added 500 mL of ether. After filtration to remove an insoluble material, evaporation gave an oily residue which was purified by flash chromatography (AcOEt/hexane, 10:1 to 3:1) to afford 28.1 g (67%) of colorless oil: IR (neat) 3410, 2983, 2940, 1713, 1648, 1605, 1568 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (1 H, br s), 1.60 (9 H, s), 2.30–2.70 (2 H, m), 3.65–3.90 (2 H, m), 5.63–6.70 (2 H, m), 7.33 (1 H, d, J = 8 Hz), 7.36 (1, H, d, J = 8 Hz), 7.91 (1 H, d, J = 8 Hz), 7.95 (1 H, d, J = 7 Hz). Anal. ($C_{15}H_{20}O_3$) C, H.

***tert*-Butyl 4-(4-Hydroxybutyl)benzoate.** A suspension of 11 (28.1 g, 113 mmol) and 10% Pd/C (3.0 g) in 150 mL of methanol was stirred vigorously under a hydrogen atmosphere for 4 h. Filtration of the mixture through a Celite pad and evaporation of the filtrate gave 28.3 g (100%) of colorless oil: IR (neat) 2980, 1940, 1720, 1710, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.10–1.85 (4 H, m), 1.59 (9 H, s), 1.67 (2 H, t, J = 7 Hz), 3.62 (2 H, t, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.88 (2 H, d, J = 8 Hz). Anal. ($C_{15}H_{22}O_3$) C, H.

***tert*-Butyl 4-(4-Oxobutyl)benzoate (13b).** A similar procedure as that described for **13a** converted *tert*-butyl 4-(4-hydroxybutyl)benzoate (10.0 g, 40.0 mmol) to 8.59 g (86%) of colorless oil: IR (neat) 2980, 2940, 1720, 1710, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.60 (9 H, s), 1.96 (2 H, tt, J = 7, 7 Hz), 2.43 (2 H, dd, J = 7, 1 Hz), 2.68 (2 H, t, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz), 9.78 (1 H, t, J = 1 Hz). Anal. ($C_{15}H_{20}O_3$) C, H.

***tert*-Butyl 4-(5-Methoxy-4-pentenyl)benzoate (14b).** A similar procedure as that described for **14a** converted **13b** (993 mg, 4.00 mmol) to 918 mg (83%) of colorless oil: IR (neat) 2980, 2940, 2860, 1710, 1660, 1603 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55–1.76 (2 H, m), 1.59 (9 H, s), 1.96 (0.6 H, dt, J = 7, 7 Hz), 2.10 (0.4 H, tdd, J = 7, 7, 7 Hz), 2.66 (2 H, t, J = 8 Hz), 3.51 (1.8 H, s), 3.59 (1.2 H, s), 4.35 (0.4 H, td, J = 7, 6 Hz), 4.73 (0.6 H, dt, J = 13, 7 Hz), 5.91 (0.4 H, dt, J = 6, 1 Hz), 6.29 (0.6 H, d, J = 13 Hz), 7.21 (2 H, d, J = 8 Hz), 7.89 (0.8 H, d, J = 8 Hz), 7.90 (1.2 H, d, J = 8 Hz). Anal. ($C_{17}H_{24}O_3$) C, H.

***tert*-Butyl 4-(5-Hydroxypentyl)benzoate.** A similar procedure as that described for *tert*-butyl 4-(3-hydroxypropyl)-benzoate converted **12**[6] (3.80 g, 12.4 mmol) to 3.28 g (100%) of colorless oil: IR (neat) 3400, 2980, 2950, 2860, 1715, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.20–1.80 (7 H, m), 1.60 (9 H, s), 2.65 (2 H, t, J = 7 Hz), 3.61 (2 H, t, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz). Anal. ($C_{16}H_{24}O_3$) C, H.

***tert*-Butyl 4-(5-Oxopentyl)benzoate (13c).** A similar procedure as that described for **13a** converted *tert*-butyl 4-(5-hydroxypentyl)benzoate (3.28 g, 12.4 mmol) to 3.25 g (100%) of colorless oil: IR (neat) 2980, 2945, 2865, 1730, 1710, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40–1.76 (4 H, m), 1.58 (9 H, s), 2.30–2.66 (2 H, m), 2.66–2.80 (2 H, m), 7.18 (2 H, d, J = 8 Hz), 7.88 (2 H, d, J = 8 Hz), 9.73 (1 H, t, J = 1 Hz). Anal. ($C_{16}H_{22}O_3$) C, H.

