indanyl 3-H), 3.60–3.71 (m, 1 H, indanyl 1-H, partially obscured by the 2 OMe peaks), 3.70–3.83 (m, 1 H, barbiturate methine H), 3.65 (s, 3 H, OMe), 3.69 (s, 3 H, OMe), 6.66 (s, 1 H, aromatic H), 6.76 (s, 1 H, aromatic H), 11.02 (br s, 1 H, NHCO), 11.30 (br s, 2 H, NHCO); MS (CI) *m/e* 177 (100). Anal. ($C_{15}H_{16}N_2O_5$) C, H, N.

2,4,6-Trichloro-5-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)pyrimidine (14). A 4.50-g portion (14.8 mmol) of **13** was dissolved in 40 mL of *N,N*-diethylaniline and heated to 120 °C. To this was added dropwise 20 mL of $POCl_3$. The resultant mixture was stirred at 120 °C for 15 h. This was then mixed with ice water and neutralized with cold, concentrated NH_4OH, followed by extraction with EtOAc. After drying ($MgSO_4$) and solvent removal, the crude product was purified by flash chromatography on silica gel with 20% EtOAc in hexane as the eluent. A 2.5-g yield (47%) of 14 was obtained as white crystals: mp 125–127 °C; NMR (Me_2SO-d_6) δ 2.10–2.30 (m, 1 H, indanyl 2-H), 2.40–2.60 (m, 1 H, indanyl 2-H, partially obscured in the Me_2SO peak), 2.90–3.10 (m, 2 H, indanyl 3-H), 3.60 (s, 3 H, OMe), 3.73 (s, 3 H, OMe), 5.00 (t, 1 H, indanyl 1-H), 6.69 (s, 1 H, aromatic H), 6.88 (s, 1 H, aromatic H); MS (CI) *m/e* 359 (M^+ + 1, 100), 362 (M^+ + 2, 99), 363 (M^+ + 3, 33). Anal. ($C_{15}H_{13}Cl_3N_2O_2$) C, H, N, Cl.

6-Chloro-2,4-diamino-5-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)pyrimidine (15). A 1.00-g portion (2.78 mmol) of 15 was dissolved in 15 mL of absolute EtOH in a glass-lined bomb. This was chilled in ice/water bath. Ammonia gas was then introduced into the ethanolic solution until saturation, and the resultant mixture was heated in the oven for 5 h at 100 °C. The solvent was removed, and the crude product was purified by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 as the eluent, yielding 0.28 g (31%) of **15**; MS (CI) *m/e* 321 (M^+ + 1, 100), 323 (M^+ + 3, 33). This was used directly in the next reaction without analysis.

2,4-Diamino-5-(5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-yl)pyrimidine (2b). A 0.2-g portion (0.62 mmol) of **15** was dissolved in a mixture of 60 mL of absolute EtOH and 5 mL of DMF in a Parr shaker. 5% Pd/C (0.05 g) was added, and H_2 was introduced. The substance was then dehalogenated overnight. The catalyst was removed and the crude product purified by flash column chromatography on silica gel with 10% MeOH in CH_2Cl_2 as the eluent. A 0.06 g (34%) yield of **2b** was obtained as a white solid: mp 206–208 °C; NMR (Me_2SO-d_6) δ 1.60–1.90 (m, 1 H, indanyl 2-H), 2.30–2.50 (m, 1 H, indanyl 2-H), 2.60–2.95 (m, 2 H, indanyl 3-H), 3.65 (s, 3 H, OMe), 3.73 (s, 3 H, OMe), 4.15 (t, 1 H, indanyl 1-H), 5.68 (br s, 2 H, NH_2), 6.10 (br s, 2 H, NH_2), 6.59 (s, 1 H, aromatic H), 6.88 (s, 1 H, aromatic H), 7.10 (s, 1 H, pyrimidyl 6-H); MS (CI) *m/e* 287 (M^+ + 1, 100). Anal. ($C_{15}H_{18}N_4O_2 \cdot 0.3H_2O$) C, H, N.

Acknowledgment. The early experimental work on this project was carried out by Dr. Edna Oppenheimer while on a postdoctoral fellowship from Israel. Although viable routes to the desired targets were not found at that time, much valuable chemistry was learned. We also express our thanks to Dr. Lee Kuyper for helpful advice with molecular modeling, to Dr. William Pendergast for valuable suggestions with chemistry, and to Mr. Robert Ferone for the enzyme assays. Ms. Mary Y. Tidwell provided expert technical assistance.

Registry No. (*S*)-**2a**, 130985-19-2; (*R*)-**2a**, 130985-20-5; (±)-**2b**, 130985-21-6; (*S*)-**2b**, 131064-20-5; (*R*)-**2b**, 131064-21-6; **3**, 16718-42-6; **4**, 130985-22-7; **5**, 130985-23-8; **6**, 130985-24-9; **7**, 130985-25-0; (±)-(*R**,*S**)-**8**, 130985-26-1; **9**, 130985-27-2; **10**, 2107-69-9; **11**, 67-52-7; **12**, 131010-57-6; **13**, 130985-28-3; (±)-**14**, 130985-29-4; (±)-**15**, 130985-30-7; ethyl cyanoacetate, 105-56-6; guanidine hydrochloride, 50-01-1.

Novel Pyrrolo[2,3-*d*]pyrimidine Antifolates: Synthesis and Antitumor Activities

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New antifolates, characterized by a 6–5 fused ring system, a pyrrolo[2,3-*d*]pyrimidine ring, and a trimethylene bridge at position 5 (**12a,b** and **13a,b**) were designed and efficiently synthesized. The synthetic method included (1) construction of the key intermediary acyclic skeleton, 5-[4-(*tert*-butoxycarbonyl)phenyl]-2-(dicyanomethyl)pentanoates (**6a,b**), (2) cyclization with guanidine, followed by reduction to the pyrrolo[2,3-*d*]pyrimidine derivatives (**8a,b** and **9a,b**), and (3) subsequent glutamate coupling and saponification. These antifolates were more growth-inhibitory by about 1 order of magnitude than methotrexate (MTX) against KB human epidermoid carcinoma cells and A549 human nonsmall cell lung carcinoma cells in in vitro culture. Growth inhibitory IC_{50} values for *N*-[4-[3-(2,4-diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic acid (**12a**) against KB and A549 were 0.27 and 4.5 ng/mL, while those for MTX were 5.0 and 35 ng/mL, respectively. Other members of this class of antifolates, **12b** and **13a,b**, showed good activities nearly equal to that of **12a**.