***tert*-Butyl 4-(6-Methoxy-5-hexenyl)benzoate (14c).** A similar procedure as that described for **14a** converted **13c** (476 mg, 1.81 mmol) to 430 mg (82%) of colorless oil: IR (neat) 2940, 1715, 1650, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.32–1.44 (2 H, m), 1.59 (9 H, s), 1.52–1.70 (2 H, m), 1.94 (1.2 H, td, J = 8, 7 Hz), 2.09 (0.8 H, td, J = 8, 7 Hz), 2.65 (2 H, t, J = 8 Hz), 3.49 (1.8 H, s), 3.58 (1.2 H, s), 4.31 (0.4 H, td, J = 7, 6 Hz), 4.70 (0.6 H, dt, J = 13, 7 Hz), 5.88 (0.4 H, d, J = 6 Hz), 6.28 (0.6 H, d, J = 13 Hz), 7.21 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz). Anal. ($C_{18}H_{26}O_3$) C, H.

***tert*-Butyl 4-[5,5-Dicyano-4-(dimethoxymethyl)pentyl]-benzoate (3b).** A CH_2Cl_2 solution (45 mL) of bromomalononitrile (872 mg, 6.00 mmol) and **14b** (1.38 g, 5.00 mmol) containing molecular sieves 3A (1.0 g) in a quartz flask was irradiated with a UV light using a filterless analytical UV lamp at 0–5 °C for 2 h. To the mixture was added 3 mL of MeOH and the solution was stirred for 10 min. The reaction mixture was poured in 40 mL of ice–water containing K_2CO_3 (484 mg, 3.5 mmol), extracted with CH_2Cl_2 (2 × 20 mL), dried (Na_2SO_4), and concentrated. Purification of the residue by flash chromatography (AcOEt/hexane, 1:10) afforded 1.49 g (98%) of colorless oil: IR (neat) 2975, 2930, 2245, 1710, 1605 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (9 H, s), 1.60–1.92 (4 H, m), 2.20–2.30 (1 H, m), 2.73 (2 H, t, J = 7 Hz), 3.40 (3 H, s), 3.45 (3 H, s), 4.11 (1 H, d, J = 4 Hz), 4.31 (1 H, d, J = 5 Hz), 7.24 (2 H, d, J = 8 Hz), 7.93 (2 H, d, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 22.97, 27.88, 28.32, 28.51, 35.82, 43.50, 55.41, 56.75, 81.11, 104.95, 112.22, 113.35, 128.64, 130.12, 130.45, 146.58, 166.27. Anal. ($C_{21}H_{28}N_2O_4$) C, H, N. The same reaction, except at 25–30 °C, afforded the byproducts **17b** (112 mg, 5%) and **18b** (156 mg, 8%) in addition to **3b** (1.49 g, 80%). **17b**: IR (neat) 2980, 2940, 2240, 1710, 1608 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.57 (9 H, s), 1.60–2.10 (4 H, m), 2.40 (1 H, td, J = 7, 4 Hz), 2.71 (2 H, t, J = 7 Hz), 3.48 (3 H, s), 3.50 (3 H, s), 4.56 (1 H, d, J = 4 Hz), 7.24 (2 H, d, J = 8 Hz), 7.92 (2 H, d, J = 8 Hz); ^{13}C NMR ($CDCl_3$) δ 27.53, 28.07, 28.32, 29.66, 51.29, 56.35, 57.32, 81.10, 105.66, 112.32, 112.54, 128.62, 130.08, 130.41, 146.61, 166.30. Anal. ($C_{21}H_{27}BrN_2O_4$) C, H, N. **18b**: IR (neat) 2990, 2945, 1717, 1610 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.59 (9 H, s), 1.60–2.10 (4 H, m), 2.60–2.75 (2 H, m), 3.43 (6 H, s), 3.92–4.03 (1 H, m), 4.37 (1 H, d, J = 5 Hz), 7.23 (2 H, d, J = 8 Hz), 7.91 (2 H, d, J = 8 Hz). Anal. ($C_{18}H_{27}BrO_4$) C, H, N.

The corresponding analogues **3a** (colorless oil, 2.08 g, 99%) and **3c** (colorless oil, 432 mg, 95%) were prepared in a similar manner starting from **14a** (1.54 g) and **14c** (340 mg), respectively. **3a**: IR (neat) 2980, 2945, 2840, 2250, 1710, 1606 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.60 (9 H, s), 1.90–2.20 (2 H, m), 2.20–2.32 (1 H, m), 2.89 (2 H, t, J = 8 Hz), 3.39 (3 H, s), 3.46 (3 H, s), 4.13 (1 H, d, J = 4 Hz), 4.36 (1 H, d, J = 5 Hz), 7.28 (2 H, d, J = 8 Hz), 7.95 (2 H, d, J = 8 Hz). Anal. ($C_{20}H_{26}N_2O_4$) C, H, N. **3c**: IR (neat) 2940, 2250, 1715, 1610, 1455 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48–1.81 (6 H, m), 1.59 (9 H, s), 2.18–2.28 (1 H, m), 2.71 (2 H, t, J = 7 Hz), 3.40 (3 H, s), 3.46 (3 H, s), 4.10 (1 H, d, J = 4 Hz), 4.31 (1 H, d, J = 5 Hz), 7.23 (2 H, d, J = 8 Hz), 7.92 (2 H, d, J = 8 Hz). Anal. ($C_{22}H_{30}N_2O_4$) C, H, N.

***tert*-Butyl 4-[4-(2,4,6-Triaminopyrimidin-5-yl)-5,5-dimethoxypentyl]benzoate (19b).** To a suspension of guanidine hydrochloride (585 mg, 6.12 mmol) in 10 mL of *t*-BuOH under an argon atmosphere was added a 1 M solution of *t*-BuOK in THF (6.12 mL) and **3b** (1.90 g, 5.10 mmol) in 20 mL of *t*-BuOH successively. The mixture was heated to reflux for 2 h. After being cooled to room temperature, the mixture was poured in 100 mL of water, extracted with CH_2Cl_2 (3 × 40 mL), dried (Na_2SO_4), and concentrated in vacuo. Purification of the residue by flash chromatography (CH_2Cl_2/MeOH, 30:1 to 15:1) afforded 2.14 g (97%) of pale yellow amorphous powder: IR (KBr) 3480, 3380, 3200, 2980, 2940, 1715, 1610, 1570, 1440 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40–1.65 (3 H, m), 1.59 (9 H, s), 1.75–2.05 (1 H, m), 2.62 (2 H, t, J = 7 Hz), 2.81 (1 H, ddd, J = 11, 3, 1 Hz), 3.46 (3 H, s), 3.50 (3 H, s), 4.36 (1 H, d, J = 3 Hz), 4.49 (4 H, br s), 5.16 (2 H, br s), 7.18 (2 H, d, J = 8 Hz), 7.88 (2 H, d, J = 8 Hz). Anal. ($C_{22}H_{33}N_5O_4$) C, H, N.

The corresponding analogues **19a** (pale yellow amorphous powder, 2.17 g, 93%) and **19c** (pale yellow amorphous powder,

433 mg, 97%) were prepared in a similar manner starting from **3a** (2.00 g) and **3c** (378 mg), respectively. **19a**: IR (KBr) 3475, 3360, 3200, 2975, 2930, 1710, 1607, 1563, 1430 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (9 H, s), 1.86–2.05 (1 H, m), 2.25–2.53 (2 H, m), 2.57–2.80 (2 H, m), 3.45 (3 H, s), 3.48 (3 H, s), 4.35 (1 H, d, J = 3 Hz), 4.36 (2 H, br s), 4.48 (2 H, br s), 5.21 (2 H, br s), 7.18 (2 H, d, J = 8 Hz), 7.88 (2 H, d, J = 8 Hz). Anal. ($C_{21}H_{31}N_5O_4$) C, H, N. **19c**: IR (KBr) 3475, 3360, 3220, 2975, 2930, 1715, 1640, 1607, 1563, 1435 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14–1.32 (2 H, m), 1.45–1.72 (3 H, m), 1.58 (9 H, s), 1.86–2.04 (1 H, m), 2.56–2.68 (2 H, m), 2.72–2.83 (1 H, m), 3.47 (3 H, s), 3.52 (3 H, s), 4.39 (1 H, d, J = 3 Hz), 4.36 (2 H, br s), 4.48 (2 H, br s), 5.21 (2 H, br s), 7.18 (2 H, d, J = 8 Hz), 7.88 (2 H, d, J = 8 Hz). Anal. ($C_{23}H_{35}N_5O_4$) C, H, N.