Methotrexate (MTX) has been an important drug in cancer chemotherapy, mainly in the treatment of acute lymphocytic leukemia, since its debut in 1953. However, it has limitations in clinical use because of toxicity to patients and lack of efficacy against most human solid tumors.[1] Although many analogues of MTX have been synthesized and tested,[2] none with better therapeutic properties has found its way into clinical practice.

With the specific aim of synthesizing a new anticancer agent with an improved therapeutic index, we concentrated our research on the design and synthesis of new antifolates having new structural features in the heteroaromatic ring and in the bridge region linking the ring to the benzoyl moiety. Because structurally close antifolates inhibit various folate cofactor-requiring enzymes,[3] an antifolate

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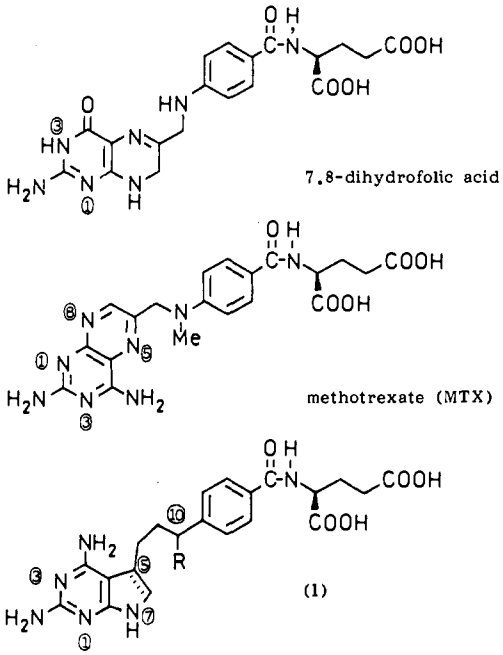
Chart I



of novel structure would be expected to possess inhibitory activities against not only long-targeted dihydrofolate reductase (DHFR) but also other folate-related enzymes.

In order to design a novel antifolate, we adopted DHFR as a mold enzyme, because it was the only folate-related enzyme for which crystallographic structure with its inhibitor, MTX, was available and well investigated. The structure of the MTX–DHFR complex has been determined by Bolin et al.[4] based on X-ray crystallographic studies, and this research group proposed the mode of binding of the substrate (7,8-dihydrofolic acid) to DHFR from theoretical considerations. While the X-ray structure of dihydrofolic acid in DHFR has not yet been determined, the structure of folic acid in DHFR was recently reported.[5] The pteridine ring of folic acid bound to DHFR in the orientation predicted for that of dihydrofolic acid, i.e. with a 180° rotation (around the axis connecting the C-2 and N-5 atoms) relative to that of MTX. The *p*-aminobenzoyl-L-glutamate moiety of folic acid was found in the same conformation as that of MTX, again as predicted. This work strengthened the postulated mode of binding of dihydrofolic acid (Chart I). We took these results together with the deduced model of the enzyme cavity[6] as our point of departure in designing new antifolates.

Structures of previously known antitumor-active antifolates are limited to those containing a pteridine or a related 6–6 fused heterocyclic ring. Thus, no antifolate with a 6–5 fused ring and having antitumor activity has been reported. The best known example, 2,6-diaminopurine antifolates, are entirely devoid of activity against L1210 leukemia.[7] With such consideration in mind, we directed our attention to searching for a 6–5 fused ring system that would be suitable for an antifolate. Molecular modeling based upon the known mode of binding of MTX and the predicted mode of binding of dihydrofolic acid led us to select 2,4-diaminopyrrolo[2,3-*d*]pyrimidine as a 6–5 fused ring. MNDO calculation indicated that the pyrrolopyrimidine would accept a proton either at N1 or at N3,[8] distinct from MTX which takes a proton only at N1.[8,9] Consequently, the pyrrolopyrimidine taking the proton at its N1 should bind to the enzyme as the pteridine of MTX, while the pyrrolopyrimidine taking the proton at its N3 should bind as the pteridine of dihydrofolic acid. Introduction of a flexible trimethylene bridge at the pyrrolopyrimidine 5-position (general structure I) would provide a totally preferred conformation that satisfies the most hydrogen-bonding and hydrophobic interaction opportunities in the enzyme cavity. When the pyrrolopyrimidine binds to DHFR in the substrate manner (protonation at N3), the conformation of its bridge and benzoyl moiety especially resembles that of MTX which binds to the enzyme.[10] In addition, it may be possible that the novel pyrrolopyrimidine antifolate inhibits other folate-related enzymes, because it has the flexible trimethylene bridge and alternative positions for protonation.

In this paper, we report on the efficient synthesis of novel antifolates having the 6–5 fused heterocycles (pyrrolo[2,3-*d*]pyrimidines) coupled with extended methylene bridges at position 5 (**12a,b** and **13a,b**) and their preliminary but potent antitumor activities superior to those of MTX.

Results and Discussion

Chemistry. There have been reports[11] on several synthetic routes for the preparation of deaza analogues of MTX and related compounds, but these procedures often involve too many laborious steps to allow extensive study of structure–activity relationships on MTX analogues. We developed facile new methods suitable for the large-scale synthesis of compounds in this series by first constructing the acyclic backbone of the antifolate molecule via 5-(4-carboxylphenyl)pentanoic acid diester (**4a,b**) and its α-dicyanomethyl derivative (**6a,b**), and its subsequent cyclization to the 6–5 fused ring system in one step. The route is shown in Scheme I.

For the synthesis of **4a,b**, *tert*-butyl 4-formylbenzoate (**1a**) and the corresponding 4-acetyl compound **1b** were selected as starting compounds. Coupling of ethyl crotonate with compound **1a** in the presence of *t*-BuOK gave a mixture of the dienoic ester **2** and the dienoic acid **2'**. Hydrogenation (10% Pd/C in AcOEt) of the mixture and subsequent esterification (DCC in EtOH, catalytic DMAP) gave the acyclic backbone diester **4a** (60% from **1a**). The methyl derivative (**4b**) was obtained by the vinylogous Reformatsky reaction[12] of ethyl 4-bromocrotonate with **1b**

(3) In addition to DHFR, much attention has been recently given to thymidylate synthase, glycinamide ribonucleotide transformylase, and 5-amino-4-imidazolecarboxyamide ribonucleotide transformylase. See: Patil, S. D.; Jones, C.; Nair, M. G.; Gallivan, J.; Maley, F.; Kisliuk, R. L.; Gaumont, Y.; Duch, D.; Ferone, R. *J. Med. Chem.* **1989**, *32*, 1284 and references cited therein. See also ref 17.