Diethyl *N*-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamate (21b). To a solution of **19b** (449 mg, 1.04 mmol) in 3 mL of TFA was added H_2O (40 mg, 2.2 mmol), and the mixture was stirred for 3 h and concentrated under reduced pressure. TFA was azeotropically removed with benzene, and the resulting residue was dried at 70 °C in vacuo. To a suspension of the residue and diethyl L-glutamate hydrochloride (374 mg, 1.56 mmol) in 4 mL of DMF was added a solution of diethyl phophorocyanidate (178 mg, 1.09 mmol) in 4 mL of DMF and triethylamine (474 mg, 4.68 mmol) in 4 mL of DMF. After the mixture was stirred at 0 °C for 30 min and at room temperature for 2 h, the solvent was removed in vacuo. Purification of the residue by flash chromatography (CH_2Cl_2 saturated with concd aqueous NH_4OH to CH_2Cl_2/8% NH_3 in EtOH, 30:1) afforded 434 mg (84%) of colorless, microcrystalline powder: mp 81–82 °C; IR (KBr) 3330, 3160, 1735, 1632 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.17 (3 H, t, J = 7 Hz), 1.20 (3 H, t, J = 7 Hz), 1.80–2.20 (4 H, m), 2.44 (2 H, t, J = 7 Hz), 2.68 (2 H, t, J = 7 Hz), 2.72 (2 H, t, J = 7 Hz), 4.05 (2 H, q, J = 7 Hz), 4.11 (2 H, q, J = 7 Hz), 4.35–4.50 (1 H, m), 5.34 (2 H, s), 5.91 (2 H, s), 6.42 (1 H, s), 7.31 (2 H, d, J = 8 Hz), 7.80 (2 H, d, J = 8 Hz), 8.66 (1 H, d, J = 8 Hz), 10.51 (1 H, s). Anal. ($C_{25}H_{32}N_6O_5$) C, H, N.

The corresponding analogues **21a** (colorless, microcrystalline powder, 1.95 g, 84%) and **21c** (colorless amorphous powder, 228 mg, 87%) were prepared in a similar manner starting from **19a** (2.00 g) and **19c** (230 mg), respectively. **21a**: mp 85–87 °C; IR (KBr) 3375, 3200, 2980, 2930, 1735, 1640, 1605, 1572 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (3 H, t, J = 7 Hz), 1.31 (3 H, t, J = 7 Hz), 2.10–2.40 (2 H, m), 2.48 (2 H, dd, J = 6, 6 Hz), 3.00 (4 H, br s), 4.12 (2 H, q, J = 7 Hz), 4.25 (2 H, q, J = 7 Hz), 4.61 (2 H, br s), 4.75–4.86 (1 H, m), 4.95 (2 H, br s), 6.40 (1 H, s), 7.13 (1 H, d, J = 7 Hz), 7.22 (2 H, d, J = 8 Hz), 7.74 (2 H, d, J = 8 Hz), 8.55 (1 H, br s). Anal. ($C_{24}H_{30}N_6O_5$) C, H, N. **21c**: IR (KBr) 3380, 3200, 2980, 2930, 1735, 1640, 1605, 1572 cm^{-1}; ^1H NMR ($CDCl_3$/CD_3OD) δ 1.22 (3 H, t, J = 7 Hz), 1.31 (3 H, t, J = 7 Hz), 1.60–1.83 (4 H, m), 2.43–2.51 (2 H, m), 2.63–2.76 (4 H, m), 4.11 (2 H, q, J = 7 Hz), 4.24 (2 H, q, J = 7 Hz), 4.74–4.86 (1 H, m), 6.45 (1 H, s), 7.24 (2 H, d, J = 8 Hz), 7.74 (2 H, d, J = 8 Hz). Anal. ($C_{26}H_{34}N_6O_5$) C, H, N.