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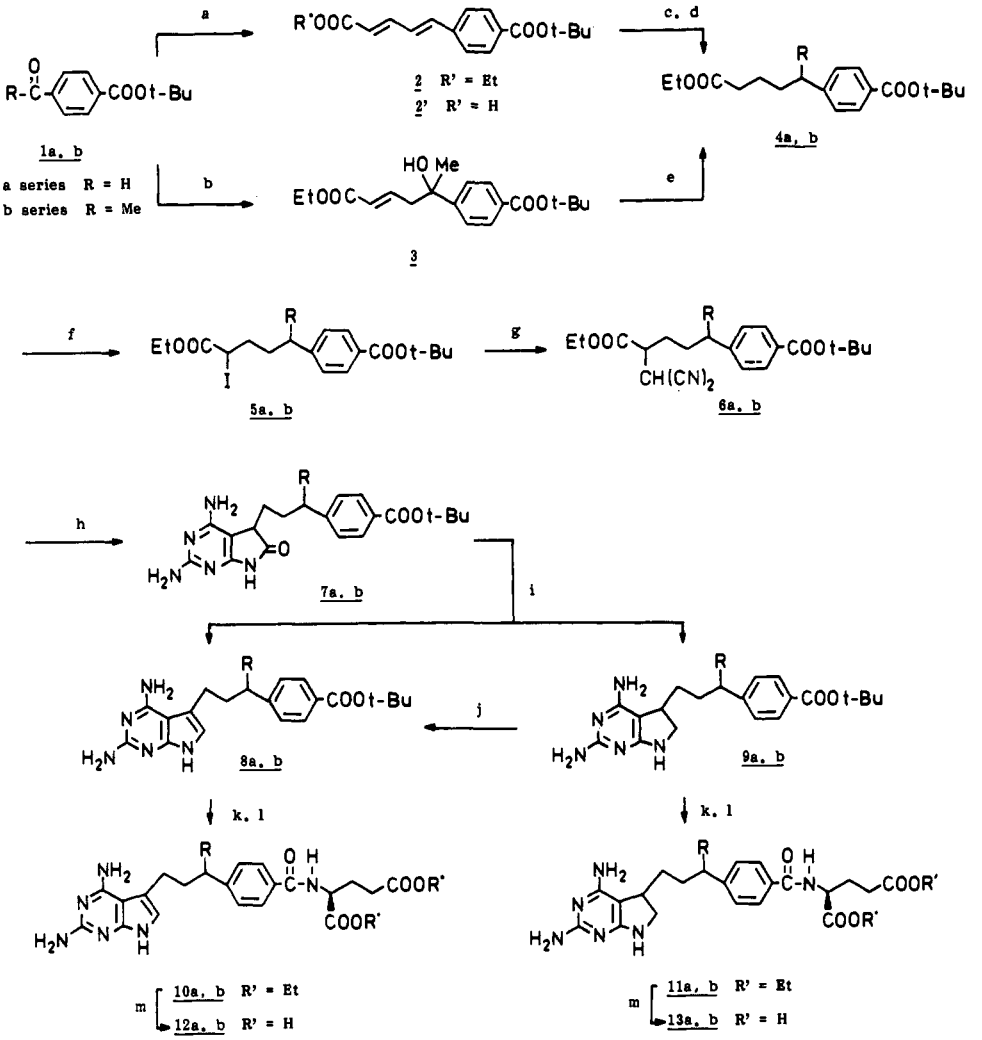
Scheme I[a]



[a] Conditions: (a) $CH_3CH{=}CHCOOEt$, *t*-BuOK, *t*-BuOH–ether; (b) $BrCH_2CH{=}CHCOOEt$, Zn, benzene–ether–THF; (c) H_2, Pd/C, AcOEt; (d) EtOH, DCC, DMAP (catalytic); (e) H_2, Pd/C, EtOH–AcOH; (f) (1) LDA, THF, (2) I_2; (g) $NCCH_2CN$, NaH, DMSO; (h) guanidine hydrochloride, *t*-BuOK, *t*-BuOH; (i) (1) BH_3–THF, THF, (2) AcOH–MeOH; (j) $RuCl_2(PPh_3)_3$, THF; (k) CF_3COOH; (l) diethyl L-glutamate hydrochloride, DPPA, Et_3N, DMF; (m) NaOH, THF–H_2O, (2) AcOH.

(Zn in benzene–ether–THF, at reflux temperature, 85%), followed by hydrogenation and hydrogenolysis (10% Pd/C in EtOH–AcOH, 85%). The key intermediary acyclic skeleton **6a** was synthesized by converting the diester **4a** by treatment with LDA and I_2[13] in THF at −78 °C (71%) to the α-iodo ester **5a**, which on treatment with the sodium salt of malononitrile in Me_2SO at 20 °C gave 2-(dicyanomethyl)pentanoate derivative **6a** in 95% yield. Heating compound **6a** with guanidine in the presence of a catalytic amount of *t*-BuOK (for 2 h) induced the cyclization, a key reaction in our strategy, which progressed smoothly to yield 6-oxopyrrolo[2,3-*d*]pyrimidine **7a** (89%). Conversion of **7a** to **8a** or to **9a** by reduction of the lactam carbonyl was best carried out with BH_3–THF.[14] Treatment of **7a** with BH_3–THF (5 equiv) in THF at 0 °C, followed by decomposition of the borane–product complex with AcOH–MeOH, gave a mixture of pyrrolo[2,3-*d*]pyrimidine **8a** and dihydropyrrolo[2,3-*d*]pyrimidine **9a**, which were separated by flash chromatography (**8a**, 45%; **9a**, 46%). The use of excess BH_3 (10 equiv) resulted in the formation of the dihydro compound **9a** exclusively (95%). In contrast, all attempts to convert **7a** selectively to **8a** by carefully choosing the borane reductants, molar ratio, reaction temperature, and quenching methods resulted in failure. However, because dehydrogenation from **9a** to pyrrolopyrimidine **8a** was found to proceed smoothly (68%) on treatment with $RuCl_2(PPh_3)_3$,[15] a practical synthetic route to **8a** was secured by connecting these two reactions (**7a** → **9a** → **8a**). Conversion of the ester group of pyrrolopyrimidine **8a** to the corresponding carboxylic acid by treatment with CF_3COOH and the following coupling with diethyl L-glutamate in the presence of diphenyl phosphorazidate (DPPA)[16] gave ester **10a**, which on alkaline hydrolysis at room temperature gave the desired pyrrolo[2,3-*d*]pyrimidine antifolate **12a** in high yield. By the similar synthetic route starting from 6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidine **9a**, the corresponding dihydropyrrolo[2,3-*d*]pyrimidine antifolate **13a** was synthesized in excellent yield.