***N*-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic Acid (1b).** To a solution of **21b** (250 mg, 0.503 mmol) in 3 mL of THF–H_2O (2:1) was added a 1 M aqueous solution of NaOH (1.5 mL). After being stirred for 1 h, the organic solvent was removed in vacuo, and to the remaining aqueous solution was added 0.5 mL of AcOH. The resulting colorless precipitate was ultrasonicated, filtered, washed with water, and dried (50 °C, 0.3 mmHg) to give 210 mg (91%): mp 180–181 °C dec; IR (KBr) 3340, 3200, 2940, 1660–1630 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.75–2.20 (4 H, m), 2.35 (2 H, t, J = 7 Hz), 2.68 (2 H, t, J = 7 Hz), 2.71 (2 H, t, J = 7 Hz), 4.30–4.47 (1 H, m), 5.53 (2 H, br s), 6.15 (2 H, s), 6.46 (1 H, s), 7.31 (2 H, d, J = 8 Hz), 7.81 (2 H, d, J = 8 Hz), 8.48 (1 H, d, J = 8 Hz), 10.51 (1 H, s); CIMS m/z 441 (MH^+). Anal. ($C_{21}H_{24}N_6O_5 \cdot H_2O$) C, H, N.

The corresponding analogues **1a** (colorless powder, 610 mg, 92%) and **1c** (colorless powder, 72 mg, 77%) were prepared in a similar manner starting from **21a** (719 mg) and **21c** (103 mg), respectively. **1a**: mp 240–242 °C dec; IR (KBr) 3320, 1660, 1637, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.85–2.20 (2 H, m), 2.46 (2 H, t, J = 8 Hz), 2.96 (4 H, br s), 4.30–4.45 (1 H, m), 5.49 (2 H, br s), 6.13 (2 H, s), 6.37 (1 H, s), 7.33 (2 H, d, J = 8 Hz), 7.80 (2 H, d, J = 8 Hz), 8.46 (1 H, d, J = 7 Hz), 10.43 (1 H, br s); CIMS m/z 427 (MH^+). Anal. ($C_{20}H_{22}N_6O_5 \cdot H_2O$) C, H, N. **1c**: mp > 230 °C dec; IR (KBr) 3340, 3200, 2930, 1650, 1635, 1540 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.45–1.76 (4 H, m), 1.88–2.19 (2 H, m), 2.29–2.43 (2 H, m), 2.58–2.76 (4 H, m), 4.32–4.46 (1 H, m), 5.54 (2 H, br s), 6.16 (2 H, br s), 6.42 (1 H, s), 7.29 (2 H, d, J = 8 Hz), 7.79 (2 H, d, J = 8 Hz), 8.52 (1 H, d, J = 7 Hz), 10.48 (1 H, br s); CIMS m/z 455 (MH^+). Anal. ($C_{22}H_{26}N_6O_5 \cdot 0.5H_2O$) C, H, N.