As certain antifolates having a lower alkyl group on the bridge atom next to the phenyl ring possess better anti-

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Table I. Growth Inhibition of the Pyrrolo[2,3-*d*]pyrimidine Antifolates in KB and A549 Cells

cell line	IC_{50} (n = 3) ± SE, ng/mL				
	12a	12b	13a	13b	MTX
KB	0.27 ± 0.03	0.59 ± 0.03	0.49 ± 0.12	0.30 ± 0.04	5.0 ± 0.3
A549	4.5 ± 0.6	2.3 ± 0.4	4.9 ± 0.3	1.6 ± 0.3	35 ± 5.0

tumor properties than the corresponding desalkyl counterparts,[17b] we added pyrrolopyrimidine antifolates bearing a methyl group in the bridge part (at position 10) to our synthetic targets. Thus, the 10-methyl congeners (**12b** and **13b**) were synthesized in a similar way from **4b** in good yields. For the synthesis of this class of antifolates, our acyclic backbone intermediate strategy proved to be efficient as it requires fewer steps and offers excellent total yields. This method enabled us to synthesize a variety of new antifolates in this class sufficiently to allow extensive biological testing.

Biology. The inhibitory effects of these new antifolates (**12a,b** and **13a,b**) against human epidermoid carcinoma KB cells and human nonsmall cell lung carcinoma A549 cells are shown in Table I. All of them showed dramatically potent in vitro activity against these tumor cells. These compounds were 7–22 times more active than MTX on the basis of direct comparison. Compounds **12a,b** and **13a,b** had similar levels of activity, suggesting that the presence of the methyl group at position 10 and the saturation of the pyrrole moiety of the pyrrolopyrimidine ring do not significantly modify the potency of the in vitro activity. As for in vivo activity, these compounds proved to be highly active against some solid tumors and MTX-resistant tumors (details will be reported elsewhere). Thus, although we molded the novel pyrrolopyrimidine antitumor agents on the DHFR cavity, the biological data attaching to them would imply that these structurally unique antifolates may have additional or other loci of action. Investigations in this regard are now in progress and will be described.

Our results show that introduction of the pyrrolo[2,3-*d*]pyrimidine ring together with the trimethylene bridge into a suitable position of the molecule greatly improves antitumor activity. Although an overwhelming number of folic acid analogues have been prepared and tested over the past 3 decades, few agents have been found that are superior to MTX.[17] Thus, the very potent antitumor activity of this class of compounds is striking and supports the validity of our approach using a working hypothesis mentioned above which can provide a positive guidance to search for a new antifolate.

Experimental Section

Melting points were determined on a Yanaco micromelting apparatus and are uncorrected. ^{1}H NMR spectra were recorded either on a Varian EM-390 (90 MHz) or on a Varian Gemini-200 (200 MHz) with tetramethylsilane as internal standard. IR spectra were obtained on a Hitachi 215 infrared spectrometer. Secondary ionization mass spectra (SIMS) were performed 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). Alumina column chromatography was performed with Woelm neutral aluminum oxide.

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Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]pentanoate (4a). Potassium (25.0 g, 639 mmol) was dissolved in *t*-BuOH (800 mL) after 3 h reflux, and the solution was diluted with ether (300 mL). To the mixture was added a solution of 72.9 g (639 mmol) of ethyl crotonate and 65.8 g (319 mmol) of *tert*-butyl 4-formylbenzoate (**1a**) in *t*-BuOH–ether (300 mL, 2:1) at 10 °C. The resulting dark brown slurry was stirred for 2 h at 12 °C. The slurry was then acidified to pH 4 by addition of 1 N aqueous $KHSO_4$ (750 mL) and extracted with ether (3 × 800 mL). The combined extracts were washed successively with water (2×) and brine (2×) and dried (Na_2SO_4), and the solvents were evaporated under reduced pressure. To the resulting oil were added 15.0 g of 5% Pd/C and AcOEt (100 mL), and the suspension was stirred vigorously under 4 kg/cm^2 of hydrogen for 3 h. Filtration of the reaction mixture through Celite followed by evaporation gave a residue. This was dissolved in EtOH–CH_2Cl_2 (400 mL, 1:1), and 30 mg of 4-(*N,N*-dimethylamino)pyridine was added. After cooling, to the mixture was added a solution of 132 g (640 mmol) of dicyclohexylcarbodiimide in CH_2Cl_2 (200 mL) slowly at 0 °C under stirring. The resulting slurry was kept stirring at 20 °C for 18 h, and AcOH (30 mL) was added at 0 °C. Further stirring was continued for 30 min at 0 °C and for 30 min at 20 °C. The mixture was filtered through a Celite pad, and the solvent was removed to give a oily residue. Purification by flash column chromatography on silica gel (ether–hexane, 1:15 to 1:5) afforded 59.0 g (60%) of the title compound as a colorless oil: bp 158 °C (0.2 mm); IR (neat) 2980, 2950, 1740, 1712, 1605 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.22 (3 H, t, J = 7 Hz), 1.50–1.75 (4 H, m), 1.58 (9 H, s), 2.15–2.45 (2 H, m), 2.50–2.75 (2 H, m), 4.10 (2 H, q, J = 7 Hz), 7.16 (2 H, d, J = 8 Hz), 7.85 (2 H, d, J = 8 Hz). Anal. ($C_{18}H_{26}O_4$) C, H.

Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]-5-hydroxy-2-hexenoate (3). Ethyl 4-bromocrotonate (17.4 g, 90.3 mmol) was slowly added to a refluxing suspension of 11.8 g (181 mmol) of zinc, 19.9 g (90.3 mmol) of *tert*-butyl 4-acetylbenzoate (**1b**), and 20 mg of iodine in benzene–ether–THF (200 mL, 3:3:2), and the mixture was refluxed for 1 h. A second portion (3.00 g, 15.5 mmol) of ethyl 4-bromocrotonate was added to the reaction mixture. After stirring for further 15 min, the reaction mixture was cooled to room temperature, poured into water (500 mL), and acidified with AcOH to pH 4.5. The aqueous layer was separated and extracted with ether (3 × 100 mL), and the combined organic extracts were washed with 5% aqueous NH_4OH (2×) and brine, dried (Na_2SO_4), and concentrated. Flash chromatography of the crude product (AcOEt–hexane, 1:5) gave 25.6 g (85%) of the title compound as a colorless oil: IR (neat) 3480, 2975, 1720, 1700, 1650, 1605 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.20 (3 H, t, J = 7 Hz), 1.53 (12 H, s), 2.64 (2 H, d, J = 7 Hz), 2.67 (1 H, br s), 4.08 (2 H, q, J = 7 Hz), 5.80 (1 H, d, J = 15 Hz), 6.80 (1 H, dt, J = 15, 7 Hz), 7.45 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz). Anal. ($C_{19}H_{26}O_5$) C, H.

Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]hexanoate (4b). To a solution of 22.3 g (66.7 mmol) of **3** in EtOH–AcOH (210 mL, 20:1) was added 5.0 g of 5% Pd/C, and the suspension was stirred vigorously under a hydrogen atmosphere for 115 h. The slurry was then filtered through Celite, followed by evaporation to give a residual oil. This was distilled under reduced pressure to give 18.2 g (85%) of the title compound as a colorless oil: bp 162–165 °C (0.3 mm); IR (neat) 2980, 2940, 1735, 1710, 1607, 848 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.20 (3 H, t, J = 7 Hz), 1.23 (3 H, d, J = 6 Hz), 1.30–1.80 (4 H, m), 1.58 (9 H, s), 2.24 (2 H, br t, J = 6 Hz), 2.77 (1 H, dq, J = 6, 6 Hz), 4.08 (2 H, q, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz). Anal. ($C_{19}H_{28}O_4$) C, H.

Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]-2-iodopentanoate (5a). Under an argon atmosphere, BuLi (24.4 mmol) in hexane (15.3 mL) was added to a solution of 2.48 g (24.5 mmol) of diisopropylamine in THF (100 mL) at 0 °C. After 10 min of stirring, the mixture was cooled to −78 °C, and 6.83 g (22.3 mmol) of **4a** in THF (50 mL) was added over 30 min. The resulting

mixture was stirred for 10 min at −78 °C and 5.66 g (22.3 mmol) of iodine in THF (30 mL) was added at a temperature below −60 °C. Stirring was continued for 20 min at −78 °C, and the temperature was allowed to warm up to 0 °C in 30 min. After addition of 1 N aqueous $KHSO_4$ solution (30 mL), the resulting aqueous phase was extracted with ether (3 × 100 mL). The combined extracts, after being washed with 1 N aqueous K_2CO_3 (2×) and brine and dried over $MgSO_4$, were evaporated to a residue. Purification by flash chromatography (ether–hexane, 1:9) gave 6.84 g (71%) of the title compound as a pale brown oil: IR (neat) 2990, 2905, 1744, 1718, 1612 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.25 (3 H, t, J = 7 Hz), 1.50–2.20 (4 H, m), 1.58 (9 H, s), 2.69 (2 H, t, J = 7 Hz), 4.20 (2 H, q, J = 7 Hz), 4.31 (1 H, t, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz). Anal. ($C_{18}H_{25}IO_4$) C, H.

Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]-2-(dicyanomethyl)pentanoate (6a). A suspension of 420 mg (17.5 mmol) of NaH in Me_2SO (3 mL) was heated at 70 °C for 1 h and cooled. To the resulting mixture was added 1.16 g (17.5 mmol) of malononitrile in Me_2SO (8 mL) at room temperature. After stirring for 5 min, a solution of 3.78 g (8.75 mmol) of **5a** in Me_2SO (8 mL) was added, and the mixture was stirred for 1 h at 20 °C. After the reaction was quenched with 1 N aqueous $KHSO_4$ (45 mL), the aqueous layer was extracted with ether (3 × 50 mL). The combined ether layer was washed with water (3×) and brine and concentrated to a residue under reduced pressure. This was purified by flash chromatography (AcOEt–hexane, 1:5) to afford 3.07 g (95%) of the title compound as a colorless oil: IR (neat) 2970, 2930, 2252, 1740, 1713, 1608 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.28 (3 H, t, J = 7 Hz), 1.50–2.05 (4 H, m), 1.58 (9 H, s), 2.70 (2 H, br t, J = 7 Hz), 2.85–3.15 (1 H, m), 4.03 (1 H, d, J = 7 Hz), 4.25 (2 H, q, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.92 (2 H, d, J = 8 Hz). Anal. ($C_{21}H_{26}N_2O_4$) C, H, N.

***tert*-Butyl 4-[3-(2,4-Diamino-6-oxo-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoate (7a).** To a suspension of 1.36 g (12.1 mmol) of *t*-BuOK and 1.07 g (11.2 mmol) of guanidine hydrochloride in *t*-BuOH (10 mL) was added a solution of 3.46 g (9.34 mmol) of **6a** in *t*-BuOH (10 mL), and the mixture was refluxed for 4 h. After being cooled to room temperature, the reaction mixture was then poured into water (150 mL), which resulted in the formation of a precipitate. Filtration followed by succesive washing with MeOH and ether and vacuum drying gave 3.19 g (89%) of the title compound as a white, crystalline solid. This was used in the following reaction without further purification. An analytical sample of **7a**, mp 224–225 °C, was obtained on recrystallization from MeOH: IR (KBr) 3430, 3360, 1710, 1627, 1583, 1432 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$–Me_2SO-d_6) δ 1.15–1.73 (2 H, m), 1.55 (9 H, s), 1.73–2.10 (2 H, m), 2.61 (2 H, t, J = 7 Hz), 3.35 (1 H, t, J = 6 Hz), 5.40 (2 H, br s), 5.51 (2 H, br s), 6.30 (1 H, br s), 7.12 (2 H, d, J = 8 Hz), 7.29 (2 H, d, J = 8 Hz). Anal. ($C_{20}H_{25}N_5O_3$) C, H, N.