Ethyl 6-[4-(*tert*-Butoxycarbonyl)phenyl]-2-cyano-3-(dimethoxymethyl)hexanoate (4b). A CH_2Cl_2 solution (50 mL) of ethyl bromocyanoacetate (576 mg, 3.0 mmol) and **14b** (691 mg, 2.5 mmol) containing molecular sieves 3A (1.0 g) in a quartz flask was irradiated with a UV light using a filterless analytical UV lamp at 0–5 °C for 2 h. To the mixture was added 1 mL of MeOH and the solution was stirred for 10 min. The reaction mixture was poured in 30 mL of ice–water containing K_2CO_3 (242 mg, 1.75 mmol), extracted with CH_2Cl_2 (2 × 15 mL), dried (Na_2SO_4), and concentrated. Purification of the residue by flash chromatography (AcOEt/hexane, 1:12) afforded the diastereomeric mixture (1:1) of the title compound (630 mg, 60%) as colorless oil: IR (neat) 2980, 2940, 2250, 1745, 1715, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.28 (1.5 H, t, J = 7 Hz), 1.31 (1.5 H, t, J = 7 Hz), 1.59 (9 H, s), 1.59–1.81 (4 H, m), 2.44–2.57 (1 H, m), 2.63–2.76 (2 H, m), 3.34 (1.5 H, s), 3.36 (1.5 H, s), 3.41 (3 H, s), 3.54 (0.5 H, d, J = 3 Hz), 3.93 (0.5 H, d, J = 3 Hz), 4.23 (1 H, q, J = 7 Hz), 4.24 (1 H, q, J = 7 Hz), 4.28 (0.5 H, d, J = 6 Hz), 4.37 (0.5 H, d, J = 8 Hz), 7.21 (1 H, d, J = 8 Hz), 7.22 (1 H, d, J = 8 Hz), 7.90 (1 H, d, J = 8 Hz), 7.91 (1 H, d, J = 8 Hz). Anal. ($C_{23}H_{33}NO_6$) C, H, N.

The corresponding analogue **4a** (colorless oil, 1.30 g, 67%) was prepared in a similar manner starting from **14a** (1.25 g): IR (neat) 2950, 2250, 1710, 1610, 1165 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (1.5 H, t, J = 7 Hz), 1.30 (1.5 H, t, J = 7 Hz), 1.59 (9 H, s), 1.77–2.14 (2 H, m), 2.46–2.61 (1 H, m), 2.66–2.83 (2 H, m), 3.29 (1.5 H, s), 3.37 (1.5 H, s), 3.40 (1.5 H, s), 3.42 (1.5 H, s), 3.61 (0.5 H, d, J = 3 Hz), 3.97 (0.5 H, d, J = 3 Hz), 4.24 (1 H, q, J = 7 Hz), 4.25 (1 H, q, J = 7 Hz), 4.33 (0.5 H, d, J = 6 Hz), 4.40 (0.5 H, d, J = 8 Hz), 7.23 (1 H, d, J = 8 Hz), 7.25 (1 H, d, J = 8 Hz), 7.92 (1 H, d, J = 8 Hz), 7.93 (1 H, d, J = 8 Hz). Anal. ($C_{22}H_{31}NO_6$) C, H, N.

***tert*-Butyl 4-[4-(2,6-Diamino-4(3*H*)-oxopyrimidin-5-yl)-5,5-dimethoxypentyl]benzoate (22b).** A similar procedure as that described for **19b** except for the amount of *t*-BuOK (1.9 equiv) converted **4b** (260 mg, 0.62 mmol) to 215 mg (80%) of colorless amorphous powder: IR (KBr) 3350, 2980, 2940, 1710, 1660–1580 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45–1.72 (2 H, m), 1.58 (9 H, s), 1.74–2.15 (3 H, m), 2.61 (2 H, t, J = 8 Hz), 3.40 (6 H, s), 4.26–4.48 (1 H, m), 5.22 (2 H, s), 5.39 (2 H, br s), 7.18 (2 H, d, J = 8 Hz), 7.86 (2 H, d, J = 8 Hz). Anal. ($C_{22}H_{32}N_4O_5$) C, H, N.

The corresponding analogue **22a** (colorless amorphous powder, 617 mg, 74%) was prepared in a similar manner starting from **4a** (807 mg): IR (KBr) 3345, 2980, 2940, 1710, 1665–1580, 1495 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.58 (9 H, s), 1.81–2.04 (2 H, m), 2.06–2.34 (1 H, m), 2.43–2.70 (2 H, m), 3.40 (6 H, s), 4.30–4.51 (1 H, m), 5.21 (2 H, s), 5.38 (2 H, br s), 7.21 (2 H, d, J = 8 Hz), 7.85 (2 H, d, J = 8 Hz). Anal. ($C_{21}H_{30}N_4O_5$) C, H, N.