***tert*-Butyl 4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoate (8a) and *tert*-Butyl 4-[3-(2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoate (9a).** To a suspension of 575 mg (1.50 mmol) of **7a** in THF (6 mL) was added 7.5 mL of 1.0 M BH_3–THF in THF under argon at 0 °C, and the resulting solution was stirred for 5 h. The reaction was quenched by addition of AcOH–MeOH (6 mL, 1:1), and the mixture was left to stand for 18 h at 20 °C. After evaporation in vacuo the residue was purified by flash chromatography (EtOH–CH_2Cl_2, eluent gradient 6:94 to 12:88) to give initially 248 mg (45%) of **8a** as a white solid and then 255 mg (46%) of **9a** as a viscous oil. **8a**: mp 172–173 °C; IR (KBr) 3335, 3180, 2975, 2935, 1710, 1607, 1287, 1163, 1110 cm^{-1}; ^1H NMR (200 MHz, Me_2SO-d_6) δ 1.54 (9 H, s), 1.77–1.90 (2 H, m), 2.68 (2 H, t, J = 8 Hz), 2.72 (2 H, t, J = 8 Hz), 5.54 (2 H, br s), 6.11 (2 H, br s), 6.45 (1 H, s), 7.33 (2 H, d, J = 8 Hz), 7.82 (2 H, d, J = 8 Hz), 10.51 (1 H, s). Anal. ($C_{20}H_{25}N_5O_2$) C, H, N. **9a**: IR (KBr) 3375, 3325, 3190, 2970, 2930, 1712, 1603 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$–Me_2SO-d_6) δ 1.20–1.70 (4 H, m), 1.54 (9 H, s), 2.55–2.70 (2 H, m), 3.00–3.15 (2 H, m), 3.43 (1 H, t, J = 9 Hz), 5.56 (4 H, br s), 6.11 (1 H, br s), 7.30 (2 H, d, J = 8 Hz), 7.80 (2 H, d, J = 8 Hz). Anal. ($C_{20}H_{27}N_5O_2$) C, H, N.

Dehydrogenation of 9a to 8a. A solution of $RuCl_2(PPh_3)_3$ (42 mg, 0.043 mmol) and **9a** (160 mg, 0.43 mmol) in THF (3 mL) was refluxed for 10 h. The resulting dark brown solution was concentrated under reduced pressure, and the residue was purified by alumina column chromatography (CH_2Cl_2 to EtOH–CH_2Cl_2, 1:4) to afford **8a** as a white solid (108 mg, 68%).

Diethyl *N*-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamate (10a). Under an argon atmosphere 381 mg (1.04 mmol) of **8a** was dissolved in CF_3COOH (3 mL), and the solution was left to stand for 2 h. After evaporation of CF_3COOH the residual oil was freed of impurities by evacuation under a pressure below 0.1 mmHg, for 2 h at 70 °C. To the mixed suspension containing the residue and 274 mg (1.14 mmol) of diethyl L-glutamate hydrochloride in DMF (4 mL) was added a solution of DPPA in DMF (4 mL) at 0 °C and the mixture stirred for 15 min. After addition of 421 mg (4.16 mmol) of triethylamine in DMF (4 mL), stirring was continued for 30 min at 0 °C and then for 63 h at room temperature. The slurry was filtered and the filtrate was concentrated to a residue. This was purified by flash chromatography (CH_2Cl_2 saturated with concentrated aqueous NH_4OH to 30:1 CH_2Cl_2–8% NH_3 in EtOH) to give 403 mg (78%) of the title compound as a white, microcrystalline powder: mp 81–82 °C (AcOEt–benzene); IR (KBr) 3330, 3160, 1735, 1632, 1575, 1540, 1500, 1200 cm^{-1}; ^1H NMR (200 MHz, Me_2SO-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.

***N*-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic Acid (12a).** To a solution of 250 mg (0.503 mmol) of **10a** in THF–water (6 mL, 2:1) was added 2.52 mL of 1 N aqueous NaOH at 20 °C, and the mixture was kept standing for 2 h. After the solution was concentrated to 2 mL in vacuo, 0.5 mL of AcOH was added dropwise. A white, crystalline precipitate was filtered and washed succesively with water, MeOH, and ether, and dried in vacuo over P_2O_5 to give 204 mg (89%) of the title compound: mp 180–181 °C dec; IR (KBr) 3340, 3200, 2940, 1660–1630, 1540, 1500, 1397 cm^{-1}; ^1H NMR (200 MHz, Me_2SO-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); SIMS m/z 441 (MH^+). Anal. ($C_{21}H_{24}N_6O_5 \cdot H_2O$) C, H, N.

Diethyl *N*-[4-[3-(2,4-Diamino-6,7-dihydro-5*H*-pyrrolo-[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamate (11a). In a similar manner as that described for **10a**, **9a** (6.83 g, 18.5 mmol) was converted to the title compound (8.22 g, 89%) of a viscous oil: IR (KBr) 3350, 2990, 2945, 1740, 1610, 1540, 1508, 1438 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.20 (3 H, t, J = 7 Hz), 1.27 (3 H, t, J = 7 Hz), 1.43–1.95 (6 H, m), 2.05–2.77 (4 H, m), 3.10–3.30 (2 H, m), 3.65 (1 H, t, J = 9 Hz), 4.12 (2 H, q, J = 7 Hz), 4.23 (2 H, q, J = 7 Hz), 4.25 (2 H, br s), 4.33 (1 H, br s), 4.56 (2 H, br s), 4.72–4.85 (1 H, m), 7.06–7.20 (1 H, m), 7.23 (2 H, d, J = 8 Hz), 7.78 (2 H, d, J = 8 Hz). Anal. ($C_{25}H_{34}N_6O_5$) C, H, N.

***N*-[4-[3-(2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)propyl]benzoyl]-L-glutamic Acid (13a).** In a similar manner as that described for **12a**, **11a** (7.85 g, 15.8 mmol) was hydrolyzed to the title compound (7.17 g, 99%), a white, microcrystalline powder: mp 180–181 °C; IR (KBr) 3700–2350, 3215, 1690–1620, 1540 cm^{-1}; ^1H NMR (200 MHz, Me_2SO-d_6) δ 1.20–1.75 (4 H, m), 1.80–2.13 (2 H, m), 2.31 (2 H, t, J = 7 Hz), 2.50–2.75 (2 H, m), 3.05–3.20 (2 H, m), 3.55 (1 H, t, J = 11 Hz), 4.15–4.45 (1 H, m), 6.38 (2 H, br s), 6.77 (2 H, br s), 6.90 (1 H, br s), 7.22 (2 H, d, J = 8 Hz), 7.74 (2 H, d, J = 8 Hz), 8.22 (1 H, d, J = 7 Hz); SIMS m/z 443 (MH^+). Anal. ($C_{21}H_{26}N_6O_5 \cdot H_2O$) C, H, N.

Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]-2-iodohexanoate (5b). Compound **4b** (6.41 g, 20.0 mmol) gave the title compound (6.25 g, 70%) as a colorless oil in the same way as that described for **5a**: IR (neat) 2980, 2940, 1738, 1715, 1610, 850 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ 1.23 (3 H, t, J = 7 Hz), 1.23 (3 H, d, J = 7 Hz), 1.40–1.95 (4 H, m), 1.60 (9 H, s), 2.75 (1 H, dq, J = 6, 6 Hz), 4.15 (2 H, q, J = 7 Hz), 4.18 (1 H, t, J = 7 Hz), 7.20 (2 H, d, J = 8 Hz), 7.90 (2 H, d, J = 8 Hz). Anal. ($C_{19}H_{27}IO_4$) C, H.

Ethyl 5-[4-(*tert*-Butoxycarbonyl)phenyl]-2-(dicyanomethyl)hexanoate (6b). Compound **5b** (3.90 g, 8.74 mmol) gave the title compound (3.19 g, 95%) as a colorless oil in the same way as that described for **6a**: IR (neat) 2980, 2930, 2250, 1735,

1710, 1605, 847 cm^{-1}; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.26 (1.5 H, t, J = 7 Hz), 1.26 (3 H, d, J = 7 Hz), 1.27 (1.5 H, t, J = 7 Hz), 1.35–1.80 (4 H, m), 1.58 (9 H, s) 2.50–3.08 (2 H, m), 4.00 (1 H, dd, J = 8, 4 Hz), 4.22 (1 H, q, J = 7 Hz), 4.23 (1 H, q, J = 7 Hz), 7.18 (2 H, d, J = 8 Hz), 7.92 (2 H, d, J = 8 Hz). Anal. ($C_{22}H_{28}N_2O_4$) C, H, N.

***tert*-Butyl 4-[3-(2,4-Diamino-6-oxo-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-1-methylpropyl]benzoate (7b).** Compound **6b** (3.18 g, 8.27 mmol) afforded the title compound (2.61 g, 79%) as white crystals in the same way as that described for **7a**: mp 240–241 °C; IR (KBr) 3360, 3235, 2975, 2700, 1715, 1625, 1584, 1438, 1290, 1163, 1118, 848 cm^{-1}; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 1.14 (3 H, d, J = 7 Hz), 1.20–1.50 (2 H, m), 1.54 (9 H, s), 1.55-1.80 (1 H, m), 1.80–2.05 (1 H, m), 2.60–2.78 (1 H, m), 3.20–3.30 (1 H, m), 5.86 (2 H, br s), 5.96 (2 H, br s), 7.25 (2 H, d, J = 8 Hz), 7.81 (2 H, d, J = 8 Hz), 10.42 (1 H, s). Anal. ($C_{21}H_{27}N_5O_3$) C, H, N.

***tert*-Butyl 4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-1-methylpropyl]benzoate (8b) and *tert*-Butyl 4-[3-(2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-1-methylpropyl]benzoate (9b).** Compound **7b** (2.00 g, 5.03 mmol) was reduced to **8b** (768 mg, 40%) and **9b** (1.02 g, 53%). Each product was a viscous oil. **8b**: IR (KBr) 3350, 3200, 2980, 2940, 1714, 1650, 1608, 1290, 1163, 1115, 848 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.13 (3 H, d, J = 7 Hz), 1.60 (9 H, s), 1.94 (2 H, dt, J = 8, 8 Hz), 2.40–2.60 (2 H, m), 2.85 (1 H, tq, J = 7, 7 Hz), 4.50–5.50 (4 h, br), 6.46 (1 H, s), 7.27 (2 H, d, J = 8 Hz), 7.96 (2 H, d, J = 8 Hz), 9.20 (1 H, br s). Anal. ($C_{21}H_{27}N_5O_2$) C, H, N. **9b**: IR (KBr) 3340, 3195, 2980, 2935, 1715, 1607, 1430, 1295, 1163, 1115, 847 cm^{-1}; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 1.18 (2.25 H, d, J = 7 Hz), 1.19 (0.75 H, d, J = 7 Hz), 1.23–1.42 (2 H, m), 1.45–1.65 (2 H, m), 1.54 (9 H, s), 2.64–2.70 (1 H, m), 2.90–3.08 (2 H, m), 3.30–3.50 (1 H, m), 5.43 (4 H, s), 5.95 (0.25 H, s), 6.00 (0.75 H, s), 7.32 (2 H, d, J = 8 Hz), 7.82 (2 H, d, J = 8 Hz). Anal. ($C_{21}H_{29}N_5O_2$) C, H, N.