Diethyl *N*-[4-[3-(2-Amino-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamate (23b). A similar procedure as that described for **21b** converted **22b** (204 mg, 0.472 mmol) to 211 mg (90%) of colorless amorphous powder: IR (KBr) 3340, 2980, 2940, 1740, 1680–1620, 1610, 1540 cm^{-1}; ^1H NMR ($CDCl_3$/CD_3OD) δ 1.20 (3 H, t, J = 7 Hz), 1.28 (3 H, t, J = 7 Hz), 1.87–2.36 (4 H, m), 2.37–2.56 (2 H, m), 2.60–2.91 (4 H, m), 4.10 (2 H, q, J = 7 Hz), 4.22 (2 H, q, J = 7 Hz), 4.60–4.88 (1 H, m), 6.37 (1 H, s), 7.23 (2 H, d, J = 8 Hz), 7.55 (1 H, d, J = 8 Hz), 7.71 (2 H, d, J = 8 Hz); CIMS m/z 498 (MH^+). Anal. ($C_{25}H_{31}N_5O_6$) C, H, N.

The corresponding analogue **23a** (colorless solid, 542 mg, 87%) was prepared in a similar manner starting from **22a** (539 mg): mp 131–133 °C; IR (KBr) 3320, 2980, 2930, 1735, 1680–1630, 1610, 1530, 1440 cm^{-1}; ^1H NMR (DMSO-d_6) δ: 1.17 (3 H, t, J = 7 Hz), 1.19 (3 H, t, J = 7 Hz), 1.89–2.18 (2 H, m), 2.44 (2 H, t, J = 7 Hz), 2.79–2.91 (2 H, m), 2.92–3.04 (2 H, m), 4.05 (2 H, q, J = 7 Hz), 4.11 (2 H, q, J = 7 Hz), 4.35–4.47 (1 H, m), 6.00 (2 H, s), 6.30 (1 H, d, J = 2 Hz), 7.29 (2 H, d, J = 8 Hz), 7.78 (2 H, d,

J = 8 Hz), 8.63 (1 H, d, J = 7 Hz), 10.14 (1 H, s), 10.61 (1 H, d, J = 2 Hz); CIMS m/z 484 (MH^+). Anal. ($C_{24}H_{29}N_5O_8$) C, H, N.

***N*-[4-[3-(2-Amino-4(3*H*)-oxo-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic acid (2b).** To a solution of **23b** (196 mg, 0.394 mmol) in 3 mL of THF–H_2O (2:1) was added a 1 M aqueous solution of NaOH (1.58 mL). After being stirred for 4 h, the organic solvent was removed in vacuo, and to the remaining aqueous solution was added a 1 M solution of HCl (1.58 mL). The resulting precipitate was collected by filtration, washed with water, and dried to give 146 mg (81%) of colorless powder: mp 191–193 °C; IR (KBr) 3390, 3300, 2940, 1690, 1650, 1545, 1510, 1405 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.80–2.17 (4 H, m), 2.23–2.40 (2 H, m), 2.53–2.83 (4 H, m), 4.27–4.56 (1 H, m), 5.92 (2 H, s), 6.33 (1 H, s), 7.27 (2 H, d, J = 8 Hz), 7.78 (2 H, d, J = 8 Hz), 8.46 (1 H, d, J = 7 Hz), 10.08 (1 H, s), 10.57 (1 H, s); CIMS m/z 442 (MH^+). Anal. ($C_{21}H_{23}N_5O_6 \cdot H_2O$) C, H, N.

The corresponding analogue **2a** (colorless powder, 388 mg, 85%) was prepared in a similar manner starting from **23a** (493 mg): IR (KBr) mp 172–174 °C; 3300, 2930, 1680–1600, 1585, 1500, 1440, 1400, cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.87–2.20 (2 H, m), 2.36 (2 H, t, J = 7 Hz), 2.80–3.05 (4 H, m), 4.34–4.47 (1 H, m), 6.00 (2 H, s), 6.31 (1 H, d, J = 2 Hz), 7.29 (2 H, d, J = 8 Hz), 7.79 (2 H, d, J = 8 Hz), 8.52 (1 H, d, J = 8 Hz), 10.15 (1 H, s), 10.61 (1 H, s); CIMS m/z 428 (MH^+). Anal. ($C_{20}H_{21}N_5O_6 \cdot H_2O$) C, H, N.

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