Diethyl *N*-[4-[3-(2,4-Diamino-7*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-L-glutamate (10b). Compound **8b** (540 mg, 1.42 mmol) gave the title compound (556 mg, 77%) as a white, microcrystalline powder in the same way as that described for **10a**: mp 87–88 °C; IR (KBr) 3340, 3180, 2935, 1735, 1640, 1610, 1580, 1200, 1095, 1018, 850 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.23 (3 H, t, J = 7 Hz), 1.30 (3 H, d, J = 7 Hz), 1.31 (3 H, t, J = 7 Hz), 1.80–2.05 (4 H, m), 2.15–2.57 (4 H, m), 2.83 (1 H, tq, J = 7, 7 Hz), 4.12 (2 H, q, J = 7 Hz), 4.25 (2 H, q, J = 7 Hz), 4.68 (2 H, br s), 4.75–4.87 (1 H, m), 4.92 (2 H, br s), 6.43 (1 H, s), 7.26 (2 H, d, J = 8 Hz), 7.37 (1 H, dd, J = 7, 3 Hz), 7.77 (2 H, d, J = 8 Hz), 8.81 (1 H, br s). 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)-1-methylpropyl]benzoyl]-L-glutamic Acid (12b).** In the same way, compound **10b** (540 mg, 1.06 mmol) gave the desired compound (436 mg, 89%) as a white, microcrystalline powder: mp 180–181 °C; IR (KBr) 3350, 3205, 1650, 1640, 1540, 1400, 850 cm^{-1}; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 1.25 (3 H, d, J = 7 Hz), 1.73–2.20 (4 H, m), 2.35 (2 H, t, J = 8 Hz), 2.40–2.68 (2 H, m), 2.85 (1 H, tq, J = 7, 7 Hz), 4.32–4.45 (1 H, m), 5.54 (2 H, br s), 6.06 (2 H, br s), 6.38 (1 H, s), 7.33 (2 H, d, J = 8 Hz), 7.83 (2 H, d, J = 8 Hz), 8.49 (1 H, d, J = 8 Hz), 10.45 (1 H, s); SIMS m/z 455 (MH^+). Anal. ($C_{22}H_{26}N_6O_5 \cdot 0.5H_2O$) C, H, N.

Diethyl *N*-[4-[3-(2,4-Diamino-6,7-dihydro-5*H*-pyrrolo-[2,3-*d*]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-L-glutamate (11b). In the same way as that described for **11a**, **9b** (581 mg, 1.51 mmol) gave the title compound (640 mg, 82%), a colorless, viscous oil: IR (KBr) 3375, 3200, 2975, 2930, 1738, 1608, 1430, 1200, 1008, 853 cm^{-1}; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 1.17 (3 H, t, J = 7 Hz), 1.18 (3 H, d, J = 7 Hz), 1.19 (3 H, t, J = 7 Hz), 1.26–1.44 (2 H, m), 1.44–1.63 (2 H, m), 1.90–2.20 (2 H, m), 2.44 (2 H, t, J = 7 Hz), 2.63–2.80 (1 H, m), 2.90–3.08 (2 H, m), 3.30–3.50 (1 H, m), 4.05 (2 H, q, J = 7 Hz), 4.11 (2 H, q, J = 7 Hz), 4.37–4.50 (1 H, m), 5.36 (2 H, s), 5.37 (2 H, s), 5.87 (0.25 H, s), 5.91 (0.75 H, s), 7.30 (2 H, d, J = 8 Hz), 7.80 (2 H, d, J = 8 Hz), 8.66 (1 H, d, J = 8 Hz). Anal. ($C_{26}H_{36}N_6O_5$) C, H, N.

***N*-[4-[3-(2,4-Diamino-6,7-dihydro-5*H*-pyrrolo[2,3-*d*]pyrimidin-5-yl)-1-methylpropyl]benzoyl]-L-glutamic Acid (13b).** In the same way, compound **11b** (600 mg, 1.17 mmol) was hydrolyzed to the title compound (508 mg, 92%), a white, microcrystalline powder: mp 187–188 °C; IR (KBr) 3350, 3200, 1690, 1680–1610, 1635, 1530, 1400, 1300, 850 cm^{-1}; ^{1}H NMR (200 MHz, Me_2SO-d_6) δ 1.20 (3 H, d, J = 7 Hz), 1.25–1.65 (4 H, m), 1.87–2.20 (2 H, m), 2.30 (2 H, t, J = 7 Hz), 2.60–2.80 (1 H, m), 3.00–3.20 (2 H, m), 3.42–3.60 (1 H, m), 4.22–4.40 (1 H, m), 6.20–7.08 (5 H, m), 7.28 (2 H, d, J = 8 Hz), 7.78 (2 H, d, J = 8 Hz), 8.28–8.38 (1 H, m); SIMS m/z 457 (MH^+). Anal. ($C_{22}H_{28}N_6O_5 \cdot 0.5H_2O$) C, H, N.

Growth Inhibition Assay. Human epidermoid carcinoma KB cells (1×10^4 cells/mL) and human lung carcinoma A549 cells (1×10^4 cells/mL) were inoculated into each well of the 96-microwell plate and cultured in MEM medium containing 10% fetal calf serum at 37 °C under 5% CO_2 for 24 h. To this culture was added a solution of each pyrrolopyrimidine antifolate in 10% MEM at final concentration of 0.15–80 ng/mL. Then the culture was continued at 37 °C under CO_2 for 72 h, and the number of cells was estimated by the MTT method[18] to determine the concentration required for 50% inhibition of growth (IC_{50}). Data are presented as means ± SE of three experiments.

Acknowledgment. We thank Drs. Y. Sugino, M. Nishikawa, and S. Terao for their encouragement and valuable advice throughout this work. We are indebted to Mr. H. Yamamoto for the growth inhibitory assay and to Mr. M. Takamoto for MNDO calculation.

Registry No. **1a**, 65874-27-3; **1b**, 105580-41-4; **2**, 130351-29-0; **2′**, 130351-34-7; **3**, 130351-30-3; **4a**, 130351-31-4; **4b**, 125991-45-9; **5a**, 130351-32-5; **5b**, 125991-46-0; **6a**, 130351-33-6; **6b**, 125991-47-1; **7a**, 126016-77-1; **7b**, 130377-72-9; **8a**, 125991-49-3; **8b**, 130377-73-0; **9a**, 125991-56-2; **9b**, 125991-61-9; **10a**, 125991-50-6; **10b**, 125991-65-3; **11a**, 125991-57-3; **11b**, 125991-63-1; **12a**, 125991-51-7; **12b**, 125991-66-4; **13a**, 125991-59-5; **13b**, 125991-64-2; CH_3CH=CHCOOEt, 10544-63-5; $BrCH_2CH$=CHCOOEt, 6065-32-3; $CH_2(CN)_2$, 109-77-3; H-Glu(OEt)-OEt·HCl, 1118-89-4; guanidine hydrochloride, 50-01-1.

(18) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.